Synthesis of (4*R*)-D,L-[4-^{2}H]- and (4*S*)-D,L-[4-^{2}H]Homoserine Lactones

Douglas M. Kalvin and Ronald W. Woodard*

College of Pharmacy, The Univeristy of Michigan, Ann Arbor, Michigan 48109

Received September 11, 1984

The (4*R*)-D,L-[4-^{2}H]- and (4*S*)-D,L-[4-^{2}H]homoserine lactone hydrochloride salts were prepared from D,L-3-amino-3-(4-methoxphenyl)propanoic acid (1) in eight steps in an overall yield of 5.0%. Amino acid 1 was converted into D,L-3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)[1-^{2}H]propanal (4) in 57% yield through protection of the amino function, esterification, and DIBAL-D reduction of the methyl ester. The deuterio aldehyde 4 was reduced in a stereospecific manner with (*R*)- and (*S*)-Alpine boranes affording (1*S*)-D,L-3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)[1-^{2}H]propanol (**5a**) (73%) and (1*R*)-D,L-3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)[1-^{2}H]propanol (**5b**) (75%), respectively. The optical purity of deuterio alcohols **5a** (88% ee) and **5b** (76% ee) was determined from the 360-MHz ^{1}H NMR spectra of the corresponding diastereomeric *O*-acetylmandelates **6a** and **6b**. Alcohol **5a** was converted into (4*S*)-D,L-[4-^{2}H]-homoserine lactone hydrochloride in four steps in 12% yield: propionation, RuO_4 oxidative cleavage, aqueous ethanolic KOH hydrolysis, and saturated ethanolic HCl hydrolysis. In an analogous manner, alcohol **5b** was converted to (4*R*)-D,L-[4-^{2}H]homoserine lactone hydrochloride. The percent ee of the lactones at the 4-position was confirmed by enzymatic resolution of the D,L-homoserines, lactonization, and ^{1}H NMR analysis.

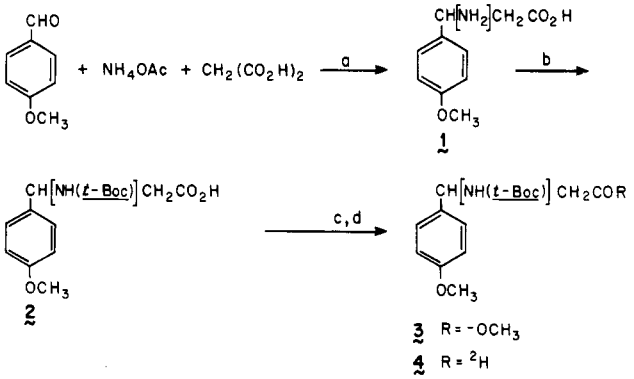
L-Homoserine (2-amino-4-hydroxybutanoic acid) is an important amino acid utilized in a variety of pyridoxal phosphate dependent enzymatic reactions[1a,b] which convert *O*-succinyl-L-homoserine, *O*-acetyl-L-homoserine, and *O*-phosphoryl-L-homoserine into L-cystathionine, L-methionine, and L-threonine, respectively. These reactions at the γ-carbon require both the abstraction of the α-hydrogen atom as a proton at an early step and the labilization of a substrate β-hydrogen atom in order to provide anchimeric assistance to eliminate the γ-substituent. The fully conjugated β,γ-unsaturated amine intermediate is common to both the γ-replacement (β-replacement in the case of L-threonine) and γ-elimination reactions.[1a]

In order to study the mechanism of these enzymatic reactions, it is necessary to prepare the (4*R*)-[4-^{2}H]- and (4*S*)-[4-^{2}H]homoserine derivatives. Fuganti et al.[2] prepared (4*R*)-D,L-[4-^{2}H]- and (4*S*)-D,L-[4-^{2}H]homoserine from the appropriate stereospecifically deuterated 3-phenylpropanols, which were derived from the reduction of cinnamaldehyde with fermenting Baker's yeast.[3] The homoserines were obtained in very poor yield from these phenylpropanols by acetylation of the alcohol function, benzylic bromination, displacement of the bromide with azide, reduction of the azide to the amine, and ozonolysis of the aromatic ring in formic acid. Detailed synthetic procedures were lacking in these reports.[2,3]

Recently Chang et al.[4] synthesized (4*R*)-L-[4-^{2}H]- and (4*S*)-L-[4-^{2}H]homoserine using a combination of both enzymatic and chemical procedures. Their key step was the reduction of aspartic semialdehyde using the enzyme L-homoserine dehydrogenase from E. coli. The stereochemistry of this enzymatic reduction was unknown. In an effort to determine the stereochemistry of this reaction the authors converted *one* of their chiral homoserines to 3-hydroxypropionate benzyl ester. The 3-hydroxylpropionate benzyl ester was subjected to oxidation with horse liver alcohol dehydrogenase (HLAD) and nicotinamide adenine dinucleotide (NAD). Their analysis of the stereochemistry of homoserine dehydrogenase, therefore, relied upon the hypothesis that HLAD removes only the *pro-R* hydrogen, not of ethanol, but of 3-hydroxylpropionate benzyl ester. This synthesis requires large amounts of relatively pure enzyme in order to produce practical quantities of chiral 4-deuteriohomoserines.

Since neither of the above procedures provided access to suitable amounts of (4*R*)-[4-^{2}H]- or (4*S*)-[4-^{2}H]homoserine, we decided to explore chemical methods which could be used to synthesize usable quantities. This paper reports the results of that study.

