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Research Article

Synthesis of d_8-geranyl diphosphate

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Summary

Multiply labelled d_8-geranyl diphosphate (3-methyl-7-$^2H_3$-methyl-[1,1,8,8,8]-$^2H_5$-2*E*,6-octadienyl diphosphate) was synthesised from geraniol in 8 steps. Geraniol was converted to [1,1]-$^2H_2$-geraniol by a three step oxidation–reduction sequence in 38% yield. Selective epoxidation of [1,1]-$^2H_2$-geranyl acetate gave 6,7-epoxy-[1,1]-$^2H_2$-geranyl acetate, which, on oxidative cleavage of the epoxide and Wittig elaboration with d_6-isopropyl triphenylphosphorane, gave d_8-geraniol (14% yield) which was, in turn, converted to the title compound.

Key Words: geraniol; geranyl diphosphate; synthesis; deuterium

Introduction

Geraniol (3,7-dimethyl-2*E*,6-octadienol) **1** is a widespread plant volatile and aroma compound which, as its diphosphate, is a biosynthetic precursor for the numerous mono and higher terpenes produced by plants.[1,2] In the course of mechanistic studies in sesquiterpene biosynthesis, we required a doubly labelled form of geranyl diphosphate.

Deuterated precursors and GCMS analysis have been used extensively in the study of terpene biosynthesis[3,4] and several relevant syntheses of deuterium labelled mono-[5], sesqui-[6] and di-[7] terpenes have been reported. The reported syntheses of d_6-geraniol (3-methyl-7-$^2H_3$-methyl-[8,8,8]-$^2H_3$-2*E*,6-octadienol)[8] and d_6-α-farnesene[6] seemed particularly relevant. We report here the synthesis of multiply labelled d_8-geranyl diphosphate (3-methyl-7-$^2H_3$-methyl-[1,1,8,8,8]-$^2H_5$-2*E*,6-octadienyl diphosphate).

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Results and discussion

3,7-Dimethyl-[1,1]-$^2H_2$-2*E*,6-octadienol (d_2-geraniol) **4** was prepared by a three step oxidation-reduction sequence (Figure 1) in 38% overall yield. Oxidation of geraniol with Cr(VI) gave an 82% yield of geranial **2** with c. 21% double bond isomerisation to neral. Oxidation of **2** with MnO_2 via the cyanohydrin[9] gave methyl ester **3** which was reduced with $LiAlD_4$ to give d_2-geraniol **4**. The ^{1}H NMR of **4** showed H-2 as a broad singlet at $\delta = 5.42$ ppm. GCMS analysis gave a molecular ion at $m/z = 156$ with characteristic fragment ions at $m/z =$ 69 (C_5H_9), 95 ($C_7H_7D_2$), 123 (C_9H_{15}) and 138 (M^+-H_2O) confirming the expected pattern of deuterium incorporation (Figure 2). The product contained c. 20% of d_2-nerol, as determined by NMR.

Conversion of d_2-geraniol **4** to d_8-geraniol **8** (14% yield) followed the methods of Fielder and Rowan[6] and of Luan *et al.*[8] Protection of **4** as the *t*-butyldimethylsilyl ether did not provide a derivative suitable for subsequent reactions. Alternatively acetylation of **4** with lipase under anhydrous conditions in vinyl acetate and pentane gave a quantitative yield of d_2-geranyl acetate **5**. Epoxidation of **5** with *m*-chloroperbenzoic acid at 0°C gave selectively the 6,7-epoxide **6** in 70% yield which was cleaved with periodic acid to give aldehyde **7** in 72% yield. Aldehyde **7** showed a characteristic low field aldehydic triplet ($J = 1.6$ Hz) at $\delta = 9.78$ ppm in the ^{1}H NMR spectrum as well as a single allylic methyl group ($\delta = 1.71$ ppm) showing a distinctive long-range coupling ($J = 1.3$ Hz) to the broad singlet of H-2 ($\delta = 5.35$ ppm). Aldehyde **7** readily trimerised on standing and was used immediately in reaction with d_6-isopropyl triphenylphosphorane **9** (prepared from d_7-isopropyl iodide), with *in situ* hydrolysis of the acetate ester to give d_8-geraniol **8** in 29% isolated yield. The ^{1}H NMR showed a single allylic methyl group ($\delta = 1.69$ ppm), again with distinctive long-range coupling ($J = 1.3$ Hz), and two olefinic signals, a broad singlet (H-2) and a triplet (H-6, $J = 6.7$ Hz) at $\delta = 5.43$ and 5.11 ppm, respectively. The ^{13}C NMR showed 4 olefinic and 3 alkyl signals. No additional methyl resonances, or resonances for H-1 and C-1, were observed. GCMS analysis of **8** gave a molecular ion

