Reactive & Functional Polymers 70 (2010) 400–407

Contents lists available at ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

Novel poly(ε-caprolactone)s bearing amino groups: Synthesis, characterization and biotinylation

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ARTICLE INFO

Article history:
Received 6 October 2009
Received in revised form 21 March 2010
Accepted 22 March 2010
Available online 27 March 2010

Keywords:
γ-(Carbamic acid benzyl ester)-ε-caprolactone
Ring-opening polymerization
Poly(ε-caprolactone)
Baeyer–Villiger reaction
Amino groups
Biotinylation

ABSTRACT

A novel functional ε-caprolactone monomer containing protected amino groups, γ-(carbamic acid benzyl ester)-ε-caprolactone (γCABεCL), was successfully synthesized. A series of copolymers [poly(CL-*co*-CABCL)] were prepared by ring-opening polymerization of ε-caprolactone (CL) and γCABεCL in bulk using tin (II)-2-ethylhexanoate [$Sn(Oct)_2$] as catalyst. The morphology of the copolymers changed from semi-crystalline to amorphous with increasing γCABεCL monomer content. They were further converted into deprotected copolymers [poly(CL-*co*-ACL)] with free amino groups by hydrogenolysis in the presence of Pd/C. After deprotection, the free amino groups on the copolymer were further modified with biotin. The monomer and the corresponding copolymers were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass, GPC and DSC analysis. The obtained data have confirmed the desired monomer and copolymer structures.

1. Introduction

In the past few decades, there has been a paradigm shift in the biomedical and pharmaceutical fields from biostable biomaterials to biodegradable biomaterials [1,2]. Among the various families of biodegradable polymers, aliphatic polyesters [3–5], such as poly (ε-caprolactone) (PCL), poly(L-lactide) (PLA) and polyglycolide (PGA), have taken a leading position over the others because of their excellent biodegradability, biocompatibility and mechanical properties. PCL especially has been widely explored due to its distinguished properties of nontoxicity and permeability to a wide range of drugs [6,7]. However, PCL degrades extremely slowly in vitro and in vivo due to its high hydrophobicity and crystallinity. Moreover, the lack of pendant reactive functional groups to which bioactive compounds can be covalently attached has severely limited its biomedical application. In recent years, different chemical methods have been applied to introduce hydroxyl [8,9], carboxylic acid [10], carbonyl [11], vinyl [12] and halogen [9,13] substituents onto the PCL chain. The introduction of these pendant functional groups regulates and adjusts the physico-chemical properties of the material, such as crystallinity, hydrophilicity, biodegradation rate, and biological activity. Therefore, modification of PCL is of great necessity.

In general, there are two main strategies available for modifying PCL with pendant functional groups. The first approach is postpolymerization modification. For example, Philippe Lecomte and coworkers have synthesized PCL bearing pendant chloride substituents, which could be easily converted into azides. These pendant azides made it possible to graft a variety of substituents onto PCL using the "click reaction" [14]. The second route is polymerization of functionalized monomers. In order to prevent inter- and intramolecular side reactions, the functional monomers have to be protected prior to polymerization and deprotected afterwards. Lavasanifar et al. synthesized a novel monomer, α-benzyl carboxylate-ε-caprolactone, from ε-caprolactone by deprotonation using LDA followed by an electrophilic substitution with benzyl chloroformate. The copolymerization of the monomer with CL was then carried out using methoxy PEO as an initiator and tin (II)-2-ethylhexanoate [$Sn(Oct)_2$] as a catalyst to get block copolymers. The block copolymers bearing carboxyl groups were finally obtained by catalytic debenzylation [10]. Moreover, these pendant functional groups open the door for further modification such as grafting, crosslinking and attaching bioactive substances or drugs for biomedical applications [15,16].

Biomolecule–polymer conjugates are widely used in tissue engineering and drug delivery [17–19]. Moreover, it would be desirable for biomaterials to have the ability to recognize and immobilize specific proteins or drugs. The biotin–avidin (or streptavidin) system is extensively used currently because of their high

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doi:10.1016/j.reactfunctpolym.2010.03.008

binding affinity ($10^{13-15}\ M^{-1}$) [20]. Once biotin is attached to a polymer, it can combine with avidin (or streptavidin) due to the specific recognition, which facilitates the combination of various proteins or drugs without complicated synthesis. For example, Narain has directly prepared well-defined protein–glycopolymer bioconjugates via avidin–streptavidin binding without many protection and deprotection steps [21]. However, the biotin is usually connected to the polymer terminus, which is quite limited in a polymer [21–23]. Therefore, conjugating biotin with the pendant groups on a polymer chain would be favorable.

In this paper, we report the following achievements: (1) synthesis of a novel functional monomer [γ-(carbamic acid benzyl ester)-ε-caprolactone, γCABεCL] with protected amino groups; (2) ring-opening copolymerization of the preprotected functional monomer with ε-caprolactone (CL) to prepare PCL-based polymers; (3) removal of the protective carbobenzoxy (Cbz) groups by hydrogenolysis in the presence of Pd/C to obtain the corresponding copolymers with free amino groups; (4) grafting of biotin onto the copolymer through amide coupling reactions. To the best of our knowledge, this functional monomer with protected amino groups is a novel compound that has not been reported, and this work is the first report introducing amino functional groups onto the PCL backbone by protection and deprotection methods.

2. Experimental

2.1. Materials

Trans-4-aminocyclohexanol (Longshan Chemical Co., Ltd., Zhejiang, China, 99.9%), benzyl chloroformate (CbzCl, 98%), chromium trioxide (CrO_3) and *N*-hydroxysuccinimide (NHS) were used as received. Palladium-coated charcoal (Pd/C, 10%) was obtained from Sinopharm Chemical Reagent Co., Ltd. *m*-Chloroperoxybenzoic acid (*m*-CPBA, Acros, 70–75%) was purified according to a method described in the literature and stored under vacuum [24]. ε-Caprolactone (ε-CL, Acros, 99%) was dried over calcium hydride for 48 h followed by vacuum distillation prior to use. Tin (II)-2-ethylhexanoate [$Sn(Oct)_2$, Sigma, 95%] and *d*-biotin (Sigma) were used without further purification. All the other solvents and reagents were of AR grade and were used as received.

2.2. Measurements

NMR spectra were recorded at room temperature on a Bruker Avance series instrument (400 MHz). The molecular weights and distributions were determined by gel permeation chromatography (GPC) measurements on a Waters GPC system equipped with a Waters 2414 HPLC solvent pump, three Ultrastyragel columns (2×10^5, 10^5, and 5×10^4 Å) in series and a refractive detector. Tetrahydrofuran (THF) was used as the eluent and delivered at a flow rate of 1.0 mL/min at 35 °C. The molecular weight was calibrated with polystyrene standards. Differential scanning calorimetry (DSC) was used to study the thermal behavior of the copolymers on a DSC2910 Modulated DSC system. FT-IR spectra were recorded on a Nicolet 5700 instrument.

