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Preparation of Enantiomerically Pure (*R*)-2-Butyryloxymethylglycidol by Lipase-Catalyzed Asymmetric Hydrolysis

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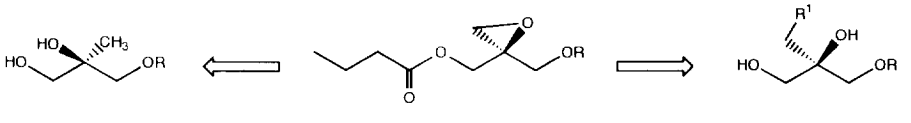
Abstract : Optically active epoxy alcohol, (*R*)-2-butyryloxymethylglycidol **3** which is the precursor of a *tert*-alcohol chiral building block was obtained in high enantiomeric purity, 98.7% e.e., by lipase-catalyzed asymmetric hydrolysis using a phosphate buffer and organic co-solvent system in 95% of chemical yield.

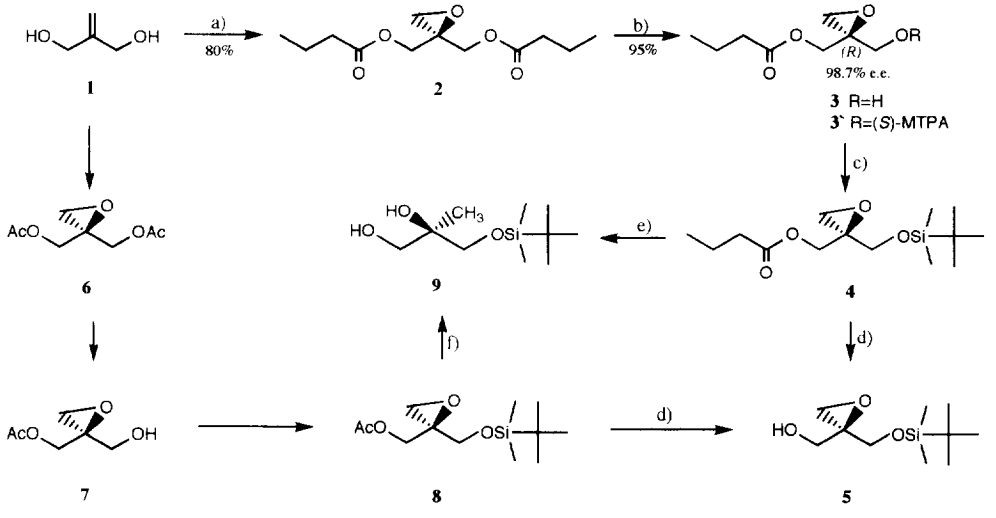
Optically active epoxy alcohol (*R*)-2-butyryloxymethylglycidol **3** (**A**, R=H) is a versatile chiral building block for the synthesis of enantiomerically pure natural products which have a stereogenic quarternary carbon center in the molecule such as α-tocopherol[1], bicyclomycin[2], frontalin[3], sordidin[4], acacialactam[5] and so on. This chiral glycidyl synthon **A** is the precursor of the chiral *tert*-alcohol building block and can be controlled as both *R*- and *S*- units in the asymmetric synthesis. Its reduction and alkylation gave chiral 2-substituted glycerol derivatives **B** and **C** which could serve as *tert*-alcohol chiral synthons. These kinds of chiral synthons bearing a quarternary stereogenic center were reported by a number of research groups, but only a few papers have been presented about a general *tert*-alcohol chiral building block. Recently, Harada *et al.* reported 2-substituted glycerol derivatives by utilizing *l*-menthone as a chiral auxiliary[6]. Asymmetric hydrolysis of [(*R,S*)-2,2,4-trimethyl-1,3-dioxolan-4-yl]methyl butyrate with lipase by the Wirz group afforded 2-methylglycerol derivatives in high enantiomeric purity[7]. Synthesis of tertiary alcohols from secondry alcohols *via* intramolecular C-H insertion of alkylidenecarbene was reported by Ohira *et al.*[8].



