

SUPPLEMENTARY MATERIAL

Asperinsuterpenes A – C from the fungus *Aspergillus insuetus* BTBU20220155

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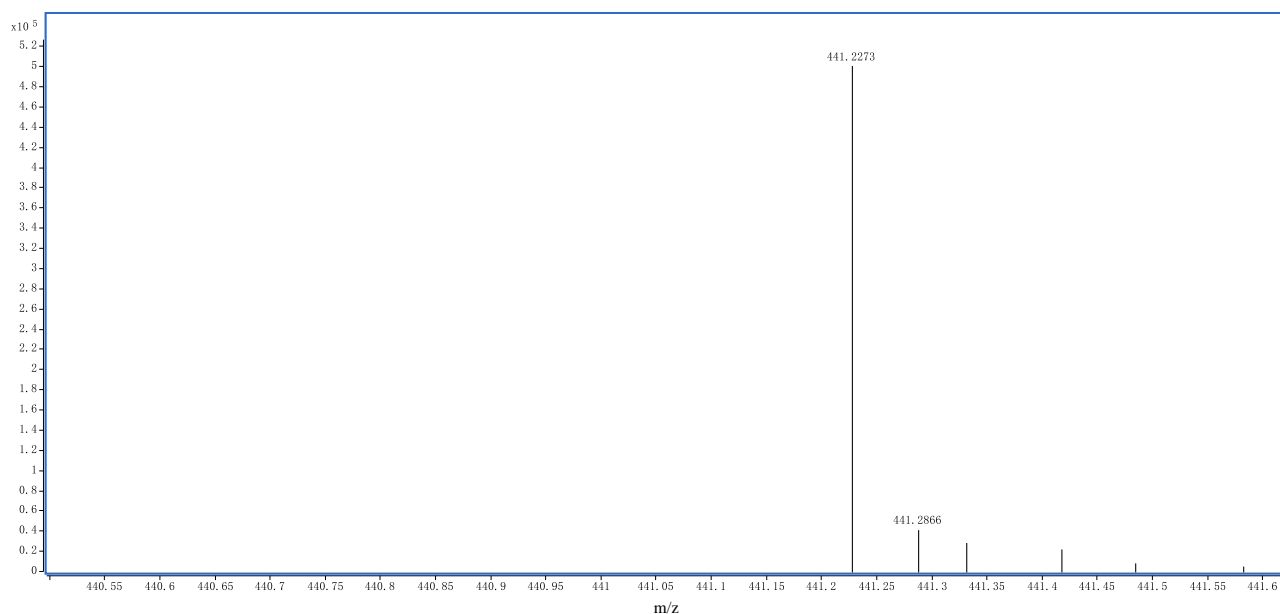


