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Modular Functionalization of Amphiphilic Block Copolymers via Radical-Mediated Thiol−Ene Reaction

Jun Yue,[†,‡] Xiaoyuan Li,[†] Guojun Mo,[†,‡] Rui Wang,[†,‡] Yubin Huang,[†] and Xiabin Jing*[,†]

[†]*State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China, and* [‡]*Graduate School of Chinese Academy of Sciences, Beijing 100049, P. R. China*

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ABSTRACT: Two kinds of amphiphilic block copolymers, allyoxyl poly(ethylene glycol)-*b*-poly(L-lactide) and methoxyl poly(ethylene glycol)-*b*-poly(L-lactide-*co*-2-methyl-2-allyloxycarbonyl-propylene carbonate), were synthesized in a controllable manner. Radical mediated thiol−ene reaction was utilized to modify these two precursor block copolymers with three model mercaptans. Highly efficient and quantitative modification under UV light without adding any photoinitiators was observed by ^{1}H NMR spectra of the products collected at specific time points. The reaction rate was mainly dependent on molar ratio of thiol to vinyl in feeding. Quantitative transformation of the vinyl groups in both block copolymers was realized in 0.5−2 h when using excessive thiol with respect to vinyl. Using commercially available mercaptans, different functional groups were introduced into the block copolymers, thus realizing modular functionalization of target block copolymers. This method of functionalization effectively avoided traditional protection/deprotection procedures, thus greatly simplifying the process of functionalization. Precipitation and dialysis procedures were proved efficient to eliminate excess mercaptans, thus endowing the material with good biocompatibility.

Introduction

Amphiphilic block copolymers which consist of at least two segments with different solubilities have attracted great interest for their ability of creating versatile self-assembled nanostructures,[1] such as polymeric micelles,[2−4] vesicles,[5−7] and large compound micelles.[8] These nanostructures have been widely studied during the past few years for their use in biomedical areas. Among various amphiphilic block copolymers, poly(ethylene glycol)-*b*-poly(lactide) (PEG-*b*-PLA) exhibit good potential for formulating drug delivery systems[9−14] for its excellent biocompatibility, biodegradability of the PLA segment and controllable loadings of hydrophobic drugs. What's more, hydrophilic PEG segments extending from the surface of nanostructures can not only improve solubility of the drugs, but also prevent opsonin adsorption and avoid subsequent uptake by reticuloendothelial systems (RES).[15,16]

However, the lack of functional groups (both in hydrophilic and hydrophobic segments) has limited its use in constructing multifunctional nanostructures. Two approaches have been made to improve this situation. One is ring-opening polymerization (ROP) of LA with appropriate functional comonomers, such as cyclic carbonates,[17−19] *N*-carboxyanhydride[20,21] and morpholine-2,5-dione derivatives,[22,23] various functional groups (carboxyl, amino, hydroxyl etc.) to form functionalized hydrophobic segments of the copolymer. The other is introduction of a functional group (R) to the hydrophilic terminal of the copolymer by using bifunctional PEG[24−30] which has a hydroxyl group at one end and a required functional group (R) at the other end to initiate ROP of LA with the hydroxyl group. In order to avoid inter- or intramolecular side reactions, the functional groups have to be protected before polymerization and deprotected after polymerization. This process is usually of low efficiency and sometimes may introduce unwanted impurities and additional toxicity, so highly efficient synthetic route without protection/deprotection procedure is a challenge to polymer functionalization.

In addition to direct polymerization, postpolymerization modification (PPM) is another approach to functionalization. Its prerequisite is the precursor polymer contains at least a reactive group. In a review paper,[31] Gauthier summarized several PPM methods that do not require protection/deprotection steps. Of these PPMs, we are interested in highly efficient and quantitative reactions that are compatible with the ring-opening polymerization (ROP), including Huisgen 1,3-dipolar cycloaddition reaction, Michael-type addition reaction, and radical thiol addition reaction. As the most important examples of click chemistry, the Cu(I)-mediated 1,3-dipolar cycloaddition reaction has been widely used to synthesize functional polymers under mild conditions both in aqueous and organic media. However, the toxicity of the copper ions precludes its use in the presence of live cells or organisms.[32] Nowadays, highly efficient reactions of thiols with reactive unsaturated carbon−carbon double bonds ("enes")[33−41] (referred as "thiol−ene reaction") have attracted more and more interest for their good properties: (1) no involvement of metallic catalysts, (2) compatible with a range of functional groups (carboxyl, hydroxyl, amine, etc.), and (3) mild reaction conditions. There are two types of thiol−ene reactions: Michael-type addition[40,42,43] and free-radical addition.[34,44−48] The former applies to α,β-unsaturated carbonyl compounds such as acrylate, maleimido, etc., and an intermediate thioanion is usually generated owing to the usage of a base for the reaction, while the latter is not limited to the conjugated double bonds. Isolated double bonds such as allyl are applicable and the free-radicals can be generated either by thermal- or photoinitiation. In this sense, radical thiol addition is superior to Michael-type addition. In 2007, our group[49] first reported an allyl-bearing poly(ester−carbonate) and its conjugation with folic acid via the radical thiol−ene addition of the allyl groups.

*Corresponding author. Telephone/Fax: +86-431-85262775. E-mail: xbjing@ciac.jl.cn.

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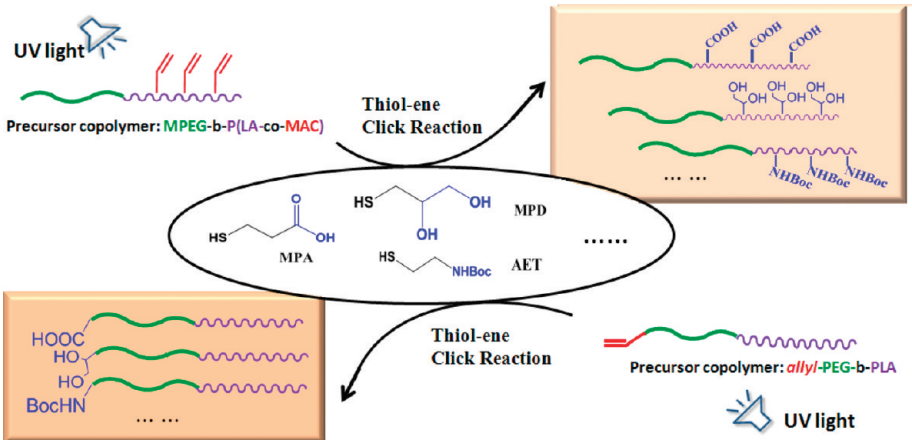
Scheme 1. Modular Functionalization of Precursor Copolymers with Different Mercaptans



Further in this direction, systematic studies were carried out to examine the feasibility, universality and advatages of this reaction in modular functionalization of biomedical polymers. In one example, allyl groups were incorporated into block copolymer methoxyl poly(ethylene glycol)-*b*-poly(L-lactide-*co*-2-methyl-2-allyloxycarbonyl-propylene carbonate) [MPEG-*b*-P(LA-*co*-MAC)] as pendant groups on the MAC units in the hydrophobic segment. In another example, the allyl group was attached to the PEG end of PEG-*b*-PLA as a terminal group of the hydrophilic segment. Three mercaptans containing COOH, OH and protected NH_2 were chosen as the thiol-containing compounds for the addition reactions. The whole project is shown in Scheme 1. Starting with a single precursor block copolymer, a series of functionalized block copolymers containing different functional groups either on the hydrophobic segment or at the terminal of the hydrophilic segment can be obtained via a single thiol−ene addition reaction. Radical thiol−ene addition is a powerful and universal strategy of functionalization of aliphatic polyesters and polycarbonates derivatives. This paper will demonstrate these results.

