Facile Chemoenzymatic Preparation of Enantiomerically Pure 2-Methylglycerol Derivatives as Versatile Trifunctional C4-Synthons

Beat Wirz,*,† Richard Barner,‡ and Joseph Hübscher‡

Hoffmann-La Roche Ltd., Pharmaceutical Research—New Technologies, Departments of Microbiology and Physics, CH-4002 Basel, Switzerland

Received November 24, 1992 (Revised Manuscript Received April 26, 1993)

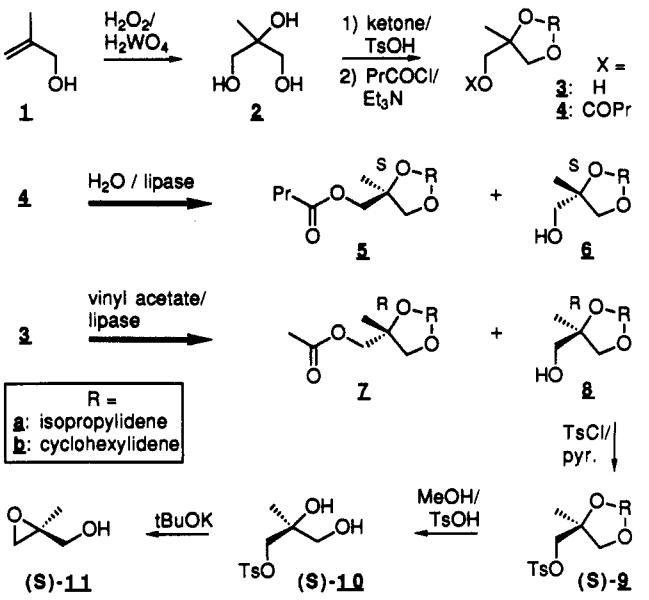
Both enantiomers of a series of synthetically valuable 2-methylglycerol derivatives have been prepared with >99% ee using a chemoenzymatic reaction sequence. The introduction of chirality was achieved by enantioselective esterification of 1,2-O-protected 2-methylglycerol 3 or enantioselective hydrolysis of its butyryl ester 4. The enzymatic reaction proceeded with unusually high selectivity and velocity for a primary alcohol (ester) substrate.

Introduction

Chiral 2-methylglycerol derivatives such as commercially available (*R*)- or (*S*)-2-methylglycidol and (*R*)- or (*S*)-2-methylglycidyl 4-nitrobenzoate are used in the construction of chiral quarternary carbons[1] encountered e.g. in *d*-α-tocopherol[2], pheromones,[3] or vitamin D3 metabolites.[4] These small highly functional chiral building blocks are produced on a large scale by enantioselective epoxidation of β-methallylic alcohol according to Sharpless.[5] The drawback of this methodology is that it requires a comparably high mole percentage of the titanium/tartrate complex for obtaining high ee-values so that its application is mostly limited to the production of high value-added compounds.

The present report describes an efficient alternative chemoenzymatic route to both enantiomers of a series of 2-methylglycidyl building blocks. Starting with H_2WO_4-catalyzed dihydroxylation of β-methallylic alcohol 1 by H_2O_2 and subsequent protection of formed triol 2 yielded acetonide alcohol 3 (Scheme I). Alcohol 3 or, alternatively, its butyryl ester 4 is enzymatically resolved with high enantioselectivity and the resulting optical antipodes 6 or 8 were converted by conventional methods to the chiral compounds 9, 10, and 11 which can serve as synthons in various syntheses of enantiomerically pure compounds (Scheme I). The enzymatic esterification as well as hydrolysis proceed with high selectivity, attaining enantiomeric ratios[6] *E* of >150 and >1000, respectively. These are unusually high values for the resolution of *primary* alcohol compounds. Recently, Fuganti et al.[7] reported the enantioselective hydrolysis of the *phenylacetyl* ester of 3 with immobilized penicillin acylase G (EC 3.5.1.11) generating the (*S*)-alcohol 6 in 90% ee (*E* ~ 60). Hydrolysis of 2-methylglycidyl butyrate with porcine pancreas lipase (EC 3.1.1.3) by Ladner and Whitesides[8] afforded the retained (*S*)-ester in ca. 51% ee (*E* ~ 3). Hydrolysis of butyl 2,2,4-trimethyl-1,3-dioxolane-4-carboxylate, an analogue of 3 on a higher oxidation state, mediated by lipase from *Candida cylindraceae* afforded both the (*S*)-acid and retained (*R*)-ester in ca. 33% ee (*E* ~ 3).[9]

Scheme I. Chemoenzymatic Routes to Chiral 2-Methylglycerol Derivatives



Results and Discussion

Strategy: For the enzymatic generation of optically active 2-methylglycerol compounds, enantiogroup selection suggested itself. However, unlike monoacylates of 2-methyl-1,3-propanediol[10] or 2-O-substituted glycerol,[11] the 2-methylglycerol monoacylates could not be obtained with satisfactory stereoselection. A screening with the 2-O-benzylated derivative was also unsuccesful. Finally, enantiogroup selection was abandoned in favor of kinetic

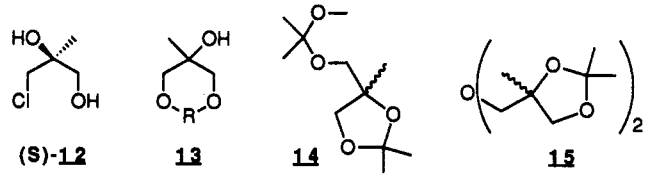
† Department of Microbiology.
‡ Department of Physics.

