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Controllable selective enzymatic synthesis of *N*-acyl and *O*-acylpropranolol vinyl esters and preparation of polymeric prodrug of propranolol

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Abstract

Controllable selective synthesis strategy of polymerizable *N*-acyl and *O*-acylpropranolol vinyl derivatives was developed by enzyme-catalyzed acylation of propranolol using divinyl dicarboxylates with different carbon chain length as acyl donor. The influence of parameters including enzyme, solvents and chain length of acyl donor on the reaction was investigated in detail. Lipase AY30 in diisopropyl ether demonstrated high selectivity towards the amino group of propranolol, while lipase M from *Mucor javanicus* in dioxane acylated selectively the hydroxyl group of propranolol. *N*-Acylpropranolol (**3a**–**3c**) and *O*-acylpropranolol vinyl (**4a**–**4c**) derivatives were obtained successfully, and can be used for preparing functional macromolecular prodrugs of beta-blockers drugs. *N*-(Vinyladipoyl)propranolol (NVAP) was copolymerized with methyl methacrylate (MMA) using AIBN as initiator. The obtained polymeric prodrug was characterized with IR, NMR and GPC. The poly(NVAP-*co*-MMA) has M_n of 3.23×10^4, and M_w/M_n of 1.66.

Keywords: Controllable selectivity; Enzymatic synthesis; Organic media; Propranolol; Copolymer

1. Introduction

The selective modification, which derivatizes some functional groups of drugs, while leaving others free for alternative modification and/or interaction with the biological target, can improve the certain applications of the pharmaceutically compounds, such as drug delivery, solubility and bioavailability [1,2]. Conventional chemical methods are generally not selective enough to allow derivatization of polyfunctional compounds without protection/deprotection schemes and require complex multistep procedures [3]. Thus, selective enzymatic acylation has played an important role in the modification of drugs due to the high selectivity and mild reaction conditions [4]. Many research groups have paid much effort in enzymatic derivatization of drugs [5–7], which has been well recognized as an excellent strategy for the preparation of pharmaceuticals.

Propranolol, 1-isopropylamino-3-(naphthalen-1-yloxy)propan-2-ol, a typical β-blockers, is used in the treatment of hypertension and cardiac arrhythmias [8]. Despite nearly complete absorption of propranolol, very low and highly variable bioavailability is observed after oral administration due to extensive presystemic first-pass metabolism, which strongly limited its application in clinic. The use of prodrug (a chemically modified parent drug) can minimize the disadvantages of a parent drug and represent a useful therapy. Many propranolol prodrugs, have been synthesized to improve the systemic delivery and enhance the skin permeability by increasing their lipophilicity [9–13]. These derivatives were mostly acylated at hydroxyl group of propranolol by traditional chemical methods. For example, Udata et al. synthesized propranolol esters using propranolol and 2-phenylbutyryl chloride [11], and Anroop et al. obtained derivatives of propranolol by adding alkyl side chains to the hydroxyl group of the propranolol [9]. In comparison, few papers have reported the acylation at the amino group of propranolol. Chiou et al. prepared *N*-acetylpropranolol with lipase from *Candida cylindracea* in diisopropyl ether without *O*-acetylated derivatives [8]. To our best knowledge, no reports about the controllable selectivity of enzymatic synthesis between hydroxyl and amino groups of propranolol were available.

In recent years, macromolecular drugs have gained interest in pharmaceutical field, because they can effectively control the rate of drug release, administrate at low dosage, improve site-

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specificity and increase therapeutic benefit [14–20]. Few propranolol derivatives which carry with polymerizable groups, such as vinyl group, have been synthesized. These pharmaceutical monomers could be homo- or copolymerized with appropriate monomers using conventional polymerization methods for preparation of polymeric prodrugs.

In our previous work, we focused on the enzymatic selective modification of pharmaceutics [16,17]. Herein, a facile control of the enzymatic acylation position at the hydroxyl or amino group of propranolol was demonstrated through optimization of reaction conditions. A series of polymerizable *N*-acyl and *O*-acylpropranolol vinyl esters derivatives were prepared, respectively, which would be available for subsequent studies of bioactivity. Moreover, *N*-(vinyladipoyl)propranolol was subjected to free radical polymerization with methyl methacrylate and polymeric prodrug of propranolol with high molecular weight was prepared.

