Synthesis of ^{13}C/D Doubly Labeled L-Leucines: Probes for Conformational Analysis of the Leucine Side-chain

Makoto Oba,* Masahito Kobayashi, Fumiyo Oikawa, and Kozaburo Nishiyama*

Department of Material Science and Technology, Tokai University, 317 Nishino, Numazu, Shizuoka, 410-0395, Japan

Masatsune Kainosho

Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, 1−1 Minami-Ohsawa, Hachioji, Tokyo 192-0397, Japan

makoto@wing.ncc.u-tokai.ac.jp

Received March 9, 2001

Introduction

Recently, the combination of stable isotope labeling and advanced multidimensional NMR spectroscopy has become a well-established method for determining the detailed three-dimensional structures of proteins in solution.[1] In particular, the vicinal spin couplings are valuable carriers of structural information on the backbone and side-chain conformation.[2] To obtain precise dihedral angle information on the protein side-chain, the stereoselective isotope labeling of only one of the diastereotopic protons and methyl groups is essential. In light of the above background, we here focus on the amino acid L-leucine because the amino acid has been recognized as an important residue which participates in the inter- and intramolecular hydrophobic interactions based on its lipophilic side-chain in polypeptides.

There are some methods for the synthesis of L-leucine stereoselectively labeled in either the diastereotopic methyl group with carbon-13 or deuterium;[3] however, reports on the synthesis of L-leucine labeled with deuterium in only one of the diastereotopic methylene protons are limited to those by us.[4,5]

The target molecules in this study are novel ^{13}C/D doubly labeled L-leucines (*2S*,*3S*,*4S*)-**7** and (*2S*,*3R*,*4R*)-**7**, in which one diastereotopic methyl group is substituted with $^{13}CH_3$ and another methyl group and one diastereotopic methylene proton at the β-position are labeled with deuteriums. The NMR spectra of these samples are expected to provide two pairs of vicinal spin coupling constants $J(H^2-H^{3S})$ and $J(H^2-H^{3R})$ about the $C_\alpha-C_\beta$ bond and $J(C^{5R}-H^{3S})$ and $J(C^{5S}-H^{3R})$ about the $C_\beta-C_\gamma$ bond straightforwardly. From these values, we can unambiguously determine the dominant conformation of the leucine side-chain, the $\chi1$ and $\chi2$ angles in peptides, as depicted in Figure 1.

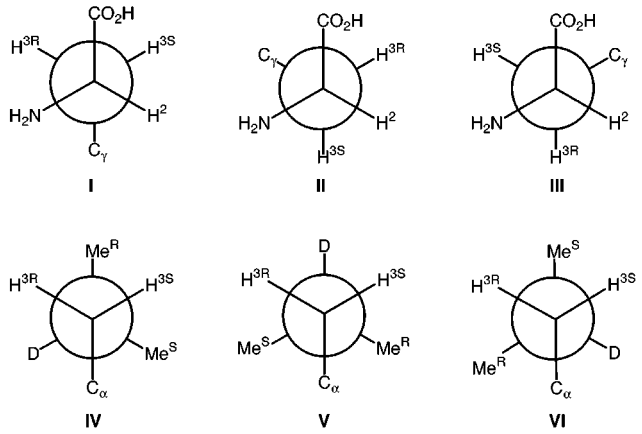


Figure 1. Newman projections for three staggered rotamers about the $C_\alpha-C_\beta$ (I, II, III) and $C_\beta-C_\gamma$ (IV, V, VI) bonds of leucine.

Results and Discussion

We previously reported the stereoselective synthesis of L-leucine, in which both the diastereotopic methyl and methylene protons were chirally labeled with deuterium, from *trans*-4-hydroxy-L-proline.[6] However, the introduction of the methyl group using the Gilman reagent, the key step in the synthesis, used the methyl sources excessively. We, therefore, planned to devise a novel protocol to access the labeled leucine in order to incorporate a carbon-13 label efficiently into one of the diastereotopic methyl groups.

