Supplementary Information for:

Ancient plant-like terpene biosynthesis in corals

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Contents

Supplementary Tables

Supplementary Table 1. Uniprot accession numbers of TC sequences used for constructing the TC profile HMM.

A0A1L7U8F2
A0A1L7VZE7
E4MYY0
A0A0H5BN61
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A0A2N3Y098
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B1W019
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S0DX56
A9FZ87
A8NU13
A7NH01
A0A0L6WB32
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A1C8C3
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Supplementary Table 2. Public genome assemblies used for genome mining.

Coral species	Number of TC genes (number including paralogs)	Acc no.	reference
Dendronephthya gigantea	2 (2)	NCBI acc no.: GCA_004324835.1	[1]
Renilla muelleri	1 (1)	Downloaded from: http://rmue.reefgenomics.org/	[2]
Renilla reniformis	1 (1)	NCBI acc no.: GCA_900177555.1	[3]
Xenia sp.	1 (2)	Downloaded from: https://cmo.carnegiescience.edu/data	[4]
Trachythela sp. YZ-2020	6 (12)	NCBI acc no.: GCA_016169945.1	[5]
Paramuricea clavata	4 (8)	Downloaded from: https://denovo.cnag.cat/pclavata	[6]

Supplementary Table 3. Public data used for transcriptome mining.

Species	Number of TC	Assembly info	SRA dataset	reference	Terpenoids reported
Alcyonium palmatum	0	Assembled in this work	SRR3407216	[7]	Yes [8]
Acanthogorgia aspera	1 (1 partial)	NCBI acc no.: GEXC01	SRR3303654	-	From genus [9]
Briareum asbestinum	2	NCBI acc no.: GHBD02	SRR7895344	[10]	Yes [11,12]
Callogorgia delta	3	Assembled in this work	SRR13925246	[13]	No
Clavularia sp.	1 (1 partial)	NCBI acc no.: GHAW01	SRR8102779, SRR8102780	[14]	From genus [15,16]
Corallium rubrum	1	Downloaded from: https://doi.org/10.5061/dryad.31f77	SRR1552945	[17]	From genus [18]
Dendronephthya gigantea	2 (1 partial)	Assembled in this work	SRR8293935	[1]	From genus [19,20]
Eleutherobia rubra	2	NCBI acc no.: GHFI01	SRR8486076	[21]	From genus [22]
Eunicea calyculata	1	Assembled in this work	SRR6039601	[23]	Yes [24]
Eunicea flexuosa	1	Assembled in this work	SRR12876635	-	From genus [24]
Eunicella cavolinii	1 (1 partial)	Assembled in this work	SRR1324943	[25]	Yes [26]
Gorgonia ventalina	3	Assembled in this work	SRR935083	-	Yes [27]
Heliopora coerulea	3	NCBI acc no.: IABP01	DRR072217, DRR072218, DRR072219	[28]	Yes [29]
Paramuricea biscaya	2	Assembled in this work	SRR7521178	[30]	From genus [31]
Paramuricea clavata	3 (1 partial)	Assembled in this work	ERR3664728	[6]	From genus [31]
Paramuricea type B3	1 (1 partial)	Assembled in this work	SRR13925244	[13]	From genus [31]
Pinnigorgia flava	0	Assembled in this work	ERX4193767	[32]	no
Renilla koellikeri	1 (1 partial)	Assembled in this work	SRR7495167	-	From genus [33]
Renilla reniformis	0	Assembled in this work	ERR2808321	[2]	Yes [33]
Sarcophyton trocheliophorum	1	Assembled in this work	SRR12573946	-	Yes [34]
Sinularia cruciata	1 (1 partial)	Assembled in this work	ERR3026434	[35]	From genus [36]
Tubipora musica	2	Assembled in this work	ERR3026435	[35]	Yes [37]
Xenia sp.	1	Assembled in this work	SRR9278441	[4]	From genus [38]

Supplementary Table 4. Details for TC coding contigs from octocoral genomes.

scaffold	Organism and reference	length (kbp)	coded TC	GC content (%)	coverage (avg. Reads per base)	Dataset used for transcript mapping	Dataset used for long read mapping
NW_021163036	*D. gigantea*[1]	2263	DgTC-1	38	90 (pacbio)	SRR8293935	SRR8293699
NW_021163042	*D. gigantea*[1]	2228	DgTC-2	37	100 (pacbio)	SRR8293935	SRR8293699
HiC_scaffold_25	*Xenia sp.*[4]	4749	XsTC-1	35	88 (ONP)	SRR9278441	SRR9278438

Supplementary Table 5. Genetic context of XsTC-1 on contig HiC_scaffold_25.

Sequence	GC%	# exons	Region (bp)	Best scoring BLAST hit:	Sequence name	organism	E-value	NCBI accession no.
XsTC-1 -1	39	8	402750-397351		uncharacterized protein LOC122962660	*Acropora millepora*	1x10-^{128}	XP_044181808.1
XsTC-1	42	1	421582-420354		terpene synthase family protein	*Streptosporangium nondiastaticum*	0.1	WP_106680131.1
XsTC-1 +1	38	1	426242-425778		hypothetical protein KOW79	*Hemibagrus wyckioides*	0.076	KAG7326961.1
XsTC-1 +2	40	1	426516-427094		no hit			
XsTC-1 +3	53	5	436341-433328		no hit			
XsTC-1 +4	50	5	442821-440118		microspherule protein 1-like	*Plakobranchus ocellatus*	8x10-^{31}	GFN97869.1
XsTC-1 +5	39	23	445833-486627		transmembrane protein 87A-like	*Actinia tenebrosa*	0.0	XP_031573117.1

Supplementary Table 6. Genetic context of DgTC-1 on contig NW_021163036.

Sequence	GC%	# exons	Region (bp)	Best scoring BLAST hit:	Sequence name	organism	E-value	NCBI accession no.
-3	47	15	1163131-1171572		rho GTPase-activating protein 39-like	*Acropora millepora*	5x10^{-154}	XP_029180732.2
-2	43	10	1178909-1174089		ubiquitin thioesterase otulin-like	*Zootermopsis nevadensis*	3x10^{-37}	XP_021939107.1
-1	45	1	1180563-1179595		retinol dehydrogenase 12-like	*Crassostrea gigas*	5x10^{-111}	XP_011420247.2
DgTC-1	46	1	1181590-1182822		HEAT repeat domain-containing protein	*Cand. Lokiarchaeota archaeon*	0.57	NMC08674.1
+1	46	14	1191885-1199119		suppressor of hairless protein	*Saccoglossus kowalevskii*	0.0	NP_001158483.1
+2	46	16	1201421-1221069		ABC transporter G family member 24	*Nematostella vectensis*	0.0	XP_001642016.2

Supplementary Table 7. Genetic context of DgTC-2 on contig NW_021163042.

Sequence	**GC%**	**# exons**	**Region (bp)**	**Best scoring BLAST hit:**	**Sequence name**	**organism**	**E-value**	**NCBI accession no.**
-2	41	10	1129642-1131952		protein-glutamine gamma-glutamyltransferase K	*Phallusia mammillata*	7x10^{-173}	CAB3266928.1
-1	42	3	1144401-1150450		uncharacterized protein LOC110068857	*Orbicella faveolata*	1x10^{-18}	XP_020631939.1
DgTC-2	39	1	1154905-1153709		nucleotidyltransferase family protein	*Oscillospiraceae bacterium*	8.9	MBR3419154.1
+1	41	6	1156399-1158527		15-hydroxyprostaglandin dehydrogenase	*Cyprinodon variegatus*	4x10^{-51}	XP_015226913.1
+2	37	6	1164817-1167808		peroxisomal biogenesis factor 19-like	*Acropora millepora*	1x10^{-56}	XP_029188873.2
+3	37	1	1169969-1171558		zinc finger protein 271-like	*Acyrthosiphon pisum*	5x10^{-108}	XP_008180596.1
+4	39	9	1172532-1177997		proton-coupled amino acid transporter 1-like	*Orbicella faveolata*	6x10-^{110}	XP_031553692.1

Supplementary Table 8. PISA server results.

Enzyme	$\Delta^i G$ (kcal/mol)	Interface (Å^2)	State in solution	reference
ErTC-2 (7s5l)	-6.5	597		This work
epi-isozizaene synthase (4lz0)	-2.2	485	monomeric	39
2-MIB synthase (4la6)	-37.2	2549	parallel homodimeric	40
trichodiene synthase (2q9z)	-40.9	2606	antiparallel homodimeric	41

Supplementary Table 9. RSMDs of ErTC2 with microbial (red) plant (green) and IDS (black) structures.

RMSD (Å)^{a}	4lz0 EIZS	4okm	4kux	5eau	3p5r TXDS	1rqj	4jzb	5ja0 FPPS
ErTC2	4.190	4.630	4.707	4.221	5.011	9.723	7.665	16.417

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Supplementary Table 10. Accession numbers for non-coral sequences used for phylogenetic analysis.

E2E2N7
B6SCF5
I6RAQ6
J7LQ09
Q5SBP3
P59287
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P22939
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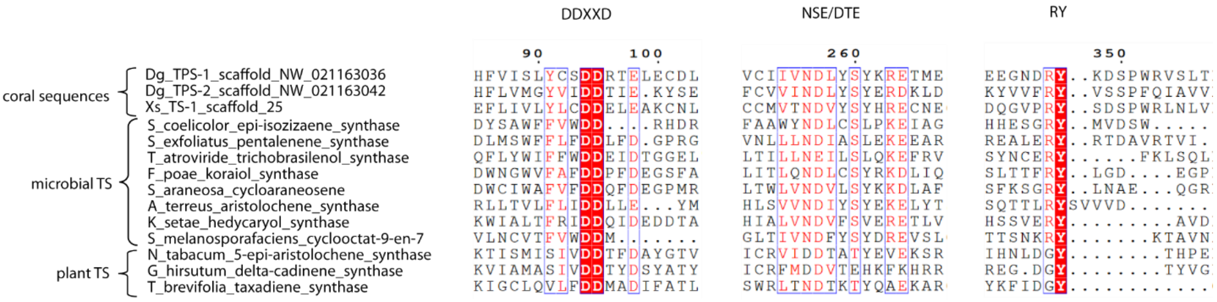
APB88779
APB88780
AVZ23977
AYD76069
ALL35411
ALL35417
ALL35420
ALL35414

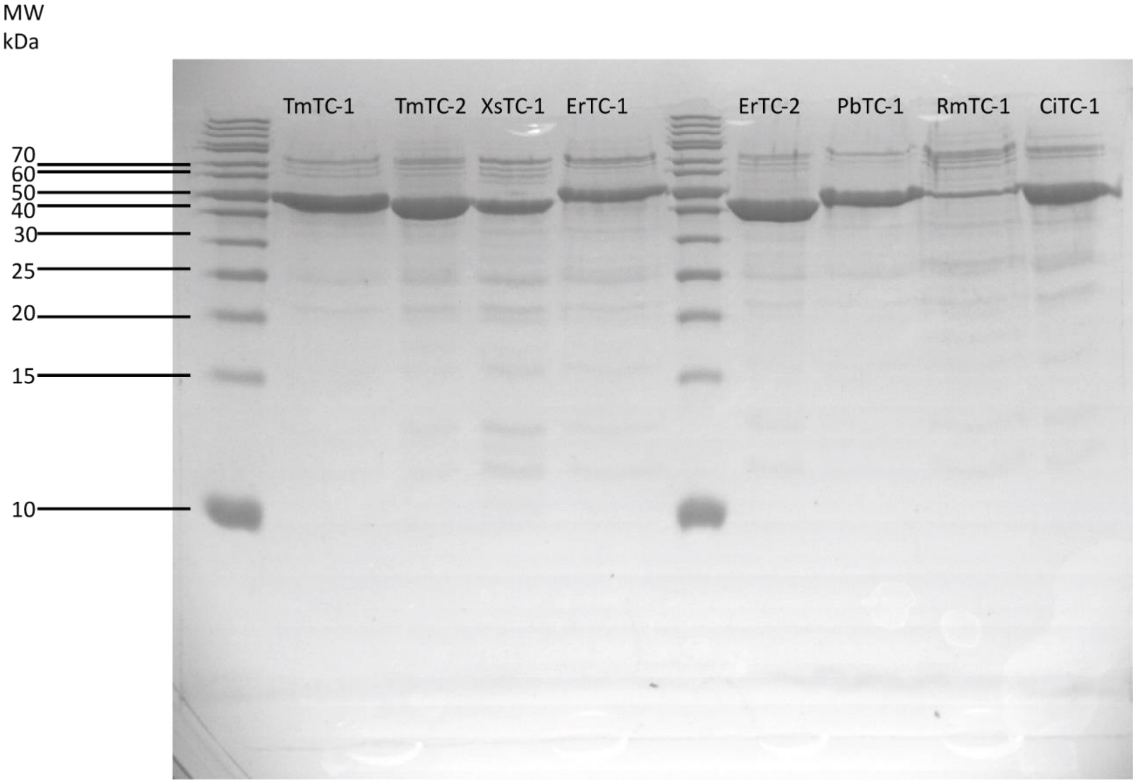
Supplementary Table 11. Data collection and model refinement statistics of coral terpene synthases.

	Cd(II)-ErTC-2	ErTC-2
PDB ID	-	7S5L
Beamline	UCSD X-ray Crystallography Facility	SSRL 12-2
Wavelength (Å)	1.54178	0.97946
Space Group	C2	C2
Cell dimensions (Å)	a = 82.75, b = 99.70, c = 46.48, β = 92.29°	a = 85.69, b = 101.65, c = 46.34, β = 90.60°
Resolution	33.98-2.57 (2.67-2.57)	40.0-1.58 (1.67-1.58)
# unique reflections	23522 (2453)	54425 (8769)
Completeness (%)	99.6 (96.8)	96.1 (94.1)
Redundancy	15.3 (8.8)	3.9 (3.9)
<I/sigI>	20.8 (3.9)	14.7 (2.6)
R_{sym}	0.073 (0.376)	0.046 (0.390)
$CC_{1/2}$	*	(0.90)
Resolution		37.97-1.58
# unique reflections		52284
R_{work} (%) / R_{free} (%)		16.1/19.2
RMS bond length (Å)		0.005
RMS bond angles (°)		0.697
# of atoms		
Protein Atoms		3152
Diphosphate		-
Ethylene glycol		12
Water molecules		188
Mg^{2+}		-
Cl^-		1
Average B-factor (Å^2)		
Protein Atoms		30
Diphosphate		-
Ethylene glycol		38
Water molecules		39
Mg^{2+}		-
Cl^-		28
Ramachandran plot		
Favored (%)		98.12
Allowed (%)		1.88
Outliers (%)		0.00
Rotamer outliers (%)		0.00
Residues modeled (1-393)		2-195, 201-381, 389-391

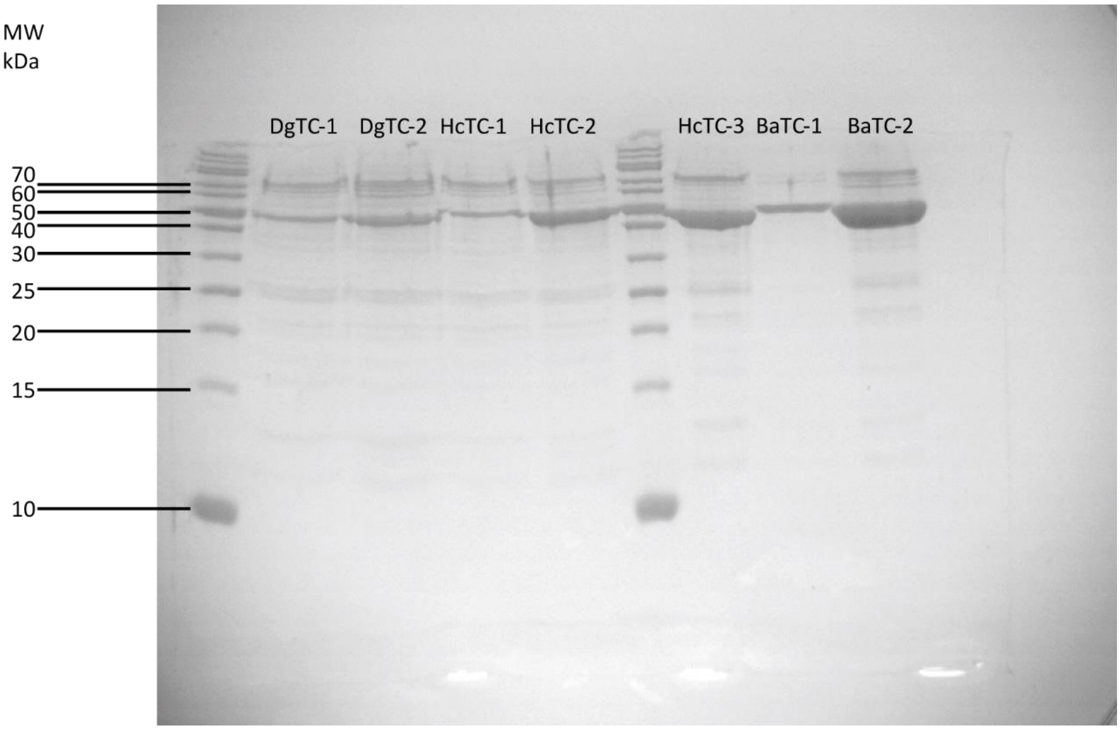
Supplementary Figures



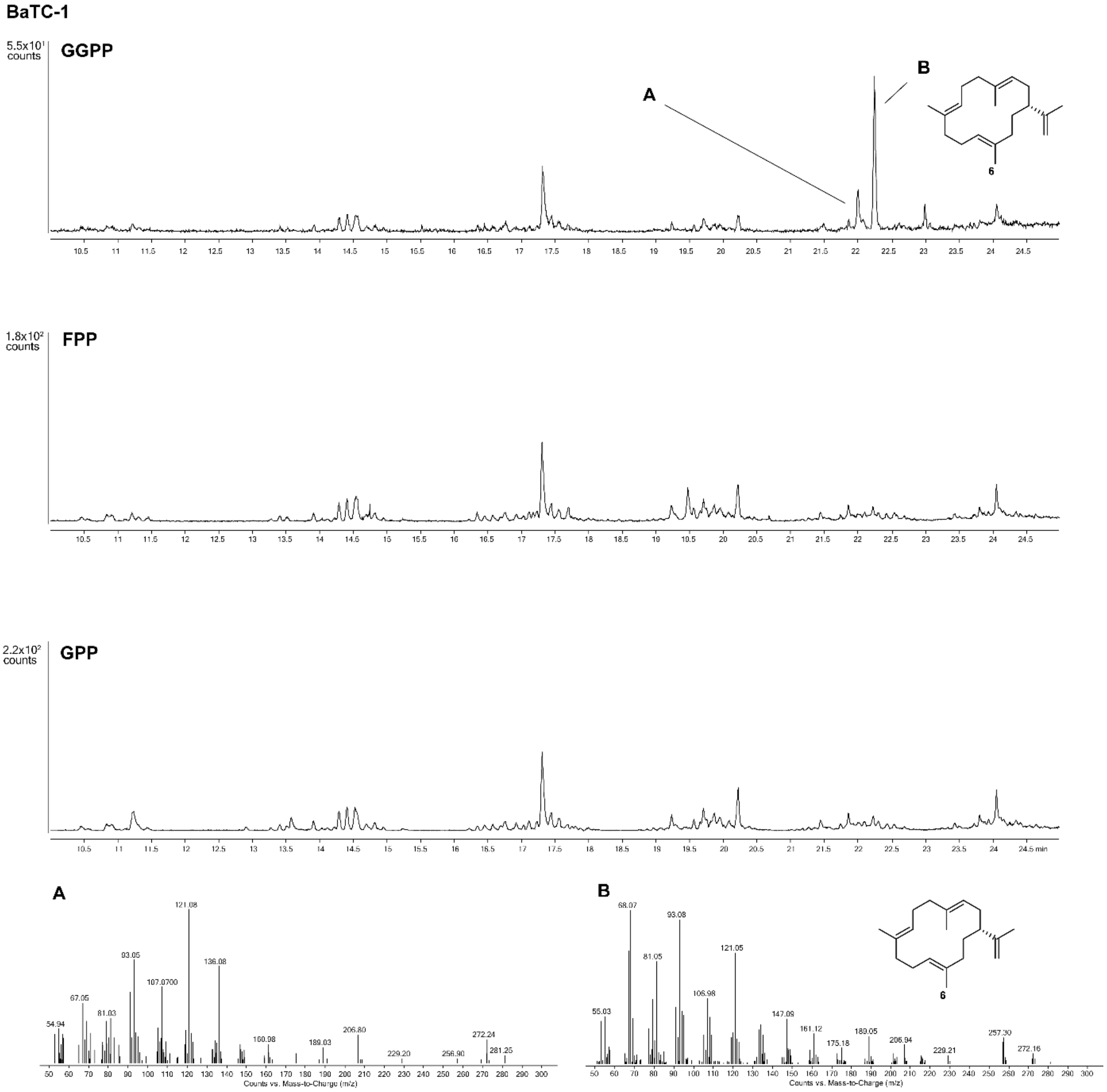
Supplementary Fig. 1. Regions from alignments with newly identified coral sequences with microbial and plant terpene synthase (TS) sequences that show the conserved motifs. Plant-like terpene synthases show more diversity in the NSD/DTE motif and do not have the RY motif.



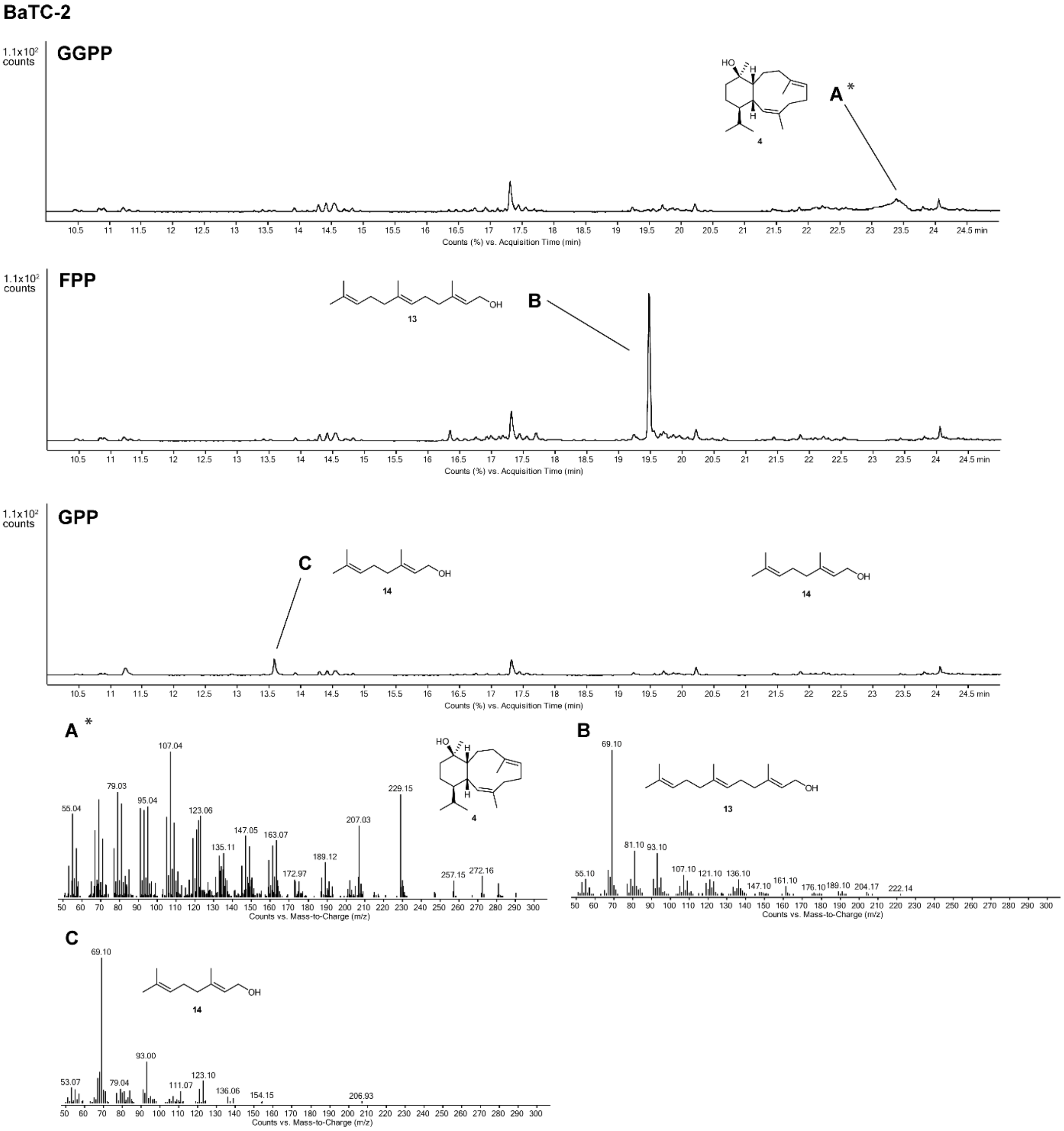
Supplementary Fig. 2. SDS PAGE analysis of recombinant TmTC-1, TmTC-2, XsTC-1, ErTC-1, ErTC-2, PbTC-1, RmTC-1, CiTC-1. The image shows representative bands after Ni-NTA purification. All expressions were carried out at least twice on different days. No attempts failed.



Supplementary Fig. 3. SDS PAGE analysis of recombinant DgTC-1, DgTC-2, HcTC-1, HcTC-2, HcTC-3, BaTC-1, BaTC-2. The image shows representative bands after Ni-NTA purification. All expressions were carried out at least twice on different days. No attempts failed.

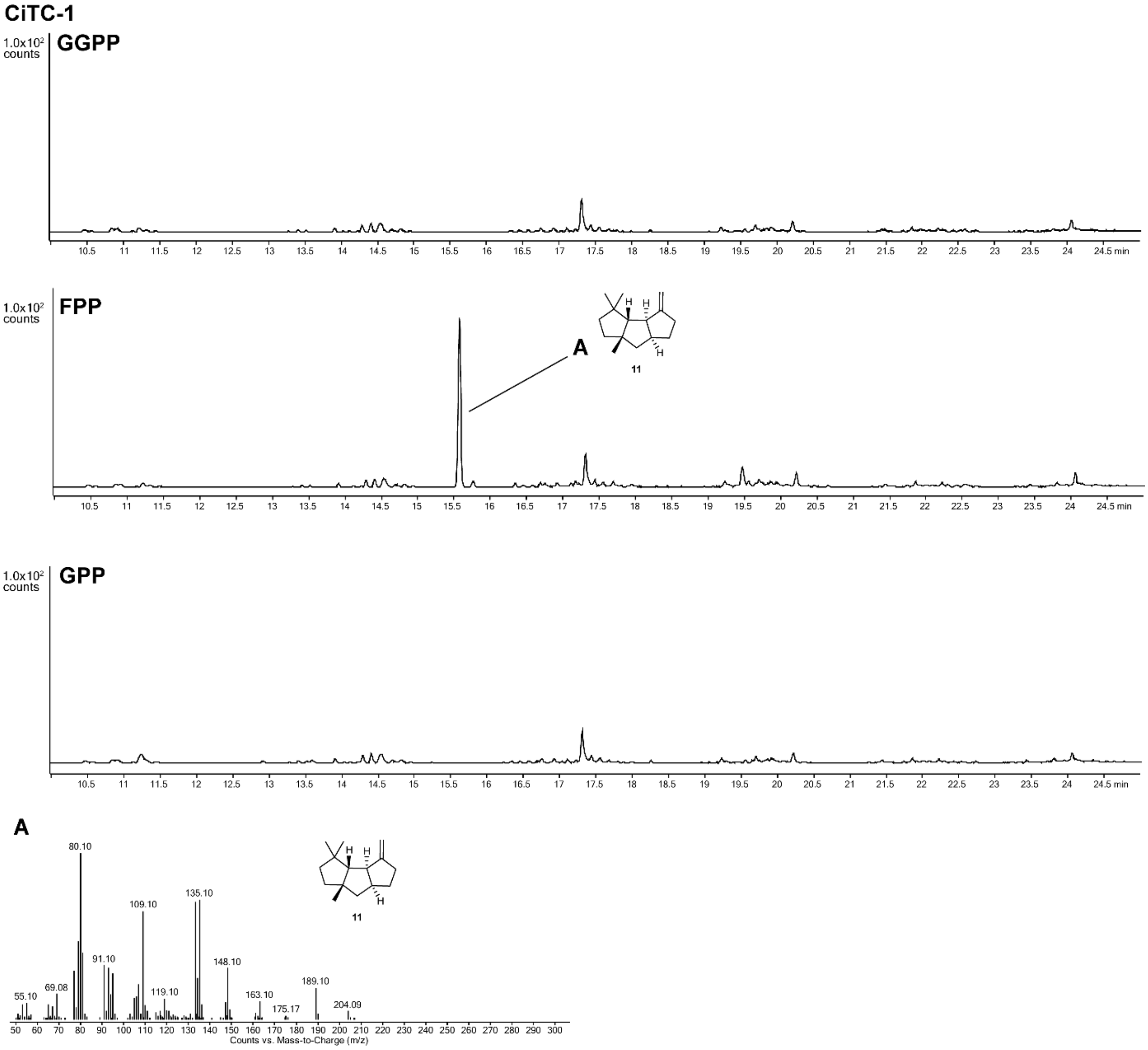


Supplementary Fig. 4. GCMS analysis of BaTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown. The absolute configuration of **6** produced by this enzyme was not determined.

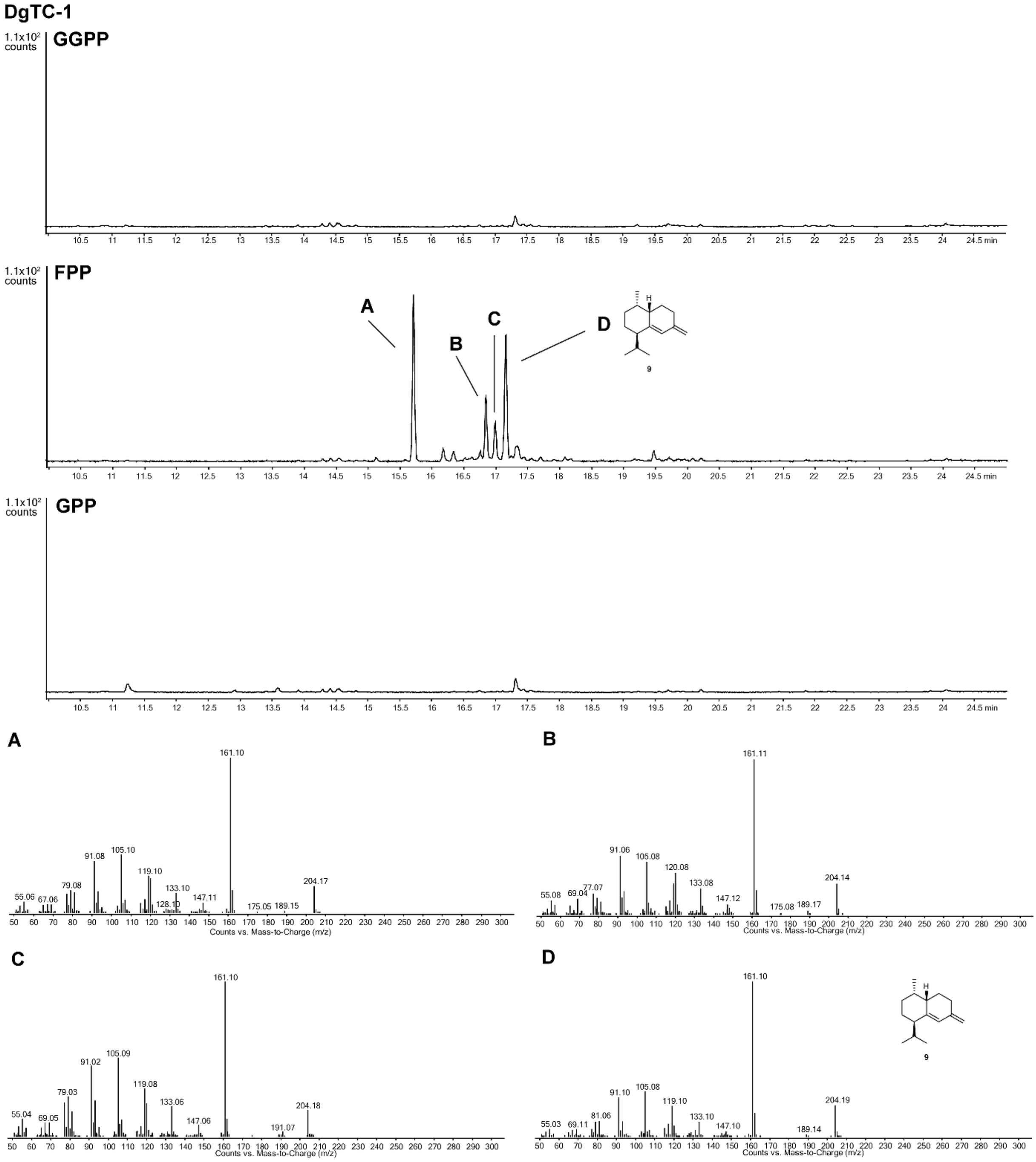


Supplementary Fig. 5. GCMS analysis of BaTC-2 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.

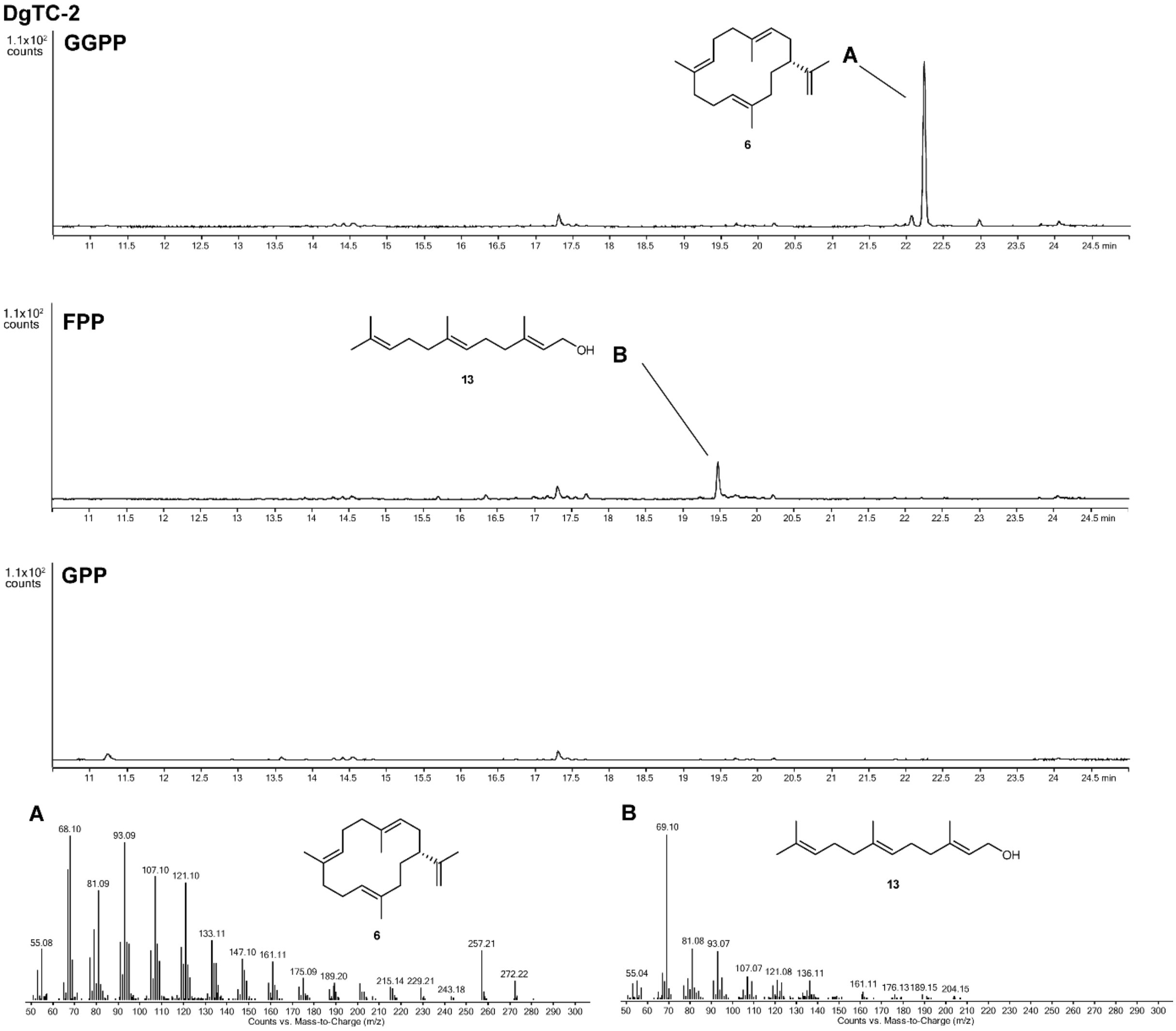
*: Compound **4** rearranges/decomposes in the GC injector and elutes as multiple products as a broad peak. Mass spectrum A is averaged over the whole signal.



