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RIMTPSL4:

MPTTDFRRRERKQVQMGTEVDRPAPVLDHSHDSIANLKPFDQRQELRMYKPPPFIPYVVKQNPYNSK
GRYSNWARWLLNLKVDEIFSTKMFEMLNRMIDIPYFANVLQPLCDEWGLQWSCVKVYFMLWIT**DDLVD**S
TKIGKSASDVLSLFLDYHLVMMWTFPDDPVLHRELPNFLSVLASQTEREQKLAHFEEILVQARTRPGT
IYEGNLSIACEVFRELFAEAYARSSRESVLQFAHCCQHLLGDLESQYREAKGMPASIEELIPI**RKR**
ACAVFVALASTDISCGLATPTEYYNSKPMKEMLDACCDFTAWH**NDVWSFKKE**IIQDKPEPFSLVVAISV
HRKLPYQEAAEVLTKMMSDRLKMDRAATDLERITPPELARNFQIYVSACQTMASGTHDWHTKTV**RYD**
V

Figure S1. Amino acid sequences of RIMTPSL4 (accession number UJU85541) from *R. lindenberiana*. Highly conserved motifs are highlighted in bold.

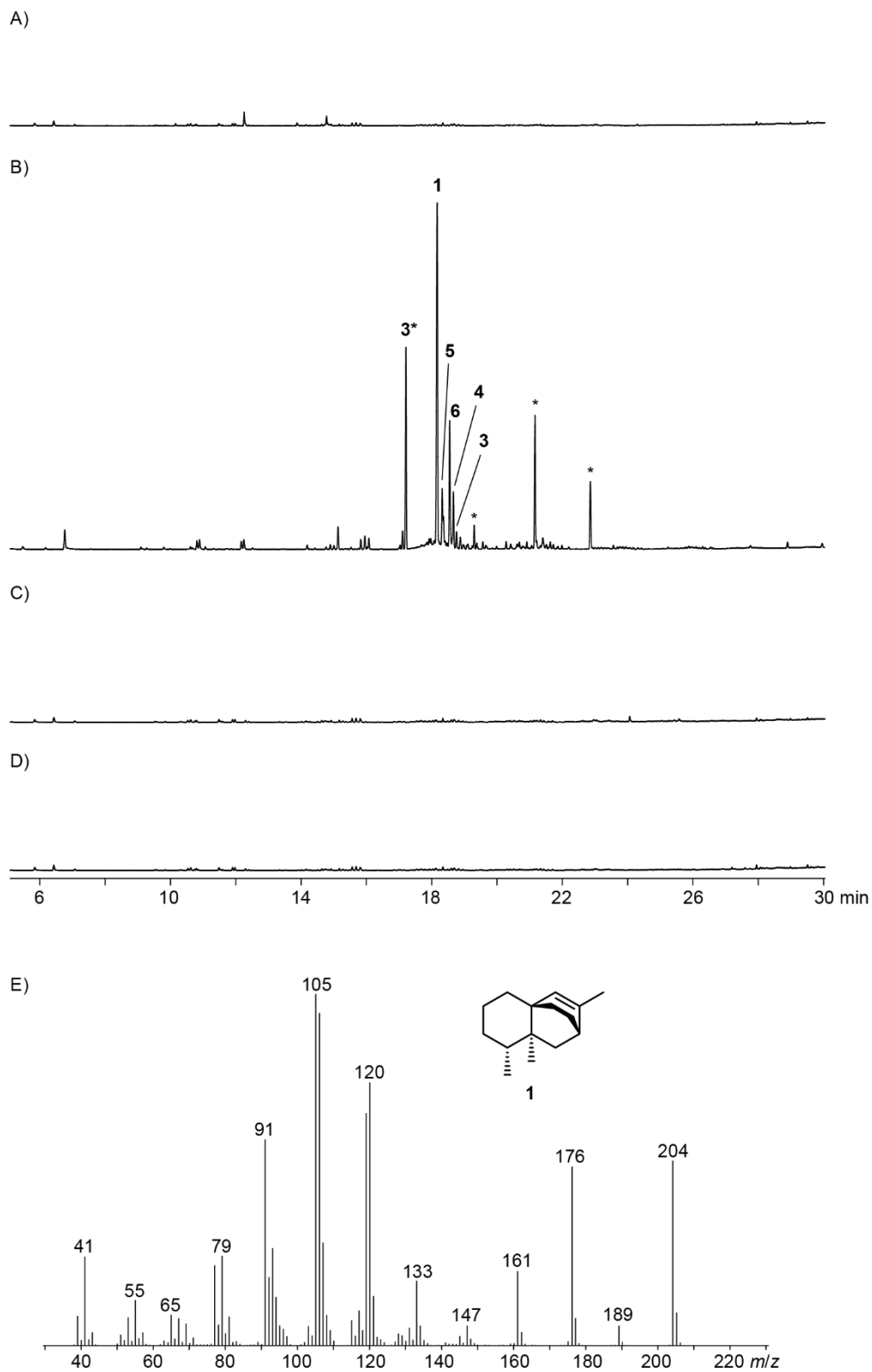


Figure S2. Total ion chromatograms of the products obtained with RIMTPSL4 from A) GPP, B) FPP, C) GGPP and D) GFPP. Asterisks indicate spontaneous hydrolysis products and contaminants from plasticisers. E) EI mass spectrum of **1**.