B **A** **C**

In previous papers we reported the preparation of (*R*)-2-acetoxymethylglycidol by lipase hydrolysis in 92% e.e. and its application to the synthesis of both the enantiomers of frontalin[9,10]. But since no satisfactory improvement in enantiomeric purity could be achieved in the present paper we wish to describe a simple and an efficient preparative method for (*R*)-2-butyryloxymethylglycidol **A** (R=H) in high enantiomeric purity with lipase-catalyzed asymmetric hydrolysis. Our strategy is a binary screening with lipase-catalyzed asymmetric hydrolysis for the induction of chirality *via* symmetry-destruction of a symmetrical substrate. The first step is screening among various lipases for the highest enantioselectivity under the general condition using a phosphate buffer at pH 7.0. And then the second stage is screening of reaction conditions under variation of pH, temperature, and composition of reaction media with the selected enzyme for the complete enantioselectivity. The substrate, prochiral epoxy dibutyrate **2** was conveniently prepared from 2-methylene-1,3-propanediol **1**[11] by esterification with butyric anhydride and pyridine, and subsequent epoxidation of the double bond with *m*-chloroperbenzoic acid (MCPBA 2.0 equiv., CH_2Cl_2) in 80% yield for the 2 steps. The enzymes employed in this screening system were 17 kinds of commercially available lipase preparations for the modest price.

We first examined the reaction in phosphate buffer (pH 7.0) at room temperature employing various enzymes as catalyst. As shown in Table I, the best enantioselectivity was obtained from lipase P (from *Pseudomonas* sp.) giving **3** in 72.5% enantiomeric excess with 91% yield. Thus we selected lipase P for further optimization in the first screening. In the case of acylase (Amano : from *Aspergillus* sp.) the enantiomeric purity (63.2% e.e., *S* configuration) was not so bad, but the reaction was much slower and the chemical yield was also lower. In general, a lipase preferentially hydrolyzes and esterifies the (*R*)-enantiomer of a substrate. On this occasion most of the tested



a) 1. butyric anhydride/pyridine 2. MCPBA/CH_2Cl_2 b) lipase P/THF+buffer c) $ClSiMe_2$-*t*-Bu, DMAP/CH_2Cl_2
d) K_2CO_3/MeOH e) LAH/THF f) **6**, **7**, **8**, **9** - Ref. 9
*$[\alpha]_D$ values ; **3**:-5.23 (c=5.93, Et_2O), **4**:-4.86 (c=5.00, $CHCl_3$), **5**:-8.98 (c=2.57, $CHCl_3$), **9**:+0.30 (c=1.35, Et_2O)

Table I. Enantioselective Hydrolysis of 2 to 3 by Various Enzymes

Enzyme	Origin	Time (h)	Yield (%)	e.e. (%)	Absolute Configuration
Lipase P (Amano)	*Pseudomonas* sp.	0.5	91	72.5	*R*
Acylase (Amano)	*Aspergillus* sp.	20	33	63.2	*S*
Urase (Amano)	*Rhizopus niveus*	3.0	56	53.6	*R*
PPL (Sigma)	Porcine pancreas	0.5	71	48.4	*R*
Lipase A (Amano)	*Aspergillus niger*	4.0	53	46.6	*R*
Pancreachin (Amano)	Hog pancreas	1.5	83	45.9	*R*
Lipase M (Amano)	*Mucor javanicus*	1.0	83	45.7	*R*
Lipase P (Nagase)	*Pseudomonas* sp.	2.0	87	45.7	*R*
Steapsin (Tokyo Kasei)	Hog pancreas	2.0	75	44.3	*R*
WG (Sigma)	Wheat Germ	17	21	43.8	*R*
Toyobo-LIP (Toyobo)	*Immobilized enzyme	1.0	72	42.7	*R*
Lipase F-AP15 (Amano)	*Rhizopus oryzae*	2.0	78	39.4	*R*
Acylase (Tokyo Kasei)	*Aspergillus* genus	20	70	37.7	*S*
Lipase MY (Meito)	*C. cylindracea* nov.sp.	0.5	68	28.6	*S*
CCL (Sigma)	*Candida cylindracea*	2.0	63	25.7	*S*
Lipase AY (Amano)	*Candida rugosa*	1.5	37	25.4	*S*
Lipase OF (Meito)	*Candida cylindracea*	1.0	29	24.1	*S*

