Straightforward Synthesis of Cysteine-Reactive Telechelic Polystyrene

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Received September 27, 2007; Revised Manuscript Received November 13, 2007

ABSTRACT: Synthesis of a thiol-reactive telechelic polystyrene and conjugation of cysteine is reported. A dimethylfulvene-protected maleimide-functionalized atom transfer radical polymerization (ATRP) initiator (**3**) was synthesized, and the thermostability was compared to the analogous furan-protected initiator (**1**) by thermogravometric analysis (TGA). The former protecting group was stable to a higher temperature than the latter in the bulk phase (143 °C vs 125 °C) and thus was investigated as an initiator for the ATRP of styrene. Kinetic studies of the polymerization of styrene mediated by copper(I)/*N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) indicated that the reaction proceeded in a controlled manner with high initiator efficiency (92%). Polystyrene with a number-average molecular weight (M_n) of 2530 Da and a narrow polydispersity index (PDI) of 1.15 was then synthesized and subjected to atom transfer radical (ATR) coupling to form the bis-functionalized polymer. Gel permeation chromatography (GPC) and ^{1}H NMR spectroscopy studies indicated that dimerization had occurred and that the end groups were intact. Reactivity of the polymer was demonstrated by an in-situ retro-Diels−Alder and thioether bond formation first with model compound benzyl mercaptan and then with the amino acid *N*-acetyl-L-cysteine methyl ester. Bis-conjugation was verified by inspection of the ^{1}H NMR spectra and by elemental analysis.

Introduction

Peptide−polymer conjugates have received considerable attention because of their wide-ranging applications in materials, biotechnology, and medicine.[1−5] In particular, amphiphilic peptide−polymer biohybrids are interesting as building blocks in nanotechnology.[6−8] Polymers of this type are known to phase separate in the bulk into ordered mesophases and exhibit liquid crystalline morphologies.[9] In addition, they aggregate into interesting vesicles and micellar aggregates in solution.[10] Many of these peptide−polymer amphiphiles contain polystyrene as the non-peptidic block. For example, protein−polystyrene giant amphiphiles have been reported.[10b,c] The terminus of polystyrene was covalently linked to lipase B from *Candida antarctica* (CAL B).[10c] The resulting conjugate aggregated into micron long fibers consisting of bundles of rods that retained some enzymatic activity. These and other self-assembling biosynthetic hybrids consisting of polystyrene possess unique properties and considerable potential for advanced materials applications. For these reasons, we were interested in developing a straightforward, general way to synthesize polystyrene with end groups that react with amino acid side chains.

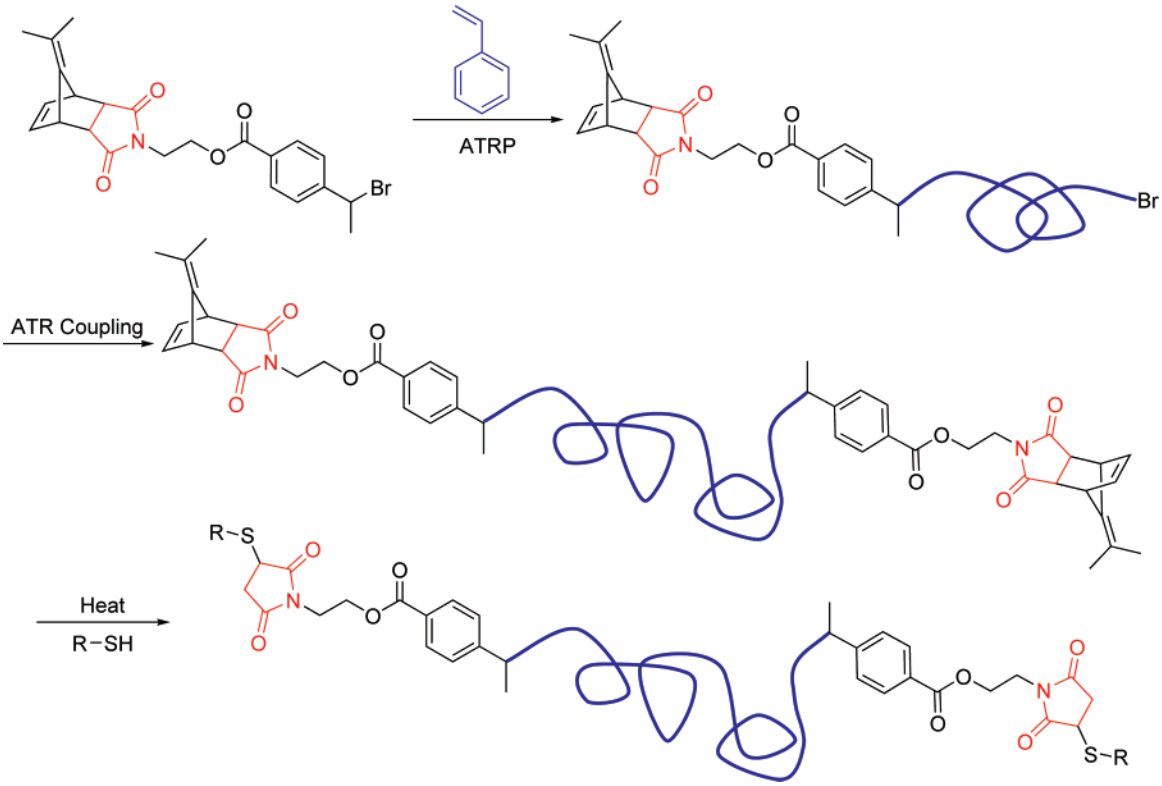
Polymers with protein and peptide reactive groups have been synthesized by a variety of methods including controlled radical polymerizations.[11,12] In particular, atom transfer radical polymerization (ATRP) is a powerful method to prepare well-defined conjugates. ATRP is a controlled/"living" polymerization consisting of a transition-metal-mediated reversible redox reaction with halogen transfer that is finely tuned by an appropriate ligand.[13−16] The process results in a low radical concentration that yields polymers with controlled molecular weights and narrow molecular weight distributions. The polymerization is also tolerant to a wide variety of functional groups. By ATRP, polymers have been synthesized in one step from initiators that react with peptides and proteins.[17] In addition, polymers have been grown directly from initiation sites on biomolecules.[10f,h,18,19] These routes have significant advantages over traditional techniques to prepare bioconjugates in that the polymers are synthesized without requiring successive reactions to install the reactive end groups. In addition, the resulting polymers have narrow molecular weight distributions. Therefore, we explored ATRP from a protein-reactive initiator to synthesize polystyrene.

