Thiol-Functionalized Poly(ethylene glycol)-*b*-polyesters: Synthesis and Characterization

Nisha C. Kalarickal,[†] Stephen Rimmer,[‡] Prodip Sarker,[‡] and Jean-Christophe Leroux*,[†]

Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, Montreal, Quebec H3C 3J7, Canada, and Polymer and Biomaterials Chemistry Laboratories, Department of Chemistry, University of Sheffield, Sheffield S3 7HF, U.K.

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ABSTRACT: This paper describes a novel synthetic strategy for the preparation of thiol end-functionalized poly(ethylene glycol) (PEG)-*b*-polyesters. Block copolymers containing an internal disulfide bond were prepared through the ring-opening polymerization of DL-lactide (LA) and ϵ-caprolactone (CL) employing a PEG disulfide [$(PEG\text{-}S)_2$] as the macroinitiator. This initiator was synthesized from α-*tert*-butanethio-ω-hydroxy-PEG (*t*Bu-S-PEG) through the deprotection of *t*Bu and the subsequent formation of a disulfide. The disulfide bond of the block copolymers was cleaved by reduction using tributylphosphine to generate block copolymers bearing a thiol at the PEG chain end. Thiolated PEG-*b*-PLA and PEG-*b*-PCL with number-average molecular weights (M_n) in the range of 3300−5800 and 3600−4600, respectively, were thereby obtained. The PLA and PCL contents could be varied according to the feed ratio and ranged between 20−47 and 15−30 mol %, respectively. Aqueous solutions of the disulfide block copolymers formed degradable gels at high concentration and underwent a gel−sol transition upon an increase in temperature. The gels were liquefied by treating with dithiothreitol, indicating that the triblock configuration is essential for the gelation.

Introduction

The thiol group is distinctive among the various functionalities found in nature, with its ability to reversibly form disulfide bonds. The thiol's unique features originate from its typical pK_a, nucleophilicity, and bonding characteristics which make it a versatile site for chemical processes. Thiols play an important role in protein folding, signal transduction, cell proliferation, and various redox processes.[1] Moreover, the strong affinity of the thiol group toward metal substrates such as gold and silver and its ability to react selectively and quantitatively with maleimides under physiological conditions have led to its extensive use in various fields. Thiol-functionalized polymers have been widely used for the derivatization of interfaces with synthetic and biological molecules in tissue engineering,[2−4] protein arrays,[5,6] molecular diagnostics,[7,8] and drug delivery.[9−13] For example, thiol end-functionalized poly(ethylene glycol)-*block*-poly(DL-lactic acid) (PEG-*b*-PDLLA) synthesized by modification of an amine end group by *N*-succinimidyl 3-maleimidopropionate has been investigated for the preparation of biomimetic surfaces.[3] Alternatively, thiol end-functionalized polymers have been applied for the preparation and stabilization of nanosized transition metals or semiconductor particles.[14−19] Thiol-modified polycarbophil,[9] chitosan,[10] and poly(acrylic acid)[11] have shown promise for the preparation of mucoadhesive drug delivery systems. Clearly, the establishment of effective synthetic procedures for the preparation of thiol-functionalized (co)polymers is of great interest.

Synthetic strategies for the end-functionalization of (co)polymers involve the use of either bifunctional spacers obeying the amidation chemistry or functionalized initiator species. The latter strategy is preferred over the standard acid/amine coupling chemistry which is associated with inherent limitations such as nonuniform labeling and formation of side products that require further purification steps. Extensive research by various groups has demonstrated the merit of using initiators with masked functionalities for the synthesis of PEGs having different functional groups at both chain ends.[20−22] When these heterotelechelic PEGs are employed as macroinitiators in the polymerization of hydrophobic monomers, new heterobifunctional block copolymers with tailored applications can be produced.[23] Nevertheless, there are only few reports available on the direct synthesis of thiol end-functionalized (co)polymers.

Reversible addition−fragmentation chain transfer polymerization has been recently evaluated for the synthesis of thiol-terminated (co)polymers via the base cleavage of terminal polymer dithioester groups.[19,24−27] Thiol-containing polymers were also prepared by atom transfer radical polymerization using either disulfide initiators or polyfunctional halide initiators with incorporated protected thiol groups.[28−30] Dufresne et al.[31] reported on the use of *tert*-butyl mercaptan to initiate the synthesis of *tert*-butyl-S-PEG and its diblock copolymer *t*Bu−S-PEG-*block*-poly((*N*,*N*-dimethylamino)ethyl methacrylate). However, to the best of our knowledge, strategies for the direct synthesis of thiol end-functionalized block copolymers of PEG with biodegradable polyesters such as PLA and poly(ϵ-caprolactone) (PCL) have not been developed so far. In this work, the synthesis and characterization of thiol-terminated PEG-*b*-PLA and PEG-*b*-PCL are described. The proposed synthetic approach takes advantage of the reversible thiol/disulfide bond formation and proceeds through the ring-opening polymerization of the respective monomers initiated by $(PEG\text{-}S)_2$. The thiol end-functionalized block copolymers could be easily generated by the reduction of the block copolymer disulfides thus obtained. This synthetic procedure would allow the in-situ modification of the generated thiol functional groups with suitable labeling or targeting agents. This work further demonstrates the possibility of forming degradable redox gels from the disulfide copolymers.

* To whom correspondence should be addressed: Tel +1 514 343-6455, Fax +1 514 343-6871, e-mail Jean-Christophe.Leroux@umontreal.ca.
[†] University of Montreal.
[‡] University of Sheffield.

