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Aminooxy and Pyridyl Disulfide Telechelic Poly(Polyethylene Glycol Acrylate) by RAFT Polymerization

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Abstract

An efficient method to synthesize telechelic, bio-reactive polymers is described. Homotelechelic polymers were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization in one step by employing bifunctional chain transfer agents (CTAs). A bis-carboxylic acid CTA was coupled to *N*-BOC-aminooxy ethanol or pyridyl disulfide ethanol resulting in a bis-*N*-BOC-aminooxy CTA and a bis-pyridyl disulfide CTA, respectively. RAFT polymerization of polyethylene glycol (PEG) acrylate in the presence of both CTAs resulted in a series of polymers over a range of molecular weights (~8.4 kDa to 35.2 kDa; polydispersity indices, PDIs of 1.11 to 1.44) with retention of end-groups post-polymerization. The polymers were characterized by ¹H NMR spectroscopy and gel permeation chromatography (GPC). Conjugations of small molecules and peptides resulted in homotelechelic polymer conjugates.

Introduction

The synthesis of materials containing bioreactive and bioorthogonal functional groups is important to researchers in the fields of biotechnology, nanotechnology, and drug delivery.¹ These types of materials facilitate the attachment of therapeutics, even those containing a wide variety of functional groups. This chemospecificity is essential when conjugating biological cargo such as proteins and peptides to polymers.^{2,3} Oxime bond formation is an attractive approach to generate biofunctionalized materials and surfaces.⁴⁻⁷ The oxime bond exhibits improved hydrolytic stability compared to hydrazones.⁸ The reaction can be performed in aqueous media and is catalyzed by aniline derivatives.⁹ Chemospecificity and mild reaction conditions have been demonstrated by labeling the surface glycans of live cells,¹⁰ as well as zebrafish.¹¹ In this report the synthesis of homotelechelic aminooxy polymers and subsequent conjugations to the chain ends via oxime chemistries are described. For comparison, we also describe synthesis of analogous homotelechelic pyridyl disulfide polymers that react with thiols in a highly selective manner.

Reactive, telechelic polymers allow for conjugation of drugs and biomolecules to polymer scaffolds.^{3,12} These types of materials provide access to functionalized nanocarriers for targeted drug delivery.¹³ Conjugation of biological cues to surfaces of highly ordered films and nanowires provides biofunctionalization and passivation for sensing applications.¹⁴⁻¹⁶ Telechelic polymers are important materials to form extended interpenetrating networks or act as crosslinkers for hydrogel formation.¹⁷⁻¹⁹ Additionally, dimeric polymer-conjugates can be synthesized from these linear telechelic polymers.^{2,3,12,20,21} Homodimeric biomolecule-conjugates promise to have higher affinity for the desired end-target as a result of multivalency.²⁰ All of these applications are accessed by precise control over the length and the properties of the polymer, in addition to the ability to effectively synthesize a telechelic polymer and efficiently conjugate to the termini. A variety of polymerization techniques have been utilized to synthesize these materials. These techniques include anionic polymerization,²² cationic polymerization,²³ ring opening polymerization (ROP),²⁴

ring opening metathesis polymerization (ROMP),²⁵ and controlled radical polymerization (CRP).^{2,3,12}

CRP techniques such as reversible addition-fragmentation chain transfer (RAFT) polymerization, atom transfer radical polymerization (ATRP), and nitroxide mediated polymerization (NMP) are established methods to prepare well-defined telechelic polymers.¹² Telechelics can be synthesized by ATRP, but a postpolymerization reaction is required. For example, we synthesized polymers with either aminooxy or maleimide groups at both ends by preparing semi-telechelic polystyrene and then dimerizing utilizing atom transfer radical coupling.^{26,27} Bifunctional RAFT CTAs or NMP initiators allow for the direct synthesis of telechelic polymers.¹² If both functional groups on the CTA or initiator are the same then homotelechelic polymers can be synthesized in one step. For example, Hawker and coworkers have synthesized α -alcohol, ω -alcohol poly(styrene) (pSt) by polymerizing in the presence of bifunctional, alcohol-NMP initiator.²⁸ Matyjaszewski and coworkers demonstrated the synthesis of α -carboxylic acid, ω -carboxylic acid pSt and pSt-block-poly(butylacrylate) by NMP.²⁹ Bifunctional CTAs have also been extensively used to synthesize homotelechelic polymers that have carboxylic acid, alcohol, alkyne, alkene, pyridyl disulfide, phthalamide, naphthalene, anthracene, terpyridine, and hydrogen-bonding functionalized termini.¹²

