A Reduction Sensitive Cascade Biodegradable Linear Polymer

MATTHEW A. DEWIT,[1] ANNELISE BEATON,[1] ELIZABETH R. GILLIES[1,2]

[1]Department of Chemistry, The University of Western Ontario, 1151 Richmond St., London, Canada N6A 5B7

[2]Department of Chemical and Biochemical Engineering, The University of Western Ontario, 1151 Richmond St., London, Canada N6A 5B9

Received 23 April 2010; accepted 7 June 2010
DOI: 10.1002/pola.24180
Published online in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Cascade degradable linear polymers offer the potential for a high degree of control over the degradation process. They comprise a backbone that is stable in the presence of an end cap, but upon removal of the end cap a cascade of intramolecular reactions is initiated that leads of depolymerization of the polymer backbone. Reported here is a new polymer backbone based on *N*,*N*′-dimethylethylenediamine and 2-mercaptoethanol linked by carbamates and thiocarbamates. A disulfide end cap was incorporated such that its cleavage under reducing conditions revealed the thiol of 2-mercaptoethanol, leading to alternating cyclizations of the 2-mercaptoethanol and *N*,*N*′-dimethylethylenediamine moieties to provide 1,3-oxathiolan-2-one and *N*,*N*′-dimethylimidazolidinone, respectively. The degradation was monitored by ^{1}H NMR and GPC. The expected products were observed, along with a portion of nondegradable polymer that was likely cyclic species. Overall, the results demonstrate the promise of this new class of polymers to degrade selectively in reducing environments such as hypoxic tumor tissue or the intracellular compartments of cells. © 2010 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 48: 3977–3985, 2010

KEYWORDS: biodegradable; biological applications of polymers; redox-sensitive polymers; self-immolative polymers; stimuli-sensitive polymers

INTRODUCTION Biodegradable polymers have been of significant interest in recent years for a wide range of applications. For example, they can serve as environmentally friendly substitutes for nondegradable polymers in materials such as food and beverage containers.[1] They have also been developed for biomedical materials such as sutures,[2] stents,[3] and tissue engineering scaffolds,[4,5] thus allowing the materials to degrade during the natural healing or tissue regeneration process, preventing the need for further interventions to remove the foreign material. Furthermore, their incorporation into drug delivery systems such as micelles,[6,7] worms,[8,9] vesicles,[10–12] and nanoparticles[13–15] facilitates the release of encapsulated drug molecules throughout the degradation process. Thus far, significant progress has been made in these areas using polymers such as polycaprolactone,[16–18] poly(lactic acid),[19,20] and poly(glycolic acid).[21–23] However, the ability to "turn on" the degradation of these polymers under specific physiological conditions has not been demonstrated as these polymer backbones exhibit gradual degradation under most physiological conditions.[24,25]

The ability to trigger the degradation of a polymer backbone under specified conditions such as photochemical or enzymatic stimuli, or changes in pH or redox potential offers the possibility to utilize polymer backbones that will be stable for extended periods but that will degrade under the desired conditions, resulting in a controlled disintegration of biomedical materials or release of drug molecules from the drug delivery system. Thus far, several polymer backbones containing acetal[26–29] or disulfide[30,31] linkages have been developed to degrade under mildly acidic or reducing conditions respectively. However, the mechanisms of degradation for these polymers involve random chain scissions throughout the polymer backbone, and many environmentally mediated cleavage events are required to completely degrade the polymer.

Inspired by elegant work on dendrimer systems that were designed to degrade by a cascade of reactions upon removal of a single trigger moiety,[32–41] the group of Shabat[42,43] as well as our group[44] have recently developed end capped cascade degradable linear polymers. As illustrated in Figure 1, these polymers comprise backbones that are stable when the end cap is intact, but upon removal of the end cap via a single bond cleavage, a functionality is revealed at the polymer terminus that initiates a cascade of intramolecular reactions leading to complete depolymerization from end to end. Like the dendrimer systems, both of these systems have used self-immolative linkers previously developed for prodrugs.[45–50] Shabat's group reported the use of a polycarbamate based on 4-aminobenzyl alcohol derivatives, that depolymerized to fluorescent monomers via a series of rapid 1,6-elimination reactions in response to an enzyme mediated end cap cleavage, thus serving as a sensor for the enzyme.[42] Our group developed a polycarbamate that degraded by alternating cyclization

Additional Supporting Information may be found in the online version of this article. Correspondence to: E. R. Gillies (E-mail: egillie@uwo.cas)
Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 48, 3977–3985 (2010)

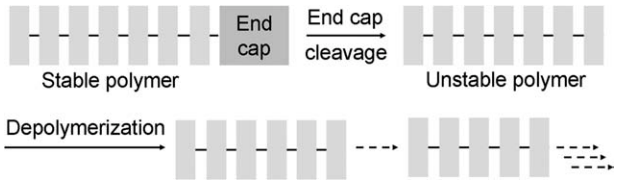


FIGURE 1 Schematic of a cascade biodegradable polymer.

and 1,6-elimination reactions and incorporated this polymer into an amphiphilic block copolymer by using a poly(ethylene glycol) derivative as an end cap.[44] It was demonstrated that the cyclization reaction could be used to control the overall rate of depolymerization and also that the block copolymer could be assembled into nanoparticles in aqueous solution. These nanoparticles were capable of encapsulating and releasing a model drug molecule in a controlled manner, thus demonstrating the promise of cascade degradable linear polymers in drug delivery applications.

To fully exploit this new class of polymers, it will be necessary to develop a series of polymer backbones with different depolymerization rates and also a series of end caps that can be removed under different conditions. This will allow for the selection of the appropriate backbone and end cap combination for the desired application. Furthermore, it has been suggested that the quinone methide intermediates involved in the 1,6-elimination reactions can potentially lead to toxicity,[51–53] so it would be desirable to develop new backbones that do not involve hydroxybenzyl alcohol or aminobenzyl alcohol. Towards this goal, we report here the first example of a cascade degradable linear polymer that degrades entirely by cyclization reactions. Furthermore, we describe the first incorporation of a disulfide end cap that can be cleaved under mildly reducing conditions. Such conditions can be encountered in hypoxic tumor tissue[54] where the concentration of the reducing agent glutathione is at least fourfold higher than in normal tissues[55] or within the intracellular environment where the concentration of glutathione is approximately 0.5–10 mM relative to 2–20 μM in the extracellular environment.[56,57]

EXPERIMENTAL

General Procedures and Materials

Solvents used were anhydrous and obtained from a solvent purification system. Chemicals were obtained from Sigma Aldrich and Alfa Aesar and were used without further purification unless otherwise noted. Glassware used in all reactions was flame dried, evacuated and put under N_2 before being charged with any materials. Silica used for column chromatography was 70–230 mesh, 0.063–0.200 mm particle size. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ (δ 7.27 and 77 ppm). ^{1}H NMR spectra were obtained on a Varian Mercury Instrument at 400 MHz and ^{13}C NMR spectra were obtained on a Varian Inova Instrument at 100 MHz. Chemical shifts are reported in ppm. Coupling constants (J) are reported in Hz. IR samples were dissolved in CH_2Cl_2 and cast as films on NaCl plates, then spectra were obtained using a Bruker Tensor 27 instrument. ESI mass spectrometry was performed using a PE-Sciex API 365 triple quadrupole instrument. Gel permeation chromatography was performed using a Waters 515 HPLC pump, equipped with Wyatt mini-DawnTREOS and Wyatt Optilab Rex detectors, and two Resi-Pore 300 $\times$ 7.5 mm, 3 μm particle size columns from Polymer Laboratories. The eluent used was THF and the calibration was performed using polystyrene standards.

