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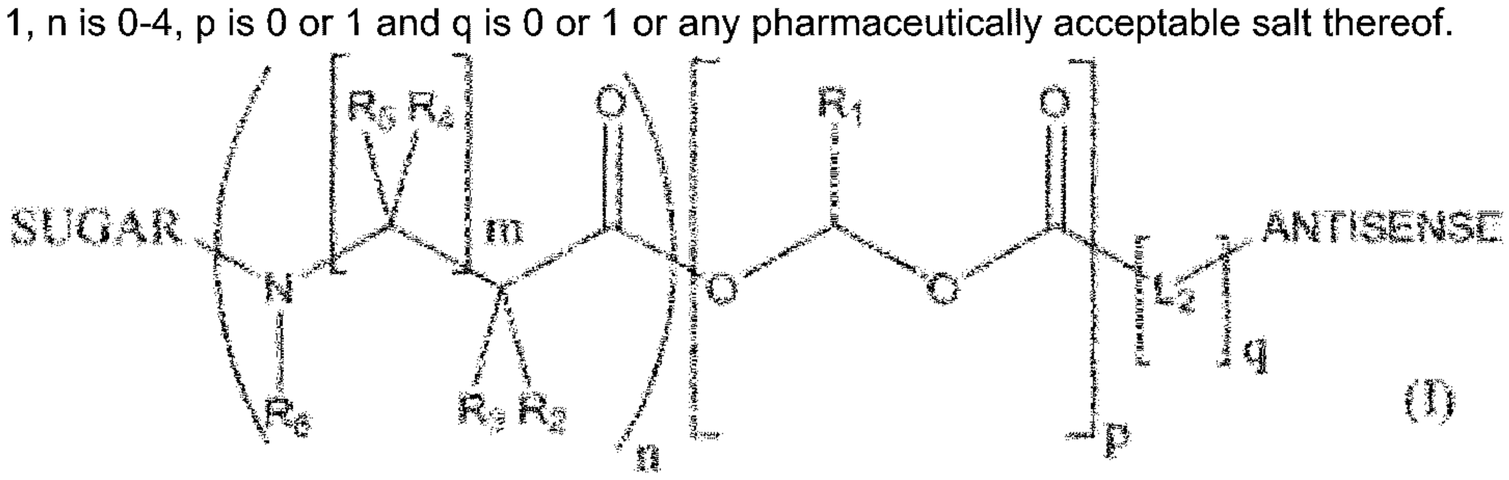
(54) Title of the Invention: **Antibacterial antisense agents**
Abstract Title: **Conjugates of antibacterial antisense oligonucleotides**

(57) Improved antisense oligonucleotide agents of formula (I) are disclosed for the treatment of pathogenic gram-negative bacterial infections. The said agents utilise an antibiotic-assisted translocation (AAT) platform to improve influx into bacterial cells through enhanced permeability, providing improved intracellular exposure of the antisense agent and superior treatment of the infection. The said agent takes the form of an acyl fragment of an N-acylmuramic acid or 1,6-anhydro-N-acylmuramic acid (SUGAR) which is conjugated to said oligonucleotide (ANTISENSE) via a linker as defined therein and L_2, R_1, R_2, R_3, R_4, R_5, R_6 are each as defined therein and m is 0 or 1, n is 0-4, p is 0 or 1 and q is 0 or 1 or any pharmaceutically acceptable salt thereof.



(I)

GB 2579253 A

ANTIBACTERIAL ANTISENSE AGENTS

FIELD OF THE INVENTION

The present invention relates to compounds that include antisense oligomers targeted against genes that contribute to virulence, antibiotic resistance, biofilm formation or essential growth and survival processes in pathogenic bacteria. The disclosure herein details compounds that include antisense oligomers that are useful in the monotherapy treatment of bacterial infections or through the use of combinations with known antibiotics to impart improved and clinically meaningful activity (minimum inhibitory concentration; MIC) against pathogenic gram-negative bacteria. Whilst not bound by a specific theory, at an equivalent dose, compounds of the invention may generate higher intracellular concentrations of the antisense oligomer in bacterial cells than can be otherwise achieved by use of the antisense oligomer alone. Compounds of the invention contain an antibiotic-assisted translocation (AAT) moiety that imparts increased influx into bacterial cells through enhanced permeability, providing improved intracellular exposure of the antisense oligomer and superior treatment of the infection.

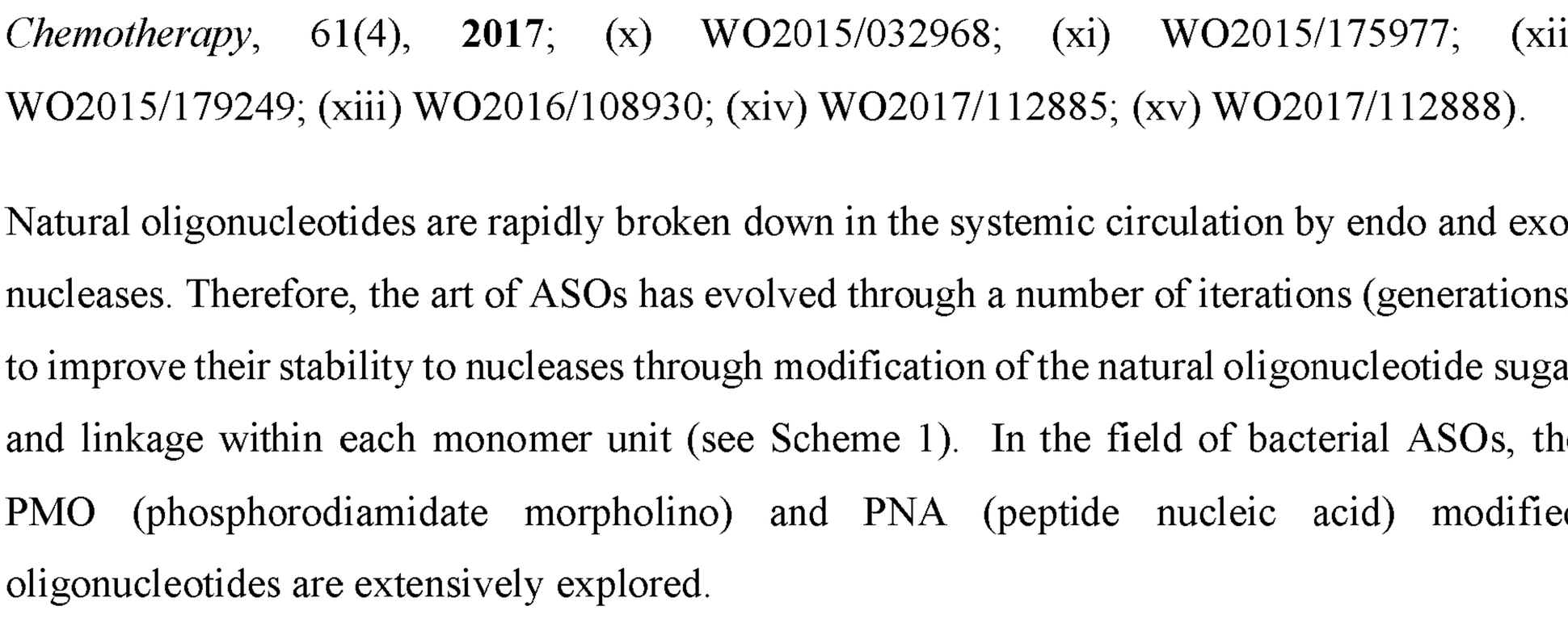
BACKGROUND TO THE INVENTION

Drug-resistant infections are already responsible for a significant number of deaths globally each year and the development of new therapeutic approaches and new antibacterial drugs is becoming an increasingly urgent requirement. Of these drug-resistant infections, those caused by MDR Gram-negative pathogens such as *Enterobacter species, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are amongst the most serious health threats. Many Gram-negative bacteria are now resistant to a significant number of old and current antibiotics and can cause infections that are difficult to treat.

A new therapeutic approach to treatment of human diseases and infections is through the use of antisense oligonucleotides (ASOs) that target protein biosynthesis at the genetic level to provide the positive therapeutic endpoint. A number of products based on these principles have been approved for use, for example Fomivirsen® (an antisense antiviral drug that was used in the treatment of cytomegalovirus retinitis (CMV) in immunocompromised patients), Kynamro® (used to treat homozygous familial hypercholesterolemia), and Alicaforsen® (that targets the mRNA for the production of human ICAM-1 protein and indicated for pouchitis).

The treatment of bacterial infections through the use of ASOs that target gene products essential for bacterial growth, survival or the development of resistance mechanisms holds enormous potential (e.g. see (i) Bai, H. *et al., Curr. Drug Disc. Tech.,* 7, 76-85, **2010**; (ii) Hatamoto, M. *et al., Appl. Microbiol. Technol.,* 86, 397-402, **2010**; (iii) Geller, B. L. *et al., J. Infec. Dis.,* 208, 1553-1560, **2013**; (iv) Good, L. & Stach, E. M., *Frontiermicrobiol.,* 2, 185, **2011**; (v) Hansen, A. M. *et al., Bioconj. Chem.* 27(4), 863-7, **2016**; (vi) Sully, E. K. & Geller, B. L. *Curr. Opin. Microbiol.,* 33, 47-55, **2016**; (vii) Hegarty, J. P. & Stewart Sr, D. B. *Appl. Microbiol & Biotech.* 102(3), 1055-65, **2018**; (viii) Geller, B. L. *et al., J. Antimicrob. Chemotherapy,* 73, 1611-1619, **2018**; (ix) Howard, J. J. et al., *Antimicrob. Agents & Chemotherapy*, 61(4), **2017**; (x) WO2015/032968; (xi) WO2015/175977; (xii) WO2015/179249; (xiii) WO2016/108930; (xiv) WO2017/112885; (xv) WO2017/112888).

Natural oligonucleotides are rapidly broken down in the systemic circulation by endo and exo-nucleases. Therefore, the art of ASOs has evolved through a number of iterations (generations) to improve their stability to nucleases through modification of the natural oligonucleotide sugar and linkage within each monomer unit (see Scheme 1). In the field of bacterial ASOs, the PMO (phosphorodiamidate morpholino) and PNA (peptide nucleic acid) modified oligonucleotides are extensively explored.



Scheme 1. Modified oligonucleotide monomer units utilised in ASO sequences

To exert their therapeutic effect, bacterial ASOs need to penetrate the bacterial membranes and transit into the cytoplasm. Unlike eukaryotic cells, bacteria have the double-strand DNA located in the bacterial nucleoid that has no nucleic membrane. RNA transcription and protein synthesis in bacteria are processed in the cytoplasm and as such antisense oligomers that reach this intracellular compartment may exert their effect. Natural and modified ASOs do not possess the physiochemical properties required to achieve this and at present virtually all intracellular delivery of ASOs requires attachment of a cell-penetrating peptide (CPP) signal. Historically, CPPs are small highly charged peptide signals (6→ 20+ amino acids in length) with origins from HIV TAT protein or penetratin, a 16-residue peptide derived from the *Drosophila Antennapedia* gene (e.g. see (i) McClorey, G & Banerjee, S., *Biomedicines,* 6(2), E51, **2018**; (ii) Shiraishi, T and Nielsen, P. E. *Methods Mol. Biol.,* 1050, 193-205, **2014**). Although effective, in general CPPs are non-discriminant and the antisense cargo that is attached to a CPP is delivered into many tissues and cells, leading to toxicity and low therapeutic windows for the disease of interest. Therefore, a mechanism of toxicity for CPP-ASOs is hybridization-dependent off-target effects in healthy cells that can potentially occur due to the binding of ASOs to complementary regions of unintended RNAs. This off-target toxicity becomes an even more important consideration as the number of complementary regions increases dramatically with tolerated mismatches (e.g. see Yoshida, T. *et al., Genes Cells,* 23(6), 448-455, **2018**). Therefore, a method to improve the preferential delivery of bacterial ASOs into bacteria, whilst minimising intracellular exposure into healthy human cells and thereby significantly reducing the potential for unwanted off-target effects would provide a major advance to the state of the art.

The bacterial uptake mechanism detailed herein exploits naturally occurring sugars, namely N-acetyl D-muramic acid (MurNAc) which is the ether of lactic acid and *N*-acetylglucosamine and is a key element in forming the backbone of the cell wall peptidoglycan of Gram-negative bacteria and a cyclic variant namely 1,6-anhydro-*N*-acetylmuramic acid (anhMurNAc). Chemical attachment of these sugars to the ASO provides the compounds of the present invention, herein termed AAT agents. Because the AAT antisense agent requires cytoplasm-based enzymes within the bacteria to release the parent ASO, only low levels of the parent ASO are ever present in the peripheral circulation. Also, since a CPP signal peptide is not used herein, the AAT ASOs detailed herein exhibit very limited penetration into healthy mammalian cells and therefore a dramatically improved opportunity for a beneficial toxicity profile. This

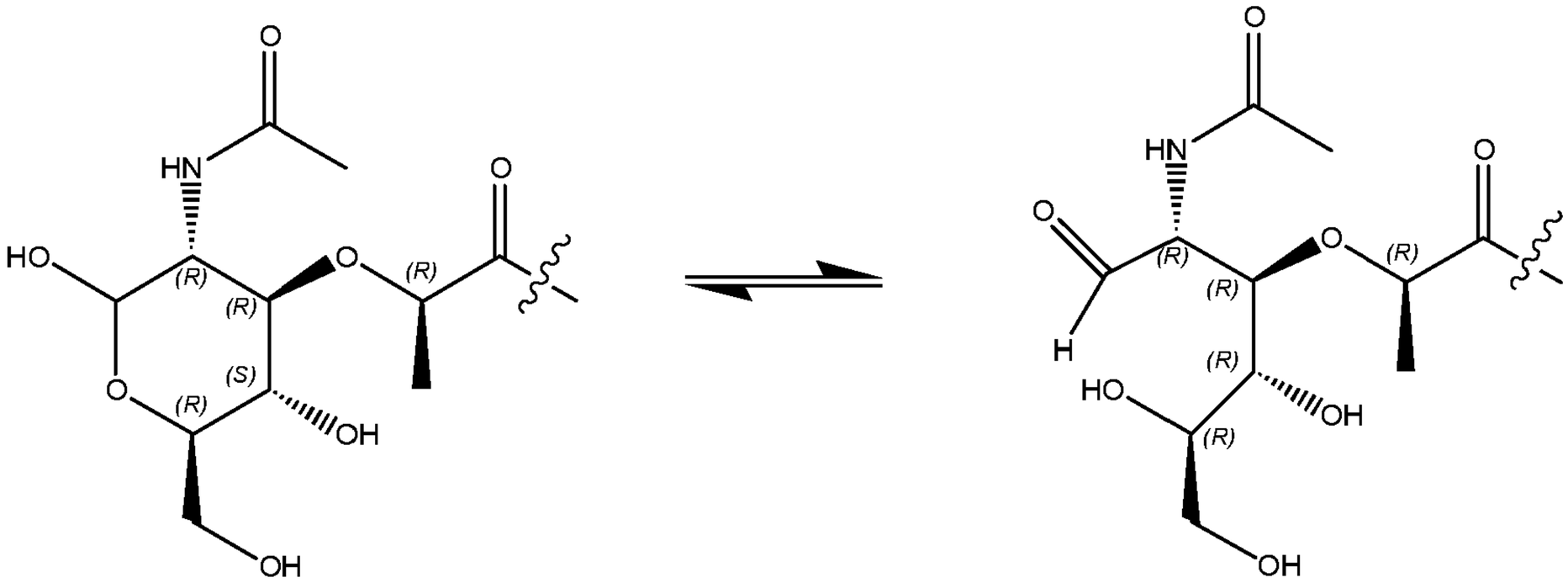
is a key aspect of the invention since the most effective AAT agent aims to provide intracellular exposure of the ASO primarily within the bacteria.

SUMMARY OF THE INVENTION

A bacterial antisense sequence is conjugated (i.e. chemically bonded) to a sugar moiety thereby providing an AAT agent with selective uptake across the bacterial membranes into the cytoplasm of Gram-negative bacteria. Depending upon the design of the AAT agent, the parent antisense may be subsequently cleaved and released through bacterial enzymatic process(s) catalysed by a selective ligase and/or amidase. Alternatively, the full AAT construct may remain intact and elicit a similar or equivalent antisense activity (e.g. see Bai, H. *et al ibid* wherein a CPP-ASO retained full potency with respect to the parent ASO).

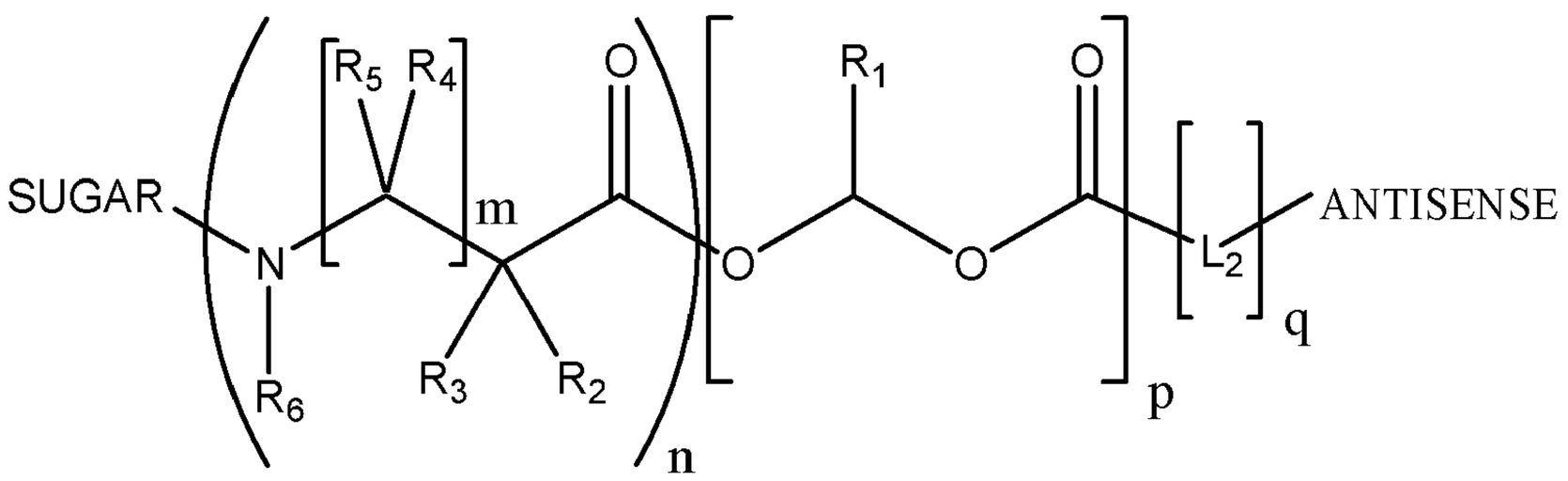
The AAT ASO compounds of the present invention may have intrinsic antibacterial activity when targeting genes that produce protein products that are essential for bacterial growth and survival. Alternatively, the AAT ASOs of the present invention may target bacterial genes that produce protein products that have evolved as resistance mechanisms for otherwise effective antibacterial drugs. In this example, then a combination of the AAT ASO and an existing antibiotic may improve the antibacterial activity of the antibiotic by concomitant antisense inhibition of the bacterial resistance mechanism.

The compounds of the present invention (AAT agents) may be any tautometric form of the sugar, including an open or cyclic (closed) form. It will be understood to those skilled in the art that when in solution the sugar groups exist in equilibrium between their open chain acyclic and closed cyclic forms. For instance, one sugar of interest in the present invention, *N*-acetyl muramic acid exists in the forms as shown in the tautomeric equilibrium (Scheme 2). Within the scope of the invention are compounds in both the open acyclic or closed cyclic form or in equilibrium between the two forms. Wherein the AAT agent is shown in one form, it is intended to include the other tautomeric form as well as both open and closed forms in equilibrium.



Scheme 2

A first aspect of the invention relates to compounds of general formula (I), and pharmaceutically acceptable salts thereof,

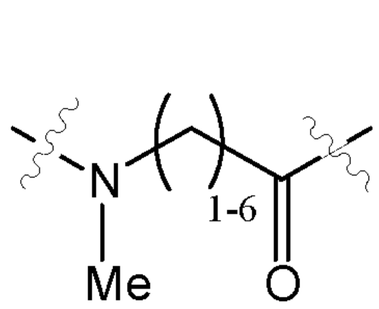


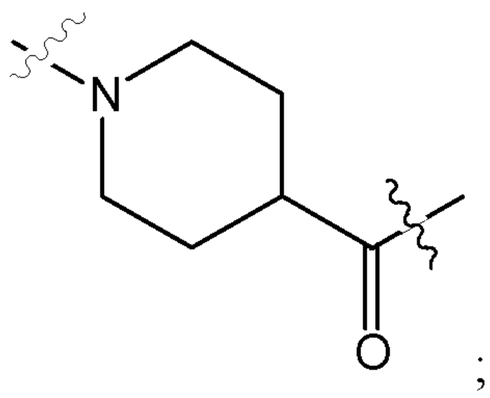
(I)

wherein,

ANTISENSE is an oligonucleotide having natural, artificial and/or modified nucleobases, the oligonucleotide selected from the group consisting of phosphodiester oligonucleotides (PDOs), phosphorothioate oligonucleotides (PSOs), phosphorodiamidate morpholino oligonucleotides (PMOs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs), 2'-O-Alkyl oligonucleotides (2'-O-Me, 2'-O-Et. 2'-O-methoxyethyl) and combinations thereof; wherein the oligonucleotide is bonded to the remainder of the molecule of formula I via a terminal amino group present within the ANTISENSE sequence; and

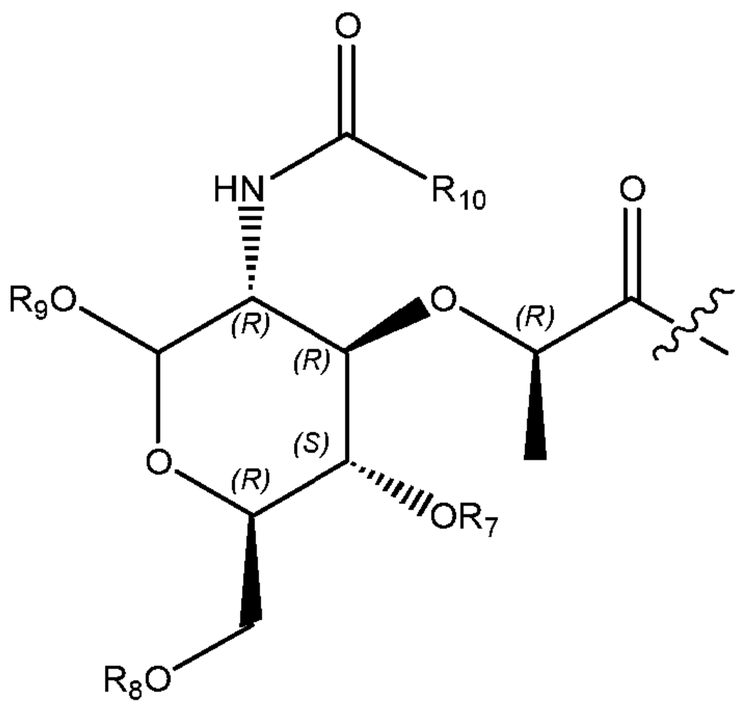
L_2 is a spacer that forms a chemical bond to a terminal amino group present within the ANTISENSE sequence and a second chemical bond to the terminal carbonyl of the remainder of the molecule of formula I and is chosen from the group consisting of:



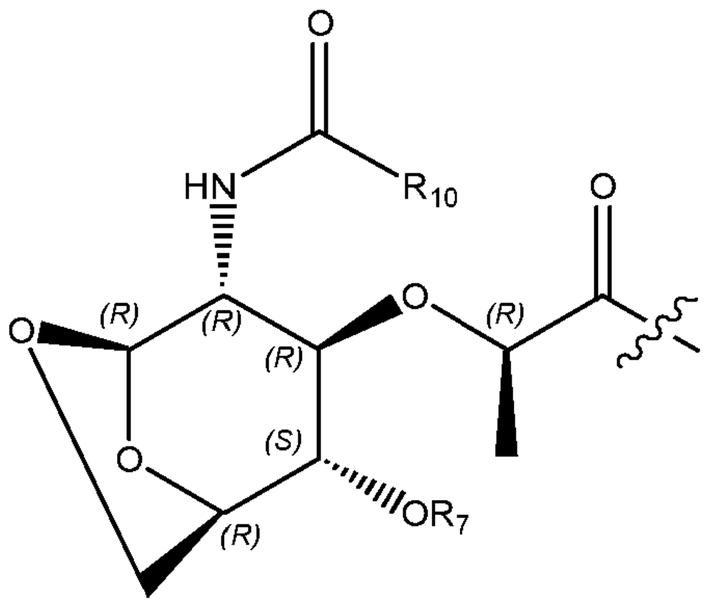


;

SUGAR is any tautomeric form of the acyl fragment of an *N*-acylmuramic acid or 1,6-anhydro-*N*-acylmuramic acid having the structure:



or



;

R_1 and R_6 are each independently selected from the group consisting of:

H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl;

R_2 and R_3 are each independently selected from the group consisting of:

H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl, or both together with the carbon atom to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms; and

R_4 and R_5 are each independently selected from the group consisting of:

H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl, or both together with the carbon atom to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms;

or

R_2 and R_4 together with the adjacent carbon atoms to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms; and R_3 and R_5 are each independently selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl, or both together with the carbon atom to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms;

R_7, R_8 and R_9 are each independently selected from the group consisting of:

H, acetyl, benzoyl; and

R_{10} is selected from the group consisting of:

methyl, ethyl, propyl; and

m is 0 or 1; and

n is 0 or 1 or 2 or 3 or 4; and

p is 0 or 1; and

q is 0 or 1.