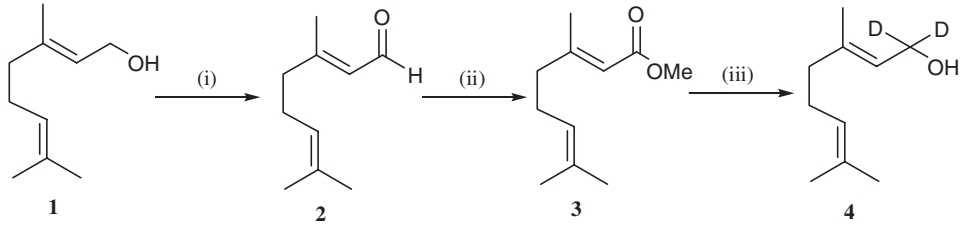

Figure 1. Synthesis of d_2-geraniol 4. Reagents and conditions: (i) Dipyridinium chromium (VI) oxide, DCM. (ii) NaCN, MnO_2, AcOH, MeOH. (iii) $LiAlD_4$, Et_2O, reflux

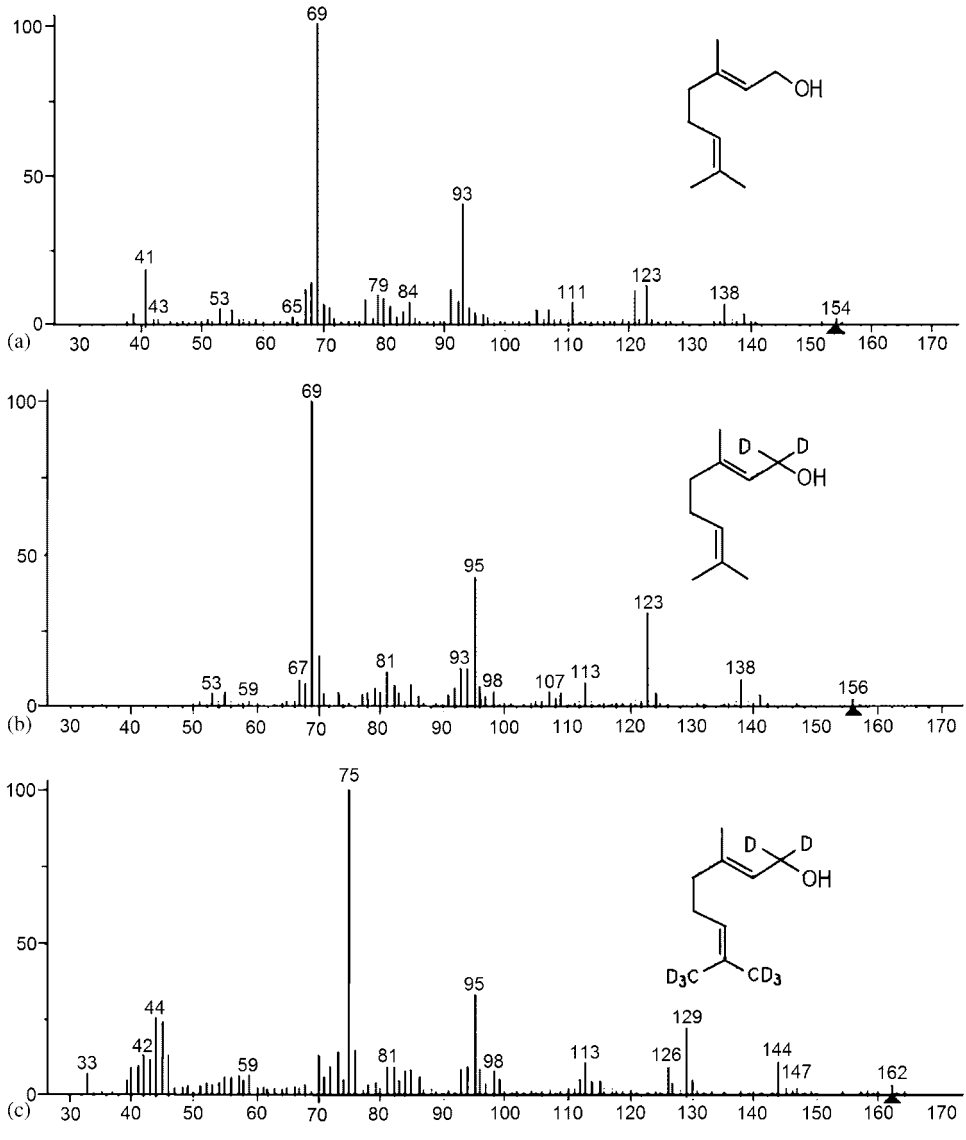

Figure 2a. GCMS EI (70 eV) MS of geraniol (a, from NIST library); d_2-geraniol (b) and d_8-geraniol (c)

$m/z = 162.1853$ ($\Delta = 4.3$ ppm) with characteristic fragment ions at $m/z = 75$ ($C_5H_3D_6$), 95 ($C_7H_7D_2$) and 129 ($C_9H_9D_6$) (Figure 2) confirming the expected pattern of deuterium incorporation. The final product **8** also contained 5% d_8-nerol resulting from double bond isomerisation in the initial step of the synthesis.

d_8-Geraniol **8** was converted to d_8-geranyl diphosphate in 32% yield, following the method of Keller and Thompson.[10] The use of this compound in biosynthetic studies will be reported elsewhere.

