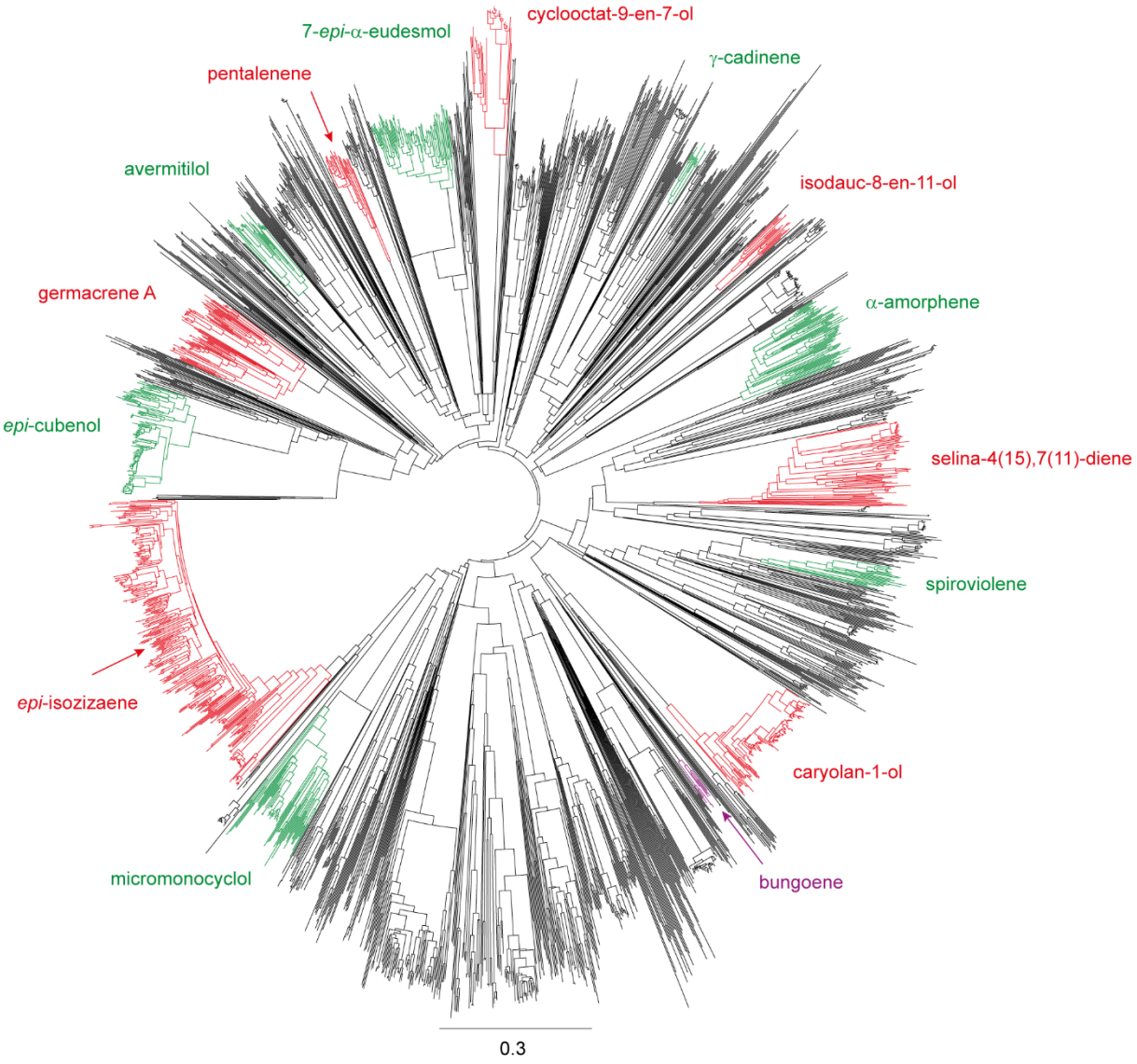


Figure S1. Phylogenetic tree of bacterial type I terpene synthases showing the largest groups of homologous enzymes with at least one characterised member (red and green). The newly characterised bungoene synthase and its closely related homologs are shown in purple. The enzymes from *S. bungoensis* identified in this study are indicated by arrows. The scale bar shows substitutions per site.

Accession: WP_061914986, locus: AQJ66_RS18560 (geosmin synthase)

MTQPFELPHFYMPYPARLNPHLDEARAHSTAWARETGMLEGSGIWEQADLEAHDYGLLCAYTHPDCDG
PALSLITDWYVWVFFFDDHFLDMYKRTQDRAAGKAHLDRLPLFMPLDPAAPVPEPENPVEAGLKDLWA
RTVPAMSVDWRRRFAVATEHLLNESLWELSNINEGRIANPVEYIEMRRKVGGAPWSAGLVEYATAEVP
AAVAGTRPLRVLMETFADAVHLRNDLFSYQREVEDEGENSNGVLVLETFFGCTTQEAAETVNDILTSR
LHQFEHTALTEVPAVALEKGLRPDEAAAVAAYTKGLQDWQSGGHEWHLRSSRYMNQGARTGSPWQLPS
GPGTSAADVGALLASAAAERLRAYAHLPFQRVGPSRLPDFRMPFPLELSPHLDRARGNLVTWSHRMGI
LHEGVWDEEKLAAYDLALCSAGLDPDAGPEALDLSAQWLAWGTYGDDYYPLVYGHRRDLAAARLTTAR
LSACMGVDGEPAVVPADAMERALVDLWTRTTAGMPPDQRRTLKAAVDTMTESWVWELSNQLQNRVPDP
VDYLEMRRSTFGSDLTLSMCRTGHGPAIPPEVYRSGPVRSLENAAIDYACLLNDVFSYQKEIEYEGEI
HNAVLVVQNFFGVDYPAALGVVHDLMTQRMAQFEHVIAHELPVLYDDFRLSEEARAAMAGYVLDLQNW
LAGILNWHRHVDRYKAEWLGRRTHTFLPDRPPAPVRLAG

Accession: WP_061917807, locus: AQJ66_RS06310 (pentalenene synthase, PS)

MPQDVDFHIPLPSRQSPDHARADAEQLVWPRSLGLIKSEAAATRHLRGGYADLASRFYPHATGADLDL
GVDLMSWFFLFDDLFDGPRGENPEETKRLTDAVAAALDGPLPDTAPPIAHGFADIWRRTREGMTPAWC
ARSARHWRSYFDGYVDEAESRFWDTPYDTAAEYLAVRRRTIGVQPTVDLAERAGRFEVPHRVFDSAVL
SAMLQIAVDVNLLLNDIASLEKEEARGEQNNMVMILRRERGWSKDRSVTHIQSEVRVRLEQYLLLESC
LPQVADIYRLDEAEHQALERYRDNAVRTVIRGSYDWHRSSGRYDAEFALAAGAQGYLEELGSTAR

Accession: WP_107118987, locus: AQJ66_RS20525 (*epi*-isozizaene synthase, EIZS)

MHAFSHGTTSSTAVAVPPALALPVIEDAFPRQLHPYWPKLQENTRDWLLEKRLMPADKVREYADGLCY
TDLMAGYYLGAPDDVLQAIADYSAWFFVWDDRHDRDIVHGRPADWRLLRNRLHAALDAPLHHLHHPDP
LVEGFADSVSRLYSFLPRTWNRRFARHFHAVIEAYDREFRNRTEGYVPKVEEYLALRRLTFAHRIWTD
LLEPSAGCELPDSVRGHPAYRRAALLSQEFAAWYNDLCSLPKEIAGDEVHNLGISLVTHEGLTLEEAV
DEVRRRIEECIAEFLEAERGALALAAEMTGETAHGEELGPAVRACVGNMRNWFSSVYWFHHESGRYMV
DSWDDRSTPPYVNNEAAGEK

Accession: WP_061922997, locus: AQJ66_RS18560 (bungoene synthase, BgS)

MDTFRIPDLCIPVPAALNPELRTATAATDAWLDEFGLVPTEEARRHVQRTRVDRLTAWLCPNASAEAL
TLITQWNAWLFQLDDQFDDDPVRGYQPERWREAFGPLFAVFDGTVAAGRLARSLADLWRRTTAVRSSA
WQRRFVPHLLWYFDSYRADMIHREEGRVPGLQQYLAHRRASFAFDAVLDLMEIGTGVDLPDRLHANPA
FADMREAVIYANACVNDLYSLRKELAHDYAFNAVTVIGHHHRCDLQEAVDRVAAMQAGYVQRILDLEQ
VLPERLTRAGLADDVADDALRCVRDFHALTAGNVAWSKETGRYAEIEAQPTYLADLFIP

Figure S2. Amino acid sequences of type I terpene synthases from *Streptomyces bungoensis* DSM 41781. Highly conserved motifs are highlighted in yellow. The old accession number for EIZS (WP_061924158) represented an amino acid sequence lacking the 23 underlined N-terminal amino acids.

Strains and culture conditions

Streptomyces bungoensis DSM 41718 was obtained from the DSMZ (Braunschweig, Germany) and was cultivated on Medium 65 GYM (4.0 g L^{-1} glucose, 4.0 g L^{-1} yeast extract, 10.0 g L^{-1} malt extract, distilled water, pH 7.2, autoclaved for 121 °C for 20 min, for agar plates 2.0 g L^{-1} $CaCO_3$ and 12.0 g L^{-1} agar were added before autoclaving). Cultures were grown at 28 °C, liquid cultures were shaken at 160 rpm. *Saccharomyces cerevisiae* FY834 was grown in liquid YPAD medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} glucose, 400 mg L^{-1} adenine sulphate, distilled water, autoclaved at 121 °C for 20 min) or on SM-URA agar plates (1.7 g L^{-1} yeast nitrogen base, 5 g L^{-1} ammonium sulphate, 20 g L^{-1} glucose, 770 mg L^{-1} nutritional supplement minus uracil, 20 g L^{-1} agar, distilled water, autoclaved for 20 min at 121 °C) at 28 °C. *Escherichia coli* BL21(DE3) was grown in LB-broth (10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 5 g L^{-1} NaCl, distilled water, autoclaved at 121 °C for 20 min, for agar plates 16 g L^{-1} agar was added before autoclaving) at 37 °C.

Preparation of CLSA headspace extracts

For CLSA analyses 65 GYM agar plates were inoculated with 1 mL taken from a *S. bungoensis* liquid culture grown for 7 days. The plates were then incubated for 7 days and subjected to the CLSA to collect the volatile compounds for 24 h. The adsorbed compounds were extracted from the activated charcoal using 10 units of dichloromethane, 10 µL each. The extract was then directly subjected to GC/MS analysis.

GC/MS and GC/MS-QToF analyses

GC/MS analyses were carried out using a 7890B GC – 5977A mass detector system (Agilent, Santa Clara, CA, USA). The GC was fitted with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 µm film). GC parameters 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 parameters 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 in comparison to a homologous series of *n*-alkanes (C_7-C_{40}).

GC/MS-QTOF analyses were performed on a 7890B GC – 7200 accurate-mass Q-TOF detector system (Agilent). The GC was equipped with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 µm film). GC parameters were 1) injection volume: 1 µL, 2) split ratio: 50:1, 60 s valve time, 3) carrier gas: He at 1 mL min^{-1}, and 4) temperature program: 5 min at 50 °C increasing at 10 °C min^{-1} to 320 °C. 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.

NMR spectroscopy

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

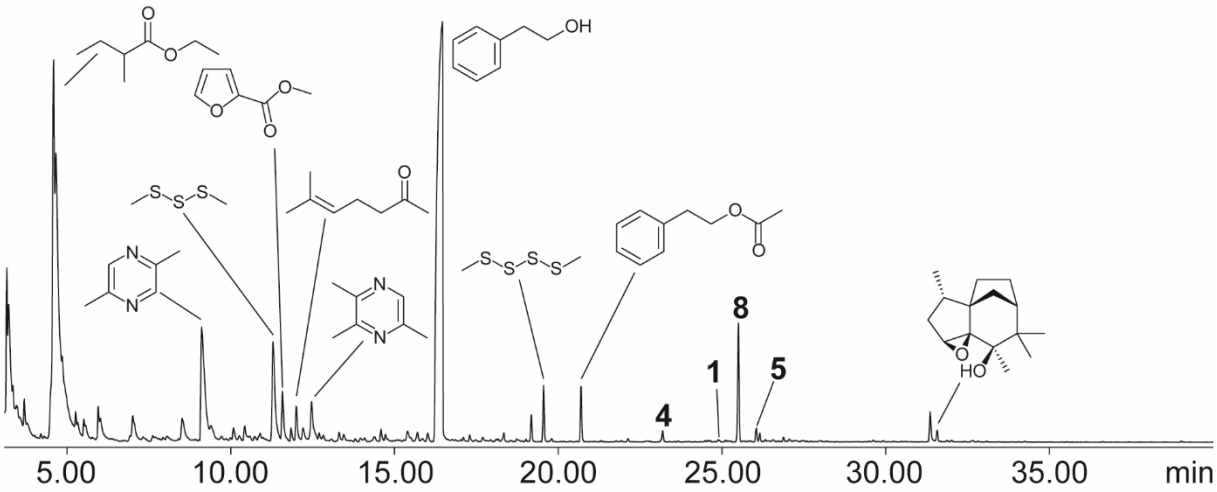


Figure S3. Total ion chromatogram of a CLSA headspace extract from an agar plate culture of *S. bungoensis*.

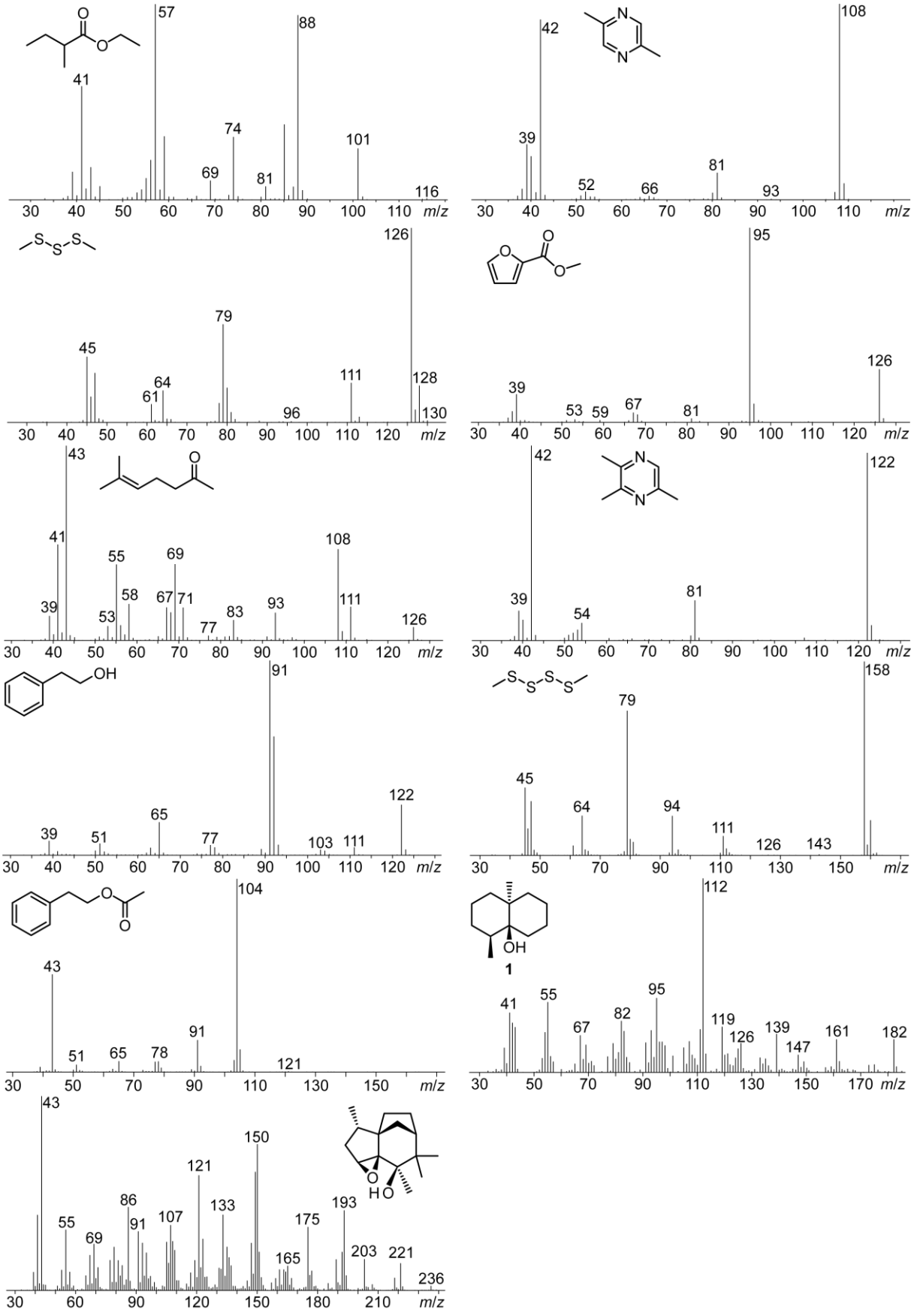


Figure S3 (continued). EI mass spectra of the compounds identified in the headspace extract of *S. bungoensis*.

Table S1. Primers used in this study.

Primer	Sequence[a]
LL097f	ATGCCCCAGGACGTCG
LL097r	TTAGCGGGCGGTGCTG
LL098f	GGCAGCCATATGGCTAGCATGACTGGTGGAATGCCCCAGGACGTCG
LL098r	TCTCAGTGGTGGTGGTGGTGGTGCTCGAGTTTAGCGGGCGGTGCTG
LL093f	GTGATCGAGGACGCCTTTCC
LL093r	TCATTTCTCACCTGCCGCTTC
LL094f	GGCAGCCATATGGCTAGCATGACTGGTGGAGTGATCGAGGACGCCTTTCC
LL094r	TCTCAGTGGTGGTGGTGGTGGTGCTCGAGTTCATTTCTCACCTGCCGCTTC
LL123f	GTGCACGCTTTCTCACACG
LL124f	GGCAGCCATATGGCTAGCATGACTGGTGGAGTGCACGCTTTCTCACACG
LL099f	ATGGACACATTCCGGATTCCGG
LL099r	CTACGGAATGAACAGGTCTGCC
LL100f	GGCAGCCATATGGCTAGCATGACTGGTGGAATGGACACATTCCGGATTCCGG
LL100r	TCTCAGTGGTGGTGGTGGTGGTGCTCGAGTCTACGGAATGAACAGGTCTGCC

[a] Homology arms for recombination in yeast matching the terminal sequences of linearised pYE-Express (HindIII and EcoRI digestion) are underlined.

Isolation of genomic DNA

Genomic DNA from *S. bungoensis* was isolated using a known phenol-chloroform extraction protocol.[2] A culture was grown for 48 h and was centrifuged with 8.000 x g. The supernatant was discarded and the pellet was resuspended in 5 mL SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris HCl, pH 8.0). A freshly mixed lysozyme solution (100 µL, 50 mg mL^{-1}) was added and the mixture was incubated at 37 °C for 30 minutes. SDS solution (600 µL, 10 %) and freshly mixed proteinase K solution (100 µL, 50 mg mL^{-1}) were added and the mixture was further incubated for 1 h at 55 °C.A premixed phenol/chloroform/isoamylacohol mixture (5 mL) was added and the resulting emulsion was shaken and centrifuged at 8000 x g for 30 minutes. The supernatant aqueous phase was carefully pipetted to a fresh tube and mixed with ethanol to a percentage of 60 % to precipitate the DNA. The DNA was spun down, the supernatant was discarded and the DNA was washed with 70 % ethanol and spun down again, this was repeated twice. The remaining DNA was dried overnight and dissolved in nuclease-free water to give a final concentration of 487 ng μL^{-1}.

Gene cloning

The desired genes were obtained from the previously isolated genomic DNA via polymerase-chain-reaction (PCR) using Q5®-High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA). PCR was performed following a 3-step-procedure using gDNA from *S. bungoensis* as a template and the primers LL097f and LL097r for PS, LL093f and LL093r for EIZS (WP_061924158), LL123f and LL093r for EIZS (WP_107118987) and LL099f and LL099r for BgS, respectively (Table S1). The PCR was conducted under standard conditions with initial denaturation at 98 °C for 1 min, a 3-step-cycle with denaturation at 98 °C for 15 sec, annealing at 70 °C for 30 sec and elongation at 72 °C for 30 seconds repeated 30 times and a final elongation step at 72 °C for 5 min. Gel electrophoresis was used to determine successful amplification, the obtained products were used as templates in a second PCR with the primers LL098f, LL098r for PS, LL094f, LL094r for EIZS (WP_061924158), LL124f, LL094r for EIZS (WP_107118987) and LL100f, LL100r for BgS, respectively to attach homology-arms for homologous recombination in yeast. This was carried out sticking to a standard protocol using PEG, LiOAc and salmon-sperm DNA to combine the PCR products with linearised pYE-Express shuttle vector.[3,4] The transformed *Saccharomyces cerevisiae* cultures were plated on SM-URA plates and grown for 3 days at 28 °C. Colonies were collected from the plates and a plasmid mixture was isolated using Zymoprep™ Yeast Plasmid Miniprep II (Zymo Research,

Irvine, USA) kit to be further used for electroporation in *E. coli* BL21(DE3) electrocompentent cells. The transformed *E. coli* were grown overnight at 37 °C on LB agar plates containing kanamycin (50 µg mL^{-1}) from which single colonies were picked to inoculate 6 mL LB with kanamycin. The resulting cultures were grown for 24 h to isolate single plasmids which were checked by analytical digest and finally by sequencing. This yielded transformants carrying the plasmids pYE_PS, pYE_EIZSshort, pYE_EIZSfull and pYE_BgS which were used for further experiments.

Gene expression and protein purification

For gene expression the previously obtained *E. coli* transformants were used to inoculate a preculture in liquid LB medium containing kanamycin (50 µg mL^{-1}) which was grown overnight at 37 °C. Larger cultures were then inoculated using 1 mL L^{-1} of the preculture. The cultures were grown at 37 °C with shaking until an OD_{600} between 0.4 and 0.6 was reached. The cultures were cooled to 18 °C and protein expression was induced by adding IPTG solution (400 mM, 1 mL L^{-1}) and the cultures were shaken overnight at 18 °C. The cultures were centrifuged at 3.600 x g, the supernatant medium was discarded and the pelleted cells were resuspended 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). The resulting suspension was subjected to ultra-sonication (50 %, 5 x 1 min) to lyse and the cell debris was removed by centrifugation (14.610 x g, 2 x 7 min). The supernatant was filtrated and loaded onto a Ni^{2+}-NTA affinity chromatography column (Super Ni-NTA, Generon, Slough, UK). The column was washed with binding buffer (2 x 10mL L^{-1} culture), and the desired His-tagged protein was eluted using elution buffer (10 mL L^{-1} culture, 20 mM Na_2HPO_4, 500 mM NaCl, 500 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C).

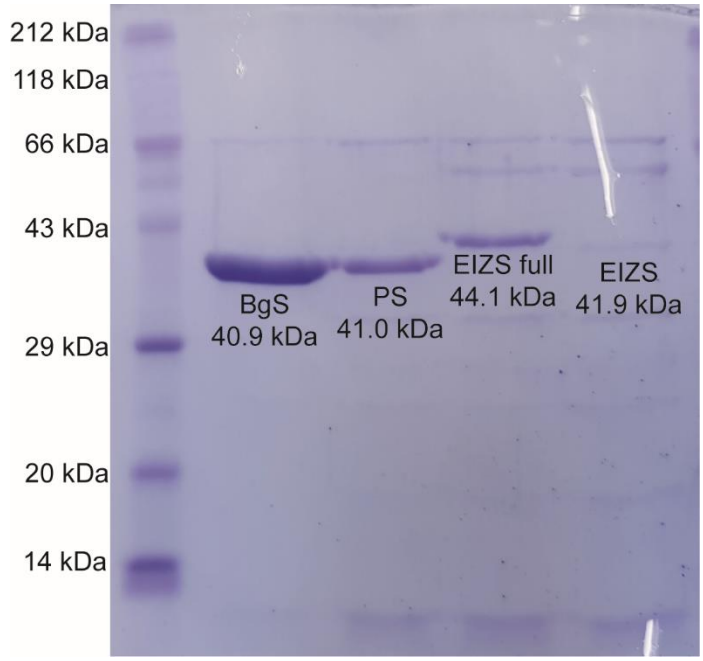


Figure S4. SDS-PAGE analysis of recombinant terpene synthases from *S. bungoensis*. From left to right: protein markers, bungoene synthase (BgS), pentalenene synthase (PS), *epi*-isozizaene synthase (EIZS, full length protein), and EIZS (short length protein).

Incubation experiments with recombinant PS, EIZS (short and full length enzyme) and BgS

Incubation experiments were carried out using the abovementioned purified proteins in elution buffer and FPP (0.5 mg mL^{-1}) in substrate buffer (25 mM NH_4HCO_3). Experiments with BgS were diluted with an equal amount of incubation buffer A (10 mM $MgCl_2$, 20 % glycerol), experiments using the other enzymes with incubation buffer B (50 mM TRIS, 10 mM $MgCl_2$, 20 % glycerol, pH 8.2). Incubation with substrates was performed shaking the mixtures at 28 °C for 3 h. Small scale incubations using 1 mg FPP were extracted with 200 µL hexane, the organic phase was dried and subjected to GC-MS. Incubation experiments for the preparative production of bungoene and pentalenene using FPP (60 mg for bungoene, 100 mg for pentalenene) were extracted with hexane (3 x 150 mL), the combined organic layers were dried with $MgSO_4$, concentrated under reduced pressure and subjected to column chromatography on SiO_2 using pentane as a solvent. Evaporation of the fractions containing the product yielded the terpenes as colourless oils.

Bungoene, (1a*S*,4a*S*,7*R*,7a*R*)-4a-isopropyl-2,7-dimethyl-1a,4,4a,5,6,7-hexahydro-1*H*-cyclopropa[*d*]indene (8). Colourless oil. Yield: 2.5 mg (0.01 mmol, 9 %). R_f (pentane) = 0.85. $[\alpha]_D^{20}$ = +68.4 (*c* 0.37, C_6D_6). HRMS (APCI): *m*/*z* = 205.1951 (calc. for $[C_{15}H_{25}]^+$ 205.1951). GC (HP5-MS): *I* = 1463. MS (EI, 70 eV): *m*/*z* (%) = 204 (9), 189 (3), 161 (59), 147 (7), 136 (25), 133 (13), 121 (50), 119 (100), 107 (16), 105 (83), 93 (24), 91 (22), 81 (24), 79 (16), 77 (11), 69 (8), 55 (11), 41 (15), see Figure S7. IR (diamond ATR): $\tilde{\nu}$ / cm^{-1} = 2955 (s), 2928 (s), 2873 (m), 1667 (w), 1461 (m), 1379 (w), 1261 (w), 1095 (m), 1079 (m), 1036 (m), 807 (w), 607 (w). NMR data are given in Table S2 and Figures S8–S14.

Pentalenene, (1*R*,3a*S*,5a*S*,8a*R*)-1,4,7,7-tetramethyl-1,2,3,3a,5a,6,7,8-octahydrocyclopenta[*c*]pentalene (4). Colourless oil. Yield: 5.2 mg (0.03 mmol, 13 %).R_f (pentane) = 0.90. $[\alpha]_D^{20}$ = +11.5 (*c* 0.39, C_6D_6), Lit.: $[\alpha]_D^{25}$ = +11.8 (*c* 6.8, $CHCl_3$)[5]. HRMS (EI): *m*/*z* = 201.1871 (calc. for $[C_{15}H_{24}]^+$ 201.1873). GC (HP5-MS): *I* = 1344. MS (EI, 70 eV): *m*/*z* (%) = 204 (100), 189 (62), 175 (17), 161 (46), 147 (75), 133 (36), 119 (46), 105 (64), 91 (46), 77 (14), 55 (10), 41 (11), see Figure S5. IR (diamond ATR): $\tilde{\nu}$ / cm^{-1} = 3027 (w), 2948 (s), 2927 (s), 2867 (m), 1461 (w), 1377 (w), 1260 (w), 1103 (w), 1019 (w), 828 (w), 812 (w). NMR data are given in Table S6 and Figures S36–S42.

