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Synthesis of polymeric prodrugs of chlorphenesin with saccharide branches by chemo-enzymatic regioselective strategy

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Abstract

A facile and regioselective enzymatic synthesis approach to prepare polymerizable lipophilic chlorphenesin vinyl esters was developed in this research. The influence of different organic solvents, enzyme sources, reaction time and the acylation reagent on the synthesis of chlorphenesin vinyl esters was investigated. Then the polymerizable monomers 1-*O*-vinylsuccinyl-chlorphenesin (OVSC) and 1-*O*-vinyladipoyl-chlorphenesin (OVAC) were homopolymerized using AIBN as the initiator. The obtained polymeric prodrugs were characterized with IR, NMR and GPC analyzes. The poly-OVSC has M_n of 1.35×10^4 and M_w/M_n of 1.95, and the poly-OVAC has M_n of 2.37×10^4 and M_w/M_n of 4.30. Moreover, 6-*O*-vinyladipoyl-D-glucose (OVAG), a biocompatible monomer, was copolymerized with OVSC and OVAC. Polymeric prodrugs of chlorphenesin with saccharide branches were successfully obtained with high molecular weight.

Keywords: Copolymer; Enzymatic synthesis; Chlorphenesin

1. Introduction

In recent years, there has been considerable interest in developing biodegradable polymeric drugs. Generally, macromolecules cannot pass through the capillary walls of normal tissue in contrast to the low-molecular weight compound diffusing into normal and pathological tissues. The endothelial layer of the capillaries in the tumor tissue is fenestrated and leaky so that the entry of macromolecules into tumor tissue takes place in the capillaries where blood flow is diminished and nutrients transfer into the tissue [1–3]. Therefore, it is an active research field to prepare macromolecular drugs for increasing therapeutic benefit, controlling the rate of the drug release and minimizing side effects [4–9]. On the other hand, as biological recognition signals and functional biomolecules in living systems, saccharides play a central role. A great number of drugs in use today rely on carbohydrates for part of their therapeutic action. These combinations of carbohydrates and other specific molecules span a wide range of drug types and diseases [10–16]. Moreover, it is particularly effective for the improvement of drugs' dissolution behavior [17]. As a result, the synthesis of polymeric prodrugs containing biocompatible molecules [18], such as saccharides, is especially desirable.

The vast majority of polymers are prepared from rather simple monomer. Nevertheless, the biodegradable polymeric drugs should include structures of complex biocompatible molecules and drugs, which usually contain a large number of functional groups that are nearly indistinguishable chemically. Then, the selective synthesis of polymerizable monomers of biocompatible molecules and drugs is extremely important. However, selective protection/deprotection of polyfunctional compounds is a challenging problem in organic synthesis. Thus, biocatalysts have been widely used to modify some pharmaceutically important compounds and biocompatible molecules due to their high selectivity, mild reaction conditions and simplified downstream processing by using immobilized enzymes [19–22]. Many research groups have

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made great effort in the area of enzymatic modification of drugs [23,24]. Combining the conventional polymerization chemistry [25] with the highly efficient and regioselective enzymatic approaches, this chemo-enzymatic synthetic method is attractive for the preparation of polymeric prodrugs.

Chlorphenesin (3-(4-chlorophenoxy)-1,2-propanediol) is an orally administered muscle relaxant. It works by blocking nerve impulses (or pain sensations) that are sent to the brain. Chlorphenesin is used, along with rest and physical therapy, to treat injuries and other painful muscular conditions [26–28]. It is also an effective antifungal agent. However, chlorphenesin exhibits a short half-life in the blood stream and a high overall clearance rate. Therefore, it is necessary to develop novel prodrugs of chlorphenesin. So far, there are only few reports about the synthesis of chlorphenesin prodrugs, especially the chlorphenesin derivatives that carry polymerizable groups, such as vinyl group. These chlorphenesin monomers could be homo- or copolymerized with appropriate monomers, especially saccharides, for the preparation of polymeric prodrugs, which increased the parent drugs' stability and prolonged their biological half-life.

In the present work, we reported an efficient enzymatic approach for regioselective esterification of chlorphenesin and obtained vinyl esters monomers of chlorphenesin. The chlorphenesin vinyl esters with lipid solubility, as monomers of polymer drugs, would be expected to render them more suitable for diffusion across the lipophilic membranes of the intestinal cells, thus improving intestinal and cellular absorptions [29]. Then, the polymerizable chlorphenesin vinyl esters were homopolymerized with AIBN. In particular, the copolymers with saccharide branches were prepared successfully. Furthermore, the property and function of copolymers would be adjusted by changing the chain length of chlorphenesin vinyl esters and different saccharides.

2. Experimental

2.1. Materials

Lipozyme® (E.C. 3.1.1.1, an immobilized preparation of lipase from *Mucor miehei*, 42 U/g), lipase from porcine pancreas (E.C. 3.1.1.3, type II, powder, 30–90 U/mg), lipase from *Candida cylindracea* (E.C. 3.1.1.3, powder, 2.8 U/mg) and lipase from hog pancreas (E.C. 3.1.1.3, powder, 2.4 U/mg) were purchased from Fluka. *Candida antarctica* lipase acrylic resin (E.C. 3.1.1.3, 10,000 U/g) and lipase type VII from *Candida rugosa* (E.C. 3.1.1.3, powder, 706 U/mg) were purchased from Sigma. Amano lipase M from *Mucor javanicus* (E.C. 3.1.1.3, powder, 10 U/mg) and Amano protease PS from *Aspergillus melleus* (E.C. 3.4.21.63, powder, 100 U/mg) were purchased from Aldrich. Lipase AY30 (E.C. 3.1.1.3, powder) was purchased from Acrös. Alkaline protease from *Bacillus subtilis* (E.C. 3.4.21.14, a crude preparation of the alkaline serine protease, 100 U/mg) was purchased from Wuxi Enzyme Co. Ltd (Wuxi, PR China). Chlorphenesin was purchased from Alfa Aesar, a Johnson Matthey Company. All the enzymes were used directly as commercial preparations without further purification. 2,2′-Azoisobutyronitrile (AIBN) was purified by recrystallization with methanol. All solvents were of analytical grade and were dried by storing over activated 3 Å molecular sieves before use. All other reagents were used as received.

