2-Methyl-1,3,4-cyclopentanetrione 4-Monothioketal (8).—To a stirred solution of 1.5 g (0.012 mole) of triketone **6** and 1.21 g (0.015 mole) of ethanedithiol in 20 ml of glacial acetic acid was added 0.5 ml of boron trifluoride etherate. After 24 hr the solid was removed by filtration and washed well with ether. Recrystallization from benzene gave 1.75 g (73%) of **8**, mp 199–201° dec, with infrared peaks at 5.95 and 6.15 μ.

Anal. Calcd for $C_8H_{10}O_2S_2$: C, 47.49; H, 4.98. Found: C, 47.39; H, 5.25.

Raney Nickel Desulfurization of 8.—A solution of 240 mg of **8** in 10 ml of absolute alcohol was refluxed for 67 hr with a large excess of Raney nickel. The mixture was worked up in the usual manner to give, after recrystallization from water, 30 mg of diketone **4a** which was identified by its infrared and nmr spectra.

Enol Acetates 9b and 10b.—The monothioketal **8** was treated with acetic anhydride and pyridine according to the procedure described earlier. Upon molecular distillation there was obtained a light yellow oil which showed infrared peaks at 5.6, 5.8, and 6.0 μ. Nmr analysis of this liquid indicated the presence of **9b** and **10b** in a ratio of 2:1.

Enol Ethers 9c and 10c.—This mixture of enol ethers was prepared by the action of diazomethane on the diketone **9**. Evaporative distillation at 100° (0.5 mm) gave an oil which showed infrared peaks at 5.8 and 6.1 μ. Nmr analysis showed that the enol ethers **9c** and **10c** were present in a ratio of 2.4:1.

2-Acetoxy-3-methyl-2-cyclopenten-1-one (5b).—This compound was prepared according to the method described by Erickson and Collins[16] and showed mp 61.5° (lit.[16] mp 62–62.5°); λ_{max} 5.6, 5.8, and 6.0 μ.

(16) J. L. E. Erickson and F. E. Collins, *J. Org. Chem.*, **30**, 1050 (1965).

2-Methoxy-3-methyl-2-cyclopenten-1-one (5c).[17]—A mixture of 6 g (0.053 mole) of diketone **5a**, 6.75 g of dimethyl sulfate, and 3.19 g of sodium methoxide in 50 ml of dry methanol was refluxed for 46 hr. The mixture was cooled, poured into 150 ml of 5% sodium hydroxide solution, and extracted with ether. Distillation gave 3.8 g (57%) of **5c**; bp 33° (0.5 mm), $n^{25}D$ 1.4866, λ_{max} 5.8 and 6.0 μ.

3-Acetoxy-2-methyl-2-cyclopenten-1-one (4b).—A solution of 2.3 g of diketone **4a** (mp 201–205°), 5 ml of acetic anhydride, and 7 ml of pyridine was kept at room temperature for 17 hr and then distilled to give 2.6 g (84%) of the acetate **4b**: bp 66–67.5° (0.5 mm); $n^{14}D$ 1.4912; λ_{max} 5.6, 5.8, and 6.0 μ.

Anal. Calcd for $C_8H_{10}O_3$: C, 62.33; H, 6.54. Found: C, 62.00; H, 6.76.

3-Methoxy-2-methyl-2-cyclopenten-1-one (4c).—A solution of 201 mg of diketone **4a** in ether–ethanol was treated with an excess of ethereal diazomethane. The solvents were removed and two sublimations *in vacuo* gave a solid: mp 61.8–62.3°, λ_{max} 5.85 and 6.1 μ.

Anal. Calcd for $C_7H_{10}O_2$: C, 66.65; H, 7.99. Found: C, 66.65; H, 7.90.

2-Methyl-3-methoxy-2-cyclohexenone.—A slurry of 500 mg (0.0035 mole) of 2-methyl-1,3-cyclohexanedione in 100 ml of ether was treated with excess ethereal diazomethane. After stirring for 3 hr, the ether was evaporated and the yellow residue (570 mg) was purified by vpc (200°, 20% DEGS on Chromosorb) to afford the enol ether which showed ν_{max} (film) 5.85 and 6.25 μ, and nmr signals at 1.72 (triplet, vinyl methyl), 1.80–2.78 [multiplets, $(CH_2)_3$], and 3.87 ppm (singlet, OCH_3).

(17) M. A. Gianturco and P. Friedel, *Tetrahedron*, **19**, 2039 (1963).

Deuterated Amino Acids. III. Synthesis of DL-Aspartic-2,3,3-d_3 Acid, L-Glutamic-2,3,3,4,4-d_5 Acid, L-Asparagine-2,3,3-d_3, and L-Glutamine-2,3,3,4,4-d_5[1,2a]

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Highly deuterated asparaginyl and glutaminyl derivatives were prepared for use in the synthesis of certain deuterio analogs of oxytocin and the vasopressins. DL-Aspartic-2,3,3-d_3 acid (96.7% D) was obtained in 59% yield in a five-step synthetic sequence from acetic acid-d_4 *via* alkylation of phthalimidomalonic ester with ethyl bromoacetate-d_2. DL-Glutamic-2,3,3,4,4-d_5 acid as well as the d_9 acid (98.6 and 96.4% D, respectively) were synthesized in an eight-step sequence from dipotassium acetylenedicarboxylate (50% over-all) *via* alkylation of acetamidomalonic ester with ethyl β-bromopropionate-d_4. Pure L-glutamic-2,3,3,4,4-d_5 acid (96.4% D) was obtained by selective enzymatic deacylation of the N-acetyl derivative of the aforementioned DL-galutamic acid-d_9 in 85% yield. DL-Asparagine-2,3,3-d_3 was prepared in 68% over-all yield from DL-aspartic acid-d_7 deuteriochloride *via* ammonolysis of the β-methyl ester. Selective enzymatic deacylation of the corresponding N-acetyl derivative produced L-asparagine-2,3,3-d_3 (97.5% D) in 87% yield. DL-N-Acetyl glutamine-2,3,3,4,4-d_5 was synthesized in 85% yield in a three-step synthetic sequence from DL-glutamic acid-d_9 deuteriochloride *via* ammonolysis of the corresponding N-acetyl γ-methyl ester. Enzymatic deacylation afforded L-glutamine-2,3,3,4,4-d_5 (65%, 96.1% D).

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,[1a] useful syntheses of the title compounds have been accomplished. More specifically, the deuterated asparaginyl and glutaminyl compounds are of interest in the synthesis of certain deuterio analogs of the peptide hormones, oxytocin, and the vasopressins. It is possible that study of a number of such analogs may provide information relative to the problem of structure and biological activity.

The syntheses presented in this report are useful in the sense that they are suitable for obtaining, in quantity, high yield, and isotopic purity, the title amino acids from relatively inexpensive materials. Further, these syntheses are considered to be the most satisfactory ones possible on the basis of our present knowledge,[3] including scouting experiments carried out in this laboratory.

DL-Aspartic-2,3,3-d_3 Acid.—Synthesis of this deuterated amino acid was best accomplished as outlined in

(1) (a) Part II: A. T. Blomquist, R. J. Cedergren, B. F. Hiscock, S. L. Tripp, and D. N. Harpp, *Proc. Natl. Acad. Sci. U. S.*, **55**, 453 (1966); (b) part I: A. T. Blomquist, B. F. Hiscock, and D. N. Harpp, *J. Org. Chem.*, **31**, 338 (1966).

(2) (a) This study was supported in part by the National Science Foundation, Grant No. G-18902; (b) National Institute of Health Molecular Biology Training Grant Predoctoral Fellow, 1965–1966; abstracted in part from the Ph.D. thesis of B. F. Hiscock, submitted to Cornell University in 1966; (c) National Institute of Health Molecular Biology Training Grant Postdoctoral Fellow, 1965–1966.

