Table S1. Primers used in this study.

Primer	Sequence	Target
DdTPS9f	GGATCCATGTATTCTCTTCATGATTTCAAATTC	pET32a::*DdTPS9*
DdTPS9r	CTCGAGTTATTTTAATAAAATTTTATCAACAATTTTTTC	pET32a::*DdTPS9*
DpTPS10f	GGATCCATGGAAGCTATAAAACAAAAAAGAAGT	pET32a::*DpTPS10*
DpTPS10r	CTCGAGTCATCGAAAAAATGTTTTTATGTTGGAC	pET32a::*DpTPS10*
DpTPS11f	GGATCCATGGATATAAATAAACAAAAAACCAAATG	pET32a::*DpTPS11*
DpTPS11r	CTCGAGTTAATTATTTTTATTGATATAGTTTTTG	pET32a::*DpTPS11*
JR098f	CATTTAACTGACCCTTTACATTTTTTAC	DdTPS9-N236D
JR098r	GTAAAAAATGTAAAGGGTCAGTTAAATG	DdTPS9-N236D
JR099f	CATTTAACTGCCCCTTTACATTTTTTAC	DdTPS9-N236A
JR099r	GTAAAAAATGTAAAGGGGCAGTTAAATG	DdTPS9-N236A
JR106f	GCTTATTGTGACACTTTTTATTTTTTAC	DpTPS10-N236D
JR106r	GTAAAAAATAAAAAGTGTCACAATAAGC	DpTPS10-N236D
JR107f	GCTTATTGTGCCACTTTTTATTTTTTAC	DpTPS10-N236A
JR107r	GTAAAAAATAAAAAGTGGCACAATAAGC	DpTPS10-N236A
JR110f	CCAGATACTATGGATTATGTAAAAATTATG	DpTPS11-N243D
JR110r	CATAATTTTTACATAATCCATAGTATCTGG	DpTPS11-N243D
JR111f	CAGATACTATGGCTTATGTAAAAATTATG	DpTPS11-N243A
JR111r	CATAATTTTTACATAAGCCATAGTATCTG	DpTPS11-N243A
JR149f	GACAGCCCAGATCTG	pET32a start forward
JR149r	CAGATCTGGGCTGTC	pET32a start reversed
JR150f	CGAATTCGAGCTCCG	pET32a end forward
JR150r	CGGAGCTCGAATTCG	pET32a end reversed

Strains and culture conditions

Dictyostelium discoideum strain AX4 (DBS0235552) and *Dictyostelium purpureum* strain AX1 (DBS0308472) were obtained from the Dictybase Stock Center (www.dictybase.org). Both *Dictyostelium* species were cultured with bacterium *Klebsiella pneumonia* on SM agar plates, which were incubated at 22 °C under continuous darkness.

Gene cloning

For both *D. discoideum* and *D. purpureum*, the cultured cells were harvested just prior to the formation of fruiting bodies and subject to RNA extraction. Total RNA was isolated using RNeasy mini kit (https://www.qiagen.com) and made into cDNAs using the First-strand cDNA synthesis kit (https://www.gelifesciences.com). Full-length cDNAs for *DdTPS9*, *DpTPS10* and *DpTPS11* were amplified using PfuUtra II fusion high-fidelity DNA polymerase (https://www.agilent.com) with modified gene specific-primers listed in Table S1. The amplified cDNAs were cloned into pGEM-T Easy vector (https://www.promega.com) and confirmed by sequencing. Next, each cDNA was excised from the pGEM-T vector with the digestion of restriction enzymes BamHI and XhoI, and the fragment was inserted into the *BamHI* and *XhoI* cloning sites of the pET32a vector.

For site-directed mutagenesis, a two-step PCR approach was pursued. In the first PCR reaction, two fragments for two target mutations were amplified from each of the three expression constructs in pET32a (first fragment using primers JR149f and JR098r or JR099r [DdTPS9], JR106r or JR107r [DpTPS10], JR110r or JR111r [DpTPS11]; second fragment using primers JR150r and JR098f or JR099f [DdTPS9], JR106f or JR107f [DpTPS10], JR110f or JR111f [DpTPS11]). PCR conditions using Q5 DNA polymerase (NEB, Ipswich, MA, USA) were as follows: initial denaturation 30 s 98 °C, denaturation 10 s 98 °C, annealing 30 s between 60 °C and 65 °C, elongation 30 s 72 °C, cycle repeated 33 times, final elongation 5 min 72 °C. PCR products were obtained from gel slices. In a second PCR, the two fragments were combined using primers JR149f and JR150r to yield one mutation-containing fragment, which was homologously recombined to linear pET32a (obtained by PCR using JR149r and JR150f) using In-Fusion HD cloning kit (Takara Bio, Shimogyō-ku, Kyoto, Japan) following the manufacturers protocol. The resulting plasmids were isolated from single colonies and the correct insertion of the gene containing the desired mutation(s) was checked by analytical digest and sequencing. Correct plasmids were shuttled to *E. coli* BL21(DE3).

Gene expression and protein purification

E. coli BL21(DE3) transformants were grown in LB medium precultures containing ampicillin (100 μg/mL) overnight at 37 °C with shaking. Main cultures were inoculated by transferring the preculture (1/1000) to culture flasks with LB-ampicillin. The cells were grown at 37 °C until OD_{600} = 0.4-0.6 was reached. The cultures were cooled to 18 °C, before protein expression was induced by addition of IPTG (400 μM). Incubation was continued overnight at 18 °C with shaking and the cells were harvested by centrifugation (10000 x g, 4 min). The cell pellet was suspended in binding buffer (10 mL L^{-1} culture; 20 mm Na_2HPO_4, 500 mM NaCl, 20 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C). Ultra-sonification on ice (5x 0.5 min; 50% power) was used to lyse the cells, followed by centrifugation (15000 x g, 4 °C, 7 min). The soluble enzyme fractions were loaded onto Ni^{2+}-NTA columns (Qiagen, Venlo, Netherlands) and the bound proteins were washed with binding buffer (2x 10 mL/L culture). The proteins were eluted by the addition of elution buffer (2x 6.25 mL/L culture; 20 mM Na_2HPO_4, 500 mM NaCl, 500 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C). Elution fractions were checked by SDS-PAGE (Figure S1) and the protein concentrations were estimated by Bradford assay[1] (typical concentrations: 1.0 mg/mL for DdTPS9, 0.34 mg/mL for DpTPS10 and 0.45 mg/mL for DpTPS11).

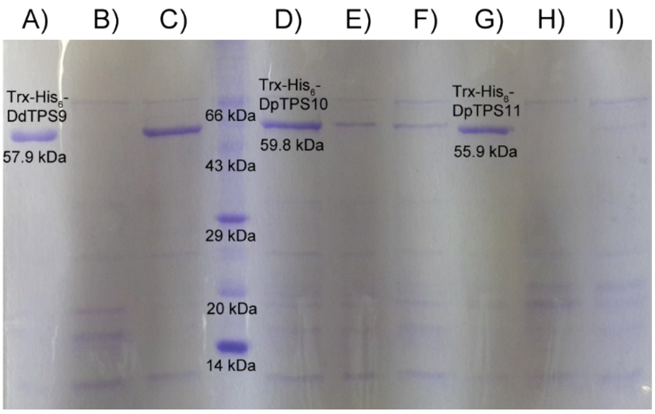


Figure S1. SDS-PAGE of recombinant protein preparations of A) (–)-β-barbatene synthase from *Dictyostelium discoideum* AX4 (DdTPS9), B) DdTPS9-N236D, C) DdTPS9-N236A, D) (–)-β-araneosene synthase from *Dictyostelium purpureum* AX1 (DpTPS10), E) DpTPS10-N249D, F) DpTPS10-N249A, G) (*S*)-(+)-nephthenol synthase from *D. purpureum* AX1 (DpTPS11), H) DpTPS11-N243D, and I) DpTPS11-N243A. Calculated molecular weights for the TrxA-fusion proteins are given.

GC/MS and GC/MS-QTOF analyses

GC/MS analyses were performed on a 7890B GC connected to a 5977A mass selective detector (Agilent, Santa Clara, CA, USA). The GC was equipped with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film). Specific GC settings were 1) inlet pressure: 77.1 kPa, He at 23.3 mL min^{-1}, 2) injection volume: 2 μL, 3) temperature program: 5 min at 50 °C 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 settings were 1) source: 230 °C, 2) transfer line: 250 °C, 3) quadrupole: 150 °C and 4) electron energy: 70 eV. Retention indices (*I*) were determined from retention times in comparison to the retention times of of *n*-alkanes (C_7-C_{40}).
A 7890B GC connected to a 7200 accurate-mass Q-TOF detector (Agilent) equipped with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film) were used for GC/MS-QTOF analyses. MS parameters were 1) inlet pressure: 83.2 kPa, He at 24.6 mL min^{-1}, 2) transfer line: 250 °C, 3) electron energy 70 eV. GC parameters were 1) temperature program: 5 min at 50 °C increasing at 5 °C min^{-1} to 320 °C, 2) injection volume: 1 μL, 3) split ratio: 50:1, 60 s valve time, and 4) carrier gas: He at 1 mL min^{-1}.

NMR spectroscopy

NMR spectra were recorded on a Bruker (Billerica, MA, USA) Avance I (300 MHz), Avance I (400 MHz), Avance I (500 MHz), Avance III HD Prodigy (500 MHz) or an Avance III HD Cryo (700 MHz) NMR spectrometer. Spectra were measured in C_6D_6 and referenced against solvent signals (^{1}H-NMR, residual proton signal: δ = 7.16; ^{13}C-NMR: δ = 128.06).[2]

Incubation experiments with recombinant TPSs

Small scale incubations for testing the substrate scope, and labelling experiments were carried out by dissolving the diphosphate substrate(s) in substrate buffer (1 mL; 25 mM NH_4HCO_3). The solutions were diluted with incubation buffer (5 mL; 50 mM Tris/HCl, 10 mM $MgCl_2$, 20% glycerol, pH = 8.2), before adding enzyme elution fractions (1 mL each). The reactions were further diluted by binding buffer (to 10 mL total reaction volume) and incubated at 28 °C with shaking for 3 h. Extraction was done by hexane (150 μL) or C_6D_6 (650 μL, 300 μL) and the extracts were analysed by GC/MS and/or NMR.

For large scale product isolation, a solution of the corresponding diphosphate substrate (100 mg) in substrate buffer (20 mL) was dropped to a slowly stirred mixture of TPS elution fraction (100 mL), incubation buffer (200 mL) and binding buffer (100 mL) over 2 h at room temperature. Incubation was further continued for 4 h at 28 °C with shaking. The reaction was extracted with pentane (2x 300 mL) and the extracts were dried with MgSO4, concentrated under reduced pressure and purified by column chromatography on silica gel ([pentane] for **4** and **5**, [pentane/Et_2O (5:1)] for **6**) to afford the target terpenes as colourless oils (**4**: 2.3 mg; **5**: 5.4 mg; **6**: 5.4 mg).

(–)-β-Barbatene, (3a*R*,4*R*,8*R*,8a*S*)-3a,4,8a-trimethyl-7-methylenedecahydro-4,8-methano-azulene (**4**). R_f (pentane) = 0.92. $[\alpha]_D^{20}$ = –11.1° (*c* 0.25, C_6D_6). HRMS (QTOF): *m*/*z* = 204.1879 (calc. for $[C_{15}H_{24}]^+$ 204.1873). GC (HP5-MS): *I* = 1456. MS (EI, 70 eV): *m*/*z* (%) = 204 (6), 189 (17), 175 (6), 161 (7), 148 (6), 133 (10), 119 (11), 111 (38), 108 (100), 96 (99), 93 (93), 81 (60), 69 (14), 55 (14), 41 (9), cf. Figure S2. NMR data are given in Table S2 and Figures S3–S9.

(–)-β-Araneosene, (3a*S*,6*E*,10*E*,12a*S*)-6,10,12a-trimethyl-3-(propan-2-ylidene)-1,2,3,3a,4,5,8,9,12,12a-decahydrocyclopenta[11]annulene (**5**). R_f (pentane) = 0.78. $[\alpha]_D^{20}$ = –133.0° (*c* 0.64, C_6D_6). HRMS (QTOF): *m*/*z* = 272.2499 (calc. for $[C_{20}H_{32}]^+$ 272.2499). GC (HP5-MS): *I* = 2025. MS (EI, 70 eV): *m*/*z* (%) = 272 (12), 257 (7), 229 (25), 216 (35), 201 (12), 189 (35), 175 (13), 161 (37), 147 (18), 136 (68), 135 (67), 121 (100), 107 (40), 93 (29), 79 (19), 67 (16), 55 (9), 41 (8), cf. Figure S15. NMR data are given in Table S3 and Figures S16–S22.

(+)-Nephthenol, 2-((*S*,3*E*,7*E*,11*E*)-4,8,12-trimethylcyclotetradeca-3,7,11-trien-1-yl)propan-2-ol (**6**). R_f ([cyclohexane/EtOAc (3:1)]) = 0.63. $[\alpha]_D^{20}$ = +45.5° (*c* 0.68, C_6D_6). HRMS (QTOF): *m*/*z* = 272.2501 (calc. for $[C_{20}H_{32}]^+$ 272.2499, $[M-H_2O]^+$). GC (HP5-MS): *I* = 2164. MS (EI, 70 eV): *m*/*z* (%) = 272 (38), 257 (26), 229 (20), 215 (7), 202 (13), 201 (13), 189 (26), 175 (18), 161 (33), 147 (36), 136 (48), 135 (49), 133 (43), 121 (88), 107 (85), 93 (100), 81 (74), 79 (46), 69 (33), 68 (47), 67 (51), 59 (42), 55 (25), 53 (14), 43 (22), 41 (22), cf. Figure S26. NMR data are given in Table S4 and Figures S27–S33.

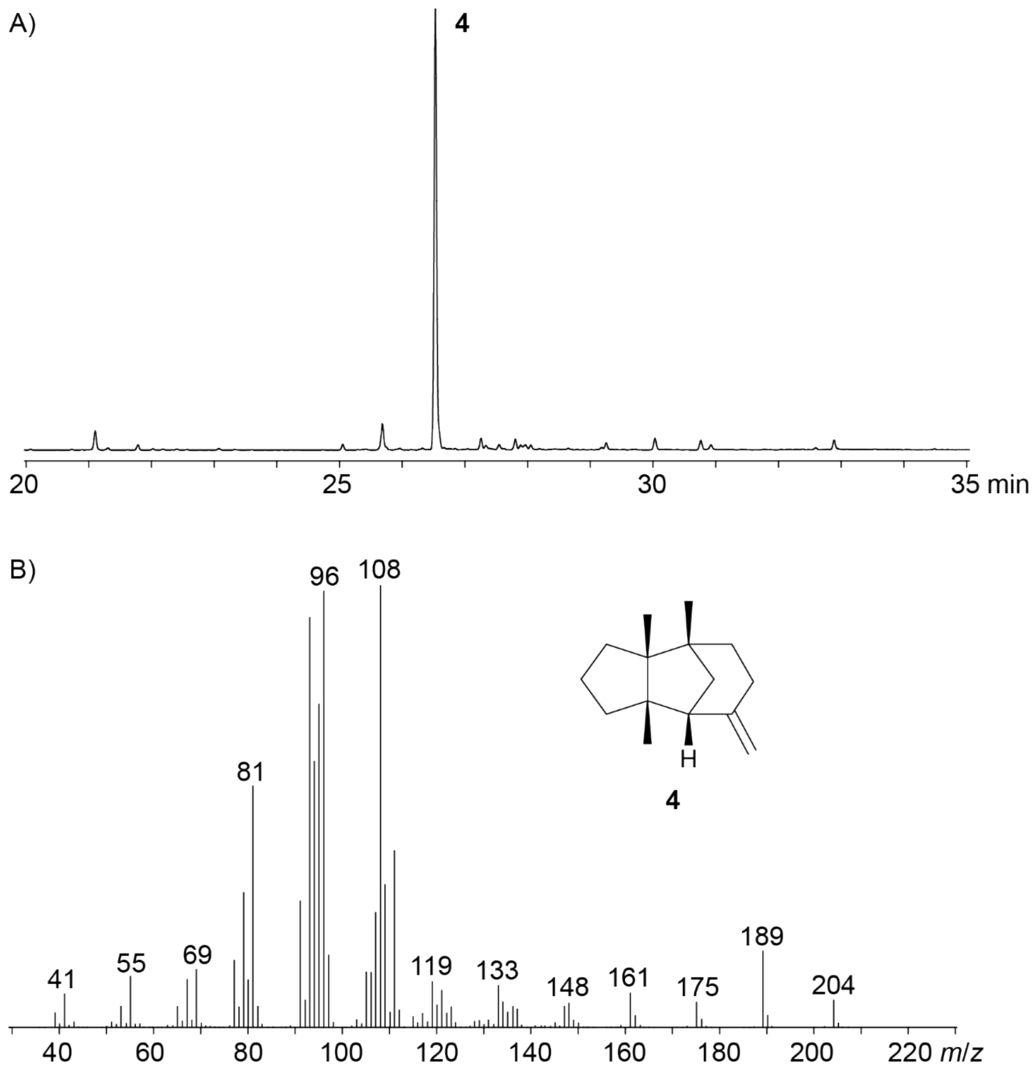
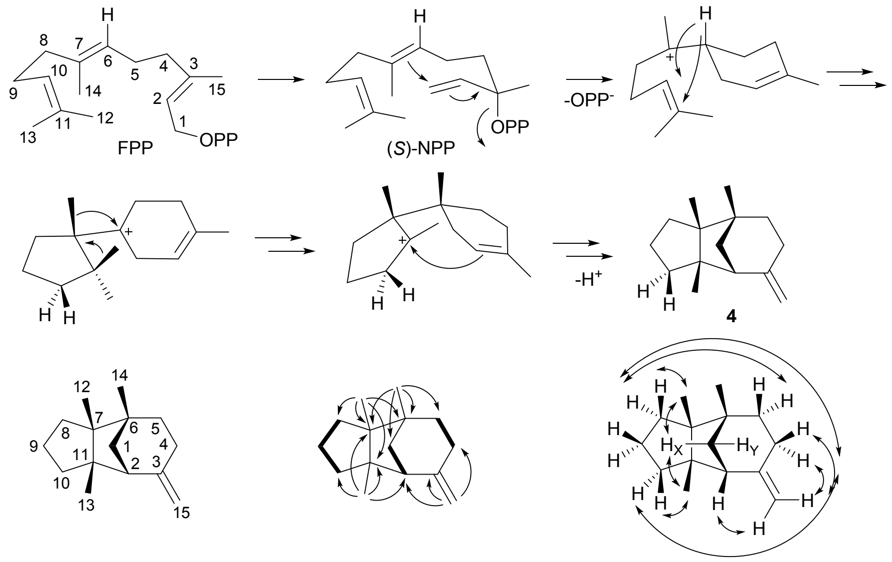


Figure S2. A) Total ion chromatogram of an extract from the incubation of FPP with DdTPS9. B) EI mass spectrum of β-barbatene (**4**).



Scheme S1. Short biosynthesis and structure elucidation of **4**. Carbon numbers refer to the corresponding positions in FPP. H,H-COSY spin systems are represented by bold bonds, single headed arrows show HMBC correlations and NOESY correlations are depicted by double headed arrows.

Table S2. NMR data of β-barbatene (**4**) in C_6D_6 recorded at 298 K.

C[a]		^{13}C[b]	^{1}H[b]	^{13}C[c]
1	CH_2	47.04	1.99 (ddd, J = 11.4, 5.0, 3.1, H_X)	46.8
			1.35 (m, H_Y)	
2	CH	56.44	2.20 (d, J = 5.0)	56.0
3	C_q	151.58	–	152.0
4	CH_2	29.07	2.41 (m, H_α)	28.7
			2.18 (dd, J = 16.6, 8.1, H_β)	
5	CH_2	38.33	1.63 (ddd, J = 13.7, 8.1, 2.5, H_α)	38.0
			1.34 (m, H_β)	
6	C_q	43.27	–	43.0
7	C_q	54.41	–	54.1
8	CH_2	35.86	1.82 (m, H_α)	35.5
			1.03 (m, H_β)	
9	CH_2	27.85	1.75 (m, 2H)	27.5
10	CH_2	37.45	1.87 (m, H_α)	37.0
			1.21 (m, H_β)	
11	C_q	55.73	–	55.4
12	CH_3	23.54	0.84 (s)	23.3
13	CH_3	27.68	0.99 (s)	27.5
14	CH_3	24.95	0.80 (s)	24.8
15	CH_2	108.28	4.74 (m, 2H)	107.5

[a] Carbon numbering indicates the origin of each carbon from FPP by identical number as shown in Scheme S1. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, br = broad, coupling constants J are given in Hertz. [c] ^{13}C-NMR data in $CDCl_3$ from ref. [3].

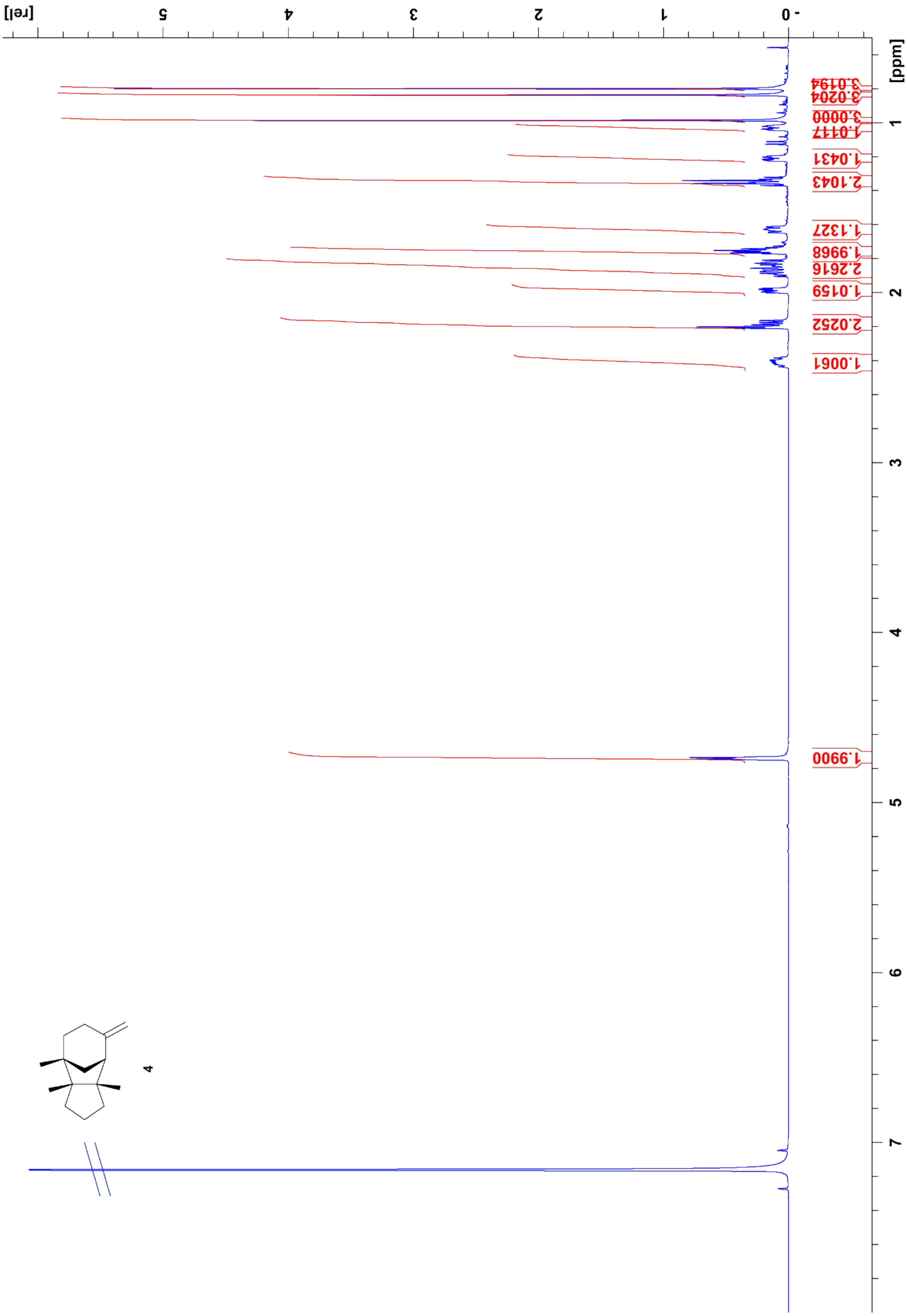


Figure S3. ^{1}H-NMR spectrum of **4** (700 MHz, C_6D_6).

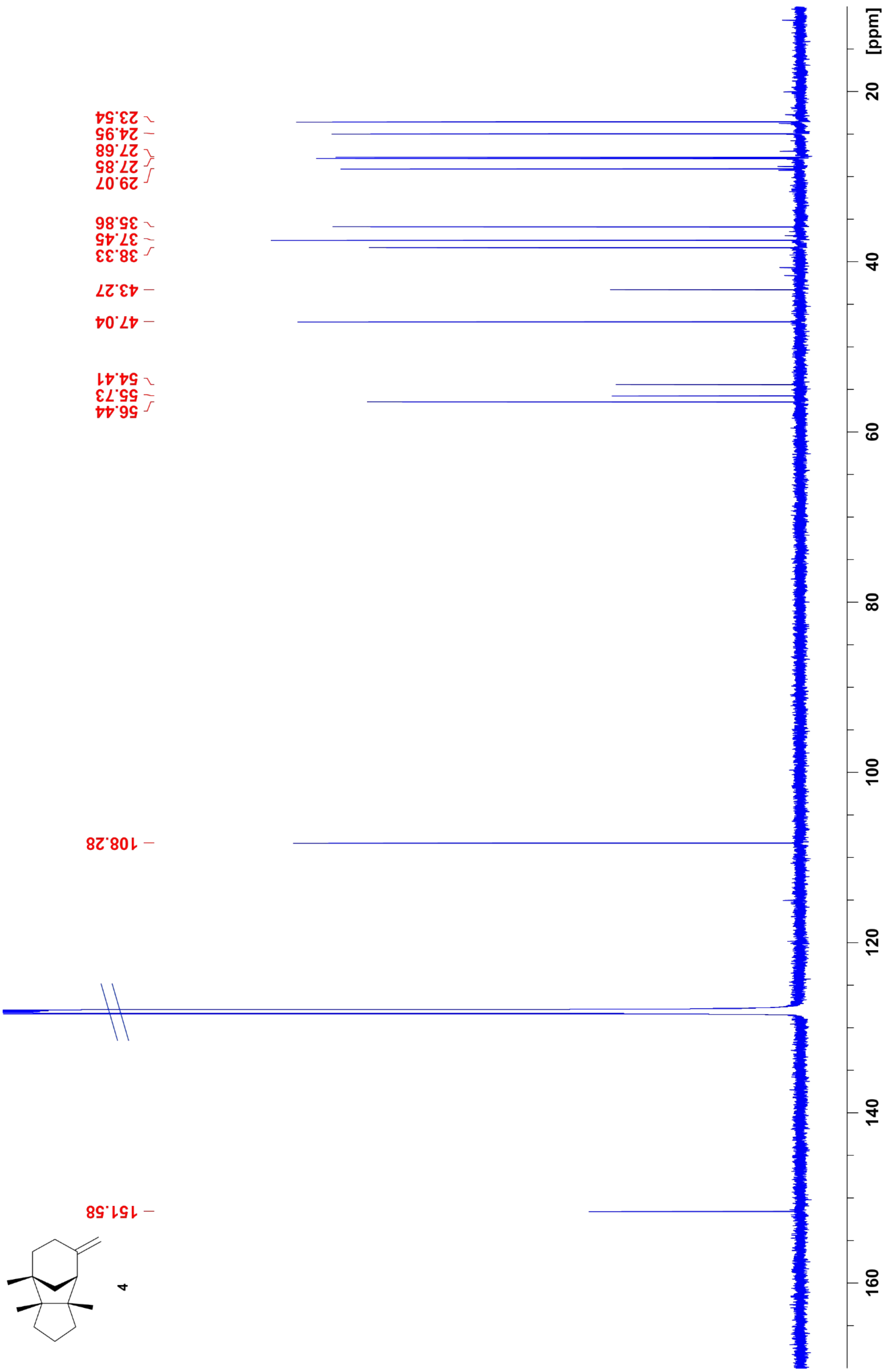


Figure S4. ^{13}C-NMR spectrum of **4** (175 MHz, C_6D_6).

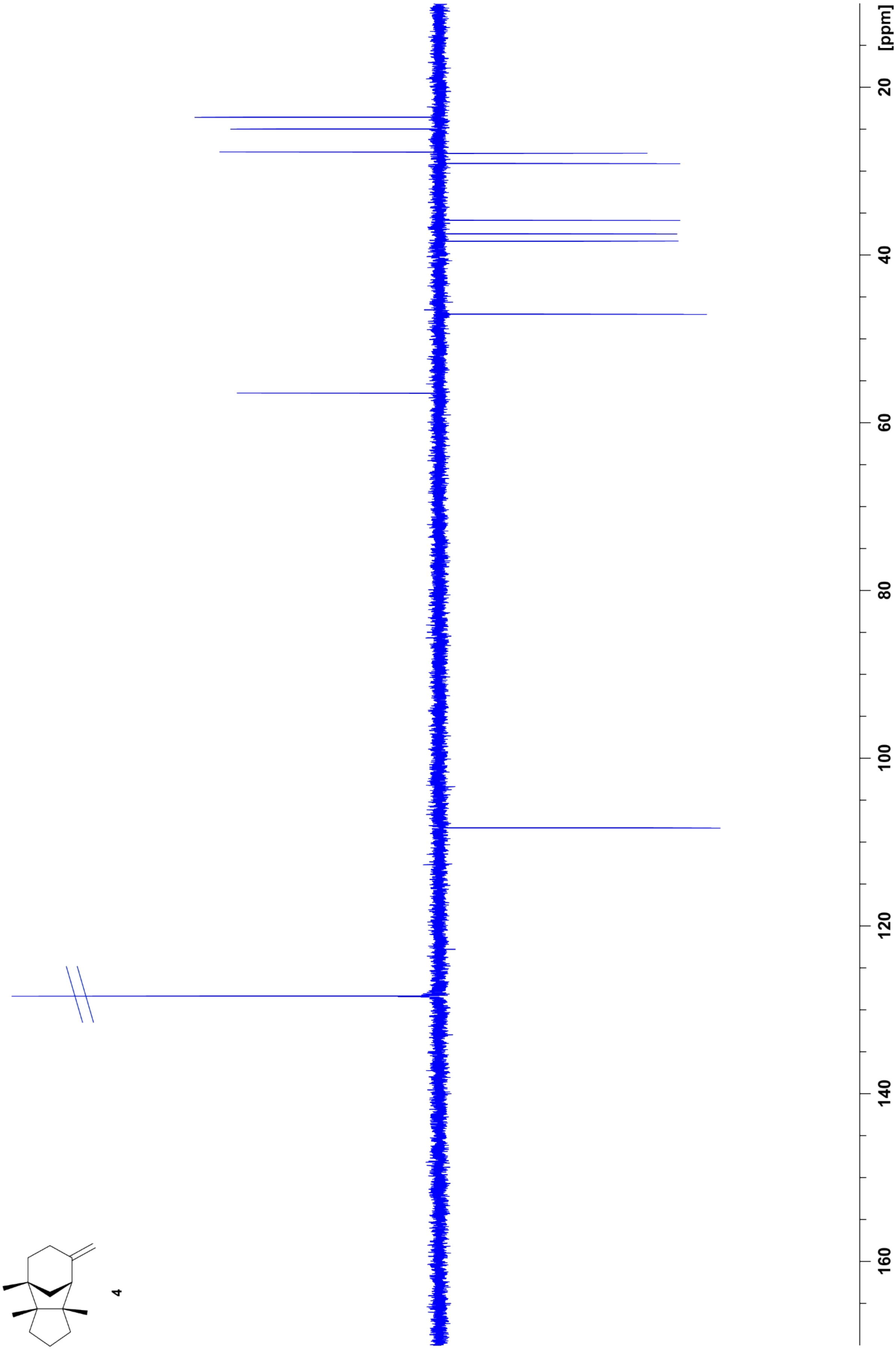


Figure S5. ^{13}C-DEPT spectrum of **4** (175 MHz, C_6D_6).

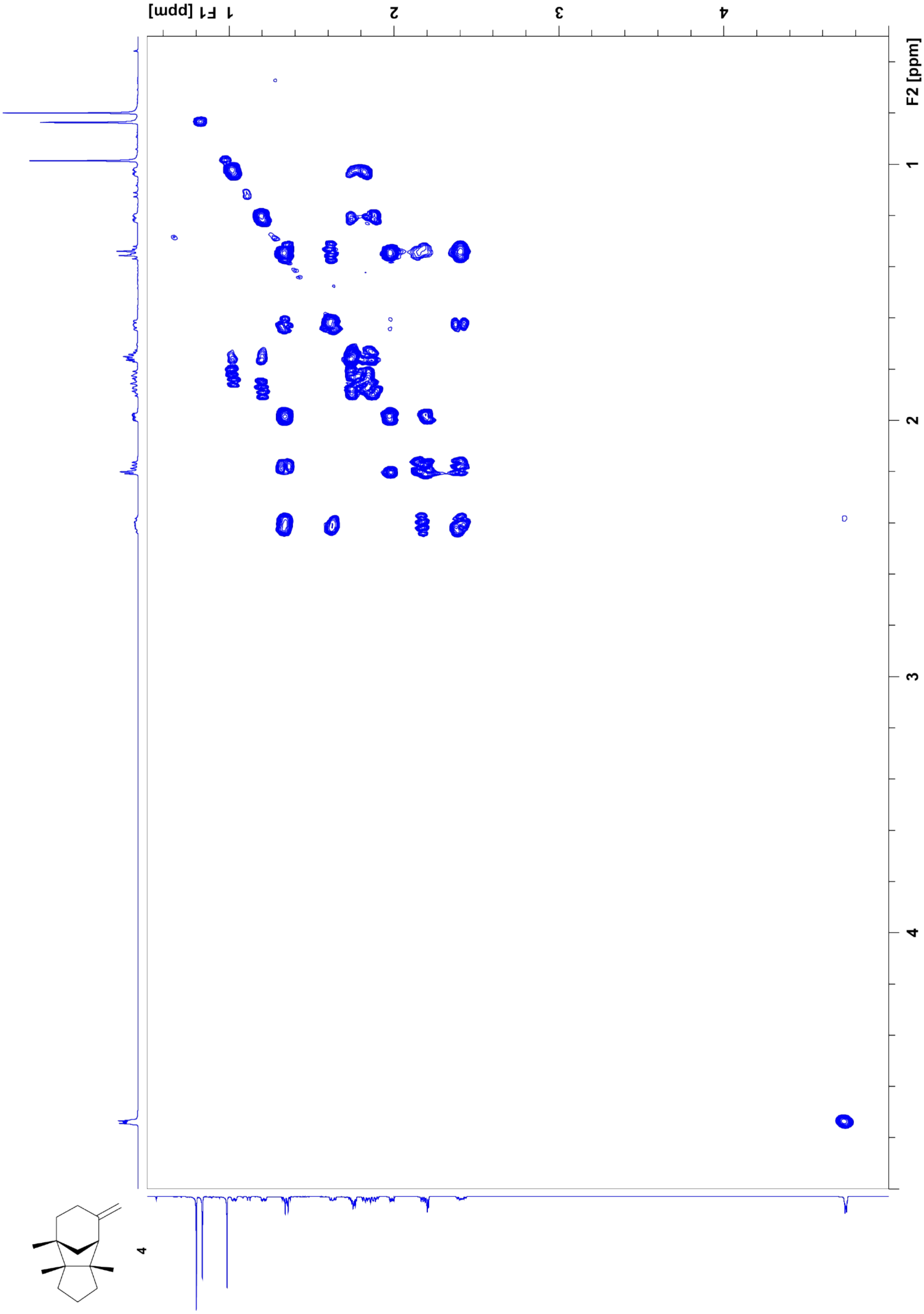


Figure S6. ^{1}H,^{1}H-COSY spectrum of **4** (700 MHz, C_6D_6).

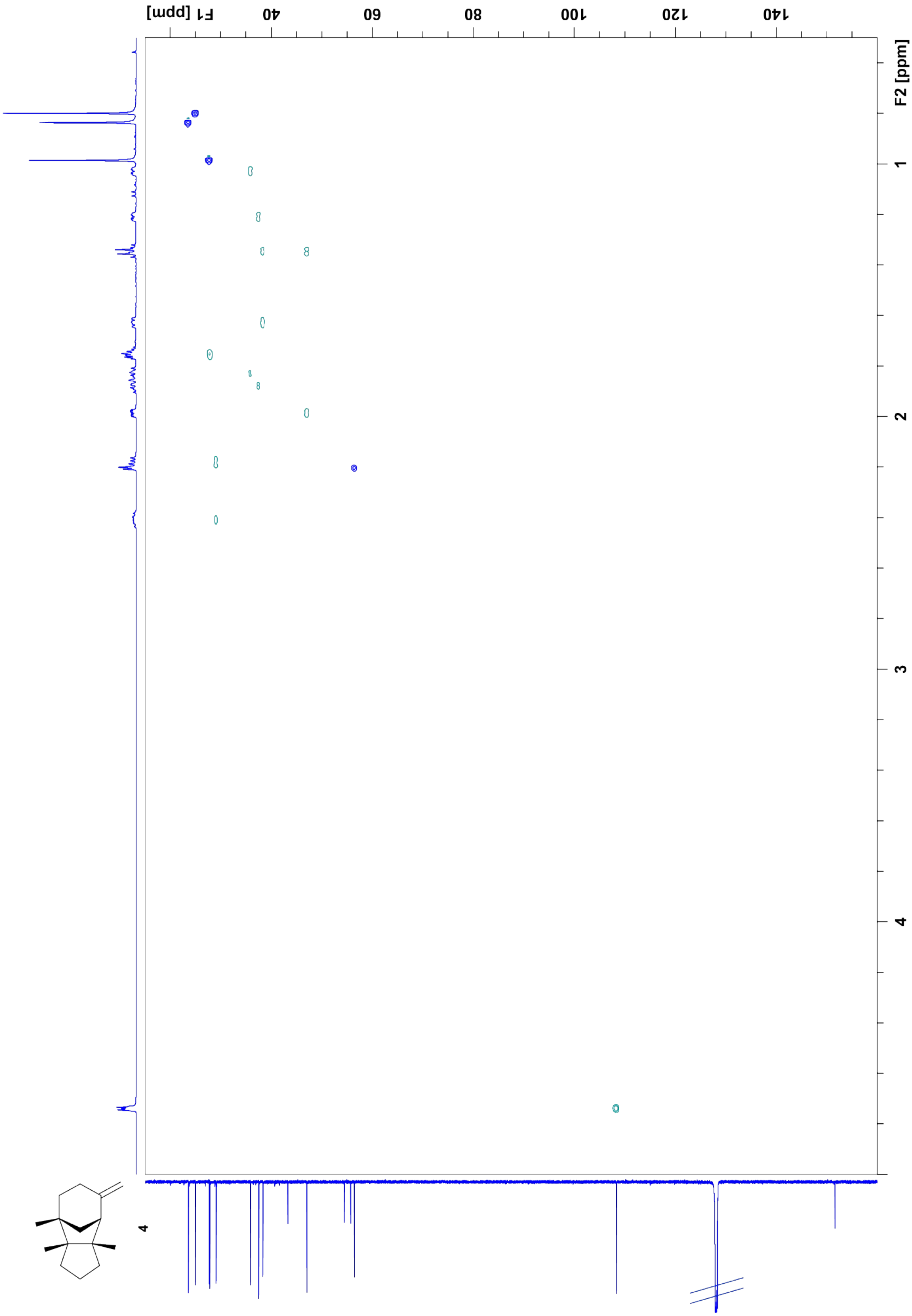


Figure S7. HSQC spectrum of **4** (700 MHz, C_6D_6).

