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EFFICIENT SYNTHESES OF DIASTEREOSELECTIVELY LABELED (3S)-[4-^{13}C]-L-VALINE, AND REGIOSELECTIVELY LABELED [3-$^{13}CH_3$]-L-ISOLEUCINE HYDROCHLORIDES

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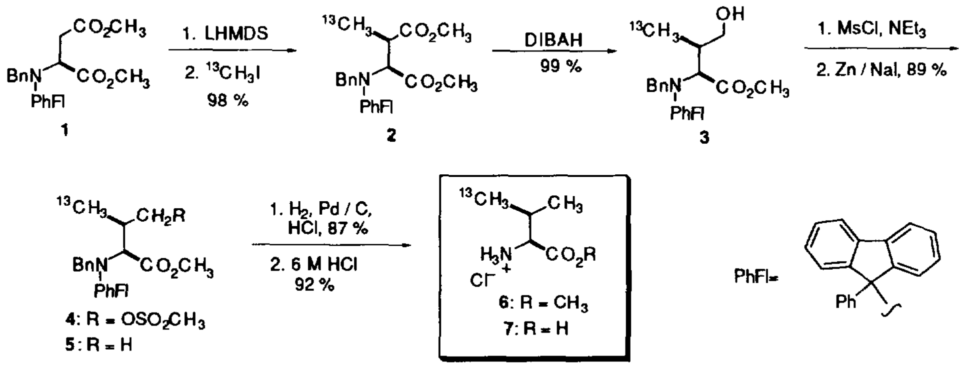
Abstract. Diastereoselectively labeled (3S)-[4-^{13}C]-L-valine and regioselectively labeled [3-$^{13}CH_3$]-L-isoleucine hydrochlorides were synthesized in 54% and 62% respective overall yields from L-aspartic acid.

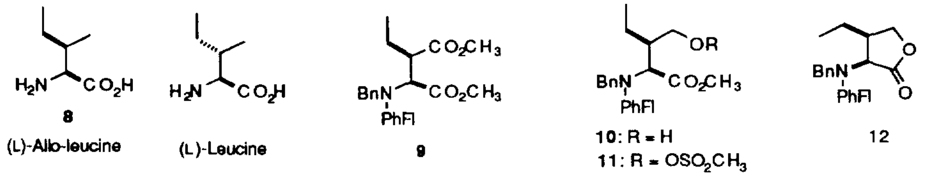
Carbon-13 labeled amino acids and their derivatives are important probes for the study of biochemical systems due to their enhanced spectroscopic detectability. In addition to acting as tracers for the elucidation of various metabolic pathways[1-8] with the aid of ^{13}C NMR[9] and/or infrared spectroscopy,[10] they are useful for NMR structural studies of proteins.[11,12] This approach requires the complete assignment of ^{1}H resonances, a task that is complicated by chemical shift overlap and consequently requires multi-dimensional heteronuclear NMR.[13-16] Many of these methods rely on ^{1}H-^{13}C or ^{13}C-^{13}C correlation, and often necessitate regio- and/or stereoselective isotopic enrichment.[15,17-19] Other NMR techniques, such as Rotational Resonance,[20-25] can provide structural information even for non-crystalline or insoluble proteins, but they also require selective isotopic enrichment.[26-30] This selective enrichment is generally accomplished by the judicious incorporation of synthetically or biosynthetically[31] derived, carbon-13 labeled amino acids. In light of the importance of such probes, we wish to report efficient syntheses of diastereoselectively labeled [^{13}C]-L-valine and regioselectively labeled [^{13}C]-L-isoleucine hydrochlorides **7** and **16** in 54% and 62% respective overall yields from L-aspartic acid.[33]

Scheme 1



We have recently reported the regio- and diastereoselective β-alkylation of **1**[34] and envisioned using this methodology to perform a similar alkylation with ^{13}C-enriched iodomethane in a sequence leading ultimately to isotopically labeled valine (Scheme 1). Accordingly, the protected aspartate **1** was alkylated (LHMDS/$^{13}CH_3I$) to give the crystalline diastereomer **2** in 98% yield (the stereochemical result of this alkylation was proven previously in the preparation of non-labeled **2**[34]). Regioselective DIBAH reduction of the distal ester through a slight modification of the literature procedure[35] gave the crystalline alcohol **3**, also in excellent yield. Quantitative conversion into the mesylate **4**[36,37] followed by Zn-I reduction[32,38] (89%) and deprotection (H_2/Pd-C, HCl, 87%; 6 M HCl, 60 °C, 92%) gave the labeled valine hydrochloride **7** in 9 steps and 54% overall yield from aspartic acid.

