5.24 (d, J = 12 Hz, 1 H), 6.03 (br s, 1 H), 6.68 (q, J = 7 Hz, 1 H), 1.2–5.2 (m signals); high-resolution MS, *m/e* 379.1961, calcd for $C_{20}H_{29}NO_6$ 379.1993.

(±)-Integerrimine (1). A solution of the MOM ether of integerrimine (**30**, 5.6 mg) in DME (1 mL) and 1 N H_2SO_4 (2 mL) was stirred at 40 °C for 2.5 h. Excess Na_2CO_3 and then acetonitrile were added, and the mixture was concentrated in vacuo. Methylene chloride was added and the solid residue was filtered off. Concentration of the filtrate and purification by preparative layer chromatography (alumina plate, eluted with chloroform–methanol, 60:1) afforded (±)-**integerrimine** (**1**, 4.2 mg, 86%); mp 162 °C (recrystallized from ethanol). IR (CH_2Cl_2 solution) 3540, 1710, 1650 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 3 H), 1.33 (s, 3 H), 1.77 (d, J = 7 Hz, 3 H), 4.13 and 5.42 (AB q, J = 12 Hz, 2 H), 4.33 (br s, 1 H), 5.03 (br s, 1 H), 6.23 (br s, 1 H), 6.55 (q, J = 7 Hz, 1 H), 2.0–4.0 (m signals); ^{13}C NMR ($CDCl_3$, ppm, relative intensity %) 178.3 (7.7), 169.2 (7.3), 136.9 (15.7), 135.3 (15.6), 134.0 (10.3), 131.8 (9.2), 77.2 (28.6), 76.6 (15.3), 75.6 (15.3), 62.8 (17.3), 61.0 (16.6), 53.2 (18.9), 39.5 (16.1), 33.9 (17.3), 29.6 (15.2), 25.2 (11.6), 14.2 (12.2), 11.9 (11.6); high-resolution MS, *m/e* 335.1773, calcd for $C_{18}H_{25}O_5N$ 335.1743.

NMR (^1H), mass spectra, and also chromatographic mobility were completely identical with those of natural integerrimine.

Acknowledgment. We are very grateful to Professor T. Mukaiyama for his helpful discussion. We are indebted to Professor C. C. J. Culvenor for his kind gift of authentic integerrimine and its NMR spectrum. We also thank Professor T. Furuya for providing the sample of retronecine and Dr. T. Murae for performing high-resolution mass spectra. This research received financial support from the Kurata Foundation and the Minstry of Education, Japanese Government (Grant-In-Aid 56209004).

Stereochemistry of the Ethanolamine Ammonia Lyase Reaction with Stereospecifically Labeled [1-$^2H_1$]-2-Aminoethanol[1]

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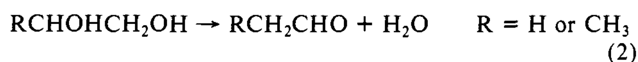
Abstract: The antipodes of [1-$^2H_1$]-2-aminoethanol (**10**) have been synthesized from (S)-[α-$^2H_1$]benzyl alcohol and separately subjected to the action of ethanolamine ammonia lyase in the presence of alcohol dehydrogenase, in order to determine whether the migration of a hydrogen atom from C-1 to C-2 during acetaldehyde formation is stereospecific. (R)-**10** reacts 4 times as fast as (S)-**10**, the isotope effect showing that the *pro-S* hydrogen migrates preferentially in each case. ^1H and ^{13}C NMR spectra of the ethanol formed show that (R)-**10** leads to CH_3CHDOH while (S)-**10** affords DCH_2CH_2OH, confirming the stereoselectivity of hydrogen transfer.

Ethanolamine ammonia lyase (ethanolamine deaminase, E. C.4.3.1.7), first described by Bradbeer[4] and purified and characterized by Kaplan and Stadtman,[5] is a clostridial cobamide dependent enzyme which catalyzes the conversion of ethanolamine to acetaldehyde and ammonia (eq 1). The reaction is directly

$$H_2NCH_2CH_2OH \rightarrow CH_3CHO + NH_3 \qquad (1)$$

analogous to the enzymatic reactions catalyzed by diol dehydrase, such as the dehydration of ethylene glycol or 1,2-propanediol (eq 2), and is related to other alkyl arrangements catalyzed by vitamin B_{12} coenzymes.



$$RCHOHCH_2OH \rightarrow RCH_2CHO + H_2O \qquad R = H \text{ or } CH_3 \qquad (2)$$