Figure S8. HMBC spectrum of **4** (700 MHz, C_6D_6).

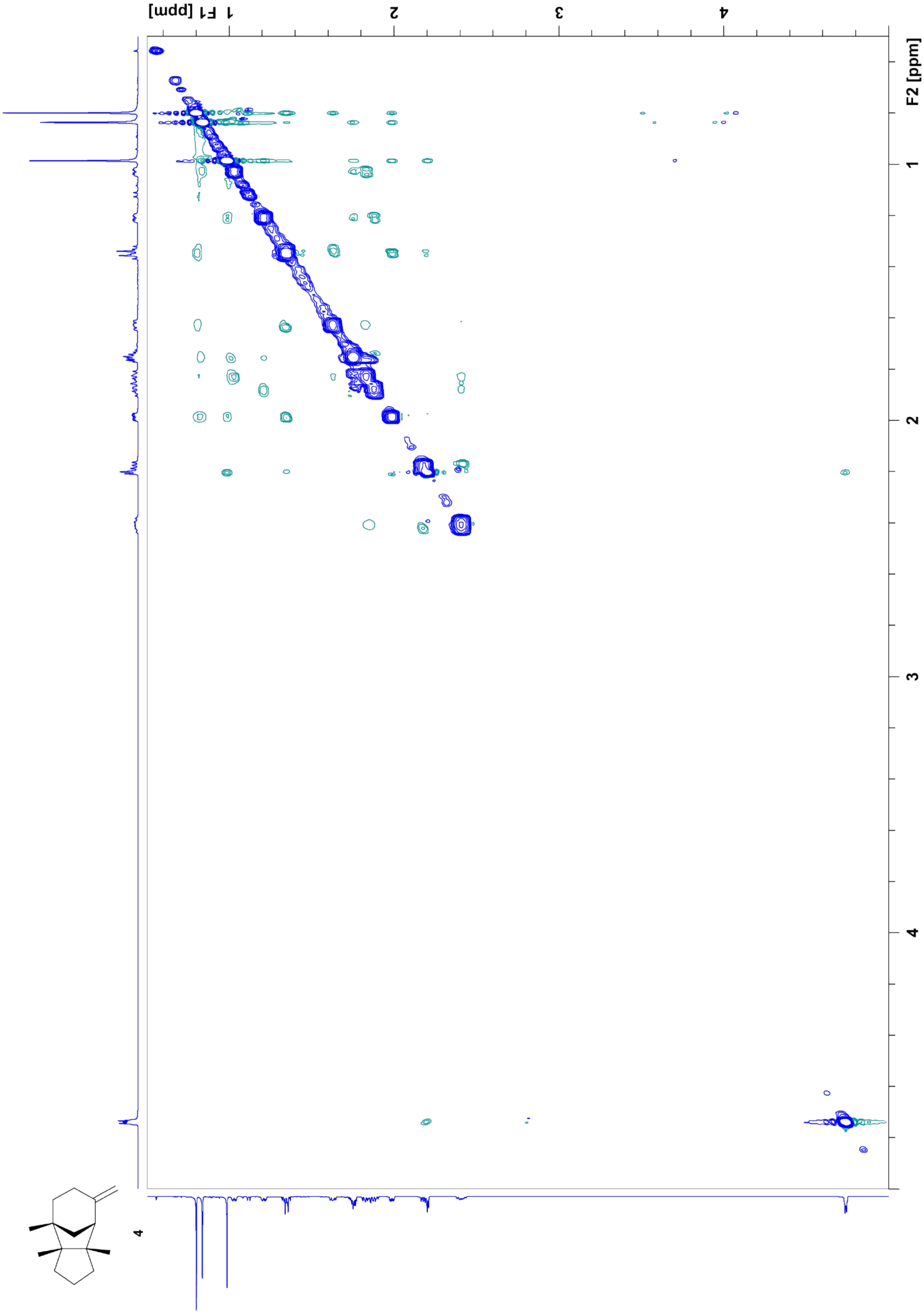


Figure S9. NOESY spectrum of **4** (700 MHz, C_6D_6).

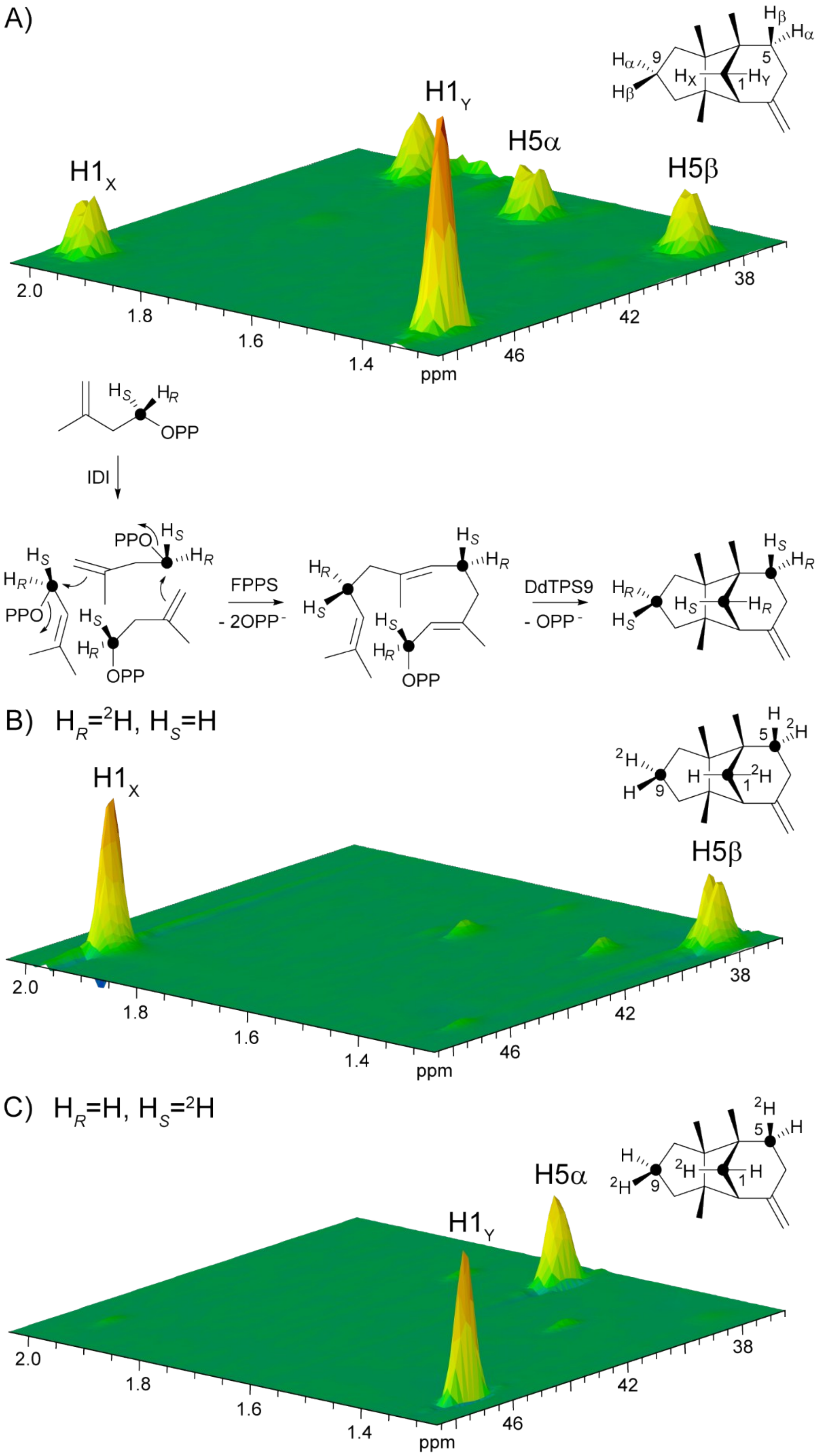


Figure S10. Partial HSQC spectra of A) unlabelled **4**, B) an incubation of (*R*)-(1-^{13}C,1-^{2}H)IPP[4] with IDI,[4] FPPS[5] and DdTPS9 and C) an incubation of (*S*)-(1-^{13}C,1-^{2}H)IPP[4] with IDI, FPPS and DdTPS9 showing the selective incorporation of deuterium into the methylene positions C1 and C5. The observed outcome is in line with the shown absolute configuration of **4**. Because of overlaying hydrogen signals, the labelled position C9 is not shown. Black dots represent ^{13}C-labelled carbon atoms.

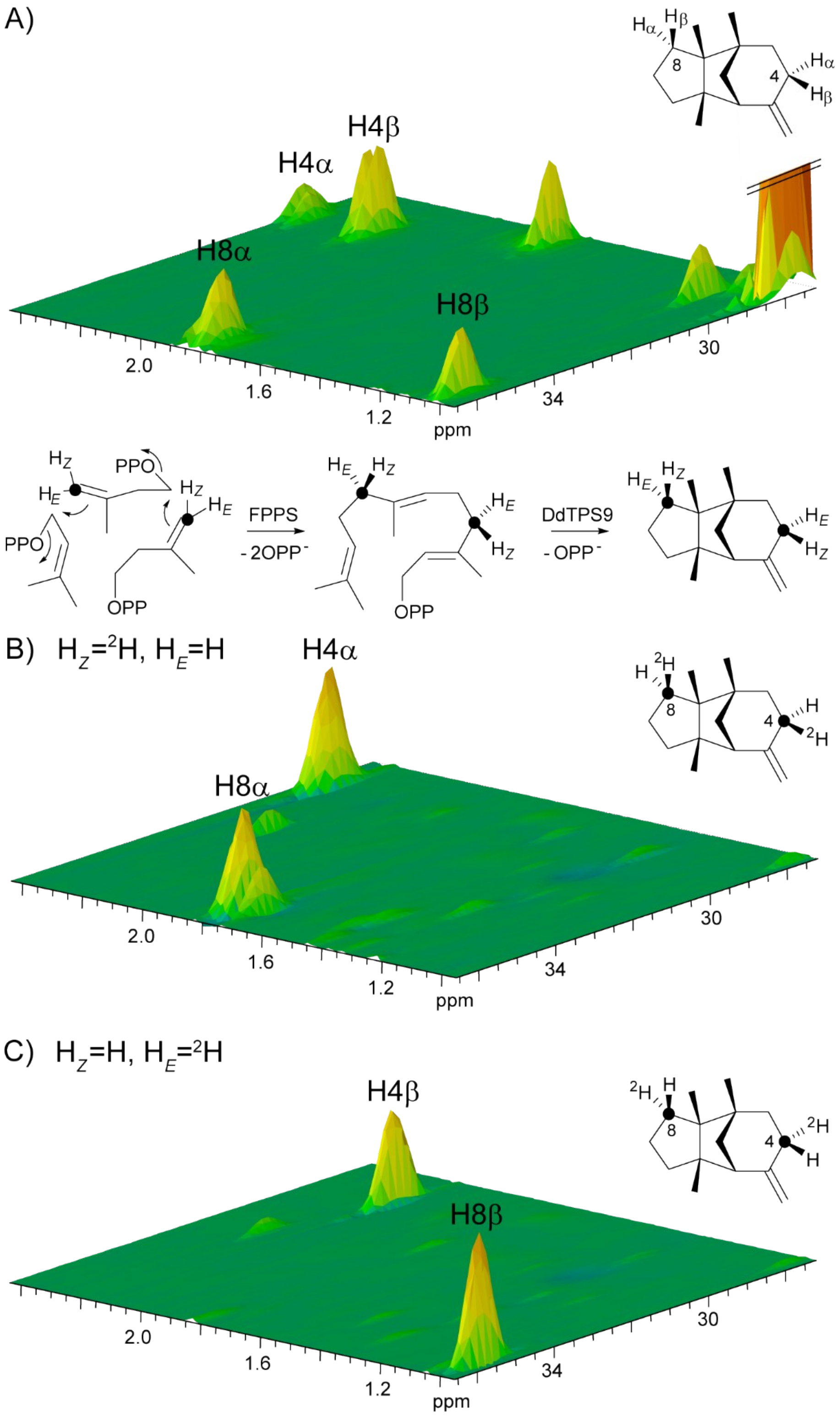


Figure S11. Partial HSQC spectra of A) unlabelled **4**, B) an incubation of (*Z*)-(4-^{13}C,4-^{2}H)IPP[6] with DMAPP, FPPS and DdTPS9 and C) an incubation of (*E*)-(4-^{13}C,4-^{2}H)IPP[6] with DMAPP, FPPS and DdTPS9 showing the selective incorporation of deuterium into the methylene positions C4 and C8. The observed outcome is in line with the shown absolute configuration of **4**. Black dots represent ^{13}C-labelled carbon atoms.

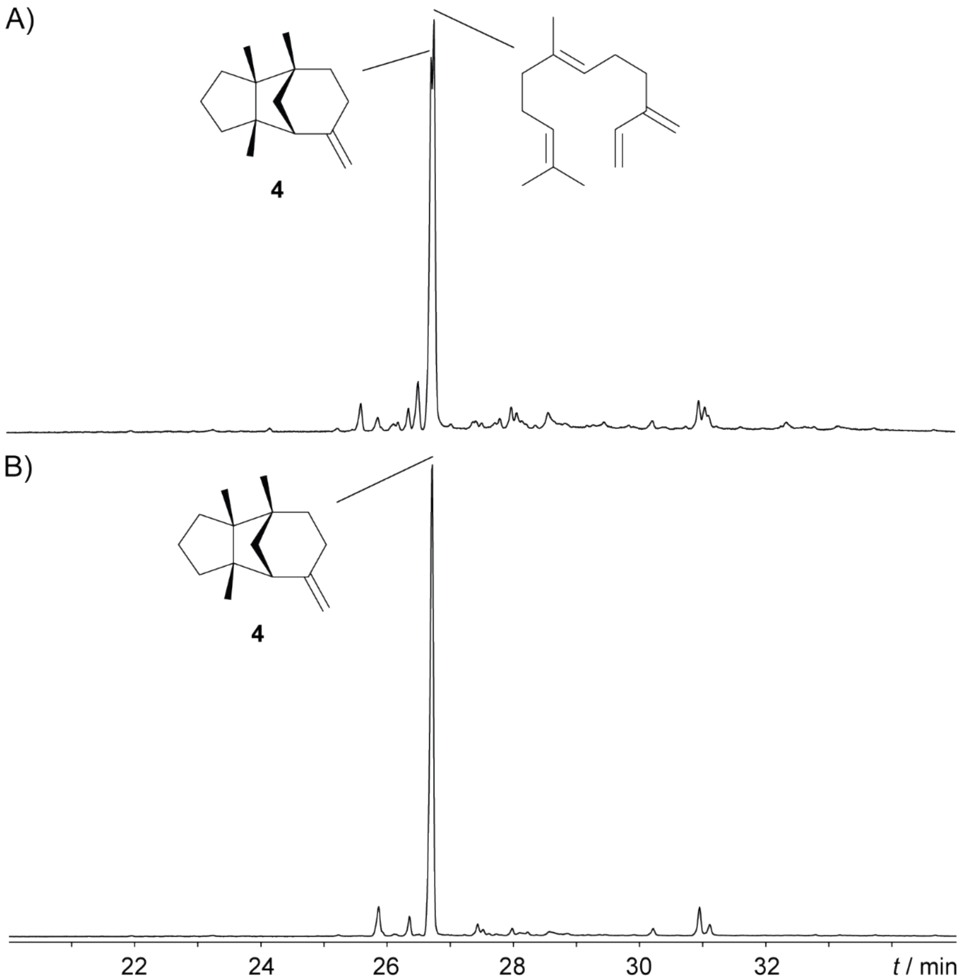


Figure S12. Total ion chromatograms of extracts from the incubation of DdTPS9 with A) (*R*)-NPP[7] and B) (*S*)-NPP.[7]

Incubation experiment of DdTPS9 with (2-^{13}C)DMAPP and (2-^{2}H)DMAPP

(2-^{2}H)DMAPP[8] (1 mg) was dissolved in substrate buffer (100 μL), diluted with binding buffer (180 μL) and incubation buffer (500 μL), before IDI elution fraction was added (220 mL). The reaction was incubated at 37 °C for 30 min, before heat inactivation was performed by keeping the sample for 10 min at 90 °C with shaking. The mixture was centrifuged (1 min, 14000 x g) and the soluble fraction was added to a mixture of (2-^{13}C)DMAPP[9] dissolved in substrate buffer (1.12 mL), incubation buffer (5 mL), binding buffer (2 mL), FPPS elution fraction (440 μL) and DdTPS9 elution fraction (440 μL). The reaction was further incubated for 3 h at 28 °C, extracted with C_6D_6 (650 μL; 250 μL) and analysed by NMR.

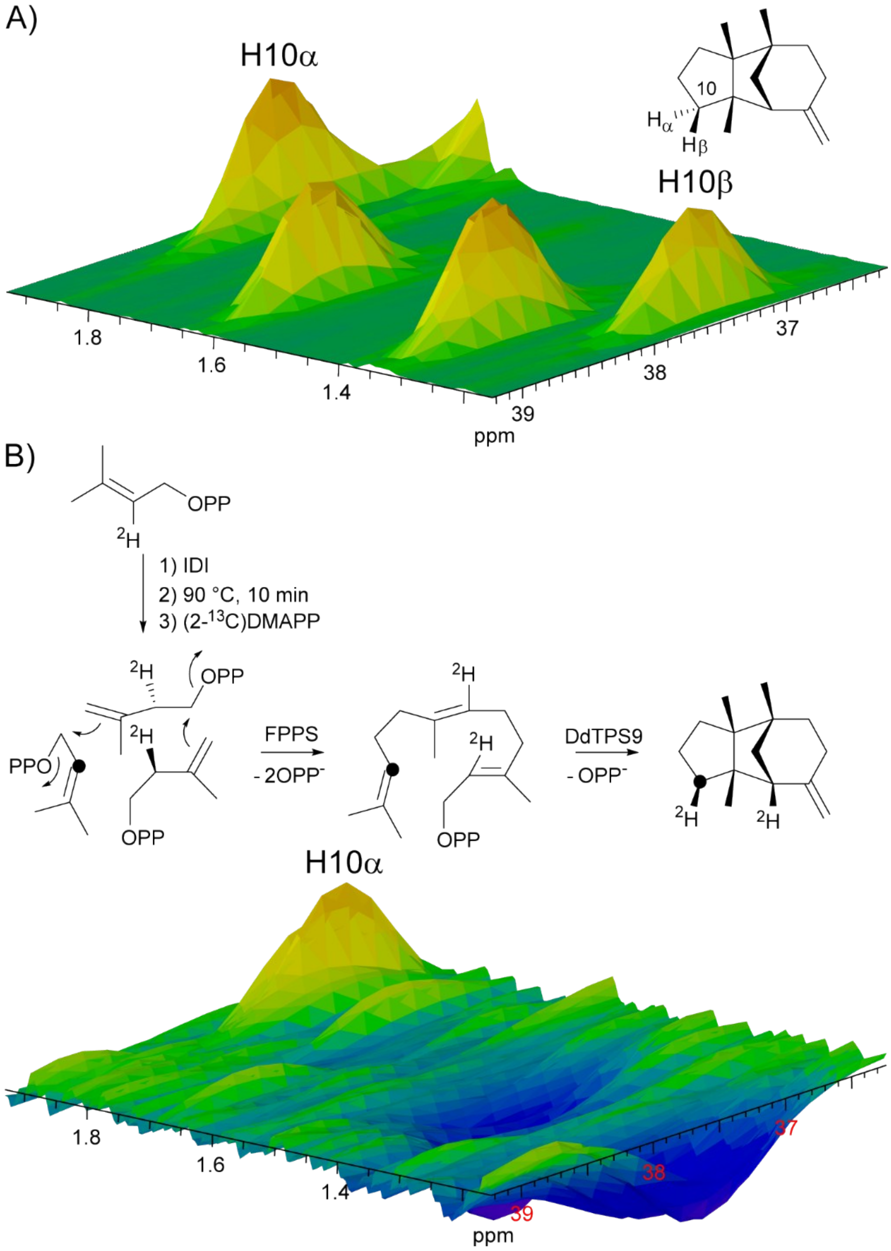


Figure S13. Movement of H6 to C10 in the cyclisation mechanism of **4**. Partial HSQC spectra of A) unlabelled **4** and B) an incubation experiment using (2-^{2}H)DMAPP with IDI, followed by heat inactivation of IDI, addition of (2-^{13}C)DMAPP, FPPS and DdTPS9. Note that HSQC B) was recorded in biphasic mode to differentiate between CH/CH_3 signals (positive crosspeaks, orange) and CH_2 signals (negative crosspeaks, blue, caused by contaminants in the extracted mixture and are not belonging to labelled **4**). Black dots represent ^{13}C-labelled carbon atoms.

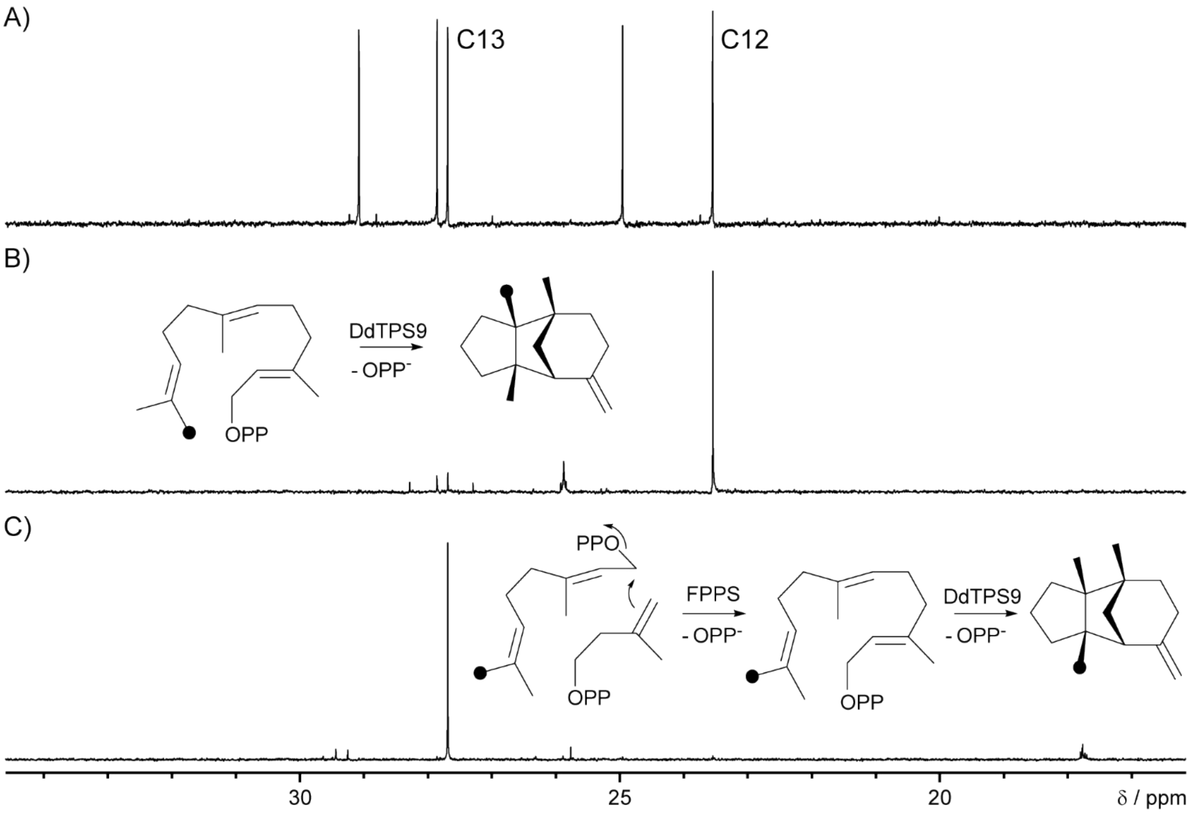


Figure S14. Partial ^{13}C-NMR spectra of A) unlabelled **4**, B) incubation of (12-^{13}C)FPP[10] with DdTPS9 and C) incubation of (9-^{13}C)GPP,[11] FPPS and DdTPS9. Black dots represent ^{13}C-labelled carbon atoms. The minor peak of C13 in B) is caused by an impure labelling of the starting material.

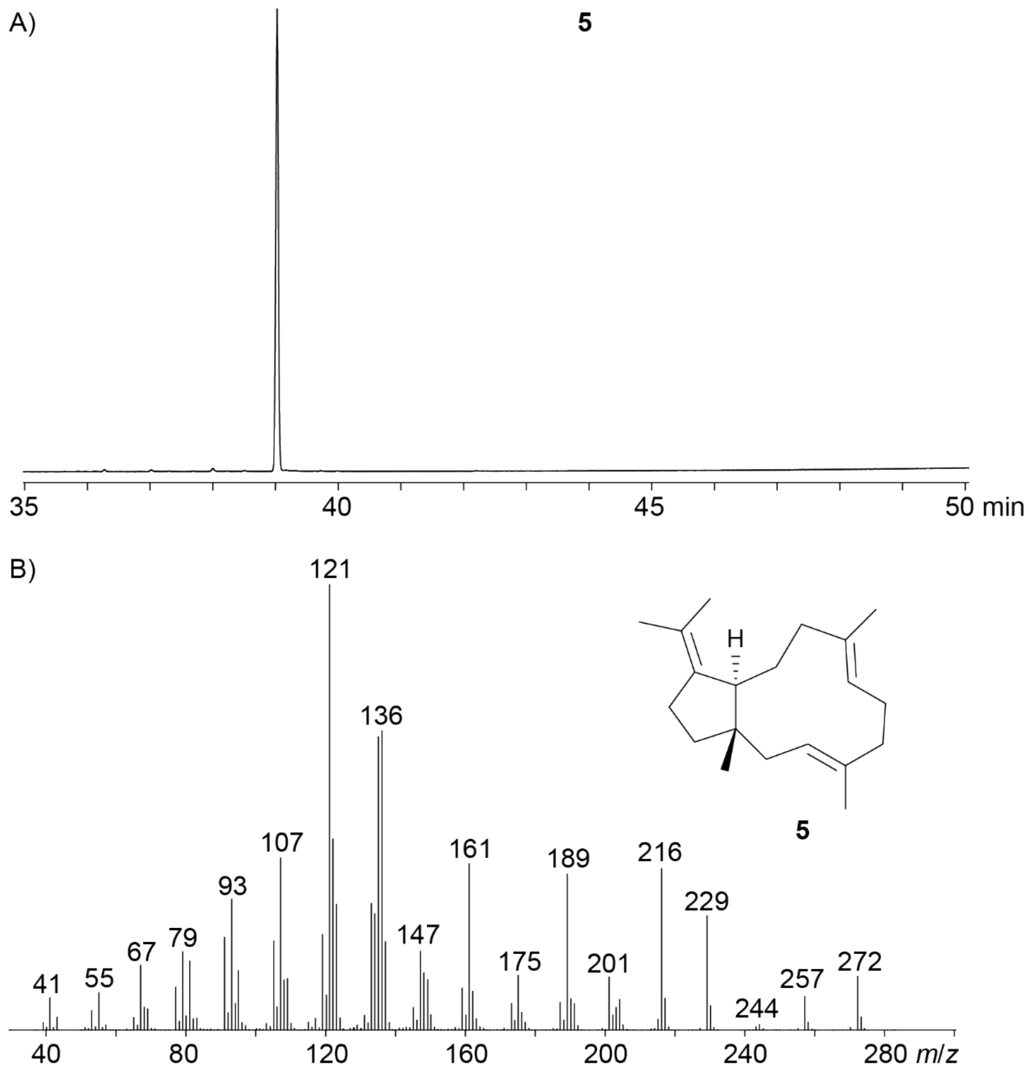
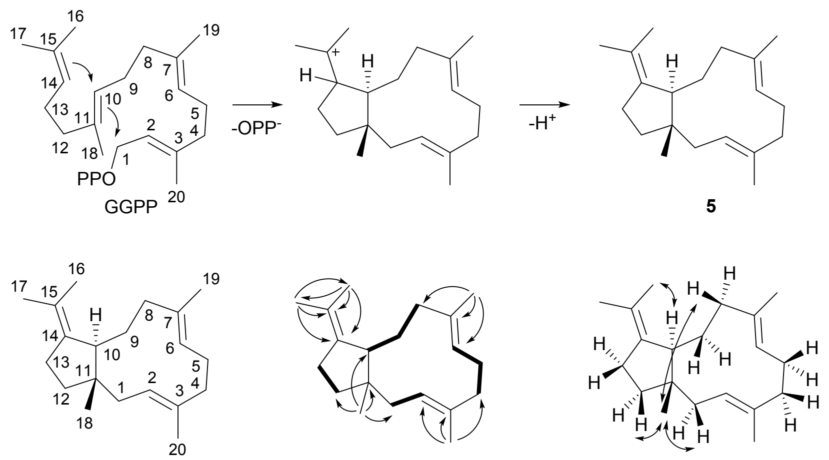


Figure S15. A) Total ion chromatogram of an extract from the incubation of GGPP with DpTPS10. B) EI mass spectrum of β-araneosene (**5**).



Scheme S2. Short biosynthesis and structure elucidation of **5**. Carbon numbers refer to the corresponding positions in GGPP. H,H-COSY spin systems are represented by bold bonds, single headed arrows show HMBC correlations and NOESY correlations are depicted by double headed arrows.

Table S3. NMR data of β-araneosene (**5**) in C_6D_6 recorded at 298 K.

C[a]		^{13}C[b]	^{1}H[b]	^{13}C[c]
1	CH_2	39.16	2.20 (dd, J = 12.5, 11.5, H_α)	38.7
			1.52 (m, H_β)	
2	CH	126.39	5.28 (ddm, J = 11.3, 5.6)	126.0
3	C_q	134.77	–	134.8
4	CH_2	40.30	2.16 (m, H_α)	40.0
			2.08 (m, H_β)	
5	CH_2	24.68	2.27 (m, H_α)	24.3
			2.04 (m, H_β)	
6	CH	129.93	4.92 (br d, J = 10.8)	129.3
7	C_q	132.27	–	132.5
8	CH_2	38.65	2.31 (ddd, J = 12.7, 12.7, 5.7, H_β)	38.2
			2.07 (m, H_α)	
9	CH_2	28.27	1.53 (m, H_β)	27.9
			1.49 (m, H_α)	
10	CH	42.43	2.52 (d, J = 11.0)	42.1
11	C_q	48.69	–	48.4
12	CH_2	40.63	1.66 (ddd, J = 12.7, 10.3, 10.3, H_β)	40.3
			1.43 (dddd, J = 12.7, 8.1, 3.0, 0.7, H_α)	
13	CH_2	28.67	2.26 (m, H_β)	28.3
			2.24 (m, H_α)	
14	C_q	143.05	–	142.5
15	C_q	122.11	–	122.0
16	CH_3	21.50	1.68 (br s)	21.2
17	CH_3	21.84	1.62 (br s)	21.7
18	CH_3	23.90	1.11 (s)	23.6
19	CH_3	16.43	1.63 (br s)	16.3
20	CH_3	15.47	1.46 (br s)	15.3

[a] Carbon numbering indicates the origin of each carbon from GGPP by identical number as shown in Scheme S2. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, br = broad, coupling constants J are given in Hertz. [c] ^{13}C-NMR data in $CDCl_3$ from ref. [12].

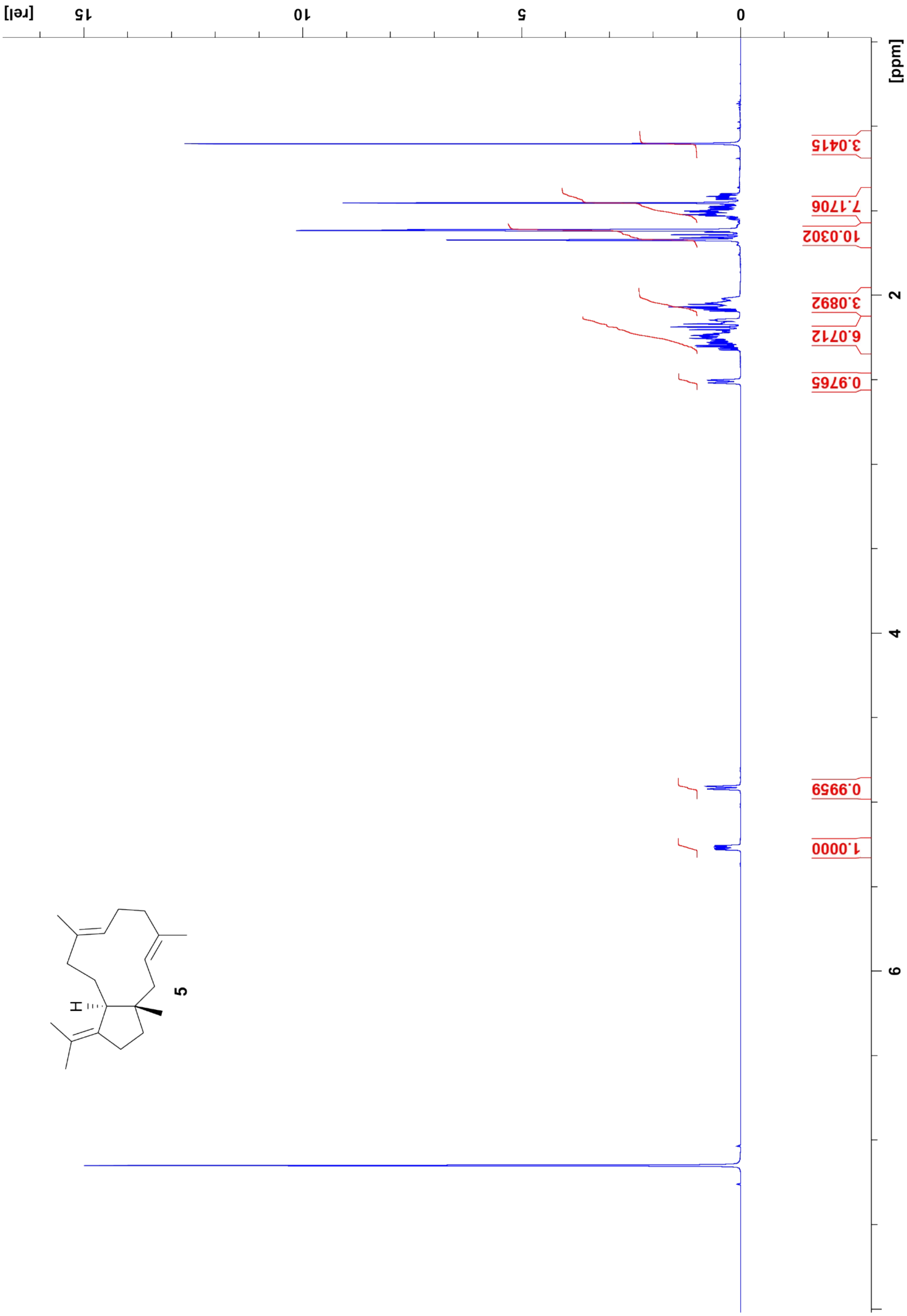


Figure S16. ^{1}H-NMR spectrum of **5** (700 MHz, C_6D_6).

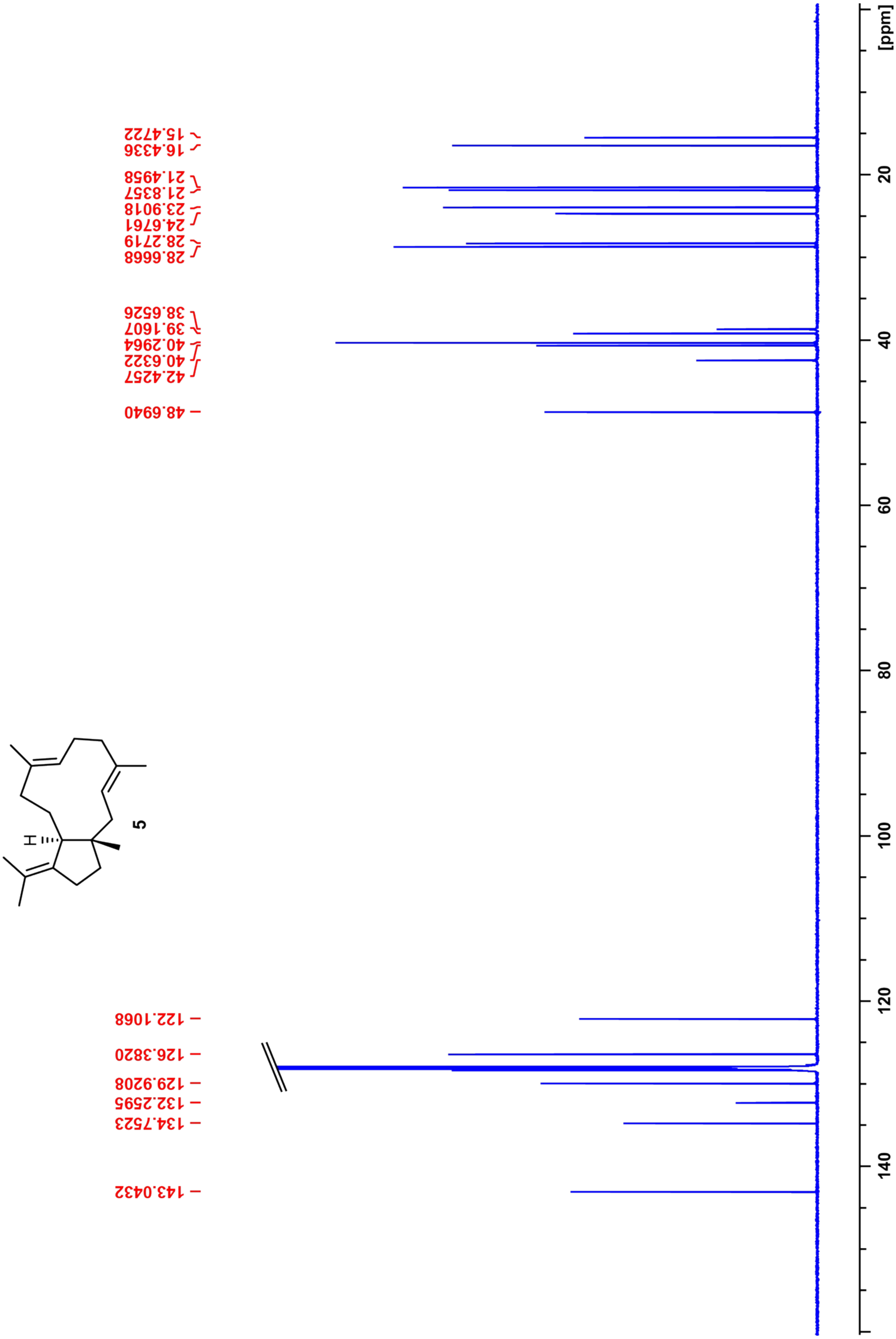


Figure S17. ^{13}C-NMR spectrum of **5** (175 MHz, C_6D_6).

