Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Design, Synthesis and Characterization of A Novel Cationic Polymer Poly(lactic acid-*b*-L-lysine)

Hui Yu[ab]; Xinyuan Shen[a]; Yaogang Li[a]; Yourong Duan[b]
[a] State Key Lab for Modification of Chemical Fibers and Polymer Materials, College of Materials Science & Engineering, Donghua University, Shanghai, China [b] Cancer Institute of Shanghai Jiao Tong University, Shanghai, China

Online publication date: 28 January 2010

To cite this Article Yu, Hui , Shen, Xinyuan , Li, Yaogang and Duan, Yourong(2010) 'Design, Synthesis and Characterization of A Novel Cationic Polymer Poly(lactic acid-*b*-L-lysine)', Journal of Macromolecular Science, Part A, 47: 3, 230 — 234
To link to this Article: DOI: 10.1080/10601320903526949
URL: http://dx.doi.org/10.1080/10601320903526949

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry (2010) **47**, 230–234

ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601320903526949

Taylor & Francis
Taylor & Francis Group

Design, Synthesis and Characterization of A Novel Cationic Polymer Poly(lactic acid-*b*-L-lysine)

HUI YU[1,2], XINYUAN SHEN[1], YAOGANG LI[1] and YOURONG DUAN[2,*]

[1]*State Key Lab for Modification of Chemical Fibers and Polymer Materials, College of Materials Science & Engineering, Donghua University, Shanghai 200051, China*
[2]*Cancer Institute of Shanghai Jiao Tong University, Shanghai 200032, China*

Received August 2009, Accepted September 2009

A novel biodegradable block copolymer poly(lactic acid-*b*-lysine) (PLA-*b*-PLL) has been synthesized and characterized in this study. This product was synthesized via a five-step reaction: Synthesis of hydroxyl-tailed poly(lactic acid) (PLA) by the ring-opening polymerization (ROP) of D,L-lactide in the presence of stannous octoate ($Sn(OCt)_2$) as initiator; coupling *N*-t-butoxycarbonyl-L-phenylalanine to hydroxyl-tailed PLA using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP); the amino-tailed PLA was obtained through de-protection of the Boc-protective group in trifluoroacetic acid (TFA) solution; and then ring-opening polymerization of N^{ε}-(Z)-lysine-*N*-carboxyanhydride (NCA) using the amino-tailed PLA as macro-initiator; finally removal of the Cbz-protective group of PLA-*b*-poly(N^{ε}-(Z)-L-lysine) (PLA-*b*-PLL(Z) in a mixed hydrobromic acid/acetic acid solution to give the target copolymer. The characterization of this copolymer and its precursors were performed by ^{1}H-NMR, FTIR and GPC. The block copolymer PLA-*b*-PLL, combining the characteristics of an aliphatic polyester and poly(amino acids), could be of potential interest in a variety of medical applications, such as the fields of targeted drug delivery and gene delivery systems.

Keywords: Biomaterials, poly(lactic acid), poly(amino acids), biodegradable, N-carboxyanhydride, ring-opening polymerization

1 Introduction

In the recent two decades, considerable efforts have been performed to develop various kinds of functional biodegradable polymers and many of them, such as amphiphilic copolymers, have been widely used in life science. Amphiphilic copolymers are well known for their excellent blood compatibility (1–4), and receive more and more attention for their potential application in tissue engineering and drug delivery (5–7).

Poly(lactic acid) (PLA) is a typical building block of amphiphilic copolymer, and a great deal of work has been carried out to modify its structures for the purpose of biological applications (8–10). Different biological properties could be achieved by grafting different functional groups to the polymer backbone. For an example, cationic poly(α-amino acid)s-modified PLA has relatively high cationic-charged surface (11–13) and therefore, could interact with the negative charged cellular membranes, such as those of malignant cells (14, 15). Moreover, the amino acid substituent could be biodegraded by either proteases or peptidases, and thus improved the polymer's biodegradation property (16).

