Published in final edited form as:

Macromolecules. 2013 January 1; 46(1): 8–14. doi:10.1021/ma302183g.

Keto-Functionalized Polymer Scaffolds As Versatile Precursors to Polymer Side Chain Conjugates

Jingquan Liu^{1,2}, Ronald C. Li¹, Gregory J. Sand¹, Volga Bulmus², Thomas P. Davis^{2,*}, and Heather D. Maynard^{1,*}

¹Department of Chemistry and Biochemistry & California Nanosystems Institute, University of California, Los Angeles, CA 90095-1569, USA

²Centre for Advanced Macromolecular Design, School of Chemical Sciences and Engineering, The University of New South Wales NSW 2052

Abstract

A new methacrylate monomer with a reactive ketone side-chain, 2-(4-oxo-pentanoate) ethyl methacrylate (PAEMA), was synthesized and subsequently polymerized by reversible addition-fragmentation chain transfer (RAFT) polymerization to give a polymer with a narrow molecular weight distribution (PDI = 1.25). The polymer was chain extended with poly(ethylene glycol methyl ether acrylate) (PEGMA) to yield a block copolymer. Aminoxy containing small molecules and oligoethylene glycol were conjugated to the ketone functionality of the side chain in high yields. Cytotoxicity of the oxime-linked tetra(ethylene glycol) polymer to mouse fibroblast cells was investigated; the polymer was found to be non-cytotoxic up to 1 mg/mL. The ease with which this polymer is functionalized, suggests that it may be useful in forming tailored polymeric medicines.

Introduction

Polymers with side chain groups that can be modified easily and in high yield are important synthetic building blocks.^{1–3} For example, reactive polymer scaffolds provide a starting point to produce side-chain functionalized polymer therapeutics.^{4–6} For many applications, post-polymerization modification avoids the need to determine new polymerization conditions for each unique monomer, yet at the same time allowing comparison of a series of macromolecules without complications due to different molecular weight backbones. Ideally, the modification reactions are highly efficient and occur without the use of harsh reaction conditions and reagents. This allows for conjugation of molecules that contain sensitive groups.

‘Click’ reactions are particularly well suited for modification of polymer chains. ‘Click’ was first used by Sharpless to describe reactions that are highly efficient without side products.⁷

*Heather D. Maynard, maynard@chem.ucla.edu, Thomas P. Davis, t.davis@unsw.edu.au.

Supporting Information

Supporting Information Available. NMR spectra of monomer, polymers not in text, kinetic evaluation of block polymerization, and CMA conjugate data. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

The quintessential example of such a reaction is Huisgen 1,3-dipolar cycloaddition.⁸ Typically this reaction is catalyzed by copper, yet copper-less forms have been described when the alkyne is strained.⁹ Other examples include thiol-ene and hetero-Diels-Alder reactions.^{7,10} Reaction of an aldehyde or ketone with an *O*-hydroxylamine is another highly efficient reaction.^{11,12} Although a side product is released, that product is benign (water). Other advantages of oxime chemistry is that ambient temperatures are required, the bond can be formed in water, is reversible under certain conditions, and no other reagents are necessary for the chemistry to occur.

For many applications of reactive polymers, narrow molecular weight distributions of the starting polymer scaffold are desired.⁴⁻⁶ For example, for use in therapeutics, broad weight distributions may result in varying toxicity or pharmacokinetics. To synthesize well-defined polymers containing reactive side chains, controlled/"living" radical polymerizations (CRPs) have been utilized such as atom transfer radical polymerizations (ATRP),^{5,13-23} nitroxide-mediated living free radical polymerization (NMP),²⁴⁻²⁸ and reversible-addition fragmentation chain transfer (RAFT) polymerization.²⁹⁻⁴¹ These polymerization techniques not only give low polydispersity indices (PDIs), they enable targeting of specific molecular weights.

