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Exploring the Effect of Amphiphilic Polymer Architecture: Synthesis, Characterization, and Self-Assembly of Both Cyclic and Linear Poly(ethylene gylcol)-*b*-polycaprolactone

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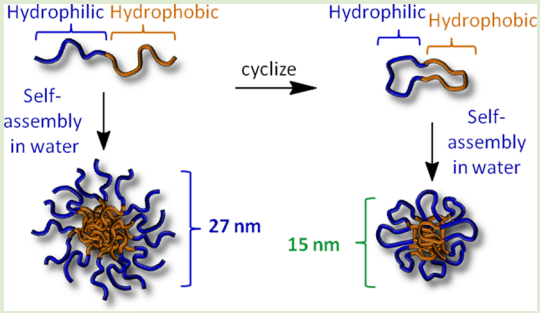
Supporting Information

ABSTRACT: While amphiphilic block copolymers have demonstrated their utility for a range of practical applications, the behavior of cyclic block copolymers remains largely unexplored due to limited synthetic access. To investigate their micelle formation, biocompatible cyclic amphiphilic poly(ethylene glycol)-polycaprolactone, c-(PEG-PCL), was synthesized by a combination of ring-opening polymerization (ROP) and click chemistry. In addition, exactly analogous linear block copolymers have been prepared as a control sample to elucidate the role of polymer architecture in their self-assembly and acid-catalyzed degradation.



Amphiphilic polymers are of interest for a range of applications because of their inherent ability to self-assemble into discrete phases in bulk or specific morphologies in solution.[1−5] Controlling the size and stability of such micellar aggregates is particularly important for drug delivery applications, as the size is one of the most critical features in determining its biodistribution, and the stability can be utilized to prevent premature release, or enable a controlled release of a therapeutic payload. A substantial body of work has already been carried out by numerous investigators in order to define and control the parameters of simple linear amphiphilic block copolymers.[6,7] For example, well-defined spherical or cylindrical micelles[8,9] can load hydrophobic drugs into the hydrophobic core of a micellar aggregate, thereby dramatically improving the drugs' aqueous solubility.[10] In addition, by controlling the micelle size, the enhanced permeability and retention effect can be used to reduce the toxicity of anticancer drugs via passively targeting cancer tissues.[11] This has been demonstrated with linear diblock copolymers of poly(ethylene glycol)-*b*-polylactide (PEG-PLA) for the controlled delivery and release of the anticancer drug, paclitaxel.[12,13] Despite the unique behavior demonstrated by cyclic polymers,[14,15] only a limited amount of work has focused on the utility of cyclic block copolymers,[16−18] owing to the difficulty in preparing such macromolecules in high purity.[19] Biocompatible and biodegradable cyclic diblock copolymers merit investigation considering that the cyclic architecture of block copolymers imparts a distinct size[15,20] and stability of their self-assembled micelles.[21] Herein, we report the first investigation of biocompatible, biodegradable amphiphilic cyclic block copolymers.

Previously, our group reported an efficient methodology for preparing well-defined macrocyclic polymers by using click cyclization. The Huisgen copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction was used to efficiently convert an α-alkynyl, ω-azido linear polystyrene into its exactly analogous cyclic polymer.[22,23] The CuAAC reaction is an extremely powerful tool for the assembly of a wide range of biological and synthetic macromolecular architectures.[24−26] The high efficiency of the click reaction ensured that the intramolecular cyclization reaction is preferred when highly dilute reaction conditions are used, such as a dropwise addition of the linear precursor to that catalyst system. This versatile technique is equally amenable to the formation of cyclic polymers from precursors prepared by atom transfer radical polymerization[22] and reversible addition−fragmentation chain transfer (RAFT) polymerization[27] as well as biodegradable polyesters prepared via ring-opening polymerization.[28,29] It has also been used to cyclize diblock polymers such as poly(methyl acrylate)-*block*-poly(styrene)[30] and even bicyclic "figure eight" block copolymers.[20]

Using a similar approach, a biocompatible and biodegradable amphiphilic cyclic diblock copolymer based on blocks of poly(ethylene glycol) and polycaprolactone has been successfully prepared (Scheme 1).

