Chemoenzymic synthesis of chiral furan derivatives: Useful building blocks for optically active structures

ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · APRIL 1988

Impact Factor: 4.72 · DOI: 10.1021/jo00243a003

60%; $[\alpha]^{25}_D$ −13.4° (c 0.7, MeOH);[24] ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, d, J = 6.0 Hz), 1.40–2.00 (3 H, m), 2.15 (2 H, br s), 3.30–3.90 (4 H, m).

Synthesis of 10. This compound was synthesized by starting from *cis*-**3Dc** as reported above for *cis*-**3Ac**. The diastereomeric ratio has been obtained by capillary GC analysis (150 °C 0 min/4 °C/min/250 °C 5 min, t_R = 13.72 (major), 14.10 (minor)). The yield is reported in Table II: ^{1}H NMR ($CDCl_3$) δ 0.77 (3 H, d, J = 7.1 Hz), 1.16 (3 H, d, J = 7.0 Hz), 2.10–2.90 (3 H, m), 3.67 (3 H, s), 3.73 (3 H, s), 4.10–4.33 (1 H, m), 5.02 (1 H, d, J = 5.9 Hz), 5.09 (1 H, d, J = 2.4 Hz), 7.3 (5 H, s); ^{13}C NMR ($CDCl_3$) selected data δ 16.2, 16.4, 33.0, 34.9, 51.5, 52.5, 56.5, 80.7, 92.2, 155.0, 173.6. Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.55; H, 7.24; N, 4.38.

The same product was obtained by adding Bu_3P (1 equiv, entry 2, Table II or 4 equiv, entry 3, Table II) prior to **3Dc** addition.

An analytical sample of **10** as a 1:1 $C_{1'}$ epimeric mixture has been obtained via hydrogenation of methyl 4,4-dimethoxy-3-methylcrotonate (E:Z = 6:4) (H_2, 1 atm, Pd/C, MeOH) and subsequent BF_3·Et_2O-catalyzed cyclization with excess **1D**: ^{1}H NMR ($CDCl_3$) δ 1.03 (3 H, d, J = 6.6 Hz, $C_{1'}$-Me *R* isomer), 1.16 (3 H, d, J = 7.0 Hz, $C_{1'}$-Me *S* isomer); ^{13}C NMR ($CDCl_3$) selected data δ 91.3 (*R* isomer) and 92.2 (S isomer).

Acknowledgment. We thank the Ministero della Pubblica Istruzione for financial support, Prof. A. I. Meyers for helpful discussion, and Enrico Caneva for the NMR spectra and NOE difference experiments.

Registry No. 1A, 104863-92-5; **1B**, 108591-33-9; **1C**, 113322-99-9; **1D**, 113323-00-5; **2a**, 6044-68-4; **2b**, 18318-79-1; **2c**, 32815-00-2; *cis*-**3Aa**, 113323-06-1; *trans*-**3Aa**, 113323-07-2; *cis*-**3Ab**, 113427-44-4; *trans*-**3Ab**, 113427-45-5; *cis*-**3Ac**, 105226-55-9; *trans*-**3Ac**, 113427-46-6; *cis*-**3Bb**, 113323-04-9; *trans*-**3Bb**, 113323-05-0; *cis*-**3Bc**, 113323-08-3; *trans*-**3Bc**, 113323-09-4; *cis*-**3Cc**, 113323-02-7; *trans*-**3Cc**, 113323-03-8; *cis*-**3Dc**, 113323-10-7; *trans*-**3Dc**, 113351-82-9; **4b** (isomer 1), 113323-11-8; **4b** (isomer 2), 113427-49-9; **4c** (isomer 1), 113323-12-9; **4c** (isomer 2), 113427-50-2; **7**, 113323-01-6; *cis*-**8**, 113427-47-7; *trans*-**8**, 113427-48-8; **9a** (isomer 1), 105140-27-0; **9a** (isomer 2), 113323-13-0; **9b**, 105140-28-1; **9c**, 105140-29-2; **9d**, 105140-32-7; **9e** (isomer 1), 105140-30-5; **9e** (isomer 2), 113323-14-1; **9f** (isomer 1), 105140-31-6; **9f** (isomer 2), 113323-15-2; **10a**, 113351-83-0; **10b**, 113351-65-8; **10c** (isomer 1), 113323-16-3; **10c** (isomer 2), 113323-18-5; **10d**, 113323-17-4; **11a**, 105226-56-0; **11b**, 71633-61-9; **11c**, 71464-83-0; **11d**, 71464-84-1; (2*S*)-2-methylbutane-1,4-diol, 70423-38-0.

Supplementary Material Available: Tables of atomic coordinates, anisotropic thermal parameters, bond distances, and bond angles (5 pages). Ordering information is given on any current masthead page.

Chemoenzymic Synthesis of Chiral Furan Derivatives: Useful Building Blocks for Optically Active Structures

Dale G. Drueckhammer, Carlos F. Barbas III, Kenji Nozaki, and Chi-Huey Wong*

Department of Chemistry, Texas A&M University, College Station, Texas 77843

Cynthia Y. Wood and Marco A. Ciufolini*

Department of Chemistry, Rice University, Houston, Texas 77251

Received April 10, 1987

Practical procedures have been developed for the enantioselective reduction of 2-acetylfuran (**6a**) and 2-(trifluoroacetyl)furan (**6b**) to the corresponding carbinols (*S*)-**1** and **7b** with 88–90% ee using *Thermoanaerobium brockii* alcohol dehydrogenase coupled with an NADPH regeneration system. Kinetic resolution of racemic (*S*)-**1** via lipase-catalyzed esterification, followed by cholesterol esterase or lipase-catalyzed hydrolysis of the ester gives (*R*)-**1** with 94% ee. Conversion of (*S*)-**1** to the dihydropyranones **4** and **5** without racemization has been illustrated. Enantioselective hydrolysis of N-protected furylglycine methyl esters catalyzed by papain gave the unreacted esters and the free acids (*S* form) both in 45% yield and 97% ee. The resolved furylglycines are excellent substrates for the synthesis of optically active synthons for alkaloids.

