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Regioselective synthesis of amphiphilic metoprolol–saccharide conjugates by enzymatic strategy in organic media

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ABSTRACT

An efficient protocol to prepare metoprolol–saccharide conjugates by a selective enzymatic synthesis method was developed. Firstly, the transesterification of metoprolol with three divinyl dicarboxylates (divinyl succinate, divinyl adipate and divinyl sebacate) was performed. The influences of organic solvents, sources of enzymes and acylating reagents on the synthesis of *N*-(vinyloxycarbonyl)metoprolol were investigated. A series of lipophilic metoprolol derivatives with vinyl group were obtained by using a lipase from porcine pancreas (PPL) in anhydrous tetrachloromethane at 50 °C. Subsequently, alkaline protease from *Bacillus subtilis* catalyzed highly regioselective acylation of three monosaccharides (glucose, mannose and galactose) and two disaccharides (maltose and sucrose) with *N*-(5-vinyloxycarbonylpentanoyl)metoprolol in anhydrous pyridine at 50 °C to give metoprolol–saccharide conjugates in good yields. The partition coefficients of the products were investigated. The results indicated that the aqueous solubility of metoprolol–monosaccharide and metoprolol–disaccharide conjugates was improved markedly compared with the parent drug of metoprolol, and the aqueous solubility of metoprolol–disaccharide conjugates was much better than that of metoprolol–monosaccharide conjugates.

1. Introduction

Metoprolol, 1-(isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol, is a typical β-blocker, which has remarkable efficacy in angina pectoris, hypertension, cardiac arrhythmias, migraine headaches and other disorders related to sympathetic nervous system [1]. However, drug candidates in developmental stage currently exhibit some limitations, such as very low and highly variable bioavailability [2,3], and rapid elimination with half-life between 3 and 4 h [4], which strongly restrict their applications in clinic. To overcome those drawbacks, much attention has been paid to searching for new derivatives and extensive modifications of metoprolol [5,6]. Among the derivatives, metoprolol–saccharide conjugates have attracted particular interest [7]. The combinations of drugs and carbohydrates for parts of their therapeutic actions have extended a wide range of drugs [8–11]. Some antitumor drugs and vaccines have been modified by galactose or lactose to improve their bioavailability [12–15]. The properties of porphyrin–saccharide conjugates have been proved to possess the abilities of targeting and incapacitating on cancer cells [16].

However, chemical routes for the synthesis of those derivatives, especially their selective modifications, are complicated because of specific protection/de-protection steps. So exploring of new approaches is highly demanded. The enzymatic method is a better protocol due to its high regioselectivity under mild conditions [17]. Since Klibanov and co-workers first demonstrated selective acylation of monosaccharides catalyzed by lipases in organic media [18], various studies concerning similar biotransformations have been reported [19,20]. Carbohydrates bearing vinyl esters can offer a new family of functional water-soluble monomers for preparing drug–saccharide conjugates by enzyme-catalyzed selective transformations. Especially divinyl dicarboxylates, which have been proved to be useful acylating reagents, have higher transesterification reactivity for the synthesis of sugar/drugs vinyl ester derivatives and drug–saccharide conjugates [21–24]. In addition, the aqueous solubility of drug–saccharide conjugates is still a valuable and interesting topic, which would provide available information for further investigation of the therapeutic benefit of drugs and their derivatives.

In this paper, based on our previous work on enzymatic modification of pharmaceutics [25–28], three *N*-(vinyloxycarbonyl) metoprolol monomers were prepared by PPL in CCl_4. The obtained derivatives were subjected to highly regioselective enzymatic transformations with three monosaccharides (glucose, mannose and galactose) and two disaccharides (maltose and sucrose) for the

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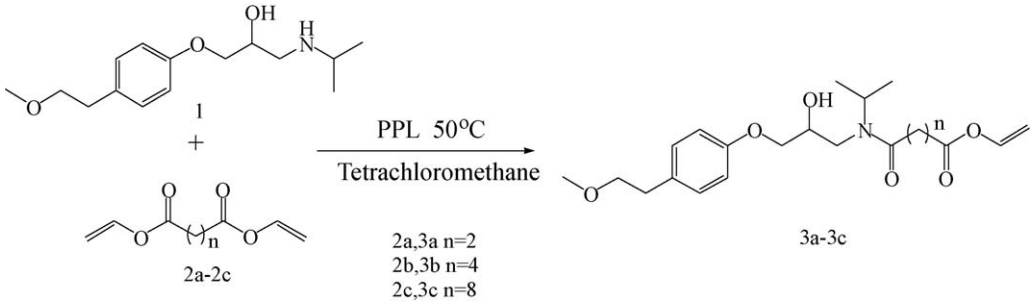


Scheme 1. Enzymatic synthesis of *N*-(vinyloxycarbonyl)metoprolol.

synthesis of metoprolol–saccharide conjugates (Scheme 1). And the aqueous solubility of five kinds of metoprolol–saccharide conjugates was also studied.