Synthesis of Compound 2

To a solution of imidazole (3.84 g, 56.3 mmol, 2.16 eq.) in DMF (40 mL) was added a solution of *tert*-butyldiphenylchlorosilane (7.75 g, 28.2 mmol, 1.08 eq.) in DMF (30 mL) and the resulting solution was stirred for 10 minutes. 2-Mercaptoethanol (**1**) (2.04 g, 26.1 mmol, 1.00 eq.) in DMF (8 mL) was then added and the reaction mixture was stirred at room temperature for 24 hours. The DMF was then removed in vacuo and the crude product was taken up in CH_2Cl_2 (30 mL), filtered, and washed with an equal volume of H_2O to remove the imidazole. The organic layer was dried with $MgSO_4$, filtered, and the solvent was removed in vacuo. The residue was purified by silica gel chromatography using 97:3 hexanes:ethyl acetate as an eluent to provide 7.74 g (24.4 mmol) of **2** as a clear colorless oil.

Yield: 94%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.67 (m, 4H, phenyl CH), 7.48–7.37 (m, 6H, phenyl CH), 3.79 (t, J = 6.35 Hz, 2H, O—CH_2), 2.68 (dt, J_1 = 8.30, J_2 = 6.30 Hz, 2H, S-CH_2), 1.60 (t, J = 8.30 Hz, 1H, SH), 1.08 (s, 9H, *t*-butyl). ^{13}C NMR (100 MHz, $CDCl_3$): 135.2, 133.1, 129.4, 127.4, 65.3, 26.8, 26.5, 18.9. IR (NaCl, thin film, cm^{-1}): 1520 (aryl C=C), 1650 (aryl C=C), 1685 (aryl C=C), 1770 (C—H bond), 1930 (S—H), 2970 (C—H), 3070 (sp^2 C—H) cm^{-1}. HRMS (*m/z*): calcd for $C_{18}H_{24}OSSi$, 315.1239; found (ESI), 315.1230 [M-H]$^+$.

Synthesis of Compound 3

Compound **2** (1.01 g, 3.20 mmol, 1.00 eq.) was dissolved in CH_2Cl_2 (40 mL). Triethylamine (2.2 mL, 15 mmol, 4.7 eq.) was added followed by 4-nitrophenyl chloroformate (1.30 g, 6.39 mmol, 2.00 eq.) and the reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was then poured onto 1 M HCl (50 mL), and the product was extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed in vacuo. The product was purified by silica gel chromatography using 1:1 CH_2Cl_2:hexanes as an eluent to provide 1.52 g (3.16 mmol) of **3** as a clear oil.

Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, J = 9.38 Hz, 2H, *p*-nitrophenyl), 7.69 (dd, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 4H, phenyl CH), 7.37–7.50 (m, 6H, phenyl CH), 7.32 (d, J = 9.4 Hz, 2H, *p*-nitrophenyl), 3.91 (t, J = 6.1 Hz, 2H), 3.18 (t, J = 6.1 Hz, 2H), 1.06–1.11 (m, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 169.5, 155.6, 135.6, 133.1, 129.8, 127.7, 125.2, 122.0, 62.2, 34.3, 26.8, 19.2. IR (NaCl, thin film, cm^{-1}): 1520 (aryl C=C), 1590 (aryl C=C), 1725 (C=O), 2850 (C—H), 2930 (C—H), 3050 (sp^2 C—H). HRMS (*m/z*) calc'd for $C_{25}H_{27}NO_5SSi$, 482.1457; found (ESI), 482.1461 [M+H]$^+$.

Synthesis of Compound 4

To a solution of compound **3** (3.17 g, 6.58 mmol, 1.00 eq.) in toluene (60 mL) were added 4-(dimethylamino)pyridine

(DMAP) (0.077 g, 0.63 mmol, 0.096 eq.), *N,N*-diisopropylethylamine (DIPEA) (1.72 g, 13.3 mmol, 2.02 eq.), and Boc protected *N,N′*-dimethylethlyenediamine[44] (1.81 g, 9.63 mmol, 1.46 eq.). The reaction mixture was heated at reflux for 5 hours. The reaction mixture was then washed 1M HCl, followed by two washes with saturated Na_2CO_3 solution. The organic layer was dried with $MgSO_4$, filtered, and the solvent was removed in vacuo to provide 3.48 g (6.55 mmol) of **4** as a clear pale yellow oil. Yield: 99%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (dd, $J_1 = 7.9$ Hz, $J_2 = 1.7$ Hz, 4H), 7.47–7.34 (m, 6H), 7.30–7.14 (m, 3H), 3.82 (m, 2H, CH_2—O), 3.57–3.32 (m, 4H, (rotamer) diamine CH_2), 3.18–3.08 (m, 2H, CH_2-S), 3.02 (br s, 3H), 2.94–2.81 (m, 3H), 1.46 (d, $J = 8.40$ Hz, 9H, Boc CH_3), 1.06 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 168.2 (rotamer), 167.5 (rotamer), 155.7, 155.4, 135.5, 133.6, 129.6, 127.6, 125.3, 115.6, 79.7, 63.3, 47.6 (rotamer), 46.7 (rotamer), 45.8 (rotamer), 35.7 (rotamer), 35.3 (rotamer), 34.8 (rotamer), 34.6 (rotamer), 32.99 (rotamer), 32.93 (rotamer), 28.4, 26.8, 19.2. IR (NaCl, thin film, cm^{-1}): 1650 (C=O), 1690 (C=O), 2850 (C—H), 2925 (C—H), 3060 (sp^2 C—H). HRMS (m/z): calcd for $C_{28}H_{42}N_2O_4SSi$, 531.2707. found (ESI), 531.2691 $[M+H]^+$.

Synthesis of Compound 5

To a solution containing **4** (1.18 g, 2.22 mmol, 1.00 eq.) in THF (20 mL) was added a 1 M solution of tetrabutylammonium fluoride (TBAF) in THF (4.43 mL, 4.43 mmol, 2.00 eq.) and the reaction mixture was stirred at room temperature for 2 hours. The solvent was then removed in vacuo and the resulting residue was purified by silica gel chromatography using 85:15 hexanes:ethyl acetate as an eluent to provide 0.50 g (1.73 mmol) of **5** as a clear, pale yellow oil.

Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75 (t, $J = 5.8$ Hz, 2H CH_2-O), 3.54–3.33 (m, 4H (rotamer) diamine CH_2), 3.05 (t, $J = 5.6$ Hz, 2H, CH_2-S), 2.99 (br s, 3H carbamate CH_3), 2.90–2.78 (m, 3H, boc protected amine CH_3), 2.00 (s, 1H, OH), 1.42 (s, 9H, boc CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 168.9 (rotamer), 168.7 (rotamer), 155.5, 80.0 (rotamer), 79.7 (rotamer), 79.5 (rotamer), 62.1 (rotamer), 61.8 (rotamer), 48.1 (rotamer), 47.8 (rotamer), 47.4 (rotamer), 47.4 (rotamer), 46.7 (rotamer), 46.5 (rotamer), 45.6 (rotamer), 35.7 (rotamer), 35.4 (rotamer), 35.1 (rotamer), 34.9 (rotamer), 34.7 (rotamer), 34.4 (rotamer), 33.2 (rotamer), 32.8, 28.2. IR (NaCl, thin film, cm^{-1}): 1650 (C=O), 1680 (C=O), 2850 (C—H), 2930 (C—H), 2960 (—CH), 3400 (O—H). HRMS (*m/z*) calc'd for $C_{12}H_{24}N_2O_4S$, 292.1457; found (ESI), 292.8123 $[M]^+$.

Synthesis of Compound 6

To a solution containing **5** (0.45 g, 1.5 mmol, 1.0 eq.) in CH_2Cl_2 (10 mL) was added pyridine (0.37 mL, 4.6 mmol, 3.0 eq.), followed by 4-nitrophenyl chloroformate (0.62 g, 3.1 mmol, 2.0 eq.) and the reaction mixture was stirred for 20 hours. The reaction mixture was then washed with 10 mL of 1M HCl, then the organic layer was dried over $MgSO_4$, filtered, and the solvent was removed in vacuo. The resulting residue was purified by silica gel chromatography using 1:1 CH_2Cl_2:ethyl acetate as an eluent to provide 0.60 g (1.31 mmol) of **6** as a clear, pale yellow oil.

Yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33–8.25 (m, 2H, *p*-nitrophenyl), 7.46–7.37 (m, 2H, *p*-nitrophenyl), 4.42 (t, $J = 6.5$ Hz, 2H, carbonate CH_2) 3.61–3.50 (m, 2H, (rotamer) diamine CH_2), 3.40 (d, $J = 6.1$ Hz, 2H, carbamate CH_2), 3.27 (d, $J = 5.5$ Hz, 2H, (rotamer) diamine CH_2), 3.04 (s, 3H, carbamate CH_3), 2.89 (br s, 3H, Boc protected amine CH_3), 1.47 (br. s. 9H, boc CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 167.0 (rotamer), 166.8 (rotamer), 166.2 (rotamer), 155.6 (rotamer), 155.4, 155.2 (rotamer), 152.1, 145.3, 125.1, 121.7, 79.7 (rotamer), 79.52 (rotamer), 79.45 (rotamer), 88.0, 53.4, 47.9 (rotamer), 47.5 (rotamer), 46.7 (rotamer), 46.4 (rotamer), 45.6 (rotamer), 35.5 (rotamer), 35.2 (rotamer), 34.7 (rotamer), 34.3 (rotamer), 28.5 (rotamer), 28.3 (rotamer). IR (NaCl, thin film, cm^{-1}): 1650 (C=O), 1690 (C=O), 1770 (C=O), 2920 (C—H), 2964 (C—H), 3070 (sp^2 C—H), 3105 (sp^2 C—H). HRMS (*m/z*) calc'd for $C_{19}H_{27}N_3O_8S$, 458.1597; found (ESI), 458.1599 $[M+H]^+$.

Synthesis of Compound 8

Compound **7**[58] (0.24 g, 1.3 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (6 mL) and pyridine (0.30 mL, 3.8 mmol, 2.9 eq.) then 4-nitrophenyl chloroformate (0.51 g, 2.5 mmol, 1.9 eq.) was added. The reaction mixture was stirred for 4 hours. Triethylamine (0.34 mL, 2.5 mmol, 1.9 eq.) and tri(ethylene glycol) monomethylether (0.30 mL, 1.9 mmol, 1.5 eq.) were then added and the reaction mixture was stirred for an additional 10 minutes. The reaction mixture was then poured into 1M HCl (5 mL), and the product was extracted twice with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$ and filtered. The solvent was removed in vacuo and the resultant residue was purified by silica gel chromatography using 9:1 hexanes:EtOAc as an eluent to provide 0.33 g (0.94 mmol) of **8** as a pale yellow oil.

Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53–8.48 (m, 1H, pyridyl), 8.32–8.26 (m, 2H, *p*-nitrophenyl), 7.70–7.63 (m, 2H, pyridyl), 7.42–7.36 (m, 2H, *p*-nitrophenyl), 7.15–7.12 (m, 1H, pyridyl), 4.57 (t, $J = 6.4$, 2H, CH_2—O), 3.17 (t, $J = 6.4$, 2H, CH_2—S). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.1, 155.3, 152.1, 149.8, 145.4, 137.1, 125.3, 121.7, 121.1, 120.2, 66.6, 36.7. IR (NaCl, thin film, cm^{-1}): 1520 (aryl C=C), 1570 (aryl C=C), 1590 (aryl C—N), 1615 (aryl C=C), 1760 (C=O), 2855 (C—H), 2960 (C—H), 3045 (sp^2 C—H), 3080 (sp^2 C—H), 3115 (sp^2 C—H). HRMS (*m/z*) calc'd for $C_{14}H_{12}N_2O_5S_2$, 352.0188; found (ESI), 352.0184 $[M]^+$.

Synthesis of Polymer 10

Compound **6** (0.60 g, 1.3 mmol, 1.0 eq.) was dissolved in 1:1 CH_2Cl_2:trifluoroacetic acid (6 mL) and the reaction mixture was stirred at room temperature for 2 hours. The solvent was evaporated in vacuo, and then CH_2Cl_2 was added and evaporated five times to remove residual TFA, providing **9**. The residue was dissolved in toluene (1 mL) and triethylamine (0.91 mL, 6.5 mmol, 5.0 eq.), DMAP (0.015 g, 0.12 mmol, 0.09 eq.) and end cap **8** (10.6 mg, 30 μmol, 0.023 eq.) were added. The resulting solution was stirred at room temperature for 18 hours. The solvent was removed in vacuo