(1) Shimazaki, M.; Hara, H.; Suzuki, K.; Tsuchihashi, G.-i. *Tetrahedron Lett.* **1987**, *28*, 5891–5894.
(2) Hübscher, J.; Barner, R. *Helv. Chim. Acta* **1990**, *73*, 1068–1086.
(3) Wershofen, S.; Classen, A.; Scharf, H.-D. *Ann. Chem.* **1989**, 9–18. Barner, R.; Hübscher, J. *Helv. Chim. Acta.* **1983**, *66*, 880–890.
(4) Barner, R.; Hübscher, J.; Daly, J. J.; Schönholzer, P. *Helv. Chim. Acta* **1981**, *64*, 915–938.
(5) Scott, J. W.; *Asymmetric Synthesis*; Morrison, J. D., Scott, J. W., Eds.; Academic: Orlando, FL, 1984; Vol. 4, pp 1–226.
(6) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
(7) Fuganti, C.; Grasselli, P.; Servi, S.; Lazzarini, A.; Casati, P. J. *Chem. Soc., Chem. Commun.* **1987**, 538–539.
(8) Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250–7251.
(9) Pottie, M.; Van der Eycken, J.; Vandewalle, M. *Tetrahedron Lett.* **1989**, *30*, 5319–5322.
(10) Wirz, B.; Schmid, R.; Walther, W. *Biocatalysis* **1990**, *3*, 159–167. Xie, Z.-F.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1988**, 1638–1639. Santaniello, E.; Ferraboschi, P.; Grisenti, P. *Tetrahedron Lett.* **1990**, *31*, 5657–5660.

0022-3263/93/1958-3980$04.00/0

resolution. 1,2-Ketals of 2-methylglycerol were chosen as synthetically readily accessible substrates, at the same time promising simple racemization of the unwanted enantiomer (for both alcohol and ester). After successfully testing the more sterically hindered spiroketal **4b**, the investigations were extended to the more economically accessible 1,2-acetonide **4a** which likewise provided positive results.

Substrate Synthesis: 2-Methylglycerol (**2**) was prepared from **1** in analogy to previous reports[12] using the H_2WO_4/H_2O_2 reagent. The subsequent ketalization step led to ca. 50% (GC) of the unwanted 1,3-ketal **13a** if solely remaining H_2WO_4 present in crude **2** was employed as acidic catalyst. Addition of TsOH reduced the formation of the unwanted isomers **13** to 6–8%, using acetone or 2,2-dimethoxypropane (DMP). If H_2WO_4 was removed completely by an additional anion exchange step (<4 ppm), the respective value was lowered to 4.5%. **13** can be readily removed in the following steps because it is excluded from



(S)-12 13 14 15

chemical (absence of DMAP) or enzymatical acylation as well as from tosylation. If **3a** was synthesized by transketalization using DMP, two side products interfering with the resolution step were formed. They were tentatively assigned structures **14** and **15** as judged by GC-MS in combination with their chemical behavior: **14** showed a *m/e* value of 203 (M – CH_3) and, exposed to water under acidic conditions, hydrolyzed to **3a** within hours, whereas the diastereomers of "dimer" **15** (double peak on GC) exhibited a peak at *m/e* 259 (M – CH_3) and were only hydrolyzed slowly. The side products could neither be silylated nor was there a simple way to remove them jointly from **3a** or **4a**. Owing to their hydrolytic decomposition to racemic **3a** in an aqueous milieu their presence in the enzymatic step noticeably affected the otherwise excellent enzymatic performance. Therefore, the method using acetone was preferred despite of its lower chemical yield. From 100 g of **1** was synthesized 110 and 135 g of crude **3a** and **3b**, respectively.

Kinetic Resolution: Quite surprisingly, *primary* alcohol esters **4a** and **4b** were hydrolyzed with excellent selectivity by several lipases (Table I). Best results were obtained with lipase P from *Pseudomonas fluorescens* (recently renamed and reclassified as lipase PS from *P. cepacia*) which showed remarkable retention of its enantioselectivity also at higher substrate concentrations (Table II).

One is inclined to associate the excellent stereoselectivity with the dioxolane ring common to both substrates. However, also the tertiary methyl group seems to play an important role, for an enzyme screening with the cyclohexanone ketal lacking this *tert*-methyl group under the conditions described in Table I afforded only modest ee-values. As reported by Sonnet and Antonian[13] also the corresponding unmethylated acetonide ester was hydrolyzed by several lipases with only moderate enantioselectivity. The authors also stated a considerably faster hydrolysis rate for this acetonide ester as compared to other glyceryl ester derivatives when normalized for a single released acid residue. The same phenomenon was observed with acetonide ester **4a** (results not shown).