Based on the above mentioned points, we proposed the design and synthesis of a novel cationic biocompatible copolymer (PLA-*b*-PLL) in this study. The synthesis outline of this copolymer was shown in Scheme 1. By combining the aliphatic polyester (PLA) and cationic poly(lysine) (PLL) segments into the copolymer via ring-opening polymerization (ROP), we expected that this new amphiphilic copolymer could achieve good capability for the delivery of bioactive drugs.

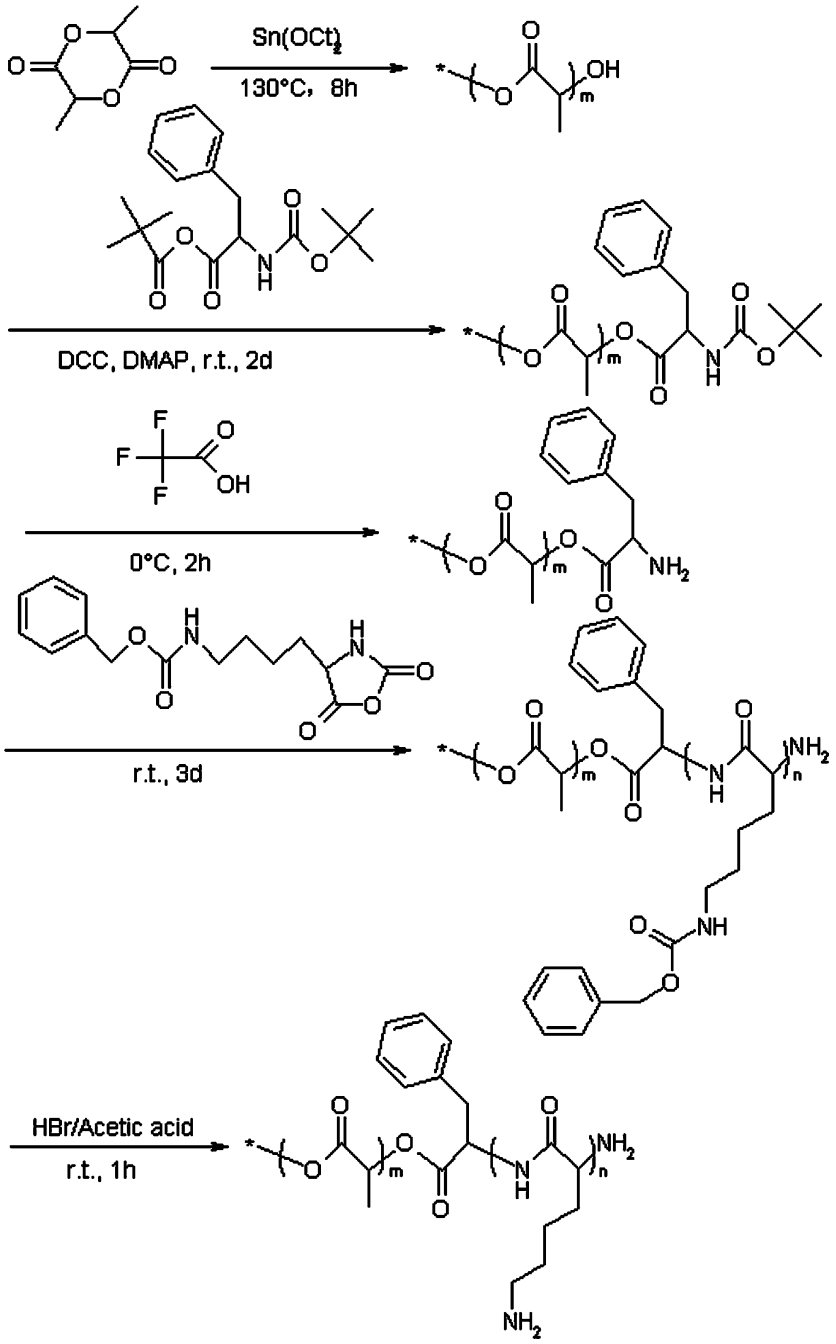
2 Experiment

2.1 Materials

D,L-lactide was purchased from GLACO (China), recrystallized from toluene and ethyl acetate and stored at −20°C before use. Trifluoroacetic acid (TFA) was purchased from Sinopharm (China) and dried over phosphors pentoxide before use.

