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Chemo-enzymatic synthesis of optically active polymeric prodrug of naproxen, ketoprofen and ibuprofen

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Abstract

A combined enzymatic resolution and chemical polymerization strategy has been used to create optically active polymeric prodrugs. (*S*)-Naproxen, (*S*)-ketoprofen and (*S*)-ibuprofen derivatives were obtained in excellent optical purity and high yield by enzymatic resolution after optimization of reaction conditions. Each optically active monomer was subjected to free radical polymerization with methyl methacrylate. The obtained optically active polymeric prodrugs bearing (*S*)-naproxen, (*S*)-ketoprofen or (*S*)-ibuprofen residue were characterized by IR, NMR and GPC. The effect of molar ratio of naproxen vinyl ester to methyl methacrylate on the polymerization was also investigated. This methodology is useful to provide a facile and clean route to optically active macromolecular prodrugs.

Keywords: Copolymer; Enzymatic resolution; Optically active

1. Introduction

In recent years, macromolecular drugs have gained prominence in pharmaceutical field, since they can effectively control the rate of drug release, administrate at low dosage, improve site-specificity and increase therapeutic benefit [1–3]. To date, several polymer-based pharmaceutical products, such as Brufen Retard® (ibuprofen) and MST Continus® (morphine sulphate) have achieved both improved clinical outcomes and considerable market success. However, for chiral drugs, there are still no reports about synthesis of their optically active polymeric prodrug, because it represents a major challenge to prepare optically active drug derivatives possessing polymerizable groups.

Ketoprofen, naproxen and ibuprofen (profens) are some interesting non-steroidal anti-inflammatory drugs (NSAIDs), whose bioactivities are mainly on the (*S*)-enantiomer [4]. Previous pharmacological studies of profens have indicated that gastrointestinal (GI) side effects are the most frequent adverse reactions due to the acidic moiety [5]. Appropriately designed polymeric prodrugs, in which NSAIDs are linked to biodegradable polysaccharides or polyhydroxy aspartamide-type polymers, have therefore been reported to reduce adverse effects and prolong pharmacological activity [6–9]. However, these prodrugs are usually *graft* polymers and almost obtained by conventionally chemical methods, which show an almost lack of enantioselectivity and invariably lead to a racemic mixture. Therefore, our interest has been devoted to prepare optically active polymeric prodrugs of profens, especially by copolymerization, which can provide a versatile means to design novel polymeric prodrugs possessing alterable properties and compositions by choosing appropriate comonomers and controlling the ratio of copolymerization [10–12]. Furthermore, this method was preferred rather than linking the drug to the polymer because this should lead to polymeric prodrug with a higher degree of substitution, which is required for higher yields of drug release [13].

Enzymes have been widely recognized as useful chiral catalysts for the production of optically active compounds, due to the simplicity of process and the high enantioselectivity of biocatalysis under mild conditions [14]. Considerable efforts

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have been made to prepare optically active profens by enzymatic resolution [15–18]. However, until recently, few efforts have been devoted for the synthesis of optically active profens with a polymerizable group.

In the present work, we have developed an efficient chemo-enzymatic method to prepare optically active polymeric prodrugs of NSAIDs with high molecular weight (Scheme 1). (*S*)-Naproxen vinyl ester (NVE), (*S*)-ketoprofen vinyl ester (KVE) and (*S*)-ibuprofen vinyl ester (IVE) were prepared by enzymatic resolution with high optical purity. These new monomers were then polymerized by radical polymerization with methyl methacrylate (MMA).

2. Materials and methods

2.1. Materials

Lipozyme® immobilized from *Mucor miehei* (Lipozyme®) was purchased from Fluka. Lipase immobilized on acrylic resin from *Candida antarctica* (CAL-B) was purchased from Sigma. Racemic naproxen (2-(6-methoxy-2-naphthyl) propionic acid) was purchased from Zhejiang Charioteer Pharmaceutical Co. Ltd (Taizhou, P.R. China). Racemic ketoprofen (2-(3-benzoylphenyl) propionic acid) was a generous gift from Zhejiang Jiuzhou Pharmaceutical Co. Ltd (Taizhou, P.R. China). Racemic ibuprofen (2-(4-isobutylphenyl) propionic acid) was purchased from Juhua Corp. Pharmaceutical Factory (Quzhou, P.R. China). 2,2′-Azo-bis-*iso*-butyronitrile (AIBN) was purified by recrystallization with methanol. All other chemicals used in this work were of analytical grade and were dried over 3 Å molecular sieves for 24 h prior to use.