2. Experimental

2.1. Materials

Lipozyme® (E.C. 3.1.1.1, an immobilized preparation of lipase from *Mucor miehei*, 42 U/g, MML), lipase from porcine pancreas (E.C. 3.1.1.3, type II, powder, 30–90 U/mg, PPL) and lipase from *Candida cylindracea* (E.C. 3.1.1.3, powder, 2.8 U/mg, CCL) were purchased from Fluka. *Candida antarctica* lipase acrylic resin (E.C. 3.1.1.3, 10,000 U/g, CAL-B) and lipase type VII from *Candida rugosa* (E.C. 3.1.1.3, powder, 706 U/mg, CRL) were purchased from Sigma. Lipase AY30 (E.C. 3.1.1.3, powder, AY30) was purchased from Acrös. Alkaline protease from *Bacillus subtilis* (E.C. 3.4.21.14, a crude preparation of alkaline serine protease, 100 U/mg) was purchased from Wuxi Enzyme Co., Ltd. (Wuxi, PR China). Metoprolol succinate was presented by the research center of Aisen (Jinhua, PR China), and the free metoprolol was prepared by neutralization of the aqueous solutions of salts with NaOH. All other chemicals used in this work were of analytical grade and all solvents were first dried over 4 Å molecular sieves.

2.2. Analytical methods

All reactions were monitored by TLC on silica gel plates eluted with petroleum ether/ethyl acetate (1.5/1, v/v). The ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-400 MHz spectrometer at 400 and 100 MHz, respectively. Infrared spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Analytical HPLC was performed using Agilent 1100 system (Agilent, USA) with a reversed-phase Shim-Pack VP-ODS column (150 mm × 4.6 mm) and a DAD detector (220 nm). Mobile phase for *N*-(vinyloxycarbonyl)metoprolol was methanol/PBS (10 mmol/L, pH = 3.0) (60/40, v/v). Mobile phase for metoprolol–saccharide conjugates was methanol/water (80/20, v/v). Flow rate was adjusted to 1.0 mL/min.

2.3. General procedure for enzymatic synthesis of *N*-(vinyloxycarbonyl)metoprolol (3a–3c)

The reaction was initiated by adding 300 mg PPL to 20 mL anhydrous tetrachloromethane containing metoprolol (2 mmol) and divinyl dicarboxylates (8 mmol) in 50 mL conical flask. The suspension was kept at 50 °C and stirred at 200 rpm for 48–96 h. The reaction was terminated by filtering off the enzyme and tetrachloromethane was evaporated. The formation of *N*-(vinyloxycarbonyl)metoprolol was monitored by TLC. The products were purified by silica gel chromatography with an eluent consisting of petroleum ether/ethyl acetate (2/1, v/v).

2.3.1. Synthesis of *N*-(3-vinyloxycarbonylpropanoyl)metoprolol (**3a**)

The reaction time was 48 h and the yield of **3a** was 57% (except special explanation, the yields were all determined by HPLC). IR (KBr, cm^{-1}): 3335 (OH), 1757 (OC=O), 1646 (C=C), 1514, 794, 776 (Ar). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.25 (dd, 1H, J = 6.6 Hz, J = 13.4 Hz, –CH=), 7.12 (d, 2H, J = 8.0 Hz, Ar–H), 6.83 (d, 2H, J = 8.4 Hz, Ar–H), 5.20 (s, 1H, OH), 4.89 (d, 1H, J = 14.0 Hz, =CH_2), 4.58 (d, 1H, J = 6.0 Hz, =CH_2), 4.13 (t, 1H, J = 6.6 Hz, NCH_2), 3.99 (q, 2H, J = 9.6 Hz, NCH_2 and NCH(CH_3)$_2$), 3.78 (q, J = 8.6 Hz, 1H, CHOH), 3.61–3.54 (m, 3H, OCH_2CH$_2$ and OCH_2CH), 3.43 (d, 1H, J = 13.4, OCH_2CH), 3.35 (s, 3H, CH_3O), 2.84–2.73 (m, 6H, CH_2 and Ar–CH_2), 1.28 (d, 3H, J = 6.8 Hz, CH_3), 1.22 (d, 3H, J = 6.8, CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.6, 170.2 (C=O), 156.9, 131.4, 129.8, 114.3 (Ar–C, metoprolol), 141.1 (OCH=), 97.9 (=CH_2), 73.8, 72.1, 46.1, 35.2, 29.0, 28.0 (CH_2), 69.5, 48.9 (CH), 58.6, 21.1, 20.6 (CH_3). ESI-MS (m/z): 416.0 [M+Na]$^+$.