Using commercial available α-azido-ω-hydroxy-PEG **1** as a initiator, caprolactone monomer was polymerized in bulk using tin(II) 2-ethylhexanoate catalyst at 130°. After 30 min, the product was precipitated from dichloromethane into cold hexane (yield 80%). The matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) spectra showed an increase of molecular weight from 1500 to 3500 Da (Figure 1), while the GPC data exhibited a shift to an

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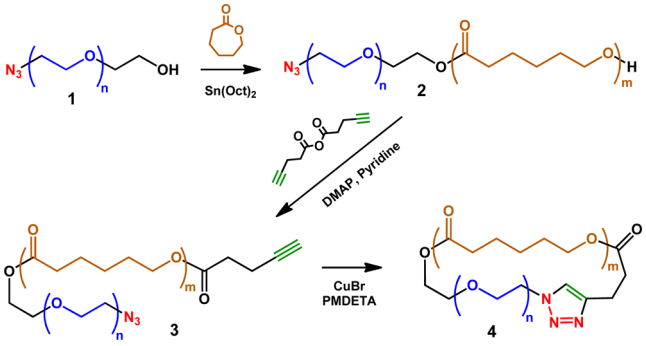
Scheme 1. Synthetic Route for Preparing Biodegradable Cyclic Block Copolymers Based on Poly(ethylene glycol) and Poly(caprolactone)



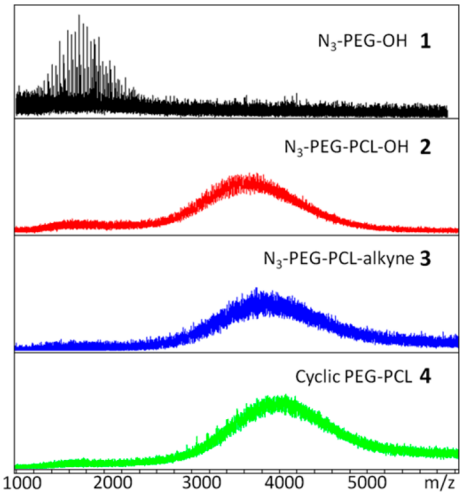


Figure 1. MALDI mass spectra of the PEG starting material **1** (black, top), the linear α-azido-ω-hydroxy PEG-PCL diblock **2** (red, second), the linear α-azido-ω-alkynyl PEG-PCL diblock **3** (blue, third), and the cyclic PEG-PCL diblock **4** (green, bottom).

earlier retention time, both of which confirm the successfully polymerization of the polycaprolactone block (Figure 2). The IR spectra exhibited a characteristic peak at 2100 cm^{-1} verifying retention of the azide end functional group (SI, Figure S1) while the ^{1}H NMR data exhibited new resonances at δ = 1.31, 1.57, 2.27, and 4.03 ppm, corresponding to the protons along the polycaprolactone backbone (SI, Figure S2). In addition, the gel permeation chromatography (GPC) trace showed a single peak with dispersity ($Đ$) less than 1.2, confirming the formation of a well-defined PEG-PCL diblock copolymer **2**.

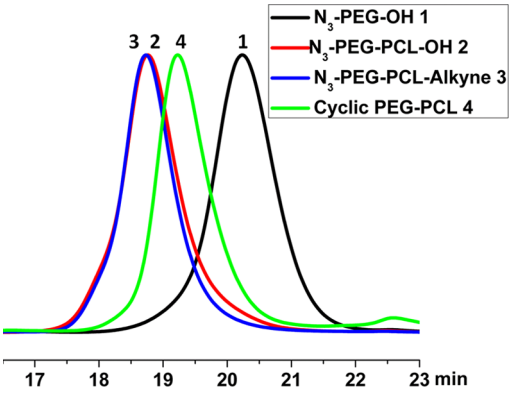


Figure 2. GPC traces of the PEG starting material **1** (black), the linear α-azido-ω-hydroxy PEG-PCL diblock **2** (red), the linear diblock α-azido-ω-alkynyl PEG-PCL diblock **3** (blue), and the cyclic PEG-PCL diblock **4** (green).