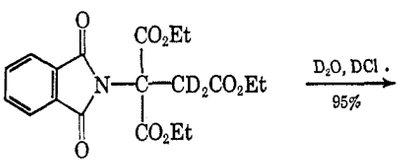
(3) (a) L-Aspartic-2,3,3-d_3 acid and L-glutamic-2,3,3,4,4-d_5 acid, among others, have been prepared by the hydrolysis of an alga grown in deuterated media: M. I. Blake, H. L. Crespi, V. Mohan, and J. J. Katz, *J. Pharm. Sci.*, **50**, 425 (1961); (b) J. S. Stekol and W. H. Hamill, *J. Biol. Chem.*, **120**, 531 (1937); N. Tamiya and T. Shimanouchi, *Spectrochim. Acta*, **18**, 895 (1962). L-Aspartic-2,3,3-d_3 acid has been prepared by these methods of exchange: (c) A. Murray, III, and D. L. Williams, "Organic Syntheses with Isotopes," Interscience Publishers, Inc., New York, N. Y., 1958; (d) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1961.

the reaction sequence given below.[4,5] With reference to this sequence, formation of ethyl bromoacetate-d_2 required ethanol-d to avoid loss of deuterium on the α-carbon atom. The over-all yields of the deuterated amino acids IIIa and IIIb from acetic acid-d_4 were *ca.* 59%.

$$CD_3CO_2D \xrightarrow[81\%]{Br_2,\ P} BrCD_2CO_2D \xrightarrow[D^+]{EtOD,} \underset{88\%}{}$$

$$BrCD_2CO_2Et \xrightarrow[\text{malonic ester in DMF}]{\text{sodio phthalimido}} \underset{94\%}{}$$

96.5% D



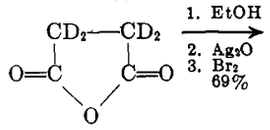
$$\xrightarrow[95\%]{D_2O,\ DCl} \text{I, 98.2\% D}$$

$$\text{DL-}DO_2CCD_2CDCO_2D \cdot ND_3^+Cl^- \text{ (II)} \xrightarrow[92\%]{Et_3N,\ D_2O \text{ or } H_2O} \text{DL-}RO_2CCD_2CDCO_2^- \cdot NR_3^+$$

IIIa, R = D (98.7% D)
b, R = H (96.7% D)

L-Glutamic-2,3,3,4,4-d_5 Acid.—After careful consideration of the various syntheses reported for protio glutamic acid[3d,6] and the special requirements set up for a useful method to obtain the deuterio analog, the synthesis that was actually worked out is given in the diagram that follows.[7]

$$KO_2C{-}C{\equiv}C{-}CO_2K \xrightarrow[2.\ HBr]{1.\ D_2O,\ Na\text{-}Hg} \underset{93\%}{HO_2CCD_2CD_2CO_2H} \xrightarrow[95\%]{AcCl} \underset{99.0\%\ D}{}$$



99.0% D

$$BrCD_2CD_2CO_2Et \xrightarrow[\text{malonic-N,2-}d_2\text{ ester (IV)}]{\text{sodio acetamido}} \underset{90\%}{AcNRCCD_2CD_2CO_2Et \cdot CO_2Et}$$

98.6% D

Va, R = D
b, R = H (99.5% D)

$$V \xrightarrow[2.\ Et_3N,\ D_2O \text{ or } H_2O]{1.\ DCl,\ D_2O} \underset{89\%}{\text{DL-}RO_2CCD_2CD_2CDCO_2^- \cdot NR_3^+} \xrightarrow[Na_2CO_3]{Ac_2O,} \underset{71\%}{}$$

VIIa, R = D (96.4% D)
b, R = H (98.6% D)

$$\text{DL-}RO_2CCD_2CD_2CDCO_2R \cdot NRAc$$

VIIIa, R = D
b, R = H (99.1% D)

$$VIII \xrightarrow[H_2O]{\text{renal acylase}} \text{L-}HO_2CCD_2CD_2CDCO_2^- \cdot NH_3^+ +$$

IX, 85% yield, 96.4% D

$$\text{D-}HO_2CCD_2CD_2CDCO_2H \cdot NHAc$$

X, 61% yield, 98.2% D

$$X \xrightarrow[2.\ Et_3N]{1.\ HCl,\ H_2O} \text{D-}HO_2CCD_2CD_2CDCO_2^- \cdot NH_3^+$$

XI

The preparation of ethyl 3-bromopropionate-d_4 was relatively straightforward but the succeeding alkylation of acetamidomalonic ester proved to be a bit troublesome. In order to obtain a high yield and maintain high deuterium content in the alkylation, it was necessary to use acetamidomalonic-N,2-d_2 ester; alkylation of phthalimidomalonic ester was completely unsuccessful.[8] Acetylation of the amino acid VII had to be done in deuterium oxide in order to avoid loss of deuterium content.[9] The deuterated L-glutamic acid, IX, was obtained in 30% yield over-all from dipotassium acetylenedicarboxylate.

L-Asparagine-2,3,3-d_3.—After careful study of the many procedures used to obtain asparagine from aspartic acid it was found that the reaction sequence given below was the most suitable one for the preparation of the desired deuterated amino acid.

$$II \xrightarrow[2.\ Et_3N]{1.\ MeOD,\ D^+} \text{DL-}CH_3O_2CCD_2CDCO_2^- \cdot NR_3^+ \xrightarrow[2.\ H_2O]{1.\ ND_3,\ MeOD} \underset{94\%}{}$$

72%

XIIa, R = D
b, R = H (97.2% D)

$$\text{DL-}H_2NCCD_2CDCO_2^- \cdot NH_3^+ \xrightarrow[72\%]{Ac_2O} \text{DL-}H_2NCCD_2CDCO_2H \cdot NHAc$$

XIII
97.8% D

XIV
96.7% D

$$XIV \xrightarrow[\text{acylase}]{\text{renal}} \text{L-}H_2NCCD_2CDCO_2^- \cdot NH_3^+ + \text{D-}H_2NCCD_2CDCO_2H \cdot NHAc$$

XV
87% yield, 97.5% D

XVI
69% yield, 97.2% D

The desired L-asparagine was obtained in 42% yield over-all from the DL salt II.

L-Glutamine-2,3,3,4,4-d_5.—Detailed preliminary studies on protio compounds indicated that of all the various procedures reported for the preparation of glutamine from glutamic acid, the method of Maschler and Lichtenstein[10] (outlined below) was most suitable for the preparation of the desired deuterated glutamine. It is of interest to note that the reactions of N-acetylation and ester ammonolysis of the β-ester amino acid were best done in an order inverse to that used in the synthesis of asparagine[11] (*vide supra*).

The over-all yield of the deuterated L-glutamine XX from the salt VI was 55%.

The Experimental Section of this report is rather

(4) In particular, the amination of diethyl bromosuccinate-d_4 (available from the ethyl hydrogen succinate-d_4) was investigated. The reaction greatly favored elimination over substitution in all instances tried.

(5) This sequence was adapted in part from [M. S. Dunn and B. W. Smart, "Organic Syntheses," Coll. Vol. 4, John Wiley and Sons, Inc., New York, N. Y., 1963, p 55] and was extensively modified giving over twice the yield originally reported.

(6) C. W. Huffman and W. G. Skelly, *Chem. Rev.*, **163**, 625 (1963).

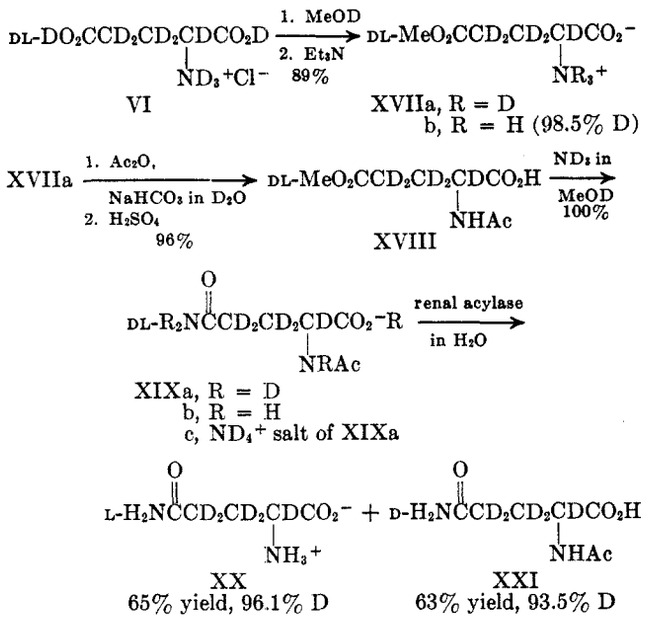
(7) This sequence was adapted in part from J. Kato, H. Ishihara, and O. Hiwatashi, *J. Agr. Chem. Soc. Japan*, **27**, 498 (1953).

(8) Only phthalimidomalonic-2-*d* ester was isolated indicating elimination had occurred, an observation previously made indirectly by C. S. Marvel and M. P. Stoddard [*J. Org. Chem.*, **3**, 198 (1938)] and O. Hiwatashi [*J. Agr. Chem. Soc. Japan*, **27**, 498 (1953)].

(9) Considerable efforts to improve the yield of this reaction were unsuccessful.

(10) I. J. Maschler and N. Lichtenstein, *Biochim. Biophys. Acta*, **57**, 252 (1952).

