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Synthesis of L-[4,5,5,5-D_4]Isoleucine: Determination of Approximate Rotamer Population About The C_β-C_γ Bond

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Summary: An asymmetric synthesis of regio- and stereoselectively deuterium-labelled L-isoleucine was examined. An unsaturated γ-lactam readily available from L-pyroglutamic acid was stereoselectively methylated by the Gilman reagent and subsequent oxidation of the hydroxymethyl moiety afforded a 3-methylpyroglutamate derivative. Introduction of a deuterium atom into the γ-position was achieved via a radical-based deuteriation of the corresponding phenyl selenide by tributyltin deuteride. Then, the deuteriated 3-methylpyroglutamate derivative was converted to L-[4,5,5,5-D_4]isoleucine via a base-promoted ring opening and a reductive deuteriation of the terminal carboxyl moiety.

Key words: isoleucine, deuterium-labelling, asymmetric synthesis, pyroglutamic acid

Introduction

In order to determine the detailed three dimensional structure of proteins in solution by NMR spectroscopy, it is first necessary to make sure of stereospecific assignments for various pairs of diastereotopic protons and methyl groups. Such assignments allow us to obtain useful structural information through spin-spin coupling, NOEs, and spin relaxation times.[1] In particular, vicinal coupling constants have been recognized as the most useful parameters for conformational analysis of the peptide main chain and side chains.[2] To achieve the above purpose, a biosynthetic incorporation of stereoselectively deuteriated amino acids into the target protein seems to be the most effective method.[3] Therefore, we have recently devised novel synthetic routes to such amino acids.[4,5]

In this paper, we focus on L-isoleucine, a branched-chain amino acid, and examine the deuterium-labelling of the terminal methyl group and one of diastereotopic protons at the γ-position so that the vicinal *J*-values, which depend on the dihedral angle about C_β-C_γ bond (χ2 angle in peptide), can be easily obtained.

Results and Discussion

Some reports on the selective isotope-labelling at the γ-position of isoleucine can be found in the literature. For example, Komatsubara *et al.* biosynthetically converted the labelled threonine or α-aminobutanoic acid into (*4S*)-L-[4-D]isoleucine.[6] The malonic ester synthesis was also applied to the preparation of (*4S*)-L-[4-T]isoleucine from 2-butyl sulfonate with a tritium of fixed configuration at

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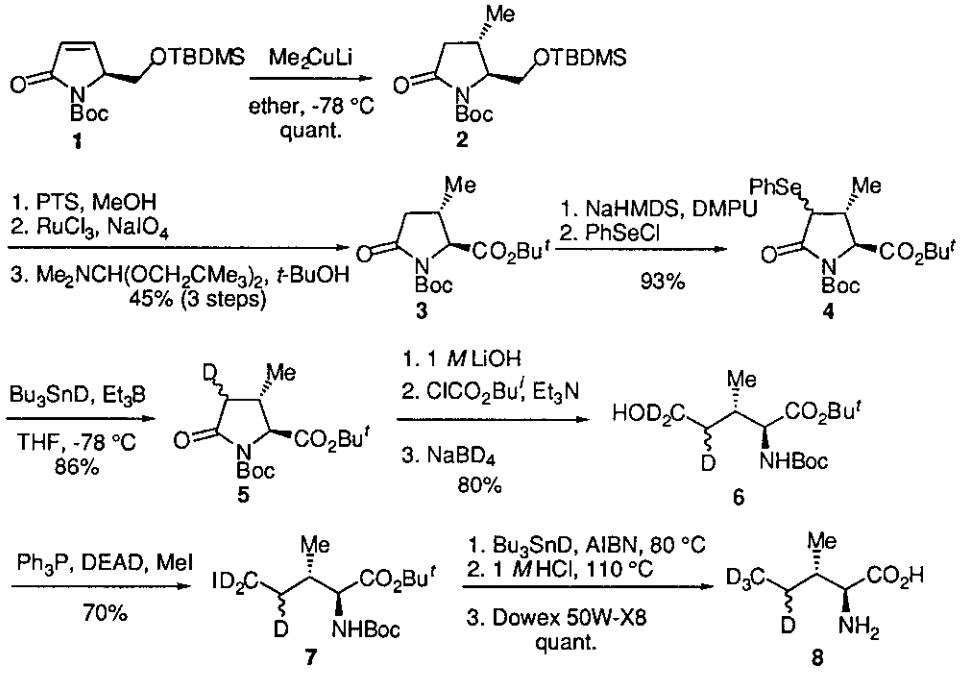
C-3.[7] Furthermore, [3,4-D_2]- or [3,4-T_2]isoleucine was prepared by a reduction of the corresponding dehydroisoleucine with [D_2]- or [T_2]diimide, respectively.[8] However, these methods were unsuitable for our purpose.

