for 1 H_8 and 1 H_9), 7.3-8.5 (H_7 and 22 aromatic protons). Mass spectrum (12 eV): m/z (relative intensity): 629 (24), 507 (7), 105 (100).

(±)-7β,8α,9α-Tris(benzoyloxy)-10β-amino-7,8,9,10-tetrahydrobenzo[a]pyrene (27). In a 25-mL two-necked round-bottomed flask were placed the BaP azido tribenzoate **26** (31.8 mg, 0.048 mmol) and Lindlar catalyst (35 mg), in absolute EtOH (3 mL). The mixture was stirred under 1 atm of hydrogen for 24 h. Due to limited solubility of **26** in EtOH, the reaction progressed very slowly. Therefore, dry THF (3 mL) was added, and the stirring was continued as before for 48 h. At this point, TLC still showed presence of starting material. Therefore, an additional 15 mg of catalyst was added, and the reaction was continued for 24 h at the end of which no starting azide was visualized by TLC. The reaction mixture was filtered through anhydrous $MgSO_4$, and the residue was washed with EtOH. The combined filtrate was evaporated under reduced pressure. Chromatography on silica gel using 5% MeOH in $CHCl_3$ afforded 22 mg (77%) of pure amino tribenzoate **27**. NMR spectrum: 5.46 (d, 1 H_{10}, J = 3.0), 6.1 (br s, 1 H_9), 6.57 (d, 1 H_8, J = 10.7), 7.3-8.5 (H_7 and 22 aromatic protons), 8.45 (d, 1 H_{11}, J = 9.3). Mass spectrum (12 eV): m/z (relative intensity) 387 (29), 105 (100).

(±)-7β,8α,9α-Trihydroxy-10β-amino-7,8,9,10-tetrahydrobenzo[a]pyrene (28). The BaP azido triol **25** (7.7 mg, 0.022 mmol), Lindlar catalyst (50 mg), and 1:1 EtOH/THF (4 mL) were placed in a two-necked 25-mL round-bottomed flask. The mixture was stirred under 1 atm of hydrogen pressure for 24 h. The mixture was filtered through anhydrous Na_2SO_4, and the filtrate was evaporated under reduced pressure. Chromatography on a 200 μm C-18 reverse-phase preparative TLC plate using 15% water in MeOH gave 4 mg (57%) of fairly pure amine **28**. NMR spectrum (acetone-d_6): 4.38 (dd, 1 H_8, J = 8.4, 2.1), 4.5 (m, 1 H_9), 4.74 (d, 1 H_{10}, J = 3.0), 5.14 (d, 1 H_7, J = 8.4), 8.0-8.4 (7 H, aromatic), 8.55 (s, 1 H_6). In CD_3OD the aliphatic protons have identical chemical shifts as in acetone-d_6: the aromatic H_6 is a singlet at 8.5 ppm and the remaining aromatic protons appear at 8.0-8.3 ppm. Mass spectrum (12 eV): m/z (relative intensity) 319 (M^+, 0.4), 303 (4.1), 302 (13.7), 286 (8.1), 284 (100), 268 (3.5).

Enzymes in Organic Synthesis: Synthesis of Highly Enantiomerically Pure 1,2-Epoxy Aldehydes, Epoxy Alcohols, Thiirane, Aziridine, and Glyceraldehyde 3-Phosphate

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This paper describes a chemoenzymatic procedure for the synthesis of (*R*)- and (*S*)-glycidaldehyde diethyl acetal [2-(diethoxymethyl)oxirane] (**4** and **5**). 2-Acetoxy-3-chloropropanal diethyl acetal (**1c**) was enantioselectively hydrolyzed by LP-80 lipase to give (*S*)-3-chloro-2-hydroxypropanal diethyl acetal (**2c**) and the unreacted acetate (**3c**), both in >95% calculated yield and >98% ee. Both products were subsequently converted to epoxides **4** and **5**, respectively. Resolutions of 2-acetoxy-1-(benzyloxy)-3-chloropropane (**11a**) and 3-(allyloxy)-2-acetoxypropyl *p*-toluenesulfonate (**14b**) were similarly carried out to give the corresponding optically active 2-hydroxy and 2-acetoxy derivatives in 90% and >95% ee. These products were subsequently converted to the corresponding 1,2-epoxides. Nucleophilic opening of epoxide **4** was exemplified by the syntheses of (*R*)-3-azido-2-hydroxypropanal and D-glyceraldehyde 3-phosphate. Conversion of the chiral epoxides to thiirane and aziridine was also described.

Optically active 1,2-epoxides are useful building blocks in organic synthesis. Asymmetric epoxidation of various allylic alcohols based on the Sharpless procedure[1] has been widely used for synthesis of this type of compounds. Resolution of epoxy alcohols[2] and epoxy acids[3] catalyzed by esterases, and asymmetric epoxidation of olefins catalyzed by monooxygenases,[4] are useful alternatives complementary to the chemical approach. In this paper, we describe a simple and practical chemoenzymatic route to highly enantiomerically pure epoxy aldehydes and epoxy alcohols and the use of these epoxides for the synthesis of other enantiomerically pure, yet not readily available, compounds including (diethoxymethyl)thiirane, (diethoxymethyl)aziridine and D-glyceraldehyde 3-phosphate.