(11) Treatment of γ-methylglutamate with ammonia affords primarily pyrrolidonecarboxylic acid; see A. F. Beecham, *J. Am. Chem. Soc.*, **76**, 4615 (1954).



detailed since it is considered to be important with reference to yield, deuterium content, etc., of the compounds of interest. Some experiments that involve protio analogs are described since they are pertinent to the general synthetic approaches.

Molecular rotations of some of the protio and deuterio compounds are given in Table I.

TABLE I
MOLECULAR ROTATIONS OF SOME OF THE PROTIO AND DEUTERIO COMPOUNDS[a]

Compound	$[\phi]_{589}$	$[\phi]_{578}$	$[\phi]_{546}$	$[\phi]_{436}$	$[\phi]_{365}$
L-Asp(NH_2)-$d_3 \cdot H_2O$[b]	41.3	43.1	49.2	85.4	136.2
L-Asp(NH_2)$\cdot H_2O$[b]	42.2	44.2	50.4	87.4	139.2
D-N-AcAsp(NH_2)-d_3[c]	6.2	7.1	8.3	18.1	34.4
D-N-AcAsp(NH_2)[c]	2.1	2.6	3.5	10.0	23.0
L-Glu-d_5[d]	46.0	47.3	54.2	96.6	160.5
L-Glu[d]	45.0	46.9	53.9	95.8	158.5
D-Glu-d_5[d]	45.7	47.2	54.2	96.6	156.1
D-Glu[d]	45.3	47.5	54.1	97.0	159.6
L-Glu(NH_2)-d_5[b]	44.6	46.7	53.5	95.7	160.3
L-Glu(NH_2)[b]	46.6	48.7	55.7	99.4	164.9
L-Glu(NH_2)-d_5[c]	9.4	10.3	11.5	22.2	41.6
L-Glu(NH_2)[c]	9.4	10.1	11.4	21.8	40.0
D-N-AcGlu(NH_2)-d_5[c]	24.4	25.3	28.4	45.6	63.4
D-N-AcGlu(NH_2)[c]	23.2	23.9	26.7	42.0	56.8

[a] As seen in the cases of protio and deuterio L-Asp(NH_2)$\cdot H_2O$, L-Glu(NH_2), and D- and L-Glu molecular rotations are generally quite similar. The large differences observed for the protio and deuterio D-N-AcAsp(NH_2) compounds are not immediately explicable. All rotations are positive excepting those for D-Glu-d_5 and D-Glu. [b] 1 *N* HCl, 25°, *c* 2. The solutions were examined immediately in order to minimize complications resulting from hydrolysis. [c] H_2O, 25°, *c* 2. [d] 5 *N* HCl, 25°, *c* 2.

Experimental Section[12]

Ethyl Bromoacetate-d_2.—Bromination of acetic acid-d_4[13] (50.0 g, 0.78 mole) *via* the procedure used for the protio analog[14] gave 89.4 g (81%) of bromoacetic acid-d_3: bp 90–96° (10 mm); λ_{max}^{KBr} 4.40, 5.75–5.90, 7.35–7.45, and 10.12 μ.

Anal.[15] Calcd for $C_2D_3BrO_2$: C, 16.92; H, 2.37; Br, 56.28; D, 100 atom %. Found: C, 16.45; H, 2.55; Br, 55.59; D, 91.0 atom %.[16]

Esterification[17] of 77.8 g (0.55 mole) of bromoacetic acid-d_3 with ethanol-d[18] and concentrated sulfuric acid-d_2[19] as catalyst afforded 82 g (88%) of ethyl bromoacetate-d_2: bp 156–157° (741 mm); $n^{24}D$ 1.4490; d^{24} 1.5164; $[R_L]D$ calcd[20] 29.84, found, 29.92; λ_{max}^{neat} 5.78, 7.94, 9.31, 9.45, and 9.77 μ.

Anal.[15] Calcd for $C_4H_5D_2BrO_2$: C, 28.42; H, 4.31; Br, 47.28; D, 28.6 atom %. Found: C, 28.60; H, 4.42; Br, 47.47; D, 27.6 atom % (96.5%).

Diethyl 2-Phthalimido-2-carbethoxysuccinate-3,3-d_2 (I).—A solution of 327 g (1.0 mole) of sodio phthalimidomalonic ester[21] and 161.5 g (0.96 mole) of ethyl bromoacetate-d_2 in 946 ml of dimethylformamide (DMF) was heated at 85–90° for 16 hr. The cooled reaction mixture was filtered and then concentrated *in vacuo*. The residue obtained was dissolved in 635 ml of dry ether. This ether solution was then filtered, washed with eight 30-ml portions of water, and dried. Removal of the ether gave 325 g (94%) of the phthalimido triester I as a pale yellow oil that crystallized slowly at room temperature. After one recrystallization from ethanol the product I had mp 67.0–67.5°; λ_{max}^{KBr} 5.61, 5.70, 5.82, 7.29, 7.93, and 13.91 μ.

Anal.[15] Calcd for $C_{19}H_{19}D_2NO_8$: C, 58.01; H, 5.38; N, 3.56; D, 9.52 atom %. Found: C, 58.21; H, 5.47; N, 3.57; D, 9.35 atom % (98.2%).

DL-Aspartic Acid-d_7 Deuteriochloride (II).—To an acidic solution, prepared by the reaction of 218 ml (3.0 moles) of freshly distilled thionyl chloride with 435 ml of 99.7% D_2O at 0°, there was added 150 g (0.38 mole) of the crude triester I. The mixture was refluxed for 20 hr, cooled, and filtered to remove the bulk of the phthalic acid-d_2 (62.63 g, 98%) that separated. The collected phthalic acid was washed with 55 ml of D_2O, the wash liquid was added to the original filtrate, and the whole was evaporated to near dryness at 40–50° *in vacuo*.[22] The residue obtained was stirred with 700 ml of 1,2-dimethoxyethane (glyme)[23] to

(12) (a) Melting points are uncorrected. Microanalyses were performed by Scandinavian Microanalytical Laboratories, Herlev, Denmark, and Micro-Tech Labs, Skokie, Ill. Deuterium analyses were carried out by Josef Nemeth, Urbana, Ill., using the falling drop technique. Whatman No. 4 paper (10-cm path) was employed for chromatography using 95% ethanol–water (7:3, v/v) as eluent. Thin layer chromatography was carried out on silica gel G coated microscope slide plates using chloroform–acetone, 7:3, iodine developer. Renal acylase and the protio amino acids were purchased from Mann Labs, New York, N. Y., magnesium nitride was obtained from K & K Labs, New York, N. Y. Optical rotations were obtained on a Perkin-Elmer Model 141 polarimeter; infrared spectra were obtained on a Perkin-Elmer Infracord spectrophotometer; nmr data were recorded on a Varian Associates Model A-60 spectrometer and mass spectra were obtained on a Consolidated Engineering Corp. Type 21-103A spectrometer. Preliminary studies employing protio materials afforded yields comparable to those reported below. Sodium amalgam (2%) was prepared as previously described; see L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1957, p 340. It was not necessary to use a flame to initiate this reaction if the sodium was cut with scissors and added directly to the mercury. (b) Care was exercised where appropriate, to avoid contamination by atmospheric moisture.

(13) Acetic acid-d_4 may be purchased from Merck Sharp and Dohme Ltd., Montreal.

(14) C. F. Ward, *J. Chem. Soc.*, **121**, 1161 (1922).

(15) The calculated values for per cent 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 = $[1.008A(18.02 + 2.016B)]/18.02C \times 100$ 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. For example Calcd for $C_4H_5D_2BrO_2$ (mol wt 169), % H = $[1.008 \times 7(18.02 + 2.016 \times {}^2/_7)]/18.02 \times 169 \times 100 = 4.31$.

(16) Analysis of this compound by nmr showed a deuterium content of at least 95%. Deuterium analyses by the falling drop method were low probably owing to the difficulty in drying and handling the low-melting, hydroscopic solid.

(17) For the preparation of the protio analog see S. Natelson and S. Gottfried, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p 381.

(18) V. J. Shiner and M. L. Smith, *J. Am. Chem. Soc.*, **83**, 593 (1961).

(19) Small amounts of sulfuric acid-d_2 can be conveniently prepared by hydrolyzing dimethyl sulfate in D_2O.

(20) In the calculation of $[R_L]D$ (from A. I. Vogel, "A Textbook of Practical Organic Chemistry Including Qualitative Organic Analysis," 3rd ed, John Wiley and Sons, Inc., New York, N. Y., 1956, p 1034) the deuterium atom is assumed to have a value of 1.004: E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 401.