Introduction

A strategy for the total synthesis of monosaccharides involves the preparation of a properly substituted furylcarbinol as a building block.[1-3] In most cases, however, a racemic carbinol is used as starting material. If an optically active furylcarbinol such as (*S*)-**1** were available, it would be useful for the synthesis of the L series of dihydropyranones (Figure 1), which would serve subsequently as substrates for the facile introduction of further functionality.[1c] We report here several preparative enzymatic routes to (*R*)- and (*S*)-furylmethylcarbinols and (*R*)-furyl(trifluoromethyl)carbinol through an asymmetric reduction of the corresponding acylfuran catalyzed by the alcohol dehydrogenase from *Thermoanaerobium brockii* (TADH)[4] and through a kinetic resolution of the racemic carbinol esters catalyzed by esterases. Compound (*S*)-**1** was converted to the 2,3,6-trideoxy-L-hex-2-enopyranosid-4-uloses (**4** and **5**) to illustrate the synthetic utility. We also report a practical procedure for the preparation of optically active (>97% ee) furylglycine derivatives such as (*S*)- and (*R*)-**9** (X = benzoyl; COOBn;

(1) (a) Achmatowicz, O. In *Organic Synthesis Today and Tomorrow*; Trost, B. M., Hutchinson, C. R., Eds.; Pergamon Press: 1981, pp 307–318. (b) Zamojski, A.; Grynkiewicz, G. In *The Total Synthesis of Natural Products*; Ap Simon, J., Ed.; Wiley: New York, 1984; pp 141–235. (c) Coppola, G. M.; Schuster, H. F. *Asymmetric Synthesis*; Wiley: 1987; p 25.

(2) Jaworska, A.; Zamojski, A. *Carbohydr. Res.* **1984**, *126*, 191.

(3) Askin, D.; Angst, C.; Danishefsky, S. *J. Org. Chem.* **1985**, *50*, 5005.

(4) The enzyme has been used for reduction of other compounds: (a) Wong, C.-H.; Drueckhammer, D. G., Sweers, H. M. *J. Am. Chem. Soc.* **1985**, *107*, 4028. (b) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *Ibid.* **1986**, *108*, 162.

0022-3263/88/1953-1607$01.50/0

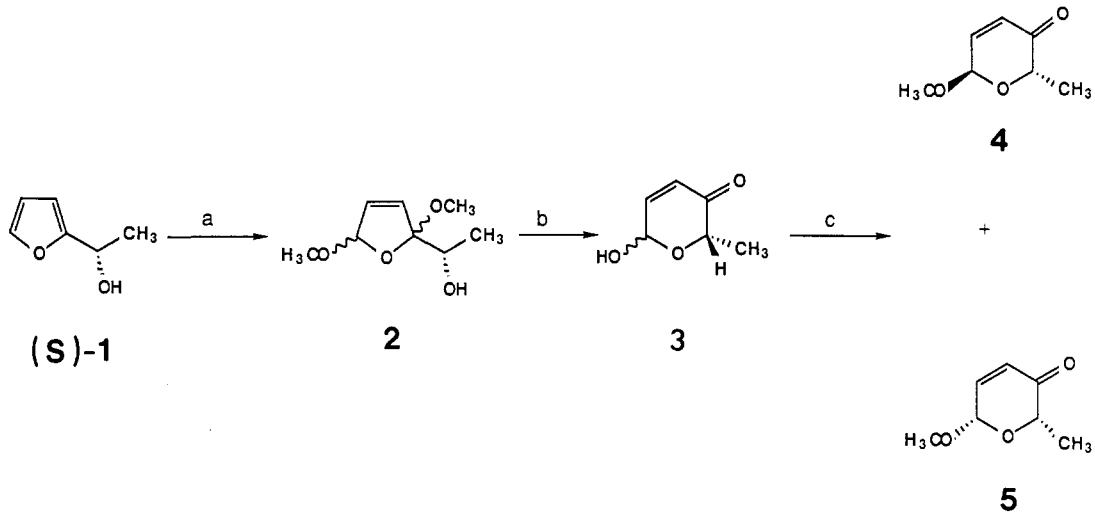


Figure 1. Conversion of compound (*S*)-1 to the dihydropyranones 4 and 5: (a) Br_2/MeOH; (b) H^+; (c) $HC(OMe)_3/SnCl_4$.