An important consideration when designing a macromolecule for biohybrid formation is the ability to achieve site-specific conjugation with proteins and peptides. For most bioconjugates such precise modification is crucial for the resulting material properties.[1−5] For example, for amphiphilic conjugates the point of attachment may dictate which self-assembled structures form. In addition, well-defined conjugates retain bioactivity to a greater extent than those with random polymer chain placement. Site-specific conjugation is typically accomplished by reaction with rare amino acids, surface accessible amino acids, ligand binding sites, and non-natural modifications.[11,12] In particular, free cysteines provide convenient reactive sites because they occur with low frequency in peptides and proteins, and the sulfhydryl group of the side chain can be selectively targeted. In this study, we designed a protected maleimide ATRP initiator to synthesize polystyrene because the deprotected polymer should readily undergo Michael addition with free cysteines. ATRP initiators with pyridyl disulfides or protected maleimides have been previously shown to be effective for the polymerization of acrylamides and methacrylates.[17a,c] However, to our knowledge, this strategy has not been employed to prepare polystyrene.

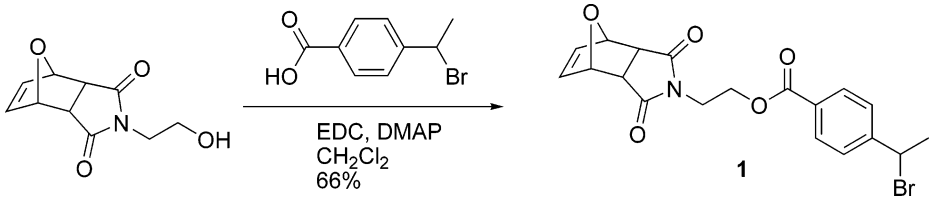
Bis-functionalized polystyrene may serve as an excellent building block for amphiphilic biohybrids with complex and interesting properties. Therefore, we investigated the synthesis of polystyrene with groups at both ends that react with free cysteines. Fortunately, ATRP can also be employed to produce telechelic polymers. The process conveniently provides a halogen end group on the ω-end of the polymer chain that is then available for postpolymerization modification by nucleophilic and electrophilic subsitutions.[20] The halogenated end group can also be utilized in atom transfer radical (ATR) coupling reactions to form polymeric dimers.[21,22] Opposite from the goal of ATRP, the radical concentration is maximized during

10.1021/ma702161q CCC: $40.75
Published on Web 12/29/2007

Scheme 1. Cysteine-Reactive Polystyrene Dimer Synthesis



Scheme 2. Synthesis of Furan-Protected ATRP Initiator



ATR coupling; in the absence of monomer this results in polymer termination. Interestingly, polystyrene has been shown to preferentially terminate by combination rather than disproportionation.[23] By polymerizing from a functionalized initiator followed by ATR coupling, telechelic polystyrene is obtainable.[21,22] In this manner, polymeric dimers have been synthesized with a variety of functional groups at both the α and ω ends, including alcohols, carboxylic acids, aminooxys, esters, and aldehydes.[24−27] We hypothesized that this same approach could be employed to install cysteine-reactive groups at both ends of a polystyrene chain.

Herein we describe a strategy to create thiol-reactive semitelechelic and telechelic polystyrene (Scheme 1). First, a dimethylfulvene-protected maleimide-functionalized ATRP initiator was synthesized and subsequently used to polymerize styrene. The resulting polystyrene was dimerized via ATR coupling to yield the telechelic polymer. Reactivity was demonstrated by conjugation of *N*-acetyl-L-cysteine methyl ester to yield the bis(amino acid)-modified macromolecule.

Experimental Section

Materials. All chemicals were purchased from Sigma-Aldrich or Acros and used as received, unless otherwise specified. Copper bromide (CuBr) was purified by stirring in glacial acetic acid for 12 h, filtering and rinsing with ethanol and diethyl ether, and drying under vacuum. Methylene chloride and styrene were distilled from calcium hydride before use. 4-Hydroxymethyl-10-oxa-4-azatricyclo-[5.2.1.0]dec-8-ene-exo-3,5-dione[28] and *N*-(2-hydroxyethyl)maleimide[29] were synthesized according to literature procedures. Deuterated solvents were obtained from Cambridge Isotope Laboratories.

Analytical Techniques. NMR spectra were recorded on a Bruker ARX500 spectrometer. Gel permeation chromatography (GPC) was conducted on a Shimazdzu HPLC system equipped with a refractive index detector RID-10A and two 300 × 4.6 mm Polymer Laboratories PLgel 5 μm mixed-D columns (with column guard). THF was used as the eluent (23 °C, flow rate 0.8 mL/min). Near-monodisperse polystyrene standards (Polymer Laboratories) were employed for calibration for molecular weight determination. Chromatograms were processed using the EZStart 7.3 chromatography software. Infrared spectroscopy was recorded using a Perkin-Elmer Spectrum 100 FT-IR equipped with a universal ATR sample accessory. Thermogravimetric analysis (TGA) was performed using a Perkin-Elmer Pyris Diamond TG/DTA (supported by the NSF IGERT Materials Creation Training Program under grant DGE-0114443) at a heating rate of 10 °C/min. Mass spectrometry and elemental analysis were performed at the UCLA Molecular Instrumentation Center (MIC).

