# NOTE

## SYNTHESIS OF (2S,4S)-[5-$^{13}$C] LEUCINE, (2R,4S)-[5-$^{13}$C] LEUCINE, AND (2RS)-[1,2-$^{13}C_2$] LEUCINE

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SUMMARY

(2RS,3S)-[4-$^{13}$C]Valine was diazotized and converted to (2RS,3S)-[4-$^{13}$C]-2-bromo-isovaleric acid, which on reduction with Zn/HCl gave (3S)-[4-$^{13}$C] isovaleric acid. This was converted to (3S)-[4-$^{13}$C] isovaleraldehyde, which was converted by a Strecker synthesis to (2RS,4S)-[5-$^{13}$C] leucine. Resolution via the N-acetate then gave (2S,4S) and (2R,4S)-[5-$^{13}$C] leucines. [1-$^{13}$C]Isovaleric acid was similarly converted to [1-$^{13}$C] isovaleraldehyde, which, in a Strecker synthesis using K$^{13}$CN, gave (2RS)-[1,2-$^{13}C_2$] leucine.

Key Words: $^{13}$C-leucines, chiral valine, Strecker synthesis

### INTRODUCTION

For the continuation of our studies on the stereochemistry of branched-chain amino acid metabolism (1-3), we required the $^{13}$C-labeled leucines named in the title. Since the syntheses of these compounds were carried out by routes having a number of steps in common, we describe them together in this paper.

### SYNTHESES OF (2R,4S) AND (2S,4S)-[5-$^{13}$C] LEUCINES

These compounds, required for studies on the stereochemistry of metabolic reactions involving the prochiral isopropyl group of leucine, were synthesized by the route outlined in Scheme 1. Since only low $^{13}$C enrichment was required in the products, the starting material, (2RS,3S)-[4-$^{13}$C] valine 1a, 90 atom % $^{13}$C, (4,5) was diluted with unlabeled DL-valine to give an enrichment at the

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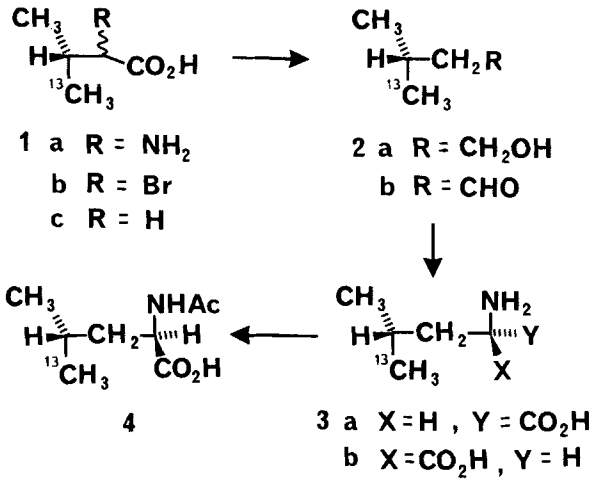
0362-4803/83/050663-06$01.00


Received September 15, 1982
Revised December 22, 1982




**SCHEME 1**

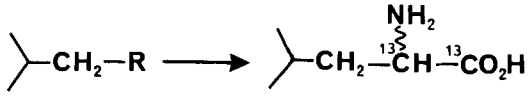
labeled position of ca 2 atom % $^{13}C$. This material was then diazotized in the presence of KBr (6) to give the α-bromo acid 1b, which was reduced in good yield by zinc in aqueous $H_2SO_4$ to (3S)-[4-$^{13}C$] isovaleric acid, 1c‡. The product was methylated ($CH_2N_2$) and reduced ($LiAlH_4$) to (3S)-[4-$^{13}C$] isoamyl alcohol, 2a. Oxidation of this using pyridinium dichromate in $CH_2Cl_2$ (9) afforded (3S)-[4-$^{13}C$] isovaleraldehyde, 2b, which was converted by the Strecker method (4) to (2RS,4S)-[5-$^{13}C$] leucine, 3a + 3b. After acetylation and resolution of the acetate with hog kidney acylase I in the usual way (10), (2S,4S)-[5-$^{13}C$] leucine, 3a, and (2R,4S)-[5-$^{13}C$] leucine N-acetate, 4, were obtained. The latter was hydrolyzed (3N HCl, reflux 2h) to (2R,4S)-[5-$^{13}C$] leucine, 3b. The $^{13}C$ NMR spectrum of 3a in $D_2O$ + $D_2SO_4$ (trace) showed enhanced intensity in the upfield methyl signal ( δ 21.70), whereas the $^{13}C$ NMR spectrum of 3b showed enhanced intensity in the downfield methyl signal ( δ 22.82). The spectra were otherwise identical with the spectrum of unlabeled leucine. The signal assignments thus derived for the diastereotopic methyls of leucine are in agreement with those reported earlier

‡The (3R) isomer of 1c has been previously synthesized in a twelve-step procedure from 2-phenylcyclopropyl bromide (7). Also the (3S) isomer, 1c, has been obtained by Ag(II) oxidation of (2S,4S)-[5-$^{13}C$] leucine derived biosynthetically in very low yield from (2S, 3S)-[4-$^{13}C$] valine (8). The present method is a far simpler, high yield method of obtaining chirally labeled isovaleric acid from "chiral valine".

by Fuganti et al. (11), who synthesized chirally labeled leucines in an alternative manner.