Table S1. Identification of terpenes formed from FPP by RIMTPSL4 by GC/MS.

Compound	<i>I</i> ^[a]	<i>I</i> (lit.) ^[b]	MS match ^[c]
β -elemene (3*)	1406	1389 ^[1]	949
4,5- <i>diepi</i> -isoishwarane (1)	1478	–	–
4,5- <i>diepi</i> -aristolochene (5)	1491	1471 ^[1]	932
eremophilene (6)	1509	1503 ^[2]	925
α -selinene (4)	1518	1498 ^[1]	945
germacrene A (3)	1527	1508 ^[1]	891

[a] Retention index on a HP5-MS GC column. [b] Retention index data from the literature on the same or a similar GC column. [c] Mass spectral match factor (0 – 999, 999 indicates identical mass spectra).

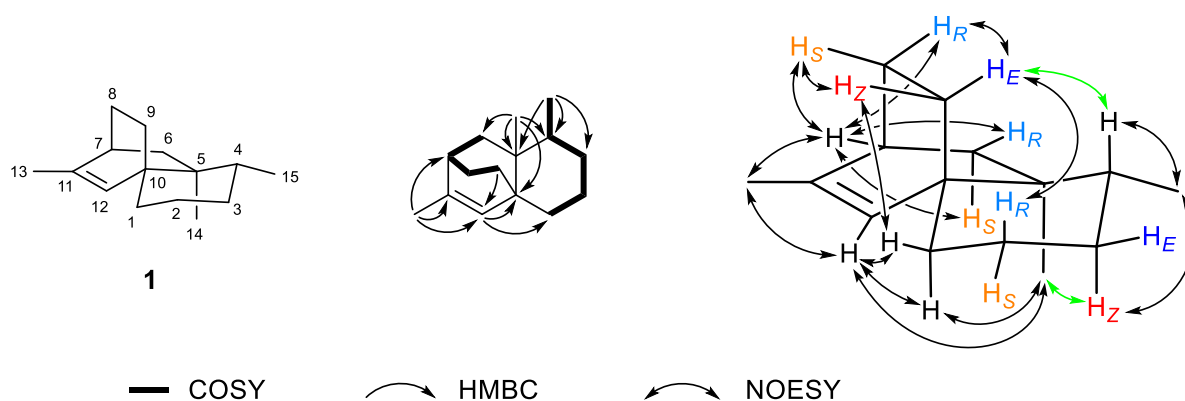


Figure S3. Structure elucidation of **1**. Bold lines show $^1\text{H}, ^1\text{H}$ -COSY correlations, single headed arrows show key HMBC correlations, and double headed arrows show key NOESY correlations. The green double headed arrows indicate NOESY correlations that were used to conclude on the absolute configuration of **1** from the labeling experiments with (*E*)- and (*Z*)-(4- $^{13}\text{C}, 4\text{-}^2\text{H}$)IPP (Figure 4 of main text).

Table S2. NMR data of 4,5-*diepi*-isoishwarane (**1**) in C_6D_6 recorded at 298 K.

C ^[a]	type	^{13}C ^[b]	^1H ^[b]
1	CH_2	30.90	1.78 (ddd, $J = 13.7, 13.7, 4.7$, H_α) 1.33 (m, H_β)
2	CH_2	21.76	1.55 (m, H_R) 1.44 (m, H_S)
3	CH_2	30.77	1.34 (m, H_E) 1.19 (m, H_Z)
4	CH	36.97	1.54 (m)
5	C_q	40.88	–
6	CH_2	42.30	1.15 (m, H_R) 1.12 (m, H_S)
7	CH	37.07	2.10 (m)
8	CH_2	28.22	1.37 (m, H_R) 1.34 (m, H_S)
9	CH_2	26.23	2.16 (ddd, $J = 13.5, 9.7, 4.3$, H_E) 0.75 (m, H_Z)
10	C_q	40.94	–
11	C_q	139.30	–
12	CH	133.22	5.50 (br s)
13	CH_3	20.19	1.71 (d, $J = 1.6$)
14	CH_3	19.14	0.76 (s)
15	CH_3	18.15	0.79 (d, $J = 6.7$)

[a] Carbon numbering 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.