Experimental Section

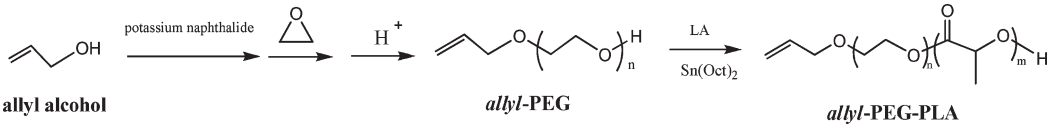
Materials. 2,2-Bis(hydroxymethyl) propionic acid (bis-HPA, Acros, 99%), Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, Strem Chemicals, 90% in 2-ethylhexanoic acid), ethyl chloroformate (Sigma-Aldrich, 97%), monomethoxyl poly(ethylene glycol) (MPEG, average M_n ~2000/5000, Sigma-Aldrich), 3-mercaptopropionic acid (MPA, Alfa Aesar, 99%), 2-(Boc-amino)ethanethiol (AET, Acros, 98%), allyl alcohol (Sigma-Aldrich, 99%) were used as received. Allyl bromide (97%), 3-mercapto-1,2-propanediol (MPD, 95%), naphthalene (99.6%), potassium (99%) were purchased from Shanghai Jingchun Chemical Reagent Co., Ltd. (Shanghai, China). L-Lactide (LA) was prepared in our own laboratory and recrystallized from ethyl acetate for three times before use. Ethylene oxide (CP) was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China) and was dried over sodium hydroxide column before use. Tetrahydrofuran (THF) and toluene were purified by distillation from sodium with benzophenone.

Synthesis of Cyclic Carbonate Monomer MAC. The cyclic carbonate monomer 2-methyl-2-(allyloxycarbonyl)propylene carbonate (MAC) was prepared according to the literature.[49] The final products were white crystals (yield: 68%). ^{1}H NMR ($CDCl_3$, δ ppm vs TMS): 1.35 (s, 3H, $-CH_2-(CH_3)C(COOCH_2CH{=}CH_2)-CH_2-$), 4.20−4.25 and 4.68−4.73 (q, 4H, $-CH_2-(CH_3)C(COOCH_2CH{=}CH_2)-CH_2-$), 4.70 (s, 2H, $-CH_2-(CH_3)C(COOCH_2CH{=}CH_2)-CH_2-$), 5.28−5.38 (t, 2H, $-CH_2-(CH_3)C(COOCH_2CH{=}CH_2)-CH_2-$), 5.8−6.0 (m, 1H, $-CH_2-(CH_3)C(COOCH_2CH{=}CH_2)-CH_2-$).

Synthesis of Block Copolymer MPEG-*b*-P(LA-*co*-MAC). The block copolymer MPEG-*b*-P(LA-*co*-MAC) was synthesized through ring-opening polymerization of LA and MAC in the presence of MPEG as the macroinitiator, with $Sn(Oct)_2$ as the catalyst. The following is a typical example of synthesis of MPEG(5K)-*b*-P(LA-*co*-MAC)20%. After a certain amount of MPEG (5.0 g, 1.0 mmol) was dried by toluene azeotropic distillation for 1 h, a mixture of LA (2.45 g, 17 mmol) and MAC (0.8 g, 4 mmol) was added into the above system, followed by argon-purging three times. After sealing the system, prescribed amount of $Sn(Oct)_2$ (0.5 mol % of the total monomers) was added using a glass syringe. The reaction mixture was then heated to 110 °C and stirred at this temperature for 12 h. Purification was performed by precipitating the reaction mixture against large excess of diethyl ether. The block copolymer was collected and dried in vacuo for 8 h. ^{1}H NMR ($CDCl_3$, δ ppm vs TMS): 1.3 (s, 9H, $-O-CH_2-(CH_3)C(COOCH_2CH{=}CH_2)-CH_2-$), 1.6 (d, 90H, $-O-CH(CH_3)-C(O)-$), 3.6 (s, 452H, $-CH_2-CH_2-O-$), 4.2−4.4 (s, 12H, $-O-CH_2-(CH_3)C(COOCH_2CH{=}CH_2)-CH_2-$), 4.6 (s, 6H, $-O-CH_2-CH{=}CH_2$), 5.2 (q, 30H, $-O-CH(CH_3)-C(O)-$), 5.25−5.35 (q, 6H, $-O-CH_2-CH{=}CH_2$), 5.9 (m, 3H, $-CH_2-CH=CH_2$).

Synthesis of Block Copolymer *allyl*-PEG-*b*-PLA. The synthesis of copolymer *allyl*-PEG-*b*-PLA with an allyl group at the end of PEG segment was conducted in two steps as shown in Scheme 2. First, α-allyl-ω-hydroxy PEG (*allyl*-PEG) was synthesized by anion ring-opening polymerization of ethylene oxide in the presence of allyl alcohol potassium as initiator. Typically, to a 250 mL round-bottom flask which contained 0.2 g of allyl alcohol (3.44 mmol) in 40 mL of THF, 1.65 mL of potassium naphthalide solution (2.0 M in THF) was added under nitrogen atmosphere and the mixture was stirred at room temperatures for 10 min to afford allyl alcohol potassium. Potassium naphthalide was synthesized according to the literature.[50] Then the mixture was cooled to 0 °C and calculated amount of ethylene oxide (17.6 g, 0.4 mol) was added to the flask using a cold glass syringe. The reaction system was kept stirring at 0 °C for about 4 h and then the ice bath was withdrawn to continue the polymerization for another 44 h at room temperature (25−30 °C). The polymerization was stopped by adding trace of acetic acid/ethanol (50/50) solution. After precipitating the mixture against large excess of diethyl ether, the polymers were collected and dried in vacuo for 8 h. Second, The block copolymer *allyl*-PEG-*b*-PLA was synthesized by ring-opening polymerization of LA in the presence of *allyl*-PEG as macroinitiator with $Sn(Oct)_2$ as the catalyst at 110 °C for 8 h in toluene. This process was similar to the synthesis of MPEG-*b*-P(LA-*co*-MAC), so detail information would not be described here any more. ^{1}H NMR ($CDCl_3$, δ ppm vs TMS): 1.6 (d, 204H, $-O-CH(CH_3)-C(O)-$), 3.6 (s, 452H, $-CH_2-CH_2-O-$), 4.0 (d, 2H, $CH_2{=}CH-CH_2-O-$), 5.2 (q, 68H, $-O-CH(CH_3)-C(O)-$), 5.3 (q, 2H, $CH_2=CH-CH_2-O-$), 5.9 (m, 1H, $CH_2{=}CH-CH_2-O-$).

