# Deuterated amino acids. IV. The synthesis of L-phenyl-$d_5$-alanine-2,3,3-$d_3$[1,2]

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The synthesis of L-phenyl-$d_5$-alanine-2,3,3-$d_3$ is described. The synthetic route involved the enzymatic deacylation of acetylphenyl-$d_5$-alanine-2,3,3-$d_3$, which was obtained by catalytic reduction (deuteriumation) of the hydrolyzed 2-methyl oxazolone derivative of benzaldehyde-$d_6$. The ultraviolet, infrared, and optical rotatory dispersion spectra of L-phenylalanine-$d_8$ are reported along with some corresponding data on the deuterated intermediates.

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With the general objective of providing highly deuterated amino acids as possible tools for use in the study of a variety of chemical and biological systems, as outlined briefly in a recent article (1), a useful synthesis for the title compound is reported. The synthesis of L-phenyl-$d_5$-alanine-2,3,3-$d_3$ is accomplished as outlined in the reaction sequence given below. With reference to this sequence, the formation of benzaldehyde-$d_6$ requires the addition of sodium chloride to the reaction mixture as a catalyst to guarantee reproducible yields.[4]

The catalytic reduction of the acid **3** in dioxane – ethyl acetate using 10% palladium on carbon as the catalyst is satisfactory but with the solvent–catalyst system of acetic anhydride, deuterium oxide, and platinum oxide (3), substantial reduction of the benzene ring occurs, giving *N*-acetylcyclohexyl-$d_{11}$-alanine-2,3,3-$d_3$ (**12**).

Because the activity of the renal acylase varies (4) it is useful to carry out the deacylation reaction simultaneously on separate solutions of the acid **4** and its protio analogue. Since the phenyl protons of the amino acid are distinguishable from the phenyl protons of the *N*-acetyl amino acid in the nuclear magnetic resonance spectrum, the enzymatic deacylation is followed by integration of the two phenyl peaks, and the necessary reaction time is easily determined.

The deuterated L-phenylalanine (**5**) is obtained in 18% yield overall from benzene-$d_6$.

The ultraviolet and optical rotatory dispersion data for the amino acid **5** are given in the Experimental section. It is to be noted that in the ultraviolet spectrum of **5** all maxima of the deuterated compound are at a lower wavelength than the corresponding maxima of the protio analogue. The higher energy needed to excite the deuterated compound results from the lowered zero point energy associated with deuterium substitution.

Condensation of the aldehyde **1** with *N*-acetylglycine, with 2-mercaptothiazole-5-one, and with hippuric acid affords as products the oxazolone **2**, 2-thio-4-benzylidene-$d_6$-thiazolone (**9**), and 2-phenyl-4-benzylidene-$d_6$-oxazolone (**8**) which contain, respectively, 96.4%, 93.9%, and 99.8% deuterium. The reduction of the oxazolone **8** and the thiazolone **9** with deuteriodic acid[5] and red phosphorus gave DL-phenyl-$d_5$-alanine-$d_3$ (**7**) in 66% and 51% yields. The product (**7**) from these reactions indicated considerable exchange (81.5%) had occurred rendering these alternate schemes unacceptable.

The observed amount of isotopic dilution of the three condensation products **2** (3.5%), **9** (6%), and **8** (0%) can be rationalized on the basis of tautomerism. Exchange with the solvent of the benzylidene positions of **2** and **9** occurs *via* their respective tautomers, 2-methylene-4-benzyl-$\Delta^3$-oxazolone (**11**) (5, 6)[6] and 2-thio-4-

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[1] Part III: A. T. Blomquist, B. F. Hiscock, and D. N. Harpp, J. Org. Chem., **31**, 4121 (1966).

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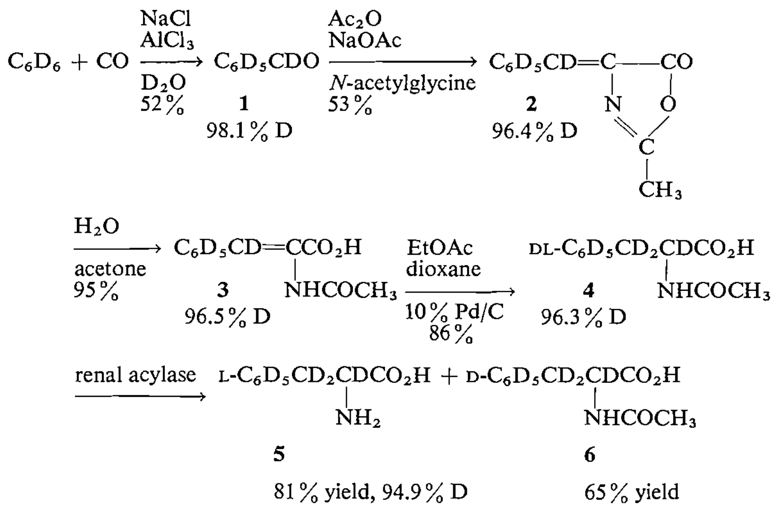
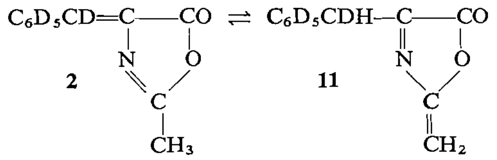
[4] Certain salts have been shown (2) to catalyze Friedel–Crafts reaction.