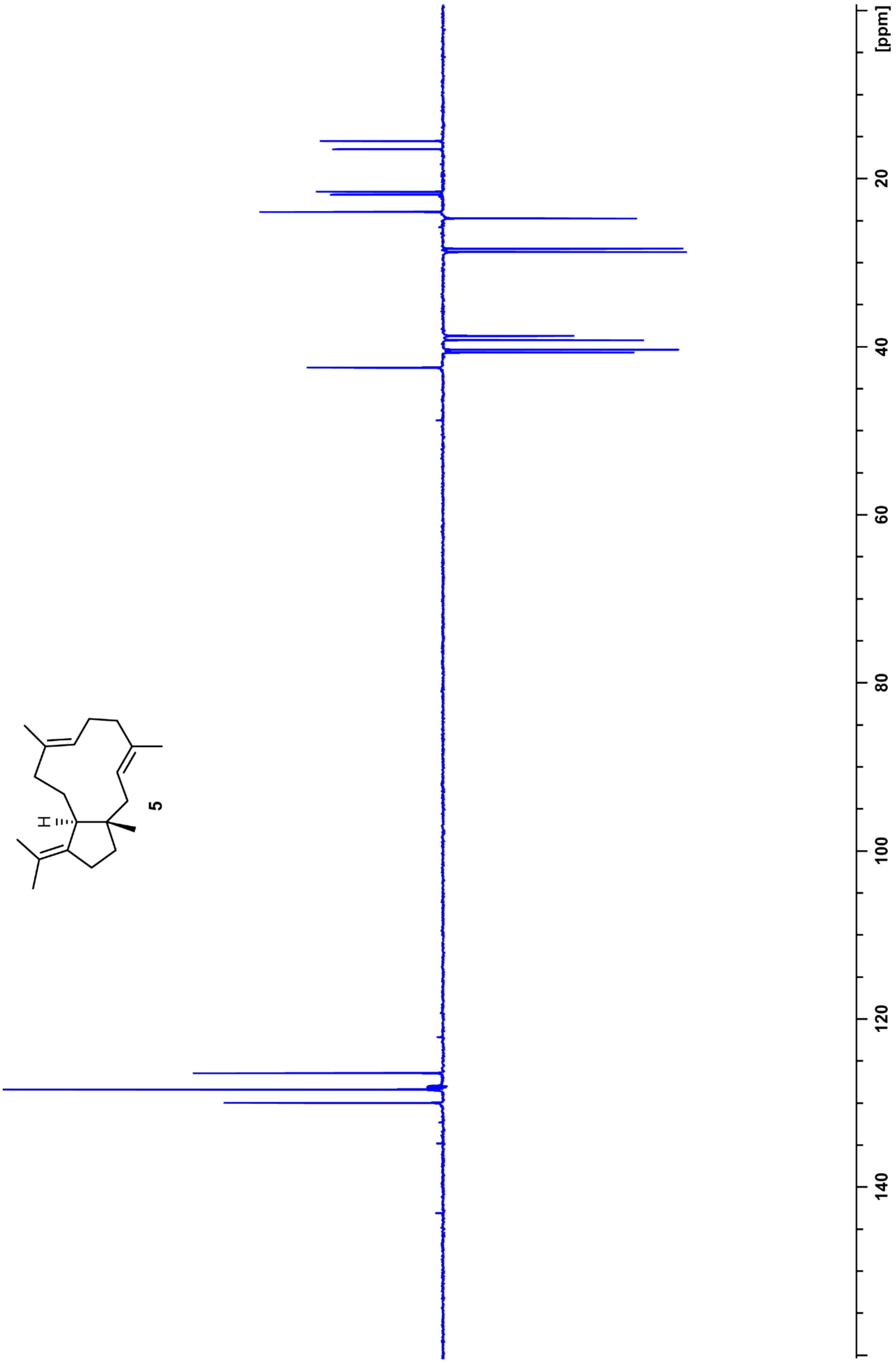


Figure S18. ^{13}C-DEPT spectrum of **5** (175 MHz, C_6D_6).

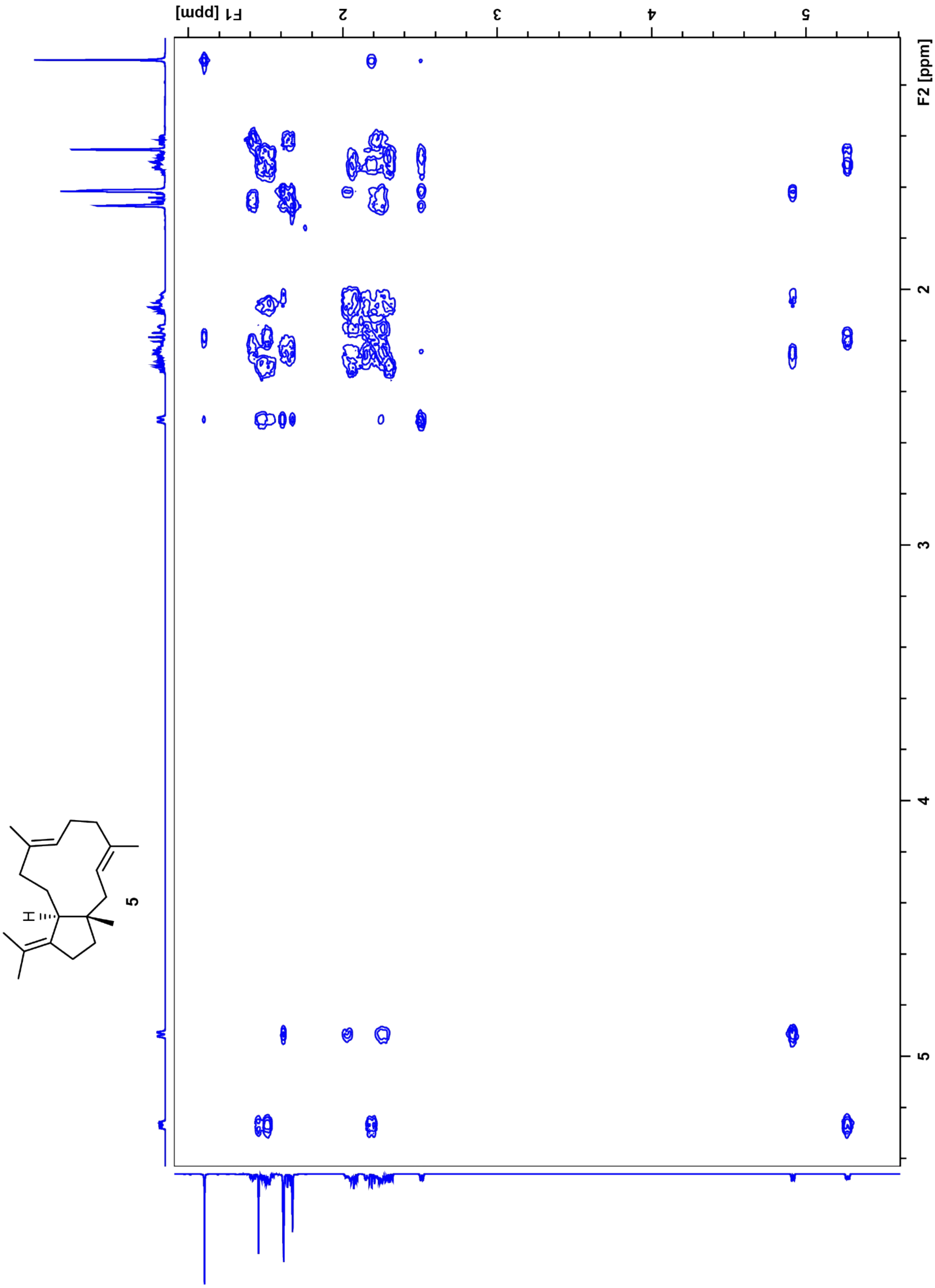


Figure S19. $^{1}H,^{1}H$-COSY spectrum of **5** (700 MHz, C_6D_6).

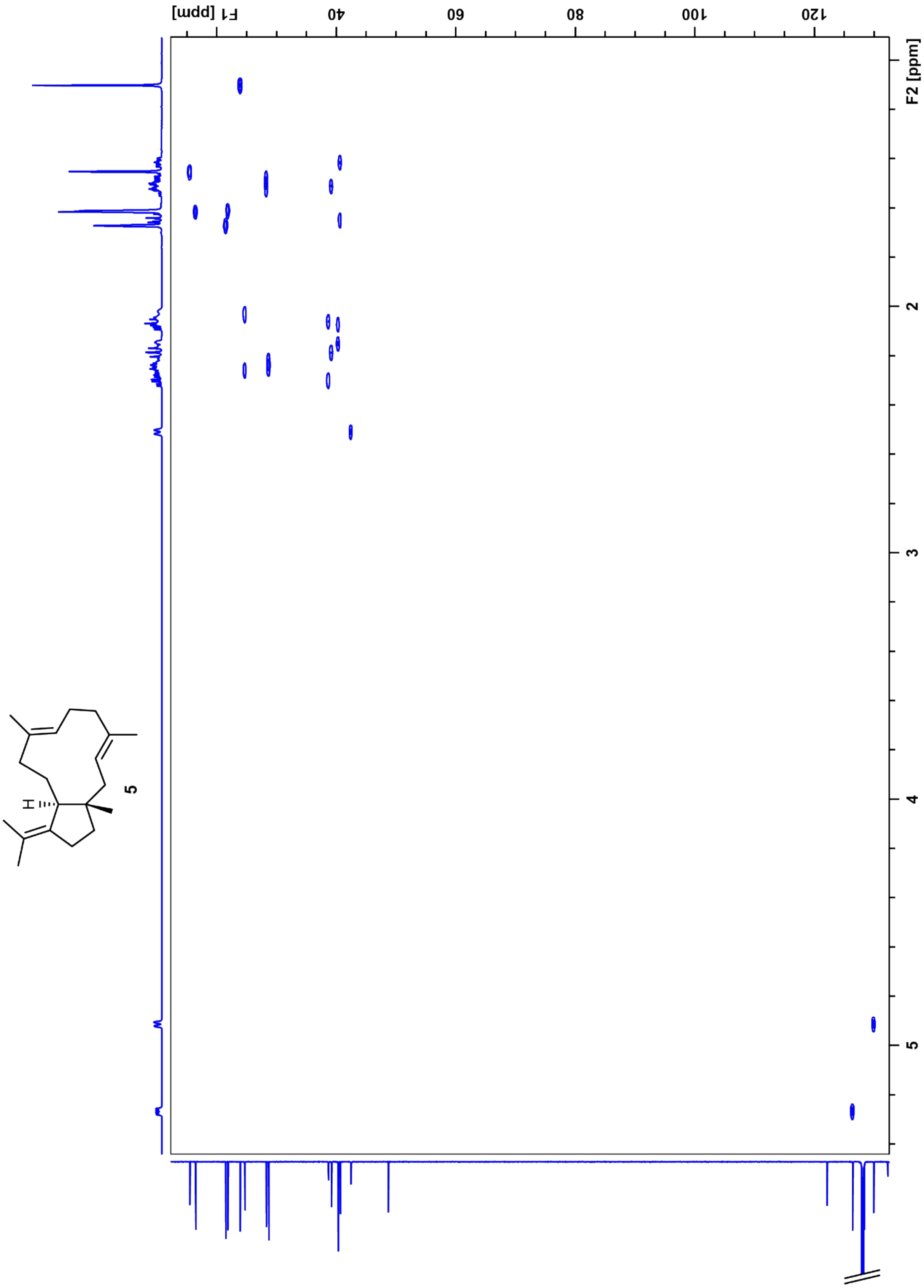


Figure S20. HSQC spectrum of **5** (700 MHz, C_6D_6).

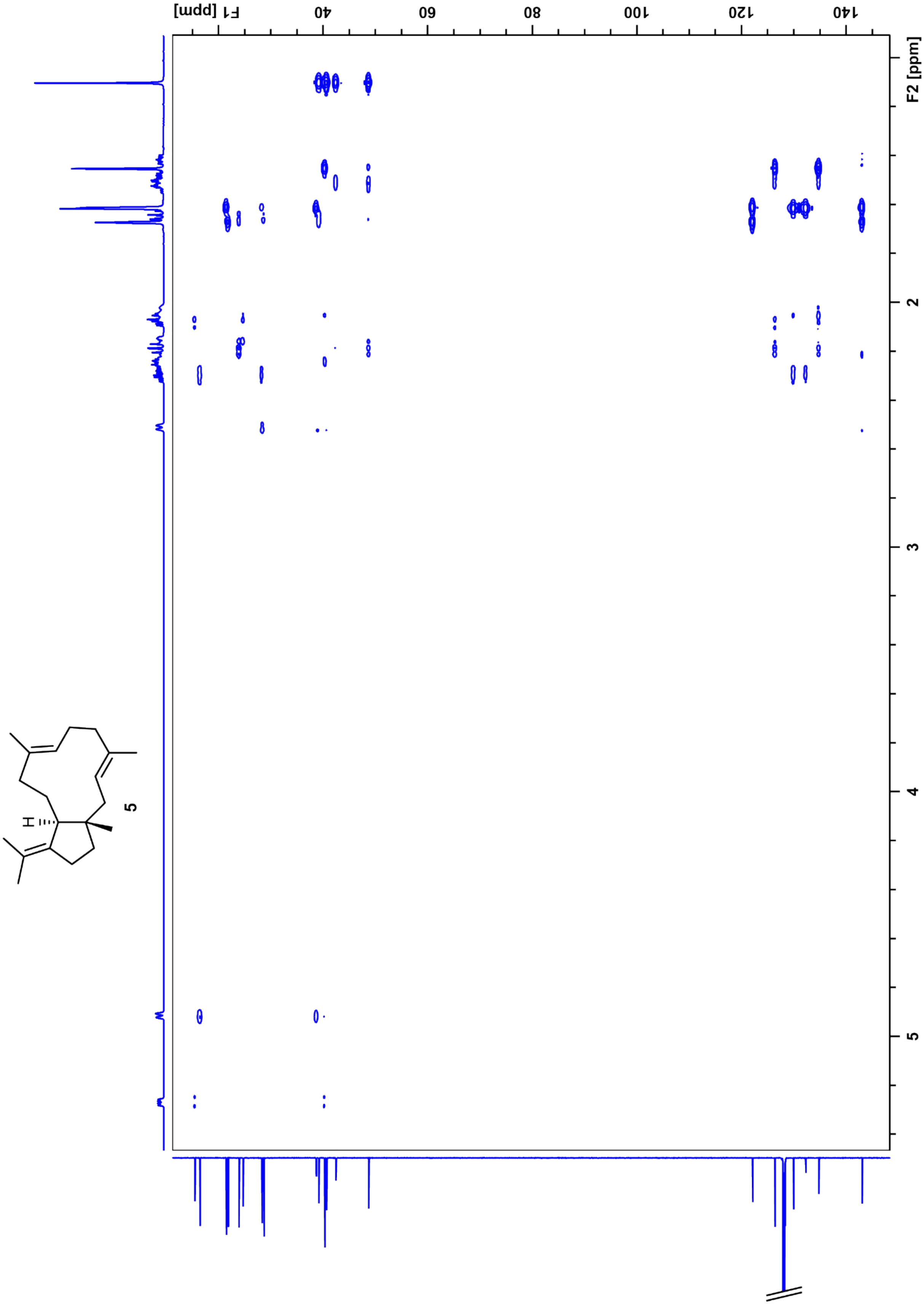
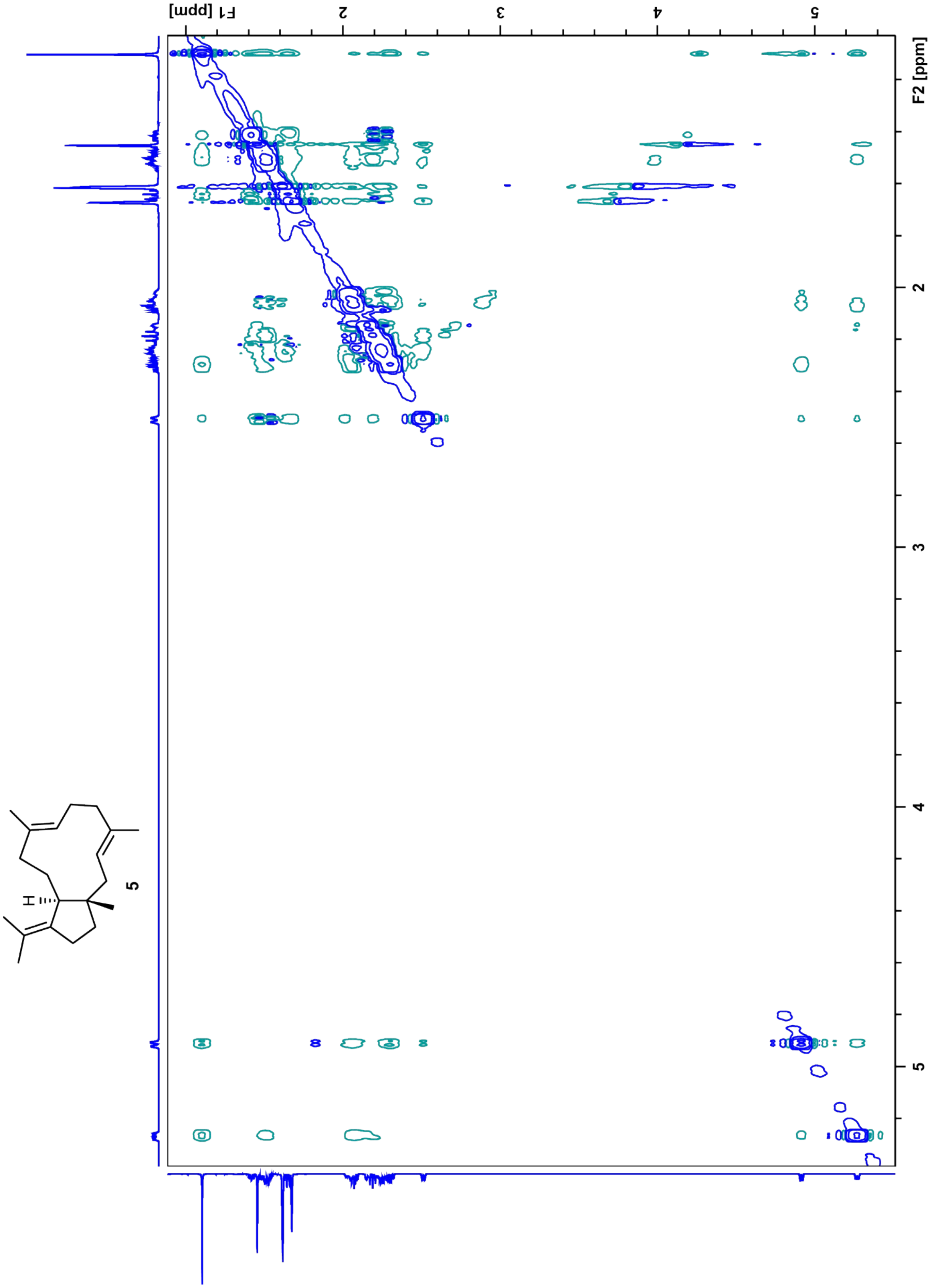


Figure S21. HMBC spectrum of **5** (700 MHz, C_6D_6).



5

Figure S22. NOESY spectrum of **5** (700 MHz, C_6D_6).

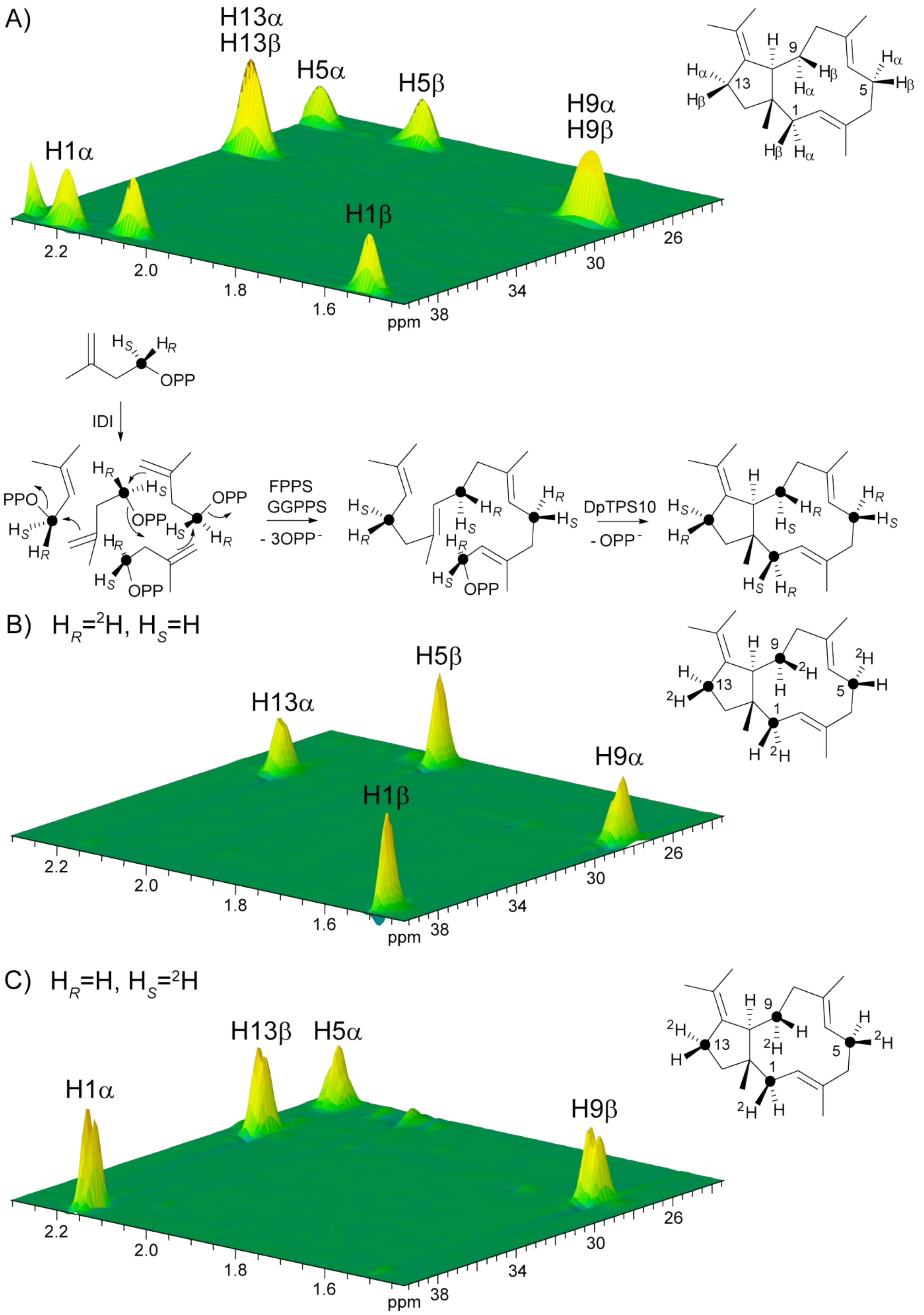


Figure S23. Partial HSQC spectra of A) unlabelled **5**, B) an incubation of (*R*)-(1-^{13}C,1-^{2}H)IPP with IDI, FPPS, GGPPS[13] and DpTPS10 and C) an incubation of (*S*)-(1-^{13}C,1-^{2}H)IPP with IDI, FPPS, GGPPS and DpTPS10 showing the selective incorporation of deuterium into the methylene positions C1, C5, C9 and C13. The observed outcome is in line with the shown absolute configuration of **5** for C1, C9 and C13. Determination of the relative configuration of C5 by NOE correlations was not possible. Black dots represent ^{13}C-labelled carbon atoms.

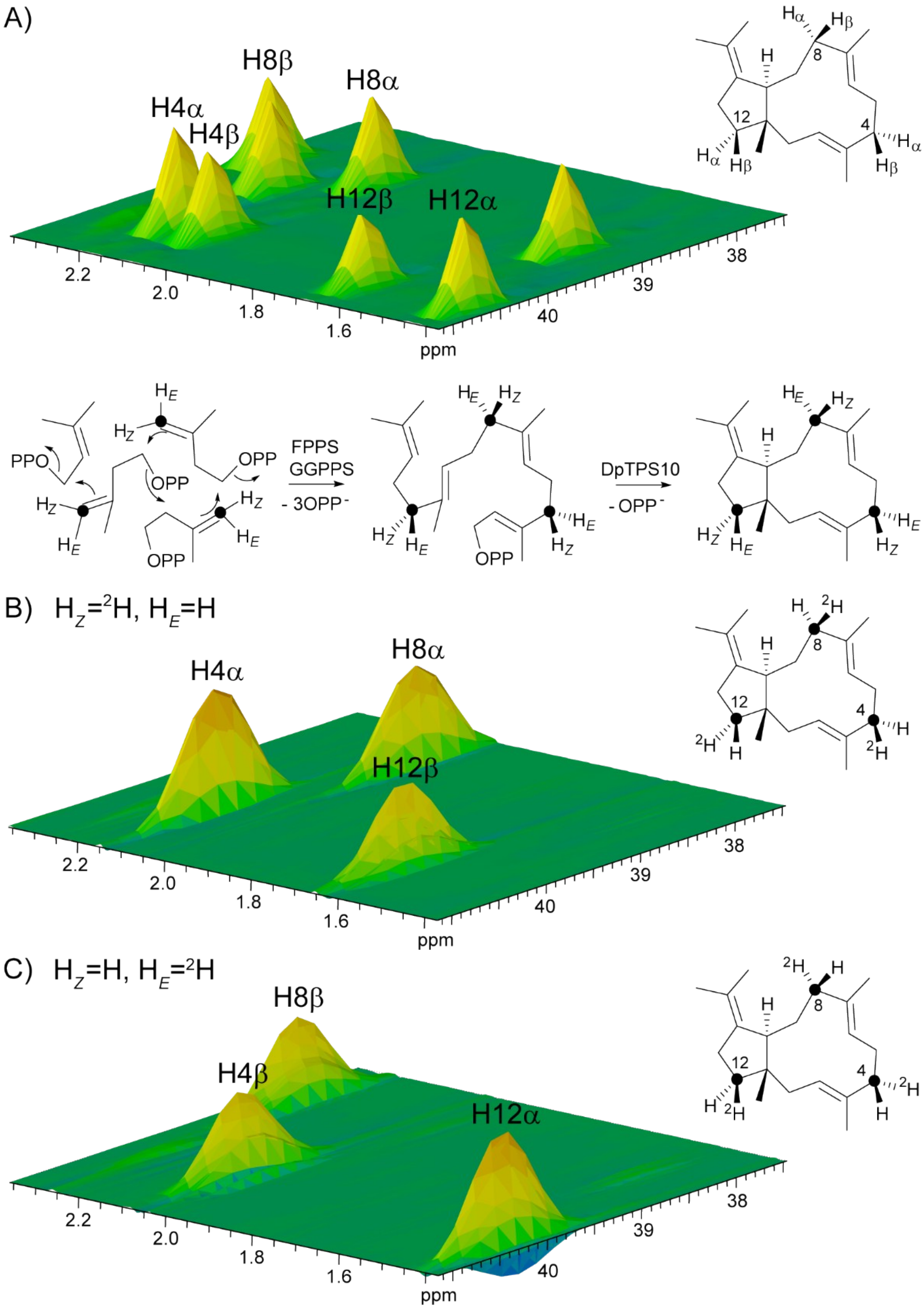


Figure S24. Partial HSQC spectra of A) unlabelled **5**, B) an incubation of (*Z*)-(4-^{13}C,4-^{2}H)IPP with DMAPP, FPPS, GGPPS and DpTPS10 and C) an incubation of (*E*)-(4-^{13}C,4-^{2}H)IPP with DMAPP, FPPS, GGPPS and DpTPS10 showing the selective incorporation of deuterium into the methylene positions C4, C8 and C12. The observed outcome is in line with the shown absolute configuration of **4** regarding C8 and C12. For C4, the relative configuration could not be obtained from the NOESY spectrum. Black dots represent ^{13}C-labelled carbon atoms.

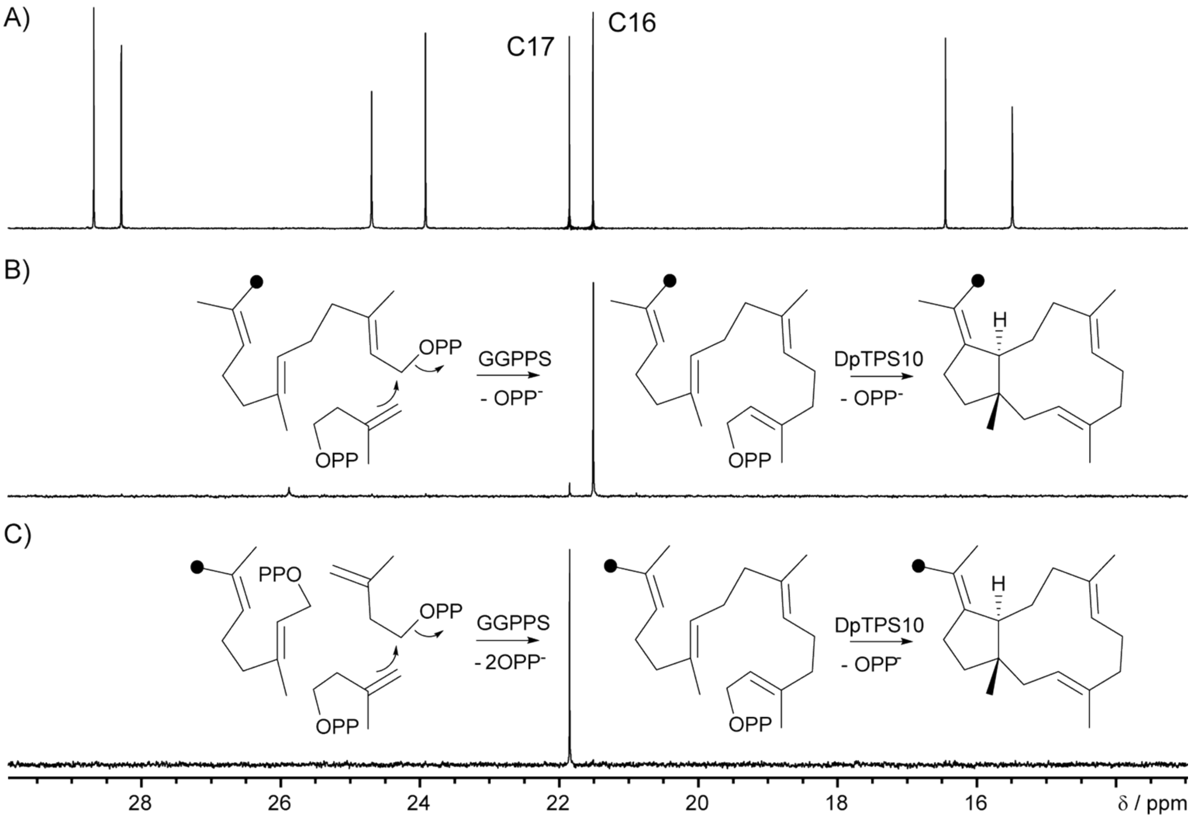


Figure S25. Partial ^{13}C-NMR spectra of A) unlabelled **5**, B) incubation of (12-^{13}C)FPP with IPP, GGPPS and DpTPS10 and C) incubation of (9-^{13}C)GPP, IPP, GGPPS and DpTPS10. Black dots represent ^{13}C-labelled carbon atoms. The minor peak of C17 in B) is caused by an impure labelling of the starting material.

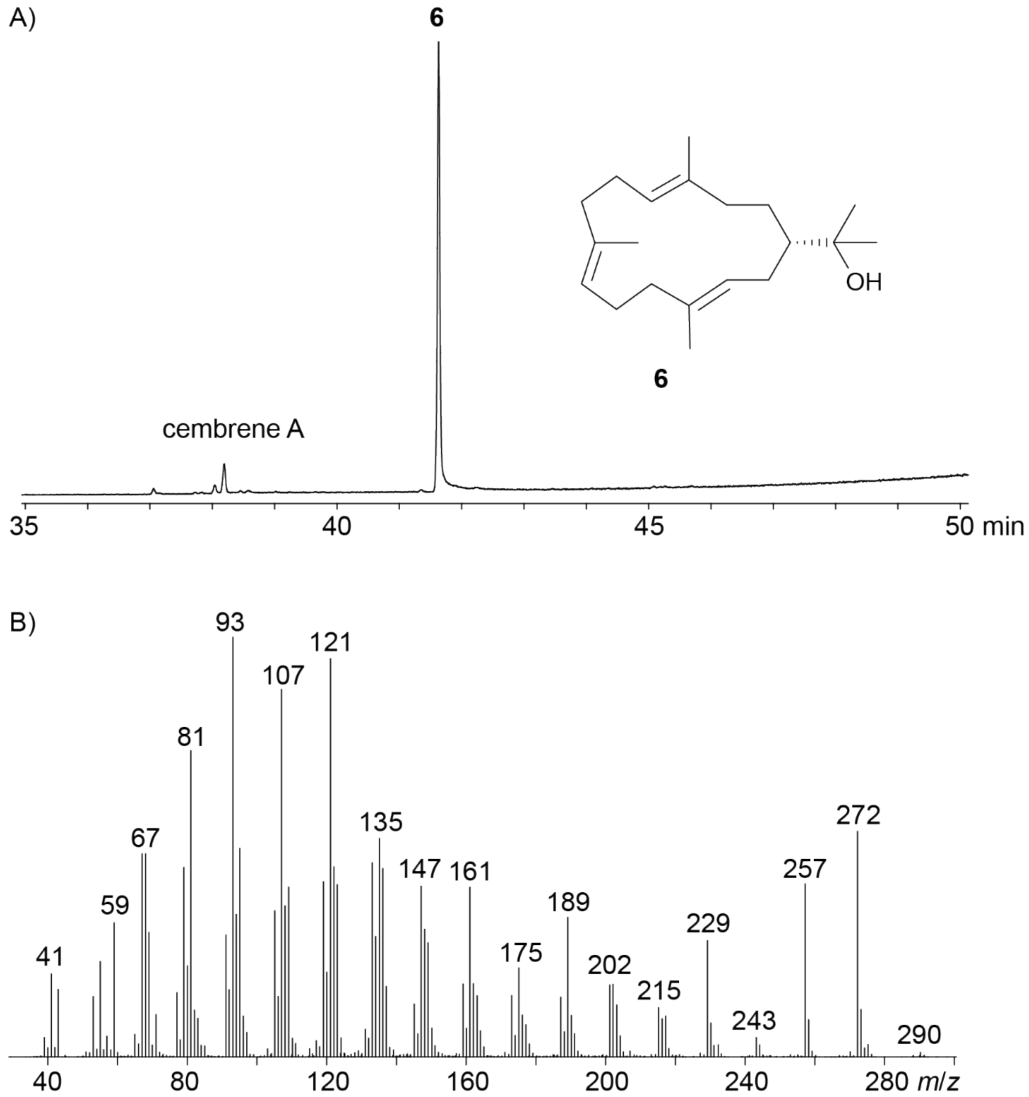
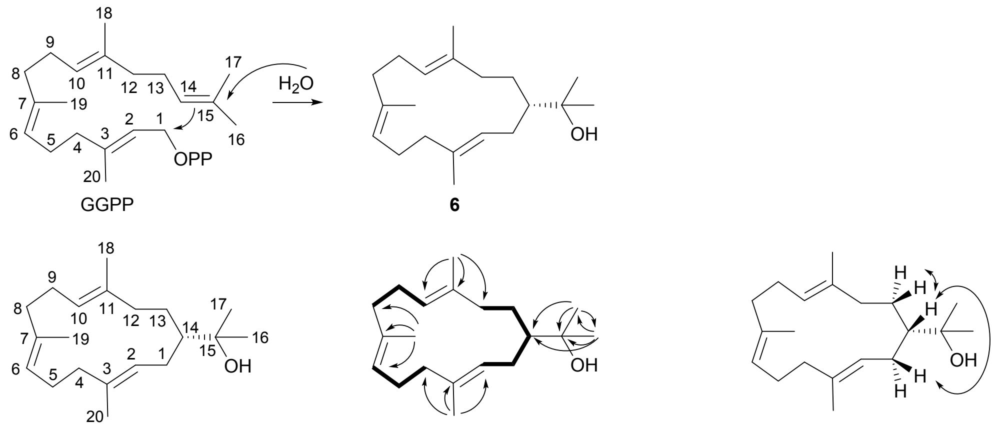


Figure S26. A) Total ion chromatogram of an extract from the incubation of GGPP with DpTPS11. B) EI mass spectrum of nephthenol (**6**).



Scheme S3. Short biosynthesis and structure elucidation of **6**. Carbon numbers refer to the corresponding positions in GGPP. H,H-COSY spin systems are represented by bold bonds, single headed arrows show HMBC correlations and NOESY correlations are depicted by double headed arrows.

Table S4. NMR data of nephthenol (**6**) in C_6D_6 recorded at 298 K.

C[a]		^{13}C[b]	^{1}H[b]	^{13}C[c]
1	CH_2	29.03	2.20 (m)	28.5
			1.91 (dddd, *J* = 7.6, 7.6, 7.6, 7.6)	
2	CH	126.92	5.26 (tm, *J* = 7.0)	125.9
3	C_q	133.03	–	134.4
4	CH_2	39.30	2.13 (m, 2H)	38.8
5	CH_2	25.15	2.18 (m, 2H)	24.7
6	CH	126.28	5.07 (tm, *J* = 6.9)	125.8
7	C_q	133.01	–	133.0
8	CH_2	39.87	2.11 (m)	39.4
			2.06 (m)	
9	CH_2	24.48	2.13 (m, 2H)	24.0
10	CH	125.32	5.16 (tm, *J* = 6.6)	125.0
11	C_q	134.27	–	134.0
12	CH_2	38.21	2.16 (m)	37.7
			2.08 (m)	
13	CH_2	28.76	1.66 (m)	28.3
			1.24 (m)	
14	CH	48.79	1.34 (m)	48.5
15	C_q	73.21	–	73.9
16	CH_3	27.83[d]	1.07 (s)[d]	27.7[d]
17	CH_3	27.86[d]	1.06 (s)[d]	27.5[d]
18	CH_3	15.78	1.59 (s)	15.6
19	CH_3	15.43	1.54 (s)	15.3
20	CH_3	15.68	1.57 (s)	15.6

[a] Carbon numbering indicates the origin of each carbon from GGPP by identical number as shown in Scheme S3. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, br = broad, coupling constants *J* are given in Hertz. [c] ^{13}C-NMR data in $CDCl_3$ from ref. [14]. [d] Signals may be interchanged.