Recently, we envisioned the use of pyroglutamic acid as a chiral template for the asymmetric synthesis of deuterium-labeled amino acids. Our previous paper demonstrated the stereoselective synthesis of [3,4,5-D_3]-proline and [4,5,5,5-D_4]isoleucine using the template.[7] Scheme 1 shows the synthetic course of (*2S*,*3S*,*4S*)-[5-^{13}C;3,4,5′,5′,5′-D_5]leucine (**7**) starting from L-pyroglutamic acid.

The synthesis of leucine **7** was begun by introduction of the [^{13}C]methyl group into γ-lactam **1**[8] readily accessible from L-pyroglutamic acid. Treatment of the lactam **1** with two equivalents of sodium hexamethyldisilazane (NaHMDS) and phenyl selenenyl chloride followed by iodo[^{13}C]methane afforded the [^{13}C]methylated phenyl

(1) Kainosho, M. *Nature Struct. Biol.* **1997**, 858−861. Roberts, G. C. K. *NMR of Macromolecules. A Practical Approach*; Oxford University Press: Oxford, 1993. Wuthrich, K. *NMR of Proteins and Nucleic Acids*; John Wiley & Sons: New York, 1986.

(2) Bystrov, V. F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41−81.

(3) Cardillo, R.; Fuganti, C.; Ghiringhelli, D.; Grasselli, P.; Gatti, G. *J. Chem. Soc., Chem. Commun.* **1977**, 474−476. Sylvester, S. R.; Lan, S. Y.; Stevens, C. M. *Biochemistry* **1981**, *20*, 5609−5611. Aberhart, D. J.; Weiller, B. H. *J. Labeled Compds. Radiopharm.* **1983**, *20*, 663−668. Kelly, N. M.; Reid, R. G.; Willis, C. L.; Winton, P. L. *Tetrahedron Lett.* **1995**, 8315−8318. Hill, R. K.; Abacherli, C.; Hagishita, S. *Can. J. Chem.* **1994**, *72*, 110−113. August, R. A.; Khan, J. A.; Moody, C. M.; Young, D. W. *Tetrahedron Lett.* **1992**, 4617−4620. August, R. A.; Khan, J. A.; Moody, C. M.; Young, D. W. *J. Chem. Soc., Perkin Trans. 1* **1996**, 507−514.

(4) Oba, M.; Ueno, R.; Fukuoka, M.; Kainosho, M.; Nishiyama, K. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1603−1609.

(5) Oba, M.; Nakajima, S.; Nishiyama, K. *J. Chem. Soc., Chem. Commun.* **1996**, 1875−1876. Oba, M.; Terauchi, T.; Owari, Y.; Imai, Y.; Motoyama, I.; Nishiyama, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1275−1281.

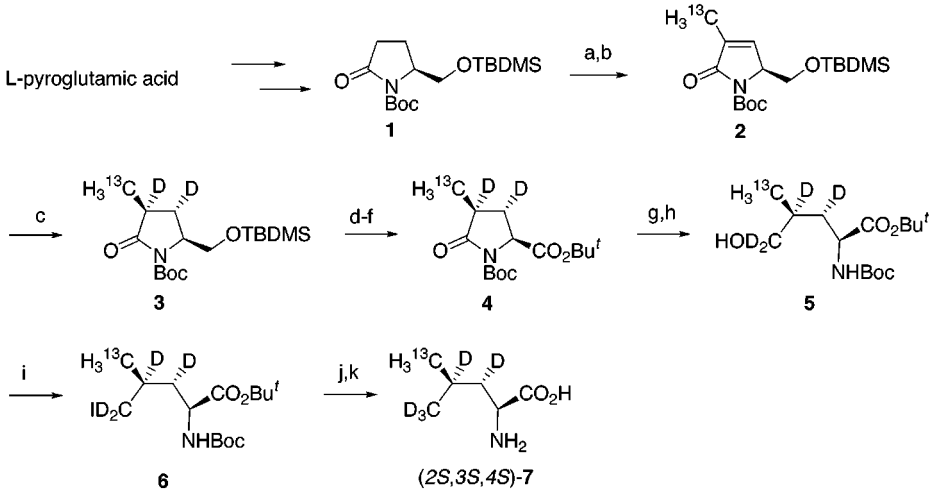
(6) Oba, M.; Terauchi, T.; Miyakawa, A.; Kamo, H.; Nishiyama, K. *Tetrahedron Lett.* **1998**, *39*, 1595−1598.