2. Experiment

2.1. Materials and methods

Lipase AY30 (700–1500 units/mg solid, one unit will hydrolyze 1.0 microequivalent of olive oil from a triglyceride in 1 h at pH 7.7 at 37 °C) was purchased from Acrös. Lipase from hog pancreas (HPL, 2.4 units/mg, 1 unit is the amount of immobilized enzyme which forms 1% octyl laurate from 0.5 mmol lauric acid and 1.0 mmol 1-octanol in 10 mL water-saturated isooctane in 1 h at 20 °C), lipase from *C. cylindracea* (CCL, 1.6 units/mg, 1 unit corresponds to the amount of enzyme which liberates 1 μmol oleic acid/min at pH 8.0 and 40 °C) and lipase from *Mucor javanicus* (MJL, 9.9 units/mg, 1 unit corresponds to the amount of enzyme which liberates 1 μmol oleinic acid from trioleoyl glycerol/min at pH 8.0 and 37 °C) were purchased from Fluka. Lipase Type VII from *Candida rugosa* (CRL, 706 units/mg, one unit will hydrolyze 1.0 microequivalent of olive oil from a triglyceride in 1 h at pH 7.7 at 37 °C) and lipase from porcine pancreas (PPL, 30–90 units/mg protein, one unit will hydrolyze 1.0 microequivalent of triacetin in 1 h at pH 7.7 at 37 °C) were purchased from Sigma. Propranolol hydrochloride was purchased from Jiangsu Xingyuan Chemical Plant (Jürong, PR China). 2,2′-Azoisobutyronitrile (AIBN) was purified by recrystallization with methanol. All other chemicals used in this work were of analytical grade and all solvents were first dried over 4 Å molecular sieves. The free base was prepared by neutralization of aqueous solutions of the salts (propranolol hydrochloride) with NaOH.

2.2. Analytical methods

All reactions were monitored by TLC on silica gel plates eluted with petroleum ether/ethyl acetate (1:1, v/v). The ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-500 MHz spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. Infrared spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Mass spectrometry data were obtained on Bruker Esquire-LC for electrospray (ESI-MS) measurements. Analytical HPLC was performed using Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150 mm × 4.6 mm) and a UV detector (289 nm). 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 2350. The mobile phase was tetrahydrofuran at a flow rate of 1.5 mL/min.

2.3. Synthesis of N-vinylpropranolol derivatives (*3a–3c*)

The reaction was initiated by adding 10 mg/mL Lipase AY30 to 20 mL diisopropyl ether containing propranolol (1 mmol), divinyl dicarboxylates (4 mmol). The suspension was kept at 50 °C and stirred at 250 rpm. The reaction was terminated by filtering of the enzyme and diisopropyl ether was evaporated. Formation of *N*-vinylpropranolol derivatives was monitored by TLC. The product was purified by silica gel chromatography with an eluent consisting of petroleum ether/ethyl acetate (3:1, v/v).

2.4. Synthesis of N-(vinylsuccinyl)propranolol (*3a*)

The reaction time was 36 h and the yield of product **3a** was 52.4%. ^{1}H NMR ($CDCl_3$, δ, ppm): 8.20 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.81 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.44–7.50 (m, 3H, Ar–H), 7.38 (t, 1H, $J = 7.5$ Hz, Ar–H), 7.29 (dd, 1H, $J = 6.3$ Hz, $J = 14.0$ Hz, –CH=), 6.86 (d, 1H, $J = 7.5$ Hz, Ar–H), 5.26 (br, 1H, –OH), 4.92 (d, 1H, $J = 14.0$ Hz, =CH_2), 4.59 (d, 1H, $J = 6.3$ Hz, =CH_2), 4.18–4.24 (m, 3H, –OCH_2–, –OCH$_2$CH–), 4.04 (m, 1H, $J = 6.7$ Hz, –CH(CH_3)$_2$), 3.73–3.78 (dd, 1H, $J = 8.5$ Hz, $J = 14.6$ Hz, –CHCH_2N–), 3.57 (d, 1H, $J = 8.5$ Hz, $J = 14.6$ Hz, –CHCH_2N–), 2.76–2.83 (m, 4H, –CH_2–), 1.36 (d, 3H, $J = 6.7$ Hz, –CH_3), 1.25 (d, 3H, $J = 6.7$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm) was shown in Table 1. IR (KBr, cm^{-1}): 3333 (OH), 1757 (O–C=O), 1610 (N–C=O), 1646 (C=C), 1508, 794, 776 (Ar). ESI-MS (*m/z*): 386.0 $[M + H]^+$, 408.0 $[M + Na]^+$.

2.5. Synthesis of N-(vinyladipoyl)propranolol (*3b*)

The reaction time was 84 h, and the yield of product **3b** was 68.2%. ^{1}H NMR ($CDCl_3$, δ, ppm): 8.21 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.81 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.60–7.51 (m, 3H, Ar–H), 7.38 (t, 1H, $J = 7.5$ Hz, Ar–H), 7.29 (dd, 1H, $J = 6.2$ Hz, $J = 14.0$ Hz, –CH=), 6.86 (d, 1H, $J = 7.5$ Hz, Ar–H), 5.58 (br, 1H, –OH), 4.89 (d, 1H, $J = 14.0$ Hz, =CH_2), 4.57 (d, 1H, $J = 6.2$ Hz, =CH_2), 4.13–4.25 (m, 3H, –OCH_2–, –OCH$_2$CH–), 4.04 (t, 1H, $J = 6.7$ Hz, –CH(CH_3)$_2$), 3.73–3.77 (dd, 1H, $J = 8.5$ Hz, $J = 14.6$ Hz, –CHCH_2N–), 3.54 (d, 1H, $J = 8.5$ Hz, $J = 14.6$ Hz, –CHCH_2N–), 2.45–2.50 (m, 4H, –CH_2–), 1.75 (m, 4H, –CH_2–), 1.43 (d, 3H, $J = 6.7$ Hz, –CH_3), 1.21 (d, 3H, $J = 6.7$, –CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm) was shown in Table 1. IR (KBr, cm^{-1}): 3335 (OH), 1754 (O–C=O), 1615 (N–C=O), 1646 (C=C), 1508, 794, 776 (Ar). ESI-MS (*m/z*): 414.0 $[M + H]^+$, 436.0 $[M + Na]^+$.