N^{ε}-(carbonybenzoxy)-L-lysine, *N*-t-butoxycarbonyl-L-phenylalanine (Boc-L-Phe) and triphosgene were purchased from GL Biochem. Dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP) and HBr

*Address correspondence to: Yourong Duan, Cancer Institute of Shanghai Jiao Tong University, Shanghai 200032, China. Tel: +86-21-6443 7139; Fax:+86-21-6417 8312; E-mail: yrduan@sci.shmu.edu.cn



Scheme 1. Synthetic scheme of poly(lactic acid-*b*-L-lysine)(PLA-*b*-PLL).

(33%wt)/HAc were purchased from Acros. N^{ε}-(Z)-lysine-*N*-carboxyanhydride (NCA) was synthesized and purified using the method reported by Dorman et al., (17) and stored at −20°C *in vacuo*. Hexane, methylene dichloride and chloroform were refluxed over calcium hydride and distilled under nitrogen. Tetrahydrofuran (THF) was dried by sodium metal immediately before use.

2.2 Measurements

^{1}H-NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. FTIR-Raman spectra were measured using a Nicolet NEXUS-670 Fourier transform infrared spectrometer. GPC measurements were carried out on a Polymer PL-GPC 50 gel permeation chromatograph system equipped with a RI detector.

2.3 Synthesis of Hydroxyl-Tailed PLA

D,L-lactide and $Sn(OCt)_2$ (0.2 wt% of D,L-lactide) solution in hexane were added into a dry tube under the protection of nitrogen and then the hexane evaporated under vacuum. After being purged three times with N_2, the tube was flame-sealed and then immersed in a 130°C oil bath for 8 h.

Afterwards, the product was dissolved in chloroform and then excess methanol was added to precipitate it, filtered and dried under vacuum to give one hydroxyl-tailed PLA on one end.

2.4 End-capping of PLA-OH with Boc-L-Phe

10.0 g (1 mmol) of PLA-OH and 2.2 g (6.3 mmol) of Boc-L-Phe were added into a nitrogen-purged flask, followed by adding 80 ml anhydrous methylene dichloride, and then cooled to −10°C, 5 min later, 1.8 g (8.4 mmol) of DCC and 0.16 g (1.3 mmol) of DMAP were added into the flask and the solution stirred at 0°C for 48 h. Afterwards, it was filtered to collect the filtered liquid, and transferred to a separating funnel, washed with saturated aqueous sodium hydrogen carbonate (2 × 100 ml) and distilled water (2 × 100 ml), respectively. Then, excess cold methanol was added to the collected CH_2Cl_2 solution to precipitate the copolymer, filtered and dried under vacuum to give Boc-L-Phe end-capped PLA.

2.5 De-protection of Boc-L-Phe End-Capped PLA

At 0°C, 15 ml trifluoroacetic acid was added to 70 ml of anhydrous methylene dichloride containing 6 g (0.6 mmol) of Boc-L-Phe end-capped PLA, and the solution stirred for 2 h under nitrogen atmosphere. Afterwards, the solvent was evaporated under vacuum and anhydrous chloroform was added to dissolve the residue. The solution was transferred to a separatory funnel, washed with saturated aqueous sodium hydrogen carbonate (2 × 100 ml) and distilled water (2 × 100 ml), the organic phase collected, and dried over sodium sulfate. In the end, the organic phase was poured into excess of cold methanol, the insoluble solid filtered and dried under vacuum to obtain the product.

2.6 Synthesis of PLA-*b*-poly(N$^{\varepsilon}$-(Z)-L-lysine) Block Copolymer

Copolymerization was done as follows: 2.0 g (6.6 mmol) of N^{ε}-(Z)-lysine-*N*-carboxyanhydride (NCA) and 1.0 g (0.1 mmol) of amino-tailed PLA were dissolved in 80 ml of anhydrous chloroform and injected into a dry flask which had been purged with nitrogen three times. After the solution had been stirred for 72 h at room temperature, it was concentrated to 30 ml by rotary evaporation and poured into an excess of cold diethyl ether to precipitate the solid, filtered and dried *in vacuo* to give PLA-*b*-poly(N^{ε}-(Z)-L-lysine) block copolymer.

