Table I. Spectroscopic Data for 9,10-Dioxa-*syn*-(hydro,chloro)bimane [*syn*-(H,Cl)B]

CH_3CN solution[a]	solid		
	1 bar[b]	11 kbar	17 kbar
3154.3[a]	*c*	*c*	*c*
3135.1	3135.1	3138.3	3139.4
d	3107.3	3108.3	3107.6
d	3063.3	3063.6	3067.1
1774.9	1749.0	1748.4	1750.4
1730.7	*e*	*e*	*e*
1695.7	1670.9	1669.1	1669.7
1577.2	1567.3	1567.7	1569.3
1560.8	1541.9	1537.1	1538.9
1442.7	1447.7	1452.2	1458.2
1422.0	1428.9	*e*	*e*
1287.3	1289.3	1293.0	1295.1
f	1273.3	1276.9	1280.7
1255.7	1254.4	1262.6	1264.6
f	1213.7	1221.3	1227.0
1186.7	1181.6	1184.6	1182.1
1119.6	1125.8	1131.7	1133.7
f	897.0	900.9	903.7
801.5	798.3	802.4	804.7
756.4	764.6	770.1	773.7
737.9	728.7	728.9	728.1

[a] Obtained for a solution in CD_3CN (1% CD_2HCN). [b] Atmospheric pressure. [c] Bands not present. [d] Bands in this region are partially obscured by solvent absorption. [e] Shoulders; frequencies uncertain. [f] Bands obscured by solvent absorption.

The C=O (1749 cm^{-1}) and C=C stretching bands do not change in position with pressure except for the band at 1448 cm^{-1} which shifts to 1458 cm^{-1} (17 kbar). One set of bending vibrations shows a modest change with pressure, the absorptions at 1254, 1126, and 897 cm^{-1} shifting to 1265, 1134, and 904 cm^{-1} (17 kbar), respectively.

The C-H bands for 1 in most solvents ($CDCl_3$, CH_3COCH_3, CH_2Cl_2) resemble those in the KBr pellet except for one band (3084-3090 cm^{-1}) which shifts from 3107 cm^{-1} in the crystal. In CH_3CN solution, on the other hand, there are bands at 3154, 3135, and around 3080 cm^{-1}. The C=O band is found at 1779 ($CDCl_3$), 1778 (CH_3COCH_3), 1777 (CH_2Cl_2), and 1775 cm^{-1} (CH_3CN) in solution.

A set of infrared spectra for syn-(H,Cl)B (1) under pressures from 0 to 17 kbar in a KBr pellet are compared to the spectrum of 1 in CH_3CN solution in Figure 1, and the observed frequencies are summarized in Table I.

Conclusions

1. The similarity of the spectra of 1 in solution and in KBr pellet indicate that the complexity of the C-H region has an intramolecular, rather than an intermolecular origin. The extra bands are most likely due to resonance enhancement of a binary combination of ring modes, probably those at 1543 cm^{-1} (in solution, 1568-1565 cm^{-1}). A similar example of such a Fermi resonance involving C=C stretching bands has been observed for benzene.[11]

2. The effect of pressure on the IR bands was to cause a small shift to higher frequencies, as expected for the increased intermolecular interaction.[12] No other changes were observed as the pressure was raised. This implies that the crystal is already very well packed (high density, crystal structure) and resistant to further change. Another bimane, syn-(CH_3,H)B, although susceptible to a phase transition on cooling from 300 to 183 K, does not exhibit any change, detectable by X-ray diffraction, under 45-kbar pressure in a diamond anvil cell.[8]

3. The shift of the C=O band (solution, 1779 cm^{-1} → crystal, 1750 cm^{-1}) may be due to either (a) the intermolecular H-bond or (b) electrostatic repulsion due to the molecules in the layers below and above the absorbing molecule. It may be noted (Figure 1) that the C=C bands approach the carbonyl band in intensity for the pellet spectra. The relative intensification of the C=C stretching band absorptions for the crystal suggests that the hydrogen bonding C=O···H—C (positions 4 and 6 in the bimane ring) enhances the contribution of the C=O resonance forms, $C^+—O^-$ and $C^+—C=C—O^-$.