Methods. *4-(1-Bromoethyl)benzoic Acid 2-(3,5-Dioxo-10-oxa-4-azatricyclo[5.2.1.0]dec-8-en-4-yl)ethyl Ester (**1**).* 4-(1-Bromoethyl)benzoic acid (252 mg, 1.10 mmol) and 4-hydroxymethyl-10-oxa-4-azatricyclo[5.2.1.0]dec-8-ene-exo-3,5-dione (200 mg, 0.96 mmol) were stirred under argon atmosphere in dry methylene chloride (20 mL). The mixture was cooled to 0 °C in an ice bath,

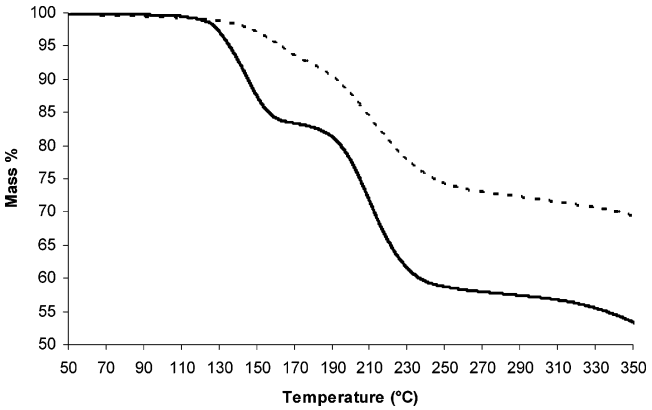


Figure 1. TGA (10 °C/min) curves of furan-adduct initiator **1** (solid) and dimethylfulvene-adduct initiator **3** (dotted).

and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) hydrochloride (211 mg, 1.10 mmol) was added. Last, 4-(dimethylamino)pyridine (DMAP) (12.2 mg, 0.1 mmol) was added, and the reaction was allowed to proceed for 1.5 h at 0 °C. The reaction was removed from the ice bath and allowed to stir for an additional 6.5 h at 23 °C before removal of the solvent under vacuum. Purification of the residue by column chromatography (5:1 methylene chloride:ethyl acetate) yielded **1** as a white, crystalline solid in 66% yield (267 mg). ^{1}H NMR ($CDCl_3$): δ 7.96 (d, J = 8.2, 2H, aromatic H ortho to ester), 7.49 (d, J = 8.2, 2H, aromatic H ortho to 1-bromoethyl), 6.50 (s, 2H, CH═CH), 5.25 (s, 2H, CHOCH), 5.19 (q, J = 6.9, 1H, CHBr), 4.45 (t, J = 5.4, 2H, CH_2-OOC), 3.90 (t, J = 5.2, 2H, CH_2N), 2.87 (s, 2H, CHCONCOCH), 2.04 (d, J = 7.0, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 176.18, 165.85, 148.26, 136.73, 130.31, 129.73, 127.13, 81.10, 61.53, 48.12, 47.59, 37.99, 26.67. IR: 2974, 1774, 1713, 1686, 1608, 1575, 1465, 1428, 1400, 1357, 1337, 1309, 1266, 1185, 1152, 1132, 1112, 1045, 1022, 987, 962, 944, 915, 871, 856, 796, 774, 722, 702, 660 cm^{-1}. Mass spec (ESI HRMS): calcd for $C_{19}H_{18}BrNO_5Na$ $(M + Na)^+$ 442.0260; found 442.0222.

*4-(2-Hydroxyethyl)-10-isopropylidene-4-aza-tricyclo[5.2.1.0]dec-8-ene-3,5-dione (**2**).* *N*-(2-Hydroxyethyl)maleimide (124 mg, 0.88 mmol) was stirred in diethyl ether (2 mL) under argon. Dimethylfulvene (318 μL, 2.64 mmol) was added, and the mixture stirred for 48 h at 23 °C before concentrating under reduced pressure. The crude product was dissolved in minimal chloroform and precipitated into rapidly stirring cold hexanes, followed by centrifugation (2×). The pellet was dried under vacuum to yield a pale yellow solid (**2**) in 60% yield (130 mg). ^{1}H NMR ($CDCl_3$): δ 6.26 (s, 2H, CH═CH), 3.83 (s, 2H, CHCCH), 3.66 (m, 2H, CH_2O), 3.59 (t, J = 5.1, 2H, NCH_2), 3.25 (s, 2H, CHCONCOCH), 2.20 (t, J = 6.0, 1H, OH), 1.60 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 178.03, 146.97, 134.91, 111.76, 61.18, 45.40, 44.71, 41.60, 19.72. IR: 3491, 3359, 2928, 2870, 1757, 1692, 1675, 1419, 1395, 1372, 1356, 1318, 1252, 1228, 1166, 1093, 1071, 1060, 993, 944, 880, 872, 852, 837, 798, 753, 743, 686 cm^{-1}. Mass spec (ESI HRMS): calcd for $C_{14}H_{18}NO_3$ $(M + H)^+$ 248.1281; found 248.2357.

*4-(1-Bromoethyl)benzoic Acid 2-(10-Isopropylidene-3,5-dioxo-4-azatricyclo[5.2.1.0]dec-8-en-4-yl)ethyl Ester (**3**).* **2** (123.8 mg, 0.50 mmol) was stirred in methylene chloride (5 mL), and 4-(1-bromoethyl)benzoic acid (83.5 mg, 0.5 mmol) was added. The mixture was cooled to 0 °C in an ice bath, and EDC was added. Last, DMAP (6.1 mg, 0.05 mmol) was added, and the reaction was allowed to proceed for 1.5 h at 0 °C. The reaction was removed from the ice bath and allowed to stir for an additional 6.5 h at 23 °C before concentrating and purifying by column chromatography (5:1 methylene chloride:ethyl acetate). The product **3** was obtained as a white, crystalline solid in 74% yield (169 mg). ^{1}H NMR ($CDCl_3$): δ 7.97 (d, J = 8.4, 2H, aromatic H ortho to ester), 7.51 (d, J = 8.3, 2H, aromatic H ortho to 1-bromoethyl), 6.12 (s, 2H, CH═CH), 5.20 (q, J = 6.8, 1H, CHBr), 4.34 (t, J = 5.2, 2H, CH_2OOC), 3.80−3.78 (m, 4H, CHCCH and NCH_2), 3.22 (s, 2H, CHCONCOCH), 2.05 (d, J = 2.8, 3H, $CBrCH_3$), 1.55 (s, 6H, bridgehead CH_3). ^{13}C NMR ($CDCl_3$): δ 177.04, 165.82, 148.29, 147.06, 134.83, 130.32, 129.72, 127.17, 111.61, 62.06, 48.09, 45.45, 44.72, 37.55, 26.69, 19.67. IR: 2910, 1771, 1716, 1695, 1611, 1419, 1393, 1331, 1272, 1181, 1100, 1042, 1018, 988, 858, 838, 797, 772, 742, 703, 682 cm^{-1}. Mass spec (ESI HRMS): calcd for $C_{23}H_{24}$-$BrNO_4Na$ $(M + Na)^+$ 480.0779; found 480.0810.