SYNTHESIS OF (2RS)-[1,2-$^{13}C_2$]LEUCINE

This compound, 6, required for studies on biochemical reactions involving the possible cleavage of the C-1,C-2 bond of leucine, was synthesized by the route outlined in Scheme 2. Carboxylation of isobutylmagnesium bromide with $^{13}CO_2$ gave [1-$^{13}$C]isovaleric acid, 5a, which was methylated ($Ch_2N_2$) to 5b, reduced ($LiAlH_4$) to 5c, and oxidized (PDC) to [1-$^{13}$C]isovaleraldehyde, 5d, 92 atom % $^{13}$C. The crude product was subjected to a Strecker synthesis using Na$^{13}$CN (90 atom % $^{13}$C) to give (2RS)-[1,2-$^{13}C_2$] leucine, 6. The product showed the expected $^{13}C$-$^{13}C$ couplings in the $^{13}$C nmr spectrum (see experimental).




5 a R = $^{13}CO_2H$
b R = $^{13}CO_2Me$
c R = $^{13}CH_2OH$
d R = $^{13}CHO$

6

**SCHEME 2**

EXPERIMENTAL

General. $^1$H NMR spectra were taken on a Varian EM-390 instrument. $^{13}$C NMR spectra were taken on a Bruker SXP 22/100 instrument at 22.63 MHz, or on a Bruker HX-270 instrument at 67.88 MHz. Samples were dissolved in $D_2O$ containing a few drops of either $D_2SO_4$ or dil NaOD/$D_2O$, plus dioxane as internal reference ($\delta$ $^{13}$C = 67.398). Gas-liquid chromatography was performed using a Varian Model 920 gas chromatograph equipped with a 6 ft x 1/4 in column of 15% SE-30 on 80-100 Supelcoport. Ba$^{13}CO_3$ and Na$^{13}$CN were obtained from Merck, Sharpe, and Dohme of Canada. Melting points were taken on a hot stage apparatus and are uncorrected. (3S)-[4-$^{13}$C]Isoamyl Alcohol, 2a. (2RS,3S)-[4-$^{13}$C]Valine, 1a (15 mg, 90 atom % $^{13}$C), was mixed with unlabeled valine (1.35 g), and dissolved in ice-cold $H_2SO_4$ (30 ml). KBr (5 g) was added, followed by $NaNO_2$ (1.32 g), added in small portions

over 45 min with stirring. Aftr 15 min additional stirring at 0°, and 1 h at 25°, the mixture was extracted with ether, and the extract was dried ($Na_2SO_4$) and evaporated to yield crude (1b) as a viscous oil, 2.21 g. This was suspended in ice-cold water (35 ml) and treated with conc $H_2SO_4$ (0.69 ml) and granular zinc (30 mesh, 2.7 g) stirring vigorously at 0° for 5 min, then 25 min at 25°. Solid NaCl was added with stirring to saturation, and the solution was extracted with ether, the extract dried ($Na_2SO_4$) and evaporated under reduced pressure at room temperature to yield 1c as an oil (1.2 g). This was treated with ethereal $CH_2N_2$, and (after removal of excess $CH_2N_2$ under reduced pressure) the solution was filtered through a short column containing silica gel (100-200 mesh), eluting with a little additional ether. The filtrate was treated with excess $LiAlH_4$ (0.25 g) at 0° for 15 min, then at reflux for 45 min. After dropwise treatment of the solution with sat. aqueous $Na_2SO_4$ and filtration, the filtrate was evaporated under reduced pressure to yield 2a, 1.04 g; glc retention and $^1H$ NMR identical with authentic isoamyl alcohol.

(2RS,4S)-[5-$^{13}$C]Leucine, 3a + 3b. (3S)-[4-$^{13}$C]Isoamyl alcohol, 2a (0.60 g), in dry $CH_2Cl_2$ (25 ml; distilled from $P_2O_5$) was treated with pyridinium dichromate (4.0 g), stirring at room temperature for 20 h. The brown solution was decanted, and filtered through a column of 10 g Florisil (100-200 mesh), eluting with 40 ml additional $CH_2Cl_2$. After evaporation of the filtrate at 25° under reduced pressure, the crude product, containing ca 0.3 g (3S)-[4-$^{13}$C] isovaleraldehyde, 2b, plus a little pyridine (as estimated by glc) was dissolved in a mixture of $H_2O$ (5 ml), $NH_4Cl$ (0.5 g), NaCN (0.5 g) and conc $NH_4OH$ (1.5 ml) and stirred at 25° for 24 h. Then conc HCl (10 ml) was added, and the mixture was refluxed for 24 h. The resultant leucine was isolated by cation exchange in the usual way (4) giving (2RS,4S)-[5-$^{13}$C] leucine, 3a + 3b, 213 mg.

