SYNTHESIS OF 1-^{13}C and 3-^{13}C ISOTOPIC ISOMERS OF ASPARTIC AND GLUTAMIC ACIDS

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SUMMARY

Convenient routes to 1-C and 3-C labeled glutamic and aspartic acids and to their precursors are described.

Key Words: [1-^{13}C] glutamic acid, [3-^{13}C] glutamic acid, [1-^{13}C] aspartic acid, [3-^{13}C] aspartic acid.

The synthesis of isotopic isomers of amino acids is often required for a variety of studies. We have sought efficient routes to ^{13}C isomers of aspartic and glutamic acids singly labeled in the 1 or 3 positions. These isomers are required to define more precisely the angle ϕ in peptides from vicinal heteronuclear NMR couplings, $^{3}\underline{J}(^{13}C^{3}\text{-}C^{2}\text{-}N'\text{-}^{1}H')$, $^{3}\underline{J}(^{13}C_{i+i}\text{-}N_{i}^{2}\text{-}^{1}H_{i}^{2})$ and $^{3}\underline{J}(^{13}C'\text{-}C^{1}\text{-}N'\text{-}H')$ (1). The efficient synthesis of 1-^{13}C amino acids using the Strecker reaction of the appropriate aldehyde with sodium or potassium cyanide is well known (2) for other amino acids. Efficient incorporation of label depends on adequate purity of the adlehyde. There do not appear to be previous reports of the Strecker synthesis of aspartic acid from malonaldehyde. Previous reports of the synthesis of the crucial intermediate for the Strecker synthesis of glutamic acid, succinic semialdehyde, are very long (3) or are impractical (4) for laboratory use (5). We describe an efficient synthesis of ethyl succinaldehydate in high yield (78%) from the oxidation of ethyl-4-hydroxybutyrate by pyridinium dichromate in dichloromethane solution (6). Routes to [1-^{13}C] aspartic and [1-^{13}C] glutamic acids are shown in schemes I and II respectively. These schemes are efficient in use of materials, and convenient for synthesis of isotopic isomers.

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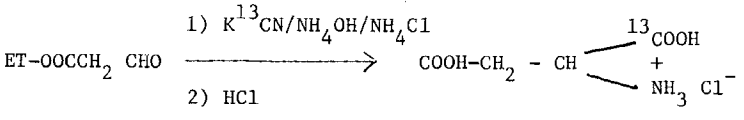
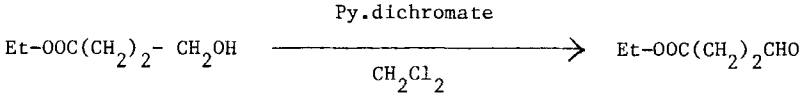
Synthesis of [3-^{13}C] amino acids can be generally accomplished by reaction of the appropriately labeled halide with aceto- or phthalamidomalonic esters (2). A more general route might use carbon-carbon bond formation between a 3-C labeled general precursor and an unlabeled reagent, but no satisfactory general precursor is available. Previous routes (7) to [3-$^{2}H_2$] isomers of glutamic and aspartic acids were modified to yield the [3-^{13}C] isomers, from the appropriate labeled halide. The syntheses of [3-^{13}C] glutamic acid is shown in scheme III. Reported synthesis of [2,3,3,4,4-$^{2}H_5$] glutamic acid (7) using propyl 3-bromo [3-^{13}C] propionate and sodium acetamidomalonic ester provided [3-^{13}C] glutamic acid.