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Table 1. Characterization of Poly(DL-lactide)-*b*-poly(ethylene glycol) Disulfide, (PLA-*b*-PEG-S)$_2$, Synthesized by Ring-Opening Polymerization of DL-Lactide in Toluene under Refluxing Conditions Using Poly(ethylene glycol) Disulfide/Stannous Octoate as the Macroinitiator/Catalyst System and the Thiolated Poly(ethylene glycol)-*b*-poly(DL-lactide) (PLA-*b*-PEG-SH) Copolymers Obtained by the Reduction of Respective Disulfide Copolymers Using Tributylphosphine

			(PLA-*b*-PEG-S)$_2$ (GPC)[a]			PLA-*b*-PEG-SH (GPC)[a]		
sample code	*t*Bu-S-PEG M_n(GPC)[a]	(PEG-S)$_2$ M_n(GPC)[a]	M_n[a]	M_w[a]	PI[a]	M_n[a]	PI[a]	PLA mol % (NMR)[b]
PEG-PLA-I	2100	4200	5700	6600	1.16	3300	1.22	20
PEG-PLA-II	2100	4000	7700	9400	1.22	4100	1.30	31
PEG-PLA-III	2100	4300	8600	10900	1.27	4400	1.38	36
PEG-PLA-IV	2100	4100	10800	14800	1.37	5300	1.58	45
PEG-PLA-V	2100	4400	12800	19600	1.53	5800	1.64	47

[a] Number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (PI) were determined by GPC using PEG standards. [b] Determined by ^{1}H NMR from the ratio of the number of protons under the peaks characteristic of PLA (δ = 5.21 ppm) and PEG (δ = 3.68 ppm) blocks.

Experimental Section

Materials. *tert*-Butyl mercaptan (*t*Bu-SH, 99%), ethylene oxide (EO, 99.5%), 3,6-dimethyl-1,4-dioxane-2,5-dione (DL-lactide, LA,), ϵ-caprolactone (CL, 99%), stannous octoate (95%), tributylphosphine (Bu_3P, 97%), deuterated chloroform ($CDCl_3$, 99.96% atom D), DL-dithiothreitol (DTT, 98%), trifluoroacetic acid (TFA, 99%), and dimethyl sulfoxide (DMSO, 99.9%) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). *t*Bu-SH was dried over calcium oxide, distilled under argon, and stored over molecular sieves. EO was passed through a sodium hydroxide column, dried over CaH_2, and cryodistilled prior to use. LA was recrystallized from ethyl acetate and then dried in vacuo at room temperature. CL was dried over CaH_2, distilled under vacuum, and stored over molecular sieves under argon. Tetrahydrofuran (THF) and toluene (purification grade, Sigma-Aldrich) were purified by filtration through drying columns on a Pure Solv system (Innovative Technology Inc., Boston, MA). All other chemicals were used as received.

Techniques. ^{1}H NMR spectra were recorded on a Bruker NMR spectrometer (Milton, ON, Canada) operating at 400 MHz, using $CDCl_3$. Gel permeation chromatography (GPC) measurements were performed in THF using an Alliance GPCV 2000 system (Waters, Milford, MA) equipped with a differential refractive index detector and the Millennium software program. Adequate molecular weight separation was achieved using three Waters Styragel columns (HR2, HR3, and HR5; bead size: 10 μm; molecular weight ranges: 100−10K, 500−30K, and 50K−4M, respectively) in series at a flow rate of 1.0 mL/min and a temperature of 40 °C. A calibration curve was obtained with near-monodisperse PEG standards. Molecular weights by relative analysis were obtained from comparison of the retention times of synthesized polymers with those of PEG standards. Raman spectra were recorded on a Renishaw InVia micro-Raman system (Renishaw, ON, Canada), which is equipped with a Leica optical microscope adapted to a double grating spectrograph and charge-coupled device (CCD) array detector. The laser excitation was provided by an argon ion laser operating at 25 mW and of 514 nm output. The sample was focused with a 50× magnification objective lens, and the laser spot at the sample was 2 μm. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were obtained using a TofSpec 2E instrument (MicroMass, Manchester, U.K.), using dithranol as the matrix. The following parameters were used to analyze the polymer samples: instrument voltage 20 kV, sampling rate 500 MHz, pulse time 39, pulse voltage 2471 V, detector voltage 1689 V, and cationizing agent NaI. A sample of polymer (5−10 mg), dithranol (10 mg), and NaI (5 mg) were dissolved in THF (0.5 mL) separately. The polymer solution (20 μL) was mixed with dithranol solution (20 μL), and 5 μL of the NaI solution was added. About 1 μL of the resulting mixture was then spotted onto the MALDI target, allowed to dry, and then loaded for MALDI-TOF analysis.

Synthetic Procedures. Synthesis of α-*tert*-Butanethio-ω-hydroxy-PEG (*t*Bu-S-PEG). The asymmetrical *t*Bu-S-PEG was synthesized using *t*Bu-SH as the initiator by following a previously reported procedure.[31] Briefly, anhydrous THF (22 mL), potassium naphthalene (0.2 M solution in THF, 12 mL), and *t*Bu-SH (0.54 mL, 4.8 mmol) were charged into a sealed 100 mL round-bottom flask. The components were stirred for at least 15 min to ensure the formation of thiolates, at which point liquid EO (11.5 mL, 0.230 mol) was added using a two-head needle. The polymerization reaction was carried out for 48 h, and the product was recovered by precipitation in cold diethyl ether. Yields after purification were in the range 90−95%. ^{1}H NMR δ (ppm): 3.68, m ((−C*H*$_2$−C*H*$_2$−O−)$_n$); 2.75, t (−C*H*$_2$−S−C(CH$_3$)$_3$); 2.35, s (−CH$_2$−CH$_2$−O*H*); 1.33, s (−CH$_2$−S−C(C*H*$_3$)$_3$). GPC: M_n − 2100; M_w/M_n: 1.05. Trifluoroacetic anhydride was added to the NMR tube in order to shift the signal of the methylene group adjacent to the terminal hydroxyl group and allow for the determination of the degree of thiol functionalization. ^{1}H NMR ($CDCl_3$ with 7.5% (v/v) trifluoroacetic anhydride) δ (ppm): 4.50, t (−C*H*$_2$−O−CO−CF$_3$); 3.68, m ((−C*H*$_2$−C*H*$_2$−O−)$_n$); 2.75, t (−C*H*$_2$−S−C(CH$_3$)$_3$); 1.33, s (−CH$_2$−S−C(C*H*$_3$)$_3$), degree of thiol functionalization 98%.