CRP techniques have been employed to prepare side chain polymers that could be used in oxime 'click' reactions. Aminooxy side chain polymers have been synthesized by CRP.⁴² Polymers containing pendent acetals that were deprotected by mildly acidic conditions to produce aldehydes were prepared both by ATRP and RAFT.^{23,36,43-45} Polymers synthesized by RAFT with ketal (dioxolane) side chains, which were deprotected to diols and oxidized to aldehydes were also described.⁴⁴ Examples of direct polymerization of aldehyde-containing monomers by RAFT has been demonstrated.^{41,46-49} The use of a ketone-containing monomer, however, not only avoids deprotection steps, but is also more stable and less likely to oxidize than aldehydes. Vinyl ketone monomers have been polymerized by RAFT and NMP.^{50,51} In this report 4-oxo-pentanoic acid ethyl methacrylate (PAEMA) was polymerized by RAFT polymerization. This results in polymers that contain a spacer between the ketone and backbone moiety, which could be important for conjugation reactions, particularly of bulky moieties. Synthesis and characterization of the polymers, conjugation of aminooxy compounds, and initial toxicity studies are described.

Experimental Section

Materials

Levulinic acid (>97%, Fluka), 2-hydroxyethyl methacrylate (HEMA) (Acros, 98%), *N,N'*-dicyclohexylcarbodiimide (DCC) (Fluka, 99.0%), 4-dimethylaminopyridine (DMAP) (Fluka, 99.0%), 2,2'-azobisisobutyronitrile (AIBN) (Aldrich, 98%), dioxane (Fisher, 99.9%), ammonium chloride (Aldrich, 99%), *O*-benzylhydroxylamine hydrochloride (BHA) (Aldrich, 99%), carboxymethoxyamine hemihydrochloride (CMA) (Aldrich, 98%), triethylamine (TEA) (Acros, 99%), and poly(ethylene glycol) methyl ether acrylate (PEGMA) (Aldrich) were used as received. The RAFT agent, 2-(ethoxycarbonyl)prop-2-yl dithiobenzoate (ECPDB) was synthesized according to a literature procedure.⁵² *O*-hydroxylamine tetra(ethylene glycol) (TEG) was synthesized as described in literature.⁵³

Methods

^1H and ^{13}C NMR spectra were acquired on an Avance DRX 400 or 500 MHz spectrometer. To obtain gel permeation chromatography (GPC) data, a Shimadzu LC-10ATvp pump was used with eluents 0.1 M LiBr in DMF (40 °C) or THF (25 °C) at a rate of 0.8 mL/min through two Polymer Laboratories 5 μm mixed-D columns (with guard column) and a RID-10A detector. The standards used for DMF and THF GPC were PMMA or PSt and PMMA, respectively. Infrared absorption spectra were recorded using a PerkinElmer FT-IR equipped with an ATR accessory.

Synthesis of 4-oxo-pentanoic acid ethyl methacrylate (PAEMA)

Levulinic acid (2.81 g, 24.2 mmol), HEMA (3 g, 23 mmol), DCC (5.23 g, 25.3 mmol), and DMAP (0.14 g, 1.15 mmol) were mixed and stirred in DMF for 15 h. The solid precipitate was removed by filtration, and the volatiles removed by evaporation. The residue was purified by silica gel column chromatography using ethyl acetate/hexanes (2/3) as the eluent to give 5.2 g (95% yield) of a pure, colorless oil. ^1H NMR (400MHz, CDCl_3) δ : 6.06 (m, 1H, $\text{CH}_2=\text{C}$), 5.52 (m, 1H, $\text{CH}_2=\text{C}$), 4.27 (m, 4H, $2 \times \text{CH}_2\text{O}$), 2.66-2.70 (t, 2H, $\text{CH}_2\text{-CO}$), 2.52-2.54 (t, 2H, OOC-CH_2), 2.10 (s, 3H, $\text{CH}_3\text{-CO}$), 1.88 (m, 3H, $\text{CH}_3\text{-C}=\text{CH}_2$). ^{13}C NMR (100 MHz, CDCl_3) δ : 206.23 ($\text{C}=\text{O}$), 172.69 ($\text{CH}_2\text{-CO-O}$), 166.87 ($\text{CO-C}=\text{CH}_2$), 136.10 ($\text{C}=\text{CH}_2$), 126.12 ($\text{C}=\text{CH}_2$), 62.09 (CH_2O), 37.97 ($\text{H}_2\text{C-CO}$), 29.93 ($\text{H}_3\text{C-CO}$), 27.99 ($\text{H}_2\text{C-COO}$), 18.29 ($\text{H}_3\text{C-C}=\text{CH}_2$).

