BIOCIDAL POLYMERS

Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION, Madison, WI (US)

Inventors: Samuel H. Gellman, Madison, WI (US); Michael A. Gelman, New York, NY (US); Bernard Weisblum, Madison, WI (US); David M. Lynn, Middleton, WI (US)

Assignee: WISCONSIN ALUMNI RESEARCH FOUNDATION, Madison, WI (US)

Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

Appl. No.: 14/687,191
Filed: Apr. 15, 2015

Prior Publication Data

Related U.S. Application Data
Continuation of application No. 10/933,987, filed on Sep. 3, 2004, now Pat. No. 9,034,309.

Provisional application No. 60/500,201, filed on Sep. 4, 2003.

Int. Cl.
A61K 31/74 (2006.01)
A61K 31/785 (2006.01)
C08F 25/07 (2006.01)
C08F 265/04 (2006.01)
C08F 265/10 (2006.01)
C08F 283/06 (2006.01)
C08G 65/04 (2006.01)
C08G 65/329 (2006.01)
C08L 25/04 (2006.01)
C08L 33/08 (2006.01)
C08L 33/26 (2006.01)
C08L 35/06 (2006.01)
C08L 51/00 (2006.01)
C08L 55/00 (2006.01)
C08L 59/04 (2006.01)

U.S. Cl.
CPC A61K 31/785 (2013.01); A61K 31/74 (2013.01); C08F 25/07 (2013.01); C08F 265/04 (2013.01); C08F 265/10 (2013.01); C08F 283/06 (2013.01); C08G 65/04 (2013.01); C08G 65/329 (2013.01); C08L 25/04 (2013.01); C08L 33/08 (2013.01); C08L 33/26 (2013.01); C08L 35/06 (2013.01); C08L 51/00 (2013.01); C08L 55/00 (2013.01); C08L 59/04 (2013.01)

Field of Classification Search
None

See application file for complete search history.

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ABSTRACT
Pharmaceutical compositions containing biocidal co-polymers of poly(styrenes), poly(acrylates), poly(acrylamides), and poly(C1-C6)alkylene glycols are disclosed, along with methods of using the compositions to treat microbial infections in mammals.

16 Claims, No Drawings

Primary Examiner — Benjamin Packard
Attorney, Agent or Firm — Daniel Blasiole; Joseph Leone, Esq.; DeWitt Ross & Stevens SC

(Continued)
(56)

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**OTHER PUBLICATIONS**


BIOCIDAL POLYMERS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 10/933,987, filed 3 Sep. 2004, which claims priority under 35 USC §119(e) to U.S. Provisional Application Ser. No. 60/500,201, filed 4 Sep. 2003, the entireties of both of which are incorporated herein by reference.

FEDERAL FUNDING

This invention was made with government support under 0140621 awarded by the National Science Foundation. The government has certain rights in the invention.

INCORPORATION BY REFERENCE

All of the references cited below are incorporated herein by reference.

FIELD OF THE INVENTION

The invention is directed to pharmaceutical compositions containing biocidal co-polymers of poly(styrenes), poly(acrylates), poly(acrylamides), and poly(C1−C6)alkylene glycols. More specifically, the invention is directed to pharmaceutical compositions and methods of treating microbial infections in mammals.

BACKGROUND

The emerging prevalence of bacteria resistant to common therapeutically agents has led to a dire need for new antimicrobial compounds. Peptide antimicrobials, a central element of the human immune system, have received increasing interest as potential new antimicrobial treatments. One reason for their potential success is that it appears to be difficult, although not impossible, for pathogenic microbes to develop resistance to these innate "host-defense" peptides. One large subset of host-defense peptides forms an amphiphilic α-helical structure. These peptides act by disrupting bacterial membranes because their net positive charge attracts the peptides to the negatively charged bacterial membrane, and the hydrophobic face of the helix allows the formation of aggregates that compromise membrane integrity. Amphiphilic topology also plays a factor in the biological activity of these molecules, as enantiomeric peptides retain full activity.

This design principle has been applied to distinct types of amphiphilic helical antimicrobial oligomers. For example, β-Amino acid oligomers ("β-peptides") can adopt discrete helical conformations. By properly arranging cationic and lipophilic residues within the β-peptide, amphiphilic helicates with antimicrobial activity can by obtained. DeGrado et al. also describe aryl amide oligomers with elongated conformations that can project lipophilic and cationic groups to opposite sides of the molecular backbone. Unlike α- or β-peptide oligomers, these aryl amide oligomers are achiral.

SUMMARY OF THE INVENTION

The invention disclosed and claimed herein arose from the inventors' interest in determining how much conformational "pre-organization" is required for antimicrobial activity in synthetic oligomers or polymers. For example, the helical, antimicrobial β-peptides developed by Gellman et al.
without limitation, t-amyl peroxybenzoate, 4,4-azo-bis-(4-cyanovuleric acid), 1,1'-azo-bis-(cyclohexane carboxonitrile), AIBN, benzoyl peroxide, 2,2-bis-(t-butylperoxy)butane, 1,1-bis-(t-butylperoxy)cyclohexane, 2,5-bis-(t-butylperoxy)-2,5-dimethylhexane, 2,5-bis-(t-butylperoxy)-2,5-dimethyl-3-hexyne, t-butyl-peracetate, t-butyl peroxide, t-butyl peroxybenzoate, t-butylperoxy-isopropyl carbonate, cumene hydroperoxide, peracetic acid, potassium persulfate, and the like. See Denisov et al. for an exhaustive treatment of free-radical initiators and free-radical-mediated polymerizations.