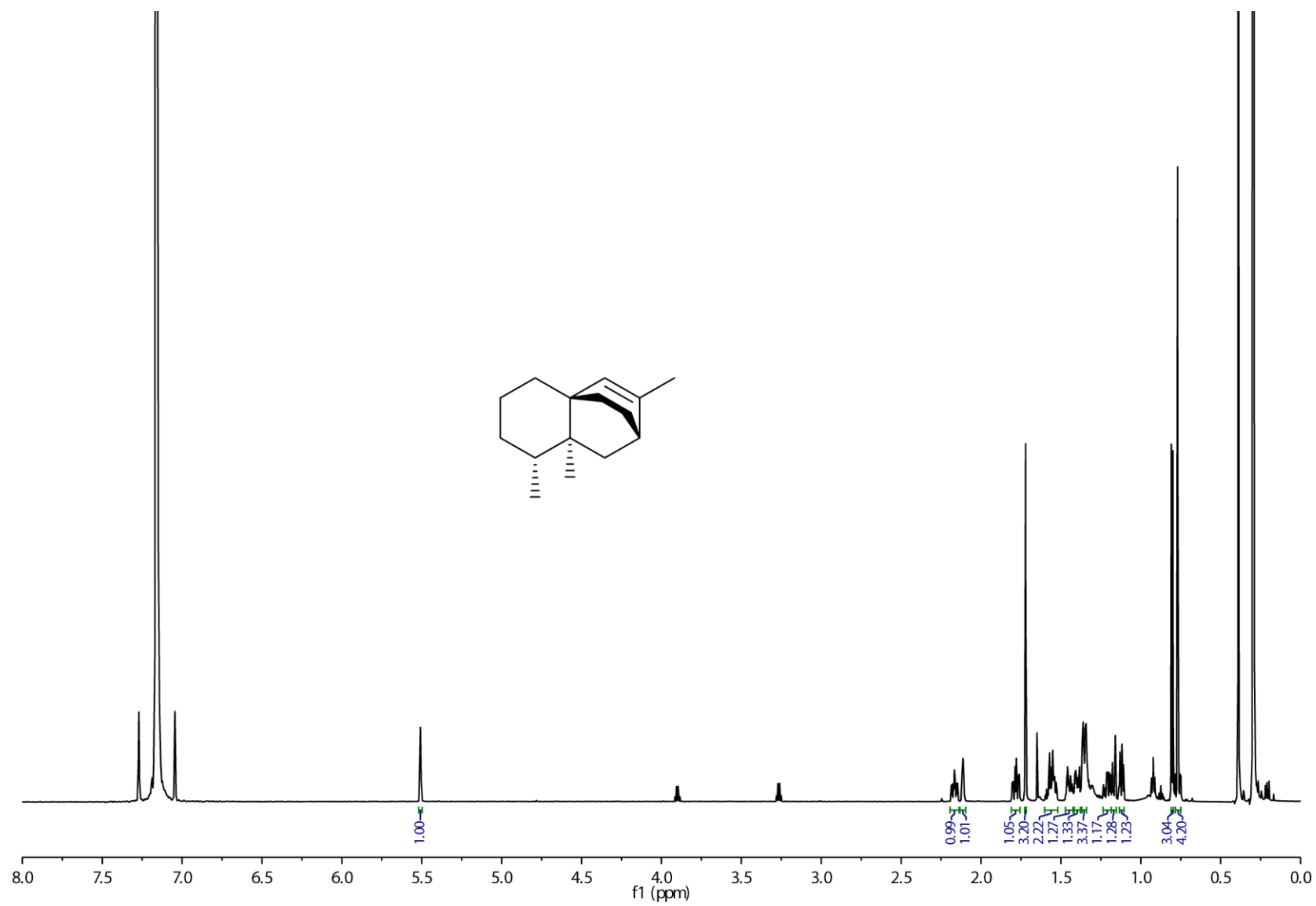


Figure S4. ¹H-NMR spectrum of **1** (700 MHz, C₆D₆).