(21) G. Barger and T. E. Weichselbaum "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 384.

(22) If moisture has been carefully excluded from the aforementioned procedures, the valuable D_2O–DCl solution containing some EtOD may be almost entirely recovered.

(23) (a) Glyme is very useful for removing traces of water and precipitating amino acid hydrohalides. (b) Residual phthalic acid-d_2 was removed in this step.

produce a fine suspension of the crude product II that was then filtered and washed successively with 100 ml of glyme and 200 ml of ether. After drying *in vacuo* there was obtained 64.4 g (95%) of the salt II which had R_f 0.46. An analytical sample of the product II was obtained by crystallization of 500 mg of II from a mixture comprised of 10 ml of ethanol and 90 ml of glyme. The infrared spectrum of this sample of II showed λ_{max}^{KBr} 4.22, 4.49, 5.75, 5.90, 7.43, 8.64, and 9.73 μ.

Anal.[15] Calcd for $C_4D_3ClNO_4$. C, 27.05; H, 5.05; Cl, 19.96; N, 7.89. Found: C, 27.24; H, 5.08; Cl, 19.64; N, 7.98.

DL-**Aspartic Acid-d_7 (IIIa).**—This amino acid was obtained from the salt II in 92% yield (0.01 mole scale)[24] *via* the same procedure used for the preparation of DL-glutamic acid-d_9 (*vide infra*). The compound IIIa showed R_f 0.45; λ_{max}^{KBr} 4.41, 5.95, 6.26, 7.45, 8.44, 9.23, and 13.20 μ.

Anal.[15] Calcd for $C_4D_7NO_4$: C, 34.30; H, 5.60; N, 10.00; D, 100 atom %. Found: C, 34.15; H, 5.66; N, 9.95; D, 98.7 atom %.

DL-**Aspartic-2,3,3-d_3 Acid (IIIb).**—This amino acid was prepared from the salt II (0.01-mole scale) by the same procedure used for the amino acid IIIa except that water was used instead of D_2O. Further, the product IIIb was recrystallized twice from water to incur complete exchange of deuterium at the labile positions. The compound IIIb showed R_f 0.45; λ_{max}^{KBr} 3.32, 5.95, 6.74, and 7.60–7.70 μ.

Anal.[15] Calcd for $C_4H_4D_3NO_4$: C, 35.29; H, 5.43; N, 10.29; D, 42.8 atom %. Found: C, 35.19; H, 5.53; N, 10.13; D, 41.4 atom % (96.7%).

Dipotassium Acetylenedicarboxylate.—A solution of potassium hydroxide (66.0 g, 1.0 mole) and monopotassium acetylenedicarboxylate (152.1 g, 1.0 mole) in 300 ml of water was allowed to evaporate at room temperature over several days. The crystals were filtered, washed with acetone, and dried *in vacuo* for 48 hr over phosphorus pentoxide. The salt was then crushed, and dried *in vacuo* for 24 hr at 95° to yield 170.0 g (89%) of a nonhygroscopic, slightly off-white powder: λ_{max}^{KBr} 6.15, 7.40, 7.53, 10,10, and 12.86 μ.

Anal. Calcd for $C_4K_2O_4$: C, 25.25; H, 0.00. Found: C, 25.19; H, 0.17.

Succinic-d_4 Acid.[25]—About 50 ml of a solution of 91.89 g (0.48 mole) of dipotassium acetylenedicarboxylate in 300 ml of 99.7% D_2O was added to 2500 g (2.1 g-atoms) of freshly prepared 2% sodium amalgam.[12] The flask was manually shaken to loosen the amalgam and the remainder of the solution was added over 45 min. The mixture was stirred vigorously for 1 hr and separated, and the mercury was washed with 10 ml of D_2O. The combined extracts were cooled,[26] 350 ml of 48% HBr was added in one portion, and the mixture was stirred for 1 hr. After evaporating the mixture to dryness *in vacuo*, the material was dried over phosphorus pentoxide and sodium hydroxide pellets *in vacuo* for 24 hr, then crushed and redried. The solid (360 g) was extracted for 48 hr with anhydrous ether in a Soxhlet apparatus. The ether was evaporated, 250 ml of dry benzene was added, and the crude product was filtered to give 54.6 g (93%), mp 185–186°. This material was used without further purification in the next step. Crystallization from water provided pure acid: mp 185–185.5° (lit.[25] mp 181–182.6°); λ_{max}^{KBr} 3.26–3.86, 4.12, 5.86, 7.09, 7.68, 9.45, and 9.84 μ.

Anal.[15] Calcd for $C_4H_2D_4O_4$: C, 39.34; H, 5.32; D, 66.67 atom %. Found: C, 39.40; H, 5.15; D, 66.0 atom % (99.0%).

Succinic Anhydride-d_4.[27]—Succinic-d_4 acid, (59.0 g, 0.48 mole) was converted into 48.2 g (96%) of succinic anhydride-d_4 as previously described for the protio analog.[28] After crystallization from purified chloroform[29] the anhydride showed mp 120–121° (lit.[27] mp 119.3–119.6°); λ_{max}^{KBr} 4.31–4.67, 5.37, 5.59, 8.05, and 10.14 μ. Mass spectrometry showed 95.6% d_4 and 4.4% d_3 species (98.9 atom % D). Analysis by the falling drop method gave 99.0 atom % D.

Anal.[15] Calcd for $C_4D_4O_3$: C, 46.15; H, 4.31; D, 100.0 atom %. Found: C, 46.37; H, 4.10; D, 99.0 atom %.

Ethyl Hydrogen Succinate-d_4.—Succinic anhydride-d_4[30] (47.27 g, 0.45 mole) was treated with absolute ethanol (32 ml) as previously described for a protio homolog.[31] Distillation yielded 65.2 g (96%) of the half-ester: bp 130–131° (1.2 mm); $n^{25}D$ 1.4300; d^{25} 1.1718; $[R_L]D$ calcd[20] 32.93, found 33.11. The major bands in the infrared spectrum were found at 3.21, 4.44–4.76, 5.73–5.83, 7.80, and 9.57 μ. Analysis by nmr revealed only signals owing to the ethyl and carboxyl protons.

Anal.[15] Calcd for $C_6H_6D_4O_4$: C, 47.99; H, 7.01; D, 40.0 atom %. Found: C, 47.89; H, 6.88; D, 39.30 atom % (98.3%).

Ethyl Silver Succinate-d_4.—Silver oxide was prepared by adding a solution of 9.45 g (0.14 mole) of potassium hydroxide in 50 ml of water to 16.99 g (0.10 mole) of silver nitrate in 50 ml of water. The brown precipitate was thoroughly stirred, filtered, washed with 200 ml of water, and finally with 10 ml of D_2O. This material was added to a beaker containing 15.02 g (0.10 mole) of ethyl hydrogen succinate-d_4 and the resulting gray paste was stirred vigorously with a spatula. (During these preparations it was sometimes necessary to add more D_2O to the reaction in order to facilitate good mixing.) The paste was dried *in vacuo* over phosphorus pentoxide for 24 hr, crushed, and redried for 24 hr. The product, 24.86 g (97%) showed λ_{max}^{KBr} 4.49, 5.79, 6.38, 7.09, 9.26, and 9.56 μ.

Anal. Calcd for $C_6H_5AgD_4O_4$. D, 44.44 atom %. Found: D, 43.4 atom % (97.7%).

Ethyl 3-Bromopropionate-d_4.—A flask equipped with stirrer, condenser, drying tube, and containing 34.53 g, (0.13 mole) of ethyl silver succinate-d_4 suspended in 180 ml of dry carbon tetrachloride was placed in an oil bath maintained at 80°. A solution of 21.45 g (0.13 mole) of bromine in 150 ml of carbon tetrachloride was added over 45 min. After an additional 15 min of reflux, the red-orange mixture was cooled to room temperature and treated with 1.0 ml of oleic acid which rendered the liquid phase colorless. The yellow residue of silver bromide (25.39 g) was collected, and the solution was concentrated *in vacuo* at 40–45°. The pale yellow oil was distilled with no appreciable forerun and gave 18.29 g (74%) of the bromo ester: bp 55–57° (6.0 mm); $n^{25}D$ 1.4487; d^{25} 1.4368; $[R_L]D$ calcd[26] 34.44, found 34.53, λ_{max}^{neat} 3.42, 4.58, 5.75, 6.83, 7.84, 9.29, and 9.75 μ. Analysis by nmr revealed only signals due to the ethyl group.

Anal.[15] Calcd for $C_5H_5BrD_4O_2$: C, 32.45; H, 5.14; Br, 43.18; D, 44.44 atom %. Found: C, 32.51; H, 5.17; Br, 42.98; D, 43.8 atom % (98.6%).