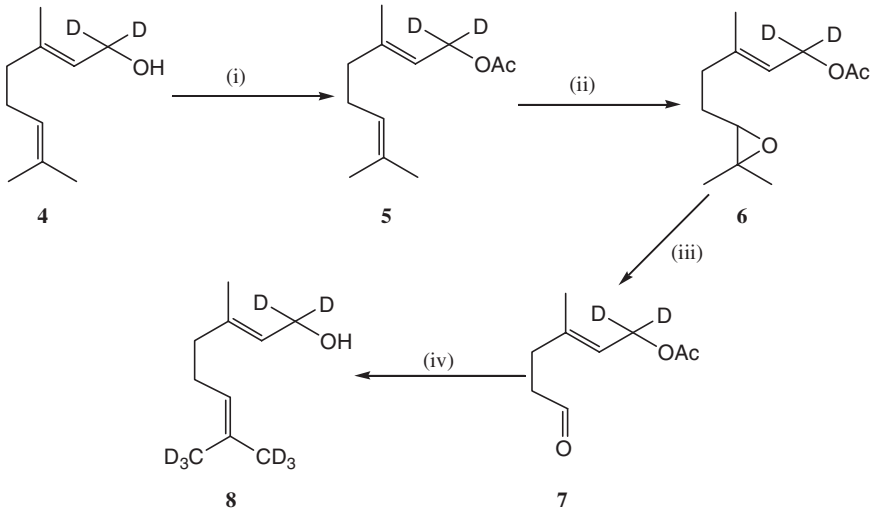

Figure 2b. Synthesis of d_8-geraniol 8. Reagents and conditions: (i) Lipase, vinyl acetate, pentane. (ii) *m*CPBA, DCM, 0°C. (iii) H_5IO_6, THF, Et_2O. (iv) [1,1,1,2,3,3,3-$^2H_7$]-isopropyl triphenylphosphonium iodide, *n*-BuLi, THF, MeOH, 0°C

Experimental

General

All reagents were of commercial grade and were used as received unless otherwise stated. Lipase AK 'amano' 20 was gifted from Amano Enzyme Inc. Nagoya, Japan. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in $CDCl_3$, except for d_8-geranyl diphosphate which was dissolved in D_2O. ^{31}P NMR were recorded with proton and carbon decoupled and the signals referenced to phosphoric acid. GCMS and high resolution MS were recorded on a Waters GCT Time of Flight mass spectrometer at 70 eV ionisation energy.