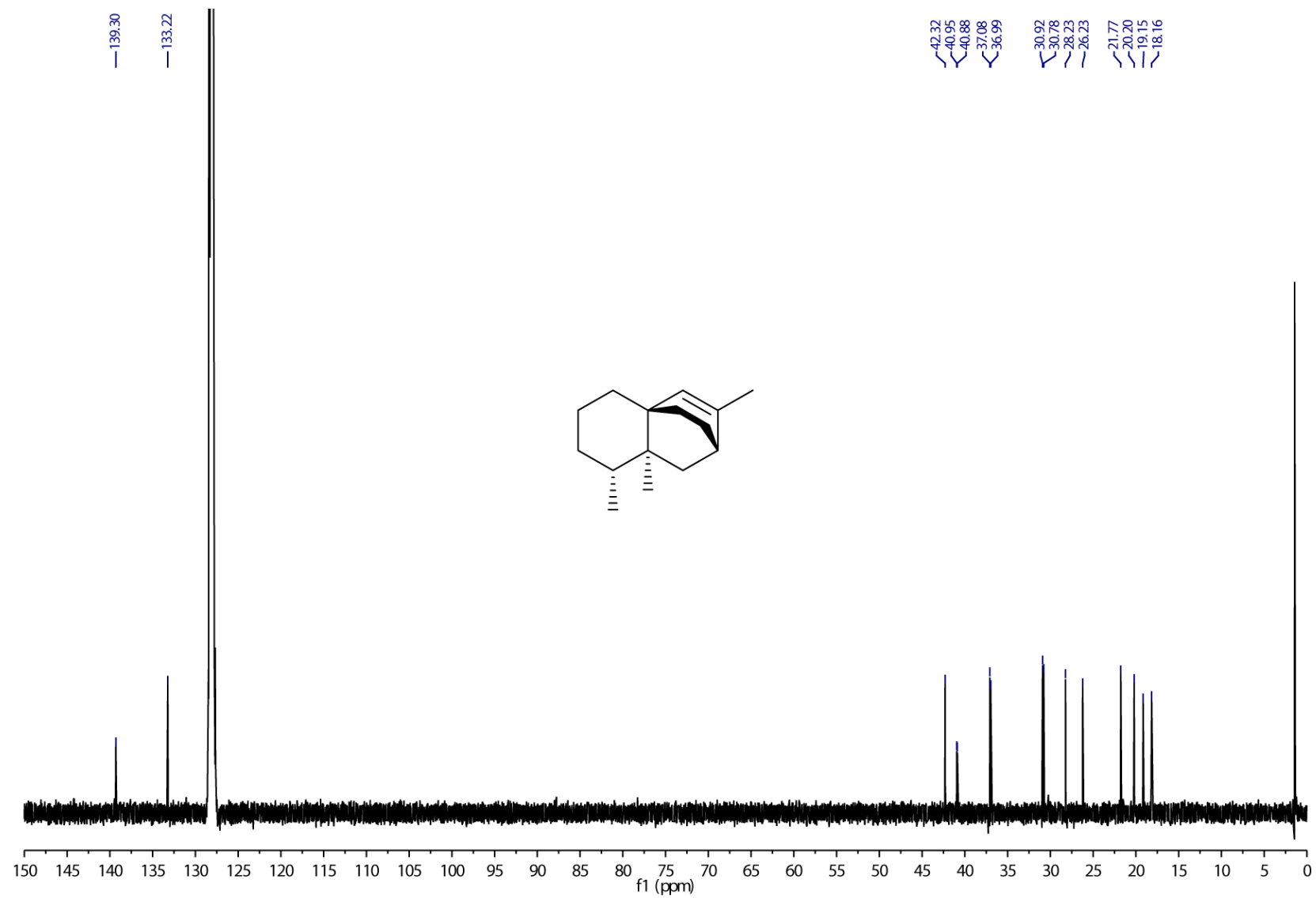


Figure S5. ^{13}C -NMR spectrum of **1** (175 MHz, C_6D_6).