2.2. Analytical methods

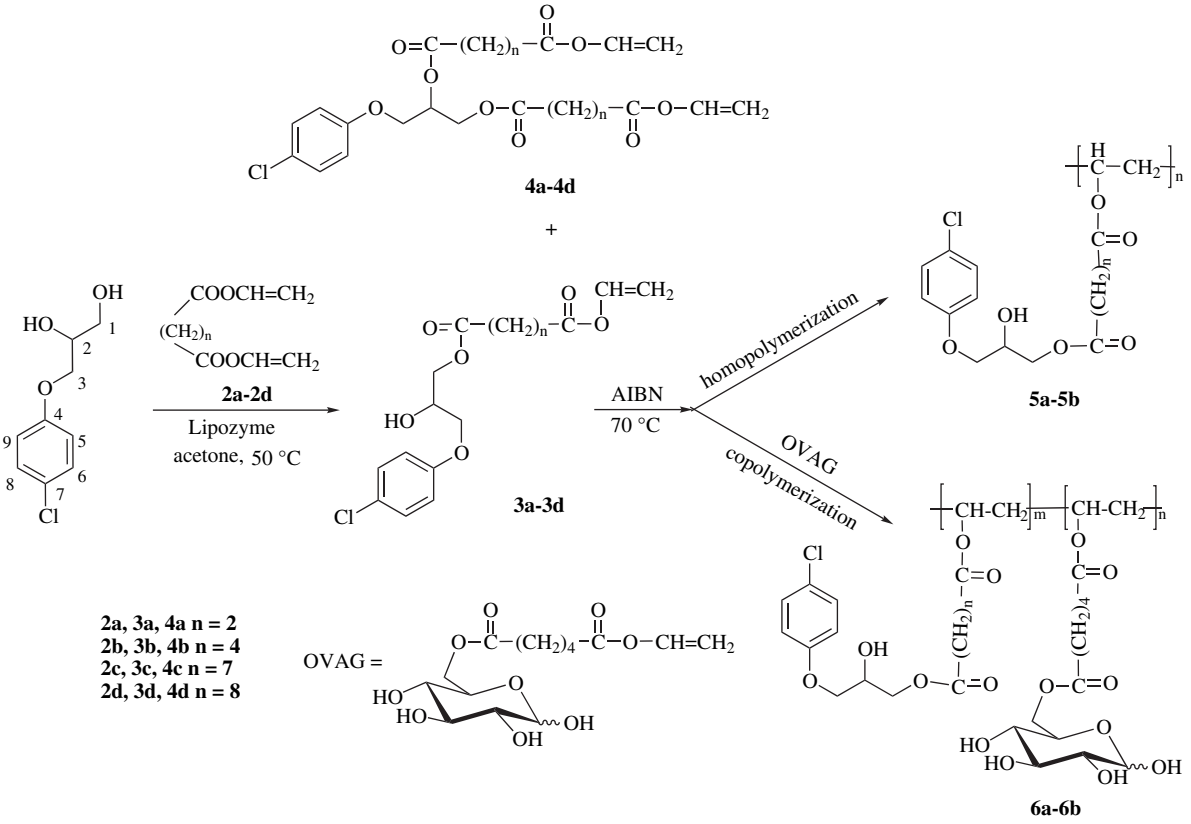
The progress of reactions was monitored by TLC on silica with petroleum ether/EtOAc (2/1, v/v) as solvent, for the monoester compounds (**3a–3d**), petroleum ether/EtOAc (3/1, v/v) for the diester compounds (**4a–4d**). The ^{1}H NMR and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-500 MHz spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. Chemical shifts were expressed in parts per million and coupling constants (J) in Hertz. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Analytical HPLC was performed using an Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150 × 4.6 mm) and a UV detector (275 nm). Methanol/water (80/20, v/v) was used as a mobile phase, while the flow rate was adjusted to 1 mL min^{-1}. 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^{-1}.

2.3. General procedure for the synthesis of chlorphenesin vinyl esters

The reaction was initiated by adding 15 mg mL^{-1} Lipozyme® to 20 mL anhydrous acetone containing 5 mmol chlorphenesin and 30 mmol divinyl dicarboxylates (**1a–1d**). The suspension was kept at 50 °C and shaken at 250 rpm. The reaction was monitored by TLC (petroleum ether/ethyl acetate 5/2, v/v). The reactions were terminated by filtering off the enzyme and then the filtrate was evaporated under reduced pressure. The products were isolated by silica gel column chromatography with an eluent consisting of petroleum ether/ethyl acetate (3/1, v/v). Enzymatic synthesis of vinyl esters of chlorphenesin are shown in Scheme 1.

2.4. Synthesis of 1-O-vinylsuccinyl-chlorphenesin (OVAC, *3a*)

The reaction time was 1 h and the product yield was 84.7%. ^{1}H NMR ($CDCl_3$): δ (ppm) 7.26 (dd, 1H, $J = 6.3$, 14.1 Hz, −CH═), 7.23 (d, 2H, Ar−H), 6.85 (d, 2H, Ar−H), 4.91 (dd, 1H, $J = 14.1$, 6.3 Hz, ═CH_2), 4.58 (dd, 1H, $J = 6.3$, 1.5 Hz, ═CH_2), 4.35–4.24 (m, 3H, −C*H*(OH)CH_2−), 4.00 (m, 2H, −OCH_2CH−), 2.74 (m, 4H, −CH_2CH_2−). ^{13}C NMR ($CDCl_3$): δ (ppm) 173.7, 171.0 (C═O), 157.2, 129.6, 129.6, 126.3, 116.0, 116.0 (Ar, chlorphenesin), 141.4 (−O−CH═), 98.1 (═CH_2), 69.2 (C-3, chlorphenesin), 68.5 (C-2, chlorphenesin), 65.5 (C-1, chlorphenesin), 29.2, 29.1



Scheme 1. Regioselective enzymatic synthesis of chlorphenesin vinyl esters and their chemical polymerization.

(−CH_2−). IR (KBr): ν (cm^{-1}) 3528 (OH), 1762, 1740 (O−C═O), 1647 (C═C), 1597, 1499, 836, 806 (Ar). ESIMS (*m/z*): 351 $[M + Na]^+$.

2.5. Synthesis of 1,2-O-divinylsuccinyl-chlorphenesin (*4a*)

The reaction time was 24 h and the product yield was 4.5%. ^{1}H NMR ($CDCl_3$): δ (ppm) 7.25 (dd, 2H, $J = 6.3$, 14.1 Hz, 2−CH═), 7.15 (d, 2H, Ar−H), 6.83 (d, 2H, Ar−H), 5.39 (m, 1H, $J = 5.0$ Hz, −CH_2CH(OH)−), 4.89 (d, 2H, $J = 14.1$ Hz, 2═CH_2), 4.57 (dd, 2H, $J = 6.3$, 1.5 Hz, 2═CH_2), 4.47 (dd, 1H, $J = 12.0$, 3.5 Hz, −$CHCH_2$O−), 4.35 (dd, 1H, $J = 12.0$, 6.0 Hz, −$CHCH_2$O−), 4.08 (d, 2H, $J = 5.0$ Hz, −OCH_2CH−), 2.71 (m, 8H, 2−CH_2CH_2−). ^{13}C NMR ($CDCl_3$): δ (ppm) 173.5, 172.7, 172.7, 171.0 (C═O), 157.1, 129.7, 129.7, 126.5, 116.1, 116.0 (Ar, chlorphenesin), 141.3 (−O−CH═), 97.9 (═CH_2), 69.8 (C-2, chlorphenesin), 66.4 (C-3, chlorphenesin), 62.7 (C-1, chlorphenesin), 29.3, 29.2, 29.1, 29.0 (−CH_2−). IR (film): ν (cm^{-1}) 1760, 1735 (O−C═O), 1647 (C═C), 1597, 1499, 835, 804 (Ar). ESIMS (*m/z*): 477 $[M + Na]^+$.

2.6. Synthesis of 1-O-vinyladipoyl-chlorphenesin (OVAC, *3b*)

The reaction time was 2 h and the product yield was 69.7%. ^{1}H NMR ($CDCl_3$): δ (ppm) 7.27 (dd, 1H, $J = 6.3$, 14.1 Hz, −CH═), 7.22 (d, 2H, Ar−H), 6.84 (d, 2H, Ar−H), 4.88 (dd, 1H, $J = 14.1$, 6.3 Hz, ═CH_2), 4.57 (dd, 1H, $J = 6.3$, 1.5 Hz, ═CH_2), 4.32−4.21 (m, 3H, −CH(OH)CH_2−), 3.98 (m, 2H, −OCH_2CH−), 3.05 (br, 1H, −CH(O*H*)−), 2.39 (m, 4H, 2−CH_2−), 1.68 (m, 4H, 2−CH_2−). ^{13}C NMR ($CDCl_3$): δ (ppm) 173.5, 170.6 (C═O), 157.1, 129.5, 129.5, 126.3, 116.0, 116.0 (Ar, chlorphenesin), 141.2 (−O−CH═), 98.0 (═CH_2), 69.2 (C-3, chlorphenesin), 68.5 (C-2, chlorphenesin), 65.4 (C-1, chlorphenesin), 33.8, 33.5, 24.3, 24.0 (−CH_2−). IR (film): ν (cm^{-1}) 3515 (OH), 1760, 1736 (O−C═O), 1647 (C═C), 1592, 1497, 836, 806 (Ar). ESIMS (*m/z*): 379 $[M + Na]^+$.