4. Further investigation of bimanes with small numbers of C-H bonds is warranted.

5. Infrared measurements as a function of pressure using a diamond anvil cell are a convenient way to probe both intramolecular behavior and intermolecular interactions in the solid state with a single set of measurements.

Experimental Section

Samples of *syn*-(H,Cl)B[9] (1) were examined with an evacuable Nicolet Model 8000 FTIR spectrometer equipped with a cooled MCT/InSb infrared detector. All spectra were recorded with a resolution of 2 cm^{-1}.

The spectra of the solids (pellets of crystalline 1 ground together with KBr) at various pressures were taken in a diamond anvil cell.[13] Pressures were established by evaluating the pressure-induced fluorescence shifts of ruby dust included in the KBr pellet.[14] At each pressure, the KBr pellet was annealed at 85 °C (a temperature previously found to be sufficient for 1) for several hours to ensure equilibration of the system. The spectra were the same before and after annealing. The spectra were resolved into Lorentzian curves by using the Nicolet curve analysis program.

Spectra of the solutions were somewhat difficult to obtain because of the low solubility of 1 (0.028 M in CH_3CN) in CH_3CN, $CDCl_3$, CH_2Cl_2, and CH_3COCH_3. Solvent bands were subtracted from the spectra of solutions of 1 by using a Nicolet subtraction routine.

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Reductive Amination of Ethyl 2-Oxo-4-phenylbutanoate with L-Alanyl-L-proline. Synthesis of Enalapril Maleate

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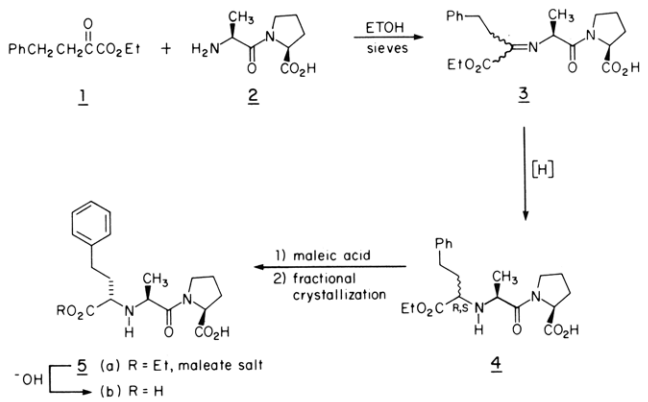
Inhibition of angiotensin-converting enzyme (EC 3.4.15.1) has been demonstrated to be an effective means

Scheme I



of controlling hypertension in man.[1] In 1980, Patchett et al. described substituted *N*-carboxymethyl dipeptides as a new class of potent, specific inhibitors of this enzyme.[2] In this note, the synthesis and some of the structural features of enalapril maleate (MK-421, **5a**) and its diacid analogue, enalaprilat (MK-422, **5b**), will be described.[3] These compounds are effective clinically in the treatment of hypertension and congestive heart failure with minimal side effects.[4]

Reductive amination reactions of substituted pyruvic acids have been of interest to chemists for many years because of their biochemical implications in the synthesis of α-amino acids. Borch in 1971 introduced sodium cyanoborohydride as a method of preparing α-amino acids from substituted pyruvic acids.[5] Harada and others have studied the reductive amination of α-keto acids and α-keto esters with α-amino acids, especially with (*S*)-phenylglycine derivatives.[6] When the adducts were subjected to hydrogenolysis, this constituted an asymmetric synthesis of (*S*)-α-amino acids.[7] In this note, the reductive amination of an α-keto ester, ethyl 2-oxo-4-phenylbutanoate (**1**), with a dipeptide unit, L-alanyl-L-proline (**2**), is described in detail.

