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(54) **Title:** ANTIMICROBIAL COATINGS AND METHODS OF MAKING AND USING THEREOF

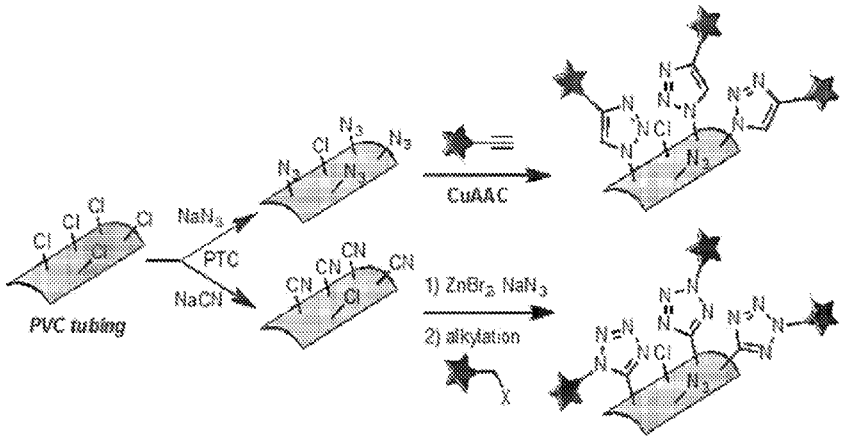


FIGURE 21

(57) **Abstract:** Disclosed are antimicrobial articles and surfaces, as well as methods of making such articles and surfaces.

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Antimicrobial Coatings and Methods of Making and Using Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 62/343,317, filed May 31, 2016, and U.S. Provisional Application No. 62/393,684, filed September 13, 2016, both of which are hereby incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government Support under Grant No. CHE 1011796 awarded by the National Science Foundation. The Government has certain rights in the invention.

BACKGROUND

Infection acquired from health care environments is one of the leading major medical complications in the present world. Studies have shown that almost 6% of patients admitted to hospitals acquire infections and the number of such cases is increasing. According to reports by the U.S. Centers for Disease Control and Prevention, hospital acquired infections account for more than 2 million cases leading to 99,000 deaths annually.

The most common hospital acquired infections include urinary tract infections, surgical wound infections, and those associated with intravascular cannulas. The mode of transmission of these infections is mostly by physical contact with infected medical devices. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* are the most common bacterial isolates that give rise to these infectious diseases. It has been observed that most of the bacterial strains develop resistance to antibiotics over a period of time. In the hospital environment, over 50% of *Staphylococcus aureus* have developed resistance to methicillin, which ultimately leads to surgical wound infection and catheter related sepsis. Some of the emerging antibiotic resistant pathogens include vancomycin resistant *enterococci*, vancomycin intermediate *staphylococcus*, and multiple antibiotic resistant Gram negative organisms like *acinetobacter*, *Enterobacter*, and mycobacterium.

Antimicrobial surfaces employed, for example, on the surface of medical devices, offer promise in helping curb the spread of infections. Towards this end, a wide range of antimicrobial agents have been applied to surfaces: antibiotics including chlorhexidine,

rifampin and monocycline and others, silver/silver ions/silver compounds, hydantoin (also known as halamine) compounds, furanone compounds, and quaternary ammonium or phosphonium polymers. There have been a smaller number of non-permanently cationic antimicrobial polymeric materials prepared for use on surfaces, generally incorporating benzoic acid derivatives.

The various agents are most often physically applied to the surface, physically impregnated into the bulk of the material, or physically incorporated into a coating that is then applied to the surface for "controlled release". In all these approaches the antimicrobial agent leaches from the surface, leading to two key problems: a limited time of effectiveness; and environmental, health and safety concerns, such as the promotion of drug resistant microbes.

Non-leaching antimicrobial surfaces have been created by covalently grafting an antimicrobial polymer to the surface, atom transfer radical polymerization of an antimicrobial polymer directly from an initiating surface, and covalent attachment of an agent to a polymer chain. In the latter case, any attachment scheme must not obscure the active moiety of the molecule. Also, particular care must be taken to ensure that the agent is actually covalently bound and is not just physically incorporated and that it is not releasing from the surface, which leads to the same issues discussed above for leaching antimicrobial agents.

While non-leaching systems address some of the shortcomings associated with the "controlled release" architectures described above, many existing non-leaching systems are not compatible commercially-viable manufacturing methods such as molding, extrusion, and other thermoplastic methods of 'conversion' or solvent-based processing. Further, many existing non-leaching coatings unacceptably alter the physiochemical and mechanical properties of the substrate to which they are applied.

SUMMARY

Provided herein are processes for rendering a polyvinyl chloride (PVC) surface antimicrobial (e.g., antibacterial and/or antifungal). The processes generally involve functionalization of the PVC surface to convert chloride residues on the PVC surface to functional groups (e.g., an alkyne or an azide) that can participate in a click reaction (e.g., a 1,3-dipolar cycloaddition reaction). Subsequently, click chemistry can be used to covalently tether one or more antimicrobial agents to the surface. The resulting surfaces

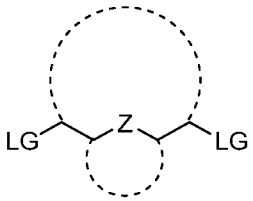
can exhibit antimicrobial activity (e.g., antibacterial activity, antifungal activity, or a combination thereof).

Importantly, these processes can employ mild reaction conditions that permit covalent attachment of the antimicrobial agents to a PVC surface without substantially altering the physiochemical and mechanical properties of the article comprising the PVC surface. As a consequence, the processes described herein can be used to impart antimicrobial activity to a surface of a formed PVC article (e.g., an extruded article such as PVC tubing) without unacceptably compromising the physiochemical and mechanical properties of the article (e.g., without damaging the PVC tubing).

In some embodiments, the process for rendering a polyvinyl chloride surface antimicrobial can comprise (a) contacting the polyvinyl chloride with an azidation reagent in the presence of a phase transfer catalyst to form an azide-substituted polyvinyl chloride; and (b) contacting the azide-substituted polyvinyl chloride with an antimicrobial agent comprising an alkyne moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

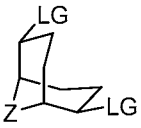
In other embodiments, the process for rendering a polyvinyl chloride surface antimicrobial can comprise (a) contacting the polyvinyl chloride with a cyanation reagent in the presence of a phase transfer catalyst to form a cyano-substituted polyvinyl chloride; and (b) covalently bonding an antimicrobial agent to the cyano-substituted polyvinyl chloride.

Also provided are antimicrobial articles. The antimicrobial articles can comprise a surface (e.g., a surface of a substrate), and a polycationic polymer derived from the condensation of a cyclic bis-electrophile and a polynucleophilic monomer immobilized on the surface. The cyclic bis-electrophile can be defined by Formula II below

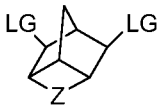


Formula II

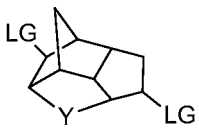
where each dotted line represents a cyclic moiety; LG represents a leaving group; Z represents S, Se, or NR^3; and R^3 represents alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy. In some embodiments, the cyclic bis-electrophile can be defined by Formula IIA, Formula IIB, or Formula IIC below



Formula IIA

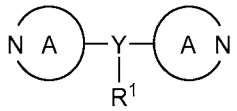


Formula IIB



Formula IIC

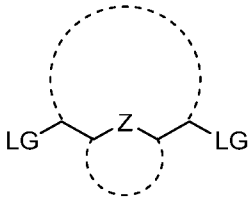
wherein LG and Z are as defined above with respect to Formula II. The polynucleophilic monomer can be defined by Formula III below



Formula III

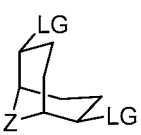
wherein A represents a heterocyclic ring comprising a tertiary nitrogen atom; Y is absent or represents a linking group; and R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

Also provided are methods for controlling biofilm formation on a substrate. Methods can comprise covalently bonding a polycationic polymer to the substrate in an amount effective to inhibit biofilm formation. The polycationic polymer can be derived from the condensation of a cyclic bis-electrophile and a polynucleophilic monomer immobilized on the surface. The cyclic bis-electrophile can be defined by Formula II below

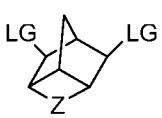


Formula II

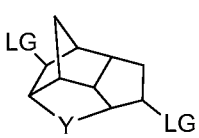
where each dotted line represents a cyclic moiety; LG represents a leaving group; Z represents S, Se, or NR^3; and R^3 represents alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy. In some embodiments, the cyclic bis-electrophile can be defined by Formula IIA, Formula IIB, or Formula IIC below



Formula IIA

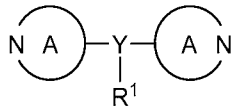


Formula IIB



Formula IIC

wherein LG and Z are as defined above with respect to Formula II. The polynucleophilic monomer can be defined by Formula III below



Formula III

wherein A represents a heterocyclic ring comprising a tertiary nitrogen atom; Y is absent or represents a linking group; and R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

DESCRIPTION OF DRAWINGS

Figure 1 shows the ^{1}H NMR spectra of WCL electrophiles. The inset shows the C1/5-H ^{1}H NMR resonances of equimolar mixtures of pairs of electrophiles after reaction with 0.5 equiv. of benzylamine, confirming the reactivity order iodide > nitrate > chloride.

Figure 2 shows the ^{1}H NMR analysis of competition experiments between WCL nitrate and chloride electrophiles for benzylamine.

Figure 3 shows the ^{1}H NMR analysis of competition experiments between WCL chloride, nitrate, and iodide electrophiles for benzylamine.

Figures 4A-4B show ^{1}H NMR spectra taken at various intervals over 100 hours as **5d** was incubated in deuterated aqueous phosphate buffer (pH 7) at 50°C. Figure 4A shows the downfield region of the ^{1}H NMR spectra. Figure 4B shows the upfield region of the ^{1}H NMR spectra.

Figure 5 shows the pseudo-first order plot obtained for the fragmentation of 5d in deuterated buffer at 50°C.

Figure 6 shows ^{1}H NMR spectra taken at various intervals over 204 hours as polymer **3i** was incubated in deuterium oxide at 37°C.

Figure 7A shows gel permeation chromatography (GPC) traces of polymer **3a** before (black trace) and after (gray trace) fragmentation in water at 37°C.

Figure 7B shows GPC traces of polymer **3c** before (black trace) and after (gray trace) fragmentation in water at 37°C.

Figure 7C shows GPC traces of polymer **3i** before (black trace) and after (gray trace) fragmentation in water at 37°C.

Figure 8A shows the results of thermogravimetric analysis (TGA) of polymers **3a**, **3c**, and **3i**.

Figure 8B shows a differential scanning calorimetry (DSC) trace obtained for polymer **3a**.

Figure 9A shows a box-and-whisker plot of viability test results for GFP-HeLa at different N/P ratios with polymer **3a**.

Figure 9B shows a box-and-whisker plot of viability test results for GFP-HeLa at different N/P ratios with polymer **3i**.

Figure 10 shows a box-and-whisker plot summarizing the results of siRNA transfection tests at various N/P ratios with polymer **3a**.

Figure 11A is a plot of cytotoxicity tests of polymer **3a** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 11B is a plot of cytotoxicity tests of polymer **3c** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 11C is a plot of cytotoxicity tests of polymer **3i** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 11D is a plot of cytotoxicity tests of polymer **3a'** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12A is a plot of cytotoxicity tests of monomer **2a** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12B is a plot of cytotoxicity tests of monomer **2c** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12C is a plot of cytotoxicity tests of monomer **2i** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12D is a plot of cytotoxicity tests of monomer **4** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12E is a plot of cytotoxicity tests of compound **5a** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12F is a plot of cytotoxicity tests of compound **5c** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12G is a plot of cytotoxicity tests of compound **5i** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 12H is a plot of cytotoxicity tests of compound **5m** towards CHO-K1. Cell viability was measured after 4h (black bars) or 24 h (red bars) treatment at the indicated concentration.

Figure 13A is a plot of the adduct half life at 50°C vs. pyridine basicity (*see also* Table 1). The pyridine basicity is indicated by the calculated pK_a of the conjugate acid. The dotted line is meant to highlight the approximate trend, and is not a mathematical fit.

Figure 13B is a plot of the adduct half life at 37°C vs. pyridine basicity (*see also* Table 2). The pyridine basicity is indicated by the calculated pK_a of the conjugate acid. The dotted line is meant to highlight the approximate trend, and is not a mathematical fit.

Figure 14 shows the ^1H NMR spectrum of an oligomer formed by reaction of **1b** and **2a** (Table 4, entry 3).

Figure 15A shows the results of agarose gel electrophoresis of plasmid DNA mixed with increasing amounts of polycation **3a**.

Figure 15B shows the results of dynamic light scattering of a PBS buffer solution containing plasmid DNA mixed with polycation **3a** at an N/P ratio of 2/1.

Figures 16 is an assessment of the cytotoxic activity of example polycations (MIC = minimum concentration required to inhibit the growth of 90% of cells in a standard starting culture). Cytotoxicity was evaluated against *B. subtilis* (a representative Gram-positive strain), *E. Coli* (a representative Gram-negative strain), and CHO-K1 (a representative mammalian cell). Certain commercially-available antibacterial agents (e.g., amoxicillin) are shown for reference. Initial studies suggest that the polycations can exhibit broadspectrum activity against bacteria with a large therapeutic window.

Figure 17 is a schematic illustration of the methods used to assess the cytotoxicity of glass substrates covalently functionalized with example polycations.

Figure 18 is a schematic illustration of methods for covalently functionalizing polyvinyl chloride (e.g., medical tubing) to improve resistance to bacterial and fungal colonization and biofilm formation.

Figure 19 is a schematic illustrated of classes of polycationic materials based on the reaction of a 9-thia/aza/selenabicyclo[3.3.1]nonyl electrophile with a nucleophile.

Figure 20 is a schematic illustration of the strategy used to immobilize the polycations on a glass substrate.

Figure 21 is a schematic illustration of the strategy used to immobilize the polycations on a PVC substrate.

DETAILED DESCRIPTION

The materials, compounds, compositions, articles, and methods described herein may be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter and the Examples and Figures included therein.

Before the present materials, compounds, compositions, and methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific synthetic methods or specific reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

Also, throughout this specification, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the disclosed matter pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

General Definitions

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings:

Throughout the description and claims of this specification the word “comprise” and other forms of the word, such as “comprising” and “comprises,” means including but not limited to, and is not intended to exclude, for example, other additives, components, integers, or steps.

As used in the description and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a composition" includes mixtures of two or more such compositions, reference to "the compound" includes mixtures of two or more such compounds, reference to "an agent" includes mixture of two or more such agents, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

By "reduce" or other forms of the word, such as "reducing" or "reduction," is meant lowering of an event or characteristic (e.g., bacterial growth, biofilm formation, etc.). It is understood that this is typically in relation to some standard or expected value, in other words it is relative, but that it is not always necessary for the standard or relative value to be referred to. For example, "reduces biofilm formation" means reducing the rate of biofilm formation relative to a standard or a control.

By "prevent" or other forms of the word, such as "preventing" or "prevention," is meant to stop a particular event or characteristic, to stabilize or delay the development or progression of a particular event or characteristic, or to minimize the chances that a particular event or characteristic will occur. Prevent does not require comparison to a control as it is typically more absolute than, for example, reduce. As used herein, something could be reduced but not prevented, but something that is reduced could also be prevented. Likewise, something could be prevented but not reduced, but something that is prevented could also be reduced. It is understood that where reduce or prevent are used, unless specifically indicated otherwise, the use of the other word is also expressly disclosed.

"Contacting," as used herein, refers to any means for providing a component or element to a surface. Contacting can include, for example, spraying, wetting, immersing, dipping, painting, flowing across, bonding or adhering or otherwise providing a surface with a the component or element.

"Biofilm" or "biofilms" refer to communities of microorganisms that are attached to a substrate. The microorganisms often excrete a protective and adhesive matrix of polymeric compounds. They often have structural heterogeneity, genetic diversity, and complex community interactions. "Biofilm preventing", "biofilm removing", "biofilm inhibiting", "biofilm reducing", "biofilm resistant", "biofilm controlling" or "antifouling" refer to prevention of biofilm formation, inhibition of the establishment or growth of a

biofilm, or decrease in the amount of organisms that attach and/or grow upon a substrate, up to and including the complete removal of the biofilm.

The phrase "effective amount" or "amount effective" refers to an amount of an antimicrobial agent (e.g., a polycationic polymer described herein) that significantly reduces the number of organisms that attach to a defined surface (cells/mm^2) relative to the number that attach to an untreated surface. Particularly preferred are amounts that reduce the number of organisms that attach to the surface by a factor of at least 2. Even more preferred are amounts that reduce the surface attachment of organisms by a factor of 4, more preferably by a factor of 6. An effective amount of an antimicrobial agent (e.g., a polycationic polymer described herein) is said to inhibit the formation of biofilms, and to inhibit the growth of organisms on a defined surface. The term "inhibit," as applied to the effect of an antimicrobial agent (e.g., a polycationic polymer described herein) on a surface includes any action that significantly reduces the number of organisms that attach thereto.

It is understood that throughout this specification the identifiers "first" and "second" are used solely to aid in distinguishing the various components and steps of the disclosed subject matter. The identifiers "first" and "second" are not intended to imply any particular order, amount, preference, or importance to the components or steps modified by these terms.

Chemical Definitions

Terms used herein will have their customary meaning in the art unless specified otherwise. The organic moieties mentioned when defining variable positions within the general formulae described herein (e.g., the term "halogen") are collective terms for the individual substituents encompassed by the organic moiety. The prefix C_n-C_m indicates in each case the possible number of carbon atoms in the group.

The term "alkyl," as used herein, refers to saturated straight, branched, primary, secondary or tertiary hydrocarbons, including those having 1 to 20 atoms. In some examples, alkyl groups will include C_1-C_{12}, C_1-C_{10}, C_1-C_8, C_1-C_6, C_1-C_5, C_1-C_4, C_1-C_3, or C_1-C_2 alkyl groups. Examples of C_1-C_{10} alkyl groups include, but are not limited to, methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl, 1-ethylpropyl, hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-

trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, heptyl, octyl, 2-ethylhexyl, nonyl and decyl groups, as well as their isomers. Examples of C_1-C_4-alkyl groups include, for example, methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylpropyl, and 1,1-dimethylethyl groups.

Optionally, alkyl groups may also contain one or more heteroatoms within the carbon backbone. The heteroatoms incorporated into the carbon backbone may be oxygen, nitrogen, sulfur, or combinations thereof. In certain embodiments, the alkyl group can include between one and four heteroatoms.

Cyclic alkyl groups or "cycloalkyl" groups include cycloalkyl groups having from 3 to 10 carbon atoms. Cycloalkyl groups can include a single ring, or multiple condensed rings. In some examples, cycloalkyl groups include C_3-C_4, C_4-C_7, C_5-C_7, C_4-C_6, or C_5-C_6 cyclic alkyl groups. Non-limiting examples of cycloalkyl groups include adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like.

Alkyl and cycloalkyl groups can be unsubstituted or substituted with one or more moieties chosen from alkyl, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, alkyl- or dialkylamino, amido, arylamino, alkoxy, aryloxy, nitro, cyano, azido, thiol, imino, sulfonic acid, sulfate, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrazine, carbamate, phosphoric acid, phosphate, phosphonate, hydrazone, carbonate, ammonium (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group), pyridinium, or any other viable functional group that does not inhibit the biological activity of the compounds of the invention, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as described in Greene, *et al.*, *Protective Groups in Organic Synthesis*, John Wiley and Sons, Third Edition, 1999, hereby incorporated by reference.

Terms including the term "alkyl," such as "alkylamino" or "dialkylamino," will be understood to comprise an alkyl group as defined above linked to another functional group, where the group is linked to the compound through the last group listed, as understood by those of skill in the art.

The term "alkenyl," as used herein, refers to both straight and branched carbon chains which have at least one carbon-carbon double bond. In some examples, alkenyl groups can include C_2-C_{20} alkenyl groups. In other examples, alkenyl can include C_2-C_{12}, C_2-C_{10}, C_2-C_8, C_2-C_6, or C_2-C_4 alkenyl groups. In one embodiment of alkenyl, the number of double bonds is 1-3, in another embodiment of alkenyl, the number of double bonds is

one or two. Other ranges of carbon-carbon double bonds and carbon numbers are also contemplated depending on the location of the alkenyl moiety on the molecule. "C_2-C_{10}-alkenyl" groups can include more than one double bond in the chain. The one or more unsaturations within the alkenyl group can be located at any position(s) within the carbon chain as valence permits. In some examples, when the alkenyl group is covalently bound to one or more additional moieties, the carbon atom(s) in the alkenyl group that are covalently bound to the one or more additional moieties are not part of a carbon-carbon double bond within the alkenyl group. Examples of alkenyl groups include, but are not limited to, ethenyl, 1-propenyl, 2-propenyl, 1-methyl-ethenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-methyl-1-propenyl, 2-methyl-1-propenyl, 1-methyl-2-propenyl, 2-methyl-2-propenyl; 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-methyl-1-butenyl, 2-methyl-1-butenyl, 3-methyl-1-butenyl, 1-methyl-2-butenyl, 2-methyl-2-butenyl, 3-methyl-2-butenyl, 1-methyl-3-butenyl, 2-methyl-3-butenyl, 3-methyl-3-butenyl, 1,1-dimethyl-2-propenyl, 1,2-dimethyl-1-propenyl, 1,2-dimethyl-2-propenyl, 1-ethyl-1-propenyl, 1-ethyl-2-propenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 1-methyl-1-pentenyl, 2-methyl-1-pentenyl, 3-methyl-1-pentenyl, 4-methyl-1-pentenyl, 1-methyl-2-pentenyl, 2-methyl-2-pentenyl, 3-methyl-2-pentenyl, 4-methyl-2-pentenyl, 1-methyl-3-pentenyl, 2-methyl-3-pentenyl, 3-methyl-3-pentenyl, 4-methyl-3-pentenyl, 1-methyl-4-pentenyl, 2-methyl-4-pentenyl, 3-methyl-4-pentenyl, 4-methyl-4-pentenyl, 1,1-dimethyl-2-butenyl, 1,1-dimethyl-3-butenyl, 1,2-dimethyl-1-butenyl, 1,2-dimethyl-2-butenyl, 1,2-dimethyl-3-butenyl, 1,3-dimethyl-1-butenyl, 1,3-dimethyl-2-butenyl, 1,3-dimethyl-3-butenyl, 2,2-dimethyl-3-butenyl, 2,3-dimethyl-1-butenyl, 2,3-dimethyl-2-butenyl, 2,3-dimethyl-3-butenyl, 3,3-dimethyl-1-butenyl, 3,3-dimethyl-2-butenyl, 1-ethyl-1-butenyl, 1-ethyl-2-butenyl, 1-ethyl-3-butenyl, 2-ethyl-1-butenyl, 2-ethyl-2-butenyl, 2-ethyl-3-butenyl, 1,1,2-trimethyl-2-propenyl, 1-ethyl-1-methyl-2-propenyl, 1-ethyl-2-methyl-1-propenyl and 1-ethyl-2-methyl-2-propenyl groups.

The term "alkynyl," as used herein, refers to both straight and branched carbon chains which have at least one carbon-carbon triple bond. In one embodiment of alkynyl, the number of triple bonds is 1-3; in another embodiment of alkynyl, the number of triple bonds is one or two. In some examples, alkynyl groups include from C_2-C_{20} alkynyl groups. In other examples, alkynyl groups can include C_2-C_{12}, C_2-C_{10}, C_2-C_8, C_2-C_6 or C_2-C_4 alkynyl groups. Other ranges of carbon-carbon triple bonds and carbon numbers are also contemplated depending on the location of the alkenyl moiety on the molecule. For example, the term "C_2-C_{10}-alkynyl" as used herein refers to a straight-chain or branched unsaturated hydrocarbon group having 2 to 10 carbon atoms and containing at least one

triple bond, such as ethynyl, prop-1-yn-1-yl, prop-2-yn-1-yl, n-but-1-yn-1-yl, n-but-1-yn-3-yl, n-but-1-yn-4-yl, n-but-2-yn-1-yl, n-pent-1-yn-1-yl, n-pent-1-yn-3-yl, n-pent-1-yn-4-yl, n-pent-1-yn-5-yl, n-pent-2-yn-1-yl, n-pent-2-yn-4-yl, n-pent-2-yn-5-yl, 3-methylbut-1-yn-3-yl, 3-methylbut-1-yn-4-yl, n-hex-1-yn-1-yl, n-hex-1-yn-3-yl, n-hex-1-yn-4-yl, n-hex-1-yn-5-yl, n-hex-1-yn-6-yl, n-hex-2-yn-1-yl, n-hex-2-yn-4-yl, n-hex-2-yn-5-yl, n-hex-2-yn-6-yl, n-hex-3-yn-1-yl, n-hex-3-yn-2-yl, 3-methylpent-1-yn-1-yl, 3-methylpent-1-yn-3-yl, 3-methylpent-1-yn-4-yl, 3-methylpent-1-yn-5-yl, 4-methylpent-1-yn-1-yl, 4-methylpent-2-yn-4-yl, and 4-methylpent-2-yn-5-yl groups.

Alkenyl and alkynyl groups can be unsubstituted or substituted with one or more moieties chosen from alkyl, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, alkyl- or dialkylamino, amido, arylamino, alkoxy, aryloxy, nitro, cyano, azido, thiol, imino, sulfonic acid, sulfate, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrazine, carbamate, phosphoric acid, phosphate, phosphonate, hydrazone, carbonate, ammonium (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group), pyridinium, or any other viable functional group that does not inhibit the biological activity of the compounds of the invention, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as described in Greene, *et al.*, *Protective Groups in Organic Synthesis*, John Wiley and Sons, Third Edition, 1999.

The term "aryl," as used herein, refers to a monovalent aromatic carbocyclic group of from 6 to 14 carbon atoms. Aryl groups can include a single ring or multiple condensed rings. In some examples, aryl groups include C_6-C_{10} aryl groups. Aryl groups include, but are not limited to, phenyl, biphenyl, naphthyl, tetrahydronaphtyl, phenylcyclopropyl and indanyl. Aryl groups can be unsubstituted or substituted by one or more moieties chosen from halo, cyano, nitro, hydroxy, mercapto, amino, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, haloalkyl, haloalkenyl, haloalkynyl, halocycloalkyl, halocycloalkenyl, alkoxy, alkenyloxy, alkynyloxy, haloalkoxy, haloalkenyloxy, haloalkynyloxy, cycloalkoxy, cycloalkenyloxy, halocycloalkoxy, halocycloalkenyloxy, alkylthio, haloalkylthio, cycloalkylthio, halocycloalkylthio, alkylsulfinyl, alkenylsulfinyl, alkynyl-sulfinyl, haloalkylsulfinyl, haloalkenylsulfinyl, haloalkynylsulfinyl, alkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, haloalkyl-sulfonyl, haloalkenylsulfonyl, haloalkynylsulfonyl, alkylamino, alkenylamino, alkynylamino, di(alkyl)amino, di(alkenyl)-amino, di(alkynyl)amino, or trialkylsilyl.

The term "alkylaryl," as used herein, refers to an aryl group that is bonded to a parent compound through a diradical alkylene bridge, $(-CH_2-)_n$, where n is 1-12 (*e.g.*, n is from 1 to 6) and where "aryl" is as defined above. The term "arylalkyl," as used herein, refers to an aryl group, as defined above, which is substituted by an alkyl group, as defined above.

The term "alkylcycloalkyl," as used herein, refers to a cycloalkyl group that is bonded to a parent compound through a diradical alkylene bridge, $(-CH_2-)_n$, where n is 1-12 (*e.g.*, n is from 1 to 6) and where "cycloalkyl" is as defined above.

The term "alkoxy," as used herein, refers to alkyl-O-, wherein alkyl refers to an alkyl group, as defined above. Similarly, the terms "alkenyloxy," "alkynyloxy," and "cycloalkoxy," refer to the groups alkenyl-O-, alkynyl-O-, and cycloalkyl-O-, respectively, wherein alkenyl, alkynyl, and cycloalkyl are as defined above. Examples of C_1-C_6-alkoxy groups include, but are not limited to, methoxy, ethoxy, C_2H_5-CH_2O-, $(CH_3)_2CHO$-, n-butoxy, C_2H_5-$CH(CH_3)O$-, $(CH_3)_2CH$-CH_2O-, $(CH_3)_3CO$-, n-pentoxy, 1-methylbutoxy, 2-methylbutoxy, 3-methylbutoxy, 1,1-dimethylpropoxy, 1,2-dimethylpropoxy, 2,2-dimethylpropoxy, 1-ethylpropoxy, n-hexoxy, 1-methylpentoxy, 2-methylpentoxy, 3-methylpentoxy, 4-methylpentoxy, 1,1-dimethylbutoxy, 1,2-dimethylbutoxy, 1,3-dimethylbutoxy, 2,2-dimethylbutoxy, 2,3-dimethylbutoxy, 3,3-dimethylbutoxy, 1-ethylbutoxy, 2-ethylbutoxy, 1,1,2-trimethylpropoxy, 1,2,2-trimethylpropoxy, 1-ethyl-1-methylpropoxy, and 1-ethyl-2-methylpropoxy.

The term "alkylthio," as used herein, refers to alkyl-S-, wherein alkyl refers to an alkyl group, as defined above. Similarly, the term "cycloalkylthio," refers to cycloalkyl-S- where cycloalkyl are as defined above.

The term "alkylsulfinyl," as used herein, refers to alkyl-S(O)-, wherein alkyl refers to an alkyl group, as defined above.

The term "alkylsulfonyl," as used herein, refers to alkyl-$S(O)_2$-, wherein alkyl is as defined above.

The terms "alkylamino" and "dialkylamino," as used herein, refer to alkyl-NH- and $(alkyl)_2N$- groups, where alkyl is as defined above.

The terms "alkylcarbonyl," "alkoxycarbonyl," "alkylaminocarbonyl," and "dialkylaminocarbonyl," as used herein, refer to alkyl-C(O)-, alkoxy-C(O)-, alkylamino-C(O)- and dialkylamino-C(O)- respectively, where alkyl, alkoxy, alkylamino, and dialkylamino are as defined above.

The term "heteroaryl," as used herein, refers to a monovalent aromatic group of from 1 to 15 carbon atoms (*e.g.*, from 1 to 10 carbon atoms, from 2 to 8 carbon atoms, from 3 to 6 carbon atoms, or from 4 to 6 carbon atoms) having one or more heteroatoms within the ring. The heteroaryl group can include from 1 to 4 heteroatoms, from 1 to 3 heteroatoms, or from 1 to 2 heteroatoms. In some examples, the heteroatom(s) incorporated into the ring are oxygen, nitrogen, sulfur, or combinations thereof. When present, the nitrogen and sulfur heteroatoms can optionally be oxidized. Heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings provided that the point of attachment is through a heteroaryl ring atom. Preferred heteroaryls include pyridyl, piridazinyl, pyrimidinyl, pyrazinyl, triazinyl, pyrrolyl, indolyl, quinolinyl, isoquinolinyl, quinazolinyl, quinoxalinnyl, furanyl, thiophenyl, furyl, pyrrolyl, imidazolyl, oxazolyl, isoxazolyl, isothiazolyl, pyrazolyl benzofuranyl, and benzothiophenyl. Heteroaryl rings can be unsubstituted or substituted by one or more moieties as described for aryl above.

The term "alkylheteroaryl," as used herein, refers to a heteroaryl group that is bonded to a parent compound through a diradical alkylene bridge, $(-CH_2-)_n$, where n is 1-12 and where "heteroaryl" is as defined above.

The terms "heterocyclyl," "heterocyclic" and "heterocyclo" are used herein interchangeably, and refer to fully saturated or unsaturated, cyclic groups, for example, 3 to 7 membered monocyclic or 4 to 7 membered monocyclic; 7 to 11 membered bicyclic, or 10 to 15 membered tricyclic ring systems, having one or more heteroatoms within the ring. The heterocyclyl group can include from 1 to 4 heteroatoms, from 1 to 3 heteroatoms, or from 1 to 2 heteroatoms. In some examples, the heteroatom(s) incorporated into the ring are oxygen, nitrogen, sulfur, or combinations thereof. When present, the nitrogen and sulfur heteroatoms can optionally be oxidized, and the nitrogen heteroatoms can optionally be quaternized. The heterocyclyl group can be attached at any heteroatom or carbon atom of the ring or ring system and can be unsubstituted or substituted by one or more moieties as described for aryl groups above.

Exemplary monocyclic heterocyclic groups include, but are not limited to, pyrrolidinyl, pyrrolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, 4-piperidonyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide,

thiamorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, triazolyl, triazinyl, and the like.

Exemplary bicyclic heterocyclic groups include, but are not limited to, indolyl, benzothiazolyl, benzoxazolyl, benzodioxolyl, benzothienyl, quinuclidinyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl]or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), tetrahydroquinolinyl and the like.

Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthrolinyl, acridinyl, phenanthridinyl, xanthenyl, and the like.

The term "alkylheterocyclyl," as used herein, refers to a heterocyclyl group that is bonded to a parent compound through a diradical alkylene bridge, $(-CH_2-)_n$, where n is 1-12 and where "heterocyclyl" is as defined above. The term "heterocyclylalkyl," as used herein, refers to a heterocyclyl group, as defined above, which is substituted by an alkyl group, as defined above.

Heretrocyclyl and heteroaryl groups can be unsubstituted or substituted with one or more moieties chosen from alkyl, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, alkyl- or dialkylamino, amido, arylamino, alkoxy, aryloxy, nitro, cyano, azido, thiol, imino, sulfonic acid, sulfate, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrazine, carbamate, phosphoric acid, phosphate, phosphonate, hydrazone, carbonate, ammonium (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group), pyridinium, or any other viable functional group that does not inhibit the biological activity of the compounds of the invention, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as described in Greene, *et al.*, *Protective Groups in Organic Synthesis*, John Wiley and Sons, Third Edition, 1999

The term "halogen," as used herein, refers to the atoms fluorine, chlorine, bromine and iodine. The prefix halo- (e.g., as illustrated by the term haloalkyl) refers to all degrees of halogen substitution, from a single substitution to a perhalo substitution (*e.g.*, as illustrated with methyl as chloromethyl ($-CH_2Cl$), dichloromethyl ($-CHCl_2$), trichloromethyl ($-CCl_3$)).

The term “polyalkyleneoxy,” as used herein, generally refers to an oligomeric or polymeric group formed from the following repeating alkyleneoxy units: —CH_2CH_2O—, $CH_2CH_2CH_2O$—, —$CH_2CH_2CH_2CH_2O$—, —$CH_2CH(CH_3)O$—, —$CH_2CH(CH_2CH_3)O$— $CH_2CH_2CH(CH_3)O$—, and any combination thereof. The number of repeat alkyleneoxy groups typically is from 2 to 50 (e.g., from 5 to 50, from 10 to 50, from 2 to 40, from 5 to 40, from 10 to 40, from 2 to 30, from 5 to 30, from 10 to 30, from 2 to 20, from 5 to 20, from 10 to 20, from 2 to 15, from 5 to 15, from 10 to 15, from 2 to 10, or from 5 to 10) repeat alkyleneoxy groups, although the number of repeat units can be outside of these ranges. The term "pendant", as used herein in reference to a polyalkyleneoxy group, refers to a polyalkyleneoxy group that is present as an end group and/or a sidechain attached to a polymeric backbone.

The term “polyamino,” as used herein, generally refers to an oligomeric or polymeric group derived from one or more monomers containing an amine group. Suitable monomers of this type include vinylamine, allylamine, and ethyleneimine. Other suitable amino-containing monomers include (meth)acrylate monomers containing one or more primary and/or secondary amine groups, such as 2-aminoethyl methacrylate, 2-aminoethyl acrylate, 2-(*tert*-butylamino)ethyl acrylate, 2-(*tert*-butylamino)ethyl methacrylate. In some cases, the polyamino group can be a polycationic polyamino group (e.g., polyethyleneimine). The term "pendant", as used herein in reference to a polyamino group, refers to a polyamino group that is present as an end group and/or a sidechain attached to a polymeric backbone.

As used herein, the term “(meth)acrylate monomer” includes acrylate, methacrylate, diacrylate, and dimethacrylate monomers.

As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, and aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, Such substituents include, but are not limited to, halogen, hydroxyl, carbonyl (such as a carboxyl, alkoxycarbonyl, formyl, or an acyl), thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), alkoxyl, phosphoryl, phosphate, phosphonate, a phosphinate, amino, amido, amidine, imine, cyano, nitro, azido, sulfhydryl, alkylthio, sulfate, sulfonate, sulfamoyl, sulfonamido, sulfonyl, heterocyclyl, aralkyl, hydrazone, carbonate, ammonium (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a

diaryldialkylammonium group, or a triarylalkylammonium group), pyridinium, or an aromatic or heteroaromatic moiety.

The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This disclosure is not intended to be limited in any manner by the permissible substituents of organic compounds. Also, the terms "substitution" or "substituted with" include the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

Unless stated to the contrary, a formula with chemical bonds shown only as solid lines and not as wedges or dashed lines contemplates each possible isomer, e.g., each enantiomer, diastereomer, and meso compound, and a mixture of isomers, such as a racemic or scalemic mixture.

Reference will now be made in detail to specific aspects of the disclosed materials, compounds, compositions, articles, and methods, examples of which are illustrated in the accompanying Examples and Figures.