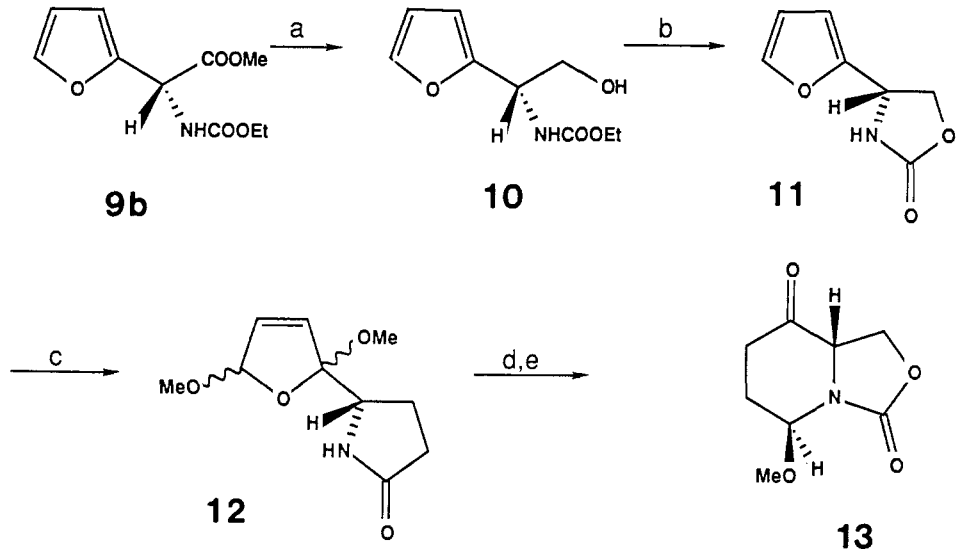


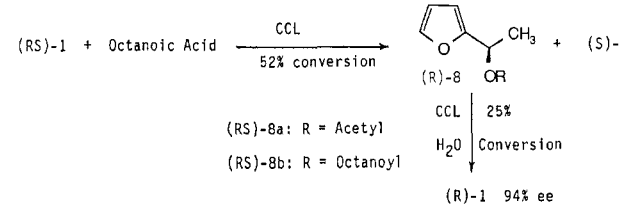
Figure 2. Conversion of (*R*)-**9b** to the alkaloid synthon **13**: (a) $LiAlH_4$, ether, −78 °C; (b) NaH, THF, 25 °C; (c) Br_2, MeOH, −40 °C; (d) H_2, $Rh(Al_2O_3)$; (e) 10 mol % TfOH, 2 mol of H_2O, THF, 25 °C.

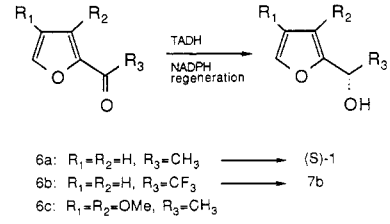
COOEt) via an enantioselective hydrolysis using papain as a catalyst[5] and a representative synthesis of the alkaloid synthon **13** from (*R*)-**9b** (Figure 2).

Results and Discussion

The TADH-catalyzed reductions of **6a** and **6b** proceeded with high enantioselectivity (88–90% ee), making these potentially useful transformations.[6] Compound **6c** was not a substrate for the enzyme. The L (*S*) configuration of (*S*)-**1** was supported by its negative sign of rotation.[7] This stereochemistry was further confirmed by oxidizing the α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA) ester of **1** to the ester of lactic acid by using RuO_2 followed by deesterification. The relative amounts of D- and L-lactate produced were then determined by doing separate assays using D- and L-lactic dehydrogenase. The L (*R*) configuration of **7b** was confirmed by its degradation following the procedure applied to **1** to give (*R*)-trifluorolactic acid, identified by comparison of its rotation with that of the same compound prepared by degradation of authentic (*R*)-(trifluoromethyl)phenylcarbinol.[4a,9]

Scheme I. Enzymatic Double Resolution





(5) (a) Resolution of the *N*-carbobenzoxy and *N*-*tert*-butoxycarbonyl derivatives of furylglycine using subtilisins was reported, but the optical purity of the products was not addressed: Schutt, H.; Schmidt-Kastner, G.; Arens, A.; Preiss, M. *Biotech. Bioeng.* **1985**, *27*, 420. (b) Ben-Ishai, D.; Sataty, I.; Bernstein, Z. *Tetrahedron* **1976**, *32*, 1571.

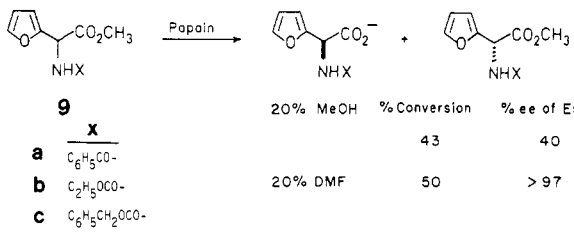
(6) The relative v_{max} values for the reduction of acetone, **6a**, and **6b** are 100, 16, and 5. The reduction of **1a** with the Mukaiyama reagent with a chiral amine of 72% optical purity as the ligand gave (*S*)-**1** with 61% ee.

(7) Kasai, M.; Ziffer, H. *J. Org. Chem.* **1983**, *48*, 2346.

(8) For conventional resolution of **1**, see: Dureen, D.; Kenyon, J. *J. Chem. Soc.* **1936**, 621.

In an attempt to prepare (*R*)-**1**, several esterases (pig liver esterase, lipases from pancreas, candida and other microbial species, and cholesterol esterase) were tested to selectively hydrolyze the racemic acetate and octanoate (**8a** and **b**) of 1 (Scheme I). The ee's of the carbinol obtained were quite low (<52% ee at 50% conversion). For the esterase- and lipase-catalyzed hydrolysis and esterification, the *R* enantiomer is the faster substrate in each direction. Therefore, it was possible to increase the optical purity of the carbinol by using a double resolution strategy. In this process, the reaction was run in the esterification direction to give ester of predominantly *R* configuration. This material was isolated and then selectively hydrolyzed to the *R* alcohol, further increasing the optical purity (Scheme I). The product of this "double resolution" procedure had an ee of 94% (52% esterification followed by 25% hydrolysis; enantioselectivity value (E) = 5[10] for both reactions). By comparison, microbial hydrolysis of (*RS*)-**8a** has given (*R*)-**1** with only 20% ee.[11] A different recycling procedure has previously been reported with pig liver esterase[10] (PLE) where the hydrolysis product was recovered, chemically esterified, and resubjected to the enzymatic hydrolysis. Resolution of (*RS*)-**1** was also attempted by hydrolysis of (*RS*)-**8b** using cholesterol esterases. The enzyme from bovine pancreas showed almost no selectivity. The cholesterol esterase from *Pseudomonas fluorescens*, however, gave (*R*)-**1** with 92% ee when the alcohol was isolated at 20% conversion (E = 20).