Acetamidomalonic-N,2-d_2 Ester (IV).—Acetamidomalonic ester (35.00 g, 0.16 mole) was added to 87 ml of 99.7% D_2O and the mixture was refluxed for 20 min. The solution was chilled for 12 hr, and the crystallized product IV was filtered in a drybox, and dried over phosphorus pentoxide to give 33.25 g (94%) of the ester IV: mp 94–95°; λ_{max}^{KBr} 3.33, 4.17, 5.70, and 6.10 μ. Analysis by nmr revealed only signals due to the ethyl and methyl groups.

Anal.[15] Calcd for $C_9H_{13}D_2NO_5$: C, 49.31; H, 7.00; N, 6.39; D, 13.33 atom %. Found: C, 49.30; H, 7.07; N, 6.38; D, 13.10 atom % (98.3%).

Diethyl N-Acetyl-2-carbethoxyglutamate-3,3,4,4-d_4 (Vb).—To a solution of 2.24 g (0.097 g-atom) of sodium in 135 ml of ethanol-d[32,33] was added 21.25 g of the ester IVb with 5 ml of ethanol-d wash. The temperature was maintained at *ca.* 50° while 18.00 g (0.097 mole) of ethyl 3-bromopropionate in 10 ml of ethanol-d was added over 5 min. Sodium bromide began to precipitate in about 1 min. The mixture was refluxed for 12 hr, and analysis by thin layer chromatography (tlc) indicated the presence of the desired triester Va as well as traces of the ester IV. Sodium ethoxide[34] in ethanol-d (1 ml) was added and continued reflux for 20 min rendered the reaction mixture homogeneous to tlc. The ethanol-d was removed by distillation (*ca.* 140 ml),[35] the

(24) Although large quantities of amino acid III may be prepared by this procedure, most of the salt VII was used for the preparation of L-asparagine-2,3,3-d_3.

(25) J. O. Halford and L. C. Anderson, *J. Am. Chem. Soc.*, **58**, 737 (1936).

(26) Most of the D_2O can be recovered at this point (prior to the addition of the HBr solution).

(27) A. McLean and R. Adams, *J. Am. Chem. Soc.*, **58**, 804 (1936).

(28) L. F. Fieser and E. L. Martin, ref 21, p 560.

(29) A. Vogel, "Practical Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1956, p 176.

(30) Owing to the fluffy nature of the crystals it was necessary to consolidate them by means of a large pellet press before reaction.

(31) J. Cason, ref. 17, p 169.

(32) A. Streitwieser, Jr., L. Verbit, and P. Stang, *J. Org. Chem.*, **29**, 3706 (1964).

(33) Rigorously dried ethanol-d must be used for satisfactory results; tetraisopropyl titanate (Alfa Inorganics, Beverly, Mass.) was used to test for moisture.

(34) Addition of base catalyzed a Michael addition between ethyl acrylate-d_3 and ester IV. These materials were formed in small amounts by an elimination reaction between the sodio salt of IV and ethyl-3-bromopropionate-d_4.

mixture was cooled to room temperature, and 130 ml of anhydrous ether was added. The sodium bromide was filtered (9.87 g, 99%). (*Anal.* Calcd for NaBr: Br, 77.66. Found: Br, 76.99.) The pale yellow ether solution[36] was placed in a continuous ether extractor for 20 hr with 70 ml of D_2O. The ether was separated, dried, and concentrated *in vacuo* to yield 28.26 g (90%) of the d_5 triester Va, mp 59–61°. This material was used without further purification in the next step. About 500 mg of the ester Va was recrystallized twice from water–ethanol (3 ml, 5:1) giving white crystals of Vb (this process effected exchange of the amido deuteron for a proton), mp 64–64.5°; the infrared spectrum showed λ_{max}^{KBr} 3.06, 3.33, 3.89–4.13, 5.75, 6.06, 9.34, and 9.69 μ.

Anal.[15] Calcd for $C_{14}H_{19}D_2NO_7$: C, 52.32; H, 7.35; N, 4.36; D, 17.39 atom %. Found: C, 52.17; H, 7.37; N, 4.24; D, 17.30 atom % (99.5%).

DL-Glutamic Acid-d_9 Deuteriochloride (VI).—The triester Va (21.75 g, 0.067 mole) was hydrolyzed by refluxing it for 3 hr in an acidic solution prepared by carefully adding 38 ml of freshly distilled thionyl chloride to 95 ml of 99.7% D_2O at 5° in a dry atmosphere. The reaction mixture was treated with decolorizing carbon, concentrated (90 ml removed) to a pale yellow, viscous solution, and treated at 5° with 140 ml of glyme.[23a] The mixture was stirred 2 hr, filtered, and washed with 20 ml of glyme and finally with 50 ml of dry ether. The white salt VI weighed 11.36 g after drying for several hours *in vacuo*. An additional 1.00 g (total yield 12.36 g, 95%) of VI was obtained by concentrating the filtrate, adding 75 ml of glyme, and treating as above. The infrared spectrum showed λ_{max}^{KBr} 4.43–4.68, 5.81, 5.95, 7.32, 9.40, and 9.65 μ; paper chromatography indicated a single spot, R_f 0.59. The salt was analyzed without further purification.

Anal.[15] Calcd for $C_5D_{10}ClNO_4$: C, 31.01; H, 5 79; Cl, 18.31; N, 7.23. Found: C, 30.82; H, 5.83; Cl, 18.27; N, 7.18.

DL-Glutamic Acid-d_9 (VIIa).[37]—A solution of 8.40 g (0.043 mole) of the deuterio chloride VI in a minimum volume of D_2O (13 ml) was chilled in an ice bath and treated with 6.7 ml (0.048 mole) of triethylamine. With continued cooling, this mixture was stirred magnetically for several minutes; partial crystallization of the free amino acid occurred during this time. To ensure maximum precipitation of the free amino acid, the reaction mixture was diluted with *ca.* five volumes of acetone, stirred thoroughly, and filtered. The filtered product was washed successively with 25 ml of acetone, three 15-ml portions of chloroform, and finally with 10 ml of anhydrous ether. The yield of glutamic acid-d_9 hydrate-d_2[38] was 7.15 g (94%). Recrystallization was effected by dissolving the product in 77 ml of hot D_2O, filtering, adding a few crystals of anhydrous VIIa,[39] and adding 33 ml of ethanol-*d*. Pure amino acid VIIa (5.93 g) was obtained in 94% yield (88% from the salt VI). Infrared bands were found at λ_{max}^{KBr} 4.31–4.60, 6.04, 6.21, and 8.43 μ. Paper chromatography indicated a single spot, R_f 0.52.

Anal.[15] Calcd for $C_5D_9NO_4$: C, 38.45; H, 6.46; N, 8.97; D, 100.0 atom %. Found: C, 38.10; H, 6.49; N, 8.93; D, 96.4 atom %.

DL-Glutamic-2,3,3,4,4-d_5 Acid (VIIb).—The deuterio chloride salt VI (1.94 g, 0.01 mole) was converted to 1.63 g (96%) of glutamic-2,3,3,4,4-d_5 acid hydrate by the same procedure as that used for the amino acid VIIa except that water was used instead of D_2O. This material was recrystallized[39] twice from water–ethanol (82% recovery) to effect complete exchange for hydrogen at the labile positions. The water of hydration was completely removed by drying in an oven at 100° *in vacuo* for 96 hr. Paper chromatography indicated a single spot, R_f 0.52; λ_{max}^{KBr} 3.25, 3.98, 4.81, 6.11, 6.61, 7.06, and 7.66 μ.

Anal.[15] Calcd for $C_5H_4D_5NO_4$: C, 39.47; H, 6.33; N, 9.21; D, 55.56 atom %. Found: C, 39.59; H, 6.38; N, 9.45; D, 54.95 atom % (98.6%).

DL-N-Acetylglutamic-2,3,3,4,4-d_5 Acid (VIIIb).—To a slurry of amino acid VIIa (5.32 g, 0.034 mole) in 20 ml of D_2O was added 12.6 g (0.12 mole) of anhydrous sodium carbonate over 5 min. The mixture was stirred magnetically for 10 min and 3.4 ml (0.036 mole) of freshly distilled acetic anhydride was added in one portion. The reactants formed a thick paste which was loosened with a stirring rod. Stirring was continued for 24 hr, the mixture was cooled, and 21 ml of a DCl–D_2O solution[40] was carefully added. The precipitated acetyl derivative VIIIa was chilled for 24 hr and filtered to give 8.60 g of a white solid. A DCl–D_2O solution (10 ml) was added to the filtrate, and the mixture was cooled for 24 hr, and filtered to afford an additional 4.18 g of solid. The solids were combined, dried *in vacuo* over phosphorus pentoxide, and placed in a Soxhlet thimble and extracted for 30 hr with dry acetone.