Our synthesis of enalapril maleate is depicted in Scheme I. Ethyl 2-oxo-4-phenylbutanoate (**1**)[8] was condensed with L-alanyl-L-proline (**2**)[9] to give Schiff base **3**, presumably as a mixture of syn and anti isomers. Since Schiff bases of α-amino acids/esters are known to be susceptible to racemization,[10] **3** was generated in the presence of the reducing agents. Excess α-keto ester was required because reduction to the corresponding α-hydroxy ester was a serious side reaction. Reduction of the imine bond in **3** resulted in the formation of a diastereomeric mixture (*SSS* and *RSS*). The *SSS* component in this mixture has previously been shown to be the biologically more active diastereomer.[2] Sodium cyanoborohydride reduction of **3** gave mixture **4** in which the *SSS* isomer predominated by only a slight margin (10% diastereomeric excess).[12,13] Mixture **4** was also obtained by catalytic hydrogenation of **3** over 10% palladium on carbon; however, under these conditions the desired *SSS* diastereomer was now favored by a 62:38 margin (24% diastereomeric excess).[14]

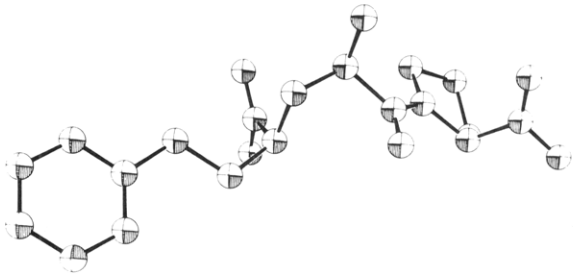


Figure 1. A computer-generated perspective drawing of **5b** showing its absolute configuration (*SSS*) and conformation in the solid state. Hydrogen atoms are omitted for clarity.

Isolation of the desired *SSS* diastereomer by preparative HPLC on a reverse-phase column was a tedious task. Consequently, many pharmaceutically acceptable acids and bases were examined for their ability to effect efficient separation of the diastereomers as their salts.[15] Maleic acid proved most efficient in this regard and gave, after recrystallization, the desired *SSS* diastereomer **5a** (>99% purity) as a nonhygroscopic crystalline salt in 32–34% overall yield.

Alkaline hydrolysis of **5a** gave diacid **5b** (enalaprilat) which, after recrystallization from water, produced small rods suitable for X-ray structural analysis. An ORTEP drawing of **5b** as determined by X-ray analysis is shown in Figure 1. Two important structural features of **5b** should be noted. The biologically more active diastereomer **5b** has *S* chirality at the new asymmetric center, and the inhibitor has crystallized in the trans rotamer about the amide bond. Recently, Thorsett et al. have shown that it is the trans rotamer that is the bioactive conformation in these inhibitors of angiotensin-converting enzyme.[16]

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(9) L-Alanyl-L-proline was prepared by standard solution peptide methodology. *N*-α-*t*-Boc-L-alanine was reacted in methylene chloride with L-proline benzyl ester with *N*,*N*′-dicyclohexylcarbodiimide (DCC) as the coupling agent. Appropriate removal of protecting groups afforded L-alanyl-L-proline as a hygroscopic solid. The dipeptide is also commercially available.

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(12) The reaction was conveniently monitored by a fluorescamine assay. Fluorescamine reacts with primary amines but not with secondary and tertiary amines to give a highly fluorescent derivative. Aliquots of the reaction mixture were periodically removed and treated with a 0.01 N HCl solution. After a few minutes, the aliquot was further diluted with a 0.2 M sodium borate buffer (pH 8.5) and then mixed with a stock solution of fluorescamine in acetone. The disappearance of L-alanyl-L-proline was easily monitored under a long wavelength UV lamp. Fluorescamine is commercially available.

(13) On a reverse-phase (RP-18) column, the *SSS* diastereomer was the faster moving component of the mixture.

(14) In both the sodium cyanoborohydride procedure and the catalytic hydrogenation procedure, the presence of molecular sieves was required for high yields.

(15) For a list of acceptable pharmaceutical salts, see: Berge, S. M.; Bighley, L. D.; Monkhouse, D. C. *J. Pharm. Sci.* **1977**, *66*, 1.

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Spectroscopic examination (^{13}C NMR) of **5b** in D_2O/NaOD (pD 11) indicated a 7:1 mixture of rotamers about the amide bond. The observed chemical shifts for the β and γ ring carbons of proline agree with published values for acylproline derivatives[17] and permitted the assignment of the trans amide linkage (as depicted in Figure 1) as the major rotamer in D_2O.

