Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Gene expression and purification of CS

The gene for casbene synthase (accession number XP002513340) was obtained codon optimised for *Escherichia coli* from Eurofins Genomics (Ebersberg, Germany). The gene was cloned into the expression vector pYE-Express through homologous recombination in yeast as reported previously to obtain plasmid pYE-XP002513340.[1] A preculture of *E. coli* BL21(DE3) transformed with pYE-XP002513340 was grown in LB medium with kanamycin (50 μg/mL) overnight with shaking at 37 °C. The gene expression cultures were inoculated with the preculture (2 %) and grown in LB medium containing kanamycin (50 μg/mL) with shaking at 37 °C until an OD_{600} = 0.4 – 0.6 was reached. After cooling the temperature to 18 °C, the enzyme expression was induced by the addition of IPTG solution (400 mM, 1 ‰). The cultures were shaken at 18 °C for 18 h. The cells were harvested via centrifugation (3500 rpm, 40 min, 4 °C), resuspended in binding buffer (30 mL/L culture; 20 mM Na_2HPO_4, 500 mM NaCl, 20 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C) and lysed by ultrasonication (10x 1 min) on ice. The cell debris was removed by centrifugation (14600 g, 7 min, 4 °C) and the supernatant was loaded on a Ni^{2+}-NTA superflow affinity chromatography column (Qiagen, Venlo, Netherlands) equilibrated with binding buffer. The column was washed with binding buffer (2x Ni^{2+}-NTA volumes) and washing buffer (2x Ni^{2+}-NTA volumes; 20 mM Na_2HPO_4, 500 mM NaCl, 100 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C). The desired protein was eluted with elution buffer (1x Ni^{2+}-NTA volume; 20 mM Na_2HPO_4, 500 mM NaCl, 500 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C).

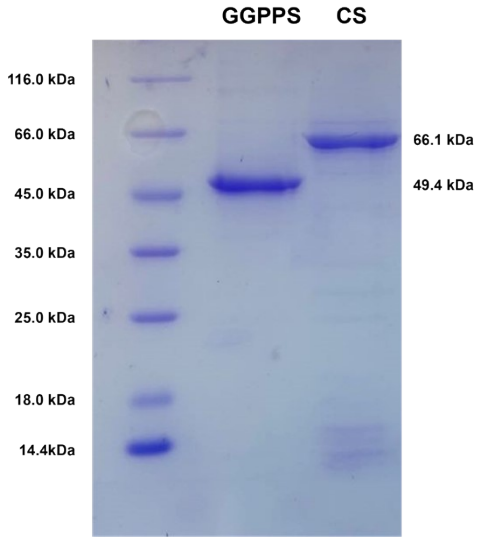


Figure S1. SDS-PAGE analysis of purified recombinant CS and geranylgeranyl diphosphate synthase (GGPPS). GGPPS was obtained as reported before.[2]

Enzyme incubation of GGPP with wildtype CS and compound isolation

Test incubations were carried out with GGPP (1 mg) dissolved in substrate buffer (100 μL; 25 mM NH_4HCO_3) and diluted with incubation buffer (700 μL; 50 mM Tris/HCl, 10 mM $MgCl_2$, 10% glycerol, pH = 8.2) and $MgCl_2$ solution (10 μL, 1 M). Wildtype CS protein solution (200 μL) obtained from 100 mL expression culture was added to the mixture, followed by incubation with shaking at 30 °C overnight. The crude product was extracted with hexane (200 μL), the extract was dried with $MgSO_4$ and directly analysed by GC/MS. Large scale preparations were done by dissolving GGPP (50 mg, 0.1 mmol) in substrate buffer (10 mL). This solution was added dropwise into reaction mixture of protein preparation (20 mL; from 4 L expression culture, 1.25 mg/mL), $MgCl_2$ solution (1 mL, 1 M) and incubation buffer (70 mL). The reaction mixture was stirred overnight at 30 °C. The reaction mixture was extracted with pentane (3x 100 mL), and the extracts were dried with $MgSO_4$ and concentrated in vacuo (600 mbar, 35 °C). Column chromatography on silica gel with

pentane yield pure casbene (**1**).

GC/MS

GC/MS analyses were carried out on a 7890B/5977A series gas chromatography/mass selective detector (Agilent, Santa Clara, CA, USA). The GC was equipped with an HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film; Agilent) and operated using the settings 1) inlet pressure: 77.1 kPa, He at 23.3 mL min^{-1}, 2) injection volume: 1 – 2 μL, 3) temperature program: 5 min at 50 °C then increasing 5 °C min^{-1} to 320 °C, 4) 60 s valve time, and 5) carrier gas: He at 1.2 mL min^{-1}. The MS was operated with settings 1) source: 230 °C, 2) transfer line: 250 °C, 3) quadrupole: 150 °C and 4) electron energy: 70 eV.

HRMS

High resolution mass spectra using APCI were recorded on an Orbitrap XL instrument (Thermo Fisher Scientific, Waltham, MA, USA).

High resolution mass spectroscopy analyses were conducted on a 7890B/7200 series gas chromatography/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 mm film). GC settings were 1) injection volume: 1 μL, 2) temperature program: 5 min at 50 °C, increasing 10 °C min^{-1} to 320 °C, 3) split ratio: 5:1, 60 s valve time and 4) carrier gas flow: He at 1 mL min^{-1}. MS settings were 1) inlet pressure: 83.2 kPa, He flow at 24.6 ml min^{-1}, 2) transfer line temperature: 250 °C, 3) ionization energy: 70 eV.

NMR spectroscopy

NMR spectra were recorded at 298 K on a Bruker (Billerica, MA, USA) 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 ppm; ^{13}C-NMR: δ = 128.06 ppm).[3] Coupling constants are given in Hz.

IR spectroscopy

IR spectra were recorded on a Bruker α infrared spectrometer with a diamond ATR probehead. Peak intensities are given as s (strong), m (medium), w (weak) and br (broad).

Optical rotations

Optical rotations were recorded on a Modular Compact Polarimeter MCP 100 (Anton Paar, Graz, Austria). The temperature setting was 25 °C; the wavelength of the light used was 589 nm (sodium D line); the path-length was 10 cm, the compound concentrations ***c*** are given in g 100 mL^{-1}.

Casbene (1). TLC (pentane): R_f = 0.56. GC (HP5-MS): *I* = 1930. IR (diamond ATR): $\tilde{\nu}$ = 2924 (s), 2854 (s), 2289 (s), 2271 (s), 1566 (s), 1439 (m), 1343 (m), 1259 (m), 1089 (s), 1012 (s), 797 (m), 626 (s), 587 (s), 541(w) cm^{-1}. HR-MS (APCI): calc. for $[C_{20}H_{32}]^+$ *m*/*z* = 272.2499; found: *m*/*z* = 272.2497. Optical rotary power: $[\alpha]_D^{25}$ = –119.8 (*c* 0.37, C_6D_6). NMR data are given in Table S1.

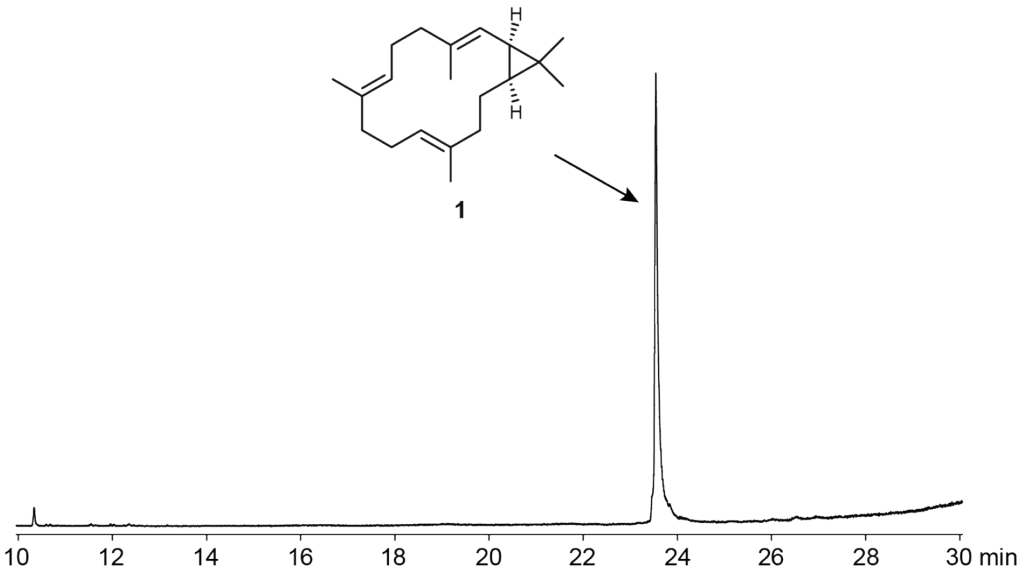
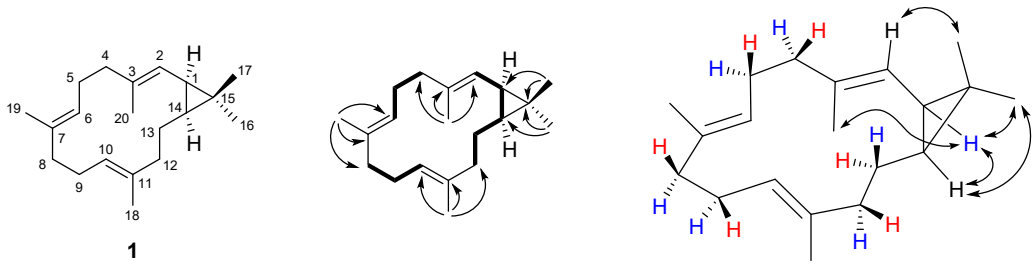


Figure S2. A) Total ion chromatogram of an extract from the incubation of GGPP with CS. B) EI mass spectrum of **1**.



1

Figure S3. Structure elucidation of **1**. Bold: ^{1}H,^{1}H-COSY, single headed arrows: key HMBC, and double headed arrows: key NOESY correlations. The colour code indicates hydrogens for which incorporations of deuterium have been observed from (*R*)-(1-^{13}C,1-^{2}H)IPP and (*E*)-(4-^{13}C,4-^{2}H)IPP (blue) or from (*S*)-(1-^{13}C,1-^{2}H)IPP and (*Z*)-(4-^{13}C,4-^{2}H)IPP (red) (Figures S12 and S13).

Table S1. NMR data of casbene (**1**) in C_6D_6 recorded at 298 K.

C[a]	type	^{13}C[b]	^{1}H[b]
1	CH	26.27	1.30 (dd, *J* = 8.4, 8.4)
2	CH	121.74	5.02 (m)
3	C_q	136.10	–
4	CH_2	39.73	2.19 (m, H)
			2.14 (m, H)
5	CH_2	25.46	2.28 (m, H)
			2.08 (m, H)
6	CH	125.98	5.03 (m)
7	C_q	133.35	–
8	CH_2	39.92	2.13 (m, H)
			1.97 (m, H)
9	CH_2	24.50	2.14 (m, H)
			2.09 (m, H)
10	CH	124.09	5.15 (m)
11	C_q	135.29	–
12	CH_2	40.86	2.23 (m, H)
			1.96 (m, H)
13	CH_2	24.43	1.74 (m, H)
			1.07 (m, H)
14	CH	31.02	0.58 (ddd, *J* = 10.4, 8.8, 1.6)
15	C_q	20.02	–
16	CH_3	29.06	1.06 (s)
17	CH_3	16.02	0.97 (s)
18	CH_3	16.75	1.62 (br s)
19	CH_3	15.87	1.53 (br s)
20	CH_3	16.47	1.66 (br s)

[a] Carbon numbering and colour code for hydrogens as shown in Figure S3. [b] Chemical shifts δ in ppm; multiplicity: s = singlet, d = doublet, m = multiplet, br = broad; coupling constants *J* are given in Hertz.

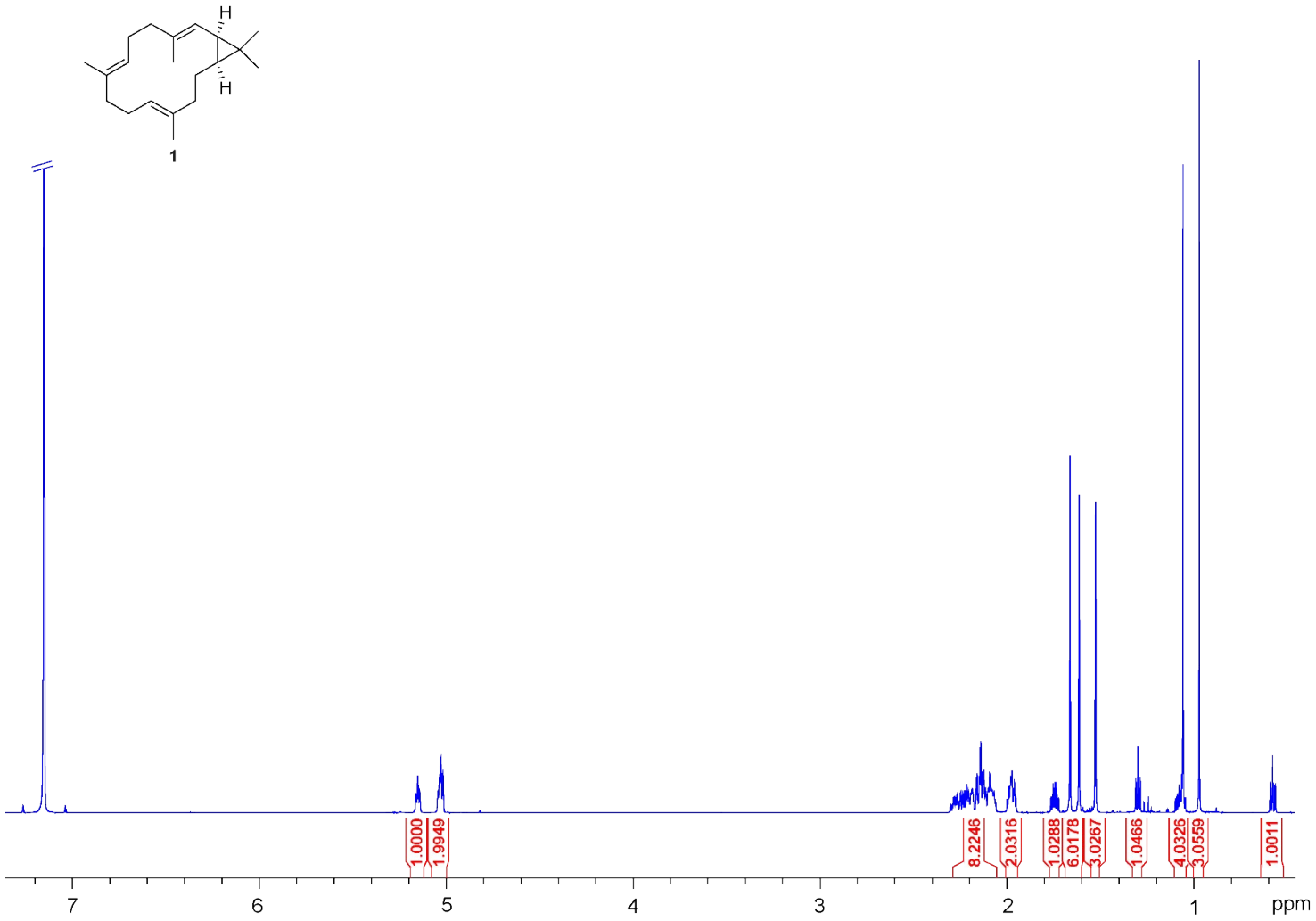


Figure S4. ^{1}H-NMR spectrum of **1** (700 MHz, C_6D_6).

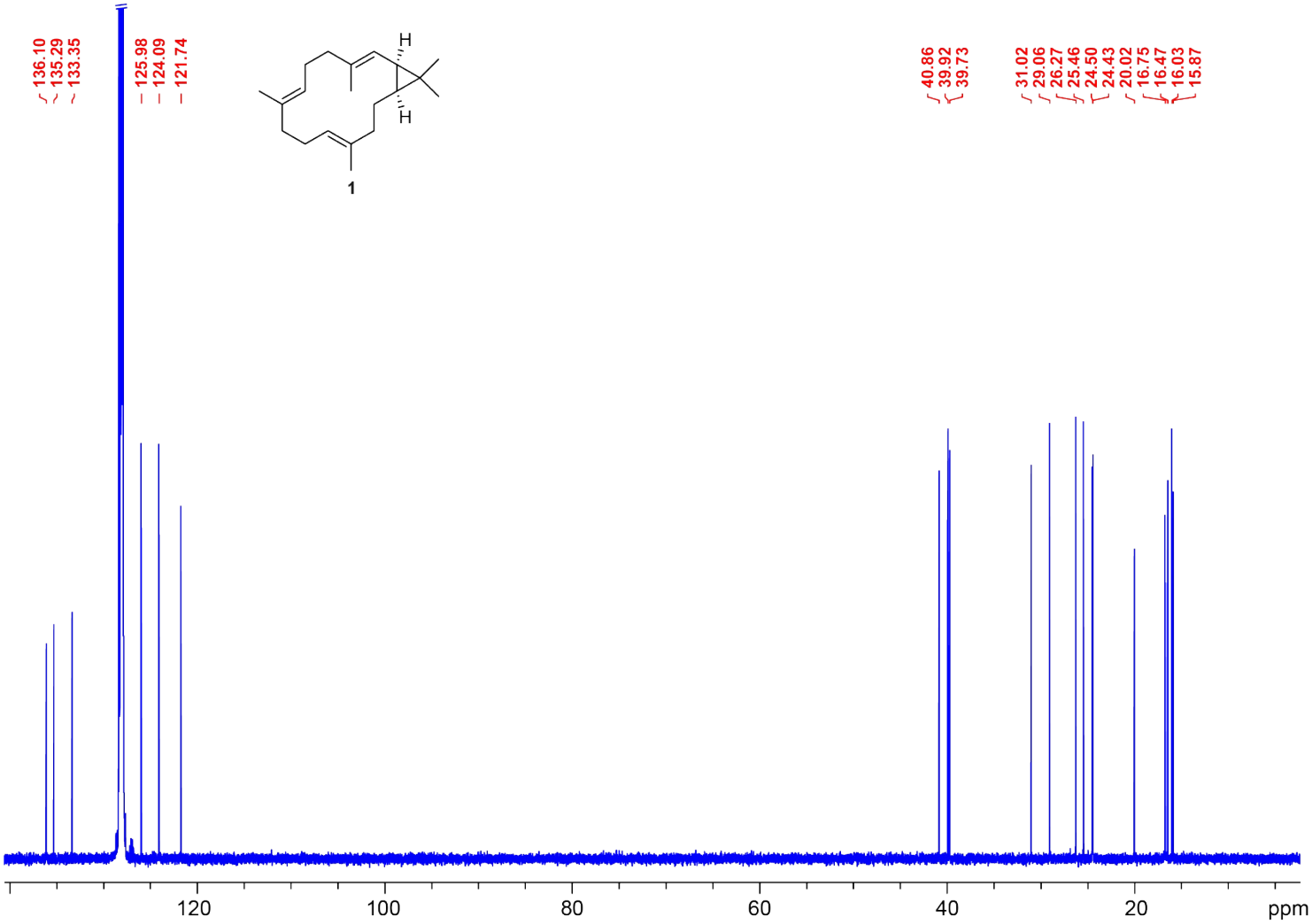


Figure S5. ^{13}C-NMR spectrum of **1** (176 MHz, C_6D_6).

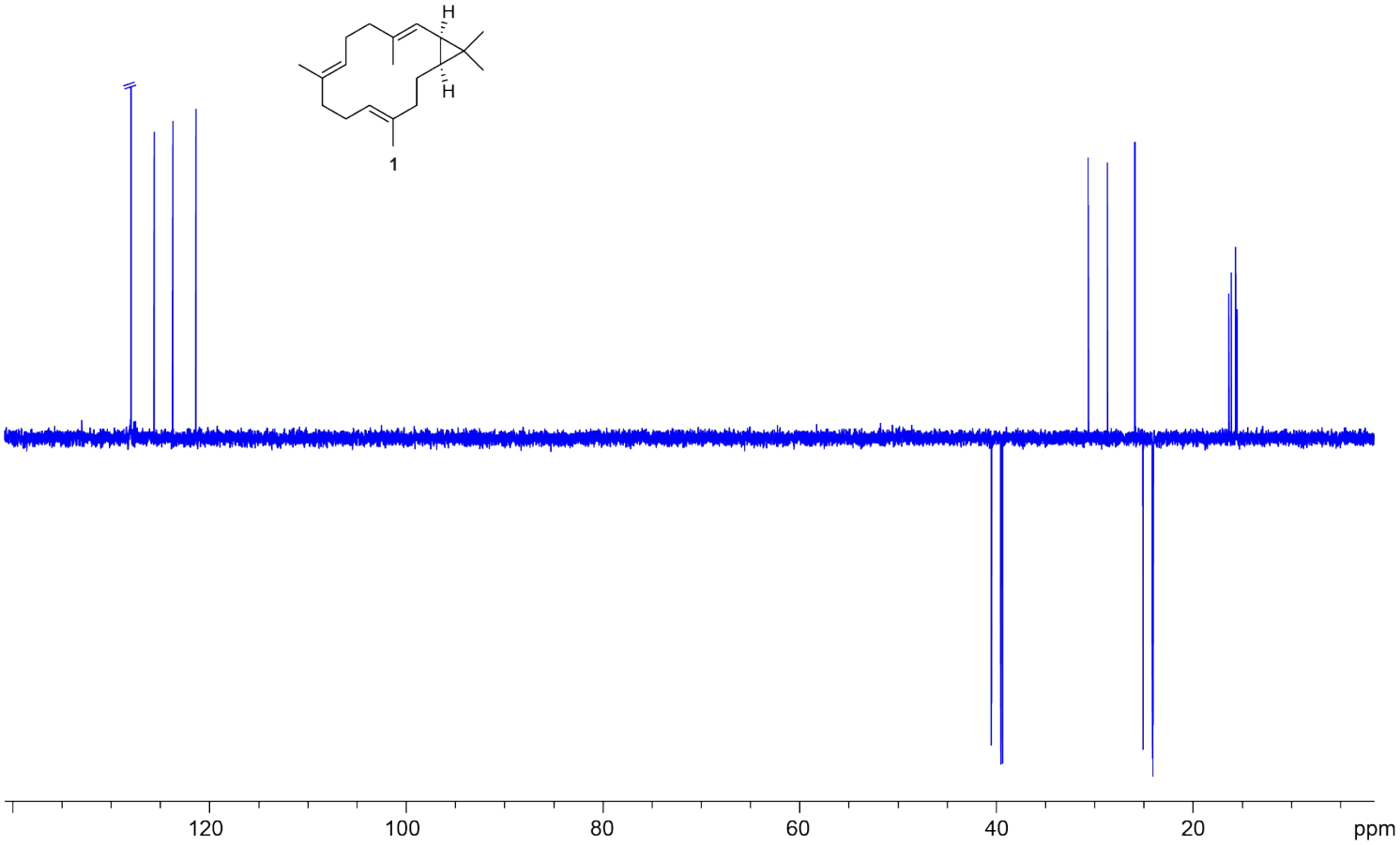


Figure S6. ^{13}C-DEPT spectrum of **1** (176 MHz, C_6D_6).

