pressure to give a pale yellow oil, which was purified by passing it through a small silica gel column (Woelm Activity I) in 10% MeOH/$CHCl_3$ (v/v) to yield 343 g (91%) of **6a**: ^1H NMR δ 2.3 (6 H, s), 4.2 (1 H, s), 5.1 (1 H, t), 5.9 (2 H, s), 6.9-7.6 (8 H, m); ^{13}C NMR (undecoupled) δ 45.3 (q, NCH_3), 66.4 (t, CH_2N), 66.7 (d, COH), 101.0 (t, OCH_2O), 106.1 (d, CH=), 106.3 (d, CH=), 123.8, 126.4, 127.9, 128.4, 133.0, 134.6, 137.3, 147.0, 147.9; IR (neat) 3330, 1470, 1240 cm^{-1}; MS, *m/e* 389 and 391 (M^+). Anal. [as HCl salt (mp 228-229 °C dec and 240-241 °C dec; polymorphs] Calcd for $C_{19}H_{21}O_3NBrCl$: C, 53.46; H, 4.92. Found: C, 53.29; H, 4.70.

Hofmann Degradation of Racemic Steporphine (1a) and Its Racemic C-4 Epimer (1b). The racemic C-4 epimers (±)-**1** and (±)-**1b** were each, individually, converted to their corresponding methiodides with excess methyl iodide in acetone at reflux. From (±)-**1a**, the racemic methiodide had mp 249-250 °C dec, and from (±)-**1b**, the methiodide had mp 235-236 °C dec. Recrystallization was effected from benzene-ethanol. The respective racemic methiodides (65 mg, 0.22 mmol) were suspended in 10% aqueous NaOH (25 mL), and the respective suspensions were heated at reflux for 10 h, during which the suspended material disappeared and was replaced by a white precipitate, which was collected from the cool solution by such filtration and repeatedly washed with distilled water until the washings were no longer basic to litmus. The precipitate was dried, in vacuum, at 80 °C for 14 h to yield 52 mg, 0.17 mmol (94% of theory), of **7b**: mp 130-131 °C (ethanol) IR (KBr) 3330, 2850, 1600, 1450 cm^{-1}; ^1H NMR δ 2.3 (6 H, s), 4.8 (1 H, s), 5.5 (1 H, t), 6.1 (2 H, s), 7.2-9.0 (7 H, m); ^{13}C NMR (undecoupled) δ 45.9 (q, NCH_3), 67.7 (t, CH_2N), 67.9 (d, HCOH), 102.2 (t, OCH_2O), 108.2 (d), 117.4, 123.0, 125.7, 125.8, 127.1, 127.7, 128.1, 128.6, 132.9, 135.3, 143.5, 146.6; MS, *m/e* 309 (M^+). Anal. Calcd for $C_{19}H_{19}O_{13}N$: C, 73.79; H, 6.15. Found: C, 74.00; H, 6.04.

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Supplementary Material Available: ORTEP drawings, details of the X-ray crystallographic procedures, and atomic coordinates for racemic steporphine methiodide, (±)-**1a**, and for the C-4 epimers of racemic 1-(*o*-bromobenzyl)-4-hydroxy-2-methyl-6,7-(methylenedioxy)-1,2,3,4-tetrahydroisoquinoline, (±)-**3a** and (±)-**3b** (13 pages). Ordering information is given on any current masthead page.

Enzymatic Routes to Enantiomerically Enriched 1-Butene Oxide[1]

H. Keith Chenault, Mahn-Joo Kim, Alan Akiyama, Toshifumi Miyazawa, Ethan S. Simon, and George M. Whitesides*

Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

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This paper compares several routes to enantiomerically enriched 1-butene oxide (**1**) in which resolution is achieved by using an enzymatic reaction (Scheme I). This research had two objectives: to enumerate several general strategies that can be followed in practical, enzyme-based routes to enantiomerically enriched epoxides and to compare the utility of these routes in the particular case of compound **1**. In general, we restrict our account to reactions that proceed with high values of enantiomeric excess (ee) and that have the potential for preparing 50-g quantities of product. Enantiomerically enriched epoxides are useful synthons in chiral synthesis. General routes are now available to only epoxy alcohols and analogues of these substances.[2,3]

All reactions were conducted on scales that generated 1-5 g of **1** as product. The chemical yields reported in Scheme I are *overall* yields for conversion of the substrate for the enzymatic reaction to **1**; these yields are not optimized. We established the optical purity of **1** by ^1H NMR analysis in the presence of $Eu(hfc)_3$.[4] With careful calibration, this method can detect a 1% enantiomeric impurity (i.e., 98% ee).

