Tandem Dynamic Kinetic Resolution and Enzymatic Polycondensation to Synthesize mPEG-Functionalized Poly(amine-*co*-ester)-Type Chiral Prodrugs

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ABSTRACT: Amphiphilic poly(amine-co-ester)s, which contain a single effective enantiomer of an asymmetric drug and thus can avoid potentially serious side effects, are difficult to prepare through nonselective chemical routes not only in the process of introducing chiral drugs to the polymer, but also in the synthesis of the polymer's backbone by metal catalysts. A model of racemic mexiletine, an important antiarrhythmic agent, was used to demonstrate the tandem combination of Candida antarctica lipase B (CAL-B)- and Pd/C-catalyzed dynamic kinetic resolution (DKR) and subsequent CAL-B-catalyzed polycondensation, as an efficient protocol to prepare poly(ethylene glycol)-functionalized poly(amine-co-ester)s containing (R)-mexiletine with 99% ee value. Chemoenzymatic DKR and enzymatic polymerization conditions were optimized, and the optical purity of incorporated (R)-mexiletine was confirmed through its hydrolysis from polyester. The copolymers can readily self-assemble into nanometer-scale-sized micelles with well-dispersed spheres, which have a size distribution that can be efficiently adjusted by changing the polymer concentration. © 2013 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2013**, *51*, 2049–2057

KEYWORDS: CAL-B; dynamic kinetic resolution; enzyme; micelles; stereospecific polymers

INTRODUCTION Macromolecular prodrugs, which generally show advantages such as high stability, prolonged half-life, enhanced permeability and retention (EPR) effect, antigenicity, and low immunogenicity, have attracted increasing attention from both academia and industry in recent years.[1–3] More research has focused on designing new macromolecular prodrugs for medical applications.[4–7] The typical approaches to incorporating small molecular drugs into macromolecular carriers include various chemically catalyzed reactions. Most are very effective. However, when the targeting molecule contains chiral centers, the chemical route usually makes it difficult to prepare macromolecular prodrugs that bear its single effective enantiomer. This is crucial for many drugs, because two enantiomers of a chiral drug candidate often have differences in physiology, pharmacokinetics, metabolic activities, and toxicology. Furthermore, the use of toxic metal catalysts, required critical conditions such as high temperature, and complicated protection/deprotection steps for the incorporation of drugs through chemical routes all cause problems of environmental or synthetic efficiency.[8,9] Poly(amine-*co*-ester), which is safe to use and easy to produce as a nonviral gene vector because of its ability to condense plasmid DNA via electrostatic interactions to form polyplexes, is attracting more scientific attention.[10] However, few efficient synthetic methods are available for the preparation of amino-containing polyesters, primarily because metal catalysts required for conventional polyester synthesis are often sensitive to and deactivated by amino groups.[11] The study of more efficient methods to synthesize poly(amine-*co*-ester) and selectively introduce one effective enantiomer of a chiral drug into a macromolecular system has thus become an important issue for pharmaceutical academia and industry.

Lipase, because of its excellent selectivity, nontoxicity, "green" energy savings, and other advantages,[12] has been widely used as a powerful tool for the preparation of chiral intermediates and the resolution of racemic drugs.[13] Gotor et al. have demonstrated *Candida antarctica* lipase B (CAL-B)-catalyzed kinetic resolution (KR) of some pharmacologically important β-substituted isopropylamines to obtain the amides with a 98% ee value.[14] Koul developed a facile route for the conversion of (*R*,*S*)-naproxen ester to (*S*)-naproxen (ee $>$ 99%, $E \sim 500$) using lipase from *Trichosporon* sp.[15] The combination of the enzymatic resolution and the macromolecular synthesis may overcome the difficulties described above by the incorporation of one effective enantiomer of racemic drugs into a macromolecular system. Our group previously reported a combinational strategy of lipase-catalyzed KR and chemically free radical polymerization to prepare nonbiodegradable poly(vinyl alcohol) prodrugs of optically active

Additional Supporting Information may be found in the online version of this article.

nonsteroidal anti-inflammatory drugs from vinyl ester monomers.[16,17] Although KR is useful for preparing optically active monomers, its yield of the desired isomer is greatly limited and cannot exceed 50%.

The dynamic kinetic resolution (DKR) of racemates by metal-catalyzed racemization combined with enzymatic KR is attracting interest, because it is a promising route to overcome the limitation of KR for the synthesis of optically pure compounds from racemic mixtures without separating the undesired enantiomer after the reaction.[18–22] Reetz and Schimossek reported the first example of chemoenzymatic DKR for the preparation of enantiopure amines,[23] obtaining (*R*)-*N*-(1-phenylethyl) acetamide (99% ee) in a 64% yield after 8 days at 50–55 °C using palladium on charcoal and CAL-B as catalysts. Kroutil and coworkers[24] reported the enzymatic DKR for the preparation of enantiopure mexiletine in 2009. Considering the high efficiency and wide application of DKR, the powerful combination of enzymatic DKR and enzymatic polymerization for the preparation of chiral macromolecules or chiral polymeric prodrugs containing the effective enantiomer is appealing. Recent pioneer works for the synthesis of chiral oligomers or polyesters by combining DKR and enzymatic polymerization have been reported by Palmans, Heise, and Meijer[25,26] and Howdle.[27] However, these works were mainly restricted to the preparation of general polyester with a chiral center at the backbone or at the ring-opening initiator. Synthesis of chiral polymeric prodrugs has seldom involved combining DKR and enzymatic polymerization.

