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# Chemozymatic synthesis and characterization of H-shaped triblock copolymer

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## Summary

The synthesis of well-defined H-shaped block copolymer based on the enzymatic ring-opening polymerization (eROP) and atom transfer radical polymerization (ATRP) is described. The dihydroxyl polycaprolactone (PCL) was synthesized by the eROP of ε-caprolactone (ε-CL) in the presence biocatalyst Novozyme 435 and initiator ethylene glycol. Subsequently, the resulting PCL was converted to tetrafunctional macroinitiator by the esterification with 2,2-dichloro acetyl chloride (DCAC). The H-shaped block copolymer was then synthesized by the ATRP of styrene. The polymers were characterized by NMR and GPC. Linear first-order kinetics, linearly increasing molecular weight with conversion, and low polydispersities observed from the ATRP of St showed that the polymerization was well controlled. $(PSt)_2$-*b*-PCL-*b*-$(PSt)_2$ block copolymers with varying molecular weight and controllable composition were obtained.

## Introduction

The design of polymers with different architectures and chemical compositions is a great challenge in polymer science. Recently, considerable progress has been made in the design and synthesis of H-shaped block copolymers. Many methods have been come forth, including living anionic polymerization (AP) [1], living cationic polymerization (CP) [2]. After the advent of controlled/"living"polymerization (i.e. atom transfer radical polymerization (ATRP) [3, 4], reversible addition–fragmentation chain transfer (RAFT) [5] and nitroxide-mediated polymerization (NMP) [6] ), it has opened a new route for preparation of well-defined block copolymer because many monomers can undergo free-radical polymerization.

In the research program on the synthesis of H-shaped polymers, the combination of various living polymerization techniques, such as ATRP and ring-opening polymerization (ROP) should be an attractive synthetic method [7-11] because various combinatorial approaches enable those polymers obtained from different types of polymerizations in one polymer. The new polymers obtained can have variable compositions and architectures, thus they may have astonishing properties.

Polymerizations catalyzed by enzymes have attracted wide attention as an important environmental friendly methodology. The green biocatalyst enzyme is a promising alternative to the conventional chemical organometallic catalyst, originating from the special properties such as its nontoxicity, recyclability, (enatio-, region- and chemo-) selectivity, biocompatibility and ability to operate under mild conditions.[12, 13] Biocatalytic approaches have been employed in the design and construction of various polymers with different structures for the increasing demands[14, 15]. However, the full exploitation of biocatalysis in polymer synthesis will require the development of mutually compatible chemo- and biocatalytic methods.

The combination of eROP and ATRP to synthesize block copolymers have been reported in the publications. Andreas Heise et al carried out the consecutive/one-pot cascade/simultaneous synthesis of the diblock copolymers by incorporating eROP with ATRP from a dual initiator, which contains a primary alcohol and single a-bromoester.[16-18] Our group has also demonstrated the feasibility of 2,2,2-trichloroethanol as another novel dual initiator, which permits a sequential two-step synthesis combining enzyme and ATRP catalysis[19, 20].

Although H-shaped block copolymers have been synthesized by many method, no investigation on the preparation of H-shaped copolymers by the combination of eROP and ATRP was reported. Base on this reason, in this article, we reported chemo-enzymatic synthesis of H-shaped triblock copolymer by this method. The block copolymer must have unique properties due to its special structure.

