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Synthesis of Antibacterial Glycosylated Polycaprolactones bearing Imidazoliums with Reduced Hemolytic Activity

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ABSTRACT

Most synthetic antimicrobial polymers are not biodegradable, thus limiting their potential for large-scale applications in personal care disinfection and environmental contaminations. Poly(ϵ -caprolactone) (PCL) is known to be both biodegradable and biocompatible, thus representing an ideal candidate biopolymer for antimicrobial applications. Here we successfully grafted

alkylimidazolium (Im) onto PCL to mimic the cationic properties of antimicrobial peptides. The Poly(ϵ -caprolactone)-graft-butylimidazolium had only moderate MICs (32 $\mu\text{g/mL}$), reasonably good red blood cell selectivity (36) and relatively good fibroblast compatibility (81% cell viability at 100 $\mu\text{g/mL}$), indicating that combining the hydrophobic PCL backbone with the most hydrophilic butylimidazolium gives a good balance of MIC and cytotoxicity. On the other hand, the PCL-graft -hexylimidazolium and -octylimidazolium demonstrated better MICs (4-32 $\mu\text{g/mL}$), but considerably worse cytotoxicity. We postulated that the worse hydrophilicity of hexylimidazolium and octylimidazolium was responsible for their higher cytotoxicity and sought to moderate their cytotoxicity with different sugar compositions and lengths. Through our screening, we identified a candidate polymer, $\text{P}(\text{C6Im})_{0.35}\text{CL-co-P}(\text{Man})_{0.65}\text{CL}$, that demonstrated both superior MIC and very low cytotoxicity. We further demonstrated that our biopolymer hit had superior antimicrobial kinetics compared to the antibiotic vancomycin. This work paves the way forward for the use of biodegradable polyesters as the backbone scaffold for biocompatible antibacterial agents, by clicking with different types and ratios of alkylimidazolium and carbohydrate moieties.

INTRODUCTION

Antimicrobial peptides (AMPs) generally possess amphiphilic properties, arising from both hydrophilic and hydrophobic side chains, which are responsible for their activities.¹ Synthetic polymers which mimic the amphiphilic structures of AMPs, have attracted great attention as a new class of disinfectants because of their ease of synthesis and low-cost of production in large quantities.²⁻⁴ The antimicrobial polymers, inspired by the cationic amino acids residues in AMPs, generally have cationic groups to facilitate their adsorption to negatively charged bacterial

1 membranes.^{5,6} For example, DeGrado and coworkers synthesized a biomimetic poly(arylamide)
2 with amphiphilic structures bearing a primary amine, which showed broad antibacterial
3 activity.^{7,8} Also, many other polymers have been synthesized and tested for activities against
4 different pathogens, for example, polynorbornenes,⁹⁻¹² polycarbonates,¹³⁻¹⁷ poly(β -lactam),¹⁸⁻²⁰
5 polypeptides^{21,22} and polyacrylate derivatives.²³⁻²⁵ However, most synthetic antimicrobial
6 polymers consist of carbon-based backbones that are not biodegradable, thus limiting their
7 potential in clinical applications. Aliphatic polyesters constitute a class of biodegradable
8 polymers and their roles as scaffolds for biodegradable antibacterial polymers have been rarely
9 investigated.^{13,17,26,27} Moreover, antibacterial polycarbonates have been well studied, which
10 presented a good example of degradable antibacterial polymers. In comparison with
11 polycarbonates, PCL (poly(ϵ -caprolactone)) has more efficient degradation behavior,²⁸⁻³⁰ and
12 little has been studied using PCL as the backbone for antibacterial polymers. Besides, some
13 reported polyesters have the ability of self-degradation despite their relatively high hemolytic
14 activity.^{31,32} Intriguingly, cationic coumarin polyesters and polyurethanes were reported to
15 exhibit selective activity against Gram-negative bacteria which is very surprising compared with
16 other cationic polymers.^{33,34} The backbone of the cationic antibacterial polymers is known to
17 profoundly affect the resultant biological properties. Therefore, these interesting and attractive
18 results had inspired us to design new antibacterial polymers based on polycaprolactones. As a
19 well-known biodegradable polyester, PCL has attracted considerable attention for the past
20 decades, but the major drawback is the lack of side functional groups which can be solved by
21 introducing functional groups to the α -position of the carbonyl.^{35,36} Interestingly, functionalized
22 ϵ -caprolactone has been prepared. It has been successfully polymerized and modified by click
23 reactions, thus implying it is a great candidate substrate for producing biodegradable

1 antimicrobial polyesters.^{35,37,38} Furthermore, imidazolium salts have gained considerable
2 attention in antimicrobial applications owing to their relative hydrophilicity, permanent charges
3 and stability.³⁹⁻⁴¹

4 Furthermore, like natural AMPs, most cationic polymers are hemolytic or cytotoxic to human
5 cells,⁴² limiting their further applications. Thus, studies have been widely pursued to manipulate
6 the antimicrobial activity and biocompatibility of the synthetic polymers through various factors
7 such as charge density,^{9,43} molecular weight,^{12,44} type of active moiety^{45,46} and alkyl chain
8 length.^{23,24,47} In particular, the balance between hydrophilicity and hydrophobicity of the entire
9 polymer is most crucial to the selectivity. This can be done by adding alkyl chains to increase
10 hydrophobicity or adding biocompatible compounds such as poly(ethylene glycol)^{48,49} and
11 sugars⁵⁰⁻⁵³ to increase the hydrophilicity. Noteworthy, the effect of glyconunits was studied on
12 antimicrobial polyacrylates systematically but with only one sugar.⁵³

13 Here, we report the synthesis of a new class of antibacterial compounds based on the
14 biodegradable PCL backbone. Imidazolium was grafted to mimic the cationic antibacterial
15 properties of antimicrobial peptides whereas the alkyl chain on the imidazoliums and
16 carbohydrates were used to balance the hydrophobicity/hydrophilicity of the resultant polymer.
17 The synthesis of the polymers could be successfully achieved through ring-opening
18 polymerization and subsequent click reaction between the azido PCL and alkyne-imidazoliums
19 (Im) and alkyne-carbohydrates in a one-pot grafting reaction (Figure 1). Subsequently, the PCL-
20 Im-carbohydrates grafts were tested for activity against both Gram-positive and Gram-negative
21 bacteria, including up to 9 MRSA strains. In addition, to evaluate selectivity against bacteria,
22 these polymers were also tested for toxicities against red blood cells and 3T3 cells.