2.7. Synthesis of 1,2-O-divinyladipoyl-chlorphenesin (*4b*)

The reaction time was 24 h and the product yield was 4.2%. ^{1}H NMR ($CDCl_3$): δ (ppm) 7.27 (dd, 1H, $J = 6.3$, 14.1 Hz, 2−CH═), 7.22 (d, 2H, Ar−H), 6.84 (d, 2H, Ar−H), 5.39 (m, 1H, $J = 5.0$ Hz, −CH_2CH(OH)−), 4.88 (d, 2H, $J = 14.1$ Hz, 2═CH_2), 4.57 (dd, 2H, $J = 6.3$, 1.5 Hz, 2═CH_2), 4.44 (dd, 1H, $J = 4.0$, 12.0 Hz, −$CHCH_2$O−), 4.28 (dd, 1H, $J = 12.0$, 6.0 Hz, −$CHCH_2$O−), 4.08 (d, 2H, $J = 5.0$ Hz, −OCH_2CH−), 2.38 (m, 8H, 4−CH_2−), 1.68 (m, 8H, 4−CH_2−). ^{13}C NMR ($CDCl_3$): δ (ppm) 173.0, 172.7, 172.7, 170.5 (C═O), 157.1, 129.7, 129.7, 126.5, 116.1, 116.1 (Ar, chlorphenesin), 141.3 (−O−CH═), 97.9 (═CH_2), 69.7 (C-2, chlorphenesin), 66.5 (C-3, chlorphenesin), 62.6 (C-1, chlorphenesin), 33.9, 33.7, 33.7, 33.6, 24.3, 24.3, 24.1, 24.0 (−CH_2−). IR (film): ν

(cm^{-1}) 1758, 1734 (O–C=O), 1647 (C=C), 1597, 1495, 835, 804 (Ar). ESIMS (*m/z*): 533 $[M + Na]^+$.

2.8. Synthesis of 1-O-Vinylazeloyl-chlorphenesin (**3c**)

The reaction time was 2 h and the product yield was 56.8%. ^1H NMR ($CDCl_3$): δ (ppm) 7.27 (dd, 1H, $J = 6.3$, 14.1 Hz, –CH=), 7.22 (d, 2H, Ar–H), 6.84 (d, 2H, Ar–H), 4.88 (dd, 1H, $J = 14.1$, 6.3 Hz, $=CH_2$), 4.57 (dd, 1H, $J = 6.3$, 1.5 Hz, $=CH_2$), 4.32–4.21 (m, 3H, $-CH(OH)CH_2-$), 3.99 (m, 2H, $-OCH_2CH-$), 3.60 (br, 1H, $-CH(OH)-$), 2.36 (m, 4H, $2-CH_2-$), 1.64 (m, 4H, $2-CH_2-$), 1.32 (m, 6H, $3-CH_2-$). ^{13}C NMR ($CDCl_3$): δ (ppm) 174.2, 170.8 (C=O), 157.1, 129.7, 129.6, 126.4, 116.0, 116.0 (Ar, chlorphenesin), 141.2 (–O–CH=), 98.0 ($=CH_2$), 69.3 (C-3, chlorphenesin), 68.4 (C-2, chlorphenesin), 65.3 (C-1, chlorphenesin), 34.3, 34.0, 29.0, 29.0, 29.0, 25.0, 24.7 ($-CH_2-$). IR (film): ν (cm^{-1}) 3520 (OH), 1757, 1734 (O–C=O), 1645 (C=C), 1592, 1497, 836, 806 (Ar). ESIMS (*m/z*): 421 $[M + Na]^+$.

2.9. Synthesis of 1,2-O-divinylazeloyl-chlorphenesin (**4c**)

The reaction time was 24 h and the product yield was 3.4%. ^1H NMR ($CDCl_3$): δ (ppm) 7.27 (dd, 1H, $J = 6.3$, 14.1 Hz, 2–CH=), 7.22 (d, 2H, Ar–H), 6.83 (d, 2H, Ar–H), 5.36 (m, 1H, $J = 5.0$ Hz, $-CH_2CH(OH)-$), 4.87 (d, 2H, $J = 14.1$ Hz, $2=CH_2$), 4.56 (dd, 2H, $J = 6.3$, 1.5 Hz, $2=CH_2$), 4.41 (dd, 1H, $J = 4.0$, 12.0 Hz, $-CHCH_2O-$), 4.27 (dd, 1H, $J = 12.0$, 6.0 Hz, $-CHCH_2O-$), 4.07 (d, 2H, $J = 5.0$ Hz, $-OCH_2CH-$), 2.34 (m, 8H, $4-CH_2-$), 1.61 (m, 8H, $4-CH_2-$), 1.28 (m, 12H, $6-CH_2-$). ^{13}C NMR ($CDCl_3$): δ (ppm) 173.4, 172.7, 172.7, 170.9 (C=O), 157.2, 129.7, 129.6, 126.4, 116.2, 116.0 (Ar, chlorphenesin), 141.2 (–O–CH=), 98.0 ($=CH_2$), 69.8 (C-2, chlorphenesin), 66.3 (C-3, chlorphenesin), 62.6 (C-1, chlorphenesin), 34.4, 34.3, 34.0, 33.8, 29.1, 29.1, 29.0, 29.0, 28.9, 28.9, 25.1, 25.1, 24.7, 24.6 ($-CH_2-$). IR (film): ν (cm^{-1}) 1754, 1737 (O–C=O), 1646 (C=C), 1595, 1498, 837, 804 (Ar). ESIMS (*m/z*): 617 $[M + Na]^+$.

2.10. Synthesis of 1-O-vinylsebacoyl-chlorphenesin (**3d**)

The reaction time was 2 h and the product yield was 51.9%. ^1H NMR ($CDCl_3$): δ (ppm) 7.27 (dd, 1H, $J = 6.3$, 14.1 Hz, –CH=), 7.22 (d, 2H, Ar–H), 6.84 (d, 2H, Ar–H), 4.88 (dd, 1H, $J = 14.1$, 6.3 Hz, $=CH_2$), 4.57 (dd, 1H, $J = 6.3$, 1.5 Hz, $=CH_2$), 4.30–4.22 (m, 3H, $-CH(OH)CH_2-$), 3.99 (m, 2H, $-OCH_2CH-$), 2.80 (br, 1H, $-CH(OH)-$), 2.36 (m, 4H, $2-CH_2-$), 1.63 (m, 4H, $2-CH_2-$), 1.27 (m, 8H, $4-CH_2-$). ^{13}C NMR ($CDCl_3$): δ (ppm) 174.2, 171.18 (C=O), 157.2, 129.5, 129.5, 126.4, 116.0, 116.0 (Ar, chlorphenesin), 141.3 (–O–CH=), 98.1 ($=CH_2$), 69.3 (C-3, chlorphenesin), 68.5 (C-2, chlorphenesin), 65.4 (C-1, chlorphenesin), 34.3, 34.1, 29.2, 29.2, 29.1, 29.1, 25.0, 24.7 ($-CH_2-$). IR (film): ν (cm^{-1}) 3519 (OH), 1757, 1734 (O–C=O), 1642 (C=C), 1592, 1497, 832, 803 (Ar). ESIMS (*m/z*): 435 $[M + Na]^+$.