2.2. Methods

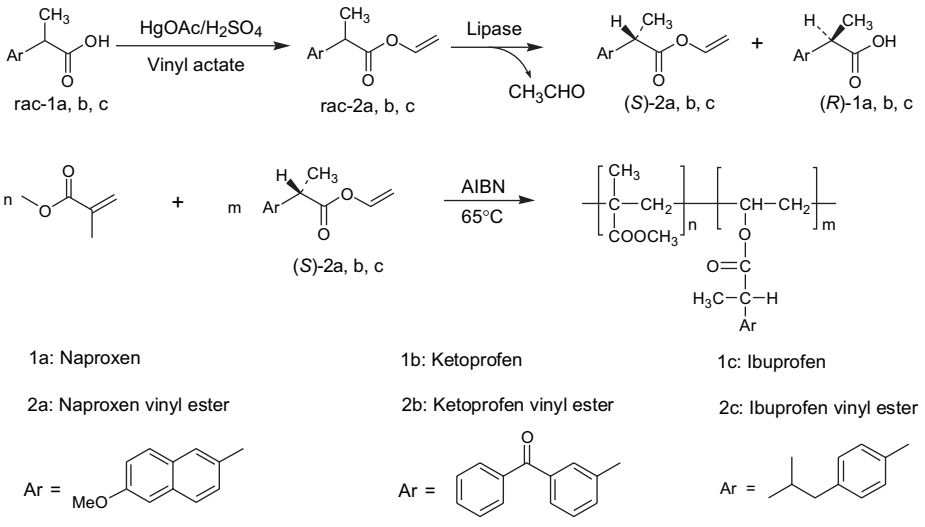
Infrared spectra were measured with a Nicolet Nexus FT-IR 470 spectrophotometer. NMR spectra were recorded with a Bruker DRX 500 NMR spectrometer. HRMS was obtained on a Bruker 7-tesla FT-ICR MS equipped with an electrospray source (Billelica, MA, USA). GPC was performed with a system equipped with refractive-index detector (Waters 2410) and Waters Styragel GPC columns. The GPC columns were standardized with narrow dispersity polystyrene in molecular weights ranging from 4.7×10^6 to 2000. The mobile phase was tetrahydrofuran at a flow rate of 1.5 ml/min. The enantiomers of ketoprofen, naproxen and ibuprofen were analyzed using an Agilent 1100 series with a chiral column ((*S*,*S*)-Whelk-O1, 250 × 4.6 mm, Regis, USA) and were detected at 250 nm, 232 nm and 220 nm, respectively. The mobile phase was *n*-hexane/isopropanol/acetic acid: 95/5/0.1, v/v/v, for ketoprofen, 85/15/0.1, v/v/v, for naproxen and 98/2/0.1, v/v/v, for ibuprofen with a flow rate of 1.5 ml/min.

2.3. Synthesis of (R,S)-profen vinyl ester

Vinyl esters were synthesized and purified as described by Yang et al. [19]. Racemic profen (3.2 g) and mercuric acetate (0.3 g) were dissolved in 30 ml of vinyl acetate. After stirring the mixture for 30 min at room temperature, 0.2 ml of concentrated sulphuric acid was added and the solution was refluxed for 3 h. Then the mixture was allowed to cool to room temperature, and sodium acetate (1.0 g) was added to quench the catalyst. The solution was filtered and concentrated. The crude products were purified by silica gel column chromatography with the mobile phase of petroleum ether/ethyl acetate (30:1, v/v). The products were identified by IR, ^{1}H NMR and HRMS.