Processes

Provided herein are processes for rendering a polyvinyl chloride (PVC) surface antimicrobial (e.g., antibacterial and/or antifungal). The processes generally involve functionalization of the PVC surface to convert chloride residues on the PVC surface to functional groups (e.g., an alkyne or an azide) that can participate in a click reaction (e.g., a 1,3-dipolar cycloaddition reaction). Subsequently, click chemistry can be used to covalently tether one or more antimicrobial agents to the surface. The resulting surfaces can exhibit antimicrobial activity (e.g., antibacterial activity, antifungal activity, or a combination thereof).

Importantly, these processes can employ mild reaction conditions that permit covalent attachment of the antimicrobial agents to a PVC surface without substantially altering the physiochemical and mechanical properties of the article comprising the PVC surface. As a consequence, the processes described herein can be used to impart antimicrobial activity to a surface of a formed PVC article (e.g., an extruded article such as

PVC tubing) without unacceptably compromising the physiochemical and mechanical properties of the article (e.g., without damaging the PVC tubing).

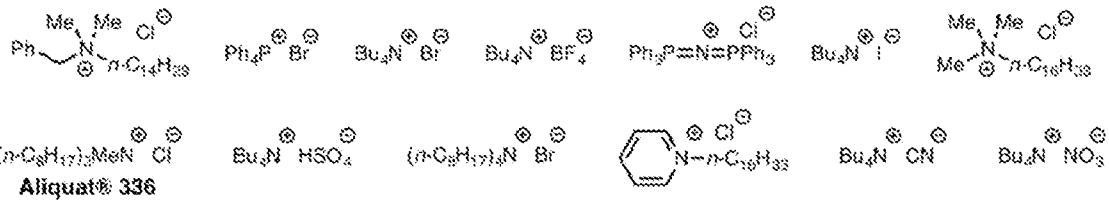
In some embodiments, the process for rendering a polyvinyl chloride surface antimicrobial can comprise (a) contacting the polyvinyl chloride with an azidation reagent in the presence of a phase transfer catalyst to form an azide-substituted polyvinyl chloride; and (b) contacting the azide-substituted polyvinyl chloride with an antimicrobial agent comprising an alkyne moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

In some embodiments, step (a) can comprise flowing an aqueous solution comprising the azidation reagent and the phase transfer catalyst across the polyvinyl chloride surface. This can be accomplished, for example, using a flow reactor. By way of example, in the case of a segment of PVC tubing, step (a) can comprise flowing an aqueous solution comprising the azidation reagent and the phase transfer catalyst through the segment of PVC tubing to form an azide-substituted polyvinyl chloride on the interior surface of the PVC tubing. Step (a) can also involve, for example, immersing the PVC surface in an aqueous solution comprising the azidation reagent and the phase transfer catalyst, or spraying an aqueous solution comprising the azidation reagent and the phase transfer catalyst onto the PVC surface.

In some embodiments, step (a) can be performed at a temperature of from 20°C to 25°C. In some embodiments, the reaction time in step (a) can be limited in order to minimize any potential impact on the physiochemical and/or mechanical properties of the article comprising the PVC surface. For example, in some embodiments, step (a) can comprise contacting the polyvinyl chloride with the azidation reagent and the phase transfer catalyst for 8 hours or less (e.g., 7 hours or less, 6 hours or less, 5 hours or less, 4 hours or less, 3 hours or less, 2 hours or less, or 1 hour or less). In certain embodiments, step (a) can comprise contacting the polyvinyl chloride with the azidation reagent and the phase transfer catalyst for from 10 minutes to 8 hours (e.g., from 10 minutes to 6 hours, from 10 minutes to 3 hours, from 10 minutes to 2 hours, or from 10 minutes to 1 hour).

The phase transfer catalyst can comprise a salt compound, such as a Group 15 salt compound (e.g., a quaternary ammonium salt, or a phosphonium salt). Examples of suitable phase transfer catalysts include (but are not limited to) tetrabutyl ammonium bromide, tetrabutyl ammonium cyanide, tetrabutyl ammonium iodide, tetrabutyl ammonium nitrate, tetrabutyl ammonium bisulfate, tetrabutyl phosphonium bromide, tetraphenyl phosphonium

bromide, or a combination thereof. In some embodiments, the phase transfer catalyst can comprise one of the following:



Examples of suitable azidation reagents include, for example, sodium azide (NaN_3), trimethylsilyl azide, diphenylphosphoryl azide, potassium azide, chlorine azide, bromine azide, iodine azide, hydrazoic acid, tetraalkylammonium azides, tetraphenylphosphonium azides, and combinations thereof. In certain embodiments, the azidation reagent can comprise NaN_3.

In some embodiments, step (b) can be performed at a temperature of from 20°C to 25°C. In some embodiments, step (a) can comprise flowing an aqueous solution comprising the antimicrobial agent comprising the alkyne moiety across the azide-substituted polyvinyl chloride surface. As described above, this can be accomplished, for example, using a flow reactor. By way of example, in the case of a segment of PVC tubing, step (b) can comprise flowing an aqueous solution comprising the antimicrobial agent comprising the alkyne moiety through the segment of PVC tubing that includes the azide-substituted polyvinyl chloride surface. Step (b) can also involve, for example, immersing the azide-substituted PVC surface in an aqueous solution comprising the antimicrobial agent, or spraying an aqueous solution comprising the antimicrobial agent onto the azide-substituted PVC surface.

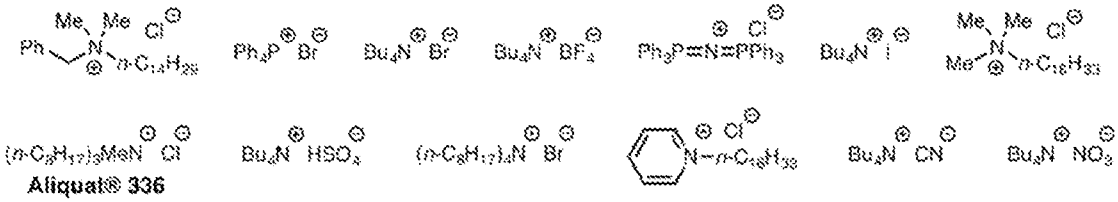
In some embodiments, the alkyne moiety present on the antimicrobial agent can be activated (e.g., activated by ring strain, activated by one or more electron withdrawing groups, or a combination thereof). In some of these embodiments, the step (b) can be performed without a catalyst. Alternatively, a Cu(I) catalyst may be used to facilitate reaction between the azide on the PVC surface and the alkyne moiety on the antimicrobial agent. In these embodiments, step (b) can comprise contacting azide-substituted polyvinyl chloride with the antimicrobial agent comprising the alkyne moiety in the presence of a Cu(I) catalyst.

In other embodiments, the process for rendering a polyvinyl chloride surface antimicrobial can comprise (a) contacting the polyvinyl chloride with a cyanation reagent in the presence of a phase transfer catalyst to form a cyano-substituted polyvinyl chloride; and (b) covalently bonding an antimicrobial agent to the cyano-substituted polyvinyl chloride.

In some embodiments, step (a) can comprise flowing an aqueous solution comprising the cyanation reagent and the phase transfer catalyst across the polyvinyl chloride surface. This can be accomplished, for example, using a flow reactor. By way of example, in the case of a segment of PVC tubing, step (a) can comprise flowing an aqueous solution comprising the cyanation reagent and the phase transfer catalyst through the segment of PVC tubing to form an cyano-substituted polyvinyl chloride on the interior surface of the PVC tubing. Step (a) can also involve, for example, immersing the PVC surface in an aqueous solution comprising the cyanation reagent and the phase transfer catalyst, or spraying an aqueous solution comprising the cyanation reagent and the phase transfer catalyst onto the PVC surface.

In some embodiments, step (a) can be performed at a temperature of from 20°C to 25°C. In some embodiments, the reaction time in step (a) can be limited in order to minimize any potential impact on the physiochemical and/or mechanical properties of the article comprising the PVC surface. For example, in some embodiments, step (a) can comprise contacting the polyvinyl chloride with the cyanation reagent and the phase transfer catalyst for 8 hours or less (e.g., 7 hours or less, 6 hours or less, 5 hours or less, 4 hours or less, 3 hours or less, 2 hours or less, or 1 hour or less). In certain embodiments, step (a) can comprise contacting the polyvinyl chloride with the cyanation reagent and the phase transfer catalyst for from 10 minutes to 8 hours (e.g., from 10 minutes to 6 hours, from 10 minutes to 3 hours, from 10 minutes to 2 hours, or from 10 minutes to 1 hour).

The phase transfer catalyst can comprise a salt compound, such as a Group 15 salt compound (e.g., a quaternary ammonium salt, or a phosphonium salt). Examples of suitable phase transfer catalysts include (but are not limited to) tetrabutyl ammonium bromide, tetrabutyl ammonium cyanide, tetrabutyl ammonium iodide, tetrabutyl ammonium nitrate, tetrabutyl ammonium bisulfate, tetrabutyl phosphonium bromide, tetraphenyl phosphonium bromide, or a combination thereof. In some embodiments, the phase transfer catalyst can comprise one of the following:



Examples of suitable cyanation reagents include, for example, cyanide salts such as NaCN, KCN, trimethylsilyl cyanide, tetraalkylammonium cyanides,

tetraphenylphosphonium cyanides, and combinations thereof. In some embodiments, the cyanation reagent can comprise NaCN, KCN, or a combination thereof.

In some embodiments, step (b) can be performed at a temperature of from 20°C to 25°C.

In some embodiments, the antimicrobial agent can comprise a hydrazone moiety or an azide moiety, and step (b) can comprise contacting the cyano-substituted polyvinyl chloride with the antimicrobial agent under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

In other embodiments, step (b) can comprise (i) converting cyano groups in the cyano-substituted polyvinyl chloride to 1*H*-tetrazole moieties, thereby forming a 1*H*-tetrazole-substituted polyvinyl chloride; and (ii) contacting the 1*H*-tetrazole-substituted polyvinyl chloride with an antimicrobial agent comprising an electrophilic moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface. In these embodiments, step (i) can comprise contacting the cyano-substituted polyvinyl chloride with an azide salt in the presence of a catalyst, such as $ZnBr_2$.

In other embodiments, step (b) can comprise (i) converting cyano groups in the cyano-substituted polyvinyl chloride to 1*H*-tetrazole moieties, thereby forming a 1*H*-tetrazole-substituted polyvinyl chloride; (ii) contacting the 1*H*-tetrazole-substituted polyvinyl chloride with an electrophile comprising an azide group to form an azide-substituted polyvinyl chloride; and (iii) contacting the azide-substituted polyvinyl chloride with an antimicrobial agent comprising an alkyne moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface. The electrophile comprising the azide group can be chosen from, for example, a benzylic halide comprising an azide group, an allylic halide comprising an azide group, and a propargylic halide comprising an azide group. In some embodiments, the alkyne moiety present on the antimicrobial agent can be activated (e.g., activated by ring strain, activated by one or more electron withdrawing groups, or a combination thereof). In some of these embodiments, the step (iii) can be performed without a catalyst. Alternatively, a Cu(I) catalyst may be used to facilitate reaction between the azide on the PVC surface and the alkyne moiety on the antimicrobial agent. In these embodiments, step (iii) can comprise contacting azide-substituted polyvinyl chloride with the antimicrobial agent comprising the alkyne moiety in the presence of a Cu(I) catalyst.

In other embodiments, step (b) can comprise (i) converting cyano groups in the cyano-substituted polyvinyl chloride to 1*H*-tetrazole moieties, thereby forming a 1*H*-

tetrazole-substituted polyvinyl chloride; (ii) contacting the 1*H*-tetrazole-substituted polyvinyl chloride with an electrophile comprising an alkynyl group to form an alkyne-substituted polyvinyl chloride; and (iii) contacting the alkyne-substituted polyvinyl chloride with an antimicrobial agent comprising an azide moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface. The electrophile comprising the alkynyl group can be chosen from, for example, a benzylic halide comprising an alkynyl group, an allylic halide comprising an alkynyl group, and a propargylic halide comprising an alkynyl group. In some embodiments, the alkyne group present on the PVC surface can be activated (e.g., activated by ring strain, activated by one or more electron withdrawing groups, or a combination thereof). In some of these embodiments, the step (iii) can be performed without a catalyst. Alternatively, a Cu(I) catalyst may be used to facilitate reaction between the alkyne on the PVC surface and the azide moiety on the antimicrobial agent. In these embodiments, step (iii) can comprise contacting alkyne-substituted polyvinyl chloride with the antimicrobial agent comprising the azide moiety in the presence of a Cu(I) catalyst.

In some embodiments of the processes described above, the PVC surface can comprise a surface of a medical article. In certain embodiments, the medical article can comprise PVC tubing (e.g., endotracheal tubing). In the case of PVC tubing, the interior surface of the tubing, the exterior surface of the tubing, or both the interior surface of the tubing and the exterior surface of the tubing can be rendered antimicrobial through the processes described above.

As discussed above, these processes can employ mild reaction conditions that permit covalent attachment of the antimicrobial agents to a PVC surface without substantially altering the physiochemical and mechanical properties of the article comprising the PVC surface. As a consequence, the processes described herein can be used to impart antimicrobial activity to a surface of a formed PVC article (e.g., an extruded article such as PVC tubing) without unacceptably compromising the physiochemical and mechanical properties of the article (e.g., without damaging the PVC tubing).

In some embodiments when the processes described above are performed on a surface of PVC tubing, the process does not significantly impact the hardness of the PVC tubing as measured using the standard method described in ASTM D-2240-15, entitled *Standard Test Method for Rubber Property—Durometer Hardness* (2015), which is hereby incorporated by reference in its entirety. For example, in some embodiments, the hardness of the PVC tubing after a process described above is performed is within 20% (e.g., within

15%, within 10%, or within 5%) of the hardness of the PVC tubing before the process described above is performed, as measured by ASTM D-2240-15.

In some embodiments when the processes described above are performed on a surface of PVC tubing, the process does not significantly impact the tensile strength of the PVC tubing as measured using the standard method described in ASTM D-638-14, entitled *Standard Test Method for Tensile Properties of Plastics* (2014), which is hereby incorporated by reference in its entirety. For example, in some embodiments, the tensile strength of the PVC tubing after a process described above is performed is within 20% (e.g., within 15%, within 10%, or within 5%) of the tensile strength of the PVC tubing before the process described above is performed, as measured by ASTM D-638-14.

In some embodiments when the processes described above are performed on a surface of PVC tubing, the process does not significantly impact the elongation of the PVC tubing as measured using the standard method described in ASTM D-638-14, entitled *Standard Test Method for Tensile Properties of Plastics* (2014), which is hereby incorporated by reference in its entirety. For example, in some embodiments, the elongation of the PVC tubing after a process described above is performed is within 20% (e.g., within 15%, within 10%, or within 5%) of the elongation of the PVC tubing before the process described above is performed, as measured by ASTM D-638-14.

In the processes described above, any suitable antimicrobial agent can be covalently bound to the PVC surface, provided that (1) the antimicrobial agent includes or can be modified to include a functional group (e.g., an azide, an alkyne, a hydrazone, etc.) which can react with a functional group introduced onto the PVC surface to form a covalent bond, and (2) covalent attachment of the antimicrobial agent to the PVC surface does not eliminate the agent's ability to function as an antimicrobial agent.

In some embodiments, the antimicrobial agent can comprise a conventional biocide. A "biocide" as used herein refers to a substance with the ability to kill or to inhibit the growth of microorganisms (e.g., bacteria, fungal cells, protozoa, etc.), such as an antibiotic. Examples of antibiotics include aminoglycosides, carbacephems (e.g., loracarbef), carbapenems, cephalosporins, glycopeptides (e.g., teicoplanin and vancomycin), macrolides, monobactams (e.g., aztreonam) penicillins, polypeptides (e.g., bacitracin, colistin, polymyxin B), quinolones, sulfonamides, tetracyclines, etc. Antibiotics treat infections by either killing or preventing the growth of microorganisms. Many act to inhibit cell wall synthesis or other vital protein synthesis of the microorganisms.

Aminoglycosides are commonly used to treat infections caused by Gram-negative bacteria such as *Escherichia coli* and *Klebsiella*, particularly *Pseudomonas aeroginosa*. Examples of aminoglycosides include, but are not limited to amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, and paromomycin.

Carbapenems are broad-specrum antibiotics, and include, but are not limited to, ertapenem, doripenem, imipenem/cilstatin, and meropenem.

Cephalosporins include, but are not limited to, cefadroxil, cefazolin, cefalotin (cefalothin), cefalexin, cefaclor, cefamandole, cefoxitin, cefprozil, loracarbef, cefuroxime, cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefepime, cefpirome, and ceftobiprole.

Macrolides include, but are not limited to, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin and spectinomycin.

Penicillins include, but are not limited to, amoxicillin, ampicillin, azlocillin, bacampicillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcillin, oxacillin, penicillin, piperacillin and ticarcillin.

Quinolones include, but are not limited to, ciprofloxacin, enoxacin, gatifloxacin, gemifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin and trovafloxacin.

Sulfonamides include, but are not limited to, mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilamide, sulfasalazine, sulfisoxazole, trimethoprim, and co-trimoxazole (trimethoprim-sulfamethoxazole).

Tetracyclines include, but are not limited to, demeclocycline, doxycycline, minocycline, oxytetracycline and tetracycline.

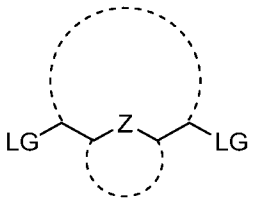
Other antibiotics include arsphenamine, chloramphenicol, clindamycin, lincomycin, ethambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampin (rifampicin), tinidazole, etc.

In some embodiments of the processes described above, the antimicrobial agent can comprise an antimicrobial polymer. Antimicrobial polymers are known in the art, and include, for example, polylactams, polyamino acids, polymers containing tertiary and/or quaternary ammonium groups, and polymers containing pyridinium sidechains. In certain embodiments, the antimicrobial polymer can comprise a polycationic polymer that includes a plurality of positively charged centers, each of which is formed by condensation of cyclic

bis-electrophile, such as a 9-thia/aza/selenabicyclo[3.3.1]nonyl electrophile. Such polycationic polymers are described in more detail below.

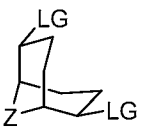
Articles

Antimicrobial articles are also provided herein. The antimicrobial articles can comprise a surface (e.g., a surface of a substrate), and a polycationic polymer derived from the condensation of a cyclic bis-electrophile and a polynucleophilic monomer immobilized on the surface. The cyclic bis-electrophile can be defined by Formula II below

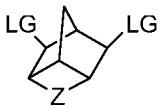


Formula II

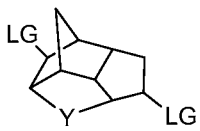
where each dotted line represents a cyclic moiety; LG represents a leaving group; Z represents S, Se, or NR^3; and R^3 represents alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy. In some embodiments, the cyclic bis-electrophile can be defined by Formula IIA, Formula IIB, or Formula IIC below



Formula IIA

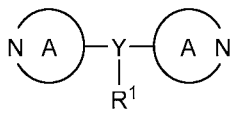


Formula IIB



Formula IIC

wherein LG and Z are as defined above with respect to Formula II. The polynucleophilic monomer can be defined by Formula III below



Formula III

wherein A represents a heterocyclic ring comprising a tertiary nitrogen atom; Y is absent or represents a linking group; and R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

Polycationic polymers of this type are discussed in more detail below. The polycationic polymers can be bound to the surface covalently or non-covalently using any suitable strategy known in the art.

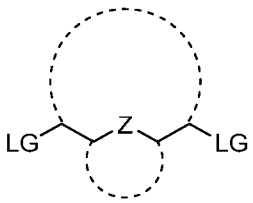
The surface can be of any composition, such as a ceramic, glass, metal, wood, or polymer. In certain embodiments, the surface comprises a polymer surface (e.g., a polyvinyl chloride surface).

The article can comprise any of the substrates described below. In some embodiments, the article can comprise a medical device. Examples of medical device include pins, screws, plates, ventriculoatrial shunts, ventriculoperitoneal shunts, dialysis shunts, heart valves, pacemakers, infusion pumps, vascular grafting prostheses, stents, sutures, surgical meshes, replacement prostheses, breast implants, tissue expanders, contact lenses, stoma appliances, artificial larynx, endotracheal tubes, tracheal tubes, gastrostomy tubes, biliary drainage tubes, biliary stents, catheters, bandages, adhesive tapes, and clear plastic adherent sheets. In certain embodiments, the article can comprise polyvinyl chloride tubing.

Methods

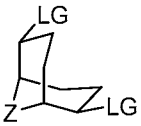
Also provided are methods for controlling biofilm formation on a substrate. Methods can comprise covalently bonding a polycationic polymer to the substrate in an amount effective to inhibit biofilm formation.

The polycationic polymer can be derived from the condensation of a cyclic bis-electrophile and a polynucleophilic monomer immobilized on the surface. The cyclic bis-electrophile can be defined by Formula II below

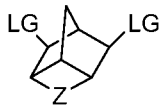


Formula II

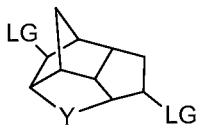
where each dotted line represents a cyclic moiety; LG represents a leaving group; Z represents S, Se, or NR^3; and R^3 represents alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy. In some embodiments, the cyclic bis-electrophile can be defined by Formula IIA, Formula IIB, or Formula IIC below



Formula IIA

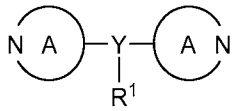


Formula IIB



Formula IIC

wherein LG and Z are as defined above with respect to Formula II. The polynucleophilic monomer can be defined by Formula III below



Formula III

wherein A represents a heterocyclic ring comprising a tertiary nitrogen atom; Y is absent or represents a linking group; and R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

Polycationic polymers of this type are discussed in more detail below. The polycationic polymers can be bound to the surface covalently or non-covalently using any suitable strategy known in the art.

The substrate can be of any composition, such as a ceramic, glass, metal, wood, or polymer. In certain embodiments, the substrate comprises a polymer substrate (e.g., a polyvinyl chloride substrate).

The substrate can comprise any of the substrates described below. In some embodiments, the substrate can comprise a medical device. Examples of medical device include pins, screws, plates, ventriculoatrial shunts, ventriculoperitoneal shunts, dialysis shunts, heart valves, pacemakers, infusion pumps, vascular grafting prostheses, stents, sutures, surgical meshes, replacement prostheses, breast implants, tissue expanders, contact lenses, stoma appliances, artificial larynx, endotracheal tubes, tracheal tubes, gastrostomy tubes, biliary drainage tubes, biliary stents, catheters, bandages, adhesive tapes, and clear plastic adherent sheets. In certain embodiments, the article can comprise polyvinyl chloride tubing.

The biofilm can comprise Gram-positive bacteria or Gram-negative bacteria. In some embodiments, the biofilm can comprise Gram-positive bacteria. Examples of Gram-positive bacteria affected by compounds described herein include, but are not limited to, bacteria of the genera *Listeria*, *Staphylococcus*, *Streptococcus*, *Bacillus*, *Corynebacterium*,

Peptostreptococcus, and *Clostridium*. For example, the bacteria can include *Listeria monocytogenes, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Bacillus cereus, Bacillus anthracis, Clostridium botulinum, Clostridium perfringens, Clostridium difficile, Clostridium tetani, Corynebacterium diphtheriae, Corynebacteruim ulcerans*, and *Peptostreptococcus anaerobius*. Other examples of Gram-positive bacteria include, for example, bacteria of the genera *Actinomyces, Propionibacterium, Nocardia* and *Streptomyces.*

In some embodiments, the biofilm can comprise Gram-negative bacteria. Examples of Gram-positive bacteria affected by compounds described herein include, but are not limited to, bacteria of the genera *Escherichia, Salmonella, Vibrio*, *Helicobacter*, *Pseudomonas, Bordetella, Vibrio, Haemophilus, Halomonas*, and *Acinetobacter*. For example, the bacteria can include *Pseudomonas aeuroginosa, Bordetella pertussis, Vibrio vulnificus, Haemophilus influenzae, Halomonas pacifica*, and *Acinetobacter baumannii.* Other examples of Gram-negative bacteria include, for example, bacteria of the genera *Klebsiella, Proteus, Neisseria, Helicobacter, Brucella, Legionella, Campylobacter, Francisella, Pasteurella, Yersinia, Bartonella, Bacteroides, Streptobacillus, Spirillum, Moraxella* and *Shigella.*

Substrates and Surfaces

The term "substrate" as used herein refers to bases on which an organism, such as those commonly found in biofilms, may live. The term "substrate," as used herein, refers to any substrate, whether in an industrial or a medical setting, that provides or can provide an interface between an object and a fluid, permitting at least intermittent contact between the object and the fluid. A substrate, as understood herein, further provides a plane whose mechanical structure, without further treatment, is compatible with the adherence of microorganisms. Substrates compatible with biofilm formation may be natural or synthetic, and may be smooth or irregular. Fluids contacting the substrates can be stagnant or flowing, and can flow intermittently or continuously, with laminar or turbulent or mixed flows. A substrate upon which a biofilm forms can be dry at times with sporadic fluid contact, or can have any degree of fluid exposure including total immersion. Fluid contact with the substrate can take place via aerosols or other means for air-borne fluid transmission.

Biofilm formation with health implications can involve those substrates in all health-related environments, including substrates found in medical environments and those substrates in industrial or residential environments that are involved in those functions

essential to human well being, for example, nutrition, sanitation and the prevention of disease. Substrates found in medical environments include the inner and outer aspects of various instruments and devices, whether disposable or intended for repeated uses. Examples include the entire spectrum of articles adapted for medical use, including scalpels, needles, scissors and other devices used in invasive surgical, therapeutic or diagnostic procedures; implantable medical devices, including artificial blood vessels, catheters and other devices for the removal or delivery of fluids to patients, artificial hearts, artificial kidneys, orthopedic pins, plates and implants; catheters and other tubes (including urological and biliary tubes, endotracheal tubes, peripherably insertable central venous catheters, dialysis catheters, long term tunneled central venous catheters, peripheral venous catheters, short term central venous catheters, arterial catheters, pulmonary catheters, Swan-Ganz catheters, urinary catheters, peritoneal catheters), urinary devices (including long term urinary devices, tissue bonding urinary devices, artificial urinary sphincters, urinary dilators), shunts (including ventricular or arterio-venous shunts); prostheses (including breast implants, penile prostheses, vascular grafting prostheses, heart valves, artificial joints, artificial larynxes, otological implants), vascular catheter ports, wound drain tubes, hydrocephalus shunts, pacemakers and implantable defibrillators, and the like. Other examples will be readily apparent to practitioners in these arts. Substrates found in the medical environment also include the inner and outer aspects of pieces of medical equipment, medical gear worn or carried by personnel in the health care setting. Such substrates can include counter tops and fixtures in areas used for medical procedures or for preparing medical apparatus, tubes and canisters used in respiratory treatments, including the administration of oxygen, of solubilized drugs in nebulizers and of anesthetic agents. Also included are those substrates intended as biological barriers to infectious organisms in medical settings, such as gloves, aprons and faceshields. Commonly used materials for biological barriers may be latex-based or non-latex based. Vinyl is commonly used as a material for non-latex surgical gloves. Other such substrates can include handles and cables for medical or dental equipment not intended to be sterile. Additionally, such substrates can include those non-sterile external substrates of tubes and other apparatus found in areas where blood or body fluids or other hazardous biomaterials are commonly encountered.

Substrates in contact with liquids are particularly prone to biofilm formation. As an example, those reservoirs and tubes used for delivering humidified oxygen to patients can bear biofilms inhabited by infectious agents. Dental unit waterlines similarly can bear biofilms on their substrates, providing a reservoir for continuing contamination of the

system of flowing an aerosolized water used in dentistry. Sprays, aerosols and nebulizers are highly effective in disseminating biofilm fragments to a potential host or to another environmental site. It is especially important to health to prevent biofilm formation on those substrates from where biofilm fragments can be carried away by sprays, aerosols or nebulizers contacting the substrate.

Other substrates related to health include the inner and outer aspects of those articles involved in water purification, water storage and water delivery, and articles involved in food processing. Substrates related to health can also include the inner and outer aspects of those household articles involved in providing for nutrition, sanitation or disease prevention. Examples can include food processing equipment for home use, materials for infant care, tampons and toilet bowls.

Substrates can be smooth or porous, soft or hard. Substrates can include a drainpipe, glaze ceramic, porcelain, glass, metal, wood, chrome, plastic, vinyl, Formica® brand laminate, or any other material that may regularly come in contact with an aqueous solution in which biofilms may form and grow. The substrate can be a substrate commonly found on household items such as shower curtains or liners, upholstery, laundry, and carpeting.

Another substrate on which biofilm preventing, removing or inhibiting is important is that of a ship hull. Biofilms, such as those of *Halomonas pacifica*, promote the corrosion of the hull of ships and also increase the roughness of the hull, increasing the drag on the ship and thereby increasing fuel costs. The biofilm can also promote the attachment of larger living structures such as barnacles on the ship hull. Fuel can account for half of the cost of marine shipping, and the loss in fuel efficiency due to biofilm formation is substantial.

Substrates on which biofilms can adhere include those of living organisms, as in the case of humans with chronic infections caused by biofilms, as discussed above. Biofilms can also form on the substrates of food contact surfaces, such as those used for processing seafood, and also on food products themselves. Examples of seafood products that may have biofilm contamination include oysters. Human infections caused by the ingestion of raw oysters has been linked to *Vibrio vulnificus* bacterium. *Vibrio* bacteria attach to algae and plankton in the water and transfer to the oysters and fish that feed on these organisms.

Other examples of substrates or devices on which biofilms can adhere can be found in U.S. Pat. Nos. 5,814,668 and 7,087,661; and U.S. Pat. Application Publication Nos. 2006/0228384 and 2006/0018945, each of which is incorporated herein by reference in its entirety.

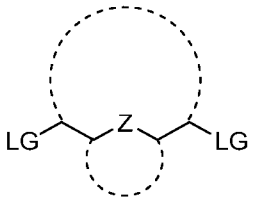
The term "surface" as used herein refers, can refer to a surface of any of the substrates described above.

Polycationic Polymers

Also provided are antimicrobial polycationic polymers that can be used in conjunction with the processes, articles, and methods described above. The polycationic polymers can include a plurality of positively charged centers, each of which is formed by condensation of cyclic bis-electrophile, such as a 9-thia/aza/selenabicyclo[3.3.1]nonyl electrophile, with a nucleophile. The resulting polycationic polymers can efficiently bind nucleic acids. The polycations can also exhibit properties of cytotoxicity and DNA transfection with interesting structure-activity characteristics.

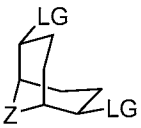
In some cases, the polycationic polymer can be formed by the condensation of cyclic bis-electrophile (e.g., a 9-thia/aza/selenabicyclo[3.3.1]nonyl dielectrophile) with a polynucleophiles (e.g., a dinucleophiles). Each bond-forming event in this condensation polymerization process generates a positively charged center within the polymer backbone. In these systems, the length of the polymer chain was found to be dependent on the reactivity of the electrophile, which can be readily tuned through variation of the leaving group β to sulfur/nitrogen/selenium atom present in the cyclic bis-electrophile. These polycations can also be biodegradable. Degradation of the polycations can occur via hydrolysis, with the rate of hydrolysis dependent on the leaving-group ability of the nucleophile (which correlates roughly with the pK_a of the nucleophile's conjugate acid). Thus, the degradation rates of the polycations can be tuned through selection of an appropriate nucleophile. Polymer decomposition occurs simultaneously throughout the length of the chains, rather than from the ends; the decomposition products can be only minimally toxic to mammalian cells.

In some embodiments, the polycationic polymer can be derived from the condensation of a cyclic bis-electrophile and a polynucleophilic monomer. The cyclic bis-electrophile can be defined by Formula II below

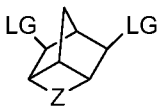


Formula II

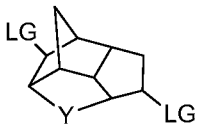
where each dotted line represents a cyclic moiety; LG represents a leaving group; Z represents S, Se, or NR^3; and R^3 represents alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy. In some embodiments, the cyclic bis-electrophile can be defined by Formula IIA, Formula IIB, or Formula IIC below



Formula IIA

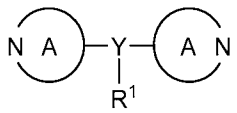


Formula IIB



Formula IIC

wherein LG and Z are as defined above with respect to Formula II. The polynucleophilic monomer can be defined by Formula III below



Formula III

wherein A represents a heterocyclic ring comprising a tertiary nitrogen atom; Y is absent or represents a linking group; and R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

In some embodiments, the polycationic polymer can be derived from the condensation of a single species of cyclic bis-electrophile defined by Formula II and a single species of polynucleophilic monomer defined by Formula III. In other embodiments, the polycationic polymer can be derived from the condensation of a single species of cyclic bis-electrophile defined by Formula II and two or more different species of polynucleophilic monomer defined by Formula III. In other embodiments, the polycationic polymer can be derived from the condensation of two or more different species of cyclic bis-electrophile defined by Formula II and a single species of polynucleophilic monomer defined by Formula III. In other embodiments, the polycationic polymer can be derived from the condensation of two or more different species of cyclic bis-electrophile defined by Formula II and two or more different species of polynucleophilic monomer defined by Formula III.

In some embodiments of Formula II, Z can be S. In other embodiments, Z can be NR^3.

In some embodiments of Formula II, Z can be NR^3, and R^3 can comprise a cationic group. For example, in certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

In some embodiments of Formula II, Z can be NR^3, and R^3 can comprise a hydrophobic group. For example, in certain embodiments, Z can be NR^3, and R^3 can comprise an aryl group or an alkylaryl group.

In some embodiments of Formula II, Z can be NR^3, and R^3 can comprise a hydrophilic group. For example, in certain embodiments, Z can be NR^3, and R^3 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

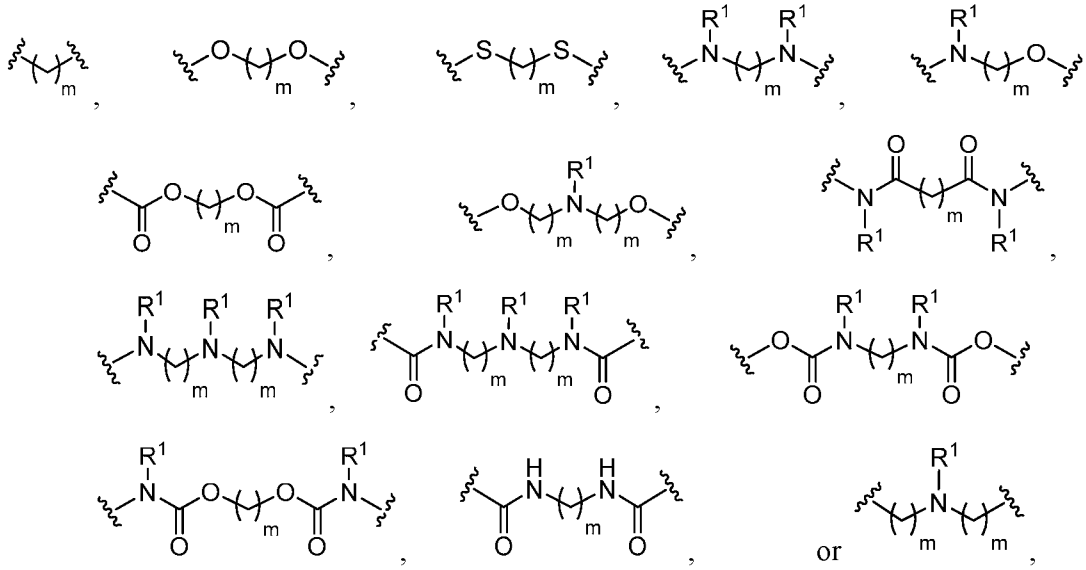
In some embodiments of Formula II, Z can be NR^3, and R^3 can comprise a reactive functional group. Reactive functional groups include moieties that can participate in a reaction with another moiety, which may also be a reactive functional group, to form a covalent bond. Examples of reactive functional groups include, but are not limited to, olefins, acetylenes, alcohols, phenols, ethers, oxides, halides, aldehydes, ketones, carboxylic acids, esters, amides, cyanates, isocyanates, thiocyanates, isothiocyanates, amines, hydrazines, hydrazones, hydrazides, diazo, diazonium, nitro, nitriles, mercaptans, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, acetals, ketals, anhydrides, sulfates, sulfenic acids isonitriles, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids thiohydroxamic acids, allenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, imines, azides, azo compounds, azoxy compounds, and nitroso compounds. Reactive functional groups also include those used to prepare bioconjugates, e.g., N-hydroxysuccinimide esters, maleimides and the like. Methods to prepare each of these functional groups are well known in the art and their application to or modification for a particular purpose is within the ability of one of skill in the art. *See*, for example, Sandler and Karo, eds. *Organic Functional Group Preparations*, Academic Press, San Diego, 1989. For example, in certain embodiments, Z can be NR^3, and R^3 can comprise an alkynyl group or an azido group. In other embodiments, Z can be NR^3, and R^3 can comprise a hydrazone group.

LG can represent any suitable leaving group. Leaving groups are well known in the art, and include substituent present within a chemical compound that can be readily displaced. Examples of leaving groups include halides (chloride, bromide, and iodide), triflates (OTf), boron moieties including boronic acids and trihaloborate salts such as trifluoroborate salts (BF_3), diazonium salts (N_2^+), tosylates (OTs) and other sulfonic esters, mesylates (OMs), nitrates (-ONO_2), and phosphates (-$OPO(OH)_2$). In some examples, LG can be a halide (e.g., chloride, bromide, or iodide). In other examples, LG can be triflate. In other examples, LG can be nitrate.