A second aspect of the invention relates to a compound of the invention for use as a medicament.

A third aspect of the invention relates to a pharmaceutical or veterinary composition comprising a compound of the invention and a pharmaceutically acceptable or veterinarily acceptable diluent, excipient and/or carrier.

A fourth aspect of the invention relates to a compound of the invention for use in the treatment of pathogenic infections.

A fifth aspect of the invention relates to a compound of the invention for use in the treatment of pathogenic multi-drug resistant (MDR) gram-negative bacterial infections.

A sixth aspect of the invention relates to a compound of the invention for use as a therapeutic in combination with any other antibiotic.

A seventh aspect of the invention relates to a method of treating pathogenic infections that involves administering to a subject in need thereof a therapeutically effective amount of a compound of the invention.

An eighth aspect of the invention relates to a method of treating pathogenic multi-drug resistant (MDR) gram-negative bacterial infections that involves administering to a subject in need thereof a therapeutically effective amount of a compound of the invention.

A ninth aspect of the invention relates to a method of reducing the adverse side-effects associated with systemic exposure to an antisense oligonucleotide through use of a compound of the invention to target preferential accumulation of the antisense oligonucleotide within multi-drug resistant (MDR) gram-negative bacteria.

A tenth aspect of the invention provides a method comprising intravenous administration to a subject of a therapeutically effective amount of a compound of the invention.

An eleventh aspect of the invention relates to intravenous administration of compounds of the invention providing direct distribution to pathogen infected tissues prior to passage and metabolism in the hepatic circulation.

A twelfth aspect of the invention provides preferential accumulation of compounds of the invention in gram-negative pathogen infected cells when compared to other mammalian cells and tissues.

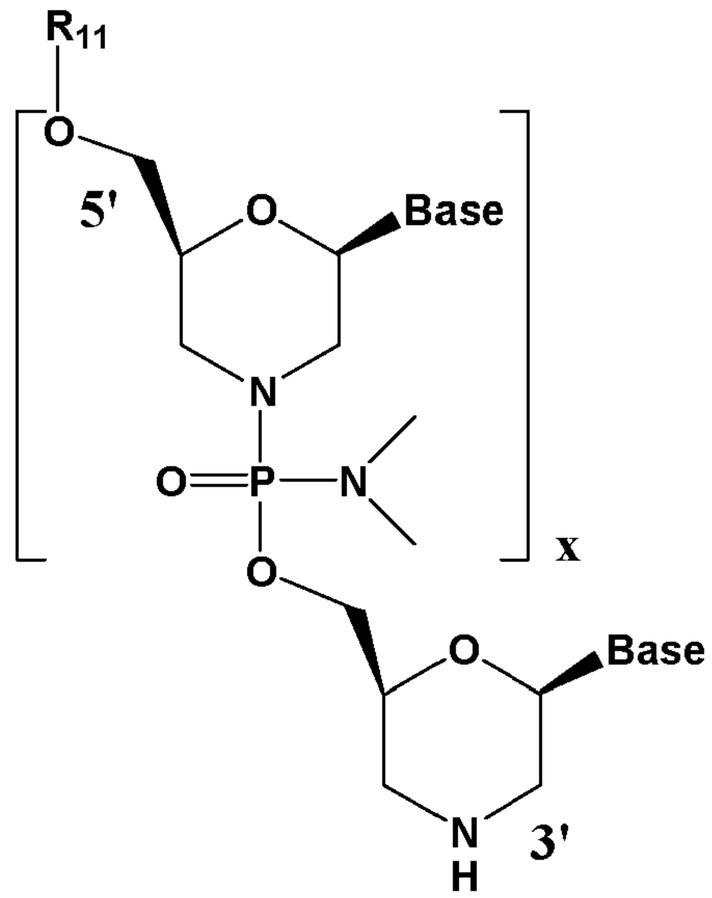
A thirteenth aspect of the invention relates to the use of a compound according to the invention in combination with an existing antibiotic towards an advantageous change in the optimal pharmacokinetic-pharmacodynamic relationship that is otherwise observed for the existing antibiotic.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

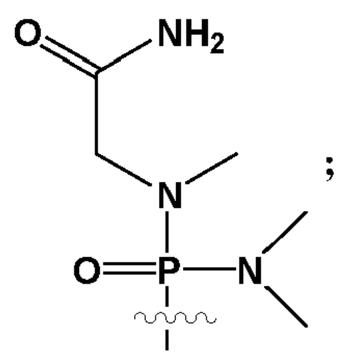
As used herein, the terms "ANTISENSE", "ASO" or "oligomer" refers to a linear sequence of nucleotides, or nucleotide analogues, which allows the nucleobases (e.g. a purine or pyrimidine) to mimic the structure of nucleic acid and bind through well characterised Watson-Crick base pairing to bacterial DNA or RNA to prevent production of protein products that are essential for bacterial growth, survival or development of resistance mechanisms. The terms "ANTISENSE", "ASO" or "oligomer" also encompass sequences that have one or more additional moieties conjugated at the 5'- or 3'-end such as a 5'-N-methylgylcinamide.

Typically, the synthetic oligomers are modified sequences termed PMOs or PNAs as depicted below.



PMO

wherein R_{11} = H;



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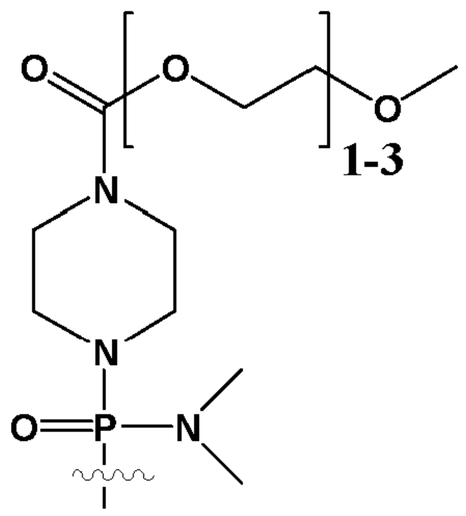
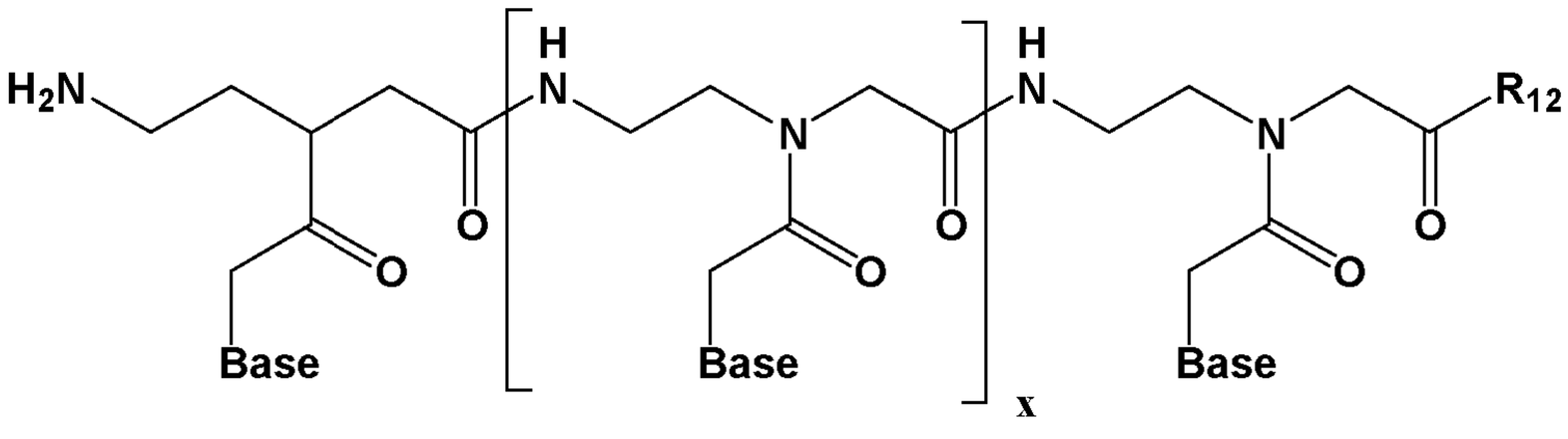




PNA

R_{12} = OH; NH_2

The term "antibiotic", “antibacterial” or “antibacterial agent”, unless otherwise indicated, refers to any of the classes of compounds that have antibacterial activity against Gram-positive or Gram-negative bacteria.

The term "SPACER”, unless otherwise indicated, refers to a fragment (L_2 in formula (I)) that chemically bonds the “ANTISENSE” to the “LINKER” that in turn is bonded to the “SUGAR”

such that the chemical bonds can be stable or cleaved by intracellular bacterial enzymatic processes.

The term "LINKER", unless otherwise indicated, refers to a fragment that chemically bonds the "SUGAR" to the "SPACER" that in turn is bonded to the "ANTISENSE" such that the chemical bonds can be stable or cleaved by intracellular bacterial enzymatic processes.

The term "SUGAR", unless otherwise indicated, refers to the acyl fragment that chemically bonds to the "LINKER" which in turn chemically bonds to "SPACER" that in turn chemically bonds to the "ANTISENSE" such that the chemical bonds can be stable or can be cleaved by intracellular bacterial enzymatic processes. Herein, the term "SUGAR" specifically refers to *N*-acetyl D-muramic acid (MurNAc), 1,6-anhydro-*N*-acetyl D-muramic acid (anhMurNAc) and the simple N-acyl variants thereof within the scope of general formula I. One or more of the functional groups within the "SUGAR" may be protected. Suitable amino-protecting groups include, for example, acetyl and azido. Suitable hydroxy-protecting groups include, for example acetyl, benzyl, benzoyl and benzylidine.

The term "SUGAR-LINKER", unless otherwise indicated, refers to the acyl fragment that is formed by chemical bonding of the "SUGAR" and "LINKER" and in turn chemically bonds to the "SPACER-ANTISENSE" to form the full "SUGAR-LINKER-SPACER-ANTISENSE AGENT".

The term "SUGAR-LINKER-SPACER", unless otherwise indicated, refers to the acyl fragment that is formed by chemical bonding of the "SUGAR" and "LINKER" and "SPACER" that in turn chemically bonds to the "ANTISENSE" to form the full "SUGAR-LINKER-SPACER-ANTISENSE AGENT".

As used herein, the term 'alkyl' includes stable straight and branched chain aliphatic carbon chains which may be optionally substituted. Preferred examples include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, pentyl, isopentyl and hexyl and any simple isomers thereof. Preferably, the alkyl group is a C_{1-4} alkyl group. Substituents for the alkyl group may be halogen, e.g. fluorine, chlorine, bromine and iodine, OH or C_1-C_4 alkoxy. Other substituents for the alkyl group may alternatively be used.

'Halogen' or 'Halo' as applied herein encompasses F, Cl, Br, I.

'Heteroatom' as applied herein encompasses O, S, P and N, more preferably, O, S and N.

As used herein, the term "cycloalkyl" refers to a cyclic alkyl group (i.e. a carbocyclic ring) which may be substituted (mono- or poly-) or unsubstituted. Substituents for the cycloalkyl group may be halogen, e.g. fluorine, chlorine, bromine and iodine, OH, C_1-C_4 alkyl or C_1-C_4 alkoxy. Other substituents for the cycloalkyl group may alternatively be used. Suitable substituents include, for example, one or more halo groups. Preferably, the cycloalkyl group is a C_{3-6}-cycloalkyl. Examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like. In addition, the carbocyclic ring itself may optionally contain one or more heteroatoms, for example, to give a heterocycloalkyl group such as tetrahydrofuran, pyrrolidine, piperidine, piperazine or morpholine.

Aromatic groups (e.g. phenyl and benzyl) may be optionally substituted, for example, by one or more C_{1-6} alkoxy, OH, COOH, COOMe, NH_2, NMe_2, NHMe, NO_2, CN, CF_3 and/or halo groups.

Heteroaromatic groups may be optionally substituted, for example, by one or more C_{1-6} alkoxy, OH, COOH, COOMe, NH_2, NMe_2, NHMe, NO_2, CN, CF_3 and/or halo groups.

The present invention includes all salts, hydrates, solvates, complexes of the compounds of this invention. The term "compound" is intended to include all such salts, hydrates, solvates, complexes and prodrugs, unless the context requires otherwise.

The present invention also includes deutero analogues of the compounds of this invention (see (a) Tung, R., "Deuterium medicinal chemistry comes of age", *Future Med. Chem.*, 8(5), 491-4, **2016**; (b) Uttamsingh, V. *et al.*, "Altering metabolic profiles of drugs by precision deuteration", *J. Pharmacol. Exp. Ther.*, 354(1), 43-54, **2015**). The term "compound" is intended to also include all deutero analogues, unless the context requires otherwise.

Abbreviations and symbols commonly used in the peptide and chemical arts are used herein to describe compounds of the present invention, following the general guidelines presented by the IUPAC-IUB Joint Commission on Biochemical Nomenclature as described in *Eur. J. Biochem.*, 158, 9-, 1984. Compounds of formula (I) and the intermediates and starting materials used in their preparation are named in accordance with the IUPAC rules of nomenclature in which the characteristic groups have decreasing priority for citation as the principle group.

The term "AAT agent", as used herein, includes but is not limited to a compound of the invention that includes the ANTISENSE sequences listed in Table 1A – Table 1D, wherein the "SUGAR-LINKER-SPACER" is covalently attached through the preferred functional groups detailed. The AAT agent may be therapeutically inactive until cleaved to release the parent ANTISENSE or may retain inherent antibacterial activity of its own.

Unless otherwise specified, the term "naturally occurring" refers to occurring in nature, for example, in bacteria or in a mammal (e.g., a human).

In one embodiment, the term "pharmaceutically acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic (e.g., trifluoroacetic acid), propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, 2-hydroxyethanesulfonic, toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, algenic, β-hydroxybutyric, salicylic, galactaric and galacturonic acid. Suitable pharmaceutically acceptable base addition salts include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenedi amine, meglumine (N-methylglucamine) and procaine. These salts may be prepared, for example, by reacting, in another embodiment, the appropriate acid or base with the compound.

In one embodiment, the term "pharmaceutically acceptable carriers" includes, but is not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer, or in another embodiment 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be in another embodiment 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. In

one embodiment the level of phosphate buffer used as a pharmaceutically acceptable carrier is between about 0.01 to about 0.1M, or between about 0.01 to about 0.09M in another embodiment, or between about 0.01 to about 0.08M in another embodiment, or between about 0.01 to about 0.07M in another embodiment, or between about 0.01 to about 0.06M in another embodiment, or between about 0.01 to about 0.05M in another embodiment, or between about 0.01 to about 0.04M in another embodiment, or between about 0.01 to about 0.03M in another embodiment, or between about 0.01 to about 0.02M in another embodiment, or between about 0.01 to about 0.015 in another embodiment.

The term "systemic administration" as used herein refers to oral, sublingual, buccal, transnasal, transdermal, rectal, intramuscular, intravenous, intraventricular, intrathecal, and subcutaneous routes.

The term "intravenous administration" includes injection and other modes of intravenous administration.

The terms "administration of" or "administering a" compound refers to providing a compound of the invention to the individual in need of treatment in a form that can be introduced into that individual's body in a therapeutically useful form and therapeutically useful amount, including, but not limited to: oral dosage forms, such as tablets, capsules, syrups, suspensions, and the like; injectable dosage forms, such as IV, IM, or IP, and the like; transdermal dosage forms, including creams, jellies, powders, or patches; buccal dosage forms; inhalation powders, sprays, suspensions, and the like; and rectal suppositories.

Techniques and compositions for making useful dosage forms using the present invention are described in one or more of the following references: Ansel, Introduction to Pharmaceutical Dosage Forms 2nd Edition (1976); Remington's Pharmaceutical Sciences, 17th ed. (Mack Publishing Company, Easton, Pa., 1985); Advances in Pharmaceutical Sciences (David Ganderton, Trevor Jones, Eds., 1992); Advances in Pharmaceutical Sciences Vol 7. (David Ganderton, Trevor Jones, James McGinity, Eds., 1995); Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms (Drugs and the Pharmaceutical Sciences, Series 36 (James McGinity, Ed., 1989); Pharmaceutical Particulate Carriers: Therapeutic Applications: Drugs and the Pharmaceutical Sciences, Vol 61 (Alain Rolland, Ed., 1993); Drug Delivery to the Gastrointestinal Tract (Ellis Horwood Books in the Biological Sciences. Series in Pharmaceutical Technology; J. G. Hardy, S. S. Davis, Clive G. Wilson, Eds.); Modern

Pharmaceutics Drugs and the Pharmaceutical Sciences, Vol 40 (Gilbert S. Banker, Christopher T. Rhodes, Eds.), and the like, relevant portions of each incorporated herein by reference.

The term "subject" refers to a mammal, such as humans, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, and cats, avian species, such as chickens, turkeys, and songbirds. Preferably, the subject is a human. The subject can be, for example, a child, such as an adolescent, or an adult.

The term "treatment" refers to any treatment of a pathologic condition in a mammal, particularly a human, and includes: (i) preventing the pathologic condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the disease condition; (ii) inhibiting the pathologic condition, i.e., arresting its development; (iii) relieving the pathologic condition, i.e., causing regression of the pathologic condition; or (iv) relieving the conditions mediated by the pathologic condition.

The term "therapeutically effective amount" refers to that amount of a compound of the invention that is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

Pharmaceutical Compositions:

The pharmaceutical composition may include one or more excipients including, but not limited to, lubricants (such as magnesium stearate, calcium stearate, zinc stearate, powdered stearic acid, hydrogenated vegetable oils, talc, polyethylene glycol, and mineral oil), colorants, binders (sucrose, lactose, gelatin, starch paste, acacia, tragacanth, povidone, polyethylene glycol, Pullulan and corn syrup), glidants (such as colloidal silicon dioxide and talc), surface active agents (such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate, triethanolamine, polyoxyethylene sorbitan, poloxalkol, and quaternary ammonium salts), preservatives, stabilizers, adhesives (such as mucoadhesives), disintegrants, bulking substances, flavorings, sweeteners, pharmaceutically acceptable carriers, and other excipients (such as lactose,

mannitol, glucose, fructose, xylose, galactose, sucrose, maltose, xylitol, sorbitol, chloride, sulfate and phosphate salts of potassium, sodium, and magnesium).

The AAT agents of the invention may be formulated into an oral dosage forms (such as tablets and capsules) by methods known in the art. Examples of dosage forms include, without limitation, chewable tablets, quick dissolve tablets, effervescent tablets, reconstitutable powders, elixirs, liquids, solutions, suspensions, emulsions, tablets, multi-layer tablets, bi-layer tablets, capsules, soft gelatin capsules, hard gelatin capsules, caplets, troches, lozenges, chewable lozenges, beads, powders, granules, particles, microparticles, dispersible granules, cachets, thin strips, oral films, transdermal patches, and combinations thereof.

The AAT agents of the invention may be formulated into an intravenous dosage form by any suitable method detailed in the techniques and composition references cited herein.

Tablets, capsules and intravenous formulations of presentation are provided in discrete units conveniently contain a daily dose, or an appropriate fraction thereof, of one or more of the AAT agents of the invention. For example, the units may contain from about 1 mg to about 1000 mg, alternatively from about 5 mg to about 500 mg, alternatively from about 5 mg to about 250 mg, alternatively from about 10 mg to about 100 mg of one or more of the AAT agents or combinations of the present invention.

Methods of Treatment:

The AAT agents and pharmaceutical compositions of the present invention alone or in combination with other antibiotics can be administered to treat bacterial infections caused by Gram-negative and Gram-positive bacteria. More preferred, the AAT agents and pharmaceutical compositions of the present invention alone or in combination with other antibiotics can be administered to treat bacterial infections caused by Gram-negative bacteria.

Typically, a therapeutically effective amount of the AAT agents or pharmaceutical composition is administered to treat the infection.

The dose range for adult or pediatric human beings will depend on a number of factors including the age, weight and condition of the patient. Suitable oral dosages of the AAT agents of the present invention can range from about 1 mg to about 2000 mg.

The AAT agents or a combination of antibacterial agent(s) and AAT agents may be administered once-a-day, or two, or three or more times a day. Preferably, the AAT agents or

combinations are administered by intravenous infusion from once-a-day to four times a day, preferably with each infusion lasting from 30 to 60 mins.

Polymorphs

The invention further relates to the compounds of the present invention in their various crystalline forms, polymorphic forms and (an)hydrous forms. It is well established within the pharmaceutical industry that chemical compounds may be isolated in any of such forms by slightly varying the method of purification and or isolation form the solvents used in the synthetic preparation of such compounds.

If different structural isomers are present, and/or one or more chiral centres are present, all isomeric forms are intended to be covered. Enantiomers are characterised by the absolute configuration of their chiral centres and described by the *R*- and *S*-sequencing rules of Cahn, Ingold and Prelog. Such conventions are well known in the art (e.g. see 'Advanced Organic Chemistry', 3rd edition, ed. March, J., John Wiley and Sons, New York, **1985**). It is also intended to include compounds of general formula (I) where any hydrogen atom has been replaced by a deuterium atom.

Oligonucleotide

The term "oligonucleotide" as used herein is defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides. Such covalently bound nucleosides may also be referred to as nucleic acid molecules or oligomers. Oligonucleotides are commonly made in the laboratory by solid-phase synthesis followed by purification. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides starting at 5'-end and finishing at the 3'-end (irrespective of the backbone of the oligonucleotide). The oligonucleotide of the invention is man-made, and is chemically synthesized, and is typically purified or isolated. The oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides.

Antisense oligonucleotides

The term "Antisense oligonucleotide" as used herein is defined as an oligonucleotide capable of modulating expression of a target gene by hybridizing to a target nucleic acid, in particular to a contiguous sequence on a target nucleic acid. The antisense oligonucleotides are not

essentially double stranded and are therefore not siRNAs or shRNAs. Preferably, the antisense oligonucleotides of the present invention are single stranded. It is understood that single stranded oligonucleotides of the present invention can form hairpins or intermolecular duplex structures (duplex between two molecules of the same oligonucleotide), as long as the degree of intra or inter self-complementarity is less than 50% across of the full length of the oligonucleotide.

PREFERRED SUB-STRUCTURES OF FORMULA (I)

In an embodiment, R_1 is selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl.

In an embodiment, R_1 is selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl.

In an embodiment, R_1 is H.

In an embodiment, R_2 and R_3 are each independently selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl.

In an embodiment, R_2 and R_3 are each independently selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl.

In an embodiment, R_2 and R_3 are each independently selected from the group consisting of: H and C_{1-6} alkyl.

In an embodiment, one of R_2 and R_3 is H and the other is C_{1-6} alkyl. Preferably, one of R_2 and R_3 is H and the other is C_{1-4} alkyl. More preferably, one of R_2 and R_3 is H and the other is Me.