2.4. Synthesis of (S)-profen vinyl ester

The synthesis of (*S*)-naproxen vinyl ester was catalyzed by CAL-B in IPE while (*S*)-ketoprofen vinyl ester was by Lipozyme® in dioxane composed of 2.5% (v/v) of water and (*S*)-ibuprofen vinyl ester was by Lipozyme® in tetrahydrofuran. The synthesis procedure was similar. The reaction was initiated by adding 150 mg lipase to 15 ml solvent containing 1.5 g racemic profen vinyl ester. The suspension was kept at 25 °C and shaken at 200 rpm. The reaction was detected by HPLC and terminated



Scheme 1. Chemo-enzymatic synthesis of optically active polymeric drugs.

by filtering off the enzyme. The reaction mixture was concentrated under reduced pressure. The products were separated by silica gel chromatography with a mobile phase consisting of petroleum ether/ethyl acetate (30:1, v/v). The product was analyzed by IR, ^{1}H NMR and HRMS. (*S*)-Naproxen vinyl ester: white solid; yield: 80%; ee ~ 95%; $[\alpha]_D^{20} = +14.5$ ($c = 1$, MeOH). ^{1}H NMR ($CDCl_3$), $\delta = 7.38-7.15$ (m, 6H, ArH), 7.29 (dd, 1H, $-CH{=}CH_2$, $J = 5.6$ Hz, $J = 14.7$ Hz), 4.88 (dd, 1H, $-CH{=}CH_2$, $J = 1.3$ Hz, $J = 13.9$ Hz), 4.58 (dd, 1H, $-CH{=}CH_2$, $J = 1.3$ Hz, $J = 6.2$ Hz), 3.94 (t, 3H, CH_3O-, $J = 10.6$ Hz), 3.90 (m, 1H, $-C_6H_4CH$), 1.64 (d, 3H, $-CH_3$, $J = 7.2$ Hz). IR (KBr): 1750 (s, C=O), 1644 cm^{-1} (s, C=C). HRMS (ESI) *m/z* calcd. for [M + Na] $C_{16}H_{16}O_3Na$ 279.0992, found 279.0987. (*S*)-Ketoprofen vinyl ester: light yellow liquid; yield: 90%; ee ~ 90%; $[\alpha]_D^{20} = +41.2$ ($c = 1$, MeOH). ^{1}H NMR ($CDCl_3$), $\delta = 7.81-7.46$ (m, 9H, ArH), 7.26 (dd, 1H, $-CH{=}CH_2$, $J = 6.3$ Hz, $J = 14.0$ Hz), 4.88 (dd, 1H, $-CH{=}CH_2$, $J = 1.6$ Hz, $J = 14.0$ Hz), 4.58 (dd, 1H, $-CH{=}CH_2$, $J = 1.6$ Hz, $J = 6.2$ Hz), 3.87 (q, 1H, $-C_6H_4CH$), 1.58 (d, 3H, $-CH_3$, $J = 7.2$ Hz). IR (KBr): 1751 (s, C=O), 1660 cm^{-1} (s, C=C). HRMS (ESI) *m/z* calcd. for [M + Na] $C_{18}H_{16}O_3Na$ 303.0992, found 303.0983. (*S*)-Ibuprofen vinyl ester: transparent liquid; yield: 74%; ee ~ 70%; $[\alpha]_D^{20} = +21.2$ ($c = 1$, MeOH). ^{1}H NMR ($CDCl_3$), $\delta = 7.26$ (dd, 1H, $-CH{=}CH_2$, $J = 6.3$ Hz, $J = 14.0$ Hz), 7.22–7.10 (m, 4H, ArH), 4.85 (dd, 1H, $-CH{=}CH_2$, $J = 1.3$ Hz, $J = 14.0$ Hz), 4.50 (dd, 1H, $-CH{=}CH_2$, $J = 1.4$ Hz, $J = 6.3$ Hz), 3.75 (q, 1H, $-C_6H_4CH$), 2.44 (d, 2H, $-C_6H_4CH_2$, $J = 7.2$ Hz), 1.84 (m, 1H, CH), 1.52 (d, 3H, $-CH_3$, $J = 7.2$ Hz), 0.89 (d, 6H, $-(CH_3)_2$, $J = 6.6$ Hz). IR (KBr): 1751 (s, C=O), 1647 cm^{-1} (s, C=C). HRMS (ESI) *m/z* calcd. for [M + Na] $C_7H_9N_3O_4Na$ 255.1356, found 250.1350.