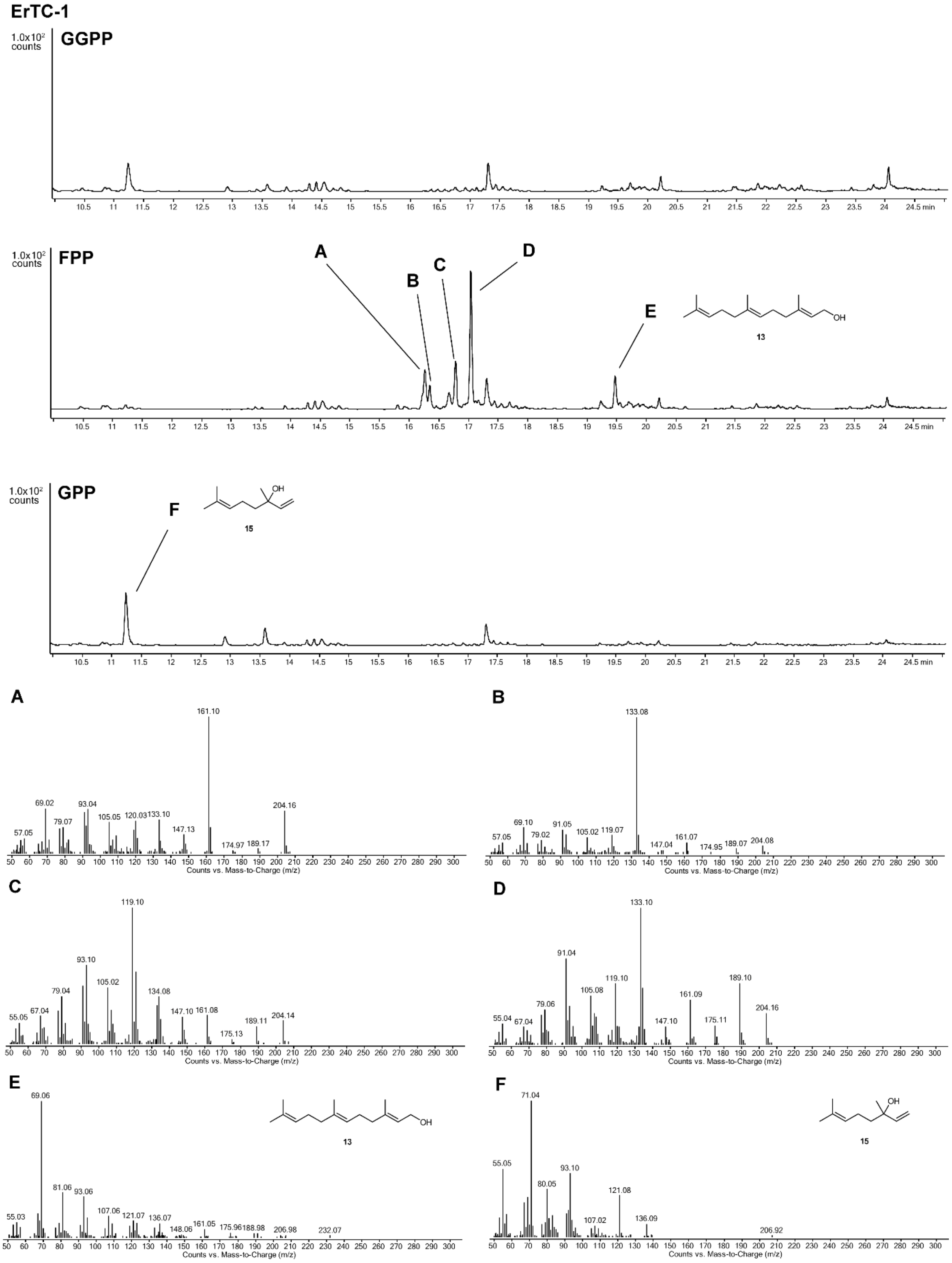
Supplementary Fig. 6. GCMS analysis of CiTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.



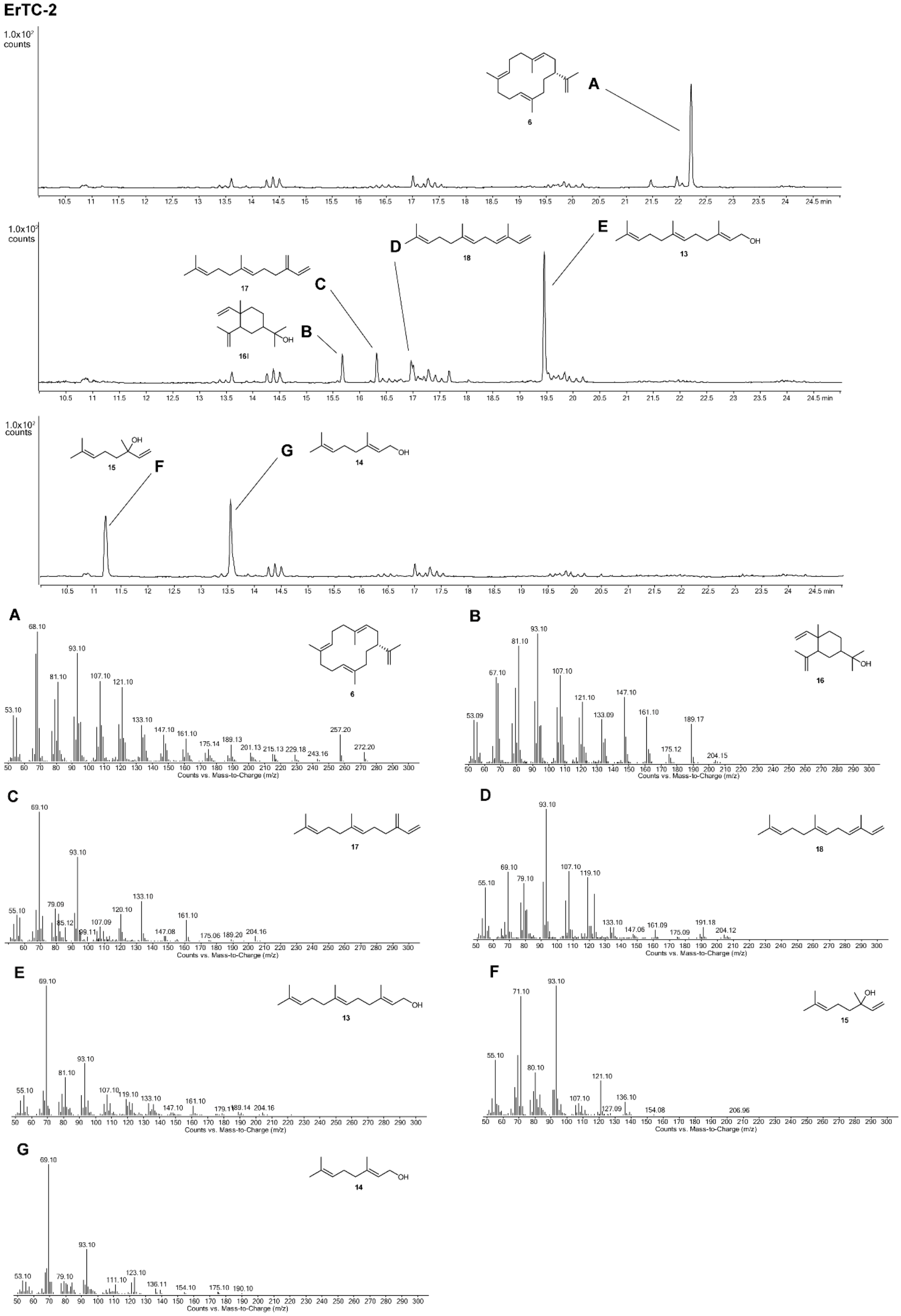
Supplementary Fig. 7. GCMS analysis of DgTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown. The absolute configuration of **9** produced by this enzyme was not determined.



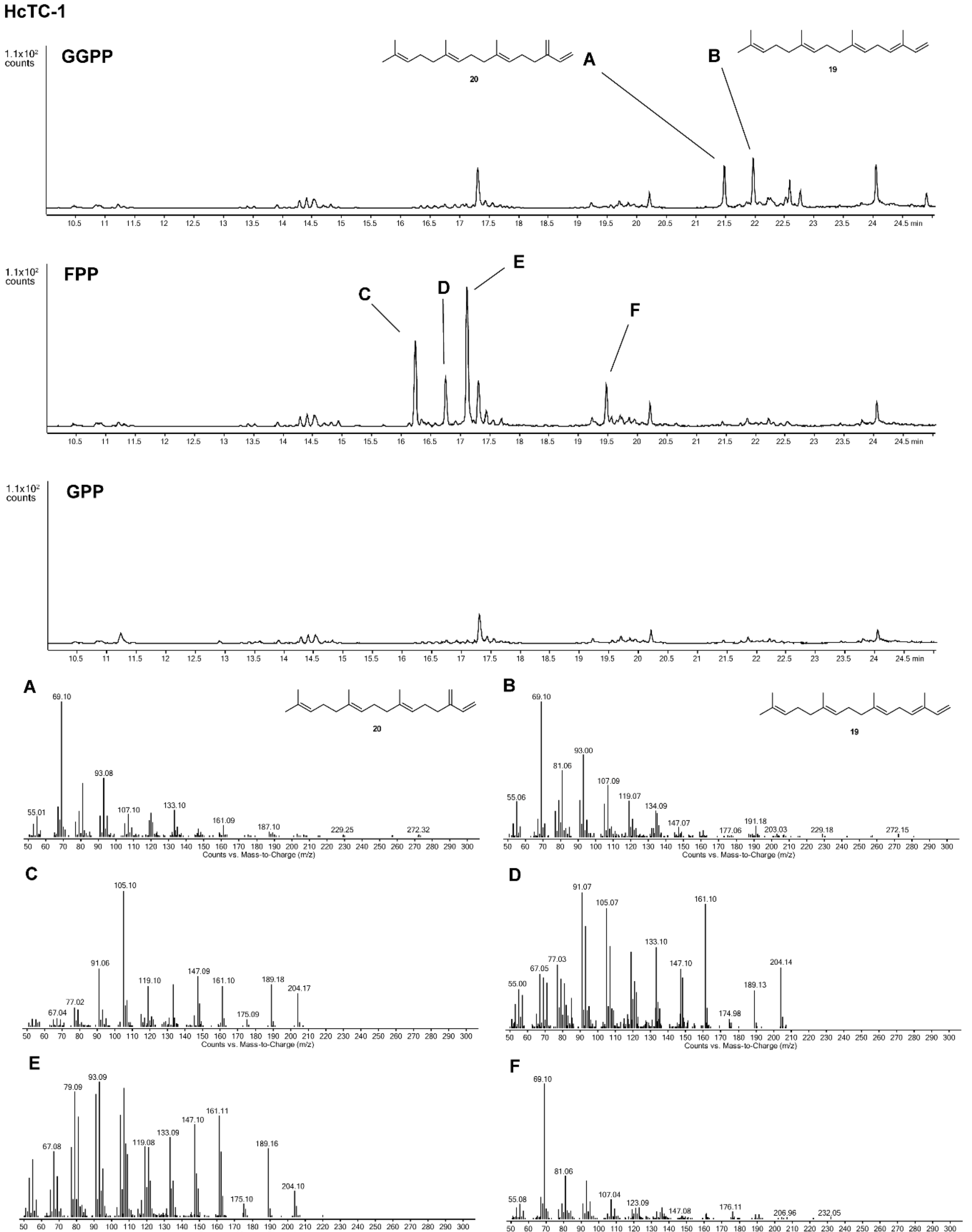
Supplementary Fig. 8. GCMS analysis of DgTC-2 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown. The absolute configuration of **6** produced by this enzyme was not determined.



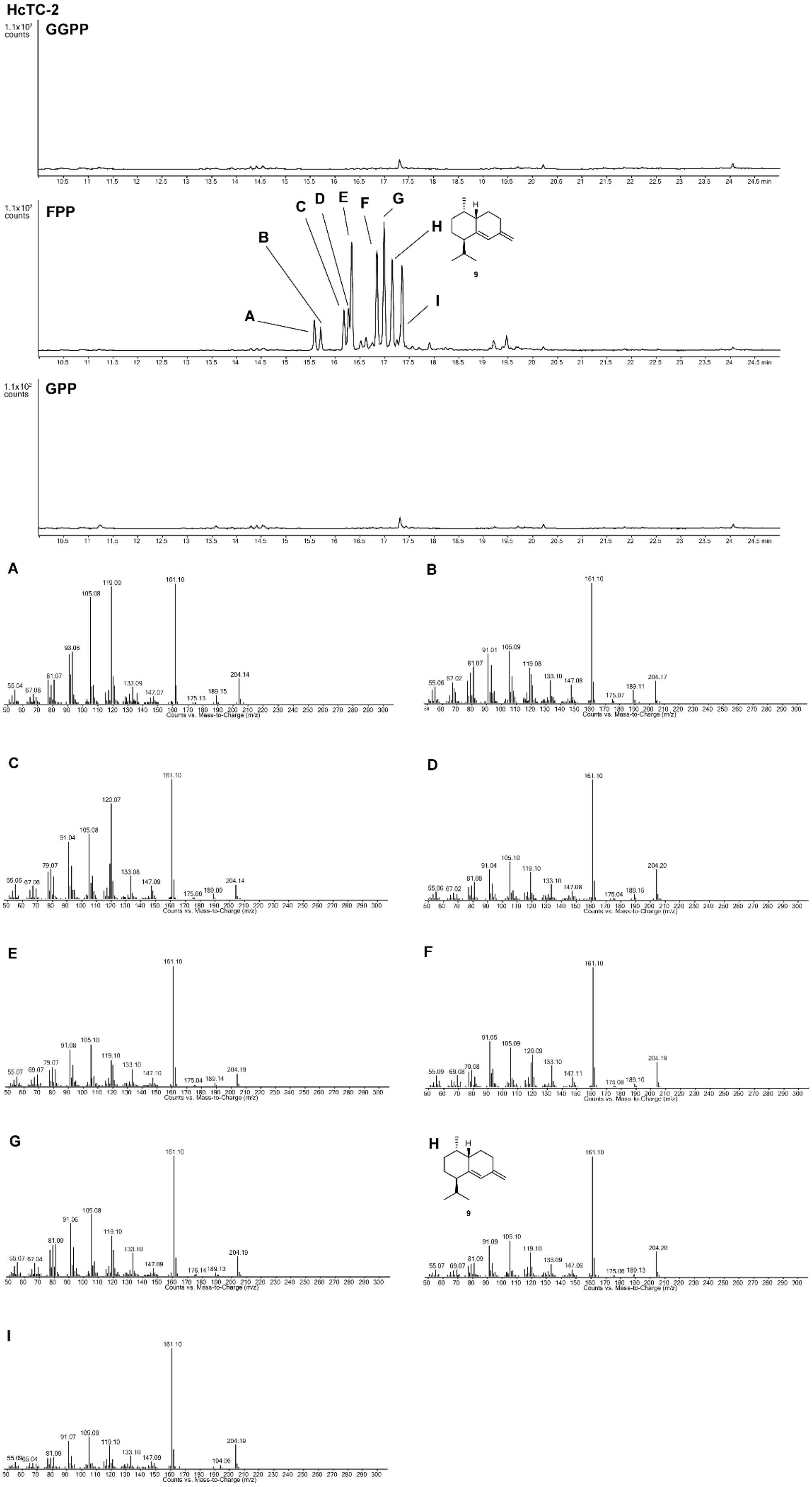
Supplementary Fig. 9. GCMS analysis of ErTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.



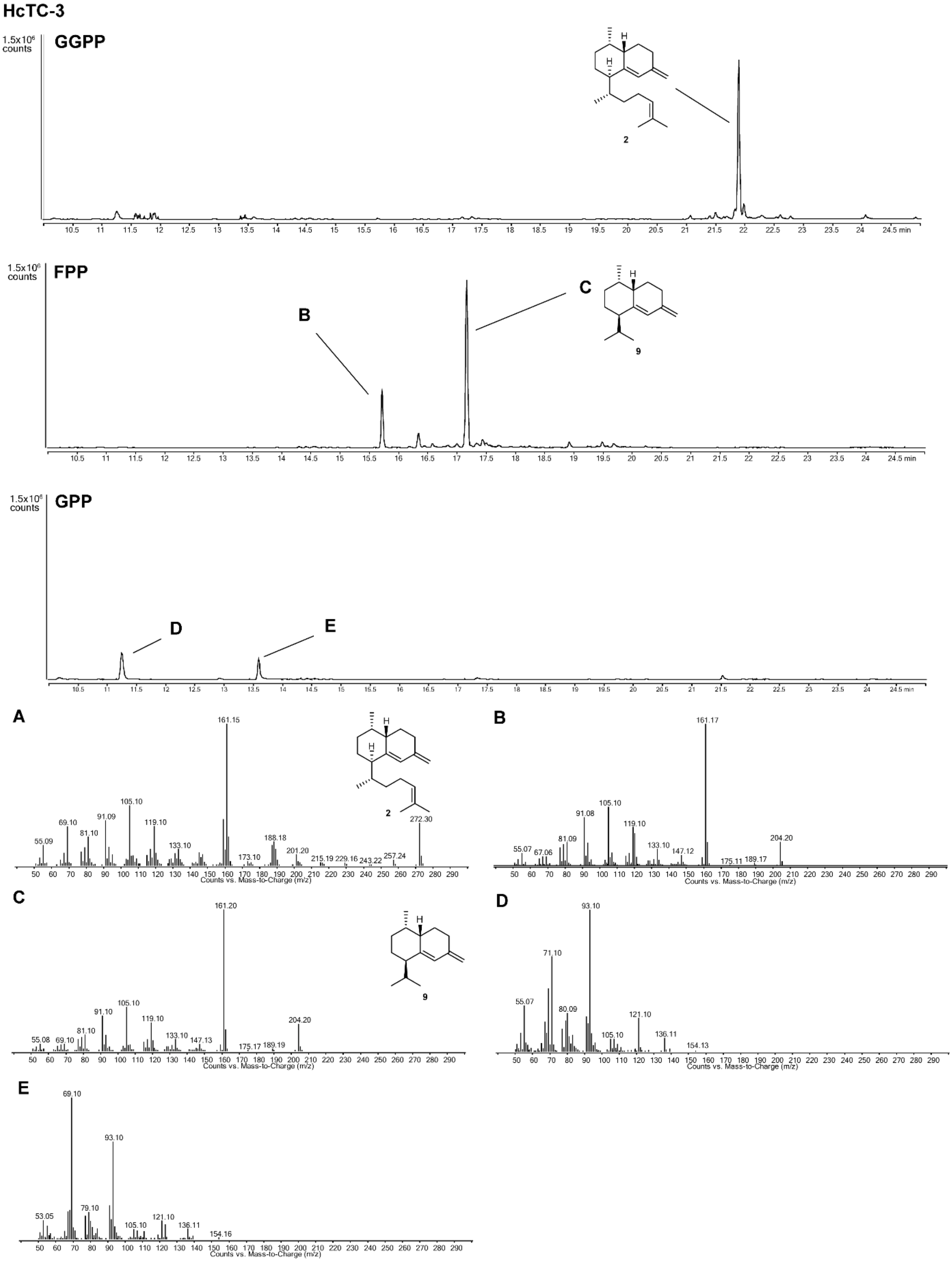
Supplementary Fig. 10. GCMS analysis of ErTC-2 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.



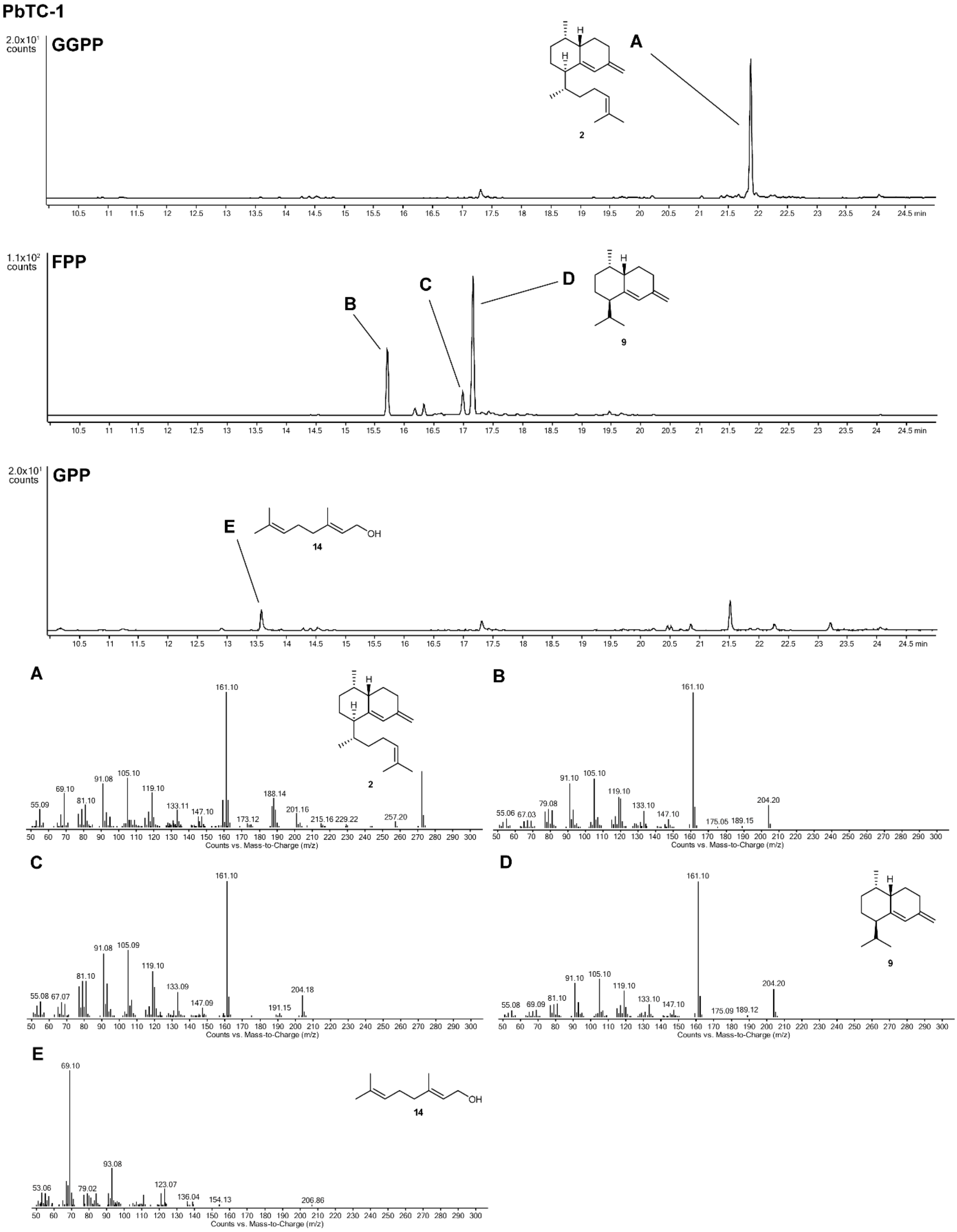
Supplementary Fig. 11. GCMS analysis of HcTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.



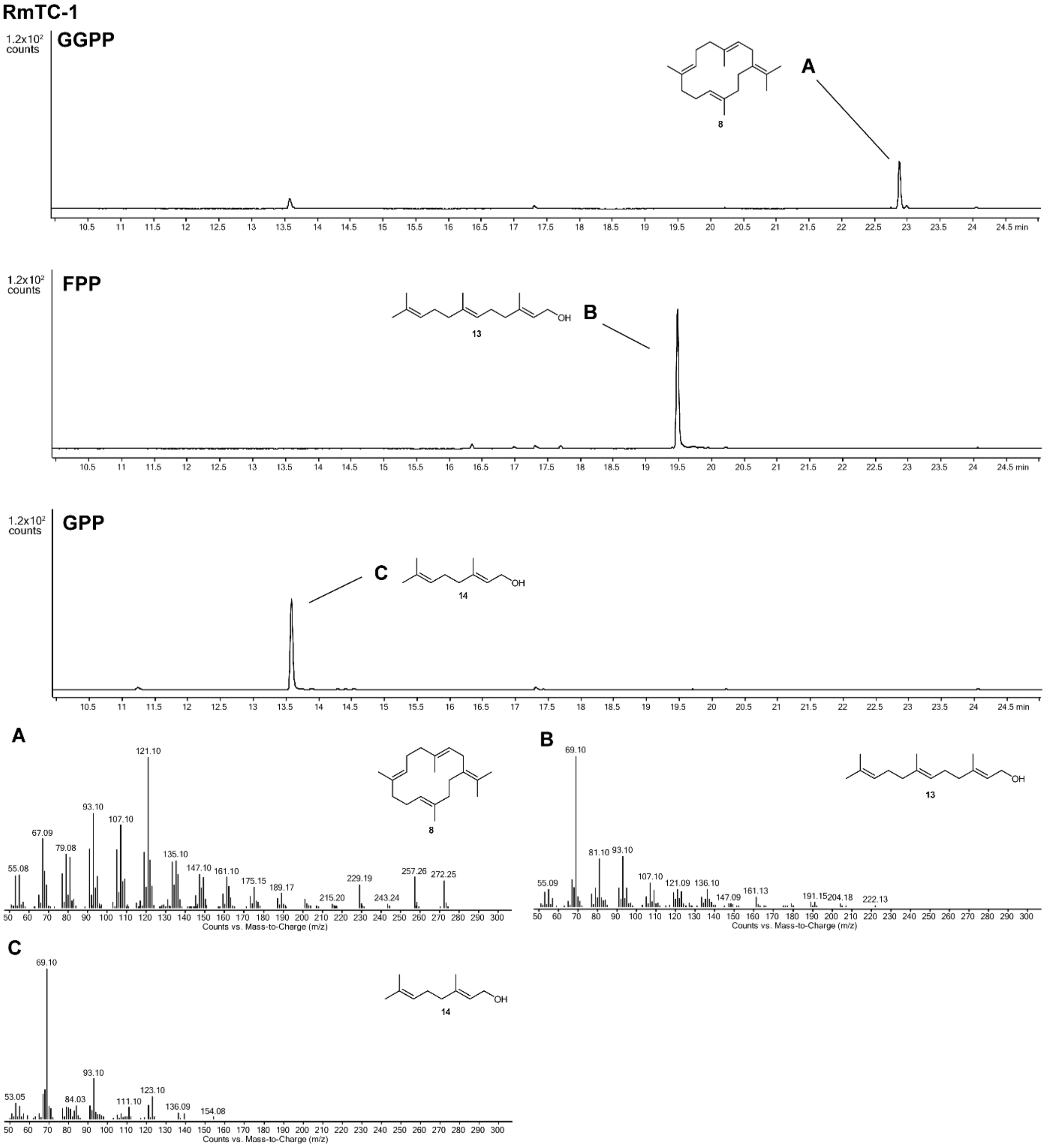
Supplementary Fig. 12. GCMS analysis of HcTC-2 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown. The absolute configuration of **9** produced by this enzyme was not determined.



Supplementary Fig. 13. GCMS analysis of HcTC-3 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.

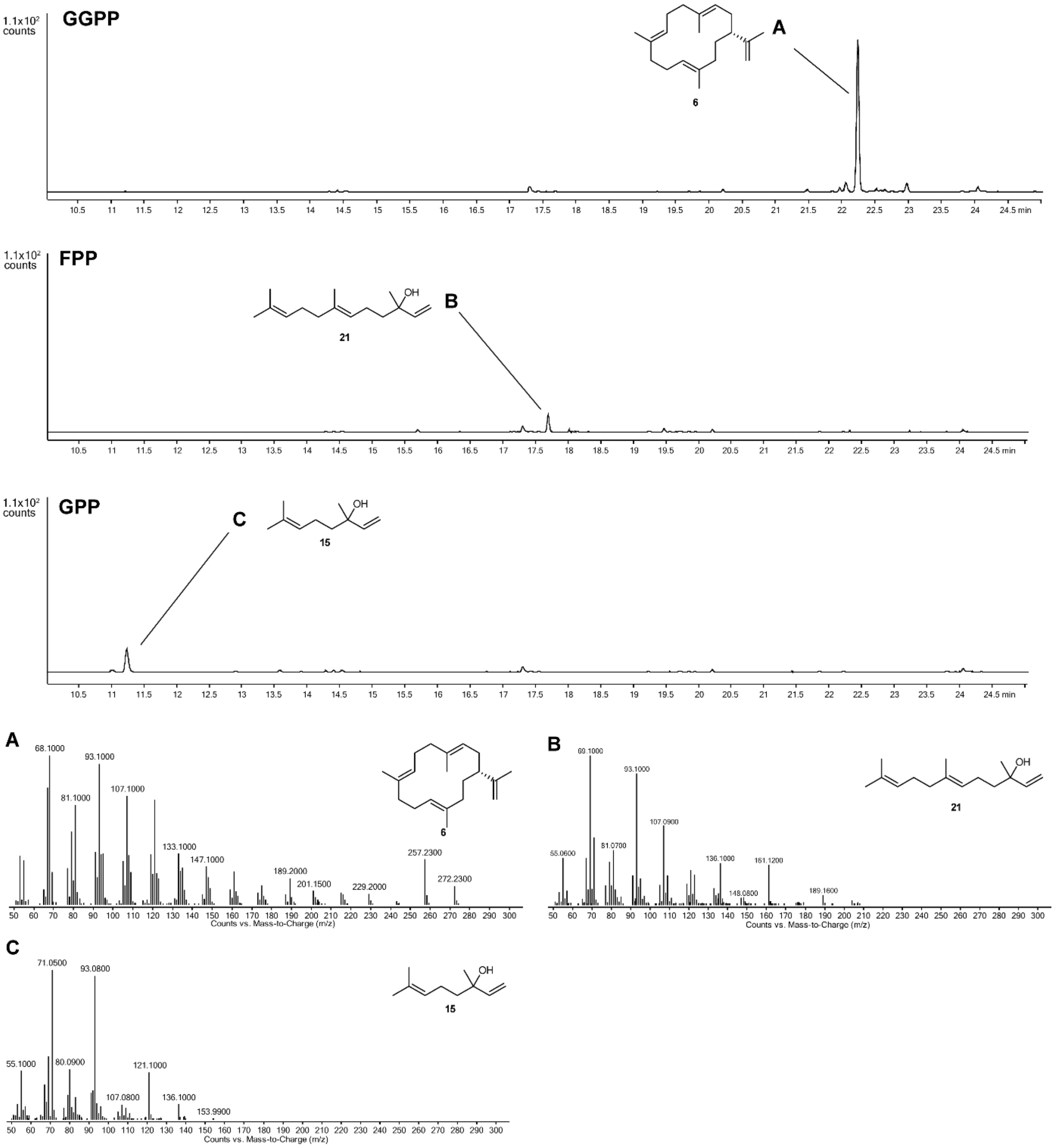


Supplementary Fig. 14. GCMS analysis of PbTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.

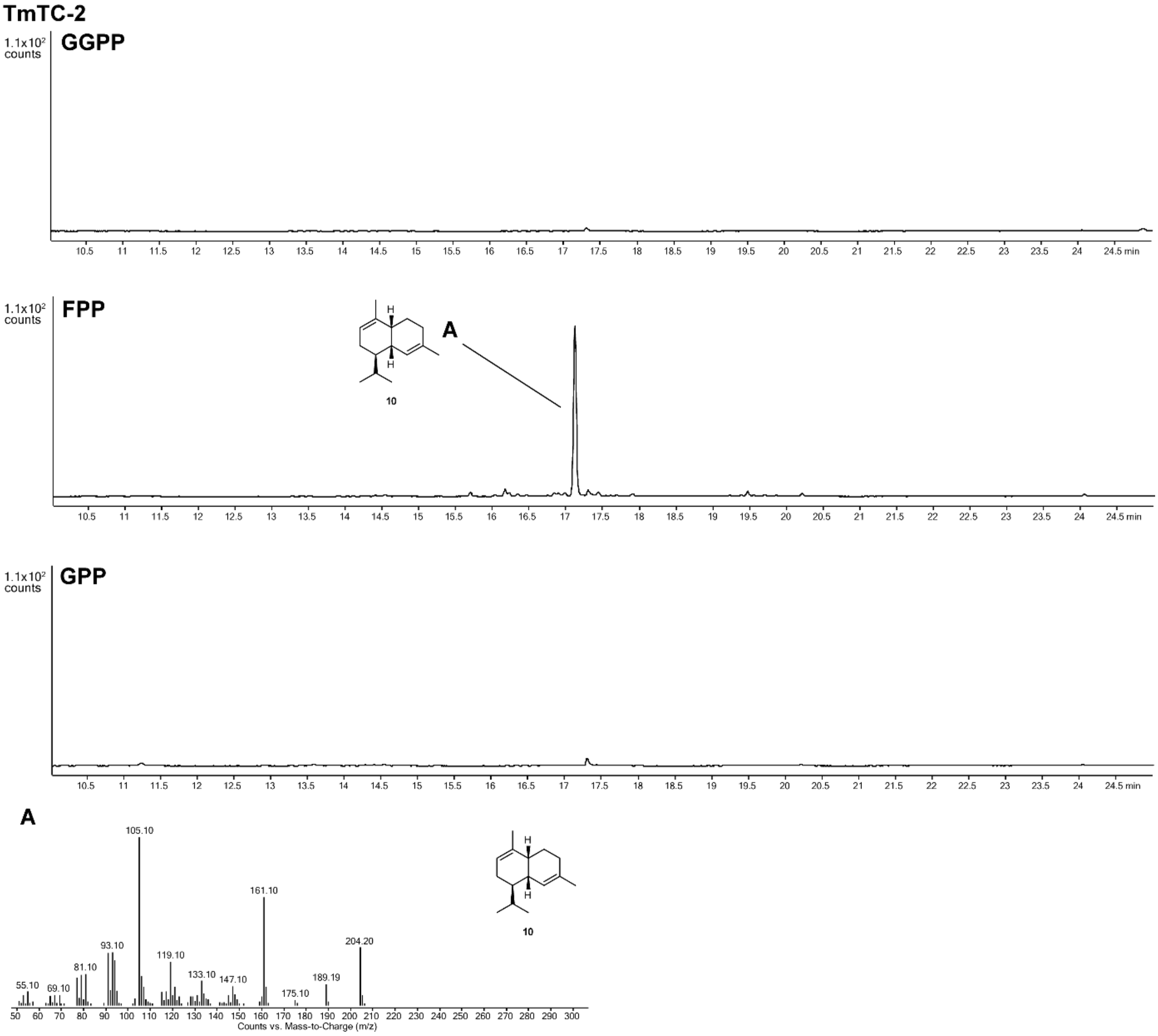


Supplementary Fig. 15. GCMS analysis of RmTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.