## Experiment

### *Materials*

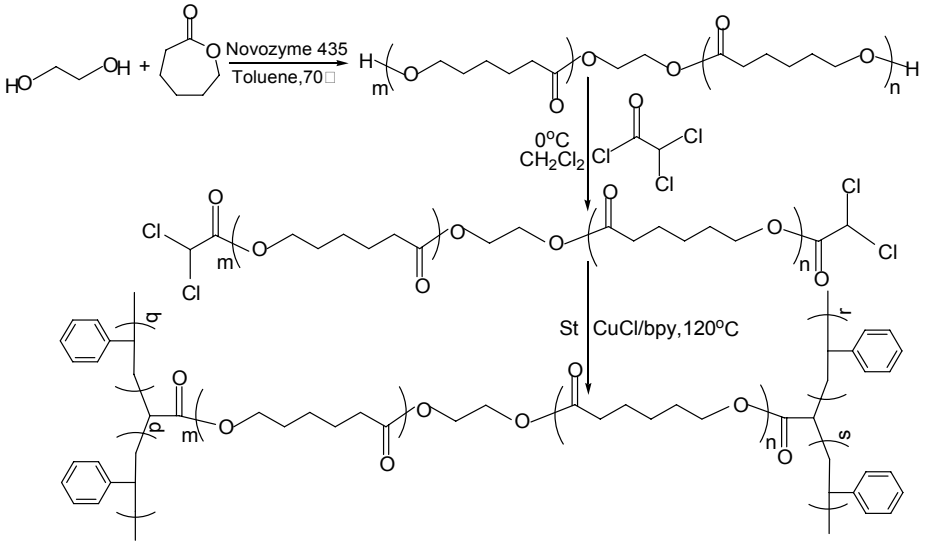
Ethylene glycol (Tianjin chemical Co.) were distilled over sodium. Novozyme-435 (activity approximately 7000PLU/g) was provided by Novozymes (Denmark). ε–CL were obtained from Aldrich Chemical Co. and distilled over calcium hydride ($CaH_2$) under vacuum. CuCl (Beijing Chemical Co.) was purified by precipitation from acetic acid to remove $Cu^{2+}$ and then dried. 2, 2′-Bipyridine (Beijing Chemical Co.) was used without further purification. 2,2-dichloro acetyl chloride (DCAC, 99%) were purchased from Aldrich Chemical Co. Styrene(Beijing chemical co) were distilled over $CaH_2$ under reduced pressure prior to use. Toluene (Tianjin Chemical Co.) and dichloromethane (Tianjin Chemical Co.) was dried with $CaH_2$ and distilled. Triethylamine (Beijing chemical co) was refluxed for 12h in the presence of $CaH_2$ and distilled. All the reagents used in this study were of analytic grade.

### *Analytical Methods*

Measurements of nuclear magnetic resonance (NMR) spectra were conducted on a Bruker ARX-500 NMR spectrometer with $CDCl_3$ as solvent, operating at 500 and 125 MHz for the corresponding $^1H$ and $^{13}C$ nuclei. Chemical shifts (ppm) were reported downfield from 0.00 ppm using trimethylsilane (TMS) as internal standard. The molecular weights and molecular weight distributions were measured on a Waters 410 Gel permeation chromatography (GPC) apparatus equipped with a 10-μm Styragel HT6E column (300 mm×7.8 mm) using linear polystyrene standards. THF was used as the eluent at a flow rate of 1 mL/min.

*Synthesis of polyester*

Novozyme-435 (0.216g), dried over $P_2O_5$ in a vacuum desiccator (0.1mmHg, 25°C, 24h), was transferred into oven-dried 50 mL reaction vial under an argon atmosphere. Reagent ε–caprolactone (2.156g, $1.89\times10^{-2}$ mol), ethylene glycol (0.035 mL, $6.3\times10^{-4}$ mol) and solvent toluene (4.3 mL, twice v/w of the monomer weight) were added via gastight syringe under argon into the reaction vial. The vial was then placed into a constant temperature (70 °C) oil bath with magnetic stirring for a predetermined time. The reaction was terminated by pouring excess cold chloroform into the reactants and the enzyme was removed via filtration. The filtrate was concentrated and further precipitated in methanol, dried in a vacuum oven. The yield was 90%. $M_{n,NMR}$=6700, $M_{n,GPC}$=13800, $M_w/M_n$=1.16. $^1$H NMR ($CDCl_3$, δ): 4.28 (s, $CH_2O$), 4.1 (m, $CH_2O$ in PCL), 3.65 (t, terminal $CH_2O$ in PCL), 2.30 (m, $COCH_2$ in PCL), 1.6 (m, $CH_2$ in PCL), 1.4 (m, $CH_2$ in PCL).