MBC=minimal bactericidal concentration; the minimum concentration of an active agent that kills all or substantially all of the selected target cell type.

MIC=minimum inhibitory concentration; the minimum concentration of an active agent that inhibits growth of the selected target cell type.

NMR=nuclear magnetic resonance spectroscopy

PDI=polydispersity index

Pharmaceutically-suitable salt: any salt conventionally used in the formulation of pharmaceutical compositions for ingestion, injection, or topical application, including, without limitation, those derived from mineral acids and organic acids, explicitly including hydrohalides, e.g., hydrochlorides and hydrobromides, sulphates, phosphates, nitrates, sulphamates, acetates, citrates, lactates, tartrates, malonates, oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene-bis-b-hydroxynaphthoates, gentisates, isethionates, di-p-toluoyltartarates, methane-sulphonates, ethanesulphonates, benzenesulphonates, p-toluenedisulphonates, cyclohexylsulphamates, quinates, and the like; and base addition salts, including those derived from alkali or alkaline earth metal bases or conventional organic bases, such as triethylamine, pyridine, piperidine, morpholine, N-methylmorpholine, and the like.

UV-Vis=ultraviolet-visible spectroscopy

Biocidal and Antimicrobial Pharmaceutical Compositions:

The biocidal and antimicrobial compositions of the present invention contain as active ingredients co-polymers of poly(styrenes), poly(acrylates), poly(acrylamides), and poly(C1-C6-alkylene glycols). Co-polymers of poly(styrenes) are preferred. Copolymers of 4-(dimethylaminomethyl)-styrene (1) and 4-octylstyrene (2) were prepared by a free-radical polymerization method. Hydrophobic size-exclusion chromatography using Sephadex LH-20 yielded samples of polymer without monomer vinyl peaks in the 1H NMR spectrum. For clarity and brevity, copolymers will be referred to herein by the molar percentages of monomers in the feed mixture. Thus, copoly(195:25) refers to the product generated by polymerization of a mixture of 95 mol% 1 and 5 mol% 2. Analysis of the copolymers indicated that their composition closely approximates that of the feed mixture.

Polymer molecular weight averages were determined by GPC using literature methods. Most polymers had molecular weights (Mw) close to 9000 Da, with polydispersity indices, PDI’s (i.e., Mw/Mn) close to 3.0, which is typical for AIBN-initiated radical polymerizations. These data yield Mn values near 3000, which are comparable to the molecular weight of the magainin derivative used as a standard.
Thus, the present invention is directed to biocidal and antimicrobial pharmaceutical compositions (homopolymers and heteropolymers), wherein the active ingredient comprises one or more polymers of the formula -(A)n-, wherein each A is a residue independently selected from the group consisting of:

- C1-C6-alkyl, -(CH2)n+1-OR1, -(CH2)n+1-SR1,
- 10

wherein each R is independently selected from the group consisting of hydrogen, linear or branched C1-C6-alkyl, alkenyl, or alkynyl, unsubstituted amino-C1-C6-alkyl, mono- or di-C1-C6-alkylamino, mono or bicyclic hydroxy having up to 5 heteroatoms selected from N, O, and S; mono or bicyclic aryl-C1-C6-alkyl, mono- or bicyclic heteroaryl-C1-C6-alkyl, mono- or bicyclic aryl-C1-C6-alkyl, mono- or bicyclic heteroaryl-C1-C6-alkyl, and pharmaceutically-suitable salts thereof; in combination with a pharmaceutically-suitable carrier.

Note that where a substituent is designated as being "independently selected" from a given set of moieties, each appearance of the stated substituent can be different. Thus, for example, the formula -(A)n- comprises homopolymers wherein each appearance of "A" is the same, and heteropolymers wherein each appearance of "A" is different (i.e., A-A'-A"-A"'). The same applies for the various R substituents.

Synthesis of the poly(styrene), poly(acrylamide), and poly(acrylate) co-polymers according to the present invention is preferably carried out via free-radical-mediated polymerization, as described in the Examples.

While poly(C1-C6-alkyl)-co-polymers can be synthesized using free-radical methods, it is not the preferred route. Using poly(ethylene glycol) (PEG) as an example, PEG is preferably synthesized via a ring-opening reaction of ethylene oxide, which is widely known in the art. Poly(alkylene glycol) can be co-polymerized with the other monomer types described herein using the methods described in, for example, Ishizu, Shen & Tsubaki (March 2000) Polymer, 41(6):2053-2057, and Cheng, Wang & Chen (February 2003) Materials Chemistry & Physics 78(3):581-590. For methods of synthesizing co-polymers of PEG and poly(acrylamides), see Auzanneau et al. (1998) Can. J. Chem. 76(8):1109-1118.

Ring-opening polymerizations of epoxides, such as ethylene oxide or propylene oxide may be accomplished using cationic ring-opening polymerization. Strong protic acids are conventionally used as a catalyst, e.g., H2SO4, CF3SO3H, and CF3CO2H. The same polymerization can also be accomplished using anionic ring-opening polymerization. Typical initiators for anionic ring-opening polymerization include, without limitation, alkali metals (Na, K), inorganic bases (NaOH, KOH), metal oxides (LiOCH3), and metal alkyls and hydrides (BuLi, NaH).