2.11. Synthesis of 1,2-O-divinylsebacoyl-chlorphenesin (**4d**)

The reaction time was 24 h and the product yield was 2.3%. ^1H NMR ($CDCl_3$): δ (ppm) 7.28 (dd, 1H, $J = 6.3$, 14.1 Hz, 2–CH=), 7.22 (d, 2H, Ar–H), 6.83 (d, 2H, Ar–H), 5.36 (m, 1H, $J = 5.0$ Hz, $-CH_2CH(OH)-$), 4.87 (d, 2H, $J = 14.1$ Hz, $2=CH_2$), 4.56 (dd, 2H, $J = 6.3$, 1.5 Hz, $2=CH_2$), 4.41 (dd, 1H, $J = 4.0$, 12.0 Hz, $-CHCH_2O-$), 4.27 (dd, 1H, $J = 12.0$, 6.0 Hz, $-CHCH_2O-$), 3.99 (d, 2H, $J = 5.0$ Hz, $-OCH_2CH-$), 2.34 (m, 8H, $4-CH_2-$), 1.61 (m, 8H, $4-CH_2-$), 1.28 (m, 16H, $8-CH_2-$). ^{13}C NMR ($CDCl_3$): δ (ppm) 173.5, 173.2, 173.2, 171.0 (C=O), 157.0, 129.7, 129.6, 126.4, 116.0, 116.0 (Ar, chlorphenesin), 141.4 (–O–CH=), 97.8 ($=CH_2$), 69.7 (C-2, chlorphenesin), 66.5 (C-3, chlorphenesin), 62.5 (C-1, chlorphenesin), 34.5, 34.4, 34.1, 29.9, 29.9, 29.3, 29.3, 29.3, 29.3, 29.2, 29.2, 29.2, 29.2, 25.1, 25.1, 24.8 ($-CH_2-$). IR (film): ν (cm^{-1}) 1756, 1733 (O–C=O), 1647 (C=C), 1598, 1500, 838, 804 (Ar). ESIMS (*m/z*): 645 $[M + Na]^+$.

2.12. Synthesis of 6-O-vinyladipoyl-D-glucose (OVAG)

OVAG was synthesized by our previous methods of regioselective acylation of sugars [30]. The isolated yield of product was 58.4%. ^1H NMR (DMSO-d_6): δ (ppm) 7.21 (1H, dd, $J = 6.3$, 14.1 Hz, –CH=), 6.71 (0.62H, br s, β1-OH of D-glucose), 6.37 (0.38H, br s, α1-OH of D-glucose), 5.20–4.45 (br m, other OH of D-glucose), 4.88 (dd, 1H, $J = 14.1$, 6.3 Hz, $=CH_2$), 4.57 (dd, 1H, $J = 6.3$, 1.5 Hz, $=CH_2$), 4.31 (1.5H, m, H-6 (1H) and βH-1 (0.5H) of D-glucose), 4.0 (1H, m, H-6′ of D-glucose), 3.75 (0.5H, m, αH-5 of D-glucose), 3.50–3.20, 3.14, 3.0 (br m, other α or βH of D-glucose), 2.89 (0.5H, m, βH-2 of D-glucose), 2.39 (m, 4H, $2-CH_2-$), 1.68 (m, 4H, $2-CH_2-$). ^{13}C NMR (DMSO-d_6): δ (ppm) 172.8, 170.4 (C=O), 141.2 (–O–CH=), 98.0 ($=CH_2$), 96.8 (C1 of β-D-glucose), 92. 2 (C1 of α-D-glucose), 76.5 (C3 of β-D-glucose), 75.3 (C2 of β-D-glucose), 73.9 (C5 of β-D-glucose), 73.0 (C3 of α-D-glucose), 72.1 (C2 of α-D-glucose), 70.1 (C4 of α-D-glucose), 70.0 (C4 of β-D-glucose), 69.9 (C5 of α-D-glucose), 63.7 (C6 α, β of D-glucose), 33.8, 33.5, 24.3, 24.0 ($-CH_2-$). IR (film): ν (cm^{-1}) 3389 (OH), 1740 (C=O). ESIMS (*m/z*): 357 $[M + Na]^+$.

2.13. Homopolymerization of 1-O-vinylsuccinyl-chlorphenesin (OVSC)

The poly-OVSC was prepared by using AIBN initiator. OVSC (1 mmol) was added to a small flame-dried flask with the addition of 0.2 mL DMF. The solution was degassed (freeze/pump/thaw cycles) and 2% AIBN (w/w) was added. The polymerization was continued for 6 h at 70 °C. Precipitating the polymer in methanol terminated the reaction and the light yellow precipitate was washed with acetone. Then the product was dried under vacuum (yield: 78.6%). ^1H NMR ($CDCl_3$): δ (ppm) 7.23 (d, 2H, Ar–H), 6.85 (d, 2H, Ar–H), 4.83 (s, 1H, $(-CHCH_2-)_n$), 4.25–4.17 (d, 3H, $-CH(OH)CH_2-$), 3.92 (s, 2H, $-OCH_2CH-$), 3.78 (s, 1H, –OH), 2.61 (s, 4H,

$-CH_2CH_2-$), 1.83–1.73 (d, 2H, $(-CHCH_2-)_n$). ^{13}C NMR ($CDCl_3$): δ (ppm) 172.8, 172.5 (C=O), 157.3, 129.7, 129.7, 126.3, 116.1, 116.1 (Ar, chlorphenesin), 69.3 (C-3, chlorphenesin), 68.3 (C-2, chlorphenesin), 66.0 ($(-CHCH_2-)_n$), 65.5 (C-1, chlorphenesin), 29.5 ($(-CHCH_2-)_n$), 29.2, 29.1 ($-CH_2-$). IR (film): ν (cm^{-1}) 3466 (OH), 1734 (O–C=O), 1596, 1492, 825 (Ar). Poly-OVSC has M_n of 1.35×10^4 and M_w/M_n of 1.95.

2.14. Homopolymerization of 1-O-vinyladipoyl-chlorphenesin (OVAC)

OVAC (1 mmol) was added to a small flame-dried flask with 2% AIBN (w/w). The polymerization was continued for 6 h at 70 °C. Precipitating the polymer in methanol terminated the reaction and the light yellow precipitate was washed with acetone. Then the product was dried under vacuum (yield: 74.2%). ^1H NMR ($CDCl_3$): δ (ppm) 7.20 (d, 2H, Ar–H), 6.80 (d, 2H, Ar–H), 4.82 (s, 1H, $(-CHCH_2-)_n$), 4.27–4.26 (d, 3H, $-CH(OH)CH_2-$), 4.01 (s, 2H, $-OCH_2CH-$), 3.79 (s, 1H, –OH), 2.35–2.27 (d, 4H, $-CH_2CH_2-$), 1.81–1.62 (m, 6H, $(-CHCH_2-)_n$, $-CH_2CH_2-$). ^{13}C NMR ($CDCl_3$): δ (ppm) 173.8, 173.2 (C=O), 157.2, 129.6, 129.6, 126.4, 116.2, 116.1 (Ar, chlorphenesin), 69.3 (C-3, chlorphenesin), 68.5 (C-2, chlorphenesin), 66.8 ($(-CHCH_2-)_n$), 65.7 (C-1, chlorphenesin), 29.5 ($(-CHCH_2-)_n$), 34.1, 33.9, 29.2, 29.1 ($-CH_2-$). IR (film): ν (cm^{-1}) 3466 (OH), 1735 (O–C=O), 1597, 1492, 828 (Ar). Poly-OVAC has M_n of 2.37×10^4 and M_w/M_n of 4.30.