Our previous success in the LP-80 lipase catalyzed resolution of 2-acetoxy-3-azidopropanal diethyl acetal (**1a**) for use in enzymatic aldol condensations[5] led us to exploit the possible synthesis of various 3-substituted 2-hydroxy

Table I. LP-80 Lipase Catalyzed Hydrolysis of 1 and 14 at pH 7.0

substrate	% convn	product, ee[a]	*E* value[b]
1a	50	**2a**, >98% **3a**, >98%	>100
1b	50	**2b**, >98% **3b**, >98%	>100
1c	50	**2c**, >98% **3c**, >98%	>100
1d	50	**2d**, 50% **3d**, 50%	4
14a	45	**15a**, 70% **16a**, 57%	10
14b	51	**15b**, 90% **16b**, 94%	60

[a] Determined with ^1H NMR (300 MHz) by measuring the shift of acetoxy group in the presence of Eu(hfc)$_3$. [b] Enantioselectivity value. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *Am. Chem. Soc.* **1982**, *104*, 7294.

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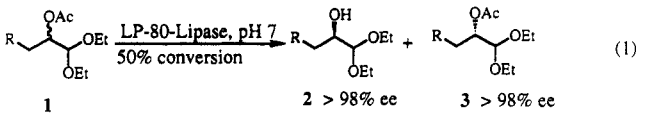
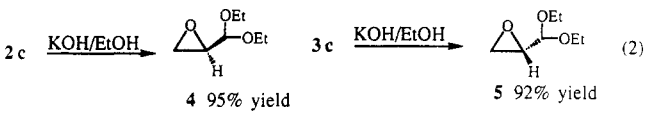
aldehydes. During the course of our investigation, we found that at 50% conversion, both 3-fluoro (**1b**) and 3-chloro (**1c**) derivatives of compound **1** were resolved with LP-80 lipase (in 0.05 N phosphate, pH 7.0) to give **2b**, **3b** and **2c**, **3c** all in >98% ee and >92% calculated yield.



(1)

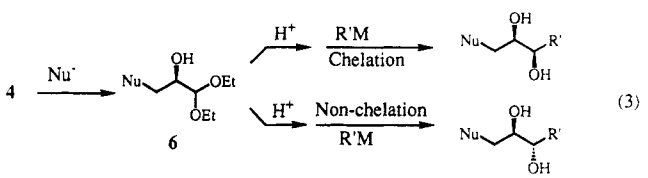
a, R = N_3; b, R = F; c, R = Cl; d, R = $C_6H_5CH_2O$-

With compounds **2c** and **3c** readily available, the epoxides **4** and **5** were prepared in 95% yield via a straightforward procedure (eq 2). Compounds **4** and **5** are



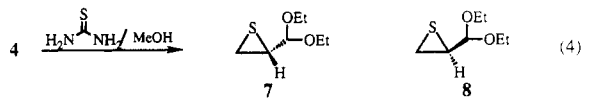
(2)

versatile synthons for various organic syntheses. Nucleophilic opening of epoxide **4**, for example, would provide optically active 2-hydroxy aldehyde **6**, which is useful for synthesis of vic-diol systems via chelation[6] or nonchelation[7] controlled addition to the carbonyl group (eq 3). The

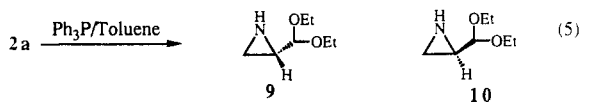


(3)

absolute stereochemistry of epoxides **4** and **5** (and thus of **2** and **3**) were established via ring opening with azide[5] to give the corresponding 3-azido-2-hydroxypropanal diethyl acetal, the absolute configuration of which was determined previously.[5] The procedures employed for epoxide formation and opening had no effect on the chirality of the stereogenic center, and no epimerization was observed. Compound **4** was then converted to (*S*)-thiirane **7** and compound **2a** was converted to (*S*)-aziridine **9** with thiourea (in 99% yield) and triphenylphosphine (in 37% yield) respectively (eqs 4 and 5). Similarly, the enantiomers **8** and **10** could be prepared from **5** and deacylated **3a**.



(4)



(5)

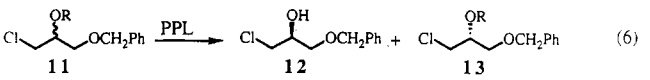
The high enantioselectivity of LP-80 lipase reaction encouraged us to test other secondary alcohols that might lead to useful epoxides. Compound **11a** was subject to the lipase-catalyzed hydrolysis. At 60% conversion, the unreacted substrate was isolated with 63% ee as determined by HPLC equipped with a Chiracel OB column. The stereochemistry of this product was determined[8] to have

(6) Reetz, M. T. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 556.
(7) Braun, M.; Mahler, H. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 896. The compound was converted to an epoxide which has the configuration as shown in **17**.
(8) Miyano, S.; Lu, L. P. L.; Viti, S. M.; Sharpless, B. K. *J. Org. Chem.* **1985**, *50*, 4350.