Polymerization can be head-to-tail and/or head-to-head, syndiotactic, isotactic, and combinations thereof.

Polymers that are cationic by virtue of quaternized nitrogen atoms and structurally related to poly(I) and copoly(1:2) have been studied as antimicrobial agents. 14-18 However, polymers prepared from 1 differ significantly from the quaternized prior art compounds in that, like host-defense peptides, polymers containing dimethylaminomethyl groups require protonation to develop positive charge. Thus, the preferred compounds for use in the present invention are those containing a fraction (e.g., from about 1 mol% to about 99 mol%) of R groups that require protonation to develop positive charge, and a fraction that or R groups (e.g., from about 99 mol% to about 1 mol%) that are neutral or anionic.

Two examples from the quaternized class of compounds were used for comparison with the protonatable polymers described herein. Poly(5) was synthesized from monomer 5 via reported methods. 15,16 Poly(7) was purchased (Sigma, St. Louis, Mo.) in a form that is >98% quaternized, having an Mn=12.0 KDa and a PDI=1.06. Both poly(5) and poly(7) are quaternized via N-methylation. These polymers have shorter alkyl chains than the octyl group of (2) but are preferred for this work because limited aqueous solubility was reported by
B. subtilis, mers were not tested at higher concentrations due to limited vancomycin-resistant related pattern. Below 20-30% (2), antimicrobial activity is poly(7) >50 25 35.4 proportion of styrene was raised; once a threshold value was 60 period, thus causing turbidity even in the absence of bacteria. benzylammonium18·19 and vinylpyridinium 17 salts have 55 poor activity of poly(5) in our brain-heart infusion broth of the polymers with the agar, 16 or cell viability counting after incubating cells with polymer in sterile water or saline. 16 The present data for copoly(l :2) show a decreased activity. 17 The low activity of the comparison polymers in the BHI encoun-ter thereof, or a pharmaceutically acceptable salt of the com-pound or isomeric form thereof, together with an acceptable composition. The data on Orange OT solubilization and ANS fluorescence, taken together, are consistent with a “molten globule-like” model of polymer conformation. In such a model, these polymers cannot present solely cationic functionality to the solvent surface at the concentrations studied. The tertiary amine-containing polymers show inhibitory activity against all four organisms tested. Because data points are taken at twofold dilutions, a twofold variation in MIC corresponds to a single data point. Therefore, even the apparent wide variation in MIC seen should be interpreted with great caution. The polymers also show bactericidal activity against both Gram-positive and Gram-negative pathogens.

### TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>E. coli JM109</th>
<th>B. subtilis BR151</th>
<th>S. aureus 5332</th>
<th>E. faecium A436</th>
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</thead>
<tbody>
<tr>
<td>poly(1)</td>
<td>25</td>
<td>12.5</td>
<td>50</td>
<td>12.5</td>
</tr>
<tr>
<td>Copoly(1a:2b)</td>
<td>50</td>
<td>12.5</td>
<td>25</td>
<td>6.3</td>
</tr>
<tr>
<td>Copoly(1b:2a)</td>
<td>50</td>
<td>12.5</td>
<td>25</td>
<td>6.3</td>
</tr>
<tr>
<td>copoly(1a:2b)</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
<td>3.2</td>
</tr>
<tr>
<td>copoly(1a:2b)</td>
<td>50</td>
<td>12.5</td>
<td>17.8</td>
<td>6.3</td>
</tr>
<tr>
<td>copoly(1b:2a)</td>
<td>50</td>
<td>24</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>copoly(1b:2a)</td>
<td>50</td>
<td>35.4</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>copoly(1a:2b)</td>
<td>&gt;50</td>
<td>50</td>
<td>&gt;50</td>
<td>50</td>
</tr>
<tr>
<td>(Ala8·18)-magainin-2-amide</td>
<td>12.5</td>
<td>6.3</td>
<td>12.5</td>
<td>3.2</td>
</tr>
<tr>
<td>poly(5)</td>
<td>&gt;50</td>
<td>50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>poly(7)</td>
<td>&gt;50</td>
<td>25</td>
<td>35.4</td>
<td>50</td>
</tr>
<tr>
<td>(1: monomer)</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

Both nonpolar and electrostatic forces are believed to be important in the interactions of host-defense peptides with bacterial membranes. Prior studies of copolymers of vinylbenzylammonium18·19 and vinylpyridinium17 salts have shown that antimicrobial activity is influenced by the proportion of cationic and lipophilic monomers. In an examination of N-benzyl-4-vinyl pyridinium/styrene copolymers, Li et al. found that sterilizing activity at first remained constant as the proportion of styrene was raised; once a threshold value was reached, however, additional styrene incorporation led to decreased activity. The present data for copoly(1:2) show a related pattern. Below 20-30% (2), antimicrobial activity is only modestly affected by the proportion of lipophilic component (2), but above this level activity drops precipitously.

GPC analysis showed that for the polymers described in the Examples, the GPC procedure used gives similar, broad molecular weight distributions, regardless of polymer composition, a phenomenon typical of AIBN-initiated free-radical polymerizations.