Preparation of PEG Disulfide (PEG-S)$_2$. The cleavage of *t*Bu and subsequent disulfide formation was achieved by treating the *t*Bu-S-PEG with a TFA/DMSO mixture. The polymer (1.0 g) was dissolved in DMSO (19 mL) followed by addition of TFA (106 mL, 15/85 v/v) to a final polymer concentration of 8 mg/mL. The reaction was conducted for 20 min, after which TFA was removed by rotary evaporation. The crude polymer extract was then precipitated twice in cold diethyl ether to recover the PEG disulfide. The product was isolated by filtration and dried in vacuo. Yields after purification were in the range 75−80%. The (PEG-S)$_2$ thus obtained was further purified by fractional precipitation. At first, the polymer (1.0 g) was dissolved in dichloromethane (100 mL), and then cold diethyl ether was added stepwise with stirring until the appearance of a precipitate. The solution was further stirred for 30 min, and the precipitated mass was isolated by filtration and dried in vacuo. The recovery yield of PEG disulfide at the end of two to three fractional precipitations was in the range 55−60%. ^{1}H NMR δ (ppm): 3.68, m ((−C*H*$_2$−C*H*$_2$-O−)$_n$); 2.86, t (−C*H*$_2$−S−S−C*H*$_2$−). GPC: M_n − 4200; M_w/M_n: 1.06.

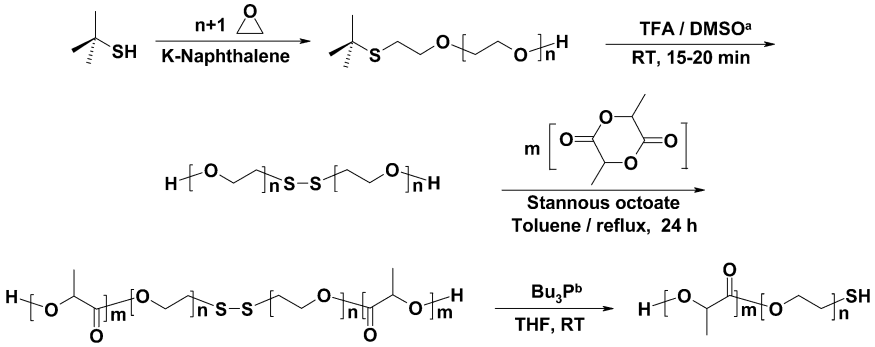
Ring-Opening Polymerization of LA in the Presence of (PEG-S)$_2$. (PLA-*b*-PEG-S)$_2$ was prepared by ring-opening polymerization of LA using (PEG-S)$_2$ as the initiator. In a typical procedure, (PEG-S)$_2$ (1.0 g, 0.25 mmol) was dissolved in 20 mL of toluene. Using a Dean−Stark trap, the polymer was dried by azeotropic distillation. Toluene was then distilled off completely. LA (0.96 g, 6.7 mmol) was charged into the reaction flask under an argon atmosphere, and the mixture was dried in vacuo at 80 °C for 3 h. Stannous octoate (0.75 wt %) dissolved in dry toluene was added to the flask. The volume was made up to 11 mL by dry toluene, and the reaction mixture was refluxed under an argon atmosphere for 24 h. (PLA-*b*-PEG-S)$_2$ was precipitated in cold diethyl ether. The polymer was collected and dried in vacuo at room temperature. Yield: 80%. ^{1}H NMR δ (ppm): 5.21, m (−CO−C*H*(CH$_3$)−O− of PLA backbone); 4.32, m (−CO−C*H*(CH$_3$)−O− of PLA chain end, −C*H*$_2$−O−CO− of PEG linked to lactide); 3.68, m (−O−C*H*$_2$−C*H*$_2$− of PEG backbone); 2.85, t (−C*H*$_2$−S−S−C*H*$_2$−); 1.52, m (−CO−CH-

Table 2. Characterization of Poly(ϵ-caprolactone)-*b*-poly(ethylene glycol) Disulfide, (PCL-*b*-PEG-S)$_2$, Synthesized by Ring-Opening Polymerization of ϵ-Caprolactone in Toluene under Refluxing Conditions Using Poly(ethylene glycol) Disulfide/Stannous Octoate as the Macroinitiator/Catalyst System and the Thiolated Poly(ethylene glycol)-*b*-poly(ϵ-caprolactone) (PCL-*b*-PEG-SH) Copolymers Obtained by the Reduction of Respective Disulfide Copolymers Using Tributylphosphine

			(PCL-*b*-PEG-S)$_2$ (GPC)[a]			PCL-*b*-PEG-SH (GPC)[a]		
sample code	*t*Bu-S-PEG M_n(GPC)[a]	(PEG-S)$_2$ M_n(GPC)[a]	M_n[a]	M_w[a]	PI[a]	M_n[a]	PI[a]	PCL mol % (NMR)[b]
PEG-PCL-I	2100	4400	5900	7400	1.25	3600	1.21	15
PEG-PCL-II	2100	4300	7700	10000	1.30	3700	1.28	18
PEG-PCL-III	2100	4400	8700	10400	1.20	4600	1.14	30

[a] Number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (PI) were determined by GPC using PEG standards. [b] Determined by ^{1}H NMR from the ratio of the number of protons under the peaks characteristic of PCL (δ = 4.0 ppm) and PEG (δ = 3.68 ppm) blocks.