Herein, we describe the synthesis of a bifunctional CTA containing aminooxy-functional groups. The CTA was synthesized by esterification of a bis-carboxylic acid CTA with *N*-BOC-aminooxy alcohol. RAFT polymerization of PEG-acrylate in the presence of this CTA resulted in a series of well-defined, telechelic aminooxy poly(poly(ethylene glycol) acrylate)s (pPEGAs). The high retention of the aminooxy group postpolymerization facilitated mild, site-selective conjugation of organic molecules and peptides to the polymer termini. For comparison, telechelic, pyridyl disulfide polymers were also synthesized from an analogous bis-pyridyl disulfide CTA. Conjugation to a thiol containing small molecule and peptide resulted in dimeric polymer conjugates containing reversible disulfide bonds.

Results and Discussion

Bis-acid CTAs and derivatives have been successfully utilized to prepare telechelic polymers.^{12,30} Thus, we synthesized a bioreactive, bis-aminooxy functionalized CTA through carbodiimide coupling of the functional alcohol to 2,2'-(thiocarbonylbis(sulfanediyl))dipropanoic acid **1**.³¹ **CTA1** was prepared in 41 % yield by *N,N'*-dicyclohexylcarbodiimide (DCC)/4-(dimethylamino)pyridine (DMAP) coupling between 2-(*N*-BOC-aminooxy) ethanol³² (**2**) and **1** (Scheme 1). The synthesis of a symmetric, bis-pyridyl disulfide CTA to prepare poly(methacrylates) has been demonstrated by a handful of groups.^{12,30} For example, Liu et al. have used a bis-pyridyl disulfide CTA to generate protein-polymer conjugates *in situ* by first attachment of the CTA to bovine serum albumin and polymerization from the protein-CTA.³⁰ The same group also used this CTA to prepare triblock copolymers, which in turn were utilized to form peptide-decorated micelles.³³ Since the CTA utilized in both cases was different in structure than **1**, we also pursued the synthesis of a pyridyl disulfide bis-functional CTA (**CTA2**). This allowed us to prepare the comparable pyridyl disulfide p(PEGA)s to the aminooxy end functionalized polymers. This was accomplished by DCC/DMAP coupling of **1** to 2-pyridyl disulfide ethanol **3** (Scheme 2) in 92% yield.³⁴

RAFT polymerization of poly(ethylene glycol) acrylate (number average molecular weight (M_n) = 454, PEGA₄₅₄) in the presence of **CTA1** was performed using a [CTA] : [PEGA₄₅₄] : [azobisisobutyronitrile] (AIBN) ratio of 1:30:0.15 at 60 °C and stopped at 68% conversion to yield **poly1a** (Scheme 3). ¹H NMR indicated a molecular weight of 13,100 g/

mol (targeted $M_n = 9,830$ g/mol) (see supporting information, Figure S1) for **poly1a** by calibrating the carbamate (ONH) protons of the end-group to 2.0 (protons **a**) and comparing to the integration of the methylene protons (protons **c**) alpha to the ester units of the polymer. Gel permeation chromatography (GPC) against poly(methylmethacrylate) (pMMA) standards gave a M_n of 18,100 g/mol and a PDI of 1.16 (see supporting information, Figure S3). The molecular weight evolution over the course of the polymerization was linear and the PDI was low indicative of a controlled polymerization (Figure 1). A series of different polymerization conditions of PEGA₄₅₄ with **CTA1** were screened and the results of these experiments (summarized in Table 1, Figure S2, and Figure S3) demonstrate the ability to target different molecular weights and obtain well-defined polymers with **CTA1** at low molecular weight. However, when targeting higher molecular weight with this monomer/CTA system ($M_n = 29,300$) an increase in PDI was observed. This result is consistent with our previous results polymerizing PEGA₄₅₄ by RAFT polymerization.³⁵