Acylase I (EC 3.5.1.14) is commercially available, inexpensive, and stable. It hydrolyzes a range of *N*-acyl-α-amino acids with high (>99% ee) enantioselectivity.[5] Both enantiomers are easily recovered, and the reaction can be run virtually to completion. In resolutions of 2-aminobutanoic acid, the reaction stops spontaneously after hydrolysis of the *S* enantiomer is complete, and losses of material occur entirely in the recovery and purification of product. Conversion of the 2-amino acid via the corresponding 2-chloro acid and chlorohydrin to the epoxide preserves chirality well.[6]

Lipase (porcine pancreas, EC 3.1.1.3) and **cholesterol esterase** (CE, EC 3.1.1.13) are broad-specificity enzymes showing substrate-dependent enantioselectivity.[3,7] For the hydrolysis of 2-bromobutyl butyrate, without extensive optimization, the best ee was obtained with CE and was 80% at 77% conversion of the racemic starting material. Although this number could, in principle, be improved by carrying the reaction to higher conversion, and probably also by varying temperature and pH, we have not done so since alternative procedures seemed preferable. We note, however, that high values of ee can be obtained in kinetic resolutions of other halohydrins (e.g. 2-bromopropyl butyrate: ≥98% ee at 60% conversion) and that this method provides a good route to certain optically active epoxides.

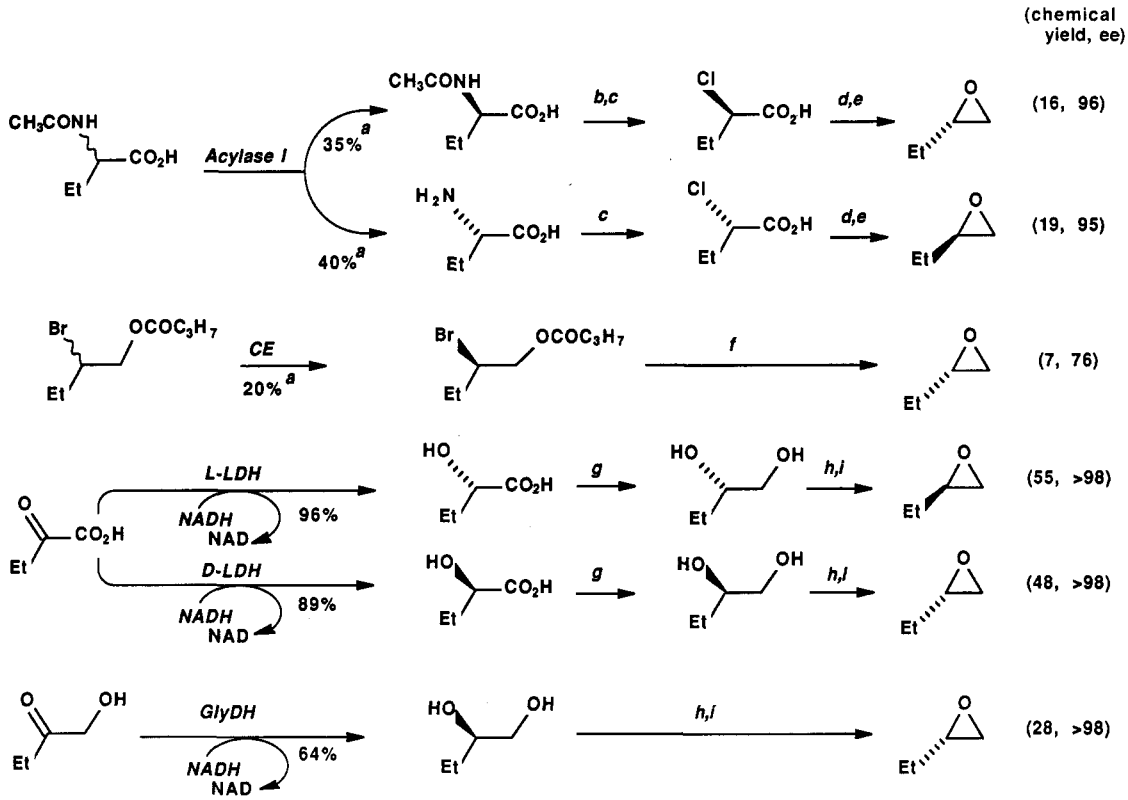
L-**Lactate dehydrogenase** (L-LDH, EC 1.1.1.27) and D-**lactate dehydrogenase** (D-LDH, EC 1.1.1.28) are both highly enantioselective.[8] The L enzyme accepts a broad range of small and medium-size unhindered α-keto acids; the D enzyme is more restrictive toward its substrates.[9] The LDH enzymes are interesting in asymmetric synthesis because they comprise one of the few systems in which enzymes catalyzing reactions having opposite enantioselectivities are both commercially available. Unfortunately, the range of products available by this method in both D and L forms is limited by the range of substrates accepted by both enzymes. The LDH-catalyzed reactions (coupled with formate/formate dehydrogenase (FDH) for in situ regeneration of NADH[10]) can be run to completion with high enantioselectivities (>99% ee) and good isolated yields (84-99%).