Scheme 1. Synthetic Route to the Preparation of Thiol-Terminated Poly(ethylene glycol)-*b*-poly(DL-lactide) Copolymers[a]



[a] a = trifluoroacetic acid/dimethyl sulfoxide; b = tributylphosphine.

(CH_3)−O− of PLA backbone). GPC: M_n − 7700; M_w/M_n: 1. 22 (Table 1, PEG-PLA-II).

Polymers of different molecular weight were synthesized according to the above-described procedure with an increasing molar ratio of LA to EO in the feed. Yields after purification were in the range 70−80%.

Ring-Opening Polymerization of CL in the Presence of (PEG-S)$_2$. (PCL-*b*-PEG-S)$_2$ was prepared by ring-opening polymerization of CL using (PEG-S)$_2$ as the initiator. (PEG-S)$_2$ (1.0 g, 0.23 mmol) was dissolved in 20 mL of toluene. Using a Dean−Stark trap, the polymer was dried by azeotropic distillation. Toluene was then distilled off completely. CL (0.84 g, 7.4 mmol) was charged into the reaction flask under an argon atmosphere, and the mixture was dried in vacuo at 50 °C for 3 h. After drying, 1 wt % stannous octoate dissolved in dry toluene was added to the flask. The volume was made up to 7 mL by dry toluene, and the reaction mixture refluxed under argon atmosphere for 24 h. (PCL-*b*-PEG-S)$_2$ was precipitated in cold diethyl ether. The polymer was collected and dried in vacuo at room temperature. Yield: 70%. ^{1}H NMR δ (ppm): 4.17, t (−CH_2−O−CO− of PEG linked to CL); 4.00, t (−CH_2−O−CO− of PCL main chain); 3.68−3.70, m (−O−CH_2−CH_2− of PEG backbone, −CH_2−OH of PCL chain end); 2.85, t (−CH_2-S−S−CH_2−); 2.25, m (−CH_2−CO−O− of PCL carbonyl); 1.59, m (−CH_2− of PCL methylene groups); 1.32, m (−CH_2− central methylene group of CL units). GPC: M_n − 7700; M_w/M_n: 1.30 (Table 2, PEG-PCL-II).

Polymers of different molecular weight were synthesized according to the above-described procedure with an increasing molar ratio of CL to EO in the feed. Yields after purification were in the range 70−80%.

Reduction of (PEG-S)$_2$ and Disulfide Block Copolymers. The PEG disulfide (0.3 g, 0.07 mmol) was dissolved in deoxygenated THF (16 mL), and Bu_3P (1.7 mL, 7.0 mmol, 100 equiv with respect to disulfide units) was added. The reaction mixture was stirred under argon at room temperature. Samples were periodically withdrawn, diluted with THF, and analyzed immediately by GPC to determine the extent of cleavage of the disulfide bond in the polymer. The thiolated polymer was recovered by precipitation in acidified cold diethyl ether followed by filtration under argon atmosphere. Yield: 53%; GPC: M_n − 2100; M_w/M_n: 1.06. PLA-*b*-PEG-SH and PCL-*b*-PEG-SH were generated by the same procedure with the exception that the thiolated copolymers were precipitated in cold diethyl ether. The recovery yields after precipitation were in the range 60−70%.

Gel-to-Sol Transition of (PLA-*b*-PEG-S)$_2$ and (PCL-*b*-PEG-S)$_2$. The aqueous solutions of synthesized copolymers were prepared by dissolving copolymers in various concentrations at temperatures above their gelling point. Phase-change behaviors of aqueous solutions of the disulfide copolymers were investigated by the inverse flow method. The temperature was increased by 2° intervals, and the gel−sol transition was determined by placing the vial horizontally after an equilibrium time of 15 min. The liquefaction of the gels was achieved by adding 10 mol % excess DTT into the gel at room temperature.

Results and Discussion

The primary objective of the present work was to develop a direct synthetic methodology to prepare block copolymers of PEG and polyesters bearing a thiol group at the extremity of the PEG segment. Typically, end-functionalized PEG block copolymers are synthesized using PEG with masked functionalities as the macroinitiator during the block copolymerization. However, this approach could not be directly used for the synthesis of thiol-functionalized biodegradable block copolymers. This is due to the fact that the standard procedures for the deprotection of thiol groups employ either highly acidic conditions or reagents which are relatively harsh for the polyester segment. Thus, the major challenge was to design a PEG macroinitiator from which the thiol functionality could be easily generated under mild conditions, after block copolymerization. (PEG-S)$_2$ was considered as a potential initiator due to the ease of reducing it into a free thiol. It proved to be useful for the synthesis of block copolymers of LA (Scheme 1) and CL. Moreover, it was found that this method also allowed for the synthesis of block copolymers with redox gelling properties in water.

Synthesis of Asymmetrical *t*Bu-S-PEG. The heterobifunctional PEG bearing a protected thiol and hydroxyl group at each

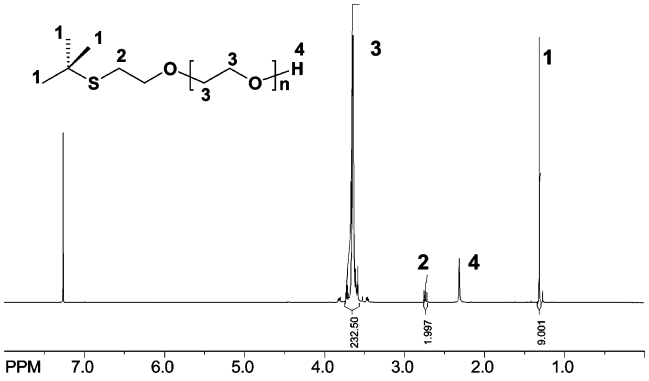


Figure 1. ^{1}H NMR spectrum in $CDCl_3$ of α-*tert*-butanethio-ω-hydroxy-PEG (*t*Bu-S-PEG) synthesized by the ring-opening polymerization of ethylene oxide using a *tert*-butyl mercaptan/potassium−naphthalene initiator system.