Figure 1



8 (L)-Allo-leucine

(L)-Leucine

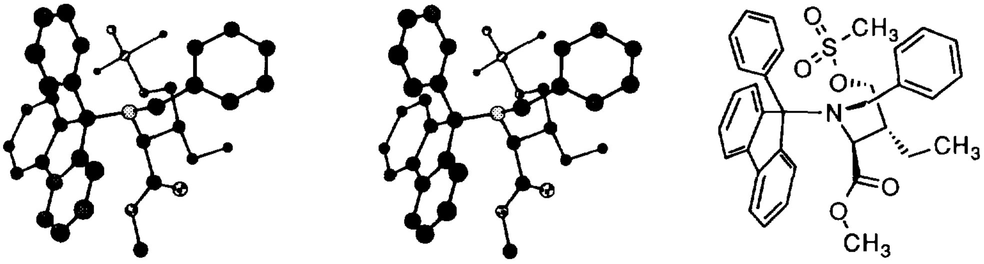
9

10: R = H
11: R = OSO_2CH_3

12

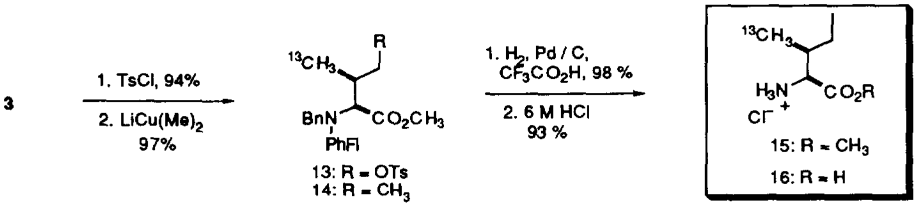
A logical extension of this route would be to substitute iodoethane[39] for iodomethane, which would give the non-natural *allo*-isoleucine **8** (Figure 1). However, while ethylation to give **9**, DIBAH reduction, and mesylation of the resultant alcohol **10** proceeded well, the zinc-iodide reduction of the mesylate **11** failed, giving mainly the lactone **12** (characterized by ^1H NMR, IR, and HRMS). While one could make a direct steric control argument (ethyl is larger than methyl) for the failure of this reduction, the X-ray crystal structure of **11** (Figure 2) suggests a more subtle explanation: the first step in the reduction is S_N2 mesylate displacement by iodide, but the required trajectory of the nucleophile is blocked by the N-benzyl group. Rotation of the mesyl group into a more accessible position would result in unfavorable steric interactions between the ethyl/mesyl groups and the phenylfluorenyl/benzyl groups.

Figure 2. Stereoview of 11.



While the attempted *allo*-isoleucine synthesis proved unfruitful, we were able to prepare labeled isoleucine **16** from **3** via a sequence that included simple O-tosyl substitution with dimethylcuprate.[40] Initial experiments showed that the O-tosyl group of **13**[41] was more prone to displacement than the O-mesyl group of **4**, since the former underwent reaction with dimethylcuprate at 0 °C but the latter was unreactive under the same conditions. Thus, treatment of **13** with 3 molar equivalents of dimethylcuprate (0 °C – rt) gave the isoleucine derivative **14** in excellent yield. Deprotection (H_2/Pd-C, 6 M HCl, 60 °C) then provided the regioselectively labeled [$^{13}CH_3$]-isoleucine hydrochloride **16**, in 9 steps and 62% overall yield from aspartic acid.