The synthetic course of L-[4,5,5,5-D_4]isoleucine is illustrated in Scheme 1. We adopted an unsaturated γ-lactam **1** derived from L-pyroglutamic acid as a chiral template for this synthesis. First of all, stereospecific introduction of the methyl group was examined. When the olefin **1** was treated with Me_2CuLi in ether at -78 °C, the conjugate addition occurred exclusively from the α-face of the γ-lactam ring to afford the corresponding methylated γ-lactam **2** in quantitative yield. This stereochemical outcome can be attributed to the effective shielding of the β-face by the bulky side chain and is very important in establishing the (*3S*)-configuration of the L-isoleucine. After removal of the silyl protecting group of the lactam **2** by treatment with *p*-toluenesulfonic acid (PTS) in MeOH, RuO_4-oxidation of the resulting primary alcohol and subsequent esterification with dimethylformamide di-*t*-butyl acetal furnished the *t*-butyl 3-methylpyroglutamate **3** in 45% yield (3 steps).



Scheme 1

In order to incorporate a deuterium atom into the γ-position, the pyroglutamate **3** was converted to the corresponding phenyl selenide **4** in 93% yield. Then the selenide **4** was carried forward in a radical-based deuteriation with Bu_3SnD-Et_3B system at -78 °C to afford the deuteriated methylpyroglutamate **5** in 86% yield. The stereoselectivity of the deuterium incorporation was determined by ^1H NMR integration (*4S* : *4R* = 64 : 36), suggesting the preferential delivery of the deuterium atom from the opposite face to the methyl substituent. Although this deuteriation procedure was also applied to the γ-lactam **2**, the selectivity of the deuterium addition was unsatisfactory.

Conversion of the 3-methyl[4-D]pyroglutamate **5** into L-[4,5,5,5-D_4]isoleucine was carried out in a similar manner to the synthesis of L-[3,4,5,5,5-D_5]leucine.[5]

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Thus, the pyroglutamate **5** was treated with 1 *M* LiOH and the resulting carboxylic acid was reduced to the deuteriated alcohol **6** with $NaBD_4$ via a mixed anhydride in 80% yield. Treatment of the alcohol **6** with PPh_3-DEAD-MeI system gave the iodide **7** in 70% yield. Finally, reductive deuteriation of the iodide **7** with Bu_3SnD-AIBN followed by the standard deprotection procedure furnished L-[4,5,5,5-D_4]isoleucine **8** in quantitative yield. The optical purity (100%ee) was checked by HPLC analysis using a chiral stationary phase column which also revealed the sample was not contaminated with alloisoleucine.