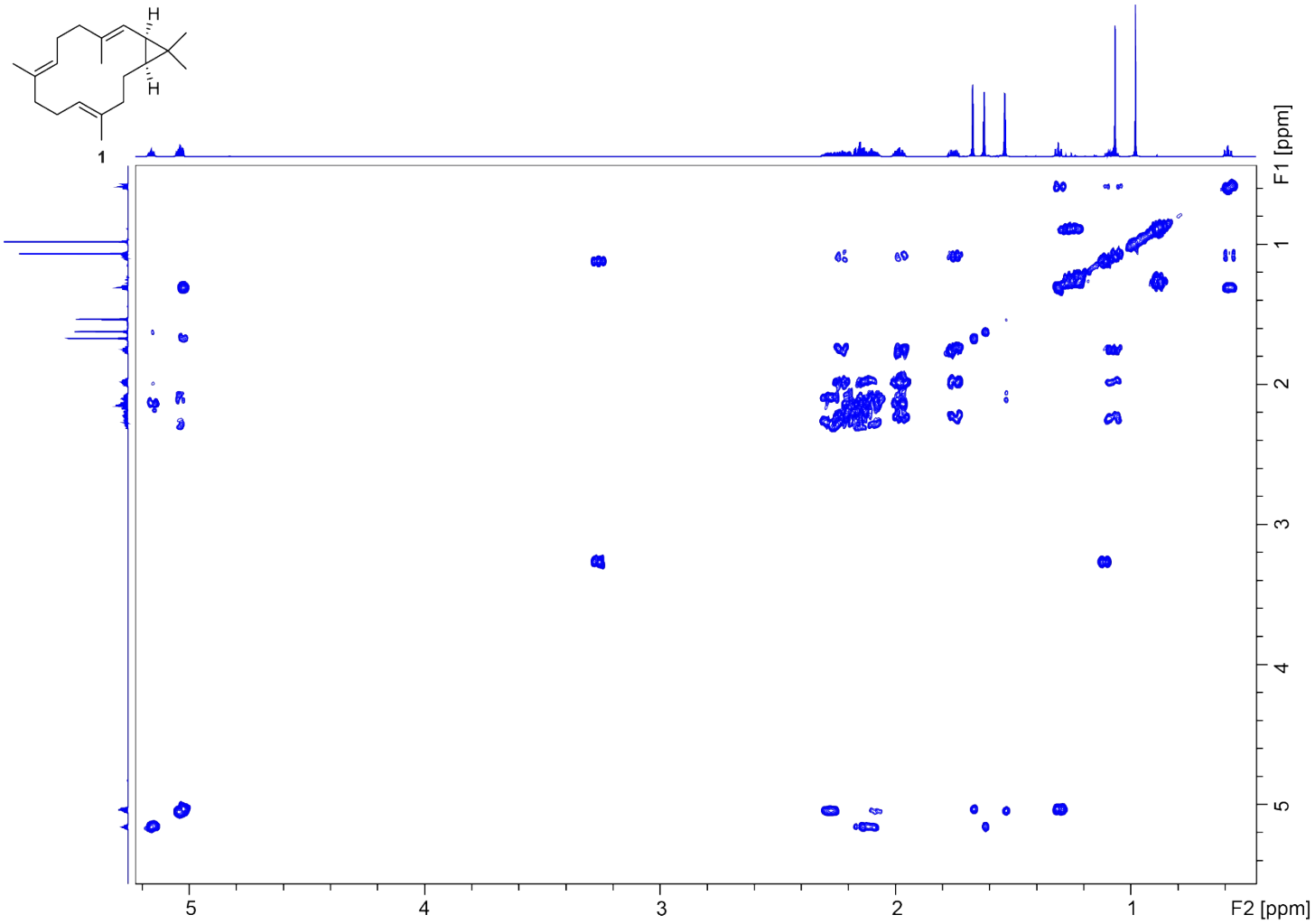


Figure S7. ^{1}H-^{1}H-COSY spectrum of **1** (700 MHz, C_6D_6).

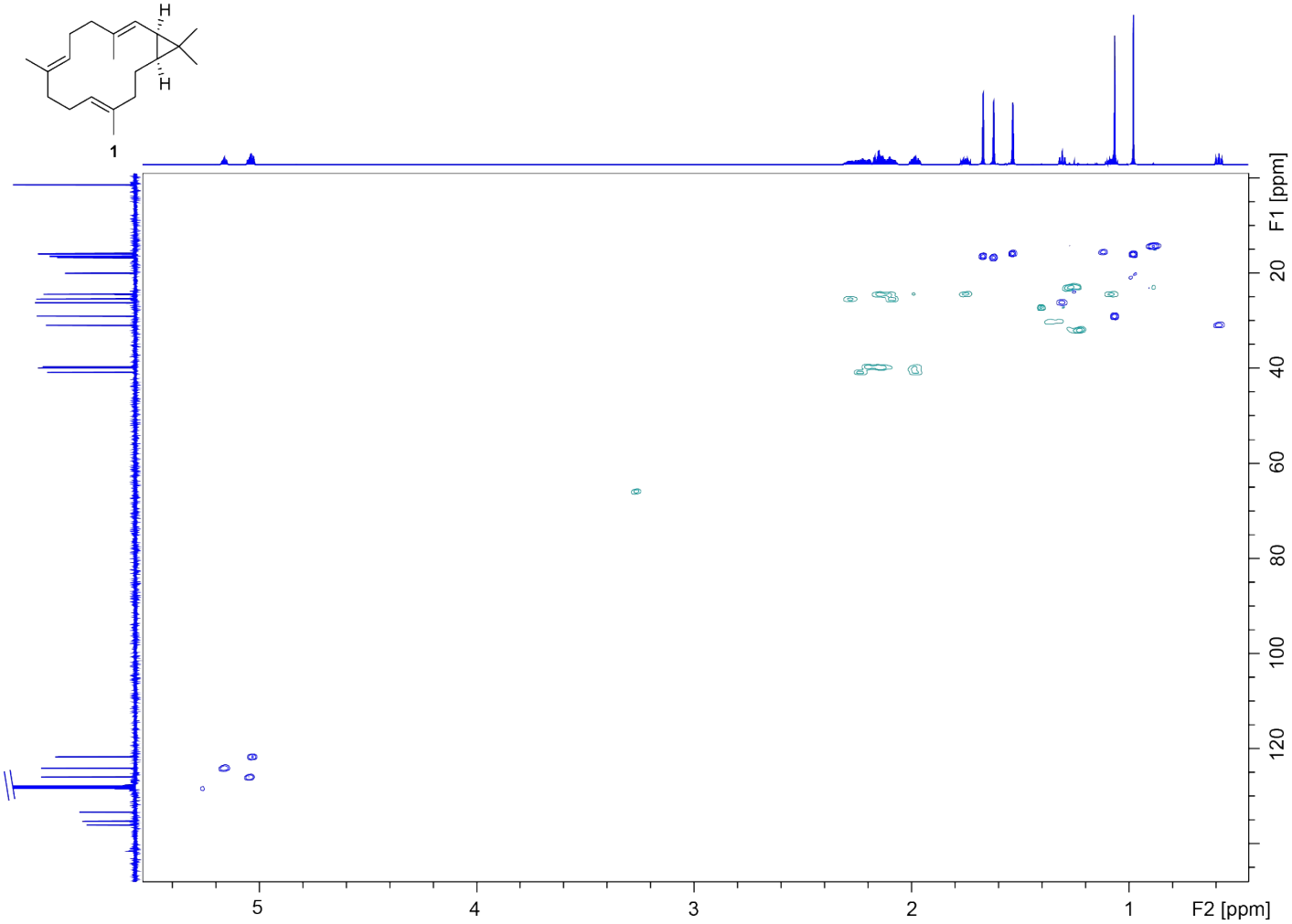


Figure S8. HSQC spectrum of **1** (C_6D_6).

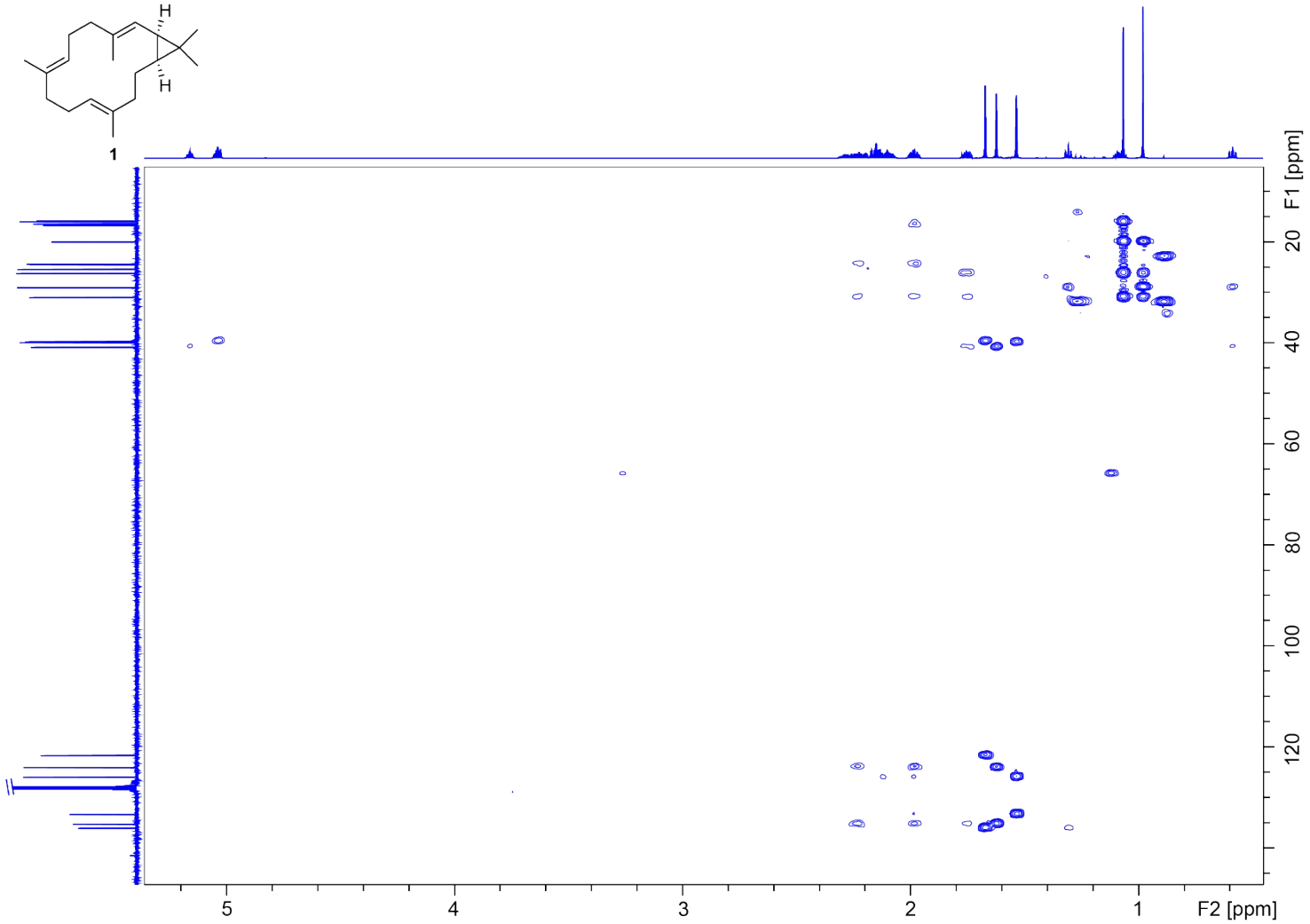


Figure S9. HMBC spectrum of **1** (C_6D_6).

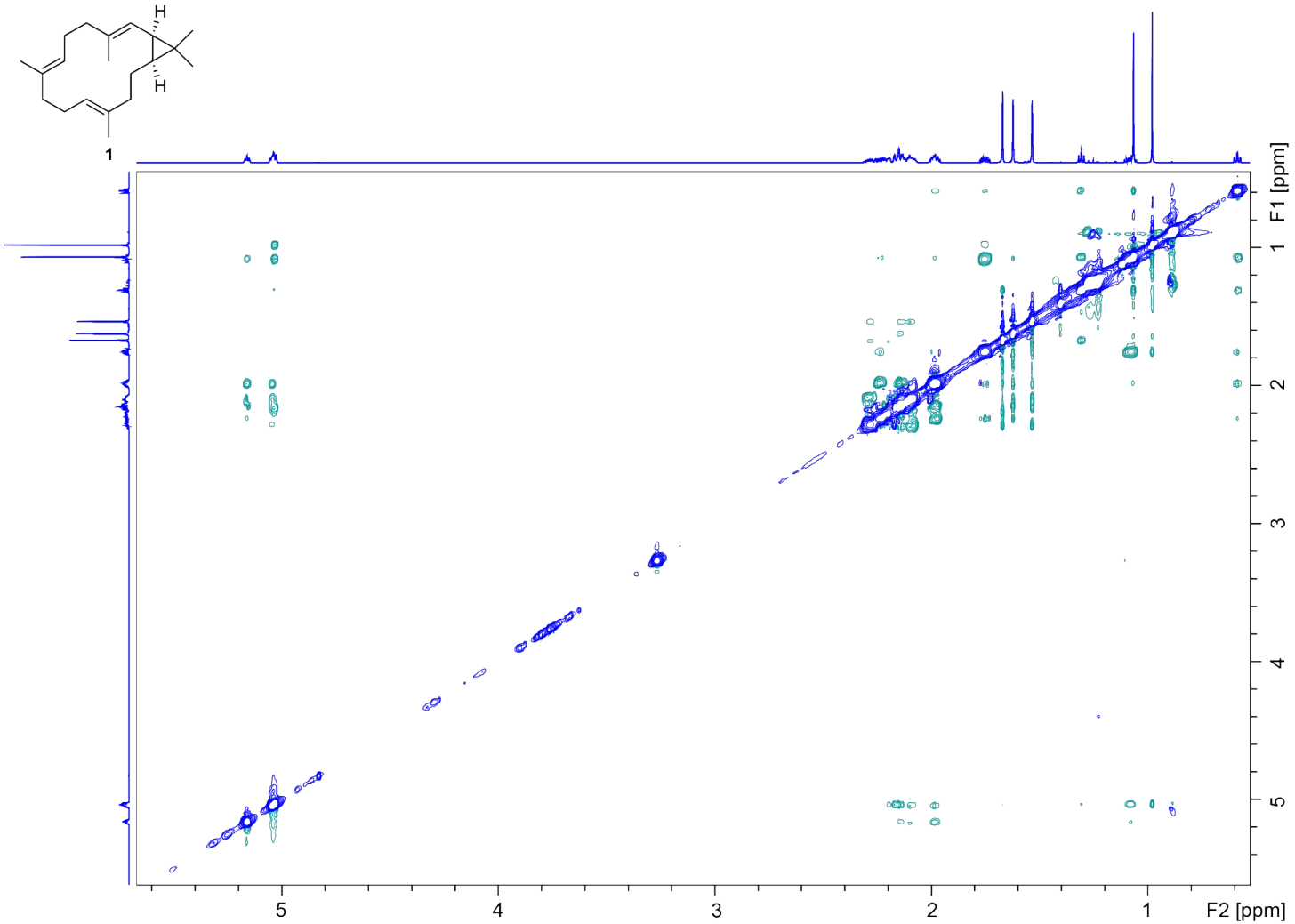


Figure S10. NOESY spectrum of **1** (C_6D_6).

Isotopic labelling experiments

Isotopic labelling experiments were performed with the substrates and enzymes as listed in Table S2.

Isotopic labelling experiments were performed with ca. 1 mg labelled GGPP or its precursors in aqueous NH_4HCO_3 (1 mL; 25 mM), incubation buffer (up to 10 mL) and purified enzyme solutions (GGPPS: 2 mL, CS: 2.5 mL, IDI: 1.5 mL) as listed in Table S2. The reaction mixtures were incubated at 30 °C overnight, and the products were extracted with C_6D_6 (200 μL, three times) or hexane (200 μL), and then analysed by NMR and or GC/MS.

Table S2. Labelling experiments with CS.

entry	substrate	enzymes	results shown in
1	(*R*)-(1-^{13}C,1-^{2}H)IPP[4]	IDI,[4] GGPPS,[2] CS	Figures S11, S12, S35
2	(*S*)-(1-^{13}C,1-^{2}H)IPP[4]	IDI, GGPPS, CS	Figures S11, S12, S35
3	DMAPP + (*E*)-(4-^{13}C,4-^{2}H)IPP[5]	GGPPS, CS	Figures S13, S35
4	DMAPP + (*Z*)-(4-^{13}C,4-^{2}H)IPP[5]	GGPPS, CS	Figures S13, S35
5	(*R*)-(1-^{2}H)iso-GGPP	CS	Figure S31
6	(*S*)-(1-^{2}H)iso-GGPP	CS	Figure S31
7	iso-FPP + (*R*)-(1-^{2}H)IPP[6]	GGPPS, CS	Figure S32
8	iso-FPP + (*S*)-(1-^{2}H)IPP[6]	GGPPS, CS	Figure S32
9	(1-^{13}C)GGPP[7]	CS	Figures S33, S34
10	(2-^{13}C)GGPP[4]	CS	Figures S33, S34
11	(3-^{13}C)GGPP[7]	CS	Figures S33, S34
12	(4-^{13}C)GGPP[7]	CS	Figures S33, S34
13	(1-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
14	(2-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
15	(3-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
16	(4-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
17	(1-^{13}C)GPP + IPP	GGPPS, CS	Figures S33, S34
18	(6-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
19	(7-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
20	(8-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
21	(9-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
22	(10-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
23	(11-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
24	(12-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
25	(9-^{13}C)GPP[9] + IPP	GGPPS, CS	Figures S33, S34
26	(14-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
27	(15-^{13}C)FPP[8] + IPP	GGPPS, CS	Figures S33, S34
28	(20-^{13}C)GGPP[4]	CS	Figures S33, S34

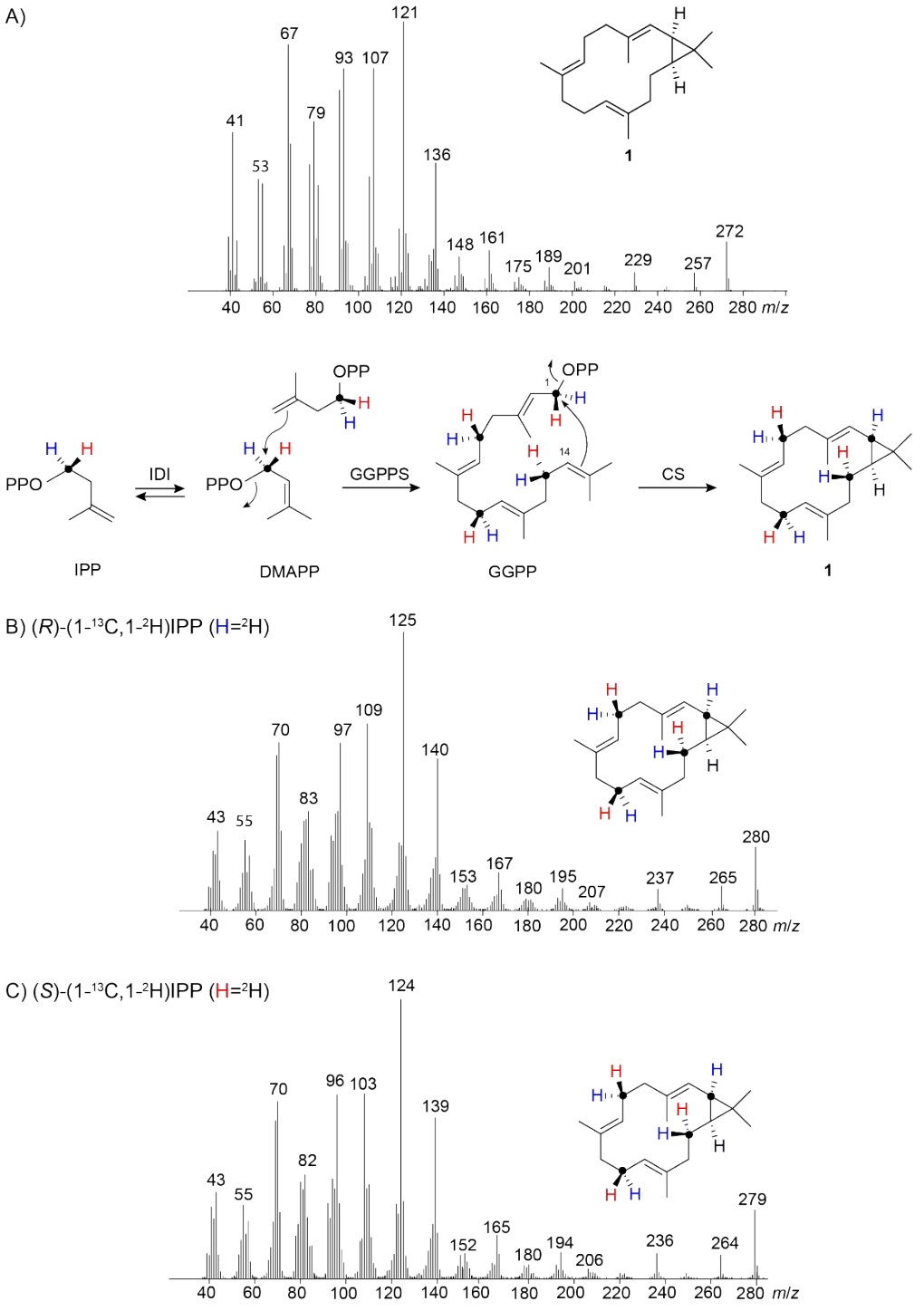


Figure S11. Conversion of (*R*)- and (*S*)-(1-^{13}C,1-^{2}H)IPP with IDI, GGPPS and CS. EI mass spectra of A) unlabelled **1**, B) labelled **1** obtained from (*R*)-(1-^{13}C,1-^{2}H)IPP (blue H = ^{2}H), and C) from (*S*)-(1-^{13}C,1-^{2}H)IPP (red H = ^{2}H), revealing the loss of the 1-*pro*-*S* hydrogen of GGPP in the cyclisation to **1**.

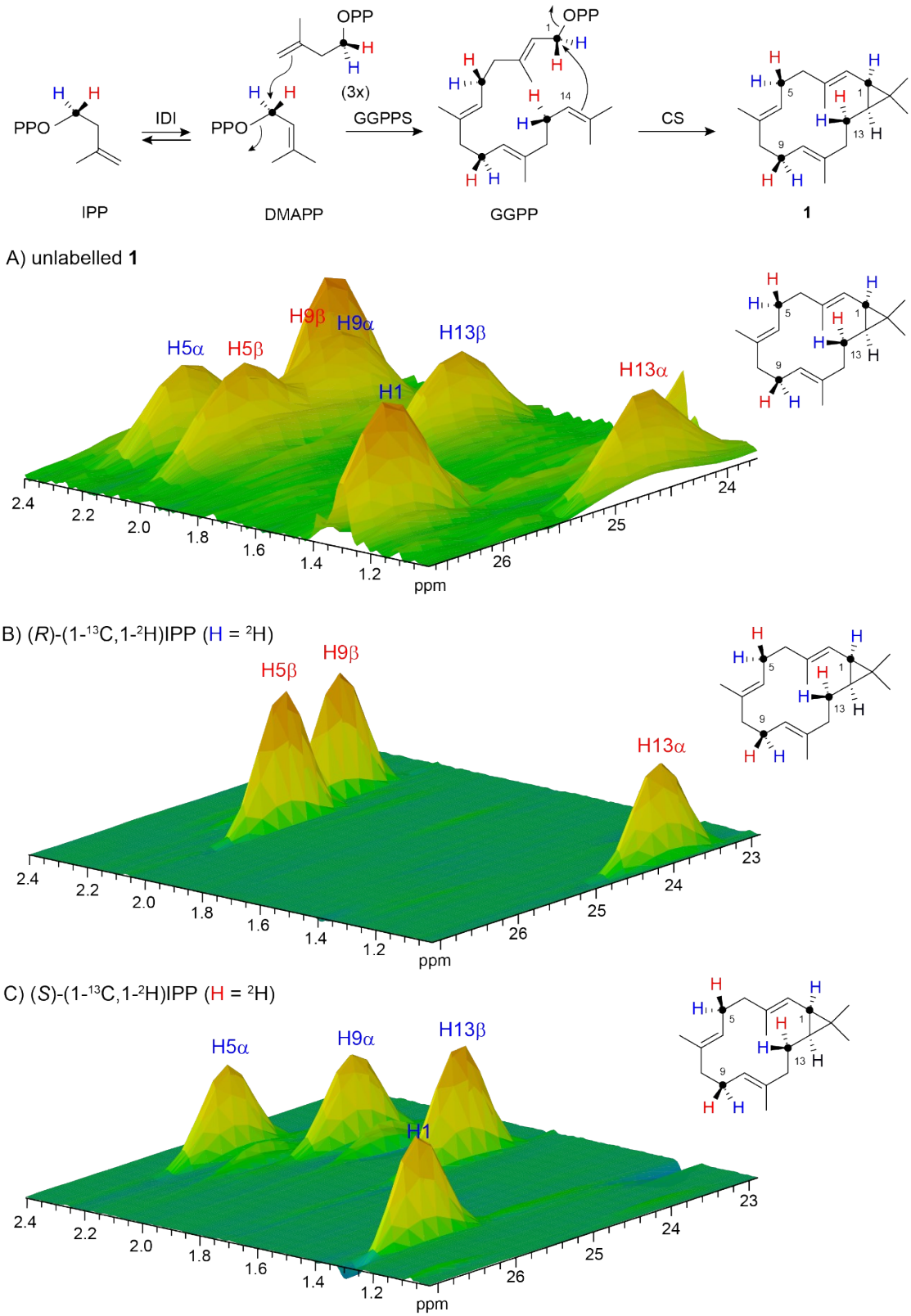


Figure S12. HSQC spectra of A) unlabelled **1**, B) labelled **1** obtained from (*R*)-(1-^{13}C,1-^{2}H)IPP with IDI, GGPPS and CS (blue H = ^{2}H), and C) labelled **1** obtained from (*S*)-(1-^{13}C,1-^{2}H)IPP with IDI, GGPPS and CS (red H = ^{2}H). The HSQC signals are strongly enhanced for hydrogens attached to the ^{13}C-labelled carbons C1, C5, C9 and C13, while the crosspeaks for stereoselective deuterations at these carbons are eradicated.

