

Description

Title of Invention: Acid-Sensitive Degradable Imidazolium Polymers for Antimicrobial Applications

Cross-Reference to Related Application

This application claims priority to Singapore application number 10201808211U filed on 20 September 2018, the disclosure of which is hereby incorporated by reference.

Technical Field

The present invention relates to imidazolium-based oligomers and polymers, specifically imidazolium based oligomers and polymers which may demonstrate antimicrobial activity. Such oligomers may be used in an antimicrobial composition for therapeutic and non-therapeutic purposes.

Background

Antimicrobial resistance (AMR) is one of the critical challenges facing modern society, with predictions estimating about 10 million deaths caused by antimicrobial resistant microorganisms annually by 2050. As a result of longer hospital stays and higher morbidity, the cost of treating antibiotic-resistant infections has been pegged at between US\$150 million to \$30 billion a year. Most worryingly, emerging reports of bacterial infections by strains resistant to all existing drugs underscore the pressing need to tackle the issue of resistance in bacteria.

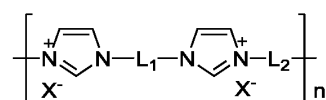
Resistant strains may be contracted directly from animals, water and air, or the community. More importantly, it has been found that resistance to existing antimicrobial treatments may develop through misuse or prolonged exposure of the microorganisms to antibiotics in the environment. The overuse of antibiotics for therapeutic and non-therapeutic applications, such as agricultural and environmental disinfection results in accumulation of low levels of antibiotics in the ecosystem over long periods of time. These antibiotic residues may eventually enter the food chain, where they stand to further contaminate downstream agriculture products. Increased exposure to these low levels of antibiotics in the environment

may lead to the development of resistant strains of bacteria, which survive and propagate through natural selection processes.

Imidazolium-based oligomers and polymers show great promise as antimicrobial compounds due to their high efficacy, selectivity and fast killing kinetics against a broad range of bacteria and fungi. However, the development of such polymers has been rather limited due to growing concerns that such polymers may accumulate in the environment over time, thereby encouraging the development of resistant strains of bacteria. As such, modification or preparation of new imidazolium polymers which may circumvent or reduce selection for resistant microbial strains, are essential. In particular, it is an object of the present invention to provide new imidazolium-based polymers for use as antimicrobials, wherein the imidazolium-based polymers may be safely produced and used without risk or with substantially reduced risk of accumulation in the environment. It is further an object of the present invention to provide such environmentally-friendly antimicrobials without compromising on their antimicrobial properties or efficacy.

Summary of Invention

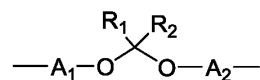
In one aspect, there is provided a polymer having the following Formula (I):



Formula (I)

wherein

L₁ has the following structure:



wherein R₁ and R₂ are, in each instance, same or different, and are independently selected from the group consisting of hydrogen, optionally substituted alkyl, and optionally substituted alkoxy;

A₁ and A₂, are in each instance, same or different, and are optionally substituted aryl;

L₂ is, in each instance, selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl;

X, in each instance, is same or different, and is a halogen;

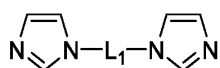
n is an integer of at least 1;
or a salt or hydrate thereof.

Advantageously, the polymer of Formula (I) as described herein may be capable of being degradable under neutral or acidic conditions. In embodiments, the polymer may be capable of being degradable at pH 6 to pH 8. In one preferred embodiment, under the condition of pH 6, the polymer may have a half-life of less than 9 hours, or preferably about 6 hours. These advantageous properties may be attributed to the polymer capable of being cleaved at the position of L₁. Accordingly, the polymer may not be retained or entrained in the natural environment. This may reduce the bioaccumulation of antimicrobials in the ecosystem, which is critical for reducing, preventing or avoiding the development of antimicrobial-resistant microorganisms.

More advantageously, the polymer may exhibit improved or comparable antimicrobial activity over known non-degradable antimicrobial analogues. This may be attributed to the structure of L₁ serving as a hydrophobic region to form an amphiphilic conformation on the imidazolium main chain of the polymer. In embodiments, the polymer may be effective against a plurality of microorganisms, including but not limited to, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. In one preferred embodiment, the microorganism activity may be reduced to about 1% within 10 minutes. In another preferred embodiment, the microorganism activity may be reduced to about 0.1% within 180 minutes. Also advantageously, the polymer may have low toxicity with less than 10% hemolysis.

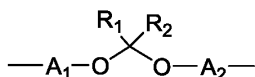
Even more advantageously, the degradation products of the polymer may have weak or substantially no antimicrobial activity as well as low toxicity with less than 10% hemolysis. This may result in little or substantially no antibiotic residues passing into the ecosystem, reducing the potential of secondary environmental contamination.

In another aspect, there is provided a method for preparing a polymer as described herein, comprising the step of:
contacting a di-imidazole having the following Formula (II):



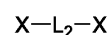
Formula (II)

wherein L_1 has the following structure:



wherein R_1 and R_2 are, in each instance, same or different, and are independently selected from the group consisting of hydrogen, optionally substituted alkyl, and optionally substituted alkoxy; and A_1 and A_2 , are in each instance, same or different, and are optionally substituted aryl;

with a dihalide having the following Formula (III):



Formula (III)

wherein L_2 is, in each instance, selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl; and X , in each instance, is same or different, and is a halogen.

In another aspect, there is provided an antimicrobial composition comprising a polymer as described herein or a salt or hydrate thereof.

In another aspect, there is provided a method for killing or inhibiting the growth of a microorganism, the method comprising contacting said microorganism *ex vivo* with an antimicrobial composition as described herein.

In another aspect, there is provided non-therapeutic use of an antimicrobial composition as described herein, for killing or inhibiting the growth of a microorganism *ex vivo*.

In another aspect, there is provided an antimicrobial composition as described herein for use as an antibiotic.

In another aspect, there is provided a method for treating a microbial infection, the method comprising administering to a subject an antimicrobial composition as described herein.

In another aspect, there is provided use of an antimicrobial composition as described herein,

in the manufacture of a medicament for treating a microbial infection.

Brief Description of Drawings

Figure 1a is a graph showing the efficiency of IBN-AP2 and IBN-OP4 against *E. Coli* at concentrations of 4 µg/ml or 8 µg/ml. *E. coli* grew in pure Mueller Hinton Broth (MHB) was used as control. The data are expressed as mean of the surviving colony forming units $\pm$ standard deviation of triplicates. Data was obtained in triplicates.

Figure 1b is a graph showing the efficiency of IBN-AP4 and IBN-OP3 against *E. coli* at concentrations of 4 µg/ml or 8 µg/ml. *E. coli* grew in pure Mueller Hinton Broth (MHB) was used as control. The data are expressed as mean of the surviving colony forming units $\pm$ standard deviation. Data was obtained in triplicates.

Figure 2a is a plot of the concentration of the acetal-linked polymer IBN-AP2 over 14 days. The graph depicts the degradation of the IBN-AP2 polymer, measured by incubation the polymer at a concentration of 4 mg/ml in 100 mM Sorenson's phosphate buffer at pH 6, 7 and 8.

Figure 2b is a plot of the concentration of the acetal-linked polymer IBN-AP4 over 14 days. The graph depicts the degradation of the IBN-AP4 polymer, measured by incubation of the polymer at a concentration of 4 mg/ml in 100 mM Sorenson's phosphate buffer at pH 6, 7 and 8.

Figure 2c is a plot of the concentration of the orthoformate-linked polymer IBN-OP2 over 14 days. The graph depicts the degradation of the IBN-OP2 polymer, measured by incubation of the polymer at a concentration of 4 mg/ml in 100mM Sorenson's phosphate buffer at pH 6, 7 and 8.

Figure 2d is a plot of the concentration of the orthoformate-linked polymer IBN-OP3 over 14 days. The graph depicts the degradation of the IBN-OP3 polymer, measured by incubation of the polymer at a concentration of 4 mg/ml in 100mM Sorenson's phosphate buffer at pH 6, 7 and 8.

Figure 2e is a plot of the concentration of the orthoformate-linked polymer IBN-OP4 over 14 days. The graph depicts the degradation of the IBN-OP4 polymer, measured by incubation of the polymer at a concentration of 4 mg/ml in 100mM Sorenson's phosphate buffer at pH 6, 7 and 8.

Figure 3a is a graph of the change in MIC values of IBN-AP4 in solutions of different pH, against *E.coli*. The MIC values were measured by dissolving the IBN-AP4 polymer in rain water and Sorenson's phosphate buffer at pH 6, 7 and 8, respectively. The relative activity of the polymer at a given point is expressed as a fraction of its MIC on day 0 against its MIC at the time of measurement.

Figure 3b is a graph of the change in MIC values of IBN-AP4 in solutions of different pH against *S.aureus*. The MIC values were measured by dissolving the IBN-AP4 polymer in rain water and Sorenson's phosphate buffer at pH 6, 7 and 8, respectively. The relative activity of the polymer at a given point is expressed as a fraction of its MIC on day 0 against its MIC at the time of measurement.

Figure 3c is a graph of the change in MIC values of IBN-OP3 in solutions of different pH against *E.coli*. The MIC values were measured by dissolving the IBN-OP3 polymer in rain water and Sorenson's phosphate buffer at pH 6, 7 and 8, respectively. The relative activity of the polymer at a given point is expressed as a fraction of its MIC on day 0 against its MIC at the time of measurement.

Figure 3d is a graph of the change in MIC values of IBN-OP3 in solutions of different pH against *S.aureus*. The MIC values were measured by dissolving the IBN-OP3 polymer in rain water and Sorenson's phosphate buffer at pH 6, 7 and 8, respectively. The relative activity of the polymer at a given point is expressed as a fraction of its MIC on day 0 against its MIC at the time of measurement.

Figure 4 is a ^1H NMR spectra of IBN-OP3 in $\text{d}^6\text{-DMSO}$ at the δ 6.5 – 10.3 ppm region. The peaks corresponding to the imidazole (δ 6.9 or 7.2 ppm) protons 401 and imidazolium protons 402 (δ 9.5–9.4 ppm) are indicated. The area under these peaks were integrated to determine the number of imidazole and imidazolium protons in the polymer.

Figure 5a is an overlay of ^1H NMR spectra of IBN-AP4 in 100mM PBS in D_2O at pH of 6, measured over the course of 4 days. The degradation of the IBN-AP4 polymer was observed via the disappearance of the dimethyl acetal protons (504) at about δ 1.5-1.7 ppm, and the appearance of the alkene protons (501) of the degradation product.

Figure 5b is a ^1H NMR spectrum of the independently prepared F-diol4 in D_2O , which is postulated to be one of the degradation products.

Figure 5c is a scheme illustrating the degradation of IBN-AP4 to F-diol4 under acidic conditions. The protons which may be used to observe the degradation of IBN-AP4 are assigned accordingly.

Figure 6a is an overlay of ^1H NMR spectra of IBN-OP3 in 100mM PBS in D_2O at pH 6, measured over 48 h. The degradation of the IBN-OP3 polymer was observed via the gradual disappearance of the CH_3 protons of the orthoformate $-\text{OCH}_2\text{CH}_3$ group (604) at about δ 1-1.2 ppm, and appearance of the $-\text{CH}_3$ protons of ethyl formate (606), the $-\text{CH}_3$ protons of ethanol (605), and the appearance of formate protons (601) at about δ 8.0 ppm.

Figure 6b is a ^1H NMR spectrum of the independently prepared F-diol3 in D_2O , which is postulated to be one of the degradation products.

Figure 6c is a scheme illustrating the degradation IBN-OP3 to F-diol3 under acidic conditions. The protons which are used to observe the degradation of IBN-OP3 are assigned accordingly.

Definitions

The following words and terms used herein shall have the meaning indicated:

In the definitions of a number of substituents below it is stated that "the group may be a terminal group or a bridging group". This is intended to signify that the use of the term is intended to encompass the situation where the group is a linker between two other portions of the molecule as well as where it is a terminal moiety. Using the term alkyl as an example, some publications would use the term "alkylene" for a bridging group and hence in these other publications there is a distinction between the terms "alkyl" (terminal group) and "alkylene" (bridging group). In the present application no such distinction is made and most

groups may be either a bridging group or a terminal group.

The term "amphiphilic conformation" as used herein refers to a structure having discrete hydrophilic and hydrophobic regions which are arranged alternately in an amphiphilic topology, i.e., the hydrophilic and hydrophobic regions are opposite facing relative to one another.