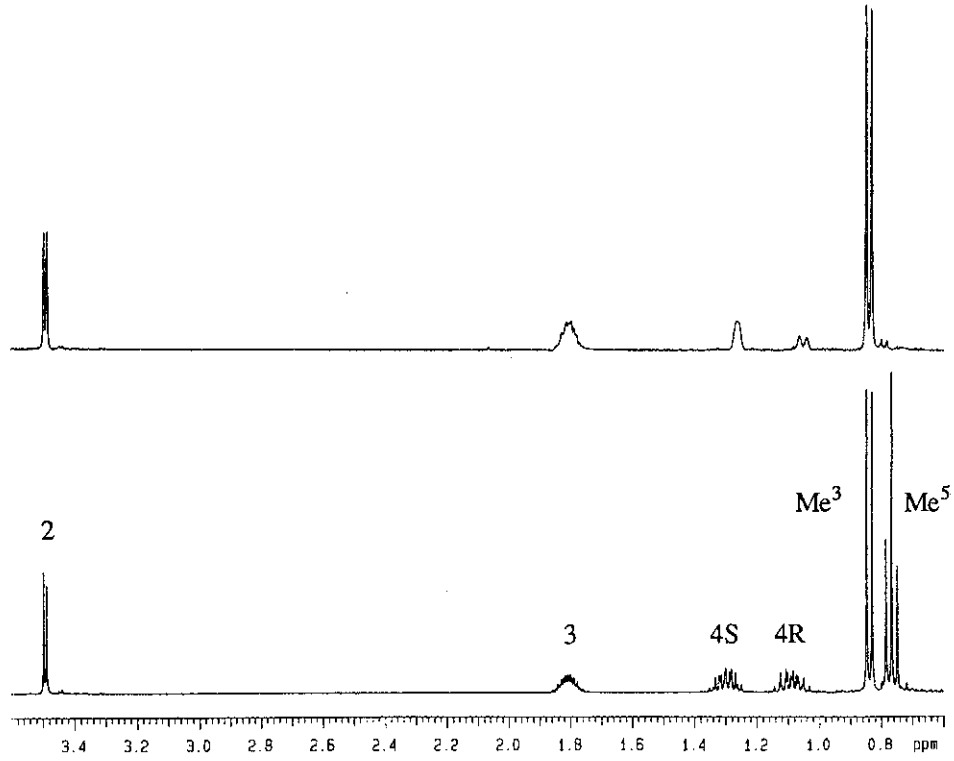

Figure 1. 400 MHz ^1H NMR spectra of deuteriated isoleucine **8** (upper) and unlabelled isoleucine (lower) in D_2O.

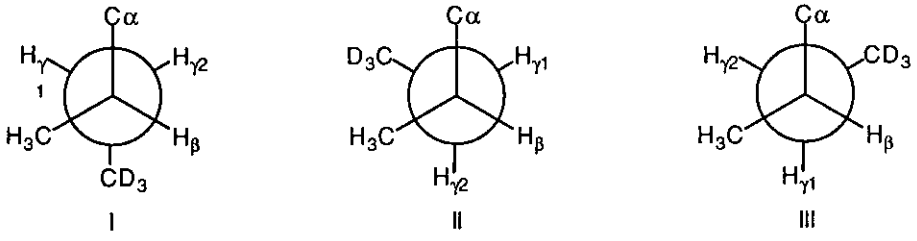

Figure 2. Newman projections for three staggered rotamers about the C_β-C_γ bond of isoleucine.

Figure 1 (upper) shows the ^1H NMR spectrum of the isoleucine **8**. The signal of the terminal methyl group has disappeared and the ratio of signal intensities for *4S*- and *4R*-proton is 64 : 36, indicating selective formation of the (*2S,3S,4R*)-isomer.

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The spectrum is simplified by deuterium substitution, as compared with that of unlabelled isoleucine (Figure 1, lower). In particular, the *4S*- and *4R*-proton show 4.0 and 9.2 Hz coupling only to the H-3, respectively. From these J values, the approximate rotamer populations (P) about the C_β-C_γ bond (Figure 2) can be calculated to be $P_I = 0.61$, $P_{II} = 0.13$, and $P_{III} = 0.26$ using Pachler's equations: $P_I = (J_{\beta\gamma1} - J_g)/(J_t - J_g)$, $P_{II} = (J_{\beta\gamma2} - J_g)/(J_t - J_g)$, $P_{II} = 1 - (P_I + P_{II})$, in which $J_t = 13.6$ Hz and $J_g = 2.6$ Hz.[9] The structure of the most predominant conformer I was in agreement with those obtained by PM3[10] calculation using a conductor-like screening model (COSMO).[11]

Experimental

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. All chemical shifts are reported as δ values (ppm) relative to residual chloroform (δ_H 7.26), sodium 3-(trimethylsilyl)[2,2,3,3-D_4]propionate (δ_H 0.00), the central peak of $CDCl_3$ (δ_c 77.0), or dioxane (δ_c 66.5). Mass spectral determinations (EI) were carried out at 30 eV. Optical purity was determined on a HPLC system equipped with chiral MCIGEL CRS10W column using 2 *mM* $CuSO_4$ solution as an eluent. All other reagents were of commercial grade and used as supplied.