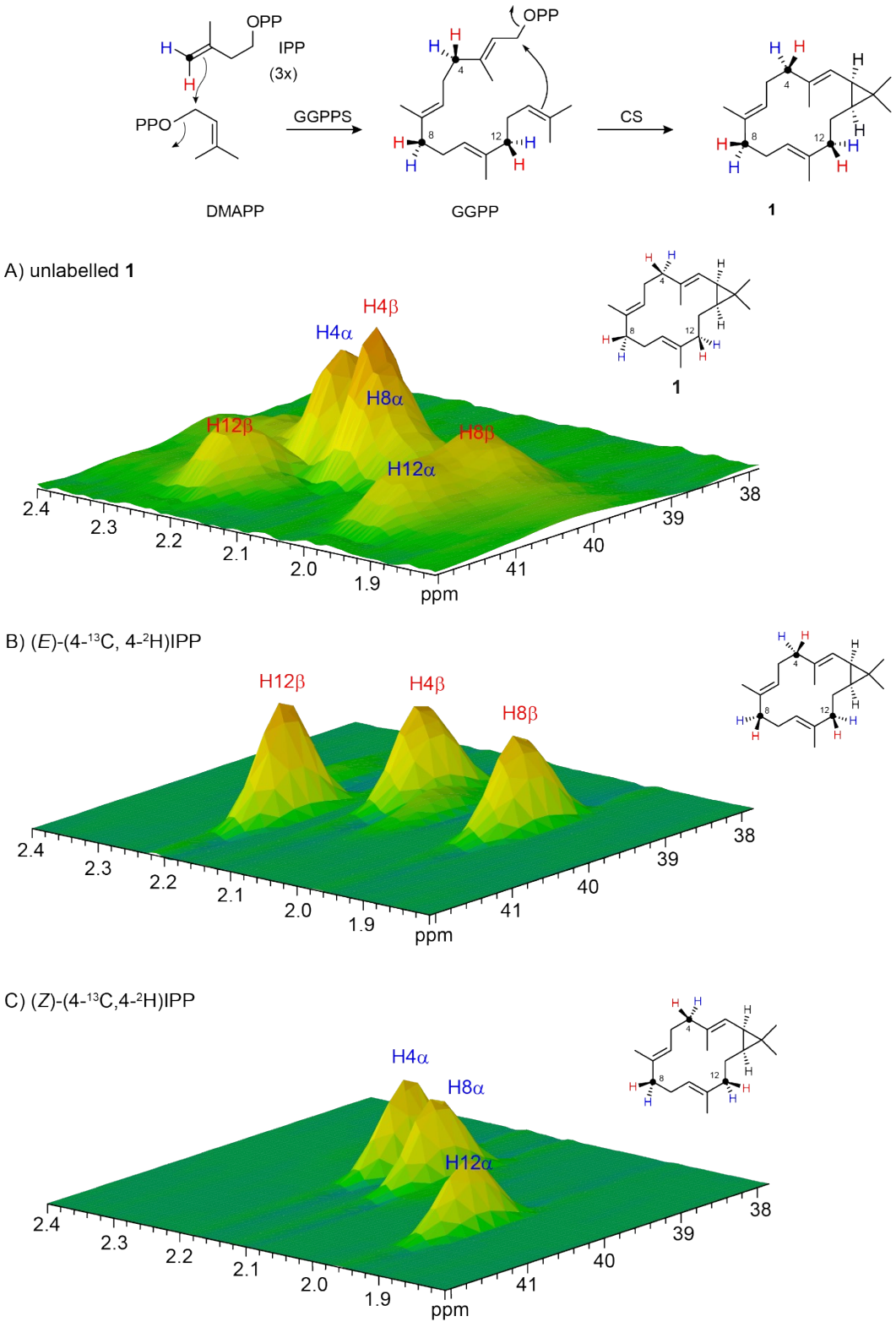


Figure S13. HSQC spectra of A) unlabelled **1**, B) labelled **1** obtained from DMAPP and (*E*)-(4-^{13}C,4-^{2}H)IPP with GGPPS and CS, and C) labelled **1** obtained from DMAPP and (*Z*)-(4-^{13}C,4-^{2}H)IPP with GGPPS and CS. The HSQC signals are strongly enhanced for hydrogens attached to the ^{13}C-labelled carbons C4, C8 and C12, while the crosspeaks for stereoselective deuterations at these carbons are eradicated.

Synthesis of iso-FPP and iso-GGPP

General procedure for synthesis of allyl bromides
To a cooled (0 °C) solution of alcohol (1.00 eq) in Et_2O (3 mL mmol^{-1}) PBr_3 (0.40 eq) was added dropwise. The mixture was stirred for 1 h at 0 °C and was transferred directly to an ice/water mixture. The aqueous layer was extracted with Et_2O three times and the organic layers were dried with $MgSO_4$, concentrated under reduced pressure and the bromides were directly used for subsequent reactions without purification.

General procedure for synthesis of alcohols 6a and 6b
To a solution of *n*-Buli (1.6 M in hexane, 2.00 eq) in hexane (0.75 mL mmol^{-1}, –15 °C) was added TMEDA (2.00 eq) and the mixture was stirred for 0.5 h under –23 °C. The mixture was warmed to room temperature and 3-methylbut-3-en-1-ol (1.00 eq) was added dropwise. After 6 h stirring, HMPA (3 mL) and a solution of the corresponding allyl bromide (1.00 eq) in Et_2O was added and the mixture was stirred at –78 °C for 2 h. The reaction was quenched by the addition of 1 M HCl (60 mL) and extracted with Et_2O (140 mL, three times). The combined organic layers were dried with $MgSO_4$, evaporated under reduce pressure and subjected to column chromatography (pentane/Et_2O, 3:1) to yield the desired alcohols.

7-Methyl-3-methyleneoct-6-en-1-ol (6a): 4.54 g, 29.5 mmol, 38%. TLC (pentane/Et_2O, 1:1): R_f = 0.50. GC (HP5-MS): *I* = 1219. EI-MS (70 eV): *m*/*z* (%) = 154 (0.05), 136 (0.5), 121 (1), 111 (6), 93 (4), 79 (2), 69 (24), 53 (3), 41 (17). ^{1}H-NMR (C_6D_6, 500 MHz): 5.15 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.83 (m, 1H), 4.77 (m, 1H), 3.48 (m, 2H), 2.10 (m, 4H), 1.97 (m, 2H), 1.65 (d, $^4J_{H,H}$ = 1.2, 3H), 1.52 (br s, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 146.48 (C_q), 131.54 (C_q), 124.57 (CH), 111.45 (CH_2), 60.78 (CH_2), 39.74 (CH_2), 36.36 (CH_2), 26.80 (CH_2), 25.82 (CH_3), 17.74 (CH_3) ppm.

(*E*)-7,11-Dimethyl-3-methylenedodeca-6,10-dien-1-ol (6b): 3.51 g, 15.8 mmol, 36%. TLC (pentane/Et_2O, 1:1): R_f = 0.48. GC (HP5-MS): *I* = 1696. EI-MS (70 eV): *m*/*z* (%) = 207 (0.4), 179 (1), 151 (1), 136 (2), 121 (2), 107 (4), 93 (5), 81 (6), 69 (12), 55 (3), 41 (10).^{1}H-NMR (C_6D_6, 500 MHz): 5.22 (m, 2H), 4.83 (q, $^4J_{H,H}$ = 1.5, 1H), 4.78 (m, 1H), 3.48 (m, 2H), 2.12 (m, 8H), 1.98 (m, 2H), 1.68 (d, $^4J_{H,H}$ = 1.1, 3H), 1.57 (m, 6H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 146.46 (C_q), 135.38 (C_q), 131.22 (C_q), 124.90 (CH), 124.48 (CH), 111.50 (CH_2), 60.77 (CH_2), 40.18 (CH_2), 39.74 (CH_2), 36.35 (CH_2), 27.18 CH_2), 26.69 (CH_2), 25.87 (CH_3), 17.77 (CH_3), 16.12 (CH_3) ppm.

General procedure for synthesis of alkyl iodides 7a and 7b
A solution of imidazole (1.20 eq) and PPh_3 (1.20 eq) in DCM (5 mL mmol^{-1}) was mixed with I_2 (1.20 eq) and the corresponding alcohol **6a** or **6b** (1.00 eq) subsequently. After stirring for 4 h at room temperature, the reaction mixture was quenched by the addition of sat. aqueous NH_4Cl and then extracted with Et_2O (three times). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was taken up in pentane, the precipitate was filtered off, and pentane was removed in vacuo. The product was purified by column chromatography (cyclohexane) to yield the desired iodides as colorless oils.

8-Iodo-2-methyl-6-methyleneoct-2-ene (7a): 3.11 g, 11.8 mmol, 61%. TLC (pentane): R_f = 0.50. GC (HP5-MS): *I* = 1401. EI-MS (70 eV): *m*/*z* (%)= 222 (1), 221 (8), 155 (1), 141 (1), 127 (2), 109 (7), 93 (4), 69 (9), 41 (9).^{1}H-NMR (C_6D_6, 500 MHz): 5.09 (thept, $^3J_{H,H}$ = 7.0, $^4J_{H,H}$ = 1.4, 1H), 4.78 (m, 1H), 4.62 (m, 1H), 2.82 (t, $^3J_{H,H}$ = 7.4, 2H), 2.29 (t, $^3J_{H,H}$ = 7.4, 2H), 2.00 (m, 2H), 1.83 (m, 2H), 1.65 (d, $^4J_{H,H}$ = 1.2, 3H), 1.49 (br s, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 147.88 (C_q), 131.63 (C_q), 124.35 (CH), 111.34 (CH_2), 40.66 (CH_2), 35.59

(CH_2), 26.58 (CH_2), 25.81 (CH_3), 17.73 (CH_3), 3.28 (CH_2) ppm. IR (diamond ATR): $\tilde{\nu}$ = 3075 (w), 2965 (m), 2924 (m), 2854 (m), 1644 (m), 1440 (s), 1376 (m), 1343 (m), 1105 (s), 893 (s), 823 (m), 612 (w), 498 (w), 443 cm^{-1}. HR-MS (APCI): calc. for $[C_{10}H_{18}I]^+$ *m*/*z* = 265.0448; found: *m*/*z* = 265.0447.

(*E*)-12-Iodo-2,6-dimethyl-10-methylenedodeca-2,6-diene (7b): 4.19 g, 12.6 mmol, 70%. TLC (pentane): R_f = 0.50. GC (HP5-MS): *I* = 1889. EI-MS (70 eV): *m*/*z* (%)= 317 (1), 289 (2), 261 (2), 248 (1), 221 (1), 177 (1), 155 (1), 136 (4), 121 (3), 93 (4), 69 (7), 41 (7). ^{1}H-NMR (C_6D_6, 500 MHz): 5.23 (thept, $^3J_{H,H}$ = 7.0, $^4J_{H,H}$ = 1.4, 1H), 5.15 (m, 1H), 4.79 (m, 1H), 4.63 (m, 1H), 2.83 (t, $^3J_{H,H}$= 7.7, 2H), 2.30 (t, $^3J_{H,H}$ = 7.7, 2H), 2.17 (m, 2H), 2.05 (m, 4H), 1.85 (m, 2H), 1.68 (d, $^4J_{H,H}$ = 1.2, 3H), 1.57 (br s, 3H), 1.54 (br s, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 147.89 (C_q), 135.48 (C_q), 131.25 (C_q), 124.87 (CH), 124.25 (CH), 111.39 (CH_2), 40.66 (CH_2), 40.16 (CH_2), 35.59 (CH_2), 27.17 (CH_2), 26.49 (CH_2), 25.89 (CH_3), 17.79 (CH_3), 16.12 (CH_3), 3.29 (CH_2) ppm. IR (diamond ATR): $\tilde{\nu}$ = 3075 (s), 2964 (m), 2922 (w), 2853 (m), 1644 (m), 1440 (w), 1376 (m), 1233 (s), 1169 (w), 1107 (s), 983 (s), 892 (w), 832 (s), 544 (s) cm^{-1}. HR-MS (APCI): calc. for $[C_{15}H_{26}I]^+$ *m*/*z* = 333.1074; found: *m*/*z* = 333.1074.

General procedure for synthesis of β-keto esters 8a and 8b

To a cooled solution of ethyl acetoacetate (2.00 eq) in THF (4 mL $mmol^{-1}$) was added NaH (2.00 eq) in small portions. The reaction mixture was allowed to reach room temperature and stirred for 1 h. The corresponding iodide **7** was added dropwise and the reaction mixture was refluxed overnight, followed by cooling to room temperature. The cooled mixture was quenched by the addition of saturated aqueous NH_4Cl solution and then extracted with Et_2O. The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was subjected to column chromatography (cyclohexane/EtOAc, 10:1) to yield the desired β-keto esters **8**.

Ethyl 2-acetyl-9-methyl-5-methylenedec-8-enoate (8a): 5.18 g, 19.5 mmol, 67%. TLC (pentane/Et_2O, 5:1): R_f = 0.46. GC (HP5-MS): *I* = 1777. ^{1}H-NMR (C_6D_6, 500 MHz): 5.18 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.83 (m, 1H), 4.81 (m, 1H), 3.880 (q, $^3J_{H,H}$ = 7.1, 1H), 3.878 (q, $^3J_{H,H}$ = 7.1, 1H), 3.28 (m, 1H), 2.14 (m, 2H), 2.08 (m, 1H), 2.06 (m, 8H), 1.87 (s, 3H), 1.66 (d, $^4J_{H,H}$ = 1.1, 3H), 1.55 (br s, 3H), 0.88 (t, $^3J_{H,H}$ = 7.1, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 201.44 (C_q), 169.69 (C_q), 148.41 (C_q), 131.53 (C_q), 124.59 (CH), 110.52 (CH_2), 61.04 (CH_2), 59.26 (CH), 36.09 (CH_2), 34.10 (CH_2), 28.54 (CH), 26.78 (CH_2), 26.53 (CH_2), 25.83 (CH_3), 17.75 (CH_3), 14.03 (CH_3) ppm.

Ethyl (*E*)-2-acetyl-9,13-dimethyl-5-methylenetetradeca-8,12-dienoate (8b): 5.05 g, 15.0 mmol, 67%. TLC (cyclohexane/EtOAc, 10:1): R_f = 0.33. GC (HP5-MS): *I* = 2233. ^{1}H-NMR (C_6D_6, 500 MHz): 5.23 (m, 2H), 4.84 (m, 1H), 4.81 (m, 1H), 3.883 (q, $^3J_{H,H}$ = 7.1, 1H), 3.881 (q, $^3J_{H,H}$ = 7.1, 1H), 3.28 (m, 1H), 2.09 (m, 12H), 1.87 (s, 3H), 1.68 (d, $^4J_{H,H}$ = 1.2, 3H), 1.59 (br s, 3H), 1.57 (br s, 3H), 0.88 (t, $^3J_{H,H}$ = 7.1, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 201.42 (C_q), 169.69 (C_q), 148.40 (C_q), 135.38 (C_q), 131.19 (C_q), 124.93 (CH), 124.49 (CH), 110.56 (CH_2), 61.04 (CH_2), 59.26 (CH), 40.19 (CH_2), 36.09 (CH_2), 34.08 (CH_2), 28.54 (CH_3), 27.20 (CH_2), 26.67 (CH_2), 26.54 (CH_2), 25.88 (CH_3), 17.77 (CH_3), 16.14 (CH_3), 14.03 (CH_3) ppm.

General procedure for synthesis of methyl ketones 9a and 9b

To a solution of the corresponding β-keto ester **8** (1.00 eq) in EtOH (2 mL $mmol^{-1}$) was added an aqueous solution of KOH (3.00 eq; 2 M) and the reaction mixture was refluxed for 3 h before cooling to room temperature. The reaction mixture was slowly acidified with 2 M HCl solution until CO_2 developed. The resulting suspension was extracted with pentane (three times). The combined layers were dried with $MgSO_4$ and concentrated under

reduced pressure. The residue was subjected to column chromatography (cyclohexane/EtOAc, 12:1) to yield the desired methyl ketones **9**.

10-Methyl-6-methyleneundec-9-en-2-one (9a): 2.73 g, 14.0 mmol, 72%. TLC (pentane/Et_2O, 5:1): R_f = 0.46. GC (HP5-MS): *I* = 1450. ^{1}H-NMR (C_6D_6, 500 MHz): 5.21 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.84 (m, 1H), 4.79 (m, 1H), 2.17 (m, 2H), 2.04 (m, 2H), 1.92 (m, 4H), 1.67 (m, 4H), 1.64 (m, 4H), 1.56 (m, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 206.01 (C_q), 149.06 (C_q), 131.45 (C_q), 124.72 (CH), 109.88 (CH_2), 42.64 (CH_2), 36.23 (CH_2), 35.73 (CH_2), 29.39 (CH_3), 26.87 (CH_2), 25.84 (CH_3), 21.95 (CH_2), 17.76 (CH_3) ppm.

(*E*)-10,14-Dimethyl-6-methylenepentadeca-9,13-dien-2-one (9b): 2.85 g, 11.0 mmol, 73%. TLC (cyclohexane/EtOAc, 10:1): R_f = 0.43. GC (HP5-MS): *I* = 1932. ^{1}H-NMR (C_6D_6, 500 MHz): 5.25 (m, 2H), 4.85 (m, 1H), 4.80 (m, 1H), 2.18 (m, 4H), 2.08 (m, 4H), 1.92 (m, 4H), 1.68 (d, $^4J_{H,H}$ = 1.2, 3H), 1.64 (m, 2H), 1.63 (s, 3H), 1.61 (br s, 3H), 1.57 (br s, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 206.00 (C_q), 149.04 (C_q), 135.30 (C_q), 131.19 (C_q), 124.93 (CH), 124.62 (CH), 109.92 (CH_2), 42.65 (CH_2), 40.21 (CH_2), 36.23 (CH_2), 35.72 (CH_2), 29.39 (CH_3), 27.21 (CH_2), 26.76 (CH_2), 25.88 (CH_3), 21.96 (CH_2), 17.77 (CH_3), 16.15 (CH_3) ppm.

General procedure for Horner-Wadsworth-Emmons reactions
A solution of diisopropylamine (1.05 eq) dissolved in dry THF (5 mL mmol^{-1}) was cooled to 0 °C. *n*-Buli (1.6 M in hexane, 1.05 eq) was added dropwise and the reaction was stirred for 1 h at 0 °C. The reaction mixture was cooled to –78 °C and triethyl phosphonoacetate (1.00 eq) was added. After stirring the reaction mixture for 1 h at –78 °C and the corresponding methyl ketone **9** (1.00 eq) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight. Water (5 mL mmol^{-1}) was added to quench the reaction. The aqueous phase was extracted with Et_2O (three times), and the combined layers were dried with $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc, 13:1) yielded pure **10a** (mixture of 2*E* and 2*Z* stereoisomers) and **10b** (separated stereoisomers) as colorless oils.

Ethyl 3,11-dimethyl-7-methylenedodeca-2,10-dienoate 10a: Yield: 2.60 g, 9.82 mmol, 70%, 6:4 mixture of 2*E* and 2*Z*-stereoisomers). TLC (pentane, 10:1): R_f = 0.59 (2*E*) and 0.68 (2*Z*). GC (HP5-MS): *I* = 1861 (2*E*) and 1801 (2*Z*). For spectroscopic characterisation a small amount of (*E*)-**10a** was purified from the mixture of stereoisomers.

Ethyl (*E*)-3,11-dimethyl-7-methylenedodeca-2,10-dienoate (2*E*)-10a: ^{1}H-NMR (C_6D_6, 500 MHz): 5.82 (hex, $^4J_{H,H}$ = 1.3, 1H), 5.21 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.84 (m, 1H), 4.77 (m, 1H), 4.06 (q, $^3J_{H,H}$ = 7.1, 2H), 2.20 (d, $^4J_{H,H}$ = 1.3, 3H), 2.16 (m, 2H), 2.00 (m, 2H), 1.84 (m, 4H), 1.67 (d, $^4J_{H,H}$ = 1.4, 3H), 1.55 (br s, 3H), 1.41 (m, 2H), 1.02 (t, $^3J_{H,H}$ = 7.1, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 166.48 (C_q), 159.56 (C_q), 148.92 (C_q), 131.47 (C_q), 124.70 (CH), 116.37 (CH), 109.79 (CH_2), 59.41 (CH_2), 40.53 (CH_2), 36.33 (CH_2), 35.83 (CH_2), 26.88 (CH_2), 25.84 (CH_3), 25.67 (CH_2), 18.73 (CH_3), 17.75 (CH_3), 14.46 (CH_3) ppm.

Ethyl (2*E*,10*E*)-3,11,15-trimethyl-7-methylenehexadeca-2,10,14-trienoate (2*E*)-10b: Yield: 1.45 g, 4.36 mmol, 45%. TLC (pentane, 10:1): R_f = 0.59. GC (HP5-MS): *I* = 2328. EI-MS (70 eV): *m*/*z* (%) = 332 (0.4), 289 (0.5), 215 (0.5), 204 (0.8), 189 (1), 175 (2), 135 (3), 121 (3), 107 (3), 93 (4), 81 (4), 69 (5), 41 (5). ^{1}H-NMR (C_6D_6, 500 MHz): 5.81 (hex, $^4J_{H,H}$ = 1.2, 1H), 5.25 (thex, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.3, 1H), 5.23 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 2H), 4.84 (m, 1H), 4.77 (m, 1H), 4.06 (q, $^3J_{H,H}$ = 7.1, 2H), 2.19 (d, $^4J_{H,H}$ = 1.3, 1H), 2.17 (m, 4H), 2.09 (m, 2H), 2.01 (m, 2H), 1.85 (m, 4H), 1.68 (d, $^4J_{H,H}$ = 1.3, 3H), 1.59 (br s, 3H), 1.57 (br s, 3H), 1.42 (m, 2H), 1.02 (t, $^3J_{H,H}$ = 7.1, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz):

166.46 (C_q), 159.55 (C_q), 148.91 (C_q), 135.30 (C_q), 131.21 (C_q), 124.91 (CH), 124.60 (CH), 116.36 (CH), 109.82 (CH_2), 59.40 (CH_2), 40.51 (CH_2), 40.21 (CH_2), 36.33 (CH_2), 35.82 (CH_2), 27.20 (CH_2), 26.76 (CH_2), 25.88 (CH_3), 25.69 (CH_2), 18.73 (CH_3), 17.77 (CH_3), 16.14 (CH_3), 14.47 (CH_3) ppm. IR (diamond ATR): $\tilde{\nu}$ = 2975 (w), 2927 (m), 2856 (w), 1715 (s), 1646 (m), 1443 (w), 1376 (w), 1223 (s), 1152 (s), 1095 (w), 1072 (w), 1037 (w), 887 (m), 857 (m) cm^{-1}. HR-MS (APCI): calc. for $[C_{22}H_{37}O_2]^+$ *m*/*z* = 333.2788; found: *m*/*z* = 333.2784.

Ethyl (2*Z*,10*E*)-3,11,15-trimethyl-7-methylenehexadeca-2,10,14-trienoate (2*Z*)-10b: Yield: 0.39 g, 1.17 mmol, 12%. TLC (pentane, 10:1): R_f = 0.68. GC (HP5-MS): *I* = 2269. EI-MS (70 eV): *m*/*z* (%) = 332 (0.4), 289 (0.4), 215 (0.4), 204 (0.6), 189 (1), 175 (1), 135 (3), 121 (3), 107 (3), 93 (4), 81 (4), 69 (11), 41 (6). ^{1}H-NMR (C_6D_6, 500 MHz): 5.75 (m, 1H), 5.28 (thex, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.3, 1H), 5.23 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 2H), 4.87 (m, 2H), 4.02 (q, $^3J_{H,H}$ = 7.1, 2H), 2.73 (m, 2H), 2.15 (m, 10H), 1.68 (d, $^4J_{H,H}$ = 1.3, 3H), 1.64 (m, 2H), 1.60 (br s, 3H), 1.56 (br s, 3H), 1.53 (d, $^4J_{H,H}$ = 1.4, 3H), 1.01 (t, $^3J_{H,H}$ = 7.1, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 166.01 (C_q), 160.07 (C_q), 149.39 (C_q), 135.14 (C_q), 131.12 (C_q), 124.99 (CH), 124.79 (CH), 116.91 (CH), 109.61 (CH_2), 59.37 (CH_2), 40.22 (CH_2), 36.58 (CH_2), 36.44 (CH_2), 33.40 (CH_2), 27.23 (CH_2), 26.82 (CH_2), 26.80 (CH_3), 25.88 (CH_2), 24.89 (CH_3), 17.77 (CH_3), 16.14 (CH_3), 14.43 (CH_3). IR (diamond ATR): $\tilde{\nu}$ = 2977 (w), 2928 (m), 2856 (w), 1716 (s), 1647 (m), 1443 (w), 1380 (w), 1220 (s), 1142 (s), 1096 (w), 1071 (w), 1039 (w), 887 (m) cm^{-1}. HR-MS (APCI): calc. for $[C_{22}H_{37}O_2]^+$ *m*/*z* = 333.2788; found: *m*/*z* = 333.2784.