2.5. Copolymerization of (S)-profen vinyl ester with methyl methacrylate

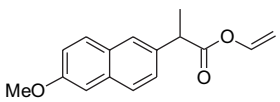
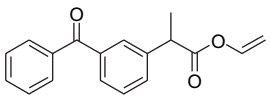
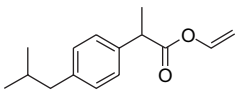
The copolymerization of (*S*)-ketoprofen vinyl ester or (*S*)-ibuprofen vinyl ester with methyl methacrylate was conducted by bulk polymerization, using AIBN initiator. A solution of 1 mmol ketoprofen monomer or ibuprofen monomer and 0.5 mmol methyl methacrylate was added to a small flame-dried flask. The solution was degassed (freeze/pump/thaw cycles) and 0.2% AIBN (w/w) was added. The polymerization was continued for 10 h at 65 °C. Precipitating the polymer in methanol terminated the reaction, and the white precipitate was washed with acetone. Poly(NVE-*co*-MMA) was synthesized by solution polymerization with the addition of 2 ml tetrahydrofuran and the general procedure was similar to the synthesis of poly(KVE-*co*-MMA).

3. Results and discussion

Optically active polymers can be prepared from optically active monomers [20–22]. We obtained three types of monomers with good optical purity after optimization of the reaction conditions, such as enzyme sources, solvent and temperature [23]. The optical purity and yield of the products are shown in Table 1. For naproxen vinyl ester, CAL-B showed good enantioselectivity (ee ~ 95%). To obtain (*S*)-ketoprofen vinyl ester, Lipozyme® was used to catalyze the hydrolysis of ketoprofen vinyl ester in dioxane. However, (*S*)-ibuprofen vinyl ester was only obtained with moderate optical purity.

Methyl methacrylate was chosen as the comonomer to study the synthesis of optically active polymeric prodrug of NSAIDs. We carried out the copolymerization using AIBN initiator. Products were analyzed by FT-IR and NMR. When taken naproxen vinyl ester as an example (Figs. 1 and 2), as expected, the double bond present in the naproxen vinyl ester monomer and methyl methacrylate was absent in the polymer. Other bands assigned, respectively, to the vinyl ester of profens and methyl methacrylate appeared in the IR and NMR spectra. The composition of the optical polymeric prodrugs was calculated from the ^{1}H NMR according to the ratio between the integral of methoxy protons of methyl methacrylate (δ 3.6 ppm) and the integral of methylene protons of ibuprofen (δ 2.4 ppm) or the

Table 1
Enantioselective synthesis of optically active vinyl ester of profens

Entry	Substrate	Enzyme	Solvent	Yield (%)	ee%[b]
1[a]		CAL-B	Isopropyl ether	80	95
2[a]		Lipozyme®	Dioxane	90	90
3[a]		Lipozyme®	Tetrahydrofuran	74	70

[a] The reaction was initiated by adding 10 mg enzyme ml^{-1} to the organic solvent, 25 °C, 200 rpm.
[b] Determined by HPLC analysis.