These polymers contain both hydrophobic and hydrophilic functional groups. Their hydrophobic portions could be either buried within micelle-like structures or exposed to solvent. Colorimetric assays, as described in the Examples, were performed to test each of these hypotheses. Specifically, solubilization of the hydrophobic dye Orange OT can indicate the formation of micelle-like structures with hydrophobic interior regions. Single polymer molecules might form such structures even at low concentrations, but the data collected to date (not shown) did not show significant, reproducible dye solubilization. Hence, at the concentrations studied, the subject polymers do not act as conventional micellar detergents.

The data on Orange OT solubilization and ANS fluorescence, taken together, are consistent with a “molten globule-like” model of polymer conformation. In such a model, these polymers cannot present solely cationic functionality to the solvent surface at the concentrations studied. The tertiary amine-containing polymers show inhibitory activity against all four organisms tested. Because data points are taken at twofold dilutions, a twofold variation in MIC corresponds to a single data point. Therefore, even the apparent wide variation in MIC seen should be interpreted with great caution.

The polymers also show bactericidal activity against both Gram-positive and Gram-negative pathogens.

The interaction between anionic bacterial membranes and antimicrobial peptides is believed to be primarily electrostatic. Nevertheless, poly(l) and its derivatives displayed anti-bacterial activity similar to that of magainin, whereas poly(5) and poly(6) showed little to no activity.

Past assays of poly(5) and poly(6) used agar plate assays (which have since been discontinued) or cell viability counting after incubating cells with polymer in sterile water or saline. The low activity of the comparison polymers in the BHI growth inhibition assay may be due to interaction between the polymers and anionic components of the broth. However, a rich medium like BHI may better represent conditions encountered during infection of a host organism.

All of the tertiary amine polymers described in the Examples are useful as broad-spectrum biocides and antibiotics. Pharmaceutical compositions of the present invention, comprise a biocidal- or antimicrobial-effective amount of an active compound as described above, an isomeric form thereof, or a pharmaceutically acceptable salt of the compound or isomeric form thereof, together with an acceptable carrier for it, and optionally other therapeutically active ingredients. The carrier must be “pharmaceutically acceptable” or “pharmaceutically suitable,” i.e., the carrier must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof.
The compositions include those suitable for oral, rectal or parenteral (including subcutaneous, intramuscular, intradermal and intravenous) nasal, or bronchial administration. Topical formulations are also included, for example, for topical antibiotic use.

It is noted that some of the compounds and isomers described herein thereof are also rather insoluble in water and, accordingly, liquid formulations which account for this factor may be made according to art-recognized pharmaceutical techniques. Examples of these techniques include an injection wherein the active compound is dissolved in a suitable solvent or co-solvent such as an appropriate polyethylene glycol, or a propylene glycol or the like; a sealed gelatin capsule enclosing an oily solution of the active compound; a suppository of the active compound in a conventional suppository base such as cocoa butter; or a liposome some formulation, for example, the active compound and a glycerophospholipid such as phosphatidylcholine. In any event, the aforementioned characteristics of the subject compounds and isomers are not uncommon in the pharmaceutical art and, accordingly, art-recognized pharmaceutical techniques are employed to prepare appropriate formulations for such compounds, isomers or pharmaceutically acceptable salts of either.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound or salt into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound or salt into association with a liquid or solid carrier and then, if necessary, shaping the product into desired unit dosage form.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets, boluses or lozenges, each containing a predetermined amount of the active compound (optionally in the form of a salt thereof); as a powder or granules; or in liquid form, e.g., as suspension, solution, syrup, elixir, emulsion, dispersion, or the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form, e.g., as a powder or granules, optionally mixed with accessory ingredients, e.g., binders, lubricants, inert diluents, surface active or dispersing agents. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active compound with any suitable carrier.

Formulations suitable for parenteral administration conveniently comprise a sterile preparation of the active compound (optionally in the form of a salt thereof) in, for example, a polyethylene glycol 200 or propylene glycol solution which is preferably isotonic with the blood of the recipient.

Useful formulations also comprise concentrated solutions or solids containing the active compound(s), any isomeric form thereof, or a pharmaceutically acceptable salt of the compound or isomeric form thereof, which upon dilution with an appropriate solvent give a solution suitable for parenteral administration.

Preparations for topical or local applications, which are, for example, conventional for preventing or treating bacterial infections of the skin, mouth, and eyes, comprise aerosol sprays, lotions, gels, ointments, etc. and pharmaceutically acceptable vehicles therefore such as, for example, lower aliphatic alcohols, polyglycerols such as glycerol, polyethylene glycol, esters of fatty acids, oils and fats, silicones, and other conventional topical carriers.

In topical formulations, the active compounds (or isomers thereof) are preferably utilized at concentrations of from about 0.1% to about 5.0% percent by weight.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more optional accessory ingredient(s) utilized in the art of pharmaceutical formulations, e.g., diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, suspending agents, preservatives (including antioxidants) and the like.

The active compounds described herein (including all isomers) and salts thereof of the invention are intended to be administered under the guidance of a physician or veterinarian.

The amount of pharmacologically active compound (or any isomer thereof) or salt thereof required to be effective for antimicrobial treatment will, of course, vary with the individual mammal being treated and is ultimately at the discretion of the medical or veterinary practitioner. The factors to be considered include the condition being treated, the route of administration, the nature of the pharmaceutical composition, the mammal’s body weight, surface area, age and general condition, and the particular compound or salt to be administered. In general, the pharmaceutical compositions of this invention contain from about 0.5 to about 500 mg and, preferably, from about 5 to about 350 mg of the active ingredient, preferably in a unit dosage form.

