Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3207–3210

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A NOVEL METHOD FOR PREPARATION OF OPTICALLY ACTIVE α-MONOBENZOYL GLYCEROL *VIA* LIPASE-CATALYZED ASYMMETRIC TRANSESTERIFICATION OF GLYCEROL

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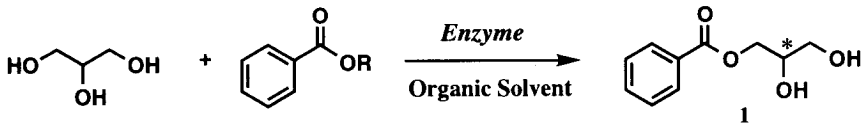
Received 6 September 1999; accepted 1 October 1999

Abstract: One-step synthesis of optically active α-monobenzoyl glycerol is described by lipase-catalyzed transesterification of benzoate derivatives with glycerol in 1,4-dioxane.

Optically active α-monoacyl glycerol (MAG) is a useful starting material for the preparation of chiral drugs, such as β-blockers[1], platelet activating factor (PAF)[2], (*S*)-carnitine, and γ-amino-β-hydroxybutyric acid (GABOB)[3]. Although MAGs are commonly prepared by chemical synthesis from chiral pool[4], there is currently a great interest in the use of chemo-enzymatic method for their synthesis[5]. It is reported that MAGs are synthesized *via* stereoselective acylation of prochiral glycerol derivatives whose β–hydroxyl group is protected[6]. In this process, the product can be obtained quantitatively but the preparation of the substrate requires many steps. Furtheremore, the transesterification of glycerol derivatives is limited to the use of acetate esters as acyl donors[5, 6], although changing the acyl donors is expected to increase the enantio- and regioselectivity[7]. Herein we report a novel and efficient method for synthesizing optically active α-monobenzoyl glycerol **1** *via* an enzymatic transesterification of glycerol with benzoate derivatives in organic solvents. In this process, optically active **1** can be theoretically obtained in one step and in a quantitative yield (Scheme 1).



Scheme 1

The optical purity and an absolute configuration of **1** was determined by HPLC with Chiralcel OJ column after derivatization to its 1,2-isopropylidene derivative by reacting with acetone dimethylacetal and catalytic amount of (+)-camphorsulfonic acid at 30°C[8].

Glycerol, an acyl acceptor of the reaction, was highly insoluble in hydrophobic organic solvents, in which the reaction mixture became biphasic. We found that hydrophilic solvents, such as DMSO, DMF, CH_3CN,

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00556-9

THF, and 1,4-dioxane, solubilized glycerol well and selected 1,4-dioxane as the most suitable solvent for the reaction by its ease to manipulate.

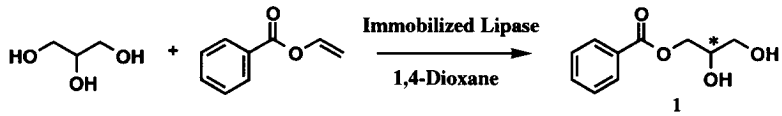
We chose vinyl benzoate as an acyl donor for the transesterification reaction. This reagent was screened against 40 commercially available lipases and hydrolases. Although almost all enzymes did not exhibit the transesterification activity due to an inactivation in 1,4-dioxane, lipases such as Lipozyme and carrier-fixed CHIRAZYME L-2 (c.-f., C-2, lyo), which are already immobilized on resins, catalyzed the reaction. We assumed that immobilization might increase the stability of enzymes towards 1,4-dioxane. The commercial enzymes were immobilized on macroporous resin by absorption and were examined for the reaction. Among them, 14 enzymes catalyzed the reaction and gave optically active **1** (Table I). As expected, we could find out the transesterification activity in the commercial enzymes by immobilization, but optical purity of the obtained **1** were low (up to 32% e.e.) compared to that of **1** synthesized by carrier-fixed CHIRAZYME L-2 (60% e.e.).