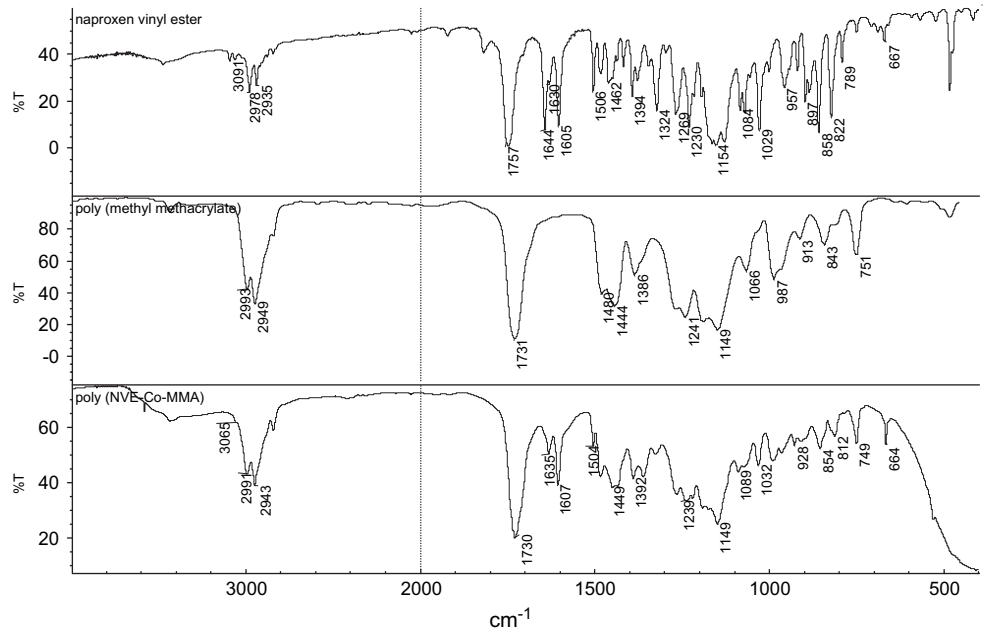


Fig. 1. IR spectra of (*S*)-naproxen vinyl ester, its methyl methacrylate copolymer and poly(methyl methacrylate).

