Supporting Information for:

Isolation and Characterization of Cyclic C_{33} Botryococcenes and a Trimethylsqualene Isomer from *Botryococcus braunii* Race B

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Table S1. ^{13}C NMR assignments for hydrocarbons from race B. δ denotes ^{13}C chemical shifts determined for carbons of each hydrocarbon molecule.

	C$_{32}$ Botry.[a]	Braunicene[b]	Showacene[c]	Wolficene[d]	Monomethyl squalene[e]	Dimethyl squalene[e]	C$_{34}$ Botry. isomer[f]	Tetramethyl squalene[e]
Position	δ$_C$, type	δ$_C$, type	δ$_C$, type	δ$_C$, type	δ$_C$, type	δ$_C$, type	δ$_C$, type	δ$_C$, type
1	109.4, CH$_2$	109.5, CH$_2$	17.9, CH$_3$	17.9, CH$_2$	109.6, CH$_2$	109.6, CH$_2$	109.3, CH$_2$	109.6, CH$_2$
2	150.2, C	ND, C	ND[g], C	ND, C	150.1, C	ND, C	ND, C	ND, C
3	40.7, CH	41.0, CH	124.7, CH	124.6, CH	40.9, CH	40.9, CH	41.2, CH	41.2, CH
4	33.3, CH$_2$	33.6, CH$_2$	26.9, CH$_2$	27.0, CH$_2$	33.6, CH$_2$	33.6, CH$_2$	33.6, CH$_2$	33.6, CH$_2$
5	37.4, CH$_2$	37.7, CH$_2$	39.9, CH$_2$	39.9, CH$_2$	37.7, CH$_2$	37.7, CH$_2$	124.1, CH	31.9, CH$_2$
6	135.0, C	ND, C	ND, C	ND, C	135.5, C	ND, C	ND, C	ND, C
7	124.5, CH	124.9, CH	125.0, CH	125.0, CH	124.5, CH	124.5, CH	34.7, CH	39.8, CH
8	23.1, CH$_2$	23.4, CH$_2$	23.3, CH$_2$	23.4, CH$_2$	26.9, CH$_2$	26.9, CH$_2$	29.5, CH$_2$	34.2, CH$_2$
9	41.4, CH$_2$	41.6, CH$_2$	41.5, CH$_2$	41.6, CH$_2$	40.0, CH$_2$	40.0, CH$_2$	39.7, CH$_2$	37.7, CH$_2$
10	41.8, C	ND, C	ND, C	ND, C	135.5, C	ND, C	ND, C	ND, C
11	135.8, CH	135.7, CH	136.0, CH	135.7, CH	124.5, CH	124.5, CH	136.0, CH	124.3, CH
12	133.8, CH	134.5, CH	134.0, CH	134.5, CH	28.5, CH$_2$	28.5, CH$_2$	134.0, CH	28.5, CH$_2$
13	36.7, CH	37.6, CH	36.9, CH	37.6, CH	28.5, CH$_2$	28.5, CH$_2$	37.5, CH	28.5, CH$_2$
14	37.5, CH$_2$	36.1, CH$_2$	37.7, CH$_2$	36.0, CH$_2$	124.5, CH	124.5, CH	35.2, CH$_2$	124.3, CH
15	25.8, CH$_2$	32.3, CH$_2$	26.0, CH$_2$	32.3, CH$_2$	135.5, C	ND, C	33.6, CH$_2$	ND, C
16	124.5, CH	56.0, CH	124.8, CH	56.0, CH	40.0, CH$_2$	40.0, CH$_2$	40.3, CH	37.8, CH$_2$
17	135.0, C	ND, C	ND, C	ND, C	26.9, CH$_2$	26.9, CH$_2$	ND, C	33.6, CH$_2$
18	37.4, CH$_2$	31.2, CH$_2$	37.6, CH$_2$	31.2, CH$_2$	124.5, CH	124.5, CH	31.8, CH$_2$	39.8, CH
19	33.3, CH$_2$	24.2, CH$_2$	33.5, CH$_2$	24.2, CH$_2$	135.5, C	ND, C	33.0, CH$_2$	ND, C
20	40.7, CH	35.0, CH	40.9, CH	35.0, CH	40.0, CH$_2$	37.7, CH$_2$	41.8, CH	31.9, CH$_2$
21	150.2, C	ND, C	ND, C	ND, C	26.9, CH$_2$	33.6, CH$_2$	ND, C	33.6, CH$_2$
22	109.4, CH$_2$	27.1, CH$_3$	109.6, CH$_2$	27.1, CH$_3$	124.5, CH	40.9, CH	109.7, CH$_2$	41.2, CH
23	19.0, CH$_3$	19.2, CH$_3$	25.9, CH$_3$	25.9, CH$_3$	150.1, C	ND, C	19.1, CH$_3$	ND, C
24	16.0, CH$_3$	16.1, CH$_3$	16.2, CH$_3$	16.1, CH$_3$	17.9, CH$_3$	109.6, CH$_2$	18.2, CH$_3$	109.7, CH$_2$
25	23.5, CH$_3$	23.8, CH$_3$	23.8, CH$_3$	23.8, CH$_3$	19.2, CH$_3$	19.2, CH$_3$	23.8, CH$_3$	19.2, CH$_3$
26	146.8, CH	147.1, CH	147.0, CH	147.1, CH	16.2, CH$_3$	16.2, CH$_3$	147.2, CH	107.5, CH$_2$
27	111.1, CH$_2$	111.3, CH$_2$	111.3, CH$_2$	111.3, CH$_2$	16.2, CH$_3$	16.2, CH$_3$	111.2, CH$_2$	16.2, CH$_3$
28	21.1, CH$_3$	21.2, CH$_3$	21.3, CH$_3$	21.2, CH$_3$	16.2, CH$_3$	16.2, CH$_3$	21.4, CH$_3$	16.2, CH$_3$
29	16.0, CH$_3$	109.4, CH$_2$	16.2, CH$_3$	109.3, CH$_2$	16.2, CH$_3$	16.2, CH$_3$	107.4, CH$_2$	107.5, CH$_2$
30	19.0, CH$_3$	21.9, CH$_3$	19.2, CH$_3$	21.9, CH$_3$	25.9, CH$_3$	19.2, CH$_3$	19.9, CH$_3$	19.2, CH$_3$
31	19.7, CH$_3$	16.9, CH$_3$	19.9, CH$_3$	16.9, CH$_3$	19.9, CH$_3$	19.9, CH$_3$	19.6, CH$_3$	19.9, CH$_3$
32	19.7, CH$_3$	19.9, CH$_3$				19.9, CH$_3$	20.0, CH$_3$	20.4, CH$_3$
33							20.5, CH$_3$	20.4, CH$_3$
34							19.8, CH$_3$	19.9, CH$_3$

a) ^{13}C chemical shifts reported here match Okada, S.; Murakami, M.; Yamaguchi, K. *Phytochemical Analysis* **1997**, *8,* 198–203.
b) ^{13}C chemical shifts reported here match Huang, Z.; Poulter, C. D.; Wolf, F. R.; Somers, T. C.; White, J. D. *J. Am. Chem. Soc*. **1988**, *110*, 3959-3964.
c) ^{13}C chemical shifts reported here match Huang, Z.; Poulter, C. D. *Phytochemistry* **1989**, *28*, 3043-3046.
d) ^{13}C chemical shifts reported here match Huang, Z.; Poulter, C. D. *J. Org. Chem.***1988**, *53*, 5390-5392.
e) ^{13}C chemical shifts reported here match Achitouv, E.; Metzger, P.; Rager, M. N.; Largeau, C. *Phytochemistry* **2004**, *65*, 3159-3165.
f) ^{13}C chemical shifts reported here match Metzger, P.; Casadevall, E.; Pouet, M. J.; Pouet, Y. *Phytochemistry* **1985**, *24*, 2995-3002.
g) ND: Not determined in this study, but has been shown in literature.

Table S2. ^{13}C assignments for previously identified hydrocarbons used for comparison to 5-7 and 8. δ denotes ^{13}C chemical shifts determined for each hydrocarbon molecule.

C_{33} Botryococcene[a]		Trimethylsqualene[b]
Position	δ_C, type	δ_C, type
1	109.3, CH_2	109.6, CH_2
2	150.1, C	150.1[c], C
3	40.8, CH	41.2, CH
4	33.4, CH_2	33.6, CH_2
5	37.5, CH_2	31.9, CH_2
6	135.0, C	155.0[c], C
7	124.6, CH	39.8, CH
8	23.1, CH_2	34.2, CH_2
9	41.3, CH_2	37.6, CH_2
10	42.0, C	135.5[c], C
11	135.7, CH	124.5, CH
12	133.9, CH	28.5, CH_2
13	37.3, CH	28.5, CH_2
14	35.0, CH_2	124.5, CH
15	33.4, CH_2	135.3[c], C
16	40.1, CH	40.0, CH_2
17	155.0, C	26.8, CH_2
18	31.6, CH_2	124.5, CH
19	33.4, CH_2	135.2[c], C
20	41.0, CH	37.7, CH_2
21	150.2, C	33.6, CH_2
22	109.5, CH_2	40.9, CH
23	19.0, CH_3	150.3[c], C
24	15.9, CH_3	109.6, CH_2
25	23.5, CH_3	19.2, CH_3
26	146.8, CH	107.5, CH_2
27	111.1, CH_2	16.2, CH_3
28	21.1, CH_3	16.2, CH_3
29	107.2, CH_2	16.2, CH_3
30	18.9, CH_3	19.2, CH_3
31	19.8, CH_3	19.9, CH_3
32	19.7, CH_3	19.9, CH_3
33	20.2, CH_3	20.4, CH_3

[a] All ^{13}C chemical shifts shown for C_{33} botryococcene taken from a previous publication[11] for comparison to **5** in Table 1.

[b] ^{13}C chemical shifts shown for trimethylsqualene were determined in the current study except where noted.

[c] These individual ^{13}C chemical shifts not determined in this study. Presented ^{13}C chemical shifts are from a previous publication[8] for comparison to **8** in Table 2.

Table S3. ^{13}C and ^{1}H NMR Spectroscopic Data for 5.

	5		
Position	$^{1}J_{C\text{-}H}$ (J in Hz), type	NOE	HMBC (^{1}H)
1	153.0, CH_2	nd[a]	3, 23
	153.0	nd	
2	-	-	1, 3, 4, 23, 32
3	127.8, CH	nd	1, 4, 23, 32
4	124.2, CH_2	nd	3, 5, 32
	129.6	nd	
5	120.6, CH_2	nd	4, 7, 24
6	-	-	5, 24
7	151.2, CH	nd	8, 9, 24
8	124.2, CH_2	nd	7, 9
9	127.8, CH_2	nd	8
10	-	-	9, 11, 12, 25, 26, 27
11	147.0, CH	nd	9, 12, 13, 25, 26
12	146.4, CH	nd	11, 13, 14, 28
13	119.4, CH	nd	11, 12 14, 28
14	125.4, CH_2	nd	13, 28
	127.2	nd	
15	121.2, CH_2	nd	14, 16, 33
	127.8	nd	
16	126.0, CH	nd	29, 33
17	-	-	15, 16, 18, 19, 22, 29, 33
18	123.0, CH_2	18a, 19a, 20, 22b, 33	19, 22, 29
	126.6	18b, 19a, 20, 22b, 29, 33	
19	125.4, CH_2	18a, 19a, 29, 33	18, 31
	130.8	18b, 19b, 20, 31	
20	124.2, CH	18b 18a, 19a, 22b, 31	18, 22, 30, 31
21	-	-	19, 22, 31
22	114.0, CH_2	18b, 20, 22a, 33	29, 30
	124.8	16, 22b, 29, 30, 31	
23	124.8, CH_3	nd	1
24	125.4 CH_3	nd	5, 7
25	126.6, CH_3	nd	11, 26
26	138.0, CH	nd	9, 11, 25, 27
27	168.0, CH_2	nd	
28	126.0, CH_3	nd	12
29	125.4, CH_3	18a, 19b, 22a, 30, 33	22
30	143.4, CH_2	22a, 29, 31	22
31	126.0, CH_3	19a, 20, 30	19
32	125.4, CH_3	nd	3, 4
33	125.4, CH_3	nd	15, 16

[a] Not determined for these positions.

Table S4. ^{13}C and ^{1}H NMR Spectroscopic Data for 6.