General procedure for DIBAL-H reductions

To a cooled (0 °C) solution of the corresponding ester (2*EZ*)-**10a** or (2*EZ*)-**10b** (from a second performed synthesis where *E* and *Z* stereoisomers were not separated) (1.00 eq) in Et_2O (10 mL $mmol^{-1}$) was added DIBAL-H (1 M in hexane, 2.20 eq) and the reaction mixture was stirred for 1 h at room temperature. The mixture was cooled to 0 °C again and a saturated solution of Na-K-tartrate was added. The resulting slurry was stirred for 2 h to dissolve the precipitate and the aqueous phase was extracted with Et_2O (three times). The organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (pentane/Et_2O, 3:1) to yield the desired alcohols **11** as colorless oils. At this stage the (2*E*)- and (2*Z*)-stereoisomers of **10a** and **10b** were completely separated.

(2*E*)-3,11-Dimethyl-7-methylenedodeca-2,10-dien-1-ol (2*E*)-11a: 1.20 g, 5.41 mmol, 62%. TLC (pentane, 1:1): R_f = 0.46. GC (HP5-MS): *I* = 1730. EI-MS (70 eV): *m*/z (%) = 204 (0.2), 189 (0.6), 161 (1), 135 (2), 121 (2), 107 (2), 93 (5), 81 (5), 69 (16), 41 (13). ^{1}H-NMR (C_6D_6, 500 MHz): 5.37 (thex, $^3J_{H,H}$ = 6.7, $^4J_{H,H}$ = 1.3, 1H), 5.22 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.86 (m, 2H), 3.96 (br d, $^3J_{H,H}$ = 5.5, 2H), 2.19 (m, 2H), 2.07 (m, 2H), 1.96 (m, 2H), 1.90 (m, 2H), 1.66 (d, $^4J_{H,H}$ = 1.2, 3H), 1.55 (br s, 3H), 1.51 (m, 2H), 1.44 (br s, 3H), 0.63 (br s, OH) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 149.46 (C_q), 138.14 (C_q), 131.42 (C_q), 125.04 (CH), 124.78 (CH), 109.55 (CH_2), 59.37 (CH_2), 39.47 (CH_2), 36.50 (CH_2), 36.10 (CH_2), 26.95 (CH_2), 26.20 (CH_2), 25.85 (CH_3), 17.76 (CH_3), 16.09 (CH_3) ppm.

(2*Z*)-3,11-Dimethyl-7-methylenedodeca-2,10-dien-1-ol (2*Z*)-11a: 0.60 g, 2.70 mmol, 31%. TLC (pentane, 1:1): R_f = 0.60. GC (HP5-MS): *I* = 1709. EI-MS (70 eV): *m*/*z* (%) = 204 (0.2), 189 (0.7), 161 (1), 135 (2), 121 (2), 107 (2), 93 (5), 81 (5), 69 (15), 41 (12). ^{1}H-NMR (C_6D_6, 500 MHz): 5.36 (br t, $^3J_{H,H}$ = 6.7, 1H), 5.23 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.87 (m, 1H), 4.83 (m, 1H), 3.97 (t, $^3J_{H,H}$ = 5.8, 2H), 2.19 (m, 2H), 2.07 (m, 2H), 1.94 (m, 4H), 1.67 (d, $^4J_{H,H}$ = 1.4, 3H), 1.59 (q, $^4J_{H,H}$ = 1.2, 3H), 1.56 (br s, $^4J_{H,H}$ = 1.2, 3H), 1.47 (m, 2H), 0.54 (t, $^3J_{H,H}$ = 5.3, OH) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 149.35 (C_q), 138.58 (C_q), 131.46 (C_q), 125.86 (CH), 124.75 (CH), 109.55 (CH_2), 59.10 (CH_2), 36.49 (CH_2), 36.15 (CH_2), 31.78 (CH_2), 26.92 (CH_2), 26.52 (CH_2), 25.85 (CH_3), 23.39 (CH_3), 17.75 (CH_3) ppm.

(2*E*,10*E*)-3,11,15-Trimethyl-7-methylenehexadeca-2,10,14-trien-1-ol (2*E*)-11b: TLC (pentane, 1:1): R_f = 0.46. GC (HP5-MS): *I* = 2204. EI-MS (70 eV): *m*/*z* (%) = 272 (0.4), 204 (0.8), 190 (0.4), 175 (0.4), 161 (0.8), 147 (1), 133 (2), 121 (3), 107 (3), 93 (5), 81 (7), 69 (11), 41 (9). ^{1}H-NMR (C_6D_6, 500 MHz): 5.38 (thex, $^3J_{H,H}$ = 6.7, $^4J_{H,H}$ = 1.3, 1H), 5.28 (thex, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.3, 1H), 5.24 (thept, $^3J_{H,H}$ = 6.8, $^4J_{H,H}$ = 1.4, 1H), 4.88 (m, 1H), 4.86 (m, 1H), 3.97 (m, 2H), 2.20 (m, 4H), 2.10 (m, 4H), 1.98 (m, 2H), 1.91 (m, 2H), 1.68 (d, $^4J_{H,H}$ = 1.3, 3H), 1.60 (bs s, 3H), 1.56 (br s, 3H), 1.52 (m, 2H), 1.45 (br s, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 149.44 (C_q), 138.16 (C_q), 135.27 (C_q), 131.20 (C_q), 125.03 (CH), 124.93 (CH), 124.68 (CH), 109.59 (CH_2), 59.38 (CH_2), 40.21 (CH_2), 39.47 (CH_2), 36.50 (CH_2), 36.09 (CH_2), 27.21 (CH_2), 26.84 (CH_2), 26.21 (CH_2), 25.88 (CH_3), 17.71 (CH_3), 16.15 (CH_3), 16.10 (CH_3) ppm. IR (diamond ATR): $\tilde{\nu}$ = 3336 (w), 3072 (w), 2966 (m), 2928 (s), 2856 (m), 1738 (w), 1668 (w), 1644 (w), 1441 (m), 1377 (m), 1229 (w), 1105 (w), 1001 (m), 887 (m), 543 (w) cm^{-1}. HR-MS (APCI): calc. for $[C_{20}H_{35}O]^+$ *m*/*z* = 291.2682; found: *m*/*z* = 291.2675.

(2*Z*,10*E*)-3,11,15-Trimethyl-7-methylenehexadeca-2,10,14-trien-1-ol (2*Z*)-11b: TLC (pentane, 1:1): R_f = 0.60. GC (HP5-MS): *I* = 2176. EI-MS (70 eV): *m*/*z* (%) = 272 (0.6), 204 (0.8), 190 (0.3), 175 (0.5), 161 (0.7), 147 (1), 133 (2), 121 (2), 107 (3), 93 (4), 81 (7), 69 (14), 41 (8). ^{1}H-NMR (C_6D_6, 500 MHz): 5.36 (br t, $^3J_{H,H}$ = 6.7, 1H), 5.27 (thex, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.3, 1H), 5.23 (thept, $^3J_{H,H}$ = 6.8, $^4J_{H,H}$ = 1.3, 1H), 4.87 (m, 1H), 4.83 (m, 2H), 3.98 (br d, $^3J_{H,H}$ = 6.3, 2H), 2.18 (m, 4H), 2.08 (m, 4H), 1.93 (m, 4H), 1.68 (d, $^3J_{H,H}$ = 1.4, 3H), 1.60 (br s, 3H), 1.59 (m, 3H), 1.56 (br s, 3H), 1.46 (m, 2H) 0.60 (br s, OH) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 149.34 (C_q), 138.56 (C_q), 135.30 (C_q), 131.20 (C_q), 125.88 (CH), 124.93 (CH), 124.65 (CH), 109.58 (CH_2), 59.10 (CH_2), 40.21 (CH_2), 36.49 (CH_2), 36.14 (CH_2), 31.80 (CH_2), 27.21 (CH_2), 26.81 (CH_2), 26.54 (CH_2), 25.88 (CH_3), 23.40 (CH_3), 17.77 (CH_3), 16.14 (CH_3) ppm. IR (diamond ATR): $\tilde{\nu}$ = 3319 (w), 2965 (m), 2927 (s), 2856 (m), 1667 (w), 1644 (w), 1444 (m), 1376 (m), 1107 (w), 1093 (w), 995 (m), 887 (m), 544 (w) cm^{-1}. HR-MS (APCI): calc. for $[C_{20}H_{35}O]^+$ *m*/*z* = 291.2682; found: *m*/*z* = 291.2675.

General procedure for synthesis of diphosphates

To solution of tris (tetra-*n*-butylammonium) hydrogen diphosphate (1.50 eq) in acetonitrile (7.5 mL mmol^{-1}) a solution of the corresponding allyl bromide (1.00 eq) was added and the mixture was stirred at room temperature overnight. Acetonitrile was removed at the rotary evaporator and the resulting residue was dissolved in aqueous NH_4HCO_3 solution (0.25 M) and loaded onto a DOWEX 50WX8 ion-exchange column (NH_4^+ form, pH ~ 7.0). The column was flushed slowly with 1.5 column volume of NH_4HCO_3 buffer (25 mM, 5% *i*PrOH) and the eluate was lyophilised to yield the diphosphates. The residue was purified by redissolving in NH_4HCO_3 solution (50 mM) followed by precipitation of inorganic diphosphate by addition of isopropanol/acetonitrile (1:1). The solids were removed by centrifugation (14.000 x g, 10 min) and the supernatant was transferred to another flask. The procedure was repeated twice and the combined supernatant fractions were lyophilised again to give the pure diphosphates as colourless hygroscopic powders.

Iso-FPP: 580 mg (from 200 mg (2*E*)-**11a**, containing ca. 20% monophosphate). ^{1}H-NMR (C_6D_6, 500 MHz): 5.47 (t, ^3J = 7.3, 1H), 5.20 (t, ^3J = 6.6, 1H), 4.48 (dd, $^3J_{H,H}$ = 6.6, $^3J_{P,H}$ = 6.6, 2H), 2.07 (m, 8H), 1.72 (br s, 3H), 1.69 (br s, 3H), 1.63 (br s, 3H), 1.60 (m, 2H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 151.52 (C_q), 143.06 (C_q), 133.55 (C_q), 124.46 (CH), 119.91 (d, $^3J_{C,P}$ = 8.6, CH), 108.76 (CH_2), 62.55 (d, $^2J_{C,P}$ = 5.2, CH_2), 38.65 (CH_2), 35.36 (CH_2), 35.01 (CH_2), 25.73 (CH_2), 25.25 (CH_2), 24.90 (CH_3), 17.04 (CH_3), 15.60 (CH_3) ppm. ^{31}P-NMR (D_2O, 203 MHz): –6.61 (d, $^2J_{P,P}$ = 22.0), –10.26 (d, $^2J_{P,P}$ = 22.0) ppm. HR-MS (APCI): calc. for $[C_{15}H_{27}O_7P_2]^-$ *m*/*z* = 381.1237; found: *m*/*z* = 381.1238.

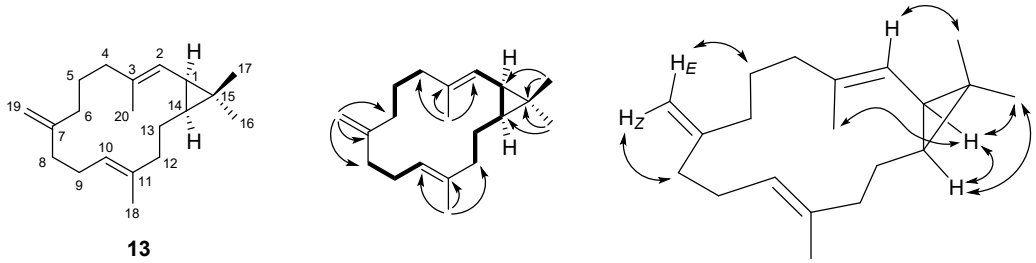
Iso-GGPP: 487 mg (from 700 mg (2*E*)-**11a**, ca. 1:1 mixture of mono- and diphosphate). ^{1}H-NMR (C_6D_6, 500 MHz): 5.45 (m, 1H), 5.37 (m, 1H), 5.11 (m, 3H), 4.76 (m, 1H), 4.72 (m, 1H), 4.48 (dd, $^{3}J_{H,H}$ = 6.6, $^{3}J_{P,H}$ = 6.6, 2H), 4.37 (dd, $^{3}J_{H,H}$ = 7.9, $^{3}J_{P,H}$ = 7.9, 1H), 2.11 (t, $^{3}J_{H,H}$ = 7.1, 2H), 2.00 (m, 8H), 1.71 (d, $^{4}J_{H,H}$ = 1.3, 2H), 1.68 (m, 1H), 1.65 (m, 6H), 1.58 (m, 3H), 1.57 (m, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 149.38 (C_q), 148.81 (C_q), 142.47 (C_q), 141.48 (C_q), 134.94 (C_q), 134.70 (C_q), 130.97 (C_q), 130.60 (C_q), 124.55 (CH), 124.31 (CH), 124.23 (CH), 120.11 (CH, $^{3}J_{P,C}$ = 6.8), 119.93 (CH, $^{3}J_{P,C}$ = 8.6), 109.05 (CH_2), 108.99 (CH_2), 62.88 (d, $^{3}J_{P,C}$ = 5.3, CH_2), 60.84 (d, $^{3}J_{P,C}$ = 3.8, CH_2), 39.76 (CH_2), 39.66 (CH_2), 39.28 (CH_2), 39.19 (CH_2), 36.23 (CH_2), 36.14 (CH_2), 35.75 (CH_2), 35.66 (CH_2), 26.78 (CH_2), 26.67 (CH_2), 26.32 (CH_2), 26.23 (CH_2), 25.75 (CH_2), 25.70 (CH_2), 25.44 (CH_3), 25.42 (CH_3), 17.42 (CH_3), 17.40 (CH_3), 15.95 (CH_3), 15.85 (CH_3), 15.82 (CH_3), 15.80 (CH_3) ppm. ^{31}P-NMR (D_2O, 203 MHz): –9.79 (d, $^{2}J_{P,P}$ = 19.6 Hz), –10.61 (d, $^{2}J_{P,P}$ = 19.6 Hz) ppm. HR-MS (APCI): calc. for $[C_{20}H_{35}O_7P_2]^-$ m/z = 449.1863; found: *m*/*z* = 449.1868.

Enzymatic conversion of iso-FPP and iso-GGPP into iso-casbenes 13 and 14

Enzymatic preparation of iso-casbene I (**13**) was done by dissolving iso-GGPP (80 mg, 0.16 mmol) in substrate buffer (25 mM NH_4HCO_3; 8 mL). This solution was added slowly within 1 h into the reaction mixture of protein preparation (20 mL; from 8 L expression culture, 1.25 mg/mL), $MgCl_2$ solution (1 mL, 1 M) and incubation buffer (50 mM Tris/HCl, 10 mM $MgCl_2$, 10% glycerol, pH = 8.2, 70 mL). Then after stirring overnight at 30 °C, the reaction mixture was extracted with pentane (3x 100 mL), and the extracts were dried with $MgSO_4$ and concentrated in vacuo (600 mbar, 35 °C). Column chromatography on silica gel with pentane yield the compound **13**. The compound iso-casbene II (**14**) was prepared from iso-FPP (60 mg, 1.39 mmol) and IPP (60 mg) using the enzyme combination of GGPPS (24 mL enzyme preparation, 1 mg/mL) and CS (30 mL enzyme preparation, 1 mg/mL).

iso-Casbene I (13): Yield: 0.4 mg (1.5 μmol, 1%). TLC (pentane): R_f = 0.61. GC (HP5-MS): *I* = 1972. IR (diamond ATR): $\tilde{\nu}$ = 2925 (s), 2857 (m), 2156 (w), 1641 (w), 1453 (w), 1019 (w), 882 (w), 806 (w), 540 (s), 445 (w) cm^{-1}. HR-MS (APCI): calc. for $[C_{20}H_{32}]^+$ *m*/*z* =272.2499; found: *m*/*z* = 272.2497. Optical rotary power: $[\alpha]_D^{25}$ = –75.0 (*c* 0.04, C_6D_6). NMR data are given in Table S3.

iso-Casbene II (14): Yield: 1.2 mg (4.4 μmol, 3%). TLC (n-Hexane): R_f = 0.69. GC (HP5-MS): *I* = 1971. IR (diamond ATR): $\tilde{\nu}$ = 2927 (s), 2859 (m), 1738 (s), 1644 (w), 1441 (m), 1374 (s), 1229 (s), 1216 (s), 1094 (w), 1020 (w), 883 (m), 808 (w), 546 (w) cm^{-1}. HR-MS (APCI): calc. for $[C_{20}H_{32}]^+$ *m*/*z* =272.2499; found: *m*/*z* = 272.2497. Optical rotary power: $[\alpha]_D^{25}$ = –70.7 (*c* 0.12, C_6D_6). NMR data are given in Table S4.



13

Figure S14. Structure elucidation of **13**. Bold: ^{1}H,^{1}H-COSY, single headed arrows: key HMBC, and double headed arrows: key NOESY correlations.

Table S3. NMR data of iso-casbene I (**13**) in C_6D_6 recorded at 298 K.

C[a]	type	^{13}C[b]	^{1}H[b]
1	CH	25.92	1.27 (dd, *J* = 8.2, 8.2)
2	CH	122.04	4.99 (br d, *J* = 7.7)
3	C_q	136.57	–
4	CH_2	39.34	2.16 (m)
			2.01 (ddd, *J* = 13.6, 11.9, 2.8)
5	CH_2	24.42	1.61 (m)
			1.51 (m)
6	CH_2	35.53	2.24 (m)
			1.74 (m)
7	C_q	152.19	–
8	CH_2	36.98	2.26 (m)
			1.92 (m)
9	CH_2	29.80	2.23 (m)
			2.12 (m)
10	CH	124.99	5.20 (br t, *J* = 7.2)
11	C_q	135.92	–
12	CH_2	41.08	2.17 (m)
			1.91 (m)
13	CH_2	25.09	1.73 (m)
			0.88 (m)
14	CH	30.93	0.55 (ddd, *J* = 10.4, 8.8, 1.5)
15	C_q	19.60	–
16	CH_3	15.85	0.99 (s)
17	CH_3	28.97	1.04 (s)
18	CH_3	15.98	1.56 (br s)
19	CH_2	109.27	4.86 (br s, H_Z)
			4.85 (m, H_E)
20	CH_3	16.65	1.67 (br s)

[a] Carbon numbering as shown in Figure S14. [b] Chemical shifts δ in ppm; multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad; coupling constants *J* are given in Hertz.

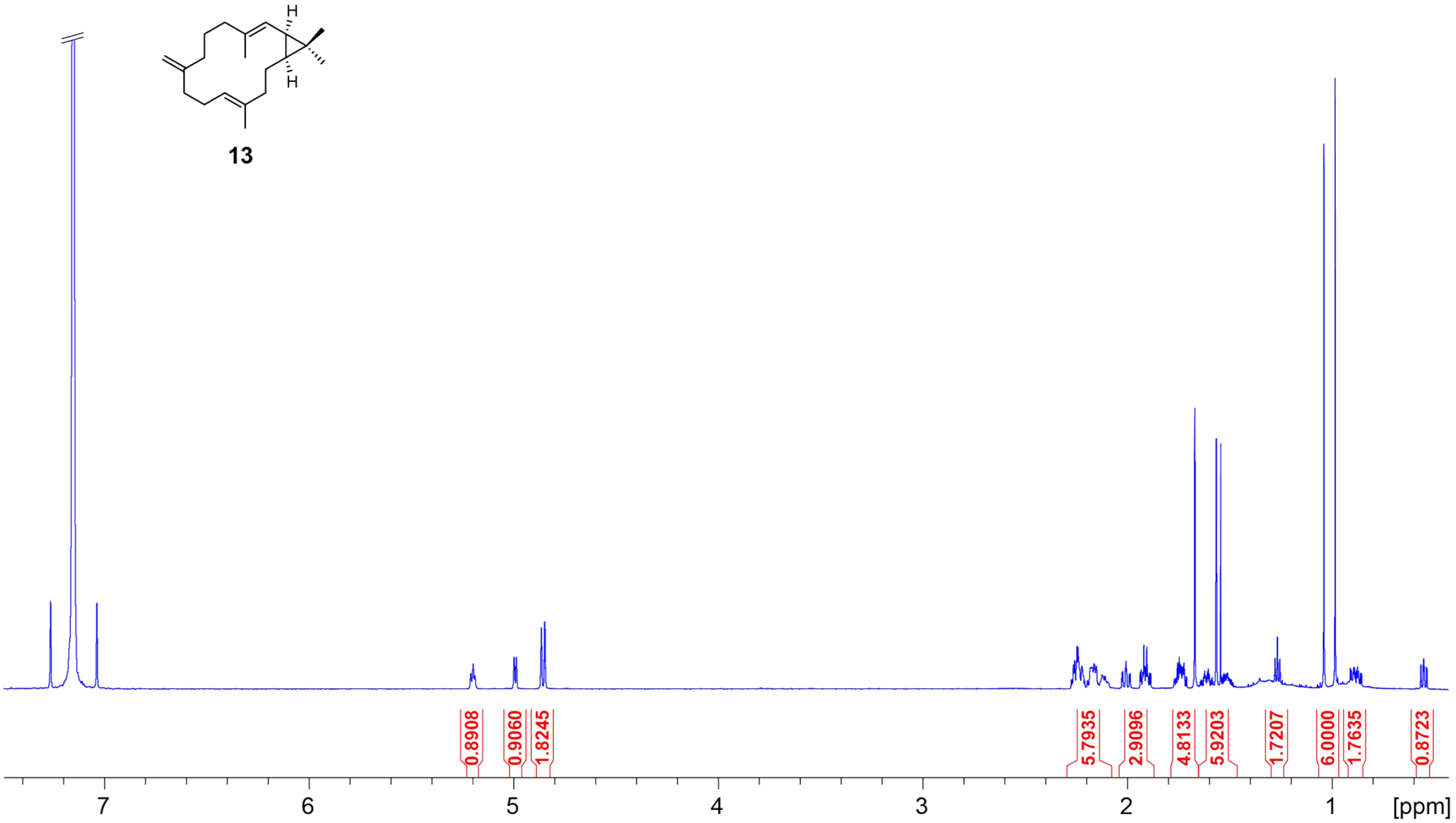


Figure S15. ^{1}H-NMR spectrum of **13** (700 MHz, C_6D_6).