An important trait of **poly1** is that it contains a trithiocarbonate in the polymer backbone. To investigate if the trithiocarbonate was in the middle of the polymer, we pursued radical cleavage following a published protocol using two equivalents of lauroyl peroxide (LPO) and 20 equiv of AIBN to **poly1c** in an 80 °C oil bath.³⁶ After the reaction the M_n by GPC of **poly1c** decreased from 15,100 g/mol (PDI = 1.28) to 9,500 g/mol (PDI = 1.30) (see supporting information, Figure S4) based on GPC analysis against pMMA standards. The M_n is not half of original value as expected; however, the PDI remained similar after radical cleavage. The PDI results indicate that the chain growth occurs equally from both R groups, and the M_n discrepancy may be a result of using pMMA standards. However, the results do not completely confirm that the polymer is symmetrical.

RAFT polymerization of PEGA₄₅₄ in the presence of **CTA2** was performed at 60 °C using a [CTA] : [PEGA₄₅₄] : [AIBN] ratio of 1:30:0.3. The reaction was taken to 63% conversion to give **poly2b** (Scheme 4). ¹H NMR indicated a molecular weight of 11,200 g/mol (targeted $M_n = 9,170$ g/mol) (see supporting information, Figure S5) and GPC against pMMA standards gave a M_n of 15,700 g/mol with a PDI of 1.18 (see supporting information, Figure S7). The M_n by ¹H NMR was calculated from the methylene protons alpha to the ester at 4.2 ppm (proton **f** in Figure S5) after calibrating proton **a** of the end group (8.5 ppm) to 2.0. Retention of the pyridyl disulfide groups by ¹H NMR was determined to be 97% from the end group aromatic protons **a-d** versus the protons of the methylene adjacent to the ester of the last monomer repeat unit **e** (Figure S5). Kinetic analysis gave a linear increase of molecular weight versus conversion while maintaining a low PDI indicating a controlled radical polymerization (Figure 2). A series of different [CTA] : [PEGA₄₅₄] : [AIBN] at 60 °C and 70 °C (for NMR and GPC results see supporting information, Figures S6 and S7) were then screened. The results in Table 1 demonstrated that **CTA2** is able to produce well-defined pPEGA₄₅₄ at a variety of different conditions. Similar to **CTA1** targeting higher molecular gave polymers with increased molecular weight distributions.

Conjugations of small molecules and a model peptide to the telechelic polymers were performed to demonstrate the versatility of this approach to synthesize bioreactive, telechelic polymers. Conjugations of 4-bromobenzaldehyde (BBA) and glutathione (GSH)-ketone³⁷ to a representative telechelic aminoxy pPEGA were pursued. The BOC group of **poly1c** was removed with 10 % trifluoroacetic acid (TFA) in acetonitrile for 1.5 h. The solvent was removed *in vacuo*, and BBA was conjugated to the polymer in 0.04 % TFA in acetonitrile for 24 h to give **poly1c-BBA**. BOC-deprotection was confirmed by ¹H NMR due to the disappearance of the ONH proton at 8.3 ppm (Figure 3). ¹H NMR for **poly1c-BBA** confirmed the presence of the oxime bond (protons **a** at 8.2 ppm, Figure 3), as well aromatic protons (protons **b**, Figure 3). Calibration of the ¹H NMR to the aromatic peaks