2.3.2. Synthesis of *N*-(5-vinyloxycarbonylpentanoyl)metoprolol (**3b**)

The reaction time was 72 h and the yield of **3b** was 74%. IR (KBr, cm^{-1}): 3335 (OH), 1752 (OC=O), 1646 (C=C), 1513, 794, 776 (Ar). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.26 (dd, 1H, J = 6.6 Hz, J = 13.8 Hz, –CH=), 7.13 (d, 2H, J = 8.4 Hz, Ar–H), 6.83 (d, 2H, J = 8.8 Hz, Ar–H), 5.51 (1H, OH), 4.87 (dd, 1H, J = 13.8 Hz, =CH_2), 4.57 (dd, 1H, J = 6.0 Hz, =CH_2), 4.11–3.99 (m, 3H, NCH_2 and NCH(CH_3)$_2$), 3.80 (d, 1H, J = 8.4 Hz, CHOH), 3.62–3.54 (m, 3H, OCH_2CH$_2$ and OCH_2CH), 3.40 (d, 1H, J = 14.8 Hz, OCH_2CH), 3.35 (s, 3H, CH_3O), 2.81 (t, 2H, J = 7.0, Ar–CH_2), 2.42 (q, 4H, J = 9.2 Hz, CH_2), 1.73 (s, 4H, CH_2), 1.26 (d, 3H, J = 6.4 Hz, CH_3), 1.19 (d, 3H, J = 6.4, CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm): 175.3, 170.4 (C=O), 156.9, 131.3, 129.8, 114.2 (Ar–C, metoprolol), 141.1 (OCH=), 97.7 (=CH_2), 73.8, 72.3, 46.1, 35.2, 33.6, 33.2, 24.6, 24.2 (CH_2), 69.5, 49.1 (CH), 58.6, 21.2, 20.7 (CH_3). ESI-MS (m/z): 444.0 [M+Na]$^+$.

2.3.3. Synthesis of *N*-(9-vinyloxycarbonylnonanoyl)metoprolol (**3c**)

The reaction time was 96 h and the yield of **3c** was 52%. IR (KBr, cm^{-1}): 3342 (OH), 1755 (OC=O), 1645 (C=C), 1512, 794, 776 (Ar). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.26 (dd, 1H, J = 6.6 Hz, J = 13.4 Hz, –CH=), 7.13 (d, 2H, J = 8.4 Hz, Ar–H), 6.83 (d, 2H, J = 8.4 Hz, Ar–H), 5.63 (s, 1H, OH), 4.86 (d, 1H, J = 14.0 Hz, =CH_2), 4.55 (d, 1H, J = 6.4 Hz, =CH_2), 4.13–3.97 (m, 3H, NCH_2 and NCH(CH_3)$_2$), 3.78 (t, 1H, J = 8.4 Hz, CHOH), 3.63–3.54 (m, 3H, OCH_2CH$_2$ and OCH_2CH), 3.39 (d, 1H, J = 14.8 Hz, OCH_2CH), 3.35 (s, 3H, CH_3O), 2.81 (t, 2H, J = 7.2 Hz, Ar–CH_2), 2.45–2.34 (m, 4H, CH_2), 1.64 (t, 4H, J = 6.8 Hz, CH_2), 1.33 (s, 8H, CH_2), 1.26 (d, 3H, J = 6.4 Hz, CH_3), 1.18 (d, 3H, J = 6.8, CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm): 176.1, 170.8 (C=O), 156.9, 131.3, 129.8, 114.2 (Ar–C, metoprolol), 141.1 (OCH=), 97.4 (=CH_2), 73.8, 72.4, 46.1, 35.2, 33.9, 33.6, 29.3, 29.1, 29.0, 28.9, 25.3, 24.5 (CH_2), 69.5, 49.1 (CH), 58.6, 21.2, 20.7 (CH_3). ESI-MS (m/z): 500.1 [M+Na]$^+$.

