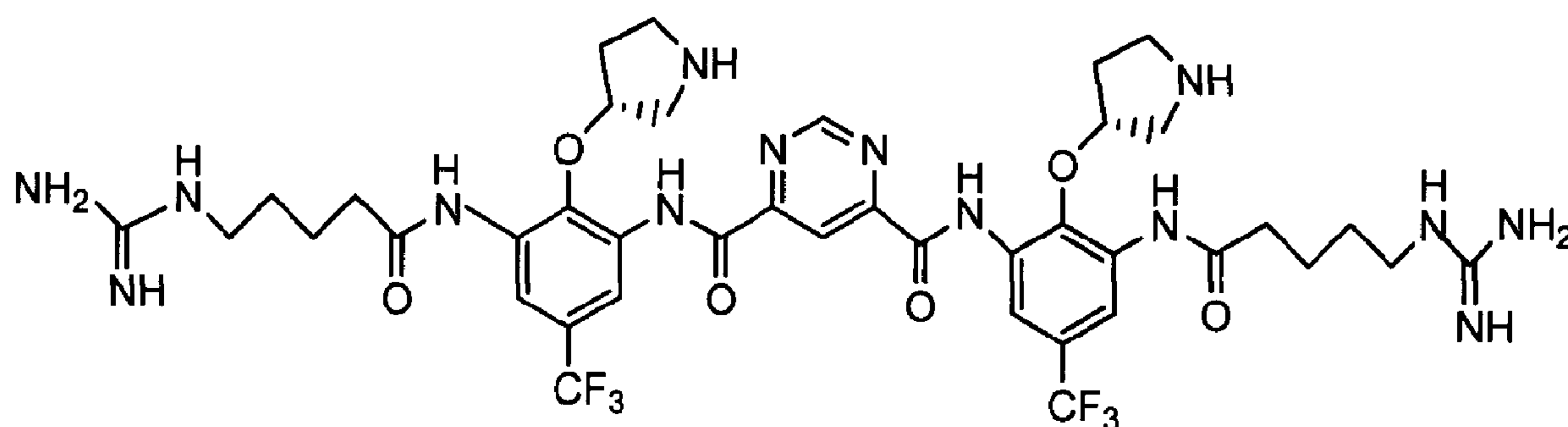




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AND USES THEREOF



(57) Abrégé/Abstract:

The present invention discloses ophthalmic and otic compositions of facially amphiphilic antimicrobial polymers and oligomers and their uses, including their use in methods for treating and preventing ophthalmic infections and otic infections in humans and animals.

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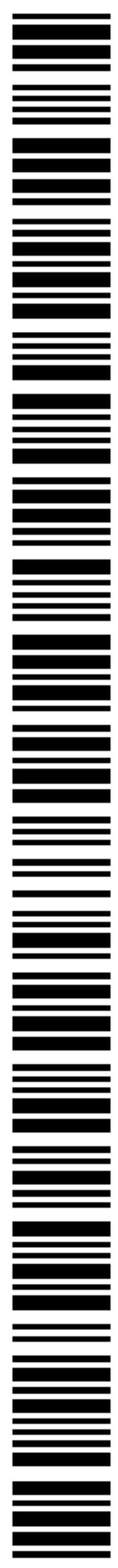
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(57) Abstract: The present invention discloses ophthalmic and otic compositions of facially amphiphilic antimicrobial polymers and oligomers and their uses, including their use in methods for treating and preventing ophthalmic infections and otic infections in humans and animals.



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OPHTHALMIC AND OTIC COMPOSITIONS OF FACIALLY AMPHIPHILIC POLYMERS AND OLIGOMERS AND USES THEREOF

Field of the Invention

5 The present invention relates to antimicrobial compositions of facially amphiphilic antimicrobial polymers and oligomers useful for the treatment or prevention of ophthalmic and otic infections. The present invention also relates to methods of using the compositions for treating and/or preventing ophthalmic and otic infections.

10 Background of the Invention

 Bacterial drug resistance is a significant current health problem throughout the world. Multiple drug resistance is being commonly seen in a number of human pathogens (see, e.g., Hiramatsu et al., J. Antimicrob. Chemother., 1998, 40, 311-313 and Montecalvo et al., Antimicro. Agents Chemother., 1994, 38, 1363-1367, and the incidence of drug-resistant hospital
15 infections is growing at a rapid rate. For example, in some U.S. hospitals, nosocomial pathogens, such as *E. faecium* and *Acinetobacter* species, have acquired multiple resistance determinants and are virtually untreatable with current antimicrobial agents. Bacterial resistance has now reached epidemic proportions and has been attributed to a variety of abuses of antibiotic treatments, including overuse (Monroe et al., Curr. Opin. Microbiol., 2000, 3, 496-501),
20 inappropriate dosing at sub-therapeutic levels (Guillemot et al., JAMA, 1998, 279, 365-370), and misuse as antimicrobial growth promoters in animal food (Lathers, J. Clin. Pharmacol., 2002, 42, 587-600). Moreover, the threat of bio-terrorism has provided a further impetus to develop novel classes of antibiotics, particularly ones against which it will be difficult to develop resistant bacterial strains.

25 The pharmaceutical scientific community is responding to this challenge by focusing on the development of new antibiotic drugs. Much of this work, however, is directed to synthesizing analogs of known drugs, such as cephalosporins and quinolones, that, while potentially useful for a short time, will inevitably also encounter bacterial drug resistance and become ineffective. Thus, therapeutically effective antimicrobial drugs that act by novel mechanisms would provide
30 an economic as well as a human health benefit.

 A series of nonpeptidic mimics of the natural antimicrobial peptides have been developed that are polymers, oligomers and small molecules comprised of non-natural building blocks. See, Tew et al., Proc. Natl. Acad. Sci. U.S.A., 2002, 99, 5110-5116; Arnt et al., J. Polym. Sci., Part A, 2004, 42, 3860-3864; and Liu et al., Angew Chem. Int. Ed. Engl., 2004, 43, 1158-

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1162. Many of these compounds are significantly smaller and easier to prepare than the natural antimicrobial peptides and peptidic mimetics, with the shortest of these oligomers having molecular weights typical of small molecule drugs. They have the same mechanism of action as magainin, are highly potent and have a broad spectrum of activity, killing gram-positive, gram-negative and antibiotic-resistant pathogens. Relative to the antimicrobial peptides, the non-peptidic mimetics are significantly less toxic towards human erythrocytes, much less expensive to prepare, and more stable.

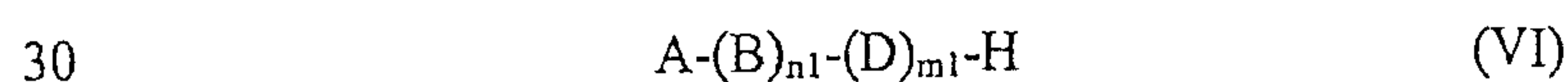
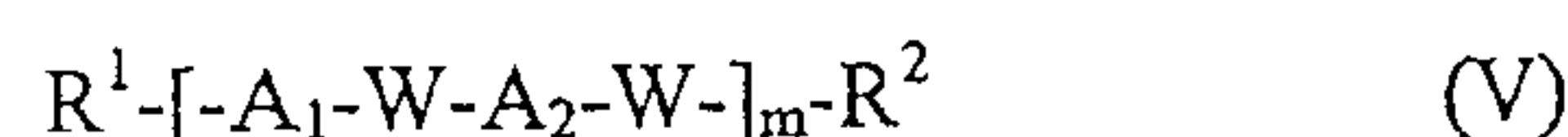
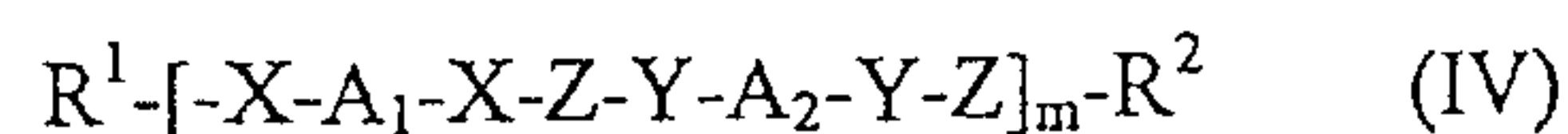
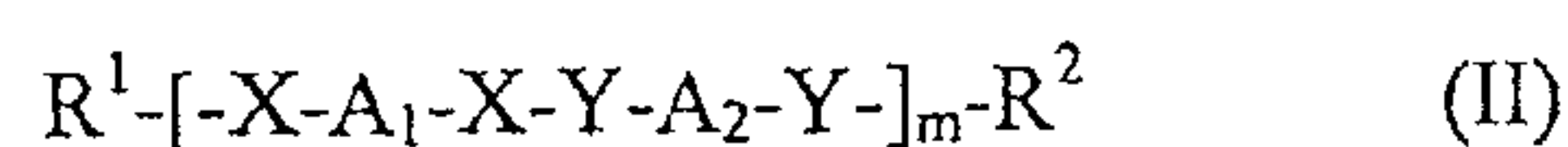
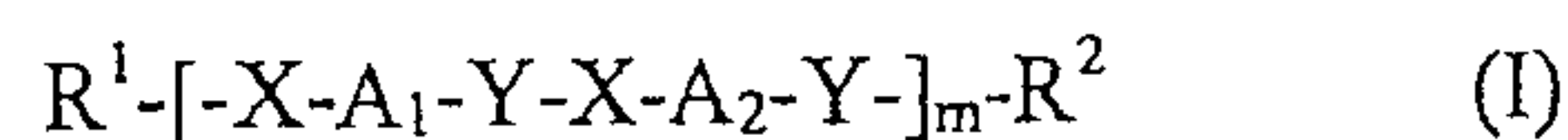
See, for example, U.S. Published Patent Appl. Nos. US 2006-0041023 A1, US 2004-0202639 A1, US 2005-0287108 A1, and US 2006-0024264 A1, and US Patent No. 7,173,102.

There is a great need for improved compositions and methods of treatment based on the use of antimicrobials that are more effective than existing agents against key ophthalmic and otic pathogens, and less prone to the development of resistance by those pathogens. In particular, there is a great need for effective compositions and methods for the treatment of otic infections, especially bacterial infections. The use of oral antibacterials to treat otic infections in children has limited efficacy and creates a serious risk of pathogen resistance to the orally administered antibacterial agent.

Thus, a need remains for improved ophthalmic and otic antimicrobial compositions, in particular, for broad-spectrum antimicrobial agents useful for the treatment of ophthalmic and otic infections that are not prone to the development of resistance by ophthalmic and/or otic pathogens and that are effective in the treatment of ophthalmic and otic pathogens that have already developed resistance to existing antimicrobial agents.

Summary of the Invention

The present disclosure provides compositions of antimicrobial, amphiphilic polymers and oligomers or Formulae I, II, IV, V, and VI,



or acceptable salts or solvates thereof, wherein R^1 , R^2 , A_1 , A_2 , A , B , D , X , Y , Z , W , m , $m1$, and $n1$ are as defined below, including antimicrobial compositions that can be administered for the treatment or prevention of ophthalmic and otic infections in humans or animals.

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The amphiphilic polymers and oligomers disclosed include, but are not limited to, polyamide and polyester compounds of Formulae I and II wherein X is O, NR³, or S, Y is C=O, C=S, or SO₂, and A₁ and A₂ are aromatic, heteroaromatic, or aliphatic moieties appropriately substituted with one or more polar and/or nonpolar groups; polyurea,
 5 polycarbamate, and polycarbonate compounds of Formula IV wherein X and Y are O, NR³, or S, Z is C=O, C=S, or SO₂, and A₁ and A₂ are aromatic, heteroaromatic, or aliphatic moieties appropriately substituted with one or more polar and/or nonpolar groups. Also useful in the present invention are amphiphilic polyaryl and polyarylalkynyl polymers and oligomers of Formula V wherein W is -CH₂-, -CH₂-CH₂-, -CH=CH-, or -C≡C-, and A₁ and A₂ are aromatic
 10 or heteroaromatic moieties appropriately substituted with one or more polar and/or nonpolar groups; and random methacrylate copolymers of Formula VI wherein R¹ and R² are end groups appropriate for the specific polymer or oligomer and are as defined below.

Thus, the present disclosure is directed to an ophthalmic composition, comprising an effective amount of an antimicrobial polymer or oligomer of Formula I as disclosed herein, or an
 15 acceptable salt or solvate thereof, and an ophthalmically acceptable excipient.

The present disclosure is also directed to an ophthalmic composition, comprising an effective amount of an antimicrobial polymer or oligomer of Formula II as disclosed herein, or an acceptable salt or solvate thereof, and an ophthalmically acceptable excipient. In some embodiments, the antimicrobial oligomer of Formula II has Formula IIa as disclosed herein.

20 The present disclosure is further directed to an ophthalmic composition, comprising an effective amount of an antimicrobial polymer or oligomer of Formula IV as disclosed herein, or an acceptable salt or solvate thereof, and an ophthalmically acceptable excipient. In some embodiments, the antimicrobial oligomer of Formula IV has Formula IVa, Formula IVb, or Formula IVc as disclosed herein.

25 The present disclosure is also directed to an ophthalmic composition, comprising an effective amount of an antimicrobial polymer or oligomer of Formula V as disclosed herein, or an acceptable salt or solvate thereof, and an ophthalmically acceptable excipient. In some embodiments, the antimicrobial oligomer of Formula V has Formula Va as disclosed herein.

The present disclosure is further directed to an ophthalmic composition, comprising an
 30 effective amount of an antimicrobial random polymer or oligomer of Formula VI as disclosed herein, or an acceptable salt or solvate thereof, and an ophthalmically acceptable excipient.

The present disclosure is also directed to an antimicrobial ophthalmic composition, the composition comprising a) an antimicrobial oligomer of Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb, Formula IVc, Formula V, Formula Va, or Formula VI

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as disclosed herein, or a pharmaceutically acceptable salt or solvate thereof, in an amount effective for treatment and/or prophylaxis of a microbial infection of an eye of an animal; and b) an ophthalmically acceptable excipient, wherein the composition is suitable for administration to one or more tissues of the eye.

5 The present disclosure is also directed to an ophthalmic composition for use in treatment or prevention of a microbial infection in an eye of an animal, wherein the improvement comprises employing an antimicrobial oligomer of Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb, Formula IVc, Formula V, Formula Va, or Formula VI as disclosed herein, or an acceptable salt or solvate thereof, in the composition in an amount
10 effective to treat or prevent the infection when the composition is administered to one or more tissues of the eye.

 The present disclosure is also directed to any of the ophthalmic compositions disclosed herein, wherein the composition is suitable for topical administration to one or more tissues of an eye of an animal.

15 The present disclosure is also directed to any of the ophthalmic compositions disclosed herein, wherein the composition is in a form selected from the group consisting of a solution, a suspension, an emulsion, a gel, an ointment, and a solid article suitable for ocular implant.

 The present disclosure is also directed to any of the ophthalmic compositions disclosed herein, wherein the oligomer is present in the composition at a concentration of from about
20 0.01% to about 20% by weight.

 The present disclosure is also directed to any of the ophthalmic compositions disclosed herein, wherein the ophthalmically acceptable excipient is selected from a preservative, a stabilizer, an antioxidant, an anti-inflammatory agent, a viscosity-enhancing agent, and an agent to prolong residence time of the oligomer in ocular tissue, or any combination thereof.

25 The present disclosure is also directed to use of the compounds and compositions of the invention in the preparation of a medicament for treating or preventing ophthalmic and/or otic infections in a human or animal.

 In some embodiments of the ophthalmic compositions of the present invention, the preservative is selected from a phenylmercuric salt, thimerosal, stabilized chlorine dioxide, a
30 quaternary ammonium compound, imidazolidinyl urea, a paraben, phenoxyethanol, chlorophenoxyethanol, phenoxypropanol, chlorobutanol, chlorocresol, phenylethyl alcohol, and sorbic acid and its salts, or any combination thereof.

 In some embodiments, the antioxidant is selected from ascorbic acid, sodium metabisulfite, sodium bisulfite, and acetylcysteine.

In some embodiments, the stabilizer is a chelating agent, such as, for example, disodium EDTA.

In some embodiments, the viscosity-enhancing agent is selected from methylcellulose, hydroxypropylmethyl cellulose, polyvinyl alcohol, and glycerol.

5 In some embodiments, the ophthalmic composition further comprises an additional ophthalmically acceptable excipient. The additional ophthalmically acceptable excipient is selected from a buffering agent, a solubilizing agent, a surfactant, a lubricating agent, and an ophthalmically acceptable salt, or any combination thereof.

10 In some embodiments, the ophthalmic composition further comprises an additional medicament. The additional medicament is selected from an anti-inflammatory agent, an antimicrobial agent, an anesthetic agent, and an anti-allergic agent.

The present disclosure is further directed to a method of treating or preventing a microbial infection in an eye of an animal, comprising administering to an eye of an animal in need of the treating or preventing an effective amount of an ophthalmic composition of the
15 present invention.

The present disclosure is also directed to a method for treating or preventing a microbial infection in an eye of an animal by administering to one or more tissues of the eye an antimicrobial ophthalmic composition, wherein the composition comprises an antimicrobial oligomer of Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb,
20 Formula IVc, Formula V, Formula Va, or Formula VI, as disclosed herein, in an amount effective to treat or prevent the infection.

In some embodiments of the methods, the antimicrobial ophthalmic composition is administered topically to one or more tissues of the eye of the animal. In some embodiments of the methods, the ophthalmic composition is in a form selected from a solution, a suspension, an emulsion, a gel, an ointment, and a solid article suitable for ocular implant. In other embodiments,
25 the ophthalmic composition is administered 2 to 4 times daily. In yet other embodiments, the oligomer in the ophthalmic composition is present in the composition at a concentration of about 0.01% to about 20% by weight.

In some embodiments of the methods, the microbial ophthalmic infection is a bacterial infection. For example, in some embodiments, the bacterial infection is caused by *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Corynebacterium*, *Moraxella*, *Haemophilia*, *Serratia*, *Pseudomonas*, or *Neisseria spp.* In other embodiments, the microbial infection is a fungal infection. For example, in some embodiments, the fungal infection is caused by *Aspergillus* or *Fusarium spp.* In yet other embodiments, the microbial infection is a viral infection. For example, in some

embodiments, the viral infection is caused by a herpes virus. In some embodiments of the methods, the ophthalmic infection is selected from bacterial keratitis, bacterial conjunctivitis, and corneal ulcers.

The present disclosure is also directed to an otic composition, comprising an effective amount of an antimicrobial oligomer or polymer of Formula I, Formula II, Formula IV, Formula V, or Formula VI, or an acceptable salt or solvate thereof, and an otically acceptable excipient.

5 The present disclosure is also directed to an antimicrobial otic composition, the composition comprising a) an antimicrobial oligomer of Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb, Formula IVc, Formula V, Formula Va, or Formula VI, or a pharmaceutically acceptable salt or solvate thereof, in an amount effective for treatment and/or prophylaxis of a microbial infection of an ear of an animal; and b) an otically acceptable excipient, wherein the composition is suitable for administration to one or more tissues of the
10 ear.

The present disclosure is also directed to an otic composition for use in treatment or prevention of a microbial infection in an ear of an animal, wherein the composition comprises an antimicrobial oligomer of Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb, Formula IVc, Formula V, Formula Va, or Formula VI as disclosed herein, or an acceptable
15 salt or solvate thereof, in an amount effective to treat or prevent the infection when the composition is administered to one or more tissues of the ear.

The present disclosure is also directed to any of the otic compositions disclosed herein, wherein the composition is suitable for topical administration to one or more tissues of an ear of an animal.

20 The present disclosure is also directed to any of the otic compositions disclosed herein, wherein the composition is in a form selected from a solution, a suspension, an emulsion, a gel, an ointment, and a solid article suitable for otic implant.

The present disclosure is also directed to any of the otic compositions disclosed herein, wherein the polymer or oligomer is present in the otic composition at a concentration of about
25 0.01% to about 20% by weight.

The present disclosure is also directed to any of the otic compositions disclosed herein, wherein the otically acceptable excipient is selected from a preservative, a stabilizer, an antioxidant, and a viscosity-enhancing agent, or any combination thereof.

In some embodiments of the otic compositions, the preservative is selected from a
30 phenylmercuric salt, thimerosal, stabilized chlorine dioxide, a quaternary ammonium compound, imidazolidinyl urea, paraben, phenoxyethanol, chlorophenoxyethanol, phenoxypropanol,

chlorobutanol, chlorocresol, phenylethyl alcohol, and sorbic acid and its salts, or any combination thereof.

In some embodiments, the antioxidant is selected from ascorbic acid, sodium metabisulfite, sodium bisulfite, and acetylcysteine.

5 In some embodiments, the stabilizer is a chelating agent, such as, for example, disodium EDTA.

In some embodiments, the viscosity-enhancing agent is selected from methylcellulose, hydroxypropylmethyl cellulose, polyvinyl alcohol, and glycerol.

10 In some embodiments, the otic composition further comprises an additional otically acceptable excipient. The additional otically acceptable excipient is selected from a buffering agent, a solubilizing agent, a surfactant, a lubricating agent, and an ophthalmically acceptable salt, or any combination thereof.

15 In some embodiments, the otic composition further comprises an additional medicament. The additional medicament is selected from an anti-inflammatory agent, an antimicrobial agent, an anesthetic agent, and an anti-allergic agent.

The present disclosure is further directed to a method of treating or preventing a microbial infection in an ear of an animal, the method comprising administering to an ear of an animal in need of the treating or preventing an effective amount of an otic composition of the present invention.

20 The present disclosure is also directed to a method for treating or preventing a microbial infection in an ear of an animal by administering to one or more tissues of the ear an antimicrobial otic composition, wherein the composition comprises an antimicrobial oligomer of Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb, Formula IVc, Formula V, Formula Va, or Formula VI, as disclosed herein, in an amount effective to treat or prevent the
25 infection.

In some embodiments of the methods, the antimicrobial otic composition is administered topically to one or more tissues of the ear of the animal.

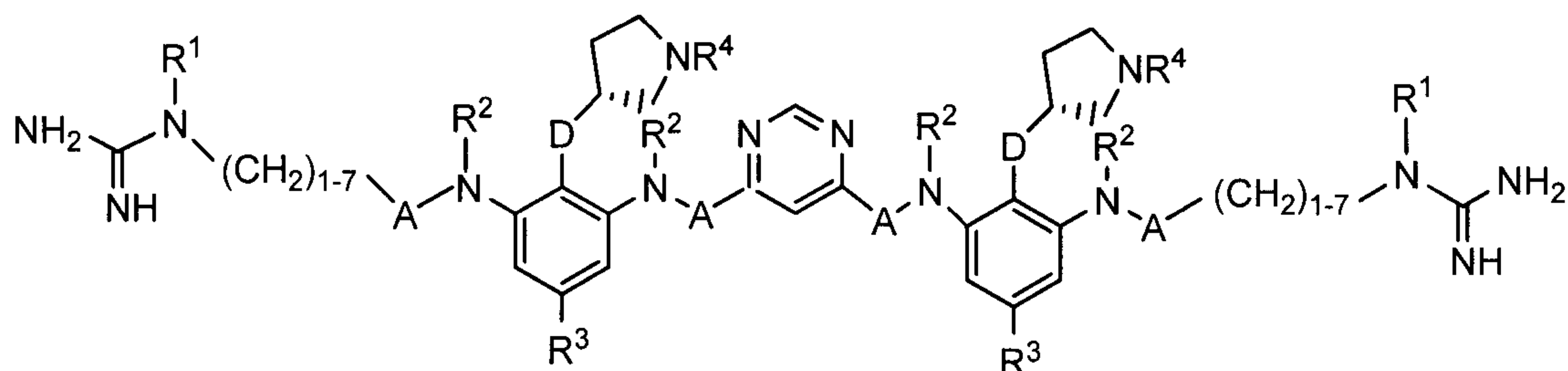
30 In some embodiments of the methods, the otic composition is in a form selected from a solution, a suspension, an emulsion, a gel, an ointment, and a solid article suitable for otic implant. In other embodiments, the otic composition is administered 2 to 4 times daily. In yet other embodiments, the polymer or oligomer is present in the otic composition at a concentration of about 0.01% to about 20% by weight.

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In some embodiments of the methods, the microbial otic infection is a bacterial infection. In other embodiments, the infection is a fungal infection. In yet other embodiments, the infection is a viral infection.

In some embodiments of the methods, the otic infection is selected from otitis externa and otitis media.

In one embodiment, there is provided a compound of Formula I

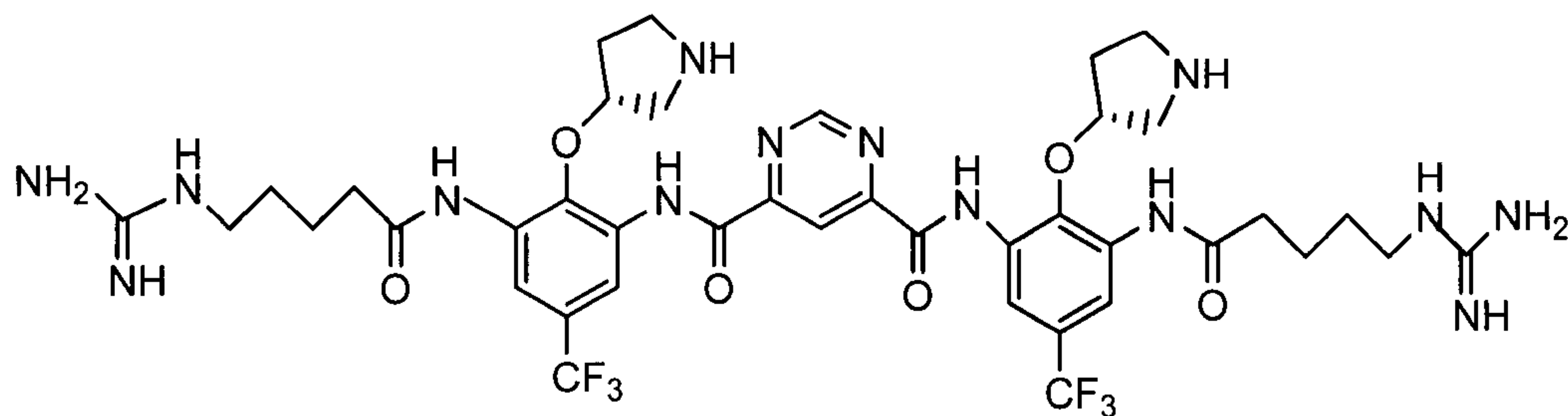


I

wherein:

- 10 each A is, independently, -C=O, -C=S, or -CH₂;
 each D is, independently, O or S;
 each R¹ is, independently, hydrogen, C₁₋₃alkyl, C₁₋₃alkoxy, halo, or haloC₁₋₃alkyl;
 each R² is, independently, hydrogen, C₁₋₃alkyl, C₁₋₃alkoxy, halo, or haloC₁₋₃alkyl;
 each R³ is, independently, hydrogen, C₁₋₄alkyl, C₁₋₄alkoxy, halo, or haloC₁₋₄alkyl; and
 15 each R⁴ is, independently, hydrogen, C₁₋₃alkyl, C₁₋₃alkoxy, halo, or haloC₁₋₃alkyl;
 or a pharmaceutically acceptable salt thereof.

In another embodiment, there is provided the compound



or a pharmaceutically acceptable salt thereof.

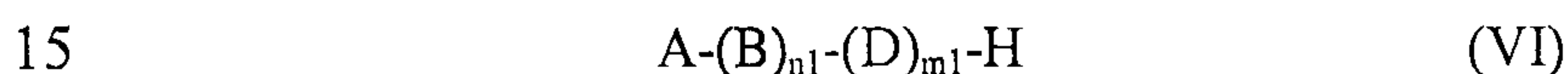
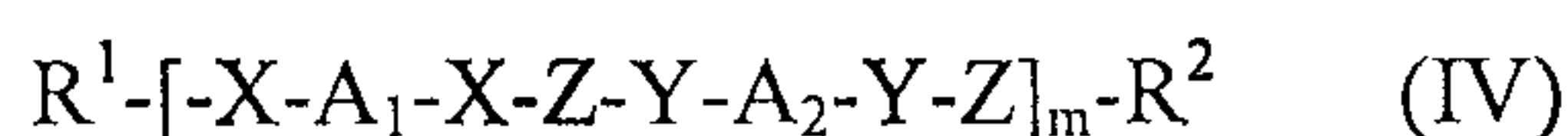
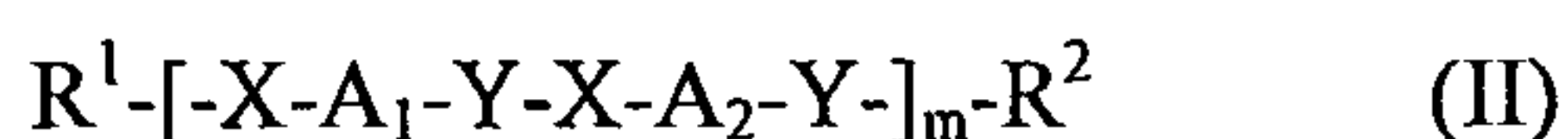
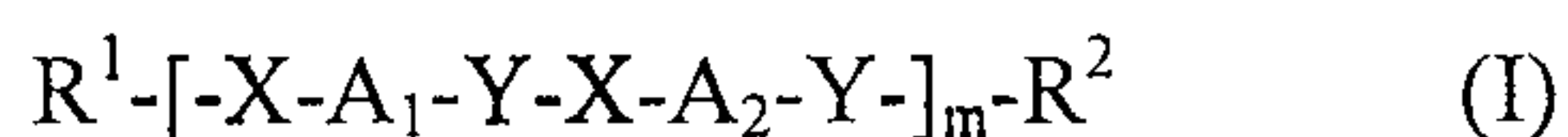
- 20 Also provided is a pharmaceutical composition comprising the above compound or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

Also provided are methods of preparing compounds of the present invention. Also provided is use of compounds of the present invention in the manufacture of a medicament for treating a microbial infection.

Description of Embodiments

5 The present invention provides compositions of amphiphilic, antimicrobial polymers, and/or oligomers that can be used in the treatment or prevention of ophthalmic and otic infections in humans and animals. The present invention also provides methods of using the compositions to treat or prevent ophthalmic and otic infections in humans and animals.

10 The antimicrobial polymers and oligomers useful in the present invention are polymers and oligomers of Formulae I, II, IV, V and VI:



or acceptable salts or solvates thereof, wherein R^1 , R^2 , A_1 , A_2 , A , B , D , X , Y , Z , W , m , $m1$, and $n1$ are as defined below.

20 The polymers and oligomers useful in the present invention are capable of adopting amphiphilic conformations that allow for the segregation of polar and nonpolar regions of the molecule into different spatial regions. This separation of charge, or facial amphiphilicity, forms the basis for the anti-microbial activity observed for these polymers and oligomers, making them useful as anti-microbial agents. Use of the polymers and oligomers of Formulae I, II, and IV generally as anti-microbial agents is described in US Published Patent Appl. No. US 2006-0041023 A1 and US Patent No. 7,173,102. Use of the polymers and oligomers of Formula 25 V generally as anti-microbial agents is described in US Published Patent Appl. Nos. US 2004-0202639 A1 and US 2005-0287108 A1. Use of the random copolymers of Formula VI generally as anti-microbial agents is described in US Published Patent Appl. No. US 2006-0024264 A1.

30 The polymers and oligomers employed in the present invention were originally designed to mimic the antimicrobial activities of host defense peptides, which were potentially exciting therapeutic agents because of their broad spectrum of activity, rapid bacteriocidal activity, and very low incidence of development of bacterial resistance, but which presented a

number of significant pharmaceutical issues, including systemic toxicity and difficulty and expense of manufacturing, that severely hampered clinical progress in their use as therapeutics.

Many of the oligomers of Formulae I, II, and IV are significantly smaller and easier to prepare than their naturally occurring counterparts. They have the same mechanism of action as
5 magainin (a naturally occurring host defense peptide) and are approximately equipotent and as broad in their spectrum of action as magainin. However, the non-peptidic polymers and oligomers of the present invention are significantly less toxic towards human erythrocytes, much less expensive to prepare, and are expected to be much more stable *in vivo*.

The present invention discloses ophthalmic and otic compositions comprising anti-
10 microbial, facially amphiphilic polymers and oligomers. Polymers are generally defined as synthetic compounds assembled from monomer subunits that are polydisperse in molecular weight, and are most commonly prepared by one-pot synthetic procedures. The term “polymer” as used herein refers to a macromolecule comprising a plurality of repeating units or monomers. The term includes homopolymers, which are formed from a single type of monomer, and
15 copolymers, which are formed from two or more different monomers. In copolymers, the monomers may be distributed randomly (random copolymer), in alternating fashion (alternating copolymers), or in blocks (block copolymer). The polymers of the present invention are either homopolymers or alternating copolymers having about 2 monomer units to about 500 monomer units, with average molecular weights that range from about 300 Daltons to about 1,000,000
20 Daltons, or from about 400 Daltons to about 120,000 Daltons. Preferred polymers are those having about 5 to about 100 monomer units, with average molecular weights that range from about 1,000 Daltons to about 25,000 Daltons.

The term “oligomer” as used herein refers to a homogenous polymer with a defined sequence and molecular weight. Modern methods of solid phase organic chemistry have allowed
25 the synthesis of homodisperse, sequence-specific oligomers with molecular weights approaching 5,000 Daltons. An oligomer, in contrast to a polymer, has a defined sequence and molecular weight and is usually synthesized either by solid phase techniques or by step-wise solution chemistry and purified to homogeneity. Oligomers of the present invention are those having about 2 monomer units to about 25 monomer units, with molecular weights that range from
30 about 300 Daltons to about 6,000 Daltons. Preferred oligomers are those having about 2 monomer units to about 10 monomer units, with molecular weights that range from about 300 Daltons to about 2,500 Daltons.

For the ophthalmic and otic compositions described herein, oligomers are the preferred species because of their defined size and structure.

The term “polymer backbone,” “oligomer backbone,” or “backbone” as used herein refers to that portion of the polymer or oligomer which is a continuous chain comprising the bonds formed between monomers upon polymerization. The composition of the polymer or oligomer backbone can be described in terms of the identity of the monomers from which it is formed without regard to the composition of branches, or side chains, of the polymer or oligomer backbone.

The term “polymer side chain,” “oligomer side chain,” or “side chain” refers to portions of the monomer which, following polymerization, forms an extension of the polymer or oligomer backbone. In homopolymers and homooligomers, all the side chains are derived from the same monomer.

The term “amphiphilic” as used herein describes a three-dimensional structure having discrete hydrophobic and hydrophilic regions. An amphiphilic polymer requires the presence of both hydrophobic and hydrophilic elements along the polymer backbone. The presence of hydrophobic and hydrophilic groups is a necessary, but not sufficient, condition to produce an amphiphilic molecule, polymer, or oligomer.

The term “facially amphiphilic” or “facial amphiphilicity” as used herein describes polymers or oligomers with polar (hydrophilic) and nonpolar (hydrophobic) side chains that adopt conformation(s) leading to segregation of polar and nonpolar side chains to opposite faces or separate regions of the structure or molecule.

The phrase “groups with chemically nonequivalent termini” refers to functional groups such as esters amides, sulfonamides, and N-hydroxyoximes where reversing the orientation of the substituents, for example, $R^1C(=O)OR^2$ versus $R^1O(O=)CR^2$, produces unique chemical entities.

The present invention is directed to antimicrobial ophthalmic and otic compositions comprising one or more of the polymers or oligomers disclosed herein, as defined below, and an ophthalmically acceptable excipient.

Thus, in some aspects of the present invention, the ophthalmic or otic composition comprises a polymer or oligomer of Formula I:



or an acceptable salt or solvate thereof,

wherein:

X is NR^8 , $-N(R^8)N(R^8)-$, O, or S;

Y is C=O, C=S, O=S=O, or $-C(=O)C(=O)-$;

R^8 is hydrogen or alkyl;

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A_1 and A_2 are, independently, optionally substituted arylene or optionally substituted heteroarylene, wherein A_1 and A_2 are, independently, optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

5 A_1 is optionally substituted arylene or optionally substituted heteroarylene and A_2 is a C_3 to C_8 cycloalkyl or $-(CH_2)_q-$, wherein q is 1 to 7, wherein A_1 and A_2 are, independently, optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

10 A_2 is optionally substituted arylene or optionally substituted heteroarylene, and A_1 is a C_3 to C_8 cycloalkyl or $-(CH_2)_q-$, wherein q is 1 to 7, wherein A_1 and A_2 are, independently, optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s);

15 R^1 is

(i) hydrogen, a polar (PL) group, or a non-polar (NPL) group, and R^2 is $-X-A_1-Y-R^{11}$, wherein R^{11} is hydrogen, a polar (PL) group, or a non-polar (NPL) group; or

(ii) R^1 and R^2 are, independently, hydrogen, a polar (PL) group, or a non-polar (NPL) group; or

20 (iii) R^1 and R^2 together are a single bond;

NPL is a nonpolar group independently selected from $-B(OR^4)_2$ and $-(NR^{3'})_{q1NPL}-U^{NPL}-(CH_2)_{pNPL}-(NR^{3''})_{q2NPL}-R^4$, wherein:

R^3 , $R^{3'}$, and $R^{3''}$ are, independently, selected from the group consisting of hydrogen, alkyl, and alkoxy;

25 R^4 and $R^{4'}$ are, independently, selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, any of which is optionally substituted with one or more alkyl or halo groups;

30 U^{NPL} is absent or selected from O, S, $S(=O)$, $S(=O)_2$, NR^3 , $-C(=O)-$, $-C(=O)-N=N-NR^3-$, $-C(=O)-NR^3-N=N-$, $-N=N-NR^3-$, $-C(=N-N(R^3)_2)-$, $-C(=NR^3)-$, $-C(=O)O-$, $-C(=O)S-$, $-C(=S)-$, $-O-P(=O)_2O-$, $-R^3O-$, $-R^3S-$, $-S-C=N-$, and $-C(=O)-NR^3-O-$, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

the $-(CH_2)_{pNPL}-$ alkylene chain is optionally substituted with one or more alkyl, amino or hydroxy groups, or is unsaturated;

$pNPL$ is 0 to 8;

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q1NPL and q2NPL are, independently, 0, 1, or 2;

PL is a polar group selected from halo, hydroxyethoxymethyl, methoxyethoxymethyl, polyoxyethylene, and $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, wherein:

R^5 , $R^{5'}$, and $R^{5''}$ are, independently, selected from hydrogen, alkyl, and alkoxy;

5 U^{PL} is absent or selected from O, S, S(=O), S(=O)₂, NR^5 , -C(=O)-, -C(=O)-N=N- NR^5 -, -C(=O)- NR^5 -N=N-, -N=N- NR^5 -, -C(=N-N(R^5)₂)-, -C(=NR⁵)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, - R^5 O-, - R^5 S-, -S-C=N-, and -C(=O)- NR^5 -O-, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

V is selected from nitro, cyano, amino, hydroxy, alkoxy, alkylthio, alkylamino, 10 dialkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, guanidino, guanyl, semicarbazone, aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, -NH(CH₂)_pNH₂ wherein p is 1 to 4, N(CH₂CH₂NH₂)₂, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, lower acylamino, or benzyloxycarbonyl, wherein p is 1 to 4;

15 the $-(CH_2)_{pPL}$ - alkylene chain is optionally substituted with one or more amino or hydroxy groups, or is unsaturated;

pPL is 0 to 8;

q1PL and q2PL are independently 0, 1 or 2; and

m is 1 to about 500;

20 and an ophthalmically or otically acceptable excipient.

US Patent Application Publ. No. US 2006-0041023 A1 discloses antimicrobial polymers and oligomers of Formula I that can be used in the compositions of the present invention.

For example, oligomers of Formula I preferred for use in the ophthalmic and otic 25 compositions of the present invention are those wherein m is 1 to about 25, 1 to about 20, 1 to 10, 2 to 8, 2 to 6, 2 to 5, or 4 or 5.

Preferred oligomers of Formula I are also those wherein X is NR^8 , O, or -N(R^8)N(R^8)-, and R^8 is hydrogen or C₁-C₆ alkyl. Especially preferred are those polymers and oligomers wherein X is NR^8 and Y is C=O. For example, oligomers of Formula I wherein X is NH and Y 30 is C=O are especially preferred.

Also preferred are those oligomers of Formula I wherein A₁ or A₂ are, independently, optionally substituted *o*-, *m*-, or *p*-phenylene. Those oligomers wherein A₁ or A₂ are optionally substituted *m*-phenylene are especially preferred.

Preferred oligomers of Formula I are also those wherein one of A_1 and A_2 is substituted arylene and the other of A_1 and A_2 is $-(CH_2)_q-$, wherein q is 1 or 2, wherein one of A_1 and A_2 is substituted with one or two polar (PL) group(s), and the other of A_1 and A_2 is substituted with one or two non-polar (NPL) group(s).

5 Preferred are oligomers of Formula I wherein (i) R^1 is hydrogen, a polar (PL) group, or a non-polar (NPL) group, and R^2 is $-X-A_1-Y-R^{11}$, wherein R^{11} is hydrogen, a polar (PL) group, or a non-polar (NPL) group. Especially preferred are oligomers of Formula I wherein R^1 is hydrogen, R^2 is $-X-A_1-Y-R^{11}$, and R^{11} is a polar (PL) group, for example, amino.

10 In some embodiments, preferred oligomers of Formula I are those wherein R^1 and R^2 are, independently, hydrogen, a polar (PL) group, or a non-polar (NPL) group. Especially preferred are oligomers of Formula I wherein R^1 is hydrogen, and R^2 is a polar group, for example, amino.

In other aspects of the invention, preferred oligomers of Formula I are those wherein NPL is $-(NR^{3'})_{q1NPL}-U^{NPL}-(CH_2)_{pNPL}-(NR^{3''})_{q2NPL}-R^{4'}$, and R^3 , $R^{3'}$, $R^{3''}$, $R^{4'}$, U^{NPL} , $pNPL$, $q1NPL$,
15 and $q2NPL$ are as defined above. Especially preferred are those oligomers of Formula I wherein $q1NPL$ and $q2NPL$ are 0, so that NPL is $-U^{NPL}-(CH_2)_{pNPL}-R^{4'}$.

Preferred values for each of R^3 , $R^{3'}$, and $R^{3''}$ are hydrogen, C_1 - C_6 alkyl, and C_1 - C_6 alkoxy. Hydrogen is an especially preferred value for R^3 , $R^{3'}$, and $R^{3''}$.

Preferred values of $R^{4'}$ are hydrogen, C_1 - C_{10} alkyl, C_3 - C_{18} branched alkyl, C_2 - C_{10}
20 alkenyl, C_2 - C_{10} alkynyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, especially phenyl, and heteroaryl, any of which is optionally substituted with one or more C_1 - C_6 alkyl or halo groups. Especially preferred values of $R^{4'}$ are C_1 - C_{10} alkyl and C_3 - C_{18} branched alkyl. Suitable C_1 - C_{10} alkyl and C_3 - C_{18} branched alkyl groups are methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, *n*-pentyl, and isopentyl.

25 Preferred values of U^{NPL} are NH, $-C(=O)-$, $-C(=O)O-$, O, and S. Especially preferred values are NH, $-C(=O)-$, O, and S, or NH, O, and S. Especially preferred oligomers of Formula I also are those wherein U^{NPL} is absent.

Preferred values of $pNPL$ are 0 to 6; values of $pNPL$ of 0 to 4 are especially preferred, with values of $pNPL$ of 0 to 2 most preferred.

30 Preferred values of $q1NPL$ and $q2NPL$ are 0 or 1. Values of $q1NPL$ and $q2NPL$ of 0 or 1 are especially preferred, with a value of 0 being the most preferred for each of $q1NPL$ and $q2NPL$.

In preferred ophthalmic and otic compositions, oligomers of Formula I wherein the

-(CH₂)_{pNPL}- alkylene chain in NPL is unsubstituted or substituted with one or more alkyl groups are preferred. More preferred are those oligomers of Formula I wherein the -(CH₂)_{pNPL}- alkylene chain in NPL is unsubstituted.

An especially preferred value of NPL for the polymers and oligomers of Formula I is C₁-C₆ alkyl or aryl C₁-C₆ alkyl. Examples of preferred values for NPL are *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, and benzyl.

In some embodiments of the invention, preferred oligomers of Formula I are those wherein PL is -(NR^{5'})_{q1PL}-U^{PL}-(CH₂)_{pPL}-(NR^{5''})_{q2PL}-V, and R⁵, R^{5'}, R^{5''}, V, U^{PL}, pPL, q1PL, and q2PL are as defined above. Especially preferred are those oligomers of Formula I wherein q1PL and q2PL are 0, so that PL is -U^{PL}-(CH₂)_{pPL}-V.

Preferred values for R⁵, R^{5'}, and R^{5''} are hydrogen, C₁-C₆ alkyl, and C₁-C₆ alkoxy. Hydrogen is an especially preferred value for each of R⁵, R^{5'}, and R^{5''}.

Preferred values of U^{PL} are O, S, NH, -C(=O)O-, and -C(=O). Especially preferred values are NH, -C(=O)-, O, and S, or NH, O, and S. Preferred oligomers of Formula I are also those wherein U^{PL} is absent.

Preferred values of V are amino, C₁-C₆ alkylamino, C₁-C₆ dialkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, guanidino, guanyl, and semicarbazone, preferably any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, -NH(CH₂)_pNH₂, -N(CH₂CH₂NH₂)₂, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, lower acylamino, or benzyloxycarbonyl.

Especially preferred values of V are amino, C₁-C₆ alkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, and guanidino. Values of V that are most preferred are amino and guanidino.

Preferred values of pPL are 0 to 6, with values of pPL of 2 to 5 especially preferred.

Preferred values of q1PL and q2PL are 0 or 1. Values of q1PL and q2PL of 0 or 1 are especially preferred, with a value of 0 being especially preferred for each of q1PL and q2PL.

In preferred ophthalmic compositions, oligomers of Formula I wherein the -(CH₂)_{pPL}- alkylene chain in PL is optionally substituted with one or more amino groups are preferred.

Thus, preferred ophthalmic or otic compositions comprise an oligomer of Formula I, or an acceptable salt or solvate thereof, wherein:

X is NR⁸, Y is C=O, and R⁸ is hydrogen;

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A_1 is optionally substituted *o*-, *m*-, or *p*-phenylene and A_2 is $-(CH_2)_q-$, wherein q is 1, and wherein one of A_1 and A_2 is substituted with one or two polar (PL) group(s), and the other of A_1 and A_2 is substituted with one or two non-polar (NPL) group(s); or

A_2 is optionally substituted *o*-, *m*-, or *p*-phenylene and A_1 is $-(CH_2)_q-$, wherein q is 1, and wherein one of A_1 and A_2 is substituted with one or two polar (PL) group(s), and the other of A_1 and A_2 is substituted with one or two non-polar (NPL) group(s);

R^1 and R^2 are, independently, hydrogen, a polar (PL) group, or a non-polar (NPL) group;

NPL is $-(NR^{3'})_{q1NPL}-U^{NPL}-(CH_2)_{pNPL}-(NR^{3''})_{q2NPL}-R^{4'}$, wherein:

$R^{4'}$ is selected from C_1 - C_{10} alkyl, C_3 - C_{18} branched alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, and C_6 - C_{10} aryl, any of which is optionally substituted with one or more alkyl or halo groups;

U^{NPL} is absent or selected from NH, $-C(=O)-$, O, and S;

the $-(CH_2)_{pNPL}-$ alkylene chain is optionally substituted with one or more amino groups;

$pNPL$ is 0 to 8;

$q1NPL$ and $q2NPL$ are 0;

PL is $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, wherein:

U^{PL} is absent or selected from O, S, NH, and $-C(=O)-$;

V is selected from amino, C_1 - C_6 alkylamino, $-NH(CH_2)_pNH_2$ wherein p is 1 to 4, $-N(CH_2CH_2NH_2)_2$, diazamino, amidino, and guanidino;

the $-(CH_2)_{pPL}-$ alkylene chain is optionally substituted with one or more amino groups;

pPL is 0 to 8;

$q1PL$ and $q2PL$ are 0; and

m is 4 or 5;

and an ophthalmically or otically acceptable excipient.