Scheme I[a]



[a] (a) 95% C_2H_5OH/H_2O, reflux 12 h. (b) (1) $(Boc)_2O$, 0 °C, 10 min, (2) room temperature, 3 h. (c) (1) DCC, (2) DMAP, CH_3OH, room temperature, 12 h. (d) (1) DIBAL-^{2}H, −78 °C, 6 min, (2) CH_3OH, NaK tartrate·$4H_2O$.

Results and Discussion

The synthetic sequence begins with the preparation of the aromatic β-amino acid, D,L-3-amino-3-(4-methoxyphenyl)propanoic acid (1). The amino acid is prepared using a Perkin-type condensation of *p*-anisaldehyde, ammonium acetate, and malonic acid in aqueous ethanol[5] (Scheme I). Although the yield of this β-amino acid was reported to be 95%,[5] yields of 50–60%[6] were more common. These lower yields are, however, comparable to other reports on the synthesis of aromatic β-amino acids by

(1) For reviews of these types of enzymatic conversions, see the following and references contained therein: (a) Walsh, C. "Enzymatic Reaction Mechanisms"; W. H. Freeman and Co.: San Francisco, 1979; p 823. (b) Mahler, H. R.; Cordes, E. H. "Biological Chemistry", 2nd ed.; Harper and Row: New York, 1971; p 778.

(2) Coggiola, D.; Fuganti, C.; Ghiringelli, D.; Graselli, P. *J. Chem. Soc., Chem. Commun.* **1976**, 143.

(3) Fuganti, C.; Ghiringelli, D.; Graselli, P. *J. Chem. Soc., Chem. Commun.* **1975**, 846.

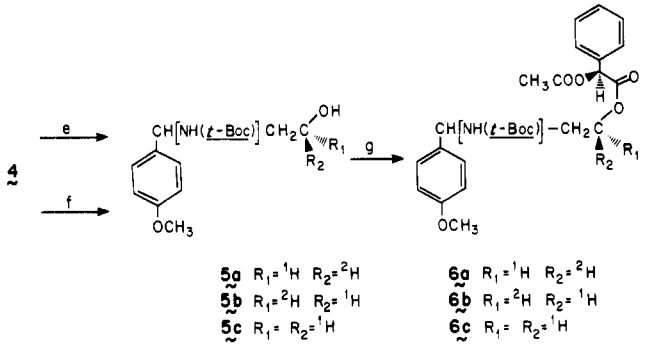
(4) Chang, M. N. T.; Walsh, C. T. *J. Am. Chem. Soc.* **1981**, *103*, 4921.

(5) Wang, J. S.; Shih, Y. E.; Chen, C. T. *Bull. Inst. Chem., Acad. Sin.* **1979**, *26*, 87.

(6) The major side product of this reaction is 4-methoxycinnamic acid, which could be readily separated from amino acid 1 by recrystallization.

0022-3263/85/1950-2259$01.50/0

Scheme II[a]



[a] (e) (*R*)-Alpine borane ((+)-α-pinanyl-β-isopinocamphenyl-9-borabicyclo[3.3.1]nonane);room temperature, 12 h, reflux 1 h. (f) (*S*)-Alpine borane ((−)-α-pinanyl-β-isopinocamphenyl-9-borabicyclo[3.3.1]nonane); room temperature, 12 h, reflux 1 h. (g) (1) DCC, (2) DMAP, (*S*)-2-acetoxy-2-phenylethanoic acid, −10 °C, 1 h; room temperature, 15 h.

similar methods.[7] At this point the β-amino acid may be formylated and resolved into the *R* (D) and *S* (L) enantiomers;[8] however, we completed the synthesis with the racemate, since the enzymes to be studied utilize only the *S* (L) enantiomer of homoserine.

The amino acid **1** was converted into the *N*-*t*-Boc derivative **2** and esterified using dicyclohexylcarbodiimide-/4-(dimethylamino)pyridine/methanol (DCC/DMAP/CH_3OH)[9a,b] to produce the *N*-*t*-Boc-β-amino acid methyl ester **3** in a yield of 77%. Compound **3** was reduced to the *N*-*t*-Boc-β-amino[1-^{2}H]aldehyde **4** with diisobutylaluminum deuteride (DIBAL-D) in 75% yield as described by Kalvin et al.[10] The mass spectral analysis of compound **4** showed 99.9% deuterium incorporation.

Deuterio aldehyde **4** (Scheme II) was reduced in a stereospecific manner with (*R*)- and (*S*)-Alpine boranes[11] to yield the chiral *S* and *R* alcohols **5a** (73%) and **5b** (75%), respectively. Midland et al.[12a,b] and other investigators[12c,d] have prepared a variety of chiral primary deuterio alcohols in enantiomeric excesses (expressed as % ee) ranging from 70 to 100%. To determine the percent ee of the alcohols, these workers have employed several methods, each of which relies on conversion of the respective alcohols into diastereomeric compounds. The alcohols **5a** and **5b** were converted into the diastereomeric mandelates **6a** and **6b**, respectively, in yields of 70–80% by the method of Parker[13] (Scheme II). Parker has reacted a series of chiral primary alcohols with (*S*)-2-acetoxy-2-phenylethanoic acid and determined their enantiomeric purity with the use of ^{1}H NMR spectroscopy. In each case studied, the *pro*-*R* hydrogen had shifted upfield relative to the *pro*-*S* hydrogen. Therefore, an *R* alcohol with its chirality due to isotopic substitution of a deuterium would have only the downfield signal due to the proton found at the *S* position. The 360-MHz ^{1}H NMR spectra of the mandelates **6a**–**c**, the