Scheme 2



The structure proof of both **7** and **16** was accomplished by comparison of their spectral data (^{1}H NMR, ^{13}C NMR, optical rotation, IR and HRMS) with that of authentic (unlabeled) samples. The 500 MHz proton NMR spectra of **7** and **16** in D_2O were similar to that of authentic valine and isoleucine, respectively, except that the isopropyl methyl region (ca. 1.0 ppm) exhibited the expected ^{13}C coupling. The 500 MHz ^{13}C NMR spectra of **7** and **16** were identical to authentic samples except for the enhanced ^{13}C resonances indicated in the experimental section. The optical rotations of the final products **7** and **16** were also consistent with those of authentic L-valine and L-isoleucine, respectively. Since each of the characterized products are crystalline solids, the synthesis is amenable to scale-up and chromatography is only necessary for improved yields.

Experimental Procedures

General. ^{1}H spectra were obtained on an Omega 500 (500 MHz) or a General Electric GN-500 (500 MHz) spectrometer. For spectra measured in organic solvents, data are reported in ppm from internal tetramethylsilane. For ^{1}H spectra taken in D_2O, data are reported in ppm relative to HDO (3.80 ppm). Data are reported as follows: chemical shift, multiplicity (app = apparent, par obsc = partially obscured, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant, and integration. For ^{13}C NMR spectra taken in D_2O, data are reported relative to internal 3-trimethylsilylpropionic acid sodium salt (0.00 ppm) under basic conditions (pH=16.0). Infrared (IR) spectra were taken with a Perkin-Elmer Model 1600 series FTIR spectrophotometer. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Melting points (mp) were obtained from a Laboratory Devices Mel-Temp melting-point apparatus and are reported uncorrected. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Dry tetrahydrofuran (THF) was distilled first from calcium hydride, and then from potassium. Carbon-13 iodomethane was purchased from Sigma-Aldrich, and LHMDS (1.0 M in THF) was purchased from Aldrich Chemical Company. Copper iodide was purified as described in the literature.[40] All inert atmosphere operations were conducted under nitrogen passed through a Drierite drying tube in oven or flame-dried glassware. Thin-layer chromatography (TLC) was performed on 0.25 mm Merck precoated silica gel plates (60 F-254). Column chromatography was performed with ICN 200-400 mesh silica gel.

L-(3R)-N-Benzyl-N-(9-phenylfluoren-9-yl)-[3-$^{13}CH_3$] methyl dicarbomethoxy aspartate (2). To 27.3 g (55.6 mmol) of **1** in 165 mL of THF at −78 °C was added dropwise over 10 min 111 mL (111 mmol) of a 1.0 M solution of LHMDS in THF. After stirring for 10 min, 9.94 g (69.6 mmol) of [^{13}C]-methyl iodide was added dropwise. After storing at −78 °C for 20 h, the reaction was quenched with 100 mL of satd aq NH_4Cl, and extracted 2X with ether. Combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated to give a white foam that provided colorless prisms (27.6 g, 98%) in two crops from ether / hexanes: mp 153-154.5 °C; IR (thin film) 3058, 2946, 1728 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.61 (dd, J = 7.0, 129.3 Hz, 3H), 2.56 (m, 1H), 2.91 (s, 3H), 3.54 (s, 3H), 3.78 (dd, J = 1.7, 11.7 Hz, 1H), 4.35 (d, J = 14.2 Hz, 1H), 4.68 (d, J = 14.2 Hz, 1H), 7.15 - 7.99 (m, 18H); HRMS *m/e* calcd for ($^{13}CC_{32}H_{32}NO_4^+$): 507.2367, found 507.2342. Analysis calcd for $^{13}CC_{31}H_{32}NO_4$: C, 78.44; H, 6.17; N, 2.77; found: C, 78.62; H, 6.09; N, 2.67.