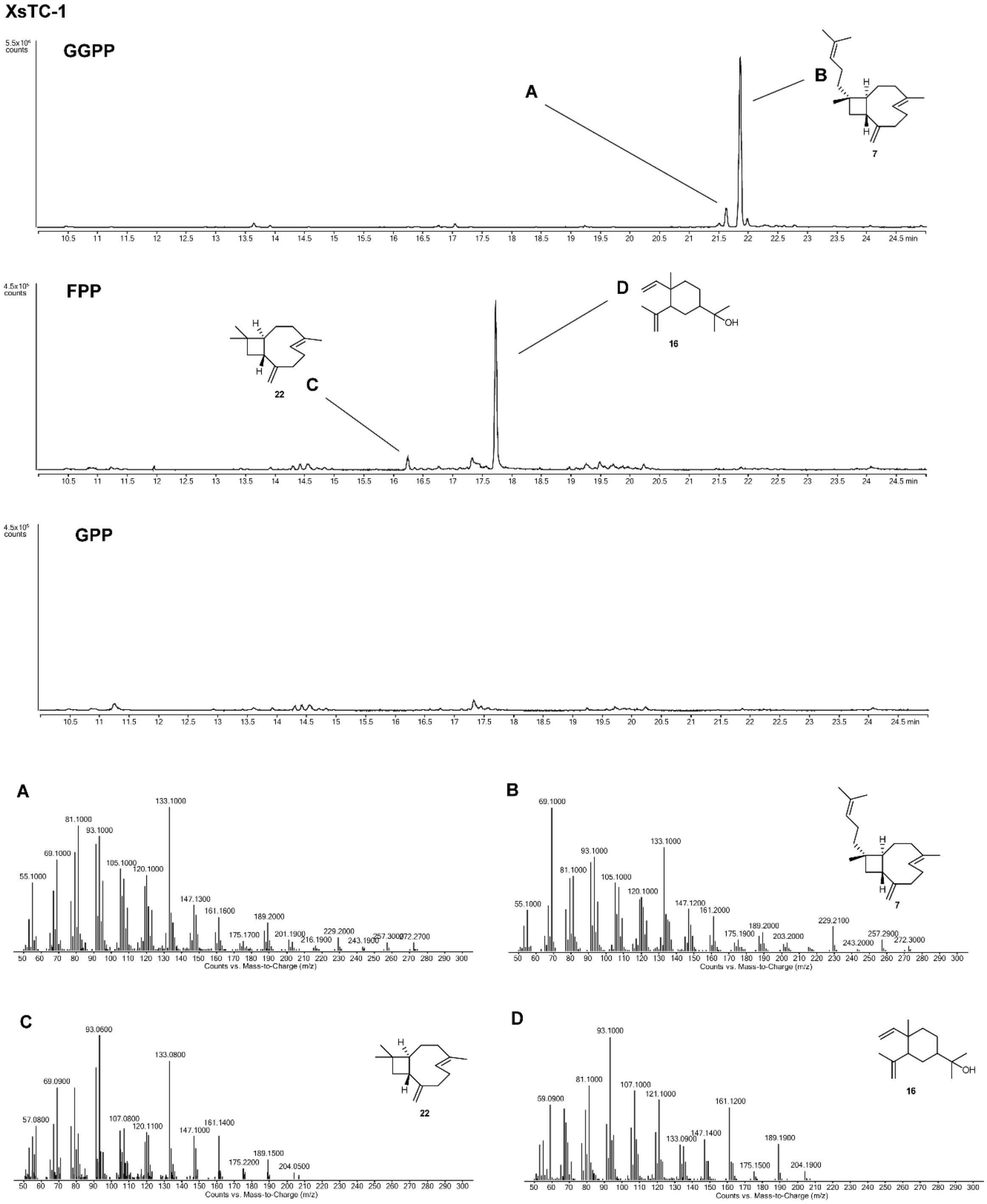
TmTC-1



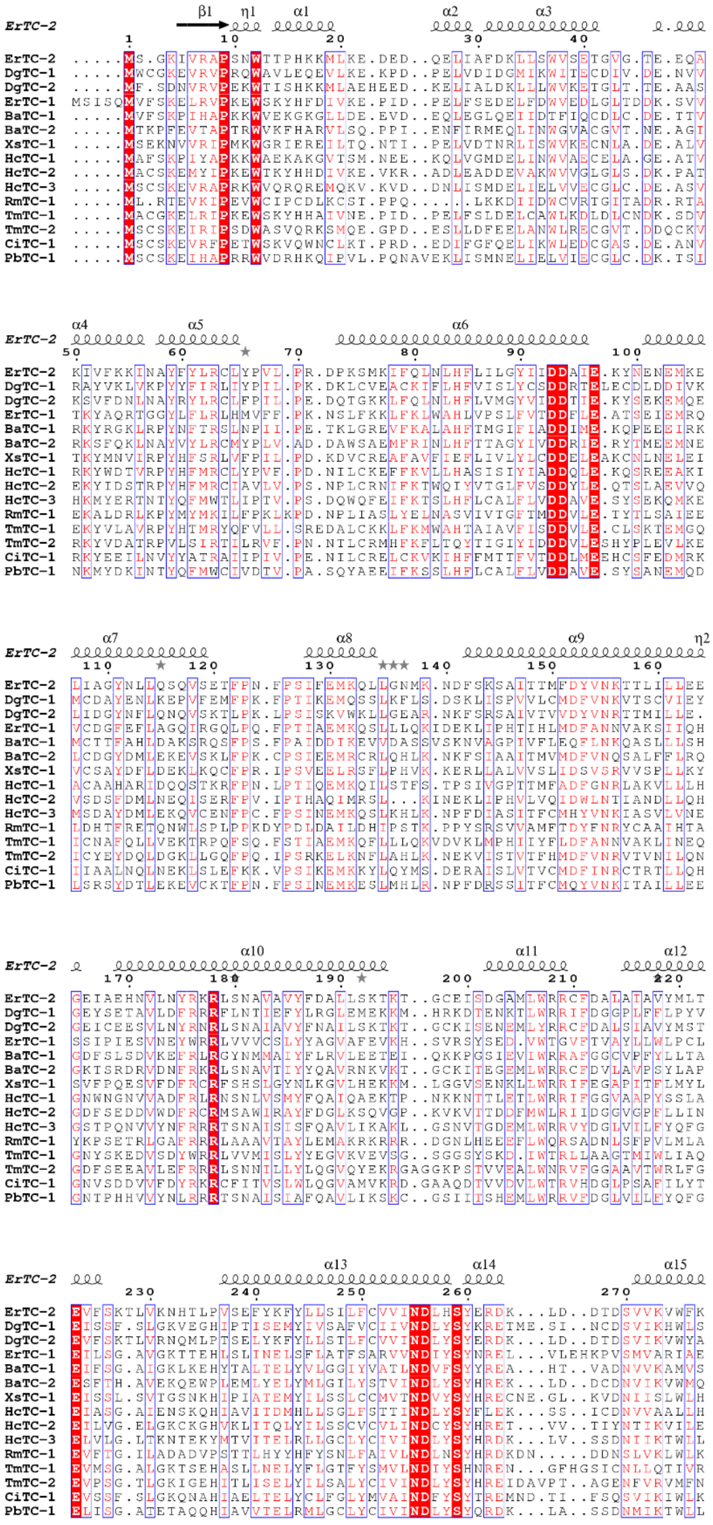
Supplementary Fig. 16. GCMS analysis of TmTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown. The absolute configuration of **6** produced by this enzyme was not determined.

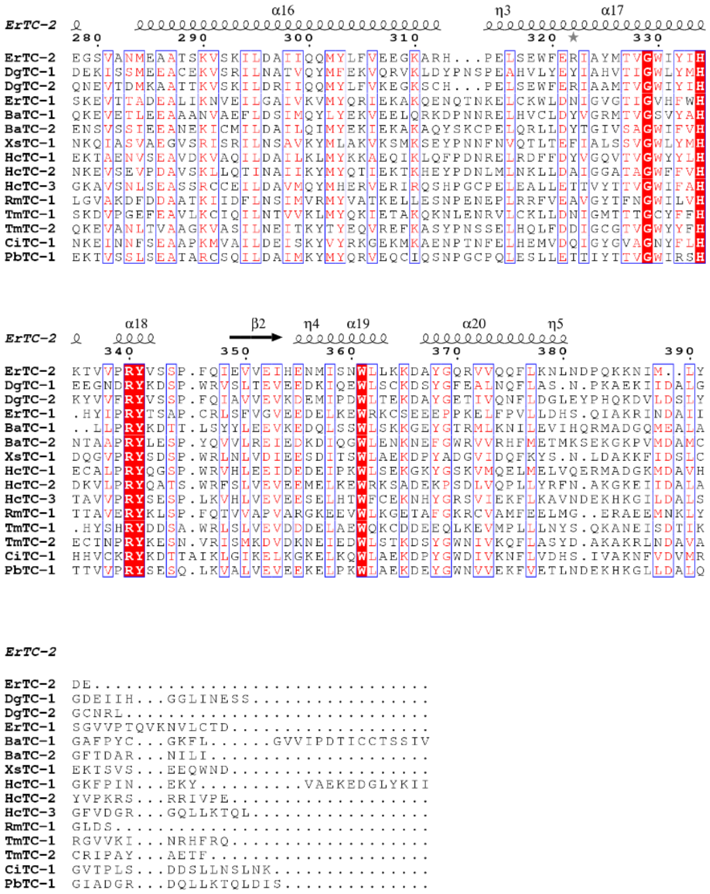


Supplementary Fig. 17. GCMS analysis of TmTC-2 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.

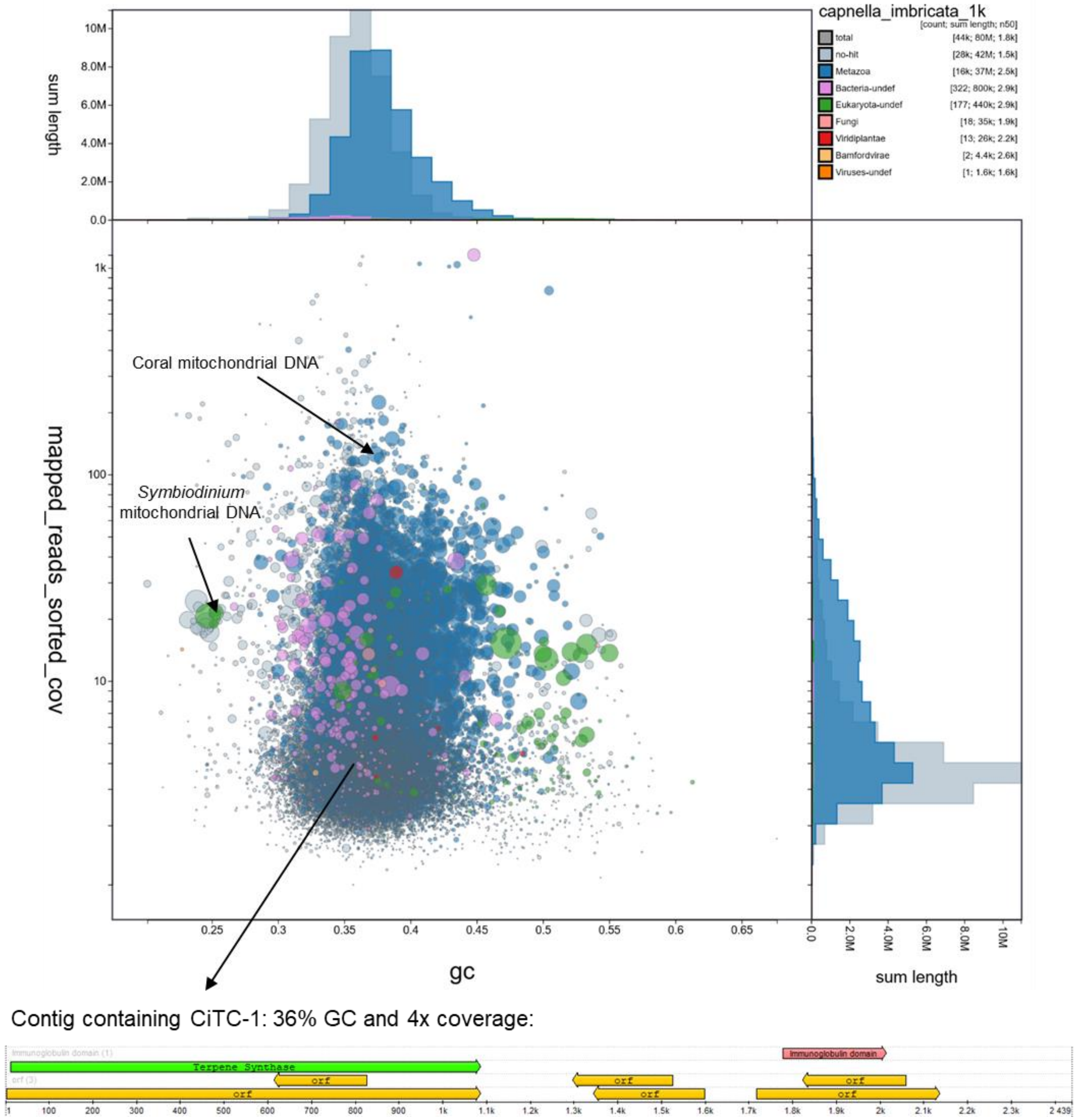


Supplementary Fig. 18. GCMS analysis of XsTC-1 with the substrates GGPP (top) FPP (middle) and GPP (bottom). Mass spectra for major peaks are shown.

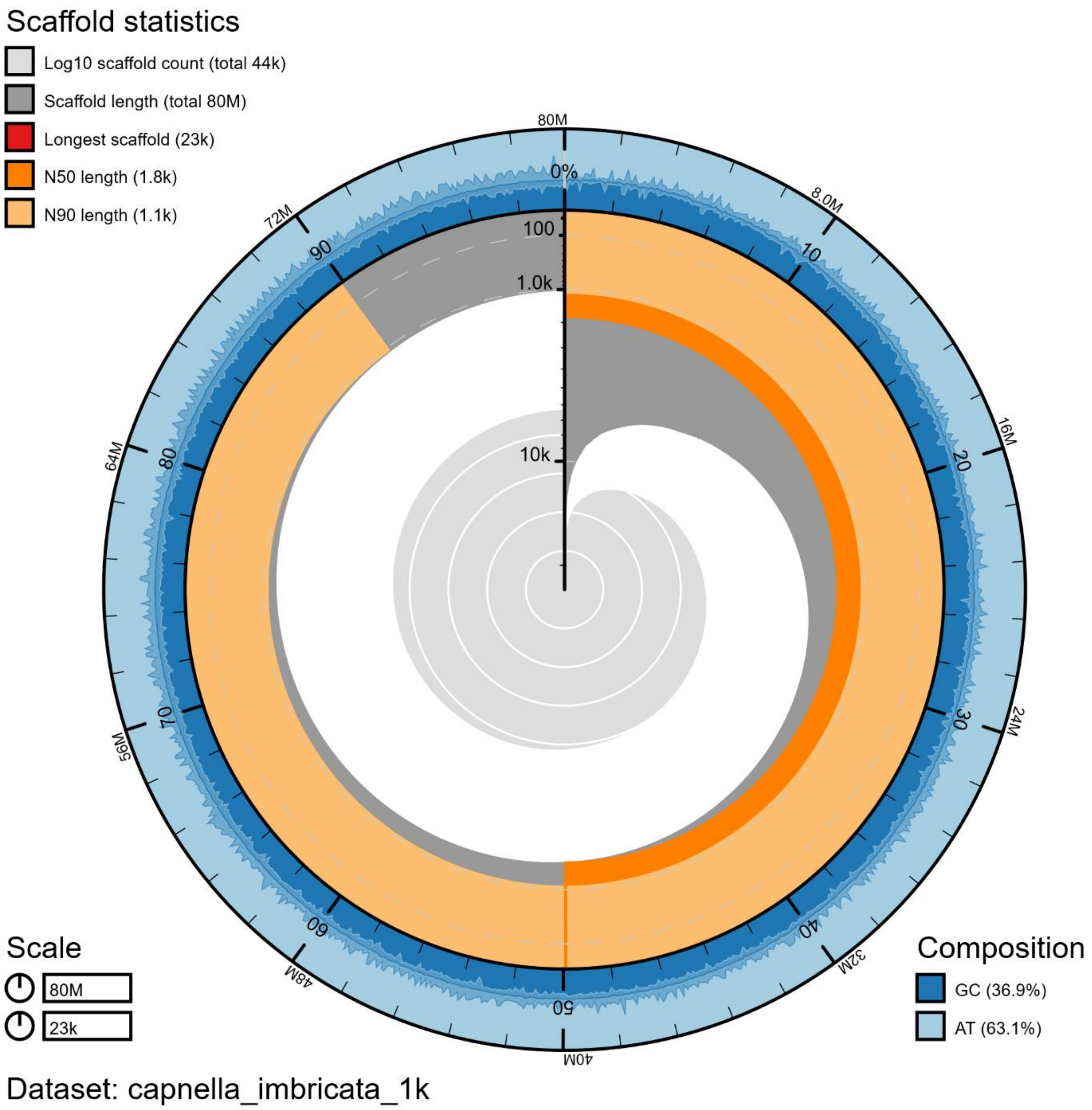

ErTC-2
β1
η1
α1
α2
α3
α4
α5
α6
α7
α8
α9
η2
α10
α11
α12
α13
α14
α15
ErTC-2
DgTC-1
DgTC-2
ErTC-1
BaTC-1
BaTC-2
XsTC-1
HcTC-1
HcTC-2
HcTC-3
RmTC-1
TmTC-1
TmTC-2
CiTC-1
PbTC-1



Supplementary Fig. 19. Sequence alignment of octocoral TC genes characterized in this work. Structural elements observed in the ErTC-2 structure are indicated at the top.



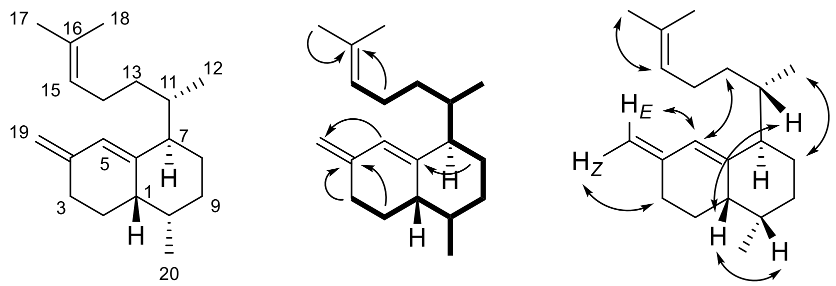
Supplementary Fig. 20: GC-vs-coverage blob plot created using Blobtools[42] for contigs 1 kilobasepairs and larger in our assembly for Capnella imbricata. Contigs are colored by kingdom as classified by Diamond[43] blastx against the NCBI nr database. Circles are sized in proportion to contig length on a square-root scale, ranging from 1,000 to 22,827. Histograms show the distribution of contig length sum along each axis. Most Symbiodinium genomic and plastid DNA did not assemble into contigs > 1 kilobasepairs. The large spread in coverage is likely due to contigs representing repetitive elements. The contig containing CiTC-1 is shown. It also contains a fragment of a gene with an immunoglobulin domain, however this is sometimes not present when assembled using other parameters or assemblers. Only one full-length terpene cyclase gene could be detected in this and any other assembly of *C. imbricata*, and the sequence of CiTC-1 synthesized was a consensus of that in various assemblies.



Supplementary Fig. 21: Snail plot created using Blobtools for contigs 1 kilobasepairs and larger in our assembly for *Capnella imbricata*. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 79,762,010 bp assembly. The distribution of record lengths is shown in dark grey with the plot radius scaled to the longest record present in the assembly (22,827 bp). Orange and pale-orange arcs show the N50 and N90 record lengths (1,772 and 1,104 bp), respectively. The pale grey spiral shows the cumulative record count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. The assembly has been filtered to only include contigs classified as Metazoa or Unclassified by Diamond blastx against the NCBI nr database.

Supplementary Note 1 – Structure elucidation

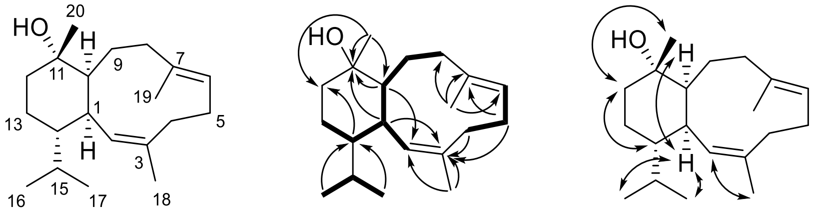
Diterpene product of HcTC-3 (**2**)



Structure of elisabethatriene (**2**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows. **Purification by column chromatography**: Pentanes (100%), **yield**: 1.9 mg. **Optical rotary power**: $[\alpha]_D^{25.0}$ = +56.8 (*c* 0.055, MeOH). **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 273 (7), 272 (31), 257 (4), 201 (8), 190 (4), 189 (11), 188 (17), 187 (14), 162 (20), 161 (100), 160 (11), 159 (33), 147 (7), 146 (6), 145 (9), 134 (4), 133 (11), 132 (5), 131 (8), 129 (5), 128 (4), 121 (4), 120 (8), 119 (27), 118 (5), 117 (11), 115 (7), 109 (5), 107 (7), 106 (6), 105 (42), 104 (3), 103 (4), 95 (7), 93 (13), 92 (4), 91 (31), 82 (5), 81 (20), 79 (12), 77 (9), 69 (27), 67 (9), 65 (4), 55 (14), 53 (5). The relative configuration in agreement with structural revision[44] after first the report [45]. The absolute configuration derived from enantioselectively synthesized oxidized derivatives [46]

C	^{13}C	^{1}H
1	37.1	2.24 (q, ^{3}J = 6.0)
2	27.5	1.63-1.58 (m) 1.53-1.44 (m)
3	29.8	2.36 (ddd, ^{2}J = 14.1, ^{3}J = 9.1, ^{3}J = 4.6) 2.21-2.15 (m)
4	143.9	-
5	127.5	6.14 (s)
6	144.02	-
7	50.0	1.83-1.79 (m)
8	23.2	1.67-1.69 (m, 2H)
9	29.6	1.75-1.67 (m) 1.21 (ddt, ^{2}J = 12.4, ^{3}J = 3.1, ^{3}J = 3.1)
10	35.5	1.79-1.75 (m)
11	31.9	1.74-1.70 (m)
12	17.7	0.9 (d, ^{3}J = 6.6, 3H)
13	35.2	1.56-1.52 1.04 (ddt, ^{2}J = 13.2, ^{3}J = 9.4, ^{3}J = 4.9)
14	25.9	2.15-2.09 (m) 2.00-1.90 (m)
15	125.6	5.12 (ddsept, ^{3}J = 7.1, ^{3}J = 7.1, ^{4}J = 1.5)
16	131	-
17	25.9	1.65 (s, 3H)
18	17.8	1.59 (s, 3H)
19	109. 0	4.86 (s, H_E) 4.78 (s, H_Z)
20	15.2	0.86 (s, 3H)

Diterpene product of BaTC-2 (**4**)

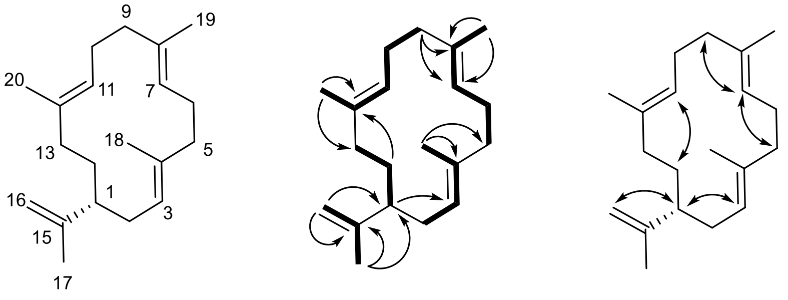


Structure of klysimplexin R (**4**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows. **Purification by column chromatography**: Hexanes/EtOAc (4:1), **yield**: 1.1 mg. **Optical rotary power**: $[\alpha]_D^{25.0}$ = -23.6 (*c* 0.09, $CHCl_3$). **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 290 (5), 281 (9), 272 (17), 257 (11), 230 (13), 229 (83), 229 (12), 207 (29), 207 (15), 205 (14), 202 (16), 201 (9), 191 (9), 190 (9), 189 (42), 187 (16), 175 (9), 173 (21), 164 (17), 164 (10), 163 (47), 162 (14), 162 (10), 161 (38), 161 (14), 160 (9), 159 (35), 155 (9), 150 (19), 149 (40), 148 (19), 148 (15), 147 (33), 147 (21), 145 (24), 137 (10), 136 (15), 136 (12), 135 (48), 134 (19), 134 (14), 133 (50), 131 (17), 127 (15), 124 (9), 123 (46), 123 (27), 122 (42), 122 (32), 121 (100), 120 (15), 119 (46), 119 (20), 117 (13), 113 (13), 111 (26), 110 (16), 109 (56), 109 (50), 108 (26), 108 (12), 107 (69), 107 (46), 106 (14), 105 (82), 99 (20), 97 (12), 96 (13), 95 (72), 94 (13), 93 (48), 93 (46), 92 (9), 92 (11), 91 (58), 91 (25), 85 (44), 84 (15), 83 (19), 83 (11), 82 (15), 81 (77), 81 (31), 80 (12), 79 (80), 77 (35), 77 (16), 71 (82), 70 (14), 69 (77), 68 (13), 68 (10), 67 (45), 67 (11), 65 (10), 58 (15), 57 (54), 56 (14), 55 (9), 55 (64), 53 (24).

Absolute configuration set after optical rotation [47]. NMR spectra show several minor conformers.

C	^{13}C	^{1}H
1	37.1	2.94-2.88 (m)
2	131.5	5.23-5.27 (m)
3	133.7	-
4	32.9	2.18-2.11 (m) 1.77-1.71 (m)
5	26.1	2.25-2.20 (m) 1.98-1.91 (m)
6	125.2	5.34-5.29 (m)
7	138.0	-
8	40.5	2.20-2.14 (m) 1.91-1.84 (m)
9	24.6	1.50-1.45 (m) 1.27-1.22 (m)
10	47.3	1.96-1.91 (m)
11	72.7	-
12	36.3	1.41-1.35 (m, 2H)
13	20.6	1.57-1.50 (m) 1.42-1.36 (m)
14	45.6	0.99-0.94 (m)
15	26.9	1.92-1.86 (m)
16	22.4	0.94 (d, ^{3}J = 6.8, 3H)
17	18.2	0.84 (d, ^{3}J = 6.7, 3H)
18	25.2	1.68 (s, 3H)
19	17.5	1.54 (s, 3H)
20	26.7	1.13 (s, 3H)

Diterpene product of ErTC-2 (**6**)

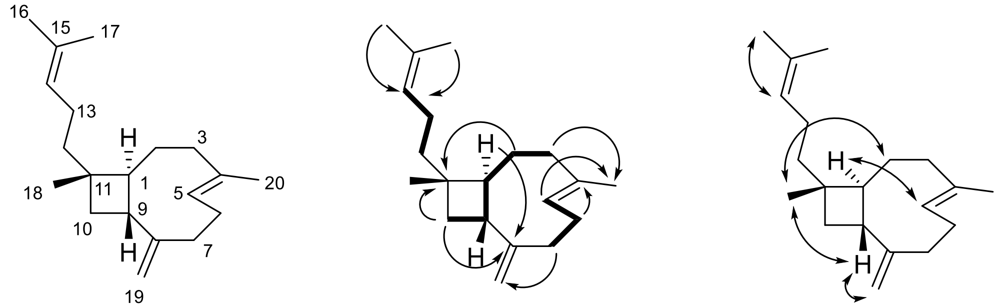


Structure of cembrene A (**6**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows.
Purification by column chromatography: Pentanes (100%), yield: 3.4 mg. **Optical rotary power**: $[\alpha]_D^{25.0}$ = -28.0 (*c* 0.02, $CHCl_3$). **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 272 (7), 272 (3), 258 (6), 257 (21), 229 (4), 216 (6), 215 (6), 207 (5), 203 (4), 201 (7), 191 (4), 190 (4), 189 (17), 187 (6), 176 (5), 175 (7), 175 (6), 173 (6), 163 (5), 162 (8), 161 (4), 161 (17), 160 (4), 159 (5), 159 (4), 149 (9), 148 (15), 147 (25), 145 (6), 137 (3), 136 (6), 136 (4), 135 (21), 134 (20), 133 (33), 131 (4), 123 (16), 122 (17), 121 (66), 120 (22), 119 (6), 119 (25), 117 (3), 109 (25), 108 (33), 107 (70), 106 (13), 105 (28), 96 (5), 95 (33), 94 (36), 93 (93), 92 (4), 92 (15), 91 (37), 82 (9), 81 (67), 80 (17), 79 (48), 78 (3), 77 (25), 69 (22), 68 (100), 67 (76), 66 (4), 65 (11), 55 (31), 53 (33).

The absolute configuration was set after optical rotation [48]

C	^{13}C (δ in ppm)	^{1}H (δ in ppm,)
1	46.5	2.25-2.19 (m)
2	33.0	2.19-2.13 (m) 2.05-1.99 (m)
3	124.5	5.35-5.30 (m)
4	134.9	-
5	39.4	2.18-2.12 (m, 2H)
6	25.3	2.32-2.24 (m) 2.17-2.10 (m)
7	126.5	5.10-5.05 (m)
8	133.5	-
9	39.9	2.12-2.06 (m, 2H)
10	24.2	2.18-2.12 (m, 2H)
11	122.3	5.28-5.23 (m)
12	137.8	-
13	34.3	2.07-2.00 (m) 1.98-1.90 (m)
14	28.5	1.84-1.77 (m) 1.45-1.36 (m)
15	149.2	-
16	110.8	4.84-4.82 (m, 2H)
17	19.4	1.68 (s, 3H)
18	15.6	1.55 (s, 3H)
19	15.4	1.54 (s, 3H)
20	18.3	1.61 (s, 3H)

Diterpene product of XsTC-1 (**7**)

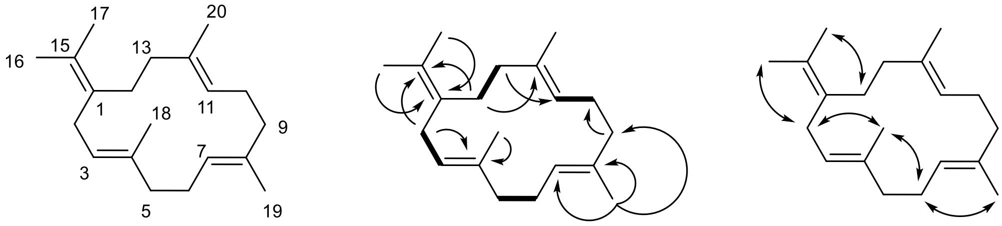


Structure of xeniaphyllene (**7**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows. **Purification by column chromatography**: Pentanes (100%), **yield**: 2.5 mg. **Optical rotary power**: $[\alpha]_D^{25.0}$ = -12.1 (*c* 0.18, $CHCl_3$). **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 272 (3), 257 (7), 230 (4), 229 (17), 203 (6), 201 (4), 190 (5), 189 (13), 188 (4), 187 (10), 175 (7), 173 (6), 162 (6), 161 (24), 160 (4), 159 (11), 149 (10), 148 (18), 147 (29), 146 (5), 145 (9), 137 (8), 136 (20), 135 (21), 134 (25), 133 (72), 132 (3), 131 (9), 123 (21), 122 (11), 121 (30), 120 (37), 119 (36), 118 (4), 117 (8), 115 (5), 109 (23), 108 (10), 107 (45), 106 (26), 105 (48), 103 (4), 96 (4), 95 (34), 94 (13), 93 (66), 92 (18), 91 (63), 83 (6), 82 (10), 81 (52), 80 (12), 79 (51), 78 (8), 77 (29), 70 (6), 69 (100), 68 (10), 67 (32), 66 (4), 65 (10), 55 (28), 53 (17).

The absolute configuration was derived from enantioselectively synthesized oxidized derivatives [49]. NMR spectra show several minor conformers.

C	^{13}C	^{1}H
1	52.6	1.85-1.78 (m)
2	30.3	1.51-1.44 (m) 1.44-1.33 (m)
3	40.3	2.08-2.02 (m) 1.98-1.92 (m)
4	135.1	-
5	124.9	5.39 (dd, ^{3}J = 9.4, 5.4)
6	29.0	2.36-2.32 (m) 2.02-1.96 (m)
7	35.0	2.16-2.11 (m) 2.00-1.94 (m)
8	154.7	-
9	49.0	2.35-2.30 (m)
10	39.7	1.79-1.69 (m, 2H)
11	36.2	-
12	44.6	1.51-1.44 (m) 1.43-1.37 (m)
13	23.4	2.06-1.98 (m, 2H)
14	125.8	5.27-5.20 (m)
15	130.8	-
16	25.2	1.70 (s, 3H)
17	17.7	1.60 (s, 3H)
18	20.1	0.98 (s, 3H)
19	112.3	5.05 (s, H_Z) 4.87 (s, H_E)
20	16.5	1.58 (s, 3H)

Diterpene product of RmTC-1 (**8**)

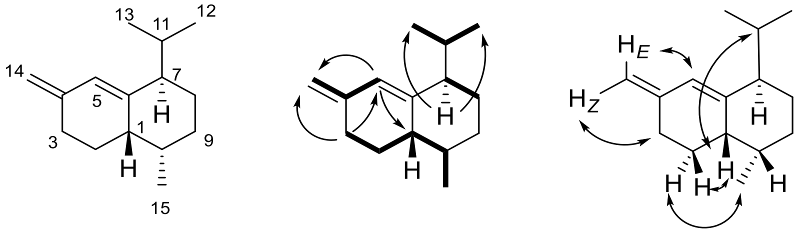


Structure of cembrene C (**8**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows.

Purification by column chromatography: Pentanes (100%), **yield**: 1.1 mg. **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 273 (4), 272 (18), 258 (3), 257 (20), 229 (15), 201 (6), 189 (9), 187 (6), 177 (4), 176 (4), 175 (14), 173 (8), 163 (6), 162 (15), 161 (21), 159 (9), 150 (5), 149 (20), 148 (13), 147 (22), 145 (8), 137 (6), 136 (22), 135 (31), 134 (16), 133 (32), 131 (5), 123 (15), 122 (32), 121 (100), 120 (13), 119 (36), 117 (5), 115 (3), 109 (19), 108 (18), 107 (55), 106 (10), 105 (39), 103 (3), 95 (21), 94 (13), 93 (64), 92 (7), 91 (39), 83 (6), 82 (4), 81 (34), 80 (8), 79 (36), 78 (3), 77 (23), 69 (15), 68 (24), 67 (46), 65 (8), 57 (3), 55 (21), 53 (22).

C	^{13}C	^{1}H
1	124.74	-
2	31.0	2.91 (d, ^{3}J = 7.0, 2H)
3	125.3	5.18 (tq, ^{3}J = 6.9, ^{4}J = 1.4)
4	133.7	-
5	39.3	2.14-2.10 (m, 2H)
6	25.3	2.17 (dt, ^{3}J = 6.3, ^{3}J = 8.7, 2H)
7	126.8	5.05 (tq, ^{3}J = 6.5, ^{4}J = 1.4)
8	133.1	-
9	40.4	2.09-2.05 (m, 2H)
10	24.3	2.11-2.08 (m, 2H)
11	124.66	5.16 (tq, ^{3}J = 6.9, ^{4}J = 1.4)
12	134.8	-
13	37.9	2.10-2.07 (m, 2H)
14	31.9	2.30 (t, ^{3}J = 7.7, 2H)
15	131.7	-
16	20.7	1.67 (s, 3H)
17	20.9	1.70 (s, 3H)
18	15.4	1.56 (s, 3H)
19	15.1	1.52 (s, 3H)
20	16.0	1.61 (s, 3H)

Sesquiterpene product of HcTC-3 (**9**)

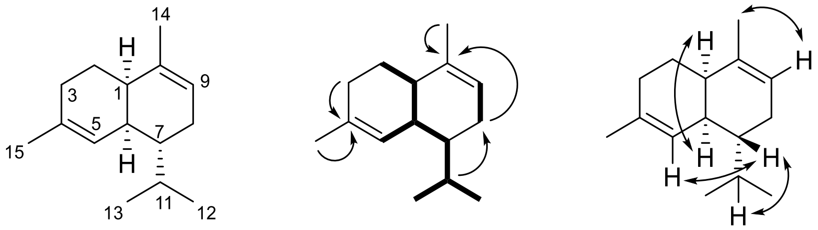


Structure of nephthene (**9**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows. **Purification by column chromatography**: Pentanes (100%), **yield**: 1.3 mg **Optical rotary power**: $[\alpha]_D^{25.0}$ = +5.3 (*c* 0.19, $CHCl_3$). **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 204 (20), 162 (15), 161 (100), 147 (5), 133 (9), 131 (3), 120 (7), 119 (19), 117 (8), 115 (6), 107 (4), 106 (4), 105 (27), 103 (3), 93 (10), 92 (4), 91 (24), 81 (9), 79 (9), 77 (8), 69 (4), 67 (4), 65 (3), 55 (4).

The absolute configuration was derived from **2**., sample contains a second sesquiterpene (~20%) of unknown structure, which is not separable by column chromatography.

C	^{13}C	^{1}H
1	37.1	2.13-2.07, (m)
2	27.5	1.62-1.53 (m) 1.53-1.44 (m)
3	30.1	2.35 (ddd, *J* = 14.5, 8.4, 4.6 Hz) 2.19-2.12 (m)
4	144.1	-
5	127.3	6.09 (s)
6	144.2	-
7	51.5	1.69-1.62 (m)
8	23.1	1.70-1.64 (m) 1.64-1.56 (m)
9	29.6	1.70-1.63 (m) 1.25-1.17 (m)
10	35.2	1.78-1.75 (m)
11	27.5	1.75-1.69 (m)
12	21.0	0.79 (d, *J* = 6.4, 3H)
13	21.5	0.88 (d, *J* = 6.3, 3H)
14	108.9	4.88 (s, H_E) 4.80 (s, H_Z)
15	15.0	0.84 (d, *J* = 7.0, 3H)

Sesquiterpene product of TmTC-2 (**10**)

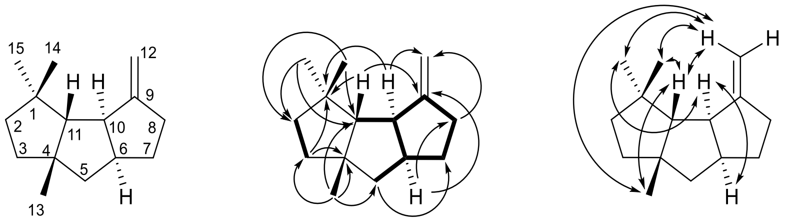


Structure of a-muuroladiene (**10**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows.
Purification by column chromatography: Pentanes (100%), **yield**: 2.5 mg. **Optical rotary power**: $[\alpha]_D^{25.0}$ = +55.0 (*c* 0.25, $CHCl_3$). **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 205 (5), 204 (33), 189 (12), 162 (10), 161 (60), 160 (5), 149 (3), 148 (6), 147 (11), 145 (6), 135 (3), 134 (6), 133 (14), 131 (6), 129 (5), 128 (5), 123 (5), 121 (10), 120 (5), 119 (25), 117 (8), 115 (7), 107 (10), 106 (17), 105 (100), 103 (3), 95 (8), 94 (27), 93 (31), 92 (8), 91 (30), 81 (18), 80 (3), 79 (18), 78 (4), 77 (16), 69 (5), 67 (5), 65 (5), 55 (8), 53 (6).

The absolute configuration was set after optical rotation [50].

