Pergamon

Tetrahedron Letters 40 (1999) 8369–8373

TETRAHEDRON LETTERS

Synthesis of [3,5,5,5-$^2H_4$]-2-*C*-methyl-D-erythritol, a substrate designed for the elucidation of the mevalonate independent route for isoprenoid biosynthesis

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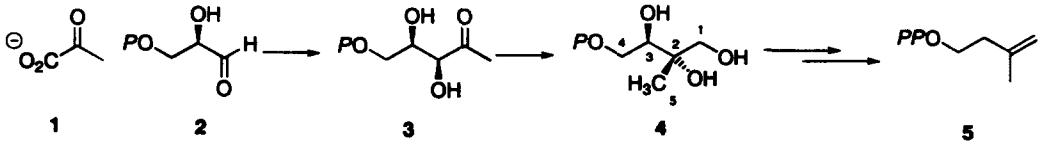
Received 31 August 1999; accepted 9 September 1999

Abstract

2-*C*-Methyl-D-erythritol 4-phosphate is a key intermediate in the mevalonate independent pathway for isoprenoid biosynthesis. In order to investigate the fate of the protons in this metabolic route, [3,5,5,5-$^2H_4$]-2-*C*-methyl-D-erythritol was synthesized. The relevant steps allowing deuterium introduction were a palladium(II)-catalyzed hydrostannation and a coupling reaction between a vinyl iodide and a methylcyanocuprate.

Keywords: biosynthesis; deuterium labeling; 2-*C*-methyl-D-erythritol; isoprenoids.

An alternative mevalonate-independent route to isopentenyl diphosphate (IPP) (**5**), the universal precursor of isoprenoids, was found in eubacteria, algae and plants (Scheme 1).[1] The first two steps yielding 1-deoxy-D-xylulose 5-phosphate (DXP) (**3**) from pyruvate (**1**) and from D-glyceraldehyde 3-phosphate (**2**) and 2-*C*-methyl-D-erythritol 4-phosphate (MEP) (**4**) from DXP are well documented.[2,3] Few data, however, are available on the following steps involved in the conversion of MEP into IPP. Detailed information on the origin of the hydrogen atoms found in isoprenic units would give useful clues for the full elucidation of this metabolic route. Valuable data have already been obtained from incubations with deuterium labeled deoxyxylulose[4] or glucose.[5] *Escherichia coli* mutants with disrupted DXP synthase and/or DXP isomero-reductase genes, accordingly requiring 2-*C*-methyl-D-erythritol



Scheme 1. The 2-*C*-methyl-D-erythritol 4-phosphate pathway (MEP) pathway for isoprenoid biosynthesis

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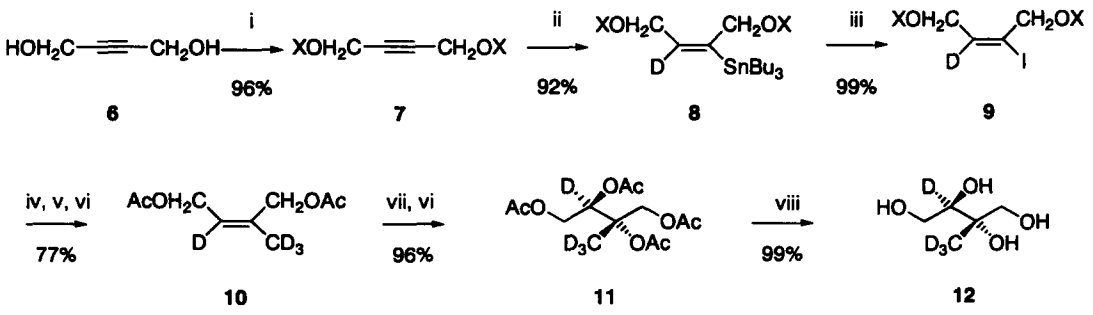
0040-4039/99/$ - see front matter
PII: S0040-4039(99)01769-4

(ME) for growth, are now available.[6] They represent essential tools for further feeding experiments with deuterium labeled ME isotopomers. Indeed, *E. coli* is the only known organism capable of incorporating free ME into isoprenoids.[7] A synthesis of ME with deuterium labeling at C-3 and/or C-5 is described in this contribution.