Herein, the resolution of mexiletine was selected as a model. Mexiletine (1-(2,6-dimethylphenoxy)-2-amino-propane) is classified as an antiarrhythmic agent, (*R*)-enantiomer, which is more potent than its (*S*)-counterpart in experimental arrhythmias and in binding studies on cardiac sodium channels.[28,29] However, mexiletine in its racemic form is a risky treatment for ventricular tachyarrhythmias. The DKR of the racemic mexiletine with methyl 3-(bis (2-hydroxyethyl) amino) propanoate (MAP) was achieved under the combinational catalysts system of CAL-B and Pd/C. The obtained ((*R*)-3-(bis (2-hydroxyethyl) amino)-*N*-(1-(2, 6-dimethylphenoxy) propan-2-yl) propanamide ((*R*)-MAPP) can be copolymerized with divinyl dicarboxylate and poly(ethylene glycol) (PEG) to prepare amphiphilic polymeric chiral prodrugs, the micellization ability of which was further investigated (Scheme 1).

EXPERIMENTAL

Materials

Diphenyl ether (99%), tetrahydrofuran (THF) (99%), and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent (China). Polyethylene glycol 1000 monomethyl ether was purchased from Fluka. (*R,S*)-mexiletine hydrochloride was purchased from Jiangsu Jintan Yabang Pharmaceutical. Lipase immobilized on acrylic resin from CAL-B (EC 3.1.1.3, $\geq$10,000 U/g) was purchased from Sigma. Lipase from *porcine pancreas* (PPL) was purchased from Fluka. Lipase Type VII from *Candida rugosa* (CRL) was purchased from Sigma. Amano lipase PS-IM was purchased from Sigma-Aldrich. The lipase catalyst was dried at 25 °C under 2.0 mmHg for 24 h before use. Pd/C (10% Pd) was purchased from China National Medicine. Pd/$BaSO_4$ (5% Pd) was purchased from Aladdin.

Methods

NMR spectra were measured with a Bruker DRX 400 NMR spectrometer at 300 Hz using $CDCl_3$ as the solvent. IR spectra were recorded on a Nicolet Nexus FTIR 470 spectrophotometer. Samples were film-cast in chloroform onto sodium chloride plates. High-resolution mass spectrometry (HRMS) was obtained on a Bruker 7-tesla FT-ICR MS equipped with an electrospray source (Billelica, MA). The number and weight average molecular weights (M_n and M_w, respectively) of copolymers were measured by gel permeation chromatography (GPC) with a system equipped with refractive-index detector (Waters 2414) and Waters Styragel GPC columns. The GPC columns were standardized with narrow dispersity polystyrene in molecular weights ranging from 6×10^5 to 500. The mobile phase was THF at a flow rate of 1.0 mL/min. The enantiomer of (*R,S*)-MAPP was analyzed using AD-H column and was detected at 220 nm. The enantiomer of (*R,S*)-mexiletine acetamide was analyzed using OD-H column and was detected at 220 nm.

General Procedure for DKR of Mexiletine

Pd/C-catalyzed DKR

A suspension containing racemic mexiletine 10 mg, Pd/C 10 mg, CAL-B 20 mg, MAP 70 mg, and Et_3N 50 μL, in 1 mL solvent was stirred at 50 °C under 1 atm H_2. After 5 days, the reaction mixture was cooled to room temperature for high-performance liquid chromatography (HPLC) analysis.