2.15. Copolymerization of OVSC with 6-O-vinyladipoyl-D-glucose (OVAG)

In a 25 mL sealed polymerization tube, a mixture containing OVSC (1 mmol), OVAG (1 mmol) with 2% AIBN (w/w) and DMF (0.3 mL) was maintained at 70 °C under nitrogen for 12 h after the solution was degassed (freeze/pump/thaw cycles). The resulting product was precipitated in acetone. The precipitated material was dried under reduced pressure. The light yellow solid of poly(OVSC-*co*-OVAG) was obtained in 65.8% yield. IR (film): ν (cm^{-1}) 3419, 1170, 1057 (OH), 1733 (O–C=O), 1596, 1582, 1493, 827 (Ar). ^{13}C NMR (DMSO-d_6 + D_2O): δ (ppm) 174.5, 173.4 (C=O), 158.5, 130.5, 130.5, 125.9, 117.5, 117.5 (Ar, chlorphenesin), 97.8 (C1 of β-D-glucose), 93.3 (C1 of α-D-glucose), 77.3 (C3 of β-D-glucose), 75.7 (C2 of β-D-glucose), 74.6 (C5 of β-D-glucose), 74.0 (C3 of α-D-glucose), 73.1 (C2 of α-D-glucose), 71.5 (C4 of α-D-glucose), 71.3 (C4 of β-D-glucose), 70.5 (C5 of α-D-glucose), 70.3 (C-3, chlorphenesin), 68.0 (C-2, chlorphenesin), 66.5 (C-1, chlorphenesin), 65.0 (C6 α, β of D-glucose), 34.3 ($(-CHCH_2-)_n$), 34.3, 29.8, 29.8, 25.0, 25.0 ($-CH_2-$). ^1H NMR (DMSO-d_6 + D_2O) is shown in Fig. 4(c). Poly(OVSC-*co*-OVAG) has M_n of 4.34×10^4 and M_w/M_n of 2.15.

2.16. Copolymerization of OVAC with 6-O-vinyladipoyl-D-glucose (OVAG)

Poly(OVAC-*co*-OVAG) was synthesized by the same method as for poly(OVSC-*co*-OVAG). The light yellow solid was obtained in 59.5% yield. IR (film): ν (cm^{-1}) 3420, 1168, 1055 (OH), 1734 (O–C=O), 1597, 1583, 1496, 826 (Ar). ^{13}C NMR (DMSO-d_6 + D_2O): δ (ppm) 174.2, 173.1 (C=O), 158.4, 130.6, 130.4, 125.9, 117.5, 117.5 (Ar, chlorphenesin), 97.6 (C1 of β-D-glucose), 93.0 (C1 of α-D-glucose), 77.4 (C3 of β-D-glucose), 75.5 (C2 of β-D-glucose), 74.3 (C5 of β-D-glucose), 74.1 (C3 of α-D-glucose), 73.0 (C2 of α-D-glucose), 71.3 (C4 of α-D-glucose), 71.0 (C4 of β-D-glucose), 70.2 (C5 of α-D-glucose), 70.1 (C-3, chlorphenesin), 67.8 (C-2, chlorphenesin), 66.2 (C-1, chlorphenesin), 46.8 (C6 α, β of D-glucose), 34.0 ($(-CHCH_2-)_n$), 34.2, 29.7, 29.7, 25.0, 24.9, 24.9 ($-CH_2-$). Poly(OVAC-*co*-OVAG) has M_n of 1.05×10^4 and M_w/M_n of 1.35.

3. Results and discussion

3.1. Enzymatic synthesis and characterization of chlorphenesin vinyl esters and D-glucose vinyl esters

Transesterification of chlorphenesin with divinyl dicarboxylates catalyzed by Lipozyme® is shown in Scheme 1. Based on the general strategy described by Yoshimoto et al. [31], acylation of a hydroxyl group of substrate will lead the *O*-acylated carbon (*CH_2OCOR) to downfield shift, while the neighboring carbon (*CCH_2OCOR) to upfield shift in ^{13}C NMR. Characterization of the product **3a** by ^{13}C NMR revealed that chlorphenesin was substituted at primary hydroxyl position. The signals for primary hydroxyl of chlorphenesin shifted downfield from 63.8 ppm to 65.5 ppm and that of neighboring secondary hydroxyl shifted upfield from 70.5 ppm to 68.4 ppm. Likewise, analysis of ^{13}C NMR spectra of other products showed that the products (**3b–3d**) substituted at the primary hydroxyl of chlorphenesin. The analysis of ^1H NMR spectra also confirmed the structure of products.

Characterization of the product OVAG by ^{13}C NMR revealed that glucose was substituted at C-6 position, since signals for C-6 shifted downfield and C-5 position shifted upfield compared with D-glucose. These results imply that alkaline protease from *B. subtilis* shows an effective regioselectivity in the transesterification of glucose with **2b**.

3.2. Enzyme screening

This study focused on the identification of suitable enzymes for high transesterification activity of chlorphenesin and divinyl dicarboxylates. Ten commercially available enzymes were tested for the transesterification of chlorphenesin with divinyl adipate (**2b**) in anhydrous acetone at 50 °C. The screening results are presented and compared in Table 1. The yield of OVAC was determined by HPLC. The corresponding control reaction in the absence of enzyme resulted in OVAC formation of less than 0.5%, demonstrating that it was the enzyme that

Table 1
Influence of enzyme on the synthesis of OVAC

Enzyme	Yield[a] (%)
Control, no enzyme	−0.8
Alkaline protease from *Bacillus subtilis*	1.9
Amano protease PS from *Aspergillus melleus*	33.9
Lipozyme® immobilized lipase from *Mucor miehei*	72.5
Lipase from porcine pancreas	70.7
Lipase from hog pancreas	67.6
Lipase from *Candida cylindracea*	24.8
Lipase type VII from *Candida rugosa*	21.8
Lipase AY30	34.5
Amano lipase M from *Mucor javanicus*	41.3
Candida antarctica lipase acrylic resin	65.4

Conditions: enzyme (15 mg mL^{-1}), chlorphenesin (1 mmol), divinyl adipate (6 mmol), anhydrous acetone (20 mL), 50 °C, 250 rpm.
[a] The yield of OVAC was determined by HPLC and the retention time of OVAC in HPLC is 2.88 min.

played a crucial role in esterification reaction. The yield of OVAC catalyzed by the ten enzymes ranged in 1.9–72.5%. The best result was obtained from Lipozyme®, while alkaline protease from *B. subtilis* had the lowest activity in the transesterification. It was found that enzymes derived from various sources showed different properties.

All investigated enzymes for the transesterification were found to catalyze exclusively the formation of the monosubstituted 1-*O*-vinyladipoyl-chlorphenesin in the initial reaction stage. 1,2-*O*-Divinyladipoyl-chlorphenesin (**4b**) was formed with the increasing reaction time. After 24 h, the yields of **4b** were in 0–4.5% range with all investigated enzymes (the data was not shown in Table 1), and had no obvious increase with extending reaction time. *C. antarctica* lipase acrylic resin gave the highest yield 4.5% of **4b** at 24 h, and Lipozyme® with the yield of 2.5%. There were no other products detected by HPLC and TLC. Therefore, we selected Lipozyme® for further investigation.

3.3. Effect of organic solvents

Reaction media plays a crucial role in maintaining enzyme catalytic activity and stability [32]. Sixteen different solvents with Log *P* value ranging from −1.3 to 4.9 were screened to optimize the reaction conditions for enzymatic transesterification. The results are shown in Table 2.