A suitable effective dose is in the range of about 0.1 to about 200 mg/kg body weight per day, preferably in the range of about 1 to about 100 mg/kg per day, calculated as the non-salt form of the active compound. The total daily dose may be given as a single dose, multiple doses, e.g., two to six times per day, or by intravenous infusion for a selected duration. Dosages above or below the range cited above are within the scope of the present invention and may be administered to the individual patient if desired and necessary.

For example, for a 75 kg mammal (preferably an human), a dose range would be about 7.5 to about 1500 mg per day, and a typical dose would be about 800 mg per day. If discrete multiple doses are indicated, treatment might typically be 200 mg of a compound disclosed herein, given 4 times per day. Topical Biocidal Compositions:

The subject compounds are also useful for sterilizing surfaces and rendering them resistant to subsequent cellular growth. Thus, for example, one or more of the active ingredients disclosed herein, optionally in combination with a suitable carrier, can be used to treat the surfaces of implantable medical devices prior to their implantation. For instance, pacemakers, stents, shunts, catheters, and the like can be treated with the compounds of the subject invention to render these items free of cellular contamination in general and bacterial contamination in particular. Likewise, optical devices that are inserted into the body, such as endoscopes, bronchoscopes, and the like, can also be treated with compounds of the subject invention, thereby rendering the surface of the device microbe-free.

In practice, using the compounds of the present invention to treat a surface is a simple matter of contacting the surface with a sufficient amount of the active ingredient, for a time sufficient to allow the compound to exert its biocidal effect. The active ingredient can be applied neat, or in combination with a suitable carrier. Insofar as the active ingredients will not be injected or ingested into the body, the carrier for purposes of treating inanimate surfaces need not be pharmaceutically suitable.
Alternatively, the subject compounds can be covalently bonded to the surface of interest. In one approach, the fully-formed compounds can be attached covalently using the same chemistries described herein. Also, the subject compounds can be synthesized directly attached to the surface via covalent bonds by polymerizing the compounds de novo from an initiator group that is covalently attached to the surface of interest. Again, this can be accomplished using the chemistries described herein.

EXAMPLES

The following Examples are included solely to provide a more clear and consistent understanding of the invention disclosed and claimed herein.

Materials: Substituted styrenes (liquid) (obtained commercially from Monomer-Polymer/Dajac Laboratories, Feasterville, Pa.) were purified by vacuum distillation (representative boiling points: DMAS 1, 73° C. at 1.7 mm Hg; 2, 122° C. at 0.75 mm Hg) and stored at −80° C. until use. Styrene 4 (Aldrich, Milwaukee, Wis.) was purified by passage through an inhibitor-removal column (SDTR-7, Scientific Polymer Products, Ontario, N.Y.) immediately before use. VBC (a 68:32 mixture of m- and p-isomers, Aldrich) was purified by vacuum distillation immediately before use. Benzene was distilled from sodium benzenophenone ketyl. Methanol was distilled from Mg(OMe)\(_2\).

AIBN, dodecyldimethylamine, and (ar-vinylbenzyl)trimethylammonium chloride 5 (Aldrich) were used immediately before use. Benzal chloride salts of polymers were obtained by dissolving the monomer-Polymer/Dajac Laboratories. Feasterville, Pa.) were purified by vacuum distillation (representative boiling points: DMAS 1, 73° C. at 1.7 mm Hg; 2, 122° C. at 0.75 mm Hg) and stored at −80° C. until use. Styrene 4 (Aldrich, Milwaukee, Wis.) was purified by passage through an inhibitor-removal column (SDTR-7, Scientific Polymer Products, Ontario, N.Y.) immediately before use. VBC (a 68:32 mixture of m- and p-isomers, Aldrich) was purified by vacuum distillation immediately before use. Benzene was distilled from sodium benzenophenone ketyl. Methanol was distilled from Mg(OMe)\(_2\).

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13

ArCH₂ octyl), 3.231 ppm (lump, 2.2H, backbone), 1.673 ppm (multiple lumps, 2.26H, backbone, water), 2.148 ppm (two lumps, 4.99H, Me₃N), 2.486 ppm (lump, 0.46H, ArCH₂ octyl), 3.207 ppm (two lumps, 1.67H, ArCH₃N), 6.407 ppm (lump, 1.75H, aromatic), 6.932 ppm (lump, 2.23H, aromatic).

Poly(1:2): 26.9 mg (156 µmol, 7.8%). 1H NMR δ (CDCl₃, 300 MHz) 0.896 ppm (lump, 0.83H, octyl methyl), 1.295 ppm (lump, 3.72H, octyl, backbone), 1.673 ppm (multiple lumps, 2.26H, backbone, water), 2.148 ppm (two lumps, 4.99H, Me₃N), 2.486 ppm (lump, 0.46H, ArCH₂ octyl), 3.207 ppm (two lumps, 1.67H, ArCH₃N), 6.407 ppm (lump, 1.75H, aromatic), 6.932 ppm (lump, 2.23H, aromatic).

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GPC Mw = 5.74 KDa, Mn = 2.85 KDa, PDI = 2.01.