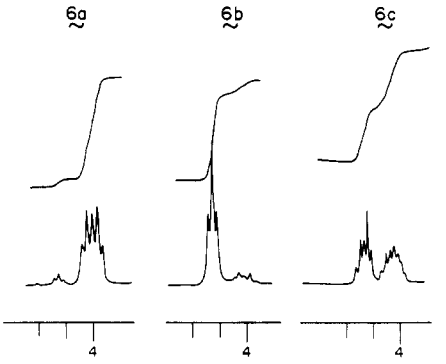
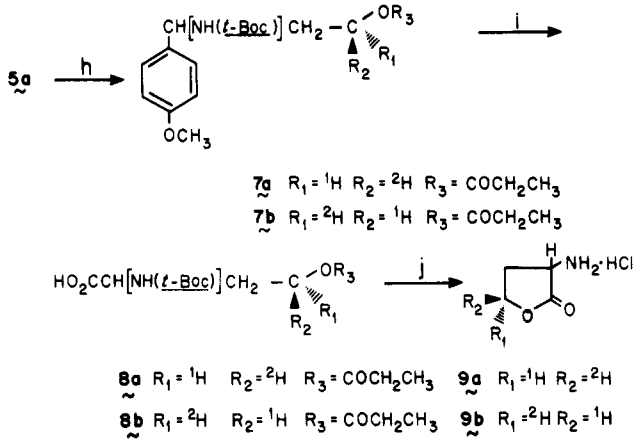


Figure 1. **6a**: 88% ee of *S*, 97% corrected for (+)-α-pinene of 91.3% ee. **6b**: 76% ee of *R*, 93% corrected for (−)-α-pinene of 81.9% ee. **6c**: achiral.

Scheme III[a]



[a] (h) $(CH_3CH_2CO)_2O$, pyridine, DMAP, 100 °C, 12 h; (i) $RuCl_3 \cdot 3H_2O$ (2.2 mol %), $NaIO_4$ (18 equiv), CH_3CN, CCl_4, H_2O (1:1:4 v/v/v); room temperature, 20 h. (j) (1) CH_3CH_2OH/H_2O/1 N KOH 1 h at room temperature, (2) CH_3CH_2OH/H_2O/HCl reflux 3 h.

achiral alcohol, are shown in Figure 1. As can be seen from these spectra, it is possible to determine the percent ee of the alcohols **5a** and **5b** and verify their configurations. From the integration of the spectra, **5a** was prepared in 88% ee and **5b** in 76% ee. These differences are due to the enantiomeric purity of the commercially available (+)-α-pinene and (−)-α-pinene (91.3% ee and 81.9% ee, respectively) which Aldrich Chemical Co. uses in the preparation of their (*R*)- and (*S*)-Alpine boranes.[11] Recently Brown et al.[14] published a method for increasing the optical purity of the pinenes used to synthesize (*R*)- and (*S*)-Alpine boranes, thus making it possible to increase the optical purity of alcohols **5a** and **5b**. The results from the ^{1}H NMR studies are consistent with Midland's results which show that (*R*)-Alpine borane reduction of a deuterated aldehyde yields the *S* alcohol predominantly, whereas (*S*)-Alpine borane reduction yields the *R* alcohol predominantly.

The final step in this sequence (Scheme III) involves converting the alcohols **5a** and **5b** into their respective homoserine lactone salts. In order to protect the alcohol from oxidation and render the protected homoserine product more extractable from an aqueous reaction mixture, the alcohol **5a** was reacted with propionic anhydride to yield the propionate **7a** (87%). With both the amine and alcohol functional groups protected with *aliphatic*-

(7) "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 91.
(8) Fischer, E.; Scheibler, H.; Groh, R. *Chem. Ber.* **1910**, *43*, 2020.
(9) (a) Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, 4475. (b) Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962.
(10) DIBAL-D was prepared from diisobutylaluminum chloride and LiD by the method of: Kalvin, D. M., Woodard, R. W. *Tetrahedron* **1985**, *40*, 3387.
(11) Trademark Aldrich Chemical Co.
(12) For examples, see: (a) Midland, M. M.; Tramantano, A.; Zderic, A. *J. Am. Chem. Soc.* **1977**, *99*, 5211. (b) Midland, M. M.; McDowell, D. C.; Hatch, R. L.; Tramantano, A. *J. Am. Chem. Soc.* **1980**, *102*, 867. (c) Parry, R. J.; Trainor, D. A. *J. Am. Chem. Soc.* **1977**, *99*, 5211. (d) Baker, R.; Rao, B. V. *J. Chem. Soc., Perkin Trans. 1*, **1982**, 69.
(13) Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1983**, 83.
(14) Brown, H. C.; Jadhau, P. K., Desai, M. C. *J. Org. Chem.* **1982**, *47*, 4583.