The extensive studies of Babior[6,7] have revealed that a hydrogen atom from C-1 of ethanolamine migrates to C-2 without exchanging with solvent protons but that it becomes transiently attached to C-5′ of the adenosine unit of the coenzyme en route to product. A deuterium kinetic isotope effect of 6.8 shows that this hydrogen transfer is part of the rate-determining step.[6] The oxygen atom of acetaldehyde was shown to originate from the substrate, not water.[6] Current understanding of the mechanism of this and related adenosylcobalamin-dependent rearrangements is summarized in recent reviews.[8]

Stereochemistry. Although neither substrate nor product in this enzymatic reaction is chiral, two "cryptic" stereochemical questions[9] originate in the prochiral methylene groups of ethanolamine. The first is the following: does the replacement of the amino group by hydrogen occur with retention, inversion, or racemization of configuration at C-2? This question has been answered by the elegant labeling experiments of Rětey et al.:[10] chirality is lost at C-2 in the rearrangement of (R)- or (S)-[2-$^2H_1$, $^3H_1$]-2-aminoethanol (eq 3), a rare example of racemization during

$$(R)\text{- or } (S)\text{-}H_2NCDTCH_2OH \rightarrow (R,S)\text{-}CHDTCHO \qquad (3)$$

biological formation of a chiral methyl group. This result has been interpreted[10] as implicating a freely rotating methylene radical intermediate at C-2. Remarkably, the related substrate

(1) Presented at the 31st Southeastern Regional Meeting of the American Chemical Society, Roanoke, VA, October 24, 1979.
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(3) (a) University of Georgia. (b) Tufts—New England Medical Center.
(4) Bradbeer, C. *J. Biol. Chem.* **1965**, *240*, 4669–4674, 4675–4681.
(5) Kaplan, B. H.; Stadtman, E. R. *J. Biol. Chem.* **1968**, *243*, 1787–1793, 1794–1803; *Method Enzymol.* **1971**, *17*, 818–824.
(6) Babior, B. M. *J. Biol. Chem.* **1969**, *244*, 449–456.
(7) Babior, B. M. *J. Biol. Chem.* **1970**, *245*, 6125–6133 and preceding papers. Silverman, R. B.; Dolphin, D.; Babior, B. M. *J. Am. Chem. Soc.* **1972**, *94*, 4028–4030.
(8) (a) Babior, B. M. *Acc. Chem. Res.* **1975**, *8*, 376–384. (b) Babior, B. M.; Krouwer, J. S. *Crit. Rev. Biochem.* **1979**, *6*, 35–102. (c) Johnson, A. W. *Chem. Soc. Rev.* **1980**, *9*, 125–141. (d) Babior, B. M. "B_{12}"; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 2, Chapter 10.
(9) Hanson, K. R.; Rose, I. A. *Acc. Chem. Res.* **1975**, *8*, 1–10.
(10) Rětey, J.; Suckling, C. J.; Arigoni, D.; Babior, B. M. *J. Biol. Chem.* **1974**, *249*, 6359–6360.

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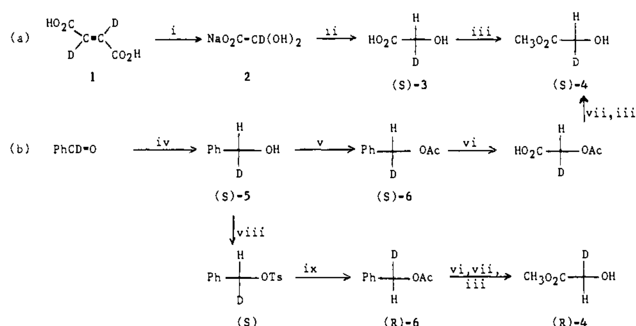
Scheme I[a]



[a] Reagents: (i) $NaIO_4$, OsO_4, Na_2CO_3; (ii) NAD, lactate dehydrogenase, alcohol dehydrogenase; (iii) CH_2N_2; (iv) bakers yeast; (v) Ac_2O, pyr; (vi) O_3, H_2O_2, AcOH; (vii) 4 N HCl; (viii) TsCl, pyr; (ix) KOAc, 18-crown-6 ether, benzene.

(2*S*)-2-amino-1-propanol undergoes enzymatic deamination with retention of configuration at C-2, while its enantiomer rearranges with inversion of configuration.[11]

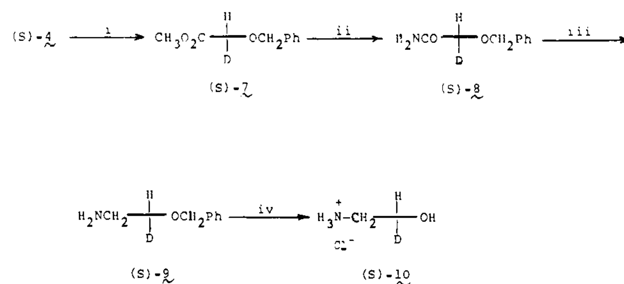
This paper addresses the second stereochemical question: is the selection of migrating hydrogen stereorandom, or does either the *pro-R* or *pro-S* hydrogen rearrange specifically? From the observation that the rate of deamination of (*R*,*S*)-H_2NCH_2CHTOH decreased with time, Babior deduced that the enzyme *does* discriminate between the enantiotopic hydrogens at C-1, and that consequently the unreacted substrate became progressively more enriched in that enantiomer in which tritium replaces the hydrogen selected for transfer.[6] We have now directly confirmed the stereoselectivity of ethanolamine ammonia lyase in distinguishing the C-1 hydrogens in the deamination of stereospecifically labeled substrates and report here the stereochemical identity of the migrating hydrogen.