Figure S1. HRESIMS spectrum for **1**

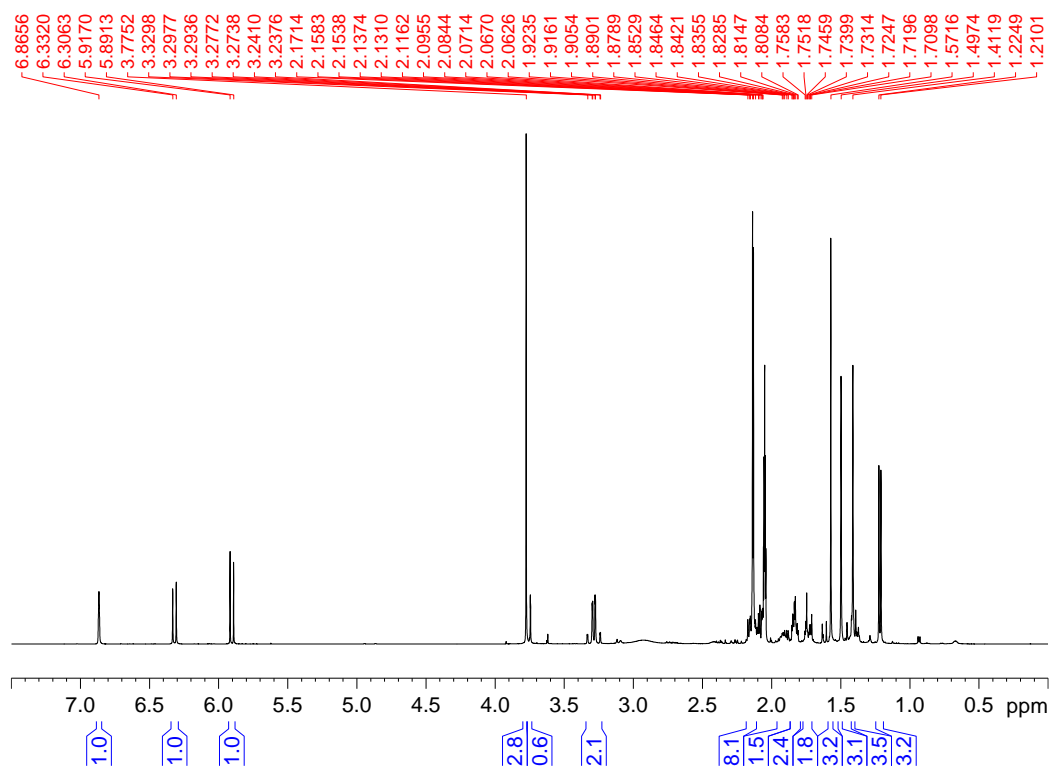


Figure S2. ¹H NMR spectrum (500 MHz, Acetone-*d*₆) of **1**

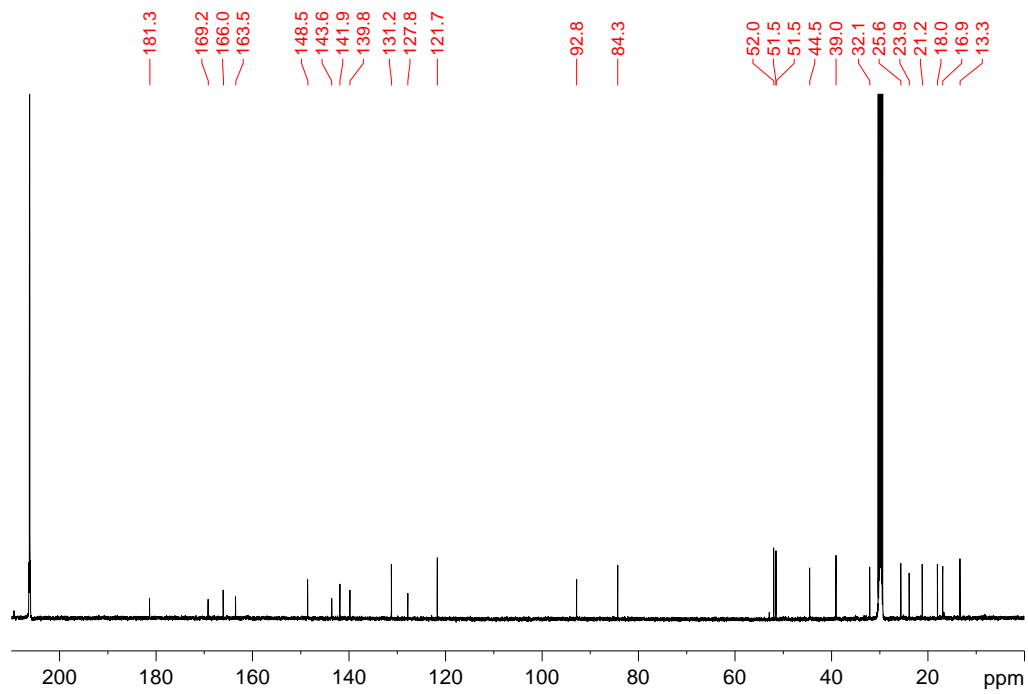


Figure S3. ¹³C NMR spectrum (125 MHz, Acetone-*d*₆) of **1**

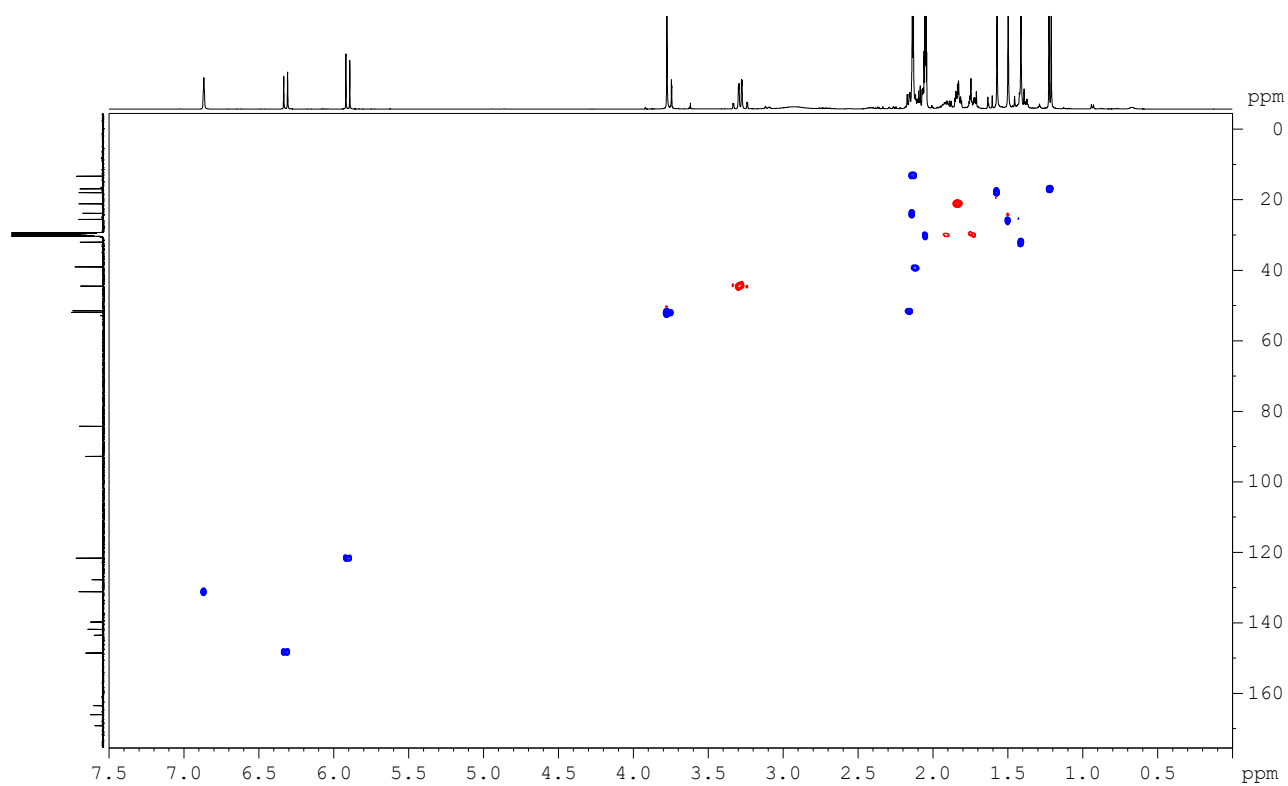


Figure S4. HSQC spectrum (500 MHz, Acetone- d_6) of **1**

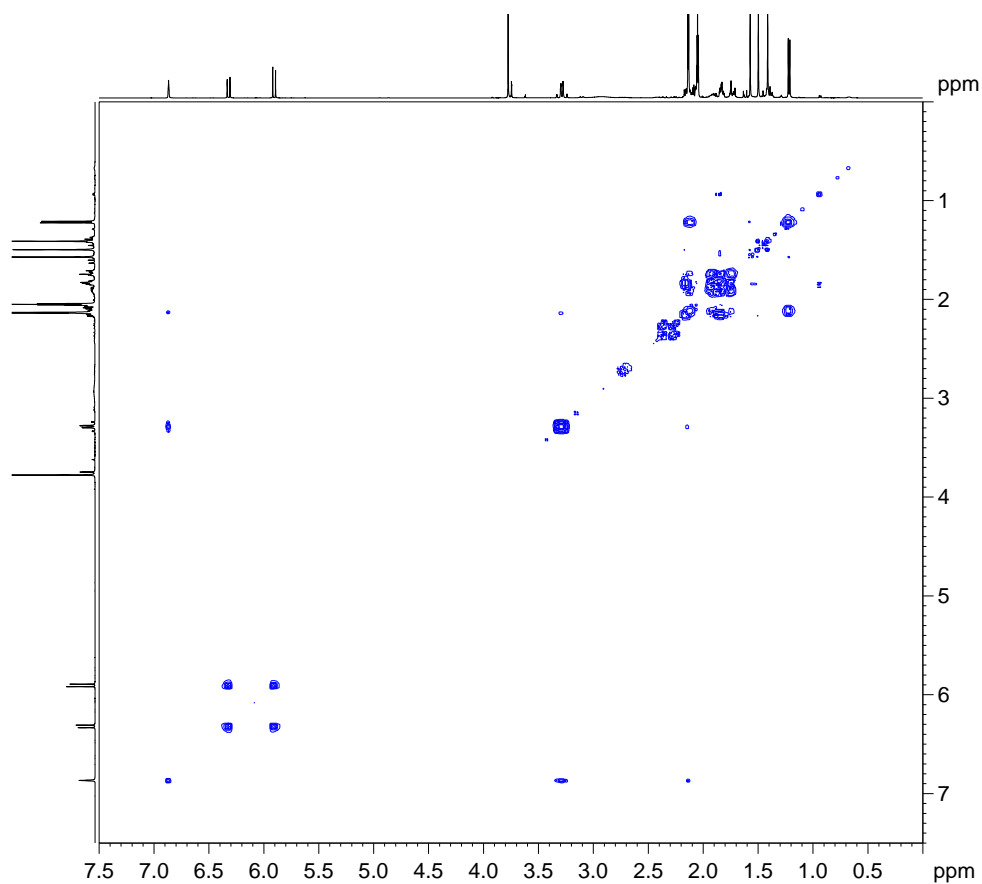


Figure S5. ^1H - ^1H COSY spectrum (500 MHz, Acetone- d_6) of **1**

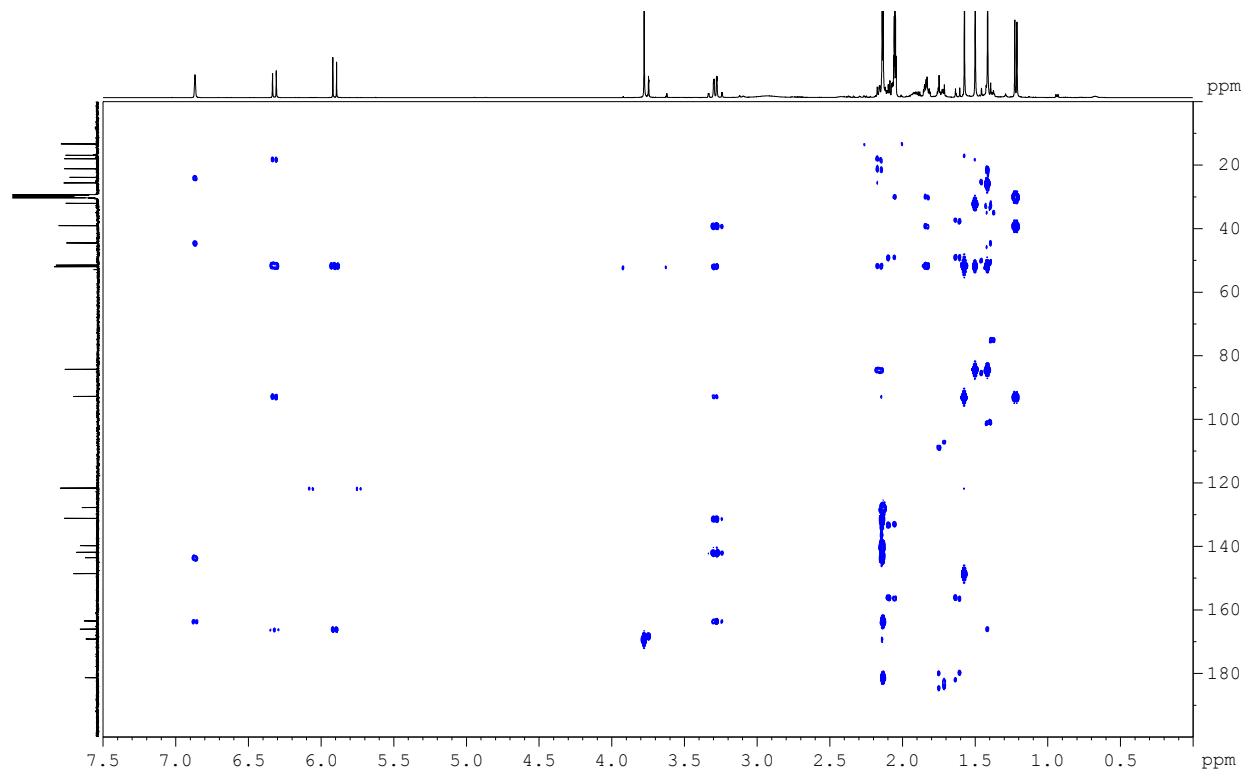


Figure S6. HMBC spectrum (500 MHz, Acetone- d_6) of **1**

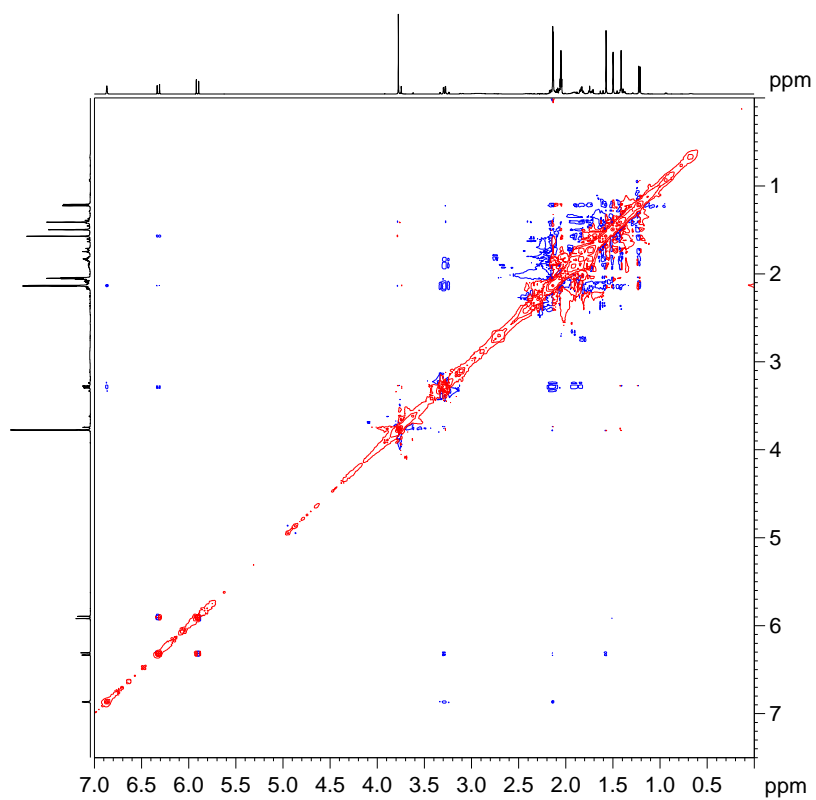