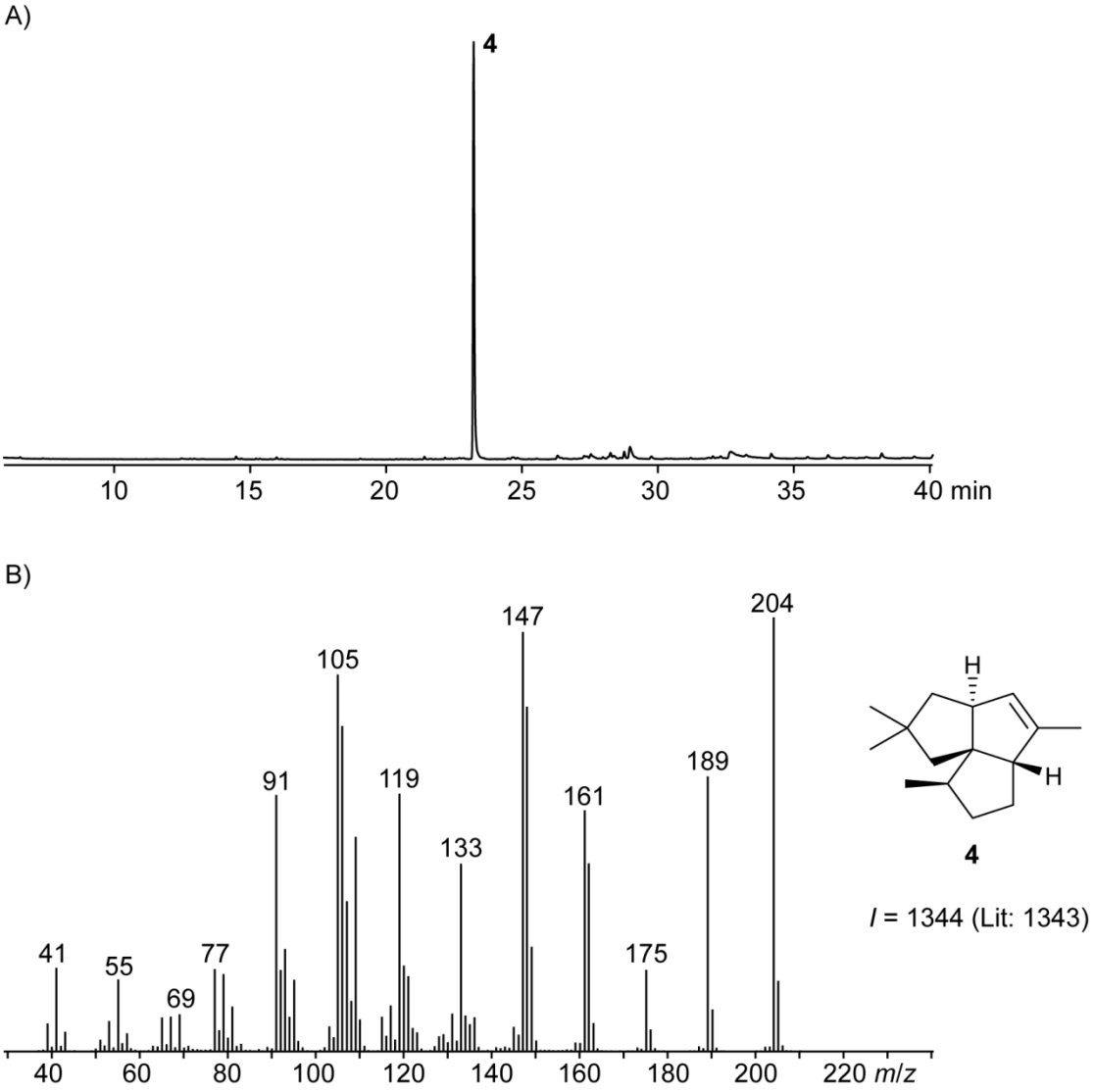


Figure S5. Characterisation of *S. bungoensis* pentalenene synthase (PS). A) Total ion chromatogram of the products obtained from FPP with PS. B) EI mass spectrum and retention index *I* of the main product **4**. For comparison literature data for the retention index of **4** were taken from reference [6].

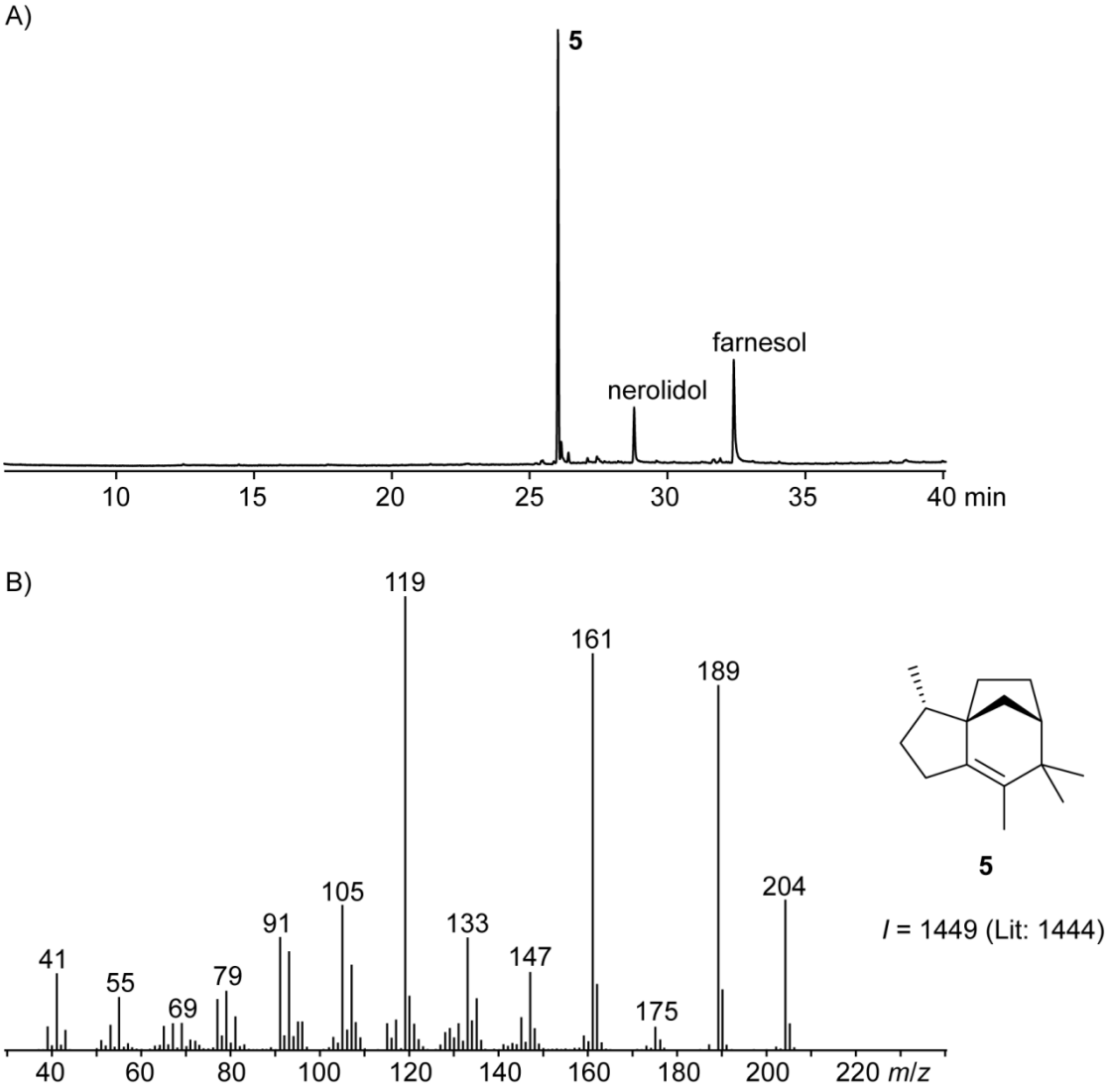


Figure S6. Characterisation of *S. bungoensis epi*-isozizaene synthase (EIZS). A) Total ion chromatogram of the products obtained from FPP with EIZS. B) EI mass spectrum and retention index *I* of the product **5**. For comparison literature data for the retention index of **5** were taken from reference [7]. Farnesol and nerolidol were unambiguously identified by comparison to authentic standards.[8]

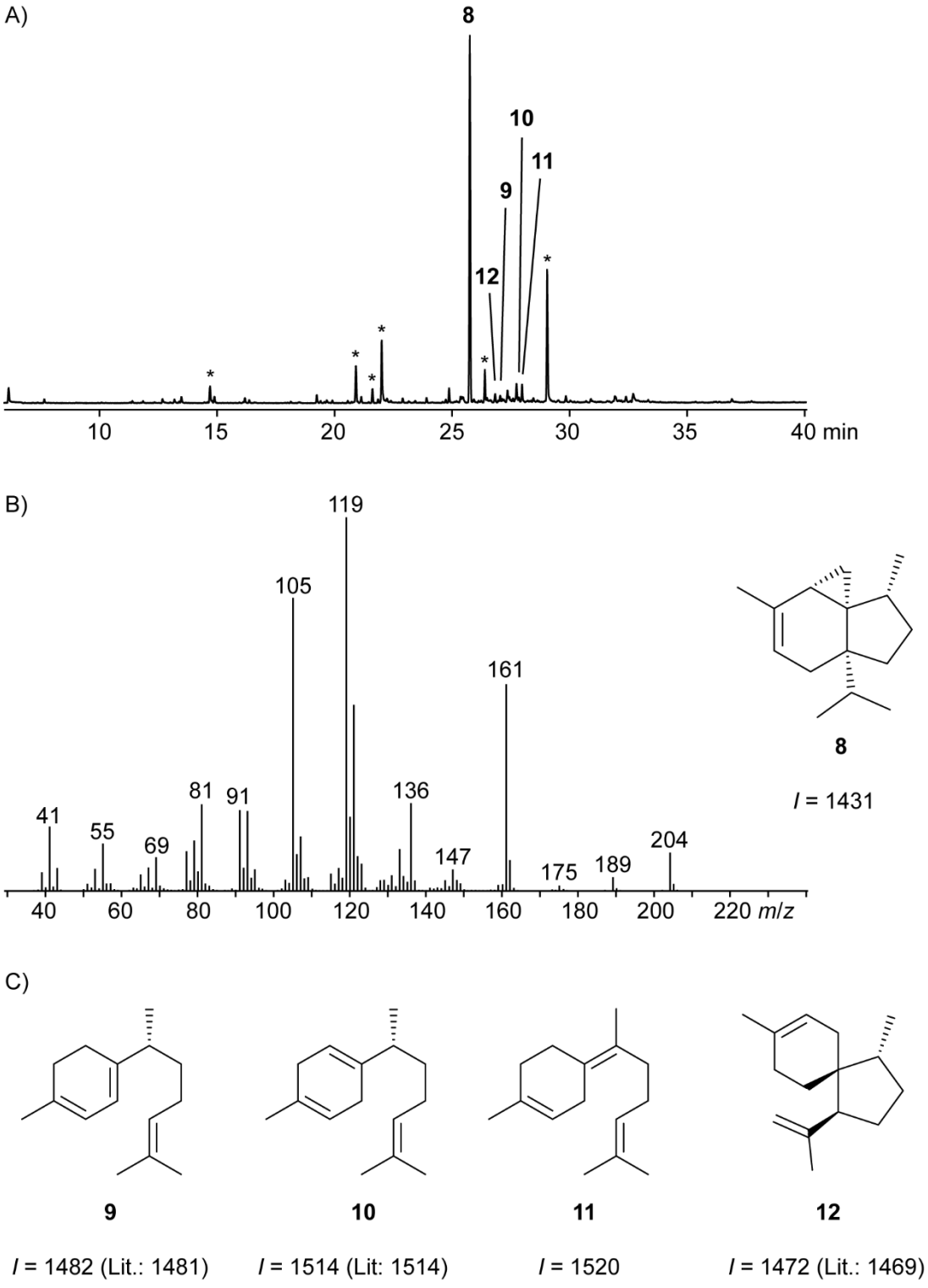


Figure S7. Characterisation of *S. bungoensis* bungoene synthase (BgS). A) Total ion chromatogram of the products obtained from FPP with BgS. B) EI mass spectrum and retention index *I* of the main product **8**. C) Structures and retention indices I of the side products **9** – **12** (the tentatively assigned absolute configurations were inferred from the pathway intermediates of Scheme 1B in the main text). For comparison literature data for the retention indices of **9**, **10** and **12** were taken from reference [9]. Compound **11** was unambiguously identified by comparison to an authentic standard obtained with the (*Z*)-γ-bisabolene synthase from *Cryptosporangium arvum*.[8] Asterisks indicate contaminants such as plasticisers.

D)

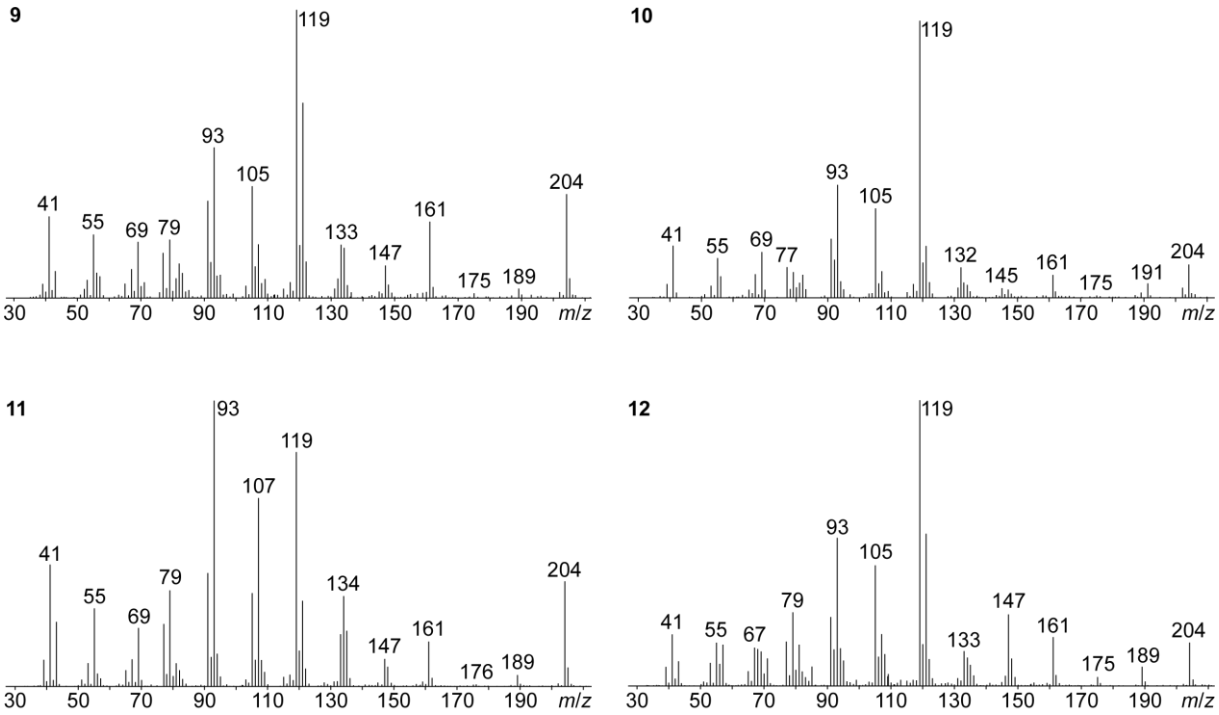


Figure S7 (continued). Characterisation of *S. bungoensis* bungoene synthase (BgS). D) EI mass spectra of side products **9** – **12**.

Table S2. NMR data of bungoene (**8**) in C_6D_6 recorded at 298 K.

C[a]		^{1}H[b]	^{13}C[b]
1	CH_2	0.90 (dd, $^2J_{H,H}$ = 4.6, $^3J_{H,H}$ = 8.9, 1 H, H_β)	17.9
		0.23 (dd, $^2J_{H,H}$ = 4.9, $^3J_{H,H}$ = 4.9, 1H, H_α)	
2	CH	0.73 (dd, $^3J_{H,H}$ = 8.9, 5.0, 1 H)	25.8
3	C_q	–	135.7
4	CH	5.31 (m, 1 H)	119.4
5	CH_2	1.89 (dm, $^2J_{H,H}$ = 15.8, 1 H, H_α)	31.9
		1.84 (dd, $^2J_{H,H}$ = 15.8, $^3J_{H,H}$ = 5.6, 1 H, H_β)	
6	C_q	–	34.9
7	CH	1.59 (dq, $^3J_{H,H}$ = 11.7, 7.2, 7.2, 7.2, 1 H)	42.4
8	CH_2	1.93 (m, 1 H, H_β)	31.3
		1.31 (m, 1 H, H_α)	
9	CH_2	1.76 (m, 1 H, H_β)	32.3
		1.28 (m, 1 H, H_α)	
10	C_q	–	47.5
11	CH	1.54 (sept, $^3J_{H,H}$ = 6.9, 1 H)	30.0
12	CH_3	0.85 (d, $^3J_{H,H}$ = 6.5, 3 H)	18.4
13	CH_3	0.85 (d, $^3J_{H,H}$ = 6.8, 3 H)	18.6
14	CH_3	0.86 (d, $^3J_{H,H}$ = 7.2, 3 H)	19.0
15	CH_3	1.79 (s, 3 H)	22.3

[a] Carbon numbering as shown in Scheme 1 of main text. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, coupling constants *J* are given in Hertz.

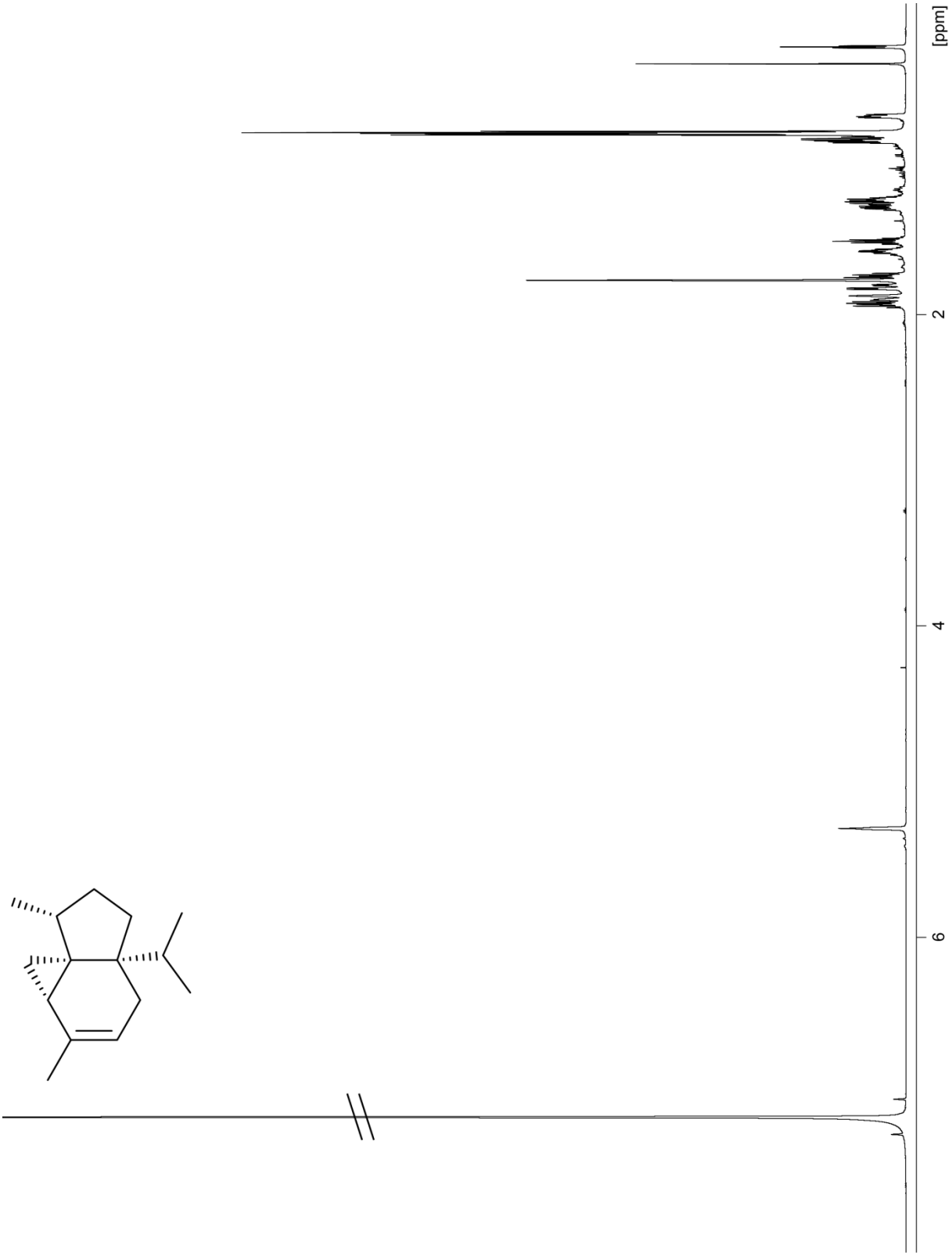


Figure S8. ^{1}H NMR spectrum of **8** (700 MHz, C_6D_6).

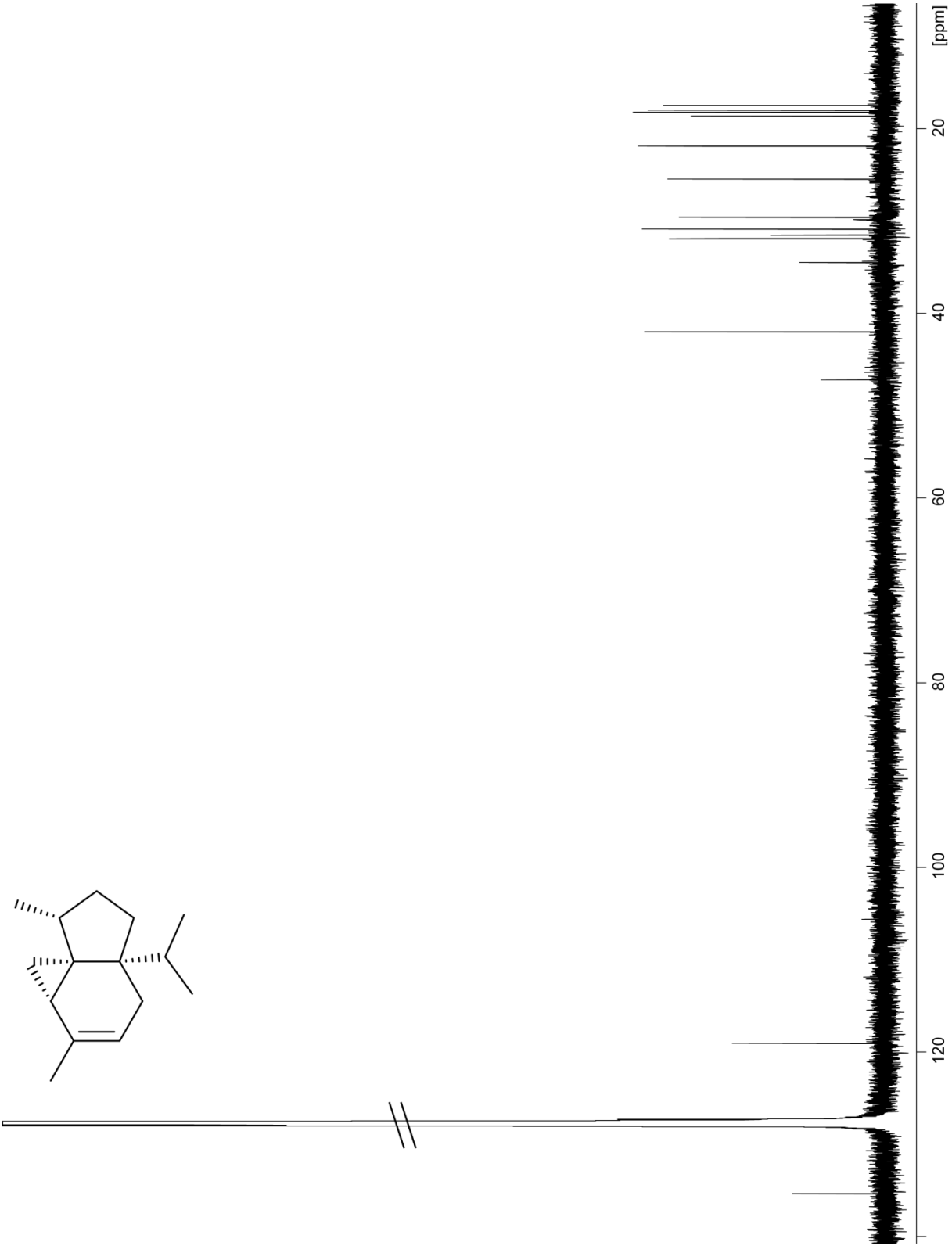


Figure S9. ^{13}C NMR spectrum of **8** (175 MHz, C_6D_6).

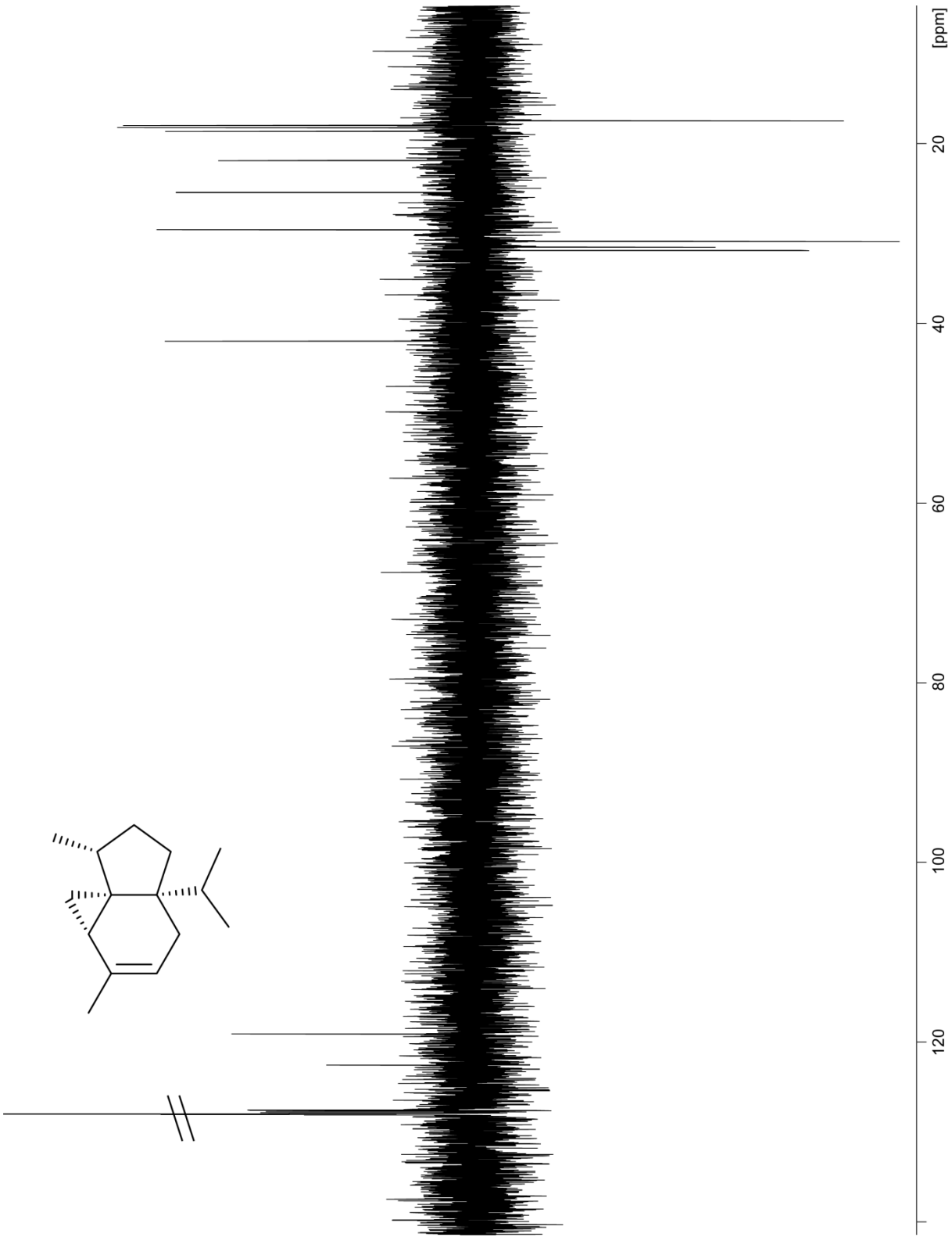


Figure S10. ^{13}C-DEPT-135 spectrum of **8** (175 MHz, C_6D_6).