RAFT Homopolymerization of PAEMA

PAEMA (1 g, 4.38×10^{-3} mol), ECPDB (39 mg, 1.45×10^{-4} mol), and AIBN (4.8 mg, 2.9×10^{-5} mol) were dissolved in DMF (2 mL). The solution was deoxygenated for 30 min by bubbling with argon then immersed in a 70 °C bath. At 0.5, 1, 1.5, 2 and 3 h, samples were syringed from the incubating mixture under air free conditions. The monomer conversion for each polymerization sample was determined by ^1H NMR in CDCl_3 by comparing the shifts of the alkene protons of the monomer at 4.60 and 6.06 ppm to the growing polymer peaks at 4.21, 4.09. The polymer was isolated by precipitation in diethyl ether to give pPAEMA. ^1H NMR (400MHz, DMSO-D_6) δ : 7.79 (end group), 7.60 (end group), 7.44 (end group), 4.21, 4.09, 2.73, 2.12, 1.83-1.73, 1.45-0.77 ppm. Number-average molecular weight (M_n) in DMF = 15,700 Da; PDI = 1.25.

Chain Extension of pPAEMA-RAFT with PEGMA

pPAEMA (160 mg, 1.01×10^{-5} mol, M_n 15,700, PDI 1.25), PEGMA (0.45 g, 9.9×10^{-4} mol), and AIBN (0.35 mg, 2.13×10^{-6} mol) were dissolved in DMF (1 mL). The solution was deoxygenated for 30 min by bubbling with argon then immersed in a 70 °C bath. At 0.5, 1, 1.5, 2 and 3 h, samples were syringed from the incubating mixture under air free conditions. The monomer conversions were determined by ^1H NMR. The pure copolymer was obtained by precipitation into diethyl ether and methylene chloride, followed by lyophilization. ^1H NMR (400MHz, DMSO-D_6) δ : 4.21, 4.09, 3.51-3.25 (overlap with H_2O), 2.74, 2.13, 1.95-1.35, 0.96, 0.78 ppm. M_n (DMF) = 30,000 Da, PDI = 1.32.

Conjugation of *O*-benzylhydroxyamine (BHA) to pPAEMA

To a mixture of pPAEMA (12 mg, 7.64×10^{-4} mmol, M_n 15,700 Da, PDI 1.25) in 1 mL $CDCl_3$ were added triethylamine (5.3 mg, 5.3×10^{-2} mmol) followed by BHA hydrochloride salt (8.4 mg, 5.3×10^{-2} mmol). BHA hydrochloride slowly dissolved. The resulting mixture was kept stirring for 3 h. The $CDCl_3$ was removed. To the resulting solid was added 10 mL of CH_2Cl_2 , which was washed with saturated NH_4Cl aqueous solution (pH 5.0) (2×10 mL). The organic layer was dried with Na_2SO_4 and evaporation gave the product. 1H NMR (400MHz, DMSO- D_6) δ : 7.26 (BHA conjugate), 4.93 (BHA conjugate), 4.09-4.00, 2.18, 1.73, 1.23-0.75.

Conjugation of carboxymethoxyamine hemihydrochloride (CMA) to pPAEMA

To a mixture of pPAEMA (11.3 mg, 7.2×10^{-4} mmol, M_n 15,700 Da, PDI 1.25) in $CDCl_3/CD_3OH$ (1:1, 2 mL) were added CMA hemihydrochloride (5.4 mg, 2.5×10^{-2} mmol) and TEA (2.5 mg, 2.5×10^{-2} mmol). The resulting mixture was stirred for 3 h. By 1H NMR, all of the ketone groups on polymer were converted into oxime bonds. The conjugate was purified by the dialysis using 6000-8000 dialysis membrane (molecular weight cut off MWCO: 6-8,000 Da) in CH_3OH for 24 h before drying to give the product. 1H NMR (500MHz, DMSO- D_6) δ : 4.5 (CMA conjugate), 4.35, 4.24, 2.71, 2.64, 2.55, 1.95, 1.07, 0.89 ppm.