EXPERIMENTAL SECTION

Materials. Chemicals and solvents are purchased from Alfa-Aesar, Sigma-Aldrich and VWR and used without further purification unless otherwise noted. Benzyl alcohol (Alfa-Aesar, 99%) was stirred with sodium at room temperature and distilled under nitrogen and stored in Schlenk flask in desiccator until further use. Anhydrous toluene (over sodium/benzophenone), tetrahydrofuran (THF, over sodium/benzophenone) and dichloromethane (DCM, over calcium hydride) were freshly distilled under nitrogen atmosphere before use. All the other anhydrous solvents were purchased from Sigma-Aldrich and used as received. Deuterated solvents are obtained from Cambridge Isotope Laboratories and used as received. Thin layer chromatography (TLC) with Merck TLC silica gel 60 F254 plate was used to monitor reaction. UV, potassium permanganate and iodine staining was used to visualize compounds on TLC plates. Regenerated cellulose dialysis tubing (MWCO 3500 Da) was purchased from Fisher Scientific. Deionized water was obtained from a Merck Millipore Integral 3 water purification system. For biological studies, NIH 3T3 fibroblasts were purchased from Millipore, Singapore. All broth and agar were purchased from Becton Dickinson Company (Franklin Lakes, US) and used as received. Bacteria strains used (*Escherichia coli* ATCC8739, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* PAO1, *Bacillus subtilis*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434 and drug-resistant *Staphylococcus aureus* MRSA BAA40, MRSA USA300) were purchased from ATCC. Drug-resistant *Staphylococcus aureus* MRSA 1-7 were clinical strains isolated from local hospital (TTSH).

Instruments for characterization

^1H and ^{13}C and 2D NMR spectra were recorded on Bruker Avance 300, Bruker Avance 400, Bruker AVIII 400 and JEOL JNM-ECA 400 spectrometers using deuterated solvents as

reference. Mass spectra were obtained using an Agilent 6230 TOF LC/MS with an electrospray (ESI) source with purine and HP-0921 as an internal calibrates. Organic phase gel permeation chromatography (GPC) was carried out on a Shimadzu liquid chromatography system equipped with a Shimadzu refractive index detector (RID-10A) and two Agilent Polargel columns operating at 40 °C using DMF (with 1 wt % LiBr) or THF as the eluent at a flow rate of 1 mL/min using polystyrene kit as standard. Aqueous phase GPC was carried out on an Agilent liquid chromatography system equipped with an Agilent refractive index detector with two Shodex OHpak columns operating at 40 °C using 0.05M NaCl solution as the eluent at a flow rate of 0.5 mL/min using pullulan kit as standard to determine M_n , M_w , and polydispersity index (PDI= M_w/M_n).

Synthesis of α -bromo- ϵ -caprolactone (α -BrCL). The α -bromo- ϵ -caprolactone (α -BrCL) was synthesized according to the literature procedure started from cyclohexanone.⁵⁴ Briefly, Cyclohexanone (14.7 g, 0.15 mol) was dissolved in dry diethyl ether (150 mL) followed by the addition of N-bromosuccinimide (NBS, 28.0 g, 0.158 mol). The solution was stirred and ammonium acetate (1.16 g, 0.015 mol) was added portion-wise. After stirring for 0.5 h at room temperature, the solid was filtered off and the filtrate was washed with water, dried over Na_2SO_4 , concentrated and subject to flash column chromatography, giving pale yellow liquid α -bromo-cyclohexanone (17.8 g, 67%). To the solution of α -bromo-cyclohexanone (10.0 g, 56 mmol) in anhydrous dichloromethane (DCM, 200 mL), 3-chloroperoxybenzoic acid (*m*-CPBA, 25.7 g, 85 mmol, ca. 70% with water) was added and the solution was stirred at room temperature for 24 h. The mixture was cooled to -20 °C and filtered. The filtrate was washed successively with $\text{Na}_2\text{S}_2\text{O}_3$ and saturated NaHCO_3 solution thoroughly until no acid could be detected by TLC. The

organic layer was dried over Na₂SO₄, concentrated and subject to flash column chromatography, affording the desired monomer α -bromo- ϵ -caprolactone in 41% yield (4.4 g). ¹H NMR (400 MHz, CDCl₃) δ 4.85 (dd, J = 6.2, 3.6 Hz, 1H), 4.77 – 4.63 (m, 1H), 4.37 – 4.20 (m, 1H), 2.20 – 2.11 (m, 2H), 2.11 – 1.93 (m, 2H), 1.92 – 1.76 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 169.8, 69.7, 48.2, 31.8, 29.1, 25.2. MS (ESI) m/z calcd. for C₆H₁₀⁷⁹BrO₂ [M+H]⁺ 192.99, found 192.96, calcd. for C₆H₁₀⁸¹BrO₂ [M+H]⁺ 194.98, found 194.96.

Synthesis of poly(α -azido- ϵ -caprolactone) (PN₃CL). The poly(α -azido- ϵ -caprolactone) (PN₃CL) was synthesized according to the reported procedure with some modifications (Scheme 2).^{37,38} To the solution of α -bromo- ϵ -caprolactone (8.7 g, 45 mmol) in anhydrous toluene (20 mL), benzyl alcohol (BnOH, 162 mg, 1.5 mmol) and tin(II) 2-ethylhexanoate (Sn(Oct)₂, 648 mg, 1.6 mmol) were added sequentially. The mixture in the Schlenk flask was subject to three cycles of freeze-pump-thaw cycles, sealed under nitrogen and stirred at 80 °C for 48 h before quenched with highly diluted HCl in methanol. The resulted mixture was diluted with DCM and precipitated with hexane, centrifuged and dried under vacuum, giving the poly(α -azido- ϵ -caprolactone) (PBrCL) in 83% yield (7.2 g). ¹H NMR (300 MHz, CDCl₃) δ 7.37 (m, *Ar-H* from Bn–), 5.21 (s, PhCH₂–), 4.53 – 3.88 (m, –OCH₂– and –CH(Br)–), 3.66 (t, –CH₂OH), 2.24 – 1.91 (m, –CH(Br)CH₂–), 1.85 – 1.37 (m, –CH(Br)CH₂CH₂CH₂–). ¹³C NMR (75 MHz, CDCl₃) δ 169.7, 65.5, 45.7, 34.3, 27.8, 23.8. M_n^{NMR} = 4.8 kDa, M_n^{GPC} = 4.4 kDa, PDI = 1.29. To a solution of PBrCL (7.5 g, 39 mmol of repeating unit) in DMF (120 mL) was added sodium azide (NaN₃, 5.1 g, 78 mmol), the mixture was stirred at room temperature overnight before concentrated under vacuum. The concentrated solution was diluted with toluene followed by centrifugation to remove the insoluble solid. The supernatant was concentrated and dried in vacuo afterwards to afford the final desired PN₃CL (5.6 g, 93%). ¹H NMR (400 MHz, CDCl₃) δ

1 7.38 (m, *Ar-H* from Bn-), 5.22 (s, PhCH_2 -), 4.40 – 4.11 (m, $-\text{OCH}_2$ -), 3.97 – 3.78 (m, –
2 $\text{CH}(\text{N}_3)$ -), 3.67 (t, $-\text{CH}_2\text{OH}$), 1.94 – 1.68 (m, $-\text{CH}(\text{Br})\text{CH}_2\text{CH}_2\text{CH}_2-$), 1.62 – 1.44 (m, –
3 $\text{CH}(\text{Br})\text{CH}_2\text{CH}_2\text{CH}_2-$). ^{13}C NMR (100 MHz, CDCl_3) δ 170.4, 65.4, 61.9, 30.9, 28.1, 22.3.
4 $M_{\text{n}}^{\text{NMR}} = 4.0$ kDa, $M_{\text{n}}^{\text{GPC}} = 4.6$ kDa, PDI = 1.30.