(1) Supported by the National Institutes of Health, Grant GM-30367, and Naval Air Systems Command, MDA 903-86-M-0505.

(2) Sharpless, K. B. *Chem. Br.* **1986**, *22*, 38-44.
(3) Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250-7251.
(4) Chiral shift reagents: Fraser, P. R. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1983; Vol. 1, pp 173-196.
(5) Birnbaum, S. M.; Levintow, L.; Kingsley, R. B.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *194*, 455-470. Greenstein, J. P. *Methods Enzymol.* **1957**, *3*, 554-570.
(6) Fu, S. C.; Birnbaum, S. M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1954**, *76*, 6054-6058. Koppenhoeffer, B.; Weber, R.; Schurig, V. *Synthesis* **1982**, 316-318.
(7) Lavagre, J.; Verrier, J.; Baratti, J. *Biotech. Bioeng.* **1982**, *24*, 2175-2188.
(8) Hirschbein, B. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1982**, *104*, 4458-4460.
(9) Kim, M.-J.; Simon, E. S.; Whitesides, G. M., unpublished results.
(10) Shaked, Z.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7105-7107. Wichmann, R.; Wandrey, C.; Bückmann, A. F.; Kula, M.-R. *Biotech. Bioeng.* **1981**, *23*, 2789-2802.

0022-3263/87/1952-2608$01.50/0

Scheme I. Enzymatic Routes to Enantiomerically Enriched 1-Butene Oxide[a]



[a] Since this reaction is a kinetic resolution, the maximum theoretical yield of one enantiomer is 50%. [b] 2 N HCl, 100 °C, 2 h (90%). [c] 6 N HCl, $NaNO_2$, −10 °C (70%). [d] BH_3·THF (90%). [e] KOH, −30 → 25 °C (75%). [f] $C_5H_{11}OK$, $C_5H_{11}OH$, room temperature (33%). [g] BH_3·THF, 0 °C, 20 h (81–91%). [h] 30% HBr–AcOH, −15 → 25 °C, 3 h (65–90%). [i] $C_5H_{11}OK$, $C_5H_{11}OH$, room temperature (70–81%).

Glycerol dehydrogenase (GlyDH, EC 1.1.1.6) reduces 1-hydroxy-2-butanone and a number of small α-keto alcohols with good enantioselectivity (94–98% ee).[11] It also requires in situ NADH regeneration.

A number of other enzymes are also, in principle, applicable to the synthesis of enantiomerically enriched epoxides. Epoxide hydratase (EC 4.2.1.64)[12] and various oxidative enzymes[13] are particularly interesting. These enzymes operate, however, with variable degrees of enantioselectivity. Further, none is commercially available, and the oxidative enzymes are too difficult to manipulate for routine laboratory use in synthesis.

We conclude that enzymatic catalysis offers a number of practical routes for the preparation of epoxides with high enantiomeric purities. For the synthesis of 1, methods based on LDH have the advantage of high enantiomeric purity and good chemical yield. Methods based on acylase I have advantages of low cost of enzyme and starting material and of operational simplicity. For other epoxides, the best route will depend upon the substance being synthesized. Acylase I accepts hindered side chains and has very high enantioselectivity; lipases are active with water-insoluble substrates; routes based on LDH or GlyDH may be favored if starting materials are particularly readily available.

Experimental Section

Materials and Methods. Enzymes from Sigma were used without further purification: acylase I (porcine kidney, lyophilized powder, grade I), CE (porcine pancreas, lyophilized powder), L-LDH (rabbit muscle, crystalline suspension in 2.1 M $(NH_4)_2SO_4$, type II), D-LDH (*Lactobacillus leichmannii*, crystalline suspension in 3.2 M $(NH_4)_2SO_4$), and GlyDH (*Cellulomonas* sp., lyophilized powder). FDH (yeast, lyophilized powder) from Boehringer Mannheim was used without further purification. α-Bromobutyryl bromide came from Fluka. Butyryl chloride came from Aldrich. 1-Hydroxy-2-butanone from BASF was distilled three times before use. All other chemicals and biochemicals came from Sigma and were used without further purification.

Water was distilled twice, the second time from a Corning AG-lb glass still. Argon (welding grade) was used without further purification. THF was freshly distilled from Na/benzophenone.

^1H NMR spectra were recorded on Bruker AM-300 and AM-250 instruments with peaks referenced to Me_4Si ($CDCl_3$, acetone-d_6) or DSS (D_2O). A Chemtrix 45AR pH controller equipped with an LKB 2120 peristaltic pump afforded pH control of certain reactions.

