

Chemically modifiable N-heterocycle-functionalized polycarbonates as a platform for diverse smart biomimetic nanomaterials†

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A series of functional aliphatic polycarbonates bearing pendant N-heterocycles has been synthesized using a facile and modular synthetic strategy. This allows rapid access to a diverse range of biomimetic nanostructured materials that show promise as non-fouling polyzwitterions, host-defense peptide mimics, and potential drug/gene-delivery vectors, all starting from a common precursor. Preliminary biological data indicate promising non-fouling properties, antimicrobial activity, and negligible toxicity in human cell lines.

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Aliphatic polycarbonates show tremendous potential as smart nanobiomaterials because of their therapeutic potential, biodegradability, biocompatibility, low toxicity, and tunability. Some important nanomedicine applications include drug and gene delivery, antimicrobial action, and *in vivo* medical imaging.¹ These functional aliphatic polycarbonates are usually synthesized by ring-opening polymerization (ROP) of cyclic carbonates *via* organocatalytic, anionic, cationic, coordination-insertion, and enzymatic methods.²

We had previously developed a number of synthetic methods based on the organocatalytic ROP of tailor-made cyclic carbonate monomers, and more recently, the facile post-polymerization functionalization of a versatile precursor polycarbonate containing substitutable pentafluorophenyl esters.³ Using a combination of these methods, we already possess the ability to synthesize wide-ranging functional polycarbonates for use as smart nanomedicines, *e.g.* drug and gene delivery vectors,⁴ host defense peptide mimics,⁵ *etc.* To date however, there has not been a satisfactory way to synthesize N-heterocycle-functionalized polycarbonates using existing methods. The motivation behind making these materials lies in the synthetic versatility of the N-heterocycles, which allow for easy access to quaternized cationic polymers with antimicrobial activity or gene/drug-delivery potential. More importantly, it

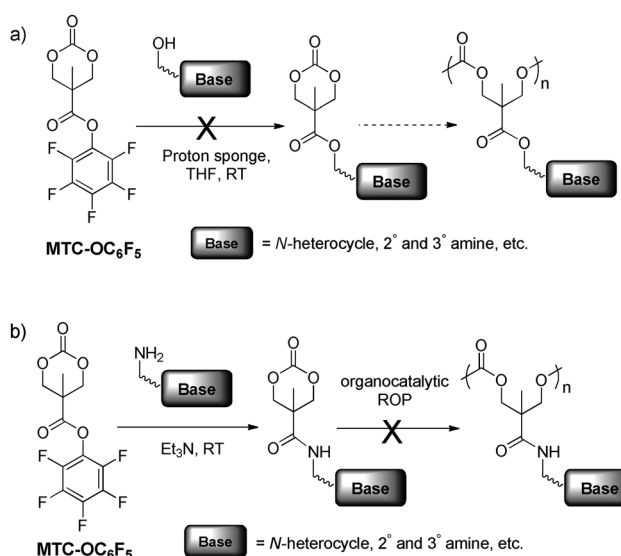
allows the facile synthesis of sulfobetaine-type polyzwitterions,⁶ thus providing another efficient route to zwitterion-functionalized polycarbonates, a class of materials that has recently been explored by others.⁷ Similar to phosphatidylcholine moieties of lipid biomembranes and related synthetic systems,⁸ sulfobetaines tend to confer antifouling properties. Thus, sulfobetaine-functionalized polycarbonates may represent a biodegradable alternative to poly(ethylene glycol) (PEG)-based stealth materials for therapeutic applications.⁹ Our previous attempts to access N-heterocycle-containing cyclic carbonate monomers from MTC-OC₆F₅ and the corresponding alcohols (Scheme 1a) were met by extremely low yields as well as purification and

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Scheme 1 Previous attempts to prepare heterocycle/amine-containing polycarbonates.

isolation difficulties. Subsequent efforts to circumvent this problem by reacting MTC-OC₆F₅ with ω -aminoalkyl-N-heterocycles (Scheme 1b) successfully afforded N-heterocycle-containing cyclic carbonates, but these could not be polymerized in a well-controlled manner using any of our organocatalytic ROP methodologies. The basic organocatalysts either showed no effect on the monomer, or proved incompatible with the amide functionality. Furthermore, our recent acid-catalyzed ROP method¹⁰ is incompatible with monomers bearing basic amine or N-heterocycle functional groups.