The term "polymer" as used herein refers to a large molecule, or macromolecule, composed of repeating units. Polymers may comprise at least one repeating unit and may comprise an infinite number of repeating units.

The term "alkyl" as a group or part of a group refers to a straight or branched aliphatic hydrocarbon group having but not limited to, from 1 to 16 carbon atoms, eg, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 carbon atoms, preferably a C₁-C₁₆ alkyl, C₁-C₁₂ alkyl, more preferably a C₁-C₁₀ alkyl, most preferably C₁-C₆ alkyl unless otherwise noted. Examples of suitable straight and branched alkyl substituents include but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, undecyl, 2,2,3-trimethyl-undecyl, dodecyl, 2,2-dimethyl-dodecyl, tridecyl, 2-methyl-tridecyl, 2-methyltridecyl, tetradecyl, 2-methyl-tetradecyl, pentadecyl, 2-methyl-pentadecyl, hexadecyl, 2-methyl-hexadecyl and the like. The group may be a terminal group or a bridging group. The alkyl may be optionally substituted with one or more groups as defined under the term "optionally substituted" below.

The term "aryl" as a group or part of a group to be interpreted broadly denotes (i) an optionally substituted monocyclic, or fused polycyclic, aromatic carbocycle (ring structure having ring atoms that are all carbon) preferably having from 5 to 12 atoms per ring, wherein the optionally substitution can be di-substitution, or tri-substitution. Examples of aryl groups include phenyl, naphthyl, and the like; (ii) an optionally substituted partially saturated bicyclic aromatic carbocyclic moiety in which a phenyl and a C₅-C₇ cycloalkyl or C₅-C₇ cycloalkenyl group are fused together to form a cyclic structure, such as tetrahydronaphthyl, indenyl or indanyl. The group may be a terminal group or a bridging group. Typically an aryl group is a C₆-C₂₀ aryl group. The aryl may be optionally substituted with one or more groups

as defined under the term "optionally substituted" below.

The term "arene" as used herein refers to hydrocarbons with sigma bonds and delocalized pi electrons between carbon atoms forming a circle. The arene may also refer to an aromatic hydrocarbon. The arene may be monocyclic or polycyclic. The arene may have but not limited to, at least 6 carbon atoms, 6 to 20 carbon atoms, or 6 to 12 carbon atoms. Examples of arene include but not limited to, benzene, methylbenzene, ethylbenzene, xylene, and diethylbenzene. The arene may be optionally substituted with one or more groups as defined under the term "optionally substituted" below.

The term "alkyloxy" or "alkoxy" refers to an alkyl-O- group to be interpreted broadly in which alkyl is as defined herein. The alkyloxy is a C₁-C₁₆ alkyloxy, C₁-C₁₂ alkyloxy, more preferably a C₁-C₁₀ alkyloxy, most preferably C₁-C₆ alkyloxy. Examples include, but are not limited to, methoxy, ethoxy and propoxy. The group may be a terminal group or a bridging group. The term alkyloxy may be used interchangeably with the term "alkoxy". The alkyloxy or alkoxy may be optionally substituted with one or more groups as defined under the term "optionally substituted" below.

The term "alkenyl" as a group or part of a group denotes an aliphatic hydrocarbon group containing at least one carbon-carbon double bond and which may be straight or branched having but not limited to, at least 2 carbon atoms, 2-20 carbon atoms, 2-10 carbon atoms, 2-6 carbon atoms, or any number of carbons falling within these ranges, in the normal chain. The group may contain a plurality of double bonds in the normal chain and the orientation about each is independently E, Z, cis or trans where applicable. Exemplary alkenyl groups include, but are not limited to, ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl and nonenyl. The group may be a terminal group or a bridging group. The alkenyl may be optionally substituted with one or more groups as defined under the term "optionally substituted" below.

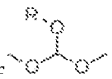
The term "alkynyl" as used herein includes within its meaning unsaturated aliphatic hydrocarbon groups having but not limited to, at least 2 carbon atoms or 2 to 20 carbon atoms, and having at least one triple bond anywhere in the carbon chain. Examples of alkynyl groups include but are not limited to ethynyl, 1-propynyl, 1-butylnyl, 2-butylnyl, 1-methyl-2-butylnyl, 3-methyl-1-butylnyl, 1-pentylnyl, 1-hexynyl, methylpentylnyl, 1-heptylnyl, 2-heptylnyl,

1-octynyl, 2-octynyl, 1-nonyl, 1-decynyl, and the like. The group may be a terminal group or a bridging group. The alkynyl may be optionally substituted with one or more groups as defined under the term "optionally substituted" below.

The term "halo" or "halogen" as used herein refers to fluorine, chlorine, bromine and iodine.

The term "dihalide" as used herein refers to compounds containing two halogen atoms, where the two halogen atoms may be same or different, and each of them may be bonded to a carbon atom.

The term "orthoformate linker" as used herein refers to functional groups containing three alkoxy groups attached to one carbon atom. The orthoformate linker may have a general

formula of , wherein R may be optionally substituted alkyl.

The term "acetal linker" as used herein refers to functional groups containing two alkoxy groups attached to one carbon atom. The acetal linker may have a general formula of

, wherein R₁ and R₂ may be optionally substituted alkyl.

The term "alcohol" as used herein refers to compounds in which the hydroxyl functional group (–OH) is bound to a carbon. The alcohol may have but not limited to, at least 1 carbon atom, 1 to 20 carbon atoms, 1 to 12 carbon atoms, 1 to 6 carbon atoms, 2 to 6 carbon atoms, or 2 to 4 carbon atoms. Examples of alcohol include but not limited to, methanol, ethanol, propan-1-ol, propan-2-ol, 2-methylpropan-1-ol, 2-methylpropan-2-ol, butan-1-ol and butan-2-ol. The alcohol may be optionally substituted with one or more groups as defined under the term "optionally substituted" below.

The term "diol" as used herein refers to compounds containing two hydroxyl groups (–OH groups).

The term "minimum inhibitory concentration (MIC)" as used herein refers to the concentration of an antimicrobial agent at which no meaningful microorganism growth was

observed. The growth of microorganisms may be detected through cell counting methods; microscopy techniques; by measuring the weight of cells isolated from culture media; or by measuring the turbidity of the culture medium. The turbidity of the culture medium may be measured using a turbidimeter, or by spectroscopic means, such as by determining optical density of the medium at a specific wavelength.

The term “hemolysis” as used herein refers to the rupturing (lysis) of red blood cells and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma). Hemolysis may occur inside or outside the body.

The term “ex vivo” as used herein refers to experimentation or measurements done in or on tissue from an organism in an external environment with minimal alteration of natural conditions.

It is understood that included in the family of compounds of Formula (I) are isomeric forms including diastereoisomers, enantiomers, tautomers, and geometrical isomers in "E" or "Z" configurational isomer or a mixture of E and Z isomers. It is also understood that some isomeric forms such as diastereomers, enantiomers, and geometrical isomers can be separated by physical and/or chemical methods and by those skilled in the art.

Some of the compounds of the disclosed embodiments may exist as single stereoisomers, racemates, and/or mixtures of enantiomers and/or diastereomers. All such single stereoisomers, racemates and mixtures thereof, are intended to be within the scope of the subject matter described and claimed.

The term “optionally substituted” as used herein refers to the group to which this term refers may be unsubstituted, or may be substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenyl, cycloalkylalkenyl, heterocycloalkyl, cycloalkylheteroalkyl, cycloalkyloxy, cycloalkenyloxy, cycloamino, halo, carboxyl, haloalkyl, haloalkynyl, alkynyloxy, heteroalkyl, heteroalkyloxy, hydroxyl, hydroxyalkyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyl, haloalkynyl, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, aminoalkyl, alkynylamino, acyl, alkyloxy, alkyloxyalkyl, alkyloxyaryl, alkyloxycarbonyl, alkyloxycycloalkyl,

alkyloxyheteroaryl, alkyloxyheterocycloalkyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocyclic, heterocycloalkenyl, heterocycloalkyl, heterocycloalkylalkyl, heterocycloalkylalkenyl, heterocycloalkylheteroalkyl, heterocycloalkyloxy, heterocycloalkenyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfinyl, alkylsulfonyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, aminosulfonyl, phosphorus-containing groups such as phosphono and phosphinyl, sulfinyl, sulfinylamino, sulfonyl, sulfonylamino, aryl, heteroaryl, heteroarylalkyl, heteroarylalkenyl, heteroarylheteroalkyl, heteroarylamino, heteroaryloxy, arylalkenyl, arylalkyl, alkylaryl, alkylheteroaryl, aryloxy, arylsulfonyl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)₂. Where the term "substituted" is used, the group to which this term refers to may be substituted with one or more of the same groups mentioned above.

The term "half-life" as used herein refers to the time required for a quantity to reduce to half of its initial value. Specifically, the half-life may be the time required for the concentration of substance to decrease to half of its initial concentration value.

The term "pharmaceutically acceptable salts" as used herein refers to salts that retain the desired biological activity of the above -identified compounds, and include pharmaceutically acceptable acid addition salts and base addition salts. Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, sulfuric, and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, heterocyclic carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, fumaric, maleic, alkyl sulfonic, arylsulfonic. Additional information on pharmaceutically acceptable salts can be found in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Co., Easton, PA 1995. In the case of agents that are solids, it is understood by those skilled in the art that the inventive compounds, agents and salts may exist in different crystalline or polymorphic forms, all of which are intended to be within the scope of the present disclosure and specified formulae.

The term "therapeutically effective amount" or "effective amount" as used herein refers to an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. An effective amount is typically sufficient to

palliate, ameliorate, stabilize, reverse, slow or delay the progression of the disease state.

The term "microorganism" as used herein, refers broadly to both eukaryotic and prokaryotic organisms possessing a cell membrane, including but not limited to, bacteria, yeasts, fungi, plasmids, algae and protozoa.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically, $\pm 2\%$ of the stated value, even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

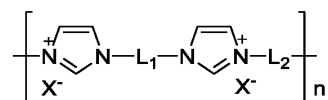
Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of

whether or not the excised material is specifically recited herein.

Detailed Disclosure of Optional Embodiments

Exemplary, non-limiting embodiments of a polymer of Formula (I) will now be disclosed.

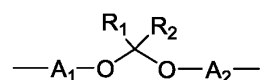
In one aspect, the present polymer may be of Formula (I):



Formula (I)

wherein

L₁ has the following structure:



wherein R₁ and R₂ are, in each instance, same or different, and are independently selected from the group consisting of hydrogen, optionally substituted alkyl, and optionally substituted alkoxy; A₁ and A₂, are in each instance, same or different, and are optionally substituted aryl; L₂ is, in each instance, selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl; X, in each instance, is same or different, and is a halogen; n is an integer of at least 1; or a salt or hydrate thereof.

In some embodiments of the polymer of Formula (I), R₁ and R₂ may, in each instance, be same or different, and be independently selected from the group consisting of hydrogen and optionally substituted alkoxy. The alkoxy may be a liner or a branched group. The alkoxy may be a C₁-C₁₆ alkoxy, a C₁-C₁₂ alkoxy, more preferably a C₁-C₁₀ alkoxy, most preferably a C₁-C₈ alkoxy. Preferably, the C₁-C₈ alkoxy may be methoxy, ethoxy, propoxy isopropoxy, 1-butoxy, 2-butoxy or isobutoxy. In preferred embodiments, at least one of R₁ and R₂ may be selected from C₁-C₈ alkoxy. In more preferred embodiments, R₁ may be hydrogen and R₂ may be selected from methoxy, ethoxy, propoxy, or isopropoxy. In most preferred embodiments, R₁ is hydrogen and R₂ is ethoxy. In embodiments, an orthoformate linker may be formed in L₁ structure in the polymer of Formula (I).

Advantageously, the polymer of Formula (I) as described herein may be capable of being cleaved at the position of the orthoformate linker so as to being degradable under a neutral to acidic condition where pH is no more than 8. In certain embodiments, under the condition of pH 6, the polymer having orthoformate linker may have a half-life of less than 24 hours, or preferably less than 9 hours. In certain embodiments, under the condition of pH 7, the polymer having orthoformate linker may have a half-life of less than 72 hours, preferably less than 15 hours, or more preferably less than 12 hours. In certain embodiments, under the condition of pH 8, the polymer having orthoformate linker may have a half-life of less than 20 days, less than 8 days, or preferably less than 6 days. In one embodiment, more than 90% of the polymer may have been degraded after 90 days.