Table 1
Chemical shifts of ^{13}C NMR ($CDCl_3$) of propranolol and products **3a**–**3c** and **4a**–**4c**

Carbon	**1**	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**
Ar–C	154.6	154.3	154.3	154.3	154.5	154.5	154.5
	134.7	134.7	134.8	134.8	134.7	134.7	134.7
	127.8	127.8	127.9	127.9	127.7	127.7	127.6
	126.7	126.6	126.6	126.6	126.7	126.7	126.6
	126.1	126.2	126.2	126.2	126.0	126.0	126.0
	125.8	125.6	125.6	125.6	125.8	125.8	125.8
	125.5	125.4	125.5	125.5	125.6	125.5	125.6
	122.1	121.8	121.8	121.8	122.2	122.2	122.1
	120.8	120.9	120.9	120.9	120.9	120.9	120.9
	105.1	105.1	105.1	105.1	105.1	105.1	105.0
Ar–O*C*H$_2$–	71.0	70.1	70.1	70.1	68.2	68.1	68.2
Ar–OCH$_2$*C*H–	68.8	72.3	72.7	72.8	72.3	72.3	72.2
–CH*C*H$_2$N–	49.8	46.4	46.5	46.6	47.6	47.6	47.6
–CH(CH$_3$)$_2$	49.2	49.2	49.3	49.4	48.9	48.9	48.8
CH$_3$	23.4	21.5	21.7	21.7	23.2	23.3	23.2
	23.3	20.9	21.0	21.1	23.1	23.1	23.1
–CH$_2$–		29.9	34.0	34.4	29.9	34.3	34.4
		29.3	33.8	34.2	29.7	33.7	34.1
			24.9	30.2		24.6	30.2
			24.5	30.0		24.2	30.0
				29.9			29.9
				29.7			29.7
				25.2			25.2
				25.0			24.9
C=O		174.1	175.9	175.5	173.3	173.2	173.1
		170.4	170.7	170.8	170.6	170.5	170.6
–CH=CH$_2$		141.4	141.4	141.3	141.3	141.3	141.4
		98.1	98.0	98.1	97.9	97.9	97.8

2.6. Synthesis of N-(vinylsebacoyl)propranolol (*3c*)

The reaction time was 96 h, and the yield of product **3c** was 45.6%. ^1H NMR ($CDCl_3$, δ, ppm): 8.21 (d, 1H, J = 7.5 Hz, Ar–H), 7.81 (d, 1H, J = 7.5 Hz, Ar–H), 7.44–7.80 (m, 3H, Ar–H), 7.38 (t, 1H, J = 7.5 Hz, Ar–H), 7.29 (dd, 1H, J = 6.3 Hz, J = 14.0, –CH=), 6.86 (d, 1H, J = 7.5 Hz, Ar–H), 5.77 (br, 1H, –OH), 4.87 (d, 1H, J = 14.0 Hz, =CH$_2$), 4.56 (d, 1H, J = 6.3 Hz, =CH$_2$), 4.14–4.25 (m, 3H, –OCH$_2$–, –OCH$_2$C*H*–), 4.04 (m, 1H, J = 6.7 Hz, –C*H*(CH$_3$)$_2$), 3.73–3.78 (dd, 1H, J = 8.5 Hz, J = 14.6 Hz, –CHCH$_2$N–), 3.56 (d, 1H, J = 8.5 Hz, J = 14.6 Hz, –CHCH$_2$N–), 2.37–2.47 (m, 4H, –CH$_2$–), 1.63–1.67 (m, 4H, –CH$_2$–), 1.31–1.28 (m, 11H, –CH$_2$–, –CH$_3$), 1.21 (d, 3H, J = 6.7 Hz, –CH$_3$). ^{13}C NMR ($CDCl_3$, δ, ppm) was shown in Table 1. IR (KBr, cm^{-1}): 3334 (OH), 1753 (O–C=O), 1616 (N–C=O), 1646 (C=C), 1508, 794, 773 (Ar). ESI-MS (*m/z*): 470.0 [M + H]$^+$, 492.0 [M + Na]$^+$.