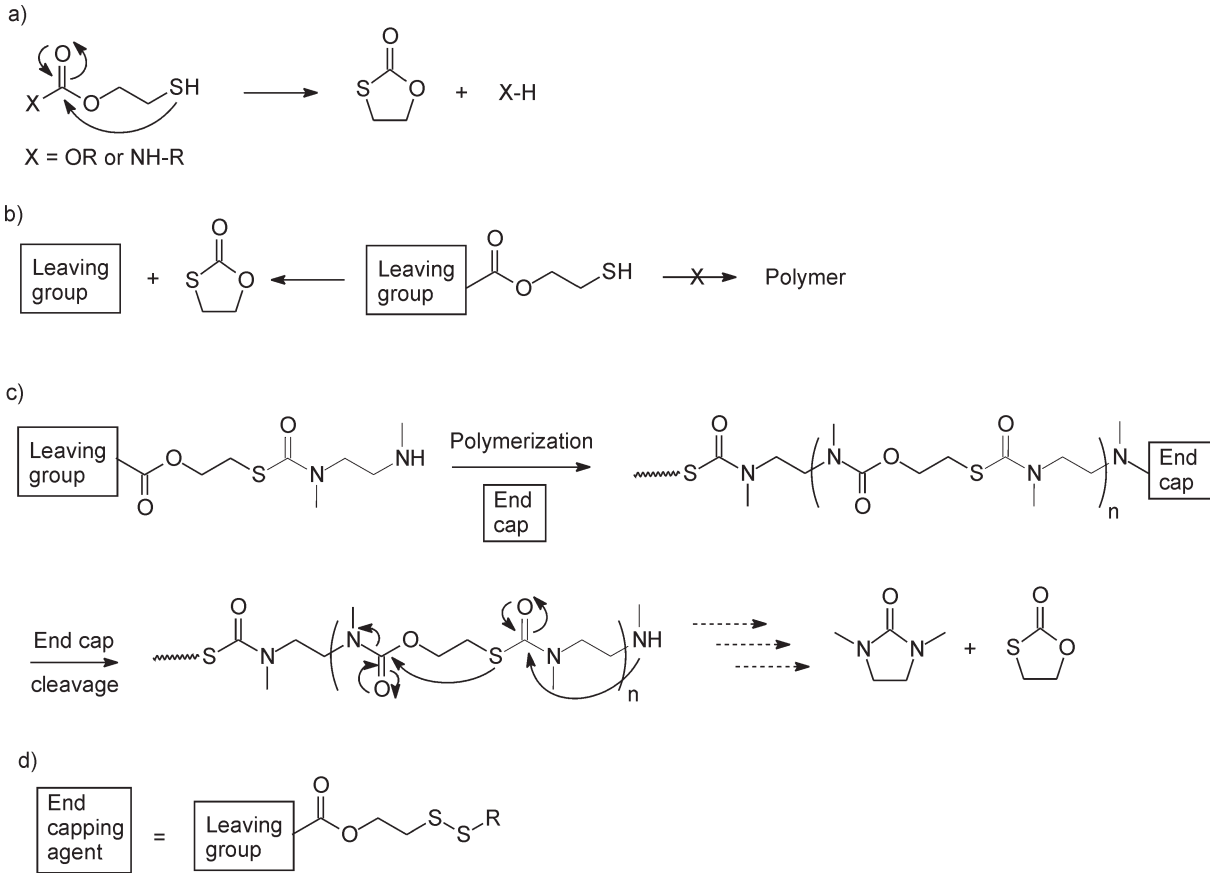


FIGURE 2 Design of the cascade degradable polymer: (a) cyclization of 2-mercaptoethanol derivatives to 1,3-oxathiolan-2-one; (b) undesired cyclization of an activated 2-mercaptoethanol based monomer prohibits polymerization; (c) proposed polymerization of an activated heterodimer and depolymerization of the resulting polymer; (d) proposed disulfide based end cap.

and the crude polymer was dissolved in 2 mL of DMF. The solution was dialyzed against DMF (200 mL, 1 solvent change) using a regenerated cellulose membrane (Spectrum Laboratories Spectra/Por, 3500 M_w cutoff). The DMF was then removed in vacuo to provide 0.10 g of polymer **10**.

Yield: 34%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (d, J = 4.7 Hz, 1H, pyridyl), 8.28 (d, J = 9.0 Hz, 1H, *p*-nitrophenyl) 7.74–7.61 (m, 2H, pyridyl), 7.41 (*d*, J = 9.0 Hz, 1H, *p*-nitrophenyl), 7.10 (br s, 1H, pyridyl), 4.39–4.28 (m, 2H, CH_2—O terminal), 4.27–4.07 (m, 76H, CH_2—O), 3.61–3.34 (m, 155H, CH_2—N), 3.23–3.10 (m, 77H, CH_2—S), 3.02 (br S, 114H, CH_3—N), 2.98–2.89 (m, 120H, CH_3—N). SEC: M_n = 1800 g/mol, M_w = 2950 g/mol, PDI = 1.6.

Degradation Study

Buffer Preparation

$NaH_2PO_4{\cdot}H_2O$ (0.069 g, 0.5 mmol) was dissolved in 5 mL of D_2O. To this, a saturated solution of NaOH in D_2O was added dropwise with stirring, while monitoring with a pH meter until the desired pH of 7.4 was obtained.

Degradation of Polymer 10

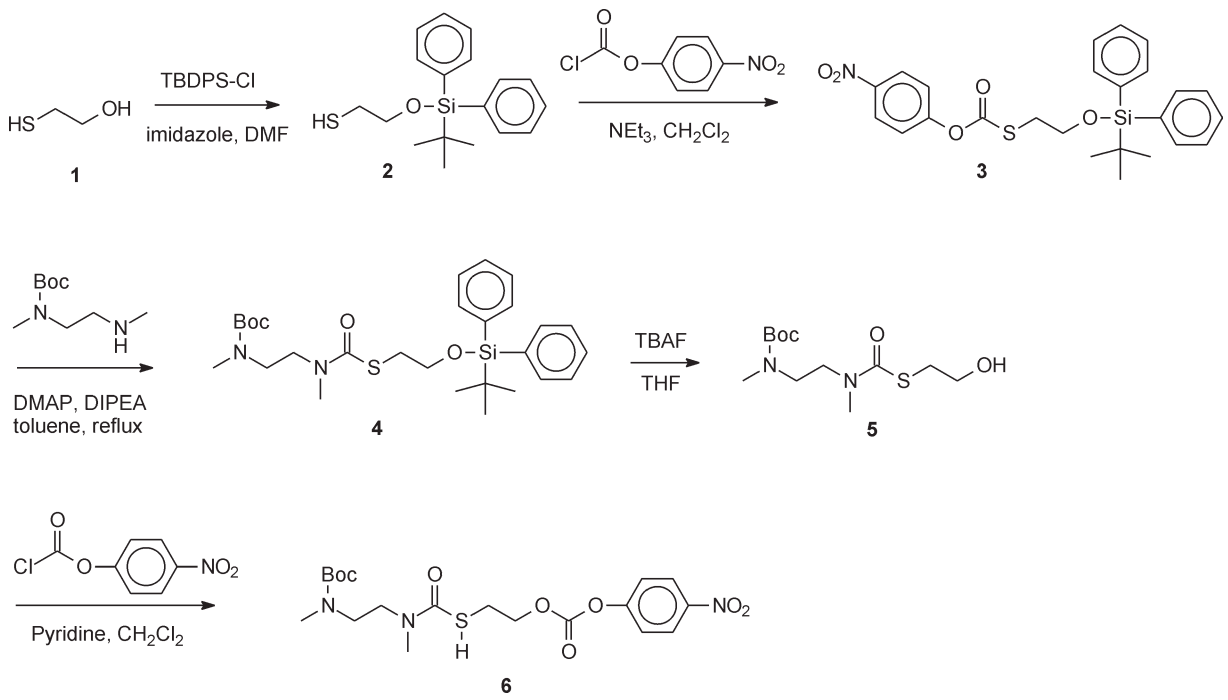
Fifteen milligrams of polymer **10** was dissolved in 1 mL of 0.1 M phosphate buffered D_2O:acetone-d_6 (3:2), and the solution was incubated at 37°C. Three milligrams of Dithiothreitol (DTT) was added at the beginning and subsequently every 7 days to maintain reducing conditions. The extent of depolymerization was quantified using ^{1}H NMR by integrating the methylene peak corresponding to the *N,N′*-dimethylimidazolidinone degradation product (3.4 ppm) relative to the peak corresponding to the methylene group adjacent to the oxygen in the polymer (4.3 ppm). A control sample was monitored under the same conditions as above, but without DTT. For SEC samples, a 0.25 mL aliquot was dried and the resulting residue was taken up in THF. The salts were removed by filtration through a 0.2 μm filter.