**Scheme 1.** Synthesis route of the H-shape block copolymer.

*Synthesis of macroinitiator*

PCL was added into a two-neck flask which was composed of triethylamine (1 mL, $7.2\times10^{-3}$ mol) and 5 mL of dichloromethane, and then cooled in an ice bath (0 °C). After the mixture was homogeneous, 5 mL dichloromethane solution containing DCAC (0.6 ml, $6.2\times10^{-3}$ mol) was transferred to the constant pressure dropping funnel and added dropwise over 0.5 h. The reaction was carried out at 0 °C for 2 h and then at room temperature for 22 h. The solution was filtrated to remove the quaternary ammonium halide $(CH_3CH_2)_3NH^+Cl^-$. The filtrate was concentrated and precipitated in methanol, dried in vacuum. The yield is 82%. $M_{n,GPC}$=14500, $M_w/M_n$=1.16. $^1$H NMR ($CDCl_3$, δ): 5.95 (s, $Cl_2HCCO$), 4.28 (s, $CH_2O$), 4.1 (m, $CH_2O$ in PCL), 2.30 (m, $COCH_2$ in PCL), 1.6 (m, $CH_2$ in PCL), 1.4 (m, $CH_2$ in PCL).

**Synthesis of H-shaped triblock copolymers by ATRP**

CuCl (0.018 g, $1.8\times10^{-4}$ mol) and bpy (0.084 g, $5.4\times10^{-4}$ mol) were added into a toasted flask containing macroinitiator PCL (0.2g, $3.6\times10^{-5}$ mol). The reaction flask was immersed in ice water/NaCl mixture at about -10 $^{o}$C and degassed by vacuum-argon for three times to remove the oxygen. Solvent toluene (2mL) and monomer styrene (2g, $2\times10^{-2}$mol) degassed by inert dry argon were introduced into the flask via an Ar-washed syringe. After PCL macroinitiator was completely dissolved, the reaction flask was placed into a constant temperature (120 $^{o}$C) oil bath with magnetic stirring for a predetermined time. Aliquots (about 0.8 mL of reaction mixture) were removed from the reaction mixture at selected time intervals to monitor the reaction progress. Finally the reaction was rapidly terminated in an ice bath. The catalyst was removed through an alumina column. The crude polymer was precipitated in