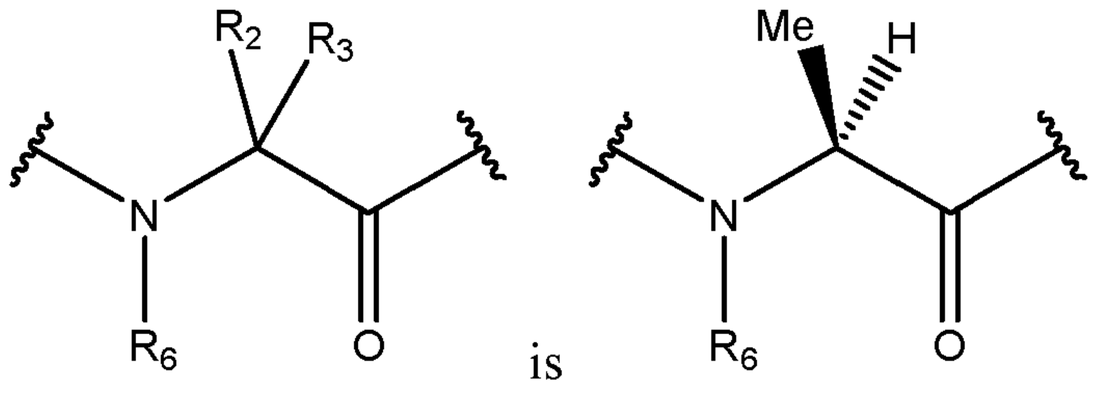
In an embodiment, R_4 and R_5 are each independently selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl.

In an embodiment, R_4 and R_5 are each independently selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl.

In an embodiment, R_4 and R_5 are each independently selected from the group consisting of: H and C_{1-6} alkyl.

In an embodiment, one of R_4 and R_5 is H and the other is C_{1-6} alkyl. Preferably, one of R_4 and R_5 is H and the other is C_{1-4} alkyl. More preferably, one of R_4 and R_5 is H and the other is Me.

In an embodiment, m is 0 and R_4 and R_5 are absent. In an embodiment, m is 0 and R_4 and R_5 are absent and one of R_2 and R_3 is H and the other is C_{1-6} alkyl. Preferably, m is 0 and R_4 and R_5 are absent and one of R_2 and R_3 is H and the other is C_{1-4} alkyl. More preferably, m is 0 and R_4 and R_5 are absent and one of R_2 and R_3 is H and the other is Me. Most preferably, m is 0 and R_4 and R_5 are absent and the R_2 and R_3 groups are selected such that the moiety:



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In an embodiment, R_6 is selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl.

In an embodiment, R_6 is selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl.

In an embodiment, R_6 is H.

In an embodiment, R_1 is selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl; R_2 and R_3 are each independently selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl; R_4 and R_5 are each independently selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl; and R_6 is selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl.

In an embodiment, R_1 is selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl; R_2 and R_3 are each independently selected from the group consisting of: H,

C_{1-6} alkyl and C_{1-6} substituted alkyl; R_4 and R_5 are each independently selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl; and R_6 is selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl.

In an embodiment, R_1 is H; R_2 and R_3 are each independently selected from the group consisting of: H and C_{1-6} alkyl; R_4 and R_5 are each independently selected from the group consisting of: H and C_{1-6} alkyl; and R_6 is H.

In an embodiment, R_1 is H; one of R_2 and R_3 is H and the other is C_{1-6} alkyl, preferably, one of R_2 and R_3 is H and the other is C_{1-4} alkyl; one of R_4 and R_5 is H and the other is C_{1-6} alkyl, preferably, one of R_4 and R_5 is H and the other is C_{1-4} alkyl; and R_6 is H.

In an embodiment, R_1 is selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl; R_2 and R_3 are each independently selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl; m is 0; and R_6 is selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl and C_{3-8} substituted cycloalkyl.

In an embodiment, R_1 is selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl; R_2 and R_3 are each independently selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl; m is 0; and R_6 is selected from the group consisting of: H, C_{1-6} alkyl and C_{1-6} substituted alkyl.

In an embodiment, R_1 is H; R_2 and R_3 are each independently selected from the group consisting of: H and C_{1-6} alkyl; m is 0; and R_6 is H.

In an embodiment, R_1 is H; one of R_2 and R_3 is H and the other is C_{1-6} alkyl, preferably, one of R_2 and R_3 is H and the other is C_{1-4} alkyl; m is 0; and R_6 is H.

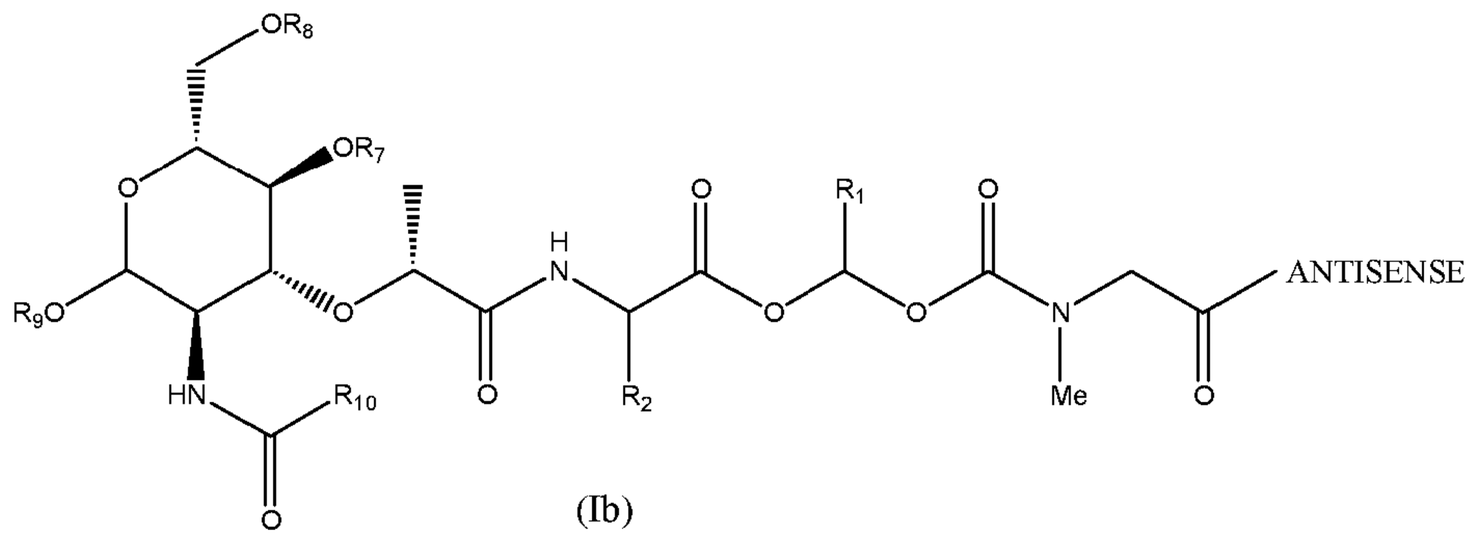
In an embodiment, R_7 is H. In an embodiment, R_7 is acetyl. In an embodiment, R_7 is benzoyl.

In an embodiment, R_8 is H. In an embodiment, R_8 is acetyl. In an embodiment, R_8 is benzoyl.

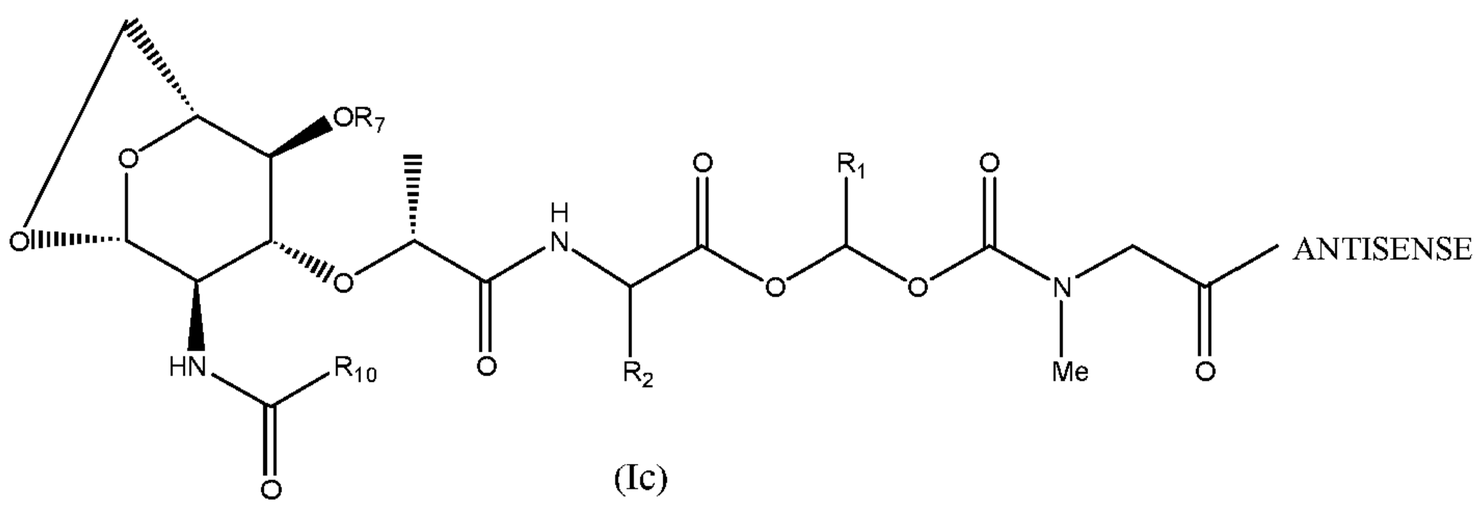
In an embodiment, R_9 is H. In an embodiment, R_9 is acetyl. In an embodiment, R_9 is benzoyl.

In an embodiment, R_{10} is methyl. In an embodiment, R_{10} is ethyl. In an embodiment, R_{10} is propyl.

By way of example, compounds according to the present invention include but are not limited from the following preferred substructures:

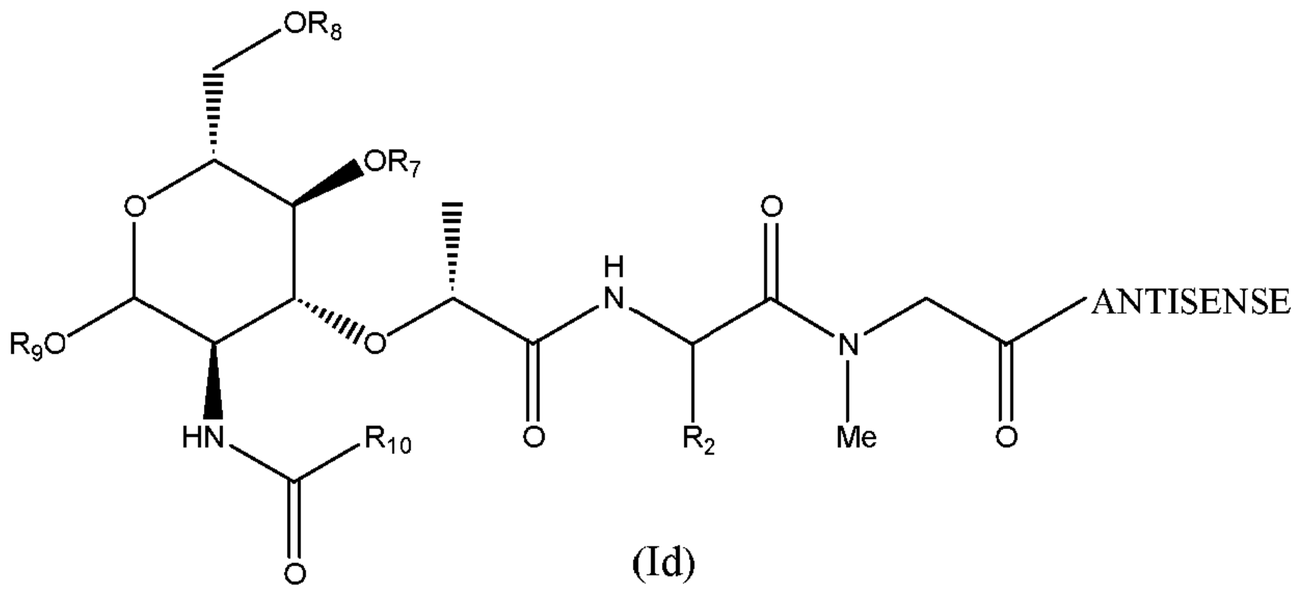


(Ib)



(Ic)

wherein m is selected as '0', n is selected as '1', p is selected as '1', q is selected as '1, R_3 is H, R_6 is H and "SPACER" is N-methylglycine and R_1, R_2, R_7, R_8, R_9 and R_{10} are as defined above; or



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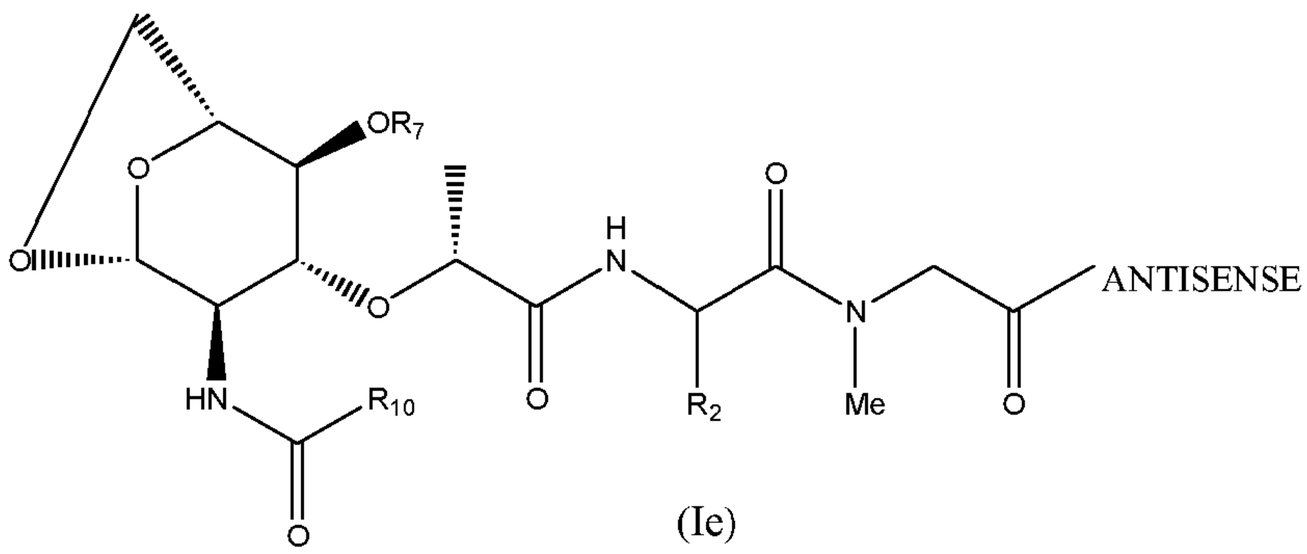
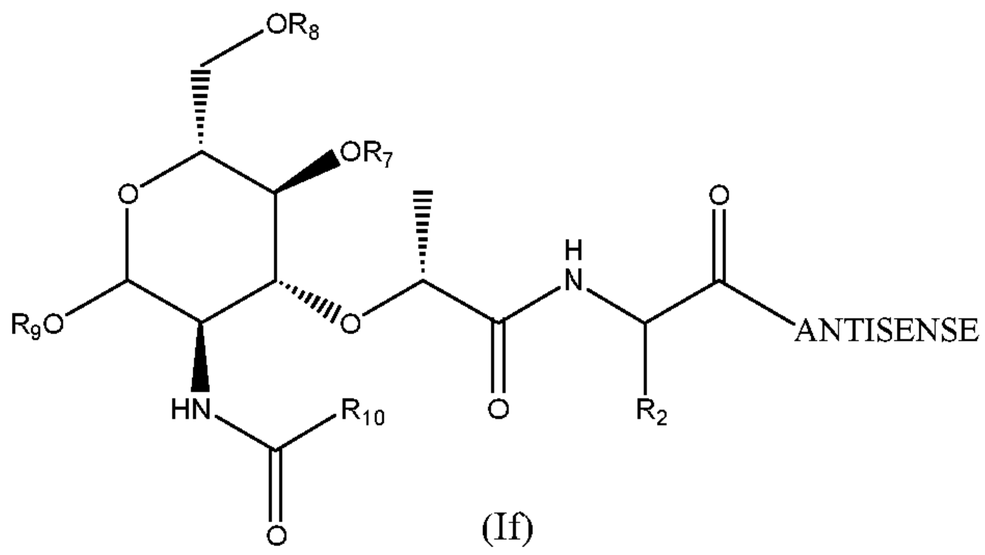
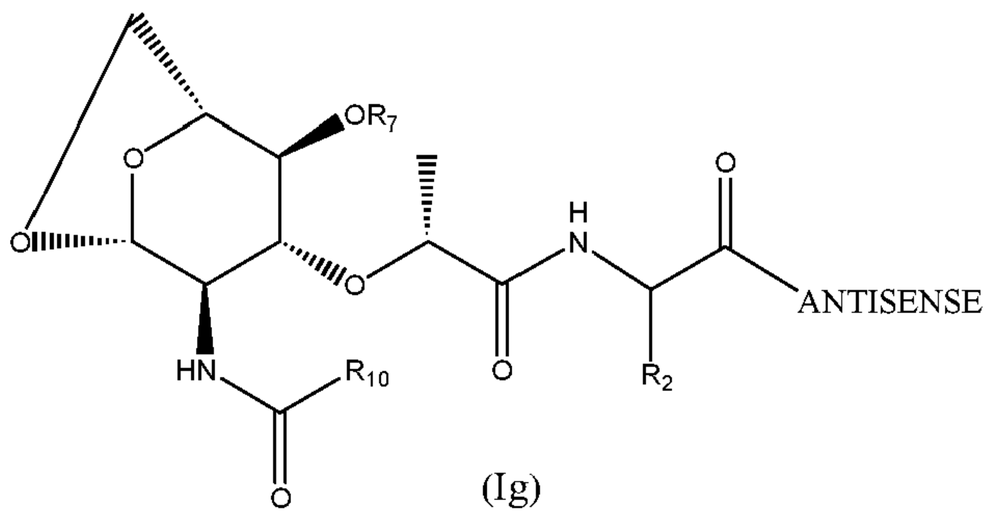


(Ie)

wherein m is selected as '0', n is selected as '1', p is selected as '0', q is selected as '1', R_3 is H, R_6 is H and "SPACER" is N-methylglycine and R_2, R_7, R_8, R_9 and R_{10} are as defined above; or

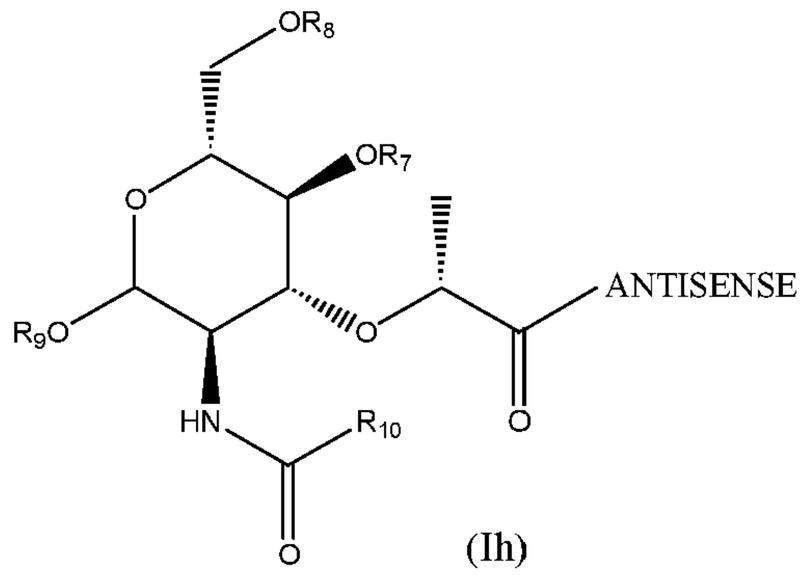


(If)

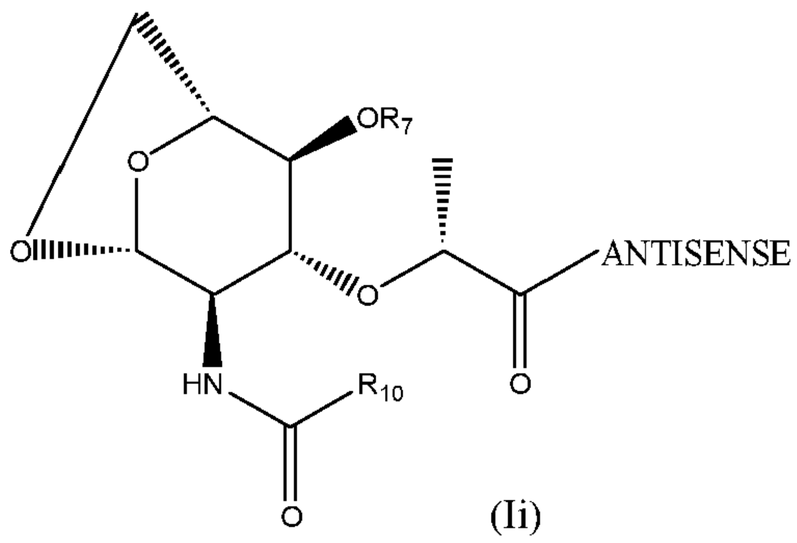


(Ig)

wherein m is selected as '0', n is selected as '1', p is selected as '0', q is selected as '0', R_3 is H, and R_2, R_7, R_8, R_9 and R_{10} are as defined above; or



(Ih)



(Ii)

wherein n is selected as '0', p is selected as '0', q is selected as '0', and R_7, R_8, R_9 and R_{10} are as defined above;

In a preferred embodiment substructures (Ic), (Ie), (Ig), and (Ii) are chosen.

In an even more preferred embodiment substructures (Ic) and (Ig) are chosen.

PREFERRED ANTISENSE AGENTS

The ANTISENSE agent contains a terminal amino functional group for chemical bond formation with the remainder of the molecule to provide molecules of general formula (I). The following Tables 1A, 1B, 1C, 1D are not intended to be an exhaustive list of potential antisense targets but detail the sequences of bases that the ANTISENSE agent might include. Preferred ASOs are of sufficient length and complementarity to specifically hybridize to a bacterial mRNA target that encodes a gene in a biochemical pathway and/or cellular process that is essential for bacterial survival and growth. General examples include cell division, murein biosynthesis, global regulatory mechanisms, fatty acid biosynthesis, DNA replication, ribosomal proteins, transcription, translation initiation, lipopolysaccharide biosynthesis, nucleic acid biosynthesis, biofilm growth and intermediary metabolism. Particular examples of genes in biochemical pathways and cellular processes include: RpsJ and RpmB (ribosomal

proteins); LpxC, WaaC, WaaG, WaaA, WaaF, LpxA, LpxB (lipopolysaccharide biosynthesis); murA, mraY, murB, murC, murE, murF, murG (murein peptidoglycan biosynthesis); acpP, accA, accB, fabG, fabZ (fatty acid biosynthesis); acpS (acyl carrier protein synthase); fabI (enoyl-acyl carrier protein reductase); fabD (malonyl coenzyme A acyl carrier protein transcyclase); folP (dihydropteroate synthase); fmhB (protein in glycine attachement); gyrA (DNA gyrase subunit); adk (adenylate kinase, cell energy homeostasis); infA (protein biosynthesis); ftsZ (cell division); rpoD (RNA synthesis); aroC (aromatic compound biosynthesis); inhA (enoyl-acyl carrier protein reductase); ompA (outer membrane protein A); blaT, cml, adeA (antibiotic resistance-associated genes); cepL, cepR, suhB, CsuE, SecA, Pg1L, PilU1, AlgZ, AlgU, LasR, FleR, PelF (biofilm formation-associated).