2.3. Benzyl 4-hydroxycyclohexanecarbamate (**1**)

A solution of sodium bicarbonate (18.8 g, 224 mmol) and trans-4-aminocyclohexanol (10.3 g, 89.4 mmol) in 600 mL of water was added into a 1000-mL flask. The solution was cooled in an ice/water bath, and benzyl chloroformate (16.6 mL, 116 mmol) was added dropwise with continuous stirring over 10 min. The reaction mixture was then heated to 45 °C, and the stirring was continued for 4 h. The mixture was extracted with ethyl acetate and butanol. After extraction, the combined organic layers were washed with 0.5 M HCl and brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated until **1** began to crystallize. The product was recrystallized overnight in the refrigerator and collected as white needles by suction filtration (17.8 g, 71.4 mmol, 80%).

^{1}H NMR ($CDCl_3$): δ = 1.12–2.15 [m, 8H, –(CH_2)$_2$–CH(OH)–(CH_2)$_2$–], 3.43 (m, 1H, –C*H*–NH–), 3.58 (m, 1H, –C*H*–OH–), 4.62 (s, 1H, –N*H*–), 5.09 (s, 2H, –CH_2–Ph), 7.30–7.37 (m, 5H, –C$_6H_5$).

IR (KBr): 3389 cm^{-1} (OH), 3341 cm^{-1} (NH), 1688 cm^{-1} (–CONH–), 780 cm^{-1}, 758 cm^{-1}, 724 cm^{-1}, 693 cm^{-1} (CH).

MS (70 eV): m/z = 249.2 (M^+), 158.1 ($M^+-C_6H_5CH_2$), 114.1 ($M^+-COOCH_2C_6H_5$), 91.1 ($C_6H_5CH_2^+$).

2.4. Benzyl 4-oxocyclohexane carbamate (**2**)

A suspension of **1** (17.4 g, 70 mmol) in acetone (250 mL) was placed in a 500-mL flask. The mixture was cooled in an ice bath, and Jones' reagent (20 mL) was added dropwise over a few minutes. When the addition of the Jones' reagent was complete, the reaction mixture was allowed to warm slowly to room temperature, stirred overnight and quenched by addition of isopropyl alcohol. After stirring for 5 min, the mixture was filtered. The filtrate was concentrated, and a proper amount of sodium bicarbonate solution was added until the pH of the reaction mixture was tested neutral. The orange aqueous mixture was extracted three times with ethyl acetate. Then the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by recrystallization (EtOAc/hexane = 1:1) to afford the white crystalline solid **2** (12.4 g, 50 mmol, 71%).

^{1}H NMR ($CDCl_3$): δ = 1.65–2.45 [m, 8H, –(CH_2)$_2$–(O)–(CH_2)$_2$–], 3.99 (m, 1H, –C*H*–NH–), 4.80 (s, 1H, –N*H*–), 5.14 (s, 2H, –CH_2–Ph), 7.30–7.38 (m, 5H, –C$_6H_5$).

IR (KBr): 3343 cm^{-1} (NH), 1718 cm^{-1} (C=O), 1688 cm^{-1} (–CONH–), 777 cm^{-1}, 756 cm^{-1}, 723 cm^{-1}, 695 cm^{-1} (CH).

MS (70 eV): m/z = 247.2 (M^+), 140.1 ($M^+-C_6H_5CH_2O$), 112.1 ($M^+-COOCH_2C_6H_5$), 91.1 ($C_6H_5CH_2^+$).

2.5. γ-(Carbamic acid benzyl ester)-ε-caprolactone (γCABεCL) (**3**)

A 500-mL, round-bottomed flask was charged with **2** (24.7 g, 0.1 mol) and 100 mL of CH_2Cl_2. The mixture was stirred vigorously in an ice bath while a suspension of *m*-chloroperoxybenzoic acid (25.3 g, 0.11 mol) in 250 mL of CH_2Cl_2 was added dropwise. After the addition of the peracid, the reaction mixture was stirred for an additional 14 h at room temperature. The solution was then washed successively with sodium thiosulfate solution (three times), bicarbonate solution (three times) and sodium chloride solution (three times). After the organic layer was dried over Na_2SO_4, it was filtered, concentrated and purified by recrystallization (EtOAc/hexane = 3:2) to obtain white needle crystals (18.41 g, 70 mmol, 70%), m.p. 116–117 °C, 99.9% purity as determined by HPLC analysis (recrystallized from dry toluene three times).

^{1}H NMR ($CDCl_3$): δ = 1.52–2.29 [m, 4H, –CH_2–CH(NH)–CH_2–], 2.53–2.62 (m, 2H, –C(O)–CH_2–CH$_2$–), 3.82 (s, 1H, –C*H*–NH–), 4.14–4.31 (m, 2H, –O–CH_2–CH$_2$–) 4.76 (s, 1H, –N*H*–), 5.09 (s, 2H, –CH_2–Ph), 7.30–7.37 (m, 5H, –C$_6H_5$).

^{13}C NMR ($CDCl_3$): δ = 29.5 [–CH_2–CH$_2$–C(O)O–], 30.9 [–CH_2–CH$_2$–O–C(O)–], 36.1 [–CH$_2$–CH_2–C(O)O–], 51.5 [–CH$_2$–C*H*(NH)–CH$_2$–], 66.1 [–CH$_2$–CH_2–O–C(O)–], 67.4 [C$_6H_5$–CH_2–O–C(O)–], 128.7, 128.8, 129.1, 136.8 (–C$_6H_5$), 156.0 (–O–C*O*–NH–), 175.6 (–CH$_2$–C*O*–O–).

IR (KBr): 3337 cm^{-1} (NH), 1719 cm^{-1} (–CO(O)–), 1688 cm^{-1} (–CONH–), 780 cm^{-1}, 758 cm^{-1}, 724 cm^{-1}, 693 cm^{-1} (CH).

MS (70 eV): m/z = 263.1 (M^+), 156.1 ($M^+-C_6H_5CH_2O$), 128.1 ($M^+-C_6H_5CH_2O$–CO), 91.1 ($C_6H_5CH_2^+$).

2.6. Copolymerization of CL and γCABεCL

The copolymer poly(CL-*co*-CABCL) was synthesized by ROP of CL and γCABεCL using trace water as initiator and $Sn(Oct)_2$ as catalyst. A typical example of the synthetic procedure is described as follows. Under the protection of argon, 1.71 g of CL (15 mmol), 0.44 g of γCABεCL (1.67 mmol) and a stirring bar were quickly put into a flame-dried polymerization tube. The tube was connected to a Schlenk line, where an exhausting–refilling process was repeated several times. The tube was then placed into an oil bath at 50 °C with vigorous stirring until the mixture became homogeneous and clear. To obtain the desired monomer/catalyst molar ratio, $Sn(Oct)_2$ in dry toluene was added to the polymerization tube by using a microliter syringe, and the exhausting–refilling process was conducted again to remove the toluene. The tube was then sealed under vacuum and placed in an oil bath at 130 °C for 24 h. The polymerization was stopped by cooling the tube to room temperature. The resulting product was dissolved in chloroform and precipitated by addition to cold methanol under vigorous stirring. The copolymer was collected and dried in vacuo before characterization.

^{1}H NMR ($CDCl_3$): δ = 1.33–1.40 (2H, –CH_2–C*H*$_2$–CH_2–CH_2–O–), 1.62–1.85 [8H, –CH_2–C*H*$_2$–CH_2–C*H*$_2$–CH_2–O–, –CH_2–C*H*$_2$–CH(NH)–C*H*$_2$–CH_2–O–], 2.27–2.40 [4H, –C(O)–C*H*$_2$–CH_2–CH_2–, –C(O)–C*H*$_2$–CH_2–CH(NH)–], 3.76 [1H, –CH_2–C*H*(NH)–CH_2–], 4.03–4.17 [4H, –CH_2–CH_2–C*H*$_2$–O–, –CH(NH)–CH_2–C*H*$_2$–O–], 4.82 (1H, –CON*H*–), 5.06–5.14 (2H, –C*H*$_2$–Ph), 7.30–7.40 (5H, –C_6H_5).