Table I. Enantioselective Hydrolysis of 4 to 6 by Various Lipases[a]

	lipase (U)[c]	hydrolysis time (min)	% ee of 6	*E*
4a:	P-30 (202)	26	>99	>1000
	SAM-2 (85)	81	>99	>1000
	Palatase M 1000 (100)	26	>99	>1000
	Rh. delemar (525)	28	98	>400
	M-AP 10 (80)	60	>97	>200
	D-20 (286)	24	97	>200
	F-AP 15 (1008)	16	97	>200
4b:	P-30 (202)	35	>99	>1000
	SAM-2[b] (85)	168	>99	>1000
	Palatase M 1000 (100)	40	>98	>800
	Rh. delemar (525)	52	>98	>600
	CE-5 (116)	10	>98	>600
	Rh. arrhizus (1480)	15	>98	>500
	D-20 (286)	38	98	>350
	M-AP10 (80)	168	96	>100
	F-AP 15[b] (1008)	176	96	>100

[a] Ester **4** (100 mg) was hydrolyzed in 26 mL of buffer, pH 7.5, to 48–50% conversion (as determined by the consumption of 0.1 N NaOH) as described in the Experimental Section. [b] At pH 7.0. [c] Units indicated as declared and defined by the producer.

Table II. Enantioselective Hydrolysis of 4 by Lipase P-30 at Different Substrate Concentrations[b]

	substrate g	substrate %	lipase (U)[a]	hydrolysis time (min)	*E*
4a:	0.1	0.4	202	26	>1000
	1.0[c]	3.7	607	28	>1000
	3.0[c]	10.3	1820	60	>700
	5.0[c]	16.1	1618	60	>700
4b:	0.1	0.4	202	35	>1000
	1.0	3.7	607	180	>1000
	1.0[c]	3.7	607	150	>700
	3.0[c]	10.3	1820	200	>700

[a] Units indicated as declared and defined by the producer. [b] Hydrolysis in 26 mL of buffer, pH 7.5, to 47–50% conversion using 0.1 N NaOH. [c] 1.0 N NaOH.

The antipodes **6** and **8** were converted following the reaction sequence outlined in Scheme I according to standard methods without noticeable loss of enantiomeric purity. The absolute configuration of the reaction products was established by chemical correlation of **8** to (*S*)-**12** and was confirmed by comparison of the specific rotation of **6a** to that obtained by Fuganti et al.[7] Racemization of the alcohol **6a** was achieved very simply just by incubating it in acetone in the presence of TsOH. Ester **5a** was racemized under similar conditions in the added presence of racemic alcohol **3a**. Racemization of the alcohol was associated with the formation of ~4% **13a**.

Continuous Hydrolysis: Hydrolysis was also carried out effectively in a *continuous* manner using enzyme immobilized on the epoxy resin Eupergit C. In order to enhance the enzyme/support ratio crude lipase P was partially purified prior to covalent immobilization. A simple ammonium sulfate precipitation from 0 to 35%

(11) Kerscher, V.; Kreiser, W. *Tetrahedron Lett.* **1987**, *28*, 531–534. Wirz, B.; Schmid, R.; Foricher, J. *Tetrahedron Asymmetry* **1992**, *3*, 137–142. Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205. Breitgoff, D.; Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 1523–1524. Terao, Y.; Murata, M.; Achiwa, K.; Nishio, T.; Akamtsu, M.; Kamimura, M. *Tetrahedron Lett.* **1988**, *29*, 5173–5176.

(12) Kirby, P. GB 74-7902 (priority Feb 21, 1974). Chrystal, E. J. T.; Haines, A. H.; Patel, R. *Carbohydrate Res.* **1989**, *186*, 320–325.

(13) Sonnet, P.; Antonian, E. *J. Agric. Food Chem.* **1988**, *36*, 856–862.

saturation (cf. Sugiura et al.[14]) gave the enzyme in 84% purity (SDS-PAGE) and nearly quantitative yield. The coupling turned out to be practically quantitative; however, the immobilized enzyme showed only 3% residual activity.[15] This result was compensated by the good stability: at 4 °C within the first 100 days the activity dropped to 80% but afterwards remained nearly constant, losing only <2% within the following 200 days. A 2.75-g column was successfully run for half a year and was only abandoned for want of substrate. Ester **4a** was converted in >12 g/d at a concentration of 7% (w/w). Since no pH-stating was possible on this small scale, a phosphate buffer with higher ionic strength (0.5 M) and higher initial pH (7.5) than that used in batch experiments had to be employed.

Esterification: Enantioselective esterification of racemic alcohol **3a** in anhydrous organic solvents was also investigated. Two lipases (Lipozyme IM-20 and lipase P), a few acyl donors (enol acylates and ethyl butyrate), and some organic solvents (*n*- and c-hexane, pentane, *i*-octane, and TBME) afforded (*R*)-configured **8a** with ee >97% (E > 150). An example of a fast and simple acetylation is the reaction of 5 g of **3a** in 50 mL of *n*-hexane by transesterification with vinyl acetate (0.55 mol equiv) using 0.24 g of lipase P adsorbed on porous glass beads reaching 45% (GC) conversion after 2 h (E > 150). Repeated batchwise use of the catalyst (five runs) did not reveal any noticeable inactivation. Using vinyl butyrate as an acyl donor under identical conditions (two runs), the reaction was completed after 200 min (49–50% conversion) providing 2.93 g (40%) (*R*)-butyryl ester of 96–97% ee (E > 150).