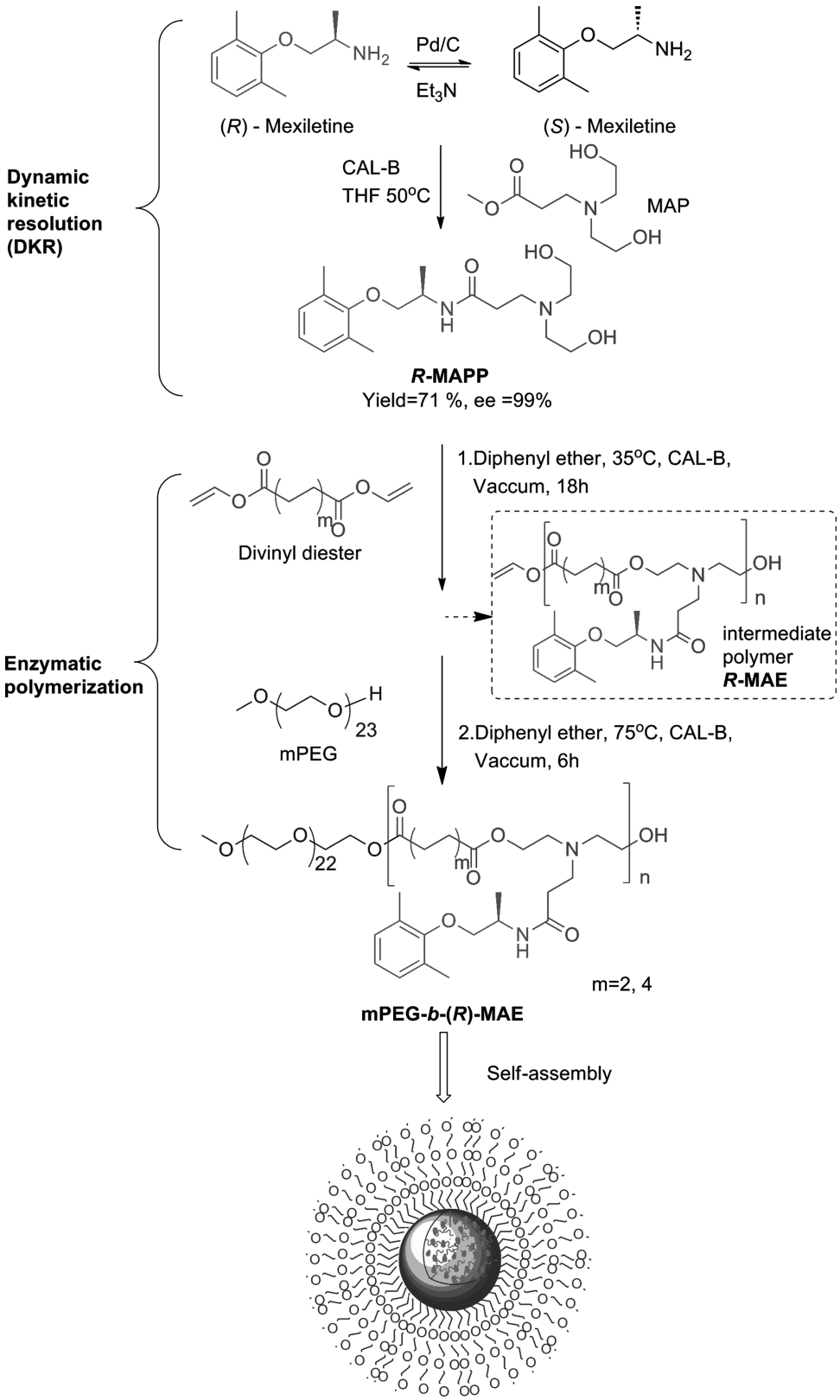
Pd/$BaSO_4$-catalyzed DKR

A suspension containing racemic mexiletine 10 mg, Pd/$BaSO_4$ 10 mg, CAL-B 20 mg, and MAP 70 mg, in 1 mL toluene was stirred at 70 °C under 1 atm H_2. After 3 days, the reaction mixture was cooled to room temperature for HPLC analysis.

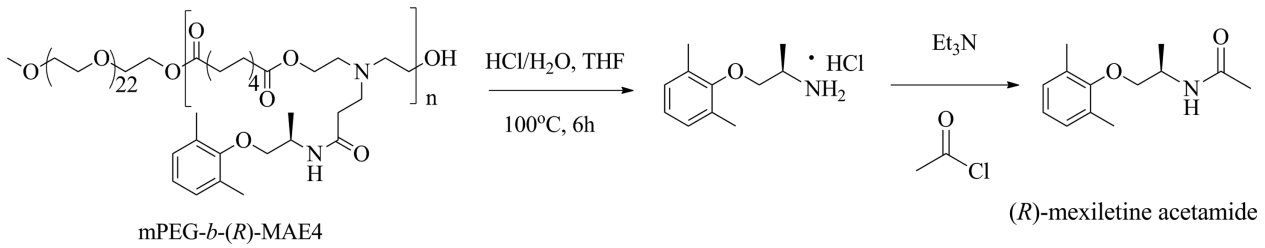
General Procedure for Synthesis of (*R*)-MAPP

A suspension containing racemic mexiletine 1 g, Pd/C 1 g, CAL-B 2 g, MAP 7 g, and Et_3N 5 mL, in 30 mL THF was stirred at 50 °C under 1 atm H_2. After 5 days, the reaction mixture was cooled to room temperature. The solution was filtered and concentrated. The crude products were purified by silica gel column chromatography with the mobile phase of ethyl acetate/methanol (6:1, v/v). The separated yield of (*R*)-MAPP was 63%.

^{1}H NMR (500 MHz, D_2O) δ 7.05 (d, J = 7.6 Hz, 2H), 7.01–6.93 (m, 1H), 4.23 (dd, J = 11.1, 6.5 Hz, 1H), 3.77 (ddd, J = 16.9, 10.0, 5.7 Hz, 2H), 3.63 (t, J = 6.1 Hz, 4H), 2.86 (dq, J = 13.7, 6.9 Hz, 2H), 2.67 (t, J = 6.0 Hz, 4H), 2.43 (t, J = 7.1 Hz, 2H), 2.20 (s, 6H), 1.21 (d, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, D_2O) δ 174.41, 154.21, 131.18, 128.99, 124.68, 74.22, 58.61, 54.71, 50.04, 45.77, 32.41, 16.07, 15.31. IR (/cm): 3292, 2948, 1643, 1552, 1202, 770. HRMS (EI) calcd for 338.2206, found: 338.2203. $[\alpha]_D^{25} = +21.1°$ in $CHCl_3$.



SCHEME 1 Combinational DKR and enzymatic polymerization for efficient synthesis of PEG-functionalized polyester enantiopure prodrugs.



SCHEME 2 Hydrolysis of mPEG-*b*-(*R*)-MAE4 and synthesis of (*R*)-mexiletine acetamide.