*Synthesis of Polystyrene from **1**.* Styrene (0.228 mL, 1.98 mmol), copper(I) bromide (5.7 mg, 0.04 mmol), anisole (23 μL, 10 vol %) as an internal standard, and **1** (16.6 mg, 0.04 mmol) were charged into a Schlenk tube and subjected to five freeze−pump−thaw cycles. *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA) (8.5 μL, 0.04 mmol) was degassed for 1 h and added to the Schlenk tube, and the mixture allowed to stir for 5 min, after which the tube was placed in an 70 °C oil bath. After 12 h air was bubbled through the reaction to oxidize and deactivate the catalyst. Molecular weight and polydispersity index (PDI) were determined by GPC.

*Kinetic Procedure for the ATRP of Styrene from **3**.* Styrene (258 μL, 2.25 mmol), copper(I) bromide (6.5 mg, 0.045 mmol), anisole as an internal standard (26 μL, 10 vol %), and **3** (20.6 mg, 0.045 mmol) were charged into a Schlenk tube and subjected to five freeze−pump−thaw cycles. PMDETA (9.5 μL, 0.045 mmol) was degassed for 1 h and added to the Schlenk tube. The mixture was stirred for 5 min, after which the tube was placed in an 80 °C oil bath. Kinetic samples were removed from the Schlenk tube and diluted with $CDCl_3$ to calculate conversion by ^{1}H NMR spectroscopy. The integration of the alkene proton of styrene at 5.34 ppm was compared to the integration of the peak of the methyl protons of anisole at 3.87 ppm before the polymerization was started to give a base value. The ratio of the two peaks was taken at subsequent time points and was compared to this base value to calculate conversion. For each sample, the solvent was removed and the residue dissolved in THF for GPC determination of molecular weight and PDI.

*Synthesis of Polystyrene **4**.* Styrene (1 mL, 9 mmol), copper(I) bromide (6.5 mg, 0.045 mmol), copper(II) bromide (0.5 mg, 0.0023 mmol), anisole (100 μL, 10% vol) as an internal standard, and **3** (20.6 mg, 0.045 mmol) were charged into a Schlenk tube and subjected to five freeze−pump−thaw cycles. PMDETA (10 μL, 0.0475 mmol) was degassed for 1 h and added to the Schlenk tube. The mixture was stirred for 5 min, after which the tube was placed in an 80 °C oil bath. After 1 h the reaction was purposely stopped at low conversion (∼15%) by bubbling air through the solution to oxidize and deactivate the catalyst. Molecular weight and PDI were determined by GPC. The polystyrene was then purified by precipitation into cold MeOH (3×) followed by freeze-drying from benzene. M_n was also calculated by ^{1}H NMR by comparison of the integrations of the peaks of the polymer side chain at 6.9−6.3 ppm (2H) and end group at 6.12 ppm (2H, alkene). ^{1}H NMR ($CDCl_3$): δ end group peaks: 7.80 (2H, aromatic H ortho to ester), 6.12 (2H, CH═CH), 4.55−4.46 (1H, CHBr of ω chain end), 4.32 (2H, CH_2OOC), 3.80 (4H, CHCCH and NCH_2), 3.22 (2H, CHCONCOCH). Polymer peaks: 7.32−6.30 (aromatic H), 2.56−0.77 (CH_2CH backbone).

*ATR Coupling of Polystyrene (**5**).* Polystyrene **4** (100 mg, 0.04 mmol) and nano-copper(0) (10.2 mg, 0.16 mmol) were subjected to five vacuum−argon−refill cycles in a two-neck round-bottom flask equipped with a septum. In a separate Schlenk flask, copper(I) bromide (5.7 mg, 0.04 mmol) and toluene (500 μL) were subjected to five freeze−pump−thaw cycles. PMDETA (8.4 μL, 0.04 mmol) was degassed for 1 h and added to the Schlenk flask, and the mixture was allowed to stir for 5 min. This catalyst complex was then transferred to the round-bottom flask under argon. The flask was inserted into a 70 °C oil bath, and the reaction was allowed to stir for 5 h. Polymer **5** was purified by precipitation into cold methanol (3×) followed by freeze-drying from benzene. The molecular weight and PDI were determined by GPC. M_n was also calculated by ^{1}H NMR by comparison of the peak integrations of the polymer side chain at 6.9−6.3 ppm (2H) and end group at 4.32 ppm (2H).

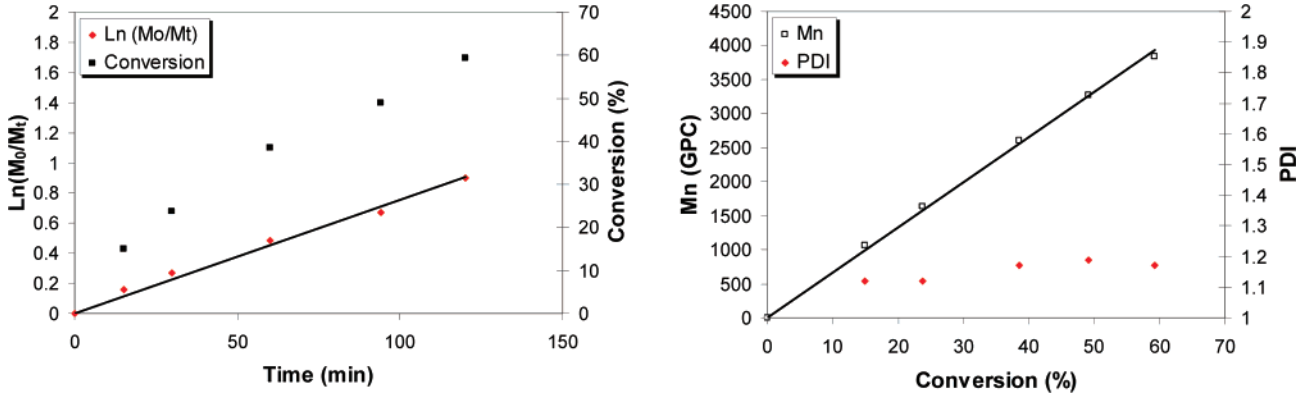


Figure 2. ATRP of styrene using **3**. Kinetic plot (left) determined from ^{1}H NMR spectra and evolution of molecular weight vs conversion plot (right) determined by GPC in THF. Reaction conditions: styrene:**3**:CuBr:PMDETA = 50:1:1:1, 80 °C.