afforded a M_n of 23,300 g/mole with 91 % of the chains containing the ONCH protons (protons **a**, Figure 3). Comparison of **poly1c** to **poly1c-BBA** via GPC indicated that the polymer backbone was preserved (Figure S8) (i.e. the trithiocarbonate was still intact post-conjugation). Conjugation of a model peptide, GSH-ketone, was also pursued. BOC-deprotected **poly1c** was reacted with GSH-ketone in 5 % TFA in water for 12 h to give **poly1c-GSH**. ^1H NMR showed the disappearance of the carbamate protons and a new peak was present (protons **b**, Figure 3). These protons overlap with the protons that correspond to the monomer units that are directly adjacent to the trithiocarbonate (protons **a**, Figure 3). The other peaks attributed to the GSH are not apparent in acetonitrile and only appear in water (vide infra). The ^1H NMR spectrum was calibrated to 4.0 for these two overlapping peaks and the molecular weight was calculated to be 25,500 g/mole, which is similar to the starting value. However, GPC analysis of **poly1c-GSH** showed a slight broadening of molecular weight against pMMA standards (PDI of 1.38 compared to 1.28 of the original polymer (see supporting information, Figure S8). This result suggests some cleavage of the polymer chain due to conjugation of the GSH. In this case the thiol is capped, therefore the partial cleavage could be due to the nucleophilic attack of amines on the trithiocarbonate within the polymer. However, the conjugation was performed under acidic conditions (5 % TFA in water), which would cause the amines to be protonated. Another possible cause of polymer degradation was decomposition of trithiocarbonate during the purification by dialysis.³⁸

Conjugations of benzyl mercaptan (BM) and GSH to bis-pyridyl disulfide pPEGA were pursued under acidic conditions to yield **poly2a-BM** and **poly2a-GSH**; respectively. The conjugation efficiencies were assessed by ^1H NMR spectroscopy (Figure 4) and the resulting polymers were analyzed by GPC (Figure S9). High conjugation efficiencies were observed for both **poly2a-BM** and **poly2a-GSH**. The aromatic protons of the pyridyl disulfide group disappeared from the ^1H NMR and were replaced by the aromatic protons of benzyl mercaptan (7.35 ppm, protons **a**, Figure 4). Calibration of these new aromatic peaks to 10.0 gave a molecular weight of 8,400 g/mol which was the same as the original molecular weight of 8,400 g/mol. GPC analysis of **poly2a-BM** showed no low/high molecular weight shoulders and overlaid with **poly2a** (Figure S9). Taken together the ^1H NMR and the GPC indicated that the trithiocarbonate core remained intact post-conjugation to BM. GSH was then conjugated to **poly2a** under acidic conditions (phosphate buffer pH 6.0). The aromatic peaks for the pyridyl disulfide were not present in ^1H NMR of **poly2a-GSH**, and a new peak (protons **b**, Figure 4) was distinguishable from the polymer backbone. This new peak correlates with the GSH peptide. In deuterated water, other peaks corresponding to the GSH were visible (Figure S10) confirming conjugation. However, GPC analysis of **poly2a-GSH** (supporting information, Figure S9) again showed a broadening of molecular weight, indicating low molecular weight chains. These low molecular weight chains are likely due to degradation of the trithiocarbonate within the polymer. The degradation of the trithiocarbonate group could be result from nucleophilic attack by the amine or thiol of the GSH or during purification by dialysis in water.

Together the data indicate that the telechelic polymers can be synthesized efficiently in one step by bis-functionalized CTAs. Conjugations are generally efficient, yet the use of the GSH peptide did result in some cleavage of the polymer chains. The telechelic pyridyl disulfide system allowed for thiol-reactive polymers to be formed in which the end-groups are conjugated via disulfides and therefore may be reversible. The telechelic aminooxy polymers form bonds that are less reversible, since the equilibrium for the reaction between hydroxylamines and ketones greatly favors oxime formation. Since thiols are available in drugs, peptides, and proteins and oxo groups can be readily introduced into such, this research may be applicable to preparing telechelic polymers with a variety of functional groups at either end of the poly(PEGA) chain.