Preparation of mPEG-*b*-(*R*)-MAE Copolymers

The mixture of divinyl diester (5 mmol), (*R*)-MAPP (5 mmol), and diphenyl ether (2 mL) were placed in a dried test tube equipped with a magnetic stir bar. After the mixture was stirred for 15 min, CAL-B was added. The tube was immersed in an oil bath thermostated at 35 °C for 18 h in a vacuum. Then mPEG (1.6 mmol) was added and kept at 75 °C for another 6 h. The polymerization was quenched by introducing a small amount of chloroform to the mixture. The insoluble CAL-B was filtered away, and the organic solution was separated by filtration. The filtrate was dropped into a large amount of ether to isolate the polymer product. The resulting oily precipitates were obtained by centrifugation, then dried in a vacuum. The products were characterized by FTIR, GPC, and NMR spectroscopy.

mPEG-b-(R)-MAE2

Yield: 73%. ^{1}H NMR (400 MHz, DMSO) δ 6.97 (d), 6.94–6.83 (m), 4.31 (s), 4.23–4.06 (m), 3.68 (d), 3.63 (s), 3.36 (s), 2.38 (d), 2.38 (d), 2.27 (s), 2.23 (s), 1.58 (d), 1.37 (d). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.09, 171.65, 154.83, 130.61, 128.86, 123.96, 77.29, 76.97, 76.66, 73.75, 70.43, 61.88, 52.32, 51.11, 45.20, 33.60, 24.08, 17.56, 16.08. M_n (GPC): 2660 g/mol.

mPEG-b-(R)-MAE4

Yield: 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d), 6.95–6.83 (m), 4.10–3.60 (m), 3.39 (s), 3.15–2.66 (m), 2.46 (s), 2.46–2.17 (m), 1.56 (s), 1.39 (d), 1.25 (s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.55, 154.93, 130.62, 128.85, 123.93, 77.27, 76.95, 76.63, 73.78, 70.45, 61.78, 52.36, 51.18, 45.14, 34.04, 28.97, 28.76, 24.71, 17.61, 16.10. M_n (GPC): 3360 g/mol.

Preparation of Micelles

The micelles of mPEG-*b*-(*R*)-MAE2 were prepared using a simple dialysis method. A certain amount of amphiphilic copolymer was dissolved in DMSO. Ultrapure water was then dropped into the organic solvents under vigorous stirring. The solution was subjected to dialysis against 2000 mL of distilled water for 48 h using a dialysis tube (molecular weight cutoff of 3500). The distilled water was replaced every 6 h to remove organic solvent. The resulting solution was finally lyophilized.

Dynamic Light Scattering Measurement

The hydrodynamic size of formed micelles in the aqueous phase was determined using dynamic light scattering (DLS) techniques with a Nanoseries (Malvern, UK) zetasizer with 90° instrument. Measurements were carried out at 25 °C.

Transmission Electron Microscopy Measurement

Transmission electron microscopy (TEM) was carried out with a JEM-1230 TEM at an accelerating voltage of 80 keV. A drop of the micelle solution was placed onto a 230-mesh copper grid coated with carbon and then dried in air.

Hydrolysis of mPEG-*b*-(*R*)-MAE4 and Analysis of Optical Purity of Mexiletine in Copolymers

A measured amount of mPEG-*b*-(*R*)-MAE4 (200 mg) was placed in a dry test tube equipped with a magnetic stir bar. Then 5 mL of THF and 10 mL of hydrochloric acid (12 mol/L) was added. The tube was immersed in an oil bath thermostated at 100 °C for 6 h. The solution was dried on the rotary evaporator to obtain the crude mexiletine hydrochloride. The product was dissolved in 30 mL of chloroform that contained 5 mL Et_3N. Because mexiletine must be derived using acetyl chloride in order to determine the optical purity of released mexiletine using HPLC, the acylation reaction of mexiletine was carried out in room temperature for 30 min after dropping 3 mL of acetyl chloride into the solution. The mixture was then washed three times by 30 mL of saturated NaCl solution. The product was prepared for chiral HPLC analysis, and the ee value was 93% (Scheme 2).

RESULTS AND DISCUSSION

Chemoenzymatic DKR of Mexiletine

MAP was selected as the acyl donor of mexiletine, because two hydroxyl groups of MAP can be further used as diols for the subsequent polycondensation. Palladium complexes such as Pd/C and $Pd/BaSO_4$ were used as racemization catalysts of chiral mexiletine. Scheme 1 shows the DKR of racemic mexiletine with MAP under the racemization by Pd/C and the transesterification by CAL-B. The influence of organic solvents on the synthesis of (*R*)-MAPP was investigated. Unfortunately, MAP was immiscible with many solvents, such as ether and hexane, because of the high polarity of two hydroxyl groups. Thus, some solvents commonly used in the resolution reactions did not show good results (data not shown). When using Pd/C as the racemization catalyst in the screened polar solvents (Table 1, entries 1–5), all obtained ee values were about 99%. Among these solvents, the reaction catalyzed by Pd/C in THF gave the highest yield of 71% after 120 h. Reactions carried out in 1,4-dioxane, and chloroform could give better results than that in 2-methyl-2-butanol or acetone (entries 2, 3, vs. 1, 5 in Table 1). Compared to Pd/C catalyst, $Pd/BaSO_4$ could more efficiently racemize the chiral mexiletine to provide a higher yield of 84% in a

TABLE 1 Dynamic Kinetic Resolution of Racemic Mexiletine[a]

Entries[a]	Solvent	ee_p (%)[b]	Yield (%)[b]
1	Acetone	99	34
2	1,4-Dione	99	40
3	Chloroform	99	46
4	THF	99	71
5	2-Methyl-2-butanol	99	21
6[c]	Toluene	92	84

[a] Reaction conditions: racemic mexiletine 10 mg, CAL-B 20 mg, Et_3N 50 μL, Pd/C 10 mg, methyl 3-(bis (2-hydroxyethyl) amino) propanoate (MAP) 70 mg, solvent 1 mL, 50 °C, 120 h, 1 atm H_2.
[b] Determined by HPLC analysis using AD-H column.
[c] Conditions: racemic mexiletine 10 mg, Pd/$BaSO_4$ 10 mg, CAL-B 20 mg, MAP 70 mg, toluene 1 mL,70 °C, 72 h, 1 atm H_2.

shorter reaction time of 72 h. However, the stereoselectivity of the Pd/$BaSO_4$-CAL-B system (ee: 92%) was lower than that of the Pd/C-CAL-B system. The high temperature of racemization (70 °C) required for Pd/$BaSO_4$ led to the decrease of enzymatic stereoselectivity. Finally, the DKR process provided (*R*)-MAPP with 99% ee value in a 71% yield.