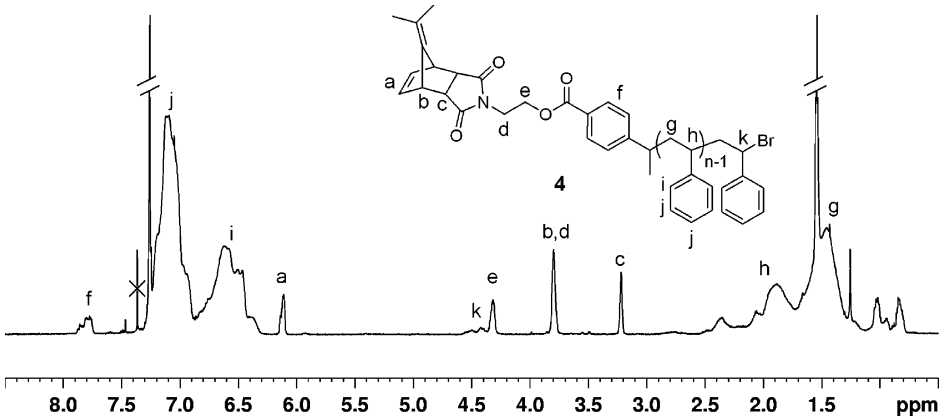


Figure 3. ^{1}H NMR spectrum ($CDCl_3$) of **4**.

*In-Situ Deprotection of **5** and Thioether Formation with Benzyl Mercaptan (**6**).* Polystyrene **5** (5 mg, 0.001 mmol) and benzyl mercaptan (50 uL, 0.43 mmol) were stirred in refluxing toluene (1 mL) under argon for 24 h. The solvent was removed under reduced pressure, and the resulting solid was purified by precipitation into cold methanol (3×) followed by freeze-drying from benzene. M_n was calculated by ^{1}H NMR by comparison of the peak integrations of the polymer side chain at 6.9−6.3 ppm (2H) and end group at 4.46 ppm (2H).

*In-Situ Deprotection of Functionalized Polystyrene and Thioether Formation with N-Acetyl-L-cysteine Methyl Ester (**7**).* Polystyrene **5** (17.8 mg, 0.003 mmol) and *N*-acetyl-L-cysteine methyl ester (10.6 mg, 0.06 mmol) were stirred in refluxing toluene (1 mL) under argon for 12 h. The solvent was removed under reduced pressure, and the resulting solid was purified by precipitation into cold methanol (3×) followed by freeze-drying from benzene. M_n was calculated by ^{1}H NMR by comparison of the peak integrations of the polymer side chain at 6.9−6.3 ppm (2H) and end group at 4.46 ppm (2H). Elemental analysis (EA): calcd for sulfur content 1.29%; found 1.28%.

Results and Discussion

Initiator. Maleimides are an excellent functional group for bioconjugate formation due to their high selectivity and reactivity toward cysteine residues at pH 6−7.[30] However, maleimides are also excellent comonomers with styrene during radical polymerization, with reactivity ratios resulting in alternating copolymers.[31] Thus, a protected maleimide initiator was designed to shield the moiety during polymerization and prevent side reactions. It was expected that after the polymerization a thermal retro-Diels−Alder deprotection would readily expose the maleimide group for bioconjugate formation.

Initially, the oxo-bridged norbornene initiator **1** was pursued due to the ease with which this moiety is deprotected.[17c] The compound was synthesized in 66% yield by EDC-mediated esterification of 4-(1-bromoethyl)benzoic acid with 4-hydroxymethyl-10-oxa-4-azatricyclo[5.2.1.0]dec-8-ene-exo-3,5-dione (Scheme 2). Polymerization of styrene was attempted with initiator **1** using a CuBr/PMDETA (1:1) catalyst system at a lower than typical temperature (70 °C) to prevent the retro-Diels−Alder reaction from proceeding. However, the resulting polymer displayed a broad, multimodal GPC trace (Figure 4a). This indicated that even at this temperature undesired deprotection and subsequent copolymerization of the unprotected maleimide with styrene occurred.

The literature was surveyed in order to find a cycloadduct that would undergo the retro-Diels−Alder reaction at a high enough temperature to withstand the polymerization, yet at a low enough temperature to be easily deprotected.[32] To meet these criteria, a new initiator with a dimethylfulvene protecting group was designed and synthesized (Scheme 3). First, *N*-(2-hydroxyethyl)maleimide was prepared and stirred in the presence of dimethylfulvene to obtain **2** in 60% yield. Initiator **3** was synthesized by EDC-mediated esterification of 4-(1-bromoethyl)benzoic acid with **2** in 74% yield.

Solid-state TGA was performed on the initiators to analyze and compare the thermostabilities of **1** and **3** (Figure 1). Deprotection of **1** was found to begin at a temperature of 125 °C in the solid state. The mass loss was measured to be 16% for the initial decomposition step. This corresponded to loss of the furan subunit. Deprotection of **3** was found to begin at 143 °C, and the mass loss was measured as 23% for the initial