5 **General procedure for modification of PN₃CL with grafting compounds.** The modification
6 of PN₃CL was modified from literature procedure.³⁷ All the modification reactions were
7 conducted using a similar procedure, therefore, the procedure of modification with 1-propargyl-
8 3-butyl-imidazolium bromide is taken as an example herein. To the solution of PN₃CL (129 mg,
9 1.0 equiv. azide group) and 1-propargyl-3-butyl-imidazolium bromide (212 mg, 1.05 equiv) in
10 DMF (5 mL), anhydrous triethylamine (8.4 mg, 0.1 equiv.) was added and the mixture was
11 subject to two freeze-pump-thaw cycles before the addition of CuI (15.8 mg, 0.1 equiv.). The
12 mixture was subject to another two freeze-pump-thaw cycles before sealed under argon and
13 stirred at 40 °C overnight. The resulted mixture was diluted with water and transferred into
14 dialysis tubing directly followed by dialyzed against highly diluted EDTA solution (0.25 mg/ L)
15 for 2 days and deionized water for another one day. Puffy solid could be obtained after
16 lyophilization.

17 **Degradability examination.** The degradation ability of polymers was conducted using the
18 selected sample as a model. Polymer was incubated in phosphate-buffered-saline (PBS, PH = 7.4)
19 at 37 °C. Aliquots were taken out at certain time intervals and monitored directly by GPC.

20 **Minimum inhibitory concentration (MIC) determination.** Minimum inhibition
21 concentrations (MICs) were measured following standard broth dilution method with minor
22 modification.⁵⁵ Bacterial cells were grown overnight in Mueller-Hinton broth (MHB, Difco®,
23 Becton, Dickinson and Company) at 37 °C. The bacteria were 1:100 subcultured in MHB to a

mid-exponential phase and diluted to 5×10^5 CFU·mL⁻¹ in fresh MHB. Stock solutions of PCLs were prepared in the MHB medium at a concentration of 1024 µg·mL⁻¹. The solutions were 2-fold serially diluted in MHB medium, and 50 µL of each dilution was placed in each well of 96-well microplates (NuncTM, ThermoScientific) followed by the addition of 50 µL of the bacterial suspension. The plate was mixed in a shaker incubator for 10 min before incubated at 37 °C for 18 h, and the absorbance at 600 nm was measured with a microplate reader (TECAN, infinite F200). A positive control without polymer and a negative control without bacteria were included. MIC was determined as the lowest concentration of the compound that inhibited the growth of bacteria by more than 90%. All tests were done in three independent tests with duplicate per test.

Hemolysis studies. Fresh human blood was collected from a health donor (IRB-2015-03-040) and used within the same day. Human blood was drawn directly into K2-EDTA-coated Vacutainer tubes to prevent coagulation of blood and stored at 4 °C for 30 min. 1 mL blood was mixed with 9mL PBS and centrifuged at 1,000 rpm for 5 min. Supernatant was discarded and red blood cells (RBCs) were collected. The RBCs were washed with PBS three times and resuspended to a final concentration of 5% (v/v) in PBS. A two-fold dilution series of polymer in PBS solution was prepared, 50 µL red blood cell suspension was mixed with 50 µL polymer solution in each well and incubated for 1 h at 37 °C in an inoculation shaker with continuous shaking at 150 rpm. The 96 well plates were centrifuged at 1,000 rpm for 10 min. After centrifugation, 80 µL centrifuge supernatant samples were transferred to a new 96-well plate and diluted with 80 µL PBS, and hemolytic activity was calculated by measuring absorbance at 540 nm using a 96-well plate spectrophotometer (Benchmark Plus, BIO-RAD). PBS buffer (pH 7.4) was used as a negative hemolysis control, and Triton X-100 (0.1% v/v in PBS) was used as a positive control. The percentage of hemoglobin release was calculated from the following

equation: Hemolysis (%) = $[(O_p - O_b)/(O_t - O_b)] \times 100\%$ where O_p is the absorbance for the polymer, O_b is the absorbance for the negative control (PBS), and O_t is the absorbance for the positive control of Triton X-100. All data were obtained from the mean value of three replicates.

Cytotoxicity assays. The mammalian cell biocompatibility study was tested towards 3T3 fibroblast cell using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in colorimetric assay. 3T3 cells (ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin). The cells in tissue culture flask were cultured at 37 °C in a humidified incubator with 5% CO₂ until 80% confluence was reached. 3T3 cells were harvested from the confluence flask by trypsinization. Cell number was determined using a hemocytometer and 10⁴ cells/well were seeded into a 96-well tissue culture plate and incubated at 37 °C in a humidified incubator with 5% CO₂ for 24h. Polymer in culture medium solutions at 100 µg/mL and 200 µg/mL were added into the 96-well plate seeded with cells and incubated at 37 °C in a humidified incubator with 5% CO₂ incubator for 24 h. Cells incubated with only DMEM were used as positive nontoxic controls. Afterwards, the culture medium containing polymer was removed, and each well was washed with PBS prior to the addition of MTT solution (1 mg·mL⁻¹ in DMEM). After another 4 h of incubation, the MTT solution was aspirated and 100 µL dimethyl sulfoxide (DMSO) was added into each well, and the plate was shaken at 150 rpm for 10 minutes, after which the absorbance of each well was measured at 570 nm using a microplate reader spectrophotometer (BIO-RAD, Benchmark Plus). The cell viability results were expressed as percentages relative to the absorbance obtained in the control experiment.

$$\% \text{ Cell viability} = \frac{\text{Average abs of treated cells}}{\text{Average abs of controls}} \times 100\%$$

Bacteria killing kinetics. Time kill study was conducted by incubating bacteria with different concentrations of polymer/antibiotics and determining CFU·mL⁻¹ at various time points. Bacterial cells were grown overnight in Mueller-Hinton broth medium at 37 °C. After subculturing to a mid-exponential phase, bacteria were diluted to 5×10⁵ CFU·mL⁻¹ in fresh MHB. Polymer or antibiotic were added to 1000 µL bacteria in MHB suspension in Eppendorf tubes to achieve a final polymer/antibiotic concentration of 4×MIC, 2×MIC, 1×MIC and 0.5×MIC respectively. Bacteria in MHB suspension without addition of polymer were used as positive control. The bacteria suspensions with polymers were incubated in an inoculation shaker at 37 °C with continuous shaking. Aliquots were taken at different time intervals (0, 0.25, 0.5, 1, 2, 4, 6, and 24 h) and ten-fold serial diluted in PBS for plating. The diluted aliquots were plated on LB agar and incubated at 37 °C and CFU of each sample was determined after 20 h. The killing model for the comparison with polymer degradation was carried out in PBS with same conditions except no medium. Two independent experiments with duplicate for each test were performed for each polymer/pathogen combination, and the resulting average values are plotted on CFU·mL⁻¹ against time.