A can be any heterocyclic ring comprising a tertiary nitrogen atom. In some embodiments, A can be a heteroaromatic ring. In some embodiments, A can comprise one or more additional heteroatoms in addition to the tertiary nitrogen atom (e.g., one or more additional nitrogen atoms, one or more oxygen atoms, one or more sulfur atoms, or a combination thereof). For example, A can be chosen from pyridine, imidazole, pyrazole, triazole, tetrazole, oxazole, isoxazole, furazan, isothioazole, and thiazole. In certain embodiments, A can represent a pyridine ring.

In some embodiments, Y can be absent (i.e., the two heterocyclic rings can be bound directly to one another). In other embodiments, Y is present. When present, the linking group can be any suitable group or moiety which is at minimum bivalent, and connects the heterocyclic rings in the nucleophile. The linking group can be composed of any assembly of atoms, including oligomeric and polymeric chains. In some cases, the total number of atoms in the linking group can be from 3 to 200 atoms (e.g., from 3 to 150 atoms, from 3 to 100 atoms, from 3 and 50 atoms, from 3 to 25 atoms, from 3 to 15 atoms, or from 3 to 10 atoms). In some embodiments, the linking group can be, for example, an alkyl, alkoxy, alkylaryl, alkylheteroaryl, alkylcycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, or polyamino group. In some embodiments, the linking group can comprises one of the groups above joined to each of the heterocyclic rings by a functional group. Examples of suitable functional groups include, for example, secondary amides (-CONH-), tertiary amides (-CONR-), secondary carbamates (-OCONH-; -NHCOO-), tertiary carbamates (-OCONR-; -NRCOO-), ureas (-NHCONH-; -NRCONH-; -NHCONR-, or -NRCONR-), carbinols (-CHOH-, -CROH-), ethers (-O-), and esters (-COO-, –CH_2O_2C-, $CHRO_2C$-), wherein R is an alkyl group, an aryl group, or a heterocyclic group. For example, in some embodiments, the linking group can comprise an alkyl group (e.g., a C_1-C_{12} alkyl group, a C_1-C_8 alkyl group, or a C_1-C_6 alkyl group) bound to each

heterocyclic ring via an ester (-COO-, $-CH_2O_2C-$, $CHRO_2C-$), a secondary amide (-CONH-), or a tertiary amide (-CONR-), wherein R is an alkyl group, an aryl group, or a heterocyclic group. In certain embodiments, Y can be chosen from one of the following:



where m is an integer from 1 to 12.

In some embodiments, R^1 can be absent. In other embodiments, R^1 can be present. When present, R^1 can comprise a halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy group.

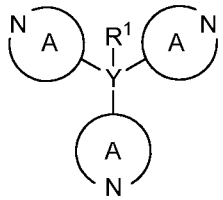
In some embodiments, R^1 can comprise a cationic group. In certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

In some embodiments, R^1 can comprise a hydrophobic group. For example, in certain embodiments, R^1 can comprise an aryl group or an alkylaryl group. In some embodiments, R^1 can comprise a hydrophilic group. For example, in certain embodiments, R^1 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

In some embodiments, R^1 can comprise a reactive functional group, such as an olefin, acetylene, alcohol, phenol, ether, oxide, halide, aldehyde, ketone, carboxylic acid,

ester, amide, cyanate, isocyanate, thiocyanate, isothiocyanate, amine, hydrazine, hydrazone, hydrazide, diazo, diazonium, nitro, nitrile, mercaptan, sulfide, disulfide, sulfoxide, sulfone, sulfonic acid, sulfinic acid, acetal, ketal, anhydride, sulfate, sulfenic acid isonitrile, amidine, imide, imidate, nitrone, hydroxylamine, oxime, hydroxamic acid, thiohydroxamic acid, allene, ortho ester, sulfite, enamine, ynamine, urea, pseudourea, semicarbazide, carbodiimide, carbamate, imine, azide, azo group, azoxy group, nitroso group, N-hydroxysuccinimide ester, or maleimides. For example, in certain embodiments, R^1 can comprise an alkynyl group or an azido group. In other embodiments, R^1 can comprise a hydrazone group.

In some embodiments, the polycationic polymer polymer can be derived from the condensation of one or more cyclic bis-electrophiles defined by Formula II, one or more polynucleophilic monomers defined by Formula III, and one or more polynucleophilic monomers defined by Formula IV below



Formula IV

wherein A represents a heterocyclic ring comprising a tertiary nitrogen atom; Y is absent or represents a linking group; and R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

In Formula IV, A can be any heterocyclic ring comprising a tertiary nitrogen atom. In some embodiments, A can be a heteroaromatic ring. In some embodiments, A can comprise one or more additional heteroatoms in addition to the tertiary nitrogen atom (e.g., one or more additional nitrogen atoms, one or more oxygen atoms, one or more sulfur atoms, or a combination thereof). For example, A can be chosen from pyridine, imidazole, pyrazole, triazole, tetrazole, oxazole, isoxazole, furazan, isothioazole, and thiazole. In certain embodiments, A can represent a pyridine ring.

In some embodiments of Formula IV, Y can be absent (i.e., the two heterocyclic rings can be bound directly to one another). In other embodiments, Y is present. When present, the linking group can be any suitable group or moiety which is at minimum

bivalent, and connects the heterocyclic rings in the nucleophile. The linking group can be composed of any assembly of atoms, including oligomeric and polymeric chains. In some cases, the total number of atoms in the linking group can be from 3 to 200 atoms (e.g., from 3 to 150 atoms, from 3 to 100 atoms, from 3 and 50 atoms, from 3 to 25 atoms, from 3 to 15 atoms, or from 3 to 10 atoms). In some embodiments, the linking group can be, for example, an alkyl, alkoxy, alkylaryl, alkylheteroaryl, alkylcycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, or polyamino group. In some embodiments, the linking group can comprises one of the groups above joined to each of the heterocyclic rings by a functional group. Examples of suitable functional groups include, for example, secondary amides (-CONH-), tertiary amides (-CONR-), secondary carbamates (-OCONH-; -NHCOO-), tertiary carbamates (-OCONR-; -NRCOO-), ureas (-NHCONH-; -NRCONH-; -NHCONR-, or -NRCONR-), carbinols (-CHOH-, -CROH-), ethers (-O-), and esters (-COO-, $-CH_2O_2C-$, $CHRO_2C-$), wherein R is an alkyl group, an aryl group, or a heterocyclic group. For example, in some embodiments, the linking group can comprise an alkyl group (e.g., a C_1-C_{12} alkyl group, a C_1-C_8 alkyl group, or a C_1-C_6 alkyl group) bound to each heterocyclic ring via an ester (-COO-, $-CH_2O_2C-$, $CHRO_2C-$), a secondary amide (-CONH-), or a tertiary amide (-CONR-), wherein R is an alkyl group, an aryl group, or a heterocyclic group.

In some embodiments of Formula IV, R^1 can be absent. In other embodiments, R^1 can be present. When present, R^1 can comprise a halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy group.

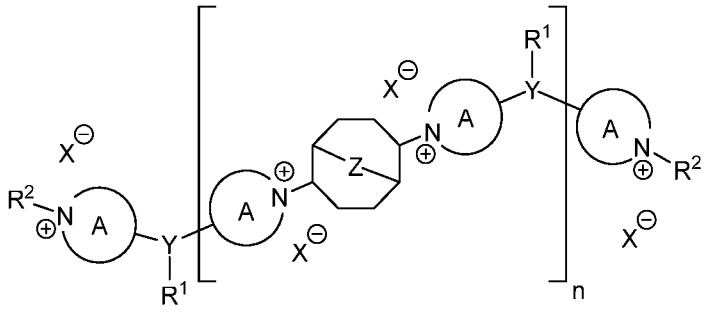
In some embodiments of Formula IV, R^1 can comprise a cationic group. In certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, an aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

In some embodiments of Formula IV, R^1 can comprise a hydrophobic group. For example, in certain embodiments, R^1 can comprise an aryl group or an alkylaryl group. In

some embodiments, R^1 can comprise a hydrophilic group. For example, in certain embodiments, R^1 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

In some embodiments of Formula IV, R^1 can comprise a reactive functional group, such as an olefin, acetylene, alcohol, phenol, ether, oxide, halide, aldehyde, ketone, carboxylic acid, ester, amide, cyanate, isocyanate, thiocyanate, isothiocyanate, amine, hydrazine, hydrazone, hydrazide, diazo, diazonium, nitro, nitrile, mercaptan, sulfide, disulfide, sulfoxide, sulfone, sulfonic acid, sulfinic acid, acetal, ketal, anhydride, sulfate, sulfenic acid isonitrile, amidine, imide, imidate, nitrone, hydroxylamine, oxime, hydroxamic acid, thiohydroxamic acid, allene, ortho ester, sulfite, enamine, ynamine, urea, pseudourea, semicarbazide, carbodiimide, carbamate, imine, azide, azo group, azoxy group, nitroso group, N-hydroxysuccinimide ester, or maleimides. For example, in certain embodiments, R^1 can comprise an alkynyl group or an azido group. In other embodiments, R^1 can comprise a hydrazone group.

In some embodiments, the polycation polymer can be a polymer defined by Formula I below

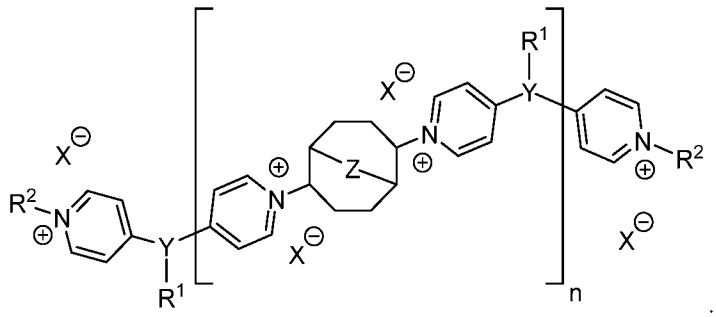


Formula I

wherein Z represents, individually for each occurrence within the polymer, S, Se, or NR^3; A represents, individually for each occurrence within the polymer, a heterocyclic ring comprising a cationic nitrogen center; X represents, individually for each occurrence within the polymer, an anion; Y is absent, or represents, individually for each occurrence within the polymer, a linking group; R^1 is absent, or represents, individually for each occurrence within the polymer, halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy; R^2 is absent, or represents, individually for each occurrence within the polymer, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl,

alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy; R^3 represents, individually for each occurrence within the polymer, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy; and n is an integer from 2 to 400.

In Formula I, A can be any heterocyclic ring comprising a tertiary nitrogen atom. In some embodiments, A can be a heteroaromatic ring. In some embodiments, A can comprise one or more additional heteroatoms in addition to the tertiary nitrogen atom (e.g., one or more additional nitrogen atoms, one or more oxygen atoms, one or more sulfur atoms, or a combination thereof). For example, A can be chosen from pyridine, imidazole, pyrazole, triazole, tetrazole, oxazole, isoxazole, furazan, isothioazole, and thiazole. In certain embodiments, A can represent a pyridine ring. In these embodiments, the polycation can be defined by Formula IA below



Formula IA

In some embodiments of Formula I and Formula IA, Z can be S. In other embodiments, Z can be NR^3.

In some embodiments of Formula I and Formula IA, Z can be NR^3, and R^3 can comprise a cationic group. For example, in certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, an aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

In some embodiments of Formula I and Formula IA, Z can be NR^3, and R^3 can comprise a hydrophobic group. For example, in certain embodiments, Z can be NR^3, and R^3 can comprise an aryl group or an alkylaryl group.

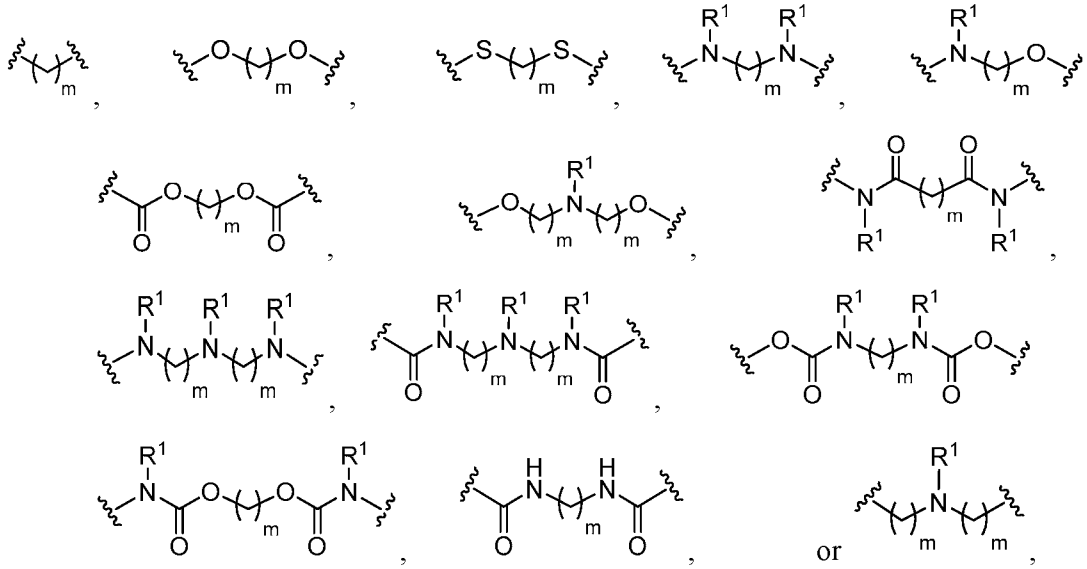
In some embodiments of Formula I and Formula IA, Z can be NR^3, and R^3 can comprise a hydrophilic group. For example, in certain embodiments, Z can be NR^3, and R^3 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

In some embodiments of Formula I and Formula IA, Z can be NR^3, and R^3 can comprise a reactive functional group, such as an olefin, acetylene, alcohol, phenol, ether, oxide, halide, aldehyde, ketone, carboxylic acid, ester, amide, cyanate, isocyanate, thiocyanate, isothiocyanate, amine, hydrazine, hydrazone, hydrazide, diazo, diazonium, nitro, nitrile, mercaptan, sulfide, disulfide, sulfoxide, sulfone, sulfonic acid, sulfinic acid, acetal, ketal, anhydride, sulfate, sulfenic acid isonitrile, amidine, imide, imidate, nitrone, hydroxylamine, oxime, hydroxamic acid, thiohydroxamic acid, allene, ortho ester, sulfite, enamine, ynamine, urea, pseudourea, semicarbazide, carbodiimide, carbamate, imine, azide, azo group, azoxy group, nitroso group, N-hydroxysuccinimide ester, or maleimides. For example, in certain embodiments, R^3 can comprise an alkynyl group or an azido group. In other embodiments, R^3 can comprise a hydrazone group.

In Formula I and Formula IA, X can comprise any suitable, stable anion. By way of example, X can be chosen from chloride, bromide, iodide, nitrate, sulfate, triflate, borate, and phosphate.

In some embodiments of Formula I and Formula IA, Y can be absent (i.e., the two heterocyclic rings can be bound directly to one another). In other embodiments, Y is present. When present, the linking group can be any suitable group or moiety which is at minimum bivalent, and connects the heterocyclic rings in the nucleophile. The linking group can be composed of any assembly of atoms, including oligomeric and polymeric chains. In some cases, the total number of atoms in the linking group can be from 3 to 200 atoms (e.g., from 3 to 150 atoms, from 3 to 100 atoms, from 3 and 50 atoms, from 3 to 25 atoms, from 3 to 15 atoms, or from 3 to 10 atoms). In some embodiments, the linking group can be, for example, an alkyl, alkoxy, alkylaryl, alkylheteroaryl, alkylcycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, or polyamino group. In some embodiments, the linking group can comprises one of the groups above joined to each of the heterocyclic rings by a functional group. Examples of suitable functional groups include, for example, secondary amides (-CONH-), tertiary amides (-

CONR-), secondary carbamates (-OCONH-; -NHCOO-), tertiary carbamates (-OCONR-; -NRCOO-), ureas (-NHCONH-; -NRCONH-; -NHCONR-, or -NRCONR-), carbinols (-CHOH-, -CROH-), ethers (-O-), and esters (-COO-, $-CH_2O_2C-$, $CHRO_2C-$), wherein R is an alkyl group, an aryl group, or a heterocyclic group. For example, in some embodiments, the linking group can comprise an alkyl group (e.g., a C_1-C_{12} alkyl group, a C_1-C_8 alkyl group, or a C_1-C_6 alkyl group) bound to each heterocyclic ring via an ester (-COO-, $-CH_2O_2C-$, $CHRO_2C-$), a secondary amide (-CONH-), or a tertiary amide (-CONR-), wherein R is an alkyl group, an aryl group, or a heterocyclic group. In certain embodiments, Y can be chosen from one of the following:



where m is an integer from 1 to 12.

In some embodiments of Formula I and Formula IA, R^1 can be absent. In other embodiments, R^1 can be present. When present, R^1 can comprise a halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy group.

In some embodiments of Formula I and Formula IA, R^1 can comprise a cationic group. In certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, a aryltrialkylammonium

group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

In some embodiments of Formula I and Formula IA, R^1 can comprise a hydrophobic group. For example, in certain embodiments, R^1 can comprise an aryl group or an alkylaryl group. In some embodiments, R^1 can comprise a hydrophilic group. For example, in certain embodiments, R^1 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

In some embodiments of Formula I and Formula IA, R^1 can comprise a reactive functional group, such as an olefin, acetylene, alcohol, phenol, ether, oxide, halide, aldehyde, ketone, carboxylic acid, ester, amide, cyanate, isocyanate, thiocyanate, isothiocyanate, amine, hydrazine, hydrazone, hydrazide, diazo, diazonium, nitro, nitrile, mercaptan, sulfide, disulfide, sulfoxide, sulfone, sulfonic acid, sulfinic acid, acetal, ketal, anhydride, sulfate, sulfenic acid isonitrile, amidine, imide, imidate, nitrone, hydroxylamine, oxime, hydroxamic acid, thiohydroxamic acid, allene, ortho ester, sulfite, enamine, ynamine, urea, pseudourea, semicarbazide, carbodiimide, carbamate, imine, azide, azo group, azoxy group, nitroso group, N-hydroxysuccinimide ester, or maleimides. For example, in certain embodiments, R^1 can comprise an alkynyl group or an azido group. In other embodiments, R^1 can comprise a hydrazone group.

In some embodiments of Formula I and Formula IA, R^2 can be absent. In other embodiments, R^2 can be present. When present, R^2 can comprise an alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy

In some embodiments of Formula I and Formula IA, R^2 can comprise a cationic group. In certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

In some embodiments of Formula I and Formula IA, R^2 can comprise a hydrophobic group. For example, in certain embodiments, R^2 can comprise an aryl group or an alkylaryl group. In some embodiments, R^2 can comprise a hydrophilic group. For example, in

certain embodiments, R^2 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

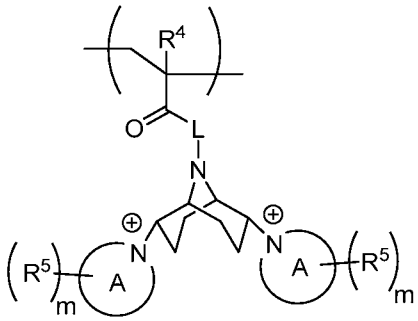
In some embodiments of Formula I and Formula IA, R^2 can comprise a reactive functional group, such as an olefin, acetylene, alcohol, phenol, ether, oxide, halide, aldehyde, ketone, carboxylic acid, ester, amide, cyanate, isocyanate, thiocyanate, isothiocyanate, amine, hydrazine, hydrazone, hydrazide, diazo, diazonium, nitro, nitrile, mercaptan, sulfide, disulfide, sulfoxide, sulfone, sulfonic acid, sulfinic acid, acetal, ketal, anhydride, sulfate, sulfenic acid isonitrile, amidine, imide, imidate, nitrone, hydroxylamine, oxime, hydroxamic acid, thiohydroxamic acid, allene, ortho ester, sulfite, enamine, ynamine, urea, pseudourea, semicarbazide, carbodiimide, carbamate, imine, azide, azo group, azoxy group, nitroso group, N-hydroxysuccinimide ester, or maleimides. For example, in certain embodiments, R^2 can comprise an alkynyl group or an azido group. In other embodiments, R^2 can comprise a hydrazone group.

In some embodiments of Formula I and Formula IA, n can be at least 2 (e.g., at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, or at least 375). In some embodiments, n can be 400 or less (e.g., 375 or less, 350 or less, 325 or less, 300 or less, 275 or less, 250 or less, 225 or less, 200 or less, 190 or less, 180 or less, 170 or less, 160 or less, 150 or less, 140 or less, 130 or less, 120 or less, 110 or less, 100 or less, 90 or less, 80 or less, 70 or less, 60 or less, 50 or less, 40 or less, 30 or less, 25 or less, 20 or less, 15 or less, 10 or less, or 5 or less).

n can range from any of the minimum values described above to any of the maximum values described above. For example, n can range from 2 to 400 (e.g., from 2 to 200, from 2 to 100, from 2 to 50, from 5 to 400, from 5 to 200, from 5 to 100, or from 5 to 50).

In other cases, the polycationic polymer can be formed by polymerization of monomers (e.g., acrylate monomers) containing pendant cyclic bis-electrophiles (e.g., pendant versions of the cyclic bis-electrophiles defined by Formula IIA, Formula IIB, or Formula IIC where Z is NR^3, and R^3 represents an acrylate group connected to the cyclic bis-electrophile via a linking group). Following polymerization, the pendant cyclic bis-electrophiles can be reacted with nucleophiles to introduce positively charged centers within the polymer sidechains.

By way of example, also provided are polycationic polymers comprising a recurring unit defined by Formula V below



Formula V

wherein R^4 represents H or methyl; L is absent or represents a linking group; A represents, individually for each occurrence, a heterocyclic ring comprising a cationic nitrogen center; R^5 is absent, or represents, individually for each occurrence, halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy; and m is an integer from 0 to 5.

In some embodiments, L can be absent. In other embodiments, L is present and represents a linking group. When present, the linking group can be any suitable group or moiety which is at minimum bivalent, and connects the pendant 9-azabicyclo[3.3.1]nonyl moiety to the polymer backbone. The linking group can be composed of any assembly of atoms, including oligomeric and polymeric chains. In some cases, the total number of atoms in the linking group can be from 3 to 200 atoms (e.g., from 3 to 150 atoms, from 3 to 100 atoms, from 3 and 50 atoms, from 3 to 25 atoms, from 3 to 15 atoms, or from 3 to 10 atoms). In some embodiments, the linking group can be, for example, an alkyl, alkoxy, alkylaryl, alkylheteroaryl, alkylcycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, or polyamino group.

In Formula V, A can be any heterocyclic ring comprising a tertiary nitrogen atom. In some embodiments, A can be a heteroaromatic ring. In some embodiments, A can comprise one or more additional heteroatoms in addition to the tertiary nitrogen atom (e.g., one or more additional nitrogen atoms, one or more oxygen atoms, one or more sulfur atoms, or a combination thereof). For example, A can be chosen from pyridine, imidazole,

pyrazole, triazole, tetrazole, oxazole, isoxazole, furazan, isothioazole, and thiazole. In certain embodiments, A can represent a pyridine ring.

In some embodiments, R^5 can be absent. In other embodiments, R^5 can be present. When present, R^5 can comprise, individually for each occurrence, a halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy group.

In some embodiments, R^5 can comprise a cationic group. In certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, a aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

In some embodiments, R^5 can comprise a hydrophobic group. For example, in certain embodiments, R^5 can comprise an aryl group or an alkylaryl group. In some embodiments, R^5 can comprise a hydrophilic group. For example, in certain embodiments, R^5 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

In some embodiments, R^5 can comprise a reactive functional group, such as an olefin, acetylene, alcohol, phenol, ether, oxide, halide, aldehyde, ketone, carboxylic acid, ester, amide, cyanate, isocyanate, thiocyanate, isothiocyanate, amine, hydrazine, hydrazone, hydrazide, diazo, diazonium, nitro, nitrile, mercaptan, sulfide, disulfide, sulfoxide, sulfone, sulfonic acid, sulfinic acid, acetal, ketal, anhydride, sulfate, sulfenic acid isonitrile, amidine, imide, imidate, nitrone, hydroxylamine, oxime, hydroxamic acid, thiohydroxamic acid, allene, ortho ester, sulfite, enamine, ynamine, urea, pseudourea, semicarbazide, carbodiimide, carbamate, imine, azide, azo group, azoxy group, nitroso group, N-hydroxysuccinimide ester, or maleimides. For example, in certain embodiments, R^5 can comprise an alkynyl group or an azido group. In other embodiments, R^5 can comprise a hydrazone group.

In some embodiments, the polycationic polymer can further comprise a recurring unit derived from the polymerization of one or more ethylenically-unsaturated monomers. Example ethylenically-unsaturated monomers include (meth)acrylate monomers, vinyl

aromatic monomers (e.g., styrene), ethylenically unsaturated aliphatic monomers (e.g., butadiene), vinyl ester monomers (e.g., vinyl acetate), (meth)acrylonitrile monomers, vinyl halide monomers, vinyl ether monomers, silane-containing monomers, (meth)acrylamide monomers (as well as (meth)acrylamide derivatives), sulfur-based monomers, and combinations thereof.

Exemplary acrylate and (meth)acrylate monomers include, but are not limited to, methyl acrylate, methyl (meth)acrylate, ethyl acrylate, ethyl (meth)acrylate, butyl acrylate, butyl (meth)acrylate, isobutyl (meth)acrylate, n-hexyl (meth)acrylate, ethylhexyl (meth)acrylate, n-heptyl (meth)acrylate, ethyl (meth)acrylate, 2-methylheptyl (meth)acrylate, octyl (meth)acrylate, isooctyl (meth)acrylate, n-nonyl (meth)acrylate, isononyl (meth)acrylate, n-decyl (meth)acrylate, isodecyl (meth)acrylate, dodecyl (meth)acrylate, lauryl (meth)acrylate, tridecyl (meth)acrylate, stearyl (meth)acrylate, glycidyl (meth)acrylate, alkyl crotonates, vinyl acetate, di-n-butyl maleate, di-octylmaleate, acetoacetoxyethyl (meth)acrylate, acetoacetoxypropyl (meth)acrylate, hydroxyethyl (meth)acrylate, allyl (meth)acrylate, tetrahydrofurfuryl (meth)acrylate, cyclohexyl (meth)acrylate, 2-ethoxyethyl (meth)acrylate, 2-methoxy (meth)acrylate, 2-(2-ethoxyethoxy)ethyl (meth)acrylate, 2-ethylhexyl (meth)acrylate, 2-propylheptyl (meth)acrylate, 2-phenoxyethyl (meth)acrylate, isobornyl (meth)acrylate, caprolactone (meth)acrylate, polypropyleneglycol mono(meth)acrylate, polyethyleneglycol (meth)acrylate, benzyl (meth)acrylate, 2,3-di(acetoacetoxy)propyl (meth)acrylate, hydroxypropyl (meth)acrylate, methylpolyglycol (meth)acrylate, 3,4-epoxycyclohexylmethyl (meth)acrylate, 1,6 hexanediol di(meth)acrylate, 1,4 butanediol di(meth)acrylate and combinations thereof.

Suitable vinyl aromatic compounds include styrene, α- and p-methylstyrene, α-butylstyrene, 4-n-butylstyrene, 4-n-decylstyrene, vinyltoluene, and combinations thereof. Vinyl esters of carboxylic acids include, for example, vinyl laurate, vinyl stearate, vinyl propionate, versatic acid vinyl esters, vinyl acetate, and combinations thereof. The vinyl halides can include ethylenically unsaturated compounds substituted by chlorine, fluorine or bromine, such as vinyl chloride and vinylidene chloride. The vinyl ethers can include, for example, vinyl ethers of alcohols comprising 1 to 4 carbon atoms, such as vinyl methyl ether or vinyl isobutyl ether. Aliphatic hydrocarbon monomers can include, for example, hydrocarbons having 4 to 8 carbon atoms and two olefinic double bonds, such as butadiene, isoprene, and chloroprene. Silane containing monomers can include, for example, vinyl silanes, such as vinyltrimethoxysilane, vinyltriethoxysilane (VTEO), vinyl tris(2-

methoxyethoxysilane), and vinyl triisopropoxysilane, and (meth)acrylatoalkoxysilanes, such as (meth)acryloyloxypropyltrimethoxysilane, γ-(meth)acryloxypropyltrimethoxysilane, and γ-(meth)acryloxypropyltriethoxysilane. (Meth)acrylamide derivatives include, for example, keto-containing amide functional monomers defined by the general structure below

$$CH_2{=}{=}CR_1C(O)NR_2C(O)R_3$$

wherein R_1 is hydrogen or methyl; R_2 is hydrogen, a C_1-C_4 alkyl group, or a phenyl group; and R_3 is hydrogen, a C_1-C_4 alkyl group, or a phenyl group. For example, the (meth)acrylamide derivative can be diacetone acrylamide (DAAM) or diacetone methacrylamide. Sulfur-containing monomers include, for example, sulfonic acids and sulfonates, such as vinylsulfonic acid, 2-sulfoethyl methacrylate, sodium styrenesulfonate, 2-sulfoxyethyl methacrylate, vinyl butylsulfonate, sulfones such as vinylsulfone, sulfoxides such as vinylsulfoxide, and sulfides such as l-(2-hydroxyethylthio) butadiene.

By way of non-limiting illustration, examples of certain embodiments of the present disclosure are given below.

EXAMPLES

Example 1: Fragmentable Polycationic Materials Based on Anchimeric Assistance

New modular, fragmentable oligo- and polycations have been developed based on the reactions of 9-thiabicyclo[3.3.1]dichloride and related compounds with substituted dipyridyl nucleophiles by an anchimeric assistance mechanism. Each bond-forming event in this condensation polymerization process generates a positive charge in the main chain. Product lengths were found to be dependent on the reactivity of the electrophile, which was tunable by changing the nature of the leaving group β to sulfur. The monomers were easily synthesized, and the resulting readily available polymers were found to be highly efficient binders of nucleic acid. The polycations also exhibited properties of cytotoxicity and DNA transfection with interesting structure-activity characteristics. The polycations decomposed by hydrolysis at rates dependent on the leaving-group ability of the pyridyl unit, which correlated roughly with the pK_a of its conjugate acid. Polymer decomposition occurs simultaneously throughout the length of the chains, rather than from the ends; the decomposition products were tested and found to be only minimally toxic to cultured cells

Background

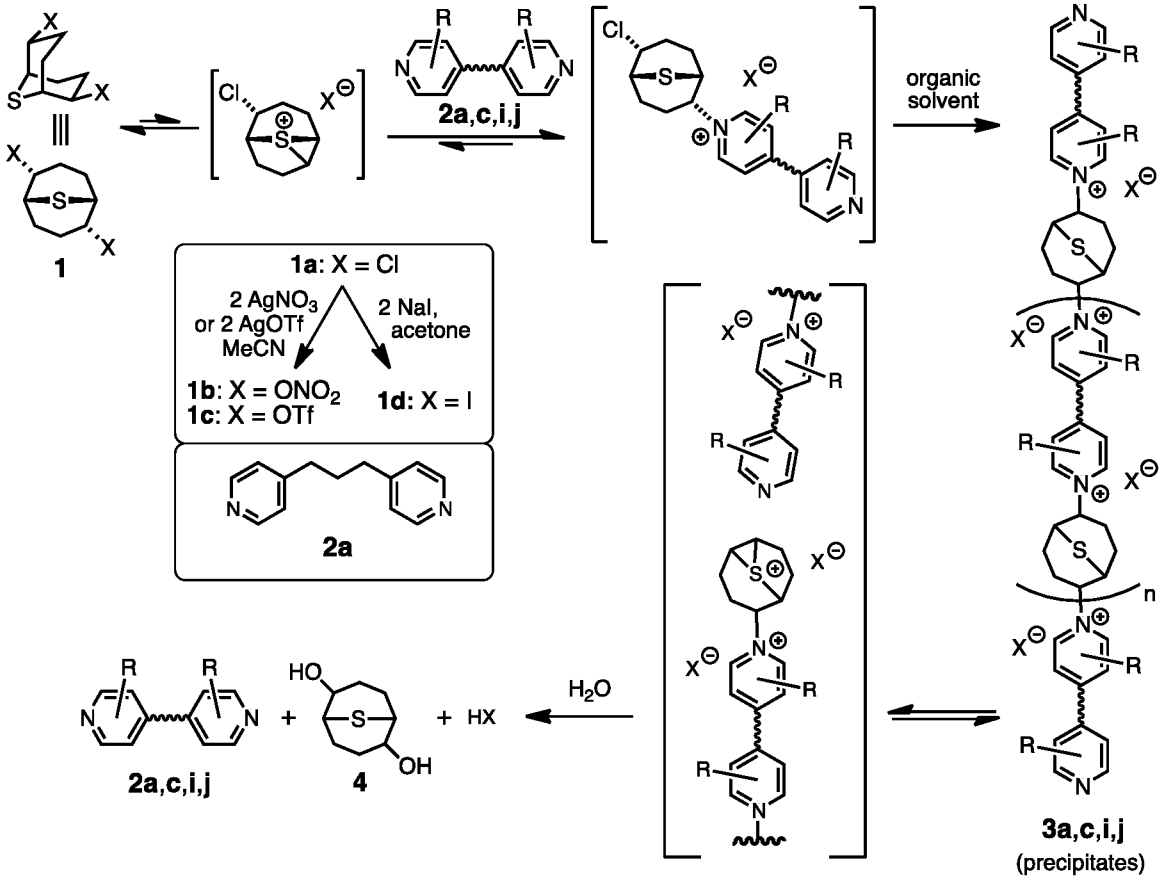
The reliable substitution chemistry of 9-thiabicyclo[3.3.1]dichloride (**1**) and related electrophiles was first described almost 40 years ago by the Weil, Corey, and Lautenschlaeger group (resulting in the designation of **1** as a "WCL" building block). The

reactivity of this scaffold has been explored for its fast and clean nature due to anchimeric assistance provided by the internal nucleophilic center. As with other reactions meeting the click chemistry standard, such reactions can potentially be used in materials science applications. Substitution chemistry accelerated by neighboring group participation has been used intermittently for many years in polymer synthesis and modification, although sometimes intramolecular effects have been mistaken for true anchimeric assistance.

Cationic polymers are of interest primarily for gene delivery and surface antimicrobial properties, with recent attention to new designs and precise control of structure-property relationships. The majority of such materials employ primary, secondary, and tertiary amines that require protonation to carry the desired charges. Polyethylenimine(PEI), synthesized by the ring opening polymerization of aziridine, is a popular example. Cleavable linkages such as esters, acetals/ketals, imines, and disulfides have been employed in constructing biodegradable transfection agents from branched PEI, often with the sacrifice of a unit positive charge at each point of linker connection. Permanently charged species such as quaternary ammonium or pyridinium ions are only rarely employed as linkages in the backbone of polycationic chains, although they have been used to modify polymer branches and are the predominant positively-charged constituents of cationic lipid head groups used as small-molecule transfection reagents. An example are the polyionenes usually synthesized by S_N2 Menschutkin reaction at comparatively high temperatures. The resulting materials show properties characteristic of other polycations, but are thermally and chemically stable at physiological temperature, and are therefore not biodegradable.

In this example, the use of WCL electrophiles in the synthesis of a new class of polycationic molecules that have a unique architecture and mode of fragmentation are described. The polycationic materials are represented schematically in Scheme 1. In these systems, positive charge is created by each substitution event and disappears when the linkage fragments in the course of anchimerically-assisted hydrolysis, rather than being carried by monomers, so the starting materials and products are uncharged (except for byproduct mineral acid). Preliminary characterization of the cytotoxic and DNA transfection properties of the polycations are also described, and demonstrate that this class of materials may be suitable for antimicrobial and gene delivery applications.

Scheme 1. Schematic representation of WCL-based condensation polymerization and depolymerization, both processes taking place via anchimeric assistance.



Materials and Methods

Reagents and solvents were purchased from commercial sources and used as received, unless otherwise stated. When dry solvents were required, solvents were passed through activated alumina columns on an MBraun solvent purification system (MB-SPS), and collected in oven-dried glassware prior to use. Water was purified on a Millipore Milli-Q Advantage A10 system. Unless otherwise stated, the reactions were performed under inert atmosphere in capped reaction vessels. Flash chromatography was performed on 60-mesh silica. Analytical TLC was performed on aluminum-backed plates and visualized by exposure to UV light and/or staining with aqueous potassium permanganate (2% $KMnO_4$ + 5% K_2CO_3).

Instrumentation

NMR spectra were obtained on Bruker AMX-400, and DRX-500 instruments in deuterated solvents (Cambridge Isotope Laboratories, Inc.) and referenced to the signals of

residual protium in the NMR solvent. Spectra were processed in MestReNova software (Mestrelab Research). Routine mass spectra were obtained on an Advion Compact Mass Spectrometer (G1946D) ESI-MSD instrument, using direct sample injection followed with 9:1 CH_3CN:H_2O containing 0.1% formic acid as mobile phase. Absorbance and fluorescence spectra were collected on a VarioskanFlash plate reader (ThermoFisher). Gel permeation chromatography (GPC) analysis was performed in DMF or Milli-Q water at 1 mL/min flow rate (LC-20AD pump) on a Shimadzu GPC setup equipped with two Phenomenex Phenogel 10 μm linear columns (300 x 7.8 mm) or PolySep-GFC-P (300 x 7.0 mm), autosampler (SIL-20A) and column oven (CTO-20A) set at 40 °C. Detection was achieved using a diode array detector (SPD---M20A), and RI detector (RID---10A), and instrument was calibrated with polystyrene standards kits (Supelco) or Dextran kit (Phenomenex ALO-2772). Dynamic light scattering measurements were taken on a DynaPro plate reader and analyzed with Dynamics® software (Wyatt Technology, Santa Barbara, CA). Transmission electron microscope images were acquired on a Hitachi HT7700 microscope operated at 120 kV.