^{13}C NMR ($CDCl_3$): δ = 24.5 (–CH_2–C*H*$_2$–CH_2–CH_2–O–), 25.5 (–CO–CH_2–C*H*$_2$–CH_2–), 28.3 (–CH_2–C*H*$_2$–CH_2–O–), 30.2 [–CO–CH_2–C*H*$_2$–CH(NH)–], 30.8 [–CH(NH)–C*H*$_2$–CH_2–O–], 34.0 (–CO–C*H*$_2$–CH_2–CH_2–), 34.2 [–CO–C*H*$_2$–CH_2–CH(NH)–], 48.6 (–NH–C*H*–), 61.2 [–CH(NH)–CH_2–C*H*$_2$–O–], 64.1 [–CH(NH)–CH_2–C*H*$_2$–O–], 66.6 (–C*H*$_2$–C_6H_5), 128.1, 128.3, 128.5, 136.5 (–C_6H_5), 156.0 (–CO–NH–), 173.3 [–CO–CH_2–CH_2–CH(NH)–], 173.5 (–CO–CH_2–CH_2–CH_2–).

IR (KBr): 3364 cm^{-1} (NH), 3064 cm^{-1}, 3034 cm^{-1} (C–H), 1732 cm^{-1} [–C(O)O–], 740 cm^{-1}, 699 cm^{-1} (C–H).

2.7. Deprotection of poly(CL-co-CABCL) by hydrogenolysis

A solution of the protected copolymer poly(CL-*co*-CABCL) (0.5 g in 20 mL of THF) was placed into a 50-mL round-bottomed flask. Then, 0.2 g of Pd/C (10%) suspension in CH_3OH (10 mL) was added to the solution. The flask was degassed and saturated with hydrogen. The reaction mixture was stirred vigorously under a hydrogen atmosphere (balloon) for 48 h at 50 °C. Then the mixture was centrifuged at 8×10^3 rpm to remove most of the catalyst. The residual catalyst was removed by filtration through Celite using THF as the eluent. The filtrate was collected, concentrated and precipitated in cold diethyl ether to obtain the deprotected copolymer, [poly(CL-*co*-ACL)] bearing γ-amino groups. The obtained product was washed with diethyl ether several times to remove impurities and dried in vacuo at room temperature (yield: 70%).

^{1}H NMR ($CDCl_3$): δ = 1.33–1.40 (2H, –CH_2–C*H*$_2$–CH_2–CH_2–O–), 1.64–1.86 [8H, –CH_2–C*H*$_2$– CH_2–C*H*$_2$–CH_2–O–, –CH_2–C*H*$_2$–CH(NH_2)–C*H*$_2$–CH_2–O–], 2.29–2.37 [4H, –C(O)–C*H*$_2$–CH_2–CH_2–, –C(O)–C*H*$_2$–CH_2–CH(NH)–], 3.77 [1H, –CH_2–C*H*(NH_2)–CH_2–], 4.03–4.15 [4H, –CH_2–CH_2–C*H*$_2$–O–, –CH(NH_2)–CH_2–C*H*$_2$–O–].

IR (KBr): 3368 cm^{-1}, 737 cm^{-1} (NH_2), 1 733 cm^{-1} (–C(O)O–).

2.8. (Cbz)reprotection of poly(CL-co-ACL)

A typical procedure for the reprotection of poly(CL-*co*-ACL) with benzyl chloroformate is described as follows: poly(CL-*co*-ACL) (0.5 g) was dissolved in 15 mL of CH_2Cl_2, then 0.5 mL of triethylamine and 0.25 mL of CbzCl were added to the solution. The mixture was stirred at room temperature for 12 h, then precipitated in cold diethyl ether, isolated by filtration and dried in vacuum (yield: 80%).

2.9. Biotinylation

A solution of biotin (50 mg, 0.21 mmol) and *N*-hydroxysuccinimide (NHS, 23.6 mg, 0.21 mmol) in 6 mL anhydrous DMF was cooled in an ice bath, and then *N*,*N*′-dicyclohexylcarbodiimide (DCC, 55 mg, 0.27 mmol) was added portion-wise. The solution was stirred overnight at room temperature, during which time a white precipitate (DCU) was formed. The precipitate was then removed by filtration. The copolymer (0.5 g) with free amino groups was dissolved in 5 mL DMF and added drop-wise to the filtrate. To the mixture was added triethylamine (0.41 mL, 2.9 mmol), and the reaction was stirred for another 24 h under N_2. The resulting reaction solution was filtered, dialyzed in DMF with a dialysis membrane (cutoff M_n 3000) and condensed in vacuum. The condensation product was precipitated in a large amount of diethyl ether, filtered and washed with diethyl ether several times. The white precipitates were dried under vacuum (yield: 70%).

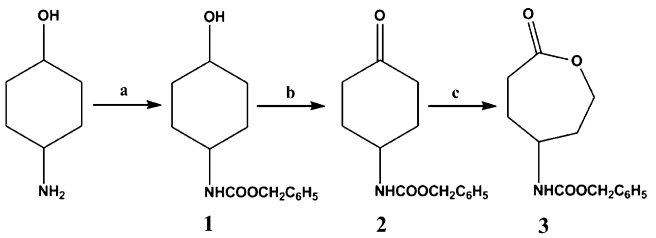
^{1}H NMR (DMSO-d_6): δ = 1.33–1.40 (2H, –CH_2–C*H*$_2$–CH_2–CH_2–O–), 1.25–1.67 (6H, biotin, –C*H*$_2$–C*H*$_2$–C*H*$_2$–CH–), 1.64–1.86 [8H, –CH_2–C*H*$_2$–CH_2–C*H*$_2$–CH_2–O–, –CH_2–C*H*$_2$–CH(NH)–C*H*$_2$–CH_2–O–], 2.21 [2H, biotin, –C(O)–C*H*$_2$–], 2.29–2.37 [4H, –C(O)–C*H*$_2$–CH_2–CH_2–, –C(O)–C*H*$_2$–CH_2–CH(NH)–], 2.54–2.86 (2H, biotin, –S–C*H*$_2$–), 3.1 (1H, biotin, –S–C*H*–), 3.77 [1H, –CH_2–C*H*(NH)–CH_2–], 4.03–4.15 [4H, –CH_2–CH_2–C*H*$_2$–O–, –CH(NH)–CH_2–C*H*$_2$–O–], 4.15–4.35 (2H, biotin, –NH–C*H*–C*H*–NH–), 6.25–6.45 [2H, biotin, –N*H*–C(O)–N*H*–], 7.6 (1H, –CON*H*–).

3. Results and discussion

In the past few years, there have been several reviews of functional PCLs based on functionalized ε-caprolactone monomers, mainly focusing on the introduction of hydroxyl, carboxylic acid, carbonyl and halogen substituents [25,26]. To date, there have been few reports on PCL modified with pendant amino groups. Our work fills this gap and could potentially broaden the applications of PCL in biomedical fields due to the presence of the amine active site.