In some other embodiments of the polymer of Formula (I), R_1 and R_2 may be, in each instance, same or different, and be independently selected from optionally substituted alkyl. The alkyl may be a linear or a branched group. The alkyl may be a C_1 - C_{16} alkyl, a C_1 - C_{12} alkyl, more preferably a C_1 - C_{10} alkyl, most preferably a C_1 - C_8 alkyl. Preferably, the C_1 - C_8 alkyl may be methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, or isobutyl. In preferred embodiments, R_1 and R_2 may be same and be selected from methyl, ethyl, 1-propyl, or isopropyl. In more preferred embodiments, R_1 and R_2 are methyl. In embodiments, an acetal linker may be formed in L_1 structure in the polymer of Formula (I).

Advantageously, the polymer of Formula (I) as described herein may be capable of being cleaved at the position of the acetal linker so as to be degradable under neutral to acidic conditions, for example, conditions where pH is no more than 8. In certain embodiments, under conditions of pH 6, the polymer having an acetal linker may have a half-life of less than 30 hours, or preferably less than 6 hours. In certain embodiments, under conditions of pH 7, the polymer having acetal linker may have a half-life of less than 72 hours, or preferably less than 10 hours. In certain embodiments, under the condition of pH 8, the polymer having acetal linker may have a half-life of less than 12 days, or preferably less than 6 days.

Also advantageously, the polymer of Formula (I) as described herein may show improved or comparable antimicrobial activity with known non-degradable antimicrobial analogues. This may be attributed to the structure of L_1 . Without being bound by theory, an imidazolium main chain in an amphiphilic conformation. The amphiphilic conformation having hydrophobic

regions and hydrophilic regions arranged alternately may be formed by imidazole rings serving as hydrophilic regions, and L₁ serving as hydrophobic regions. Such imidazolium main chain in an amphiphilic conformation may facilitate the antimicrobial activity of the polymer.

In the polymer of Formula (I), A₁ and A₂ may be, in each instance, same or different, and be independently selected from optionally substituted aryl. In preferred embodiments, A₁ and A₂ may be selected from C₈-C₂₀ aryl group. The C₈-C₂₀ aryl may be derived from an arene. The arene may be a monocyclic arene or a polycyclic arene. Preferably, the arene may be a monocyclic arene. Preferably, the monocyclic arene may be selected from benzene, methylbenzene, ethylbenzene, xylene, or diethylbenzene. In more preferred embodiments, A₁ and A₂ may be independently selected from xylylenediyl, which is an aryl group derived from diethylbenzene. In most preferred embodiments, A₁ and A₂ are same and are selected from ortho-xylylenediyl or para-xylylenediyl.

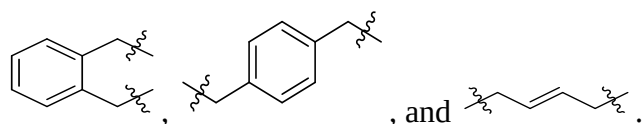
Advantageously, the polymer of Formula (I) where A₁ and A₂ are para-xylylenediyl may exhibit a shorter degradation period under neutral or acidic condition compared to the polymer where A₁ and A₂ are ortho-xylylenediyl. Under acidic condition, specifically at pH of 6, the half-life of the polymer having para-xylylenediyl groups may be about 60%-80% shorter than that of the polymer having ortho-xylylenediyl groups, showing a faster degradation speed. Under the condition of pH 8, the polymer of Formula (I) may display similar stability, where A₁ and A₂ groups may have less influences on the half-lives of the polymers.

In one preferred embodiment, L₁ may be provided where R₁ is H, R₂ is ethoxy, and A₁ and A₂ are para-xylylenediyl. In another preferred embodiment, L₁ may be provided where R₁ is H, R₂ is ethoxy, and A₁ and A₂ are ortho-xylylenediyl. In a further preferred embodiment, L₁ may be provided where R₁ and R₂ are methyl and A₁ and A₂ are para-xylylenediyl. In yet another preferred embodiment, L₁ may be provided where R₁ and R₂ are methyl, and A₁ and A₂ are ortho-xylylenediyl.

Advantageously, the present polymer of Formula (I) having the linker L₁ as described herein may exhibit retained or improved antimicrobial activity against a broad range of microbes and possess degradation properties under neutral or acidic condition compared to the known non-degradable antimicrobial analogues. In preferred embodiments, the minimum inhibitory

concentration (MIC) value of the polymer as described herein may be substantially equal to that of known non-degradable antimicrobial analogues. In more preferred embodiments, the MIC value may be at least 50%, or at least 75%, or preferably about 90% less than that of known non-degradable antimicrobial analogues.

In the polymer of Formula (I), L_2 may be, in each instance, selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl. In embodiments, L_2 may be selected from optionally substituted alkenyl or optionally substituted aryl. The alkenyl may be a C_2 - C_{16} alkenyl, a C_2 - C_{12} alkenyl, more preferably a C_2 - C_{10} alkenyl, most preferably a C_2 - C_8 alkenyl. The C_2 - C_8 alkenyl may be butenyl. Preferably, the butenyl may be 2-trans-butenyl or 2-cis-butenyl. The aryl may be a C_8 - C_{20} aryl group. Preferably, the C_8 - C_{20} aryl substituent may be xylylenediyl. In preferred embodiments, L_2 may be xylylenediyl or butenyl. In more preferred embodiments, L_2 is selected from the group consisting of:

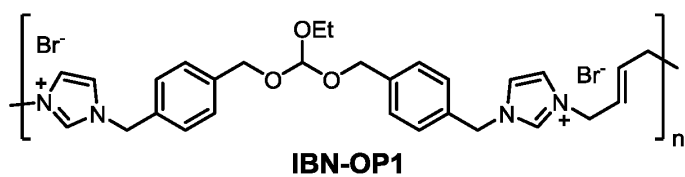
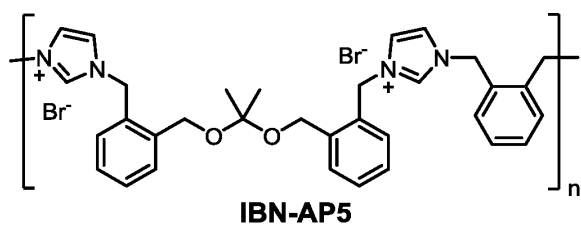
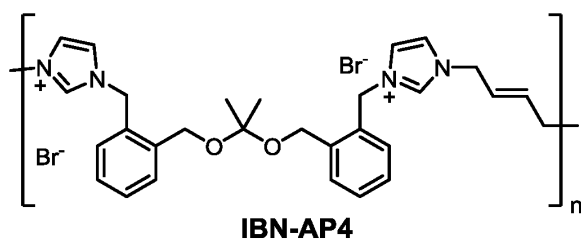
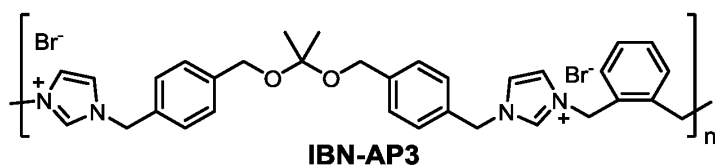
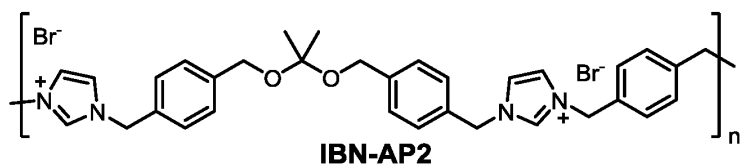
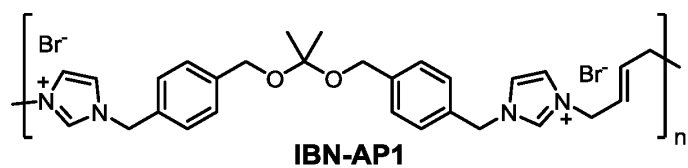


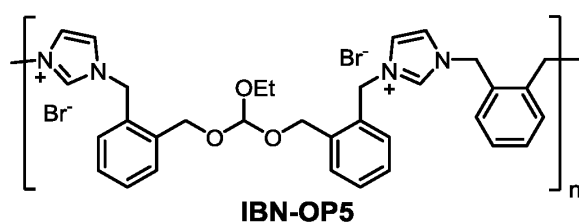
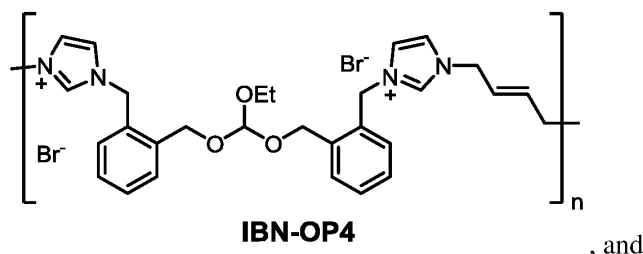
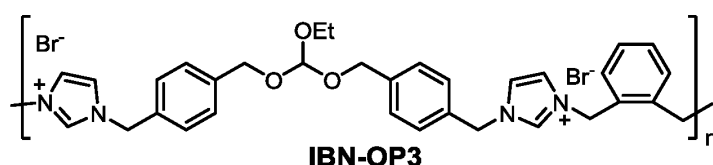
Advantageously, L_2 as described herein may provide a hydrophobic region to form an amphiphilic conformation in the present polymer. Without being bound to theory, this may further contribute to the antimicrobial activity of the polymer.

In the polymer of Formula (I), X may be, in each instance, same or different, and be a halogen. The halogen may be selected from the group consisting of chlorine, fluorine, bromine and iodine. In one embodiment, X is bromine.

In the polymer of Formula (I), n may be an integer of at least 1. Preferably, n may be an integer from 1 to 100, 1 to 50, 1 to 30, 1 to 15, 2 to 15, or 3 to 15. More preferably, n may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. Most preferably, n may be 7.

In embodiments, the polymer of Formula (I) as described herein may be selected from the group consisting of:





Advantageously, the polymers IBN-AP1 to IBN-AP5 and IBN-OP1 to IBN-OP5 as described herein may be capable of being degraded under neutral or acidic conditions. Accordingly, the polymer may not be retained or entrained in the natural environment. This may reduce the bioaccumulation of antimicrobials in the ecosystem, which is critical for reducing, preventing or avoiding the development of antimicrobial-resistant microbes. More advantageously, the disclosed polymer has been found to exhibit retained or improved antimicrobial activity against a plurality of microorganisms compared to known non-degradable antimicrobial analogues.

In preferred embodiments, the polymers IBN-AP2, IBN-AP4, IBN-OP2, IBN-OP3 and IBN-OP4 may advantageously exhibit retained or improved antimicrobial activity. Specifically, the MIC values of these polymers may be substantially equal to or lower than that of known non-degradable antimicrobial analogues against the same species of microorganism, showing improved antimicrobial activity. In preferred embodiments, the MIC value may be about 50-90% lower than that of the known non-degradable antimicrobial analogues. In more preferred embodiments, the MIC value may be about 90% lower than that of the known non-degradable antimicrobial analogues. More specifically, the polymers may display antimicrobial activity even at a low concentration of 4 µg/ml. Also advantageously, the

polymers as described herein may have less than 10% hemolysis even at a highest concentration of 2000 µg/ml, demonstrating low toxicity.

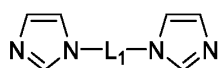
In more preferred embodiments, the polymers IBN-AP4 and IBN-OP3 may advantageously show improved or comparable antimicrobial activity to that of known non-degradable antimicrobial analogues, while possessing degradation properties under neutral or acidic condition. Specifically, the polymers may show a faster killing speed against the microorganisms. In one embodiment, the microorganism population may be reduced to about 1% within 10 minutes. In another embodiment, the microorganism population may be reduced to about 0.1% within 180 minutes.

Advantageously, the polymer of Formula (I) as described herein may be capable of being cleaved at L₁ under neutral or acidic conditions. The neutral condition may be at a pH of no less than 7 and no more than 8. Preferably, the neutral condition may be at pH 7 or pH 8. The acidic condition may be at a pH of less than 7. Preferably, the acidic condition may be at pH 6, pH 5, pH 4, pH 3, pH 2, or pH 1. More preferably, the acidic condition may be mildly acidic. Most preferably, the mildly acidic condition may be at a pH of 6.