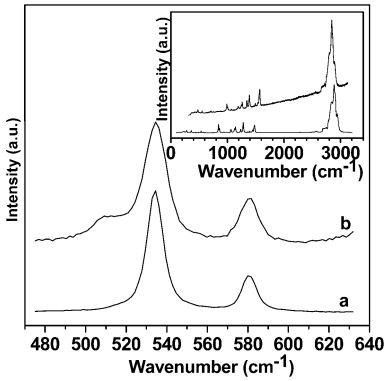


Figure 2. Raman spectra in the region 470−640 cm^{-1} for PEG (a) and $(PEG\text{-}S)_2$ (b). Inset: the complete range Raman spectra of these samples in the same order.

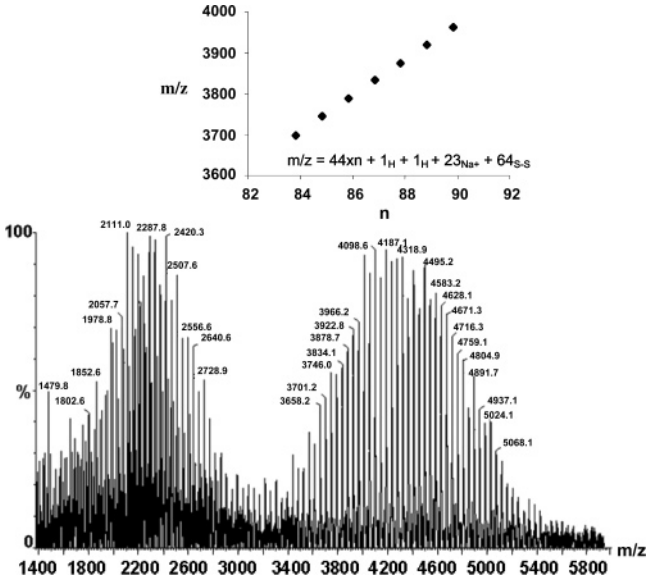


Figure 3. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrum of poly(ethylene glycol) disulfide, $(PEG\text{-}S)_2$. Inset: *m*/*z* vs *n* spectrum for the $(PEG\text{-}S)_2$ species. Experimental conditions: instrument voltage 20 kV, sampling rate 500 MHz, pulse time 39, pulse voltage 2471 V, detector voltage 1689 V, and cationizing agent NaI.

end was obtained by initiating the anionic ring-opening polymerization of EO using the *t*Bu-SH/potassium−naphthalene system.[31] This procedure yielded PEG with nearly quantitative thiol functionalization and low molecular weight distributions (≤1.05). A typical ^{1}H NMR spectrum of *t*Bu-S-PEG in $CDCl_3$ is presented in Figure 1 with all peaks assigned. The degree of end-functionalization (>95%) was estimated by derivatizing the hydroxyl group of *t*Bu-S-PEG to a trifluoroacetate using trifluoroacetic anhydride as described in the Experimental Section.

Preparation of $(PEG\text{-}S)_2$ and Subsequent Purification by Fractional Precipitation. The PEG disulfide was obtained by reacting the *t*Bu-S-PEG with a mixture of TFA/DMSO. ^{1}H NMR analysis on the product showed a complete removal of the *t*Bu unit as indicated by the disappearance of the peak at 1.32 ppm (Figure S2A, Supporting Information). GPC analysis showed a 2-fold increase in the molecular weight, which also confirmed the formation of disulfide (Table 1). By controlling the TFA/DMSO ratio, polymer concentration, and reaction time, $(PEG\text{-}S)_2$ with 75−80% purity could be achieved (straight line, Figure S3, Supporting Information). However, a further purification step was deemed necessary as low molecular weight species which could potentially influence the outcome of block copolymerization remained. Typically, two to three fractional precipitations were necessary to achieve a purity of 95% (Figure S3, Supporting Information). All the $(PEG\text{-}S)_2$ used in this study for further block copolymerization with LA and CL was ∼95% pure in disulfide.

Characterization of $(PEG\text{-}S)_2$. $(PEG\text{-}S)_2$ was analyzed by Raman spectroscopy and MALDI-TOF mass spectrometry. Figure 2 shows the Raman spectra of $(PEG\text{-}S)_2$ along with PEG control. As observed from the inset figure, the overall features of both spectra were essentially the same. The main absorption peaks were consistent with the Raman spectra of PEG.[32,33] However, a close observation of the $(PEG\text{-}S)_2$ spectrum revealed the presence of a shoulder at 508 cm^{-1}, the characteristic absorption band corresponding to −S−S− stretching (ν_{S-S}) which is absent in the control sample. MALDI-TOF analysis also confirmed the disulfide formation. Figure 3 shows a typical spectrum of $(PEG\text{-}S)_2$, where two distinct populations corresponding to the disulfide and the fragmented low molecular weight PEG are clearly visible. The end-functional group analysis clearly showed that the PEG chains bore hydroxyl groups at both chain ends along with a disulfide bond in the molecule (Figure 3, equation shown in inset). The presence of the disulfide bond was further confirmed by the end-functional group analysis of the population corresponding to the low molecular weight species. The MALDI-TOF mass spectrum showed that the mass of one chain end in these species corresponded to that of disulfide plus the sodium ion. MALDI-TOF analysis was also performed on a sample of *t*Bu-S-PEG treated with TFA to obtain further insight into the possible side reactions occurring during the deprotection step. Even though the end-functional group analysis showed the presence of a hydroxyl group, it did not reveal any sulfur-containing functional groups such as −SOH, −SOOH, or SO_3H. The presence of free thiol groups was also ruled out by a negative Ellman's test result. Hence, the exact nature of side reactions could not be established from the present experimental results.