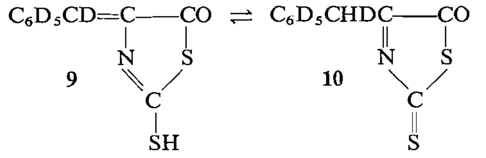
[5] Prepared from phosphorus triiodide and deuterium oxide.

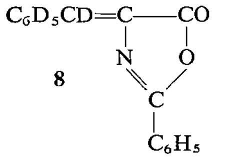
[6] An analogous isomerization of certain 2-vinyl-4-benzyloxazolones to 2-ethyl-4-benzylideneoxazolones is described (6). The vinyl oxazolones were prepared by dehydration of acryloyl α-amino acids.



benzyl-$\Delta^3$-thiazolone (**10**); no tautomer is possible for the azlactone **8**.







Analysis by mass spectrometry shows the thiazolone **9** to be a mixture of -$d_5$ (38.9%) and -$d_6$ (61.1%)[7] species, an indication that exchange occurs at only one position on the molecule. Exchange at the ring positions would give rise to additional species containing only 2, 3, and 4 deuteriums; these were not detected.

## Experimental

### *Materials*

Benzene-$d_6$ was purchased from Ciba Chemical Company, Summit, New Jersey. Phosphorus triiodide was supplied by K and K Fine Chemicals, Jamaica, New York, and renal acylase was obtained from Mann Assayed Biochemicals, New York, New York.

### *Equipment*

The following machines were used in the course of this research: model A-60 Varian nuclear magnetic resonance spectrometer, Cary 14 recording spectrophotometer, Cary 60 spectropolarimeter, Consolidated Engineering model 21-103A mass spectrometer, and a Perkin-Elmer Infracord. A controlled atmosphere box was used throughout this research whenever a possibility of exchange with water vapor in the atmosphere arose. The box was kept dry by maintaining a positive pressure of dry nitrogen in the box, and by placing an evaporation dish containing 1:1 Celite : phosphorus pentoxide in the box. Dry nitrogen referred to in this Experimental section was dried by passing through columns of 1:1 Celite : phosphorus pentoxide.

### *Analyses*

Deuterium analyses were carried out by J. Nemeth, Urbana, Illinois, the B. F. Goodrich Co., Brecksville, Ohio, and by one of the authors (R. J. C.). Microanalyses were performed at Scandinavian Microanalytical Laboratories, Herlev, Denmark, and Galbraith Laboratories, Inc., Knoxville, Tennessee.

### *Benzaldehyde-$d_6$ (1)*

To a stirred metal autoclave was added 63.8 g (0.760 mole) of benzene-$d_6$, 112 g of aluminium chloride, 20 g of sodium chloride, and 1.0 ml of deuterium oxide (a modification of the procedure described in ref. 7) under an atmosphere of dry nitrogen. This mixture, pressured with carbon monoxide to 1000 p.s.i., was stirred for 3 h at room temperature; the carbon monoxide was then vented. Deuterium oxide (200 ml) and ether (200 ml) were added slowly and simultaneously to the opened bomb; stirring was continued until evolution of gas ceased. Distillation of the washed and dried ether layer gave 44.1 g (52%) of the aldehyde **1**: b.p. 68° (15 mm), 179° (760 mm); $n_D^{25}$ 1.5414; $d_4^{25}$ 1.1026; $\lambda_{max}$ (neat) 4.43, 4.79, 6.01 μ.

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[7] Deuterium analysis was carried out by the B. F. Goodrich Co., Brecksville, Ohio.

Anal. Calcd. for $C_7D_6O$: C, 74.95; H, 5.94;[8] D, 100 atom %. Found: C, 75.11, 74.96; H, 6.00, 6.07; D, 98.1 atom %.

### *2-Methyl-4-benzylidene-$d_6$-oxazolone (2)*

Following the procedure of Herbst and Shemin (3), 39.3 g (0.350 mole) of the aldehyde **1**, 44.7 g (0.38 mole) of *N*-acetylglycine, 22.4 g of sodium acetate, and 105 ml of acetic anhydride afforded 35.8 g (53%) of the recrystallized azlactone **2**, m.p. 149–151°.