The product was converted by published methods (10) to the N-acetate which was resolved using hog kidney acylase I (4,10), followed by separation of the products by cation exchange to yield (2S,4S)-[5-$^{13}$C] leucine, 3a, 105 mg, plates from EtOH-$H_2O$, mp 230-250°C (subl) (lit (12) mp 337°C), and (2R,4S)-5-$^{13}$C] leucine N-acetate, 4, 99 mg. The latter was hydrolyzed by refluxing in 3N HCl (10 ml, 2 h). After purification by cation exchange, (2R,4S)-[ 5-$^{13}$C] leucine,

3b, 55 mg, was isolated as plates from EtOH-$H_2O$, mp 230-250°C (subl).

(2RS)-[1,2-$^{13}C_2$]Leucine, 6. [1-$^{13}$C]Isovaleric acid, 5a (4.49 g, 92 atome % $^{13}$C) was prepared by reaction of isobutylmagnesium bromide with $^{13}CO_2$ (from $Ba^{13}CO_3$, 10 g, 92 atom % $^{13}$C) in the usual way (13). The product was methylated ($CH_2N_2$-ether). After removing the excess $CH_2N_2$ under reduced pressure, the ether solution (ca 250 ml) was filtered through a short column of silica gel (100-200 mesh, 25 g), and washed through with 50 ml additional ether. The filtrate was treated with $LiAlH_4$ (1 g) at 25°, 30 min, then at reflux for 2 h. After treatment with sat $Na_2SO_4$, the mixture was filtered and the ether evaporated under reduced pressure to yield an oil, which was distilled in a short-path apparatus to yield [1-$^{13}$C] isoamyl alcohol, 5c (2.37 g, 92 atom % $^{13}$C), glc identical with authentic isoamyl alcohol; $^1$H NMR ($CDCl_3$ δ 0.95 (3H,d,J = 6 Hz), 1.2-2.2 (4H, m, including -OH), 3.60 (ca 1.8 H, d,t, $J_{^{13}C-H}$ = 140 Hz, $J_{HH}$ = 6 Hz, with a small multiplet, ca 0.2 H for -$^{12}CH_2$ OH).

The product, 5c, was oxidized with PDC as described above to yield crude [1-$^{13}$C] isovaleraldehyde, 5d (ca 1.0 g estimated by glc, containing a little pyridine and ether). The crude product was treated with $NH_4Cl$ (1 g), conc $NH_4OH$ (3 ml) and $Na^{13}CN$ (1 g, 90 atom % $^{13}$C) in $H_2O$ (10 ml) stirring at 25° for 23 h. Then conc HCl (20 ml) was added, and the mixture refluxed for 24 h. After isolation by cation exchange on Dowex 50W-X8, 50-100 mesh, $H^+$ form in the usual way (4), followed by crystallization of the product from EtOH-$H_2O$, (2RS)-[1,2-$^{13}$C] leucine, 6, 794 mg, was obtained: plates from EtOH-$H_2O$, mp 240-250°C (subl), lit (14) mp 278-283°C. $^{13}$C NMR ($D_2O$ + few drops dil NaOD/$D_2O$):

| Carbon | δ | Rel. Intensity | $J_{CC}$ (Hz) |
|---|---|---|---|
| 1 (uncoupled) | 185.174 | 8.1 | |
| 1 (coupled) | 185.174 | 66.5 | 51.79 |
| 2 (uncoupled) | 55.440 | 27.6 | |
| 2 (coupled) | 55.413 | 183.3 | 51.79 |
| 3 (coupled)ǂ | 45.142 | 4.8 | 33.29 |
| 4 | 25.227 | 5.2 | |
| 5 | 22.320 | 5.2 | |
| 5' | 22.230 | 5.1 | |

ǂ Uncoupled signal invisible

ACKNOWLEDGMENT

This work was supported by grants GM 24420 and GM 25919 from the National Institute of General Medical Sciences. We thank Mr. Frank Shea, Clark University, Worcester, Mass., for some $^{13}C$ NMR spectra taken on the Bruker SXP 22/100 instrument, supported in part by National Science Foundation equipment grant CHE-77-09059, Mr. Peter Demou, Yale University for $^{13}C$ NMR spectra taken on the Bruker HX-270 instrument, supported in part by National Science Foundation grant CHE-79-16210, and Mr. Cliff McDonald for $^{1}H$ NMR spectra.

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