(*4S,5S*)-1-*t*-Butoxycarbonyl-5-*t*-butyldimethylsiloxymethyl-4-methyl-2-pyrrolidone (**2**)

To a stirred suspension of CuI (2.63 g, 13.8 mmol) in ether (15 ml) at -15 °C under an argon atmosphere was added 1.14 *M* MeLi in ether (24.2 ml, 27.6 mmol) and the mixture was stirred for 10 min. To the resulting solution was added a solution of the unsaturated γ-lactam **1**[12] (1.50 g, 4.60 mmol) in ether (5 ml) at -78 °C. After 4 h, saturated aqueous NH_4Cl was added and the reaction mixture was warmed to room temperature and extracted with ether. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the oily 4-methyl-γ-lactam **2** (1.60 g, quant.) which was used without purification. ^{1}H NMR ($CDCl_3$) δ 0.01 and 0.02 (2s, 6 H), 0.85 (s, 9 H), 1.11 (d, J = 3 and 7 Hz, 3 H), 1.51 (s, 9 H), 1.99 (dd, J = 2 and 17 Hz, 1 H), 2.29-2.37 (m, 1 H), 2.88 (dd, J = 9 and 18 Hz, 1 H), 3.67-3.71 (m, 2 H), 3.87 (dd, J = 4 and 11 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ - 5.64 and - 5.63, 18.1, 21.4, 25.7, 28.0, 28.2, 40.5, 63.7, 66.3, 82.7, 150.3, 174.5. HRMS *m/z* 344.2263 [(M + 1)$^+$, calcd for $C_{17}H_{34}NO_4Si$ 344.2257].

t-Butyl (*2S,3S*)-1-*t*-Butoxycarbonyl-3-methylpyroglutamate (**3**)

To a solution of the γ-lactam **2** (1.49 g, 4.33 mmol) in MeOH (20 ml) was added *p*-toluenesulfonic acid (75.0 mg, 0.433 mmol) and the resulting solution was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of the solvent afforded the oily 5-hydroxymethyl-3-methyl-2-pyrrolidone (889 mg, 90%) which was used in the next step without purification. ^{1}H NMR ($CDCl_3$) δ 1.16 (d, J = 7 Hz, 3 H), 1.54 (s, 9 H), 2.08 (dd, J = 18 and 4 Hz, 1 H), 2.21-2.29 (m, 1 H), 2.83 (dd, J = 18 and 9 Hz, 1 H), 3.71-3.79 (m, 2 H), 3.82-3.87 (m, 1 H). HRMS (EI, 30 eV) *m/z* 230.1408 [(M + 1)$^+$, calcd for $C_{11}H_{20}O_4N$ 230.1392].

To a suspension of sodium metaperiodate (8.29 g, 38.8 mmol), $RuCl_3 \cdot nH_2O$ (276 mg) in H_2O (23 ml) was added a solution of the obtained alcohol (889 mg, 3.88 mmol) in acetone (20 ml). The resulting two-phase mixture was vigorously stirred at room temperature for 1 h. The layers were separated and to the organic phase was added 2-propanol (12 ml) and stirred for 1 h. After removal of the precipitated RuO_2

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using a Celite pad, the filtrate was concentrated, extracted with chloroform, and dried over $MgSO_4$. Evaporation of the solvent gave oily 3-methylpyroglutamic acid (953 mg) in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 1.28 (d, J = 7 Hz, 3 H), 1.50 (s, 9 H), 2.19 (dd, J = 17 and 4 Hz, 1 H), 2.43-2.52 (m, 1 H), 2.81 (dd, J = 17 and 8 Hz, 1 H), 4.22 (d, J = 4 Hz, 1 H). HRMS (EI, 30 eV) m/z 244.1137 [(M + 1)$^+$, calcd for $C_{11}H_{18}O_5N$ 244.1185].

To a refluxing solution of the crude pyroglutamic acid (953 mg, 3.88 mmol) in benzene (20 ml) was added a mixture of *N,N*-dimethylformamide dineopentyl acetal (1.62 g, 6.98 mmol) and *t*-butanol (860 mg, 11.6 mmol), and the reaction mixture was stirred for 0.5 h. Then the cooled reaction mixture was diluted with ethyl acetate, washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel. Elution with a mixture of hexane and ethyl acetate (90 : 10) afforded the oily *t*-butyl 3-methylpyroglutamate **3** (590 mg, 51%). ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J = 7 Hz, 3 H), 1.48 (s, 9 H), 1.50 (s, 9 H), 2.12 (dd, J = 17 and 4 Hz, 1 H), 2.28-2.37 (m, 1 H), 2.76 (dd, J = 17 and 9 Hz, 1 H), 4.05 (d, J = 3 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 20.7, 27.9, 27.9, 29.7, 39.4, 66.6, 82.2, 83.3, 149.5, 169.9, 173.0. HRMS (EI, 30 eV) m/z 300.1829 [(M + 1)$^+$, calcd for $C_{15}H_{26}O_5N$ 300.1811].