2.4. General procedure for the synthesis of metoprolol–saccharide conjugates

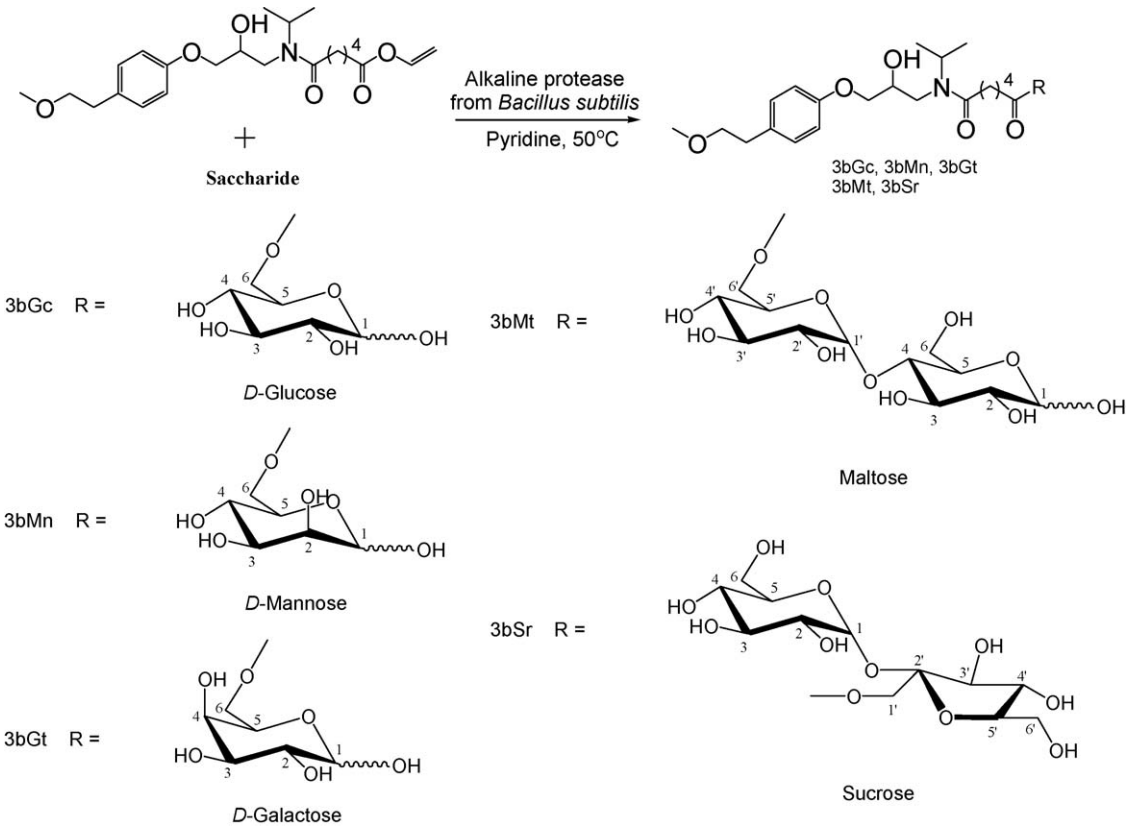
A mixture of *N*-(5-vinyloxycarbonylpentanoyl)metoprolol (**3b**) (1 mmol), saccharides (4 mmol), alkaline protease from *B. subtilis* (250 mg) and 10 mL anhydrous pyridine in 50 mL conical flask was kept at 50 °C and stirred at 200 rpm for 3 days. The reaction was terminated by filtering off the enzyme and pyridine was evaporated. The formation of metoprolol–saccharide conjugates was monitored by TLC. The products were isolated by silica gel chromatography with an eluent consisting of ethyl acetate/methanol/water (17/2/1, v/v/v). The regioselective enzymatic synthesis of metoprolol–saccharide conjugates was shown in Scheme 2.