Scheme 2. Synthetic Route of *allyl*-PEG-*b*-PLA Block Copolymer



Radical Thiol−Ene Addition Reactions of MPEG-*b*-P(LA-*co*-MAC) and *allyl*-PEG-*b*-PLA with Mercaptans. Three mercaptans (MPA, MPD, and AET) were selected to investigate the universality of this reaction. As an example, the reaction of block copolymer MPEG(5K)-*b*-P(LA-*co*-MAC)20% with MPA is described here. Other reactions were conducted in a similar way. The block copolymer MPEG(5K)-*b*-P(LA-*co*-MAC)20% (1.0 g, 0.12 mmol, ~3 MAC units per chain) and 3-mercaptopropionic acid (MPA) (0.21 g, 2.0 mmol) were dissolved in 30 mL of THF in a 250 mL round-bottom quartz flask, followed by N_2 bubbling with a gentle flow for 30 min to eliminate dissolved oxygen. Then the mixture was stirred at room temperature under UV light (254 nm, 1.29 mW/cm^2). After 2 h, the light source was turned off and the mixture was concentrated by evaporating part of the solvent. Residues were poured into large amounts of cold diethyl ether to get precipitates (this process was repeated for three times). The precipitates were collected and redissolved in 10 mL of DMF. Then the solution was placed in a dialysis bag (cutoff Mn: 3.5 kDa) and dialyzed against water for 4 days to remove residual MPD. The solution outside the bag was replaced with fresh water every 12 h and finally, the mixture in the dialysis bag was freeze-dried to give final product (yield: 90%). ^{1}H NMR ($CDCl_3$, δ ppm vs TMS): 1.3 (s, 9H, $-O-CH_2-(CH_3)C(COOCH_2CH_2CH_2SCH_2CH_2COOH)-CH_2-$), 1.6 (d, 90H, $-O-CH(CH_3)-C(O)-$), 1.9 (m, 6H, $-CH_2-CH_2-CH_2-S-$), 2.6 (t, 12H, $-CH_2-S-CH_2-$), 2.8 (t, 6H, $-S-CH_2-CH_2-COOH$), 3.4 (t, 6H, $-OCH(O)-CH_2-$), 3.6 (s, 452H, $-CH_2-CH_2-O-$), 4.2−4.4 (s, 12H, $-O-CH_2-(CH_3)C(COOCH_2CH_2-CH_2SCH_2CH_2COOH)-CH_2-$), 5.2 (q, 30H, $-O-CH(CH_3)-C(O)-$). To test the efficiency of the thiol−ene reaction, conversion rates of the allyl groups were measured at specified time points (4, 8, 15, 30, 60, 120, 240 min). A small amount of the reaction mixture was taken out and precipitated against enough amount of diethyl ether. After purification and drying procedures, the sample was collected and ^{1}H NMR experiments were done to determine the allyl content in the sample.

Micellization of the Copolymers MPEG-*b*-P(LA-*co*-MAC)s Modified with Mercaptans. Dialysis technology was used to prepare micelles from three copolymers MPEG-*b*-P(LA-*co*-MAC)s modified with different mercaptans (MPA, MPD, AET). To a 100 mL conical flask which contained 10 mL of copolymer solution (10 mg/mL in THF) was added 25 mL of water, dropwise, under magnetic stirring at the same time. Then the mixture was placed in a dialysis bag (cutoff Mn: 3.5 kDa) and dialyzed against water for 3 days to remove THF. The water was replaced every 12 h, and finally, the mixture in the dialysis bag was freeze-dried to give sponge-like micelles.

Characterization. ^{1}H NMR (300 MHz, field strength: 7.05 T) spectra were recorded on a Bruker AV300 M in $CDCl_3$ at 25 °C. Chemical shifts were given in parts per million from that of tetramethylsilane (TMS) as an internal reference. Gel permeation chromatography (GPC) measurements were conducted with a Waters 410 GPC instrument equipped with a Waters Styragel HT3 column (bead size, 10 μm; molecular weight range, 500−30 000) and a differential refractometer detector. $CHCl_3$ (for block copolymers or THF for *allyl*-PEGs) was used as eluent at a flow rate of 1 mL/min at 35 °C. The molecular weights were calibrated with polystyrene standards (molecular weight range: 1790−200 000). Matrix-assisted laser desorption/ionization time-of-flight mass (MALDI-TOF MS) spectra were recorded using Bruker REFLEX III. α-Cyano-4-hydroxycinnamic acid (HCCA) was used as the matrix for the ionization. Two solutions were prepared before detection: the sample solution (1 mg/mL in 33% acetonitrile/water) and the matrix solution (10 mg/mL of HCCA in 33% acetonitrile/water). Two solutions were mixed together (volume ratio 1:1) and 1 μL of the mixture was introduced to the probe (laser repetition rate, 200 Hz; ion source voltage, 120 kV; linear detector voltage, 1.572 kV).

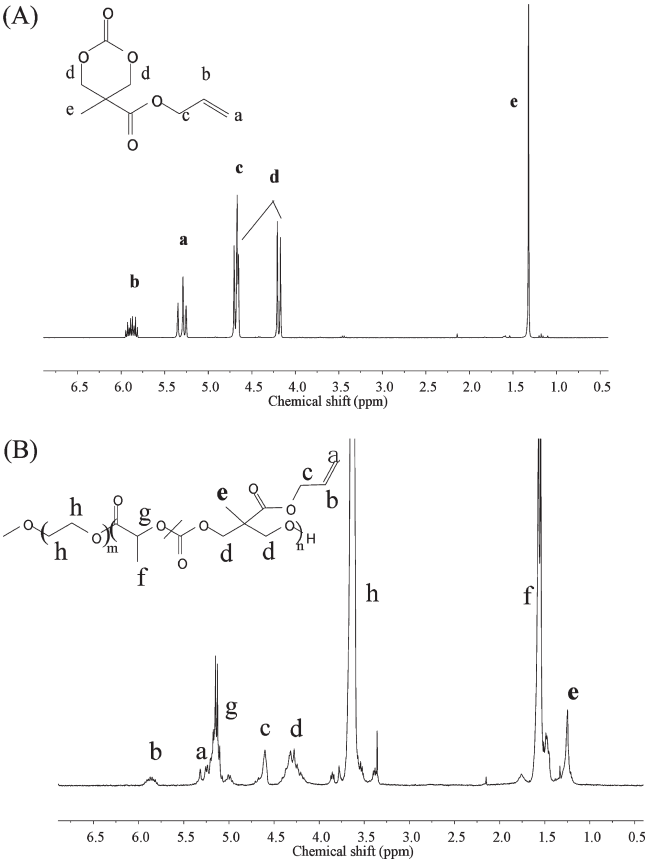


Figure 1. ^{1}H NMR spectra (300 MHz, $CDCl_3$) of MAC monomer (A) and MPEG-*b*-P(LA-*co*-MAC) block copolymer (B).

Biocompatibility of the Micelles Prepared from Three Different Functional Copolymers. L929 cells were used to evaluate the biocompatibility of three different micelles. L929 cells were seeded in 96-well plate at 10 000 cells/well 12 h prior to incubation with samples. Each well was filled with 100 μL of Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 mg/L streptomycin. Then the micellar samples at four concentrations (500, 250, 125, and 62.5 μg/mL) were added to the wells, respectively (four parallel wells for each sample at a specific concentration). After coincubation with L929 cells for 24 h, inverted fluorescence microscope (TE2000-U, Nikon) was used to observe the status of the cells and three pictures of the cells for each sample were taken with a DXM1200F digital camera (Nikon).