Conjugation of *O*-hydroxylamine tetra(ethylene glycol) to pPAEMA

To a solution of pPAEMA (50 mg, 3.2×10^{-3} mmol, M_n 15,700 Da, PDI 1.25) in methanol/DCM (1:1, 2 mL) were added *O*-hydroxylamine tetra(ethylene glycol) (TEG) (0.5 g, 2.24 mmol) and NaOAc (0.360 g, 4.39 mmol). The reaction was allowed to stir for 24 hours. The solvent was then evaporated, and the residue was redissolved in deionized water. The aqueous solution was purified using centrifugation filtration (MWCO: 10,000 Da) for 10 minutes times 4 cycles. The filtrate was lyophilized to yield a clear oil. 1H NMR (500MHz, $CDCl_3$) δ : 4.26, 4.13, 3.65, 3.56, 3.38, 2.58, 2.50, 2.02-1.62, 1.01-0.86 ppm.

Analysis of TEG-pPAEMA cytotoxicity to mouse fibroblast cells

A 10 mg/mL stock solution of polymer in 10% calf serum in Dulbecco's modified eagle medium (DMEM) was prepared. This stock solution was diluted to make concentrations of 1.0, 0.1, and 0.01 mg/mL TEG-pPAEMA polymer in 10% calf serum in DMEM. Mouse fibroblast cells (NIH 3T3, ATCC) were allowed to adhere to 96 well plates prior to adding 100 μ L per well of the polymer solutions. For each concentration, five replicates were used and the control was repeated 10 times. The fibroblasts were incubated with various polymer concentrations at 37 $^{\circ}C$, 5% CO_2 for 24 hours. Upon removal from the incubator, (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) bromide (MTT) solutions were added according to the manufactures protocol. The supernatant was then aspirated from each well, followed by the addition of 50 μ L of DMSO to dissolve the purple crystals. UV analysis at 570 nm was undertaken. The results were divided by that of the control to determine the percent viability

Results and Discussion

The monomer with the required ketone moiety, PAEMA, was synthesized by esterification of levulinic acid with hydroxyl ethyl methacrylate (Scheme 1a). Polymerization of this monomer using ECPDB as the chain transfer agent (CTA) in DMF was initiated by thermolysis of AIBN at 70 °C (Scheme 1b). The kinetics of PAEMA polymerization are shown in Figure 1a. A linear relationship was observed between the semi-logarithmic plot and time. In addition, the molecular weight increased linearly with monomer conversion up to 92%, with a corresponding decrease in PDI over the course of the reaction, consistent with a living polymerization. The distributions by GPC with increasing conversions are shown in Figure 2. The final polymer was purified by precipitation into diethyl ether, and the ¹H NMR spectrum of the purified homopolymer is provided as Figure 3. The molecular weight of the final polymer was 15,700 Da and the PDI was 1.25. Both the kinetic plots and low PDI suggest that this system is a controlled radical polymerization.

As a result of RAFT polymerization with ECPDB, a dithioester was present at the ω -end of the polymer chain end. Although this moiety was not clearly visible in the spectrum taken in CDCl₃, the chain end was clearly observed in DMSO-D₆ (see Supporting Information, Figure S3) as resonances at 7.79, 7.60 and 7.44 ppm. Because the dithioester group remained intact after polymerization, the homopolymer could potentially be used as a macro-chain transfer agent for chain extension reaction reactions to form block copolymers. To investigate this, poly(ethylene glycol) methacrylate (PEGMA) was polymerized in the presence of pPAEMA, forming a block copolymer (Scheme 2). The polymerization conditions of [monomer]:[macroRAFT-CTA]:[initiator] of 465:5:1 resulted in the formation of PPAEMA-*b*-PEGMA.

Similar to the behavior of the homopolymerization of PAEMA, the polymerization of PEGMA using pPAEMA as the macroRAFT-CTA resulted in a controlled radical polymerization up to 60% conversion (Figure S4b,c). GPC chromatograms of the initial PPAEMA and the subsequent block copolymer formation clearly showed increasing molecular weight with increasing reaction times (Figure S4a). Large shift of retention times between the two polymers was observed and may be attributed to the dramatic change in the polarity of the copolymer after the addition of PEGMA fragments. As these hydrophilic fragments are added, the solubility of the macromolecule is expected to increase in polar media, resulting in an increase of the hydrodynamic volume as measured by GPC.

