SYNTHESIS OF L-[4,4-$^2H_2$] and D,L-[3,3,4,4-$^2H_4$] METHIONINE

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SUMMARY

The syntheses of D,L-[3,3,4,4-$^2H_4$]methionine from [2,2,2-$^2H_3$] acetic acid in seven steps in an overall yield of 26% and L-[4,4-$^2H_2$]methionine from N-t-BOC-L-aspartic acid α-benzyl ester in five steps in an overall yield of 40% are described.

Key words: L-[4,4-$^2H_2$]methionine, D,L-[3,3,4,4-$^2H_4$]methionine, Biosynthesis, Deuterium labeling, Amino Acids.

INTRODUCTION

Our interest in the origin of a number of natural products, 1-aminocyclopropane-1-carboxylic acid(1); 3-(3-amino-3-carboxypropyl)-N^6-Δ^2-isopentenyladenine (Discadenine)(2), L-azetidine-2-carboxylic acid(3) and 3-(3-amino-3-carboxypropyl)uridine(4), whose biosyntheses utilize the C_1-C_4 carbons from L-methionine has led us to synthesize [3,3,-$^2H_2$](5), [2,3,3-$^2H_3$](5), [4,4-$^2H_2$] and [3,3,4,4-$^2H_4$] methionine. Currently these methionines are being utilized in our laboratories to study the biosynthesis of 1-aminocyclopropane-1-carboxylic acid, L-azetidine-2-carboxylic acid(4) and to study the ^1H and ^{13}C-nmr of various methionine derivatives.

RESULTS AND DISCUSSION

The reaction sequence for the preparation of L-[4,4-$^2H_2$]methionine 4 is shown in Scheme 1. The reduction of N-t-BOC-L-aspartic acid α-benzyl ester 1 via the "mixed carbonic anhydride" (6) with sodium borodeuteride afforded the N-t-BOC-L-[4,4-$^2H_2$]homoserine α-benzyl ester 2 in a 55.0% yield with no detectable formation of the reduction product of the α-benzyl

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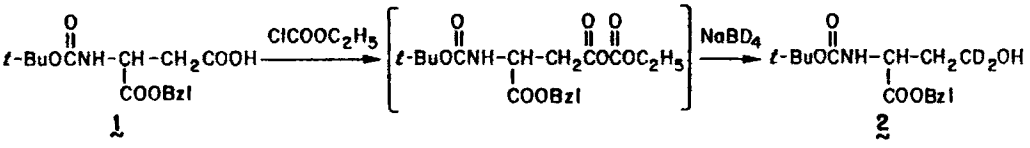
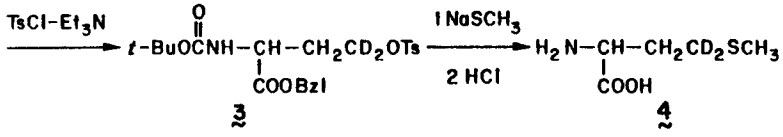
ester. Treatment of 2 with p-toluenesulfonyl chloride in CH_2Cl_2/Et_3N afforded the desired tosylate 3 in 84.0% yield.

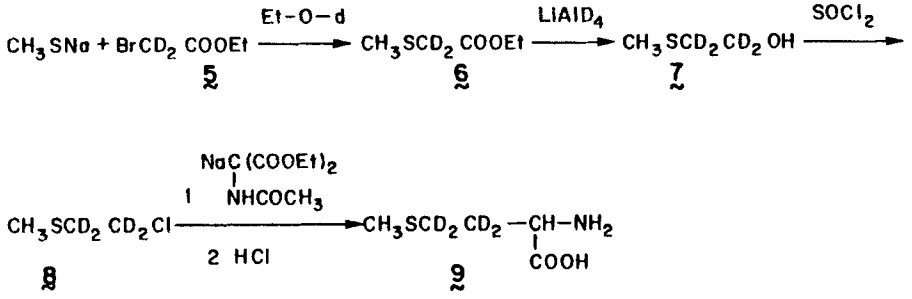
Displacement of the tosylate function with sodium methyl mercaptide in ethanol followed by simultaneous cleavage of the N-t-BOC and the benzyl ester groups with 6 N HCl gave the desired methionine 4.