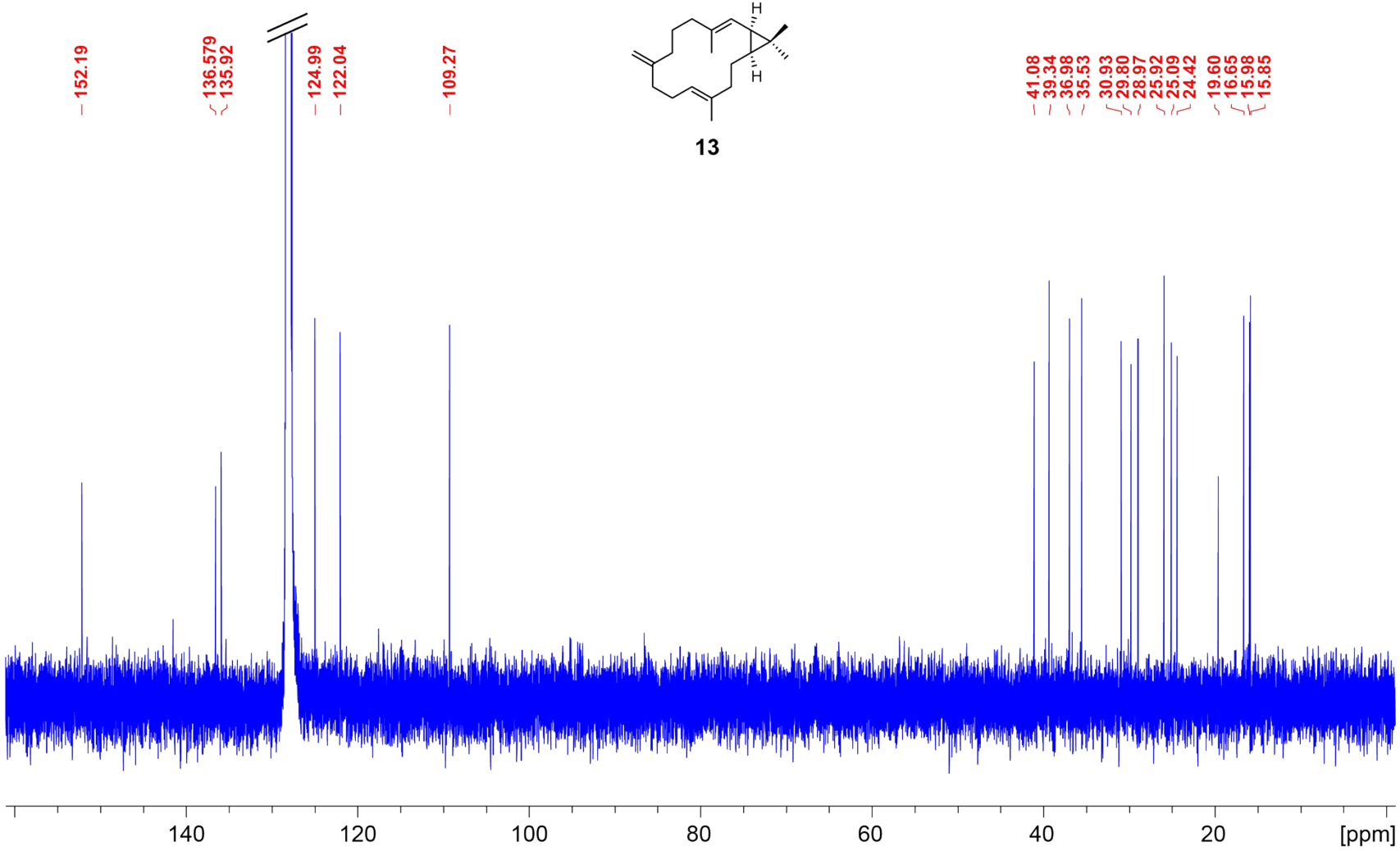
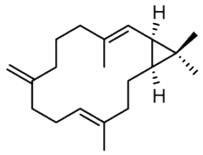


Figure S16. ^{13}C-NMR spectrum of **13** (176 MHz, C_6D_6).



13

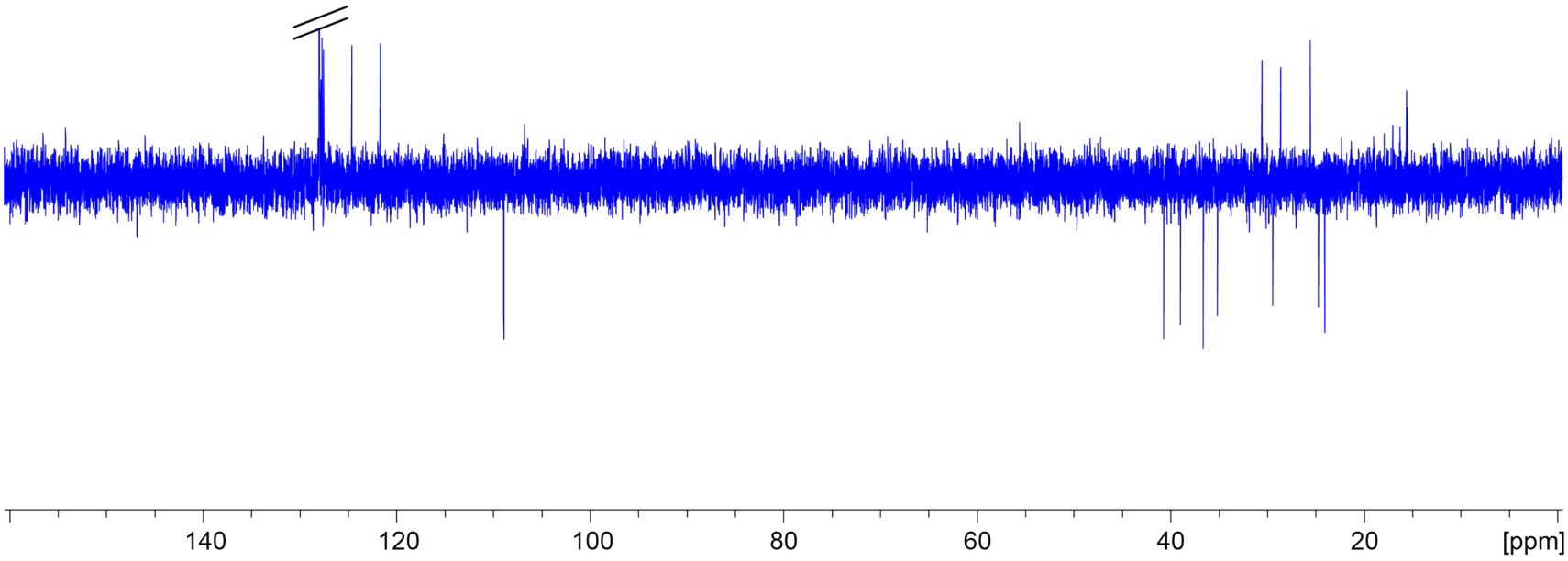


Figure S17. ^{13}C-DEPT spectrum of **13** (176 MHz, C_6D_6).

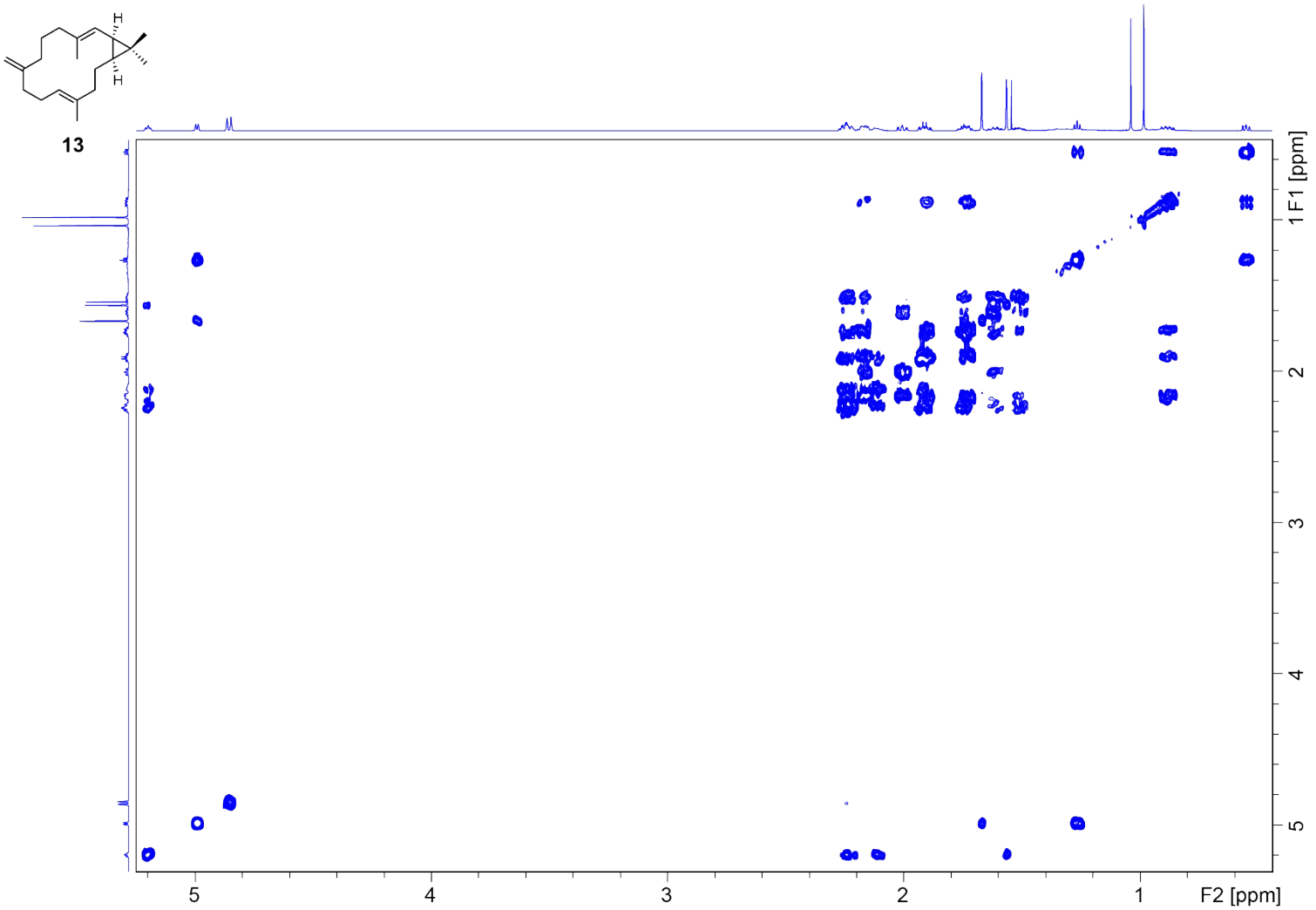


Figure S18. ^{1}H-^{1}H-COSY spectrum of **13** (700 MHz, C_6D_6).

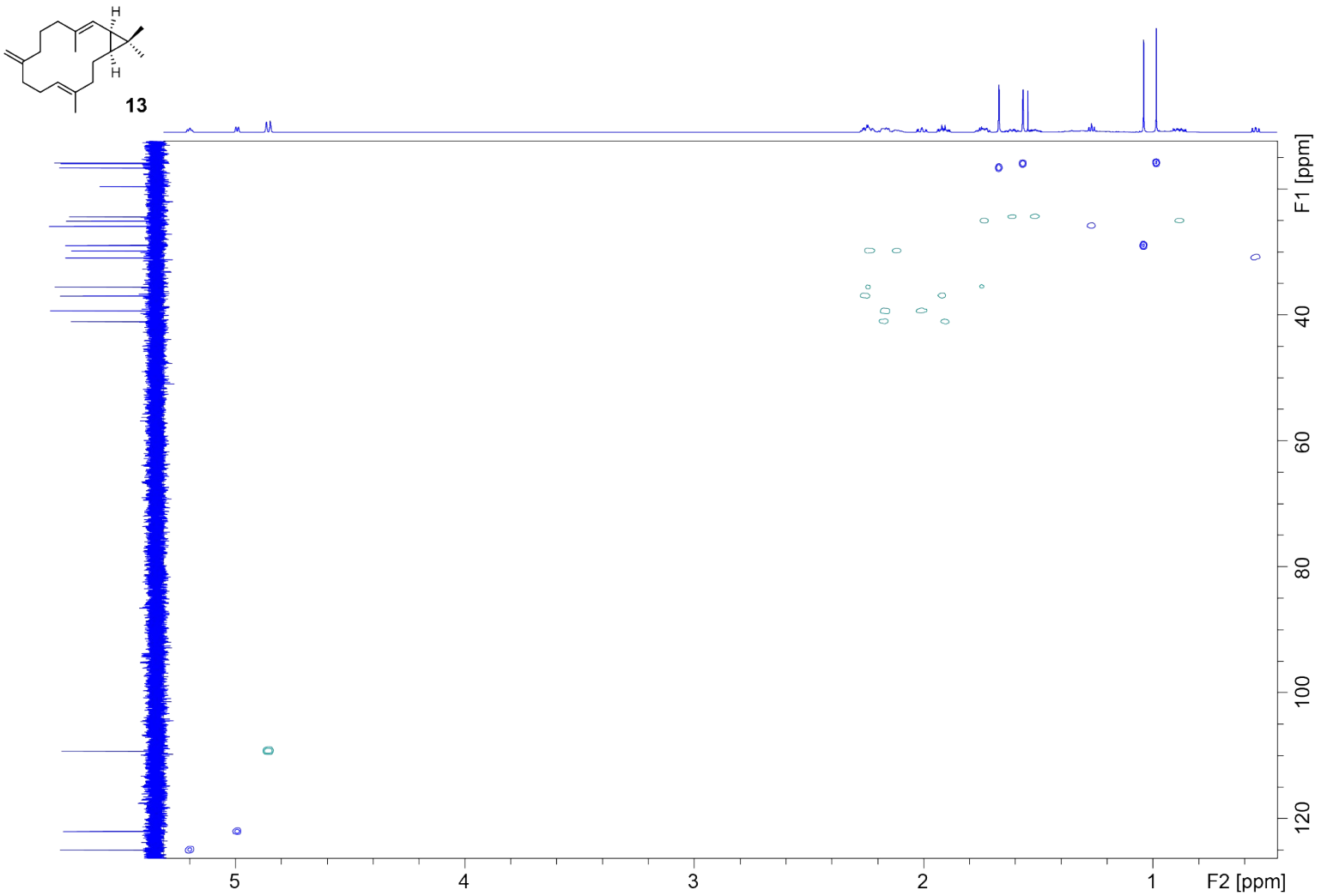


Figure S19. HSQC spectrum of **13** (C_6D_6).

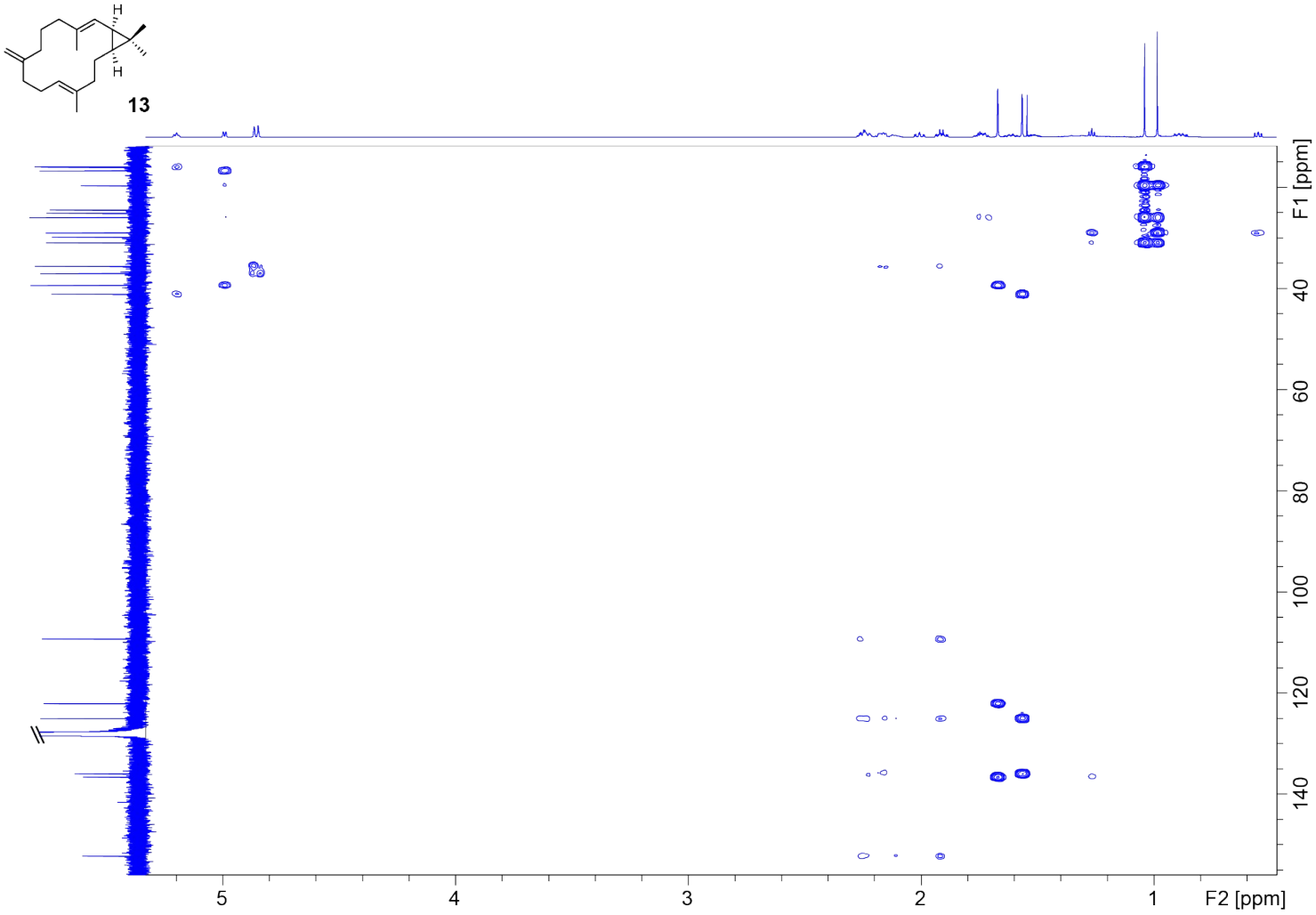


Figure S20. HMBC spectrum of **13** (C_6D_6).

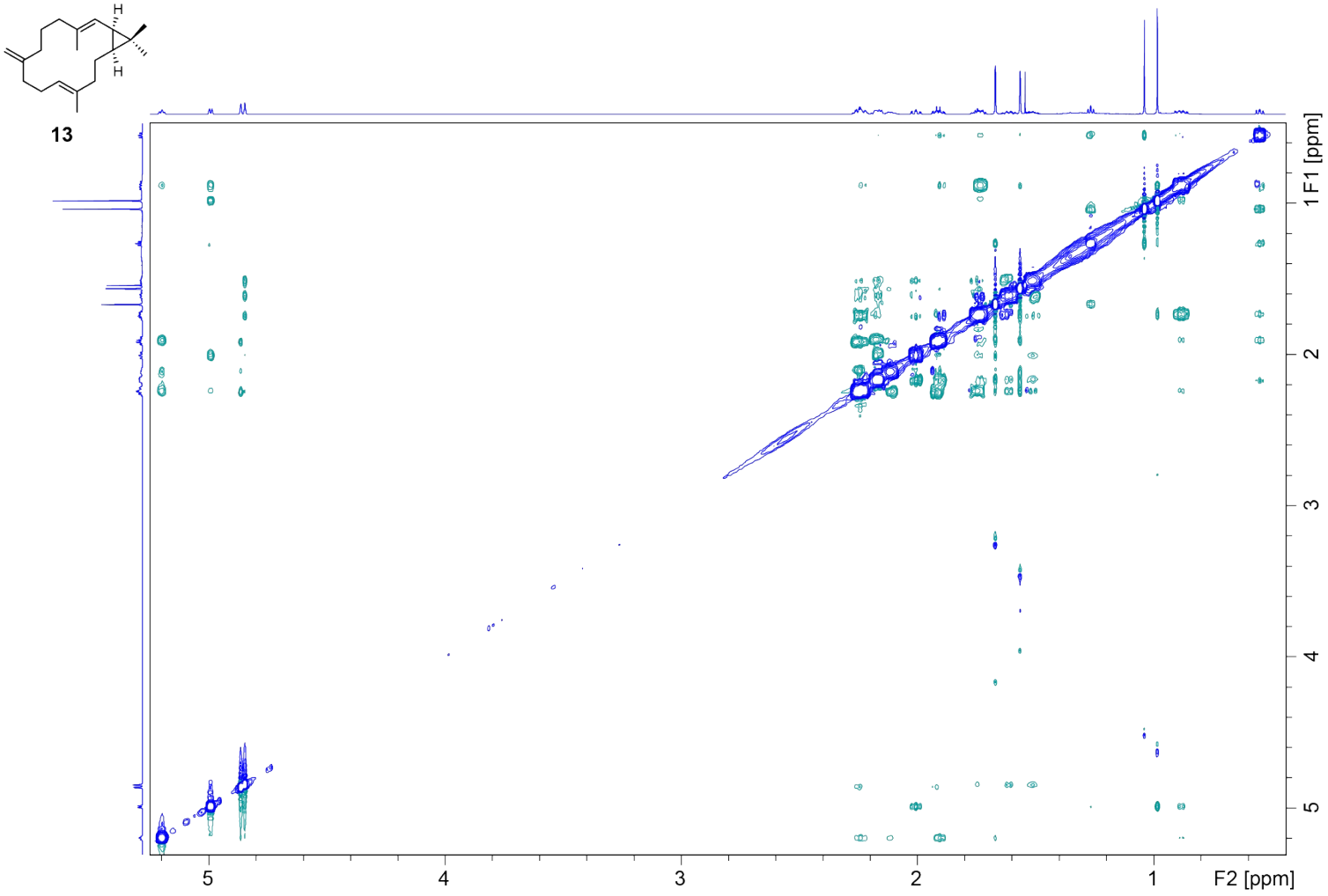


Figure S21. NOESY spectrum of **13** (C_6D_6).

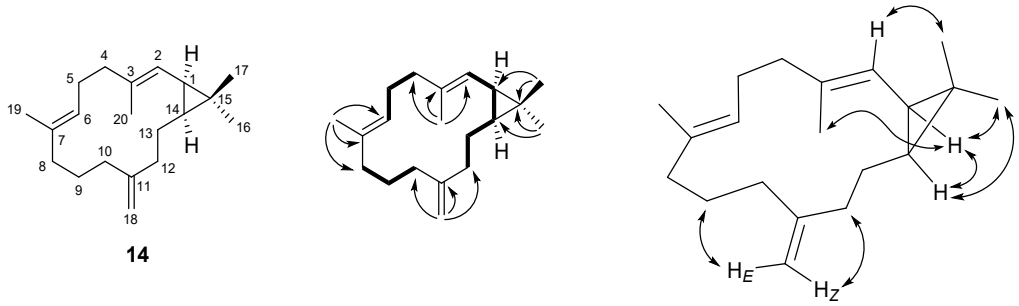


Figure S22. Structure elucidation of **14**. Bold: ^{1}H,^{1}H-COSY, single headed arrows: key HMBC, and double headed arrows: key NOESY correlations.

Table S4. NMR data of iso-casbene II (**14**) in C_6D_6 recorded at 298 K.

C[a]	type	^{13}C[b]	^{1}H[b]
1	CH	26.08	1.32 (dd, *J* = 9.6, 9.0)
2	CH	122.05	4.99 (br d, *J* = 9.9)
3	C_q	134.78	–
4	CH_2	40.45	2.20 (m)
			2.07 (m)
5	CH_2	25.38	2.21 (m)
			2.12 (m)
6	CH	124.69	5.11 (tq, *J* = 7.1, 1.2)
7	C_q	133.90	–
8	CH_2	37.77	2.08 (m)
			1.94 (ddd, *J* = 12.2, 9.4, 3.2)
9	CH_2	26.10	1.60 (m, 2H)
10	CH_2	33.19	2.10 (m)
			1.86 (ddd, *J* = 14.1, 8.9, 6.2)
11	C_q	150.94	–
12	CH_2	38.28	2.18 (m)
			2.06 (m)
13	CH_2	24.43	1.58 (m)
			1.30 (m)
14	CH	30.16	0.64 (ddd, *J* = 11.2, 8.8, 2.6)
15	C_q	20.34	–
16	CH_3	29.28	1.04 (s)
17	CH_3	15.86	1.00 (s)
18	CH_2	109.36	4.92 (m, H_Z)
			4.85 (m, H_E)
19	CH_3	16.57	1.53 (br s)
20	CH_3	15.77	1.64 (br s)

[a] Carbon numbering as shown in Figure S22. [b] Chemical shifts δ in ppm; multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants *J* are given in Hertz.