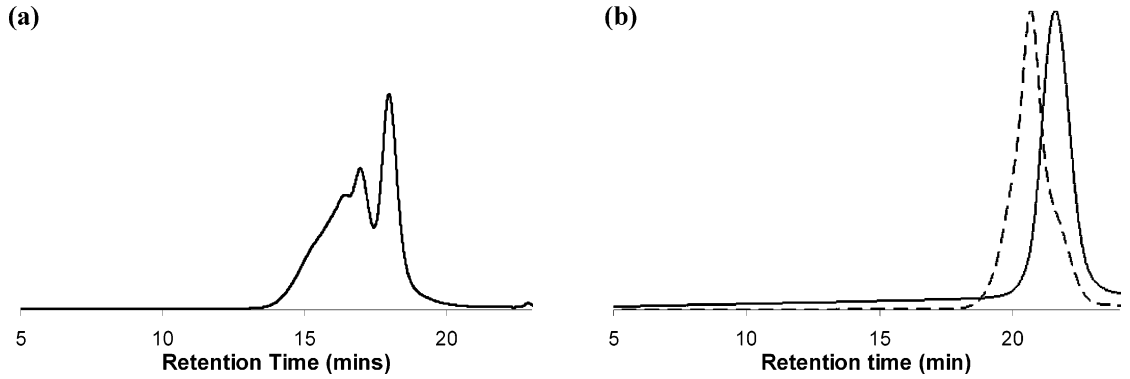
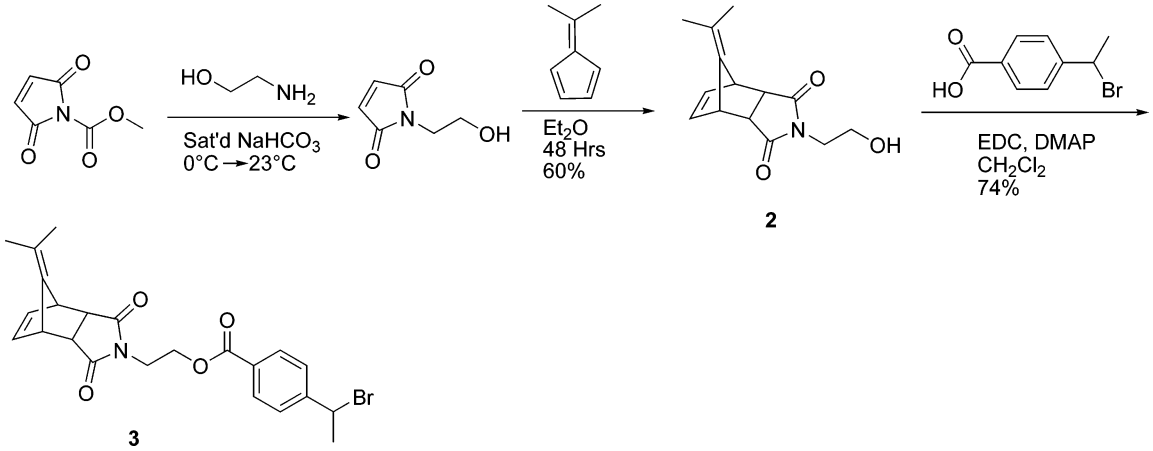


Figure 4. (a) GPC trace of polystyrene synthesized by **1**. (b) GPC traces of polystyrene synthesized by **3** before (solid) and after (dashed) ATR coupling.

Scheme 3. Synthesis of Dimethylfulvene-Protected ATRP Initiator



decomposition step, the loss of a dimethylfulvene subunit. Thus, the dimethylfulvene adduct was more stable to retro-Diels−Alder than the furan adduct. It was anticipated that this increased stability would allow us to effectively polymerize styrene using **3**.

Polymer Synthesis. In order to investigate **3** as an initiator for styrene, kinetic studies were undertaken. ATRP of neat styrene was attempted with initiator **3** using a CuBr/PMDETA (1:1) catalyst system at 80 °C with 10 vol % anisole as an internal standard. Progression of the polymerization was monitored by ^{1}H NMR spectroscopy and GPC. The polymerization proceeded in a controlled manner to ∼60% conversion as indicated by the linear pseudo-first-order logarithmic plot (Figure 2). In addition, the molecular weight was found to increase linearly with conversion, and the molecular weight distribution remained narrow throughout the polymerization with a final PDI of 1.17. The M_n obtained was 3840 Da while the expected was 3530 Da. Thus, the initiator efficiency was 92%, indicating that **3** was an effective initiator for the ATRP of styrene.

We next sought to create a bis-end-functionalized polystyrene for chemoselective reaction with thiols. The method of ATR coupling was chosen as a convenient route to provide the desired product. First, the mono-end-functionalized polymer was prepared as above with the addition of 5% $CuBr_2$ (Scheme 4). It is known from literature that adding copper(II) results in better retention of the halogen chain end during the ATRP of styrene compared to polymerization with copper(I) alone.[22,33] Indeed, low ATR coupling efficiencies were observed when the polymer was prepared without $CuBr_2$ (data not shown). The M_n of the polymer synthesized for ATR coupling was 2310 Da, and the PDI was low (1.15). The polymer was isolated after repeated precipitation, and the molecular weight was estimated by ^{1}H NMR spectroscopy. The M_n (2470 Da) was close to the value determined by GPC, indicating that the end group was intact. Importantly, the presence of the brominated chain end was evident from the presence of the peak at ∼4.55 ppm (Figure 3).

Dimerization via ATR coupling proceeded using a CuBr/PMDETA (1:1) catalyst system with 4 equiv of nano-Cu(0) at 70 °C in toluene. There was a clear shift to lower retention time of the GPC trace, indicating the majority of the polymer chains had coupled (Figure 4b). The molecular weight of the dimeric polymer was found to be 4200 Da, and the PDI was broadened to 1.32. This broadening was a result of a low molecular weight shoulder which indicated that some of the chains had not reacted. This could have been due to lack of the halogen end group for these chains or from disproportionation of some of the chains. ^{1}H NMR spectroscopy indicated that the Diels−Alder protected chain end was intact. Peaks were observed at 6.12, 3.80, and 3.22 ppm corresponding to the dimethylfulvene cycloadduct (Figure 5). Signals at 7.77, 4.32, and 3.80 for the phenyl protons, ester, and amide methylene hydrogens, respectively, of the initiator were also observed. All peaks integrated correctly with respect to one another. The absense of a peak at ∼4.55 ppm corresponding to the CHBr group of the ω chain end indicated that the terminal bromine was no longer present and had reacted. The M_n was estimated by comparison of the polymer and end-group peak integrations to be 5040 Da, essentially double the molecular weight of the monomeric polystyrene (2470 Da). This also indicated that the end group was unaffected by the coupling reaction. Taken together, these results demonstrated that bis-end-functionalized