3,7-Dimethyl-2E,6-octadienal (geranial) **2**. Chromium (VI) oxide (40 g, 400 mmol) was added in one portion to a solution of pyridine (63 g, 796 mmol) in DCM (1 l) at 5°C. This was stirred and allowed to warm to room temperature over one hour. To this solution was added a solution of geraniol (10 g, 64.9 mmol) in DCM (100 ml). After stirring for 30 min the reaction mixture was washed sequentially with 200 ml 5% NaOH, 5% HCl, 5% $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give **2** as a colourless oil (8.13 g, 82%). ^1H NMR: δ (ppm) 9.99 (1H, d, $J = 8.1$ Hz), 5.88 (1H, dq, $J = 8.1, 1.3$ Hz), 5.08 (1H, m),

2.23 (4H, m), 2.16 (3H, d, $J = 1.3$ Hz), 1.68 (3H, s), 1.61 (3H, s). ^{13}C NMR: δ (ppm) 191.3, 163.90, 132.9, 127.4, 122.5, 40.6, 25.7, 25.7, 17.7, 17.6. MS *m/z* (relative intensity %) 152.1223 (15, M^+, $C_{10}H_{16}O$ requires 152.1201), 137 (22), 123 (18), 109 (23), 94 (33), 84 (55), 69 (78), 41 (100).

Methyl 3,7-dimethyl-2E,6-octadienoate **3**. Sodium cyanide (9.52 g, 194 mmol) followed by manganese dioxide (50 g, 575 mmol) and acetic acid (3.4 ml, 3.5 g, 58.8 mmol) was added to a solution of **2** (5.6 g, 36.8 mmol) in MeOH (500 ml) at room temperature. After stirring for 20 hours, the reaction mixture was filtered through celite, concentrated *in vacuo*, then taken up in 100 ml water and extracted twice with 200 ml diethyl ether. The organic phase was dried over $MgSO_4$ and the crude product purified by flash chromatography (petroleum ether/diethylether 9:1) to give **3** as a colourless oil (3.7 g, 55%). ^{1}H NMR: δ (ppm) 5.68 (1H, br s), 5.09 (1H, m), 3.69 (3H, s), 2.16 (7H, m), 1.69 (3H, s), 1.61 (3H, s). ^{13}C NMR: δ (ppm) 167.3, 160.2, 132.5, 122.9, 115.8, 50.8, 40.9, 25.7, 18.8, 17.7. MS *m/e* (relative intensity %) 182.1327 (3, M^+, $C_{11}H_{18}O_2$ requires 182.1307), 151 (11), 139 (13), 123 (47), 114 (48), 83 (25), 69 (100), 41 (41).

3,7-Dimethyl-[2,2]-$^2H_3$-2E,6-octadienol (d_2-geraniol) **4**. A solution of **3** (3.7 g, 20.3 mmol) in diethyl ether (20 ml) was added to a stirred slurry of lithium aluminium deuteride (1.1 g, 25.4 mmol) in diethyl ether (60 ml), at a rate sufficient to maintain reflux. The reaction mixture was stirred for a further hour before dropwise quenching with 15% NaOH and further dilution with water. This was then extracted twice with 50 ml diethyl ether, dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (petroleum ether/ethyl acetate 9:1) to give **4** as a colourless oil (2.7 g, 17.3 mmol, 85%). ^{1}H NMR: δ (ppm) 5.42 (1H, br s), 5.10 (1H, m), 2.08 (4H, m) 1.69 (3H, s), 1.68 (3H, s), 1.61 (3H, s). ^{13}C NMR: δ (ppm) 139.9, 131.8, 123.9, 123.2, 39.6, 26.4, 25.7, 17.7, 16.3. MS *m/e* (relative intensity %), 156.1513 (4, M^+, $C_{10}H_{16}D_2O$ requires 156.1483), 138 (17), 123 (49), 113 (13), 95 (100), 81 (30), 69 (48), 53 (16), 41 (92).