The reaction of the hydroxyl end group of the N_3-l-PEG-PCL-OH **2**, with pentynoic anhydride yielded the desired linear PEG-PCL precursor, N_3-l-PEG-PCL-alkyne **3**, with complementary click functionalities on opposite ends (yield 70%). GPC and MALDI-TOF analysis verified that while the mass and hydrodynamic volume did not shift noticeably, the narrow polydispersity was maintained during this transformation. ^{1}H NMR analysis revealed new resonances corresponding to the pentynoic group at δ = 2.45 ppm (J and H, SI, Figure S2) and δ = 1.98 ppm (I, SI, Figure S2), while the IR data confirmed retention of the azide peak at 2100 cm^{-1} after esterification (SI, Figure S1).

The slow addition "click" cyclization technique was then used to cyclize the linear precursor PEG-PCL diblock. A solution of the linear PEG-PCL **3** diblock solution (1 mg/mL) was added dropwise into a deoxygenate CuBr catalyst solution by using a syringe pump to regulate the addition rate at 2 mL/h. This use of dilute Ruggli-Ziegler conditions has been demonstrated to encourage α-,ω-functionalized polymers to favor intramolecular cyclization (yield 95%).[31,32] Higher molecular weight macromolecular cyclizations have been achieved in previous studies with homopolymers, but required slower addition and more dilute solutions.[28] After cyclization, the MALDI-TOF MS spectra verifies the mass of diblock remains unaltered while the GPC data shows the increase of retention time expected for cyclic structures due to their decreased hydrodynamic volume. The GPC trace shows the cyclic PEG-PCL **4** maintained a low dispersity and confirms the nearly quantitative cyclization. In the ^{1}H NMR, the alkyne proton resonance at about δ = 2.0 ppm disappears (I in SI, Figure S2), while a single proton resonance appears at about δ = 7.75 ppm (J in SI, Figure S2), corresponding to the proton on the triazole formed upon cyclization. Furthermore, a shift in the proton resonances immediately adjacent to the triazole is observed, from δ = 2.45 ppm and δ = 3.37 ppm to δ = 2.70 and 3.00 ppm, respectively (H and A_1 in SI, Figure S2). In addition, the IR data shows the complete disappearance of the azide peak at 2100 cm^{-1} (SI, Figure S1). All the above data provide strong evidence for the successfully synthesis of a well-defined cyclic PEG-PCL diblock.

The self-assembly behavior of the cyclic diblock PEG-PCL in water was investigated by using light scattering and compared to its linear precursor. The sizes of the micelles formed by both linear and cyclic diblocks were measure five times using static light scattering in aqueous solution to determine an average diameter. Light scattering data confirms that both the linear and the cyclic PEG-PCL form micelles in aqueous solution, though with an appreciable difference in size. The linear block copolymer exhibits an average diameter of 27 nm, while the cyclic block copolymer has a diameter of only 15 nm (Figure 3). This is in agreement with studies of phase behavior of cyclic diblocks in bulk that suggests that the cyclic topology results in more compact self-assembled units.[33]

The degradation behavior of cyclic amphiphilic block copolymers is of interest because their degradation in vivo may result in stimulus responsive behavior. This is of particular interest because in addition to the cyclic polyester's reduced rate of weight loss during degradation,[22] the initial degradation

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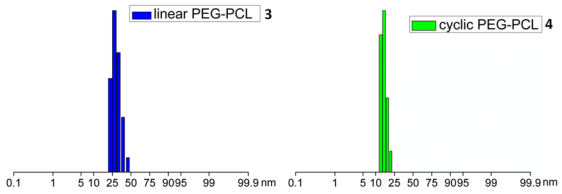

Figure 3. Light scattering data in water (number distribution) for the linear diblock α-azido-ω-alkynyl precursor (blue and left) and the cyclic diblock (green and right).

product will be a linear polymer that should exhibit different self-assembly properties than its cyclic precursor. The degradation studies of linear and cyclic PEG-PCL were carried out in a dichloromethane/methanol solution with 0.15% by weight sulfuric acid. As seen with the PCL homopolymers, the cyclic architecture does result in a distinctly slower degradation profile than its linear analogs because the first ester cleavage in each chain changes the polymer topology from cyclic to linear, but does not result in a loss of mass. While the GPC data shows measurable degradation of the linear PEG-PCL in just 2 h, the cyclic PEG-PCL exhibits a clear *increase* in hydrodynamic volume during the first 6 h because the change of topology from cyclic to linear (Figure 4). The GPC degradation trends