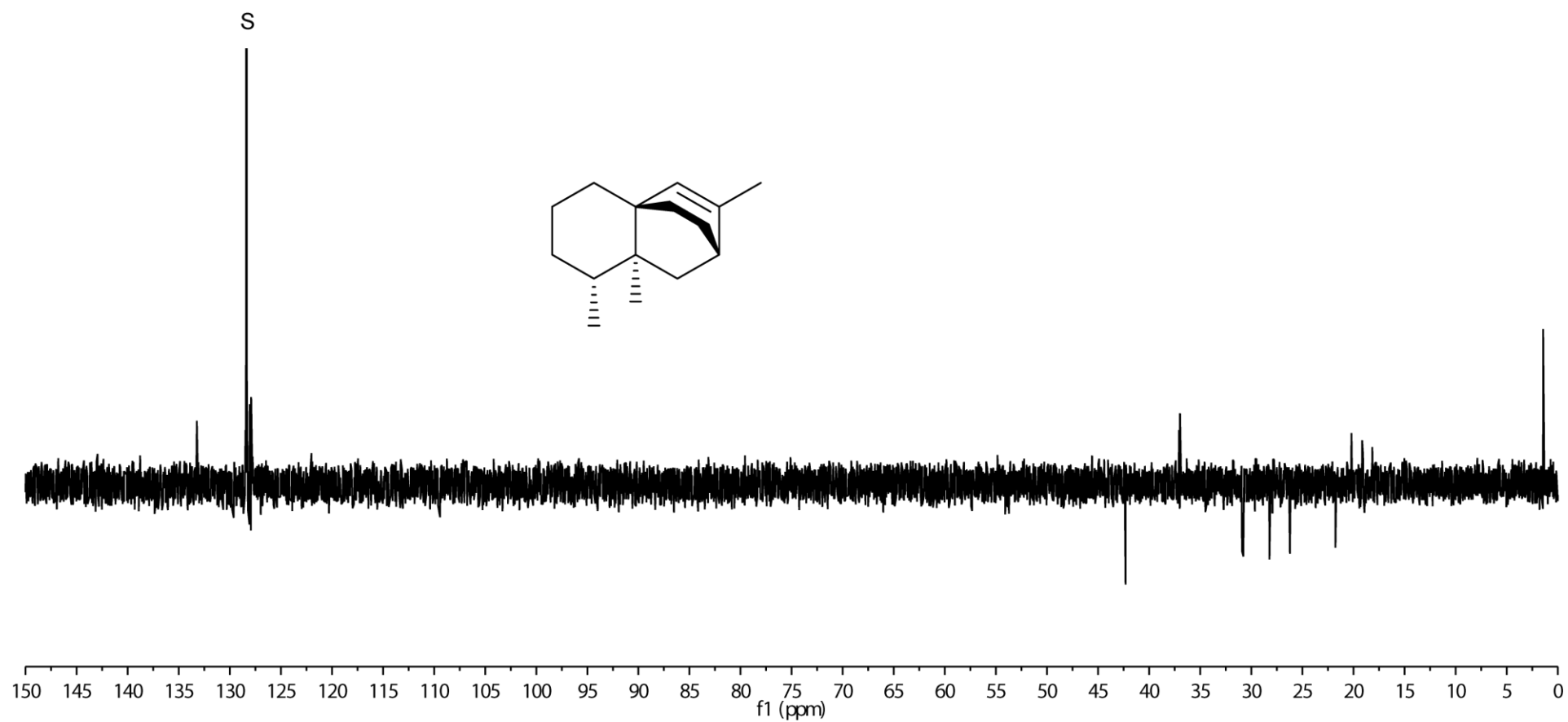


Figure S6. ^{13}C -DEPT-135 spectrum of **1** (175 MHz, C_6D_6). S indicates residual solvent signal.