C	^{13}C	^{1}H
1	39.4	2.02-1.96 (m)
2	25.0	1.82-1.74 (m) 1.53-1.43 (m)
3	30.7	1.90-1.79 (m, 2H)
4	134.4	-
5	124.7	5.59-5.55 (m)
6	37.2	2.16-2.10 (m)
7	41.5	1.57-1.48 (m)
8	24.9	1.91-1.84 (m) 1.82-1.74 (m)
9	122.6	5.47-5.44 (m)
10	136.1	-
11	27.0	2.07 (dsept, ^3J = 6.9, ^3J = 4.4)
12	21.6	0.88 (d, ^3J = 6.9, 3H)
13	16.1	0.87 (d, ^3J = 6.8, 3H)
14	21.9	1.70 (s, 3H)
15	24.1	1.67 (s, 3H)

Sesquiterpene product of CiTC-2 (**11**)



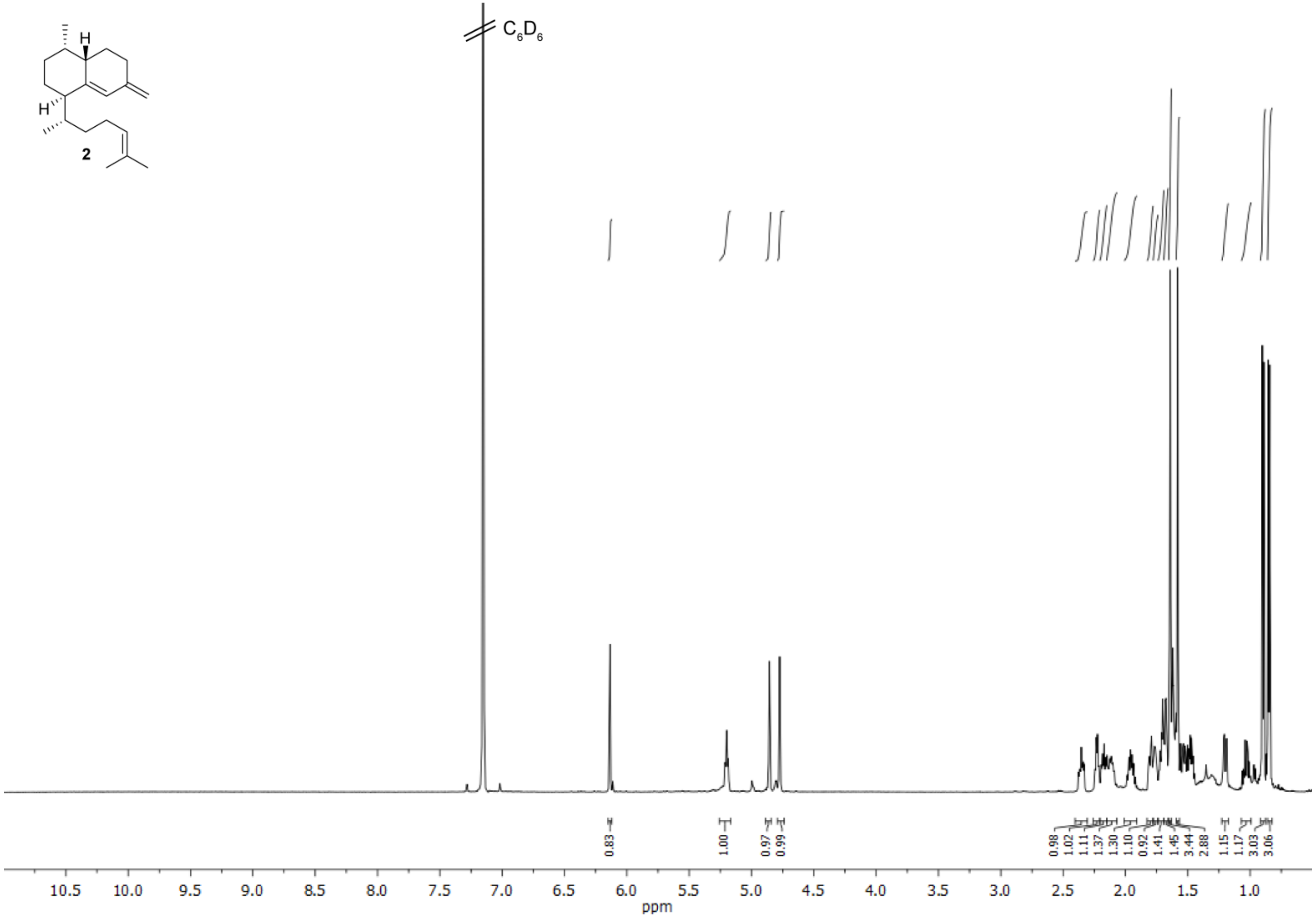
Structure of capnellene (**11**). COSY correlations are shown as bold lines, key HMBC correlations are shown as single headed arrows. Key NOE correlations are shown as double headed arrows.

Purification by column chromatography: Pentanes (100%), **yield**: 2 mg. **Optical rotary power**: $[\alpha]_D^{25.0}$ = -93.7 (*c* 0.16, $CHCl_3$). **NMR**: See table. **EI-MS** (70 eV): m/z (%) = 204 (6), 189 (14), 163 (7), 149 (3), 148 (21), 147 (6), 136 (6), 135 (46), 134 (18), 133 (45), 121 (3), 120 (3), 119 (12), 117 (4), 115 (4), 111 (4), 110 (6), 109 (42), 107 (13), 106 (9), 105 (15), 95 (18), 94 (9), 93 (19), 92 (5), 91 (28), 81 (25), 80 (100), 79 (30), 78 (5), 77 (18), 69 (10), 67 (9), 65 (6), 55 (11), 53 (7).

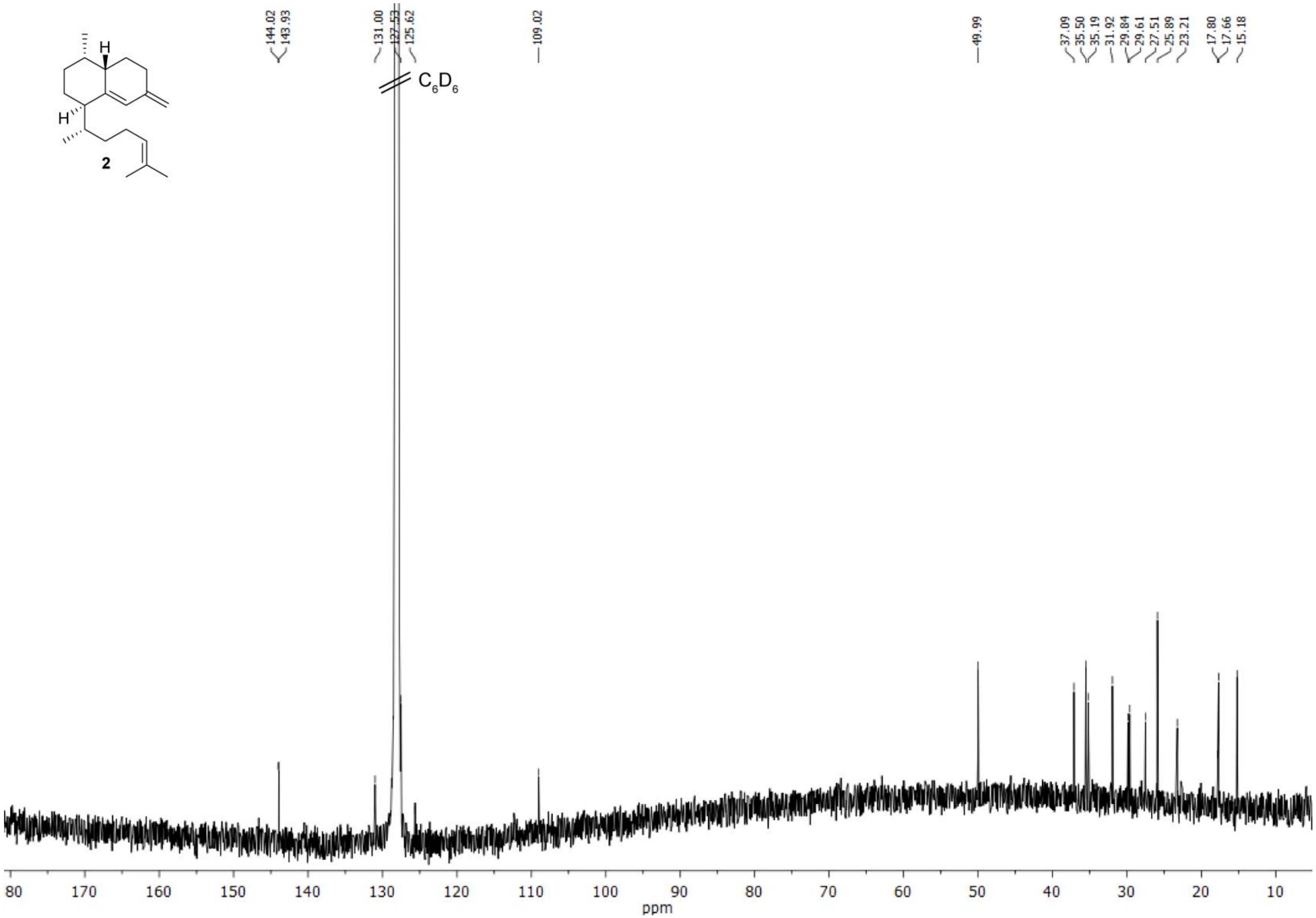
The absolute configuration set after optical rotation [51].

C	^{13}C	^{1}H
1	42.2	-
2	41.7	1.44 (m, 2H)
3	40.6	1.52 (m, 2H)
4	53.2	-
5	47.9	1.61 (m) 1.23 (dd, *J* = 13.1, 9.4 Hz)
6	46.1	2.44 (m)
7	29.0	1.63 (m) 1.43 (m)
8	31.5	2.46 (m) 2.34 (m)
9	158.3	-
10	52.3	2.67 (dt, *J* = 7.8, 2.6 Hz)
11	69.0	1.8 (d, *J* = 2.2 Hz)
12	105.4	5.04 (q, *J* = 2.4, H_E) 4.97 (q, *J* = 2.4, H_Z)
13	31.8	1.22 (s, 3H)
14	30.7	1.07 (s, 3H)
15	25.8	0.96 (s, 3H)

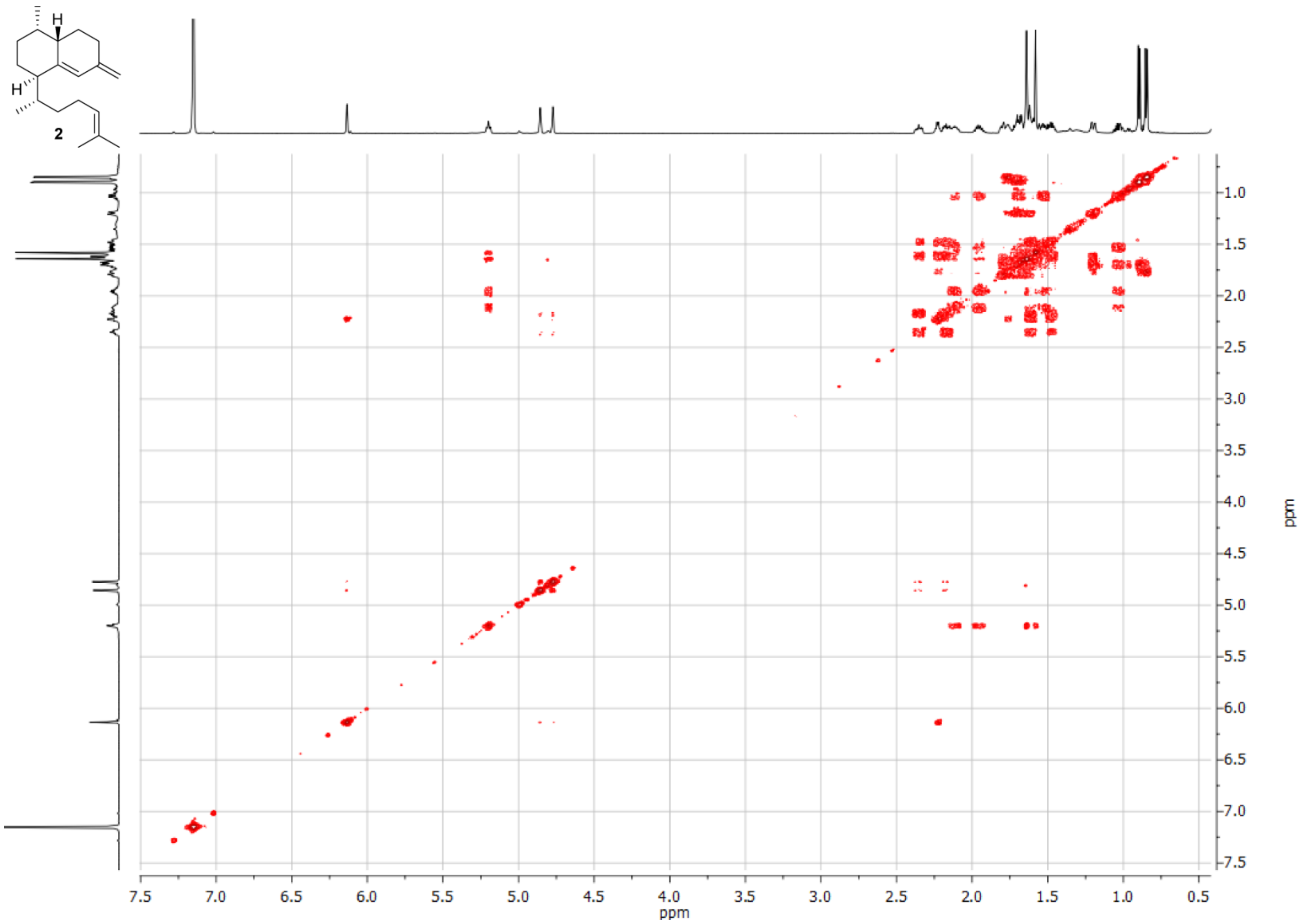
NMR spectra



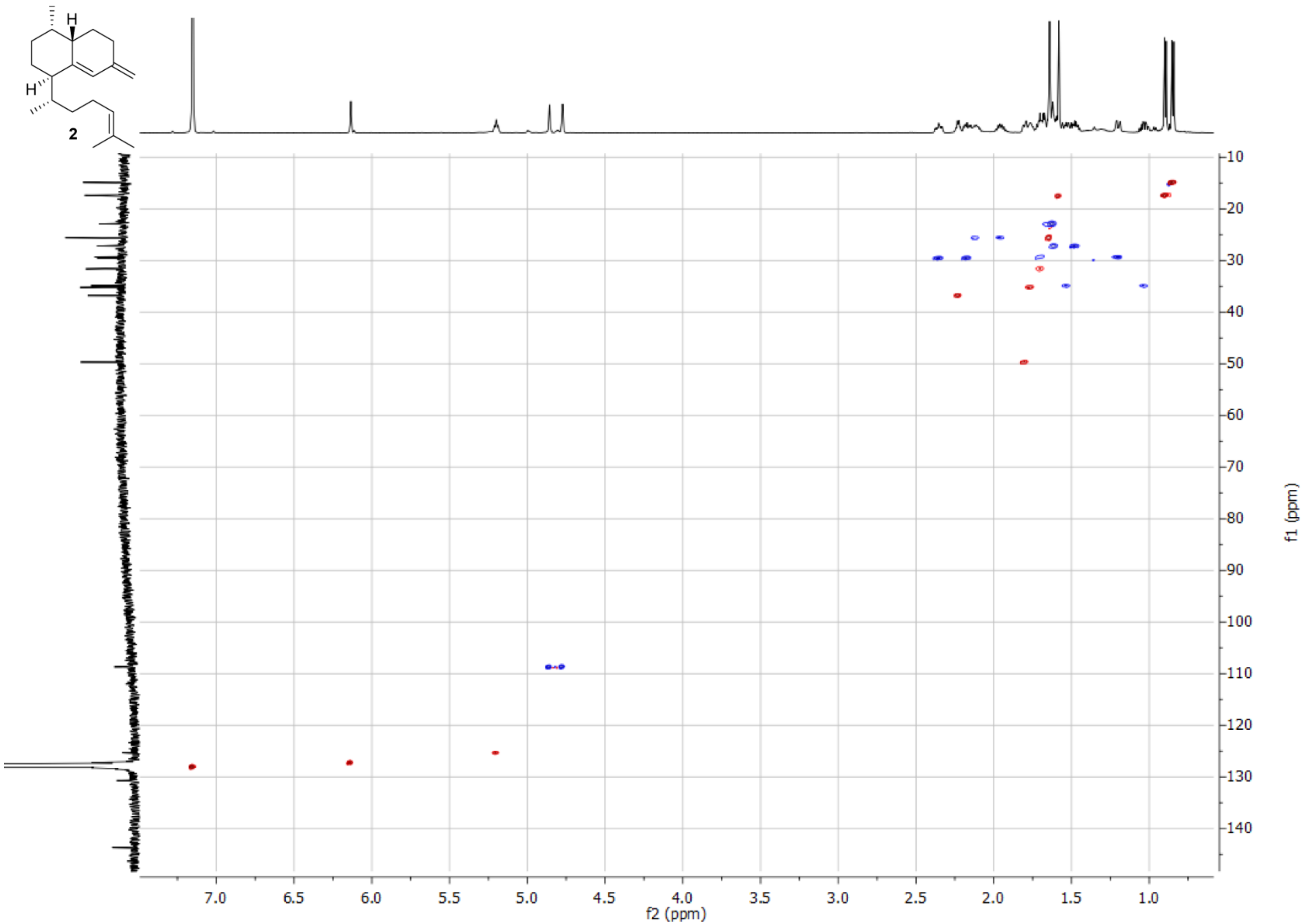
^{1}H NMR spectrum (600 MHz) of **2**.



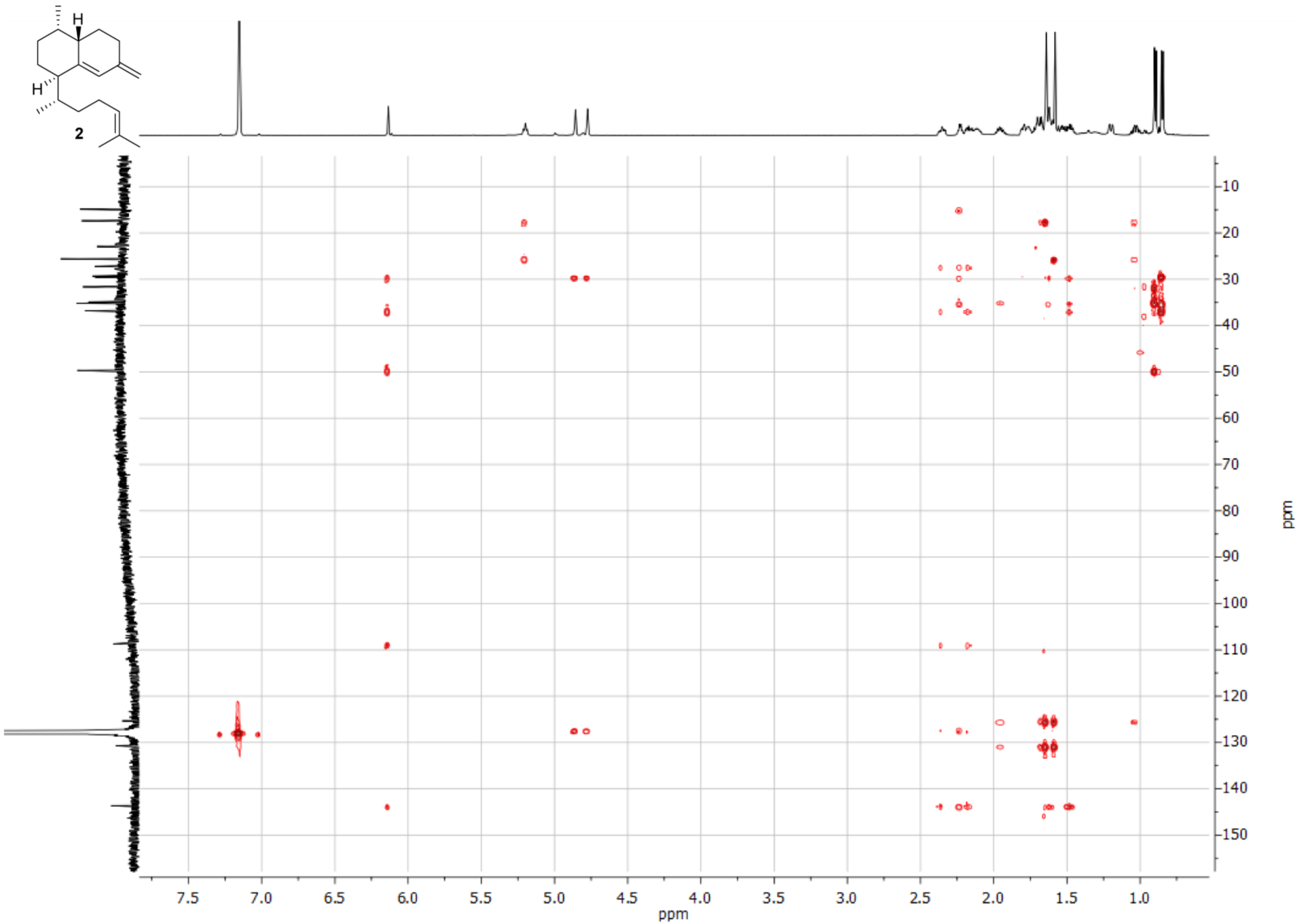
^{13}C NMR spectrum (125 MHz) of **2**.



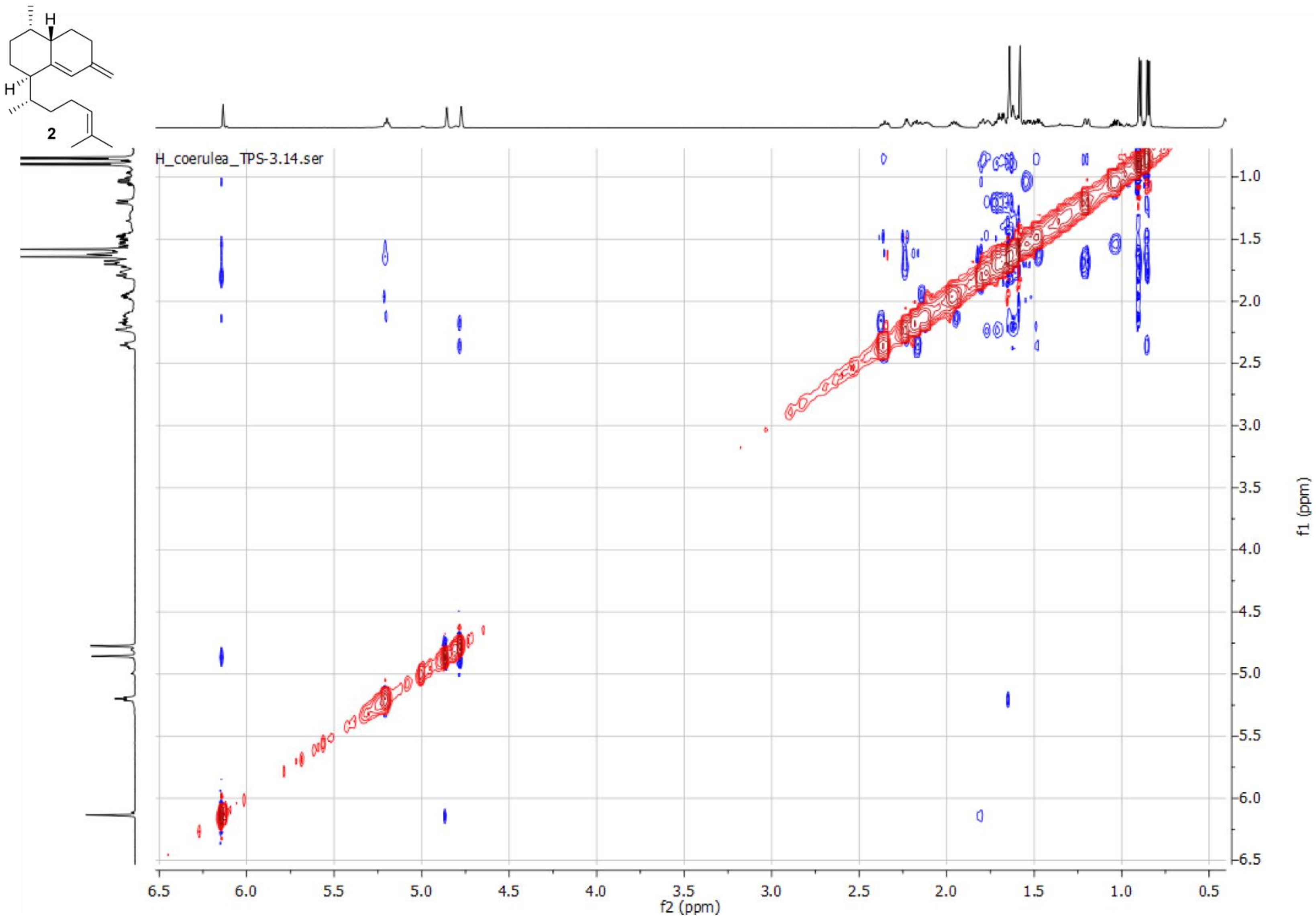
^{1}H, ^{1}H COSY spectrum (600 MHz) of **2**.



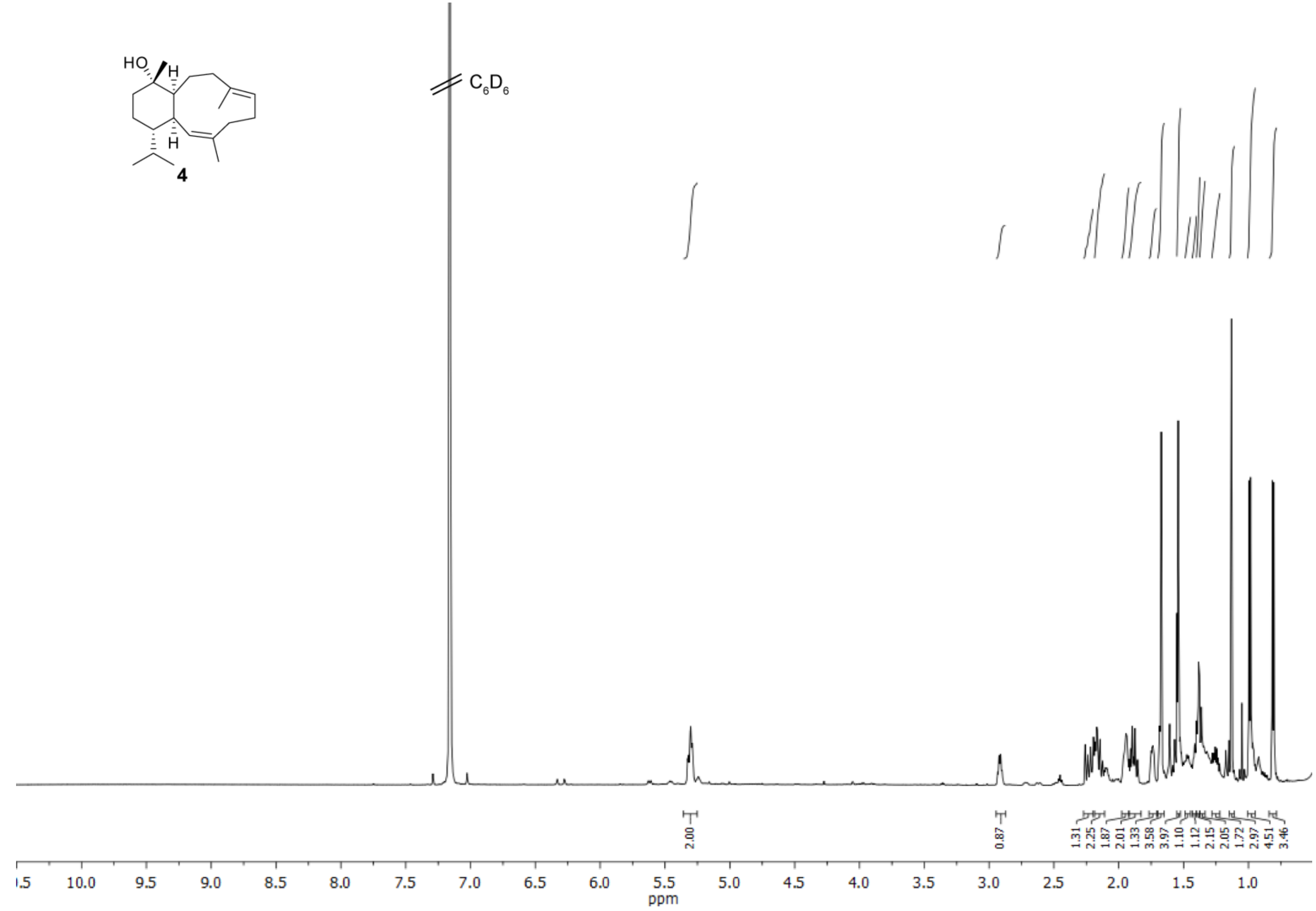
^{1}H, ^{13}C HSQC spectrum (600 MHz) of **2**



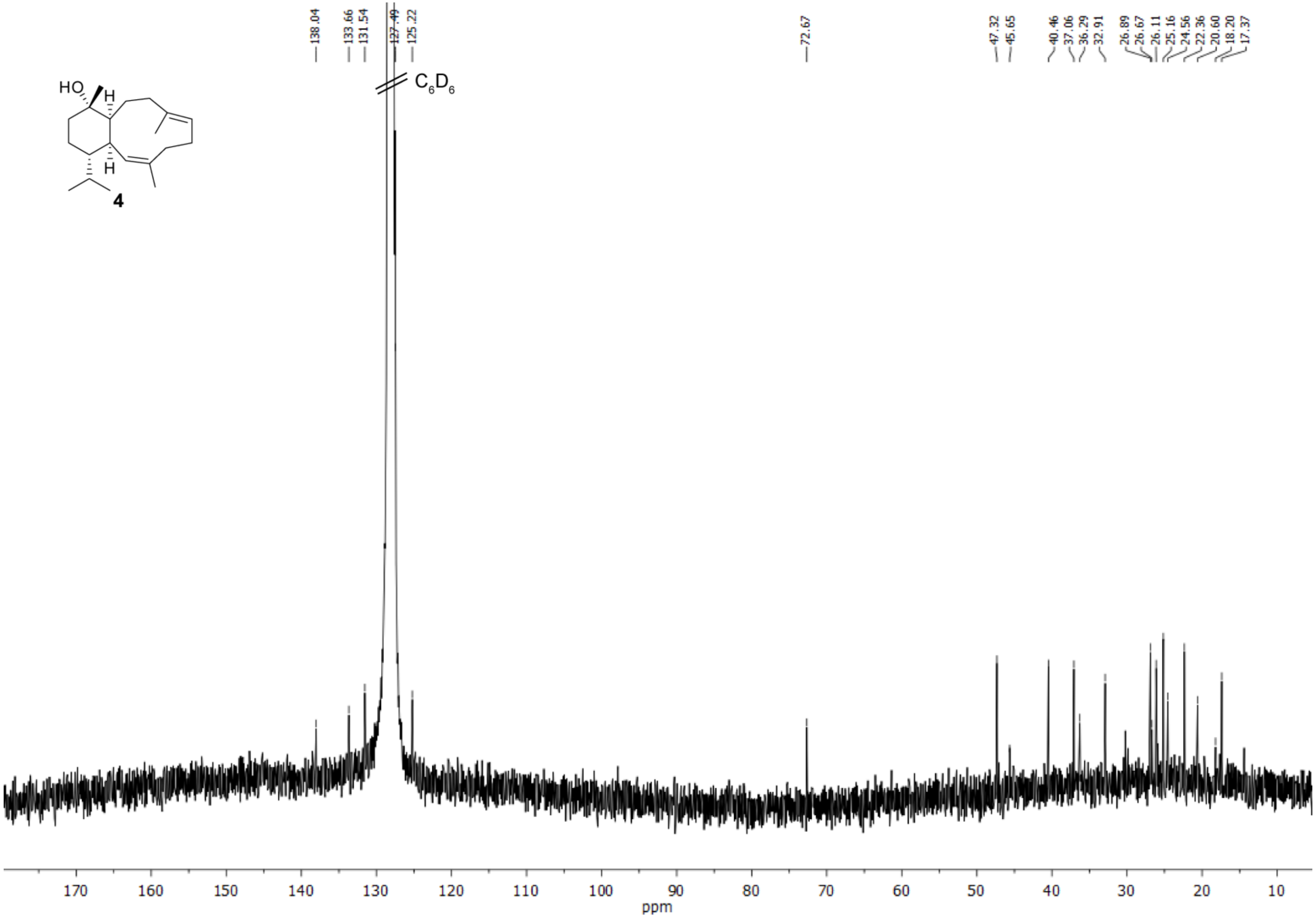
^{1}H, ^{13}C HMBC spectrum (600 MHz) of **2**



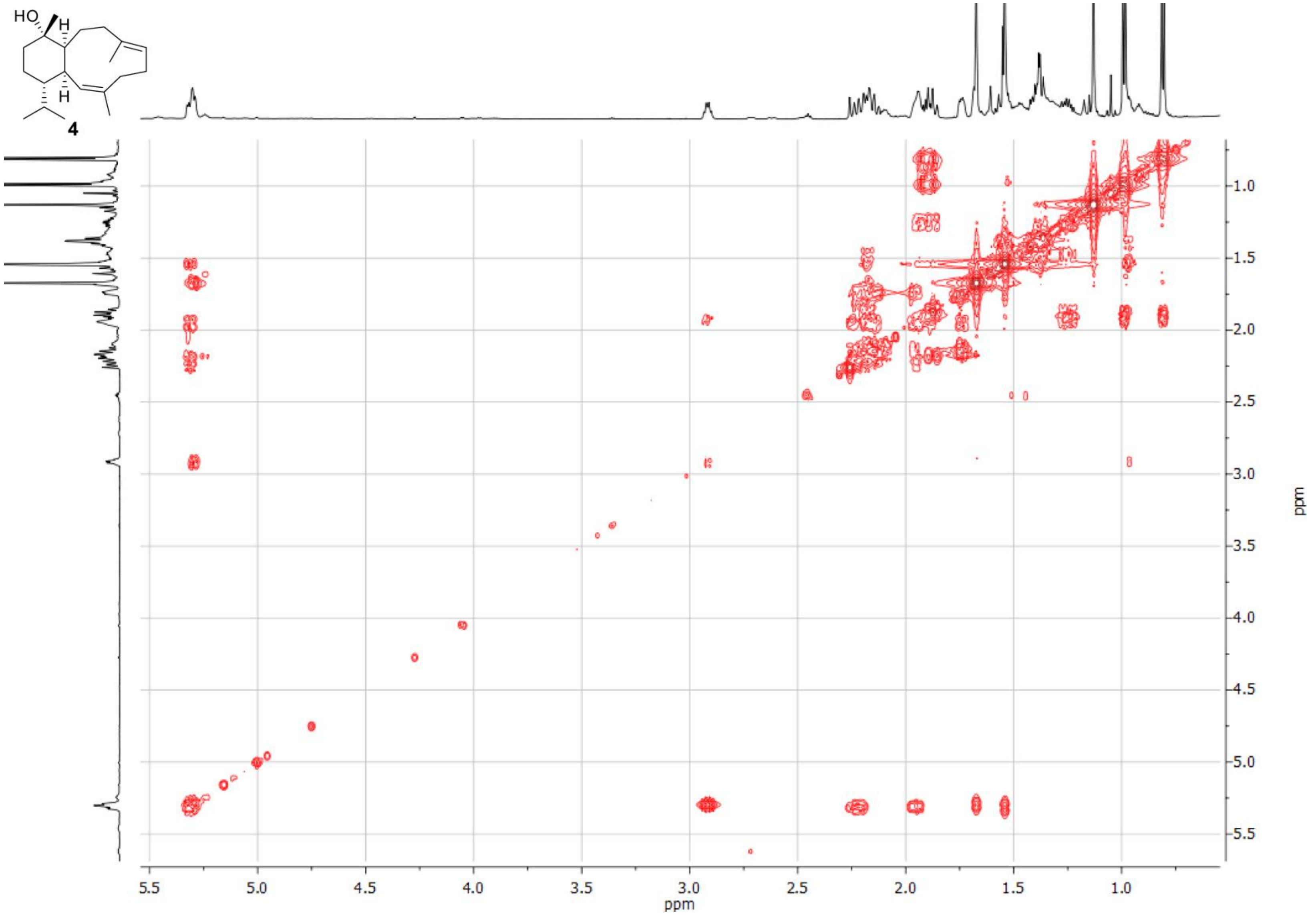
^{1}H, ^{1}H NOESY spectrum (600 MHz) of **2**.