Table II. LP-80 Lipase and Pancreatic Lipase (PPL) Catalyzed Hydrolysis of 11

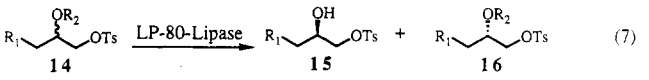
substrate	enzyme	% convn	product, ee[a]	*E*
11a	LP-80	60	**12**, - **13a**, 63%	4.5
	PPL	25	**12**, 92% **13a**, -	33
	PPL	60	**12**, 75% **13a**, >98%	33

the *R* configuration as shown in **13**. At this stage, several lipases were tested in order to improve the enantioselectivity. Of the enzymes tested, porcine pancreatic lipase (PPL) was the best (eq 6). As shown in Table II, both **12** and **13a** can be prepared in 92–98% ee when the reaction was stopped at 25% and 60% conversion, respectively.



(6)

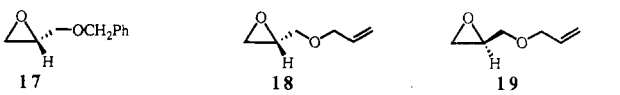
a, R = CH_3CO-; b, R = H



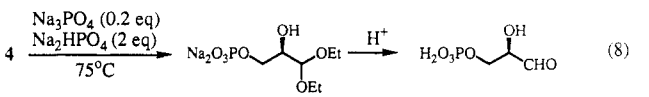
(7)

a, R_1 = H; R_2 = CH_3CO-
b, R_1 = CH_2=$CHCH_2O$-; R_2 = CH_3CO-

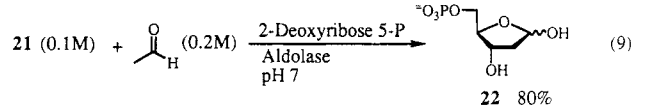
Conversion of **13a** to epoxide **17** and **12** to its enantiomer was carried out as described above. Similarly, compound **14**, particularly **14b**, was resolved with LP-lipase to give **15** and **16** with high enantioselectivity (eq 7). Both **15b** and **16b** prepared in high ee (90 and 94% ee, respectively) were converted to epoxides **18** and **19**, respectively.



Equation 8 illustrates the use of **4** for the synthesis of D-glyceraldehyde 3-phosphate[9] **21**, a valuable biochemical currently not readily available, and eq 9 illustrates the use of **21** for the synthesis of 2-deoxyribose 5-phosphate catalyzed by 2-deoxyribose 5-phosphate aldolase, a key intermediate for the synthesis of deoxy nucleosides and phosphates.



(8)



(9)

In summary, this paper describes a simple and practical enzymatic approach to the preparaton of both *R* and *S* enantiomers of glycidaldehyde diethyl acetal and 3-(benzyloxy)- and 3-(allyloxy)-1,2-epoxypropane useful for organic synthesis. The starting materials and enzymes used for the syntheses are relatively inexpensive, and the procedures described are applicable to large-scale processes. These chiral synthons should find use in numerous organic reactions.

Experimental Section

Enantiomer excesses were determined using Eu(hfc)$_3$ chiral shift reagent or by chiral HPLC analysis with a Daicel Chiralcel OB

(9) Ballou, C. E.; MacDonald, D. C. *Methods Carbohydr. Chem.* **1963**, *2*, 289.

column. GC analyses were performed with a dimethylsilicone column (5 m × 0.53 mm) using the following conditions: 40 °C, 3 min to 250 °C at 10 °C/min. PPL was purchased from Sigma, and LP-80 lipase was purchased from Amano.

2-Acetoxy-3-chloropropanal Diethyl Acetal (1c). To a 1-L round-bottomed flask containing 500 mL of dry CH_2Cl_2 was added 100 g (85% pure, 255 mmol) of Ph_3PCl_2,[10] and the solution was cooled to 0 °C under N_2. Glycidaldehyde diethyl acetal[11] (37.28 g, 255 mmol) in 40 mL of CH_2Cl_2 was added dropwise, and the mixture was stirred overnight. The solution was added to 500 mL of ice/water containing 42.0 g (500 mmol) of $NaHCO_3$, and the mixture was stirred for 90 min. The aqueous layer was separated, saturated with NaCl, and extracted with 2 × 200 mL of CH_2Cl_2. The organic fractions were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to 200 mL. Pyridine (31.64 g, 400 mmol) was added to the mixture at 0 °C under N_2. Acetic anhydride (30.62 g, 300 mmol) was then added dropwise, and the mixture was stirred for 12 h. The reaction mixture was poured into 400 mL of ice/water and stirred for 30 min. The solution was extracted with 3 × 200 mL of CH_2Cl_2. The organic fractions were combined and washed with 200 mL each of 2 N HCl, $NaHCO_3$ (saturated), and H_2O. The organic fraction was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The liquid was distilled to yield 47.9 g (213 mmol, 84%) of **1c**: $bp_{0.9-1.0}$ 76–80 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 1.2 (2 t, 6 H, 2 CH_3), 2.1 (s, 3 H, CH_3CO), 3.4–3.8 (m, 6 H, 2 CH_2O, $ClCH_2$), 4.5 (d, 1 H, anomeric), 5.1 (m, 1 H, CHOAc). Anal. Calcd for $C_9H_{17}O_4Cl$: C, 48.00; H, 7.56. Found: C, 48.01; H, 7.60.

Immobilization of LP-80 Lipase. The procedure was the same as reported earlier,[5] except with the following modifications. In a 50-mL Erlenmeyer flask with a magnetic stirbar were added 10 g of XAD-8 resin, 25 mL of 0.05 N phosphate buffer at pH 7.0, and 150 mg of LP-80 lipase. The mixture was stirred slowly for 36 h at 4 °C. The resin was filtered and rinsed with 100 mL of water and 50 mL ether. The immobilized enzyme was used without further purification.