We selected carrier-fixed CHIRAZYME L-2 as the most suitable catalyst for the transesterification of glycerol and the reaction conditions were optimized. We set the concentrations of acyl donor and acceptor to 100 mM since the yield was decreased when these concentrations were over 150 mM probably due to the substrate inhibitions. The optical purity of the synthesized **1** was gradually decreased in the reaction mixture by a 1,3-rearrangement of **1** catalyzed by a basic functional group of resins of the enzyme. To prevent the base-catalyzed racemization, the enzyme reaction was carried out at 15°C, at which the side-reaction was suppressed although the enzyme activity was decreased to a half of which at 30°C. A kind of acyl donor for the reaction is varied and benzoic anhydride was selected as the best acyl donor, since the reaction rate was markedly increased when it was used as an acyl donor. Other benzoate derivatives, such as methyl benzoate, phenyl benzoate, 2-chloroethyl benzoate, and trifluoromethyl benzoate were not suitable for the reaction. Based on the results, we optimized the condition was as follows: 100 mM each of glycerol and benzoic anhydride was incubated with carrier-fixed CHIRAZYME L-2 in 1,4-dioxane at 15°C.

A multigram scale-synthesis (100 mmol scale) of (*R*)-**1** by the transesterification reaction under the optimized conditions was examined[9]. Reaction was completed within 24 hr. After an extraction and a purification with column chromatography on silica-gel, the reaction product was obtained in 94% yield and its structure was confirmed to be (*R*)-**1** (54% e.e.) by its NMR, IR, and mass spectrum[10] and chiral HPLC analysis. Its optical purity was elevated up to 95% e.e. by one recrystallization from hexane / ^{i}PrOH.

In conclusion, we have described a novel efficient method preparing optically active **1** by the transesterification of glycerol with benzoate derivatives in 1,4-dioxane. By using carrier-fixed CHIRAZYME L-2, (*R*)-**1** having > 95% e.e. was synthesized in one step.

Table I Enzymatic transesterification of glycerol by commercial lipases and immobilized lipases on macroporous resin[a]



1

Lipase from (lipase name)	Yield (%)	Optical purity (% e.e.)	Absolute configuration
Commercial immobilized lipases			
Candida antharctica (CHIRAZYME L-2)[b, c]	62	60	*R*
Mucor miehei (Lipozyme)[d]	87	15	*R*
Lipases immobilized on macroporous resin in this study[e]			
Pseudomonas sp. (Lipase PS)[f]	100	37	*R*
Rhizopus sp. (Lipase D)[f]	10	32	*S*
Pseudomonas sp. (CHIRAZYME L-4)[b]	6.2	27	*R*
Pseudomonas sp. (CHIRAZYME L-6)[b]	17	24	*R*
Rhizopus sp. (Lipase F-AP15)[f]	17	24	*S*
Mucor sp. (Lipase M-AP)[f]	4.8	18	*S*
Pseudomonas fluorescens (Lipase AK)[f]	13	11	*R*
Rhizopus delemar[g]	7.6	11	*S*
Pseudomonas sp. (Toyozyme LIP)[d]	30	11	*S*
Aspergillus sp. (Lipase PZ-6)[f]	11	5.4	*R*
Humicola sp. (CHIRAZYME L-8)[b]	68	4.2	*S*
Burkholderia sp. (CHIRAZYME L-1)[b]	18	2.3	*R*
Penicillium aurantiogriseum (Lipase G)[f]	13	2.3	*S*

[a] The reaction mixture (2 ml) containing 100 mg of an enzyme, 200 μmol each of glycerol and vinyl benzoate in 1,4-dioxane, was shaken at 170 rpm for 1 - 7 days at 30°C. [b] Roshe Diagnostics K.K. (Tokyo, Japan) [c] L-2, c.-f., C-2, lyo. [d] Toyo Jozo Co Ltd. (Shizuoka, Japan) [e] One hundred mg of lipase was dissolved in 25 ml of distilled water and to this was added 400 mg of macroporous resin (DIAION HPA 25, Mitsubishi Chem., Tokyo, Japan). The mixture was gently shaken at 100 rpm at 10°C for 3 - 24 hr. The resins were collected by filtration and dried under reduced pressure over silica-gel for 8 hr at room temperature. [f] Amano Pharmaceutical Co. (Nagoya, Japan) [g] Seikagaku Kogyo. (Tokyo, Japan)