Preferred ophthalmic or otic compositions also comprise an oligomer of Formula I, or an acceptable salt or solvate thereof, wherein:

X is NR^8 , Y is $C=O$, and R^8 is hydrogen;

A_1 is optionally substituted *o*-, *m*-, or *p*-phenylene and A_2 is $-(CH_2)_q-$, wherein q is 1 or 2, and wherein one of A_1 and A_2 is substituted with one polar (PL) group, and the other of A_1 and A_2 is substituted with one non-polar (NPL) group; or

A_2 is optionally substituted *o*-, *m*-, or *p*-phenylene and A_1 is $-(CH_2)_q-$, wherein q is 1 or 2, and wherein one of A_1 and A_2 is substituted with one polar (PL) group, and the other of A_1 and A_2 is substituted with one non-polar (NPL) group;

R^1 and R^2 are, independently, hydrogen or amino;

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NPL is $-U^{NPL}-(CH_2)_{pNPL}-R^{4'}$, wherein:

$R^{4'}$ is selected from C_1 - C_{10} alkyl and C_3 - C_{18} branched alkyl, any of which is optionally substituted with one or more alkyl or halo groups;

U^{NPL} is absent or selected from NH, $-C(=O)-$, O, and S;

5 the $-(CH_2)_{pNPL}-$ alkylene chain is unsubstituted;

$pNPL$ is 0 to 8;

$q1NPL$ and $q2NPL$ are 0;

PL is $-U^{PL}-(CH_2)_{pPL}-V$, wherein:

U^{PL} is absent or selected from O, S, NH, and $-C(=O)-$;

10 V is selected from amino, C_1 - C_6 alkylamino, $-NH(CH_2)_pNH_2$ wherein p is 1 to 4,

$-N(CH_2CH_2NH_2)_2$, diazamino, amidino, and guanidino;

the $-(CH_2)_{pPL}-$ alkylene chain is optionally substituted with one or more amino groups;

pPL is 0 to 8;

$q1PL$ and $q2PL$ are 0; and

15 m is 4 or 5;

and an ophthalmically or otically acceptable excipient.

In other aspects of the present invention, the ophthalmic or otic composition comprises a polymer or oligomer of Formula II:



20 or an acceptable salt or solvate thereof,

wherein:

X is NR^8 , O, S, $-N(R^8)N(R^8)-$, $-N(R^8)-(N=N)-$, $-(N=N)-N(R^8)-$, $-C(R^7R^{7'})NR^8-$, $-C(R^7R^{7'})O-$, or $-C(R^7R^{7'})S-$; and

Y is $C=O$, $C=S$, $O=S=O$, $-C(=O)C(=O)-$, $C(R^6R^{6'})C=O$ or $C(R^6R^{6'})C=S$; or

25 X and Y are taken together to be pyromellitic diimide;

wherein

R^8 is hydrogen or alkyl;

R^7 and $R^{7'}$ are, independently, hydrogen or alkyl, or

R^7 and $R^{7'}$ together are $-(CH_2)_p-$, wherein p is 4 to 8; and

30 R^6 and $R^{6'}$ are, independently, hydrogen or alkyl, or

R^6 and $R^{6'}$ together are $(CH_2)_2NR^{12}(CH_2)_2$, wherein R^{12} is hydrogen, $-C(=N)CH_3$ or $C(=NH)-NH_2$;

A_1 and A_2 are, independently, optionally substituted arylene or optionally substituted heteroarylene, wherein A_1 and A_2 are, independently, optionally substituted with one or more

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polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s);

R^1 is

(i) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is

5 -X-A₁-X- R^1 , wherein A₁ is as defined above and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(ii) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is

10 -X-A'-X- R^1 , wherein A' is aryl or heteroaryl and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s);

(iii) -Y-A₂-Y- R^2 , and R^2 is hydrogen, a polar group (PL), or a non-polar group (NPL); or

(iv) -Y-A' and R^2 is -X-A', wherein A' is aryl or heteroaryl and is optionally

15 substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(v) R^1 and R^2 are, independently, a polar group (PL) or a non-polar group (NPL);

or

20 (vi) R^1 and R^2 together form a single bond;

NPL is a nonpolar group independently selected from -B(OR⁴)₂ and

-(NR^{3'})_{q1NPL}-U^{NPL}-(CH₂)_{pNPL}-(NR^{3''})_{q2NPL}-R^{4'}, wherein:

R³, R^{3'}, and R^{3''} are independently selected from hydrogen, alkyl, and alkoxy;

R⁴ and R^{4'} are, independently, selected from hydrogen, alkyl, alkenyl, alkynyl,

25 cycloalkyl, aryl, and heteroaryl, any of which is optionally substituted with one or more alkyl or halo groups;

U^{NPL} is absent or selected from O, S, S(=O), S(=O)₂, NR³, -C(=O)-, -C(=O)-N=N-NR³-, -C(=O)-NR³-N=N-, -N=N-NR³-, -C(=N-N(R³)₂)-, -C(=NR³)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, -R³O-, -R³S-, -S-C=N-, and -C(=O)-NR³-O-, wherein groups with two chemically

30 nonequivalent termini can adopt both possible orientations;

the -(CH₂)_{pNPL}- alkylene chain is optionally substituted with one or more alkyl, amino, or hydroxy groups, or is unsaturated;

pNPL is 0 to 8;

q1NPL and q2NPL are, independently, 0, 1, or 2;

PL is a polar group selected from halo, hydroxyethoxymethyl, methoxyethoxymethyl, polyoxyethylene, and $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, wherein:

R^5 , $R^{5'}$, and $R^{5''}$ are, independently, selected from hydrogen, alkyl, and alkoxy;

U^{PL} is absent or selected from O, S, $S(=O)$, $S(=O)_2$, NR^5 , $-C(=O)-$, $-C(=O)-N=N-NR^5-$,
 5 $-C(=O)-NR^5-N=N-$, $-N=N-NR^5-$, $-C(=N-N(R^5)_2)-$, $-C(=NR^5)-$, $-C(=O)O-$, $-C(=O)S-$, $-C(=S)-$,
 $-O-P(=O)_2O-$, $-R^5O-$, $-R^5S-$, $-S-C=N-$ and $-C(=O)-NR^5-O-$, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

V is selected from the group consisting of nitro, cyano, amino, hydroxy, alkoxy, alkylthio, alkylamino, dialkylamino, $-NH(CH_2)_pNH_2$ wherein p is 1 to 4, $-N(CH_2CH_2NH_2)_2$,
 10 diazamino, amidino, guanidino, guanyl, semicarbazone, aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy,
 $-NH(CH_2)_pNH_2$ wherein p is 1 to 4, $-N(CH_2CH_2NH_2)_2$, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkylthio, lower acylamino, or benzyloxycarbonyl;

the $-(CH_2)_{pPL}-$ alkylene chain is optionally substituted with one or more amino or
 15 hydroxy groups, or is unsaturated;

pPL is 0 to 8;

q1PL and q2PL are, independently, 0, 1, or 2; and

m is 1 to about 500;

and an ophthalmically or otically acceptable excipient.

20 US Patent Publ. No. US 2006-0041023 A1 discloses antimicrobial polymers and oligomers of Formula II that can be used in the compositions of the present invention. For example, oligomers of Formula II that are preferred for use in the ophthalmic or otic compositions of the present invention are those wherein m is 1 to about 25, 1 to about 20, 1 to about 10, 1 to about 5, or 1, 2, or 3.

25 Thus, preferred ophthalmic or otic compositions of the present invention also comprise an oligomer of Formula IIa:



or an acceptable salt or solvate thereof,

wherein:

30 X is NR^8 , O, S, or $-N(R^8)N(R^8)-$; and Y is C=O, C=S, or O=S=O; wherein R^8 is hydrogen or alkyl;

A_1 and A_2 are, independently, optionally substituted arylene or optionally substituted heteroarylene, wherein A_1 and A_2 are, independently, optionally substituted with one or more

polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s);

R^1 is a polar group (PL) or a non-polar group (NPL); and R^2 is R^1 ;

NPL is a nonpolar group $-(NR^{3'})_{q1NPL}-U^{NPL}-(CH_2)_{pNPL}-(NR^{3''})_{q2NPL}-R^{4'}$, wherein:

5 R^3 , $R^{3'}$, and $R^{3''}$ are, independently, selected from hydrogen, alkyl, and alkoxy;

R^4 and $R^{4'}$ are, independently, selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, any of which is optionally substituted with one or more alkyl or halo groups;

10 U^{NPL} is absent or selected from O, S, S(=O), S(=O)₂, NR^3 , -C(=O)-, -C(=O)-N=N- NR^3 -, -C(=O)- NR^3 -N=N-, -N=N- NR^3 -, -C(=N-N(R^3)₂)-, -C(=NR³)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, -R³O-, -R³S-, -S-C=N-, and -C(=O)- NR^3 -O-, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

the $-(CH_2)_{pNPL}$ - alkylene chain is optionally substituted with one or more alkyl, amino, or hydroxy groups, or is unsaturated;

15 $pNPL$ is 0 to 8;

$q1NPL$ and $q2NPL$ are, independently, 0, 1, or 2;

PL is a polar group selected from halo, hydroxyethoxymethyl, methoxyethoxymethyl, polyoxyethylene, and $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, wherein:

R^5 , $R^{5'}$, and $R^{5''}$ are, independently, selected from hydrogen, alkyl, and alkoxy;

20 U^{PL} is absent or selected from O, S, S(=O), S(=O)₂, NR^5 , -C(=O)-, -C(=O)-N=N- NR^5 -, -C(=O)- NR^5 -N=N-, -N=N- NR^5 -, -C(=N-N(R^5)₂)-, -C(=NR⁵)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, -R⁵O-, -R⁵S-, -S-C=N-, and -C(=O)- NR^5 -O-, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

25 V is selected from nitro, cyano, amino, hydroxy, alkoxy, alkylthio, alkylamino, dialkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, guanidino, guanyl, semicarbazone, aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, lower acylamino, or benzyloxycarbonyl;

30 the $-(CH_2)_{pPL}$ - alkylene chain is optionally substituted with one or more amino or hydroxy groups, or is unsaturated;

pPL is 0 to 8; and

$q1PL$ and $q2PL$ are, independently, 0, 1, or 2;

and an ophthalmically or otically acceptable excipient.

Preferred oligomers of Formula IIa for use in the ophthalmic or otic compositions of the present invention are those wherein X is NR^8 and Y is $\text{C}=\text{O}$. For example, oligomers of Formula IIa wherein X is NH and Y is $\text{C}=\text{O}$ are especially preferred.

Preferred also are those oligomers of Formula IIa wherein A_1 and A_2 are independently optionally substituted *o*-, *m*-, or *p*-phenylene. Those oligomers wherein A_1 and A_2 are optionally substituted *m*-phenylene are especially preferred.

Also preferred are those oligomers of Formula IIa wherein one of A_1 and A_2 is *o*-, *m*-, or *p*-phenylene, and the other of A_1 and A_2 is *o*-, *m*-, or *p*-heteroarylene. Preferred heteroarylene groups include, but are not limited to, pyridinylene, pyrimidinylene, and pyrazinylene. An especially preferred heteroarylene group is pyrimidinylene, in particular, *m*-pyrimidinylene.

Also preferred are oligomers of Formula IIa wherein A_1 and A_2 are, independently, optionally substituted arylene or optionally substituted heteroarylene, and (i) one of A_1 and A_2 is substituted with one or more polar (PL) group(s) and one or more nonpolar (NPL) group(s) and the other of A_1 and A_2 is unsubstituted; or (ii) one of A_1 and A_2 is substituted with one or more polar (PL) group(s) and one or more nonpolar (NPL) group(s) and the other of A_1 and A_2 is substituted with one or more polar (PL) group(s). Especially preferred are oligomers in which either (i) one of A_1 and A_2 is substituted with one polar (PL) group and one nonpolar (NPL) group, and the other of A_1 and A_2 is unsubstituted, or (ii) one of A_1 and A_2 is substituted with one polar (PL) group and one nonpolar (NPL) group and the other of A_1 and A_2 is substituted with one or two polar (PL) group(s), as defined above.

Preferred oligomers of Formula IIa are also those wherein R^1 is hydrogen or a polar group (PL). Especially preferred oligomers are those wherein R^1 is $-(\text{NR}^{5'})_{q1\text{PL}}-\text{U}^{\text{PL}}-(\text{CH}_2)_{p\text{PL}}-(\text{NR}^{5''})_{q2\text{PL}}-\text{V}$, wherein R^5 , $\text{R}^{5'}$, $\text{R}^{5''}$, V, U^{PL} , and $p\text{PL}$ are as defined above, and $q1\text{PL}$ and $q2\text{PL}$ are each 0, so that especially preferred oligomers of Formula IIa are those wherein R^1 is $-\text{U}^{\text{PL}}-(\text{CH}_2)_{p\text{PL}}-\text{V}$. Preferred R^1 polar groups are those wherein U^{PL} is absent or is O, S, NH, $-\text{C}(=\text{O})\text{O}-$, or $-\text{C}(=\text{O})$; $p\text{PL}$ is 0 to 6, especially 1 to 4; and V is amino, aminoalkyl, amidino, guanidino, aryl, or heteroaryl optionally substituted with one or more amino, guanidino, amidino, or halo groups.

Preferred values for each of R^3 , $\text{R}^{3'}$, and $\text{R}^{3''}$ are hydrogen, C_1 - C_6 alkyl, and C_1 - C_6 alkoxy. Hydrogen is an especially preferred value for R^3 , $\text{R}^{3'}$, and $\text{R}^{3''}$.

Preferred values of R^4 are hydrogen and alkyl optionally substituted with one or more alkyl or halo groups. More preferred values of R^4 are hydrogen, C_1 - C_{10} alkyl, C_3 - C_{18} branched alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, and C_6 - C_{10} aryl, especially phenyl. Especially preferred

values of R^4 are C_1 - C_{10} alkyl and C_3 - C_{18} branched alkyl. Suitable C_1 - C_{10} alkyl and C_3 - C_{18} branched alkyl groups are methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, and *n*-pentyl.

Preferred oligomers of Formula IIa are those wherein U^{NPL} is absent. In other embodiments, preferred oligomers of Formula IIa are those wherein U^{NPL} is O, S, NH, $-C(=O)-$,
 5 $-C(=O)O-$, $-R^3S-$, or $-R^3O-$. Especially preferred values of U^{NPL} are O, $-C(=O)-$, and $-C(=O)O-$.

Preferred values of pNPL are 0 to 6; values of pNPL of 0 to 4 are especially preferred, with values of pNPL of 0, 1 or 2 most preferred.

Preferred values of q1NPL and q2NPL are 0 or 1. Values of q1NPL and q2NPL of 0 or 1 are especially preferred, with a value of 0 being the most preferred for each of q1NPL and
 10 q2NPL.

In preferred oligomers of Formula IIa, the $-(CH_2)_{pNPL}-$ alkylene chain in NPL is unsubstituted or substituted with one or more alkyl groups.

An especially preferred value of NPL for oligomers of Formula II is C_1 - C_6 alkyl optionally substituted with one or more halo groups. Examples of preferred values for NPL are
 15 *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, and trifluoromethyl.

Preferred oligomers of Formula IIa are those wherein PL is $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, and R^5 , $R^{5'}$, $R^{5''}$, V, U^{PL} , pPL, q1PL and q2PL are as defined above.

Preferred values for R^5 , $R^{5'}$, and $R^{5''}$ are hydrogen, C_1 - C_6 alkyl, and C_1 - C_6 alkoxy.
 20 Hydrogen is an especially preferred value for each of R^5 , $R^{5'}$, and $R^{5''}$.

Preferred values of U^{PL} are O, S, NR^5 , $-C(=O)-$, $-C(=O)-N=N-NH-$, $-C(=O)-NH-N=N-$, $-N=N-NH-$, $-C(=N-N(R^5)_2)-$, $-C(=NR^5)-$, $-C(=O)O-$, $-R^5S-$, and $-R^5O-$, wherein R^5 is hydrogen. Especially preferred values of U^{PL} are O, S, NH, $-C(=O)O-$, and $-C(=O)$. Preferred oligomers of Formula IIa are also those wherein U^{PL} is absent.

Preferred values of V are nitro, cyano, amino, hydroxy, C_1 - C_6 alkoxy, C_1 - C_6 alkylthio, C_1 - C_6 alkylamino, C_1 - C_6 dialkylamino, $-NH(CH_2)_pNH_2$ wherein p is 1 to 4, $-N(CH_2CH_2NH_2)_2$, diazamino, amidino, guanidino, guanyl, semicarbazone, C_6 - C_{10} aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy,
 25 $-NH(CH_2)_pNH_2$ wherein p is 1 to 4, $-N(CH_2CH_2NH_2)_2$, amidino, guanidino, guanyl,
 30 aminosulfonyl, aminoalkoxy, lower acylamino, or benzyloxycarbonyl.

Suitable heteroaryl groups include indolyl, 3*H*-indolyl, 1*H*-isoindolyl, indazolyl, benzoxazolyl, pyridyl, and 2-aminopyridyl. Suitable heterocycle groups include piperidinyl, piperazinyl, imidazolidinyl, pyrrolidinyl, pyrazolidinyl, and morpholinyl.

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Values of V that are more preferred are amino, C₁-C₆ alkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, and guanidino, preferably any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, amidino, guanyl, guanidino, or aminoalkoxy. Values of V that are most preferred are amino and guanidino.

Preferred values of pPL are 0 to 6; values of pPL of 0 to 4 are especially preferred, with values of pPL of 2 to 4 especially preferred.

Preferred values of q1PL and q2PL are 0 or 1. Values of q1PL and q2PL of 0 or 1 are especially preferred, with a value of 0 being especially preferred for each of q1PL and q2PL.

In preferred polymers and oligomers of Formula IIa, the -(CH₂)_{pPL}- alkylene chain in PL is optionally substituted with one or more amino or hydroxy groups.

Thus, preferred ophthalmic or otic compositions comprise an oligomer of Formula IIa, or an acceptable salt or solvate thereof, wherein:

X is NR⁸, and Y is C=O; wherein R⁸ is hydrogen or (C₁-C₄)alkyl;

A₁ and A₂ are, independently, optionally substituted phenylene or optionally substituted pyrimidinylene, wherein A₁ is substituted with one or more polar (PL) group(s) and one or more non-polar (NPL) group(s), and A₂ is substituted with one or more polar (PL) group(s) or is unsubstituted;

R¹ is a polar group (PL); and R² is R¹;

NPL is a nonpolar group -(NR^{3'})_{q1NPL}-U^{NPL}-(CH₂)_{pNPL}-(NR^{3''})_{q2NPL}-R^{4'}, wherein:

R⁴ and R^{4'} are, independently, selected from hydrogen and alkyl optionally substituted with one or more alkyl or halo groups;

U^{NPL} is absent or selected from O, S, NR³, and -C(=O)-;

pNPL is 0 to 6;

q1NPL and q2NPL are, independently, 0;

PL is a polar group -(NR^{5'})_{q1PL}-U^{PL}-(CH₂)_{pPL}-(NR^{5''})_{q2PL}-V, wherein:

U^{PL} is absent or selected from O, S, NR⁵, and -C(=O)-;

V is selected from amino, alkylamino, dialkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, and guanidino, any of which is optionally substituted with one or more of amino, halo, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, and lower acylamino;

pPL is 0 to 8; and

q1PL and q2PL are, independently, 0;

and an ophthalmically or otically acceptable excipient.

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In some embodiments, preferred ophthalmic or otic compositions of the present invention comprise an oligomer of Formula IIa, or an acceptable salt or solvate thereof, wherein:

A₁ is phenylene substituted with one (PL) group and one non-polar (NPL) group, and A₂ is unsubstituted pyrimidinylene or pyrimidinylene substituted with one or two polar (PL) group(s);

NPL is R^{4'}, wherein R^{4'} is (C₁-C₆)alkyl optionally substituted with one or more halo groups;

PL is -U^{PL}-(CH₂)_{pPL}-V, wherein:

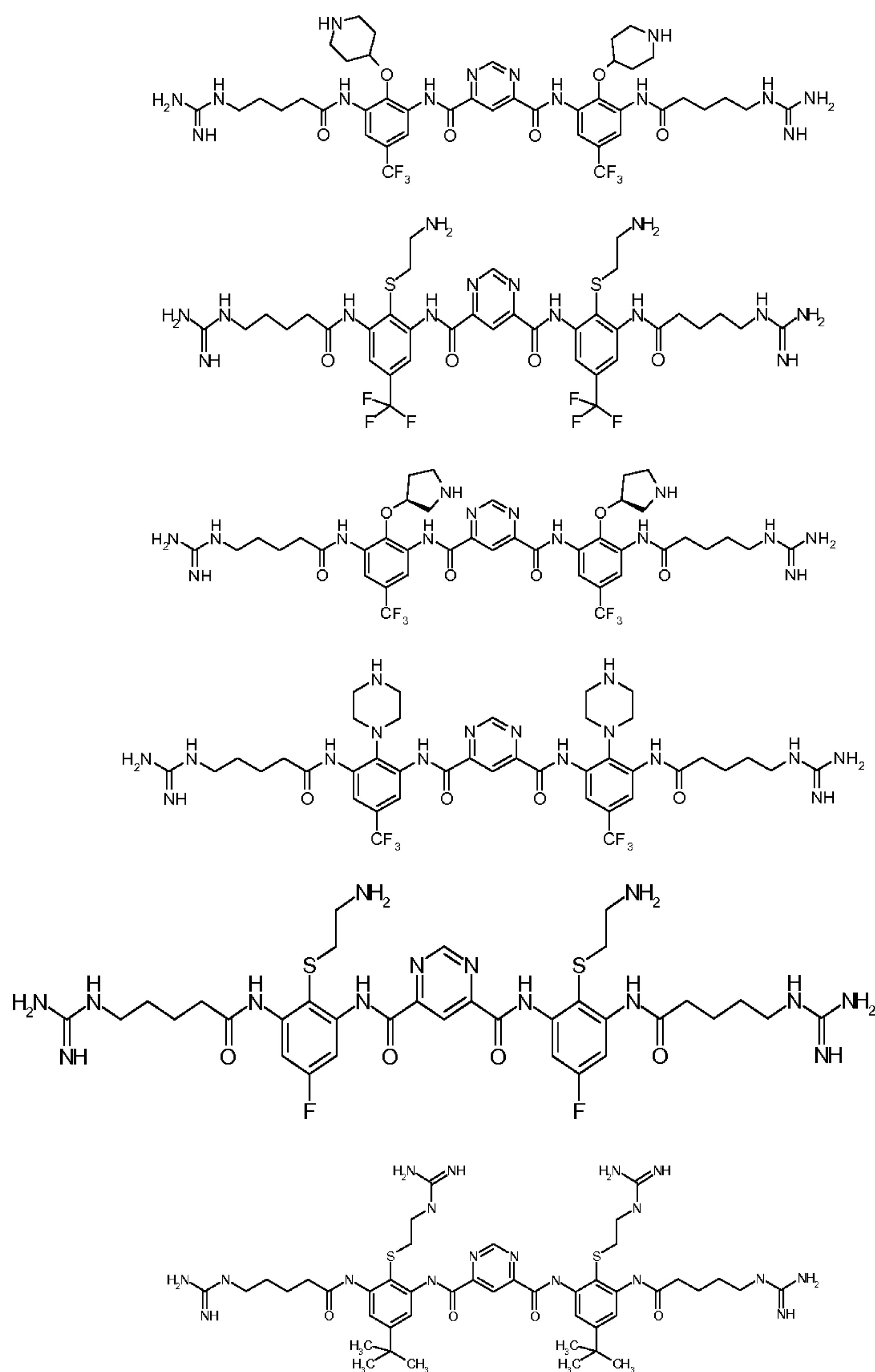
U^{PL} is O or S;

V is selected from amino, amidino, and guanidino; and

pPL is 0 to 6;

and an ophthalmically or otically acceptable excipient.

Examples of oligomers of Formula IIa for use in the ophthalmic or otic compositions of the present invention include



and

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and ophthalmically or otically acceptable salts thereof.

In other embodiments, preferred ophthalmic or otic compositions comprise an oligomer of Formula IIa, or an acceptable salt or solvate thereof, wherein:

A₁ is phenylene substituted with one (PL) group and one non-polar (NPL) group, and

5 A₂ is unsubstituted phenylene or phenylene substituted with one or two polar (PL) group(s);

NPL is R^{4'}, wherein R^{4'} is (C₁-C₆)alkyl optionally substituted with one or more halo groups;

PL is -U^{PL}-(CH₂)_{pPL}-V, wherein:

U^{PL} is O or S;

10 V is selected from amino, amidino, and guanidino; and

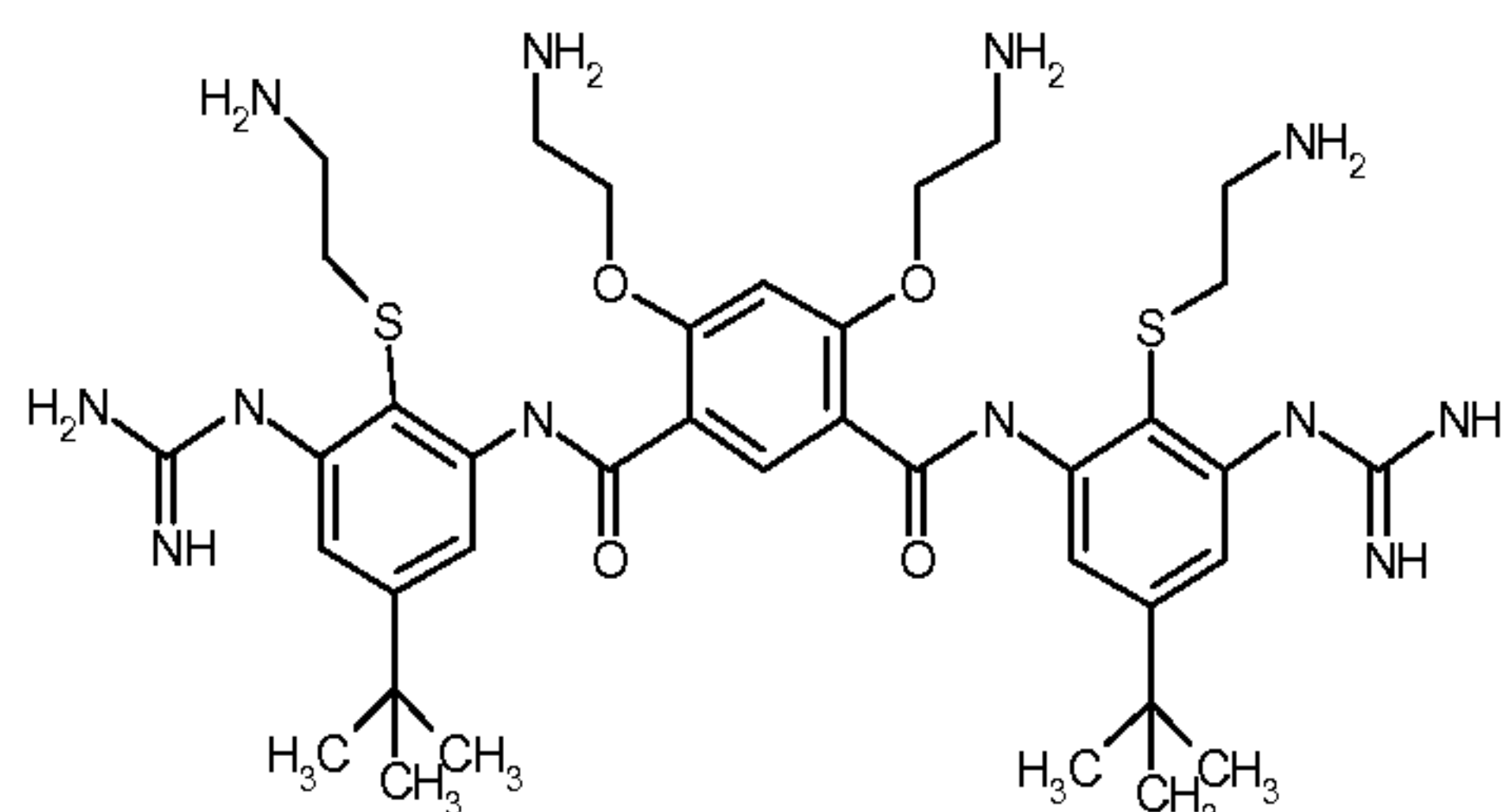
pPL is 0 to 6; and

an ophthalmically or otically acceptable excipient.

In some of these embodiments, preferred ophthalmic or otic compositions comprise oligomers of Formula IIa wherein A₁ is phenylene substituted with one (PL) group and one non-

15 polar (NPL) group, and A₂ is phenylene substituted with one or two polar (PL) group(s).

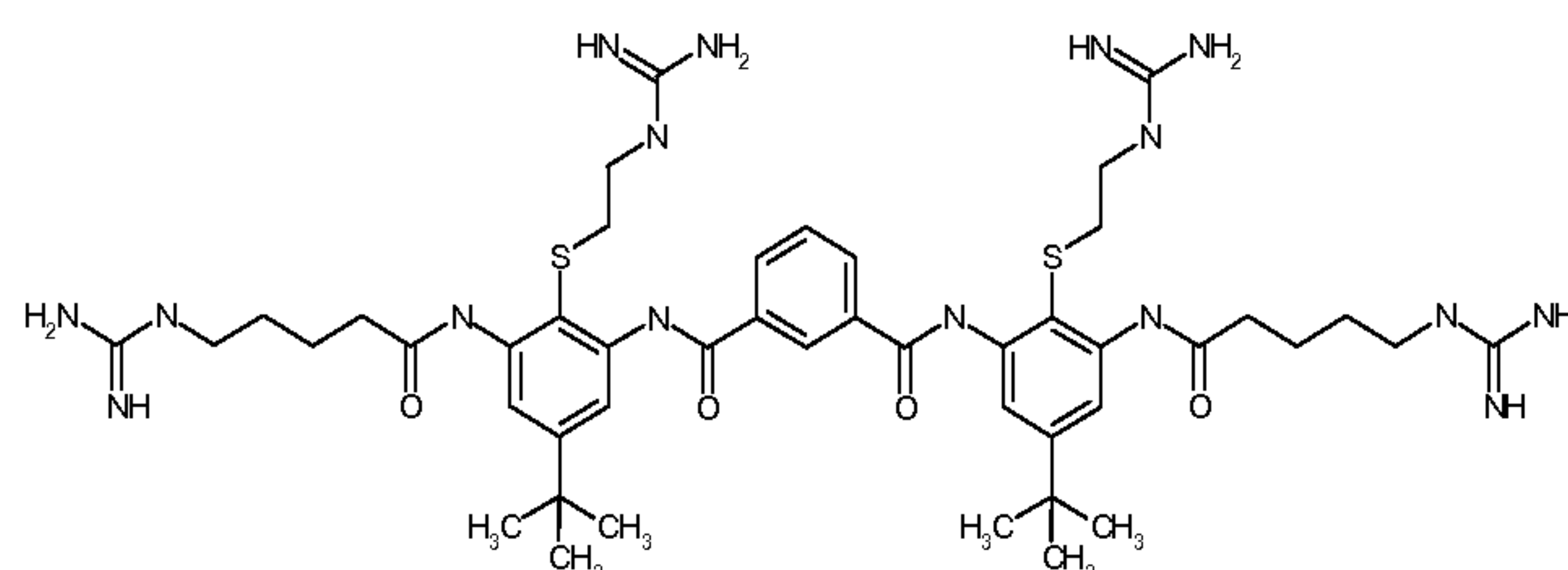
Oligomers of Formula IIa falling within the scope of these embodiments include to following:



and ophthalmically or otically acceptable salts thereof.

In other embodiments, preferred ophthalmic or otic compositions comprise oligomers

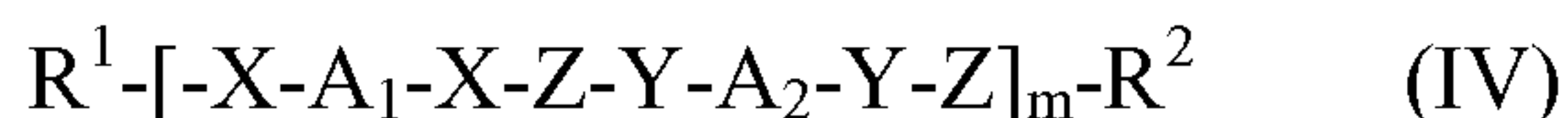
20 wherein A₁ is phenylene substituted with one (PL) group and one non-polar (NPL) group, and A₂ is unsubstituted phenylene. Oligomers falling within the scope of these embodiments include the following:



and ophthalmically or otically acceptable salts thereof.

25 In other aspects, the ophthalmic or otic compositions of the present invention comprise a polymer or oligomer of Formula IV:

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or an acceptable salt or solvate thereof,

wherein:

X is NR^8 , $-NR^8NR^8-$, $C=O$, or O ; Y is NR^8 , $-NR^8NR^8-$, $C=O$, S , or O ; and R^8 is

5 hydrogen or alkyl;

Z is $C=O$, $C=S$, $O=S=O$, $-NR^8NR^8-$, or $-C(=O)C(=O)-$;

A_1 and A_2 are, independently, optionally substituted arylene or optionally substituted heteroarylene, wherein A_1 and A_2 are, independently, optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more

10 polar (PL) group(s) and one or more non-polar (NPL) group(s);

R^1 is

(i) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is $-X-A_1-X-R^1$, wherein A_1 is as defined above and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of

15 one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(ii) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is $-X-A_1-X-Z-Y-A_2-Y-R^1$, wherein A_1 and A_2 are as defined above, and each of which is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more

20 non-polar (NPL) group(s); or

(iii) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is $-X-A'-X-R^1$, wherein A' is aryl or heteroaryl and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(iv) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is $-X-A_1-X-Z-Y-A'-Y-R^1$, wherein A_1 is as defined above, A' is aryl or heteroaryl, and each of A_1 and A' is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(v) $-Z-Y-A'$ and R^2 is hydrogen, a polar group (PL), or a non-polar group (NPL), wherein A' is aryl or heteroaryl and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

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(vi) $-Z-Y-A'$, and R^2 is $-X-A''$, wherein A' and A'' are, independently, aryl or heteroaryl, and each of A' and A'' is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

5 (vii) R^1 and R^2 are, independently, a polar group (PL) or a non-polar group (NPL); or

(viii) R^1 and R^2 together form a single bond;

NPL is a nonpolar group independently selected from $-B(OR^4)_2$ and $-(NR^{3'})_{q1NPL}-U^{NPL}-(CH_2)_{pNPL}-(NR^{3''})_{q2NPL}-R^{4'}$, wherein:

10 R^3 , $R^{3'}$, and $R^{3''}$ are, independently, selected from hydrogen, alkyl, and alkoxy;

R^4 and $R^{4'}$ are, independently, selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, any of which is optionally substituted with one or more alkyl or halo groups;

U^{NPL} is absent or selected from O, S, $S(=O)$, $S(=O)_2$, NR^3 , $-C(=O)-$, $-C(=O)-N=N-NR^3-$,
15 $-C(=O)-NR^3-N=N-$, $-N=N-NR^3-$, $-C(=N-N(R^3)_2)-$, $-C(=NR^3)-$, $-C(=O)O-$, $-C(=O)S-$, $-C(=S)-$,
 $-O-P(=O)_2O-$, $-R^3O-$, $-R^3S-$, $-S-C=N-$, and $-C(=O)-NR^3-O-$, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

the $-(CH_2)_{pNPL}-$ alkylene chain is optionally substituted with one or more alkyl, amino, or hydroxy groups, or is unsaturated;

20 $pNPL$ is 0 to 8;

$q1NPL$ and $q2NPL$ are, independently, 0, 1, or 2;

PL is a polar group selected from halo, hydroxyethoxymethyl, methoxyethoxymethyl, polyoxyethylene, and $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, wherein:

R^5 , $R^{5'}$, and $R^{5''}$ are, independently, selected from the group consisting of hydrogen,
25 alkyl, and alkoxy;

U^{PL} is absent or selected from O, S, $S(=O)$, $S(=O)_2$, NR^5 , $-C(=O)-$, $-C(=O)-N=N-NR^5-$,
 $-C(=O)-NR^5-N=N-$, $-N=N-NR^5-$, $-C(=N-N(R^5)_2)-$, $-C(=NR^5)-$, $-C(=O)O-$, $-C(=O)S-$, $-C(=S)-$,
 $-O-P(=O)_2O-$, $-R^5O-$, $-R^5S-$, $-S-C=N-$, and $-C(=O)-NR^5-O-$, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

30 V is selected from nitro, cyano, amino, hydroxy, alkoxy, alkylthio, alkylamino, dialkylamino, $-NH(CH_2)_pNH_2$ wherein p is 1 to 4, $-N(CH_2CH_2NH_2)_2$, diazamino, amidino, guanidino, guanyl, semicarbazone, aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, $-NH(CH_2)_pNH_2$ wherein p is

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1 to 4, -N(CH₂CH₂NH₂)₂, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, lower acylamino, or benzyloxycarbonyl;

the -(CH₂)_{pPL}- alkylene chain is optionally substituted with one or more amino or hydroxy groups, or is unsaturated;

5 pPL is 0 to 8;

q1PL and q2PL are, independently, 0, 1, or 2; and

m is 1 to about 500;

and an ophthalmically or otically acceptable excipient.

10 US Application Publ. No. US 2006-0041023 A1 discloses antimicrobial polymers and oligomers of Formula IV that can be used in the compositions of the present invention.

For example, oligomers that are preferred for use in the ophthalmic or otic compositions of the present invention are those oligomers of Formula IV wherein m is 1 to about 25, 1 to about 20, 1 to about 10, 1 to about 5, or 1, 2, or 3.

15 Thus, preferred ophthalmic or otic compositions also comprise an oligomer of Formula IV having Formula IVa, Formula IVb, or Formula IVc:



or an acceptable salt or solvate thereof,

20 wherein:

X is NR⁸, -NR⁸NR⁸-, C=O, or O; Y is NR⁸, -NR⁸NR⁸-, C=O, S, or O; and R⁸ is hydrogen or alkyl;

Z is C=O, C=S, O=S=O, -NR⁸NR⁸-, or -C(=O)C(=O)-;

25 A₁ and A₂ are, independently, optionally substituted arylene or optionally substituted heteroarylene, wherein A₁ and A₂ are, independently, optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s);

R¹ is hydrogen, a polar group (PL), or a non-polar group (NPL), and R² is R¹;

NPL is a nonpolar group -(NR^{3'})_{q1NPL}-U^{NPL}-(CH₂)_{pNPL}-(NR^{3''})_{q2NPL}-R^{4'}, wherein:

30 R³, R^{3'}, and R^{3''} are, independently, selected from hydrogen, alkyl, and alkoxy;

R⁴ and R^{4'} are, independently, selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, any of which is optionally substituted with one or more alkyl or halo groups;

U^{NPL} is absent or selected from O, S, S(=O), S(=O)₂, NR³, -C(=O)-, -C(=O)-N=N-NR³-, -C(=O)-NR³-N=N-, -N=N-NR³-, -C(=N-N(R³)₂)-, -C(=NR³)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, -R³O-, -R³S-, -S-C=N-, and -C(=O)-NR³-O-, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

5 the -(CH₂)_{pNPL}- alkylene chain is optionally substituted with one or more amino or hydroxy groups, or is unsaturated;

pNPL is 0 to 8;

q1NPL and q2NPL are, independently, 0, 1, or 2;

10 PL is a polar group selected from halo, hydroxyethoxymethyl, methoxyethoxymethyl, polyoxyethylene, and -(NR^{5'})_{q1PL}-U^{PL}-(CH₂)_{pPL}-(NR^{5'})_{q2PL}-V, wherein:

R⁵, R^{5'}, and R^{5''} are, independently, selected from hydrogen, alkyl, and alkoxy;

15 U^{PL} is absent or selected from O, S, S(=O), S(=O)₂, NR⁵, -C(=O)-, -C(=O)-N=N-NR⁵-, -C(=O)-NR⁵-N=N-, -N=N-NR⁵-, -C(=N-N(R⁵)₂)-, -C(=NR⁵)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, -R⁵O-, -R⁵S-, -S-C=N-, and -C(=O)-NR⁵-O-, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

20 V is selected from nitro, cyano, amino, hydroxy, alkoxy, alkylthio, alkylamino, dialkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, guanidino, guanyl, semicarbazone, aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, lower acylamino, or benzyloxycarbonyl;

the -(CH₂)_{pPL}- alkylene chain is optionally substituted with one or more amino or hydroxy groups, or is unsaturated;

pPL is 0 to 8; and

25 q1PL and q2PL are, independently, 0, 1, or 2;

and an ophthalmically or otically acceptable excipient.

US Application Publ. No. US 2006-0041023 A1 discloses antimicrobial polymers and oligomers of Formulae IVa, IVb, and IVc that can be used in the compositions of the present invention.

30 Preferred ophthalmic or otic compositions comprise oligomers of Formulae IVa, IVb and IVc wherein X and Y are, independently, NR⁸, C=O, or O; Z is C=O or -NR⁸NR⁸; and R⁸ is hydrogen or C₁-C₆ alkyl. Especially preferred for use in the ophthalmic or otic compositions are those oligomers wherein X and Y are each NR⁸, Z is C=O, and R⁸ is hydrogen. Also preferred

are oligomers wherein X and Y are each C=O, and Z is -N(R⁸)N(R⁸)-, especially wherein R⁸ is hydrogen.

Also preferred for use in the ophthalmic or otic compositions are those oligomers of Formulae IVa, IVb and IVc wherein A₁ and A₂ are independently optionally substituted *o*-, *m*-, or *p*-phenylene. Those oligomers wherein A₁ and A₂ are optionally substituted *m*-phenylene are especially preferred. Also preferred are polymers and oligomers of Formula IV wherein one of A₁ and A₂ is *o*-, *m*-, or *p*-phenylene, and the other of A₁ and A₂ is heteroarylene. Preferred heteroarylene groups include, but are not limited to, pyridinylene, pyrimidinylene, and pyrazinylene.

Also preferred are oligomers of Formulae IVa, IVb and IVc wherein A₁ and A₂ are, independently, optionally substituted arylene or optionally substituted heteroarylene, and (i) each of A₁ and A₂ is substituted with one or two polar (PL) group(s) and one or two nonpolar (NPL) group(s); or (ii) one of A₁ and A₂ is substituted with one or two polar (PL) group(s) and the other of A₁ and A₂ is substituted with one or two nonpolar (NPL) group(s).

Preferred ophthalmic or otic compositions also comprise oligomers of Formulae IVa, IVb and IVc are those wherein R¹ is hydrogen or a polar group (PL). Especially preferred oligomers are those wherein R¹ is -(NR^{5'})_{q1PL}-U^{PL}-(CH₂)_{pPL}-(NR^{5''})_{q2PL}-V, wherein R⁵, R^{5'}, R^{5''}, V, U^{PL}, and pPL are as defined above, and q1PL and q2PL are each 0, so that especially preferred oligomers of Formulae IVa, IVb and IVc are those wherein R¹ is -U^{PL}-(CH₂)_{pPL}-V. Preferred R¹ polar groups are those wherein U^{PL} is absent or is O, S, NH, -C(=O)O-, or -C(=O); pPL is 0 to 6, especially 1 to 4; and V is amino, aminoalkyl, amidino, guanidino, aryl, or heteroaryl optionally substituted with one or more amino, guanidino, amidino, or halo groups.

Preferred values for each of R³, R^{3'}, and R^{3''} are hydrogen, C₁-C₆ alkyl, and C₁-C₆ alkoxy. Hydrogen is an especially preferred value for R³, R^{3'}, and R^{3''}.

Preferred values of R⁴ are hydrogen, C₁-C₁₀ alkyl, C₃-C₁₈ branched alkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, and C₆-C₁₀ aryl, especially phenyl. Especially preferred values of R⁴ are C₁-C₁₀ alkyl and C₃-C₁₈ branched alkyl. Suitable C₁-C₁₀ alkyl and C₃-C₁₈ branched alkyl groups are methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, and *n*-pentyl.

Preferred values of U^{NPL} are O, S, NH, -C(=O)-, -C(=O)O-, -R³S- and -R³O-. Preferred oligomers of Formulae IVa, IVb and IVc are also those wherein U^{NPL} is absent.

Preferred values of pNPL are 0 to 6; values of pNPL of 0 to 4 are especially preferred, with values of pNPL of 0, 1, or 2 most preferred.

Preferred values of q1NPL and q2NPL are 0 or 1. Values of q1NPL and q2NPL of 0 or 1 are especially preferred, with a value of 0 being the most preferred for each of q1NPL and q2NPL.

In preferred ophthalmic or otic compositions, in the oligomers of Formulae IVa, IVb and IVc, the $-(CH_2)_{pNPL}-$ alkylene chain in NPL is unsubstituted or substituted with one or more alkyl groups. More preferred are those oligomers wherein the $-(CH_2)_{pNPL}-$ alkylene chain in NPL is unsubstituted.

An especially preferred value of NPL for polymers and oligomers of Formulae IVa, IVb and IVc is C₁-C₆ alkyl. Examples of preferred values for NPL are *n*-propyl, isopropyl, *n*-butyl, and *tert*-butyl.

Preferred oligomers of Formulae IVa, IVb and IVc for use in the ophthalmic or otic compositions are also those wherein PL is $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, and R⁵, R^{5'}, R^{5''}, V, U^{PL}, pPL, q1PL, and q2PL are as defined above.

Preferred values for R⁵, R^{5'}, and R^{5''} are hydrogen, C₁-C₆ alkyl, and C₁-C₆ alkoxy. Hydrogen is an especially preferred value for each of R⁵, R^{5'}, and R^{5''}.

Preferred values of U^{PL} are O, S, NH, -C(=O)-, -C(=O)O-, -R⁵S-, and -R⁵O-, wherein R⁵ is hydrogen or C₁-C₆ alkyl. Especially preferred values of U^{PL} are O, S, and -C(=O).

Preferred values of V are nitro, cyano, amino, hydroxy, C₁-C₆ alkoxy, C₁-C₆ alkylthio, C₁-C₆ alkylamino, C₁-C₆ dialkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, guanidino, guanyl, semicarbazone, C₆-C₁₀ aryl, heterocycle, and heteroaryl, preferably any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, lower acylamino, or benzyloxycarbonyl.

Suitable heteroaryl groups include indolyl, 3*H*-indolyl, 1*H*-isoindolyl, indazolyl, benzoxazolyl, pyridyl, and 2-aminopyridyl. Suitable heterocycle groups include piperidinyl, piperazinyl, imidazolidinyl, pyrrolidinyl, pyrazolidinyl, and morpholinyl.

Especially preferred values of V are amino, C₁-C₆ alkylamino, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, diazamino, amidino, and guanidino, preferably any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxy, -NH(CH₂)_pNH₂ wherein p is 1 to 4, -N(CH₂CH₂NH₂)₂, amidino, guanyl, guanidino, or aminoalkoxy. Values of V that are most preferred are amino and guanidino.

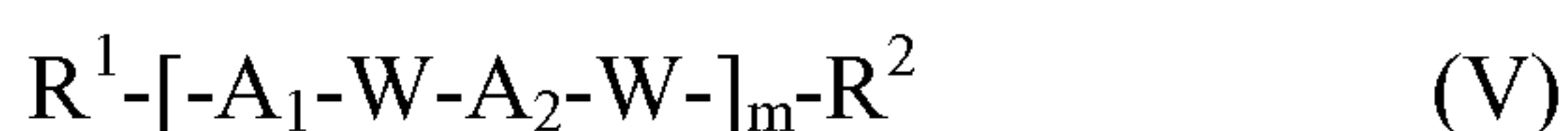
Preferred values of pPL are 0 to 6; values of pPL of 0 to 4 are especially preferred, with values of pPL of 2 to 4 especially preferred.

Preferred values of q1PL and q2PL are 0 or 1. Values of q1PL and q2PL of 0 or 1 are especially preferred, with a value of 0 being especially preferred for each of q1PL and q2PL.

In the preferred ophthalmic or otic compositions of the invention, in the oligomers of Formulae IVa, IVb, and IVc, the $-(CH_2)_{pPL}-$ alkylene chain in PL is optionally substituted with one or more amino or hydroxy groups.

Examples of oligomers of Formulae I, II, IIa, IV, IVa, IVb, and IVc that can be used in the ophthalmic or otic compositions of the present invention include, but are not limited to, the individual oligomers disclosed in US Application Publ. No. 2006-0041023 A1 and US Patent No. 7,173,102.