The methodology formerly developed for the synthesis of [1,1-$^2H_2$]- and [1,1,4,4-$^2H_4$]ME does not allow access to [3,5,5,5-$^2H_4$]ME (**12**),[7] and a different approach had to be found. All reactions were first performed with unlabeled reagents, and later with deuterium labeled compounds. Only the synthesis of the deuterium labeled ME isotopomer (**12**) is described (Scheme 2).



Scheme 2. Synthesis of [3,5,5,5-$^2H_4$]-2-*C*-methyl-D-erythritol (**12**), (X=TBDPSi-). Reagents: (i) TBDPSiCl, DMAP, NEt_3, CH_2Cl_2; (ii) Bu_3SnD, $PdCl_2(PPh_3)_2$, THF; (iii) I_2, Et_2O; (iv) $(CD_3)_2CuLi \cdot LiCN$, Et_2O, −70°C; (v) Bu_4NF, THF; (vi) Ac_2O, DMAP, NEt_3, CH_2Cl_2; (vii) AD-mix β, H_2O/*t*-BuOH, −15°C to 4°C; (viii) Amberlyst A-26 (OH^-), MeOH

Butyne-1,4-diol (**6**) was protected with a *t*-butyldiphenylsilyl group to give the bisilyl ether (**7**).[8] A palladium(II)-catalyzed hydrostannation of the protected symmetric acetylenic derivative afforded the vinylstannane (**8**).[9] The deuterium atom on the carbon atom, which corresponds to C-3 of methylerythritol, was introduced at this stage using deuterated tributylstannane. This reaction is a *syn* addition, leading to the vinylstannane (**8**) with an *E* configuration with up to 98% selectivity. The latter compound (**8**) was treated with an iodine solution in ether to give, in a quantitative yield, the vinyl iodide (**9**).[10] The deuterium labeled methyl group was introduced by exposing the vinyl iodide (**9**) to cyanocuprate coupling conditions. The crude methylated product was deprotected with tetrabutylammonium fluoride and acetylated to yield the diacetate (**10**) with the required *Z* configuration.[11] Attempts to introduce the trideuterated methyl group by a palladium catalyzed Grignard coupling[12] surprisingly failed and left the starting material unchanged when a deuterium was present on the double bond. In contrast, this method afforded with the non-deuterated vinyl iodide the expected olefin with a satisfactory yield (82%). Enantioselective Sharpless dihydroxylation[13] of (**10**) followed by acetylation afforded the ME tetraacetate (**11**) with an 80% enantiomeric excess, identical with that obtained for the unlabeled compound.[7] Basic Amberlyst A-26 (OH^-) in methanol removed the acetate groups in an almost quantitative yield to directly give [3,5,5,5-$^2H_4$]ME (**12**) (Scheme 2).[14,15]

Acknowledgements

We wish to thank Dr. R. Graf and Mr. J.-D. Sauer for the NMR analyses. This work was supported by a grant to M.R. from the 'Institut Universitaire de France'. L.C. was a recipient of a grant from the 'Ministère de l'Education Nationale et de la Recherche Scientifique'.

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15. **Experimental data.** Unless otherwise indicated, all reactions were carried out under argon and at room temperature. THF and ether were dried and freshly distilled from sodium/benzophenone. Dichloromethane was freshly distilled over CaH_2. Flash chromatography was conducted on Merck silica gel (40–63 μm). Thin-layer plates were developed by heating at 100°C after spraying with an ethanol solution of *p*-anisaldehyde (2.5%), H_2SO_4 (3.5%), AcOH (1.6%). ^1H and ^{13}C NMR spectra were recorded on a Bruker AC200 spectrometer. Chemical shifts were reported in ppm with reference to chloroform (7.26 ppm) for ^1H NMR and deuteriochloroform (77.03 ppm) for ^{13}C NMR, or methanol (3.30 ppm) for ^1H NMR and perdeuteriomethanol (49.00 ppm) for ^{13}C NMR. ^2H NMR spectra were recorded in $CHCl_3$ solution at 300 K on a Bruker AV400 spectrometer equipped with a Silicon Graphics station using the following conditions: 30° pulse, 4 sec repetition time, 20.03 ppm spectra width, 0.15 Hz fid resolution, digital acquisition mode, ^1H decoupling by WALTZ 16 during acquisition and relaxation.