Synthetic Procedures and Characterization of New Compounds

2,6-Dichloro-9-thiabicyclo[3.3.1]nonane (**1a**) was synthesized using the methods described in Diaz, D.D. et al. "2,6-Dichloro-9-thiabicyclo[3.3.1]nonane: Multigram Display of Azide and Cyanide Components on a Versatile Scaffold," *Molecules*, **2006**, *11*, 212-218, which is hereby incorporated herein by reference. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.71 (d, J = 3.3 Hz, 2H), 2.85 (dd, J = 6.8, 3.6 Hz, 2H), 2.72 – 2.61 (m, 2H), 2.36 – 2.18 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 62.34, 37.10, 32.40, 28.12.

2,6-Dinitro-9-thiabicyclo[3.3.1]nonane (**1b**). **1a** (21 mg, 0.1 mmol, 1 equiv) and silver nitrate (34 mg, 0.2 mmol, 2 equiv) were mixed in 0.5 mL MeCN and stirred overnight at room temperature. The resulting AgCl was removed by centrifugation and the solvent was evaporated to give **1b** as a colorless oil (22 mg, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 – 5.50 (m, 2H), 3.02 (d, J = 3.1 Hz, 2H), 2.51 (dd, J = 14.5, 6.7 Hz, 2H), 2.32 (dd, J = 9.8, 3.0 Hz, 2H), 2.20 (dt, J = 13.5, 6.7 Hz, 2H), 2.11 – 1.92 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 82.24, 32.72, 26.92, 24.84.

2,6-Diiodo-9-thiabicyclo[3.3.1]nonane (**1d**). Compound **1a** (252 mg, 1.2 mmol, 1 equiv) and sodium iodide (540 mg, 3.6 mmol, 3 equiv) were mixed in 6 mL acetone and stirred overnight at room temperature. NaCl was filtered off and the solution was evaporated. The resulting solid was dissolved in CH_2Cl_2, leaving behind excess sodium iodide. Evaporation of this solution gave **1d** as a yellow powder (270 mg, 58% yield). ^{1}H

NMR (500 MHz, $CDCl_3$) δ 5.26 – 5.12 (m, 2H), 3.11 (t, J = 3.4 Hz, 2H), 2.95 – 2.81 (m, 2H), 2.71 (ddd, J = 11.6, 6.1, 4.0 Hz, 2H), 2.58 – 2.42 (m, 2H). ^{13}C NMR (126 MHz, CDCl3) δ 38.50, 36.11, 35.75, 32.35.

Representative procedure for the synthesis of pyridine adducts of both small molecules and oligomers (Table 2, Table 3): Compound **1a** (21 mg, 0.1 mmol, 1 equiv), silver nitrate (34 mg, 0.2 mmol, 2 equiv) and the pyridine of interest (0.2 mmol, 2 equiv) were mixed in 0.5 mL MeCN and stirred overnight at room temperature under inert atmosphere. The precipitate was collected and washed with MeCN. The charged adduct was dissolved in water and any remaining solids were removed by centrifugation, repeating the centrifugation step until the solution was not cloudy. The resulting solution was frozen and lyophilized to obtain the product as a colorless solid.

2,6-di-3-methoxycarbonylpyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5b**): ^1H NMR (400 MHz, D_2O) δ 9.58 (s, 2H), 9.35 (d, J = 4.9 Hz, 2H), 9.23 – 9.08 (m, 2H), 8.47 – 8.27 (m, 2H), 5.85 (dd, J = 8.3, 4.0 Hz, 2H), 3.41 (s, 2H), 3.25 (q, J = 13.0 Hz, 2H), 2.49 (t, J = 14.5 Hz, 4H), 2.36 (d, J = 14.9 Hz, 2H). ^{13}C NMR (126 MHz, D_2O) δ 163.09, 146.47, 146.01, 144.54, 131.16, 129.01, 74.35, 53.86, 35.67, 26.25, 24.27.

2,6-di-4-methoxycarbonylpyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5c**): ^1H NMR (400 MHz, D_2O) δ 9.31 (d, J = 6.7 Hz, 4H), 8.65 (d, J = 6.6 Hz, 4H), 5.92 – 5.77 (m, 2H), 3.40 (s, 2H), 3.19 (dd, J = 12.9, 5.5 Hz, 2H), 2.60 – 2.43 (m, 4H), 2.43 – 2.31 (m, 2H). ^{13}C NMR (126 MHz, D_2O) δ 163.50, 145.43, 144.84, 128.20, 74.39, 54.22, 35.92, 26.52, 24.68.

2,6-di-3-carbamylpyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5d**): ^1H NMR (400 MHz, D_2O) δ 9.47 (s, 2H), 9.28 (d, J = 6.0 Hz, 2H), 8.98 (dd, J = 16.1, 7.3 Hz, 2H), 8.48 – 8.28 (m, 2H), 5.94 – 5.74 (m, 2H), 3.41 (s, 2H), 3.36 – 3.13 (m, 2H), 2.66 – 2.40 (m, 4H), 2.36 (d, J = 15.7 Hz, 2H). ^{13}C NMR (101 MHz, D_2O) δ 165.66, 145.33, 144.82, 143.44, 134.42, 128.93, 74.42, 35.82, 26.42, 24.44.

2,6-di-4-carbamylpyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5e**): ^1H NMR (400 MHz, D_2O) δ 9.36 (d, J = 6.8 Hz, 4H), 8.53 (d, J = 6.7 Hz, 4H), 5.87 (dd, J = 10.5, 6.7 Hz, 2H), 3.45 (d, J = 2.7 Hz, 2H), 3.36 – 3.17 (m, 2H), 2.62 – 2.46 (m, 4H), 2.40 (dd, J = 9.4, 6.4 Hz, 2H). ^{13}C NMR (126 MHz, D_2O) δ 166.24, 149.13, 144.47, 126.79, 74.08, 35.78, 26.41, 24.59,

2,6-di-3-bromopyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5f**): ^1H NMR (400 MHz, D_2O) δ 9.33 (d, J = 0.9 Hz, 2H), 9.10 (d, J = 5.2 Hz, 2H), 8.93 – 8.72 (m, 2H), 8.11 (dd, J = 8.3, 6.2 Hz, 2H), 5.90 – 5.61 (m, 2H), 3.34 (s, 2H), 3.13 (dd, J = 13.0, 5.7 Hz, 2H),

2.58 – 2.25 (m, 6H). ^{13}C NMR (126 MHz, D_2O) δ 149.30, 144.72, 141.88, 129.23, 123.63, 74.22, 35.83, 26.38, 24.27.

2,6-di-3-trifluoromethylpyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5g**): ^{1}H NMR (500 MHz, D_2O) δ 9.86 (s, 2H), 9.53 (d, J = 6.4 Hz, 2H), 9.13 (d, J = 8.1 Hz, 2H), 8.53 – 8.48 (m, 2H), 6.01 – 5.89 (m, 2H), 3.52 (d, J = 2.4 Hz, 2H), 3.32 (dd, J = 12.9, 6.2 Hz, 2H), 2.66 – 2.48 (m, 4H), 2.42 (dd, J = 15.2, 4.3 Hz, 2H). ^{13}C NMR (101 MHz, D2O) δ 149.76, 147.21, 147.17, 129.58, 114.67, 113.24, 75.11, 35.64, 26.23, 24.36.

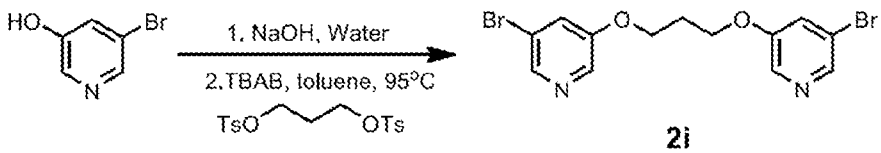
2,6-di-3-cyanopyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5h**): ^{1}H NMR (500 MHz, D_2O) δ 9.69 (s, 2H), 9.54 (d, J = 6.3 Hz, 2H), 9.07 (d, J = 8.2 Hz, 2H), 8.61 – 8.44 (m, 2H), 5.94 (dd, J = 8.3, 3.7 Hz, 2H), 3.50 (d, J = 3.0 Hz, 2H), 3.37 (dd, J = 12.9, 6.4 Hz, 2H), 2.66 – 2.45 (m, 4H), 2.45 – 2.33 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 146.26 (s), 144.09 (d, J = 3.0 Hz), 142.46 (d, J = 4.0 Hz), 131.43 (q, J = 36.5 Hz), 121.26 (q, J = 273.2 Hz), 75.01 (s), 35.82 (s), 26.42 (s), 24.34 (s).

2,6-di-3-bromo-5-methoxylpyridinium-9-thiabicyclo[3.3.1]nonane dinitrate (**5i**): ^{1}H NMR (400 MHz, D_2O) δ 9.00 (s, 2H), 8.87 (s, 2H), 8.49 (s, 2H), 5.79 – 5.63 (m, 2H), 3.41 (d, J = 2.6 Hz, 2H), 3.27 (dd, J = 13.0, 6.1 Hz, 2H), 2.49 (td, J = 11.3, 6.4 Hz, 2H), 2.45 – 2.31 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ 159.03, 135.88, 133.04, 131.50, 123.55, 74.47, 57.72, 35.92, 26.48, 24.03.

2,6-di-3- bromo-5-carbamylpyridinium-9-thiabicyclo[3.3.1]nonane ditriflate (**5j**): ^{1}H NMR (500 MHz, D_2O) δ 9.47 (s, 2H), 9.41 (s, 2H), 9.17 (s, 2H), 5.77 (dd, J = 8.4, 4.0 Hz, 2H), 3.37 (s, 2H), 3.19 (dd, J = 13.1, 6.1 Hz, 2H), 2.46 (t, J = 11.3 Hz, 2H), 2.38 (dd, J = 13.2, 6.1 Hz, 2H), 2.34 – 2.25 (m, 2H). ^{13}C NMR (126 MHz, D_2O) δ 164.26, 147.54, 146.69, 141.73, 134.73, 123.69, 74.79, 35.62, 26.07, 23.87.

2,6-di-3,5-dimethoxycarbonylpyridinium-9-thiabicyclo[3.3.1]nonane ditriflate (**5k**): ^{1}H NMR (400 MHz, D_2O) δ 9.70 (s, 4H), 9.50 (s, 2H), 5.89 (d, J = 12.5 Hz, 2H), 3.37 (s, 2H), 3.30 (dd, J = 12.7, 5.2 Hz, 2H), 2.53 – 2.21 (m, 6H).

2,6-di-3-bromo-5-methoxycarbonylpyridinium-9-thiabicyclo[3.3.1]nonane ditriflate (**5l**): ^{1}H NMR (400 MHz, D_2O) δ 9.51 (s, 2H), 9.48 (s, 2H), 9.28 (s, 2H), 5.75 (d, J = 12.1 Hz, 2H), 3.32 (s, 2H), 3.17 (ddd, J = 25.6, 13.2, 6.3 Hz, 2H), 2.51 – 2.18 (m, 6H).



2i

Synthesis of bispyridine linker **2i**: 5-Bromopyridin-3-ol (570 mg, 3.3 mmol, 4 equiv) was dissolved in 5 mL of 1 M NaOH with vigorous stirring for 1 h. Propane-1,3-diyl bis(4-methylbenzenesulfonate) (320 mg, 0.83 mmol, 1 equiv) and tetra-*n*-butylammonium bromide (367 mg 1.14 mmol, 1.3 equiv) in 10 mL toluene were added. The solution was heated to 95 °C overnight. The mixture was cooled, the solvent removed by rotary evaporation, and the product was purified by column chromatography (2:1 hexanes/EtOAc) elution. The desired product, 1,3-bis((5-bromopyridin-3-yl)oxy)propane (**2i**), was obtained as a white powder (216 mg, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 1.6 Hz, 1H), 8.27 (d, J = 2.5 Hz, 1H), 7.41 (s, 1H), 4.23 (t, J = 5.9 Hz, 2H), 2.39 – 2.29 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.99, 143.04, 136.22, 123.70, 120.26, 64.48, 28.71.

Representative procedure for the synthesis of polymers (entries 4-10 in Table 4 and Table 5). Compound **1a** (21 mg, 0.1 mmol, 1 equiv), silver salt (0.2 mmol, 2 equiv) and the bispyridine of interest (0.1 mmol, 1 equiv) were mixed in 0.5 ml MeCN and stirred overnight at room temperature under inert atmosphere. For aqueous soluble polymers (entries 3, 4, 5, and 7 in Table 4; entries 1-3 in Table 5), the precipitate was collected and washed with MeCN. The charged adduct was dissolved in water and any remaining solids were removed by centrifugation, repeating the centrifugation step until the solution was not cloudy. The resulting solution was frozen and lyophilized to obtain the product as a colorless solid. For organic soluble polymers (entries 6, 8, 9, and 10 in Table 4; entry 4 in Table 5), any solid material was filtered out and the resulting solution was added to 10ml CH_2Cl_2 to precipitate the desired product. The material was isolated by filtration and dried to give an off-white solid.

Alternative Strategies for Accelerating the Substitution Chemistry of Compound 1

Different halophilic transition metals were tested for their ability to speed the substitution reactions of **1** by interaction with the leaving group, accelerating the rate-limiting formation of the strained episulfonium intermediate. Representative Al, Sn, and Pd species showed no effect (Table 1). Silver salts were quite effective, prompting the isolation of the resulting intermediates as described below.

Table 1. Survey of halophilic metal complexes for the acceleration of reactions of **1** with $PhCH_2NH_2$ (10 equiv) in acetonitrile at room temperature.

Entry	Halophilic Metal Complex	Reaction Time
1	None	16h
2	AgOTf	2h
3	$AgNO_3$	4h
4	$AlCl_3$	>16h
5	$SnCl_2$	14h
6	$PdCl_2$	12h
7	$Pd(OAc)_2$	14h
8	$Zr(Cp)_2Cl_2$	>16h
9	$Pd(PPh_3)_2Cl_2$	>16h

Comparison of Reactivities between Different WCL Intermediates

Compound **1a** (10.5 mg, 0.05 mmol, 1 equiv) and **1b** (0.2 mL of 0.25 M MeCN solution, 0.05 mmol, 1 equiv) were combined in 1 mL MeCN. The solvent was then evaporated and the residue analyzed by ^{1}H NMR in $CDCl_3$ (Figure 2, spectra A). In parallel, a separate identical [**1a** + **1b**] mixture in MeCN was treated with benzylamine (8.1 µL, 0.1 mmol, 1 equiv) and triethylamine (69.6 µL, 0.5 mmol, 5 equiv, previously shown to be unreactive towards WCL compounds and used to soak up any HCl produced). After 24 hours, the mixture was evaporated, redissolved in $CDCl_3$, and analyzed by ^{1}H NMR (Figure 2, spectra B). The data show almost complete reaction of the amine with the nitrate electrophile rather than the chloride.

An internal competition between all three easy-to-handle electrophiles (chloride, nitrate, iodide; **1a**, **1b**, and **1d**) was performed in a similar manner, in which 0.05 mmol of each was combined in acetonitrile and treated with slightly less than one equivalent of $BnNH_2$ (0.125 mmol) and 5 equiv. (5 mmol) of Et_3N (total volume 1 mL). NMR spectra recorded immediately and after 24 h (in each case, an aliquot was removed, evaporated, and redissolved in CDCl3) are shown in Figure 3. Integration of the α-protons for each electrophile showed nearly equimolar ratios at the start, and a highly skewed distribution after reaction. The relative reactivities thus revealed are iodide > nitrate > chloride.

Monitoring Fragmentation of Representative WCL-Substituted Adducts and WCL-Based Oligocations by ^{1}H NMR

The fragmentation of cationic adducts was followed by ^{1}H NMR in deuterated buffer, with the relative concentration of each species established by integration of unique

resonances. Each adduct in Table 3 and Table 4 was thus examined in three independent runs.

Figures 4A-4B show representative fragmentation data for compound **5d**, with the relative concentration of each species established by integration of unique resonances, labeled in the ^{1}H NMR spectra. The first-order kinetic treatment is shown in Figure 5.

Figure 6 shows representative fragmentation data for polymer **3i**, with the relative concentration of each species established by integration of unique resonances, labeled in the ^{1}H NMR spectra.

GPC Analysis of WCL Polymers Before and After Fragmentation

Three WCL based polymers were dissolved in pure water at 1 mg/mL concentration solution and characterized by aqueous gel permeation chromatography (GPC, black traces in Figures 7A-7C). Separate identical solutions were heated at 37 °C for 36 hours and analyzed again by aqueous GPC (gray traces in Figures 7A-7C). The major peaks between 8 and 10 mins were assigned to polymers; after heating, small molecule components were evident as peaks at longer retention times. Polymer **3a** was confirmed as being a particularly stable molecule, showing no difference in GPC trace after heating. Polymers **3c** and **3i** underwent partial degradation as expected from NMR experiments; interestingly, the remaining polymer peaks shifted modestly but reproducibly to shorter retention time (higher molecular weight, Figures 7B-7C). This could suggest (a) some ligation of oligomers (bearing pyridine nucleophiles at the end) with active episulufonium electrophilic intermediates formed in the decomposition process, and/or (b) the presence of cyclic structures of sufficient size to result in a shift to higher molecular weight upon partial cleavage. Calculation of molecular weight parameters by comparison to dextran standards proved to be nonsensical, so Mn, Mw, and polydispersities could not be assigned by this method.

Thermal Analysis of Representative WCL Polymers

The thermal decomposition temperature of WCL polymers were measured on a Perkin-Elmer Pyris 1 thermogravimetric analyzer (TGA) in a nitrogen atmosphere (flow rate 25 mL/min) with a heating rate of 10°C/min. Thermal transitions of WCL polymer **3a** were measured with a TA Q200 Differential Scanning Calorimeter (DSC) in a nitrogen atmosphere (flow rate 50 mL/min) with a heating/cooling rate of 10°C/min. Each sample was scanned for three cycles and the 2nd cycle is presented in Figure 8B.

Polymer **3a** began extensive decomposition at approximately 220 °C while the decomposition of **3c** and **3i** was initiated at approximately 180-185 °C, with two obvious

stages of mass loss. A glass transition of **3a** was observed at 67 °C, comparable to Tg values previously reported for representative polyionenes.

Cell Culture

All cell culture reagents were acquired from Life Technologies (Carlsbad, CA) unless noted otherwise. HeLa cells were purchased from ATCC (Manassas, VA). GFP-HeLa cells were a gift from the Schmid lab (University of Texas, Southwestern, Dallas, TX). Cells were grown and maintained in complete growth media: Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 5% Fetal bovine serum (FBS), sodium pyruvate (1 mM), penicillin (100 units/mL), streptomycin (100 μg/mL), and GlutaMAX (2 mM). Cells were grown at 37°C under humidified air with 5% CO_2. Glass-bottomed culture dishes were purchased form MatTek (Ashland, MA). Accutase was purchased from Innovative Cell Technologies, Inc. (San Diego, CA). 96-well microtiter plates were purchased from Thermo Fisher (Waltham, MA).

siRNAs were a gift from Integrated DNA Technologies (Coralville, IA), with the following sequences. GFP sense: 5'-CAAGCUGACCCUGAAGUUCUU, GFP antisense: 5'-GAACUUCAGGGUCAGCUUGUU. Approximately 1×10^4 GFP-HeLa cells/well were plated in a 96-well plate in 100 μL of complete growth media, and adhered overnight at 37 °C. For postitive control experiments, 10 nM double-stranded siRNA was transfected with Lipofectamine RNAiMAX according to the manufacturer's protocol. After 24 h, the media was replaced with complete growth media (100 μL/well). Fluorescence was measured 48 h post-transfection. For knockdown experiments with polyplexes, cells were plated as described. 100 μL of oligomer/ds-siRNA complexes at indicated N/P ratios were added to cells. Media was again replenished after 24 h. Fluorescence was measured 48 h post-treatment.

Viability of Mammalian Cells in the Presence of WCL Monomers and Polymers

Approximately 1×10^4 CHO-K1 cells/well were plated in a 96-well plate in 100 μL of complete growth media, and adhered overnight at 37 °C. The media was then replaced by 100 μL of polymer or monomer in serum-free media at the indicated concentration. Full media was replenished after 4 or 24 h. Viability was measured by MTT assay, with non-treated cells assigned as 100% viability. The results are plotted in Figures 11A-11D and 12A-12H.

Results and Discussion

Fundamental Reactivity

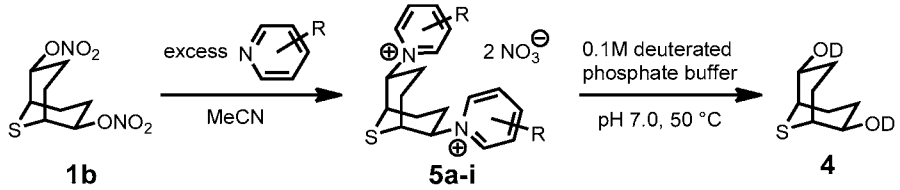
While the substitution chemistry of compound **1a** (Scheme 1, X = Cl) is quite clean, its reaction rate at room temperature (rate constant ≈ 2.4 x 10^{-4} $M^{-1}s^{-1}$) and thermodynamic driving force were thought to be incompatible with the generation of high molecular weight polymers by the simple condensation route outlined in Scheme 1. Accordingly, more reactive forms of **1** were prepared, focusing on the rate-limiting formation of episulfonium ion intermediate. Treatment of **1a** with silver nitrate or silver triflate in acetonitrile afforded **1b** or **1c** in high yield after filtration of AgCl. The triflate **1c** proved to be the most reactive, but may have limited utility under practical conditions because of its high sensitivity to hydrolysis with atmospheric moisture or trace water in the solvent. The nitrate derivative **1b**, in contrast, was stable enough to survive standard isolation, handling, NMR characterization. The apparent Finkelstein reaction of **1a** with sodium iodide in acetone gave **1d** in high yield, which was similarly stable to isolation by filtration and characterization in air. The NMR spectra revealed significant differences between the chemical shifts of the H atoms adjacent to the leaving groups (on C1 and C5) among the different derivatives, suggesting that the order of reactivity would be iodide (**1d**) ≥ nitrate (**1b**) > chloride (**1a**), correcting for the upfield shift characteristic of C-H bonds next to iodine due to factors unrelated to the electron density of the C-halogen bond.

A preliminary assessment of substitution reactivity was obtained by competition reactions in which an equimolar mixture of two electrophiles was treated with half an equivalent of benzylamine (Figure 1). These pairwise comparisons, as well as reaction of all three electrophiles together (Figure 2, Figure 3) confirmed the above order of reactivity, with nitrate approximately 10 times more reactive than chloride, and iodide at least 5 times more reactive than nitrate. These differences are expected to be general since the reaction rate is only weakly dependent on the nature of the capturing nucleophile.

The parent pyridine derivative **5a** was highly stable toward fragmentation in water (less than 5% hydrolysis observed after 1 week at 50°C), so pyridine nucleophiles were chosen to include primarily electron-withdrawing groups to enhance this rate. Such pyridine derivatives are necessarily poorer nucleophiles for the forward step, so the enhanced reactivity of **1b** was important for the efficient synthesis of the corresponding substituted pyridine dicationic adducts, as shown in Table 2. Each adduct dinitrate was isolated, characterized, and heated at 50°C in deuterated buffer; following the disappearance of the adduct by NMR, first-order rate constants of fragmentation were obtained. The NMR

spectra also confirmed the clean nature of the reaction, giving diol **4** and the released pyridines.

Table 2. Comparison of pseudo first order fragmentation of pyridine adducts at 50°C; pK_a refers to the conjugate acid of the pyridine calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2015 ACD/Labs).



1b **5a-i** **4**

Compound	R	pK_a	k_{frag} (h^{-1})	k_{rel}	half life (h)
5a	H	5.23	$< 1 \times 10^{-4}$	-	-
5b	3-CO_2Me	3.19	$(3.03 \pm 0.38) \times 10^{-2}$	1.86	23.2 ± 3.1
5c	4-CO_2Me	3.16	$(3.66 \pm 0.33) \times 10^{-2}$	2.25	19.1 ± 1.8
5d	3-CO_2NH_2	3.54	$(3.18 \pm 0.32) \times 10^{-2}$	1.95	21.7 ± 1.8
5e	4-CO_2NH_2	3.39	$(1.62 \pm 0.10) \times 10^{-2}$	1.00	42.7 ± 2.7
5f	3-Br	2.87	$(6.59 \pm 0.58) \times 10^{-2}$	4.05	9.9 ± 1.1
5g	3-CN	1.78	$(74.8 \pm 5.4) \times 10^{-2}$	46.2	0.9 ± 0.1
5h	3-CF_3	2.8	$(10.8 \pm 0.98) \times 10^{-2}$	6.68	6.4 ± 0.8
5i	3-Br, 5-OMe	2.26	$(7.51 \pm 0.53) \times 10^{-2}$	4.64	9.2 ± 0.8

The stability of pyridine adducts to fragmentation in water was found to roughly correlate with calculated pyridine basicity (Figures 13A-13B). This is consistent with the idea that more nucleophilic pyridine groups are more difficult to eject from the pyridinium adduct structure, and that Brønsted basicity is indicative of nucleophilicity. In practice, half-lives were shown to vary over a 40-fold range (Table 2). Decomposition rates at 37°C, tested for potential applications *in vivo*, were found to be much slower than at 50°C, as expected for a fragmentation reaction. For example, **5c** underwent hydrolysis approximately 23 times faster at 50°C than at 37°C. To tune these potential release rates to a reasonably fast range, two electron-withdrawing groups were used, leaving the *ortho* positions unsubstituted to avoid steric problems in adduct formation (Table 3). Some additional limits were imposed on the process by the observation that pyridines having too many (or too potent) electron-withdrawing substituents were found not to react cleanly with **1b** or **1d**. For example, the strongly electron-deficient 3-bromo-5-amidopyridine (calculated $pK_a = 1.22$) did not form a clean adduct unless reacted with the most reactive ditriflate electrophile, **1c**. The resulting compound **5j** underwent relatively fast

fragmentation (half life less than a day), as expected. Carboxylate-based electron-withdrawing groups provide for easy linkage of pyridine units together to make higher-valent monomers for the synthesis of fragmentable polymers and crosslinked materials.

Table 3. Comparison of pseudo first order fragmentation of pyridine adducts at 37°C.

Compound	R	pK_a	k_{frag} (h^{-1})	k_{rel}	half life (h)
5c	4-CO_2Me	3.16	$(0.16 \pm 0.01) \times 10^{-2}$	0.3	422±41
5g	3-CN	1.78	$(5.87 \pm 0.71) \times 10^{-2}$	11	11.8±1.9
5h	3-CF_3	2.8	$(1.14 \pm 0.16) \times 10^{-2}$	2.1	61±8
5i	3-Br, 5-OMe	2.26	$(0.55 \pm 0.04) \times 10^{-2}$	1	127±10
5j	3-Br, 5-CO_2NH_2	1.22	$(9.34 \pm 0.87) \times 10^{-2}$	17	7.4±0.5
5k	3-Br, 5-CO_2Me	0.84	$(11.9 \pm 1.13) \times 10^{-2}$	22	0.24±0.01
5l	3-CO_2Me, 5-CO_2Me	1.16	$(10.9 \pm 1.81) \times 10^{-2}$	20	0.27±0.01

Oligo- and Polycation Synthesis

The condensation polymerization of a dipyridine nucleophile and WCL electrophile was explored using the parent compound **2a** (Scheme 1, Table 4). Reaction of **1a** and **2a** in MeCN solvent at 50°C, conditions that produce good yields of adducts with pyridine itself, give only small oligomers (average n = 3). In contrast, dinitrate **1b**, generated *in situ* from **1a** and $AgNO_3$, gave copious quantities of precipitate within two minutes of mixing with an equimolar amount of **2a**, all in MeCN. Filtration and washing provided the corresponding species **3a**•$(NO_3)_{2n}$. Characterization of this material was aided by its high solubility in water and by the fact that the second substitution event on each WCL core is usually faster than the first, so that chains are capped by the pyridine nucleophile. ^1H NMR in D_2O therefore showed distinct residues for non-alkylated pyridines at each end, enabling an estimate of average chain length of approximately n=10 for **3a** prepared under these conditions (molecular weight approximately 4000). Gel permeation chromatography (GPC) analysis also showed minor fractions with longer retention time, consistent with shorter chains or cyclic oligomers. Somewhat higher molecular weights were obtained with heating to 50°C (n ≈ 13), or with the use solvents better able to support the developing polycation and therefore to delay precipitation until longer chain lengths are achieved (Table 3). In a brief survey of activating silver salts and solvents, the use of $AgPF_6$ in DMSO was found to give the largest average molecular weights as determined by NMR end-group analysis (Table 4); polydispersities could not be assessed because of the lack of suitable GPC standards (values obtained against dextran standards in water were wildly

divergent from estimates made by NMR) and because m/z values for molecules of different chain lengths are close enough to make deconvolution of the mass spectra of these mixtures difficult. Values of hydrodynamic radii determined by dynamic light scattering (Table 6) were comparable with those of ammonium ionenes with similar degrees of polymerization. Analysis by TGA showed representative oligomers to have the anticipated stability (decomposition initiation at approximately 200°C), and DSC revealed discrete glass transitions to be rare, both consistent with materials that are potentially dynamic at higher temperatures. Note that the WCL electrophiles are used here in racemic form, making for polymeric structures that are likely mixtures of many diastereomers.

Table 4. Condensation polymerization of **1a** + **2a**.

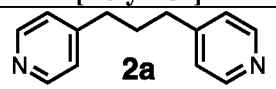
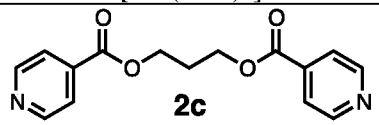
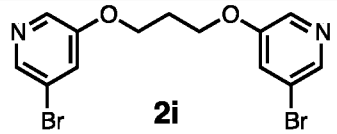
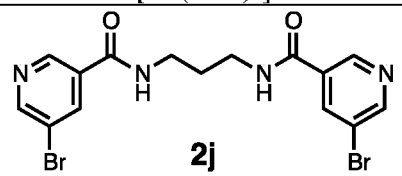
Entry	[1a] = [2a] (M)	Additive (2 equiv)	Solvent	Temperature	M_n (kDa)	n[a]
1	0.1	None	MeCN	50 °C	1.5	3
2	0.1	None	THF/H_2O	RT	--	--
3	0.1	$AgNO_3$	MeCN	RT	4-5	8-10
4	0.1	$AgNO_3$	MeCN	50 °C	6	13
5	0.1	$AgNO_3$	THF/H_2O	RT	--	--
6	0.1	$AgBF_4$	DMF	RT	5.3	10
7	0.2	$AgNO_3$	DMSO	RT	9	18
8	0.2	AgOTf	DMSO	RT	11.5	23
9	0.4	$AgPF_6$	DMSO	RT	22.2	35
10	0.2	$AgBF_4$	DMSO	RT	11.4	22

(a) n = average degree of polymerization

Using the conditions of Table 4, three additional oligocations were prepared from the bis(pyridine) linkers as shown in Table 5. The synthetic procedure proved to be modular, giving oligomers of similar size ($n \approx 10$) with the same methods of isolation and analysis. The stabilities of these compounds toward fragmentation under aqueous conditions were assessed by NMR in deuterated buffer, following the disappearance of the thiabicyclononane C-H resonance adjacent to the pyridine group (position *g* in Figure 14) and the appearance of the corresponding resonance (4.3 ppm) for the diol product **4** (Figure 6). The observed half lives of the WCL-pyridine bonds in these oligomers corresponded reasonably well (within a factor of 2) with the analogous small-molecule bis(pyridine) adducts described above, ranging from several hours to a month at physiological

temperature and pH. Note of course that the oligomer structures decompose much faster than this, since the rupture of only a fraction of the bonds (occurring at approximately the same first-order rate at each position of the chain) would be necessary to chop the linear structure into shorter pieces. Indeed, these materials are also very likely to be dynamic in nature, since breakage of a C-N(pyridyl) bond is probably followed by re-formation of that same bond more often than by irreversible capture of the intermediate episulfonium ion with water.

Table 5. Representative oligocations and half lives ($t_{1/2}$) of C-N bond fragmentation in aqueous buffer (pH 7).

Nucleophilic Monomer [Polymer]	**n** [a]	**T** [b]	$t_{1/2}$	$t_{1/2}$ **of Small Molecule Analogue**
 **2a** [**3a**•$(NO_3)_x$]	10	37 °C	n.d.	n.d.
		50 °C	n.d.	n.d.
 **2c** [**3c**•$(NO_3)_x$]	10	37 °C	624 h	422 h (**5c**)
		50 °C	36 h	19 h (**5c**)
 **2i** [**3i**•$(NO_3)_x$]	6	37 °C	70 h	127 h at 37 °C (**5i**)
 **2j** [**3j**•$(OTf)_x$]	6	37 °C	9.2 h	5.8 h (**5j**)

(a) n = average degree of polymerization. (b) Temperature at which polymer decomposition was measured in aqueous buffer. Polymer nitrates (**3a**, **3c**, **3i**) were prepared as in Table 4, entry 3; the triflate **3j** was prepared as in Table 4, entry 8. "n.d." = no decomposition observed by NMR (< 5%) after 1 week at the indicated temperature.

Table 6. Characterization of polymer/polyplexes formed from the indicated polycations (1 mg/ml in PBS) or polycations and double-stranded plasmid DNA (pcDNA3-EGFP, 6.2 kb, 20 ng/μL) at the indicated N/P ratio. Radius and polydispersity were determined by dynamic light scattering. "pd" = polydispersity; "n/o" = no aggregates were observed.

Entry	Cationic Oligomer	N/P	Conditions	radius (nm)	pd (%)
1	**3a**•$(NO_3)_n$	2	H_2O, RT, freshly prepared	82 ± 2	19.7
2	**3a**•$(NO_3)_n$	2	Entry 1, heated at 37 °C for 48 h	80 ± 3	16.5
3	**3a**•$(NO_3)_n$	2	PBS buffer, RT, freshly prepared	147 ± 9	15.5
4	**3i**•$(NO_3)_n$	2	H_2O, RT, freshly prepared	116 ± 6	30.5
5	**3i**•$(NO_3)_n$	2	Entry 4, heated at 37 °C for 48 h	n/o	n/o
6	**3i**•$(NO_3)_n$	2	PBS buffer, RT, freshly prepared	210±18	37.8
7	**5a**•$(NO_3)_2$	150	H_2O or PBS	n/o	n/o
8	none	--	Plasmid DNA alone	n/o	n/o
9	**3a**•$(NO_3)_n$	--	PBS buffer, RT, no DNA	31 ± 2	17.6
10	**3c**•$(NO_3)_n$	--	PBS buffer, RT, no DNA	56± 4	21.4
11	**3i**•$(NO_3)_n$	--	PBS buffer, RT, no DNA	49± 3	12.4
12	**3i**•$(NO_3)_n$	--	Entry 11, heated at 50 °C for 4 h	n/o	n/o

Binding and Transfection of DNA and siRNA

Binding of WCL oligocations to DNA was examined by agarose gel shift assay; a representative example is shown in Figure 15A. Polyplex formation at a level sufficient to completely inhibit the electrophoretic migration of a 6.2-kb double-stranded plasmid was observed with polymer **3a** at a low N/P ratio of approximately 1.0. A similar result was observed for **3i**, but polymer **3c** was less efficient, showing significant but incomplete retardation of electrophoresis at N/P = 50. Dynamic light scattering analysis showed the resulting polyplexes to be of reasonable size and narrow size distribution (Table 6), with larger structures formed in PBS buffer *vs.* water. The presence of small-molecule adduct **5a** in large excess had no effect on the electrophoresis of DNA nor was an observable polyplex formed. The polyplex formed with **3a** resisted heating at 37°C for 48 hours, whereas particles formed with **3i** were decomposed after 24 hours of such treatment (Table 6, entries

2,5), corresponding to the fragmentation stabilities observed for the polycations alone (Table 5).

The efficiency of both stable and fragmentable oligomers for the transfection of siRNA into HeLa cells was surveyed as shown in Figures 16A-16B. Much higher N/P ratios were required for siRNA transfection than for DNA complexation, and were constrained by the cytotoxicity of these new materials.

As shown in Figures 11A-11C, polymers **3a**, **3c**, and **3i** engendered significant toxicity after 24 hours exposure at 2 μM, 200 μM and 12 μM respectively (concentrations refer to that of the chains, not the monomeric units, calculated based on average molecular weights established by NMR); toxicities were much lower for 4 h exposure. The milder toxicity of **3c** could be due to ester cleavage in the cell or culture media. Polymers **3a** and **3c** should not fragment significantly by anchimeric substitution during 24 h incubation at 37°C; **3i** may experience significant fragmentation over that time, since C-N bond breakage should occur to approximately 21% after 24 h. In any event, the dipyridine monomers from which these polymers are constructed (**2a**, **2c**, and **2i**) were found to be nontoxic at concentrations below 500 μM, and the other decomposition product (diol **4**) had no effect on cellular viability up to 1 mM. Interestingly, the dicationic monomeric adducts **5a**, **5c**, and **5i** were much less toxic than their corresponding polymers (no effect up to 500 μM or greater), and a longer polymer (**3a'**, approximately 18 repeat units; Table 4, entry 7) was significantly more toxic than **3a** (approximately 10 repeat units) at 4 h exposure. These observations suggest that cytotoxicity is associated with the polymeric nature of these materials and therefore results from interactions with the cell membrane, rather than being a function of DNA alkylation.