In some aspects, the ophthalmic or otic compositions of the present invention comprise a polymer or oligomer of Formula V:



or an acceptable salt or solvate thereof,

wherein:

A_1 and A_2 are, independently, optionally substituted arylene or optionally substituted heteroarylene, wherein:

(i) A_1 and A_2 are independently optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(ii) one of A_1 or A_2 is as defined above and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); and the other of A_1 or A_2 is the group $-C\equiv C(CH_2)_pC\equiv C-$, wherein p is 0 to 8, and the $-(CH_2)_p-$ alkylene chain is optionally substituted with one or more amino or hydroxyl groups;

W is absent, or represents $-CH_2-$, $-CH_2-CH_2-$, $-CH=CH-$, or $-C\equiv C-$;

R^1 is

(i) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is $-A_1-R^1$, wherein A_1 is as defined above and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(ii) hydrogen, a polar group (PL), or a non-polar group (NPL), and R^2 is $-A_1-W-A_2-R^1$, wherein each of A_1 and A_2 is as defined above and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL)

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group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(iii) A'-W- and R² is -A₁-W-A', wherein A' is aryl or heteroaryl, either of which is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(iv) A'-W- and R² is -A', wherein A' is aryl or heteroaryl, either of which is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) groups(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or

(v) R¹ and R² together form a single bond;

NPL is a nonpolar group independently selected from -B(OR⁴)₂ and -(NR^{3'})_{q1NPL}-U^{NPL}-(CH₂)_{pNPL}-(NR^{3''})_{q2NPL}-R⁴, wherein:

R³, R^{3'}, and R^{3''} are, independently, selected from hydrogen, alkyl, and alkoxy;

R⁴ is selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, any of which is optionally substituted with one or more alkyl or halo groups;

U^{NPL} is absent or selected from O, S, S(=O), S(=O)₂, NR³, -(C=O)-, -(C=O)-N=N-NR³-, -(C=O)-NR³-N=N-, -N=N-NR³-, -C(=N-N(R³)₂)-, -C(=NR³)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, -R³O-, -R³S-, -S-C=N-, and -(C=O)-NR³-O-, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

the -(CH₂)_{pNPL}- alkylene chain is optionally substituted with one or more alkyl, amino or hydroxyl groups, or the alkylene chain is unsaturated;

pNPL is 0 to 8;

q1NPL and q2NPL are, independently, 0 to 2;

PL is a polar group selected from halo, hydroxyethoxymethyl, methoxyethoxymethyl, polyoxyethylene, and -(NR^{5'})_{q1PL}-U^{PL}-(CH₂)_{pPL}-(NR^{5''})_{q2PL}-V, wherein:

R⁵, R^{5'}, and R^{5''} are, independently, selected from hydrogen, alkyl, and alkoxy;

U^{PL} is absent or selected from O, S, S(=O), S(=O)₂, NR⁵, -(C=O)-, -(C=O)-N=N-NR⁵-, -(C=O)-NR⁵-N=N-, -N=N-NR⁵-, -C(=N-N(R⁵)₂)-, -C(=NR⁵)-, -C(=O)O-, -C(=O)S-, -C(=S)-, -O-P(=O)₂O-, -R⁵O-, -R⁵S-, -S-C=N-, and -(C=O)-NR⁵-O-, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

V is selected from nitro, cyano, amino, hydroxyl, alkoxy, alkylthio, alkylamino, dialkylamino, -NH(CH₂)_pNH₂, -N(CH₂CH₂NH₂)₂, diazamino, amidino, guanidino, guanyl, semicarbazone, aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one

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or more of amino, halo, cyano, nitro, hydroxyl, $-\text{NH}(\text{CH}_2)_p\text{NH}_2$, $-\text{N}(\text{CH}_2\text{CH}_2\text{NH}_2)_2$, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, lower acylamino, or benzyloxycarbonyl;

the $-(\text{CH}_2)_{p\text{PL}}-$ alkylene chain is optionally substituted with one or more amino or hydroxyl groups, or the alkylene chain is unsaturated;
 5 pPL is 0 to 8;
 q1PL and q2PL are, independently, 0 to 2; and
 m is 1 to at least about 500;

with the proviso that if A_1 and A_2 are thiophene, the polar groups cannot be 3-(propionic acid) or methoxy(diethoxy)ethyl and the nonpolar group cannot be n-dodecyl;
 10 and an ophthalmically or otically acceptable excipient.

US Appl. Publ. No. US 2005-0287108 A1 discloses antimicrobial polymers and oligomers of Formula V that can be used in the compositions of the present invention.

For example, oligomers that are preferred for use in the ophthalmic or otic compositions of the present invention are those oligomers of Formula V wherein m is 1 to about 25, 1 to about 20, 1 to about 10, 1 to about 7, 1 to about 5, or 1, 2, or 3.
 15

Thus, preferred ophthalmic or otic compositions of the invention also comprise oligomers of Formula Va:



20 or an acceptable salt or solvate thereof,
 wherein:

A_1 and A_2 are, independently, optionally substituted arylene or optionally substituted heteroarylene, wherein:

(i) A_1 and A_2 are independently optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); or
 25

(ii) one of A_1 or A_2 is as defined above and is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s); and the other of A_1 or A_2 is the group $-\text{C}\equiv\text{C}(\text{CH}_2)_p\text{C}\equiv\text{C}-$, wherein p is 0 to 8, and the $-(\text{CH}_2)_p$ -alkylene chain is optionally substituted with one or more amino or hydroxyl groups;
 30 W is $-\text{C}\equiv\text{C}-$;

R^1 is hydrogen, a polar group (PL), a non-polar group (NPL), or $-\text{W-A}'$,

wherein A' is aryl or heteroaryl, either of which is optionally substituted with one or more polar (PL) group(s), one or more non-polar (NPL) group(s), or a combination of one or more polar (PL) group(s) and one or more non-polar (NPL) group(s);

R^2 is R^1 ;

5 NPL is a nonpolar group $-(NR^{3'})_{q1NPL}-U^{NPL}-(CH_2)_{pNPL}-(NR^{3''})_{q2NPL}-R^4$, wherein R^3 , $R^{3'}$, and $R^{3''}$ are, independently, selected from hydrogen, alkyl, and alkoxy;

R^4 is selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, any of which is optionally substituted with one or more alkyl or halo groups;

10 U^{NPL} is absent or selected from O, S, S(=O), S(=O)₂, NR^3 , $-(C=O)-$, $-(C=O)-N=N-NR^3-$, $-(C=O)-NR^3-N=N-$, $-N=N-NR^3-$, $-C(=N-N(R^3)_2)-$, $-C(=NR^3)-$, $-C(=O)O-$, $-C(=O)S-$, $-C(=S)-$, $-O-P(=O)_2O-$, $-R^3-O-$, $-R^3-S-$, $-S-C=N-$ and $-(C=O)-NR^3-O-$, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

the alkylene chain $-(CH_2)_{pNPL}-$ is optionally substituted with one or more alkyl, amino, or hydroxyl groups, or the alkylene chain is unsaturated;

15 $pNPL$ is 0 to 8;

$q1NPL$ and $q2NPL$ are, independently, 0 to 2;

PL is a polar group selected from halo, hydroxyethoxymethyl, methoxyethoxymethyl, polyoxyethylene, and $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, wherein:

R^5 , $R^{5'}$, and $R^{5''}$ are, independently, selected from hydrogen, alkyl, and alkoxy;

20 U^{PL} is absent or selected from O, S, S(=O), S(=O)₂, NR^5 , $-(C=O)-$, $-(C=O)-N=N-NR^5-$, $-(C=O)-NR^5-N=N-$, $-N=N-NR^5-$, $-C(=N-N(R^5)_2)-$, $-C(=NR^5)-$, $-C(=O)O-$, $-C(=O)S-$, $-C(=S)-$, $-O-P(=O)_2O-$, $-R^5O-$, $-R^5S-$, $-S-C=N-$, and $-(C=O)-NR^5-O-$, wherein groups with two chemically nonequivalent termini can adopt both possible orientations;

25 V is selected from nitro, cyano, amino, hydroxyl, alkoxy, alkylthio, alkylamino, dialkylamino, $-NH(CH_2)_pNH_2$, $-N(CH_2CH_2NH_2)_2$, diazamino, amidino, guanidino, guanyl, semicarbazone, aryl, heterocycle, and heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxyl, $-NH(CH_2)_pNH_2$, $-N(CH_2CH_2NH_2)_2$, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, lower acylamino, or benzyloxycarbonyl;

30 the alkylene chain $-(CH_2)_{pPL}-$ is optionally substituted with one or more amino or hydroxyl groups, or the alkylene chain is unsaturated;

pPL is 0 to 8; and

$q1PL$ and $q2PL$ are, independently, 0 to 2;

and an ophthalmically or otically acceptable excipient.

Preferred oligomers of Formula Va for use in the ophthalmic or otic compositions are those oligomers of Formula Va wherein A_1 and A_2 are, independently, optionally substituted *o*-, *m*-, or *p*-phenylene, with *m*-phenylene being especially preferred. Also preferred are oligomers of Formula Va wherein one of A_1 or A_2 is *o*-, *m*-, or *p*-phenylene, and the other of A_1 or A_2 is
 5 heteroarylene. Preferred heteroarylene groups include, but are not limited to, pyridinyl, pyrimidinyl, and pyrazinyl.

Preferred oligomers of Formula Va are also those wherein A_1 is substituted with one or two polar (PL) group(s) and A_2 is unsubstituted. Especially preferred are those oligomers wherein A_1 is substituted with one polar (PL) group and A_2 is unsubstituted.

10 Preferred ophthalmic or otic compositions also comprise oligomers of Formula Va wherein R^1 is hydrogen, a polar group (PL), or a non-polar group (NPL); and R^2 is R^1 . More preferred are oligomers of Formula Va wherein R^1 is selected from hydrogen, halo, nitro, cyano, C_1 - C_6 alkoxy, C_1 - C_6 alkoxycarbonyl, and benzyloxycarbonyl. Oligomers of Formula Va wherein R^1 and R^2 are halo are especially preferred.

15 Preferred R^3 , $R^{3'}$, and $R^{3''}$ groups include hydrogen and C_1 - C_4 alkyl. Especially preferred are those oligomers of Formula Va wherein R^3 , $R^{3'}$, and $R^{3''}$ are each hydrogen.

Preferred R^4 groups include hydrogen, C_1 - C_{10} alkyl, C_3 - C_{18} branched alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, or C_6 - C_{10} aryl, especially phenyl. Oligomers wherein R^4 is hydrogen, C_1 - C_{10} alkyl, and C_3 - C_{18} branched alkyl, any of which is optionally substituted with one or more
 20 C_1 - C_4 alkyl or halo groups, are especially preferred.

Preferred oligomers of Formula Va are also those wherein U^{NPL} is O, S, NH, $-(C=O)-$, $-C(=O)O-$, $-R^3O-$, or $-R^3S-$. Oligomers of Formula Va wherein U^{NPL} is O, S, or $-(C=O)-$ are especially preferred. Oligomers of Formula Va wherein U^{NPL} is absent are also preferred.

Preferred oligomers of Formula Va also include those oligomers wherein the alkylene chain $-(CH_2)_{pNPL}-$ is optionally substituted with one or more alkyl groups. Especially preferred
 25 are those oligomers in which the alkylene chain is unsubstituted. Also preferred are those oligomers of Formula Va wherein $pNPL$ is 0 to 8, or 1 to 6, or, more preferably, 2 to 4.

Preferred oligomers of Formula Va are those wherein $q1NPL$ and $q2NPL$ are independently 0 or 1.

30 In some embodiments, preferred ophthalmic or otic compositions comprise oligomers wherein NPL is *n*-pentoxy, *n*-butoxy, *sec*-butoxy, *tert*-butoxy, propyloxy, ethyloxy, methoxy, or phenoxy.

Preferred ophthalmic or otic compositions also comprise oligomers of Formula Va wherein one or more PL are halo, especially bromo or iodo.

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Preferred oligomers of Formula Va for use in the ophthalmic or otic compositions include those wherein PL is $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, and R^5 , $R^{5'}$, $R^{5''}$, V , U^{PL} , and pPL , and $q1PL$ and $q2PL$ are as defined above.

Preferred values for R^5 , $R^{5'}$, and $R^{5''}$ are hydrogen, C_1 - C_6 alkyl, and C_1 - C_6 alkoxy.

5 Hydrogen is an especially preferred value for each of R^5 , $R^{5'}$, and $R^{5''}$.

Preferred values of U^{PL} are O, S, NH, $-(C=O)-$, $-C(=O)O-$, $-R^5O-$, and $-R^5S-$. Also preferred are oligomers of Formula Va wherein U^{PL} is absent.

Preferred oligomers of Formula Va also are those wherein $q1PL$ and $q2PL$ are, independently, 0 or 1.

10 Preferred ophthalmic or otic compositions also comprise oligomers of Formula Va wherein V is nitro, cyano, amino, hydroxyl, C_1 - C_6 alkoxy, C_1 - C_6 alkylthio, C_1 - C_6 alkylamino, C_1 - C_6 dialkylamino, $-NH(CH_2)_pNH_2$, $-N(CH_2CH_2NH_2)_2$, diazamino, amidino, guanidino, guanyl, semicarbazone, heterocycle, or heteroaryl, any of which is optionally substituted with one or more of amino, halo, cyano, nitro, hydroxyl, $-NH(CH_2)_pNH_2$, $-N(CH_2CH_2NH_2)_2$, amidino, 15 guanyl, guanidine, or aminoalkoxy. Suitable heteroaryl groups include indolyl, 3*H*-indolyl, 1*H*-isoindolyl, indazolyl, benzoxazolyl, pyridyl, and 2-aminopyridyl. Suitable heterocycle groups include piperidinyl, piperazinyl, imidazolidinyl, pyrrolidinyl, pyrazolidinyl, and morpholinyl. Especially preferred values of V include amino, C_1 - C_6 alkylamino, C_1 - C_6 dialkylamino, $-NH(CH_2)_pNH_2$, $-N(CH_2CH_2NH_2)_2$, diazamino, amidino, guanidino, and guanyl, any of which is 20 optionally substituted with one or more of amino, halo, $-NH(CH_2)_pNH_2$, $-N(CH_2CH_2NH_2)_2$, amidino, guanyl, guanidine, or aminoalkoxy.

Especially preferred oligomers of Formula Va for use in the preferred ophthalmic compositions are those wherein PL is halo, guanidinomethyl, guanidinoethyl, guanidinopropyl, aminomethyl, aminoethyl, aminopropyl, aminoethylaminocarbonyl, or 25 aminomethylaminocarbonyl.

Preferred oligomers of Formula Va are also those wherein pPL is 0 to 4. Especially preferred are those oligomers wherein pPL is 0 to 2.

Thus, in some embodiments, especially preferred ophthalmic or otic compositions of the present invention comprise an oligomer of Formula Va wherein:

30 A_1 and A_2 are, independently, optionally substituted *m*-phenylene, wherein A_1 is optionally substituted with two polar (PL) groups, and A_2 is unsubstituted;

R^1 is a polar group;

PL is $-(NR^{5'})_{q1PL}-U^{PL}-(CH_2)_{pPL}-(NR^{5''})_{q2PL}-V$, wherein:

U^{PL} is absent or selected from O, S, NR^5 , and $-C(=O)-$;

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V is selected from amino, amidino, and guanidino, any of which is optionally substituted with one or more of amino, halo, $-\text{NH}(\text{CH}_2)_p\text{NH}_2$ wherein p is 1 to 4, $-\text{N}(\text{CH}_2\text{CH}_2\text{NH}_2)_2$, amidino, guanidino, guanyl, aminosulfonyl, aminoalkoxy, aminoalkythio, and lower acylamino;

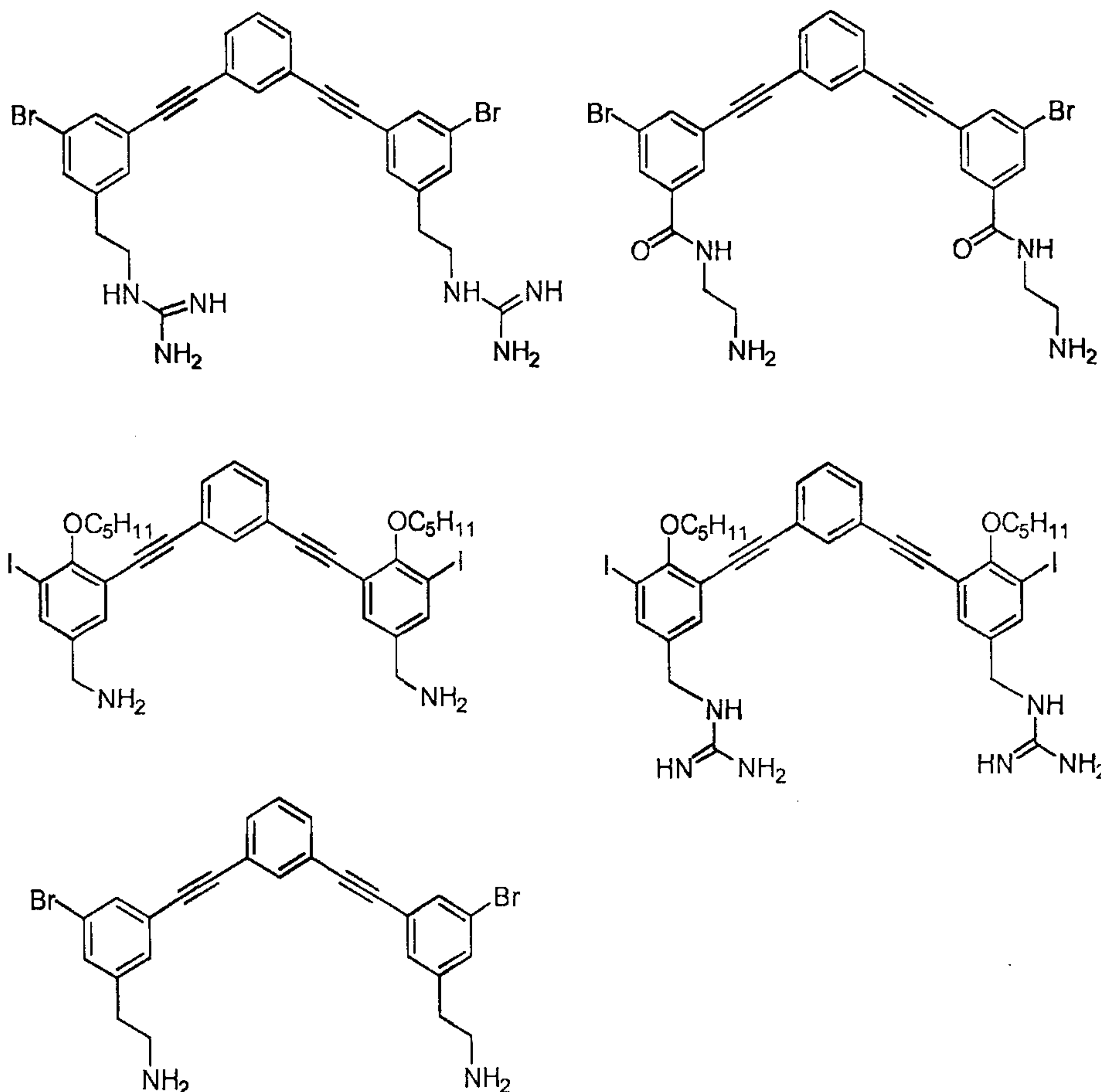
5 pPL is 0 to 8; and

q1PL and q2PL are, independently, 0;

and an ophthalmically acceptable excipient.

Especially preferred are oligomers of Formula Va wherein R^1 is halo, and PL is $-\text{U}^{\text{PL}}-(\text{CH}_2)_{p\text{PL}}-\text{V}$, wherein U^{PL} is absent; V is selected from amino, amidino, and guanidino, any
 10 of which is optionally substituted with one or more of amino or halo; and pPL is 0 to 6.

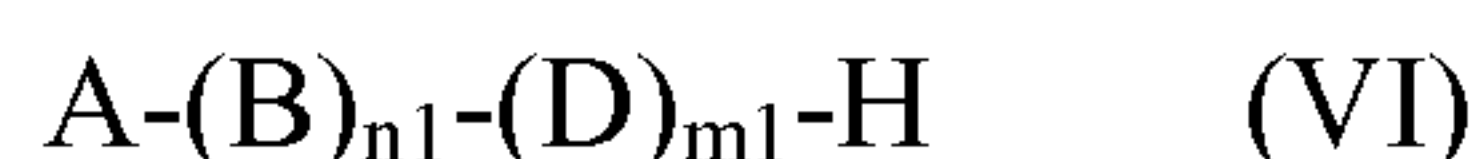
Exemplary structures of oligomers of Formula Va within the scope of the invention include the following, as well as those individual oligomers disclosed in U.S. Application Publication No. 2005-0287108, :



15 and ophthalmically or otically acceptable salts thereof.

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In some aspects, the ophthalmic or otic compositions of the present invention comprise a random copolymer of Formula VI:



or an acceptable salt or solvate thereof,

5 wherein:

A is the residue of a chain transfer agent;

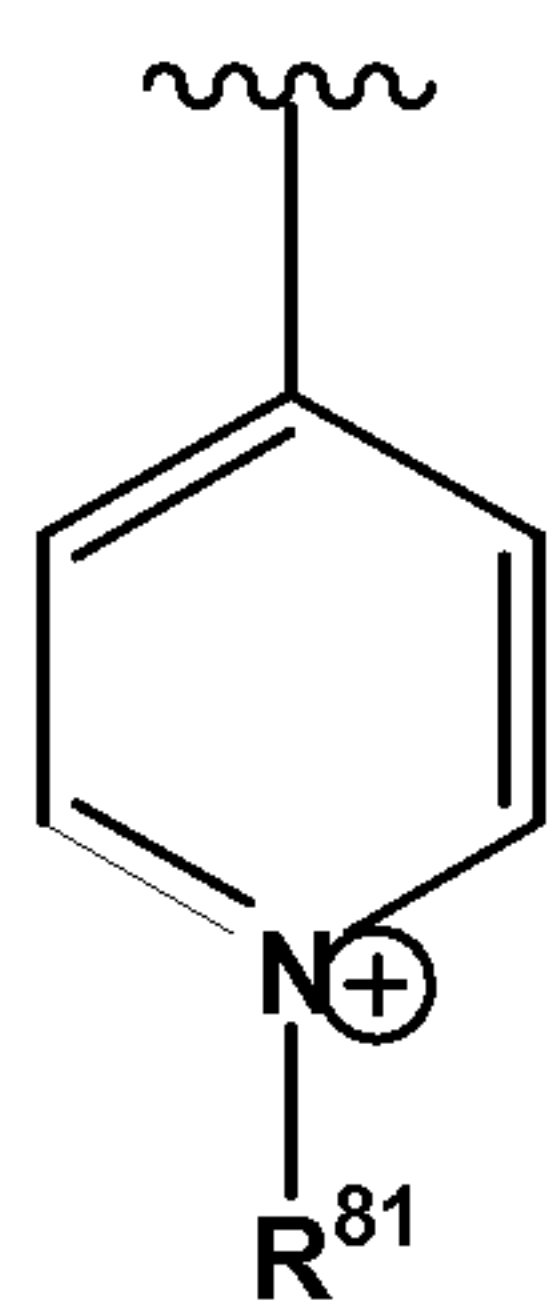
B is $-\text{[CH}_2\text{-C(R}^{11}\text{)(B}_{11}\text{)]-}$ wherein B₁₁ is $-\text{X}_{11}\text{-Y}_{11}\text{-Z}_{11}$, wherein

X₁₁ is carbonyl ($-\text{C(=O)-}$) or optionally substituted C₁₋₆ alkylene; or X₁₁ is absent;

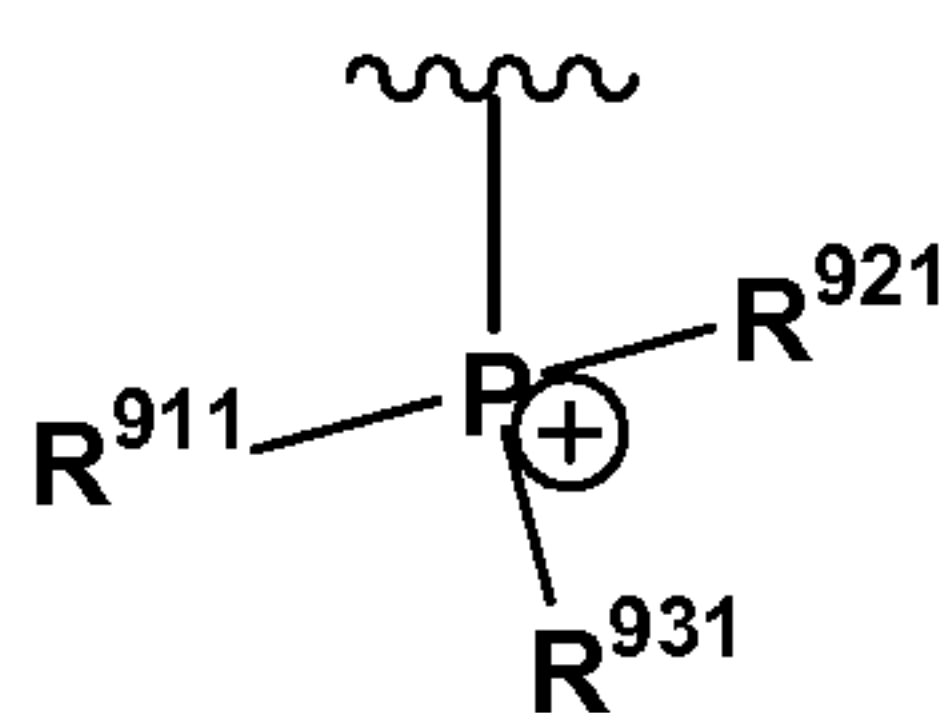
Y₁₁ is O, NH, or optionally substituted C₁₋₆ alkylene; or Y₁₁ is absent;

10 Z₁₁ is $-\text{Z}_{11A}\text{-Z}_{11B}$, wherein Z_{11A} is alkylene, arylene, or heteroarylene, any of which is optionally substituted; or Z_{11A} is absent; and Z_{11B} is -guanidino, -amidino, $-\text{N(R}^3\text{)(R}^4\text{)}$, or $-\text{N}^+(\text{R}^3\text{)(R}^4\text{)(R}^5\text{)}$, wherein R³, R⁴, and R⁵ are, independently, hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, heterocyclic, or aralkyl; or

Z₁₁ is pyridinium



15 , or phosphonium



wherein

R⁸¹, R⁹¹¹, R⁹²¹, and R⁹³¹ are, independently, hydrogen or alkyl;

R¹¹ is hydrogen or C₁₋₄ alkyl;

D is $-\text{[CH}_2\text{-C(R}^{21}\text{)(D}_{21}\text{)]-}$, wherein D₂₁ is $-\text{X}_{21}\text{-Y}_{21}\text{-Z}_{21}$, wherein

20 X₂₁ is carbonyl ($-\text{C(=O)-}$) or optionally substituted C₁₋₆ alkylene; or X₂₁ is absent;

Y₂₁ is O, NH, or optionally substituted C₁₋₆ alkylene, or Y₂₁ is absent;

Z₂₁ is alkyl, cycloalkyl, alkoxy, aryl, or aralkyl, any of which is optionally substituted;

R²¹ is hydrogen or C₁₋₄ alkyl;

m₁, the mole fraction of D monomer, is about 0.1 to about 0.9; and

25 n₁, the mole fraction of B monomer, is 1-m₁;

wherein the copolymer is a random copolymer of B and D monomers, and

wherein the copolymer has a degree of polymerization of about 5 to about 50;

and an ophthalmically or otically acceptable excipient.

US Application Publ. No. US 2006/0024264 A1 discloses random antimicrobial copolymers of Formula VI that can be used in the compositions of the present invention. Preferred ophthalmic or otic compositions comprise a random copolymer of Formula VI wherein:

- 5 A is C₁₋₄ alkoxy carbonyl(C₁₋₄)alkylthio;
- X₁₁ and X₂₁ are carbonyl;
- Y₁₁ and Y₂₁ are O;
- Z₁₁ is -Z_{11A}-Z_{11B}, wherein Z_{11A} is C₁₋₆ alkylene optionally substituted with C₁₋₄ alkyl or aryl; and Z_{11B} is -N(R³¹)(R⁴¹) or -N⁺(R³¹)(R⁴¹)(R⁵¹), wherein R³¹, R⁴¹, and R⁵¹ are, independently,
- 10 hydrogen C₁₋₄ alkyl;
- Z₂₁ is C₁₋₆ alkyl, C₁₋₆ aryl, or C₁₋₆ ar(C₁₋₄)alkyl;
- R¹¹ and R²¹ are independently hydrogen or methyl;
- m₁ is about 0.35 to about 0.60; and
- wherein the copolymer has a degree of polymerization of about 5 to about 10;
- 15 and an ophthalmically or otically acceptable excipient.

When any variable occurs more than one time in any constituent or in any of the polymers or oligomers recited for any of the general Formulae above (for example, in Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb, Formula IVc, Formula V, Formula Va, or Formula VI), its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

It is understood that the present invention encompasses the use of stereoisomers, diastereomers and optical isomers of the polymers and oligomers disclosed herein, as well as mixtures thereof, for use in the ophthalmic or otic compositions and methods of the present invention. Additionally, it is understood that stereoisomers, diastereomers and optical isomers of the disclosed polymers and oligomers, and mixtures thereof, are within the scope of the present invention. By way of non-limiting example, the mixture can be a racemate or the mixture may comprise unequal proportions of one particular stereoisomer over the other. Thus, in some aspects of the invention, the disclosed polymers and oligomers are provided as mixtures that are racemates. Additionally, the polymers and oligomers can be provided as a substantially pure stereoisomers, diastereomers and optical isomers. Thus, in some aspects of the invention, the polymers and oligomers in the compositions of the invention are provided as substantially pure stereoisomers, diastereomers, or optical isomers.

In other aspects of the present invention, the polymers and oligomers in the ophthalmic or otic compositions are provided in the form of an acceptable salt (for example, a pharmaceutically acceptable salt) for treating microbial infections. Polymer or oligomer salts can be provided for pharmaceutical use, or as an intermediate in preparing the pharmaceutically
5 desired form of the polymer or oligomer. One polymer or oligomer salt that is considered to be acceptable is the hydrochloride acid addition salt. Since one or more of the disclosed polymers and oligomers may be polyionic, such as a polyamine, the acceptable polymer or oligomer salt can be provided in the form of a poly(amine hydrochloride). Examples of other acceptable salts include, but are not limited to, those having sodium, potassium, or ammonium cations, and/or
10 those having chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate, bisulfite, mesylate, esylate, napsydisylate, tosylate, besylate, orthophosphate, acetate, gluconate, glutamate, lactate, malonate, fumarate, tartrate, maleate, or trifluoroacetate anions. In some embodiments, acceptable salts are those having mesylate, chloride, sulfate, esylate, napsydisylate, tosylate, besylate, phosphate, orthophosphate, acetate, gluconate, glutamate,
15 lactate, malonate, citrate, fumarate, tartrate, maleate, or trifluoroacetate anions. In other embodiments, acceptable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite, and ammonium sulfate.

In some aspects of the invention, the disclosed polymers and oligomers (such as the polymers and/or oligomers of Formulae I, II, IIa, IV, IVa, IVb, IVc, V, Va, and VI) are
20 derivatives referred to as prodrugs. The expression “prodrug” denotes a derivative of a known direct acting drug, which derivative has enhanced delivery characteristics and therapeutic value as compared to the drug, and is transformed into the active drug by an enzymatic or chemical process.

Unless otherwise defined, the terms below have the following meanings.

25 The term “alkyl” as used herein by itself or as part of another group refers to both straight and branched chain radicals from 1 to 12 carbons, such as methyl, ethyl, propyl, isopropyl, butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, and dodecyl.

The term “alkenyl” as used herein refers to a straight or branched chain radical of 2 to
30 20 carbon atoms, unless the chain length is limited thereto, including, but not limited to, ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, and the like. Preferably, the alkenyl chain is 2 to 10 carbon atoms in length, more preferably, 2 to 8 carbon atoms in length most preferably from 2 to 4 carbon atoms in length.

The term “alkynyl” as used herein refers to a straight or branched chain radical of 2 to 20 carbon atoms, unless the chain length is limited thereto, wherein there is at least one triple bond between two of the carbon atoms in the chain, including, but not limited to, acetylene, 1-propylene, 2-propylene, and the like. Preferably, the alkynyl chain is 2 to 10 carbon atoms in length, more preferably, 2 to 8 carbon atoms in length, most preferably from 2 to 4 carbon atoms in length.

The term “alkylene” as used herein refers to an alkyl linking group, for example, an alkyl group that links one group to another group in a molecule.

The term “alkoxy” as used herein refers to mean a straight or branched chain radical of 1 to 20 carbon atoms, unless the chain length is limited thereto, bonded to an oxygen atom, including, but not limited to, methoxy, ethoxy, n-propoxy, isopropoxy, and the like. Preferably the alkoxy chain is 1 to 10 carbon atoms in length, more preferably 1 to 8 carbon atoms in length, and even more preferred 1 to 6 carbon atoms in length.

The term “aryl” as used herein by itself or as part of another group refers to monocyclic or bicyclic aromatic groups containing from 6 to 12 carbons in the ring portion, preferably 6 to 10 carbons in the ring portion, such as the carbocyclic groups phenyl, naphthyl or tetrahydronaphthyl. The term “aryl” can represent carbocyclic aryl groups, such as phenyl, naphthyl or tetrahydronaphthyl, as well as heterocyclic aryl (“heteroaryl”) groups, such as pyridyl, pyrimidinyl, pyridazinyl, furyl, and pyranlyl.

The term “arylene” as used herein by itself or as part of another group refers to an aryl linking group, for example, an aryl group that links one group to another group in a molecule.

The term “cycloalkyl” as used herein by itself or as part of another group refers to cycloalkyl groups containing 3 to 9 carbon atoms, more preferably, 3 to 8 carbon atoms. Typical examples are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and cyclononyl.

The term “halogen” or “halo” as used herein by itself or as part of another group refers to chlorine, bromine, fluorine, or iodine.

The term “hydroxy” or “hydroxyl” as used herein by itself or as part of another group refers to an -OH group.

The term “heteroaryl” as used herein refers to groups having 5 to 14 ring atoms; 6, 10, or 14 π -electrons shared in a cyclic array; and containing carbon atoms and 1, 2, or 3 oxygen, nitrogen or sulfur heteroatoms. Examples of heteroaryl groups include, but are not limited to, thienyl, imadizolyl, oxadiazolyl, isoxazolyl, triazolyl, pyridyl, pyrimidinyl, pyridazinyl, furyl, pyranlyl, thianthrenyl, pyrazolyl, pyrazinyl, indoliziny, isoindolyl, isobenzofuranyl,

benzoxazolyl, xanthenyl, 2H-pyrrolyl, pyrrolyl, 3H-indolyl, indolyl, indazolyl, purinyl, 4H-quinoliziny, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinazolinyl, phenanthridinyl, acridinyl, perimidinyl, phenanthrolinyl, phenazinyl, isothiazolyl, phenothiazinyl, isoxazolyl, furazanyl, and phenoxazinyl groups. Especially preferred heteroaryl groups include 1,2,3-
5 triazole, 1,2,4-triazole, 5-amino-1,2,4-triazole, imidazole, oxazole, isoxazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 3-amino-1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, pyridine, and 2-aminopyridine.

The term “heteroarylene” as used herein by itself or as part of another group refers to a heteroaryl linking group, such as, a heteroaryl group that links one group to another group in a
10 molecule.

The term “heterocycle” or “heterocyclic ring”, as used herein except where noted, represents a stable 5- to 7-membered mono- or bicyclic or stable 7- to 10-membered bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from N, O, and S, and wherein the
15 nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. Especially useful are rings containing one oxygen or sulfur, one to three nitrogen atoms, or one oxygen or sulfur combined with one or two nitrogen atoms. The heterocyclic ring may be attached at any heteroatom or carbon atom which
20 results in the creation of a stable structure. Examples of such heterocyclic groups include, but are not limited to, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, pyrrolyl, 4-piperidonyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, imidazolyl, imidazolidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl,
25 quinuclidinyl, isothiazolidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, thiadiazoyl, benzopyranyl, benzothiazolyl, benzoxazolyl, furyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzothienyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl. Morpholino is the same as morpholinyl.

The term “alkylamino” as used herein by itself or as part of another group refers to an
30 amino group which is substituted with one alkyl group having from 1 to 6 carbon atoms. The term “dialkylamino” as used herein by itself or as part of an other group refers to an amino group which is substituted with two alkyl groups, each having from 1 to 6 carbon atoms.

The term “alkylthio” as used herein by itself or as part of an other group refers to a thio group which is substituted with one alkyl group having from 1 to 6 carbon atoms.

The term “lower acylamino” as used herein by itself or as part of an other group refers to an amino group substituted with a C₁-C₆ alkylcarbonyl group.

The term “chemically nonequivalent termini” as used herein refers to a functional group such as an ester, amide, sulfonamide, or N-hydroxyoxime that, when reversing the orientation of the functional group (for example, -(C=O)O-) produces different chemical entities (for example, -R¹C(=O)OR²- versus -R¹OC(=O)R²-).

The polymers and oligomers employed in the ophthalmic compositions of the present invention (e.g., the polymers and/or oligomers of Formulae I, II, IIa, IV, IVa, IVb, IVc, V, Va, and VI) can be prepared as described in the following patents and patent publications: US Published Patent Appl. Nos. US 2006-0041023 A1, US 2004-0202639 A1, US 2005-0287108 A1, and US 2006-0024264 A1, as well as US Patent No. 7,173,102. For example, US Pat. Appl. Publ. No. US 2006-0041023 A1 discloses methods for the design, synthesis, and testing of polymers and oligomers of Formulae I, II, IIa, IV, IVa, IVb, and IVc. US Pat. Appl. Publ. No. US 2005/0287108 A1 discloses methods for the design, synthesis, and testing of polymers and oligomers of Formula V and Formula Va.

Examples of the design, synthesis, and testing of arylamide oligomers, a subgroup of oligomers of Formula II and Formula IIa, are also presented in Tew et al., Proc. Natl. Acad. Sci. USA, 2002, 99, 5110-5114 and in WIPO Publication No. WO 2004/082634.

The oligomers can be synthesized by solid-phase synthetic procedures well known to those of skill in the art. See, for example, Tew et al., Proc. Natl. Acad. Sci. USA, 2002, 99, 5110-5114; Barany et al., Int. J. Pept. Prot. Res., 1987, 30, 705-739; Solid-phase Synthesis: A Practical Guide, Kates, S.A., and Albericio, F., eds., Marcel Dekker, New York (2000); and Dörwald, F.Z., Organic Synthesis on Solid Phase: Supports, Linkers, Reactions, 2nd Ed., Wiley-VCH, Weinheim (2002).

The ophthalmic or otic compositions can be tested for anti-microbial activity by methods known to those of skill in the art. For example, anti-microbial assays suitable for testing the antimicrobial activity of the ophthalmic or otic compositions of the invention are described, for example, US Pat. Appl. Publ. No. US 2006-0041023 A1; Tew et al., Proc. Natl. Acad. Sci. USA, 2002, 99, 5110-5114; and Liu et al., J. Amer. Chem. Soc., 2001, 123, 7553-7559.

Compositions

The ophthalmic and otic compositions of the present invention can take the form of a liquid or solid, including, e.g., but not limited to, a solution, a suspension, an emulsion, a gel, an ointment, or a solid article that can be inserted in a suitable location in the eye.

In some embodiments, a composition of the present invention is in the form of a liquid wherein the active agent (*i.e.*, one of the facially amphiphilic polymers or oligomers disclosed herein) is present in solution, in suspension, as an emulsion, or as a “solution/suspension.” The term “solution/suspension” as used herein refers to a liquid composition wherein a first portion
5 of the active agent is present in solution and a second portion of the active agent is present in particulate form, in suspension in a liquid matrix. In some embodiments, the liquid composition is in the form of a gel. In other embodiments, the liquid composition is aqueous. In other embodiments, the composition is in the form of an ointment.

In yet other embodiments, the composition is in the form of a solid article. For example,
10 in some embodiments, the ophthalmic composition is a solid article that can be inserted in a suitable location in the eye, such as between the eye and eyelid or in the conjunctival sac, where it releases the active agent as described, for example, U.S. Pat. No. 3,863,633; U.S. Pat. No. 3,867,519; U.S. Pat. No. 3,868,445; U.S. Pat. No. 3,960,150; U.S. Pat. No. 3,963,025; U.S. Pat. No. 4,186,184; U.S. Pat. No. 4,303,637; U.S. Pat. No. 5,443,505; and U.S. Pat. No. 5,869,079.
15 Release from such an article is usually to the cornea, either via the lacrimal fluid that bathes the surface of the cornea, or directly to the cornea itself, with which the solid article is generally in intimate contact. Solid articles suitable for implantation in the eye in such fashion are generally composed primarily of polymers and can be bioerodible or non-bioerodible. Bioerodible polymers that can be used in the preparation of ocular implants carrying one or more of the anti-
20 microbial, facially amphiphilic polymer or oligomer active agents in accordance with the present invention include, but are not limited to, aliphatic polyesters such as polymers and copolymers of poly(glycolide), poly(lactide), poly(epsilon-caprolactone), poly-(hydroxybutyrate) and poly(hydroxyvalerate), polyamino acids, polyorthoesters, polyanhydrides, aliphatic polycarbonates and polyether lactones. Suitable non-bioerodible polymers include silicone
25 elastomers.

The present invention provides anti-microbial ophthalmic or otic compositions comprising a polymer of an oligomer of Formula I, Formula II, Formula IIa, Formula IV, Formula IVa, Formula IVb, Formula IVc, Formula V, Formula Va, or Formula VI and an ophthalmically or otically acceptable excipient.

30 The polymer or oligomer is typically present in the ophthalmic or otic composition in an “effective amount” or “effective concentration.” The terms “effective amount,” “effective concentration,” or “amount effective,” as used herein in reference to a polymer or oligomer in a composition of the present invention, refers to the amount of the polymer or oligomer sufficient

to treat or prevent an ophthalmic infection in an eye of an animal, or to treat or prevent an otic infection in an ear of an animal.

The “effective amount” or concentration of the polymer or oligomer in the composition will vary and depends, among other factors, on the particular facially amphiphilic polymer or oligomer (active agent) being administered (e.g., on the relative antimicrobial activity of the specific polymer or oligomer); the mode of administration; the residence time provided by the particular formulation of the polymer or oligomer; the species, age and body weight of the subject; the intended use of the composition (e.g., treatment of existing infections or prevention of post-surgical infections); the particular condition for which treatment or prophylaxis is sought; and the severity of the condition.

The activity of antimicrobials is generally expressed as the minimum concentration of a compound (active agent) required to inhibit the growth of a specified pathogen. This concentration is also referred to as the “minimum inhibitory concentration” or “MIC.” The term “MIC₉₀” refers to the minimum concentration of an antimicrobial active agent required to inhibit the growth of ninety percent (90%) of the tested isolates for one particular organism. The concentration of a compound required to totally kill a specified bacterial species is referred to as the “minimum bactericidal concentration” or “MCB.”

The “effective amount” or concentration of the polymer or oligomer in the compositions of the invention will generally be an amount sufficient to provide a concentration on or in the affected eye or ear tissue equal to or greater than the MIC₉₀ level for the selected polymer or oligomer, relative to the microbes commonly associated with the infection. Thus, the “effective amount” or concentration of the polymer or oligomer in the ophthalmic or otic composition will generally be the amount of the polymer or oligomer sufficient to provide a concentration on or in the eye or ear tissue(s) equal to or greater than the MIC₉₀ level for the polymer or oligomer, relative to microbes commonly associated with the ophthalmic or otic infection.

Thus, for example, in the ophthalmic and otic compositions of the present invention, an effective concentration of the antimicrobial polymer or oligomer in the composition will generally be from about 0.01% to about 20% by weight (*i.e.*, wt%) of the composition. More typically, it will be about 0.05% to about 10% by weight, about 0.1% to about 8.0% by weight, about 0.5% to about 5.0% by weight, about 1.0% to about 5.0% by weight, or about 2.0% to about 4.0% of the composition. For example, in ophthalmic compositions in the form of solid suspensions, such as ointments, an effective concentration of the antimicrobial polymer or oligomer will generally be from about 1% to about 5% by weight (wt%) of the composition.

The ophthalmic and otic compositions of the invention are preferably sterile and have physical properties (e.g., osmolality and pH) that are specially suited for application to ophthalmic or otic tissues, including tissues that have been compromised as the result of preexisting disease, trauma, surgery or other physical conditions. For example, aqueous
5 compositions of the invention typically have a pH in the range of 4.5 to 8.0, more preferably, 6.0 to 8.0, or 6.5 to 8.0, or 7.0 to 8.0.

In addition to one or more of the polymers or oligomers disclosed herein, the ophthalmic or otic compositions of the invention can also comprise one or more ophthalmically or otically acceptable excipients.

10 The term “ophthalmically acceptable” as used herein means having no persistent detrimental effect on the treated eye or the functioning thereof, or on the general health of the subject being treated. However, it will be recognized that transient effects such as minor irritation or a “stinging” sensation are common with topical ophthalmic administration of drugs and the existence of such transient effects is not inconsistent with the composition, formulation,
15 or ingredient (e.g., excipient) in question being “ophthalmically acceptable” as herein defined. However, preferred ophthalmically acceptable compositions, formulations, and excipients are those that cause no substantial detrimental effect, even of a transient nature.

Similarly, the term “otically acceptable,” as used herein, means having no persistent detrimental effect on the treated ear or the functioning thereof, or on the general health of the
20 subject being treated. Preferred otically acceptable compositions, formulations, and excipients are those that cause no substantial detrimental effect, even of a transient nature.

Ophthalmically and otically acceptable excipients include, but are not limited to, viscosity-enhancing agents, preservatives, stabilizers, antioxidants, suspending agents, solubilizing agents, buffering agents, lubricating agents, ophthalmically or otically acceptable
25 salts, and combinations thereof.

For example, aqueous ophthalmic compositions of the present invention, when in suspension or solution form, are preferably viscous or mucoadhesive, or both viscous or mucoadhesive, and thus comprise a viscosity-enhancing agent. Examples of suitable viscosity-enhancing agents include, but are not limited to, glycerin, polyvinyl alcohol, polyvinyl
30 pyrrolidone, methylcellulose, hydroxypropylmethylcellulose, hydroxyethyl-cellulose, carboxymethylcellulose, hydroxypropylcellulose, and/or various gelling agents. For example, in some embodiments, the viscosity-enhancing agent is selected from methylcellulose, hydroxypropyl-methylcellulose, polyvinyl alcohol, and glycerol. Such agents are generally

employed in the compositions of the invention at a concentration of about 0.01% to about 3% by weight.

Thus, for ophthalmic compositions of the present invention, in some embodiments, the ophthalmically acceptable excipient is a viscosity-enhancing agent or a promoter of mucoadhesion, such as carboxymethylcellulose. In such embodiments, the concentration of carboxymethylcellulose in the aqueous suspension or solution is 0.1% to 5% by weight or about 0.1% to about 2.5% by weight. The carboxymethylcellulose is preferably in the form of sodium carboxymethylcellulose substituted to a degree that the sodium content of the sodium carboxymethylcellulose is about 1% to about 20%.

In other embodiments, the ophthalmic composition is an *in situ* gellable aqueous composition, more preferably, an *in situ* gellable aqueous solution. Such a composition comprises a gelling agent in a concentration effective to promote gelling upon contact with the eye or with lacrimal fluid in the exterior of the eye, enabling the composition to remain in the eye for a prolonged period without loss by lacrimal drainage. Suitable gelling agents non-restrictively include thermosetting polymers such as tetra-substituted ethylene diamine block copolymers of ethylene oxide and propylene oxide (e.g., poloxamine 1307); polycarbophil; and polysaccharides such as gellan, carrageenan (e.g., kappa-carrageenan and iota-carrageenan), chitosan and alginate gums.

The phrase “*in situ* gellable” as used herein is to be understood as embracing not only liquids of low viscosity that form gels upon contact with the eye or with lacrimal fluid in the exterior of the eye, but also more viscous liquids such as semi-fluid and thixotropic gels that exhibit substantially increased viscosity or gel stiffness upon administration to the eye.