Table 1A; Exemplary Antibiotic Resistance Targeting Sequences

Target Gene	Antisense sequence (5'-3')	Sequence ID
NDM-1	TCA AGT TTT CC	1
NDM-1	TCC TTT TAT TC	2
NDM-1	CCA TCA AGT TT	3
NDM-1	GGC AAT TCC AT	4
adeA	ATA CTG TCC AA	5
OmpA	CAT GGA TAT CC	6
AcrA	ATG TAA ACC TC	7
AcrA	GTT CAT ATG TA	8
AcrA	ACC CCT CTG TT	9
AcrA	TGT TCA TAT GT	10
AcrB	GTC TTA ACG GC	11
AcrB	AGG CAT GTC TT	12
AcrB	TAG GCA TGT CT	13
AcrR	TAT GTT CGT GA	14
TolC	TTC ATT TGC AT	15
TolC	ATT CCT TGT GG	16
TolC	TTT GCA TTC CT	17
KPC	GAT ACA GTG AC	18
KPC 1-4	AAC GAT ATT CC	19

Table 18; Exemplary Biofilm Formation Targeting Sequences		
Target Gene	Antisense sequence (5'-3')	Sequence ID
cepI	AAG GTC TGC AT	20
cepI	TCG GAT CTG TG	21
cepI	CAT GGA TGT CC	22
cepI	CGT GAA CGA AG	23
cepI	CGT GTG GCA AC	24
cepI	GCC CGA GAT CC	25
cepI	CTT TCG TTC GC	26
subB	ATG CAT GAG CC	27
subB	GGA TGC ATG AG	28
CsuE	TTA TAT TCA TGG	29
CsuE	TCA TGG CAA AG	30
CsuE	TTT CCT GTC AA	31
SecA	TTG CCA ACA TG	32
PgiL	CAT TAC CCA AG	33
PilU1	TTA AAA TCC AT	34
Alg2	TAG GCA TCG AC	35
AlgU	AAA GCT CCT CT	36
LasR	AGG CCA TAG CG	37
FleR	TTA CTC CTG AA	38
PelF	TTC GGT CAT GT	39

Table 1C; Exemplary Fatty Acid Biosynthesis-associated Targeting Sequences		
Target Gene	Antisense sequence (5'-3')	Sequence ID
acpP	GTC CAT TAC CC	40
acpP	CAT TAC CCC TC	41
acpP	CCA TTA CCC CT	42
acpP	TCC ATT ACC CC	43
acpP	TGT CCA TTA CC	44
acpP	TTG TCC ATT AC	45
acpP	GTT GTC CAT TA	46
acpP	TGT TGT CCA TT	47
acpP	ATG TTG TCC AT	48
acpP	TTT ACA AGT GC	49
acpP	CCT CCG AGG GA	50
acpP	ACA CGT TGT TC	51
acpP	AGT TCA GCG AC	52
acpP	CTC ATA CCT TG	53
acpP	TGC TCA TAC TC	54
acpP	CTC ATA CTC T	55
acpP	CTC ATA CTA T	56
acpP	CTT CGA TAG TG	57
acpP	ATA TCG CTC AC	58
acpP	ATT CTC CTC AT	59
acpP	CAC AGG AAT TC	60
acpP	CAT TGC TTG TG	61
acpP	CAT ACC TTG TT	62
acpS	TTG CCA TTA GC	63
acp-5	CTG TAG TGA TTT CAC CA	64
fabA	TTA TCT ACC AT	65
fabB	CGT TTC ATT AA	66
fabB	GCA CGT TTC AT	67
fabI	AGA AAA CCC AT	68
fabI	GCT TTA ATC C	69
fabI	CCC ATA GCT T	70
fabI	CAT GTA AGA T	71
fabI	AGA TAA CTC C	72
gapA	TTG ATA GTC AT	73
accA	GCT TTT TTC AT	74
accA	AGG CTT CCG TC	75
fabD	GTC ATG TTT T	76
inhA	GTC ATT TGG T	77
inhA	CAT TTG GTG ACT	78

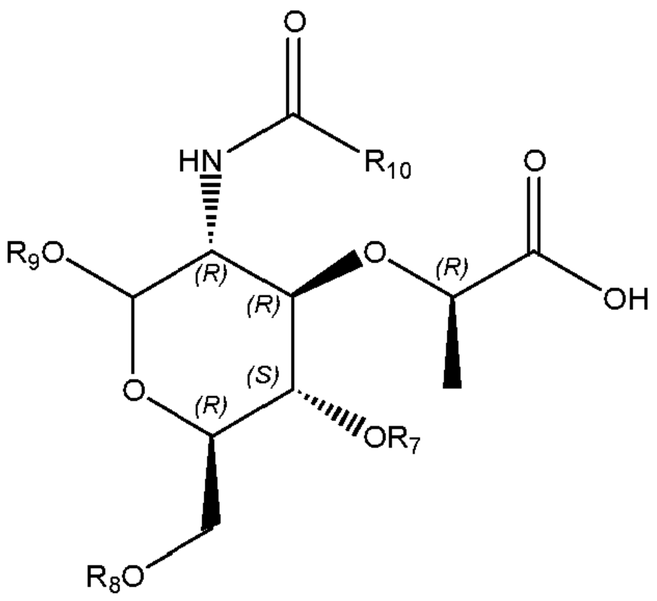
Table 1D; Exemplary Targeting Sequences associated with other pathways or cellular processes		
Target Gene	Antisense sequence (5'-3')	Sequence ID
RpoD	TCA TCT TTG CT	79
RpoD	TTT TGC TCC AT	80
PolB	AGT AAC TCC AC	81
murA	TTT ATC CAT TG	82
rpsJ	GCA TTT GAC CT	83
rpsJ	TAG ACA TAC CA	84
rpsJ	TAC CAG TAA AC	85
rpsJ	TGG TTC TGC AT	86
rpsJ	CCT CAG ACT CC	87
rpsJ	GCA TTT GAC CT	88
ftsZ	AGT TTC TCT CC	89
ftsZ	GTT CAA ACA TA	90
ftsZ	TCA AAT GAG GC	91
ftsZ	AAT GAG GCC AT	92
ftsZ	ATA GTT TCT CTC C	93
gyrA	CGC TCA TCT AA	94
gyrA	CTA TAC ATA GAC	95
gyrA	GCC ATC TCG GAC ATC	96
gyrA	ATA CCA GGT GTT ATC T	97
dnaB	TTC CTG CCA TA	98
LpxC	TTT GAT CAT CG	99
LpxC	TGT TTG ATC AT	100
LpxC	TGT TTC ACC AT	101
LpxC	GTT GTT TGA TC	102
23S rRNA	AGT GCT CTA CC	103
23S rRNA	GCC TGT TAT CC	104
16S rRNA	CCA TGC AGC AC	105
16S rRNA	TTG CGC TCG TT	106
16S rRNA	GGC TGC TGG CA	107
fmhB	CCA TGA AAA A	108
polA	TTC ATG CCT GT	109
murA	ATC CAT TTA GT	110
murA	CAT TTA GTT TG	111
murA	AAT TTA TCC AT	112
murA	AAA TTT ATC CA	113
rpmB	ACT CGG GAC AT	114
rpmB	CTA TTC TCC AA	115
rpmB	GGC AGA CTC GG	116
rpmB	CTT AGA CAT GG	117
adk	ATG ATA CGC AT	118
adk	AGT GCC CTC C	119
infA	TCT TTG GCC AT	120

Table 1D; Exemplary Targeting Sequences associated with other pathways or cellular processes		
Target Gene	Antisense sequence (5'-3')	Sequence ID
aroC	TTT CCA GCC AT	121
aroC	TTC CCT GCC AT	122
murF	ACG CTA ATC AT	123
murF	ACC TCC CAG GC	124
kdtA	AAT TCG AGC AT	125
boxA	TGT TTA AGA GC	126
boxA	CTC TTA ATG AT	127
boxC	ATC CAC CAC AG	128
rpoD-E	CTT GTA ACC ACA CCA	129
rpoD-E	TCC ACC AAG TCA CCA	130
pryC	GGT GCA GTC AT	131
pryC	AGA GTT CAA GG	132
pryA	GAC TTA ATC AA	133
lgt	CTA CTG GTC AT	134
folA	CAT TGA GAT TT	135
lolB	ACA TCT GTC AT	136
nrdA	TTC TGA TTC AT	137
nrdB	GTA TAT GCC AT	138
zipA	TCC TGC ATC AT	139
casA	ATA TAC CTC AT	140
gyrA-E	GTT ACC CTG ACC GAC CA	141
gyrA-E	GTT ACC CTG ACC ACC A	142
mrdA	TGT TTC ATA CG	143
Lpx8	GGT TTG CCA AG	144
Lpx8	TAA TCC GTC AG	145
carA	GGT GCT CAA AC	146
adeA	ATA CTG TAA AA	147
blaT	CTC TTC CTT TT	148
crmI	TCC TTC TGA TT	149
folP	ATG TTA TCC C	150
fabB	CCA TGA TTT A	151
hmr8	TCC ACG TCG A	152
rpm8	GTC TAT TCT CC	153
rpm8	GAC ATG TCT AT	154
hmr8	TCC ACG TCG A	155
FabG	TTC TCT CCT TT	156
fpm8	CTC TAG ACA TG	157
WaeC	AGC ACC CTC AT	158
MryA	TGA CTC TCC TC	159
MurC	CCA CCT CCA GG	160
LpxA	ATC AAA CTC AT	161
WaaG	GCC AGG GTC AT	162
WaaA	GTACGG TTC AT	163
mur8	CAG TCG CCC CT	164
murE	AGG CTC ATA GG	165
AccB	CTA GCA CTC CC	166
FabZ	ATG TCC ATC AT	167
MurG	GCA AAG TCC TC	168
AmpR	GTC GAA CCA AT	169

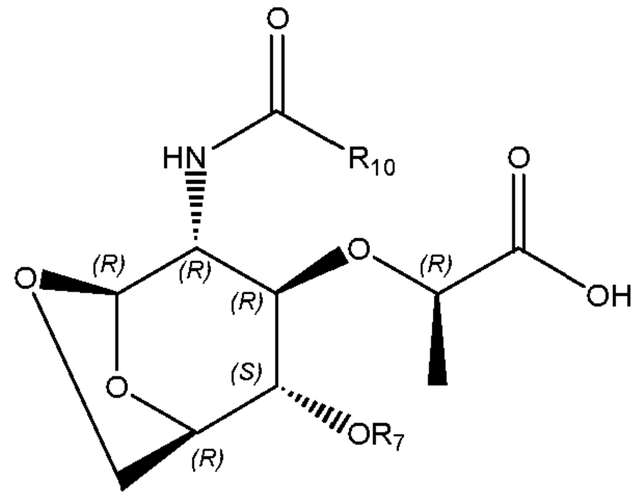
One skilled in the art will appreciate that the sequences listed in Tables 1A, 1B, 1C and 1D describe targeting antisense sequences and these may be increased in length through the addition of extra monomer units to either or both the 5'- and 3'-ends. Also, the targeting sequences listed in Tables 1A, 1B, 1C and 1D may differ by one, two or three monomer units and still retain the ability to bind to the bacterial gene of interest.

PREFERRED "Sugar Reagents"

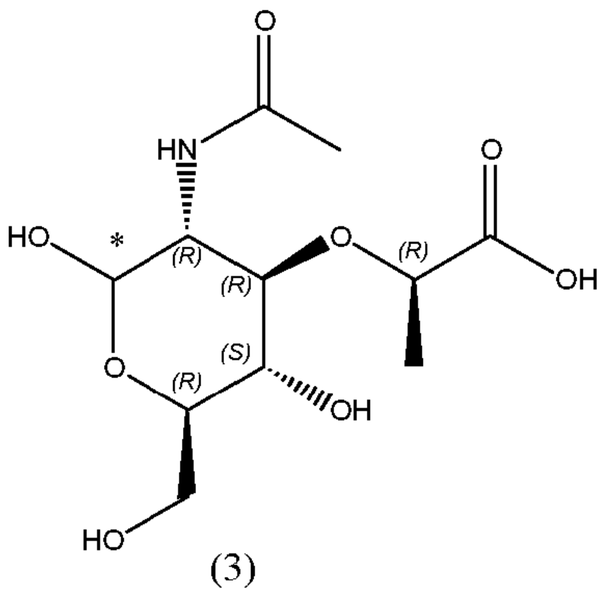
The "SUGAR" of the present invention is prepared through the utilisation of the "Sugar Reagents" Utilisation of the "Sugar Reagents" provides chemoselective formation of the chemical bond (primarily an amide bond formation) between the α-carbonyl of the terminal carboxylic acid of the "Sugar Reagent" and the remainder of the molecule of general formula I. When the terminal carboxylic acid of the "Sugar Reagent" is the α-carbonyl of the lactyl residue of the "SUGAR", the following are known and preferred reagents **(2-6)** for these steps;



(1)

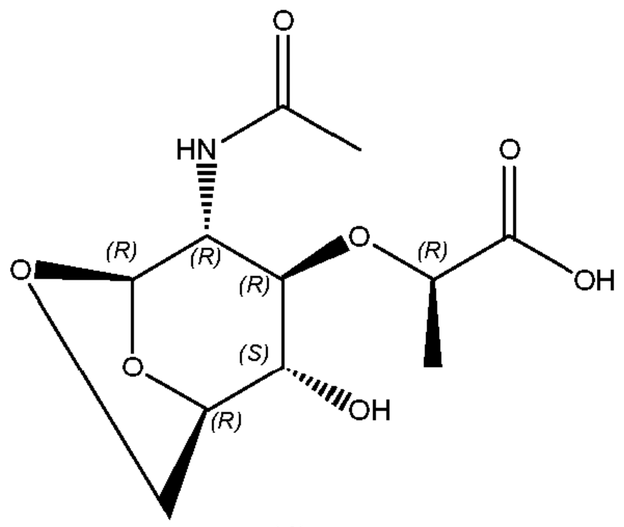


(2)



(3)

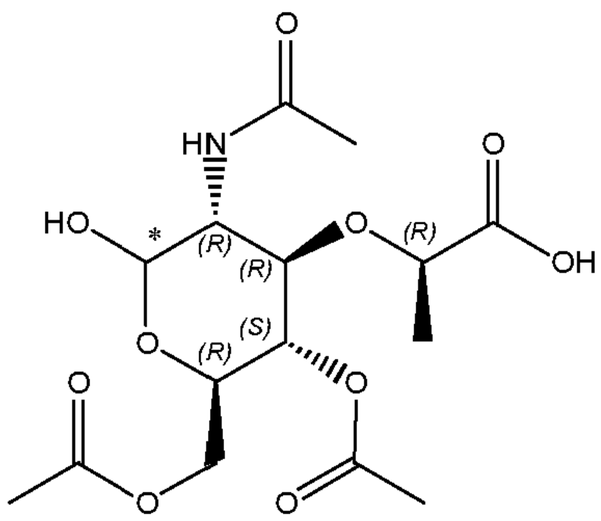
* anomeric (S);
CAS 61633-77-8



(4)

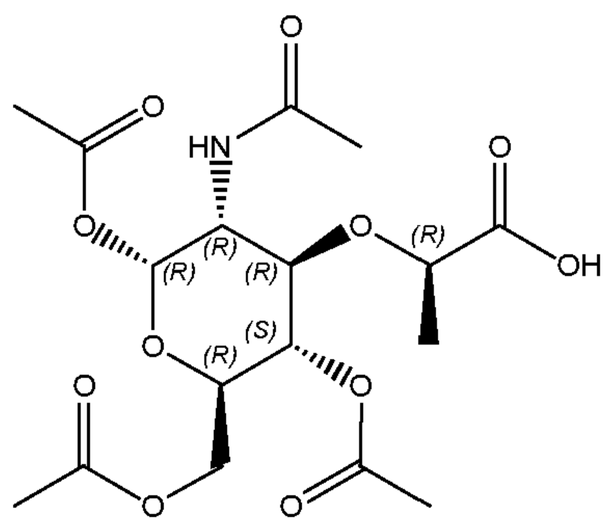
* anomeric (R);
CAS 61665-31-4

CAS 104430-66-2



(5)

CAS 25605-74-7
* anomeric (S); CAS 149713-67-7

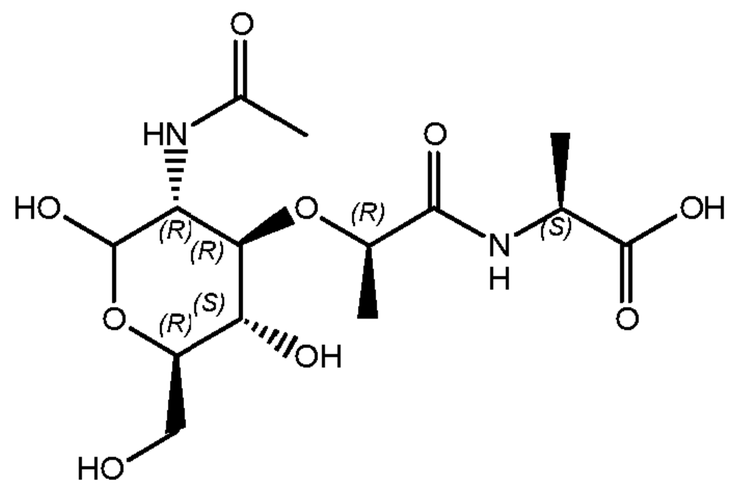


(6)

CAS 149622-52-6

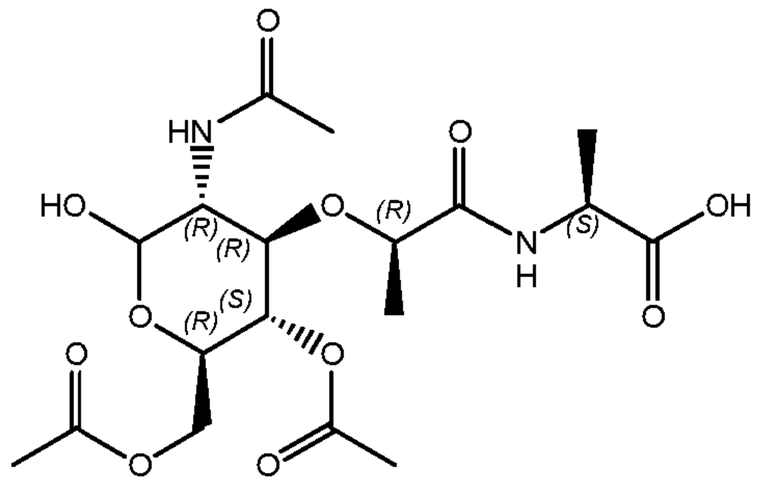
These reagents are commercially available or full synthetic procedures have been described in the literature (e.g. for **(3)**, commercially available and see Merten, H and Brossmer, R., *Carbohydrate Res.*, 191(1), 144-9, **1991**; for **(4)** see Paulsen, H *et al., Liebigs Annalen der Chemie*, 4, 664-74, **1986**; Hesek, D. *et al., JACS*, 131(14), 5187-93, **2009**; Wang, Q. *et al., Org. Biomol. Chem*. 14(3), 1013-23, **2016**; Calvert, M. B. *et al.,Beilstein J. Org. Chem.* 13, 2631–2636, **2017** for **(5)** see Osawa, T *et al., Biochemistry*, 8(8), 3369-75, **1969**; for **(6)** see Wacker, O and Traxler, P. EP541486.

In the variation wherein 'n' is chosen as 1, wherein for example the terminal carboxylic acid of the "Sugar Reagent" is the α-carbonyl of an *L*-Alanine residue, it may be advantageous to extend reagents **(2-6)** and use these further intermediates in subsequent reactions. In this tactical variation, the following are preferred reagents **(7** to **9b)** for these steps;



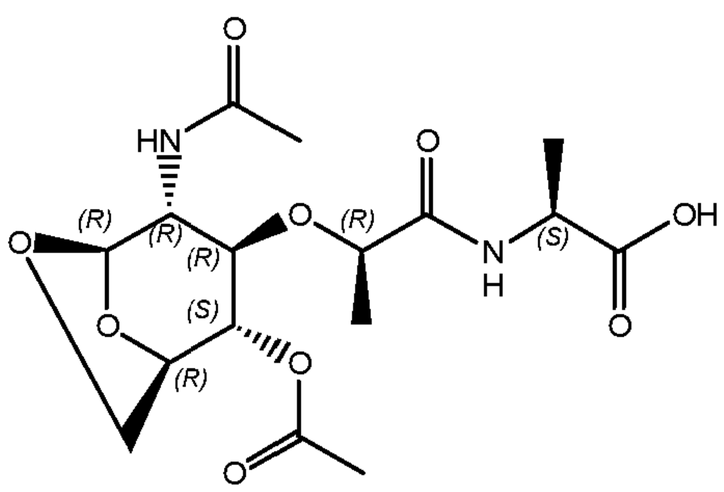
(7)

* anomeric (S);
CAS 14468-72-5

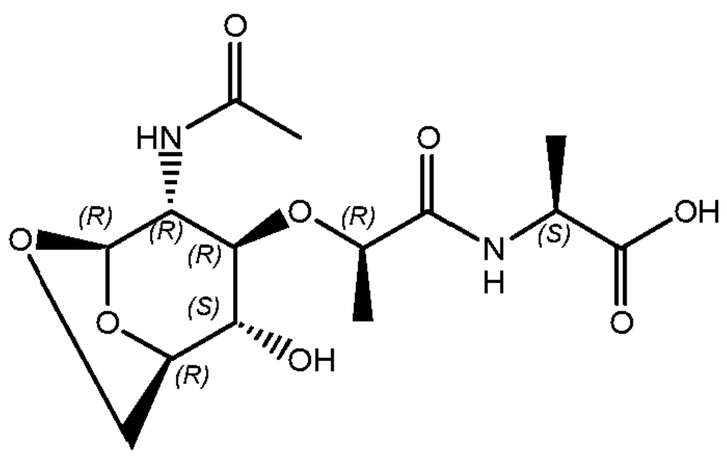


(8)

* anomeric (R);
CAS 80996-07-2



(9a)



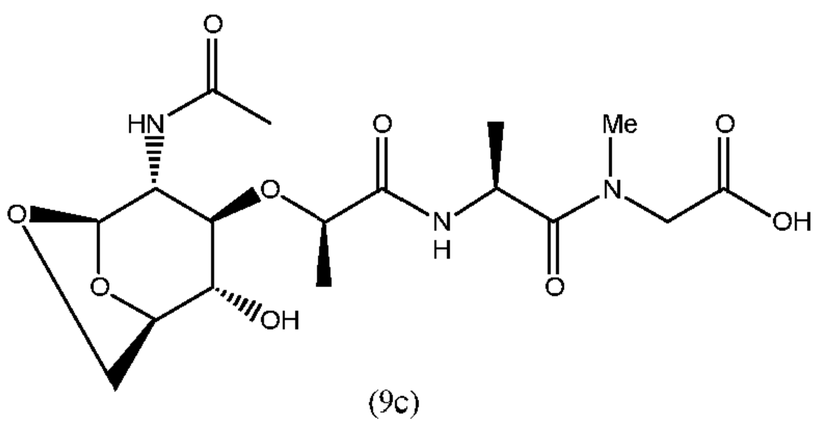
(9b)

These reagents have full synthetic procedures described in the literature or are available by simple adaptations (e.g. for **(7)** see Chaturvedi, N. C. *at al, J. Med. Chem.*, 9, 971-3, **1966**; Klaic, B. *Carbohydrate Res.*, 110(2), 320-5, **1982**; for **(8)** see Bacic, A. and Pecar, S. *Tet. Assym.*, 19, 2265-71, **2008**; for **(9b)** see WO2014/002039, cpd 17 pg 87 wherein (*S*)-amphetamine can simply be replaced by *L*-alanine benzyl ester and synthesis commences from commercially available CAS 55682-47-8 (2*S*, 3*R*, 4*R*)-4-azido-2-(benzyloxy)-6,8-dioxabicyclo[3.2.1]octan-3-ol). Also see WO2016/172615 wherein routes to N-acyl variants of the N-acetyl reagent **(7-9b)** are detailed.

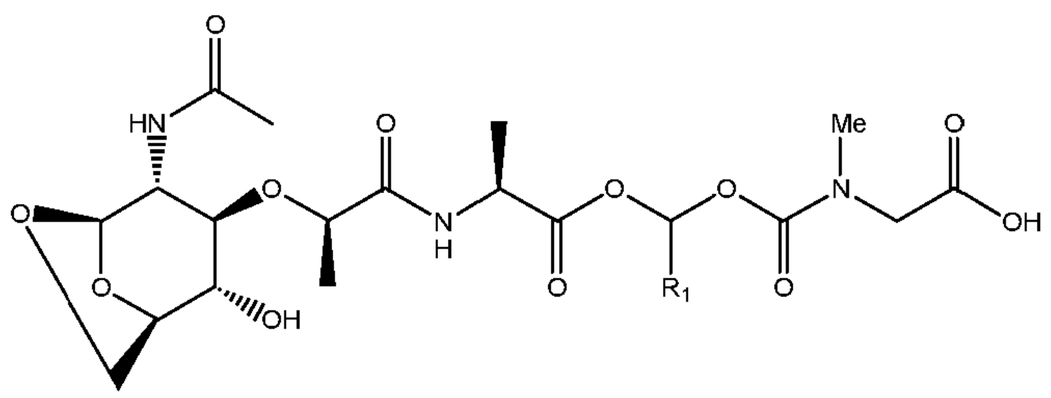
In a further variation wherein 'n' is chosen as 1, it may be advantageous to prepare a LINKER-SPACER-ANTISENSE intermediate and then utilise reagents such as (**1-4**).