At optimal N/P ratios of 55-65, polymer **3a** was able to mediate transfection with efficiencies near that of a standard commercial reagent (Lipofectamine, RNAiMAX) with similarly modest cytotoxicity (Figure 16A), but these materials are, as discussed above, more toxic overall toward cultured cells. Polymer **3i** exhibited no separation between cellular toxicity and apparent siRNA knockdown (Figure 16B). Preliminary show that transfection and expression of plasmid DNA in HeLa cells can also be mediated by polymer **3a**.

Conclusions

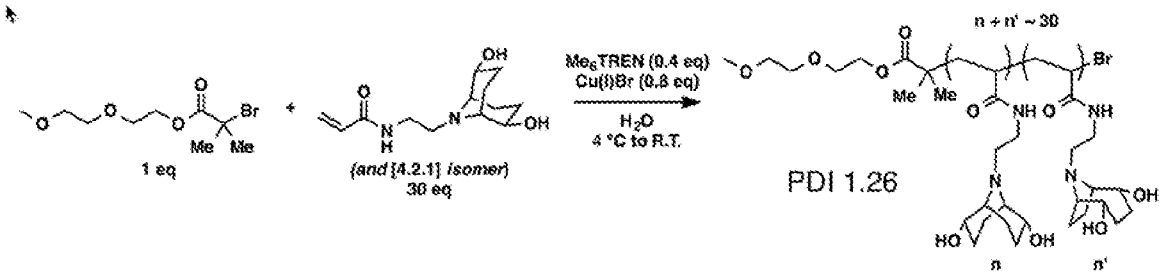
A new class of fragmentable polycations has been developed through facile nucleophilic substitution of WCL scaffolds by divalent pyridines. The efficiency of this process depends on the leaving group ability of the group being substituted, consistent with

rate-limiting activation of the electrophile by formation of a highly reactive episulfonium ion. Degrees of polymerization from approximately 3 to 30 were observed for these condensation polymerization processes. A range of polymer stabilities could be engineered by tuning the nucleophilicity and leaving group ability of the pyridine units, with pyridine basicity serving as a reasonably good guide to these properties. The resulting polymers were highly efficient in complexing DNA and showed functional behavior (transfection and cytotoxicity) associated with some other classes of polycationic materials.

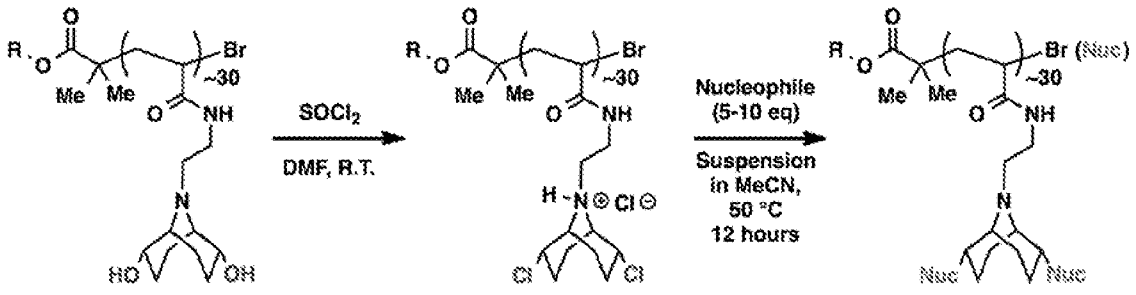
Example 2: Preparation of Polycations Bearing Cationic Side Chains Formed by the Condensation of 9-azabicyclo[3.3.1]nonyl Electrophiles with Nucleophiles

Polycationic polymers bearing cationic side chains formed by the condensation of a 9-azabicyclo[3.3.1]nonyl electrophile with nucleophiles were prepared using the synthetic methodology outlined in Schemes 2 and 3.

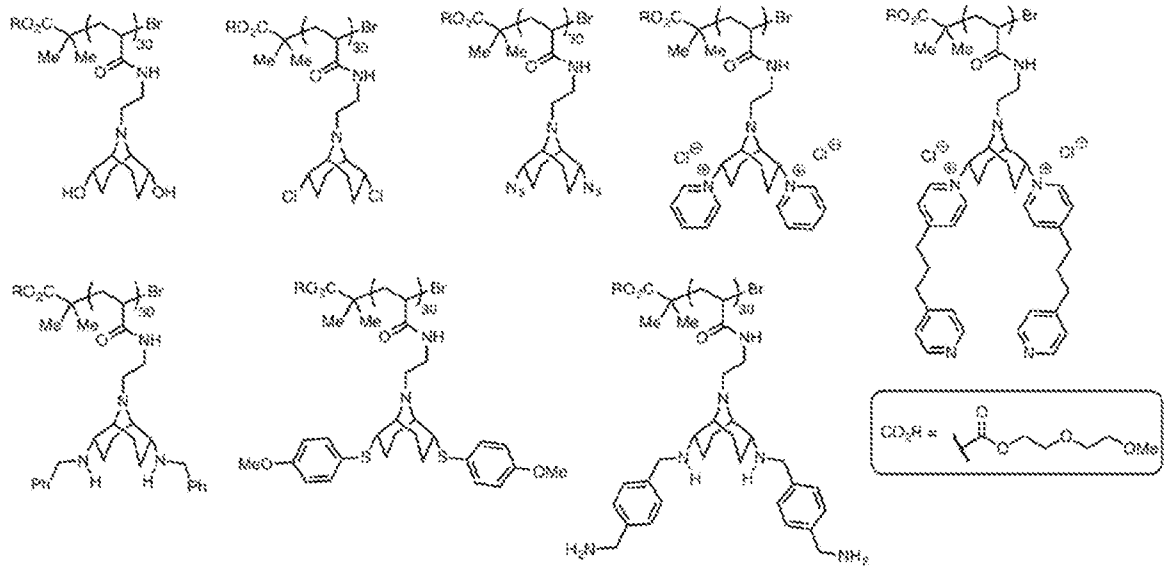
Scheme 2. Synthetic methodology used to prepare precursor polymers bearing 9-azabicyclo[3.3.1]nonyl diol sidechains.



Scheme 3. Preparation polycationic polymers from precursor polymers bearing 9-azabicyclo[3.3.1]nonyl diol sidechains.



Using the synthetic methodology detailed in schemes 2 and 3, the polymers below were successfully prepared.



Example 3: Cytotoxicity Activity of Polycationic Materials

As detailed in Example 1, condensation of 9-thia/aza/selenabicyclo[3.3.1]nonyl electrophiles with polynucleophiles, with each bond-forming event creating a positively charged center, provides access to a wide range of polycationic materials. These materials are subject to fragmentation by the reverse process, in which the internal nucleophile ejects a heterocyclic nucleophile as a leaving group. In the case of pyridines, fragmentation rates can be efficiently tuned from hours to years at 37°C.

The cytotoxic function of these polycations was evaluated by two methods:

Method A

Bacteria suspensions (*E. coli* and *B. subtilis* ATCC 8037) were grown in Mueller-Hinton Broth (MHB) overnight at 37° C. The resulting culture was used to inoculate a second culture in 2 mL of MHB medium until an optical density of 0.8 at 600 nm was reached. The suspension was diluted with fresh MHB to an optical density of 0.001 (approximately) at 600 nm (OD_{600}). This suspension was mixed with different concentrations of freshly prepared polymer solutions in TRIS saline (pH 7.0) in a 96-well plate, and incubated overnight at 37 °C. The OD_{600} was measured for bacteria suspensions that were incubated in the presence of polymer solution or only buffer solutions as controls. Antibacterial activity was expressed as minimal inhibitory concentration (MIC), the concentration at which more than 90% inhibition of growth was observed. All experiments were run in triplicate.

Method B

The bacteria were collected by centrifugation at 4,000 x g for 3 min at 4 °C, washed with sterile PBS (pH 7.4) and finally suspended in PBS to get a final concentration of 6 X 10^6 cells mL^{-1}. The polymer solution was added into the bacteria suspension and shaken for 0.5-4 hours. Then 25 μL of suspension was spread onto a sterile Petri dishes covered with a layer of LB medium containing 0.8% agar (previously autoclaved, and cooled to 37 °C). After overnight incubation at 37°C, bacterial colonies became visible and were counted and compared with the untreated bacteria plate. The MIC was defined as the minimum concentration in the diluted series when the CFU number on the agar plate reached no more than 10% of the control plate.

As shown in Figure 16, the polycations exhibited extraordinary cytotoxic activity, with *E. coli* MIC values for the polymers in solution at or below 0.1 μg/mL. These polycations are more than two orders of magnitude more potent than standard antibacterial polymers or compounds. Measurements conducted against Gram-positive *B. subtilis* gave similar results. Polycations employing the same type of positive charge carrier arranged on the side chains of acrylate monomers (as opposed to within the polymer backbone) were much less active, even though the charge density per molecular weight is nearly identical. Furthermore, the polycations were less toxic toward a representative mammalian cell line by 100-fold or more.

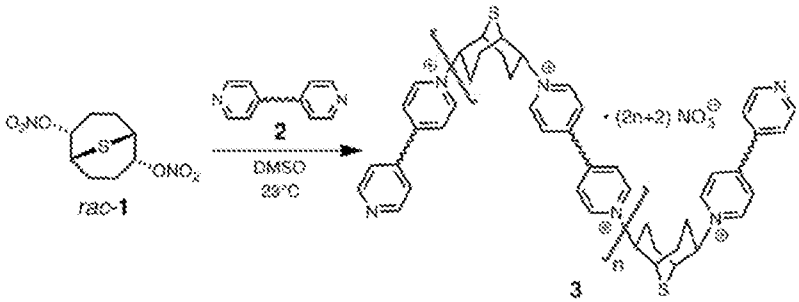
Members of this class are also efficient at binding polynucleotides, forming discrete polyplexes at 1:1 positive:negative charge ratios, which are far lower than most polycationic transfection agents. Monomeric adducts analogous to some of the active polymers were also toxic, but significantly less so.

Example 4: Covalent Attachment of Polycations to Glass Substrates

Remarkable levels of bacterial cytotoxicity were also observed when the polycations described above were covalently attached to the surface of substrates. In this example, the polycations were covalently attached to a glass substrate. The strategy used to immobilize the polycations on a glass substrate are schematically illustrated in Figure 20.

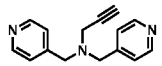
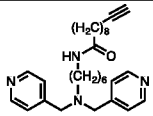
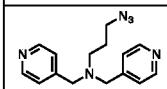
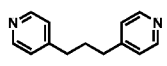
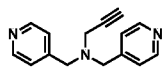
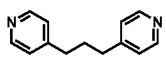
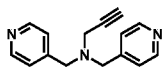
To facilitate covalent attachment to the glass substrate, polycations bearing alkynyl moieties were prepared. These polycations could then participate in click reactions with azide moieties introduced onto the glass substrate, thereby covalently bonding the polycation to the glass substrate. Polycations bearing alkynyl moieties were prepare using the synthetic methodology outlined in Scheme 4 below.

Scheme 2. Synthetic methodology used to prepare precursor polymers bearing 9-azabicyclo[3.3.1]nonyl diol sidechains.



Briefly, the cyclic bis-electrophile 1 (21 mg, 0.1 mmol, 1 equiv), silver nitrate (34 mg, 0.2 mmol, 2 equiv) and a bispyridine dinucleophile of interest (0.1 mmol, 1 equiv) were mixed in 0.5 mL DMSO and stirred overnight at room temperature under inert atmosphere. After centrifugation, any solid material was filtered out and the resulting solution was added to 15 mL CH_2Cl_2 or toluene to precipitate the desired product. The material was isolated by filtration, washed with CH_2Cl_2 and dried to give a yellow sticky solid. The structure of polycations bearing alkynyl moieties prepared using this methodology, along with preliminary characterization data, are include

Table 7. Examples of polycations bearing alkynyl moieties prepared using the methodology outlined in Scheme 2 (but with varying nucleophilic monomers.

nucleophile monomer	Polymer	n[a]	Mn (Da)	R_h[b] (nm)	nucleophile monomer	Polymer	n[a]	Mn (Da)	R_h[b] (nm)
 **2b**	3b	12	6200	77	 **2c**	3c	8	5800	-
 **2d**	3d	10	5500	35		3i	18	8800	151
 **2a** (1 equiv)  **2b** (9 equiv)	3k	18	8400	-	 **2a** (6 equiv)  **2b** (4 equiv)	3l	18	8600	-

One prepared, the polycations were covalently attached to the surface of glass cover slips using the procedures detailed below. First, the glass cover slips were rendered amenable to click chemistry modification by initial attachment of azide groups via standard siloxide coating methods. Briefly, 1 cm x 1cm silicon or glass chips or glass beads were cleaned with piranha solution for 30 min, sonicated with deionized water for another 30 min, rinsed with methanol and dried with N_2. The resulting clean substrate was immersed in a solution of (3-azidopropyl) triethoxysilane in toluene (20 mM) for 12 hours. The glass or silicon substrate was removed from the reaction mixture, washed with methanol, and dried.

Subsequently, the azide-functionalized slips were reacted with a polycation bearing alkyne groups in a Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. Briefly, the azide-functionalized glass was immersed in a solution of the polycation of interest (1mg/mL in 80/20 water/t-BuOH mixture) containing copper sulfate (5% equivalents with respect to alkyne), sodium ascorbate (10 mM) and THPTA ligand (1 equivalent with respect to Cu) for 12 hours. After the reaction solution was away from the material, the surface was further cleaned by immersion in a 1mM solution of cysteine to remove metal ions that may be bound to the surface, followed by washing with methanol and drying.

The cytotoxicity of the polycations deposited on the glass cover slips was then evaluated. Briefly, the bacteria were collected by centrifugation at 4,000 x g for 3 min at 4 °C, washed with sterile PBS (pH 7.4) and finally suspended in PBS to get a final concentration of 6×10^6 cells mL^{-1}. The glass/silicon substrate (0.01-0.04 cm^2 in area) was added into the bacteria suspension and shaked for 0.5-4 hours. Then 25ul of suspension was spread onto a sterile Petri dishes covered with a layer of LB medium containing 0.8% agar (previously autoclaved, and cooled to 37 °C). After overnight incubation at 37°C, bacterial colonies became visible and were counted and compared with the untreated bacteria plate. The MIC was defined as the minimum concentration in the diluted series when the CFU number on the agar plate reached no more than 10% of the control plate.

The results are shown in Figure 17. Polycation-functionalized glass cover slips, approximately 0.01 cm^2 in area, were found to abolish (*E. coli*) or significantly inhibit (*P. aeruginosa*) the ability of bacteria to grow on agar plates after exposure. If dispersed in solution, the overall concentration of the polycations would have been approximately 0.01-0.3 μg/mL.).

As a control, azide-functionalized glass cover slips were similarly reacted with a small quaternary alkyne (*N,N,N*-trimethylprop-2-yn-1-ammonium bromide). These control

slips were not effective at reducing bacterial growth, demonstrating that that cytotoxicity resulted from the attached polycationic polymer.

To confirm that cytotoxicity did not result from leaching of the polycation into the culture, the polycation-functionalized glass cover slip was vigorously washed before being immersed in the bacterial culture. The pre-washed polycation-functionalized glass cover slip still deactivated the bacterial culture, suggesting that leaching is not a major component of cytotoxic activity.

Example 5. Covalent Attachment of Polycations to Polyvinyl Chloride (PVC) Substrates

Numerous conditions, including ventilator-associated pneumonia and catheter-related urinary tract infections, are linked to the formation of biofilms and colonization of bacteria on medical tubing, which then leads to a bacterial infection. These conditions are associated with high mortality rates, as well as increased hospital stay lengths and medical care costs. Currently, using silver-coated tubing is the main effort to minimize these infections in intubated patients, however, this is not FDA approved for pediatric use.

To address these needs, the covalent linkage of anti-microbial agents to existing medical tubing to reduce/eliminate the growth of pathogenic bacteria was investigated. Covidien Mallinckrodt cuffless endotracheal tubing (4.00 mm ID; PVC) was used as a substrate for proof of principle studies. Anti-microbial agents were covalently attached to the surface of the medical tubing. The functionalized materials was then be tested for bacterial adhesion using hospital-relevant strains: *P. aeruginosa*, *S. aureus*, *E. coli*. In this way, different modifications of the substrate could be screened in a time-efficient manner to develop alternative strategies for minimizing biofilm and colonization of bacteria and fungi on medical tubing. These strategies are schematically illustrated in Figure 18.

In a first example, the PVC of an example segment of medical tubing was functionalized with example polycations using a CuAAC) reaction. In the first step, one or more PVC surfaces of the medical tubing (the interior surface, the exterior surface, or both the interior surface and the exterior surface) were derivatized with azide. Subsequently, the azides on the derivatized PVC were reacted with alkynes present in the polycationic polymer in an azide-alkyne cycloaddition reaction to covalently attach the polycationic polymer to the PVC surface.

Strategies used to immobilize the polycations described herein on a PVC substrate (in this case PVC tubing) are outlined in Figure 21.

To introduce azide moieties onto the surface of the PVC, a phase-transfer catalyst was used to facilitate the substitution reaction of chloride for azide on the solvent-exposed surface. Briefly, initial studies were performed using commercial plain endotracheal tubing (ET) (Covidien Mallinckrodt Oral/Nasal Tracheal Tube Cuffless, 4.0 mm inner diameter, 5.6 mm outer diameter) and catheter tubing (CT) (GentleCath Intermittent Urinary Catheter, Size 14 Male Nelaton 16") purchased from the indicated vendors. Test reactions were performed as follows.

Small small pieces of clear, cut tubing (approximately 0.5 x 0.5 cm) were placed in 4 mL of an aqueous solution of 450 mM NaN_3 and 9 mM phase transfer catalyst in sealed vials. Each mixture was heated to 60°C, 80°C, or 100°C for periods from 12 to 48 hours while being shaken vigorously on an orbital shaker. The samples were then placed in water and sonicated for 1 hour to rinse, changing the water at 30 minutes, and then dried in an oven at 60 °C for 4-8 hours. Using this methodology, the amount of azide introduced onto the surface of the PVC tubing could be readily controlled without leaching plasticizers or changing the bulk mechanical properties of the PVC.

Subsequently, anti-bacterial and anti-fungal molecules were covalently attached to the PVC. In these proof of principle studies, polycationic polymers based on cationic centers formed by the reaction of a 9-thia/aza/selenabicyclo[3.3.1]nonyl electrophile with a nucleophile. Each bond-forming event generates a positive charge. Figure 19 illustrates examples of polycationic polymers that can be prepared through the reaction of a 9-thia/aza/selenabicyclo[3.3.1]nonyl electrophile with a nucleophile. Type I materials are prepared through the condensation of 9-thia/aza/selenabicyclo[3.3.1]nonyl dielectrophiles with polynucleophiles (e.g., dinucleophiles). Each bond-forming event in this condensation polymerization process generates a positively charged center within the polymer backbone. Type II materials contain cationic sidechains. Type II materials can be formed, for example, by polymerization of monomers (e.g., acrylate monomers) containing pendant 9-azabicyclo[3.3.1]nonyl electrophiles. Following polymerization, the pendant 9-azabicyclo[3.3.1]nonyl electrophiles can be reacted with nucleophiles to introduce positively charged centers within the polymer sidechains. If desired, conventional anti-microbial compounds (e.g., antibiotics) can be used in place of (or in addition to) these polycationic polymers.

In a second example, the PVC was derivatized with alternative functional groups (other than azides) that could subsequently be reacted with anti-bacterial and/or anti-fungal molecules. Other moieties that can serve as reactive functional groups to facilitate surface functionalization include, for example, thiolate, thiocyanate (SCN^-), hydroxide, iodide, and

nitrile groups. In a second example, the PVC was cyano-functionalized. Briefly, small pieces of clear, cut tubing (approximately 0.5 x 0.5 cm) were placed in 4 mL of an aqueous solution of 450 mM NaCN and 9 mM phase transfer catalyst in sealed vials. Each mixture was heated to 60°C, 80°C, or 100°C for periods from 12 to 48 hours while being shaken vigorously on an orbital shaker. The samples were then placed in water and sonicated for 1 hour to rinse, changing the water at 30 minutes, and then dried in an oven at 60 °C for 4-8 hours.

Subsequently, the nitrile groups on the derivatized PVC were reacted sodium azide to produce 1*H*-tetrazoles. Briefly, the cyanated PVC samples were immersed in an aqueous solution of sodium azide (485 mM) zinc bromide (440 mM) at 100 °C for 72 h to form 5-substituted 1*H*-tetrazoles on the material surface. These surfaces were then alkylated with an antimicrobial compound or with a compound containing an azide or alkyne group to which subsequent connection can be made by azide-alkyne cycloaddition. By way of example, the samples were further immersed in a solution of 360 mM potassium carbonate and 48 mM of the electrophile 2,3,4,5,6-pentafluorobenzyl bromide, and shaken at 80 °C for 12 hours. Alternatively, nitrile groups on the derivatized PVC could be reacted with polycationic polymers bearing hydrazone moieties (which condense with the nitrile groups to form 1,2,4-triazoles).

The compositions, devices, systems, and methods of the appended claims are not limited in scope by the specific compositions, devices, systems, and methods described herein, which are intended as illustrations of a few aspects of the claims. Any compositions, devices, systems, and methods that are functionally equivalent are intended to fall within the scope of the claims. Various modifications of the compositions, devices, systems, and methods in addition to those shown and described herein are intended to fall within the scope of the appended claims. Further, while only certain representative compositions, devices, systems, and method steps disclosed herein are specifically described, other combinations of the compositions, devices, systems, and method steps also are intended to fall within the scope of the appended claims, even if not specifically recited. Thus, a combination of steps, elements, components, or constituents may be explicitly mentioned herein or less, however, other combinations of steps, elements, components, and constituents are included, even though not explicitly stated.

The term "comprising" and variations thereof as used herein is used synonymously with the term "including" and variations thereof and are open, non-limiting terms. Although

the terms "comprising" and "including" have been used herein to describe various embodiments, the terms "consisting essentially of" and "consisting of" can be used in place of "comprising" and "including" to provide for more specific embodiments of the invention and are also disclosed. Other than where noted, all numbers expressing geometries, dimensions, and so forth used in the specification and claims are to be understood at the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, to be construed in light of the number of significant digits and ordinary rounding approaches.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

WHAT IS CLAIMED IS:

1. A process for rendering a polyvinyl chloride surface antimicrobial, the process comprising:

(a) contacting the polyvinyl chloride with an azidation reagent in the presence of a phase transfer catalyst to form an azide-substituted polyvinyl chloride; and

(b) contacting the azide-substituted polyvinyl chloride with an antimicrobial agent comprising an alkyne moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

2. The process of claim 1, wherein step (a) comprises flowing an aqueous solution comprising the azidation reagent and the phase transfer catalyst across the polyvinyl chloride surface.

3. The process of any of claims 1-2, wherein step (a) comprises contacting the polyvinyl chloride with the azidation reagent and the phase transfer catalyst for 3 hours or less.

4. The process of any of claims 1-3, wherein the alkyne moiety is activated by ring strain, electron withdrawing groups, or a combination thereof.

5. The process of any of claims 1-4, wherein step (b) comprises contacting the azide-substituted polyvinyl chloride with the antimicrobial agent comprising the alkyne moiety in the presence of a Cu(I) catalyst.

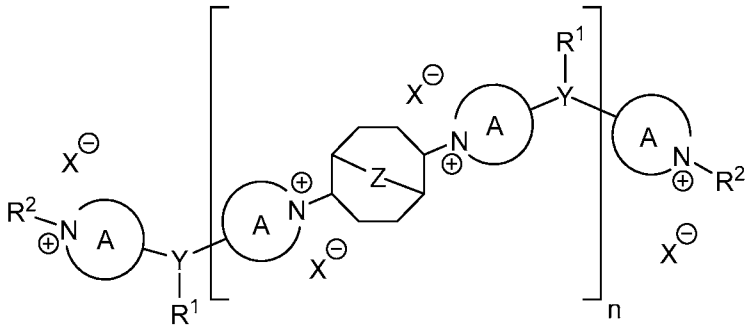
6. The process of any of claims 1-5, wherein the polyvinyl chloride surface comprises a surface of a medical article.

7. The process of claim 6, wherein the medical article comprises tubing.

8. The process of claim 7, wherein the surface of the medical article comprises an interior surface of the tubing, an exterior surface of the tubing, or both an interior surface of the tubing and an exterior surface of the tubing.

9. The process of any of claims 1-8, wherein the antimicrobial agent comprises a polycationic polymer.

10. The process of claim 9, wherein the polycationic polymer comprises a polymer defined by Formula I below



Formula I

wherein

Z represents, individually for each occurrence, S, Se, or NR^3;

A represents, individually for each occurrence, a heterocyclic ring comprising a cationic nitrogen center;

X represents, individually for each occurrence, an anion;

Y is absent, or represents, individually for each occurrence, a linking group;

R^1 is absent, or represents, individually for each occurrence, halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy;

R^2 is absent, or represents, individually for each occurrence, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy;

R^3 represents, individually for each occurrence, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy; and

n is an integer from 2 to 400,

wherein at least one of R^1, R^2, or R^3 comprises an alkynyl group.

11. The process of claim 10, A is chosen from pyridine, imidazole, pyrazole, triazole, tetrazole, oxazole, isoxazole, furazan, isothioazole, and thiazole.

12. The process of any of claims 10-11, wherein Z is S.

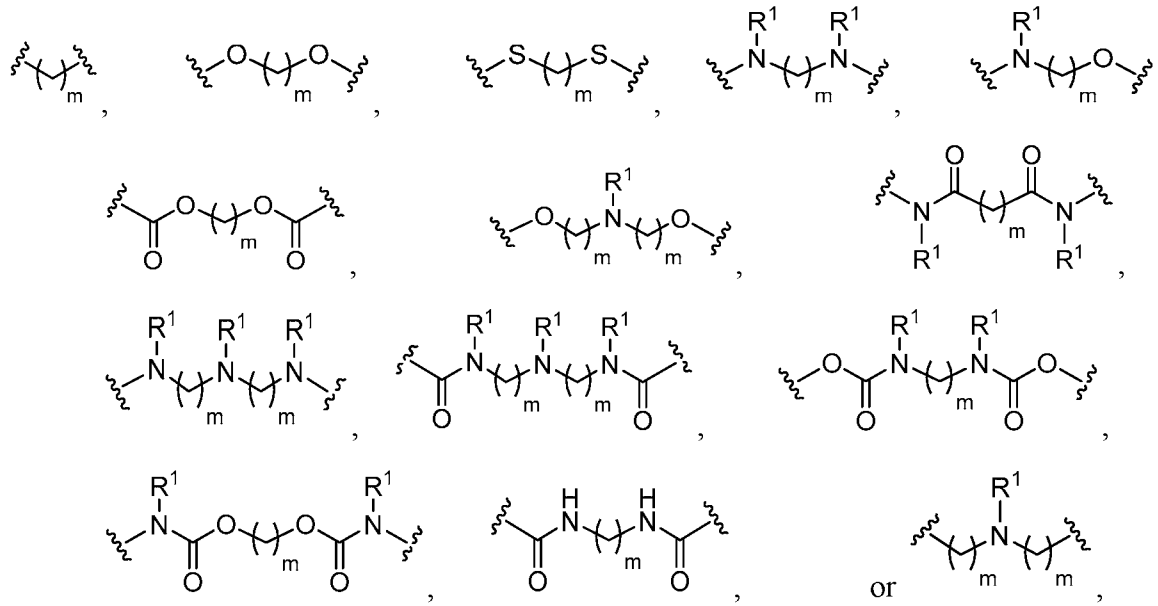
13. The process of any of claims 10-11, wherein Z is NR^3.

14. The process of any of claims 10-13, wherein R^1, R^2, R^3, or a combination thereof comprise a cationic moiety.

15. The process of any of claims 10-14, wherein R^1 comprise an alkynyl group.

16. The process of any of claims 10-15, wherein R^2 comprises an alkynyl group.

17. The process of any of claims 10-16, wherein Y is chosen from one of the following:

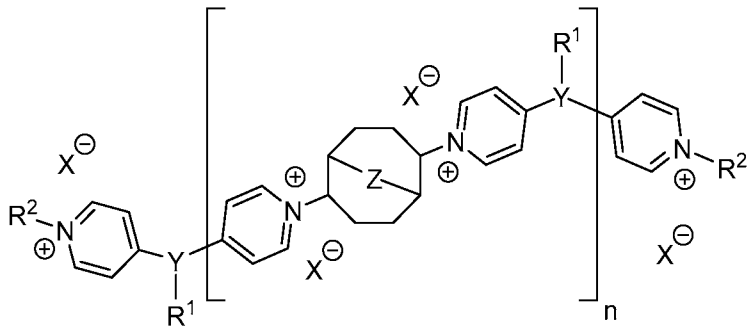


where m is an integer from 1 to 12.

18. The process of any of claims 10-17, where X is chosen from chloride, bromide, iodide, nitrate, sulfate, triflate, borate, and phosphate.

19. The process of any of claims 10-18, wherein n is from 10 to 50.

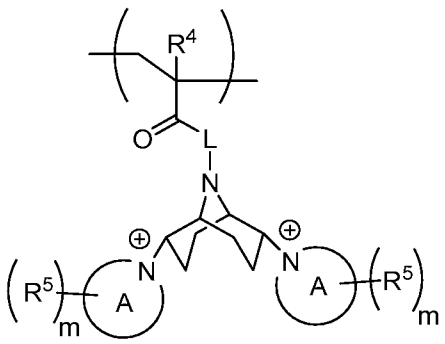
20. The process of any of claims 10-19, wherein the polycationic polymer comprises a polymer defined by Formula IA below



Formula IA

wherein Z, X, Y, R^1, R^2, R^3, and n are as defined above with respect to Formula I.

21. The process of claim 9, wherein the polycationic polymer comprises a recurring unit defined by Formula V below



Formula V

wherein

R^4 represents H or methyl;

L is absent or represents a linking group;

A represents, individually for each occurrence, a heterocyclic ring comprising a cationic nitrogen center;

R^5 is absent, or represents, individually for each occurrence, halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy; and

m is an integer from 0 to 5.

22. The process of claim 21, wherein the polycationic polymer further comprises a recurring unit derived from the polymerization of one or more ethylenically-unsaturated monomers.

23. The process of claim 22, wherein the one or more ethylenically-unsaturated monomers are chosen from the group consisting of styrene, butadiene, meth(acrylate) monomers, vinyl acetate, vinyl ester monomers and combinations thereof.

24. The process of any of claims 1-23, wherein the phase transfer catalyst comprises a quaternary ammonium salt.

25. The process of any of claims 1-24, wherein the azidation reagent comprises NaN_3.

26. A process for rendering a polyvinyl chloride surface antimicrobial, the process comprising:

(a) contacting the polyvinyl chloride with a cyanation reagent in the presence of a phase transfer catalyst to form a cyano-substituted polyvinyl chloride; and

(b) covalently bonding an antimicrobial agent to the cyano-substituted polyvinyl chloride.

27. The process of claim 26, wherein the antimicrobial agent comprises a hydrazone moiety or an azide moiety, and wherein step (b) comprises contacting the cyano-substituted polyvinyl chloride with the antimicrobial agent under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

28. The process of claim 26, wherein step (b) comprises: (i) converting cyano groups in the cyano-substituted polyvinyl chloride to 1*H*-tetrazole moieties, thereby forming a 1*H*-tetrazole-substituted polyvinyl chloride; and (ii) contacting the 1*H*-tetrazole-substituted polyvinyl chloride with an antimicrobial agent comprising an electrophilic moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

29. The process of claim 28, wherein step (i) comprises contacting the cyano-substituted polyvinyl chloride with an azide salt in the presence of a catalyst.

30. The process of claim 26, wherein step (b) comprises: (i) converting cyano groups in the cyano-substituted polyvinyl chloride to 1*H*-tetrazole moieties, thereby forming a 1*H*-tetrazole-substituted polyvinyl chloride; (ii) contacting the 1*H*-tetrazole-substituted polyvinyl chloride with an electrophile comprising an azide group to form an azide-substituted polyvinyl chloride; and (iii) contacting the azide-substituted polyvinyl chloride with an antimicrobial agent comprising an alkyne moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

31. The process of claim 30, wherein the electrophile comprising the azide group is chosen from a benzylic halide comprising an azide group, an allylic halide comprising an azide group, and a propargylic halide comprising an azide group.

32. The process of any of claims 30-31, wherein the alkyne moiety is activated by ring strain, electron withdrawing groups, or a combination thereof.

33. The process of any of claims 30-32, wherein step (iii) comprises contacting the azide-substituted polyvinyl chloride with the antimicrobial agent comprising the alkyne moiety in the presence of a Cu(I) catalyst.

34. The process of claim 26, wherein step (b) comprises: (i) converting cyano groups in the cyano-substituted polyvinyl chloride to 1*H*-tetrazole moieties, thereby forming a 1*H*-tetrazole-substituted polyvinyl chloride; (ii) contacting the 1*H*-tetrazole-substituted polyvinyl chloride with an electrophile comprising an alkynyl group to form an alkyne-substituted polyvinyl chloride; and (iii) contacting the alkyne-substituted polyvinyl chloride with an antimicrobial agent comprising an azide moiety under conditions effective to covalently bond the antimicrobial agent to the polyvinyl chloride surface.

35. The process of claim 34, wherein the electrophile comprising the alkynyl group is chosen from a benzylic halide comprising an alkynyl group, an allylic halide comprising an alkynyl group, and a propargylic halide comprising an alkynyl group.

36. The process of any of claims 34-35, wherein the alkyne group is activated by ring strain, electron withdrawing groups, or a combination thereof.

37. The process of any of claims 34-36, wherein step (iii) comprises contacting the alkyne-substituted polyvinyl chloride with the antimicrobial agent comprising the azide moiety in the presence of a Cu(I) catalyst.

38. The process of any of claims 26-37, wherein step (a) comprises flowing an aqueous solution comprising the cyanation reagent and the phase transfer catalyst across the polyvinyl chloride surface.

39. The process of any of claims 26-38, wherein step (a) comprises contacting the polyvinyl chloride with the cyanation reagent and the phase transfer catalyst for 3 hours or less.

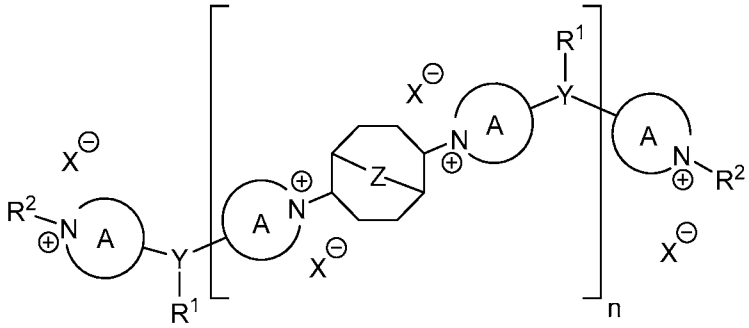
40. The process of any of claims 26-39, wherein the polyvinyl chloride surface comprises a surface of a medical article.

41. The process of claim 40, wherein the medical article comprises tubing.

42. The process of claim 41, wherein the surface of the medical article comprises an interior surface of the tubing, an exterior surface of the tubing, or both an interior surface of the tubing and an exterior surface of the tubing.

43. The process of any of claims 26-42, wherein the antimicrobial agent comprises a polycationic polymer.

44. The process of claim 43, wherein the polycationic polymer comprises a polymer defined by Formula I below



Formula I

wherein

Z represents, individually for each occurrence, S, Se, or NR^3;

A represents, individually for each occurrence, a heterocyclic ring comprising a cationic nitrogen center;

X represents, individually for each occurrence, an anion;

Y is absent, or represents, individually for each occurrence, a linking group;

R^1 is absent, or represents, individually for each occurrence, halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio,

alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy;

R^2 is absent, or represents, individually for each occurrence, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy;

R^3 represents, individually for each occurrence, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy; and

n is an integer from 2 to 400.

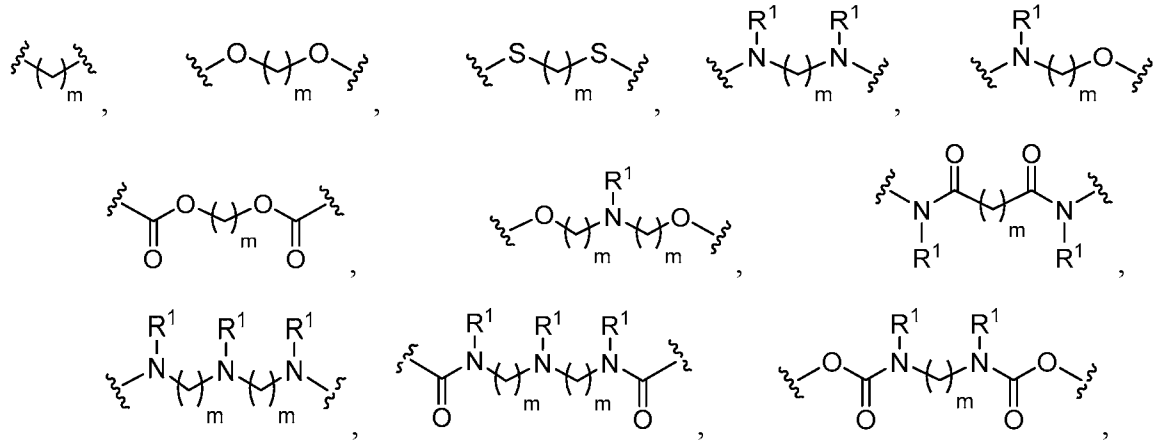
45. The process of claim 44, A is chosen from pyridine, imidazole, pyrazole, triazole, tetrazole, oxazole, isoxazole, furazan, isothioazole, and thiazole.