Kinetic Resolution of 1c. To a 500-mL Erlenmeyer flask were added the immobilized enzyme, 250 mL of 0.05 N phosphate buffer at pH 7.0, and 14.81 g (65.9 mmol) of **1c**. The solution was stirred with an overhead stirrer, with the pH maintained at 7.0 by addition of 1.0 N NaOH via a peristaltic pump. After 19.5 h, the reaction consumed 33.0 mL of base. The resin was filtered and washed with 3 × 100 mL of $CHCl_3$. The aqueous phase was saturated with NaCl and extracted with 2 × 200 mL of $CHCl_3$. The chloroform layers were combined and dried over anhydrous Na_2SO_4. After the solvent was removed under reduced pressure, the products were separated by column chromatography (hexane → 9:1 hexane/ether → 7:1 hexane/ether) to yield 7.18 g (32.0 mmol, 48.5% yield) of (*R*)-2-acetoxy-3-chloropropanal diethyl acetal (**3c**), $[\alpha]^{25}_D$ = −19.3° (*c* = 1.04, $CHCl_3$), and 5.49 g (30.1 mmol, 46% yield) of (*S*)-3-chloro-2-hydroxypropanal diethyl acetal (**2c**), $[\alpha]^{25}_D$ = +23.6° (*c* = 1.06, $CHCl_3$). Both products were used subsequently without further characterization.

(*R*)-Glycidaldehyde Diethyl Acetal (4). To a 250-mL round-bottomed flask containing 100 mL of anhydrous ethanol were added 5.49 g (30.1 mmol) of **2c** and 1.74 g (32 mmol) of KOH pellets. The mixture was stirred at 0 °C for 3 h. GC analysis showed the reaction was >99% complete. The solution was filtered through a pad of Celite, and the ethanol was removed under reduced pressure. The product was run down a short column of silica gel, using hexane as the mobile phase, to yield 4.17 g (28.5 mmol, 95%) of **4** as a liquid, $[\alpha]^{25}_D$ = +5.3° (*c* = 1.06, ethanol). ^1H and ^{13}C NMR data were consistent with the reported values.[11]

(*S*)-Glycidaldehyde Diethyl Acetal (5). To 100 mL of anhydrous ethanol were added 7.18 g (32 mmol) of **3c** and 1.83 g (33 mmol) of KOH pellets. The reaction was stirred at 0 °C for 3 h. The solution was worked up as described above to yield 4.30 g (29.4 mmol, 92%) of **5** as a liquid: $[\alpha]^{25}_D$ = −5.4° (*c* = 1.04, ethanol). ^1H and ^{13}C NMR data were consistent with the reported values.[11]

2-Acetoxy-3-fluoropropanal Diethyl Acetal (1b). To 8.2 g (49.5 mmol) of 3-fluoro-2-hydroxypropanal diethyl acetal[11] in 20 mL of dry CH_2Cl_2 was added 8 mL (100 mmol) of pyridine, and the solution was cooled to 0 °C under N_2. Acetic anhydride (7.1 g, 75 mmol) was added dropwise, and the solution was stirred overnight at room temperature. The solution was poured into 200 mL of ice/water, and the mixture was stirred for 30 min. This mixture was extracted with 2 × 200 mL of ether. The ether fractions were combined and washed with 100 mL each of 1 N HCl (twice), $NaHCO_3$ (saturated), and H_2O. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield 10.1 g (48.3 mmol, 98% yield) of **1b** as a liquid: ^1H NMR (200 MHz, $CDCl_3$) δ 1.1–1.4 (overlapping triplets, 6 H, 2 CH_3), 2.1 (s, 3 H, CH_3CO), 3.4–3.8 (m, 4 H, 2 OCH_2), 4.6 (dd, 2 H, CFH_2, $^1J_{HF}$ = 48 Hz, $^2J_{HF}$ = 22 Hz), 4.6 (dd, 1 H, anomeric, J_{HH} = 7.0 Hz, J_{HH} = 2.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.23, 15.08 (2 CH_3), 20.87 (CH_3CO), 64.07, 63.01 (2 OCH_2), 72.08 (CHOAc, $^2J_{CF}$ = 17.9 Hz), 83.45 (CFH_2, $^1J_{CF}$ = 169 Hz), 99.92 (anomeric, $^3J_{CF}$ = 8.4 Hz), 170.10 (OCOMe). Anal. Calcd for $C_9H_{17}O_4F$: C, 60.00; H, 9.44. Found: C, 60.08; H, 9.20.

Resolution of 1b. This was done with the same procedure as described above, except that 100 mg of lipase immobilized in XAD-8 and 10 mmol of **1b** were used. The reaction was stopped after 5 mL of 1 N NaOH was consumed. The mixture was worked up and chromatographed as described above to yield 0.96 g (4.6 mmol, 46%) of (*R*)-2-acetoxy-3-fluoropropanal diethyl acetal (**3b**), $[\alpha]^{25}_D$ = −46.1° (*c* = 2.0, $CHCl_3$) (the NMR data are the same as **1b**), and 0.664 g (4.1 mmol, 41%) of (*S*)-3-fluoro-2-hydroxypropanal diethyl acetal (**2b**), $[\alpha]^{25}_D$ = +23.0° (*c* = 2.0 $CHCl_3$), both as liquids.