Bis-1,4-*t*-butyldiphenylsiloxybut-2-yne (7). A solution of butyne-1,4-diol (**6**) (2 g, 23.3 mmol), triethylamine (6.5 ml, 48.8 mmol, 2.1 equiv.), 4-dimethylaminopyridine (0.14 g, 1.10 mmol, 0.05 equiv.) in CH_2Cl_2 (30 ml) was stirred for 5 min. *t*-Butyldiphenylchlorosilane (12.5 ml, 48.8 mmol, 2.1 equiv.) was added to the mixture and the reaction was followed by TLC. As soon as the starting material had disappeared, the reaction was quenched with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, and the solvent was evaporated in vacuo. Flash chromatography on silica gel (hexane:ethyl acetate, 95:5) afforded the bis-silylether (**7**) (12.0 g, 92%). ^1H NMR ($CDCl_3$): δ (ppm)=1.06 (18H, s), 4.32 (4H, s, 1-H and 4-H), 7.39 (12H, m), 7.73 (8H, m). ^{13}C NMR ($CDCl_3$): δ (ppm)=19.21 (quaternary C), 26.76 (CH_3), 52.80 (CH_2), 83.42 (acetylenic C), 127.72, 129.77, 132.56, 133.20 and 135.64 (aromatic C).

[3-^{2}H]-Bis-1,4-*t*-butyldiphenylsiloxy-2-(tributyltin)-but-2-ene (8). To a solution of (**7**) (6.42 g, 11.4 mmol) in THF (30 ml) containing $PdCl_2(PPh_3)_2$ (0.160 g, 0.23 mmol, 0.02 equiv.), tributyltin deuteride was added dropwise with a syringe during 1 min. After the addition of tributyltin deuteride (3.70 ml, 13.7 mmol, 1.2 equiv.), the initially light yellow solution abruptly turned orange-brown. After 10 min, the THF was evaporated in vacuo. The oily residue was purified by flash

chromatography (hexane:ethyl acetate, 98:2) and gave (**8**) (8.98 g, 92%). The reported $J_{Sn,H}$ values are approximate mean values of $J(^{117}Sn,H)$ and $J(^{119}Sn,H)$. *Non-labeled compound:* ^1H NMR ($CDCl_3$): δ (ppm)=0.88 (9H, t, *J*=7,1 Hz), 0.98 (9H, s), 0.99 (9H, s), 1.16–1.51 (12H, m), 4.05 (2H, dt, $J_{1,4}$=1.0 Hz, $J_{3,4}$=2.0 Hz, 4-H), 4.17 (2H, dt, $J_{1,4}$=1.0 Hz, $J_{1,3}$=5.4 Hz, and broad d, $^3J_{Sn,H}$=35 Hz, 1-H), 5.71 (1H, tt, $J_{3,4}$=2.1 Hz, $J_{1,3}$=5.4 Hz, and broad d, $^3J_{Sn,H}$=70 Hz, 3-H), 7.33 (12H, m), 7.64 (8H, m); ^{13}C NMR ($CDCl_3$): δ (ppm)=10.20 (CH_2), 13.77 (CH_3), 19.17 (2×quaternary C), 26.82 (CH_3), 26.94 (CH_3), 27.47 (CH_2), 29.27 (CH_2), 61.66 (CH_2, s+d, $^2J_{Sn,C}$=61 Hz, C-1), 65.53 (CH_2, s+d, $^3J_{Sn,C}$=21 Hz, C-4), 127.60, 129.51, 133.43, 133.88 and 135.55 (aromatic C), 138.51 (CH, C-3), 145.90 (quaternary C, C-2). *Labeled compound:* ^1H NMR ($CDCl_3$): δ (ppm)=0.88 (9H, t, *J*=7.1 Hz), 0.98 (9H, s), 1.00 (9H, s), 1.22–1.52 (12H, m), 4.06 (2H, s+d, $^4J_{Sn,H}$=11 Hz, H-4), 4.18 (2H, s+d, $^3J_{Sn,H}$=37 Hz, H-1), 7.33 (12H, m), 7.57 (8H, m). ^2H NMR ($CDCl_3$): δ (ppm)=5.91 (1D, 3-D); ^{13}C NMR ($CDCl_3$): δ (ppm)/^2H induced shift (ppb)=10.26 (CH_2), 13.80 (CH_3), 19.07 (quaternary C), 19.17 (quaternary C), 26.84 (CH_3), 26.97 (CH_3), 27.53 (CH_2), 29.33 (CH_2), 61.62 (CH_2, s+d, $^2J_{Sn,C}$=61 Hz, γ-shift: −35 ppb, C-1), 65.59 (CH_2, s+d, $^3J_{Sn,C}$=21 Hz, β-shift: −57 ppb, C-4), 127.64, 129.54, 133.47, 133.94 and 135.61 (aromatic C), 138.17 (CD, α-shift: −346 ppb, t, $J_{C,D}$=23.1 Hz, C-3), 145.80 (quaternary C, β-shift: −90 ppb, C-2).