Scheme I

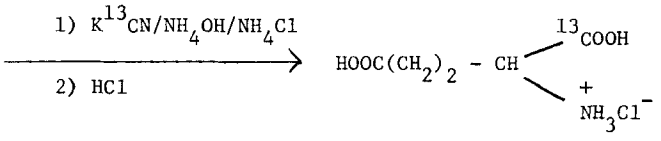


1 2

Scheme II



3 4



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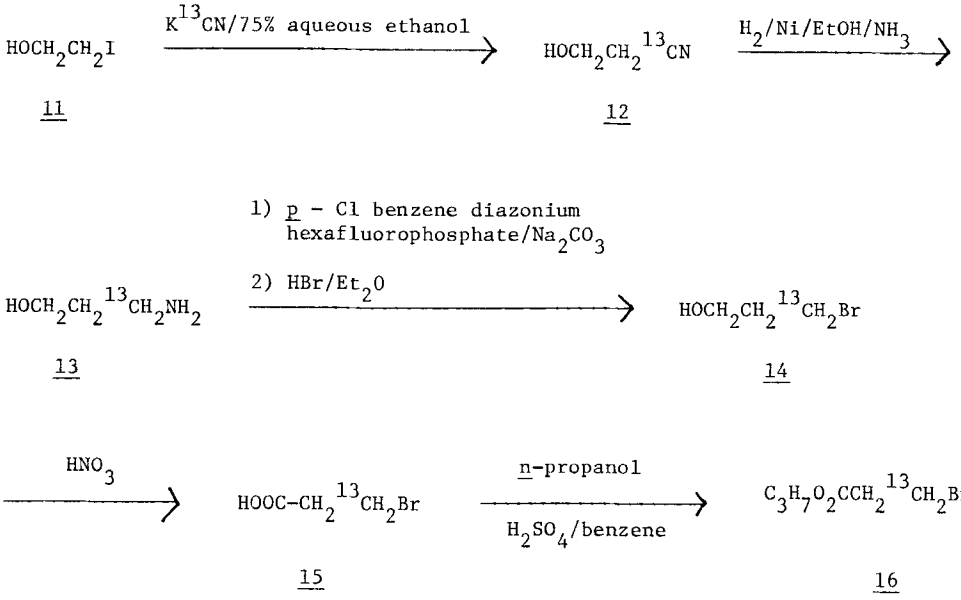
EXPERIMENTAL

NMR spectra were recorded on a Varian T60A or a Varian/Nicolet HR/TT-220 spectrometer. Using the relative peak heights in ^{13}C-split multiplets, ^{13}C labeling at the 90% level was incorporated at the correct sites, within the precision of measurement (± 10%) (8). All amino acid products are DL. Amino acid analysis used the modified Stein-Moore procedure (9); quantitation of amino acids by this procedure is estimated to be better than 5%.

Scheme III



[1-^{13}C] Aspartic acid (2)

Ethyl malonaldehydate (1), was prepared from ethyl 3,3-diethoxypropionate by boiling with 2 M HCl for 15 mins, and extracting the free aldehyde into diethyl ether (10). Ammonium chloride (2.12 g) and 1.3 g K ^{13}CN (Stohler isotopes, 90% ^{13}C) were dissolved in 20 ml of water, and 12 ml of 30% aqueous ammonia added. Ethyl malonaldehydate (4.5 g) was added to the ammoniacal solution and stirred

for 12 h at room temperature. The reaction mixture was rotary evaporated to dryness, 80 ml 12 M aqueous HCl added, and the mixture refluxed 24 h. The amino acid product was isolated by AG50WX8 cation exchange chromatography (2), yield 1.19 g (45%); amino acid analysis--only aspartic acid detected, quantitatively correct; NMR (DMSO, 19°) δ 4.4 (q, 1 H), 2.92 (t, 2 H), no impurities detected.

[1-^{13}C] glutamic acid (5) was prepared by the Strecker synthesis using ethyl succinaldehydate.

Ethyl succinaldehydate (4)

Ethyl 4-hydroxybutyrate prepared via (11) was slightly contaminated with γ-butyrolactone. Fractional distillation removed the contaminant. Sixteen g of (3) in 50 ml CH_2Cl_2 was oxidized with 66 g of pyridinium dichromate (6) added slowly with vigorous stirring. After stirring for 12 h, 20 ml dry ether was added, the resulting suspension filtered, and the filtrate evaporated, yield 12.3 g (78%), bp. 47-51° at 1 mm; IR (oil) 1720 (s), 1760 (m); NMR ($CDCl_3$, 19°) δ 1.27 (t, 3 H), 2.62 (t, 2 H), 2.80 (t, 2 H), 4.15 (q, 2 H), 9.81 (s, 1 H).

[1-^{13}C] glutamic acid (5)

Ammonium chloride (2.12 g, 0.04 mmole) and K ^{13}CN (1.3 g, 0.02 mole) were dissolved in 20 ml of water and 12 ml of 30% aqueous ammonia. The aldehyde (4) (5.2 g, 0.04 mole) was added slowly. After 12 h, the reaction mixture was evaporated to dryness, 200 ml of 25% aqueous HCl added, and the mixture refluxed for 24 h. The solution was rotary evaporated to dryness, and the product purified using cation exchange chromatography (2), yield 2.3 g (85%), amino acid analysis only glutamic acid detected, quantitatively correct; NMR (D_2O) 2.62 (t, 2 H), 4.02 (m, $^2J(H^{13}C)$ = 4.9).