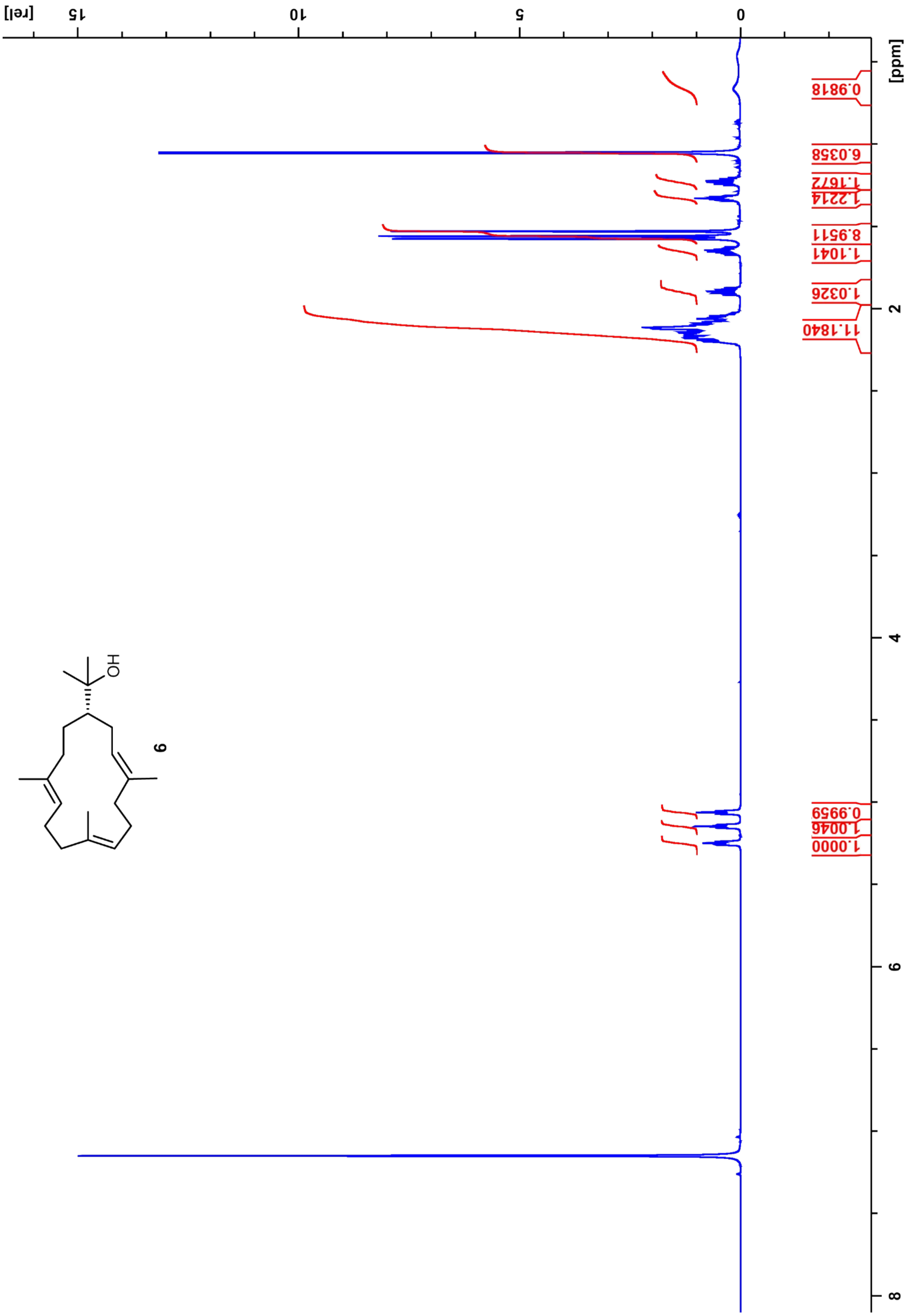


Figure S27. [1]H-NMR spectrum of **6** (700 MHz, C_6D_6).

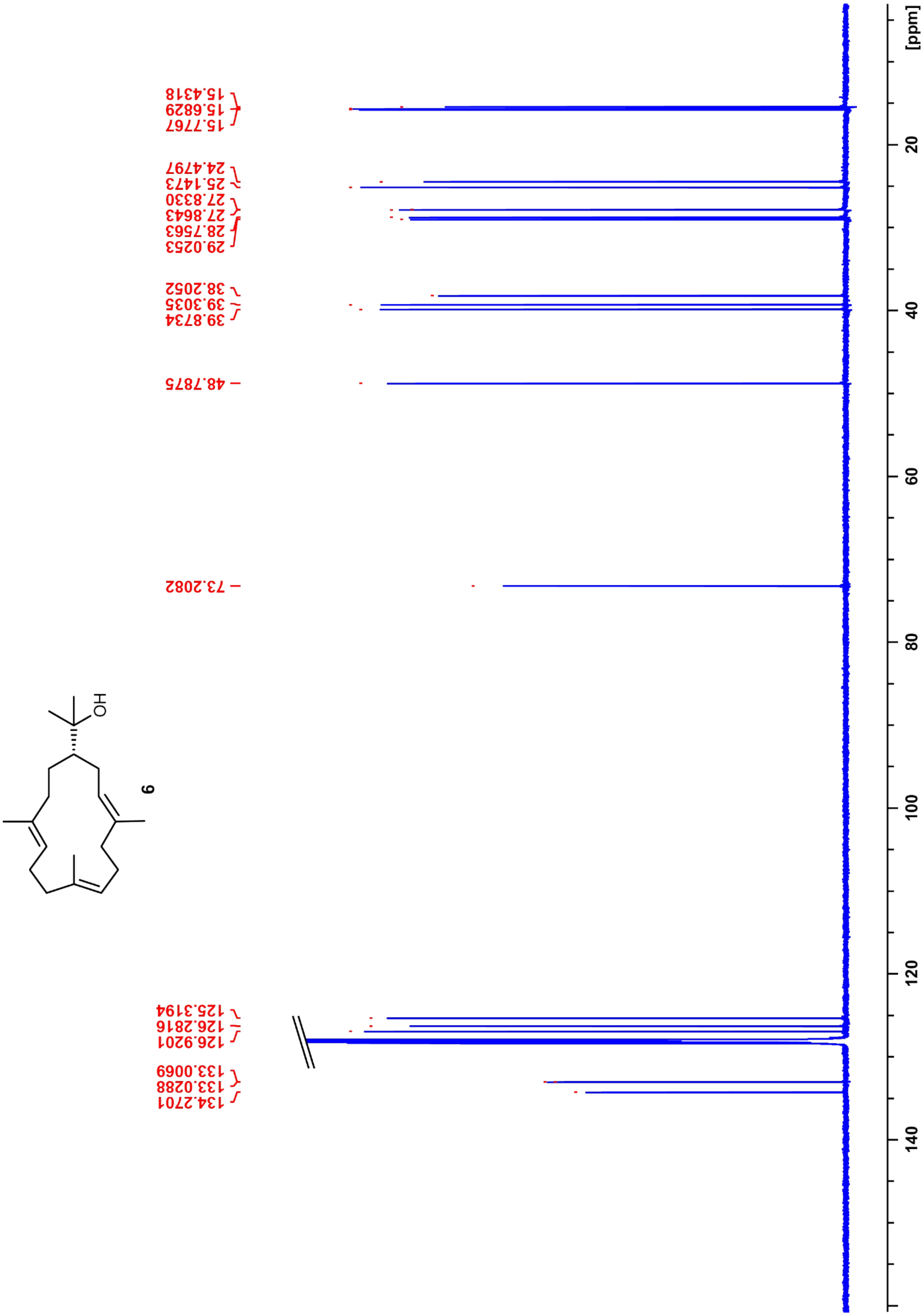


Figure S28. ^{13}C-NMR spectrum of **6** (175 MHz, C_6D_6).

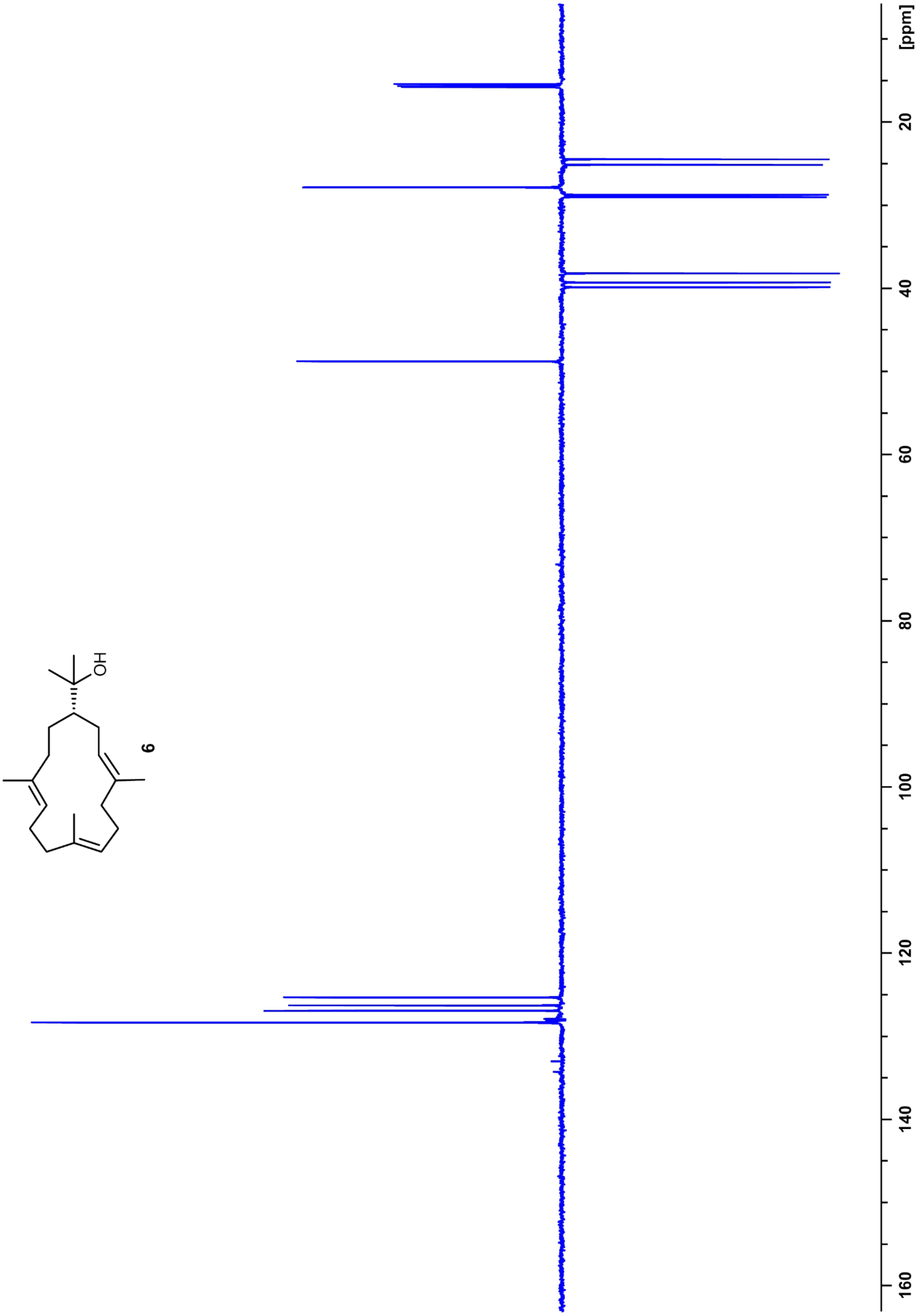


Figure S29. ^{13}C-DEPT spectrum of **6** (175 MHz, C_6D_6).

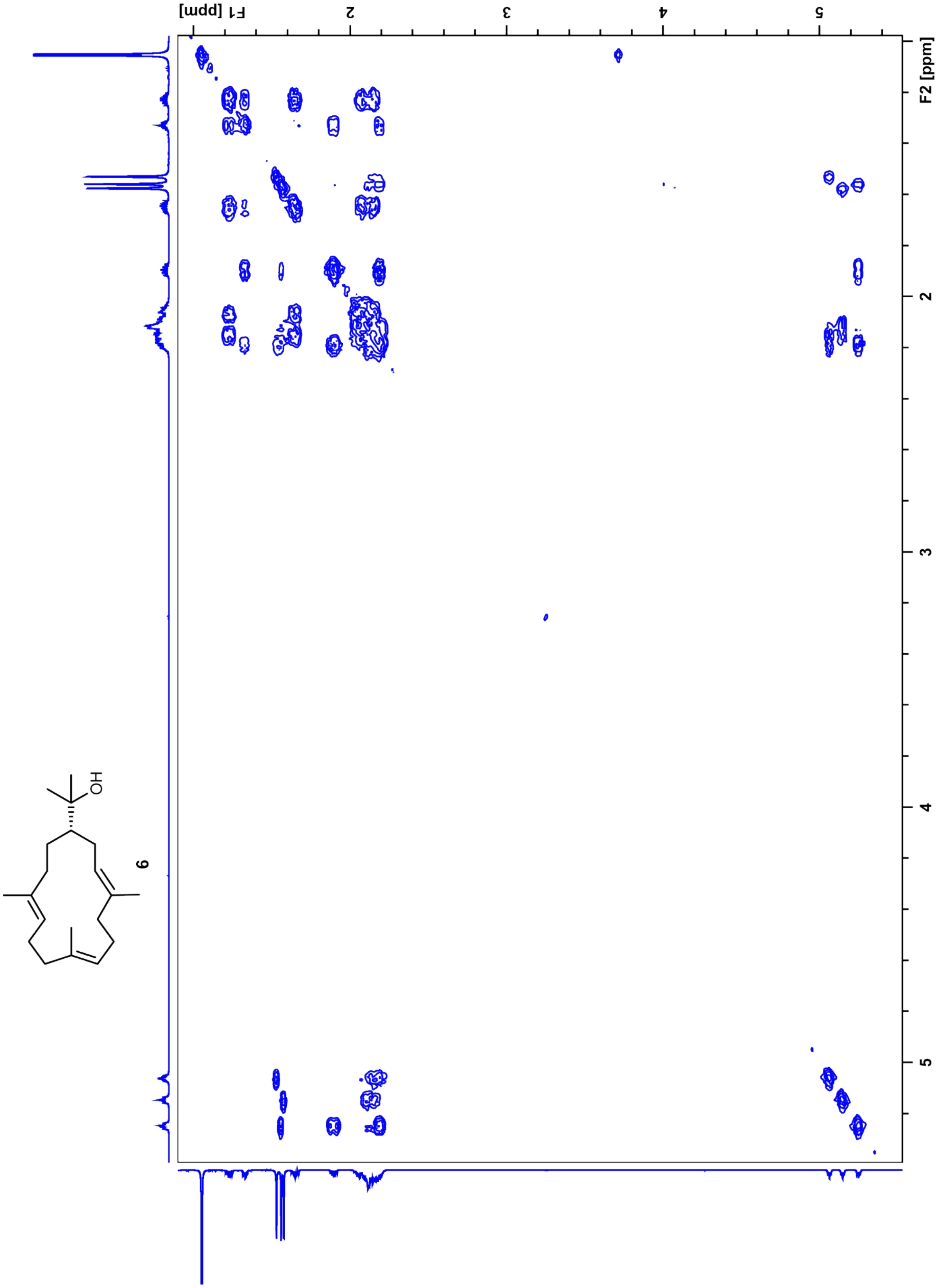


Figure S30. $^{1}H,^{1}H$-COSY spectrum of **6** (700 MHz, C_6D_6).

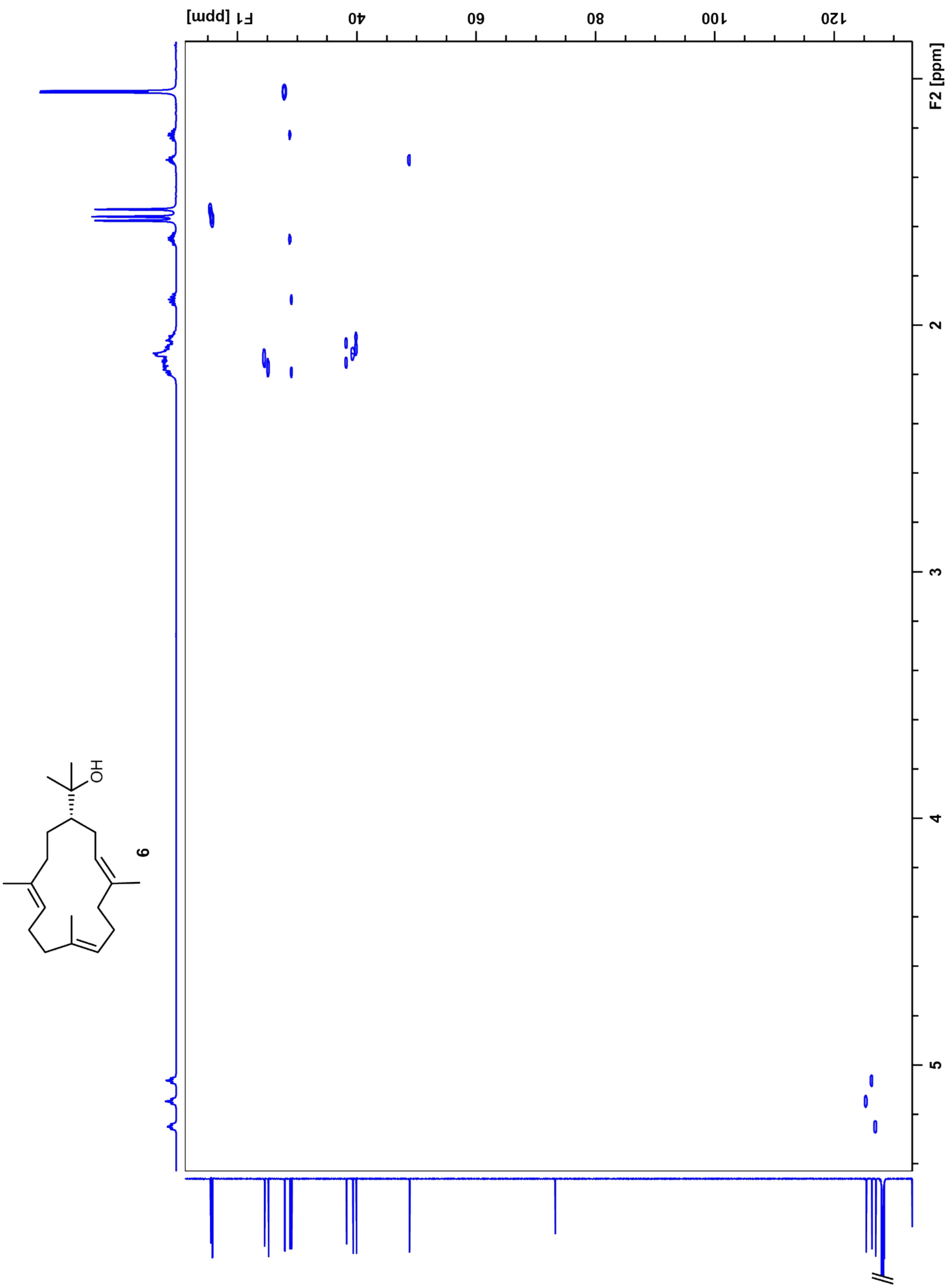


Figure S31. HSQC spectrum of **6** (700 MHz, C_6D_6).

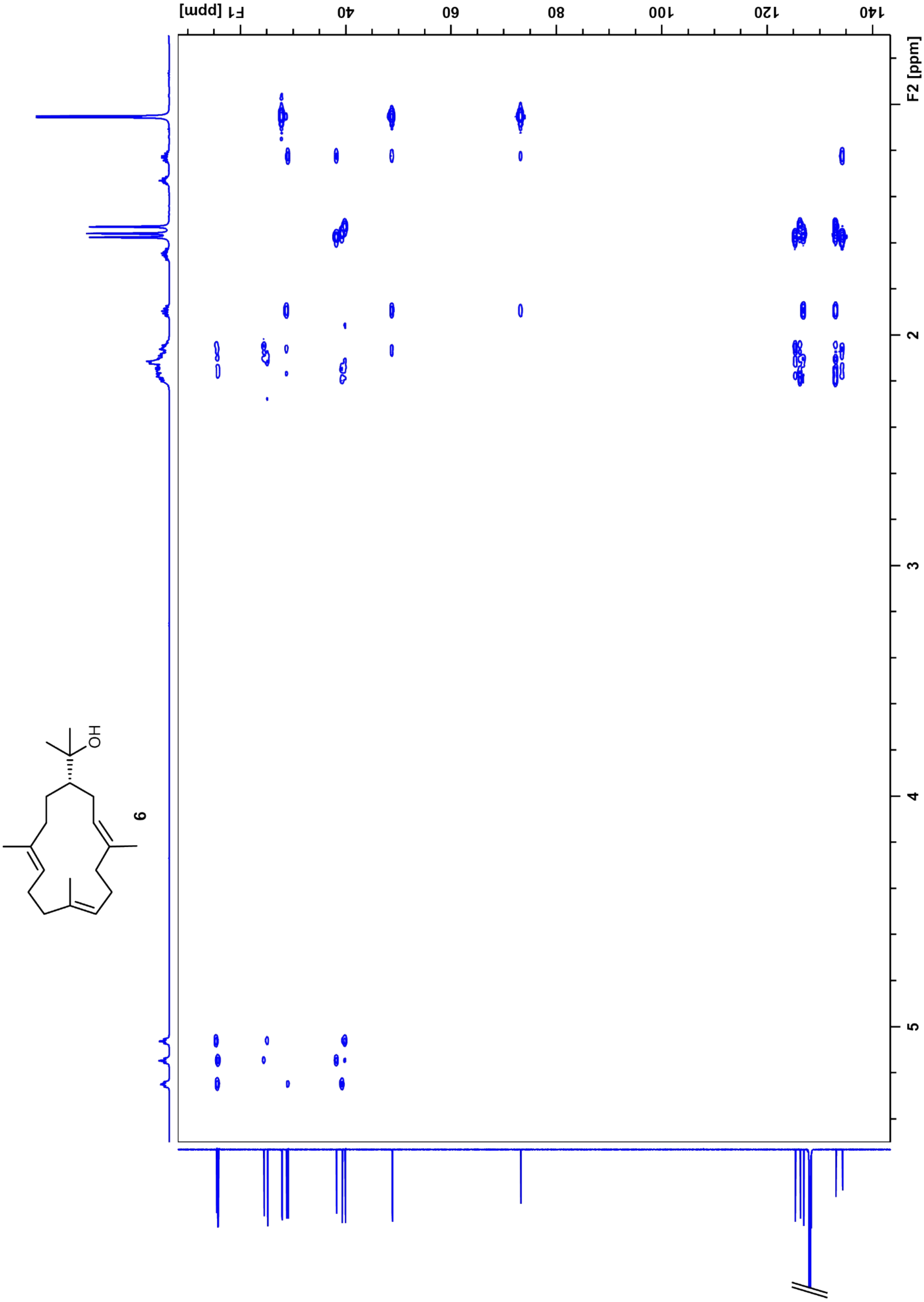


Figure S32. HMBC spectrum of **6** (700 MHz, C_6D_6).

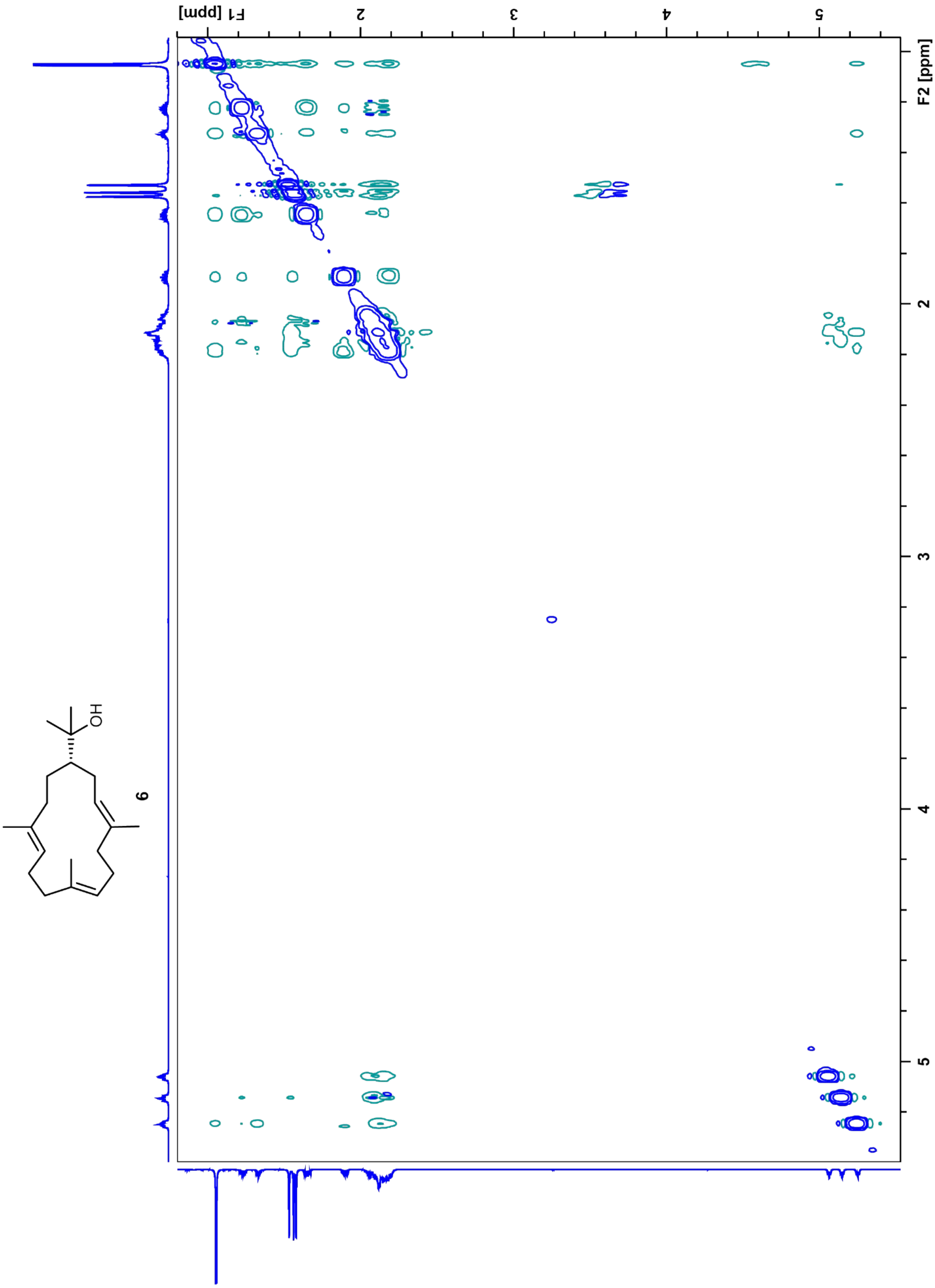


Figure S33. NOESY spectrum of **6** (700 MHz, C_6D_6).

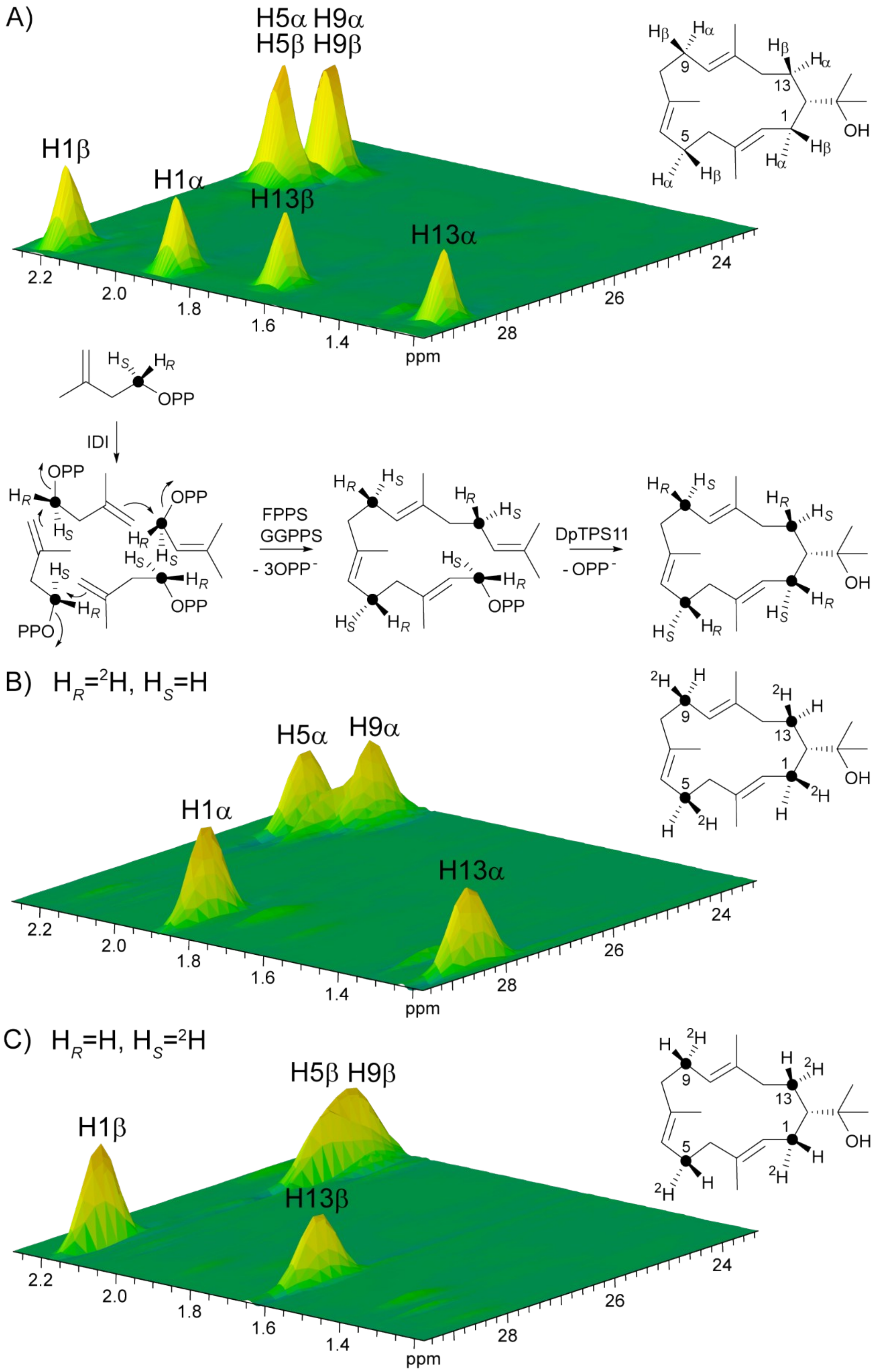


Figure S34. Partial HSQC spectra of A) unlabelled **6**, B) an incubation of (*R*)-(1-^{13}C,1-^{2}H)IPP with IDI, FPPS, GGPPS and DpTPS11 and C) an incubation of (*S*)-(1-^{13}C,1-^{2}H)IPP with IDI, FPPS, GGPPS and DpTPS11 showing the selective incorporation of deuterium into the methylene positions C1, C5, C9 and C13. The observed outcome is in line with the shown absolute configuration of **6** for C1 and C13. Determination of the relative configuration of C5 and C9 by NOE correlations was not possible. Black dots represent ^{13}C-labelled carbon atoms.