[3-^{2}H]-Bis-1,4-*t*-butyldiphenylsiloxy-2-iodo-but-2-ene (9). To a solution of (**8**) (8.98 g, 10.5 mmol) in ether (50 ml) was added dropwise a solution of iodine (2.66 g, 10.5 mmol, 1 equiv.) in ether (20 ml) at room temperature. The reaction was monitored by TLC until the starting material had disappeared. The reaction mixture was evaporated to dryness. Flash chromatography (hexane:ethyl acetate, 98:2) afforded (**9**) in a nearly quantitative yield. *Non-labeled compound:* ^1H NMR ($CDCl_3$): δ (ppm)=0.98 (9H, s), 1.00 (9H, s), 3.97 (2H, d, $J_{1,3}$=6.3 Hz, 1-H), 4.00 (2H, s, 4-H), 6.42 (1H, t, $J_{3,1}$=6.2 Hz, 3-H), 7.33 (12H, m), 7.57 (8H, m); ^{13}C NMR ($CDCl_3$): δ (ppm)=19.14 (quaternary C), 19.24 (quaternary C), 26.75 (2×CH_3), 61.92 (CH_2, C-4), 66.15 (CH_2, C-1), 103.55 (quaternary C, C-2), 127.72, 129.76, 133.01, 133.20 and 135.60 (aromatic C), 141.34 (CH, C-3). *Labeled compound:* ^1H NMR ($CDCl_3$): δ (ppm)=0.98 (9H, s), 1.00 (9H, s), 3.97 (2H, s, 1-H), 4.00 (2H, s, 4-H), 7.34 (12H, m), 7.58 (8H, m); ^2H NMR ($CHCl_3$): δ (ppm)=6.76 (1D, 3-D); ^{13}C NMR ($CDCl_3$): δ (ppm)/^2H induced shift (ppb)=19.11 (quaternary C), 19.21 (quaternary C), 26.75 (CH_3), 26.94 (CH_3), 61.82 (CH_2, β-shift: −98 ppb, C-4), 66.12 (CH_2, γ-shift: −33 ppb, C-1), 103.42 (quaternary C, β-shift: −131 ppb, C-2), 127.71, 129.74, 132.99, 133.21 and 135.51 (aromatic C), 140.92 (CD, α-shift: −417 ppb, t, $J_{C,D}$=25 Hz, C-3).