Conclusions

Herein was described the synthesis of poly(PEGA) polymers with aminooxy groups or pyridyl disulfide groups at the chain ends by RAFT polymerization in the presence of the corresponding bifunctional CTAs. The *N*-BOC-protected aminooxy CTA was synthesized in 41% yield and the pyridyl disulfide CTA in 92% yield. Various molecular weight polymers were prepared. The molecular weight distributions were narrow and broadened as the molecular weight increased. Representative small molecules and peptides were conjugated to the polymers post-polymerization with high efficiencies resulting functionalized telechelics either through oxime or disulfide bond formation.

Experimental section

Materials

Chemicals were purchased from Sigma-Aldrich and Fisher Scientific and were used as received. AIBN was recrystallized from ethanol prior to use.

Analytical techniques

NMR spectra were obtained on Bruker Avance 500 & 600 MHz DRX spectrometers. ¹H NMR spectra were acquired with a relaxation delay of 2 sec for small molecules and a relaxation delay of 30 sec for all polymers. UV-Vis spectra were obtained on a Biomate 5 Thermo Spectronic UV-Vis spectrometer with quartz cells or a Thermo Scientific Nanodrop 2000. Mass spectra were obtained by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) spectrometry on an Applied Biosystems Voyager-DE STR and operated in linear mode with an external calibration. Infrared absorption spectra were recorded using a PerkinElmer FT-IR equipped with an ATR accessory. TLC plates were pre-coated with silica gel 60 F254 and were developed in the indicated solvent systems. Merck 60 (230-400 Mesh) silica gel was used for column chromatography. GPC was conducted on a Shimadzu HPLC system equipped with a refractive index detector RID-10A, one Polymer Laboratories PLgel guard column, and two Polymer Laboratories PLgel 5 μ m mixed D columns. LiBr (0.1 M) in DMF at 40 °C was used as an eluent (flow rate: 0.80 mL/min). Calibration was performed using near-monodisperse pMMA standards from Polymer Laboratories.

Methods

Synthesis of 2,2'-(thiocarbonylbis(sulfanediyl))dipropanoic acid (1)—1 was synthesized following a literature procedure.³¹ In summary, KOH (6.60 g, 0.12 mol) was dissolved in 75 mL of water. Carbon disulfide (6.35 mL, 0.11 mol) was added and the color became light brown followed by drop wise addition of 2-bromopropionic acid (4.73 mL, 0.05 mol). After 72 h the solution was washed with methylene chloride (25 mL) five times to remove unreacted carbon disulfide. The aqueous layer was acidified to pH 5 with concentrated hydrochloric acid and then extracted with methylene chloride until the aqueous layer was no longer yellow. The organic layer was dried with magnesium sulfate, and the solvent removed *in vacuo* to give a yellow solid. The yellow solid was dissolved in a minimal amount of methylene chloride and precipitated in 1,2-dichloroethane 5 times followed by filtering to give the product as a yellow powder in 83 % yield. ¹H NMR (500 MHz in MeOD) δ : 13.24 (s, 2H), 4.69-4.62 (q, J = 8.00, 6.50 Hz, 2H), 1.55-1.49 (d, J = 6.50 Hz, 6H). ¹³C NMR (500 MHz in CDCl₃) δ : 221.96, 173.96, 49.71, 17.30.

Synthesis of tert-butyl 2-hydroxyethoxycarbamate (2)—2 was synthesized by modifying a literature procedure.³² *N*-BOC-hydroxylamine (3.26 g, 24.5 mmol) and 2-bromoethanol (0.69 mL, 9.79mmol) were dissolved in dry methylene chloride (30 mL).

DBU (1.49 g, 9.79 mmol) was then added drop wise and stirred for 72 h. Methylene chloride (20 mL) was then added, and the solution was washed with 3 × 40 mL water. The organic layer was dried with MgSO₄, filtered, and solvent was removed *in vacuo*. The crude product was purified *via* silica gel chromatography using a 2 : 1 = hexanes : EtOAc eluent. **2** was obtained in 77 % yield. ¹H NMR (500 MHz, CDCl₃) δ: 7.24 (s, 1H), 3.92-3.88 (m, 2H), 3.76-3.71 (m, 2H), 1.49 (s, 9H). ¹³C NMR (500 MHz, CDCl₃) δ: 158.66, 82.59, 78.16, 59.61, 28.28. IR: 3299, 2977, 2933, 1704, 1482, 1454, 1392, 1367, 1277, 1251, 1162, 1116, 1071, 1049, 1016, 904, 871, 838, 771 cm⁻¹. ESI-MS (+/- 1.0) observed (predicted): Na+ 200.09 (200.09).