N-Benzoyl-DL-furylglycine methyl ester (**9a**) is a poor substrate for PLE and the lipase from *Candida cylindracea* (CCL). However, one enantiomer (*S*) of this compound is a good substrate for papain, allowing the other (*R*) to be prepared by recovery after selective hydrolysis of the racemate in a reaction mixture containing 20% DMF at pH 7.0. A much lower ee was obtained when methanol was used as organic cosolvent. Papain was also found to catalyze enantioselective hydrolysis of *N*-ethoxycarbonyl (**9b**) and *N*-benzyloxycarbonyl (**9c**) furylglycine methyl esters with the same chemical and optical yields obtained in the case of the *N*-benzoyl compound. The configuration of the recovered ester **9b** was determined to be *R* after correlation with L-serine, indicating papain is specific for the *S* isomer. To illustrate the



9				
	X	20% MeOH	% Conversion	% ee of Ester
a	C_6H_5CO-		43	40
b	C_2H_5OCO-	20% DMF	50	>97
c	$C_6H_5CH_2OCO$-			

synthetic utility of (*R*)-**9b**, we have converted it to compound **13** (Figure 2) in 98% ee, thus establishing a new chemoenzymatic route to optically active, N-containing structures.

Experimental Section

Materials and Methods. All enzymes and biochemicals were from Sigma. Organic solvents were reagent grade. ^{1}H NMR was determined with a Varian XL200 instrument (200 MHz). Gas chromatography was done on a Hewlett-Packard Model 5830A instrument with an OV17 column at 175 °C. The column size was $^1/_8$ in. × 12 ft and the N_2 flow rate was 32 mL/min. Enzyme assays were carried out at room temperature (25 °C) following Bergmeyer procedures[12] with a Beckman DU-6 UV/vis spectrophotometer. TLC plates were developed with CH_2Cl_2/ether = 3:1 v/v with a silica gel plate coated on plastic film and the compound was detected with anisaldehyde reagent.[13] Microanalyses (C and H) were carried out at Galbraith Laboratories, Inc. 2-Acetylfuran (**6a**) was prepared by the method of Heid and Levine.[14] 2-(Trifluoroacetyl)furan (**6b**) was prepared by the method of Clementi and Marino.[15] Reduction of **6a** to (*RS*)-**1** was done with $NaBH_4$/aqueous EtOH as usual. (+)-MPTA esters were prepared according to the procedures described previously.[16]

2-Acetyl-3,4-dimethoxyfuran (6c). *N,N*-Dimethylacetamide (5 mL, freshly distilled from CaH_2) was cooled to 0 °C under N_2. Phosphorus oxychloride was added dropwise (3.1 g, 20.3 mmol) and the resulting mixture was stirred for 15 min. A solution of 3,4-dimethoxyfuran[17] (1.3 g, 10 mmol) in *N,N*-DMA (5 mL) was added to the preformed "Vilsmeier" reagent. The mixture was warmed to room temperature and then to 55 °C (oil bath) for 6 h. The dark reaction mixture was poured into aqueous 10% $NaHCO_3$ solution and extracted with ether. The combined ether extracts were processed as usual, giving the crude ketone as a dark solid. Purification by column chromatography (silica gel, Baker 60–200 mesh; 20% EtOAc/hexane) gave 0.45 g of the ketone as yellow-orange prisms, mp 49–50 °C, in 28% yield: 90-MHz ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3 H), 3.80 (s, 3 H), 4.12 (s, 3 H), 6.14 (s, 1 H); 22.5-MHz ^{13}C NMR δ 26.21, 58.19, 60.04, 126.81, 137.76, 143.29, 144.10, 185.24; IR (film) 3110, 2950, 1650, 1550, 1465, 1430, 1370, 1320, 1190, 1010 cm^{-1}. Anal. Calcd for $C_8H_{10}O_4$: C, 56.47; H, 5.88. Found: C, 56.88; H, 6.01.

Enzymatic Reduction of 6a and 6b: Preparation of (*S*)-1 and 7b. A solution of glucose-6-phosphate (G-6-P, 9.5 mmol) and triethanolamine hydrochloride (1.0 g) in water (70 mL) was adjusted to pH 7.7 with aqueous NaOH. Compound **6a** (1 g, 9.1 mmol), NADP (50 mg, 0.05 mmol), aqueous $MgCl_2$ (0.5 mL, 1 M), TADH (300 mg), and G-6-P dehydrogenase (2 mg) were added. The solution was stirred at room temperature and the pH was periodically adjusted to 7.7 with aqueous NaOH. After 24 h enzymatic assay of G-6-P indicated that the reaction was complete. The solution was extracted with ether (3 × 20 mL) and the combined ether extracts were evaporated under reduced pressure. Methylene chloride (25 mL) was added, the layers were separated, and the organic layer was dried over Na_2SO_4. Removal of solvent under reduced pressure gave (*S*)-**1** (0.9 g, 8 mmol, 88%): 90-MHz ^{1}H NMR ($CDCl_3$) δ 7.3 (d, 1 H), 6.1–6.3 (m, 2 H), 4.8 (q, 1 H), 2.3 (br s, 1 H), 1.5 (d, 3 H); $[\alpha]^{25}_D$ −18.4° (*c* 6, EtOH), 89% ee (lit.[8] −17.0). Anal. Calcd for $C_6H_8O_2$: C, 64.29; H, 7.14. Found: C, 64.31; H, 7.10. 200-MHz ^{1}H NMR of the (+)-MPTA ester: 7.2–7.5 (m, 6 H), 6.3 (m, 2 H), 6.2 (q, 1 H), 3.5 (m, 3 H); (*S*)-(+) isomer 1.69 (d, 3 H), (*R*)-(−) isomer 1.62 (d, 3 H). A similar procedure was used to prepare (*R*)-**7b** (0.9 g, 5.36 mmol, 90%): 90-MHz ^{1}H NMR ($CDCl_3$) δ 7.43 (d, 1 H, H-5, $J_{4,5}$ = 1.93 Hz), 6.49 (d, 1 H, H-3, $J_{3,4}$ = 3.3 Hz), 6.39 (q, 1 H, H-4), 5.02 (q, 1 H, $CHCF_3$, $J_{H,F}$ = 6.53 Hz), 3.68 (br s, 1 H, OH). Anal. Calcd for $C_6H_5O_2F_3$: C, 43.37; H, 3.01. Found: C, 43.30; H, 3.08. GC: the major diastereomer of the MPTA ester of (*R*)-**7b** had a retention time of 17.54 min, while the minor isomer, (*S*)-**7b**, had a retention time of 19.27 min.