2.4.1. Synthesis of *N*-(5-(6-deoxy-D-glucopyranose-6-yloxy)carbonylpentanoyl)metoprolol (**3bGc**)

The isolated yield of **3bGc** was 69%. IR (liquid film, cm^{-1}): 3373 (OH), 1732 (C=O). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.12 (t, 2H, J = 7.8 Hz, Ar–H), 6.81 (dd, 2H, J = 8.4 Hz, J = 16.8 Hz, Ar–H), 6.43 (s, 1H, H of D-glucose), 5.26 (d, 1H, J = 8.8 Hz, H of D-glucose), 4.88 (s, 1H, H of D-glucose), 4.67 (s, 8H, H of D-glucose), 4.25 (q, 2H, J = 14.4 Hz, H of D-glucose), 4.12–4.09 (t, J = 6.6 Hz, 1H, NCH_2), 4.00 (q, 2H, J = 9.2, NCH_2 and NCH(CH_3)$_2$), 3.92–3.78 (m, 3H, CHOH and H of D-glucose), 3.71 (t, 2H, J = 8.0 Hz, OCH_2CH$_2$), 3.52–3.41 (m, OCH_2CH and H of D-glucose), 3.23 (s, 3H, CH_3O), 2.71 (q, 2H, J = 6.6 Hz, Ar–CH_2), 2.52 (s, 4H, CH_2), 1.15 (d, 3H, J = 6.4 Hz, CH_3), 1.12 (d, 3H, J = 7.6, CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm): 175.9, 173.9 (C=O), 156.9, 131.3, 129.8, 114.3 (Ar–C, metoprolol), 92.3, 73.7, 73.2, 71.6, 70.3, 63.7 (C of D-glucose), 75.3, 74.4, 45.8, 35.1, 33.7, 33.2, 24.5, 24.5 (CH_2), 69.4, 49.2 (CH), 58.5, 21.1, 20.7 (CH_3). ESI-MS (m/z): 580.1 [M+Na]$^+$.

2.4.2. Synthesis of *N*-(5-(6-deoxy-D-mannopyranose-6-yloxy)carbonylpentanoyl)metoprolol (**3bMn**)

The isolated yield of **3bMn** was 57%. IR (liquid film, cm^{-1}): 3374 (OH), 1732 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.11 (t, 2H, J = 7.8 Hz, Ar–H), 6.80 (dd, 2H, J = 8.4, J = 18.0, Ar–H), 6.38 (s, 0.7H, H of D-mannose), 5.33 (s, 0.4H, H of D-mannose), 5.21 (s, 0.5 H, H of D-mannose), 4.86 (s, 2H, H of D-mannose), 4.58 (d, 8H, H of D-mannose), 4.29 (d, 2H, J = 10.0, H of D-mannose), 4.03–3.80 (m, 4H, NCH_2, NCH(CH_3)$_2$ and CHOH), 3.70 (d, 1H, J = 8.0, H of D-mannose), 3.55 (s, 1H, OCH_2CH$_2$), 3.49–3.30 (m, OCH_2CH$_2$, OCH_2CH and H of D-mannose), 3.22 (s, 3H, CH_3O), 2.74–2.71 (q, 2H, J = 7.0 Hz, Ar–CH_2), 2.40–2.26 (m, 4H, CH_2), 1.49 (d, 4H, J = 9.2 Hz, CH_2), 1.15 (d, 3H, J = 6.4 Hz, CH_3), 1.11 (d, 3H, J = 7.2, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 173.6, 172.9 (C=O), 157.0, 131.1, 130.0, 114.4 (Ar–C, metoprolol), 94.2, 71.5, 70.8, 70.6, 70.2, 68.9, 67.4, 64.4 (C of D-mannose), 73.3, 72.4, 44.6, 34.7, 33.6, 33.2, 24.6, 24.4 (CH_2), 68.9, 48.1 (CH), 58.0, 21.0, 20.5 (CH_3). ESI-MS (m/z): 580.2 [M+Na]$^+$.



Scheme 2. Synthesis of metoprolol–saccharide conjugates.

*2.4.3. Synthesis of N-(5-(6-deoxy-D-galactopyranose-6-yloxy)carbonylpentanoyl)metoprolol (**3bGt**)*

The isolated yield of **3bGt** was 41%. IR (liquid film, cm^{-1}): 3373 (OH), 1733 (C=O). ^1H NMR (DMSO-d_6, δ, ppm): 7.11 (t, 2H, J = 7.6, Ar–H), 6.80 (dd, 2H, J = 8.4, J = 17.6, Ar–H), 6.27 (s, 0.5H, H of D-galactose), 5.22 (s, 1H, H of D-galactose), 4.99–4.91 (m, 1H, H of D-galactose), 4.61 (s, 7H, H of D-galactose), 4.23 (t, 2H, J = 8.4, H of D-galactose), 4.09–3.98 (m, 3H, NCH_2 and NCH(CH_3)$_2$), 3.91–3.80 (m, 3H, CHOH and H of D-galactose), 3.67–3.55 (m, 3H, OCH_2CH$_2$ and OCH_2CH), 3.50–3.41 (m, OCH_2CH and H of D-galactose), 3.23 (s, 3H, CH_3O), 3.17 (s, 1H, CH_2), 2.74–2.71 (t, 2H, J = 6.8 Hz, Ar–CH_2), 2.39–2.28 (m, 4H, CH_2), 1.49 (d, 3H, J = 18.4 Hz, CH_2), 1.15 (d, 3H, J = 6.4 Hz, CH_3), 1.11 (d, 3H, J = 7.2, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 173.7, 173.2 (C=O), 157.1, 131.2, 130.0, 114.4 (Ar–C, metoprolol), 92.8, 68.8, 68.7, 67.8, 67.5, 64.2 (C of D-galactose), 73.2, 72.4, 47.1, 34.6, 33.5, 33.1, 24.6, 24.3 (CH_2), 68.5, 48.2 (CH), 58.0, 21.0, 20.5 (CH_3). ESI-MS (m/z): 580.1 $[M+Na]^+$.