For example, in some embodiments of the present invention, the ophthalmic composition is an *in situ* gellable aqueous solution, suspension or solution/suspension, comprising about 0.1% to about 6.5%, preferably about 0.5% to about 4.5%, by weight, based on the total weight of the composition, of one or more lightly cross-linked carboxyl-containing polymers as gelling agents. A preferred gelling agent in this embodiment is polycarbophil. In other embodiments, the composition is an *in situ* gellable aqueous solution, suspension or solution/suspension, preferably a solution, comprising about 0.1% to about 2% by weight of a polysaccharide that gels when it contacts an aqueous medium having the ionic strength of lacrimal fluid. A preferred polysaccharide is gellan gum, more preferably a low acetyl clarified grade of gellan gum such as that sold under the trademark Gelrite[®]. Suitable partially deacylated gellan gums are disclosed in U.S. Pat. No. 5,190,927.

In yet other embodiments, the composition is an *in situ* gellable aqueous solution, suspension or solution/suspension, comprising about 0.2% to about 3%, preferably about 0.5% to about 1%, by weight of a gelling polysaccharide, preferably selected from gellan gum, alginate gum and chitosan, and about 1% to about 50% of a water-soluble film-forming polymer, preferably selected from alkylcelluloses (e.g., methylcellulose, ethylcellulose), hydroxyalkylcelluloses (e.g., hydroxyethylcellulose, hydroxypropyl methylcellulose), hyaluronic acid and salts thereof, chondroitin sulfate and salts thereof, polymers of acrylamide, acrylic acid and polycyanoacrylates, polymers of methyl methacrylate and 2-hydroxyethyl methacrylate, polydextrose, cyclodextrins, polydextrin, maltodextrin, dextran, polydextrose, gelatin, collagen, natural gums (e.g., xanthan, locust bean, acacia, tragacanth and carrageenan gums and agar), polygalacturonic acid derivatives (e.g., pectin), polyvinyl alcohol, polyvinylpyrrolidone and polyethylene glycol. The composition can optionally contain a gel-promoting counterion such as calcium in latent form, for example encapsulated in gelatin.

In yet other embodiments, the composition is an *in situ* gellable aqueous solution, suspension or solution/suspension comprising about 0.1% to about 5% of a carrageenan gum, e.g., a carrageenan gum having no more than 2 sulfate groups per repeating disaccharide unit, such as e.g., kappa-carrageenan, having 18-25% ester sulfate by weight, iota-carrageenan, having 25-34% ester sulfate by weight, and mixtures thereof.

In still other embodiments, the composition comprises a bioerodible polymer substantially as disclosed in U.S. Pat. No. 3,914,402.

In some embodiments, the composition comprises an ophthalmically acceptable mucoadhesive polymer, selected, for example, from hydroxypropylmethylcellulose, carboxymethylcellulose, carbomer (acrylic acid polymer), poly(methylmethacrylate), polyacrylamide, polycarbophil, polyethylene oxide, acrylic acid/butyl acrylate copolymer, sodium alginate, and dextran.

Ophthalmic compositions of the invention preferably incorporate means to inhibit microbial growth, for example through preparation and packaging under sterile conditions and/or through inclusion of an antimicrobially effective amount of an ophthalmically acceptable preservative.

Suitable preservatives include, but are not limited to, mercury-containing substances such as phenylmercuric salts (e.g., phenylmercuric acetate, borate and nitrate) and thimerosal; stabilized chlorine dioxide; quaternary ammonium compounds such as benzalkonium chloride, cetyltrimethylammonium bromide and cetylpyridinium chloride; imidazolidinyl urea; parabens such as methylparaben, ethylparaben, propylparaben and butylparaben, and salts thereof;

phenoxyethanol; chlorophenoxyethanol; phenoxypropanol; chlorobutanol; chlorocresol; phenylethyl alcohol; disodium EDTA; and sorbic acid and salts thereof.

Several preservatives may precipitate in the presence of other excipients in the composition and/or in the presence of the polymers and oligomers in the ophthalmic compositions of the present invention. For example, benzalkonium chloride can precipitate in a composition using iota-carrageenan as a gelling agent. Thus, in those embodiments of the invention in which a preservative is present, the preservative is one that does not precipitate but remains in solution in the composition.

Optionally one or more stabilizers can be included in the compositions of the invention to enhance chemical stability where required. Suitable stabilizers include, but are not limited to, chelating agents or complexing agents, such as, for example, the calcium complexing agent ethylene diamine tetraacetic acid (EDTA). For example, an appropriate amount of EDTA or a salt thereof, e.g., the disodium salt, can be included in the composition to complex excess calcium ions and prevent gel formation during storage. EDTA or a salt thereof can suitably be included in an amount of about 0.01% to about 0.5%. In those embodiments containing a preservative other than EDTA, the EDTA or a salt thereof, more particularly disodium EDTA, can be present in an amount of about 0.025% to about 0.1% by weight.

One or more antioxidants can also be included in the ophthalmic compositions of the invention. Suitable antioxidants include ascorbic acid, sodium metabisulfite, polyquaternium-1, benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, or other agents known to those of skill in the art. Such preservatives are typically employed at a level of from about 0.001% to about 1.0% by weight. In some embodiments of the present invention, the facially amphiphilic polymer(s) or oligomer(s) of the compositions are solubilized at least in part by an ophthalmically acceptable solubilizing agent. The term "solubilizing agent" herein includes agents that result in formation of a micellar solution or a true solution of the drug. Certain ophthalmically acceptable nonionic surfactants, for example polysorbate 80, can be useful as solubilizing agents, as can ophthalmically acceptable glycols, polyglycols, e.g., polyethylene glycol 400 (PEG-400), and glycol ethers.

Particularly preferred solubilizing agents for solution and solution/suspension compositions of the invention are cyclodextrins. Suitable cyclodextrins can be selected from α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, alkylcyclodextrins (e.g., methyl- β -cyclodextrin, dimethyl- β -cyclodextrin, diethyl- β -cyclodextrin), hydroxyalkylcyclodextrins (e.g., hydroxyethyl- β -cyclodextrin, hydroxypropyl- β -cyclodextrin), carboxy-alkylcyclodextrins (e.g.,

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carboxymethyl- β -cyclodextrin), sulfoalkylether cyclodextrins (e.g., sulfobutylether- β -cyclodextrin), and the like. Ophthalmic applications of cyclodextrins have been reviewed in Rajewski et al., Journal of Pharmaceutical Sciences, 1996, 85, 1155-1159.

5 An ophthalmically acceptable cyclodextrin can optionally be present in an ophthalmic composition of the invention at a concentration of about 1 to about 200 mg/ml, preferably about 5 to about 100 mg/ml and more preferably about 10 to about 50 mg/ml.

In some embodiments, the ophthalmic composition optionally contains a suspending agent. For example, in those embodiments in which the ophthalmic composition is an aqueous suspension or solution/suspension, the composition can contain one or more polymers as
10 suspending agents. Useful polymers include water-soluble polymers such as cellulosic polymers, for example, hydroxypropyl methylcellulose, and water-insoluble polymers such as cross-linked carboxyl-containing polymers. However, preferred ophthalmic compositions of the invention do not contain substantial amounts of solid particulate matter, whether of the anti-microbial polymer or oligomer active agent, an excipient, or both, as solid particulate matter, if present, can cause
15 discomfort and/or irritation of a treated eye.

One or more ophthalmically acceptable pH adjusting agents and/or buffering agents can be included in the ophthalmic compositions of the invention, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-
20 hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an ophthalmically acceptable range.

One or more ophthalmically acceptable salts can be included in the compositions of the invention in an amount required to bring osmolality of the composition into an ophthalmically
25 acceptable range. Such salts include, but are not limited to, those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; preferred salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate, with sodium chloride being especially preferred.

30 Optionally an ophthalmically acceptable xanthine derivative such as caffeine, theobromine or theophylline can be included in the compositions of the invention, e.g., as disclosed in U.S. Pat. No. 4,559,343. Inclusion of the xanthine derivative can reduce ocular discomfort associated with administration of the composition.

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Optionally one or more ophthalmically acceptable surfactants, preferably nonionic surfactants, or co-solvents can be included in the compositions of the invention to enhance solubility of the components of the compositions or to impart physical stability, or for other purposes. Suitable nonionic surfactants include, but are not limited to, polyoxyethylene fatty acid
5 glycerides and vegetable oils, e.g., polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, e.g., octoxynol 10, octoxynol 40; polysorbate 20, 60 and 80; polyoxyethylene/polyoxypropylene surfactants (e.g., Pluronic® F-68, F84 and P-103); cyclodextrin; or other agents known to those of skill in the art. Typically, such co-solvents or surfactants are employed in the compositions at a level of from about 0.01% to
10 about 2% by weight.

One or more ophthalmic lubricating agents can also be included optionally in the compositions of the invention to promote lacrimation or as a “dry eye” medication. Such agents include, but are not limited to, polyvinyl alcohol, methylcellulose, hydroxypropyl
methylcellulose, polyvinylpyrrolidone, and the like. It will be understood that promotion of
15 lacrimation is beneficial in the present invention only where lacrimation is naturally deficient, to restore a normal degree of secretion of lacrimal fluid. Where excessive lacrimation occurs, residence time of the composition in the eye can be reduced.

Ophthalmic compositions of the present invention typically include a combination of one or more of the optional excipients listed above. For example, in some embodiments of the
20 invention, the ophthalmic composition can optionally further comprise glycerin in an amount of about 0.5% to about 5%, more preferably about 1% to about 2.5%, for example about 1.5% to about 2%, by weight. Glycerin can be useful to increase viscosity of the composition and for adjustment of osmolality. Independently of the presence of glycerin, the composition can also further comprise a cyclodextrin, preferably hydroxypropyl- β -cyclodextrin, in an amount of about
25 0.5% to about 25% by weight, as a solubilizing agent, and an antimicrobially effective amount of a preservative, e.g., imidazolidinyl urea in an amount of about 0.03% to about 0.5%; methylparaben in an amount of about 0.015% to about 0.25%; propylparaben in an amount of about 0.005% to about 0.01%; phenoxyethanol in an amount of about 0.25% to about 1%; disodium EDTA in an amount of about 0.05% to about 0.2%; thimerosal in an amount of 0.001%
30 to about 0.15%; chlorobutanol in an amount of about 0.1% to about 0.5%; and/or sorbic acid in an amount of about 0.05% to about 0.2%; all by weight.

The otic compositions of the present invention also optionally comprise one or more otically acceptable excipients. Otically acceptable excipients include, but are not limited to, one or more of the preservatives, stabilizers, antioxidants, viscosity-enhancing agents, buffering

agents, solubilizing agents, surfactants, lubricating agents, or acceptable salts described above, or combinations thereof, as described above for the ophthalmic compositions of the invention.

Thus, for example, in some embodiments, an otic composition of the present invention optionally comprises one or more buffering agents, solubilizing agents, and antioxidants,
5 typically in an aqueous solution. In some embodiments, the otic composition further comprises glycerin (e.g., anhydrous glycerin) or propylene glycol as a viscosity-enhancing agent. The otic composition may also comprise a surfactant in combination with the glycerin or propylene glycol to aid in the removal of cerum (ear wax). Sodium bicarbonate may also be used if wax is to be removed from the ear.

10 Thus, e.g., in some embodiments, the otic composition of the present invention is a sterile aqueous solution comprising one or more of the disclosed polymers or oligomers, glycerin, sodium bicarbonate, and, optionally, a preservative, in purified water.

The ophthalmic and otic compositions of the present invention can be prepared by methods known in the art and described in patents and publications cited herein and incorporated
15 herein by reference.

Methods of Treatment and Administration

The ophthalmic or otic compositions of the present invention possess anti-microbial activity and can be used in methods of treating or preventing ophthalmic infections in an eye of
20 an animal, or otic infections in the ear of an animal.

The term “animal” as used herein includes, but is not limited to, humans and non-human vertebrates such as wild, domestic and farm animals. Preferably, the animal is a warm-blooded, mammalian subject, including, but not limited to, domestic, farm and exotic mammals, and humans. The methods of the present invention can be useful, for example, in the treatment of
25 eye infections in dogs, cats, horses, cattle, sheep and/or pigs, but is more particularly useful where the subject is human.

The phrases “treating an ophthalmic infection” and “treatment of an ophthalmic infection” refer to both the prevention and the therapeutic treatment, e.g., the alleviation or amelioration, of an ophthalmic infection, wherein the object is to prevent or slow down (lessen)
30 the progress of an ophthalmic infection, or obtain beneficial or desired clinical results. For example, “beneficial or desired clinical results” include, but are not limited to, alleviation of the symptoms of an ophthalmic infection; diminishment of the extent of an ophthalmic infection; stabilization (for example, not worsening) of the state of an ophthalmic infection; delay in the onset or the slowing of an ophthalmic infection or its progression; amelioration of an ophthalmic

infection or remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of an ophthalmic infection. Treatment includes eliciting a clinically significant response without excessive levels of side effects.

Similarly, the phrases “treating an otic infection” and “treatment of an otic infection”
5 refer to both the prevention and the therapeutic treatment, e.g., the alleviation or amelioration, of an otic infection, wherein the object is to prevent or slow down (lessen) the progress of an otic infection, or obtain beneficial or desired clinical results. For example, “beneficial or desired clinical results” include, but are not limited to, alleviation of the symptoms of an otic infection; diminishment of the extent of an otic infection; stabilization (for example, not worsening) of the
10 state of an otic infection; delay in the onset or the slowing of an otic infection or its progression; amelioration of an otic infection or remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of an otic infection. Treatment includes eliciting a clinically significant response without excessive levels of side effects.

Ophthalmic infections for which the compositions and methods of the present invention
15 are useful include, but are not limited to, infections of one or more tissues of the eye, including, for example, conjunctivitis, keratitis (including ulcerative keratitis with bacterial infection), keratoconjunctivitis (including, e.g., keratoconjunctivitis sicca (KCS) commonly found in dogs), blepharitis, blepharoconjunctivitis, dacryocystitis, hordeolum, corneal ulcers, orbital and preseptal cellulitis, and endophthalmitis

20 In preferred methods of the invention, the infected tissue is one that is directly bathed by the lacrimal fluid, as in conjunctivitis, keratitis, keratoconjunctivitis, blepharitis, and blepharoconjunctivitis.

The ophthalmic compositions of the present invention may also be used prophylactically in connection with various ophthalmic surgical procedures that create a risk of
25 infection.

Otic infections for which the compositions and methods of the present invention are useful include, but are not limited to, otitis externa and otitis media. With respect to the treatment of otitis media, the compositions of the present invention are primarily useful in cases where the tympanic membrane has ruptured or tympanostomy tubes have been implanted. The otic
30 compositions may also be used to treat infections associated with otic surgical procedures, such as tympanostomy, or to prevent such infections.

The ophthalmic and otic compositions of the invention are effective in killing or inhibiting the growth of a broad spectrum of pathogens or microbes often associated with

ophthalmic and/or otic infections, including a range of bacteria (both gram-positive and gram-negative), fungi and viruses.

For example, the ophthalmic and otic compositions are useful in killing or inhibiting the growth of any of the following clinically relevant ocular or otic pathogens, and can be
5 administered topically to treat and/or prevent ophthalmic or otic infections caused by the following pathogens or mixtures of the following pathogens: *Staphylococcus* spp. (e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*), *Streptococcus* spp. (e.g., *Streptococcus viridans*, *Streptococcus pneumoniae*), *Enterococcus* spp., *Bacillus* spp., *Corynebacterium* spp., *Propionibacterium* spp., *Chlamydia* spp., *Moraxella* spp. (e.g., *Moraxella lacunata* and
10 *Moraxella catarrhalis*), *Haemophilus* spp. (e.g., *Haemophilus influenza* and *Haemophilus aegyptius*), *Pseudomonas* spp. (e.g., *Pseudomonas aeruginosa*, and, for otic infections, *Pseudomonas otitidis*), *Serratia* spp. (e.g., *Serratia marcescens*), *Neisseria* spp., and *Mycoplasma* spp., as well as *Enterobacter* spp. (e.g., *Enterobacter aerogenes*), *Escherichia* spp. (e.g., *Escherichia coli*), *Klebsiella* spp. (e.g., *Klebsiella pneumoniae*), *Proteus* spp. (e.g., *Proteus*
15 *mirabilis* and *Proteus vulgaris*), *Acinetobacter* spp. (e.g., *Acinetobacter calcoaceticus*), *Prevotella* spp., *Fusobacterium* spp., *Porphyromonas* spp., and *Bacteroides* spp. (e.g., *Bacteroides fragilis*). This list of microbes is purely illustrative and is in no way to be interpreted as restrictive.

Thus, for example, the ophthalmic compositions of the present invention can be
20 administered to treat or prevent a bacterial infection of the eye caused by one or more of the following species: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Enterococcus faecalis*, *Corynebacterium* spp., *Propionibacterium* spp., *Moraxella catarrhalis* and *Haemophilus influenzae*.

25 For example, treatment of bacterial conjunctivitis by administering an ophthalmic composition of the present invention is appropriate where infection with one or more of the following species is present: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Enterococcus faecalis*, *Corynebacterium* spp., *Propionibacterium* spp., *Moraxella catarrhalis* and *Haemophilus*
30 *influenzae*.

Similarly, treatment of bacterial blepharitis by administering an ophthalmic composition of the present invention is appropriate where infection with one or more of the following species is present: *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pneumoniae*. Treatment of bacterial keratitis by administering an ophthalmic composition of the present

invention is also appropriate where infection with one or more of the following species is present: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae* and *Streptococcus viridans*.

The otic compositions of the present invention, for example, can also be administered to
5 treat or prevent a bacterial infection of the ear caused by one or more of the following species: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Pseudomonas otitidis*, and *Proteus* spp. (e.g., *Proteus mirabilis* and *Proteus vulgaris*), as well as one or more of the following anaerobes: *Prevotella* spp., *Fusobacterium* spp., *Porphyromonas* spp., and *Bacteroides* spp. (e.g., *Bacteroides fragilis*).
10 Thus, for example, treatment of chronic suppurative otitis media by administering an otic composition of the present invention is appropriate where infection with one or more of the following species is present: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp. (e.g., *Klebsiella pneumoniae*), *Proteus* spp. (e.g., *Proteus mirabilis* and *Proteus vulgaris*), *Prevotella* spp., *Fusobacterium* spp., *Porphyromonas* spp., and *Bacteroides* spp. (e.g.,
15 *Bacteroides fragilis*).

The ophthalmic or otic compositions are also useful in killing or inhibiting the growth of clinically relevant ocular or otic fungi, and can be administered topically to treat and/or prevent ophthalmic or otic infections caused by one or more species of fungi, or a mixture of species of fungi, including, but not limited to, *Aspergillus* spp. (e.g., *Aspergillus fumigatus*,
20 *Aspergillus favus*, *Aspergillus niger* and *Aspergillus terreus*), *Fusarium* spp. (e.g., *Fusarium solani*, *Fusarium moniliforme* and *Fusarium proliferatum*), *Malessezia* spp. (e.g., *Malessezia pachydermatis*), and/or *Candida* spp. (e.g., *Candida albicans*), as well as *Chrysosporium parvum*, *Metarhizium anisopliae*, *Phaeoisaria clematidis*, and *Sarcopodium oculorum*. This list of microbes is purely illustrative and is in no way to be interpreted as restrictive.

25 Thus, the ophthalmic compositions of the present invention can be administered to treat or prevent a fungal infection of the eye caused by one or more of the following species: *Aspergillus* spp., *Fusarium* spp., *Chrysosporium parvum*, *Metarhizium anisopliae*, *Phaeoisaria clematidis*, and *Sarcopodium oculorum*. For example, the ophthalmic composition can be administered to treat fungal keratitis caused by one or more *Aspergillus* spp. and/or *Fusarium*
30 spp.

The otic compositions of the present invention, for example, can also be administered to treat or prevent a fungal infection of the ear caused by one or more of the following species: *Candida* spp., *Aspergillus* spp., and/or *Malessezia* spp. (e.g., *Malessezia pachydermatis*).

The ophthalmic or otic compositions are also useful in killing or inhibiting the growth of clinically relevant ocular or otic viruses and can be administered topically to treat and/or prevent ophthalmic or otic infections caused by one or more viruses, including, but not limited to, adenoviruses and herpes viruses (including, e.g., Herpes simplex 1 virus and/or varicella-
5 zoster virus), Enteroviruses and Cytomegaloviruses.

Thus, for example, the ophthalmic compositions of the present invention can be administered to treat or prevent a viral infection of the eye, e.g., Herpes keratitis, caused by Herpes simplex 1 virus.

In some embodiments, the ophthalmic or otic compositions of the invention are useful
10 and effective in killing and/or preventing the growth of microbes that have developed significant levels of resistance to anti-microbial agents other than the disclosed polymers and oligomers. For example, in some embodiments, the ophthalmic compositions and otic compositions are especially effective in methods of treating ophthalmic infections or otic infections caused by bacterial strains that have developed resistance to ciprofloxacin, e.g., Ciprofloxacin Resistant
15 (CR) *S. aureus* and CR *S. epidermidis*, or to fluoroquinolone, or bacterial strains that have developed resistance to penicillin.

In some embodiments, the compositions of the invention are administered topically to one or more tissues of the eye or ear to treat an existing microbial infection, or as a prophylactic measure to prevent a microbial infection.

20 Thus, for example, in some embodiments, an ophthalmic composition of the present invention is administered topically to one or more tissues of the eye to treat an existing microbial infection, e.g., conjunctivitis, keratitis, blepharitis, or blepharoconjunctivitis.

In other embodiments, an ophthalmic composition of the present invention is administered topically to one or more tissues of the eye as a prophylactic measure. That is, the
25 compositions are administered for prophylactic uses, e.g., in connection with various ophthalmic surgical procedures that create a risk of infection. Thus, for example, a composition of the invention can be administered in a method of post-traumatic prophylaxis, especially post-surgical prophylaxis, to prevent infection after ocular surgery, or in a method of prophylaxis prior to ocular surgery, for example, administered prior to surgery to prevent infection as a
30 consequence of surgery.

The ophthalmic and otic compositions of the present invention possess broad-spectrum anti-microbial activity due to the facially amphiphilic and cationic properties of the facially amphiphilic polymers and oligomers in the compositions. As a consequence, an ophthalmic infection or an otic infection can be treated or prevented by administering only one of the

compositions of the present invention, rather than by administering two or more separate antimicrobial compositions or one antimicrobial composition containing a combination of antimicrobial agents.

For example, because the ophthalmic compositions of the invention can be used to treat
5 or prevent both viral and bacterial ophthalmic infections in an eye, only one of the present compositions needs to be administered to the eye to treat a viral ophthalmic infection where there is a risk of a secondary bacterial infection. Similarly, for an eye infection caused by multiple strains of bacteria (e.g., by both gram-positive bacteria and gram-negative bacteria), only one composition containing one of the disclosed amphiphilic oligomers needs to be administered,
10 rather than a composition containing multiple anti-microbial agents, or a combination of separate treatments administered concurrently.

In some embodiments, the ophthalmic or otic compositions of the present invention are administered with an additional anti-microbial agent, such as, e.g., an anti-bacterial, anti-fungal, or anti-viral agent. For example, the additional anti-microbial agent can be a second facially
15 amphiphilic polymer or oligomer disclosed herein, or the additional anti-microbial agent can be another anti-microbial agent such as, for example, an antibiotic selected from the group consisting of aminoglycosides, cephalosporins, diaminopyridines, fluoroquinolones, sulfonamides and tetracyclines. Examples of useful antibiotics which can serve as additional anti-microbials include, but are not limited to, amikacin, azithromycin, cefixime, cefoperazone,
20 cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, colistin, domeclocycline, doxycycline, erythromycin, gentamicin, mafenide, methacycline, minocycline, neomycin, norfloxacin, ofloxacin, oxytetracycline, polymyxin B, pyrimethamine, silver sulfadiazine, sulfacetamide, sulfisoxazole, tetracycline, tobramycin, and trimethoprim.

In those embodiments in which the ophthalmic or otic composition is administered with
25 another anti-microbial agent, the present invention provides a method of treating or preventing multiple bacterial infections in an eye or an ear, the method comprising application to the eye or ear in co-therapy (including co-formulation) one or more facially amphiphilic polymers or oligomers disclosed herein and one or more additional anti-microbial agents. "Co-therapy" herein means administration to the eye or ear, at the same time or sequentially, of an
30 ophthalmically or otically acceptable composition comprising one or more of the facially amphiphilic polymers or oligomers disclosed herein and a separate ophthalmically or otically acceptable composition of the additional anti-microbial agent, in a treatment regimen intended to provide a beneficial effect from co-action of the two types of antimicrobial agents. "Co-formulation" herein means that the facially amphiphilic polymer or oligomer active agent and the

additional anti-microbial agent are administered to the eye or ear as components of a single ophthalmically or otically acceptable composition.

The ophthalmic or otic compositions of the present invention also can be used in co-therapy with one or more drugs, or medicaments, other than anti-microbial agents. Such medicaments other than anti-microbial agents can be co-administered to the eye or ear together with a composition of the invention. Thus, e.g., an ophthalmic composition of the present invention can further comprise, in co-formulation with the facially amphiphilic polymer or oligomer active agent, a therapeutically and/or prophylactically effective amount of one or more medicaments that are other than anti-microbial agents.

These additional medicaments other than anti-microbial agents can cooperate with the anti-microbial facially amphiphilic polymer or oligomer active agent(s) in treating and/or preventing an infective disease of the eye or ear, or can be used to treat a related or unrelated condition simultaneously affecting the eye or ear.

Any medicament having utility in an ophthalmic or otic application can be used in co-therapy, co-administration or co-formulation with an ophthalmic or otic composition of the present invention as described above. Such additional medicaments include, but are not limited to, anti-inflammatory agents (e.g., steroidal anti-inflammatory agents, non-steroidal anti-inflammatory agents (NSAIDs), and selective cyclooxygenase-2 inhibitors); topical and/or regional anesthetic agents; anti-allergic agents (e.g., anti-histamines); demulcents; acetylcholine blocking agents; adrenergic agonists, beta-adrenergic blocking agents and other anti-glaucoma agents; anti-hypertensives; and anti-cataract agents.

For example, ophthalmic and otic infections are frequently accompanied by inflammation of the infected ophthalmic and/or otic tissues and surrounding tissues. In addition, ophthalmic and otic surgical procedures that create a risk of microbial infections frequently also causes inflammation of the affected tissues. Thus, the ophthalmic and otic compositions of the present invention can be co-formulated with an anti-inflammatory agent to combine the anti-infective activity of one or more antibiotics with the anti-inflammatory activity of one or more steroid or non-steroid agents in a single composition.

The anti-inflammatory agents can be steroidal or non-steroidal. Examples of suitable steroidal anti-inflammatory agents include, but are not limited to, dexamethasone; dexamethasone derivatives such as those disclosed in US pat. No. 5,223,492; rimexolone; prednisolone; fluorometholone; and hydrocortisone.

Examples of suitable non-steroidal anti-inflammatory agents include, but are not limited to, prostaglandin H synthetase inhibitors (Cos I or Cox II), also referred to as cyclooxygenase

type I and type II inhibitors, such as diclofenac, flurbiprofen, ketorolac, suprofen, nepafenac, amfenac, indomethacin, naproxen, ibuprofen, bromfenac, ketoprofen, meclofenamate, piroxicam, sulindac, mefanamic acid, diflusal, oxaprozin, tolmetin, fenoprofen, benoxaprofen, nabumetome, etodolac, phenylbutazone, aspirin, oxyphenbutazone, tenoxicam and carprofen; cyclooxygenase type II selective inhibitors, such as viox, celecoxib, etodolac; PAF antagonists, such as apafant, bepafant, minopafant, nupafant and modipafant; PDE IV inhibitors, such as ariflo, torbafylline, rolipram, fluticasone, piclamilast, cilomafylline, and roflumilast; inhibitors of cytokine production, such as inhibitors of the NFkB transcription factor; or other anti-inflammatory agents known to those skilled in the art.

Examples of suitable topical or regional anesthetic agents include, but are not limited to, benzocaine.

Examples of suitable anti-allergic agents include, but are not limited to, pemirolast, olopatadine, and the corticosteroids (prednisolone, fluorometholone, loteprenol and dexamethasone).

The additional medicament can be administered in co-therapy (including co-formulation) with the one or more facially amphiphilic polymers of the ophthalmic or otic composition. For example, in some embodiments, an ophthalmic composition of the present invention comprising one of the anti-microbial oligomers disclosed herein is administered in co-therapy with an anti-inflammatory agent, e.g., a glucocorticoid. The glucocorticoid can be co-formulated with the oligomer in a single ophthalmically acceptable composition, which is administered to one or more tissues of an eye, to not only treat or prevent an ophthalmic infection but also to treat and/or prevent inflammation.

The ophthalmic or otic compositions can be administered by any appropriate route of administration. In some aspects of the invention, the ophthalmic and otic compositions are administered topically, for example, the composition is topically administered in an antimicrobially effective amount to one or more tissues of the eye of the animal, or to one or more tissues of the ear of an animal.

An appropriate dosage, frequency and duration of administration, for example, treatment regimen, to be used in any particular situation will be readily determined by one of skill in the art without undue experimentation, and will depend, among other factors, on the particular polymer(s) or oligomer(s) present in the composition, on the particular ophthalmic infection being treated, on the age, weight and general physical condition of the subject, and on other medication being administered to the subject. It is preferred that response of the ophthalmic

or otic infection to treatment according to the present methods be monitored and the treatment regimen be adjusted if necessary in light of such monitoring.

Frequency of administration is typically such that the dosing interval, for example, the period of time between one dose and the next, during waking hours is about 2 to about 12 hours, more typically about 3 to about 8 hours, for example about 4 to about 6 hours. It will be understood by those of skill in the art that an appropriate dosing interval is dependent to some degree on the length of time for which the selected composition is capable of maintaining a concentration of the anti-microbial polymer(s) or oligomer(s) in the lacrimal fluid and/or in the target tissue (e.g., the conjunctiva) above the MIC₉₀ (the minimum concentration of the oligomer or polymer which inhibits microbial growth by 90%). Ideally the concentration remains above the MIC₉₀ for at least 100% of the dosing interval. Where this is not achievable it is desired that the concentration should remain above the MIC₉₀ for at least about 60% of the dosing interval, in a worst case at least about 40% of the dosing interval.

For example, in some embodiments of the ophthalmic compositions of the invention, the ophthalmic composition is formulated as an *in situ* gellable aqueous liquid and is administered as eye drops. Typically each drop, generated by a conventional dispensing means, has a volume of about 10 to about 40 μ L. From 1 to about 6 such drops typically provides a suitable dose of the oligomer active agent in about 25-150 μ L of the composition. For example, preferably no more than 3 drops, more preferably no more than 2 drops, and most preferably no more than 1 drop, should contain the desired dose of the active agent for administration to an eye. Where the composition is administered in a form other than eye drops, for example, as an ophthalmic ointment or as a solid implant, an equivalent dose is provided. Such a dose can be administered as needed, but typically administration to the eye 1 to about 6 times per day, in most cases 2 to 4 times a day, provides adequate continuing relief or prevention of the infective disease indicated.

The ophthalmic compositions of the invention, e.g., the aqueous suspension compositions, can be packaged in single-dose non-reclosable containers. Such containers can maintain the composition in a sterile condition and thereby eliminate need for preservatives such as mercury-containing preservatives, which can sometimes cause irritation and sensitization of the eye. Alternatively, multiple-dose reclosable containers can be used, in which case it is preferred to include a preservative in the composition.

For example, in some embodiments, the ophthalmic composition is an aqueous solution, suspension or solution/suspension which is administered in the form of eye drops. In these embodiments, a desired dosage of the active agent can be administered by means of a suitable

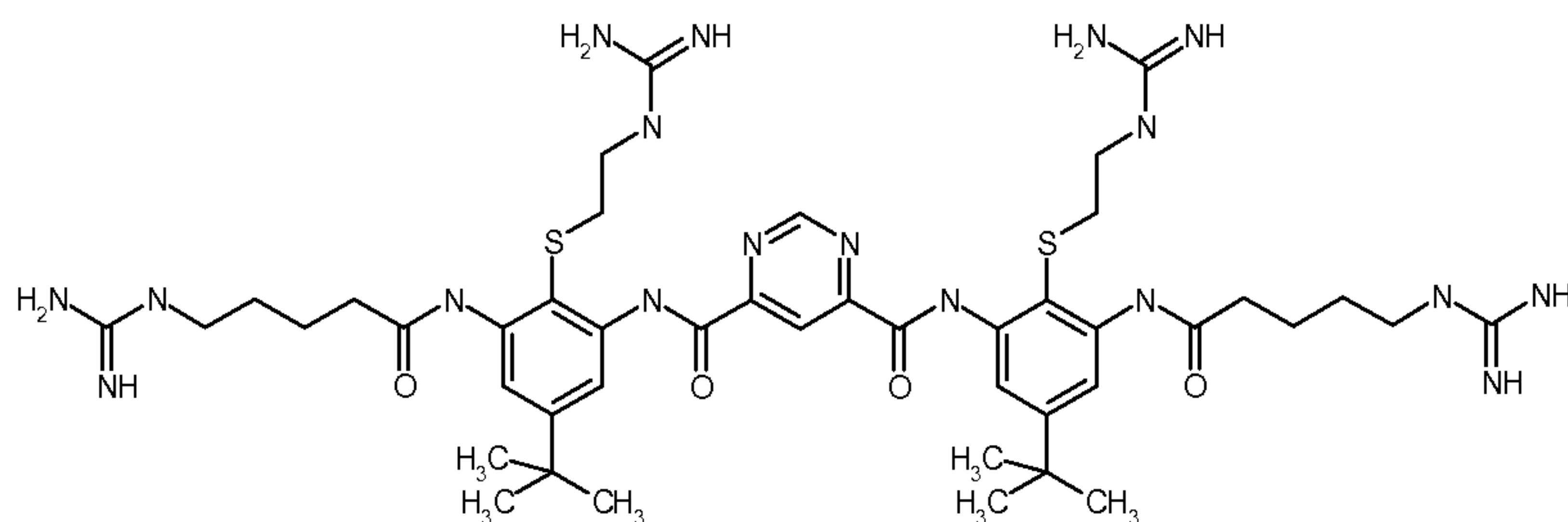
dispenser as a known number of drops into the eye. Examples of suitable dispensers are disclosed in International Patent Publication No. WO 96/06581.

The following examples will serve to further typify the nature of this invention but should not be construed as a limitation in the scope thereof, which scope is defined solely by the appended claims. In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner.

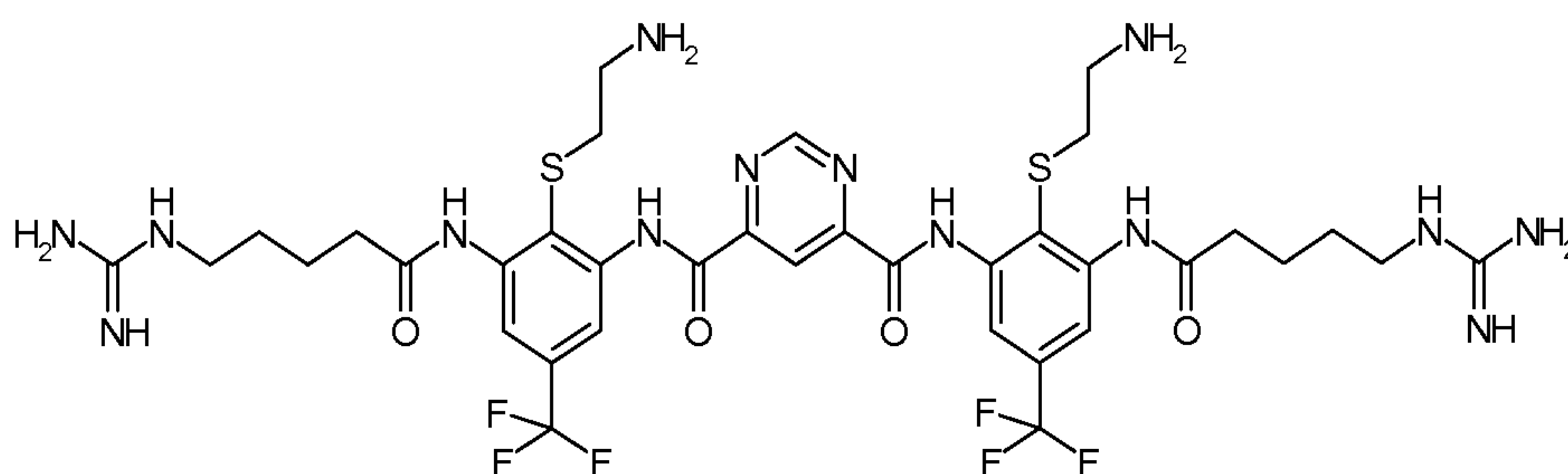
EXAMPLES

10 Example 1: Antimicrobial Activity - Minimum Inhibitory Concentrations

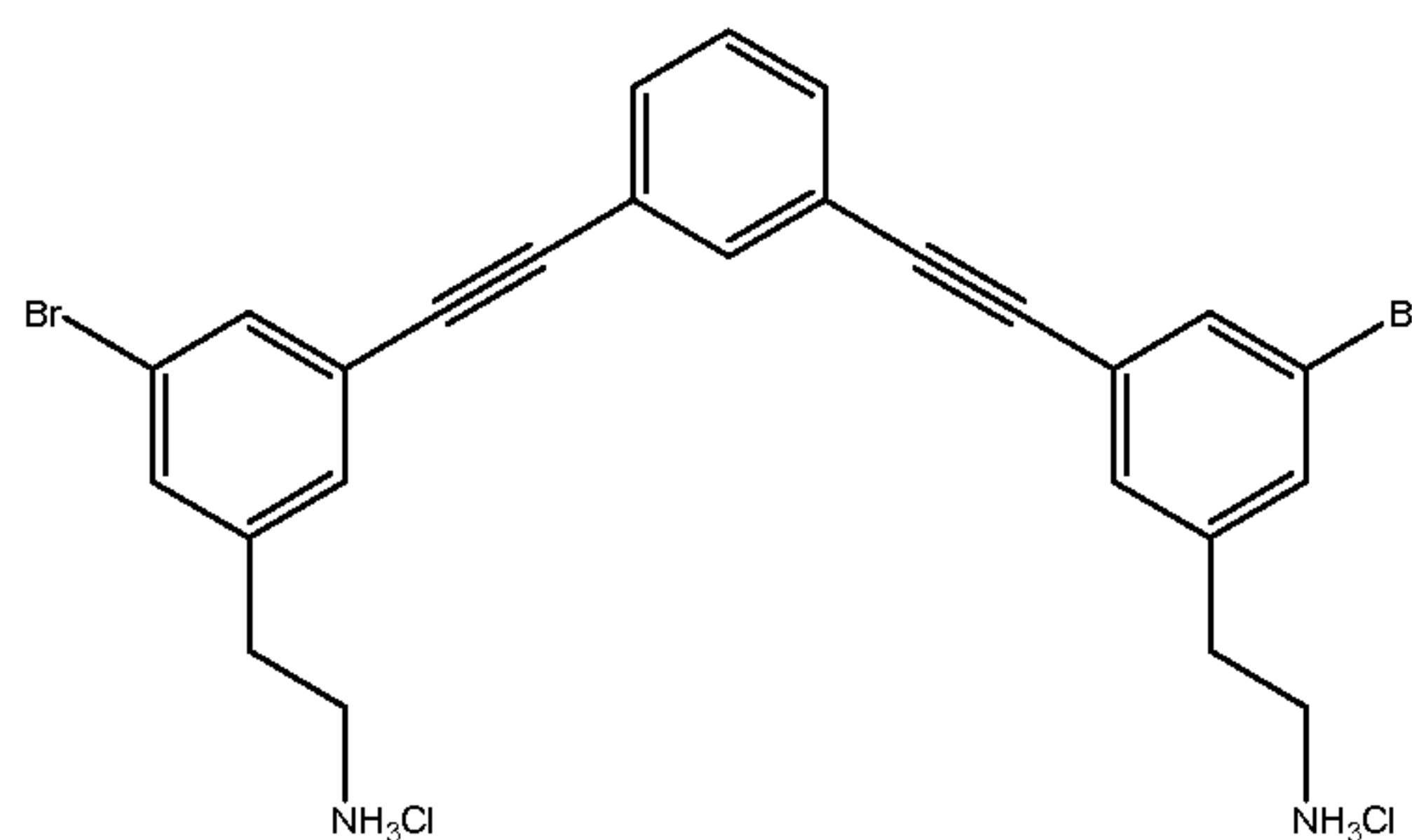
The following three oligomers of the invention were screened for antimicrobial activity against a number of clinically relevant ocular pathogens.



Oligomer 1



Oligomer 2



Oligomer 3

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Minimum Inhibitory Concentrations (MIC) of each of the 3 oligomers were determined using standard procedures for clinical ocular isolates of Ciprofloxacin Susceptible (CS) *S. aureus* (CSSA) (n=27), Ciprofloxacin Resistant (CR) *S. aureus* (CRSA) (n=28), CS *S. epidermidis* (CSSE) (n=26), CR *S. epidermidis* (CRSE) (n=26), *St. pneumoniae* (SP) (n=27), *St. viridans* group (SV), *Moraxella* Species (MS) (n=25), *H. influenzae* (HI) (n=26), *P. aeruginosa* (PA) (n=26), and *Serratia marcescens* (SM) (n=27).

The results are presented in Table 1. Data is expressed as MIC₅₀, MIC₉₀, in µg/ml for Oligomer 1, Oligomer 2, and Oligomer 3, respectively.

Table 1.

Microbial Strain	Oligomer 1		Oligomer 2		Oligomer 3	
	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
CSSA	0.125	0.25	0.125	0.25	0.5	0.5
CRSA	0.25	0.25	0.25	0.25	0.5	0.5
CSSE	0.03	0.125	0.03	0.03	0.25	0.25
CRSE	0.03	0.03	0.03	0.03	0.25	0.25
SP	0.5	1	1	2	2	2
SV	4	16	4	32	4	8
MS	0.5	0.5	0.25	0.5	1	2
HI	16	32	8	16	4	8
PA	4	8	4	8	4	4
SM	16	64	16	32	64	256

Oligomers 1, 2, and 3 demonstrated broad spectrum *in vitro* activity against a number of clinically relevant ocular pathogens.

Example 2: Ophthalmic Ointment Formulation

The following represents an example of a typical ophthalmic ointment formulation comprising an antimicrobial oligomer of the invention (oligomer 1 in Example 1 above).

Ophthalmic Ointment

<i>Ingredient</i>	<i>Amount (weight %)</i>
Oligomer 1	0.35
Mineral Oil, USP	2.0
5 White petrolatum, USP	q.s. 100

Example 3: Ophthalmic Ointment Formulation

The following represents an example of a typical ophthalmic ointment formulation comprising an antimicrobial oligomer of the invention (oligomer 2 in Example 1 above) and an
10 anti-inflammatory agent.

Ophthalmic Ointment

<i>Ingredient</i>	<i>Amount (weight %)</i>
Oligomer 2	0.3
15 Dexamethasone	0.1
Chlorobutanol, Anhydrous, NF	0.5
Mineral Oil, USP	5.0
White petrolatum, USP	q.s. 100

20 Example 4: Ophthalmic/Otic Solution Formulation

The following represents an example of a typical ophthalmic/otic solution formulation comprising an antimicrobial oligomer of the invention (oligomer 3 in Example 1 above).

Ophthalmic/Otic Solution

<i>Ingredient</i>	<i>Amount (weight %)</i>
25 Oligomer 3	0.35
Sodium Acetate	0.3
Acetic Acid	0.04
Mannitol	4.60
30 EDTA	0.05
Benzalkonium chloride	0.006
Water	q.s. 100

Example 5: Ophthalmic/Otic Suspension Formulation

The following represents an example of a typical ophthalmic/otic suspension formulation comprising an antimicrobial oligomer of the invention (oligomer 3 in Example 1 above) and an anti-inflammatory agent (dexamethasone).

5

*Ophthalmic/Otic Suspension**Ingredient**Amount (weight %)*

	Oligomer 3	0.3
	Dexamethasone, micronized USP	0.10
10	Benzalkonium chloride	0.01
	Edetate Disodium USP	0.01
	Sodium chloride USP	0.3
	Sodium sulfate USP	1.2
	Tyloxapol USP	0.05
15	Hydroxyethylcellulose	0.25
	Sulfuric Acid and/or	
	Sodium hydroxide, NF	q.s. for pH adjustment to 7.0-8.0
	Purified sterilized water	q.s. to 100

20 Example 6: Toxicity

The ocular toxicity of several concentrations of Oligomer 2, using the Draize ocular toxicity scoring system, in the NZW rabbit ocular toxicity model was carried out.

Nine rabbits were received from Myrtles' Rabbitry, Thompson Station, TN and were subsequently divided into 5 groups:

Group	Oligomer 2 Concentration	N Rabbits	N Eyes	Rabbit Numbers
I	1% Oligomer 2	2	4	1-2
II	0.25% Oligomer 2	2	4	3-4
III	0.1% Oligomer 2	2	4	5-6
IV	0.01% Oligomer 2	2	4	7-8
V	Tris-Buffered Saline	1	2	9

25

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Rabbits were treated in both eyes with (37 μ l) topical drops every 30 minutes for 3 hours (7 total doses). One rabbit was treated with Tris-Buffered Saline and served as a negative control.

Rabbits were evaluated in a masked fashion for ocular toxicity by an ophthalmologist with specialty training in corneal and external disease. Ocular toxicity was evaluated using the Draize scoring system after treatment on Day 0 and on Day 3 post treatment for any delayed toxicity. (Draize et al., J. Pharmacol. Exp. Ther., 1944, 82, 377-390).

IACUC Protocol #0701145 "The In Vivo Evaluation of Biomimetics as Topical Ocular Antibiotics". Formulations: 1) 1% Oligomer 2: 31.36 mg of Oligomer 2 in powder form was stored at -20°C until use. The vial containing Oligomer 2 was removed from the freezer and 3.126 ml of Tris-Buffered Saline (TBS) was added to the vial to yield 3.126 ml of 1% (10 mg/ml) Oligomer 2; 2) 0.25% Oligomer 2: 0.5 ml of 1% Oligomer 2 was added to 1.5 ml of TBS to yield 2 ml of 0.25% Oligomer 2; 3) 0.1% Oligomer 2: 0.2 ml of 1% Oligomer 2 was added to 1.8 ml of TBS to yield 2 ml of 0.1% Oligomer 2; 4) 0.01% Oligomer 2: 0.2 ml of 0.1% Oligomer 2 was added to 1.8 ml of TBS to yield 2 ml of 0.01% Oligomer 2; and 5) Tris-Buffered Saline: 25 ml of Tris-Buffered Saline (10mM TRIS, 150mM NaCl, pH=7.4) was filter sterilized prior to use in preparation of the above samples and use in rabbits. The following schedule was adhered to.

Ocular Toxicity Evaluation

Drop Schedule

Drop	Elapsed Time	Time of Day	Group I 1% Oligomer 2	Group II 0.25% Oligomer 2	Group III 0.1% Oligomer 2	Group IV 0.01% Oligomer 2	Group V TBS
1	0	11:40 am	X	X	X	X	X
2	:30	12:10 pm	X	X	X	X	X
3	1:00	12:40 pm	X	X	X	X	X
4	1:30	1:10 pm	X	X	X	X	X
5	2:00	1:40 pm	X	X	X	X	X
6	2:30	2:10 pm	X	X	X	X	X
7	3:00	2:40 pm	X	X	X	X	X
Examine	3:20	3:00 pm	X	X	X	X	X

A brief summary of the Draize scoring system for ocular lesions is provided below

1. Cornea

A. Opacity-degree of density (area most dense taken for reading)

No Opacity.....0

5 Scattered or diffuse area, details of iris clearly visible 1

Easily discernible translucent areas, details of iris slightly obscured 2

Opalescent areas, no details of iris visible, size of pupil barely discernible 3

Opaque, iris invisible 4

B. Area of cornea involved

10 One quarter (or less) but not zero 1

Greater than one quarter, but less than half 2

Greater than half. but less than three quarters 3

Greater than three quarters, up to whole area 4

A x B x 5

Total Maximum = 80

15 2. Iris

A Values

Normal 0

20 Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive).....1

No reaction to light, hemorrhage, gross destruction (any or all of these) 2

A x 5

Total Maximum =10

3. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivas excluding cornea and iris)

25 Vessels normal 0

Vessels definitely injected above normal 1

More diffuse, deeper crimson red, individual vessels not easily discernible 2

Diffuse beefy red 3

B. Chemosis

30 No swelling 0

Any swelling above normal (includes nictitating membrane) 1

Obvious swelling with partial eversion of lids 2

Swelling with lids about half-closed 3

Swelling with lids about half-closed to completely closed 4

C. Discharge

	No discharge	0
	Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
5	Discharge with moistening of the lids and hairs just adjacent to lids	2
	Discharge with moistening of the lids and hairs, and considerable area around the eye	3
Score (A + B + C) x 2		Total Maximum = 20

- 10 Total Maximum Score: 110 represents the sum of all scores obtained for the cornea, iris and conjunctivae.