After 60% conversion the PDI increased dramatically and the polymerization slowed significantly. The GPC trace of the polymer at 70% conversion was bimodal and the molecular weight of the block copolymer was 55,000 Da with a PDI 1.6 (Figure S4a). The shape of the curve indicated early termination and coupling, contributing to the larger molecular weight distribution. The homopolymer/macro chain transfer agent was formed at 92% conversion. Under these conditions some of the RAFT end groups can be lost. Efficient block formation has been often observed for RAFT polymers, especially if the original block was isolated from polymers taken to a limited conversion.⁵⁴

The polymer stopped at 60% conversion was isolated and had a M_n of 30,000 Da and a PDI of 1.32. Given the unique polarities of the side chains, molecular weights were determined by comparing protons of the pPAEMA and pPEGMA side chains. The ^1H NMR spectrum for the purified copolymer is depicted in Figure 4. As expected, the copolymer was significantly more water soluble than pPAEMA due to the hydrophilic PEG side chains. A water-soluble copolymer is particularly useful for conjugating biomolecules via oxime coupling reactions for applications in the biological field.

Next the reactivity of the ketone moiety was investigated. Given the ubiquity of hydrophobic drugs, BHA was first used as a model system to conjugate to pPAEMA (Scheme 3). Monitoring the conjugation by ^1H NMR spectroscopy, within three hours 100% of the ketone side chains reacted. Also, the signals of the phenyl group protons in CDCl_3 shifted from 7.23 to 7.36 ppm, corresponding to the formation of the oxime bond. In addition, a shift of molecular weight by GPC was observed (Scheme 5a).

To isolate the functionalized polymer, the conjugate was purified by washing with an aqueous NH_4Cl solution to remove trace excess of unreacted BHA and the triethylene amine hydrochloride salt generated from neutralizing the BHA. The ^1H NMR spectrum in deuterated DMSO of the polymer showed the presence of the benzyl group at 7.26 and 4.93 (Figure 5b). Comparison of the integration values of the protons in the conjugate revealed that all the ketone groups of the polymer reacted, confirming complete conjugation. In addition, the peak for the methyl protons adjacent to the ketone at 2.18 ppm was no longer visible. A peak from the dithioester group was still visible in the ^1H NMR at 7.83 ppm; however, the integration value was reduced from the starting polymer, indicating that some cleavage had occurred. The conversion was quantitative, the reaction rate was relatively fast, and there appeared to be no side reactions. Thus, the oxime coupling reaction is comparable to that of the click coupling of azide and alkynes groups.¹² The coupling was also confirmed by FTIR as shown in Figure 5c. A previously unobserved band at 1629 cm^{-1} in the starting polymer appeared in the functionalized macromolecule, corresponding to the $\text{C}=\text{N}$ absorption band of the oxime bond. This resonance is typically weak for oxime bonds. In addition, the $\text{C}=\text{O}$ absorption of the conjugate was shifted from 1719 cm^{-1} of the pPAEMA to 1731 cm^{-1} of the modified polymer. The decrease in the intensity and the peak shift may be due to the absence of the ketone groups in the conjugate compared to the homopolymer.

To demonstrate the versatility of the conjugation, carboxymethoxyamine (CMA), a hydrophilic aminoxy compound was also conjugated to pPAEMA (Scheme 4). This small molecule is an amino acid mimic and addition of the compound to N-termini of peptides enables chemoselective attachment to form oxime chemistry without the need for protection of the side chains.³⁶ Using a mixed media of CDCl_3 and CH_3OH (1:1) to solubilize the reagents, the conjugation of CMA to pPAEMA was also found to be efficient and completed within three hours. As for BHA, the molecular weight increased after conjugation (Figure S5a). The ^1H NMR spectrum of the polymer showed an extra peak at 4.5 ppm corresponding to the methylene of CMA (Figure S5b). Again the peak at 2.18 corresponding to the ketone, was not visible, indicating complete conjugation. The dithioester group was still visible. The addition of the carboxylic acid moiety on the side chains increased the polymer hydrophilicity significantly so that the polymer was soluble in water.