In a further variation wherein 'n' is chosen as 1, it may be advantageous to prepare a SPACER-ANTISENSE intermediate and then utilise reagents such as (**7-9b**).

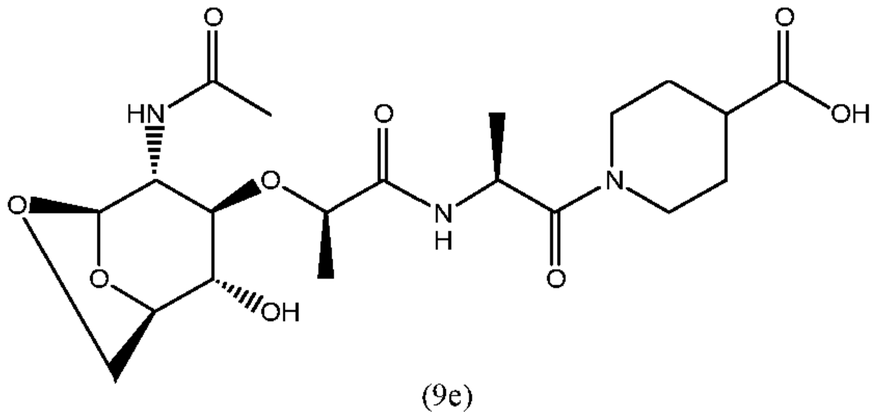
In yet a further variation, it may be advantageous to prepare an ANTISENSE intermediate and then utilise reagents such as (**1-4**, **7-9b** or **9c**-f).



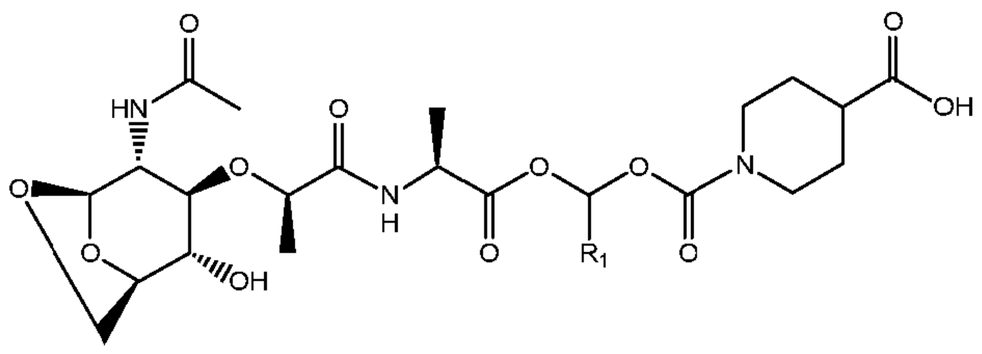
(9c)



(9d)

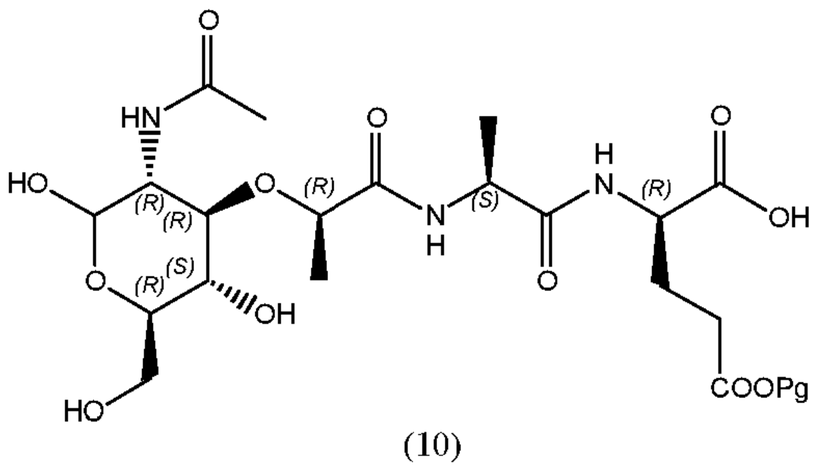


(9e)

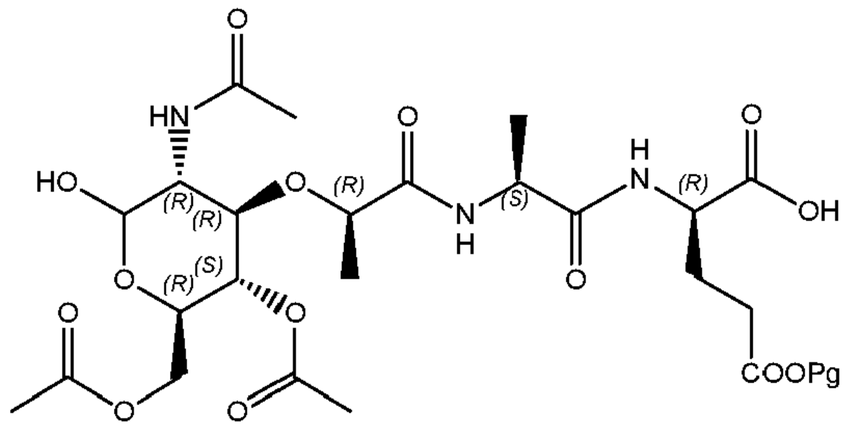


(9f)

In additional variations wherein 'n' is chosen as 2, 3 or 4 it may be advantageous to extend "Sugar Reagents" **(7-9b)** and use these further intermediates that contain increasing similarity to the full structure of the bacterial cell wall peptide (NAc-Mur-*L*-Ala-*D*-Glu-*meso*-DAP-*D*-Ala) in subsequent reactions. In this tactical variation, the following are preferred reagents **(10 to 18)** for these steps. Reagents **10** to **18** may be prepared from the reagents such as **1** to **9** by standard peptide synthesis methods well known to those in the art. In order to provide chemoselective reaction with the terminal α-carboxylic acid of the "Sugar Reagents" **10** to **18**, the side-chain carboxylic acid functional group of *D*-Glutamic acid and where present the side-chain carboxylic acid functional group and the sidechain amino functional group of *meso*-diaminopimelic acid (DAP) are protected with a protecting group Pg. Preferred protecting groups are the benzyl or *tert*-butyl ester and the benzyloxycarbonyl (Cbz) and *tert*-butoxycarbonyl (Boc) urethanes.



(10)



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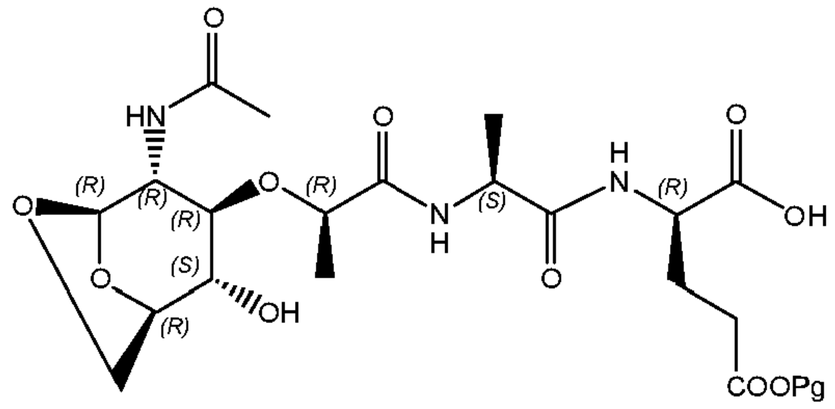

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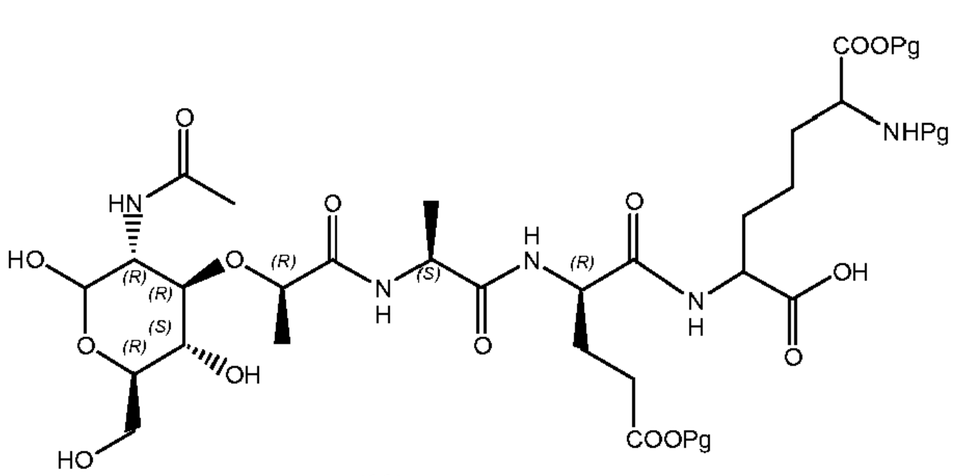

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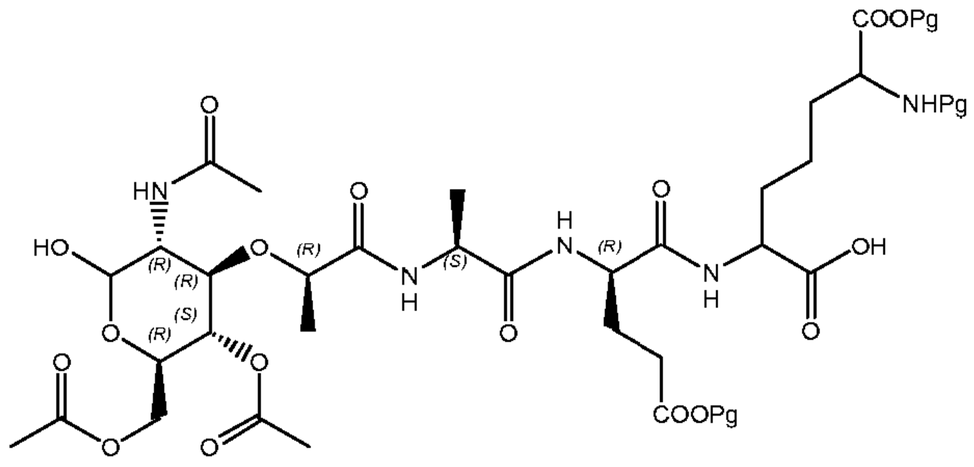

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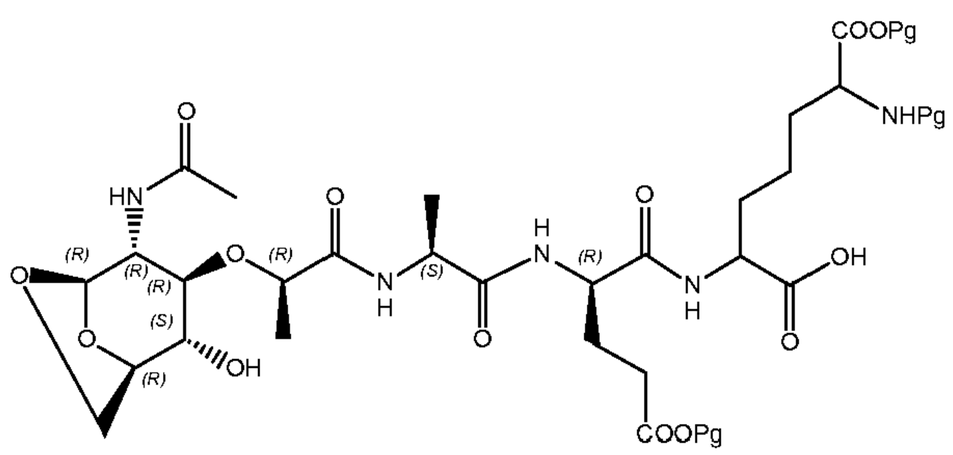

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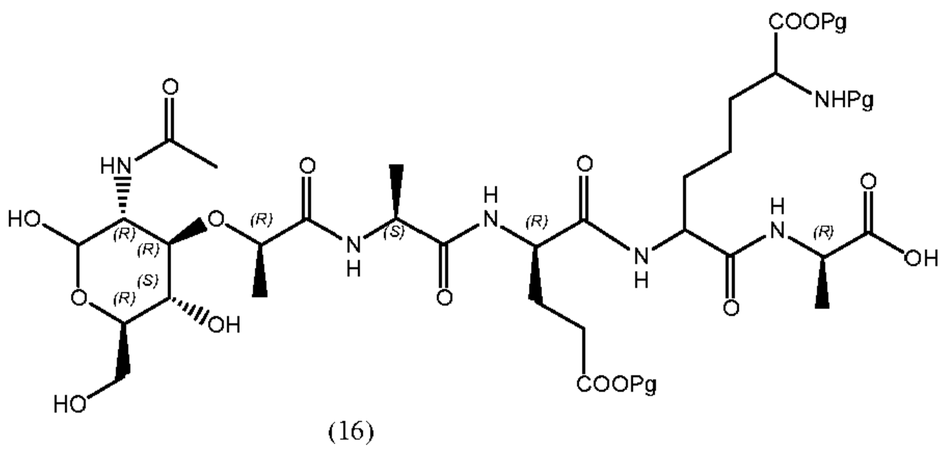

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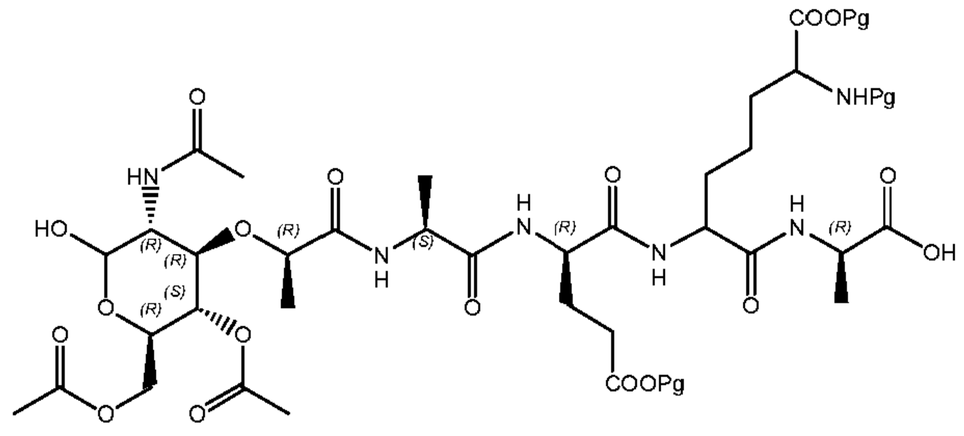

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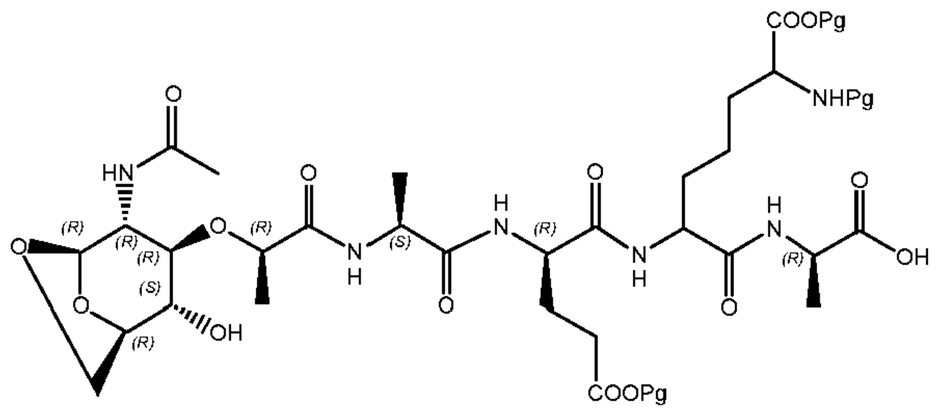
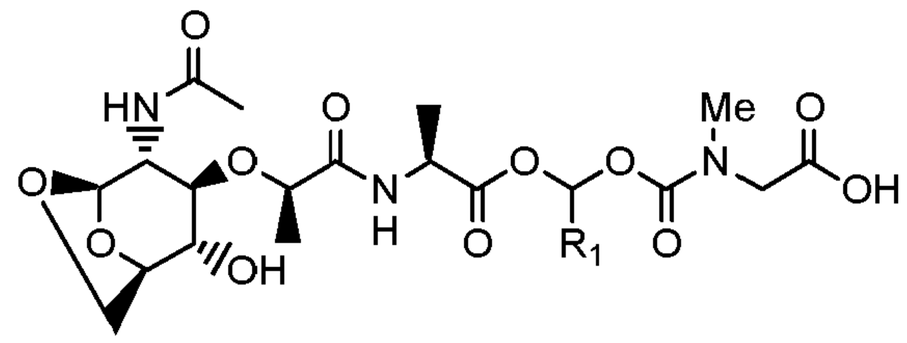


(18)

Preparation of the 'SUGAR-LINKER-SPACER-ANTISENSE AGENT' of the Invention

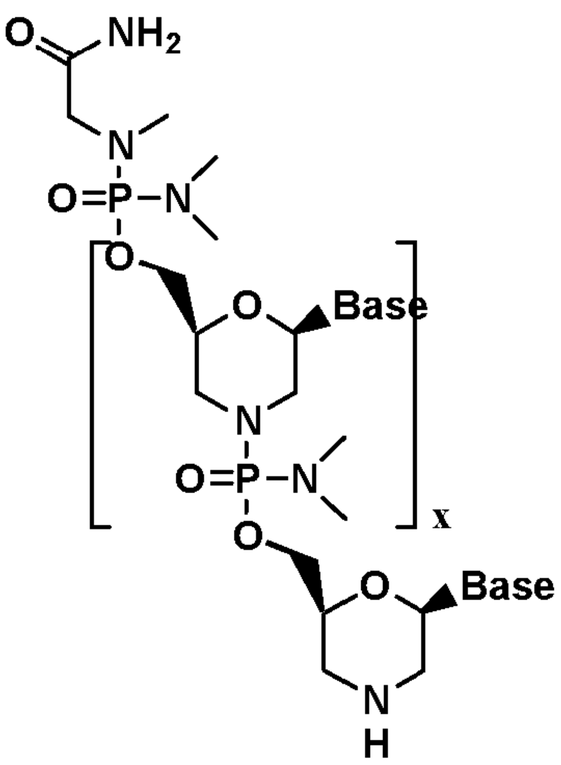
Compounds of the present invention are prepared by the general methods provided herein and detailed in Schemes 3 and 4. One skilled in the art will recognize that the methods are by no means an exhaustive description and other routes may be available to make similar compounds. One skilled in the art will also appreciate that the vast majority of ANTISENSE agents contain more than one functional group that may participate in chemical reactions. Therefore, within the schemes "ANTISENSE" refers to intermediates wherein the nucleobases are temporarily protected during synthesis. As a final step in all schemes, the selective removal of the protecting group(s) "PGs" provides the compounds of general formula (I). For a description of an example synthesis of PNAs see Lee, H. *et al., Org. Lett.* 9(17), 3291-3, **2007**. For a description of an example synthesis of PMOs see Summerton, J. and Weller, D. *Antisense & Nucl. Acid Drug Dev.*, 7, 187-195, **1997**. For a description of an example coupling to PMOs see O'Donovan, L. et al., *Nucl. Acid Ther.*, 25(1), 1-10, **2015**.

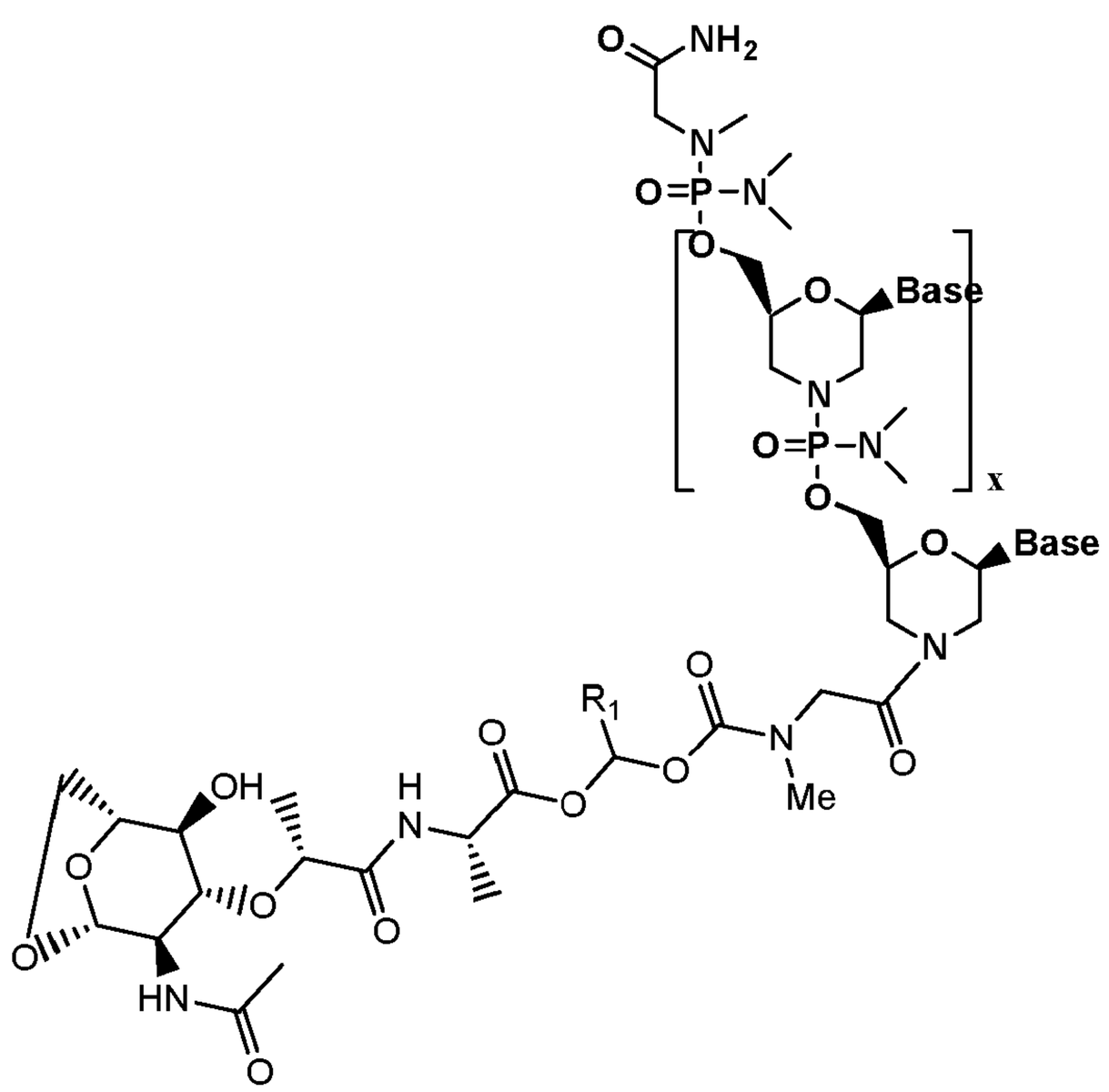


(9d)

(i) Activation, coupling

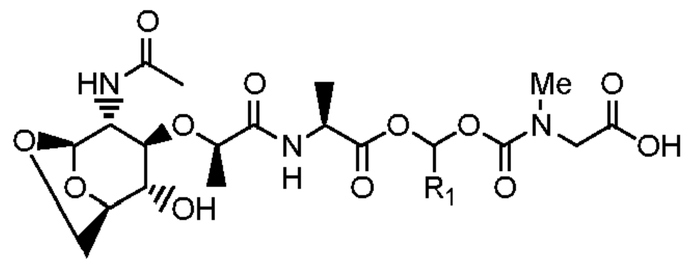
(ii)





Scheme 3. General synthesis of compounds of formula (I) wherein "ANTISENSE" is a PMO conjugated at the 3'-end by a SUGAR-LINKER-SPACER. In an analogous manner, sugar

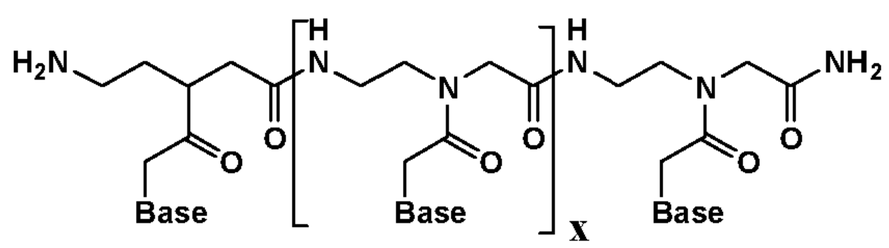
reagent **(9d)** can readily be replaced by sugar reagents such as **1** to **18** to provide variants covering the range of definitions within formula (I).