Anal. Calcd. for $C_{11}H_3D_6NO_2$: C, 68.37; H, 5.00; D, 66.7 atom %. Found: C, 68.43; H, 5.13; D, 64.2 atom % (96.4%).

### *3-Phenyl-$d_5$-2-acetamidopropenoic-3-d Acid (3)*

Using the reported procedure (3) 38.0 g (0.197 mole) of the azlactone **2** was refluxed in 400 ml of acetone and 150 ml of water for 5 h. After crystallization from water there was obtained 40.1 g (95%) of the acid **3**: m.p. 189–190°; $\lambda_{max}$ (KBr) 2.85, 3.05, 4.00, 4.40, 5.95, and 6.10 μ.

Anal. Calcd. for $C_{11}H_5D_6NO_3$: C, 62.54; H, 5.53; D, 54.5 atom %. Found: C, 62.74; H, 5.70; D, 52.6 atom % (96.5%).

### DL-*N-Acetylphenyl-$d_5$-alanine-2,3,3-$d_3$ (4)*

A solution of 9.31 g (0.0437 mole) of the acid **3** in 75 ml of ethyl acetate and 200 ml of dioxane was added to a pressure bottle that contained 1.3 g of 10% palladium on carbon. The bottle was placed in a Parr apparatus and connected directly to an inverted tank of deuterium gas.[9] (The initial deuterium gas pressure was 40 p.s.i.) After 1 h at room temperature, the theoretical amount of deuterium was absorbed. The catalyst was filtered, solvent removed under reduced pressure, and the residual oil crystallized from water to give 8.11 g (86%) of the acetyl derivative **4**: m.p. 147–149°; $\lambda_{max}$ (KBr) 3.00, 3.50, 4.40, and 5.85 μ.

Anal. Calcd. for $C_{11}H_5D_8NO_3$: C, 61.38; H, 6.50; D, 61.6 atom %. Found: C, 61.19; H, 6.66; D, 59.2 atom % (96.3%).

### *Reduction of the Acid 3*

Following a modification of the reported procedure (3), 34.9 g (0.164 mole) of the acid **3** in 196 ml of acetic anhydride and 42 ml of deuterium oxide was reduced in a Parr apparatus, using 0.83 g of platinum oxide, and a deuterium gas pressure maintained at 40 p.s.i. for 3 h. Filtration followed by evaporation of the filtrate gave 30.6 g of a mixture that comprised 70% compound **4** and 30% *N*-acetylcyclohexyl-$d_{11}$-alanine-2,3,3-$d_3$ (**12**).[10] The mixture, after two recrystallizations from 400 ml of water, gave 7.1 g of the compound **12**: m.p. 172–173°; $\lambda_{max}$ (KBr) 3.00, 3.50, 4.60, 4.75, and 5.90 μ.

Anal. Calcd. for $C_{11}H_5D_{14}NO_3$: C, 58.09; H, 9.04; D, 73.6 atom %. Found: C, 58.10; H, 8.80; D, 72.5 atom % (98.5%).

### *2-Phenyl-4-benzylidene-$d_6$-oxazolone (8)*

Using a modification of Buck and Ide's procedure (8), 12.9 g (0.115 mole) of **1**, 20.7 g (0.115 mole) of hippuric acid, 8.4 g of sodium acetate, and 37 ml of acetic anhydride were heated on a steam bath for 2 h. Ethanol (48 ml) was then added, the solvents evaporated, and the solid residue extracted with hot benzene. Concentration of the benzene solution to small volume gave 15.7 g (54%) of the azlactone **8**: m.p. 161–163°; $\lambda_{max}$ (KBr) 4.42, 5.54, and 6.05 μ.

Anal. Calcd. for $C_{16}H_5D_6NO_2$: C, 75.27; H, 4.57; D, 54.5 atom %. Found: C, 75.14; H, 4.61; D, 54.4 atom % (99.8%).

### *2-Thio-4-benzylidene-$d_6$-thiazolone (9)*

According to the procedure of Billimoria and Cook (9) from 56.4 g (0.503 mole) of the aldehyde **1** and 56 g (0.421 mole) of 2-mercaptothiazole-5-one,[11] in 500 ml of acetic acid there is obtained 93.1 g (82% based on **1**) of the product **9**, m.p. 208–210°.

Anal. Calcd. for $C_{10}HD_6NOS_2$: C, 53.00; H, 3.38; D, 85.6 atom %. Found: C, 52.85; H, 3.43; D, 80.3 atom % (93.6%), 80.6 atom % (94.2%).