	6	
Position	$^{1}J_{C\text{-}H}$ (J in Hz), type	NOE
1	154.8, CH_2	nd[a]
	153.6	nd
2	-	-
3	127.8, CH	nd
4	122.4, CH_2	nd
	130.2	nd
5	150.0, CH	nd
6	-	-
7	124.2, CH	nd
8	124.2, CH_2	nd
9	125.4, CH_2	nd
10	-	-
11	146.4, CH	nd
12	147.6, CH	nd
13	126.0, CH	nd
14	120.6, CH_2	nd
	123.6	nd
15	112.2, CH_2	nd
	137.4	nd
16	130.8, CH	14, 14a, 15, 15a, 18, 22, 29b, 30
17	-	-
18	128.4, CH_2	15b, 29b, 29a
19	112.8, CH_2	18, 19a, 20, 22
	124.2	18, 19b, 20, 22
20	124.2, CH	18, 19b 19a, 22, 30, 31
21	-	-
22	125.4, CH_3	16, 19b, 19a, 20, 30, 31
23	125.4, CH_3	nd
24	125.4, CH_3	nd
25	126.6, CH_3	nd
26	135.6, CH	nd
27	153.6, CH_2	nd
	156.0	nd
28	126.6, CH_3	nd
29	153.6, CH_2	29a
	154.8	29b
30	124.8, CH_3	16, 19b, 20, 22
31	124.8, CH_3	19b, 19a, 20, 22
32	124.8, CH_3	nd
33	125.4, CH_3	nd

[a]Not determined for this positions

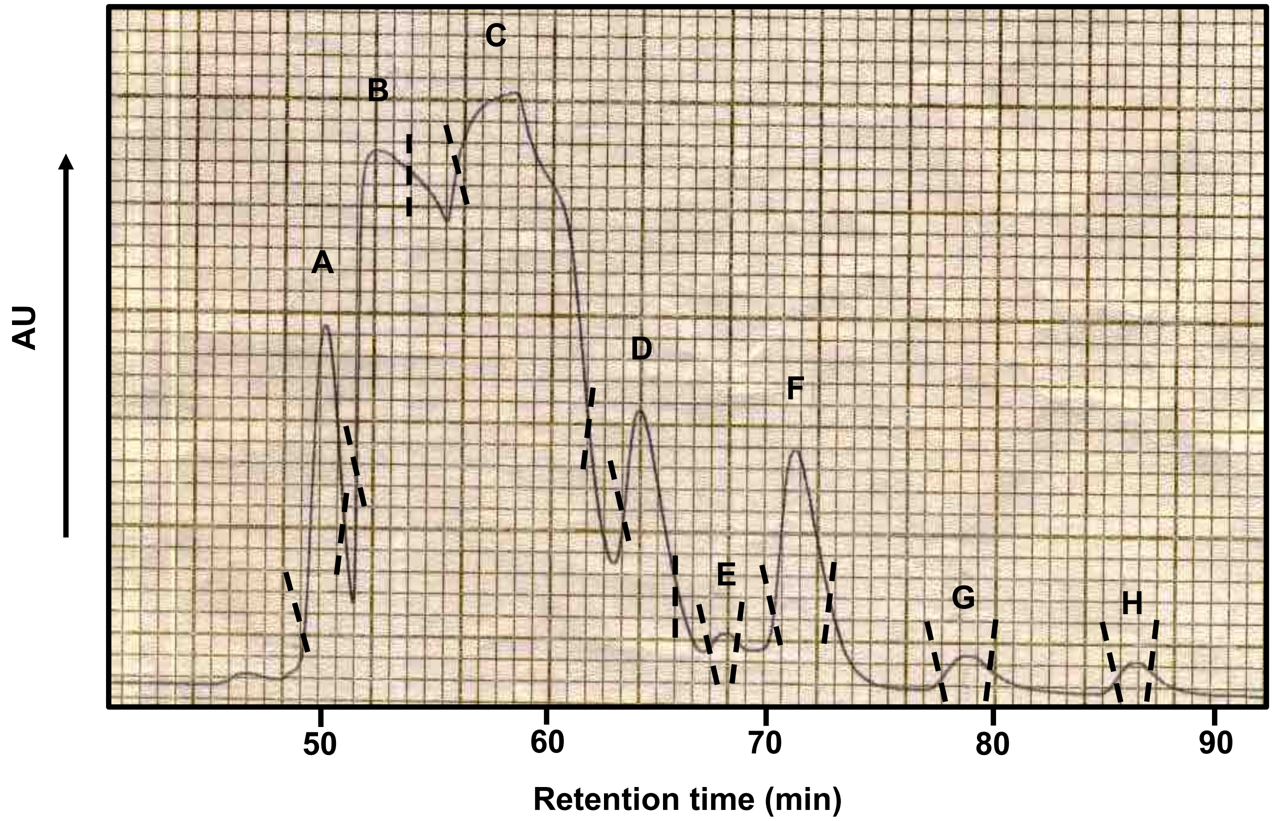


Figure S1. Chromatograph of *B. braunii* race B hydrocarbons from C_{18} HPLC. The hydrocarbon peaks labeled A to H indicate the collected fractions, and dashed vertical lines indicate the portion of each peak that was collected.

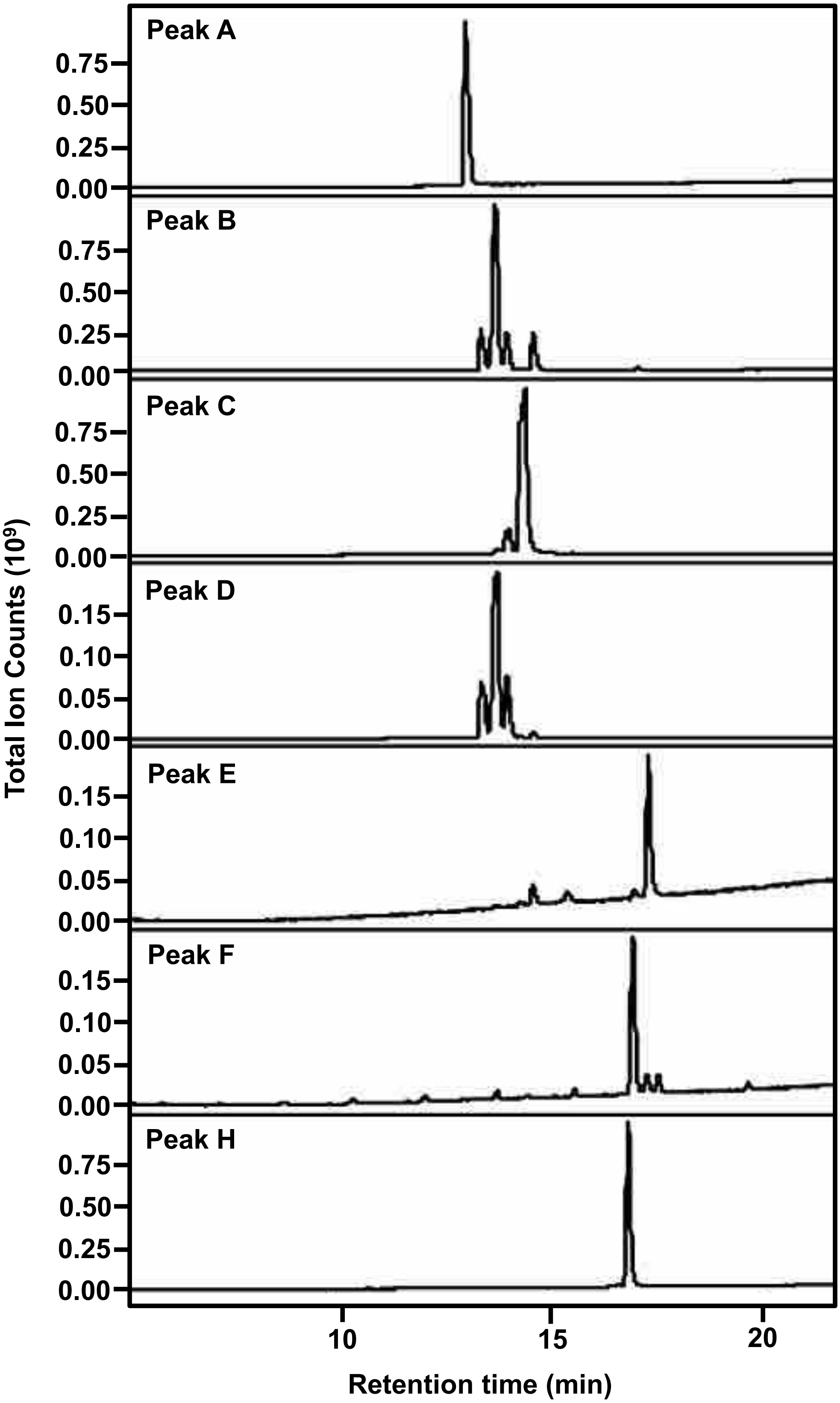
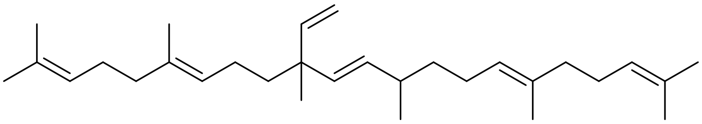
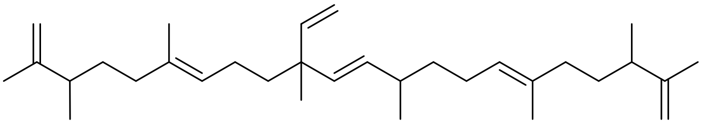


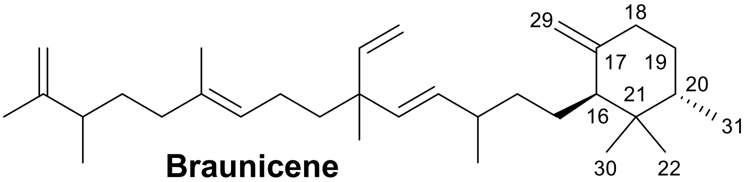
Figure S2. GC-MS total ion count profiles of each C_{18} HPLC fraction (A-H) shown in Fig. S1.



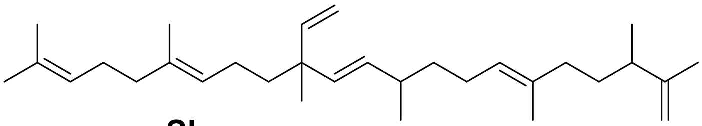
C_{30} Botryococcene 1



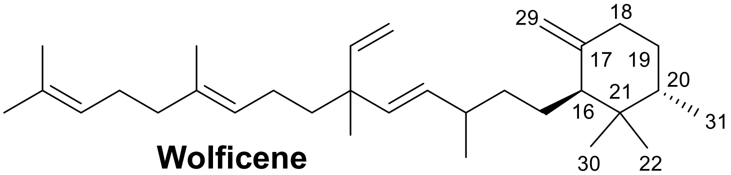
C_{32} Botryococcene



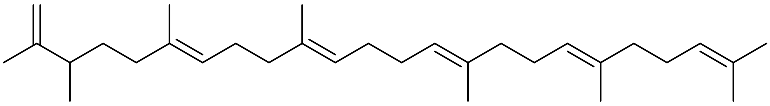
Braunicene



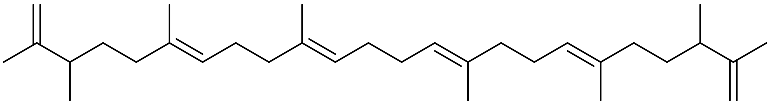
Showacene



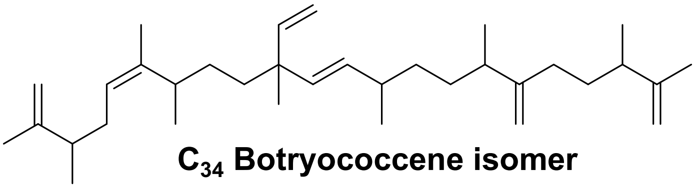
Wolficene



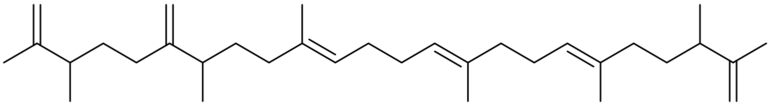
Monomethylsqualene



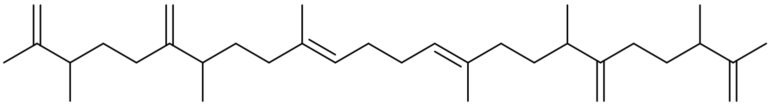
Dimethylsqualene



C_{34} Botryococcene isomer



Trimethylsqualene



Tetramethylsqualene

Figure S3. Structures for known botryococcenes and methylsqualenes isolated in this study.