Figure S7. ROESY spectrum (500 MHz, Acetone- d_6) of **1**

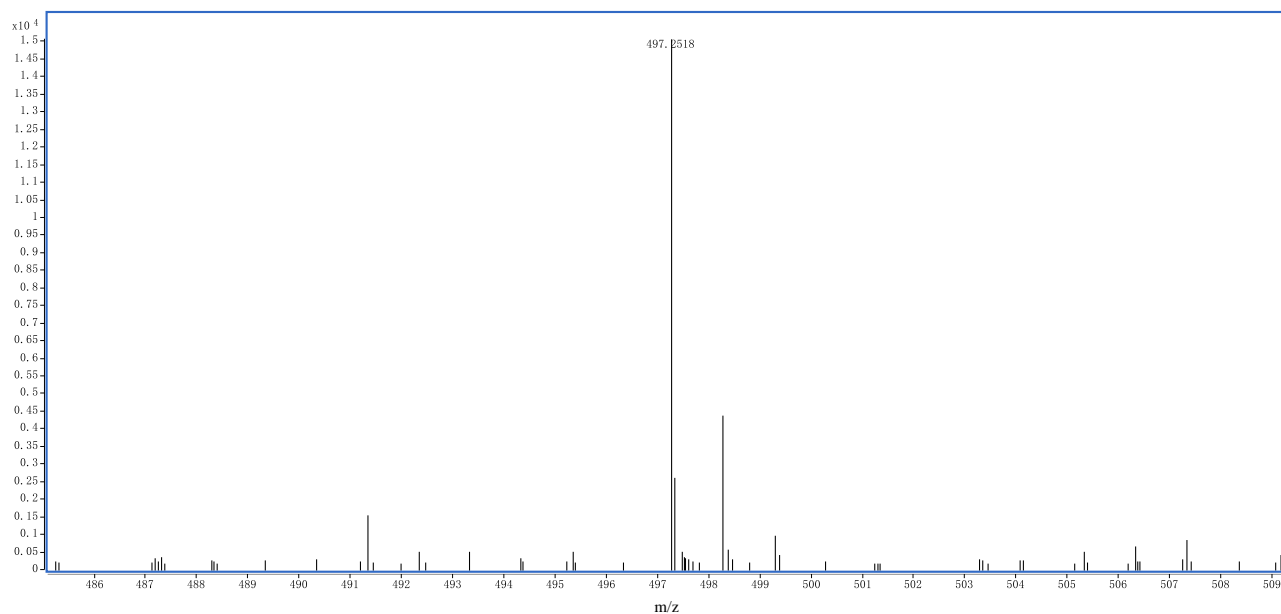


Figure S8. HRESIMS spectrum of **2**

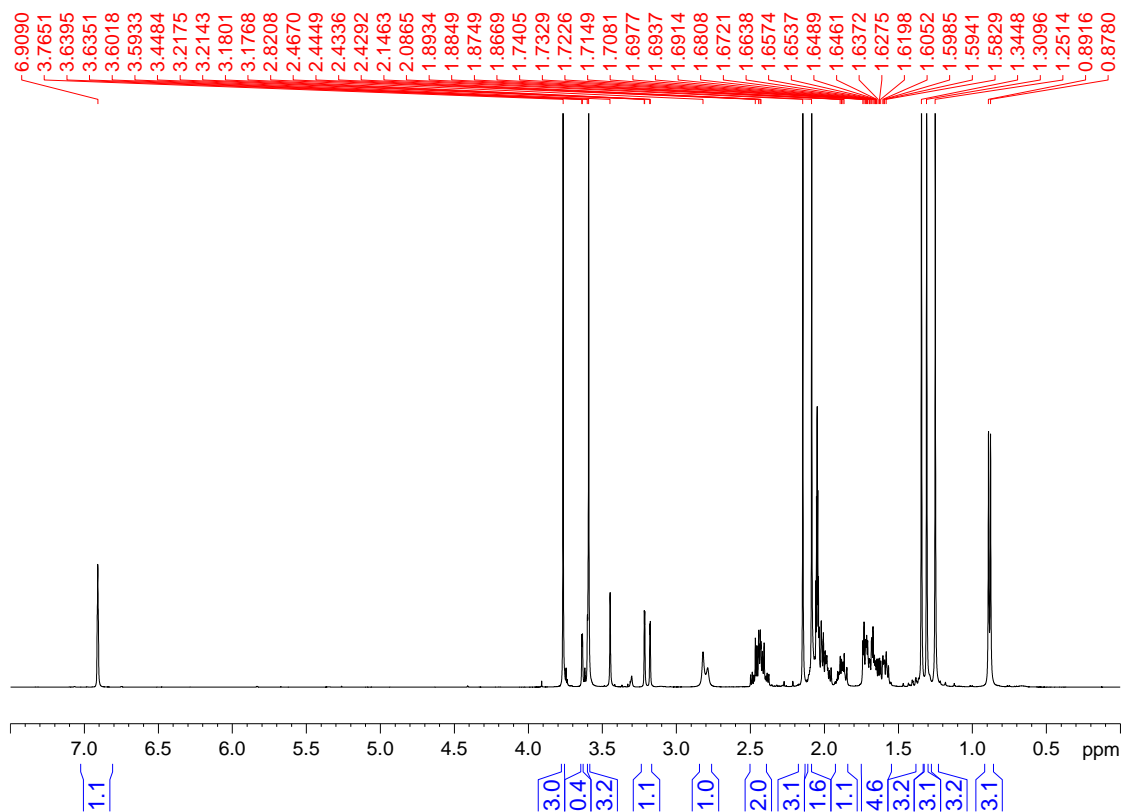


Figure S9. ^1H NMR spectrum (500 MHz, Acetone- d_6) of **2**

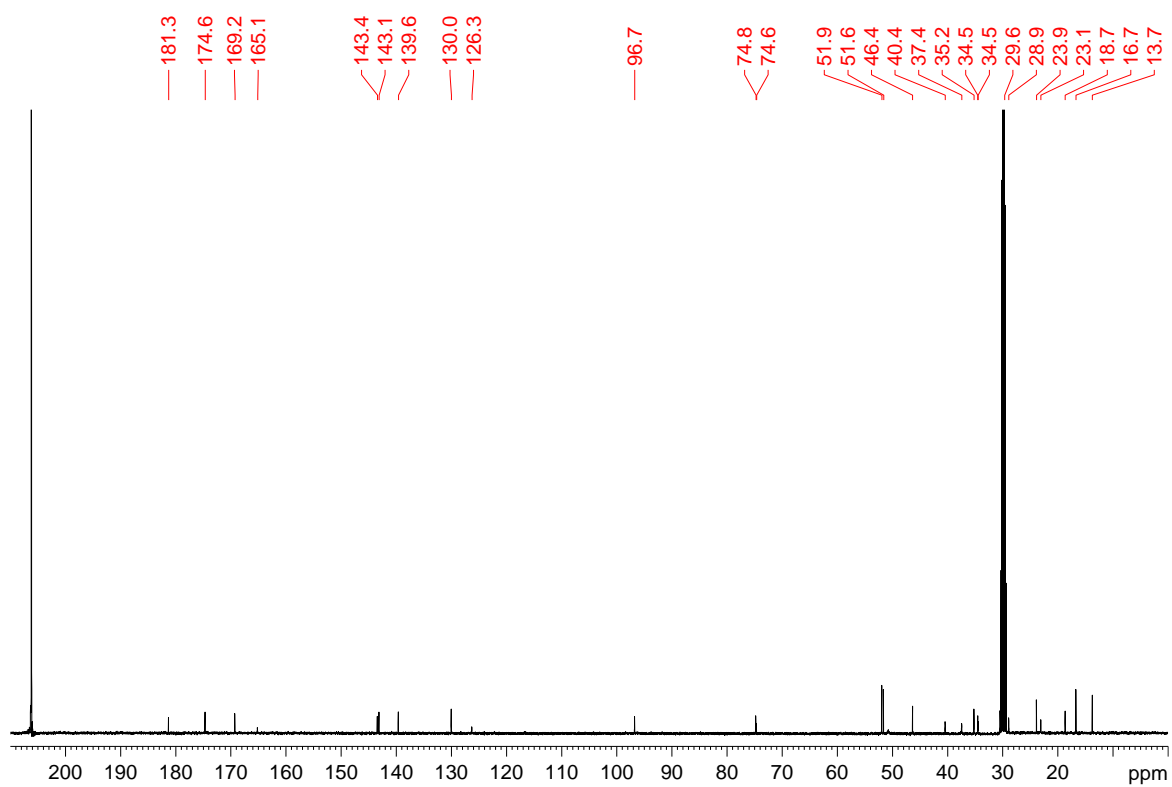


Figure S10. ¹³C NMR spectrum (125 MHz, Acetone-*d*₆) of **2**

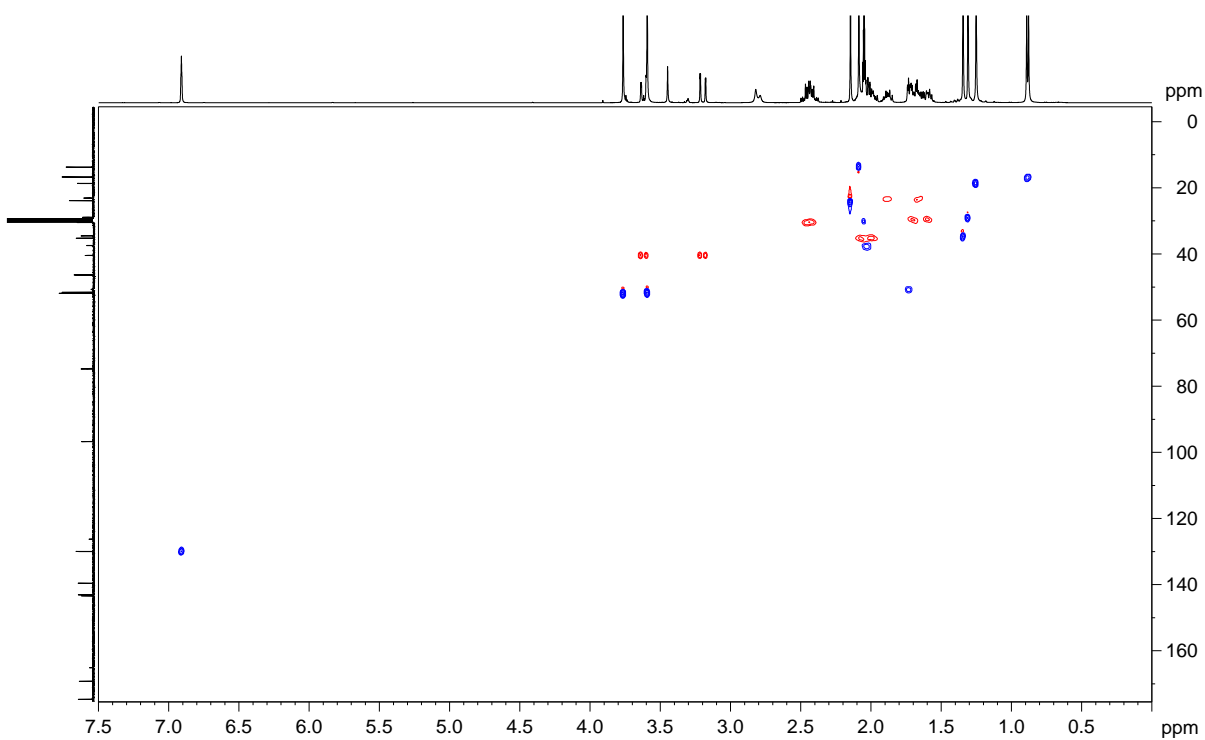


Figure S11. HSQC spectrum (500 MHz, Acetone-*d*₆) of **2**

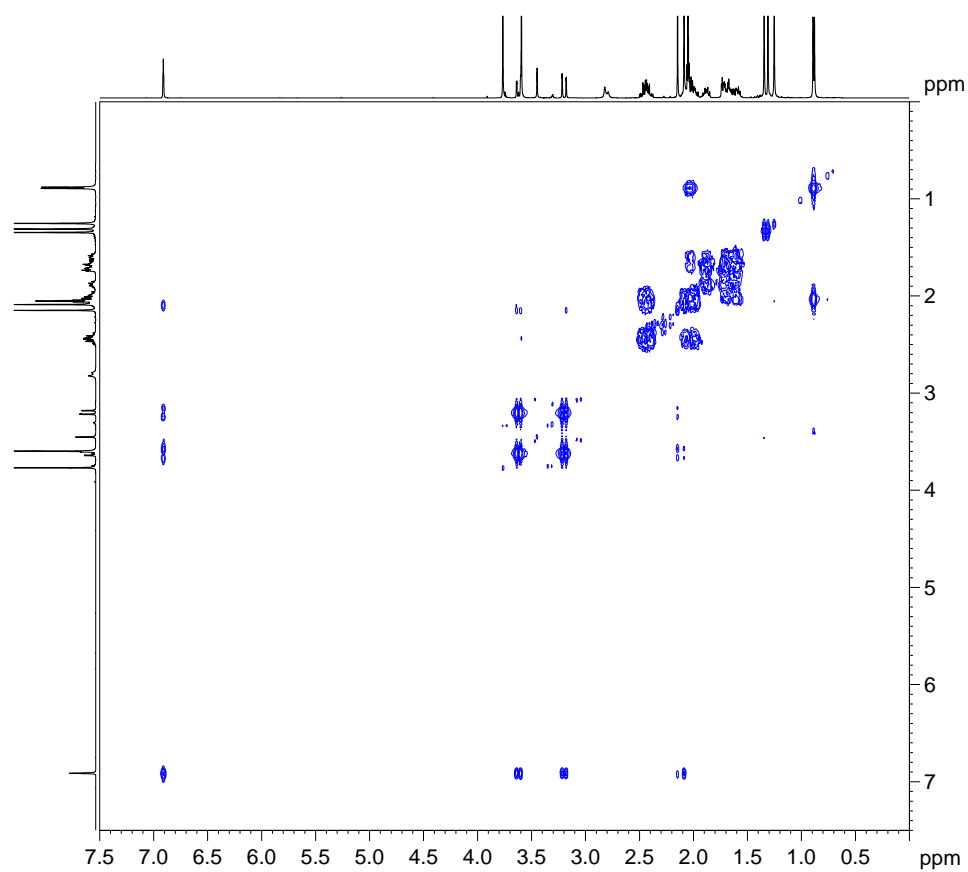


Figure S12. ^1H - ^1H COSY spectrum (500 MHz, Acetone- d_6) of **2**

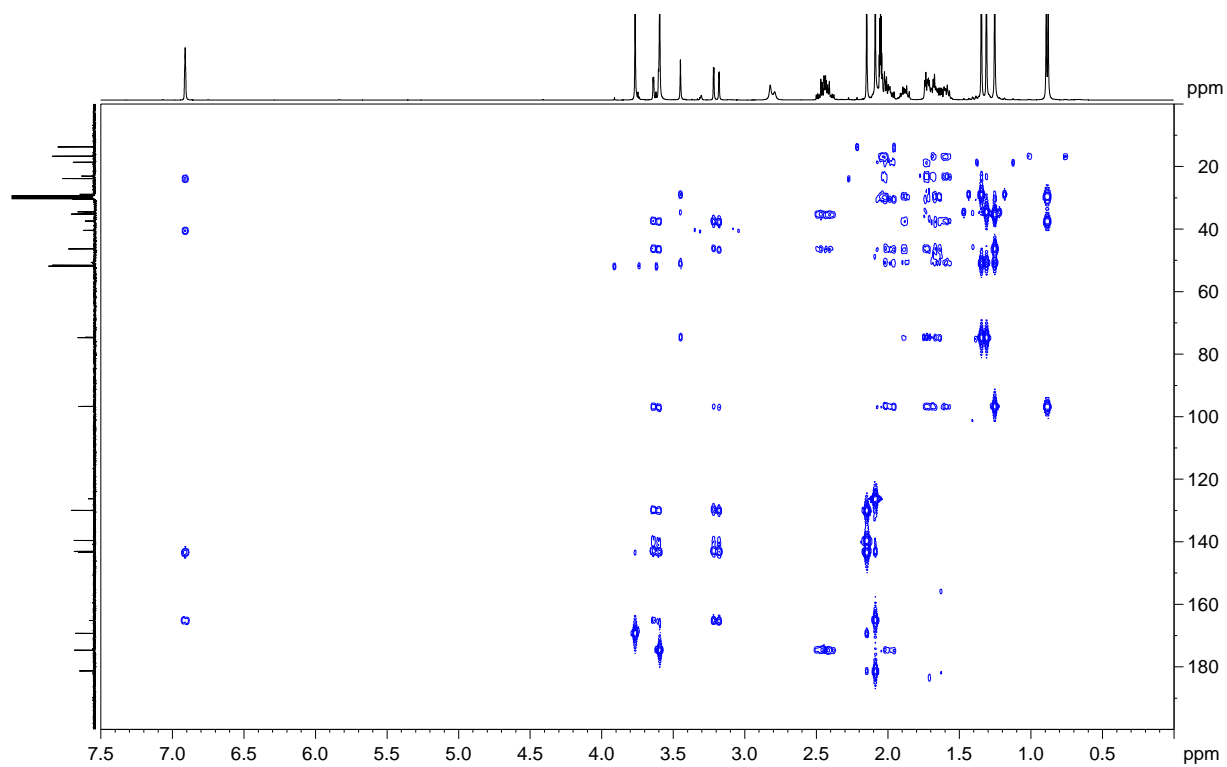