Bis-N-BOC-aminoxy CTA1—1 (0.43 g, 1.69 mmol) was dissolved in methylene chloride (17 mL) in a flame dried flask with a stir bar and cooled in an ice bath. **2** (0.89 g, 5.02 mmol) was added and stirred in an ice bath for 10 min. DCC (0.83 g, 4.04 mmol) and DMAP (0.04 g, 0.33 mmol) were dissolved in dry methylene chloride (1 mL) and added slowly to the solution containing **1** and **2** while in the ice bath. After 2 h, the reaction flask was moved to 23 °C and stirred for 4 h. The resulting crude product was filtered and the solvent removed *in vacuo*. The crude product was purified by silica column chromatography with Hex : EtOAc = 4 : 1. The yellow spot was isolated as a yellow oil in 41 % yield. ¹H NMR (500MHz, in CDCl₃) δ : 7.61 (s, 1H), 7.56 (s, 2H), 4.74-4.67 (qd, J = 2.05, 7.45 Hz, 2H), 4.37-4.26 (m, 4H), 3.98-3.91 (m, 4H), 1.58-1.51 (d, J = 7.40 Hz, 6H), 1.40 (s, 18H). ¹³C NMR (500 MHz, in CDCl₃) δ: 219.31, 171.22, 157.01, 82.05, 73.59, 62.79, 48.09, 28.05, 16.48. IR: 3296, 2977, 2933, 2360, 1727, 1450, 1392, 1367, 1246, 1150, 1110, 1028, 916, 811, 773 cm⁻¹. MALDI-TOF MS (+/- 1.0) observed (predicted): Na+ 595.72 (595.72). UV-Vis (EtOAc): λ_{max} = 304 nm.

Representative RAFT polymerization of PEGA with CTA1 (poly1a)—CTA1 (10 mg, 1.7 × 10⁻² mmol) was dissolved in DMF (0.78 mL), and PEGA (0.22 mL, 0.53 mmol) and AIBN (0.43 mg, 2.62 × 10⁻³ mmol) was added. Freeze-pump-thaw cycles were repeated five times and the flask was immersed in a 60 °C oil bath to initiate the polymerization. Time points were taken to determine monomer conversion. The polymerization was stopped at 68 % monomer conversion by cooling in liquid nitrogen and then the flask was opened and allowed to return to room temperature. The polymer was purified by dialysis against H₂O for 3 days then MeOH for 3 h (MWCO 3,500 g/mol). M_n by ¹H NMR was 13,100 g/mol (targeted 9,830 g/mol), by GPC was 18,100 g/mol, and PDI was 1.16. Series of **poly1s** were synthesized in same procedure, changing CTA concentration from 0.01 M (**poly1b** and **poly1d**) to 0.02 M (**poly1c**). M_n's by ¹H NMR of **poly1b-d** are listed in Table 1 and GPC traces are in the supporting information, Figure S3.

Radical cleavage of the trithiocarbonate group—A literature procedure for trithiocarbonate radical cleavage was followed.³⁶ Briefly, **poly1b** (10 mg, 1.30 × 10⁻³ mmol) was dissolved in DMF (3.6 mg). LPO (1.03 mg, 2.58 × 10⁻³ mmol) and AIBN (4.28 mg, 0.03 mmol) were added and the flask was freeze-pumped-thawed five times to remove oxygen and then immersed in an 80 °C oil bath. After 16 h, LPO and AIBN were removed by dialysis against water for 72 h (MWCO 3,500 g/mol).