*2.4.4. Synthesis of N-(5-(6′-deoxy-(4-yloxy-(D-glucopyranosyl)-D-glucopyranose))carbonylpentanoyl)metoprolol (**3bMt**)*

The isolated yield of **3bMt** was 62%. IR (liquid film, cm^{-1}): 3355 (OH), 1734 (C=O). ^1H NMR (DMSO-d_6, δ, ppm): 7.11 (t, 2H, J = 7.0, Ar–H), 6.80 (d, 2H, J = 8.4, J = 17.6, Ar–H), 5.70 (s, 1H, H of maltose), 5.37 (d, 0.5H, H of maltose), 4.98–4.90 (t, 1.5H, J = 15.2, H of maltose), 4.53 (s, 2H, H of maltose), 4.31–4.25 (dd, 1.5H, J = 8.6, J = 15.0, H of maltose), 4.09–3.99 (t, 3H, J = 20.6, NCH_2 and NCH(CH_3)$_2$), 3.89–3.80 (m, 2.5H, CHOH and H of maltose), 3.70–3.64 (m, 3H, H of maltose), 3.53–3.37 (m, OCH_2CH$_2$, OCH_2CH and H of maltose), 3.22 (s, 7H, CH_3O and H of maltose), 2.70 (t, 2H, J = 6.8 Hz, Ar–CH_2), 2.37–2.28 (m, 4H, CH_2), 1.48 (t, 4H, J = 9.4 Hz, CH_2), 1.14 (d, 3H, J = 6.0 Hz, CH_3), 1.11 (d, 3H, J = 6.8, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 173.7, 173.0 (C=O), 157.1, 131.3, 130.1, 114.6 (Ar–C, metoprolol), 101.4, 97.1, 92.4, 81.0, 76.8, 75.4, 74.5, 73.3, 72.8, 72.6, 70.7, 70.3, 64.0, 60.6 (C of maltose), 73.4, 72.1, 47.2, 34.8, 33.6, 33.3, 24.8, 24.5 (CH_2), 68.1, 48.3 (CH), 58.1, 21.2, 20.7 (CH_3). ESI-MS (m/z): 742.1 $[M+Na]^+$.

*2.4.5. Synthesis of N-(5-(1′-deoxy-(4-yloxy-(D-fructofuranosyl)-D-glucopyranoside))carbonylpentanoyl)metoprolol (**3bSr**)*

The isolated yield of **3bSr** was 63%. IR (liquid film, cm^{-1}): 3363 (OH), 1736 (C=O). ^1H NMR (DMSO-d_6, δ, ppm): 7.11 (d, 2H, J = 7.6 Hz, Ar–H), 6.80 (d, 2H, J = 8.6 Hz, J = 17.4 Hz, Ar–H), 5.35 (s, 1H, H of sucrose), 5.17 (d, 1H, H of sucrose), 4.93 (s, 2H, H of sucrose), 4,46 (d, 4H, J = 13.6 Hz, H of sucrose), 4.27–4.24 (t, 1H, J = 6.6 Hz, H of sucrose), 4.17–4.08 (m, 2H, NCH_2 and NCH(CH_3)$_2$), 3.95 (d, 2H, J = 12.0 Hz, NCH_2 and H of sucrose), 3.90–3.77 (m, 4H, CHOH and H of sucrose), 3.65–3.62 (m, 2H, OCH_2CH$_2$), 3.56 (s, 4H, OCH_2CH and H of sucrose), 3.52–3.39 (m, OCH_2CH and H of sucrose), 3.22 (s, 3H, CH_3O), 2.70 (t, 2H, J = 6.8 Hz, Ar–CH_2), 2.40–2.30 (m, 4H, CH_2), 1.49 (t, 4H, J = 9.2 Hz, CH_2), 1.14 (d, 3H, J = 6.0 Hz, CH_3), 1.11 (d, 3H, J = 7.6, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 173.5, 172.8 (C=O), 157.0, 131.1, 130.0, 114.4 (Ar–C, metoprolol), 103.3, 92.3, 82.9, 76.7, 73.5, 71.6, 70.2, 62.2 (C of sucrose), 73.3, 72.4, 47.0, 34.7, 33.6, 33.1, 24.6, 24.4 (CH_2), 69.9, 48.1 (CH), 58.0, 21.1, 20.5 (CH_3). ESI-MS (m/z): 742.1 $[M+Na]^+$.