In our study, the yields of product varied with solvents. Results indicated that the solvents can be classified into three categories. In the first category of solvents with Log $P < 0.6$ (entry 1–7, Table 2), which can dissolve chlorphenesin well, better yields from 72.5% to 40.2% were obtained. It was noteworthy that the highest yield was obtained in anhydrous acetone, which was a solvent with lower toxicity and easier processing. In the second category of solvents with Log *P* ranging from 0.65 to 2.9, there is no obvious relationship between the yields and Log *P* of solvents. The yields ranged from 10.8% in toluene to 46.2% in 1,2-dichloroethane. In the last category, which includes more hydrophobic solvents (Log $P > 3.0$), the reaction was slow and the yield of OVAC

Table 2
Influence of solvent on the synthesis of OVAC

Entry	Solvent	Log *P*[a]	Yield[b] (%)
1	DMSO	−1.3	41.1
2	DMF	−1.0	50.6
3	Dioxane	−0.5	52.3
4	Acetonitrile	−0.39	48.4
5	Acetone	−0.23	72.5
6	Tetrahydrofuran	0.46	40.2
7	Dichloromethane	0.6	44.8
8	Pyridine	0.65	27.8
9	2-Methyl-2-propanol	0.79	27.1
10	1,2-Dichloroethane	1.2	46.2
11	Isopropyl ether	1.9	14.9
12	Toluene	2.2	10.8
13	Tetrachloromethane	2.9	22.6
14	Cyclohexane	3.4	3.2
15	Hexane	3.9	4.1
16	Octane	4.9	5.2

Conditions: Lipozyme® (15 mg mL^{-1}), chlorphenesin (1 mmol), divinyl adipate (6 mmol), solvent (20 mL), 50 °C, 250 rpm.
[a] Log *P* is the logarithm of the partition coefficient of a given compound in the octanol–water two-phase system.
[b] The yield of OVAC was determined by HPLC.

was low. It was verified that nonpolar solvents were unsuitable because of poor solubility of chlorphenesin.

3.4. Influence of water content in acetone on the synthesis of OVAC

The water content of the reaction medium would be an important factor affecting the activity of enzyme and the yields of products [33]. In the transesterification, more residual water would be favoring the hydrolysis of the ester products. To obtain high yields, the level of the water in the reactions should be maintained as low as possible, but under those conditions, many enzymes are not active.

Fig. 1 shows the effect of water content in acetone on the transesterification of chlorphenesin with divinyl adipate catalyzed by Lipozyme®. In anhydrous acetone, Lipozyme® brought about the highest yield of OVAC. Then, the yield of OVAC decreased when the content of water increased and

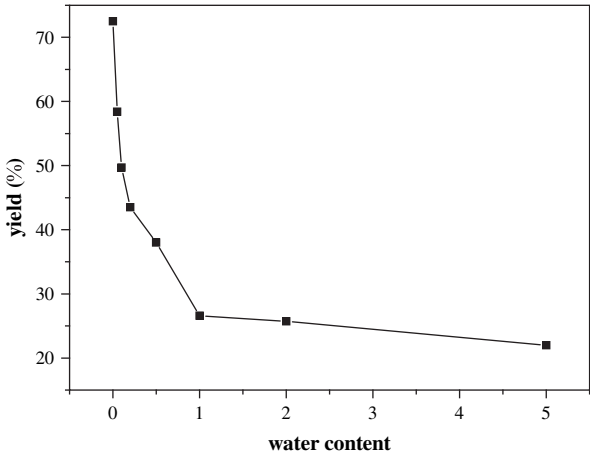


Fig. 1. Effect of water content in acetone on the synthesis of OVAC.

the yield was 22.2% with the water content of 5% in acetone. It was presumable that the immobilized enzymes had more stability and less influence on the water content in solvents.

Table 3
Influence of the structures of acylation reagents on initial reaction rate and yield

Acylation agents	Time (min)	Yields (%)[a]	Initial rate (mM min^{-1})
2a	60	84.7	4.73
2b	120	69.7	1.85
2c	120	56.8	1.43
2d	120	51.9	0.88

Conditions: Lipozyme® (15 mg mL^{-1}), chlorphenesin (1 mmol), divinyl dicarboxylates (6 mmol), anhydrous acetone (20 mL), 50 °C, 250 rpm.
[a] Determined by HPLC.

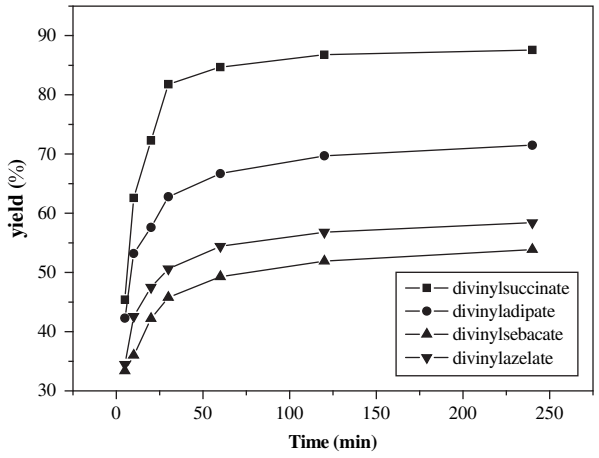


Fig. 2. Time process curve of enzymatic synthesis of chlorphenesin vinyl esters catalyzed by Lipozyme® in anhydrous acetone.

3.5. Influence of the structures of acylation reagents on initial reaction rate and yields

The influence of the structures of acylation reagent was evaluated. The enzymatic reactivity decreased as the length of carbon chains of the divinyl dicarboxylates increased. As shown in Table 3, the initial reaction rate was the highest with 4.73 mM min^{-1} when divinyl succinate was chosen as acylation reagent. However, compared with divinyl succinate, the reactivity of divinyl adipate and divinyl azelate was lower, and the initial reaction rate was 1.85 mM min^{-1} and 1.43 mM min^{-1}, respectively. The activity of divinyl sebacate in the transesterification was the lowest with the initial reaction rate of 0.88 mM min^{-1} (Fig. 2).

Similarly, the yield of **3a** had already been greater than 81% after 30 min. The yields of **3c–3d** were much lower than that of **3a**, which were 58.4% and 53.9% after 4 h, respectively. The yields of **3c–3d** showed no obvious increase with extending reaction time. It was possible that the more sterically hindered divinyl dicarboxylates provided lower yield.

3.6. Preparation of the homopolymer of poly-OVSC and poly-OVAC

The use of macromolecular prodrugs allows longer circulation times and gives access to additional chemical functionality or delivery that is more precise. In this study, we carried out the homopolymerization using AIBN as initiator. Products were analyzed by FTIR and NMR. In IR spectra of poly-OVSC (Fig. 3) 3101 cm^{-1} and 1646 cm^{-1} assigned to the vibration bands of double bond in the OVSC disappeared, and the absorption at 3466 cm^{-1}, 1596 cm^{-1}, 1582 cm^{-1},