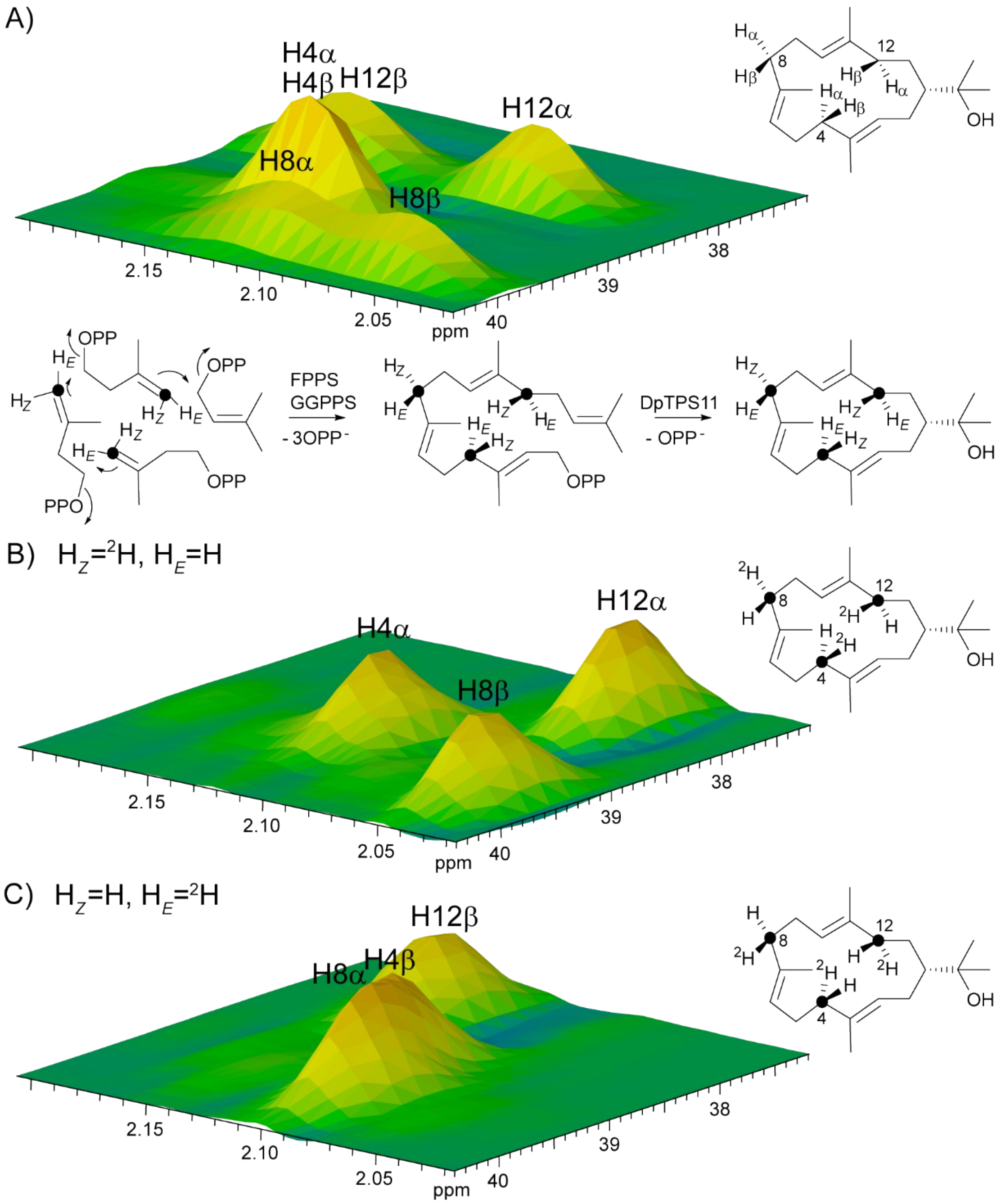


Figure S35. Partial HSQC spectra of A) unlabelled **6**, B) an incubation of (*Z*)-(4-^{13}C,4-^{2}H)IPP with DMAPP, FPPS, GGPPS and DpTPS11 and C) an incubation of (*E*)-(4-^{13}C,4-^{2}H)IPP with DMAPP, FPPS, GGPPS and DpTPS11 showing the selective incorporation of deuterium into the methylene positions C4, C8 and C12. Because of inconclusive NOE correlations on these positions, no conclusion on the absolute configuration was drawn. Black dots represent ^{13}C-labelled carbon atoms.

```
                                                                                      1         10        20        30        40        50        60
XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)                             -----------------------------------------------------------M
XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)                        --------------------------------------------------MEAIKQKRSL
XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)                          -----------------------------------------MDINKQKTKWDISIFKNEN
XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)                      -------------------------------------------------------MDIKN
XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)                            ----------------------------------------------------------MS
WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)                  -------------------------------------MSTTHEEIALAGPDGIPAVDLRD
CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)                     -------------------------------------------------------MTVRA
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene)    --------------------------------------------------------MEPE
WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)                               -----------------------------------------MPAFPHSTARAQTTSVPEA
WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)                         ---------------------------------------------------------MSQ
WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)                           ------------------------------------------------------------
WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol)             -----------------------------------------------------------M
Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)                                  -----------------------------------------------------------M
WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)                          -----------------------------------------------------------M
AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)                          ------------------------------------------------MHAHASRPQARQ
WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)                   ---------------------------------------MAHETTSGRRLPDPTSPSDPT
WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)                              ------------------------------------------------------------
WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)                             ---------------------------------------------------------MPT
FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)                                 ---------------------------------MPHKDLPIRPLVRAFDPVGPDTLGPPD
XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)                                  ------------------------MVPSLITPPPSRSGEATPQKDACLNPVNIAEPEGHW
XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)                 -------MVKFDSGSESEMTNGDELHINSKHEVKSRMANGNGVHNVPDHDQFQDRAEMEV
XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)                              -------------MDSLWSIVVTIRDIALKRTVGTDHLHKAAAEVLDERKDVIRRALNKK
Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)                         MAIPALEPQLHDADTSSNNMSSNSTDSGYDTNSTTPLEKSEKPNTQELKQQQLDPKRPPF

                                                                                      61        70        80        90        100       110       120
XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)                             YSLHD----FKFPE-DWIEPPANDKCI---YTCYKEVVDFKLF--EENKKTLEYYYG---
XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)                        ISLKE---SFIYPH-EWNHEPCDNKFI---LECFKESVNLGIF--QIDDKKSFSYIN---
XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)                          FTLPV----IKSPF-NTYYNKYIDSVI---NDIEEWHRECCFL--GREKLKGYIES----
XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene) ***                  LSLKD----IKFPK-SWETKPSKIEYM---QFVYQEAIDMKVW--RKDNEIDILTHH---
XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene) ***                        LSLGD----IKFPD-NWDLIPNEKNYI---DYVYKESIELEVW--RPNNKRDLMAHN---
WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)                  LIDAQ----LYMPF-PFERNPHASEAA---AGVDHWLSTWGLT--DDPAVAAMISCT---
CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)                     VELPQ----LWMPY-PLRVNPYLSALR---EESETWAREMGML--GGEGPSARGAIWTRS
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene)    LTVPP----LFSPI-RQAIHPKHADID---VQTAAWAETFRI---GSEELRGKLVTQ---
WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)                               LALPV----IEQAF-PRHRHEYWPKLQ---VESRAWLLEKRLM--PADKVTRYADEL---
WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)                         ITLPA----FHMPFQSAGCHPGLAETR---EAAWEWAAAEGLD--LSVPARRKMIRT---
WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)                           ----------------------MAHTR---GHLDSWTRRTGLV--HRESARNRFEQA---
WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol)             PQDVR----FDLPF-ETPVSKHLESAR---ARHLRWVWEMRLV--HSREGFEEYRSW---
Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)                                  PQDVD----FHIPL-PGRQSPDHARAE---AEQLAWPRSLGLI--RSDAAAERHLRG---
WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)                          PQDID----FGLPA-PAGISPGLEATR---RHNLGWVRRLGLV--GDGPSLAWYTSW---
AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)                          TTLLRRAALFDFPA-SADLSPGTEAAR---HHTIQWLSRFGVF--EGHESVAEYDAL---
WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)                   RRTAA----IRIPF-PARLNPHAERAR---QHTLQWVQETGLL--TGDEATAEYDTL---
WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)                              -MIPR----FDFPW-PSACHPHARQAE---QGALAFAERHGLV--PTAAYRSRLERT---
WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)                             ITLPR----IIYPF-PSLINQFVTAAH---EQNRQWVADFGFI--TTPEAMARFDRS---
FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol ***)                             LDFASLFRERNVPE-DAPLTLYPEQLNVPWHTSLPWTRQSKWWVQGEAAGRDLVNRISAD
XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol ***)                              IKLPEALFSSIMAV-EPEVNPLYRTSK---ALSDEWLKTALRM---NDKTAVIWSRL---
XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene ***)             LILPD-LFSSLMSV-PARENPHYASVK---ADADEWI-SFVIN--ADAKWASRNKRV---
XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol ***)                          VLVPD--ILALMPEWPSEFQPDIDEVN---LEIDEWLKTVKVA--EEKKAKHR-ARG---
Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol ***)                     VRVPD-LFGSIMST-KPVVNPNYFAAK---ARGDRWIARVMNF---NKAVAARNSKV---
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Figure S36. Amino acid sequence alignment of DdTPS9, DpTPS10 and DpTPS11 with characterised bacterial and fungal terpene synthases.[5,15-30]

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                                                                          121       130       140       150       160       170       180
XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)                 --------------------------TISS-TIYLYPLCNYEQLLVASRYLTVCFVVDD-
XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)            --------------------------SCLSSCAYMWPLCNHSQSLMTGRFIQWSFLIDD-
XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)              --------------------------KPYLFSAYYYCHLNEKVLPFIIKFVDMFSIYDDE
XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)          --------------------------HVTDLSRFFWPNADFEGLVLGAELMVWFFAFDD-
XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)                --------------------------NVVSLAKYFWPHVDFNRLVMGGELMVWFFSFDD-
WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)      --------------------------RPAELAAFNGPDMDSGLLQIAANQIAYQFVFDDR
CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)         QFHAM---------------------TVELLTAWTLPDASLAGLRLNHRFNIWALAWDDY
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene) --------------------------DIGTFSARILPEGREEVVSLLADFILWLFGVDDG
WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)                   --------------------------RYTDLIAGYYVGAPRDVISAISDFSTWFFVWDD-
WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)             --------------------------RPELWISLIFPQATQAHLDLFCQWLFWAFLVDD-
WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)               --------------------------DFGAFVGMVYPTADEEHLDLVADWFVWLFLVDD-
WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol) --------------------------DLPQAAARTYPHASADDMVVLMNWFSLAFLFDD-
Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)                      --------------------------GYADLASRFYPHATGADLDLGVDLMSWFFLFDD-
WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)              --------------------------DMPRLAACGFPHARGAALDLCADAMAFFFVFDD-
AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)              --------------------------RFDVLAGLFYPRATGADLNLGSDLVGWYFVFDD-
WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)       --------------------------RLERLMAYFYPDASAGDLELAADFNAWFFIFDD-
WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)                  --------------------------RYGWLAARCYPDADDVLLQLCADYFIWFFIVDDL
WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)                 --------------------------RFAWLAARAFPHAGFHELCTIANFNTWLFMLDD-
FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)                     KASERGALPVEFMDERRKGKIDELVEDAVSCAVYLYPSSSPTRIELLTQALLLLFFHDD-
XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)                      --------------------------DIAYMSAICAPHADLETLKLMNDWNGWVFAFDD-
XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)     --------------------------DFTYLASIWAPDCSAFALRTSADWNSWAFLFDD-
XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)                  --------------------------NYTLLAGIYYPHCKKDKLLVLSQFLYWIFFWDDE
Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)             --------------------------DLCFLASMWAPDAPEDRLVMMLDWNHWVFLFDD-

                                                                          181       190       200       210       220       230       240
XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)                 FLE--SKLTNPDDSRELIKKLEHIFMDGNFYDSNNISNIEKY--VLYFRETTKQFV----
XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)            YLD--SLEIDDKKTDSTVLNVEKALI--NGTITNKNSKLEEY--TVFFRNKLFEYC----
XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)              YLE------KTNCSEDVINQFLDKNY----------KDKNIY--GVEWFKIVEGLK----
XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)          LFDGGFIDDNENEQYRLVNRMNKVFL--EGTIEDDSTGAERM--GYYLRNKVRAIC----
XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)                VLDAGIYTDE--KQMDLVKRMDNVFI--NGTVESDATGPEKM--ALHLRKKCEVMC----
WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)      AEDIGR--HSPGRLLPMLSESVAILR-------DGQPPTTPL--GAALADLHRQVQ----
CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)         FASTFKQTGDLPGALDFTARLHAFLR--PEADARSPEPVNAV--ERGIADLQKHLF----
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene) HCEEGELGHRPGDLAGLLHRLIRVAQ----NPEAPMMQDDPL--AAGLRDLRMRVD----
WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)                   QHDRDAVHGRRTAWYRLSAELHTALD-APGDHLAHPE---PL--VAAFAETVHRLN----
WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)             EFDDGPAGRDPLMCERAIARLVDVFD--------GAAPNGPM--ERALAGLRDRTC----
WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)               QLDDGHLGRSPDRVRDVVDRMRAVVD---GSAPEVLPDEDAPAAVTALVDLWKRTM----
WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol) QFD-ASRPDRADRIAEVARELIVTPL---RPAGTPPRVACPI--TLAWTEVWKHLS----
Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)                      LFD-GPRGENPEDTKQLTDQVAAALD------GPLPDTAPPI--AHGFADIWRRTC----
WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)              QFD-GPLGRDPARAARVCRRLTGIVH-----GAGPGPGADAC--SAAFADVWARST----
AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)              QFD-GELGSRPEAVARLVADVIRITE-EDTAHGRAQDGEGPL--LESFRDLWRRIS----
WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)       QFD-GGLGTRPHEIRGVVDALVGTMT-TDGAPRPADVRDTPL--VRAFRDIWLRST----
WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)                  FVD--RVDTLSERTIPNLTAMIDVLD-------HHRPGAEPVFGEHAWLDVCTRLR----
WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)                 QCDEAQLGKKAVYLEHVTDGFMNILK-------HNTPVDTVL--GRSFTDIWERMQ----
FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)                     VMERGATQDDATVCDDFVTMI---------------PKNKHM--KRYFAEVL--------
XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)                      PFDEGTFANDPIKAAEEVIYTLATLD---NIHPVVSPDENPL--RHTLQSCWMRFR----
XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)     QFDEGHLSNDLEGAINEIARTREIME--GTAPRYTADSEHPI--RYVFQTLCDRVKQNPE
XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)                   IDTGGELTHDRQGTLQCCAETNKCIDDCLGPNPNYSPPPGSRGTVEMFYPILRDLR---
Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)             QFDEGHLKEDPAAAAEEVKQTIAIMG---GNAPRYTAESNPI--RYVFQQCWDRLK----
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Figure S36. Amino acid sequence alignment of DdTPS9, DpTPS10 and DpTPS11 with characterised bacterial and fungal terpene synthases.[5,15-30]

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                                                                                 241       250       260       270       280       290       300
XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)                        -------GEKIEFFNQFLKFIIDWINSINPFNRADNL----------NNYD-SYNFFKR-
XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)                   ------GRERLDAFNLLINELVICLWTLVPFSKIHSK-----EKDFYPSYQ--LYRCIRT
XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)                     ------KYGNKQSVNKFLKEFEFFIKNVHSIHLKENA--------NYTNINFEEYTNTRS
XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)                 ----GEKRQSTFHRFNSSCVQWVDSIIPFIKLKRN-----------QKSLDFNLYIHHRK
XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)                       ----GKRRKDTFNRFISSCVQWVDSIIPFNKLRSA--------QGTSPHLE--LYSYLRK
WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)             ----ERCTPAQAARWAWNSREYVHGLLY-EAVAQA----------HPAPVESGLCRSIRS
CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)                ------PPRLAHWRQEFNLALAHYIDAGVQ-ELTNSR------GGRVPHLI--EYAPFRR
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene) ----RFGTAGQTARWVDALREYFFSVVW-EAAHRR--------AGTVPDLN--DYTLMRL
WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)                          ----GFLGPAWNERFARHFHAIVGAYDQ-EFENRV------T-KVT-PKVA--EYIELRR
WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)                    -----RGRSPQWNRQFRRDTAAWLWTYYA-EAVERA-------AGQVPSRA--EFAKHRR
WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)                      ------PEAAPHWRTRFAWHLVTYLTTATTWEAGNRA------EDVVPSEE--TYIAKRR
WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol)        ----HGMSLTWQSRFAASWGRFLEAHCE-EVDLAA--------RGLEGTLGLVEFTEFRR
Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)                             ----EGMTPAWCARSARHWRNYFDGYVD-EAESRF---------WNAPCDSAAQYLAMRR
WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)                     ----DGAHPGWVARTAHEWEYYFAAQAH-EAINRL--------RGTPGDME--SYLQVRR
AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)                     ------SGRPQVWRDRFRHHWLEYLHSYHREALERTGALPGAGGDAPRSVE--AVLALRR
WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)              ------AGAPYAWRLRFRDHWQAYLAAHVG-EAHHRN------ADRLPSLE--QFLEVRR
WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)                         ----AYLSDEHFQRFAHGMRMWAATAGL-QIANHL--------GADTVDVA--PYETIRR
WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)                        ----ALGDTAWQTRFIRSMEEYFTSCHW-EAGNRA--------ADIVPTVA--EYVTMRP
FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)                            -------ECDPILGPGLLRAIGLFVNAGRKKSPFKQD--------KYATLA--EYLDYRR
XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)                             ----ERSSPSLQYRWKKHLTMYCVGVLQ-QVGVQH--------RATRPTIE--EYMDMRA
XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)            GFYAGKPSSERFYRRWMWAHELYWEGLVA-QVRTNV-------EGRSFTRGPEEYLAMRR
XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)                         ----SGLGPVSTERLRQELHDYVNGAAN-QQGVRE--------EDHLPDPW--VHFQMRA
Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)                    ----AVSSQEMQQRWIDQHKRYFDQLLV-QVDQQV--------GGENFTRDVEAYMDLRR

                                                                                 301       310       320       330       340       350       360
XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)                        TNSGTYVSLSVAM-LLYPNSKIDPKIWINPRFDRFATNGGYQMATMNDCASYAKEIRNNN
XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)                   INVGIIACCALNF-ILFK--DLDVKLWLNPRFRKILNRASIQISIVNDAVSYAKEILNEN
XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)                     IDFGFDLVVSAAI-IDCE--EPSKEIRESSLFLTLNTKSSIICVLVNDIYSFVKESKQ-P
XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)                 FNIGAIPCFLVSEIILDPMSNIECFIWLDSRWIKMSEIICEIIALVNDCVSYEKEIKENG
XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)                       VNIGAYPCVTLTE-VMLDH-EIEYYIWSDPKWIKMNEDIAIITTLINDLVSYEKEVNDQA
WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)             LIAGVEPFYPLCE-AAQRC-ELAPEELHHPAMRRLSRLSADAAVWIPDLFSAVKEQRA-G
CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)                ESFAAHTAPYSVE-LATGA-RIPEQLRHTRTVHALLDAFMDVMGLANDVASYEREVHEER
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene) YDGATSVVLPMLE-MGHGY-ELQPYERDRTAVRAVAEMASFIITWDNDIFSYHKERRGSG
WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)                          HTFGHSVWIDLLE-PTAGR-EIPADLRTSGPFLAAARHCQDFSAWYNDLCSLPKELAG-D
WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)                    DSVAMQPFLCLHE-ITAGI-DLPDSARSLPAYIALRNAVTDHSGLCNDICSFEKEAAL-G
WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)                      HTGAIHVCMDLIE-IVAGI-DAPESVHNDPRFITALEAACNHVCWANDVYSFEKEQVL-G
WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol)        RTVGIHHSIDAGE-RSRGF-EVPAQAMAHPVMERMRDLAADTIGFMNDIHSFEREKRR-G
Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)                             HTIGVQPTVDLAE-RAGRF-EVPHRVFDSAVMSAMLQIAVDVNLLLNDIASLEKEEAR-G
WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)                     GIAGTDLPLSLGE-RAAGI-TVPAAAFHSPQLRIMREAAIDVTLMCNDVYSLEKEEAR-G
AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)                     HSIGVQPCLDLNE-PFGGY-TLPPALHGGFPMARMREATDDVVVFTNDIASLDKELAV-G
WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)              HSIGVQPCLDFTE-RCGGY-ALPDELYRSFPLREMREITGDVVIFVNDIVSLVKELAA-G
WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)                         HTSGTNPCLALAD-AAKHG-PVTPAEYHSPPVQRLVLHANNVVCWSNDVQSLKMELNQPG
WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)                        YTGALFADVEAIE-IIEKV-YLPAHILQHFIVQRLVLACNNIVCWANDIFSCAKEARQ-G
FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)                            HDIAKPFMIAAIR-FGSGVRQTPEE---TAPFAELEDLYVQHSILINDLYSYDKEMYE-A
XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)                             GCVGAYPCIGLME-FAEGI-DIPQNVMDHPSMQAISRITCDLVTLQNDLCSYRKDLIQ-G
XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)            GSLGAYPALVNNE-WAYGI-DLPEEVADHPLVFEIMIIMSDQILLVKDILSYEKDLRL-G
XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)                         DDVGVIPSITQNE-YAMEF-ELPEWIRRHEAMEEIVLECTKLTILLNEVLSLQKEFRV-S
Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)                    GTIGVYPAISLSE-YGAGV-NVPQHVYDHPSLQECMKVSADLVTLVNDVLSYRKDLEL-G
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Figure S36. Amino acid sequence alignment of DdTPS9, DpTPS10 and DpTPS11 with characterised bacterial and fungal terpene synthases.[5,15-30]

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                                                                                   361       370       380       390       400       410       420
                                XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)  HLTNPLHFLQNQVG-------SFDNVYKVILKFNDEIMNQICED-ERILLLECPIE----
                             XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)  AYCNTFYFLQKDST----KFSTFDQVCEYLFNEANTYIKDIITD-EPLLLHEFEDV----
                              XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)  DTMNYVKIMANKKK-------SIQKALNHTNKIINNTLKEIISI-ENQIKMQY-------
                          XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)  APLNSLKFIQIEKN------LNLQESFEYISNYLNELINQYIEL-ETSFIKSYKPITSN-
                             XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)  GDLNPLYFLQNQKN------IPLPDSYKQVVDLIDFWVKDYQTM-EQSLLNEMEFKDSK-
                    WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)  GMINLALAYRRTHR------CSLPAAVTLAVRHINSTIREFEDL-YGEVRPELSPSGIG-
                     CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)  DVNNLVVVIGTSLG------ITLHQAVPAALHMVNARMRDFEHLRHHELPPLVDRFGLRP
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene)  YYLNALRVLEQERG------LTPAQALDAAISQRDRVMCLFTTV-SEQLAEQGSP-----
                               WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)  DQHNLGISLIRHEG------LSLEEAVVEVRKRVENCVTEFEVA-EKELRELLAGHLAAL
                         WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)  YEHNAVRLIQRDRG------STLQEAVDEAGIQLARIAERVQRA-ERELIEEIEAAGIDG
                           WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)  EIHNLVHLVRHHRG------LGEQQALDHVAERLAMETERFLTA-EDELLELYPE-----
                WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol)  DGHNLIAVLRRERG------CSWQEATDEAYRMTIARLDEYLEL-QERVPQMCDELRLDE
                                  Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)  EQNNMVMILRREHG------WSKSRSVSHMQNEVRARLEQYLLL-ESCLPKVGEIYQLDT
                          WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)  DMDNLVLVIEHARR------CTRDEAVTAARGEVARRVIRFEQL-AREVPALCAQLGLSA
                          AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)  DVHNSVIVQWERAG------GELEDAVRHIADLANARYRWFEET-AARLPALLTEAGADP
                   WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)  DINNSVVIEREHKG------CTLEESVEHITALANARTARFARL-AASLPGTLADLGVPA
                            WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)  QYWNMAAIYAH-RG------LSLQQAVDLVALRVRGEIASFQSLALTLEPH---------
                            WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)  DVHNLVLVLQHERN------STLQEAVNETARMHNEEVKLFTAL-EKLLPSFGAE-----
                                FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)  RTINGSVVNAVHVIEKLMC-VPPHLAKTITRTMSFDVEKKYYAESERFMRDPA-------
                                XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)  EESNIIFIL-KDQG------MTDQQAVDQIGEMLYDCYRRWHMA-LANLPFWGEG-----
                     XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)  VDHNMVRLL-KAKG------LSTQQAINEVGVMINNCYRRYYRA-LSELPCFGEE-----
                            XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)  QLENLCLLFMNTDN------VSIEEAIDKILGLLQEHYEICVAA-EARLPWSETDEK---
                       Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)  VDHNLMSLLMQRDN------LSAQQAVDVIGDMVNECYRRWYLA-LAELPSYGEK-----

                                                                                   421       430       440       450       460       470       480
                                XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)  -----------QRDDLKLL-TRSMKLILGGNYLW-SLQCSRYVDINSPFIEQRSN-----
                             XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)  -----------EDRKVVQSLLNHVHYLISGNFVWSIENNQRYQSSISPFIETNKK-----
                              XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)  -----------KENNLYQY-IERLNSVISATIYL-HQNHKRYSVHNKNYINKNN------
                          XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)  -----------YNSNFIAI-VEHLHNMSFANVSW-STQTPRYLSQTQPFLELRRN-----
                             XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)  -----------QRSDMEFI-LEHLRYLASGSKKW-SMQTPRYCSPTSPFIEMRTKPSTPV
                    WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)  --------------------VEGMAGWIRGCYFW-SRTVPRYADTLTAPAGL--------
                     CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)  D----------EREELETW-LGGAASFLSGLHAW-YTGAPRYAPTDHSVPEQRQRGGSEG
WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene)  --------------QLRQY-LHSLRCFIRGAQDW-GISSVRYTTPDDPANMPSVFTDVPT
                               WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)  PGGAGTAEARSVAEAVGSA-VFNMRNWFSSVY-WFHHESGRYRVDSWDD-----------
                         WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)  P----------TRTALERC-VRDYRGLVRGDFDY-HARAERYTRPDLVELDER--------
                           WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)  -----------LSGMLVPY-LDGMRSWMRGNLDW-SRQTPRYNPADVGQYEEPEEYLEET
                WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol)  A----------QRDGVRLG-VEAIQHWINGNYEW-ALTSGRYAAAKEGAVATAE------
                                  Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)  A----------EREALERYRTDAVRTVIRGSYDW-HRSSGRYDAEFALA-----------
                          WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)  V----------ERAHVDTY-LGVMEAWMSGYHAW-QTQTRRYTGAPHVLP----------
                          AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)  G----------THHAVGRY-VDGMRHVMTGNLGW-SVRTARYDERGTEAVSGGRQRPWAQ
                   WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)  P----------SREHVSHY-VDGMRHVMAGNLSW-SLATSRYDETGIAAVSGGRRRPWDG
                            WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)  -----------ASRPLRGF-VDGLRHWMRGYQDWVENDTLRYADAFIAE-----------
                            WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)  -----------MDRELERF-MAVLRSWITANYDWSYHDTGRYQVKEVEVVINS-------
                                FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)  -----------LNDKQRTY-VIALFDCLTGNLFH-HATLGRYSRYAEYVFDCKT------
                                XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)  -----------IDRDVIKF-VTGCRNIALGNLHW-SLYTFRYLGNDGPEVK---------
                     XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)  -----------ADRALLGY-LEVEKNHALGSLLW-SYNTGRYFKSKEDGARVR-------
                            XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)  -----------LNEDIREY-VRGANRLATGTACW-SYNCERYFKLSQVN-----------
                       Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)  -----------IDYNVMKF-VEICRAVAQGNLYW-SFQTGRYLGPEGHEVH---------
```

Figure S36. Amino acid sequence alignment of DdTPS9, DpTPS10 and DpTPS11 with characterised bacterial and fungal terpene synthases.[5,15-30]