More advantageously, the degradation products of the polymers as described herein may have weak or substantially no antimicrobial activity against microorganisms, resulting in little or substantially no antibiotic residues passing into the ecosystem. Even more advantageously, the degradation products may demonstrate low toxicity with less than 10% hemolysis even at a highest concentration of 2000 µg/ml.

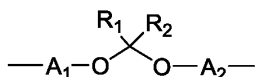
Without being bound by theory, the degradation products of the polymer may comprise di-imidazolium fragments and small molecules. Preferably, the di-imidazolium fragment may be a diol. The small molecule may be a ketone, ester, carboxylic acid or alcohol. Preferably, the small molecule may be acetone, ethyl formate, formic acid and ethanol.

In another aspect, there is provided a method for preparing the polymer of Formula (I) as described herein, comprising the step of:
contacting a di-imidazole having Formula (II):



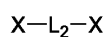
Formula (II)

wherein L_1 has the following structure:



wherein R_1 and R_2 are, in each instance, same or different, and are independently selected from the group consisting of hydrogen, optionally substituted alkyl, and optionally substituted alkoxy; and A_1 and A_2 , are in each instance, same or different, and are optionally substituted aryl;

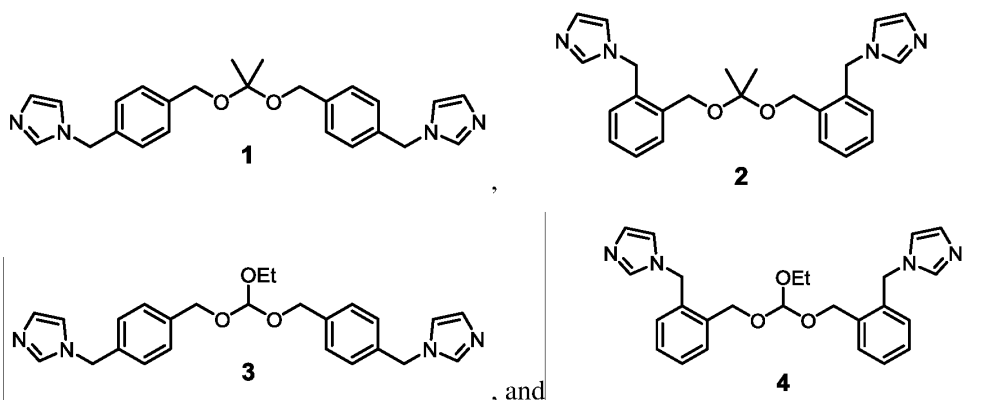
with a dihalide having Formula (III):



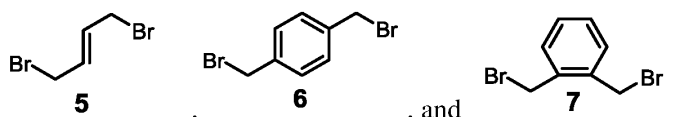
Formula (III)

wherein L_2 is, in each instance, selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl; and X , in each instance, is same or different, and is a halogen.

In embodiments, the di-imidazole of Formula (II) may be selected from the group consisting of:



In embodiments, the dihalide of Formula (III) may be selected from the group consisting of:



In another aspect, an antimicrobial composition may comprise the polymer of Formula (I) as described herein.

In some embodiments, the antimicrobial composition may be used for non-therapeutic applications. Preferably, the non-therapeutic applications may comprise environmental disinfections. More preferably, the non-therapeutic applications may comprise pesticidal, agricultural, or horticultural use.

In preferred embodiments, the present application provides a method for killing or inhibiting the growth of a microorganism *ex vivo*. The method may comprise contacting said microorganism *ex vivo* with the antimicrobial composition as described herein in an effective amount. Preferably, the method may comprise contacting the antimicrobial composition with inanimate surfaces. Non limiting examples of inanimate surfaces may include surfaces of medical devices, hospital interior surfaces, textiles, food packaging, children's toys, or electrical appliances.

In preferred embodiments, the present application provides non-therapeutic use of the antimicrobial composition as described herein. The non-therapeutic use may comprise killing or inhibiting the growth of a microorganism *ex vivo*. Preferably, the non-therapeutic use may comprise contacting the antimicrobial composition with inanimate surfaces. Non-limiting examples of inanimate surfaces may include surfaces of medical devices, hospital interior surfaces, textiles, food packaging, children's toys, or electrical appliances.

The microorganism may be selected from a bacterium, a fungus, or a mixture thereof. The bacterium may be a Gram-positive bacterium or a Gram-negative bacterium. The Gram-positive bacterium may be selected from *Staphylococcus aureus*, *Staphylococcus argenteus*, *Staphylococcus schweitzeri*, *Staphylococcus simiae*, or the mixture thereof. The Gram-negative bacterium may be selected from *Pseudomonas aeruginosa*, *Pseudomonas polycolor*, *Pseudomonas vendrelli*, *Escherichia coli*, *Escherichia fergusonii*, *Escherichia hermannii*, *Escherichia vulneris*, or the mixture thereof. The fungus may be yeast selected from *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, or the mixture thereof. In preferred embodiments, the microorganism may be selected from *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, or the mixture thereof.

When the microorganism is contacted with the antimicrobial composition in an effective amount, the microorganism population may be reduced to in the range of about 1% to about 0.1%, in the range of about 0.5% to about 0.1%, preferably to about 0.5%, more preferably to about 0.1%.

The concentration of the antimicrobial composition as described herein may be in the range of about 1 µg/ml to about 300 µg/ml, about 1 µg/ml to about 200 µg/ml, about 1 µg/ml to about 100 µg/ml, about 1 µg/ml to about 80 µg/ml, about 1 µg/ml to about 62 µg/ml, about 1 µg/ml to about 31 µg/ml, about 1 µg/ml to about 16 µg/ml, about 1 µg/ml to about 8 µg/ml, about 2 µg/ml to about 300 µg/ml, about 2 µg/ml to about 200 µg/ml, about 2 µg/ml to about 100 µg/ml, about 2 µg/ml to about 80 µg/ml, about 2 µg/ml to about 62 µg/ml, about 2 µg/ml to about 31 µg/ml, about 2 µg/ml to about 16 µg/ml, about 2 µg/ml to about 8 µg/ml, about 4 µg/ml to about 300 µg/ml, about 4 µg/ml to about 200 µg/ml, about 4 µg/ml to about 100 µg/ml, about 4 µg/ml to about 80 µg/ml, about 4 µg/ml to about 62 µg/ml, preferably about 4 µg/ml to about 31 µg/ml, more preferably about 4 µg/ml to about 16 µg/ml, or most preferably about 4 µg/ml to about 8 µg/ml. Preferably, the concentration may be 4 µg/ml or 8 µg/ml.

The reduction of the microorganism population may be achieved within a duration of about 0.5 minutes to about 360 minutes, about 0.5 minutes to about 180 minutes, about 0.5 minutes to about 60 minutes, about 0.5 minutes to about 10 minutes, about 1 minute to about 360 minutes, about 1 minute to about 180 minutes, about 1 minute to about 60 minutes, about 1 minute to about 10 minutes, about 2 minutes to about 360 minutes, about 2 minutes to about 180 minutes, about 2 minutes to about 60 minutes, about 2 minutes to about 10 minutes, about 5 minutes to about 360 minutes, about 5 minutes to about 180 minutes, about 5 minutes to about 60 minutes, or about 5 minutes to about 10 minutes. Preferably, the reduction of the microorganism activity may be achieved within 10 minutes.

In some other embodiments, the antimicrobial composition may be used for therapeutic applications. In preferred embodiments, the antimicrobial composition as described herein may further comprise pharmaceutically acceptable salts. Preferably, the antimicrobial composition may be used as an antibiotic to treat a microbial infection. The antimicrobial composition may kill or inhibit the growth of a microorganism so as to treat the microbial infection.

In preferred embodiments, the present application provides a method for treating a microbial infection. The method may comprise administering to a subject the antimicrobial composition as described herein in a therapeutically effective amount. The subject may be a human or animal body. The microbial infection may be caused by one or more microorganism.

In preferred embodiments, the present application provides use of the antimicrobial composition as described herein, in the manufacture of a medicament for treating a microbial infection. The microbial infection may be caused by one or more microorganism.

The microorganism may be selected from a bacterium, a fungus, or the mixture thereof. The bacterium may be a Gram-positive bacterium or a Gram-negative bacterium. The Gram-positive bacterium may be selected from *Staphylococcus aureus*, *Staphylococcus argenteus*, *Staphylococcus schweitzeri*, *Staphylococcus simiae*, or the mixture thereof. The Gram-negative bacterium may be selected from *Pseudomonas aeruginosa*, *Pseudomonas polycolor*, *Pseudomonas vendrelli*, *Escherichia coli*, *Escherichia fergusonii*, *Escherichia hermannii*, *Escherichia vulneris*, or the mixture thereof. The fungus may be yeast selected from *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, or the mixture thereof. In preferred embodiments, the microorganism may be selected from *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, or the mixture thereof.

When the microorganisms are contacted with the antimicrobial composition in a therapeutically effective amount, the microorganism population may be reduced to in the range of about 1% to about 0.1%, in the range of about 0.5% to about 0.1%, preferably to about 0.5%, more preferably to about 0.1%.

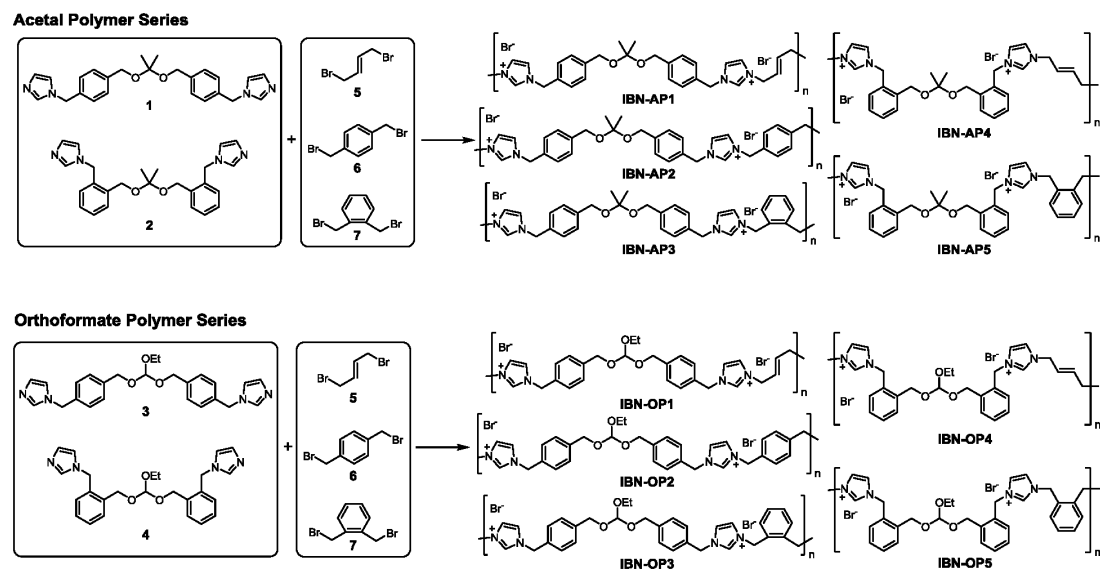
The concentration of the antimicrobial composition as described herein may be in the range of about 1 µg/ml to about 300 µg/ml, about 1 µg/ml to about 200 µg/ml, about 1 µg/ml to about 100 µg/ml, about 1 µg/ml to about 80 µg/ml, about 1 µg/ml to about 62 µg/ml, about 1 µg/ml to about 31 µg/ml, about 1 µg/ml to about 16 µg/ml, about 1 µg/ml to about 8 µg/ml, about 2 µg/ml to about 300 µg/ml, about 2 µg/ml to about 200 µg/ml, about 2 µg/ml to about 100 µg/ml, about 2 µg/ml to about 80 µg/ml, about 2 µg/ml to about 62 µg/ml, about 2 µg/ml to about 31 µg/ml, about 2 µg/ml to about 16 µg/ml, about 2 µg/ml to about 8 µg/ml, about 4 µg/ml to about 300 µg/ml, about 4 µg/ml to about 200 µg/ml, about 4 µg/ml to about 100

$\mu\text{g/ml}$, about 4 $\mu\text{g/ml}$ about 80 $\mu\text{g/ml}$, about 4 $\mu\text{g/ml}$ to about 62 $\mu\text{g/ml}$, preferably about 4 $\mu\text{g/ml}$ to about 31 $\mu\text{g/ml}$, more preferably about 4 $\mu\text{g/ml}$ to about 16 $\mu\text{g/ml}$, or most preferably about 4 $\mu\text{g/ml}$ to about 8 $\mu\text{g/ml}$. Preferably, the concentration may be 4 $\mu\text{g/ml}$ or 8 $\mu\text{g/ml}$.