RESULTS AND DISCUSSION

Design

A diverse array of intramolecular cyclization reactions have been reported, potentially allowing the rate of depolymerization to be controlled by the choice of the cyclization reaction.[48,59,60] In this particular work, the cyclizations of 2-mercaptoethanol derivatives to the corresponding cyclic thiocarbonate [Fig. 2(a)] were of interest as they have been recently reported as components of traceless self-immolative spacers in fluorescent protease sensors.[59]

The development of monomers capable of undergoing polymerization to form cascade degradable polymers requires



SCHEME 1 Synthesis of the protected monomer.

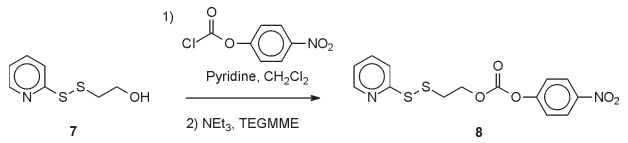
careful design. In particular, in the preparation of polymers designed to degrade by cyclization mechanisms, cyclization of the activated monomer [Fig. 2(b)] must be avoided. In our previous work, it has been found that the synthesis and polymerization of activated dimers is an effective approach, as the activated leaving group is distant from the nucleophilic moiety such that the resulting ring size is not particularly favorable for cyclization.[44] This allows polymerization to be a highly competitive reaction at high concentrations. In particular, the use of alternating monomers, and thus the preparation of activated heterodimers as polymerizable "monomers" has been found to be an effective strategy for overcoming the challenges associated with the synthesis of both the activated monomers and their corresponding polymers.[44] Therefore, an activated heterodimer based on 2-mercaptoethanol and *N*,*N*′-dimethylethylenediamine units was proposed. Carbamate derivatives of *N*,*N*′-dimethylethylenediamine are known to spontaneously cyclize to form *N*,*N*′-dimethylimidazolidinone[49] and this spacer has been incorporated into our previously reported linear cascade degradable polymer[44] as well and some of the previously reported cascade degradable dendrimers.[32,36,39,40] As shown in Figure 2(c), polymerization of this activated heterodimer in the presence of an end cap should lead to a polymer based on 2-mercaptoethanol and *N*,*N*′-dimethylethylenediamine with alternating carbamate and thiocarbamate linkages. Removal of an end cap would lead to alternating cyclization reactions resulting in end to end depolymerization with the release of *N*,*N*′-dimethylimidazolidinone and 1,3-oxathiolan-2-one.

The end cap selected for the target polymer was a disulfide [Fig. 2(d)]. Disulfide linkages are known to be cleaved by biological reducing agents such as glutathione, and it has been shown that the incorporation of disulfide linkages into gene and drug delivery systems can provide a selective release of the cargo under the reducing conditions within cells, leading to enhanced therapeutic efficacy.[61–66] In addition, because of the incorporation of the 2-mercaptoethanol cyclization reaction in the degradation cascade, the disulfide was a natural choice for an end cap as the thiol moiety can be readily converted to a disulfide which upon cleavage can directly initiate the depolymerization cascade.

Synthesis

As shown in Scheme 1, the synthesis of the target activated heterodimer began by the selective protection of the alcohol group on 2-mercaptoethanol (**1**) using *tert*-butyldiphenylchlorosilane in the presence of imidazole to provide the *tert*-butyldiphenylsilyl (TBDPS) protected derivative **2**. The thiol of **2** was then treated with 4-nitrophenylchloroformate to provide the activated thiocarbonate **3**. The mono *tert*-butylcarbamate (Boc) protected derivative of *N*,*N*′-dimethylethylenediamine[44] was reacted with **3** using 4-(dimethylamino)-pyridine (DMAP) as a catalyst and *N*,*N*-diisopropylethylamine (DIPEA) as a base to give the thiocarbamate **4**, and then the TBDPS protecting group was removed using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) to provide the alcohol **5**. The alcohol was then converted to the activated carbonate **6** by reaction with 4-nitrophenyl chloroformate, providing the protected version of the polymerization monomer.

For the synthesis of the target end cap, the alcohol group of the previously reported thiopyridyl derivative **7**[58] was treated with 4-nitrophenyl chloroformate to provide the activated carbonate **8** as shown in Scheme 2. This activated carbonate



SCHEME 2 Synthesis of the end cap.

allows for incorporation of the end cap onto the polymer. Tri(ethylene glycol) monomethyl ether (TEGMME) was used to quench the excess chloroformate in this reaction as it was otherwise chromatographically inseparable from the product.

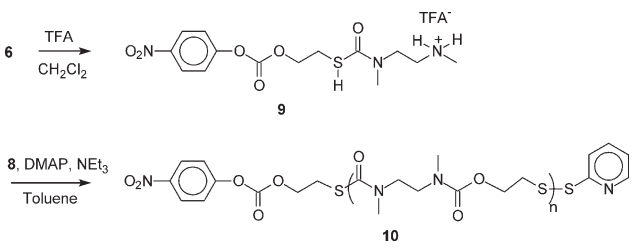
In preparation for the polymerization, the Boc group was removed from compound **6** using trifluoroacetic acid (TFA), providing the corresponding amine **9**, isolated as its TFA salt (Scheme 3). This molecule was sufficiently stable that as long as the deprotection was performed within hours before the polymerization reaction it could be isolated and transferred to the polymerization conditions without premature polymerization or cyclization. In contrast, upon deprotonation **9** is capable of self-condensing via reaction of the amine with the activated carbonate to form a polycarbamate, releasing *p*-nitrophenol. The polymerization was carried out by reacting **9** with 0.02 equivalents of the end cap **8** in toluene in the presence of DMAP as a catalyst and triethylamine as a base.

The resulting polymer **10** was purified by dialysis in *N*,*N*-dimethylformamide (DMF) using a regenerated cellulose membrane with a molecular weight (MW) cut-off of 3500 g/mol to remove small molecule byproducts. The material isolated from the dialysis was pure and free of low MW impurities as determined by ^{1}H NMR and size exclusion chromatography (SEC). In the analysis of the dialysate, cyclization products were not detected (Supporting Information), indicating that the cyclization of the monomer was not a competing reaction during the polymerization. In addition, no monomer was detected, indicating that the polymerization proceeded to completion. However, some polymeric material was lost into the dialysate. It should be noted that the MW cut-off of 3500 g/mol is an estimate as it depends on the macromolecule's size and shape. In addition, the MW cut-off corresponds to aqueous conditions and is likely lower in DMF due to the decreased swelling of the membrane in DMF. However, we have routinely observed that linear polymers above the MW cut-off can pass through the membrane, likely via reptation.[67] Although the yield for this polymer following dialysis was relatively low, ~35%, this method is much less labor intensive than preparative SEC, which was previously used to purify our cascade degradable linear polymers. Using ^{1}H NMR spectroscopy a ratio of end cap to monomer of ~35:1 was determined (Supporting Information). Using SEC the polymer was found to have a number average MW (M_n) of 1800 g/mol, a weight average MW (M_w) of 2950 g/mol, and a polydispersity index (PDI) of 1.6 relative to polystyrene standards.