Conclusions

An efficient kinetic racemic resolution step for the generation of valuable 2-methylglyceryl synthons of high enantiomeric purity has been established. By starting from **1**, the substrates **3** or **4** were readily obtained in satisfactory yields. The side product **13** formed in the synthesis of **3** is discriminated in the subsequent chemical or enzymatic acylation or in the tosylation step later on. The excellent enantiomeric ratios obtained for the hydrolysis of **4** (E > 1000) and the acylation of **3** (E > 150), together with the comparatively large number of successful enzymes exhibiting this selectivity, qualify the present enzymatic kinetic resolution as extraordinary for a *primary* alcohol ester. Depending on the chosen reaction sequence (Scheme I), both enantiomers, as well the alcohols of the corresponding acylates, can be produced and the unwanted isomers racemized and recycled. The high reaction rate and enantioselectivity are essentially retained also at higher substrate concentration (e.g. 10%). Continuous enzymatic hydrolysis over 6 months and repeated batchwise esterification experiments did not reveal any appreciable inactivation of the catalyst. All these features suggest also a technical potential of this enzymatic procedure.[16]

Experimental Section

General: Lipases P (32700 U/g), CE, D, M-AP and F-AP were kindly provided by Amano Pharmaceutical Co., Nagoya, Japan, and lipases Palatase and Lipozyme from Novo Industri, Bagsvaerd, DK. Lipase from *Rhizopus arrhizus* was purchased from Sigma, lipase from *Rhizopus delemar* from Seikagaku Kogyo, Tokyo, and lipase SAM from Fluka. Unless otherwise stated, reagents were from Fluka or Merck and used as received. After an extraction or washing step, the combined organic phases were dried (Na_2SO_4, $MgSO_4$) and evaporated. Purifications by flash chromatography were carried out on Merck silica gel 60 (0.040–0.063 mm) using the slurry method of column packing. ee-Values were determined on a chiral stationary phase as described below.

2-Methyl-1,2,3-propanetriol (2): Alcohol **1** (300 g, 4.16 mol) and H_2WO_4 (7.9 g) were dissolved in twice-distilled H_2O (300 mL) by heating to 70 °C. The oil bath was replaced by an ice bath and the temperature maintained at 70–77 °C by the controlled addition of 30% H_2O_2 (exothermic). After a consumption of 550 g, the reaction mixture was kept for 1 h at 75 °C and, in order to destroy residual H_2O_2, for another 2.5 h at 97 °C. The reaction mixture was cooled to rt, filtered, and passed through a Dowex 1-X4 column (800 mL; OH^--form; equilibrated in twice-distilled H_2O) in order to remove H_2WO_4. The eluate was set to pH 6 by adding some drops of 30% H_2SO_4, concentrated, and dried *in vacuo* (70 °C, stripped with toluene) to yield crude **2** (360 g) as slightly yellowish and opaque oil: 82% (GC) (90% (GC) as TMS derivative); ^{1}H-NMR (DMSO) δ 0.97 (s, 3 H), 3.21 (m, 4 H), 4.02 (s, 1 H), 4.37 (t, 2 H).

(*R,S*)-2,2,4-Trimethyl-1,3-dioxolane-4-methanol (3a). (A) Ketalization: Crude **2** (100 g, 84% GC), TsOH·H_2O (2.0 g), and Na_2SO_4 (25 g) were stirred in acetone (250 mL) overnight. For neutralization the reaction mixture was stirred with $NaHCO_3$ (10 g, 0.5 h), the filtrate concentrated, and the residue distilled to yield **3a** (91.7 g) as a colorless oil (22 mbar/85–89 °C): 95% (4.7% of isomer **13a**, GC on OV 1701 capillary column); ^{1}H-NMR ($CDCl_3$) δ 1.30 (s, 3 H), 1.43 (d, 6 H), 1.88 (bs, 1 H), 3.47/3.52 (AB, 2 H, J_{AB} = 11.3 Hz), 3.73/3.98 (A'B', 2 H, $J_{A'B'}$ = 8.5 Hz), [**13a**: 1.05 (s), ~1.44 (~d)]; EI-MS *m/e* 131 (M – CH_3); IR (film) 3453, 1114, 1059 cm^{-1}. Anal. Calcd for $C_7H_{14}O_3$: C, 57.51; H, 9.65. Found: C, 57.37; H, 9.86. **(B) Transketalization**: Crude **2** (50 g, 84% GC), DMP (50 g, 0.48 mol), and TsOH·H_2O (1.0 g) in acetone (100 mL) were stirred overnight. Neutralization (5.0 g of $NaHCO_3$) and workup were carried out as described yielding crude **3a** (53.7 g, 90% GC) containing 4.5% (GC) of the isomer **13a** and 4% of **14**.