2.5. Determination of distribution coefficient

The distribution coefficients ($D_{7.4}$) of drugs and drug–saccharide conjugates were determined by dissolving 5 mg of different metoprolol–saccharide conjugates in 2.5 mL of *n*-octanol and an equal volume of PBS (pH = 7.4) in screw-capped test tube. The solutions were then mixed for 15 min, and centrifuged at 1×10^4 rpm for 5 min. The layers were separated and aliquots of 50 μL were diluted to 500 μL with methanol. Samples were taken with 20 μL and determined by HPLC. The value of $D_{7.4}$ was the peak area ratio of up-layer to down-layer [29,30]. All experiments were conducted in triplicate and the mean values were taken. The $\lambda_{detection}$ of HPLC was 220 nm. The values of $D_{7.4}$ for different metoprolol–saccharide conjugates were then calculated and the results were listed in Table 3.

3. Results and discussion

3.1. Effects of enzymes and solvents on the synthesis of N-(vinyloxycarbonyl)metoprolol

To identify suitable enzymes with high transesterification activity in the synthesis of *N*-(vinyloxycarbonyl)metoprolol, five commercially available enzymes were tested for the transesterification of metoprolol with divinyl adipate at 50 °C as shown in Scheme 1. The reaction between metoprolol and divinyl adipate could take place in most of organic solvents. In the absence of enzymes, the reaction yields were all less than 2% in THF, DMF and CCl_4, so the three solvents were selected as the reaction media. As seen from Table 1, the reaction media played an important role in the enzymatic transesterification. PPL showed the highest catalytic ability in CCl_4 (entry 6, Table 1). The yield of **3b** was

Table 1
The influences of enzymes and solvents on the reaction of *N*-(vinyloxycarbonyl) metoprolol[a].

Entry	Enzyme	Solvent	Yield (%)[b]
1	AY30	THF	25
2		DMF	10
3		CCl_4	26
4	PPL	THF	< 1
5		DMF	6
6		CCl_4	74
7	CRL	THF	13
8		DMF	8
9		CCl_4	3
10	MML	THF	2
11		DMF	8
12		CCl_4	16
13	CCL	THF	19
14		DMF	1
15		CCl_4	21

[a] Reaction conditions: enzyme (15 mg/mL), metoprolol (0.1 mmol/mL), divinyl adipate (0.4 mmol/mL), solvent (1.0 mL), 50 °C, 200 rpm, 3 days.
[b] Yields were determined by HPLC. Eluent: methanol/PBS (10 mmol/L, pH = 3.0) (60/40, v/v).

74% after 72 h. However, no positive result was observed in other two solvents (entries 4 and 5, Table 1). The enzyme sources also affected the transesterification. The other four lipases could not catalyze the reaction effectively. Therefore, PPL was employed as the catalyst and three *N*-(vinyloxycarbonyl)metoprolol (**3a–3c**) were synthesized in CCl_4 by the reaction of metoprolol with divinyl dicarboxylates.

3.2. Effects of the chain length of acylating reagents on the synthesis of N-(vinyloxycarbonyl) metoprolol

The influences of the chain length of acylating reagents on reaction yield were evaluated. Under the same reaction conditions, the yield of *N*-(3-vinyloxycarbonylpropanoyl)metoprolol was 34% after 24 h, while that of *N*-(5-vinyloxycarbonylpentanoyl)metoprolol and *N*-(9-vinyloxycarbonylnonanoyl)metoprolol were 31% and 17%, respectively. The yields decreased as the chain length of divinyl dicarboxylates increased. It may be due to the more steric influences as the length increased.