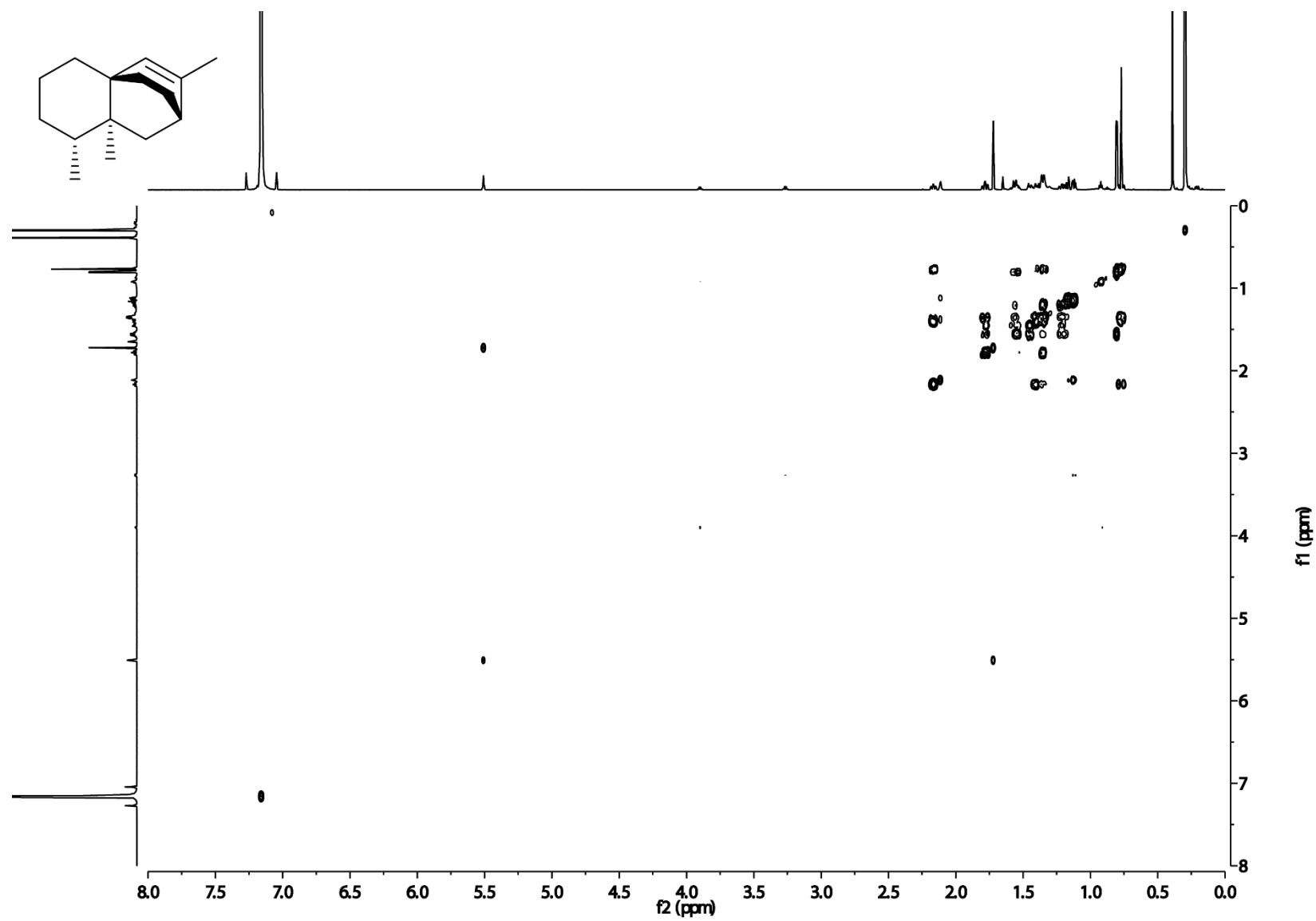


Figure S7. ^1H , ^1H -COSY spectrum of **1** (C_6D_6).