Oligo(ethylene glycol) side chain units were introduced into the polymer side chains in order to increase the biocompatibility of the polymer. *O*-hydroxylamine tetra(ethylene glycol) was incubated with pPAEMA in the presence of sodium acetate. As for BHA and CMA, the conjugation was efficient. Peaks corresponding to the tetra(ethylene glycol) were observed at 3.65–3.38 ppm (Figure 6). A minor peak was observed corresponding to residual ketone. The dithioester group was no longer visible, potentially due to the increased time used to conjugate the TEG in this case.

The cytotoxicity of this polymer was then determined for mouse fibroblast cells. The cells were incubated with increasing concentrations of polymer 0.01, 0.1, and 1 mg/mL for 24 hours before undertaking the MTT assay. The conjugate was found to be non-cytotoxic at all concentrations tested for this cell line as no loss in cell viability was observed (Figure 7). This data indicates that this approach to synthesize polymers may result in biocompatible materials, applicable in medicine. It can be envisioned that many different small molecules, drugs, and peptides can be readily conjugated to this versatile and stable polymer backbone utilizing the efficient oxime click reaction.⁵⁵

Conclusion

A ketone containing monomer, PAEMA, was synthesized and polymerized to form homo- and block copolymers by RAFT polymerization. The synthesis of the homopolymer was found to be well-controlled and yielded a low dispersity polymer. The macroRAFT CTA resulted in controlled polymerization of PEGMA up to 60% conversion, after which bimodal molecular weight distributions were observed. The resulting block copolymer, pPAEMA-*b*-PEGMA, was soluble in water. *O*-hydroxylamine compounds were readily attached to the polymer backbone with high efficiency. Both hydrophobic (BHA) and hydrophilic moieties (CMA and tetra(ethylene glycol)) were conjugated. Initial cytotoxicity work with mouse fibroblast cells revealed that an oligo(ethylene glycol) substituted pPAEMA was non-cytotoxic up to at least 1 mg/mL. This work demonstrated that the oxime covalent coupling to this keto polymer can be a very useful methodology to prepare conjugates. Future work will focus on the utilization of the ketone bearing polymers to form drug and peptide-polymer conjugates for therapeutic delivery and personalized medicine applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the NIH (R21 CA 137506-01) and the Australian Research Council (ARC Discovery Project Grant, DP 0770818) for funding the research. GJS thanks the UC Nanotoxicology Research and Training Program for a fellowship. Dr. Erhan Bat is thanked for help with the NMR figures.

References

1. Coessens V, Pintauer T, Matyjaszewski K. Prog. Polym. Sci. 2001; 26:337–377.
2. Hawker CJ, Wooley KL. Science. 2005; 309:1200–1205. [PubMed: 16109874]

