**Gene cloning, expression and protein purification of DpTPS9**

Cloning of the cDNA for DpTPS9 into an expression vector was described previously.[1] A preculture of *Escherichia coli* BL21 harbouring the expression plasmid for DpTPS9 (1 mL) was used to inoculate expression cultures in LB medium (1 L) containing ampicillin (100 mg $mL^{-1}$) that were grown with shaking until the $OD_{600}$ reached 0.4 – 0.6. The cultures were cooled to 18 °C and IPTG solution (1 mL, 400 mM) was added to induce protein expression. The cultures were grown at 18 °C with shaking overnight and then cells were harvested by centrifugation (14.000 x g, 15 min). The pelleted cells were resuspended in binding buffer (10 mL $L^{-1}$ of expression culture; 20 mM $Na_2HPO_4$, 500 mM NaCl, 20 mM imidazole and 1 mM $MgCl_2$, pH = 7.4 ) and lysed by ultra-sonication (5 x 30 s). The cell debris was removed by centrifugation (14600 g, 7 min, 4 °C) and the soluble protein fraction was loaded onto $Ni^{2+}$-NTA affinity chromatography column (Protino Ni-NTA, Macherey-Nagel, Düren, Germany). The column was washed with binding buffer (20 mL $L^{-1}$) and the desired $His_6$-tagged protein was eluted by elution buffer (10 mL $L^{-1}$; 20 mM $Na_2HPO_4$, 500 mM NaCl, 500 mM imidazole and 1 mM $MgCl_2$, pH = 7.4). The enzyme concentration was determined by Bradford assay[2] and was ca. 6 mg/mL.

**Preparative scale incubation of FPP with DpTPS9**

FPP (trisammonium salt, 150 mg) was dissolved in substrate buffer (20 mL; 25 mM $NH_4HCO_3$) and the solution was added dropwise within 2 h to a slowly stirred mixture of incubation buffer (300 mL; 50 mM Tris, 10 mM $MgCl_2$ and 20% glycerol, pH = 8.2), water (150 mL) and DpTPS9 elution fraction (150 mL) obtained from 12 L *E. coli* expression culture. The reaction mixture was stirred overnight at 28 °C and then extracted with pentane (3x 100 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography on silica gel followed by HPLC purification using a Chiralpak IA column and elution with hexane/iPrOH (98:2) yielded (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**; 0.3 mg).

**NMR spectroscopy**

NMR data were recorded on a Bruker (Billerica, MA, USA) Avance III HD Cryo NMR spectrometer (700 MHz). Spectra were measured in $C_6D_6$ and referenced against solvent signals ($^1$H-NMR, residual proton signal: $\delta$ = 7.16 ppm; $^{13}$C-NMR: $\delta$ = 128.06 ppm).[3]

**GC/MS analysis**

GC/MS analyses were performed on an Agilent (Santa Clara, CA, USA) 7890B GC connected to a 5977A mass detector. A HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 $\mu$m film) was used for gas chromatographic separation. GC parameters were 1) inlet pressure: 77.1 kPa, He at 23.3 mL $min^{-1}$, 2) injection volume: 1 µL, 3) temperature program: 5 min at 50 °C, then increasing at 5 °C $min^{-1}$ to 320 °C, 4) 60 s valve time, and 5) carrier gas: He at 1.2 mL $min^{-1}$. MS parameters were 1) source: 230 °C, 2) transfer line: 250 °C, 3) quadrupole: 150 °C and 4) electron energy: 70 eV.

**GC analyses on a chiral stationary phase**

Enantioselective GC analyses of the side product **2** from DpTPS9 and the essential oil from *Solidago canadensis* known to contain (–)-**2** and (+)-**2** in a ca. 3:1 ratio[4] were performed on an Agilent 7820A GC system (Agilent, Santa Clara, CA, USA) equipped with an FID detector and an Agilent Cyclosil-B capillary column (30 m, 0.25 mm i. d., 0.25 $\mu$m film). The temperature program was: 5 min at 70 °C, increasing at 2 °C $min^{-1}$ to 170 °C, followed by increasing at 10 °C $min^{-1}$ to 200 °C. Inlet temperature was 250 °C, inject volume was 1 µL, the carrier gas was $H_2$ at 2.3 mL $min^{-1}$.

**Isotopic labelling experiments**

Isotopic labelling experiments were performed with the substrates and enzymes as listed in Table S1. Reaction mixtures contained substrates (0.5 mg each) in aqueous $NH_4HCO_3$

solution (0.1 mL, 25 mM), enzyme elution fractions (0.2 mL each) and incubation buffer (0.5 – 0.9 mL to a total volume of 1.2 mL). After incubation with shaking at 28 °C overnight, the reaction mixtures were extracted with $C_6D_6$ (0.4 mL). The extracts were dried with $MgSO_4$ and directly analysed by NMR and GC/MS.

**Table S1.** Isotopic labelling experiments with DpTPS9.

| No. | substrates | enzymes | results shown in |
|---|---|---|---|
| 1 | DMAPP + (*E*)-(4-$^{13}$C, 4-$^{2}$H)IPP[5] | FPPS[6] + DpTPS9 | Figure S9 |
| 2 | DMAPP + (*Z*)-(4-$^{13}$C, 4-$^{2}$H)IPP[5] | FPPS + DpTPS9 | Figure S9 |
| 3 | (*R*)-(1-$^{13}$C,1-$^{2}$H)IPP[7] | IDI[8] + FPPS + DpTPS9 | Figures S10, S12, S13 |
| 4 | (*S*)-(1-$^{13}$C,1-$^{2}$H)IPP[7] | IDI + FPPS + DpTPS9 | Figures S10, S12, S13 |
| 5 | (3-$^{13}$C,1,1-$^{2}H_2$)FPP[6] | DpTPS9 | Figures S14, S15 |

**Site-directed mutagenesis of DpTPS9**

The QuickChange site-directed mutagenesis kit (Agilent) was used to generate mutants according to the manufacturer's protocol. The initial plasmid template was a pET32a vector (Agilent) containing a wildtype full-length cDNA of *DpTPS9*. After the generation of single mutants, the single mutant plasmids were used as the plasmid template for the production of double mutants. The triple mutant was generated using one of the double mutant as the plasmid template. Target-specific primers are listed in Table S2. All variants were fully sequenced to verify the target mutation(s).

**Table S2.** Primers used for site-direct mutagenesis.

| Primer name | Target | Nucleotide sequence (5' → 3') |
|---|---|---|
| DpTPS9C60PF | C60P | GTTGTTGTGCATATTTCTGGCCTAAATGTTCAAGATCCGAAATG |
| DpTPS9C60PR | C60P | CATTTCGGATCTTGAACATTTAGGCCAGAAATATGCACAACAAC |
| DpTPS9M67LF | M67L | GTAAATGTTCAAGATCCGAACTGAAATTAATTGGCCATCTAATG |
| DpTPS9M67LR | M67L | CATTAGATGGCCAATTAATTTCAGTTCGGATCTTGAACATTTAC |
| DpTPS9F78VF | F78V | CCATCTAATGTTATGGACAGTTTTATTAGATGATATTTTAG |
| DpTPS9F78VR | F78V | CTAAAATATCATCTAATAAAACTGTCCATAACATTAGATGG |
| DpTPS9F78IF | F78I | CCATCTAATGTTATGGACAATTTTATTAGATGATATTTTAG |
| DpTPS9F78IR | F78I | CTAAAATATCATCTAATAAAATTGTCCATAACATTAGATGG |
| JR102f_Dp9_N239D | N239D | GGTTTCATCGATTCACTATTAATTTTAC |
| JR102r_Dp9_N239D | N239D | GTAAAATTAATAGTGAATCGATGAAACC |
| JR103f_Dp9_N239A | N239A | GTTTCATCGCTTCACTATTAATTTTAC |
| JR103r_Dp9_N239A | N239A | GTAAAATTAATAGTGAAGCGATGAAAC |

**Gene expression and protein purification of DpTPS9 enzyme variants**

*E. coli* BL21 was transformed with the expression plasmids for each enzyme variant by electroporation. Cells were plated on LB agar plates containing ampicillin (100 mg $mL^{-1}$) and incubated at 37 °C overnight. A single colony was selected from the plate and used to inoculate LB liquid medium (10 mL) followed by incubation at 37 °C overnight. These precultures (0.1 mL) were used to inoculate expression cultures (100 mL) that were grown until the $OD_{600}$ reached 0.4 – 0.6. The cultures were cooled to 18 °C and IPTG solution (0.1 mL, 400 mM) was added to induce protein expression. The cultures were grown overnight and then cells were harvested by centrifugation (14000 g, 15 min, 4 °C). The pelleted cells were resuspended in binding buffer (1 mL; 20 mM $Na_2HPO_4$, 500 mM NaCl, 20 mM imidazole and 1 mM $MgCl_2$, pH = 7.4 ) and lysed by ultra-sonication (5 x 30 s) on ice. The supernatant was collected by centrifugation (14000 g, 15 min, 4 °C) and loaded onto a $Ni^{2+}$ NTA affinity chromatography column (Protino Ni-NTA, Macherey-Nagel, Düren, Germany). The column was washed with binding buffer (2 mL) and the desired $His_6$-tagged protein was eluted by elution buffer (1 mL; 20 mM $Na_2HPO_4$, 500 mM NaCl, 500 mM imidazole and 1 mM $MgCl_2$, pH = 7.4). Enzyme concentrations for the soluble enzymes (DdTPS9 wildtype and the enzyme variants C60P, M67L, C60P-M67L, F78V, F78I, C60P-F78V, M67L-F78V, and C60P-M67L-F78V) were determined by Bradford assay[2] and were ca. 6 mg/mL in all cases. Only for the enzyme variants N239A and N239D no soluble protein was obtained.