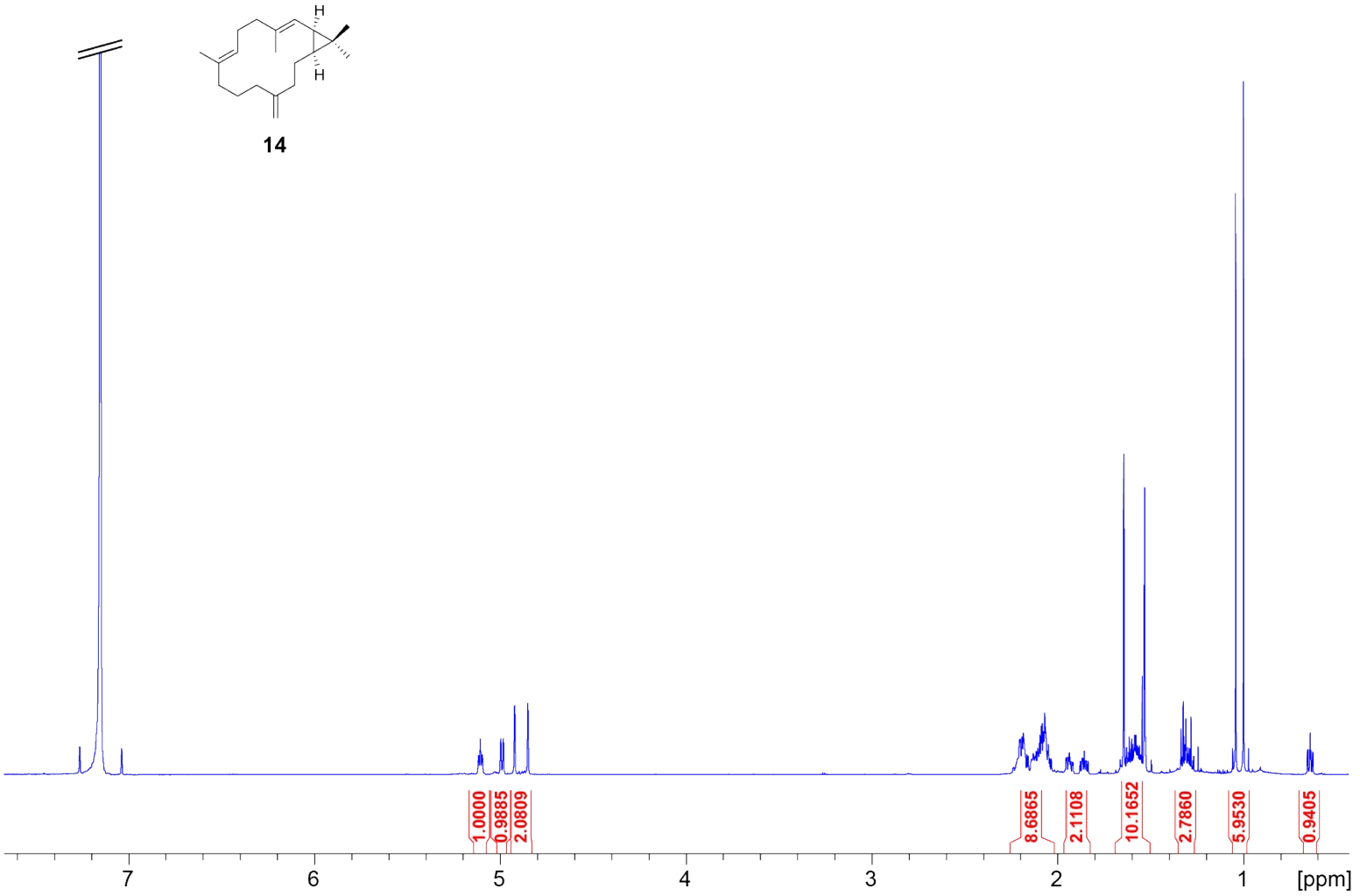


Figure S23. ^{1}H-NMR spectrum of **14** (700 MHz, C_6D_6).

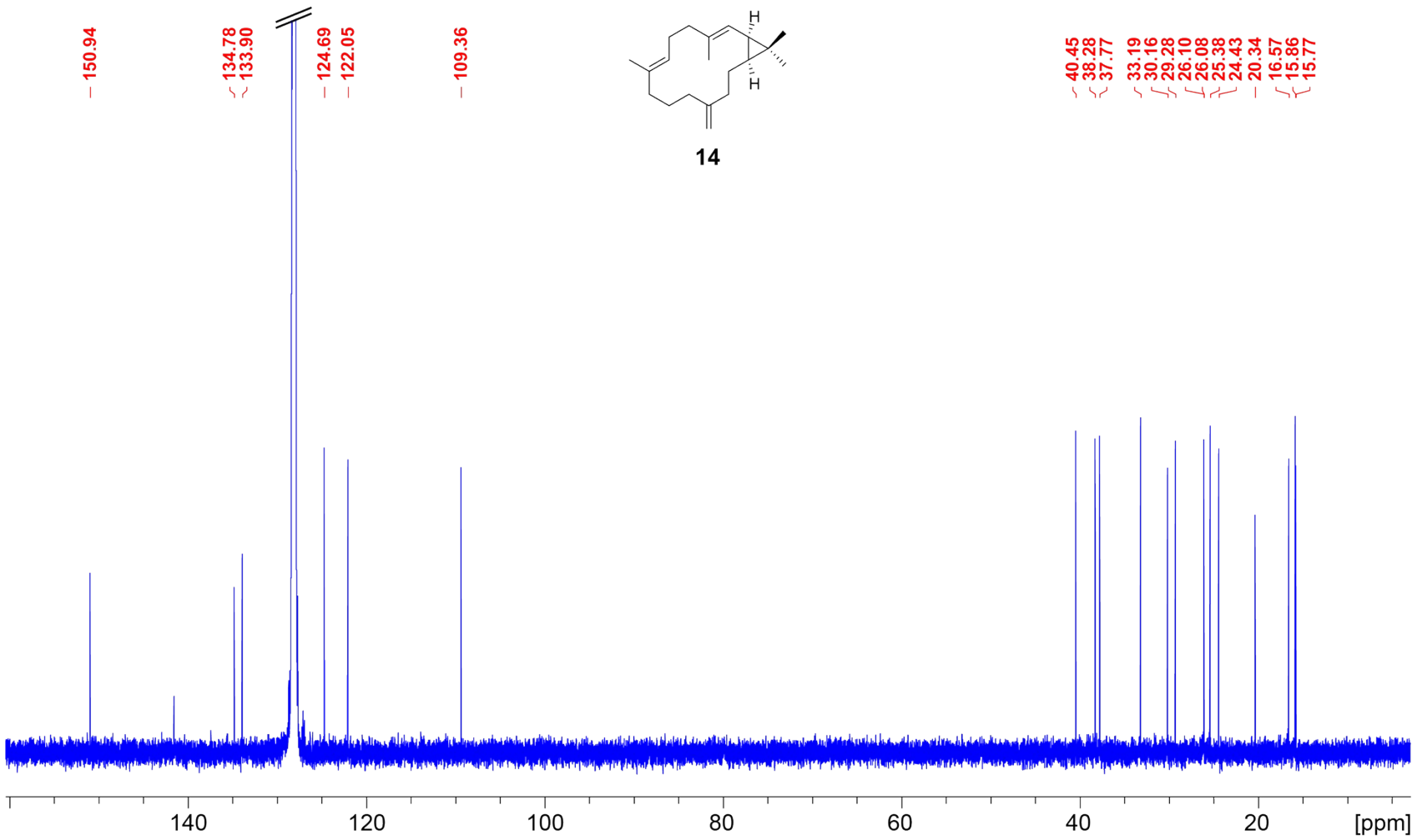


Figure S24. ^{13}C-NMR spectrum of **14** (176 MHz, C_6D_6).

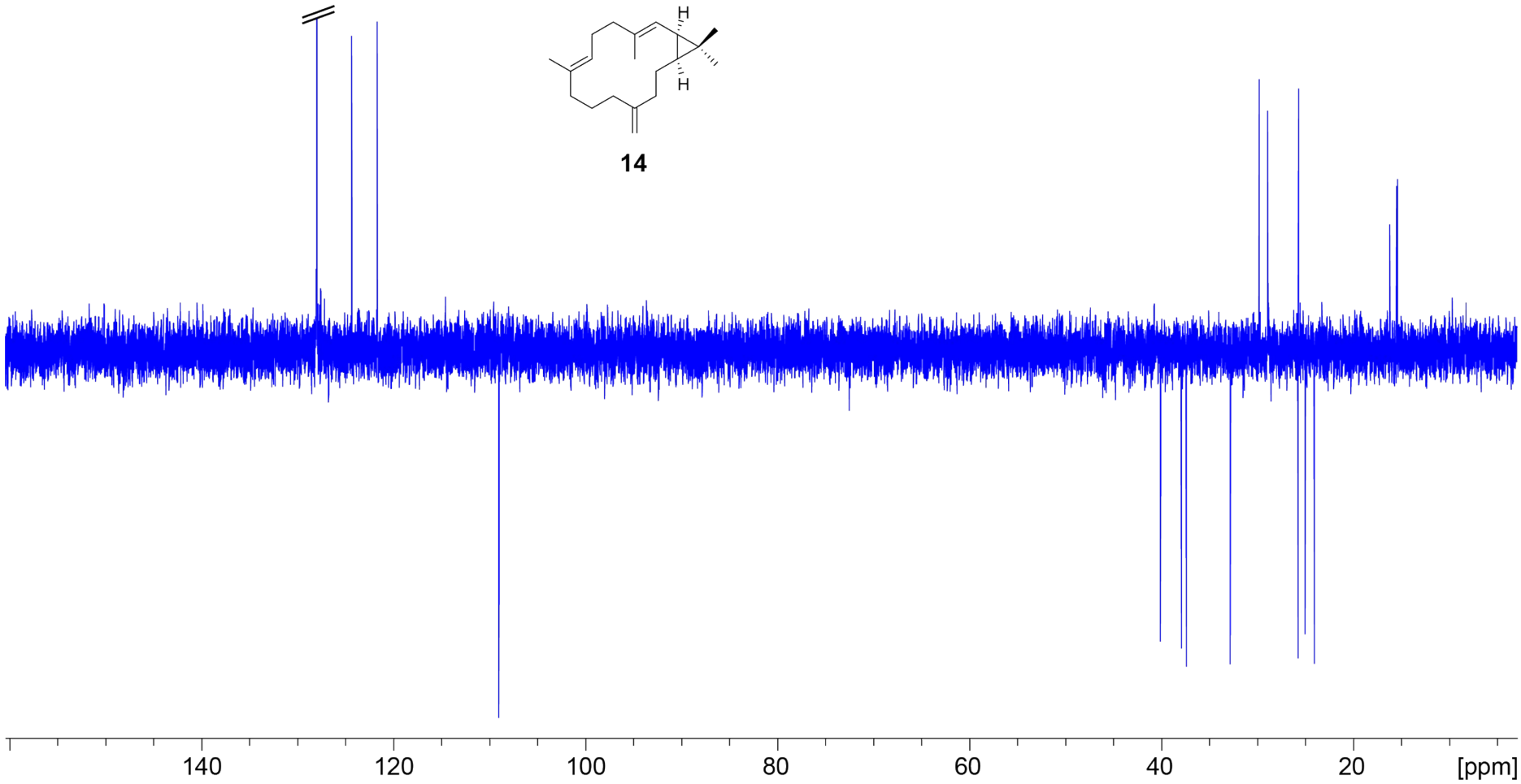


Figure S25. ^{13}C-DEPT spectrum of **14** (176 MHz, C_6D_6).

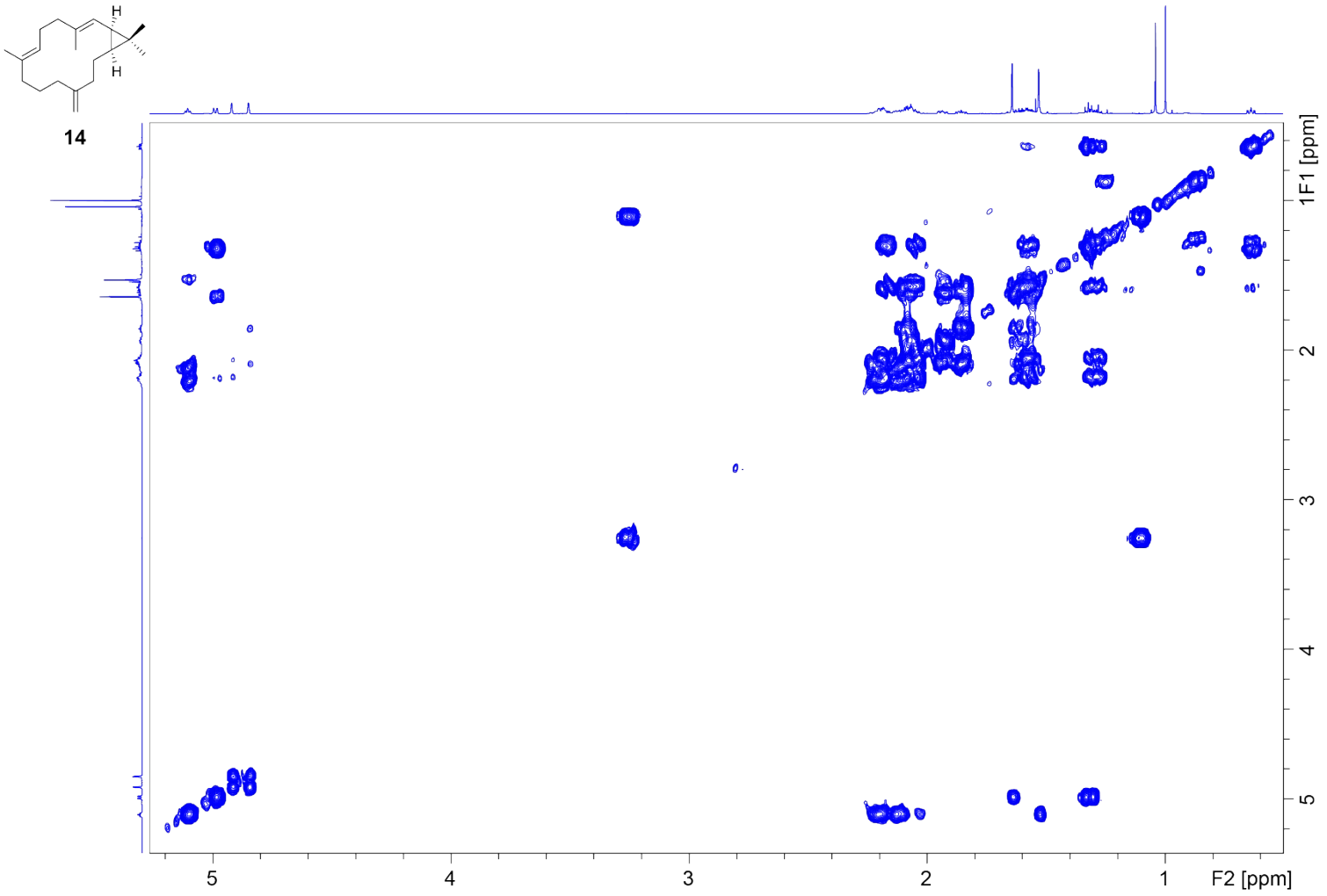


Figure S26. ^{1}H-^{1}H-COSY spectrum of **14** (700 MHz, C_6D_6).

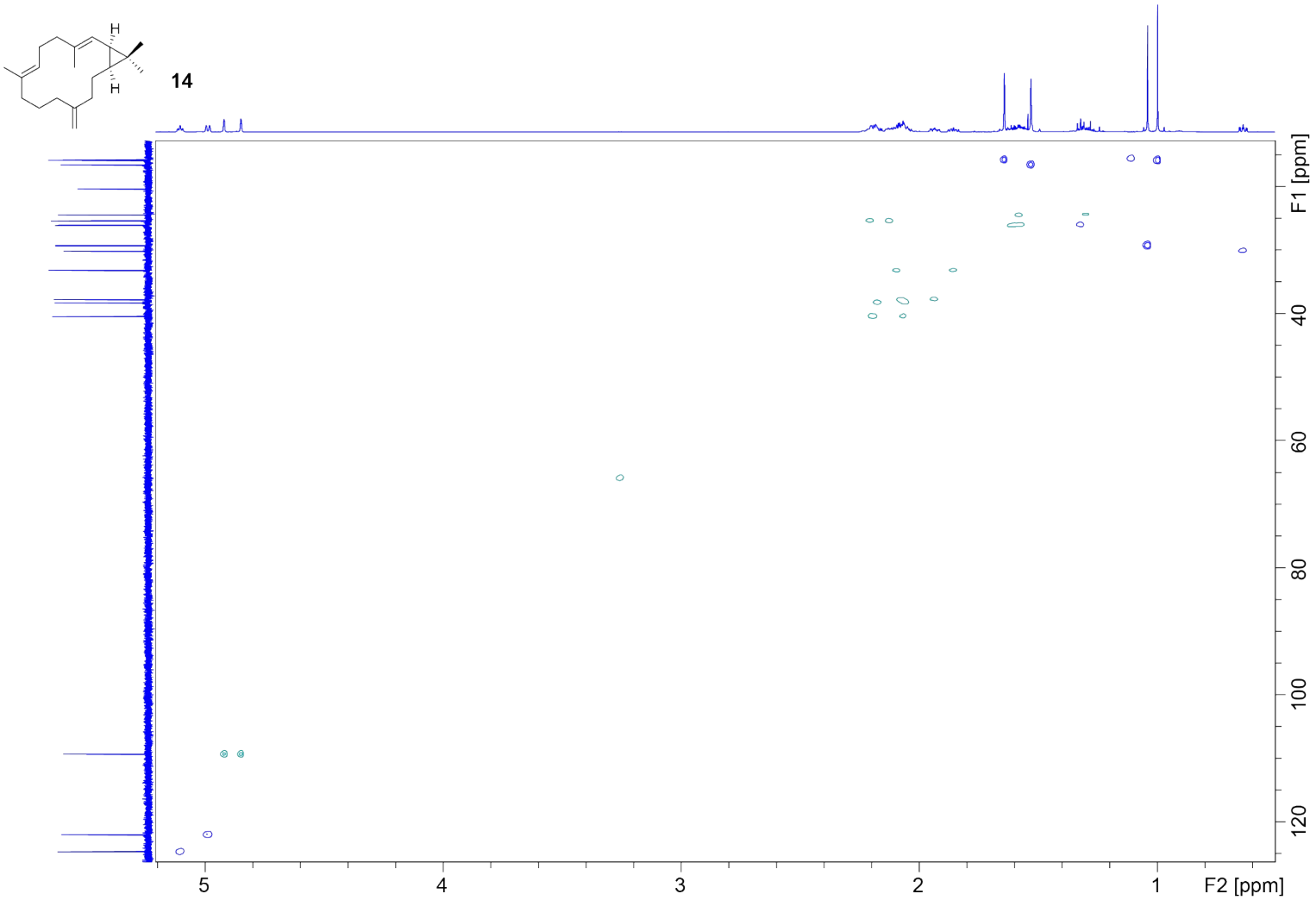


Figure S27. HSQC spectrum of **14** (C_6D_6).

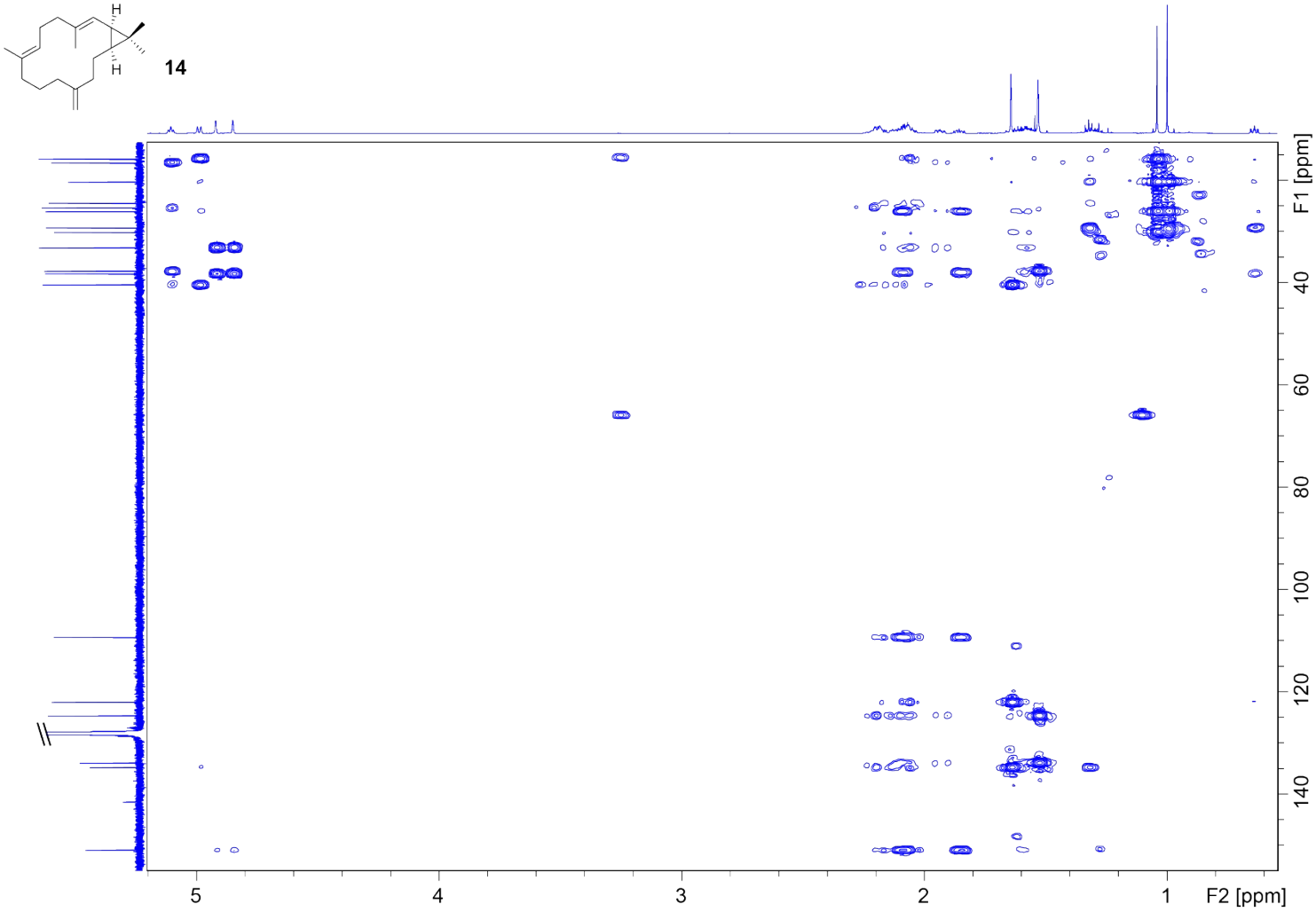


Figure S28. HMBC spectrum of **14** (C_6D_6).

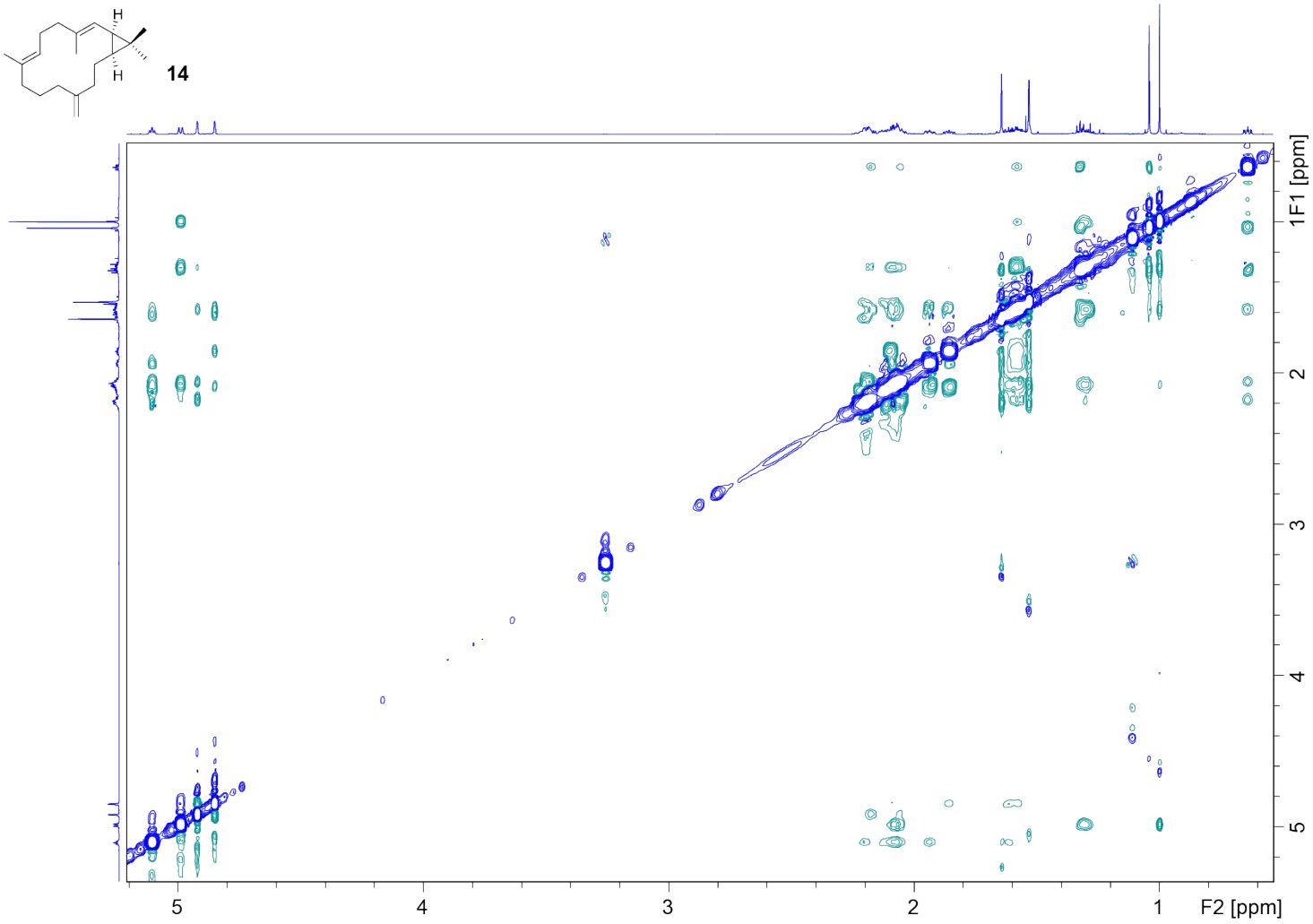
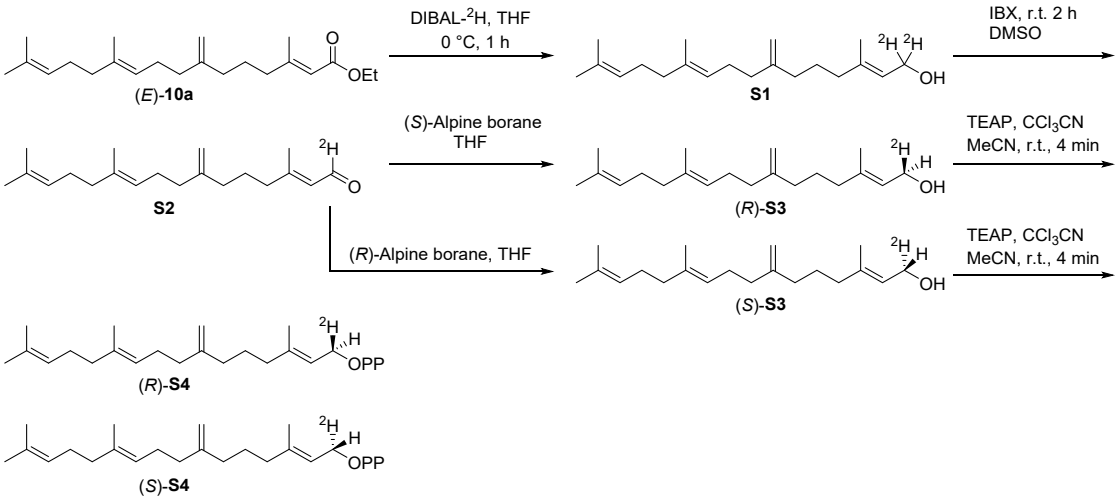


Figure S29. NOESY spectrum of **14** (C_6D_6).



