*Biosci. Biotechnol. Biochem.*, **70** (1), 276–278, 2006

*Note*

# Improved Isotopic Deuterium Labeling at the Diastereotopic Methyl Group of Leucine: a Synthetic Route to (4*S*)- and (4*R*)-[5-$^2H_1$]Leucine

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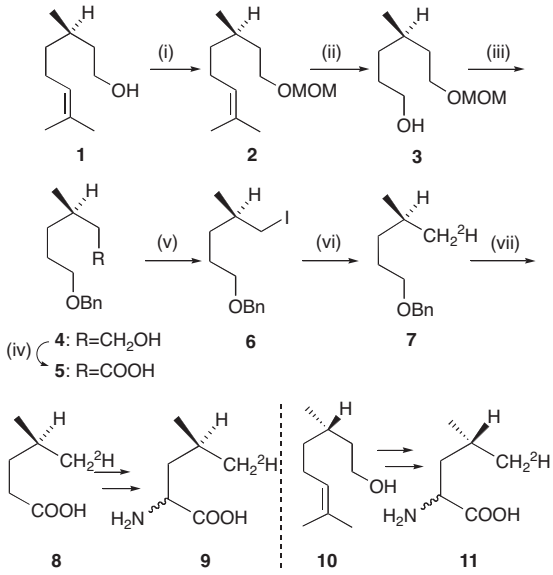
Received August 10, 2005; Accepted October 9, 2005

**Two isotopomers of deuterium-labeled leucine in the diastereotopic methyl group were synthesized from inexpensively available (*R*)- and (*S*)-citronellol in a more convenient way than by previous methods.**

**Key words:** leucine; diastereotopic methyl group; deuterium labeling

Deuterium-labeled amino acids are important in bioorganic chemistry for trace experiments in metabolic studies of amino acids and related compounds. Thanks to NMR spectroscopy, the importance of these compounds has recently increased to facilitate the elucidation of the 3-D structures of peptides and proteins.[1] Among these, the leucine residue is important in the three-dimensional structure of proteins with hydrophobic interaction. Assignment of the diastereotopic methyl groups of leucine by NMR could allow the protein structures and substrate-enzyme interaction in the hydrophobic region to be ascertained more precisely.

Several methods have been reported for the specific chemical and chemo-enzymatic synthesis of deuterium-labeled leucine in the diastereotopic methyl group. Young *et al.* have reported the first synthesis of (2*S*,4*R*)-[5,5,5-$^2H_3$]leucine from pyroglutamate.[2] Oba and Nishiyama disclosed the synthesis of stereo- and regiospecifically deuterium-labeled leucine by a similar approach.[3] Hill *et al.* described the synthesis of (2*S*,4*S*)- and (2*R*,4*S*)-[5,5,5-$^2H_3$]leucine, starting from (*R*)-pulegone as the chiral source.[4] Willis *et al.* described the chemo-enzymatic synthesis of the labeled leucine at the diastereotopic methyl group.[5,6] Their stereogenic center at C-4 was established by using Evans's chiral auxiliaries and the camphor sultam chiral auxiliary. However, their method had several problems for scaled-up synthesis of the product or of another isotopomer of the labeled compound by their use of an unnatural pyroglutamate isomer for one isotopomer, a quantitative amount of the expensive ruthenium-containing Wilkinson catalyst for decarbonylation of the intermediate, and the requirement for purifying the labeled isopropanol and isopropyl bromide.




**Scheme 1.** Reagents and conditions: (i) MOMCl, *i*Pr$_2$NEt, $CH_2Cl_2$ (90%); (ii) $O_3$, EtOH, then $NaBH_4$ (89%); (iii) (a) BnCl, NaOH, DMSO; (b) HCl, MeOH (70% for 2 steps); (iv) Jones reagent, acetone (80%); (v) $I_2$, PhI(OAc)$_2$, $CCl_4$, W-lamp irr. (100 W ×2) (87%); (vi) Li$Al^2H_4$, THF (80%); (vii) (a) $H_2$/Pd-C, *i*PrOH; (b) Jones reagent, acetone (46% for 2 steps).

In our biosynthetic studies of archaeal characteristic isoprenoidal lipid, we planned to determine the role of the metabolism of leucine after publication of the results of lysine metabolism and isoprenoidal lipid biosynthesis.[7] We first improved the synthesis for the diastereotopic labeling of leucine at the specific position of the methyl group.