(*R,S*)-2-Methyl-1,4-dioxaspiro[4.5]decane-2-methanol (3b): Crude triol **2** (5.00 g, 87% GC), TsOH·H_2O (0.4 g), and Na_2SO_4 (1 g) in cyclohexanone (10 mL) were stirred for 6 h. After neutralization (1 g of $NaHCO_3$ and 25 μL of Et_3N) the suspension was filtered and concentrated and the residue chromatographed (300 g; Et_2O/CH_2Cl_2 1:1) to yield **3b** (5.64 g, 30.3 mmol) as a slightly yellowish oil: 92% (GC) containing 7% of **13b**; ^{1}H-NMR ($CDCl_3$) δ 1.17 (s, 3 H), 1.2–1.3 (stack, ~10 H), 3.14–1.32 (ddd, 2 H), 3.55/3.88 (AB, 2 H, J_{AB} = 9.1 Hz), 4.83 (t, 1 H), [**13b**: 1.08 (s), 3.47 (s), 4.66 (s)]; MS *m/e* 186 (M).

[(*R,S*)-2,2,4-Trimethyl-1,3-dioxolan-4-yl]methyl Butyrate (4a): To a solution of **3a** (205 g, containing 6% **13a**, 1.32 mol) and Et_3N (215 mL, 156.2 g, 1.54 mol) in CH_2Cl_2 (1 L) was slowly added butyryl chloride (154 mL, 1.47 mol) keeping the temperature at 20 °C. The suspension formed was stirred for 4 h and washed (3 × 1 L water). The organic phase was concentrated and the residue distilled *in vacuo*. **4a** (227.6 g, 1.05 mol, 80%) was obtained as colorless oil (0.6 mbar/58–60 °C): >99% (GC); ^{1}H-NMR ($CDCl_3$) δ 0.96 (t, J = 7.4 Hz, 3 H), 1.32 (s, 3 H), 1.41 (s, 6 H), 1.67 (m, J = 7.4 Hz, 2 H), 2.33 (t, J = 7.4 Hz, 2 H), 3.73/3.96 (AB, J_{AB} = 8.8 Hz, 2 H) overlapped by 4.00/4.03 (A'B', $J_{A'B'}$ ~10 Hz, 2 H); MS *m/e* 201 (M – CH_3); IR (film) 1742, 1212, 1174, 1122 cm^{-1}. Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 60.86; H, 9.33.

[(*R,S*)-2-Methyl-1,4-dioxaspiro[4.5]dec-2-yl]methyl Butyrate (4b): Spiroketal **3b** (5.62 g containing 7% **13b**, 28.1 mmol), Et_3N (4.6 mL, 33 mmol), and butyric anhydride (4.9 mL, 30 mmol) in CH_2Cl_2 (20 mL) was stirred overnight and washed (H_2O) and the organic phase concentrated and chromatographed (300 g; EtOAc/hexane 1:4) providing **4b** (6.55 g, 25.5 mmol, 91%) as a colorless oil: >99% (GC); ^{1}H-NMR ($CDCl_3$) δ 0.95 (t, 3 H), 1.24-1.75 (stack, ~17 H (H_2O-overlapping)), 2.32 (t, 3 H), 3.70/3.94 (AB, 2 H, J_{AB} = 8.8 Hz) overlapped by 3.99/4.01 (A'B'~t, 2 H,

(14) Sugiura, M.; Oikawa, T.; Hirano, K.; Inukai, T. *Biochim. Biophys. Acta* **1977**, *488*, 353–358.

(15) An improved method for immobilization of lipase P on Eupergit C, and samples are available from Röhm Pharma (Darmstadt, Germany). Personal communication from D. Krämer and T. Boller.

(16) Wirz, B.; Barner, R.; Hübscher, J. Eur. Patent 388778, 1990.

$J_{A'B'}$ = 10.7 Hz); MS *m/e* 256 (M); IR (film) 1741, 1165, 1097 cm^{-1}. Anal. Calcd for $C_{14}H_{24}O_4$: C, 65.60; H, 9.44. Found: C, 65.30; H, 9.60.

(*S*)-2,2,4-Trimethyl-1,3-dioxolane-4-methanol (6a): Ester **4a** (5.01 g, 23.2 mmol) was emulsified in 0.1 M NaCl, 4 mM sodium phosphate buffer 7.5 (26 mL) under vigorous stirring and hydrolyzed with 50 mg of lipase (pH-stat using 1.0 N NaOH, 46.6% conversion after 60 min). After extraction (100 + 50 + 50 mL of CH_2Cl_2) using short centrifugation for faster phase separation and chromatography (80 g; hexane/EtOAc 2:1), **5a** (2.52 g, 11.7 mmol, 50%) and **6a** (1.33 g, 9.1 mmol, 39%) were obtained as a colorless oil: $[\alpha]_{365}$ = −24.2° (1% in $CHCl_3$); $[\alpha]_{365}$ = +13.2° (1% in EtOH); >99% ee; >99% (GC). On a larger scale **5a** (10 mbar/~112 °C) and **6a** (10 mbar/~80 °C) can be separated by distillation[17] (deterioration for both compounds ≤0.1% ee).

[(*S*)-2,2,4-Trimethyl-1,3-dioxolan-4-yl]methyl Butyrate (5a): Final hydrolysis of optically impure **5a** (2.52 g, 11.7 mmol) overnight (26 mL buffer, 12 mg lipase P), extraction (50 + 20 mL of CH_2Cl_2), and chromatography (hexane/EtOAc 3:1) yielded **5a** (2.04 g, 9.4 mmol, 81%) as a slightly yellowish oil: $[\alpha]_{365}$ = −18.4° (1% in EtOH); >99% ee; >99% (GC).