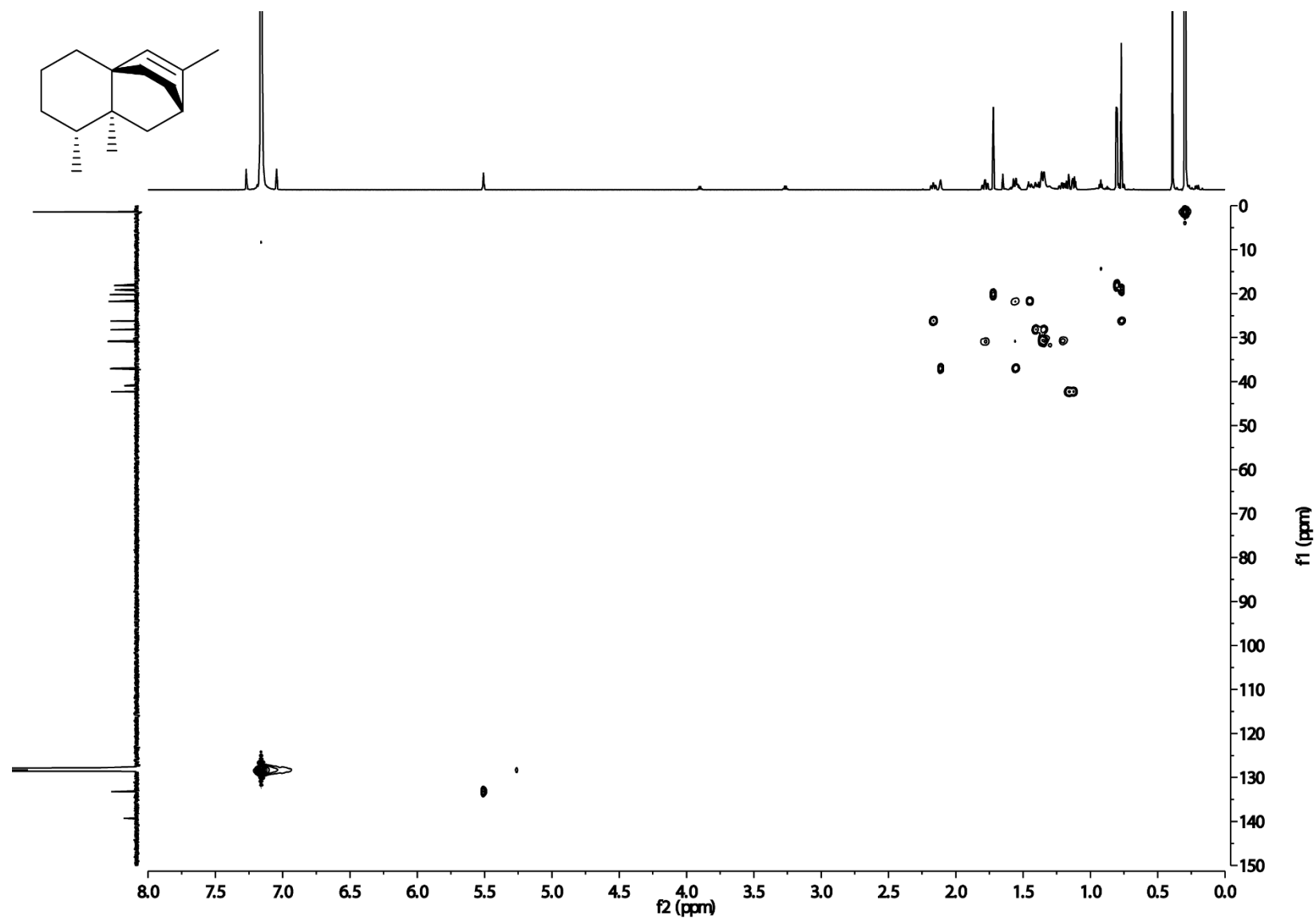
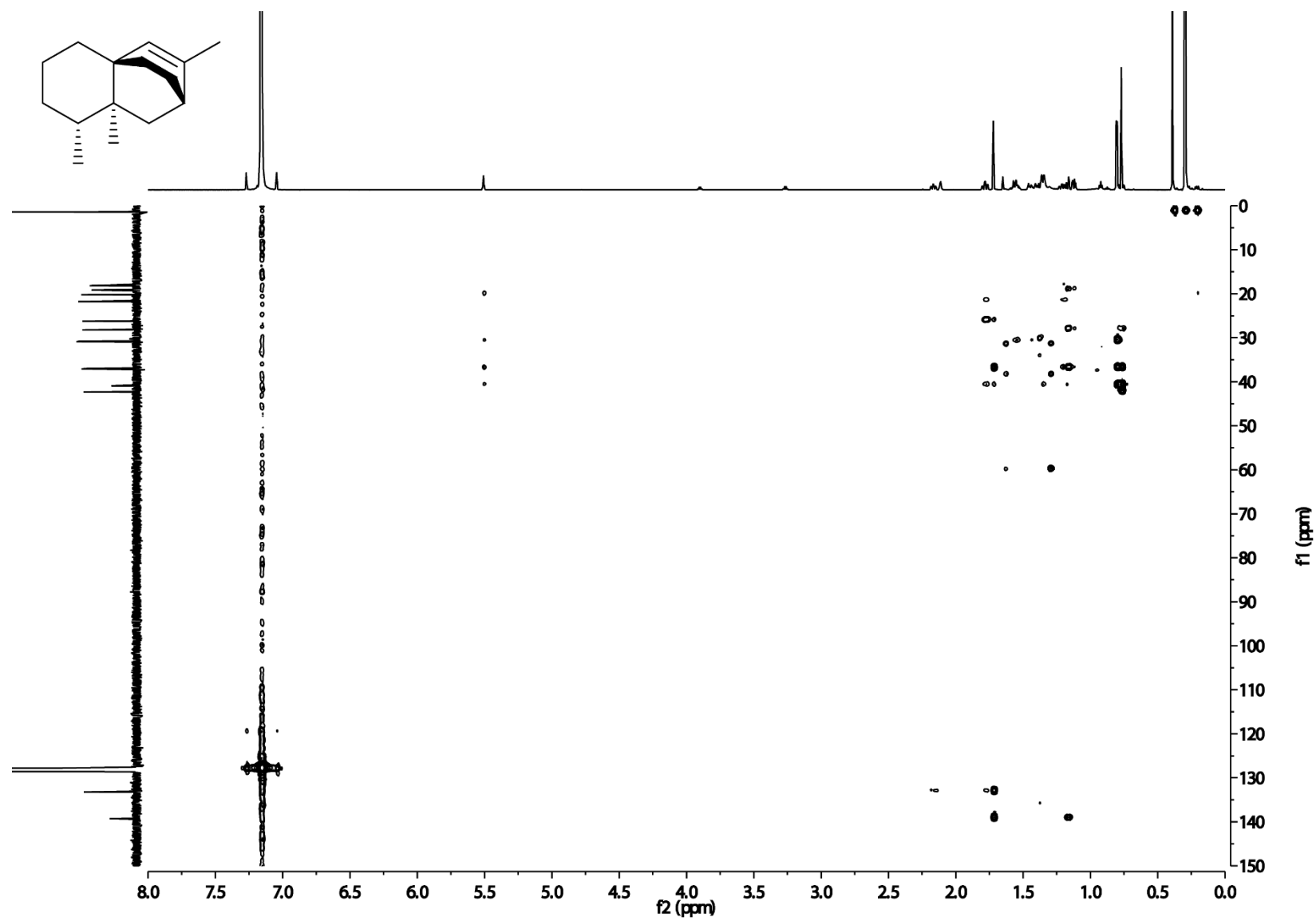


Figure S8. HSQC spectrum of 1 (C_6D_6).