RESULTS AND DISCUSSION

Synthesis of Poly(azido-ε-caprolactone) (PN₃CL) with Clickable Side Groups. The functionalizable monomer of α-bromo-ε-caprolactone was synthesized using previously reported procedure⁵⁴ and functionalizable poly(ε-caprolactone) were synthesized by reported procedure with some modifications (Figure 1).^{37,38} Cyclohexanone was converted into α-bromocyclohexanone by bromination with N-bromosuccinimide (NBS) followed by Baeyer-Villiger oxidation with 3-chloroperoxybenzoic acid (*m*-CPBA) as the oxidant, producing the monomer α-bromo-ε-caprolactone (α-BrCL) in moderate yield (41%). With the monomer in

1 hand, the poly(α -bromo- ϵ -caprolactone) (PBrCL) was prepared by ring-opening polymerization
2 using benzyl alcohol (BnOH) as initiator and tin(II) 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$) as catalyst. The
3 antibacterial activities and toxicity of cationic polymers have been found to be influenced by
4 molecular weights. Polymers with moderate molecular weights have generally been found to be
5 active against bacteria with moderate toxicities.^{21,42,56} Thus, various functionalized
6 polycaprolactone derivatives with moderate molecular weight of about 4 kDa to 5 kDa were
7 targeted by controlling the feeding ratio and a single peak was observed in GPC for each
8 polymer, indicating a well- controlled polymerization. For example, a PBrCL of 4.8 kDa (DP of
9 25) was obtained by controlling the feeding ratio of benzyl alcohol and monomer at 1:30.
10 Considering the 83% yield, we can conclude that the targeted molecular weight was successfully
11 obtained in a controlled manner (Supporting information, Figure S1 and S2). Subsequently, the
12 bromo substituents on PBrCL were converted into azido groups by reaction with sodium azide
13 (NaN_3) in DMF at room temperature. The resulting poly(α -azido- ϵ -caprolactone) (PN_3CL) was
14 characterized with ^1H NMR and the results indicated the complete conversion of bromo to azido
15 groups (Supporting Information, Figure S1).

Figure 1. Design and synthesis of new antibacterial agent with biodegradable poly(ϵ -caprolactone) (PCL). (a) Synthesis of PCLs: i) NBS, NH_4OAc , Et_2O , rt, 30 min; ii) m -CPBA, DCM, rt, 24 h; iii) BnOH, Sn(Oct)_2 , 80°C , 48 h; iv) NaN_3 , DMF, rt, overnight. (b) Synthesis of $\text{P(C6Im)}_{0.64}\text{-co-P(Glc)}_{0.36}\text{CL}$ in a one-pot reaction: i) CuI, Et_3N , DMF, 40°C , overnight (Glc = Glucose); c) Calculation of the ratio of grafted imidazolium and carbohydrate by the integrals in $^1\text{H-NMR}$, $\text{Ratio}_{(\text{C6Im/Glc})} = I_d / (I_{c+f} - I_d)$, which should be 0.64 and 0.36 for $\text{P(C6Im)}_{0.64}\text{-co-P(Glc)}_{0.36}\text{CL}$ (Predicted ratio is 70:30); d) The characteristic signals in resulting PCLs: signals d and e are from imidazolium ring while c and f are from the formed triazole rings.

Conjugation of Poly(α -azido- ϵ -caprolactone) with Alkyne-Imidazoliums and Alkyne-Carbohydrates via CuAAC Click Chemistry. The post-modification of the obtained PN₃CL was conducted using CuAAC click reaction via a modified literature procedure.³⁷ Briefly, the click reaction was carried out utilizing the CuI/triethylamine combination in anhydrous and degassed DMF at a slightly elevated temperature of 40 °C because of poor solubility of cationic compounds and carbohydrates at room temperature. Initially, as a first trial, a functionalized PCL modified with 1-propargyl-3-hexyl-imidazolium bromide (C6Im) and prop-2-ynyl-D-glucopyranoside (Glc) was synthesized successfully in one-pot reaction (Figure 1). In order to investigate the structure-activity relationship especially the carbohydrate effects, a library of PCLs was synthesized. The signal of proton on triazole ring (8.2 ppm for sugars and 8.3 for imidazolium) is different from the signals of protons on the imidazolium ring (8.9 ppm and 7.6 ppm), and thus the ratios of sugars and imidazoliums can be calculated from the integrals in ¹H NMR of resultant polymers. (This was calculated to possess 64% imidazolium and 36% glucoside and coded as P(C6Im)_{0.64}CL-*co*-P(Glc)_{0.36}CL in Figure 1). Also, some proton signals on the main chain of PCL are isolated from the others, which could be confirmed by 2D NMR and applied in calculating the click efficiency as well as molecular weight (Figure 2, full spectra see Supporting Information). The HMBC of P(Glc)CL indicates H _{β} coupled with the adjacent carbonyl C _{α} and C _{γ} , proving H _{β} is on the adjacent carbon C _{β} of carbonyl group. With the information from HMQC of P(Glc)CL, H _{γ} has coupled with only C _{γ} , proving it is the only proton connected with carbon C _{γ} . Furthermore, the H _{γ} only coupled with the C _{γ} in the HMQC of PCLs bearing imidazolium as well, indicating H _{γ} is the isolated proton signal in the main chain. The molecular weight of the polymers was calculated from both ¹H NMR and GPC, and the results are summarized in Table S1. (The GPC traces were unimodal (Figure S2).) Moreover, the actual

1 content of resulting polymers is very close to the designed feeding ratio of grafting agents (Table
2 S2).

3
4 **Figure 2.** Confirmation of the characteristic protons from the main chain of PCL with 2D NMR
5 spectra. (a) Confirmation of C_γ from the main chain; (b) Confirmation of H_γ from the main chain;
6 (c,d). Confirmation that the H_γ is isolated from other signals in imidazolium bearing PCLs.

7 **Antibacterial Activities and Toxicity of the Resulting Poly(ϵ -caprolactone).** The
8 antibacterial efficacy of the resulting PCLs was first evaluated by employing both Gram-negative
9 (*Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)) and Gram-positive

bacteria (*Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *Staphylococcus aureus* (MRSA)). The minimum inhibitory concentration (MIC), or the lowest concentration of compound needed to prevent visible growth of bacteria, of various polymer derivatives were measured against the different bacterial strains. We measured hemolytic toxicity as the concentration causing 50% hemolysis of human red blood cells (HC₅₀). We measured the selectivity of the functionalized PCLs as the ratio between HC₅₀ and MIC values (Herein, MIC values against *S. aureus* was used). Toxicity towards a typical mammalian cell (3T3 fibroblasts) at 100 and 200 µg/mL (several times the MICs) was also measured.