(*R*)-3-Fluoro-2-hydroxypropanal Diethyl Acetal [(*R*)-2b]. To 0.960 g (4.6 mmol) of **3b** in 10 mL of anhydrous ethanol was added 16 mg of (60%, 0.4 mmol) NaH. The reaction was stirred at room temperature for 6 h. GC analysis indicated two peaks, (*S*)-glycidaldehyde diethyl acetal with t_R 3.40 min and 3-fluoro-2-hydroxypropanal diethyl acetal with t_R 4.2 min. The solution was filtered, and the solvent was removed under reduced pressure. The product was chromatographed as described above to produce 532 mg (3.2 mmol, 70%) of (*R*)-**2b**, $[\alpha]^{25}_D$ = −23.0° (*c* = 2.0, $CHCl_3$), and 146.1 mg (1.0 mmol, 23%) of **5**, $[\alpha]^{25}_D$ = −5.4° (*c* = 2.0, $CHCl_3$), both as liquids.

(*S*)-Epithioglycidaldehyde Diethyl Acetal [(*S*)-2-(Diethoxymethyl)thiirane] (7). To a 100-mL round-bottomed flask containing 25 mL of anhydrous methanol were added 1.46 g (10 mmol) of **4** and 0.76 g (10 mmol) of thiourea[12] (recrystallized from ethanol) were added, and the mixture was stirred at room temperature under N_2 for 3 days. GC analysis indicated >99% product formed, t_R 5.30 min. The ethanol was removed under reduced pressure, and the remaining residue was run down a short column of silica gel, with hexane as the mobile phase, to yield 1.62 g (99.8%) of **7**, $[\alpha]^{25}_D$ = +9.6° (*c* = 2.07, $CDCl_3$), as a liquid: ^1H NMR (200 MHz, $CDCl_3$) δ 1.14, 1.15 (2 t, 6 H, 2 CH_3), 2.30 (ddd, 2 H, SCH_2), 3.0 (q, 1 H, SCH), 3.4–3.7 (m, 4 H, 2 OCH_2), 4.0 (d, 1 H, anomeric); ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.19 (2 CH_3), 21.01 (SCH_2), 34.26 (SCH), 62.25 (2 OCH_2), 105.29 (anomeric). Anal. Calcd for $C_7H_{14}SO_2$: C, 51.85; H, 8.64. Found: C, 51.80; H, 8.66.

(*S*)-Aziridinoglycidaldehyde Diethyl Acetal [(*S*)-2-(Diethoxymethyl)aziridine] (9). To a 50-mL round-bottomed flask containing 20 mL of anhydrous toluene and 1.0 g (5.3 mmol) **2a**[13] was added 2.62 g (10 mmol) triphenylphosphine.[14] The solution was stirred at room temperature until bubbling of N_2 stopped. The mixture was then heated to 120 °C for 3 h. The solvent was removed under reduced pressure, and the remaining residue was distilled under reduced pressure to yield 0.290 g (2.0 mmol, 37%) of **9**: $[\alpha]^{25}_D$ = +4.89° (*c* = 0.47, THF); $bp_{3.3}$ 61–62 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 0.51 (br s, 1 H, NH), 1.15 (2 t, 6 H, 2 CH_3), 1.55 (dd, 2 H, NCH_2), 2.14 (q, 1 H, NCH), 3.4–3.7 (m, 4 H, 2 OCH_2), 4.31 (d, 1 H, anomeric); ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.18 (2 CH_3), 21.39 (NCH_2), 31.50 (NCH), 61.96, 61.59 (2 OCH_2), 101.45

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(anomeric). Anal. Calcd for $C_7H_{15}NO_2$: C, 57.98; H, 10.43; N, 9.66. Found: C, 59.16; H, 10.21; N, 9.52.

Determination of Enantiomeric Excess and Absolute Stereochemistry. By HPLC. HPLC equipped with a Chiralcel OB column (0.46 × 25 cm) and a UV detector (254 nm) was used. The mobile phase used was hexane/2-propanol (9:1 v/v), and the flow rate was 0.5 mL/min. Compound **12** obtained after 25% conversion with pancreatic lipase was acetylated with acetic anhydride/pyridine, and the acetylated product was injected into the column. The peak at retention time 24 min was the *S* enantiomer and that at 21.5 min was the *R* enantiomer. Based on the relative intensities, the product was established to have 92% ee. Compound **13** obtained after 60% conversion with the same enzyme was analyzed. It was determined to be >98% ee.

By NMR. (i) 18 mg of **1c** was added to an NMR tube and diluted with 1.0 mL of $CDCl_3$. Tris[3-(heptafluoropropyl-hydroxymethylene)-(+)-camphorato]europium(III) derivative [Eu(hfc)$_3$] was added until the acetyl peaks at 3.18 and 3.23 ppm were well separated. Integration of these peaks yields a 1:1 ratio. (ii) Compound **3c** recovered from lipase-catalyzed resolution was mixed with Eu(hfc)$_3$ as described and determined to have >98% ee. (iii) Compound **2c** obtained from the lipase reaction of **1c** was acetylated with acetic anhydride/pyridine. The acetylated product (15 mg) was mixed with the shift reagent as described and was determined to have >98% ee.