**Activity assay of DpTPS9 and its mutants**

For activity testings of DpTPS9 and its mutants enzymatic conversions of FPP were performed in a total volume of 1 mL, containing 0.5 mg FPP dissolved in 0.1 mL aqueous $NH_4HCO_3$ solution (25 mM), enzyme elution fraction in elution buffer (0.3 mL), binding buffer (0.1 mL) and incubation buffer (0.5 mL). After incubation with shaking at 28 °C overnight, the reaction mixtures were extracted with hexane (0.2 mL). The extracts were dried with $MgSO_4$ and analysed by GC/MS. Each enzymatic experiment was conducted in triplicates. The enzyme activity was assessed by peak integration of the product **1**.

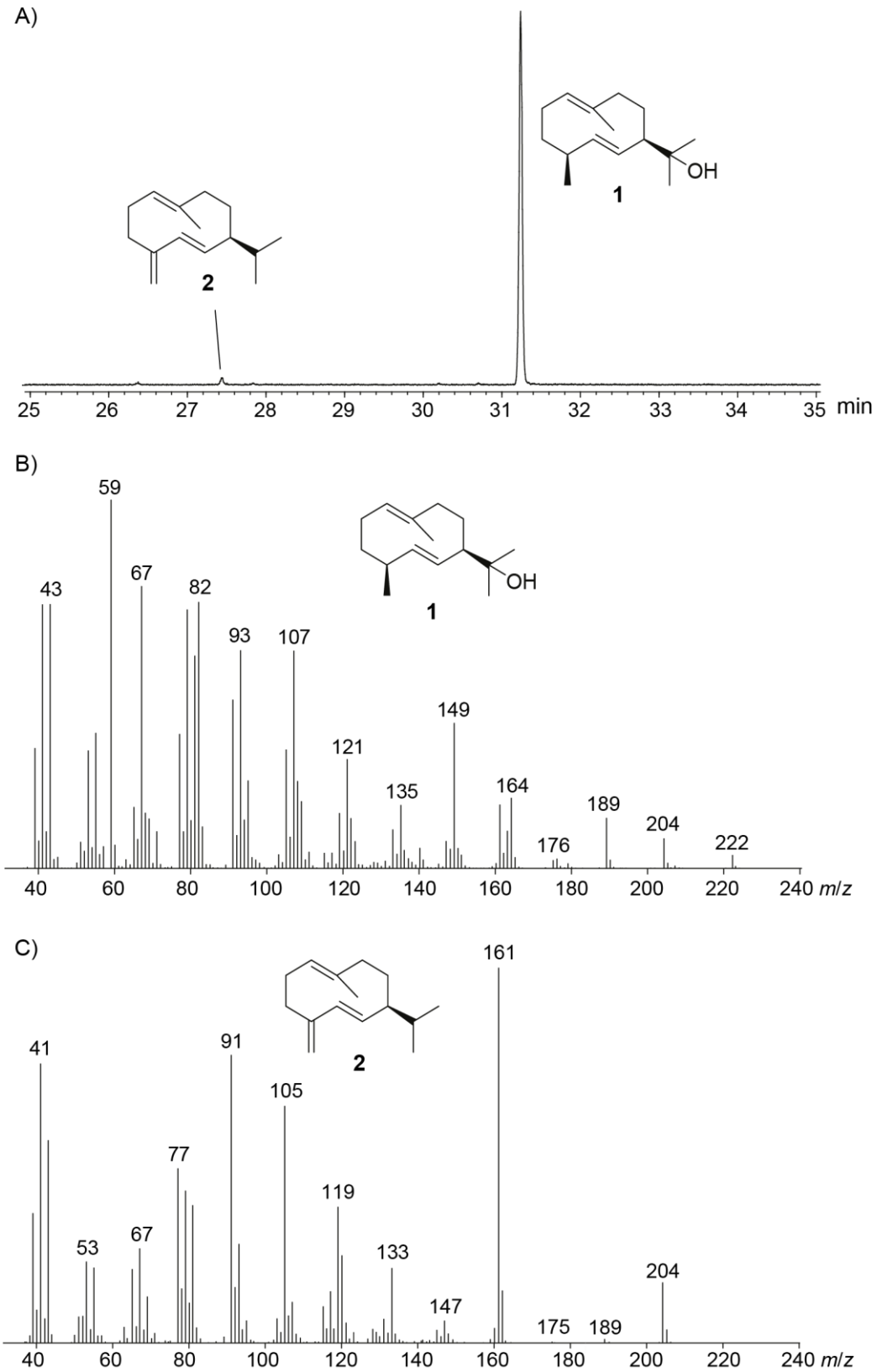


**Figure S1.** Products of DpTPS9 from FPP.

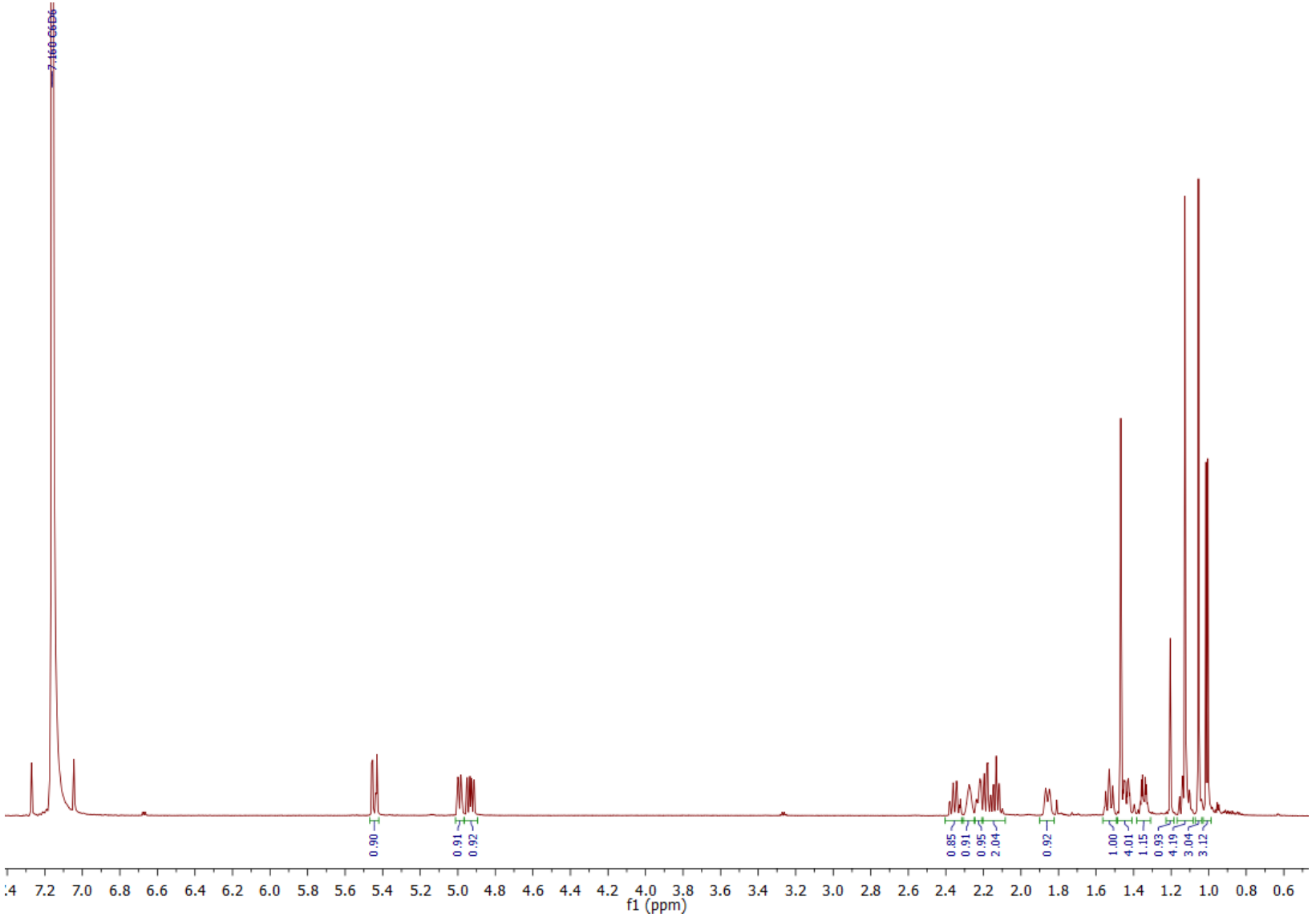


**Figure S2.** $^{1}$H-NMR spectrum (700 MHz, $C_6D_6$) of (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**).

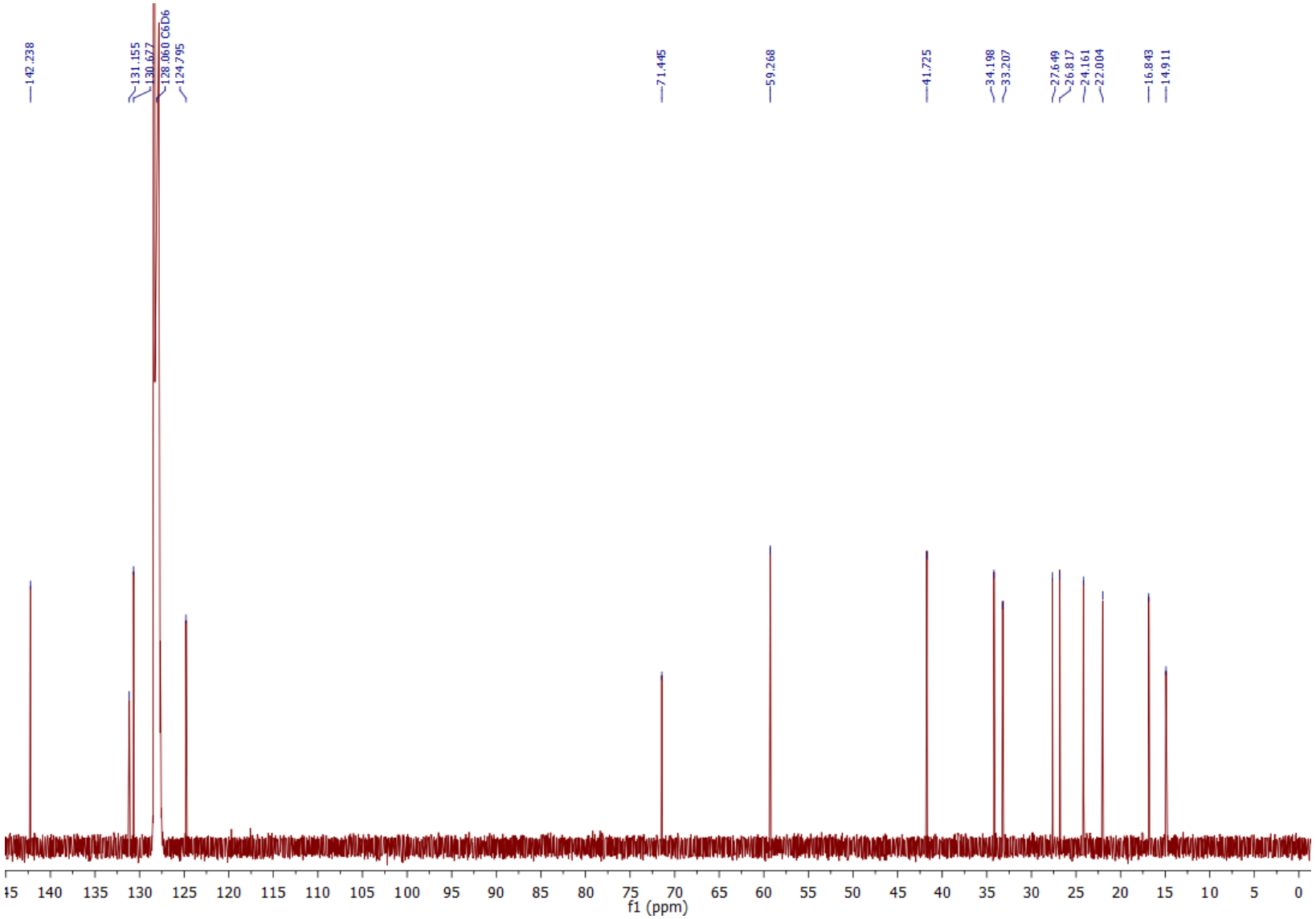


**Figure S3.** $^{13}$C-NMR spectrum (175 MHz, $C_6D_6$) of (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**).

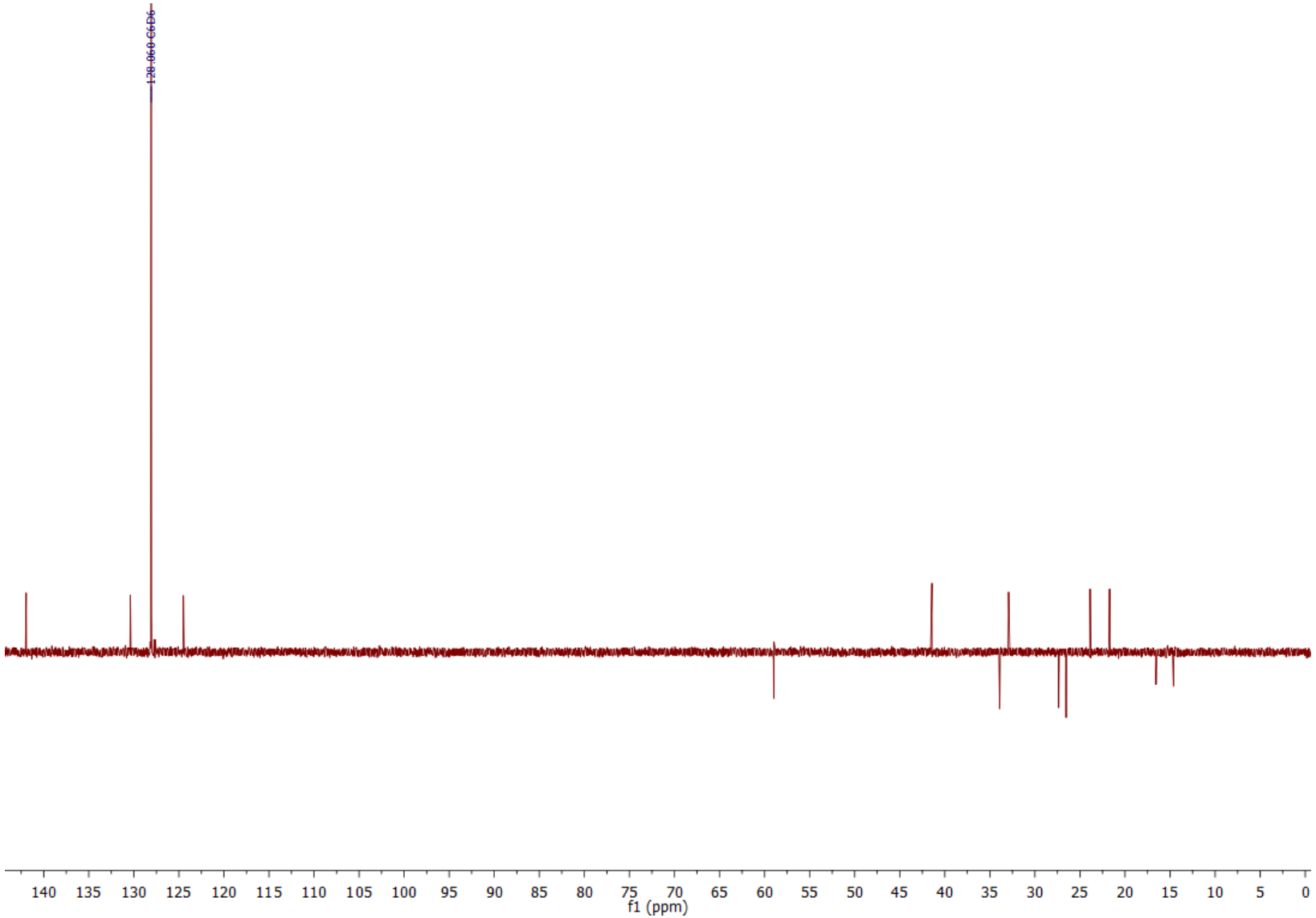


**Figure S4.** $^{13}$C-DEPT-135 spectrum (175 MHz, $C_6D_6$) of (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**).