2.7 De-protection of PLA-*b*-poly(N$^{\varepsilon}$-(Z)-L-lysine) Block Copolymer

1.0 g of PLA-*b*-poly(N^{ε}-(Z)-L-lysine) was added to a dry flask, purged with nitrogen three times and then 30 ml of hydrobromic acid/acetic acid was injected. After the slurry had been stirred for 1 h under the N_2 protection at room temperature, it was poured into excess cold diethyl ether, the insoluble solid filtered and dried under vacuum to give the di-block copolymer PLA-*b*-PLL.

3 Results and Discussion

3.1 Synthesis and Characterization of Hydroxyl-Tailed PLA

The hydroxyl-tailed PLA was characterized by both IR and ^{1}H-NMR spectroscopy and the results are shown in Figures 1(a) and 2(a), respectively. From Figure 1(a), the major peaks at 2900–3000 cm^{-1}, 1725 cm^{-1}, and 1080 cm^{-1}

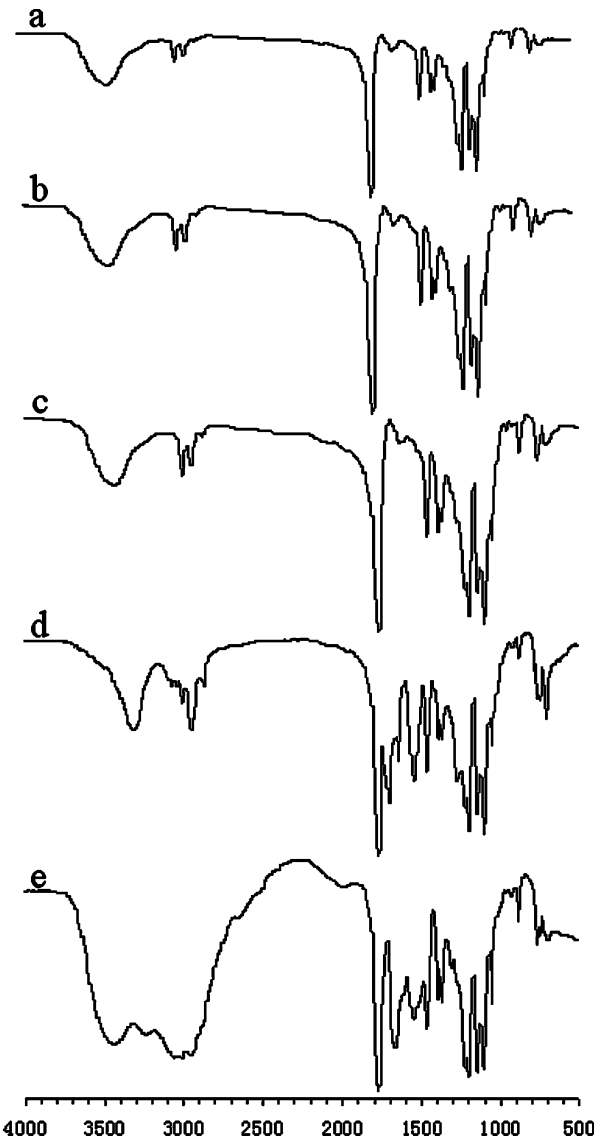


Figure 1. FITR spectra of hydroxyl-tailed PLA (a), BOC-L-Phe-tailed PLA (b), amino-terminated PLA (c), PLA-*b*-PLL(Z) (d), and PLA-*b*-PLL (e).