3.1. Synthesis of γ-(carbamic acid benzyl ester)-ε-caprolactone (γCABεCL)

The novel functional monomer, γ-(carbamic acid benzyl ester)-ε-caprolactone (γCABεCL), was prepared by a three-step reaction from trans-4-aminocyclohexanol. The reaction pathway is shown in Scheme 1. Benzyl chloroformate was used to protect the amino group. Then the resultant alcohol **1** was oxidized into the corresponding ketone **2** by the use of Jones' reagent. By a Baeyer–Villiger oxidation, ketone **2** was converted into the corresponding lactone **3** by using *m*-chloroperoxybenzoic acid at room temperature. The products in each step were easily purified by recrystallization, and the yields were between 70% and 80%. The structure of the



Scheme 1. The general synthetic route for the monomer (γCABεCL): (a) CbzCl, $NaHCO_3$, H_2O; (b) acetone, Jones' reagent, 0 °C; (c) CH_2Cl_2, *m*-CPBA, 0 °C.

monomer was confirmed by the combined analysis of ^{1}H NMR, ^{13}C NMR, FT-IR, and mass spectrometry. The ^{1}H NMR spectrum of γCABεCL, shown in Fig. 1A, was consistent with the desired structure: peaks g and h correspond to the benzyl protons in the Cbz group, peak f is the proton of amide bond, and the remaining peaks are assigned to the corresponding protons on the ε-caprolactone ring. The chemical structure was also confirmed by the ^{13}C NMR spectrum, as shown in Fig. 1B. The peak at 175.57 ppm is the signal of the ester carbonyl carbon, and the peak at 156.04 ppm is the amide carbonyl carbon. The remaining peaks are assigned to the carbons of the benzyl group and the ε-caprolactone ring. The infrared spectrum (Fig. 1C) showed two sharp peaks at 1719 and 1688 cm^{-1} corresponding to the carbonyl groups of the ester and amide bonds, respectively. In mass spectrometry, the presence of the molecular ion (M^{+}) peak at $m/z = 263.1$ and other fragment ion peaks at m/z 156.1, m/z 128.1, m/z 91.1 (data not shown) provided additional evidence for the chemical structure of the monomer. Based on the HPLC analysis, the purity (99.9%) of γCABεCL was proved to be high enough for the successful polymerization.

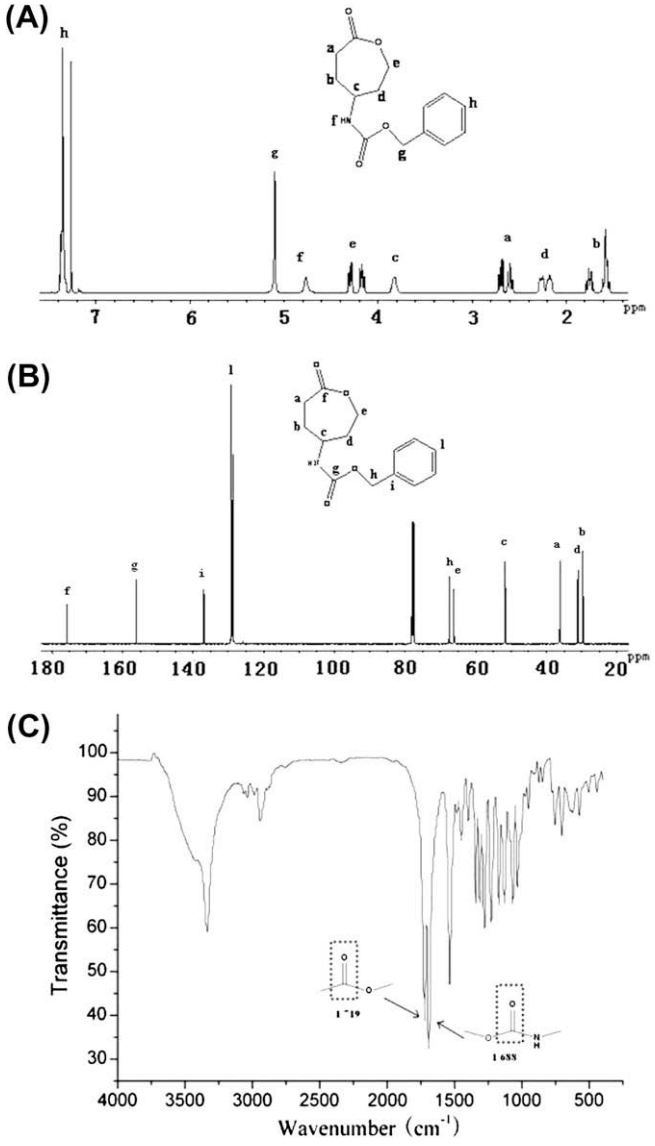
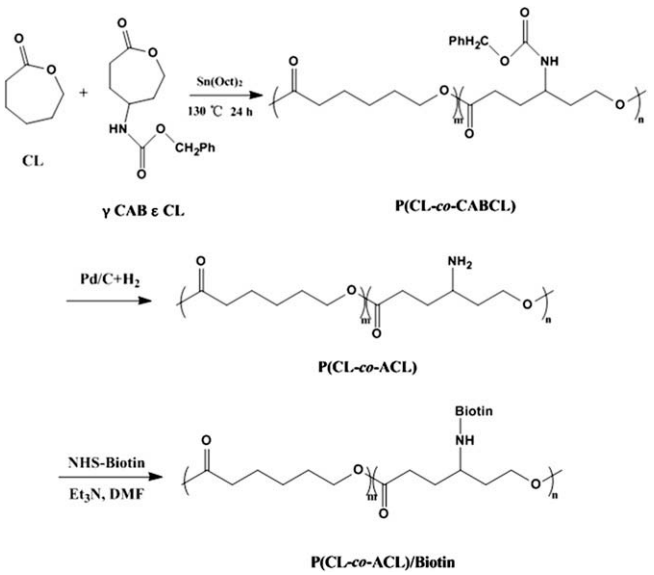


Fig. 1. (A) ^{1}H NMR spectrum of γCABεCL monomer in $CDCl_3$. (B) ^{13}C NMR spectrum of γCABεCL monomer in $CDCl_3$. (C) IR spectrum of γCABεCL monomer. The arrows indicate the characteristic peaks of the monomer.