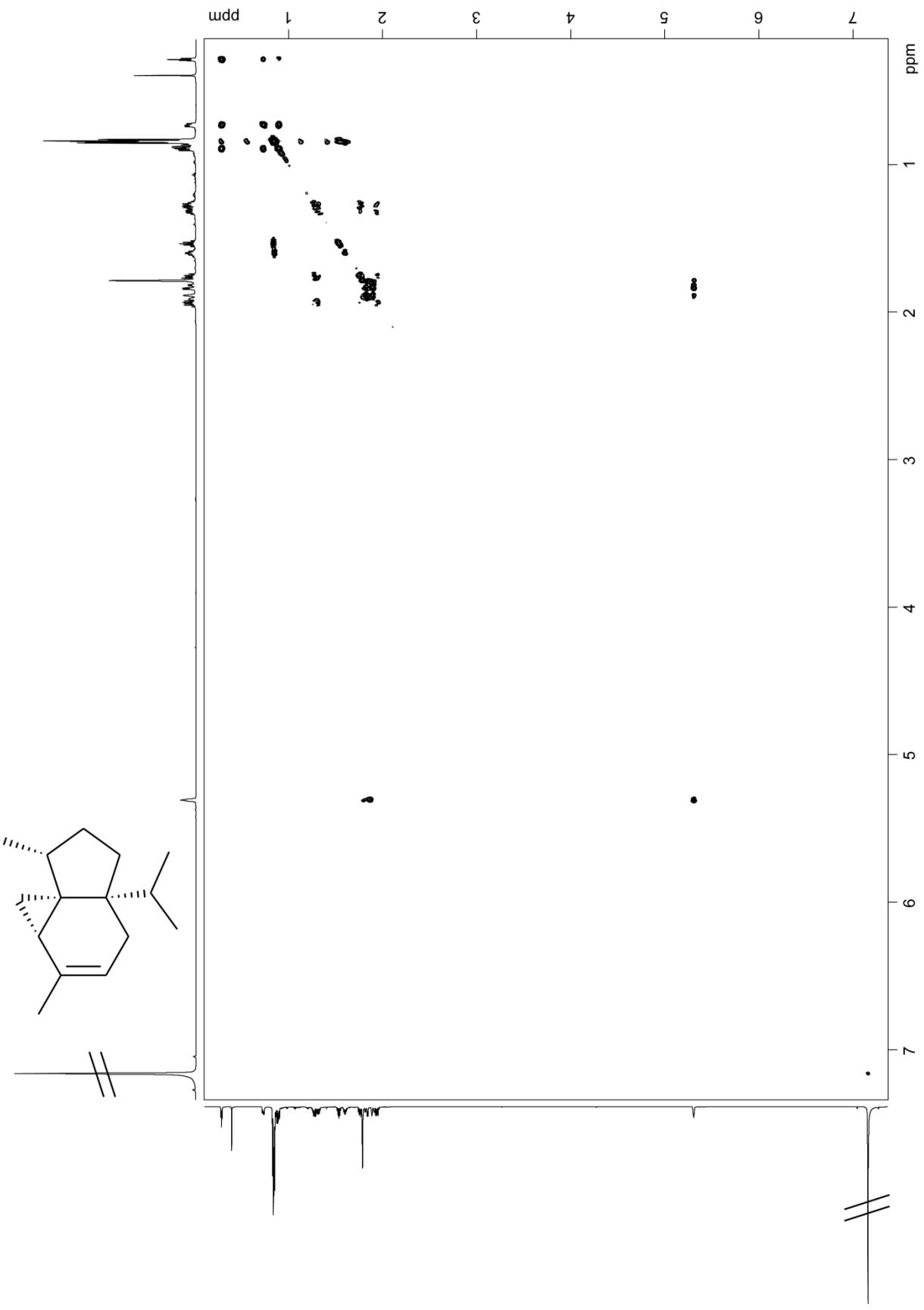


Figure S11. $^{1}H,^{1}H$-COSY spectrum of **8** (C_6D_6).

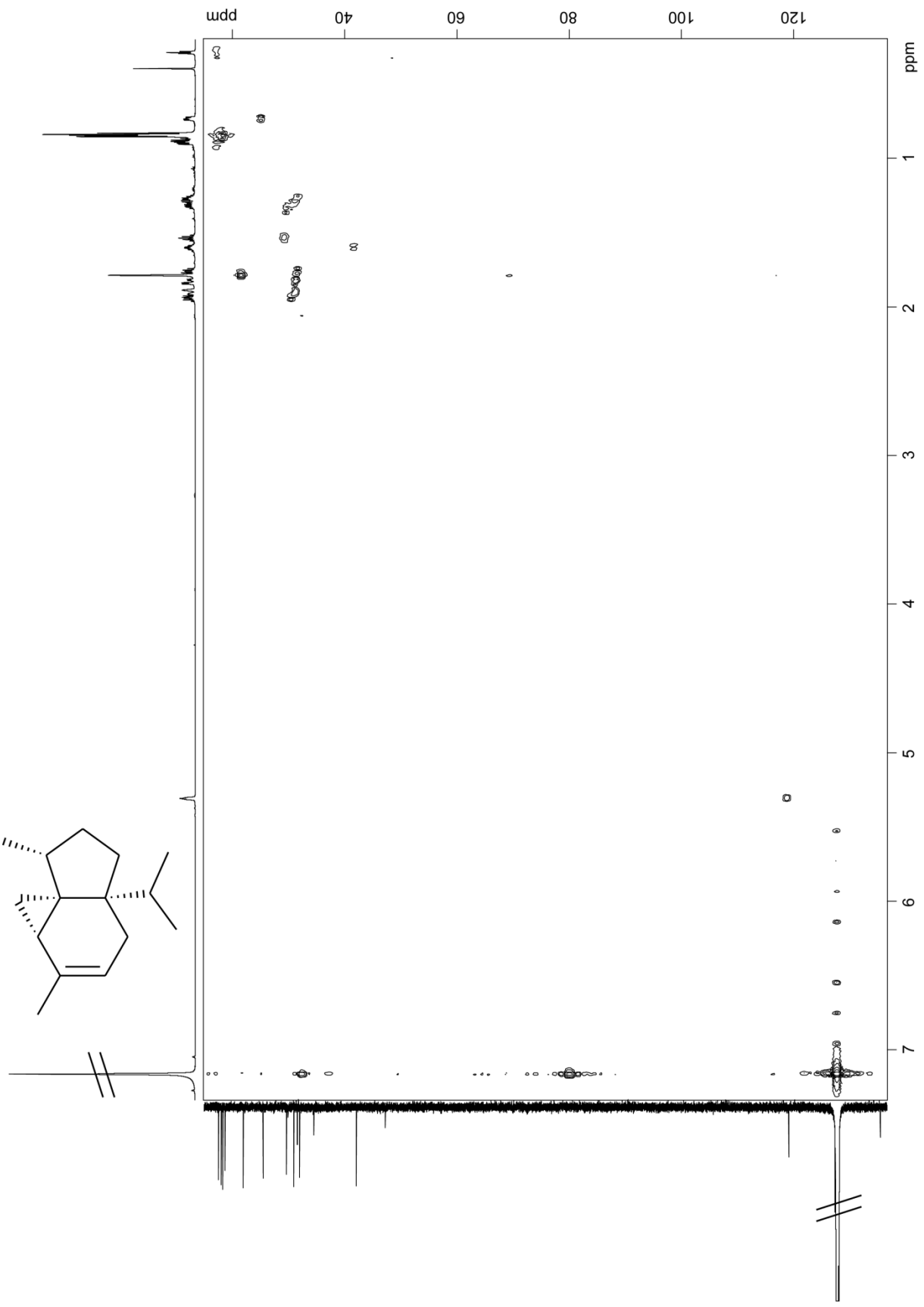


Figure S12. HSQC spectrum of **8** (C_6D_6).

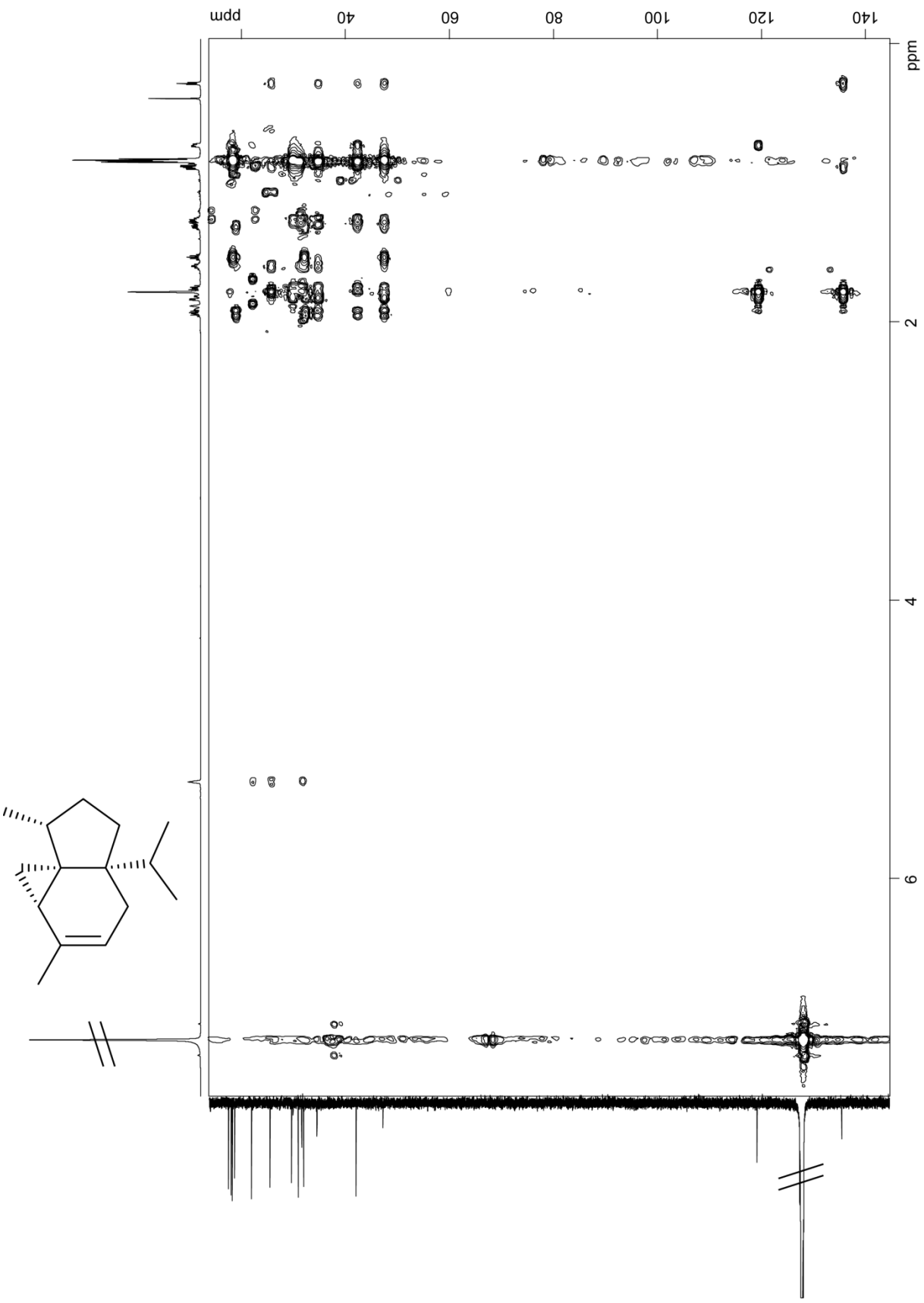


Figure S13. HMBC spectrum of **8** (C_6D_6).

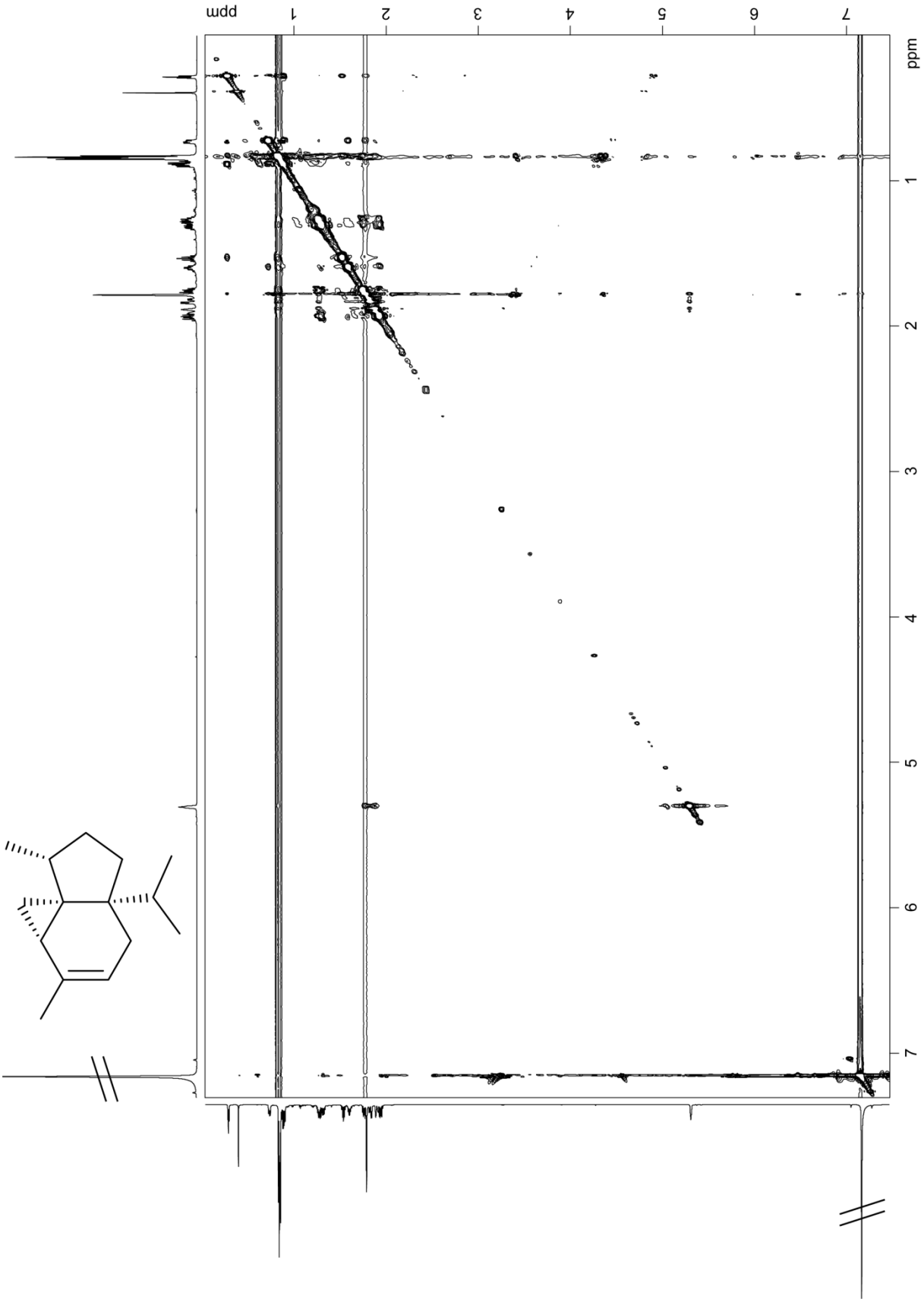


Figure S14. NOESY spectrum of **8** (C_6D_6).

Incubation experiments with isotopically labelled substrates and BgS

Isotopic labelling experiments were performed with amounts of ca. 1 mg labelled FPP (or its precursors) in substrate buffer (1 mL), incubation buffer A (4 mL), enzyme elution fractions (final concentration of BgS : 0.1 mg mL^{-1}, 8 mL total volume) with the substrates and enzyme preparations as listed in Table S3. After incubation with shaking at 28 °C for 3 h the products were extracted with C_6D_6 (650 μL) or *n*-hexane (200 μL) for NMR or GC/MS experiments, respectively. The extracts were dried with $MgSO_4$ and analysed by NMR and/or GC/MS.

Table S3. Isotopic labelling experiments with BgS.

entry	substrate(s)	enzyme(s)	results shown in
1	DMAPP + (*E*)-(4-^{13}C,4-^{2}H)IPP[10]	BgS + FPPS[11]	Figure S15
2	DMAPP + (*Z*)-(4-^{13}C,4-^{2}H)IPP[10]	BgS + FPPS	Figure S15
3	(*R*)-(1-^{13}C,1-^{2}H)IPP[12]	BgS + FPPS + IDI[13]	Figure S16
4	(*S*)-(1-^{13}C,1-^{2}H)IPP[12]	BgS + FPPS + IDI	Figure S16
5	(1-^{13}C)FPP[14]	BgS	Figure S17
6	(2-^{13}C)FPP[14]	BgS	Figure S17
7	(3-^{13}C)FPP[14]	BgS	Figure S17
8	(4-^{13}C)FPP[14]	BgS	Figure S17
9	(5-^{13}C)FPP[14]	BgS	Figure S17
10	(6-^{13}C)FPP[14]	BgS	Figure S17
11	(7-^{13}C)FPP[14]	BgS	Figure S17
12	(8-^{13}C)FPP[14]	BgS	Figure S17
13	(9-^{13}C)FPP[14]	BgS	Figure S17
14	(10-^{13}C)FPP[14]	BgS	Figure S17
15	(11-^{13}C)FPP[14]	BgS	Figure S17
16	(12-^{13}C)FPP[14]	BgS	Figure S17
17	(9-^{13}C)GPP[15] + IPP	BgS + FPPS	Figure S17
18	(10-^{13}C)GPP[16] + IPP	BgS + FPPS	Figure S17
19	(15-^{13}C)FPP[14]	BgS	Figure S17
20	(3-^{13}C,2-^{2}H)GPP[17] + IPP	BgS + FPPS	Figure S19
21	(2-^{2}H)DMAPP[18] + IPP	BgS + FPPS	Figure S20
22	GPP + (*E*)-(4-^{2}H)IPP[13]	BgS + FPPS	Figure S21
23	GPP + (*Z*)-(4-^{2}H)IPP[13]	BgS + FPPS	Figure S21

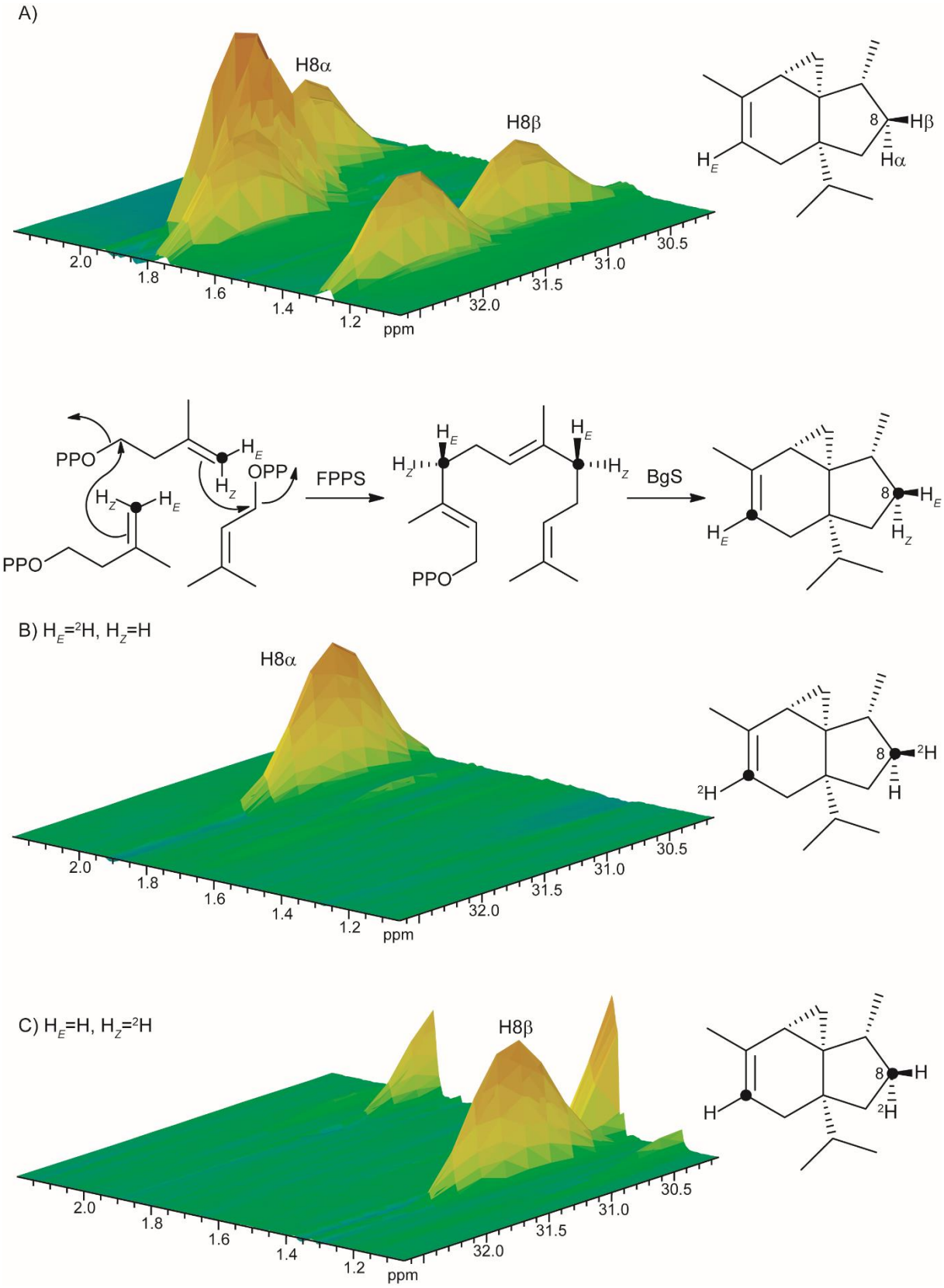


Figure S15. Determination of the absolute configuration of **8** using the substrates (*E*)- and (*Z*)-(4-^{13}C,4-^{2}H)IPP.

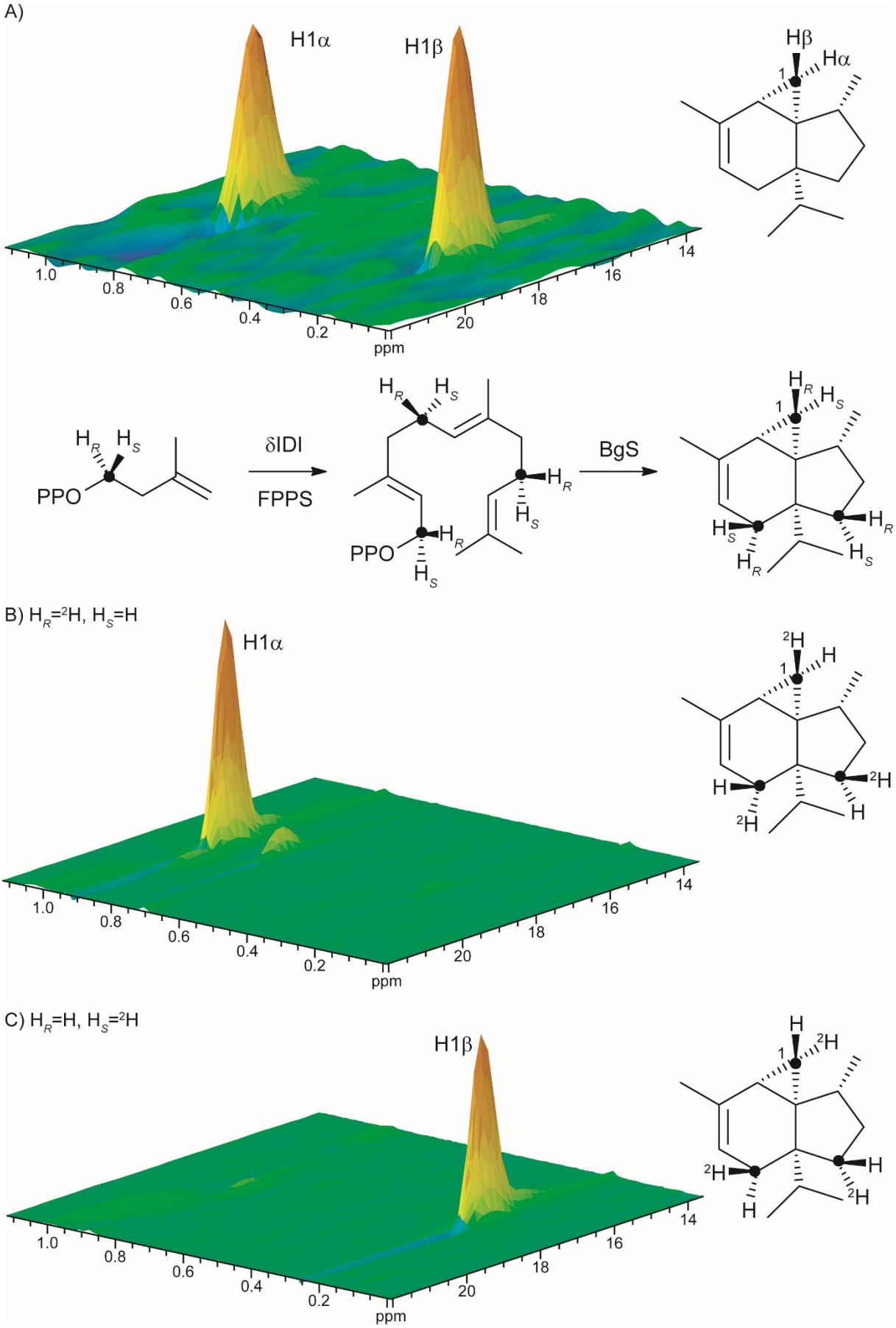


Figure S16. Determination of the absolute configuration of **8** using the substrates (*R*)- and (*S*)-(1-^{13}C,1-^{2}H)IPP.

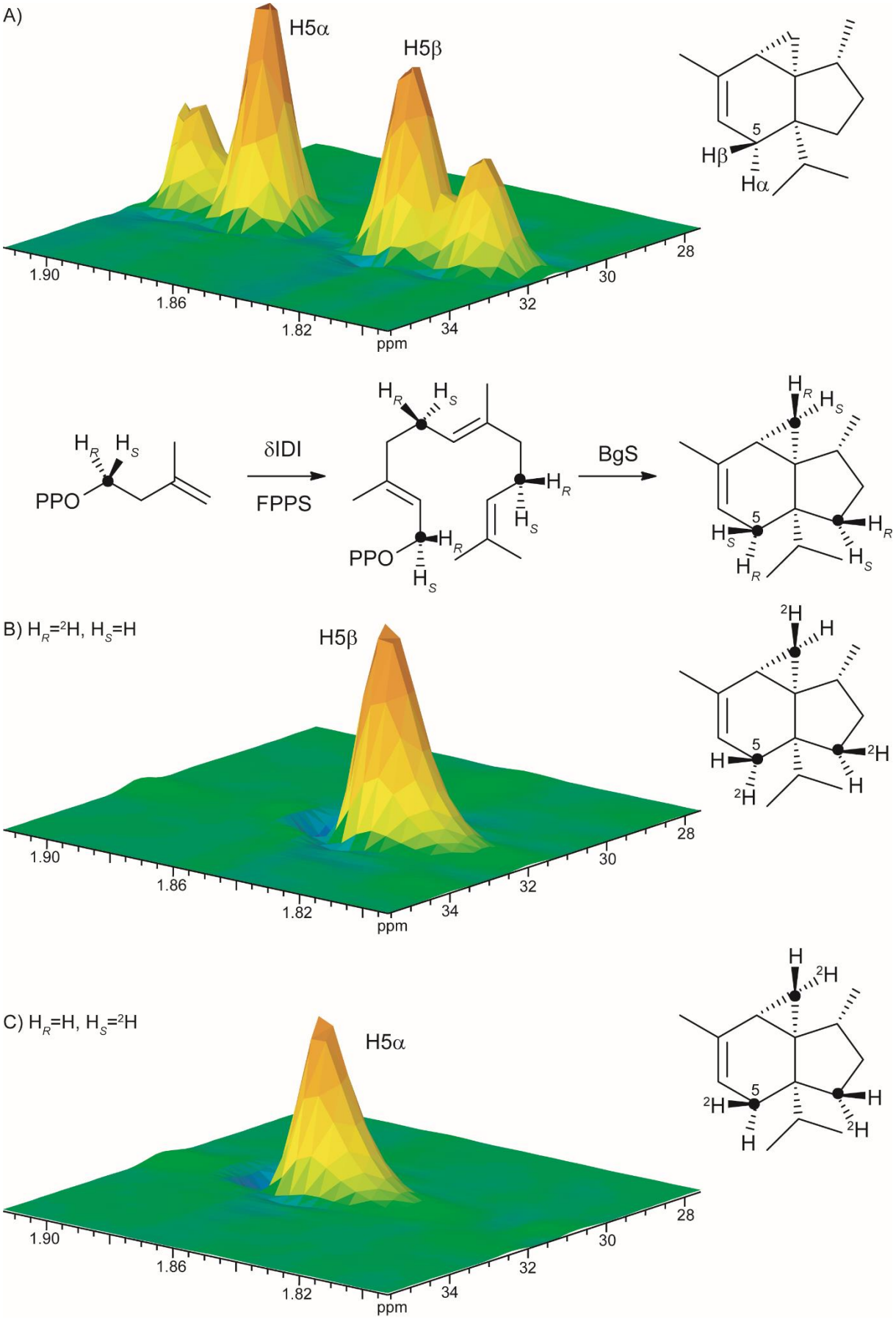


Figure S16. Determination of the absolute configuration of **8** using the substrates (*R*)- and (*S*)-(1-^{13}C,1-^{2}H)IPP.