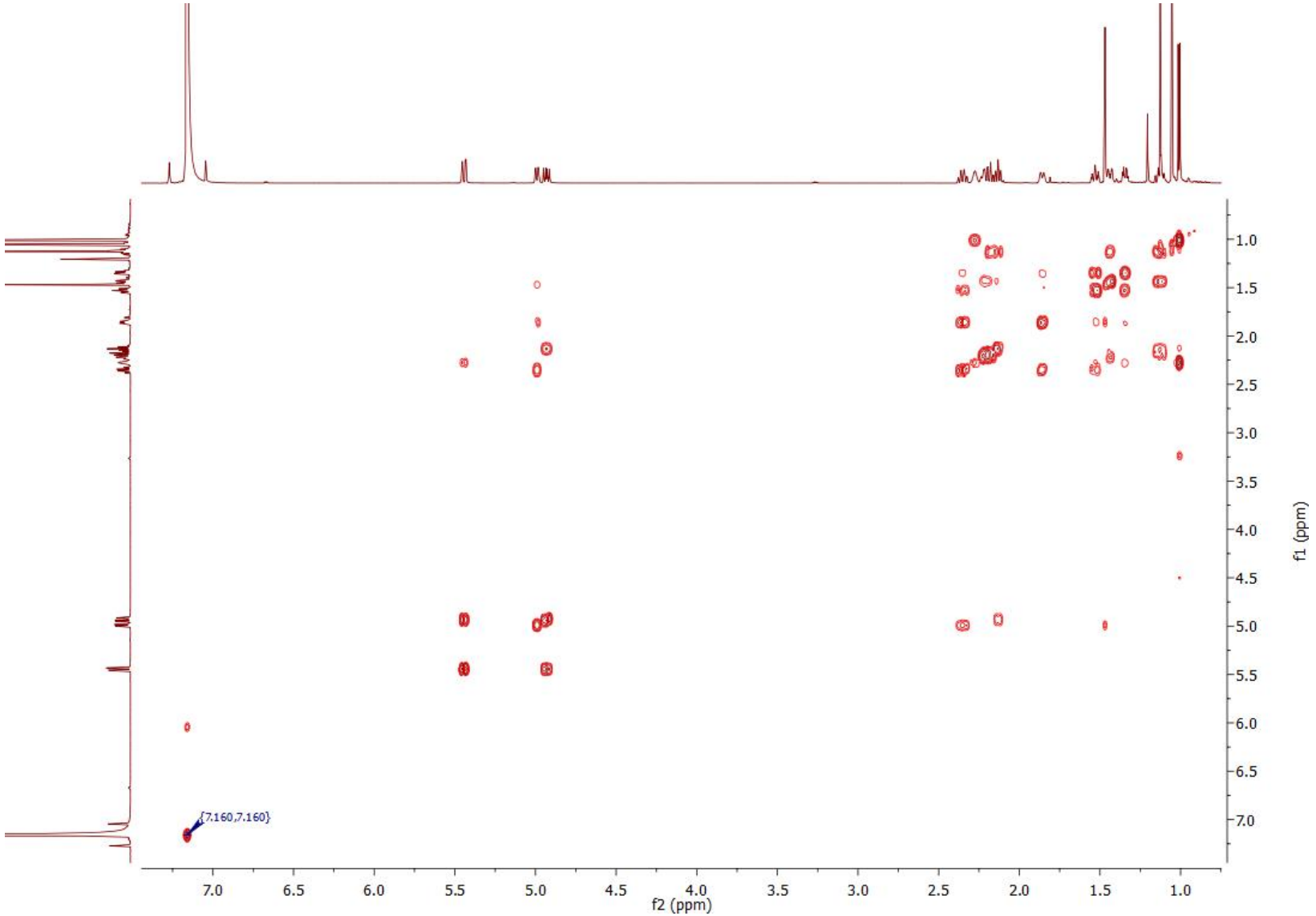


**Figure S5.** $^{1}$H,$^{1}$H-COSY spectrum ($C_6D_6$) of (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**).

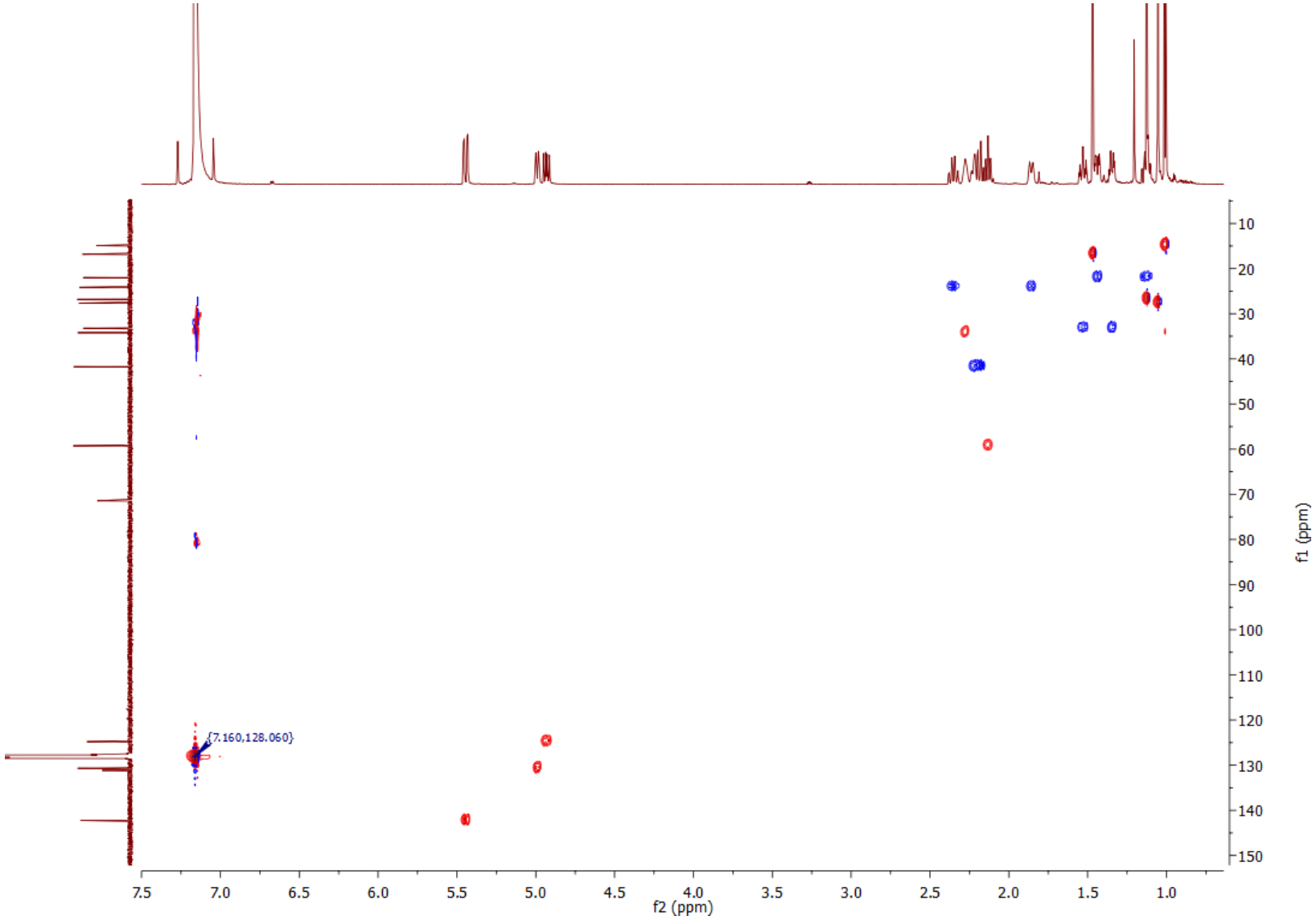


**Figure S6.** HSQC spectrum ($C_6D_6$) of (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**).

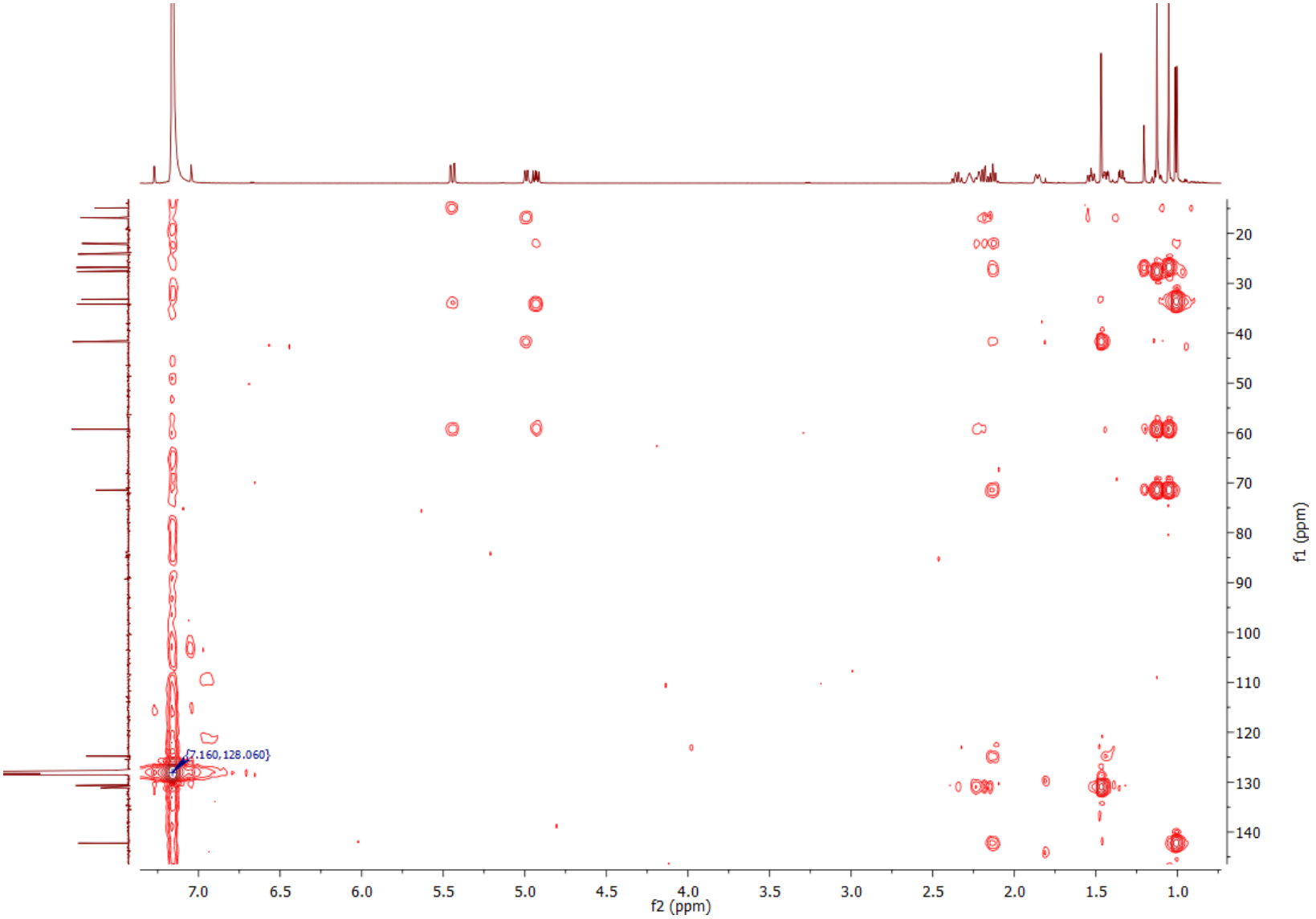