Table II. Enantioselective Hydrolysis of 2 to 3 by Lipase P at Different Reaction Conditions Using Various Co-Solvents

Co-Solvent[a]	Time (h)	Yield (%)	(*R*)-3 e.e. (%)[b]
Tetrahydrofuran(THF)	0.3	95	98.7[c]
Isobutyl alcohol	1.0	69	98.0[c]
n-Butyl alcohol	1.0	76	96.4[c]
Ethyl acetate	4.0	58	95.3
Methylene chloride	27	44	93.7
Diethyl ether	3.0	76	93.3
Acetone	2.0	85	91.3
Methyl alcohol	0.5	75	89.5
Chloroform	27	45	87.8
Ethyl alcohol	1.0	91	87.3
2-Methyl-2-propanol	0.5	82	87.1
Benzene	4.0	12	86.4
Isopropyl ether	2.0	27	84.6
Acetonitrile	1.5	83	84.3
Dioxane	1.0	82	83.9
Toluene	2.0	12	82.2
Dimethylformamide	0.5	88	80.0

[a] 0.25g **2**, 17mg lipase P - 0.1M Phosphate buffer pH6.5 / co-solvent = 20ml / 10ml
[b] Determined by HPLC analysis of **3**
[c] Tested in an ice-bath

enzymes gave the (*R*)-enantiomer except lipases from *Candida* sp. and acylases. In the second approach, in order to find the influence of the organic solvent we studied the augmentation of the enantioselectivity for the selected enzyme lipase P in organic co-solvent system with phosphate buffer. Surprisingly, as shown in Table II the enantioselectivity of lipase P in phosphate buffer and organic co-solvent conditions was dramatically different from that in phosphate buffer only. In THF and phosphate buffer at pH 6.5 system lipase P hydrolyzed **2** to give (*R*)-**3** {$[\alpha]_D^{25}$-5.23 (c=5.93, Et_2O)} in 98.7% e.e. and 95% chemical yield (Table II). Under the same condition with isobutyl alcohol gave also good enantioselectivity (98.0% e.e.). When organic solvent was added in buffer system all of the cases revealed enhanced enantioselectivity, but there seems to exist no general rule on the influence of the structure, solubility and polarity of the solvent. The enantiomeric purity of hydrolyzed product **3** was calculated by converting it to the corresponding (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetate[12] (MTPA ester) **3`**. HPLC analysis of **3`** revealed it to be of 98.7% e.e., and therefore the enantiomeric purity of **3** was estimated to be as high as 98.7% e.e.. The absolute configuration of 2-substituted glycidol **3** was determined by comparison of specific rotation values of the reduced alcohol **9**[9] {$[\alpha]_D^{25}$+0.30 (c=1.35, Et_2O), lit[9] (*R*)-**9** : $[\alpha]_D^{22}$+0.2 (c=5.0, Et_2O)} and a new epoxy alcohol **5** {$[\alpha]_D^{25}$-8.98 (c=2.57, $CHCl_3$)} derived from **4** and **8**, respectively. From the comparision of our measured $[\alpha]_D$ values, the stereochemistry of the stereogenic quarternary carbon at 2-butyryloxymethylglycidol **3** was assigned as *R*.

In summary, an efficient lipase-catalyzed hydrolysis in a biphasic system has been established for the generation of valuable chiral 2-substituted glycidol which yields *tert*-alcohol chiral building block in high enantiomeric purity. The asymmetric hydrolysis of achiral substrate **2** with lipase P (*Pseudomonas* sp.) in pH 6.5 phosphate buffer and THF (1/1) at 0°C system produced (*R*)-2-butyryloxymethylglycidol **3** in a high enantiomeric purity of 98.7% e.e. with the *R* configuration in 95% chemical yield.

EXPERIMENTAL

^1H NMR (300 MHz) spectra were obtained on Varian UNITY 300 spectrometer using deuteriochloroform ($CDCl_3$) as solvent and TMS as internal reference. Infrared spectra were recorded as film on RFX-65 FTIR spectrometer. Optical rotations were measured on JASCO DIP 370 digital polarimeter at the wave length of the sodium D-line (589 nm). 2-Methylene-1,3-propanediol **1** was purchased from Aldrich Chemical Co. Merck Kieselgel 60 Art. 7734 was used for a column chromatography.