[3-^{13}C] aspartic acid was synthesized from the condensation of ethyl [2-^{13}C] 2-bromoacetate with sodiophthalamidomalonic ester, a scaled-down duplication of a previous synthesis (2).

[3-^{13}C] glutamic acid was synthesized from propyl [3-^{13}C] 3-bromo propionate and diethyl acetamidomalonate (scheme III).

3-Hydroxy [1-^{13}C] propionitrile (12)

Iodoethanol (8.6 g) was added drop by drop to 2.5 g of $K^{13}CN$ dissolved in 30 ml 75% ethanol/water, and refluxed overnight. The product was evaporated to dryness in vacuum, extracted with 2 x 100 ml ether. After filtration, the product was obtained by evaporation, yield 3.15 g (88%); IR superimposable with commercial unlabeled material.

3-Amino-[3-^{13}C] propanol (13)

Hydroxypropionitrile (12) (2.4 g in 50 ml saturated ammoniacal ethanol) was reduced for 4 h at 50 psi in the presence of 500 mg of Raney nickel (12). After filtration, the product was isolated by evaporation, yield 2 g (80%); IR superimposable with commercial unlabeled product; NMR (D_2O) δ 1.67 (m, 2 H), 2.68 (^{13}C split m, ^1J = 135 (14), 2 H), 3.64 (m, 2 H), shifts identical to unlabeled product.

3-Bromo [3-^{13}C] propanol (14)

A solution of p-chlorobenzenediazonium hexafluorophosphate (9.6 g in 50 ml DMF) was added slowly to a stirred mixture of aminopropanol (13) and 50.6 g powdered Na_2CO_3 in 100 ml DMF at -5°. The temperature was slowly raised to 0°, and after 30 m, 200 ml of ether was added. The solution was filtered and the filtrate was washed thoroughly with water, dried over $MgSO_4$ and the triazine isolated by evaporation (13). Ether saturated with HBr was added to it until the mixture was acidic. The resulting tar was extracted with ether. The ether washings were neutralized with sodium bicarbonate, and filtered. The crude product was isolated by evaporation, and purified on a silica gel column, washing with hexane, and eluting with ether, yield 1.8 g (49%); IR superimposable with commercial unlabeled material; NMR ($CDCl_3$) δ 2.05 (m, 2 H), 3.50 (^{13}C split m, ^1J = 152 (14)), 3.73 (m, 2 H), trace contamination with precursors.

3-Bromo [3-^{13}C] propionic acid (15)

Following a procedure for 6-bromohexanoic acid (15), 1.8 g of (14) was added drop by drop to 48 ml fuming nitric acid at 5°C, stirred 4 h at room temperature, then heated at 90° for 45 m. The mixture was diluted with 200 ml of H_2O,

and then evaporated to dryness, yield 1.15 g (60%); IR superimposable with commercial unlabeled product; NMR ($CDCl_3$) δ 3.00 (m, 2 H), 3.57 (^{13}C split m, ^1J = 154 (14), 2 H), 10.76 (v br. s), trace contamination with precursor alcohol.

Propyl-3-bromo-[3-^{13}C] propionate (16)

The free acid (15) (150 mg) was dissolved in 4 ml 1-propanol; two drops of concentrated sulfuric acid and 12.5 ml benzene were added, and the mixture refluxed for 12 h. Ethyl acetate (100 ml) was added, the solution washed with aqueous sodium bicarbonate, and with water, and then excess solvent was distilled off, yield 156 mg (78%); IR superimposable with unlabeled material; NMR ($CDCl_3$) δ 0.96 (t), 1.67 (m), 2.92 (q), 3.59 (^{13}C split t, ^1J = 154 (14)), 4.09 (t), shifts same as unlabeled material.

[3-^{13}C] Glutamic acid (18)

The Blomquist procedure (7) was followed on a scale of 1/100, without isolation of the carbethoxy intermediate, yield 53 mg (47%); amino acid analysis only glutamic acid detected, quantitatively correct; NMR (D_2O) δ 2.21 (^{13}C split m, ^1J = 131 (14)), 2.62 (m), 4.02 (m).

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