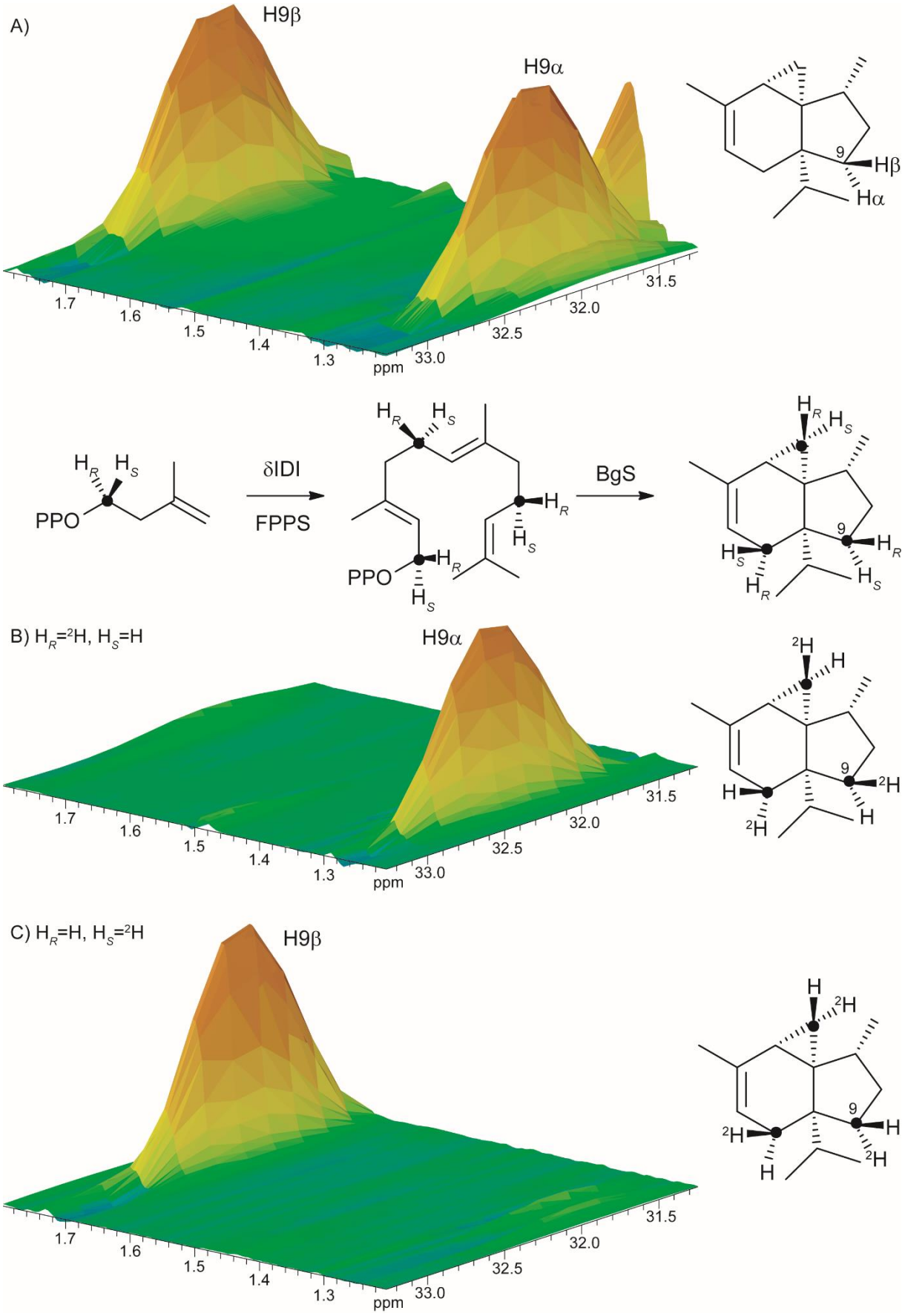


Figure S16. Determination of the absolute configuration of **8** using the substrates (*R*)- and (*S*)-(1-^{13}C,1-^{2}H)IPP.

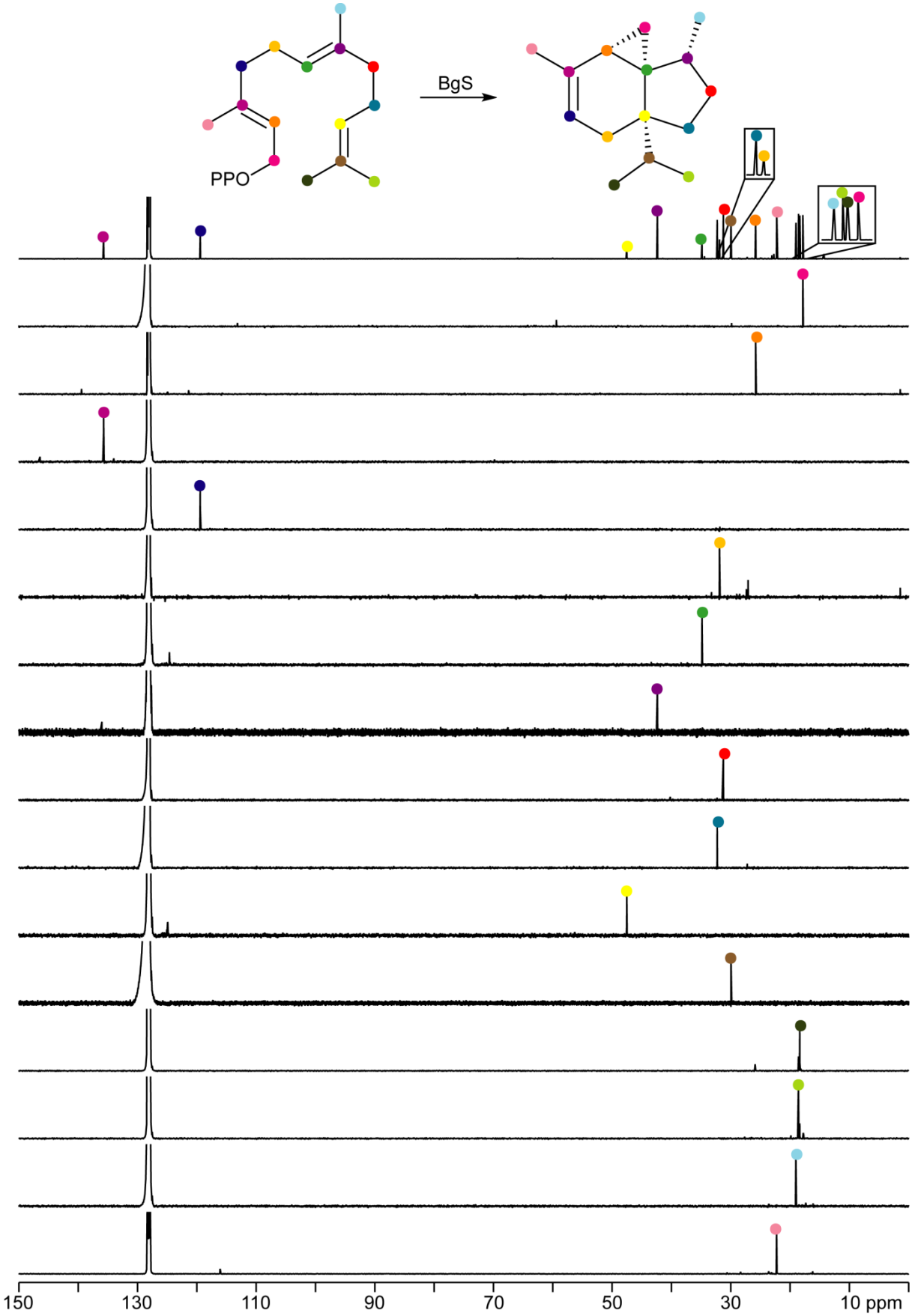
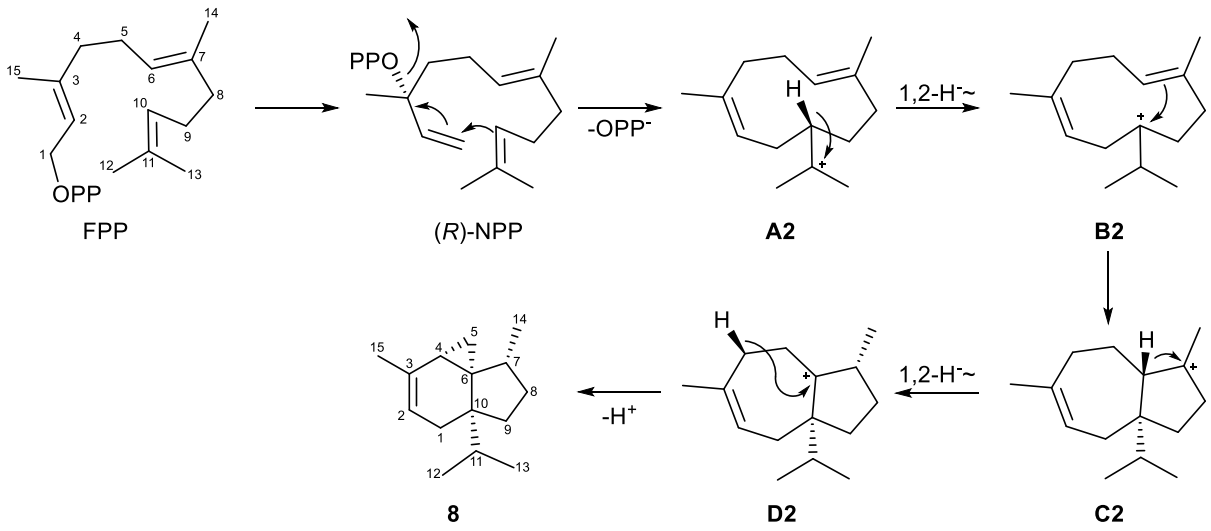


Figure S17. ^{13}C-NMR spectra of labelled **8** obtained from all 15 isotopomers of (^{13}C)FPP with BgS. Coloured dots correlate the observed ^{13}C signals to the individual carbons of **8**.



Scheme S1. Alternative cyclisation mechanism of BgS from FPP to **8** that is excluded by the labelling experiments of Figure S17. This alternative mechanism also cannot explain the formation of the observed side products **9** – **12**.

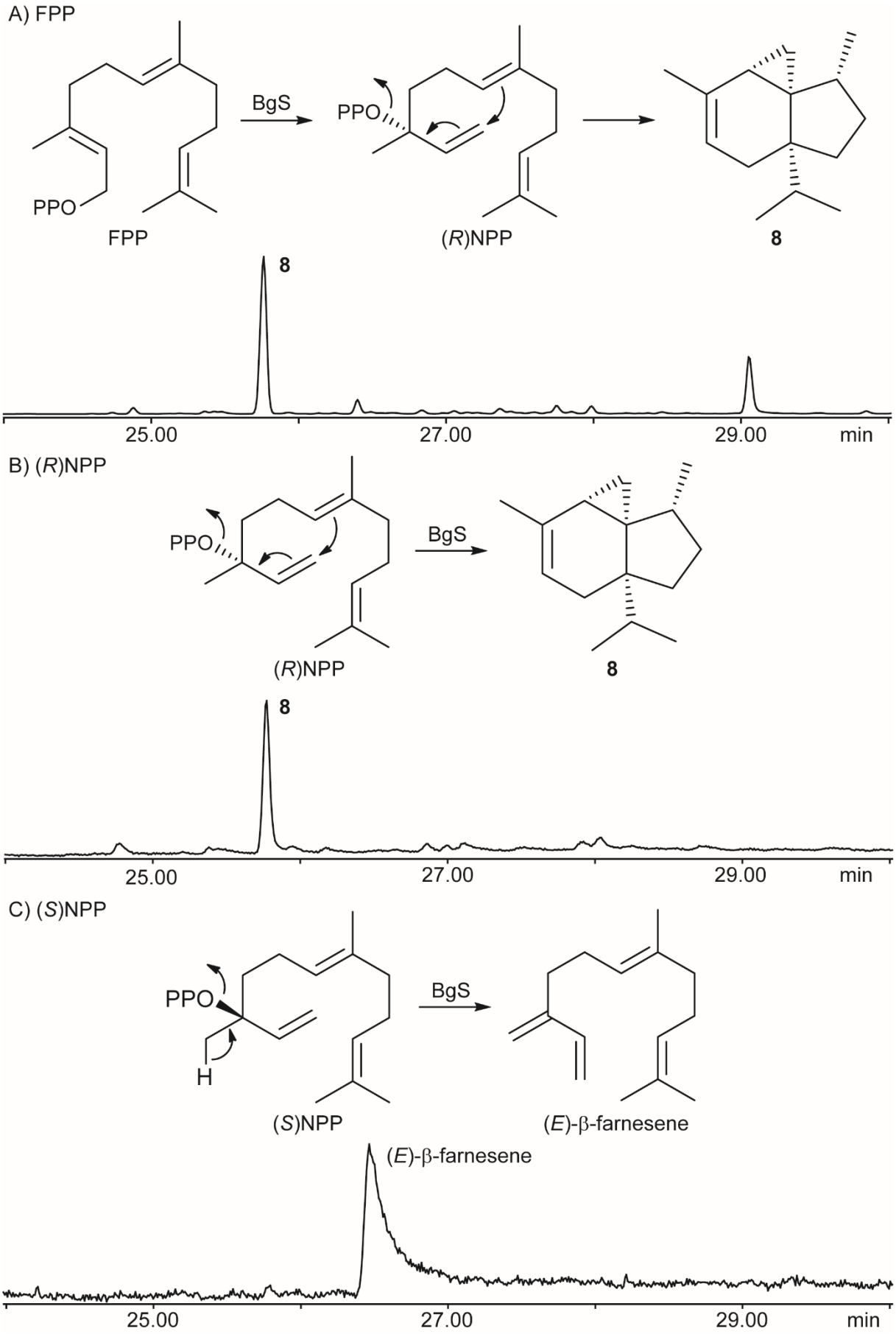


Figure S18. Incubation experiments with enantiomerically pure (*R*)- and (*S*)-NPP[8] with BgS.

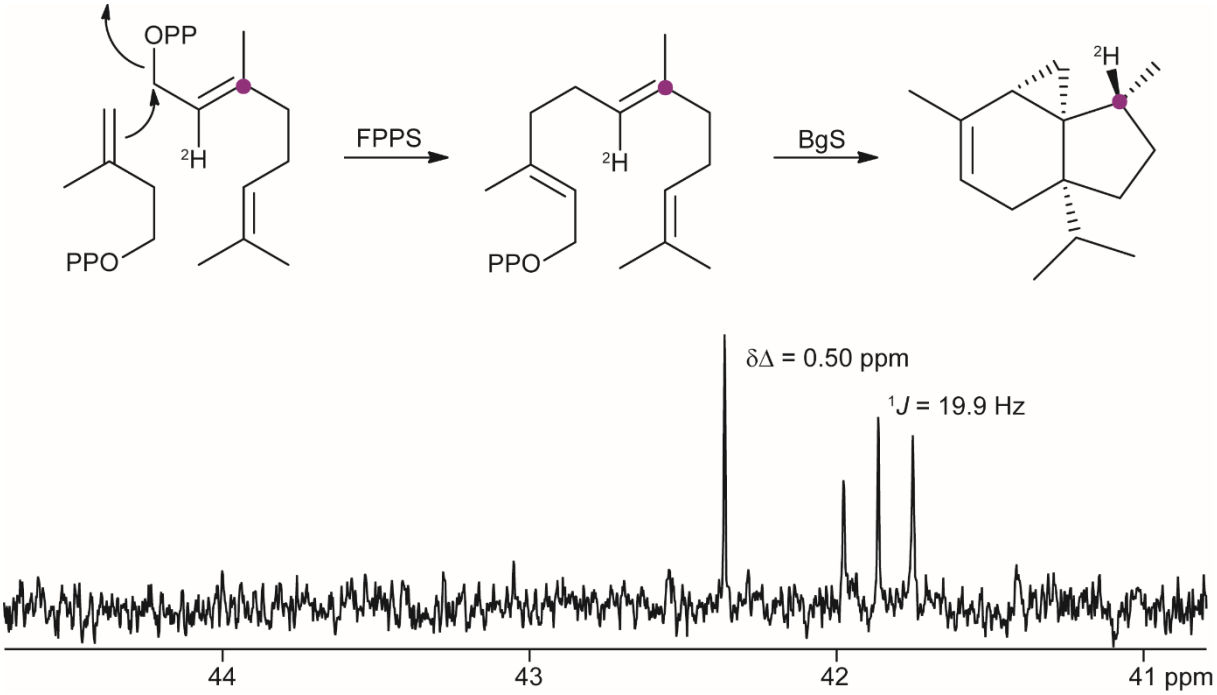


Figure S19. Investigation of the 1,2-hydride shift from **A** to **B** by incubation of (3-^{13}C,2-^{2}H)GPP and IPP with FPPS and BgS. The triplet at 41.8 ppm clearly indicates a direct ^{13}C-^{2}H bond in bungoene, while the residual singlet at 42.3 ppm is observed because of an incomplete deuteration of the substrate (3-^{13}C,2-^{2}H)GPP.

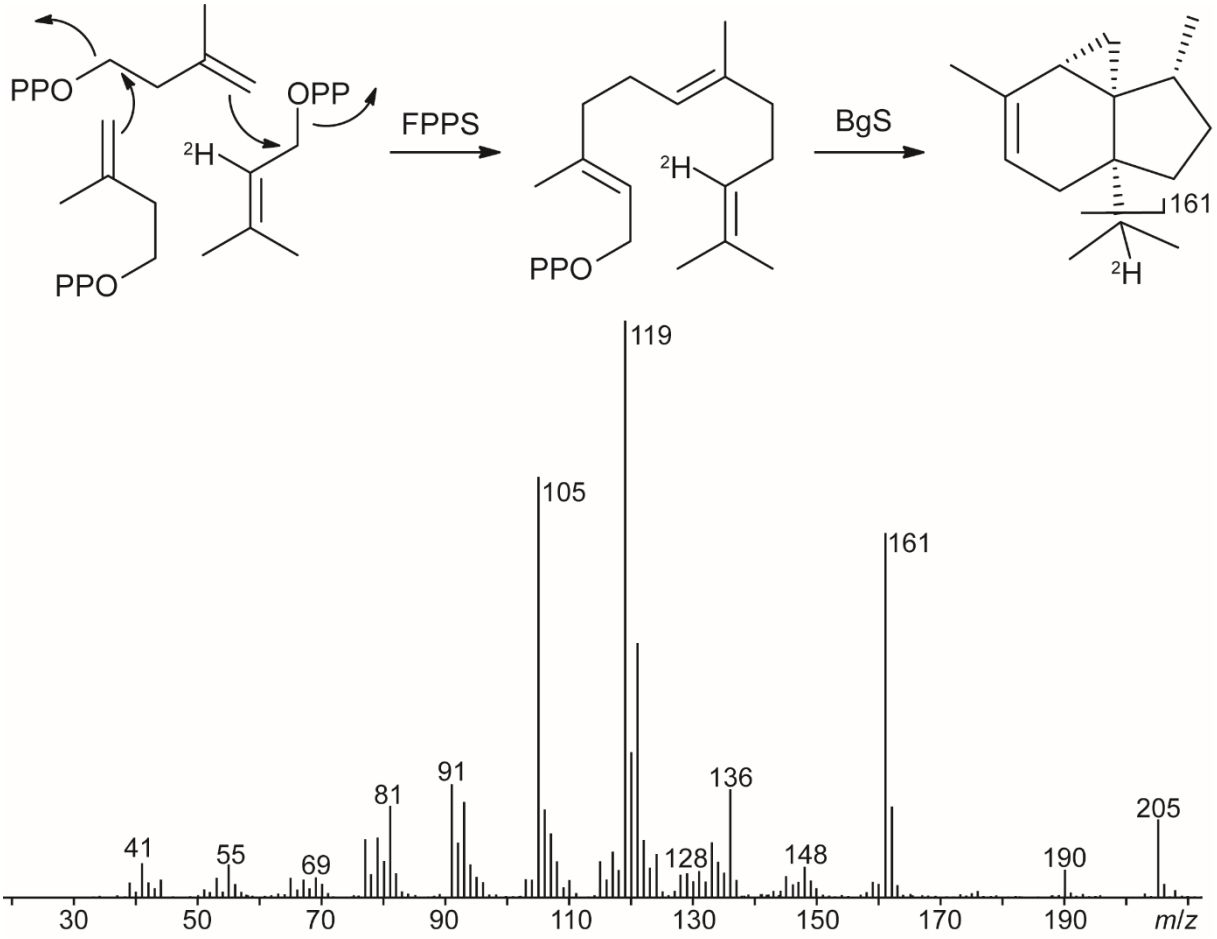


Figure S20. Investigation of the 1,2-hydride shift from **C** to **D** by incubation of (2-^{2}H)DMAPP and IPP with FPPS and BgS.

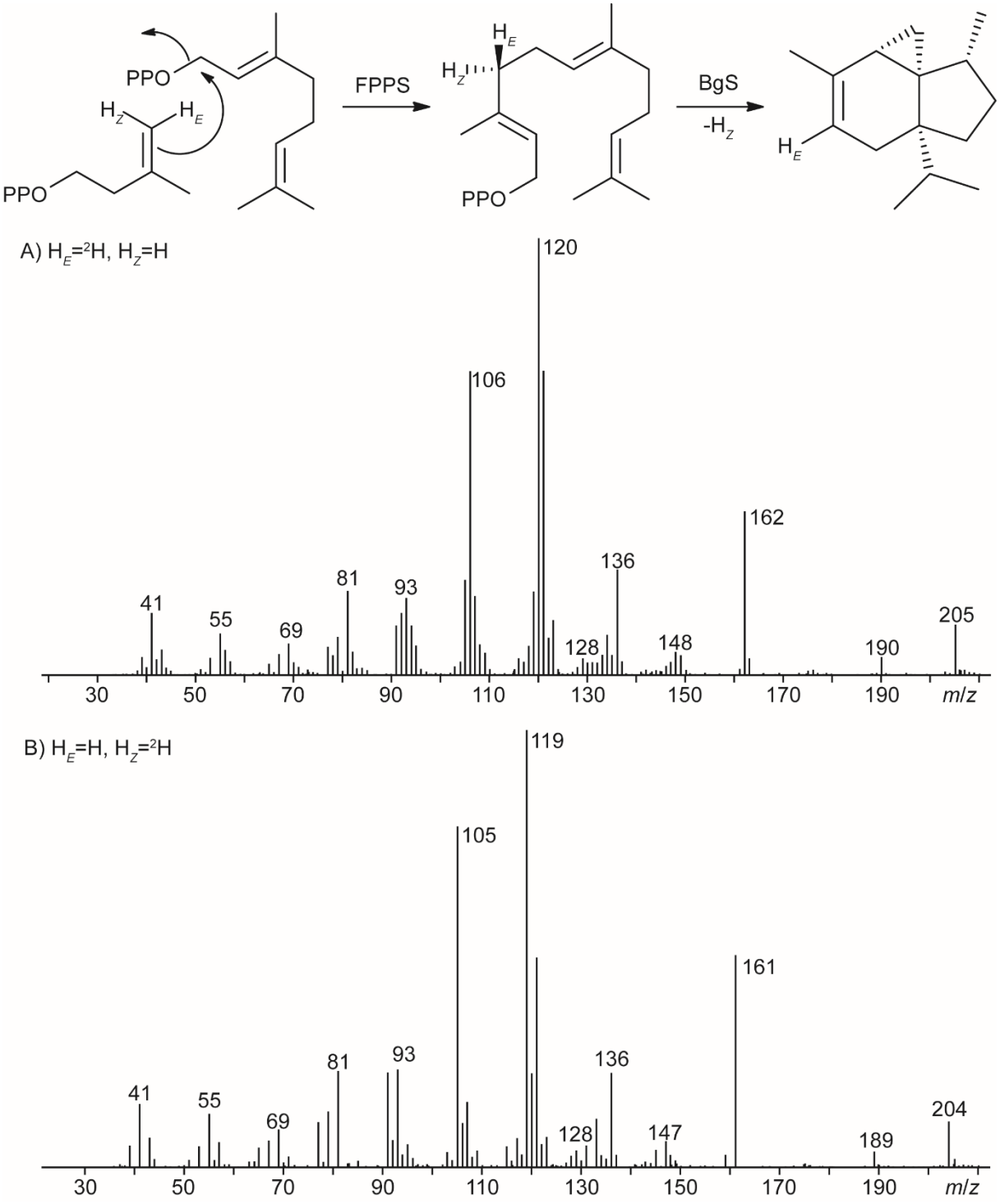


Figure S21. Investigation of the stereochemical course for the deprotonation of **F** to **8** by incubation of GPP and (*E*)- and (*Z*)-(4-^{2}H)IPP with FPPS and BgS.

Epoxidation of 8 with *m*CPBA

Bungoene (**8**, 4.5 mg, 0.02 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and the solution was cooled to 0 °C. *m*CPBA (3.8 mg, 0.02 mmol, 1.0 eq.) was added and the mixture was stirred for 1.5 h. Water was added and the mixture was extracted three times with diethyl ether. The combined organic phases were dried with $MgSO_4$ concentrated under reduced pressure and subjected to column chromatography on silica gel (pentane:Et_2O, 2:1) to yield the products **9** (1.5 mg, 0.007 mmol, 31 %) and **10** (0.6 mg, 0.003 mmol, 12 %).

Bungoan-4-one, (1a*S*,2*R*,4a*S*,7*R*,7a*R*)-4a-isopropyl-2,7-dimethylhexahydro-1*H*-cyclopropa[d]inden-3(4H)-one (14). Colourless oil. TLC (pentane:Et_2O, 2:1): R_f = 0.7. $[\alpha]_D^{25}$ = +2.5 (*c* 0.17, C_6D_6). HRMS (APCI): *m*/*z* = 221.1899 (calc. for $[C_{15}H_{25}O]^{+\cdot}$ *m*/*z* = 221.1900). GC (HP5-MS): *I* = 1631. MS (EI, 70 eV): *m*/*z* (%) = 220 (6), 205 (2), 178 (19), 177 (74), 163 (9), 149 (100), 137 (20), 135 (34), 123 (24), 121 (29), 107 (49), 105 (12), 95 (31), 93 (73), 91 (27), 81 (48), 79 (27), 77 (18), 69 (27), 67 (15), 55 (58), 43 (16), 41 (32), 39 (11). IR (diamond ATR): $\tilde{\nu}$ / cm^{-1} = 2956 (m), 2924 (s), 1854 (m), 1715 (w), 1461 (w), 1377 (w), 1261 (w), 1091 (w), 1022 (w), 800 (w). NMR data are given in Table S4 and Figures S22–S28.

3-Hydroxybungoan-4-one, (1a*R*,2*S*,4a*S*,7*R*,7a*R*)-2-hydroxy-4a-isopropyl-2,7-dimethylhexahydro-1*H*-cyclopropa[d]inden-3(4H)-one (15). Colourless oil. TLC (pentane:Et_2O, 2:1): R_f = 0.3. $[\alpha]_D^{25}$ = +1.0 (*c* 0.06, C_6D_6). HRMS (APCI): *m*/*z* = 237.1850 (calc. for $[C_{15}H_{25}O_2]^{+\cdot}$ *m*/*z* = 237.1849). GC (HP5-MS): *I* = 1657. MS (EI, 70 eV): *m*/*z* (%) = 236 (0.1), 218 (0.2), 193 (100), 175 (15), 149 (5), 137 (12), 135 (13), 133 (13), 123 (88), 119 (8), 109 (17), 107 (22), 105 (12), 97 (9), 95 (21), 93 (18), 91 (22), 83 (8), 81 (23), 79 (11), 77 (9), 69 (21), 55 (23), 43 (51), 41 (16). IR (diamond ATR): $\tilde{\nu}$ / cm^{-1} = 3360 (w), 2955 (m), 2925 (s), 2854 (m), 1719 (w), 1664 (w), 1461 (w), 1376 (w), 1261 (w), 1107 (m), 1021 (m), 799 (m). NMR data are given in Table S5 and Figures S29–S35.

Table S4. NMR data of bungoan-4-one (**14**) in C_6D_6 recorded at 298 K.

C[a]		^{1}H[b]	^{13}C[b]
1	CH_2	0.48 (dd, $^2J_{H,H}$ = 5.9, $^3J_{H,H}$ = 9.6, 1 H, H_β)	12.4
		0.03 (dd, $^2J_{H,H}$ = 5.9, $^3J_{H,H}$ = 6.0, 1 H, H_α),	
2	CH	0.96 (ddd, $^3J_{H,H}$ = 9.7, 7.4, 6.3, 1 H)	31.1
3	CH	2.28 (dq, $^3J_{H,H}$ = 7.6, 6.9, 1 H)	42.0
4	C_q	–	213.0
5	CH_2	2.22 (d, $^2J_{H,H}$ = 11.3, 1 H, H_α)	49.0
		2.10 (d, $^2J_{H,H}$ = 11.3, 1 H, H_β)	
6	C_q	–	35.7
7	CH	1.54 (m, 1 H)	43.0
8	CH_2	1.77 (m, 1 H, H_β)	32.2
		1.19 (m, 1 H, H_α)	
9	CH_2	1.72 (m, 1 H, H_α)	32.3
		1.02 (m, 1 H, H_β)	
10	C_q	–	52.6
11	CH	1.08 (t, $^3J_{H,H}$ = 6.9, 6.9, 1 H)	31.7
12	CH_3	0.72 (d, $^3J_{H,H}$ = 6.8, 3 H)	19.3
13	CH_3	0.65 (d, $^3J_{H,H}$ = 7.2, 3 H)	17.4
14	CH_3	0.84 (d, $^3J_{H,H}$ = 6.6, 3 H)	17.3
15	CH_3	1.11 (d, $^3J_{H,H}$ = 6.7, 3 H)	13.6

[a] Carbon numbering in analogy to the numbering of **8** as shown in Scheme 1 of main text. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, coupling constants *J* are given in Hertz.