type moieties, propionate **7a** was subjected to an oxidative cleavage reaction which resulted in the oxidative cleavage of the five carbons of the aromatic ring (C-2–6) and the oxidation of C-1 to a carboxylic acid. The reagent of choice for effecting this type of transformation is RuO_4. Since it had been reported by Ayres[15] that a para-substituted methoxy group enhanced the rate and the extent of oxidative cleavage by RuO_4, we chose anisaldehyde rather than benzaldehyde as the starting aldehyde used in the preparation of the β-amino acid **1**. The conditions employed in this conversion were those of Carlsen et al.[16] which utilize the mixed solvent system CH_3CN, CCl_4, and H_2O (1:1:4 v/v/v). This oxidation procedure was selected since it had been used to synthesize a variety of chiral acids and amino acids in which the oxidation of the aromatic ring occurred adjacent to the asymmetric center.[17a,b] Since these conditions did not affect the chirality of the adjacent asymmetric center, there is little probability that they would affect a chiral center three carbon atoms away. The oxidative cleavage of propionate **7a** yielded (4*S*)-*O*-propionyl-*N*-*t*-Boc-D,L-[4-^{2}H]homoserine (**8a**) in 76% yield. The crude acid **8a** was used without further purification to complete the synthesis. The protecting groups were removed by stepwise hydrolysis ((a) EtOH/1 N KOH aqueous; (b) EtOH/HCl (saturated)) to afford the lactonized homoserine salt **9a** (18%).[18] The analogous reactions of **5b** gave similar results.

In order to complete the study, it was desirable to confirm the enantiomeric purity of lactones **9a** and **9b** at the four position. We had hypothesized that there would be little or no racemization in any step following our determination of the enantiomeric purity of **5a** and **5b**. Since we had chosen not to resolve the starting β-amino acid our lactones **9a** and **9b** are racemic at the 2- or α-position and are therefore diastereomeric. Since the enzymes to be studied will utilize only the isomer with the *S* configuration (L) at the amino acid center, we would not normally resolve **9a** or **9b**. The ^{1}H NMR shows peaks at both δ 4.27 and 4.42[19] which integrate for one-half a proton each for each diastereomeric mixture. This integration pattern is due to one deuterium at the four position (either *R* or *S* or *R,S*) and the racemic 2-position of the homoserine lactones (**9a** and **9b**).

To determine the enantiomeric purity at the 4-position of lactone **9a** (a diastereomeric mixture (4*S*,2*R* and 4*S*,2*S*)), we treated a basic buffered solution (pH 8.5) of **9a** with D-amino acid oxidase and catalase.[22] After separation of the amino acid from the keto acid, we were left with (4*S*,2*S*)-homoserine which was subjected to high-field ^{1}H NMR spectroscopy and its spectrum was found to be virtually identical with the spectrum of (4*S*,2*S*)-homoserine reported previously by Chang et al.[21] The homoserine was then lactonized and subjected to ^{1}H NMR analysis. The same procedure was performed for the diastereomeric mixture **9b** (4*R*,2*S* and 4*R*,2*R*). The enantiomeric purity of these resolved compounds was equivalent to the enantiomeric purity of the starting alcohols **5a** and **5b**, which confirms our hypothesis that none of the ensuing reactions, particularly the RuO_4 oxidation, occur with racemization of the 4-position.

The product chiral homoserines may be converted to the desired homoserine derivatives, first by tritylation to afford the (4*R*)-*N*-trityl- and (4*S*)-*N*-trityl-D,L-[4-^{2}H]homoserine lactones.[23] The tritylated lactones may then be opened by basic hydrolysis.[23] Since the *N*-trityl group both protects the amino function and prevents lactonization, the *N*-tritylhomoserines may be O-acetylated, O-phosphorylated, or O-succinylated directly. The *N*-trityl may be removed by catalytic hydrogenation to yield the desired homoserine substrates chirally labeled with deuterium in the 4-position.

Conclusion

This paper describes a chemical synthesis of (4*R*)- and (4*S*)-D,L-[4-^{2}H]homoserine lactones from readily available and inexpensive starting materials. This synthesis does not require microorganisms[3] (fermenting baker's yeast) or isolated enzymes (homoserine dehydrogenase)[4] and therefore constitutes the first total chemical synthesis of homoserine lactones and homoserines that are chiral in the four (γ) position due to isotopic substitution. The key step in the introduction of asymmetry at the 4-position involves reduction of 1-deuterio aldehyde **4** with (*R*)- and (*S*)-Alpine boranes. The optical purity of the resulting primary alcohols **5a** and **5b** (76–88% ee) although quite good could be increased by improving the percent ee of the pinenes used in the preparation of the Alpine boranes.[14] The synthesis, accomplished in 5% overall yield, 12% after the introduction of the deuterium atom, resulted in the production of (4*R*)- and (4*S*)-D,L-[4-^{2}H]homoserine lactones with high enantiomeric purity. The enantiomeric purity of these compounds was confirmed by enzymatic resolution of the (4*R*)- and (4*S*)-D,L-[4-^{2}H]homoserines with D-amino acid oxidase followed by ^{1}H NMR spectroscopy of the resolved homoserine lactones and homoserines. The synthetic methodology presented here is readily applicable to the preparation of O-acetylated, O-phosphorylated, and O-succinylated homoserine derivatives that are chirally labeled with deuterium in the 4-position.