Figure S13. HMBC spectrum (500 MHz, Acetone- d_6) of **2**

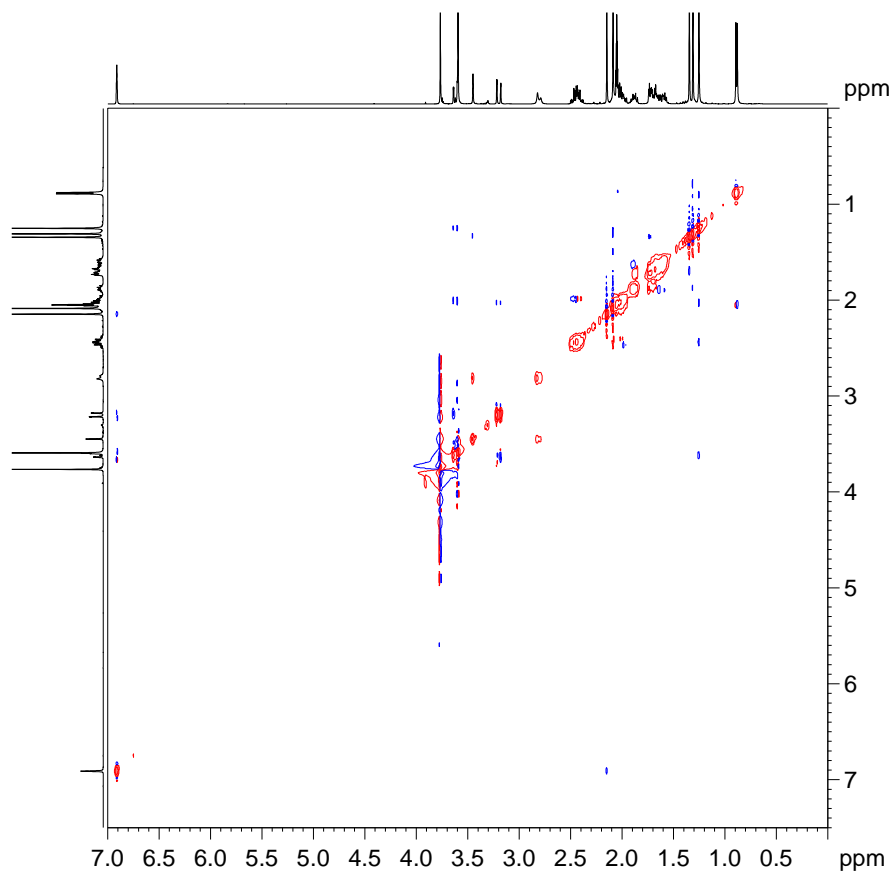


Figure S14. ROESY spectrum (500 MHz, Acetone- d_6) of **2**

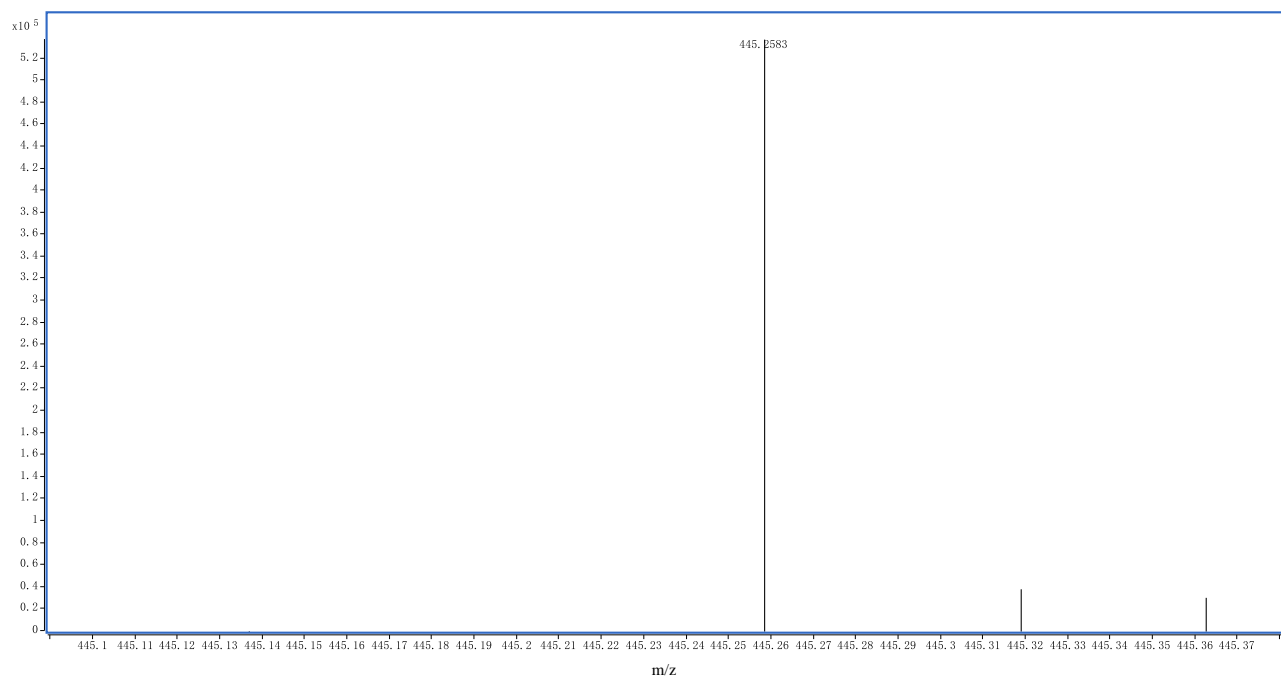


Figure S15. HRESIMS spectrum of **3**

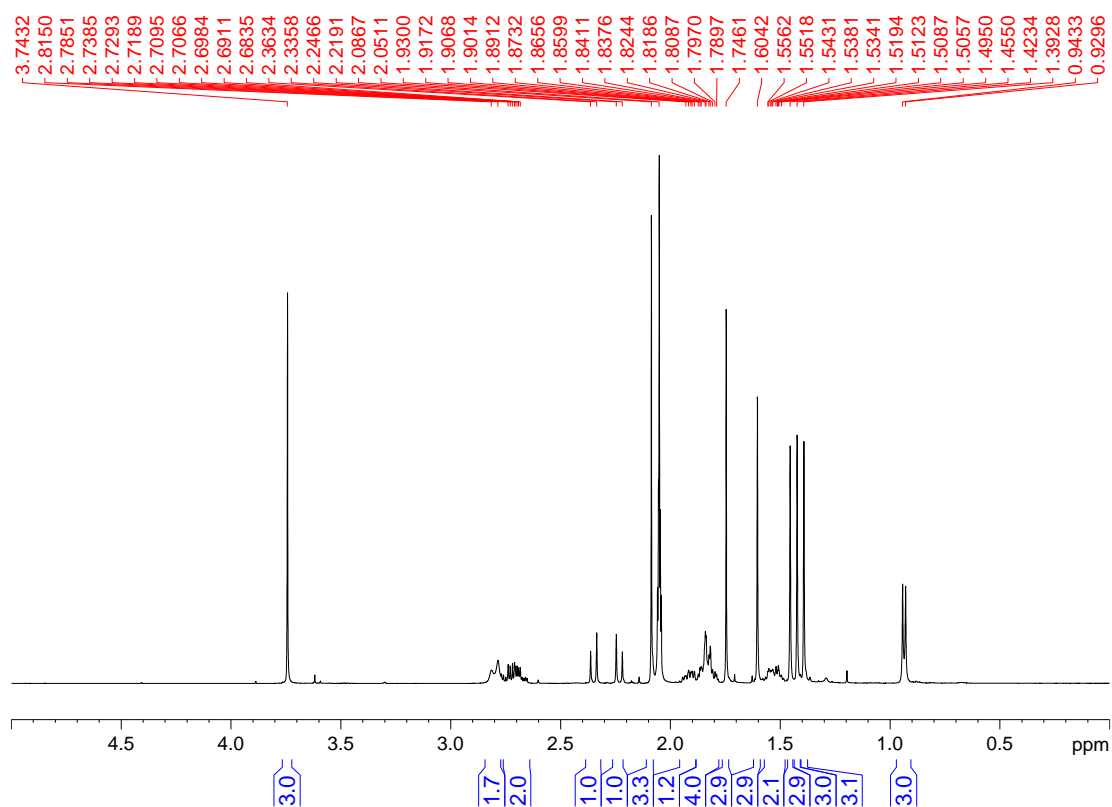


Figure S16. ¹H NMR spectrum (500 MHz, Acetone-*d*₆) of **3**

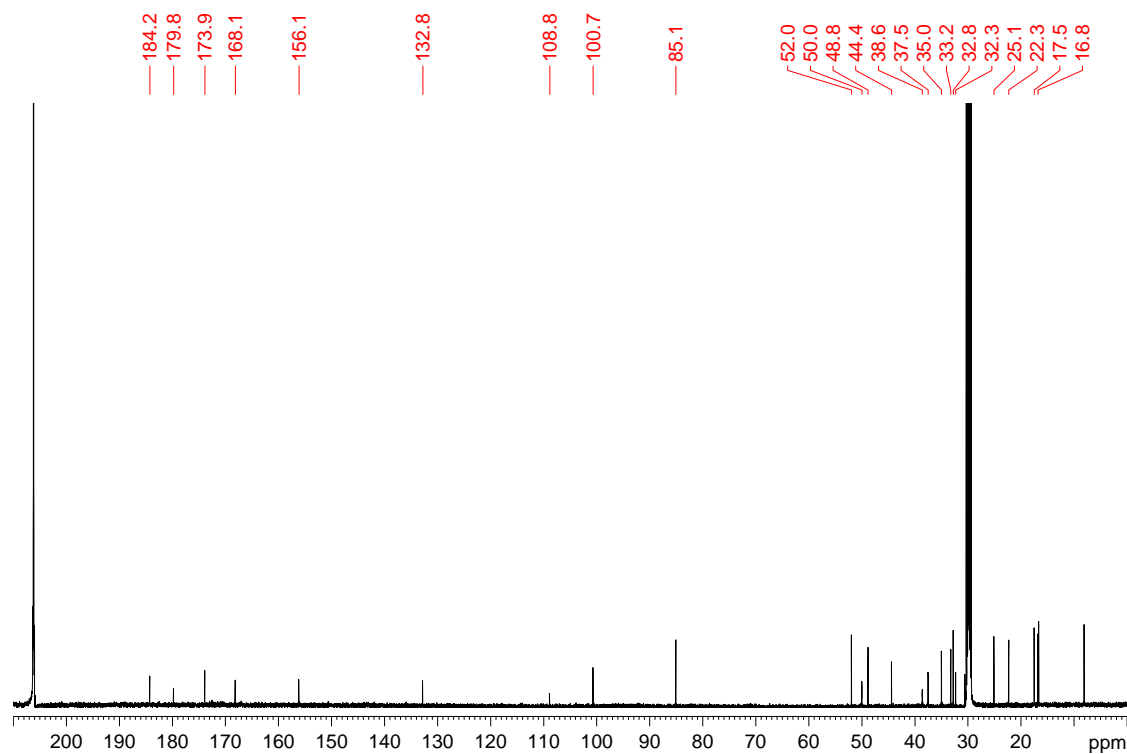


Figure S17. ¹³C NMR spectrum (125 MHz, Acetone-*d*₆) of **3**

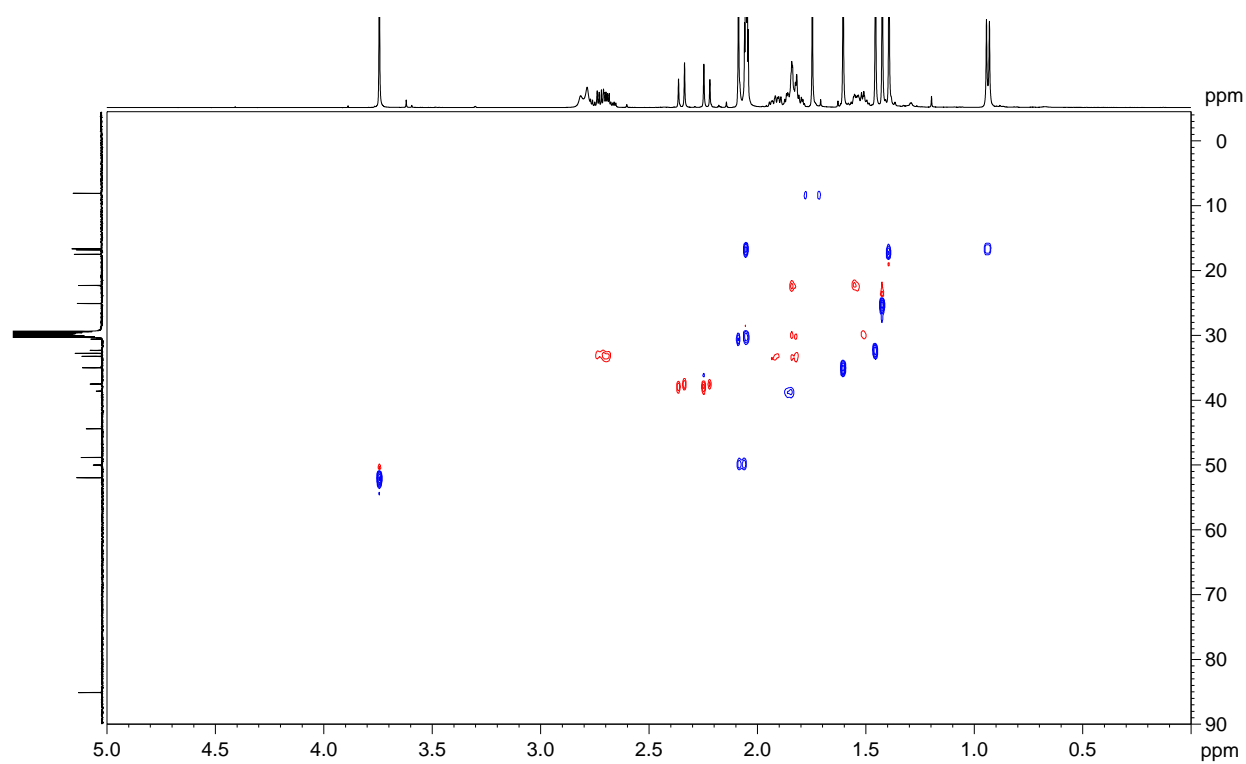


Figure S18. HSQC spectrum (500 MHz, Acetone- d_6) of **3**

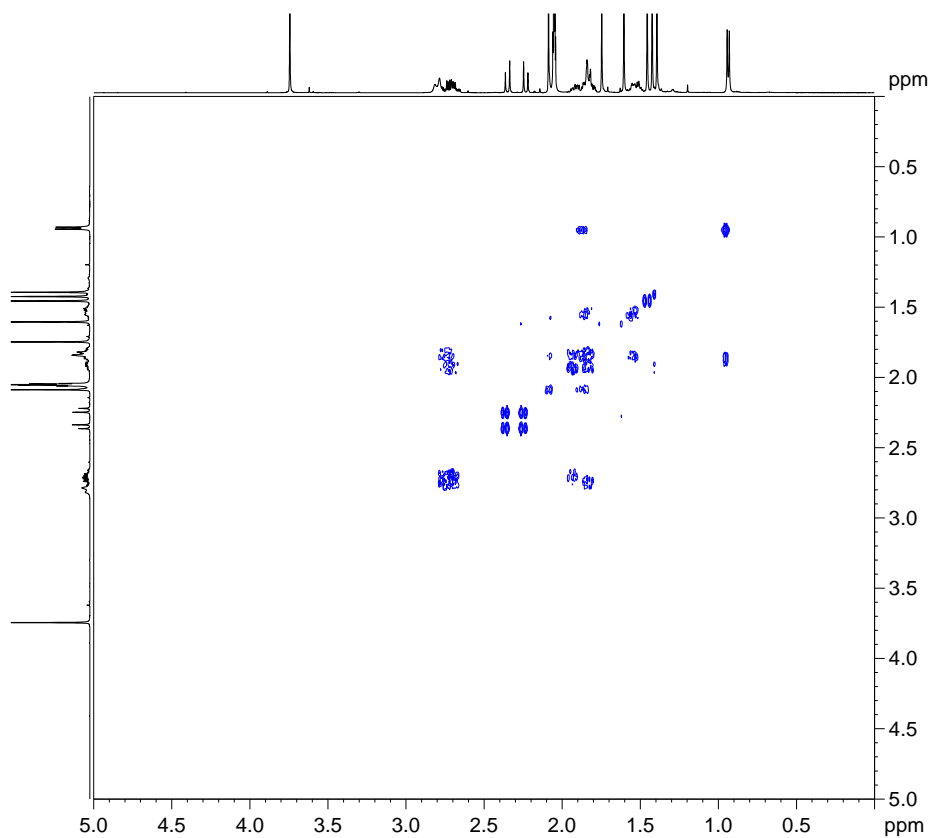