Absolute Stereochemistry of 2c. Compound **2c**, $[\alpha]^{25}_D$ = +23.6° (*c* = 1.06, $CHCl_3$), obtained from the lipase resolution of **1c** was converted to **4**, $[\alpha]^{25}_D$ = +5.4° (*c* = 1.06, ethanol), as stated earlier. Compound **4** was further converted[5] to 3-azido-2-hydroxypropanal diethyl acetal, $[\alpha]^{25}_D$ = +45.5° (*c* = 1.5, $CHCl_3$), which has been proven to have the *R* configuration.[5] Therefore, compound **2c** has *S* stereochemistry and **4** has *R* stereochemistry.

To determine the absolute stereochemistry of **3b**, $[\alpha]^{25}_D$ = −46.1° (*c* = 2, $CHCl_3$), it was deacetylated as described above. During the deacetylation, a small portion of **3b** was converted to glycidaldehyde diethyl acetal, $[\alpha]^{25}_D$ = −5.4° (*c* = 2.0, $CHCl_3$). This product had the opposite rotation of (*R*)-glycidaldehyde diethyl acetal; therefore, the absolute stereochemistry of **3b** is *R* and that of **2b** is *S*.

1-(Benzyloxy)-3-chloro-2-propanol (11b). To a 250-mL flask was added 54.0 g (0.5 mol) of benzyl alcohol, and the mixture was heated to 90 °C under a nitrogen atmosphere. Tin tetrachloride (250 mg) was added followed by a dropwise addition of 23.1 g of epichlorohydrin. The mixture was heated at 90–100 °C for 8 h until the reaction was complete according to GC analysis of epichlorohydrin. Vacuum distillation ($bp_{1.5-2.0}$ = 85 °C) yielded 42.1 g of **11b** as a colorless liquid (84% yield) (lit.[15] bp_2 = 126–127 °C).

2-Acetoxy-1-(benzyloxy)-3-chloropropane (11a). To 33.0 g (165 mmol) of **11b** in 50 mL ofdry pyridine, cooled to 0 °C, was added dropwise 25 mL of acetic anhydride. The solution was allowed to warm to room temperature and stirred for 5 h until TLC showed complete consumption of the chlorohydrin. The mixture was extracted with ether. The organic phase was washed with 1.0 N HCl, saturated sodium bicarbonate, and distilled water and dried over sodium sulfate. Evaporation of the ether yielded 39.85 g of **11a** as a clear liquid (99.7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (s, 3 H, OAc), 3.6–3.8 (m, 4 H), 4.55 (d, 2 H, CH_2Ph), 5.17 (t, 1 H), 7.25–7.4 (s, 5 H, arom). Anal. Calcd for $C_{11}H_{13}O_3Cl$: C, 57.77; H, 5.69. Found: C, 57.80; H, 5.50.

Lipase-Catalyzed Resolution of 11a. To a solution of **11a** (2.43 g, 10 mmol) in 0.05 M phosphate buffer (pH 7.0) was added 10 mg of LP-80 lipase. With stirring, the reaction mixture was maintained at pH 7 by addition of 0.1 N NaOH solution. After addition of 6 mmol of base (60% conversion) the reaction was stopped by extraction of the starting material and hydrolysis product into ether. The organic phase was washed with saturated sodium bicarbonate and dried over sodium sulfate. After evaporation of the solvent, the acetate and the alcohol were separated by silica gel column chromatography (hexane/ether, 9:1 to 4:1), $[\alpha]^{25}_D$ for the acetate = −4.2° (*c* = 2.1, $CHCl_3$). The two acetate isomers were separated by HPLC with a chiral column (Chiracel OB). The enantiomeric excess for the unreacted substrate was calculated to be 63%. Treatment of the acetate with KOH as described above yielded the epoxide. The major isomer remaining was determined to be the *R* isomer based on rotation.[8] Replacement of the enzyme with crude porcine pancreatic lipase (PPL, 500 mg) gave the unreacted *R* substrate in >98% ee at 60% conversion, which was treated with KOH to yield compound **17**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.73 (ddd, 2 H), 3.19 (m, 1 H), 3.45 (dd, 1 H), 3.78 (dd, 1 H), 4.6 (d, 2 H), 7.25–7.4 (m, 5 H); $[\alpha]_D$ +14.0° (*c* = 1, EtOH). The NMR data were consistent with the reported values.[8]

Preparation of 2-Acetoxypropyl *p*-Toluenesulfonate (14a). To a solution of dry pyridine (30 mL) and 1,2-propanediol (1.85 g, 24.3 mmol) was added *p*-toluenesulfonyl chloride (5 g, 26.3 mmol). The solution was stirred at room temperature for 4 h, and then ice water was added to stop the reaction. The mixture was extracted with diethyl ether (3 × 40 mL), and the extracts were washed with $NaHCO_3$(saturated) (1 × 40 mL), 2 N HCl (3 × 40 mL) and dried with Na_2SO_4. Evaporation of the solvent yielded the tosylate. The crude tosylate was treated with dry pyridine (30 mL) and acetic anhydride (3.5 mL) at room temperature. After 6 h, ice water was added to quench the reaction. The solution was extracted with diethyl ether (3 × 40 mL), and the extracts were washed with 2 N HCl (3 × 40 mL) and $NaHCO_3$(saturated) (1 × 40 mL). Evaporation of the solvent yielded 5.1 g (18.8 mmol, overall yield 77.4%) of **14a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.24 (3 H, d, *J* = 6.4), 1.98 (3 H, s), 2.45 (3 H, s), 4.03 (2 H, d, *J* = 5.2) 4.83–5.18 (1 H, m), 7.34 (2 H, d, *J* = 8.2), 7.78 (2 H, d, *J* = 8.2). Anal. Calcd for $C_{13}H_{18}O_5S$: C, 54.55; H, 6.30. Found: C, 54.50; H, 6.01.