Experimental Section

General Procedures. All melting points were obtained on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$, Me_2SO-d_6, and D_2O. Samples dissolved in $CDCl_3$ and Me_2SO-d_6 were reported in parts per million downfield from tetramethylsilane, while samples dissolved in D_2O were reported in parts per million downfield from 3-(tri-

(15) Ayres, D. C. *J. Chem. Soc., Chem. Commun.* **1975**, 440.
(16) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
(17) For examples of this, see: (a) Weller, H. N.; Gordon, E. M. *J. Org. Chem.* **1982**, *47*, 4160. (b) Kasai, M.; Ziffer, H. *J. Org. Chem.* **1983**, *48*, 2346.
(18) The low yields of the homoserine lactone hydrochlorides **9a** and **9b** may be explained by the fact that in acidic aqueous ethanolic solution the homoserine lactone is in equilibrium with homoserine. It has been reported by Mudd (see: Mudd, S. H. *J. Biol. Chem.* **1959**, *241*, 87) that when D,L-homoserine was incubated in 6 N HCl for 1 h at room temperature an equilibrium mixture of lactone (60%) and homoserine (40%) was obtained. Armstrong (see: Armstrong, M. D. *J. Am. Chem. Soc.* **1949**, *71*, 3399) has also reported that homoserine is in equilibrium with its lactone in acidic solutions. We have observed comparable results in the preparation of D,L-homoserine lactone hydrochloride from commercially available D,L-homoserine. In this case we isolated D,L-homoserine lactone hydrochloride in 56% yield from D,L-homoserine after treatment with boiling ethanolic HCl (saturated).
(19) We have previously shown[20] that the resonances of the 4*R*- and 4*S*-protons of the homoserine lactones differ by 54 Hz (*pro-R* ^{1}H 1591.2 Hz and *pro-S* ^{1}H 1537.2 Hz) as opposed to only 1.8-Hz difference in the case of homoserine (*pro-R* ^{1}H 1029.3 Hz and *pro-S* ^{1}H 1027.5 Hz) itself as reported by Chang et al.[21] We therefore believe that in order to have greater confidence in the assignment of configuration one should measure the ^{1}H NMR of homoserine lactone and not homoserine.
(20) Subramanian, P. K.; Woodard, R. W. *Org. Magn. Reson.*, manuscript in preparation.
(21) Chang, M. N.; Walsh, C. *J. Am. Chem. Soc.* **1980**, *102*, 2499.
(22) For examples of the use of D-amino acid oxidase in the selective oxidation of D-amino acids, see: (a) Greenstein, J. P.; Birnbaum, S. M.; Otey, C. M. *J. Biol. Chem.* **1953**, *204*, 307. (b) Meister, A.; Wellner, D. In "The Enzymes", 2nd ed.; Boyer, P. D., Lardy, H., Myrbuck, K., Ed.; Academic Press: New York, 1963; Vol. 7, p 609 and references contained within.
(23) Barlos, K.; Theodoropoulos, D. *Z. Naturforsh.* **1982**, *37*, 886.

methylsilyl)propionic acid sodium salt. All spectral and physical properties of deuterated compounds were found to be comparable to those of the non-deuterated compounds.

Diisobutylaluminum deuteride (DIBAL-D) was prepared by the method of Kalvin et al.[10] All organic and inorganic reagents were purchased from the usual chemical sources and were used without further purification. Organic solvents were dried by standard methods.[24] TLC plates (silica) were purchased from Analtech. The plates were visualized by ultraviolet irradiation from a Mineralight shortwave UV lamp, by spraying with an aqueous ethanolic H_2SO_4 solution containing (2,4-dinitrophenyl)hydrazine or by spraying with an ethanolic solution of ninhydrin. Medium grade silica gel (E. Merck, 70–230 mesh) was used for column chromatography.

Air- and moisture-sensitive reactions were performed in round-bottom flasks fitted with rubber septa and nitrogen inlets and outlets. The reaction vessels were flame dried in a steady stream of prepurified nitrogen. Reagents and solvents were transferred into these flasks under nitrogen using glass syringes fitted with metal needles. A nitrogen atmosphere was maintained in these vessels during the course of the reaction. All organic solvent extracts were dried over $MgSO_4$ and the solvents were removed in vacuo using a rotary evaporator (water aspirator vacuum) unless stated otherwise.

D,L-3-Amino-3-(4-methoxyphenyl)propanoic Acid (1). Amino acid 1 was prepared by a modification of the published procedure.[5] Ammonium acetate (46.25 g, 0.60 mol) was added to a solution of *p*-anisaldehyde (40.84 g, 0.30 mol) dissolved in 95% C_2H_5OH/H_2O (50 mL). The reaction mixture was warmed to 45 °C with stirring. Malonic acid (62.44 g, 0.60 mol) and an additional 25 mL of 95% C_2H_5OH/H_2O were added to the reaction mixture. The suspension was heated to reflux. All the solid material dissolved after 1 h and a yellow solution remained. The reaction was refluxed for an additional 12 h. The amino acid 1, which precipitated from the reaction mixture as a white solid, was collected by filtration. The product was recrystallized from 70% C_2H_5OH/H_2O to yield 30.0 g (51%): mp 240–241 °C (lit.[5] mp 237–239 °C); ^1H NMR (D_2O) δ 2.80 (d, 2 H, J = 6 Hz, $CHCH_2COOH$), 3.80 (s, 3 H, OCH_3), 4.80 (t, 1 H, J = 6 Hz, $CHCH_2COOH$), 6.80–7.40 (m, 4 H, Ar H).