Synthesis of $(PLA\text{-}b\text{-}PEG\text{-}S)_2$. Typically, ring-opening polymerization of LA is initiated by an alcohol using various catalysts such as stannous octoate, metal hydrides, or metal alkoxides.[34−37] In the present study, stannous octoate was selected as the catalyst since it was observed that the metal hydrides reduced the disulfide bond in the $(PEG\text{-}S)_2$ macroinitiator (data not shown). Polymers with increasing molar feed ratios of LA to EO were synthesized and purified as described in the Experimental Section. A typical ^{1}H NMR spectrum of $(PLA\text{-}b\text{-}PEG\text{-}S)_2$ is shown in Figure 4A. The characteristic

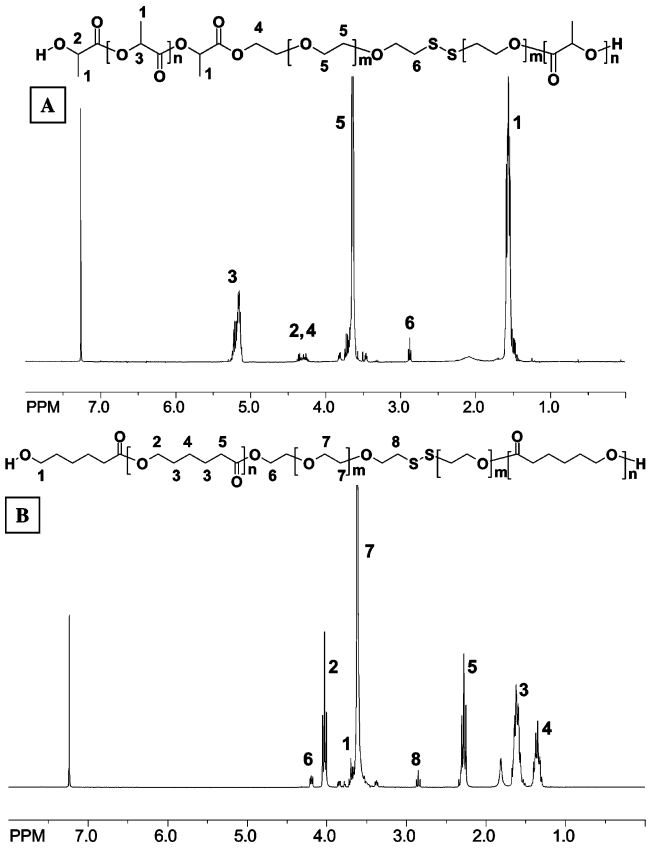


Figure 4. ^{1}H NMR spectra of poly(DL-lactide)-*b*-poly(ethylene glycol) disulfide, (PLA-*b*-PEG-S)$_2$ **(A)**, and poly(ϵ-caprolactone)-*b*-poly(ethylene glycol) disulfide, (PCL-*b*-PEG-S)$_2$ **(B)**, in $CDCl_3$. The block copolymers were synthesized by ring-opening polymerization of DL-lactide or ϵ-caprolactone in toluene under refluxing conditions using poly(ethylene glycol) disulfide/stannous octoate as the macroinitiator/catalyst system.

resonances of both PLA and PEG were observed in the spectrum, which correlates well with the reported data on PLA-*b*-PEG-*b*-PLA.[35] The peaks at 5.21 and 1.52 ppm corresponded to the $-CH(CH_3)-$ and $-CH_3$ groups of PLA, respectively. The resonance at 3.68 ppm was characteristic of main-chain methylene units within the PEG blocks. The α-methylene protons of the EO unit adjacent to PLA ($PLA-CO-O-CH_2-$) appeared at 4.32 ppm, together with the $-CH(CH_3)-$ protons of the hydroxylated lactyl end units. The presence of a peak at 2.85 ppm clearly showed that the block copolymers contained a disulfide bond.

The PLA content (mol %) in the block copolymers was estimated from the ratio of the number of protons under the peaks characteristic of PLA and PEG blocks in ^{1}H NMR spectra. The LA/EO molar ratio in the synthesized (PLA-*b*-PEG-S)$_2$ varied between 0.25 and 0.89. Molecular weights and polydispersity indices (PI) of the polymers estimated by GPC relative to PEG standards are presented in Table 1. M_n values were in the range 5700−12 800, and PI ranged between 1.16 and 1.53. A slight increase in the PI was observed for the block copolymers compared to that of (PEG-S)$_2$. This could be related to the fact that the (PEG-S)$_2$ used for the block copolymerization of LA is only 95% pure. The remaining 5−6% includes the unmodified PEG with at least one hydroxyl group which could also initiate the polymerization of LA, resulting in a small fraction of diblock copolymers and a consequent increase in the PI.

Synthesis of (PCL-*b*-PEG-S)$_2$. In order to demonstrate the general applicability of (PEG-S)$_2$ as a macroinitiator, block copolymers of CL were synthesized following similar reaction conditions as those used for LA. A series of polymers with increasing molar feed ratios of CL to EO (0.22−0.53) were obtained. The ^{1}H NMR spectrum of (PCL-*b*-PEG-S)$_2$ in $CDCl_3$ (Figure 4B) revealed peaks characteristic of both polymer blocks. The methylene protons of EO units corresponded to the peak at 3.68 ppm. The methylene peaks of the CL main chain appeared at 4.0, 2.25, 1.59, and 1.32 ppm (labeled 2, 5, 3, and 4, respectively). The α-methylene protons of the EO unit adjacent to PCL ($PCL-CO-O-CH_2-$) appeared at 4.17 ppm. The presence of a peak at 2.85 ppm indicates that the block copolymers contained a disulfide bond. The PCL content (mol %) in the block copolymers was calculated on the basis of ratios of the peak areas characteristic of PCL and PEG blocks. Copolymers with M_n values ranging from 5900 to 8700 were obtained with increasing CL to EO molar feed ratios. The results in Table 2 showed that the molar ratio of CL/EO in synthesized (PCL-*b*-PEG-S)$_2$ were in the range 0.18−0.43.