Chiral Purity Determination on (*S*)-1 and 7b.[18] To a solution of $NaIO_4$ (0.54 g) in H_2O (2 mL) were added CH_3CN (3 mL), CCl_4 (3 mL), CCl_4 (2 mL), and $RuO_2 \cdot nH_2O$ (1.0 mg). This mixture was stirred at room temperature for 20 min and the MTPA ester of (*S*)-**1** (0.15 mmol in 0.5 mL CH_3CN) was added

(9) Peters, H. M.; Feigl, D. M.; Mosher, H. S. *J. Org. Chem.* **1968**, *33*, 4245.
(10) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294. Chen, C. S.; Sih, C. J. *Ibid.* **1982**, *104*, 7294.
(11) Ziffer, H.; Kawai, K.-I.; Kasai, M.; Imuta, M.; Froussios, C. *J. Org. Chem.* **1983**, *48*, 3017.
(12) Bergmeyer, H. U. *Methods of Enzymatic Analysis*; Academic Press: New York, 1974.
(13) Gordon, A. J.; Ford, R. A. *The Chemist's Companion*; Wiley-Interscience: New York, 1972; p 379.
(14) Heid, J. V.; Levine, R. *J. Org. Chem.* **1948**, *13*, 409.
(15) Clementi, S.; Marino, G., *J. Chem. Soc., D* **1970**, 1642.
(16) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(17) McDonald, E.; Suksamrarn, A.; Wylie, R. D. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1823. Iten, P. X.; Hoffman, A. A.; Eugster, C. H. *Helv. Chim. Acta* **1978**, *35*, 430.
(18) Danishefsky, S. J.; Pearson, W. H.; Segmuller, B. E. *J. Am. Chem. Soc.* **1985**, *107*, 1280.

followed by more H_2O (0.5 mL). After being stirred for 2 h at room temperature, water (10 mL) was added, the mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with ether (2 × 6 mL) and the extracts were combined with the first organic layer. The solution was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was dissolved in methanol (10 mL) containing NaOH (0.5 g). The solution was allowed to stir at room temperature overnight after which the solvent was removed under reduced pressure. To the residue was added water (10 mL) and the pH of the solution was adjusted to 7.0 with 1 N HCl. This solution was then assayed enzymatically[12] for D- and L-lactate to determine the optical purity of 1. For compound **7b**, chiral purity was determined by comparison of the sign of the optical rotation of the MTPA derivative obtained before NaOH hydrolysis of a sample of the same material prepared from authentic (*R*)-(trifluoromethyl)phenylcarbinol.[4a]

Esters of Furylmethylcarbinol (*RS*)-8a and (*RS*)-8b. To a solution of furylmethylcarbinol (3.5 g, 31 mmol) in pyridine (20 mL) was added acetic anhydride (3.5 mL, 37 mmol). The solution was stirred for 12 h at room temperature, then 15 mL of pyridine was removed under reduced pressure, and the remaining residue was dissolved in $CHCl_3$ (100 mL). The solution was washed with aqueous $CuSO_4$ solution (0.2 M, 4 × 30 mL) and water (30 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give (*RS*)-**8a** (4.05 g, 26 mmol, 84%): 90-MHz ^1H NMR δ 7.3 (s, 1 H) 6.3 (s, 2 H), 5.9 (q, 1 H), 2.05 (s, 3 H), 1.6 (d, 3 H). To prepare (*RS*)-**8b**, a similar procedure was followed except that octanoyl chloride was used and the reaction was done in 10 min (0.42 g, 82%): 90-MHz ^1H NMR ($CDCl_3$) δ 7.37 (m, 1 H), 6.32 (s, 2 H), 5.59 (q, 1 H), 2.30 (t, 2 H), 1.55 (d, 3 H), 1.26 (b, 10 H), 0.90 (t, 3 H). Anal. Calcd for $C_{14}H_{22}O_3$: C, 76.19; H, 7.48. Found: C, 76.38; H, 7.55.