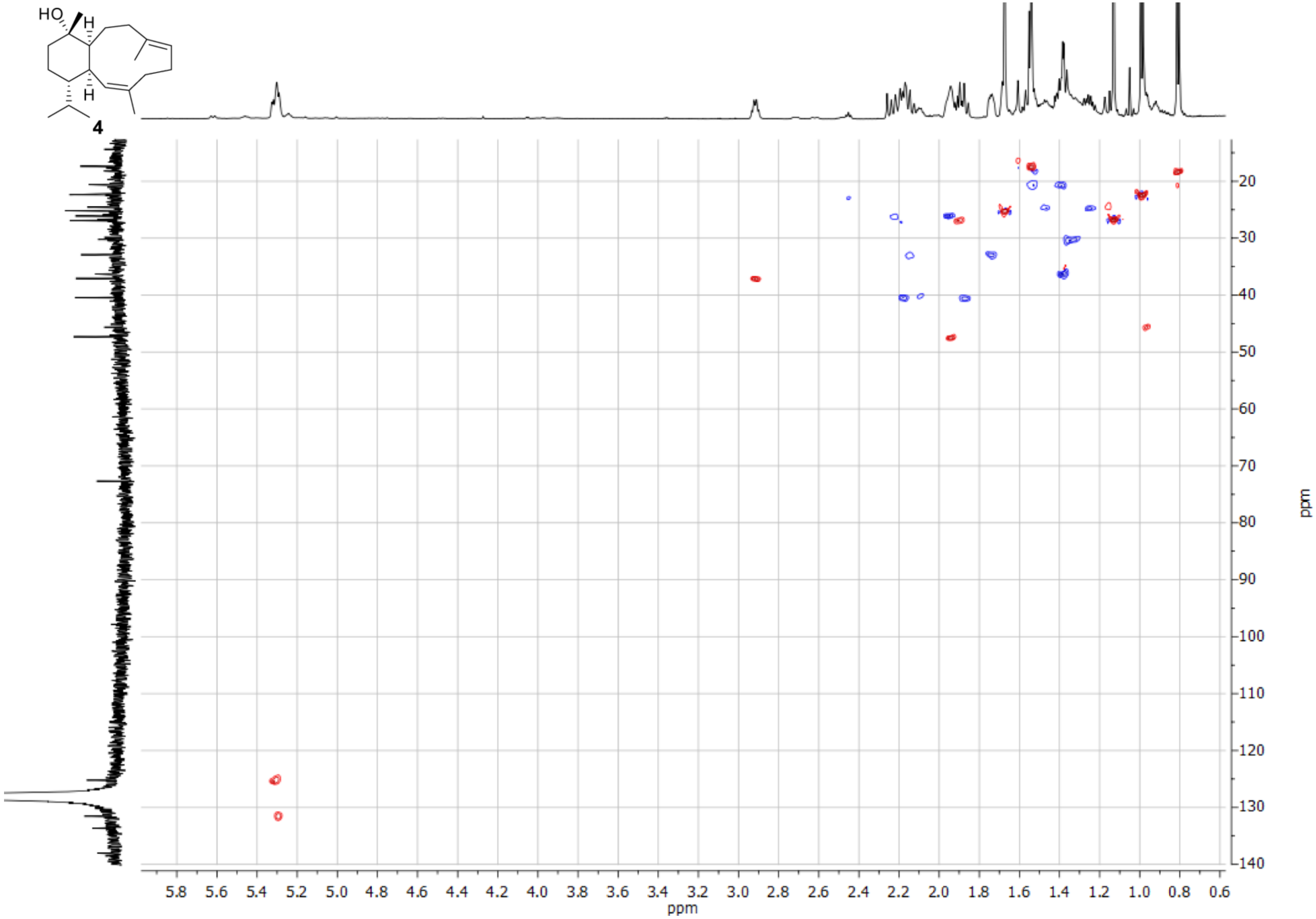
^{1}H NMR spectrum (600 MHz) of **4**.



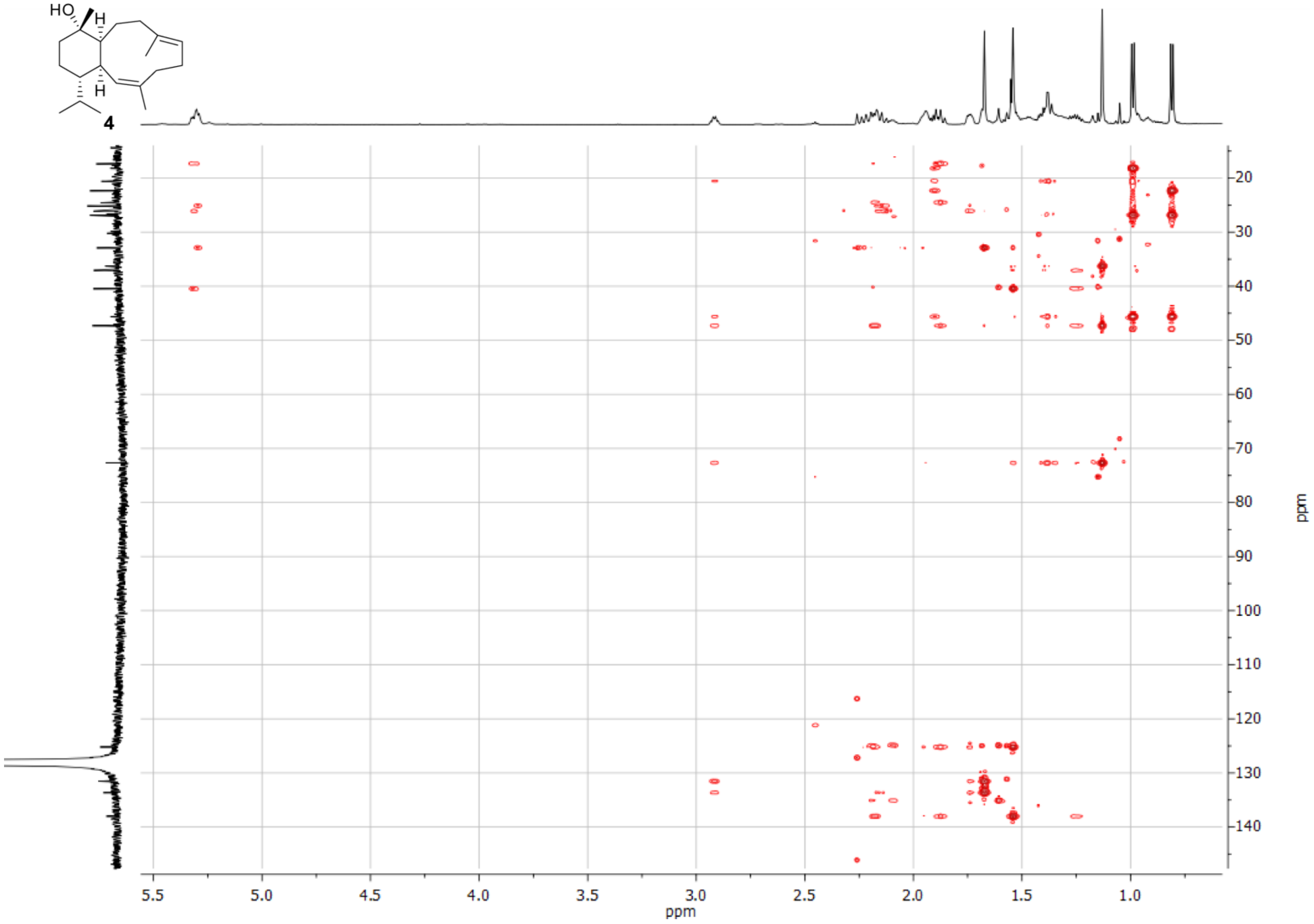
^{13}C NMR spectrum (125 MHz) of **4**.



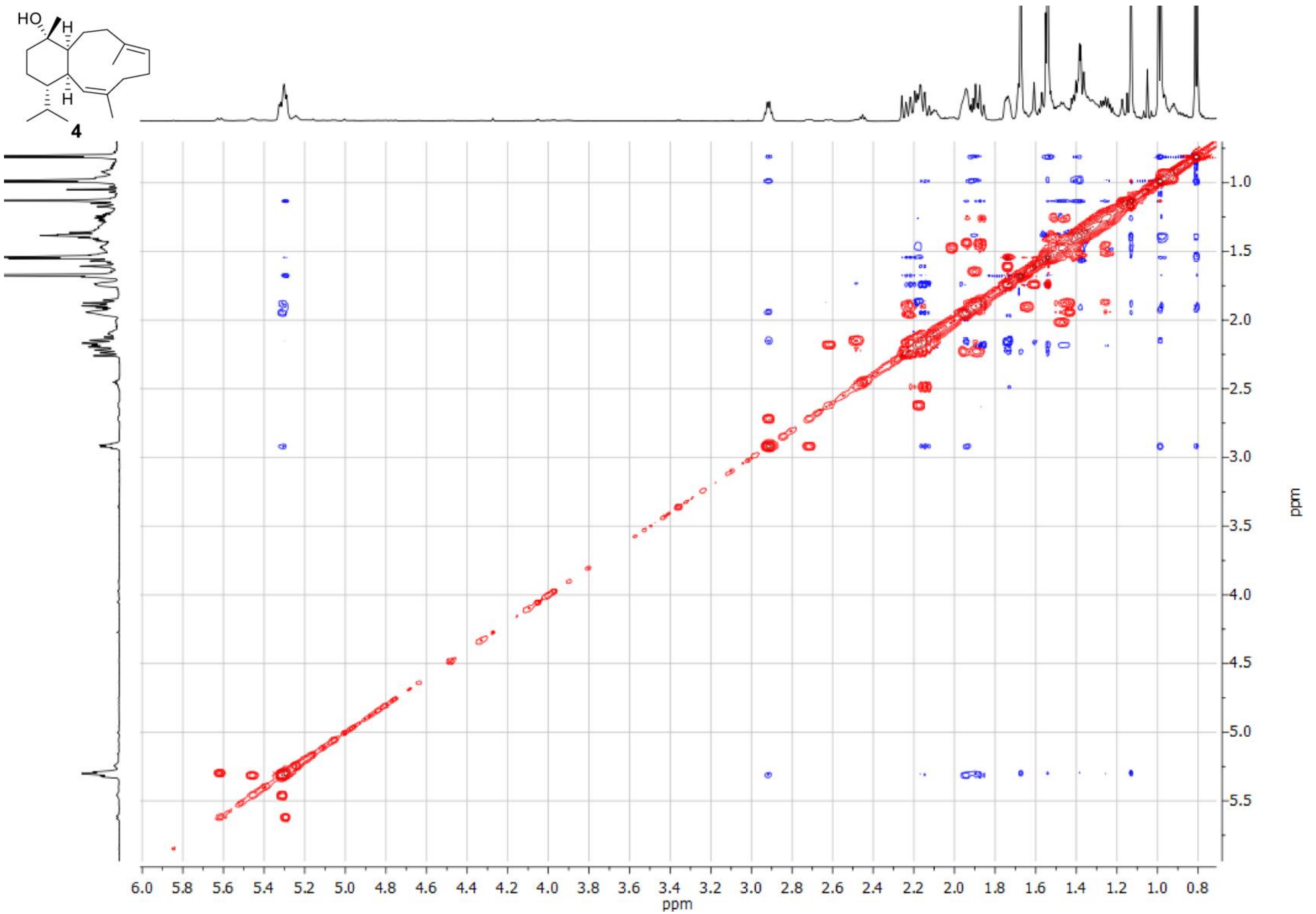
^{1}H, ^{1}H COSY spectrum (600 MHz) of **4**.



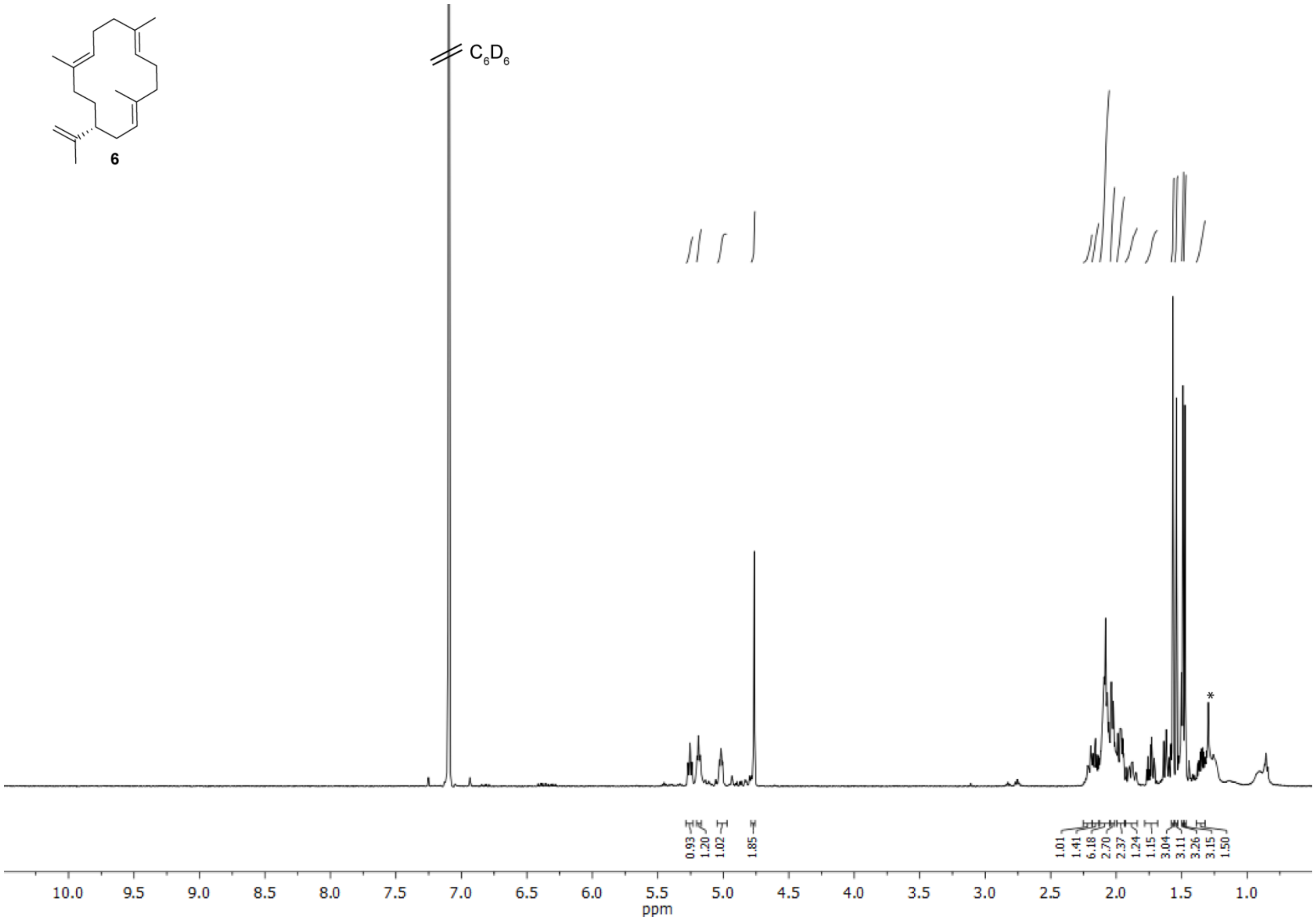
^{1}H, ^{13}C HSQC spectrum (600 MHz) of **4**.



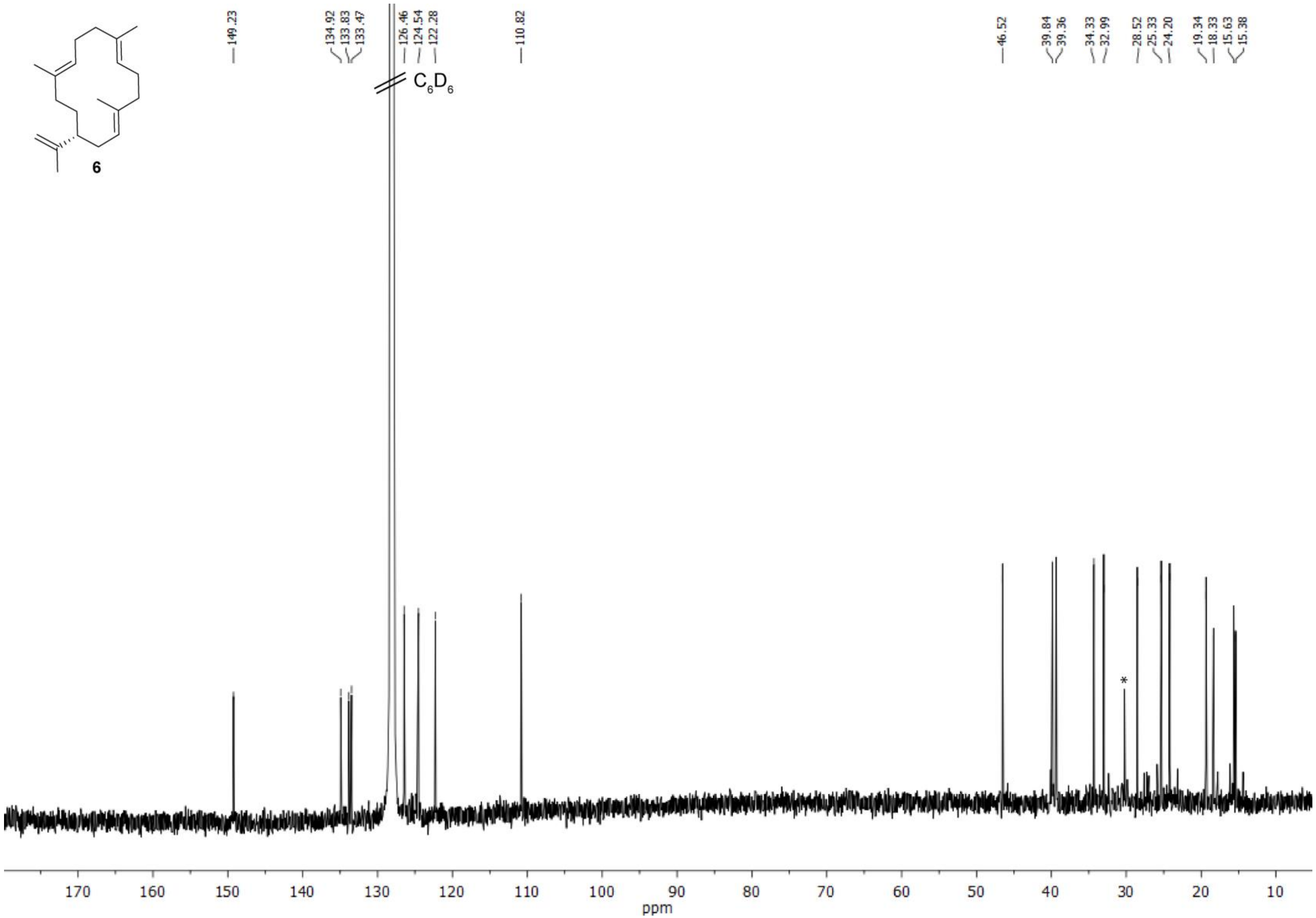
^{1}H, ^{13}C HMBC spectrum (600 MHz) of **4**.



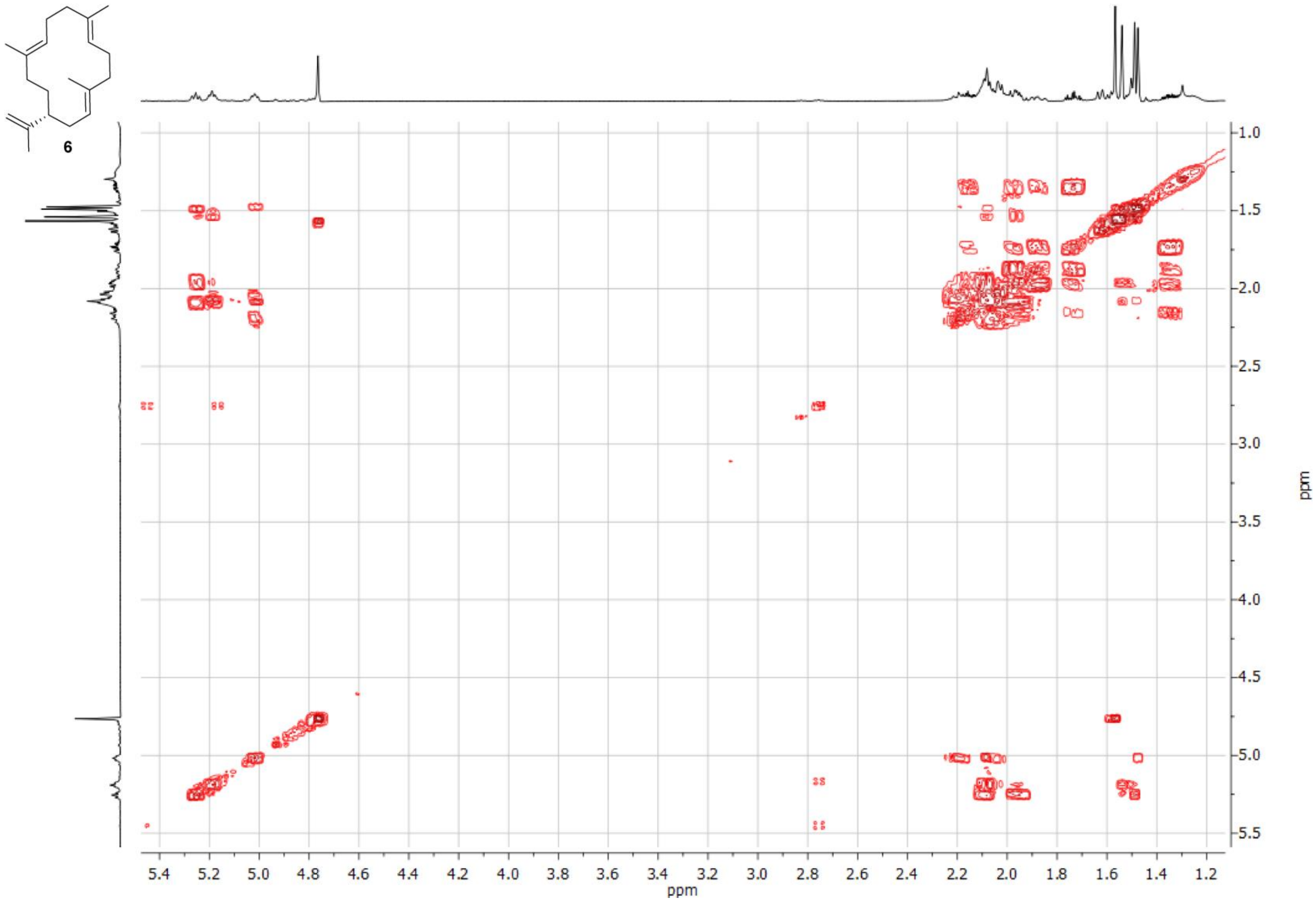
^{1}H, ^{1}H NOESY spectrum (600 MHz) of **4**.



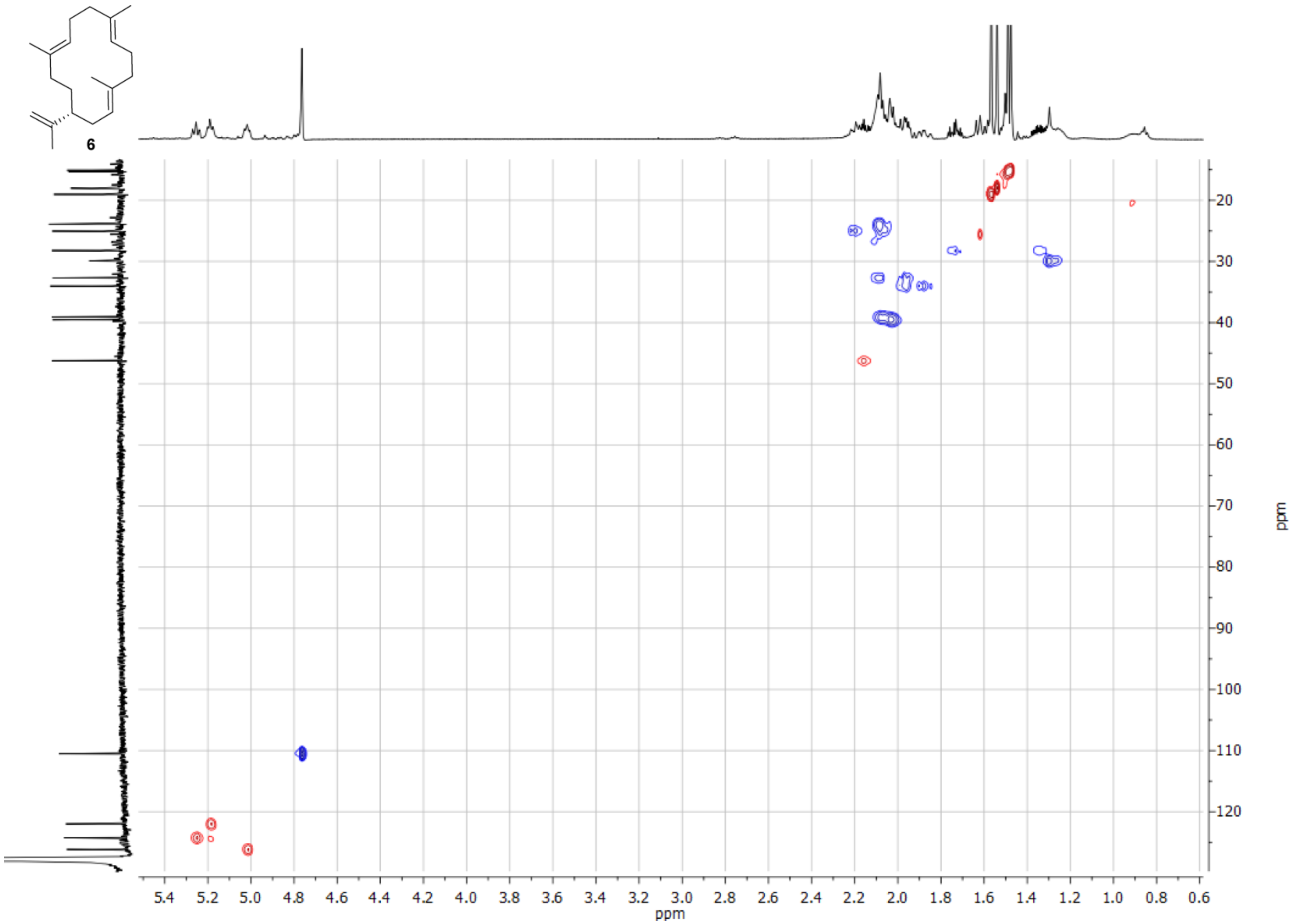
^{1}H NMR spectrum (500 MHz) of **6**.



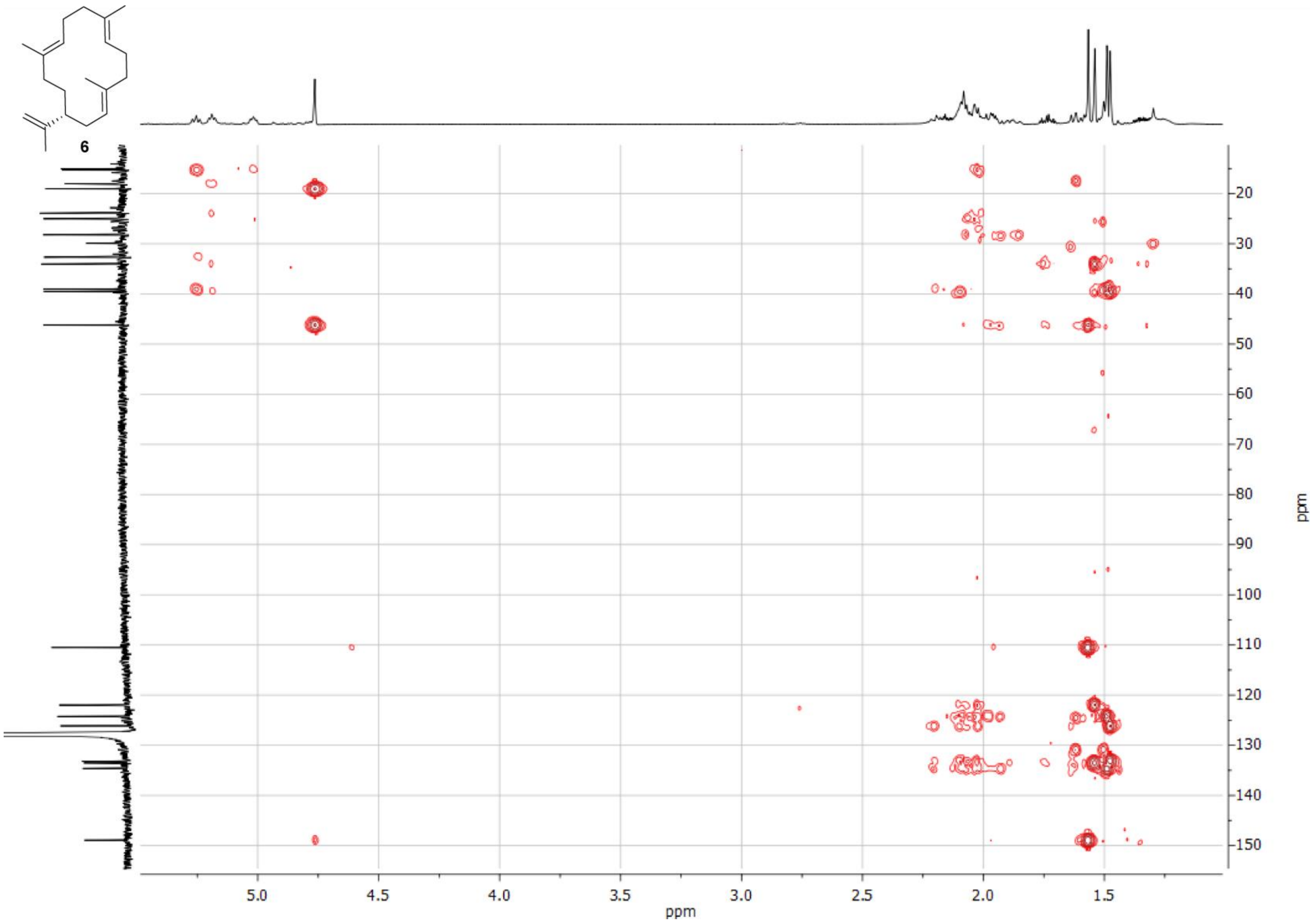
^{13}C NMR spectrum (125 MHz) of **6**.



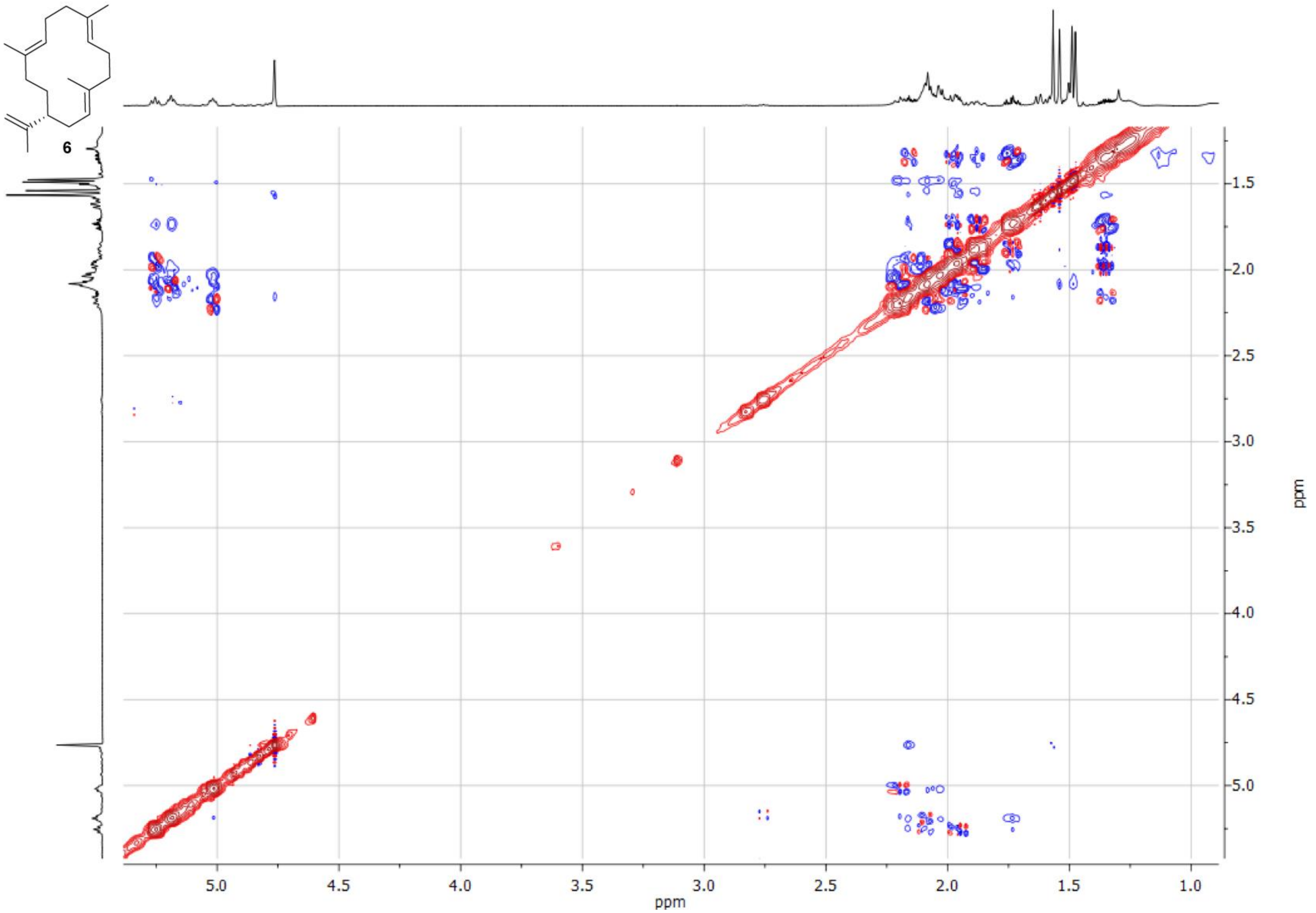
^{1}H, ^{1}H COSY spectrum (500 MHz) of **6**.



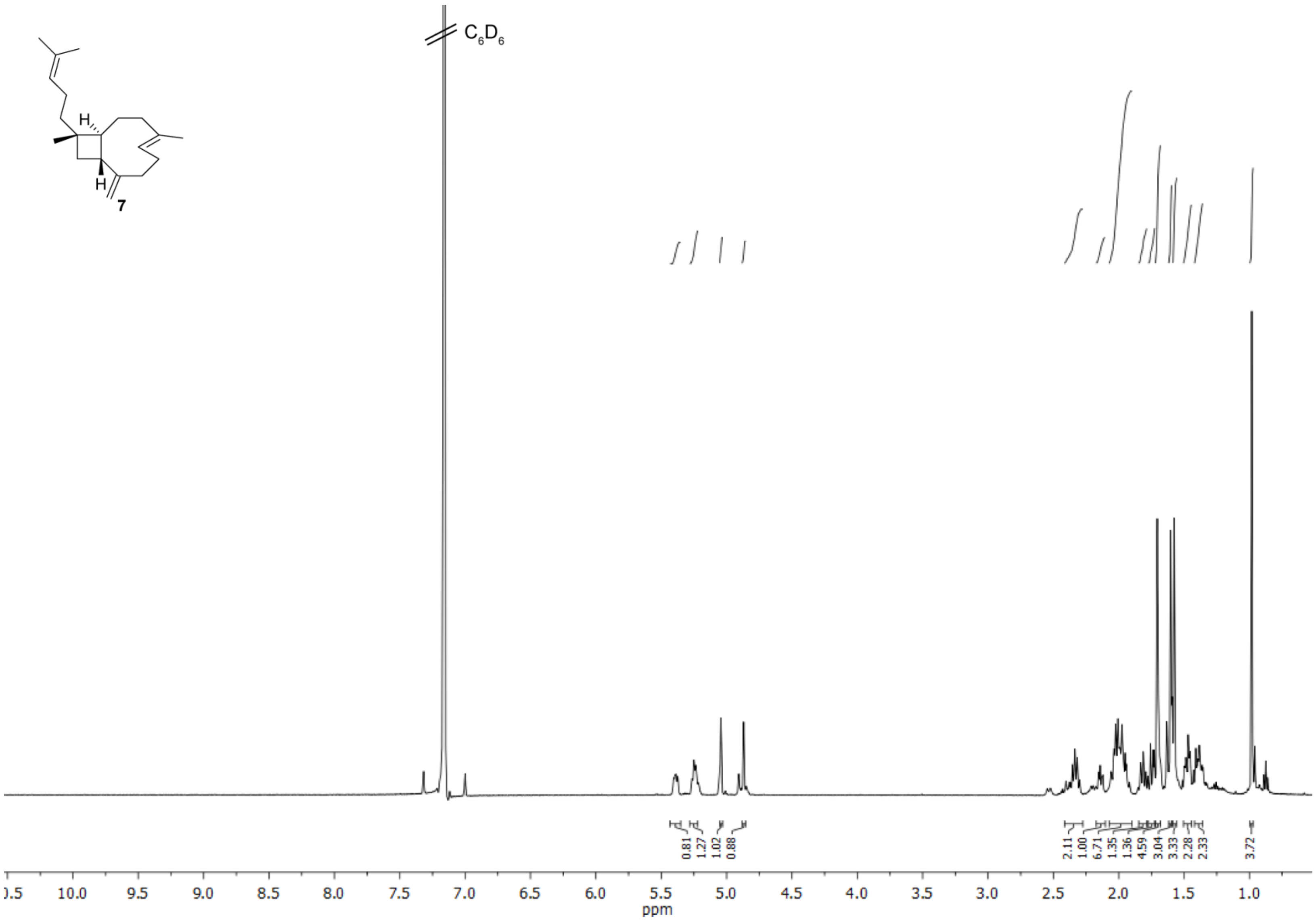
^{1}H, ^{13}C HSQC spectrum (500 MHz) of **6**.