D,L-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)propanoic Acid (2). Amino acid 1 (12.00 g, 0.06 mol) was dissolved in dioxane (123 mL) H_2O (61.5 mL), and 1 N NaOH (61.5 mL). To the cooled solution (0 °C) di-*tert*-butyl dicarbonate (14.80 g, 0.068 mol) was added in portions with stirring. The reaction mixture was stirred for 10 min at 0 °C and an additional 3 h at room temperature. The dioxane was removed in vacuo. The remaining aqueous solution was overlayed with ethyl acetate (700 mL), cooled in an ice water bath, and acidified with stirring to pH 1–2 with $KHSO_4$. The ethyl acetate was separated from the aqueous layer and evaporated in vacuo to afford a white crystalline solid 2. Compound 2 was recrystallized from 50% C_2H_5OH/H_2O to yield 16.0 g (88%): mp 168–169 °C; ^1H NMR ($CDCl_3$, Me_2SO-d_6) δ 1.40 (s, 9 H, O-t-C_4H_9), 2.70 (d, 2 H, J = 6 Hz, $CHCH_2COOH$), 3.80 (s, 3 H, OCH_3), 4.85 (m, 2 H, $CHCH_2CO_2H$, NH-t-Boc), 6.80–7.40 (m, 4 H, Ar H). Anal. Calcd for $C_{15}H_{21}NO_5$: C, 60.99; H, 7.18. Found: C, 60.85; H, 7.29.

Methyl D,L-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)propanoate (3). To a suspension of the D,L-*N*-*t*-Boc-protected amino acid 2 (7.00 g, 0.024 mol) in dry CH_2Cl_2 (140 mL) was added successively dry CH_3OH (2.88 mL, 0.071 mol), 4-(dimethylamino)pyridine (DMAP) (0.290 g, 0.002 mol), and dicyclohexylcarbodiimide (DCC) (5.37 g, 0.026 mol). The reaction was stirred at room temperature for 12 h. The precipitated dicyclohexylurea (DCU) was removed by filtration. The filtrate was evaporated to afford an oil. The oil was redissolved in dry ether (500 mL) and the residual DCU was filtered. The ether filtrate was evaporated to afford a white solid. Recrystallization of this white solid from hexanes–cyclohexane yielded 6.35 g of 3 (87%): mp 88–89 °C; ^1H NMR ($CDCl_3$) δ 1.45 (s, 9 H, O-t-C_4H_9), 2.80 (d, 2 H, J = 6 Hz, $CHCH_2COOCH_3$), 3.60 (s, 3 H $COOCH_3$), 3.80 (s, 3 H, OCH_3), 5.00–5.30 (m, 2 H, $CHCH_2COOCH_3$, NH-t-Boc), 6.80–7.30 (m, 4 H, Ar H). Anal. Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49. Found: C, 62.17; H, 7.53.

D,L-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)[1-^{2}H]propanal (4). A round-bottom flask was charged with D,L-*N*-*t*-Boc amino acid methyl ester 3 (2.00 g, 6.47 × 10^{-3} mol) and dry toluene (50 mL). The solution was stirred and cooled to −78 °C. To the cooled solution was then added dropwise a solution (7.10 mL, 0.014 mol) of 2.02 M DIBAL-D in hexane/ether. The reaction mixture was stirred for 6 min at −78 °C, quenched at −78 °C with dry CH_3OH (8 mL) and saturated aqueous sodium potassium tartrate tetrahydrate (15 mL), and finally allowed to warm to room temperature. The reaction mixture was diluted with ether (150 mL). The aqueous layer was extracted with ether (3 × 150 mL). The combined ether extracts were dried, the solvent was removed in vacuo, and the residue was placed under high vacuum at room temperature. The residue was dissolved in ethyl acetate (200 mL) and evaporated onto 3.5 g of silica gel. The preadsorbed product 4 was then placed on a chromatography column containing 40 g of silica gel and eluted with hexane–ethyl acetate (4:1). The fractions containing 1-deuterio aldehyde 4 were combined and the solvent was removed in vacuo to yield 1.36 g (75%) of a white crystalline solid: mp 90–91 °C; ^1H NMR ($CDCl_3$) δ 1.50 (s, 9 H, O-t-C_4H_9), 2.95 (d, 2 H, J = 6 Hz, $CHCH_2CDO$), 3.80 (s, 3 H, OCH_3), 5.20 (m, 2 H, $CHCH_2CDO$, NH-t-Boc), 6.80–7.40 (m, 4 H, Ar H); deuterium incorporation (MS analysis) 99.9%. Anal. Calcd for $C_{15}H_{20}DNO_4$: C, 64.27; H, 7.19. Found: C, 64.47; H, 7.55.

(1*S*)-D,L-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)[1-^{2}H]propanol (5a). A flask fitted with a reflux condenser was charged with 4.74 mL of a 0.50 M (*R*)-Alpine borane/THF[11] (2.37 × 10^{-3} mol), dry THF (15 mL), and 1-deuterio aldehyde 4 (0.600 g, 2.14 × 10^{-3} mol). The reaction mixture was stirred for 16 h at room temperature, heated to reflux for 1 h, and allowed to cool to room temperature. Acetaldehyde (3 mL) was added to the reaction mixture and the THF was removed in vacuo to afford a yellow-green residue. The residue was redissolved in dry ether; the ethereal solution was placed under a nitrogen atmosphere and cooled to 0 °C. Ethanolamine (2.5 mL) was added to the ether solution whereupon a white precipitate formed. The mixture was filtered through a Celite pad and the Celite was washed with ether (150 mL). The combined ether filtrates were washed with water (2 × 50 mL) and dried, and the ether was removed in vacuo. The residue was dissolved in ethyl acetate (200 mL), evaporated onto silica gel (1.70 g), and the preadsorbed product **5a** was placed onto a chromatography column containing 20 g of silica gel. The column was eluted with 400 mL of hexane and then with 400 mL of hexane–ethyl acetate (1:1). Those fractions containing *S* deuterio alcohol **5a** were collected and the solvent removed in vacuo. The residue was triturated with petroleum ether (30–65 °C), and the resulting crystalline white solid was collected to yield 0.441 g (73%) of **5a**, mp 92–93 °C. In an analogous manner, 1-deuterio aldehyde 4 was reacted with 0.5 M (*S*)-Alpine borane/THF[11] to afford *R* deuterio alcohol **5b** (75%): ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H, O-t-C_4H_9), 1.90 (m, 2 H, $CHCH_2CDHOH$), 2,80 (br s, 1 H, OH), 3.70–3.80 (m, 1 H, CH_2CDHOH; s, 3 H, OCH_3) 4.90 (m, 2 H, $CHCH_2CDHOH$, NH-t-Boc), 6.70–7.30 (m, 4 H, Ar H). Anal. Calcd for $C_{15}H_{23}NO_4$: C, 64.02; H, 8.26. Found: C, 64.12; H, 8.39.