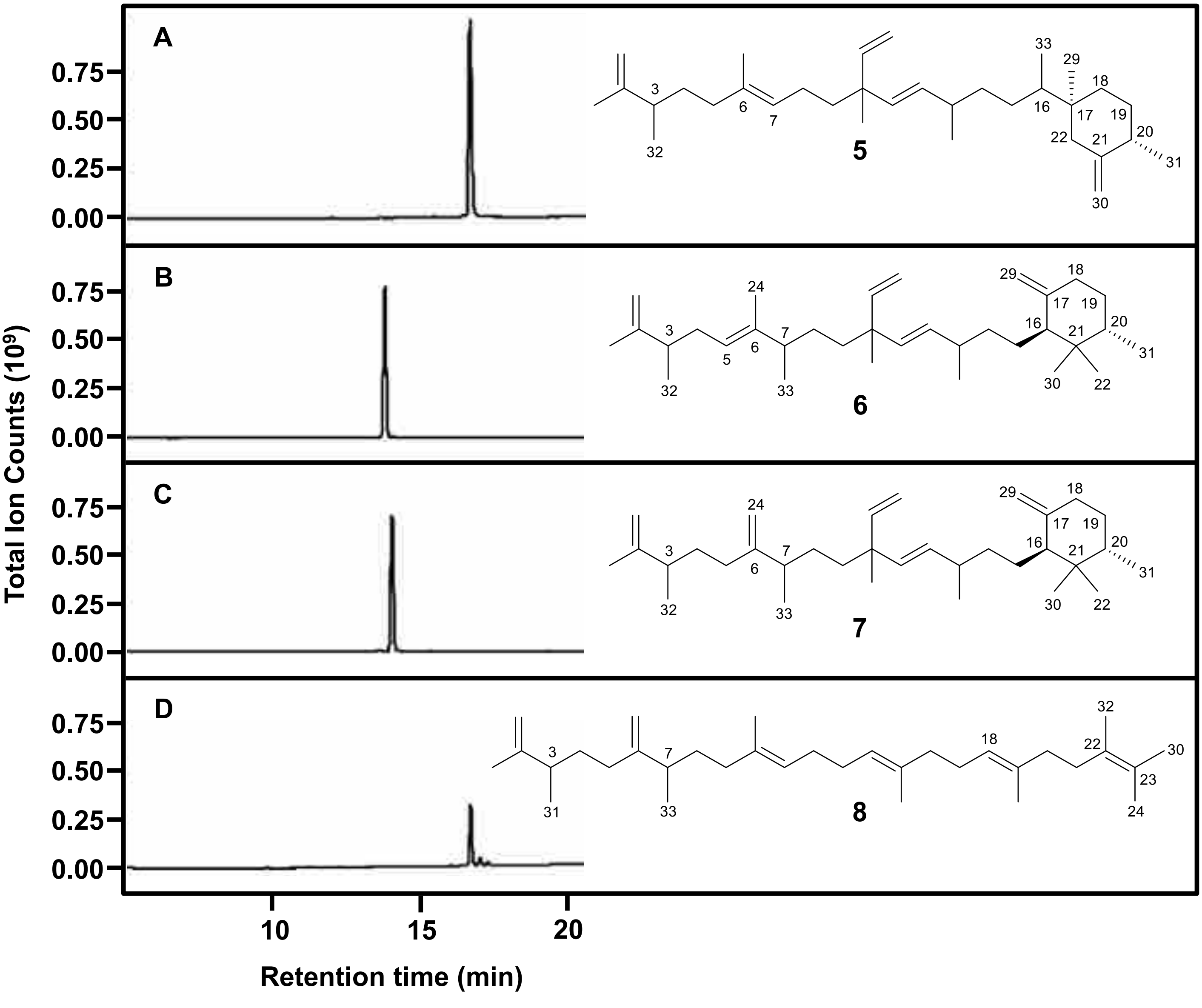


Figure S4. GC-MS total ion count profiles and structures for **5** (A), **6** (B), **7**, (C) and **8** (D).

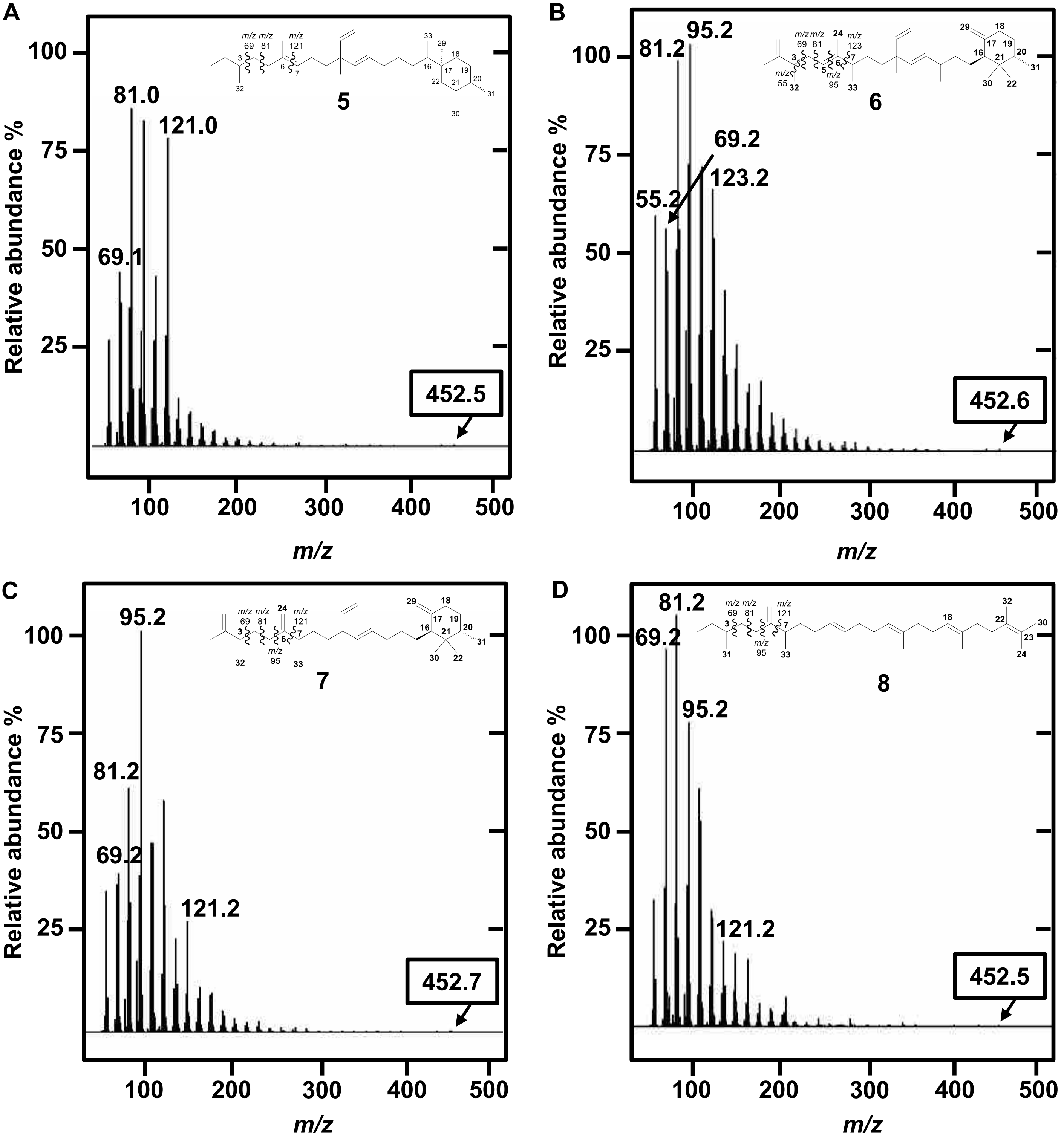


Figure S5. The EI mass spectra from GC-MS and structures for **5** (A), **6** (B), **7** (C) and **8** (D).

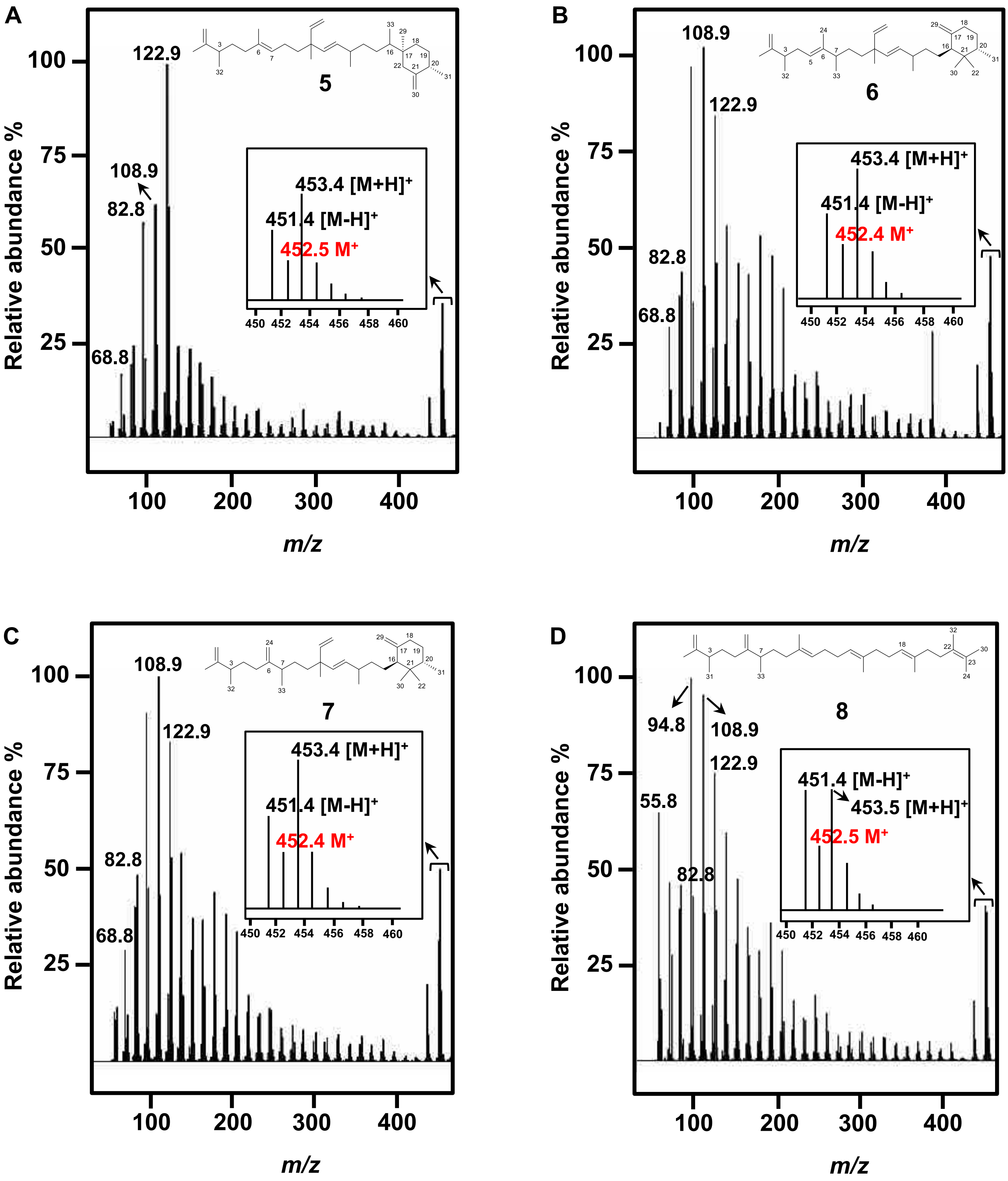


Figure S6. The mass spectra from chemical ionization GC-MS for **5** (A), **6** (B), **7** (C), and **8** (D). Spectra in boxes shows a blow up of the parent ion region indicating the parent ion (shown in red) and other ionic species.

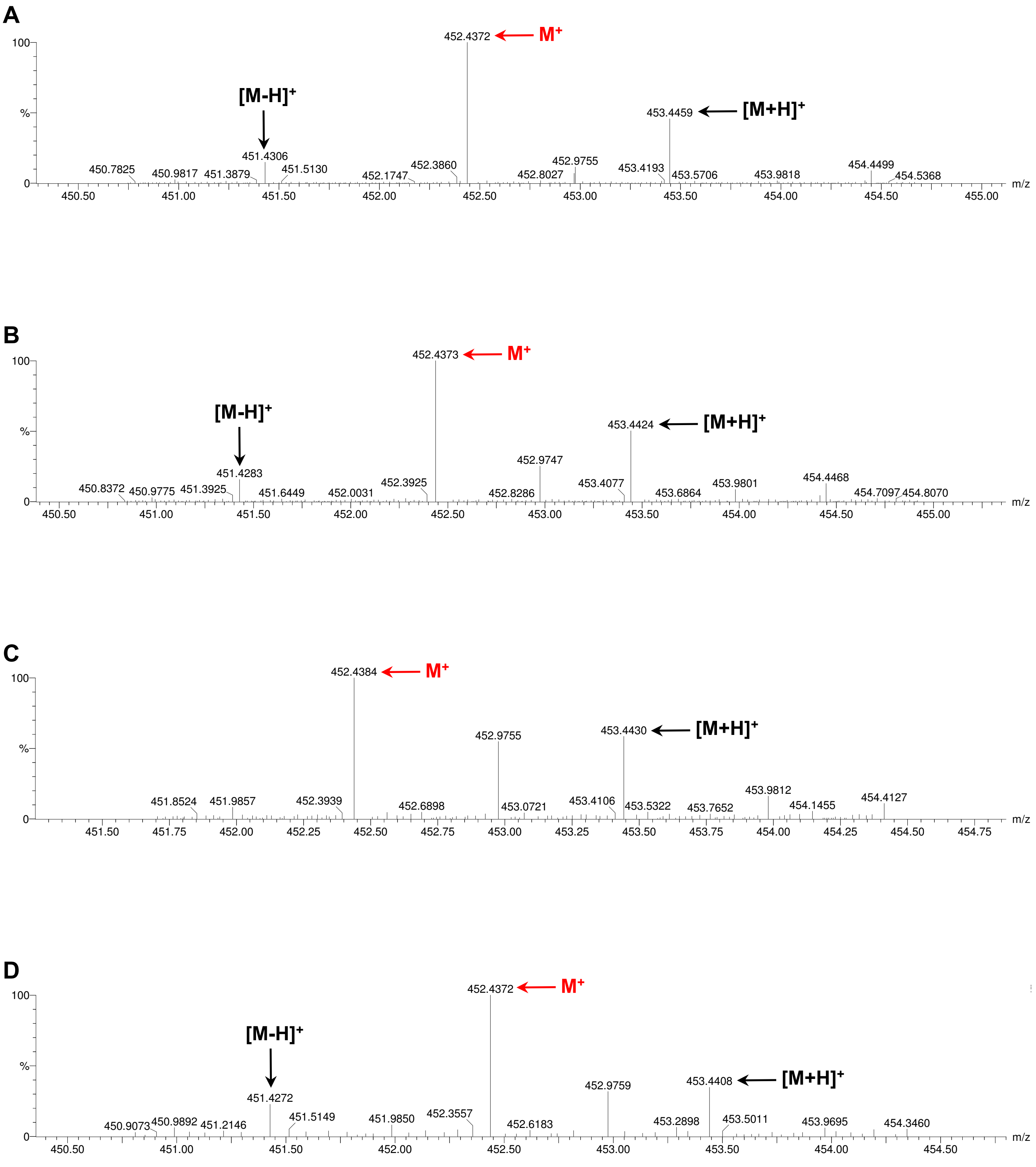


Figure S7. High resolution mass spectra from chemical ionization MS for **5** (A), **6** (B), **7** (C), and **8** (D). The parent ion is shown in red and other ionic species are shown in black.