### L-*Phenyl-$d_5$-alanine-2,3,3-$d_3$ (5) and* D-*N-Acetylphenyl-$d_5$-alanine-2,3,3-$d_3$ (6)*

In accordance with the method of Greenstein and Winitz (11), renal acylase (0.245 g) was added to a solution of 6.15 g (0.0286 mole) of the DL-acetyl derivative **4** in 100 ml of water that had been neutralized to pH 7.1 with lithium hydroxide. After standing at 37° for 1 day, 10 drops of acetic acid were added, the mixture heated to 60° and filtered with the aid of charcoal. After addition of 20 ml of ethanol, the filtrate was evaporated to 5 ml. This gave 1.61 g of the L-amino acid **5**. An additional 0.21 g of **5** was obtained by the addition of ethanol to the above ethanol–water filtrate after it had been concentrated to small volume by evaporation.

Acidification to pH 1 of the final aqueous ethanol filtrate gave, after filtration, 2.00 g (65%) of the D-*N*-acetyl derivative **6**: m.p. 167–179°; $[\alpha]_D^{25}$ −36° (*c*, 1, MeOH); $\lambda_{max}$ (KBr) 3.00, 3.50, 4.42, and 5.95 μ.

The following procedure afforded an additional 0.20 g of the product **5**. The filtrate from the separation of **6** was adjusted to pH 5 and 0.30 g of cupric acetate added. The filtered copper salt was added to 10 ml of water, through which hydrogen sulfide gas was bubbled. The solution of the acid **5** was filtered, concentrated to small volume, and worked up with ethanol as reported above. The total yield of the amino acid **5** was 2.02 g (81%): m.p. 260–262° (decomp.); $[\alpha]_D^{25}$ −34° (*c*, 1, $H_2O$); $\lambda_{max}$ (KBr) 3.35, 3.40, 6.15, and 6.50 μ. Ultraviolet

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[8] The calculated value for %H on all compounds containing deuterium is that figure which would be obtained in a routine analysis which assumes all water of combustion to be $H_2O$. The following formula was used, $\%\,H = [1.008\,A\,(18.02 + 2.016\,B)]/18.02\,C \times 100$ where A equals the total number of H and D atoms in the molecular formula, B is the theoretical atom fraction of D, and C equals the formula weight, including the mass of deuterium.

[9] The ballast tank of the Parr apparatus is not used so as to prevent contamination of the deuterium gas by adsorbed hydrogen.

[10] Ratio determined by the ultraviolet absorption at 258 mμ.

[11] Obtained in 36% yield from ethyl chloroacetate using the procedure described in ref. 10.

absorption spectrum, ε's in parentheses:[12a] $\lambda_{max}$ ($H_2O$) 241.8 (77.2), 246.4 (114.2), 251.2 (161.0), 256.8 (189), 262.5 (143.5), and 265.6 mμ (92.6). Molecular rotation (at 25°; *c*, 1; 1 *N* HCl):[12b] [φ] 589, −13.3°; [φ] 470, −18.0°; [φ] 360, 0°; [φ] 275, +313°; [φ] 255, +837°; [φ] 240, +6050°.

Anal. Calcd. for $C_9H_3D_8NO_2$: C, 62.40; H, 6.88; D, 72.7 atom %. Found: C, 62.18; H, 6.99; D, 68.9 atom % (94.9%).

A pilot experiment was used to monitor the above reaction indirectly. An appropriate amount of the protio acetylphenylalanine with renal acylase was reacted in an nuclear magnetic resonance tube. The reaction was followed by the increase in the n.m.r. signal at τ 2.70 due to the phenyl protons of L-phenylalanine and the decrease of the signal at τ 2.80 due to the phenyl protons of acetylphenylalanine. Integration of the peaks gave the percentage of reaction.

### *Racemization of the Acetyl Derivative **6***

The labile protons were exchanged by stirring 2.00 g of the derivative **6** in 50 ml of deuterium oxide at room temperature. The heavy water was evaporated, 50 ml of acetic anhydride added, and the resulting solution refluxed for 3 h. After this time the solvents were removed *in vacuo* and 15 ml of deuterium oxide added; the evaporation procedure was repeated twice. Finally, the oil was crystallized from 20 ml of water to give 1.27 g (64%) of the racemized derivative **4**; m.p. 142–145°.

Anal. Calcd. for $C_{11}H_5D_8NO_3$: D, 61.6 atom %. Found: D, 65.3 atom % (106%).

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[12] The following data were obtained for protiophenylalanine. (*a*) $\lambda_{max}$ ($H_2O$), ε's in parentheses: 242.2 (70.6); 247.1 (106); 251.9 (153.2); 257.5 (189); 263.5 (147.5); and 266.8 mμ (82.5). (*b*) [φ] 589, −14.0°; [φ] 470, −18.6°; [φ] 360, 0°; [φ] 275, +312°; [φ] 255, +824°; [φ] 240, +6350°.

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