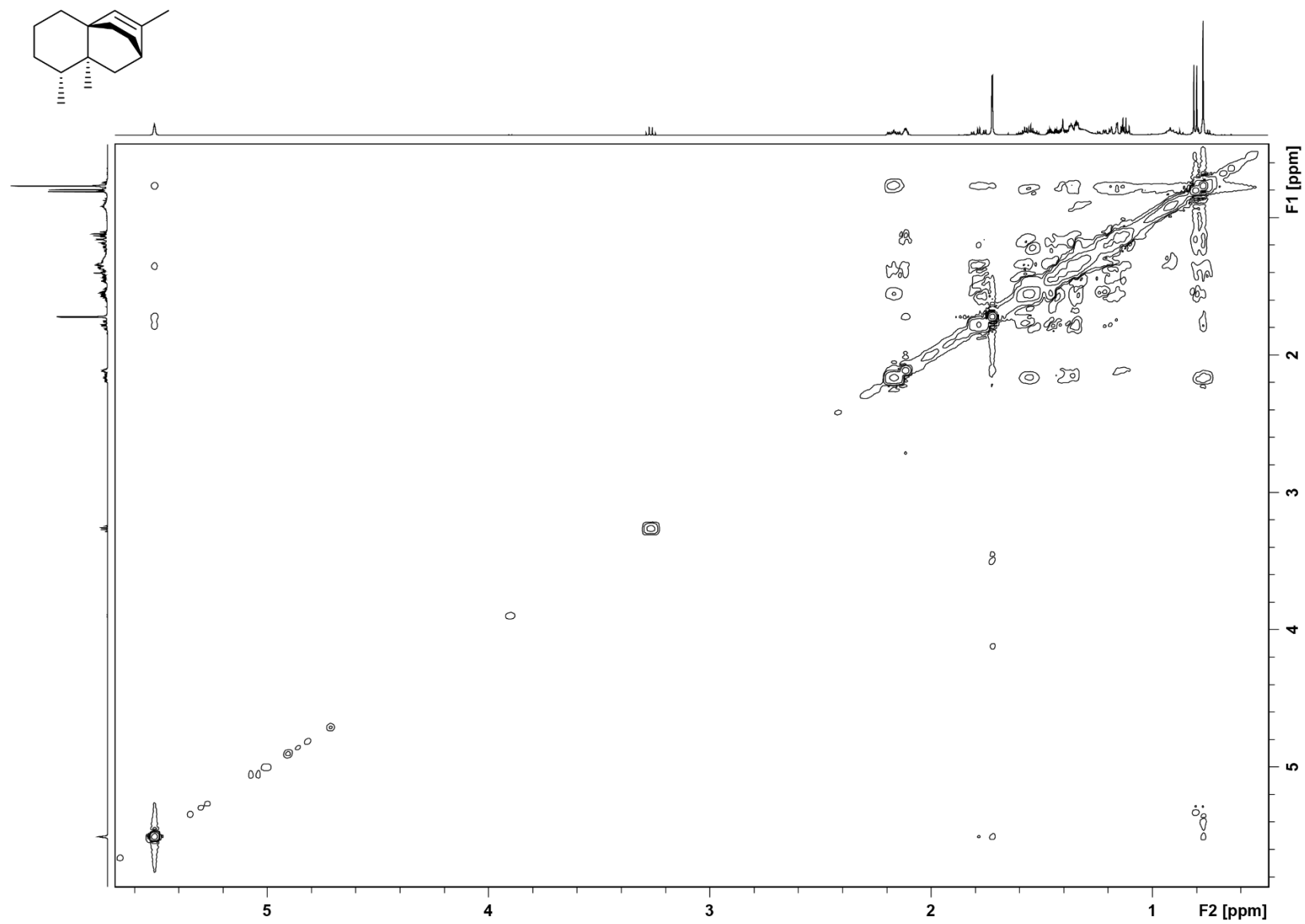


Figure S10. NOESY spectrum of **1** (C_6D_6).

Table S3. Labelling experiments with RIMTPSL4.

entry	substrate	enzyme(s)	results shown in
1	(12- ¹³ C)FPP ^[3]	RIMTPSL4	Figure 1
2	(9- ¹³ C)GPP, ^[4] IPP	FPPS, ^[5] RIMTPSL4	Figure 1
3	(6- ¹³ C)FPP ^[3]	RIMTPSL4	Figure 2
4	(3- ¹³ C,2- ² H)FPP ^[6]	RIMTPSL4	Figure 3
5	DMAPP, (<i>E</i>)-(4- ¹³ C,4- ² H)IPP ^[7]	FPPS, RIMTPSL4	Figure 4
6	DMAPP, (<i>Z</i>)-(4- ¹³ C,4- ² H)IPP ^[7]	FPPS, RIMTPSL4	Figure 4
7	(<i>R</i>)-(1- ¹³ C,1- ² H)IPP ^[8]	IDI, ^[10] FPPS, RIMTPSL4	Figure 4
8	(<i>S</i>)-(1- ¹³ C,1- ² H)IPP ^[8]	IDI, FPPS, RIMTPSL4	Figure 4

References

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