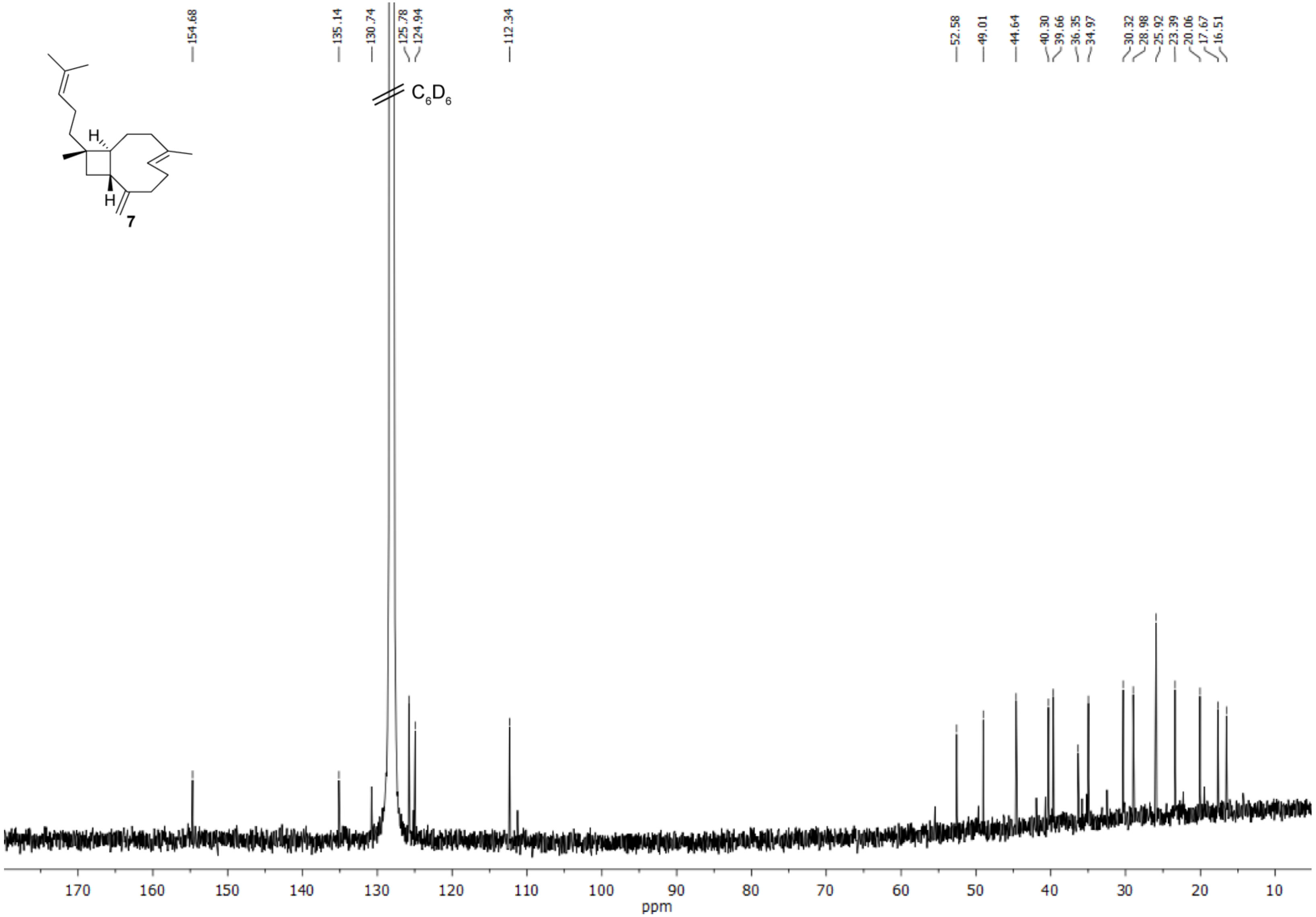
^{1}H, ^{13}C HMBC spectrum (500 MHz) of **6**.



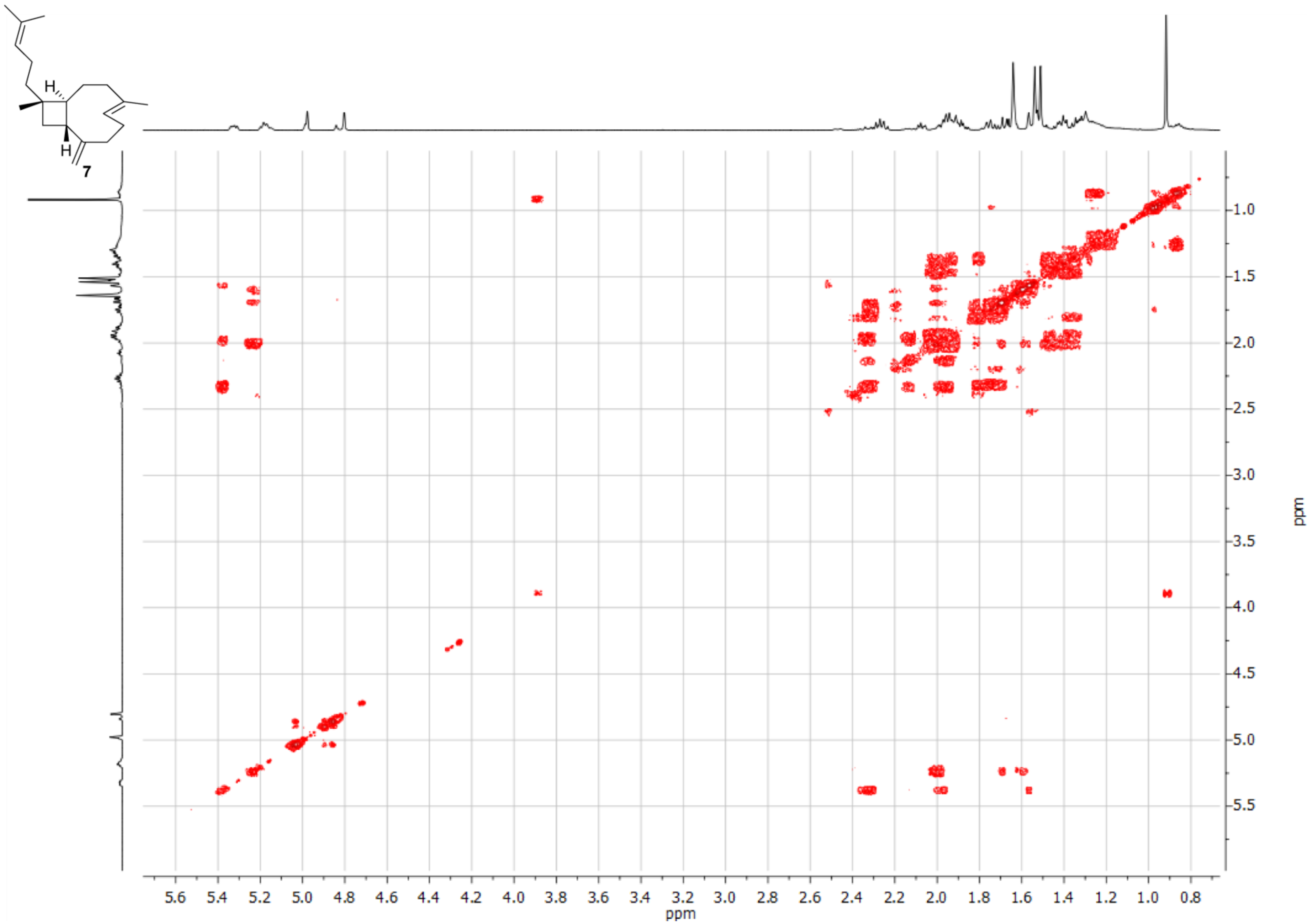
^{1}H, ^{1}H NOESY spectrum (600 MHz) of **6**.



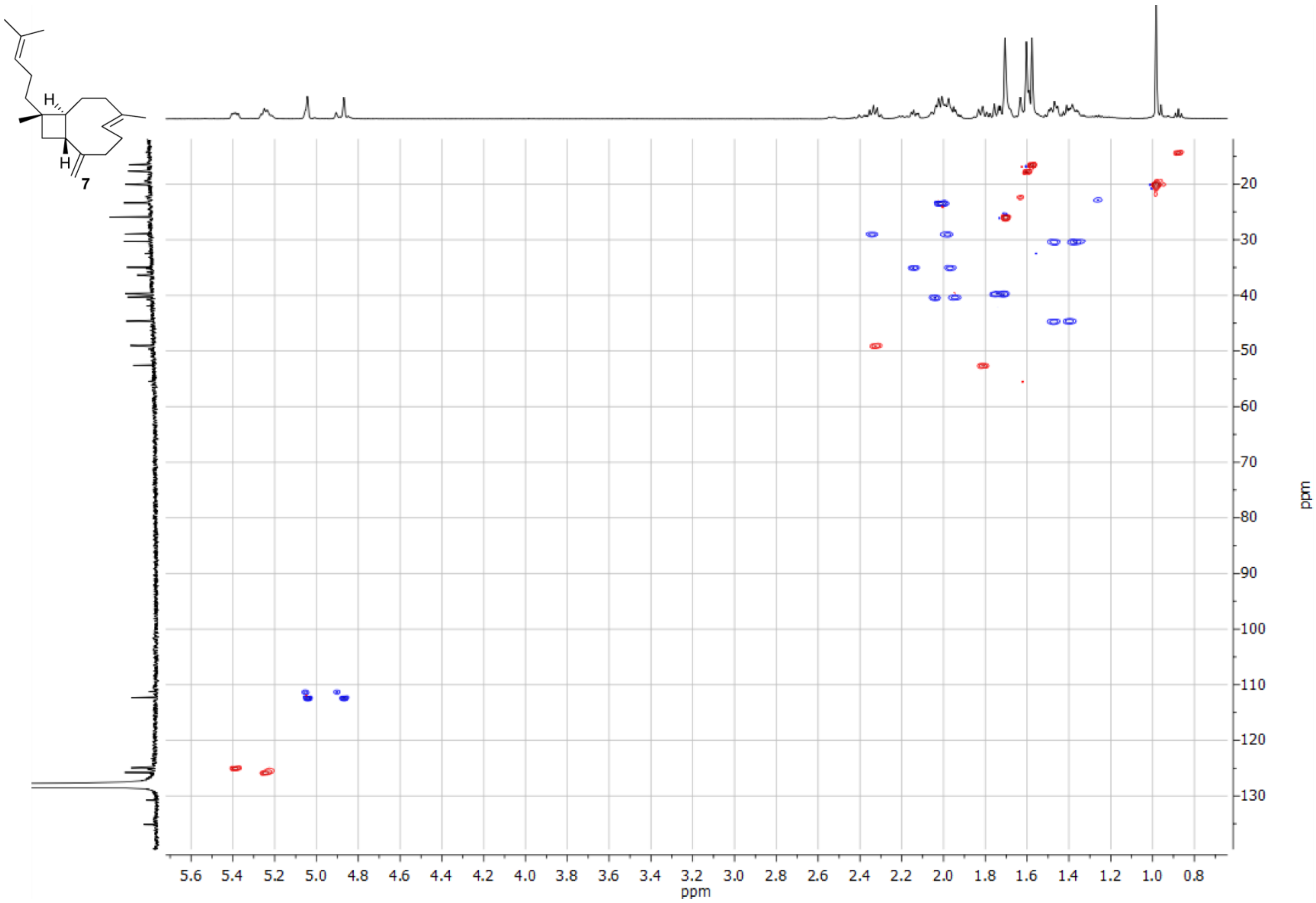
^{1}H NMR spectrum (600 MHz) of **7**.



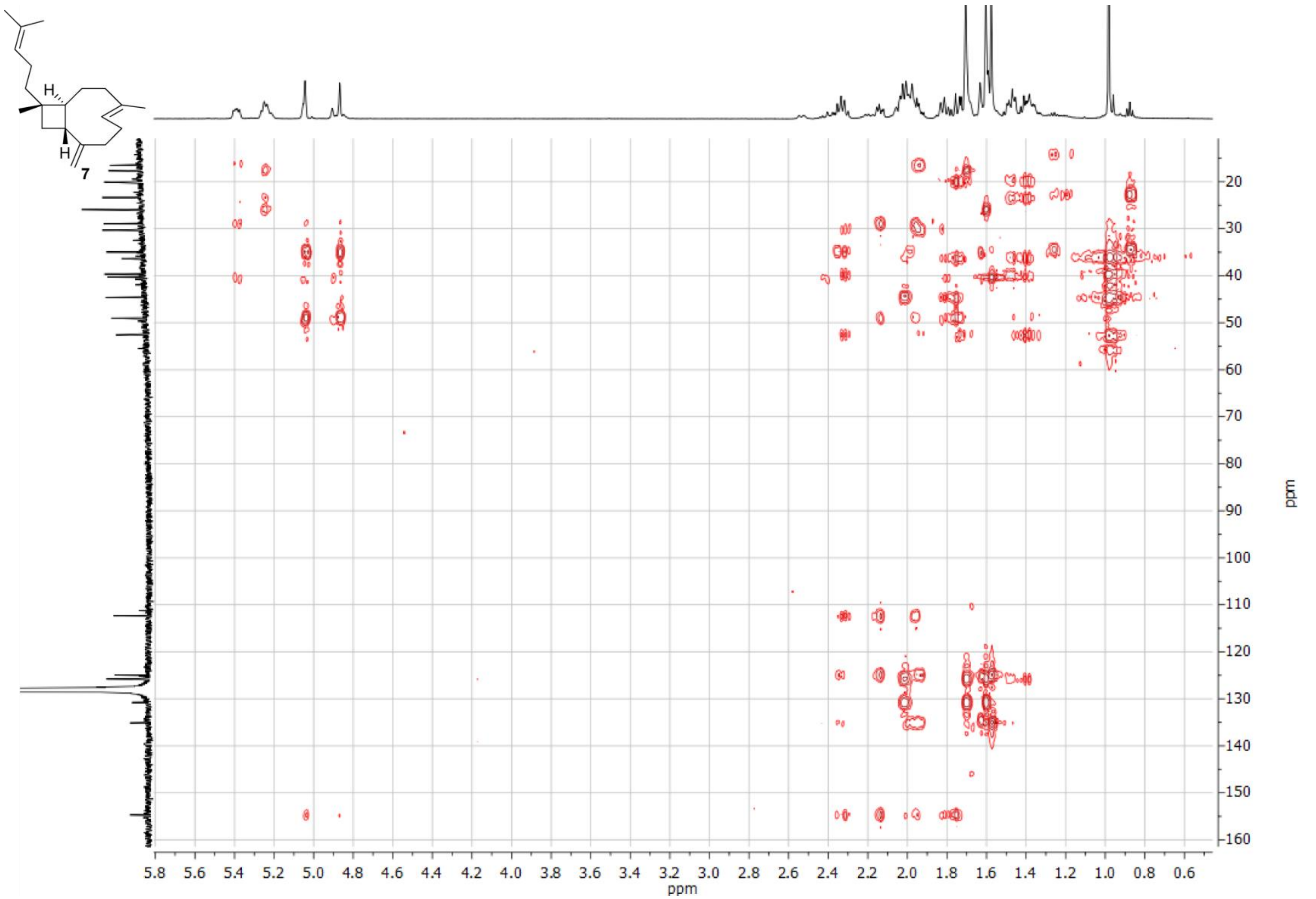
^{13}C NMR spectrum (125 MHz) of **7**.



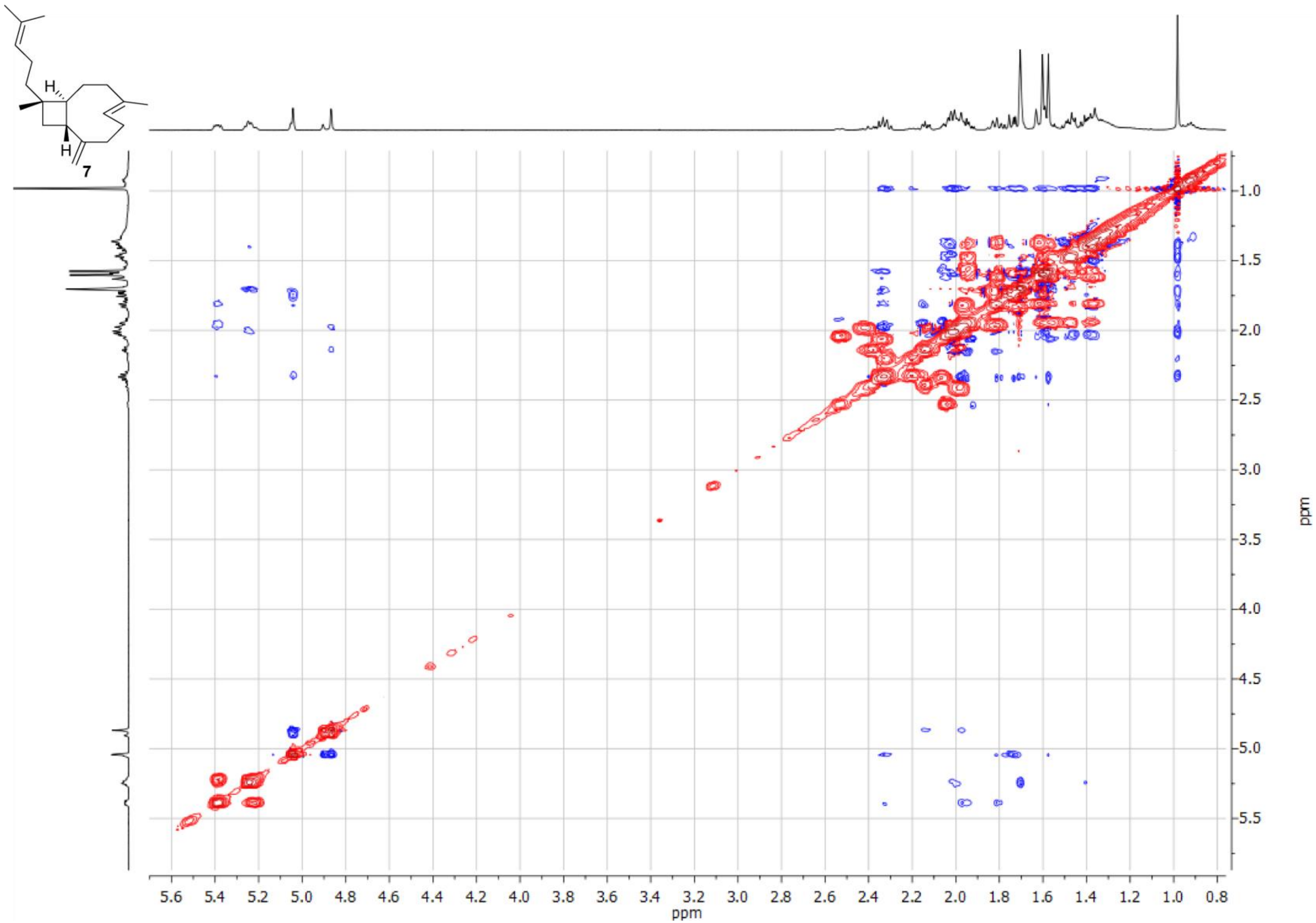
^{1}H, ^{1}H COSY spectrum (600 MHz) of **7**.



^{1}H, ^{13}C HSQC spectrum (600 MHz) of **7**.



^{1}H, ^{13}C HMBC spectrum (600 MHz) of **7**.



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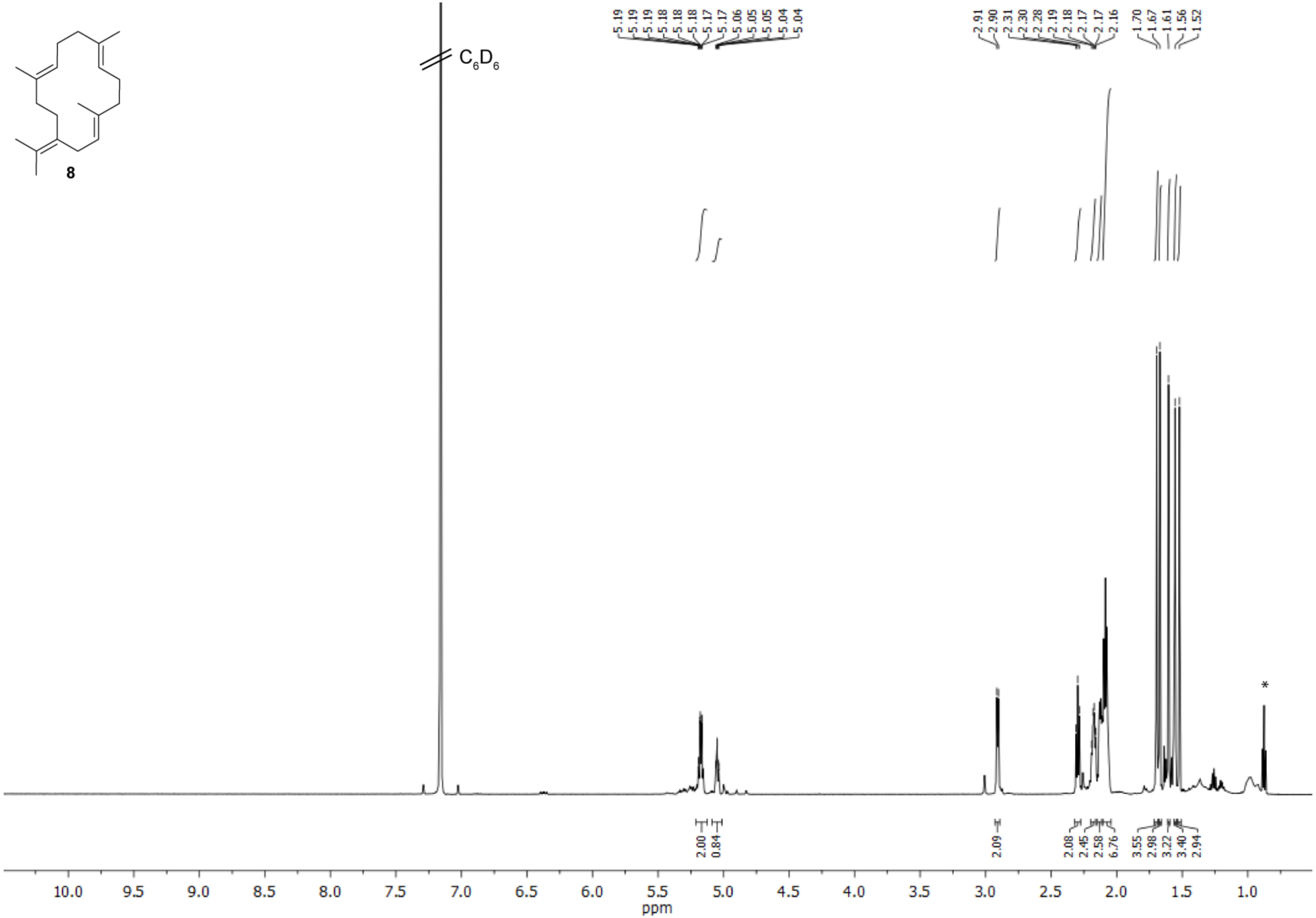
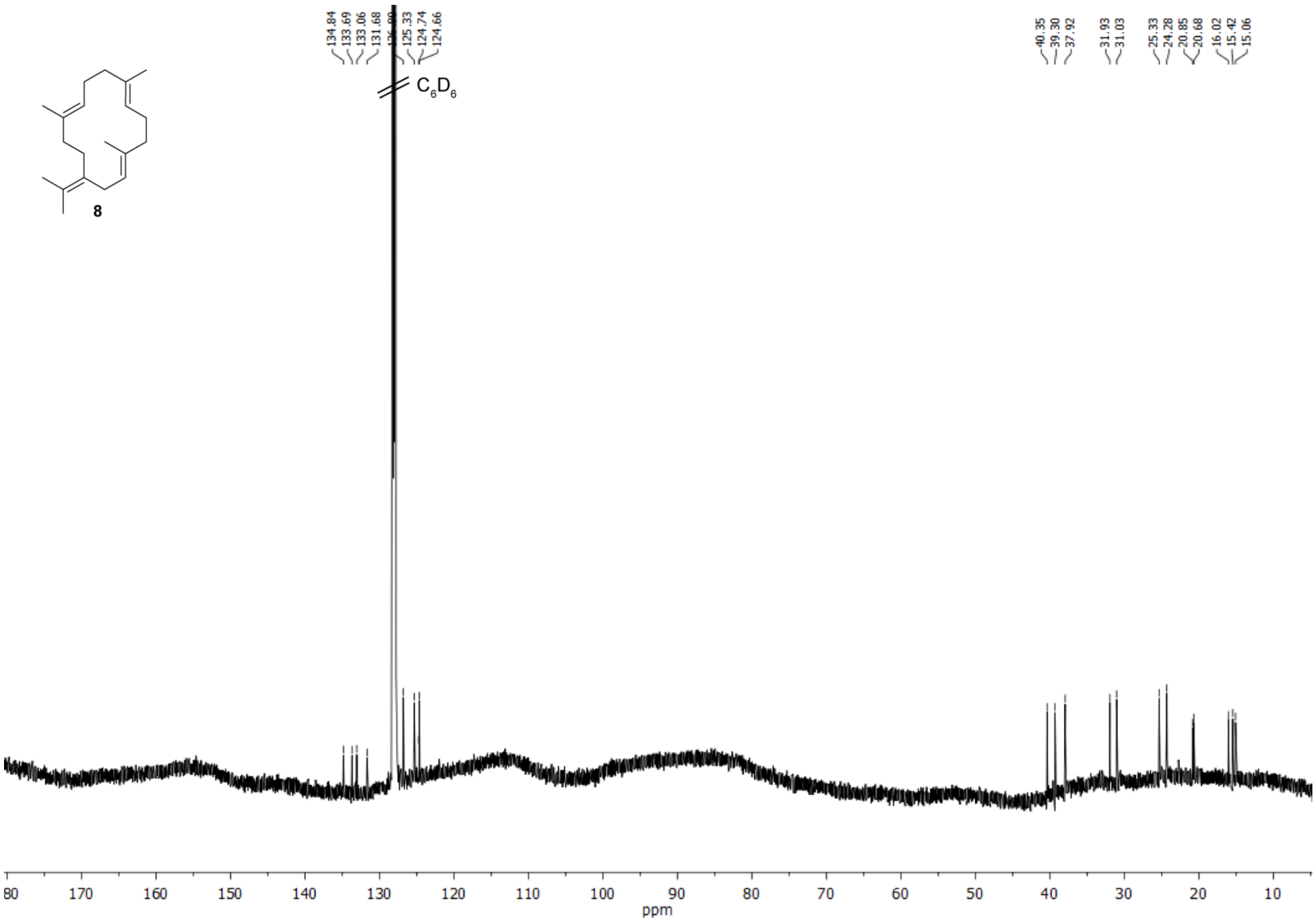
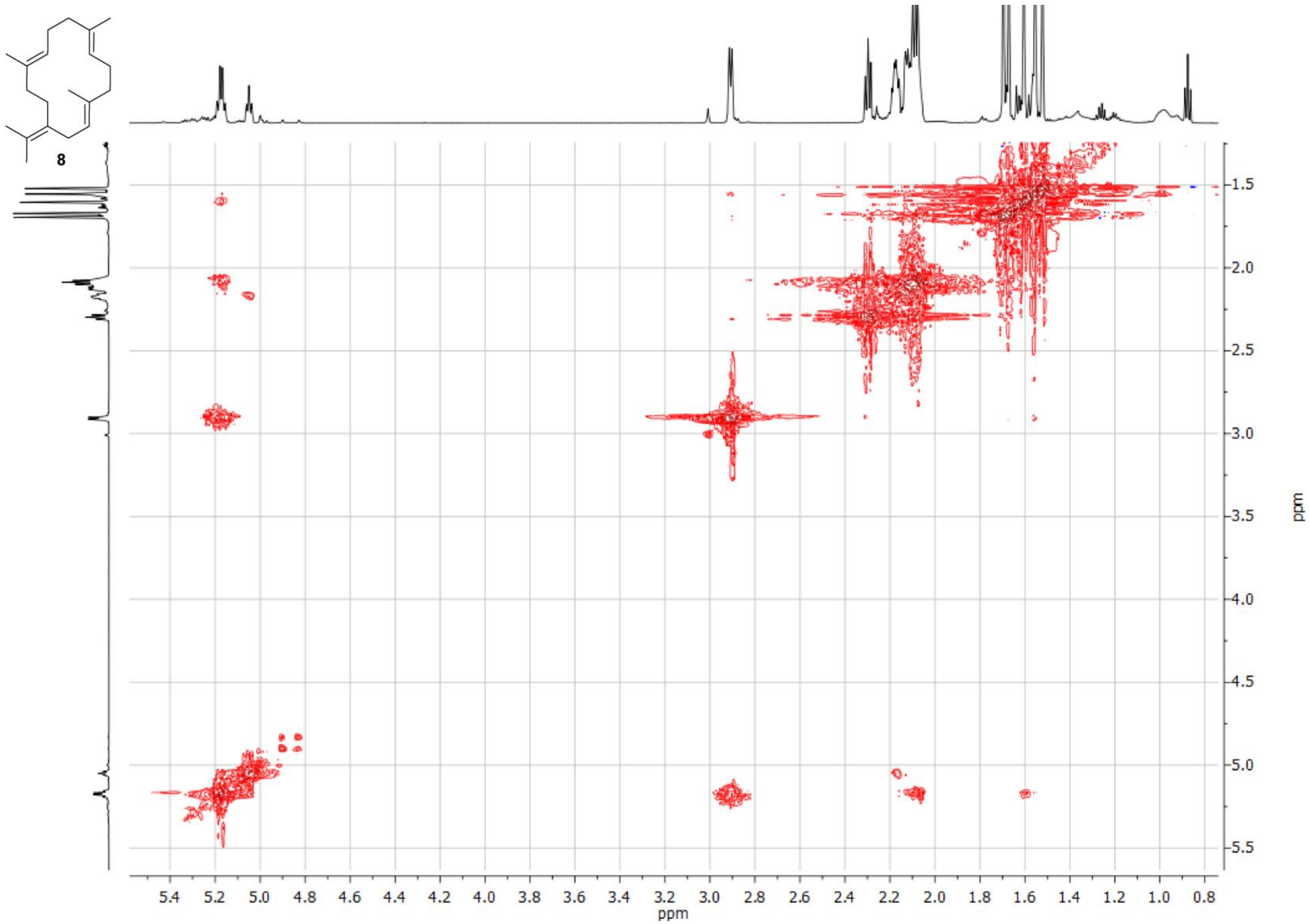


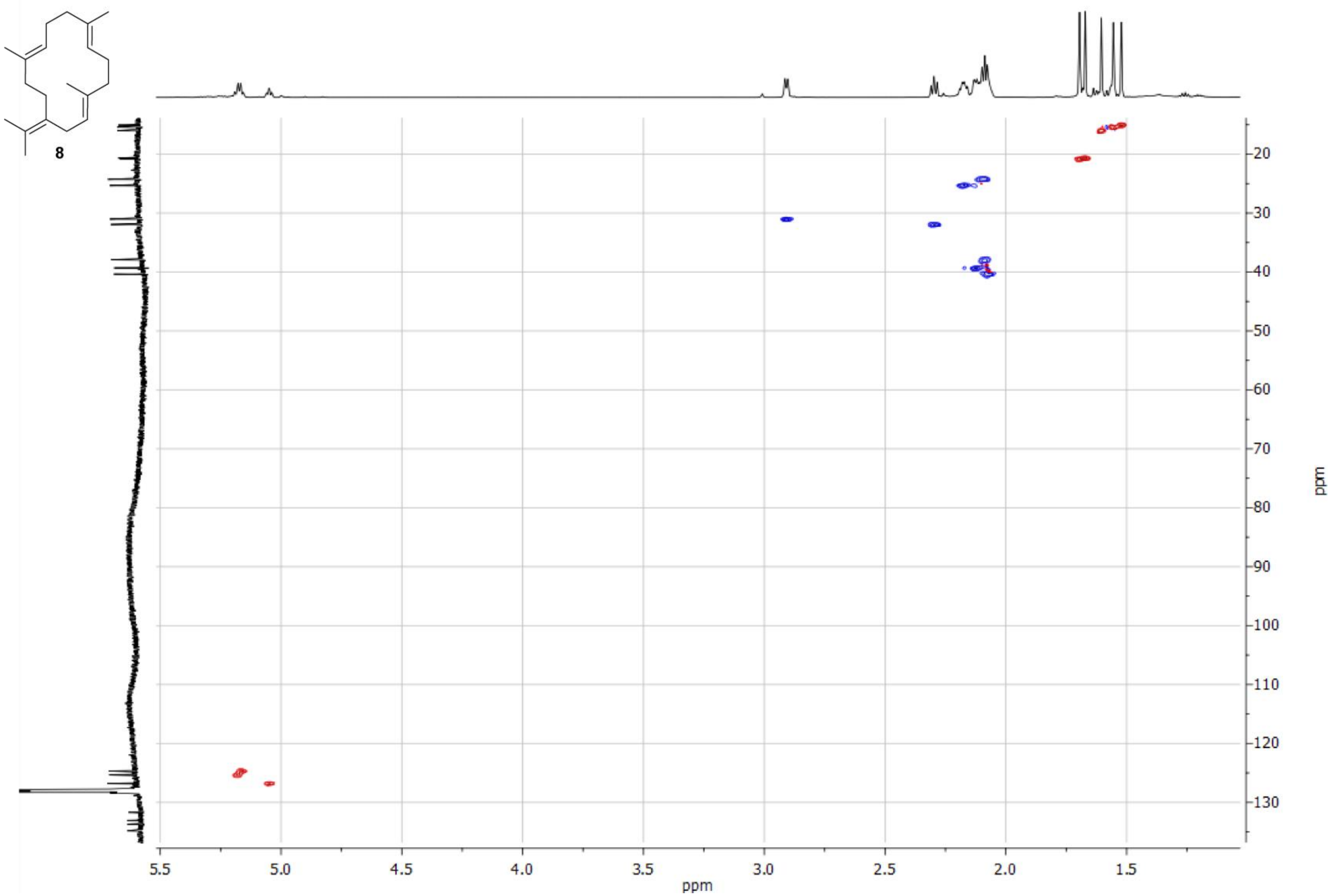
^{1}H NMR spectrum (600 MHz) of **8**. Asterisk denotes residual pentanes.



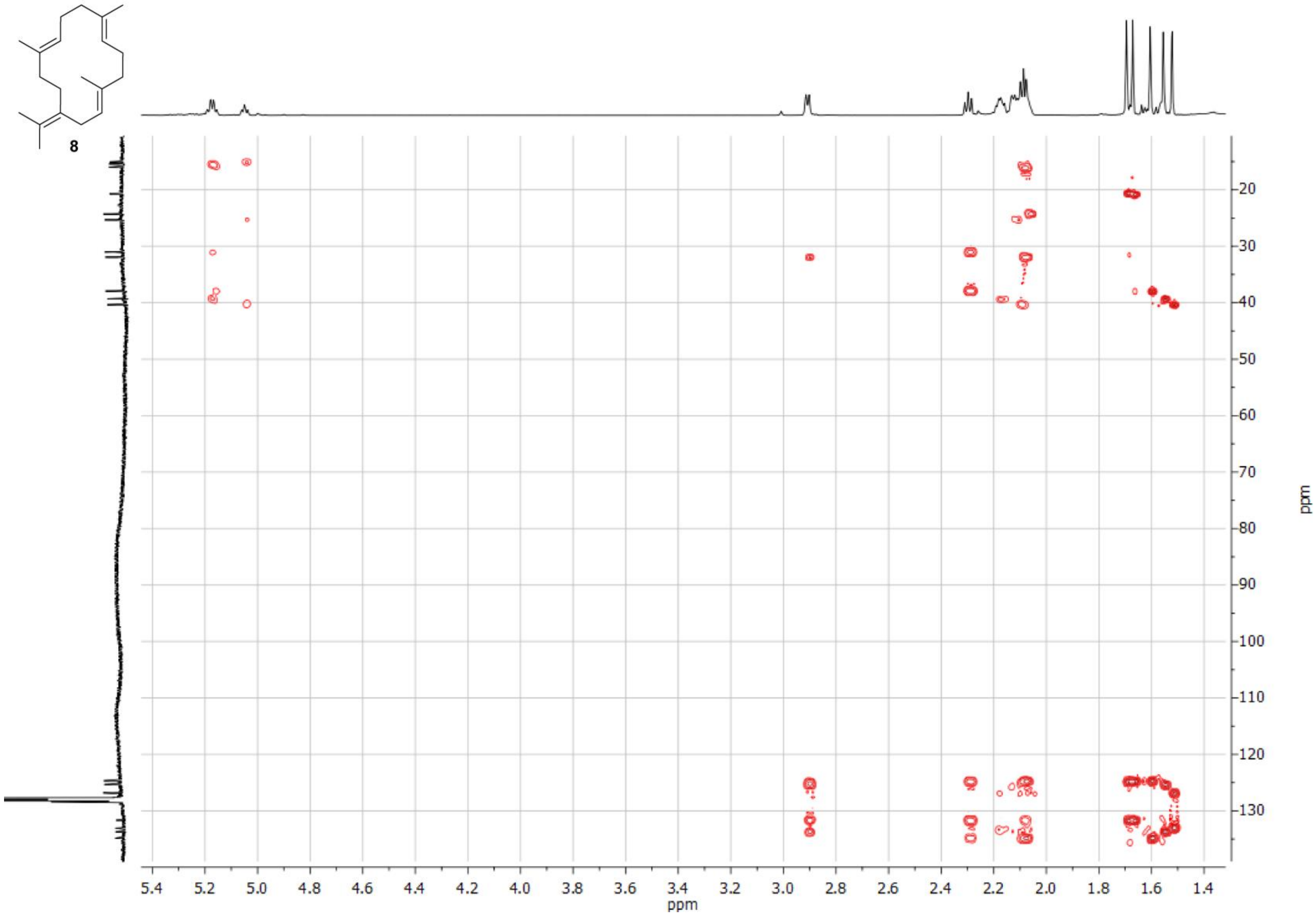
^{13}C NMR spectrum (125 MHz) of **8**.