Poly(1:2): 26.9 mg (156 µmol, 7.8%). 1H NMR δ (CDCl₃, 300 MHz) 0.896 ppm (lump, 0.83H, octyl methyl), 1.295 ppm (lump, 3.72H, octyl, backbone), 1.673 ppm (multiple lumps, 2.26H, backbone, water), 2.148 ppm (two lumps, 4.99H, Me₃N), 2.486 ppm (lump, 0.46H, ArCH₂ octyl), 3.207 ppm (two lumps, 1.67H, ArCH₃N), 6.407 ppm (lump, 1.75H, aromatic), 6.932 ppm (lump, 2.23H, aromatic).

GPC Mw = 5.74 KDa, Mn = 2.85 KDa, PDI = 2.01.
(lump, 2.52H, aromatic). $^{13}$C NMR δ (CDCl$_3$, 75 MHz) 12.644 ppm (broad, positive to DEPT-135, aromatic), 127.096 ppm (broad, positive to DEPT-135, aromatic), 127.816 ppm (broad, positive to DEPT-135, aromatic), 128.522 ppm (broad, positive to DEPT-135, aromatic), 40.189 ppm (positive to DEPT-135, backbone), 45.183 ppm (positive to DEPT-135, NMe$_2$), 63.991 ppm (negative to DEPT-135, ArchN). GPC M$_w$=8.17 KDa, M$_n$=3.12 KDa, PDI=2.47.

Poly(1):47: 15.6 mg (99.2 µmol, 5.0%). $^1$H NMR δ (CDCl$_3$, 300 MHz) 0.895 ppm (lump, 0.18H, backbone), 1.32 ppm (lump, 1.91H, backbone), 1.826 ppm (lump, 1.6H, backbone), 2.145 ppm (two lumps, 5.71H, Me$_2$N), 3.27 ppm (lump, 1.85H, ArCH$_2$N), 6.326 ppm (lump, 1.75H, aromatic), 6.865 ppm (lump, 2.25H, aromatic). $^{13}$C NMR δ (CDCl$_3$, 75 MHz) 127.344 ppm (multiple positive peaks on DEPT-135, aromatic), 45.285 ppm (positive to DEPT-135, backbone), 1.7 ppm (lump, 1.19H, backbone), 2.149 ppm (two lumps, 5.89H, Me$_2$N), 3.226 ppm (double lump, 2H, ArCH$_2$N), 6.408 ppm (lump, 1.85H, aromatic), 6.946 ppm (lump, 2.46H, aromatic). $^{13}$C NMR δ (CDCl$_3$, 75 MHz) 127.82 ppm (broad, positive to DEPT-135, aromatic), 40.255 ppm (positive to DEPT-135, backbone), 45.227 ppm (positive to DEPT-135, NMe$_2$), 64.015 ppm (negative to DEPT-135, ArCH$_2$N), 64.275 ppm (negative to DEPT-135, ArCH$_2$N).

Determination of Apparent Molecular Weight: GPC was carried out at ambient temperature (23-25°C) using a Shimadzu HPLC system and pair (in series) of 300×7.5 mm PLgel Mixed D columns (5 µm pore size, Polymer Laboratories, Amherst, Mass.). Calibration was performed using polystyrene standards (EasiCal PS-1, Polymer Laboratories) of M$_w$: 841.7, 320, 148, 59.5, 28.5, 10.85, 2.93, and 0.58 KDa. Polymers were dissolved in ether (0.1% TEA, freshly distilled from CaH$_2$, in HPLC-grade THF, Aldrich) to concentrations of ~5 mg/mL, and 30 µL injections were made. Ultraviolet absorbance data was collected using Shimadzu CLASS-VP software (version 7.1.1) and exported to Microsoft Excel. A baseline was determined by linear regression analysis of peak-free regions and subtracted from the trace. The baseline-corrected trace was then, integrated according to the method described by Yau et al. 30 to determine M$_w$ and M$_n$. Correction for peak spreading was not performed. PDI was calculated as M$_w$/M$_n$.

Separate GPC analysis was performed on a representative subset of samples using a variety of PLgel columns using ELS detection, showed similar apparent MW. However, GPC performed on a Waters instrument in 2% NMP/THF, using a pair of Waters Styragel HT6E columns and RI detection, gave consistently larger apparent MW values (by approximately a factor of 3). This may be due to the 5 KDa lower detection limit of the Styragel columns and is mentioned as a caution that GPC-determined molecular weights are apparent.

Partial Fractionation: Samples of poly(1) and copoly(l$_{16}$:2$_3$), (l$_{16}$:2$_2$), (l$_{16}$:2$_1$), (l$_{16}$:2$_{10}$), (l$_{16}$:2$_{13}$), (l$_{16}$:2$_{20}$), and (l$_{16}$:2$_{30}$) were dissolved in 0.2N HCl (prepared by dilution of sterile-filtered 1.0N HCl with Millipore water) to a concentration of 1-2 mg/mL. One to ten mL (1-10 mL) of these solutions were added to Amicon Centrifplus YM-50 filter units (Millipore, Bedford, Mass.), which had been rinsed once with Millipore water. The filter units were centrifuged at 3000g for 30 min. The retentates were collected by inverted spin filtration. The filtrates were then transferred to YM-30 filter units and centrifuged for 30 min. Further serial filtration through YM-10 (90 min) and YM-3 (250 min) filter units gave five fractions (i.e., the fractions retained by YM-50, retained by YM-30, retained by YM-10, retained by YM-3, and filtrate), which were dried by centrifugal evaporation. GPC analysis was performed as described above.