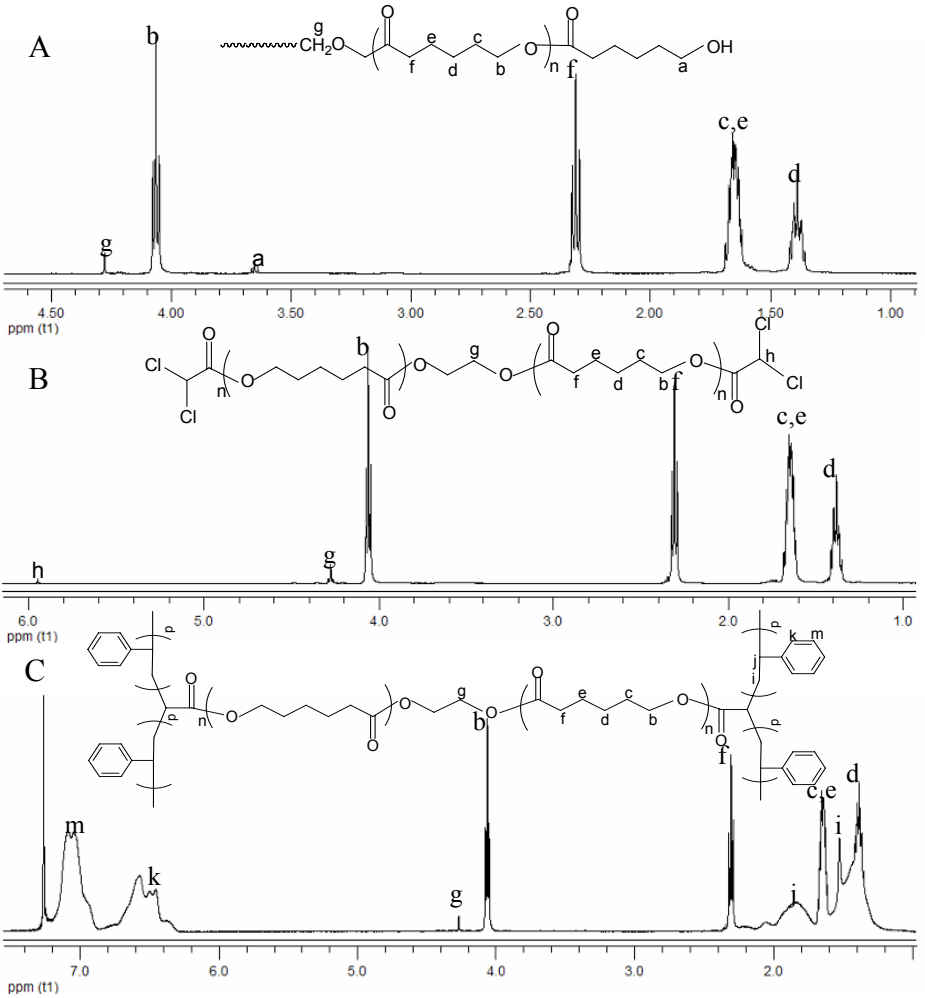



**Figure 1.** $^{1}$H-NMR spectrum of PCL 1 (A) macroinitiator 2 (B) and H-shaped $(PSt)_2$-*b*-PCL-*b*-$(PSt)_2$ 3 (C) were recorded at room temperature in $CDCl_3$.

methanol, and then dried under vacuum overnight. GPC data after 780 min reaction: $M_{n,NMR}$=29000, $M_{n,GPC}$=39500, $M_w/M_n$=1.28. $^1$H NMR ($CDCl_3$, δ): 6.3-7.0 (m, aromatic protons), 4.28 (s, $CH_2O$), 4.1 (m, $CH_2O$ in PCL), 2.30 (m, $COCH_2$ in PCL), 0.90-2.18 (m, CH and $CH_2$ in PSt), 1.6 (m, $CH_2$ in PCL), 1.4 (m, $CH_2$ in PCL).

## Result and discussion

Linear block copolymer has been synthesized and studied widely. Chemoenzymatic synthesis of PCL-*b*-PSt and PSt-*b*-PCL-*b*-PSt was reported by Heise and our group. As the extending of this technique, in this paper, we investigate the synthesis of H-shaped block copolymer. The general synthetic route used for the preparation of the H-shaped block copolymers is presented in scheme 1. The advantages of this approach include the mild conditions used in each step, the use of cheap, readily available starting materials, and the avoidance of protecting group chemistry.

### *Synthesis of dihydroxyl PCL*

Enzymatic ROP of ε-CL was performed at 70$^o$C in toluene, using biocatalyst Novozyme 435 and initiator ethylene glycol. The control of the polymer structure strongly depends on the frequency of side reactions caused by the water activity. Since water is an effective initiator for eROP. A water-involving transesterification reaction (hydrolysis) will result in a polyester end-functionalized not with an initiator but with carboxylic acid. Hence it is necessary to dry the reagents (especially the biocatalyst Novozyme 435) thoroughly in order to minimize the water initiation.

**Table 1.** Results of PCL, Macroinitiator and block copolymers.

| | | Carboxyl Terminal[a] | Monomer[b] | $M_{n,th}$[c] | $M_{n.nmr}$[d] | | $M_{n,GPC}$[e] | |
|---|---|---|---|---|---|---|---|---|
| PCL | $[M]_0/[I]_0$ | Chains (mol %) | conversion | (g/mol) | (g/mol) | EI[d] | (g/mol) | $M_w/M_n$[e] |
| 1 | 30/1 | <2% | 90% | 3100 | 6700 | 56% | 13800 | 1.16 |
| | | The Degree of End | | | | | $M_{n,GPC}$[e] | |
| Macroinitiator | | functionalization (mol%) | | | | | (g/mol) | $M_w/M_n$[e] |
| 2 | | >98% | | | | | 14500 | 1.16 |
| | | Time | Monomer[b] | $M_{n,th}$[c] | $M_{n.nmr}$[a] | | $M_{n,GPC}$[e] | |
| Copolymer | $[M]_0/[I]_0$ | (min) | conversion | (g/mol) | (g/mol) | CL/St[f] | (g/mol) | $M_w/M_n$[e] |
| 3 | 970/1 | 780min | 60% | 67000 | 29000 | 12/50 | 39500 | 1.28 |

[a] Determined by $^1$H-NMR analysis.

[b] The conversion was determined gravimetrically.