The reduction of the microorganism activity may be achieved within a duration of about 0.5 minutes to about 360 minutes, about 0.5 minutes to about 180 minute, about 0.5 minutes to about 60 minutes, about 0.5 minutes to about 10 minutes, about 1 minute to about 360 minutes, about 1 minute to about 180 minutes, about 1 minute to about 60 minutes, about 1 minute to about 10 minutes, about 2 minutes to about 360 minutes, about 2 minutes to about 180 minutes, about 2 minutes to about 60 minutes, about 2 minutes to about 10 minutes, about 5 minutes to about 360 minutes, about 5 minutes to about 180 minutes, about 5 minutes to about 60 minutes, or about 5 minutes to about 10 minutes. Preferably, the reduction of the microorganism activity may be achieved within 10 minutes.

Examples

Synthesis of Imidazolium Polymers



Scheme 1. Synthetic schemes of acid-sensitive degradable imidazolium polymers.

The synthesis of new imidazolium polymers was carried out via the preparation of di-imidazole monomer units (compounds **1-4**) containing potential acid-sensitive degradable

linkers. The final polymers were prepared by condensing di-imidazoles with dibromides **5**, **6** or **7** to give imidazolium polymers **IBN-API**, **2**, **3**, **4** and **5**, and **IBN-OP1**, **2**, **3**, **4** and **5**. Further details are provided in the examples below.

General Information

All anhydrous solvents were purchased from Sigma-Aldrich and used without further purification. All other reagents were used as received, except where otherwise noted in the experimental text.

Analytical thin layer chromatography (TLC) was performed using Merck 60 F-254 silica gel plates with visualization by ultraviolet light (254 nm) and/or heating the plate after staining with a solution of 20% KMnO₄ w/v in H₂O. Flash column chromatography was carried out on Kieselgel 60 (0.040-0.063 mm) supplied by Merck under positive pressure.

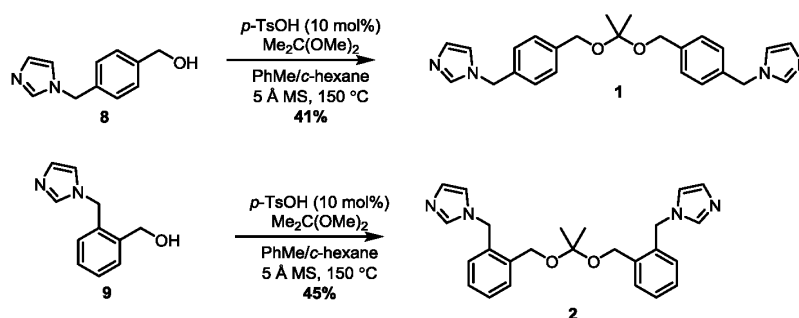
¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV-400 (400 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with the residual solvent peak of tetramethylsilane used as the internal standard at 0.00 ppm. ¹H NMR data are reported in the following order: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet), coupling constants (J, Hz), integration and assignment. High resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF-Q system. The samples were directly injected into the chamber at 20 μL·min⁻¹. Typical instrument parameters: capillary voltage, 4 kV; nebulizer, 0.4 bars; dry gas, 2 L·min⁻¹ at 120 °C; m/z range, 40 – 3000.

List of Abbreviations

THF	-	Tetrahydrofuran
PTFE	-	Polytetrafluoroethylene
TsOH	-	para-toluenesulfonic acid
DMSO	-	dimethylsulfoxide
MHB	-	Mueller Hinton Broth
YM	-	Yeast Mold broth
CFU	-	Colony Forming Units
PBS	-	Phosphate Buffer Saline

MeOH - Methanol
 OD - Optical Density
 eq. - equivalent

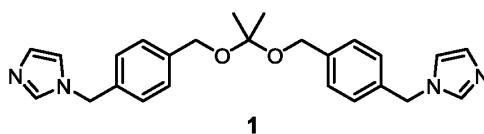
Example 1 - Synthesis of Acetal Linkers



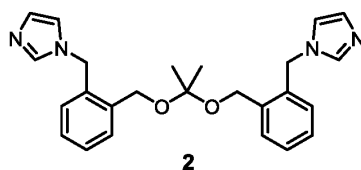
General Procedure:

Alcohol **8** or **9** (1.0 eq), 2,2-dimethoxypropane (0.8-1.0 eq) and *p*-TsOH·H₂O (10 mol%) were suspended in toluene/cyclohexane (1:1 v/v, 0.25 M) in a round-bottom flask fitted with a pressure-equalising dropping funnel loaded with 5 Å molecular sieves (370 mg/mmol). A reflux condenser was fitted over the dropping funnel and the atmosphere within the reaction apparatus was exchanged for Ar. The mixture was heated at 150 °C, ensuring the refluxing solvent condensed over the molecular sieves before returning to the reaction mixture. After 16 h, the reaction mixture was cooled to room temperature and concentrated *in vacuo*. The solids were purified by column chromatography to obtain the acetal linkers as colourless oils.

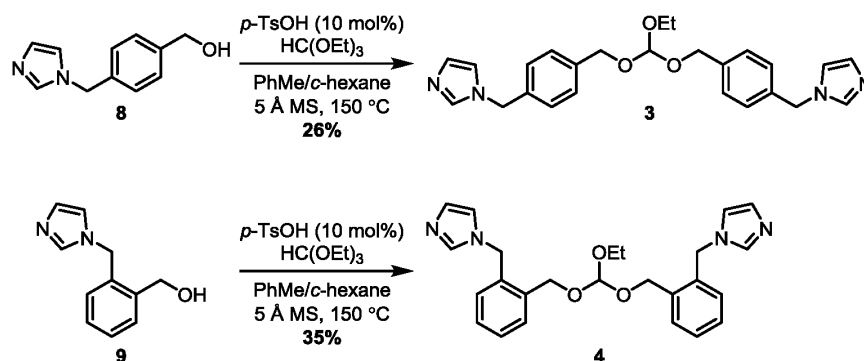
Example 1.1 Synthesis of Acetal Linker 1



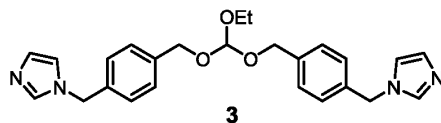
Acetal linker **1** was prepared by the general procedure from alcohol **8** (527 mg, 2.80 mmol) and 2,2-dimethoxypropane (295 µL, 2.39 mmol) and isolated by column chromatography (2% MeOH/CHCl₃) as a yellow oil (225 mg, 41%). ¹H NMR (400 MHz, CDCl₃) δ 7.54 (s, 2H, ImH), 7.32 (d, 4H, *J* = 7.5 Hz, ArH), 7.12 (d, 4H, *J* = 7.5 Hz, ArH), 7.08 (s, 2H, ImH), 6.89 (t, 2H, *J* = 1.0 Hz, ImH), 5.10 (s, 4H, 2 x NCH₂), 4.54 (s, 4H, 2 x OCH₂), 1.51 (s, 6H, 2 x CH₃); ¹³C NMR (101 MHz, CDCl₃) δ 139.0, 137.4, 135.2, 129.8, 127.9, 127.4, 119.2, 100.9, 62.7, 50.5, 25.2.

Example 1.2 Synthesis of Acetal Linker 2

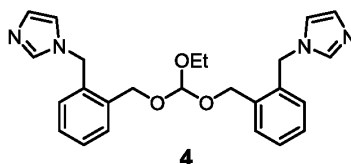
Acetal linker **2** was prepared by the general procedure from alcohol **9** (1.00 g, 5.31 mmol) and 2,2-dimethoxypropane (650 μ L, 5.31 mmol) and isolated by column chromatography (5% MeOH/CHCl₃) as a colourless oil (502 mg, 45%). ¹H NMR (400 MHz, CDCl₃) δ 7.47 (s, 2H, ImH), 7.33-7.28 (m, 6H, ArH), 7.07 (t, 2H, J = 1.0 Hz, ImH), 7.00-6.98 (m, 2H, ArH), 6.85 (t, 2H, J = 1.0 Hz, ImH), 5.15 (s, 4H, 2 x NCH₂), 4.44 (s, 4H, 2 x OCH₂), 1.49 (s, 6H, 2 x CH₃); ¹³C NMR (101 MHz, CDCl₃) δ 137.6, 136.3, 134.6, 129.7, 129.1, 128.5, 119.5, 101.3, 61.3, 48.1, 25.1.

Example 2 – Synthesis of Orthoformate Linker*General Procedure:*

Alcohol **8** or **9** (1.0 eq), 2,2-dimethoxypropane (0.8-1.0 eq) and *p*-TsOH·H₂O (10 mol%) were suspended in toluene/cyclohexane (1:1 v/v, 0.25 M) in a round-bottom flask fitted with a pressure-equalising dropping funnel loaded with 5Å molecular sieves (370 mg/mmol). A reflux condenser was fitted over the dropping funnel and the atmosphere within the reaction apparatus was exchanged for Ar. The mixture was heated at 150 °C, ensuring the refluxing solvent condensed over the molecular sieves before returning to the reaction mixture. After 16 h, the reaction mixture was cooled to room temperature and concentrated *in vacuo*. The solids were purified by column chromatography to obtain the acetal linkers as colourless oils.

Example 2.1 Synthesis of Orthoformate Linker 3

Orthoformate linker **3** was prepared by the general procedure from alcohol **8** (500 mg, 2.66 mmol) and triethylorthoformate (440 μ L, 2.66 mmol) and isolated by column chromatography (2 to 10% MeOH/CH₂Cl₂) as a colourless oil (148 mg, 26%). ¹H NMR (400 MHz, CDCl₃) δ 7.54 (s, 2H, ImH), 7.33 (d, 4H, J = 7.5 Hz, ArH), 7.13 (d, 4H, J = 7.5 Hz, ArH), 7.09 (s, 2H, ImH), 6.89 (s, 2H, ImH), 5.35 (s, 1H, CH(OEt)), 5.11 (s, 4H, 2 x NCH₂), 4.64 (s, 4H, 2 x OCH₂), 3.65 (q, 2H, J = 7.0 Hz, OCH₂CH₃), 1.23 (t, 3H, J = 7.0 Hz, OCH₂CH₃); ¹³C NMR (101 MHz, CDCl₃) δ 137.7, 137.4, 135.6, 129.8, 128.3, 127.4, 119.2, 111.8, 65.3, 60.6, 50.5, 14.9.

Example 2.2 Synthesis of Orthoformate Linker 4

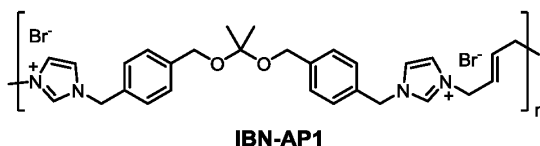
Orthoformate linker **4** was prepared by the general procedure from alcohol **9** (500 mg, 2.66 mmol) and triethylorthoformate (440 μ L, 2.66 mmol) and isolated by column chromatography (3 $\rightarrow$ 5% MeOH/CHCl₃) as a colourless oil (200 mg, 35%). ¹H NMR (400 MHz, CDCl₃) δ 7.48 (s, 2H, ImH), 7.33-7.30 (m, 6H, ArH), 7.06 (t, 2H, J = 1.0 Hz, ImH), 7.02-6.99 (m, 2H, ArH), 6.85 (t, 2H, J = 1.0 Hz, ImH), 5.31 (s, 1H, CH(OEt)), 5.19 (s, 4H, 2 x NCH₂), 4.59-4.58 (m, 4H, 2 x OCH₂), 3.60 (q, 2H, J = 7.0 Hz, OCH₂CH₃), 1.23 (t, 3H, J = 7.0 Hz, OCH₂CH₃); ¹³C NMR (101 MHz, CDCl₃) δ 137.6, 135.0, 134.9, 129.9, 129.6, 129.0, 128.6, 128.5, 119.4, 112.0, 63.9, 61.0, 47.9, 14.9.

