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K-5263-M1

Supplementary Material for the Microfilm Edition

The labeled leucine was synthesized by condensation of ethyl acetamidocyanoacetate and labeled isobutyl bromide and subsequent hydrolysis. The halide was made from diethyl malonate and labeled methyl iodide as discussed below. All products were characterized by proton magnetic resonance at 60 and 200 MHz and carbon magnetic resonance at 50 MHz.

I. *Diethyl 2,2-Di-[$^{13}C_1$]-methylpropandioate*

Sodium metal (4.83 g, 0.21 mol) was dissolved in absolute ethanol (150 mL) in a 500 mL round-bottomed flask cooled in an ice-water bath. Diethyl malonate (15.0 g, 0.094 mol) dissolved in absolute ethanol (20 mL) was added dropwise over 30 minutes and the reaction was stirred for an additional hour. The mixture was cooled to -10°C in an ice-salt water bath and [^{13}C]-methyl iodide (33.0 g, 0.23 mol, MSD isotopes) dissolved in absolute ethanol (5 mL) was added dropwise over 45 minutes. The reaction was warmed to room temperature, refluxed for 16 hours and evaporated under reduced pressure. The resulting oil was taken up in ether, treated with decolorizing carbon and filtered. Removal of the ether under reduced pressure gave I (15.15 g, 85.1%).

II. *2-[$^{13}C_1$]-Methyl-3-[$^{13}C_1$]-propanoic acid*

I (15.0 g, .080 mol) dissolved in 12 M sulfuric acid (50 mL) in a 250 mL round-bottomed flask was refluxed for 8 1/2 hours. It was then cooled to room temperature, diluted with water (ca. 200 mL), extracted with ether, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to yield II (6.4 g, 88.9%).

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III. *2-[$^{13}C_1$]-Methyl-3-[$^{13}C_1$]-1-propanol*

II (6.4 g, 0.071 mol) dissolved in anhydrous ethyl ether (15 mL) was added dropwise over 45 minutes to an ice-water cooled 250 mL round-bottomed flask containing lithium aluminum hydride (3.14 g, 0.082 mol) stirred in anhydrous ethyl ether (100 mL). After addition was complete, the reaction was warmed to room temperature and refluxed for two hours. The reaction mixture was cooled in an ice-water bath. Excess hydride was destroyed with a small amount of water, and the reaction mixture was transferred to a separatory funnel with the aid of 9 M sulfuric acid. The aqueous layer was washed several times with ethyl ether. All ether layers were combined and dried over magnesium sulfate and the ether removed under reduced pressure to give III (3.4 g, 63.0%).

IV. *1-Bromo-2[$^{13}C_1$]-methyl-propane*

Phosphorous tribromide (5.87 g, 0.022 mol) in a 50 mL two-necked round-bottomed flask equipped with magnetic stirrer and thermometer was cooled to -8°C in an ice-salt water bath. III (3.2 g, 0.042 mol) was added dropwise over an 80 minute period so that the temperature never exceeded 0°C. The reaction was then warmed to room temperature, stirred overnight, and distilled under reduced pressure.

All material distilling below 50°C at a pressure of ca. 100 mm Hg was collected, dissolved in ethyl ether and washed with concentrated sulfuric acid. The ether layer was dried over anhydrous potassium carbonate and the ether removed under reduced pressure to yield IV (1.0 g, 17.1%). [Unreacted labelled alcohol that did not distill below 50°C at 100 mm Hg was recovered and subjected again to treatment with PBr_3, but the product of this reaction was not utilized in the succeeding steps].

V. *Ethyl 2-Acetamido-2-cyano-4-[$^{13}C_1$]-methyl-5-[$^{13}C_1$]-pentanoate*

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Sodium metal (0.21 g, 0.0091 mol) dissolved in absolute ethanol (5 mL) was added dropwise over 20 minutes to a solution of ethyl acetamidocyanoacetate (1.30 g, 0.0076 mol) in absolute ethanol (15 mL) in a 50 mL round-bottomed flask. V (1.0g, 0.0072 mol) was also dissolved in absolute ethanol (3 mL) and added dropwise over 15 minutes. The reaction mixture was refluxed for 16 hours and the ethanol was then removed under vacuum. The solid residue was taken up in methylene chloride and extracted with water. The organic layer was dried over anhydrous magnesium sulfate and the solvent evaporated under reduced pressure to yield V (0.48 g, 29.3%).

VI.*2-Amino-4-[$^{13}C_1$]-methyl-5-[$^{13}C_1$]-pentanoic Acid*

V (0.48 g, 0.0021 mol) was refluxed for 3 hours with concentrated hydrochloric acid (10 mL). All solvent was evaporated under reduced pressure and the residue was taken up in water and passed over a short column containing AG3-X4A resin (5.0 g, Bio-Rad) to produce VI (0.185 g, 66.1%).