The synthetic procedure is shown in Scheme 1.[8] The starting material was citronellol; either the (*R*) or (*S*) isomer is inexpensively available commercially as a chiral source. The hydroxyl group of (*R*)-citronellol **1**, ($[\alpha]_D^{25} = +3.89$ (neat)) was protected with MOM-ether and double-bond oxidative fission by ozonolysis, and the resulting aldehyde was reduced with $NaBH_4$ *in situ* to give alcohol **3**. The newly-formed hydroxyl group was protected with a benzyl ether, while deprotection of

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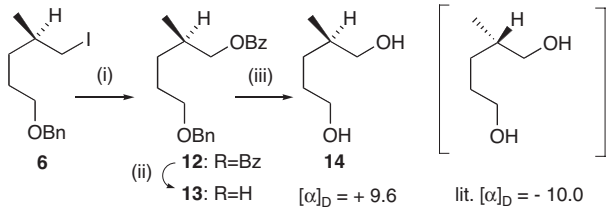


**Scheme 2.** Reagents and conditions: (i) NaOBz, DMF (99%); (ii) NaOH, EtOH (61%); (iii) $H_2$/Pd-C (73%).

MOM-ether gave alcohol **4**. Jones oxidation of **4** then yielded carboxylic acid **5**. The key step in the synthetic scheme is the decarboxylation-iodide formation of carboxylic acid with iodosobenzene diacetate-iodine as developed by Suarez *et al.*[9] This reaction avoids some of the difficulties of the modified Hunsdiecker reaction that needs the use of a quantitative amount of a heavy metal (*e.g.* $Pb(OAc)_4$ or $Cu(OAc)_2$). The reaction proceeded cleanly, and iodide **6** was obtained in good yield.[9] The experimental details for this key step are shown in the experimental section.

Deuterium was then introduced with the reduction of **6** by $LiAl^2H_4$ to give **7**. Deprotection and Jones oxidation of the resulting alcohol yielded labeled 4-methylvaleric acid **8** which is the $CH_2{}^2H_1$ derivative of Hill's intermediate;[4] the product was converted to a mixture of labeled (2*S*,4*S*)- and (2*R*,4*S*)-[5-$^2H_1$]leucine **9** by their procedure. Another isotopomeric mixture **11** could also be synthesized from (*S*)-citronellol **10** ($[\alpha]_D^{25} = -3.39$ (neat)) in the same manner.

Possible stereochemical ambiguity was then elucidated. There was the possibility of racemization in the decarboxylation-iodide formation step; **6** was converted to (*R*)-2-methylpentane-1,5-diol **14** ($[\alpha]_D^{23} = +9.6$ ($c = 1.2$, ether)) in the three steps shown in Scheme 2, and the optical rotation of **14** was compared with known (−)-(*S*)-2-methylpentane-1,5-diol ($[\alpha]_D^{25}$ −10.0 ($c = 0.025$, ether),[10] or −8.5 ($c = 2.0$, ether).[11] Thus, racemization was determined not to have occurred in this step. This method would provide a pathway for tritium labeling of leucine in the diastereotopic methyl group. Utilizing the enzymatic hydrolysis of racemic leucine acetate, a D or L (2*R* or 2*S*) amino acid could also be obtained.[4]

*Experimental*

Infrared spectra were obtained with a Perkin Elmer 1600 FT-IR spectrometer, $^1$H-NMR spectra were recorded with a Jeol EX-90 spectrometer, and HR-MS was recorded with a Jeol D-300 mass spectrometer. Chromatographic separation was carried out with Merck Kieselgel 60, 70–230 mesh columns.