Synthesis of Labeled Substrates. An unambiguous solution to this stereochemical question requires the use of substrates in which the *pro-R* and *pro-S* hydrogens of the C-1 methylene group are differentiated by isotopic labeling. We chose to approach the synthesis of chirally labeled ethanolamine through [2-$^2H_1$]glycolic acid (3) as the key intermediate. Optically active 3 can be prepared enzymatically by reduction of sodium [$^2H_1$]glyoxylate dihydrate (2) with muscle lactate dehydrogenase and NADH,[12] and the absolute configuration of the enzymatically formed (−) acid was shown to be *S* by neutron and X-ray diffraction of the ^6Li salt,[12] taking advantage of the anomalous neutron scattering amplitude of ^6Li. An independent configurational assignment was achieved by Arigoni and Weber[13] by chemical conversion of (*S*)-(−)-3 to (*S*-(−)-[1-$^2H_1$]ethanol; thus 3 is a chiral deuterated primary alcohol of firmly established configuration.

While we were able to repeat the enzymatic preparation of (*S*)-3 without difficulty (Scheme I, path a), this method is limited to relatively small-scale preparations and affords only one enantiomer. We therefore devised a new route to 3 (Scheme I, path b) which allows the inexpensive preparation of both *R* and *S* antipodes in gram quantities.[14] Our synthesis begins with (*S*)-(+)-[α-$^2H_1$]-benzyl alcohol 5, available on a large scale by reduction of [α-$^2H_1$]benzaldehyde with either fermenting yeast,[15] glucose 6-phosphate dehydrogenase,[16] or 3-pinanyl-9-BBN.[17] The absolute configuration of 5 has been conclusively established.[15,18] Ozonolysis of its acetate 6 followed by oxidative workup gave (*S*)-[2-$^2H_1$]glycolic acid, isolated in 27–31% yield as its methyl ester 4. The deuterated benzyl alcohol prepared by yeast reduction is essentially enantiomerically pure[15] but contains only 90% deuterium, and the methyl glycolate 4 prepared by this short sequence is assumed to be of the same high optical purity.

Access to the antipodal series was gained by inverting the configuration of the deuterated benzyl alcohol. Clean inversion was effected by displacement of the tosylate with potassium acetate, using a crown ether to solubilize the nucleophile in a nonpolar solvent (benzene) to assure bimolecular substitution.[19] The acetate obtained had an optical rotation equal in magnitude but opposite in sign to that of the *S* enantiomer, and was ozonized to (*R*)-3.

Scheme II[a]



[a] Reagents: (i) $PhCH_2Br$, Ag_2O; (ii) NH_3; (iii) BH_3·THF; (iv) H_2/Pd, HCl.

Table I

substrate	rate[a]
$H_2NCH_2CH_2OH$	9.85
(*R*)-H_2NCH_2CHDOH	8.83
(*S*)-H_2NCH_2CHDOH	2.23
$H_2NCH_2CD_2OH$	1.95

[a] μmol/min/mg enzyme. Each run contained 350 nmol NADH (Sigma), 13 nmol of 5′-deoxyadenosylcobalamine (Sigma), 17.5 units of yeast alcohol dehydrogenase (Sigma), 175 μmol of potassium phosphate buffer, pH 7.4, and 350 nmol of substrate in a total volume of 3.5 mL. The enzyme (22 μg, 0.22 units) was added, and the decrease in absorbance of NADH at 340 nm was followed. The decrease remained linear for the first 50% of reaction.