Dye Solubilization: Orange OT (Aldrich) was dissolved in ACS reagent-grade acetone, precipitated with Millipore water, recrystallized twice from absolute EtOH, and dried overnight under vacuum. Stock solutions of polymers (2 mg/mL), prepared as described above, were diluted with Mil-
lipase samples at each concentration were prepared; a small dilution was measured (1.0 cm path length cell, HP 8452 UV/vis to be examined; mock dilutions of Millipore water were added to one, and the other (treated identically but containing no Orange OT) was used as a spectroscopic blank to correct for absorbance of the polymer. All vials were agitated gently for 3 d at room temperature on a blood-rocker and then filtered through cotton to remove undissolved orange OT. An aliquot (200 µL) of each sample was diluted with 800 µL of absolute EtOH, and absorbance of each solution was measured (1.0 cm path length cell, HP 8452 UV/vis spectrophotometer) at 500 nm (Orange OT absorbance) and 340 nm (background, to correct for baseline drift). Net absorbance (OD$_{500}$-OD$_{340}$), using the Orange OT-free samples as spectroscopic blanks and corrected for net absorbance of the polymer-free samples, was determined.

ANS Fluorescence: A 10 mM stock solution of high-purity ANS (Molecular Probes, Eugene Oreg.) was prepared in Millipore water and transferred to a disposable vial. Vials were charged with 382.5 µL of 50 mM freshly prepared Tris buffer (pH 6.8 at 23° C. in Millipore water, to avoid the pH dependence of ANS fluorescence, which arises below pH 6.0); 7.5 µL of polymer stock solutions (2 mg/mL), prepared as described above, or Millipore water (as a polymer-free blank) were added to pairs of vials. Ten µL of ANS stock solution was then added to one (1) vial from each pair, and 10.0 µL of Millipore water to the other, to give 400 µL of solutions that were ~250 µM in monomer residue and either 0 µM (ANS-free blank) or 250 µM in ANS.

Fluorescence spectra were measured in a single session on a Hitachi F-4500 fluorescence spectrophotometer. Excitation and emission slit widths were set to 5 nm. Samples were excited at 350 nm; emission spectra were collected from 370 nm to 650 nm. Polymer fluorescence in the ANS-free blanks was of negligible intensity compared to the fluorescence of the ANS-containing samples, but the corresponding ANS-free blank spectrum was subtracted from each ANS-containing sample spectrum.

Biological Properties:

MIC, MBC, and hemolysis assays were performed based upon standard protocols, as described herein.

Minimal Inhibitory Concentration (MIC): Stock solutions of polymers (5.00 µL at 2 mg/mL, prepared as described above), commercial synthetic (Ala$^{\text{K}},$ Ala$^{\text{K}}$)-1-magainin-2-amide (Sigma, dissolved in Millipore water to 1 mg/mL, 10.0 µL, as positive control), or solvent blank (5.00 µL) were diluted into brain-heart infusion (BHI) broth in row A of 96-well sterile assay plates (B-D Falcon 35-3075, Fisher Scientific) and seven serial twofold dilutions were performed to give 50 µL of peptide solution (at double the final assay concentration) in each well. Bacterial strains (Escherichia coli JM109, Bacillus subtilis BR151, 24 Staphylococcus aureus 5332 (a clinical MRSA isolate from the Weisblum laboratory strain collection), and Enterococcus faecium A436$^{\text{K}}$) were grown on 2% bacteriological agar in BHI medium. Cells from these cultures were suspended in BHI broth at a concentration of approximately 10$^9$ colony-forming units (CFU)/mL, and 50 µL of cell suspension was added to each well to give a total of 100 µL in each well.

Plates were then incubated at 37° C. for 6 h. Bacterial growth gives rise to turbidity and light scattering, which was detected by measuring 0$_{465}$, using a Molecular Devices Emax microplate reader connected to a Windows computer running SOFTmax v.2.34. OD$_{465}$ values from two simultaneous experiments were averaged. MIC, the concentration at which growth is completely inhibited, is reported as the median value of at least three experiments from separately diluted bacterial suspensions, at least two of which were performed on different days. If an even number of experiments was performed and the two median values were not identical, the geometric mean is reported as the MIC.

Minimal Bactericidal Concentration (MBC): To determine cell viability, 10 µL from each of the MIC, 2xMIC, and 4xMIC wells (up to the maximum concentration of 50 µL/mL) was diluted 100-fold. Of this, 100 µL (containing at most 5x10$^5$ CFU) was plated on 2% bacteriological agar in BHI medium and incubated at 37° C. overnight (for B. subtilis) or for 4 d (for E. coli, because no growth was seen after 10 h; colonies appeared on some plates after extended incubation). The absence of colonies was considered indicative of >99% bacterial killing, and the lowest assay well concentration showing no colonies is reported as the MBC.