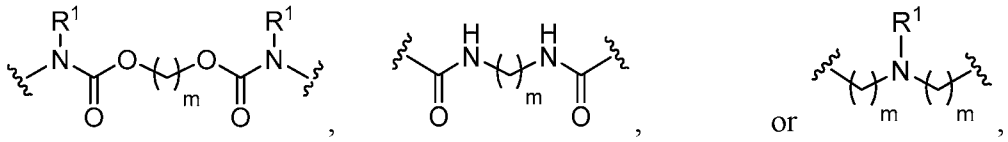
46. The process of any of claims 44-45, wherein Z is S.

47. The process of any of claims 44-45, wherein Z is NR^3.

48. The process of any of claims 44-47, wherein R^1, R^2, R^3, or a combination thereof comprise a cationic moiety.

49. The process of any of claims 44-48, wherein Y is chosen from one of the following:



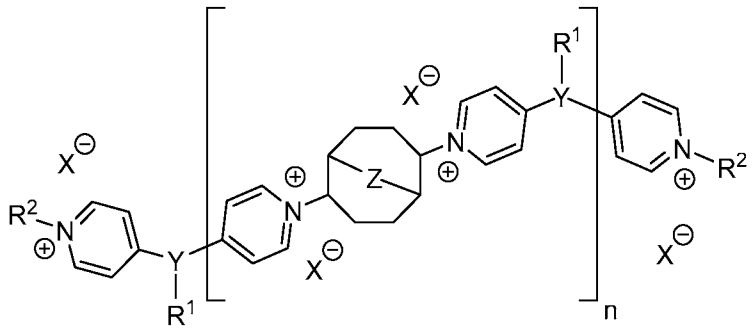


where m is an integer from 1 to 12.

50. The process of any of claims 44-49, where X is chosen from chloride, bromide, iodide, nitrate, sulfate, triflate, borate, and phosphate.

51. The process of any of claims 44-50, wherein n is from 10 to 50.

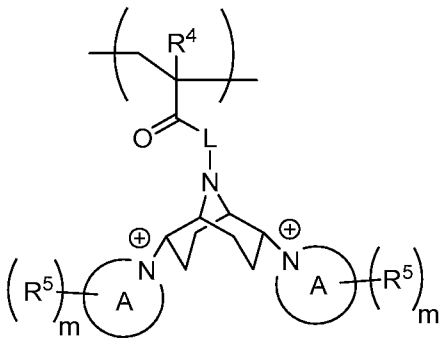
52. The process of any of claims 44-51, wherein the polycationic polymer comprises a polymer defined by Formula IA below



Formula IA

wherein Z, X, Y, R^1, R^2, R^3, and n are as defined above with respect to Formula I.

53. The process of claim 43, wherein the polycationic polymer comprises a recurring unit defined by Formula V below



Formula V

wherein

R^4 represents H or methyl;

L is absent or represents a linking group;

A represents, individually for each occurrence, a heterocyclic ring comprising a cationic nitrogen center;

R^5 is absent, or represents, individually for each occurrence, halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy; and

m is an integer from 0 to 5.

54. The process of claim 53, wherein the polycationic polymer further comprises a recurring unit derived from the polymerization of one or more ethylenically-unsaturated monomers.

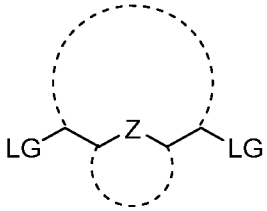
55. The process of claim 54, wherein the one or more ethylenically-unsaturated monomers are chosen from the group consisting of styrene, butadiene, meth(acrylate) monomers, vinyl acetate, vinyl ester monomers and combinations thereof.

56. The process of any of claims 26-55, wherein the phase transfer catalyst comprises a quaternary ammonium salt.

57. The process of any of claims 1-24, wherein the cyanation reagent comprises NaCN, KCN, or a combination thereof.

58. An antimicrobial article comprising a surface and a polycationic polymer derived from the condensation of a cyclic bis-electrophile and a polynucleophilic monomer immobilized on the surface,

wherein the cyclic bis-electrophile is defined by Formula II below



Formula II

wherein

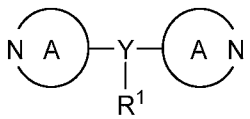
each dotted line represents a cyclic moiety;

LG represents a leaving group;

Z represents S, Se, or NR^3; and

R^3 represents alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy; and

wherein the polynucleophilic monomer is defined by Formula III below



Formula III

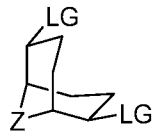
wherein

A represents a heterocyclic ring comprising a tertiary nitrogen atom;

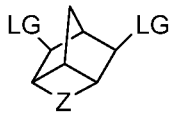
Y is absent or represents a linking group;

R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

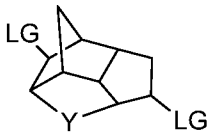
59. The article of claim 58, wherein the cyclic bis-electrophile is defined by Formula IIA, Formula IIB, or Formula IIC below



Formula IIA



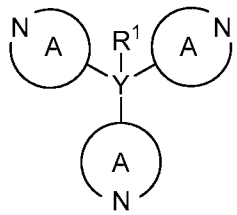
Formula IIB



Formula IIC

wherein LG and Z are as defined above with respect to Formula II.

60. The article of any of claims 58-59, wherein the polycationic polymer is derived from the condensation of the cyclic bis-electrophile defined by Formula II, the polynucleophilic monomer defined by Formula III, and a polynucleophilic monomer defined by Formula IV below



Formula IV

wherein

A represents a heterocyclic ring comprising a tertiary nitrogen atom;

Y is absent or represents a linking group;

R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

61. The article of any of claims 58-60, wherein the polycationic polymer is covalently bound to the surface.

62. The article of any of claims 58-60, wherein the polycationic polymer is non-covalently bound to the surface.

63. The article of any of claims 58-62, wherein the surface comprises a glass surface.

64. The article of any of claims 58-62, wherein the surface comprises a polymer surface.

65. The article of claim 64, wherein the polymer surface comprises polyvinyl chloride.

66. The article of any of claims 58-65, wherein the article comprises a medical device.

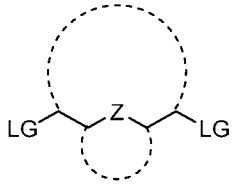
67. The article of claim 66, wherein the medical device is chosen from pins, screws, plates, ventriculoatrial shunts, ventriculoperitoneal shunts, dialysis shunts, heart valves, pacemakers, infusion pumps, vascular grafting prostheses, stents, sutures, surgical meshes, replacement prostheses, breast implants, tissue expanders, contact lenses, stoma appliances, artificial larynx, endotracheal tubes, tracheal tubes, gastrostomy tubes, biliary drainage tubes, biliary stents, catheters, bandages, adhesive tapes, and clear plastic adherent sheets.

68. The article of any of claims 58-67, wherein the article comprises polyvinyl chloride tubing.

69. A method of controlling biofilm formation on a substrate, the method comprising covalently bonding a polycationic polymer to the substrate in an amount effective to inhibit biofilm formation,

wherein the polycationic polymer derived from the condensation of a cyclic bis-electrophile and a polynucleophilic monomer,

wherein the cyclic bis-electrophile is defined by Formula II below



Formula II

wherein

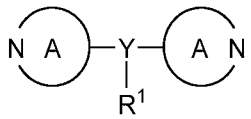
each dotted line represents a cyclic moiety;

LG represents a leaving group;

Z represents S, Se, or NR^3; and

R^3 represents alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, peptidyl, polyamino, or polyalkyleneoxy; and

wherein the polynucleophilic monomer is defined by Formula III below



Formula III

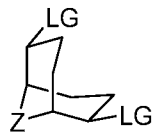
wherein

A represents a heterocyclic ring comprising a tertiary nitrogen atom;

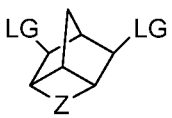
Y is absent or represents a linking group;

R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

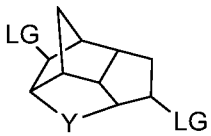
70. The method of claim 69, wherein the cyclic bis-electrophile is defined by Formula IIA, Formula IIB, or Formula IIC below



Formula IIA



Formula IIB

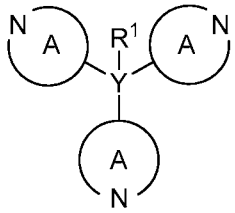


Formula IIC

wherein LG and Z are as defined above with respect to Formula II.

71. The method of any of claims 69-70, wherein the polycationic polymer is derived from the condensation of the cyclic bis-electrophile defined by Formula II, the polynucleophilic

monomer defined by Formula III, and a polynucleophilic monomer defined by Formula IV below



Formula IV

A represents a heterocyclic ring comprising a tertiary nitrogen atom;

Y is absent or represents a linking group;

R^1 is absent, or represents halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy.

72. The method of any of claims 69-71, wherein the substrate comprises a glass surface.

73. The method of any of claims 69-71, wherein the substrate comprises a polymer surface.

74. The method of claim 73, wherein the polymer surface comprises polyvinyl chloride.

75. The method of any of claims 69-74, wherein the substrate comprises a medical device.

76. The method of claim 75, wherein the medical device is chosen from pins, screws, plates, ventriculoatrial shunts, ventriculoperitoneal shunts, dialysis shunts, heart valves, pacemakers, infusion pumps, vascular grafting prostheses, stents, sutures, surgical meshes, replacement prostheses, breast implants, tissue expanders, contact lenses, stoma appliances, artificial larynx, endotracheal tubes, tracheal tubes, gastrostomy tubes, biliary drainage tubes, biliary stents, catheters, bandages, adhesive tapes, and clear plastic adherent sheets.

77. The method of any of claims 69-76, wherein the substrate comprises polyvinyl chloride tubing.

78. The method of any of claims 69-77, wherein the biofilm comprises Gram-positive bacteria.

79. The method of claim 78, wherein the biofilm comprises bacteria of a genus *Staphylococcus*.

80. The method of claim 79, wherein the biofilm comprises bacteria of the species *Staphylococcus aureus.*

81. The method of any of claims 69-77, wherein the biofilm comprises Gram-negative bacteria.

82. The method of claim 81, wherein the biofilm comprises bacteria of a genus chosen from *Pseudomonas, Bordetello, Vibrio, Haemophilus, Halomonas,* and *Acinetobacter.*

83. The method of claim 82, wherein the biofilm comprises bacteria of the species selected from the group consisting of: *Pseudomonas aeuroginosa, Bordetello pertussis, Vibrio vulnificus, IIaemophilus influenzae, Halomonas pacifica,* and *Acinetobacter baumannii.*

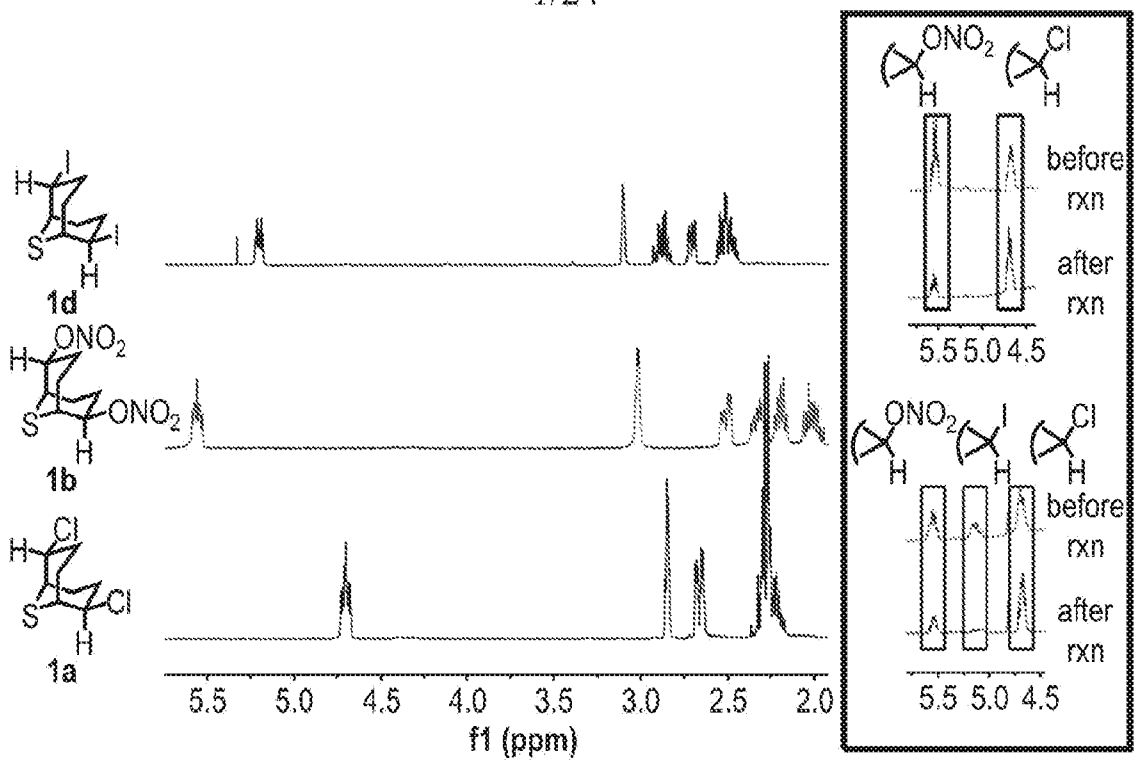


FIGURE 1

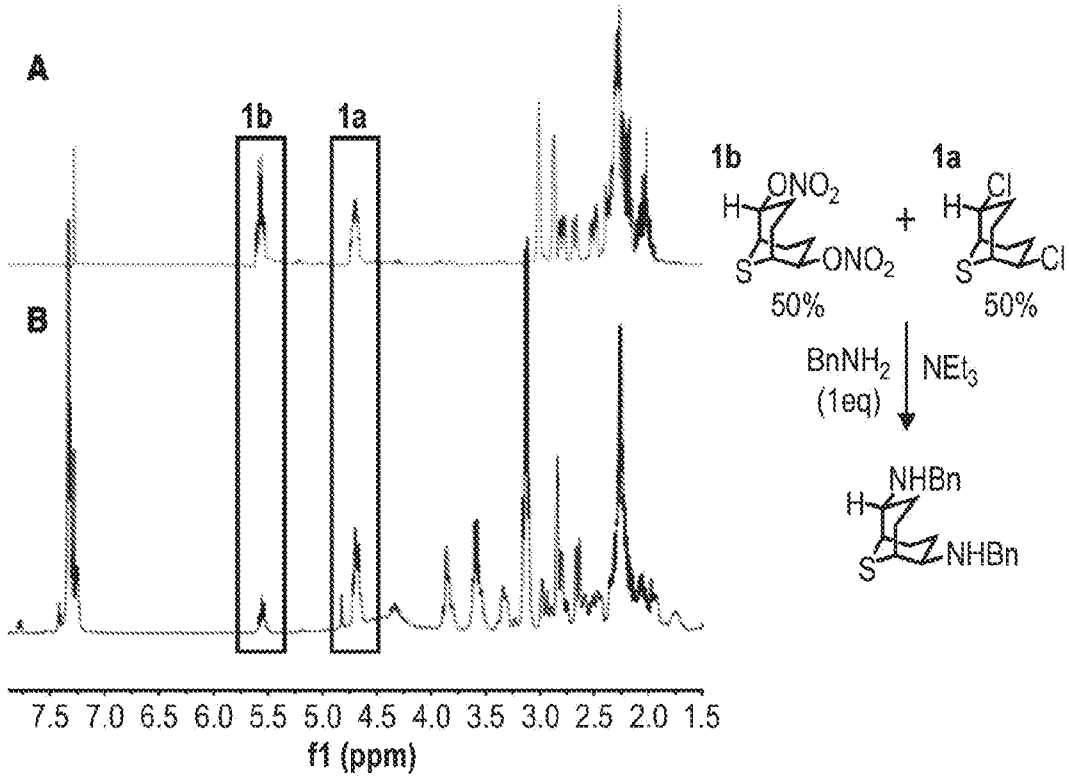


FIGURE 2

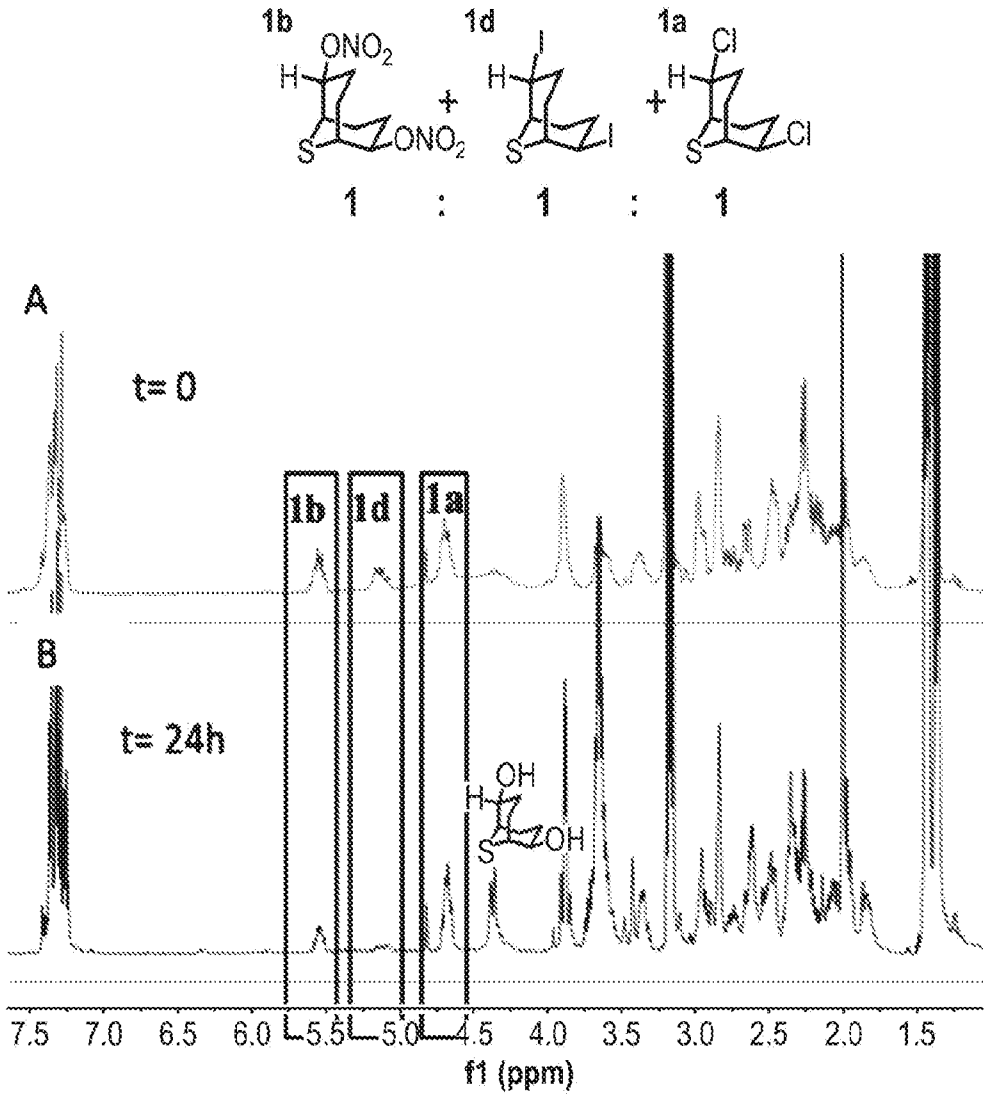

1b
1d
1a
ONO2
I
Cl
1 : 1 : 1
A
t= 0
B
t= 24h
OH
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)

FIGURE 3

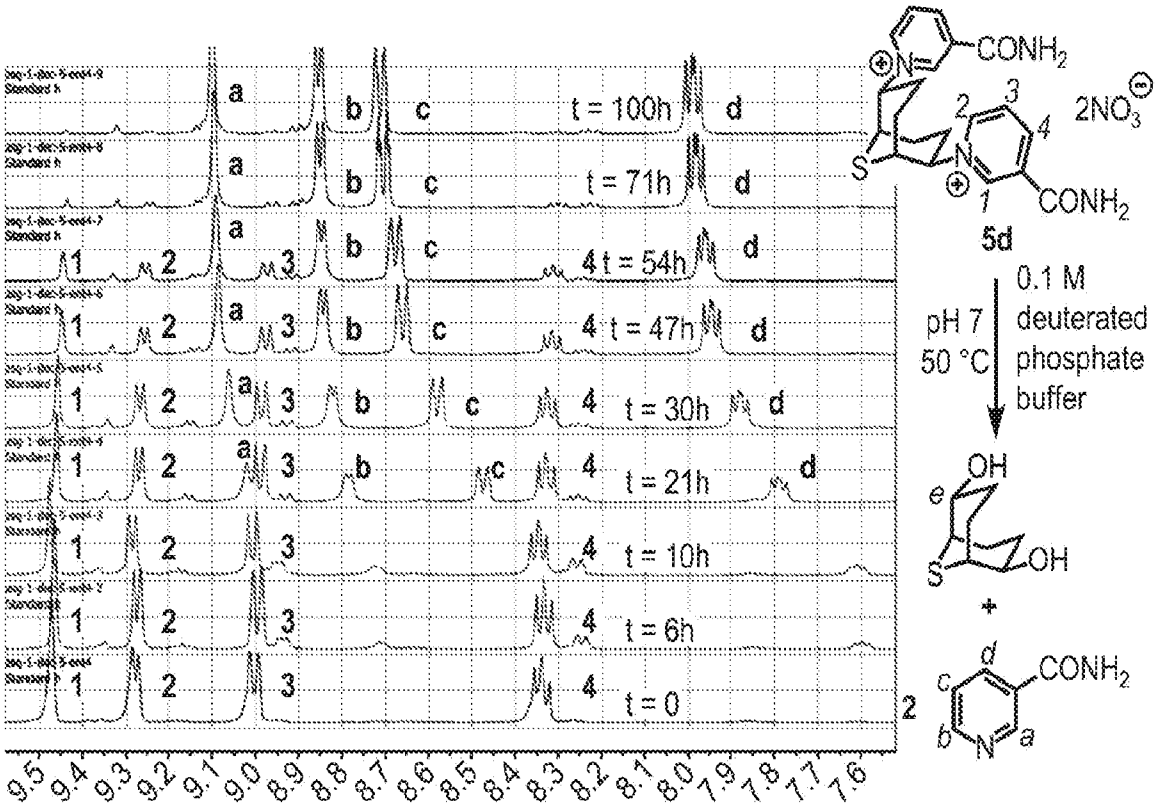


FIGURE 4A

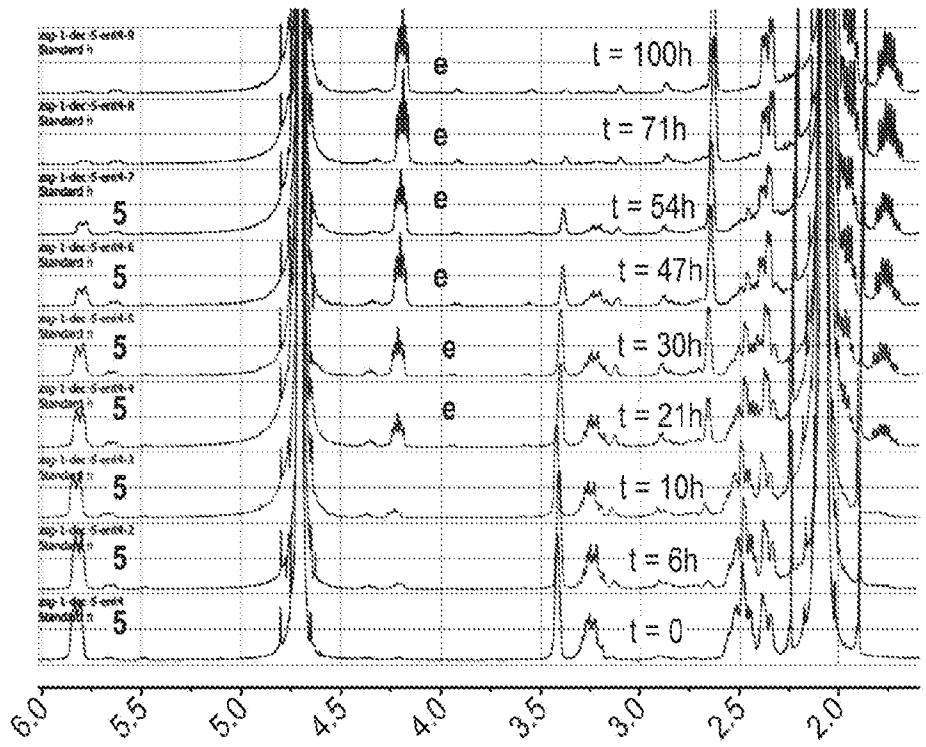


FIGURE 4B

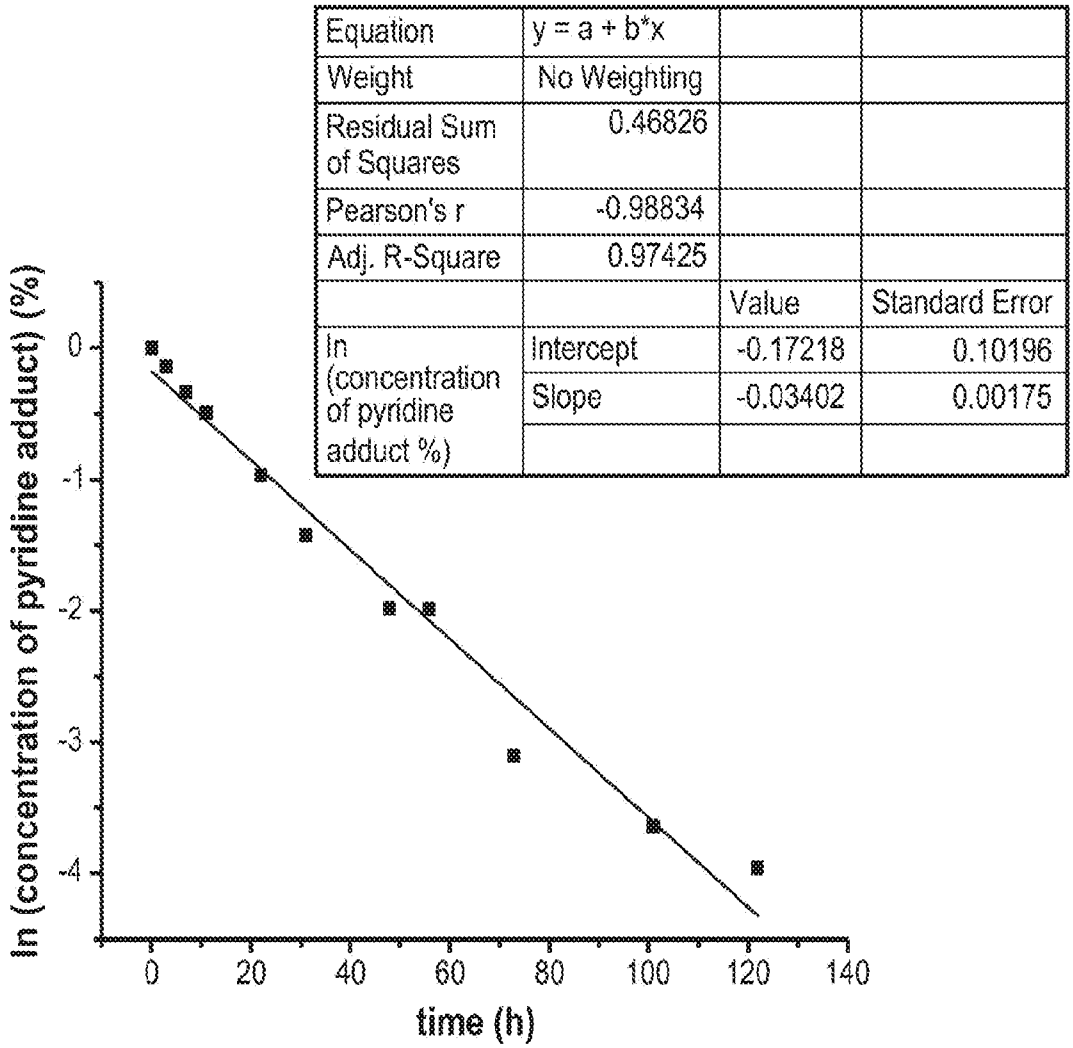
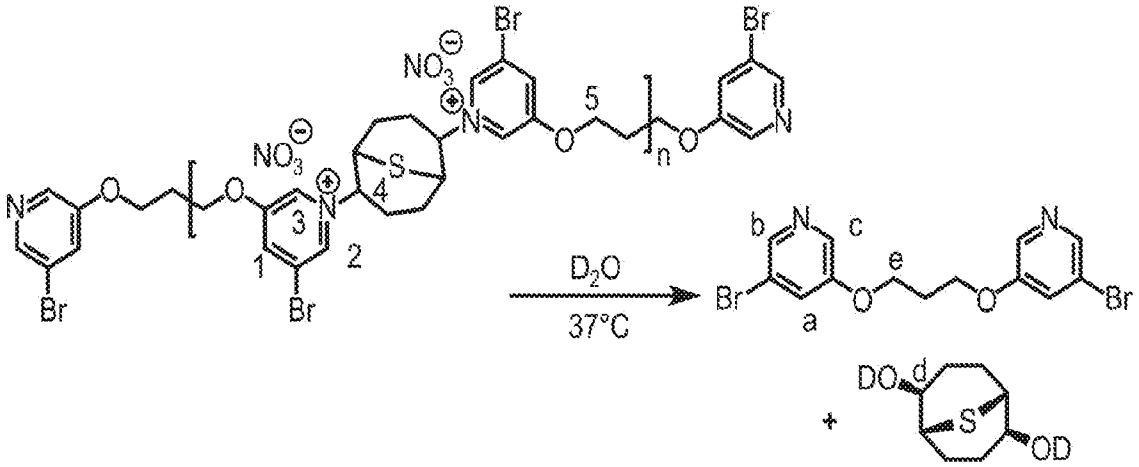

Equation
y = a + b*x
Weight
No Weighting
Residual Sum of Squares
0.46826
Pearson's r
-0.98834
Adj. R-Square
0.97425
Value
Standard Error
ln (concentration of pyridine adduct %)
Intercept
-0.17218
0.10196
Slope
-0.03402
0.00175
ln (concentration of pyridine adduct) (%)
0
-1
-2
-3
-4
0
20
40
60
80
100
120
140
time (h)

FIGURE 5


Br
NO3
5
n
Br
N
O
S
4
3
1
2
Br
Br
D2O
37°C
b
c
e
a
DO
d
OD

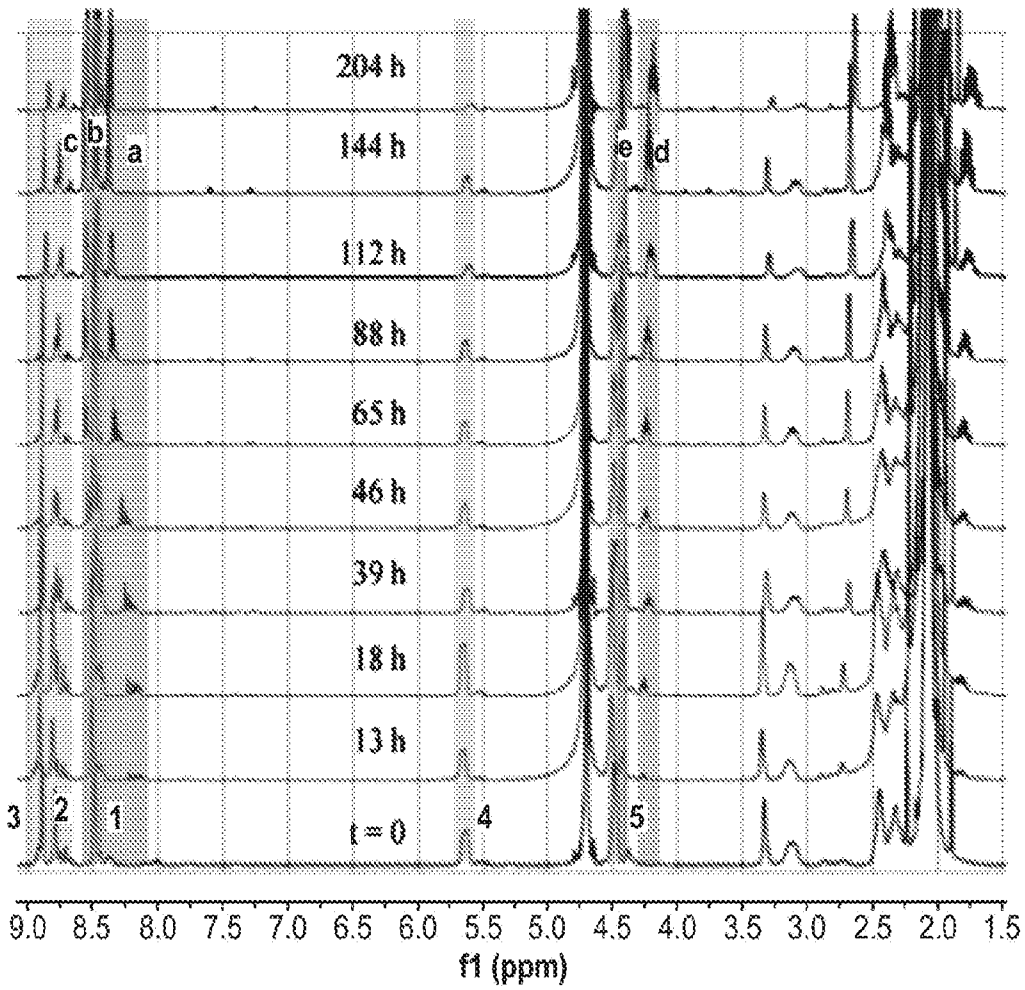

204 h
144 h
112 h
88 h
65 h
46 h
39 h
18 h
13 h
t = 0
a
b
c
d
e
1
2
3
4
5
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)