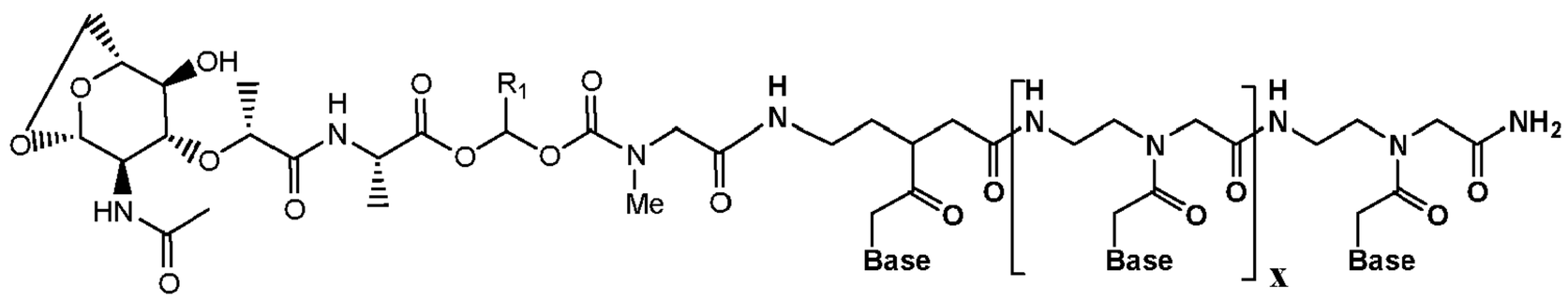


(9d)

(i) Activation, coupling

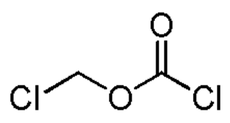
(ii)



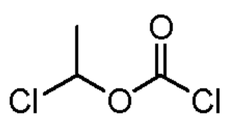


Scheme 4. General synthesis of compounds of formula (I) wherein "ANTISENSE" is a PNA conjugated at the N-terminus by a SUGAR-LINKER-SPACER. In an analogous manner, sugar reagent **(9d)** can readily be replaced by sugar reagents such as **1** to **18** to provide variants covering the range of definitions within formula (I).

For variants of the R_1 group, a range of chloroalkoxyacylchlorides are known or commercially available, *e.g.*,



CAS 22128-62-7



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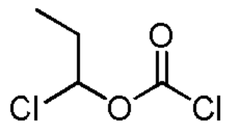
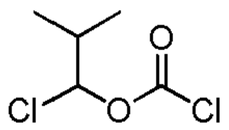
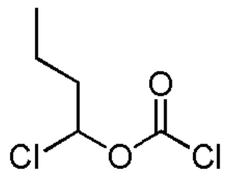


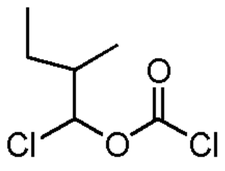
CAS 92600-20-9



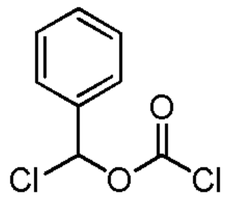
CAS 92600-11-8



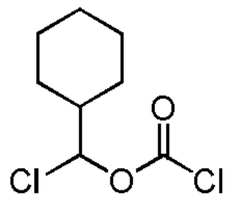
CAS 103057-35-8



CAS 882572-70-5



CAS 81363-09-9



CAS 81363-12-4

EXAMPLES

The present invention is further illustrated by reference to the following Examples. However, it should be noted that these Examples, like the embodiments described above, are illustrative and are not to be construed as restricting the enabled scope of the invention in any way.

The following examples serve to more fully describe the manner of making and using the above-described invention. It is understood that these examples in no way serve to limit the true scope of the invention, but rather are presented for illustrative purposes.

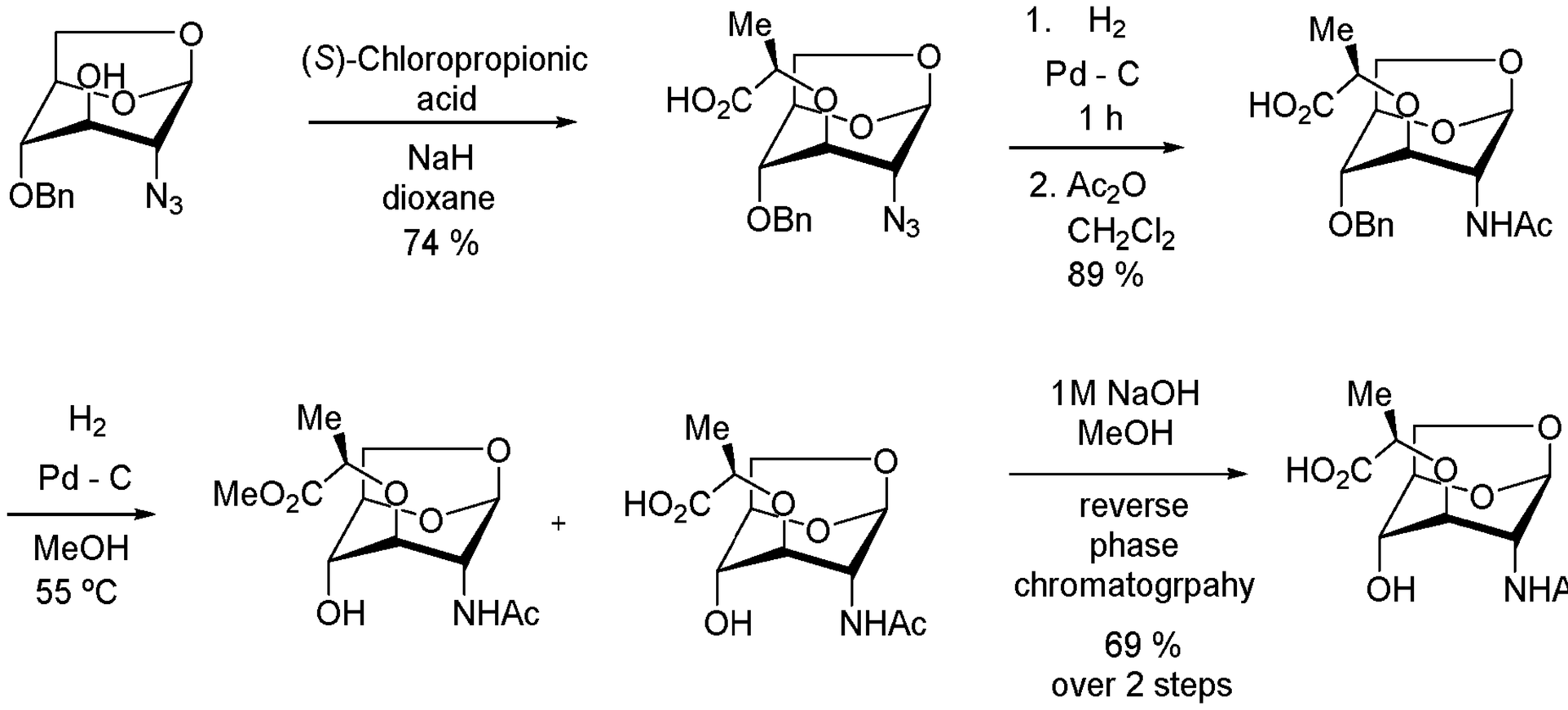
Synthetic Chemistry

In the examples and the synthetic schemes below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

AcOH	acetic acid
Ac_2O	acetic anhydride
aq	aqueous
BOC (Boc)	N-tert-butoxycarbonyl or tert-butyloxycarbonyl
CBz	carboxybenzyl
DCE	dichloroethane
DCM	dichloromethane
DCM/EA	dichloromethane/ethanol
DIPEA (or DIEA)	N,N-diisopropylethylamine, or Hünig's base
DME	dimethoxyethane
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO-d6	deuterated dimethylsulfoxide
DMSO	dimethylsulfoxide
EC_{50}	50% effective concentration
EDTA	ethylenediaminetetraacetic acid
Et	ethyl
Et_2O	diethyl ether
EtOH	ethanol
EtOAc, EA, AcOEt	ethyl acetate

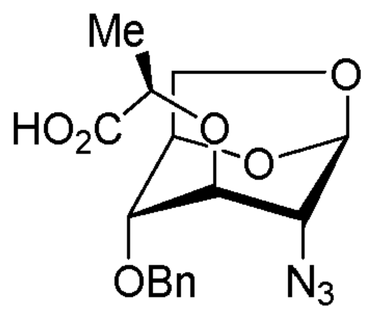
h	hour(s)
HPLC	high performance liquid chromatography
IC_{50}	50% inhibition concentration
iPrOH	isopropyl alcohol or isopropanol
LCMS	Liquid chromatography mass spectroscopy
LDA	lithium di-isopropyl amide
Me	methyl
MeOH	methanol
$NaBH(OAc)_3$	sodium triacetoxyborohydride
NMR	Nuclear Magnetic Resonance spectroscopy
$Pd_2(dba)_3$	Tris(dibenzylideneacetone)dipalladium(0)
PE	petroleum ether or petrol
PPh_3	triphenylphosphine
Pr	propyl
SFC	supercritical fluid chromatography
T3P	1-Propanephosphonic anhydride solution, 2,4,6-Tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide
TFA	trifluoroacetic acid
THF	tetrahydrofuran
uv	ultraviolet

Part 1: Synthesis of 1,6-Anhydro-*N*-acetylmuramic acid



Scheme 5. Synthesis of 1,6-Anhydro-*N*-acetylmuramic acid

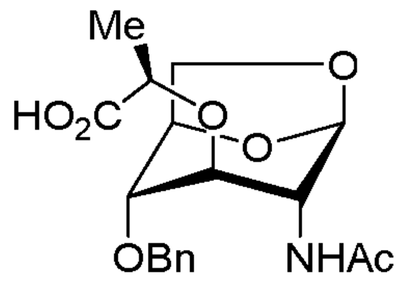
Step 1. Preparation of (*R*)-2-(((1*R*,2*S*,3*R*,4*R*,5*R*)-4-Azido-2-(benzyloxy)-6,8-dioxabicyclo[3.2.1]octan-3-yl)oxy) propanoic acid



To a solution of (1*R*,2*S*,3*R*,4*R*,5*R*)-4-azido-2-(benzyloxy)-6,8-dioxabicyclo[3.2.1]octan-3-ol (200 mg, 0.72 mmol) in anhydrous 1,4-dioxane (4 mL) was added sodium hydride (60 %dispersion in oil; 191 mg, 4.76 mmol) and the mixture was heated at 45 ℃ for 10 min. The suspension was cooled to room temperature, (*S*)-2-chloropropioninc acid (188 mg, 148 µL, 1.73 mmol) was added and the mixture was heated at 90 ℃ for 2 h. The brown suspension was cooled to room temperature and concentrated to give a pale brown solid. This material was cautiously quenched with water (10 mL) and the solution was acidified to pH 3 with conc. hydrochloric acid. The aqueous suspension was extracted with dichloromethane (7 × 10 mL). The combined organic layers were washed with water (25 mL), dried ($MgSO_4$) and concentrated to give a green oil. This material was purified using a Biotage Isolera automated chromatography system under normal phase conditions (silica column, gradient of 0 → 100 % methanol in dichloromethane) with detection at 254 nm to give titled acid (185 mg, 74 %) as a green oil. R_f = 0.40 (methanol - dichloromethane, 8 : 92 v/v)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 5H, 5 × ArH), 5.57 (m, 1H, CH), 4.69 (m, 3H, CH and benzylic CH_2), 4.00 (m, 2H, 2 × CH), 3.76 (dd, *J* = 7.5, 5.6 Hz, 1H, CH), 3.63 (m, 1H, CH), 3.38 (m, 1H, CH), 3.31 (m, 1H, CH), 1.40 (d, *J* = 6.9 Hz, 3H, CH_3).

Step 2. Preparation of (*R*)-2-(((1*R*,2*S*,3*R*,4*R*,5*R*)-4-Acetamido-2-(benzyloxy)-6,8-dioxabicyclo[3.2.1]octan-3-yl)oxy) propanoic acid



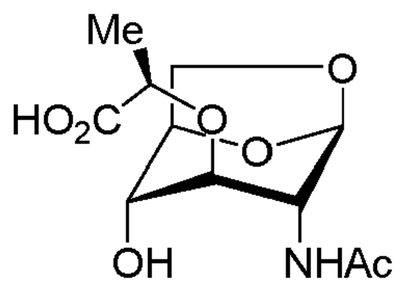
To suspension of 10 % Pd-C (19 mg, 10 % w/w) in anhydrous methanol (1 mL) under nitrogen was added a solution of Step 1 acid (185 mg, 0.53 mmol) in anhydrous methanol (3 mL) and

the reaction mixture was stirred under a hydrogen atmosphere for 1 h 15 min. The suspension was filtered through Celite and the filtrate was concentrated to give a white solid (172 mg). This material was dissolved in anhydrous dichloromethane (3.8 mL), acetic anhydride (1.56 mL) was added and the reaction mixture was stirred at room temperature overnight. The solution was concentrated and the residue was partitioned between dichloromethane (10 mL) and water. The layers were separated and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with saturated brine (25 mL), dried ($MgSO_4$) and concentrated to give colourless oil. This material was purified using a Biotage Isolera automated chromatography system under normal phase conditions (silica column, gradient of 0 → 100 % methanol in dichloromethane) with detection at 254 nm to give titled acid (173 mg, 89 %) as a white foam.

LCMS r.t. = 7.5 min, ESI-MS (m/z): 364 $[M-H]^-$

^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H, 5 × ArH), 6.17 (d, J = 6.9 Hz, 1H, NH), 5.34 (m, 1H, CH), 4.61 (m, 3H, CH and benzylic CH_2), 4.23 (q, J = 6.8 Hz, 1H, CH), 4.12 (m, 2H, 2 × CH), 3.72 (dd, J = 7.3, 5.9 Hz, 1H, CH), 3.47 (m, 1H, CH), 3.39 (m, 1H, CH), 1.94 (s, 3H, CH_3), 1.41 (d, J = 6.9 Hz, 3H, CH_3).

Step 3. Preparation of 1,6-Anhydro-*N*-acetylmuramic acid



To suspension of 10 % Pd-C (26 mg, 15 % w/w) in methanol (1 mL) under nitrogen was added a solution of Step 2 acid (171 mg, 0.47 mmol) in methanol (3 mL) and the reaction mixture was heated under hydrogen atmosphere at 55 °C for 4 h. The suspension was cooled to room temperature, filtered through Celite and the filtrate was concentrated to give a white solid (136 mg, mixture of 1,6-anhydro-*N*-acetylmuramic acid and 1,6-anhydro-*N*-acetylmuramic acid methyl ester). This material was dissolved in methanol (4 mL), 1M aqueous solution of NaOH (4 mL) was added and the mixture was stirred for 1 h at room temperature. The methanol was removed and the aqueous solution was acidified to pH 3 with conc. hydrochloric acid and concentrated. The material was purified using a Biotage Isolera automated chromatography system under reversed-phase conditions (C_{18} column, gradient of 10 → 100 % acetonitrile in

water) with detection at 210 nm to give 1,6-anhydro-*N*-acetylmuramic acid (87 mg, 67 %) as a white solid.

LCMS r.t. = 1.3 min ESI-MS (m/z): 276.00 $[M+H]^+$

^{1}H NMR (300 MHz, CD_3CN) δ 6.76 (d, J = 8.7 Hz, 1H, NH), 5.51 (m, 1H, CH), 4.68 (m, 1H, CH), 4.44 (q, J = 6.9 Hz, 1H, CH), 4.30 (dd, J = 7.4, 1.0 Hz, 1H, CH), 4.05 (m, 1H, CH), 3.85 (m, 2H, 2 × CH), 3.56 (m, 1H, CH), 2.10 (s, 3H, CH_3), 1.56 (d, J = 6.9 Hz, 3H, CH_3).

Stability Testing procedures

(i) Plasma Stability (Human, mouse and/or Rat)

To quantify the degradation of the test compound in plasma over a 1 hour period. The percent of parent compound present at 0, 30 and 60 mins after initiating incubations in plasma is determined. Compounds are taken from 10 mM DMSO stock solutions and added to plasma, which has previously been incubated at 37°C, to give a final concentration of 25 μM and re-incubated. Aliquots are removed at the appropriate timepoints and quenched with an equal volume of cold acetonitrile. After mixing vigorously, the precipitated protein matter are removed by filtration (Multiscreen Solvinert filter plates, Millipore, Bedford, MA, USA) and the filtrate analysed by reverse phase HPLC with mass spectrometric detection, using single ion monitoring of the $[M+H]^+$ species. Metabolic turnover is determined by comparison of peak areas from the ion chromatograms of the parent before and after incubation and expressed as percent remaining at each timepoint.

(ii) Microsomal Metabolic Stability (Human, mouse and/or Rat)

Test compound (3μM) is incubated with pooled liver microsomes. Test compound is incubated at 5 time points over the course of a 45 min experiment and the test compound is analysed by LC-MS/MS. An intrinsic clearance value (CL_{int}) with standard error and $t_{½}$ value are calculated.

Microsomes (final protein concentration 0.5mg/mL), 0.1M phosphate buffer pH7.4 and test compound (final substrate concentration 3μM; final DMSO concentration 0.25%) are pre-incubated at 37 C prior to the addition of NADPH (final concentration 1mM) to initiate the reaction. The final incubation volume is 50μL. A minus cofactor control incubation is included for each compound tested where 0.1M phosphate buffer pH7.4 is added instead of NADPH

(minus NADPH). Two control compounds are included with each species. All incubations are performed singularly for each test compound. Each compound is incubated for 0, 5, 15, 30 and 45min. The control (minus NADPH) is incubated for 45min only. The reactions are stopped by transferring 20µL of incubate to 60µL methanol at the appropriate time points. The termination plates are centrifuged at 2,500rpm for 20min at 4 C to precipitate the protein. Following protein precipitation, the sample supernatants are combined in cassettes of up to 4 compounds and analysed using generic LC-MS/MS conditions. From a plot of ln peak area ratio (compound peak area/internal standard peak area) against time, the gradient of the line is determined. Subsequently, half-life and intrinsic clearance are calculated using the equations below:

Elimination rate constant (k) = (- gradient)

$$\text{Half-life } (t_{½}) \text{ (min)} = \frac{0.693}{k}$$

$$\text{Intrinsic clearance } (CL_{int}) \text{ (µL/min/mg protein)} = \frac{V \times 0.693}{t_{1/2}}$$

where V = Incubation volume (µL)/Microsomal protein (mg)

Relevant control compounds are assessed, ensuring intrinsic clearance values fall within the specified limits.

(iii) Hepatocyte Stability (Human, mouse and/or Rat)

Test compound (3µM) is incubated with cryopreserved hepatocytes in suspension. Samples are removed at 6 time points over the course of a 60 min experiment and test compound is analysed by LC-MS/MS. An intrinsic clearance value (CL_{int}) with standard error and half-life ($t_{½}$) are calculated. Cryopreserved pooled hepatocytes are stored in liquid nitrogen prior to use. Williams E media supplemented with 2mM L-glutamine and 25mM HEPES and test compound (final substrate concentration 3µM; final DMSO concentration 0.25 %) are pre-incubated at 37 C prior to the addition of a suspension of cryopreserved hepatocytes (final cell density $0.5x10^6$ viable cells/mL in Williams E media supplemented with 2mM L-glutamine and 25mM HEPES) to initiate the reaction. The final incubation volume is 500µL. A control incubation is included for each compound tested where lysed cells are added instead of viable cells. Two control compounds are included with each species.

The reactions are stopped by transferring 50µL of incubate to 100µL methanol containing internal standard at the appropriate time points. The control (lysed cells) is incubated for 60min only. The termination plates are centrifuged at 2500rpm at 4°C for 30min to precipitate the protein. Following protein precipitation, the sample supernatants are combined in cassettes of up to 4 compounds and analysed using generic LC-MS/MS conditions. From a plot of ln peak area ratio (compound peak area/internal standard peak area) against time, the gradient of the line is determined. Subsequently, half-life ($t_{½}$) and intrinsic clearance (CL_{int}) are calculated using the equations below:

Elimination rate constant (k) = (- gradient)

$$\text{Half-life } (t_{½}) \text{ (min)} = \frac{0.693}{k}$$

$$\text{Intrinsic clearance } (CL_{int}) \text{ (µL/min/million cells)} = \frac{V \times 0.693}{t_{1/2}}$$

where V = Incubation volume (µL)/Number of cells

Two control compounds for each species are included in the assay and if the values for these compounds are not within the specified limits the results are rejected and the experiment repeated.

(iv) Whole Human Blood Stability (Human, mouse and/or Rat)

The test compound is incubated with fresh human (mixed sex) blood at 37 C at 5 time points over a 60min period. The samples are analysed by LC-MS/MS and the percent of parent compound remaining is calculated for each time-point. The percent parent compound remaining at each time point is determined.

Fresh human (mixed sex) blood is used. Single incubations are performed at a test or control compound concentration of 1µM in blood at 37 °C. The final DMSO concentration in the incubation is 0.25%. A control compound is included with each species. Reactions are terminated following 0, 5, 15, 30 and 60min by acetonitrile containing internal standard. The sampling plate is centrifuged (3000rpm, 45min, 4 C) and the supernatants from each time point analysed for parent compound by LC-MS/MS. The percentage of parent compound remaining at each time point relative to the 0min sample is then calculated from LC-MS/MS peak area ratios (compound peak area/internal standard peak area).

(v) LogD Determinations:

$LogD_{(PBS)}$ determinations is performed in 96 well microtitre plates using a miniaturised "shake-flask" method. In brief, compounds are taken from 10 mM DMSO stock solutions and added to wells containing equal volumes of phosphate buffered saline (10 mM; pH 7.4) (PBS) and 1-octanol (Sigma-Aldrich, Poole, Dorset, UK) to give a final concentration of 50 μM. The plates are then capped and mixed vigorously for 1 hour on a microtitre plate shaker, after which they were left to stand, allowing the PBS and octanol phases to separate. The PBS layer is analysed by reverse phase HPLC with mass spectrometric detection, using single ion monitoring of the $[M+H]^+$ species. $LogD_{(PBS)}$ is determined by comparison of the peak area from the ion chromatogram of the compound in the PBS phase with that of a 50μM standard of the same compound dissolved in acetonitrile/water (50:50) and calculated using the following formula:

$$LogD = Log\left[\frac{AUCstd - AUCpbs}{AUCpbs}\right]$$

Where *AUCstd* and *AUCpbs* are the peak areas from the standard and test ion chromatograms respectively. $LogD_{(PBS)}$ determinations were also made using PBS at pH6.9 and 5.5 by adjusting the pH of the buffer prior to the start of the assay, with 0.1 M HCL

Determination of Chemical Stability as a Function of pH

The chemical stability of the compounds of the invention is studied as a function of pH vs time. The loss of the compound and formation of released parent is quantified by RP-HPLC as appropriate.

General Procedure for HPLC stability tests

All chemical stability tests (0.2-2.0mg/mL) are performed at 37°C in duplicate with or without co-solvent (MeCN or DMSO) depending on the solubility in the following pH buffered solutions. The results are presented as the mol % of both the compound and parent antibacterial present initially and at the final time point (measured by HPLC peak integration). In order to calculate the concentration of the parent antibacterial formed, it is necessary to calibrate the HPLC using a pure standard to take into account any difference in the extinction coefficients.