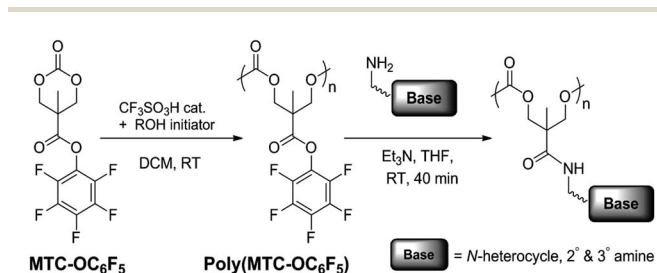
Following our recently reported postpolymerization functionalization of poly(MTC-OC₆F₅) with primary amines,³ we explored the possibility of grafting pendant N-heterocycles onto a polycarbonate backbone, followed by the appropriate chemical modification of the N-heterocycles for different biomedical applications. We envisioned that the desired polymers could be accessed by reacting poly(MTC-OC₆F₅) with bifunctional ω -aminoalkyl-N-heterocycles containing a nucleophilic primary amine (NH₂) on one end of the molecule, connected by an aliphatic linker to a weakly nucleophilic N-heterocycle (e.g. imidazole, pyridine) on the other (Scheme 2).

The key attraction of installing pendant N-heterocycles on the polymer backbone is that the rings can serve as versatile

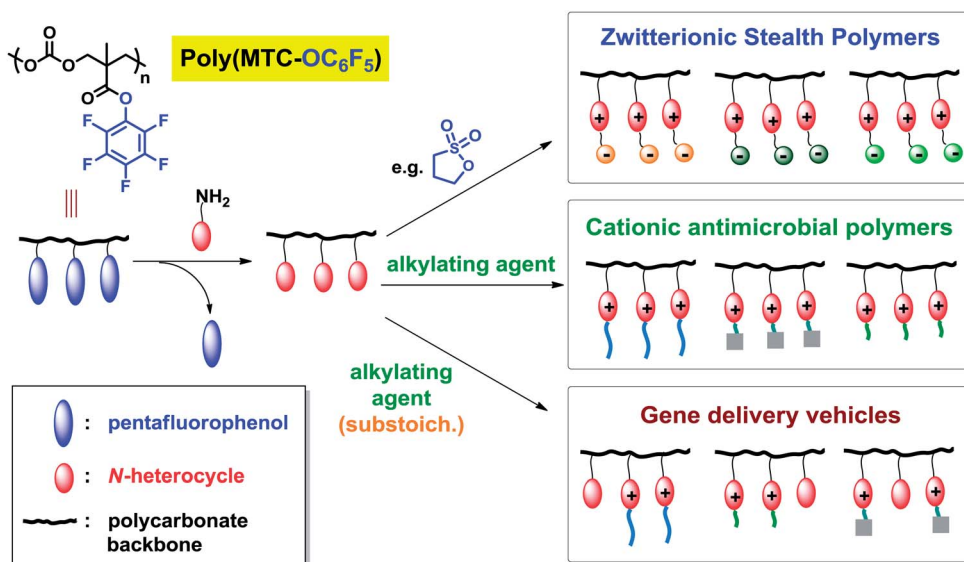
synthetic handles. Then, selective chemical modification of the heterocycles can be carried out and customized based on the polymer's intended application. This enables the generation of diverse polycarbonates with unprecedented control over the polymer structure–property–function relationships (Scheme 3). This approach also converges upon a single heterocycle-containing polycarbonate as the common precursor to more complex functional materials. Structural diversity can be conveniently incorporated at either stage of the synthetic sequence, where one can vary: (1) the type of N-heterocycle being grafted, and (2) the nature of the chemical modification performed on the heterocycle. Herein we report a highly modular and diversity-oriented synthesis of N-heterocycle-functionalized polycarbonates that can be customized and fine-tuned for different nanomedicine applications, allowing for high throughput screening.