Lipase-Catalyzed Esterification of (*RS*)-1 with Octanoic Acid and Hydrolysis of the Ester Product 8b. Compound (*RS*)-1 (6.0 g, 50 mmol) and octanoic acid (10 mL, 63 mmol) were added to water-saturated hexane (85 mL). Candida lipase (CCl, 2.0 g) was added and the mixture was stirred at room temperature. After 5 days, NMR integration showed that the reaction was 52% completed. The enzyme was removed by centrifugation. The solution was extracted with water (3 × 40 mL). The combined aqueous layers were extracted with ether (3 × 60 mL). The combined ether layers were dried over Na_2SO_4, evaporated, and distilled (92–95 °C/50 mmHg) to give (*S*)-1 (1.8 g, 30% yield, 52% ee). ^1H NMR data are the same as the racemic form described above. The hexane layer was separated and distilled to give (*R*)-**8b** (125 °C/0.75 mmHg), 4.8 g, 40% yield, $[\alpha]^{25}_D$ +80.93° (*c* 1.23, $CHCl_3$). ^1H NMR data are the same as the racemic form obtained above. This ester (*R*)-**8b** (2.5 g) was subjected to CCL-catalyzed hydrolysis in a phosphate buffer (0.5 mM, pH 7.5, 1 g of enzyme) until 25% conversion. The carbinol (*R*)-1 was isolated as before in 25% yield (0.6 g), $[\alpha]^{25}_D$ +19.09° (*c* 1.10, $CHCl_3$), 94% ee. When (*RS*)-**8b** (1 g) was used as a substrate for the cholesterol esterase from *Pseudomonas fluorescens* (0.4 g), the reaction reached 20% completion in 20 min to give (*R*)-1 in 92% ee.

Papain-Catalyzed Hydrolysis of 9a–c: (*R*)- and (*S*)-9a–c. Compound **9a**[5] (7.5 g, 24 mmol) was dissolved in 8 mL of DMF. Water (300 mL), phosphate buffer (2 mL, 0.1 M), and β-mercaptoethanol (0.2 mL) were added and the solution was adjusted to pH 7.0 with 2 M HCl. Papain (800 mg × 2 crystallized) from Sigma was added. The pH was kept at 7.0 with the addition of 0.1 M NaOH. After 13 h and 50% hydrolysis the pH was adjusted to 8.0 and the mixture was extracted twice with ethyl acetate (150 mL). The ethyl acetate extracts were combined and washed with 100 mL of 5% potassium bicarbonate and drived over anhydrous $MgSO_4$. The mixture was evaporated to give 3.4 g (45% yield) of (*R*)-**9a** $[\alpha]^{25}_D$ −109.5° (*c* 1, $CHCl_3$). Enantiomeric excess was determined by using 200-MHz ^1H NMR and the chiral shift reagent tris[[(3-heptafluoropropyl)hydroxymethylene]-(+)-camphorato]europium(III) derivative ($Eu(hfc)_3$) from Aldrich with the addition of approximately 1 mg of shift reagent/mg of sample. The methoxy protons of the D and L esters were clearly separated by 20 Hz at 4.15 and 4.05 ppm, respectively. Subsequent integration allowed the determination of a 97% ee. The aqueous layer of the 50% conversion portion was acidified to pH 3 and extracted with ether (2 × 100 mL). After drying, the solution was treated with a slight excess of diazomethane (12 mmol) in ether for 30 min and evaporated to give 2.4 g (31% yield) of (*S*)-**9a**: $[\alpha]^{25}_D$ +108.6° (*c* 1, CHCl); 97% ee. The NMR data of both (*S*)-**9a** and (*R*)-**9a** are identical with those of the racemate reported previously.[5,15] The same procedure was applied to the syntheses of compound **9b** and **9c**. The physical data are the following.

(***R***)-**9b**: 42% yield, $[\alpha]^{25}_D$ −135.2° (*c* 1.4, $CHCl_3$), 97% ee, mp 55–57 °C; ^1H NMR ($CDCl_3$) δ 1.23 (t, 3 H), 3.75 (s, 3 H), 4.12 (q, 2 H), 5.51 (d, 1 H), 5.67 (d, 1 H), 6.35 (s, 2 H), 7.38 (s, 1 H). Anal. Calcd for $C_{10}H_{13}O_5N$: C, 52.86; H, 5.73. Found: C, 52.91; H, 5.41.

(***R***)-**9c**: 44% yield, $[\alpha]^{25}_D$ −50.5° (*c* 1, CH_3OH), mp 76–78 °C; ^1H NMR (DMSO) δ 3.66 (s, 3 H), 5.07 (s, 2 H), 5.40 (d, 1 H), 6.43 (s, 2 H), 7.35 (s, 5 H), 7.63 (s, 1 H), 8.36 (s, 1 H). Anal. Calcd for $C_{15}H_{15}O_5N$: C, 62.28; H, 5.23. Found: C, 62.44; H, 4.91.