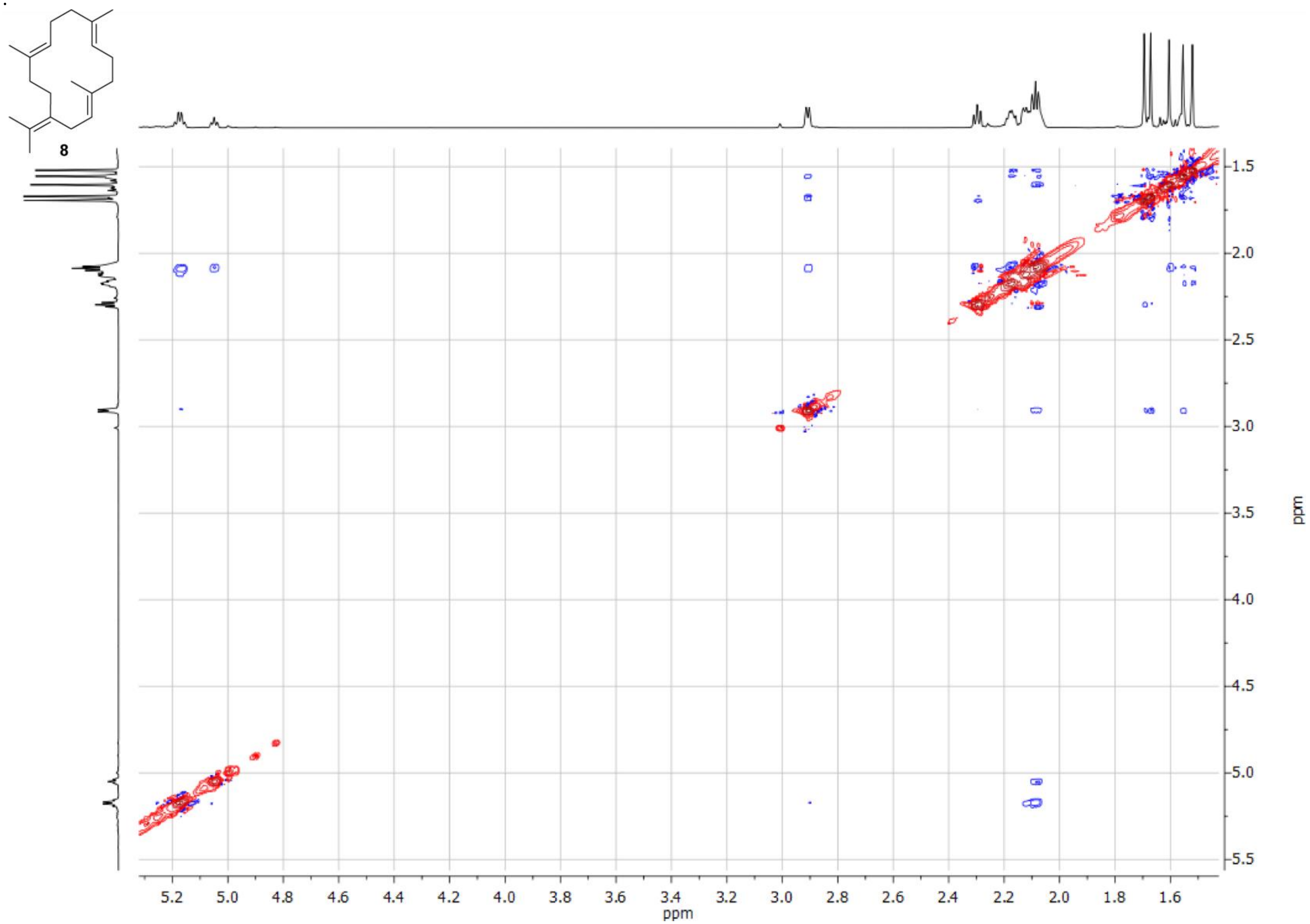
^{1}H, ^{1}H COSY spectrum (600 MHz) of **8**.



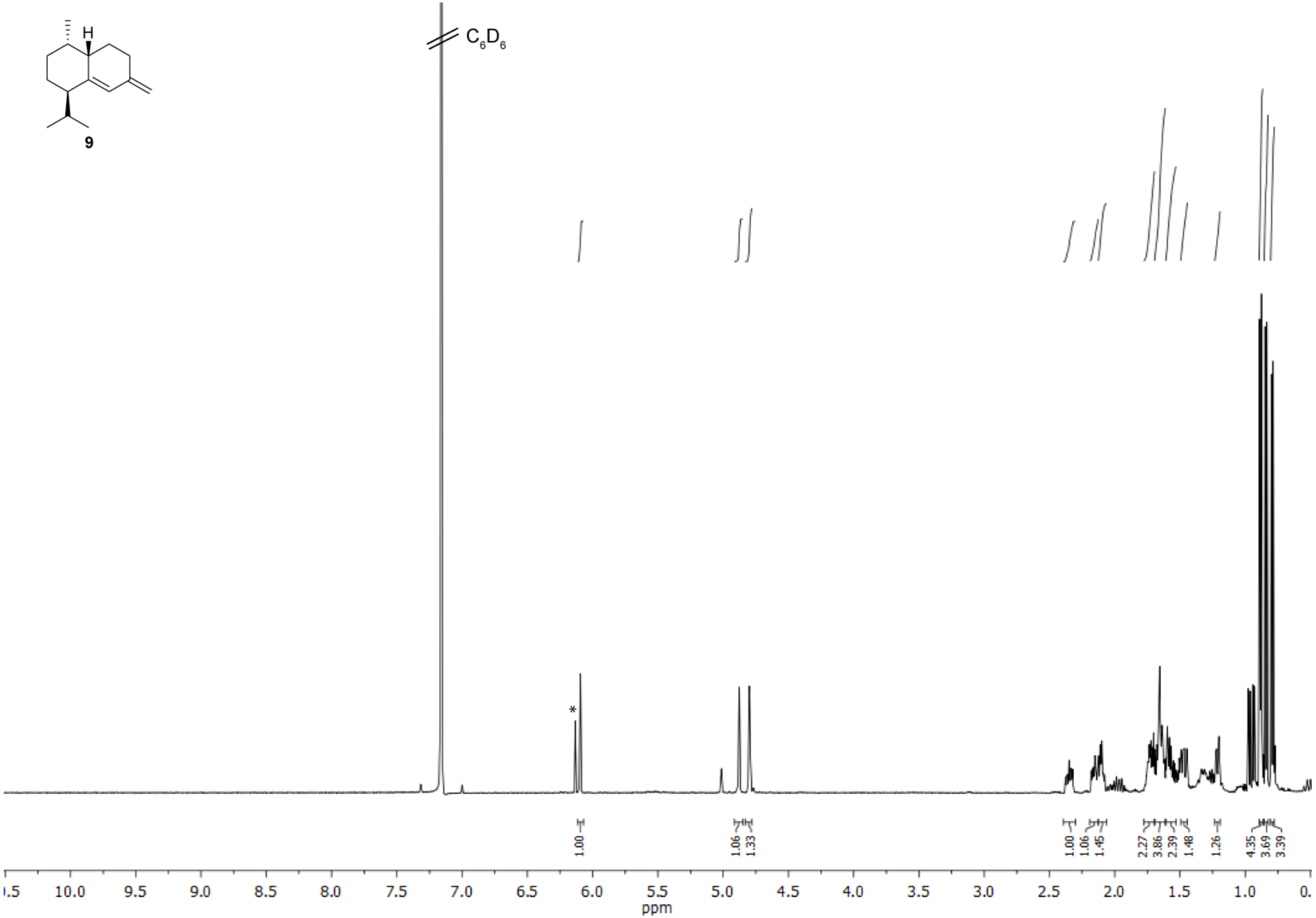
^{1}H, ^{13}C HSQC spectrum (600 MHz) of **8**.



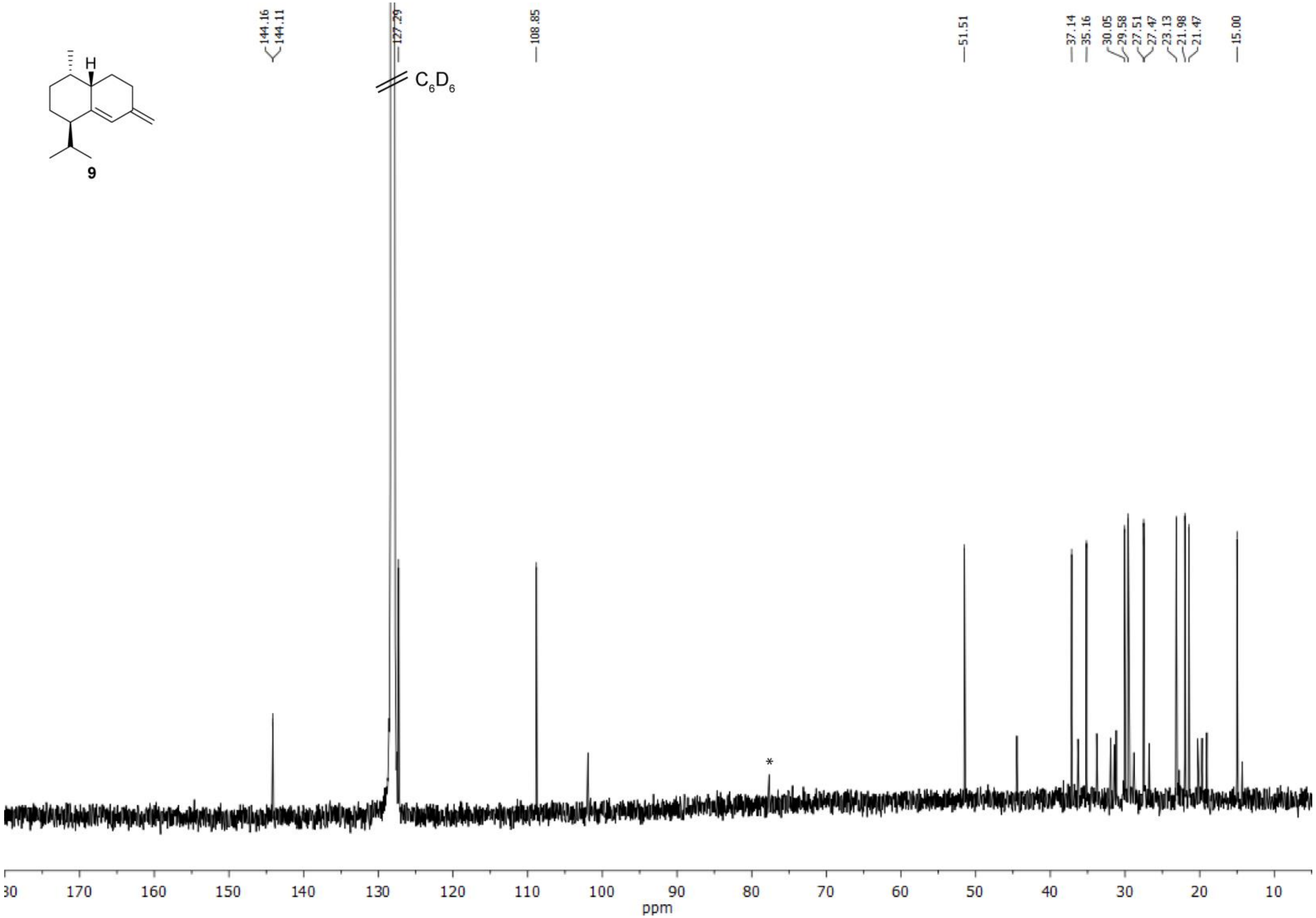
^{1}H, ^{13}C HMBC spectrum (600 MHz) of **8**



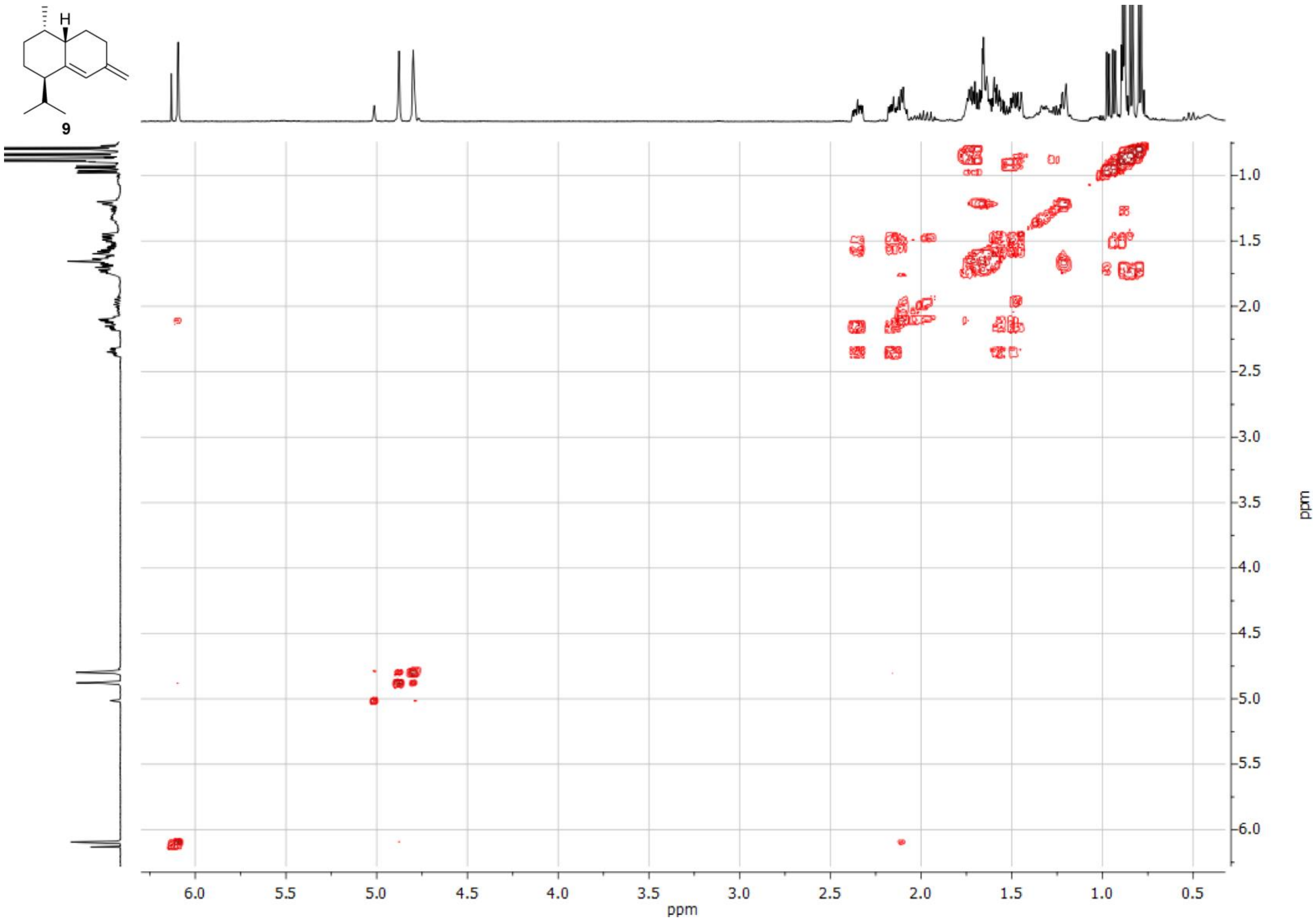
^{1}H, ^{1}H NOESY spectrum (500 MHz) of **8**.



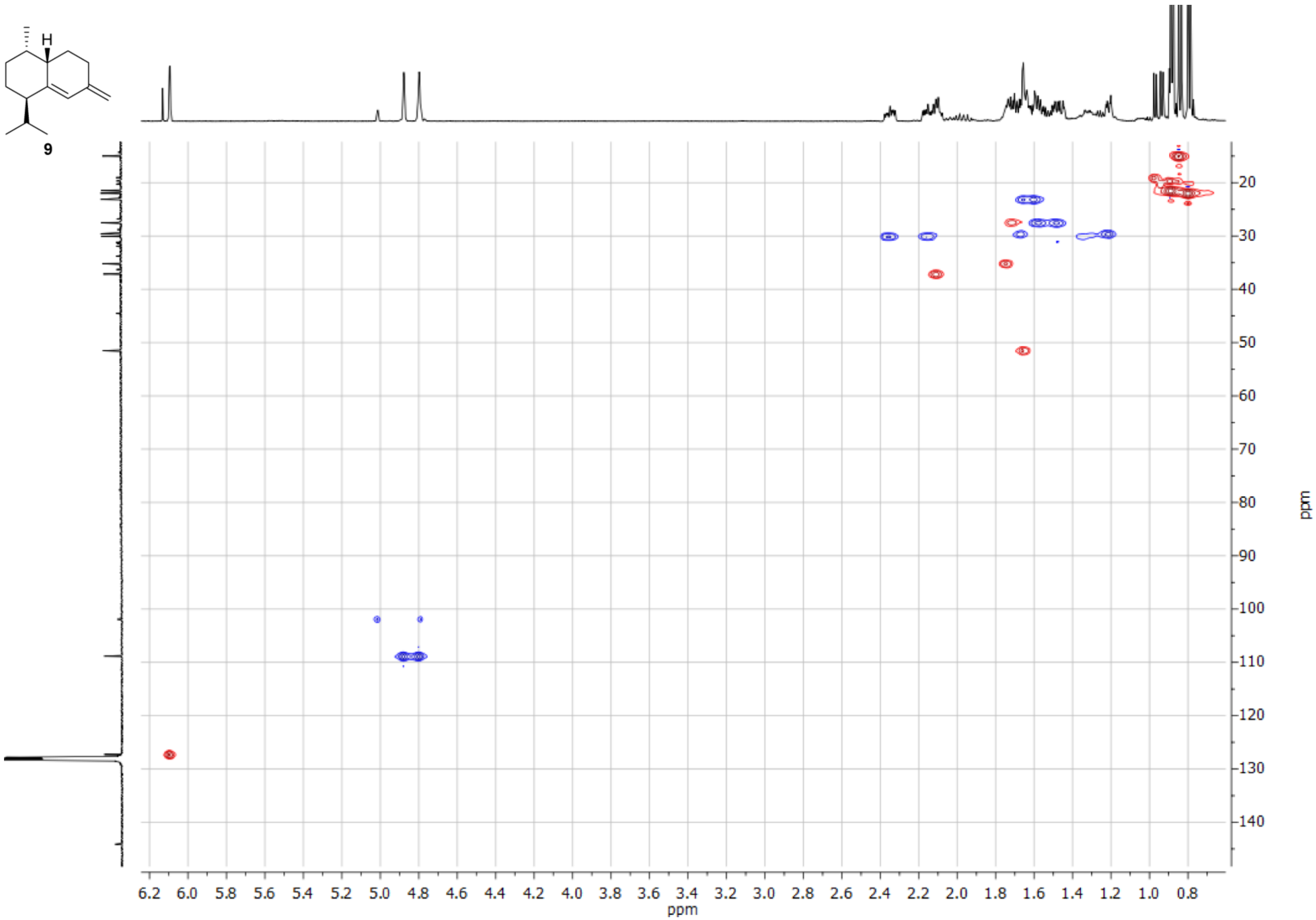
^{1}H NMR spectrum (500 MHz) of **9**. Asterisk denotes residual chloroform. Other minor signals originate from an inseparable second unidentified sesquiterpene.



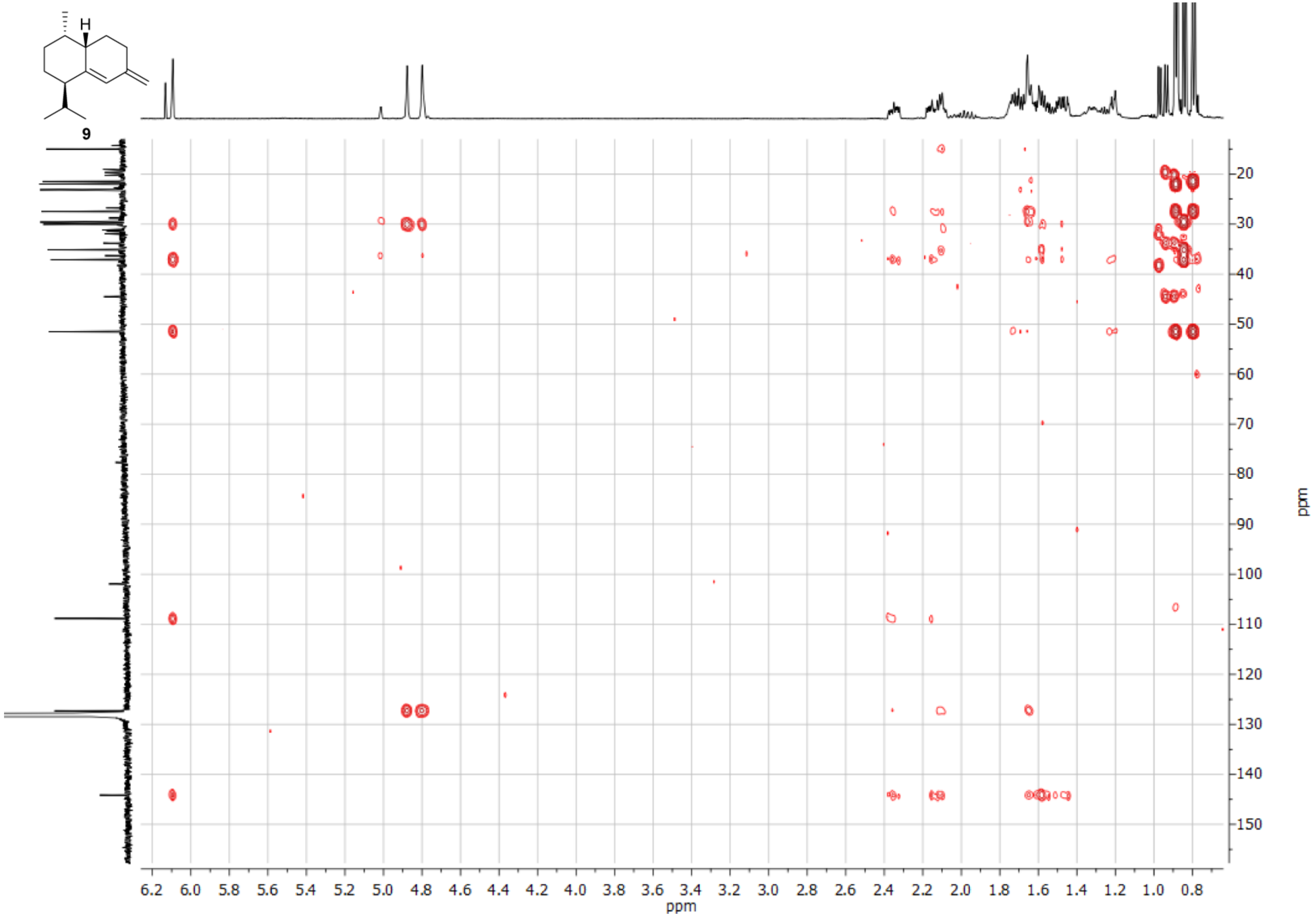
^{13}C NMR spectrum (125 MHz) of **9**. Asterisk denotes residual chloroform. Other minor signals originate from an inseparable second unidentified sesquiterpene.



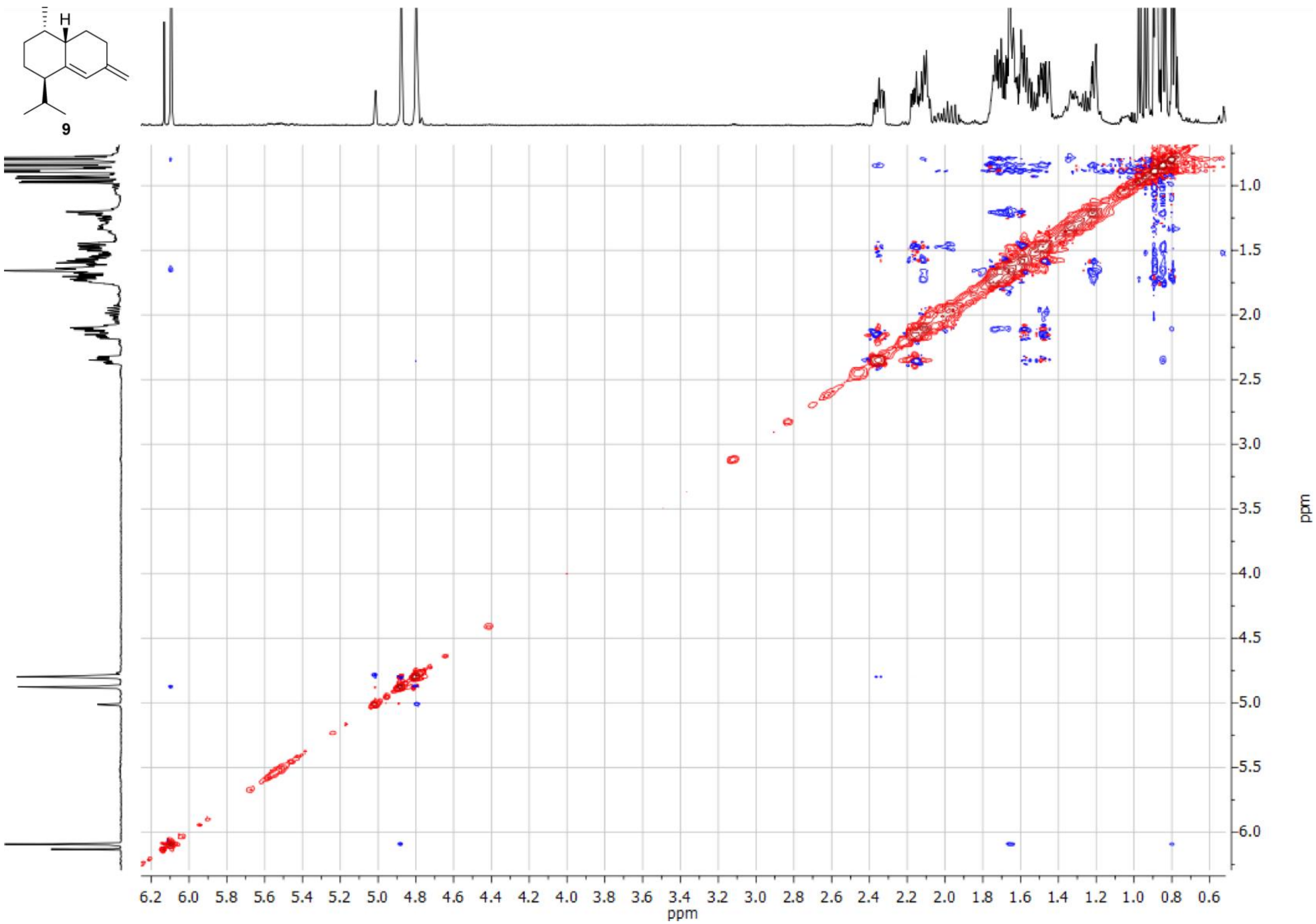
^{1}H, ^{1}H COSY spectrum (500 MHz) of **9**.



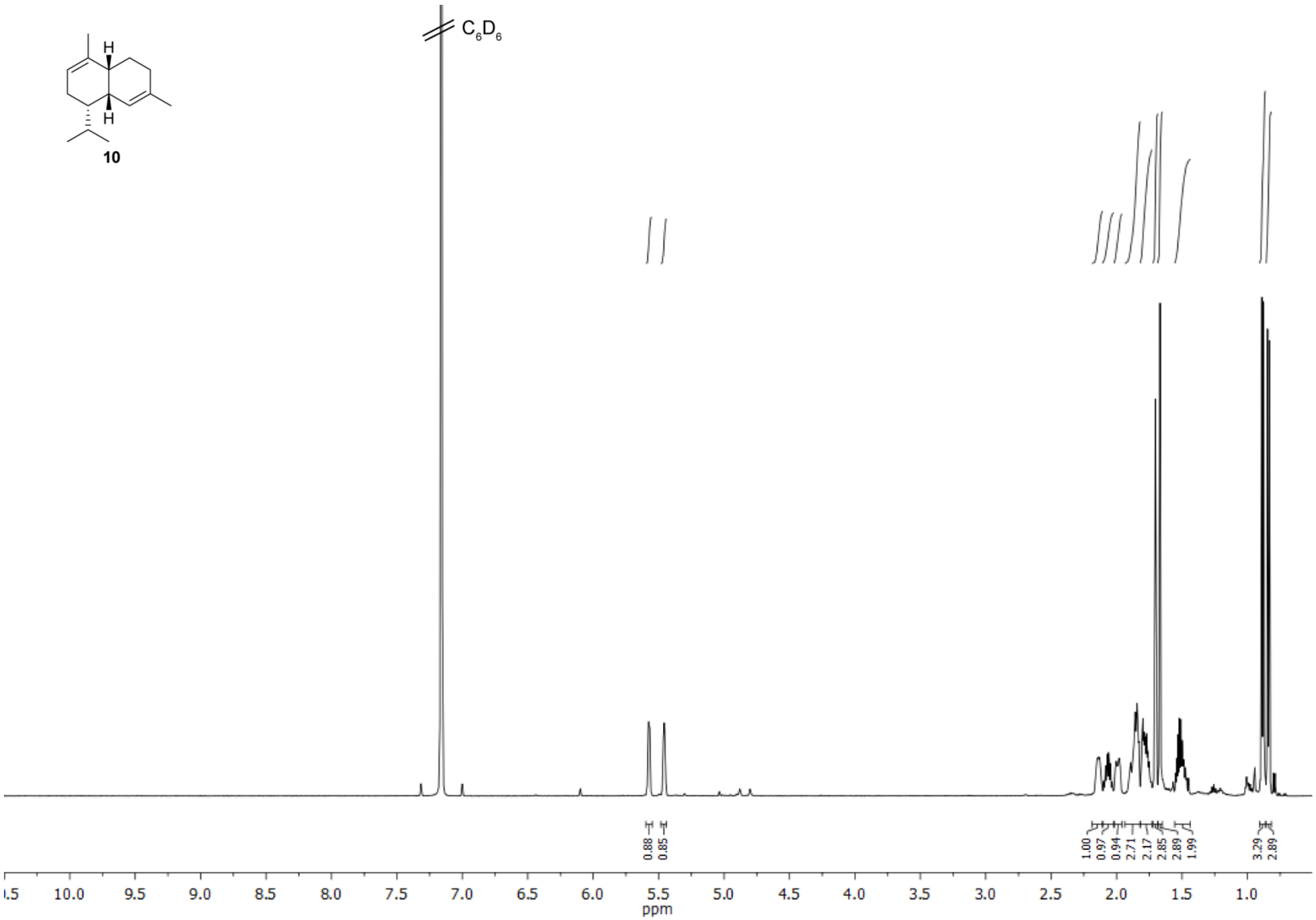
^{1}H, ^{13}C HSQC spectrum (500 MHz) of **9**.



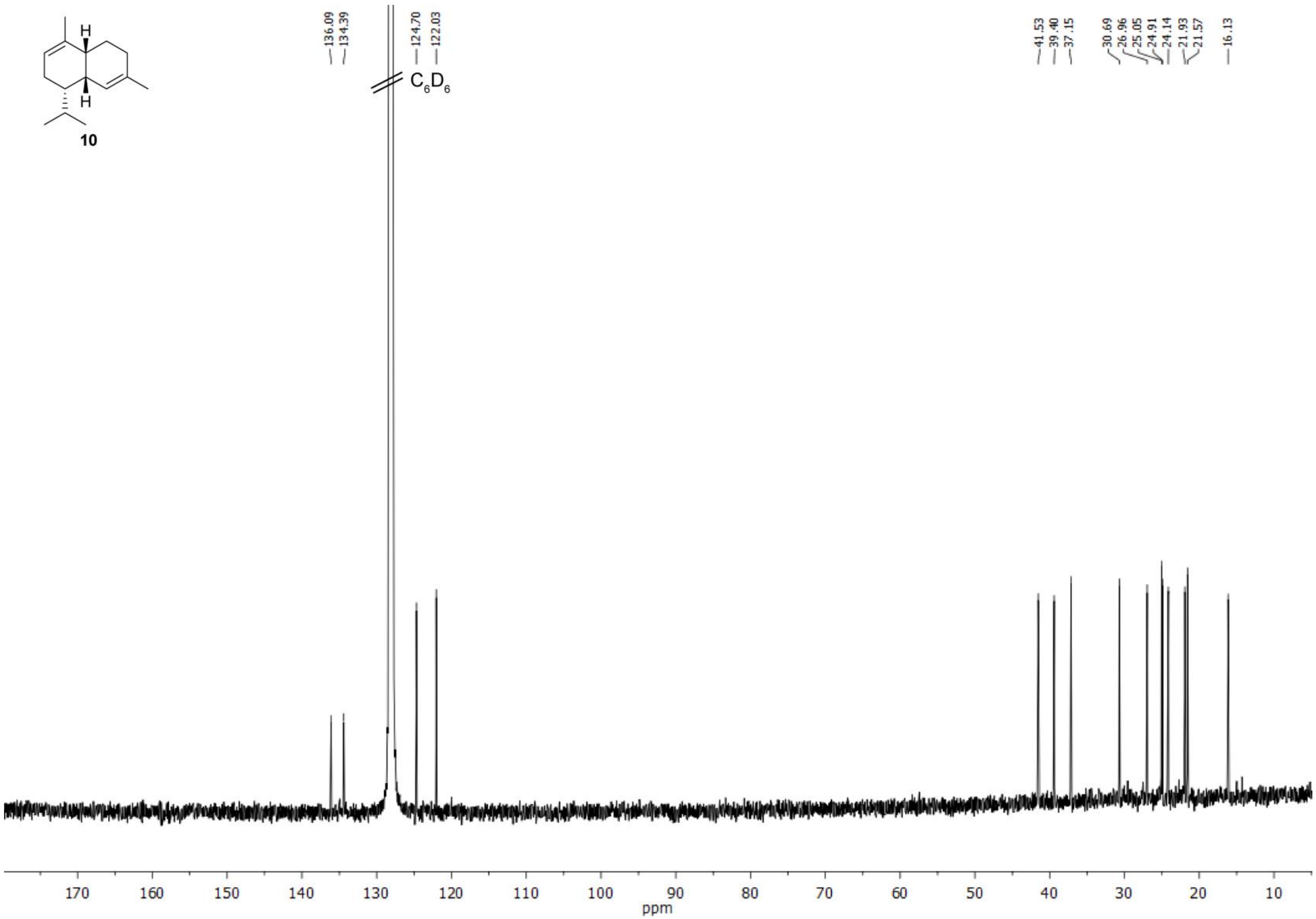
^{1}H, ^{13}C HMBC spectrum (500 MHz) of **9**.