3,7-Dimethyl-[2,2]-$^2H_3$-2E,6-octadienyl acetate (d_2-geranyl acetate) **5**. Lipase AK 'Amano' 20 Lot No. LAKY0950502 (5 g), followed by vinyl acetate (6.4 ml, 69.2 mmol), was added to a solution of **4** (2.7 g, 17.3 mmol) in pentane (300 ml) at room temperature. The reaction mixture was stirred for 2 hours before removal of the enzyme by filtration. Concentration followed by flash chromatography (petroleum ether/diethyl ether 9:1) gave **5** as a colourless oil (3.24 g, 95%). ^{1}H NMR: δ (ppm) 5.34 (1H, br s), 5.09 (1H, m), 2.10 (2H, m), 2.06 (3H, s), 2.05 (2H, m), 1.71 (3H, d, $J = 1.3$ Hz), 1.69 (3H, s), 1.61 (3H, s). ^{13}C NMR: δ (ppm) 171.6, 132.2, 124.1, 123.9, 118.5, 39.9, 32.6, 26.7, 26.1,

21.5, 18.1, 16.9. MS *m/e* (relative intensity %) 156.1491 (4, M^+-CH_2CO, $C_{10}H_{16}D_2O$ requires 156.1483), 138 (42), 123 (47), 109 (10), 95 (100), 81 (26), 69 (99), 43 (26), 41 (31).

6,7-Epoxy-3,7-dimethyl-[2,2]-$^2H_3$-2E,6-octadienyl acetate (d_2-6,7-epoxygeranyl acetate) **6**. *m*-Chloroperoxybenzoic acid (*m*CPBA) (3.2 g, 18.2 mmol) in DCM (35 ml) was added over 10 min to a solution of **5** (3.2 g, 16.2 mmol) in DCM (60 ml) cooled to 0°C. After stirring for an hour at 0°C the reaction mixture was treated with portions of *m*CPBA until the starting material disappeared as indicated by thin-layer chromatography. The reaction mixture was filtered and the filtrate washed sequentially with 50 ml saturated $NaHCO_3$ solution, water and brine and then dried over $MgSO_4$. The solution was concentrated *in vacuo* and the crude product purified by flash chromatography (petroleum ether/ethyl acetate 17:3) to give **6** as a colourless oil (2.42 g, 70%). ^{1}H NMR: δ (ppm) 5.37 (1H, br s), 2.71 (1H, t, $J = 6.3$ Hz), 2.20 (2H, m), 2.05 (3H, d, $J = 0.5$ Hz), 1.72 (3H, d, $J = 1.5$ Hz), 1.66 (2H, m), 1.30 (3H, s), 1.26 (3H, s). ^{13}C NMR: δ (ppm) 171.5, 141.8, 119.1, 64.4, 58.9, 36.6, 27.4, 25.2, 21.4, 19.1, 16.8. MS *m/e* (relative intensity %) 154.1336 (15, M^+-CH_3COOH, $C_{10}H_{14}D_2O$ requires 154.1327), 139 (12), 127 (8), 111 (34), 96 (30), 85 (94), 83 (83), 71 (78), 59 (46), 43 (100).

6-Acetoxy-4-methyl-[2,2]-$^2H_3$-4-hexenal **7**. A solution of periodic acid (2.8 g, 12.4 mmol) in THF (40 ml) was slowly added to a vigorously stirred solution of **6** (2.4 g, 11.3 mmol) in diethyl ether (40 ml) at room temperature. After an hour the reaction mixture was quenched with 40 ml ice water. The organic layer was separated and the aqueous layer extracted with 50 ml diethyl ether. The combined organic fractions were washed sequentially with 50 ml sat. $NaHCO_3$ solution, water and brine then dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (petroleum ether/diethyl ether 7:3) to give **7** as a yellow oil (1.4 g, 72%), ^{1}H NMR: δ (ppm) 9.78 (1H, t, $J = 1.6$ Hz), 5.35 (1H, br s), 2.58 (2H, m), 2.38 (2H, t, $J = 7.6$ Hz), 2.05 (3H, s), 1.71 (3H, d, $J = 1.3$ Hz). ^{13}C NMR: δ (ppm) 202.1, 171.5, 140.6, 119.5, 42.1, 31.8, 21.5, 17.0. MS *m/e* (relative intensity %) 127.0745 (40, M^+-CH_3CDO, $C_7H_9DO_2$ requires 127.0744), 112 (25), 110 (28), 97 (15), 85 (96), 83 (68), 69 (36), 55 (18), 43 (100).