SCHEME I

The route devised for the synthesis of D,L-[3,3,4,4-$^2H_4$]methionine is shown in Scheme II. Following the procedure described for 2-bromo-[2-^{13}C] acetic acid(7), [2,2,2,-$^2H_3$]acetic acid was reacted with red phosphorus and bromine to give 2-bromo-[2,2-$^2H_2$]acetic acid which was converted into ethyl 2-bromo-[2,2-$^2H_2$]acetate(8) in high yield. The key starting material is the dideuterated methylthioester 6, which should be obtainable from the reaction of ethyl 2-bromo-[2,2-$^2H_2$]acetate 5 and sodium methyl mercaptide. However, attempted condensation of 5 with CH_3SNa in absolute ethanol afforded exclusively the undeuterated $CH_3SCH_2COOC_2H_5$ apparently by exchange of deuterons with the solvent protons. The methylthioester 6 was successfully prepared by reacting sodium methyl mercaptide with 5 in ethanol-O-d as solvent. Our attempts to condense non-deuterated ethyl bromoacetate with sodium methyl mercaptide in ethanol-O-d as solvent, however, did not yield a totally deuterated methylthioester 6. The reduction of ester 6 with lithium aluminum deuteride gave the desired 2-methylthio-[1,1,2,2-$^2H_4$]ethanol 7 which was converted to 1-chloro-2-methylthio[1,1,2,2-$^2H_4$]ethane 8 with thionyl chloride in 80% yield. The chloro compound 8 was condensed with sodium diethyl



SCHEME II

acetamidomalonate (prepared from sodium ethoxide and diethyl acetamidomalonate). Hydrolysis and decarboxylation of the condensation product with 6N hydrochloric acid permitted the isolation of D,L-[3,3,4,4-$^2H_4$]methionine.

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^1H nmr spectra at 60 MHz were recorded on a Varian Associates Model EM-360 spectrometer and chemical shift values are expressed in δ units relative to TMS in organic solvents and TMSP in D_2O. For the presentation of nmr spectra, the following abbreviations are used: s=singlet, bs=broad singlet; m=multiplet, t=triplet, and q=quartet. N-t-BOC-L-aspartic acid α-benzyl ester was purchased from United States Biochemical Corporation.

N-t-BOC-L-[4,4-$^2H_2$]Homoserine α-benzyl ester (2).

A solution of ethyl chloroformate (0.76 g, 0.007 mol) in dry THF (20.0 mL) was added to a stirred solution of N-t-BOC-L-aspartic acid α-benzyl ester (2.26 g, 0.007 mol) and triethylamine (0.71 g, 0.007 mol) in THF (20 mL) at -5°C and the reaction mixture was stirred for 0.5 hr. The triethylamine hydrochloride was removed by filtration and washed with 10 mL of THF. The combined filtrate was added over a period of 30 min. to a stirred solution of $NaBD_4$ (0.586 g, 0.014 mol) in D_2O (7.0 mL) at 10-15°C. After the addition was complete, the mixture was stirred at room temperature for 4 hr, cooled to 0-5°C, acidified with 2N HCl and extracted with ether. The ethereal layer was washed with 10% $NaHCO_3$, water and dried (Na_2SO_4). The solvent was evaporated and the residue

was dissolved in ethyl acetate (15 ml) and evaporated onto 2.0 g of silica gel. The preadsorbed product was then placed on a column containing 25 g of silica gel (Merck Kieselgel-60 ; 70-230 mesh ASTM) and eluted with hexane-acetone 7:3. The fractions containing the alcohol 2 were combined and the solvent was removed on a rotary evaporator to give 1.2 g (55.3%) 2 as an oil.

Nmr ($CDCl_3$): δ 1.45 (s, 9H, t-$C_4\underline{H}_9$), 1.66-1.98 (m, 2 H, -$C\underline{H}_2$-CD_2-), 2.82 (bs, 1 H, O$\underline{H}$), 4.2-4.50 (m, 1 H, α-C$\underline{H}$), 5.02 (s, 2 H,-$C\underline{H}_2C_6H_5$), 5.42 (m, 1 H, N$\underline{H}$), 7.32 (s, 5 H, Ar-$\underline{H}$).

L-[4,4-$^2H_2$]methionine (4).

A solution of p-toluenesulfonyl chloride (0.76 g, 0.004 mol) in CH_2Cl_2 (5 mL) was added to a mixture of N-t-BOC-L-[4,4-$^2H_2$]homoserine α-benzyl ester (1.2g, 0.0038 mol) and triethylamine (0.43 g, 0.6 mL) in 7 mL of dry CH_2Cl_2 at 0°. The reaction mixture was then stirred at room temperature for 24 hr. The triethylamine hydrochloride was filtered and the CH_2Cl_2 was removed on a rotary evaporator to yield 1.52 g (84%) of the tosylate 3 as a clear viscous oil. The tosylate was used without further purification.