Enzyme Assays. Acylase I was assayed with racemic *N*-acetyl-2-aminobutyric acid (40 mM) in 0.1 M potassium phosphate buffer, pH 7.5, 40 °C. The rate of release of free amino acid was determined by removing aliquots, precipitating the protein by adding an equal volume of 14% $HClO_4$, and allowing a portion of each acidified aliquot to react with ninhydrin.[14] L- and D-lactate dehydrogenases were assayed with 2-oxobutanoic acid following the literature method.[15] Glycerol dehydrogenase and formate dehydrogenase were assayed by known procedures.[16] Enzymes immobilized on PAN gel were assayed by using a procedure analogous to that for the soluble enzymes except that the cuvettes were inverted every 10 s to keep the gel suspended.[17] One unit

(11) Lee, L. G.; Whitesides, G. M. *J. Org. Chem.* **1986**, *51*, 25–36.
(12) Armstrong, R. N.; Kedzierski, B.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1981**, *256*, 4726–4733. Yang, S. K.; McCourt, D. W.; Leultz, J. C.; Gelboin, H. V. *Science (Washington DC)* **1977**, *196*, 1199–1201.
(13) May, S. W.; Schwartz, R. D. *J. Am. Chem. Soc.* **1974**, *96*, 4031–4032. May, S. W.; Stelten-Kamp, M. S.; Schwartz, R. D.; McCoy, C. J. *J. Am. Chem. Soc.* **1976**, *98*, 7856–7858.
(14) Rosen, H. *Arch. Biochem. Biophys.* **1957**, *67*, 10–15.
(15) *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Bergmeyer, J., Grassl, M., Eds.; Verlag Chemie: Weinheim, Germany, 1983; Vol. 2, pp 232–233.
(16) *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Bergmeyer, J., Grassl, M., Eds.; Verlag Chemie: Weinheim, Germany, 1983; Vol. 2, pp 213–215 (glycerol dehydrogenase), 183–184 (formate dehydrogenase).

of enzyme refers to that amount of enzyme which produces 1 μmol min^{-1} of product under assay conditions.

Determination of Enantiomeric Excesses. Enantiomeric excesses of (*R*)- and (*S*)-2-aminobutanoic acids were determined by ^1H NMR analysis of the (+)-MTPA amides[18] of the corresponding amino acid methyl esters.[19] Calibration showed that a 0.5% diastereomeric impurity could be detected. The enantiomeric excesses of (*R*)- and (*S*)-2-hydroxybutanoic acids were likewise measured by ^1H NMR analysis of the *O*-(+)-MTPA derivatives[18] of the corresponding hydroxy acid methyl esters.[20] ^1H NMR analysis of (*R*)-2-bromobutyl butyrate and of (*R*)- and (*S*)-1-butene oxides in the presence of $Eu(hfc)_3$[4] determined their enantiomeric excesses. In the case of 1-butene oxide, calibration showed that a 1% enantiomeric impurity could be detected.

Resolution of 2-Aminobutanoic Acid. *N*-Acetyl-2-aminobutanoic acid [29 g, 0.20 mol, mp 132–133 °C (lit.[21] mp 132 °C)], obtained by reaction of acetyl chloride with 2-aminobutanoic acid,[5] was combined with 250 mL of H_2O, 100 mL of 2 N LiOH, and 272 mg (2 mmol) of KH_2PO_4 in a 1-L, round-bottomed flask with a stir bar. Additional solid LiOH adjusted the pH of the solution to pH 7.6. An aliquot was removed and acidified with an equal volume of 14% $HClO_4$. After the precipitated protein had settled or been removed by centrifugation, a portion of the aliquot was reacted with ninhydrin[14] to measure the concentration of free amino acid. Acylase I (46 mg, 800 U) was added to the reaction. During the first few hours, addition of solid LiOH maintained the pH at 7.6. Periodic assays for free amino acid measured the progress of the reaction. After 24 h, hydrolysis was 50% complete. After 48 h, acylase I had retained 70% of its original activity; no further hydrolysis past 50% conversion occurred.

The reaction was acidified to pH 2.5 with concentrated HCl and concentrated under reduced pressure to half its volume. Flocculated protein was removed by filtration, and the solution was acidified to pH 1.7 with concentrated HCl. Continuous extraction of the solution (EtOAc, 3.5 days) followed by drying ($MgSO_4$) and removal of solvent from the EtOAc fractions yielded a solid residue, which was dissolved in acetone, filtered, stripped of solvent, and then redissolved in acetone until no further LiCl precipitated upon solution in acetone. Removal of acetone under reduced pressure gave (*R*)-*N*-acetyl-2-aminobutanoic acid (10 g, 69% of theoretical): ^1H NMR (acetone-d_6) δ 0.93 (t, J = 7.4 Hz, 3 H, γ-CH_3), 1.63–1.90 (m, 2 H, CH_2), 1.93 (s, 3 H, CH_3CO), 4.37 (m, 1 H, CH), 7.28 (br, 1 H, NH); IR (KBr) 3360 (NH), 1720 (C=O acid), 1600 (C=O amide) cm^{-1}.