Classification of Eye Irritation Scores:

MMTS	Classification	Symbol
15 0.0 - 0.5	Non-Irritating	N
0.6 - 2.5	Practically Non-Irritating	PN
2.6 - 15.0	Minimally Irritating	M1
15.1 - 25.0	Mildly Irritating	M2
25.1 - 50.0	Moderately Irritating	M3
20 50.1 - 80.0	Severely Irritating	S
80.1 - 100.0	Extremely Irritating	E
100.1 - 110.0	Maximally Irritating	Mx

MMTS = Maximum Mean Total Score (The mean total score per group)

- 25 Kay et al., J. Soc. Cos. Chem., 1962, 13, 281-289.

Acute Ocular Toxicity Evaluation

Observations of Rabbit Behavior After Instillation of Test Drugs on Day 0:

Group	Oligomer 2 Concentration
I	1% Oligomer 2
II	0.25% Oligomer 2
III	0.1% Oligomer 2

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IV	0.01% Oligomer 2
V	Tris-Buffered Saline

Drop 1 (11:40 am)

No adverse behavior observed after instillation of ALL test drugs.

Drop 2 (12:10 pm)

No adverse behavior observed after instillation of ALL test drugs.

5 Drop 3 (12:40 pm)

No adverse behavior observed after instillation of ALL test drugs.

Group I - 1% Oligomer 2 - Eyes have developed noticeable conjunctivitis.

Drop 4 (1:10 pm)

No adverse behavior observed after instillation of ALL test drugs.

10 Group I - 1% Oligomer 2 - Eyes have developed noticeable discharge.

Drop 5 (1:40 pm)

No adverse behavior observed after instillation of ALL test drugs.

Drop 6 (2:10 pm)

No adverse behavior observed after instillation of ALL test drugs.

15 Drop 7 (2:40 pm)

No adverse behavior observed after instillation of ALL test drugs.

Group: I 1% Oligomer 2

	Day 0				Day 3			
Test/Eye	1L	1R	2L	2R	1L	1R	2L	2R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	1	1	1	1	0	0	0	0
II. Tot	5	5	5	5	0	0	0	0
III. A.	2	2	1	1	0	0	0	0
III. B.	2	2	1	2	0	0	0	0
III. C.	3	2	3	2	0	0	0	0
III. Tot	14	12	10	10	0	0	0	0
Score	19	17	15	15	0	0	0	0

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MMTS	16.5 - M ₂	0.0 – N
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Group: II 0.25% Oligomer 2

	Day 0				Day 3			
Test/Eye	3L	3R	4L	4R	3L	3R	4L	4R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	1	2	1	0	0	0	0	0
III. B.	1	1	0	0	0	0	0	0
III. C.	2	1	1	0	0	0	0	0
III. Tot	8	8	4	0	0	0	0	0
Score	8	8	4	0	0	0	0	0
MMTS	5.0 - M ₁				0.0 – N			

Group: III 0.1% Oligomer 2

	Day 0				Day 3			
Test/Eye	5L	5R	6L	6R	5L	5R	6L	6R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	0	0	0	0	0	0	0	0
III. B.	0	0	0	0	0	0	0	0
III. C.	0	0	0	0	0	0	0	0
III. Tot	0	0	0	0	0	0	0	0

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Score	0	0	0	0	0	0	0	0
MMTS	0.0 - N				0.0 – N			

Group: IV 0.01% Oligomer 2

	Day 0				Day 3			
Test/Eye	7L	7R	8L	8R	7L	7R	8L	8R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	0	0	1	0	0	0	1	0
III. B.	0	0	0	0	0	0	0	0
III. C.	0	0	0	0	0	0	0	0
III. Tot	0	0	2	0	0	0	2	0
Score	0	0	2	0	0	0	2	0
MMTS	0.5 - N				0.5 – N			

Group: V TBS Control

	Day 0		Day 3	
Test/Eye	9L	9R	9L	9R
I. A.	0	0	0	0
I. B.	0	0	0	0
I. Tot	0	0	0	0
II. A.	0	0	0	0
II. Tot	0	0	0	0
III. A.	0	0	0	0
III. B.	0	0	0	0
III. C.	0	0	0	0

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III. Tot	0	0	0	0
Score	0	0	0	0
MMTS	0.0 - N		0.0 - N	

Summary of MMTS Results

Group	Day 0	Day 3
1% Oligomer 2	16.5 - M ₂ Mildly Irritating	0.0 - N Non-Irritating
0.25% Oligomer 2	5.0 - M ₁ Minimally Irritating	0.0 - N Non-Irritating
0.1% Oligomer 2	0.0 - N Non-Irritating	0.0 - N Non-Irritating
0.01% Oligomer 2	0.5 - N Non-Irritating	0.5 - N Non-Irritating
Tris-Buffered Saline	0.0 - N Non-Irritating	0.0 - N Non-Irritating

Oligomer 2 demonstrated dose dependent ocular toxicity after 7 topical instillations (every 30 minutes for 3 hours) in the NZW rabbit ocular toxicity model. 1% Oligomer 2 was determined to be Mildly Irritating, 0.25% Oligomer 2 was determined to be Minimally Irritating, while 0.1% and 0.01% Oligomer 2 were determined to be Non-Irritating.

There were no acute reactions by the rabbits (flinching, immediate wiping of eyes, vocalization, hopping to rear of cage) upon instillation of any of the Oligomer 2 concentrations suggesting that Oligomer 2 does not sting upon instillation.

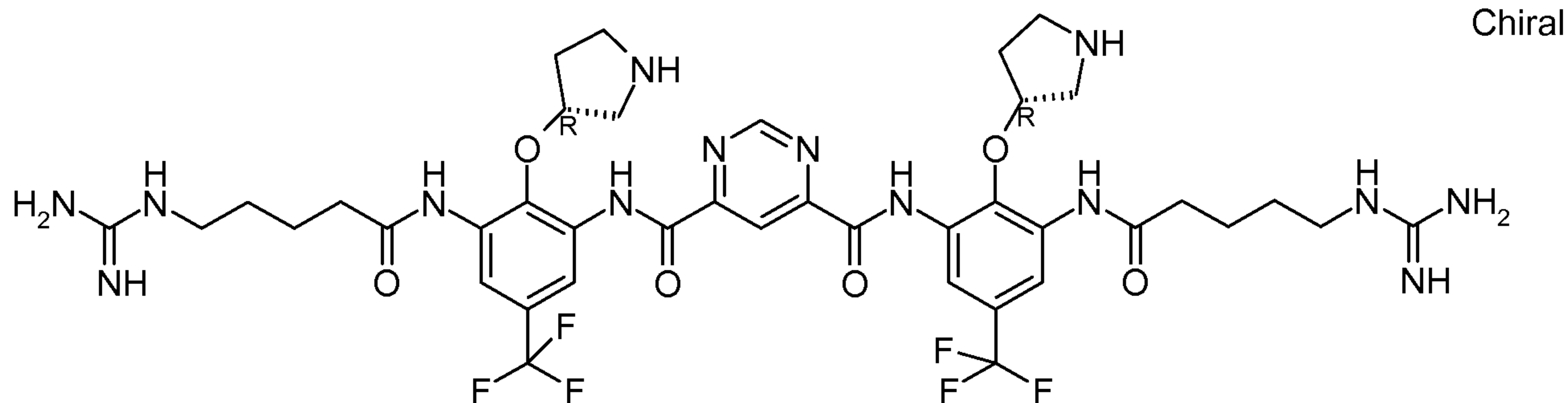
There was no prolonged toxicity (3 days after drops) demonstrated in any treatment group.

1% Oligomer 2, though Mildly Irritating, is suitable for use to determine whether Oligomer 2 demonstrates efficacy in the *Staphylococcus aureus* keratitis model.

Example 7: Toxicity

The ocular toxicity of several formulations of Oligomer 4 with and without farnesol, using the Draize ocular toxicity scoring system, in the NZW rabbit ocular toxicity model was carried out.

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Oligomer 4

Fifteen rabbits were received from Myrtles' Rabbitry, Thompson Station, TN and were divided into 8 groups:

Group	Formulation	N Rabbits	N Eyes	Rabbit Numbers
I	0.25% Oligomer 4 in Tris Buffered Saline (TBS)	2	4	1-2
II	0.5% Oligomer 4 Tris Buffered Saline (TBS)	2	4	3-4
III	100µM Farnesol in 1% Propylene Glycol (PG) and TBS	2	4	5-6
IV	200µM Farnesol in 1% Propylene Glycol (PG) and TBS	2	4	7-8
V	0.25% Oligomer 4 + 100µM Farnesol in 1% PG and TBS	2	4	9-10
VI	0.5% Oligomer 4 + 100µM Farnesol in 1% PG and TBS	2	4	11-12
VII	1% Propylene Glycol in TBS	2	4	13-14
VIII	Tris-Buffered Saline	1	2	15

5

Rabbits were treated in both eyes with (37 µl) topical drops every 30 minutes for 3 hours (7 total doses). One rabbit was treated with Tris-Buffered Saline and served as a negative control.

Rabbits were evaluated in a masked fashion for ocular toxicity by an ophthalmologist with specialty training in corneal and external disease 30 minutes after the final dose. Ocular toxicity was evaluated using the Draize scoring system (see above) after treatment on Day 0 and on Day 2 post treatment for any delayed toxicity.

10

Formulations: 1) 0.25% Oligomer 4: Vial 1 of Oligomer 4 in powder form was stored at 4°C until use. The vial was removed from the refrigerator and 1.04 ml of sterile water for

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injection was added and vortexed until solid was completely dissolved. Then, 1.04 ml of Solution A (2X TBS) was added and vortexed for 10 seconds; 2) 0.5% Oligomer 4: Vial 2 of Oligomer 4 in powder form was stored at 4°C until use. The vial was removed from the refrigerator and 1.04 ml of sterile water for injection was added and vortexed until solid was completely dissolved. Then, 1.04 ml of Solution A (2X TBS) was added and vortexed for 10 seconds; 3) 100 µM Farnesol in 1% Propylene Glycol (PG) and TBS: Vial 3 containing about 2 ml of 100µM Farnesol in 1% Propylene Glycol (PG) and TBS was stored at 4°C until use; 4) 200 µM Farnesol in 1% Propylene Glycol (PG) and TBS: Vial 4 containing about 2 ml of 200µM Farnesol in 1% Propylene Glycol (PG) and TBS was stored at 4°C until use; 5) 0.25% Oligomer 4 + 100µM Farnesol in 1% PG and TBS: Vial 5 of Oligomer 4 in powder form was stored at 4°C until use; at the time of use, the vial was removed from the refrigerator and 1.016 ml of sterile water for injection was added and vortexed until solid was completely dissolved; then 1.016 ml of Solution B (2% PG, 2X TBS, 200 µM Farnesol) was added and vortexed for 10 seconds; 6) 0.5% Oligomer 4 + 100µM Farnesol in 1% PG and TBS: Vial 6 of Oligomer 4 in powder form was stored at 4°C until use; at the time of use, the vial was removed from the refrigerator and 1.02 ml of sterile water for injection was added and vortexed until solid was completely dissolved; then 1.02 ml of Solution B (2% PG, 2X TBS, 200 µM Farnesol) was added and vortexed for 10 seconds; 7) 1% Propylene Glycol in TBS: Vial 7 containing about 2 ml of 1% Propylene Glycol was stored at 4°C until use; and 8) Tris-Buffered Saline: Vial 8 containing about 2 ml of Tris-Buffered Saline (10mM TRIS, 150mM NaCl, pH=7.4) was stored at 4°C until use.

IACUC Protocol #0701145-1 “The In Vivo Evaluation of Biomimetics as Topical Ocular Antibiotics”.

Ocular Toxicity Evaluation

Drop Schedule

Drop	Elapsed Time	Time of Day	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
1	0	10:45	X	X	X	X	X	X	X	X
2	:30	11:15	X	X	X	X	X	X	X	X
3	1:00	11:45	X	X	X	X	X	X	X	X
4	1:30	12:15	X	X	X	X	X	X	X	X
5	2:00	12:45	X	X	X	X	X	X	X	X
6	2:30	1:15	X	X	X	X	X	X	X	X

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7	3:00	1:45	X	X	X	X	X	X	X	X
Exam	3:30	2:15	X	X	X	X	X	X	X	X

Acute Ocular Toxicity Evaluation

Observations of Rabbit Behavior After Instillation of Test Drugs on Day 0

Group	Formulation
I	0.25% Oligomer 4 in Tris Buffered Saline (TBS)
II	0.5% Oligomer 4 Tris Buffered Saline (TBS)
III	100µM Farnesol in 1% Propylene Glycol (PG) and TBS
IV	200µM Farnesol in 1% Propylene Glycol (PG) and TBS
V	0.25% Oligomer 4 + 100µM Farnesol in 1% PG and TBS
VI	0.5% Oligomer 4 + 100µM Farnesol in 1% PG and TBS
VII	1% Propylene Glycol in TBS
VIII	Tris-Buffered Saline

Drop 1 (10:45 am)

5 No adverse behavior observed after instillation of ALL test drugs.

Drop 2 (11:15 am)

No adverse behavior observed after instillation of ALL test drugs.

Drop 3 (11:45 am)

No adverse behavior observed after instillation of ALL test drugs.

10 Drop 4 (12:15 am)

No adverse behavior observed after instillation of ALL test drugs.

Drop 5 (12:45 pm)

No adverse behavior observed after instillation of ALL test drugs.

Drop 6 (1:15 pm)

15 No adverse behavior observed after instillation of ALL test drugs.

Drop 7 (1:45 pm)

No adverse behavior observed after instillation of ALL test drugs.

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Group: I 0.25% Oligomer 4

	Day 0				Day 2			
Test/Eye	1L	1R	2L	2R	1L	1R	2L	2R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	0	0	0	0	0	0	0	0
III. B.	0	0	0	0	0	0	0	0
III. C.	0	1	1	1	0	1	0	0
III. Tot	0	2	2	2	0	2	0	0
Score	0	2	2	2	0	2	0	0
MMTS	1.5 - PN Practically Non-Irritating				0.5 - N Non-Irritating			

Group: II 0.5% Oligomer 4

	Day 0				Day 2			
Test/Eye	3L	3R	4L	4R	3L	3R	4L	4R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	1	1	1	0	0	0	0	0
III. B.	1	1	1	0	0	0	0	0
III. C.	2	2	2	1	0	0	1	1
III. Tot	8	8	8	2	0	0	2	2

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Score	8	8	8	2	0	0	2	2
MMTS	6.5 - M ₁ Minimally Irritating				1.0 - N Practically Non-Irritating			

Group: III 100 µM Farnesol in 1% Propylene Glycol (PG) and TBS

	Day 0				Day 2			
Test/Eye	5L	5R	6L	6R	5L	5R	6L	6R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	0	0	0	0	0	0	0	0
III. B.	0	0	0	0	0	0	0	0
III. C.	0	0	0	0	1	0	1	1
III. Tot	0	0	0	0	2	0	2	2
Score	0	0	0	0	2	0	2	2
MMTS	0.0 - N Non-Irritating				1.5 - PN Practically Non-Irritating			

Group: IV 200 µM Farnesol in 1% Propylene Glycol (PG) and TBS

	Day 0				Day 2			
Test/Eye	7L	7R	8L	8R	7L	7R	8L	8R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0

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III. A.	0	0	0	0	0	0	0	0
III. B.	0	0	0	0	0	0	0	0
III. C.	0	0	0	1	0	0	0	1
III. Tot	0	0	0	2	0	0	0	2
Score	0	0	0	2	0	0	0	2
MMTS	0.5 - N Non-Irritating				0.5 - N Non-Irritating			

Group: V 0.25% Oligomer 4 + 100 μ M Farnesol in 1% PG and TBS

	Day 0				Day 2			
Test/Eye	9L	9R	10L	10R	9L	9R	10L	10R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	0	1	0	0	0	0	0	0
III. B.	0	1	0	0	0	0	0	0
III. C.	0	2	1	1	0	1	1	1
III. Tot	0	8	2	2	0	2	2	2
Score	0	8	2	2	0	2	2	2
MMTS	3.0 - M ₁ Minimally Irritating				1.5 - PN Practically Non-Irritating			

Group: VI 0.5% Oligomer 4 + 100 μ M Farnesol in 1% PG and TBS

	Day 0				Day 2			
Test/Eye	11L	11R	12L	12R	11L	11R	12L	12R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0

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I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	2	2	2	2	0	0	0	0
III. B.	1	2	1	1	0	0	0	0
III. C.	2	2	2	2	1	0	1	0
III. Tot	10	12	10	10	2	0	2	0
Score	10	12	10	10	2	0	2	0
MMTS	10.5 - M ₁ Minimally Irritating				1.0 - PN Practically Non-Irritating			

Group: VII 1% Propylene Glycol in TBS

	Day 0				Day 2			
Test/Eye	13L	13R	14L	14R	13L	13R	14L	14R
I. A.	0	0	0	0	0	0	0	0
I. B.	0	0	0	0	0	0	0	0
I. Tot	0	0	0	0	0	0	0	0
II. A.	0	0	0	0	0	0	0	0
II. Tot	0	0	0	0	0	0	0	0
III. A.	0	0	0	0	0	0	0	0
III. B.	0	0	0	0	0	0	0	0
III. C.	0	1	0	0	1	1	0	1
III. Tot	0	2	0	0	2	2	0	2
Score	0	2	0	0	2	2	0	1
MMTS	0.5 - N Non-Irritating				1.5 - PN Practically Non-Irritating			

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Group: VIII TBS Treated Control

	Day 0		Day 2	
Test/Eye	15L	15R	15L	15R
I. A.	0	0	0	0
I. B.	0	0	0	0
I. Tot	0	0	0	0
II. A.	0	0	0	0
II. Tot	0	0	0	0
III. A.	0	0	0	0
III. B.	0	0	0	0
III. C.	1	1	1	1
III. Tot	2	2	2	2
Score	2	2	2	2
MMTS	2.0 - PN Practically Non- Irritating		2.0 - PN Practically Non- Irritating	

Summary of MMTS Results

Group	Day 0	Day 2
0.25% Oligomer 4 in Tris Buffered Saline (TBS)	1.5 - PN Practically Non-Irritating	0.5 - N Non-Irritating
0.5% Oligomer 4 Tris Buffered Saline (TBS)	6.5 - M ₁ Minimally Irritating	1.0 - N Practically Non-Irritating
100 µM Farnesol in 1% Propylene Glycol (PG) and TBS	0.0 - N Non-Irritating	1.5 - PN Practically Non-Irritating
200 µM Farnesol in 1% Propylene Glycol (PG) and TBS	0.5 - N Non-Irritating	0.5 - N Non-Irritating
0.25% Oligomer 4 + 100µM Farnesol in 1% PG and TBS	3.0 - M ₁ Minimally Irritating	1.5 - PN Practically Non-Irritating

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0.5% Oligomer 4 + 100µM Farnesol in 1% PG and TBS	10.5 - M ₁ Minimally Irritating	1.0 - PN Practically Non-Irritating
1% Propylene Glycol in TBS	0.5 - N Non-Irritating	1.5 - PN Practically Non-Irritating
Tris-Buffered Saline	2.0 - PN Practically Non-Irritating	2.0 - PN Practically Non-Irritating

Oligomer 4 demonstrated dose dependent ocular toxicity after 7 topical instillations (every 30 minutes for 3 hours) in the NZW rabbit ocular toxicity model. 0.5% Oligomer 4 was determined to be Mildly Irritating, while 0.25% was determined to be Practically Non-Irritating.

5 The addition of 100 µM Farnesol in 1% Propylene Glycol to the Oligomer 4 concentrations increased the toxicity of both 0.5% and 0.25% Oligomer 4. Both formulations were determined to be Mildly Irritating. This was the same category as 0.5% Oligomer 4 alone, but the scores were higher. This classification was an increase for 0.25% Oligomer 4. 100 µM Farnesol, 200 µM Farnesol, and 1% Propylene Glycol individually were determined to be Non-Irritating. Tris-
10 buffered Saline was determined to be Practically Non-Irritating. Rabbits demonstrated no adverse behavior upon instillation of any the test drugs. This indicates all of the test drugs did not sting upon instillation. There was really no prolonged or delayed toxicity (2 days after drops) demonstrated in any treatment group. The only finding on Day 2 was a slight discharge in some of the eyes which accounted for all of the scores. Although the complete formulations of 0.5%
15 Oligomer 4 and 0.25% Oligomer 4 (including 100 µM Farnesol and 1% Propylene Glycol) were both classified as Mildly Irritating, the MMTS score for the 0.5% Oligomer 4 formulation was at the higher end of the classification whereas 0.25% Oligomer 4 formulation was at the lower end of the classification. It appears that the complete 0.5% Oligomer 4 formulation (including 100 µM Farnesol and 1% Propylene Glycol), though Mildly Irritating in uninfected eyes is probably
20 not as suitable as other formulations for use in the efficacy studies in the *Staphylococcus aureus* keratitis model. The complete formulation of 0.25% Oligomer 4 (including 100 µM Farnesol and 1% Propylene Glycol) may be acceptable from a toxicity point of view. Experience with other formulations have generally shown that ocular toxicity can increase when instilled more frequently (21 drops vs. 7 drops) in infected eyes in the *Staphylococcus aureus* keratitis efficacy
25 model.

Example 8: MIC

One purpose of the following experiments was to determine the MICs of two biomimetic compounds vs. 25 ocular isolates of *Staphylococcus aureus* fluoroquinolone-susceptible, *Staphylococcus aureus* fluoroquinolone-resistant, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-susceptible, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-resistant, *Serratia marcescens*, *Streptococcus pneumoniae*, *Streptococcus viridans* group, *Moraxella* species (including *Moraxella catarrhalis*) and *Pseudomonas aeruginosa* and *Haemophilus influenzae*.

General Procedures:

10 Mueller-Hinton Broth in tubes was inoculated with 25 ocular isolates of *Staphylococcus aureus* fluoroquinolone-susceptible, *Staphylococcus aureus* fluoroquinolone-resistant, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-susceptible, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-resistant, *Pseudomonas aeruginosa* and *Serratia marcescens*, plus two controls (*Staphylococcus aureus* and *E. coli*) and incubated at 37°C overnight on a shaker set at 250 rpm.

Mueller-Hinton Broth supplemented with 2% lysed horse blood in tubes was inoculated with 25 ocular isolates of *Streptococcus pneumoniae*, *Streptococcus viridans* group, and *Moraxella* species (including *Moraxella catarrhalis*) plus two controls (*Staphylococcus aureus* and *E. coli*) and incubated at 37°C overnight. Additionally, Mueller-Hinton Broth in tubes was inoculated with two controls (*Staphylococcus aureus* and *E. coli*) and incubated at 37°C overnight on a shaker set at 250 rpm.

HTM (*Haemophilus* Test Medium) in tubes was inoculated with 25 ocular isolates of *Haemophilus influenzae* plus two controls (*Staphylococcus aureus* and *E. coli*) and incubated at 37°C overnight. Additionally, Mueller-Hinton Broth in tubes was inoculated with two controls (*Staphylococcus aureus* and *E. coli*) and incubated at 37°C overnight on a shaker set at 250 rpm.

On the day of testing, a 640 µg/ml (1280 µg/ml for *Serratia marcescens* and *Pseudomonas aeruginosa*) concentration was prepared from a 1% stock solution in 0.01% acetic acid, 0.2% BSA in polypropylene tubes.

Serial doubling dilutions in 0.01% acetic acid, 0.2% BSA in 96 well polypropylene plates, which are used as reservoirs for the inoculation of the test plates, were carried out to obtain serial dilutions of test agents at 10 times the required test concentrations: 640, 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 µg/ml (1280, 640, 320, 160, 80, 40, 20, 10, 5, 2.5, and 1.25 µg/ml for *Serratia marcescens* and *Pseudomonas aeruginosa*).

Ten μ l of diluted 10x test agents was added to each well of one row of the 96 well polypropylene plates from column 2 to column 12 (column 1 is a control for bacteria alone, with no peptide). Test agent concentrations in columns 2-12 were as follows: 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 μ g/ml (128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 μ g/ml for *Serratia marcescens* and *Pseudomonas aeruginosa*). The same peptide was in each of the 8 rows. One plate contained dilutions of one test agent and 8 bacterial isolates.

On the day of testing, the overnight bacterial broth cultures of *Staphylococcus aureus* fluoroquinolone-susceptible, *Staphylococcus aureus* fluoroquinolone-resistant, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-susceptible, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-resistant, *Serratia marcescens*, and *Pseudomonas aeruginosa*, plus two controls (*Staphylococcus aureus* and *E. coli*) were diluted in 5 ml of trypticase soy broth to yield turbidity equal to a 0.5 McFarland standard. The final inoculum for MIC testing for *Staphylococcus aureus* fluoroquinolone-susceptible, *Staphylococcus aureus* fluoroquinolone-resistant, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-susceptible, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-resistant, *Serratia marcescens*, and *Pseudomonas aeruginosa* was achieved by placing 0.05 ml of the turbidity adjusted sample to 5 ml of Mueller-Hinton broth.

Control Bacteria - The two control bacteria (*Staphylococcus aureus* and *E. coli*) were treated as above.

On the day of testing, the overnight bacterial broth cultures of *Streptococcus pneumoniae*, *Streptococcus viridans* and *Moraxella* species (including *Moraxella catarrhalis*) plus two controls (*Staphylococcus aureus* and *E. coli*) were diluted in 5 ml of trypticase soy broth to yield turbidity equal to a 0.5 McFarland standard. The final inoculum for MIC testing for *Streptococcus pneumoniae*, *Streptococcus viridans* and *Moraxella* species (including *Moraxella catarrhalis*) was achieved by placing 0.1 ml of the turbidity adjusted sample to 5 ml of Mueller-Hinton broth containing 2% lysed horse red blood cells.

Control Bacteria Set #1 - this set of control bacteria were treated as the *Streptococcus pneumoniae*, *Streptococcus viridans* and *Moraxella* species (including *Moraxella catarrhalis*) test isolates above; the control bacteria underwent the same conditions as the test *Streptococcus pneumoniae*, *Streptococcus viridans* and *Moraxella* species (including *Moraxella catarrhalis*) isolates. This set of control bacteria was to determine whether there was a difference in the MICs by performing the MIC determinations in 2% lysed horse red blood cells with the standard method performed in Mueller-Hinton broth.

Control Bacteria Set #2 - the control bacteria were added to 5 ml of Mueller-Hinton Broth without the 2% lysed horse red blood cells to achieve the standard inoculum concentration. This set of control bacteria is the normal control to determine whether the PMX compounds are at the target MICs.

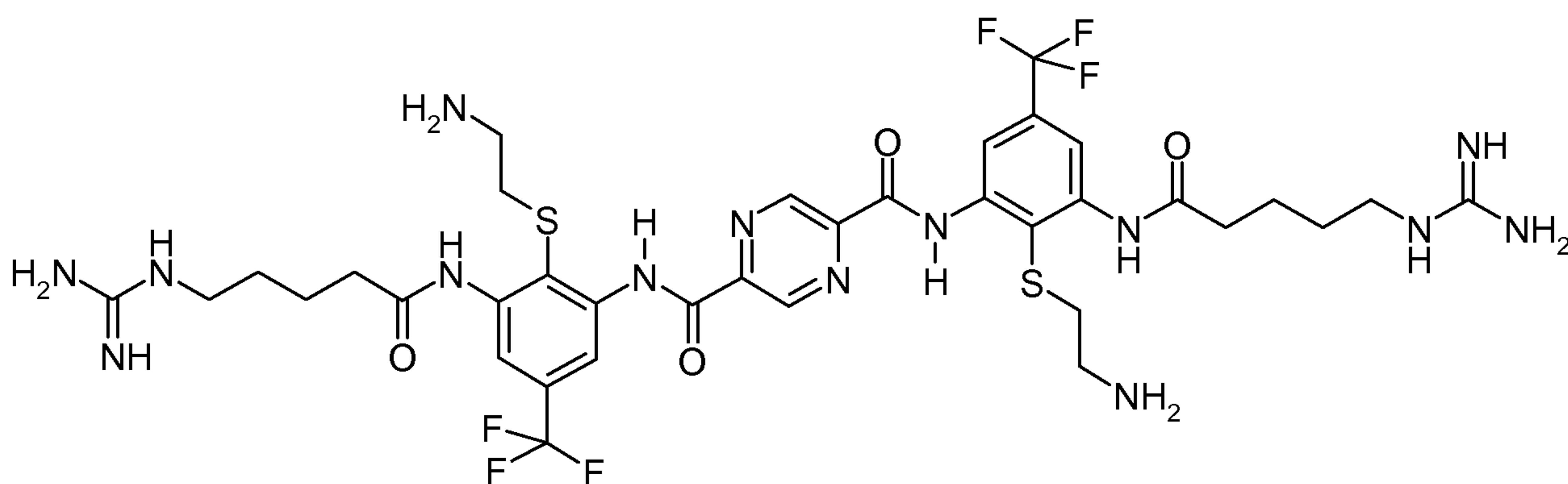
5 On the day of testing, the overnight bacterial broth cultures of *Haemophilus species* was diluted in 5 ml of trypticase soy broth to yield turbidity equal to a 0.5 McFarland standard. The final inoculum for MIC testing for *Haemophilus species* was achieved by placing 0.1 ml of the turbidity adjusted sample to 5 ml of HTM medium.

10 Control Bacteria Set #1 - this set of control bacteria were treated as the *Haemophilus influenzae* test isolates above; the control bacteria underwent the same conditions as the test *Haemophilus influenzae* isolates. This set of control bacteria is to determine whether there was a difference in the MICs by performing the MIC determinations in HTM broth with the standard method performed in Mueller-Hinton broth.

15 Control Bacteria Set #2 - the control bacteria were added to 5 ml of Mueller-Hinton Broth to achieve the standard inoculum concentration. This set of control bacteria is the normal control to determine whether the PMX compounds are at the target MICs.

20 Ninety µl of the bacterial suspensions was dispensed in each well from column 1 to column 12. Each bacterial isolate was placed in one row of a 96 well polypropylene plate containing the test agents. The plates were placed on shaker at 15 minutes at room temperature, and then incubated at 37°C overnight. MICs were determined visually as the lowest concentration of drug that inhibits visible bacterial growth.

The MICs of the 2 compounds Oligomer 4 and Oligomer 5 were compared statistically with the Kruskal-Wallis ANOVA with Duncan's Multiple Comparisons Test using True Epistat statistical software (True Epistat, Richardson, TX).



Oligomer 5

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Oligomer	MIC (ug/mL)	
	E.coli D31	S.aureus ATCC27660
Oligomer 4	0.78	0.098
Oligomer 5	1.56	0.78

Compound	MIC				
	<i>E. coli</i> Lab Strain D31	<i>S. aureus</i> ATCC 27660	<i>E. faecalis</i> ATCC 29212	<i>P. aeruginosa</i> ATCC 10145	<i>K. pneumoniae</i> Lab Strain KP10
Oligomer 4	0.78	0.098	0.78	12.5	0.78
Oligomer 5	1.56	0.78	1.56	>100	1.56

Isolate numbers with a “K” before the number indicates they have been isolated from cases of
5 Keratitis. Isolate numbers with an “E” before the number indicates they have been isolated from
cases of Endophthalmitis. Isolate numbers with a “B” before the number indicates they have
been isolated from cases of Blepharitis and or Conjunctivitis. Most *Streptococcus pneumoniae*
isolates are from cases of conjunctivitis. “Fluoroquinolone-resistant” indicates the bacteria are
resistant to the second generation fluoroquinolones ciprofloxacin and ofloxacin but, not
10 necessarily resistant to the fourth generation fluoroquinolones gatifloxacin and moxifloxacin by
CLSI serum standards.

S. aureus fluoroquinolone-susceptible MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 - E402	0.25	0.5
2 - E1512	0.25	0.25
3 - E253	0.25	0.25
4 - K1518	0.25	0.125
5 - K1525	0.125	0.125
6 - K1663	0.5	0.125
7 - K1648	0.25	0.125
8 - K1646	0.25	0.25
9 - K1642	0.5	0.25
10 - K1638	0.5	0.25
11 - K1628	0.25	0.25

12 - K1618	0.5	0.125
13 - K1617	0.25	0.25
14 - K1611	0.25	0.25
15 - K1607	0.25	0.25
16 - K1600	0.25	0.125
17 - K1591	0.25	0.5
18 - K1585	0.25	0.25
19 - K1583	0.25	0.25
20 - K1574	0.25	0.25
21 - K1566	0.25	0.25
22 - K1551	0.25	0.125
23 - K1545	0.25	0.25
24 - K1540	0.25	0.25
25 - K1530	0.25	0.5
<i>E. coli</i> D31	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	2 (0.098)	16 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

S. aureus fluoroquinolone-susceptible

MIC₅₀ and MIC₉₀ Determinations and Statistics

5

10

15

	Oligomer 4	Oligomer 5
Row	QSSA-A	QSSA-A
1	0.125	0.125
2	0.250	0.125
3	0.250	0.125
4	0.250	0.125
5	0.250	0.125
6	0.250	0.125
7	0.250	0.125
8	0.250	0.250
9	0.250	0.250
10	0.250	0.250

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	11	0.250	0.250
	12	0.250	0.250
	13	0.250	0.250 MIC ₅₀
	14	0.250	0.250
5	15	0.250	0.250
	16	0.250	0.250
	17	0.250	0.250
	18	0.250	0.250
	19	0.250	0.250
10	20	0.250	0.250
	21	0.250	0.250
	22	0.500	0.250 MIC ₉₀
	23	0.500	0.500
	24	0.500	0.500
15	25	0.500	0.500

Descriptive Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 QSSA	25	0	0.2850	0.0198	0.0990	0.1250	0.2500	0.5000
20 Olig 5 QSSA	25	0	0.2450	0.0222	0.1111	0.1250	0.2500	0.5000

Summary of Results

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	0.25 µg/ml	0.25 µg/ml	0.25 µg/ml	0.125 - 0.5 µg/ml
25 Oligomer 5	0.25 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 - 0.5 µg/ml

Mann-Whitney Test and CI: Oligomer 4 QSSA, Oligomer 5 QSSA

	N	Median
Olig 4 QSSA	25	0.2500
30 Olig 5 QSSA	25	0.2500

Point estimate for ETA1-ETA2 is 0.0000

95.2 Percent CI for ETA1-ETA2 is (-0.0000, 0.1250)

W = 712.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1483

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The test is significant at 0.0731 NS (adjusted for ties)

S. aureus fluoroquinolone-resistant MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 - E504	0.25	0.5
2 - E475	0.25	0.25
3 - E442	0.25	0.25
4 - E427	0.5	0.5
5 - E425	0.25	0.25
6 - E424	0.25	0.25
7 - E417	1	0.25
8 - E407	0.25	0.125
9 - E401	0.25	0.25
10 - K1659	0.25	0.25
11 - E96	0.125	0.25
12 - E379	0.5	0.5
13 - E369	0.125	0.5
14 - E361	0.25	0.25
15 - E339	0.25	0.25
16 - E333	0.25	0.125
17 - E332	0.25	0.25
18 - E327	0.5	0.25
19 - E325	0.5	0.25
20 - K950	0.5	0.25
21 - K839	0.25	0.25
22 - K1679	0.25	0.25
23 - K1677	0.25	0.5
24 - K1672	0.25	0.25
25 - K1670	0.25	0.25
<i>E. coli</i> D31	1 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	1 (0.098)	8 (0.78)

5 MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

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S. aureus fluoroquinolone-resistant
MIC₅₀ and MIC₉₀ Determinations and Statistics

		Oligomer 4	Oligomer 5	
	Row	QRSA-A	QRSA-A	
5	1	0.125	0.125	
	2	0.125	0.125	
	3	0.250	0.250	
	4	0.250	0.250	
	5	0.250	0.250	
10	6	0.250	0.250	
	7	0.250	0.250	
	8	0.250	0.250	
	9	0.250	0.250	
	10	0.250	0.250	
15	11	0.250	0.250	
	12	0.250	0.250	
	13	0.250	0.250	MIC ₅₀
	14	0.250	0.250	
	15	0.250	0.250	
20	16	0.250	0.250	
	17	0.250	0.250	
	18	0.250	0.250	
	19	0.250	0.250	
	20	0.500	0.250	
25	21	0.500	0.500	
	22	0.500	0.500	MIC ₉₀
	23	0.500	0.500	
	24	0.500	0.500	
	25	1.000	0.500	
30	Descriptive Statistics			
	Variable	N	N*	Mean SE Mean StDev Minimum Median Maximum
	Olig 4 QRSA	25	0	0.3200 0.0361 0.1807 0.1250 0.2500 1.0000
	Olig 5 QRSA	25	0	0.2900 0.0225 0.1125 0.1250 0.2500 0.5000

Summary of Results

	MIC50	MIC90	Median MIC	Range of MICs
Oligomer 4	0.25 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 - 1.0 µg/ml
Oligomer 5	0.25 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 - 0.5 µg/ml

5

Mann-Whitney Test and CI: Oligomer 4 QRSA, Oligomer 5 QRSA

	N	Median
Olig 4 QRSA	25	0.2500
Olig 5 QRSA	25	0.2500

10

Point estimate for ETA1-ETA2 is -0.0000

95.2 Percent CI for ETA1-ETA2 is (-0.0000, 0.0000)

W = 651.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7934

15 The test is significant at 0.7450 NS (adjusted for ties)

Control Bacteria

During the first sets of MICs performed with the *S. aureus* fluoroquinolone-susceptible and *S. aureus* fluoroquinolone-resistant, the MICs for the control bacteria (*E. coli* D31, and *S. aureus* ATCC 27660) for both Oligomer 4 and Oligomer 5 were much higher than those shown below.

20

Control Isolate	Control for MIC Test	Oligomer 4	Oligomer 5
<i>E. coli</i> D31	SA-FQS	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	SA-FQS	2 (0.098)	16 (0.78)
<i>E. coli</i> D31	SA-FQR	1 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	SA-FQR	1 (0.098)	8 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

25 A new set of MICs were performed with new batches of both Oligomer 4 and Oligomer 5, and control bacteria, in quadruplicate. The results from the experiment is as follows:

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Control Isolate	Control for MIC Test	Oligomer 4	Oligomer 5
<i>E. coli</i> D31	Control Only 1	1 (0.78)	8 (1.56)
<i>S. aureus</i> ATCC 27660	Control Only 1	0.25 (0.098)	0.25 (0.78)
<i>E. coli</i> D31	Control Only 2	1 (0.78)	8 (1.56)
<i>S. aureus</i> ATCC 27660	Control Only 2	0.25 (0.098)	0.25 (0.78)
<i>E. coli</i> D31	Control Only 3	1 (0.78)	8 (1.56)
<i>S. aureus</i> ATCC 27660	Control Only 3	0.25 (0.098)	0.5 (0.78)
<i>E. coli</i> D31	Control Only 4	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	Control Only 4	0.5 (0.098)	0.5 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

Although the MICs for Oligomer 5 for *E. coli* D31 remained high, the MICs for *S. aureus* ATCC 27660 were for both Oligomer 4 and Oligomer 5 and Oligomer 4 for *E. coli* D31 were within the acceptable range (1-2 doubling dilutions) of the MICs previously obtained. It was decided to continue with the MIC determinations using the new batches of Oligomer 4 and Oligomer 5 for all subsequent MIC determinations.

Since the MICs for both Oligomer 4 and Oligomer 5 with the *S. aureus* fluoroquinolone-susceptible and *S. aureus* fluoroquinolone-resistant were similar to that of the control *S. aureus* ATCC 27660 MIC performed previously, these MICs performed with the first batch of drugs would not be repeated using the new batches of compounds.

Staphylococcus epidermidis (Coagulase-negative *Staphylococcus*)

fluoroquinolone-susceptible MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 - E511	0.25	0.25
2 - E489	0.125	0.125

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3 - E491	0.125	0.125
4 - E476	0.25	0.25
5 - E473	0.25	0.125
6 - E462	0.125	0.125
7 - E460	0.125	0.125
8 - E453	0.125	0.125
9 - E448	0.125	0.125
10 - E443	<0.0625	<0.0625
11 - E441	<0.0625	0.125
12 - E438	0.125	0.125
13 - E437	0.125	0.125
14 - E434	0.125	0.125
15 - E433	0.125	0.125
16 - E430	<0.0625	0.125
17 - E420	0.125	0.125
18 - E419	0.125	0.125
19 - E403	0.125	0.125
20 - E394	0.125	0.125
21 - E393	0.125	0.125
22 - E328	0.25	0.25
23 - E382	0.125	0.125
24 - E381	0.125	0.25
25 - E372	0.25	<0.0625
<i>E. coli</i> D31	1 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	0.25 (0.098)	0.25 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

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Staphylococcus epidermidis (Coagulase-negative *Staphylococcus*)
 fluoroquinolone-susceptible MIC₅₀ and MIC₉₀ Determinations and Statistics
 For Statistical Calculation Purposes, <0.0625 was Replaced with 0.03125.

		Oligomer 4	Oligomer 5
5	Row	QSSE-A	QSSE-A
	1	0.03125	0.03125
	2	0.03125	0.03125
	3	0.03125	0.12500
	4	0.12500	0.12500
10	5	0.12500	0.12500
	6	0.12500	0.12500
	7	0.12500	0.12500
	8	0.12500	0.12500
	9	0.12500	0.12500
15	10	0.12500	0.12500
	11	0.12500	0.12500
	12	0.12500	0.12500
	13	0.12500	0.12500 MIC ₅₀
	14	0.12500	0.12500
20	15	0.12500	0.12500
	16	0.12500	0.12500
	17	0.12500	0.12500
	18	0.12500	0.12500
	19	0.12500	0.12500
25	20	0.12500	0.12500
	21	0.25000	0.12500
	22	0.25000	0.25000 MIC ₉₀
	23	0.25000	0.25000
	24	0.25000	0.25000
30	25	0.25000	0.25000

Descriptive Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 QSSE	25	0	0.1388	0.0129	0.0645	0.0313	0.1250	0.2500

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Olig 5 QSSE 25 0 0.1375 0.0113 0.0563 0.0313 0.1250 0.2500

Summary of Results

		MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
5	Oligomer 4	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.25 µg/ml
	Oligomer 5	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.25 µg/ml

Mann-Whitney Test and CI: Oligomer 4 QSSE, Oligomer 5 QSSE

N Median

10 Olig 4 QSSE 25 0.12500

Olig 5 QSSE 25 0.12500

Point estimate for ETA1-ETA2 is 0.00000

95.2 Percent CI for ETA1-ETA2 is (-0.00002,0.00000)

15 W = 638.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.9923

The test is significant at 0.9902 NS (adjusted for ties)

Staphylococcus epidermidis (Coagulase-negative *Staphylococcus*)

20

fluoroquinolone-resistant MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 - E515	0.125	0.125
2 - E514	<0.0625	0.125
3 - E513	0.125	0.125
4 - E510	<0.0625	0.125
5 - E509	0.125	0.125
6 - E508	0.125	0.125
7 - E505	0.125	0.125
8 - E503	0.125	0.125
9 - E502	0.125	0.25
10 - E499	0.125	0.25
11 - E498	0.125	0.125

12 - E494	<0.0625	0.125
13 - E493	0.125	0.125
14 - E485	0.125	0.125
15 - E487	0.125	<0.0625
16 - E486	<0.0625	0.125
17 - E480	0.125	0.125
18 - E475	0.25	0.125
19 - E471	0.125	0.125
20 - E458	0.125	0.125
21 - E452	0.25	0.5
22 - E450	0.125	0.125
23 - E440	0.25	0.125
24 - E446	0.125	<0.0625
25 - E444	0.25	0.25
<i>E. coli</i> D31	1 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	0.25 (0.098)	0.25 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

Staphylococcus epidermidis (Coagulase-negative *Staphylococcus*) fluoroquinolone-resistant

5 MIC₅₀ and MIC₉₀ Determinations and Statistics

For Statistical Calculation Purposes, <0.0625 was Replaced with 0.03125.

Row	Oligomer 4	Oligomer 5
	QRSE-A	QRSE-A
10 1	0.03125	0.03125
2	0.03125	0.03125
3	0.03125	0.12500
4	0.03125	0.12500
5	0.12500	0.12500
15 6	0.12500	0.12500

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5	7	0.12500	0.12500	
	8	0.12500	0.12500	
	9	0.12500	0.12500	
	10	0.12500	0.12500	
	11	0.12500	0.12500	
10	12	0.12500	0.12500	
	13	0.12500	0.12500	MIC ₅₀
	14	0.12500	0.12500	
	15	0.12500	0.12500	
	16	0.12500	0.12500	
15	17	0.12500	0.12500	
	18	0.12500	0.12500	
	19	0.12500	0.12500	
	20	0.12500	0.12500	
	21	0.12500	0.12500	
	22	0.25000	0.25000	MIC ₉₀
	23	0.25000	0.25000	
	24	0.25000	0.25000	
	25	0.25000	0.50000	

20 *Descriptive Statistics*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 QRSE	25	0	0.1300	0.0127	0.0636	0.0313	0.1250	0.2500
Olig 5 QRSE	25	0	0.1475	0.0179	0.0895	0.0313	0.1250	0.5000

25 *Summary of Results*

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.25 µg/ml
Oligomer 5	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.5 µg/ml

30 *Mann-Whitney Test and CI: Oligomer 4 QRSE, Oligomer 5 QRSE*

	N	Median
Olig 4 QRSE	25	0.12500
Olig 5 QRSE	25	0.12500

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Point estimate for ETA1-ETA2 is -0.00000

95.2 Percent CI for ETA1-ETA2 is (0.00001,-0.00002)

W = 614.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6624

5 The test is significant at 0.5800 NS (adjusted for ties)

Serratia marcescens MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 - K1681	32	>128
2 - K1674	32	>128
3 - K1558	4	>128
4 - K1538	16	>128
5 - K1503	32	>128
6 - K1216	4	>128
7 - K1496	8	>128
8 - K1481	2	>128
9 - K1470	32	>128
10 - K1468	2	>128
11 - K1467	32	>128
12 - K1462	16	>128
13 - K1461	8	128
14 - K1413	16	>128
15 - K1402	0.25	8
16 - K1357	1	>128
17 - K1351	0.5	64
18 - K1327	8	>128
19 - K1321	8	>128
20 - K1315	16	>128
21 - K1306	8	>128
22 - K1290	8	>128

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23 - K1265	8	>128
24 - K1263	8	>128
25 - K1239	8	>128
<i>E. coli</i> D31	0.5 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	0.25 (0.098)	0.5 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

Serratia marcescens

5

MIC₅₀ and MIC₉₀ Determinations and Statistics

For Statistical Calculation Purposes, > 128 was Replaced with 256.

		Oligomer 4	Oligomer 5
	Row	SM-A	SM-A
	1	0.25	8
10	2	0.50	64
	3	1.00	128
	4	2.00	256
	5	2.00	256
	6	4.00	256
15	7	4.00	256
	8	8.00	256
	9	8.00	256
	10	8.00	256
	11	8.00	256
20	12	8.00	256
	13	8.00	256 MIC ₅₀
	14	8.00	256
	15	8.00	256
	16	8.00	256
25	17	16.00	256
	18	16.00	256
	19	16.00	256
	20	16.00	256

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21	32.00	256	
22	32.00	256	MIC ₉₀
23	32.00	256	
24	32.00	256	
25	32.00	256	

Descriptive Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 SM	25	0	12.39	2.21	11.04	0.25	8.00	32.00
Olig 5 SM	25	0	233.3	13.0	65.1	8.0	256.0	256.0

Summary of Results

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	8 µg/ml	32 µg/ml	8 µg/ml	0.25 - 32 µg/ml
Oligomer 5	256 µg/ml	256 µg/ml	256 µg/ml	8 - 256 µg/ml

Mann-Whitney Test and CI: Oligomer 4 SM, Oligomer 5 SM

N Median

Olig 4 SM 25 8.00

Olig 5 SM 25 256.00

Point estimate for ETA1-ETA2 is -248.00

95.2 Percent CI for ETA1-ETA2 is (-247.98,-239.99)

W = 338.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000

The test is significant at 0.0000 (adjusted for ties)

Oligomer 4 > Oligomer 5 (More Potent > Less Potent)

Pseudomonas aeruginosa MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 – K1673	2	32
2 – K1668	2	64
3 - K1662	2	64
4 - K1657	2	64

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5 - K1651	4	128
6 - K1649	4	64
7 - K1564	8	>128
8 - K1636	0.5	4.0
9 - K1634	2	128
10 – K1633	4	64
11 – K1632	4	64
12 – K1631	8	64
13 – K1629	4	64
14 – K1627	2	64
15 – K1626	8	128
16 – K1625	4	64
17 – K1562	4	128
18 – K1613	4	32
19 – K1553	2	128
20 – K1594	2	64
21 – K1588	4	128
22 – K1554	4	128
23 – K1580	2	32
24 – K1577	2	64
25 – K1576	4	128
<i>E. coli</i> D31	0.5 (0.78)	8 (1.56)
<i>S. aureus</i> ATCC 27660	0.5 (0.098)	0.25 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

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*Pseudomonas aeruginosa*MIC₅₀ and MIC₉₀ Determinations and Statistics

For Statistical Calculation Purposes, > 128 was Replaced with 256.