Evaporation of the solvent afforded 4.72 g (71%) of DL-N-acetylglutamic-N,2,3,3,4,4-d_6 acid-d_2 (VIIIa), mp 178–180°. This material was used without further purification in the preparation of L-glutamic-2,3,3,4,4-d_5 acid (IX). An analytical sample (as VIIIb) was prepared by two crystallizations of the d_8 derivative VIIIa from water (0.3 g/ml of H_2O). This gave white crystals: mp 183–184°; λ_{max}^{KBr} 3.03, 3.84–4.17, 5.17, 5.84, 6.38, and 7.16 μ.

Anal.[22] Calcd for $C_7H_6D_5NO_5$: C, 43.29; H, 6.00; N, 7.21; D, 45.45 atom %. Found: C, 43.19; H, 6.01; N, 7.17; D, 45.05 atom % (99.1%).[41]

L-Glutamic-2,3,3,4,4-d_5 Acid (IX).—A solution of 3.94 g (0.02 mole) of compound VIIIa in 160 ml of distilled water (resulting pH, 2.31) was adjusted to pH 7.00 by the addition of 2 *N* NH_4OH, and diluted to 200 ml. Renal acylase[42] (10 mg) was added and the solution was stirred for 5 min and incubated at 37° for 24 hr. Charcoal (0.2 g) was added, the mixture was stirred 15 min and filtered through paper, then through sintered glass, and the pH was adjusted to 3.22 with 5 *N* HCl. The clear solution was evaporated to dryness at 30–35° (*ca.* 20 mm). An ethanol–water solution (30 ml, 2:1) was added and the mixture was stirred magnetically for 15 min at room temperature. The product was filtered (the filtrate containing NH_4Cl and the acetyl derivative X) and washed with 5 ml of ethanol, 30 ml of warm acetone, and finally with 30 ml of dry ether. Drying *in vacuo* afforded 1.38 g of crude amino acid IX. On standing for 24 hr, an additional 0.06 g of compound IX precipitated from the above filtrate giving 1.44 g (95%) of IX, $[\alpha]^{24}D$ +29.5° (*c* 2, 5 *N* HCl). One gram was recrystallized[39] to afford 0.90 g of pure amino acid IX: mp 189–190.5° (lit.[43,44] mp 208–209°, 198°), with authentic protio L-glutamic acid mmp 190–191°; $[\alpha]^{24}D$ +30.2° (*c* 2, 5 *N* HCl) (lit.[3a,44] $[\alpha]D$ +29.3°, 28.9°); paper chromatography showed one spot, R_f 0.52; λ_{max}^{KBr} 3.25, 4.77, 6.08, 6.31, 6.61, 7.05, 7.66, and 7.93 μ. This spectrum was identical with one taken of "crude" IX initially isolated.

Anal.[15] Calcd for $C_5H_4D_5NO_4$: C, 39.47; H, 6.33; N, 9.21; D, 55.56 atom %. Found: C, 39.50; H, 6.44; N, 9.25; D, 53.55 atom % (96.4%).

D-N-Acetylglutamic-2,3,3,4,4-d_5 Acid (X).—The filtrate of the previous experiment was evaporated to dryness *in vacuo* and the resulting 3.55 g of white powder was placed in a Soxhlet cup and extracted for 30 hr with dry acetone. Evaporation of the solvent, filtration, and an ether wash gave 1.18 g (61%) of the acetyl derivative X as a light tan powder: mp 188–191°, $[\alpha]^{25}D$ +13.9° (*c* 2, water). Two recrystallizations from water (25% recovery) gave an analytical sample: mp 192–194.5°, $[\alpha]^{25}D$ +16.4° (*c* 2, water). The infrared spectrum showed λ_{max}^{KBr} 3.03, 3.75–4.30, 5.20, 5.84, 6.37, and 7.18 μ.

(35) Analysis by nmr indicated that the isotopic integrity of the ethanol-*d* was not diminished (>95%).

(36) The color can be removed by filtration through alumina causing partial exchange of the amide deuteron, apparently from the water present in the alumina. No significant isotopic dilution results in the subsequent products.

(37) Converting an amino acid hydrohalide to the more readily purified free amino acid was of real concern in developing the best possible syntheses of labeled amino acids. This procedure appears superior to others in the literature in that it is rapid, reliable, and affords amino acids in uniformly high yield (*ca.* 95%) and purity (satisfactory C and H analyses) with no detectable racemization. The following amino acids have been obtained from their salts in this manner: alanine, aspartic acid, β-methylaspartate, cysteine, glutamic acid, glycine, histidine, leucine, phenylalanine, and serine.

(38) The D_2O of hydration could be removed by drying in an oven at 100° *in vacuo* for several hours.

(39) The product could be obtained as either VIIb of VIIb·H_2O by seeding the hot, aqueous solution (1 g of amino acid/11 ml of water) with the parent material, and adding 6 ml of ethanol.

(40) This solution (*ca.* 12 *N*) was prepared by adding 55 ml of thionyl chloride to 93 ml of chilled D_2O.

(41) When the acetyl derivative VIIIb was prepared as above in water as a reaction medium, the product VIIIb had mp 180–182°, and analyzed for 41.40 atom % D (91.1%).

(42) (a) Reference 3d, p 1948; (b) S. M. Birnbaum, L. Vevintow, R. B. Kingsley, and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952).

(43) H. J. Rhodes, S. M. Fang, and M. I. Blake, *J. Pharm. Sci.*, **54**, 1440 (1965).

(44) S. L. Lin, M. I. Blake, and F. P. Siegel, *ibid.*, **54**, 354 (1965).

Anal.[15] Calcd for $C_7H_6D_5NO_5$: C, 43.29; H, 6.00; N, 7.21; D, 45.45 atom %. Found: C, 43.11; H, 6.08; N, 7.22; D, 44.65 atom % (98.2%).

D-Glutamic-2,3,3,4,4-d_5 Acid (XI).—The acetyl derivative X (150 mg, 0.78 mmole) was refluxed for 1 hr in 1 ml of 5 *N* HCl solution. The mixture was evaporated to dryness *in vacuo* to yield 135 mg (92%) of the hydrochloride of D-glutamic-2,3,3,4,4-d_5 acid XI. The salt was converted to the free amino acid XI as previously described for compound VIIb. Two recrystallizations from water–ethanol yielded 40 mg (34%): mp 188.5–190°; $[\alpha]^{25}D$ −30.0° (*c* 2, 5 *N* HCl); paper chromatography showed one spot at R_f 0.53; λ_{max}^{KBr} 3.25, 4.76, 6.06, 6.59, 7.04, and 7.56 μ.

Anal.[15] Calcd for $C_5H_4D_5NO_4$: C, 39.47; H, 6.33; N, 9.21. Found: C, 39.40; H, 6.48; N, 9.18.

DL-β-Methyl Aspartate-2,3,3-d_3 (XIIb).[45]—A solution of 53.2 g (0.30 mole) of the salt II in a mixture of 3.0 ml (0.041 mole) of thionyl chloride and 240 ml of dry methanol-d^{32} was stirred at 25 ± 1° for 16.5 hr. Triethylamine (60 ml, 0.43 mole) was then added, and the mixture was cooled, and then stirred with 900 ml of acetone for 15 min. The product XIIa, which separated, was filtered and washed with 400 ml of acetone, three 400-ml portions of chloroform, and 400 ml of dry ether. After being dried *in vacuo* there was obtained 33.17 g (72%) of the deuterated ester XIIa: R_f 0.63, mp 194–195°. The ester XIIa was crystallized twice from methanol (1.0 g/50 ml) to give the d_3 ester XIIb: mp 195–196.5°; λ_{max}^{KBr} 3.30–3.40, 5.76, 6.35, 7.17, and 7.76 μ.

Anal.[15] Calcd for $C_5H_6D_3NO_4$: C, 39.96; H, 6.27; N, 9.33; D, 33.33 atom %. Found: C, 39.77; H, 6.31; N, 9.39; D, 32.35 atom % (97.2%).

The protio ester was prepared as above in 71% yield, R_f 0.63, mp 191–192° (lit.[46] mp 175–181°).

Anal. Calcd for $C_5H_9NO_4$: C, 40.82; H, 6.17; N, 9.52. Found: C, 40.91; H, 6.30; N, 9.56.