[1,1,1,2,3,3.3-$^2H_7$]-Isopropyl triphenylphosphonium iodide **9**. A mixture of triphenylphosphine (0.77 g, 2.94 mmol) and 2-iodo-[1,1,1.2,3,3,3-$^2H_7$]-propane (0.5 g, 2.82 mmol) was heated at 140°C for 3.5 hours in a thick walled tube sealed with a screw cap. The crude product was recrystallised from ethyl acetate/ethanol 2:1 to give **9** as white crystals (925 mg, 75%). m.p. 194.5–198°C (lit.[11] 194–197°C for the unlabelled compound).

3-Methyl-7-[$^2H_3$]-methyl-[2,2,8,8.8-$^2H_5$]-2E,6-octadienol (d_8-geraniol) **8**. *n*-Butyl lithium (1.6 M in hexanes, 0.66 ml, 1.05 mmol) was added dropwise to a solution of **9** (460 mg, 1.05 mmol) in THF (5 ml) cooled to 0°C. The reaction mixture was stirred for 30 min at 0°C before the addition of **7** (180 mg, 1.05 mmol) in THF (5 ml). This was stirred for a further hour at 0°C before quenching with 5 ml MeOH. The reaction mixture was concentrated *in vacuo*, taken up in 10 ml water, extracted with petroleum ether (4×20 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate 4:1) to give **8** as a colourless oil (49 mg, 29%). ^{1}H NMR: δ (ppm) 5.43 (1H, br s), 5.11 (1H, t, $J = 6.7$ Hz), 2.08 (4H, m), 1.69 (3H, d, $J = 1.3$ Hz). ^{13}C NMR: δ (ppm) 140.3, 132.0, 124.3, 123.6, 39.9, 26.7, 16.7. MS *m*/*e* (relative intensity) 162.1853 (1, M^+, $C_{10}H_{10}D_8O$ requires 162.1860), 144 (6), 129 (11), 126 (8), 113(6), 95 (47), 81 (15), 75 (100), 55 (12), 44 (59).

3-Methyl-7-[$^2H_3$]-methyl-[2,2,8,8,8-$^2H_5$]-2E,6-octadienyl diphosphate (d_8-geranyl diphosphate). d_8-Geraniol **8** (42 mg, 0.26 mmol) was taken up in trichloroacetonitrile (1.14 ml) in a 30 ml polypropylene Oak Ridge centrifuge tube with a magnetic stirrer bar, and heated to 37°C. Concentrated phosphoric acid (2.5 ml) was added to CH_3CN (9.5 ml) to produce solution A. Et_3N (11 ml) was added to CH_3CN (10 ml) to produce solution B. Solution A (9.1 ml) was added, dropwise, to solution B (15 ml) to create the bis-triethylammonium phosphate (TEAP) solution, and immediately thereafter TEAP (0.76 ml) solution was added to the d_8-geraniol solution. Two more additions of TEAP solution were made at 5 min intervals. The reaction mixture was stored at −18°C overnight. d_8-Geranyl diphosphate was separated from the mono and triphosphate by flash chromatography on silica using *i*-PrOH/conc NH_3/H_2O, 6:2.5:0.5. Fractions (15 ml) were collected after elution of a yellow pigment. d_8-Geranyl diphosphate was identified by TLC (*i*-PrOH/conc NH_3/H_2O, 6:3:2) against an authentic sample of geranyl diphosphate and was visualised using vanillin. The eluent volume was reduced to 20 ml *in vacuo* at 30°C, before freeze drying to yield the title compound as a white solid (31.5 mg, 0.08 mmol, 32%). ^{1}H NMR: δ (ppm) 5.32 (1H, br s), 5.08 (1H, t, $J = 6.8$ Hz), 2.00 (4H, m), 1.59 (3H, s). ^{31}P NMR: δ (ppm) −9.29 (d, $J = 53$ Hz), −9.94 (d, $J = 51$ Hz).

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