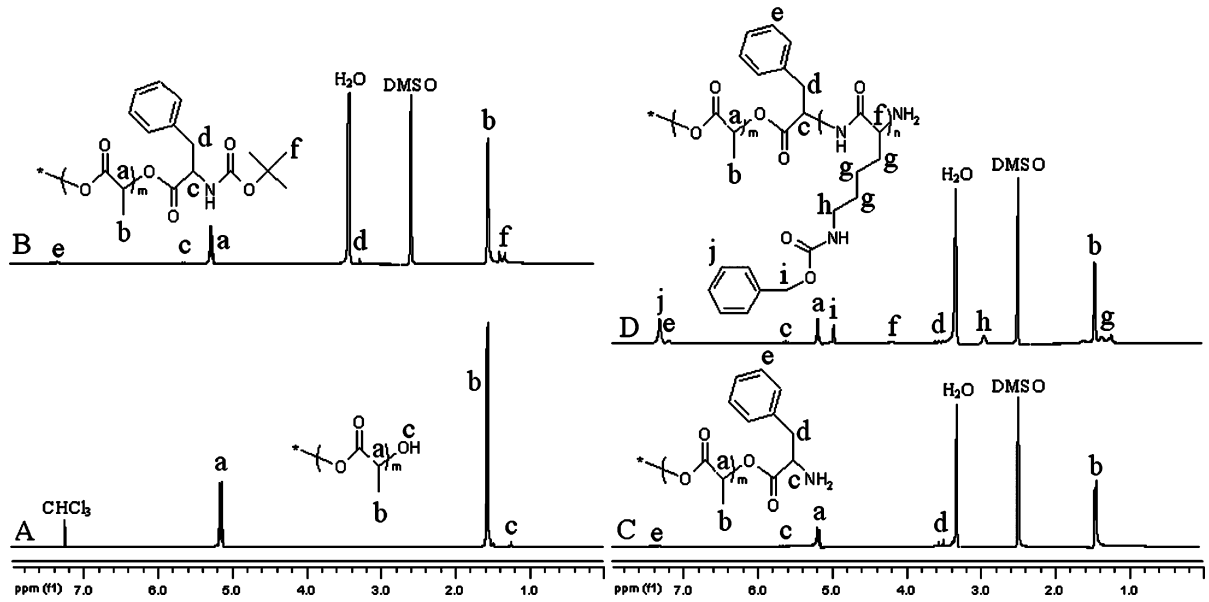


Figure 2. ^{1}H-NMR spectra of hydroxyl-tailed PLA (a), BOC-L-Phe-tailed PLA (b), amino-tailed PLA (c), PLA-*b*-PLL(Z) (d).

are assigned to C-H stretching of the polymer, its ester C=O stretching and its O-CH_2 stretching, respectively. In Figure 2(a), the chemical shifts at 1.59 and 5.09 ppm are assigned to the lactic backbone protons of this typical polymer.

3.2 Preparation and Characterization of Boc-L-Phe End-Capped PLA

Figure 2(b) shows the ^{1}H-NMR spectrum of this product. There are two new peaks, appearing at 1.29 ppm (f, $(CH_3)_3C$-) and at 7.26 ppm (g, singlet, C_6H_5-) in this ^{1}H NMR spectrum, compared with that of PLA-OH, indicating that Boc-L-Phe has been successfully conjugated to the PLA-OH backbone. Moreover, it can also be seen from this figure that almost all the hydroxyl groups of PLA were conjugated with Boc-L-Phe, judged by the ratio of peak a/peak f (*tert*-butoxycarbonyl-/CH_3O-). The GPC analysis in Figure 3(a, b) showed that the polymeric backbone was not affected because the retention time of the peak did not change too much after the reaction.

3.3 Synthesis and Characterization of Amino-tailed PLA

The amino-tailed PLA was successfully prepared by removing the protective group of Boc-L-Phe end-capped PLA in the presence of TFA. The ^{1}H-NMR spectrum of amino-tailed PLA and its GPC result are shown in Figures 2(c) and 3(c), respectively. The disappearance of the peak at 1.29 ppm (Fig. 2(c)) indicated that the *tert*-butoxycarbonyl group had been totally removed. Figure 3(c) shows that the GPC trace of PLA-NH_2 remained unchanged, indicating the polymeric backbone did not change under these conditions. Furthermore, the same absorptions at 1725 cm^{-1}(C=O), 2900 cm^{-1}, 2960 cm^{-1}(-CH_3) for all the PLA-derived compounds, as shown in Figure 1(a,b,c), confirmed that the structure of the PLA backbone was not affected.