Preparation of 3-(Allyloxy)-2-acetoxypropyl *p*-Toluenesulfonate (14b). To a solution of dry pyridine (35 mL) and 3-(allyloxy)-1,2-propanediol (4.83 g, 36.5 mmol) was added *p*-toluenesulfonyl chloride (7.5 g, 39.5 mmol), and the mixture was stirred at room temperature for 5 h. The reaction was stopped and worked up as previously described. The tosylate obtained was added to dry pyridine (35 mL) containing acetic anhydride (5.2 mL), and the mixture was stirred at room temperature for 6 h. The product was worked up as described previously to give 3-(allyloxy)-2-acetoxypropyl *p*-toluenesulfonate (**14b**) (9.07 g, 27.5 mmol, overall yield 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01 (3 H, s), 2.47 (3 H, s), 3.51 (2 H d, *J* = 5.3), 3.92 (2 H, d, *J* = 5.2), 4.19 (2 H, m), 5.15–5.45 (2 H, m), 5.65–5.85 (1 H, m), 7.35 (2 H, d, *J* = 8.1), 7.77 (2 H, d, *J* = 8.1). Anal. Calcd for $C_{15}H_{20}O_6S$: C, 54.88; H, 6.10. Found: C, 54.55; H, 6.20.

LP-80 Lipase Catalyzed Hydrolysis of 2-Acetoxypropyl *p*-Toluenesulfonate (14a). A solution of **14a** (563 mg, 2.07 mmol) in 15 mL of 0.05 N phosphate buffer, pH 7, was mixed with 40 mg of LP-80 lipase at room temperature with stirring. The reaction was stopped after 30 min when the reaction reached 45% conversion based on the consumption of base. The reaction mixture was worked up as described earlier. The products were separated by column chromatography (ethyl acetate–*n*-hexane = 1:3 v/v) on silica gel to give 285 mg of (*S*)-**16a**: $[\alpha]^{23}_D$ = −7.45° (*c* = 1.88, $CHCl_3$); 57% ee. The alcohol product (*R*)-**15a** was obtained in 150 mg: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (3 H, d, *J* = 6), 2.45 (3 H, s), 3.70–4.18 (3 H, m), 7.34 (2 H, d, *J* = 8.4); $[\alpha]^{23}_D$ = −8.34° (*c* = 1.2, $CHCl_3$); 70% ee. The stereochemistry and ee of **16a** and **15a** were determined by comparing with literature value[16] ((*S*)-**16a**, $[\alpha]^{23}_D$ = −13.0° (*c* = 2, $CHCl_3$), >99% ee; (*R*)-**15a**, $[\alpha]^{23}_D$ = −12.0° (*c* = 2, $CHCl_3$), >99% ee).

LP-80 Lipase Catalyzed Hydrolysis of 3-(Allyloxy)-2-acetoxypropyl *p*-Toluenesulfonate (14b). A solution of **14b** (1.26 g, 3.84 mmol) in 20 mL of 0.05 N phosphate buffer, pH 7, was mixed with LP-80 (50 mg) at room temperature with stirring. After 3.5 h, the conversion of reaction was determined to be 51%, and the reaction was stopped. The mixture was worked up as described earlier. The products were separated by column chromatography (ethyl acetate–*n*-hexane = 1:5 v/v) on silica gel to give 514 mg of (+)-**16b**: $[\alpha]^{23}_D$ = +1.86° (*c* = 2.69, $CHCl_3$). The ee was determined to be 94% in the presence of Eu(hfc)$_3$. The relative intensities of the acetyl groups at 3.45 and 3.59 ppm were determined to establish the enantiomeric excess. The alcohol (−)-**15b** was obtained in 449 mg: $[\alpha]^{23}_D$ = −4.93° (*c* = 2.23, $CHCl_3$);

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^{1}H NMR (300 MHz, $CDCl_3$) δ 2.47 (3 H, m), 3.51 (2 H, d, J = 5.3), 3.92–4.04 (3 H, m), 4.19 (2 H, m), 5.15–5.45 (2 H, m), 7.34 (2 H, d, J = 8.2), 7.80 (2 H, d, J = 8.2). The ee of compound (−)-**15b** was determined to be 90% by comparing the optical rotation of the acetylated product with (+)-**16b**.