Preparation of Optically Active 13. Compound (*R*)-**9b** (2.531 g, 11.88 mmol) was dissolved in 15 mL of anhydrous THF and added to a cold (−60 °C) solution of LAH (0.451 g, 11.88 mmol) in 15 mL of ether. The reduction was complete after stirring for 1 h at 50 °C (TLC, 50% ethyl acetate/hexanes). The reaction was quenched at −60 °C by the sequential addition of distilled water (0.5 mL), 20% aqueous NaOH solution (0.5 mL), and distilled water (1.35 mL); then it was warmed to room temperature. Suction filtration removed the white precipitate of aluminum compounds, which were thoroughly triturated with ethyl acetate and with ethanol. Evaporation (Rotavap) of the combined organic phases afforded 2.06 g of crude produce (**10**) in 93% yield; $[\alpha]^{25}_D$ −36° (*c* 0.25, EtOH). Without further purification, the alcohol was dissolved in 10 mL of dry THF and added to a suspension of NaH (0.401 g, 16.71 mmol, 1.5 equiv) in 10 mL of dry THF. Hydrogen was evolved and cyclization was complete in 20 min (TLC, 90% ethyl acetate/hexane). The reaction was quenched with saturated aqueous NH_4Cl solution, and the solvent was removed in vacuo. The residue was taken up with ethyl acetate. The combined extracts were dried by passage through Na_2SO_4 and evaporated to afford 1.31 g (83%) of oxazolone 11. A chromatographed sample of 11 had $[\alpha]^{25}_D$ +9.3° (*c* 0.61, EtOH). Not unexpectedly, its IR, ^1H (90 MHz) and ^{13}C (22.5 MHz) NMR, and mass spectra were indistinguishable from those of the racemic compound.[19] Oxazolone 11 (85 mg, 0.55 mmol) was dissolved in 2 mL of dry MeOH and 2 mL of dry ether and cooled to 60 °C. Next, bromine (88.7 mg, 0.55 mmol, 29 μL) was added. After 3 h the reaction was complete (TLC, 50% EtOAc/hexane). Anhydrous NH_3 was bubbled through the solution for 2 min; then the mixture was warmed to room temperature. The MeOH was evaporated and the residue was taken up with CH_2Cl_2 to give 115 mg (96%) of the dihydrofuran **12** as a mixture of four stereoisomers. This crude mixture retained optical activity: $[\alpha]^{23}_D$ +8.40° (*c* 1.1, EtOH). Without further purification, the compound was dissolved in 10 mL of ethyl acetate and hydrogenated by using 100 mg of 5% $Rh(Al_2O_3)$, at room temperature, under 1500 psi of H_2 (Parr bomb). Upon completion of the reaction (3 h), the catalyst was filtered off and the solvent was evaporated to afford 102.5 mg (92%) of the tetrahydrofuran. Without purification, the latter was dissolved in 1.15 mL of freshly distilled THF (LAH) containing 17.0 μL of distilled water (2 molar equiv vs substrate). Trifluoromethanesulfonic acid was added (10.7 mg, 0.0071 mmol, 6.3 μL, 15 mol ° vs substrate) and the solution was stirred at room temperature for 2 h. After this time, the reaction was complete (TLC, 100% EtOAc). Saturated aqueous $NaHCO_3$ solution was added (1 mL) and the mixture was extracted with ethyl acetate. Evaporation of the extracts and chromatography gave 67 mg of compound **13** (77%). Recrystallization from THF (−78 °C) gave pure compound: mp 111–112 °C; $[\alpha]^{25}_D$ 64.0° (*c* 0.29, EtOH). The optical purity of **13** was determined by a 300-MHz ^1H NMR shift study. Using $Eu(hfc)_3$ as the chiral shift reagent, the resonances of the methoxy groups in the (+) and in the (−) antipode of **13** were base line separated, and readily integrable, in both racemic and optically active **13**. An enantiomeric purity of 98% was thus determined, corresponding to 96% ee. Anal. Calcd for $C_8H_{11}O_4$: C, 52.17; H, 5.98. Found: C, 52.20; H, 5.89. This material is now available for the synthesis of other alkaloids.[19]

Stereochemical Correlation of (+)-11 with L-Serine. Ruthenium dioxide monohydrate (4.3 mg, 0.033 mmol) was added

(19) Ciufolini, M. A.; Wood, C. Y. *Tetrahedron Lett.* **1986**, *27*, 5085.

to a stirred mixture of sodium metaperiodate (10.13 g, 47 mmol), water (40 mL), carbon tetrachloride (40 mL), and acetonitrile (60 mL). After 30 min, oxazolone (+)-11 (500 mg, 3.3 mmol) in 2 mL of acetonitrile was added.[20] After 2 h at room temperature, the mixture was evaporated to dryness (Rotavap) and the solid residue was extracted three times with hot ethyl acetate (50-mL portions). The yield of 4-carboxy-2-oxazolidone was 64 mg. This crude compound had $[\alpha]^{25}_D$ −14.0° (c 0.64, H_2O). The rotation of the same acid obtained by phosgenation of L-serine has been reported as $[\alpha]^{25}_D$ −17°,[21] thus establishing the S,D absolute stereochemistry for (+)-11.

Methyl 2,3,6-Trideoxy-L-hex-2-enopyranosid-4-ulose (4 and 5). To a solution of (S)-1 (0.5 g, 4 mmol) in a mixture of anhydrous ether (2 mL) and absolute methanol (3 mL) kept at 23 °C (dry ice–hexanes) was added bromine (1 g, 5 mmol) in MeOH (4 mL) gradually with stirring. The reaction mixture was stirred for another 30 min, then saturated with gaseous NH_3 to pH 8, and allowed to warm to room temperature. After filtration to remove NH_4Br, the solution was filtered off and was evaporated to give 0.5 g of compound 2 (85% yield): ^1H NMR ($CDCl_3$) δ 6.22 (m, 1 H), 5.79 (m, 1 H, 4.62 (s, 1 H), 4.38 (m, 1 H), 3.80 (m, 1 H), 3.20, (d, 3 H), 3.10, (d, 3 H), 1.38 (m, 3 H). Crude 2 (0.5 g) prepared above was dissolved in 2% H_2SO_4 (2 mL) and the solution left for 90 min at room temperature. The reaction mixture was brought to pH 4 with $NaHCO_3$. Water was removed in vacuo below 30 °C and the residue dissolved in 20 mL of ether. The ether was dried ($MgSO_4$) and evaporated to give 3: ^1H NMR δ 6.88 (m, 1 H), 6.08 (m, 1 H), 5.58 (m, 1 H), 4.64 (m, 1 H), 3.90 (br, 1 H) 1.32 (d, 3 H). A solution of 3 (0.6 g) and methyl orthoformate (0.32 g, 5 mmol) in absolute ether (20 mL) was chilled to 0 °C and 5 drops of $SnCl_4$ slowly added with stirring. After 45 min the reaction was quenched with triethylamine. The ethereal layer was washed three times with water and dried with anhydrous $MgSO_4$. Evaporation of solvent left 0.22 g of crude product which was purified by silica gel chromatography (2 × 50 cm) with CH_2Cl_2 as eluent. Fractions of the major component (R_f 0.45) were collected to give 0.23 g of 4 and 5 in a 2/1 ratio: ^1H NMR ($CDCl_3$) δ 6.88 (m, 1 H), 6.10 (m, 1 H), 5.28 (s, β-1H), 5.08 (d, α-1H), 3.52 (s, β-3H), 3.50 (s, α-3H), 1.50 (d, β-3H), 1.38 (d, α-3H); ^{13}C NMR ($CDCl_3$) δ 146.43 (C-3), 143.15 (C-2), 96.70 (β, C-1), 94.34 (α, C-1), 75.29 (β, 1c), 55.39 and 55.93 (OCH_3), 17.09 (C-6). Anal. Calcd for $C_7H_{10}O_3$: C, 59.12; H, 7.10. Found: C, 59.20; H, 7.11.