3. Roth, PJ.; Wiss, KT.; Theato, P. In *Polymer Science: A Comprehensive Reference*. Krzysztof, M.; Martin, M., editors. Elsevier; Amsterdam: 2012. p. 247-267.
4. Kim, SH.; Nguyen, TH.; Maynard, HD. In *Comprehensive Biomaterials*. Paul, D., editor. Elsevier; Oxford: 2011. p. 377-388.
5. Godwin A, Hartenstein M, Müller AHE, Brocchini S. *Angew. Chem. Int. Ed.* 2001; 113:614–617.
6. Duncan R. *Nature Rev. Cancer.* 2006; 6:688–701. [PubMed: 16900224]
7. Kolb HC, Finn MG, Sharpless KB. *Angew. Chem. Int. Ed.* 2001; 40:2004–2021.
8. Huisgen R. *Angew. Chem. Int. Ed.* 1963; 75:604–637.
9. Debets MF, Van Berkel SS, Dommerholt J, Dirks AJ, Rutjes FPJT, Van Delft FL. *Accounts Chem. Res.* 2011; 44:805–815.
10. Barner-Kowollik C, Du Prez FE, Espeel P, Hawker CJ, Junkers T, Schlaad H, Van Camp W. *Angew. Chem. Int. Ed.* 2011; 50:60–62.
11. Maynard, HD.; Broyer, RM.; Kolodziej, CM. In *Click Chemistry for Biotechnology and Materials Science*. Lahann, J., editor. John Wiley & Sons Ltd; West Sussex: 2009. p. 53-68.
12. Prescher JA, Bertozzi CR. *Nature Chem. Bio.* 2005; 1:13–21. [PubMed: 16407987]
13. Hu ZC, Liu Y, Hong CY, Pan CY. *J. Appl. Polym. Sci.* 2005; 98:189–194.
14. Liu Y, Wang LX, Pan CY. *Macromolecules.* 1999; 32:8301–8305.
15. Theato P, Kim JU, Lee JC. *Macromolecules.* 2004; 37:5475–5478.
16. Griffith BR, Allen BL, Rapraeger AC, Kiessling LL. *J. Am. Chem. Soc.* 2004; 126:1608–1609. [PubMed: 14871072]
17. Shunmugam R, Tew GN. *J. Polym. Sci. Poly. Chem.* 2005; 43:5831–5843.
18. Sumerlin BS, Tsarevsky NV, Louche G, Lee RY, Matyjaszewski K. *Macromolecules.* 2005; 38:7540–7545.
19. Ladmiral V, Mantovani G, Clarkson GJ, Cauet S, Irwin JL, Haddleton DM. *J. Am. Chem. Soc.* 2006; 128:4823–4830. [PubMed: 16594719]
20. Fournier D, Pascual S, Fontaine L. *Macromolecules.* 2004; 37:330–335.
21. Matyjaszewski K, Coca S, Jasieczek CB. *Macromol. Chem. Physic.* 1997; 198:4011–4017.
22. De La Fuente JL, Canamero PF, Fernandez-Garcia MJ. *Polym. Sci. Poly. Chem.* 2006; 44:1807–1816.
23. Li RC, Broyer RM, Maynard HD. *J. Polym. Sci. Poly. Chem.* 2006; 44:5004–5013.
24. Sessions LB, Miinea LA, Ericson KD, Glueck DS, Grubbs RB. *Macromolecules.* 2005; 38:2116–2121.
25. Malkoch M, Thibault RJ, Drockenmuller E, Messerschmidt M, Voit B, Russell TP, Hawker CJ. *J. Am. Chem. Soc.* 2005; 127:14942–14949. [PubMed: 16231951]
26. Tully DC, Roberts MJ, Geierstanger BH, Grubbs RB. *Macromolecules.* 2003; 36:4302–4308.
27. Jones RG, Yoon S, Nagasaki Y. *Polymer.* 1999; 40:2411–2418.
28. Benoit D, Chaplinski V, Braslau R, Hawker CJ. *J. Am. Chem. Soc.* 1999; 121:3904–3920.
29. Schilli CM, Muller AHE, Rizzardo E, Thang SH, Chong YK. *Advances in Controlled/Living Radical Polymerization.* 2003; 854:603–618.
30. Vosloo JJ, Tonge MP, Fellows CM, D'Agosto F, Sanderson RD, Gilbert RG. *Macromolecules.* 2004; 37:2371–2382.
31. Hu YC, Liu Y, Pan CY. *J. Polym. Sci. Poly. Chem.* 2004; 42:4862–4872.
32. Favier A, D'Agosto F, Charreyre MT, Pichot C. *Polymer.* 2004; 45:7821–7830.
33. Savariar EN, Thayumanavan SJ. *Polym. Sci. Poly. Chem.* 2004; 42:6340–6345.
34. Ghosh S, Basu S, Thayumanavan S. *Macromolecules.* 2006; 39:5595–5597.
35. O'Reilly RK, Joralemon MJ, Hawker CJ, Wooley KL. *New J. Chem.* 2007; 31:718–724.
36. Hwang JL, R. C. Maynard HD. *J. Control. Rel.* 2007; 122:279–286.
37. Hwang JY, Li RC, Maynard HD. *J. Control. Rel.* 2007; 122:279–286.
38. Wong LJ, Boyer C, Jia ZF, Zareie HM, Davis TP, Bulmus V. *Biomacromolecules.* 2008; 9:1934–1944. [PubMed: 18564875]
39. Krieg A, Becer CR, Richard H, Schubert US. *Macromolecular Symposia.* 2009:275–276. 73–81.