2.7. Synthesis of O-vinylpropranolol derivatives (*4a–4c*)

The reaction was initiated by adding 10 mg/mL MJL to 20 mL dioxane containing propranolol (1 mmol) and divinyl dicarboxylates (4 mmol). The suspension was kept at 50 °C and stirred at 250 rpm. The reaction was terminated by filtering of the enzyme and dioxane was evaporated. Formation of *O*-vinylpropranolol derivatives was monitored by TLC. The product was purified by silica gel chromatography with an eluent consisting of petroleum ether/ethyl acetate (1:1, v/v).

2.8. Synthesis of O-(vinylsuccinyl)propranolol (*4a*)

The reaction time was 18 h and the yield of product **4a** was 17.6%. ^1H NMR ($CDCl_3$, δ, ppm): 8.20 (d, 1H, J = 7.5 Hz, Ar–H), 7.80 (d, 1H, J = 7.5 Hz, Ar–H), 7.43–7.49 (m, 3H, Ar–H), 7.36 (t, 1H, J = 7.5 Hz, Ar–H), 7.25 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH=), 6.82 (d, J = 7.5 Hz, Ar–H), 5.49 (m, 1H, –OCH$_2$C*H*–), 4.86 (d, 1H, J = 14.0 Hz, =CH$_2$), 4.55 (d, 1H, J = 6.3 Hz, =CH$_2$), 4.33 (m, 2H, –OC*H*$_2$–), 3.11 (m, 2H, –CHC*H*$_2$N–), 2.87 (m, 1H, J = 6.7 Hz, –C*H*(CH$_3$)$_2$), 2.67 (m, 4H, –CH$_2$–), 1.13 (d, 6H, J = 6.7 Hz, –CH$_3$). ^{13}C NMR ($CDCl_3$, δ, ppm) was shown in Table 1. IR (KBr, cm^{-1}): 3281 (NH), 1754, 1738 (O–C=O), 1646 (C=C), 1509, 794, 773 (Ar). ESI-MS (*m/z*): 386.0 [M + H]$^+$.

2.9. Synthesis of O-(vinyladipoyl)propranolol (*4b*)

The reaction time was 36 h and the yield of product **4b** was 20.2%. ^1H NMR ($CDCl_3$, δ, ppm): 8.20 (d, 1H, J = 7.5 Hz, Ar–H), 7.80 (d, 1H, J = 7.5 Hz, Ar–H), 7.43–7.49 (m, 3H, Ar–H), 7.36 (t, 1H, J = 7.5 Hz, Ar–H), 7.24 (dd, 1H, J = 6.2 Hz, J = 14.7 Hz, –CH=), 6.82 (d, J = 7.5 Hz, Ar–H), 5.49 (m, 1H, –OCH$_2$C*H*–), 4.86 (d, 1H, J = 14.0 Hz, =CH$_2$), 4.55 (d, 1H, J = 6.2 Hz, =CH$_2$), 4.33 (m, 2H, –OCH$_2$–), 3.09 (m, 2H, –CHC*H*$_2$N–), 2.86 (m, 1H, J = 6.7 Hz, –C*H*(CH$_3$)$_2$), 2.35–2.41 (m, 4H, –CH$_2$–), 1.69 (m, 4H, –CH$_2$–), 1.12 (d, 6H, J = 6.7 Hz, –CH$_3$). ^{13}C NMR ($CDCl_3$, δ, ppm) was shown in Table 1. IR (KBr, cm^{-1}): 3283 (NH), 1754, 1739 (O–C=O), 1646 (C=C), 1509, 792, 771 (Ar). ESI-MS (*m/z*): 414 [M + H]$^+$.

2.10. Synthesis of O-(vinylsebacoyl)propranolol (*4c*)

The reaction time was 48 h, and the yield of product **4c** was 15.8%. ^1H NMR ($CDCl_3$, δ, ppm): 8.20 (d, 1H, J = 7.5 Hz, Ar–H), 7.80 (d, 1H, J = 7.5 Hz, Ar–H), 7.43–7.49 (m, 3H, Ar–H), 7.36 (t, 1H, J = 7.5 Hz, Ar–H), 7.29 (dd, 1H, J = 6.3 Hz, J = 14.0Hz, –CH=), 6.82 (d, 1H, J = 7.5 Hz, Ar–H), 5.48 (m, 1H, –OCH$_2$C*H*–), 4.88 (d, 1H, J = 14.0 Hz, =CH$_2$), 4.56 (d, 1H, J = 6.3 Hz, =CH$_2$), 4.32 (m, 2H, –OCH$_2$–), 3.12 (m, 2H, –CHC*H*$_2$N–), 2.90 (m, 1H, J = 6.7 Hz, C*H*(CH$_3$)$_2$), 2.30–2.40 (m, 4H, –CH$_2$–), 1.58–1.60 (m, 4H, –CH$_2$–), 1.31–1.21 (m, 8H, –CH$_2$–), 1.14 (d, 6H, J = 6.7 Hz, –CH$_3$). ^{13}C NMR ($CDCl_3$, δ, ppm) was shown in Table 1. IR (KBr, cm^{-1}): 3284 (NH), 1757, 1739 (O–C=O), 1646 (C=C), 1509, 793, 772 (Ar). ESI-MS (*m/z*): 470.0 [M + H]$^+$.