FIGURE 6

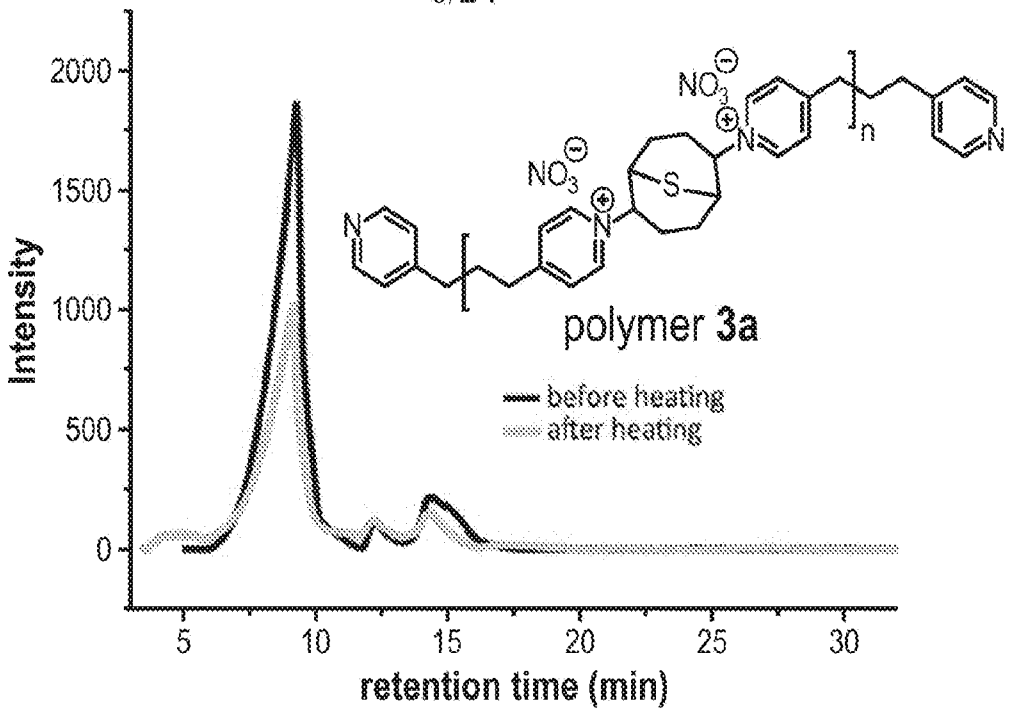


FIGURE 7A

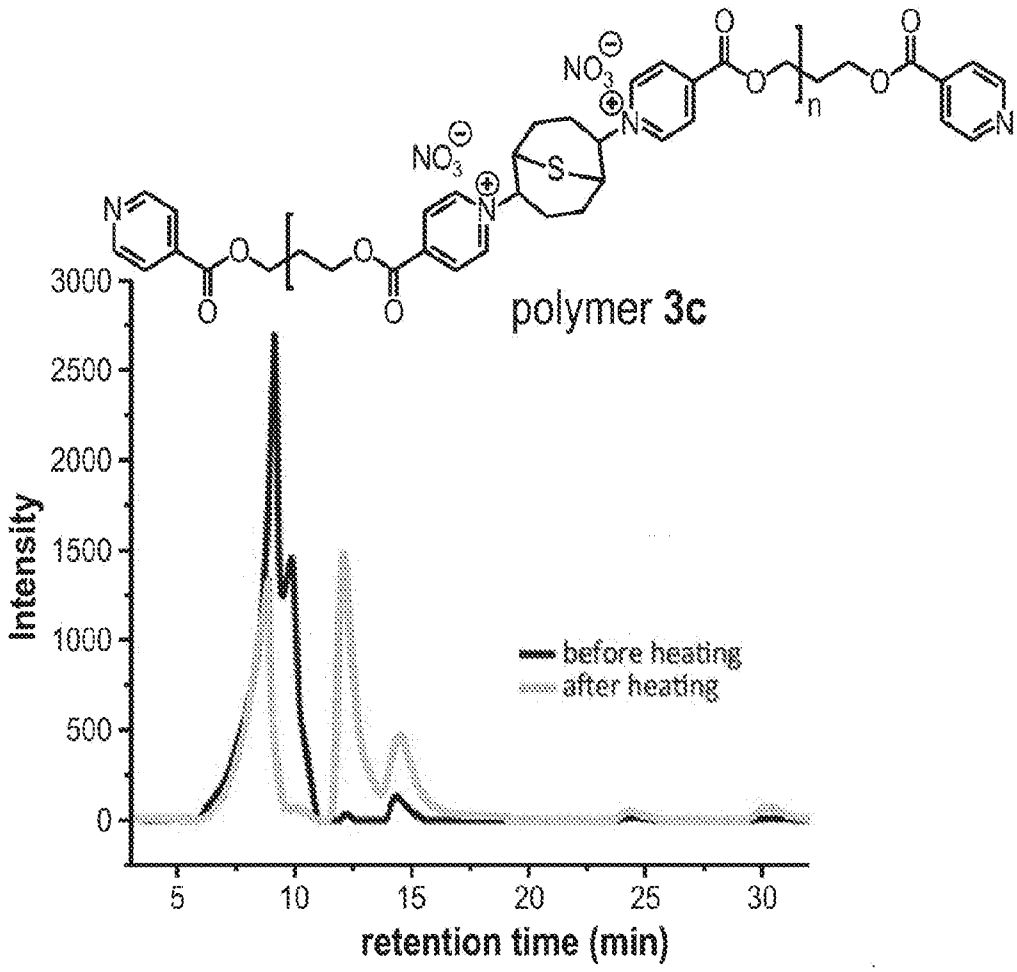


FIGURE 7B

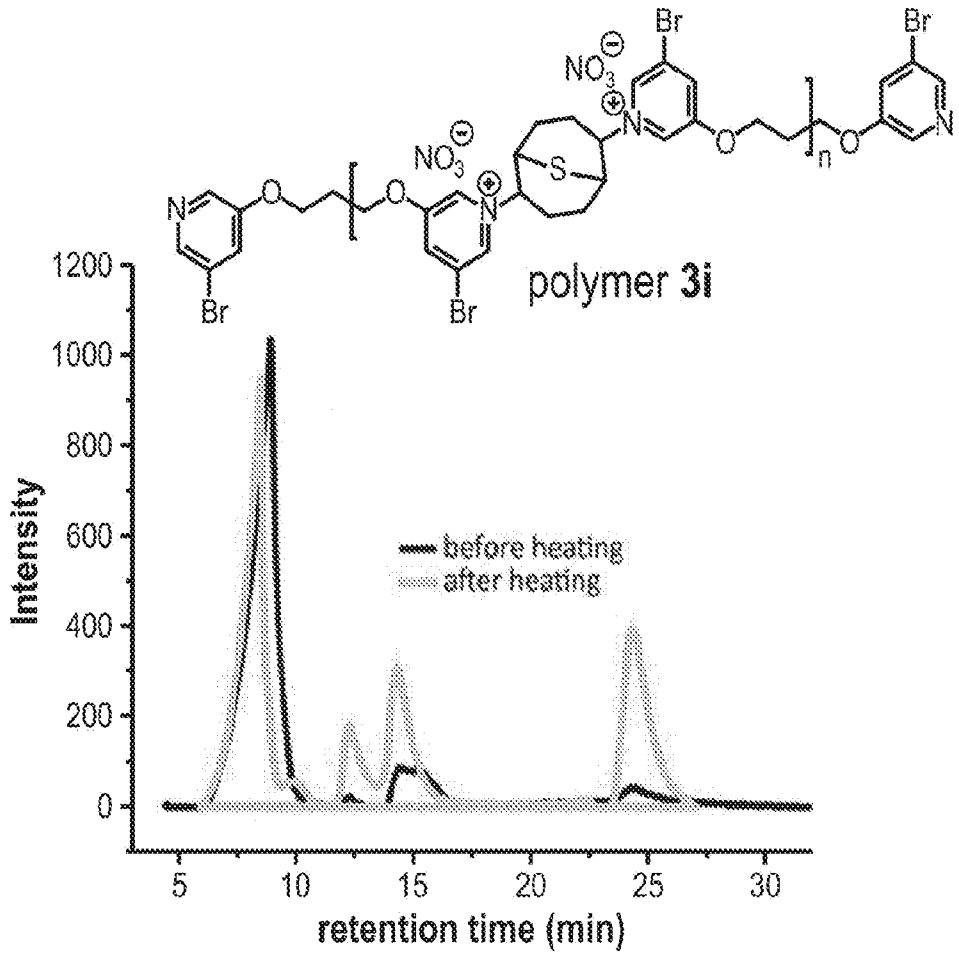


FIGURE 7C

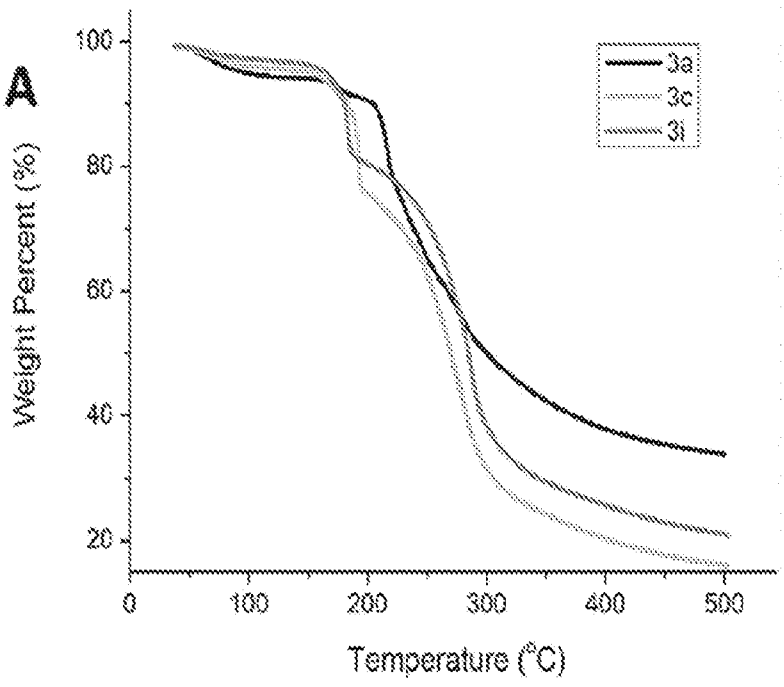


FIGURE 8A

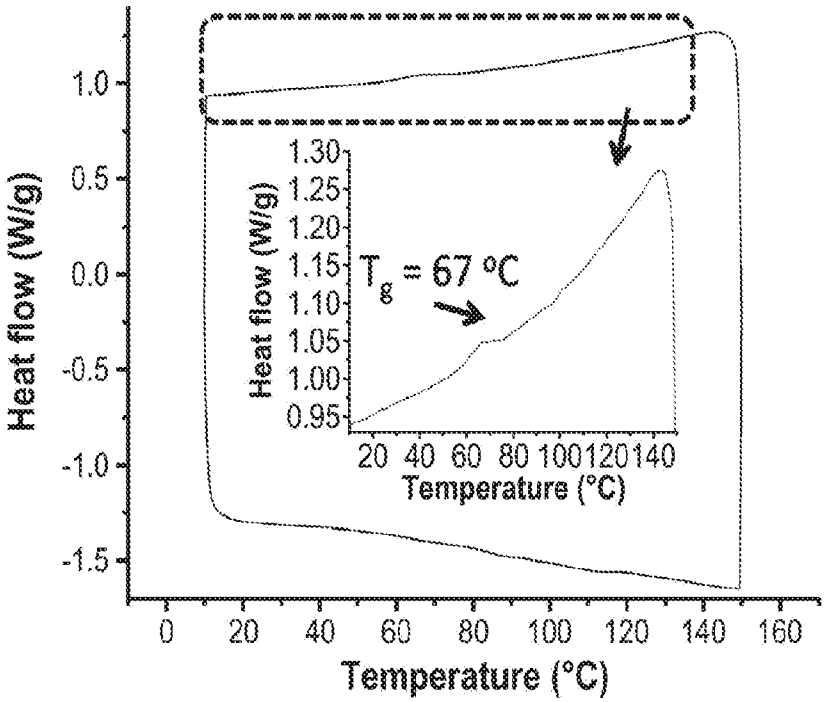


FIGURE 8B

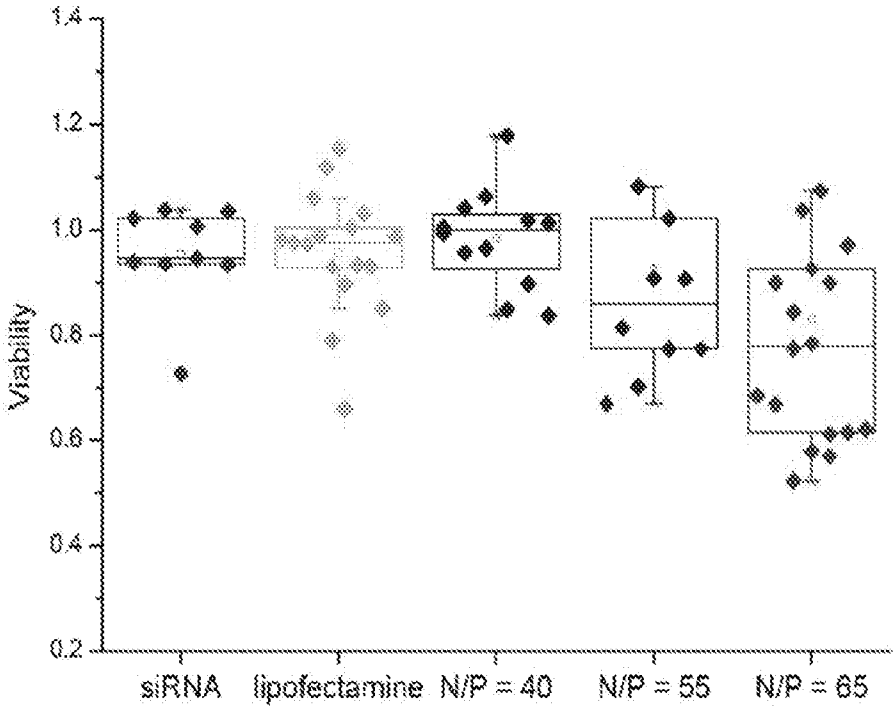


FIGURE 9A

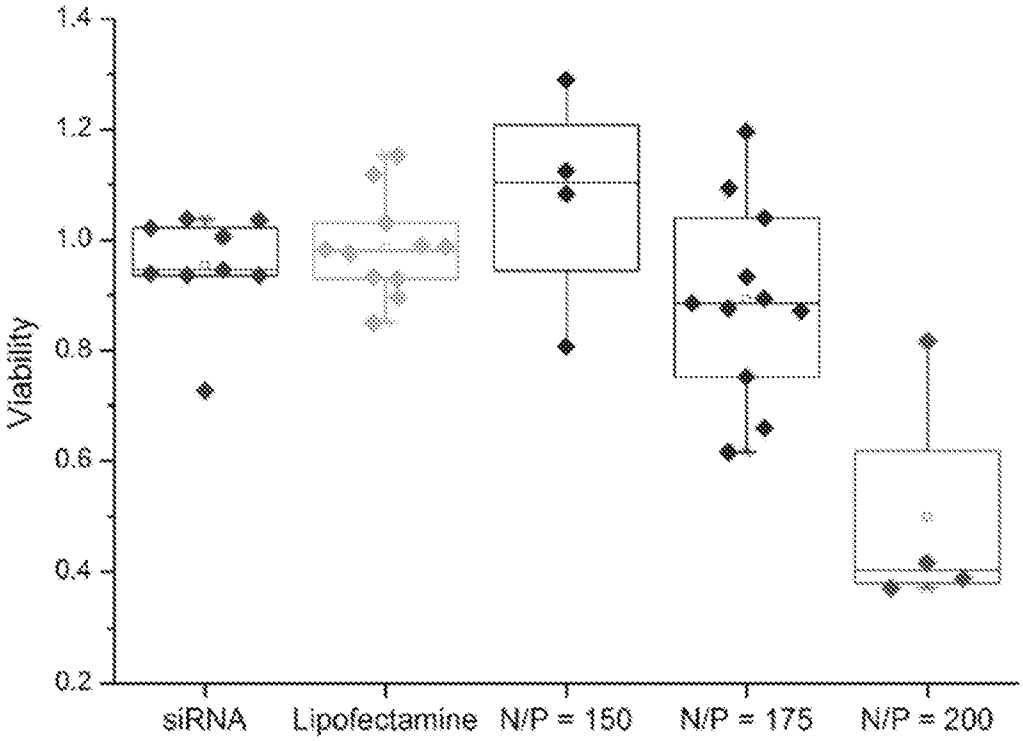


FIGURE 9B

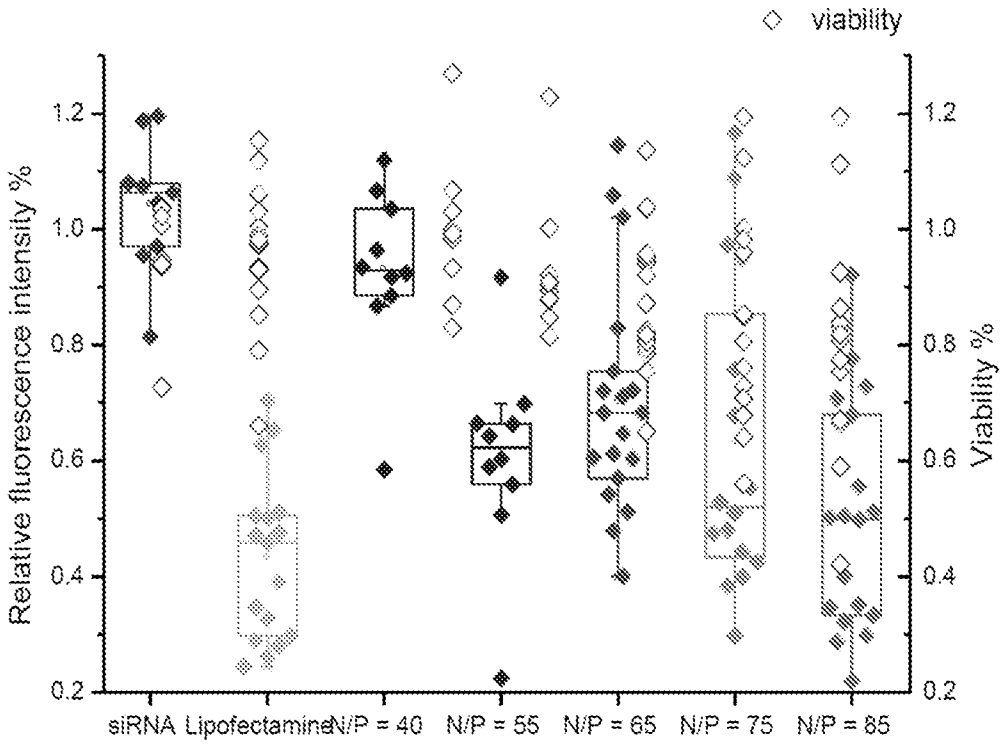


FIGURE 10

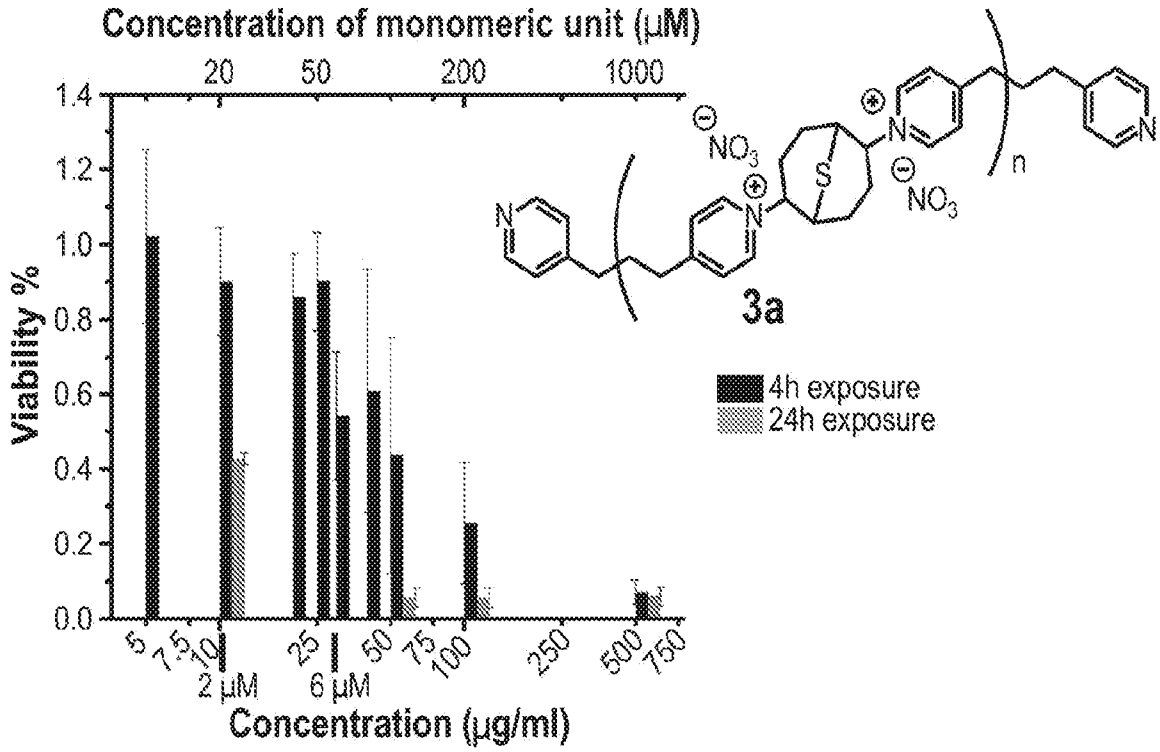


FIGURE 11A

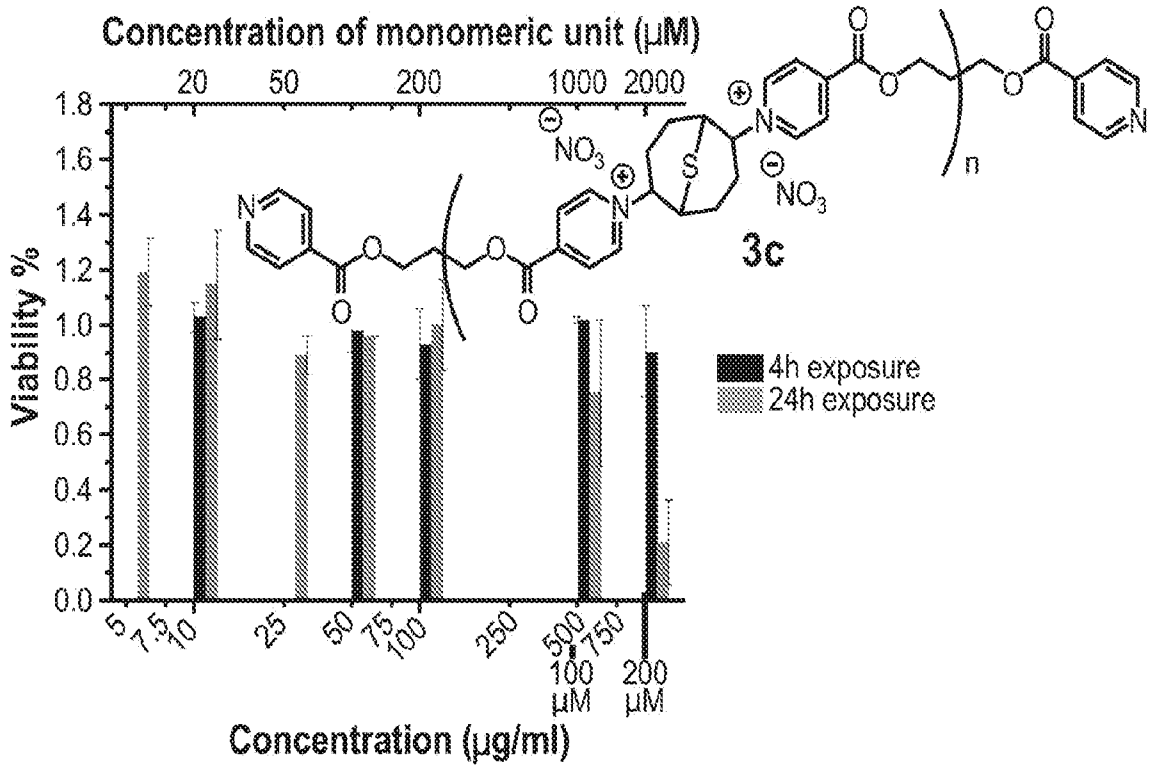


FIGURE 11B

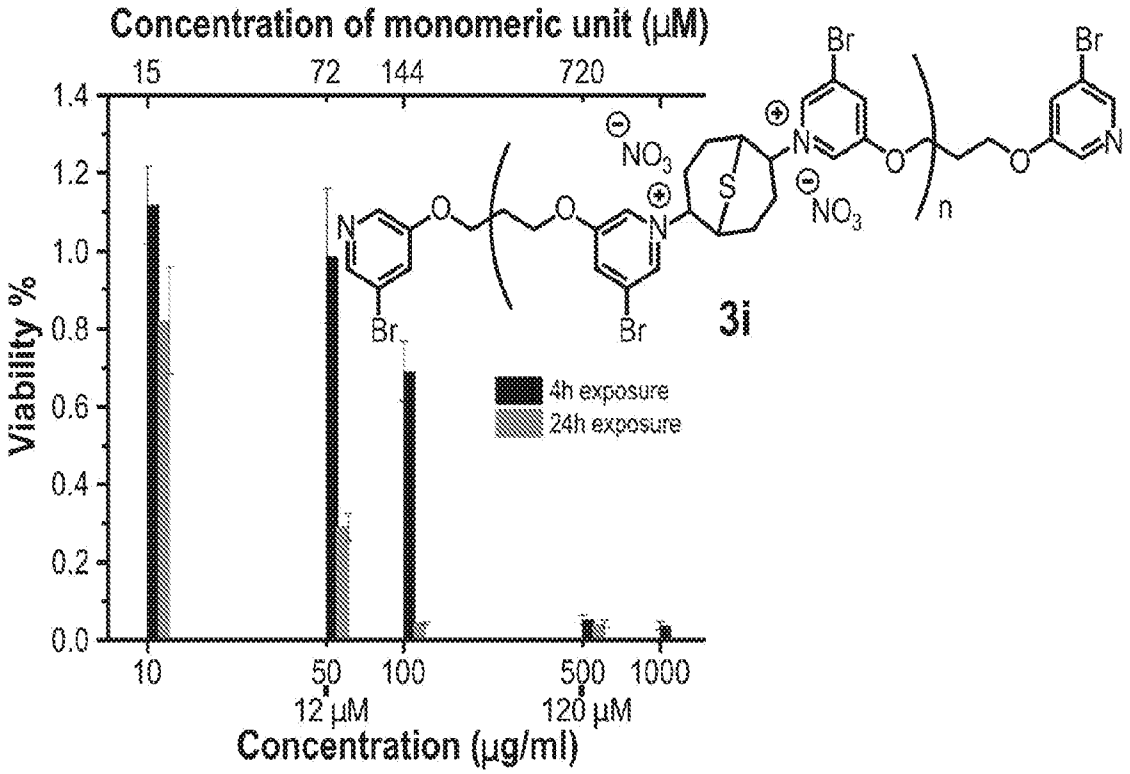


FIGURE 11C

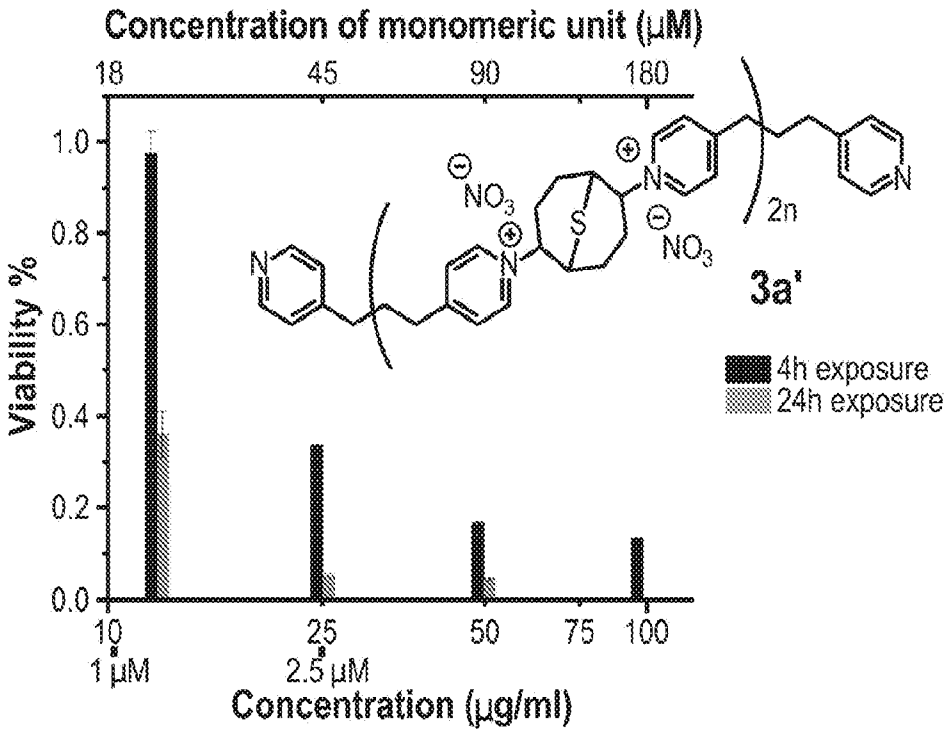


FIGURE 11D

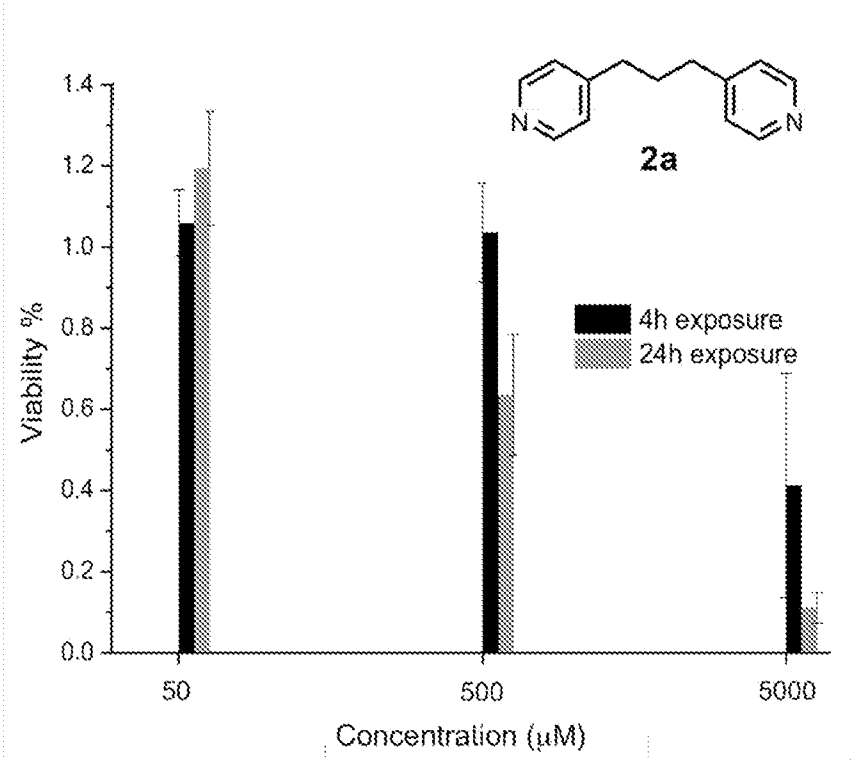


FIGURE 12A

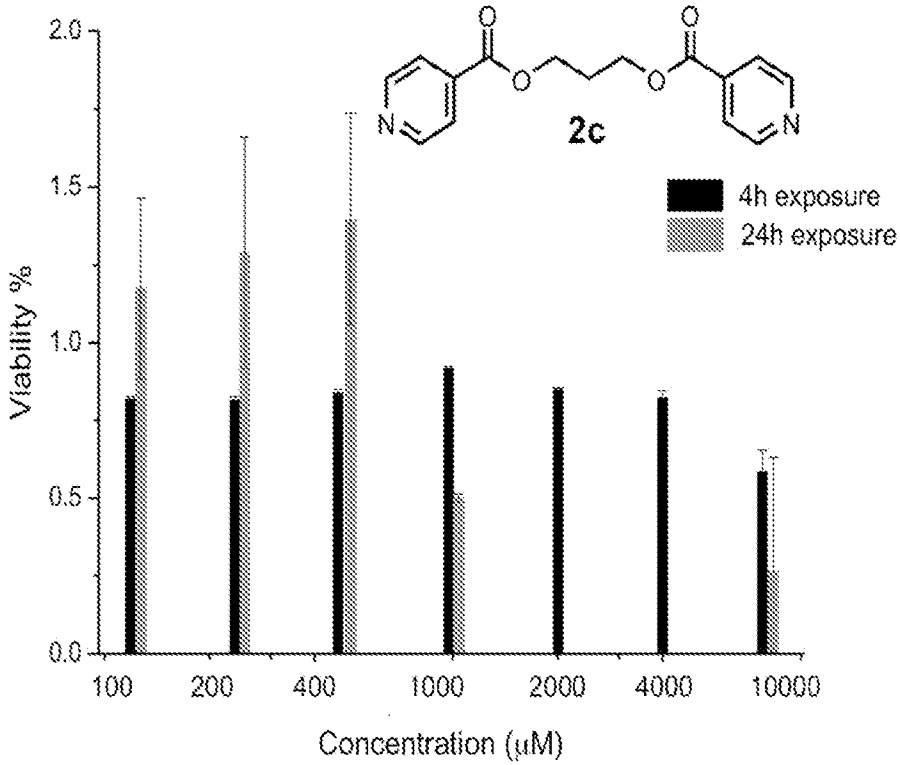


FIGURE 12B

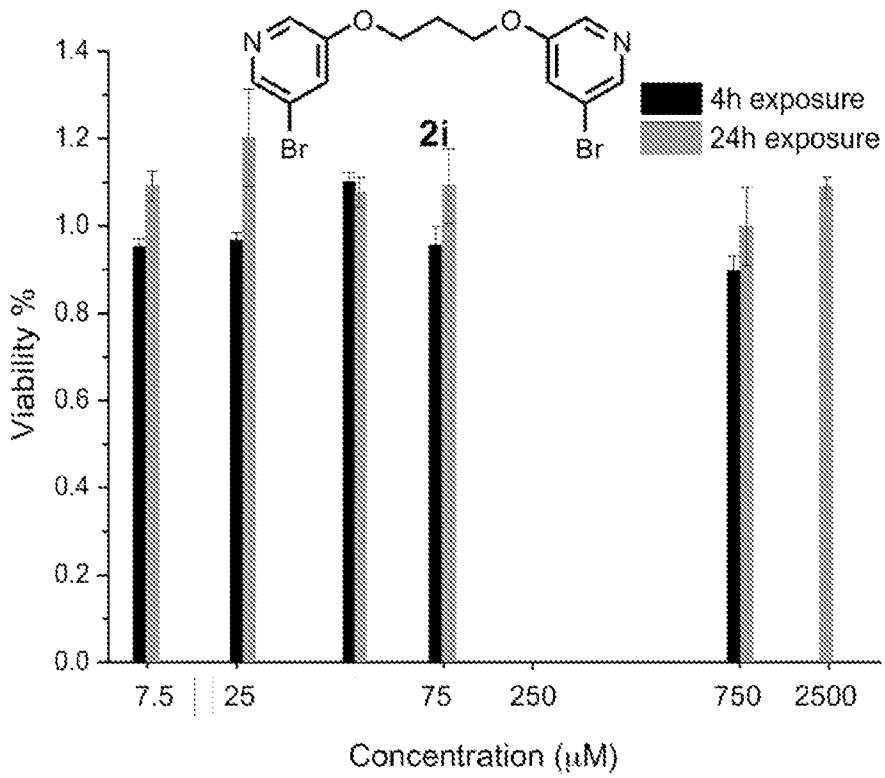


FIGURE 12C

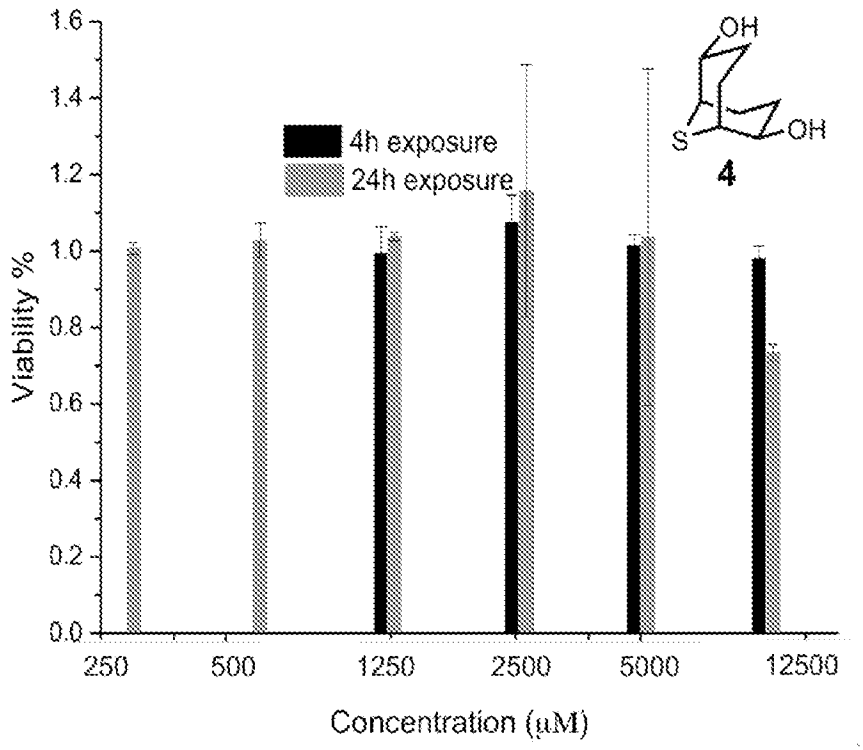


FIGURE 12D

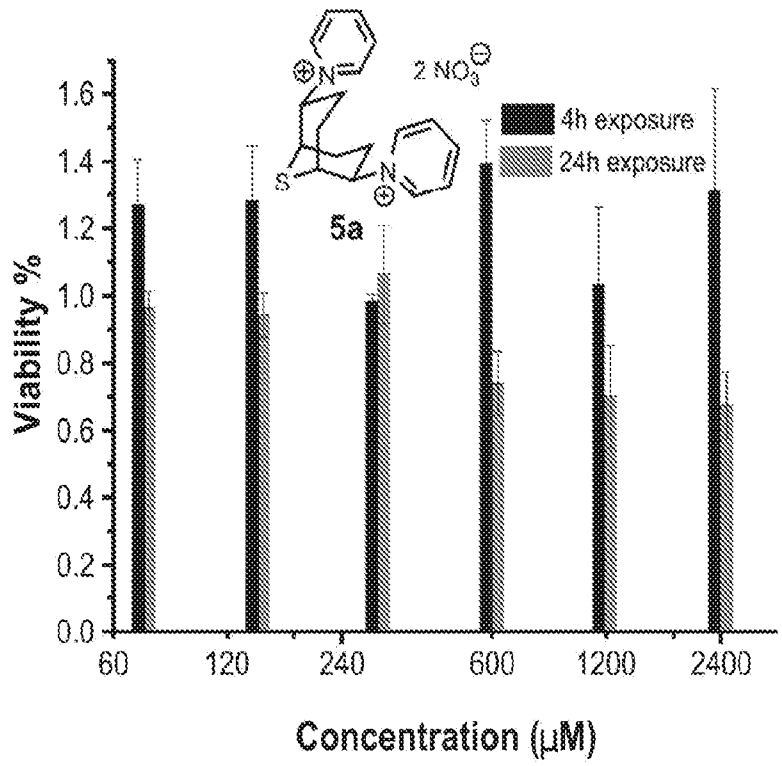


FIGURE 12E

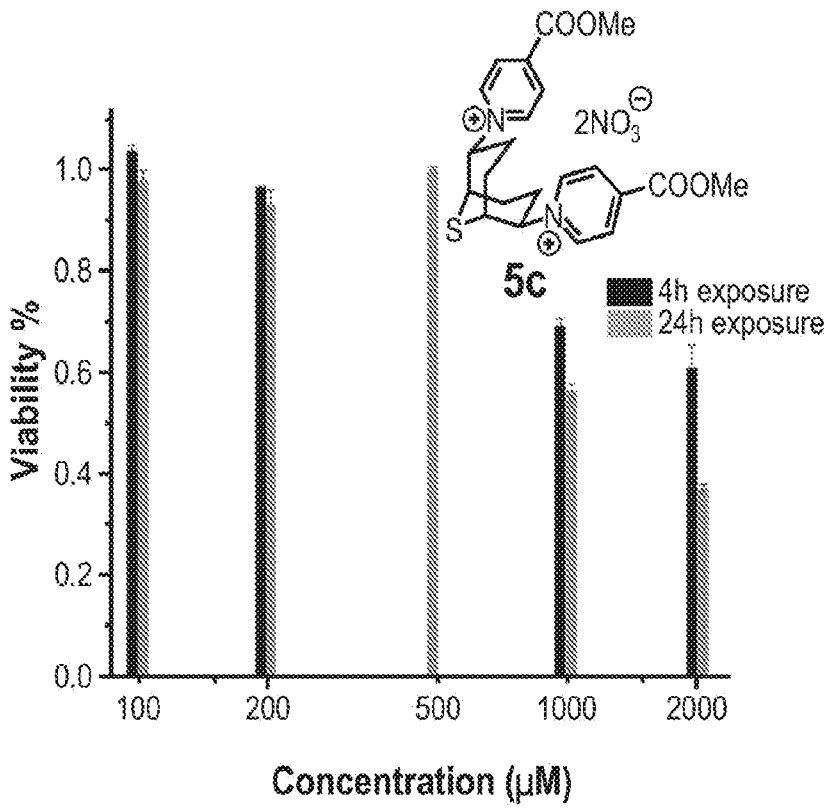


FIGURE 12F

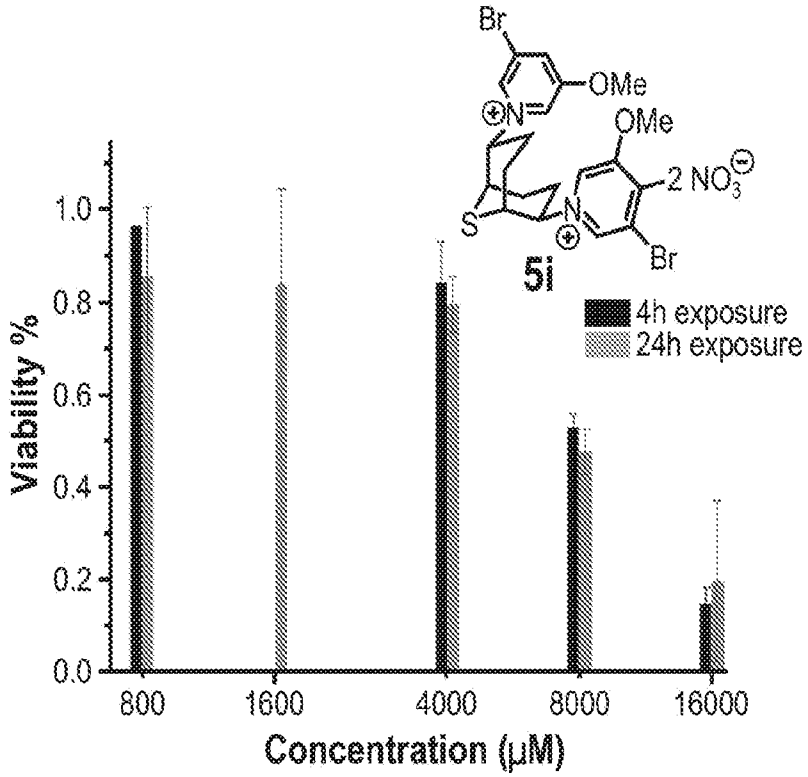


FIGURE 12G

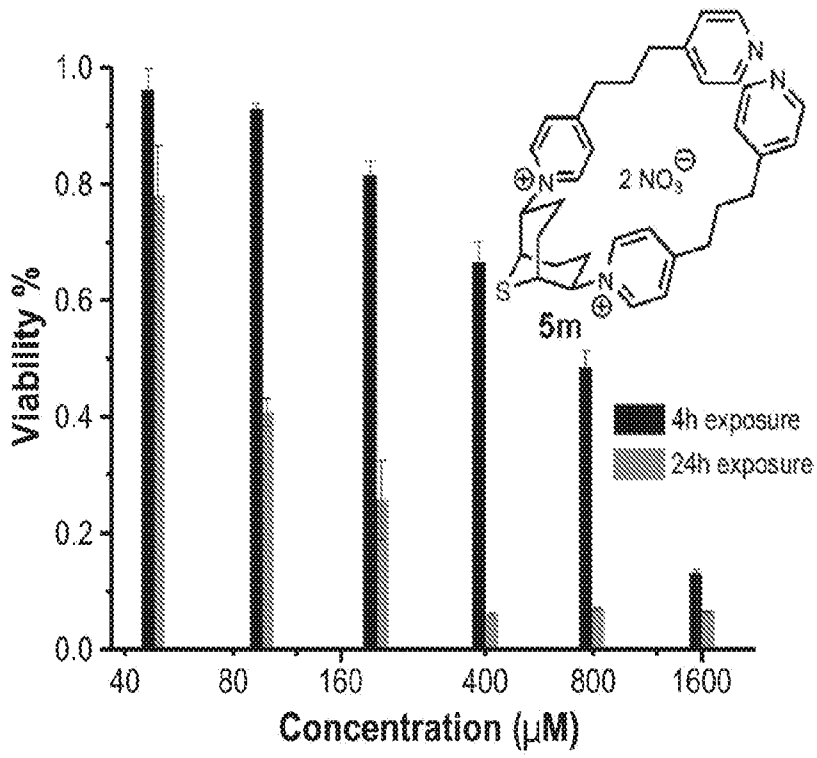


FIGURE 12H

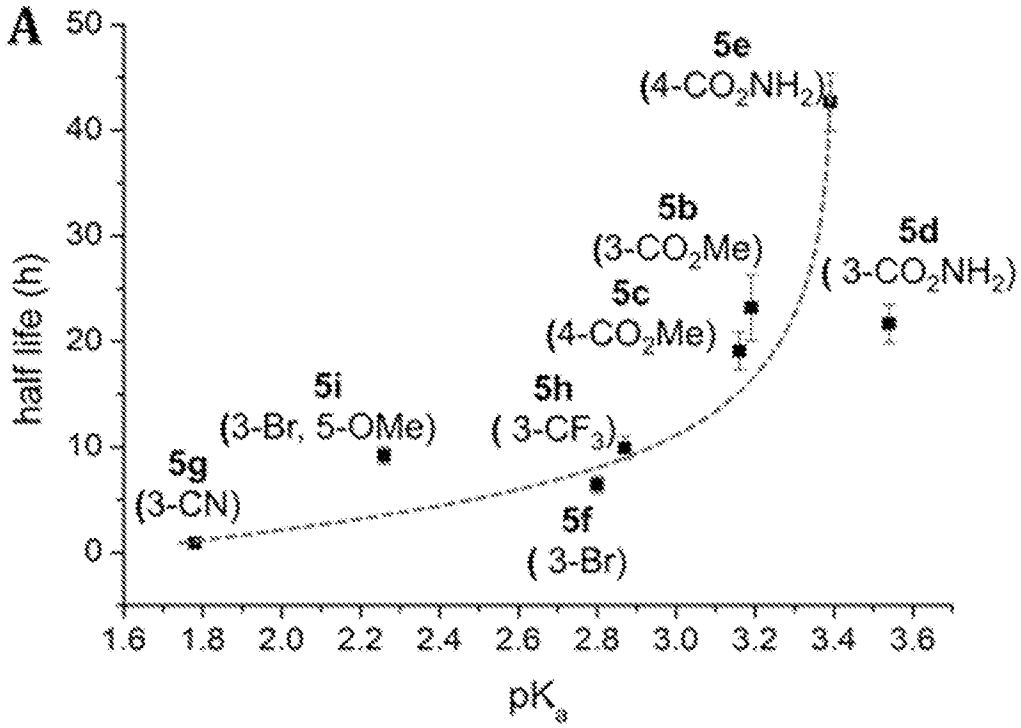


FIGURE 13A

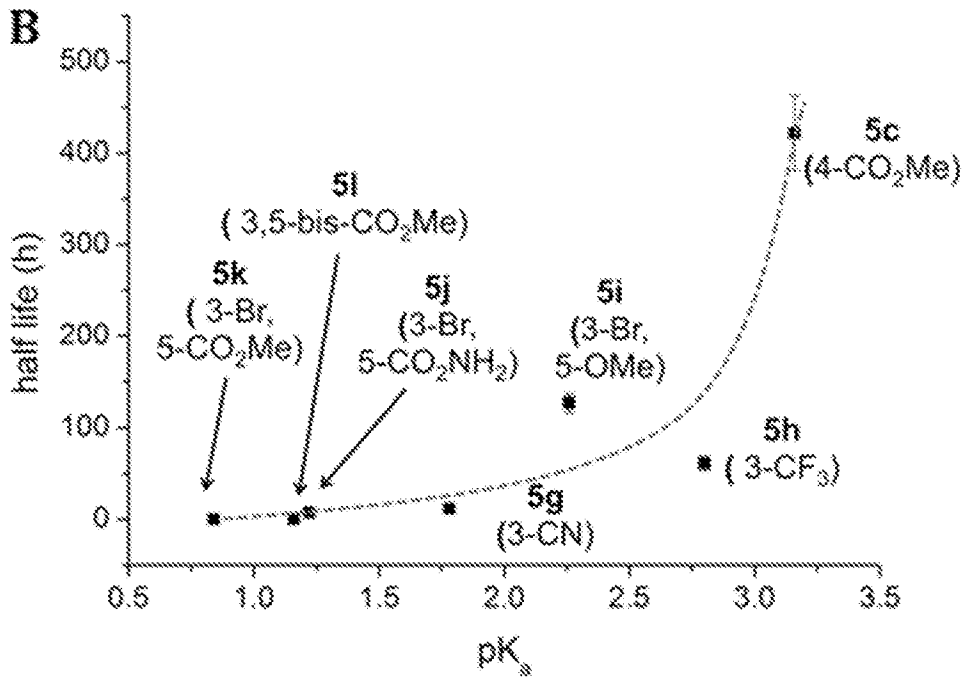


FIGURE 13B

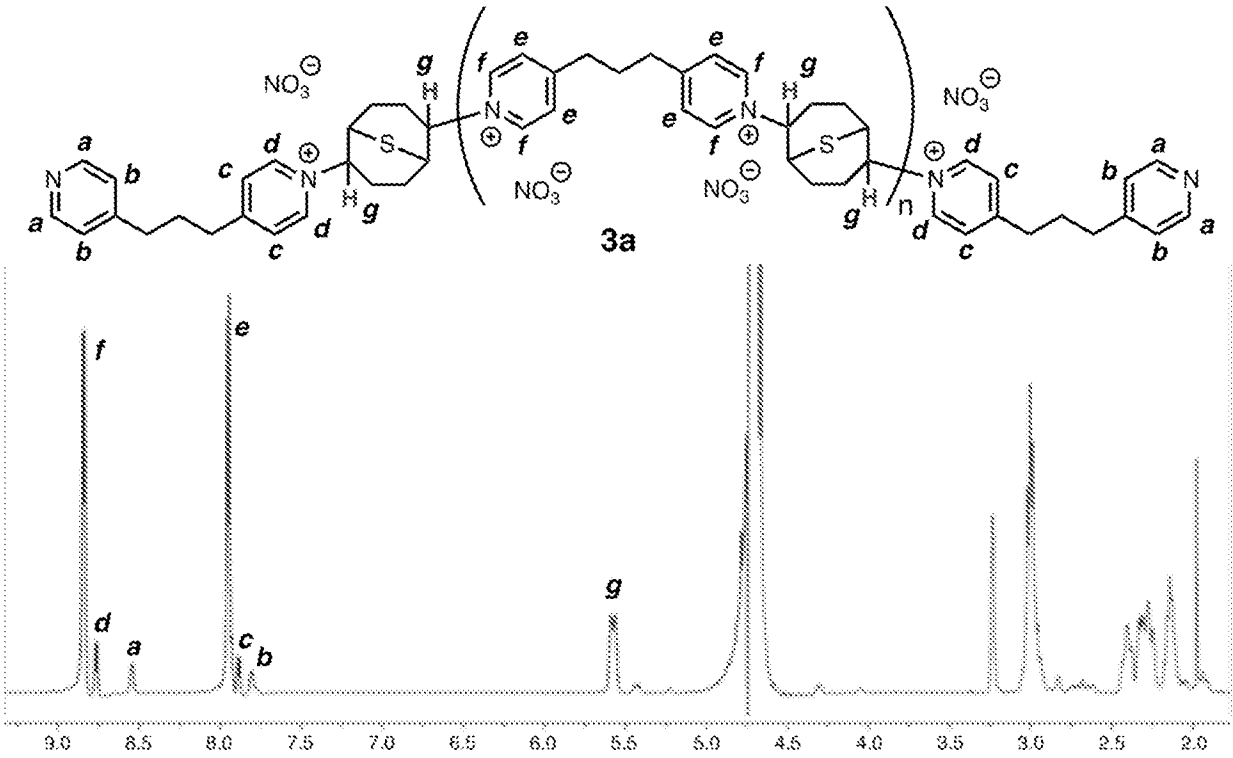


FIGURE 14

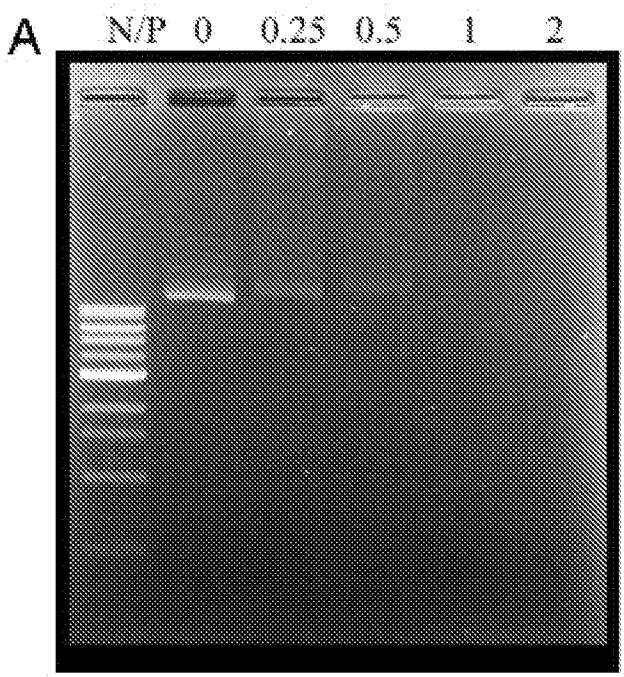


FIGURE 15A

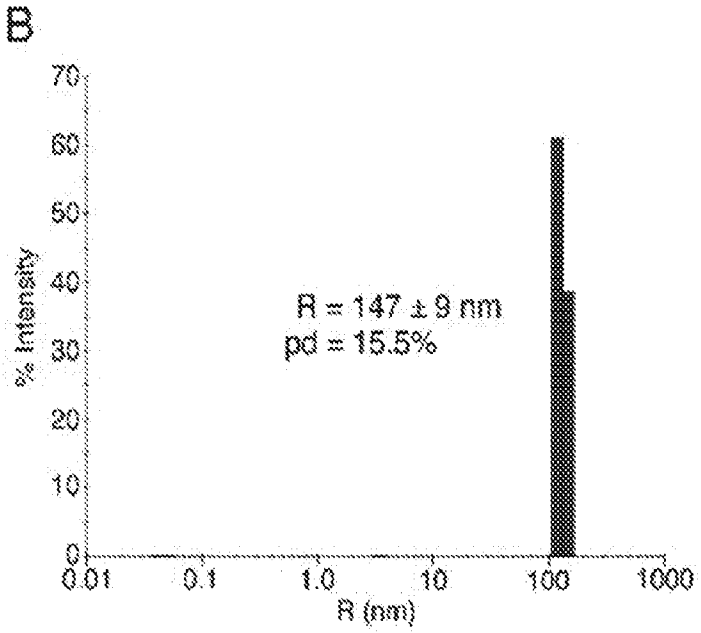

B
70
60
50
40
30
20
10
0
% Intenaity
R = 147 ± 9 nm
pd = 15.5%
0.01
0.1
1.0
10
100
1000
R (nm)

FIGURE 15B

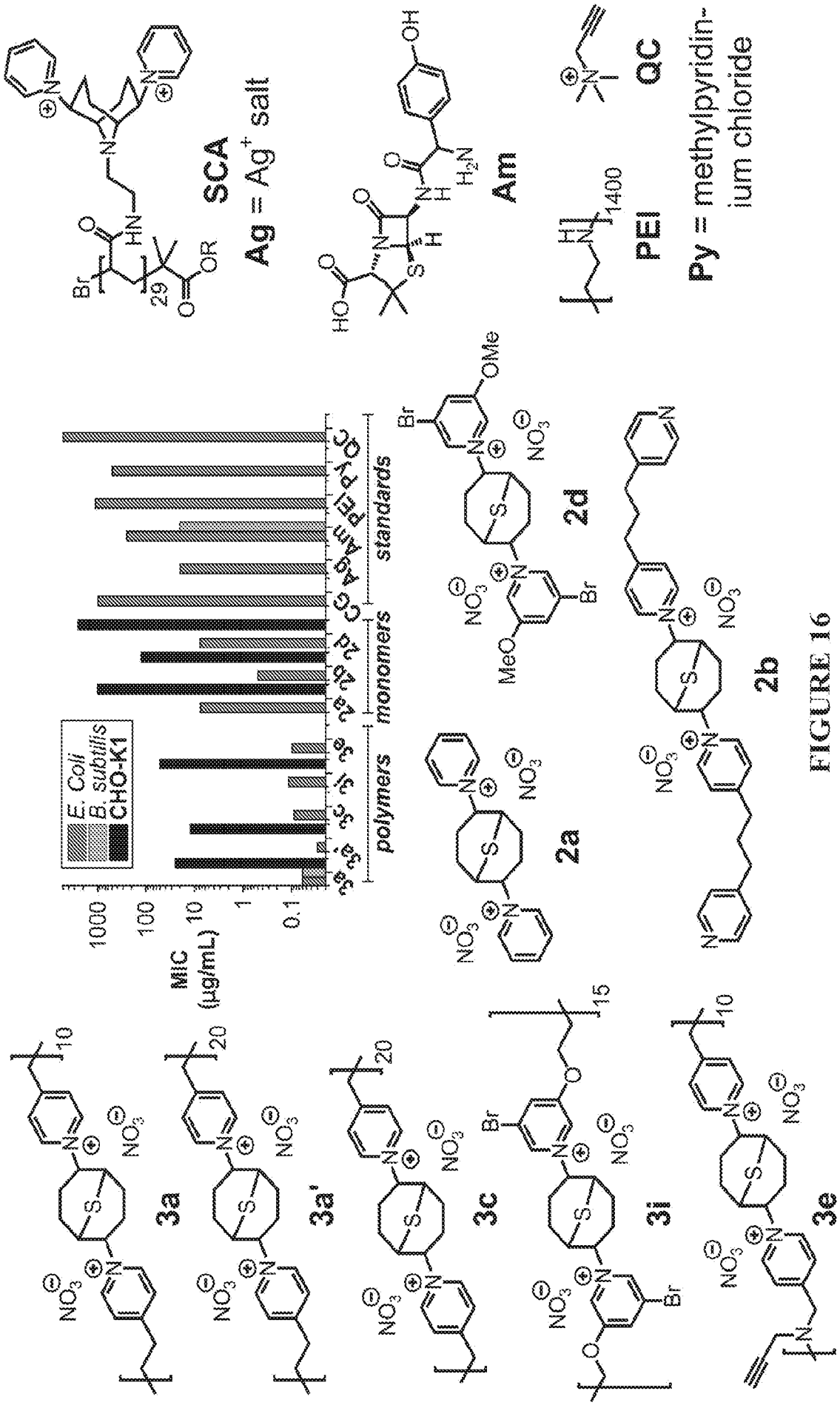

SCA
Ag = Ag+ salt
Am
PEi
QC
Py = methylpyridinium chloride
E. Coli
B. subtilis
CHO-K1
MIC (μg/mL)
1000
100
10
1
0.1
3a
3a'
3c
3i
3e
monomers
standards
polymers
2a
2b
2d
3a
3a'
3c
3i
3e

FIGURE 16

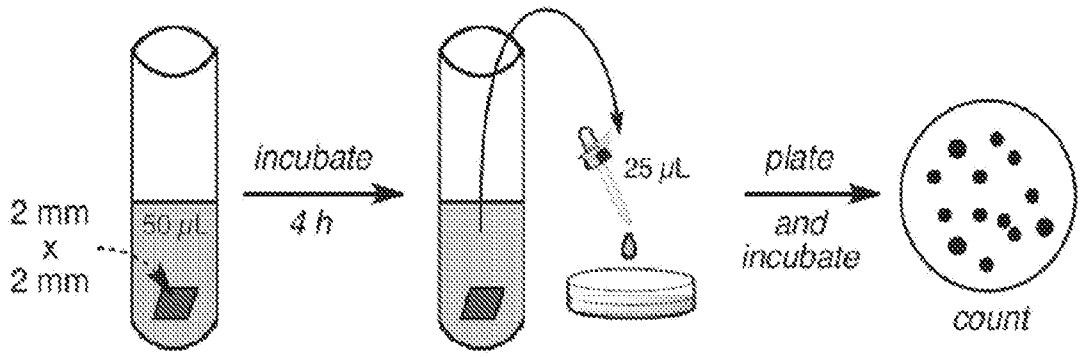


FIGURE 17

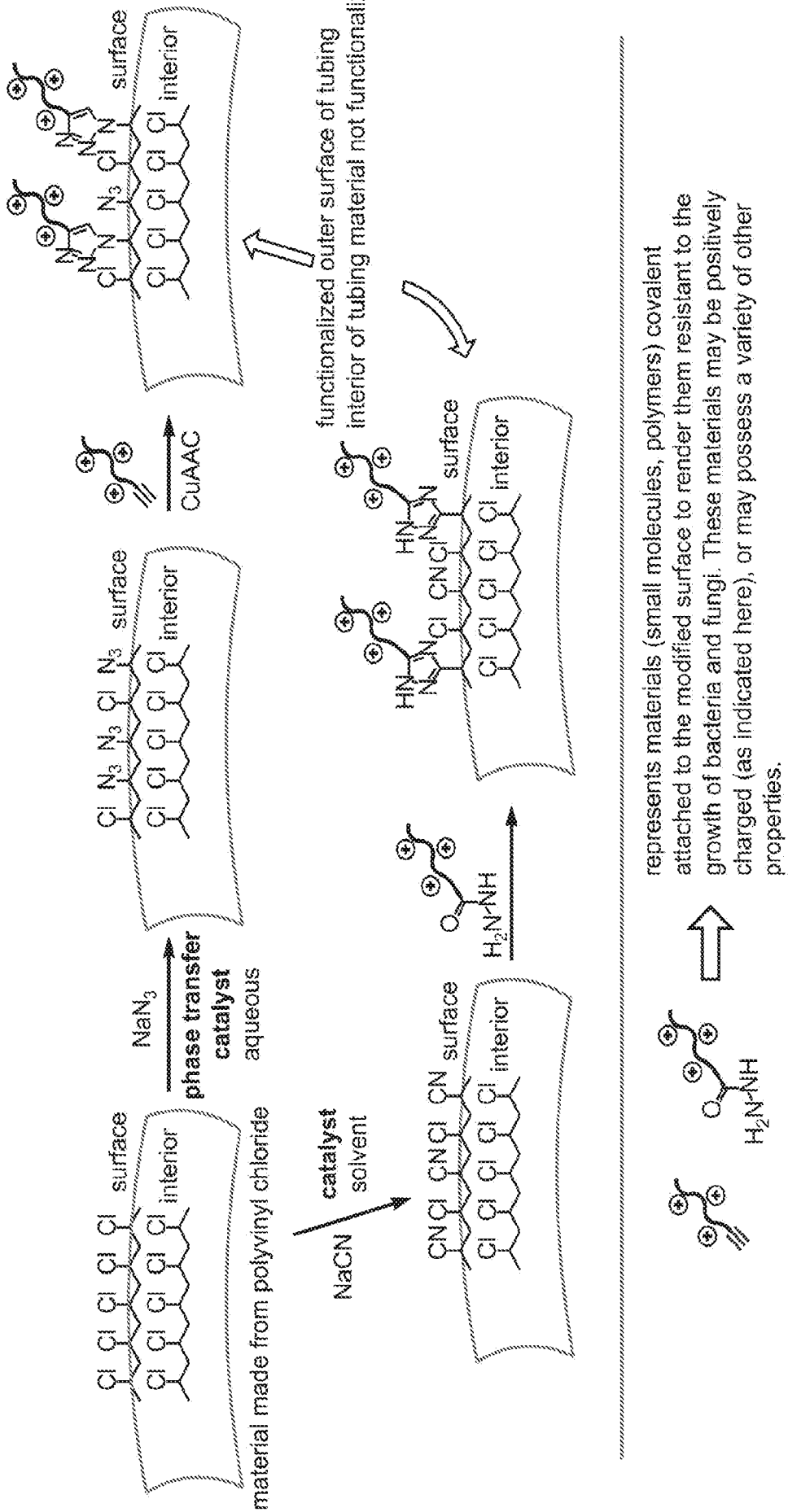

material made from polyvinyl chloride
NaCN
catalyst
solvent
NaN3
phase transfer
catalyst
aqueous
CuAAC
functionalized outer surface of tubing
interior of tubing material not functionalized
surface
interior
represents materials (small molecules, polymers) covalent attached to the modified surface to render them resistant to the growth of bacteria and fungi. These materials may be positively charged (as indicated here), or may possess a variety of other properties.