**Figure S7.** HMBC spectrum ($C_6D_6$) of (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**).

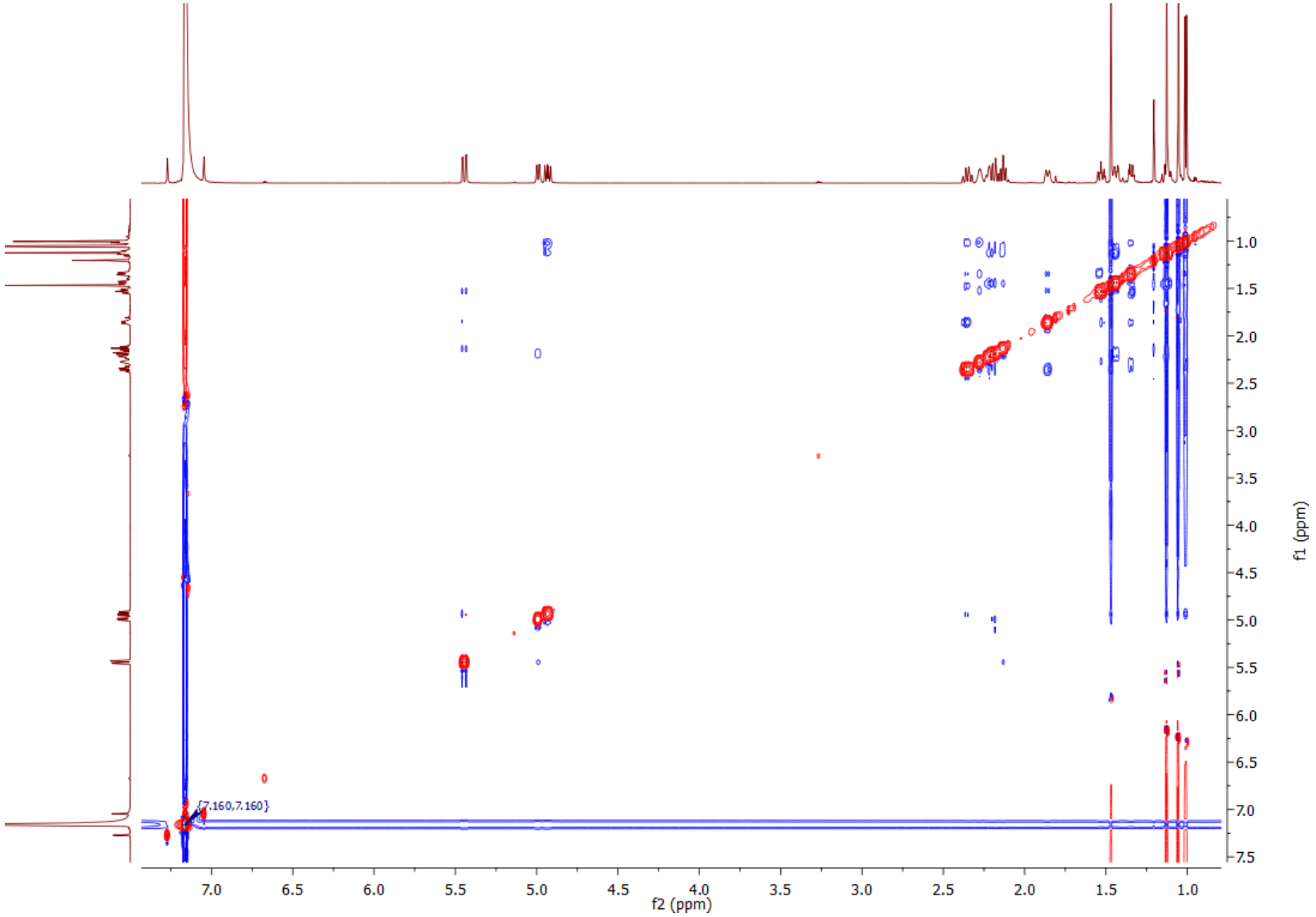


**Figure S8.** NOESY spectrum ($C_6D_6$) of (4*S*,7*R*)-germacra-(1(10)*E*,5*E*)-dien-11-ol (**1**).

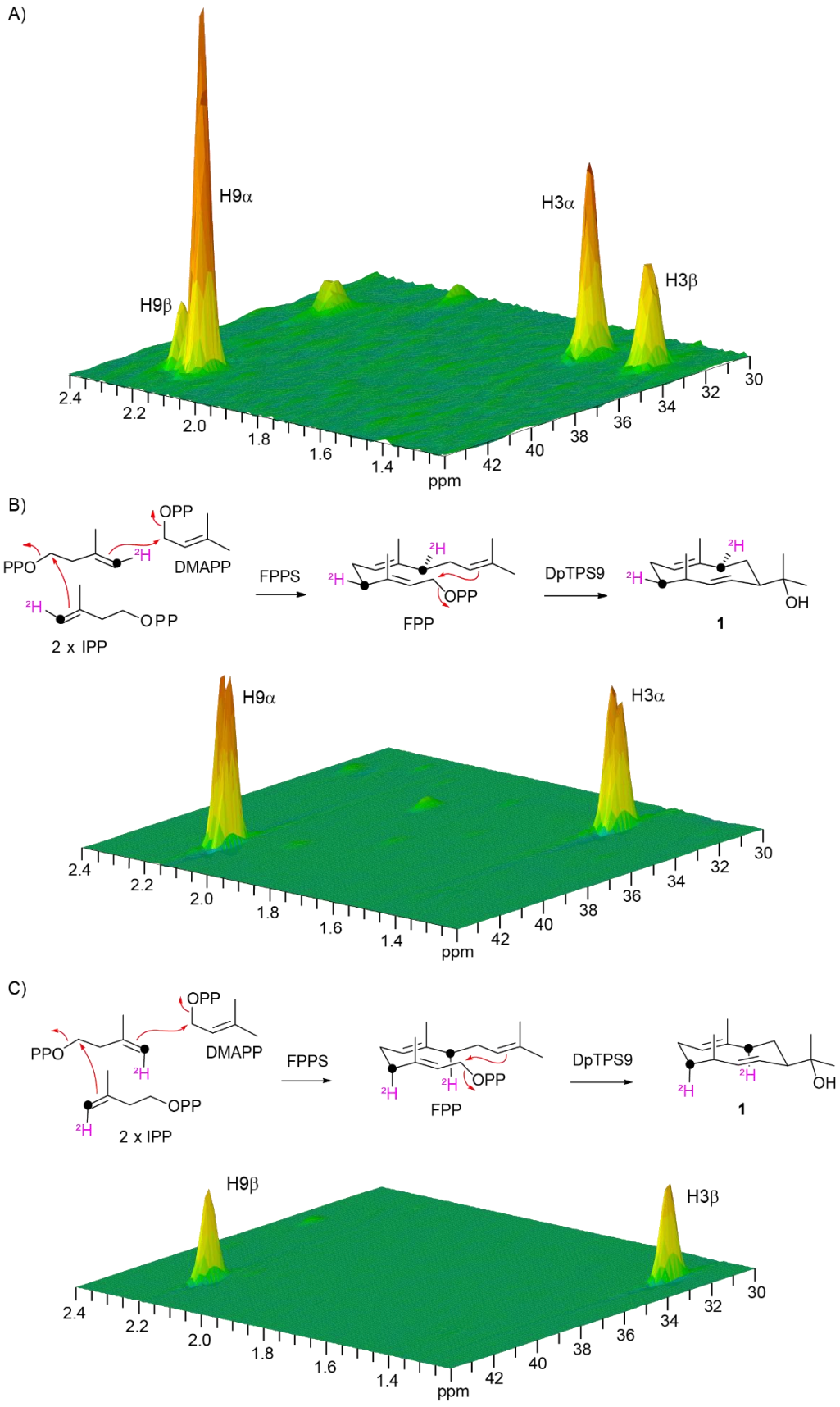


**Figure S9.** HSQC analyses of **1** obtained by the enzymatic conversions. A) FPP and DpTPS9, B) DMAPP and (*E*)-(4-$^{13}$C,4-$^{2}$H)IPP with FPPS and DpTPS9, C) DMAPP