(*S*)-D,L-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)propyl 2-Acetoxy-2-phenylethanoate (6c). To a solution of DMAP (0.005 g, 4.09 × 10^{-5} mol) in dry CH_2Cl_2 (10 mL) was added (*S*)-2-acetoxy-2-phenylethanoic acid (0.063 g, 3.24 × 10^{-4} mol). After the solution was cooled to −10 °C, D,L-3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)propanol (**5c**)[25] (0.100 g, 3.56 × 10^{-4} mol) and DCC (0.073 g, 3.54 × 10^{-4} mol) were added. The reaction mixture was stirred for 16 h and allowed to warm to room temperature. The DCU was filtered, the CH_2Cl_2 was removed in vacuo, and the residue was dissolved in dry ether. The ether was filtered to remove additional DCU. The solvent was removed in vacuo; the residue was dissolved in ethyl acetate (50 mL) and evaporated onto silica gel (1.06 g). The preadsorbed product **6c** was placed onto a

(24) Gordon, A. J.; Ford, R. A. "The Chemist's Companion"; Wiley: New York, 1972; p 429.

(25) Prepared by reaction of D,L-3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)propanoic acid with excess BH_3/THF; e.g., see: Rittle, K. E.; Homnick, C. F.; Ponticello, G. S.; Evans, B. E. *J. Org. Chem.* **1982**, *47*, 3016.

chromatography column containing 5.0 g of silica and eluted with hexane–ethyl acetate (4:1). Column fractions which contained **6c** were combined and the solvent was evaporated to yield 0.113 g (70%) of **6c** as an oil. In an analogous manner, chiral primary deuterio alcohols **5a** and **5b** were esterified with (*S*)-2-acetoxy-2-phenylethanoic acid (70–80% yield): 360-MHz ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9 H, O-*t*-C_4H_9), 2.00 (m, 2 H, $CHCH_2CH_2O$ mandelate), 2.20 (s, 3 H, $OCOCH_3$), 3.80 (s, 3 H OCH_3), 4.00 (m, 1 H, $CH_2CH_RH_SO$ mandelate), 4.15 (m, 1 H, $CH_2CH_RH_SO$ mandelate), 4.65 (br s, 1 H, N*H*-*t*-Boc), 4.90 (m, 1 H, $CHCH_2CH_2O$ mandelate), 5.90 (s, 1 H, $OCOCH(OCOCH_3)(C_6H_5)$), 6.70–7.60 (m, 9 H, Ar H). Anal. Calcd for $C_{25}H_{31}NO_7$: C, 65.63; H, 6.83. Found: C, 65.46; H, 6.59.

(1*S*)-D,L-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(4-methoxyphenyl)[1-^{2}H]propyl Propanoate (7a). A round-bottom flask, fitted with a reflux condenser, was charged with dry pyridine (15 mL, 0.185 mol), *S* deuterio alcohol **5a** (1.15 g, 4.08×10^{-3} mol), DMAP (0.050 g, 4.09×10^{-4} mol), and propionic anhydride (1.30 mL, 0.010 mol). The reaction mixture was heated to 100 °C with stirring for 12 h, cooled to room temperature, poured into an ice–water slurry (80 mL), and stirred vigorously. The white solid that precipitated was collected, recrystallized from hexanes, and dried over P_2O_5 to yield 1.20 g (87%) of **7a**: mp 82–83 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3 H, J = 6 Hz, $OCOCH_2CH_3$), 1.40 (s, 9 H, O-*t*-C_4H_9), 1.90–2.40 (m, 4 H, $CHCH_2CDHOCOCH_2CH_3$), 3.80 (s, 3 H, OCH_3), 4.05 (m, 1 H, $CHCH_2CDHOCOC_2H_5$), 4.80 (m, 2 H, $CHCH_2CDHOCOC_2H_5$, N*H*-*t*-Boc), 6.70–7.30 (m, 4 H, Ar H). Anal. Calcd for $C_{18}H_{27}NO_5$: C, 64.07; H, 8.06; N, 4.15. Found: c, 64.19; H, 8.29; N, 4.20.