[2,2,2,3-$^2H_4$]-Bis-1,4-acetoxy-but-2-ene (10). Deuterium labeled lithium dimethyl copper(I) (12.5 mmol) was prepared from copper(I) cyanide (1.12 g, 12.5 mmol, 5.0 equiv.) and [1,1,1-$^2H_3$]methyl lithium (25 mmol, 0.5 M in ether) at −60°C in ether (50 ml) for 30 min and at −10°C for 10 min. To this vigorously stirred cuprate suspension was carefully added (**9**) (1.74 g, 2.49 mmol) in ether (10 ml) at −78°C. The reaction mixture was stirred at −70°C for 1 h, warmed up gradually to room temperature and stirred for additional 2 h. The reaction mixture was poured into a saturated aqueous NH_4Cl solution and the product was extracted with ethyl acetate. The combined organic layers were washed with water and brine and dried (Na_2SO_4). Solvents were evaporated in vacuo. The crude product was dissolved in THF, and tetrabutylammonium fluoride (1.73 g, 5.48 mmol, 2.2 equiv.) was added. After 10 min, the THF was removed in vacuo. The crude product was acetylated with acetic anhydride (0.53 g, 5.23 mmol, 0.5 ml, 2.1 equiv.), triethylamine (0.76 ml, 5.48 mmol, 2.2 equiv.), and 4-dimethylaminopyridine (0.01 g, 0.12 mmol, 0.05 equiv.) in CH_2Cl_2 (50 ml) for 1 h at room temperature. The reaction was quenched with an aqueous solution of NH_4Cl, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. Flash chromatography (hexane:ethyl acetate, 85:15) afforded (**10**) (0.37 g, 77% for the three steps). *Non-labeled compound:* ^1H NMR ($CDCl_3$): δ (ppm)=1.81 (3H, d, $J_{3,5}$=0.9 Hz, 5-H), 2.04 and 2.05 (6H, s, 2×CH_3CO), 4.62 (2H, s, 1-H), 4.63 (2H, d, $J_{4,3}$=7.0 Hz, 4-H), 5.55 (1H, dt, $J_{3,4}$=7.0 Hz and $J_{3,5}$=0.9 Hz, 3-H); ^{13}C NMR ($CDCl_3$): δ (ppm)=20.81 (CH_3CO), 20.94 (CH_3CO), 21.43 (CH_3), 60.31 (CH_2, C-1), 62.67 (CH_2, C-4), 123.94 (CH, C-3), 136.26 (quaternary C, C-2), 170.81 (2×CO). *Labeled compound:* ^1H NMR ($CDCl_3$): δ (ppm)=2.05 (3H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 4.62 (2H, s, 1-H), 4.64 (2H, s, 4-H); ^2H NMR ($CHCl_3$): δ (ppm)=1.28 (3D, 5-D), 5.09 (1D, 3-D); ^{13}C NMR ($CDCl_3$): δ (ppm)/^2H induced shift (ppb)=20.75 (CH_3CO), 20.81 (CH_3CO), 60.15 (CH_2, γ-shifts: −164 ppb, C-1), 62.57 (CH_2, β-shift: −98 ppb, C-4), 123.54 (CD, α- and γ-shifts: −385 ppb, t, $J_{C,D}$=23.7 Hz, C-3), 136.00 (quaternary C, β-shifts: −262 ppb, C-2), 170.68 (2×CO).

[3,5,5,5-$^2H_4$]-2-*C*-methyl-D-erythritol tetraacetate (11). A 50 ml round-bottomed flask equipped with a magnetic stirrer was charged with *t*-butylalcohol (5 ml), H_2O (5 ml) and AD-mix β (3.32 g, 1.6 g/mmol). After stirring at room temperature two clear phases were produced: the lower aqueous phase appeared bright yellow. Methanesulfonamide (0.198 g, 2.07 mmol, 1 equiv.) was added at this stage. The mixture was cooled to 0°C. [2,2,2,3-$^2H_4$]-Bis-1,4-acetoxy-but-2-ene (**10**) (0.37 g, 2.07 mmol) was added, and the heterogeneous slurry was vigorously stirred at −15°C. The reaction was started at −15°C and the temperature was raised to 4°C for 12 h. Solid sodium sulfite (3.5 g) was added under stirring at 0°C, and the mixture was allowed to warm up to room temperature and stirred for an additional 60 min. The reaction product was extracted with ethyl acetate (5×15 ml). The combined organic layers were washed with 2N KOH (10 ml), dried over anhydrous Na_2SO_4 and concentrated. The crude product was acetylated using acetic anhydride (0.46 g, 4.55 mmol,

