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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.

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. These functional aliphatic polycarbonates are usually synthesized by ring-opening polymerization (ROP) of cyclic carbonates via organocatalytic, anionic, cationic, coordination-insertion, and enzymatic methods.

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 postpolymerization functionalization of a versatile precursor polycarbonate containing substitutable pentfluorophenol esters. 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, host defense peptide mimics, 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 quarternized cationic polymers with antimicrobial activity or gene/drug-delivery potential. More importantly, it allows the facile synthesis of sulfobetaine-type polyzwitterions, thus providing another efficient route to zwitterion-functionalized polycarbonates, a class of materials that has recently explored by others. Similar to phosphatidyl choline moieties of lipid biomembranes and related synthetic systems, 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. 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 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 method is incompatible with monomers bearing basic amine or N-heterocycle functional groups.

Scheme 1. Previous attempts to prepare heterocycle/amine-containing polycarbonates.
The key attraction of installing pendant with bifunctional synthetic handles. Then, selective chemical modification of the polymer backbone is that the rings can serve as versatile functionalization of poly(MTC-OC₆F₅) with primary amines, following our recently reported postpolymerization functionalized polycarbonates.

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 done 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).

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

Following our recently reported postpolymerization functionalization of poly(MTC-OC₆F₅) with primary amines, 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).

proton signals as the ring became positively charged. The imidazolium moieties. The zwitterionic poly(sulfobetaine)s are quantitative conversion of all pendant imidazoles to zwitterion resulted in the downfield shift of all three imidazole 7.75 ppm (see Supporting Information). Conversion to the alternatives bioaccumulation-related toxicity. Hence, the polyzwitterions system versus PEG lies in its biodegradability, which avoids immune system. The advantage of a polycarbonate-based which minimizes their detection and clearance by the patient's antibody opsonization, and non-specific protein interactions, refers to the ability of the materials to resist cell adhesion, modularity of our synthetic method allows arbitrary alkylating toxicity toward neutral mammalian cell membranes. The disadvantage 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 bearing positively-charged imidazolium sidechains. Such polymers may 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 the high throughput preparing 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 Supporting Information).

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 zwitterization 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 5kDa-PEG block and a hydrophobic

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).

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

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 nanoparticle sizes in those solutions were subsequently monitored to see if any aggregation resulted from 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) was stable over a period of 48 h (Figure 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.

Figure 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 Figure 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 conclusion, we have synthesized N-heterocycle-containing polycarbonates using a modular approach, allowing for facile postpolymerization modifications. This strategy allows for the

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 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 (D₂O).

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 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 (D₂O). (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 nonfouling 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 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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Notes and references