Example 3 – Synthesis of Imidazolium Polymers*General Procedure:*

The acetal or orthoformate degradable linker (**1-4**) (1.0 eq) and butenyl or xylyl dibromide (**5-7**) (1.0 eq) were dissolved in THF (0.2 M) in a 20-mL vial sealed with a PTFE crimp-on cap. The solution was stirred for 1 hour with heating in a pre-heated DrySyn® heating block. The reaction mixture was transferred to a 15-mL Falcon® tube, dissolved in the minimum

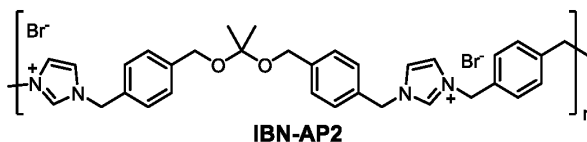
volume of methanol, then precipitated with ether to form a milky white suspension. The solids were spun down in a centrifuge (7000 rpm, 3 min), and the supernatant decanted. The solids were washed once more and the resulting solids were dried in a vacuum oven (50 °C, 10 mbar) for 16 h to yield the imidazolium polymers as white solids.

Example 3.1 Synthesis of IBN Acetal Polymer 1, IBN-AP1



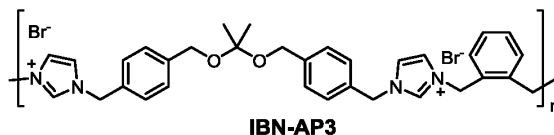
IBN Acetal Polymer 1, **IBN-AP1** was prepared by the general procedure from acetal linker **1** (100 mg, 0.24 mmol) and *trans*-1,4-dibromobutene (52 mg, 0.24 mmol) and isolated as a white crushable foam (132 mg, 87%). ¹H NMR (400 MHz, *d*₆-DMSO) δ 9.52 (br s, 2H, ImH), 7.92-7.87 (m, 2H, ImH), 7.84-7.80 (m, 2H, ImH), 7.46-7.31 (m, 8H, 2 x PhH), 6.07 (br s, 2H, 2 x CH), 5.49 (br s, 4H, 2 x NCH₂), 4.93 (br s, 4H, 2 x CHCH₂), 4.52 (br s, 4H, 2 x OCH₂), 1.43 (s, 6H, 2 x CH₃); ¹³C NMR (101 MHz, *d*₆-DMSO) δ 140.1, 136.7, 134.2, 129.7, 129.0, 128.4, 123.3, 123.1, 100.9, 62.6, 52.1, 50.1, 25.4.

Example 3.2 Synthesis of IBN Acetal Polymer 2, IBN-AP2



IBN Acetal Polymer 2, **IBN-AP2** was prepared by the general procedure from acetal linker **1** (100 mg, 0.24 mmol) and *α,α'*-dibromo-*p*-xylylene (63 mg, 0.24 mmol) and isolated as a white crushable foam (154 mg, 96%). ¹H NMR (400 MHz, *d*₆-DMSO) δ 9.50 (br s, 2H, ImH), 7.83 (br s, 4H, ImH), 7.50-7.42 (m, 12H, 3 x *p*-PhH), 5.43 (m, 8H, 4 x NCH₂), 4.51 (m, 4H, 2 x OCH₂), 1.42 (s, 6H, 2 x CH₃).

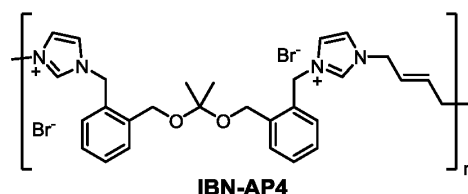
Example 3.3 Synthesis of IBN Acetal Polymer 3, IBN-AP3



IBN Acetal Polymer 3, **IBN-AP3** was prepared by the general procedure from acetal linker **1** (100 mg, 0.24 mmol) and *α,α'*-dibromo-*o*-xylylene (63 mg, 0.24 mmol) and isolated as a

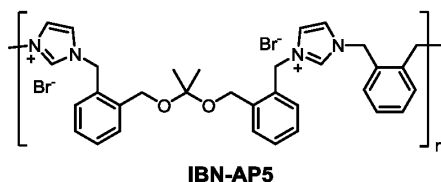
white crushable foam (157 mg, 96%). ^1H NMR (400 MHz, d_6 -DMSO) δ 9.48-9.33 (m, 2H, ImH), 7.86-7.72 (m, 4H, ImH), 7.49-7.22 (m, 12H, 2 x *p*-PhH + *o*-PhH), 5.66-5.59 (m, 4H, 2 x NCH₂), 5.45-5.37 (m, 4H, 2 x NCH₂), 4.51 (br s, 4H, 2 x OCH₂), 1.41 (s, 6H, 2 x CH₃).

Example 3.4 Synthesis of IBN Acetal Polymer 4, IBN-AP4



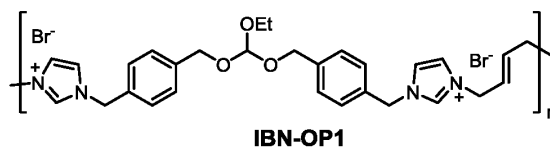
IBN Acetal Polymer 4, **IBN-AP4** was prepared by the general procedure from acetal linker **2** (100 mg, 0.24 mmol) and *trans*-1,4-dibromobutene (52 mg, 0.24 mmol) and isolated as a white crushable foam (74 mg, 49%). ^1H NMR (400 MHz, d_6 -DMSO) δ 9.42 (br s, 2H, ImH), 7.89-7.84 (m, 4H, ImH), 7.48-7.37 (m, 6H, *o*-PhH), 7.19-7.17 (m, 2H, *o*-PhH), 6.12 (br s, 2H, 2 x CH), 5.60 (br s, 4H, 2 x NCH₂), 4.98 (br s, 4H, 2 x CHCH₂), 4.61 (br s, 4H, 2 x OCH₂), 1.46 (s, 6H, 2 x CH₃); ^{13}C NMR (101 MHz, d_6 -DMSO) δ 137.2, 133.7, 129.8, 129.3, 128.9, 123.6, 123.2, 101.4, 61.5, 50.1, 49.8, 25.2.

Example 3.5 Synthesis of IBN Acetal Polymer 5, IBN-AP5



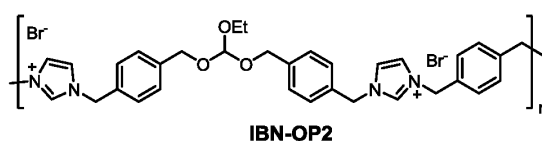
IBN Acetal Polymer 5, **IBN-AP5** was prepared by the general procedure from acetal linker **2** (100 mg, 0.24 mmol) and α,α' -dibromo-*o*-xylylene (63 mg, 0.24 mmol) and isolated as a white crushable foam (78 mg, 48%). ^1H NMR (400 MHz, d_6 -DMSO) δ 9.51 (br s, 2H, ImH), 7.92-7.86 (m, 4H, ImH), 7.49-7.38 (m, 8H, *o*-PhH), 7.30-7.28 (m, 2H, *o*-PhH), 7.19-7.16 (m, 2H, *o*-PhH), 5.77 (br s, 4H, 2 x NCH₂), 5.61 (br s, 4H, 2 x NCH₂), 4.60 (br s, 4H, 2 x OCH₂), 1.44 (s, 6H, 2 x CH₃).

Example 3.6 Synthesis of IBN Orthoformate Polymer 1, IBN-OP1



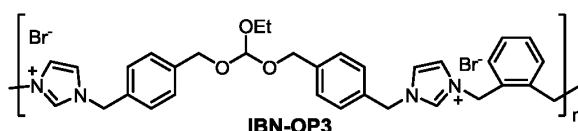
IBN Orthoformate Polymer 1, **IBN-OP1** was prepared by the general procedure from orthoformate linker **3** (77 mg, 0.18 mmol) and *trans*-1,4-dibromobutene (38 mg, 0.18 mmol) and isolated as an off-white foam (94 mg, 82%). ^1H NMR (400 MHz, d_6 -DMSO) δ 9.55-9.32 (m, 2H, ImH), 7.87-7.74 (m, 4H, ImH), 7.47-7.30 (m, 8H, 2 x PhH), 6.05 (br s, 2H, 2 x CH), 5.48-5.41 (m, 5H, CH(OEt) + 2 x NCH₂), 4.91 (br s, 4H, 2 x CHCH₂), 4.64-4.49 (m, 4H, 2 x OCH₂), 3.58 (q, 2H, J = 7.0 Hz, OCH₂CH₃), 1.12 (t, 3H, J = 7.0 Hz, OCH₂CH₃); ^{13}C NMR (101 MHz, d_6 -DMSO) δ 138.9, 136.7, 134.5, 129.7, 129.0, 128.6, 123.3, 123.1, 112.5, 65.4, 60.2, 52.1, 50.2, 15.4.

Example 3.7 Synthesis of IBN Orthoformate Polymer 2, **IBN-OP2**

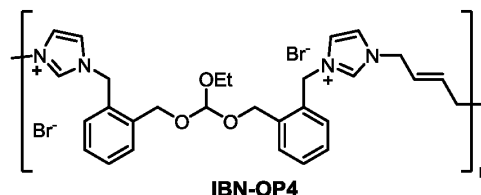


IBN Orthoformate Polymer 2 (**IBN-OP2**) was prepared by the general procedure from orthoformate linker **3** (65 mg, 0.15 mmol) and α,α' -dibromo-*p*-xylylene (40 mg, 0.15 mmol) and isolated as an off-white foam (65 mg, 62%). ^1H NMR (400 MHz, d_6 -DMSO) δ 9.54 (br s, 2H, ImH), 7.85 (br s, 4H, ImH), 7.50-7.40 (m, 12H, 3 x *p*-PhH), 5.45 (br s, 9H, CH(OEt) + 4 x NCH₂), 4.64-4.55 (m, 4H, 2 x OCH₂), 3.58 (q, 2H, J = 7.0 Hz, OCH₂CH₃), 1.12 (t, 3H, J = 7.0 Hz, OCH₂CH₃); ^{13}C NMR (101 MHz, d_6 -DMSO) δ 139.0, 136.8, 135.9, 134.4, 129.5, 129.0, 128.6, 123.4, 123.3, 112.5, 65.4, 60.2, 52.2, 52.0, 15.4.

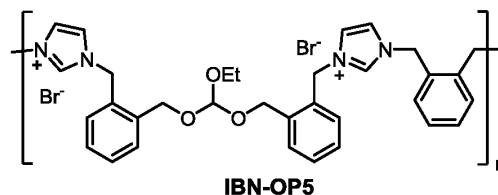
Example 3.8 Synthesis of IBN Orthoformate Polymer 3, **IBN-OP3**



IBN Orthoformate Polymer 3, **IBN-OP3** was prepared by the general procedure from orthoformate linker **3** (99 mg, 0.23 mmol) and α,α' -dibromo-*o*-xylylene (61 mg, 0.23 mmol) and isolated as white flakes (149 mg, 93%). ^1H NMR (400 MHz, d_6 -DMSO) δ 9.50-9.18 (m, 2H, ImH), 7.86-7.74 (m, 4H, ImH), 7.50-7.29 (m, 12H, 2 x *p*-PhH + *o*-PhH), 5.68-5.60 (m, 4H, 2 x NCH₂), 5.47-5.41 (m, 5H, CH(OEt) + 2 x NCH₂), 4.64-4.56 (m, 4H, 2 x OCH₂), 3.61-3.54 (m, 2H, OCH₂CH₃), 1.14-1.10 (m, 3H, OCH₂CH₃); ^{13}C NMR (101 MHz, d_6 -DMSO) δ 138.5, 136.7, 134.0, 133.0, 129.7, 129.6, 128.5, 128.1, 123.2, 122.8, 112.0, 64.9, 59.7, 51.8, 49.2, 15.0.