Preparation of (+)-3-(Allyloxy)-1,2-epoxypropane (18) from (−)-15b. To a solution of (−)-**15b** (294 mg, 1.03 mmol) in dry THF (6 mL) was added sodium hydride (72 mg) at 0 °C over a period of 10 min, and the mixture was stirred at room temperature for 30 min. Ice water was added, and the mixture was extracted with ethyl acetate. After removing the solvent under reduced pressure, the product was purified by column chromatography (ethyl acetate–n-hexane = 1:4 v/v) on silica gel to give 3-(allyloxy)-1,2-epoxypropane (**18**) (110 mg, 94% yield): $[\alpha]^{23}_D$ = +1.97° (c = 3.78, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66 (1 H, m), 2.85 (1 H, m), 3.17 (1 H, m), 3.44 (1 H, dd, J = 3, 11.4 Hz), 4.01–4.11 (2 H, m), 5.12–5.30 (2 H, m), 5.80–6.01 (1 H, m). Anal. Calcd for $C_6H_{10}O_2$: C, 63.16; H, 8.77. Found: C, 63.20; H, 8.18. On the basis of the optical purity of the starting material, the epoxide should have 90% ee.

Preparation of (−)-3-(Allyloxy)-1,2-epoxypropane (19) from (+)-16b. To a solution of (+)-**16b** (370 mg, 1.16 mmol) in 5 mL of methanol was added KOH (78 mg, 1.39 mmol) with stirring at room temperature. After 1 h, water was added, the solution was extracted with ethyl acetate, and the solvent was removed under reduced pressure. The product was purified by column chromatography (ethyl acetate–n-hexane = 1:4 v/v) on silica gel to yield 3-(allyloxy)-1,2-epoxypropane (**19**) (115 mg, 87% yield): $[\alpha]^{23}_D$ = −2.08° (c = 2.31, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.65 (1 H, m), 2.85 (1 H, m), 3.16 (1 H, m), 3.44 (1 H, dd, J = 3, 11.4) 4.01–4.12 (2 H, m), 5.15–5.38 (2 H, m), 5.85–6.00 (1 H, m). Anal. Calcd for $C_6H_{10}O_2$: C, 63.16; H, 8.77. Found: C, 63.20; H, 8.66. On the basis of the optical purity of the starting material, the epoxide should have 94% ee.

Absolute Stereochemistry of 18 and That of 15b, 16b, and 19. Authentic (2S)-(+)-glycidol tosylate was converted to (2R)-(+)-3-(allyloxy)-1,2-epoxypropane via reaction with allyl alcohol. The product had $[\alpha]^{23}_D$ = +2.1° (c = 2.4, $CHCl_3$) and had the same optical rotation as **18**. Therefore, compound **18** was established to have R configuration and **15b**, **16b**, and **19** should be R, S, and S as indicated.

D-Glyceraldehyde 3-Phosphate. To a solution of water containing 24.4 mmol of Na_2HPO_4 and 1.2 mmol of Na_3PO_4 (250 mL) was added to compound **4** (12.2 mmol).[9] The solution was heated to 75 °C for 30 h. After cooling, the solution was adjusted to pH 1.1 with 1 N HCl and incubated at 37 °C for 24 h. Enzymatic analysis[17] indicated that the solution contained 7.3 mmol (60% yield) of D-glyceraldehyde 3-phosphate. The solution was adjusted to pH 3.5 with 1 N NaOH and stored in the refrigerator. No further purification was attempted. This preparation has been used in the synthesis of 2-deoxyribose-5-phosphate using 2-deoxyribose-5-phosphate aldolase as catalyst[18] (eq 9).

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Supplementary Material Available: ^{13}C NMR spectra for compounds **7** and **9** (2 pages). Ordering information is given on any current masthead page.

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Preparative Separation of the Diastereoisomers of Dioxindolyl-L-alanine and Assignment of Stereochemistry at C-3[1]

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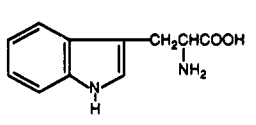
The diastereoisomers of dioxindolyl-L-alanine are obtained in a 1:1 ratio by air oxidation of oxindolyl-L-alanine. Preparative separation of the diastereoisomers is achieved by reverse-phase HPLC. Stereochemistry of the hydroxyl group at C-3 is assigned by transformation to a related pair of diastereoisomers of known stereochemistry.

The biosynthesis of tryptophan by tryptophan synthase (TSase), as well as its degradation by tryptophanase (TPase), are considered to involve an enzyme-bound intermediate—the indolenine tautomer (**2**) of L-tryptophan (**1**). Two analogues of **2**, which contain an sp^3 carbon at C-3 (**3** and **4**), were found to be significant inhibitors of both enzymes while analogues of **1** were not inhibitory.[2] The diastereoisomers of **3** were separated by HPLC and were given stereochemical designations on the basis of literature data.[3] Unexpectedly, the αS,3S isomer was found to inhibit only TSase, while the αS,3R isomer inhibited only TPase. Thus, a pair of enzymes, which catalyze essentially the same reaction in opposite directions, are found to have mirror-image stereochemical requirements for the heterocyclic portion of the substrate or the product. Although oxindolyl-L-alanine (**4**) also has a chiral center at C-3, no effort had been made in our earlier work to extend the mirror-image test, since it was already known that H-3 in **4** undergoes facile isotope exchange in mildly acidic D_2O.[4] Furthermore, chiral 3-hydroxyoxindole had been found to racemize rapidly in mild base.[5] We have



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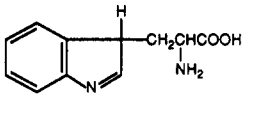

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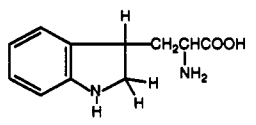

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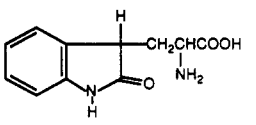

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