Polymer Degradation

To study the depolymerization initiated by end cap cleavage, polymer **10** was dissolved in pH 7.4 phosphate buffered D_2O:acetone-d_6 (3:2). DTT was added to cleave the disulfide end cap, thus initiating the degradation cascade and the sample was incubated at 37 °C. The reducing conditions were maintained by periodic additions of DTT. The degradation was monitored by ^{1}H NMR spectroscopy. As shown in Figure 3, over time, characteristic peaks appeared corresponding to *N*,*N*′-dimethylimidazolidinone at 2.8 and 3.4 ppm and 1,3-oxathiolan-2-one at 3.2 and 3.7 ppm. The presence of these products is a strong indicator that the degradation proceeds by the proposed cascade of cyclization reactions as random chain scissions would lead to *N*,*N*′-dimethylethylenediamine and 2-mercaptoethanol, products that were not detected in the NMR spectra. In addition, a control sample incubated under the same conditions except in the absence of DTT did not reveal the appearance of any degradation products, thus indicating the end cap cleavage was required to initiate the degradation (Supporting Information). Furthermore, an additional control polymer having a Boc end cap was also prepared and was demonstrated to undergo depolymerization in pH 7.4 phosphate buffered D_2O:acetone-d_6 (3:2) following prior treatment with TFA/CH_2Cl_2 to remove the Boc group. This confirmed that the polymer degradation could not be attributed to random polymer backbone cleavage by the DTT (Supporting Information).

The percentage of degradation was determined by the relative integration of the peak at 4.3 ppm assigned to the methylene group adjacent to the oxygen in the polymer and the peak at 3.4 ppm corresponding to the methylene unit of *N*,*N*′-dimethylimidazolidinone. As shown in Figure 4, the degradation reached 80% completion after 10–14 days. However, no significant further degradation was observed, even after 30 days. Size exclusion chromatograms were also obtained at different time points during the degradation process. As shown in Figure 5, before degradation, the chromatogram exhibited a peak at an elution volume of 16.7 mL as well as a distinct shoulder at 18.5 mL. As the degradation progressed, the peak at 16.7 mL decreased in intensity, consistent with the degradation progress observed by ^{1}H NMR spectroscopy. On the other hand, no change in the intensity of the peak at 18.5 mL was observed. Therefore, it is possible that the peak at 18.5 mL corresponds to cyclic polymers. These cyclic species would not be end capped and thus degradation would only be initiated by a random chain scission of the polymer backbone. As observed for the control sample such cleavages are extremely slow under the degradation conditions. This may explain why the depolymerization did



SCHEME 3 Polymerization.

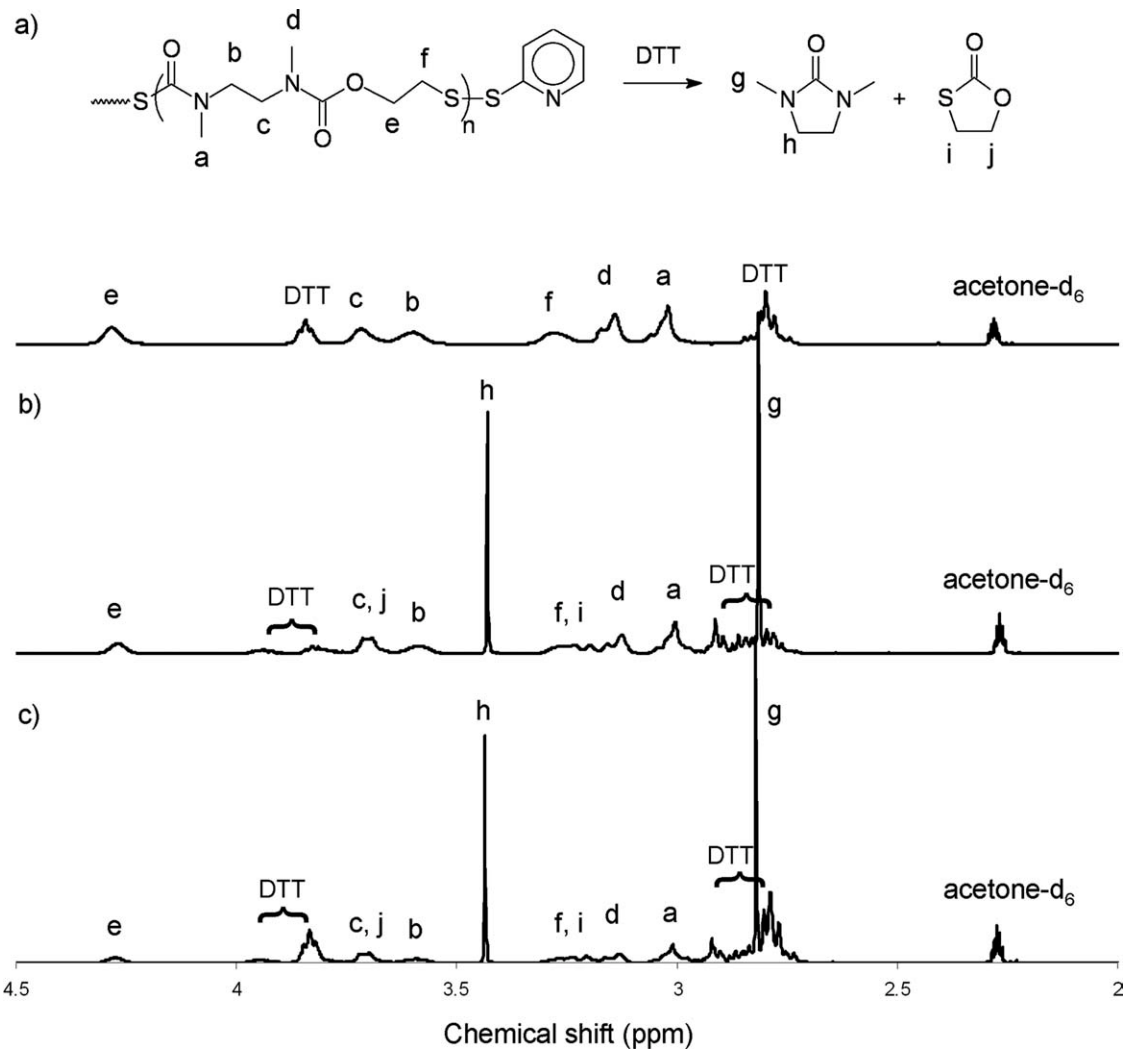


FIGURE 3 ^{1}H NMR spectra of polymer **10** and its degradation products in pH 7.4 phosphate buffered D_2O:acetone-d_6 (3:2) at different time points following the addition of DTT: (a) immediately following DTT addition; (b) after 4 days; (c) after 8 days.

not reach 100% completion according to ^{1}H NMR spectroscopy.