Conversion of the enantiomers of deuterated glycolic acids to labeled ethanolamines was straightforward (Scheme II). Methyl glycolate (4) was protected as the benzyl ether 7 and converted to the crystalline amide 8. BH_3·THF smoothly reduced the amide function to the primary amine 9, from which the benzyl group was removed by catalytic hydrogenolysis. The synthetic samples of (*R*)- and (*S*)-[1-$^2H_1$]-2-aminoethanol, isolated and stored as the crystalline hydrochlorides 10, had optical rotations of equal magnitude but opposite sign. Gani and Young have reported the synthesis of (*R*)-10 and (1*S*,2*RS*)-[1,2-$^2H_1$]-2-aminoethanol by decarboxylation of appropriately deuterated serine samples.[20]

Enzymatic Deamination. Enzymatic runs were carried out with purified enzyme[5,6] as described earlier,[6] with yeast alcohol dehydrogenase and NADH added to the lyase, substrate, and coenzyme in order to immediately reduce the acetaldehyde product to ethanol. The rate of reaction was followed spectrophotometrically by the decrease of absorption of NADH at 340 nm. Immediate evidence of stereoselectivity in the ammonia lyase reaction was seen in the relative rates of reaction of the enantiomeric

(11) Diziol, P.; Haas, H.; Rētey, J.; Graves, S. W.; Babior, B. M. *Vitam. B12, Proc. Eur. Symp., 3rd* **1979** 485–488; *Chem. Abstr.* **1980**, *92*, 176459; *Eur. J. Biochem.* **1980**, *106*, 211–224.

(12) Johnson, C. K.; Gabe, E. J.; Taylor, M. R.; Rose, I. A. *J. Am. Chem. Soc.* **1965**, *87*, 1802–1804.

(13) Weber, H. Ph.D. Dissertation No. 3591, Eidg. Tech. Hochschule, Zürich, 1965.

(14) For a preparation of both enantiomers of [2-$^3H_1$]glycolic acid, see: Meloche, H. P.; Luczak, M. A.; Wurster, J. M. *J. Biol. Chem.* **1972**, *247*, 4186–4191.

(15) Althouse, V. E.; Feigl, D. M.; Sanderson, W. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1966**, *88*, 3595–3599.

(16) Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890–4899.

(17) Midland, M. M.; Greer, S.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352–2355.

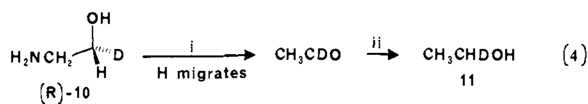
(18) (a) Horeau, A.; Nouaille, A. *Tetrahedron Lett.* **1966**, 3953–3959. (b) Streitwieser, A., Jr.; Wolfe, J. R., Jr.; Schaeffer, W. D. *Tetrahedron* **1959**, *6*, 338–344. (c) Brewster, J. H. *Tetrahedron Lett.* **1959**, 23–28.

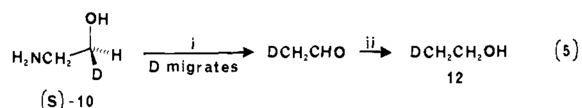
(19) Acetolysis of the tosylate of (−)-PhCHDOH in aqueous acetic acid leads to inverted alcohol whose optical rotation is less than one-third that of the starting material: Streitwieser, A., Jr.; Walsh, T. D.; Wolfe, J. R., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 3682–3685.

(20) Gani, D.; Young, D. W. *J. Chem. Soc., Chem. Commun.* **1982**, 867–869.

substrates (*R*)- and (*S*)-**10** compared with unlabeled and dideuterated substrates (Table I). (*R*)-**10** underwent deamination at approximately the same rate as unlabeled ethanolamine, 4–5 times faster than (*S*)-**10**, which reacted at about the same rate as the dideuterated substrate. The kinetic isotope effect shows that the migrating atom is mostly hydrogen in (*R*)-**10**; but mostly deuterium in (*S*)-**10**; in each case it is the *pro-S* hydrogen isotope that preferentially rearranges.

To confirm this conclusion, larger scale runs were made in which the ethanol was isolated by lyophilization and converted to the crystalline 3,5-dinitrobenzoate (DNB) for NMR analysis.[6] The spectra confirm the results shown in eq 4 and 5.





(i) ethanolamine ammonia lyase; (ii) alcohol dehydrogenase

In the proton NMR spectrum of **11**–DNB, the methylene group appears as the normal quartet, though of diminished intensity, while the methyl signal is a doublet, broadened by coupling with deuterium. Conversely, in the spectrum of **12**–DNB, the methyl signal is a triplet of decreased intensity while the methylene is a broad triplet. The carbon-13 spectra provide corroboration: the methyl signal at 14 ppm is a singlet in the spectrum of **11**–DNB but a 1:1:1 triplet in that of **12**–DNB, while the methylene signal at 63 ppm is a 1:1:1 triplet in the spectrum of **11**–DNB and a singlet in **12**–DNB.[21] The spectra show that, within the limits of NMR detection (<5%), hydrogen preferentially migrates in the rearrangement of (*R*)-**10** while deuterium preferentially migrates in the rearrangement of (*S*)-**10**. These results confirm the recent findings of Young and co-workers,[22] published while this paper was in preparation.