L-(3R)-N-Benzyl-N-(9-phenylfluoren-9-yl)-[3-$^{13}CH_3$]-4-hydroxy valine (3). To 2.77 g (5.47 mmol) of **2** in 27 mL of THF at –23 °C was added dropwise over 10 min 16.4 mL (16.4 mmol) of a 1.0 M solution of DIBAH in toluene (Aldrich). After storing at –20 °C for 24 h, 5 mL of methanol was added and 5 min later the mixture was warmed to rt and partitioned 2X between 100 mL of 1.0 M H_3PO_4 and 100 mL of ether. Combined ether portions were washed with brine, dried ($MgSO_4$), and evaporated to give a white foam that provided 1.95 g (71%) of **3** from ether / hexanes. Chromatography of the mother liquor supplied an additional 650 mg (99% total yield): mp 129-130 °C; IR (thin film) 3422br, 2948, 1726, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.34 (dd, J = 6.6, 126.2 Hz, 3H), 1.11 (m, 1H), 1.38 (br s, 1H), 2.94 (s, 3H), 3.03 (d, J = 11.2 Hz, 1H), 3.32 (m, 1H), 3.85 (m, 1H), 4.38 (d, J = 13.7 Hz, 1H), 4.69 (d, J = 13.7 Hz, 1H), 7.21-7.79 (m, 18H); HRMS *m/e* calcd for $^{13}CC_{31}H_{32}NO_3^+$: 479.2509, found 479.2441. Analysis calcd for $^{13}CC_{31}H_{31}NO_3$: C, 80.52; H, 6.53; N, 2.93; found: C, 80.51; H, 6.52; N, 2.78.

L-(3R)-N-Benzyl-N-(9-phenylfluoren-9-yl)-[4-^{13}C]-valine (5). A 1 L Morton flask equipped with a mechanical stirrer was charged with 13.2 g (20.9 mmol) of **4**, 210 mL glyme, 15.7 g (105 mmol) of sodium iodide, and 68.3 g (104 mmol) of zinc powder. The mixture was stirred at reflux for 24 h, then filtered through celite. After rinsing the filter cake with copious EtOAc, the filtrate was concentrated to provide a white solid that was partitioned 2X between 100 mL each of ether and water. Combined organic portions were washed with brine, dried over K_2CO_3, and concentrated to give a white foam. The foam was dissolved in 30 mL of methylene chloride, and 40 mL of methanol was added to initiate the formation of colorless prisms (7.1 g, 74%). Chromatography of the mother liquor gave an additional 1.5 g (89% total yield) of **5**: mp 100-101.5 °C; IR (thin film) 2949, 1726, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.29 (dd, J = 6.5, 125.3 Hz, 3H), 0.87 (dd, J = 6.0, 6.0 Hz, 3H), 1.32 (m, 1H), 2.84 (d, J = 11.1 Hz, 1H), 2.92 (s, 3H), 4.35 (d, J = 13.8 Hz, 1H), 4.58 (d, J = 13.8 Hz, 1H), 7.2 - 7.8 (m, 18H); HRMS *m/e* calcd for $^{13}CC_{32}H_{32}NO_2^+$: 463.2467, found 463.2422.

L-(3S)-[4-^{13}C]-Valine methyl ester hydrochloride (6). To 8.42 g (18.2 mmol) of **5** in 36 mL of methylene chloride was added 24 mL of methanol, 3.0 mL of concd HCl, and 4.0 g of 10% palladium on carbon. The mixture was hydrogenated at 52 psi for 20 h, filtered through celite and concd to give a white solid residue. After washing twice with ether, the aqueous layer was basified to a pH of ca 10 with satd aq Na_2CO_3 and extracted 4X with ethyl acetate. Combined extracts were dried ($MgSO_4$), filtered and evaporated to give a colorless oil that was dissolved in dry ether and precipitated by the dropwise addition of a satd solution of HCl gas in dry ether to give 2.7 g (87%) of a white solid. IR (thin film) 3406br, 2958, 2632, 1737 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.16 (dd, J = 7.0, 126.8 Hz, 3H), 1.14 (dd, J = 5.1, 6.9 Hz, 3H), 2.48 (m, 1H), 3.83 (s, 3H), 3.95 (dd, J = 4.0, 4.0 Hz, 1H), 8.88 (br s, 3H); HRMS *m/e* calcd for $^{13}CC_5H_{15}NO_2$ (M - Cl^-): 133.1058, found 133.1056.