Ammonia-d_3 in Methanol-d.—The apparatus used for this preparation was similar to the one described for the generation of DCl.[47] The reaction flask was charged with 1-methoxy-2-ethoxy ethane (500 ml) and magnesium nitride (180 g, 1.78 moles) while 600 ml of dry methanol-d^{32} was placed in the collection flask and chilled in an ice bath. After thoroughly flushing the apparatus with nitrogen, 99.7% heavy water (174 ml, 9.55 moles) was added over 1.5 hr, maintaining a brisk reflux which was continued for an additional 45 min by heating. The yield of ammonia-d_3 was 53 g (83%).

DL-Asparagine-2,3,3-d_3 (XIII).—A solution of the ester XIIa (31.0 g, 0.20 mole) in the ammonia-d_3–methanol-d solution prepared above was stirred for 12 days at room temperature. The mixture was evaporated to dryness *in vacuo* (550 ml of solvent was recovered) and thoroughly dried at room temperature (2 mm). The residue was dissolved in 68 ml of boiling water and the product XIII precipitated by the addition, with stirring, of 630 ml of absolute ethanol. After chilling, filtering, and washing with absolute ethanol (200 ml), and ether (200 ml), 29.06 g (94%) of the amino acid XIII was obtained as the monohydrate. Crystallization twice from water to ensure exchange at the labile positions followed by drying at 25° (1.5 mm) over phosphorus pentoxide for 3 days afforded a pure sample of the monohydrate of XIII: R_f 0.32; λ_{max}^{KBr} 2.91, 3.20, 3.39, 5.95, 6.10, 6.54, 7.18, and 8.35 μ.

Anal.[15] Calcd for $C_4H_7D_3N_2O_4$: C, 31.37; H, 6.79; N, 18.29; D, 30.0 atom %. Found: C, 31.19; H, 6.99; N, 18.00; D, 29.35 atom % (97.8%).

DL-N-Acetylasparagine-2,3,3-d_3 (XIV).—A slurry of 24.5 g (0.16 mole) of the asparagine XIII monohydrate in 40 ml of water[48] was mixed with 21.2 g of sodium carbonate. To the stirred paste there was added 16.0 ml (0.17 mole) of acetic anhydride in one portion. After 15 min the pH was lowered to 1.6 by the addition of 31.7 ml of concentrated hydrochloric acid. The precipitated mixture was chilled for 3 hr, filtered, and washed successively with two 100-ml portions of ethanol and three 200-ml portions of ether. The dried product XIV (29.76 g) contained some sodium chloride. Crystallization of this material from ethanol–water (1:1) gave 20.03 g (72%) of the pure N-acetylasparagine XIV: mp 176.0–177.5°; λ_{max}^{KBr} 2.90, 3.05, 5.80, 5.97, 6.23, and 7.88 μ.

Anal.[15] Calcd for $C_6H_7D_3N_2O_4$: C, 40.68; H, 5.88; N, 15.81; D, 30.0 atom %. Found: C, 40.62; H, 6.06; N, 15.79; D, 29.0 atom % (96.7%).

L-Asparagine-2,3,3-d_3 (XV).—A solution of 17.7 g (0.10 mole) of the acetyl compound XIV in 800 ml of water was adjusted to pH 7.2 with ammonium hydroxide. After dilution to a total volume of 1 l., a few drops of chloroform was added (to prevent bacterial growth) and the solution was incubated with 500 mg of hog kidney acylase[12] for 4 days at 37°.[49] The pH of the solution was then lowered to 5.4 with hydrochloric acid, *ca.* 2 g of charcoal was added, and the mixture was warmed to 70° and filtered through Celite. To the filtrate, concentrated to 70 ml at 27° (25–30 mm), 280 ml of ethanol was added slowly. The mixture was then chilled, filtered, and washed with two 25-ml portions of ethanol and two 25-ml portions of ether to give 6.92 g of the monohydrate of the product XV. The filtrate and washings were set aside for the preparation of the D-N-acetyl asparagine XVI. To remove traces of protein from the product XV it was dissolved in 20 ml of hot water, treated with charcoal, and filtered through sintered glass; two 2.5-ml washes with hot water were used. The filtrate, in which some of the product had crystallized, was warmed to effect complete solution and 225 ml of ethanol was added. From this solution the pure product XV was isolated by the procedure described above. There was obtained 6.65 g (87%) of pure deuterated L-asparagine as its monohydrate: R_f 0.32; $[\alpha]^{25}D$ +29.6 (*c* 2, 1 *N* HCl); λ_{max}^{KBr} 2.95, 3.21, 3.40, 5.98, 6.11, 6.66, 7.05, 7.20, and 8.36 μ. This product was dried as described for the racemate XIII.

Anal.[15] Calcd for $C_4H_7D_3N_2O_4$: C, 31.37; H, 6.79; N, 18.29; D, 30.0 atom %. Found: C, 31.56; H, 6.96; N, 18.34; D, 29.25 atom % (97.5%).

D-N-Acetylasparagine-2,3,3-d_3 (XVI).—The filtrate from the previous preparation was concentrated to 17 ml *in vacuo* at a temperature not exceeding 35°. The residue was acidified to pH 1.0 with concentrated HCl, chilled for several hours, filtered, and washed with two 25-ml portions of ethanol and two 25-ml portions of ether to give 6.84 g of crude product XVI. Crystallization from 175 ml of methanol afforded, in two crops, 6.14 g (69%) of the pure acetyl derivative XVI: mp 159.0–159.5°; $[\alpha]^{25}D$ +3.4° (*c* 2, 1 *N* HCl); λ_{max}^{KBr} 2.91, 3.10, 5.82, 5.98, 6.06, and 7.73 μ.

Anal.[15] Calcd for $C_6H_7D_3N_2O_4$: C, 40.67; H, 5.88; N, 15.81; D, 30.0 atom %. Found: C, 40.72; H, 5.92; N, 15.81; D, 29.15 atom % (97.2%).

DL-γ-Methyl Glutamate-2,3,3,4,4-d_5 (XVIIb).—The salt VI (13.55 g, 0.07 mole) was added to 84 ml of methanol-d^{32} and stirred at 25 ± 1° for 4 days. The clear solution was cooled and 12 ml (0.086 mole) of triethylamine was added over 5 min. The mixture was stirred for 15 min, dry acetone (70 ml) was added, and stirring was continued for an additional 15 min. The white solid was collected using 70 ml of acetone to aid transfer, and washed with chloroform (three 70-ml portions) and finally with anhydrous ether (70 ml). The material was dried at 60° to give 10.54 g (89%) of chromatographically homogeneous ester XVIIb: R_f 0.66, mp 167.5–169.5°. This material was used without further purification for the preparation of the acetyl derivative XVIII. Pure ester XVIId was prepared by crystallization from 90% methanol (77% recovery): mp 173.5–175.5°; λ_{max}^{KBr} 3.37, 4.75, 5.78, 7.15, and 9.38 μ.

Anal.[15] Calcd for $C_6H_6D_5NO_4$: C, 43.36; H, 7.01; N, 8.43; D, 45.45 atom %. Found: C, 43.15; H, 7.09; N, 8.32; D, 44.75 atom % (98.5%).

The racemic protio methyl ester was prepared as follows. Thionyl chloride (14.4 ml, 0.20 mole) was carefully added to 240 ml of anhydrous methanol at 5°. The solution was warmed to 25°, DL-glutamic acid (29.43 g, 0.20 mole) was added in one portion, and the resulting solution was stirred for 22 min. After cooling to 5°, triethylamine (70 ml, 0.50 mole) was added over 5 min. Work-up as above afforded 29.29 g (91%) of ester: mp 169.5–172° (lit.[50] mp 183°); paper chromatography indicated a single spot, R_f 0.67.

(45) In paper chromatographic studies of this reaction at different temperatures, and acid concentrations, the presence of dimethyl aspartate (R_f 0.82) was always observed for the complete disappearance of aspartic acid (R_f 0.48). Under these reaction conditions aspartic acid was completely consumed before work-up.

(46) Z. L. Braun and R. M. Leyton, *J. Med. Chem.*, **8**, 500 (1965).

(47) H. C. Brown and C. Groot, *J. Am. Chem. Soc.*, **64**, 2223 (1942).

(48) When H_2O was employed as solvent, no detectable exchange of deuterium bound to carbon was observed.

(49) The pH of the solution was periodically examined and maintained at 7.1–7.3 by the addition of dilute NH_4OH.

(50) F. E. King, B. S. Jackson, and D. A. A. Kidd, *J. Chem. Soc.*, 243 (1951).

Anal. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.89; H, 6.96; N, 8.30.