Figure S19. ^1H - ^1H COSY spectrum (500 MHz, Acetone- d_6) of **3**

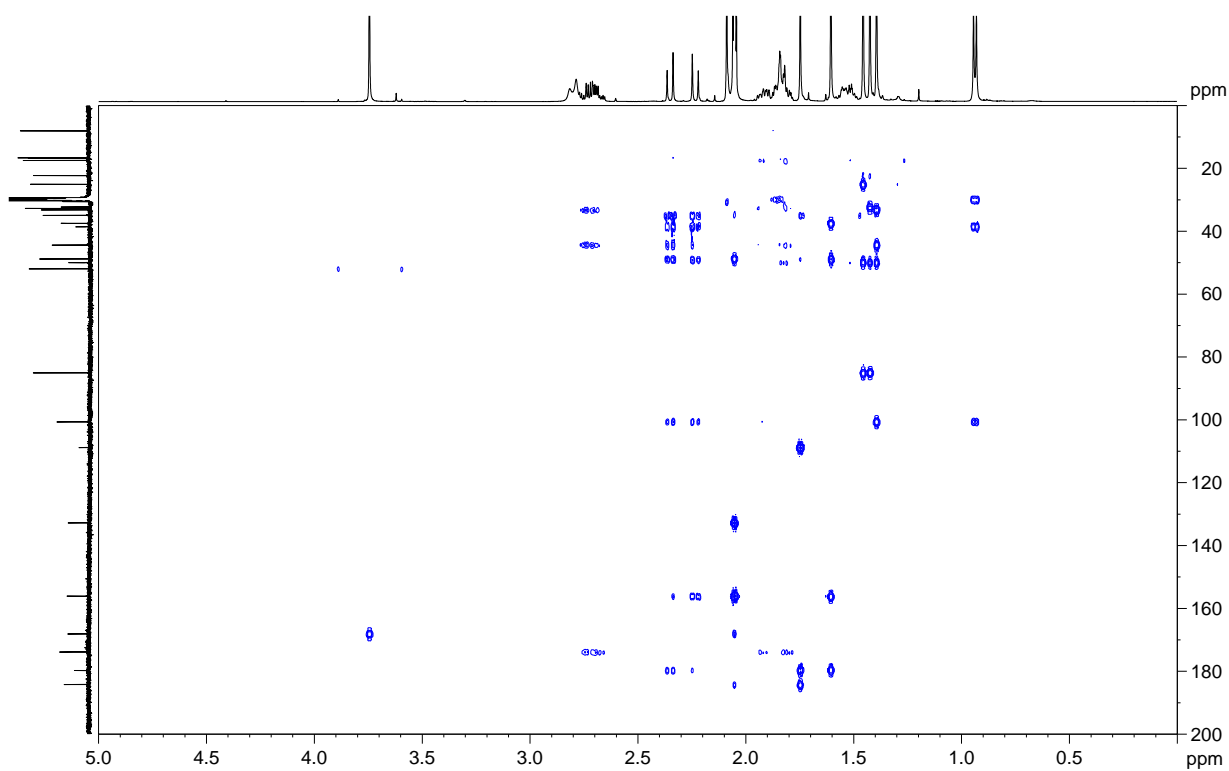


Figure S20. HMBC spectrum (500 MHz, Acetone- d_6) of **3**

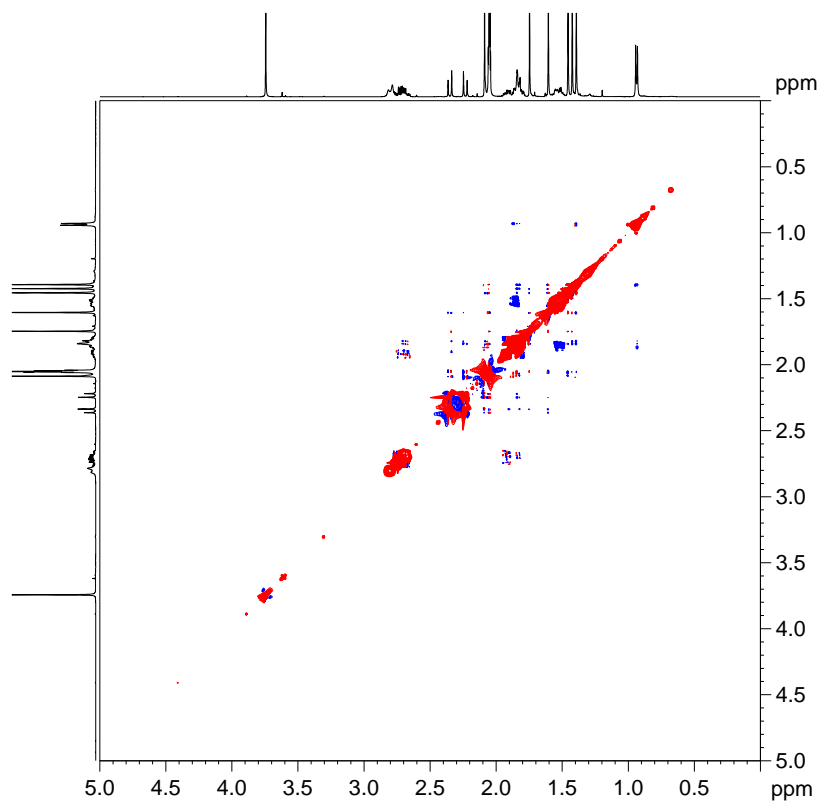


Figure S21. ROESY spectrum (500 MHz, Acetone- d_6) of **3**

Table S1. ^{13}C NMR data for compound **4**

Position	Compound 4 (Acetone- d_6)	Asnovolin I (CDCl $_3$)
	δ_{C}	δ_{C}
1	34.8, CH $_2$	34.4, CH $_2$
2	29.8, CH $_2$	29.1, CH $_2$
3	174.6, C	174.2, C
4	75.0, C	75.0, C
5	49.7, CH	48.1, CH
6	23.5, CH $_2$	23.0, CH $_2$
7	29.3, CH $_2$	28.8, CH $_2$
8	39.6, CH	39.2, CH
9	101.2, C	99.8, C