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                                                                                     481      490       500       510
                                 XP_642260 (Dictyostelium discoideum AX4, (-)-b-Barbatene)   -----------DPNVIAYEKIVDKILLK----------
                           XP_003289490 (Dictyostelium purpureum QSDP1, (-)-b-Araneosene)   -----------ENKIEKYNTLISTIFKQ----------
                             XP_003289235 (Dictyostelium purpureum QSDP1, (+)-Nephthenol)   --------------------------------------
                         XP_645125 (Dictyostelium discoideum AX4, Asterisca-2(9),6-diene)   -----------KKIITKIYDLKNK--------------
                               XP_638489 (Dictyostelium discoideum AX4, Protoillud-7-ene)   MNSTKKQKIDHVPSQSFISTPIDLNN------------
                    WP_039931950 (Streptomyces viridochromogenes DSM 40736, a-Amorphene)   --------------------------------------
                     CCA53839 (Streptomyces venezuelae ATCC 10712, Isodauc-8-en-11-ol)   AADRHAPAASSLARGAEAQTCGPTASAGSA--------
 WP_005317515 (Streptomyces pristinaespiralis ATCC 25486, Selina-4(15),7(11)-diene)   DDSTEPLDI--PAVSWWWDLLAEDARSVRRQVPAQRSA
                            WP_003950762 (Streptomyces albus J1074, epi-Isozizaene)   -----------RSTPPYVSDLAGDA-------------
                       WP_042496076 (Streptomyces griseus NBRC 13350, Caryolan-1-ol)   -----------DSLSRHFAA------------------
                         WP_003970379 (Streptomyces griseus NBRC 13350, epi-Cubenol)   VLGVPPA----RSETAAPAPCGAEAPRAR---------
          WP_003994861 (Streptomyces viridochromogenes DSM 40736, 7-epi-a-Eudesmol)   -----------LAGRGSVDDLLTV--------------
                               Q55012 (Streptomyces exfoliatus UC5319, Pentalenene)   -----------AGAQGYLEELGSSAH------------
                        WP_010981512 (Streptomyces avermitilis MA-4680, Avermitilol)   -----------STGPGYFDEVLPT--------------
                        AEM85259 (Streptomyces violaceusniger Tü 4113, Isoafricanol)   LTGAEELIR--AGRGAPLPPLGSGSGSR----------
              WP_005320742 (Streptomyces pristinaespiralis ATCC 25486, Pristinol)   L----------TTATGTASPRHPRRA------------
                            WP_014134444 (Kitasatospora setae KM-6054, Corvol ether)   -----------DADDTAVRT------------------
                          WP_012792334 (Chitinophaga pinensis DSM 2588, g-Cadinene)   --------------------------------------
                            FFUJ_10353 (Fusarium fujikuroi IMI 58289, a-Acorenol)   --------------------------------------
                               XP_023434772 (Fusarium fujikuroi IMI 58289, Koraiol)   -----------RTRMMKLP-------------------
                XP_023437750 (Fusarium fujikuroi IMI 58289, (-)-Guaia-6,10(14)-diene)   -----------KTRELLIPKKMAAL-------------
                          XP_006969402 (Trichoderma reesei QM6a, Trichobrasilenol)   -----------EKRELLLDLSYR---------------
                  Q6WP50 (Botrytis cinerea B05.10, Presilphiperfolan-8-beta-ol)   -----------ETGIMYLPPAANLVVA-----------
```

Figure S36. Amino acid sequence alignment of DdTPS9, DpTPS10 and DpTPS11 with characterised bacterial and fungal terpene synthases.[5,15-30]

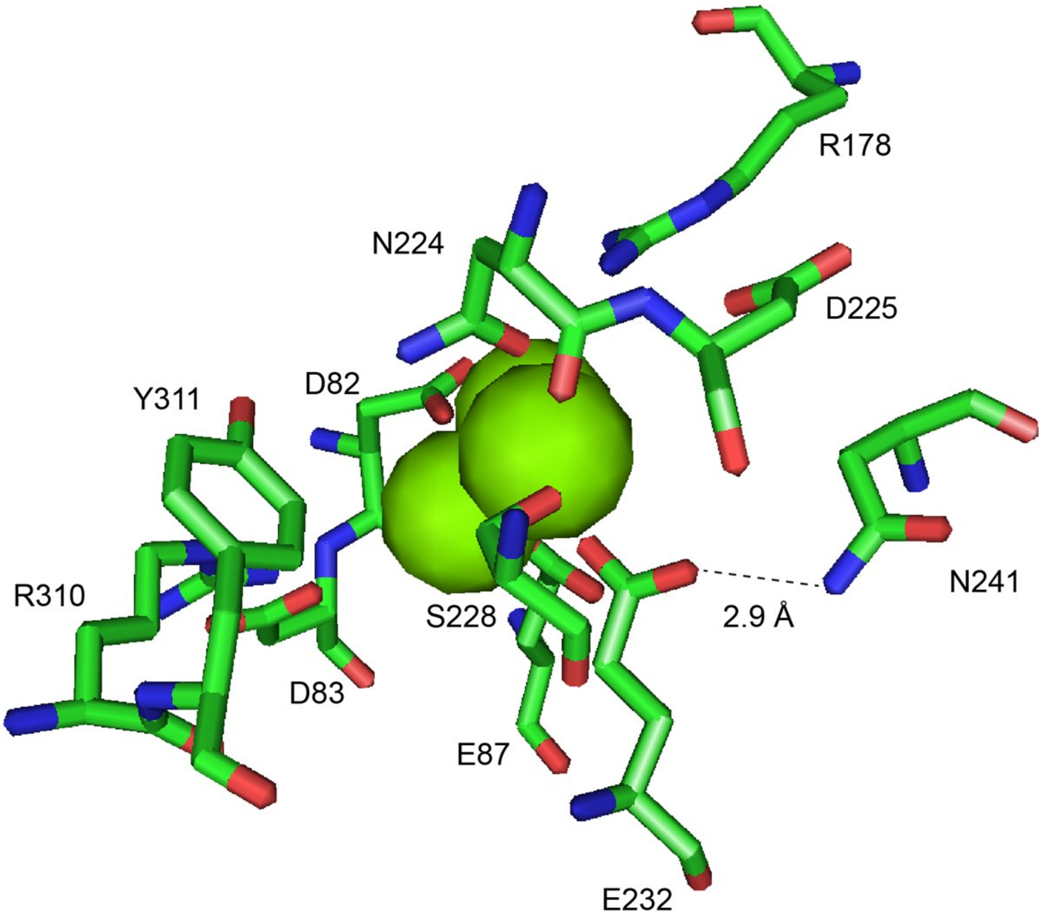

Figure S37. Active site residues observed in the crystal structure of selinadiene synthase (SdS, PDB: 4OKZ).[31] Green spheres represent Mg^{2+} cations.

Qualitative conversion assay with TPS variants

For comparing the constructed enzyme variants with their corresponding wild types, small scale reactions were conducted using 0.2 mg of diphosphate substrate (FPP or GGPP) dissolved in 100 μL substrate buffer. The solutions were diluted with incubation buffer (300 μL) and the reactions were started adding TPS elution fraction (600 μL, protein concentrations were adjusted to 0.2 mg/mL by Bradford assay, cf. Figure S1). The reactions were incubated for 30 min at 28 °C with shaking, before the mixtures were extracted with hexane (150 μL). The extracts were dried with $MgSO_4$ and analysed by GC/MS. All reactions were conducted in triplicates.

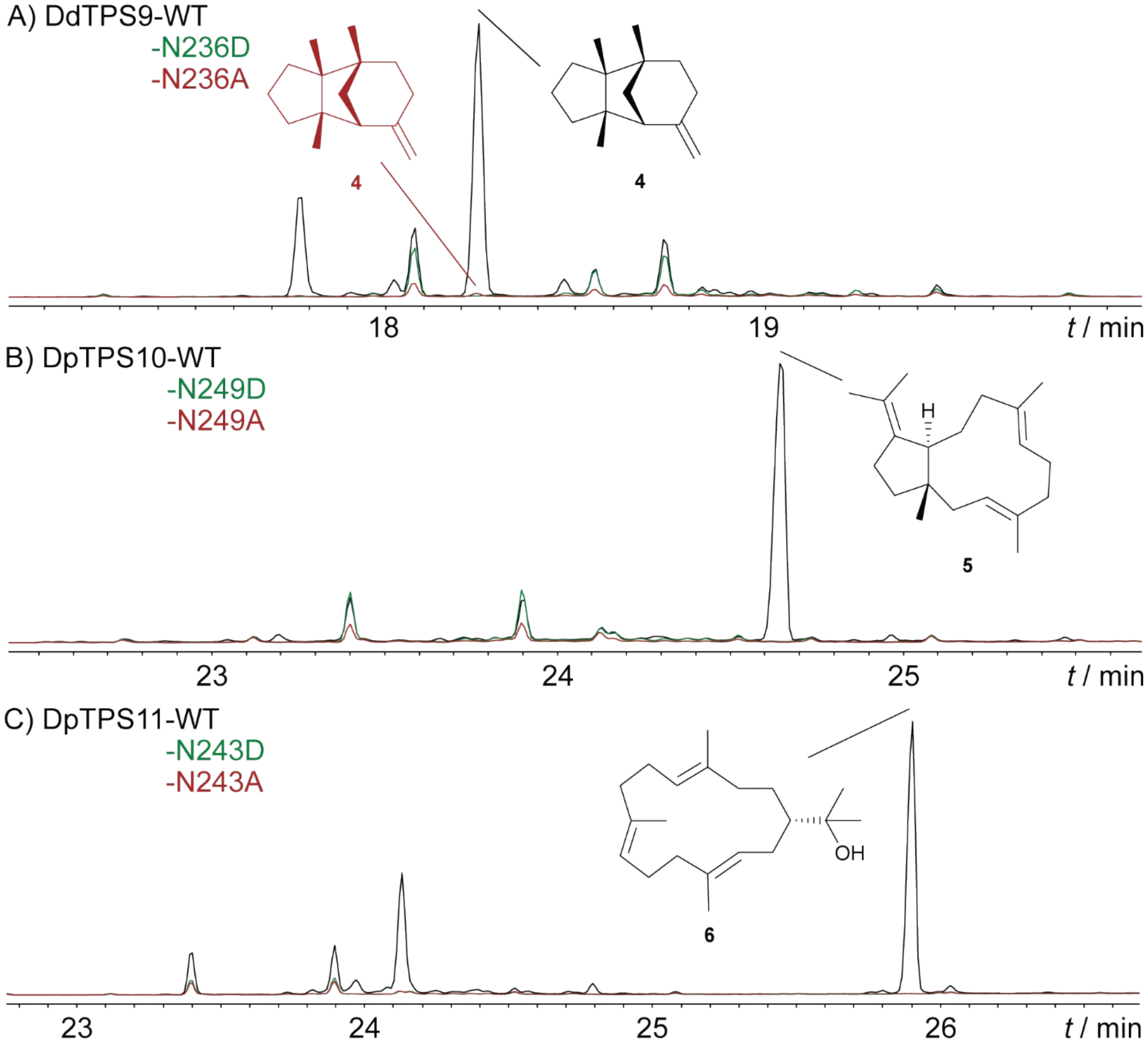

Figure S38. Comparing total ion chromatograms from incubation experiments using wild-type (WT) TPSs (black) and their corresponding Asn to Asp (green) and Asn to Ala (red) variants for A) DdTPS9 with FPP, B) DpTPS10 with GGPP and C) DpTPS11 with GGPP. Only for the variant DdTPS9-N236A, residual conversion to the native product was observed (3.0 ± 1.0)% compared to normalised WT conversion (100.0 ± 15.3)%. Standard deviations were calculated from triplicates.

HPLC conditions

Analytical scale HPLC purifications were carried out using an PLATINblue-series UHPLC system (Knauer, Berlin, Germany), equipped with a photo diode array detector PDA-1 (190-1000 nm) and a Macherey-Nagel (Düren, Germany) Nucleodur® 110-1.8 Gravity C18 column (1.8 μm; 2.0 x 100 mm) using an isocratic solvent mixture of [acetonitrile/water (90:10)] with 0.5 mL min^{-1} (334 bar). The UV-vis absorption was monitored at 220 nm. Observed elution times were: **12**: 11.39 min; **13**: 9.20 min; **14**: 8.43 min; **15**: 7.42 min.

Semi-preparative scale HPLC purifications were performed on an Smartline-series HPLC system (Knauer) with a UV-Vis detector S-2550 (190-900 nm) using a Macherey-Nagel (Düren, Germany) Nucleodur® 110-5 Gravity C18 column (5 μm; 10 x 250 mm). The solvent mixture [acetonitrile/water (90:10)] was used at 8 mL min^{-1} (113 bar) and monitoring was done at 220 nm.

Reaction of 5 with NBS