Example 3.9 Synthesis of IBN Orthoformate Polymer 4, IBN-OP4

IBN Orthoformate Polymer 4, **IBN-OP4** was prepared by the general procedure from orthoformate linker **4** (70 mg, 0.16 mmol) and *trans*-1,4-dibromobutene (35 mg, 0.16 mmol) and isolated by precipitation with THF as an off-white foam (65 mg, 62%). ¹H NMR (400 MHz, *d*₆-DMSO) δ 9.47-9.25 (m, 2H, ImH), 7.89-7.81 (m, 4H, ImH), 7.48-7.40 (m, 6H, *o*-PhH), 7.21-7.19 (m, 2H, *o*-PhH), 6.11-6.04 (m, 2H, 2 x CH), 5.62-5.59 (m, 5H, CH(OEt) + 2 x NCH₂), 4.96 (br s, 4H, 2 x CHCH₂), 4.79 (br s, 4H, 2 x OCH₂), 3.63-3.54 (m, 2H, OCH₂CH₃), 1.14-1.11 (m, 3H, OCH₂CH₃); ¹³C NMR (101 MHz, *d*₆-DMSO) δ 136.8, 135.7, 133.4, 129.8, 129.4, 128.8, 128.5, 123.1, 122.8, 112.1, 63.8, 59.9, 49.7, 49.2, 15.0.

Example 3.10 Synthesis of IBN Orthoformate Polymer 5, IBN-OP5

IBN Orthoformate Polymer 5, **IBN-OP5** was prepared by the general procedure from orthoformate linker **4** (70 mg, 0.16 mmol) and α,α'-dibromo-*o*-xylylene (43 mg, 0.16 mmol) and isolated by precipitation with THF as a white foam (52 mg, 46%). ¹H NMR (400 MHz, *d*₆-DMSO) δ 9.53-9.27 (m, 2H, ImH), 7.90-7.82 (m, 4H, ImH), 7.49-7.40 (m, 8H, *o*-PhH), 7.30-7.26 (m, 2H, *o*-PhH), 7.20-7.18 (m, 2H, *o*-PhH), 5.74-5.53 (m, 9H, CH(OEt) + 4 x NCH₂), 4.79 (br s, 4H, 2 x OCH₂), 3.58-3.54 (m, 2H, OCH₂CH₃), 1.13-1.08 (m, 3H, OCH₂CH₃); ¹³C NMR (101 MHz, *d*₆-DMSO) δ 137.2, 135.7, 133.4, 133.0, 129.8, 129.6, 129.2, 128.9, 128.8, 128.4, 123.4, 123.1, 112.1, 63.8, 60.0, 49.3, 49.2, 15.0.

Example 4 - Calculating Polymer Length by ¹H NMR Spectroscopy

IBN-AP and **IBN-OP** polymers were found to have imidazole end groups based on analysis of the ¹H NMR spectrum of purified polymers. Average polymer length, in terms of number

of imidazole and imidazolium units per chain, was calculated based on the ratio of imidazolium (δ 9.5–9.4 ppm) to imidazole (δ 6.9 or 7.2 ppm) integral values, as illustrated in Figure 4.

$$\begin{aligned}\text{Average polymer chain length} &= 2 \frac{\int_{\text{imidazolium}}}{\int_{\text{imidazole}}} + 2 \\ &= 2 \frac{2.00}{0.33} + 2 = 14 \text{ imidazole and imidazolium units}\end{aligned}$$

Evaluation of antimicrobial properties

The antimicrobial activities of novel imidazolium polymers were evaluated against four different and clinically relevant microbes: *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*.

Example 5 – Determining Minimum Inhibitory Concentration

Staphylococcus aureus (ATCC 6538, Gram-positive), *Escherichia coli* (ATCC 8739, Gram-negative), *Pseudomonas aeruginosa* (Gram-negative), and *Candida albicans* (ATCC 10231, fungus) were used as representative microorganisms to challenge the antimicrobial functions of the imidazolium salts. All bacteria and fungus were stored frozen at -80 °C, and were grown overnight at 37°C in Mueller Hinton Broth (MHB) prior to experiments. Fungus was grown overnight at 22°C in Yeast Mold (YM) broth. Subsamples of these cultures were grown for a further 3 h and diluted to give an optical density value of 0.07 at 600 nm, corresponding to 3×10^8 CFU mL⁻¹ for bacteria and 10^6 CFU mL⁻¹ for fungus (McFarland's Standard 1; confirmed by plate counts).

The polymers were dissolved in MHB or YM broth at a concentration of 4 mg mL⁻¹ and the minimal inhibitory concentrations (MICs) were determined by microdilution assay. Bacterial solutions (100 μ L, 3×10^5 CFU mL⁻¹) were mixed with 100 μ L of oligomer solutions (ranging from 4 mg mL⁻¹ to 2 μ g mL⁻¹ in serial two-fold dilutions) in each well of the 96-well plate. The plates were incubated at 37 °C for 24 h with constant shaking speed at 300 rpm. Bacterial growth was determined by measuring the optical density of the culture solution at a wavelength of 600 nm, using a microplate reader. The MIC measurement against *Candida albicans* is similar to bacteria except that the fungus solution is 10^6 CFU mL⁻¹ in YM and the plates were incubated at room temperature.

The minimum inhibitory concentrations were taken as the concentration of the antimicrobial agent at which no microbial growth was observed with the microplate reader. PBS solution containing microbial cells alone were used as negative controls. The assay was performed in four replicates and the experiments were repeated at least two times.

The minimum inhibitory concentration (MIC) values of the polymers are presented in Table 1. The MIC values of the polymers were also compared against a non-degradable imidazolium polymer PIM-45, the structure of which is depicted below:

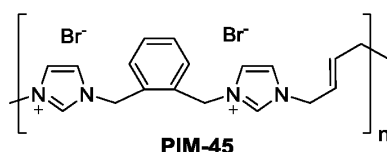


Table 1. Minimum inhibitory concentrations (MIC) of the acid-sensitive degradable polymers.

Entry	Compound	MIC (μg/ml)				HC ₁₀ (μg/ml)
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	
1	IBN-AP1	31	16	31	62	>2000
2	IBN-AP2	4	4	250	62	>2000
3	IBN-AP3	16	16	125	62	1000
4	IBN-AP4	4	4	31	62	>2000
5	IBN-AP5	31	16	31	62	>2000
6	IBN-OP1	8	16	31	16	>2000
7	IBN-OP2	4	8	31	31	>2000
8	IBN-OP3	2	2	16	31	>2000
9	IBN-OP4	4	4	31	4	>2000
10	IBN-OP5	4	8	62	16	>2000
11	PIM-45	8	8	31	31	>2000
12	Vancomycin	2	-	-	-	>2000

All ten polymers demonstrated high antimicrobial activity when tested against the four microbes. Among the acetal-linked polymer series, **IBN-AP2** exhibited the best performance. **IBN-AP2** had lower MIC values against *E. coli* and *S. aureus*, but higher MIC values against *P. aeruginosa* and *C. albicans* compared with non-degradable imidazolium polymer **PIM-45**. Among the orthoformate-linked polymer series, **IBN-OP2**, **3** and **4** were identified as the

most active polymers. All three polymers had MIC values against *E. coli*, *S. aureus* and *P. aeruginosa* and *C. albicans* lower than or comparable to that of non-degradable imidazolium polymer **PIM-45**.

Example 6 – Determining Antimicrobial Efficiency of Polymers

Time kill studies of 4 effective imidazolium polymers, **IBN-AP2**, **IBN-AP4**, **IBN-OP3** and **IBN-OP4** were carried out against *E. coli*.

The microbes were treated with polymers at MIC concentration, and samples were taken out of each well at different intervals. 100 µl of cell suspension was removed, rescued by a series of 10- fold dilutions with growth medium. For plating, 100 µl of the diluted samples was spread on growth medium agar plates and colonies were counted after overnight incubation at 37 °C.

All the polymers displayed bactericidal properties even at low concentration of 4 µg/ml (Figure 1). **IBN-AP4** and **IBN-OP3** showed higher efficiency than the other 2 polymers. For **IBN-OP3**, more than 99% reduction in cell population was observed within 10 minutes at 8 µg/ml. A 99.9% reduction in cell population can be obtained for **IBN-AP4** and **IBN-OP3** in 3 h.

Example 7 – Determining Toxicity of Polymers

The toxicities of these compounds were also evaluated by measuring the extent of hemolysis induced by the polymers.

Fresh rat red blood cells (RBCs) were diluted with PBS buffer to give an RBC stock suspension (4 vol% blood cells). A 100 µL aliquot of RBC suspension was added into a 96-well plate containing 100 µL polymer solutions of various concentrations (ranging from 4 mg mL⁻¹, to 2 µg mL⁻¹ in serial two-fold dilutions in PBS). After 1 h incubation at 37°C, the contents of each well were pipetted into a micro-centrifuge tube and centrifuged at 2000 rpm for 5 min. Aliquots (100 µl) of the supernatant were transferred to a new 96-well plate. Hemolytic activity was determined as a function of hemoglobin release by measuring the optical density at 576 nm (OD₅₇₆) of 100 µL of the supernatant using a microplate reader (TECAN). A control solution that contained only PBS was used as a reference for 0%

hemolysis. Absorbance of red blood cells lysed with 0.5% Triton-X was taken as 100% hemolysis. The % hemolysis was calculated as below:

$$\% \text{ Hemolysis} = \frac{OD576 (\text{polymer}) - OD576 (PBS)}{OD576 (\text{Triton-X}) - OD576 (PBS)} \times 100$$

The data were expressed as mean and standard deviation of four replicates, and the tests were repeated two times. Data are expressed as means $\pm$ standard deviation of the mean (S.D. is indicated by error bars). Student's *t*-test was used to determine significance among groups. A difference with $P \leq 0.05$ was considered statistically significant.

All polymers, with the exception of **IBN-AP3**, demonstrated low toxicity with less than 10% hemolysis even at 2000 $\mu\text{g/ml}$, the highest concentration that was tested.

Example 8 - Degradation of imidazolium polymers

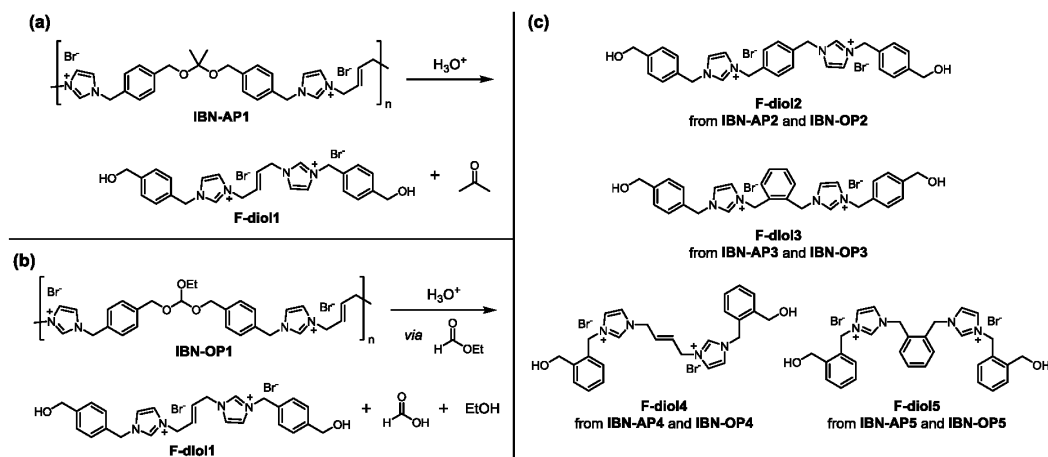
Sorenson's phosphate buffer (pH, 6.0, 7.0, 8.0) was prepared in deionized water at concentrations of 100 mM. Stock solutions of the buffers were divided into 1 mL portions which were freeze-dried and dissolved in 1 mL of D_2O . A 4 mg sample of imidazolium polymer was dissolved in deuterated buffer solution with care taken to ensure complete solution of the compounds. The solution was stored at 25 $^\circ\text{C}$ in NMR tubes and ^1H NMR spectra were obtained at specific time points. It was found that 128 scans were sufficient to obtain good signal-to-noise ratio.

Degradation of the acetal polymers (**IBN-AP**) was observed by disappearance of the signal of the dimethyl acetal protons (504). An overlay of ^1H NMR spectra of **IBN-AP4** taken in D_2O , PBS pH 6 over 4 days is shown in Figure 5 as a representative example. The ^1H NMR spectra of an independently synthesized sample of **F-diol4** is included for comparison.