The ratio of trans/cis rotamers in **5b** was found to be pH dependent with lower trans ratios (4:1) occurring in acidic solution (pD 2.5). This is surprising since for most peptides containing proline, e.g., L-alanyl-L-proline, spectroscopic studies have found that the trans rotamer is more highly favored at low pH (proline carboxylate is protonated) while the cis rotamer is proportionally more favored in alkaline solution proline carboxylate is deprotonated).[18] This effect is due to electrostatic repulsion in the trans rotamer between the amide carbonyl and the negatively charged carboxylate. This repulsion is minimized in the cis rotamer.

In order to explain the abnormal behavior (greater trans ratio) for **5b** in alkaline solution, possible interactions with the second carboxylate function must be considered. Examination of the solid-state conformation of **5b** in Figure 1 shows that the two carboxylate groups are farthest apart in the trans rotamer. Rotation about the amide bond in Figure 1 generates the cis rotamer in which the two negatively charged carboxylates are quite close in space. Assuming that the preferred solution conformation is very similar to the solid-state conformation, the abnormal behavior of **5b** is easily understood in terms of electrostatic repulsion between the carboxylates in the cis rotamer.[19]

To test this rationale, the spectroscopic behavior of **5a** was investigated. In **5a** the second carboxylate is an ester; therefore, the electrostatic repulsion between the two carboxylate groups in the cis rotamer should be greatly reduced relative to **5b** and the proportion of cis rotamer should increase on going from acid solution to a more basic medium. Spectroscopic examination (^{13}C NMR) of **5a** in D_2O revealed a predominance of trans rotamer (2:1) in acidic solution (pD 2.5). However, at pD 7.5 (proline carboxylate is ionized) **5a** existed as a 5:4 mixture of trans/cis rotamers. This result is in excellent agreement with the above argument and provides support for the assumption that the preferred solution conformation of **5b** is very similar to its solid-state conformation. In fact, variable-temperature studies on **5b** in CD_3OD (−44 to +49 °C) and in D_2O (22–94 °C) showed only minor chemical shift displacements, thereby indicating that no perceptible conformational changes occurred over this range. Since no significant exchange broadening was noted at 94 °C, the coalescence temperature of the two rotameric forms of **5b** would have to be at least 120 °C.

The reductive amination conditions described in this note for ethyl 2-oxo-4-phenylbutanoate and L-alanyl-L-proline have proven to be quite general for a variety of α-keto esters and dipeptides.

Experimental Section

Melting points were determined in open capillaries with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Cary Model 118 spectrometer. Optical rotations were determined with a Perkin-Elmer 241 polarimeter in 10-cm cells at 25 °C. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian Associates Model SC300 spectrometer. ^{13}C NMR spectra were recorded on a Varian Associates Model XL-200 spectrometer. Chemical shifts are reported in parts per million (δ) relative to an internal reference: tetramethylsilane for CD_3OD solutions and sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP) or dioxane for D_2O solutions. Thin-layer chromatography (TLC) was performed on Analtech silica gel GF plates (250 μm) in a solvent system consisting of ethyl acetate, *n*-butyl alcohol, water, and acetic acid (1:1:1:1). HPLC analyses were performed on a reverse-phase (RP-18) analytical column at 50 °C with a solvent mixture consisting of 45% methanol and 55% of a 0.005 M tetra-*n*-butylammonium phosphate buffer (pH 7.2). Microanalyses were performed by the Merck Sharp & Dohme Microanalytical Laboratory under the direction of Mr. J. Gilbert.