L-(3S)-[4-^{13}C]-Valine hydrochloride (7). 2.7 g (16.0 mmol) of **6** was dissolved in 200 mL of 6 M HCl and heated at 60 °C for 12 h. The solvent was removed in vacuo at 40 °C to provide 2.7 g of an off-white solid. Crystallization from EtOAc / methanol supplied 2.27 g (92%) of **7** in three crops: mp 223–224 °C; $[\alpha]_D^{25}$ = +26.4° (*c* 3.05, 6 M HCl);[42] ^1H NMR (500 MHz, D_2O) δ 0.91-1.18 (dd, J = 7.0, 126.6 Hz, 3H), 1.02 (dd, J = 1.1, 5.5 Hz, 3H), 2.34 (m, 1H), 3.93 (app t, J = 3.7 Hz, 1H); ^{13}C NMR (500 MHz, D_2O, pH~14)[43] δ 19.5, **21.8**, 34.5, 64.6, 186.0; HRMS *m/e* calcd for $^{13}CC_4H_{12}NO_2$ (M - Cl^-): 119.0902, found 119.0902.

L- (3R)- N- Benzyl- N- (9-phenylfluoren-9-yl)- [3-$^{13}CH_3$]- 4-hydroxy valine- O- p- toluene sulphonate (13). To a stirred solution of 2.20 g (4.60 mmol) of **3** in 9.2 mL of freshly distilled $CHCl_3$ at 0 °C was added 2.20 mL (17.6 mmol) of dry pyridine, followed by the dropwise addition of 2.50 g (18.4 mmol) of p-toluene sulphonylchloride as a 1 M solution in $CHCl_3$. The reaction was warmed to rt and stirred until complete by TLC (ca. 6 h). After partitioning between 20 mL of ether and 5 mL of water, the organic portion was washed successively with 2M HCl, 5% $NaHCO_3$ and brine, dried over magnesium sulfate and concentrated. Column chromatography (10% EtOAc in hexanes) afforded 2.74 g (94%) of a pale yellow foam that yielded colorless crystals from trace EtOAc in hexanes: mp 135-140 °C dec; $[\alpha]_D^{26}$ = –285° (*c* 2.00, $CHCl_3$); IR (thin film) 3060, 2947 br, 1727, 1361, 1178, 969, 741 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.25 (dd, J = 6.6, 127.2 Hz, 3 H), 1.58 (m, 1H), 2.49 (s, 3H), 2.90 (s, 3H), 3.09 (d, J = 11.4 Hz, 1H), 3.89 (m, 1H), 4.05 (m, 1H), 4.33 (d, J = 13.9 Hz, 1H), 4.55 (d, J = 13.9 Hz, 1H), 7.21-7.81 (m, 22 H); HRMS *m/e* calcd for $^{13}CC_{38}H_{37}NO_5S^+$: 633.2513, found 633.2402.

N-benzyl-N-(9-phenylfluoren-9-yl)-[3-$^{13}CH_3$]-isoleucine methyl ester (14). To a stirred mixture of 940 mg (4.94 mmol) of purified CuI[40] in 16.5 mL of dry ether at 0 °C was added dropwise over 15 min 6.02 mL (9.87 mmol) of a 1.64 M solution of CH_3Li (LiBr complex) in ether. To the resulting clear solution of $LiCu(Me)_2$ was added dropwise a solution of 1.04 g (1.65 mmol) of **13** in 25 mL of dry ether. The mixture was allowed to warm to rt, and after stirring for 18 h was quenched with 50 mL of saturated aqueous NH_4Cl. After stirring rapidly for 15 min, the phases were separated and the aqueous phase was extracted with two fifty mL portions of ether. Combined organic portions were washed with brine, dried over anhydrous magnesium sulfate and evaporated to give a yellow foam. The foam was purified by column chromatography (10% EtOAc in hexanes), affording 0.762 g (97%) of **14** : mp 137-138 °C; $[\alpha]^{25}_D = -340°$ (*c* 0.885, $CHCl_3$); IR (thin film) 3061, 2960, 2872, 1726, 1450, 1139, 910, 738 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.25 (dd, J = 127.8, 6.6 Hz, 3H), 0.37 (t, J = 7.3 Hz, 3H), 0.62 (m, 1H), 1.07 (m, 1H), 2.28 (m, 1H), 2.90 (s, 3H), 2.93 (obsc d, 1H), 4.35 (d, J = 13.7 Hz, 1H), 4.64 (d, J = 13.7 Hz, 1 H), 7.34 - 7.46 (m, 33 H) HRMS *m/e* calcd for $^{13}CC_{32}H_{34}NO_2^+$: 477.2625, found 477.2581.