40. Mellon W, Rinaldi D, Bourgeat-Lami E, D'Agosto F. *Macromolecules*. 2005; 38:1591–1598.
41. Sun G, Cheng C, Wooley KL. *Macromolecules*. 2007; 40:793–795. [PubMed: 19066633]
42. Hill MR, Mukherjee S, Costanzo PJ, Sumerlin BS. *Poly. Chem.* 2012; 3:1758–1762.
43. Li RC, Hwang J, Maynard HD. *Chem. Comm.* 2007:3631–3633. [PubMed: 17728876]
44. Rossi NAA, Zou Y, Scott MD, Kizhakkedathu JN. *Macromolecules*. 2008; 41:5272–5282.
45. Stukel JM, Li RC, Maynard HD, Caplan MR. *Biomacromolecules*. 2010; 11:160–167. [PubMed: 19924844]
46. Xiao N-Y, Li A-L, Liang H, Lu J. *Macromolecules*. 2008; 41:2374–2380.
47. Shi M, Li A-L, Liang H, Lu J. *Macromolecules*. 2007; 40:1891–1896.
48. Sun G, Fang H, Cheng C, Lu P, Zhang K, Walker AV, Taylor J-SA, Wooley KL. *ACS Nano*. 2009; 3:673–681. [PubMed: 19309173]
49. Murray BS, Jackson AW, Mahon CS, Fulton DA. *Chem. Comm.* 2010; 46:8651–8653. [PubMed: 20957257]
50. Cheng C, Sun G, Khoshdel E, Wooley KL. *J. Am. Chem. Soc.* 2007; 129:10086–10087. [PubMed: 17661467]
51. Hepperle JAM, Luftmann H, Studer A. *J. Polym. Sci. Poly. Chem.* 2012; 50:2150–2160.
52. Zheng GH, Pan CY. *Macromolecules*. 2006; 39:95–102.
53. Grover GN, Lam J, Nguyen TH, Segura T, Maynard HD. *Biomacromolecules*. 2012; 13:3013–3017. [PubMed: 22970829]
54. Albertin L, Stenzel MH, Barner-Kowollik C, Foster LJR, Davis TP. *Macromolecules*. 2005; 38:9075–9084.
55. Boyer C, Bulmus V, Davis TP, Ladmiral V, Liu JQ, Perrier S. *Chem. Rev.* 2009; 109:5402–5436. [PubMed: 19764725]

Scheme 1.

(a) Synthesis of monomer and (b) Homopolymerization of PAEMA.

Figure 1. RAFT polymerization of PAEMA. (a) Semi-logarithmic kinetic plot and (b) evolution of molecular weight (MW) and PDI with respect to conversion.

Figure 2. DMF GPC traces of the evolution of molecular weight at different time intervals for the polymerization of PAEMA.

Figure 3.
¹H NMR spectrum (CDCl₃) of purified pPAEMA.

Scheme 2.

Chain extension of PPAEMA-RAFT with PEGMEA.

Figure 4.
¹H NMR spectrum (DMSO-*d*₆) of block copolymer PPAEMA-*b*-PPEGMA.

Scheme 3.
Coupling of *O*-benzylhydroxylamine to pPAEMA.

Figure 5. Coupling of BHA to the homopolymers. a) THF GPC monitoring of the MW increase after coupling of BHA. b) ^1H NMR ($\text{DMSO}-\text{D}_6$) of polymer after conjugation and purification; c) FTIR analysis of the oxime conjugation.

Scheme 4.

Coupling of carboxymethoxyamine to ketone-homopolymer.

Figure 6.
 ^1H NMR (CDCl_3) of pPAEMA with tetra(ethylene glycol) side chains.

Figure 7.

Mouse fibroblasts were subjected to 0.01, 0.1, and 1.0 mg/mL of pPAEMA-tetra(ethylene glycol) for 24 h before undertaking a MTT assay. The results are shown as percent viability compared to the control with media only. Each sample was replicated 5 times and the control with no polymer 10 times. Student's t-test revealed no statistical difference between the concentrations.