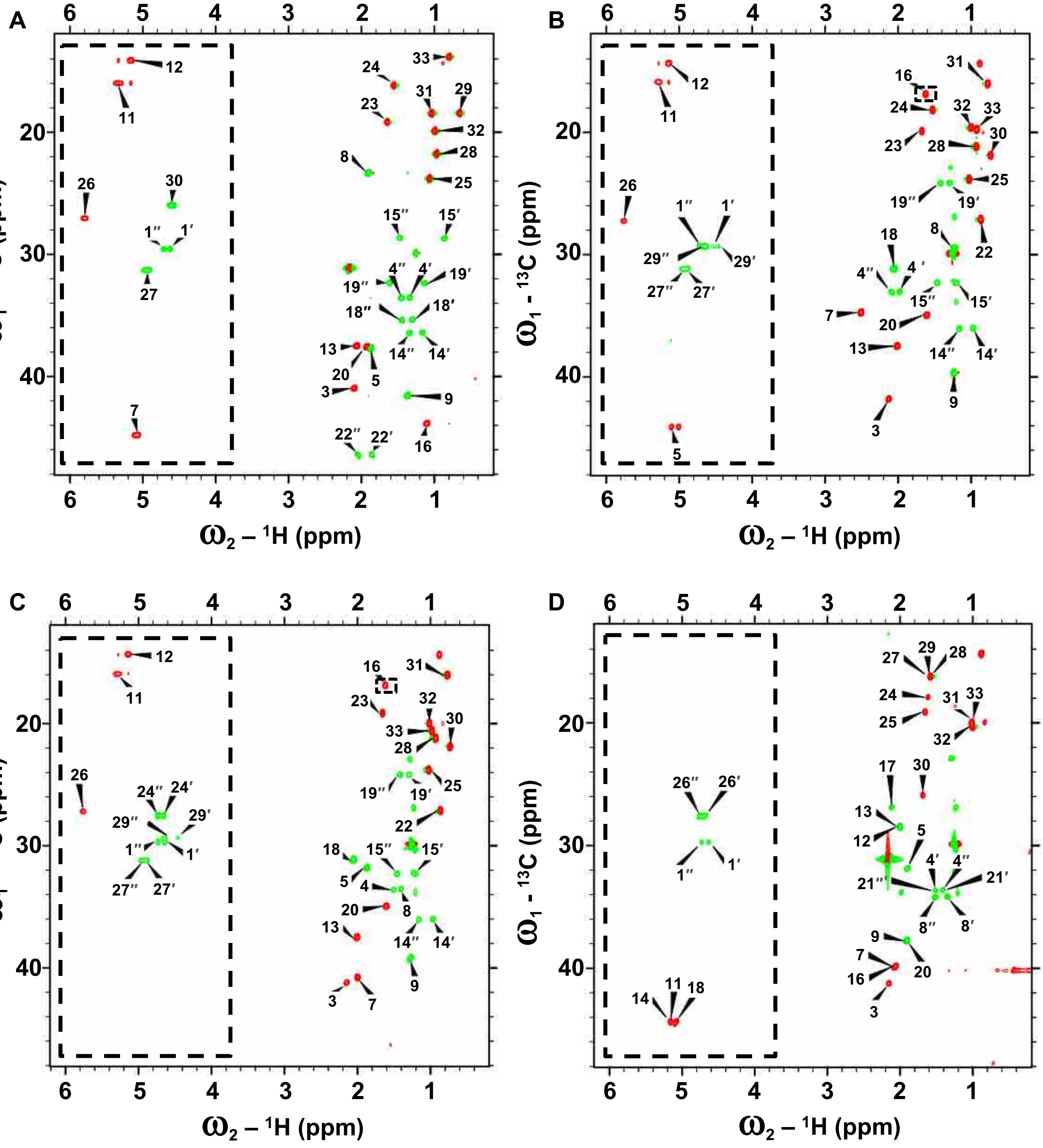


Figure S8. Multiplicity edited ^{13}C-HSQC spectra for **5** (A), **6** (B), **7** (C) and **8** (D). Positive (red) contours represent -CH or -CH_3 correlations, while negative (green) contours indicate -CH_2 correlations. Peaks originating from the molecule of interest are marked with resonance assignment, while those from impurities are left unmarked. The spectra were acquired at reduced spectral width in the indirect dimension for deliberate aliasing of ^{13}C resonances.[23] Aliased peaks are shown in dashed line boxes and the true ^{13}C chemical shifts were calculated as described in the Experimental section.

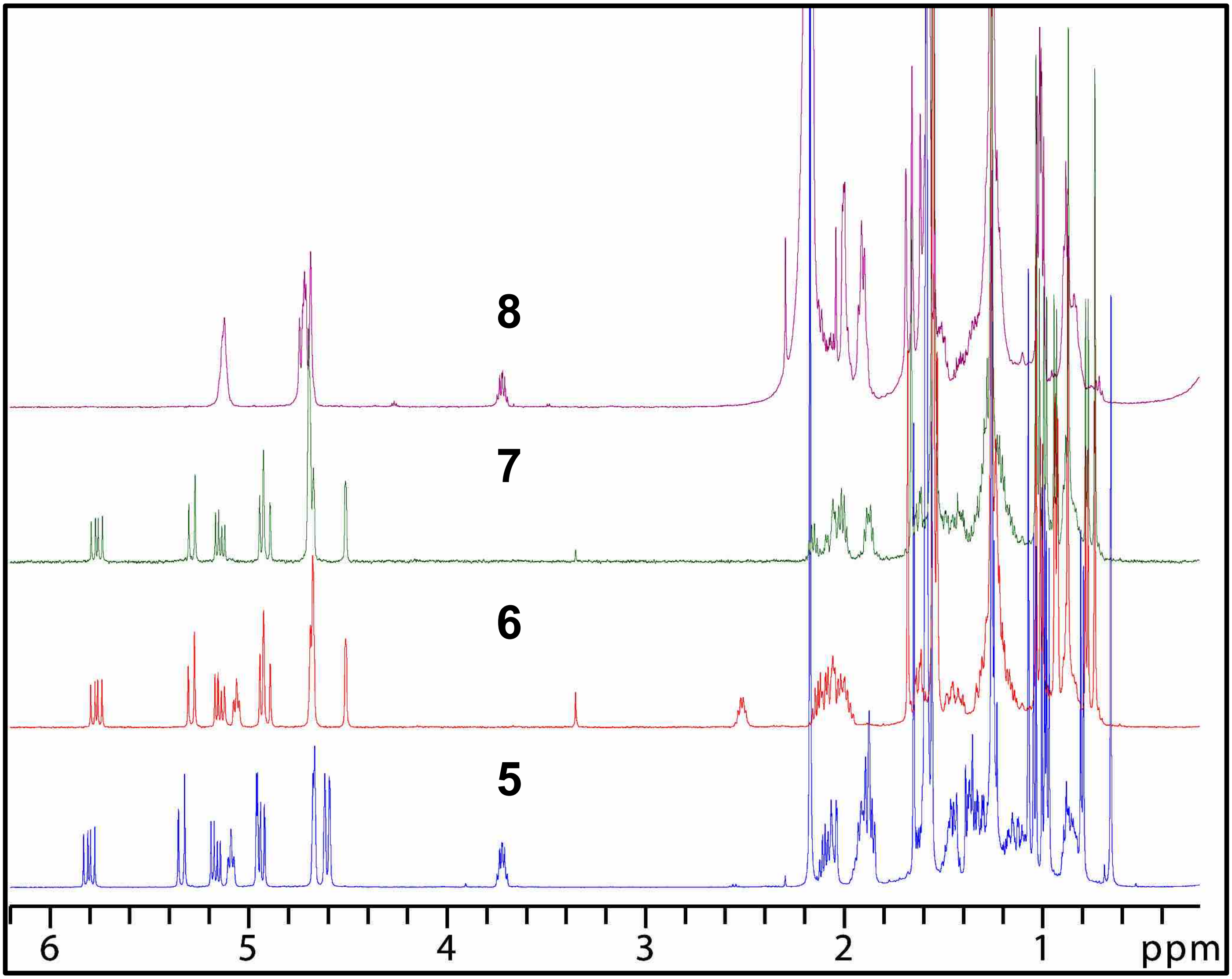


Figure S9. Overlay of 1D ^{1}H NMR spectra for **8** (top, magenta), **7** (green), **6** (red), and **5** (bottom, blue) is shown here.