Acknowledgment. Work at Texas A&M was supported by the NSF (CHE-8318217) and the Robert A. Welch Foundation (A-100A); that at Rice University was supported by the Robert A. Welch Foundation (C-1007). D.G.D. and C.F.B. are NSF Graduate Fellows.

(20) Asami, M.; Ohno, H.; Kobayashi, S.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1869.
(21) Kaneko, T.; Takeuchi, I.; Inui, T. *Bull. Chem. Soc. Jpn.* **1986**, *41*, 974.

Enzymes in Organic Synthesis. 41.[1] Stereoselective Horse Liver Alcohol Dehydrogenase Catalyzed Reductions of Heterocyclic Bicyclic Ketones[2]

Lister K. P. Lam, Iain A. Gair, and J. Bryan Jones*

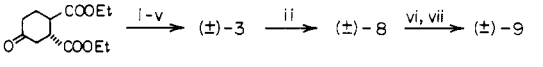
Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Received October 26, 1987

Preparative-scale horse liver alcohol dehydrogenase catalyzed reductions of racemic cis and trans bicyclic O- and S-heterocyclic ketones proceed with high enantiomeric selectivity. The diastereotopic selectivity for the pro-R faces of the carbonyl groups is also very high. The ee's of all but one of the product alcohols are >97%. The ee's of the recovered ketones are in the 52–60% range. The results confirm that an ether-oxygen or -sulfur substituent does not alter the enzyme's overall structural specificity or stereospecificity toward its ketone substrates.

While the application of enzymes in asymmetric synthesis is now well-documented, it remains a rapidly developing field.[3] One of the most versatile enzymes in this regard is horse liver alcohol dehydrogenase (HLADH[4]), which is a commercially available nicotinamide cofactor dependent enzyme that catalyzes stereospecific C=O ⇌ CH(OH) interconversions of a broad structural range of ketone and alcohol substrates.[2a,5] So far, relatively few HLADH-specificity studies have included substrates containing heteroatoms.[6] The present study examines the

Scheme I[a]



[a] (i) H^+, $HC(OEt)_3$; (ii) $LiAlH_4$; (iii) TsCl, pyr; (iv) Na_2S; (v) H^+, H_2O; (vi) Ph_3P, $C_6H_5CO_2H$, DEAD; (vii) $Ba(OH)_2$.

Table I. Relative Rates[a] of HLADH-Catalyzed Reductions of (±)-1–3

substr	rel rate	substr	rel rate
cyclohexanone	100	(±)-2	53
(±)-1	9	(±)-3	1.4

[a] Reduction rates were measured spectrophotometrically at 25 °C in 0.1 M phosphate buffer (pH 7) with [S] > 2 × 10^{-2} M and [NADH] = 1.75 × 10^{-4} M.

(1) Part 40: Toone, E. J.; Jones, J. B. *Can. J. Chem.*, in press.
(2) Abstracted from the University of Toronto Ph.D. Thesis of L.K. P.L. (1986) and M.Sc. Thesis of I.A.G. (1983).
(3) (a) Jones, J. B.; Beck, J. F. *Tech. Chem. (N.Y.)* **1976**, *10*, 107. Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (b) Wong, C., H.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. (c) Klibanov, A. M. *Science (Washington, D.C.)* **1983**, *219*, 722; *Chem. Technol.* **1986**, 354. (d) *Enzymes in Organic Synthesis*, Ciba Foundation Symposium 111; Porter, R., Clark, S., Eds.; Pitman: London, 1985. (e) *Biocatalysis in Organic Synthesis*; Tramper, J., van der Plas, H. C., Linko, P. Eds.; Elsevier: Amsterdam, 1985. (f) *Enzymes as Catalysts in Organic Synthesis*; Schneider, M., Ed.; Reidel: Dordrecht, Netherlands. (g) Sih, C. J.; Chen, C. S. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 570. (h) Butt, S; Roberts, S. M. *Natural Products Reports*; Royal Society of Chemistry: London, 1986; pp 489–503. (i) Sariaslanc, F. S.; Rosazza, J. P. N. *Enz. Microb. Technol.* **1984**, *6*, 242.
(4) Abbreviations used: HLADH, horse liver alcohol dehydrogenase; $Eu(fod)_3$, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)-europium(III); MTPA, (+)-(2R)-α-methoxy-α-(trifluoromethyl)-α-phenylacetate; NADH, reduced form of nicotinamide adenine dinucleotide.
(5) Dodds, D. R.; Jones, J. B. *J. Am. Chem. Soc.* in press, and leading references therein.

0022-3263/88/1953-1611$01.50/0