1-[*N*-[1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline (4). Sodium Cyanoborohydride Procedure. Ethyl 2-oxo-4-phenylbutanoate (44.4 g, 0.215 mol) and L-alanyl-L-proline (8 g, 0.043 mol) were dissolved in 150 mL of absolute ethanol. Powdered 4A molecular sieves (70 g) were added. To this mixture was added a solution of purified sodium cyanoborohydride (2.5 g, 0.04 mol) in 45 mL of absolute ethanol dropwise over 5.5 h under nitrogen. The reaction mixture was then stirred overnight.[12] The sieves were removed by filtration, and the filtrate was concentrated to afford an oil that was then dispersed in 300 mL of water. The pH of this solution was adjusted to 8.5 with solid dipotassium hydrogen phosphate (K_2HPO_4). Extraction with ethyl acetate (5 × 100 mL) removed unreacted α-keto ester and α-hydroxy ester. The pH of the aqueous solution was then adjusted (in hood) to 2.0 with 1 M phosphoric acid and maintained at this pH for 30 min in order to decompose any borate complexes. The pH of the solution was adjusted once again with solid K_2HPO_4 to 4.2 (isoelectric point of **4**). Sodium chloride (60 g) was added and the mixture extracted with ethyl acetate (5 × 100 mL).[20] The organic extracts were dried with anhydrous sodium sulfate and then concentrated under reduced pressure ($T < 35$ °C)[21] to afford **4** as a foam, 14.5 g (90%). HPLC analysis indicated a 55:45 diastereomeric mixture (*SSS*/*RSS*).[13] TLC showed two overlapping spots, R_f 0.66 and 0.63.

Hydrogenation Procedure. To a solution of ethyl 2-oxo-4-phenylbutanoate (4.54 g, 22 mmol) and L-alanyl-L-proline (1.86 g, 10 mmol) in 150 mL of absolute ethanol was added 16 g of powdered 4A molecular sieves and 1.0 g of 10% palladium on carbon. The mixture was hydrogenated (40 psi) for 15 h at room temperature on a Paar hydrogenation apparatus. The reaction mixture was filtered through Celite and the filtrate concentrated under reduced pressure to give a pale yellow oil, 6.45 g. This residual oil was dispersed in 200 mL of water containing 40 g of sodium chloride. The pH of the solution was adjusted to 8.5 with K_2HPO_4 and then the solution extracted with ethyl acetate (2 × 200 mL). The aqueous solution was then acidified with 1 M phosphoric acid to pH 4.2 and extracted with ethyl acetate (4 × 100 mL). The extracts were dried with anhydrous sodium sulfate and then concentrated to yield 3.47 g of an oil. This oil was dissolved in warm water (200 mL, 60 °C) and filtered. The filtrate was freeze-dried to give a white, amorphous material (**4**), 2.90 g (77%). HPLC analysis revealed a diastereomeric ratio of 62:38 (*SSS*/*RSS*).

1-[*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline (*Z*)-2-Butenedioate (Enalapril Maleate, 5a). A solution containing 2.90 g (7.7 mmol) of the diastereomeric mixture **4** (62:38, SSS/RSS) in 8.5 mL of acetonitrile was added to a hot solution of 0.90 g (7.7 mmol) of maleic acid in 13.5 mL of acetonitrile. The hot solution was filtered and then cooled to room temperature with ice. To this solution was added a seed crystal, and the mixture was then stirred for 1 h. The precipitate was collected and rinsed with acetonitrile (2 × 1 mL) and ether (5 mL) to give 1.88 g of **5a** that contained 3% of the *RSS* dia-

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(19) Calculations at Merck by Drs. Graham Smith and Tom Halgren with a modified MM-2 program (OPTIMOL) show that the trans rotamer is 0.8 kcal/mol more stable than the cis rotamer when both carboxylates in **5b** are ionized. However, the trans rotamer is 0.16 kcal/mol less stable than the cis rotamer when the carboxylates are protonated.

(20) Alternatively, **4** can be isolated by ion-exchange chromatography on a Dowex 50W-2X (50–100 mesh) column.

(21) Excessive heating of **4** in organic solvents should be avoided due to diketopiperazine formation.