It should also be noted that the cyclic polymers would not be distinguishable from the linear end capped polymers by NMR spectroscopy. Therefore, based on the degradation plateau at 80% completion, it is possible that the ratio of monomer to end cap in the linear polymers is closer to 30:1 than the 35:1 ratio mentioned earlier. Altering the concentration of the polymerization reaction did not appear to change the content of the possible cyclic species significantly, nor the polymer MW. Interestingly, such nondegradable species were not observed in our previously reported cascade degradable polymers based on *N*,*N*′-dimethylethylenediamine and 4-hydroxybenzyl alcohol.[44] This may be explained by the increased rigidity imparted by the aromatic groups of 4-hydroxybenzyl alcohol, making cyclization less favorable. Nevertheless, this somewhat unexpected result provides additional evidence of the polymer backbone's inherent stability and the specificity of the degradation process mediated

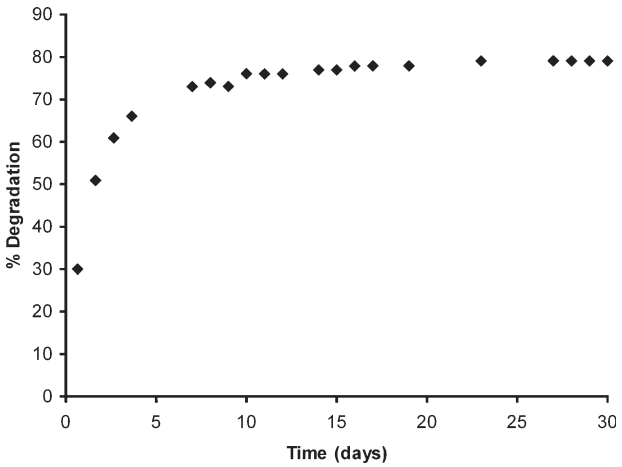


FIGURE 4 Kinetics of depolymerization of polymer **10**, as measured by ^{1}H NMR spectroscopy in pH 7.4 phosphate buffered D_2O:acetone-d_6 (3:2), following addition of DTT.

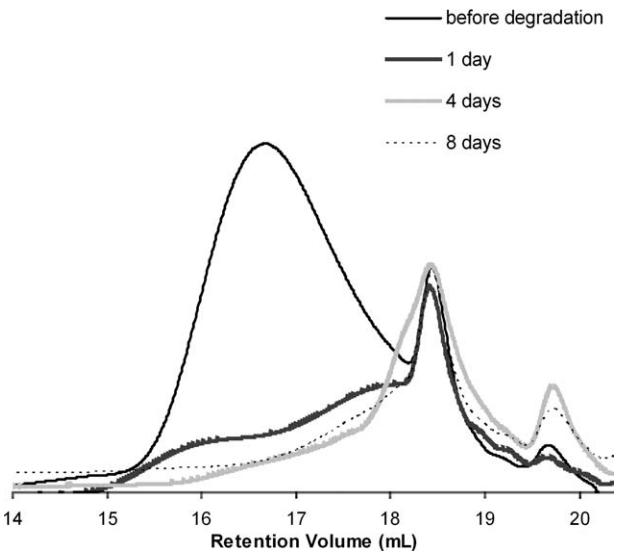


FIGURE 5 Size exclusion chromatograms of polymer **10** before degradation and after 1 day, 4 days, and 8 days of incubation in pH 7.4 phosphate buffered D_2O:acetone-d_6 (3:2) in the presence of DTT.

by end cap cleavage. It is possible that in the future, the extent of the possible cyclic species could be decreased by tuning the reactivity of the activated carbonate in the polymerization monomer.

CONCLUSIONS

In conclusion, a new polymer designed to degrade by a cascade of intramolecular cyclization reactions was prepared for the first time. A disulfide end cap was incorporated such that the degradation could be selectively initiated under reducing conditions. The degradation was initiated by the addition of DTT, a known thiol based reducing agent and was monitored by ^{1}H NMR and size exclusion chromatography. The data supported the proposed degradation mechanism, and also revealed that the polymer contained ~20% of a proposed cyclic species that did not degrade as they were lacking the labile linkage to the end cap. Overall, this new class of polymers offers a high degree of control over the degradation process as the polymer backbone is very stable under physiological conditions (pH 7.4 buffer) in the absence of the trigger required to cleave the end cap. In addition, the degradation mechanism of this polymer avoids the potentially undesirable quinone methide species generated in the degradation of the previously reported cascade degradable linear polymers. Future work on this polymer will focus on its biological properties and applications in biomedical materials.

The authors thank the Natural Sciences and Engineering Research Council of Canada, the Canada Research Chairs Program, the Canada Foundation for Innovation, and the Ontario Research Fund for support of the research.

REFERENCES AND NOTES

1 Vargas, M.; Pastor, C.; Chiralt, A.; Mcclements, J.; Gonzalez-Martinez, C. Crit Rev Food Sci Nutr 2008, 48, 496–511.

2 Chu, C. C. Encycl Biomat Biomed Eng 2004, 2, 1432–1448.

3 Ziberman, M.; Eberhart, R. C. Ann Rev Biomed Eng 2006, 8, 153–180.

4 Martina, M.; Hutmacher, D. W. Polym Int 2007, 56, 145–157.

5 Nair, L. S.; Laurencin, C. T. Adv Biochem Eng Biotech 2006, 102, 47–90.

6 Allen, C.; Yu, Y.; Maysinger, D.; Eisenberg, A. Bioconjugate Chem 1998, 9, 564–572.

7 Jongpaiboonkit, L.; Zhou, Z.; Ni, X.; Wang, Y. Z.; Li, J. J Biomater Sci Polym Ed 2006, 17, 747–763.

8 Yan, G.; Discher, D. E. J Am Chem Soc 2005, 127, 12780–12781.

9 Younghoon, K.; Dalhaimer, P.; Christian, D. A.; Discher, D. E. Nanotechnology 2005, 16, 484–491.

10 Meng, F.; Hiemstra, C.; Engbers, G. H. M.; Feijen, J. Macromolecules 2003, 36, 3004–3006.

11 Ahmed, F.; Discher, D. E. J Controlled Release 2004, 96, 37–53.

12 Ahmed, F.; Pakunlu, R. I.; Srinivas, G.; Brannan, A.; Bates, F.; Klein, M. L.; Minko, T.; Discher, D. E. Mol Pharm 2006, 3, 340–340.

13 Krause, H. J.; Schwarz, A.; Rohdewald, P. Int J Pharm 1985, 27, 145–155.

14 Vasir, J. K.; Labhasetwar, V. Adv Drug Del Rev 2007, 59, 718–728.

15 Kumari, A.; Yadav, S. K.; Yadav, S. C. Colloids Surf B 2010, 75, 1–18.

16 Bezwada, R. S.; Jamiolkowski, D. D.; Lee, I.; Vishvaroop, A.; Persivale, J.; Treka-Benthin, S.; Erneta, M.; Suryadevara, J.; Yang, A.; Lui, S. Biomaterials 1995, 16, 1141–1148.