(*R*)-*N*-Acetyl-2-aminobutanoic acid (10 g) was hydrolyzed by refluxing in 2 N HCl (200 mL) for 2 h. The solution was cooled and evaporated under reduced pressure. Twice, water was added to the residue and evaporated again under reduced pressure to remove HCl. Recrystallization of the dried residue from H_2O gave (*R*)-2-aminobutanoic acid (6.4 g, 90%): >99% ee; $[\alpha]_D^{22}$ −21.0° (*c* 4, 6 N HCl) (lit.[22] $[\alpha]_D^{23}$ −20.7° (*c* 4, 6 N HCl)); ^1H NMR (D_2O) δ 0.97 (t, J = 7.5 Hz, 3 H, CH_3), 1.89 (m, 2 H, CH_2), 3.70 (t, J = 5.8 Hz, 1 H, CH).

The acidic aqueous layer left from the continuous extraction was adjusted to pH 6.0 with solid LiOH. Concentration of the solution under reduced pressure and addition of EtOH precipitated the amino acid. Recrystallization of the precipitation from H_2O gave (*S*)-2-butanoic acid (8.1 g, 79%): ee >99%; $[\alpha]^{22}$ +20.8° (*c* 4, 6 N HCl) (lit.[22] $[\alpha]_D^{23}$ +20.6° (*c* 4, 6 N HCl)); the ^1H NMR spectrum agreed with that of the *R* antipode.

Conversion of (*R*)- and (*S*)-2-Aminobutanoic Acids to (*R*)- and (*S*)-2-Chlorobutanoic Acids. (*R*)-2-Aminobutanoic acid (10 g, 97 mmol) and 120 mL of 6 N HCl were combined in a 500-mL, round-bottomed flask with a thermometer and stir bar. $NaNO_2$ (10.5 g, 152 mmol) was added over a 3-h period to the vigorously stirred and chilled (−10 to −5 °C) solution. The reaction was kept at −10 °C overnight and then extracted with 3 × 120 mL of ether. The ethereal extracts were washed with H_2O, dried ($CaCl_2$), and evaporated under reduced pressure to give 8.3 g (70%) of (*R*)-2-chlorobutanoic acid: bp 46–50 °C (0.25 torr) [lit.[23] bp 99–101 °C (16 torr)]; ^1H NMR ($CDCl_3$) δ 1.05 (t, J = 7.3 Hz, 3 H, CH_3), 2.02 (m, 2 H, CH_2), 4.26 (dd, J = 5.8, 7.6 Hz, 1 H, CH); IR (neat) 3500–2500 (OH), 1720 (C=O) cm^{-1}.

(*S*)-2-Aminobutanoic acid was converted to (*S*)-2-chlorobutanoic acid (70%) in a similar manner.

Conversion of (*R*)- and (*S*)-2-Chlorobutanoic Acids to (*R*)- and (*S*)-2-Chloro-1-butanol. BH_3·THF[24] reduced the crude *R* and *S* chloro acids to the corresponding chlorohydrins (90%): ^1H NMR ($CDCl_3$) δ 1.02 (t, J = 7.4 Hz, 3 H, CH_3), 1.61–1.88 (m, 2 H, CH_3CH_2), 2.34 (br s, 1 H, OH), 3.58–3.77 (m, 3 H, CH_2O, CH), in agreement with lit.[25]; IR (neat) 3370 (br, OH) cm^{-1}.

Conversion of (*R*)- and (*S*)-2-Chloro-1-butanol to (*S*)- and (*R*)-1-Butene Oxide.[6] Finely ground, dry KOH (6.8 g, 122 mmol) was added slowly to the chilled (−30 °C) (*R*)-chlorohydrin (6.6 g, 61 mmol). KCl precipitated. After the addition of KOH, (*S*)-1-butene oxide was distilled from the reaction through a short-path distillation head to give 3.3 g (75%) of product: 96% ee; bp 59–61 °C; ^1H NMR ($CDCl_3$) δ 0.98 (t, J = 7.5 Hz, 3 H, CH_3), 1.55 (m, 2 H, CH_3CH_2), 2.45 (m, 1 H) and 2.71 (m, 1 H) (CH_2O), 2.87 (m, 1 H, CH).

Similar reaction of the (*S*)-chlorohydrin gave (*R*)-1-butene oxide (75% yield, 95% ee).