Hemolysis Assay: Sterile TBS (Tris buffered saline: 10 mM Tris, 150 mM NaCl, pH 7.2) was prepared and used to dilute stock solutions of polymers (5.00 µL at 2 mg/mL, prepared as described above), commercial synthetic melittin (Sigma, dissolved in Millipore water to 1 mg/mL, 10.0 µL, as control), or solvent blank (5.00 µL) in row A of 96-well sterile assay plates. Seven serial twofold dilutions were performed to give 20 µL of peptide solution (at five times the final assay concentration) in each well. Freshly drawn human red blood cells (hRBC, blood type A, collected 10 h; colonies appeared on some plates after extended incubation). The absence of colonies was considered indicative of >99% bacterial killing, and the lowest assay well concentration showing no colonies is reported as the MBC.

The results are presented in Tables 2 and 3:

<table>
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<tr>
<th>Comonomer</th>
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<th>4</th>
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<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<td>E. coli JM109</td>
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<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
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<td>25</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
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<tr>
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<td>50</td>
<td>50</td>
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### TABLE 2-continued

<table>
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<th>Comonomer</th>
<th>E. coli IM109</th>
<th>B. subtilis BR151</th>
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<td>&gt;50 25</td>
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<td>20%</td>
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<td>&gt;50</td>
</tr>
<tr>
<td>35%</td>
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| 15% [Ala8,13,18]-magainin 2 amide | 25 |

---

### TABLE 3

<table>
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<tr>
<th>Comonomer</th>
<th>E. coli IM109</th>
<th>B. subtilis BR151</th>
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<th>E. faecium A436</th>
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<tr>
<td>20%</td>
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<td>35.4 8.8 17.8 25</td>
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<td>35% [Ala8,13,18]-magainin 2 amide</td>
<td>12.5 6.3</td>
<td>12.5 6.3</td>
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</table>

poly(5) | >50 | >50 |
poly(6) | >50 | >50 |
water blank | >50 | >50 |
aq. DMF/EtOH | >50 | >50 |
1 (monomer) | >50 | >50 |

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### REFERENCES

13. Pertinent data may be found in the Supporting Information.
What is claimed is:

1. A pharmaceutical composition for treating microbial infection in a subject in need thereof, the composition comprising an antimicrobial-effective amount of a compound selected from the group consisting of a polymer of formula -(A)n-, wherein each A is a residue independently selected from the group consisting of:

   - substituted amino-C1-C6-alkyl;
   - mono- or bicyclic aryl;
   - mono- or bicyclic hetero aryl-C1-C6-alkyl;
   - unsubstituted amino-C1-C6-alkyl; disubstituted amino-C1-C6-alkyl; mono- or bicyclic aryl; mono- or bicyclic hetero aryl having up to 5 heteroatoms selected from N, O, and S;
   - wherein each R is independently selected from the group consisting of:

2. The pharmaceutical composition of claim 1, wherein at least one A is a residue containing a nitrogen atom, the nitrogen atom being capable of being quaternized, or a pharmaceutically suitable salt thereof.

3. The pharmaceutical composition of claim 2, wherein the pharmaceutically suitable carrier is suitable for oral, rectal, parenteral, nasal or bronchial administration.

4. The pharmaceutical composition of claim 1, wherein the compound has a molecular weight of between about 4.5 kDa and 11.5 kDa as determined by gel permeation chromatography.

5. The pharmaceutical composition of claim 1, wherein each R is independently selected from the group consisting of hydrogen; linear or branched C1-C30-alkyl, alkenyl, or alkynyl; unsubstituted amino-C1-C6-alkyl; mono-substituted amino-C1-C6-alkyl; disubstituted amino-C1-C6-alkyl; mono- or bicyclic aryl; mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S.

6. The pharmaceutical composition of claim 1, wherein n is an integer ≥6.

7. A method of preventing and treating microbial infections in a subject in need thereof, the method comprising administering to the subject an effective anti-microbial amount of a compound selected from the group consisting of formula -(A)n-, wherein each "A" is a residue independently selected from the group consisting of:

   - substituted amino-C1-C6-alkyl;
   - mono- or bicyclic aryl;
   - mono- or bicyclic hetero aryl-C1-C6-alkyl;

8. The method of claim 7, wherein the compound is administered to a mammalian subject.

9. The method of claim 7, wherein the compound is administered to a human subject.

10. The method of claim 7, wherein the compound is administered in combination with a pharmaceutically suitable carrier.
11. The method of claim 7, wherein the compound is administered in combination with a pharmaceutically suitable carrier suitable for oral, rectal, parenteral, nasal or bronchial administration.

12. The method of claim 7, wherein a compound having a molecular weight of between about 4.5 kDa and 11.5 kDa as determined by gel permeation chromatography is administered to the subject.

13. The method of claim 7, wherein a compound where each R is independently selected from the group consisting of hydrogen; linear or branched C₅₋C₂₀-alkyl, alkenyl, or alkynyl; unsubstituted amino-C₅₋C₂₀-alkyl; mono-substituted amino-C₅₋C₂₀-alkyl; disubstituted amino-C₅₋C₂₀-alkyl; mono- or bicyclic aryl; and mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S is administered to the subject.

14. The method of claim 7, wherein a compound wherein n is an integer ≥6 is administered to the subject.

15. The method of claim 7, wherein the amount of compound administered to the subject is sufficient to provide a concentration of the compound, at point of contact with a microbial cell, of from about 1 µM to about 100 µM.

16. The method of claim 7, wherein the amount of compound administered to the subject is sufficient to provide a concentration of the compound, at point of contact with a microbial cell, of from about 1 µM to about 10 µM.