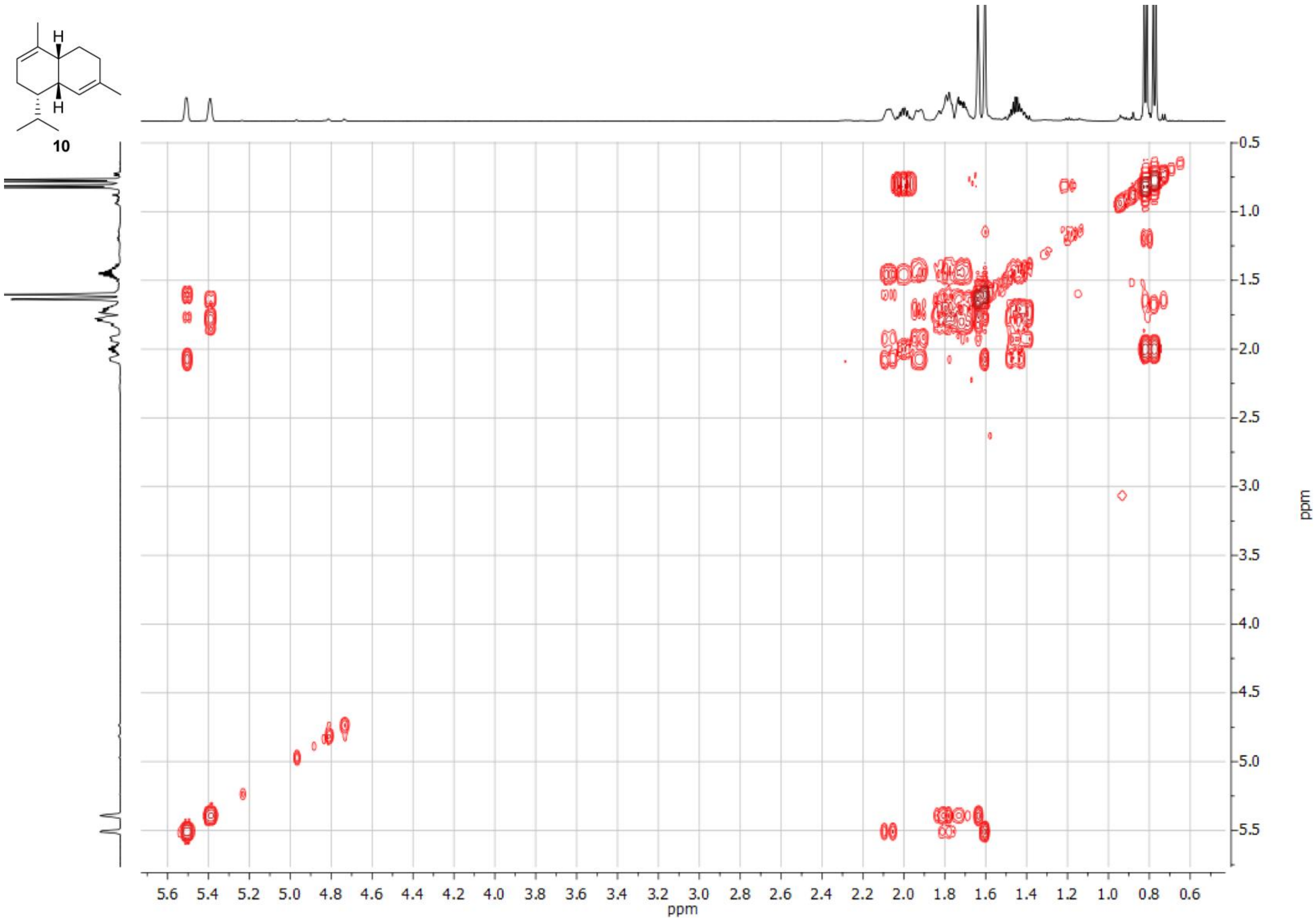
^{1}H, ^{1}H NOESY spectrum (500 MHz) of **9**.



^{1}H NMR spectrum (500 MHz) of **10**.



^{13}C NMR spectrum (125 MHz) of **10**.



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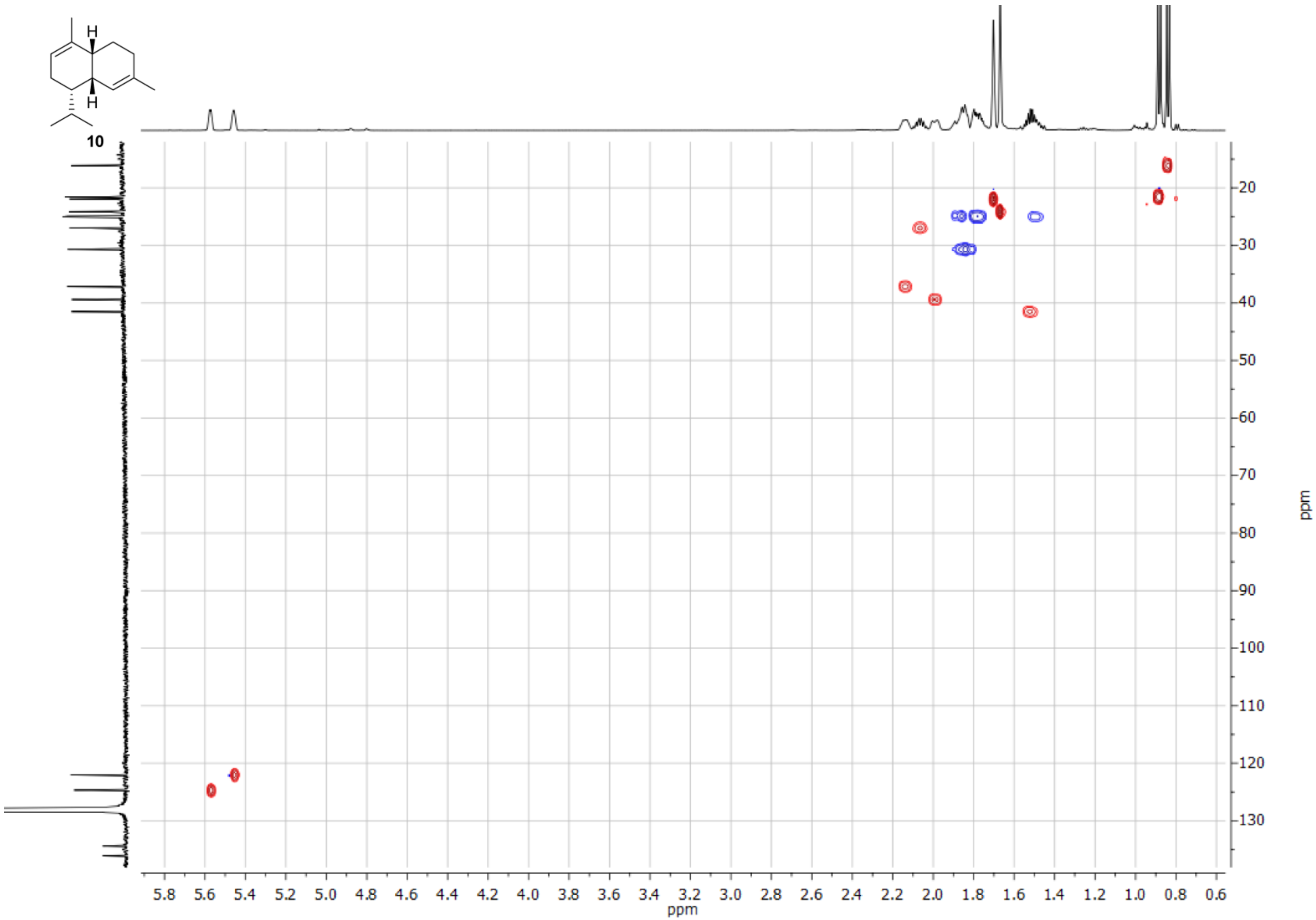
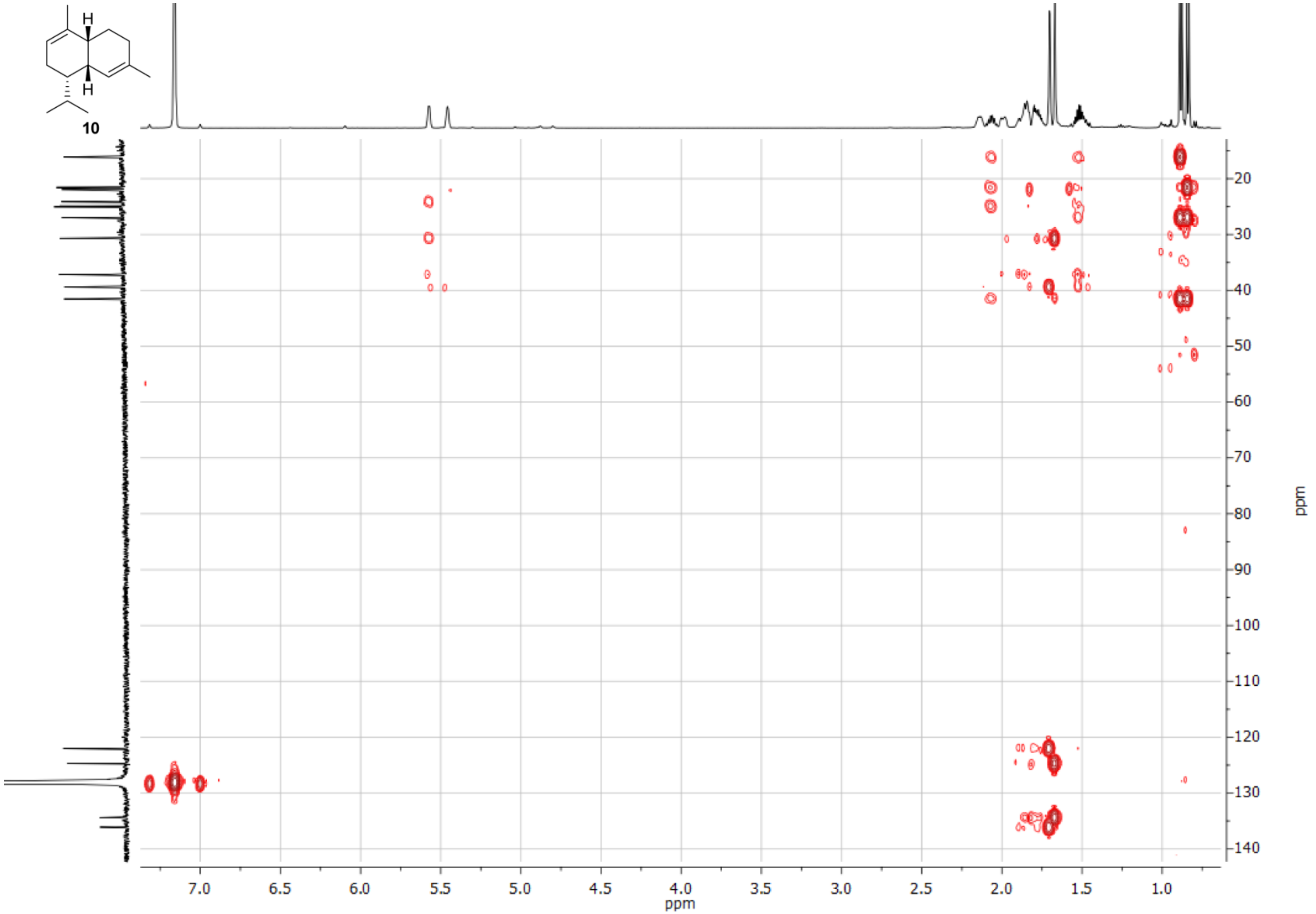
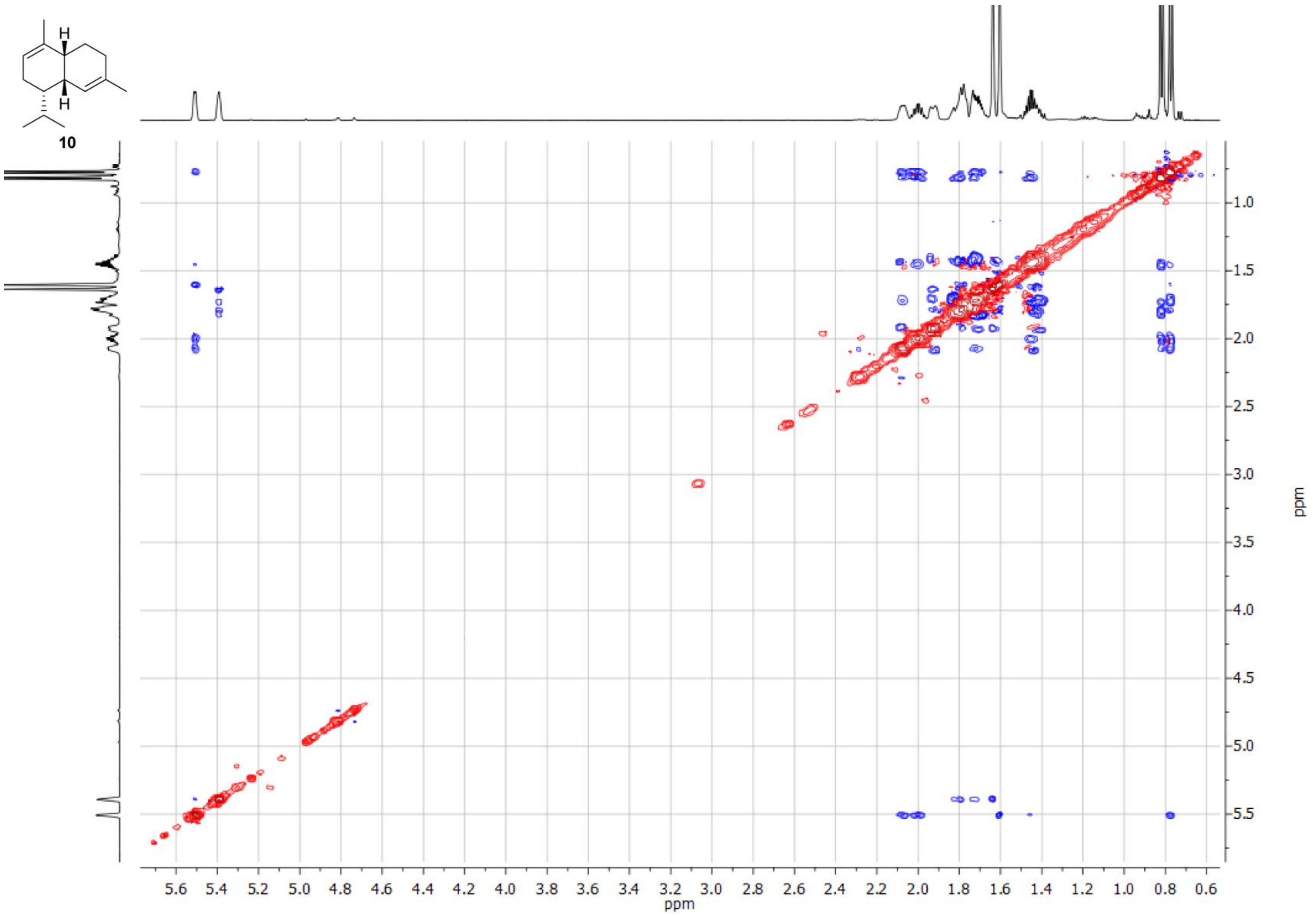


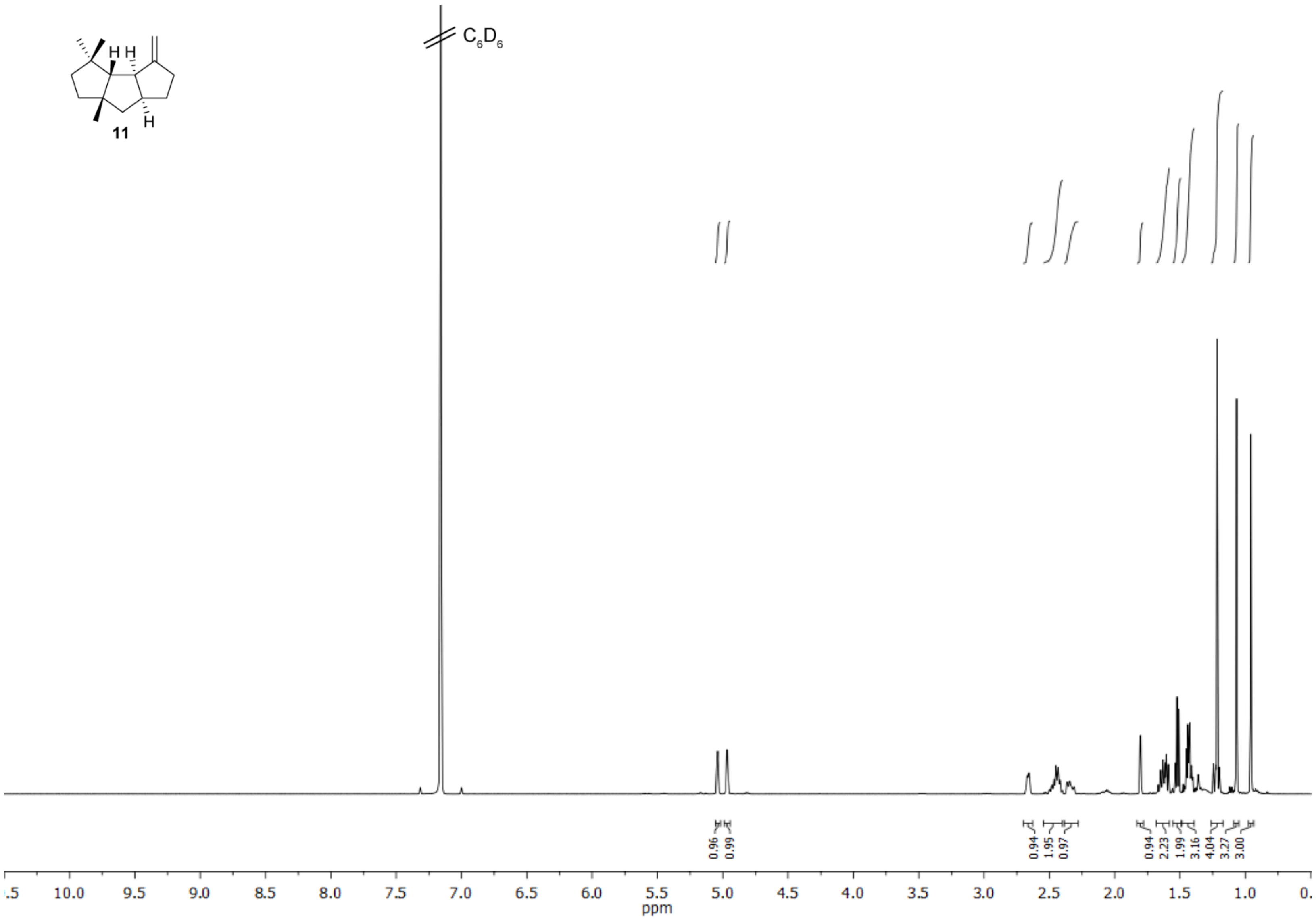
^{1}H, ^{13}C HSQC spectrum (500 MHz) of **10**.



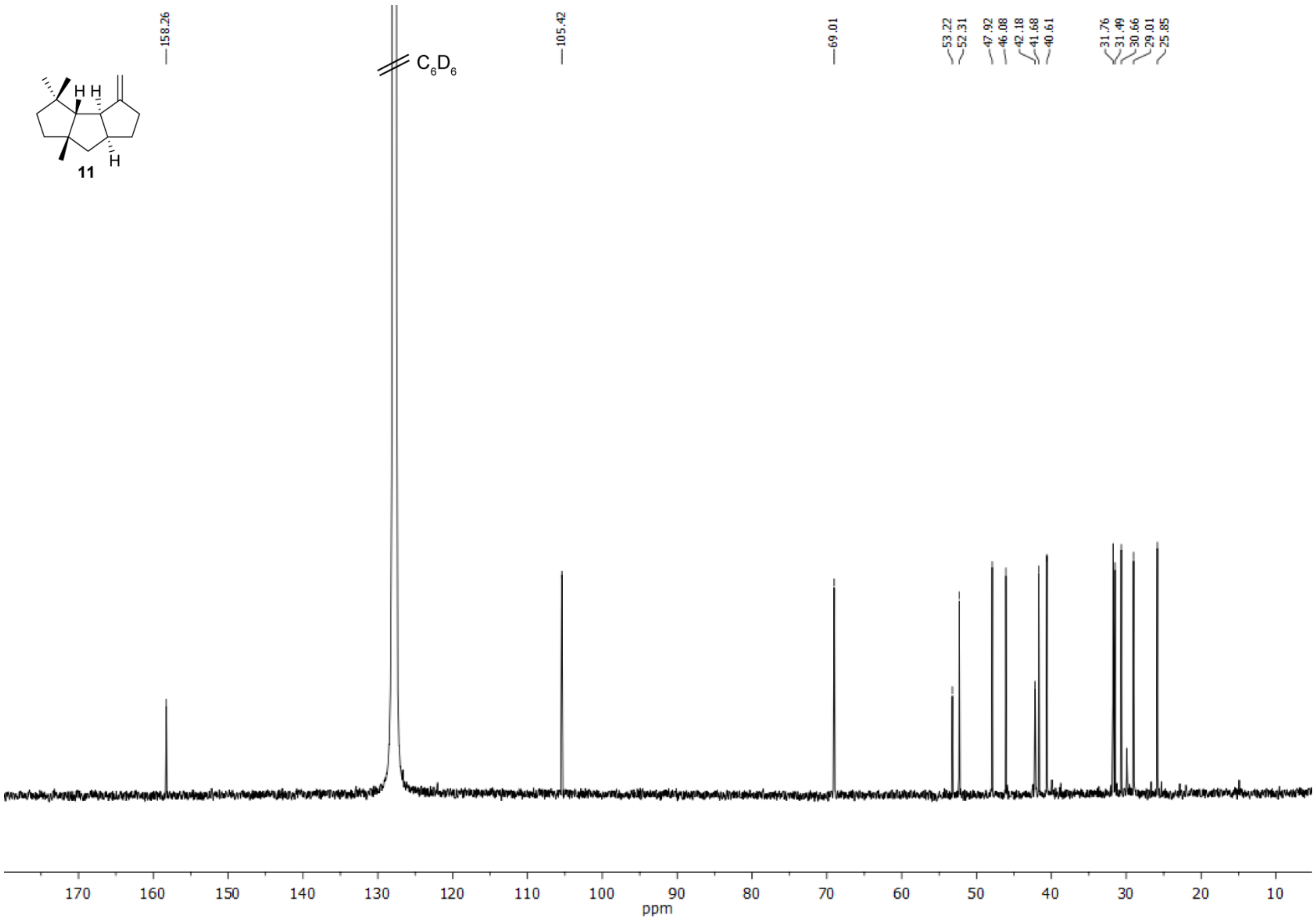
^{1}H, ^{13}C HMBC spectrum (500 MHz) of **10**.



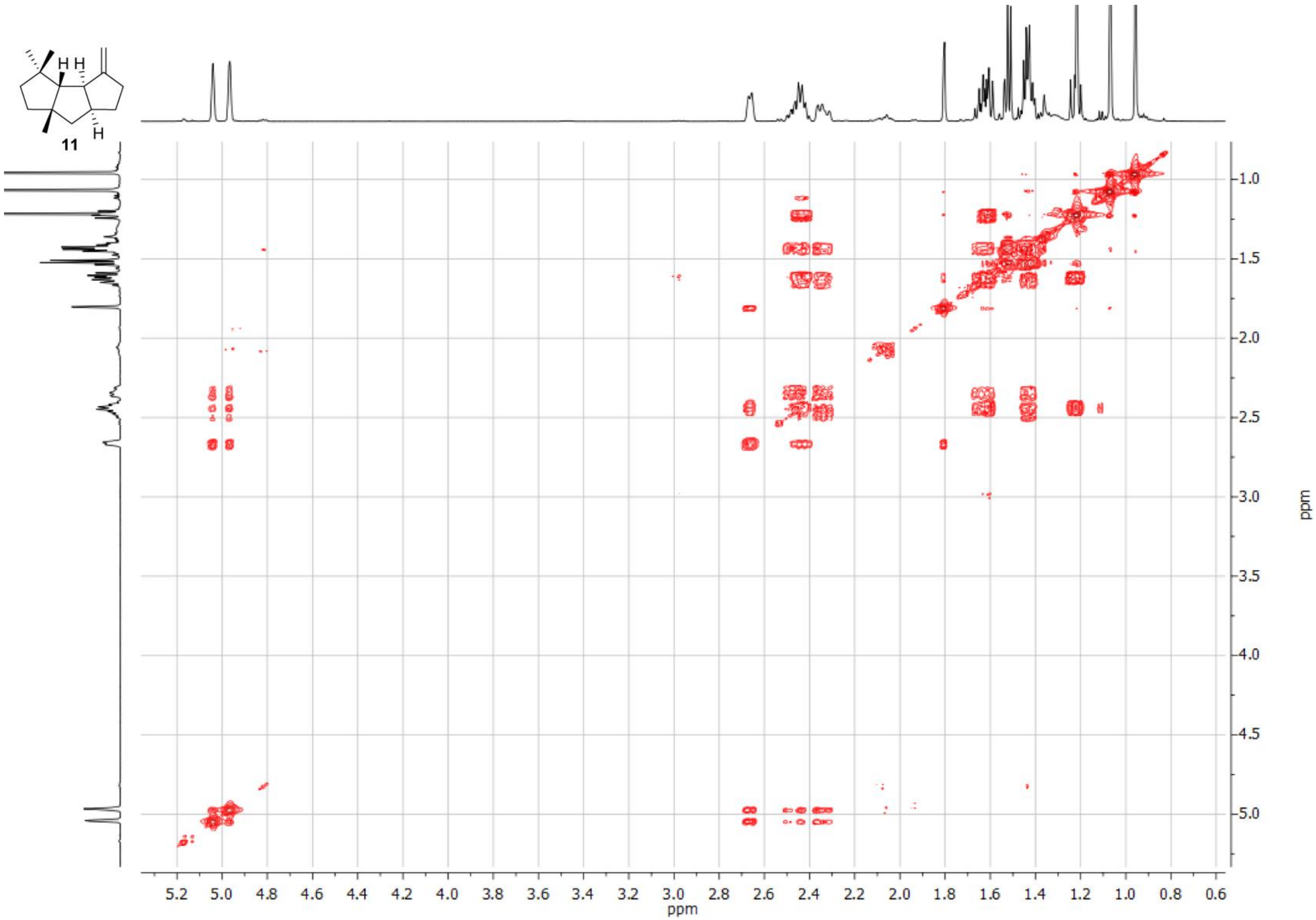
^{1}H, ^{1}H NOESY spectrum (500 MHz) of **10**.



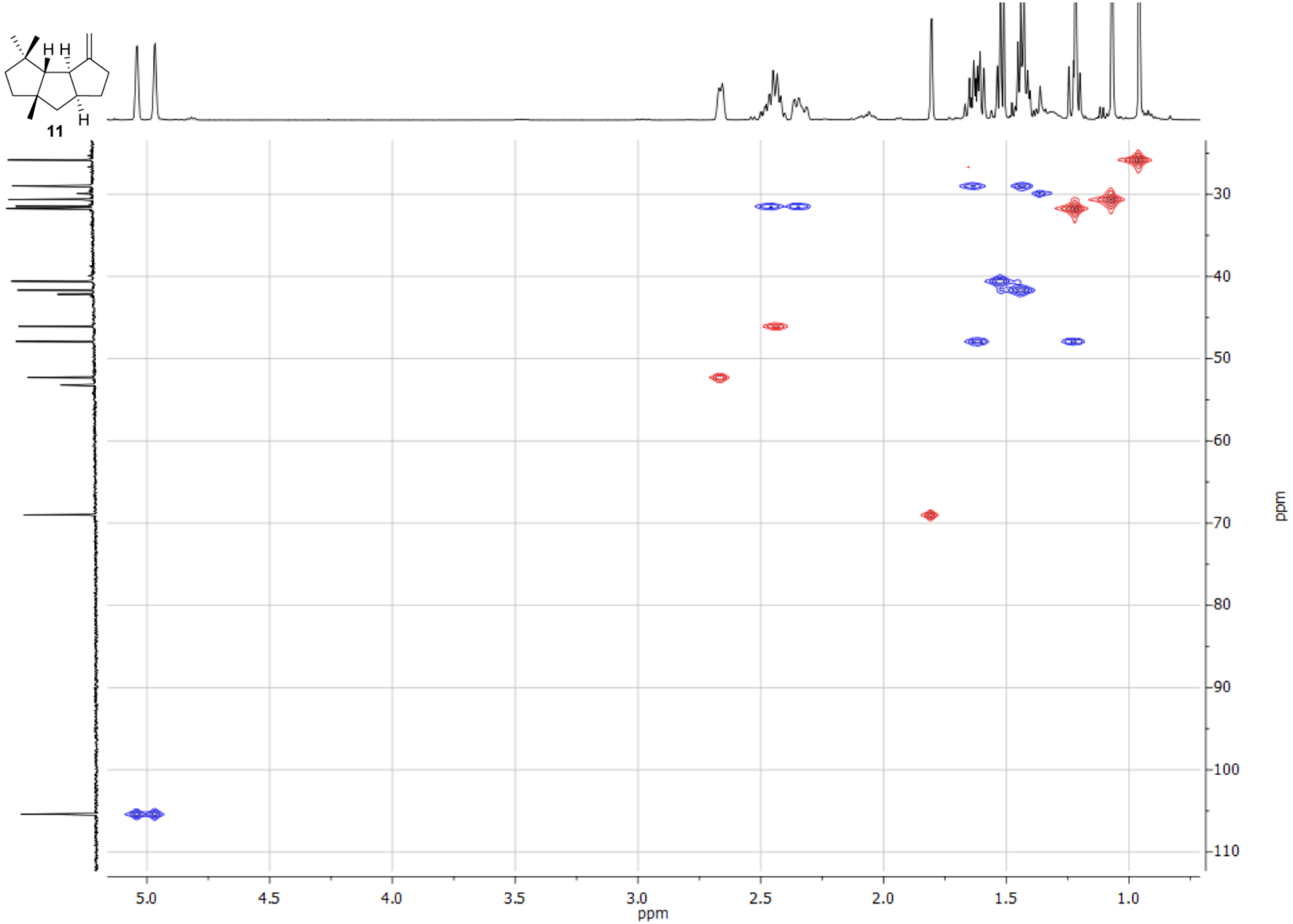
^1H NMR spectrum (500 MHz) of **11**.



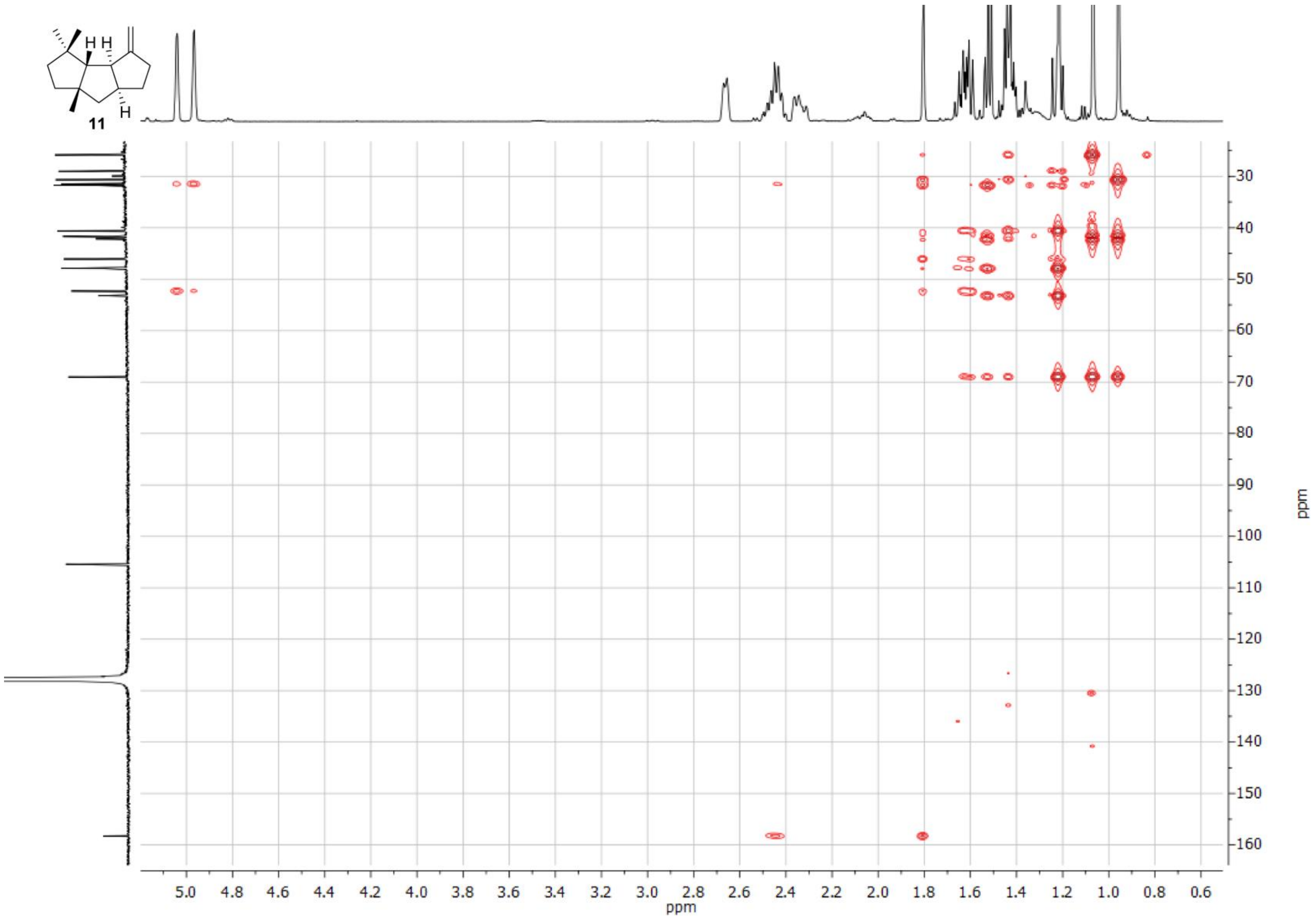
^{13}C NMR spectrum (125 MHz) of **11**.



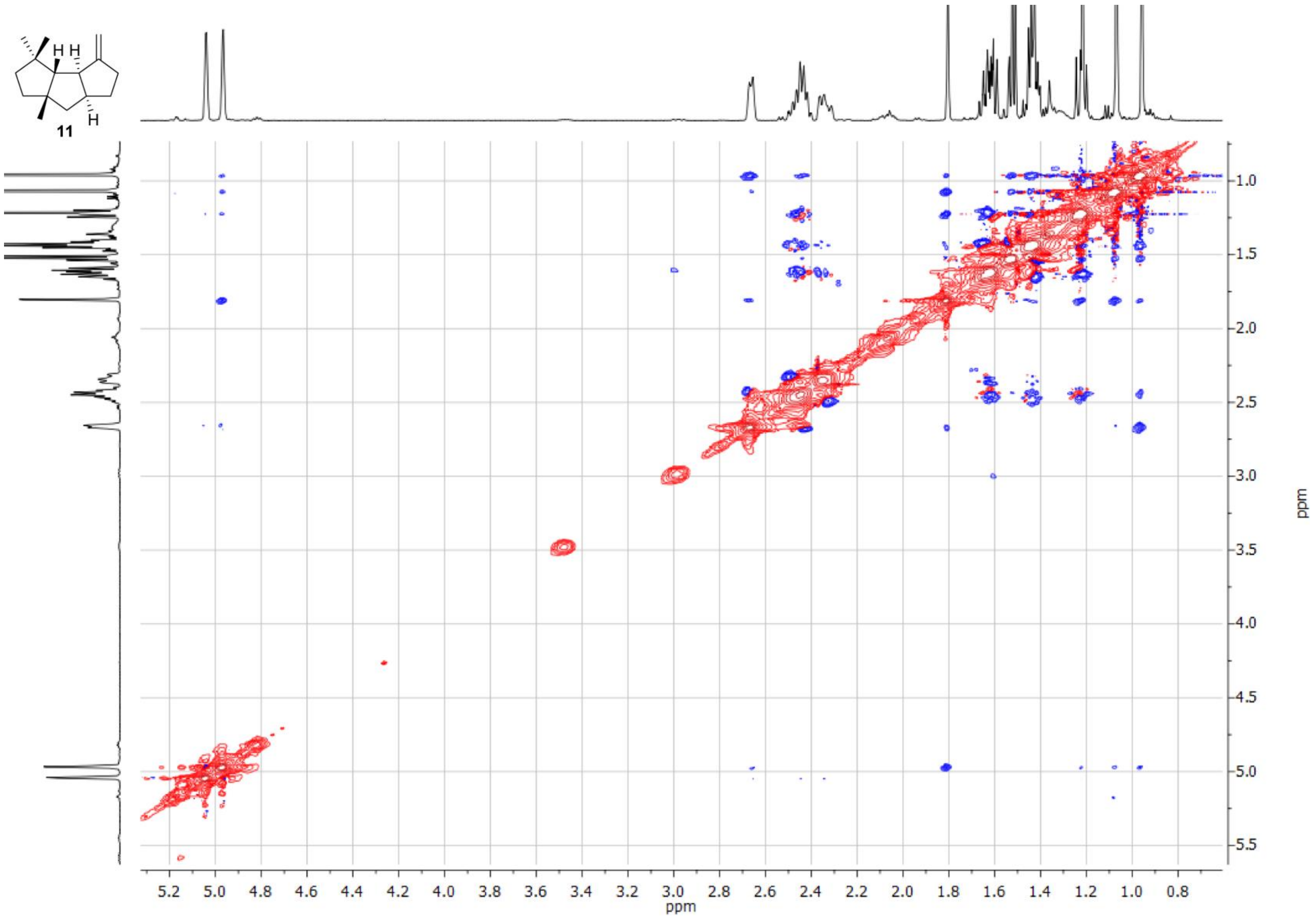
^{1}H, ^{1}H COSY spectrum (500 MHz) of **11**.



^{1}H, ^{13}C HSQC spectrum (500 MHz) of **11**.



^{1}H, ^{13}C HMBC spectrum (500 MHz) of **11**.



^{1}H, ^{1}H NOESY spectrum (500 MHz) of **11**

Supplementary Note 2 – Sequences

Capnella imbricata mutS sequence.

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TC Sequences

All characterized sequences are given as nucleotide sequences that are codon optimized for *E. coli* as used in this work and as amino acid sequences of the resulting proteins.

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