(i) pH 1.2 0.1 M Chloride Buffer

This is prepared by dissolving NaCl (0.2 g) in 90 mL of distilled water and adjusting the pH to 1.2 with approximately 5 mL of 1M hydrochloric acid. The volume is made up to 100 mL with distilled water and if required, adjusted to pH 1.2 with a few drops of 1M hydrochloric acid. The test conditions are 37 °C and a total time of 1 hour.

(ii) **pH 3.0 0.1 M Citrate Buffer**

This is prepared by adding 1 M sodium hydroxide (4 - 5 mL) to100 mL of 0.1 M aqueous citric acid until a pH of 3.0 is obtained. The test conditions are 20 °C and a total time of 2 hours.

(iii) pH 6.8 0.1 M Phosphate buffer

This is prepared by adding 1 M sodium hydroxide to 100 mL of 0.1 M aqueous sodium dihydrogen phosphate until a pH of 6.8 is obtained. The test conditions are 37 °C and a total time of 2 hours.

(iv) pH 7.4 0.1 M Phosphate buffer

This is prepared by adding 1 M sodium hydroxide to 100 mL of 0.1 M aqueous sodium dihydrogen phosphate until a pH of 7.4 is obtained. The test conditions are 37 °C and a total time of 2 hours.

(v) pH 8.0 0.1 M Phosphate buffer

This is prepared by adding 1 M sodium hydroxide to 100 mL of 0.1 M aqueous sodium dihydrogen phosphate until a pH of 8.0 is obtained. The test conditions are 20 °C and a total time of 2 hours.

MIC and MBC Test Procedure

MIC testing is used to determine *in vitro* activity of antimicrobial compounds. MIC testing is conducted in 96-well plates containing the test samples in serial 2-fold dilutions. The assay plates are incubated in an ambient-air incubator at 35±2 °C for 20 hrs, and bacterial growth is observed and recorded. The lowest concentration that inhibits visible growth of a microorganism is defined as the MIC of an antimicrobial compound.

MBC is defined as the lowest concentration that kills 99.9% of a microorganism. In this study, all the contents of no-growth wells from the MIC assay plates are transferred into new 96-well

plates, and 10 µL of the content is transferred onto TSA with liquid handler. The CFUs on the TSA plate are summarized after 20 hrs incubation. When the number of colonies from single 10 µL sample is equal to or less than the rejection value, the concentration of compound is declared bactericidal.

Bacterial strain preparation

Revive the selected strains from single-use frozen vials (-80 °C) one day before the testing. Streak onto surface of TSA plate, and incubate the plate for 20-24 hrs at 35±2 °C in ambient air.

Organisms used for MIC

Pseudomonas aeruginosa NCTC 13457 (MDR β-lactams and aminoglycosides)
ATCC-BAA-2108 (Carbapenem-resistant non-MBL producing)
PA-01 (susceptible strain)

Acinetobacter baumannii NCTC 13420 (MDR and ESBL positive)
NCTC 19606 (susceptible strain)

E.coli NCTC 13353 (ESBL positive CTX-M-15 producing)
ATCC-BAA-2340 (ESBL positive KPC producing)
ATCC 25922 wild-type

Klebsiella pneumoniae ATCC 700603, (ESBL positive and SHV-18)
ATCC-BAA-2146 (NDM-1 producing)
ATCC- 13882 (MDR).

Bacterial inoculum preparation

Take out medium broth from 4 °C fridge and allow it to warm to room temperature. Resuspend 5-10 colonies into 500 µL sterile saline (0.9% NaCl) and adjust OD_{600} to 0.1~0.15. Dilute bacterial inocula 1:300 for Gram-negative strains into 1.02x CAMHB.

Assay plate preparation

Transfer 2 µL of compound from compound plate to the corresponding well of each assay plate. After that add 98 µL of the bacterial inocula (~2×10^5 CFU/mL) to each well of the assay plates by liquid handler Cybio96. Stack the assay plates together and cover with a sterile plate lid. Spin plates at 1000 rpm for 30 secs and incubate in an ambient-air incubator at 35±2 °C for 20 hrs.

Viability determination

Dilute the bacterial inocula serially 10^{-1} to 10^{-3} in medium broth (e.g. 100 µl bacterial inocula + 900 µL of CAMHB). Spread 100 µL of each dilution onto TSA plates in duplicates. Allow the plates to dry and incubate them for 20 hrs at 35±2 ºC. Count colonies and calculate CFU of bacterial inocula.

MIC determination

Place the assay plate on the top of MIC reader, and adjust the magnification mirror to read each wells. Observe and record growth status of bacteria as raw data and determine MIC break points according to CLSI guideline M100-S26 [1].

Perform MBC test and record MBCs

Transfer all the content of no-growth wells from the MIC plates of selected strains of assay II intonew 96-well plates. Transfer 10 µl of test sample from new 96-well plates onto TSA plates with liquid handler CyBio96 in biosafety cabinets. Incubate the TSA at 35±2 ºC for 20 hrs and count MBC colonies. Determine MBCs according to the rejection values of MBC coloniesof CLSI guideline M26-A [2]

REFERENCES

[1] M100-S26 Performance Standards for Antimicrobial Susceptibility Testing. 2016. Clinical and Laboratory Standard Institute.

[2] M26-A Methods for Determining Bactericidal Activity of Antimicrobial Agents. Approved guideline, 1999. Clinical and Laboratory Standard Institute.

Spontaneous Mutation Frequency

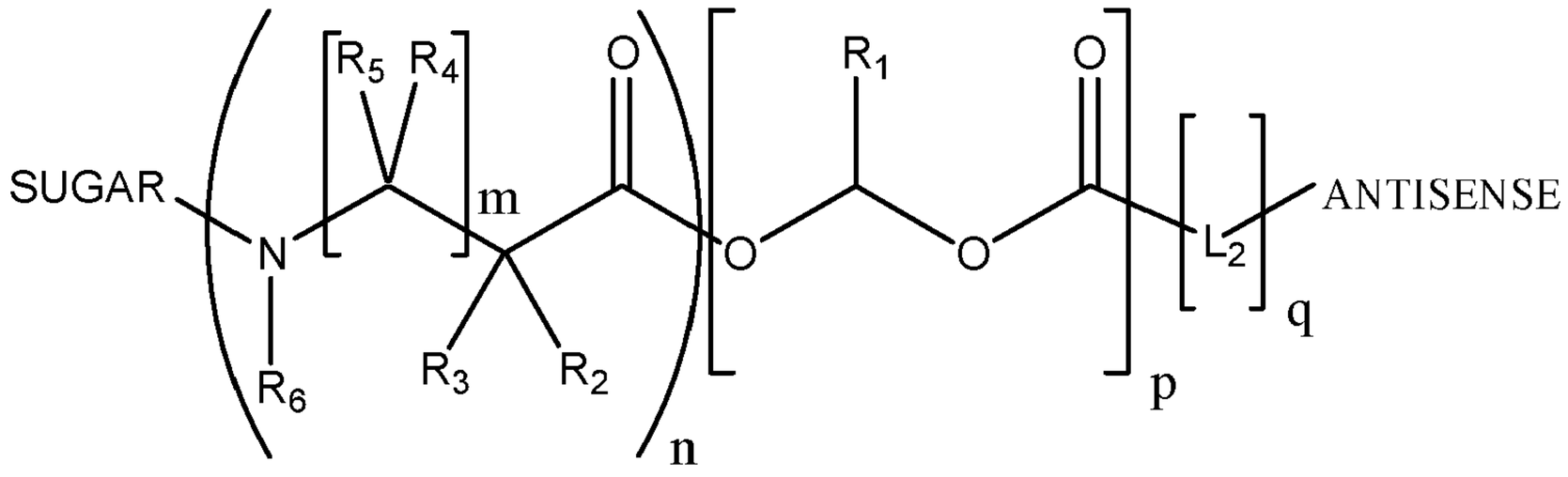
Spontaneous mutation frequency is used as a measure of the development of resistance to the compounds using a method described by Zurenko (Zurenko, G.E., et al. 1996).

Agar plates are poured with 2x, 4x and 8x MIC concentrations of the antimicrobial compounds. After setting, 100µl of each concentrated bacterial culture (~10^{11}) is plated onto the plates which were incubated for 48 hours at 37°C.

The spontaneous mutation frequency is calculated as the ratio of the number of colony forming units (cfu) per ml that grew from a known inoculation after 48 hours incubation at 37°C.

CLAIMS

1. A compound of formula (I), or a pharmaceutically acceptable salt thereof,

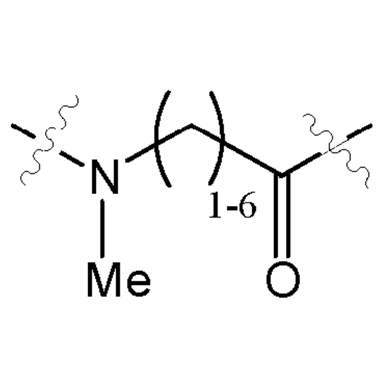


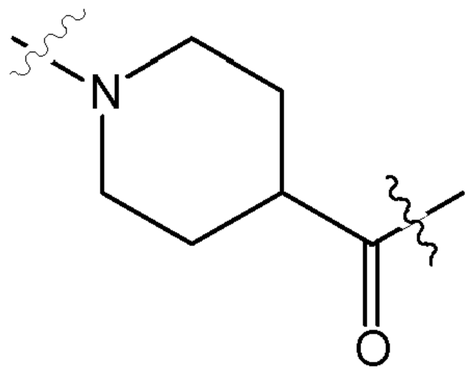
(I)

wherein,

ANTISENSE is an oligonucleotide having natural, artificial and/or modified nucleobases, the oligonucleotide selected from the group consisting of phosphodiester oligonucleotides (PDOs), phosphorothioate oligonucleotides (PSOs), phosphorodiamidate morpholino oligonucleotides (PMOs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs), 2'-O-Alkyl oligonucleotides (2'-O-Me, 2'-O-Et. 2'-O-methoxyethyl) and combinations thereof; wherein the oligonucleotide is bonded to the remainder of the molecule of formula I via a terminal amino group present within the ANTISENSE sequence; and

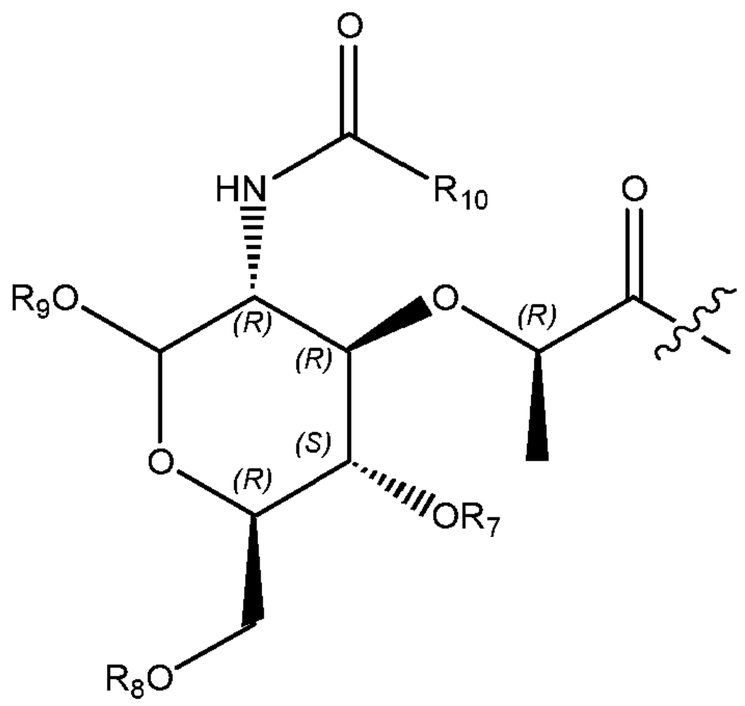
L_2 is a spacer that forms a chemical bond to a terminal amino group present within the ANTISENSE sequence and a second chemical bond to the terminal carbonyl of the remainder of the molecule of formula I and is chosen from the group consisting of:



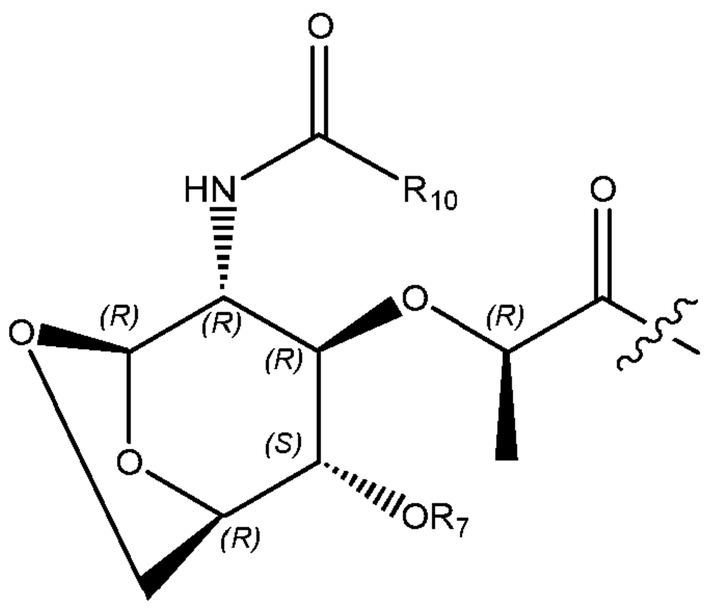


;

SUGAR is any tautomeric form of the acyl fragment of an *N*-acylmuramic acid or 1,6-anhydro-*N*-acylmuramic acid having the structure:



or



;

R_1 and R_6 are each independently selected from the group consisting of:

H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl;

R_2 and R_3 are each independently selected from the group consisting of:

H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl, or both together with the carbon atom to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms; and

R_4 and R_5 are each independently selected from the group consisting of:

H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl, or both together with the carbon atom to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms;

or

R_2 and R_4 together with the adjacent carbon atoms to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms; and R_3 and R_5 are each independently selected from the group consisting of: H, C_{1-6} alkyl, C_{1-6} substituted alkyl, C_{3-8} cycloalkyl, C_{3-8} substituted cycloalkyl, phenyl and benzyl, or both together with the carbon atom to which they are attached form a ring containing 3, 4, 5 or 6 carbon atoms;

R_7, R_8 and R_9 are each independently selected from the group consisting of:

H, acetyl, benzoyl; and

R_{10} is selected from the group consisting of:

methyl, ethyl, propyl; and

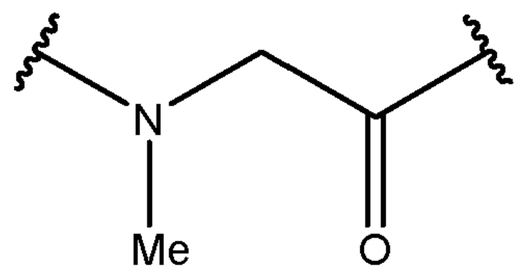
m is 0 or 1; and

n is 0 or 1 or 2 or 3 or 4; and

p is 0 or 1; and

q is 0 or 1.

2. A compound according to claim 1 wherein the ANTISENSE is a phosphorodiamidate morpholino oligonucleotide (PMO) or a peptide nucleic acid (PNA).

3. A compound according to claim 1 or claim 2 wherein m is 0, each of n and p and q are 1, R_3 is H, R_6 is H and L_2 is N-methylglycyl (i.e. 

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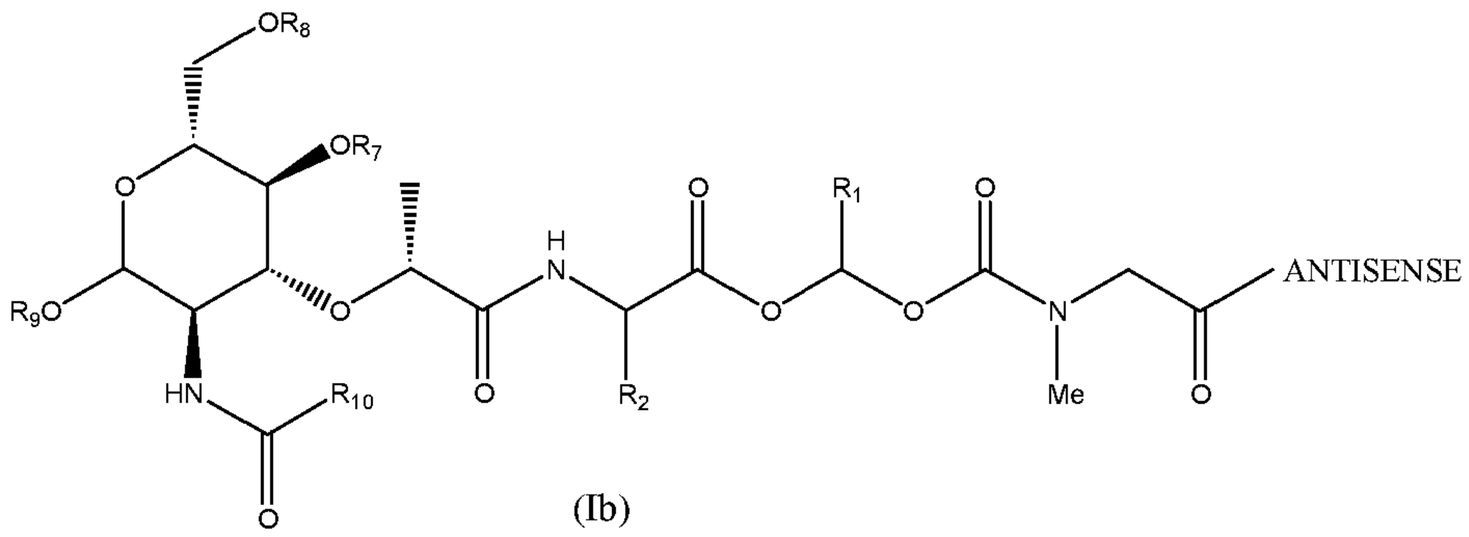
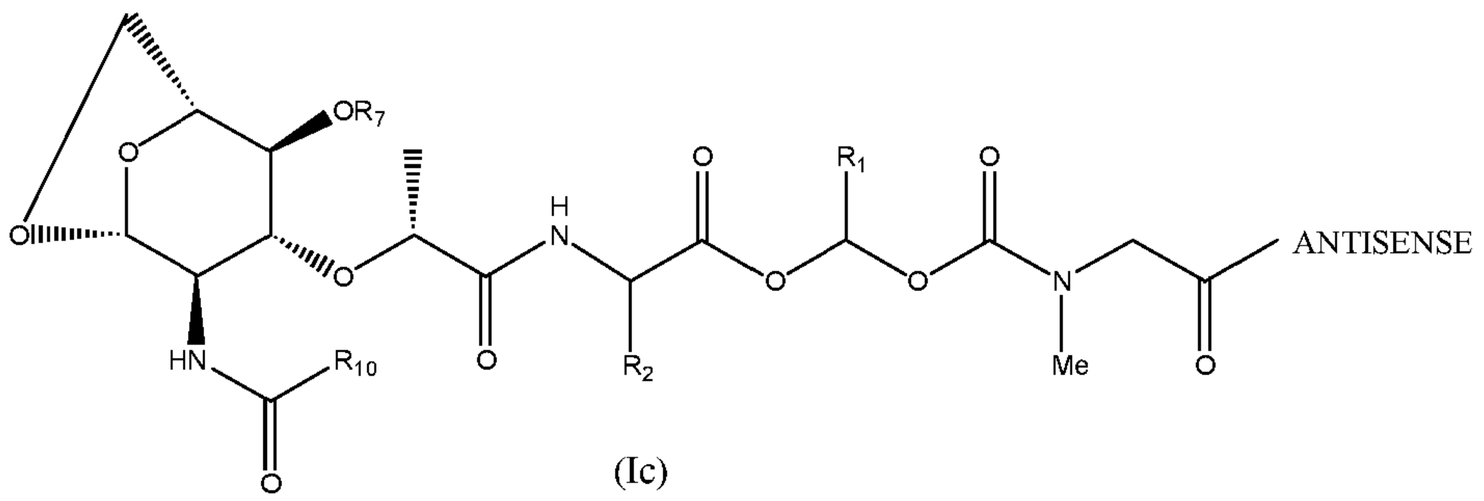
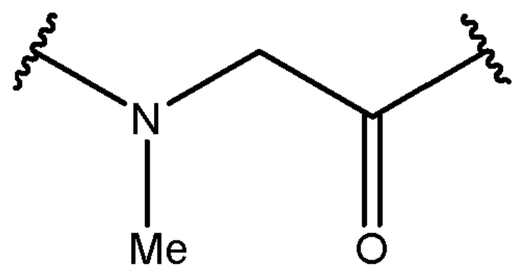


(Ib)



(Ic)

R_1, R_2, R_7, R_8, R_9 and R_{10} are as defined in claim 1.

4. A compound according to claim 1 or claim 2 wherein m is 0, n is 1, p is 0 and q is 1, R_3 is H, R_6 is H and L_2 is N-methylglycyl (i.e. 