		Oligomer 4	Oligomer 5	
	Row	PA-A	PA-A	
5	1	0.5	4	
	2	2.0	32	
	3	2.0	32	
	4	2.0	32	
10	5	2.0	64	
	6	2.0	64	
	7	2.0	64	
	8	2.0	64	
	9	2.0	64	
15	10	2.0	64	
	11	2.0	64	
	12	4.0	64	
	13	4.0	64	MIC ₅₀
	14	4.0	64	
20	15	4.0	64	
	16	4.0	64	
	17	4.0	128	
	18	4.0	128	
	19	4.0	128	
25	20	4.0	128	
	21	4.0	128	
	22	4.0	128	MIC ₀
	23	8.0	128	
	24	8.0	128	
30	25	8.0	256	

Descriptive Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 PA	25	0	3.540	0.398	1.989	0.500	4.000	8.000
Olig 5 PA	25	0	85.9	10.4	51.8	4.0	64.0	256.0

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Summary of Results

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	4 µg/ml	4 µg/ml	4 µg/ml	0.5 - 8 µg/ml
Oligomer 5	64 µg/ml	128 µg/ml	64 µg/ml	4 - 256 µg/ml

5

Mann-Whitney Test and CI: Oligomer 4 PA, Oligomer 5 PA

N Median

Olig 4 PA 25 4.00

Olig 5 PA 25 64.00

10

Point estimate for ETA1-ETA2 is -62.00

95.2 Percent CI for ETA1-ETA2 is (-120.00,-60.00)

W = 333.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000

15 The test is significant at 0.0000 (adjusted for ties)

Oligomer 4 > Oligomer 5 (More Potent > Less Potent)

Streptococcus pneumoniae MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 - B1386	>64	>64
2 - B1380	1	4
3 - B1378	1	0.5
4 - B1377	2	8
5 - B1373	1	8
6 - B1367	1	16
7 - B1355	2	8
8 - B1353	1	4
9 - B1351	1	1
10 - B1339	1	2
11 - B1337	0.5	1
12 - B1335	2	1

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13 - B1334	1	1
14 - B1333	1	1
15 - B1255	0.5	1
16 - B1288	1	8
17 - B1287	1	16
18 - B1272	0.5	1
19 - B1264	0.5	1
20 - B1252	1	16
21 - B1245	0.5	2
22 - B1211	1	8
23 - B1213	1	16
24 - B1208	0.5	8
25 - B1214	1	4
<i>E. coli</i> D31*	2	2
<i>S. aureus</i> ATCC 27660*	1	1
<i>E. coli</i> D31**	0.5 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660**	0.25 (0.098)	2 (0.78)

* Control Bacteria Set #1; ** Control Bacteria Set #2; (MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.)

5

Streptococcus pneumoniae

MIC₅₀ and MIC₉₀ Determinations and Statistics

For Statistical Calculation Purposes, > 64 was Replaced with 128.

10

	Oligomer 4	Oligomer 5
Row	SP-A	SP-A
1	0.5	0.5
2	0.5	1.0
3	0.5	1.0
4	0.5	1.0

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5	5	0.5	1.0	
	6	0.5	1.0	
	7	1.0	1.0	
	8	1.0	1.0	
	9	1.0	1.0	
10	10	1.0	2.0	
	11	1.0	2.0	
	12	1.0	4.0	
	13	1.0	4.0	MIC ₅₀
	14	1.0	4.0	
15	15	1.0	8.0	
	16	1.0	8.0	
	17	1.0	8.0	
	18	1.0	8.0	
	19	1.0	8.0	
20	20	1.0	8.0	
	21	1.0	16.0	
	22	2.0	16.0	MIC ₉₀
	23	2.0	16.0	
	24	2.0	16.0	
	25	128.0	128.0	

Descriptive Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 SP	25	0	6.08	5.08	25.40	0.50	1.00	128.00
25 Olig 5 SP	25	0	10.58	5.01	25.05	0.50	4.00	128.00

Summary of Results

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	1 µg/ml	2 µg/ml	1 µg/ml	0.5 - 128 µg/ml
30 Oligomer 5	4 µg/ml	16 µg/ml	4 µg/ml	4 - 128 µg/ml

Mann-Whitney Test and CI: Oligomer 4 SP, Oligomer 5 SP

N Median

Olig 4 SP 25 1.000

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Olig 5 SP 25 4.000

Point estimate for ETA1-ETA2 is -3.000

95.2 Percent CI for ETA1-ETA2 is (-6.999,-0.499)

W = 457.5

5 Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0005

The test is significant at 0.0002 (adjusted for ties)

Oligomer 4 > Oligomer 5 (More Potent > Less Potent)

Streptococcus viridans group MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 – K1684	2	8
2 – K1680	4	64
3 - E546	1	8
4 - E272	2	16
5 - E506	16	>64
6 - E496	1	0.5
7 - E456	4	16
8 - E432	4	8
9 - E423	4	>64
10 - E418	8	>64
11 - E412	2	8
12 - E409	8	32
13 - E405	4	>64
14 - E404	32	>64
15 - E396	16	32
16 - E262	1	4
17 - E362	4	16
18 - E359	4	32
19 - E348	8	16
20 - E344	4	4

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21 - E308	4	4
22 - E294	4	2
23 - E292	4	0.5
24 - E285	4	0.5
25 - E265	1	8
<i>E. coli</i> D31*	2	2
<i>S. aureus</i> ATCC 27660*	2	1
<i>E. coli</i> D31**	0.5 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660**	1 (0.098)	1 (0.78)

* Control Bacteria Set #1; ** Control Bacteria Set #2; (MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.)

5

Streptococcus viridans groupMIC₅₀ and MIC₉₀ Determinations and Statistics

For Statistical Calculation Purposes, > 64 was Replaced with 128.

		Oligomer 4	Oligomer 5
	Row	SV-A	SV-A
10	1	1	0.5
	2	1	0.5
	3	1	0.5
	4	1	2.0
	5	2	4.0
15	6	2	4.0
	7	2	4.0
	8	4	8.0
	9	4	8.0
	10	4	8.0
20	11	4	8.0
	12	4	8.0
	13	4	16.0
			MIC ₅₀

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5	14	4	16.0	
	15	4	16.0	
	16	4	16.0	
	17	4	32.0	
	18	4	32.0	
	19	4	32.0	
	20	8	64.0	
	21	8	128.0	
10	<u>22</u>	<u>8</u>	<u>128.0</u>	<u>MIC₉₀</u>
	23	16	128.0	
	24	16	128.0	
	25	32	128.0	

Descriptive Statistics

	Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
15	Olig 4 SV	25	0	5.84	1.34	6.72	1.00	4.00	32.00
	Olig 5 SV	25	0	36.78	9.72	48.59	0.50	16.00	128.00

Summary of Results

		MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
	Oligomer 4	4 µg/ml	8 µg/ml	4 µg/ml	1 - 32 µg/ml
20	Oligomer 5	16 µg/ml	128 µg/ml	16 µg/ml	0.5 - 128 µg/ml

Mann-Whitney Test and CI: Oligomer 4 SV, Oligomer 5 SV

	N	Median
Olig 4 SV	25	4.00
25 Olig 5 SV	25	16.00

Point estimate for ETA1-ETA2 is -7.00

95.2 Percent CI for ETA1-ETA2 is (-23.99,-3.01)

W = 487.5

30 Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0037

The test is significant at 0.0031 (adjusted for ties)

Oligomer 4 > Oligomer 5 (More Potent > Less Potent)

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Moraxella species & *Moraxella catarrhalis* CombinedMS = *Moraxella* species; MC = *Moraxella (Branhamella) catarrhalis*

Isolate	Oligomer 4	Oligomer 5
1 – K1614 - MS	16	64
2 – K1661 - MS	32	16
3 - K1643 - MS	64	0.125
4 - K1640 - MS	8.0	8.0
5 - B1431 - MS	32	0.5
6 - B1429 - MS	1	1
7 - B1418 - MS	32	0.25
8 - K1784 - MS	64	0.25
9 - K1773 - MS	64	0.25
10 - K1369 - MS	2.0	2.0
11 - B1275 - MS	2.0	0.125
12 - B1221 - MS	2.0	0.125
13 - B1172 - MS	>64	>64
14 - E542 - MS	2.0	2.0
15 - K678 - MS	2.0	0.5
16 - K660 - MS	2.0	0.25
17 - K599 - MC	0.5	0.25
18 - K1650 - MC	64	0.25
19 - K1373 - MC	1.0	0.125
20 - K1553 - MC	4.0	2.0
21 - K1453 - MC	4.0	64
22 - K1227 - MC	2.0	1.0
23 - B1102 - MC	1.0	0.5
24 - K1819 - MC	4.0	32
25 - K1855 - MC	2.0	8.0
<i>E. coli</i> D31*	4	2

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<i>S. aureus</i> ATCC 27660*	1	1
<i>E. coli</i> D31**	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660**	0.5 (0.098)	0.5 (0.78)

* Control Bacteria Set #1; ** Control Bacteria Set #2; (MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.)

5

Moraxella species & *Moraxella catarrhalis* Combined

MIC₅₀ and MIC₉₀ Determinations and Statistics

For Statistical Calculation Purposes, > 64 was Replaced with 128.

		Oligomer 4	Oligomer 5
	Row	MS-A	MS-A
10	1	0.5	0.125
	2	1.0	0.125
	3	1.0	0.125
	4	1.0	0.125
	5	2.0	0.250
15	6	2.0	0.250
	7	2.0	0.250
	8	2.0	0.250
	9	2.0	0.250
	10	2.0	0.250
20	11	2.0	0.500
	12	2.0	0.500
	13	4.0	0.500 MIC ₅₀
	14	4.0	1.000
	15	4.0	1.000
25	16	8.0	2.000
	17	16.0	2.000
	18	32.0	2.000
	19	32.0	8.000
	20	32.0	8.000

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	21	64.0	16.000	
	<u>22</u>	<u>64.0</u>	<u>32.000</u>	<u>MIC₉₀</u>
	23	64.0	64.000	
	24	64.0	64.000	
5	25	128.0	128.000	

Descriptive Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 MS	25	0	21.42	6.43	32.13	0.50	4.00	128.00
Olig 5 MS	25	0	13.26	6.00	30.00	0.13	0.50	128.00

10

Summary of Results

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	4 µg/ml	64 µg/ml	4 µg/ml	0.5 - 128 µg/ml
Oligomer 5	0.5 µg/ml	32 µg/ml	0.5 µg/ml	0.125 - 128 µg/ml

15

Mann-Whitney Test and CI: Oligomer 4 MS, Oligomer 5 MS

N Median

Olig 4 MS 25 4.00

Olig 5 MS 25 0.50

20 Point estimate for ETA1-ETA2 is 1.75

95.2 Percent CI for ETA1-ETA2 is (0.75,6.00)

W = 785.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0043

The test is significant at 0.0040 (adjusted for ties)

25 Oligomer 4 > Oligomer 5 (More Potent > Less Potent)

Haemophilus influenzae MICs µg/ml

Isolate	Oligomer 4	Oligomer 5
1 – B1359	8	>64
2 – B1346	8	>64
3 – B1345	8	>64
4 – B1343	8	>64

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5 – B1338	4	16
6 – B1332	8	64
7 – B1331	8	>64
8 – B1330	8	>64
9 – B1379	16	8
10 – B1378	8	4
11 – B1313	4	2
12 – B1477	8	4
13 – B1286	8	2
14 – B1282	32	8
15 – B1291	8	16
16 – B1280	8	16
17 – B1279	16	64
18 – B1260	8	16
19 – B1238	2	8
20 – B1209	4	8
21 – B1249	4	16
22 – B1248	8	4
23 – B1244	8	32
24 – B1419	4	32
25 – B1222	8	>64
<i>E. coli</i> D31	8	16
<i>S. aureus</i> ATCC 27660	4	4
<i>E. coli</i> D31	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	0.5 (0.098)	0.5 (0.78)

* Control Bacteria Set #1; ** Control Bacteria Set #2; (MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.)

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*Haemophilus influenzae*MIC₅₀ and MIC₉₀ Determinations and Statistics

For Statistical Calculation Purposes, > 64 was Replaced with 128.

		Oligomer 4	Oligomer 5
	Row	HI-A	HI-A
5	1	2	2
	2	4	2
	3	4	4
	4	4	4
10	5	4	4
	6	4	8
	7	8	8
	8	8	8
	9	8	8
15	10	8	16
	11	8	16
	12	8	16
	13	8	16
	14	8	16
20	15	8	32
	16	8	32
	17	8	64
	18	8	64
	19	8	128
25	20	8	128
	21	8	128
	22	8	128
	23	16	128
	24	16	128
30	25	32	128

Descriptive Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Median	Maximum
Olig 4 HI	25	0	8.56	1.16	5.82	2.00	8.00	32.00
Olig 5 HI	25	0	48.6	10.6	53.0	2.0	16.0	128.0

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Summary of Results

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	8 µg/ml	8 µg/ml	8 µg/ml	2 - 32 µg/ml
Oligomer 5	16 µg/ml	128 µg/ml	16 µg/ml	2 - 128 µg/ml

5

Mann-Whitney Test and CI: Oligomer 4 HI, Oligomer 5 HI

N Median

Olig 4 HI 25 8.00

Olig 5 HI 25 16.00

10

Point estimate for ETA1-ETA2 is -8.00

95.2 Percent CI for ETA1-ETA2 is (-56.00,0.00)

W = 493.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0054

15 The test is significant at 0.0038 (adjusted for ties)

Oligomer 4 > Oligomer 5 (More Potent > Less Potent)

Summary of Results

MIC Determinations of Control Bacteria from Each Day of MIC Testing

20

MICs [µg/ml]

Control Isolate	Control for MIC Test	Oligomer 4	Oligomer 5
<i>E. coli</i> D31	SA-FQS	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	SA-FQS	2 (0.098)	16 (0.78)
<i>E. coli</i> D31	SA-FQR	1 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	SA-FQR	1 (0.098)	8 (0.78)
<i>E. coli</i> D31	Control Only 1	1 (0.78)	8 (1.56)
<i>S. aureus</i> ATCC 27660	Control Only 1	0.25 (0.098)	0.25 (0.78)
<i>E. coli</i> D31	Control Only 2	1 (0.78)	8 (1.56)

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<i>S. aureus</i> ATCC 27660	Control Only 2	0.25 (0.098)	0.25 (0.78)
<i>E. coli</i> D31	Control Only 3	1 (0.78)	8 (1.56)
<i>S. aureus</i> ATCC 27660	Control Only 3	0.25 (0.098)	0.5 (0.78)
<i>E. coli</i> D31	Control Only 4	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	Control Only 4	0.5 (0.098)	0.5 (0.78)
<i>E. coli</i> D31	SE-FQS	1 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	SE-FQS	0.25 (0.098)	0.25 (0.78)
<i>E. coli</i> D31	SE-FQR	1 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	SE-FQR	0.25 (0.098)	0.25 (0.78)
<i>E. coli</i> D31	SM	0.5 (0.78)	4 (1.56)
<i>S. aureus</i> ATCC 27660	SM	0.25 (0.098)	0.5 (0.78)
<i>E. coli</i> D31	PA	0.5 (0.78)	8 (1.56)
<i>S. aureus</i> ATCC 27660	PA	0.5 (0.098)	0.25 (0.78)
<i>E. coli</i> D31	SP	0.5 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	SP	0.25 (0.098)	2 (0.78)
<i>E. coli</i> D31	SV	0.5 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	SV	1 (0.098)	1 (0.78)
<i>E. coli</i> D31	MS	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	MS	0.5 (0.098)	0.5 (0.78)
<i>E. coli</i> D31	HI	1 (0.78)	16 (1.56)
<i>S. aureus</i> ATCC 27660	HI	0.5 (0.098)	0.5 (0.78)

MICs for Control Bacteria (*E. coli*, *S. aureus*) are within the parentheses.

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Summary of MIC Results (n = 25 per group)

S. aureus fluoroquinolone-susceptible

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	0.25 µg/ml	0.25 µg/ml	0.25 µg/ml	0.125 - 0.5 µg/ml
5 Oligomer 5	0.25 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 - 0.5 µg/ml

S. aureus fluoroquinolone-resistant

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	0.25 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 - 1.0 µg/ml
10 Oligomer 5	0.25 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 - 0.5 µg/ml

Staphylococcus epidermidis (Coagulase-negative *Staphylococcus*) FQ-susceptible

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.25 µg/ml
15 Oligomer 5	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.25 µg/ml

Staphylococcus epidermidis (Coagulase-negative *Staphylococcus*) FQ-resistant

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.25 µg/ml
20 Oligomer 5	0.125 µg/ml	0.25 µg/ml	0.125 µg/ml	0.03125 - 0.5 µg/ml

Serratia marcescens

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	8 µg/ml	32 µg/ml	8 µg/ml	0.25 - 32 µg/ml
25 Oligomer 5	256 µg/ml	256 µg/ml	256 µg/ml	8 - 256 µg/ml

Pseudomonas aeruginosa

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	4 µg/ml	4 µg/ml	4 µg/ml	0.5 - 8 µg/ml
30 Oligomer 5	64 µg/ml	128 µg/ml	64 µg/ml	4 - 256 µg/ml

Streptococcus pneumoniae

	MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
Oligomer 4	1 µg/ml	2 µg/ml	1 µg/ml	0.5 - 128 µg/ml

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Oligomer 5	4 µg/ml	16 µg/ml	4 µg/ml	4 - 128 µg/ml
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Streptococcus viridans group

		MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
5	Oligomer 4	4 µg/ml	8 µg/ml	4 µg/ml	1 - 32 µg/ml
	Oligomer 5	16 µg/ml	128 µg/ml	16 µg/ml	0.5 - 128 µg/ml

Moraxella species (Including *Moraxella catarrhalis*)

		MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
10	Oligomer 4	4 µg/ml	64 µg/ml	4 µg/ml	0.5 - 128 µg/ml
	Oligomer 5	0.5 µg/ml	32 µg/ml	0.5 µg/ml	0.125 - 128 µg/ml

Haemophilus influenzae

		MIC ₅₀	MIC ₉₀	Median MIC	Range of MICs
15	Oligomer 4	8 µg/ml	8 µg/ml	8 µg/ml	2 - 32 µg/ml
	Oligomer 5	16 µg/ml	128 µg/ml	16 µg/ml	2 - 128 µg/ml

Oligomer 4 and Oligomer 5 demonstrated the lowest MICs for *Staphylococcus aureus* fluoroquinolone-susceptible, *Staphylococcus aureus* fluoroquinolone-resistant, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-susceptible, *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*) fluoroquinolone-resistant. Median MIC determinations were less than or equal to 0.25 µg/ml for the compounds against the ocular isolates of these species. The median MICs for Oligomer 4 and Oligomer 5 against *Streptococcus pneumoniae* and *Moraxella* species (including *Moraxella catarrhalis*) were less than or equal to 4 µg/ml. The median MIC for Oligomer 4 against *Streptococcus viridans* group was 4 µg/ml whereas the median MIC for Oligomer 5 was 16 µg/ml. Oligomer 4 and Oligomer 5 demonstrated the highest MICs against the Gram-negative pathogens *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. The median MIC of Oligomer 4 to *Pseudomonas aeruginosa*, *Serratia marcescens* and *Haemophilus influenzae* were 4, 8, and 8 µg/ml respectively. The median MICs of Oligomer 5 to *Pseudomonas aeruginosa*, *Serratia marcescens* and *Haemophilus influenzae* were 64, 128, and 16 µg/ml respectively. Overall, MICs for the Control Bacteria (*E. coli* D31 and *S. aureus* ATCC 27660) for each date on which MICs were performed were within the acceptable standard of a 1-2 dilution range in MICs from the MICs previously obtained for those compounds and between different preparation days. The

addition of 2% lysed horse red blood cells to the Mueller-Hinton broth for MIC testing with *Streptococcus pneumoniae*, *Moraxella* species (including *Moraxella catarrhalis*), and *Streptococcus viridans* group appeared to decrease the activity of the Oligomer 4 against the Control Bacteria (*E. coli* D31 and *S. aureus* ATCC 27660) approximately 4 fold. It is unknown
5 whether the 2% lysed horse red blood cells had the same effect on the test isolates. The addition of 2% lysed horse red blood cells to the Mueller-Hinton broth for MIC testing with *Streptococcus pneumoniae*, *Moraxella* species (including *Moraxella catarrhalis*), and *Streptococcus viridans* group generally appeared to increase or have no effect on the activity of the Oligomer 5 against the Control Bacteria (*E. coli* D31 and *S. aureus* ATCC 27660). It is
10 unknown whether the 2% lysed horse red blood cells had the same effect on the test isolates. The use of HTM broth for the MIC testing of *Haemophilus influenzae* appeared to decrease the activity of the Oligomer 4 and Oligomer 5 against the Control Bacteria *S. aureus* ATCC 27660 approximately 8 fold. The use of HTM broth for the MIC testing of *Haemophilus influenzae* appeared to decrease the activity of the Oligomer 4 against the Control Bacteria *E. coli* D31
15 approximately 8 fold but appeared to have no effect on the activity of Oligomer 5 against the Control Bacteria *E. coli* D31.

Oligomer 4 and Oligomer 5 demonstrated the lowest MICs against a variety of Gram-positive ocular bacterial isolates and at least one Gram-negative ocular bacterial species (*Moraxella*). Oligomer 4 and Oligomer 5 demonstrated varying *in vitro* antibacterial activity
20 against the three species that are the leading causes of conjunctivitis (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*). The order of the lower MICs for Oligomer 4 and Oligomer 5 against the species was: *Staphylococcus aureus* < *Streptococcus pneumoniae* < *Haemophilus influenzae*. (< = lower MICs). Oligomer 4 demonstrated lower MICs than Oligomer 5 for all bacterial species tested except for the Staphylococcal species
25 (equipotent) and for *Moraxella* species (less potent).

Example 9: Ker-1

One purpose of the following experiments was to compare the efficacy of 1% Oligomer 2, 0.5% Oligomer 2, and 5% vancomycin in the treatment of a fluoroquinolone-resistant,
30 methicillin-resistant *Staphylococcus aureus* infection in the NZW rabbit keratitis model with or without intact corneal epithelia.

Fifteen rabbits were received from Myrtles' Rabbitry, Thompson Station, TN. The clinical isolate of fluoroquinolone-resistant, methicillin-resistant (MRSA) *Staphylococcus aureus* (K950) was subcultured on 5% sheep blood agar and incubated at 37°C in 6% CO₂ overnight.

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The next morning, the MRSA strain was suspended in sterile trypticase soy broth to a 0.5 McFarland Standard, containing approximately 5×10^8 cfu/ml of bacteria. The absorbance of the suspension was measured at 650 nm using a Beckman DU-70 spectrophotometer. OD readings of 0.07 corresponded to 5×10^8 cfu/ml of bacteria. This concentration was appropriately diluted in sterile trypticase soy broth to provide the inoculum of approximately 1,000 (1.0×10^3) cfu/eye in 25 μ l. Colony counts were performed on the inoculum to determine the actual cfu inoculated. Following general anesthesia with ketamine and xylazine and topical anesthesia with proparacaine and prior to bacterial inoculation in the left eyes, 6 mm areas of the corneal epithelia was removed centrally with an Amoils epithelial scrubber. Nothing was done to the right eyes. The 15 rabbits were then inoculated intrastromally in both eyes with 25 μ l of the bacterial dilution of approximately 10^3 cfu/eye of the bacteria. The bacterial inoculation of the left eyes was directly under the epithelial defect created by the Amoils epithelial scrubber. The epithelia were removed in the left corneas in order to determine whether this layer of the cornea is a barrier for Oligomer 2 penetration when compared to the right cornea with an intact epithelium. A colony count was done on the inoculum to determine the actual cfu inoculated. The rabbits were immediately treated with analgesia in the form of and intramuscular injection of ketoprofen, 1.5 mg/kg. After 4 hours, the 15 rabbits were divided into 4 treatment groups and one untreated control group sacrificed at the onset of therapy. Both eyes of each rabbit of the treatment groups were treated with one 37 μ l drop of the coded solutions or control Saline or 1 drop of vancomycin from its dropper bottle. The Oligomer 2 concentrations were masked and labeled as 1 and 2. The masked concentrations were 1% Oligomer 2 and 0.5% Oligomer 2 but the specific concentrations of solutions were not known to the lab workers who carried out the experiment. The vancomycin and control (Tris-Buffered Saline) were not masked.

Groups:

Group	Left Eye	Right Eye	Rx - Both Eyes	Treatment Regimen	Rabbit #
I	Abraded Epithelium	Intact Epithelium	Oligomer 2 (PMX-1)	Every 15 minutes for 5 hours (21 total doses)	1-3
II	Abraded Epithelium	Intact Epithelium	Oligomer 2 (PMX-2)	Every 15 minutes for 5 hours (21 total doses)	4-6

III	Abraded Epithelium	Intact Epithelium	Vancomycin (50 mg/ml) (Van)	Every 15 minutes for 5 hours (21 total doses)	7-9
IV	Abraded Epithelium	Intact Epithelium	Tris-Buffered Saline (Con)	Every 15 minutes for 5 hours (21 total doses)	10-12
V	Abraded Epithelium	Intact Epithelium	Sacrifice at Onset of Therapy (4 hours PI) (ONSET)	None	13-15

Treatment was scheduled for every 15 minutes for 5 hours (21 total doses). However, PMX-1 and PMX-2 were exhausted after the 19th dose. Therefore, the actual treatment was 15 minutes for 4.5 hours (19 total doses). The 3 rabbits in group V were sacrificed 4 hours PI and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of PBS and kept on ice. The corneal buttons were homogenized for 25 seconds on ice using the motorized homogenizer. After homogenization, colony counts were done on the homogenates using 5% sheep blood agar plates to determine the amount of bacteria contained in the corneas at the onset of therapy.

Following the completion of therapy, the eyes were examined for clinical signs of infection. One hour after the final treatment, the treated rabbits (Groups I-IV) were sacrificed and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of PBS and kept on ice. The corneal buttons were homogenized for 25 seconds on ice using the motorized homogenizer. After homogenization, colony counts were performed on the homogenates using 5% sheep blood agar plates to determine the amount of bacteria contained in the corneas after treatment. The next morning, the plates were counted and the number of cfu/eye of *Staphylococcus aureus* was determined for each cornea.

Formulations: 1) Oligomer 2 (PMX-1): Oligomer 2 powder, on the day of treatment, was dissolved in 5 ml of Tris-Buffered Saline (TBS) before use. The solution was stored at room temperature during the 5 hours of use. 37 µl drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode. This solution was designated PMX-1. 2) Oligomer 2 (PMX-2): Oligomer 2 powder, on the day of treatment, was dissolved in 5 ml of Tris-Buffered Saline (TBS) before use. The solution was stored at room temperature during the 5 hours of use. 37 µl drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode. This solution was designated PMX-2. 3) 5% Vancomycin (50 mg/ml): Vancomycin (50 mg/ml) eye drops was purchased from the UPMC pharmacy as the fortified preparation used in patients.

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Vancomycin was administered using is supplied dropper bottle. 4) Control (Tris-Buffered Saline): 37 µl drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode.

IACUC Protocol #0701145 “The *In Vivo* Evaluation of Biomimetics as Topical Ocular Antibiotics”.

MIC Characterization of Fluoroquinolone-Resistant
Methicillin-Resistant *Staphylococcus aureus* Strain K950

	Antibiotic	MIC [µg/ml] (Minimum Inhibitory Concentration)
	Oligomer 2	0.25 µg/ml
10	Vancomycin	2 µg/ml

Drop Schedule

	Drop #	Time	Time of Day
	1	0	9:30
15	2	:15	9:45
	3	:30	10:00
	4	:45	10:15
	5	1:00	10:30
	6	1:15	10:45
20	7	1:30	11:00
	8	1:45	11:15
	9	2:00	11:30
	10	2:15	11:45
	11	2:30	12:00
25	12	2:45	12:15
	13	3:00	12:30
	14	3:15	12:45
	15	3:30	1:00
	16	3:45	1:15
30	17	4:00	1:30
	18	4:15	1:45
	19	4:30	2:00**

**Drops were stopped after Drop 19 because all of the PMX-1 and PMX-2 solutions were used at that time. Sacrifice rabbits 1 hour after final drop (3:00).

	PMX-Ker-1	Definitions of Abbreviations
	PMX-1-IE	Oligomer 2 with Intact Epithelium
	PMX-1-AE	Oligomer 2 with Abraded Epithelium
5	PMX-2-IE	Oligomer 2 with Intact Epithelium
	PMX-2-AE	Oligomer 2 with Abraded Epithelium
	VAN-IE	5% Vancomycin with Intact Epithelium
	VAN-AE	5% Vancomycin with Abraded Epithelium
	CON-AE	Tris-Buffered Saline Control with Abraded Epithelium
10	CON-IE	Tris-Buffered Saline Control with Intact Epithelium

Clinical Evaluation - Results

Eye	Group	Conj.	Chemosis	Discharge	Iritis	Corneal Edema	Corneal Infiltrate	Total Score
1R	PMX-1-IE	2.5	2.5	3.0	1.5	2.0	2.5	14.0
2R	PMX-1-IE	3.0	3.0	2.5	0.5	1.0	2.5	12.5
3R	PMX-1-IE	3.0	3.0	3.0	0.5	1.5	2.5	14.5
1L	PMX-1-AE	2.5	2.5	3.0	1.5	2.5	1.0	13.0
2L	PMX-1-AE	3.0	3.0	3.0	0.5	1.0	0	10.5
3L	PMX-1-AE	2.0	2.0	3.0	0.5	1	0.5	9.0
4R	PMX-2-IE	3.0	3.0	3.0	2.0	2.5	2.0	15.5
5R	PMX-2-IE	3.0	3.0	3.0	2.0	2.0	2.5	15.5
6R	PMX-2-IE	3.0	3.0	3.0	0.5	2.0	2.0	13.5
4L	PMX-2-AE	3.0	3.0	3.0	2.0	1.0	0	12.0
5L	PMX-2-AE	3.0	3.0	2.5	1.0	2.0	0	11.5
6L	PMX-2-AE	3.0	3.0	2.5	1.0	1.0	0	10.5

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7R	VAN-IE	2.5	3.0	3.0	2.0	1.0	0	11.5
8R	VAN-IE	3.0	3.0	3.0	2.0	1.0	0	12.0
9R	VAN-IE	3.0	3.0	2.5	1.0	1.0	0.5	11.0
7L	VAN-AE	3.0	3.0	3.0	1.0	0.5	0.5	11.0
8L	VAN-AE	2.5	2.5	2.0	1.5	1.0	0	9.5
9L	VAN-AE	2.5	2.5	2.5	1.5	1.0	0	10.0
10R	CON-IE	1.5	2.0	0.5	1.0	1.0	2.5	8.5
11R	CON-IE	1.0	0	0	0.5	2.0	2.5	6.0
12R	CON-IE	0	0	0	1.5	1.0	2.5	5.0
10L	CON-AE	1.5	2.0	1.0	1.5	1.0	0.5	7.5
11L	CON-AE	1.5	2.0	0.5	0.5	0	0.5	5.0
12L	CON-AE	0.5	0.5	1.0	1.0	1.5	2.0	6.5

Scale 0 = Normal; 0.5 = Trace; 1.0 = Mild; 1.5 = Mild/Moderate; 2.0 = Moderate;
2.5 = Moderate/Severe; 3.0 = Severe

5 Clinical Evaluation - Statistics

Descriptive Statistics		Total Ocular Score				
Variable	N	Mean	Median	TrMean	StDev	SE Mean
PMX-1-IE Score	3	13.667	14.000	13.667	1.041	0.601
<u>PMX-1-AE Score</u>	<u>3</u>	<u>10.83</u>	<u>10.50</u>	<u>10.83</u>	<u>2.02</u>	<u>1.17</u>
10 PMX-2-IE Score	3	14.833	15.500	14.833	1.155	0.667
<u>PMX-2-AE Score</u>	<u>3</u>	<u>11.333</u>	<u>11.500</u>	<u>11.333</u>	<u>0.764</u>	<u>0.441</u>
VAN-IE Score	3	11.500	11.500	11.500	0.500	0.289
<u>VAN-AE Score</u>	<u>3</u>	<u>10.167</u>	<u>10.000</u>	<u>10.167</u>	<u>0.764</u>	<u>0.441</u>
CON-IE Score	3	6.50	6.00	6.50	1.80	1.04
15 <u>CON-AE Score</u>	<u>3</u>	<u>6.333</u>	<u>6.500</u>	<u>6.333</u>	<u>1.258</u>	<u>0.726</u>

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Duncan Multiple Comparisons Test

Total Score

Row #	Group/Level	Mean Rank	C.I. Overlaps	
1	CON-IE Sco	3.5000	2,	
2	CON-AE Sco	3.5000	1,	
5	3	VAN-AE Sco	9.8333	4, 5, 6,
4	PMX-1-AE S	12.1667	3, 5, 6,	P=0.05
5	PMX-2-AE S	13.8333	3, 4, 6,	
6	VAN-IE Sco	14.5000	3, 4, 5,	
7	PMX-1-IE S	20.3333	8,	
10	8	PMX-2-IE S	22.3333	7,

CON IE = CON AE < VAN AE = PMX-1AE = PMX-2AE = VAN IE < PMX-1 IE = PMX-2 IE

Microbiological Results

Inoculum = 1048 cfu/cornea

15	Data Display	CFU/ml					
	Row	PMX-1-IE	PMX-1-AE	PMX-2-IE	PMX-2-AE		
	1	1700000	35	130000	5		
	2	16400	2380	1550000	100		
	3	10300000	750	15600000	0		
20	Row	VAN-IE	VAN-AE	CON-IE	CON-AE	Onset-IE	Onset-AE
	1	550	200	16000000	1200000	90000000	79500
	2	450	700	3550000	85000	140000	32000
	3	600	750	8700000	7500000	98000	110000

25 Log₁₀ CFU/ml

Row	PMX-1-IE Log	PMX-1-AE Log	PMX-2-IE Log	PMX-2-AE Log
1	6.23045	1.54407	5.11394	0.69897
2	4.21484	3.37658	6.19033	2.00000
3	7.01284	2.87506	7.19312	0.00000

30

Row	VAN-IE Log	VAN-AE Log	CON-IE Log	CON-AE Log
1	2.74036	2.30103	7.20412	6.07918
2	2.65321	2.84510	6.55023	4.92942
3	2.77815	2.87506	6.93952	6.87506

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	Row	Onset-IE Log		Onset-AE Log			
	1	7.95424		4.90037			
	2	5.14613		4.50515			
	3	4.99123		5.04139			
5	Descriptive Statistics			Log ₁₀ CFU/ml			
	Variable	N	Mean	Median	TrMean	StDev	SE Mean
	PMX-1-IE	3	5.819	6.230	5.819	1.444	0.833
	<u>PMX-1-AE</u>	<u>3</u>	<u>2.599</u>	<u>2.875</u>	<u>2.599</u>	<u>0.947</u>	<u>0.547</u>
	PMX-2-IE	3	6.166	6.190	6.166	1.040	0.600
10	<u>PMX-2-AE</u>	<u>3</u>	<u>0.900</u>	<u>0.699</u>	<u>0.900</u>	<u>1.015</u>	<u>0.586</u>
	VAN-IE Log	3	2.7239	2.7404	2.7239	0.0641	0.0370
	<u>VAN-AE Log</u>	<u>3</u>	<u>2.674</u>	<u>2.845</u>	<u>2.674</u>	<u>0.323</u>	<u>0.187</u>
	CON-IE Log	3	6.898	6.940	6.898	0.329	0.190
	<u>CON-AE Log</u>	<u>3</u>	<u>5.961</u>	<u>6.079</u>	<u>5.961</u>	<u>0.978</u>	<u>0.565</u>
15	Onset-IE Log	3	6.031	5.146	6.031	1.668	0.963
	Onset-AE Log	3	4.816	4.900	4.816	0.278	0.160

Microbiological Results - Intact Epithelium

One-way Analysis of Variance

Log₁₀ CFU/ml

20 Analysis of Variance for Counts I

Source	DF	SS	MS	F	P
Rx Corn	4	31.45	7.86	6.49	0.008
Error	10	12.12	1.21		
Total	14	43.57			

25 Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+--
CON	3	6.898	0.329	(-----*-----)
Onset	3	6.031	1.668	(-----*-----)
30 PMX-1	3	5.819	1.444	(-----*-----)
PMX-2	3	6.166	1.040	(-----*-----)
VAN	3	2.724	0.064	(-----*-----)
				-----+-----+-----+-----+--
Pooled StDev =		1.101		2.0 4.0 6.0 8.0

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Fisher's pairwise comparisons

Family error rate = 0.245

Individual error rate = 0.0500

5 Critical value = 2.228

Intervals for (column level mean) - (row level mean)

		CON	Onset	PMX-1	PMX-2
10	Onset	-1.135			
		2.870			
	PMX-1	-0.924	-1.791		
		3.081	2.214		
	PMX-2	-1.270	-2.138	-2.349	
		2.735	1.867	1.656	
15	VAN	2.172	1.304	1.093	1.439
		6.177	5.309	5.098	5.444

VAN < PMX-1 = ONSET = PMX-2 = CON

Microbiological Results - Intact Epithelium

20 Power and Sample Size

One-way ANOVA

Sigma = 1.101 Alpha = 0.05 Number of Levels = 5

Corrected Sum of Squares of Means = 10.4840

Means = 6.898, 6.031, 5.819, 6.166, 2.724

25 Sample

Size Power

3 0.9137

	Duncan Multiple Comparisons Test			Log ₁₀ CFU/ml
30	Row #	Group/Level	Mean Rank	C.I. Overlaps
	1	VAN-IE Log	2.0000	
	2	PMX-1-IE L	8.3333	3, 4, 5,
	3	PMX-2-IE L	9.0000	2, 4, 5, P=0.05
	4	Onset-IE L	9.0000	2, 3, 5,

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5 CON-IE Log 11.6667 2, 3, 4,

VAN < PMX-1 = PMX-2 = ONSET = CON

5 Microbiological Results - Abraded Epithelium

One-way Analysis of Variance

Log₁₀ CFU/ml

Analysis of Variance for Counts A

Source	DF	SS	MS	F	P
Rx Corn	4	47.954	11.989	19.55	0.000
Error	10	6.131	0.613		
Total	14	54.086			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-+-----+-----+-----+-----
15 CON	3	5.9612	0.9782	(----*----)
Onset	3	4.8156	0.2780	(----*----)
PMX-1	3	2.5986	0.9470	(----*----)
PMX-2	3	0.8997	1.0150	(----*----)
VAN	3	2.6737	0.3231	(----*----)
20				-+-----+-----+-----+-----
Pooled StDev =		0.7830		0.0 2.0 4.0 6.0

Fisher's pairwise comparisons

Family error rate = 0.245

25 Individual error rate = 0.0500

Critical value = 2.228

Intervals for (column level mean) - (row level mean)

	CON	Onset	PMX-1	PMX-2
30 Onset	-0.2788			
	2.5700			
PMX-1	1.9382	0.7926		
	4.7871	3.6415		
PMX-2	3.6371	2.4916	0.2745	

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6.4860 5.3404 3.1233
 VAN 1.8631 0.7175 -1.4996 -3.1985
 4.7119 3.5663 1.3493 -0.3496

PMX-2 < PMX-1 = VAN < ONSET = CON

5

Microbiological Results - Abraded Epithelium

Power and Sample Size

One-way ANOVA

Sigma = 0.783 Alpha = 0.05 Number of Levels = 5

10 Corrected Sum of Squares of Means = 16.0707

Means = 5.9612, 4.8456, 2.5986, 0.8997, 2.6737

Sample

Size Power

15 3 1.0000

Duncan Multiple Comparisons Test Log₁₀ CFU/ml

Row #	Group/Level	Mean Rank	C.I. Overlaps
1	PMX-2-AE L	2.3333	2, 3,
20 2	VAN-AE Log	6.1667	1, 3,
3	PMX-1-AE L	6.5000	1, 2, P=0.05
4	Onset-AE L	11.3333	5,
5	CON-AE Log	13.6667	4,

PMX-2 = VAN = PMX-1 < ONSET = CON

25

Microbiological Results - Intact vs. Abraded Epithelium

Two Sample T-Test and Confidence Interval - PMX-1

Two sample T for PMX-1-IE Log vs PMX-1-AE Log

	N	Mean	StDev	SE Mean
30 PMX-1-IE	3	5.82	1.44	0.83
PMX-1-AE	3	2.599	0.947	0.55

95% CI for mu PMX-1-IE - mu PMX-1-AE: (0.05, 6.39)

T-Test mu PMX-1-IE = mu PMX-1-AE (vs not =): T = 3.23 P = 0.048 DF = 3

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Abraded < Intact

Two Sample T-Test and Confidence Interval - PMX-2

Two sample T for PMX-2-IE Log vs PMX-2-AE Log

5		N	Mean	StDev	SE Mean
	PMX-2-IE	3	6.17	1.04	0.60
	PMX-2-AE	3	0.90	1.01	0.59

95% CI for mu PMX-2-IE - mu PMX-2-AE: (2.60, 7.94)

10 T-Test mu PMX-2-IE = mu PMX-2-AE (vs not =): T = 6.28 P = 0.0082 DF = 3

Abraded < Intact

Two Sample T-Test and Confidence Interval - VAN

Two sample T for VAN-IE Log vs VAN-AE Log

		N	Mean	StDev	SE Mean
15	VAN-IE L	3	2.7239	0.0641	0.037
	VAN-AE L	3	2.674	0.323	0.19

95% CI for mu VAN-IE L - mu VAN-AE L: (-0.768, 0.87)

T-Test mu VAN-IE L = mu VAN-AE L (vs not =): T = 0.26 P = 0.82 NS DF = 2

20

Two Sample T-Test and Confidence Interval - CON

Two sample T for CON-IE Log vs CON-AE Log

		N	Mean	StDev	SE Mean
	CON-IE L	3	6.898	0.329	0.19
25	CON-AE L	3	5.961	0.978	0.56

95% CI for mu CON-IE L - mu CON-AE L: (-1.63, 3.50)

T-Test mu CON-IE L = mu CON-AE L (vs not =): T = 1.57 P = 0.26 NS DF = 2

30 Microbiological Results - Intact vs. Abraded Epithelium

Two Sample T-Test and Confidence Interval - Onset

Two sample T for Onset-IE Log vs Onset-AE Log

		N	Mean	StDev	SE Mean
	Onset-IE	3	6.03	1.67	0.96

Onset-AE 3 4.816 0.278 0.16

95% CI for mu Onset-IE - mu Onset-AE: (-2.99, 5.42)

T-Test mu Onset-IE = mu Onset-AE (vs not =): T = 1.24 P = 0.34 NS DF = 2

5

Summary of Statistical Comparisons for Microbiological Data

< = Significantly Fewer Colony Counts

Effect of Abraded Epithelium on Effectiveness of Each Test Solution or Onset Control

10	PMX-1	Abraded < Intact
	PMX-1	Abraded < Intact
	Vancomycin	Abraded = Intact
	Saline Control	Abraded = Intact
	Onset of Therapy Control	Abraded = Intact

15

Effect of Test Solutions on Corneas with Intact Epithelium

PMX-1 = PMX-2

PMX-1 = Saline Control

PMX-2 = Saline Control

20 Vancomycin < Saline Control

Vancomycin < PMX-1

Vancomycin < PMX-2

Effect of Test Solutions on Corneas with Abraded Epithelium

25 PMX-1 < Saline Control

PMX-2 < Saline Control

PMX-2 < PMX-1

Vancomycin < Saline Control

PMX-1 = Vancomycin

30 PMX-2 < Vancomycin

Summary of Results

PMX-1 and PMX-2 were effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model only when the

corneal epithelium was removed from the corneas. PMX-2 was more effective than 5% vancomycin in reducing colony counts fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model only when the corneal epithelium was removed from the corneas. PMX-1 was as effective as 5% vancomycin in
5 reducing colony counts fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model only when the corneal epithelium was removed from the corneas. PMX-1 and PMX-2 induced toxicity similar to 5% vancomycin as manifested by higher Total Ocular Scores compared with the Saline treated eyes in eyes with intact corneal epithelia. Removal of the epithelium increased the Total Ocular Scores of eyes treated 1% and
10 0.5% Oligomer 2 compared with the Total Ocular Scores of eyes treated with of 1% and 0.5% Oligomer 2 with intact epithelia.

Conclusions

The biomimetic Oligomer 2 was effective in reducing the number fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis
15 model. However, Oligomer 2 was effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts only when the corneal epithelium was removed. This suggests that Oligomer 2 does not penetrate the corneal epithelium into the corneal stroma. In the current study, PMX-1 and PMX-2 induced greater toxicity in infected rabbit eyes
20 compared with the Mildly Irritating toxicity induced in uninfected rabbit eyes in experiment PMX-Tox-1.

Example 10: Ker-2

One purpose of the following experiments was to compare the efficacy of 0.25%
25 Oligomer 2, with and without 0.005% benzalkonium chloride, and 5% vancomycin in the treatment of a fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* infection in the NZW rabbit keratitis model with or without intact corneal epithelia. The 0.005% benzalkonium chloride has been added to try to increase the penetration of 0.25% Oligomer 2 through the corneal epithelium.

30 Fifteen rabbits were received from Myrtles' Rabbitry, Thompson Station, TN. The clinical isolate of fluoroquinolone-resistant, methicillin-resistant (MRSA) *Staphylococcus aureus* (K950) was subcultured on 5% sheep blood agar and incubated at 37°C in 6% CO₂ overnight. The next morning, the MRSA strain was suspended in sterile trypticase soy broth to a 0.5 McFarland Standard, containing approximately 5×10^8 cfu/ml of bacteria. The absorbance of the

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suspension was measured at 650 nm using a Beckman DU-70 spectrophotometer. OD readings of 0.07 corresponded to 5×10^8 cfu/ml of bacteria. This concentration was appropriately diluted in sterile trypticase soy broth to provide the inoculum of approximately 1,000 (1.0×10^3) cfu/eye in 25 μ l. Colony counts were performed on the inoculum to determine the actual cfu inoculated.

5 Following general anesthesia with ketamine and xylazine and topical anesthesia with proparacaine and prior to bacterial inoculation in the left eyes, 6 mm areas of the corneal epithelia was removed centrally with an Amoils epithelial scrubber. Nothing was done to the right eyes. The 15 rabbits were then inoculated intrastromally in both eyes with 25 μ l of the bacterial dilution of approximately 10^3 cfu/eye of the bacteria. The bacterial inoculation of the

10 left eyes was directly under the epithelial defect created by the Amoils epithelial scrubber. The epithelia were removed in the left corneas in order to determine whether this layer of the cornea is a barrier for Oligomer 2 penetration when compared to the right cornea with an intact epithelium. A colony count was done on the inoculum to determine the actual cfu inoculated. The rabbits were immediately treated with analgesia in the form of and intramuscular injection

15 of ketoprofen, 1.5 mg/kg. After 4 hours, the 15 rabbits were divided into 4 treatment groups and one untreated control group sacrificed at the onset of therapy. Both eyes of each rabbit of the treatment groups were treated with one 37 μ l drop of the PMX solutions or control Saline or 1 drop of vancomycin from its dropper bottle.