(7) Oba, M.; Miyakawa, A.; Nishiyama, K.; Terauchi, T.; Kainosho, M. *J. Org. Chem.* **1999**, *64*, 9275−9278. Oba, M.; Miyakawa, A.; Shionoya, M.; Nishiyama, K. *J. Labeled Compds. Radiopharm.* **2001**, *44*, 141−147.

(8) Ohfune, Y.; Tomita, M. *J. Am. Chem. Soc.* **1982**, *104*, 3511−3513.

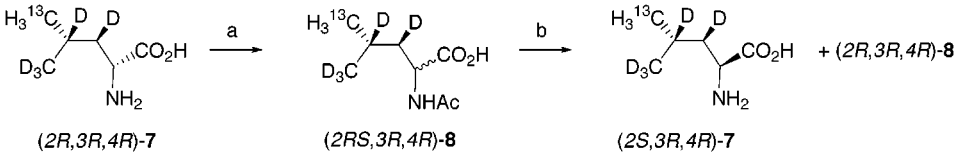
10.1021/jo010263r CCC: $20.00
Published on Web 07/31/2001

Scheme 1[a]



[a] Reagents and Conditions: (a) $NaN(TMS)_2$, PhSeCl, $^{13}CH_3I$, quant.; (b) H_2O_2, 78%; (c) D_2 (5 kgf/cm^2), PtO_2, MeOD, 98%; (d) TsOH, MeOH, 88%; (e) RuO_2, $NaIO_4$, 89%; (f) $Me_2NCH(OCH_2CMe_3)_2$, tert-BuOH, 55%; (g) 1 M LiOH, quant.; (h) $ClCO_2Bu^i$, Et_3N, then, $NaBD_4$, 64%; (i) PPh_3 (polystyrene-supported), I_2, 88%; (j) Bu_3SnD, AIBN; (k) 1 M HCl, 110 °C, then Dowex 50W-X8, 65%.

Scheme 2[a]



[a] Reagents and Conditions: (a) Ac_2O, 2 M NaOH, 70 °C; (b) *Aspergillus* Acylase, $CoCl_2$, pH 8, 37 °C, 29% (2 steps).

selenide as a mixture of diastereisomers (syn:anti = 64:36). The selenide was treated with 30% hydrogen peroxide to give [^{13}C]methylated unsaturated lactam **2** in 78% yield. Only a trace amount of the isomeric exomethylene compound, which could be removed by column chromatography, was detected in the reaction mixture by ^{1}H NMR spectroscopy, indicating the regioselective syn elimination of the selenenic acid occurred.

Catalytic deuteration of the olefin **2** was carried out using deuterium gas at medium pressure (5 kgf/cm^2) in MeOD. The use of PtO_2 as a catalyst proved advantageous, resulting in clean formation of 3,4-dideuterated γ-lactam **3** as a single diastereomer. In this case, palladium on carbon was not suitable, causing considerable H−D scrambling and olefin isomerization. After removal of the silyl protecting group of the lactam **3** by treatment with *p*-toluenesulfonic acid in MeOH, RuO_4-oxidation of the resulting primary alcohol and subsequent esterification with dimethylformamide di-*tert*-butyl acetal afforded *tert*-butyl 4-[^{13}C]methyl[3,4-D_2]pyroglutamate **4**.