0.43 ml, 2.1 equiv.), triethylamine (0.73 ml, 5.17 mmol, 2.5 equiv.) and 4-dimethylaminopyridine (0.01 g, 0.10 mmol, 0.05 equiv.) in refluxing CH_2Cl_2 (10 ml) overnight. Flash chromatography (hexane:ethyl acetate, 45:55) afforded the ME tetraacetate (**11**) (0.61 g, 96% for the two steps). *Non-labeled compound:* ^{1}H NMR ($CDCl_3$): δ (ppm)=1.54 (3H, s, CH_3, 5-H), 2.04 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 4.14 (1H, dd, $J_{4a,4b}$=11.9 Hz, $J_{3,4a}$=8.1 Hz, 4-H_a), 4.30 (1H, d, $J_{1a,1b}$=12.1 Hz, 1-H_a), 4.48 (1H, dd, $J_{4a,4b}$=11.9 Hz, $J_{3,4b}$=2.7 Hz, 4-H_b), 4.55 (1H, d, $J_{1a,1b}$=12 Hz, 1-H_b), 5.48 (1H, dd, $J_{3,4a}$=8.1 Hz, $J_{3,4b}$=2.7 Hz, 3-H); ^{13}C NMR ($CDCl_3$): δ (ppm)=17.39 (CH_3, C-5), 20.76 (3×CH_3), 21.99 (CH_3), 62.47 (CH_2, C-4), 63.36 (CH_2, C-1), 70.50 (CH, C-3), 81.16 (quaternary C, C-2), 169.62 (2×CO), 170.37 (CO), 170.71 (CO). *Labeled compound:* ^{1}H NMR ($CDCl_3$): δ (ppm)=2.04 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 4.14 (1H, d, $J_{4a,4b}$=11.8 Hz, 4-H_a), 4.30 (1H, d, $J_{1a,1b}$=12.2 Hz, 1-H_a), 4.48 (1H, d, $J_{4a,4b}$=11.8 Hz, 4-H_b), 4.55 (1H, d, $J_{1a,1b}$=12.2 Hz, 1-H_b); ^{2}H NMR ($CHCl_3$): δ (ppm)=1.53 (3D, 5-D), 5.50 (1D, 3-D); ^{13}C NMR ($CDCl_3$): δ (ppm)/^{2}H induced shift (ppb)=20.71 (3×CH_3CO), 21.93 (CH_3CO), 62.34 (CH_2, β-shift: −131 ppb, C-4), 63.29 (CH_2, γ-shifts: −65 ppb, C-1), 70.14 (CD, α- and γ-shifts: −361 ppb, t, J_{C-D}=23 Hz, C-3), 80.90 (quaternary C, β-shifts: −261 ppb, C-2), 169.53 (2×CO), 170.35 (CO), 170.65 (CO).

[3,5,5,5-$^2H_4$]-2-*C*-methyl-D-erythritol (12). A solution of (**11**) (0.61 g, 1.99 mmol) in methanol (10 ml) was stirred overnight at room temperature in the presence of Amberlyst A-26 (OH^-) (0.5 g).[14] The reaction mixture was filtered through Celite. After evaporation of the solvent [3,5,5,5-$^2H_4$]-2-*C*-methyl-D-erythritol (**12**) was obtained in quantitative yield. *Unlabeled compound:* ^{1}H NMR (CD_3OD): δ (ppm)=1.10 (3H, s, 5-H), 3.42 (1H, d, $J_{1a,1b}$=11.1 Hz, 1-H_a), 3.53 (1H, d, $J_{1a,1b}$=10.82 Hz, 1-H_b), 3.57 (1H, dd, $J_{4a,4b}$=16.3 Hz, $J_{3,4a}$=8.0 Hz 4-H_a), 3.59 (1H, t, $J_{3,4a}$=8.0 Hz, $J_{3,4b}$=8.5 Hz, 3-H) 3.80 (1H, dd, $J_{4a,4b}$=16.3 Hz, $J_{3,4b}$=8.5 Hz, 4-H_b). ^{13}C NMR (CD_3OD): δ (ppm)=19.72 (CH_3, C-5), 63.78 (CH_2, C-4), 68.41 (CH_2, C-1), 74.96 (quaternary C, C-2), 76.13 (CH, C-3). *Labeled compound:* ^{1}H NMR (CD_3OD): δ (ppm)=3.42 (1H, d, $J_{1a,1b}$=11.1 Hz, 1-H_a), 3.52 (1H, d, $J_{1a,1b}$=11.1 Hz, 1-H_b), 3.56 (1H, d, $J_{4a,4b}$=11.3 Hz, 4-H_a), 3.79 (1H, d, $J_{4a,4b}$=11.3 Hz, 4-H_b); ^{2}H NMR (CH_3OH): δ (ppm)=1.02 (3D, 5-D), 3.54 (1D, 3-D); ^{13}C NMR (CD_3OD): δ (ppm)=63.75 (CH_2, β shifts: −30 ppb, C-4), 68.40 (CH_2, γ shifts: −10 ppb, C-1), 74.76 (quaternary C, β shifts: −200 ppb, C-2), 75.65 (CD, α shift: −480 ppb, t, J_{C-D}=23 Hz, C-3).