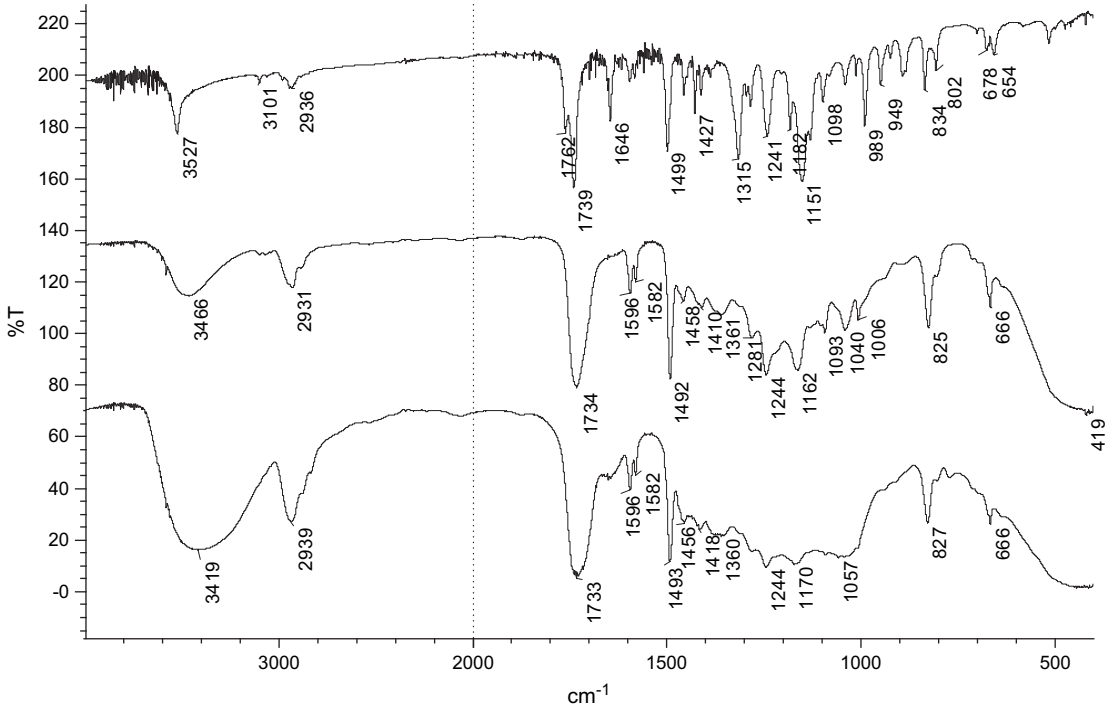


Fig. 3. IR spectra of 1-*O*-vinylsuccinyl-chlorphenesin (OVSC), poly-OVSC and poly(OVSC-*co*-OVAG).

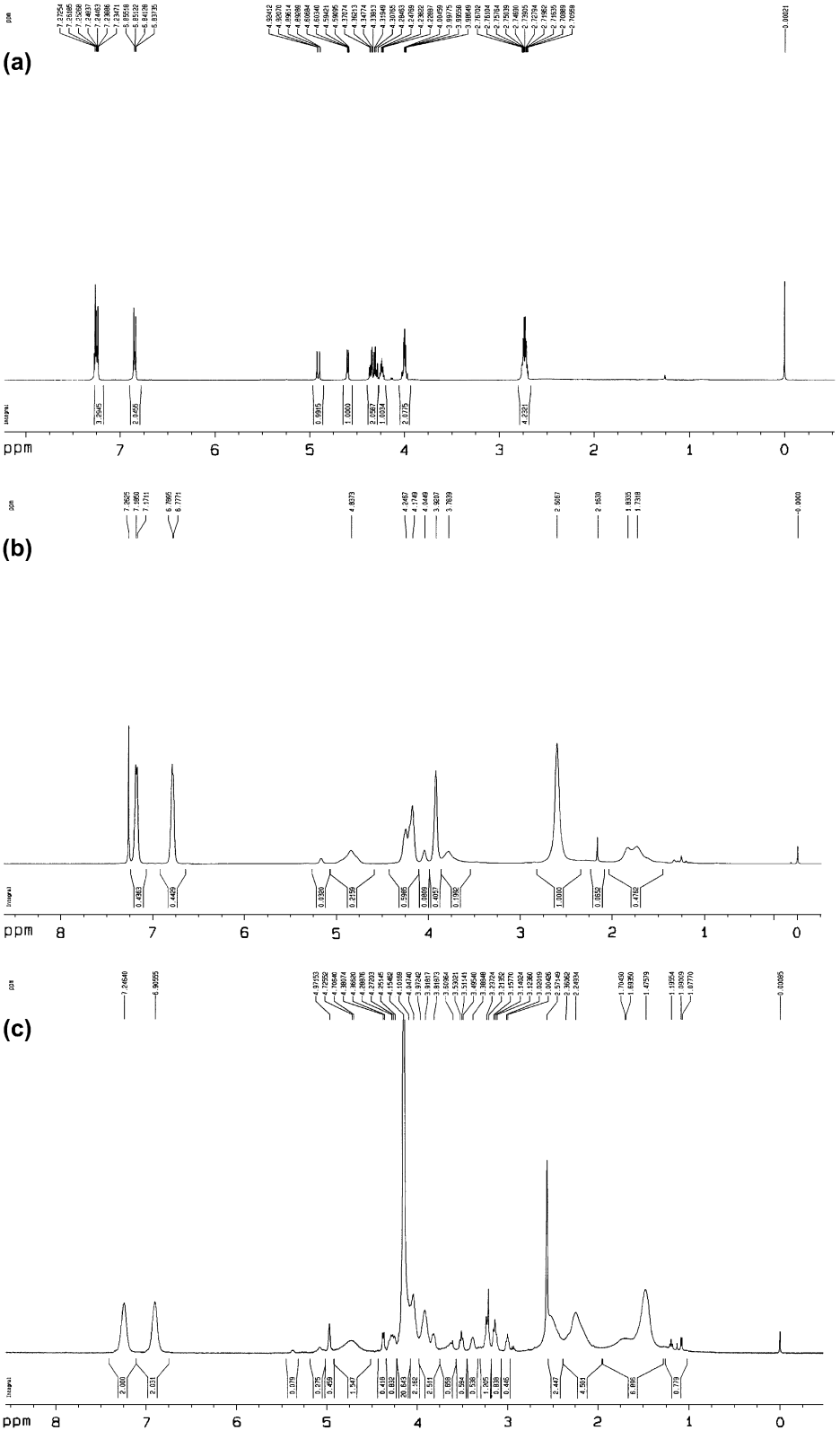


Fig. 4. ^{1}H NMR spectra of OVSC (a), poly-OVSC (b) in $CDCl_3$ and poly(OVSC-*co*-OVAG) (c) in DMSO-$d_6 + D_2O$.

1162 cm^{-1} and 825 cm^{-1} was assigned to the aromatic ring of OVSC. As shown in ^{1}H NMR spectra (Fig. 4(b)), as expected, the double bonds of the OVSC monomer was absent in the polymer. The aromatic protons appeared at around 7.23–6.85 ppm in ^{1}H NMR of poly-OVSC. Analysis of IR and NMR spectra confirmed the structure of homopolymers. Gel permeation chromatographic (GPC) analysis of the polymer from OVSC indicated an M_w of 1.35×10^4 and polydispersity

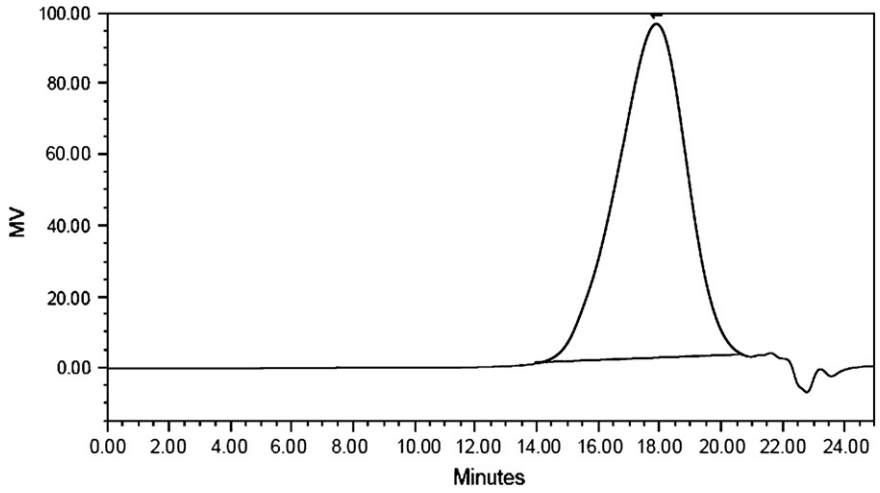


Fig. 5. GPC of poly-OVSC in THF.

of 1.95 (as shown in Fig. 5). The polymeric prodrug poly-OVAC has high molecular weight with M_n of 2.37×10^4 and M_w/M_n of 4.30. The loadup of chlorphenesin in poly-OVSC was 61 wt% and that in poly-OVAC was 56 wt%.