3.2. Copolymerization of γCABεCL with CL

Novel copolymers poly(CL-*co*-CABCL) with different compositions were synthesized by ring-opening polymerization of γCABεCL and CL at 130 °C in bulk. $Sn(Oct)_2$ was chosen as the catalyst because of its high efficiency for lactones and low toxicity. Scheme 2 shows the synthesis and the subsequent deprotection of poly(CL-*co*-CABCL). Table 1 summarizes the results of the copolymerization of γCABεCL and CL with various molar ratios of the two monomers. It has been reported that the percentage contents of the functional monomer for the further modification of polyesters by attaching bioactive substances or drugs, such as RGD, biotin and paclitaxel, was normally between 5 mol% and 10 mol% [27–30]. In this article, with the goal of modifying PCL by incorporating a certain amount of amino groups, we focused on the investigation of the molar feed ratios of γCABεCL/(γCABεCL + CL) below 25 mol%, which was sufficient for the further biomodification [29]. Using ^{1}H NMR (Fig. 2A), the copolymer composition could be calculated from the relative peak areas of the phenyl ring ($-C_6H_5$) protons at 7.30–7.40 ppm of the γCABεCL repeat units and the methylene ($-CH_2-O-$) protons at 1.3–1.43 ppm of the CL repeat units. It can be seen that the molar fraction of the γCABεCL unit in the copolymer was slightly lower than the monomer feed ratio, indicating that the reaction activity of CL was a little higher than that of γCABεCL under the reaction conditions we used. The polymerization yields were relatively high, ranging from 80% to 95%. The products we obtained were white solids [γCABεCL/(γCABεCL + CL) < 19 mol%] or transparent viscous liquids [γCABεCL/(γCABεCL + CL) > 19 mol%]. The GPC curves of all the copolymers with different compositions exhibited a unimodal peak, indicating that the copolymers had been exclusively obtained without homopolymers of PCL or PCABCL. The molecular weight of the copolymers was in the range of $1.9–2.4 \times 10^{4}$, and the polydispersity was 1.6–2.4. The polydispersities of these polymers were relatively high, which may be attributed to the difference in the reactivities of the two monomers. The Cbz substituent at the γ position of the monomer γCABεCL may cause steric hindrance and lead to a decrease in reactivity accordingly. Fig. 3 [C(I)] displays a typical GPC trace (Table 1, entry 1).

The structure of the copolymers was characterized by measuring ^{1}H and ^{13}C NMR spectra in $CDCl_3$. Fig. 2A shows a typical ^{1}H NMR spectra of copolymer P(CL-*co*-CABCL) (Table 1, entry 3). The



Scheme 2. Synthesis and biotinylation of poly(CL-*co*-ACL).

Table 1
Bulk copolymerization of CL with γCABεCL catalyzed by Sn(Oct)2.[a]

Entry	γCABεCL Content (mol%)		Copolymer			Yield (%)
	In feed	In polymer[b]	M_n[c]	M_w[c]	M_w/M_n[c]	
1	5	4.8	23,100	43,400	1.88	95
2	7.5	6.1	19,100	32,200	1.68	88
3	12.5	11.8	21,900	41,800	1.91	90
4	14	13.4	21,200	35,900	1.69	89
5	19.5	18.6	23,500	58,300	2.40	85
6	24.5	22.2	21,200	41,900	1.97	80

[a] Copolymerization was carried out in bulk at 130 °C for 24 h. Molar ratio of $Sn(Oct)_2$ to monomer (CL + γCABεCL) was 1/1000.
[b] Calculated using ^{1}H NMR ($CD Cl_3$).
[c] Calculated using GPC (THF as eluent).

signals at 7.30–7.40 ppm and 5.10 ppm are assigned to the protons of the Cbz group present in the γCABεCL repeat units. The signal at 4.8 ppm is assigned to the protons of the amide bond. The remaining peaks are the signals for the corresponding protons of the copolymer backbone. In order to determine the sequence distribution of the copolymer, we also synthesized the homopolymers of PCL and PCABCL. The distribution of the major resonances was based on their ^{13}C NMR spectra analysis. The full ^{13}C NMR spectrum of the copolymer (Table 1, entry 3) is shown in Fig. 2B, and all the corresponding peaks have been marked. As reported in previous literature studies [31,32], the sequences formed by the copolymerization were thought to be the following four possible diads: CL–CL, CABCL–CL, CL–CABCL, CABCL–CABCL. Fig. 2C shows the comparison of the expanded ^{13}C NMR spectrum of the ester carbonyl carbons of both homopolymers and copolymer (Table 1, entry 6). Four resonance signals are observed in the carbonyl region (173.48, 173.33, 173.29 and 173.07 ppm). The peak at 173.48 ppm is assigned to the diad CL-CL, which is the major sequence distribution in the copolymer. The peaks at 173.33 and 173.29 ppm are due to the CABCL–CL and CL–CABCL diads. The equal intensity of these two diads illustrates the identical probability of CL and CABCL linkage. The diad CABCL–CABCL corresponds to the peak at 173.07 ppm, which shows a low intensity because of the low γCABεCL content in the feed composition in the present study. In addition, the sequence distribution of the copolymer can also be assessed by ^{13}C NMR analysis in the range of 60–65 ppm for the oxymethylene carbon atom, displayed in Fig. 2D. Four resonance peaks were found (64.32, 64.07, 61.36 and 61.15 ppm). Compared with the homopolymers of PCL (diad CL–CL: 64.06 ppm) and PCABCL (diad CABCL–CABCL: 61.39 ppm) [the