2.11. Copolymerization of N-(vinyladipoyl)propranolol with methyl methacrylate

The copolymerization of *N*-(vinyladipoyl)propranolol (NVAP) with methyl methacrylate (MMA) was conducted by solution polymerization using AIBN initiator. 0.5 mmol NVAP and 1 mmol MMA was added to a small flame-dried flask with 0.3 mL DMF. The solution was degassed (freeze/pump/thaw

cycles) and 2% AIBN (w/w) was added. The polymerization was continued for 10 h at 70 °C. Precipitating the polymer in methanol terminated the reaction, and the white precipitate was washed with acetone.

3. Results and discussion

3.1. Enzymatic synthesis of propranolol vinyl derivatives

By choosing appropriate enzymes and organic solvents, controllable selective acylation at hydroxyl or amino group of propranolol, respectively was achieved and six polymerizable propranolol prodrugs, *N*-acyl and *O*-acylpropranolol vinyl derivatives, were synthesized by enzymatic reaction of propranolol and divinyl dicarboxylates (**2a**–**2c**). The reaction route was shown in Scheme 1. The acylation position of propranolol was determined by ^{13}C NMR. According to the general strategy described by Yoshimoto et al. [21], acylation of a hydroxyl group of substrate results in a downfield shift of the peak corresponding to the *O*-acylated carbon and an upfield shift of the peak corresponding to the neighboring carbon. The ^{13}C NMR ($CDCl_3$) data of the products **3a**–**3c**, **4a**–**4c** was shown in Table 1. The chemical shift for carbon (–OCH_2*C*H–) of product **4b** was downfield from 68.8 to 72.3 ppm, and that for carbons (–OCH_2– and –$CHCH_2$N–) of product **4b** was upfield from 71.0 to 68.1 ppm and from 49.8 to 47.6 ppm, respectively.

The ^{1}H NMR spectra also provided substitutional information of products. For the product **3b**, the chemical shift for H (–$CHCH_2$N–) of propranolol was splitted into double groups peaks and downfield from 2.85 to 3.75 and 3.54 ppm, and the chemical shift for H (Ar–OCH_2CH–) was unchanged; while for the products **4b**, the chemical shift for H (Ar–OCH_2CH–) was downfield from 4.19 to 5.49 ppm.

3.2. Effect of enzyme source

The catalytic activity and selectivity for the enzymatic synthesis of pharmaceutical derivatives depends markedly on the critical role of the enzyme [22,23]. In order to choose the appropriate enzymes for the selective acylation at hydroxyl or amino group of propranolol, respectively, six commercially available enzymes were tested in three different solvents. Lipases show a higher activity in hydrophobic solvent of lower polarity (log $P > 2$) [24], while nonpolar solvents, such as *n*-hexane, were unsuitable because of poor solubility of propranolol in this reaction. Hence, we selected diisopropyl ether, chloroform and toluene as reaction media, whose log *P* values are around 2. The yields of propranolol vinyl derivatives were determined by HPLC. The results were shown in Table 2. In the absence of enzyme, the yields were less than 2%. It can be found that different lipases indicated different catalytic activity in three solvents. Lipase AY30 had the higher reaction activity for the amino group

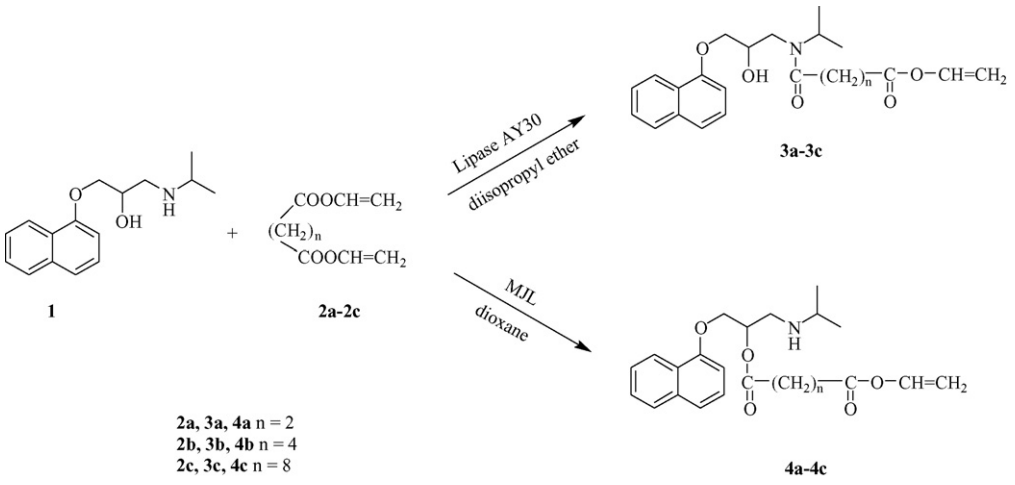
Table 2
Influence of enzyme sources on the reaction in different solvents