Conversion of the pyroglutamate **4** into the desired leucine **7** was carried out using the procedure previously reported by us.[6] Thus, the pyroglutamate **4** was treated with 1 M LiOH and the resulting carboxylic acid was reduced to the deuterated alcohol **5** using $NaBD_4$ via a mixed anhydride with isobutyl chloroformate in 64% yield. Iodination of the alcohol **5** was carried out using iodine and polystyrene-supported triphenylphosphine to give 5-iodoleucine **6** in 88% yield.[9] Finally, radical-based reduction of the iodide **6** with Bu_3SnD-AIBN followed by the standard deprotection procedure furnished (*2S*,*3S*,*4S*)-[5-^{13}C;3,4,5′,5′,5′-D_5]leucine (**7**) in 65% yield. The enantiomeric purity (97% ee) at the α-position was determined by HPLC analysis using a chiral stationary phase column.

To obtain the isotopomeric (*2S*,*3R*,*4R*)-[5-^{13}C;3,4,5′,5′,5′-D_5]leucine (**7**), we next examined epimerization of (*2R*,*3R*,*4R*)-[5-^{13}C;3,4,5′,5′,5′-D_5]leucine (**7**) at the α-position followed by enzymatic resolution as shown in Scheme 2. Thus, treatment of the (*2R*,*3R*,*4R*)-**7**, prepared from D-pyroglutamic acid according to the Scheme 1, with an excess of acetic anhydride at 70 °C gave a mixture of (*2S*,*3R*,*4R*)- and (*2R*,*3R*,*4R*)-*N*-acetyl[5-^{13}C;3,4,5′,5′,5′-D_5]-leucine (**8**). The mixture was then subjected to stereospecific hydrolysis using *Aspergillus* acylase to afford the (*2S*,*3R*,*4R*)-**7**. The enantiomeric purity (100% ee) at the α-position was checked by a chiral HPLC.

Figure 2 shows the 400 MHz ^{1}H NMR spectra of the labeled leucines (*2S*,*3S*,*4S*)-**7** (upper) and (*2S*,*3R*,*4R*)-**7** (middle) along with the unlabeled leucine (bottom). It is evident that the regio- and stereoselective incorporation of the stable isotopes into the leucine framework has been accomplished. From these spectra, we can easily obtain the vicinal spin coupling constants between the α- and β-positions and between the β- and γ-positions. By taking $J(H^2-H^{3S}) = 5.6$ Hz and $J(H^2-H^{3R}) = 8.4$ Hz, the approximate rotamer populations P_I (0.53), P_{II} (0.20), and P_{III} (0.27) about the $C_\alpha-C_\beta$ bond can be given by Pachler's equations: $P_I = [J(H^2-H^{3R}) - J_g]/(J_t - J_g)$, $P_{II} = 1 - (P_I + P_{III})$, $P_{III} = [J(H^2-H^{3S}) - J_g]/(J_t - J_g)$, in which $J_t = 13.6$ Hz and $J_g = 2.6$ Hz.[10] These results are in good agreement with the reported data.[4] Using the heteronuclear vicinal spin coupling constants $J(C^{5R}-H^{3S}) = 3.9$

(9) Quagliato, D. A.; Andrae, P. M.; Matelan, E. M. *J. Org. Chem.* **2000**, *65*, 5037−5042.

(10) Pachler, K. G. R. *Spectrochim. Acta* **1964**, *20*, 581−587.