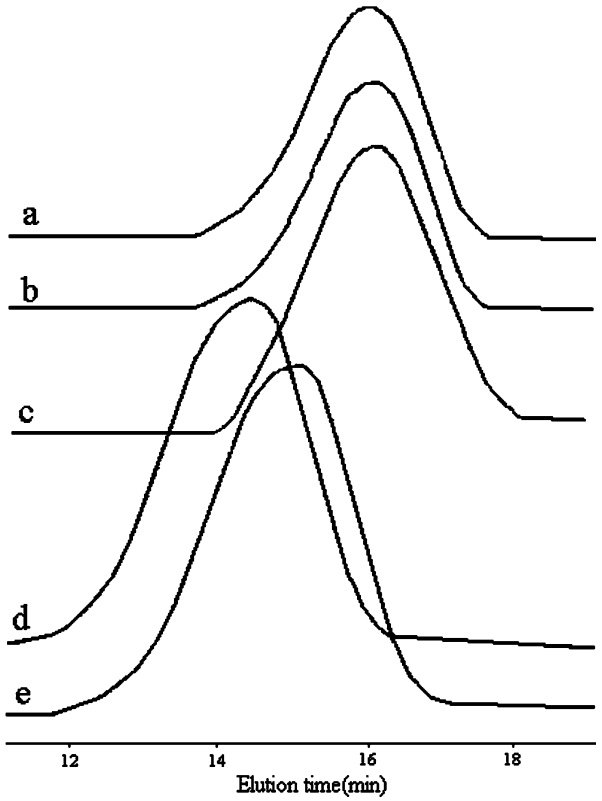


Figure 3. GPC spectra of hydroxyl-tailed PLA (a), BOC-L-Phe-terminated PLA (b), amino-terminated PLA (c), PLA-*b*-PLL(Z) (d), PLA-*b*-PLL (e).

3.4 Copolymerization and Characterization of Amino-tailed PLA and N$^{\varepsilon}$-(Z)-lysine-N-carboxyanhydride

Figures 1(d) and 2(d) show the IR spectrum of the PLA-*b*-poly(N^{ε}-(Z)-L-lysine) and its ^{1}H-NMR spectrum. As shown in Figure 1(d) the product contains two amide bands (at 1640 and 1700 cm^{-1}), amide bands (at 1540 cm^{-1}) and –NH-(at 3340 cm^{-1}). As shown in Figure 2(d), a new peak at 7.35 ppm was observed, compared with the ^{1}H-NMR of PLA-NH_2, which was attributed to the benzene ring of the protective group of (Z)-lysine and the peaks at 5.02 (i, -CH_2-), 4.20 (f, -CH-), 2.95 (h, -CH_2-) and 1.35 (g, -CH_2-) ppm were assigned to protons of the lysine segment. Therefore, both IR and ^{1}H-NMR spectra showed that the copolymerization had been successfully completed.

Based on the integral ratio of CH_3O- (b at 1.59 ppm) to –$C_6H_5CH_2OCOCH_2$- (i at 5.02 ppm) (Fig. 2(d)), the degree of polymerization (DP) of the block copolymer was calculated to be 59, which was in agreement with the starting ratio of NCA monomer to the macro-initiator. The GPC trace of the block copolymer, shown in Figure 3(d), showed that this product has a higher molecular weight because peak shifted to a shorter retention time, which meant that the co-polymerization occurred.

3.5 De-protection of PLA-*b*-poly(N$^{\varepsilon}$-(Z)-L-lysine)

The de-protection of PLA-*b*-poly(N^{ε}-(Z)-L-lysine) was carried out based on reference 18. The IR spectrum of the product was recorded and is shown in Figure 1(e). The peaks at 750 cm^{-1} and 700 cm^{-1} (δ_{CH} vibration of the benzyl group) disappeared and those at 3335 cm^{-1}, 1700 cm^{-1}, 1640 cm^{-1} and 1540 cm^{-1} remained unaffected. The results showed that the benzyl groups had been removed completely and the block copolymer backbone remained unaffected. Moreover, the GPC analysis, shown in Figure 3(e), also confirmed that the de-protection successfully occurred and the backbone was not cleaved under such conditions, since no any other new signal was detected after de-protection and the molecular weight decreased slightly.