Results and Discussion

Synthesis of Block Copolymer MPEG-*b*-P(LA-*co*-MAC). First, cyclic carbonate monomer MAC containing allyl group was synthesized according to the ref 51 with the synthetic route shown in Scheme S1 (Supporting Information). The structure of MAC was confirmed by ^{1}H NMR spectrum (Figure 1A). Then MPEG was utilized to initiate the ring-opening

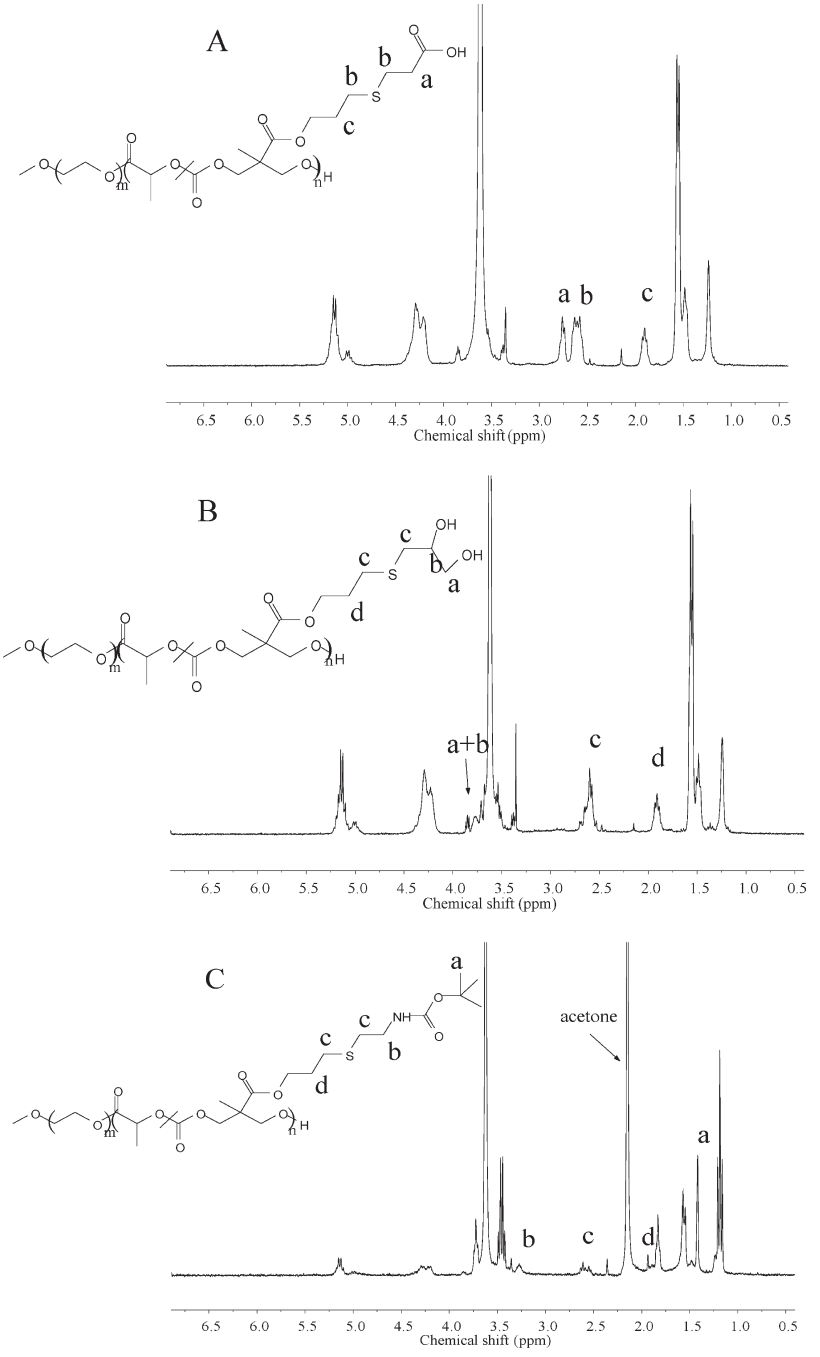


Figure 2. ^{1}H NMR spectra of MPEG-*b*-P(LA-*co*-MAC)20% modified with MPA (A), MPD (B), and AET (C).

copolymerization of LA and MAC with the catalyst $Sn(Oct)_2$. As can be seen from Figure 1B, the allyl group was intact (resonances at 4.6, 5.25−5.35 and 5.85−5.95 ppm) during polymerization. As indicated in our previous papers,[49,51] MAC was random-copolymerized with LA, resulting in random sequence distribution of MAC units in the hydrophobic segment. Different copolymerization ratios of MAC with LA can be achieved by varying the feeding ratio of them (Table S1 in Supporting Information) and the molecular weight was controllable over a large range of composition.

Modification of MPEG-*b*-P(LA-*co*-MAC) Block Copolymers by Radical Thiol−Ene Reaction. Nowadays, a large variety of mercaptans have become commercially available, thus making the postpolymerization modifications convenient. In the present study, three model mercaptans (Table S2 in Supporting Information) were selected: 3-mercaptopropionic acid (MPA), 3-mercapto-1,2-propanediol (MPD), and 2-(Boc-amino)-ethanethiol (AET) (AET was selected instead of 2-amino-ethanethiol because the free amino group can cause partial aminolysis of the hydrophobic segment) to investigate the adaptability of the modification method. The mechanism of radical thiol−ene addition[52] is shown in Scheme S2 (Supporting Information). In this study, the UV light (254 nm) was used to irradiate mercaptans to bring out active radicals (known as "thiyl") without adding any photoinitiators. The reaction was conducted at room temperature, thus making the modifications easy to handle. As can be seen from Figure 2, resonances at 4.6 ppm which are attributed to the methylene adjacent to the vinyl group all shifted to 3.4 ppm after the reaction with the three mercaptans. Disappearance of the resonances at 5.25−5.35 ppm and 5.85−5.95 ppm attributed to the vinyl group and appearance of the new resonances at

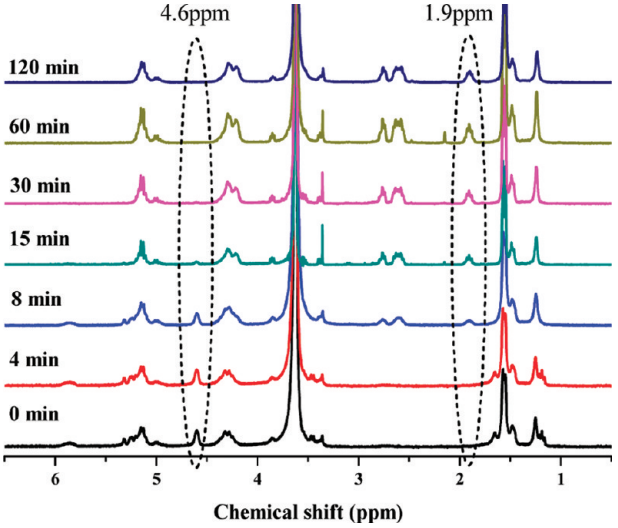


Figure 3. ^{1}H NMR spectra of MPEG-*b*-P(LA-*co*-MAC)20% modified with MPA (thiol/ene molar ratio was 10:1) at specific time points.