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8. To a 10 μl of reaction mixture were added 10 μl of 10 mM (+)-10-camphorsulfonic acid in 1,4-dioxane and 10 μl of 20% acetone dimethylacetal solution in ethylacetate, and incubated at 30°C for 1 hr. To this was added hexane (1 ml) and $NaHCO_3$ (2 mg), and centrifuged (12,000 x *g*, 3 min). The supernatant was directly analyzed by HPLC using a CHIRALCEL OJ column (Daicel Chemical Industries. Ltd.) at a flow rate of 0.3 ml/min with hexane / ^{i}PrOH= 95 / 5 as a solvent, monitored at 254 nm. (*S*)- and (*R*)-**1** were detected at retention times of 23.5 min and 25.6 min, respectively.
9. To a 1,4-dioxane solution (1 liter) containing of 9.2 g (100 mmol) of glycerol and 22.6 g (100 mmol) of benzoic anhydride, 15 g of carrier-fixed CHIRAZYME L-2 was added, and the mixture was stirred for 24 hr at 15°C. After a removal of the enzyme by filtration, the reaction mixture was evaporated under reduced pressure and the residue was partitioned between saturated brine and ethyl acetate (100 ml each), and extracted with ethyl acetate for 3 times. The combined organic layer was washed with 5% $NaHCO_3$ solution and saturated brine, and dried over anhydrous Na_2SO_4. Purification by short column chromatography on silica-gel (ethyl acetate) afforded 18.4 g of (*R*)-**1** (94% yield, 54% e.e.) as a colorless oil. The **1** was dissolved in 2 liters of hexane / ^{i}PrOH (95 / 5) at 65°C and to this was added a trace amount of optically pure (*R*)-**1**. The solution was left at room temperature for 1 hr, and the precipitate formed was filtered, washed with hexane / ^{i}PrOH (95 / 5), and dried under reduced pressure over silica-gel. (*R*)-**1** was obtained as colorless needles in a yield of 6.90 g (35.2%) whose optical purity was 95.4% e.e. ^{1}H-NMR (400 MHz, DMSO-d_6), δ = 8.00-7.98 (m, 2H; ArH), 7.67-7.63 (m, 1H; ArH), 7.54 - 7.50 (m, 2H; ArH), 5.02 (d, 1H, *J* = 5.4; $HOCH_2CH(\mathbf{OH})CH_2OBnz$), 4.70 (t, 1H, *J* = 5.6; $\mathbf{HOCH_2}CH(OH)CH_2OBnz$), 4.30 (dd, 1H, *J* = 3.9, 11.2; $\mathbf{CH_2}OBnz$), 4.16 (dd, 1H, *J* = 6.3, 11.2; $\mathbf{CH_2}OBnz$), 3.81 - 3.77 (m, 1H; $HOCH_2\mathbf{CH}(OH)CH_2OBnz$), 3.46 - 3.43 (m, 2H; $\mathbf{CH_2}OH$): ^{13}C-NMR (100 MHz, DMSO-d_6), δ = 167.0, 133.3, 129.8, 129.7, 129.6, 70.3, 65.6, 63.5: FAB-Mass *m/z*: 77 (rel. int. 89.2%), 93 (59.8%), 105 (100%), 106 (100%), 123 (71.7%), 179 (100%), 197 (100%, M + H), 198 (94.1%, M + 2H): m.p. 66 - 67°C [lit[10]. for (*S*)-**1** 65 - 66°C]: $[\alpha]^{23}_{D}$ +18.8° (c = 1.00, EtOH) [lit[10]. for (*S*)-**1** $[\alpha]^{23}_{D}$ -19.0°]: IR (KBr), 3600 - 3100, 3050, 1700, 1300, 690 cm^{-1}
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