L-[3-^{13}CH$_3$]-isoleucine methyl ester hydrochloride (15). To 931 mg (1.96 mmol) of **14** in 3.9 mL of methylene chloride was added 7.8 mL of methanol, 0.30 mL (3.9 mmol) of TFA, and 470 mg of 10% palladium on carbon. After hydrogenating at 52 psi for 7.5 h, the mixture was filtered through celite and concentrated. To the white solid residue was added 5 mL of methanol and 3 mL of a concentrated solution of dry HCl gas in anhydrous ether. Solvent and TFA removal under vacuum gave the crude product as an off-white foam. Column chromatography (5% MeOH in CH_2Cl_2) afforded 351 mg (98%) of **15**: mp 97-98° (C); IR (thin film) 3405, 2959, 2349, 1742, 1622, 1519, 1447, 1237 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, J = 7.5 Hz, 3 H), 1.11 (dd, J = 7.0, 127.6 Hz, 3 H), 1.45 (m, 1H), 1.55 (m, 1H), 2.21 (m, 1H), 3.82 (s, 3H), 4.05 (app s, 1H), 8.82 (br s, 3H); HRMS *m/e* calcd for $^{13}CC_5H_{14}NO_2$ (M - Cl$^-$): 133.1059, found 133.1056.

L-3-[^{13}CH$_3$]-isoleucine hydrochloride (16). 240 mg (1.32 mmol) of **15** in 17 mL of 6 M HCl was stirred at 60 °C for 12 h. The solvent was removed in vacuo at 40 °C to provide 222 mg of an off-white solid. Crystallization from ethyl acetate / methanol provided 221 mg (93%) in three crops. Crystals obtained from the first crop (189 mg, 85%) gave the following physical and spectral data: mp 171-172 °C; $[\alpha]^{25}_D = +39°$ (*c* 1.60, 6 M HCl)[42]; IR (KBr) 3422, 2966, 2356, 1736, 1517, 1206 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 0.96 (t, J = 7.5 Hz, 3 H), 1.04 (dd, J = 7.1 126.7 Hz, 3 H), 1.35 (m, 1H), 1.51 (m, 1H), 2.08 (m, 1H), 4.05 (dd, J = 4.2, 4.2 Hz, 3H); ^{13}C NMR (500 MHz, D_2O, pH ~14)[43] δ 13.8, 18.3, 26.8, 41.4, 63.8, 186.0; HRMS *m/e* calcd for $^{13}CC_4H_{12}NO_2$ (M - Cl$^-$): 133.1059, found 133.1056.

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32. A similar ester-alcohol-hydrocarbon reduction sequence (diborane reduction / conversion to iodide / hydrogenolysis) was reported by Kluender et. al in 1973 (see reference 2).
33. (a) Kluender et al. have prepared (2S, 3R)-[4-^{13}C] valine from dimethyl acetylenedicarboxylate in a sequence involving a chiral resolution with β-methylaspartase: see reference 2. (b) (2S, 3R)-[4-^{13}C] Valine has also been derived from (2S)-[5-^{13}C] leucine isolated from (RS)-[2-$^{13}CH_3$] acetolactate-fed E. coli: see Sylvester et al., reference 3. (c) Aberhart and group have synthesized (2RS, 3S)-[4-^{13}C] valine from trans-2,3-epoxybutan-1-ol by treatment with [^{13}C] iodomethane followed by diol cleavage and a Strecker synthesis: see reference 8. (d) Baldwin et al. describe the preparation of (2RS, 3R)-[4-^{13}C] valine in a number of steps from trans-(1S, 2S)-[1-$^{13}CH_3$]-2-cyclopropanecarboxylic acid: see reference 4. It should also be noted that the stereochemical assignments of the stereogenic center at position 3 within most of these publications are incorrect according to Cahn-Ingold-Prelog convention. The correct assignments are given in this footnote. See also *J. Am. Chem. Soc.* **1973** *95*, 6511-6512 (additions and corrections).
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36. The mesylate co-spots with the starting alcohol by TLC, so the reaction must be monitored by NMR. The reaction should be complete within 2 h, and purification beyond workup is not necessary.
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43. The sample pH was raised with NaOH to prevent solubility problems. The ^{13}C-enhanced carbon resonance is given in bold print.

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