In our study, we carried out postpolymerization modifications of poly(MTC-OC₆F₅) by treating the polymer separately with either 1-(3-aminopropyl)imidazole or 2-(2-pyridyl)ethylamine using our recently published protocol. Imidazole- and pyridine-functionalized polycarbonates were obtained respectively, in under an hour (Scheme 4). Polymer purification was easily achieved by precipitation in a non-solvent, followed by washing/sonication and centrifugation. Different chemical modifications can be performed on the pendant heterocycles to give, for example, zwitterionic sulfobetaines and N-alkylimidazolium salts (Scheme 5).

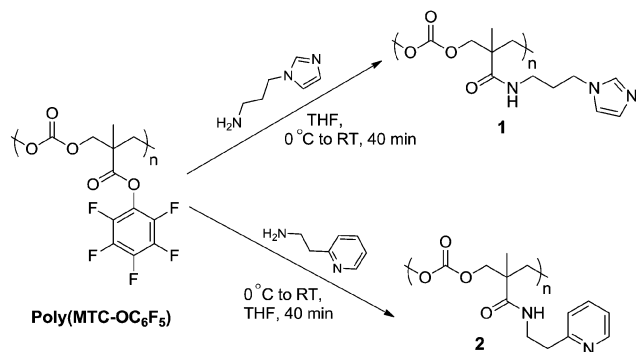
In the former case, zwitterionization was achieved by reacting the polymer with 1,3-propanesultone overnight at room temperature, resulting in a water soluble poly(sulfobetaine) 3. The chemical transformations on the polymer sidechains were verified by ¹H NMR spectroscopy. The three imidazole protons of polymer 1 produce characteristic singlets between 6.80 and 7.75 ppm (see ESI[†]). Conversion to the zwitterion resulted in the downfield shift of all three imidazole proton signals as the ring



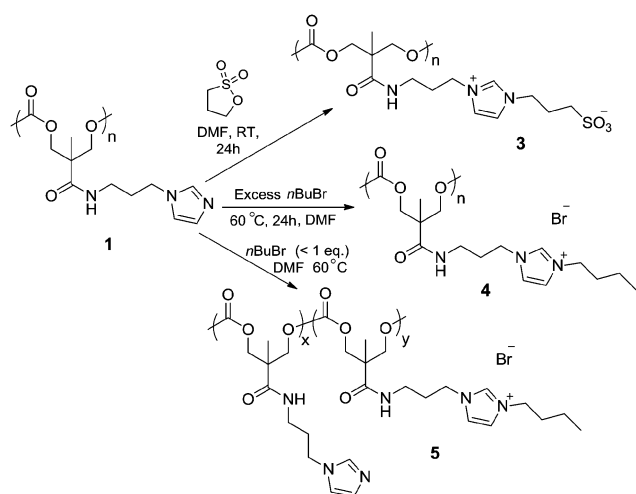
Scheme 2 General synthesis of N-heterocycle/amine-functionalized polycarbonates.



Scheme 3 Rapid generation of a functional polycarbonate library via a highly modular and diversity-oriented synthetic approach.



Scheme 4 Preparation of N-heterocycle-containing polymers from a common polycarbonate precursor.