A mixture of tosylate 3 (1.0 g, 0.0021 mol), sodium methyl mercaptide (0.364 g, 0.0052 mol) and ethanol (20.0 mL) was refluxed for 16 hr. After removal of the solvent, the residue was dissolved in 6N HCl (20 mL) and heated to reflux for 10 hr. The brown reaction mixture was evaporated to dryness under reduced pressure and the solid residue was dissolved in water (5.0 mL) and directly applied to a 100 mL column of Dowex 50 x 4-200 (NH_4^+). The column washed with water (200 mL) and eluted with 1N NH_4OH (200 mL). The ninhydrin positive fractions were concentrated in vacuo and lyophilized. The methionine was recrystallized from ethanol/water (10:1) to yield 220 mg (69.5%): m.p. 275-277°C.

Nmr (D_2O): δ 2.12 (b.s., 5 H, $C\underline{H}_3$-S-CD_2-$C\underline{H}_2$), 3.85 (t, 1 H, Cα-$\underline{H}$).

Ethyl methylthio[2,2-$^2H_2$]acetate (6).

To a solution of sodium methyl mercaptide (14.0 g, 0.2 mol) in ethanol-O-d

(50 mL) was added slowly a solution of ethyl 2-bromo-[2,2-$^2H_2$]acetate (16.9 g, 0.1 mol) in 25 mL of ethanol-O-d. The reaction mixture was heated under reflux for 3 hr, the ethanol-O-d was removed by distillation and the residue was extracted with ether and dried (Na_2SO_4). Evaporation of the ether and distillation gave 9.5 g (69.8%) of ethyl methylthio[2,2-$^2H_2$]acetate b.p. 70-72°/25 mm.

Nmr ($CDCl_3$): δ 1.29 (t, 3 H, $C\underline{H}_3CH_2$-), 2.2 (s, 3 H, $C\underline{H}_3S$), 4.22 (q, 2 H, -$C\underline{H}_2CH_3$).

2-Methylthio-[1,1,2,2-$^2H_4$]ethanol (7).

To a well stirred slurry of $LiAlD_4$ (1.1 g, 0.025 mol) in dry THF was added dropwise a solution of ethyl methylthio[2,2-$^2H_2$]acetate (5.0 g, 0.0367 mol) in dry THF at room temperature. The mixture was heated under reflux for 6 hr. The excess hydride was carefully destroyed by dropwise addition of ethyl acetate and the resultant mixture was treated with 3N HCl (20 mL). The reaction mixture was extracted with ether and treated with solid sodium bicarbonate and dried (Na_2SO_4). Removal of the solvent and vacuum distillation of the residue gave 3.34 g (94%) of 2-methylthio-[1,1,2,2-$^2H_4$]ethanol: b.p. 75°/22 mm.

Nmr ($CDCl_3$): δ 2.08 (s, 3 H, -$SC\underline{H}_3$), 2.52 (s, 1 H, $\underline{OH}$).

1-Chloro-2-methylthio-[1,1,2,2-$^2H_4$]ethane (8).

To a solution of 2-methylthio[1,1,2,2-$^2H_4$]ethanol (1.8 g, 0.0187 mol) in dry chloroform (2.5 mL) at 0°C was added dropwise over a period of 30 min, 3.0 g, (0.0252 mol) of thionyl chloride in chloroform (2.5 mL). The colorless mixture was then heated at reflux for a period of 4 hr. After removal of chloroform and excess thionyl chloride, the residue was distilled to give pure 1-chloro-2-methylthio-[1,1,2,2-$^2H_4$]ethane 1.72 g (80%), b.p. 55-56°/30 mm.

Nmr ($CDCl_3$): δ 2.18 (s, 3 H, $C\underline{H}_3S$-).

D,L-[3,3,4,4,-$^2H_4$]methionine (9).

Sodium (0.12 g, 0.0052 mol) was dissolved in absolute ethanol (20 mL) and diethyl acetamidomalonate (1.085 g, 0.005 mol) was added and the mixture was

heated under reflux for 0.5 hr. After cooling to room temperature, 1-chloro-2-methylthio[1,1,2,2-$^{2}H_4$]ethane (0.86 g, 0.0075 mol) was added and the reaction mixture was boiled for 6 hr. Ethanol was removed under diminished pressure(water aspirator) and the residue was treated with 6N HCl (10 mL) and the mixture was refluxed for 6 hr. The brown solution which resulted was evaporated to dryness under reduced pressure. The residue was taken up in water (15 mL) and applied to a 100 mL column of Dowex 50x4-200 (NH_4^+) and eluted with water (200 mL) followed by 1N NH_4OH (200 ml). The ninhydrin positive fractions were concentrated in vacuo and lyophilized. The crude methionine was dissolved in boiling water (5 mL) and hot absolute ethanol (150 mL) was added and cooled in an ice bath. The precipitated solid was filtered off under suction. Yield. 400 mg (53.6%) m.p. 274-276°.

Nmr (D_2O): δ 2.08 (s, 3 H, CH_3S-) 3.81 (s, 1 H, -CαH).

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