Crystallization from 80% methanol gave lustrous crystals, mp 175–176°. (Found: C, 45.08; H, 7.00; N, 8.41.) Chromatographically homogeneous L-γ-methyl glutamate (R_f 0.67) was prepared as described above (28-min reaction time) in 86% yield: mp 173.5–174.5°, $[\alpha]^{25}D$ +13.5° (*c* 2, H_2O), $[\alpha]^{25}D$ +32.7° (*c* 2, 0.5 *N* HCl) [lit.[51] mp 175–176°, $[\alpha]^{25}D$ +31.87° (0.5 *N* HCl)].

Anal. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.75; H, 7.01; N, 8.63. Crystallization from 80% methanol (73% recovery) gave crystals: mp 173.5–174.5°, $[\alpha]^{25}D$ +13.7° (*c* 2, H_2O), $[\alpha]^{25}D$ +33.0° (*c* 2, 0.5 *N* HCl). (Found: C, 44.82; H, 6.95; N, 8.56.) L-γ-Ethyl glutamate was prepared (0.1-mole scale) as above employing 175 ml of absolute ethanol and a 1-hr reaction time. An analytical sample was prepared by crystallization from 90% ethanol (64% recovery): R_f 0.72, mp 174–176°, $[\alpha]^{25}D$ +12.8° (*c* 1, H_2O) (lit.[52] mp 194°).

Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00. Found. C, 47.64; H, 7.51; N, 8.05.

DL-**N-Acetyl-γ-methyl Glutamate-2,3,3,4,4-d_5 (XVIII).**—Ester XVIIa (10.15 g, 0.06 mole) was dissolved in an ice-cold solution of 11.2 g (0.13 mole) of sodium bicarbonate in 123 ml of 99.7% D_2O. With continued cooling, 8.22 ml (0.087 mole) of acetic anhydride was added over 25 min. The solution was stirred for 1 hr in the cold and for 1 hr at room temperature. After addition of 26.7 ml of 5 *N* H_2SO_4, the solution was evaporated to dryness (30–40° at *ca.* 15 mm), and heated for 15 min with 145 ml of absolute ethanol. The sodium sulfate (9.33 g, 99%) was collected and the filtrate was concentrated to a clear oil which solidified on drying *in vacuo*. The crude acetyl derivative XVIII was washed with hexane (three 50-ml portions) and ether (three 30-ml portions). Drying yielded 11.99 g (96%) of a white solid, mp 101–106°, which displayed singlets of equal area in the nmr spectrum (D_2O). This material was used without further purification for the preparation of the glutamine derivative XIX. A sample of XVIII[53] was prepared for analysis by crystallization from dioxane–hexane at 15°: mp 111.5–112.5°; λ_{max}^{KBr} 2.97, 3.47, 3.97, 5.80, 6.18, 7.72, and 9.44 μ.

Anal.[15] Calcd for $C_8H_8D_5NO_5$: C, 46.14; H, 6.56; N, 6.73; D, 38.46 atom %. Found: C, 44.95; H, 6.59; N, 6.69; D, 36.0 atom % (93.7%).

DL-**N-Acetylglutamine-2,3,3,4,4-d_5 (XIXb).**—Ester XVIII (11.45 g, 0.055 mole) was added to a solution of 40 g of ammonia-d_3 in 350 ml of methanol-*d* and stirred at 40 ± 2° for 13 days.[54] Evaporation to dryness *in vacuo* (40–45°) gave the ammonium-d_4 salt of the acetyl derivative of XIXa (XIXc, 11.94 g, 100%). This material was used without further purification in the enzymatic deacylation reaction. A pure sample of XIXb was prepared as follows. The crude ammonium salt XIXc (0.98 g) was added to 34 ml of hot absolute ethanol, with continued stirring, 0.91 ml of 5 *N* H_2SO_4 was added, and the mixture was heated for 5 min. The ammonium sulfate (0.282 g, 98%) was collected and the filtrate was evaporated to dryness *in vacuo*. Ethanol was added (20 ml) and the mixture was reevaporated to dryness.[55] The derivative XIXb was washed with ether and crystallized from absolute ethanol affording 0.43 g, mp 180.5–181.5° of pure acetyl derivative XIXb; λ_{max}^{KBr} 2.89–3.44, 5.22, 5.98, 6.34, and 9.49 μ.

Anal.[15] Calcd for $C_7H_7D_5N_2O_4 \cdot 0.75H_2O$: C, 40.67; H, 6.85; N, 13.55; D, 37.04 atom %. Found: C, 40.81; H, 6.61; N, 13.96; D, 36.45 atom % (98.4%).

L-**Glutamine-2,3,3,4,4-d_5 (XX).**—The acetyl derivative XIX (10.85 g, 0.05 mole) was dissolved in 400 ml of H_2O, filtered through sintered glass, and adjusted to pH 7.15 with 2 *N* NH_4OH. Renal acylase (50 mg) was added; the resulting solution was incubated at 37° for 27 hr.[50] The pH was adjusted to 5.65 with 1 *N* HCl, charcoaled, and filtered through Celite. The clear solution was concentrated to half-volume *in vacuo* (40–45°), filtered through sintered glass, and evaporated to *ca.* 40 ml. Absolute ethanol (300 ml) was added with stirring and the mixture was chilled overnight. The white solid was filtered, washed with 10 ml of absolute ethanol and 30 ml of anhydrous ether, and dried, affording 2.88 g (76%) of crude amino acid XX: mp 179–181°; $[\alpha]^{25}D$ +5.7° (*c* 2, H_2O); $[\alpha]^{25}D$ +27.5° (*c* 2, 1 *N* HCl). Paper chromatography indicated one main spot at R_f 0.45 and a faint trace at R_f 0.68. Crystallization of 2.70 g of this material was effected by dissolving it in 50 ml of 40% ethanol, filtering, adding 20 ml of absolute ethanol, and chilling which afforded 2.14 g of chromatographically homogeneous material, R_f 0.44. A second crop (0.15 g) was obtained by evaporating the previous filtrate to dryness and crystallizing the residue as above: 65% total yield, 55% over-all from amino acid VI; mp 179.5–181.5°, with protio L-glutamine mmp 180–182°; $[\alpha]^{25}D$ +6.2° (*c* 2, H_2O); $[\alpha]^{25}D$ +29.5° (*c* 2, 1 *N* HCl); λ_{max}^{KBr} 2.81, 3.13, 3.35, 5.92, 6.13, 6.30, 8.28, 9.39, and 9.92 μ.

Anal.[15] Calcd for $C_5H_5D_5N_2O_3$: C, 39.72; H, 7.04; N, 18.53; D, 50.0 atom %. Found: C, 39.24; H, 7.03; N, 18.16; D, 48.05 atom % (96.1%).

L-Glutamic-2,3,3,4,4-d_5 acid was prepared by hydrolyzing amino acid XV (0.151 g, 0.001 mole) for 1 hr in 1 ml of 5 *N* HCl. The product was isolated as described in this section; $[\alpha]^{25}D$ +29.8° (*c* 2, 5 *N* HCl).

D-**N-Acetylglutamine-2,3,3,4,4-d_5 (XXI).**—The alcoholic filtrate from the previous experiment was evaporated to a syrup *in vacuo* (35–40°). The residue was acidified with 5.0 ml of 5 *N* HCl precipitating a white solid. The mixture was chilled overnight, filtered, and washed with 30 ml of absolute alcohol and 100 ml of anhydrous ether affording the acetyl derivative XXI in 63% yield (3.04 g, mp 190–192°). The product was crystallized from 150 ml of absolute ethanol affording pure XXI (76% recovery): mp 193–195°; $[\alpha]^{25}D$ +12.6° (*c* 2, H_2O); λ_{max}^{KBr} 2.88–3.48, 5.22, 5.99, and 9.50 μ.

Anal.[15] Calcd for $C_7H_7D_5N_2O_4$: C, 43.51; H, 6.55; N, 14.50; D, 41.67 atom %. Found: C, 43.70; H, 6.79; N, 13.99; D, 39.00 atom % (93.5%).

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(51) M. Goodman, E. E. Schmitt, and D. A. Yphantis, *J. Am. Chem. Soc.*, **84**, 1283 (1962).

(52) H. K. Miller and H. Waelsch, *Arch. Biochem. Biophys.*, **35**, 176 (1952).

(53) This material was difficult to crystallize and despite thorough drying, was somewhat sticky perhaps accounting for the anomalous analyses.

(54) The reaction was followed to completion by observing the disappearance of the methyl ester singlet in the nmr spectrum.

(55) Ethanol was added at this point to ensure complete exchange of H for D at the labile positions.