Enzymes : The enzymes used in this screening system were as follows - Lipase A, AY, F-AP15, M, P, and acylase, urase, and pancreachin (Amano Seiyaku) ; lipase MY and OF (Meito Sangyo) ; lipase P (Nagase) ; Toyobo-LIP (Toyobo) ; lipase CCL, PPL, and WG (Sigma) ; acylase and steapsin (Tokyo Kasei). These enzymes were used without further purification.

General Procedure - Enzymatic Hydrolysis of **2** (Table I) : To a solution of **2** (250 mg, 1 mmol) in 0.1M phosphate buffer (pH 7.0, 20 ml) was added the enzyme (lipase P, MY, OF : 17 mg ;

lipase A, M, F-AP15, CCL, AY, and acylase, urase, pancreachin, steapsin and Toyobo-LIP : 25 mg ; PPL and WG : 170 mg). The solution was stirred vigorously for the specified time (Table I) at room temperature. The mixture was filtered through a Celite pad and the filtrate was extracted with EtOAc (X3). The organic solution was washed with sat. aq. $NaHCO_3$, H_2O, brine, and dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with *n*-hexane/EtOAc (2/1) afforded **3** as optically active form.

(R)-2-butyryloxymethylglycidol **3** : To a solution of **2** (0.98 g, 4 mmol) in 0.1M phosphate buffer (pH 6.5, 15ml) and THF (15 ml) was added lipase P (50 mg) at 0°C and the mixture was stirred vigorously for 20 min at 0°C. The mixture was filtered through a Celite pad. Then THF in the filtrate was evaporated *in vacuo* and the remained aqueous layer was extracted with EtOAc (X3). The combined organic extract was washed with sat. aq. $NaHCO_3$, H_2O and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed on SiO_2. Elution with *n*-hexane/ EtOAc (2/1) afforded 0.66 g (95%) of (-)-**3** as a colourless oil, $[\alpha]_D^{25}$-5.23 (c=5.93, Et_2O) ; n_D^{25} 1.4456. IR (film) ν_{max} 3467 (s, O-H), 1739 (s, C=O), 1255(m), 1176 (s, C-O), 1047 (m, C-O), 889 (w, C-O), 823 (w) cm^{-1}. ^1H NMR ($CDCl_3$) : δ = 0.96 (t, 3H, *J* = 7.2 Hz), 1.672 (qt, 2H, *J* = 7.2, 7.2 Hz), 2.345 (t, 2H, *J* = 7.2 Hz), 2.820 (d, 1H, *J* = 5.1 Hz), 2.943 (d, 1H, *J* = 5.1 Hz), 3.726 (d, 1H, *J* = 12.6 Hz), 3.815 (d, 1H, *J* = 12.6 Hz), 4.167 (d, 1H, *J* = 12.0 Hz), 4.357 (d, 1H, *J* = 12.0 Hz).

Determination of the enantiomeric purity of **3** : The glycidol **3** was converted to the corresponding (*S*)-MTPA ester **3'** {or (*RS*)-MTPA ester} in the conventional manner[12] by starting from (*R*)-**3** (17 mg) and the acyl chloride (30 μl) derived from (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid {(*S*)-MTPA, or (*RS*)-MTPA} in triethylamine/CH_2Cl_2. HPLC analysis of (*S*)-MTPA ester **3'** on Merck column {LiChrosorb® Si60, 4.0 x 250 mm x 2, heptane/EtOAc (10/1), 1 ml/min} : t_R = 91.66 min (0.65%), t_R = 94.54 min (99.35%). The enantiomeric purity of (*R*)-**3** was therefore 98.7% e.e..