stereomer. Recrystallization once again from acetonitrile (19 mL) gave 1.68 g of pure **5a** (>99%). An overall yield of 34% was obtained for the hydrogenation procedure, while the sodium cyanoborohydride procedure gave a 32% overall yield of **5a**: mp 143–144.5 °C; $[\alpha]^{25}_D$ −42.2° (*c* 1.0, CH_3OH); UV (0.1 N HCl in CH_3OH) λ_{max} 258 nm (% *A* 10.5), 263 (7.40), 267 (5.64); ^1H NMR (D_2O) δ 1.30 (t, *J* = 7 Hz, OCH_2CH_3), 1.54 (minor),[22] 1.59 (major, d, *J* = 6.5 Hz, Ala-CH_3), 1.75 (minor), 2.02 (major, m, Pro γ-CH_2), 2.02 (m, Pro β-H), 2.33 (m, Pro β-H, $PhCH_2CH_2$), 2.82 (m, $PhCH_2$), 3.48 (minor), 3.60 (major, m, Pro δ-CH_2), 3.96 (m, $PhCH_2CH_2CH$), 4.09 (minor), 4.33 (major, q, *J* = 6.5 Hz, Ala α-H), 4.27 (q, *J* = 7 Hz, OCH_2CH_3), 4.44 (m, Pro α-H), 6.37 (s, CH=CH), 7.39 (m, ArH_{2-6}); ^{13}C NMR (CD_3OD) 16.2, 17.4 (major), 18.1 (minor), 24.9 (minor), 27.7, 31.8, 33.7, 35.0, 35.2 (minor), 49.8, 57.9, 58.4 (minor), 61.7, 61.8, 62.3, 62.4, 65.6, 65.7, 129.3, 131.2, 131.4, 138.0, 142.9, 170.5, 171.2, 171.6, 172.0, 172.4, 176.5, 176.7 ppm. Anal. Calcd for $C_{24}H_{32}N_2O_9$: C, 58.53; H, 6.55; N, 5.69. Found: C, 58.49; H, 6.53; N, 5.61.

1-[*N*-[1(*S*)-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline (Enalaprilat, 5b). Enalapril maleate, **5a** (50 g, 0.102 mol), and sodium hydroxide (20 g, 0.5 mol) were dissolved in 500 mL of water, and the mixture was permitted to stir overnight at room temperature. The reaction mixture was then added to a Dowex 50W-2X ion-exchange column (1.2 L, 50–100 mesh). The column was washed with water until the eluent was near neutrality. Then the product was removed from the column with 8 L of 2% pyridine in water. The appropriate fractions were concentrated, and the resulting solid was slurried in 300 mL of acetone. Filtration and drying gave 33.8 g of pure **5b**: R_f 0.55; mp 149–151 °C dec; $[\alpha]^{25}_D$ −67.0° (*c* 2.0, 0.1 N HCl); $[\alpha]^{25}_D$ −53.5° (*c* 1.0, CH_3OH); UV (0.1 N HCl) λ_{max} 257 (ε 179); ^1H NMR (D_2O) δ 1.55 (minor),[22] 1.59 (major, d, *J* = 6.5 Hz, Ala-CH_3), 1.82 (minor), 2.04 (major, m, Pro γ-CH_2), 2.04 (m, Pro β-H), 2.32 (m, Pro β-H), 2.18 (m, $PhCH_2CH_2$), 2.75 (m, $PhCH_2$), 3.54 (t, *J* = 6 Hz, $PhCH_2CH_2CH$), 3.63 (m, Pro δ-CH_2), 4.08 (minor), 4.29 (major, q, *J* = 6.5 Hz, Ala α-H), 4.51 (minor), 4.46 (major, dd, *J* = 5 Hz, *J* = 8 Hz, Pro α-H), 7.35 (m, ArH_{2-6}); ^{13}C NMR (D_2O, NaOD) 18.2, 22.9 (minor, Pro γ), 25.2 (major, Pro γ), 30.2 (major, Pro β), 32.3 (minor, Pro β), 32.6, 36.3, 48.3, 53.7 (major, Ala α), 55.2 (minor, Ala α), 62.3 (minor, Pro α), 62.6 (major, Pro α), 63.1, 126.9, 129.4, 129.5, 143.1, 175.3, 180.4, 182.2 ppm. Anal. Calcd for $C_{18}H_{24}N_2O_5 \cdot 0.5\ H_2O$: C, 60.49; H, 7.05; N, 7.84. Found: C, 60.60; H, 7.01; N, 7.79.