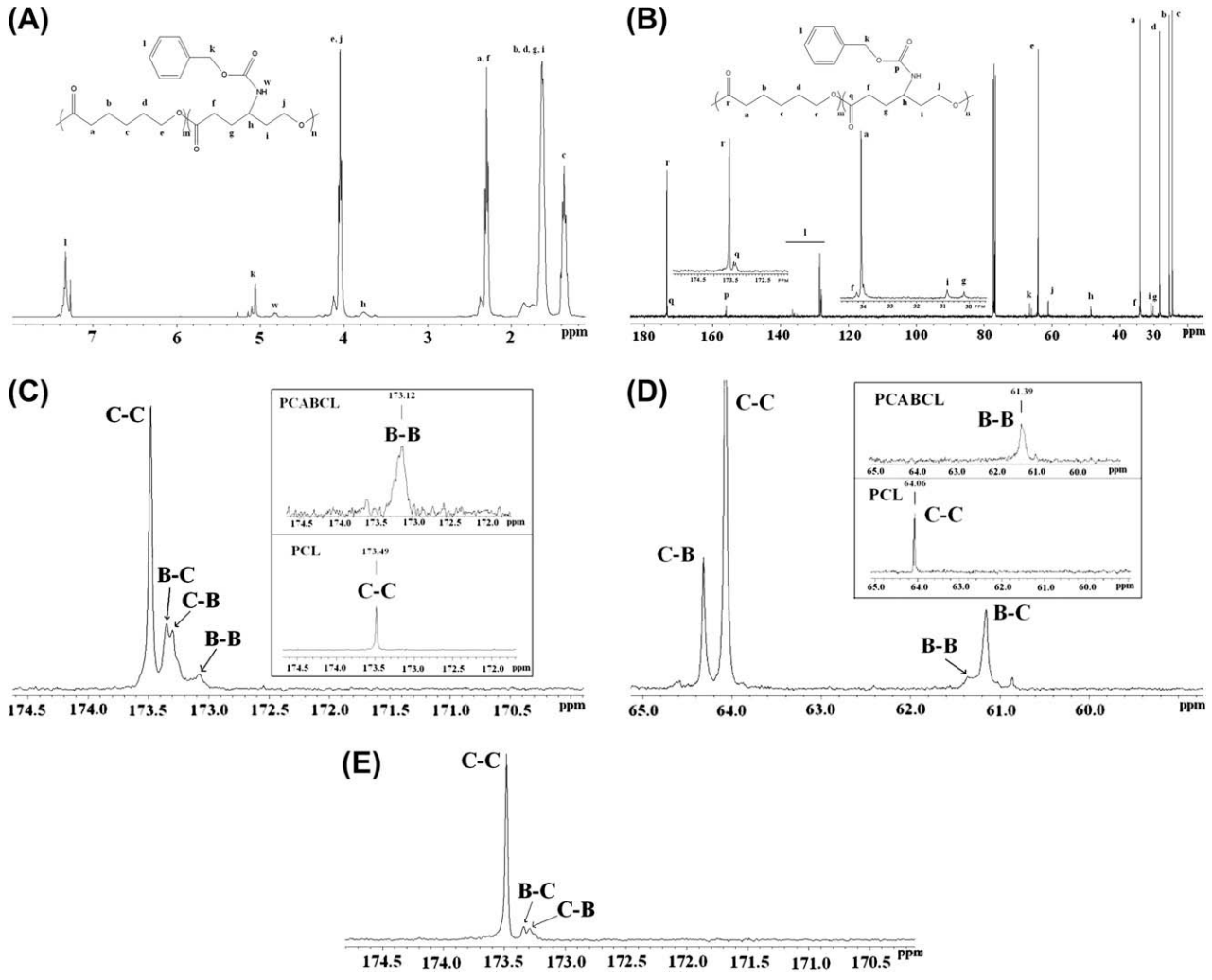


Fig. 2. (A) ^{1}H NMR spectrum of copolymer P(CL-*co*-CABCL) in $CDCl_3$ (Table 1, entry 3). (B) ^{13}C NMR spectrum of P(CL-*co*-CABCL) in $CDCl_3$ (Table 1, entry 3). (C) Expanded ^{13}C NMR spectrum in the carbonyl carbon region (Table 1, entry 6) compared with the homopolymers of PCL and PCABCL. (D) Expanded ^{13}C NMR spectrum in the oxymethylene carbon region (Table 1, entry 6) compared with the homopolymers of PCL and PCABCL. (E) Expanded ^{13}C NMR spectrum in the carbonyl carbon region (Table 1, entry 3). C represents CL, B represents CABCL.

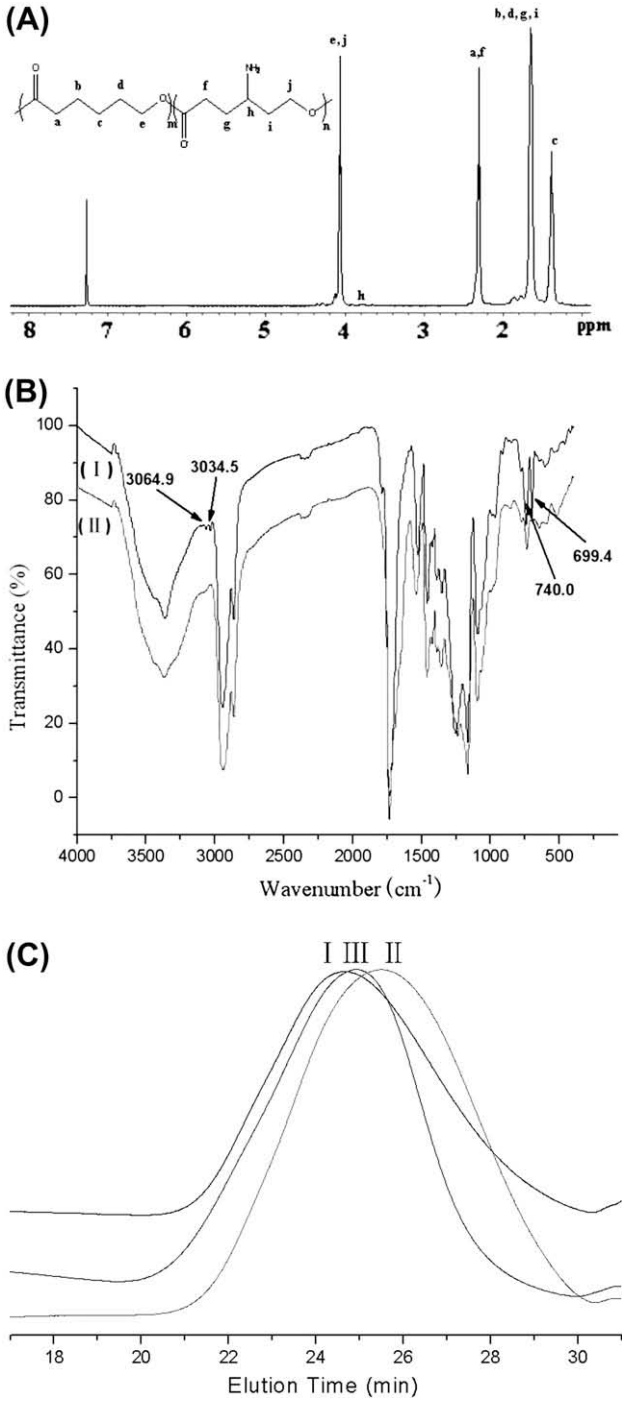


Fig. 3. (A) ^{1}H NMR spectrum of copolymer P(CL-*co*-CABCL) (Table 1, entry 1) after deprotection in $CDCl_3$. (B) FT-IR spectra of (I) P(CL-*co*-CABCL) and (II) P(CL-*co*-ACL) (Table 1, entry 5). (C) GPC curves of (I) P(CL-*co*-CABCL), (II) P(CL-*co*-ACL) and (III) reprotection of P(CL-*co*-ACL) (Table 1, entry 1).

illustrations in Fig. 2D], the additional peaks observed in the expanded ^{13}C NMR spectrum of the copolymer are assigned to the diads of CL–CABCL (64.32 ppm) and CABCL–CL (61.15 ppm) due to the sensitivity of the resonance of the oxymethylene carbon of each unit. The expanded carbonyl group region of the ^{13}C NMR spectrum of the copolymer (Table 1, entry 3) is shown in Fig. 2E. Compared with Fig. 2C, we found that the signal intensity of the CABCL–CL and CL–CABCL diads weakened with decreasing the γCABεCL molar content to 12% in copolymers, and no CABCL–CABCL diad was observed at this level. From these results, the copolymers poly(CL-*co*-CABCL) are considered to be statistical copolymers [28].

3.3. Deprotection by hydrogenolysis

The Cbz group is one of the most commonly used amino protecting groups in a variety of synthetic strategies. Several methods to remove Cbz groups have been reported in the literature [33,34]. In this paper, deprotection of the NH_2 groups was carried out by hydrogenolysis with Pd/C (10%) as the catalyst and H_2 as the hydrogen donor in THF/CH_3OH. ^{1}H NMR and FT-IR spectra confirmed the successful removal of the Cbz groups in the copolymer. As shown in Fig. 3A, no proton signals are observed at 7.30–7.40 and 5.10 ppm, which are the regions corresponding to the aromatic and the methylene protons of the Cbz group, respectively, yet the other signals are basically unchanged. In the FT-IR spectra (Fig. 3B), the ν_{CH} vibration at 3064.9, 3034.5 cm^{-1} and the γ_{CH} vibration at 740.0, 699.4 cm^{-1} of the benzene rings disappeared after hydrogenation, also indicating the absence of the Cbz group in the P(CL-*co*-ACL) copolymer [27]. The vibration at 737.5 cm^{-1} is the wagging vibration of the primary amine. Reprotection of poly(CL-*co*-ACL) with benzyl chloroformate was carried out to investigate the possibility of degradation during the reaction. Typical GPC curves of these copolymers are shown in Fig. 3C. The slight change in the molecular weight between the protected and deprotected polymer (from 23,100 to 18,000) could be attributed to either the loss of Cbz groups or the interaction between the amino groups of polymer and the filler (PS) of GPC [35]. After reprotection, the reprotected copolymer had a similar molecular weight to the protected copolymer. Additionally, the GPC trace of the deprotected copolymer still exhibits a unimodal peak, which hints that the degradation of the copolymer could be negligible [27]. In summary, there is no obvious chain scission occurring during hydrogenation. All these results demonstrated that the Cbz groups were completely removed, and the deprotection was successful.