Entry	Enzyme	Solvent[a]	Yield (%) **3b**	Yield (%) **4b**	Selectivity[b]
1	Lipase AY30	Diisopropyl ether	68.2	4.1	16.6
2		Chloroform	52.1	18.2	2.9
3		Toluene	83.6	15.1	5.7
4	CRL	Diisopropyl ether	50.3	19.8	2.5
5		Chloroform	51.3	16.6	3.1
6		Toluene	74.8	17.4	4.3
7	PPL	Diisopropyl ether	25.2	22.0	1.1
8		Chloroform	50.4	14.8	3.4
9		Toluene	78.4	20.1	3.9
10	HPL	Diisopropyl ether	21.4	23.1	0.9
11		Chloroform	51.6	20.1	2.6
12		Toluene	54.5	15.4	3.5
13	MJL	Diisopropyl ether	52.0	24.9	2.1
14		Chloroform	4.2	20.3	0.2
15		Toluene	32.3	12.2	2.6
16	CCL	Diisopropyl ether	47.2	18.1	2.6
17		Chloroform	51.4	17.3	3.0
18		Toluene	72.6	18.1	4.0

Conditions: enzyme (10 mg mL^{-1}), propranolol (0.1 mmol), divinyl adipate (0.4 mmol), solvent (2 mL), 50 °C, 250 rpm, 3 days. Yields were determined by HPLC.

[a] log *P* of diisopropyl ether, chloroform and toluene is 1.9, 2.0 and 2.2, respectively.

[b] The molar ratio **3b** to **4b**.



Scheme 1. Controllable selective enzymatic synthesis of *N*-acyl and *O*-acylpropranolol vinyl derivatives.

Table 3
Influence of organic solvent on selectivity and yield in different enzymes

Entry	Solvent	log *P*	Lipase AY30			MJL		
			Yield (%), **3b**	Yield (%), **4b**	Selectivity[a]	Yield (%), **3b**	Yield (%), **4b**	Selectivity[a]
1	DMF	−1.0	0	4.6	0.0	2.6	10.7	0.2
2	Dioxane	−0.5	0.9	14.2	0.1	0.8	20.5	0.04
3	THF	0.49	4.2	9.3	0.5	12.5	6.1	2.0
4	*tert*-Butyl-alcohol	0.79	21.0	10.4	2.0	8.3	9.1	0.9
5	MTBE	1.36	10.7	15.6	0.7	18.2	15.4	1.2
6	IPE	1.9	68.2	4.1	16.6	52.0	24.9	2.1
7	Chloroform	2.0	42.1	18.2	2.3	4.2	20.3	0.2
8	Toluene	2.2	85.6	15.1	5.7	32.3	12.2	2.6
9	Cyclohexane	3.4	26.4	4.9	5.4	35.2	9.8	3.6
10	*n*-Hexane	3.9	19.2	3.8	5.1	37.1	6.2	6.0
11	*n*-Octane	4.9	18.4	4.5	4.1	35.7	8.3	4.3

Conditions: Lipase AY30 (10 mg mL^{-1}) (a) or MJL (10 mg mL^{-1}) (b), propranolol (0.1 mmol), divinyl adipate (0.4 mmol), organic solvent (2 mL), 50 °C, 250 rpm, 4 days, yields were determined by HPLC.
[a] The molar ratio **3b** to **4b**.

of propranolol in chloroform, diisopropyl ether and toluene than other five enzymes. Its selectivity for the amino group was the highest (entry 1, Table 2).

Most of lipases facilitated the reaction of amino group of propranolol. However, the MJL showed a preference for the acylation on hydroxyl group of propranolol in chloroform, and the highest selectivity value and yield of **4b** catalyzed with MJL is 0.2 and 20.3%, respectively (entry 14, Table 2). Then the selectivity of CRL, CCL, PPL and HPL were low and the values were ranged from 4.3 to 0.9. Therefore, we selected Lipase AY30 and MJL for further investigation.

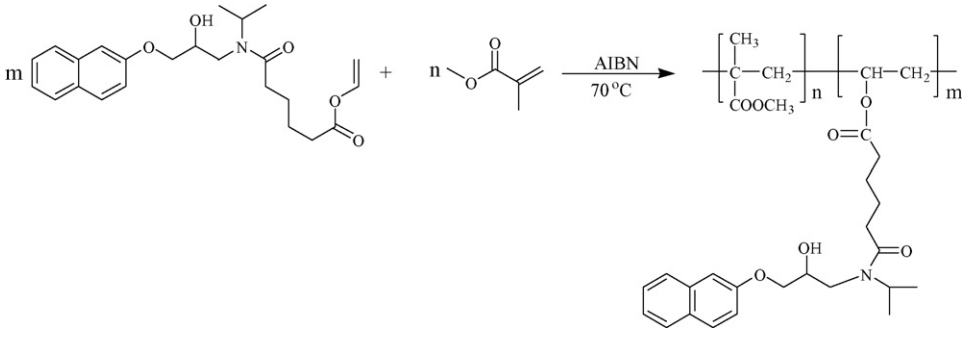
3.3. Effect of solvent

Reaction media plays a crucial role on maintaining enzyme catalytic activity and stability [24]. It is reported that selectivity of enzymes may be sometimes predictably controlled by changing the reaction medium, which creates opportunities for controllable enzymatic selective synthesis [25].