Cholesterol Esterase Catalyzed Hydrolysis of 2-Bromobutyl Butyrate. 2-Bromobutyl butyrate (30.9 g, 138 mmol), obtained by the lithium aluminum hydride reduction[26] of 2-bromobutyryl bromide and subsequent esterification of 2-bromo-1-butanol with butyryl chloride,[27] was mixed with 40 mL of 0.05 M potassium phosphate buffer, pH 7.0, and 40 mL of H_2O in a 250-mL three-neck round-bottomed flask equipped with a stir bar and pH electrode. Addition of 5 N NaOH adjusted the pH to 7.0. CE (0.15 g in 1 mL of buffer) was added. Addition of 5 N NaOH using a pH controller kept the reaction at pH 7.0; the volume of base used indicated the extent of reaction. After 21 mL of base had been added (77% conversion), the reaction was poured into 400 mL of ether. The layers were separated, and the aqueous layer was extracted with 4 × 100 mL of ether. The combined ethereal layers were evaporated under reduced pressure. The residue was dissolved in 250 mL of pentane and washed with 8 × 25 mL of 75% aqueous MeOH to remove the alcohol. The organic layer was dried over Na_2SO_4, and the pentane was evaporated under reduced pressure. Kugelrohr distillation of the residual oil gave (*R*)-2-bromobutyl butyrate (6.1 g, 84% yield based on 77% conversion): 80% ee; bp 44–47 °C (0.20 torr).

Conversion of (*R*)-2-Bromobutyl Butyrate to (*S*)-1-Butene Oxide. (*R*)-2-Bromobutyl butyrate (3.0 g, 80% ee) and 6 mL of freshly distilled 1-pentanol were combined in a 100 mL round-bottomed flask equipped with a dropping funnel and Vigreaux column leading to a condenser and receiving flask. The condenser was connected to a circulating cold bath set at 0 °C, and the receiving flask was cooled in an ice–EtOH bath. A 1.2 M solution of $C_5H_{11}OK$ in 1-pentanol (11.2 mL, 13.5 mmol) was added dropwise to the mixture. After the solution had stirred for approximately 15 min, it was heated in an oil bath. When the temperature of the oil bath reached 170 °C, the epoxide began distilling. Its yield was 0.32 g (33%): 76% ee; bp 56–58 °C; the ^1H NMR spectrum ($CDCl_3$) agreed with that of 1 obtained from 2-chloro-1-butanol.

(*R*)- and (*S*)-2-Hydroxybutanoic Acids. To a 1-L, three-neck round-bottomed flask equipped with a stir bar, pH electrode, and septa were transferred 2-oxobutanoic acid (15.3 g, 150 mmol), sodium formate (11.7 g, 170 mmol), 2-mercaptoethanol (50 μL, 0.75 mmol), and Tris-HCl (0.45 g, 3.8 mmol) in 300–400 mL of H_2O. Concentrated NaOH adjusted the pH to 7.5. The solution was degassed by bubbling Ar through it for 15 min. NAD (0.75

(17) Pollak, A.; Blumenfeld, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324–6336.
(18) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
(19) Uhle, F. C.; Harris, L. S. *J. Am. Chem. Soc.* **1956**, *78*, 381–384.
(20) Methyl esters formed by reaction with CH_2N_2.
(21) Rao, K. R.; Birnbaum, S. M.; Kingsley, R. B.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *198*, 507–524.
(22) Greenstein, J. P.; Gilbert, J. B.; Fodor, P. J. *J. Biol. Chem.* **1950**, *182*, 451–456.
(23) Reeve, W.; Steckel, T. F. *Can. J. Chem.* **1980**, 58, 2784–2788.
(24) Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38*, 2786–2792.
(25) Nakajima, T.; Nakamoto, Y.; Suga, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 960–965.
(26) Fickett, W.; Garner, H. K.; Lucas, H. J. *J. Am. Chem. Soc.* **1951**, *73*, 5063–5067. Stewart, C. A.; Vanderwerf, C. A. *J. Am. Chem. Soc.* **1954**, *76*, 1259–1264.
(27) Sonntag, N. O. V. *Chem. Rev.* **1953**, *52*, 237–416.

mmol) was added. Then, L- or D-LDH (150–400 U) and FDH (40–50 U) immobilized on PAN gel[17] were added as a suspension in 100–200 mL of degassed H_2O. The flask was capped with septa, and Ar bubbled through the solution. HCl (2.56 N) added by a pH controller maintained the reaction near pH 7.5. The reaction progress was followed by measuring the volume of HCl consumed. Within 5 days the reaction was complete. The enzyme containing gel was isolated by centrifugation and washed with degassed H_2O. The aqueous layers were combined and concentrated by rotary evaporation to 70–80 mL, acidified to pH 2.0 with 6 N HCl, and extracted with 4 × 170 mL of ether. The ethereal layers were combined, dried over $MgSO_4$, and evaporated under reduced pressure to give (*S*)-2-hydroxybutanoic acid (L-LDH used) (15.0 g, 95%) [>99% ee; mp 54.5–55.5 °C dec (lit.[28] mp 52.7–53.5 °C); $[\alpha]_D^{21}$ +7.15° (*c* 8.13, $CHCl_3$) (lit.[28] $[\alpha]_D^{16}$ +6.4° (*c* 11.03, $CHCl_3$)); ^1H NMR ($CDCl_3$) δ 1.00 (t, *J* = 7.4 Hz, 3 H, CH_3), 1.75 and 1.88 (m, 1 H each, CH_2), 4.24 (dd, *J* = 4.5, 6.9 Hz, 1 H, CH), 6.72 (br, OH); IR (Nujol) 3500–2650 (OH), 1730 (C=O) cm^{-1}] or (*R*)-2-hydroxybutanoic acid (D-LDH used) (13.9 g, 89%) [>99% ee; mp 53–55 °C dec; $[\alpha]_D^{20}$ −5.6° (*c* 3.71, $CHCl_3$)]; the ^1H NMR and IR spectra were in agreement with those for the *S* enantiomer.