Scheme 5 Formation of polyzwitterions and polyelectrolytes through chemical modification of pendant heterocycles (e.g. imidazoles, as shown).

became positively charged. The absence of unshifted imidazole proton peaks indicated quantitative conversion of all pendant imidazoles to imidazolium moieties. The zwitterionic poly-(sulfobetaine)s are potentially biomimetic since they share structural similarities with the phosphatidylcholine-based moieties of biological membranes. Just like PEG, these highly hydrophilic polymers are surrounded by a hydration layer when dissolved in aqueous media. Binding water molecules in this fashion is one of the key requisites for ‘stealth’ properties in biomaterials. Stealth refers to the ability of the materials to resist cell adhesion, antibody opsonization, and non-specific protein interactions, which minimizes their detection and clearance by the patient’s immune system. The advantage of a polycarbonate-based system *versus* PEG lies in its biodegradability, which avoids bioaccumulation-related toxicity. Hence, the polyzwitterions synthesized herein may be promising as biodegradable alternatives¹¹ to PEG and related polymers¹² for the stealth delivery of peptide/small molecule-based drugs.

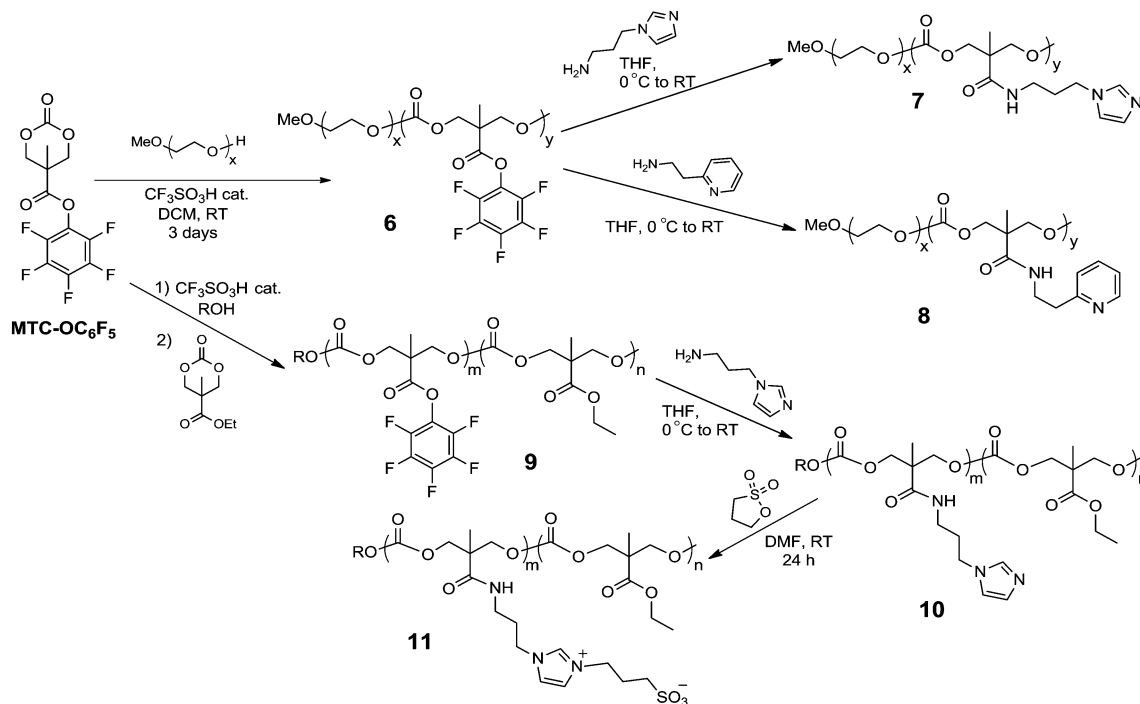
The N-heterocycles can also be easily quaternized by alkylating agents to yield water soluble polycationic materials that

mimic host defense peptides (Scheme 5). For example, exhaustive quaternization of the pendant imidazoles with excess 1-bromobutane afforded the water-soluble polymer **4** bearing positively-charged imidazolium sidechains. Such polymers can be used as biodegradable antimicrobial agents. It is known that such positively-charged macromolecules can disrupt anionic bacterial membranes, while showing negligible toxicity toward neutral mammalian cell membranes. The modularity of our synthetic method allows arbitrary alkylating agents to be employed, e.g. methyl iodide, benzyl bromide, or *n*-alkyl halides of any chain length. This enables high throughput preparation and screening of numerous candidate polymers, and also facilitates the fine-tuning of the polymer characteristics *via* manipulation of structure–property–function relationships.