Enzymatic Polymerization of Poly(amine-*co*-ester) and Structure Characterization

To combine the advantages of polymer-drug conjugates and polymer micelles carriers of drugs,[30–32] we focused on the macromolecular prodrugs micelles, which can provide a much more effective way to control the level of drug-loading and the rate of drug release than the general polymer micelles. An amphiphilic macromolecular prodrug containing *R*-mexiletine was designed, and its preparation starting from (*R*)-MAPP, divinyl diesters, and mPEG ($M_w = 1000$) is shown in Scheme 1.

Enzymatic polymerization was performed in two steps: the lipase-catalyzed polycondensation, and the sequential transesterification. First, polycondensation of (*R*)-MAPP and divinyl diesters provided the polyester (*R*)-MAE, which contained a vinyl ester group at one end of the chain; meanwhile the byproduct vinyl alcohol was *in situ* transformed into aldehyde, pushing forward the equilibrium of transesterification. The vinyl ester end of the polyester (*R*)-MAE was easily reacted with mPEG through further transesterification to prepare the final amphiphilic polymer. Generally, the first step was not necessarily separated or purified. After suitable reaction time, mPEG was added, and the second transesterification was directly carried out under the specific conditions. For example, when using CAL-B as the catalyst, the first step was carried out at 35 °C for 18 h, and after the addition of mPEG, the temperature was increased to 75 °C for 6 h for further copolymerization. To confirm the success of the first polycondensation step, the intermediate polymer (*R*-MAE) was separated for structure analysis. The average molecule weight of *R*-MAE determined by GPC was about 2950 g/mol (polydispersity = 1.32) (Supporting Information Fig. S5). Both the separated process and cascade process can provide the final copolymer.

In the whole cascade process without intermediate separation, both solvents must be brought into contact with each other for the first or second step. Considering the boiling point and polymerization effect, diphenyl ether was selected. The influence of the enzyme source on the copolymerization was also investigated. Of all the lipase tested, CAL-B showed the highest activity, and the molecule weight of the obtained polymer was about 3360 g/mol (entry 4, Table 2) (GPC was shown in Supporting Information Fig. S11). Compared with CAL-B, CRL, PPL, and PS-IM may be unsuitable for the polymerization, as the M_n of the obtained copolymers were not more than 1800 g/mol (entries 1, 2, 3, Table 2). The polydispersities of the polymers were all among 1.1–1.3, which were moderately narrow. Furthermore, divinyl adipate also can be used as acyl donor, and the molecular weight of the forming copolymer (mPEG-*b*-(*R*)-MAE2) was a bit lower than that of divinyl sebacate (entries 4, 5, Table 2) (GPC was shown in Supporting Information Fig. S7). Under the catalysis of CAL-B, mPEG-*b*-(*R*)-MAE2 and mPEG-*b*-(*R*)-MAE4 were obtained in yields of 73 and 86%, respectively.

The chemical structures of the intermediate and final products were characterized by ^1H and ^{13}C NMR spectroscopy. Figure 1 compares ^1H NMR spectra of (*R*)-MAPP, the first CAL-B-catalyzed polymerization product ((*R*)-MAE), and final product (mPEG-*b*-(*R*)-MAE4). In the spectrum of (*R*)-MAPP [Fig. 1(A)] recorded in D_2O, peaks ***a*** and ***g*** belong to the protons of benzene ring and methyl group, and peaks ***p*** and ***u*** belong to the protons of $—N(CH_2CH_2OH)_2$. All of the above specific signals of mexiletine appeared in the spectra of the obtained (*R*)-MAE [Fig. 1(B)] and mPEG-*b*-(*R*)-MAE4

TABLE 2 Enzymatic Synthesis of Amphiphilic Copolymer

Entries	Block Copolymer	Lipase	Diesters (m)	M_n[a] (g/mol)	Yield (%)	PDI[a]
1	mPEG-*b*-(*R*)-MAE4a	CRL	4	1,770	24	1.2
2	mPEG-*b*-(*R*)-MAE4b	PPL	4	1,470	17	1.3
3	mPEG-*b*-(*R*)-MAE4c	PS-IM	4	1,710	21	1.1
4	mPEG-*b*-(*R*)-MAE4	CAL-B	4	3,360	73	1.3
5	mPEG-*b*-(*R*)-MAE2	CAL-B	2	2,660	86	1.2

[a] Determined by GPC.