20 Groups:

Group	Left Eye	Right Eye	Rx - Both Eyes	Treatment Regimen	Rabbit #
I	Abraded Epithelium	Intact Epithelium	0.25% Oligomer 2 (PMX)	Every 15 minutes for 5 hours (21 total doses)	1-3
II	Abraded Epithelium	Intact Epithelium	0.25% Oligomer 2 with 0.005% BAK (PMX-B)	Every 15 minutes for 5 hours (21 total doses)	4-6
III	Abraded Epithelium	Intact Epithelium	Vancomycin (50 mg/ml) (Van)	Every 15 minutes for 5 hours (21 total doses)	7-9
IV	Abraded Epithelium	Intact Epithelium	Tris-Buffered Saline (Con)	Every 15 minutes for 5 hours (21 total doses)	10-12
V	Abraded Epithelium	Intact Epithelium	Sacrifice at Onset of Therapy (4 hours PI) (ONSET)	None	13-15

Treatment was scheduled for every 15 minutes for 5 hours (21 total doses). The 3 rabbits in group V were sacrificed 4 hours PI and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of PBS and kept on ice. The corneal buttons were homogenized for 25 seconds on ice using the motorized homogenizer. After homogenization, colony counts were done on the homogenates using 5% sheep blood agar plates to determine the amount of bacteria contained in the corneas at the onset of therapy. Following the completion of therapy, the eyes were examined for clinical signs of infection. One hour after the final treatment, the treated rabbits (Groups I-IV) were sacrificed and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of PBS and kept on ice. The corneal buttons were homogenized for 25 seconds on ice using the motorized homogenizer. After homogenization, colony counts were performed on the homogenates using 5% sheep blood agar plates to determine the amount of bacteria contained in the corneas after treatment. The next morning, the plates were counted and the number of cfu/eye of *Staphylococcus aureus* was determined for each cornea.

Formulations: 1) 0.25% Oligomer 2 (PMX): Oligomer 2 Powder (Lot 8 - 15.1 mg), on the day of treatment, was dissolved in 6.04 ml of Tris-Buffered Saline (TBS) to yield 0.25% Oligomer 2. The solution was stored at room temperature during the 5 hours of use. 37 μ l drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode. This solution was designated PMX. 2) 0.25% Oligomer 2 with 0.005% Benzalkonium Chloride (BAK) (PMX-B): Oligomer 2 Powder (Lot 8 - 15.8 mg), on the day of treatment, was dissolved in 6.288 ml of Tris-Buffered Saline (TBS) before use. Then, 0.032 ml (32 μ l) of 1% Benzalkonium Chloride was added to the solution to yield a total volume of 6.32 ml of 0.25% Oligomer 2. The solution was stored at room temperature during the 5 hours of use. 37 μ l drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode. This solution was designated PMX-B. 3) 5% Vancomycin (50 mg/ml): Vancomycin (50 mg/ml) eye drops were purchased from the UPMC pharmacy as the fortified preparation used in patients. Vancomycin was administered using is supplied dropper bottle. 4) Control (Tris-Buffered Saline): 37 μ l drops of Tris-Buffered Saline were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode.

IACUC Protocol #0701145 "The *In Vivo* Evaluation of Biomimetics as Topical Ocular Antibiotics".

MIC Characterization of Fluoroquinolone-Resistant, Methicillin-Resistant

Staphylococcus aureus Strain K950

Antibiotic	MIC [μ g/ml] (Minimum Inhibitory Concentration)
Oligomer 2	0.25 μ g/ml
Vancomycin	2 μ g/ml

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Drop Schedule

	Drop #	Time	Time of Day
	1	0	9:30
	2	:15	9:45
5	3	:30	10:00
	4	:45	10:15
	5	1:00	10:30
	6	1:15	10:45
	7	1:30	11:00
10	8	1:45	11:15
	9	2:00	11:30
	10	2:15	11:45
	11	2:30	12:00
	12	2:45	12:15
15	13	3:00	12:30
	14	3:15	12:45
	15	3:30	1:00
	16	3:45	1:15
	17	4:00	1:30
20	18	4:15	1:45
	19	4:30	2:00
	20	4:45	2:15
	21	5:00	2:30

Sacrifice rabbits 1 hour after final drop (3:00).

25 Definitions of Abbreviations

PMX-IE	0.25% Oligomer 2 with Intact Epithelium
PMX-AE	0.25% Oligomer 2 with Abraded Epithelium
PMX-B-IE	0.25% Oligomer 2 with 0.005% BAK with Intact Epithelium
PMX-B-AE	0.25% Oligomer 2 with 0.005% BAK with Abraded Epithelium
30 VAN-IE	5% Vancomycin with Intact Epithelium
VAN-AE	5% Vancomycin with Abraded Epithelium
CON-AE	Tris-Buffered Saline Control with Abraded Epithelium
CON-IE	Tris-Buffered Saline Control with Intact Epithelium
PMX	0.25% Oligomer 2

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PMX-B 0.25% Oligomer 2 with 0.005% BAK

Clinical Evaluation - Results

Eye	Group	Conj.	Chemosis	Discharge	Iritis	Corneal Edema	Corneal Infiltrate	Total Score
1R	PMX-IE	3.0	3.0	2.5	1.0	1.5	2.5	13.5
2R	PMX-IE	2.5	2.5	2.5	2.0	2.0	2.0	13.5
3R	PMX-IE	2.5	3.0	2.5	1.5	1.5	2.5	13.5
1L	PMX-AE	3.0	2.0	2.5	1.0	1.0	0.5	10.0
2L	PMX-AE	2.5	2.5	2.0	2.0	1.5	0	10.5
3L	PMX-AE	2.5	2.5	2.5	0.5	0.5	0	8.5
4R	PMX-B-IE	3.0	3.0	3.0	1.5	1.5	2.5	14.5
5R	PMX-B-IE	3.0	3.0	3.0	2.0	0.5	2.5	14.0
6R	PMX-B-IE	3.0	3.0	3.0	1.0	1.0	2.5	13.5
4L	PMX-B-AE	2.5	2.5	2.5	2.0	1.0	0	10.5
5L	PMX-B-AE	2.5	2.5	2.5	1.0	1.0	0	9.5
6L	PMX-B-AE	2.5	2.5	3.0	1.0	0.5	0	9.5
7R	VAN-IE	2.5	2.5	3.0	1.0	0.5	0.5	10.0
8R	VAN-IE	2.5	2.5	2.5	1.5	0.5	0	9.5
9R	VAN-IE	2.5	2.5	2.5	0.5	0.5	0	8.5
7L	VAN-AE	2.5	2.5	2.5	1.0	0.5	0.5	9.5
8L	VAN-AE	2.5	2.5	2.5	1.0	1.0	0	9.5
9L	VAN-AE	2.5	2.5	2.5	0.5	1.0	0	9.0
10R	CON-IE	0.5	0	1.0	0.5	0.5	3.0	5.5
11R	CON-IE	2.0	0.5	2.0	2.5	3.0	2.0	12.0
12R	CON-IE	1.5	0	2.5	2.0	0.5	3.0	9.5
10L	CON-AE	2.0	1.5	2.0	1.5	2.0	2.5	11.5
11L	CON-AE	2.0	2.0	1.5	1.5	1.0	0.5	8.5
12L	CON-AE	2.5	2.5	2.5	1.5	1.0	0.5	10.5

5 Scale 0 = Normal; 0.5 = Trace; 1.0 = Mild; 1.5 = Mild/Moderate; 2.0 = Moderate;
2.5 = Moderate/Severe; 3.0 = Severe

Clinical Evaluation - Statistics

Descriptive Statistics		Total Ocular Score				
Variable	N	Mean	Median	TrMean	StDev	SE Mean
5 PMX-IE Score	3	13.500	13.500	13.500	0.000	0.000
PMX-AE Score	3	9.667	10.000	9.667	1.041	0.601
PMX-B-IE Score	3	14.000	14.000	14.000	0.500	0.289
PMX-B-AE Score	3	9.833	9.500	9.833	0.577	0.333
VAN-IE Score	3	9.333	9.500	9.333	0.764	0.441
10 VAN-AE Score	3	9.333	9.500	9.333	0.289	0.167
CON-IE Score	3	9.00	9.50	9.00	3.28	1.89
CON-AE Score	3	10.167	10.500	10.167	1.528	0.882

Duncan Multiple Comparisons Test			Total Score
Row #	Group/Level	Mean Rank	C.I. Overlaps
15 1	VAN-AE Sco	7.3333	2, 3, 4, 5, 6,
2	VAN-IE Sco	8.0000	1, 3, 4, 5, 6,
3	CON-IE Sco	9.1667	1, 2, 4, 5, 6, P=0.05
4	PMX-AE Sco	10.1667	1, 2, 3, 5, 6,
20 5	PMX-B-AE S	10.6667	1, 2, 3, 4, 6,
6	CON-AE Sco	11.6667	1, 2, 3, 4, 5,
7	PMX-IE Sco	20.5000	8,
8	PMX-B-IE S	22.5000	7,

VAN-AE = VAN-IE = CON-IE = PMX-AE = PMX-B-AE = CON AE < PMX-IE = PMX-B-IE

25

Median Total Ocular Score

Treatment	Abraded (score)	Intact (score)
PMX	10	13.5
PMX-B	9.5	14
Vancomycin	9.5	9.5
Control	10.5	9.5

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Microbiological Results

Inoculum = 1371 cfu/cornea

Data Display

CFU/ml

	Row	PMX-IE	PMX-AE	PMX-B-IE	PMX-B-AE		
5	1	4750000	5	11000000	13500		
	2	4450000	8900	15350000	80		
	3	9650000	1200	12850000	190		
	Row	VAN-IE	VAN-AE	CON-IE	CON-AE	Onset-IE	Onset-AE
10	1	71000	550	5250000	3300000	100500	63000
	2	2200	200	13200000	510000	77000	74500
	3	350	600	14600000	965000	93500	44500

Data Display

Log₁₀ CFU/ml

15	Row	PMX-IE Log	PMX-AE Log	PMX-B-IE Log	PMX-B-AE Log
	1	6.67669	0.69897	7.04139	4.13033
	2	6.64836	3.94939	7.18611	1.90309
	3	6.98453	3.07918	7.10890	2.27875

20	Row	VAN-IE Log	VAN-AE Log	CON-IE Log	CON-AE Log
	1	4.85126	2.74036	6.72016	6.51851
	2	3.34242	2.30103	7.12057	5.70757
	3	2.54407	2.77815	7.16435	5.98453

25	Row	Onset-IE Log	Onset-AE Log
	1	5.00217	4.79934
	2	4.88649	4.87216
	3	4.97081	4.64836

30 Descriptive Statistics

Log₁₀ CFU/ml

Variable	N	Mean	Median	TrMean	StDev	SE Mean
PMX-IE Log	3	6.770	6.677	6.770	0.186	0.108
<u>PMX-AE Log</u>	<u>3</u>	<u>2.576</u>	<u>3.079</u>	<u>2.576</u>	<u>1.683</u>	<u>0.971</u>
PMX-B-IE Log	3	7.1121	7.1089	7.1121	0.0724	0.0418

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	PMX-B-AE Log	3	2.771	2.279	2.771	1.192	0.688
	VAN-IE Log	3	3.579	3.342	3.579	1.172	0.676
	VAN-AE Log	3	2.607	2.740	2.607	0.265	0.153
	CON-IE Log	3	7.002	7.121	7.002	0.245	0.141
5	CON-AE Log	3	6.070	5.985	6.070	0.412	0.238
	Onset-IE Log	3	4.9532	4.9708	4.9532	0.0598	0.0345
	Onset-AE Log	3	4.7733	4.7993	4.7733	0.1142	0.0659

Microbiological Results - Intact Epithelium

10 One-way Analysis of Variance Log_{10} CFU/ml

Analysis of Variance for Counts I

Source	DF	SS	MS	F	P
Rx Corn	4	29.162	7.290	24.69	0.000
Error	10	2.953	0.295		
15 Total	14	32.115			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	+-+-----+-----+-----+-----
CON	3	7.0017	0.2448	(----*---)
20 Onset	3	4.9532	0.0598	(----*----)
PMX	3	6.7699	0.1864	(----*----)
PMX-B	3	7.1121	0.0724	(---*----)
VAN	3	3.5792	1.1717	(----*----)

			+-+-----+-----+-----+-----
25 Pooled StDev =	0.5434	3.0	4.5 6.0 7.5

Fisher's pairwise comparisons

Family error rate = 0.245

Individual error rate = 0.0500

30 Critical value = 2.228

Intervals for (column level mean) - (row level mean)

	CON	Onset	PMX	PMX-B
Onset	1.0600			
	3.0370			

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	PMX	-0.7567	-2.8052		
		1.2203	-0.8282		
	PMX-B	-1.0989	-3.1475	-1.3308	
		0.8781	-1.1705	0.6462	
5	VAN	2.4339	0.3854	2.2021	2.5444
		4.4110	2.3624	4.1791	4.5214

VAN < ONSET = PMX = CON = PMX-B

Microbiological Results - Intact Epithelium

10	Duncan Multiple Comparisons Test			Log ₁₀ CFU/ml	
	Row #	Group/Level	Mean Rank	C.I. Overlaps	
	1	VAN-IE Log	2.0000	2,	
	2	Onset-IE L	5.0000	1,	
	3	PMX-IE Log	8.3333	4, 5, P=0.05	
15	4	CON-IE Log	12.0000	3, 5,	
	5	PMX-B-IE L	12.6667	3, 4,	

VAN = ONSET < PMX = CON = PMX-B

Microbiological Results - Abraded Epithelium

	One-way Analysis of Variance					Log ₁₀ CFU/ml
20	Analysis of Variance for Counts A					
	Source	DF	SS	MS	F	P
	Rx Corn	4	30.226	7.556	8.38	0.003
	Error	10	9.013	0.901		
	Total	14	39.238			
25	Individual 95% CIs For Mean					
	Based on Pooled StDev					
	Level	N	Mean	StDev	----+-----+-----+-----+--	
	CON	3	6.0702	0.4122	(-----*-----)	
	Onset	3	4.7733	0.1142	(-----*-----)	
30	PMX	3	2.5758	1.6827	(-----*-----)	
	PMX-B	3	2.7707	1.1923	(-----*-----)	
	VAN	3	2.6065	0.2652	(-----*-----)	
					----+-----+-----+-----+--	
	Pooled StDev =	0.9493		2.0	4.0	6.0 8.0

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Fisher's pairwise comparisons

Family error rate = 0.245

Individual error rate = 0.0500

Critical value = 2.228

5 Intervals for (column level mean) - (row level mean)

	CON	Onset	PMX	PMX-B
Onset	-0.4301			
	3.0239			
PMX	1.7673	0.4704		
10	5.2214	3.9244		
PMX-B	1.5725	0.2756	-1.9219	
	5.0265	3.7296	1.5321	
VAN	1.7367	0.4398	-1.7577	-1.5628
	5.1907	3.8938	1.6963	1.8912

15 VAN = PMX = PMX-B < ONSET = CON

Microbiological Results - Abraded Epithelium

Duncan Multiple Comparisons Test Log₁₀ CFU/ml

Row #	Group/Level	Mean Rank	C.I. Overlaps
20	1 PMX-B-AE L	4.6667	2, 3, 4,
	2 VAN-AE Log	5.0000	1, 3, 4, P=0.05
	3 PMX-AE Log	5.3333	1, 2, 4,
	4 Onset-AE L	11.0000	1, 2, 3, 5,
	5 CON-AE Log	14.0000	4,

25 PMX-B = VAN = PMX < CON;

All Groups = ONSET

Microbiological Results - Intact vs. Abraded Epithelium

Two Sample T-Test and Confidence Interval - PMX

30 Two sample T for PMX-IE Log vs PMX-AE Log

	N	Mean	StDev	SE Mean
PMX-IE L	3	6.770	0.186	0.11
PMX-AE L	3	2.58	1.68	0.97

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95% CI for mu PMX-IE L - mu PMX-AE L: (-0.01, 8.40)

T-Test mu PMX-IE L = mu PMX-AE L (vs not =): T = 4.29 P=0.050 DF = 2

Abraded < Intact

5 Two Sample T-Test and Confidence Interval - PMX-B

Two sample T for PMX-B-IE Log vs PMX-B-AE Log

	N	Mean	StDev	SE Mean
PMX-B-IE	3	7.1121	0.0724	0.042
PMX-B-AE	3	2.77	1.19	0.69

10

95% CI for mu PMX-B-IE - mu PMX-B-AE: (1.374, 7.31)

T-Test mu PMX-B-IE = mu PMX-B-AE (vs not =): T = 6.29 P=0.024 DF = 2

Abraded < Intact

15 Two Sample T-Test and Confidence Interval - VAN

Two sample T for VAN-IE Log vs VAN-AE Log

	N	Mean	StDev	SE Mean
VAN-IE L	3	3.58	1.17	0.68
VAN-AE L	3	2.607	0.265	0.15

20

95% CI for mu VAN-IE L - mu VAN-AE L: (-2.01, 3.96)

T-Test mu VAN-IE L = mu VAN-AE L (vs not =): T = 1.40 P=0.30 NS DF = 2

Two Sample T-Test and Confidence Interval - CON

25 Two sample T for CON-IE Log vs CON-AE Log

	N	Mean	StDev	SE Mean
CON-IE L	3	7.002	0.245	0.14
CON-AE L	3	6.070	0.412	0.24

30 95% CI for mu CON-IE L - mu CON-AE L: (0.05, 1.81)

T-Test mu CON-IE L = mu CON-AE L (vs not =): T = 3.37 P=0.044 DF = 3

Abraded < Intact

Two Sample T-Test and Confidence Interval - Onset

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Two sample T for Onset-IE Log vs Onset-AE Log

	N	Mean	StDev	SE Mean
Onset-IE	3	4.9532	0.0598	0.035
Onset-AE	3	4.773	0.114	0.066

5

95% CI for mu Onset-IE - mu Onset-AE: (-0.057, 0.417)

T-Test mu Onset-IE = mu Onset-AE (vs not =): T = 2.42 P=0.094 NS DF = 3

Microbiological Results - 0.25% Oligomer 2 w/o BAK vs. w/ BAK - Intact Epithelium

10

Two Sample T-Test and Confidence Interval - PMX-IE vs. PMX-B-IE

Two sample T for PMX-IE Log vs PMX-B-IE Log

	N	Mean	StDev	SE Mean
PMX-IE L	3	6.770	0.186	0.11
PMX-B-IE	3	7.1121	0.0724	0.042

15

95% CI for mu PMX-IE L - mu PMX-B-IE: (-0.84, 0.155)

T-Test mu PMX-IE L = mu PMX-B-IE (vs not =): T = -2.96 P=0.097 NS DF = 2

20

Microbiological Results - 0.25% Oligomer 2 w/o BAK vs. w/ BAK - Abraded Epithelium

Two Sample T-Test and Confidence Interval - PMX-AE vs. PMX-B-AE

Two sample T for PMX-AE Log vs PMX-B-AE Log

	N	Mean	StDev	SE Mean
PMX-AE L	3	2.58	1.68	0.97
PMX-B-AE	3	2.77	1.19	0.69

25

95% CI for mu PMX-AE L - mu PMX-B-AE: (-3.98, 3.59)

30 T-Test mu PMX-AE L = mu PMX-B-AE (vs not =): T = -0.16 P=0.88 NS DF = 3

Summary of Statistical Comparisons for Microbiological Data

< = Significantly Fewer Colony Counts

Effect of Abraded Epithelium on Effectiveness of Each Test Solution or Onset Control

	PMX	Abraded < Intact
	PMX-B	Abraded < Intact
5	Vancomycin	Abraded = Intact
	Saline Control	Abraded < Intact
	Onset of Therapy Control	Abraded = Intact

Effect of Test Solutions on Corneas with Intact Epithelium

10

PMX = PMX-B

PMX = Saline Control

PMX-B = Saline Control

Vancomycin < Saline Control

15 Vancomycin < PMX

Vancomycin < PMX-B

Effect of Test Solutions on Corneas with Abraded Epithelium

PMX < Saline Control

20 PMX-B < Saline Control

PMX-B = PMX

Vancomycin < Saline Control

PMX = Vancomycin

PMX-B = Vancomycin

25

Effect of BAK on 0.25% Oligomer 2 on Corneas with Intact Epithelium

PMX = PMX-B

Effect of BAK on 0.25% Oligomer 2 on Corneas with Abraded Epithelium

30 PMX = PMX-B

Summary of Results

0.25% Oligomer 2 (PMX) and 0.25% Oligomer 2 with 0.005% benzalkonium chloride (BAK) (PMX-2) were effective in reducing fluoroquinolone-resistant, methicillin-resistant

Staphylococcus aureus colony counts in the NZW rabbit keratitis model only when the corneal epithelium was removed from the corneas. There was no difference in fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model between 0.25% Oligomer 2 (PMX) and 0.25% Oligomer 2 with 0.005% benzalkonium chloride (BAK) (PMX-2) with intact or abraded corneal epithelium. 0.25% Oligomer 2 (PMX) and 0.25% Oligomer 2 with 0.005% benzalkonium chloride (BAK) (PMX-2) were as effective as 5% vancomycin in reducing colony counts fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model only when the corneal epithelium was removed from the corneas. 0.25% Oligomer 2 (PMX) and 0.25% Oligomer 2 with 0.005% benzalkonium chloride (BAK) (PMX-2) induced toxicity that was worse than 5% vancomycin as manifested by higher Total Ocular Scores compared with the vancomycin and Saline treated eyes in eyes with intact corneal epithelia.

The biomimetic Oligomer 2 was effective in reducing the number fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model. This result was achieved using a lower concentration (0.25%) than in previous studies (1% and 0.5%). As in the previous studies, Oligomer 2 was effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts only when the corneal epithelium was removed. The addition of 0.005% benzalkonium chloride (BAK) did not aid in the penetration of 0.25% Oligomer 2 through the intact corneal epithelium to the site of the infection in the corneal stroma. In the current study, 0.25% Oligomer 2 (PMX) and 0.25% Oligomer 2 with 0.005% benzalkonium chloride (BAK) (PMX-2) induced greater toxicity in infected rabbit eyes with intact corneal epithelium compared with 5% vancomycin and the Saline treated Control with intact corneal epithelium. As suggested in the previous study, additional studies using much lower concentrations of Oligomer 2 and/or different formulations should be considered in order to reduce its toxicity, yet retain efficacy in the fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* NZW rabbit keratitis model.

Example 11: Ker-3

One purpose of the following experiments was to determine the efficacy of 0.25% Oligomer 4, with and without 200 μ M Farnesol, and 200 μ M Farnesol in the treatment of a fluoroquinolone-resistant and methicillin-resistant *Staphylococcus aureus* infection in the NZW rabbit keratitis model with or without intact corneal epithelia. The 200 μ M Farnesol has been added to try to increase the efficacy and penetration of 0.25% Oligomer 4 through the corneal epithelium.

Fifteen rabbits were received from Myrtles' Rabbitry, Thompson Station, TN. The clinical isolate of fluoroquinolone-resistant and methicillin-resistant (MRSA) *Staphylococcus aureus* (K950) was subcultured on 5% sheep blood agar and incubated at 37°C in 6% CO₂ overnight. The next morning, the MRSA strain was suspended in sterile trypticase soy broth to a 0.5 McFarland Standard, containing approximately 5×10^8 CFU/ml of bacteria. The absorbance of the suspension was measured at 650 nm using a Beckman DU-70 spectrophotometer. OD readings of 0.07 corresponded to 5×10^8 CFU/ml of bacteria. This concentration was appropriately diluted in sterile trypticase soy broth to provide the inoculum of approximately 1,000 (1.0×10^3) CFU/eye in 25 μ l. Colony counts were performed on the inoculum to determine the actual CFU inoculated. Following general anesthesia with ketamine and xylazine and topical anesthesia with proparacaine and prior to bacterial inoculation in the left eyes, 6 mm areas of the corneal epithelia were removed centrally from the left eyes with an Amoils epithelial scrubber. Nothing was done to the right eyes. The 15 rabbits were then inoculated intrastromally in both eyes with 25 μ l of the bacterial dilution of approximately 10^3 cfu/eye of the bacteria. The bacterial inoculation of the left eyes was directly under the epithelial defect created by the Amoils epithelial scrubber. The epithelia were removed in the left corneas in order to determine whether this layer of the cornea is a barrier for drug penetration when compared to the right cornea with an intact epithelium. A colony count was done on the inoculum to determine the actual CFU inoculated. The rabbits were immediately treated with analgesia in the form of an intramuscular injection of ketoprofen, 1.5 mg/kg. After 4 hours, the 15 rabbits were divided into 4 treatment groups and one untreated control group sacrificed at the onset of therapy. Both eyes of each rabbit of the treatment groups were treated with one 37 μ l drop of the PMX solutions or control Saline.

25 Groups:

Group	Left Eye	Right Eye	Rx - Both Eyes	Treatment Regimen	Rabbit #
I	Abraded Epithelium	Intact Epithelium	0.25% Oligomer 4 (PMX)	Every 15 minutes for 5 hours (21 total doses)	1-3
II	Abraded Epithelium	Intact Epithelium	0.25% Oligomer 4 + 200 μ M Farnesol (P+F)	Every 15 minutes for 5 hours (21 total doses)	4-6

III	Abraded Epithelium	Intact Epithelium	200 μ M Farnesol (FARN)	Every 15 minutes for 5 hours (21 total doses)	7-9
IV	Abraded Epithelium	Intact Epithelium	Tris-Buffered Saline (CON)	Every 15 minutes for 5 hours (21 total doses)	10-12
V	Abraded Epithelium	Intact Epithelium	Sacrifice at Onset of Therapy (4 hours PI) (ONSET)	None	13-15

Treatment was scheduled for every 15 minutes for 5 hours (21 total doses). The 3 rabbits in group V were sacrificed 4 hours PI and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of PBS and kept on ice. The corneal buttons were homogenized for 25 seconds on ice using the motorized homogenizer. After homogenization, colony counts were done on the homogenates using 5% sheep blood agar plates to determine the amount of bacteria contained in the corneas at the onset of therapy. Following the completion of therapy, the eyes were examined for clinical signs of infection. One hour after the final treatment, the treated rabbits (Groups I-IV) were sacrificed and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of PBS and kept on ice. The corneal buttons were homogenized for 25 seconds on ice using the motorized homogenizer. After homogenization, colony counts were done on the homogenates using 5% sheep blood agar plates to determine the amount of bacteria contained in the corneas after treatment. The next morning, the plates were counted and the number of CFU/eye of *Staphylococcus aureus* was determined for each cornea.

Formulations: 1) 0.25% Oligomer 2 (PMX): Tube G1 of Oligomer 2 powder was stored at 4°C until use. Upon use, the tube was removed from the refrigerator and 3.28 ml of S1 (sterile water for injection) was added and vortexed until the solid was completely dissolved. Then 3.28 ml of S2 (2X TBS) was added and vortexed for 10 seconds. This solution was designated PMX. 37 μ l drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode; 2) 0.25% Oligomer 2 with 200 μ M Farnesol (P+F): Tube G2 of Oligomer 2 powder was stored at 4°C until use. Upon use, the tube was removed from the refrigerator and 3.33 ml of S1 (sterile water for injection) was added and vortexed until the solid was completely dissolved. Then 3.33 ml of S3 (400 μ M Farnesol + 2% Propylene Glycol in 2X TBS) was added and vortexed for 10 seconds. This solution was designated P+F. 37 μ l drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode; 3) 200 μ M Farnesol

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(FARN): Tube G3 containing about 8 ml of 200 μ M Farnesol in 1% Propylene Glycol (PG) and TBS was stored at 4°C until use. This solution was designated FARN. 37 μ l drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode; 4) Control (Tris-Buffered Saline, CON): Tube G4 containing about 8 ml of Tris-Buffered Saline (10mM TRIS, 150mM NaCl, pH=7.4) was stored at 4°C until use. This solution was designated CON. 37 μ l drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode.

IACUC Protocol #0701145-1. “The *In Vivo* Evaluation of Biomimetics as Topical Ocular Antibiotics”.

	MIC Characterization of Fluoroquinolone-Resistant, Methicillin-Resistant <i>Staphylococcus aureus</i> Strain K950
Antibiotic	MIC [μ g/ml] (Minimum Inhibitory Concentration)
Oligomer 4	0.5 μ g/ml

	Drop Schedule		
15	Drop #	Time	Time of Day
	1	0	9:30
	2	:15	9:45
	3	:30	10:00
	4	:45	10:15
20	5	1:00	10:30
	6	1:15	10:45
	7	1:30	11:00
	8	1:45	11:15
	9	2:00	11:30
25	10	2:15	11:45
	11	2:30	12:00
	12	2:45	12:15
	13	3:00	12:30
	14	3:15	12:45
30	15	3:30	1:00
	16	3:45	1:15
	17	4:00	1:30
	18	4:15	1:45
	19	4:30	2:00

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20	4:45	2:15
21	5:00	2:30

Sacrifice rabbits 1 hour after final drop (3:30).

5 Definitions of Abbreviations

PMX-IE 0.25% Oligomer 4 with Intact Epithelium
 PMX-AE 0.25% Oligomer 4 with Abraded Epithelium
 P+F-IE 0.25% Oligomer 4 + 200 μ M Farnesol with Intact Epithelium
 P+F-AE 0.25% Oligomer 4 + 200 μ M Farnesol with Abraded Epithelium
 10 FARN-IE 200 μ M Farnesol with Intact Epithelium
 FARN-AE 200 μ M Farnesol with Abraded Epithelium
 CON-AE Tris-Buffered Saline Control with Abraded Epithelium
 CON-IE Tris-Buffered Saline Control with Intact Epithelium

15 Clinical Evaluation - Results

Eye	Group	Conj.	Chemosis	Discharge	Iritis	Corneal Edema	Corneal Infiltrate	Total Score
1R	PMX-IE	2.5	2.5	2.0	2.0	1.0	2.0	12.0
2R	PMX-IE	2.0	2.0	2.0	2.0	0.5	0.5	9.0
3R	PMX-IE	2.0	2.0	2.0	2.0	0.5	1.0	9.5
1L	PMX-AE	2.0	2.5	3.0	2.0	1.5	0	11.0
2L	PMX-AE	2.0	2.0	3.0	2.0	0.5	0	9.5
3L	PMX-AE	2.0	2.0	2.5	1.5	1.0	0	9.0
4R	P+F-IE	1.5	1.5	1.5	1.0	0.5	0.5	6.5
5R	P+F-IE	2.0	1.5	1.5	2.0	1.0	2.5	10.5
6R	P+F-IE	2.0	2.0	2.5	2.0	1.0	1.5	11.0
4L	P+F-AE	2.0	2.0	2.0	1.5	1.0	0	8.5
5L	P+F-AE	2.5	2.5	2.5	2.0	1.0	0	10.5

6L	P+F-AE	2.0	2.5	3.0	2.0	1.0	0	10.5
7R	FARN-IE	1.5	1.5	1.5	1.5	1.0	2.0	9.0
8R	FARN-IE	1.5	1.0	1.0	1.5	0.5	1.5	7.0
9R	FARN-IE	1.5	1.5	1.5	2.0	1.0	2.0	9.5
7L	FARN-AE	2.0	2.0	2.0	2.0	2.0	1.0	11.0
8L	FARN-AE	1.5	1.5	1.5	1.5	1.0	0.5	7.5
9L	FARN-AE	1.5	1.5	1.5	1.5	1.0	1.0	8.0
10R	CON-IE	1.5	1.0	1.0	1.0	1.0	1.0	6.5
11R	CON-IE	1.0	1.0	1.0	1.5	1.0	1.0	6.5
12R	CON-IE	1.5	1.5	1.0	2.0	1.0	2.0	9.0
10L	CON-AE	1.0	1.5	2.0	1.0	0.5	0	6.0
11L	CON-AE	1.5	1.5	2.0	1.5	1.5	1.0	9.0
12L	CON-AE	1.5	1.5	2.0	1.5	1.5	1.0	9.0

Scale 0 = Normal; 0.5 = Trace; 1.0 = Mild; 1.5 = Mild/Moderate; 2.0 = Moderate;
 2.5 = Moderate/Severe; 3.0 = Severe

5 Clinical Evaluation - Statistics

Descriptive Statistics Total Ocular Score
 Total

	Variable	Count	Mean	SE Mean	StDev	Minimum	Median	Maximum
	PMX-IE Score	3	10.167	0.928	1.607	9.000	9.500	12.000
10	PMX-AE Score	3	9.833	0.601	1.041	9.000	9.500	11.000
	P+F-IE Score	3	9.33	1.42	2.47	6.50	10.50	11.00
	P+F-AE Score	3	9.833	0.667	1.155	8.500	10.500	10.500
	FARN-IE Score	3	8.500	0.764	1.323	7.000	9.000	9.500
	FARN-AE Score	3	8.83	1.09	1.89	7.50	8.00	11.00

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CON-IE Score	3	7.333	0.833	1.443	6.500	6.500	9.000
CON-AE Score	3	8.00	1.00	1.73	6.00	9.00	9.00

Duncan Multiple Comparisons Test Total Score

5	Row #	Group/Level	Mean Rank	C.I. Overlaps	
	1	CON-IE Sco	5.8333	2, 3, 4, 5, 6, 7, 8,	
	2	CON-AE Sco	8.0000	1, 3, 4, 5, 6, 7, 8,	
	3	FARN-IE Sc	10.8333	1, 2, 4, 5, 6, 7, 8,	
	4	FARN-AE Sc	11.6667	1, 2, 3, 5, 6, 7, 8,	P=0.05
10	5	P+F-IE Sco	14.6667	1, 2, 3, 4, 6, 7, 8,	
	6	P+F-AE Sco	15.3333	1, 2, 3, 4, 5, 7, 8,	
	7	PMX-AE Sco	16.5000	1, 2, 3, 4, 5, 6, 8,	
	8	PMX-IE Sco	17.1667	1, 2, 3, 4, 5, 6, 7,	

N0 Differences Among the Groups

15

Microbiological Results

Inoculum = 1098 CFU/cornea

Data Display CFU/ml

	Row	PMX-IE	PMX-AE	P+F-IE	P+F-AE	FARN-IE	FARN-AE	CON-IE	CON-AE
20	1	1650000	0	50	9500	45200000	7750000	115000000	30500
	2	12500	12500	13600000	50	18600000	6650000	253000000	69000000
	3	92000	350	5200000	8050	21400000	8250000	15000000	176000000

Row Onset-IE Onset-AE

25	1	75000	118000
	2	59000	61000
	3	55500	2500

Data Display Log₁₀ CFU/ml

	Row	PMX-IE Log	PMX-AE Log	P+F-IE Log	P+F-AE Log	FARN-IE Log	FARN-AE Log
30	1	6.21748	0.00000	1.69897	3.97772	7.65514	6.88930
	2	4.09691	4.09691	7.13354	1.69897	7.26951	6.82282
	3	4.96379	2.54407	6.71600	3.90580	7.33041	6.91645

		Onset-IE		Onset-AE	
Row		CON-IE Log	CON-AE Log	Log	Log
1		8.06070	4.48430	4.87506	5.07188
2		8.40312	7.83885	4.77085	4.78533
5	3	7.17609	8.24551	4.74429	3.39794

Descriptive Statistics			Log ₁₀ CFU/ml					
Total								
	Variable	Count	Mean	SE Mean	StDev	Minimum	Median	Maximum
10	PMX-IE Log	3	5.093	0.616	1.066	4.097	4.964	6.217
	<u>PMX-AE Log</u>	<u>3</u>	<u>2.21</u>	<u>1.19</u>	<u>2.07</u>	<u>0.00</u>	<u>2.54</u>	<u>4.10</u>
	P+F-IE Log	3	5.18	1.75	3.02	1.70	6.72	7.13
	<u>P+F-AE Log</u>	<u>3</u>	<u>3.194</u>	<u>0.748</u>	<u>1.295</u>	<u>1.699</u>	<u>3.906</u>	<u>3.978</u>
15	FARN-IE Log	3	7.418	0.120	0.207	7.270	7.330	7.655
	<u>FARN-AE Log</u>	<u>3</u>	<u>6.8762</u>	<u>0.0278</u>	<u>0.0482</u>	<u>6.8228</u>	<u>6.8893</u>	<u>6.9165</u>
	CON-IE Log	3	7.880	0.366	0.633	7.176	8.061	8.403
	<u>CON-AE Log</u>	<u>3</u>	<u>6.86</u>	<u>1.19</u>	<u>2.06</u>	<u>4.48</u>	<u>7.84</u>	<u>8.25</u>
	Onset-IE Log	3	4.7967	0.0399	0.0691	4.7443	4.7709	4.8751
	<u>Onset-AE Log</u>	<u>3</u>	<u>4.418</u>	<u>0.517</u>	<u>0.895</u>	<u>3.398</u>	<u>4.785</u>	<u>5.072</u>

20

Microbiological Results - Intact Epithelium

Kruskal-Wallis ANOVA with Duncan Multiple Comparisons Test - Log₁₀ CFU/ml

Row #	Group/Level	Mean Rank	C.I. Overlaps	
25	1	Onset-IE L	4.0000	2, 3,
	2	PMX-IE Log	5.0000	1, 3,
	3	P+F-IE Log	6.0000	1, 2, P=0.05
	4	FARN-IE Lo	12.0000	5,
	5	CON-IE Log	13.0000	4,

ONSET = PMX = P+F < FARN = CON

30

Microbiological Results - Abraded Epithelium

Kruskal-Wallis ANOVA with Duncan Multiple Comparisons Test - Log₁₀ CFU/ml

Row #	Group/Level	Mean Rank	C.I. Overlaps
1	PMX-AE Log	3.6667	2, 3,

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2	P+F-AE Log	4.3333	1, 3,
3	Onset-AE L	7.6667	1, 2, P=0.05
4	FARN-AE Lo	12.0000	5,
5	CON-AE Log	12.3333	4,
5	PMX = P+F = ONSET < FARN = CON		

Microbiological Results - 0.25% Oligomer 4 w/o FARN vs. w/ FARN - Intact Epithelium

Mann-Whitney Test and CI: PMX-IE Log, P+F-IE Log

10	N	Median
	PMX-IE Log	3 4.964
	P+F-IE Log	3 6.716

Point estimate for ETA1-ETA2 is -0.916

15 91.9 Percent CI for ETA1-ETA2 is (-3.034,4.518)

W = 9.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6625 NS

Microbiological Results - 0.25% Oligomer 4 w/o FARN vs. w/ FARN - Abraded Epithelium

20 Mann-Whitney Test and CI: PMX-AE Log, P+F-AE Log

	N	Median
	PMX-AE Log	3 2.544
	P+F-AE Log	3 3.906

25 Point estimate for ETA1-ETA2 is -1.362

91.9 Percent CI for ETA1-ETA2 is (-3.977,2.399)

W = 10.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000 NS

30 Microbiological Results - Intact vs. Abraded Epithelium

Mann-Whitney Test and CI: PMX-IE Log, PMX-AE Log

	N	Median
	PMX-IE Log	3 4.964
	PMX-AE Log	3 2.544

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Point estimate for ETA1-ETA2 is 2.420

91.9 Percent CI for ETA1-ETA2 is (0.001,6.218)

W = 14.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1266

5 The test is significant at 0.1212 NS (adjusted for ties)

Mann-Whitney Test and CI: P+F-IE Log, P+F-AE Log

N Median

P+F-IE Log 3 6.716

10 P+F-AE Log 3 3.906

Point estimate for ETA1-ETA2 is 2.810

91.9 Percent CI for ETA1-ETA2 is (-2.277,5.436)

W = 12.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5127

15 The test is significant at 0.5066 NS (adjusted for ties)

Mann-Whitney Test and CI: FARN-IE Log, FARN-AE Log

N Median

FARN-IE Log 3 7.3304

20 FARN-AE Log 3 6.8893

Point estimate for ETA1-ETA2 is 0.4467

91.9 Percent CI for ETA1-ETA2 is (0.3532,0.8323)

W = 15.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809 NS

25

Mann-Whitney Test and CI: CON-IE Log, CON-AE Log

N Median

CON-IE Log 3 8.061

CON-AE Log 3 7.839

30 Point estimate for ETA1-ETA2 is 0.222

91.9 Percent CI for ETA1-ETA2 is (-1.070,3.917)

W = 12.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6625 NS

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Mann-Whitney Test and CI: Onset-IE Log, Onset-AE Log

N Median

Onset-IE Log 3 4.771

Onset-AE Log 3 4.785

5 Point estimate for ETA1-ETA2 is -0.015

91.9 Percent CI for ETA1-ETA2 is (-0.328,1.477)

W = 10.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000 NS

10 Summary of Statistical Comparisons for Microbiological Data

< = Significantly Fewer Colony Counts

Effect of Abraded Epithelium on Effectiveness of Each Test Solution or Onset Control

PMX Abraded = Intact

15 P+F Abraded = Intact

FARN Abraded = Intact

Saline Control Abraded = Intact

Onset of Therapy Control Abraded = Intact

20 Effect of Test Solutions on Corneas with Intact Epithelium

ONSET = PMX = P+F < FARN = CON

Effect of Test Solutions on Corneas with Abraded Epithelium

PMX = P+F = ONSET < FARN = CON

25

Effect of Farnesol on 0.25% Oligomer 4 on Corneas with Intact Epithelium

PMX = P+F

Effect of Farnesol on 0.25% Oligomer 4 on Corneas with Abraded Epithelium

30 PMX = P+F

Summary of Results

0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) were effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus*

colony counts compared with the Saline Control in the NZW rabbit keratitis model when the corneal epithelium was intact or removed from the corneas. 0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) were not effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts compared with the Onset of Therapy Control in the NZW rabbit keratitis model when the corneal epithelium was intact or removed from the corneas. There was no difference in fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model between 0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) with intact or abraded corneal epithelium. 200 mM Farnesol alone was NOT effective in reducing colony counts fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts compared with the Saline Control in the NZW rabbit keratitis model. 0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) and 200 mM Farnesol alone did not induce statistically greater toxicity (as manifested by higher Total Ocular Scores) compared with the Saline treated eyes in eyes with intact or abraded corneal epithelia.

The biomimetic Oligomer 4 alone or in combination with 200 mM Farnesol were effective in reducing the number fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model compared with the Saline Control compared with the Saline Control. However, Oligomer 4 alone or in combination with 200 mM Farnesol were not effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts whether when the corneal epithelium was intact or removed compared with the Onset of Therapy Control in the fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* NZW rabbit keratitis model indicating the compounds did not significantly reduce the bacterial load present at the onset of therapy. The addition of 200 mM Farnesol did not appear aid in the penetration of 0.25% Oligomer 4 through the intact corneal epithelium to the site of the infection in the corneal stroma nor enhance its antibacterial efficacy in the fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* NZW rabbit keratitis model. In the current study, Oligomer 4 alone or in combination with 200 mM Farnesol did not induced significantly greater toxicity in infected rabbit eyes compared with the Saline treated Control in the fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* NZW rabbit keratitis model. The results from this study essentially reproduce those obtained in previous studies.