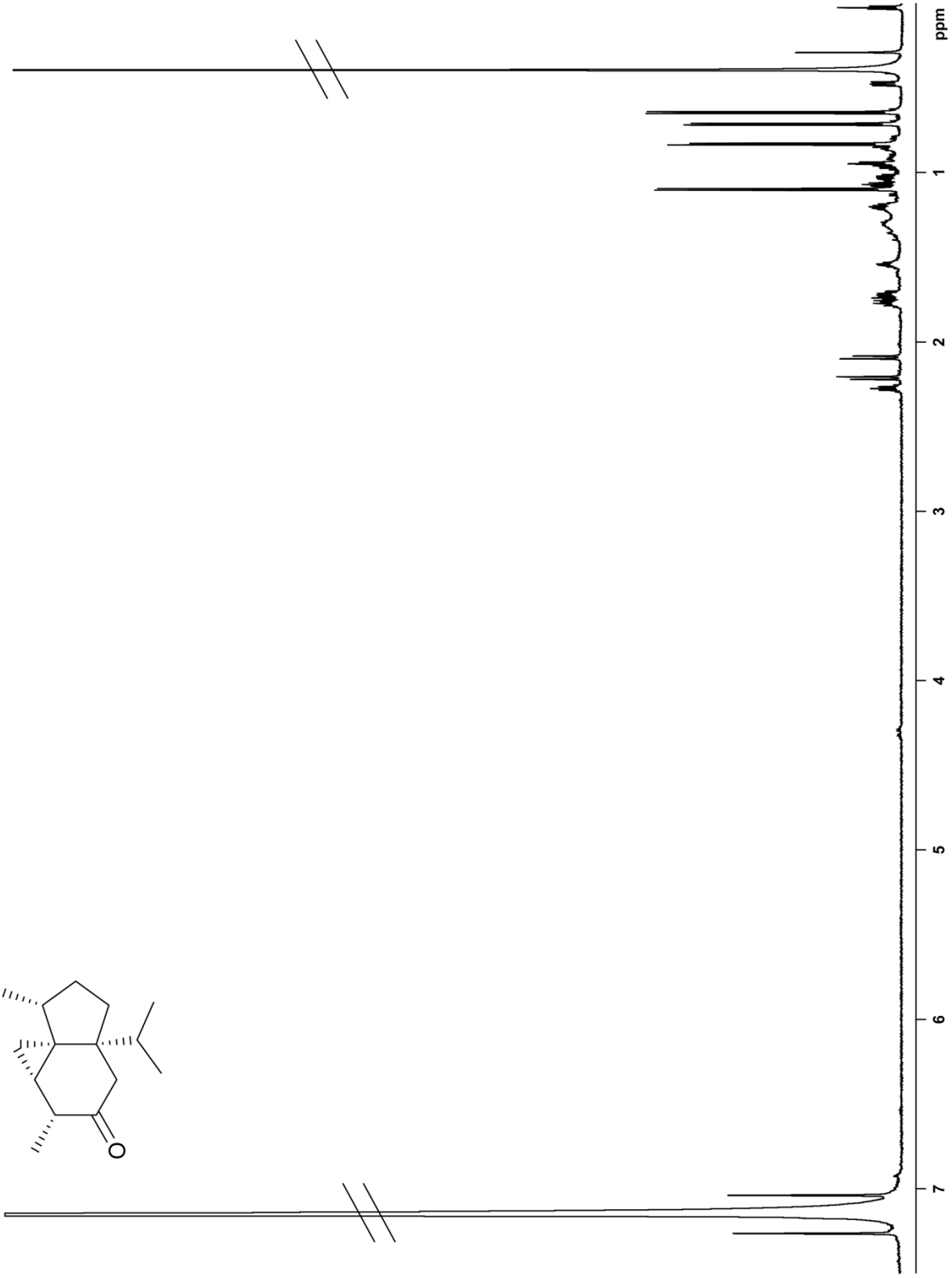


Figure S22. ^{1}H NMR spectrum of **14** (700 MHz, C_6D_6).

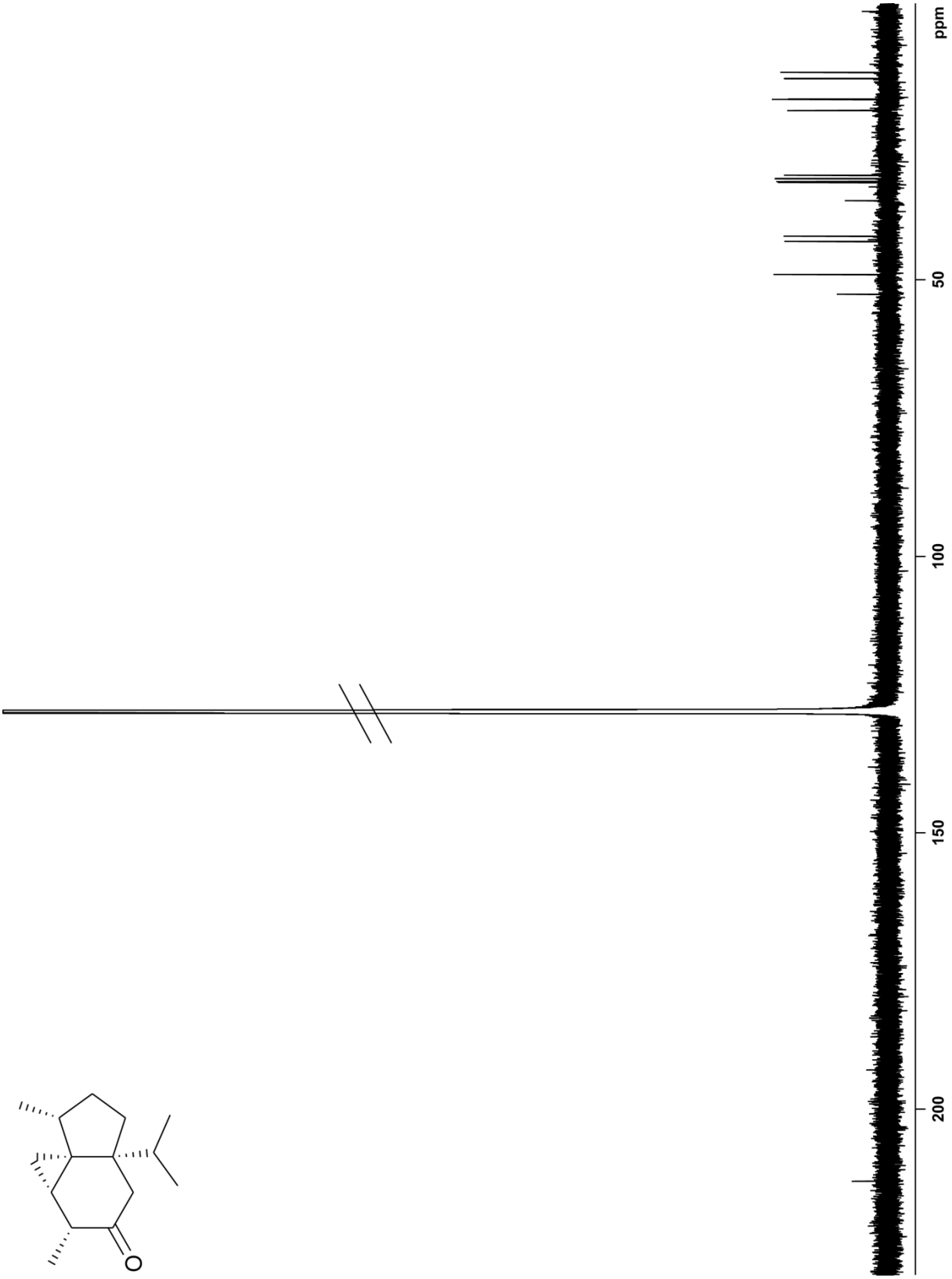


Figure S23. ^{13}C NMR spectrum of **14** (175 MHz, C_6D_6).

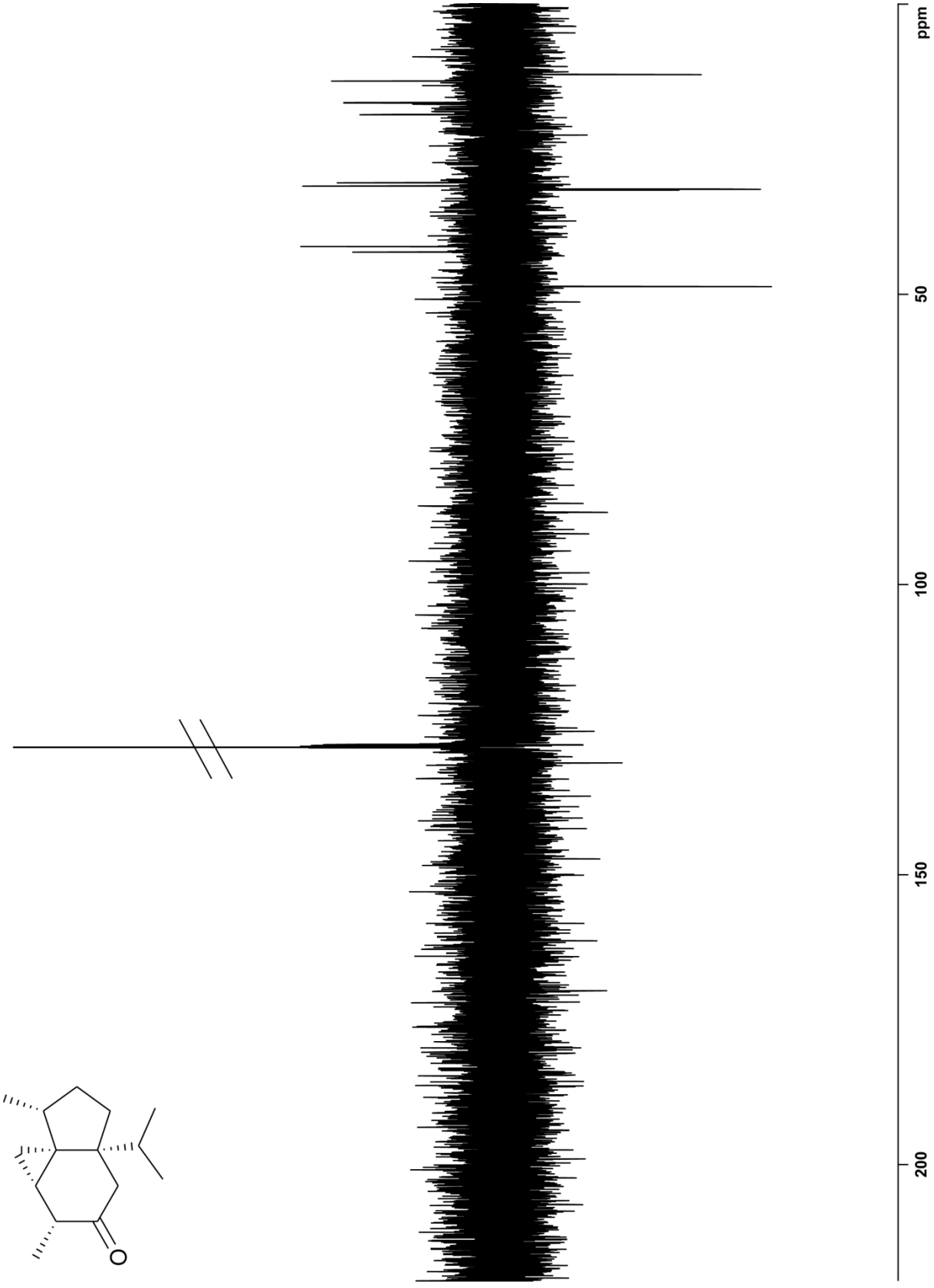


Figure S24. ^{13}C-DEPT-135 spectrum of **14** (175 MHz, C_6D_6).

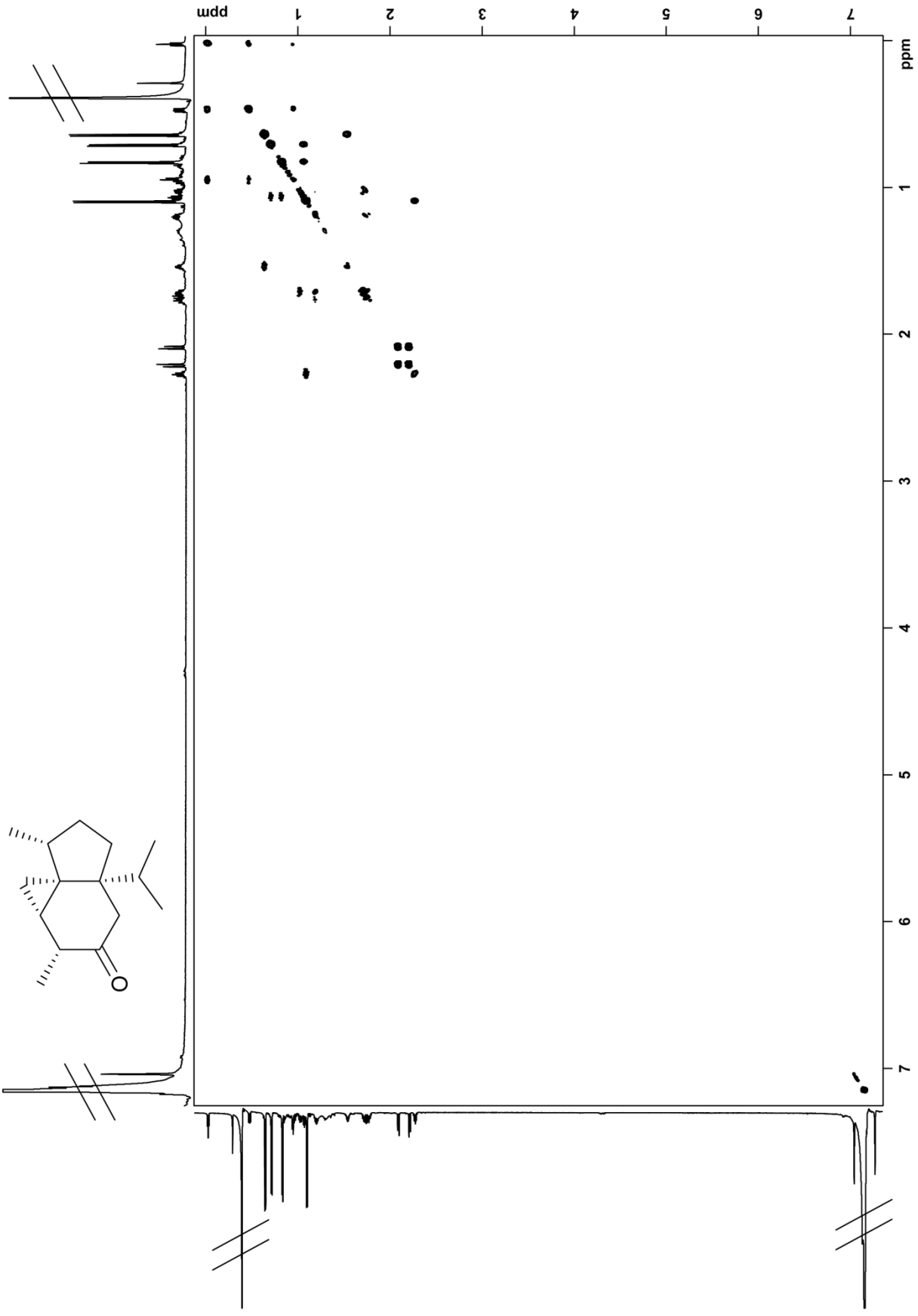


Figure S25. $^{1}H,^{1}H$-COSY spectrum of **14** (C_6D_6).

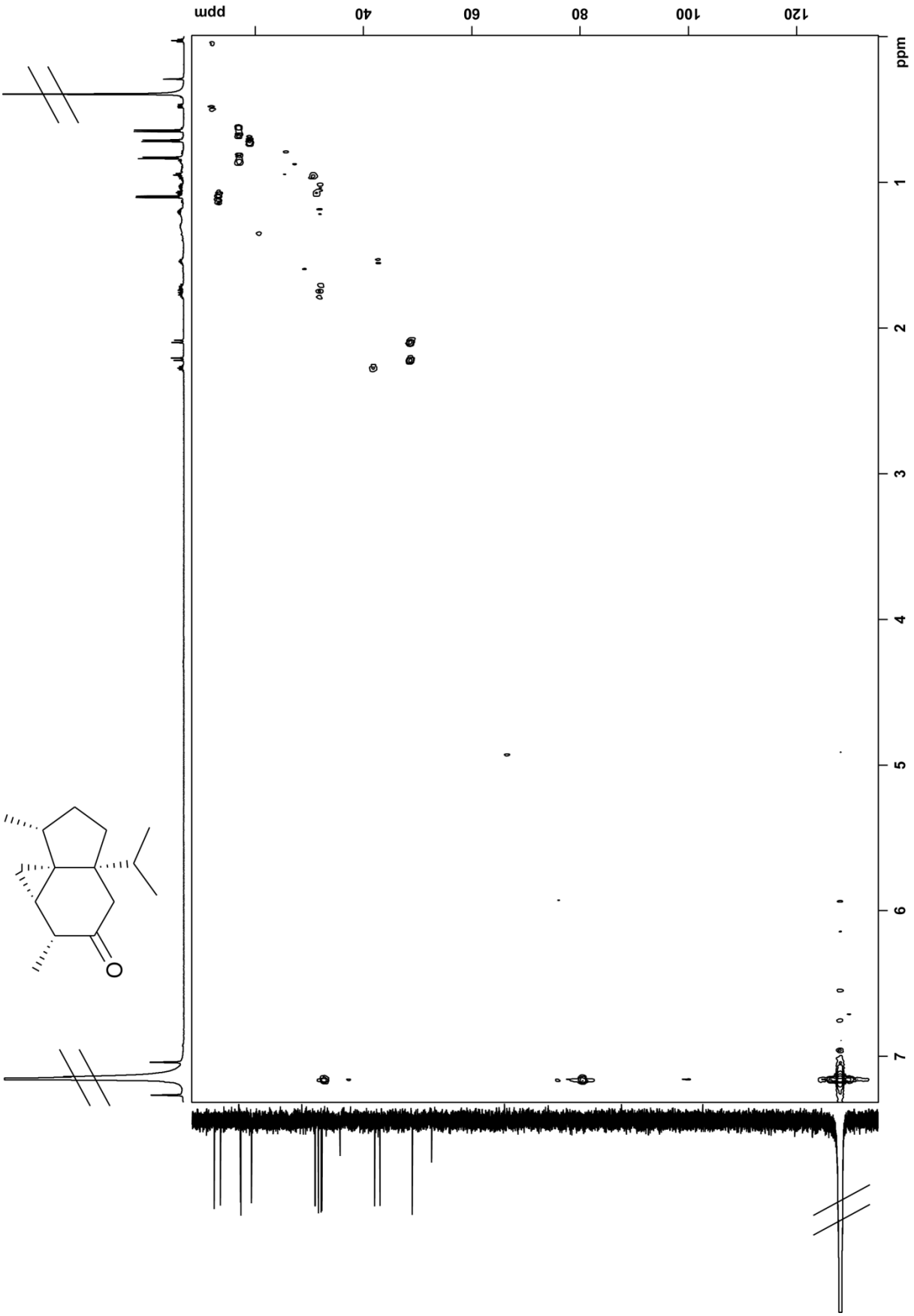


Figure S26. HSQC spectrum of **14** (C_6D_6).

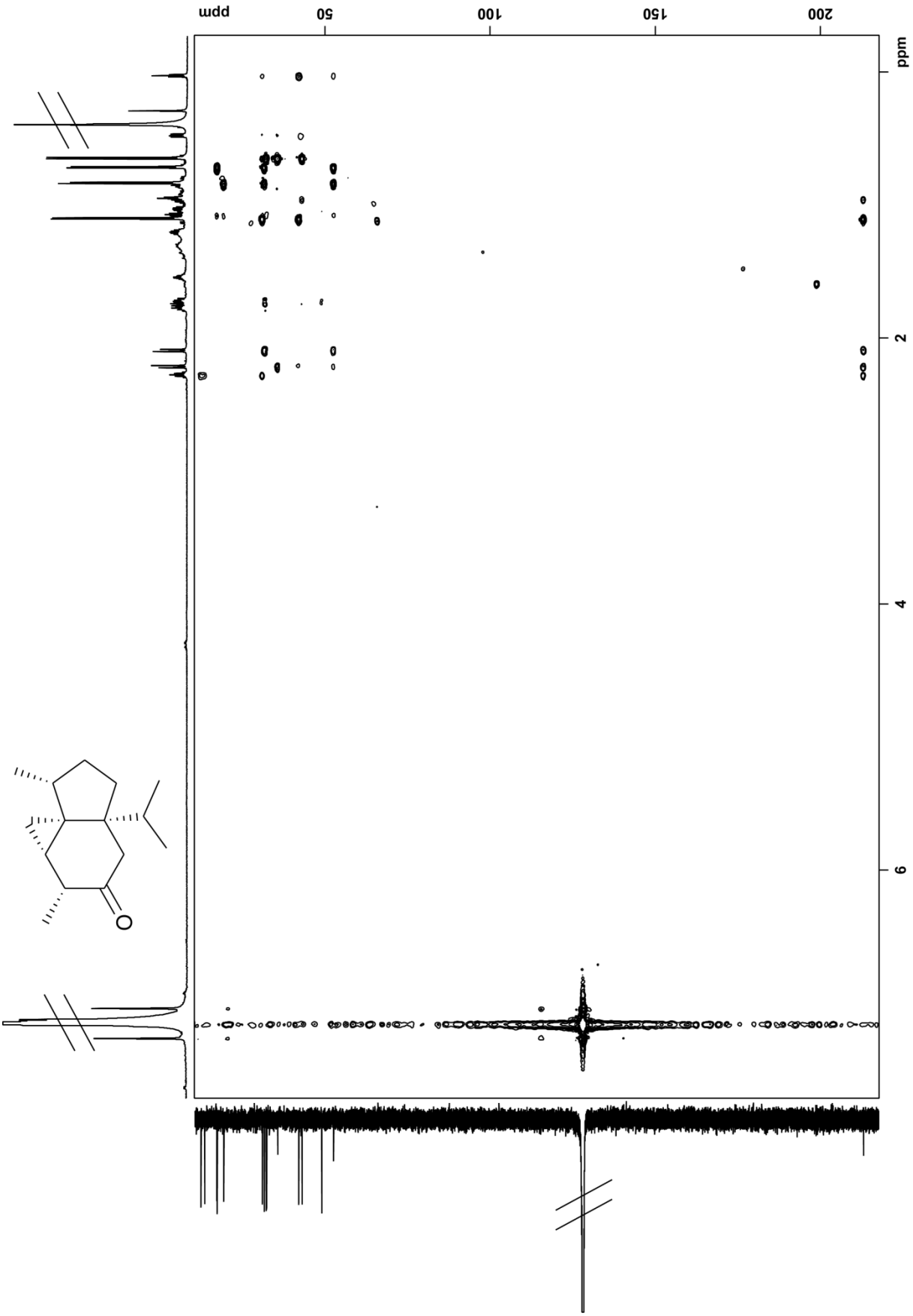


Figure S27. HMBC spectrum of **14** (C_6D_6).

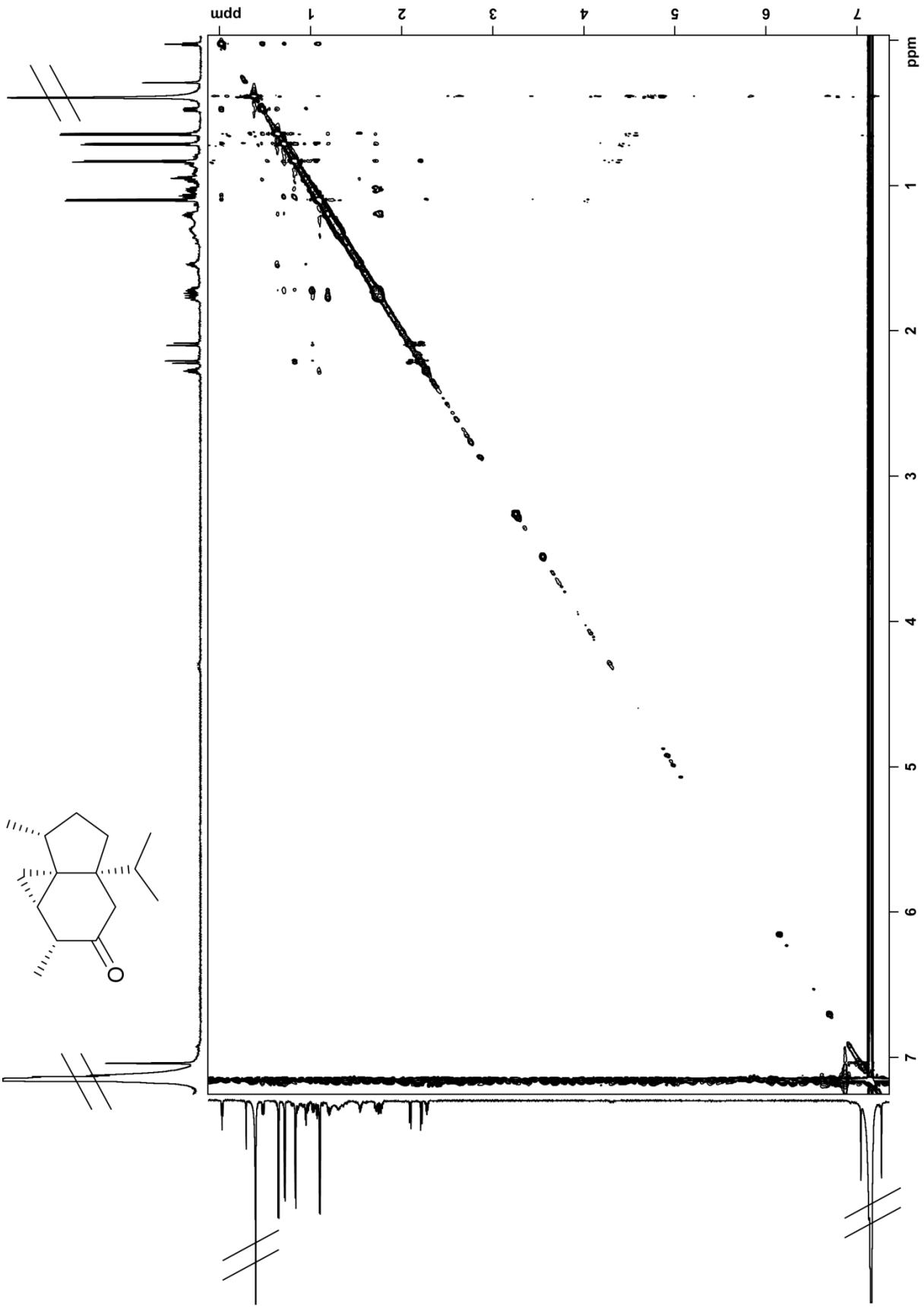


Figure S28. NOESY spectrum of **14** (C_6D_6).

Table S5. NMR data of 3-hydroxybungoan-4-one (**15**) in C_6D_6 recorded at 298 K.

C[a]		^{1}H[b]	^{13}C[b]
1	CH_2	0.61 (dd, $^2J_{H,H}$ = 6.4, $^3J_{H,H}$ = 10.2, 1 H, H_β)	11.4
		0.03 (dd, $^2J_{H,H}$ = 6.4, $^3J_{H,H}$ = 6.4, 1 H, H_α)	
2	CH	1.05 (dd, $^3J_{H,H}$ = 10.2, 6.4, 1 H)	36.2
3	C_q	–	73.0
4	C_q	–	211.8
5	CH_2	2.77 (d, $^2J_{H,H}$ = 12.0, 1 H, H_α)	41.8
		2.04 (d, $^2J_{H,H}$ = 12.0, 1 H, H_β)	
6	C_q	–	34.7
7	CH	1.55 (m, 1 H)	42.5
8	CH_2	1.86 (m, 1 H, H_β)	31.9
		1.17 (m, 1 H, H_α)	
9	CH_2	1.70 (m, 1 H, H_α)	33.3
		1.17 (m, 1 H, H_β)	
10	C_q	–	51.5
11	CH	1.20 (dd, $^3J_{H,H}$ = 7.3, 6.8, 1 H)	32.0
12	CH_3	0.65 (d, $^3J_{H,H}$ = 6.9, 3 H)	18.7
13	CH_3	0.80 (d, $^3J_{H,H}$ = 6.7, 3 H)	17.5
14	CH_3	0.63 (d, $^3J_{H,H}$ = 7.2, 3 H)	18.0
15	CH_3	1.21 (s, 3 H)	23.5

[a] Carbon numbering in analogy to the numbering of **8** as shown in Scheme 1 of main text. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, coupling constants *J* are given in Hertz.