17 Benoit, M.-A.; Baras, B.; Gillard, J. Int J Pharm 1999, 184, 73–84.

18 Pektok, E.; Nottelet, B.; Tiller, J. C.; Gurny, R.; Kalagos, A.; Moeller, M.; Walpoth, B. H. Circulation 2008, 118, 2563–2570.

19 Langer, R.; Vacanti, J. P. Science 1993, 260, 920–926.

20 Vert, M.; Schwach, G.; Engel, R.; Coudane, J. J Controlled Release 1998, 30, 85–92.

21 Kulkarni, R. K.; Moore, E. G.; Hegyeli, A. F.; Leonard, F. J Biomed Mater Res 1971, 5, 169–181.

22 Bala, I.; Hariharan, S.; Kumar, M. N. Crit Rev Ther Drug Carrier Syst 2004, 21, 387–422.

23 Astete, C. E.; Sabliov, C. M. J Biomater Sci Polym Ed 2006, 17, 247–289.

24 Sun, Y.; Watts, D. C.; John, J. R.; Shukla, A. J. Am Pharm Rev 2001, 8, 10–16.

25 Vert, M. Biomacromolecules 2005, 6, 538–546.

26 Hefferman, M. J.; Murthy, N. Bioconjugate Chem 2005, 16, 1340–1342.

27 Yang, S. C.; Bhide, M.; Crispe, I. N.; Pierce, R. H.; Murthy, N. Bioconjugate Chem 2008, 19, 1164–1169.

28 Jain, R.; Standley, S. M.; Frechet, J. M. J Macromol 2007, 40, 452–457.

29 Murthy, N.; Thng, Y. X.; Schuck, S.; Xu, M.; Frechet, J. M. J. J Am Chem Soc 2002, 124, 12398–12399.

30 Emiltiri, E.; Ranucci, E.; Ferruti, P. J Polym Sci Part A: Polym Chem 2005, 43, 1404–1416.

31 Christensen, L. V.; Chang, C.-W.; Kim, W.-J.; Kim, S. W.; Zhong, Z.; Lin, C. C.; Engbersen, J. F. J.; Feijen, J. Bioconjugate Chem 2006, 17, 1233–1240.

32 Amir, R. J.; Pessah, N.; Shamis, M.; Shabat, D. Angew Chem Int Ed 2003, 42, 4494–4499.

33 De Groot, F. M. H.; Albrecht, C.; Koekkoek, R.; Beusker, P. H.; Scheeren, H. W. Angew Chem Int Ed 2003, 42, 4490–4494.

34 Li, S.; Szalai, M. L.; Kevwitch, R. M.; McGrath, D. V. J Am Chem Soc 2003, 125, 10516–10517.

35 Szalai, M. L.; Kevwitch, R. M.; McGrath, D. V. J Am Chem Soc 2003, 125, 15688–15689.

36 Gopin, A.; Pessah, N.; Shamis, M.; Rader, C.; Shabat, D. Angew Chem Int Ed 2003, 42, 327–332.

37 Szalai, M. L.; McGrath, D. V. Tetrahedron 2004, 60, 7261–7266.

38 Amir, R. J.; Popkov, M.; Lerner, R. A.; Barbas, C. F., III; Shabat, D. Angew Chem Int Ed 2005, 44, 4378–4381.

39 Shamis, M.; Lode, H. N.; Shabat, D. J Am Chem Soc 2004, 126, 1726–1731.

40 Haba, K.; Popkov, M.; Shamis, M.; Lerner, R. A.; Barbas, C. F., III; Shabat, D. Angew Chem Int Ed Engl 2005, 44, 716.

41 McGrath, D. V. Mol Pharm 2005, 2, 253–263.

42 Sagi, A.; Weinstain, R.; Karton, N.; Shabat, D. J Am Chem Soc 2008, 130, 5434–5435.

43 Weinstain, R.; Sagi, A.; Karton, N.; Shabat, D. Chem Eur J 2008, 14, 6857–6861.

44 Dewit, M. A.; Gillies, E. R. J Am Chem Soc 2009, 131, 18327–18334.

45 Wakselman, M. Nouv J Chem 1983, 7, 439–447.

46 Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A. J Med Chem 1981, 24, 479–480.

47 Bundgaard, H. Adv Drug Del Rev 1989, 3, 39–65.

48 Shan, D.; Nicolaou, M. G.; Borchardt, R. T.; Wang, B. J Pharm Sci 1997, 86, 765–767.

49 Saari, W. S.; Schwering, J. E.; Lyle, P. A.; Smith, S. J.; Engelhardt, E. L. J Med Chem 1989, 33, 97–101.

50 Warnecke, A.; Kratz, F. J Org Chem 2008, 73, 1546–1552.

51 Monks, T. J.; Jones, D. C. Curr Drug Metab 2002, 3, 425–438.

52 Guyton, K. Z.; Thompson, J. A.; Kensler, T. W. Chem Res Toxicol 1993, 6, 731–738.

53 Thompson, D. C.; Barhoumi, R.; Burghardt, R. C. Toxicol Appl Pharmacol 1998, 149, 55–63.

54 Kuppusamy, P.; Afeworki, M.; Shankar, R. A.; Coffin, D.; Krishna, M. C.; Hahn, S. M.; Mitchell, J. B.; Zweier, J. L. Cancer Res 1998, 58, 1562–1568.

55 Kuppusamy, P.; Li, H.; Ilangovan, G.; Cardounel, A. J.; Zweier, J. L.; Yamada, K.; Krishna, M. C.; Mitchell, J. B. Cancer Res 2002, 62, 307–312.

56 Wu, G.; Fang, Y. Z.; Yang, S. C.; Lupton, J. R.; Turner, N. D. J Nutr 2004, 134, 489–492.

57 Saito, G.; Swanson, J. A.; Lee, K. D. Adv Drug Del Rev 2003, 55, 199–215.

58 Murthy, N.; Campbell, J.; Fausto, N.; Hoffman, A. S.; Stayton, P. S. Bioconjugate Chem 2003, 14, 412–419.

59 Meyer, Y.; Richard, J. A.; Massonneau, M.; Renard, P. Y.; Romieu, A. Org Lett 2008, 10, 1517–1520.

60 Vrudhula, V. M.; Mcmaster, J. F.; Li, Z.; Kerr, D. E.; Senter, P. D. Bioorg Med Chem Lett 2002, 12, 3591–3594.

61 Meng, F.; Hennick, W. E.; Zhong, Z. Biomaterials 2009, 30, 2180–2198.

62 Lin, C.; Zhong, Z.; Lok, M. C.; Jiang, X.; Hennick, W. E.; Feijan, J.; Engbersen, J. F. J. Bioconjugate Chem 2007, 18, 138–145.

63 Mckenzie, D. L.; Kwok, K. Y.; Rice, K. G. J Biol Chem 2000, 275, 8617–8624.

64 Segura, T.; Hubbell, J. A. Bioconjugate Chem 2007, 18, 736–745.

65 Kakizawa, Y.; Harada, A.; Kataoka, K. Biomacromolecules 2001, 2, 491–497.

66 Ghosh, S. K.; Irvin, K.; Thayumanavan, S. Langmuir 2007, 23, 7916–7919.

67 De Gennes, P. G. J Chem Phys 1971, 55, 572–579.