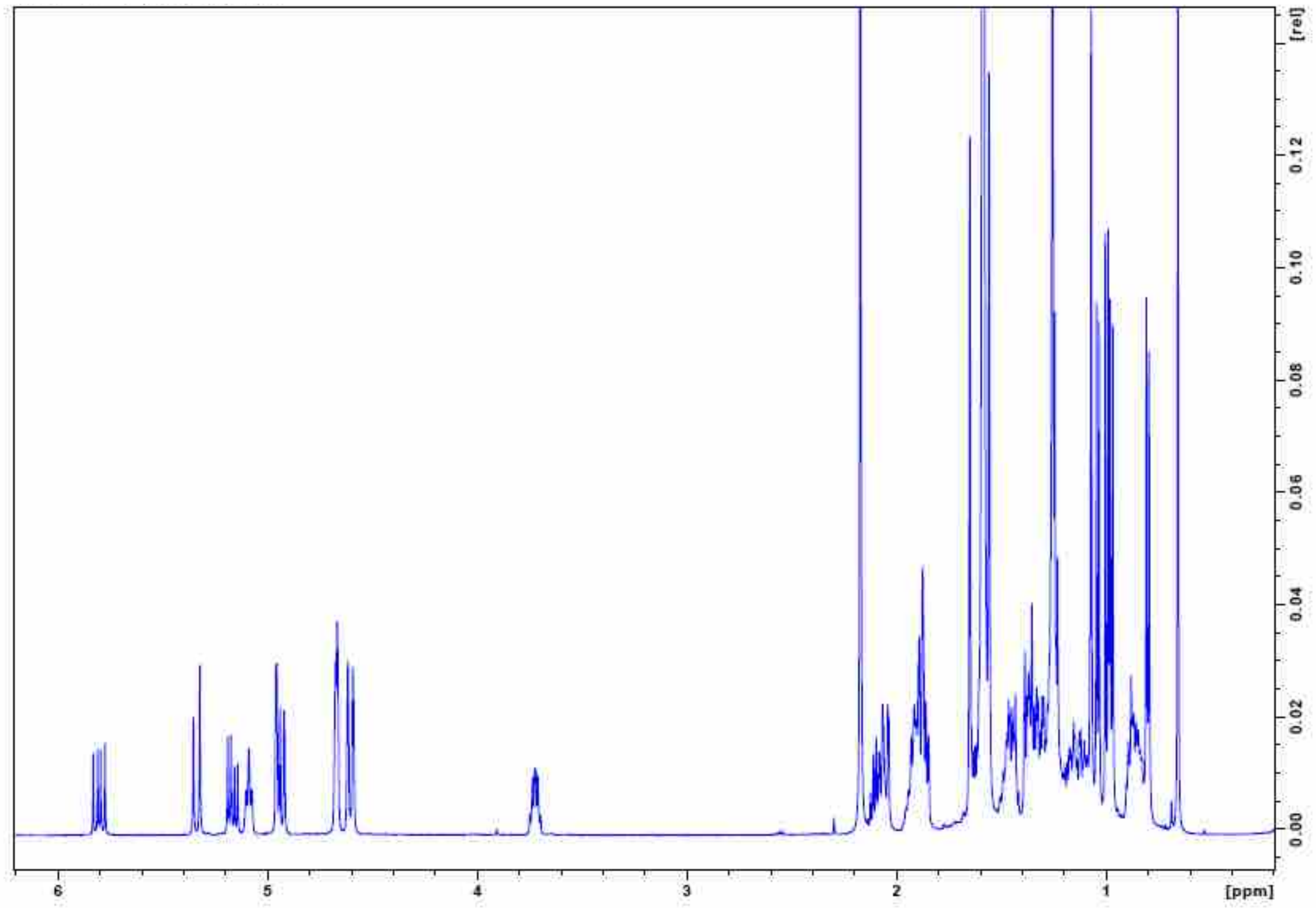


Figure S10. 1D ^{1}H NMR spectra for **5.**

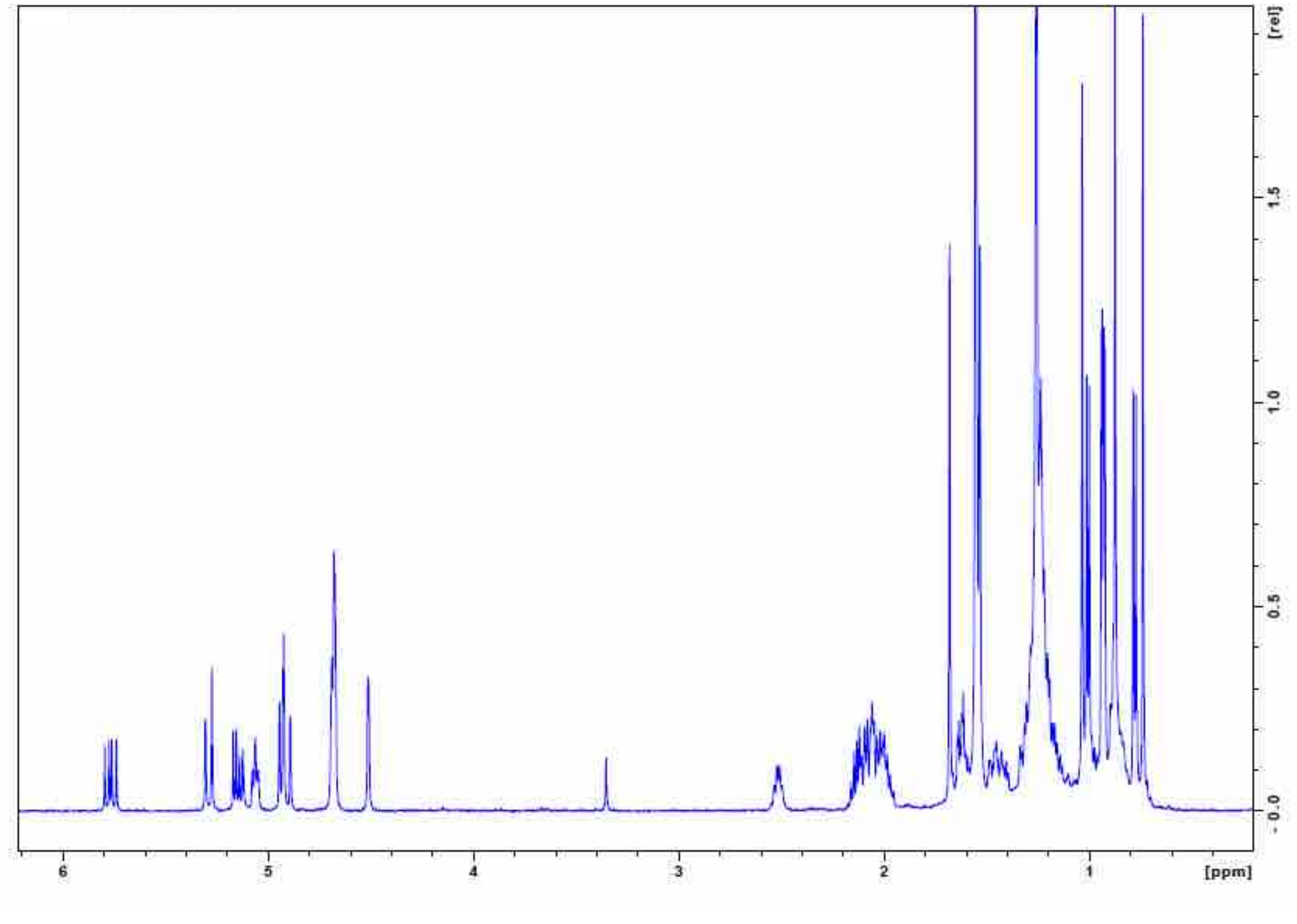


Figure S11. 1D ^{1}H NMR spectra for **6.**

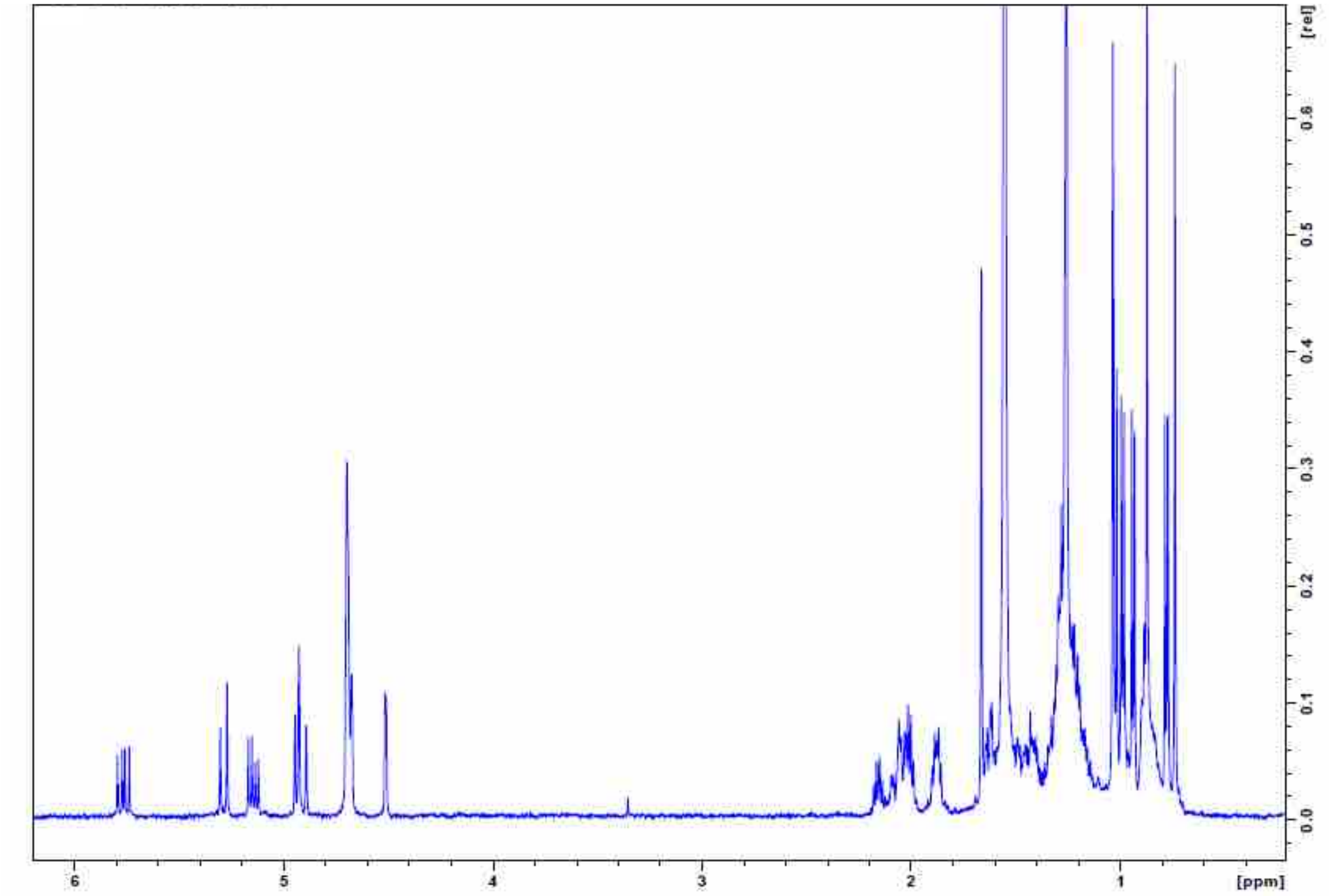


Figure S12. 1D ^{1}H NMR spectra for **7.**

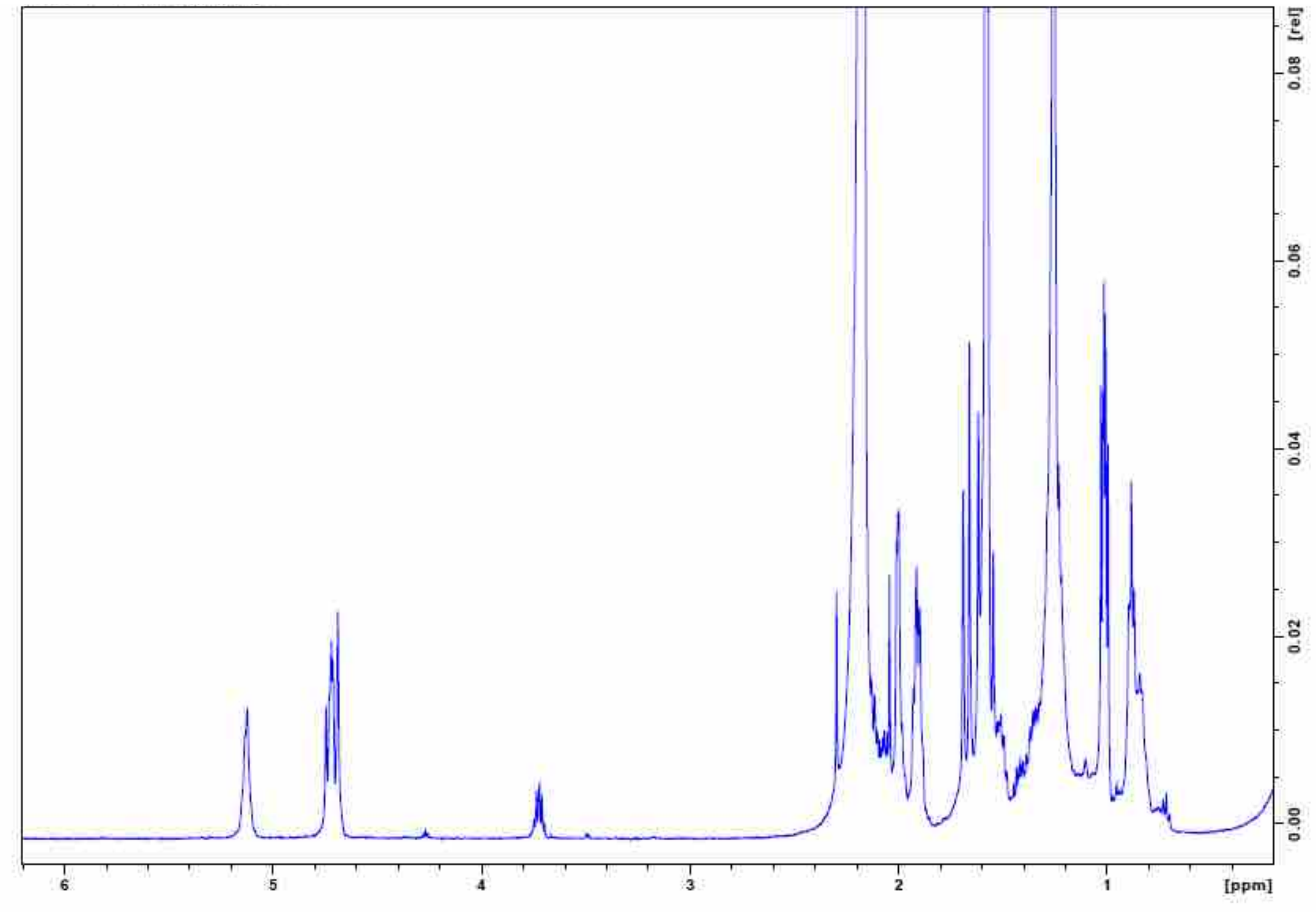


Figure S13. 1D ^{1}H NMR spectra for **8.**

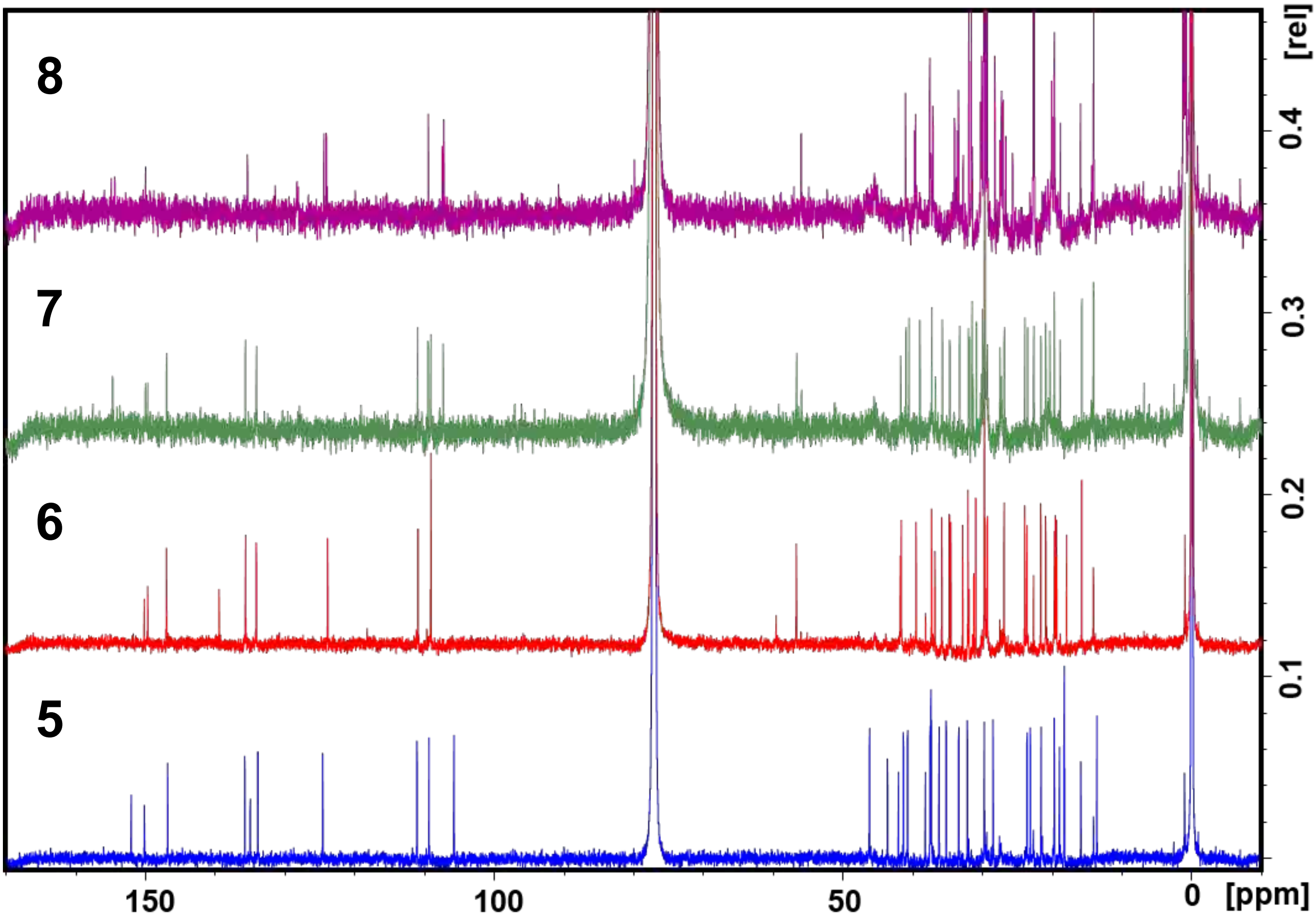


Figure S14. Overlay of 1D ^{13}C NMR spectra for **8** (top, magenta), **7** (green), **6** (red), and **5** (bottom, blue) is shown here.