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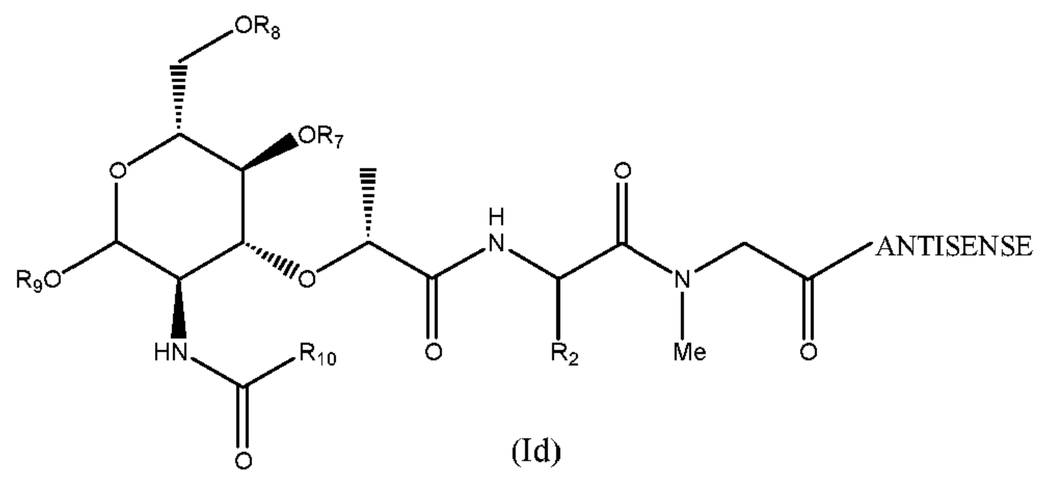
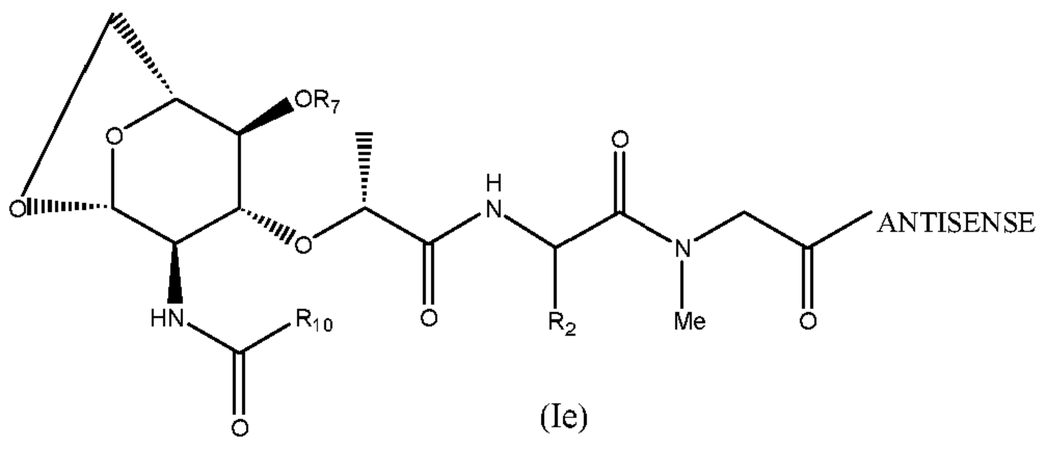


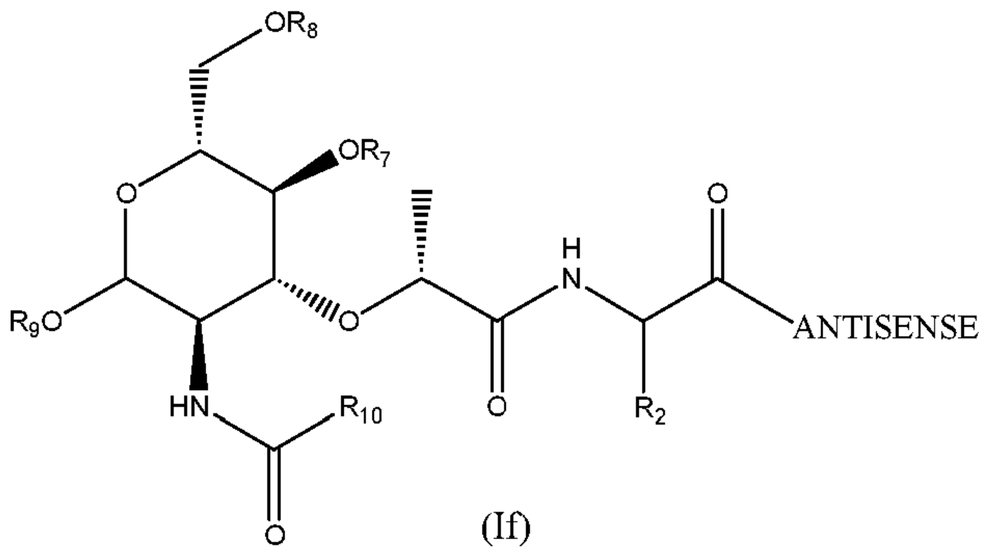
(Id)



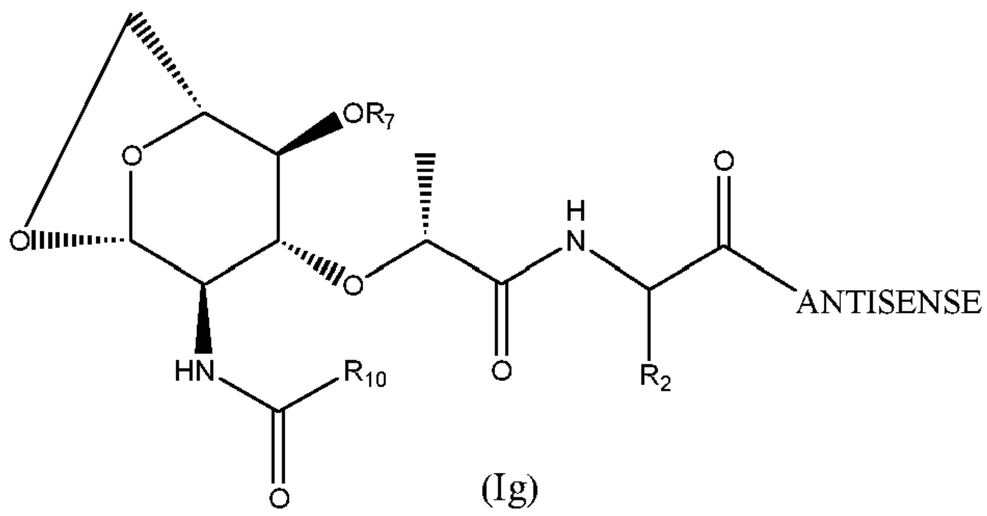
(Ie)

R_2, R_7, R_8, R_9 and R_{10} are as defined in claim 1.

5. A compound according to claim 1 or claim 2 wherein m is 0, n is 1, p is 0, q is o and R_3 is H:



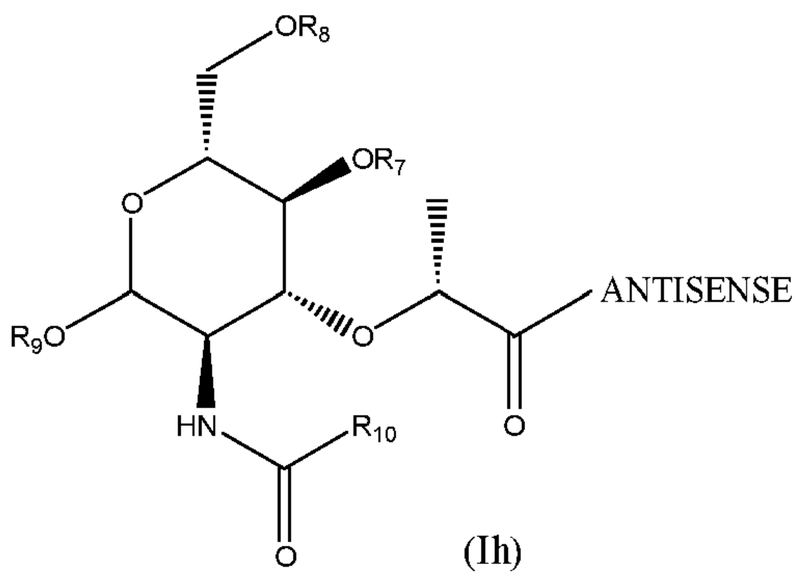
(If)



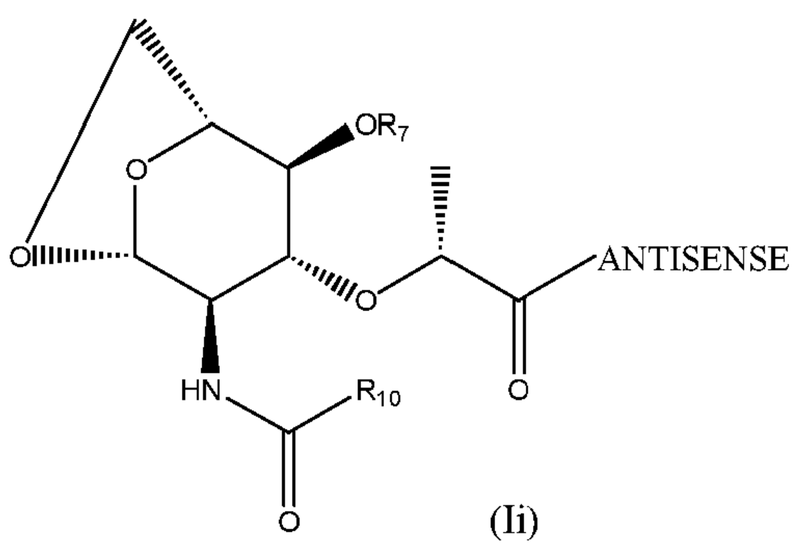
(Ig)

R_2, R_7, R_8, R_9 and R_{10} are as defined as defined in claim 1.

6. A compound according to claim 1 or claim 2 wherein m is 0, n is 0, p is 0 and q is o:



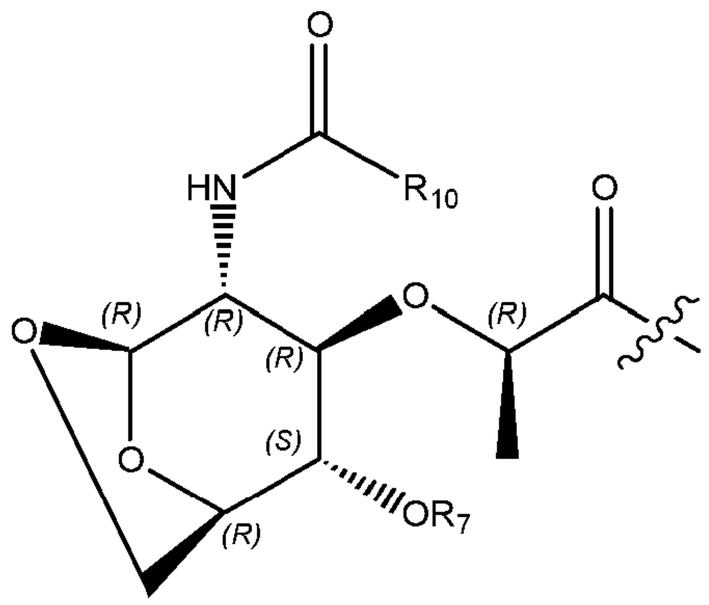
(Ih)



(Ii)

R_7, R_8, R_9 and R_{10} are as defined as defined in claim 1.

7. A compound according to any preceding claim wherein the SUGAR is



8. A compound according to any preceding claim wherein the ANTISENSE includes a sequence that is selected from the group consisting of:

Table 1A; Exemplary Antibiotic Resistance Targeting Sequences

Target Gene	Antisense sequence (5'-3')	Sequence ID
NDM-1	TCA AGT TTT CC	1
NDM-1	TCC TTT TAT TC	2
NDM-1	CCA TCA AGT TT	3
NDM-1	GGC AAT TCC AT	4
adeA	ATA CTG TCC AA	5
OmpA	CAT GGA TAT CC	6
AcrA	ATG TAA ACC TC	7
AcrA	GTT CAT ATG TA	8
AcrA	ACC CCT CTG TT	9
AcrA	TGT TCA TAT GT	10
AcrB	GTC TTA ACG GC	11
AcrB	AGG CAT GTC TT	12
AcrB	TAG GCA TGT CT	13
AcrR	TAT GTT CGT GA	14
TolC	TTC ATT TGC AT	15
TolC	ATT CCT TGT GG	16
TolC	TTT GCA TTC CT	17
KPC	GAT ACA GTG AC	18
KPC 1-4	AAC GAT ATT CC	19

Table 18; Exemplary Biofilm Formation Targeting Sequences		
Target Gene	Antisense sequence (5'-3')	Sequence ID
cepI	AAG GTC TGC AT	20
cepI	TCG GAT CTG TG	21
cepI	CAT GGA TGT CC	22
cepI	CGT GAA CGA AG	23
cepI	CGT GTG GCA AC	24
cepI	GCC CGA GAT CC	25
cepI	CTT TCG TTC GC	26
suhB	ATG CAT GAG CC	27
suhB	GGA TGC ATG AG	28
CsuE	TTA TAT TCA TGG	29
CsuE	TCA TGG CAA AG	30
CsuE	TTT CCT GTC AA	31
SecA	TTG CCA ACA TG	32
PgiL	CAT TAC CCA AG	33
PilU1	TTA AAA TCC AT	34
AlgZ	TAG GCA TCG AC	35
AlgU	AAA GCT CCT CT	36
LasR	AGG CCA TAG CG	37
FleR	TTA CTC CTG AA	38
PelF	TTC GGT CAT GT	39

Table 1C; Exemplary Fatty Acid Biosynthesis-associated Targeting Sequences		
Target Gene	Antisense sequence (5'-3')	Sequence ID
acpP	GTC CAT TAC CC	40
acpP	CAT TAC CCC TC	41
acpP	CCA TTA CCC CT	42
acpP	TCC ATT ACC CC	43
acpP	TGT CCA TTA CC	44
acpP	TTG TCC ATT AC	45
acpP	GTT GTC CAT TA	46
acpP	TGT TGT CCA TT	47
acpP	ATG TTG TCC AT	48
acpP	TTT ACA AGT GC	49
acpP	CCT CCG AGG GA	50
acpP	ACA CGT TGT TC	51
acpP	AGT TCA GCG AC	52
acpP	CTC ATA CCT TG	53
acpP	TGC TCA TAC TC	54
acpP	CTC ATA CTC T	55
acpP	CTC ATA CTA T	56
acpP	CTT CGA TAG TG	57
acpP	ATA TCG CTC AC	58
acpP	ATT CTC CTC AT	59
acpP	CAC AGG AAT TC	60
acpP	CAT TGC TTG TG	61
acpP	CAT ACC TTG TT	62
acpS	TTG CCA TTA GC	63
acp-E	CTG TAG TGA TTT CAC CA	64
fabA	TTA TCT ACC AT	65
fabB	CGT TTC ATT AA	66
fabB	GCA CGT TTC AT	67
fabI	AGA AAA CCC AT	68
fabI	GCT TTA ATC C	69
fabI	CCC ATA GCT T	70
fabI	CAT GTA AGA T	71
fabI	AGA TAA CTC C	72
gapA	TTG ATA GTC AT	73
accA	GCT TTT TTC AT	74
accA	AGG CTT CCG TC	75
fabD	GTC ATG TTT T	76
inhA	GTC ATT TGG T	77
inhA	CAT TTG GTG ACT	78

Table 1D; Exemplary Targeting Sequences associated with other pathways or cellular processes		
Target Gene	Antisense sequence (5'-3')	Sequence ID
RpoD	TCA TCT TTG CT	79
RpoD	TTT TGC TCC AT	80
Pol8	AGT AAC TCC AC	81
murA	TTT ATC CAT TG	82
rpsJ	GCA TTT GAC CT	83
rpsJ	TAG ACA TAC CA	84
rpsJ	TAC CAG TAA AC	85
rpsJ	TGG TTC TGC AT	86
rpsJ	CCT CAG ACT CC	87
rpsJ	GCA TTT GAC CT	88
ftsZ	AGT TTC TCT CC	89
ftsZ	GTT CAA ACA TA	90
ftsZ	TCA AAT GAG GC	91
ftsZ	AAT GAG GCC AT	92
ftsZ	ATA GTT TCT CTC C	93
gyrA	CGC TCA TCT AA	94
gyrA	CTA TAC ATA GAC	95
gyrA	GCC ATC TCG GAC ATC	96
gyrA	ATA CCA GGT GTT ATC T	97
dna8	TTC CTG CCA TA	98
LpxC	TTT GAT CAT CG	99
LpxC	TGT TTG ATC AT	100
LpxC	TGT TTC ACC AT	101
LpxC	GTT GTT TGA TC	102
23S rRNA	AGT GCT CTA CC	103
23S rRNA	GCC TGT TAT CC	104
16S rRNA	CCA TGC AGC AC	105
16S rRNA	TTG CGC TCG TT	106
16S rRNA	GGC TGC TGG CA	107
fmh8	CCA TGA AAA A	108
polA	TTC ATG CCT GT	109
murA	ATC CAT TTA GT	110
murA	CAT TTA GTT TG	111
murA	AAT TTA TCC AT	112
murA	AAA TTT ATC CA	113
rpm8	ACT CGG GAC AT	114
rpm8	CTA TTC TCC AA	115
rpm8	GGC AGA CTC GG	116
rpm8	CTT AGA CAT GG	117
adk	ATG ATA CGC AT	118
adk	AGT GCC CTC C	119
infA	TCT TTG GCC AT	120

Table 10; Exemplary Targeting Sequences associated with other pathways or cellular processes		
Target Gene	Antisense sequence (5'-3')	Sequence ID
aroC	TTT CCA GCC AT	121
aroC	TTC CCT GCC AT	122
murF	ACG CTA ATC AT	123
murF	ACC TCC CAG GC	124
kdtA	AAT TCG AGC AT	125
lpxA	TGT TTA AGA GC	126
lpxA	CTC TTA ATG AT	127
lpxC	ATC CAC CAC AG	128
rpoD-E	CTT GTA ACC ACA CCA	129
rpoD-E	TCC ACC AAG TCA CCA	130
pryC	GGT GCA GTC AT	131
pryC	AGA GTT CAA GG	132
pryA	GAC TTA ATC AA	133
lgt	CTA CTG GTC AT	134
folA	CAT TGA GAT TT	135
infB	ACA TCT GTC AT	136
nrdA	TTC TGA TTC AT	137
nrdB	GTA TAT GCC AT	138
zipA	TCC TGC ATC AT	139
cysA	ATA TAC CTC AT	140
gyrA-E	GTT ACC CTG ACC GAC CA	141
gyrA-E	GTT ACC CTG ACC ACC A	142
mrdA	TGT TTC ATA CG	143
LpxB	GGT TTG CCA AG	144
LpxB	TAA TCC GTC AG	145
carA	GGT GCT CAA AC	146
adeA	ATA CTG TAA AA	147
blaT	CTC TTC CTT TT	148
crmI	TCC TTC TGA TT	149
folP	ATG TTA TCC C	150
fmhB	CCA TGA TTT A	151
hmrB	TCC ACG TCG A	152
rpmB	GTC TAT TCT CC	153
rpmB	GAC ATG TCT AT	154
hmrB	TCC ACG TCG A	155
FabG	TTC TCT CCT TT	156
RpmB	CTC TAG ACA TG	157
WaaC	AGC ACC CTC AT	158
MrpA	TGA CTC TCC TC	159
MurC	CCA CCT CCA GG	160
LpxA	ATC AAA CTC AT	161
WaaG	GCC AGG GTC AT	162
WaaA	GTACGG TTC AT	163
murB	CAG TCG CCC CT	164
murE	AGG CTC ATA GG	165
AccB	CTA GCA CTC CC	166
FabZ	ATG TCC ATC AT	167
MurG	GCA AAG TCC TC	168
AmpR	GTC GAA CCA AT	169

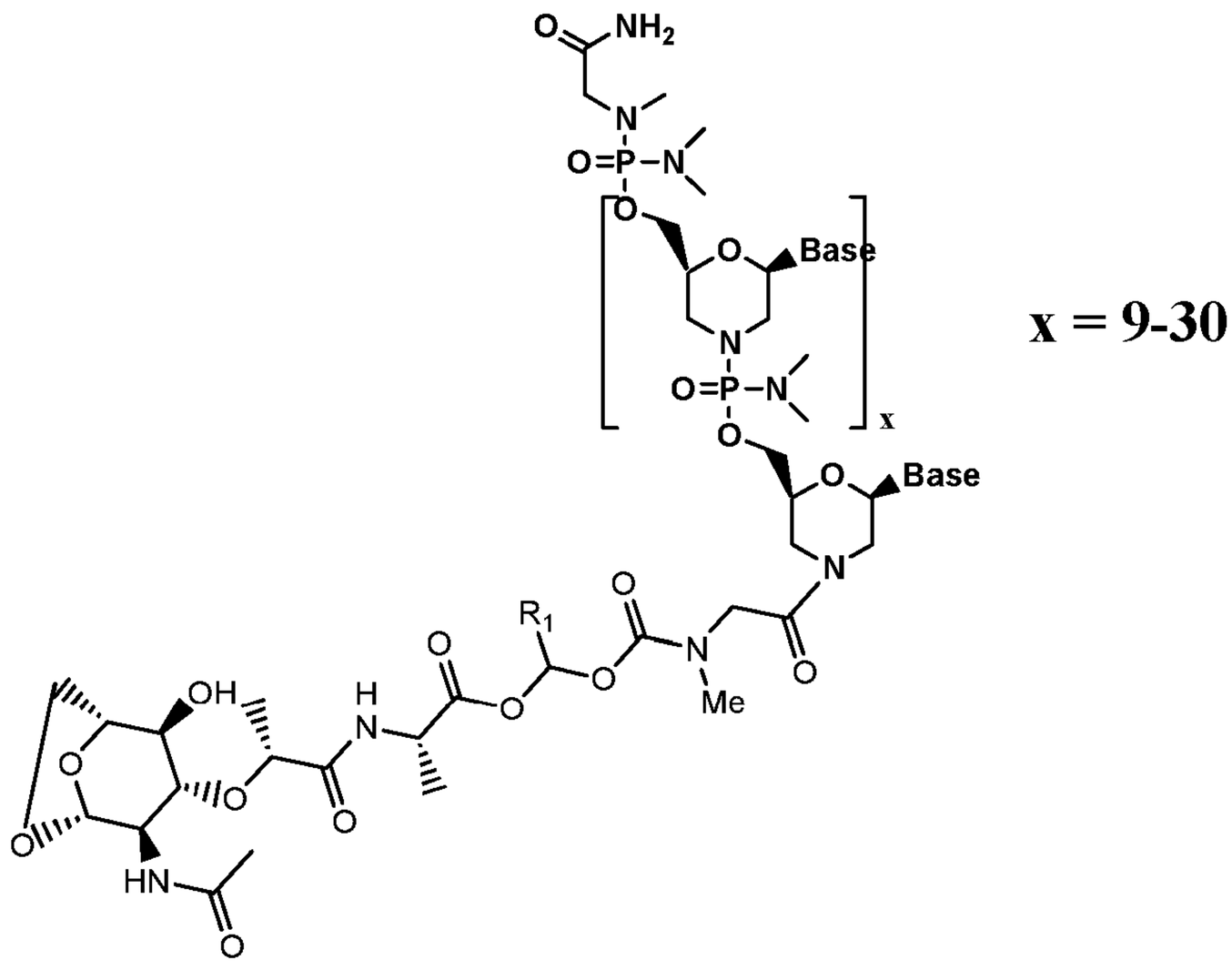
9. A compound according to claim 8 wherein the ANTISENSE sequence is selected from the group consisting of antibiotic resistance targeting sequences as listed in Table 1A.

10. A compound according to claim 8 wherein the ANTISENSE sequence is selected from the group consisting of biofilm formation targeting sequences as listed in Table 1B.

11. A compound according to claim 8 wherein the ANTISENSE sequence is selected from the group consisting of fatty acid biosynthesis-associated targeting sequences as listed in Table 1C.

12. A compound according to claim 8 wherein the ANTISENSE sequence is selected from the group consisting of targeting sequence associated with other pathways or cellular processes as listed in Table 1D.

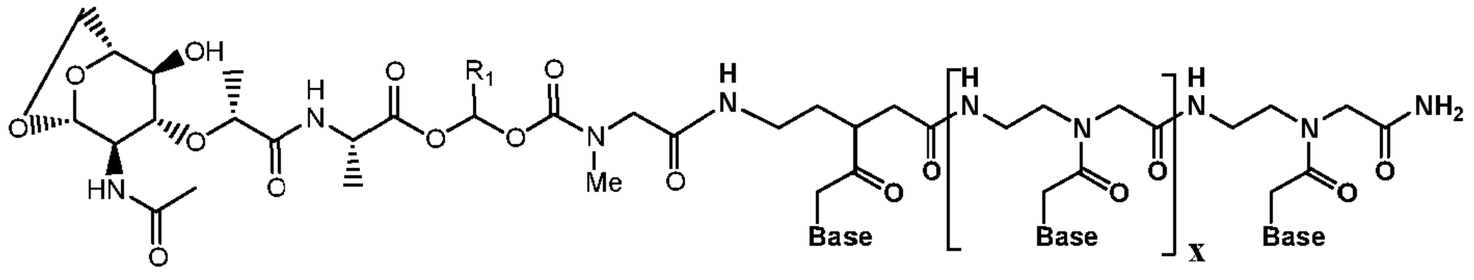
13. A compound according to claim 1 having the structure:



x = 9-30

wherein R_1 is as defined in claim 1 and wherein each "base" is an independently selected natural, artificial and/or modified nucleobase.

14. A compound according to claim 1 having the structure:



x = 9-30

wherein R_1 is as defined in claim 1 and wherein each "base" is an independently selected natural, artificial and/or modified nucleobase.

15. A compound according to any of the preceding claims for use as a medicament.

16. A pharmaceutical or veterinary composition comprising a compound according to any of claims 1 to 14 and a pharmaceutically acceptable or veterinarily acceptable diluent, excipient and/or carrier.

17. A compound according to any of claims 1 to 14 for use in the treatment of pathogenic infections.

18. A compound according to any of claims 1 to 14 for use in the treatment of pathogenic multi-drug resistant (MDR) gram-negative bacterial infections.

19. A compound according to any of claims 1 to 14 for use as a therapeutic in combination with an effective amount any other antibiotic.

Intellectual Property Office

Application No:	GB1819399.5	**Examiner:**	Dr Bill Thomson
Claims searched:	1-19	**Date of search:**	9 May 2019

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	-	GB 2020665 A (YAMAMURA ET AL) - See whole document, in particular page 2, line 33 - page 3, line 15
A	-	WO 2014/205451 A1 (ISIS PHARMACEUTICALS, INC.) - See whole document, in particular pages 77-104
A	-	WO 2015/006740 A2 (ALNYLAM PHARMACEUTICALS, INC.) - See whole document, in particular page 2, line 6 - page 33, line 16 and the Examples
A	-	WO 2018/165564 A1 (IONIS PHARMACEUTICALS, INC.) - See whole document, in particular page 38, line 16 - page 54, line 10 and the Examples

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
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Worldwide search of patent documents classified in the following areas of the IPC

A61K; C12N

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI, Patent Fulltext, CAS Online, Research Disclosure & IP.COM

International Classification:

Subclass	Subgroup	Valid From
A61K	0047/54	01/01/2017
A61P	0031/04	01/01/2006
C12N	0015/113	01/01/2010