We also show that it is possible to controllably quaternize only a fraction of the heterocycles using sub-stoichiometric amounts of alkylating agent, giving polymers with both charged and uncharged rings. These polymers are currently being evaluated as siRNA/DNA transfective agents that function based on the ‘proton-sponge’ effect.¹³ The idea is to have the positively-charged pendant rings bind to negatively-charged polynucleotides, while the uncharged rings would provide buffering capacity within the acidic endosomes. If shown to be effective, these N-heterocycle-containing polycarbonates will offer a biodegradable and biocompatible platform for gene-delivery. To that end, we have synthesized imidazole- and pyridine-functionalized polycarbonates containing both charged/quaternized and uncharged/unquaternized rings (see ESI†).

Our modular synthetic strategy also lends itself well to diblock copolymer systems. In one case, we first prepared a diblock precursor polymer comprised of a hydrophobic poly(MTC-OEt) segment and a chemically-modifiable poly(MTC-OC₆F₅) block (Scheme 6). Subjecting poly(MTC-OEt)-*b*-(MTC-OC₆F₅) to a two-stage post-polymerization modification involving initial N-heterocycle introduction and subsequent zwitterionization of the rings afforded a diblock copolymer with a hydrophobic block and a hydrophilic polyzwitterionic block. Such a polymer could form stealth micelles that may prove useful in encapsulating hydrophobic anticancer drugs (e.g. Taxol, Doxorubicin, etc.) for slow release and delivery to tumor tissues in the body. Water-soluble diblock copolymers composed of a hydrophilic 5 kDa-PEG block and a hydrophobic polycarbonate block with pendant N-heterocycles were also readily synthesized by treating precursor diblock copolymer **6** with N-heterocycle-containing amines (Scheme 6). Polymers **7** and **8** offer the possibility of forming micelles when self-assembled in aqueous solution. The resulting micelles may serve as therapeutic nanocarriers with pH-triggered release capabilities.

Preliminary tests pertaining to antimicrobial activity and non-fouling properties were carried out on some of the polymers described above. For example, to determine if polymers **3** and **11** had non-fouling characteristics, they were dissolved in serum-containing phosphate-buffered saline (PBS, pH of 7.4) and the size of nanoparticles in those solutions was subsequently monitored to see if any aggregation resulted from



Scheme 6 Synthesis of various diblock copolymers with N-heterocycle sidechains.

polymer–protein interactions. Similar to serum in PBS, with no added polycarbonate, the particle sizes of the zwitterionic homopolymers (*i.e.* polymer 3; $n = 50, 100$) in PBS containing 10% fetal bovine serum (FBS) were stable over a period of 48 h (Fig. 1), indicating the absence of aggregation. For comparison, we also prepared the amphiphilic diblock copolymer **11** ($x = 50, y = 17$) containing a hydrophobic MTC-OEt segment of significant length. This polymer induced aggregation after just 2 h of incubation, likely as a result of non-specific interactions between the hydrophobic component and the protein.

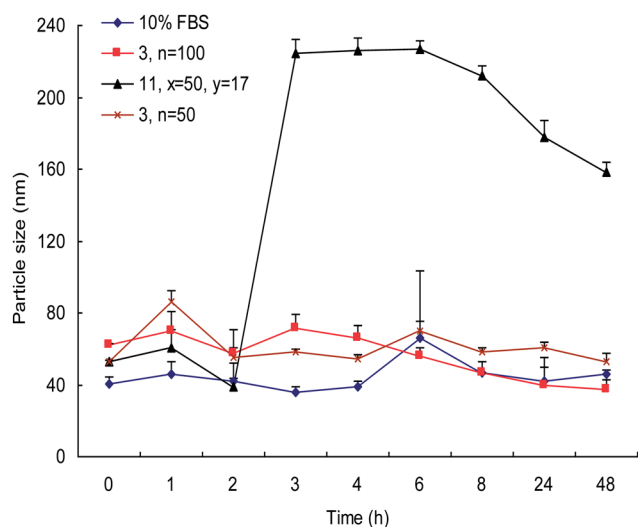


Fig. 1 Particle size change of polyzwitterions over time in PBS buffer containing 10% fetal bovine serum.