*Synthesis of (R)-1-O-benzyl-5-iodo-4-methylpentane-1-ol (**6**).* A solution of **5** (5.00 g, 21.1 mmol) in carbon tetrachloride (450 ml) containing iodosobenzene diacetate (10.20 g, 26.9 mmol) and iodine (5.35 g, 21.1 mmol) was irradiated with two 100-W tungsten lamps for 2 h at reflux temperature. The reaction mixture was cooled to room temperature, and then washed successively with a dilute sodium thiosulfate solution and water. The organic phase was dried, evaporated and chromatographed by silica gel (hexane:$CHCl_3$ = 2:1) to give 5.86 g (18.4 mmol, 87.2%) of product **6**. IR $\nu_{max}$ ($CHCl_3$) $cm^{-1}$: 3032, 2859, 1454, 1097. $^1$H-NMR (90 MHz) $\delta_H$ ($CDCl_3$): 0.98 (3H, d, $J = 6.2$ Hz), 1.30–1.82 (5H, m), 3.25 (1H, dd, $J = 5.9$, 9.1 Hz), 3.29 (1H, dd, $J = 5.9$, 9.1 Hz), 3.57 (2H, t, $J = 7.5$ Hz), 4.52 (2H, s), 7.32 (5H, aromatic). $^{13}$C-NMR (22 MHz) $\delta_C$ ($CDCl_3$): 17.54, 20.49, 27.15, 32.96, 34.54, 70.23, 72.88, 127.48, 127.57 (×2), 128.32 (×2), 138.49. HRMS $m/z$ ($M^+$): calcd. for $C_{13}H_{19}IO$, 318.0481 found, 318.0495.

*Synthesis of (S)-[5-$^2H_1$]-1-O-benzyl-4-methylhexane-1-ol (**7**).* A solution of **6** (2.42 g, 7.6 mmol) in THF (40 ml) containing $LiAl^2H_4$ (340 mg, 8.1 mmol) was refluxed for 2 h. The reaction mixture was cooled to room temperature, and then the excess reagent was quenched by adding a small amount of water. The solution was dried by adding anhydrous $Na_2SO_4$, filtered, evaporated and chromatographed by silica gel (hexane:$CHCl_3$ = 2:1) to give 1.18 g (6.1 mmol, 80.3%) of product **7**. Deuterium incorporation was >96% from the $^1$H-NMR and MS data ($m/z$ 101 and 102, ($M^+$–OBn)). IR $\nu_{max}$ ($CHCl_3$) $cm^{-1}$: 3032, 2951, 2867, 1454, 1094 $cm^{-1}$. $^1$H-NMR (90 MHz) $\delta_H$ ($CDCl_3$): 0.87 (2H, m, $CH_2{}^2H$), 0.88 (3H, d, $J = 6.3$ Hz), 1.10–1.80 (5H, m), 3.45 (2H, t, $J = 6.6$ Hz), 4.50 (2H, s), 7.32 (5H, aromatic). $^{13}$C-NMR (22 MHz) $\delta_C$ ($CDCl_3$): 22.25 (t, $J = 19$ Hz), 22.52, 27.65, 27.83, 35.29, 70.85, 72.85, 127.42, 127.60 (×2), 127.60 (×2), 138.72. EIMS $m/z$: 193 ($M^+$), 102 ($M^+$–OBn), 91. *Anal.* Found: C, 80.59; H (+$^2$H), 10.38. Calcd. for $C_{13}H_{19}{}^2H_1O$: C, 80.78; H (+$^2$H), 10.42.

## Acknowledgments

We thank Prof. Katsuki and Assoc. Prof. Irie (Kyushu University) for measuring the optical rotation. This study was supported in part by grant-aid from the Kurita Water and Environmental Foundation.

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8) All new compounds were characterized by $^1$H-NMR, IR and elemental analyses (or HR-MS). For example, **8**: $^1$H-NMR (90 MHz) $\delta_H$ ($CDCl_3$): 1.00 (2H, m, C$H_2{}^2$H), 1.01 (3H, d, $J = 6.3$ Hz), 1.60–1.82 (3H, m), 3.50 (1H, t, $J = 6.7$ Hz). *Anal.* Found: C, 61.17; H (+$^2$H), 10.17. Calcd. for $C_6H_{11}{}^2H_1O_2$: C, 61.51; H (+$^2$H), 10.32. **9**: $^1$H-NMR (90 MHz) $\delta_H$ ($D_2O$/3% NaOD): 0.64 (5H, br d), 1.09–1.23 (3H, m), 2.98 (1H, t, $J = 7.7$ Hz); *Anal.* Found: C, 54.60; H (+$^2$H), 9.89; N, 10.62. Calcd. for $C_6H_{12}{}^2H_1O_2N$: C, 54.53; H (+$^2$H), 9.91; N, 10.60.
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