(R)-2-butyryloxymethylglycidol t-butyldimethylsilyl ether **4** : *tert*-Butyldimethylsilyl chloride (0.91 g, 6 mmol) was added to a solution of **3** (0.87 g, 5mmol) and imidazole (0.68 g, 10 mmol) in CH_2Cl_2 (20 ml) at 0°C, and the mixture was stirred for 12 h at room temperature. It was then poured into water and extracted with Et_2O (X2). The organic layer was washed with water and then with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2. Elution with *n*-hexane/EtOAc (10/1) gave 1.02 g of silyl ether **4** (71%) as a colourless oil, $[\alpha]_D^{25}$-4.86 (c=5.00, $CHCl_3$) ; n_D^{25} 1.4394. IR (film) ν_{max} 1743 (s, C=O), 1467 (m, $SiCH_3$), 1255 (s, $SiCH_3$), 1176 (s, C-O), 1099 (s, C-O-Si), 839 (s, $SiCH_3$) cm^{-1}. ^1H NMR ($CDCl_3$) : δ = 0.06 (s, 3H), 0.07 (s, 3H), 0.90 (s, 9H), 0.956 (t, 3H, *J* = 7.5 Hz), 1.665 (qt, 2H, *J* = 7.5, 7.5 Hz), 2.325 (t, 2H, *J* = 7.5 Hz), 2.785 (d, 1H, *J* = 6.9 Hz), 2.801 (d, 1H, *J* = 6.9 Hz), 3.713 (d, 1H, *J* = 11.4 Hz), 3.797 (d, 1H, *J* = 11.4 Hz), 4.166 (d, 1H, *J* = 12.3 Hz), 4.329 (d, 1H, *J* = 12.3 Hz).

(S)-2-t-Butyldimethylsilyloxymethylglycidol **5** : A 0.2M solution of K_2CO_3 in 50% aqueous methanol (5 ml) was added to a solution of **4** (500 mg, 1.7 mmol) in MeOH (10 ml) with stirring and ice-cooling in an ice-bath. The mixture was stirred for 2 h at 0°C. To this was added water, and the mixture was stirred for further 30 min at room temperature. The mixture was then extracted with Et_2O. The organic layer was washed with water and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2. Elution with *n*-hexane/EtOAc (5/1) gave 300 mg of epoxy alcohol **5** (80%) as a colourless oil, $[\alpha]_D^{25}$-8.98 (c=2.57, $CHCl_3$) ; n_D^{25} 1.4439. IR (film) ν_{max} 3437 (m, O-H), 1468 (m, $SiCH_3$), 1255 (s, $SiCH_3$), 1097 (s, C-O-Si), 1047 (m, C-O), 839 (s, $SiCH_3$) cm^{-1}. ^1H NMR ($CDCl_3$) : δ = 0.07 (s, 3H), 0.08 (s, 3H), 0.90 (s, 9H), 1.955 (br. dd, 1H, *J* = 7.5, 5.1 Hz), 2.76 (d, 1H, *J* = 5.1 Hz), 2.88 (d, 1H, *J* = 5.1 Hz), 3.747 (dd, 1H, *J* = 12.0, 7.5 Hz), 3.792 (s, 2H), 3.899 (dd, 1H, *J* = 12.0, 4.2 Hz).

(R)-3-t-Butyldimethylsilyloxy-2-methyl-1,2-propanediol **9** : A solution of **4** (500 mg, 1.7 mmol) in THF (20 ml) was added slowly to a suspension of $LiAlH_4$ (200 mg) in THF at 0°C. This was stirred for 2 h at room temperature, and to this was then added Et_2O (60 ml), water. After the mixture had been filtered through a Celite pad, the organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2. Elution with *n*-hexane/EtOAc (3/1) gave 300 mg of diol **5** (78%) as a colourless oil, $[\alpha]_D^{25}$+0.30 (c=1.35, Et_2O) {lit[9] (*R*)-9 $[\alpha]_D^{22}$+0.2 (c=5.0, Et_2O)} ; n_D^{25} 1.4432. IR (film) ν_{max} 3410 (s, O-H), 1468 (m, $SiCH_3$), 1255 (s, $SiCH_3$), 1097 (s, C-O-Si), 1051 (s, C-O), 836 (s, $SiCH_3$) cm^{-1}. ^1H NMR ($CDCl_3$) : δ = 0.09 (s, 6H), 0.91 (s, 9H), 1.11 (s, 3H), 2.28 (br. s, 2H), 3.45 (d, 1H, *J* = 11.0 Hz), 3.49 (d, 1H, *J* = 9.9 Hz), 3.61 (d, 1H, *J* = 11.0 Hz), 3.63 (d, 1H, *J* = 9.9 Hz).

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