X-ray Crystal Analysis of 5b. Small rods of **5b** formed from water at room temperature. Preliminary X-ray experiments revealed cell constants of *a* = 9.798 (2) Å, *b* = 10.452 (2) Å, and *c* = 19.819 (5) Å and a space group of $P2_12_12_1$ for *Z* = 4. Of the 1619 unique reflections measured, 1360 were observed ($I \geq 3\sigma(I)$) by using Cu Kα radiation and an automatic four-circle diffractometer. The structure was solved by using direct method procedures[23] and refined by using full-matrix least-squares techniques. Anisotropic temperature parameters were refined for all non-hydrogen atoms while isotropic parameters were added but not refined for the hydrogen atoms. The function minimized was $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = 1/(\sigma(F_o))^2$ to give an unweighted residual index of 0.046. Three molecules of water were found in the crystal lattice. Figure 1 is a perspective drawing of **5b** showing its absolute configuration and conformation in the solid state.

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Registry No. **1**, 64920-29-2; **2**, 13485-59-1; **3**, 90414-32-7; (*SSS*)-**4**, 75847-73-3; (*RSS*)-**4**, 76420-74-1; **5a**, 76095-16-4; **5b**, 76420-72-9.

Supplementary Material Available: X-ray structural analysis for **5b**, Table I, fractional coordinates and temperature factors, Table II, bond distances, Table III, bond angles, and Figure 2, X-ray numbering diagram (5 pages). Ordering information is given on any current masthead page.

(22) The rotameric distribution is indicated by *minor/major* terminology.

(23) The following library of crystallographic programs were used: MULTAN 80, University of York, York, England (1980); XRAY-72, University of Maryland, College Park, MD (1972); Structure Determination Package 17.0, Enraf-Nonius Corporation, Delft, Holland (1981); ORTEP-II, Oak Ridge National Laboratory, Oak Ridge, TN (1970).

Facile, Economical Synthesis of L-[α-^{2}H]-α-Amino Acids[1]

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Three methods have been suggested for preparation of L-[α-^{2}H]-α-amino acids. The method of Wong and Whitesides[2] employs NAD^2H to introduce the label in an enzymic reductive amination reaction. Chiral and isotopic purities are high, but an enzyme specific for the production of a particular amino acid must be used. Exchange without loss of chirality has been effected with transaminases such as aspartate aminotransferase,[3] but with amino acids which are poor substrates, large amounts of enzyme may be required or the reaction may fail. The potential also exists in this system for exchange of hydrogen at the β-carbon. A different approach is to prepare the DL-[α-^{2}H]amino acid and then resolve, most easily by an enzymic method. For example, Yamamoto, Upson, Linn, and Hruby[4] prepared DL-[α-^{2}H]phenylalanine from diethyl 2-benzyl-2-acetamidomalonate by simultaneous deacetylation and ester hydrolysis, followed by decarboxylation in D_2O. The product was then reacetylated and porcine kidney acylase was used for resolution by selective deacetylation of the L derivative.

We present here a two-step procedure which begins with the commercially available *N*-acetyl α-amino acids and produces high levels of deuteration in a first step under mild conditions. The product of this step is the *N*-acetyl DL-[α-^{2}H]amino acid, which is then resolved by porcine-kidney acylase I in the second step. In other experiments, deuteration of DL-phenylalanine was achieved quickly and simply with pyridoxal phosphate or pyridoxal chloride in basic D_2O.

Results

In a typical experiment, *N*-acetyl-DL-phenylalanine was dissolved at room temperature in deuterium oxide containing sodium hydroxide. Then acetic anhydride was added, producing a heterogeneous mixture; stirred and heated gently, the mixture became homogeneous and was allowed to stand for several hours. *N*-Acetyl-[α-^{2}H]-phenylalanine was precipitated with hydrochloric acid (yield 92%; deuteration >97% by NMR, no deuterium exchange was observed at other than α-position). Resolution with porcine kidney acylase I in protium oxide then produced L-[α-^{2}H]phenylalanine with >97% deuteration.

The generality of the procedure was demonstrated by deuteration of four other *N*-acetyl α-amino acids: leucine, glutamic acid, methionine, and tyrosine. The α-deuteration of these amino acids was carried out by the procedure given above for *N*-acetylphenylalanine. α-Deuterated leucine and glutamic acid were obtained in 86% and 82% yield, respectively. The deuterium incorporation in these compounds was >97% by NMR. Similarly, the deuterium exchange reaction of methionine and tyrosine was followed

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(3) Gout, E.; Chesne, S.; Beguin, C. G.; Pelmont, J. *Biochem. J.* **1978**, *171*, 719–723.

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