Conversion of (*R*)- and (*S*)-2-Hydroxybutanoic Acids to (*R*)- and (*S*)-Butane-1,2-diol. BH_3·THF[24] reduced the *R* and *S* hydroxy acids to (*R*)-butane-1,2-diol (9.7 g, 81%) [bp 122–125 °C (30 torr); $[\alpha]_D^{21}$ +12.6° (*c* 3.23, EtOH); ^1H NMR ($CDCl_3$) δ 0.90 (t, *J* = 7.5 Hz, 3 H, CH_3), 1.41 (m, 2 H, CH_3CH_2), 3.37 (m, 1 H, CH), 3.58 (m, 2 H, CH_2O), 3.67 (br s, 2 H, 2 × OH); IR (neat) 3350 (br, OH), 1045 (C–O) cm^{-1}] and (*S*)-butane-1,2-diol (11.7 g, 91%) [bp 94–96 °C (9 torr); $[\alpha]_D^{22}$ −15.35° (*c* 2.60, EtOH)]; the ^1H NMR and IR spectra were in agreement with those of the *R* enantiomer. Analytical data for both enantiomers agreed with the literature values.[29]

Conversion of (*R*)- and (*S*)-Butane-1,2-diol to (*R*)- and (*S*)-2-Acetoxy-1-bromobutane. Reaction of the diols with 30% HBr–AcOH[29,30] gave (*R*)-2-acetoxy-1-bromobutane (17.3 g, 82%) [bp 87–91 °C (21 torr); $[\alpha]_D^{21}$ +17.8° (*c* 2.73, ether); ^1H NMR ($CDCl_3$) δ 0.90 (t, *J* = 7.4 Hz, 3 H, CH_3CH_2), 1.69 (m, 2 H, CH_3CH_2), 2.07 (s, 3 H, CH_3CO), 3.45 (m, 2 H, CH_2O), 4.91 (m, 1 H, CH); IR (neat) 1735 (C=O) cm^{-1}] and (*S*)-2-acetoxy-1-bromobutane (22.3 g, 91%) [bp 68–70 °C (9 torr); $[\alpha]_D^{22}$ −23.16° (*c* 4.14, ether)]; ^1H NMR and IR spectra were in agreement with those for the *R* enantiomer. Analytical data for both enantiomers agreed with the literature values.[29,30] ^1H NMR spectroscopy indicated that the products contained approximately 7% 1-acetoxy-2-bromobutane.

Conversion of (*R*)- and (*S*)-2-Acetoxy-1-bromobutane to (*R*)- and (*S*)-1-Butene Oxide. Treatment of (*R*)- and (*S*)-2-Acetoxybromobutanes (5 M in dry 1-pentanol) with 1 equiv of 1.18 M $C_5H_{11}OK$ in 1-pentanol (added over 30–60 min at 0 °C) followed by distillation of the product through a 15-cm Vigreaux column equipped with a condenser cooled to −10 °C gave (*R*)-1-butene oxide (5.2 g, 81%) [>98% ee; bp 59–62 °C; $[\alpha]_D^{22}$ +14.80 (*c* 1.18, ether) (lit.[29] $[\alpha]_D^{21}$ +13.6° (*c* 1.135, ether)); ^1H NMR spectrum in agreement with that of 1 obtained from 2-chloro-1-butanol] and (*S*)-1-butene oxide (5.86 g, 71%) [>98% ee; bp 59–62 °C; $[\alpha]^{22}_D$ −12.00° (*c* 4.90, dioxane) (lit.[31] $[\alpha]^{16}_D$ −12.25 (*c* 6, dioxane)); ^1H NMR spectrum in agreement with that for the *S* enantiomer].