and (*Z*)-(4-$^{13}$C,4-$^{2}$H)IPP with FPPS and DpTPS9. Black dots indicate $^{13}$C-labellings.

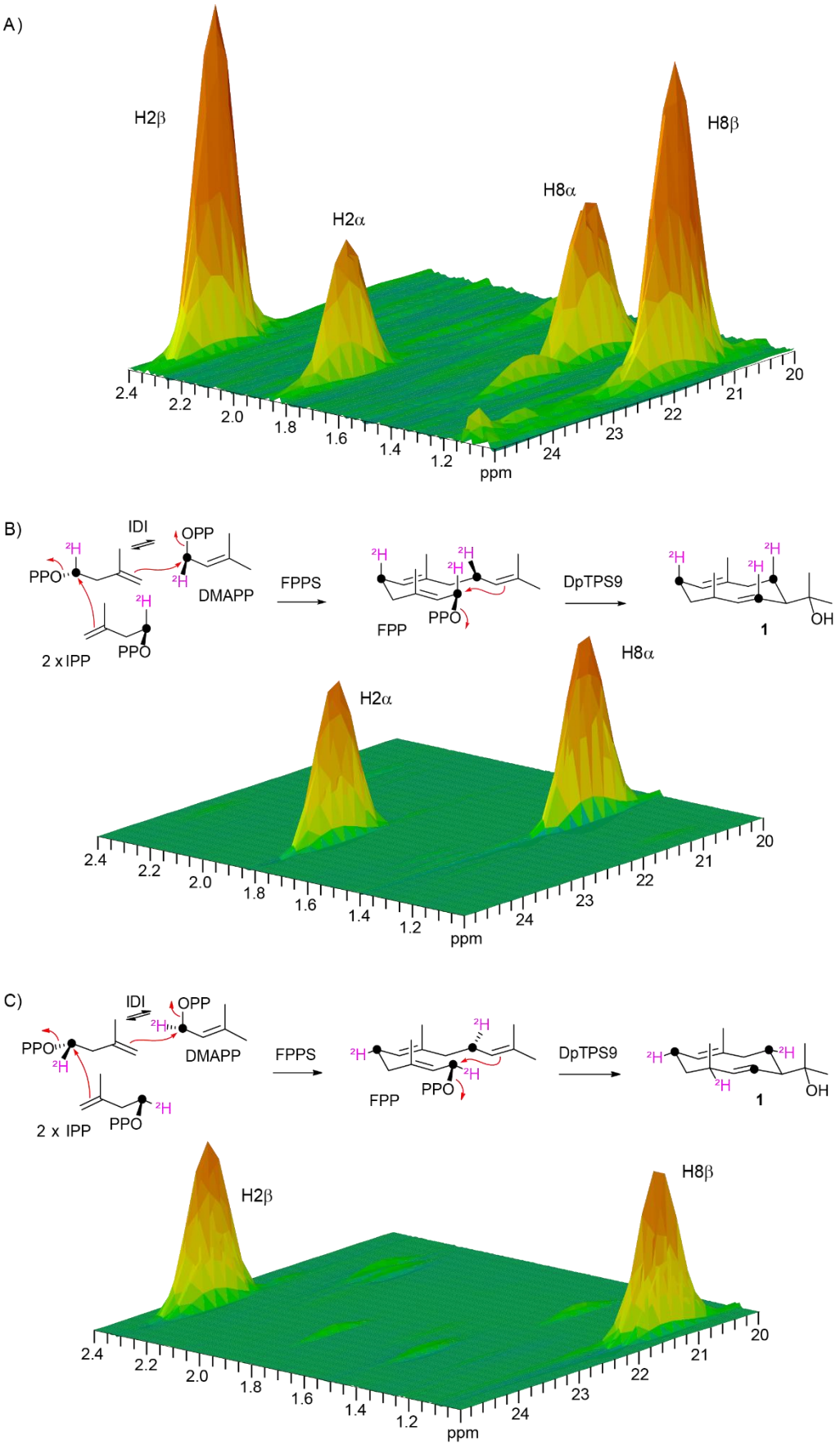


**Figure S10.** HSQC analyses of **1** obtained by the enzymatic conversions. A) FPP and DpTPS9, B) (*R*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9, C) (*S*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9. Black dots indicate $^{13}$C-labellings.