Polymer cytotoxicity was evaluated by analyzing the viability of HEK293 cells (as a model cell line) after incubation with polymers for 48 h. PEG polymers (5 and 10 kDa) were used as controls. Just like the PEG controls, all three polyzwitterions showed negligible cytotoxicity (see Fig. 2). Given these encouraging results for non-fouling behavior and marginal toxicity, further studies to determine if stealth properties can be observed *in vivo* have been planned.

In our preliminary antimicrobial studies, the cationic polymer **4** was tested against Gram-positive (*S. aureus*) and negative (*E. coli*) bacteria but found to be inactive against both (MIC > 500 mg L⁻¹). We reasoned that the hydrophobicity-to-charge balance conferred by the short *n*-butyl chains was too low to disrupt the bacterial membranes,¹⁴ and consequently modified the polymer backbone to include 20% *n*-hexyl sidechains (polymer **12** in the ESI†). Gratifyingly, this rational modification resulted in activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of 62.5 mg L⁻¹ (see ESI† for details on the biological tests and chemical synthesis associated with the polymer).

Experimental section

Representative functionalization of polymer with N-heterocycles

Polymer 1. A 20 mL glass vial containing a magnetic stir-bar was charged with poly(MTC-OC₆F₅) (0.787 g, 2.41 mmol repeat units), anhydrous THF (5.0 mL) and triethylamine (0.131 g, 1.30 mmol), and the solution was cooled to 0 °C in an ice-water bath. Next, a solution of 1-(3-aminopropyl)imidazole (0.155 g, 1.24 mmol) in THF (2 mL) was added dropwise with vigorous