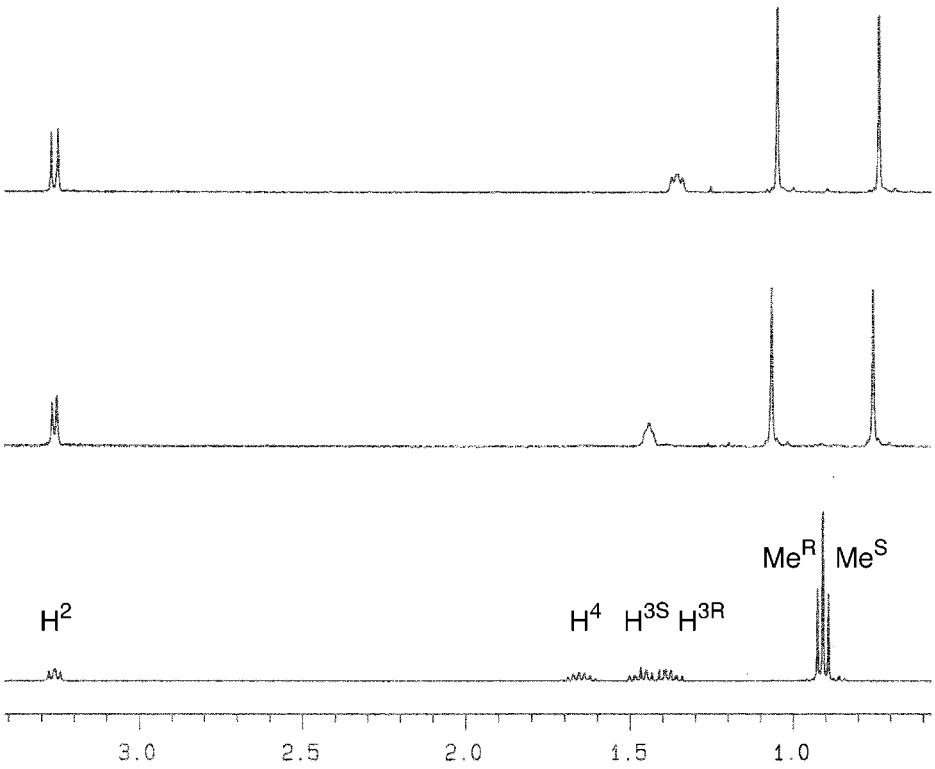


Figure 2. 400 MHz ^{1}H NMR Spectra of labeled leucines (*2S,3S,4S*)-**7** (upper) and (*2S,3R,4R*)-**7** (middle) and unlabeled leucine (bottom) in 5% NaOD/D_2O.

Hz and $J(C^{5S}-H^{3R}) = 5.2$ Hz, the fractional populations P_{IV} (0.46), P_V (0.15), and P_{VI} (0.31) about the $C_\beta-C_\gamma$ bond can be also obtained by the following equations: $P_{IV} = [J(C^{5S}-H^{3R}) - J_g]/(J_t - J_g)$, $P_V = 1 - (P_{IV} + P_{VI})$, $P_{VI} = [J(C^{5R}-H^{3S}) - J_g]/(J_t - J_g)$, $Jt = 9.8$ Hz, $Jg = 1.3$ Hz.[11] To the best of our knowledge, this is the first example of the estimation of the rotamer population about the $C_\beta-C_\gamma$ bond of leucine with the help of selective isotope labeling.

In conclusion, we have completed the synthesis of L-leucine in which both the diastereotopic methyl and methylene protons were chirally labeled with carbon-13 and deuterium. This approach has a further advantage in that it enables the preparation of uniformly carbon-13 and nitrogen-15 labeled samples starting from the commercially available [$^{13}C_5$;^{15}N]glutamic acid, which should prove useful for structural determination of larger proteins.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra were measured 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 deuteriochloroform (δ_C 77.0), or dioxane (δ_C 66.5). High-resolution mass spectra (EI) were obtained at an ionization potential of 70 eV unless otherwise noted. Enantiomeric purity was determined on an HPLC system (monitored at 254 nm) equipped with a chiral column (MCIGEL CRS10W) using 2 mM $CuSO_4$ solution as an eluent. Solvents and reagents were of commercial grade and were purified if necessary.

(*5S*)-1-*tert*-Butoxycarbonyl-3-[^{13}C]methyl-5*H*-2-pyrrolinone (2). A solution of 1 M NaHMDS in THF (26.9 mL, 26.9 mmol) was treated with *N,N′*-dimethylpropyleneurea (3.3 mL) at 0 °C under an argon atmosphere for 10 min, cooled to −78 °C, and treated with a solution of the 2-pyrrolidone **1** (4.20 g, 12.8 mmol) in THF (20 mL). After 0.5 h, a solution of PhSeCl (2.69 g, 14.1 mmol) in THF (15 mL) was added and the mixture was stirred for 2 h. Then, $^{13}CH_3I$ (2.01 g, 14.1 mmol) was added. 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 to give 3-[^{13}C]methyl-3-phenylseleno-2-pyrrolidone in quantitative yield.