3.3. Synthesis and characterization of metoprolol–saccharide conjugates

The influences of the structures of *N*-(vinyloxycarbonyl) metoprolol on the synthesis of metoprolol–glucose conjugates were investigated by using *N*-(5-vinyloxycarbonylpentanoyl) metoprolol and *N*-(9-vinyloxycarbonylnonanoyl)metoprolol as acylating reagents. The yield of *N*-(5-vinyloxycarbonylpentanoyl)metoprolol with glucose was higher (69%) than that of *N*-(9-vinyloxycarbonylnonanoyl)metoprolol (30%) after 72 h under the same reaction conditions. It indicated that the yields decreased as the chain length of the alkyl of *N*-(vinyloxycarbonyl)metoprolol increased. Thus, *N*-(5-vinyloxycarbonylpentanoyl)metoprolol was selected as the substrate to investigate the influences of the structures of saccharides on the preparation of metoprolol–saccharide conjugates.

Table 2
The selectivity and yields of metoprolol–saccharide conjugates[a].

Entry	Product	Yield (%)[b]	Acylation position
1	3bGc	69	6
2	3bMn	57	6
3	3bGt	41	6
4	3bMt	62	6′
5	3bSr	63	1′

[a] Reaction conditions: *N*-(5-vinyloxycarbonylpentanoyl)metoprolol (0.1 mmol/mL), saccharides (0.4 mmol/mL), Alkaline protease from *Bacillus subtilis* (25 mg/mL), pyridine (10.0 mL), 50 °C, 3 days.
[b] Yields were isolated yields.

Table 3
The lipophilic parameters of metoprolol–saccharide conjugates and the parent drug.

Entry	Compound	Lipophilicity[a]	Relative solubility (mg/mL)[b]	Log *P*[c]
1	Metoprolol	2.1[d]	–	0.32
2	3bGc	0.73	2.9	−0.14
3	3bMn	0.76	2.8	−0.12
4	3bGt	0.73	2.9	−0.14
5	3bMt	0.031	66.7	−1.51
6	3bSr	0.043	48.8	−1.37

[a] It was characterized by $D_{7.4}$, distribution coefficient in *n*-octanol/phosphate buffer (pH = 7.4); Eluent: methanol/water (80/20, v/v).
[b] The solubility of drug–saccharide conjugates/the solubility of the parent drug.
[c] Log *P* is the logarithm of partition coefficient in *n*-octanol/phosphate buffer (pH = 7.4).
[d] The data was from Ref. [31].

The reactions involving *N*-(5-vinyloxycarbonylpentanoyl)metoprolol and saccharides, such as monosaccharides (glucose, mannose and galactose) and disaccharides (maltose and sucrose) were carried out. The selectivity and yields of enzymatic synthesis of metoprolol–saccharide conjugates were listed in Table 2. D-glucose exhibited higher reactivity than other monosaccharides, resulting in a yield of 69% after 72 h (entry 1, Table 2). The structure of monosaccharides showed no influence on the selectivity of the transesterification. All monosaccharides were acylated at C-6 position (entries 1–3, Table 2). For disaccharides, the yields were the same under the identical conditions. Maltose was acylated at C-6′ position (entry 4, Table 2), while sucrose was acylated at C-1′ position (entry 5, Table 2).

3.4. Partition coefficients of metoprolol–saccharide conjugates

Finally, the apparent partition coefficients (log *P*) of metoprolol and its glycolipids were investigated, and the results are shown in Table 3. The metoprolol–saccharide conjugates exhibited better aqueous solubility compared with the parent drug. The aqueous solubility of metoprolol–monosaccharide conjugates was ~2.80-fold (entries 2–4, Table 3) of the parent drug. For metoprolol–maltose and metoprolol–sucrose conjugates, the aqueous solubility were 66.7-fold and 48.8-fold of metoprolol (entries 5 and 6, Table 3), respectively. It was notable that the aqueous solubility of metoprolol–disaccharide conjugates was at least 16-fold more than that of metoprolol–monosaccharide conjugates. It demonstrated that the aqueous solubility of metoprolol–disaccharide conjugates was much better than that of metoprolol–monosaccharide conjugates. Thus, the aqueous solubility of metoprolol derivatives could be regulated by conjugating with different saccharides.

4. Conclusion

In conclusion, a selective and facile strategy to prepare metoprolol–saccharide conjugates by enzymatic reaction was developed. The influences of enzyme sources, organic solvents and the chain length of acylating reagents on the reaction for the synthesis of *N*-(vinyloxycarbonyl) metoprolol were systematically investigated. PPL showed the best activity in tetrachloromethane and was used to synthesize *N*-(vinyloxycarbonyl)metoprolol. Then, a series of metoprolol–saccharide conjugates were obtained by regioselective acylation with three monosaccharides and two disaccharides. The aqueous solubility of metoprolol–saccharide

conjugates was greatly improved compared with the parent drug. Further study about the potential targeting of the prodrugs is in progress.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.procbio.2010.07.028.

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