(4*S*)-*O*-Propionyl-*N*-[(1,1-dimethylethoxy)carbonyl]-D,L-[4-^{2}H]homoserine (8a). To a biphasic reaction mixture consisting of CH_3CN (4 mL) CCl_4 (4 mL), and H_2O (8.1 mL) was added successively *S* deuterio propionate **7a** (1.10 g, 3.25×10^{-3} mol), $NaIO_4$ (12.5 g, 0.0584 mol), and $RuCl_3 \cdot 3H_2O$ (0.019 g, 7.27×10^{-5} mol). The reaction mixture was stirred for 20 h at room temperature and filtered. The precipitated iodate salts were washed with ethyl acetate (200 mL). The ethyl acetate was separated from the aqueous layer, dried, and allowed to stand for 12 h for precipitation of residual ruthenium salts. The inorganic salts were filtered and the solvent was removed in vacuo to afford 0.682 g (76%) of crude **8a** as an oil. The crude acid **8a** was used in the next synthetic step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3 H, J = 6 Hz, $OCOCH_2CH_3$), 1.50 (s, 9 H O-*t*-C_4H_9), 2.10–2.60 (m, 4 H, $CHCH_2CHDOCOCH_2CH_3$), 4.10–4.60 (m, 2 H, $CH_2CHDOCOCH_2CH_3$, $CHCH_2CDHOCOCH_2CH_3$), 5.30 (m, 1 H, N*H*-*t*-Boc), 10.3 (s, 1 H, COOH).

(4*S*)-D,L-[4-^{2}H]Homoserine Lactone Hydrochloride (9a). To an aqueous ethanol solution (2 mL) containing (4*S*)-*O*-propionyl-*N*-[(1,1-dimethylethoxy)carbonyl]-D,L-[4-^{2}H]homoserine **8a** (0.075 g, 2.72×10^{-4} mol) was added aqueous 1 N KOH (5 mL) and 95% C_2H_5OH/H_2O (2 mL). The reaction was stirred at room temperature for 1 h and the solvent was removed in vacuo. The residue was resuspended in water (5 mL) and the resulting aqueous solution was extracted with ether (2 × 20 mL). The aqueous layer was freeze dried, dissolved in water (2 mL), and added to a saturated ethanolic HCl solution (5 mL). The reaction mixture was heated to reflux for 3 h, allowed to cool to room temperature, and the solvent was removed in vacuo. The residue was dissolved in water (2 mL) and directly applied to a column of Dowex 50 X W-200 (H^+ form, 7 mL). The column was eluted with water (20 mL) and then with 1 N HCl (20 mL), and those fractions that contained ninhydrin-positive material were combined and freeze dried. The residue was triturated with absolute ethanol (1–2 mL). The off-white crystalline solid was collected, washed with anhydrous ether, and air dried to afford 6.64 mg (18%) of lactone hydrochloride **9a**: mp 188–191 °C (lit.[26] mp 196–197; 198–199.5 °C); 360-MHz ^{1}H NMR (Me_2SO-d_6) δ 2.30 and 2.55 (m, 2 H, 3-CH_2, 4.27 and 4.42 (m and d, 1 H, J = 8.9 Hz, 4-CHD) 4.32 (dd, 1 H, J = 11.02, 8.9 Hz, α-CH); 8.50–9.00 (m, 2 H, $NH_2 \cdot HCl$).

In an analogous manner, (4*R*)-D,L-[4-^{2}H]homoserine lactone hydrochloride (**9b**) was prepared from chiral deuterio alcohol **5b**. This lactone was obtained in 16% yield from (4*R*)-*O*-propionyl-*N*-[(1,1-dimethylethoxy)carbonyl]-D,L-[4-^{2}H]homoserine (**8b**): mp 188–190.5 °C; 360-MHz ^{1}H NMR (Me_2SO-d_6) δ 2.30 and 2.55 (m, 2 H, 3-CH_2), 4.27 and 4.42 (m and d, 1 H, J = 8.8 Hz, 4-CHD), 4.32 (dd, 1 H, J = 11.02, 8.9 Hz, α-CH), 8.50–9.00 (m, 2 H, $NH_2 \cdot HCl$).

Enzymatic Resolution of (4*S*)-D,L-[4-^{2}H]Homoserine Lactone Hydrochloride (9a). Lactone hydrochloride **9a** (~20 mg, 1.45×10^{-4} mol) was dissolved in 0.250 mL of 0.100 M sodium pyrophosphate buffer (pH 8.50) and allowed to stand for 2 h at ambient temperature. To this concentrated solution now containing the opened lactone **9a** ((4*S*)-D,L-[4-^{2}H]homoserine), distilled water (0.250 mL) and a 0.050 M sodium pyrophosphate solution (1.50 mL, pH 8.50) containing D-amino acid oxidase (100 mg, Sigma) plus catalase (1 mg, Sigma) were added. The reaction mixture was incubated for 20 h at ambient temperature, adjusted to pH 7.0, and applied to a Dowex 50W×4-200 (H^+ form) ion-exchange column (8 mL). The column was initially eluted with water (30 mL) and then eluted with 1 N NH_4OH (20 mL). The ninhydrin-positive fractions were freeze dried and subjected to 360-MHz ^{1}H NMR spectroscopy. Fractions containing (4*S*)-L-[4-^{2}H]homoserine were treated with ethanolic HCl (3 h, reflux) and purified using ion-exchange chromatography in the manner already described. The resulting (4*S*)-L-[4-^{2}H]homoserine lactone hydrochloride was subjected to 360-MHz ^{1}H NMR spectroscopy. The lactone hydrochloride **9b** was resolved in an analogous manner.

Acknowledgment. The financial support for this work was provided by NIH Grant GM 30097-01 and a predoctoral training fellowship to D.M.K. from NIH Grant GM 07767-06. We thank Dr. K. Ramalingam for helpful discussions.

(26) Fillman, J.; Albertson, N. *J. Am. Chem. Soc.* **1948**, *70*, 171.