To a solution of the obtained crude phenylselenide (6.21 g, 12.88 mmol) in THF (51 mL) was added dropwise H_2O_2 (14.5 g, 12.8 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 1 h. The mixture was extracted with ether and the organic layer was washed with saturated aqueous $NaHCO_3$ 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 title compound **2** (3.29 g, 78%) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 3 H), 0.04 (s, 3 H), 0.86 (s, 9 H), 1.55 (s, 9 H), 1.88 (ddd, J = 128, 2 and 2 Hz, 3 H), 3.65 (dd, J = 10 and 4 Hz, 1 H), 4.13 (dd, J = 10 and 7 Hz, 1 H), 4.45 (m, 1 H), 6.88 (m, 1 H). ^{13}C NMR ($CDCl_3$) δ −5.52, −5.60, 10.81 (enhanced), 16.08, 25.65, 28.07, 61.21, 62.49, 82.64, 134.98 (d, J = 48 Hz), 142.39, 149.71, 170.11. HRMS *m/z* 343.2122 [(M + H)$^+$, calcd for $C_{16}{}^{13}CH_{32}NO_4Si$ 343.2134].

(*3S,4S,5S*)-1-*tert*-Butoxycarbonyl-5-*tert*-butyldimethylsiloxymethyl-3-[^{13}C]methyl-2-[3,4-D_2]pyrrolidone (3). A mixture of the olefin **2** (3.29 g, 9.92 mmol) and PtO_2 (160 mg) in MeOD (100 mL) was stirred at room temperature for 1 h under medium pressure (5 kgf/cm^2) of deuterium gas. After removal of the catalyst using a Celite pad, evaporation of the solvent gave the title compound **3** (3.27 g, 98%) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 3 H), 0.04 (s, 3 H), 0.87 (s, 9 H), 1.24 (d, J = 127 Hz, 3 H), 1.53 (s, 9 H), 1.66 (dd, J = 6 and 6 Hz, 1 H), 3.70 (dd, J = 10 and 2 Hz, 1 H), 3.91 (dd, J = 10 and 5 Hz, 1 H), 4.05 (m, 1 H). ^{13}C NMR ($CDCl_3$) δ −5.50, 16.51 (enhanced), 18.22, 25.80, 27.19 (t, J = 20 Hz), 28.00, 36.80 (dt, J = 37 and 20 Hz), 56.81, 63.37, 82.56, 150.37, 177.24. HRMS (30 eV) *m/z* 347.2410 [(M + H)$^+$, calcd for $C_{16}{}^{13}CH_{32}D_2NO_4Si$ 347.2416].

***tert*-Butyl (*2S,3S,4S*)-*N*-*tert*-Butoxycarbonyl-4-[^{13}C]-methyl-[3,4-D_2]pyroglutamate (4).** To a solution of the com-

(11) Cowburn, D.; Live, D. H.; Fischman, J.; Agosta, W. C. *J. Am. Chem. Soc.* **1983**, *105*, 7435−7442.

pound **3** (2.79 g, 8.29 mmol) in MeOH (80 mL) was added *p*-toluenesulfonic acid (140 mg, 0.829 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 desilylated product (1.67 g, 88%).