Degradation of the orthoformate polymers (**IBN-OP**) was observed by disappearance of the signal of the CH_3 protons of the OEt group (604), and appearance of the signal of the terminal protons of ethyl formate (606) and ethanol (605). An overlay of ^1H NMR spectra of **IBN-OP3** taken in D_2O , PBS pH 6 over 48 hours is shown in Figure 6 as a representative example. The ^1H NMR spectra of an independently synthesized sample of **F-diol3** is included for comparison.

Both the acetal and orthoformate-linked polymer series are expected to degrade under acidic conditions to form di-imidazolium fragments and small molecules. The degradation pathways

for acetal polymer **IBN-API** and orthoformate polymer **IBN-OP1** are illustrated in Scheme 4a and 4b respectively.



Scheme 2. Degradation pathway for (a) acetal polymer **IBN-API** and (b) orthoformate polymer **IBN-OP1**. (c) Degradation products for remaining imidazolium polymers.

Upon complete degradation, acetal-linked polymer **IBN-API** forms di-imidazolium fragment **F-diol1** and an equivalent number of moles of acetone (Scheme 2a). The same di-imidazolium fragment **F-diol1** is expected from the degradation of orthoformate-linked polymer **IBN-OP1**, accompanied by an equivalent number of moles of ethyl formate which further decomposes to formic acid and ethanol (Scheme 2b). These pathways apply to the remaining polymers, **IBN-AP2-5** and **IBN-OP2-5**, giving rise to the corresponding di-imidazolium fragments **Fdiol2-5** (Scheme 2c).

The degradation products, **Fdiol1-5**, were independently synthesized in order to evaluate their antimicrobial properties and toxicity (Table 2). The antimicrobial properties and toxicity of these degradation products was determined according to methods described in Example 5 and 7 above. **F-diol2** was found to be inactive against all four strains of pathogenic microbes, while **F-diol1**, **F-diol3** and **F-diol4** only showed weak activity against *S. aureus* and no activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa* and fungus *C. albicans*. **F-diol5** was found to have weak activity against *S. aureus* and *E. coli*, and showed no inhibitory effect on *P. aeruginosa* and *C. albicans*. All the di-imidazolium fragments demonstrated low toxicity with less than 10% hemolysis even at 2000 $\mu\text{g/ml}$, the highest concentration that was tested.

Table 2. Minimum inhibitory concentrations (MIC) of the imidazolium fragments

Entry	Compound	MIC ($\mu\text{g/ml}$)				HC ₁₀ ($\mu\text{g/ml}$)
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	
1	F-diol1	125	>2000	2000	>2000	>2000
2	F-diol2	2000	>2000	>2000	>2000	>2000
3	F-diol3	500	>2000	>2000	>2000	>2000
4	F-diol4	250	1000	>2000	>2000	>2000
5	F-diol5	125	250	>2000	>2000	>2000

The degradation profiles of the most active polymers were investigated under pH 6, 7, and 8 conditions using NMR analysis. All five polymers (**IBN-AP2**, **AP4**, **OP2**, **OP3** and **OP4**) were found to be degradable under these conditions, with degradation occurring most rapidly at pH 6 and slowly at pH 8 (Figure 2). Analysis of the ^1H NMR spectra of fully degraded polymer samples confirmed the formation of di-imidazolium fragments, **F-diol2**, **F-diol 3** and **F-diol4**, along with acetone or ethyl formate.

Polymers **IBN-AP2**, **IBN-OP2** and **IBN-OP3** with *p*-xylylene spacers adjacent to the degradable linker were found to degrade on a shorter timescale compared to polymers **IBN-AP4** and **IBN-OP4** with linkers flanked by *o*-xylylene spacers. At pH 6, acetal polymer **IBN-AP2** had a half-life of less than 6 hours (Figure 2a), while the half-life of **IBN-AP4** is approximately 30 hours (Figure 2b). This trend was mirrored in the orthoformate polymer series: **IBN-OP2** and **OP3** both contain *p*-xylylene spacers adjacent to the orthoformate group and both have half-lives of 9 hours under acidic conditions (Figures 2c and 2d). **IBN-OP4**, on the other hand, was determined to have a half-life of 24 hours under the same conditions (Figure 2e).

All the polymers displayed greater stability under pH 8 buffered conditions, but ultimately degrade to the inactive di-imidazolium fragments. Under alkaline conditions, the influence of the spacer was less pronounced with **IBN-AP2**, **AP4**, **OP2** and **OP3** registering half-lives of 11 to 15 days (Figures 2a-d). After 30 days, only 10-20% of the acetal or orthoformate linkers remained intact. In contrast, **IBN-OP4** was found to have a half-life of 24 days under identical conditions, and was more than 90% degraded after 90 days (Figure 2e).

Over the course of degradation, conversion of the active polymers to inactive degradation products was expected to be reflected in changes to the MIC values. To determine if this was indeed the case, **IBN-AP4** and **IBN-OP3** were dissolved in buffer solution and their MIC values against *E. coli* and *S. aureus* were monitored at different time points. The relative activity of the polymer sample at a given point in time was expressed as a fraction of its MIC on day 0 over its MIC at the point of measurement (Figure 3).

In Sorenson's phosphate buffer (pH 6), the degradation of **IBN-AP4** and **IBN-OP3** was very fast. The activity of **IBN-AP4** against *E. coli* and *S. aureus* dropped to quarter after 5 h and almost lost activity at day 4 (MIC > 250 µg/ml; Figure 3a and 3b). When **IBN-OP3** was subjected to similar conditions, the activity of the degraded sample was less than 10% of the original polymer after day 1 (Figure 3c and 3d). Both polymers showed higher stability under pH 7 and 8 conditions, retaining half of their original activity in the first 2 days. By day 18, **IBN-OP3** lost activity faster than **IBN-AP4**, implying that **IBN-AP4** is more stable under neutral and basic conditions. Samples stored in rain water (pH 6) showed similar degradation profiles to samples stored in pH 7 phosphate buffer. The activity of the degraded sample was halved after 5 h and then degrade slowly afterwards. On the whole, these MIC changes were in good agreement with the degradation profile of **IBN-AP4** and **IBN-OP3** in different pH buffer solutions (Figure 2).

Industrial Applicability

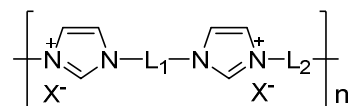
The imidazolium oligomers or polymers as defined above may be included in antimicrobial compositions. Such antimicrobial compositions may be used as therapeutic compositions or medicaments for the treatment of a broad range of microbial or fungal infections. Examples of microbial or fungal infections which may be treated include *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* infections. Such compositions may be applied externally on affected areas as topical creams, ointments or gels.

In addition, the imidazolium oligomers or polymers may be included in antimicrobial compositions for non-therapeutic applications as well. The fast killing kinetics of the present imidazolium oligomers makes it particularly useful for general disinfecting purposes. As such, the oligomers and polymers as defined above may be added to sanitizers, sterilizing solutions, decontaminants, disinfectants, and household cleaners. The oligomers may also be

added to impart an antimicrobial property to fabrics and materials and may be used for the manufacture of sterile consumables such as gloves.

Claims

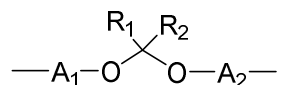
1. An oligomer or polymer having the following Formula (I):



Formula (I)

wherein

L₁ has the following structure:



wherein R₁ and R₂ are, in each instance, same or different, and are independently selected from the group consisting of hydrogen, optionally substituted alkyl, and optionally substituted alkoxy;

A₁ and A₂, are in each instance, same or different, and are optionally substituted aryl or xylylenediyl;

L₂ is, in each instance, selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl;

X, in each instance, is same or different, and is a halogen;

n is an integer of at least 2;

or a salt or hydrate thereof.

2. The oligomer or polymer according to claim 1, wherein at least one of R₁ and R₂ is selected from C₁-C₈ alkoxy group.
3. The oligomer or polymer according to claim 2, wherein R₁ and R₂ are same or different, and are independently selected from hydrogen and ethoxy.
4. The oligomer or polymer according to claim 1, wherein R₁ and R₂ are, in each instance, same or different, and are independently selected from C₁-C₈ alkyl.

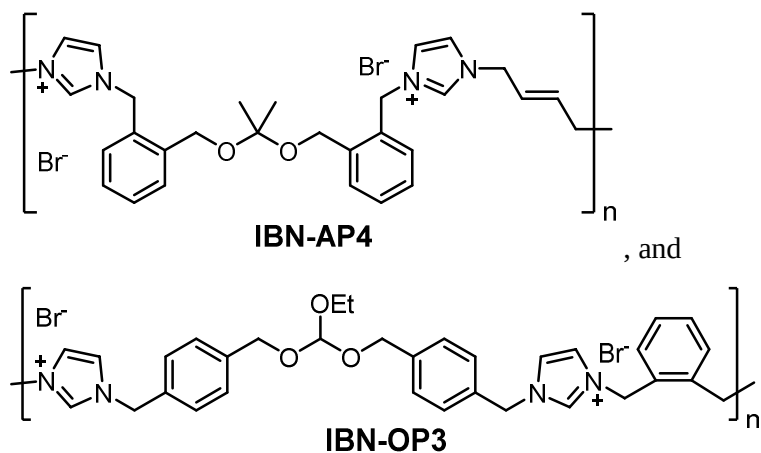
5. The oligomer or polymer according to claim 4, wherein R₁ and R₂ are methyl.
6. The oligomer or polymer according to any one of the preceding claims, wherein A₁ and A₂, are same or different, and are an aryl group derived from alkylbenzene or a xylylenediyl group.
7. The oligomer or polymer according to any one of the preceding claims, wherein A₁ and A₂, are same or different, and are xylylenediyl.
8. The oligomer or polymer according to claim 7, wherein said xylylenediyl is ortho-xylylenediyl or para-xylylenediyl.
9. The oligomer or polymer according to any one of the preceding claims, wherein R₁, R₂, A₁, and A₂ are provided in one of the following combinations:

R₁ and R₂ are methyl; and A₁ and A₂ are para-xylylenediyl;

R₁ and R₂ are methyl, and A₁ and A₂ are ortho-xylylenediyl;

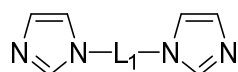
R₁ is H, R₂ is ethoxy; and A₁ and A₂ are para-xylylenediyl; and

R₁ is H, R₂ is ethoxy; and A₁ and A₂ are ortho-xylylenediyl.
10. The oligomer or polymer according to any one of the preceding claims, wherein L₂ is selected from the group consisting of alkenyl and aryl.
11. The oligomer or polymer according to any one of the preceding claims, wherein X is selected from the group consisting of chlorine, fluorine, bromine and iodine.
12. The oligomer or polymer according to any one of the preceding claims, wherein n is an integer from 1 to 100, 1 to 50, 1 to 30, 1 to 15, 3 to 15, or 7.
13. The oligomer or polymer according to any one of the preceding claims, wherein the polymer is selected from the group consisting of:



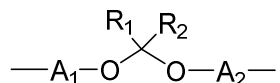
14. A method for preparing an oligomer or polymer according to any one of claims 1 to 13, comprising the step of:

contacting a di-imidazole having the following Formula (II):



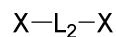
Formula (II)

wherein L_1 has the following structure:



wherein R_1 and R_2 are, in each instance, same or different, and are independently selected from the group consisting of hydrogen, optionally substituted alkyl, and optionally substituted alkoxy; and A_1 and A_2 , are in each instance, same or different, and are optionally substituted aryl or xylylenediyl;

with a dihalide having the following Formula (III):



Formula (III)

wherein L_2 is, in each instance, selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl; and X , in each instance, is same or different, and is a halogen.

15. An antimicrobial composition comprising an oligomer or polymer according to any one of claims 1 to 13, or a salt or hydrate thereof.

16. A method for killing or inhibiting the growth of a microorganism, the method comprising contacting said microorganism *ex vivo* with an antimicrobial composition according to claim 15.
17. Non-therapeutic use of an antimicrobial composition according to claim 15, for killing or inhibiting the growth of a microorganism *ex vivo*.
18. An antimicrobial composition according to claim 15, for use as an antibiotic.
19. Use of an antimicrobial composition according to claim 15, in the manufacture of a medicament for treating a microbial infection.
20. The use according to claim 19, wherein the microbial infection is caused by one or more microorganism.
21. The method according to claim 16, use according to claim 17, or use according to claim 20, wherein the microorganism is a bacterium, fungus, or a mixture thereof.