[c] The theoretical molecular weights ($M_{n.th}$) calculated from the ratio of the monomer to the initiator $[M]_0/[I]_0$ and the monomer conversion.

$$M_{n.th} = ([M]_0/[I]_0) \times M_{monomer} \times \text{con.-\%} + M_{n(macro)initiator}. \quad (1)$$

[d] EI represents the efficiency of initiator, $EI = M_{n.th}/M_{n.nmr}$.

[e] Determined by GPC measurements.

[f] The degree of polymerization of PCL:PSt calculated from the $^1$H-NMR spectra.

A typical $^1$HNMR spectrum of the resulting copolymer 1 was shown in Figure 1A. Besides the dominant PCL signals, centered at 1.4, 1.6, 2.3, and 4.1 ppm, the characteristic peak a corresponding to methylene protons of initiator ethylene glycol, could be pointed out clearly at 4.28ppm, which clarified ethylene glycol initiated successfully eROP of ε-CL. The absence of any resonance at 2.4 ppm in Figure 1A

also suggested the initiation of the PCL chains was carried out quantitatively by ethylene glycol and the amount of water-initiated PCL could reduce to less than 2% (the limitation of detection by NMR analysis), which is further supported with the absence of a $^{13}C$ NMR signal at 177 ppm corresponding to the carbon atom of the terminal carboxylic acid. Moreover, the fact that PCL shows a unimodal and symmetrical shape also proves this conclusion. Combining GPC analysis, it was clear that the theoretical molecular weight $M_{n,th}$ (3100g/mol) was lower than those (13800g/mol) obtained by GPC (Table 1). The discrepancy could be mainly ascribed to GPC analysis, in which polystyrene was used for calibration.

*Synthesis of macroinitiator*

The macroinitiator $(Cl)_2PCL(Cl)_2$ for ATRP was prepared by an esterification reaction between terminal OH group of the resulting PCL 1 and 2,2-dichloro acetyl chloride. In order to avoid cleavage of the polymer chain, the reaction was carried out at 0°C in dried $CH_2Cl_2$. In the process, triethylamine was used as the catalyst and absorbed HCl from the solution to generate a precipitate of quaternary ammonium halide $(CH_3CH_2)_3NH^+Cl^-$, which benefited the esterification. The complete substitution of the hydroxyl groups was proved by $^1$HNMR. The OH signal of PCL at 3.65ppm had disappeared after esterification and a new signal was appeared in 4.28ppm, the signals h at 5.95ppm assigned to the >CH- protons closed to the active chloride was also able to be detected (Figure 1B), which indicated that the 2,2-dichloro acetyl group was attached to the PCL chain end. Based on the above result, it was obvious that the macroinitiator had been prepared. Moreover, according to the exclusive disappearance of the signal a, it was concluded that the degree of end functionalization of the PCL 1 was more than 98%. The polydispersity after the esterification reaction was lower than those of the starting PCL, whereas $M_n$ was slightly higher (Figure 3). This could be due to inevitable fractionation of macroinitiator during the course of precipitation after esterification.