3.7. Preparation of the copolymers poly(OVSC-co-OVAG) and poly(OVAC-co-OVAG)

As known to all, biopolymers play an essential role. In previous research, we have synthesized a series of polymerizable glycolipids by regioselective enzymatic methods. In this research, we selected polymerizable glucose vinyl esters as monomers for further polymerization, which had good water-solubility and dissolution behavior. The copolymers poly-(OVSC-*co*-OVAG) and poly(OVAC-*co*-OVAG) were prepared using radical polymerization. In IR spectra of poly-OVSC (Fig. 3), 3101 cm^{-1} and 1646 cm^{-1} assigned to the vibration bands of double bond in the OVSC disappeared, and the absorption at 1596 cm^{-1}, 1582 cm^{-1} and 827 cm^{-1} were assigned to the aromatic ring of OVSC. Absorption at 3419 cm^{-1}, 1170 cm^{-1} and broad 1057 cm^{-1} were assigned to 6-*O*-vinyladipoyl-D-glucose (OVAG). The result showed that poly(OVSC-*co*-OVAG) was prepared successfully. ^1H NMR and ^{13}C NMR of poly(OVSC-*co*-OVAG) revealed the disappearance of vinyl group and existence of chlorphenesin, D-glucose groups and poly(vinyl alcohol) main chain. The polymeric prodrug poly(OVSC-*co*-OVAG) has high molecular weight with M_n of 4.34×10^4 and M_w/M_n of 2.15, and poly-(OVAC-*co*-OVAG) has molecular weight with M_n of 1.05×10^4 and M_w/M_n of 1.35. According to the calculation from the NMR spectra, the ratio of OVSC (or OVAC) to OVAG monomers in copolymer was 0.85:1. The loadup of chlorphenesin of the two polymeric prodrugs was 26.2 wt% (poly(OVSC-*co*-OVAG)), and 25.2 wt% (poly(OVAC-*co*-OVAG)).

4. Conclusion

In the present study, we described a highly regioselective enzymatic synthesis approach for the preparation of polymerizable chlorphenesin vinyl esters through the Lipozyme®-catalyzed transesterification of chlorphenesin with divinyl dicarboxylates in anhydrous acetone. High selectivity and yields can be obtained in short reaction time. The influences of enzyme source, organic solvent, reaction time and the structures of acylation agent on the transesterification were systematically investigated. Moreover, protease-catalyzed regioselective acylation of D-glucose with divinyl adipate provided a biocompatible monomer 6-*O*-vinyladipoyl-D-glucose (OVAG). The monomers of chlorphenesin were homopolymerized successfully. The obtained polymeric prodrugs were characterized with IR, NMR and GPC. The poly-OVSC and poly-OVAC have M_n of 1.35×10^4 and 2.37×10^4, and M_w/M_n of 1.95 and 4.30, respectively. Furthermore, the copolymers of chlorphenesin vinyl esters and glucose vinyl ester were also prepared successfully. The loadup of chlorphenesin in the two polymeric prodrugs was 26.2 wt% (poly(OVSC-*co*-OVAG)), and 25.2 wt% (poly-(OVAC-*co*-OVAG)). The investigation on the controlled release of the above-mentioned polymeric drugs and the preparation of chlorphenesin copolymers containing other biocompatible comonomers are in process.

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References

[1] Park OJ, Kim DY, Dordick JS. Biotechnol Bioeng 2000;70(2):208–16.
[2] Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. J Controlled Release 2000;65:271–84.
[3] Haag R, Kratz F. Angew Chem Int Ed 2006;45(8):1198–215.
[4] Gross RA, Klumar A, Kalra B. Chem Rev 2001;101(7):2097–124.
[5] Cavallaro G, Pitarresi C, Licciardi M, Giammona G. Bioconjugate Chem 2001;12(2):143–51.
[6] Swarts JC. Macromol Symp 2002;186:123–8.

[7] van der Merwe T, Boneschans B, Zorc B, Breytenbach J, Zovko M. Int J Pharm 2002;241(2):223–30.
[8] Cai XQ, Wang N, Lin XF. Polymer 2006;47(19):6491–5.
[9] Ulbricht M. Polymer 2006;47(7):2217–62.
[10] Krishnamoorthy R, Mitra AK. Adv Drug Delivery Rev 1998;29:135–46.
[11] Secundo F, Carrea G. Chem Eur J 2003;9(14):3194–9.
[12] Hölemann A, Seeberger PH. Curr Opin Biotechnol 2004;15(6):615–22.
[13] Yuan XF, Yamasaki Y, Harada A, Kataoka K. Polymer 2005;46(18): 7749–58.
[14] Kiesewetter J, Arikan B, Kaminsky W. Polymer 2006;47(10):3302–14.
[15] Zhou Y, Zhuo RX, Liu ZL. Polymer 2004;45(16):5459–63.
[16] Hernandez OS, Soliman GM, Winnik FM. Polymer 2007;48(7):921–30.
[17] Davis BG, Robinson MA. Curr Opin Drug Discovery Dev 2002;5(2): 279–88.
[18] Huynh DP, Shim WS, Kim JH, Lee DS. Polymer 2006;47(23):7918–26.
[19] Mozhaev VV, Budde CL, Rich JO, Usyatinsky AY, Michels PC, Khmelnitsky YL, et al. Tetrahedron 1998;54(16):3971–82.
[20] Bertau M. Curr Org Chem 2002;6(11):987–1014.
[21] Garcia-Junceda E, Garcia-Garcia JF, Bastida A, Fernandez-Mayoralas A. Bioorg Med Chem 2004;12(8):1817–34.
[22] He F, Li SM, Garreau H, Vert M, Zhuo RX. Polymer 2005; 46(26):12682–8.
[23] Rooseboom M, Commandeur JNM, Vermeulen NPE. Pharmacol Rev 2004;56(1):53–102.
[24] Ferrero M, Gotor V. Chem Rev 2000;100(12):4319–47.
[25] Huang CF, Kuo SW, Lin FJ, Wang CF, Hung CJ, Chang FC. Polymer 2006;47(20):7060–9.
[26] Bridiau N, Taboubi S, Marzouki N, Legoy MD, Maugard T. Biotechnol Prog 2006;22(1):326–30.
[27] Okuyama S, Aihara H. Res Commun Chem Pathol Pharmacol 1987; 55(2):147–60.
[28] Kurachi M, Aihara H. Jpn J Pharmacol 1984;30(1):29–36.
[29] Lambert DM. Eur J Pharm Sci 2000;11:s15–27.
[30] Wu Q, Wang N, Xiao YM, Lv DS, Lin XF. Carbohydr Res 2004; 339(12):2059–67.
[31] Yoshimoto K, Itatani Y, Tsuda Y. Chem Pharm Bull 1980;28: 2065–74.
[32] Carrea G, Ottolina G, Riva S. Trends Biotechnol 1995;13(2):63–70.
[33] Fan H, Kitagawa M, Raku T, Tokiwa Y. Macromol Biosci 2003;3(8): 420–4.