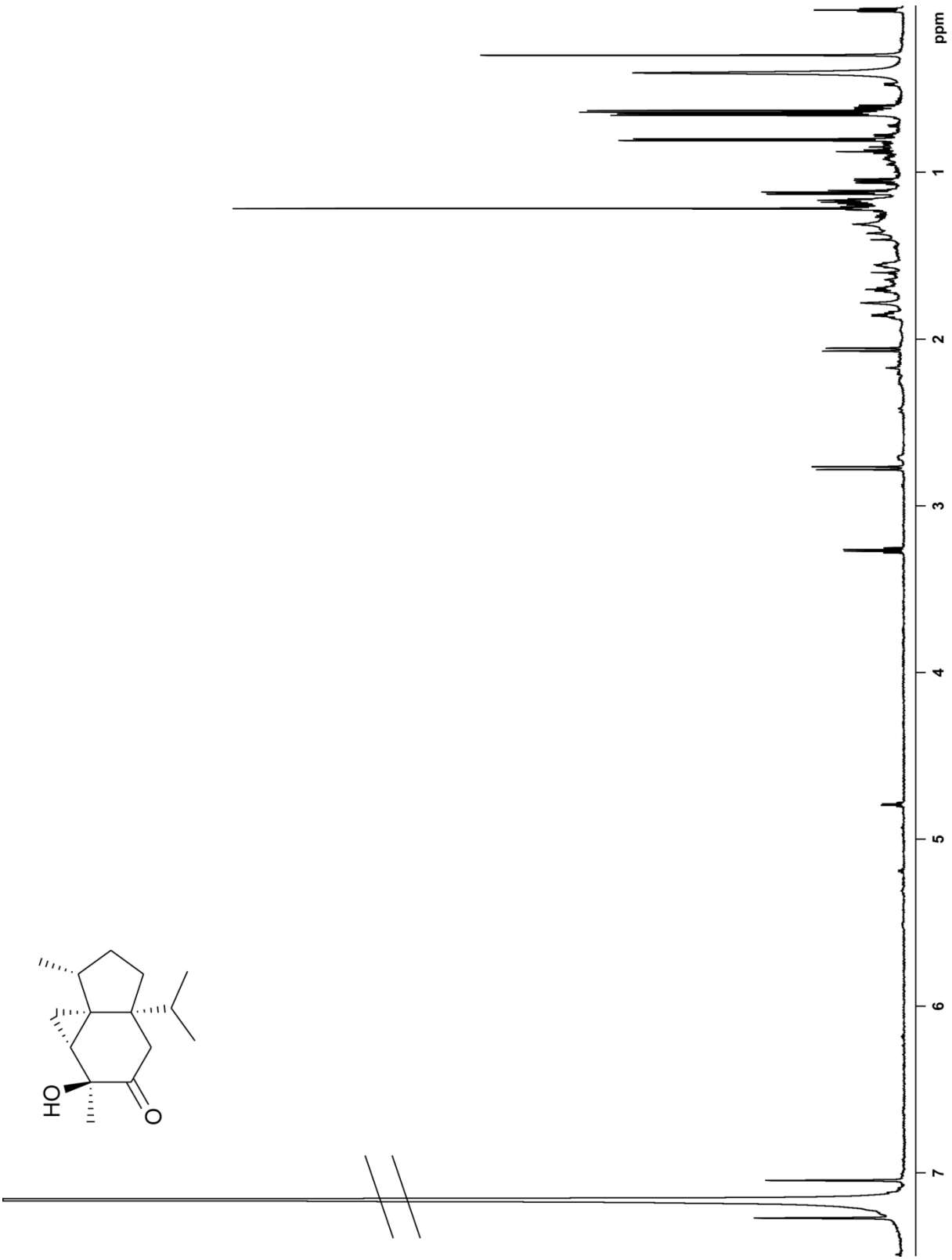


Figure S29. ^{1}H NMR spectrum of **15** (700 MHz, C_6D_6).

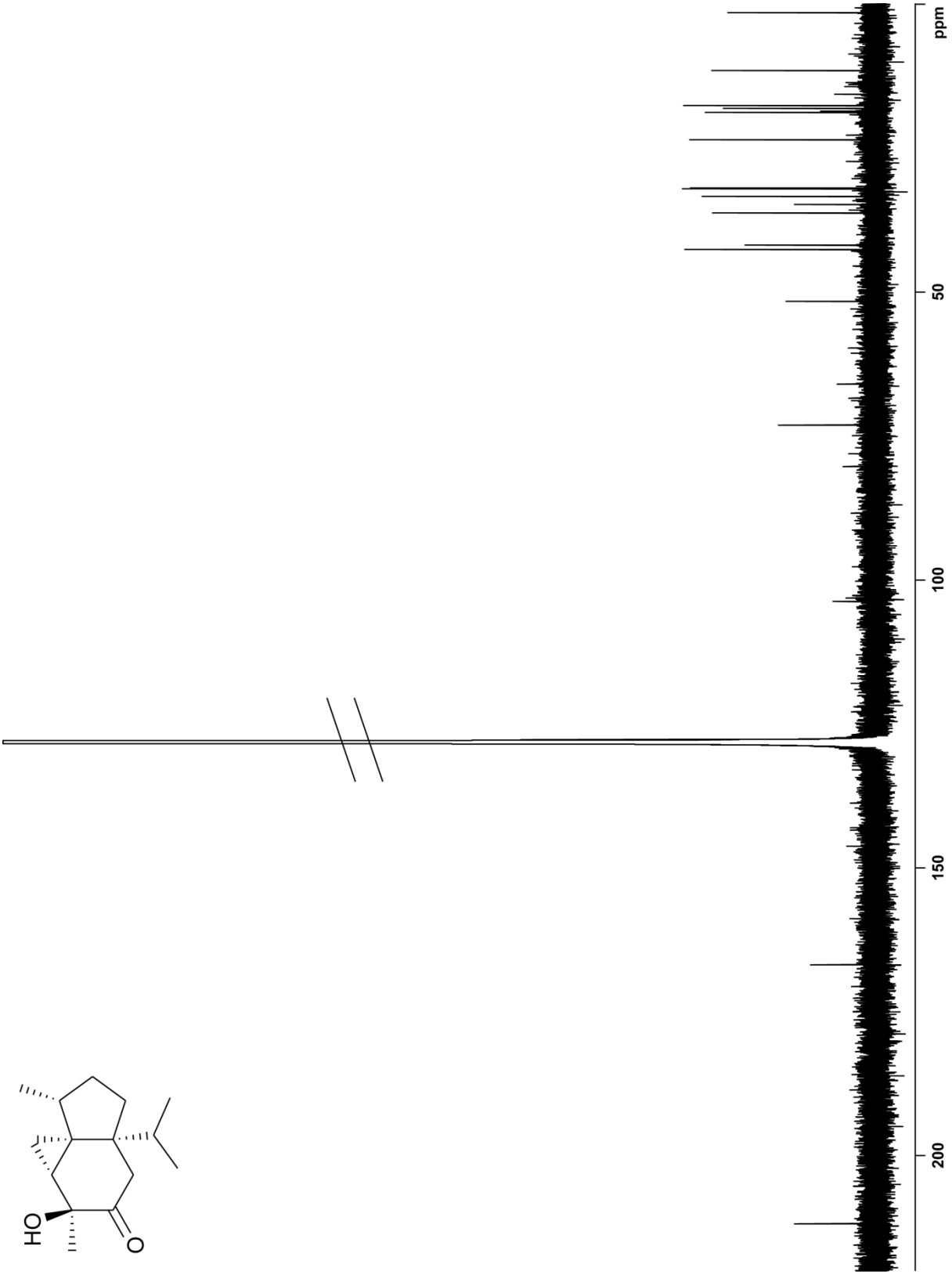


Figure S30. ^{13}C NMR spectrum of **15** (175 MHz, C_6D_6).

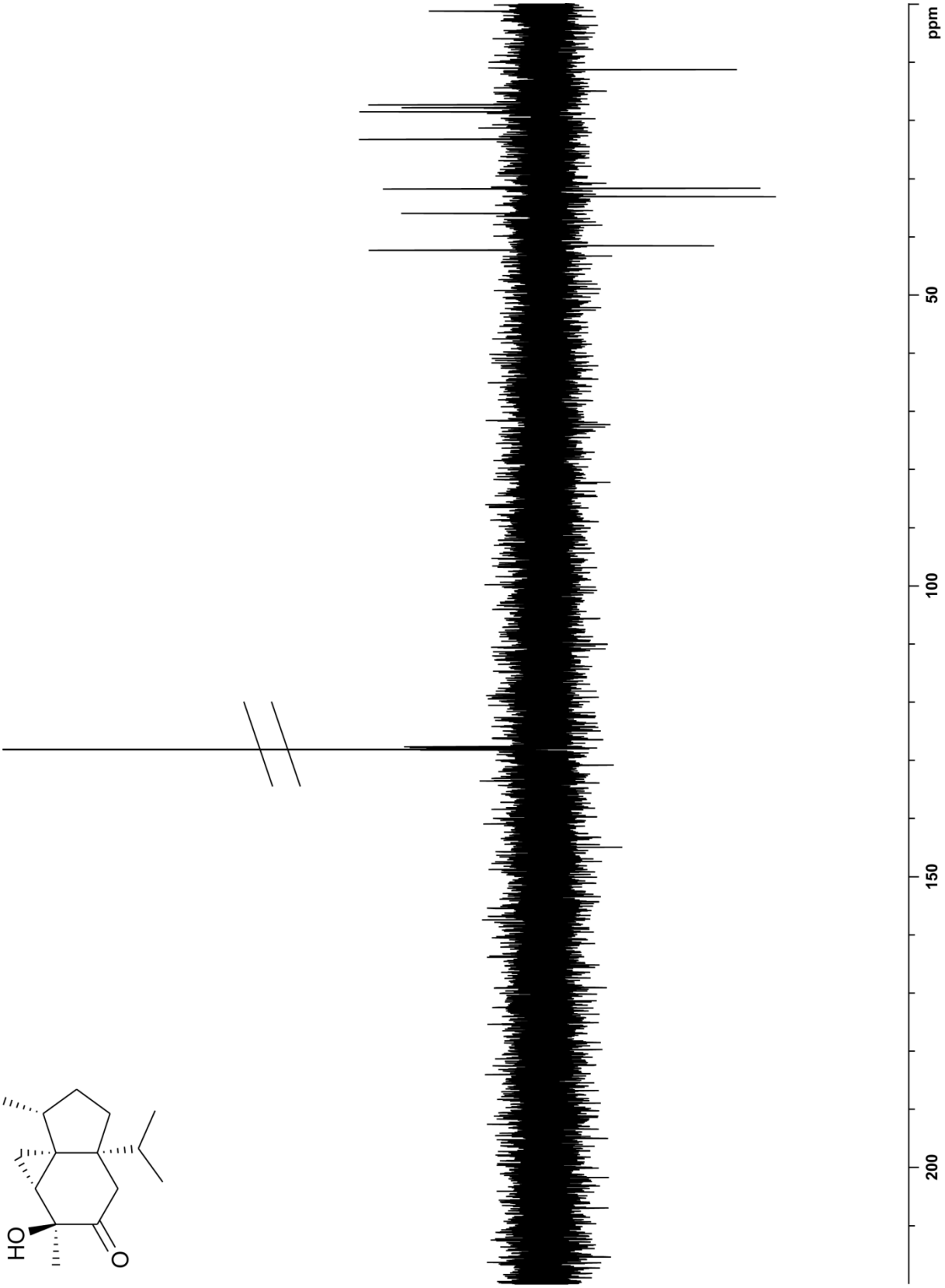


Figure S31. ^{13}C-DEPT-135 spectrum of **15** (175 MHz, C_6D_6).

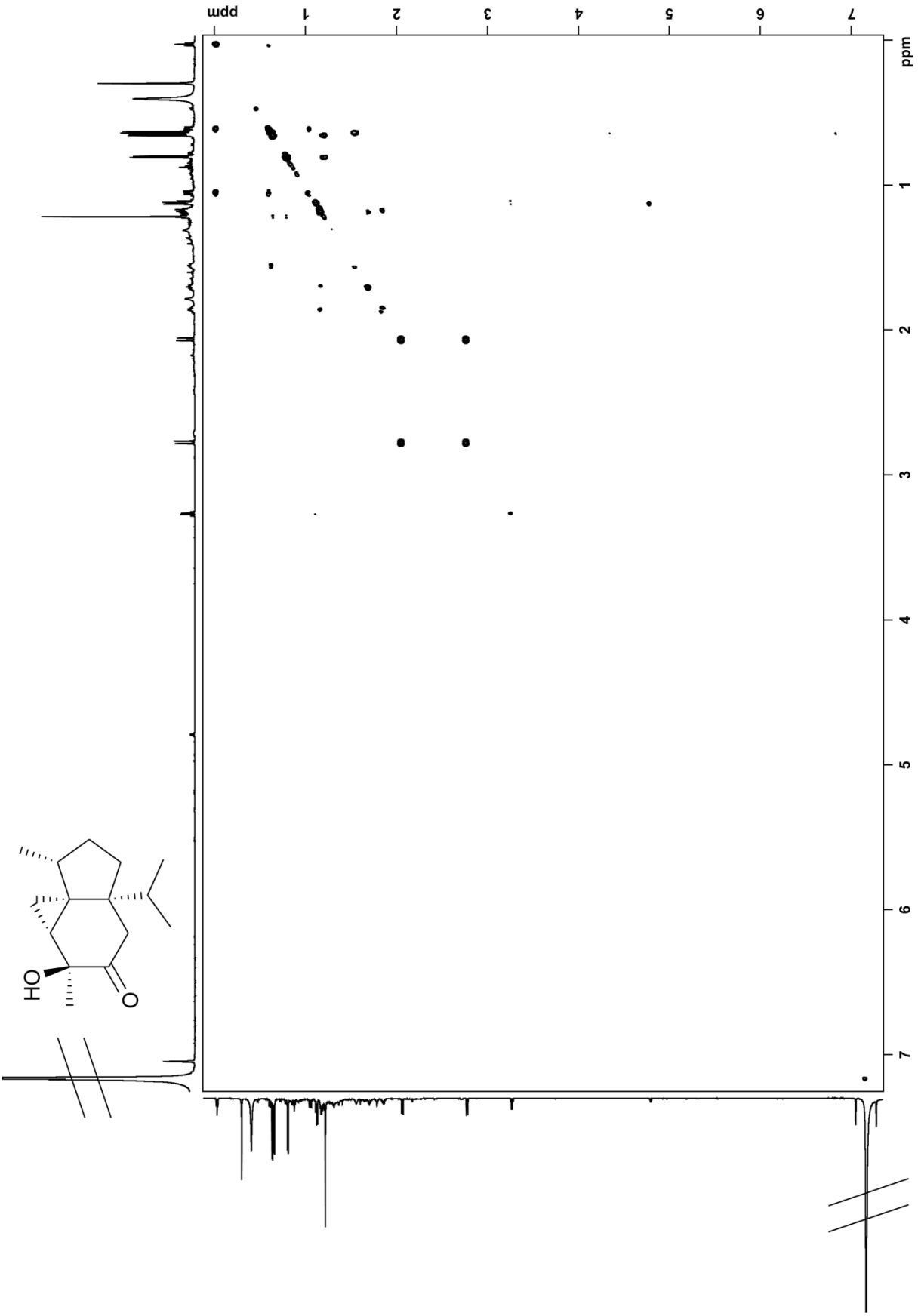


Figure S32. $^{1}H,^{1}H$-COSY spectrum of **15** (C_6D_6).

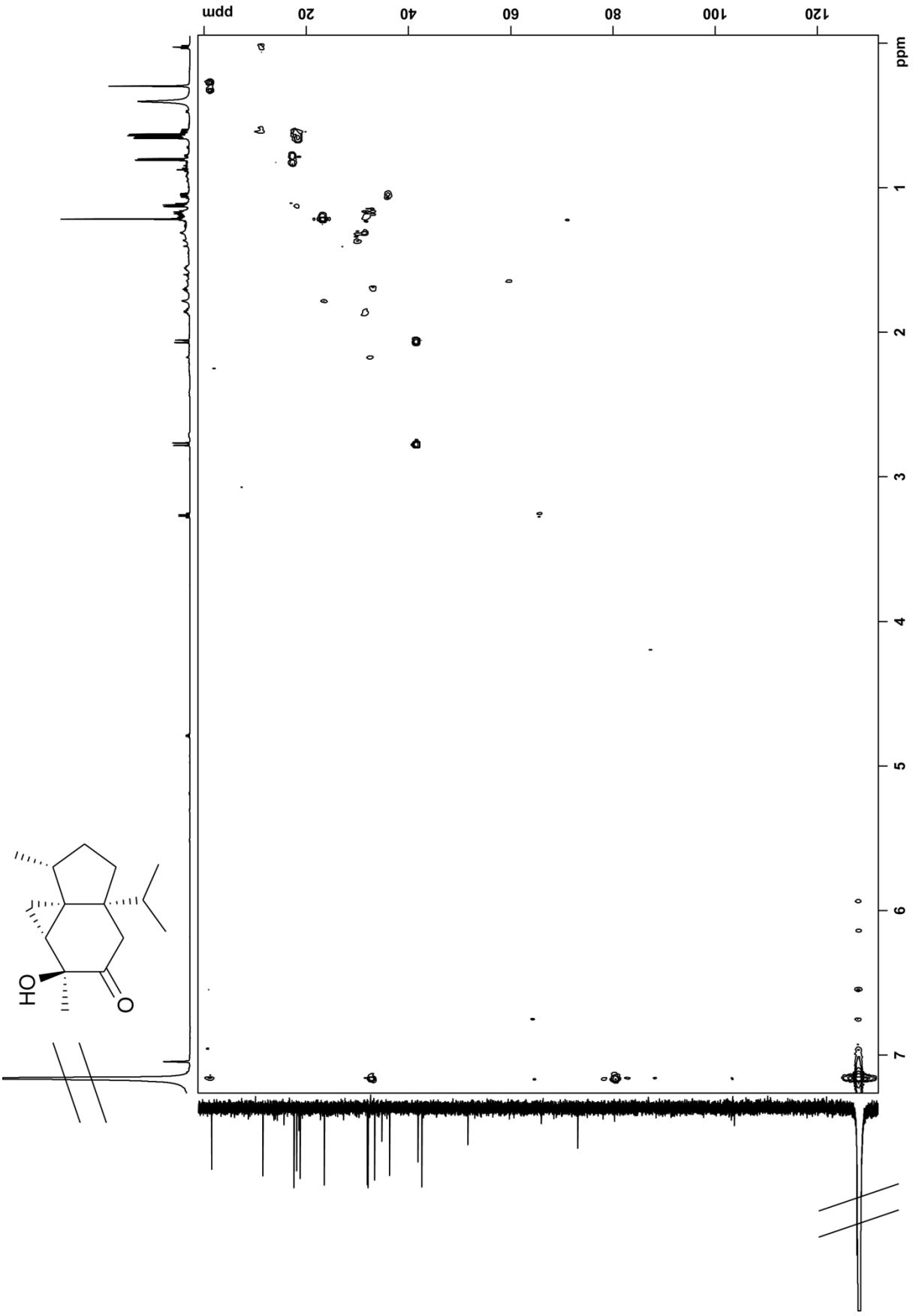


Figure S33. HSQC spectrum of **15** (C_6D_6).

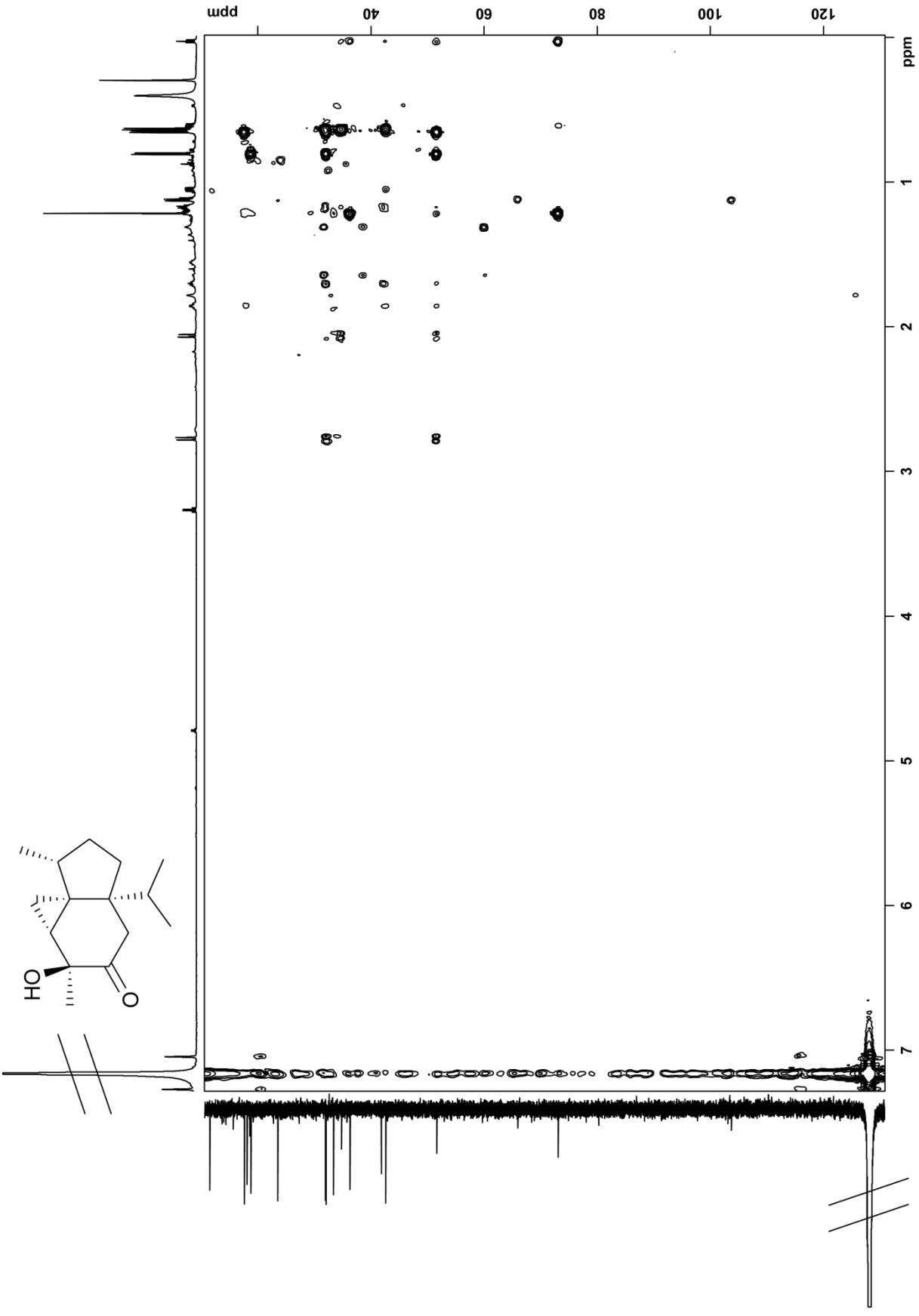


Figure S34. HMBC spectrum of **15** (C_6D_6).

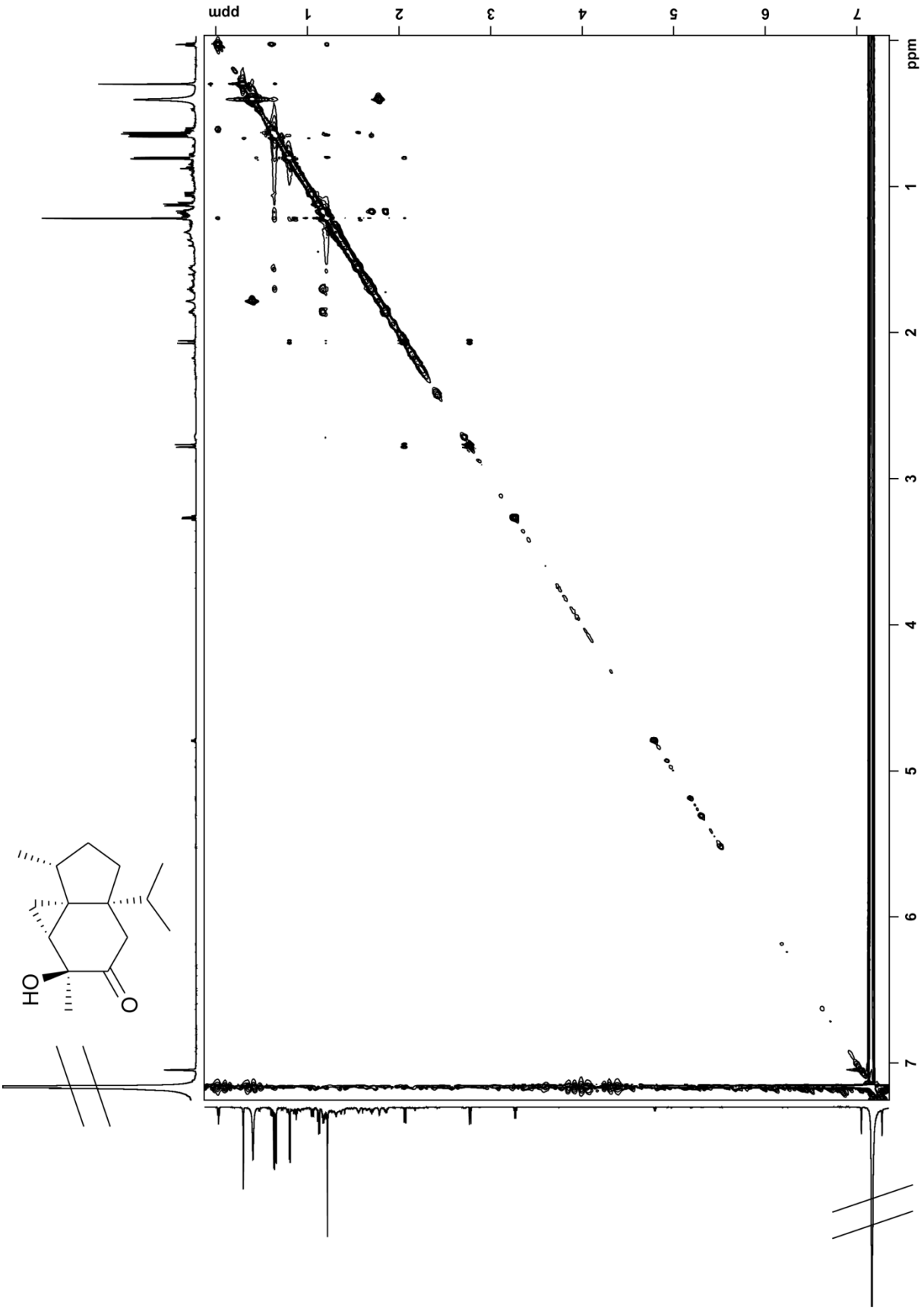


Figure S35. NOESY spectrum of **15** (C_6D_6).

Table S6. NMR data of pentalenene (**4**) in C_6D_6 recorded at 298 K.

C[a]		^{1}H[b]	^{13}C[b]
1	CH_2	1.76 (d, $^2J_{H,H}$ = 13.0, 1 H) 1.39 (d, $^2J_{H,H}$ = 13.0, 1 H)	49.3
2	C_q	–	65.1
3	CH	1.80–1.85 (m, 1 H))	45.0
4	CH_2	1.68 (dddd, $^2J_{H,H}$ = 11.9, $^3J_{H,H}$ = 10.1; 6.0; 6.0, 1 H) 1.28–1.31 (m, 1 H)	33.8
5	CH_2	1.78 (dddd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 9.8; 8.9; 6.4, 1 H) 1.34–1.38 (m, 1 H)	28.0
6	CH	2.53 (d, $^3J_{H,H}$ = 9.1, 1 H)	62.4
7	C_q	–	140.7
8	CH	5.22 (m, 1 H)	130.1
9	CH	2.70 (m, 1 H)	59.9
10	CH_2	1.65 (ddd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 9.3, $^4J_{H,H}$ = 1.0, 1 H) 1.28–1.31 (m, 1 H)	47.1
11	C_q	–	40.7
12	CH_3	1.08 (s, 3 H)	29.3
13	CH_3	1.03 (s, 3 H)	30.2
14	CH_3	0.91 (d, $^3J_{H,H}$ = 7.1, 3 H)	15.6
15	CH_3	1.60 (s, 3 H)	17.2

[a] Carbon numbering shown in Scheme 2 of main text. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, coupling constants *J* are given in Hertz.