A stirred solution of **5** (5.4 mg, 19.8 μmol, 1.0 eq.) in dry dichloromethane (1 mL) was cooled to -78 °C, before *N*-bromosuccinimide (NBS, 3.5 mg, 19.8 μmol, 1.0 eq. was added in one portion. Stirring was continued for 20 min at the same temperature and the solution was allowed to warm to room temperature over 1 h. After stirring for 30 min at room temperature, saturated NH_4Cl solution (2 mL) was added and the mixture was extracted with pentane (3x 3 mL). The organic extracts were dried with $MgSO_4$, concentrated under reduced pressure, fractionated by column chromatography on silica gel [pentane] and further purified by HPLC (conditions are described above) to yield bromodolastadienes **12** (1.4 mg, 4.0 μmol, 20%) and **13** (0.5 mg, 1.4 μmol, 7%), and bromodolastatrienes **14** (0.6 mg, 1.7 μmol, 9%) and **15** (1.8 mg, 5.2 μmol, 26%) as colourless solids.

(2*S*,6*R*,7*R*,11*S*)-6-Bromodolasta-3,10(14)-diene, (3a*S*,4a*S*,8*R*,8a*R*)-8-bromo-1-isopropyl-3a,5,8a-trimethyl-2,3,3a,4,4a,7,8,8a,9,10-decahydrobenzo[*f*]azulene (**12**). R_f (pentane) = 0.70. $[\alpha]_D^{20}$ = +3.2° (*c* 0.15, C_6H_6). HRMS (QTOF): *m*/*z* = 350.1606 (calc. for $[C_{20}H_{31}Br]^+$ 350.1604). GC (HP5-MS): *I* = 2283. MS (EI, 70 eV): *m*/*z* (%) = 352 (13), 350 (14), 337 (25), 335 (26), 309 (42), 307 (43), 281 (2), 271 (4), 255 (9), 227 (23), 213 (7), 199 (9), 190 (23), 175 (25), 161 (17), 151 (55), 133 (77), 121 (100), 107 (55), 95 (84), 81 (56), 69 (16), 55 (16), 41 (16). IR (diamond ATR): ν / cm^{-1} = 3033 (w), 2955 (s), 2926 (s), 2853 (m), 1460 (m), 1377 (w), 1174 (w), 853 (w), 795 (w), 749 (w), 596 (w). NMR data are given in Table S5 and Figures S40–S46.

(2*S*,6*R*,7*R*,11*S*)-6-Bromodolasta-3(20),10(14)-diene, (3a*S*,4a*S*,8*R*,8a*R*)-8-bromo-1-isopropyl-3a,8a-dimethyl-5-methylene-2,3,3a,4,4a,5,6,7,8,8a,9,10-dodecahydrobenzo[*f*]azulene (**13**). R_f (pentane) = 0.56. $[\alpha]_D^{20}$ = +11.3° (*c* 0.05, C_6H_6). HRMS (QTOF): *m*/*z* = 350.1597 (calc. for $[C_{20}H_{31}Br]^+$ 350.1604). GC (HP5-MS): *I* = 2249. MS (EI, 70 eV): *m*/*z* (%) = 352 (13), 350 (14), 337 (44), 335 (45), 309 (82), 307 (85), 281 (7), 271 (13), 255 (16), 241 (2), 229 (19), 227 (36), 201 (14), 187 (14), 175 (16), 161 (38), 149 (51), 133 (71), 121 (100), 107 (80), 93 (83), 79 (58), 69 (26), 55 (33), 41 (33). IR (diamond ATR): ν / cm^{-1} = 3083 (w), 2953 (s), 2926 (s), 2853 (m), 1462 (m), 1379 (w), 1208 (w), 891 (w), 726 (w). NMR data are given in Table S6 and Figures S48–S54.

(2*S*,6*R*,7*R*,11*S*)-6-Bromodolasta-3,9,13-triene, (3a*S*,4a*S*,8*R*,8a*R*)-8-bromo-1-isopropyl-3a,5,8a-trimethyl-3,3a,4,4a,7,8,8a,9-octahydrobenzo[*f*]azulene (**14**). R_f (pentane) = 0.56. $[\alpha]_D^{20}$ = +8.6° (*c* 0.07, C_6H_6). HRMS (QTOF): *m*/*z* = 348.1450 (calc. for $[C_{20}H_{29}Br]^+$ 348.1447). GC (HP5-MS): *I* = 2309. MS (EI, 70 eV): *m*/*z* (%) = 350 (15), 348 (15), 281 (4), 269 (6), 225 (4), 207 (7), 185 (4), 175 (8), 159 (8), 148 (55), 133 (100), 119 (57), 105 (42), 95 (36), 91 (35), 79 (15), 69 (7), 55 (8), 41 (10). IR (diamond ATR): ν / cm^{-1} = 3048 (w), 3027 (w), 2956 (m), 2923 (s), 2852 (m), 1459 (m), 1378 (w), 1173 (w), 998 (w), 849 (w), 821 (w), 795 (w), 751 (w). NMR data are given in Table S7 and Figures S56–S62.

(2*S*,6*R*,7*R*,11*S*)-6-Bromodolasta-3(20),9,13-triene, (3a*S*,4a*S*,8*R*,8a*R*)-8-bromo-1-isopropyl-3a,8a-dimethyl-5-methylene-3,3a,4,4a,5,6,7,8,8a,9-decahydrobenzo[*f*]azulene (**15**). R_f (pentane) = 0.49. $[\alpha]_D^{20}$ = +44.0° (*c* 0.20, C_6H_6). HRMS (QTOF): *m*/*z* = 348.1446 (calc. for $[C_{20}H_{29}Br]^+$ 348.1447). GC (HP5-MS): *I* = 2294. MS (EI, 70 eV): *m*/*z* (%) = 350 (39), 348 (39), 335 (4), 333 (5), 307 (10), 305 (10), 269 (27), 225 (9), 213 (7), 199 (6), 185 (7), 175 (9), 161 (24), 148 (45), 133 (100), 119 (36), 105 (43), 91 (40), 79 (19), 69 (7), 55 (9), 41 (11). IR (diamond ATR): ν / cm^{-1} = 3086 (w), 3047 (w), 2954 (s), 2924 (s), 2851 (m), 1460 (m), 1379 (m), 1207 (w), 1172 (w), 1001 (w), 976 (w), 901 (w), 852 (w), 820 (w), 726 (w), 615 (w). NMR data are given in Table S8 and Figures S64–S70.

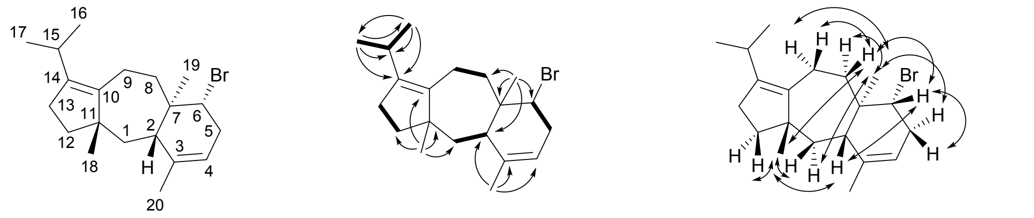

Figure S39. Carbon numbering and structure elucidation of **12**. H,H-COSY spin systems are represented by bold bonds, single headed arrows show HMBC correlations and NOESY correlations are depicted by double headed arrows.

Table S5. NMR data of (2*S*,6*R*,7*R*,11*S*)-6-bromodolasta-3,10(14)-diene (**12**) in C_6D_6 recorded at 298 K.

C[a]		^{13}C[b]	^{1}H[b]
1	CH_2	41.35	1.65 (m, H_α)
			1.37 (m, H_β)
2	CH	46.97	2.06 (m)
3	C_q	136.35	–
4	CH	121.67	4.99 (br s)
5	CH_2	36.39	2.65 (m, H_β)
			2.50 (m, H_α)
6	CH	67.03	4.03 (dd, *J* = 11.4, 5.8)
7	C_q	41.72	–
8	CH_2	37.41	2.29 (ddd, *J* = 14.7, 6.2, 4.2, H_β)
			1.54 (m)
9	CH_2	22.11	2.38 (m, H_β)
			1.98 (m, H_α)
10	C_q	139.07	–
11	C_q	50.25	–
12	CH_2	41.85	1.62 (m, H_α)
			1.56 (m, H_β)
13	CH_2	27.69	2.18 (m, 2H)
14	C_q	139.45	–
15	CH	27.09	2.54 (sept, *J* = 6.9)
16[c]	CH_3	20.79	0.941 (d, *J* = 7.0)
17[c]	CH_3	21.34	0.932 (d, *J* = 7.0)
18	CH_3	24.66	1.04 (s)
19	CH_3	11.39	0.924 (s)
20	CH_3	22.80	1.46 (s)

[a] Carbon numbering indicates the origin of each carbon from GGPP by identical number as shown in Figure S39. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, br = broad, coupling constants *J* are given in Hertz. [c] Signals may be interchanged.

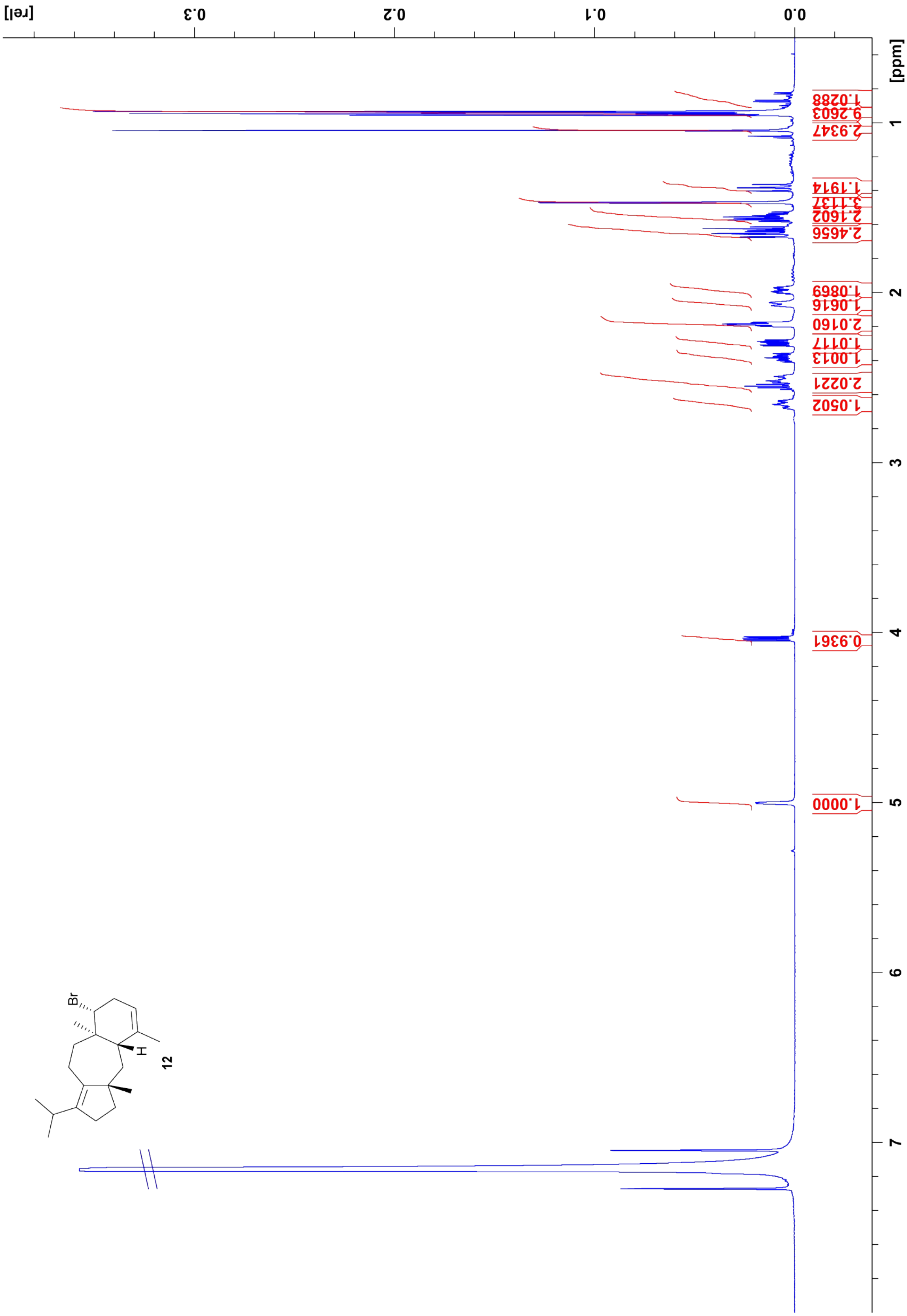

Figure S40. ^{1}H-NMR spectrum of **12** (700 MHz, C_6D_6).

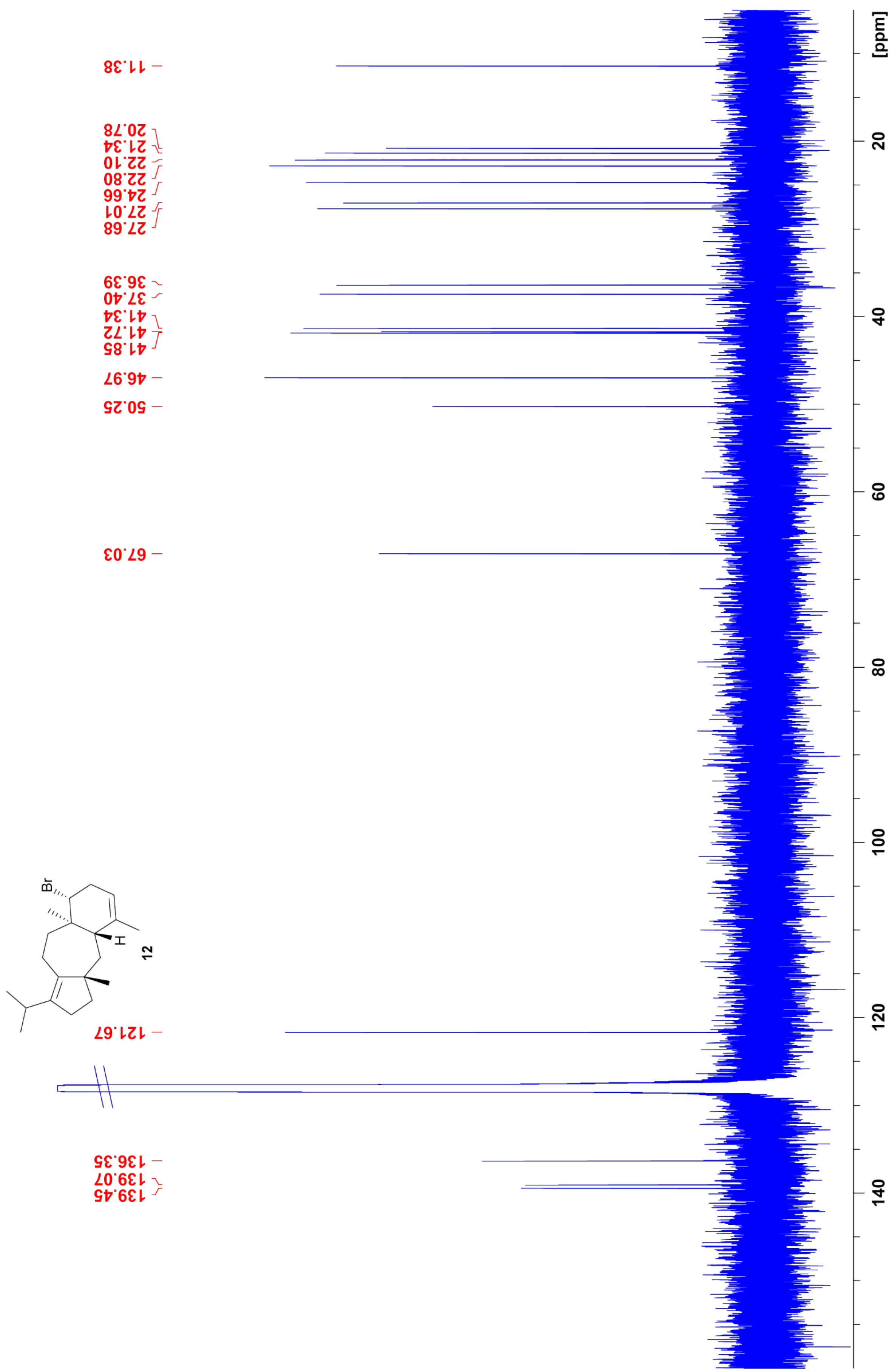

Figure S41. ^{13}C-NMR spectrum of **12** (175 MHz, C_6D_6).

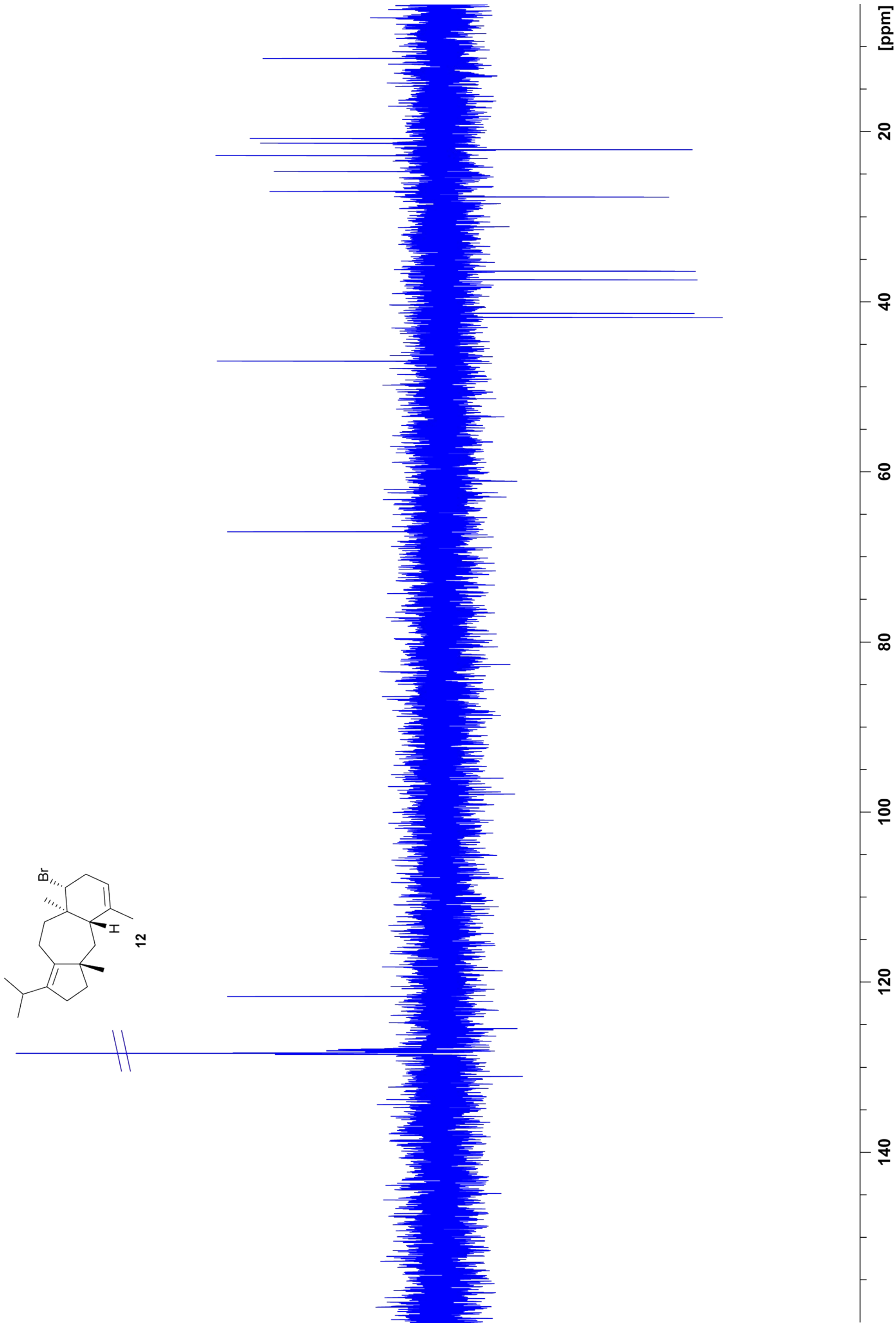

Figure S42. ^{13}C-DEPT spectrum of **12** (175 MHz, C_6D_6).

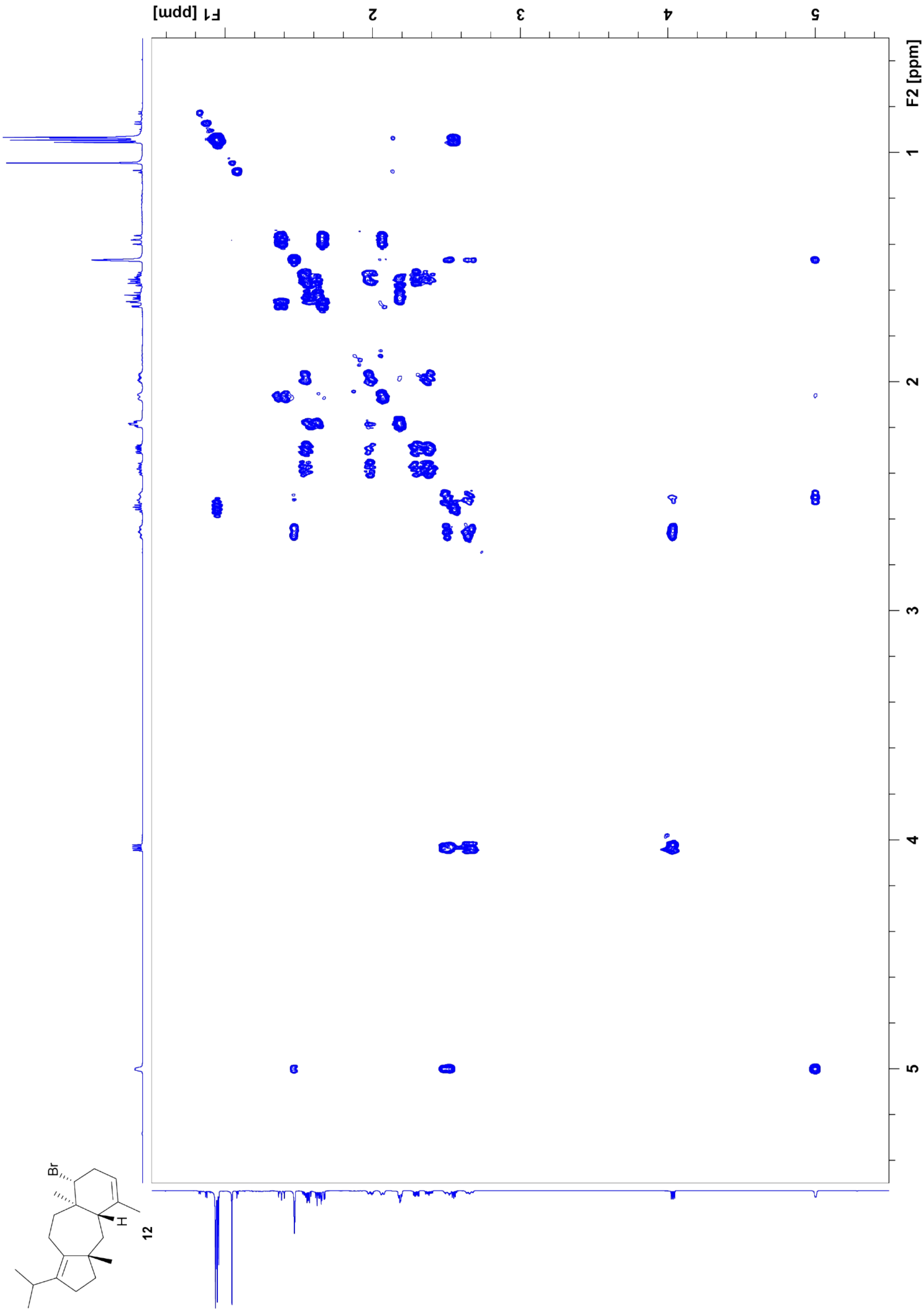

Figure S43. ^{1}H,^{1}H-COSY spectrum of **12** (700 MHz, C_6D_6).

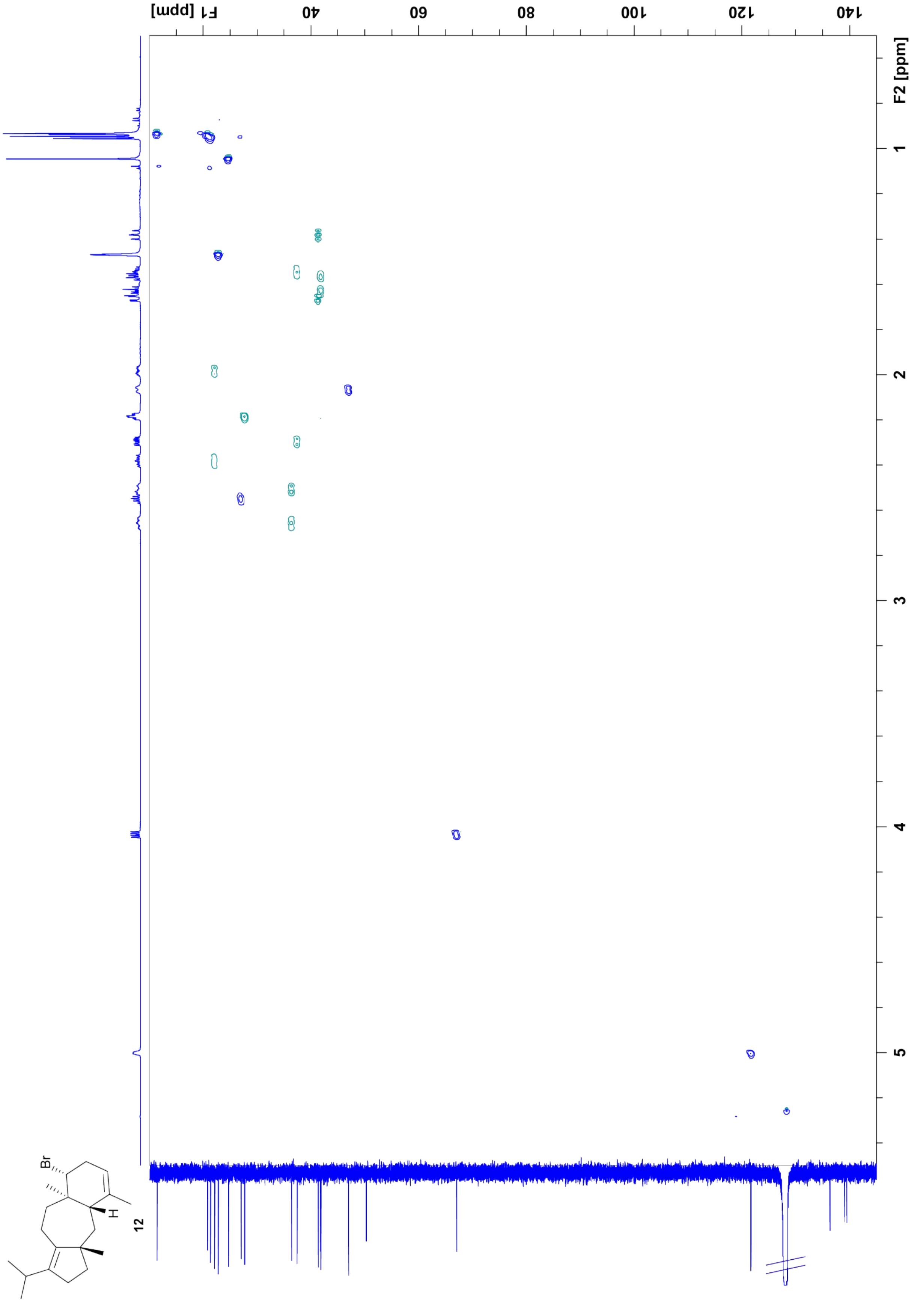

Figure S44. HSQC spectrum of **12** (700 MHz, C_6D_6).

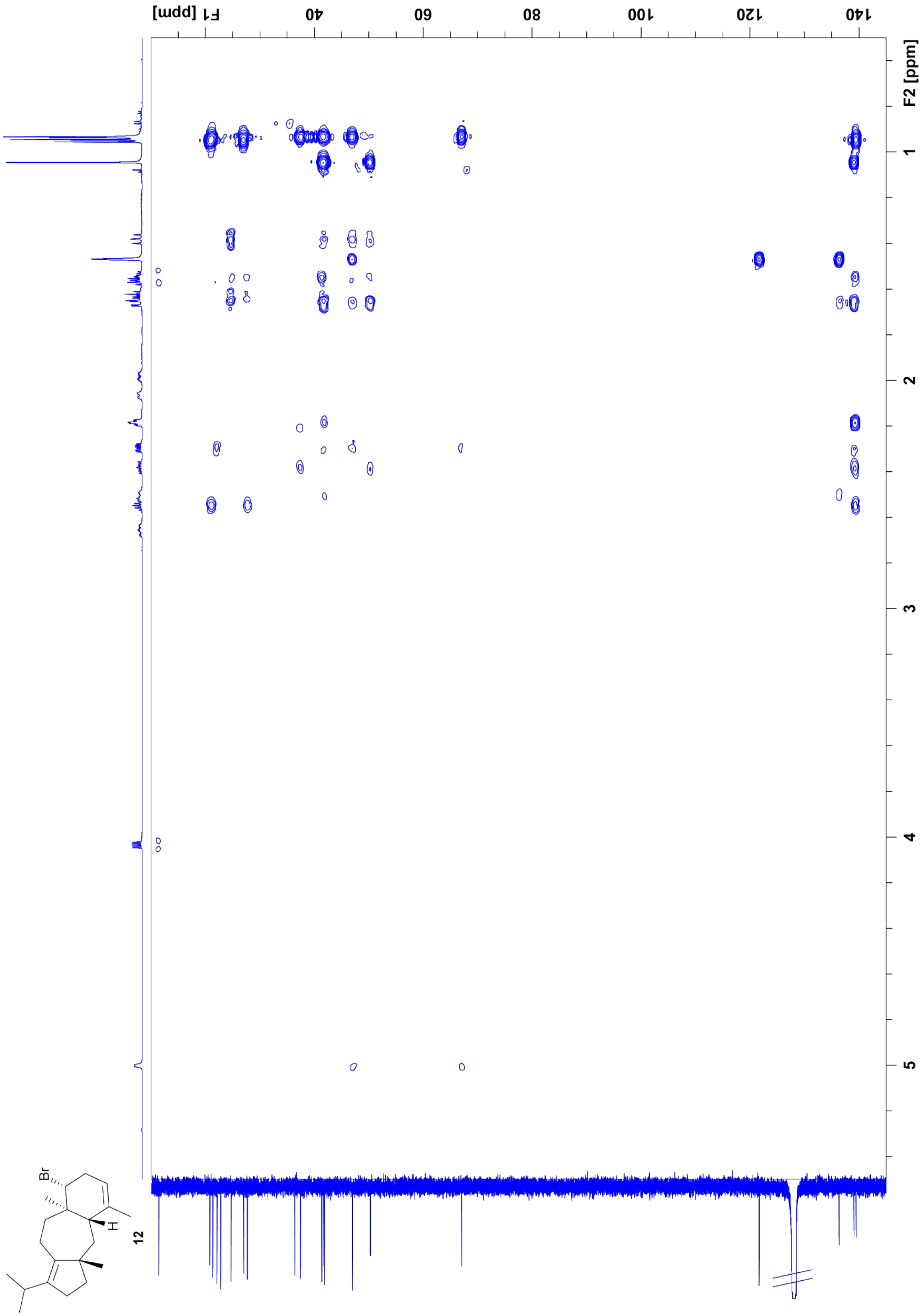

Figure S45. HMBC spectrum of **12** (700 MHz, C_6D_6).

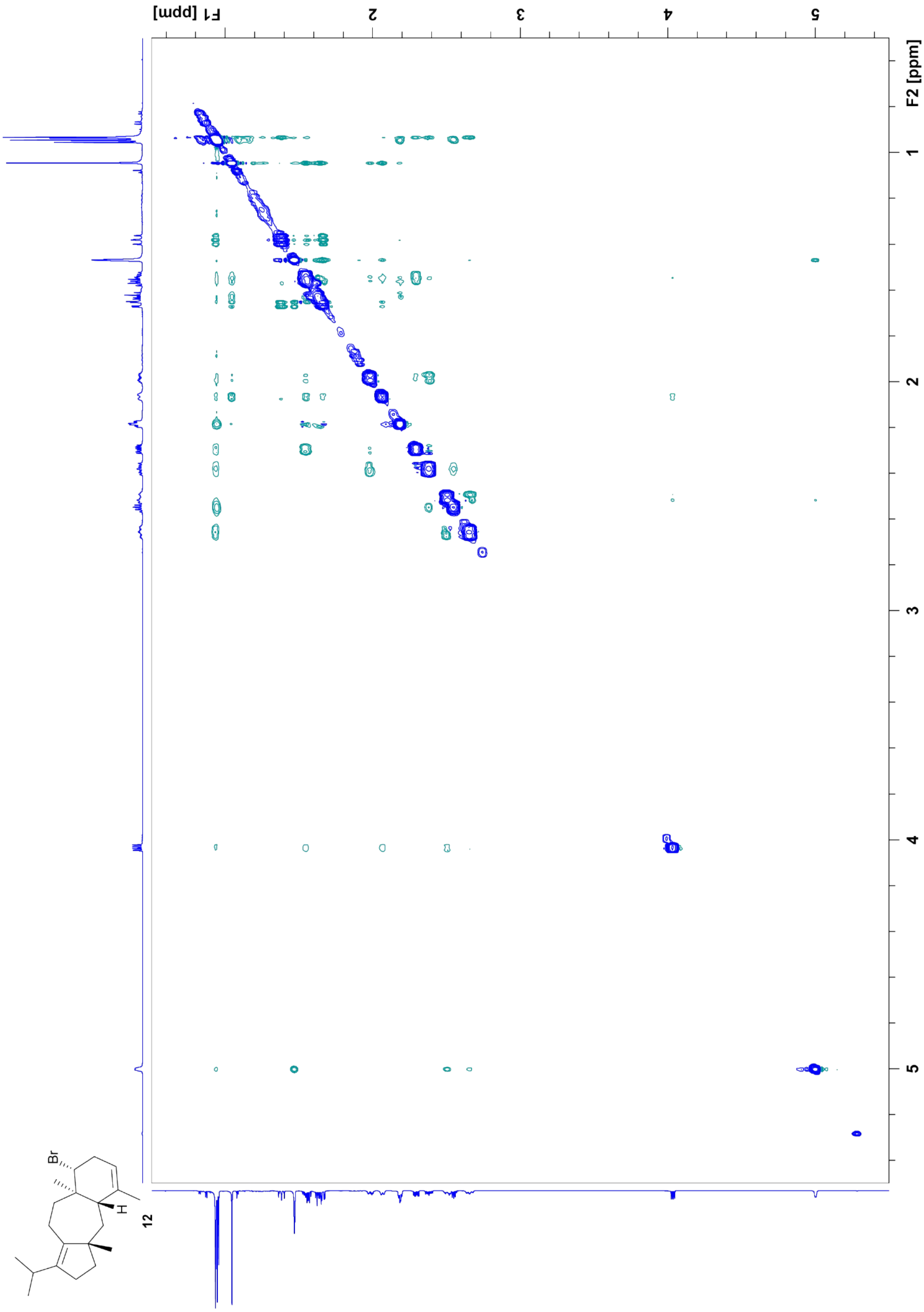

Figure S46. NOESY spectrum of **12** (700 MHz, C_6D_6).

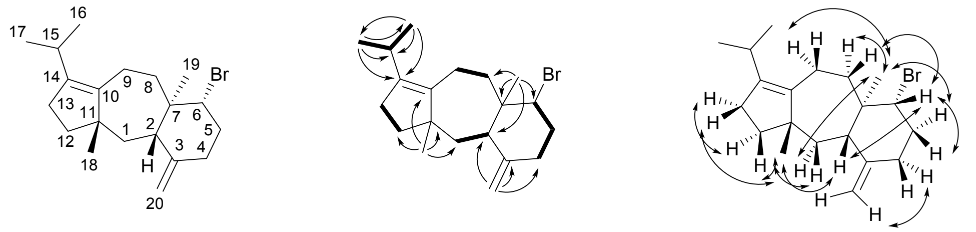

Figure S47. Carbon numbering and structure elucidation of **13**. H,H-COSY spin systems are represented by bold bonds, single headed arrows show HMBC correlations and NOESY correlations are depicted by double headed arrows.