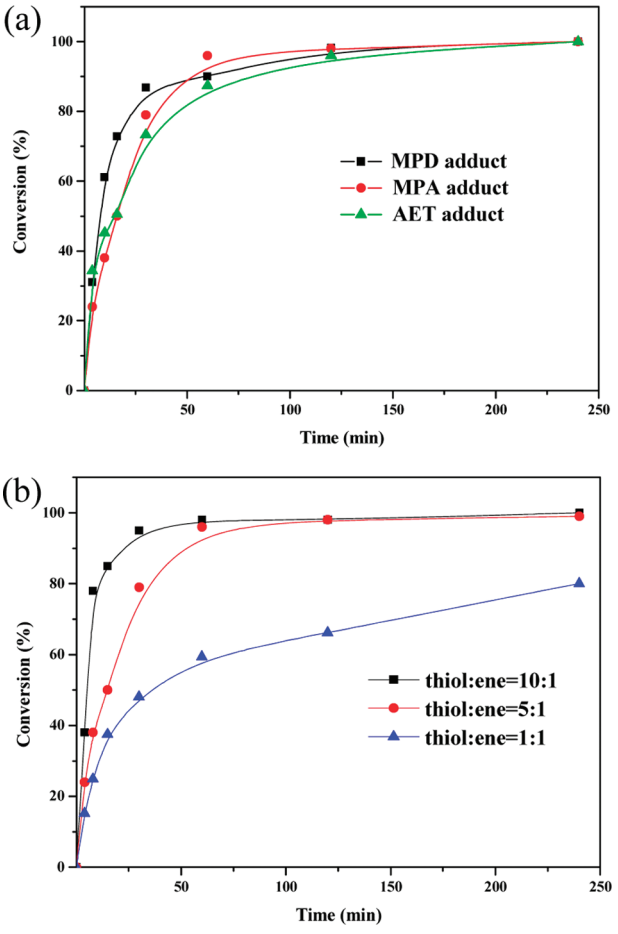


Figure 4. Conversion of allyl groups in MPEG(5K)-*b*-P(LA-*co*-MAC)20% as a function of irradiation time. (a) Molar ratio of thiol/ene was fixed at 5:1. (b) Reactions between MPEG(5K)-*b*-P(LA-*co*-MAC)20% and MPA at various thol/ene ratios as indicated.

1.9 and 2.6−2.8 ppm (Figure 2A), 1.9 and 2.6 ppm (Figure 2B), 1.9, 2.6, and 3.3 ppm (Figure 2C) indicated successful modifications of MPEG-*b*-P(LA-*co*-MAC) with MPA, MPD, and AET, respectively. Success in these reactions indicates that free COOH and OH groups do not interfere with the

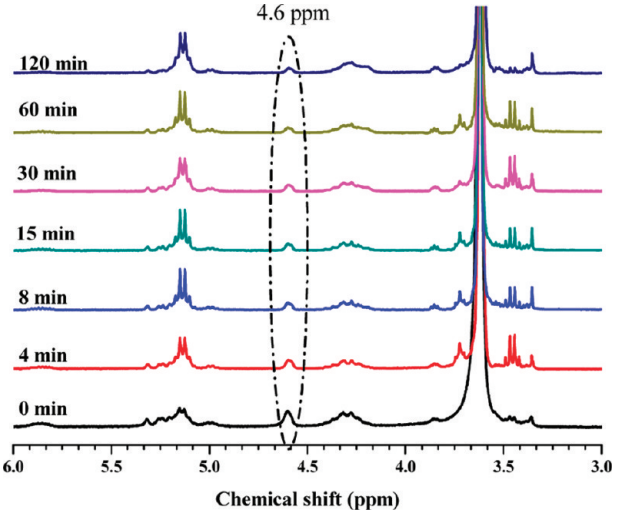


Figure 5. ^{1}H NMR spectra of MPEG-*b*-P(LA-*co*-MAC)20% modified with MPA (thiol/ene molar ratio was 1:1) at specific time points.

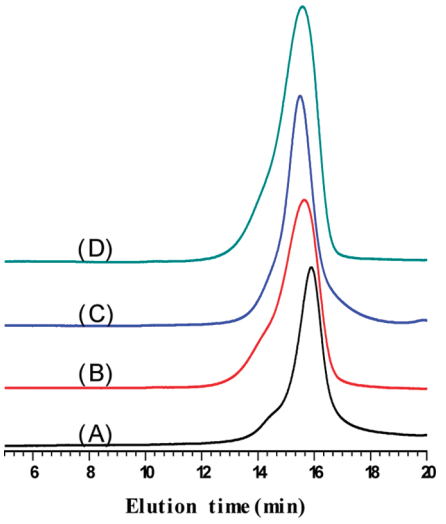


Figure 6. GPC spectra of MPEG-*b*-P(LA-*co*-MAC)20% (A) and its products after thiol−ene reaction with MPA (B), MPD (C), and AET (D), respectively.

thiol−ene reaction and protection/deprotection is not needed. Obviously, this reaction is applicable to many other functionalized mercaptans.

To test the efficiency of thiol−ene reactions, a small amount of samples were collected at different time points and ^{1}H NMR experiments were done to determine the conversion of allyl groups. As an example, Figure 3 shows the ^{1}H NMR spectra of MPEG-*b*-P(LA-*co*-MAC)20% modified with MPA at specific time points. The relative integral area of the peak at 4.6 ppm which represents the unreacted allyl groups gradually reduces with the irradiation time, while the relative integral area of the peak at 1.9 ppm (CH_2 protons from the vinyl after thiol−ene addition) which represents the addition product gradually increases with the irradiation time. The similar trends also exist in MPD and AET modifications (data not shown). In order to quantitatively illustrate the transformation of allyl groups, the conversions of allyl groups [calculated from the integral areas of the peaks at 4.6 ppm ($A_{4.6\ \mathrm{ppm}}$) and 1.9 ppm ($A_{1.9\ \mathrm{ppm}}$): $A_{1.9\ \mathrm{ppm}}/(A_{1.9\ \mathrm{ppm}} + A_{4.6\ \mathrm{ppm}})$] were plotted as a function of time (Figure 4). Given the same block copolymer, MPEG(5K)-*b*-P(LA-*co*-MAC)20%, and at the same thiol/ene molar ratio of 5:1 (Figure 4a), no significant differences exist

among the three curves, indicating similar chemical environments of the thiol groups (electronic effect, steric effect, etc.) in the three mercaptans.[53] However, the conversion curves display significant thiol/ene molar ratio dependence. As shown in Figure 4b, when the thiol/ene molar ratio decreases, the reaction rate decreases correspondingly. In order to semiquantitatively compare the conversion rates, a new parameter, T_{90}, was defined as the time required to achieve 90% conversion of allyl groups. Corresponding to thiol/ene molar ratios of 10:1 and 5:1, T_{90} values are 23 and 53 min, respectively. When the thiol/ene molar ratio decreased to 1:1, incomplete conversion of the allyl group (< 80%) was observed during the entire experiment (Figure 5). This incomplete conversion of allyl groups is attributed to the possible side reactions[53] which might consume active thiyls (Schemes S3a and S3b in Supporting Information). Because of this additional consumption of thiyl radicals, excessive amount of mercaptans is required for complete allyl conversion.[54] Therefore, a thiol/ene molar ratio of 5:1 is used in the following studies. Another possible side reaction is polymer−polymer coupling (Scheme S3c in Supporting Information), which may broaden molecular weight distribution of the copolymers obtained. In principle, increasing the thiol/ene molar ratio would suppress the polymer−polymer coupling.[35,54] As shown in Scheme S3 in the Supporting Information, both thiyl-polymer coupling and polymer−polymer coupling result in complicated derivative structures of the double bond (−CH−CH_2−SR_1) as shown in the squares in Scheme S3 in the Supporting Information, compared with that in Scheme S2 in the Supporting Information (−CH_2−CH_2−SR_1). They could be distinguished by ^{1}H NMR. Under our experimental conditions (thiol:ene = 10:1 or 5:1), the integral area ratio of the peak at 2.6 ppm (−CH_2−S−CH_2−) to 1.9 ppm (−CH_2−CH_2-CH_2−S) was 2:1 for MPA, MPD, or AET modification. In other words, neither thiyl-polymer coupling nor polymer−polymer coupling took place under our reaction conditions (otherwise, the intergral area ratio may be > 2:1). Moreover, GPC analysis