The preferential rearrangement of the 1-*pro-S* hydrogen in ethanolamine is consistent with the recent observation that ethanolamine ammonia lyase acts upon both enantiomers of 2-amino-1-propanol with migration of the 1-*pro-S* hydrogen, and that a tritiated hologenzyme introduces tritium into the 1-*pro-S* position of this substrate.[11] By contrast, the migration of hydrogen in the closely related diol dehydratase reaction is not so stereochemically consistent. In 1,2-propanediol the *pro-S* hydrogen of the 2*S* substrate migrates while the *pro-R* hydrogen of the 2*R* substrate migrates;[23] in glycerol the enzyme transfers the *pro-S* hydrogen of the *pro-R* branch,[24] while in ethylene glycol (of much higher symmetry) the enzyme does not differentiate between the enantiotopic hydrogens.[25] Nevertheless, these apparently contradictory results have been used by Rêtey and his collaborators to construct a mechanism rich in stereochemical detail[11,25] for the vitamin B_{12} catalyzed rearrangements of 1,2-diols and 1,2-amino alcohols, a picture that the present study supports in its finding of high stereoselectivity in the enzymic selection of the migrating hydrogen in ethanolamine.

(21) The NMR spectra show the presence of unlabeled ethanol, in amounts roughly equal to the deuterated ethanol produced in the enzymatic reaction, in both runs. This apparent loss of isotope has been observed by others[10,22] and attributed to dilution by ethanol present in commerical NADH preparations; in Young's study[22] of the ethanolamine ammonia lyase reaction, 45–48% of the ethanol isolated was concluded to originate in the NADH used. However, the NMR signals of the unlabeled ethanol are easily distinguished from those of the deuterated products and do not obscure the assignment of isotope location.

(22) Gani, D.; Wallis, O. C.; Young, D. W. *J. Chem. Soc., Chem. Commun.* **1982**, 898–899; *Eur. J. Biochem.* **1983**, *136*, 303–311.

(23) Zagalak, B.; Frey, P. A.; Karabatsos, G. L.; Abeles, R. H. *J. Biol. Chem.* **1966**, *241*, 3028–3035. Rêtey, J.; Umani-Ronchi, A.; Arigoni, D. *Experientia* **1966**, *22*, 72–73.

(24) Bonetti, V. Ph.D. Dissertation No. 5366, ETH Zürich, 1974.

(25) Rêtey, J. In "Stereochemistry"; Tamm, Ch., Ed.; Elsevier Biomedical Press: Amsterdam, 1982; Chapter 6.

Experimental Section

Melting points were determined in a Hoover apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 257 spectrometer. NMR spectra were recorded on Varian HA-100 and T-60 instruments, using tetramethylsilane as internal standard; the ^1H and ^{13}C spectra of the deuterated ethanol dinitrobenzoates were obtained on a JEOL FX-90Q FT spectrometer. Optical rotations were measured in a 1-cm cell using a Perkin-Elmer model 141 polarimeter. Mass spectra were obtained on a Finnigan 4023 GC–mass spectrometer.

[2,3-$^2H_2$]Fumaric Acid (1). Dimethyl [2,3-$^2H_2$]fumarate was prepared in 42% yield by reaction of dimethyl acetylenedicarboxylate with triphenylphosphine and ^2H_2O as described by Richards et al.[26] Hydrolysis of the ester with refluxing 1 N HNO_3 for 10 h gave the acid, mp 285 °C, in 67% yield.

Sodium [2-$^2H_1$]Glyoxylate Monohydrate[13] (2). [2,3-$^2H_2$]Fumaric acid (0.5 g) was dissolved in a solution of 0.25 g of sodium carbonate in 10 mL of water, 28 mg of OsO_4 was added, and the mixture was stirred until it dissolved. Sodium periodate (2.0 g) was added in small portions over 45 min to the stirred solution; by the end of the addition the dark brown color had lightened and a white solid precipitated. The mixture was stirred for an additional 1.5 h and filtered, and the solid was washed with a little water. The brown filtrate and washings were extracted with 10 mL of ether to remove the colored material, and the aqueous solution was continuously extracted with ether for 3 days. Concentration of the extract left an oily residue, which was taken up in 2 mL of water and brought to pH 7 with NaOH (total volume 7 mL) then diluted with 21 mL of acetone. The colorless crystals that precipitated on standing were filtered and dried to give 0.23 g (25%) of sodium [2-$^2H_1$]glyoxylate monohydrate: IR (KBr) 3240 (br, O—H), 2220, 2160 (weak, C—D), 1610 (C═O), 1395 (C—0) cm^{-1}. The NMR spectrum (D_2O) showed the O–H signal at δ 4.79 and only a small signal at δ 5.19 corresponding to 1–2% C–H.