Table 1. Antibacterial, Hemolytic Activity and Mammalian Cell Biocompatibility of Resulting PCLs

Entry	Sample	MIC (µg/mL)				HC ₅₀ ^a (µg/mL), RBC	Selectivity ^b HC ₅₀ /MIC	Cell viability (%), 3T3	
		<i>S. aureus</i> 29213	MRSA BAA40	<i>E. coli</i> 8739	<i>P. aeruginosa</i> PAO1			100 µg/mL	200 µg/mL
P1	P(C4Im)CL	32	32	32	128	1158	36	81	27
P2	P(C6Im)CL	4	4	8	32	687	172	20	6
P3	P(C8Im)CL	8	8	16	32	332	42	18	9
P4	P(C10Im)CL	32	16	64	128	24	1	4	4
P(3)5	P(C8Im) _{0.63} CL-co-P(Glc) _{0.37} CL	8	8	32	32	149	19	31	6
P(3)6	P(C8Im) _{0.48} CL-co-P(Glc) _{0.52} CL	8	16	32	64	346	43	32	5
P(3)7	P(C8Im) _{0.37} CL-co-P(Glc) _{0.63} CL	16	32	16	256	386	24	48	7
P(3)8	P(C8Im) _{0.2} CL-co-P(Glc) _{0.8} CL	128	256	512	>512	1361	11	98	95
P(2)9	P(C6Im) _{0.76} CL-co-P(Glc) _{0.24} CL	4	8	16	64	139	35	33	6
P(2)10	P(C6Im) _{0.64} CL-co-P(Glc) _{0.36} CL	8	8	16	64	337	42	38	6
P(2)11	P(C6Im) _{0.5} CL-co-P(Glc) _{0.5} CL	16	32	64	256	1497	47	80	35
P(2)12	P(C6Im) _{0.38} CL-co-P(Glc) _{0.62} CL	32	32	128	512	1796	56	84	45
P(2)13	P(C6Im) _{0.29} CL-co-P(Glc) _{0.71} CL	64	128	256	>512	>12500	>195	92	72
P(2)14	P(C6Im) _{0.33} CL-co-P(Gal) _{0.67} CL	32	64	64	256	1380	43	85	51
P(2)15	P(C6Im) _{0.35} CL-co-P(Man) _{0.65} CL	64	64	128	512	>12500	>195	90	82
P(2)16	P(C6Im) _{0.33} CL-co-P(GlcNAc) _{0.67} CL	64	64	64	512	5782	90	96	93
P17	P(Glc) CL	>512	>512	>512	>512	>12500	-	99	96

^a Values obtained from the plotted hemolysis curve; ^b Calculated based on the MIC values of *S. aureus* 29213.

As imidazolium salts were reported as good antibacterial agents,^{57,58} imidazolium salts with variable alkyl tail length were grafted onto the PCLs. The resultant PCL derivatives have good efficacy against both Gram-negative and Gram-positive bacteria (Table 1, P1-P4). Among these PCLs, polymers possessing imidazolium with intermediate length (6 or 8) carbon tails have better antibacterial activities than those possessing shorter and longer carbon chains (P1 and P4). With the exception of P1, the other imidazolium functionalized PCLs had relatively high toxicity towards mammalian cells. The hemolytic activities of P1 to P3 were reasonable, with a selectivity index of 171 and 41 for P2 and P3 respectively. P4 is too hemolytic, likely because the side chain with 10 carbons is too long.

Figure 3. Dose-dependent hemolytic activity of resultant PCLs. (a) PCLs bearing different ratios of Glucose. (b) PCLs bearing different carbohydrates (Glucose, Galactose, Mannose and Glucosamine). Glc = Glucose, Gal = Galactose, Man = Mannose, GlcNAc = Glucosamine.

To determine whether increasing the hydrophilicity of the polymers can improve the toxicity profile of the polymers⁵², carbohydrates were introduced into two series with 8 and 6 alkyl side chains (*i.e.* P5 to P8 and P9 to P13 respectively) and their effects were systematically studied with different molar ratios and various carbohydrates.

Comparing P3 with their glycosylated derivatives (P5 to P8), the MIC values against Gram-negative strains increased significantly with increasing ratios of carbohydrate in the polymers whereas the MIC values went up less significantly for Gram-positive bacteria. It is probably due to the impermeability of the outer membranes of Gram-negative bacteria to large hydrophilic molecules (Table 1, P5-P8).^{59,60} However, the C8-Im derivatives' hemolysis and cytotoxicity did not decrease until the sugar content is rather large (i.e. with P8).

Table 2. Antibacterial Activity of Selected Samples against other Gram-Positive Strains Including Resistant Strains

Entry	MIC ^a (μg/mL)										
	<i>B. subtilis</i> 6633	<i>E. faecalis</i> 29212	<i>E. faecium</i> 19434	MRSA USA300	MRSA 1	MRSA 2	MRSA 3	MRSA 4	MRSA 5	MRSA 6	MRSA 7
P(3)6	8	16	8	8	8	8	16	8	16	16	8
P(3)7	32	32	32	32	16	32	64	16	64	32	32
P(2)11	8	64	16	32	16	16	128	16	32	32	32
P(2)12	16	64	32	32	16	16	128	16	32	64	32
P(2)13	64	256	64	128	64	64	256	64	128	128	128
P(2)14	16	64	32	32	32	32	128	32	32	64	64
P(2)15	32	128	32	64	64	64	256	32	64	64	64
P(2)16	32	128	32	64	32	32	128	32	64	64	64

^a *B. subtilis*, *E. faecalis*, *E. faecium*, MRSA BAA40, MRSA USA300 were purchased from ATCC. MRSA 1-7 were clinical strains isolated from local hospital (TTSH).

On the other hand, for similar ratios of carbohydrate and imidazolium, PCLs bearing C6-Im has higher MICs than those possessing C8-Im despite their lower hemolysis and lower 3T3 toxicity (Table 1, P6 vs P11 and P7 vs P12). As with P3 derivatives (i.e. P5-P8), the MIC increased much less significantly with Gram-positive bacteria when the ratio of glycosylated substitution was increased. However, their toxicity to 3T3 cells, as well as their hemolytic toxicity, was significantly reduced. For example, P13 has fairly balanced profiles of bactericidal activities against Gram-positive bacterial and good selectivity indices for 3T3 and red blood cells. Additionally, more Gram-positive strains including *B. subtilis*, *Enterococcus faecalis*, *Enterococcus faecium* and MRSA USA300, together with a panel of other clinical MRSA strains from local hospitals, were used to evaluate selected compounds. Comparable results (Table 2) to

those in Table 1 were obtained, indicating they (including P11 which has good 3T3 and red blood cell compatibility, together with MICs) are potentially bactericidal towards deadly MRSA strains. Thus, we can conclude, the alkyl side chain length, and content of imidazolium in these PCL-backbone polymers are crucial to the antibacterial activity. Interestingly, only the shorter C6-Im can achieve a more balanced MIC and selectivity values possibly because the PCL backbone is relatively hydrophobic. Also, with the decreasing ratios of imidazolium in the intermediate range (Table 1, P5-P7, and P9-P12), the MIC values increased moderately for Gram-positive bacteria but dramatically for Gram-negative bacteria. Overall, the hemolysis of the PCL-Im derivatives are generally quite good (with selectivity ratios of at least 40 even for P3).