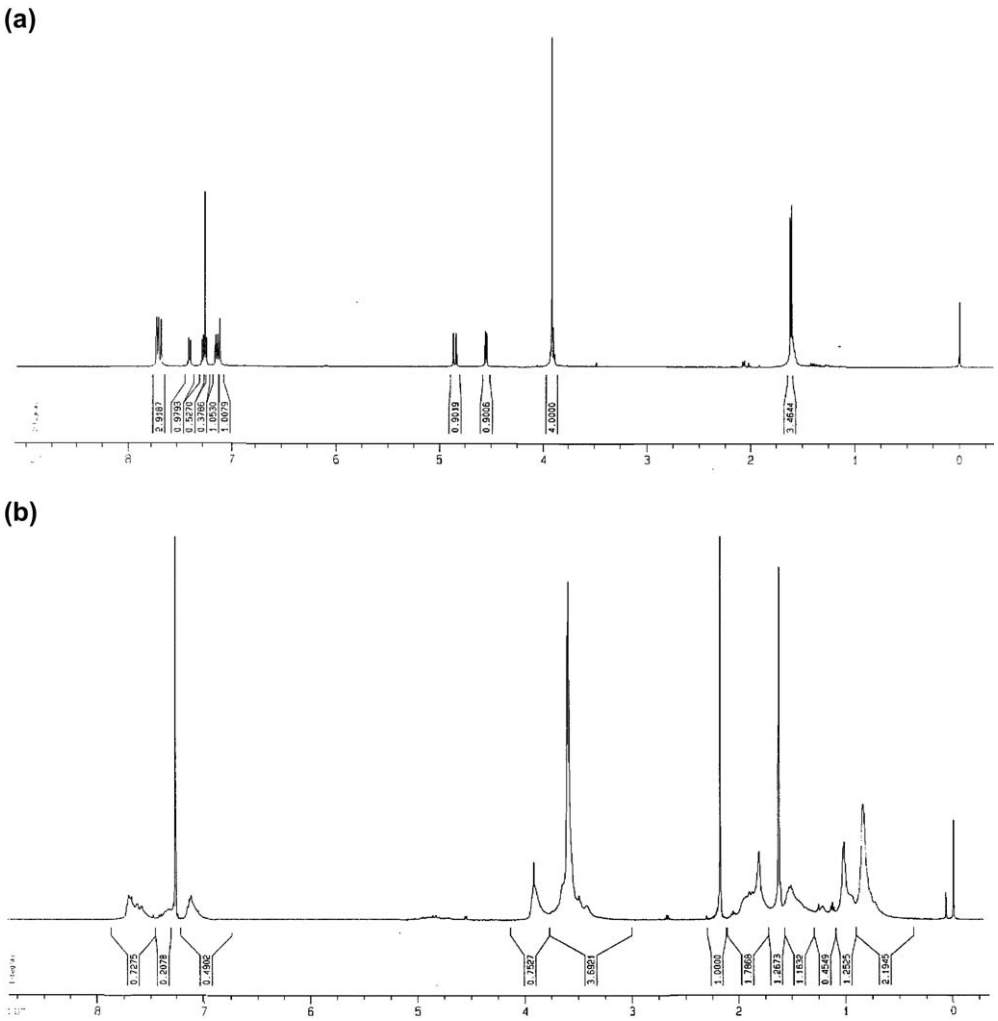


Fig. 2. ^{1}H NMR spectra of (*S*)-naproxen vinyl ester (a) and poly(NVE-*co*-MMA) (b) (entry 3 in Table 3) in $CDCl_3$.

Table 2
Copolymerization of (*S*)-profen vinyl ester

Entry	Substrate	Profen in the feed (mol%)	Yield (%)	Profen in the copolymer[a] (mol%)	M_n[b] $\times 10^{-4}$	M_w/M_n[b]
1	**1a**	67	52	35.2	2.42	2.7
2	**1b**	67	44	57.6	8.06	1.6
3	**1c**	67	85	54.0	5.80	1.9

[a] Determined by the integration ratios of ^{1}H NMR spectra.
[b] Determined by GPC analysis.

Table 3
Composition for (*S*)-naproxen vinyl ester/methyl methacrylate copolymers

Entry	Naproxen in the feed (mol%)	Yield (%)	Naproxen in the copolymer[a] (mol%)	M_n[b] $\times 10^{-4}$	M_w/M_n[b]	$[\alpha]_D^{20}$[c]
1	67	52	35.2	2.42	2.7	18.8
2	50	64	28.7	2.67	2.9	16.3
3	33	72	16.9	3.70	2.5	10.7
4	25	85	10.6	3.99	2.5	4.0

[a] Determined by the integration ratios of ^{1}H NMR spectra.
[b] Determined by GPC analysis.
[c] $c = 1.0$ in chloroform.

integral of methoxy protons of naproxen (δ 3.9 ppm) or the integral of methine protons of ketoprofen (δ 4.2 ppm).

The molecular weight of optically active polymeric prodrug was determined and is shown in Table 2. As shown in the GPC profile (see Supporting information), the polymeric prodrugs have moderately high molecular weight and good polydispersity. (*S*)-Naproxen vinyl ester and 33 mol% methyl methacrylate were copolymerized to produce poly(NVE-*co*-MMA) having M_n of 2.42×10^4 and M_w/M_n of 2.7. The other two products were poly(KVE-*co*-MMA) (33 mol% MMA) with an M_w of 8.06×10^4 and M_w/M_n of 1.6 and poly(IVE-*co*-MMA) (33 mol% MMA) with an M_w of 5.80×10^4 and M_w/M_n of 1.9. The results shown in Table 2 indicate that the preparation of poly(KVE-*co*-MMA) and poly(IVE-*co*-MMA) by bulk polymerization gave the products with higher molecular weight, lower polydispersity and higher drug amount, compared to poly(NVE-*co*-MMA), which was prepared by bulk polymerization.

The effect of molar ratio of naproxen vinyl ester to methyl methacrylate was also investigated. Polymeric prodrugs with different compositions were generated with 67, 50, 33, and 25 mol% of naproxen vinyl ester monomer in the feed (Table 3). It was found that the molecular weight increased with increasing methyl methacrylate concentration. And when an increasing amount of methyl methacrylate was used, the specific rotation of the resulting polymer tended to decrease. This reconfirmed that the drug amount of the polymeric prodrug did decrease with the increase of comonomer concentration of methyl methacrylate.

4. Conclusion

We have developed a facile and clean chemo-enzymatic method to prepare optically active polymeric prodrugs of NSAIDs with high molecular weight. The corresponding polymeric prodrugs bearing (*S*)-naproxen, (*S*)-ketoprofen or (*S*)-ibuprofen residue were obtained with good optical activity and were characterized by IR, NMR and GPC. The profen monomers can also be copolymerized with other active agents to obtain optically active macromolecular prodrugs with some special properties, such as good water solubility and site-specificity. Further researches concerning the copolymerization with other functional comonomers are in progress.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.polymer.2006.06.043.

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