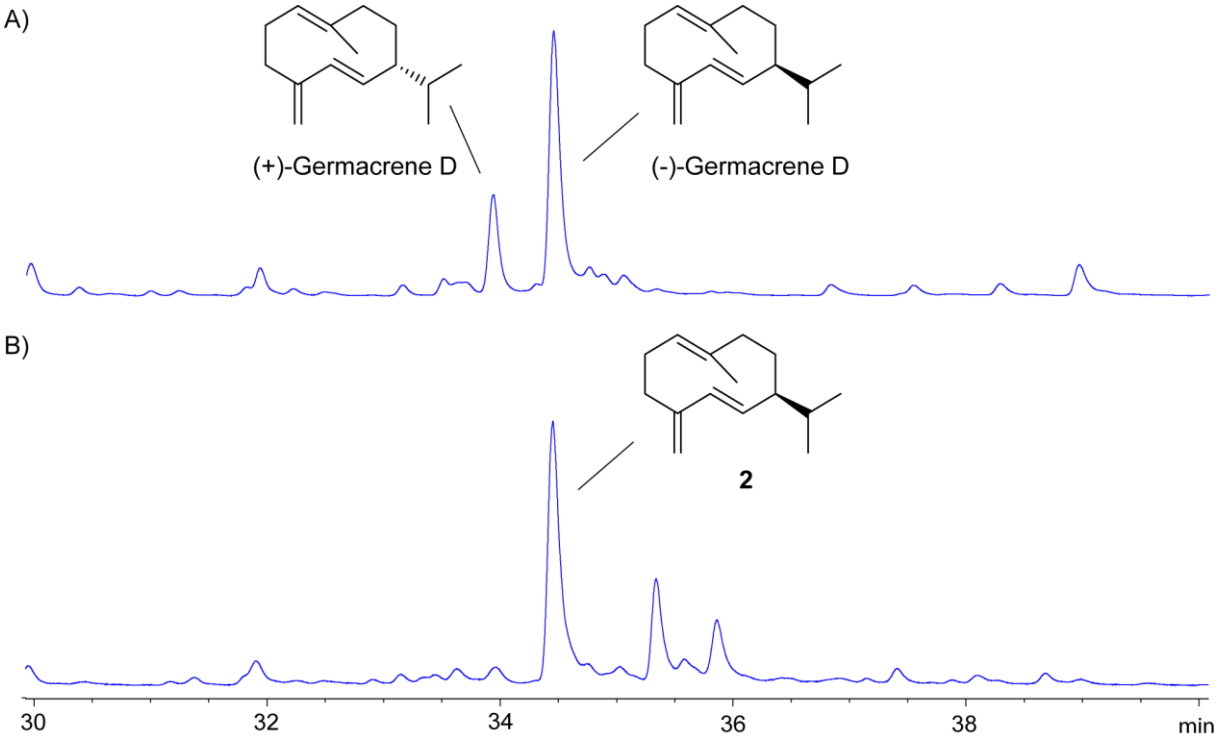


**Figure S11.** Enantioselective GC analyses using an Agilent Cyclosil-B column. A) Essential oil of *Solidago canadensis*, B) products of DpTPS9.

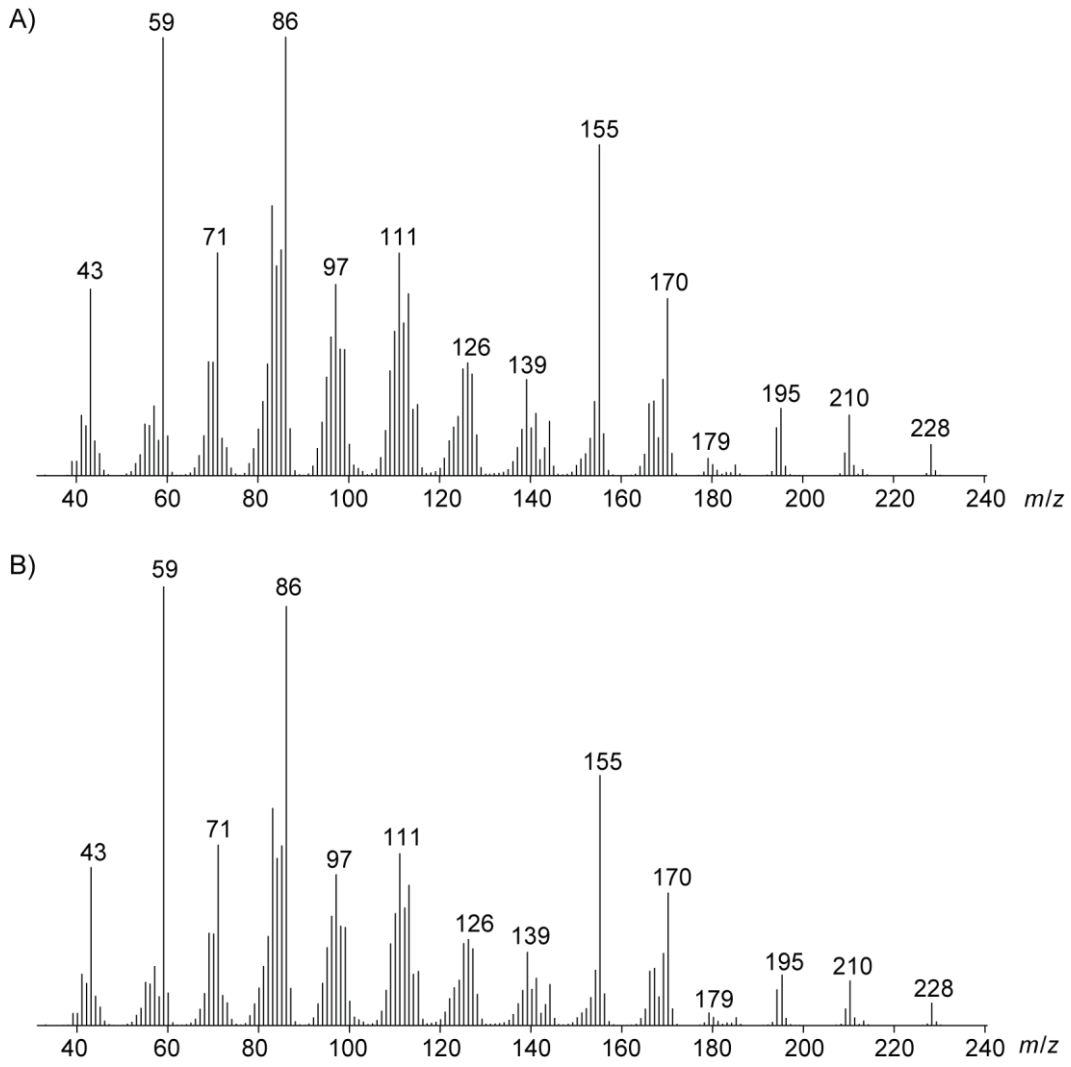


**Figure S12.** MS analyses of **1** obtained by the enzymatic conversions of A) (*R*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9, B) (*S*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9.

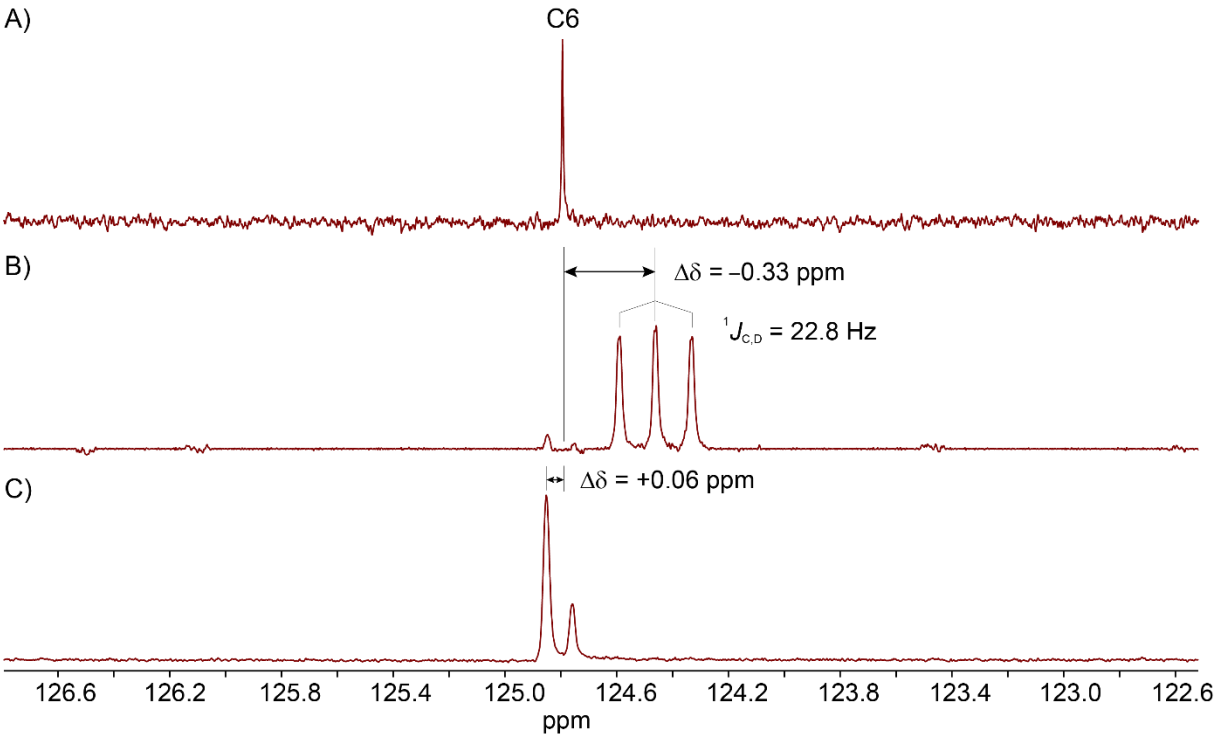


**Figure S13.** NMR analyses of C6 in **1** obtained by the enzymatic conversions. A) FPP and DpTPS9, B) (*R*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9, C) (*S*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9.