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Example 12: Ker-4

Definitions of Abbreviations

	PMX-IE	0.25% Oligomer 4 with Intact Epithelium
	PMX-AE	0.25% Oligomer 4 with Abraded Epithelium
5	P+F-IE	0.25% Oligomer 4 + 200 μ M Farnesol with Intact Epithelium
	P+F-AE	0.25% Oligomer 4 + 200 μ M Farnesol with Abraded Epithelium
	FARN-IE	200 μ M Farnesol with Intact Epithelium
	FARN-AE	200 μ M Farnesol with Abraded Epithelium
	CON-AE	Tris-Buffered Saline Control with Abraded Epithelium
10	CON-IE	Tris-Buffered Saline Control with Intact Epithelium

Clinical Evaluation - Statistics

Data Display Total Ocular Score

Row	PMX-IE	PMX-AE	P+F-IE	P+F-AE	FARN-IE	FARN-AE	CON-IE	CON-AE	
15	1	6.5	9.5	13.0	9.5	10.0	11.0	9.5	10.0
	2	13.0	10.5	8.0	8.5	10.0	8.5	11.0	14.0 Ker-3
	3	16.5	12.0	12.5	10.0	8.5	8.5	9.5	10.5
	4	12.0	11.0	6.5	8.5	9.0	11.0	6.5	6.0
	5	9.0	9.5	10.5	10.5	7.0	7.5	6.5	9.0 Ker-4
20	6	9.5	9.0	11.0	10.5	9.5	8.0	9.0	9.0

Descriptive Statistics Total Ocular Score

		Total						
Variable	Count	Mean	SE Mean	StDev	Minimum	Median	Maximum	
25	PMX-IE Score	6	11.08	1.43	3.51	6.50	10.75	16.50
	PMX-AE Score	6	10.250	0.461	1.129	9.000	10.000	12.000
	P+F-IE Score	6	10.25	1.04	2.54	6.50	10.75	13.00
	P+F-AE Score	6	9.583	0.375	0.917	8.500	9.750	10.500
	FARN-IE Score	6	9.000	0.465	1.140	7.000	9.250	10.000
30	FARN-AE Score	6	9.083	0.625	1.530	7.500	8.500	11.000
	CON-IE Score	6	8.667	0.738	1.807	6.500	9.250	11.000
	CON-AE Score	6	9.75	1.06	2.60	6.00	9.50	14.00

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Duncan Multiple Comparisons Test Total Score

	Row #	Group/Level	Mean Rank	C.I. Overlaps	
5	1	CON-IE Sco	18.5833	2, 3, 4, 5, 6, 7, 8,	
	2	FARN-AE Sc	19.5833	1, 3, 4, 5, 6, 7, 8,	
	3	FARN-IE Sc	19.7500	1, 2, 4, 5, 6, 7, 8,	
	4	P+F-AE Sco	24.2500	1, 2, 3, 5, 6, 7, 8,	P=0.05
	5	CON-AE Sco	24.4167	1, 2, 3, 4, 6, 7, 8,	
	6	P+F-IE Sco	29.0833	1, 2, 3, 4, 5, 7, 8,	
	7	PMX-IE Sco	30.1667	1, 2, 3, 4, 5, 6, 8,	
	10	8	PMX-AE Sco	30.1667	1, 2, 3, 4, 5, 6, 7,

No Differences Among the Groups

Microbiological Results

15 Data Display CFU/ml

	Row	PMX-IE	PMX-AE	P+F-IE	P+F-AE	FARN-IE	FARN-AE
20	1	0	0	11950000	255	15200000	7500000
	2	16750000	0	415000	1100000	18150000	1285000 Ker-3
	3	5800000	995000	16650000	35500	30100000	1400000
	4	1650000	0	50	9500	45200000	7750000
	5	12500	12500	13600000	50	18600000	6650000 Ker-4
	6	92000	350	5200000	8050	21400000	8250000

	Row	CON-IE	CON-AE	Onset-IE	Onset-AE	
25	1	467000000	1650000	15000	1635000	
	2	221500000	23500000	107000	130000	PMX-Ker-3
	3	202000000	5400000	132500	133000	
	4	115000000	30500	75000	118000	
	5	253000000	69000000	59000	61000	PMX-Ker-4
30	6	15000000	176000000	55500	2500	

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Data Display

Log₁₀ CFU/ml

Row	PMX-IE Log	PMX-AE Log	P+F-IE Log	P+F-AE Log	FARN-IE Log	FARN-AE Log
1	0.00000	0.00000	7.07737	2.40654	7.18184	6.87506
2	7.22401	0.00000	5.61805	6.04139	7.25888	6.10890 K-3
5	3	6.76343	5.99782	7.22141	4.55023	7.47857
	4	6.21748	0.00000	1.69897	3.97772	7.65514
	5	4.09691	4.09691	7.13354	1.69897	7.26951
	6	4.96379	2.54407	6.71600	3.90580	7.33041

Row	CON-IE Log	CON-AE Log	Log	Log
1	8.66932	6.21748	4.17609	6.21352
2	8.34537	7.37107	5.02938	5.11394
3	8.30535	6.73239	5.12222	5.12385
15	4	8.06070	4.48430	4.87506
	5	8.40312	7.83885	4.77085
	6	7.17609	8.24551	4.74429

Descriptive Statistics

Log₁₀ CFU/ml

Variable	Count	Mean	SE Mean	StDev	Minimum	Median	Maximum
PMX-IE Log	6	4.88	1.08	2.66	0.00	5.59	7.22
PMX-AE Log	6	2.11	1.04	2.55	0.00	1.27	6.00
P+F-IE Log	6	5.911	0.876	2.147	1.699	6.897	7.221
P+F-AE Log	6	3.763	0.632	1.548	1.699	3.942	6.041
FARN-IE Log	6	7.3624	0.0712	0.1744	7.1818	7.3000	7.6551
FARN-AE Log	6	6.626	0.158	0.388	6.109	6.849	6.916
CON-IE Log	6	8.160	0.212	0.520	7.176	8.325	8.669
CON-AE Log	6	6.815	0.554	1.356	4.484	7.052	8.246
Onset-IE Log	6	4.786	0.136	0.333	4.176	4.823	5.122
Onset-AE Log	6	4.951	0.370	0.906	3.398	5.093	6.214

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Microbiological Results - Intact Epithelium

Kruskal-Wallis ANOVA with Duncan Multiple Comparisons Test - Log₁₀ CFU/ml

Row #	Group/Level	Mean Rank	C.I. Overlaps
1	Onset-IE L	6.8333	2, 3,
2	PMX-IE Log	9.6667	1, 3,
3	P+F-IE Log	12.6667	1, 2, P=0.05
4	FARN-IE Lo	22.1667	5,
5	CON-IE Log	26.1667	4,

ONSET = PMX = P+F < FARN = CON

10

Microbiological Results - Abraded Epithelium

Kruskal-Wallis ANOVA with Duncan Multiple Comparisons Test - Log₁₀ CFU/ml

Row #	Group/Level	Mean Rank	C.I. Overlaps
1	PMX-AE Log	6.5000	2,
2	P+F-AE Log	9.3333	1,
3	Onset-AE L	14.3333	P=0.05
4	FARN-AE Lo	23.5000	5,
5	CON-AE Log	23.8333	4,

PMX = P+F < ONSET < FARN = CON

20

Microbiological Results - 0.25% Oligomer 4 w/o FARN vs. w/ FARN - Intact Epithelium

Mann-Whitney Test and CI: PMX-IE Log, P+F-IE Log

N Median

PMX-IE Log 6 5.591

25 P+F-IE Log 6 6.897

Point estimate for ETA1-ETA2 is -0.757

95.5 Percent CI for ETA1-ETA2 is (-3.124,1.607)

W = 34.0

30 Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4712 NS

Microbiological Results - 0.25% Oligomer 4 w/o FARN vs. w/ FARN - Abraded Epithelium

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Mann-Whitney Test and CI: PMX-AE Log, P+F-AE Log

N Median

PMX-AE Log 6 1.272

P+F-AE Log 6 3.942

5

Point estimate for ETA1-ETA2 is -1.822

95.5 Percent CI for ETA1-ETA2 is (-4.549,1.690)

W = 32.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2980

10 The test is significant at 0.2946 NS (adjusted for ties)

Microbiological Results - Intact vs. Abraded Epithelium

Mann-Whitney Test and CI: PMX-IE Log, PMX-AE Log

N Median

15 PMX-IE Log 6 5.591

PMX-AE Log 6 1.272

Point estimate for ETA1-ETA2 is 3.400

95.5 Percent CI for ETA1-ETA2 is (0.001,6.764)

W = 50.0

20 Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0927

The test is significant at 0.0864 NS (adjusted for ties)

Mann-Whitney Test and CI: P+F-IE Log, P+F-AE Log

N Median

25 P+F-IE Log 6 6.897

P+F-AE Log 6 3.942

Point estimate for ETA1-ETA2 is 2.705

95.5 Percent CI for ETA1-ETA2 is (-0.423,4.727)

W = 50.5

30 Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0782

The test is significant at 0.0776 NS (adjusted for ties)

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Mann-Whitney Test and CI: FARN-IE Log, FARN-AE Log

N Median

FARN-IE Log 6 7.3000

FARN-AE Log 6 6.8489

FARN-AE < FARN-IE

5 Point estimate for ETA1-ETA2 is 0.5964

95.5 Percent CI for ETA1-ETA2 is (0.3588,1.1843)

W = 57.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0051

10 Mann-Whitney Test and CI: CON-IE Log, CON-AE Log

N Median

CON-IE Log 6 8.325

CON-AE Log 6 7.052

CON-AE < CON-IE

Point estimate for ETA1-ETA2 is 1.003

15 95.5 Percent CI for ETA1-ETA2 is (0.100,2.691)

W = 53.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0306

Mann-Whitney Test and CI: Onset-IE Log, Onset-AE Log

20 N Median

Onset-IE Log 6 4.823

Onset-AE Log 6 5.093

Point estimate for ETA1-ETA2 is -0.218

95.5 Percent CI for ETA1-ETA2 is (-1.091,0.778)

25 W = 32.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2980 NS

Summary of Statistical Comparisons for Microbiological Data

< = Significantly Fewer Colony Counts

30 Effect of Abraded Epithelium on Effectiveness of Each Test Solution or Onset Control

PMX Abraded = Intact

P+F Abraded = Intact

FARN Abraded < Intact

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Saline Control Abraded < Intact

Onset of Therapy Control Abraded = Intact

Effect of Test Solutions on Corneas with Intact Epithelium

5 ONSET = PMX = P+F < FARN = CON

Effect of Test Solutions on Corneas with Abraded Epithelium

PMX = P+F < ONSET < FARN = CON

10 Effect of Farnesol on 0.25% Oligomer 4 on Corneas with Intact Epithelium

PMX = P+F

Effect of Farnesol on 0.25% Oligomer 4 on Corneas with Abraded Epithelium

PMX = P+F

15

Summary of Results

0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) were effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts compared with the Saline Control in the NZW rabbit keratitis model when the corneal epithelium was intact or removed from the corneas. 0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) were effective in reducing fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts compared with the Onset of Therapy Control in the NZW rabbit keratitis model when the corneal epithelium was removed but not when the epithelium was intact. There was no difference in fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts in the NZW rabbit keratitis model between 0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) with intact or abraded corneal epithelium. 200 mM Farnesol alone was not effective in reducing colony counts fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* colony counts compared with the Saline Control in the NZW rabbit keratitis model. Eyes treated with 200 mM Farnesol alone and Saline demonstrated significantly fewer colony counts in eyes with the corneal epithelium removed compared to those with intact epithelium. 0.25% Oligomer 4 (PMX) and 0.25% Oligomer 4 with 200 mM Farnesol (P+F) and 200 mM Farnesol alone did not

induce statistically greater toxicity (as manifested by higher Total Ocular Scores) compared with the Saline treated eyes in eyes with intact or abraded corneal epithelia.

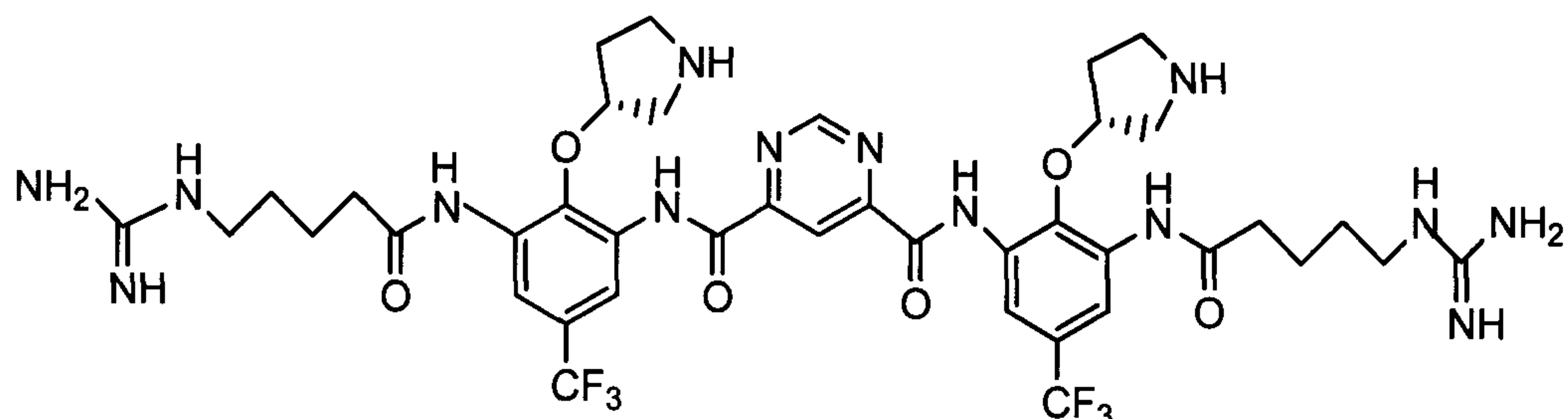
The biomimetic Oligomer 4 was effective in significantly reducing colony counts in a fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus* NZW rabbit
5 keratitis model. Oligomer 4 formulations were effective when the corneal epithelium was removed suggesting that epithelium appears to be barrier for penetration of Oligomer 4 to the site of infection in the corneal stroma. The addition of 200 mM Farnesol did nothing to promote penetration Oligomer 4 through intact corneal epithelium, nor did it enhance its antibacterial efficacy. In fact, a trend toward antagonism was observed.

10 Mechanical abrasion of the corneal epithelium alone reduced the bacterial colony counts in the control eyes. Therefore, the lower colony counts observed in the Oligomer 4-treated abraded eyes does not necessarily indicate greater drug efficacy. No significant ocular toxicity was observed for any formulation in this rabbit keratitis model.

Having now fully described this invention, it will be understood to those of
15 ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations, and other parameters without affecting the scope of the invention or any embodiment thereof. Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the
20 appended claims.

CLAIMS:

1. The compound



or a pharmaceutically acceptable salt thereof.

2. The compound according to claim 1 for use in the preparation of a medicament for the treatment of a microbial infection.
3. Use of the compound according to claim 1 in the preparation of a medicament for treating a bacterial infection in a mammal.
4. The use according to claim 3 wherein the bacterial infection is chosen from *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Eschericia coli*, *Staphylococcus aureus*, *Streptococcus viridans*, and *Streptococcus pneumonia*.
5. A pharmaceutical composition comprising the compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
6. The composition according to claim 5, which is in the form of a powder.
7. The composition according to claim 5, further comprising an additional medicament.
8. The composition according to claim 7, wherein the additional medicament is chosen from an antibiotic, an anti-inflammatory agent, an anesthetic agent, an anti-allergic agent, an

acetylcholine blocking agent, an adrenergic agonist, a beta-adrenergic blocking agent, an anti-glaucoma agent, and an anti-hypertensive agent.

9. The composition according to claim 8, wherein the antibiotic is chosen from an aminoglycoside, a cephalosporin, a diaminopyridine, a fluoroquinolone, a sulfonamide, and a tetracycline.

10. The composition according to claim 8, wherein the antibiotic is chosen from amikacin, azithromycin, cefixime, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, colistin, domeclocycline, doxycycline, erythromycin, gentamicin, mafenide, methacycline, minocycline, neomycin, norfloxacin, ofloxacin, oxytetracycline, polymyxin B, pyrimethamine, silver sulfadiazine, sulfacetamide, sulfisoxazole, tetracycline, tobramycin, and trimethoprim.

11. The composition according to claim 8, wherein the anti-inflammatory agent is a steroidal agent or a non-steroidal agent.

12. The composition according to claim 11, wherein the steroidal agent is chosen from dexamethasone, rimexolone, prednisolone, fluorometholone, and hydrocortisone, and wherein the non-steroidal agent is chosen from a cyclooxygenase type I or type II inhibitor, a PAF antagonist, a PDE IV inhibitor, and an inhibitor of cytokine production.

13. The composition according to claim 12, wherein the cyclooxygenase type I or type II inhibitor is chosen from diclofenac, flurbiprofen, ketorolac, suprofen, nepafenac, amfenac, indomethacin, naproxen, ibuprofen, bromfenac, ketoprofen, meclofenamate, piroxicam, sulindac, mefanamic acid, diflusal, oxaprozin, tolmetin, fenoprofen, benoxaprofen, nabumetome, etodolac, phenylbutazone, aspirin, oxyphenbutazone, tenoxicam, carprofen, viox, celecoxib, and etodolac.

14. The composition according to claim 12, wherein the PAF antagonist is chosen from apafant, bepafant, minopafant, nupafant, and modipafant.
15. The composition according to claim 12, wherein the PDE IV inhibitor is chosen from ariflo, torbafylline, rolipram, flaminast, piclamilast, cipamfylline, and roflumilast.
16. The composition according to claim 8, wherein the anti-allergic agent is pemirolast or olopatadine.
17. The composition according to claim 8, wherein the anti-allergic agent is a corticosteroid.
18. The composition according to claim 17, wherein the corticosteroid is chosen from prednisolone, fluorometholone, loteprenol, and dexamethasone.
19. The composition according to claim 5, further comprising a cyclodextrin.
20. Use of the composition according to claim 5 in the preparation of a medicament for treating a bacterial infection in a mammal.
21. The use according to claim 20 wherein the bacterial infection is chosen from *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Eschericia coli*, *Staphylococcus aureus*, *Streptococcus viridans*, and *Streptococcus pneumonia*.
22. A composition comprising the compound according to claim 1, or pharmaceutically acceptable salt thereof, wherein the formulation comprises an excipient chosen from purified water, propylene glycol, PEG 400, glycerin, DMA, ethanol, benzyl alcohol, citric acid/sodium citrate (pH3), citric acid/sodium citrate (pH5), tris(hydroxymethyl)amino methane HCl (pH7.0), 0.9% saline, and 1.2% saline, or any combination thereof.

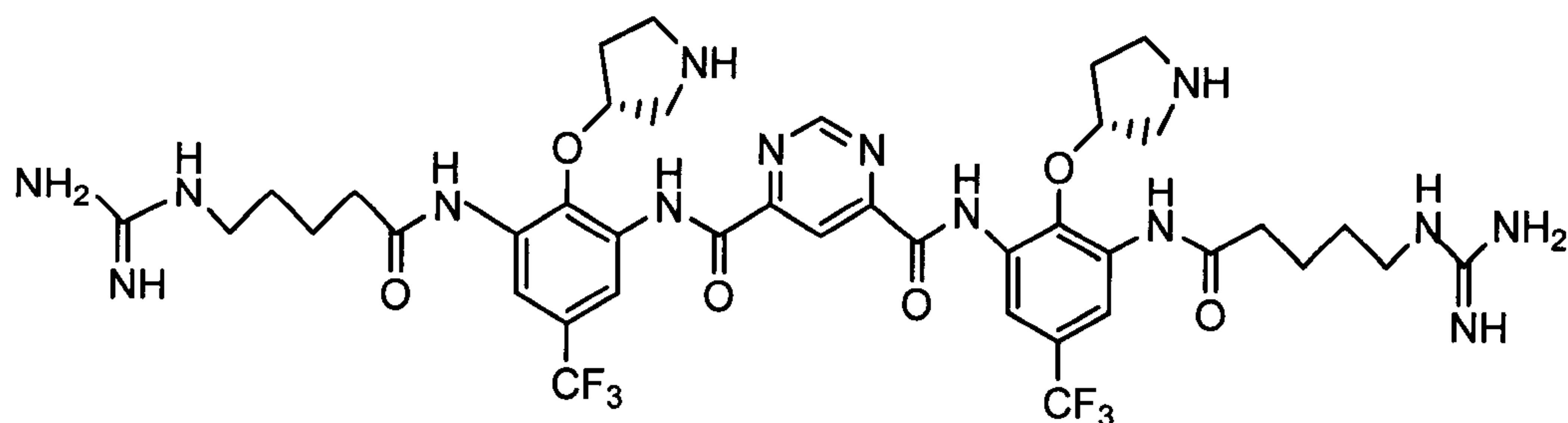
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23. A composition comprising the compound according to claim 1, or pharmaceutically acceptable salt thereof, wherein the formulation comprises an excipient chosen from 20% w/v propylene glycol in saline, 30% w/v propylene glycol in saline, 40% w/v propylene glycol in saline, 50% w/v propylene glycol in saline, 15% w/v propylene glycol in purified water, 30% w/v propylene glycol in purified water, 50% w/v propylene glycol in purified water, 30% w/v propylene glycol and 5 w/v ethanol in purified water, 15% w/v glycerin in purified water, 30% w/v glycerin in purified water, 50% w/v glycerin in purified water, 20% w/v Kleptose in purified water, 40% w/v Kleptose in purified water, and 25% w/v Captisol in purified water.

24. A composition comprising the compound according to claim 1, or a pharmaceutically acceptable salt thereof, and an aminoglycoside, a cephalosporin, or a fluoroquinolone.

25. The composition according to claim 24 wherein the aminoglycoside is gentamicin, the cephalosporin is ceftazidime or ceftriaxone, and the fluoroquinolone is ciprofloxacin.

26. A composition comprising

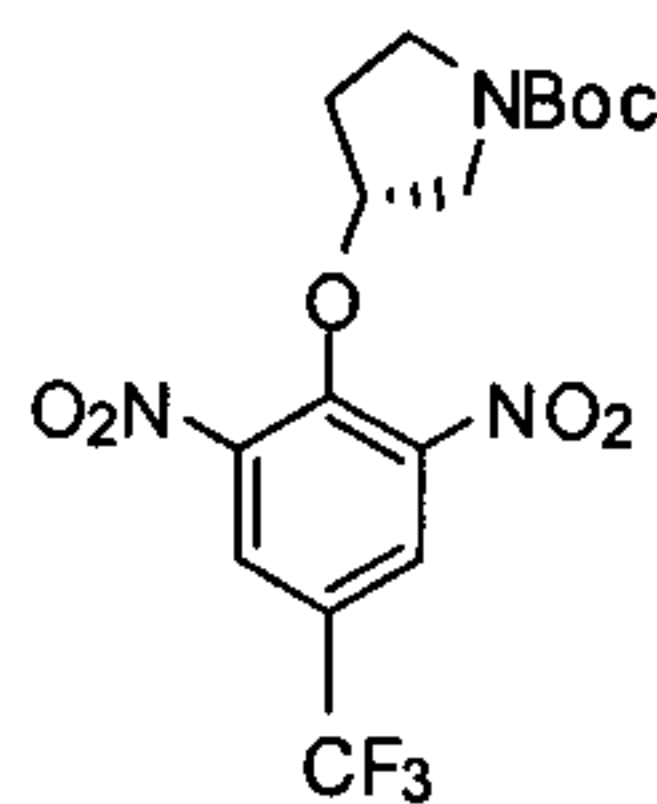


or a pharmaceutically acceptable salt thereof, in 20% w/v Kleptose in purified water.

27. A method of preparing the compound according to claim 1 comprising:

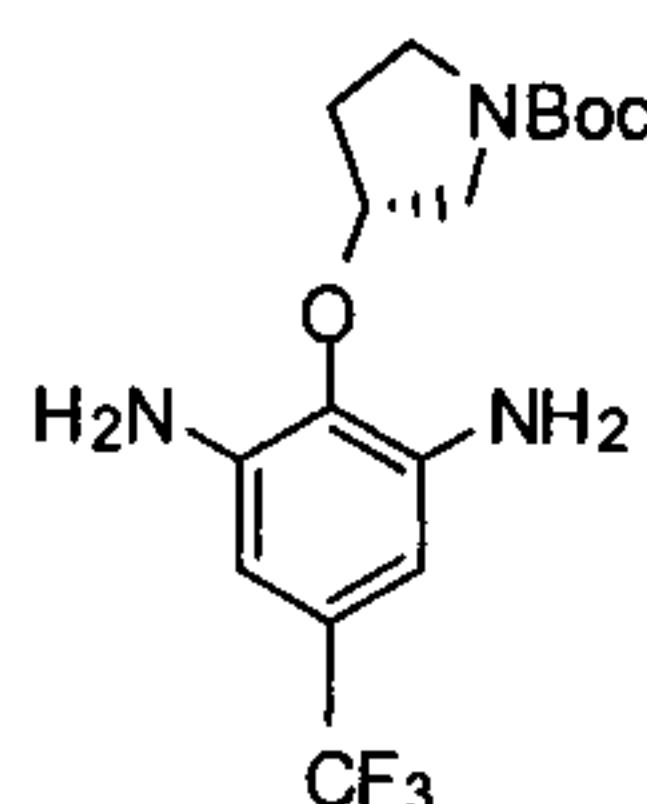
a) reacting (R)-(-)-N-Boc-3-pyrrolidinol with a strong base to form a mixture; further reacting the mixture with 2-chloro-5-(trifluoromethyl)-1,3-dinitrobenzene to form a compound having Formula II

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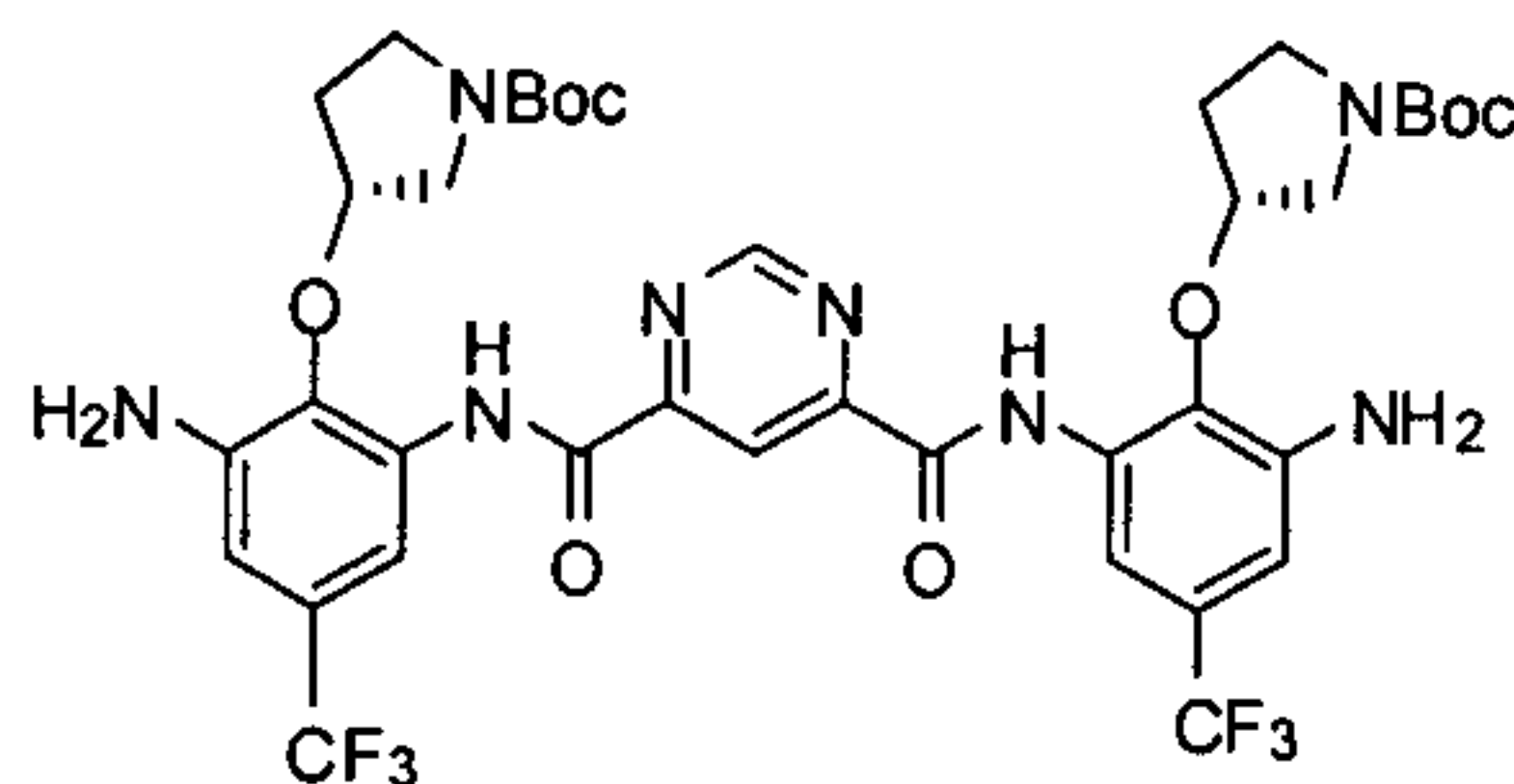
II;

b) reacting the compound of Formula II with an alcohol and a transition metal catalyst in the presence of hydrogen to form a compound of Formula III



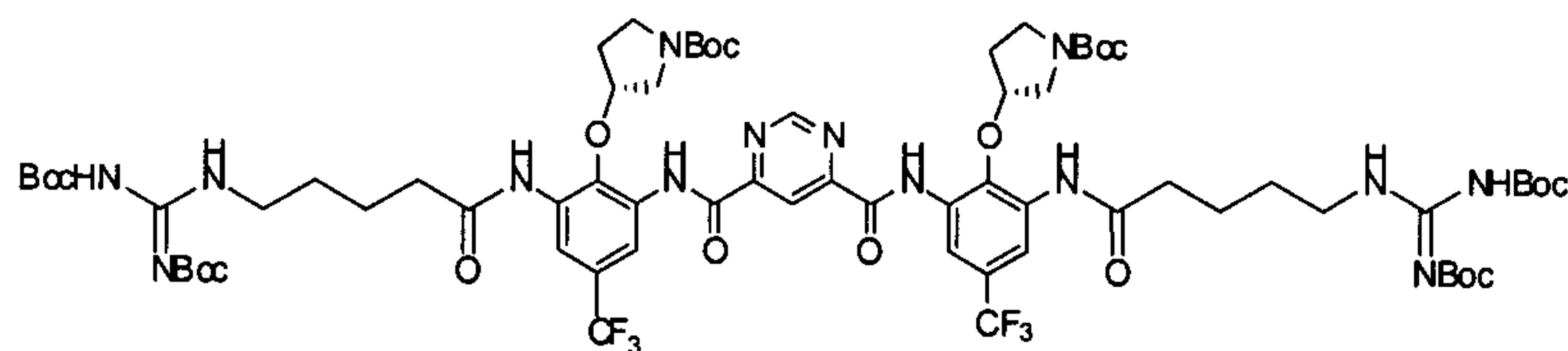
III;

c) adding the compound of Formula III and pyrimidine-4,6-dicarboxylic acid to a mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine and N-methylmorpholine to form a compound of Formula IV



IV;

d) reacting the compound of Formula IV with N-Boc- guanidine butyric acid to form a compound of Formula V



V; and

e) deprotecting the compound of Formula V to produce the compound of claim 1.

28. A method of preparing the compound according to claim 1 comprising:

a) deprotonating (R)-3-Hydroxypyrrolidine-1-carboxylic acid *tert*-butyl ester, and reacting the resultant compound with 2-chloro-1,3-dinitro-5-trifluoromethylbenzene to form (R)-3-(2,6-dinitro-4-trifluoromethylphenoxy)pyrrolidine-1-carboxylic acid *tert*-butyl ester;

b) reducing (R)-3-(2,6-dinitro-4-trifluoromethylphenoxy)pyrrolidine-1-carboxylic acid *tert*-butyl ester in the presence of an alcohol, a transition metal catalyst, and hydrogen to form (R)-3-(2,6-diamino-4-trifluoromethylphenoxy)-pyrrolidine-1-carboxylic acid *tert*-butyl ester;

c) coupling (R)-3-(2,6-diamino-4-trifluoromethylphenoxy)-pyrrolidine-1-carboxylic acid *tert*-butyl ester with pyrimidine-4,6-dicarboxylic acid in the presence of 1-[(3-(dimethylamino)-propyl)]-3-ethylcarbodiimide hydrochloride to form pyrimidine-4,6-dicarboxylic acid bis- {[3-amino-2-((R)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]amide};

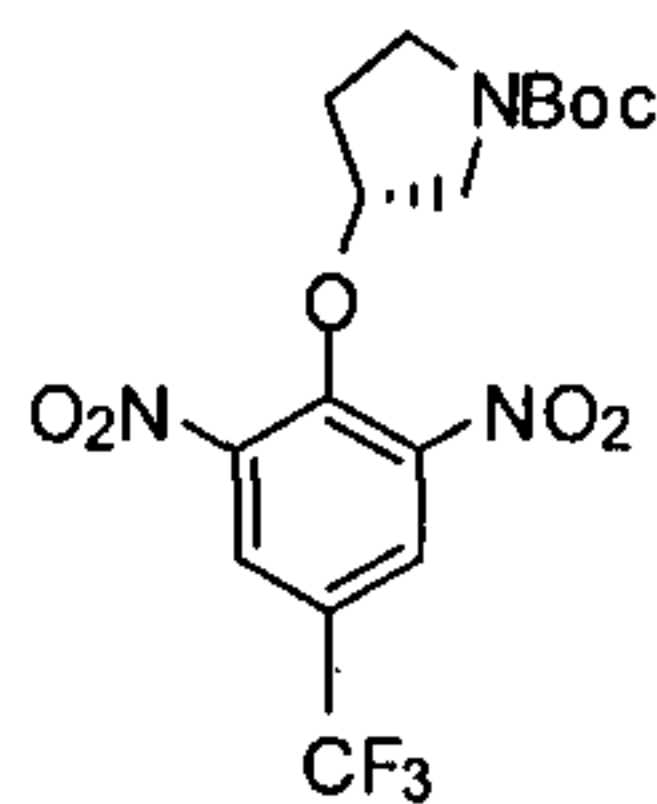
d) reacting pyrimidine-4,6-dicarboxylic acid bis- {[3-amino-2-((R)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]amide} with ({[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl} amino)pentanoic acid in the presence of phosphorous oxychloride to form pyrimidine-4,6-dicarboxylic acid bis- {[3-(5- ({[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl} amino)-pentanoylamino)-2-((R)- 1-(*tert*-butoxycarbonyl)-pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]-amide};

e) deprotecting pyrimidine-4,6-dicarboxylic acid bis- {[3-(5- ({[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl} amino)-pentanoylamino)-2-((R)-1-(*tert*-butoxycarbonyl)-pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]-amide} to form crude pyrimidine-4,6-dicarboxylic acid bis- {[3-(5-guanidino-pentanoylamino)-2-((R)-pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]-amide} tetrahydrochloride; and

f) purifying crude pyrimidine-4,6-dicarboxylic acid bis- {[3-(5-guanidino-pentanoylamino)-2-((R)-pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]-amide} tetrahydrochloride by reverse-phase chromatography.

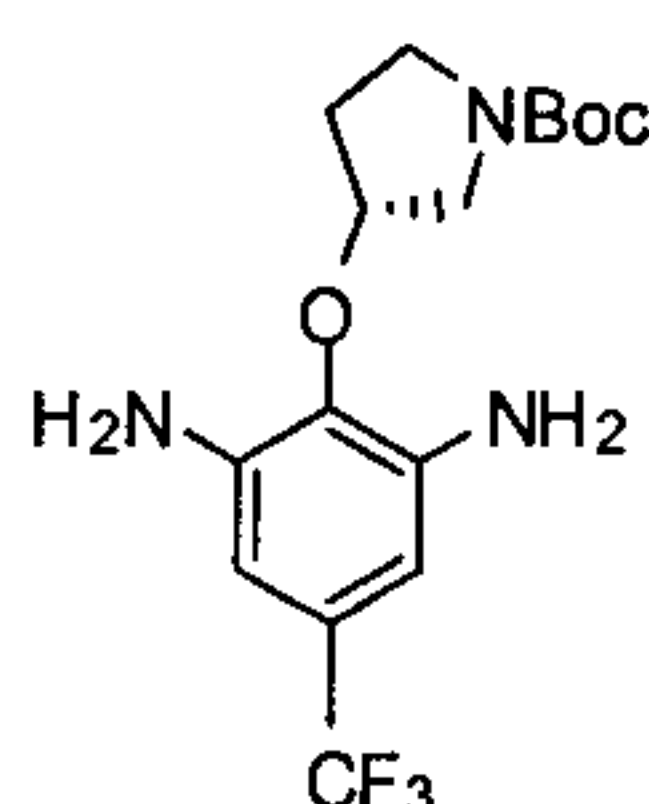
29. A method of preparing the compound according to claim 1 comprising:
- a) deprotonating (R)-3-Hydroxypyrrolidine-1-carboxylic acid *tert*-butyl ester and further reacting the resultant compound with 2-chloro-1,3-dinitro-5-trifluoromethylbenzene to form (R)-3-(2,6-dinitro-4-trifluoromethylphenoxy)pyrrolidine-1-carboxylic acid *tert*-butyl ester;
 - b) reducing (R)-3-(2,6-dinitro-4-trifluoromethylphenoxy)pyrrolidine-1-carboxylic acid *tert*-butyl ester in the presence of an alcohol, a transition metal catalyst, and hydrogen, to form (R)-3-(2,6-diamino-4-trifluoromethylphenoxy)-pyrrolidine-1-carboxylic acid *tert*-butyl ester;
 - c) coupling (R)-3-(2,6-diamino-4-trifluoromethylphenoxy)-pyrrolidine-1-carboxylic acid *tert*-butyl ester with pyrimidine-4,6-dicarboxylic acid in the presence of 1-[(3-(dimethylamino)-propyl)]-3-ethylcarbodiimide hydrochloride to form pyrimidine-4,6-dicarboxylic acid bis- {[3-amino-2-((R)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]amide};
 - d) reacting pyrimidine-4,6-dicarboxylic acid bis- {[3-amino-2-((R)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yloxy)-5-trifluoromethyl-phenyl]amide} with N-Cbz acid in the presence of thionyl chloride;
 - e) reducing the resultant compound of d) in the presence of an alcohol, a transition metal catalyst, and hydrogen;
 - f) reacting the resultant compound of e) with di-Boc pyrazole;
 - g) deprotecting the resultant compound of f) to produce the compound of claim 1.
30. A method of preparing a pharmaceutically acceptable salt of the compound according to claim 1 comprising:
- a) reacting (R)-(-)-N-Boc-3-pyrrolidinol with a strong base to form a mixture; further reacting the mixture with 2-chloro-5-(trifluoromethyl)-1,3-dinitrobenzene to form a compound having Formula II

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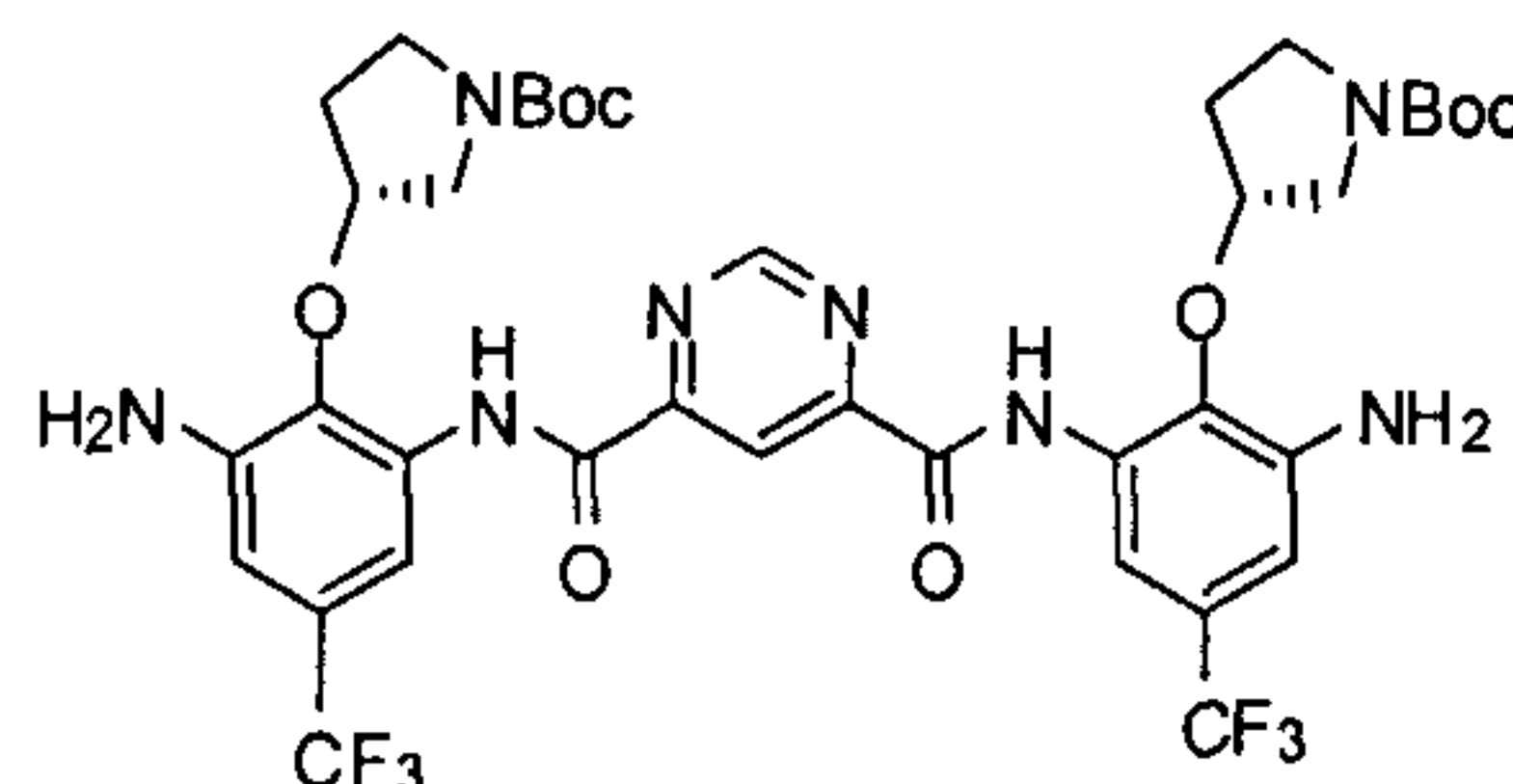
II;

b) reacting the compound of Formula II with an alcohol and a transition metal catalyst in the presence of hydrogen to form a compound of Formula III



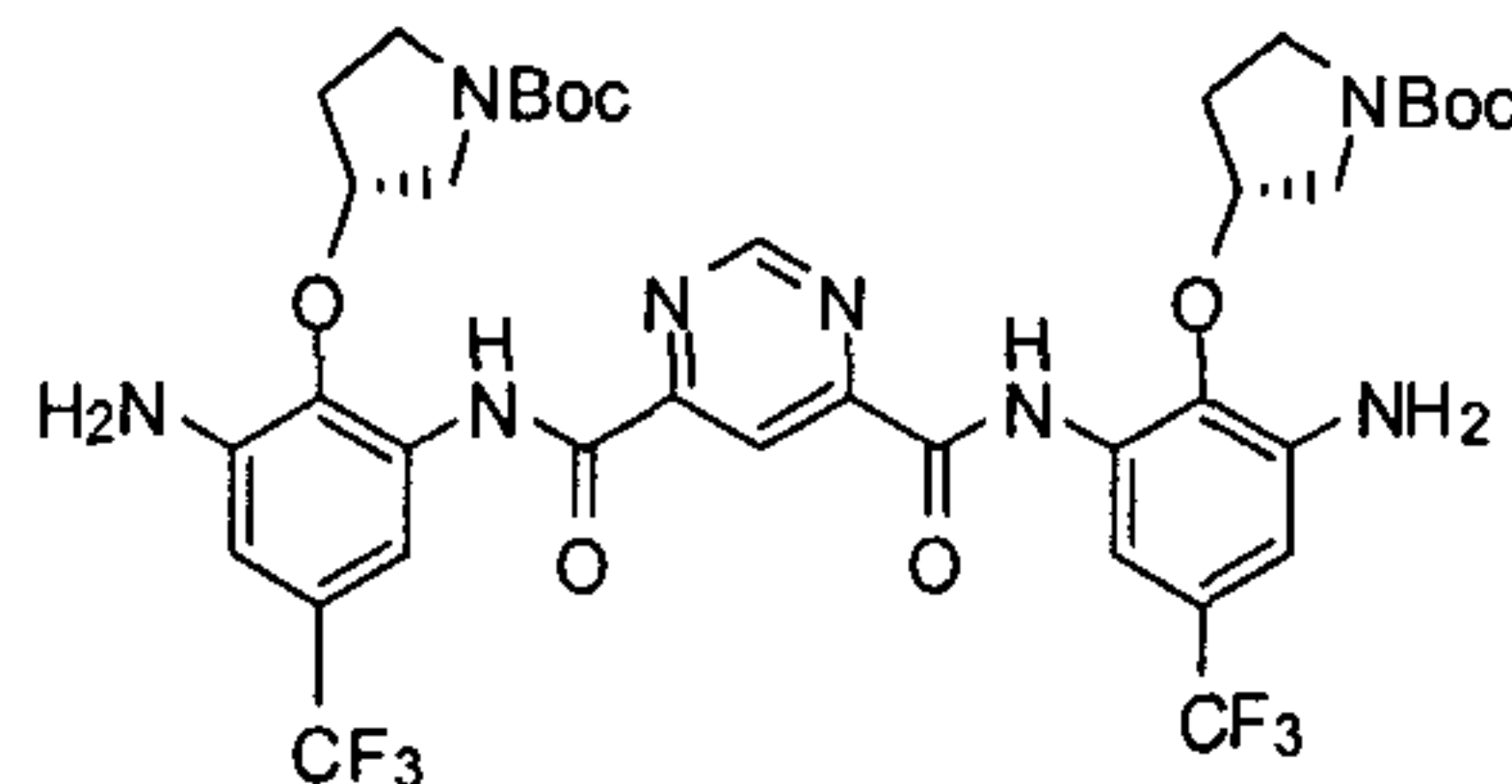
III;

c1) adding the compound of Formula III and pyrimidine-4,6-dicarboxylic acid to a mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine and N-methylmorpholine to form a compound of Formula IV



IV; or

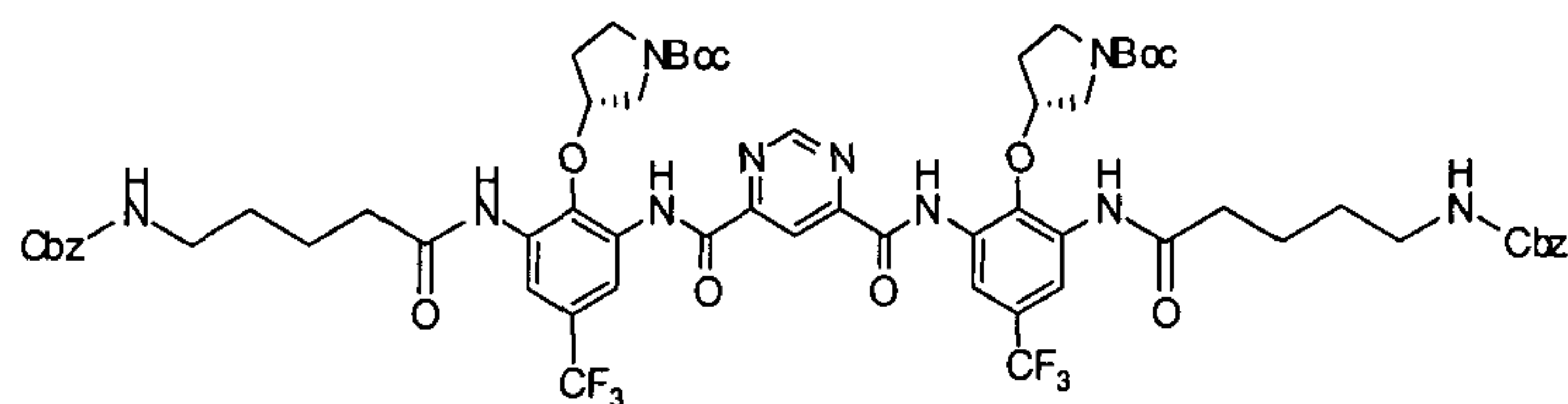
c2) adding the compound of Formula III and pyrimidine-4,6-dicarboxylic acid to a mixture of 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (EDCI) and anhydrous pyridine to form a compound of Formula IV



IV;

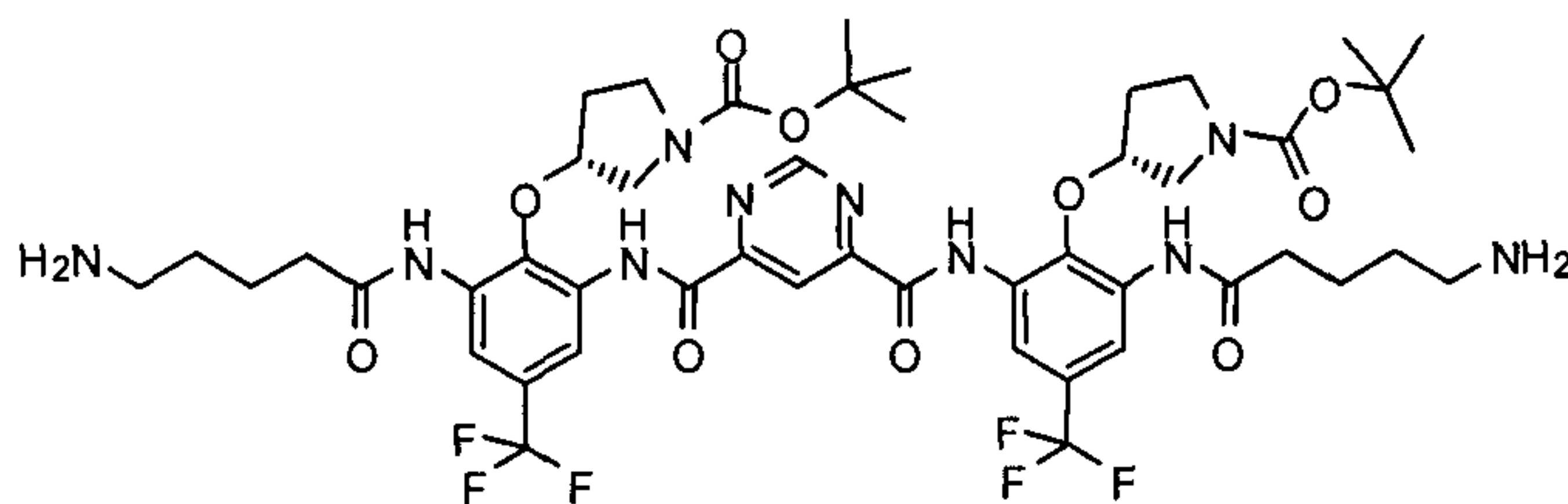
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d) adding the compound of Formula IV with an N-Cbz acid to a solution comprising anhydrous pyridine, dimethylaminopropylamine, and any one of thionyl chloride, POCl_3 , $(\text{EtO})_2\text{POCl}$, or oxalyl chloride to form a compound of Formula Va



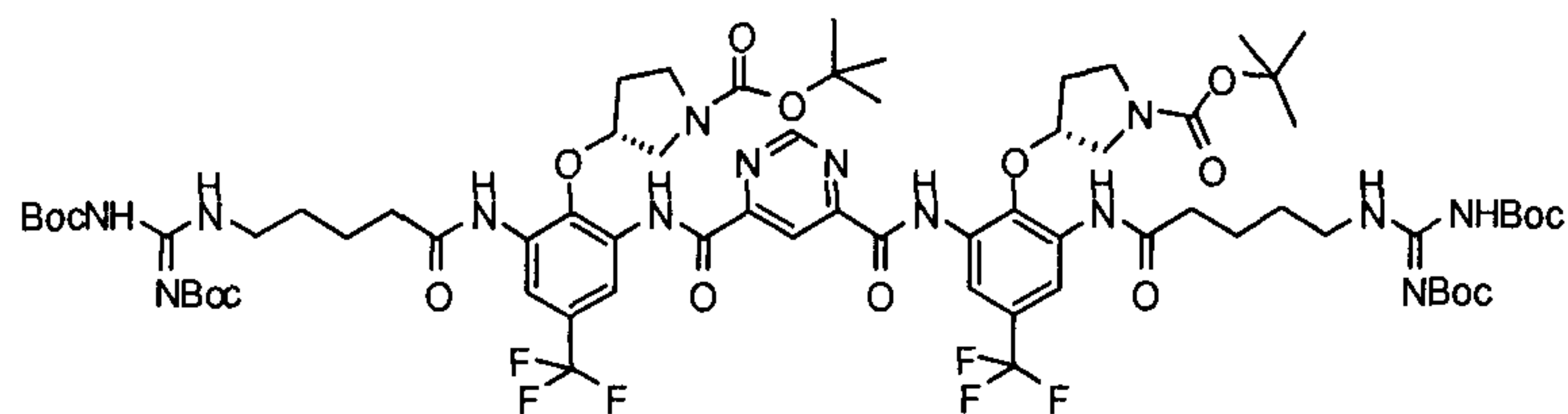
Va;

e) hydrogenolysis of the Cbz group of the compound of Formula Va to produce the compound of formula VI



VI;

f) protecting the compound of Formula VI to produce the compound of formula VII



VII; and

g) deprotecting the compound of Formula VII to produce a pharmaceutically acceptable salt of the compound of claim 1.