Synthesis of 2-(pyridin-2-ylsulfanyl)ethanol (3)—Synthesis was performed as previously described.³⁴ Aldrithiol (5.00 g, 0.02 mol) was dissolved in methanol (50 mL) and acetic acid (3 mL) was added to the solution. 2-Mercaptoethanol (0.80 mL, 0.01 mol) was dissolved in methanol (20 mL) and added drop wise. The reaction was then stirred at 23 °C for 24 h. The solvent was removed *in vacuo* and the crude product was purified by silica column chromatography with Hex : EtOAc = 3 : 2 (R_f = 0.35) and collected as a white solid in 93 % yield. ¹H NMR (500 MHz, in DMSO) δ : 8.45-8.44 (m, 1H), 7.85-7.79 (m, 2H),

7.25-7.22 (m, 1H), 5.02-4.98 (m, 1H), 3.64-3.59 (m, 2H), 2.94-2.89 (m, 2H). ^{13}C NMR (500 MHz in CDCl_3) δ : 159.55, 149.59, 137.82, 121.18, 119.36, 59.18, 41.33.

Synthesis of bis-pyridyl disulfide CTA2—1 (0.55 g, 0.002 mol) was dissolved in methylene chloride (20 mL) in a flame dried flask with a stir bar and cooled in an ice bath. **3** was added (1.22 g, 0.007 mol) and stirred in an ice bath for 10 min. DCC (1.12 g, 0.005 mol) and DMAP (0.05 g, 0.41 mmol) were dissolved in dry THF (2 mL) and added slowly to the solution containing **1** and **3** in the ice bath. After 2 h, the reaction was warmed to 23 °C and stirred for 4 h. The resulting crude product was filtered and the solvent removed *in vacuo*. The crude product was purified by silica column chromatography with Hex : EtOAc = 3 : 2. The yellow spot was collected as yellow oil in 92 % yield. ^1H NMR (500 MHz in CDCl_3) δ : 8.48-8.39 (m, 2H), 7.72-7.57 (m, 4H), 7.10-7.05 (m, 2H), 4.76-4.69 (m, 2H), 4.39-4.33 (m, 4H), 3.04-2.98 (m, 4H), 1.59-1.55 (dd, J = 2.85, 7.40 Hz, 6H). ^{13}C NMR (500 MHz in CDCl_3) δ : 219.31, 170.62, 159.40, 137.03, 120.82, 119.74, 63.29, 48.13, 36.98, 16.68, 16.53. IR: 3049, 2929, 2851, 2361, 1732, 1624, 1572, 1560, 1445, 1416, 1378, 1296, 1244, 1224, 1147, 1116, 1078, 1042, 1028, 985, 957, 875, 809, 750, 716 cm^{-1} , MALDI-TOF MS (+/- 1.0) observed (predicted): H^+ 593.62 (593.62), UV-Vis (EtOAc): λ_{max} = 300 nm.

Representative RAFT polymerization of PEGA with CTA2 (poly2b)—CTA2 (20 mg, 3.4×10^{-2} mmol) was dissolved in DMF (1.00 mL), and PEGA (0.42 mL, 1.02 mmol) and AIBN (1.6 mg, 9.74×10^{-3} mmol) were added. Freeze-pump-thaw cycles were repeated five times and the reaction was initiated by immersion in an oil bath at 60 °C. Time points were taken to determine monomer conversion. Polymerization was stopped at 63 % monomer conversion by cooling in liquid nitrogen and then flask was opened and allowed to warm to room temperature. The product was purified by dialyzing against H_2O for 72 h then MeOH for 3 h (MWCO 3,500 g/mol). M_n by ^1H NMR was 11,200 g/mol (targeted 9,170 g/mol), by GPC was 15,700 g/mol, and PDI was 1.18. Other **poly2s** were synthesized with different CTA concentrations through 0.02 M (**poly2d**) and 0.03 M (**poly2a** and **poly2c**). M_n 's by ^1H NMR of **poly2a,c-d** are listed in Table 1 and GPC traces are in the supporting information, Figure S7.