10	45.6, C	45.0, C
11	37.1, CH $_2$	37.1, CH $_2$
12	48.9, C	48.1, C
13	155.9, C	155.2, C
14	133.1, C	132.4, C
15	184.2, C	184.2, C
16	106.9, C	107.9, C
17	181.7, C	179.6, C
18	35.2, CH $_3$	35.0, CH $_3$
19	16.7, CH $_3$	16.9, CH $_3$
20	168.1, C	167.6, C
21	52.0, OCH $_3$	52.4, CH $_3$
22	8.3, CH $_3$	8.3, CH $_3$
23	21.3, CH $_3$	22.8, CH $_3$
24	16.7, CH $_3$	16.3, CH $_3$
25	30.6, CH $_3$	32.4, CH $_3$
26	34.8, CH $_3$	32.8, CH $_3$
27	51.7, OCH $_3$	52.0, OCH $_3$

Table S2. ^{13}C NMR data for compound **5**

Position	Compound 5 (Acetone- d_6)	(2' <i>E</i> ,4' <i>E</i> ,6' <i>E</i>)-6-(1'-carboxyocta-2',4',6'-triene)-9-hydroxydim-7-ene-11,12-olide (DMSO- d_6)
	δ_{C}	δ_{C}
1	30.7, CH ₂	29.4, CH ₂
2	18.6, CH ₂	17.3, CH ₂
3	45.6, CH ₂	44.3, CH ₂
4	32.8, C	33.2, C
5	45.7, CH	44.1, CH
6	66.9, CH	65.7, CH
7	123.1, CH	121.3, CH
8	136.1, C	136.5, C
9	74.7, C	73.0, C
10	38.6, C	37.2, C
11	174.9, C	174.2, C
12	69.0, CH ₂	68.1, CH ₂
13	34.4, CH ₃	32.0, CH ₃
14	25.1, CH ₃	24.1, CH ₃
15	19.0, CH ₃	18.2, CH ₃
1'	166.4, C	165.2, C
2'	120.9, CH	119.5, CH
3'	146.4, CH	145.4, CH
4'	128.5, CH	127.3, CH
5'	142.6, CH	141.7, CH
6'	132.2, CH	131.3, CH
7'	137.6, CH	135.5, CH
8'	18.6, CH ₃	18.2, CH ₃