Furthermore, to study the effects of different carbohydrates, various sugars (Glucose, Galactose, Mannose, Glucosamine) were attached in comparable molar ratios. No significant difference was observed in MIC values (Table 1, P13-P16). However, it is noteworthy that PCLs with C6-Im bearing Glucose and Mannose are relatively less hemolytic probably attributed to their difference in polarity (Table 1 and Figure 3b), and C6-Im with Glucose, Mannose and Glucosamine had markedly improved 3T3 compatibility.

With regard to selectivity against bacteria over red blood cells, P13 and P15 showed the best results (>195), followed by P2 (>172) and P16 (>90). However, considering both the hemolytic selectivity and cytotoxicity, P15 (P(C6Im)_{0.35}CL-*co*-P(Man)_{0.65}CL) was believed to have the most satisfactory balance in this polymer system.

With this promising compound in hand, the killing kinetics of the compound was studied and compared against vancomycin, which is the last resort antibiotics against MRSA infection. By exposing MRSA BBA40 to a series of concentrations of synthesized polymer and vancomycin,

1 rapid bactericidal effects were observed at 1×MIC concentration of our polymer whereas
2 vancomycin could not kill the bacteria within 6 h even at 4×MIC concentration (Figure 4a and
3 4b). Another MRSA strain tested (USA300) has revealed the same trend although some regrowth
4 could be observed at lower concentration probably due to slight degradation of antibacterial
5 agents (Figure 4c and 4d). Besides, to examine the degradability of our polymers, a model study
6 was carried out with P15 in physiological pH condition at 37 °C. The preliminary results
7 indicated that the polymers are hydrolytically degradable. In addition, no noticeable degradation
8 occurred before inhibiting bacteria and significant loss of activity after degradation was observed
9 (Figure S5). This feature endows the polymers with great advantages over enzymatically
10 degradable polymers, rendering such polymers more promising in further applications.

Figure 4. Killing kinetics against (a) MRSA BAA40. (i) P(2)15. (ii) vancomycin; and (b) MRSA USA300. (i) P(2)15. (ii) vancomycin.

Compared with bacteriostatic antibiotics, cationic polymers are preferred in many situations against resistant bacteria, biofilm bacteria or persistent bacteria where clinical studies have proven the therapeutic efficacy of bactericidal agents.⁶¹ Moreover, cationic polymers could minimize the emergence of resistance in early clinical usage, due to minimal bacteria survival and mutation.⁶² This could be an advantage of our polymer series due to its true sterilization effect within a short period of time at a relatively low concentration.⁶² Thus, the PCL bearing Mannose and imidazolium could be a potentially effective antibacterial agent in clinical applications.

CONCLUSIONS

We have successfully synthesized a new biodegradable series of polymers: poly(ϵ -caprolactone)-graft-alkylimidazolium-graft-carbohydrates via ring-opening polymerization followed by clicking with alkyne-imidazoliums and alkyne-carbohydrates. The actual ratio of alkyne-Im to alkyne-carbohydrates clicked on PCL was achieved through careful control of reagent ratios. The synthesized PCLs have good antibacterial efficacy towards Gram-positive bacteria. However, the hemolytic and 3T3 toxicities of these PCL derivatives were generally high for the PCL graft alkyl-Im polymers. These toxicities can be improved upon addition of carbohydrates, at the expense of some bacteria activity. Through our screening efforts, we have successfully identified a candidate polymer, P15, $P(C6Im)_{0.35}CL-co-P(Man)_{0.65}CL$, that demonstrated good MICs with very low toxicities against red blood cells and 3T3 cells. This candidate compound was demonstrated to have faster killing kinetics when compared against vancomycin. In conclusion, we have developed a new class of clinically relevant antibacterial polymers that is biodegradable, potent and biocompatible, paving the way for clinical applications of antibacterial polymers in the future.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Experimental details, supplementary figures and tables, NMR spectra (PDF)

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Notes

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Synthesis of Antibacterial Glycosylated Polycaprolactones bearing Imidazoliums with Reduced Hemolytic Activity

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Figure S1. ^1H -NMR spectra of synthesized PCLs. (a) ^1H -NMR of PBrCL. (b) ^1H -NMR of PN_3CL .

In order to obtain moderate molecular weight polymer for further antibacterial materials, polymer with molecular weight of 4.0 kDa was targeted. Typically, the feeding ratio of α -BrCL and BnOH was 30 and the resulted polymer poly(α -bromo- ϵ -caprolactone) (PBrCL) was obtained in 83% yield. Furthermore, the degree of polymerization (DP) was calculated from the integration of signals at 7.37 ppm (benzyl end groups) and 3.88-4.53 ppm ($-\text{OCH}_2-$ and $-\text{CH}(\text{Br})-$) in ^1H NMR, and the DP was 25 which is consistent with the calculated result with the feeding ratio and conversion (Figure S4a). The GPC results has shown the polymer gave unimodal signal and the PDI was 1.29 (Figure S5a). Subsequently, the bromo substituents on PBrCL were converted into azido groups by reacting with sodium azide (NaN_3) in DMF at room temperature. The resulted poly(α -azido- ϵ -caprolactone) (PN_3CL) was characterized with ^1H NMR and the ratio of the integration of signals at 4.41-4.40 ppm ($-\text{OCH}_2-$) and 3.78-3.97 ppm ($-\text{CH}(\text{N}_3)-$) indicates the complete conversion of bromo groups to azido groups (Figure S1b). The DP calculated from ^1H NMR and PDI determined by GPC had almost no change which were 25 and 1.30 (Figure S2a), respectively suggesting the elimination of transesterification in the process.

Figure S2. GPC traces of synthesized PCLs. (a) THF as eluent; (b) DMF (1% LiBr) as eluent; (c) 0.05M NaCl solution as eluent.