(*R*)-2,2,4-Trimethyl-1,3-dioxolane-4-methanol (8a): Ester **5a** (4.00 g, 18.5 mmol) was hydrolyzed in MeOH (25 mL) and 1.0 N NaOH (19 mL) overnight. The reaction mixture was concentrated, taken up in H_2O (25 mL), and extracted with EtOAc (3 × 50 mL) to yield **8a** (2.50 g, 17.1 mmol, 92%): >99% ee; 99% (GC).

Racemization of the Unwanted Enantiomers. Ester: 5a (4.50 g, 20.8 mmol) and **3a** (3.00 g, 20.7 mmol) were incubated in refluxing acetone (25 mL) for 20 h in the presence of $TsOH \cdot H_2O$ (100 mg). After neutralization (1 g $NaHCO_3$, 0.5 h), the filtrate was concentrated and the residue chromatographed (70 g; hexane/EtOAc 9:1) to give **4a** (4.18 g, 19.3 mmol, 93%) of 12% ee and 99% GC purity. **Alcohol: 6a** (10 g) and $TsOH \cdot H_2O$ (200 mg) in acetone (100 mL) was stirred overnight providing racemic **3a** (containing ~4% **13a**).

[(*S*)-2-Methyl-1,4-dioxaspiro[4.5]dec-2-yl]methyl Butyrate (5b): Ester **4b** (20.0 g, 78.0 mmol) was hydrolyzed in 184 mL of buffer (containing $MgCl_2$ instead of NaCl) with 100 mg lipase P as described above (1.0 N NaOH, 51.5% conversion, 24 h). Extraction (250 + 100 mL of CH_2Cl_2) and chromatography (220 g; EtOAc/hexane 1:2) yielded **5b** (9.7 g, 37.8 mmol, 48%) as a colorless oil: $[\alpha]_{365}$ = −13.5° (1% in $CHCl_3$); >99% ee; >99% (GC).

(*S*)-2-Methyl-1,4-dioxaspiro[4.5]decane-2-methanol (6b): Hydrolysis of **4b** (5.0 g, 19.5 mmol) at pH 7.0 (104 mL of buffer) with 74 mg of lipase P (1.0 N NaOH, 46% conversion, 2.3 h) followed by extraction (2 × 50 mL of CH_2Cl_2) and chromatography [100 g; CH_2Cl_2 (500 mL), CH_2Cl_2/Et_2O 1:1 (500 mL)] provided **6b** (1.79 g, 8.5 mmol, 44%) as a colorless oil: $[\alpha]_{365}$ = −20.1° (1% in $CHCl_3$); $[\alpha]_{365}$ = +20.9° (1% in EtOH); $[\alpha]_D$ = +7.5° (1% in EtOH); >99% ee; >99% (GC).

(*R*)-2-Methyl-1,4-dioxaspiro[4.5]decane-2-methanol (8b): **5b** (9.0 g, 35.1 mmol) was incubated in MeOH (50 mL) and 1.0 N NaOH (36 mL) for 4 h. The reaction mixture was concentrated and extracted with EtOAc/H_2O to give **8b** (6.24 g, 33.5 mmol, 95%): $[\alpha]_{365}$ = +19.4° (1% in $CHCl_3$); >99% ee; >99% (GC).

***Continuous Hydrolysis of 4a. Partial purification of* lipase P**: In analogy to Sugiura et al.[18] a solution/suspension of lipase P-30 (12.5 g) in 10 mM sodium phosphate buffer pH 7.1 (100 mL) at 0 °C (106 mL, 100% activity) was centrifuged for 20 min (30000*g*; 0 °C). To the supernatant (89% activity, readjusted to 100 mL) was added $(NH_4)_2SO_4$ (20.9 g) at 0 °C within 0.5 h (35% saturation). The suspension formed was stirred for 2.5 h at 0 °C and centrifuged (see above, supernatant ~0% activity), and the pellet was redissolved in a minimum amount of 50 mM sodium phosphate buffer pH 7.5 and dialyzed at 4 °C against the same buffer (5 × 1 L) yielding dialyzate (61 mL) containing 90% of the initial activity. According to SDS-PAGE, the purity of the lipase preparation amounted to 84%. **Immobilization of lipase P[19]**: The dialyzate (61 mL, 100% activity) was contacted with Eupergit C beads (15.0 g, Röhm) and the mixture left standing for 50 h at rt. The beadlets were washed with 0.1 M NaCl containing 0.02% NaN_3 (4 × 500 mL, 0.9% activity in the combined washing solutions), partially dried *in vacuo* (25 mbar, ~15 min) to a final weight of 45.0 g (3.1% residual activity), and stored at 4 °C for several months. **Column Experiment**: Through a column (7 mm diameter) filled with immobilized lipase (2.75 g of moist beadlets, corresponding to 0.76 g commercial lipase P-30) were pumped 0.5 M sodium phosphate buffer pH 7.5 (containing 0.02% NaN_3) and **4a** with a flow rate of 6.5 g/h and ca. 0.5 g/h, respectively, corresponding to a substrate concentration of 7% (w/w). The two liquids were mixed just before entering the column. The pH of the eluate was 6.4–6.5. **6a** was released wtih 99% ee at ~50% conversion (GC). **Enzyme Assay**: Enzyme solution (100–200 μL) or beadlets (200 mg) were assayed titrimetrically (0.1 N NaOH) in a vigorously stirred emulsion of tributyrin (250 μL) in 0.1 M NaCl, 4 mM sodium phosphate buffer pH 7.0 (26 mL). Determination of lipase activity was based on the time-dependent consumption of titrating agent in the maximum (linear) range.