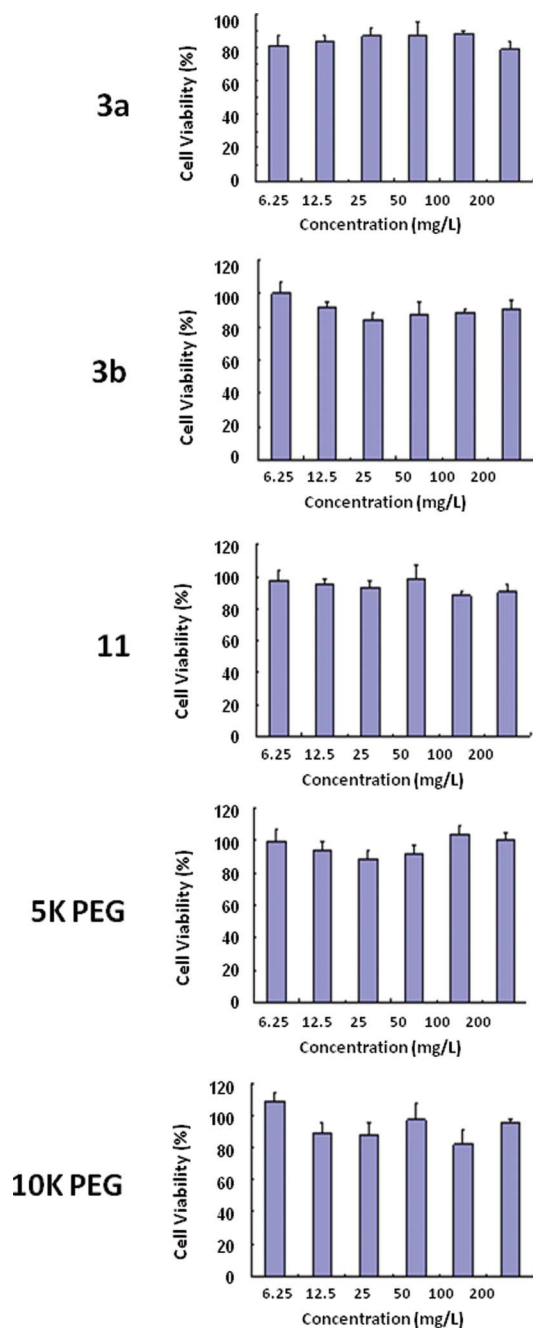


Fig. 2 HEK293 cell viability vs. polymer concentration after 2 day incubation with polyzwitterions and PEG.

stirring. Turbidity was observed within minutes, followed by the gradual formation of an off-white precipitate. The ice bath was removed and the mixture was allowed to stir for an additional 30 minutes, after which excess diethyl ether (15 mL) was added. The mixture was briefly sonicated and then centrifuged. The mother liquor was decanted and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded a white solid which was then dried under high vacuum for 24 h. This was characterized by ^1H NMR (MeOD). (Note: in cases where precipitate did not form spontaneously during the reaction, precipitation was achieved by

pipetting the crude polymer solution into stirred diethyl ether, after which purification was carried out as above).

Representative poly(sulfobetaine) synthesis

Polymer 3. A 20 mL scintillation vial containing a magnetic stir-bar was charged with polymer 1 (0.650 g, 2.43 mmol repeat units), 1,3-propanesultone (0.446 g, 3.65 mmol, 1.5 equivalents), and anhydrous DMF (2.0 mL). The reaction mixture was stirred at 18 °C for 24 h, during which gelation occurred. The gel was dissolved by addition of a minimal volume of water (~0.5 mL), and the resulting solution was precipitated into stirred diethyl ether. The suspension was then centrifuged and the solvent decanted to give the target polymer as a white solid. The poly(sulfobetaine) was washed with additional portions of ether, dried under high vacuum for 24 h, and characterized by ^1H NMR (D_2O).

Representative quaternization of pendant N-heterocycles

Polymer 4. A 20 mL glass vial containing a magnetic stir-bar was charged with polymer 1 (0.746 g, 2.79 mmol repeat units), 1-bromobutane (0.573 g, 4.18 mmol, 1.5 equivalents), and anhydrous DMF/acetonitrile (2 : 1 v/v, 6.0 mL). The reaction mixture was heated overnight at 60 °C in the sealed vial, after which it was concentrated under reduced pressure and then precipitated into diethyl ether. The suspension was centrifuged and the mother liquor was decanted off, leaving a white solid which was dried under high vacuum for 24 h and subsequently characterized by ^1H NMR (D_2O). (Note: partial quaternization to polymer 5 can be accomplished by simply using a sub-stoichiometric quantity of 1-bromobutane).

Conclusions

In conclusion, we have synthesized N-heterocycle-containing polycarbonates using a modular approach, allowing for facile postpolymerization modifications. This strategy allows for the rapid generation of diverse functional polycarbonates that can be easily customized and fine-tuned for different biomedical applications. Promising preliminary results on our biomimetic polyzwitterions point toward an ideal combination of non-fouling properties and low toxicity, and future animal studies to investigate *in vivo* stealth behavior have been planned. Similarly, preliminary antimicrobial data suggests promising activity against *S. aureus* bacteria, which is often implicated in nosocomial infections. The high degree of control afforded by our new synthetic approach allows for the unprecedented ease with which the hydrophobicity-to-charge balance can be fine-tuned, thus allowing facile adjustment of antibacterial activity *versus* toxicity/hemolytic capacity. As such, these N-heterocycle-containing polycarbonates provide an ideal platform from which a multitude of rationally designed nanomedicines can be realized. Further biological studies (*e.g.* *in vivo* activity, stealth, toxicity, degradability) on the polymers are currently underway.

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