Enzymatic Preparation of (*R*)-Butane-1,2-diol. A three-necked, 500-mL, round-bottomed flask was charged with ammonium formate (3.78 g, 60 mmol), 1-hydroxy-2-butanone (4.56 g, 50 mmol), Tris–HCl (47 mg, 0.5 mmol), and 50 mL of water. The pH was adjusted to 7.5 with 1 N KOH. The flask was sealed with septa and fitted with an Ar inlet and outlet, a pH probe, and an inlet for 2.1 N HCl. The solution was degassed by bubbling Ar through it for 1 h and NAD (0.15 mmol) was added. FDH (67 U) and GDH (100 U) immobilized on PAN gel[17] were added as a suspension in 50 mL of H_2O. A pH controller maintained the pH at 7.7 ± 0.1 by adding 2.1 N HCl; Ar bubbled through the reaction. After 14 days, the enzyme-containing gels were removed by centrifugation (51 U of FDH and 44 U of GDH were recovered). The aqueous portion was continuously extracted with ether for 3 days, saturated with K_2CO_3, and extracted with 3 × 100 mL of ether. Concentration of the ethereal portions after drying over K_2CO_3 yielded a pale yellow liquid (3.5 g). Distillation through a short-path column [122–125 °C (30 torr)] yielded the diol (2.84 g, 64%), identified by ^1H NMR spectroscopy.

Conversion of (*R*)-Butane-1,2-diol to (*R*)-1-Butene Oxide. (*R*)-Butane-1,2-diol (from the GDH-catalyzed reaction) was converted to (*R*)-1-butene oxide by the same two-step method used with butane-1,2-diol from the LDH-catalyzed reactions. The yield was 3.90 g (47% from the diol): >98% ee; $[\alpha]_D^{21}$ +13.38° (*c* 1.225, ether).

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(28) Horn, D. H. S.; Pretorius, M. Y. Y. *J. Chem. Soc.* **1954**, 1460–1464.
(29) Mori, K.; Sasaki, M.; Tamada, S.; Suguro, T.; Masuda, S. *Tetrahedron* **1979**, *35*, 1601–1605.
(30) Ellis, M. K.; Golding, B. T. *Org. Synth.* **1984**, *63*, 140–144.
(31) Schmidt, U.; Talbiensky, J.; Barkowiak, F.; Wild, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 198–199.

Substituent Effects on Rates of Inter- and Intramolecular Cycloaddition Reactions of Isobenzofurans

David Tobia and Bruce Rickborn*

Department of Chemistry, University of California, Santa Barbara, California 93106

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Isobenzofurans appear to be the *most reactive isolable dienes* known, and they exhibit unparalleled versatility in [4 + 2] reactions. Cycloadducts have been formed under relatively mild conditions with dienophiles ranging from the very poor (cyclohexene,[1] ethyl vinyl ether[1]) through common carbonyl-activated olefins (maleic anhydride etc.) to the extremely reactive arynes[2] and benzocyclobutadiene.[3] Recently Wege and Moursounidis[4] have determined that the parent unsubstituted isobenzofuran is ca. 10^6 times more reactive than 1,3-butadiene[5] with maleic anhydride.

Primarily because of advances in methodology, many new substituted isobenzofurans have recently become available. The ability to predict changes in reactivity imposed by substituents can be important in using these materials. Although relative rate data for cycloaddition reactions are not expected to transfer precisely from one dienophile to another, the literature[5] suggests that approximately parallel behavior would be found for similar dienophiles. *N*-Methylmaleimide (NMM) was chosen as a representative common dienophile for the present study. Its advantages are that it gives products cleanly and in high yield, and these materials are not susceptible to facile

(1) Mir-Mohamad-Sadeghy, B.; Rickborn, B. *J. Org. Chem.* **1983**, *48*, 2237.
(2) (a) Shepard, K. L. *Tetrahedron Lett.* **1975**, 3371. (b) Reddy, G. S.; Bhatt, M. V. *Tetrahedron Lett.* **1980**, 3627. (c) Crump, S. L.; Netka, J.; Rickborn, B. *J. Org. Chem.* **1985**, *50*, 2746. (d) Netka, J.; Crump, S. L.; Rickborn, B. *J. Org. Chem.* **1986**, *51*, 1189. (e) Camenzind, R.; Rickborn, B. *J. Org. Chem.* **1986**, *51*, 1914. (f) Pollart, D. J.; Rickborn, B. *J. Org. Chem.* **1986**, *51*, 3155. (g) Mirsadeghi, S.; Rickborn, B. *J. Org. Chem.* **1987**, *52*, 787. (h) Pollart, D. J.; Rickborn, B. *J. Org. Chem.* **1987**, *52*, 792.
(3) (a) Cava, M. P.; Hsu, A. C. *J. Am. Chem. Soc.* **1972**, *94*, 6641. (b) Moss, R. J.; Rickborn, B. *J. Org. Chem.* **1984**, *49*, 3694. (c) Unpublished work of P. da Silva and B. Rickborn.
(4) Personal communication from Prof. Dieter Wege, University of W. Australia, Nedlands; we are indebted to Prof. Wege for permission to quote this unpublished result.
(5) Sauer, J.; Sustmann, R. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 779.

0022-3263/87/1952-2611$01.50/0