3.4. Thermal properties of the copolymers

The thermal behavior of the copolymers before and after deprotection was studied by DSC analysis. Table 2 summarizes the composition dependence of T_g, T_m and ΔH_m of the polymers measured by DSC. Fig. 4A shows the DSC curves of some typical samples before protection. As expected, the homopolymer PCL was a semi-crystalline material with a melting temperature (T_m) at 58.1 °C and a glass transition temperature (T_g) at −62.9 °C. The homopolymer PCABCL was completely amorphous, with a T_g at −2.4 °C. The melting temperature was found to decrease with the increase of the γCABεCL content in the copolymer. Especially when the content of γCABεCL increased to about 20 mol%, no melting peaks were observed, and the morphology of the copolymers changed from semi-crystalline to amorphous. This change might be attributable to the random γCABεCL component in the copolymers intensively disrupting the crystallinity of the PCL chain [29].

In addition, we also studied the thermal properties of the deprotected copolymers, as shown in Fig. 4B. The T_g of the deprotected polymers was higher than that of PCL (T_g = −62.8 °C). This result was due to the formation of intermolecular hydrogen bonds between the amino groups, which hindered the movement of the PCL chain [37]. The fact that T_m of the deprotected copolymer was sharper and higher than that of the protected one was attributed to the increase of the weight percentage of CL units after removal of Cbz group [38]. As shown in Fig. 4, a unique T_g was detected for both the protected and deprotected copolymers, which further indicates that the copolymers have a random structure [27,36].

Table 2
Melting temperature, glass transition temperature, and enthalpy melting for copolymers P(CL-*co*-CABCL) and P(CL-*co*-ACL).

Entry	FγCABεCL[a]	P(CL-*co*-CABCL)			P(CL-*co*-ACL)		
		T_m (°C)[b]	T_g (°C)[b]	ΔH_m (J/g)[b]	T_m (°C)[b]	T_g (°C)[b]	ΔH_m (J/g)[b]
1	0	58.1	−62.9	83.8			
2	4.5	55.3	−55.5	77.4	60.9	−52.4	83.5
3	9.5	43.6	−44.9	47.5	49.9	−50.4	59.4
4	14	38.2	−43.0	20.2	42.7	−41.4	23.2
5	18.5	–	−40.7	–	16.0	−39.5	3.6
6	22.5	–	−35.6	–	–	−39.0	–
7	100	–	−2.4	–	–	−11.8	–

[a] The molar content of γCABεCL in the copolymer determined by ^{1}H NMR.
[b] DSC carried out under N_2 at 10 °C/min from −80 to 100 °C (second heating run).

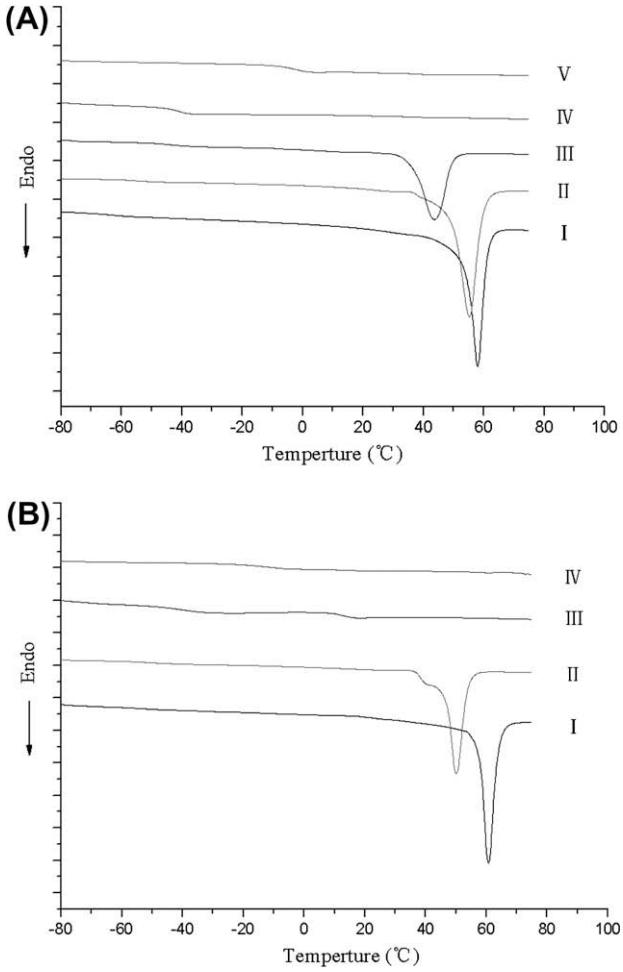


Fig. 4. (A) DSC trace of P(CL-*co*-CABCL) polymers. (I) Entry 1, (II) entry 2, (III) entry 3, (IV) entry 5, (V) entry 7 from Table 2. (B) DSC trace of P(CL-*co*-ACL) polymers. (I) Entry 2, (II) entry 3, (III) entry 5, (IV) entry 7 from Table 2.

3.5. Biotinylation

The free amino groups on the copolymer chains are capable of functionalization, which provides opportunities for covalent attachment of biological and drug molecules. To explore the activity of the free amino groups, biotin was chosen to conjugate with the amino groups of the copolymer. Fig. 5 shows the ^{1}H NMR spectra of biotin (A) and the P(CL-*co*-ACL)/biotin conjugate (B). Comparing Figs. 3A and 5A, the characteristic peaks of biotin and P(CL-*co*-ACL) could all be observed in the ^{1}H NMR spectrum of biotinylated P(CL-*co*-ACL). In Fig. 5B, the signals at 11.8 ppm for the

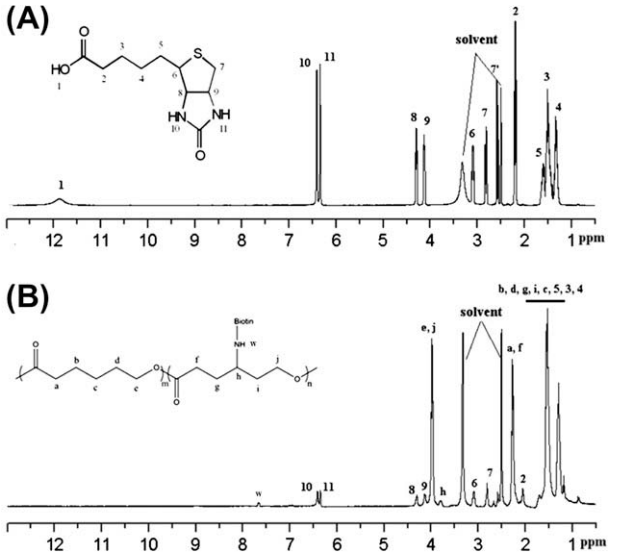


Fig. 5. ^{1}H NMR spectra of biotin (A) and the P(CL-*co*-ACL)/biotin conjugate (B) in DMSO-d_6 (Table 1, entry 4).

carboxyl groups of biotin disappeared, and the proton peaks of the amide bond appeared at 7.6 ppm. Because the unreacted biotin or NHS-biotin could be removed by dialysis, the ^{1}H NMR spectrum indicates that biotin was grafted onto the copolymer P(CL-*co*-ACL) successfully [28,39]. Analogously, other bioactive molecules could also be introduced onto PCL.