To a suspension of sodium metaperiodate (15.8 g, 72.7 mmol) and $RuCl_3 \cdot nH_2O$ (440 mg) in H_2O (45 mL) was added the obtained alcohol in acetone (38 mL). The resulting two-phase mixture was vigorously stirred at room temperature for 1 h. The layers were separated. To the organic phase was added 2-propanol (30 mL) and the mixture was stirred for 1 h. After removal of the precipitated RuO_2 using a Celite pad, the filtrate was concentrated, extracted with chloroform, and dried over $MgSO_4$. Evaporation of the solvent gave 4-[^{13}C]methyl-[3,4-D_2]pyroglutamic acid (1.58 g, 89%). ^{1}H NMR ($CDCl_3$) δ 1.27 (d, J = 128 Hz, 3 H), 1.50 (s, 9 H), 1.69 (dd, J = 6 and 6 Hz, 1 H), 4.53 (d, J = 6 Hz, 1 H). HRMS m/z 247.1366 [(M + H)$^+$, calcd for $C_{10}{}^{13}CH_{16}D_2NO_5$ 247.1344].

To a refluxing solution of the obtained pyroglutamic acid in benzene (25 mL) was added a mixture of *N*,*N*-dimethylformamide dineopentyl acetal (2.68 g, 11.6 mmol) and *tert*-butanol (1.43 g, 19.3 mmol), and the reaction mixture was refluxed 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 (80:20) afforded the title compound **4** (1.06 g, 55%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (d, J = 129 Hz, 3 H), 1.48 (s, 9 H), 1.51 (s, 9 H), 1.56 (dd, J = 6 and 5 Hz, 1 H), 4.38 (d, J = 6 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 16.31 (enhanced), 27.79 (2 C), 29.10 (t, J = 21 Hz), 36.91 (dt, J = 37 and 20 Hz), 57.91, 81.97, 83.12, 149.48, 170.60, 176.03. HRMS m/z 303.1995 [(M + H)$^+$, calcd for $C_{14}{}^{13}CH_{24}D_2NO_5$ 303.1970].

***tert*-Butyl (*2S,3S,4S*)-*N*-*tert*-Butoxycarbonyl-5-hydroxy-[5-^{13}C;3,4,5′,5′-D_4]leucine (5).** To a solution of the pyroglutamate **4** (772 mg, 2.56 mmol) in THF (15 mL) was added dropwise 1 M LiOH (3.07 mL) at 0 °C over a period of 15 min. After being stirred for an additional 30 min, the mixture was acidified to pH 4 with 10% aqueous citric acid and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and the solvent was evaporated to afford the 4-[^{13}C]methyl[3,4-D_2]-glutamate (819 mg) in quantitative yield.

The obtained acid and Et_3N (340 mg, 3.33 mmol) were dissolved in THF (25 mL) and the solution was cooled to −40 °C under an argon atmosphere. Isobutyl chloroformate (420 mg, 3.07 mmol) was added dropwise to the solution and the reaction mixture was stirred for 1 h. The precipitated $Et_3N \cdot HCl$ was filtered off and to the filtrate was added a mixture of $NaBD_4$ (330 mg, 7.68 mmol) in THF (20 mL) and D_2O (15 mL) at 0 °C under an argon atmosphere. After being stirred for 1.5 h at room temperature, the resulting suspension was extracted with ethyl acetate, 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 ethyl acetate (75:25) afforded the title compound **5** (506 mg, 64%) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.96 (d, J = 125 Hz, 3 H), 1.44 (s, 9 H), 1.46 (s, 9 H), 1.66 (dd, J = 8 and 5 Hz, 1 H), 1.92 (br s, 1 H), 4.21 (dd, J = 8 and 8 Hz, 1 H), 5.06 (d, J = 8 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 16.35 (enhanced), 27.92, 28.27, 31.81 (m), 36.25 (m), 52.19, 66.53 (m), 79.66, 81.73, 155.68, 172.51. HRMS (30 eV) m/z 309.2360 [(M + H)$^+$, calcd for $C_{14}{}^{13}CH_{26}D_4NO_5$ 309.2409].