(*S*)-[2-$^2H_1$]Glycolic Acid (3). A solution of 0.95 g of **2** in 15 mL of water, 50 mL of 0.02 M sodium phosphate buffer solution (pH 7.5), and 9.2 g of absolute ethanol was diluted with distilled water to 100 mL. To this solution was added 20 mg of NADH (Sigma), 17.5 mg of crystalline horse liver alcohol dehydrogenase (Worthington), and 0.1 mL (equivalent to 1 mg of protein) of muscle lactic dehydrogenase (Sigma), and the solution was incubated at 37 °C for 18 h. The mixture was heated at 85 °C for 5 min, filtered onto an ion-exchange column (16-g dry weight of AG-1 X8, acetate form), and washed successively with 120 mL of water, 120 mL of 0.5 N acetic acid, 120 mL of 1 N acetic acid, and 225 mL of 2 N acetic acid. This final fraction was concentrated to dryness and the residue dried over $CaCl_2$ in a vacuum desiccator to afford 0.21 g (32%) of the solid deuterated glycolic acid,[12,13] IR 1725 cm^{-1}.

(*S*)-[1-$^2H_1$]Benzyl Alcohol (5). [α-$^2H_1$]Benzaldehyde was prepared in 68% yield by the method of Kirby et al.;[27] NMR showed the deuterium content to be >98%. The deuterated aldehyde was reduced with fermenting bakers' yeast as described by Mosher et al.[15] The crude product was purified via the crystalline acid phthalate, $[\alpha]^{20}{}_D$ −0.504° (*c* 16.5, acetone), to afford the alcohol, $\alpha^{23}{}_D$ +1.508° (neat, *l* = 1) in 28% yield; Mosher et al.[15] report $\alpha^{24}{}_D$ + 1.43° for alcohol of 86% deuterium content. The methylene signal in the NMR at δ 4.46 appeared as a 1:1:1 triplet; integration showed the deuterium content to be 90 ± 2%. Corrected for 100% deuterium content, the calculated rotation of this sample is 1.68°, in close agreement with Mosher's calculated value of 1.66°. Our sample of PhCHDOH is thus optically pure, but contaminated with 10% of undeuterated benzyl alcohol.

(*S*)-[1-$^2H_1$]Benzyl Acetate (6). A solution of 8.6 g of (*S*)-**5**, 12 mL of acetic anhydride, and 30 mL of pyridine in 25 mL of ether was refluxed overnight. The mixture was poured into 100 mL of ice water and extracted with ether; the extracts were washed successively with dilute HCl, 2% $NaHCO_3$, and water and then dried over $MgSO_4$. Distillation gave 10.2 g (85%) of the *S* acetate: bp 58–61 °C (4 mm); $\alpha^{27}{}_D$ +0.385° (neat, *l* = 1). Streitwieser and Wolfe[28] report that alcohol of α_D −0.860° afforded acetate of α_D −0.208°, bp 99–100 °C (10–11 mm). The methylene signal in the NMR at δ 5.05 again appeared as a 1:1:1 triplet.

(*R*)-[1-$^2H_1$]Benzyl Acetate (6). A solution of 55 g of p-toluenesulfonyl chloride in 160 mL of pyridine was added dropwise to a solution of 16.8 g of (*S*)-**5** in 30 mL of pyridine held at −40 °C. After stirring for 6 h at −40 °C the solution was added to 1.5 L of ice water containing 125 mL of concentrated HCl. The solution was extracted with ether (3 × 300 mL), and the extracts were washed with cold 1 N HCl and brine,

(26) Richards, E. M.; Tebby, J. C.; Ward, R. S.; Williams, D. H. *J. Chem. Soc. C* **1969**, 1542–1544.

(27) Bennett, D. J.; Kirby, G. W.; Moss, V. A. *J. Chem. Soc. (C)* **1970**, 2049–2051.

(28) Streitwieser, A., Jr.; Wolfe, J. R., Jr. *J. Am. Chem. Soc.* **1957**, *79*, 903–907.

dried over $MgSO_4$ below 0 °C, and concentrated to about 100 mL. Hexane (300 mL) was added and the tosylate[29] was precipitated by cooling to -40 °C. The solid was collected and washed with hexane. Concentration of the filtrate gave a second crop, which was washed with 1:5 ether–hexane.

The combined crops of tosylate were immediately added to a suspension of 65 g of potassium acetate in 500 mL of benzene containing 3.6 g of 18-crown-6 ether, and the mixture was stirred at room temperature under nitrogen for 60 h. The mixture was washed with water, 2% $NaHCO_3$, and brine, dried over $MgSO_4$, and distilled to yield 13.9 g (60%) of the (*R*)-acetate: bp 82–84 °C (5 mm), α^{26}_D -0.392° (neat, *l* = 1).