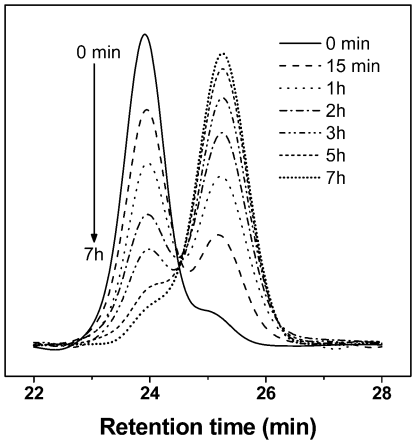


Figure 5. Gel permeation chromatogram traces recorded at various reaction time intervals during the reduction of poly(ethylene glycol) disulfide, (PEG-S)$_2$, by tributylphosphine.

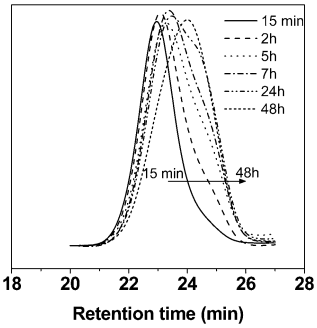


Figure 6. Gel permeation chromatogram traces recorded at various reaction time intervals during the reduction of poly(DL-lactide)-*b*-poly(ethylene glycol) disulfide, (PLA-*b*-PEG-S)$_2$, by tributylphosphine.

Reduction of (PEG-S)$_2$ and Disulfide Block Copolymers. PEG-SH was easily generated through the reductive cleavage of the respective disulfides. Numerous reagents have been described in the literature for the reduction of disulfides into thiols, mostly for applications in the field of biochemistry.[38,39] Among them, phosphines (particularly the tributylphosphine) have been increasingly used in both protein chemistry and polymer synthesis. Phosphines are preferred over conventional reducing agents like DTT mainly due to their high affinity for disulfide groups and their relative resistance toward autoxidation. In the present study, tributylphosphine was used to generate thiolated polymers from their respective disulfides. The progress of the reaction could be easily followed by GPC as the reduction

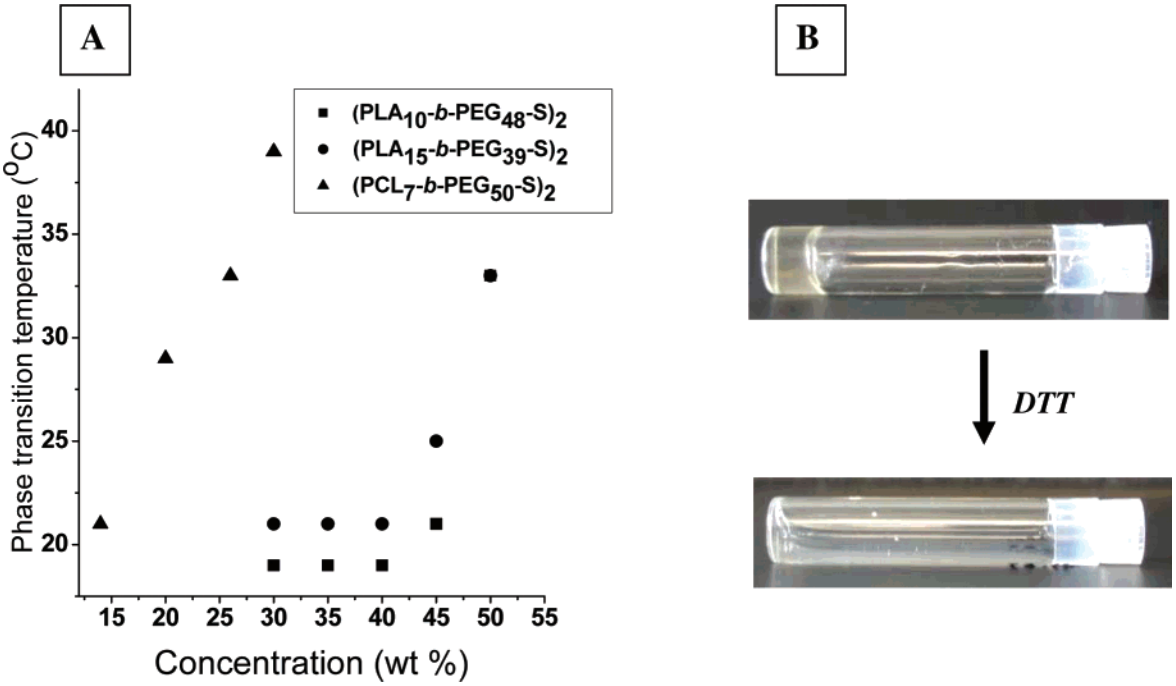


Figure 7. Gel−sol phase diagrams for aqueous solutions of poly(DL-lactide)-*b*-poly(ethylene glycol) disulfide, (PLA-*b*-PEG-S)$_2$, and poly(ϵ-caprolactone)-*b*-poly(ethylene glycol) disulfide, (PCL-*b*-PEG-S)$_2$ **(A)**. Liquefaction of the gel prepared from 50 wt % (PLA$_{10}$-PEG$_{48}$-S)$_2$ through the reduction with DL-dithiothreitol (DTT) **(B)**.

decreased the molecular weight by 2-fold. Figure 5 shows the degree of reduction with time for (PEG-S)$_2$. As clearly seen from the figure, more than 90% of the reduction was completed within 7 h of reaction. The PEG-SH thus obtained had the same PI as the starting *t*Bu-S-PEG. This shows the effectiveness of the procedure in converting the *t*Bu-S-PEG to a free sulfhydril-containing PEG though an intermediate disulfide formation.