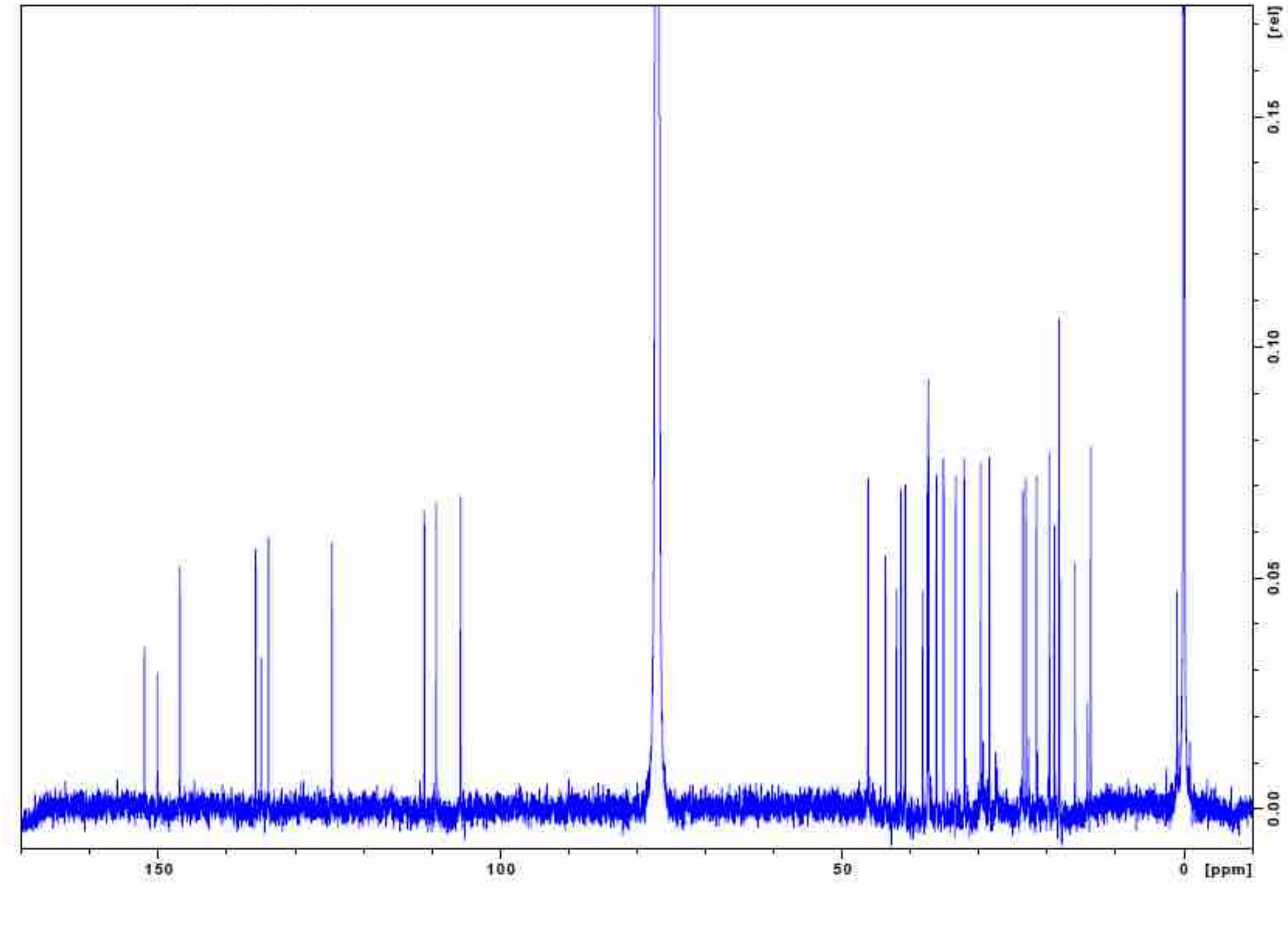


Figure S15. 1D ^{13}C NMR spectra for **5.**

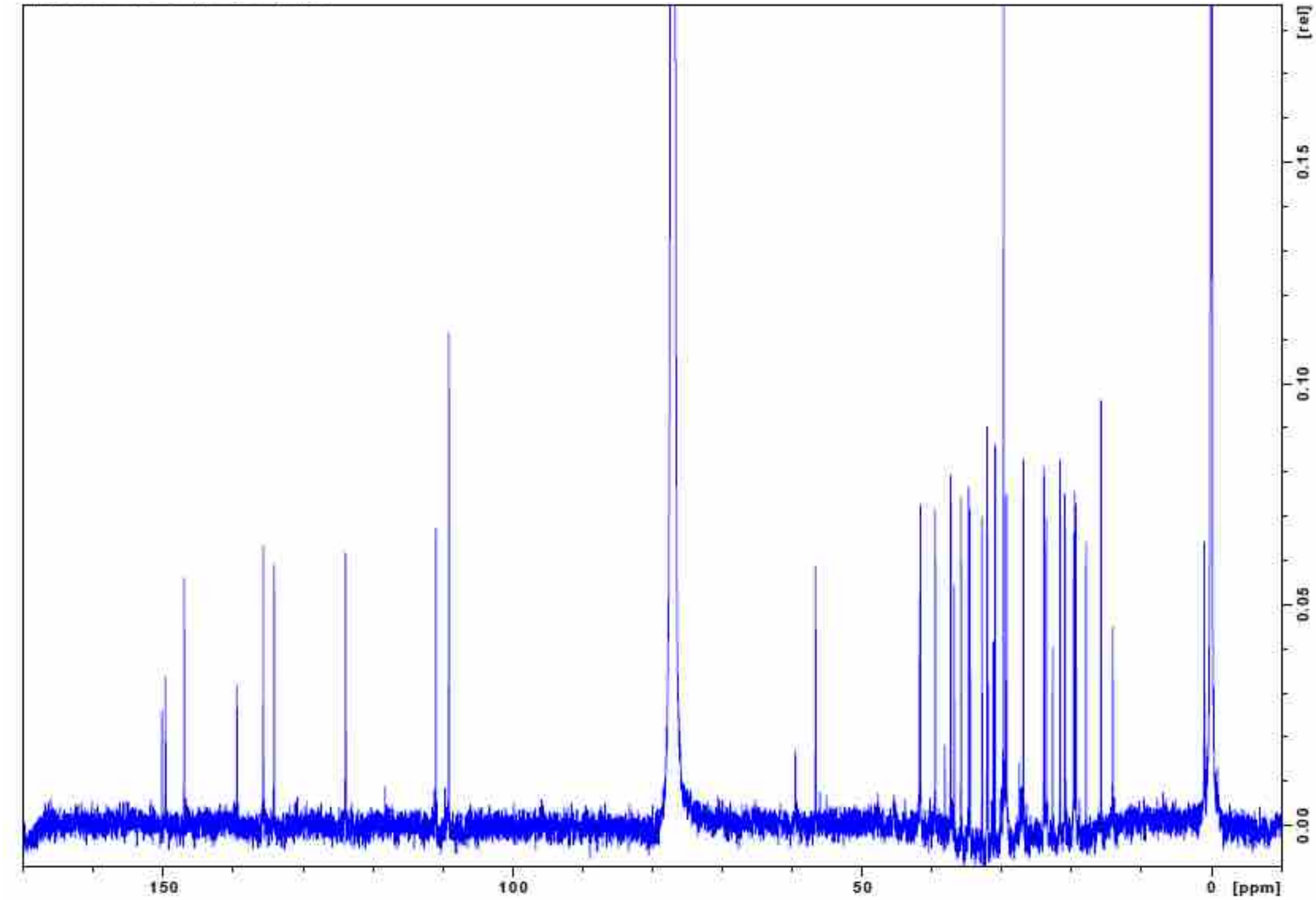


Figure S16. 1D ^{13}C NMR spectra for **6.**

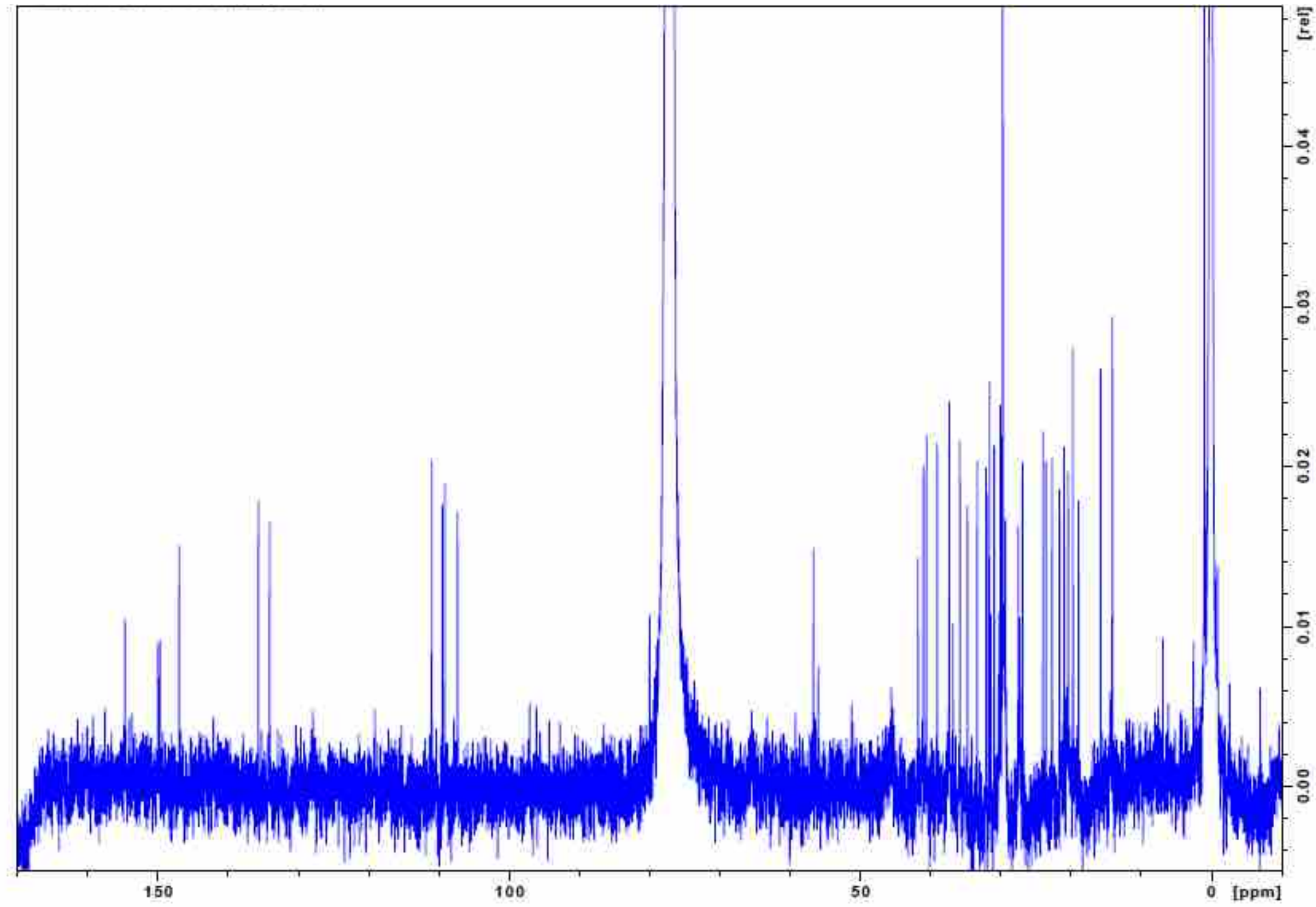


Figure S17. 1D ^{13}C NMR spectra for **7.**

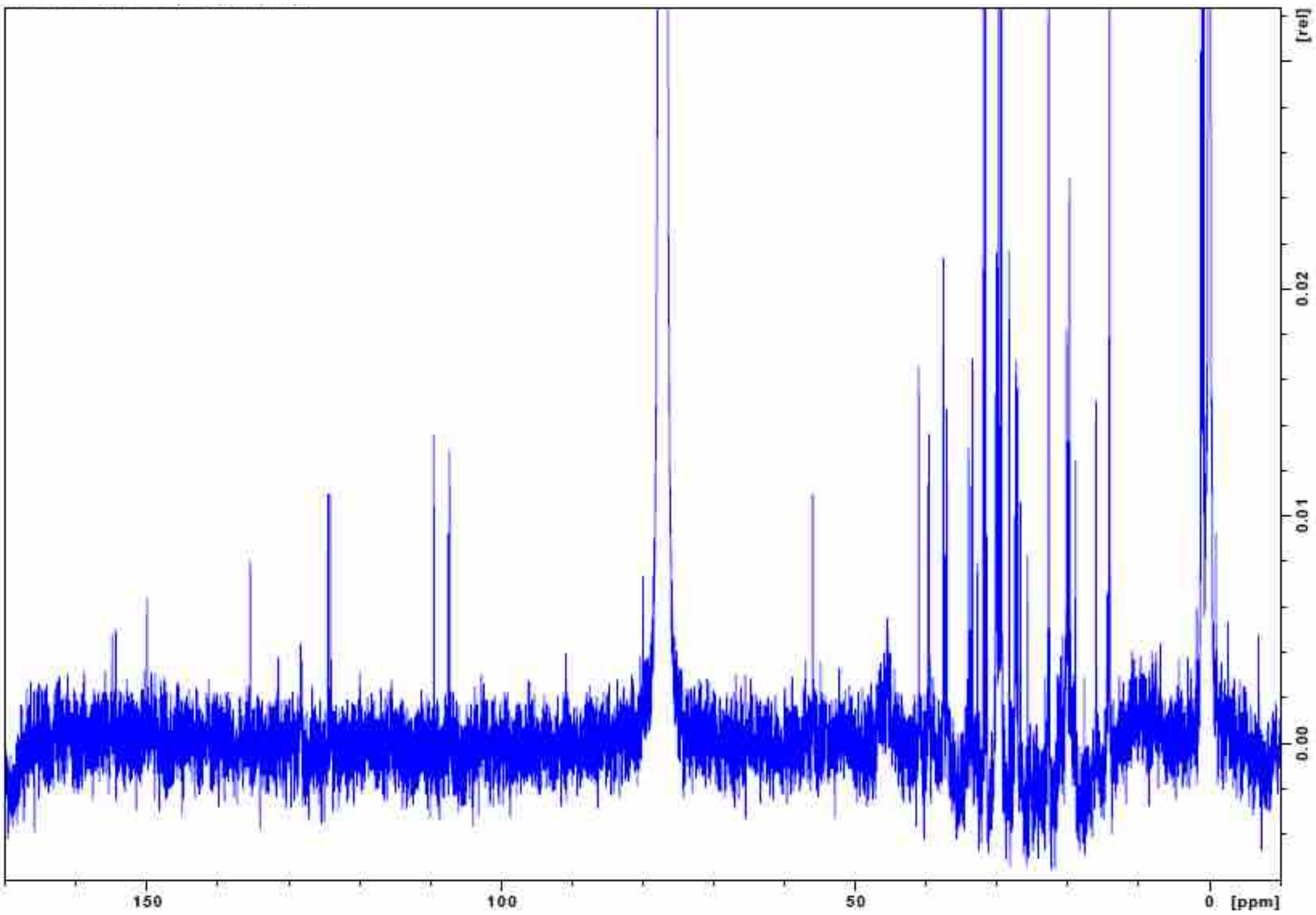


Figure S18. 1D ^{13}C NMR spectra for **8.**