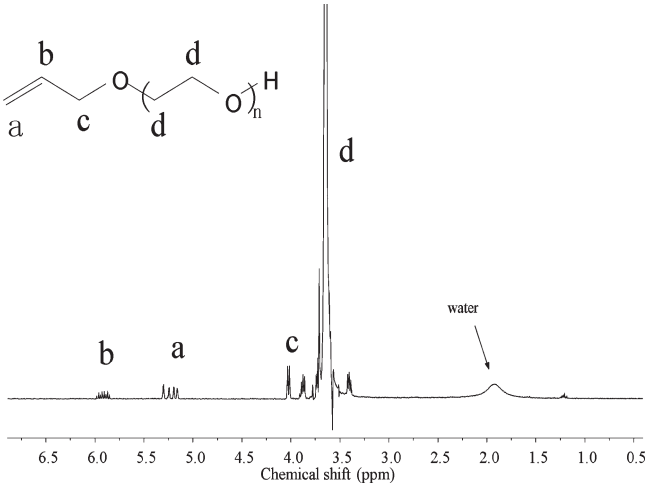


Figure 7. ^{1}H NMR spectrum of *allyl*-PEG(5K).

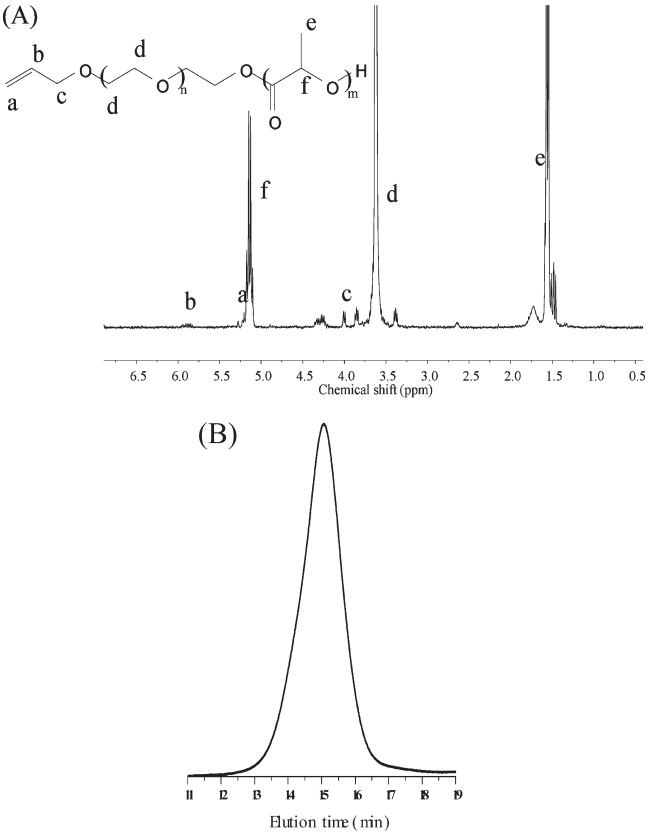


Figure 9. ^{1}H NMR (A) and GPC (B) spectra of *allyl*-PEG-*b*-PLA_{34}.

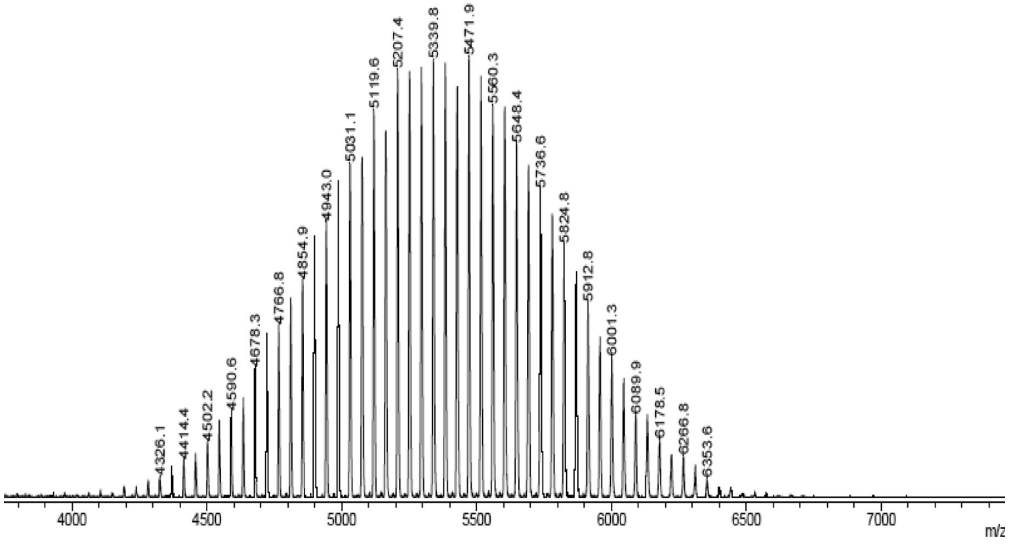


Figure 8. MALDI−TOF MS spectrum of *allyl*-PEG(5K).