Scheme S1. Synthesis of (*R*)- and (*S*)-(1-^{2}H)iso-GGPP.

Synthesis of (*R*)- and (*S*)-(1-^{2}H)iso-GGPP

Synthesis of (1-^{2}H)-(2*E*,10*E*)-3,11,15-trimethyl-7-methylenehexadeca-2,10,14-trien-1-ol (S1)

To a cooled (0 °C) solution of **10a** (1.00 g, 3 mmol, 1.00 eq) in THF (15 mL) was added DIBAL-^{2}H (0.7 M in toluene, 2.20 eq) and the reaction mixture was stirred for 1 h at room temperature. The mixture was cooled to 0 °C again and a saturated solution of Na-K-tartrate was added. The resulting slurry was stirred for 2 h to dissolve the precipitate and the aqueous phase was extracted with Et_2O (three times). The organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (pentane/Et_2O, 3:1) to yield the alcohol **S1** (667 mg, 2.28 mmol, 76%) as a colorless oil.

S1: TLC (petroleum/EtOAc, 1:1): R_f = 0.48. GC (HP5-MS): *I* = 2204. ^{1}H-NMR (C_6D_6, 500 MHz): 5.37 (br s, 1H), 5.28 (thex, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.2, 1H), 5.23 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.88 (m, 1H), 4.86 (m, 1H), 2.19 (m, 4H), 2.09 (m, 4H), 1.98 (t, $^3J_{H,H}$ = 7.7, 2H), 1.91 (t, $^3J_{H,H}$ = 7.7, 2H), 1.68 (d, $^3J_{H,H}$ = 1.0, 3H), 1.60 (br s, 3H), 1.56 (br s, 3H), 1.52 (m, 2H), 1.45 (d, $^4J_{H,H}$ = 1.4, 3H), 0.55 (br s, OH) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 149.45 (C_q), 138.30 (C_q), 135.27 (C_q), 131.20 (C_q), 124.93 (CH), 124.89 (CH), 124.68 (CH), 109.59 (CH_2), 58.66 (quin, C^2H_2), 40.21 (CH_2), 39.47 (CH_2), 36.50 (CH_2), 36.09 (CH_2), 27.21 (CH_2), 26.84 (CH_2), 26.21 (CH_2), 25.88 (CH_3), 17.77 (CH_3), 16.15 (CH_3), 16.09 (CH_3) ppm.

Synthesis of (1-^{2}H)-(2*E*,10*E*)-3,11,15-trimethyl-7-methylenehexadeca-2,10,14-trienal (S2)

To a solution of IBX (0.89 g, 3.19 mmol, 1.40 eq) in DMSO (16 mL) was slowly added **S1** (667 mg, 2.28 mmol, 1.00 eq; in 4 mL DMSO) and the reaction mixture was stirred for 2 h at room temperature. The reaction was monitored by TLC analysis. Et_2O (30 mL) was added and the organic layer was washed with sat. NH_4Cl. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (petroleum/EtOAc, 10:1) yielded the aldehyde **S2** as a colorless oil.

S2: TLC (petroleum/EtOAc, 10:1): R_f = 0.38. GC (HP5-MS): *I* = 2230. ^{1}H-NMR (C_6D_6, 500 MHz): 5.82 (m, 1H), 5.27 (thex, $^3J_{H,H}$ = 7.0, $^4J_{H,H}$ = 1.2, 1H), 5.24 (thept, $^3J_{H,H}$ = 7.0, $^4J_{H,H}$ = 1.4, 1H), 4.84 (m, 1H), 4.75 (m, 1H), 2.18 (m, 4H), 2.10 (m, 2H), 2.01 (m, 2H), 1.80 (t, $^3J_{H,H}$ = 7.5, 2H), 1.68 (d, $^4J_{H,H}$ = 1.2, 3H), 1.67 (m, 2H), 1.61 (br s, 3H), 1.57 (br s, 3H), 1.50 (d, ^4J = 1.4, 3H), 1.31 (m, 2H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 189.49 (t, $^1J_{C,D}$ = 26.0, C^2HO),

161.47 (C_q), 148.73 (C_q), 135.45 (C_q), 131.27 (C_q), 124.86 (CH), 124.48 (CH), 109.94 (CH_2), 40.20 (CH_2), 39.97 (CH_2), 36.31 (CH_2), 35.78 (CH_2), 27.19 (CH_2), 26.75 (CH_2), 25.88 (CH_2), 25.30 (CH_3), 17.77 (CH_3), 16.82 (CH_3), 16.15 (CH_3) ppm. IR (diamond ATR): $\tilde{\nu}$ = 2965 (w), 2926 (m), 2856 (w), 2093 (w), 1665 (s), 1441 (w), 1380 (w), 1189 (w), 1155 (w), 995 (w), 834 (w) cm^{-1}. HR-MS (APCI): calc. for $[C_{20}H_{31}D_1O_1]^+$ *m*/*z* = 290.2589; found: *m*/*z* = 290.2588.

Synthesis of (*R*)- and (*S*)-(1-^{2}H)-(2*E*,10*E*)-3,11,15-trimethyl-7-methylenehexadeca-2,10,14-trien-1-ol (S3)

To a cooled solution (0 °C) of (*R*)- and (*S*)-Alpine borane (1.25 eq; 0.5 M in THF) in dry THF (1.5 mL $mmol^{-1}$) was added aldehyde **S2** (1.00 eq) slowly and the reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched by adding freshly distilled acetaldehyde (50 µL $mmol^{-1}$), solvents were removed and the residue was subjected to high vacuum for 1 h. The residue was dissolved in Et_2O (2 mL $mmol^{-1}$) and the resulting solution was cooled to 0 °C. After addition of ethanolamine (1.10 eq), stirring was continued for 10 min until a white precipitate was formed. The precipitate was filtered off and washed three times with Et_2O. The combined organic layers were washed with H_2O and brine, followed by drying with $MgSO_4$. The solvent was removed under reduce pressure and the residue was purified by column chromatography (pentane/Et_2O, 3:1) to yield the alcohols **S3** as a colorless oil. The enantiomeric purity was checked by conversion of a small sample (ca. 5 mg) into the Mosher ester with (*R*)-(+)-Mosher chloride (5 mg) and pyridine (1 µL) in C_6D_6 (500 µL). After a reaction time of 2 h, the products were directly analysed by ^{1}H-NMR (Figure S30).

(*R*)-S3: Yield: 130 mg (0.45 mmol, 49%). TLC (petroleum/EtOAc, 1:1): R_f = 0.48. GC (HP5-MS): *I* = 2204. ^{1}H-NMR (C_6D_6, 500 MHz): 5.38 (br d, $^3J_{H,H}$ = 6.8, 1H), 5.28 (thex, $^3J_{H,H}$ = 6.8, $^4J_{H,H}$ = 1.2, 1H), 5.24 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, 1H), 4.88 (m, 1H), 4.86 (m, 1H), 3.95 (br d, $^3J_{H,H}$ = 6.1, 1H), 2.20 (m, 4H), 2.10 (m, 4H), 1.98 (t, $^3J_{H,H}$ = 7.7, 2H), 1.91 (t, $^3J_{H,H}$ = 7.7, 2H), 1.68 (d, $^4J_{H,H}$ = 1.4, 3H), 1.60 (br s, 3H), 1.56 (br s, 3H), 1.52 (m, 2H), 1.45 (br s, 3H), 0.54 (br s, OH) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 149.45 (C_q), 138.24 (C_q), 135.27 (C_q), 131.20 (C_q), 124.96 (CH), 124.93 (CH), 124.68 (CH), 109.59 (CH_2), 59.02 (t, $^1J_{C,D}$ = 21.3, C^{2}HH), 40.21 (CH_2), 39.47 (CH_2), 36.50 (CH_2), 36.09 (CH_2), 27.21 (CH_2), 26.84 (CH_2), 26.21 (CH_2), 25.88 (CH_3), 17.77 (CH_3), 16.15 (CH_3), 16.09 (CH_3) ppm.

(*S*)-S3: Yield: 115 mg (0.40 mmol, 43%). TLC (petroleum/EtOAc, 1:1): R_f = 0.48. GC (HP5-MS): *I* = 2204. Spectroscopic data were the same as for (*R*)-**S3.**

Synthesis of (*R*)- and (*S*)-(1-^{2}H)-iso-GGPP trisammonium salt

Alcohols (*R*)- and (*S*)-**S3** (1.00 eq) were dissolved in CCl_3CN (2.5 mL $mmol^{-1}$) and a solution of bis-triethylammonium phosphate (TEAP; prepared by adding 3.64 mL of a solution of H_3PO_4 (2.5 mL) in MeCN (9.4 mL) to a mixture of NEt_3 (11 mL) and MeCN (10 mL); 2.5 mL $mmol^{-1}$) was added dropwise at room temperature to the stirred solution within 1 min. After stirring for 2 min another portion of TEAP solution was added dropwise (same amount). Stirring was continued with a total reaction time of 4 min (not longer to suppress formation of triphosphate). The reaction mixture was directly subjected to silica gel chromatography (^{i}PrOH/25% NH_3 H_2O/H_2O=6:2.5:0.5). Fractions containing the product were lyophilised to yield the title compounds (ca. 50% purity) as light brown solids.

(*S*)-S4: Yield: 53 mg (from 107 mg (*S*)-**S3**, 96% *ee*). ^{1}H-NMR (D_2O, 500 MHz): 5.38 (d, $^3J_{H,H}$ = 6.1 1H), 5.10 (m, 2H), 4.74 (br s, 1H), 4.71 (br s, 1H), 4.47 (m, 1H), 2.03 (m, 12H), 1.67 (br s, 3H), 1.65 (br s, 3H), 1.59 (br s, 3H), 1.57 (br s, 3H) ppm. ^{13}C-NMR (D_2O, 125 MHz): 149.03 (C_q), 141.54 (C_q), 134.74 (d, *J* = 30.3, C_q), 130.74 (C_q), 124.51 (CH), 124.26 (CH), 120.19 (d, $^3J_{P,C}$ = 9.5, CH), 108.99 (CH_2), 39.90 (CH_2), 39.75 (CH_2), 39.25 (CH_2),

36.23 (CH_2), 35.73 (CH_2), 26.75 (CH_2), 26.30 (CH_2), 25.75 (CH_2), 25.47 (CH_3), 17.43 (CH_3), 15.93 (CH_3), 15.84 (CH_3) ppm. ^{31}P-NMR (D_2O, 203 MHz): –10.5 (d, $^2J_{P,P}$ = 18.3 Hz), –11.2 (d, $^2J_{P,P}$ = 18.2 Hz) ppm.

(*R*)-S4: Yield: 30 mg (from 130 mg (*S*)-**S3**, 96% *ee*). Spectroscopic data were the same as for (*S*)-**S4**.

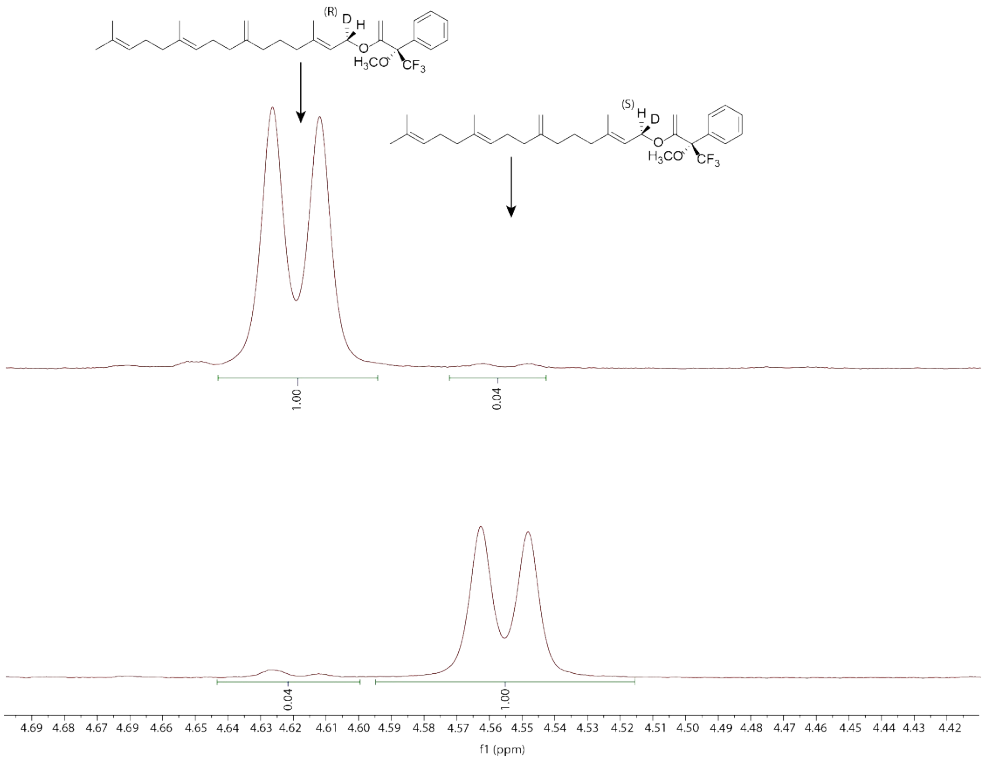
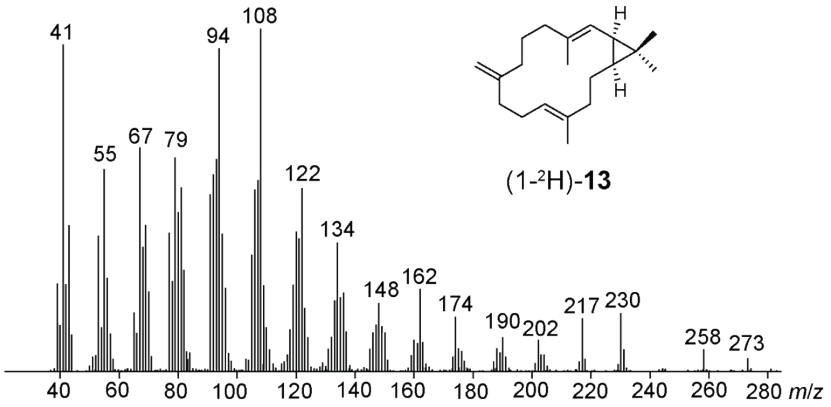


Figure S30. Mosher ester analysis of (*R*)- and (*S*)-(1-^{2}H)iso-GGPP.

A) (*R*)-(1-^{2}H)GGPP



B) (*S*)-(1-^{2}H)-GGPP

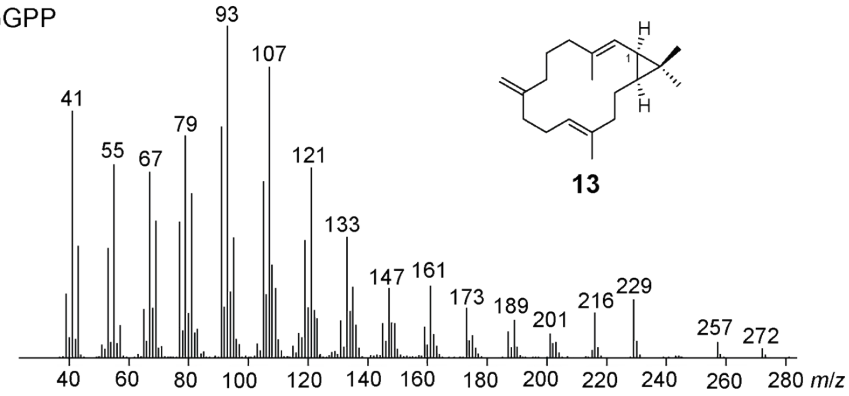
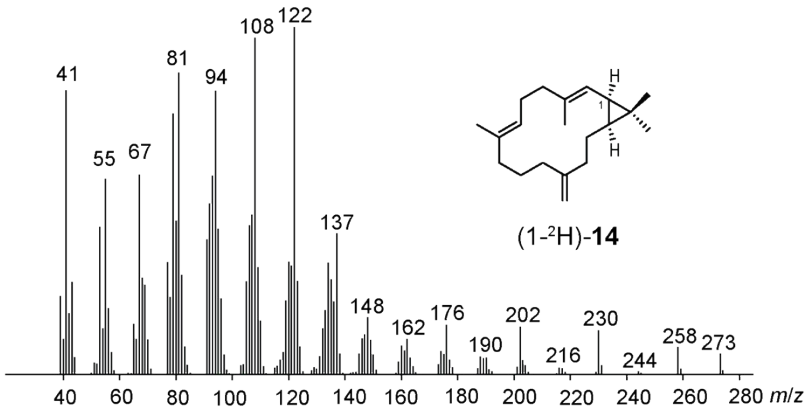


Figure S31. Conversion of (*R*)- and (*S*)-(1-^{2}H)iso-GGPP with CS. EI mass spectrum of A) (1-^{2}H)-**13** obtained from (*R*)-(1-^{2}H)iso-GGPP and B) unlabelled **13** obtained from (*S*)-(1-^{2}H)iso-GGPP.

A) iso-FPP + (*R*)-(1-^{2}H)IPP



B) iso-FPP + (*S*)-(1-^{2}H)IPP

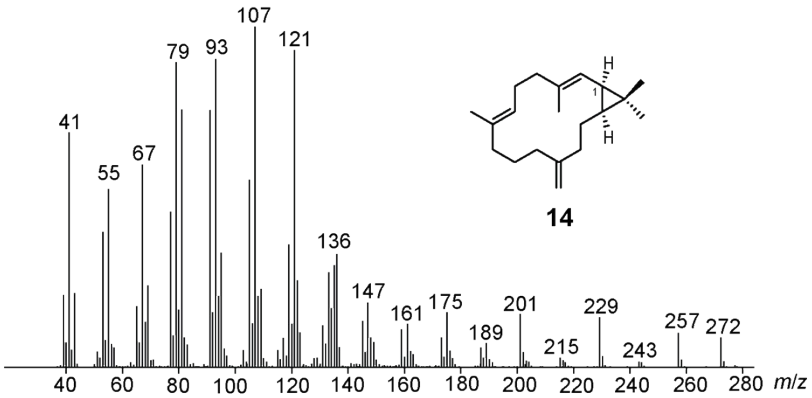
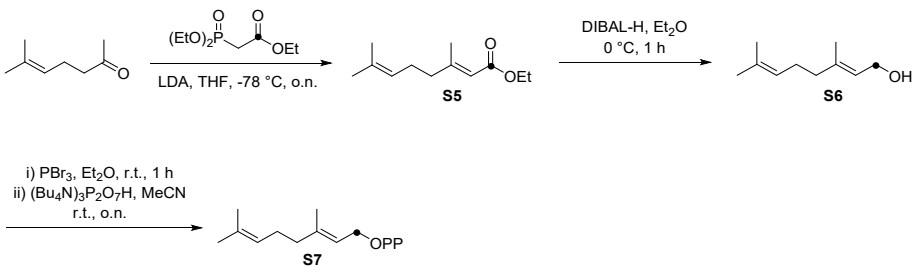


Figure S32. Conversion of iso-FPP and (*R*)- and (*S*)-(1-^{2}H)IPP with GGPPS and CS. EI mass spectrum of A) (1-^{2}H)-**14** obtained from iso-FPP and (*R*)-(1-^{2}H)IPP and B) unlabelled **14** obtained from iso-FPP and (*S*)-(1-^{2}H)IPP.



Scheme S2. Synthesis of (1-^{13}C)GPP. Black dots indicate ^{13}C-labelled carbons.

Synthesis of (1-^{13}C)GPP

Synthesis of ethyl (3-^{13}C)-(*E*)-3,7-dimethylocta-2,6-dienoate (S5)

A solution of diisopropylamine (3.15 mmol; 1.05 eq) dissolved in dry THF (16 mL) was cooled to 0 °C. *n*-Buli (1.95 mL; 1.6 M in hexane, 1.05 eq) was added dropwise and the reaction was stirred for 1 h at 0 °C. The reaction mixture was cooled to –78 °C and triethyl (3-^{13}C)phosphonoacetate (3 mmol; 1.00 eq) was added. After stirring the reaction mixture for 1 h at –78 °C, sulcatone (3 mmol; 1.00 eq) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight. Water (15 mL) was used to quench the reaction. The aqueous phase was extracted with Et_2O (three times), and the combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc, 20:1) yielded pure **S5** (7:3 mixture of *E* and *Z* stereoisomers) as colorless oils.

S5: Yield: 336 mg (1.59 mmol, 53%; 7:2 mixture of *E*/*Z*-stereoisomers). TLC (cyclohexane/EtOAc, 20:1): R_f = 0.35 (*E*-stereoisomer) and 0.42 (*Z*-stereoisomer). GC (HP5-MS): *I* = 1333 (*Z*-stereoisomer) and 1376 (*E*-stereoisomer). ^{1}H-NMR (C_6D_6, 500 MHz): 5.82 (dhex, $^2J_{C,H}$ = 2.3, $^4J_{H,H}$ = 1.2, *E*, 1H). 5.75 (m, *Z*, 1H), 5.28 (thept, $^3J_{H,H}$ = 7.3, $^4J_{H,H}$ = 1.5, *Z*, 1H), 5.02 (thept, $^3J_{H,H}$ = 6.9, $^4J_{H,H}$ = 1.4, *E*, 1H), 4.04 (dq, $^3J_{C,H}$ = 3.2, $^3J_{H,H}$ = 7.1, *E*, 2H), 4.03 (dq, $^3J_{C,H}$ = 3.1, $^3J_{H,H}$ = 7.2, *Z*, 2H), 2.79 (t, $^3J_{H,H}$ = 7.7, *Z*, 2H), 2.22 (m, *Z*, 2H), 2.20 (dd, $^4J_{H,H}$ = 1.2, $^4J_{C,H}$ = 1.2, *E*, 3H), 1.99 (m, *E*, 2H), 1.90 (m, *E*, 2H), 1.64 (d, $^4J_{H,H}$ = 1.0, *Z*, 3H), 1.60 (br s, *E* and *Z*, 3H), 1.54 (d, $^4J_{H,H}$ = 1.1, *Z*, 3H), 1.45 (br s, *E*, 3H), 1.01 (t, $^3J_{H,H}$ = 7.1, *E*, 3H), 1.00 (t, $^3J_{H,H}$ = 7.1, *Z*, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 166.49 (*E*, ^{13}C, C_q), 166.04 (*Z*, ^{13}C, C_q), 159.87 (d, $^2J_{C,C}$ = 2.3, *Z*, C_q), 159.35 (d, $^2J_{C,C}$ = 2.0, *E*, C_q), 132.14 (*E*, C_q), 132.03 (*Z*, C_q), 124.41 (*Z*, CH), 123.65 (*E*, CH), 116.9 (d, $^1J_{C,C}$ = 76.0, *Z*, CH), 116.34 (d, $^1J_{C,C}$ = 75.6, *E*, CH), 59.38 (d, $^2J_{C,C}$ = 2.3, *E*, CH_2), 59.36 (d, $^2J_{C,C}$ = 2.3, *Z*, CH_2), 41.00 (d, $^3J_{C,C}$ = 7.1, *E*, CH_2), 33.75 (d, $^3J_{C,C}$ = 1.3, *Z*, CH_2), 27.34 (*Z*, CH_2), 26.34 (*E*, CH_2), 25.83 (*Z*, CH_3), 25.74 (*E*, CH_3), 25.07 (d, $^3J_{C,C}$ = 7.6, *Z*, CH_3), 18.79 (d, $^3J_{C,C}$ = 1.6, *E*, CH_3), 17.70 (*Z*, CH_3), 17.65 (*E*, CH_3), 14.44 (d, $^3J_{C,C}$ = 2.2, *E*, CH_3), 14.43 (d, $^3J_{C,C}$ = 2.2, *Z*, CH_3) ppm.