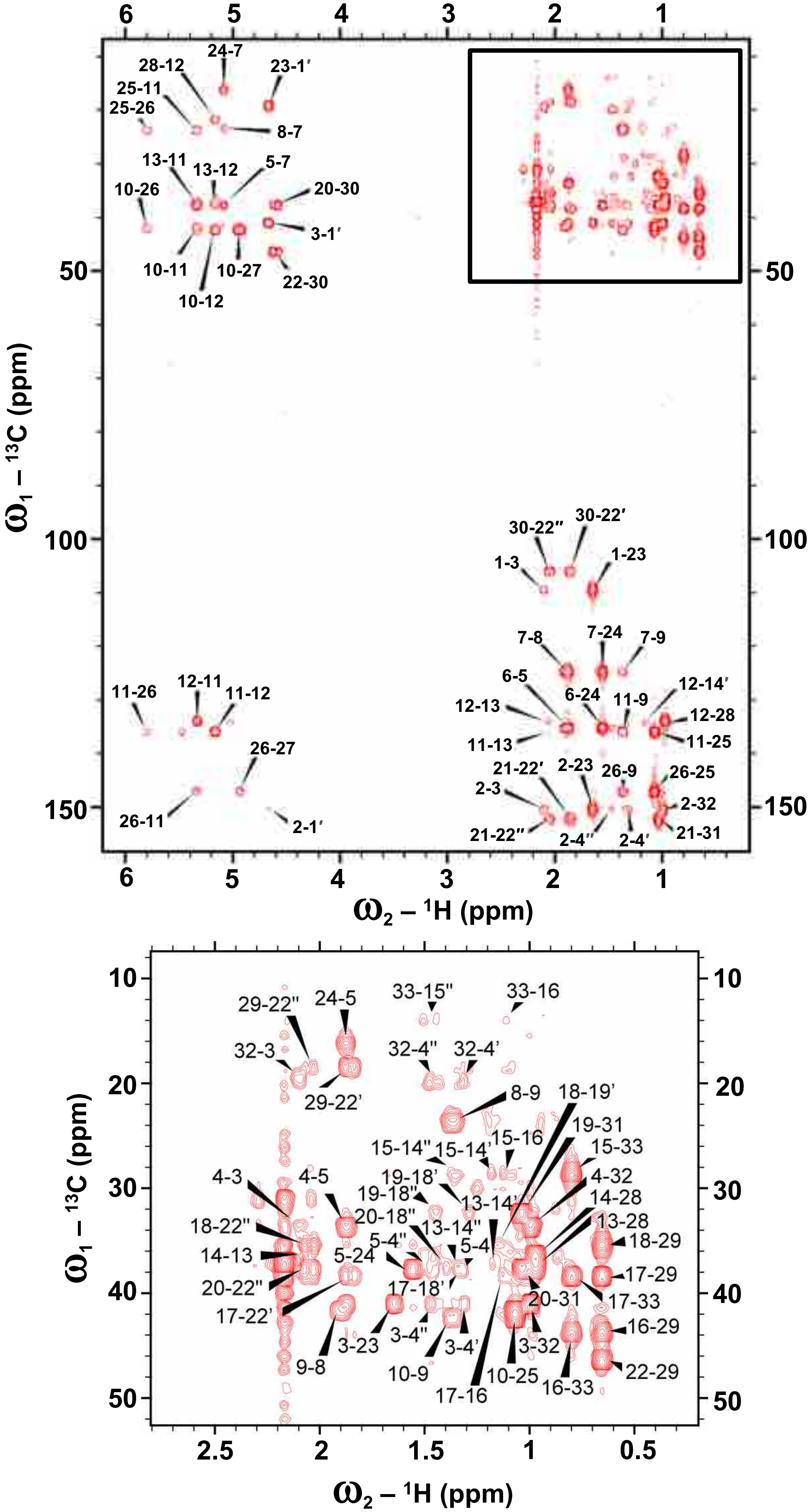


Figure S19. ^{13}C-HMBC spectrum acquired on **5**. The bottom panel is a blow up of the boxed region in the top panel.

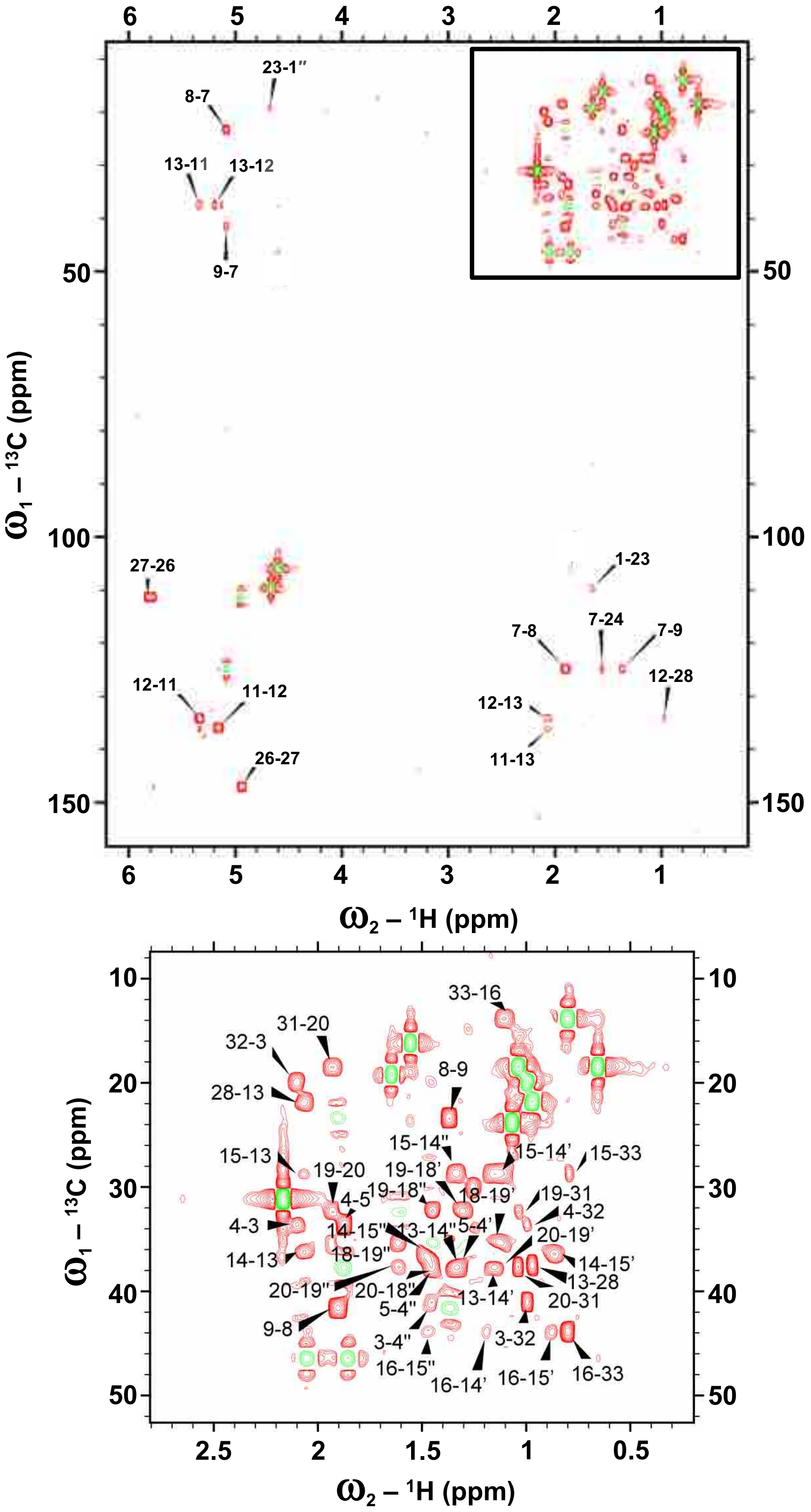


Figure S20. ^{13}C-TOCSY-HSQC spectrum acquired on **5**. The bottom panel is a blow up of the boxed region in the top panel.