FIGURE 18

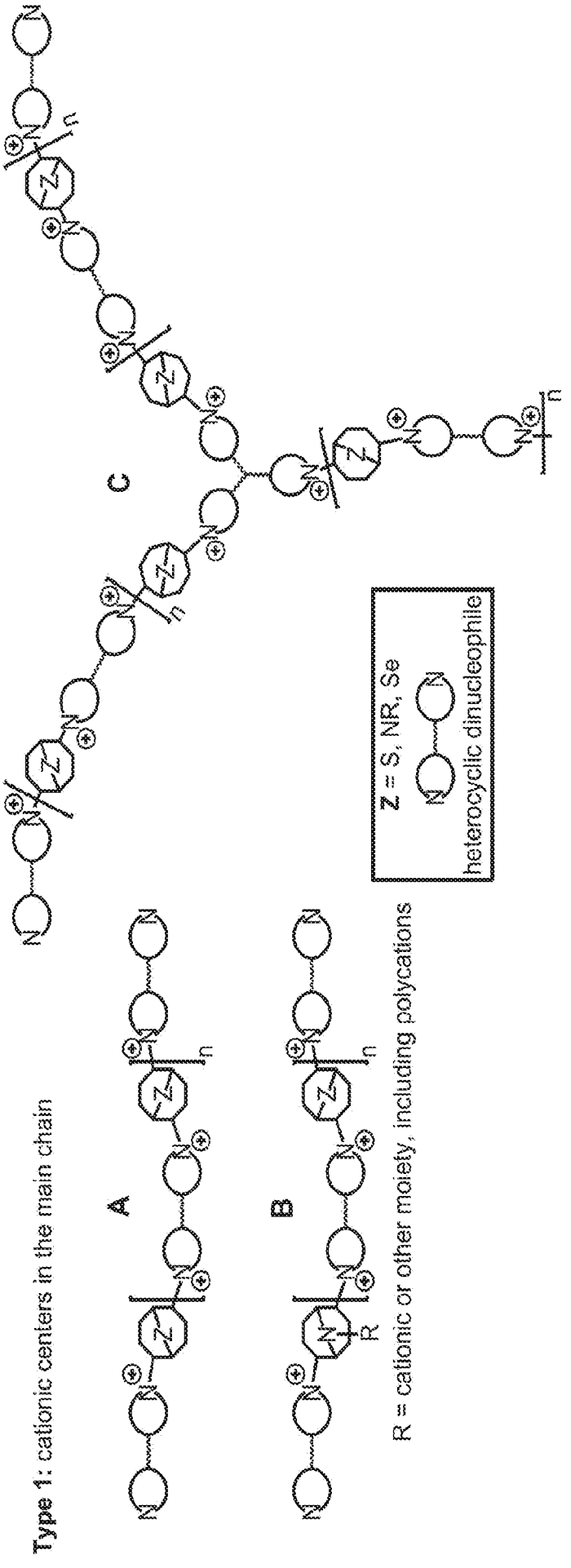

Type 1: cationic centers in the main chain
A
B
C
R = cationic or other moiety, including polycations
Z = S, NR, Se
heterocyclic dinucleophile

FIGURE 19

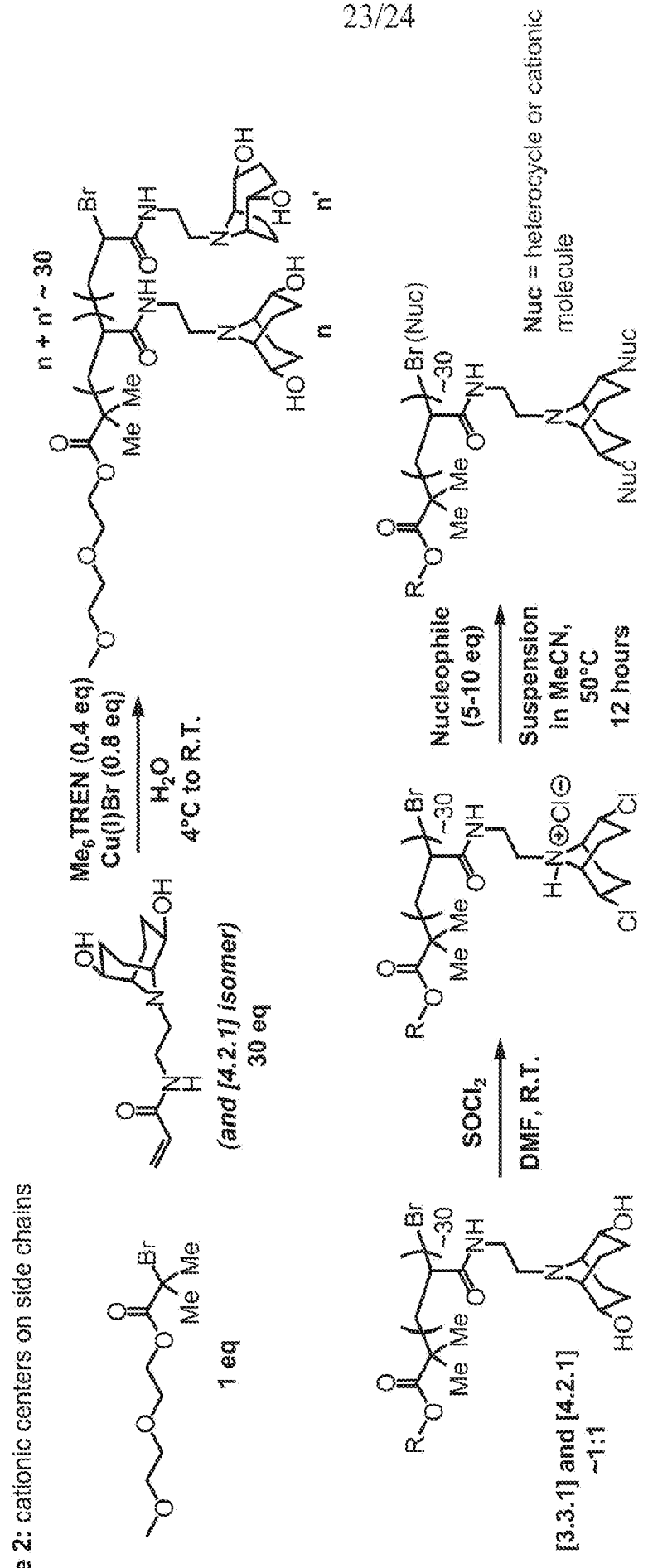


FIGURE 19 continued

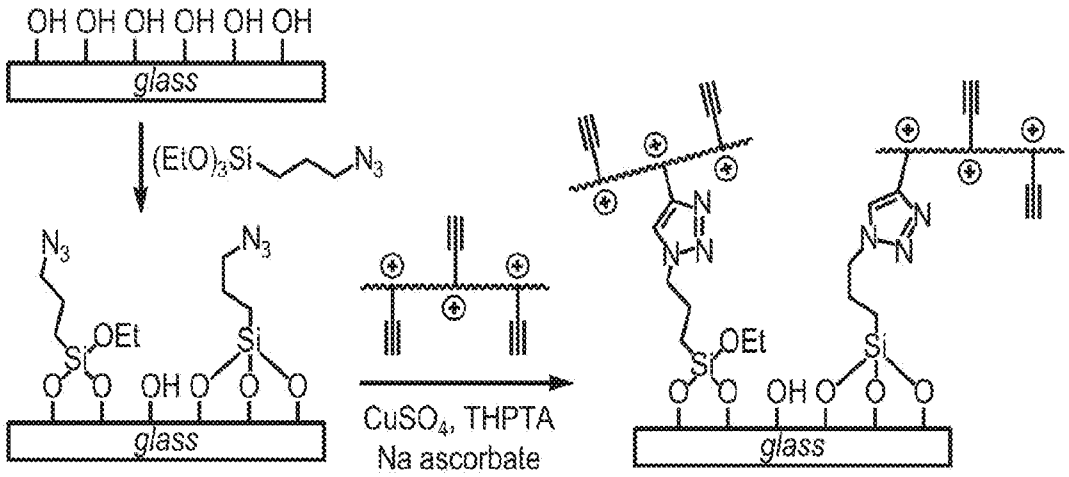


FIGURE 20

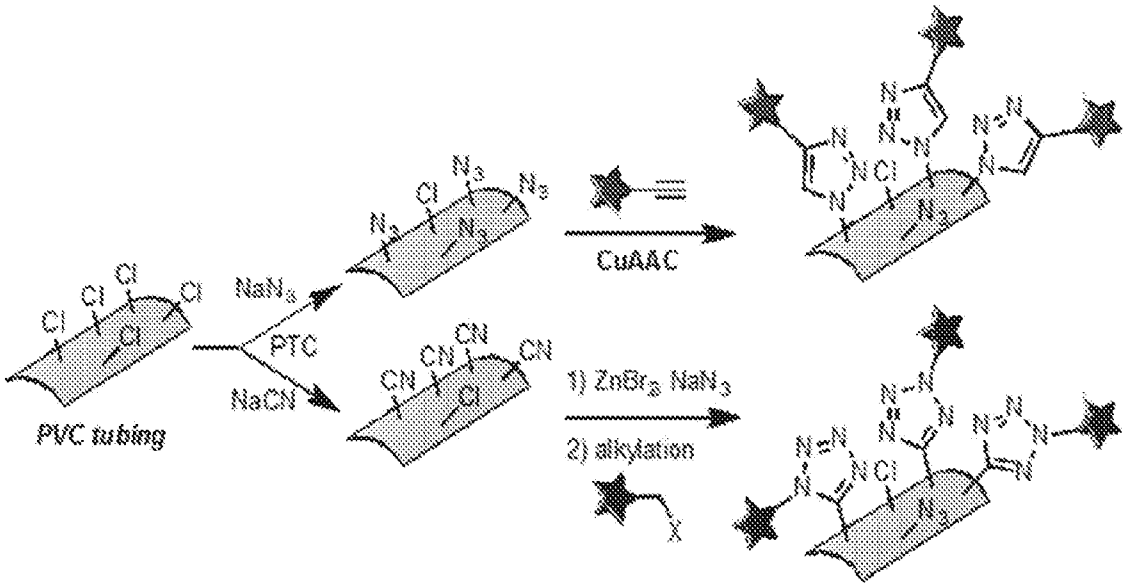


FIGURE 21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/35291

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/40; A61K 31/4425; C09D 5/16 (2017.01)
CPC - C09D5/16; A61K31/4425; C08J7/04; A01N43/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0238939 A1 (KASHER et al.) 28 August 2014 (28.08.2014) para [0020], [0023], [0048] -[0053], [0080]	1-3
---		---
A		26-32, 34-36
Y	LAKSHMI et al. 'Bacterial adhesion onto azidated poly(vinyl chloride) surfaces', Journal of Biomedical Materials Research Part A, 2002, Vol.61(1), pages 26-32. abstract; pg 27, col 1, para 4 to col 2, para 1	1-3
Y	US 2011/0294384 A1 (LOCKLIN et al.) 01 December 2011 (01.12.2011) para [0031]-[0036], [0058], [0066]	58-60, 69-71
---		---
A		26-32, 34-36
Y	GENG et al. 'Fragmentable Polycationic Materials Based on Anchimeric Assistance', Chem. Mater. 2016, Vol.28 (1), pages 146-152. [published December 16 2015] abstract; pg 146, col 1, para 2 to col 2, para 1; pg 147, Fig 1; pg 149, Table 3; Fig 4; pg 150, col 2, para 3	58-60, 69-71
Y	PUBCHEM CID 223672 Create Date: 26 March 2005 (26.03.2005) pg 3, Fig	60, 71
A	US 2009/0264317 A1 (OFIR et al.) 22 October 2009 (22.10.2009) para [0008]-[0009], [0030]-[0031], [0042], Table 1	26-32, 34-36
A	US 2009/0047517 A1 (CARUSO et al.) 19 February 2009 (19.02.2009) para [0052], [0055]-[0056], [0064], [0114]	26-32, 34-36
A	BINDER et al. "Click' Chemistry in Polymer and Materials Science', Macromol. Rapid Commun. 2007, Vol.28, pages 15-54. pg 16, scheme 1, (b); pg 19, Table 1, Entry 4a, 4b; pg 23, col 1, para 3, scheme 6	26-32, 34-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2017	06 SEP 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/35291

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-25, 33, 37-57, 61-68 and 72-83
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.