Hydrolysis of 2.1 g of (*R*)-**6** with 2 N NaOH gave 1.1 g of (*R*)-[1-$^2H_1$]benzyl alcohol, α^{22}_D -1.399° (neat, *l* = 1).

Methyl (*R*)-[2-$^2H_1$]Glycolate (4). A stream of 3% ozone in oxygen was bubbled through a solution of 15.0 g of (*R*)-**6** in 250 mL of acetic acid and 25 mL of acetic anhydride at 25 °C at the rate of 0.6 mol/min for 15 h. The solution was stirred with 50 mL of 30% H_2O_2 for 24 h then stirred for 2 days with 100 mg of PtO_2 to destroy the excess peroxide. The mixture was filtered and concentrated at reduced pressure; the residue was taken up in water and extracted continuously with ether overnight. The extracts were dried ($MgSO_4$) and concentrated, and 3.8 g of unreacted starting material was recovered by distillation, bp 68–70 °C (2 mm). The residue from the distillation was taken up in ether and extracted with 5% $NaHCO_3$. The alkaline extracts were acidified and continuously extracted with ether overnight. Concentration of the extracts left a colorless oil, which was heated to reflux with 50 mL of 4 N HCl for 3 h. The acidic solution was continuously extracted with ether for 36 h, and the extracts were treated with ethereal diazomethane until the yellow color persisted. The solution was dried ($MgSO_4$), concentrated, and distilled bulb to bulb to afford 2.12 g (31%) of the ester: bp 100–140 °C (85 mm) [lit.[13] bp 90–100 °C (60–65 mm)]; IR 3500 (br, O—H), 2140 (C—D), 1720 (s, C—O) cm^{-1}; NMR ($CDCl_3$) δ 3.15 (br s, 1 H, OH), 3.80 (s, 3 H, OCH_3), 4.30 (br m, H, CHD).

Ozonolysis of (*S*)-**6** was carried out in the same manner and gave a 27% yield of (*S*)-**4**.

Methyl (*S*)-[2-$^2H_1$]-*O*-benzylglycolate[13] (7). A mixture of 0.38 g of (*S*)-**4**, 1.03 g of redistilled benzyl bromide, 1.0 g of silver oxide, and 15 mL of ether was stirred under reflux at 42 °C for 1 h. An additional 0.1 g of silver oxide was added and stirring continued at 42 °C for 2.5 h and 25 °C overnight. The solid was filtered and washed with 15 mL of ether, and the combined filtrate and washings were concentrated, taken up in 1 mL of hexane, and chromatographed on 15 g of silica gel. Elution with 850 mL of hexane gave mostly dibenzyl ether with a little glycolate benzyl ether, and elution with 125 mL of 4:1 benzene:hexane gave 0.45 g (59%) of pure **7**: $[\alpha]^{21}_D$ -1.43° (*c* 21.8, ethanol); IR (neat) 1750 (s, C═O), 1730 (sh), 1200, 1130 (C—O), 730, 690 cm^{-1}; NMR ($CDCl_3$) δ 7.38 (s, 5 H, aromatic), 4.64 (s, 2 H, $PhCH_2O$), 4.05 (t, *J* = 2 Hz, 1 H, CHD), 3.76 (s, 3 H, OCH_3); mass spectrum, *m/z* 181.

(*S*)-[2-$^2H_1$]-*O*-Benzylglycolamide. A solution of 0.42 g of (*S*)-**7**, 1 mL of methanol, and 50 mL of liquid ammonia was sealed in a pressure bottle and kept at 25 °C for 6 days. Evaporation of the solvents left 0.37 g (98%) of crude amide, which was recrystallized from 6 mL of water to give 0.31 g of colorless crystals: mp 90.5–91.5 °C (lit.[30] mp 92–93 °C), $[\alpha]^{23}_D$ +0.202° (*c* 10.9, $CHCl_3$); IR (KBr) 3370s, 3190s (*N*—H), 2140w (C—D), 1655s (C═O), 1460m, 1435m, 1325m, 1110s, 730m, 690m cm^{-1}; NMR ($CDCl_3$) δ 7.36 (s, 5 H, aromatic), 6.0–6.8 (br, 2 H, NH_2), 4.56 (s, 2 H, $PhCH_2O$), 3.95 (t, *J* = 2 Hz, 1.1 H, CHD).

Amide **8** could be prepared somewhat more conveniently in 54% overall yield from **4** without purification of ester **7**.

The *R* amide, mp 88–90 °C, $[\alpha]^{26}_D$ -0.10° (*c*, 8.7, $CHCl_3$), was prepared similarly from (*R*)-**4** in 44% yield.