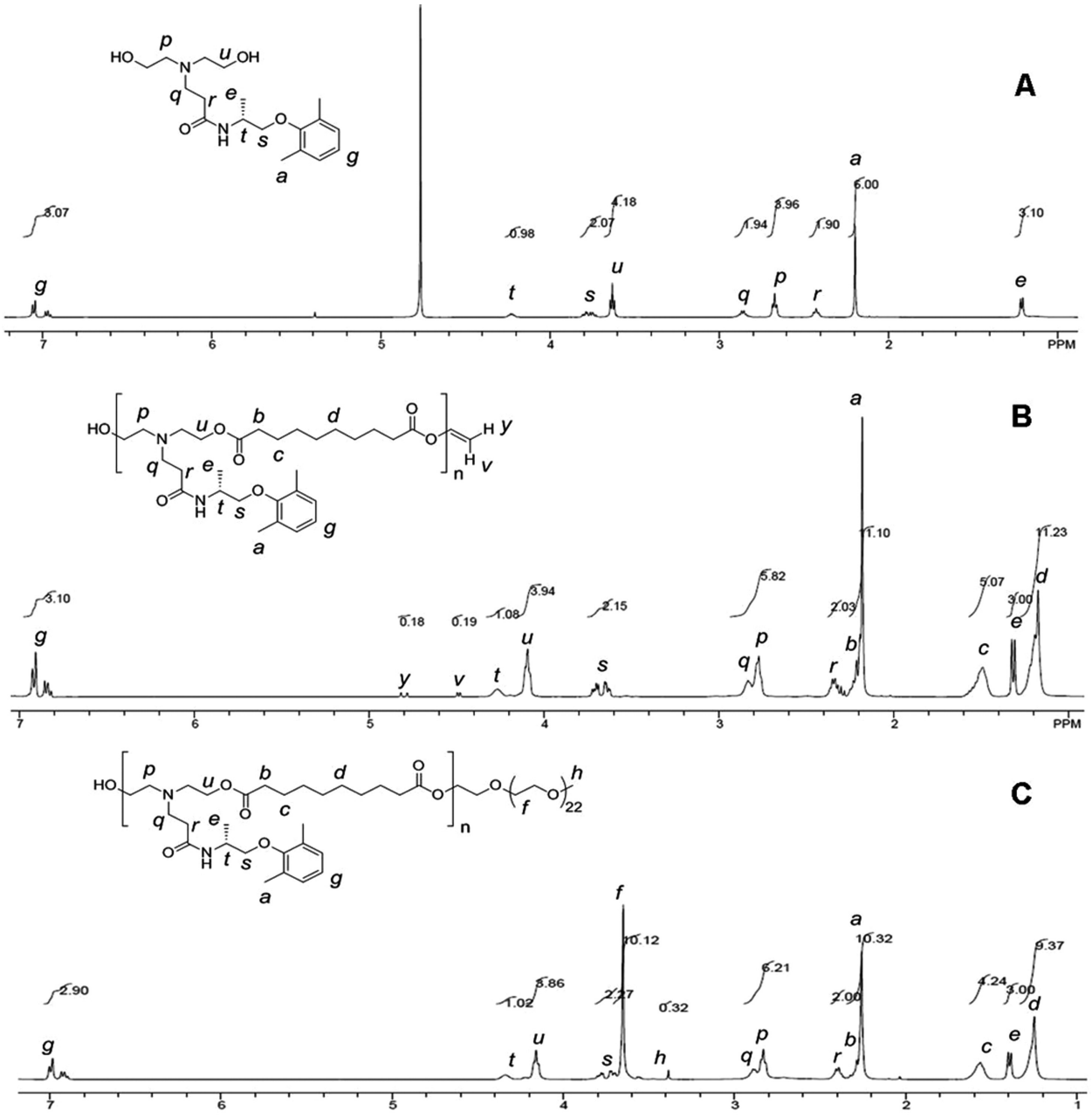


FIGURE 1 ^{1}H NMR spectra of (A) (*R*)-MAPP in D_2O, (B) (*R*)-MAE, and (C) mPEG-*b*-(*R*)-MAE4 in $CDCl_3$.

[Fig. 1(C)]. In Figure 1(B), the appearance of the peaks ***f*** and ***h*** was attributed to protons of —CH_2—O— and terminal methoxy group of mPEG, respectively. The signals of protons in sebacate or adipate units (peaks ***b***, ***c***, and ***d***) were also found in the spectra. All these results confirmed the structure of mPEG-functionalized poly(amine-*co*-ester). To completely remove the unreacted mPEG1000 and the first polycondensation product (*R*)-MAE4, the final products were rigorously separated and purified. ^{1}H NMR spectroscopy [Fig. 1(C)] of the obtained copolymer gave information to determine whether the mPEG was removed. The peak of protons of —CH_2OH end group in residual mPEG should appear at 3.44 ppm. The peak of CH_3O— end group (peak ***h*** at 3.37 ppm) was clear, but there was almost no peak at 3.44 ppm, which confirmed the absence of mixed PEG in the copolymer. Similarly, the vinyl protons at the end group of the first polycondensation product (*R*)-MAE4 [Fig. 1(B)] were not observed in ^{1}H NMR spectra of copolymers, ruling out the existence of mixed (*R*)-MAE4 in the copolymer.

Hydrolysis of Copolymers

After the preparation of amphiphilic polymeric prodrugs containing *R*-mexiletine, how well the configuration of *R*-mexiletine kept during the polymerization was investigated. The hydrolysis of mPEG-*b*-(*R*)-MAE4 in hydrochloride acid was performed to identify the configuration of mexiletine in the