[(*R*)-(2,2,4-Trimethyl-1,3-dioxolan-4-yl)]methyl Acetate (7a). Preparation of the Catalyst: 25 g of Siran Carrier (type SIKUG 041/02/120/A, Schott) were added to a solution/suspension of 0.5 g lipase P-30 in 17 mL of twice-distilled water. The wet porous glass beads were briefly agitated and then dried first on a vacuum aspirator (30 °C, no foaming occurred) and finally on a high vacuum aspirator for 5 d (residual water content 0.3% according to KF-determination). **Transesterification**: A suspension of **3a** (5.0 g, 34.2 mmol, free of six-ring acetonide), vinyl acetate (1.74 mL, 19 mmol, 0.55 mol equiv), and 12.5 g catalyst (0.24 g of enzyme) in 50 mL of *n*-hexane was incubated on a roller device and the reaction monitored on calibrated GC. After ~50% conversion (5 h), the catalyst was filtered off, washed with hexane (3 × 50 mL), and dried *in vacuo* for further reuse (seven runs). The filtrate was concentrated and chromatographed (50 g; hexane/EtOAc 9:1) providing 2.0 g (13.7 mmol, 40%) of **8a** (>99% GC; 98% ee at 51% conversion) and 2.52 g (13.3 mmol, 39%) of **7a** at 45% conversion: $[\alpha]_{365}$ = +16.4° (1% in EtOH), $[\alpha]_D$ = +5.8° (1% in EtOH); $[\alpha]_{365}$ = −2.2° (1% in $CHCl_3$); 97% ee; 99% (GC); ^{1}H-NMR ($CDCl_3$) 1.33 (s, 3 H), 1.41 (s, 6 H), 2.10 (s, 3 H), 3.73/3.96 (AB, 2 H, J_{AB} = 8.8 Hz) overlapped by 4.01 (q, 2 H); EI-MS *m/e* 173 (M − CH_3); IR (film) 1747, 1236, 1060 cm^{-1}. Anal. Calcd for $C_9H_{16}O_4$: C, 57.43; H, 8.57. Found: C, 57.48; H, 8.78.

[(*S*)-2,2,4-Trimethyl-1,3-dioxolan-4-yl]methyl p-Toluenesulfonate [(*S*)-9a]: A solution of **8a** (44.8 g, 306 mmol) in pyridine (50 mL) and CH_2Cl_2 (230 mL) was cooled to −20 °C. TsCl (73 g, 383 mmol) was added in portions and the temperature kept at −20 °C for 2 h. The reaction mixture was stirred at rt overnight and washed with 20% (w/v) sodium citrate buffer, pH 2.5 (2 × 500 mL) and saturated NaCl (500 mL). The organic residue was subjected to chromatography (800 g; hexane/EtOAc 9:1) to yield (*S*)-**9a** (78.3 g, 260 mmol, 85%) as amorphous crystals: $[\alpha]_{365}$ = +38.6° (0.7% in $CHCl_3$), $[\alpha]_D$ = +11.7° (0.7% in $CHCl_3$); >99% ee; ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 3 H), 1.31 (s, 3 H), 1.34 (s, 3 H), 2.46 (s, 3 H), 3.65–3.96 (AB overlapped by A'B', 4 H), 7.34–7.81 (~AB, 4 H); EI-MS *m/e* 285 (M − CH_3); IR (film) 1598, 1494, 1367, 1177, 1060, 836, 813 cm^{-1}. Anal. Calcd for $C_{14}H_{20}O_5S$: C, 55.98; H, 6.71; S, 10.67. Found: C, 55.79; H, 6.88; S, 10.45.

[(*R*)-2-Methyl-1,4-dioxaspiro[4.5]dec-2-yl]methyl p-Toluenesulfonate [(*R*)-9b]: To a solution of **6b** (1.67 g, 7.96 mmol) and Et_3N (3 mL) in CH_2Cl_2 (10 mL) was added TsCl (1.63 g, 8.54 mmol) in portions. The reaction mixture, having become solid overnight, was redissolved in CH_2Cl_2 (100 mL) and washed with H_2O (2 × 100 mL) and the organic residue chromatographed (70 g; CH_2Cl_2) to give (*R*)-**9b** (2.33 g, 6.84 mmol, 86%) as a slightly yellowish oil: $[\alpha]_{365}$ = −29.0° (1% in $CHCl_3$); $[\alpha]_D$ = −7.2° (1% in $CHCl_3$); >99% ee; 98% (GC); ^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 3 H) overlapped by 1.2–1.6 (stack, 10 H), 2.45 (s, 3 H), 3.63–3.95 (AB overlapped by A'B', 4 H), 7.34–7.81 (~AB, 4 H); MS *m/e* 340 (M); IR (film) 1598, 1365, 1178, 1096, 812 cm^{-1}. Anal. Calcd for $C_{17}H_{24}O_5S$: C, 59.98; H, 7.11. Found: C, 59.25; H, 7.22.