(*R*)-[2-$^2H_1$]-2-Benzyloxyethylamine (9). A solution of 32 mL of 0.98 M borane in THF was added dropwise to a solution of 1.55 g of (*R*)-**8** in 30 mL of dry THF, and the mixture was refluxed 12 h. After cooling, 25 mL of 2 N HCl was added carefully, and the THF was removed under reduced pressure. The aqueous residue was made alkaline with 3.5 g of NaOH and extracted continuously with ether overnight. The ether extracts were dried over $MgSO_4$ and distilled bulb to bulb to give 1.12 g (79%) of the amine: bp 80–130 °C (1.5 mm) [lit.[31] bp 96–100 °C (4 mm)]; $[\alpha]^{20}_D$ +1.24° (*c* 28.8, methanol); $[\alpha]^{20}_D$ +1.316° (neat, *l* = 1); IR (neat) 3380m, 3040m, 2940m, 2870s, 2120w, 1600w, 1500m, 1460s, 1380w, 1205w, 1120s, 1025m, 850w, 730s, 690s cm^{-1}; NMR ($CDCl_3$) δ 1.81 (s, 2 H, NH_2), 2.82 (d, *J* = 5.5 Hz, 2 H, CH_2N), 3.45 (m, 1.15 H, CHDO*H*), 4.49 (s, 2 H, $PhCH_2O$), 7.30 (s, 5 H, aromatic).

The *S* antipode, $[\alpha]^{21}_D$ -1.260° (*c* 28.9, methanol), was prepared in the same way from (*S*)-**8** in 41% yield.

(*R*)-[1-$^2H_1$]-2-Aminoethanol Hydrochloride (10). A mixture of 600 mg of (*R*)-**9**, 50 mg of 10% Pd/C, 8 mL of methanol, and 0.5 mL of concentrated HCl was shaken under 3 atm of H_2 for 48 h. The catalyst was filtered and washed with methanol (6 × 2 mL). Concentration of the filtrate and washings left a solid residue, which was recrystallized from methanol–ethyl acetate to yield 247 mg (63%) of colorless solid: mp 77–81 °C; $[\alpha]^{22}_D$ +0.30° (*c* 9.2, water); IR (KBr) 3300s, 3060s, 2940w, 2160w, 1625m, 1585w, 1495s, 1465w, 1350m, 1330w, 1290w, 1270w, 1155m, 1110w, 1070m, 1055m, 988s, 955m, 940w, 925w cm^{-1}.

The *S* antipode, mp 77–81 °C, $[\alpha]^{25}_D$ -0.25° (*c* 9.5, water), was obtained in 68% yield in a similar reaction starting from (*S*)-**9**.

[1-$^2H_2$]-2-Aminoethanol was prepared as reported previously[6] by reduction of glycine ethyl ester with $LiAlD_4$.

Isolation of Deuterated Ethanol. Each reaction mixture contained 40 mg of the labeled 2-aminoethanol hydrochloride, 300 mg of NADH, 4500 units of yeast alcohol dehydrogense (Sigma), and 0.24 mg of coenzyme B_{12} (Sigma) in 40 mL of potassium phosphate buffer, 0.05 M (pH 7.4). This solution was incubated in the dark with 2 units of ethanolamine ammonia lyase at 35 °C. After 60 min, an additional 0.05 mg of coenzyme B_{12} and 1 unit of lyase were added. After another 35 min, an additional 0.06 mg of coenzyme B_{12}, 2 mg of alcohol dehydrogenase, 15 mg of NADH, and 1 unit of lyase were added, and reaction was continued for 3.5 h at 25 °C. The mixture was lyophilized and the aqueous ethanol collected was divided in half. Each portion was treated with 4 g of NaOH and 1 g of sodium acetate, cooled, and shaken vigorously for 35 min with a solution of 1.5 g of freshly distilled 3,5-dinitrobenzoyl chloride in 12 mL of benzene and 3 mL of petroleum ether. The reaction mixture was diluted with 40 mL of water and extracted with three 50-mL portions of ether. The extracts were washed with 1 N NaOH, water, 1 N HCl, and water, then dried over $MgSO_4$, and concentrated. The residue was recrystallized from methanol–water and dried over P_2O_5, giving 36 mg of ethyl 3,5-dinitrobenzoate, mp 89.0–89.5 °C. The ester was further purified by thick-layer chromatography on silica gel, elution with ether, and recrystallization from aqueous methanol.

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(29) Streitwieser, A., Jr.; Wolfe, J. R., Jr. *J. Am. Chem. Soc.* **1959**, *81*, 4912–4914.
(30) Benington, F.; Morin, R. D. *J. Org. Chem.* **1961**, *26*, 194–197.
(31) Harder, U.; Pfeil, E.; Zenner, K. F. *Chem. Ber.* **1964**, *97*, 510–519.