Synthesis of grafting compounds

Prop-2-ynyl-D-glucopyranoside¹

H₂SO₄-silica was prepared according to the protocol described in Singh's report: to a slurry of silica gel (10 g, silica gel 60, 0.010-0.063 mm) in ether (50 mL) was added conc. H₂SO₄ (3 mL) with shaking for 5 min. The solvent was evaporated under reduced pressure, resulting in free flowing H₂SO₄-silica which was dried at 110 °C overnight and stored in desiccator until further use. D-Glucose (10.0 g, 55.5 mmol) was suspended in propargyl alcohol (16.1 mL, 278 mmol) and stirred at 65°C. 550 mg of H₂SO₄-silica catalyst was added to the mixture and the reaction mixture was stirred at 65°C overnight and monitored with TLC. After the complete conversion of D-glucose, the reaction mixture was transferred to a silica gel column and the excess propargyl alcohol was eluted with pure dichloromethane followed by elution of the desired glycoside with CH₂Cl₂-MeOH mixture, affording the product in 37% yield. ¹H NMR (400 MHz, Methanol-*d*₄, α/β = 3:1) δ 5.04 (d, *J* = 3.7 Hz, α-H1, 1H), 4.50 (d, *J* = 7.9 Hz, β-H1, 1H), 4.36 – 4.33 (m, 2H, both isomers), 3.96 – 3.81 (m, 1H, both isomers), 3.77 – 3.64 (m, 2H, both isomers), 3.64 – 3.57 (m, 1H, both isomers), 3.52 – 3.22 (m, 2H, both isomers), 2.93 – 2.90 (m, 1H, both isomers). MS (ESI) *m/z* calcd for C₁₈H₂₈NaO₁₂ [2M+Na]⁺ 459.15, found 459.25. ¹H NMR and MS revealed formation of the desired glycoside anomeric mixture matching the spectral data reported in literature.

Prop-2-ynyl-D-mannopyranoside²

Prop-2-ynyl-D-mannopyranoside was prepared using the same procedure for Prop-2-ynyl-D-glucopyranoside. Prop-2-ynyl-D-mannopyranoside was obtained as white solid (Yield: 34%). ¹H NMR (400 MHz, Methanol-*d*₄) δ 4.97 (s, 1H), 4.38 – 4.17 (m, 2H), 3.96 – 3.77 (m, 2H), 3.77 – 3.59 (m, 3H), 3.56 – 3.46 (m, 1H), 2.95 – 2.81 (m, 1H). MS (ESI) *m/z* calcd for C₁₈H₂₈NaO₁₂ [2M+Na]⁺ 459.15, found 459.16. ¹H NMR and MS revealed formation of the desired glycoside anomeric mixture.

Prop-2-ynyl-D-galactopyranoside³

Prop-2-ynyl-D-galactopyranoside was prepared using the same procedure for Prop-2-ynyl-D-glucopyranoside. Prop-2-ynyl-D-galactopyranoside was obtained as white solid (Yield: 31%). ¹H NMR (400 MHz, Methanol-*d*₄) δ 5.07 (d, *J* = 3.8 Hz, 1H), 4.51 – 4.26 (m, 1H), 3.96 (d, *J* = 2.9 Hz, 1H), 3.90 – 3.72 (m, 4H), 3.63 – 3.50 (m, 1H), 2.91 (t, *J* = 2.5 Hz, 1H). MS (ESI) *m/z* calcd for C₁₈H₂₈NaO₁₂ [2M+Na]⁺ 459.15, found 459.20. ¹H NMR and MS revealed formation of the desired glycoside anomeric mixture.

Prop-2-ynyl-2-acetylamino-2-deoxy-D-glucopyranoside⁴

Prop-2-ynyl-2-acetylamino-2-deoxy-D-glucopyranoside was prepared using the same procedure for Prop-2-ynyl-D-glucopyranoside. Prop-2-ynyl-2-acetylamino-2-deoxy-D-glucopyranoside was obtained as pale yellow solid (Yield: 23%). ¹H NMR (400 MHz, Methanol-*d*₄, α/β = 5:1) δ 5.01 (d, J = 3.6 Hz, α -H1, 1H), 4.62 (d, J = 8.4 Hz, β -H1, 1H), 4.40 – 4.22 (m, 2H), 4.01 – 3.90 (m, 1H), 3.90 – 3.80 (m, 1H), 3.76 – 3.64 (m, 2H), 3.64 – 3.57 (m, 1H), 3.44 – 3.36 (m, 1H), 2.88 (t, J = 2.5 Hz, 1H), 2.01 (s, 3H). MS (ESI) m/z calcd for C₂₂H₃₄N₂NaO₁₂ [2M+Na]⁺ 541.20, found 541.28. ¹H NMR and MS revealed formation of the desired glycoside anomeric mixture.

1-Propargyl-3-butyl-1,3-diazanyl-2,4-cyclopentadiene bromide⁵

N-Butyl imidazole was prepared following the method in literature with modifications⁶. To the suspension of imidazole (6.0 g, 88 mmol) in acetonitrile (50 mL) was added KOH pellets (9.9 g, 177 mmol), the mixture was stirred for 30min followed by the addition of 1-bromobutane (9.7 mL, 90 mmol). The reaction mixture was refluxed for 4 h before cooled to room temperature. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and washed with water and brine. The organic phase was dried over Na₂SO₄, filtered and concentrated under reduced pressure and subjected to flash column using MeOH-EtOAc as eluent affording the desired product in 81% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.42 (s, 1H), 7.01 (s, 1H), 6.87 (s, 1H), 3.89 (t, J = 7.1 Hz, 2H), 1.83 – 1.67 (m, 2H), 1.37 – 1.23 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H).

The 1-Propargyl-3-butyl-1,3-diazanyl-2,4-cyclopentadiene bromide was synthesized according to the procedure in the literature with modification.⁷ To the 1-butylimidazole (1.0 g, 8.1 mmol) solution in ethanol (40 mL), propargyl bromide (1.0 g, 8.5 mmol) was added and the reaction mixture was refluxed for 24h before cooled down to room temperature. The solvent was removed under reduced pressure and the residue was washed with diethyl ether and dried in vacuo, giving pale yellow oil. ¹H NMR (400 MHz, Methanol-*d*₄) δ 9.29 (s, 1H), 7.93 – 7.53 (m, 2H), 5.27 (d, J = 2.6 Hz, 2H), 4.36 (t, J = 7.4 Hz, 2H), 3.38 (t, J = 2.6 Hz, 1H), 2.03 – 1.92 (m, 2H), 1.56 – 1.40 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H). MS (ESI) m/z calcd for C₁₀H₁₅N₂ [M-Br]⁺ 163.12, found 163.20.

1-Propargyl-3-hexyl-1,3-diazanyl-2,4-cyclopentadiene bromide⁷

1-Propargyl-3-hexyl-1,3-diazanyl-2,4-cyclopentadiene bromide was produced in the same procedure. ¹H NMR (400 MHz, Methanol-*d*₄) δ 9.44 (s, 1H), 8.06 – 7.71 (m, 2H), 5.40 (d, J = 2.6 Hz, 2H), 4.46 (t, J = 7.4 Hz, 2H), 3.47 (t, J = 2.6 Hz, 1H),

2.25 – 1.90 (m, 2H), 1.61 – 1.33 (m, 4H), 1.06 (t, $J = 7.4$ Hz, 3H). MS (ESI) m/z calcd for $C_{12}H_{19}N_2$ [M-Br] $^+$ 191.15, found 191.23.