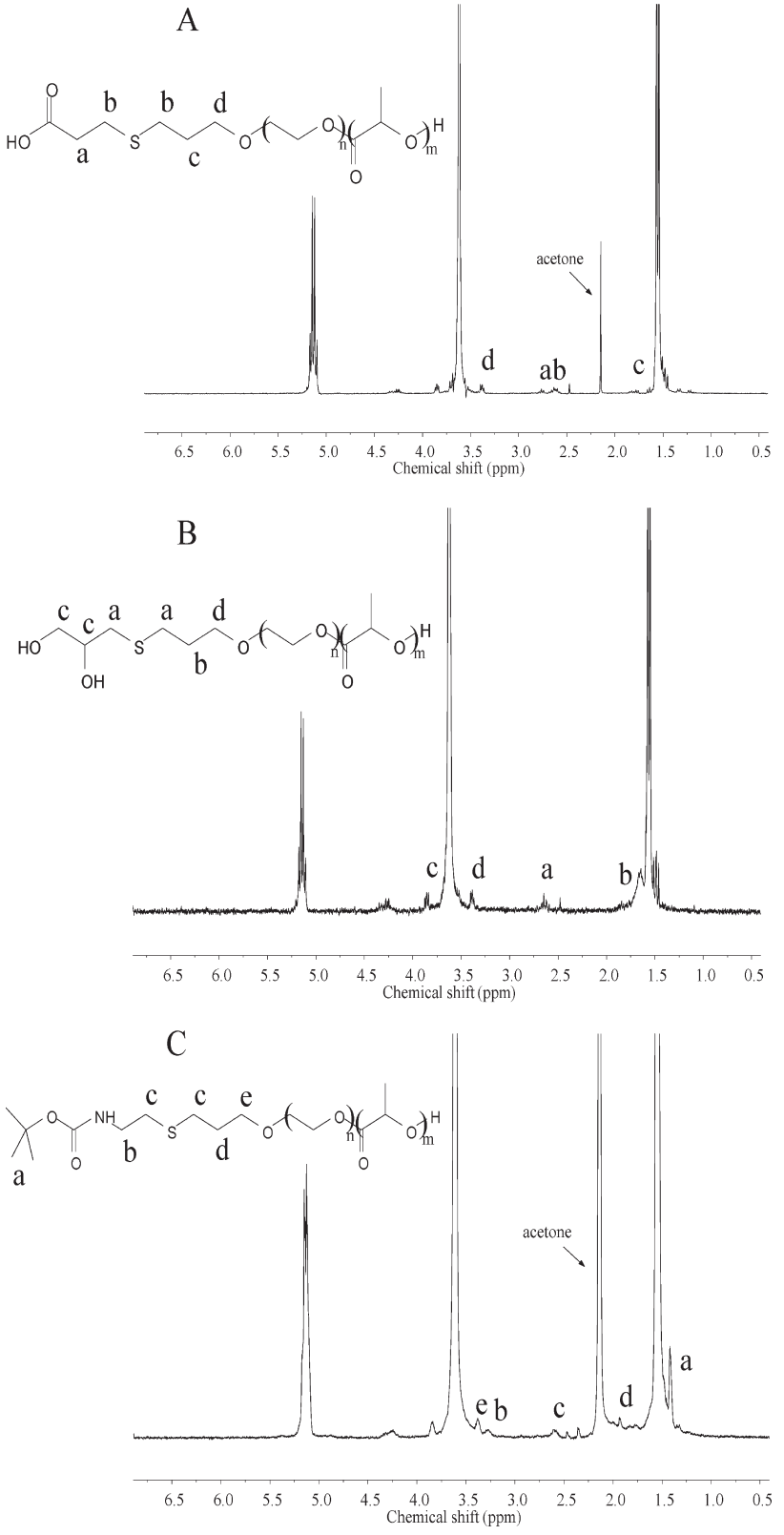


Figure 10. ^{1}H NMR spectra of *allyl*-PEG-*b*-PLA$_{34}$ modified with MPA (A), MPD (B), and AET (C), respectively.

of the modified block copolymers (Figure 6) showed little change in molecular weight or molecular weight distribution after the reactions, indicating that there were no or undetectable polymer−polymer coupling products. In addition, when the concentration of MPEG(5K)-*b*-P(LA-*co*-MAC)20% was changed from 4.0 mM to 2.0 mM in THF, little change in reaction rate has been observed (data not shown).

Synthesis of Block Copolymer *allyl*-PEG-*b*-PLA. Amphiphilic block copolymer *allyl*-PEG-*b*-PLA was synthesized generally in two steps as shown in Scheme 2. First, through allyoxyl anion ring-opening polymerization of ethylene oxide (EO), the heterobifunctional PEG having an allyl group at one end and a hydroxyl group at the other end was obtained. In Figure 7, resonances at 4.0, 5.2−5.3, and 5.8−5.9 ppm are

attributed to the end allyl group, while resonance at 3.6 ppm is attributed to the repeating unit of PEG backbone. In order to get the exact molecular weight information, MALDI-TOF MS experiment was carried out. Figure 8 shows the MS spectrum of *allyl*-PEG. The major series of the molecular masses of the product is expressed in the following equation: $M_w = 57.071\,(CH_2{=}CH{-}CH_2O) + 44.053n\,(EO) + 39.098\,(K)$, where n represents the number of repeating units in PEG. Then ROP of LA using this macroinitiator was carried out with $Sn(Oct)_2$ as catalyst. ^1H NMR spectrum (Figure 9A) clearly indicated that the allyl group was intact during polymerization, and the molecular weight distributions (Figure 9B) were relatively narrow. The degree of polymerization can be controlled by varying the molar ratio of LA to *allyl*-PEG in feeding (Table S3 in the Supporting Information).

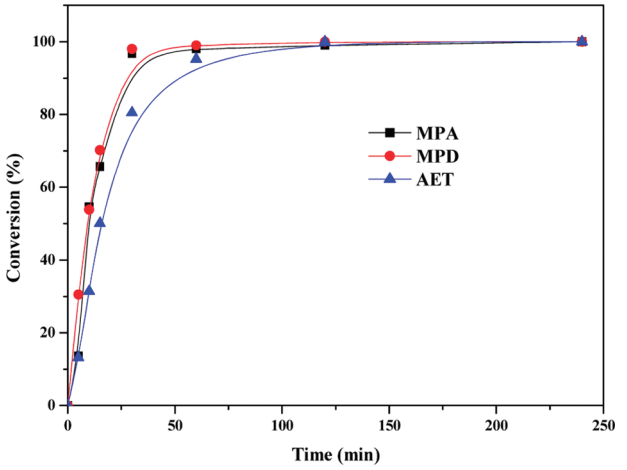


Figure 11. Conversion−time curves of the three thiol−ene reactions of *allyl*-PEG-*b*-PLA_{34} with MPA, MPD, and AET, respectively (the ratio of thiol to ene was fixed at 5:1).

Radical Thiol−Ene Addition Reactions of *allyl*-PEG-*b*-PLA with Mercaptans. In a similar way, described in the situation of MPEG-*b*-P(LA-*co*-MAC) modifications, radical thiol−ene reactions between *allyl*-PEG-*b*-PLA and the three mercaptans (MPA, MPD, and AET) were carried out in THF under UV light at room temperature. Successful modifications were confirmed by the appearance of the characteristic resonances of MPA, AET, and MPD derivatives in the ^1H NMR spectra of corresponding reaction products (Figure 10). Then, block copolymer *allyl*-PEG-*b*-PLA_{34} was selected to investigate the conversion of allyl groups [calculated from the integral area of the peak at 4.0 ppm ($A_{4.0\ ppm}$, $CH_2{=}CH{-}CH_2{-}O$) and 1.8 ppm ($A_{1.8\ ppm}$, $S{-}CH_2{-}CH_2{-}CH_2{-}O$): $A_{1.8\ ppm}/(A_{1.8\ ppm} + A_{4.0\ ppm})$] as a function of irradiation time under different reaction conditions. When the thiol/ene molar ratio was fixed at 5:1, as shown in Figure 11, no significant difference in reaction rate was observed for the three mercaptans. Compared with the situation of MPEG-*b*-P(LA-*co*-MAC) (Figure 4a), the reaction rate of allyl group at the end of PEG segment was slightly faster. The T_{90} values in Figure 11 are 33, 28, and 53 min, while those in Figure 4a are 53, 60, and 83 min for MPA, MPD, and AET, respectively. This enhanced reactivity of the terminal allyl group on PEG segment may be attributed to its terminal position and the higher mobility of the PEG segment. As in the case of MPEG-*b*-P(LA-*co*-MAC), the T_{90} of *allyl*-PEG-*b*-PLA decreased for a given mercaptan with increasing thiol/ene molar ratio but was not significantly influenced by the polymer concentration in THF (data not shown). Anyway,