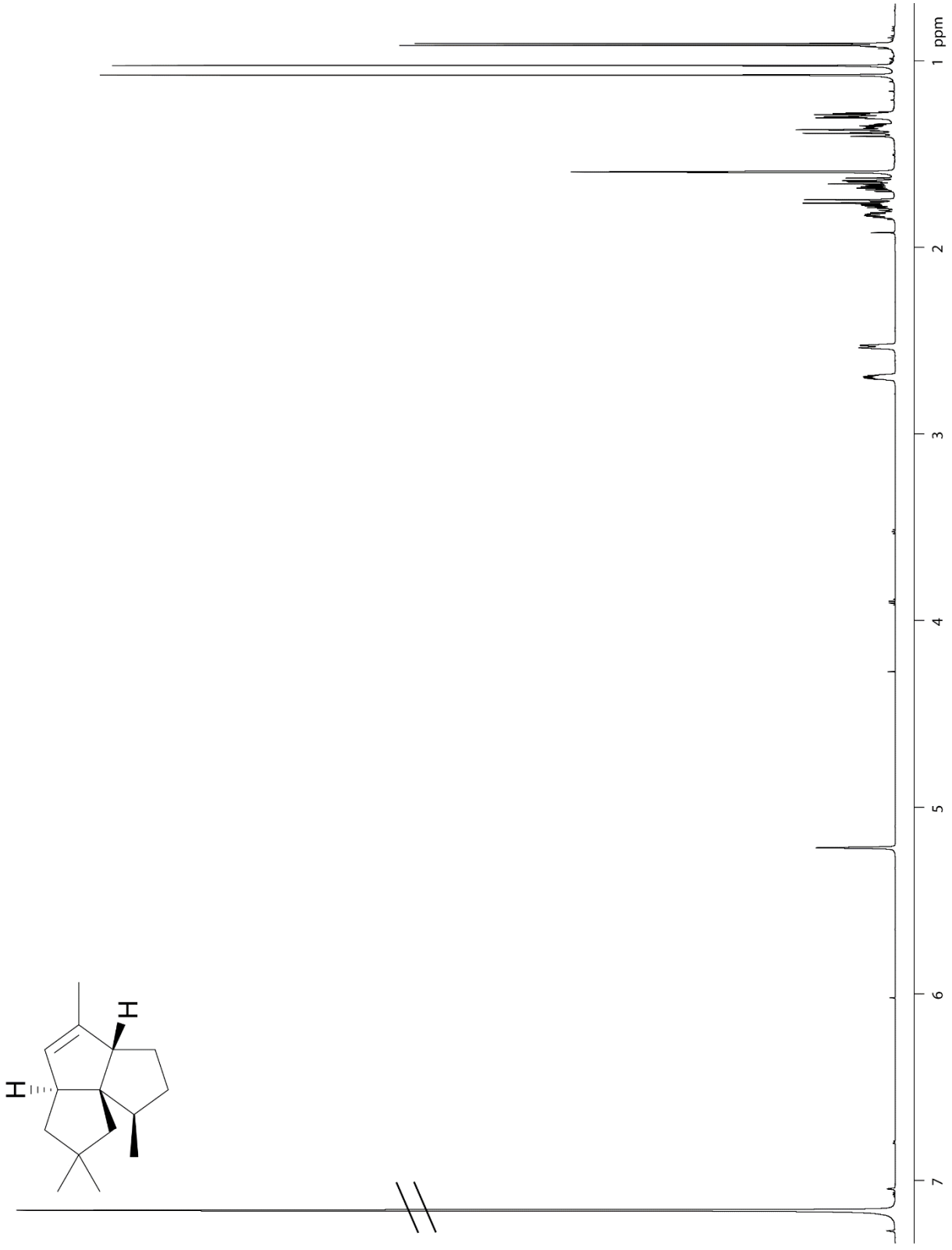


Figure S36. ^{1}H NMR spectrum of **4** (700 MHz, C_6D_6).

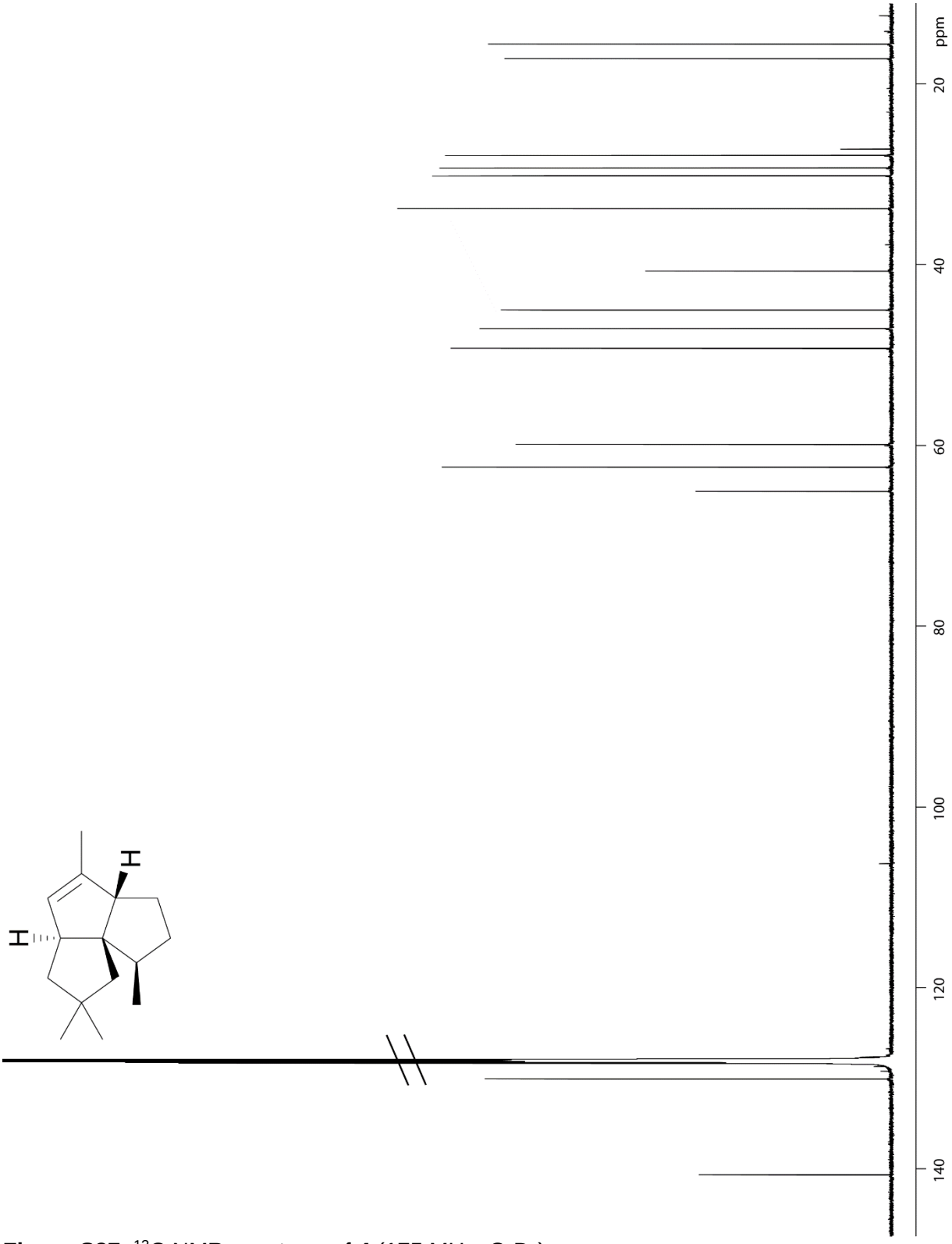


Figure S37. ^{13}C NMR spectrum of **4** (175 MHz, C_6D_6).

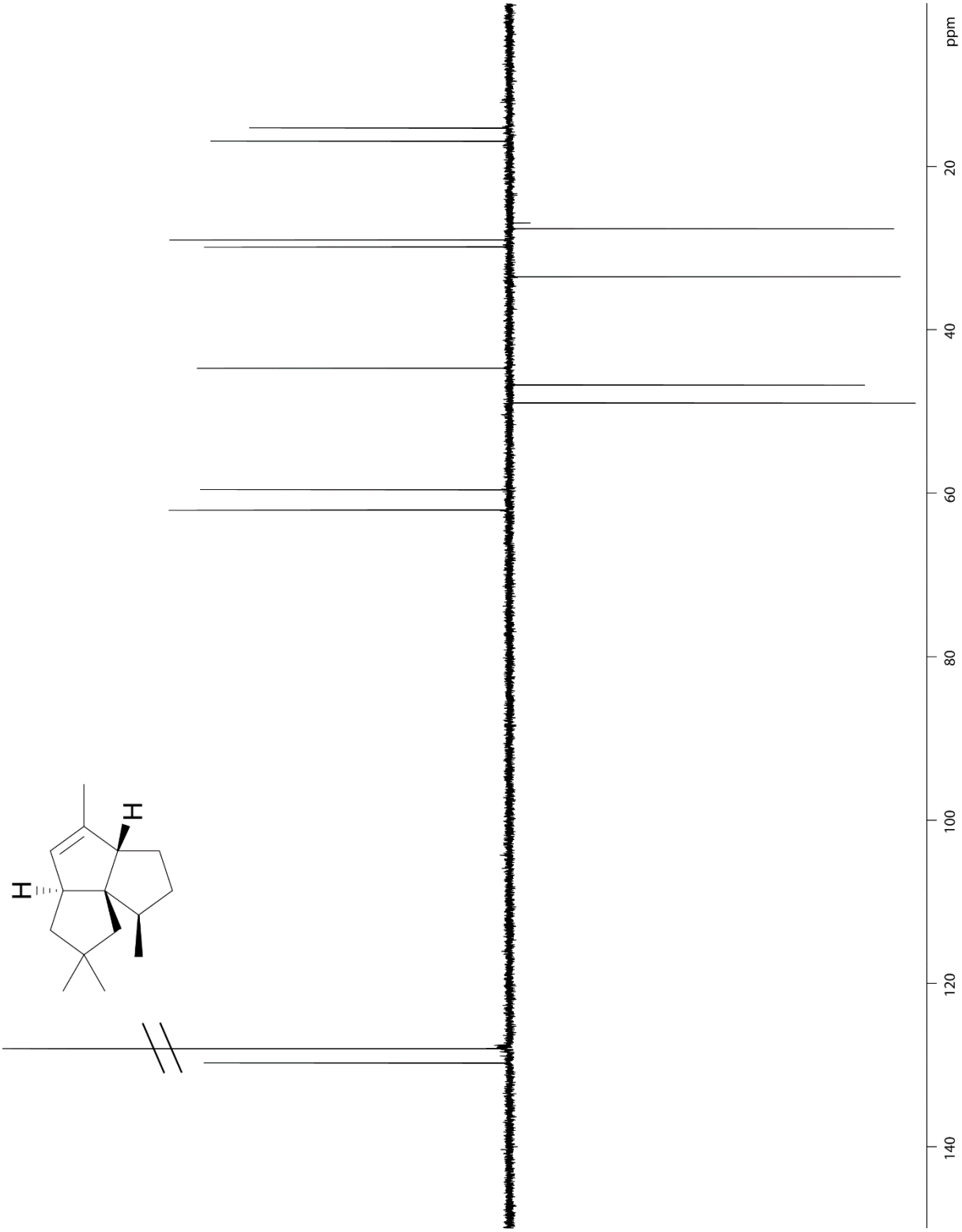


Figure S38. ^{13}C-DEPT-135 spectrum of **4** (175 MHz, C_6D_6).

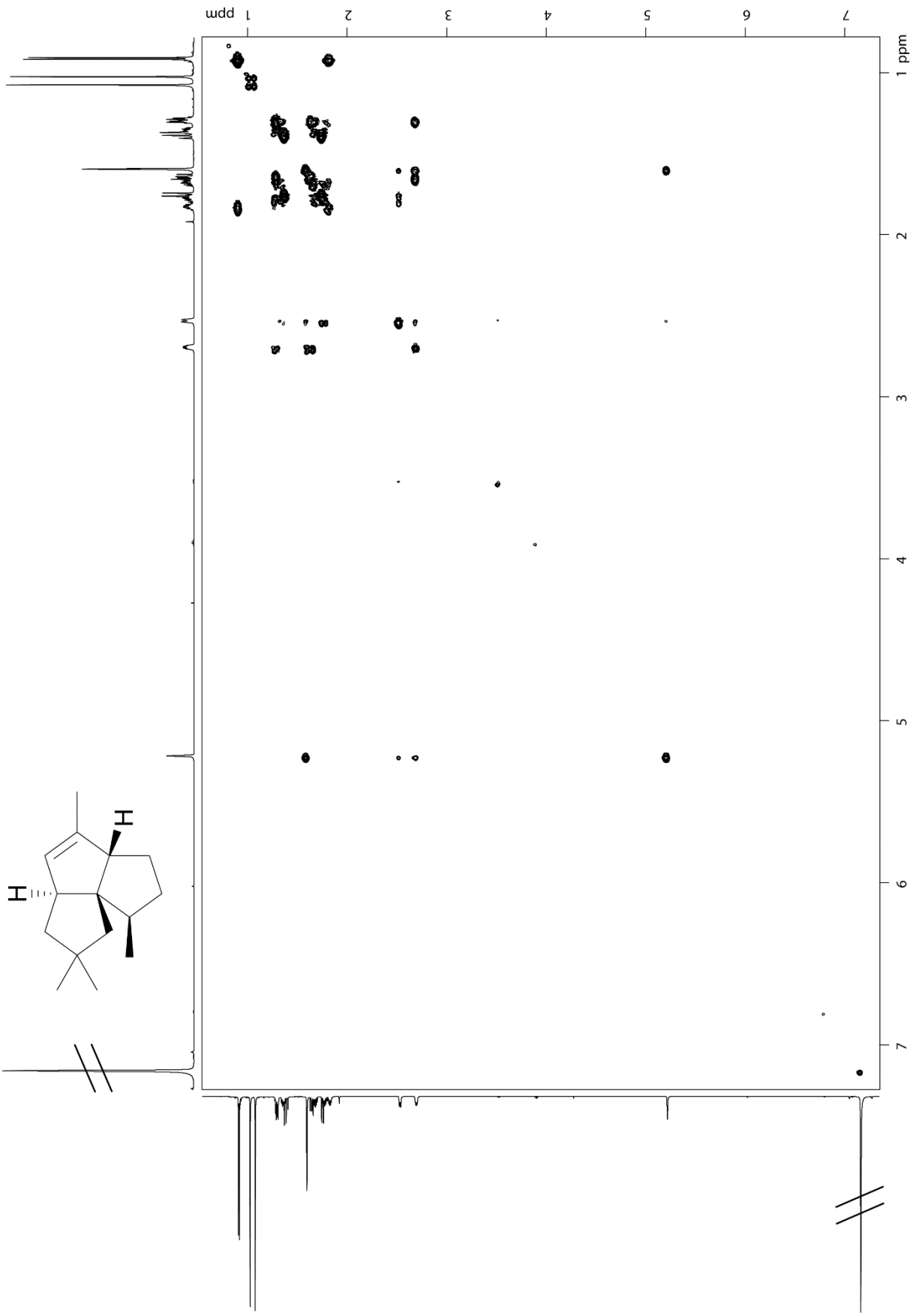


Figure S39. $^{1}H,^{1}H$-COSY spectrum of **4** (C_6D_6).

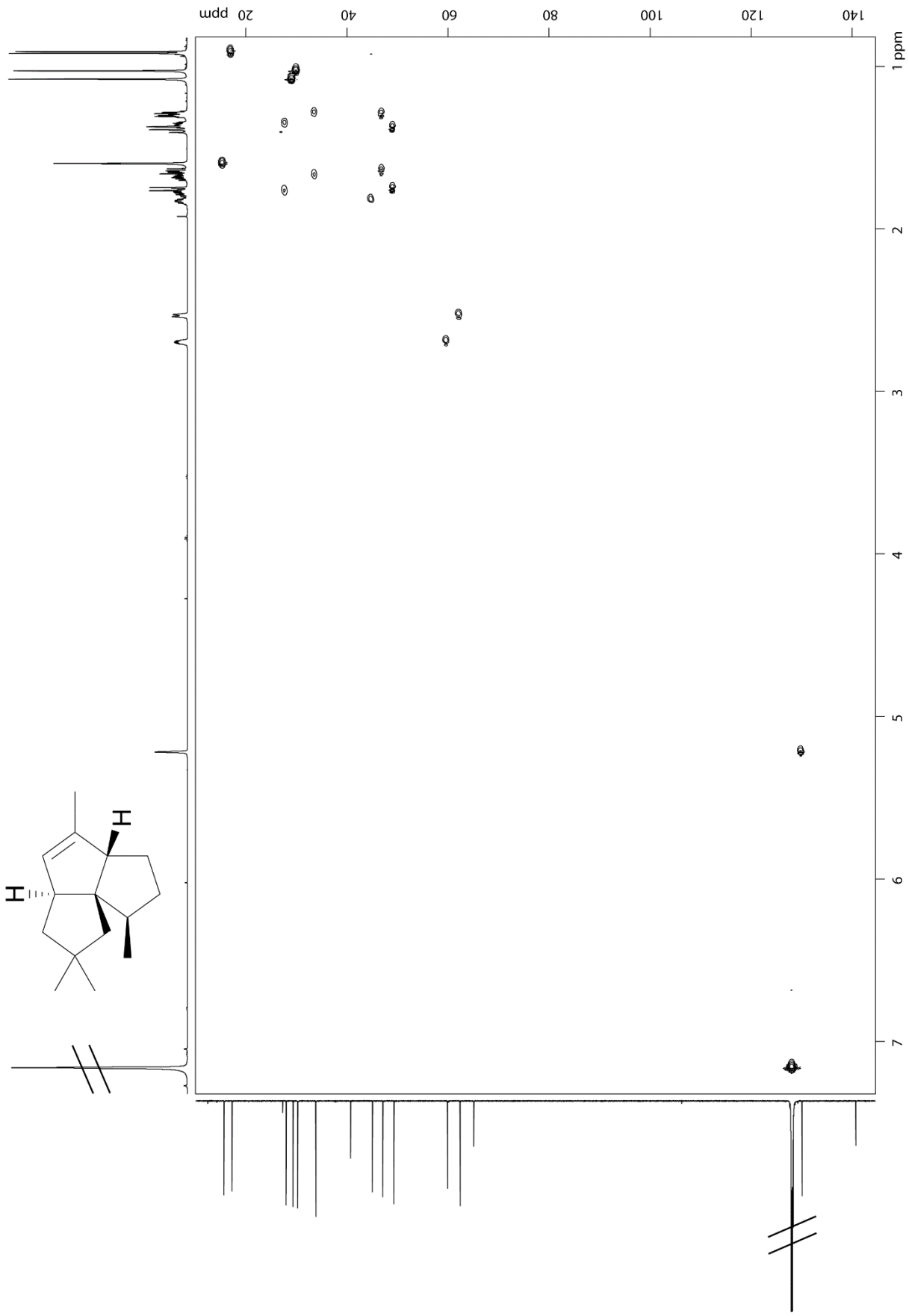


Figure S40. HSQC spectrum of **4** (C_6D_6).

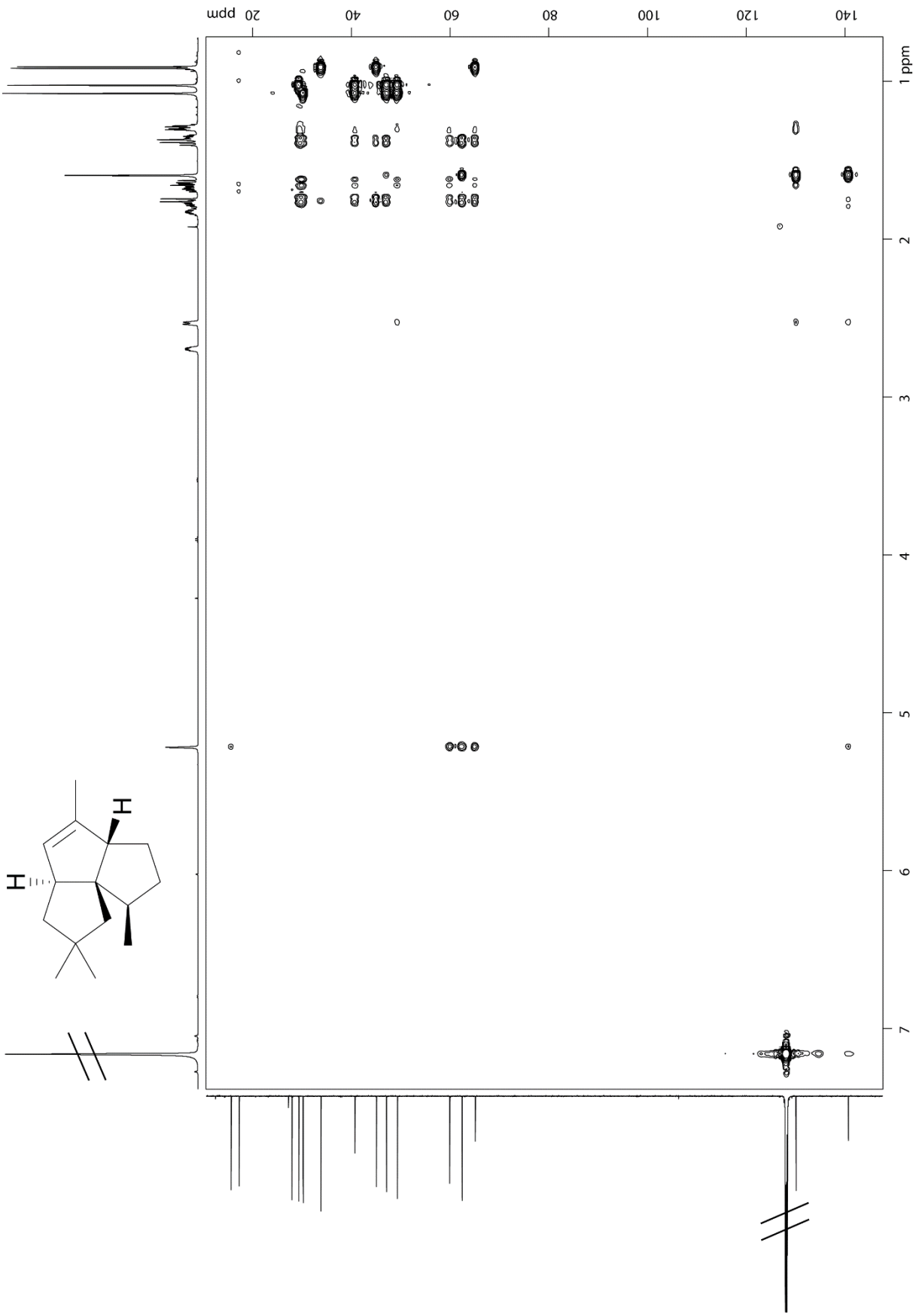


Figure S41. HMBC spectrum of **4** (C_6D_6).

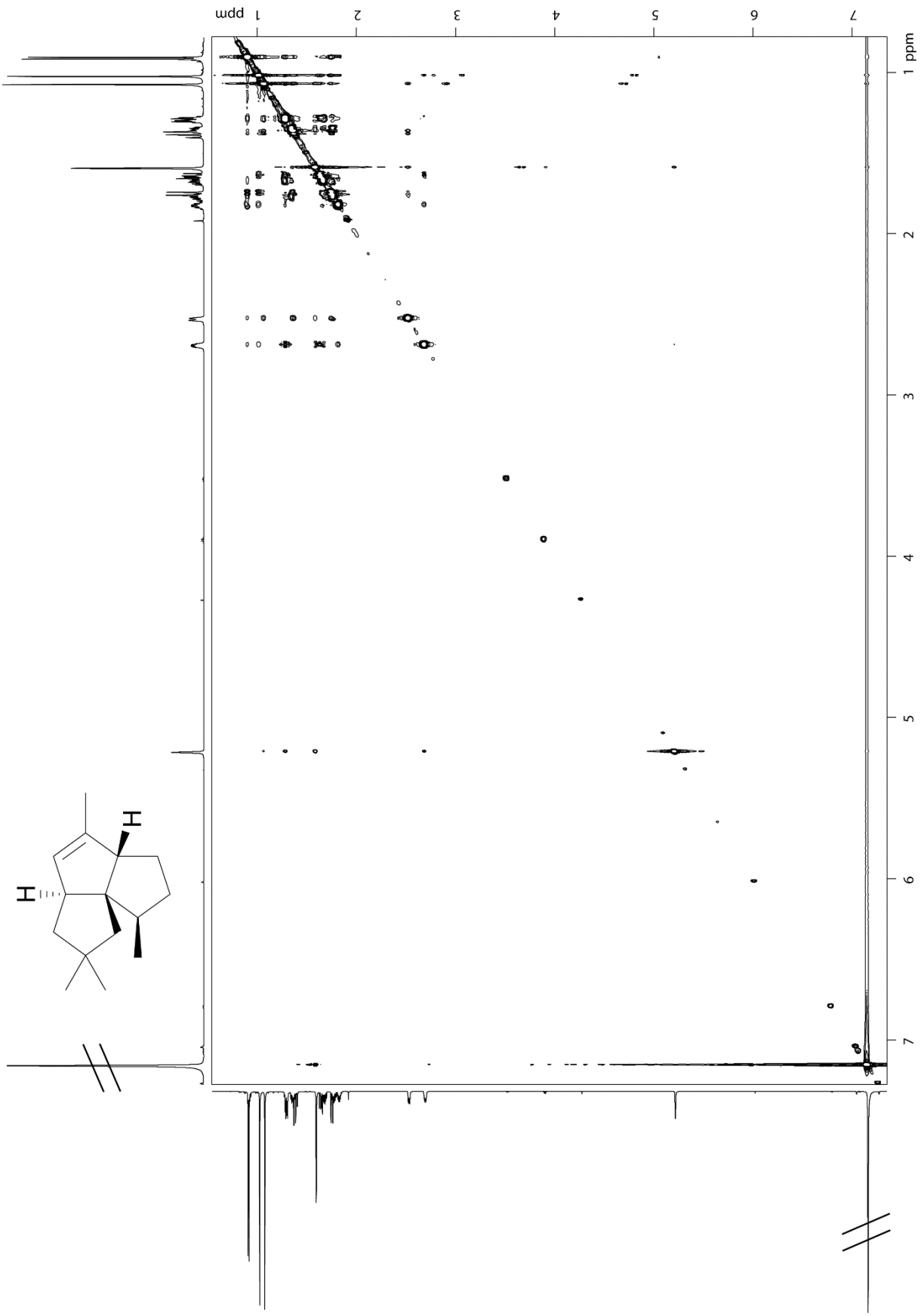


Figure S42. NOESY spectrum of **4** (C_6D_6).

Oxidation of 4 with SeO_2

To a solution of pentalenene (**4**, 5.14 mg, 25 μmol, 1.0 eq) in a 1,4-dioxane/water mixture (4:1, 0.6 mL) was added SeO_2 (5.55 mg, 50 μmol, 2.0 eq) and the mixture was refluxed. After 7 hours the same amount of SeO_2 was added again and the mixture was refluxed for 13 hours. After cooling to room temperature the mixture was diluted with saturated NH_4Cl solution (10 mL) and extracted with Et_2O (3 x 10 mL). The separated organic layer was dried with $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, pentane:Et_2O, 10:1) to yield the desired aldehyde **15** as a yellow oil (4.84 mg, 22 μmol, 88 %).

(–)-Pentalenal, (1R,3aR,5aS,8aR)-1,7,7-trimethyl-1,2,3,3a,5a,6,7,8-octahydrocyclopenta[c]pentalene-4-carbaldehyde (15). TLC (pentane:Et_2O, 10:1): R_f = 0.5. $[\alpha]_D^{20}$ = –5.5 (*c* 0.51, C_6D_6). HRMS (EI): *m*/*z* = 218.1669 (calc. for $[C_{15}H_{22}O]^{+\cdot}$ *m*/*z* = 218.1665). GC (HP5-MS): *I* = 1626. MS (EI, 70 eV): *m*/*z* (%) = 218 (100), 203 (37), 189 (19), 175 (31), 162 (60), 147 (40), 133 (34), 119 (30), 105 (40), 91 (47), 77 (18), 65 (7), 55 (11), 41 (12), see Figure S43. IR (diamond ATR): $\tilde{\nu}$ / cm^{-1} = 2951 (m), 2926 (m), 2863 (m), 2798 (w), 2709 (w), 1738 (w), 1679 (s), 1618 (w), 1461 (m), 1378 (w), 1365 (w), 1260 (m), 1171 (m), 1084 (s), 1016 (s), 865 (w), 795 (s), 739 (w), 701 (w), 644 (w). NMR data are given in Table S7 and Figures S44–S50.

Table S7. NMR data of pentalenal (**17**) in C_6D_6 recorded at 298 K.

C[a]		^{1}H[b]	^{13}C[b]
1	CH_2	1.60 (d, $^2J_{H,H}$ = 13.4, 1 H, H_α)	48.7
		1.18 (d, $^2J_{H,H}$ = 13.4, 1 H, H_β)	
2	C_q	–	64.9
3	CH	1.61 (m, 1 H)	44.6
4	CH_2	1.39–1.43 (m, 1 H, H_α)	33.4
		1.16–1.21 (m, 1 H, H_β)	
5	CH_2	2.00 (ddddd, $^2J_{H,H}$ = 13.0, $^3J_{H,H}$ = 10.1; 6.8; 6.4, $^4J_{H,H}$ = 2.7, 1 H, H_β)	29.1
		1.54–1.58 (m, 1 H, H_α)	
6	CH	3.05 (d, $^5J_{H,H}$ = 9.2, 1 H)	55.3
7	C_q	–	148.8
8	CH	5.97 (dd, $^3J_{H,H}$ = 2.5, $^4J_{H,H}$ = 1.1, 1 H)	154.9
9	CH	2.52 (dddd, $^3J_{H,H}$ =5.8; 5.5; 2.5, $^5J_{H,H}$ = 9.0, 1 H)	60.0
10	CH_2	1.50 (ddd, $^2J_{H,H}$ = 12.7, $^3J_{H,H}$ = 9.8, $^4J_{H,H}$ = 1.1, 1 H, H_α)	45.9
		1.03 (dd, $^2J_{H,H}$ = 12.8, $^3J_{H,H}$ = 6.0, 1 H, H_β)	
11	C_q	–	40.6
12	CH_3	0.88 (s, 3 H)	29.3
13	CH_3	0.86 (s, 3 H)	29.1
14	CH	9.59 (s, 1 H)	189.3
15	CH_3	0.76 (d, $^2J_{H,H}$ = 7.2, 3 H)	17.1

[a] Carbon numbering in analogy to the numbering of **4** as shown in Scheme 2 of main text. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, coupling constants *J* are given in Hertz.