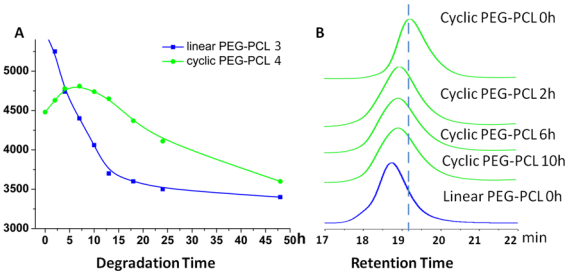

Figure 4. (A) GPC data of both linear and cyclic PEG-PCL number average molecular weight (as measured by linear polystyrene standard MW equivalents) vs degradation time while undergoing acid-catalyzed degradation. (B) GPC traces of cyclic PEG-PCL degradation exhibit a shift to earlier retention times (larger sizes) during the first 10 h of degradation.

confirm that the cyclic PEG-PCL exhibits a substantial reduced rate of hydrodynamic volume loss with respect to the linear analog. The reduction in M_n can also be more directly measured by the MALDI-TOF MS spectra of degradation aliquots at different time points. In the first 6 h, the cyclic diblock exhibited minimal loss of molecular weight and, afterward, a relatively slow loss of mass. Even after 18 h, most of the original molecular mass was maintained. The linear analog, on the other hand, showed appreciable mass loss in just 2 h, and after 18 h the linear PEG-PCL was degraded to the point that the spectra resembled that of the PEG precursor (Figure 5). As expected, based on the studies of PCL homopolymers, the acid catalyzed methanolysis of the ester bonds within the cyclic diblock PEG-PCL shows a much slower degradation than its linear analog due to its macromolecular topology.

These cyclic amphiphilic diblocks exhibit two unique behaviors with respect to the more commonly studied linear diblocks, which are relevant to their use as self-assembled drug delivery systems. First of all, the cyclic diblocks exhibit an extended degradation profile, which may extend the lifetime of

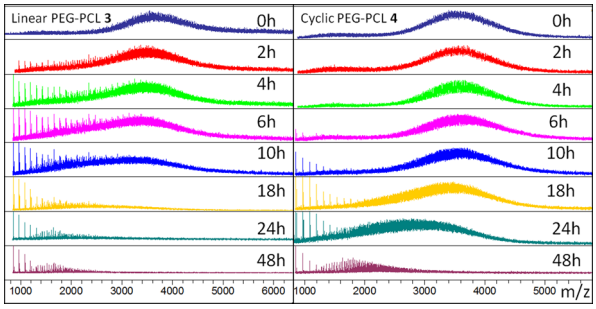

Figure 5. MALDI-TOF mass spectra of the acid-catalyzed degradation of PEG-PCL block copolymers with respect to time: linear PEG-PCL (left) and cyclic PEG-PCL (right).

the carriers and reduce premature drug release. Second, the observed size of the cyclic micelles is significantly smaller than those seen for exact linear analogs. The ability to control the size of micelles is of critical importance as the in vivo biodistribution in both organs and tumor tissue is influenced predominantly by the size of the carriers. Additional studies are under investigation to fully elucidate the effect this cyclic architecture has on the size, stability, and dynamic behavior of their resultant self-assembled micelles.

In conclusion, cyclic amphiphilic PEG-PCL diblock polymers were successfully prepared by combining ring-opening polymerization and click cyclization. The cyclic PEG-PCL shows a longer degradation time than linear analogs due to unique properties of the cyclic topology. The self-assembly of cyclic and linear PEG-PCL block copolymers show that both linear and cyclic PEG-PCL can form micelles in aqueous solution, though the cyclic micelles are notably smaller. The combination of reduced size and unique degradation behavior suggests that they may be useful stimuli-responsive materials in the field of drug delivery carriers and these will be explored in future studies.

■ ASSOCIATED CONTENT

Supporting Information

The synthetic protocol for cyclic PEG-PCL, preparation of micelles, acid catalyst degradation study, and NMR and IR data for both linear precursor and cyclic product (Figures S1 and S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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