Synthesis of (1-^{13}C)geraniol (S6)

The procedure is the same with “General procedure for DIBAL-H reactions”. The *E* and *Z* stereoisomers were separated by column chromatography, yielding pure (*E*)-**S6** (153 mg, 0.99 mmol, 58%) and (*Z*)-**S6** (44 mg, 0.28 mmol, 17%). TLC (pentane/Et_2O, 1:1): R_f = 0.57 (*E*-stereoisomer) and 0.68 (*Z*-stereoisomer).

(*E*)-S6: GC (HP5-MS): *I* = 1241. ^{1}H-NMR (C_6D_6, 500 MHz): 5.39 (m, 1H), 5.17 (m, 1H), 4.11 (m, 1H), 3.82 (m, 1H), 2.10 (m, 2H), 1.98 (m, 2H), 1.66 (t, *J* = 1.4, 3H), 1.53 (m, 3H), 1.46 (m, 3H) ppm. ^{13}C-NMR (C_6D_6, 125 MHz): 138.12 (d, $^2J_{C,C}$ = 1.4, C_q), 131.45 (C_q), 124.96 (d, $^1J_{C,C}$ = 47.5, CH), 124.60 (CH), 59.39 (^{13}C, CH_2), 39.89 (d, $^3J_{C,C}$ = 4.7, CH_2), 26.86 (CH_2), 25.83 (CH_3), 17.73 (CH_3), 16.17 (d, $^3J_{C,C}$ = 4.2, CH_3) ppm.

Synthesis of (1-^{13}C)GPP (S7)

The procedure is the same with “General procedure for synthesis of diphosphates”. Yield: 349 mg (0.96 mmol, 97%, 1:1 mixture of diphosphate and monophosphate). The compound identity was shown by the possible conversion of the material plus IPP using GGPPS and CS into labelled casbene **1**. ^{1}H-NMR (D_2O, 500 MHz, mixture of mono- and diphosphate): 5.46 (t, $^{3}J_{H,H}$ = 6.6, 1H), 5.41 (t, $^{3}J_{H,H}$ = 7.1, 1H), 5.21 (t, $^{3}J_{H,H}$ = 6.4, 1H), 5.18 (t, $^{3}J_{H,H}$ = 7.1, 1H), 4.46 (ddd, $^{1}J_{C,H}$ = 146.2, $^{3}J_{H,H}$ = 6.6, $^{3}J_{P,H}$ = 6.6, 1H), 4.39 (ddd, $^{1}J_{C,H}$ = 146.7, $^{3}J_{H,H}$ = 8.1, $^{3}J_{P,H}$ = 8.1, 1H) ppm. ^{13}C-NMR (D_2O, 125 MHz): 143.2 (d, $^{2}J_{C,C}$ = 1.7, C_q), 142.7 (d, $^{2}J_{C,C}$ = 1.5), 133.8 (C_q), 124.2 (CH), 124.1 (CH), 120.0 (dd, $^{1}J_{C,C}$ = 49.8, $^{3}J_{P,C}$ = 8.3, CH), 119.3 (dd, $^{1}J_{C,C}$ = 49.1, $^{3}J_{P,C}$ = 6.4, CH), 62.5 (d, $^{2}J_{P,C}$ = 5.3, $^{13}CH_2$), 60.9 (d, $^{2}J_{P,C}$ = 4.1, $^{13}CH_2$), 38.8 (d, $^{3}J_{C,C}$ = 4.9, CH_2), 38.7 (d, $^{3}J_{C,C}$ = 4.9, CH_2), 25.6 (CH_2), 25.5 (CH_2), 24.88 (CH_3), 24.86 (CH_3), 17.0 (CH_3), 15.6 (d, $^{3}J_{C,C}$ = 4.3, CH_3), 15.5 (d, $^{3}J_{C,C}$ = 4.2, CH_3) ppm. ^{31}P-NMR (D_2O, 203 MHz): +6.05 (d, $^{2}J_{P,C}$ = 4.1, 1P), –6.56 (d, $^{2}J_{P,P}$ = 22.0, 1P), –10.26 (dd, $^{2}J_{P,P}$ = 22.0, $^{2}J_{P,C}$ = 5.1, 1P) ppm.

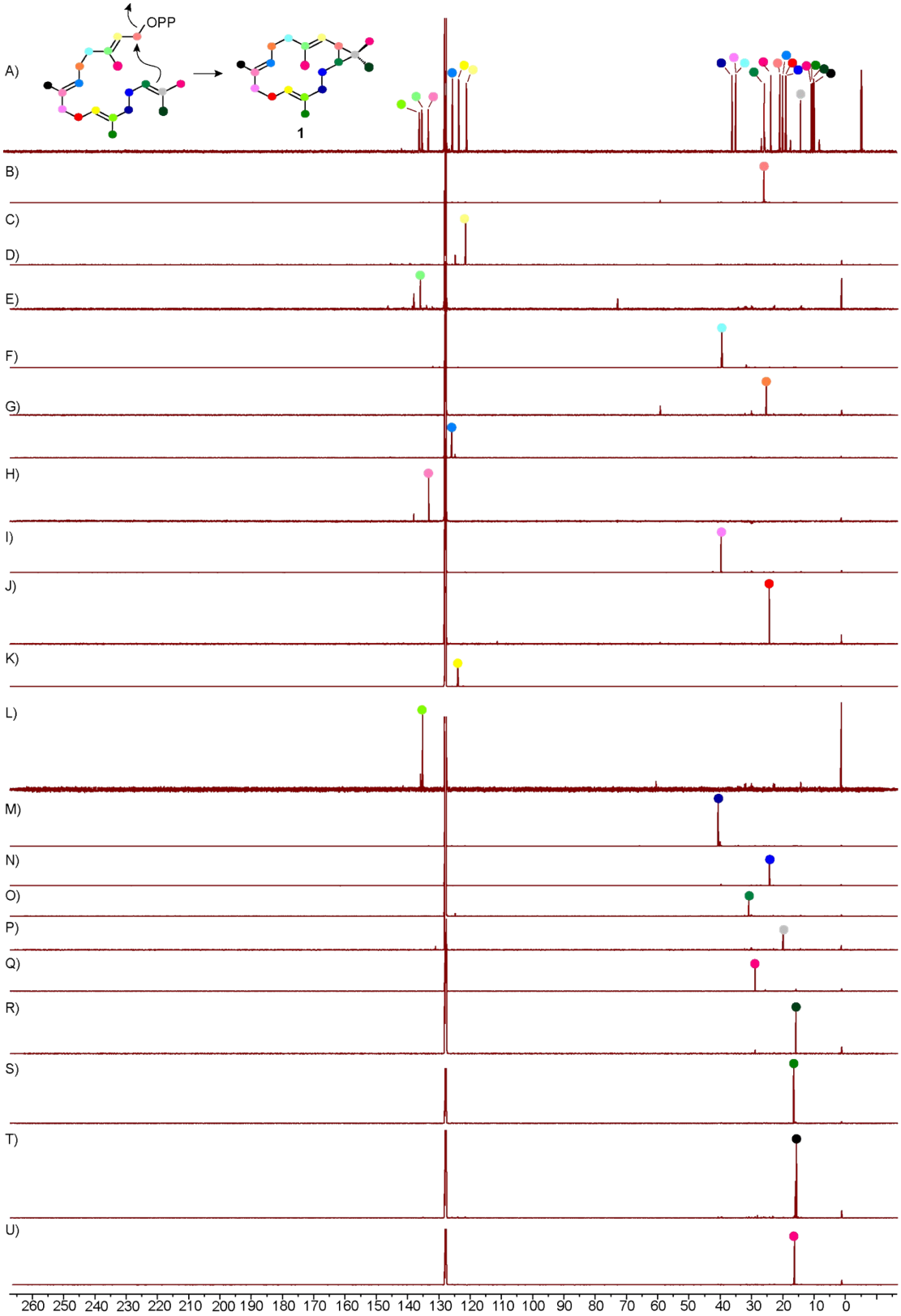


Figure S33. ^{13}C-NMR spectra of A) unlabelled **1**, and B) to U) labelled **1** from the twenty isotopomers of (^{13}C)GGPP. Coloured dots correlate peaks to the individual carbons of **1**.

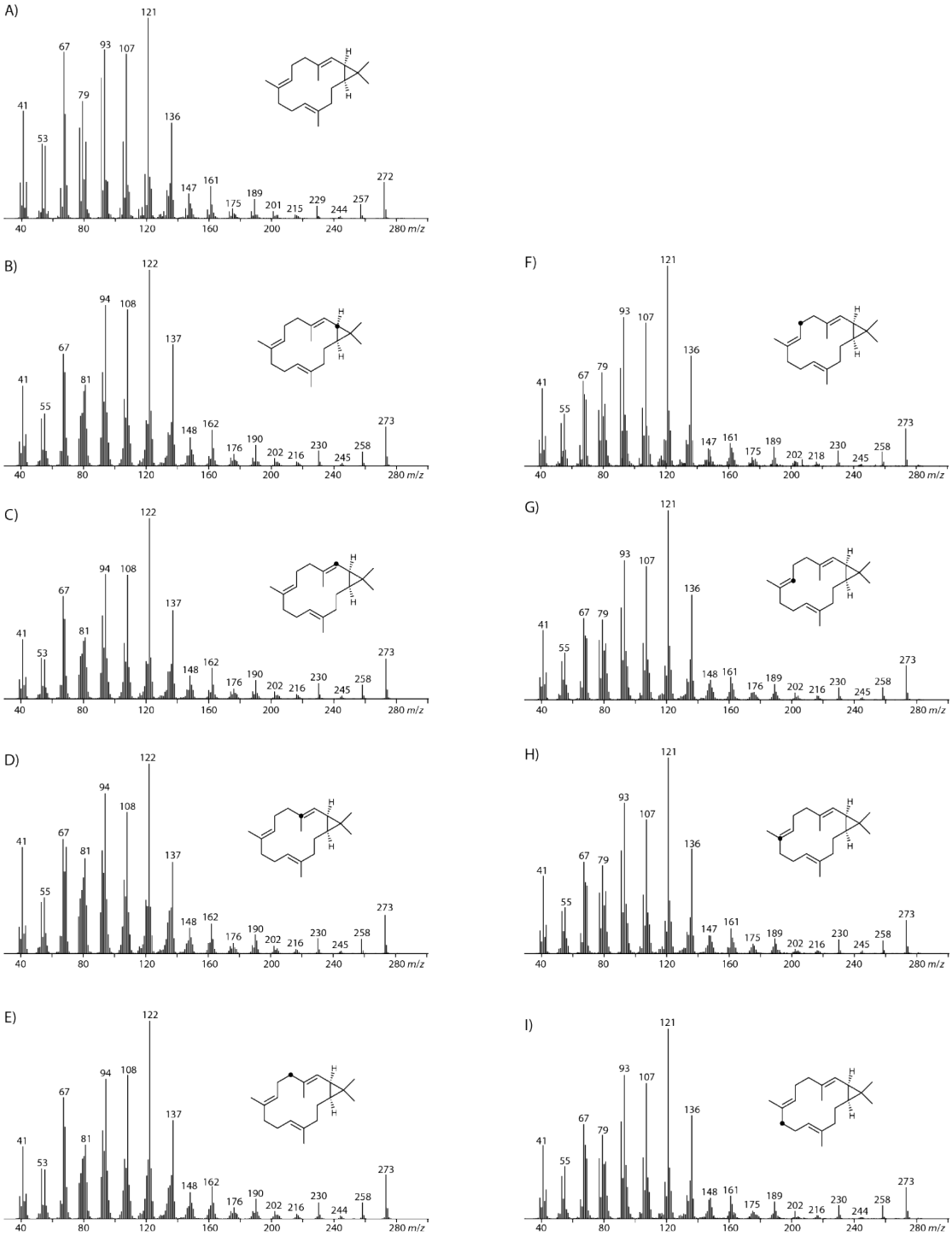


Figure S34. EI mass spectra of A) unlabelled **1**, B) (1-^{13}C)-**1**, C) (2-^{13}C)-**1**, D) (3-^{13}C)-**1**, E) (4-^{13}C)-**1**, F) (5-^{13}C)-**1**, G) (6-^{13}C)-**1**, H) (7-^{13}C)-**1**, and I) (8-^{13}C)-**1**. Labelled compounds were prepared enzymatically from the corresponding isotopomers of (^{13}C)GGPP with CS. Black dots indicate labelled carbons of **1**.

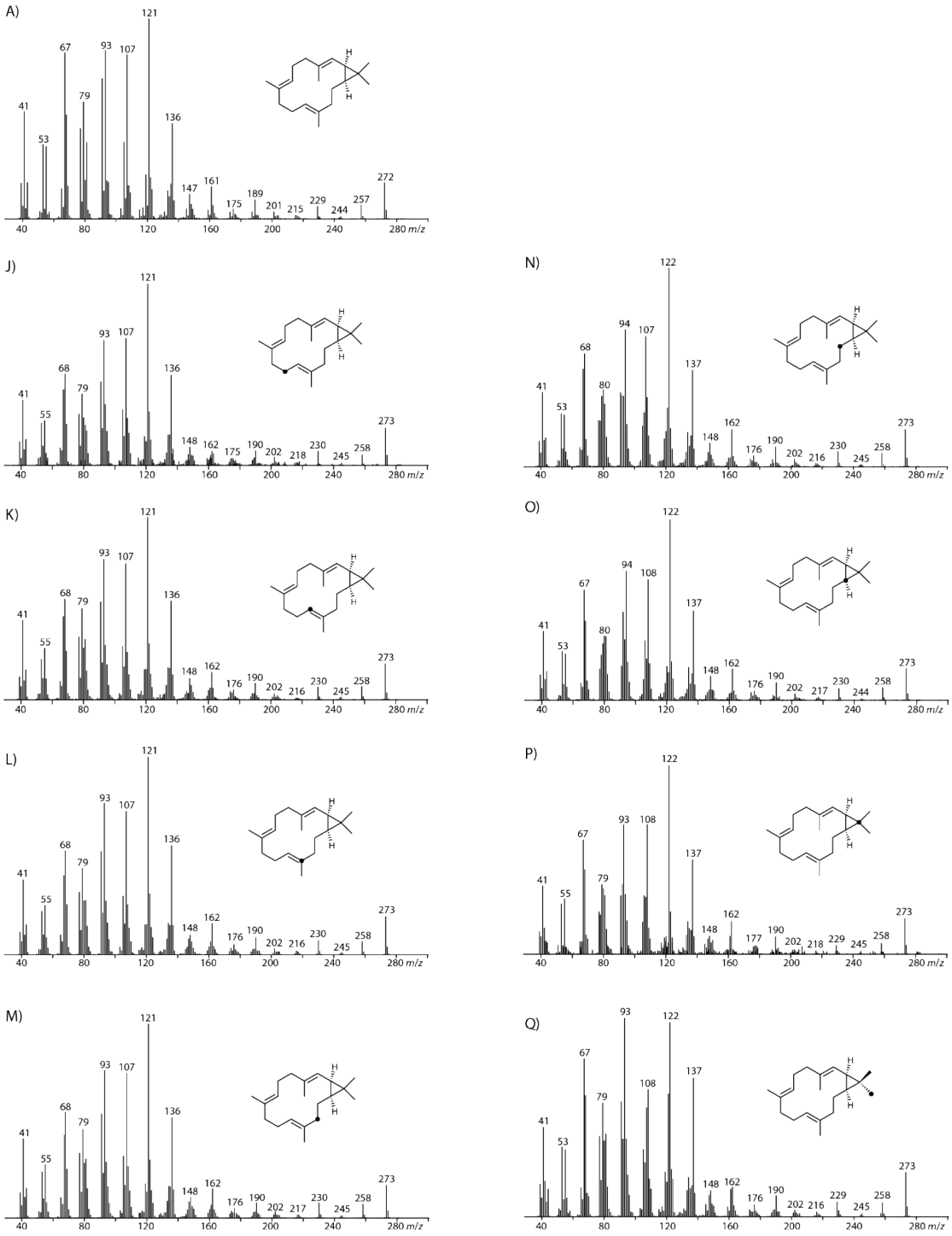


Figure S34 (continued). EI mass spectra of A) unlabelled **1**, J) (9-^{13}C)-**1**, K) (10-^{13}C)-**1**, L) (11-^{13}C)-**1**, M) (12-^{13}C)-**1**, N) (13-^{13}C)-**1**, O) (14-^{13}C)-**1**, P) (15-^{13}C)-**1**, and Q) (16-^{13}C)-**1**. Labelled compounds were prepared enzymatically from the corresponding isotopomers of (^{13}C)GGPP with CS. Black dots indicate labelled carbons of **1**.

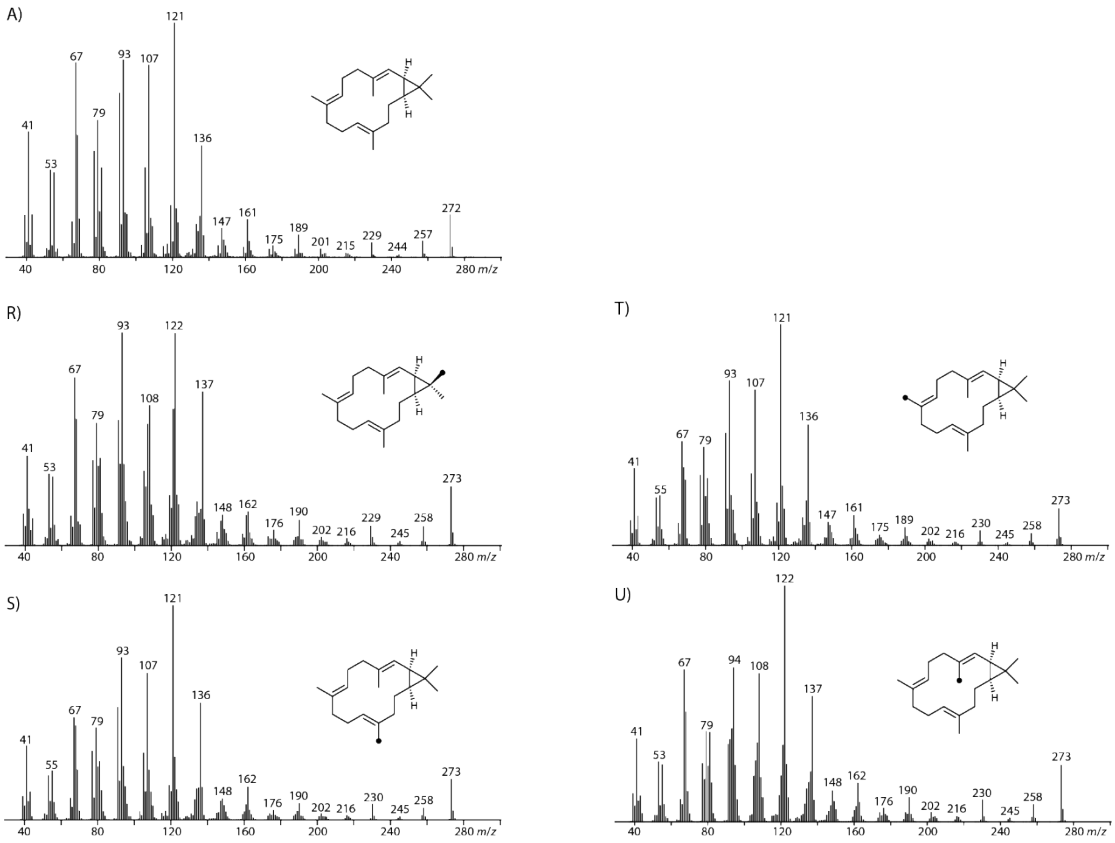


Figure S34 (continued). EI mass spectra of A) unlabelled **1**, R) (17-^{13}C)-**1**, S) (18-^{13}C)-**1**, T) (19-^{13}C)-**1**, and U) (20-^{13}C)-**1**. Labelled compounds were prepared enzymatically from the corresponding isotopomers of (^{13}C)GGPP with CS. Black dots indicate labelled carbons of **1**.

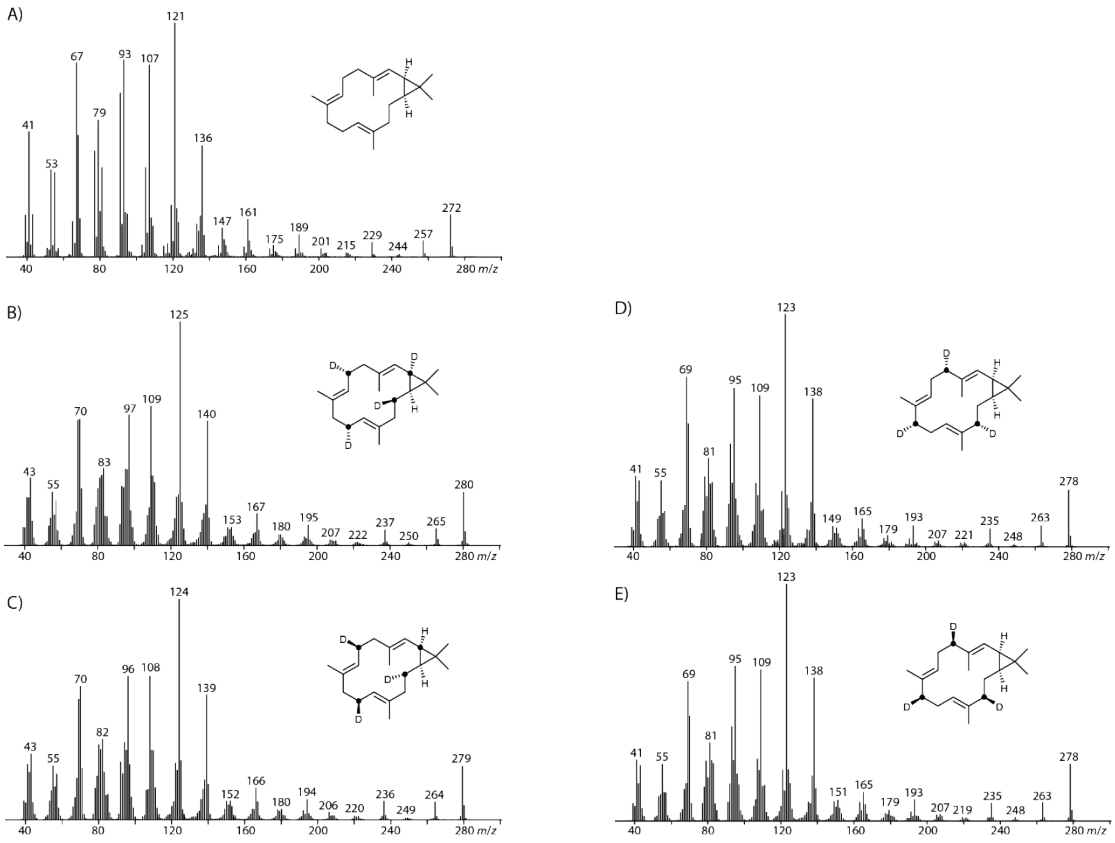


Figure S35. EI mass spectra of A) unlabelled **1**, labelled **1** obtained with IDI, GGPPS and CS from B) (*R*)-(1-^{13}C,1-^{2}H)IPP and C) from (*S*)-(1-^{13}C,1-^{2}H)IPP, labelled **1** obtained with GGPPS and CS from DMAPP and D) (*E*)-(4-^{13}C,4-^{2}H)IPP and F) (*Z*)-(4-^{13}C,4-^{2}H)IPP.

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