t-Butyl (*2S,3S,4RS*)-1-*t*-Butoxycarbonyl-3-methyl-4-phenylselenopyroglutamate (**4**)

A solution of 1 *M* NaHMDS in THF (4.20 ml, 4.20 mmol) was treated with DMPU (0.5 ml) at 0 °C under an argon atmosphere for 10 min, cooled to -78 °C, and treated with a solution of the 3-methylpyroglutamate **3** (590 mg, 1.98 mmol) in THF (5 ml). After 0.5 h, a solution of PhSeCl (416 mg, 2.17 mmol) in THF (5 ml) was added and the mixture was stirred for 2 h. The reaction was quenched with saturated aqueous NH_4Cl and the mixture was extracted with ether. The organic layer was washed with saturated aqueous NH_4Cl, dried over $MgSO_4$, and evaporated. Chromatography of the residue on silica gel with a mixture of hexane and AcOEt (92 :8) gave the selenide **4** (833 mg, 93%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.27 (d, J = 7 Hz, 3 H), 1.45 (s, 9 H), 1.51 (s, 9 H), 2.29 (ddq, J = 5, 6 and 7 Hz, 1 H), 3.48 (d, J = 6 Hz, 1 H), 4.03 (d, J = 5 Hz, 1 H), 7.26-7.33 (m, 3 H), 7.65-7.68 (m, 2 H). ^{13}C NMR ($CDCl_3$) δ 19.5, 27.8, 27.8, 37.6, 49.1, 65.2, 82.4, 83.6, 128.0 128.5, 129.3, 135.2, 149.3, 169.1, 171.4. HRMS (EI, 30 eV) m/z 300.1829 [(M + 1)$^+$, calcd for $C_{15}H_{27}O_5N$ 300.1811].

t-Butyl (*2S,3S,4RS*)-1-*t*-Butoxycarbonyl-3-methyl[4-D]pyroglutamate (**5**)

To a solution of the selenide **4** (830 mg, 1.83 mmol) and Bu_3SnD (1.07 g, 3.67 mmol) in THF (10 ml) was added 1 *M* Et_3B in hexane (2.00 ml, 2.00 mmol) at -78 °C under an argon atmosphere and dry air (10 ml) was introduced into the solution. After being stirred for 0.5 h, the mixture was warmed to room temperature and treated with saturated aqueous $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. Chromatography of the residue on silica gel with a mixture of hexane and AcOEt (86 : 14) gave the deuteriated 3-methylpyroglutamate **5** (470 mg, 86%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J = 7 Hz, 3 H), 1.48 (s, 9 H), 1.50 (s, 9 H), 2.11 (m, 0.64 H), 2.28-2.36 (m, 1 H), 2.74 (d, J = 9 Hz, 0.36 H), 4.05 (d, J = 3 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 20.6, 27.8, 27.9, 29.6, 39.0 (t, J = 21 Hz, 0.36 C), 39.1 (t, J = 21 Hz, 0.64 C), 66.6, 82.1, 83.3, 149.4, 169.9, 173.0. HRMS (EI, 30 eV) m/z 301.1842 [(M + 1)$^+$, calcd for $C_{15}H_{25}O_5N$ 301.1874].

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t-Butyl (2*S*,3*S*,4*RS*)-1-*t*-Butoxycarbonyl-5-hydroxy[4,5,5-D_3]isoleucine (**6**)

To a solution of the pyroglutamate **5** (470 mg, 1.58 mmol) in THF (10 ml) was added dropwise 1 *M* LiOH (1.89 ml) at 0 °C over a period of 15 min. After being stirred for an additional 15 min, the mixture was acidified to pH 4 with 10% aqueous citric acid and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and the solvent was evaporated to afford the 3-methylglutamic acid (550 mg, quant.) as a colorless solid, mp 133-135 °C. ^{1}H NMR ($CDCl_3$) δ 1.02 (d, *J* = 7 Hz, 3 H), 1.44 (s, 9 H), 1.48 (s, 9 H), 2.22 (d, *J* = 9 Hz, 0.36 H), 2.43 (m, 1.64 H), 4.16 (dd, *J* = 8 and 6 Hz, 1 H), 5.17 (d, *J* = 8 Hz, 1 H). HRMS (EI, 30 eV) *m/z* 319.1939 [(M + 1)$^+$, calcd for $C_{15}H_{27}DO_6N$ 319.1979].