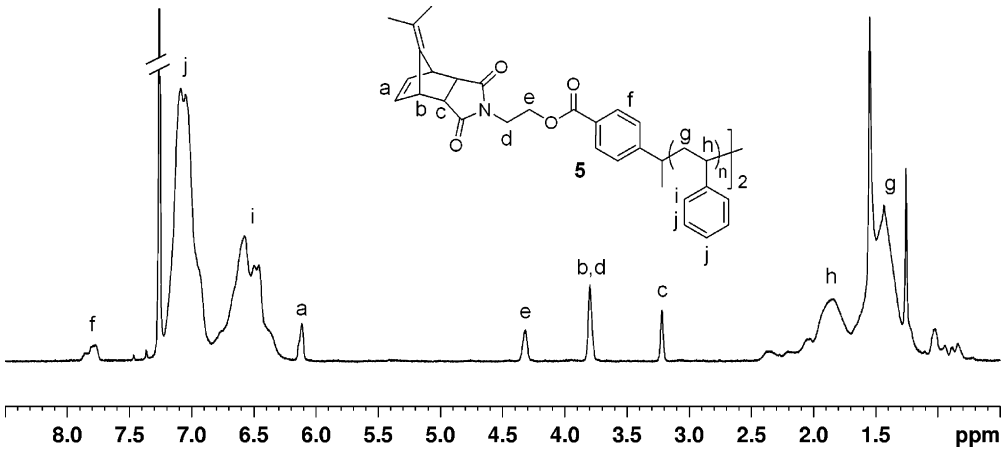
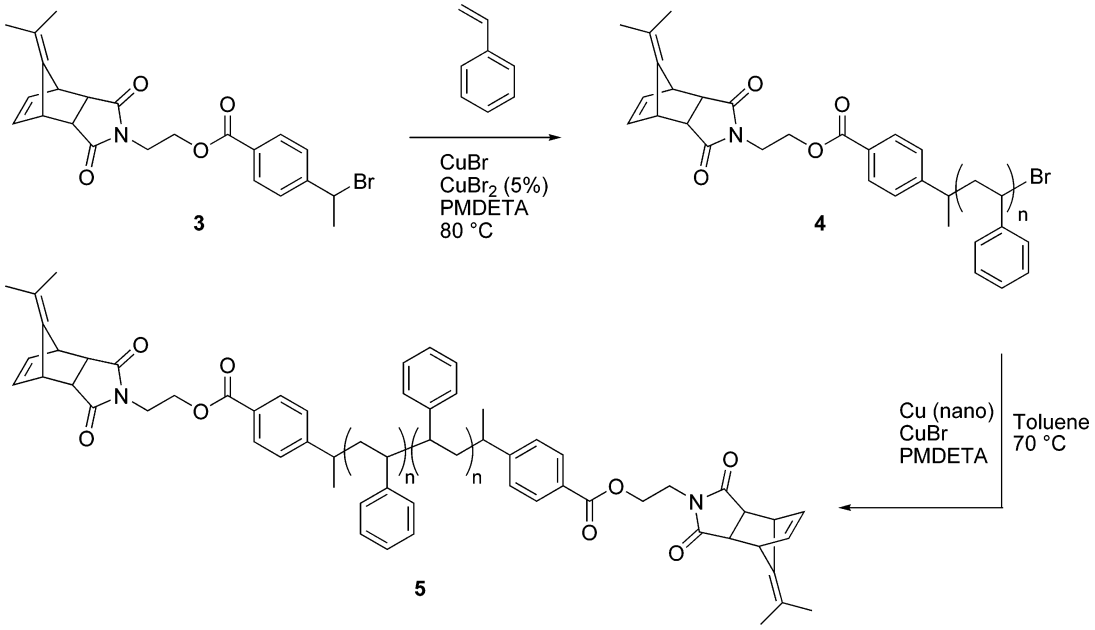


Figure 5. ^{1}H NMR spectrum ($CDCl_3$) of **5**.

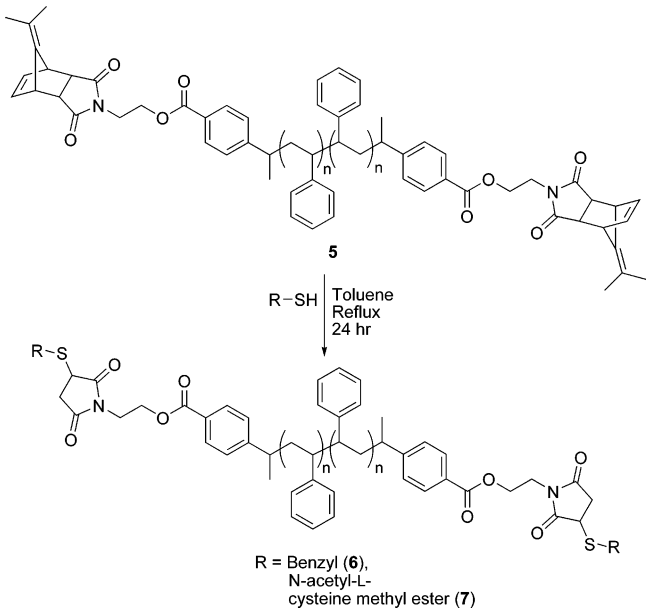
Scheme 4. Synthesis of Telechelic Polystyrene 5



protected maleimide polystyrene **5** had been successfully synthesized.

Reactivity. Modification of the polystyrene was next investigated. Deprotection of the maleimide chain ends of **5** and the thioether bond formation were performed in situ by heating the polymer in refluxing toluene in the presence of benzyl mercaptan as a model system (Scheme 5). The resulting polymer (**6**) was purified by repeated precipitation into methanol and freeze-drying from benzene. ^{1}H NMR analysis of the purified polymer confirmed the loss of dimethylfulvene by the disappearance of the signals at 6.12, 3.80, and 3.22 ppm (Figure 6). Modification with benzyl mercaptan was also apparent due to the appearance of signals at 4.15 and 3.75 ppm corresponding to the benzyl methylene protons and signals at 3.50, 2.95, and 2.45 ppm corresponding to the maleimide protons after thioether formation. It was also important to note the presence of the peaks corresponding to the end-group aromatic protons at 7.75 ppm and the ester and amide methylene proton signals at 4.45 and 3.95 ppm, respectively. Integration of the end group and comparison to the side chains indicated complete conjugation of benzyl mercaptan to the polymer. The M_n calculated by ^{1}H NMR was 4890 Da, essentially the same as before reaction (5040 Da).