Table S6. NMR data of (2*S*,6*R*,7*R*,11*S*)-6-bromodolasta-3(20),10(14)-diene (**13**) in C_6D_6 recorded at 298 K.

C[a]		^{13}C[b]	^{1}H[b]
1	CH_2	41.51	1.63 (m, H_α)
			1.57 (dd, J = 13.9, 10.4, H_β)
2	CH	46.16	2.03 (m)
3	C_q	149.33	–
4	CH_2	38.22	1.96 (ddd, J = 13.1, 4.9, 2.3, H_β)
			1.72 (m, H_α)
5	CH_2	36.11	2.10 (m, H_α)
			2.08 (m, H_β)
6	CH	66.74	3.90 (dd, J = 12.0, 5.0)
7	C_q	44.97	–
8	CH_2	38.38	2.03 (m, H_α)
			1.72 (m, H_β)
9	CH_2	20.51	2.25 (m, H_β)
			1.87 (m, H_α)
10	C_q	140.85	–
11	C_q	49.59	–
12	CH_2	41.68	1.63 (m, H_α)
			1.52 (ddd, J = 11.9, 8.4, 8.4, H_β)
13	CH_2	27.72	2.23 (m, H_α)
			2.18 (m, H_β)
14	C_q	138.85	–
15	CH	26.83	2.56 (sept, J = 6.9)
16[c]	CH_3	21.38	0.96 (d, J = 6.9)
17[c]	CH_3	21.26	0.95 (d, J = 6.9)
18	CH_3	23.68	1.00 (s)
19	CH_3	14.25	0.88 (s)
20	CH_2	108.16	4.73 (m, H_E)
			4.59 (m, H_Z)

[a] Carbon numbering indicates the origin of each carbon from GGPP by identical number as shown in Figure S47. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, br = broad, coupling constants J are given in Hertz. [c] Signals may be interchanged.

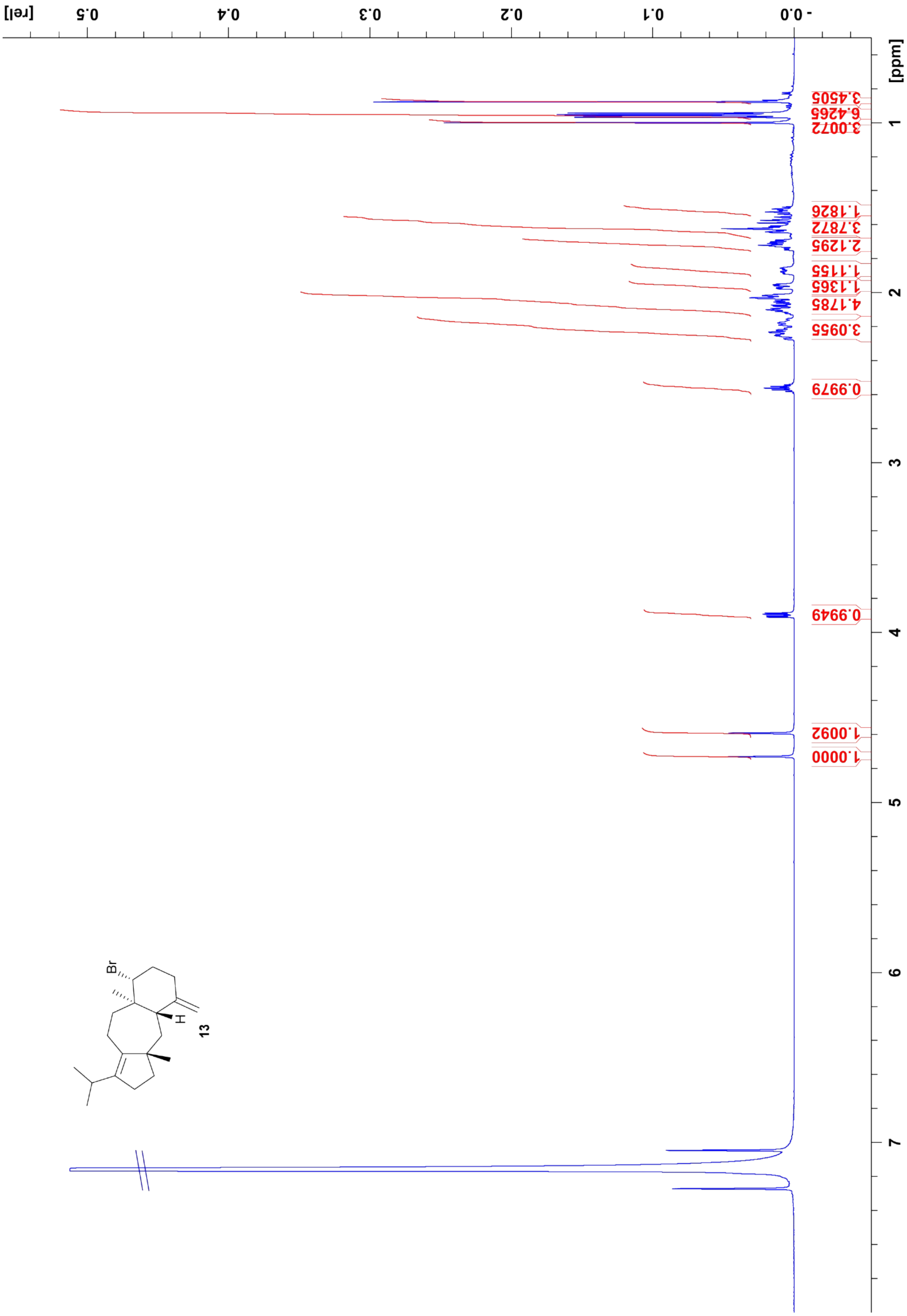

Figure S48. ^{1}H-NMR spectrum of **13** (700 MHz, C_6D_6).

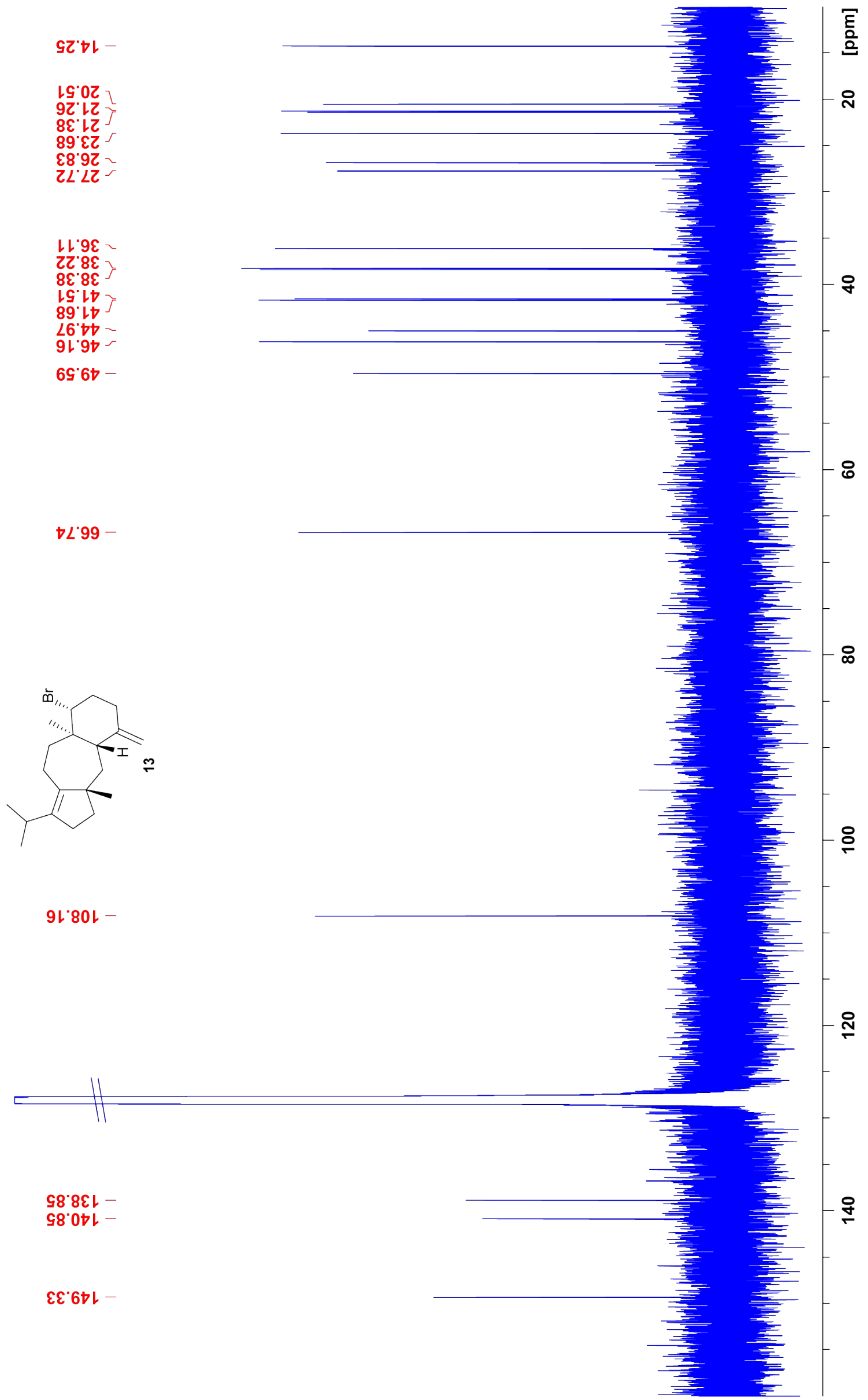

Figure S49. ^{13}C-NMR spectrum of **13** (175 MHz, C_6D_6).

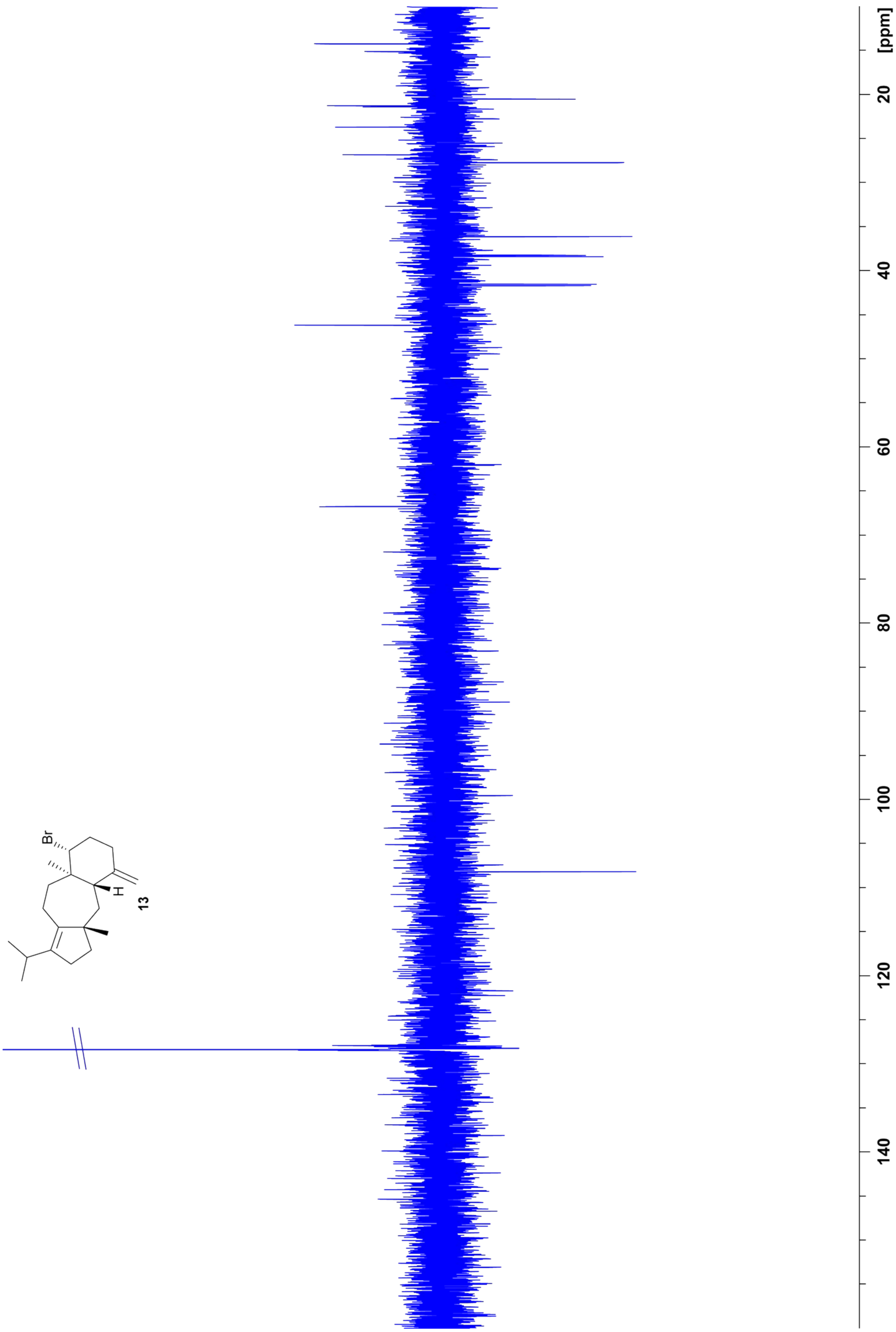

Figure S50. ^{13}C-DEPT spectrum of **13** (175 MHz, C_6D_6).

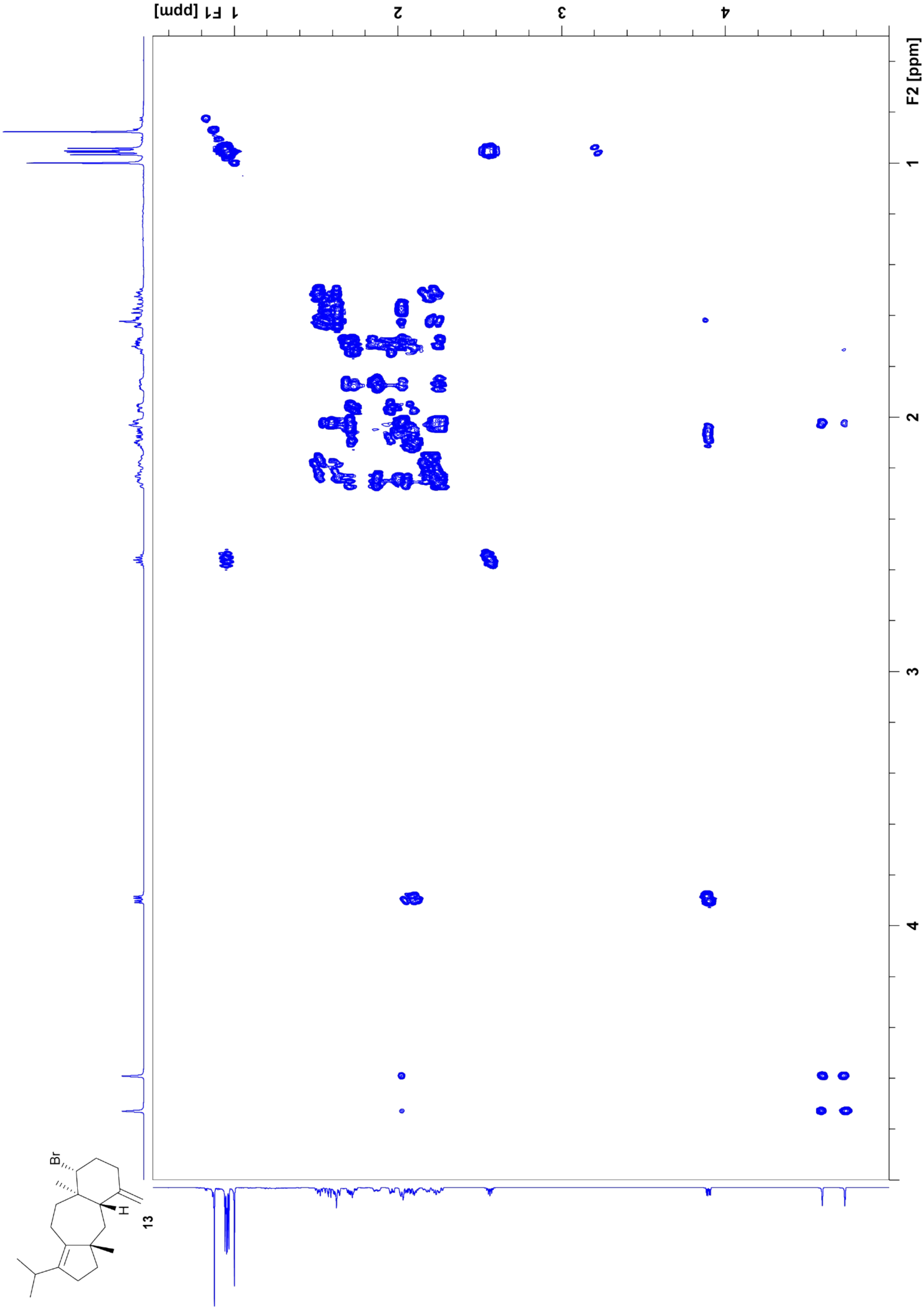

Figure S51. $^{1}H,^{1}H$-COSY spectrum of **13** (700 MHz, C_6D_6).

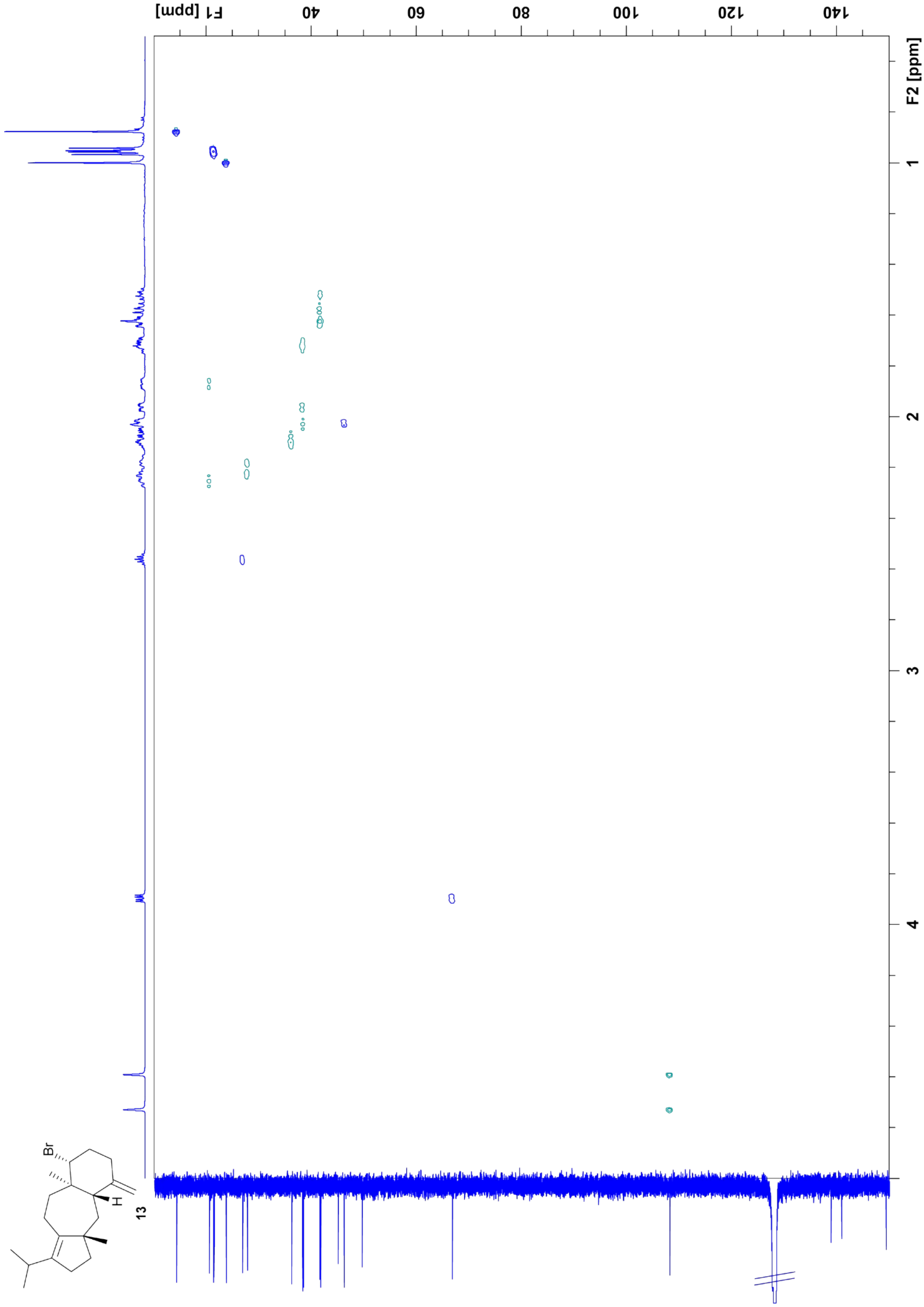

Figure S52. HSQC spectrum of **13** (700 MHz, C_6D_6).

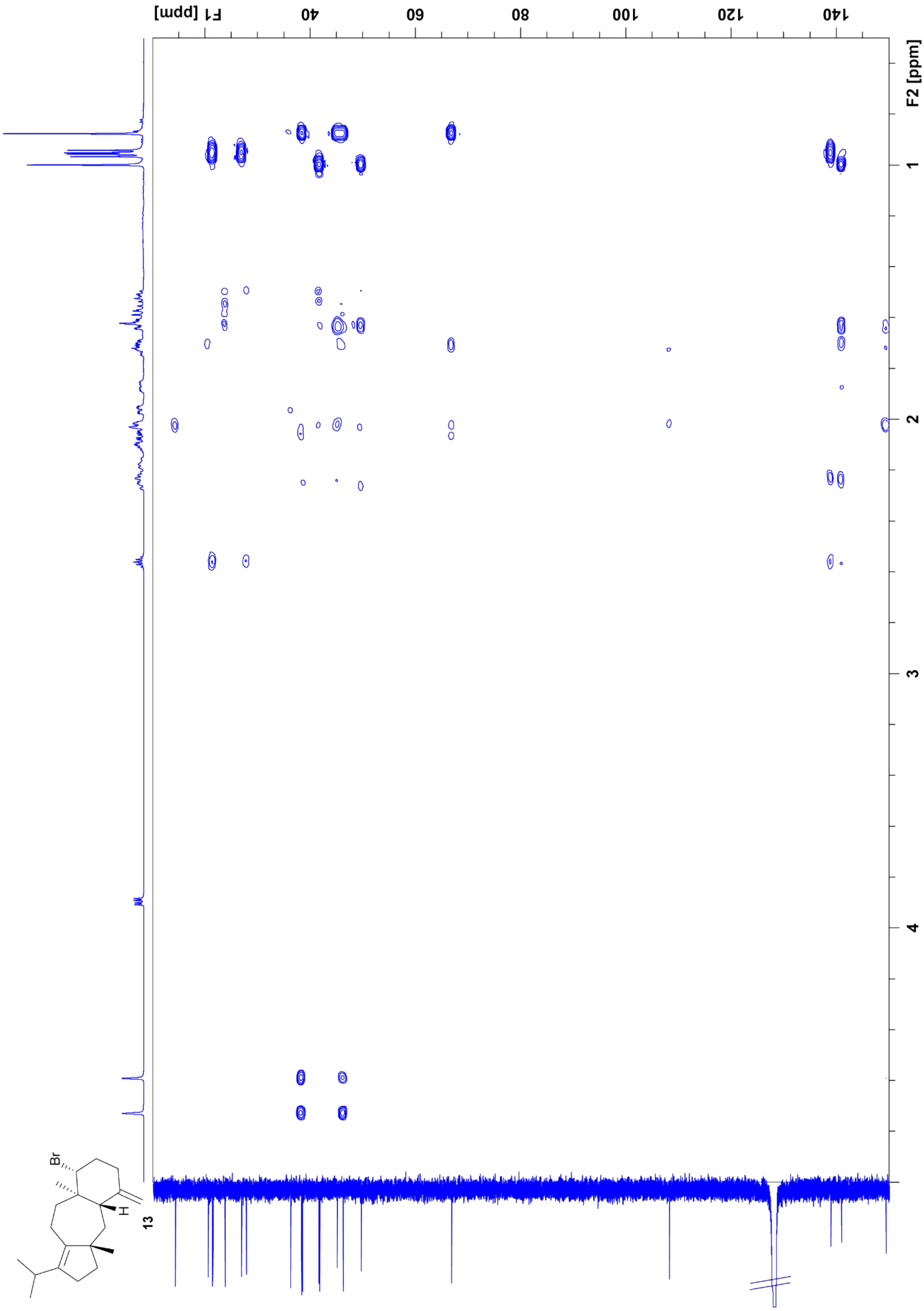

Figure S53. HMBC spectrum of **13** (700 MHz, C_6D_6).

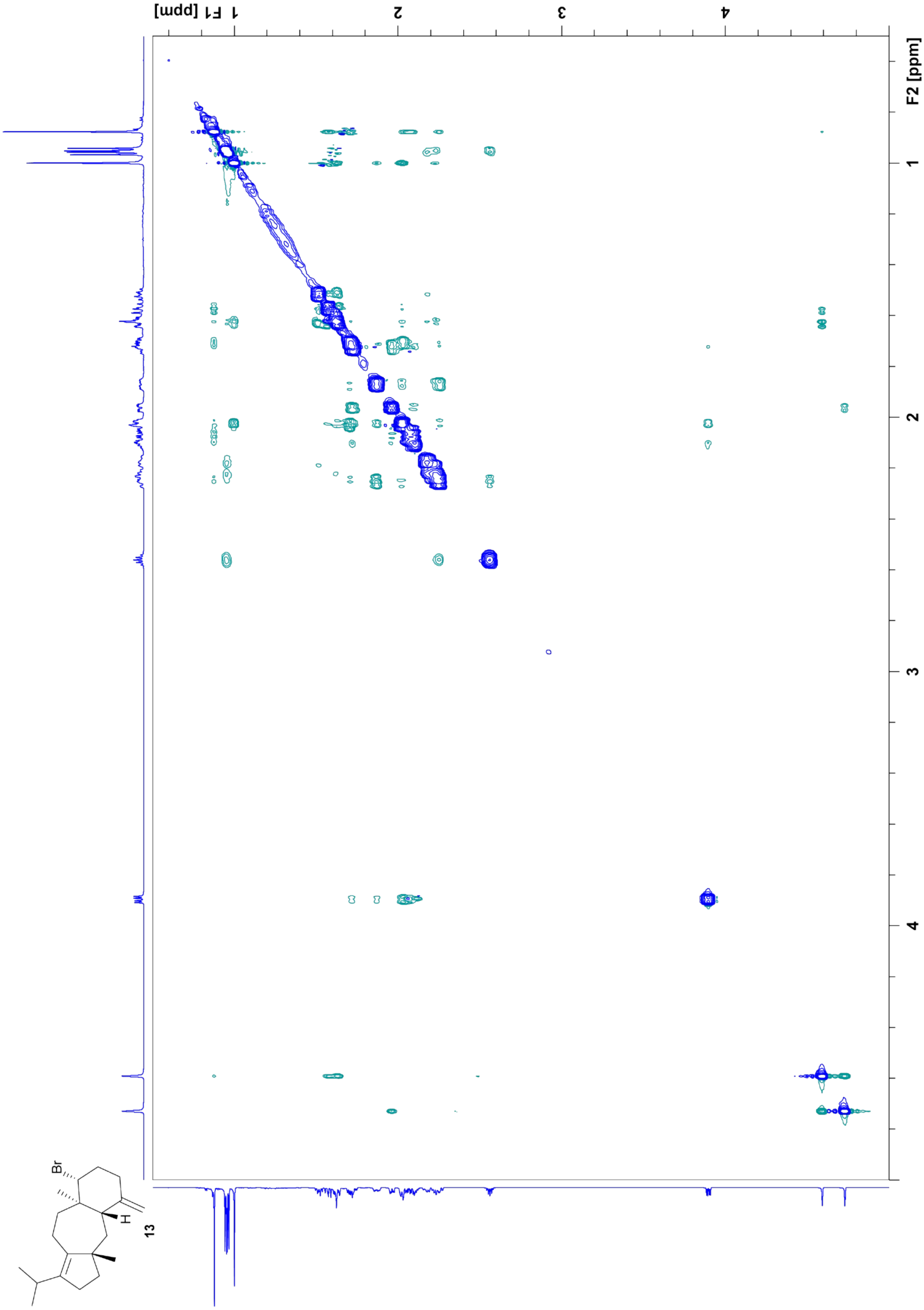

Figure S54. NOESY spectrum of **13** (700 MHz, C_6D_6).

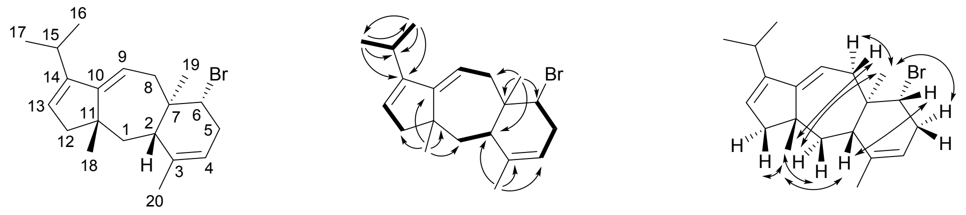

Figure S55. Carbon numbering and structure elucidation of **14**. H,H-COSY spin systems are represented by bold bonds, single headed arrows show HMBC correlations and NOESY correlations are depicted by double headed arrows.

Table S7. NMR data of (2*S*,6*R*,7*R*,11*S*)-6-bromodolasta-3,9,13-triene (**14**) in C_6D_6 recorded at 298 K.

C[a]		^{13}C[b]	^{1}H[b]
1	CH_2	37.38	1.71 (dd, J = 14.1, 2.0, H_α)
			1.48 (dd, J = 14.1, 12.9, H_β)
2	CH	47.87	2.25 (m)
3	C_q	136.27	–
4	CH	122.04	5.02 (m)
5	CH_2	36.47	2.66 (m, H_β)
			2.51 (m, H_α)
6	CH	66.28	4.00 (dd, J = 10.4, 5.8)
7	C_q	41.41	–
8	CH_2	39.33	2.99 (dd, J = 15.3, 9.8, H_β)
			2.02 (dd, J = 15.4, 4.0, H_α)
9	CH	113.53	5.36 (dd, J = 9.8, 4.2)
10	C_q	154.90	–
11	C_q	45.46	–
12	CH_2	49.78	2.20 (d, J = 16.7, H_β)
			2.09 (dd, J = 16.7, 2.7, H_α)
13	CH	125.80	5.53 (m)
14	C_q	150.30	–
15	CH	25.83	2.34 (sept, J = 6.8)
16[c]	CH_3	22.23	1.10 (d, J = 6.8)
17[c]	CH_3	22.29	1.07 (d, J = 6.8)
18	CH_3	24.28	1.07 (s)
19	CH_3	11.51	1.01 (s)
20	CH_3	22.68	1.44 (m)

[a] Carbon numbering indicates the origin of each carbon from GGPP by identical number as shown in Figure S55. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, br = broad, coupling constants J are given in Hertz. [c] Signals may be interchanged.

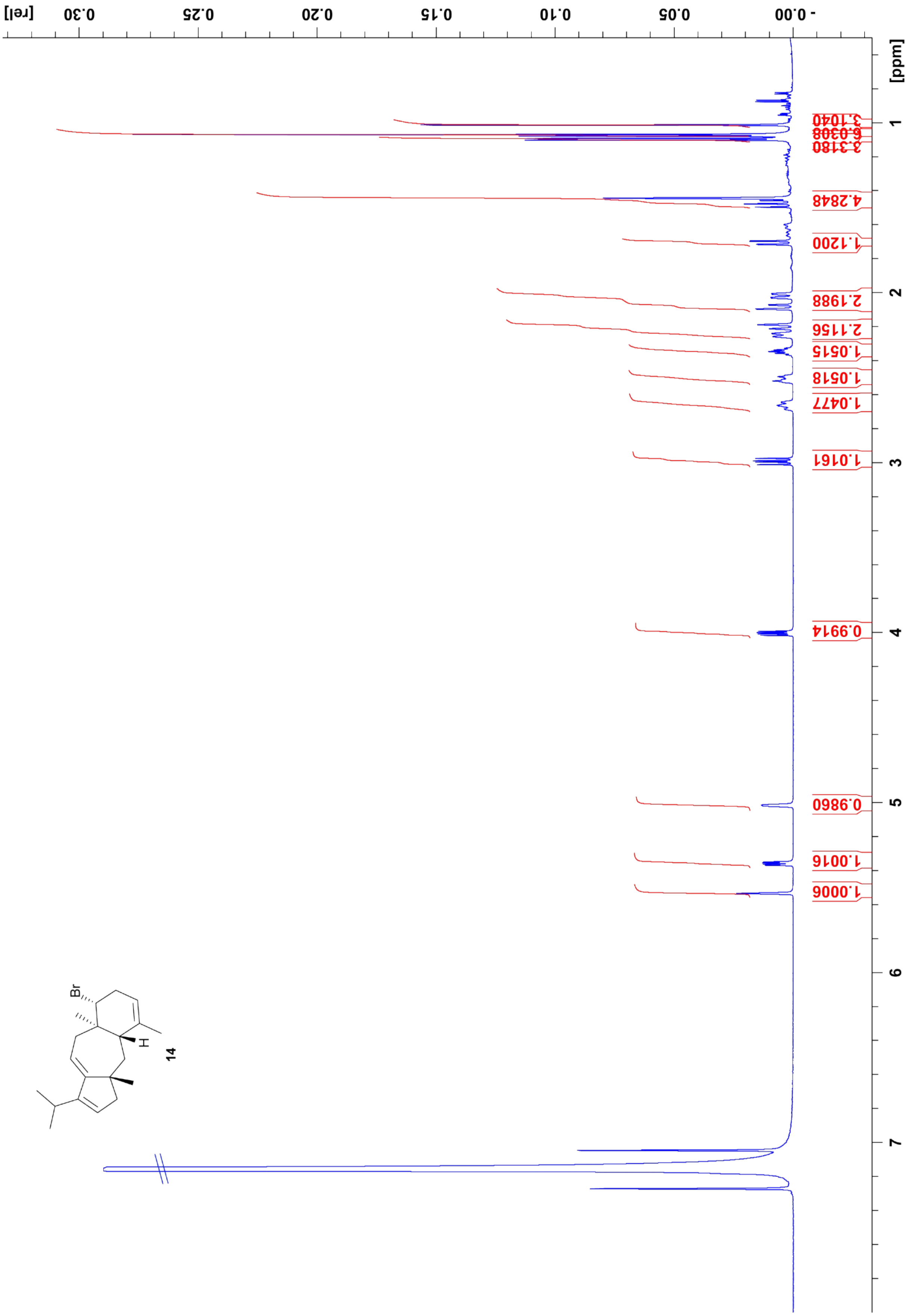

Figure S56. ^{1}H-NMR spectrum of **14** (700 MHz, C_6D_6).

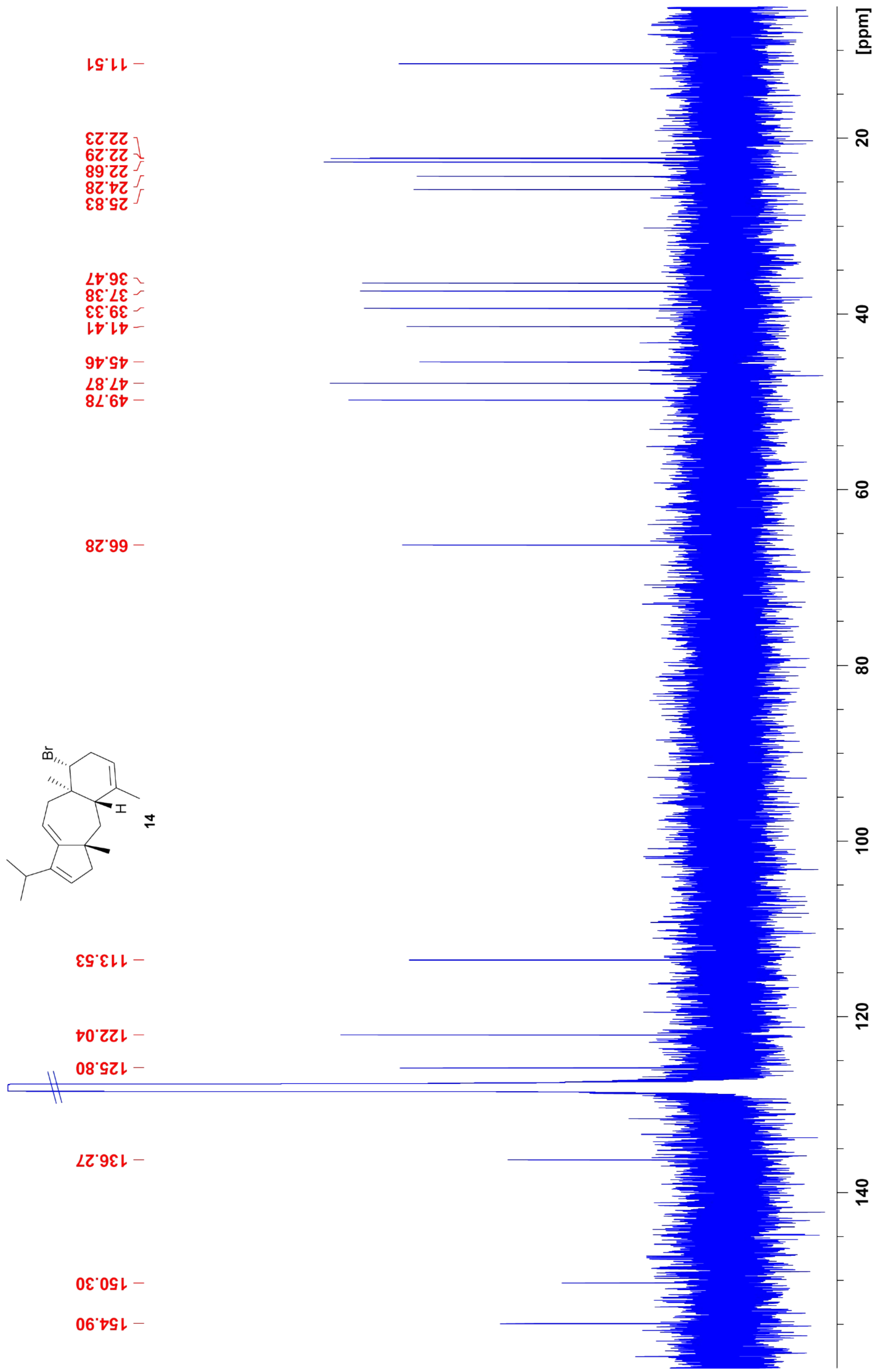

Figure S57. ^{13}C-NMR spectrum of **14** (175 MHz, C_6D_6).

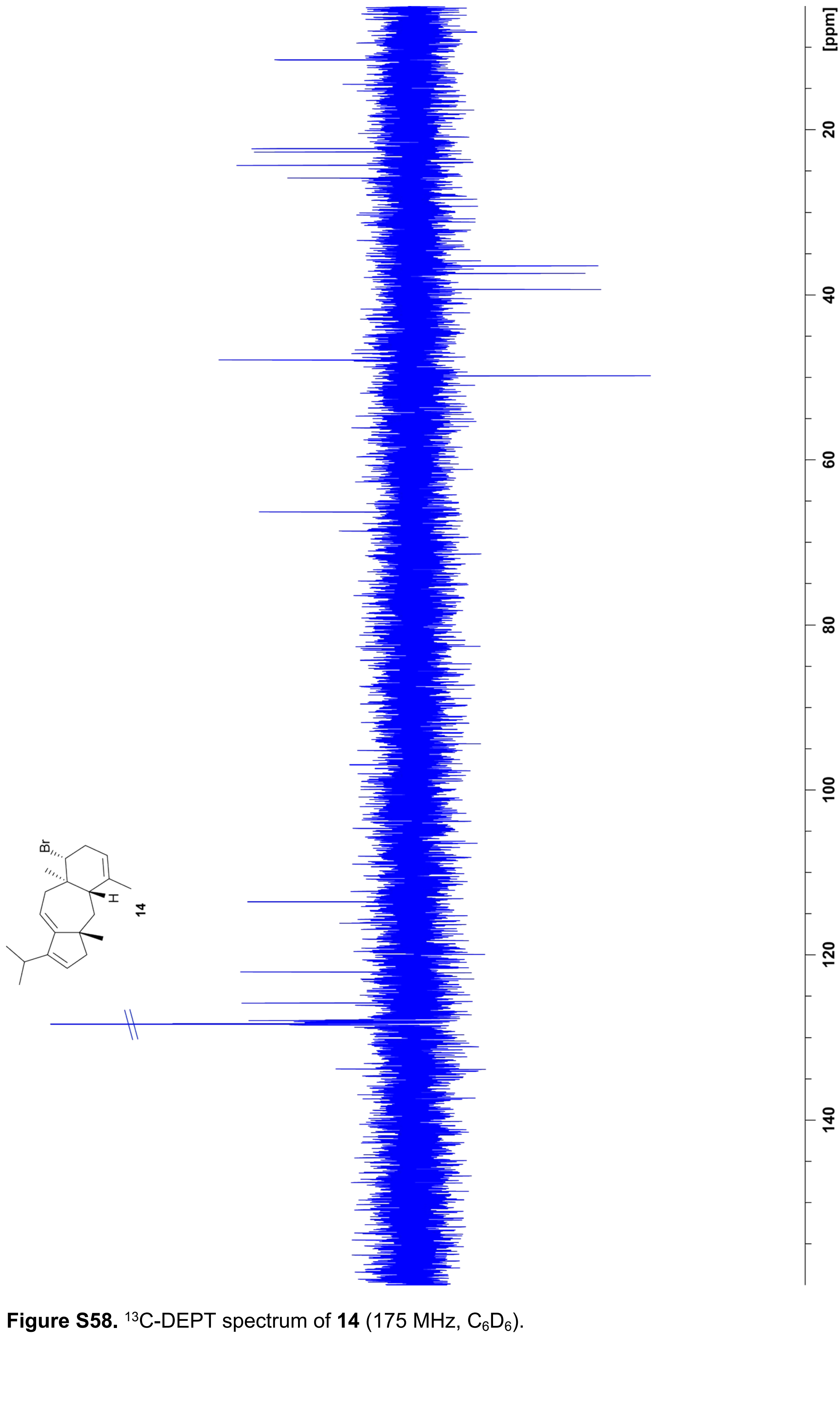

Figure S58. ^{13}C-DEPT spectrum of **14** (175 MHz, C_6D_6).

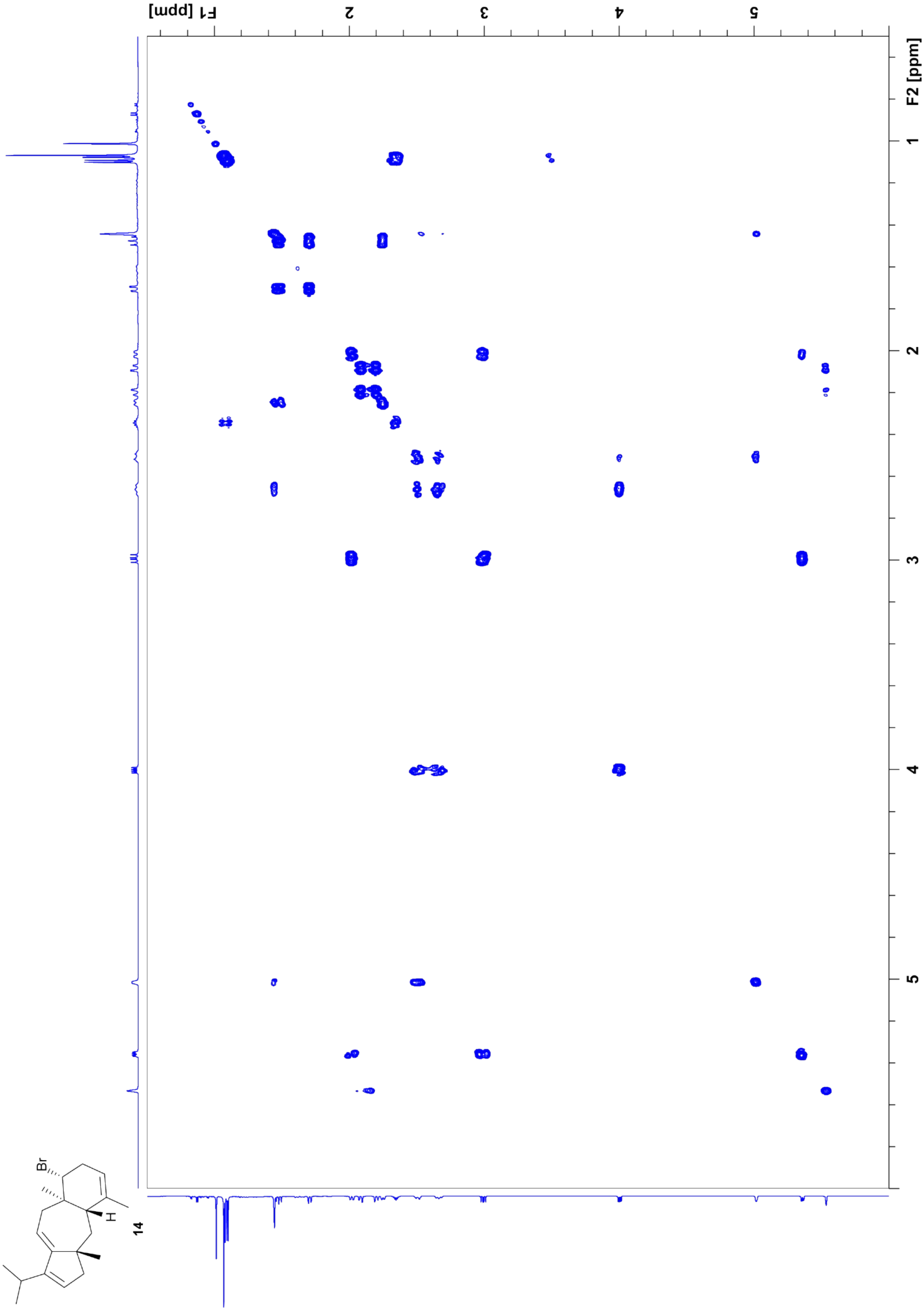

Figure S59. $^{1}H,^{1}H$-COSY spectrum of **14** (700 MHz, C_6D_6).

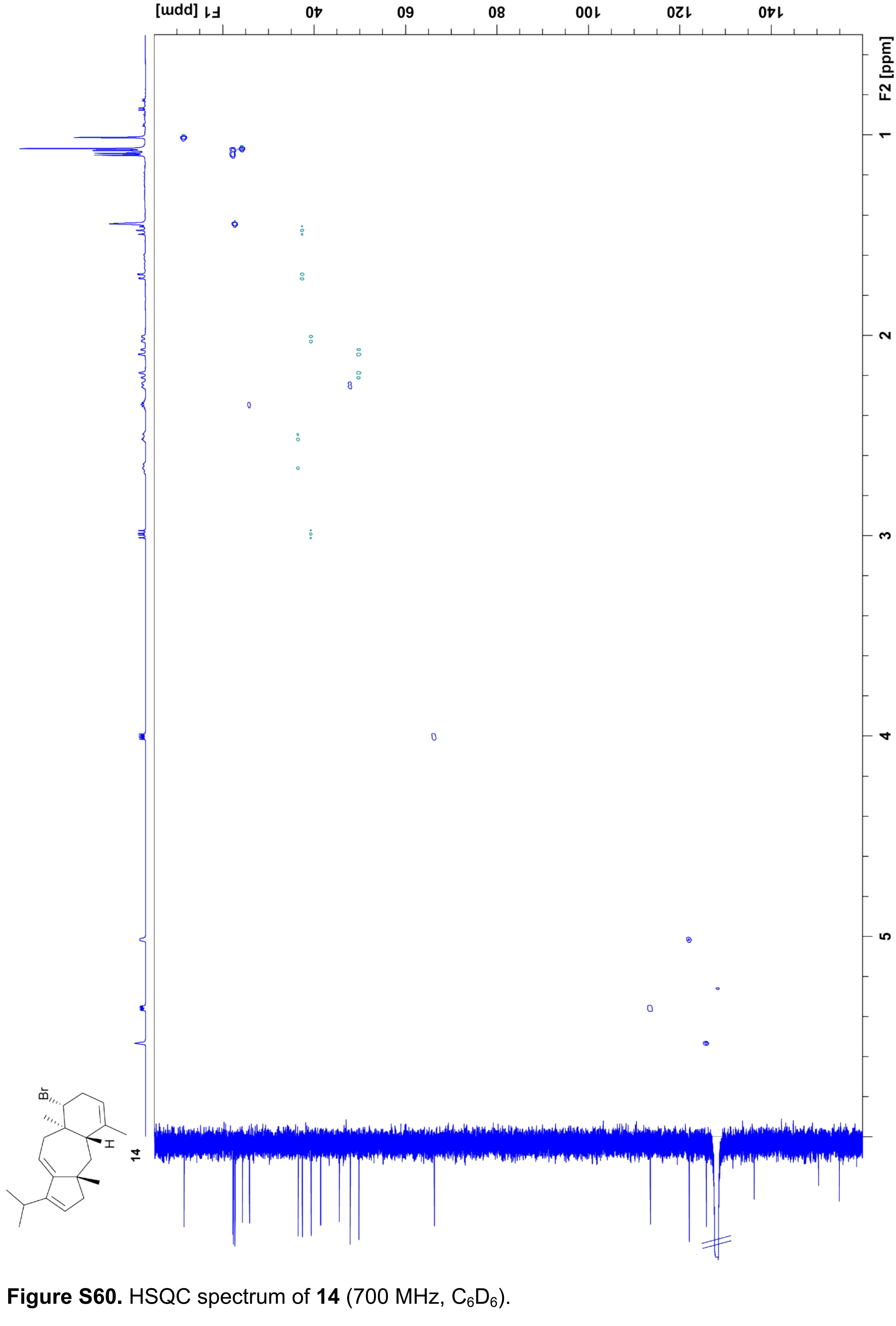

Figure S60. HSQC spectrum of **14** (700 MHz, C_6D_6).

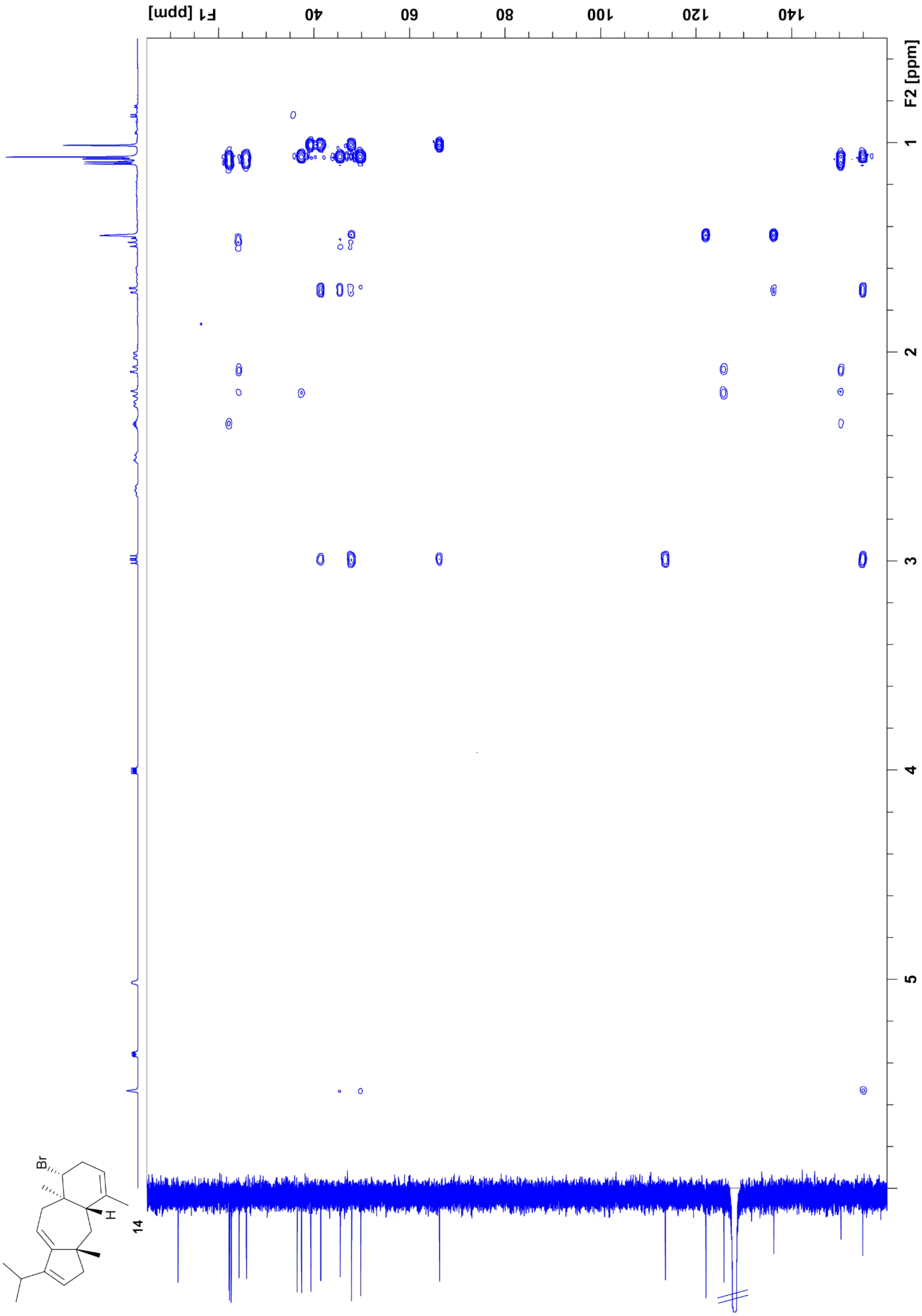

Figure S61. HMBC spectrum of **14** (700 MHz, C_6D_6).

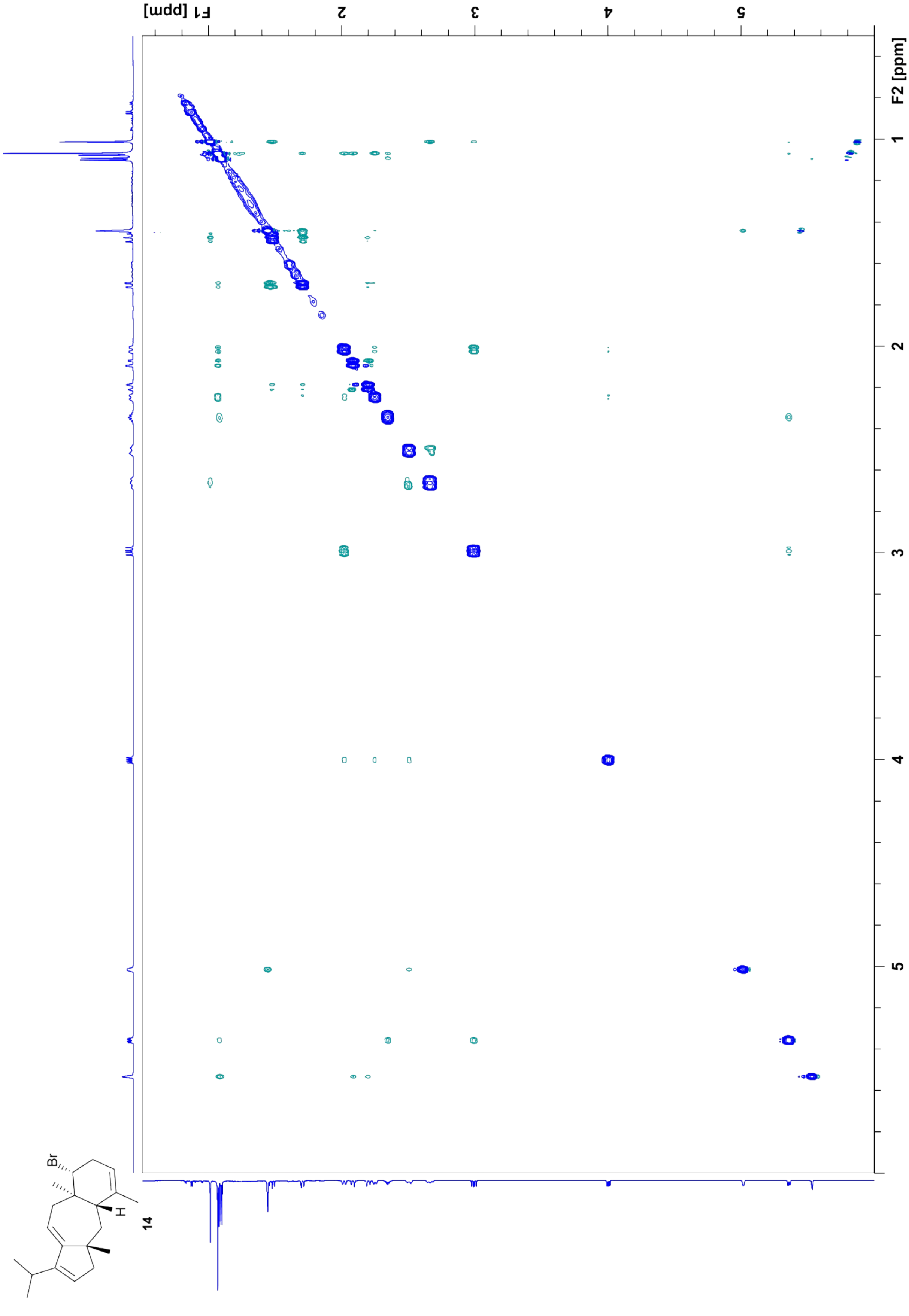

Figure S62. NOESY spectrum of **14** (700 MHz, C_6D_6).

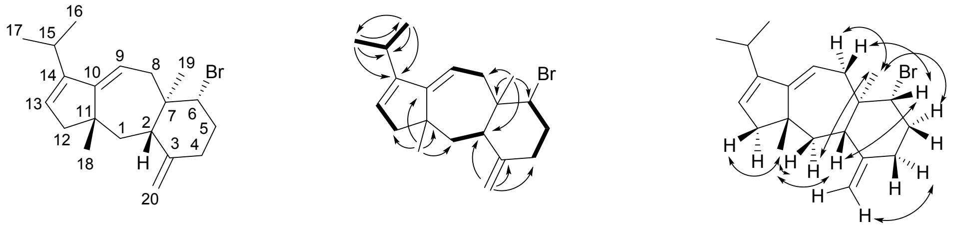

Figure S63. Carbon numbering and structure elucidation of **15**. H,H-COSY spin systems are represented by bold bonds, single headed arrows show HMBC correlations and NOESY correlations are depicted by double headed arrows.

Table S8. NMR data of (2*S*,6*R*,7*R*,11*S*)-6-bromodolasta-3(20),9,13-triene (**15**) in C_6D_6 recorded at 298 K.

C[a]		^{13}C[b]	^{1}H[b]
1	CH_2	39.24	1.71 (m, H_β)
			1.57 (dd, J = 13.8, 2.3, Hα)
2	CH	48.45	2.12 (m)
3	C_q	149.55	–
4	CH_2	38.57	1.96 (ddd, J = 12.9, 4.6, 2.6, H_β)
			1.72 (m, H_α)
5	CH_2	36.56	2.10 (m, H_α)
			2.02 (m, H_β)
6	CH	68.19	3.75 (dd, J = 12.4, 4.5)
7	C_q	45.23	–
8	CH_2	38.88	2.86 (dd, J = 14.8, 9.8, H_β)
			2.18 (dd, J = 14.8, 4.6, H_α)
9	CH	112.98	5.33 (dd, J = 9.8, 4.7)
10	C_q	155.69	–
11	C_q	44.70	–
12	CH_2	50.26	2.21 (d, J = 18.4, H_β)
			2.09 (m, H_α)
13	CH	126.26	5.54 (br s)
14	C_q	149.85	–
15	CH	25.84	2.33 (m)
16	CH_3	22.26	1.07 (d, J = 6.8)
17	CH_3	22.34	1.09 (d, J = 6.8)
18	CH_3	23.06	1.12 (s)
19	CH_3	11.92	0.96 (s)
20	CH_2	109.00	4.71 (m, H_E)
			4.50 (br s, H_Z)

[a] Carbon numbering indicates the origin of each carbon from GGPP by identical number as shown in Figure S63. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, br = broad, coupling constants J are given in Hertz.

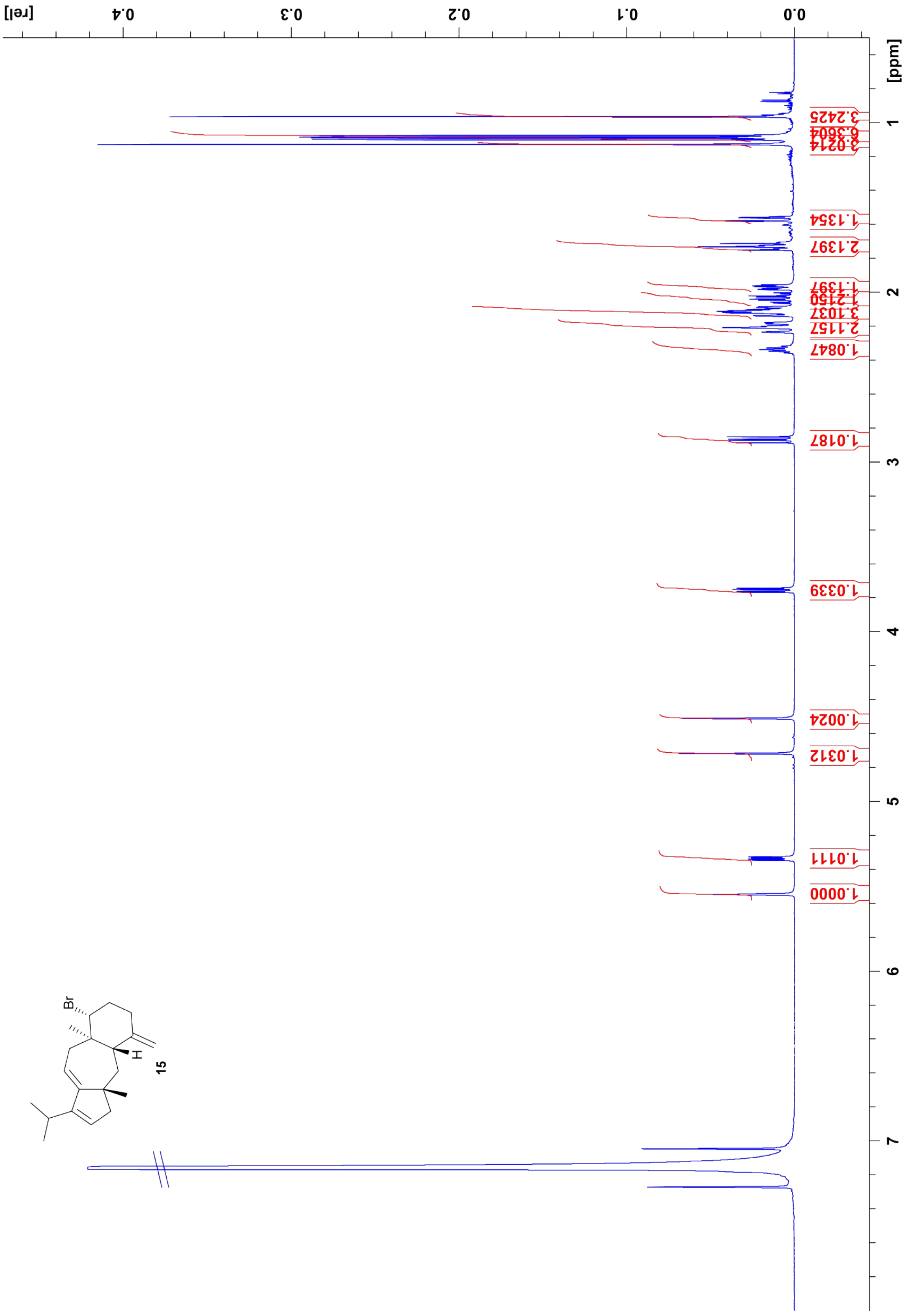

Figure S64. ^{1}H-NMR spectrum of **15** (700 MHz, C_6D_6).

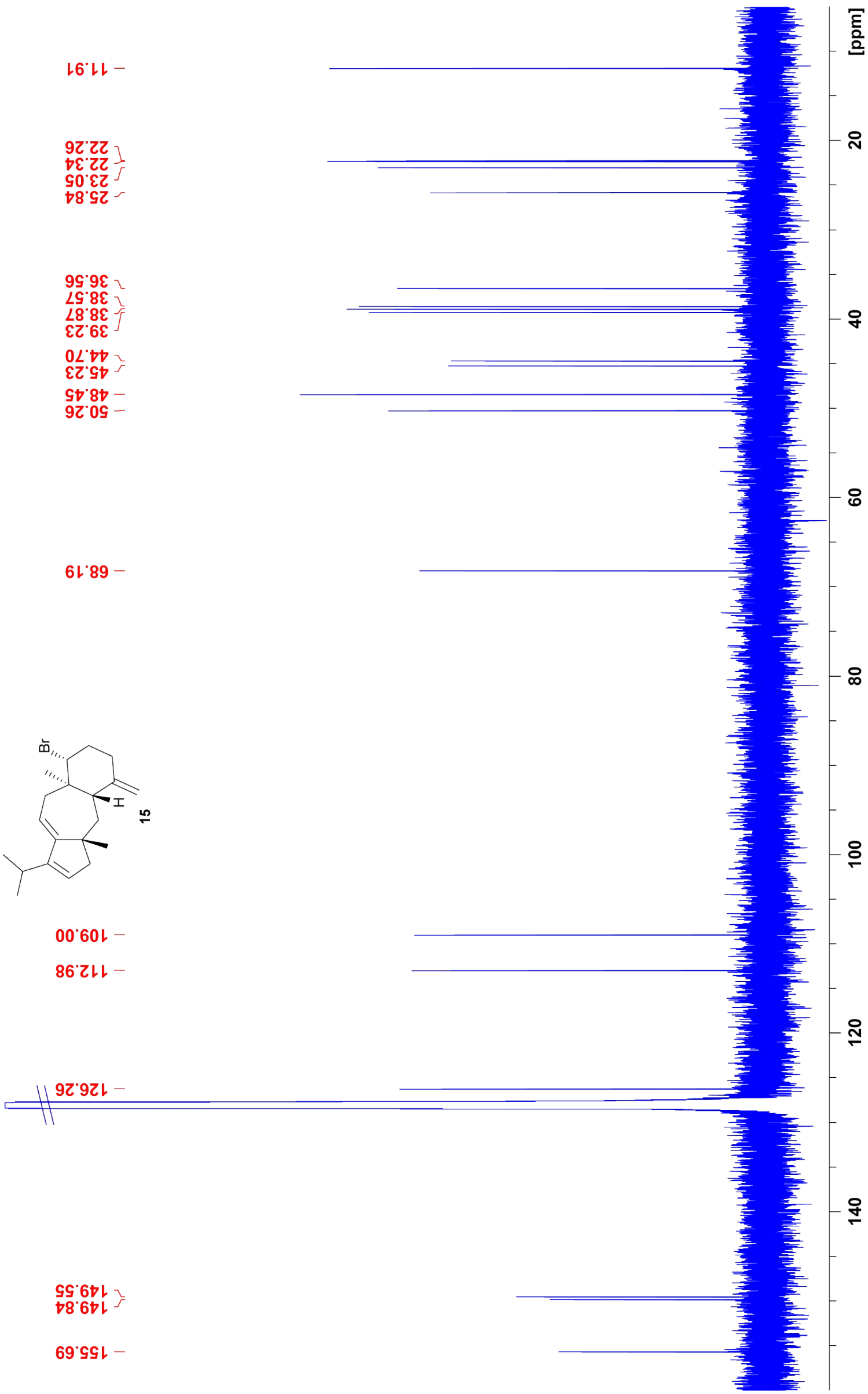

Figure S65. [13]C-NMR spectrum of **15** (175 MHz, C_6D_6).

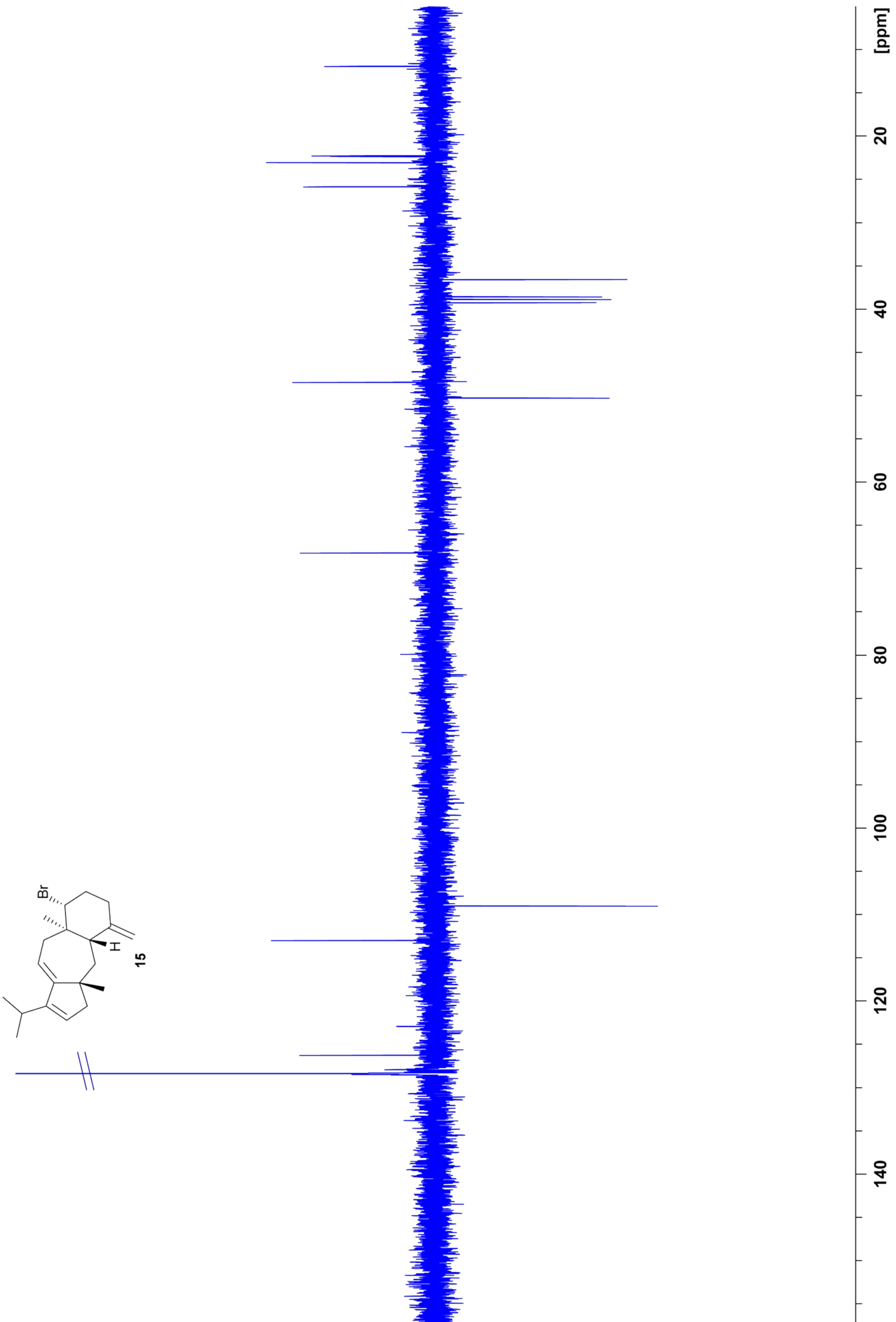

Figure S66. ^{13}C-DEPT spectrum of **15** (175 MHz, C_6D_6).

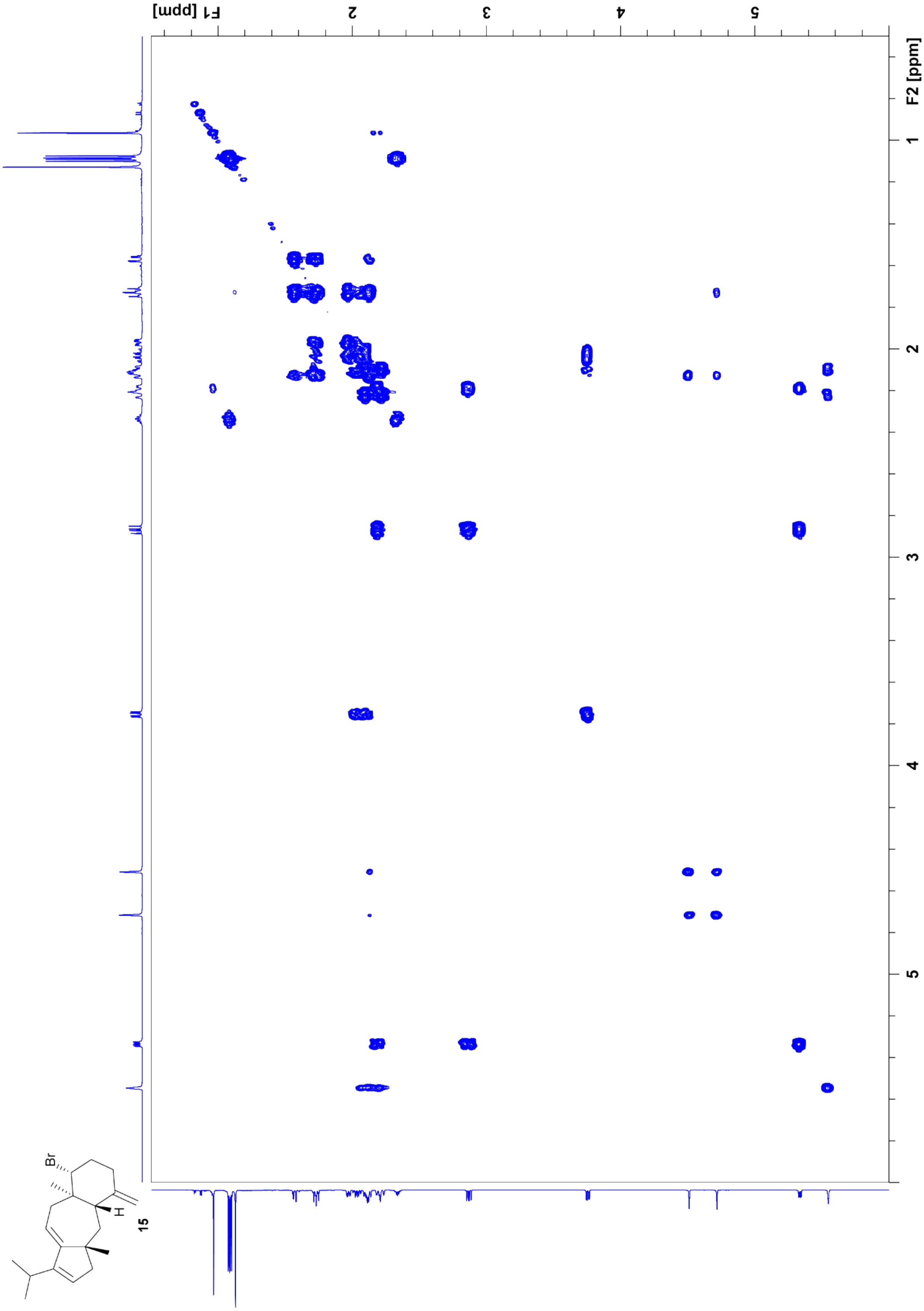

Figure S67. ^{1}H,^{1}H-COSY spectrum of **15** (700 MHz, C_6D_6).

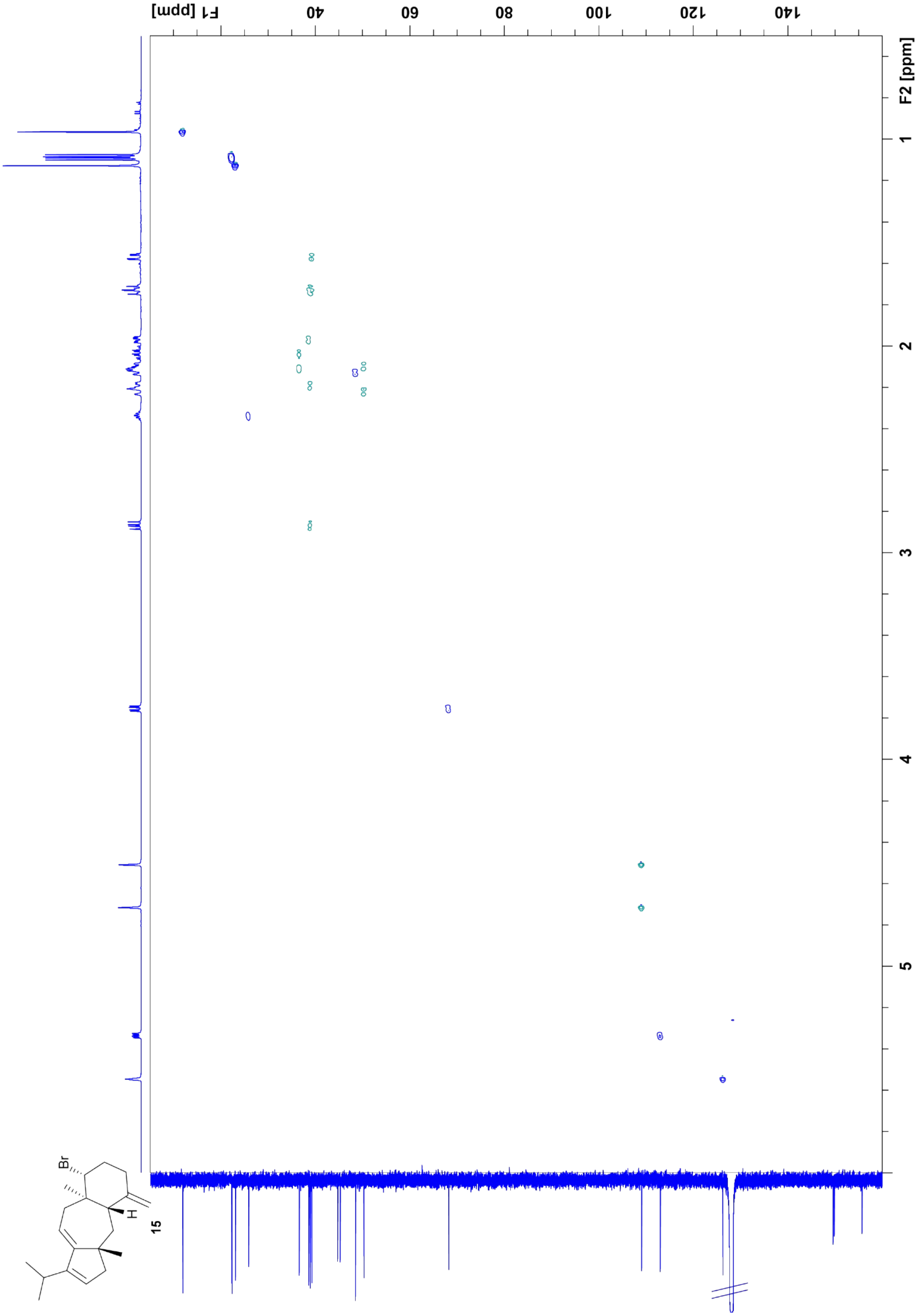

Figure S68. HSQC spectrum of **15** (700 MHz, C_6D_6).

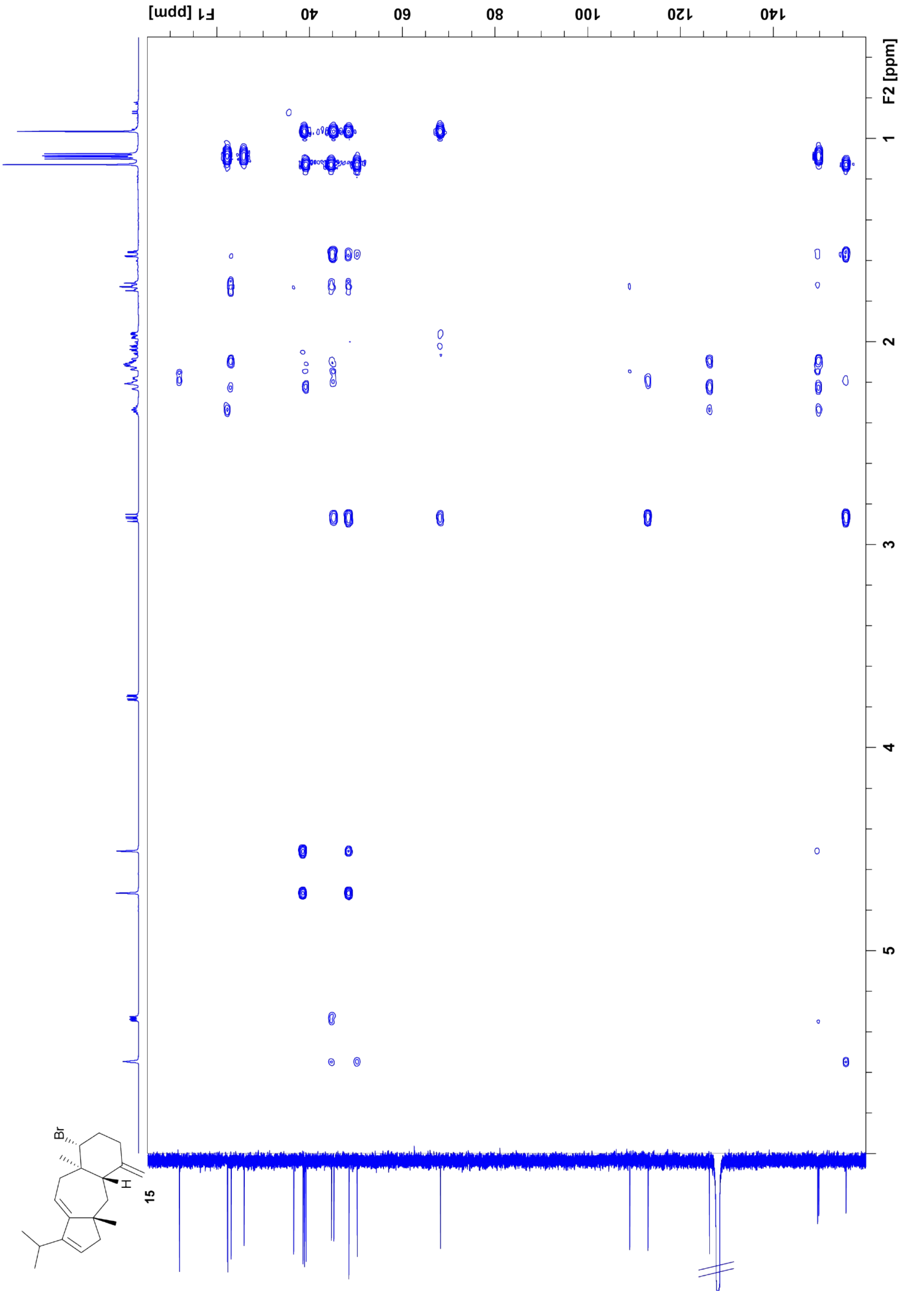

Figure S69. HMBC spectrum of **15** (700 MHz, C_6D_6).

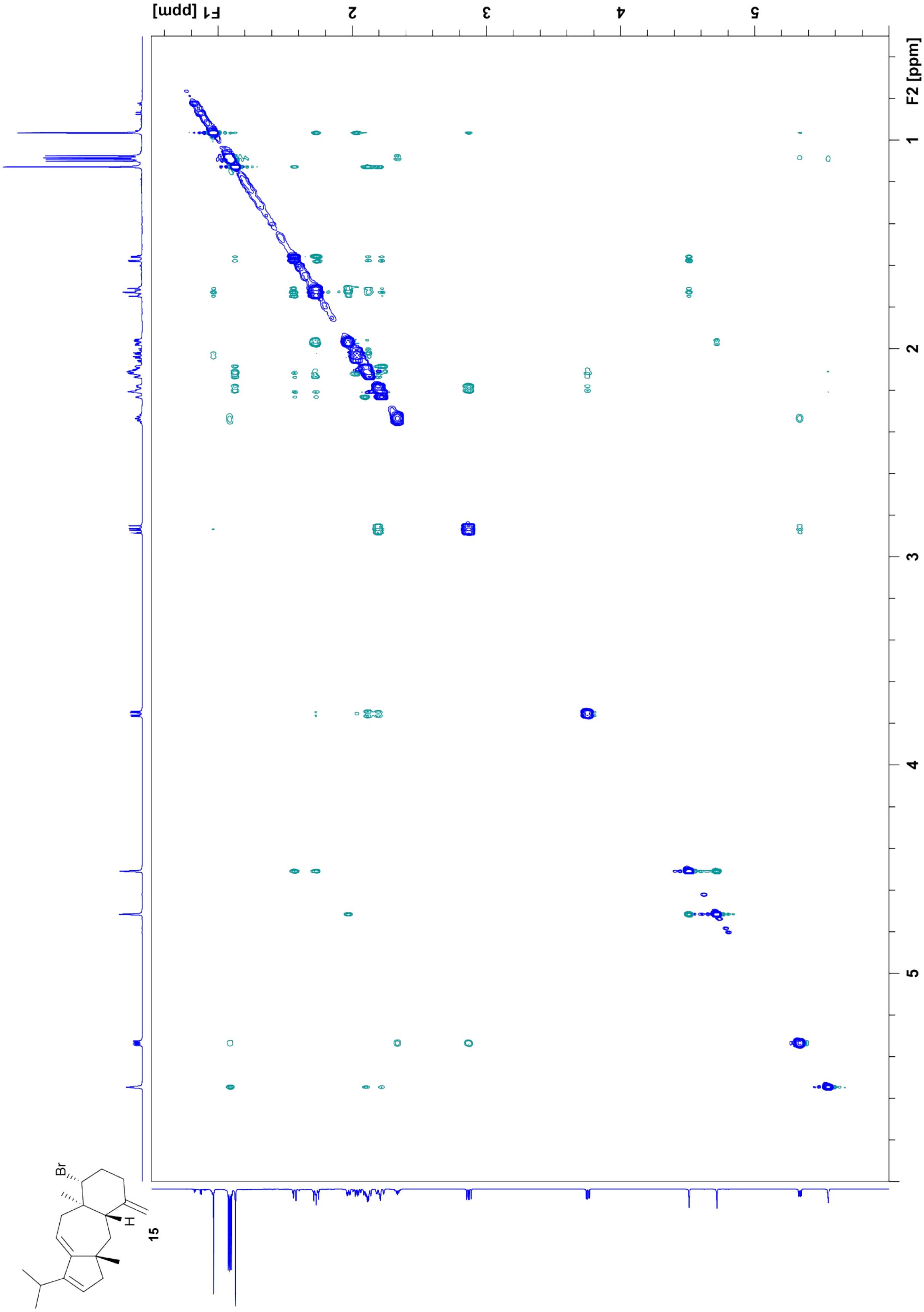

Figure S70. NOESY spectrum of **15** (700 MHz, C_6D_6).

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