4 Conclusions

In this study we presented a facile synthesis of a novel block copolymer PLA-*b*-PLL. First, the PLA-OH was prepared through ring opening polymerization of D,L-lactide in the presence of $Sn(OCt)_2$ and then it was coupled with Boc-L-Phe and de-protected to give the intermediate PLA-NH_2. The final product of PLA-*b*-PLL was prepared via the ring-opening polymerization of this amino tailed with NCA, followed by removal of the protective Cbz group.

All the structures, including the intermediates and the block copolymer, were characterized by ^{1}H NMR, FTIR and GPC. On going work is directed to study biological properties of this novel functional copolymer.

Acknowledgements

This research was supported by National Natural Science Foundation of China (Grant 50673058), by Science and Technology Develop Foundation of Shanghai (Grant 054119539) and by Major State Basic Research Development Program of China (973 program) (No. 2007CB935801).

References

1. Bulychev, N., Van, C.W., Dervaux, B., Kirilina, Y., Dirnberger, K., Zubov, V., Du, P.E.E. and Eisenbach, C.D. (2009) *Macromol. Chem.*, 210, 287.
2. Yu, H., Naka, Y., Shishido, A., Iyoda, T. and Ikeda, T. (2009) *Mol. Cryst. Liq. Cryst.*, 498, 29.
3. Letchford, K., Liggins, R., Wasan, K.M. and Burt, H. (2009) *Eur. J. Pharm. Biopharm.*, 71, 196.
4. Nagy, M., Szollosi, L., Keki, S., Faust, R. and Zsuga, M. (2009) *J. Macromol. Sci. A*, 46, 331.
5. Onaca, O., Enea, R., Hughes, D.W. and Meier, W. (2009) *Macromol. Biosci.*, 9, 129.
6. Volet, G. and Amiel, C. (2009) *Eur. Polym. J.*, 45, 852.
7. Zhang, Y.F., Liu, H., Dong, H.F., Li, C.H. and Liu, S.Y. (2009) *J. Polym. Sci. Pol. Chem.*, 47, 1636.
8. Pan, J., Zhao, M.M., Liu, Y., Wang, B., Mi, L. and Yang, L. (2009) J. *Biomed. Mater. Res. A*, 89, 160.
9. Zhou, Q.Y. and Xanthos, M. (2009) *Polym. Degrad. Stabil.*, 94, 327.
10. Ro, A.J., Huang, S.J. and Weiss, R.A. (2009) *Polymer*, 50, 1134.
11. Barrera, D.A., Zylstra, E., Lansbury, P.Jr. and Langer, R. (1993) *J. Am. Chem. Soc.*, 115, 11010.
12. Li, Y., Li, Q., Li, F., Zhang, H., Jia, L., Yu, J., Fang, Q. and Cao, A. (2006) *Biomacromolecules*, 7, 224.
13. Sun, J., Deng, C., Chen, X., Yu, H., Tian, H., Sun, J. and Jing, X. (2007) *Biomacromolecules*, 8, 1013.
14. Fischer, D., Bieber, T., Li, Y., Elsasser, H.P. and Kissel, T. (1999) *Pharmacol. Res.*, 16, 1273.
15. Mislick, K.A. and Baldeschwieler, J.D. (1996) *Proc. Natl. Acad. Sci. USA*, 93, 12349.
16. Fan, Y., Chen, G., Tanaka, J. and Tateishi, T. (2005) *Biomacromolecules*, 6, 3051.
17. Dorman, L.C., Shiang, W.R. and Meyers, P.A. (1992) *Synthetic Commun.*, 22, 3257.
18. Rodriguez, H.J.R., Gatti, M. and Klok, H.A. (2003) *Biomacromolecules*, 4, 249.