4-Bromobenzaldehyde conjugation to poly1c—BOC deprotection was performed by dissolving **poly1c** (11 mg, 4.7×10^{-4} mmol) in 250 μL acetonitrile followed by addition of TFA (25 μL). After 1.5 h at 23 °C, solvent was removed *in vacuo* to give the aminooxy polymer. The aminooxy polymer was dissolved in 500 μL of acetonitrile. TFA (0.19 μL) and 4-bromobenzaldehyde (BBA) (1 mg, 5.4×10^{-3} mmol) was added. The reaction was stirred at 23 °C for 24 h and dialyzed against MeOH (MWCO 3,500 g/mol) to give **poly1c-BBA**.

GSH conjugation to poly1c—GSH-ketone was synthesized by following a literature procedure to modify GSH with a ketone and used for conjugation.³⁷ BOC deprotection was performed by dissolving **poly1c** (20 mg, 0.86×10^{-3} mmol) in 100 μL of methylene chloride with 10 % TFA. After 40 min at 25 °C the solvent was removed *in vacuo* to give the aminooxy polymer. The aminooxy polymer and GSH-ketone (7.00 mg, 1.74×10^{-3} mmol) were dissolved in 200 μL of water with 5 % TFA and stirred at 25 °C for 12 h. The crude reaction was dialyzed against deionized H_2O (MWCO 2,000 g/mol) for 72 h to give **poly1c-GSH**.

Benzyl mercaptan conjugation to poly2a—**Poly2a** (10 mg, 1.20 mmol) was dissolved in 250 μL of MeOH. Benzyl mercaptan (BM) (0.60 μL , 4.6 mmol) and acetic acid (5 μL) was dissolved in 250 μL of MeOH in a different vial and slowly added to **poly2a**. After 24 h

of stirring at 23 °C, the crude product was dialyzed against 500 mL of MeOH (MWCO 3,500 g/mol) and dried *in vacuo* to give **poly2a-BM**.

GSH conjugation to poly2a—A pH 6.0 PB buffer was degassed with argon gas for 30 min. **Poly2a** (15mg, 1.79 mmol) was dissolved in 500 μ L of the degassed buffer. Glutathione (GSH) (3 mg, 9.76 mmol) was dissolved in 500 μ L of degassed buffer and added to **poly2a**. The reaction was allowed to stir at 23 °C for 24 h before dialyzing against H₂O (MWCO 1,000 g/mol) to give **poly2a-GSH**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Supporting Information Available Polymer NMR spectra assignments, GPC data, GSH conjugation NMR in D₂O. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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Scheme 1.

Synthesis of bifunctional, symmetric *N*-BOC-aminoxy trithiocarbonate (**CTA1**).

Scheme 2.

Synthesis of bifunctional, symmetric pyridyl disulfide trithiocarbonate (**CTA2**).

Scheme 3.
RAFT polymerization of PEGA₄₅₄ mediated by **CTA1**.

Figure 1.
Kinetic study of **poly1a**.

Scheme 4.RAFT polymerization of PEGA₄₅₄ mediated by **CTA2**.

Figure 2.
Kinetic study of **poly2b**.

Figure 3.
 ^1H NMR spectroscopy (CD₃CN) of **poly1c**, **poly1c-BBA**, and **poly1c-GSH**.

Figure 4.
¹H NMR spectroscopy (CD₃CN) of **poly2a**, **poly2a-BM**, and **poly2a-GSH**.

Table 1Polymerization of PEGA₄₅₄ by RAFT polymerization.

Polymer	CTA:M:I	Temp (°C)	target M _n	Conv(%)	M _n NMR	PDI
Poly1a	1:30:0.15	60	9,800	68	13,100	1.16
Poly1b	1:45:0.15	70	14,700	69	23,200	1.26
Poly1c	1:60:0.15	65	18,800	67	23,300	1.28
Poly1d	1:90:0.15	65	29,200	70	29,300	1.44
Poly2a	1:15:0.15	70	5,300	69	8,400	1.11
Poly2b	1:30:0.3	60	9,200	63	11,200	1.18
Poly2c	1:60:0.1	60	19,100	68	20,200	1.27
Poly2d	1:93:0.15	70	32,300	75	35,200	1.44