4. Conclusion

In conclusion, we have designed and synthesized a novel functional lactone, γ-(carbamic acid benzyl ester)-ε-caprolactone (γCABεCL), in three steps. The copolymers poly(CL-*co*-CABCL) were prepared by ring-opening polymerization of γCABεCL and CL using $Sn(Oct)_2$ as catalyst in bulk at 130 °C. In addition, the structures of the monomer and copolymers were extensively investigated. The incorporation of the γCABεCL unit into PCL resulted in an alteration of the morphology from semi-crystalline to amorphous. The Cbz protecting groups were successfully removed by hydrogenolysis in the presence of Pd/C to obtain PCL with free primary amino groups along the backbone. The pendant amino groups were used for the attachment of biotin to the polymer. Further investigation of the biological properties of the novel polymer is still in progress.

Acknowledgements

This research was supported by the Fundamental Research Funds for the Central Universities, the National Natural Science

Foundation of China (No. 20674019, 20804015), "Shu Guang" Project of Shanghai Municipal Education Commission, Specialized Research Fund for the Doctoral Program of Higher Education (200802511021), the Natural Science Foundation of Shanghai (08ZR1406000), Shanghai Leading Academic Discipline Project (B502) and Shanghai Key Laboratory Project (08DZ2230500).

References

[1] L.L. Hench, J.M. Polak, Science 295 (2002) 1014.
[2] R. Langer, Acc. Chem. Res. 33 (2000) 94.
[3] L.S. Nair, C.T. Laurencin, Prog. Polym. Sci. 32 (2007) 762.
[4] Y. Nakayama, S. Okuda, H. Yasuda, T. Shiono, React. Funct. Polym. 67 (2007) 798.
[5] A. Celik, N. Kemikli, R. Ozturk, A.E. Muftuoglu, F. Yilmaz, React. Funct. Polym. 69 (2009) 705.
[6] C. Allen, J. Han, Y. Yu, D. Maysinger, A. Eisenberg, J. Control. Release 63 (2000) 275.
[7] S.K. Saha, H. Tsuji, React. Funct. Polym. 66 (2006) 1362.
[8] M. Trollsas, V.Y. Lee, D. Mecerreyes, P. Lowenhielm, M. Moller, R.D. Miller, J.L. Hedrick, Macromolecules 33 (2000) 4619.
[9] S. Gautier, V. D'aloia, O. Halleux, M. Mazza, P. Lecomte, R. Jérôme, J. Biomater. Sci. Polym. Ed. 14 (2003) 63.
[10] A. Mahmud, X.B. Xiong, A. Lavasanifar, Macromolecules 39 (2006) 9419.
[11] J.P. Latere, P. Lecomte, P. Dubios, R. Jérôme, Macromolecules 35 (2002) 7857.
[12] D. Mecerreyes, J. Humes, R.D. Miller, J.L. Hedrick, C. Detrembleur, P. Lecomte, R. Jérôme, J. San Roman, Macromol. Rapid Commun. 21 (2000) 779.
[13] S.E. Habnouni, V. Darcos, J. Coudane, Macromol. Rapid Commun. 30 (2009) 165.
[14] R. Riva, S. Schmeits, C. Jérôme, R. Jérôme, P. Lecomte, Macromolecules 40 (2007) 796.
[15] A. Mahmud, X.B. Xiong, A. Lavasanifar, Eur. J. Pharm. Biopharm. 69 (2008) 923.
[16] X.B. Xiong, H. Uludag, A. Lavasanifar, Biomaterials 30 (2009) 242.
[17] R. Langer, J.P. Vacanti, Science 260 (1993) 920.
[18] R. Duncan, Nat. Rev. Drug Discovery 2 (2003) 347.
[19] X. Wang, X. Geng, L. Ye, A.Y. Zhang, Z.G. Feng, React. Funct. Polym. 69 (2009) 857.
[20] A. Senecal, J. Magnone, P. Marek, K. Senecal, React. Funct. Polym. 68 (2008) 1429.
[21] R. Narain, React. Funct. Polym. 66 (2006) 1589.
[22] A.K. Salem, S.M. Cannizzaro, M.C. Davies, S.J.B. Tendler, C.J. Roberts, P.M. Williams, K.M. Shakesheff, Biomacromolecules 2 (2001) 575.
[23] J.D. Clapper, M.E. Pearce, C. Allan Guymon, A.K. Salem, Biomacromolecules 9 (2008) 1188.
[24] N.N. Schwartz, J.H. Blumberg, J. Org. Chem. 29 (1964) 1976.
[25] X.D. Lou, C. Detrembleur, R. Jérôme, Macromol. Rapid Commun. 24 (2003) 161.
[26] W.F. Dai, M.L. Ru, Y.Y. He, M.D. Lang, Chin. Polym. Bull. 9 (2008) 10.
[27] X.L. Hu, X.S. Chen, Z.G. Xie, H.B. Cheng, X.B. Jing, J. Polym. Sci., Part A: Polym. Chem. 46 (2008) 7022.
[28] Z.G. Xie, X.L. Hu, X.S. Chen, J. Sun, Q. Shi, X.B. Jing, Biomacromolecules 9 (2008) 376.
[29] D.A. Barrera, E. Zylstra, P.T. Lansbury, R. Langer, J. Am. Chem. Soc. 115 (1993) 11010.
[30] D.E. Noga, T.A. Petrie, A. Kumar, M. Weck, A.J. Garcia, D.M. Collard, Biomacromolecules 9 (2008) 2056.
[31] D. Mecerreyes, B. Atthoff, K.A. Boduch, M. Trollsas, J.L. Hedrick, Macromolecules 32 (1999) 5175.
[32] J.L. Dwan'Isa, P. Lecomte, P. Dubois, R. Jérôme, Macromolecules 36 (2003) 2609.
[33] Y.B. Lim, Y.H. Choi, J.S. Park, J. Am. Chem. Soc. 121 (1999) 5633.
[34] J. Sun, X.S. Chen, T.C. Lu, S. Liu, H.Y. Tian, Z.P. Guo, X.B. Jing, Langmuir 24 (2008) 10099.
[35] H. Seyednejad, T. Vermonden, N.E. Fedorovich, R. van Eijk, M.J. van Steenbergen, W.J.A. Dhert, C.F. van Nostrum, W.E. Hennink, Biomacromolecules 10 (2009) 3048.
[36] S. Lenoir, R. Riva, X. Lou, C. Detrembleur, R. Jerome, P. Lecomte, Macromolecules 37 (2004) 4055.
[37] B. He, J.Z. Bei, S.G. Wang, Polymer 44 (2003) 989.
[38] Y. Kimura, K. Shirotani, H. Yanmane, T. Kitao, Polymer 34 (1993) 1741.
[39] T.C. Lu, J. Sun, X.S. Chen, P.B. Zhang, X.B. Jing, Macromol. Biosci. 9 (2009) 1059.