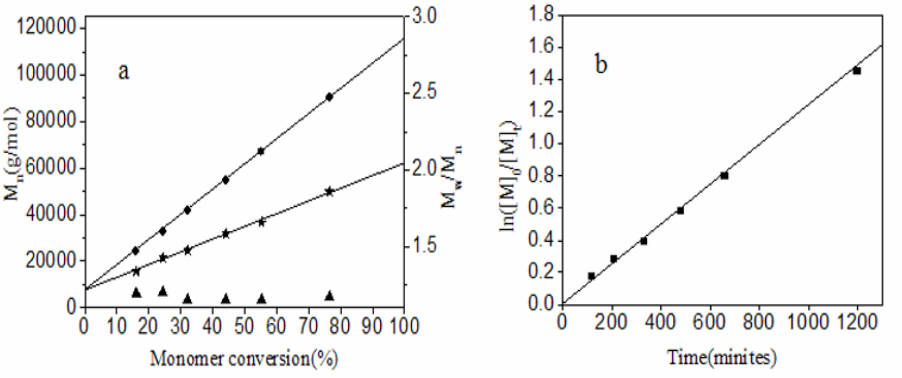



**Figure 2.** a) Dependence of $M_{n,th}$ (♦), $M_n$ (★) and polydispersity index (▲) on monomer conversion for ATRP of St initiated by PCL macroinitiator (I); b) $\ln([M]_0/[M]_t)$ versus time for ATRP of St initiated by PCL macroinitiator. $[M]_0$ and $[M]_t$ represent the initial monomer concentration and the monomer concentration after time t, respectively. [St]:[I]:[CuCl]:[bpy]=970:1:5:15, reaction temperature: 120 °C. $M_n$ and polydispersity were determined by GPC calibrated with polystyrene. The theoretical molecular weights ($M_{n,th}$) were calculated from eq 1 (Table 1).

*Synthesis of H-shaped triblock copolymer*

Y-shaped polymer was prepared from macroinitiator 2 according to literature reports [11], carried out at 120 $^{o}$C, using CuCl/bpy as the catalyst system and toluene as the solvent. The GPC-determined number average molecular weight ($M_n$), theoretical molecular weight ($M_{n,th}$) and polydispersity index ($M_w/M_n$) versus monomer conversion for ATRP were depicted in Figure 2 (a). $M_n$ increases linearly with conversion while the polydispersity index varies only a few degrees. The $M_{n,th}$ values are higher than the experimental, resulting from the GPC technique underestimating $M_n$. In addition, the $M_{n,th}$ was higher than the $M_{n,nmr}$ determined by $^1$H-NMR, which was possibly caused by the partial volatilization of the toluene (boiling point: 108°C) in the course of the reaction at 120$^{o}$C. This will result in the increase of conversion in the calculation and further the magnification of molecular weight. Figure 2 (b) plots the time dependence of $\ln([M]_0/[M]_t)$. The linear relationship indicates that the polymerization is first-order with respect to monomer concentration, and the number of active species remains constant throughout the reaction. The kinetic behavior of ATRP proves that polymerization of St is a 'living'/controlled radical process.

From the $^1$H-NMR spectra of the H-shaped triblock copolymer 3 (Figure 1C), it can be seen that besides the dominant $(Cl)_2PCL(Cl)_2$ signals, the occurrence of the signal at 6.3-7.0 ppm corresponding to aromatic protons m and k of the PSt block revealed the formation of the block copolymer 3 $(PSt)_2$-*b*-PCL-*b*-$(PSt)_2$. Moreover, the unimodal and symmetrical shape on the GPC plot of the block copolymer suggested the absence of a homopolymer composed of either St or ε-CL and the complete initiation of the macroinitiator during the ATRP process (Figure 3). The poly-dispersities is a litter higher, the reason may be that the initiation reaction of the ATRP from $(Cl)_2PCL(Cl)_2$ is slower than the propagation of the monomer.

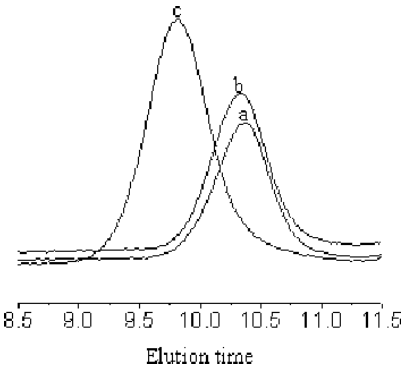



**Figure 3.** GPC traces of PCL 1 ($M_n$ = 13800 g/mol, polydispersity = 1.16), macroinitiator 2 (14500 g/mol, 1.16) and H-shaped triblock copolymer 3 (39500 g/mol, 1.28). The molecular weight and polydispersity were determined by GPC calibrated with PSt.

## Conclusions

We have synthesized well-defined H-shaped triblock copolymer by the combination of eROP and ATRP. The dihydroxyl polycaprolactone obtained from the eROP of CL was esterified with 2,2-dichloro acetyl chloride, subsequently the resulting macro-

initiator was used in the ATRP of styrene. The kinetics analysis of ATRP indicated a living/controlled radical polymerization. The structure of the resulting H-shaped block copolymer was confirmed by NMR and GPC. The versatility of the reaction pathway shown in Scheme 1 is currently exploited in our laboratory in the frame of the macromolecular engineering of H-shaped mutiblock copolymers.

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