Scheme 5. In-Situ Deprotection and Conjugation of Thiols to 5



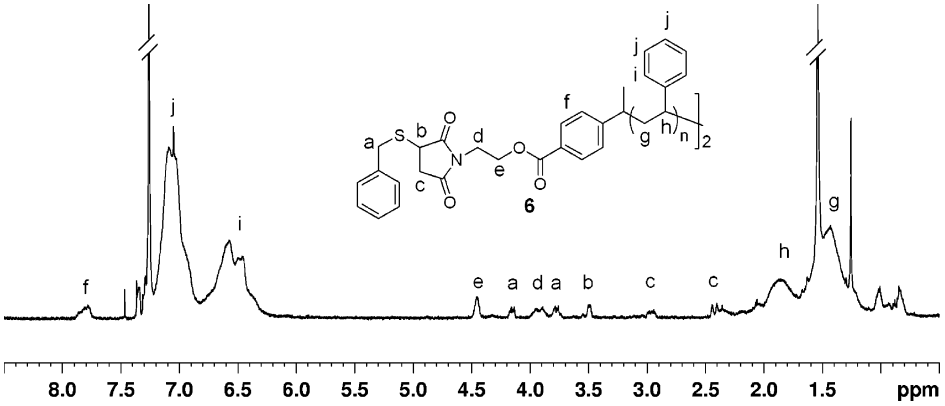


Figure 6. ^{1}H NMR spectrum ($CDCl_3$) of **6**.

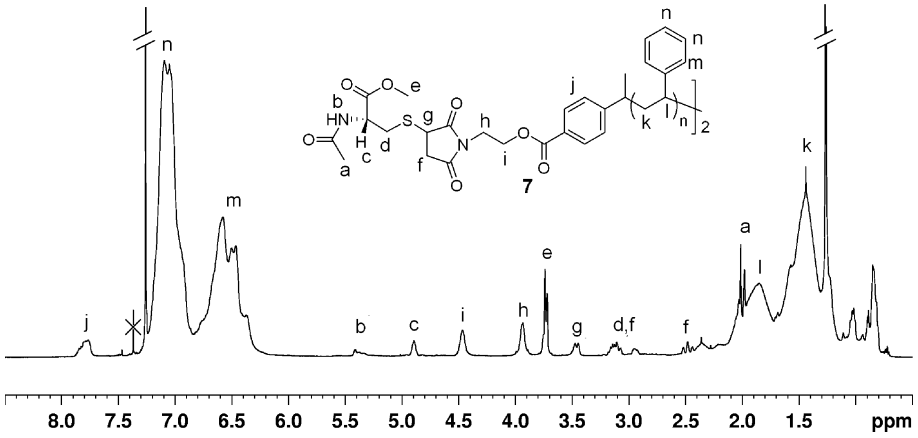


Figure 7. ^{1}H NMR spectrum ($CDCl_3$) of **7**.

With these promising results, modification with the amino acid cysteine was pursued. Polymer **5** was again subjected to in-situ deprotection and conjugation conditions, this time in the presence of *N*-acetyl-L-cysteine methyl ester. The thiol moiety was expected to undergo Michael addition with the free maleimide following deprotection. The nonconjugated *N*-acetyl-L-cysteine methyl ester was removed by precipitation of the polymer into methanol and freeze-drying from benzene. Once again, conjugation was confirmed by ^{1}H NMR spectrum. The loss of the dimethylfulvene signals and appearance of the signals at 5.40, 4.89, 3.73, 3.12, and 2.01 ppm indicated the presence of the *N*-acetyl-L-cysteine methyl ester (Figure 7). Integration of the peaks indicated complete conjugation of the amino acid to the chain end; the calculated M_n of the polymer was unchanged (4960 Da). Elemental analysis was also performed to quantify the amount of sulfur in the sample. Polymer **7** was found to contain 1.28% sulfur, while the expected value was calculated to be 1.29%. This data provided further evidence that complete conjugation of the amino acid to the polymer chain ends had occurred.

These results together demonstrated that a thiol-reactive telechelic polystyrene was readily synthesized using a combination of ATRP and ATR coupling. Maleimides are excellent comonomers for styrene in radical polymerizations and must be protected. Effective polymerization of styrene requires elevated temperatures, and thus the chosen protecting group must be able to withstand the reaction temperatures. We found that a dimethylfulvene protecting group withstood the polymerization temperatures and yet was easily removed when desired. A dimethylfulvene and maleimide cycloadduct initiator was found to be efficient for the ATRP of styrene utilizing a Cu/PMDETA catalyst system. To produce polymers with a halogen chain end available for ATR coupling, temperatures of 80 °C were used and 5% copper(II) was added. These conditions had been reported to maximize retention of the halogen, which was critical for the subsequent step.[22,33] With this polymer, ATR coupling with nano-copper(0) using conditions reported by Matyjaszewski et al. produced the dimeric polymer.[22] GPC analysis demonstrated that most of the chains had undergone dimerization. It was possible that a small portion of the chains underwent disproportionation or were not halogenated. Both of these would result in less than quantitative coupling. NMR analysis demonstrated that the end group was unaffected by the coupling reaction and that the desired telechelic polymer had been synthesized. In order to illustrate reactivity, an in-situ deprotection and Michael addition was performed utilizing the model compound benzyl mercaptan. Conjugation with the amino acid *N*-acetyl-L-cysteine methyl ester was then demonstrated. ^{1}H NMR analysis indicated that both reactions were efficient and the conjugation was complete. For example, the molecular weight estimated from the spectra did not change before and after conjugation. These results demonstrated that cysteine-reactive polystyrene was easily synthesized by a combination of ATRP and coupling to form biohybrid materials.

Conclusions

In this report we have demonstrated a simple approach to the synthesis of mono- and bis-functionalized thiol-reactive polystyrene. A novel Diels−Alder protected maleimide-functionalized ATRP initiator was synthesized and successfully used in a combination of ATRP and ATR coupling to form the end-functionalized polymers. Conjugation of cysteine was efficient,

suggesting that this strategy will be effective to prepare biohybrids from biomolecules with thiol functionality. Because free cysteines are rare, this approach may be utilized to synthesize site-specific amphiphilic conjugates with unique triblock structures. Such materials may provide unprecedented solution and solid-phase structures. Thus, we anticipate that the synthesis described here will be a convenient route to cysteine-reactive polystyrene for a variety of applications in bio- and nanotechnology.

Acknowledgment. This work was financially supported by the NSF (CHE-0416359) and an Alfred P. Sloan Research Fellowship (H.D.M.). Z.P.T. thanks the NIH-sponsored Biotechnology Training in Biomedical Sciences and Engineering Program for funding. The authors thank Dr. Amir Liba for performing elemental analysis.

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MA702161Q