A similar procedure was used for the reduction of disulfide-containing LA and CL block copolymers. Figure 6 shows the kinetics of the reduction of (PLA-*b*-PEG-S)$_2$ to the corresponding thiolated block copolymer. Compared to (PEG-S)$_2$, the reduction of disulfide-containing block copolymers proceeded relatively slowly. It took 48 h for the reduction of a block copolymer with M_n of 7700 to be completed. On the basis of the kinetics of the reaction, all the block copolymer reductions were carried out for 48 h. The slower rate may be attributed to the fact that the reactions were carried out in THF. Typically, shorter reaction times were reported for the reduction with tributylphosphine in more polar solvents such as DMF.[29] In the present study, THF was used owing to the ease of monitoring the progress of the reaction by GPC. The coordinating tendency of tributylphosphine with the ester groups present in the polyester backbone could also be another possibility for the reduced reaction rate observed in the case of disulfide block copolymers. In all cases, block copolymers with approximately 2-fold decreased M_n were obtained, indicating the complete conversion of the disulfide polymers to sulfhydril end-functionalized copolymers. However, an increase in PI was observed with the reduction of the disulfide block copolymers. The presence of free sulfhydril groups in the block copolymers was confirmed by a positive Ellman's test (data not shown).

Gel-to-Sol Transition of (PLA-*b*-PEG-S)$_2$ and (PCL-*b*-PEG-S)$_2$. Block copolymers containing PEG (A) and biodegradable polyesters (B), such as PLA, polyglycolide (PGA), PLGA, and PCL, have extensively been studied for their temperature-induced gelation.[40−42] The diblock and triblock (both ABA and BAB) copolymers show either a gel-to-sol or sol-to-gel transition depending on various factors such as polymer composition, concentration, and molecular weight of the individual blocks. In the present work, the possibility of obtaining degradable redox gels from the synthesized disulfide block copolymers was explored. The disulfide block copolymers show similarity to conventional BAB type block copolymers. The copolymers (PLA$_{10}$-*b*-PEG$_{48}$-S)$_2$ and (PLA$_{15}$-*b*-PEG$_{39}$-S)$_2$ with central PEG disulfide blocks of M_n = 4200 or 3400 and PLA blocks of M_n = 1500 or 2100, respectively, were completely soluble throughout the concentration range studied and thus were investigated for their gelling properties. However, copolymers with a higher weight proportion of PLA were insoluble in water and could not be investigated for their gelling behavior. As illustrated in Figure 7A, the copolymer aqueous solutions showed a transition from gel to sol with increasing temperature. It was observed that the gel−sol transition was independent of the concentration up to 40 wt % for both the LA copolymers. The transition temperature then gradually rose with an increase in the polymer concentration. At each concentration the gel−sol transition temperature increased as the LA/EO ratio increased. The CL block copolymer with a PEG disulfide and CL blocks of M_n = 4400 and 1500 [(PCL$_7$-*b*-PEG$_{50}$-S)$_2$], respectively, also formed solid gel in a concentration range 14−30 wt %. However, in this case, the gel−sol transition temperature steadily increased with an increase in the polymer concentration. The transition temperature curve for the CL copolymer showed a steeper slope, and also the transitions occurred at lower concentrations compared to that of LA copolymers. This could be attributed to the higher hydrophobicity of the PCL segment. The gelation of these kinds of block copolymer solutions at high concentration is attributed to the association of micelles.[38] In the present study, it was also observed that the triblock architecture through the central disulfide bond was necessary for the gelation. The gels could be degraded by the reduction of the disulfide bond using DTT. Figure 7B shows the physical state of 50 wt % aqueous solution of disulfide copolymer (PLA$_{10}$-*b*-PEG$_{48}$-S)$_2$ at room temperature before and after the addition of DTT. The gel was completely liquefied upon the addition of DTT, which indicates that the

gelation is a result of the particular triblock disulfide molecular architecture. Once the disulfide bonds were completely reduced, the copolymer solution remained in the sol state even at a temperature as low as 4 °C.

Conclusions

This work proposes a novel synthetic strategy for the preparation of thiol-functionalized PEG-*b*-PLA and PEG-*b*-PCL copolymers. $(PEG\text{-}S)_2$ was prepared and efficiently used as a macroinitiator for the synthesis of LA and CL block copolymers with an internal disulfide bond. The thiolated block copolymers were generated by the subsequent reduction of the disulfide bond using tributylphosphine. The PLA and PCL contents in the copolymers could be controlled by varying the feed ratio of monomers. The aqueous solutions of disulfide block copolymers showed a gel−sol transition upon an increase in temperature. The gels were degraded in reductive conditions (DTT), indicating the significance of the triblock structure in the gelation process. The thiol end-functionalized block copolymers would also be advantageous for the easy coupling of various targeting or labeling ligands through the thiol group.

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Supporting Information Available: ^1H NMR spectrum of $(PEG\text{-}S)_2$, ^{13}C NMR spectra of *t*Bu-S-PEG, $(PEG\text{-}S)_2$, $(PLA\text{-}PEG\text{-}S)_2$, and $(PCL\text{-}PEG\text{-}S)_2$, and the GPC traces showing the effect of fractional precipitation in purifying $(PEG\text{-}S)_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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