Eleven organic solvents with log *P* value ranging from −1.0 to 4.9 were tested to enhance the selectivity exhibited by Lipase AY30 and MJL in enzymatic reaction of propranolol with divinyl adipate. As shown in Table 3, the nature of the solvent affected the yields of the reaction. After evaluating eleven organic solvents for the influence of the reaction, it was in diisopropyl ether that *N*-(vinyladipoyl)propranolol can be obtained with better yield and best selectivity using lipase AY30 (entry 6, Table 3). In addition, the high catalytic activity was given in toluene with moderate selectivity (entry 8, Table 3). However, entry 2 in Table 3 showed that *O*-(vinyladipoyl)propranolol can be obtained with highest yield and best selectivity using MJL in dioxane, and the yield of *N*-(vinyladipoyl)propranolol was less than 1%. Furthermore, it was observed that in the nonpolar solvent, such as *n*-hexane and *n*-octane, both MJL and Lipase AY30 had higher reactive activity for the amino group of propranolol than hydroxyl group. But the yields of the main *N*-(vinyladipoyl)propranolol were low (entries 10 and 11, Table 3) because of the poor solubility of propranolol in these nonpolar solvent.

3.4. Effect of the chain length of acylating agent

In addition to divinyl adipate, we also selected divinyl succinate and divinyl sebacate as acylating agents to investigate the influence of the chain length. According to the HPLC analysis, under the same reaction conditions, the selectivity ratio of amino group to hydroxyl group in the reaction of propranolol with divinyl succinate or divinyl sebacate was near to that of divinyl adipate, it showed that the chain length of acylating agents had no obvious influence on the selectivity. However, the enzymatic



Scheme 2. Copolymerization of *N*-(vinyladipoyl)propranolol with MMA.

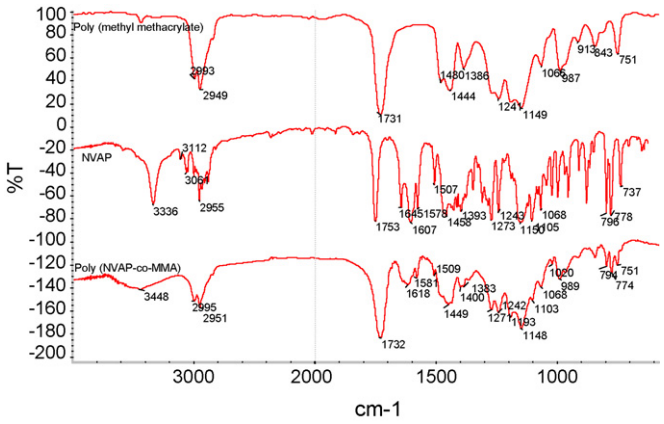


Fig. 1. IR spectra of *N*-(vinyladipoyl)propranolol (NVAP), poly(NVAP-*co*-MMA) and poly(methyl methacrylate).

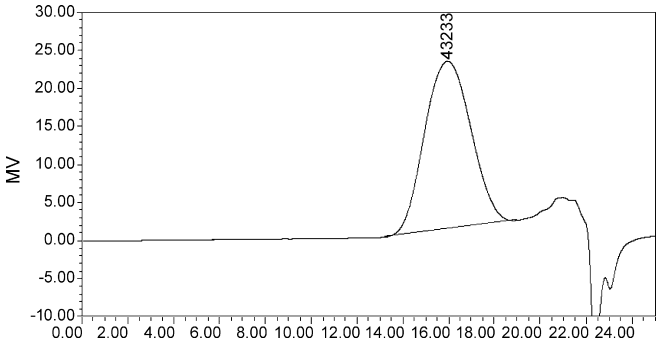


Fig. 3. GPC of poly(NVAP-*co*-MMA) in THF.

reactivity decreased as the chain of the divinyl dicarboxylates increased. The yield of *N*-(vinylsuccinyl)propranolol was 34.8% in 12 h, while the yields of *N*-(vinyladipoyl)propranolol and *N*-(vinylsebacoyl) propranolol were 21.5% and 17.6%, respectively in 12 h. In initial reaction time, divinyl dicarboxylates with longer chain length provided a lower yield due to the more steric influence. The equilibrium time of reaction prolonged with the chain length increasing. The optimum reaction time of propranolol and divinyl succinate was 36 h, while divinyl adipate and divinyl sebacate was 84 and 96 h, respectively. The yields of **3a**–**3c** had no obviously increase with extending the reaction time.