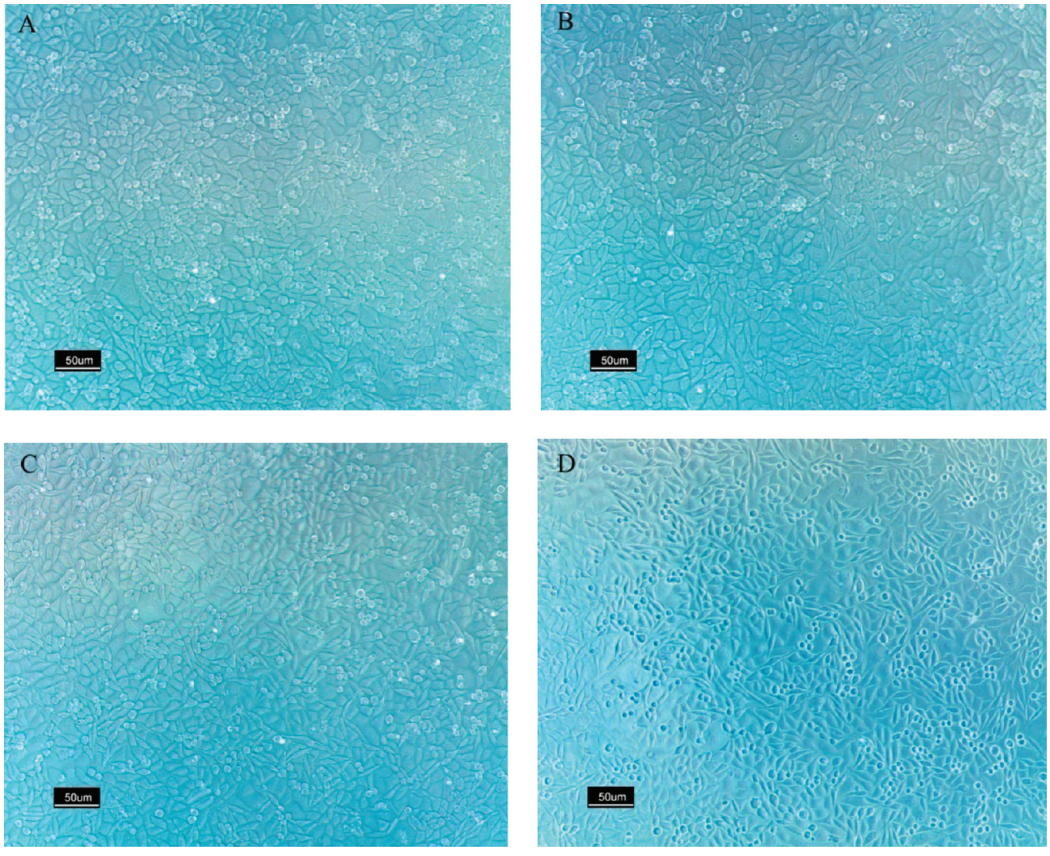


Figure 12. Microscopic images of L929 cells cultured with culture medium (A, control) or cocultured with micelles (0.5 mg/mL) functionalized with AET (B), MPA (C) and MPD (D). Incubation time: 24 h.

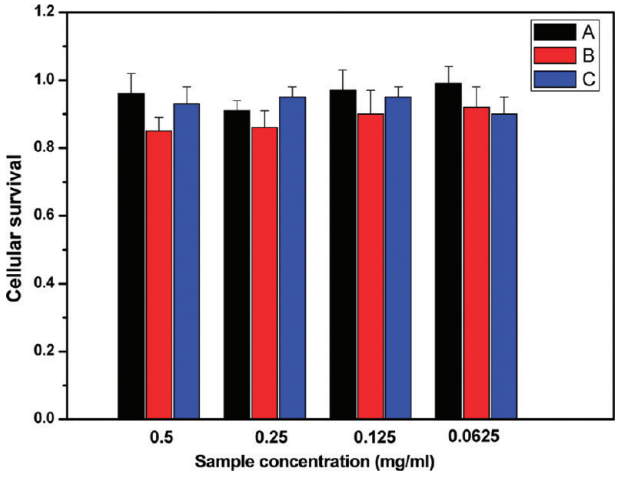


Figure 13. Cellular survival rates of L929 cells cultured with MPEG-*b*-P(LA-*co*-MAC)s modified with AET (A), MPA (B), and MPD (C) at four concentrations, deterimined by MTT method. Data are expressed as mean ±SD (n = 4).

nearly 100% conversion could be achieved in just 1−2 h under the reaction conditions employed.

Biocompatibility of the Materials after Radical Thiol−Ene Reactions. In the previous study,[49] the precursor block copolymer MPEG-*b*-P(LA-*co*-MAC) has been proved nontoxic to living cells. In the present study, mercaptans were attached to the copolymers and excessive amounts of them were introduced to the reaction system to ensure high conversion of the allyl groups and to suppress possible side reactions. Therefore, unreacted mercaptans or their reaction products may exhibit biotoxicity. To eliminate this toxicity, special purification measures were taken to remove residual marcaptans or possible impurities, including repeated precipitation and dialysis (as described in Experimental Section). In order to test the efficiency of purification and evaluate the safety of materials, cellular biocompatibility experiments were carried out using MTT method with L929 as test cell line. The modified MPEG-*b*-P(LA-*co*-MAC) materials were tested in the form of micelles to mimic their practical applications as drug carriers. Figure 12 collects pictures taken after 24 h of culture of the cells in the presence of the micelles at the highest concentration (500 μg/mL) for each sample. As seen in the pictures, the cells occupied nearly the whole microscopic horizon for the three kinds of micelles, as good as the control. Quantitative analysis of cellular survival is shown in Figure 13. Comparing with the blank cells without any other materials added (the survival rate was defined as 100%), the survival rates of L929 coincubated with the three materials at four different concentrations all exceeded 80%, indicating that incorporation of mercaptans does not significantly influence the growth of cells. This conclusion is also applicable to the situation of block copolymer *allyl*-PEG-*b*-PLA because the mounts of mercaptans added were relatively less than those added to MPEG-*b*-P(LA-*co*-MAC). In conclusion, our materials were safe enough to be used as potential drug delivery carriers.

Conclusion

Via radical thiol−ene addition reaction, modular functionalization of two precursor amphiphilic block copolymers (MPEG-*b*-P(LA-*co*-MAC) and *allyl*-PEG-*b*-PLA) was successfully realized. Under mild conditions, the reaction was highly efficient and quantitative and the conversion of vinyl groups was mainly dependent on the thiol/ene molar ratio. Generally speaking, the higher thiol/ene molar ratio, the more quickly and completely the reaction takes place. Usage of excessive mercaptans can suppress possible side reactions and unreacted mercaptans can be effectively removed by precipitation and dialysis so that appreciable cytotoxicity of the final products is not detected by MTT. The carrier polymers MPEG-*b*-P(LA-*co*-MAC) and *allyl*-PEG-*b*-PLA and mercaptans MPA, MPD and AET in the present study are only model ones. Any allyl-bearing polymers, no matter hydrophilic or hydrophobic, can be functionalized via thiol−ene addition and other functionalized mercaptans (unprotected or protected if necessary) can be used as the modifiers. Starting from a single precursor polymer, a variety of functional groups can be introduced just by changing the structure of the mercaptans. Therefore, radical thiol−ene addition reaction is a universal, highly efficient, and safe approach to biopolymer functionalization, and can find wide applications in biomedical areas.

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Supporting Information Available: Schemes showing the synthetic route of comonomer MAC, mechanisms of radical thiol−ene reaction, and possible side reactions and tables giving data for copolymerization of LA and MAC, modifications of MPEG-(5K)-*b*-P(LA-*co*-MAC)20%,and polymerization of *allyl*-PEG and *allyl*-PEG-*b*-PLA. This material is available free of charge via the Internet at http://pubs.acs.org.

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