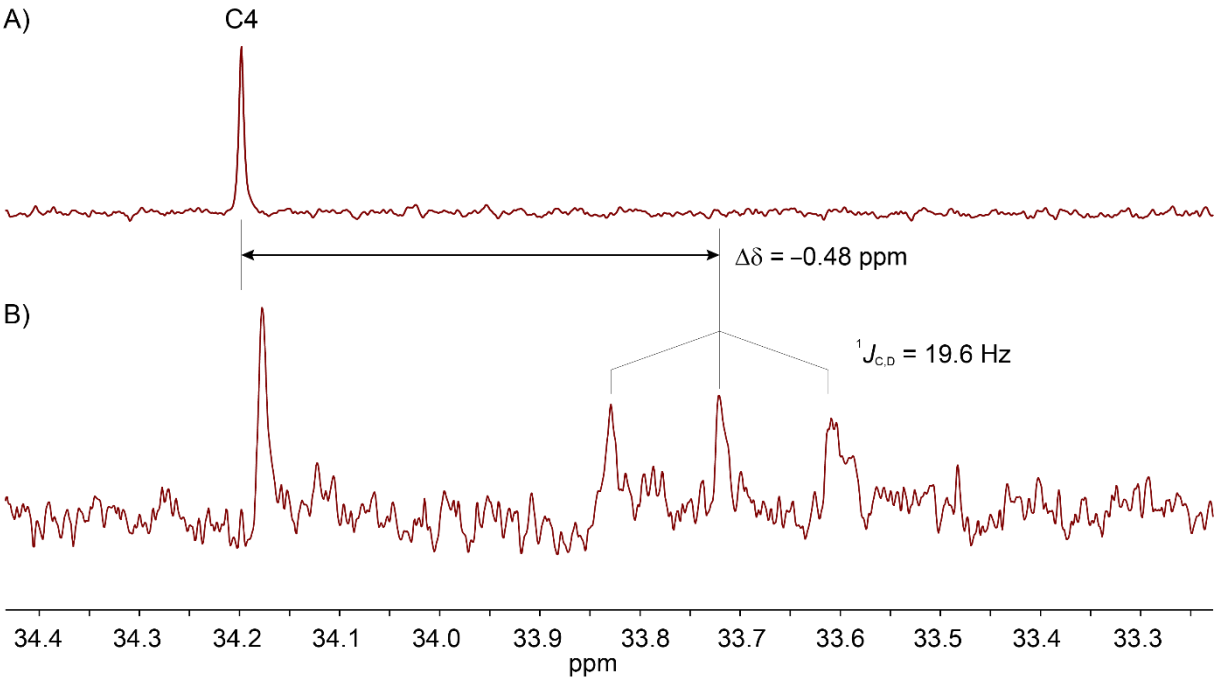


**Figure S14.** NMR analyses of C4 in **1** obtained by the enzymatic conversions. A) FPP and DpTPS9, B) (3-$^{13}$C,1,1-$^{2}H_2$)FPP and DpTPS9.

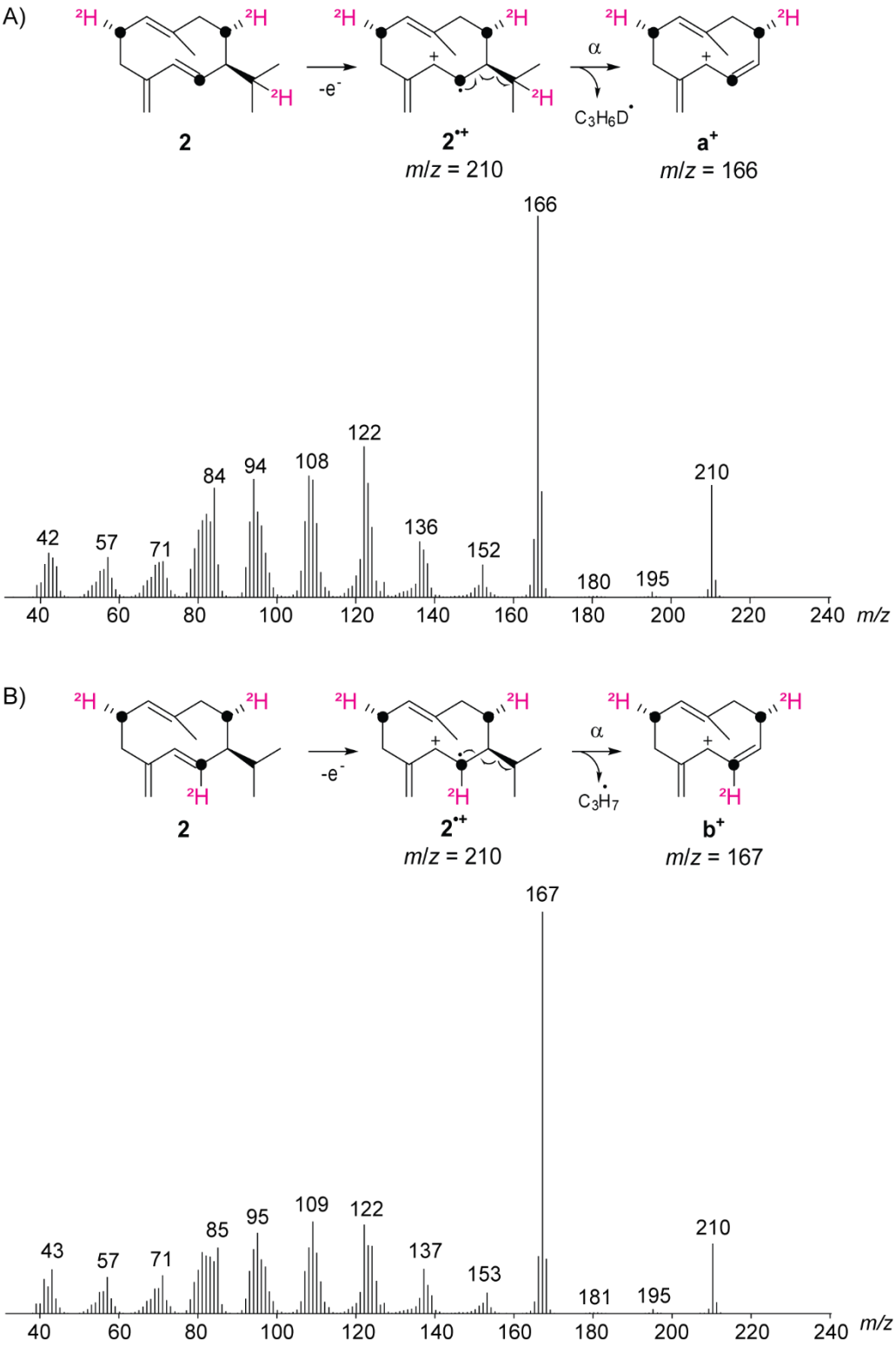


**Figure S15.** MS analyses of **2** obtained by the enzymatic conversions. A) (*S*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9, B) (*R*)-(1-$^{13}$C,1-$^{2}$H)IPP with IDI, FPPS and DpTPS9. Black dots indicate $^{13}$C-labellings.

MSLSFKNIVFPEEWQVPPNDYIFIDDCYEEALQFNLFERGDEKSYTWMYHTISCCAYFWCKCSRSEMK
LIGHLMLWTFLLDDILDSDKVNDAEAIEMIKRTEFIFIEGKLPENPTDLEKYTCYLRNEGLKIAGDRE
DMFNMFLTNSIQWILSIIPLNKSMEHKLPPHLQLHGYLRKLNVGVELCQGFTYLIFANNKVNPAIFNS
PRYKKMLECTSMVVSHVNDMASYCKEVKNGGGFINSLLILQKRADPLSIEHSYQVIAEQTNAFIRDFI
YQEKMLLESISDEQQNEVKVFLDHMKYLMKGNYLWSGTTARYASKSSPFVEMQKSLNVHLDNEIDASS
L

**Figure S16.** Amino acid sequence of DpTPS9. Highly conserved motifs are highlighted in yellow, residues targeted by site-directed mutagenesis in this study are highlighted in green.

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