3.5. Preparation of the copolymer poly(NVAP-co-MMA)

Methyl methacrylate was chosen as the comonomer to study the synthesis of polymeric prodrug of NVAP. We carried out the copolymerization using AIBN initiated system. The polymerization reaction route was shown in Scheme 2. Products were analyzed by FTIR and NMR. In IR spectra of poly(NVAP-*co*-MMA) (Fig. 1), 3061 and 1645 cm^{-1} assigned to the vibration bands of double bond in the NVAP disappeared, and the absorption at 1581, 1509, 1103, 794 and 774 cm^{-1} which were assigned to the aromatic ring presented. As shown in NMR spectra (Fig. 2), as expected, the double bonds present in the NVAP monomer and MMA was absent in the polymer. The aromatic protons appear around 6.8–8.2 ppm in ^{1}H NMR of poly(NVAP-*co*-MMA). Analysis of IR and NMR spectra confirmed the structure of copolymer. According to the calculation from the

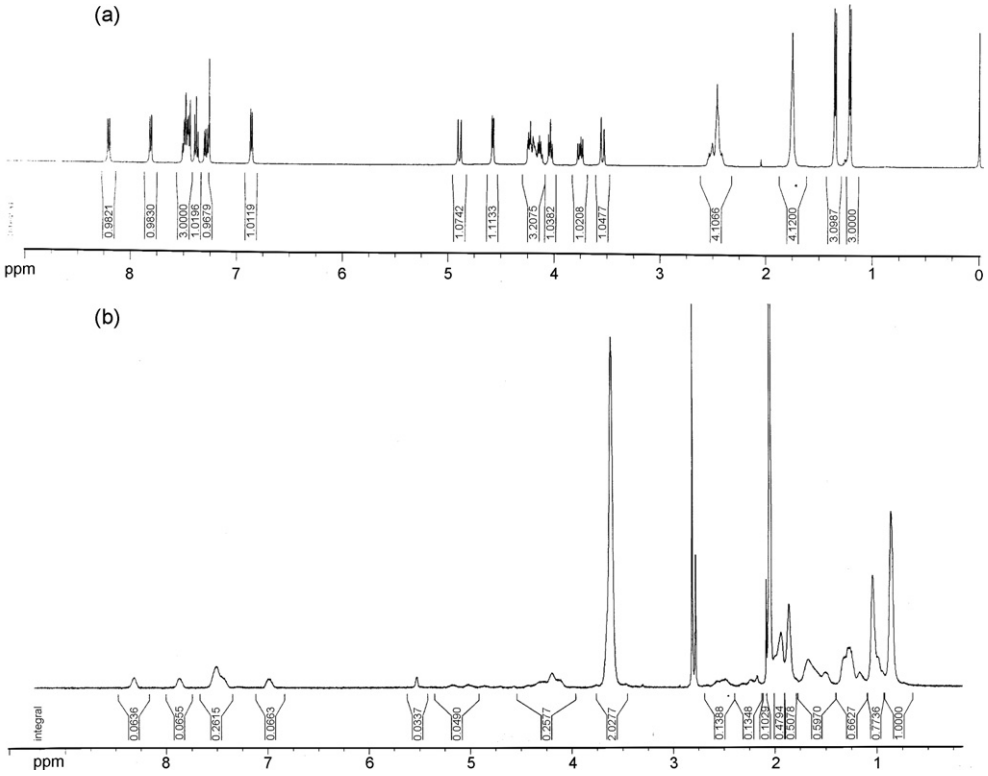


Fig. 2. ^{1}H NMR spectra of *N*-(vinyladipoyl)propranolol (NVAP) (a) in $CDCl_3$ and poly(NVAP-*co*-MMA) (b) in acetone-d_6.

NMR spectrum, the ratio of NVAP and MMA monomers in the copolymer was 1/9. From Fig. 3 (the GPC profile), the polymeric prodrugs have high molecular weight with M_n of 3.23×10^4 and narrow polydispersity with M_w/M_n of 1.66.

4. Conclusion

The controllable selective enzymatic synthesis of polymerizable propranolol derivatives with different carbon chain length was described. Lipase AY30 in diisopropyl ether demonstrated high selectivity towards the amino group of propranolol, while lipase M from *M. javanicus* in dioxane acylated selectively the hydroxyl group of propranolol. *N*-(Vinyladipoyl)propranolol was copolymerized with methyl methacrylate using AIBN as initiator. The obtained polymeric prodrug was characterized with IR, NMR and GPC. The poly(NVAP-*co*-MMA) has M_n of 3.23×10^4, and M_w/M_n of 1.66. The preparation of propranolol copolymers containing other bioactive comonomers and the investigation on the controlled release of polymeric β-blockers drugs are in progress.

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