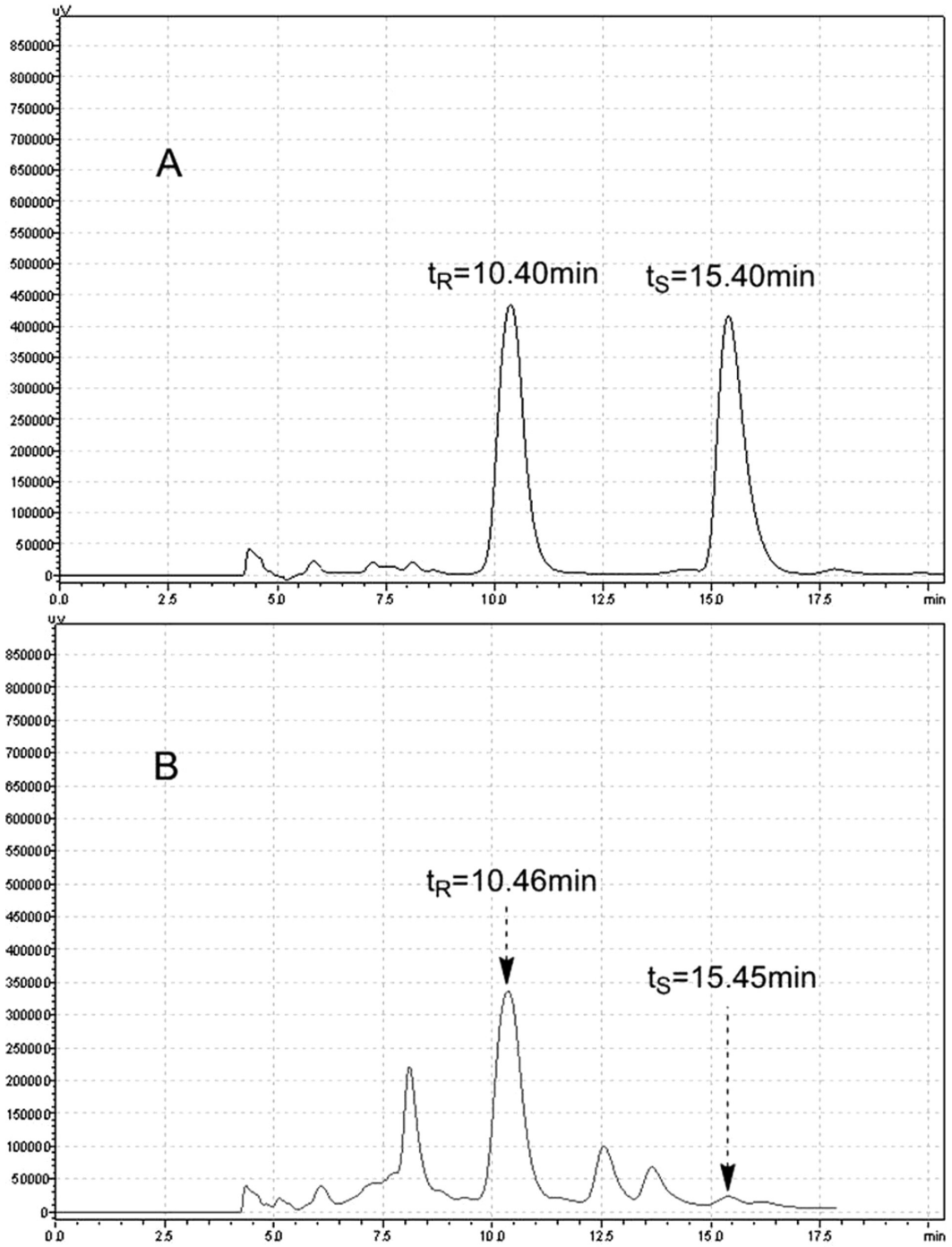


FIGURE 2 Chiral HPLC of acetamide derivatives of racemic mexiletine (A) and released (*R*)-mexiletine from polymeric prodrug mPEG-*b*-(*R*)-MAE4 (B).

polymeric prodrug. Considering the separation difficulty of mexiletine using chiral HPLC,[33] the derivation of the released mexiletine was carried out using acetyl chloride to form corresponding acetamide, which was analyzed by chiral HPLC (Fig. 2). The analysis data showed that the ee value of obtained mexiletine acetamide was greater than 93%, demonstrating that the optical purity of *R*-mexiletine was kept stable in the enzymatic polymerization, and the amphiphilic polymeric chiral prodrugs were successfully developed.

Micellization of Copolymers in Aqueous Solution

The obtained amphiphilic block copolymers, consisting of hydrophilic mPEG and hydrophobic MAE blocks, can self-assemble to form micelles in water. Nanoparticles were prepared from the amphiphilic copolymer using a dialysis method. Specific solvents that could both dissolve the copolymer and were miscible with water, such as DMSO, were selected. The morphology and size distribution of copolymer micelles were investigated by TEM. As TEM

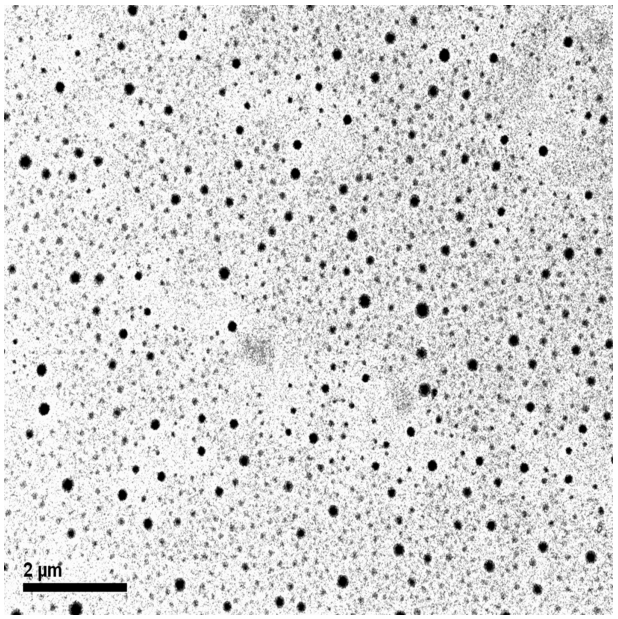


FIGURE 3 The TEM image of mPEG-*b*-(*R*)-MAE4 self-assemblies in water.