(*S*)-2,3-Dihydroxy-2-methylpropyl p-Toluenesulfonate [(*S*)-10]: A MeOH solution of (*S*)-**9a** (5.0 g, 16.6 mmol) and

(17) On a silver-foiled, high-vacuum insulated glass column of 1 m length and 3 cm diameter filled with a Sulzer Laborpackung, Type E (Sulzer, Switzerland) and fitted with a vapor-reflux-partitioner pure fractions (>99% GLC, >99% ee) of **5a** and **6a** were obtained: internal communication by M. Zingg and W. Oesch.

TsOH·H_2O (125 mg) was repeatedly evaporated (6 × 50 mL) until no more substrate was present. Chromatography (12 g; hexane/EtOAc 4:1, EtOAc) of the residue afforded (*S*)-**10** (3.95 g, 15.2 mmol, 91%) as white crystals: $[\alpha]_{365}$ = +18.3° (0.9% in $CHCl_3$); >99% ee; ^{1}H-NMR ($CDCl_3$) δ 1.17 (s, 3 H), 2.40 (bs, 2 H), 2.46 (s, 3 H), 3.46/3.59 (AB, 2 H, J_{AB} = 11.4 Hz), 3.91/3.97 (A′B′, 2 H, J_{AB} = 9.9 Hz), 7.35–7.81 (~AB, 4 H); EI-MS *m/e* 261 ($M + H^+$); IR (KBr) 1597, 1361, 1174, 976, 844 cm^{-1}. Anal. Calcd for $C_{11}H_{16}O_5S$: C, 50.76; H, 6.20; S, 12.32. Found: C, 50.56; H, 6.12; S, 12.13.

(*R*)-2,3-Dihydroxy-2-methylpropyl *p*-Toluenesulfonate [(*R*)-10]: (*R*)-**9b** (2.22 g, 6.52 mmol) and TsOH·H_2O (200 mg) were dissolved in MeOH (100 mL) and the solvent evaporated at 16 mbar. This was repeated several times until the substrate had disappeared (TLC). The residue was chromatographed (55 g; CH_2Cl_2/Et_2O 2:1) yielding (*R*)-**10** (829 mg, 3.18 mmol, 49%): $[\alpha]_{365}$ = −19.9° (1% in $CHCl_3$); $[\alpha]_D$ = −5.8° (1% in $CHCl_3$); >99% ee; 98% (GC).

(*R*)-α-Methyl-2-oxiranemethanol [(*R*)-11]: (*R*)-**10** (816 mg; 3.13 mmol) was dissolved in anhydrous Et_2O (50 mL), and *t*-BuOK (1.08 g, 9.68 mmol) was added in portions. After stirring for 3 h, twice-distilled H_2O (100 μL, 5.55 mmol) was added. After stirring for 0.5 h, precipitated KOH and TsOK was filtered off and the filtrate cautiously concentrated *in vacuo*. The concentrate (510 mg) was subjected to chromatography (20 g; pentane/Et_2O 3:2 → 1:3) to yield 72 mg of crude (*R*)-**11** with >99% ee (still containing Et_2O and a *t*-Bu component) as judged by ^{1}H-NMR ($CDCl_3$; δ 1.30 (s, CH_3), 2.64/2.78 (AB, $J_{AB} \approx 5$ Hz), 3.55/3.67 (A′B′, $J_{A'B'} \approx 11$ Hz)) and confirmed by GC analysis in the presence of a reference compound (see below).

For chemical reference and determination of the absolute configuration, racemic and chiral **11** were prepared from racemic and chiral 3-chloro-2-methyl-1,2-propanediol **12**, respectively, as follows: **12** (250 mg) was dissolved in Et_2O (25 mL) and KOH (4 pellets) was added. After 2 h stirring, the solution was distilled and the distillate (dry ice cooling trap) cautiously concentrated *in vacuo* for direct GC.

Determination of the Enantiomeric Excess: The enantiomeric excess of **6b** and **8b** was determined by directly injecting them on a chiral-phase GC capillary column (26 m, permethylated β-cyclodextrin, 120–155 °C with 1 °C/min). **6a** and **8a** were benzoylated prior to application on the same column (130–200 °C with 1 °C/min). (*R*)- and (*S*)-**11** were directly applied also on the same column (90–120 °C with 1 °C/min). The enantiomeric excess of the chiral esters **5a,b** and **7a** was determined via hydrolysis to their corresponding chiral alcohols, and that of the tosyl compounds **9a,b** and **10** by converting them to **11**.

Acknowledgment. We are grateful for the skilful technical assistance of Franzisco Vazquez, Eveline Schumacher, and Patrik Stocker. We also wish to express our cordial thanks to our colleagues from the Analytical Services for running our samples, especially to Willy Walther for carrying out numerous ee determinations. Furthermore, we are indepted to Urs Ettlin for running an SDS-PAGE, to Jeannot Schelcher for measuring tungsten, and to Dr. R.K. Müller for a generous gift of substrate **4a**.