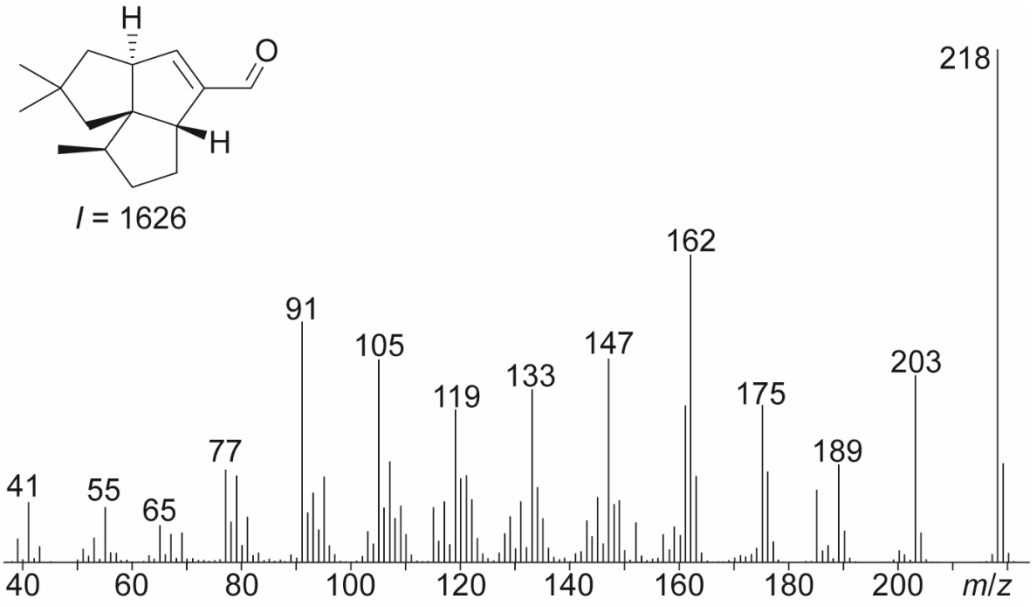


Figure S43. Mass spectrum of pentalenal (**17**).

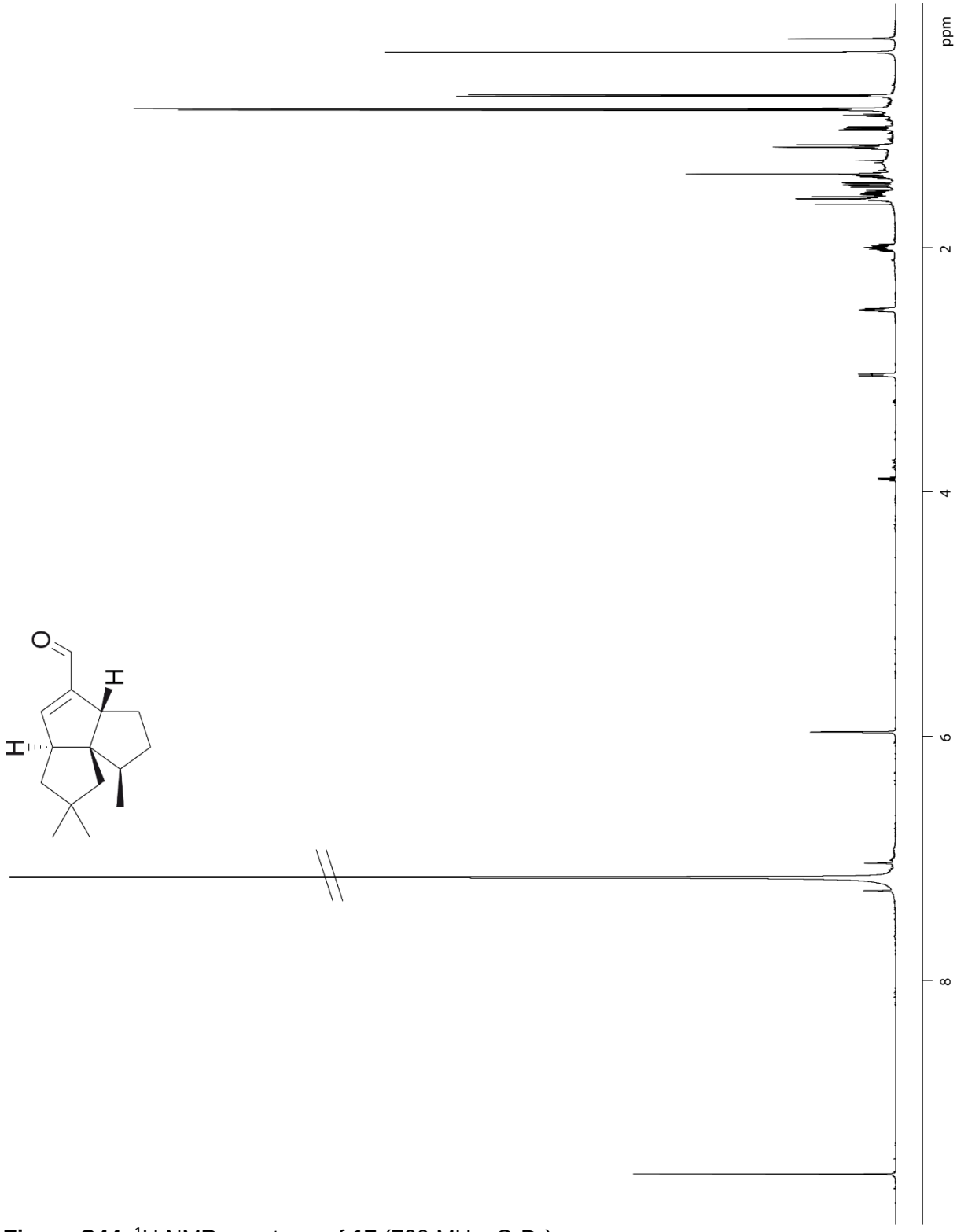


Figure S44. ^{1}H NMR spectrum of **17** (700 MHz, C_6D_6).

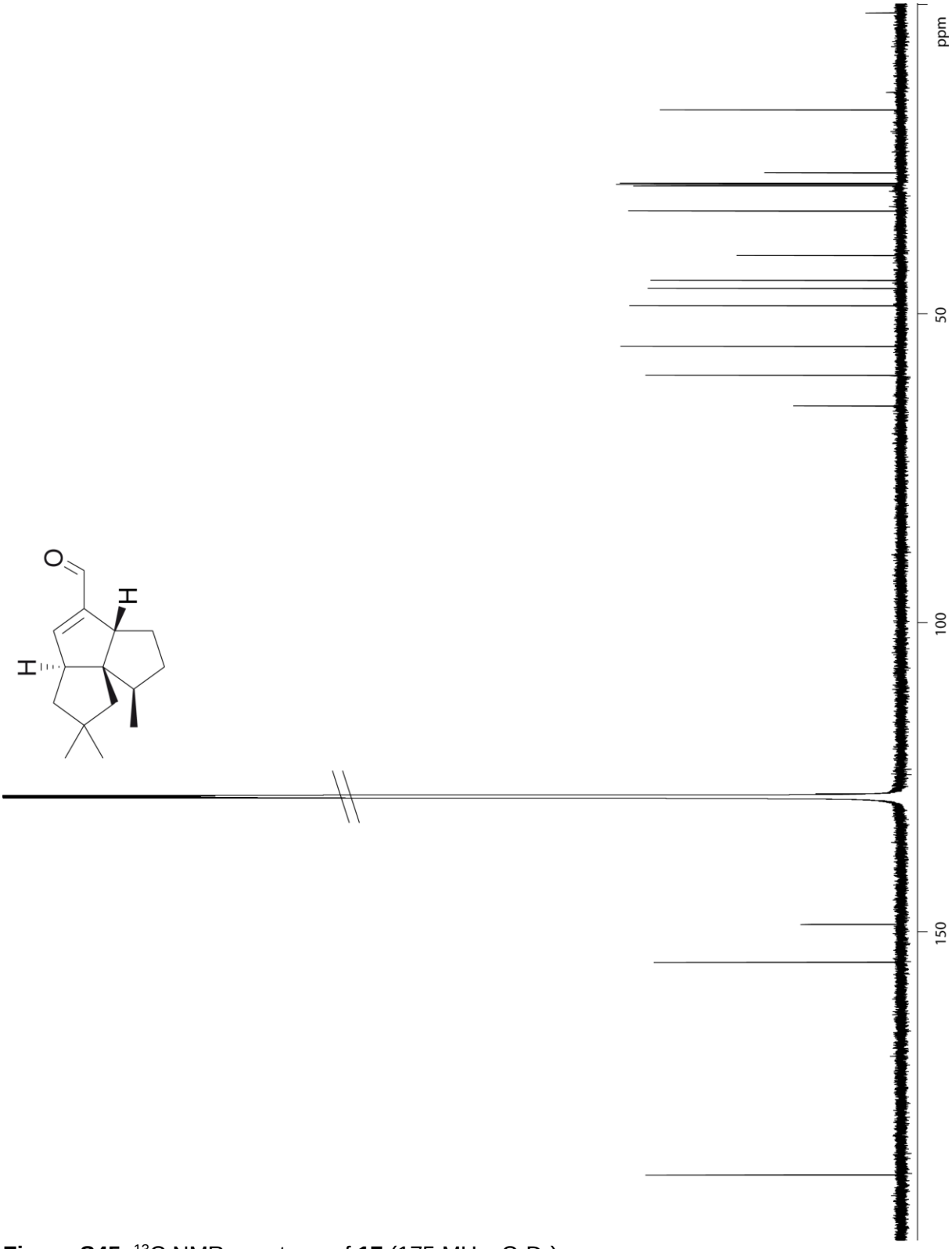


Figure S45. ^{13}C NMR spectrum of **17** (175 MHz, C_6D_6).

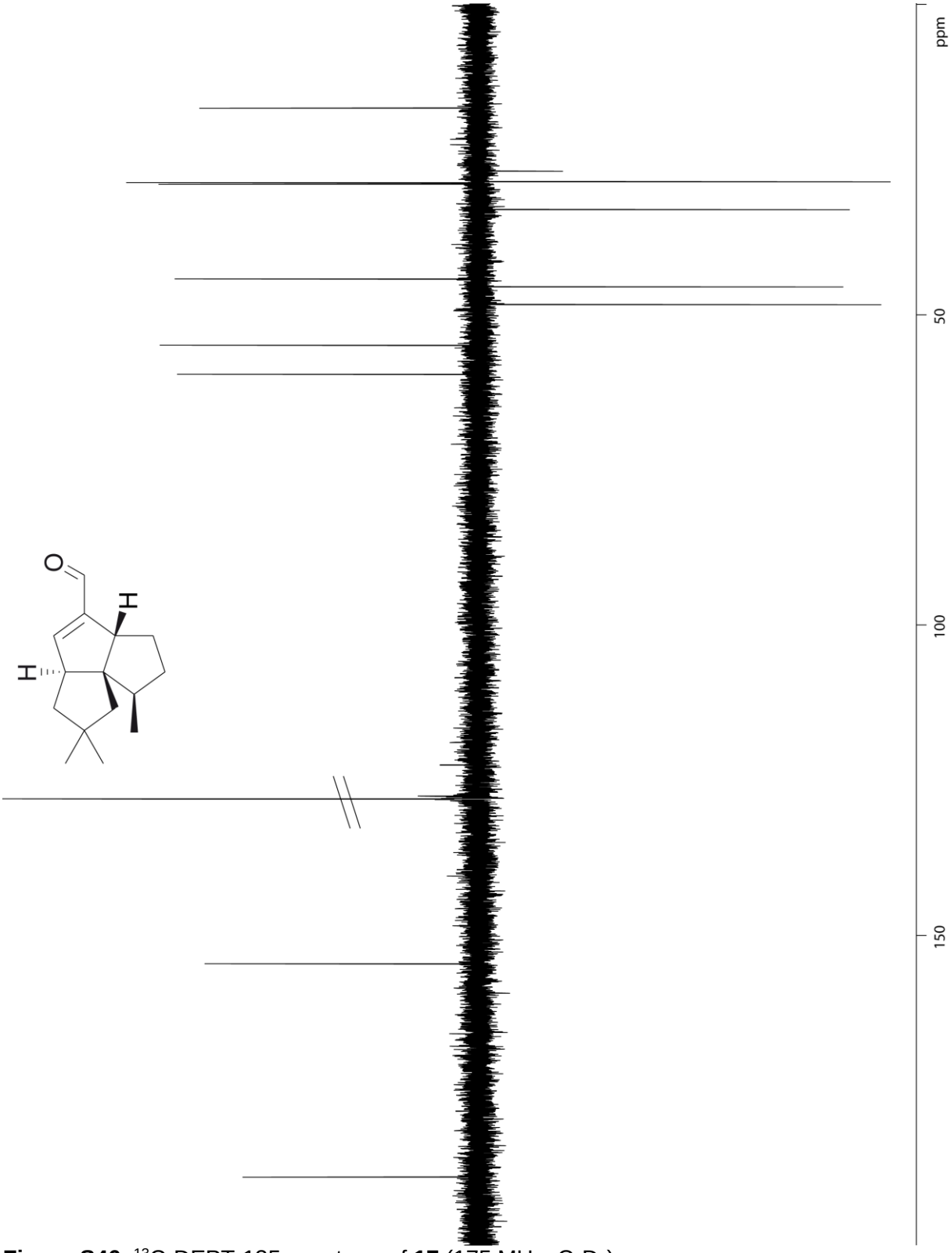


Figure S46. ^{13}C-DEPT-135 spectrum of **17** (175 MHz, C_6D_6).

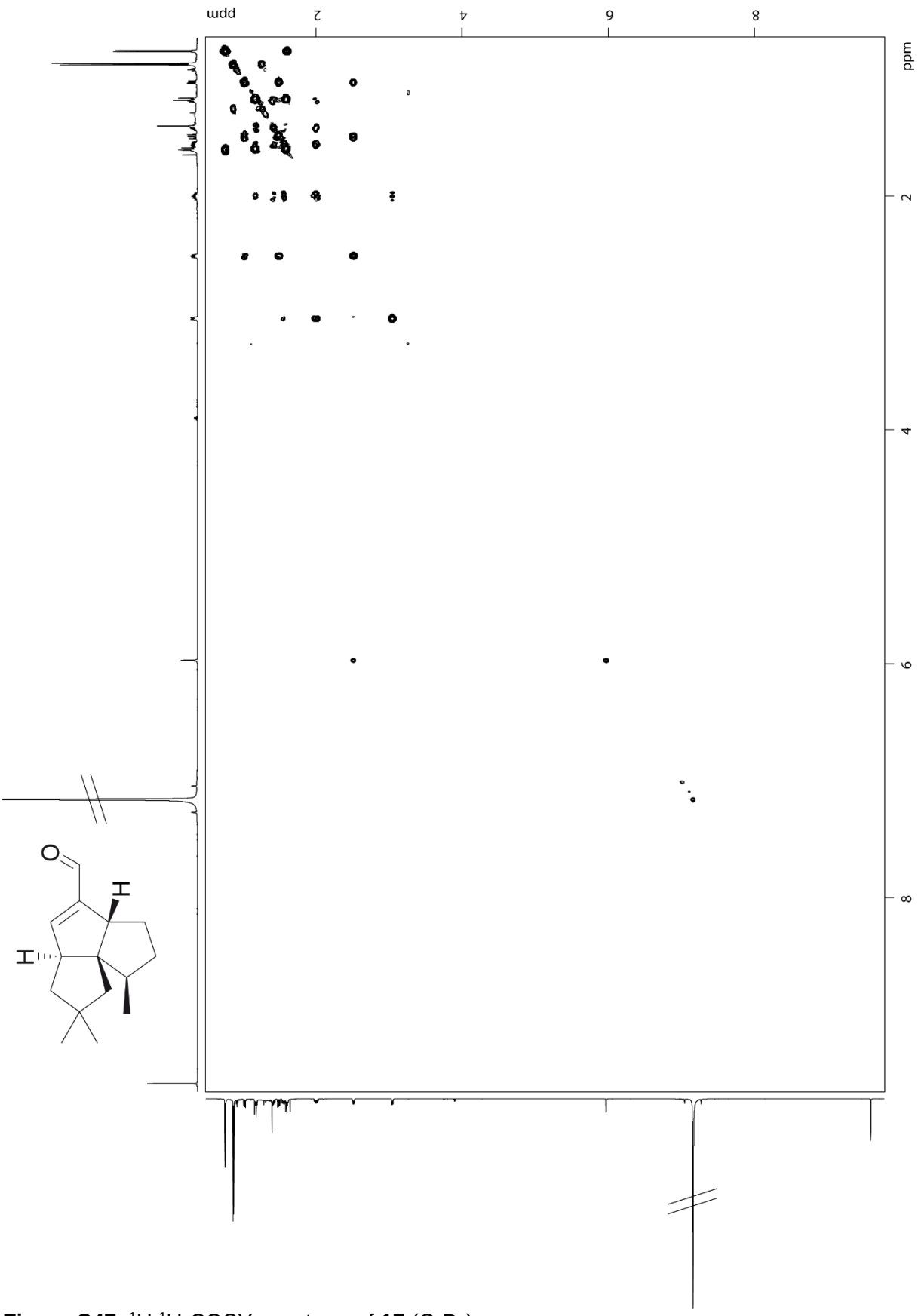


Figure S47. $^{1}H,^{1}H$-COSY spectrum of **17** (C_6D_6).

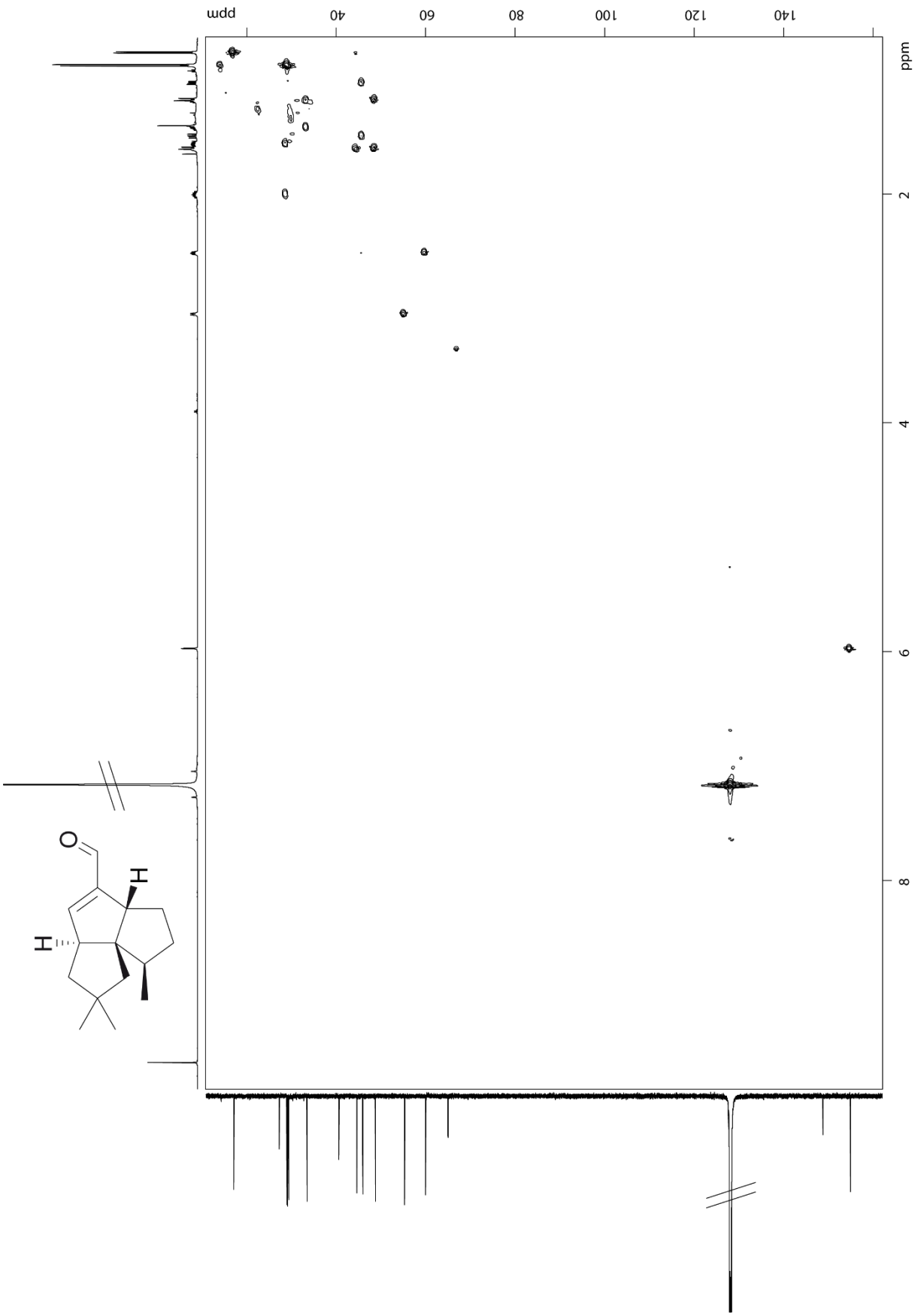


Figure S48. HSQC spectrum of **17** (C_6D_6).

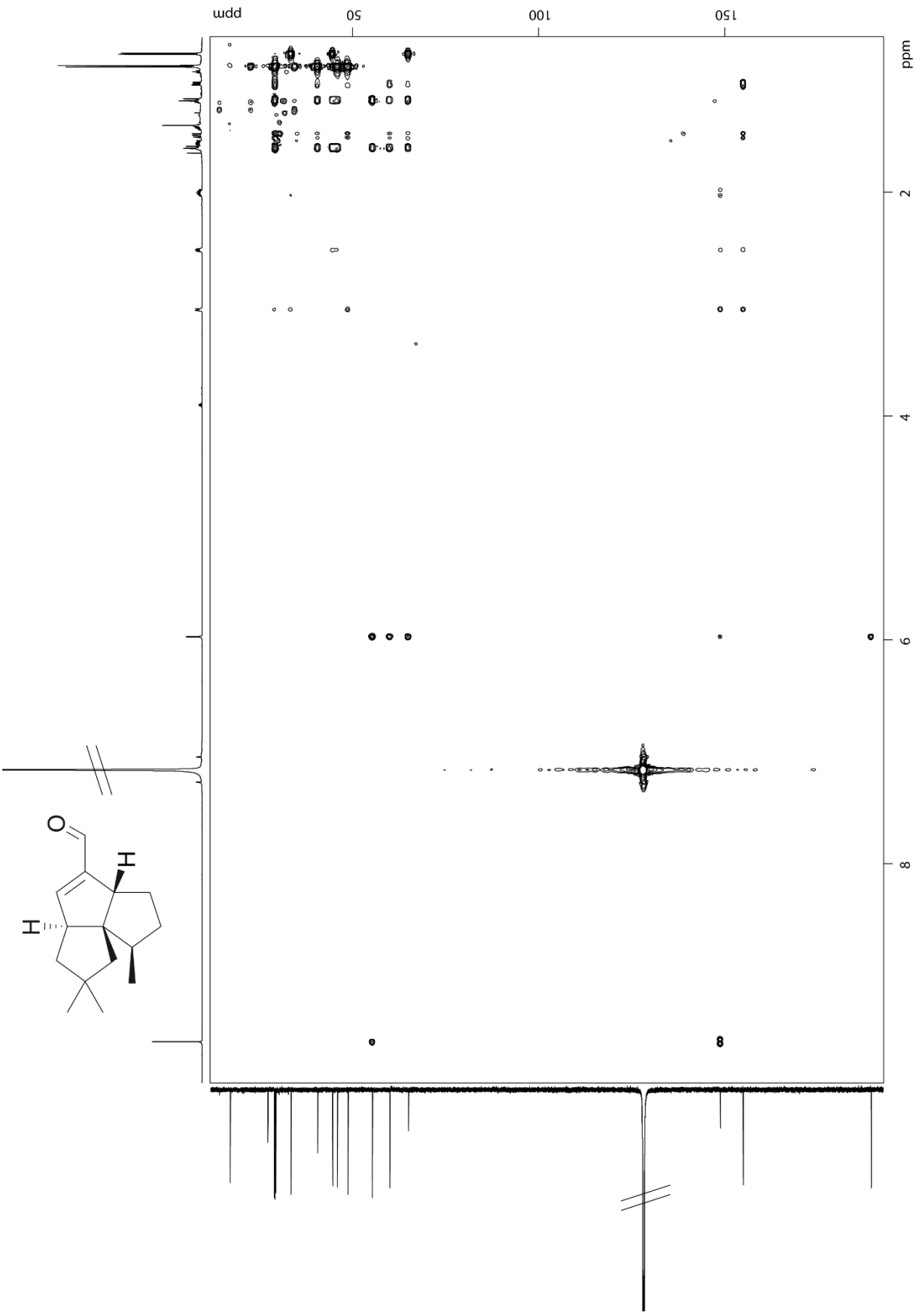


Figure S49. HMBC spectrum of **17** (C_6D_6).

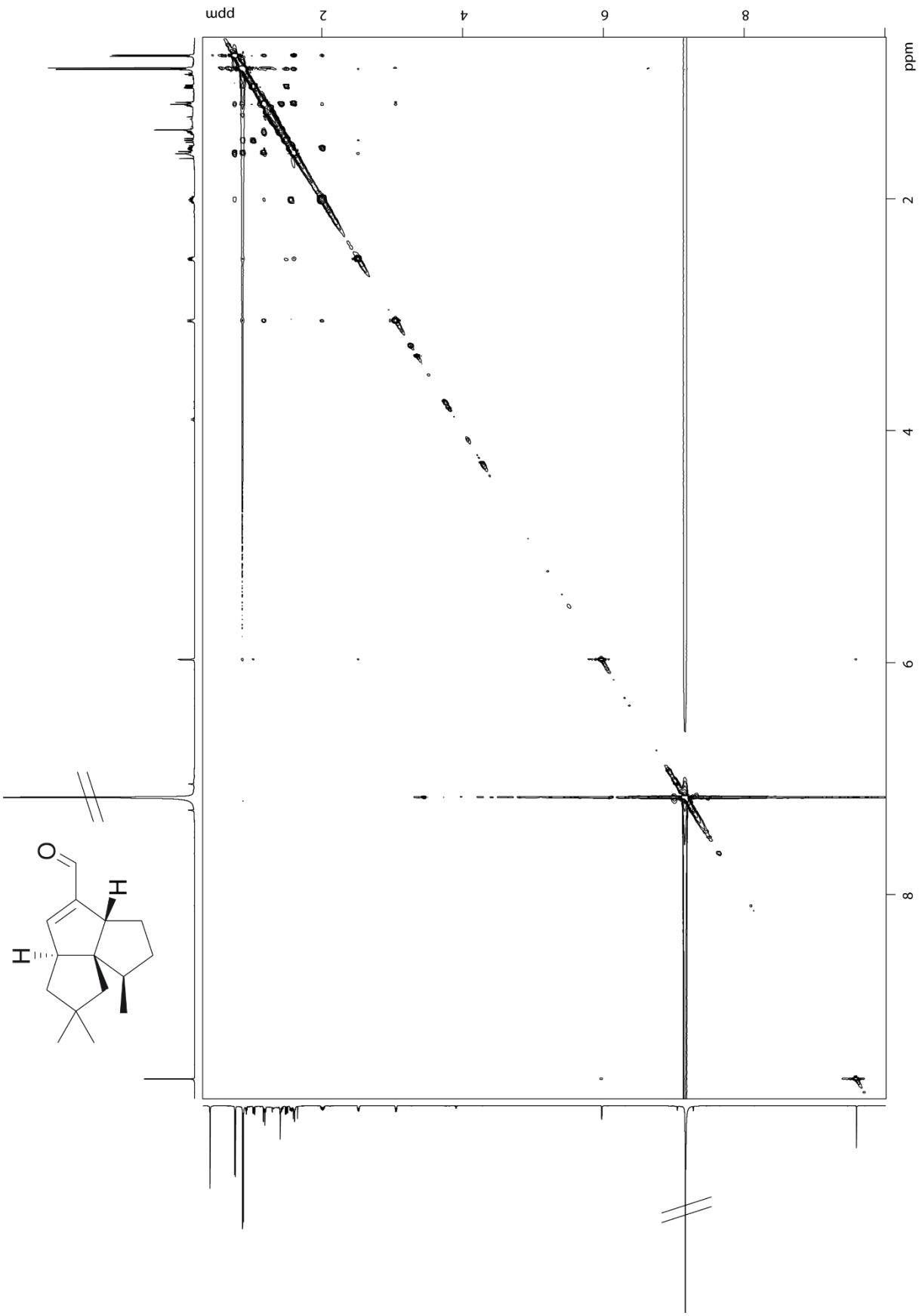


Figure S50. NOESY spectrum of **17** (C_6D_6).

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