***tert*-Butyl (*2S,3S,4S*)-*N*-*tert*-Butoxycarbonyl-5-iodo[5-^{13}C;3,4,5′,5′-D_4]leucine (6).** To a mixture of polystyrene-supported Ph_3P (1.20 g, 3.61 mmol) in CH_2Cl_2 (15 mL) was added I_2 (920 mg, 3.61 mmol) under an argon atmosphere and the mixture was stirred at room temperature for 10 min. To the reaction mixture was added imidazole (250 mg, 3.61 mmol) followed by a solution of the alcohol **5** (510 mg, 1.64 mmol) in CH_2Cl_2 (45 mL) and the resulting suspension was refluxed for 2 h. After filtration of the insoluble materials, the filtrate was washed with dilute aqueous NaS_2O_3 solution and brine, and dried over $MgSO_4$. Evaporation of the solvent afforded the title compound **6** (610 mg, 88%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.06 (d, J = 126 Hz, 3 H), 1.44 (s, 9 H), 1.47 (s, 9 H), 1.63 (dd, J = 9 and 6 Hz, 1 H), 4.18 (dd, J = 9 and 9 Hz, 1 H), 4.91 (d, J = 9 Hz, 1 H). HRMS m/z 419.1453 [(M + H)$^+$, calcd for $C_{14}{}^{13}CH_{25}D_4NO_4I$ 419.1426].

***(2S,3S,4S)*-[5-^{13}C;3,4,5′,5′,5′-D_5]Leucine (7).** A solution of the iodide **6** (264 mg, 0.630 mmol), Bu_3SnD (280 mg, 0.950 mmol), and AIBN (10 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 (20 mL) at 110 °C for 3 h. The cooled aqueous solution was washed with chloroform and concentrated to dryness. The residue was submitted to ion-exchange column chromatography on Dowex 50W−X8 and elution with 1 M NH_4OH gave the title compound **7** (45.0 mg, 65%) as a colorless solid. ^{1}H NMR (5% NaOD in D_2O) δ 0.87 (d, J = 125 Hz, 3 H), 1.33 (dd, J = 8.4 and 5.2 Hz, 1 H), 3.24 (d, J = 8.4 Hz, 1 H). HRMS (30 eV) m/z 138.1338 [(M + H)$^+$, calcd for $C_5{}^{13}CH_9D_5NO_2$ 138.1372].

***(2S,3R,4R)*-[5-^{13}C;3,4,5′,5′,5′-D_5]Leucine (7).** To a solution of the (*2R,3R,4R*)-**7** (183 mg, 1.33 mmol) in 1 M NaOH (1 mL) was added dropwise acetic anhydride (228 mg, 2.24 mmol) over 3 h at 70 °C. The progress of the epimerization was monitored by ^{1}H NMR. After an additional heating for 30 min, the reaction mixture was evaporated and the residue containing (*2S,3R,4R*)- and (*2R,3R,4R*)-**8** was directly subjected to enzymatic resolution. The obtained *N*-acetylleucine and $CoCl_2 \cdot 6H_2O$ (2.38 mg) were dissolved in 2.5 M NaOH (10 mL) and the pH was adjusted to 8.0−8.5 using 1 M HCl. The solution was added a crude powder of *Aspergillus* acylase (13 mg) and was incubated at 37 °C for 72 h. The reaction mixture was concentrated to dryness and submitted to ion exchange column chromatography on Dowex 50W−X8 and the resin was washed with water. (*2R,3R,4R*)-**8** was recovered from the aqueous washings. Elution with 1 M NH_4OH and evaporation of appropriate fractions (monitored by ninhydrin spray) gave (*2S,3R,4R*)-**7** (26.4 mg, 29%) as a colorless solid. ^{1}H NMR (5% NaOD in D_2O) δ 0.89 (d, J = 124 Hz, 3 H), 1.42 (dd, J = 5.6 and 3.9 Hz, 1 H), 3.24 (d, J = 5.6 Hz, 1 H). HRMS (30 eV) m/z 138.1372 [(M + H)$^+$, calcd for $C_5{}^{13}CH_9D_5NO_2$ 138.1372].

Acknowledgment. This work has been supported by CREST (Core Research for Evolutional Science and Technology) of the Japan Science and Technology Corporation (JST).

JO010263R