The crude acid and Et_3N (210 mg, 2.05 mmol) was dissolved in THF (10 ml) and the solution cooled to -40 °C under an argon atmosphere. Isobutyl chloroformate (260 mg, 1.89 mmol) was added dropwise to the solution and the reaction mixture was stirred for 1 h. The precipitated Et_3N•HCl was filtered off and to the filtrate was added a mixture of $NaBD_4$ (197 mg, 4.73 mmol) in THF (5 ml) and D_2O (1 ml) at 0 °C under an argon atmosphere. After being stirred for 1.5 h at room temperature, the resulting suspension was extracted with AcOEt, and the organic layer was washed successively with 10% aqueous citric acid and brine, and dried over $MgSO_4$. The solvent was evaporated and the residue was chromatographed on silica gel. Elution with a mixture of hexane and AcOEt (70 : 30) afforded the oily hydroxylisoleucine **6** (390 mg) in 80% yield. ^{1}H NMR ($CDCl_3$) δ 0.93 (d, *J* = 7 Hz, 3 H), 1.43 (s, 9 H), 1.46 (s, 9 H), 1.63 (d, *J* = 6 Hz, 0.36 H), 2.11 (m, 1 H), 2.31 (br s, 0.64 H), 4.19 (dd, *J* = 8 and 4 Hz, 1 H), 5.31 (d, *J* = 8 Hz, 1 H). HRMS (EI, 30 eV) *m/z* 307.2343 [(M + 1)$^+$, calcd for $C_{15}H_{27}D_3O_5N$ 307.2312].

t-Butyl (2*S*,3*S*,4*RS*)-1-*t*-Butoxycarbonyl-5-iodo[4,5,5-D_3]isoleucine (**7**)

To a solution of the alcohol **6** (390 mg, 1.26 mmol) and Ph_3P (460 mg, 1.76 mmol) in THF (10 ml) was added DEAD (439 mg, 2.52 mmol) followed by MeI (360 mg, 2.52 mmol) at 0 °C under an argon atmosphere. After being stirred for 1 h at room temperature, the solvent was evaporated in vacuo. Chromatography of the residue on silica gel with a mixture of hexane and AcOEt (96 : 4) afforded the oily iodoisoleucine derivative **7** (370 mg) in 70% yield. ^{1}H NMR ($CDCl_3$) δ 0.92 (d, *J* = 7 Hz, 3 H), 1.44 (s, 9 H), 1.49 (s, 9 H), 1.66 (d, *J* = 10 Hz, 0.36 H), 1.87 (br s, 0.64 H), 2.09 (m, 1 H), 4.20 (m, 1 H), 5.07 (d, *J* = 8 Hz, 1 H). HRMS (EI, 30 eV) *m/z* 417.1295 [(M + 1)$^+$, calcd for $C_{15}H_{26}D_3O_4N$ 417.1330].

(2*S*,3*S*,4*RS*)-[4,5,5,5-D_4]isoleucine (**8**)

A solution of the iodide **7** (229 mg, 0.551 mmol), Bu_3SnD (241 mg, 0.827 mmol), and AIBN (9 mg) in dry benzene (10 ml) was heated at 80 °C under an argon atmosphere for 1 h. After removal of the solvent, the residue was treated with 1 *M* HCl (15 ml) at 110 °C for 3 h. The cooled aqueous solution was washed with chloroform and concentrated to dryness. The residue was submitted for ion-change column chromatography on Dowex 50W-X8 and elution with 1 *M* NH_4OH gave (2*S*,3*S*,4*RS*)-[4,5,5,5-D_4]isoleucine (**8**, 75.0 mg) in quantitative yield, mp >300 °C dec. (commercial L-isoleucine, 288 °C dec.). ^{1}H NMR (D_2O) δ 0.84 (d, *J* = 7 Hz, 3 H), 1.06 (d, *J* = 9.2 Hz, 0.36 H), 1.27 (d, *J* = 4.0 Hz, 0.64 H), 1.81 (m, 1 H), 3.49 (d, *J* = 4 Hz, 1 H). ^{13}C NMR (D_2O) δ 14.5, 23.7 (t, *J* = 19 Hz), 35.6, 59.4, 174.1. HRMS (EI, 30 eV) *m/z* 136.1248 [(M + 1)$^+$, calcd for $C_6H_{10}D_4NO_2$ 136.1276].

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