shows in Figure 3, the self-assembled micelles of mPEG-*b*-(*R*)-MAE4 in the H_2O system were regularly spherical and well dispersed as individual nanoparticles. In order to know whether the concentration of copolymer affects the size of the formed micelles, a series of polymer concentrations from 0.01 to 1 mg/mL in DMSO were studied. The size distribution was measured by DLS, and its number-size plots are shown in Figure 4. When the concentration of the copolymer decreased from 1 to 0.2 mg/mL [Fig. 4(A–C)], the diameter of micelles was nearly the same, most of which were among 40–60 nm. When the concentration decreased from 0.2 to 0.1 mg/mL [Fig. 4(C,D)], the diameter increased slightly. Further decrease of the concentration to 0.01 mg/mL caused the diameter of nanoparticles to increase to 90–110 nm. In the DLS intensity size distribution plots (Supporting Information Fig. S12), the micellar size similarly increased when reducing the copolymer concentrations. This result indicates that the size distribution of the micelles can be adjusted by changing the concentration of copolymers. Concerning the effect of concentration on the micellar size, the copolymer mPEG-*b*-(*R*)-MAE2 at the lower concentration possibly had a slower micellization rate, and the large aggregates formed were possibly loose compound micelles or micellar clusters, while the smaller aggregates formed at high polymer concentration were well-defined micelles.[34,35] More data regarding the effect of concentration are needed to confirm this.

CONCLUSIONS

The powerful combination of chemoenzymatic DKR and lipase-catalyzed polymerization has been demonstrated for the preparation of macromolecular chiral prodrugs. The DKR of mexiletine was efficiently achieved using Pd/C or Pd/$BaSO_4$, which are inexpensive and easy to obtain for racemization together with CAL-B as the resolution catalyst. Mexiletine was transformed to the corresponding amide monomer with high yields and above 99% optical purities. The optically active drug monomer was copolymerized with mPEG and divinyl dicarboxylates under the catalysis of CAL-B. The obtained amphiphilic polymers can self-assemble into nanometer-scale-sized particles in water. The diameters of the micelles determined by DLS depend on the polymer concentration in DMSO. The chiral HPLC analysis of released mexiletine demonstrates the stable optical purity of *R*-mexiletine during the polymerization, and successful preparation of amphiphilic polymeric chiral prodrugs.

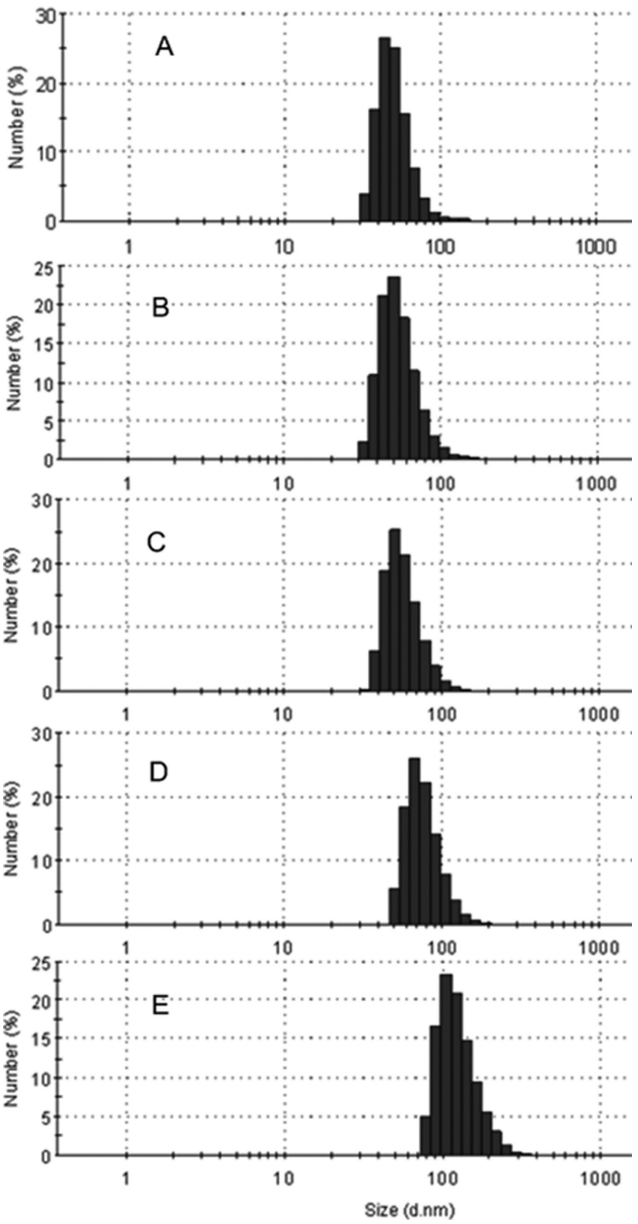


FIGURE 4 The number-size distribution of mPEG-*b*-(*R*)-MAE2 self-assemblies in water measured by DLS. The concentrations of copolymer in DMSO are (A) 1 mg/mL, (B) 0.5 mg/mL, (C) 0.2 mg/mL, (D) 0.1 mg/mL, and (E) 0.01 mg/mL.

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