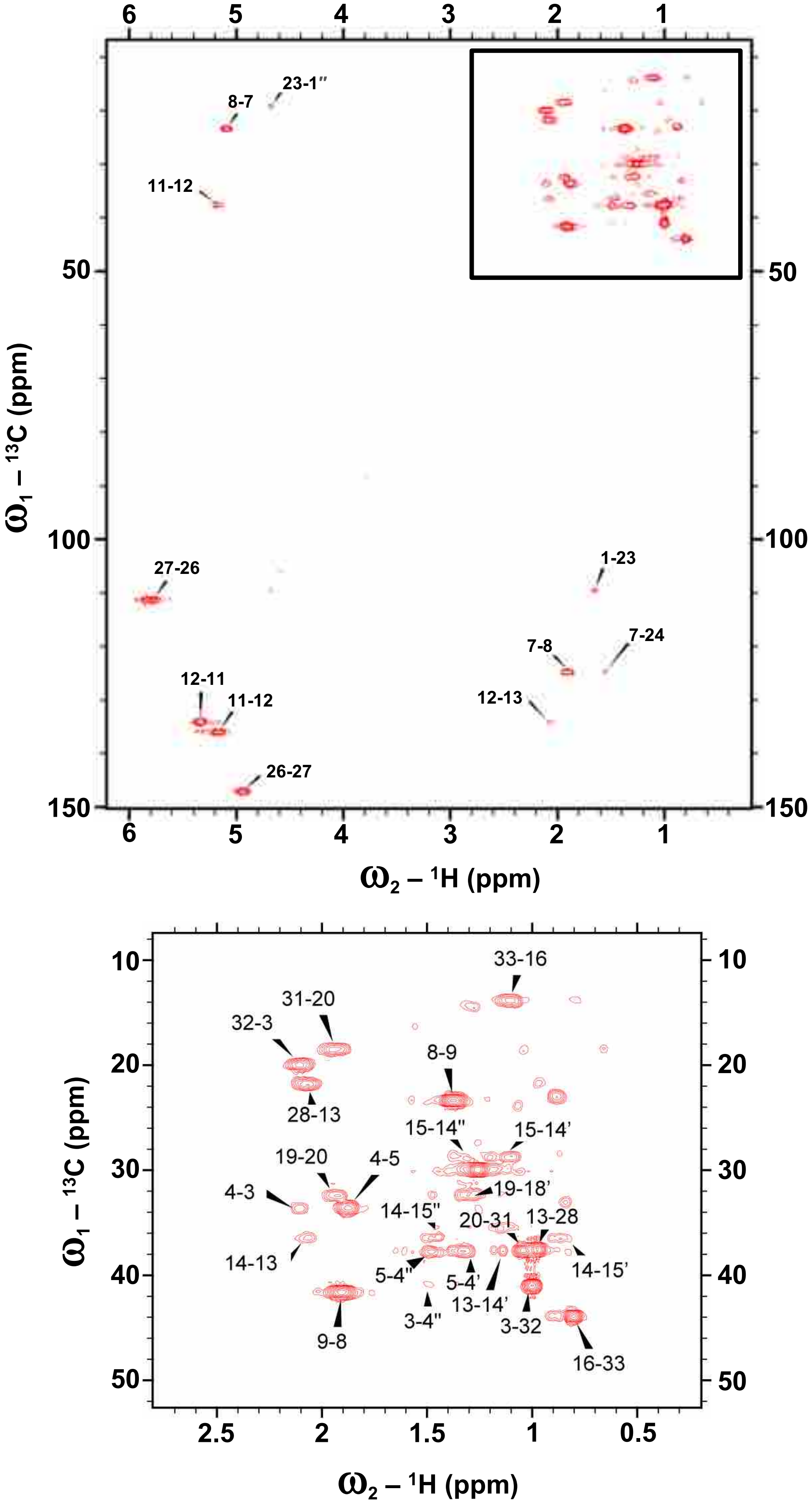


Figure S21. ^{13}C-H2BC spectrum acquired on **5**. The bottom panel is a blow up of the boxed region in the top panel.

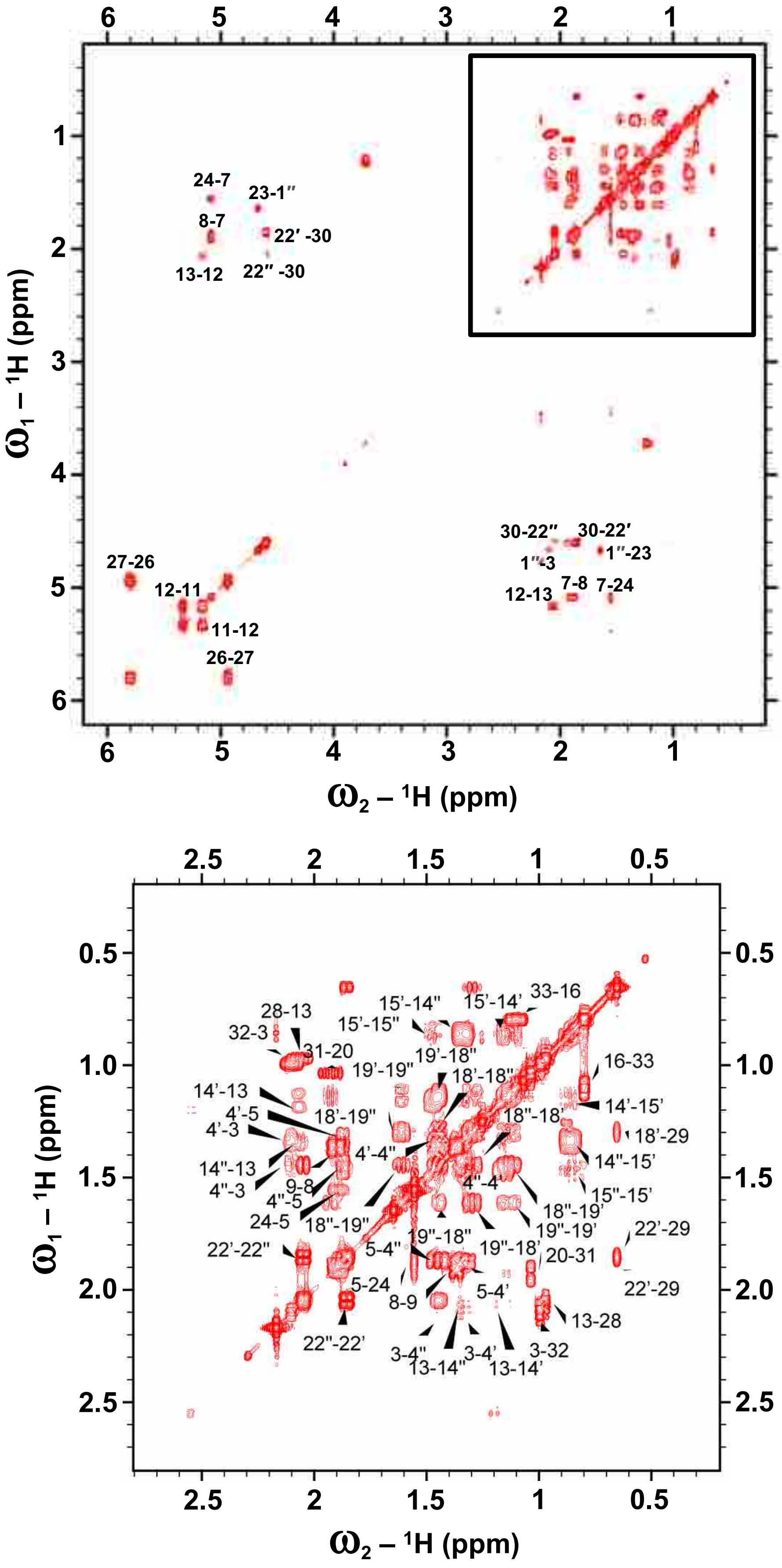
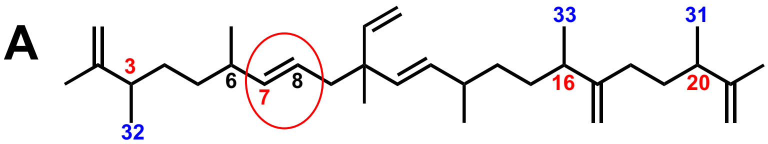
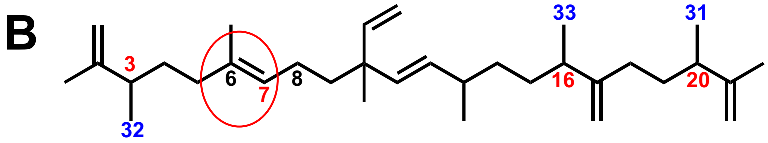


Figure S22. ^{1}H-COSY spectrum acquired on **5**. The bottom panel is a blow up of the boxed region in the top panel.



Incorrect C_{33} botryococcene structure, molecule 3-2, as reported in Okada, S.; Murakami, M.; Yamaguchi, K. *Phytochemical Analysis* **1997**, *8,* 198–203.



Corrected C_{33} botryococcene structure, molecule 3-2. All NMR chemical shifts reported in Okada, S.; Murakami, M.; Yamaguchi, K. *Phytochemical Analysis* **1997**, *8,* 198–203 agree with this structure.

Figure S23. Correction of C_{33} botryococcene, molecule 3-2, structure reported in Okada, S.; Murakami, M.; Yamaguchi, K. *Phytochemical Analysis* **1997**, *8,* 198–203. (A) The incorrect structure. (B) The corrected structure.

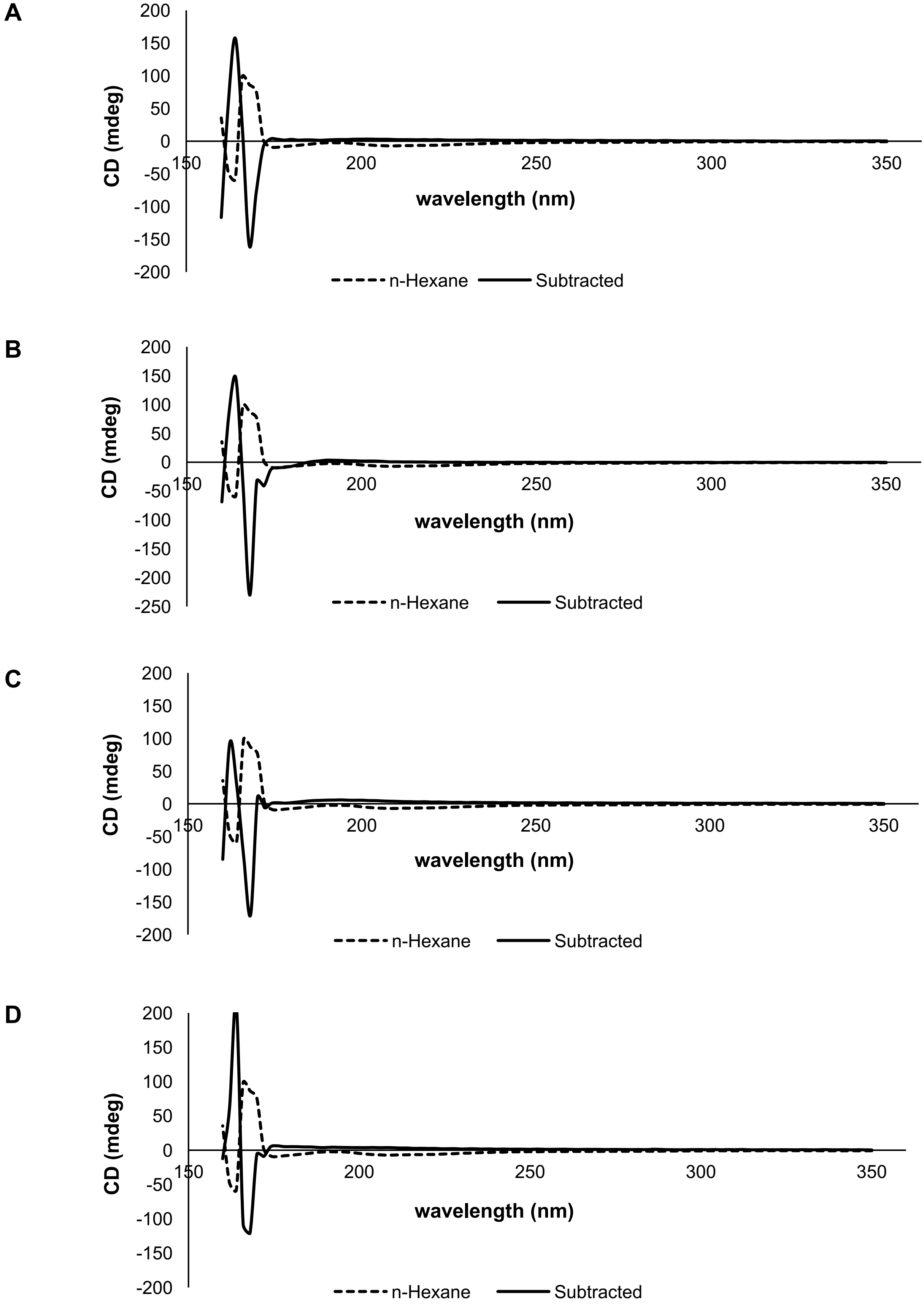


Figure S24. ECD spectra for molecules **5** (A), **6** (B), **7** (C), and **8** (D).

Supporting Methods

NMR Analysis. All NMR experiments were carried out at 25 ° C using 500 or 800 MHz Bruker Avance III HD spectrometers equipped with a 5 mm inverse detection TXI probe. Analysis was performed in 5 mm NMR tubes by dissolving purified hydrocarbon samples in deuterated chloroform with 0.03% (v/v) trimethylsilane as an internal standard. The typical sample concentrations ranged from 0.5 to 2 mg/mL and samples were $\geq$95% pure with residual *n*-hexane being the most common impurity. The 1D ^{1}H and ^{13}C spectra were acquired on the 800 MHz spectrometer as 16,384 and 65,536 complex points, respectively. The 2D NMR experiments were acquired on the 500 MHz spectrometer as a matrix of 2,048 x 256 complex points using standard pulse-programs provided by the instrument manufacturer. The resultant data was processed using the Topspin software version 3.2 and analyzed using the Sparky software version 3.115. HSQC spectra were acquired in multiplicity edited mode to derive additional information about the number of protons attached to the respective carbons. The spectra were also acquired at narrower spectral width covering only the aliphatic region such that the olefinic resonances were aliased in the indirect dimension and their true chemical shift was calculated by adding multiples of spectral width to the observed chemical shift.[23] This setup allowed for the acquisition of HSQC at much higher digital resolution. For the molecules **5-8**, short-range, three or four bond correlations were obtained by magnitude mode COSY and H2BC experiments. This was supported by long connectivities established using HMBC and HSQCTOCSY. The HMBC was performed using a long-range coupling constant of 8 Hz, and had a low-pass J-filter to suppress one-bond correlations. The HSQC-TOCSY was acquired with one-bond correlations appearing anti-phase to TOCSY peaks and a mixing time of 18 ms. The 1D NOE data was acquired with a 450 ms mixing time and a selective shape pulse. The homonuclear *J*-couplings were derived by 1D selective TOCSY whereas heteronuclear *J*-couplings were obtained by removing the decoupling scheme from HSQC.