1-Propargyl-3-octyl-1,3-diazanyl-2,4-cyclopentadiene bromide⁷

1-Propargyl-3-hexyl-1,3-diazanyl-2,4-cyclopentadiene bromide was produced in the same procedure. 1H NMR (400 MHz, Methanol- d_4) δ 9.33 (s, 1H), 7.95 – 7.60 (m, 2H), 5.29 (d, $J = 2.3$ Hz, 2H), 4.36 (t, $J = 7.4$ Hz, 2H), 3.37 (t, $J = 2.6$ Hz, 1H), 2.15 – 1.80 (m, 2H), 1.50 – 1.23 (m, 6H), 0.96 (t, $J = 7.4$ Hz, 3H). MS (ESI) m/z calcd for $C_{14}H_{23}N_2$ [M-Br] $^+$ 219.19, found 219.29.

1-Propargyl-3-decyl-1,3-diazanyl-2,4-cyclopentadiene bromide⁷

1-Propargyl-3-decyl-1,3-diazanyl-2,4-cyclopentadiene bromide was produced in the same procedure. 1H NMR (400 MHz, Methanol- d_4) δ 9.28 (s, 1H), 7.81 (s, 2H), 5.26 (d, $J = 2.5$ Hz, 2H), 4.34 (t, $J = 7.4$ Hz, 2H), 3.37 (t, $J = 2.6$ Hz, 1H), 2.18 – 1.88 (m, 2H), 1.56 – 1.25 (m, 8H), 0.96 (t, $J = 7.4$ Hz, 3H). MS (ESI) m/z calcd for $C_{16}H_{27}N_2$ [M-Br] $^+$ 247.22, found 247.34.

Figure S3. Calculation of the click efficiency and molecular weight of the resulted polymers by NMR. Click Efficiency = $I_{c+f} / (I_b/2)$; $M_{n,NMR} = [155 * I_b/2] + [I_d * MW(\text{imidazolium})] + [(I_{c+f} - I_d) * MW(\text{glycoside})]$, which should be 96% and 10.3 kDa for $P(\text{C6Im})_{0.64}\text{-co-}P(\text{Glc})_{0.36}\text{CL}$, respectively.

Figure S4. Full spectra of 2D NMR. (a) Confirmation of C_γ from the main chain; (b) Confirmation of H_γ from the main chain; (c,d). Confirmation the H_γ is isolated from other signals in imidazolium bearing PCLs.

Figure S5. The comparison of degradation and killing kinetics of P15. a) Degradation in PBS at 37 °C monitored by aqueous GPC. b) Killing kinetics against MRSA BAA40 in PBS; c) Killing kinetics against MRSA BAA40 in PBS of degraded mixture after 5 days.

Figure S6. The resultant polymer P15 after lyophilization.

Table S1. Molecular weight and PDI determined by NMR and GPC.

Entry	Sample	Molecular Weight		PDI ^a
		M _n , NMR (kDa)	M _n , GPC ^a (kDa)	
P1	P(C4Im)CL	9.1	2.0	1.06
P2	P(C6Im)CL	9.9	1.8	1.09
P3	P(C8Im)CL	10.8	1.9	1.07
P4	P(C10Im)CL	11.5	1.8	1.07
P(3)5	P(C8Im) _{0.63} CL- <i>co</i> -P(Glc) _{0.37} CL	11.5	1.8	1.08
P(3)6	P(C8Im) _{0.48} CL- <i>co</i> -P(Glc) _{0.52} CL	9.0	1.7	1.07
P(3)7	P(C8Im) _{0.37} CL- <i>co</i> -P(Glc) _{0.63} CL	7.8	1.6	1.13
P(3)8	P(C8Im) _{0.2} CL- <i>co</i> -P(Glc) _{0.8} CL	9.8	4.6 ^b	1.47 ^b
P(2)9	P(C6Im) _{0.76} CL- <i>co</i> -P(Glc) _{0.24} CL	8.6	1.9	1.06
P(2)10	P(C6Im) _{0.64} CL- <i>co</i> -P(Glc) _{0.36} CL	10.3	1.9	1.08
P(2)11	P(C6Im) _{0.5} CL- <i>co</i> -P(Glc) _{0.5} CL	8.5	1.7	1.10
P(2)12	P(C6Im) _{0.38} CL- <i>co</i> -P(Glc) _{0.62} CL	8.3	1.5	1.20
P(2)13	P(C6Im) _{0.29} CL- <i>co</i> -P(Glc) _{0.71} CL	9.4	1.2	1.27
P(2)14	P(C6Im) _{0.33} CL- <i>co</i> -P(Gal) _{0.67} CL	10.6	1.7	1.15
P(2)15	P(C6Im) _{0.35} CL- <i>co</i> -P(Man) _{0.65} CL	10.2	1.4	1.23
P(2)16	P(C6Im) _{0.33} CL- <i>co</i> -P(GlcNAc) _{0.67} CL	10.1	1.6	1.23
P17	P(Glc)CL	9.3	12.8 ^b	1.49 ^b

^a Determined by aqueous GPC (0.05M NaCl solution) system using pullulan standards as the reference without otherwise noted. ^b Determined by DMF GPC (1% LiBr) system using narrow polystyrene standards as the reference.

Table S2. Designed ratio and actual ratio of resulting polymers by one-pot click reaction.

Entry	Sample	Ratio (IM: Carbohydrate)	
		Designed	Actual ^a
P1	P(C4Im)CL	100:0	100:0
P2	P(C6Im)CL	100:0	100:0
P3	P(C8Im)CL	100:0	100:0
P4	P(C10Im)CL	100:0	100:0
P(3)5	P(C8Im) _{0.63} CL- <i>co</i> -P(Glc) _{0.37} CL	67:33	63:37
P(3)6	P(C8Im) _{0.48} CL- <i>co</i> -P(Glc) _{0.52} CL	50:50	48:52
P(3)7	P(C8Im) _{0.37} CL- <i>co</i> -P(Glc) _{0.63} CL	33:67	37:63
P(3)8	P(C8Im) _{0.2} CL- <i>co</i> -P(Glc) _{0.8} CL	20:80	20:80
P(2)9	P(C6Im) _{0.76} CL- <i>co</i> -P(Glc) _{0.24} CL	80:20	76:24
P(2)10	P(C6Im) _{0.64} CL- <i>co</i> -P(Glc) _{0.36} CL	70:30	64:36
P(2)11	P(C6Im) _{0.5} CL- <i>co</i> -P(Glc) _{0.5} CL	50:50	50:50
P(2)12	P(C6Im) _{0.38} CL- <i>co</i> -P(Glc) _{0.62} CL	40:60	38:62
P(2)13	P(C6Im) _{0.29} CL- <i>co</i> -P(Glc) _{0.71} CL	30:70	29:71
P(2)14	P(C6Im) _{0.33} CL- <i>co</i> -P(Gal) _{0.67} CL	40:60	33:67
P(2)15	P(C6Im) _{0.35} CL- <i>co</i> -P(Man) _{0.65} CL	40:60	35:65
P(2)16	P(C6Im) _{0.33} CL- <i>co</i> -P(GlcNAc) _{0.67} CL	40:60	33:67
P17	P(Glc)CL	0:100	0:100

^a Determined by NMR.

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NMR Spectra