Table S3. ^{13}C NMR data for compound **6**

Position	Compound 6 (Acetone- <i>d</i> ₆)	(2' <i>E</i> ,4' <i>E</i> ,6' <i>E</i>)-6-(1'-carboxyocta-2',4',6'-triene)-11,12-epoxy-9,11-dihydroxydrim-7-ene (DMSO- <i>d</i> ₆)
	δ_c	δ_c
1	33.2, CH ₂	33.3, CH ₂
2	19.1, CH ₂	19.4, CH ₂
3	45.6, CH ₂	32.8, C
4	32.8, C	32.8, C
5	45.7, CH	45.7, CH
6	67.5, CH	68.7, CH
7	121.3, CH	121.2, CH
8	143.2, C	143.9, C
9	78.0, C	78.5, C
10	39.1, C	39.9, C
11	98.5, CH	99.5, CH
12	67.1, CH ₂	67.9, CH ₂
13	32.6, CH ₃	33.6, CH ₃
14	25.0, CH ₃	25.5, CH ₃
15	18.8, CH ₃	19.7, CH ₃
1'	166.5, C	168.4, C
2'	119.0, CH	120.2, CH
3'	146.0, CH	147.3, CH
4'	128.6, CH	128.9, CH
5'	142.4, CH	143.4, CH
6'	132.3, CH	132.8, CH
7'	135.9, CH	136.8, CH
8'	18.6, CH ₃	18.9, CH ₃

Table S4. ^{13}C NMR data for compound **7**

Position	Compound 7 (CDCl_3)	cinereain (CDCl_3)
	δ_{C}	δ_{C}
1	60.7, CH	60.7, CH
2	163.0, C	162.9, C
4	124.8, C	124.6, C
5	161.0, C	160.9, C
7	164.6, C	165.1, C
9	143.2, CH	143.1, CH
10	117.3, CH	117.2, CH
11	127.8, CH	127.6, CH
12	125.8, CH	125.7, CH
13	110.6, C	110.3, C
14	149.6, C	149.6, C
16	33.6, CH	33.4, CH
17	22.1, CH_3	22.1, CH_3
18	17.1, CH_3	17.0, CH_3
20	128.7, C	129.2, C
21	26.4, CH	26.2, CH
22	22.2, CH_3	22.1, CH_3
23	19.9, CH_3	19.7, CH_3

Table S5. ^{13}C NMR data for compound **8**

Position	Compound 8 (Acetone- d_6)	carnequinazoline A (CDCl ₃)
	δ_{C}	δ_{C}
1	164.6, C	164.6, C
3	126.0, C	125.6, C
4	146.6, C	144.9, C
6	148.1, C	147.2, C
7	128.0, CH	127.5, CH
8	135.1, CH	134.7, CH
9	127.1, CH	126.9, CH
10	127.1, CH	127.0, CH
11	120.8, C	120.2, C
12	160.9, C	160.8, C
14	60.8, CH	60.3, CH
15	33.6, CH	33.5, CH
16	17.8, CH ₃	17.5, CH ₃
17	19.7, CH ₃	19.7, CH ₃
18	126.1, CH	125.5, CH
19	26.1, CH	26.3, CH
20	22.3, CH ₃	22.2, CH ₃
21	22.1, CH ₃	22.1, CH ₃

Table S6. ^{13}C NMR data for compound **9**

Position	Compound 9 (CDCl_3)	carnequinazoline B (CDCl_3)
	δ_{C}	δ_{C}
1	165.3, C	165.0, C
3	125.4, C	125.4, C
4	144.6, C	144.4, C
6	135.7, C	135.6, C
7	151.5, C	151.4, C
8	117.7, CH	117.3, CH
9	128.1, CH	127.9, CH
10	117.6, CH	117.5, CH
11	120.2, C	120.1, C
12	160.5, C	160.3, C
14	60.5, CH	60.4, CH
15	33.7, CH	33.5, CH
16	17.6, CH_3	17.5, CH_3
17	19.9, CH_3	19.7, CH_3
18	126.6, CH	126.1, CH
19	26.3, CH	26.2, CH
20	22.4, CH_3	22.2, CH_3
21	22.3, CH_3	22.1, CH_3

Table S7. ^{13}C NMR data for compound **10**

Position	Compound 10 (CDCl_3)	carnemycin B (CD_3OD)
	δ_{C}	δ_{C}
1	159.2, C	164.8, C
2	110.4, C	113.1, C
3	159.4, C	163.0, C
4	109.7, CH	106.3, CH
5	142.9, C	148.4, C
6	108.0, C	111.3, C
7	170.3, C	173.4, C
8	34.7, CH_2	36.4, CH_2
9	33.9, CH_2	36.3, CH_2
10	131.2, CH	132.8, CH
11	130.7, CH	132.7, CH
12	130.4, CH	132.5, CH
13	132.3, CH	134.0, CH
14	34.1, CH_2	36.4, CH_2
15	22.0, CH_2	24.3, CH_2
16	13.6, CH_3	14.6, CH_3
17	51.9, CH_3	53.0, CH_3
1'	74.2, CH	76.3, CH
2'	71.3, CH	73.7, CH
3'	78.6, CH	80.7, CH
4'	69.9, CH	72.3, CH
5'	81.3, CH	83.2, CH
6'	60.8, CH_2	63.3, CH_2

Table S7. ^{13}C NMR data for compound **11**

Position	Compound 11 (CDCl_3)	stromemycin ($\text{DMSO}-d_6$)
	δ_{C}	δ_{C}
1	164.2, C	158.29, C
2	112.6, C	110.56, C
3	164.4, C	159.32, C
4	111.0, CH	109.58, CH
5	148.0, C	142.46, C
6	109.3, C	108.83, C
7	170.6, C	167.36, C
8	37.7, CH_2	34.27, CH_2
9	36.2, CH_2	34.02, CH_2
10	131.9, CH	130.79, CH
11	131.9, CH	130.72, CH
12	131.6, CH	130.24, CH
13	133.3, CH	132.22, CH
14	36.9, CH_2	34.02, CH_2
15	23.6, CH_2	21.96, CH_2
16	14.0, CH_3	13.50, CH_3
17	76.1, CH	74.40, CH
18	73.1, CH	71.53, CH
19	80.0, CH	78.38, CH
20	71.6, CH	69.65, CH
21	82.6, CH	81.21, CH
22	62.6, CH_2	60.51, CH_2
1'	163.0, C	157.17, C
2'	105.8, CH	107.31, CH
3'	154.8, C	151.55, C
4'	113.3, CH	113.18, CH
5'	147.8, C	142.25, C
6'	116.4, C	118.40, C
7'	173.8, C	169.73, C
8'	35.8, CH_2	33.44, CH_2
9'	35.8, CH_2	33.61, CH_2
10'	132.4, CH	130.79, CH
11'	132.6, CH	130.79, CH
12'	131.7, CH	130.31, CH
13'	133.7, CH	132.38, CH
14'	37.7, CH_2	34.02, CH_2
15'	23.7, CH_2	21.97, CH_2
16'	14.0, CH_3	13.50, CH_3

Preparation of tested compounds and positive controls

Compounds were dissolved in dimethyl sulfoxide (DMSO) with final concentrations ranging from 4,000 to 15.625 µg/mL using a 2-fold serial dilution method. Amphotericin B, ciprofloxacin, or vancomycin dissolved in DMSO with final concentrations of 160, 80, 40, 20, 10, 5, 2.5, 1.25 µg/mL using a 2-fold serial dilution method.

Positive controls were added by a column respectively, while the tested compounds were diluted 2-fold by row in a 96-well microtiter plate.

Antibacterial assay

The MICs against *S. aureus* and *E. coli* were determined according to the CLSI broth microdilution method and our previous report. *S. aureus* or *E. coli* culture was grown at 37°C with shaking in MHB broth to midlogarithmic phase [optical density at 600 nm = 0.3] and diluted 10 times by MHB broth to give a final concentration of approximately 1×10^6 CFU/ml. Twofold serial dilutions of the test compounds were prepared in a 96-well microtiter plate by row containing 78 µL of MHB media and 2 µL diluted compounds (The final concentrations were 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625 µg/mL, respectively.), while the positive controls were diluted 2-fold by row in a 96-well microtiter plate (The final concentrations were 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 µg/mL, respectively). The MIC was defined as the lowest concentrations of samples with no visible growth of bacteria after incubation at 37°C for 18 hours. All MIC assays were performed in two independent experiments.

Antifungal assay

MICs against *C. albicans* were determined according to the CLSI guideline and our previous report. *C. albicans* culture was grown at 30°C with shaking in RPMI 1640 medium to midlogarithmic phase [optical density at 530 nm = 0.5] and diluted 500 times by RPMI 1640 medium to give a final concentration of approximately 1×10^4 CFU/ml. Twofold serial dilutions of the test compounds were prepared in a 96-well microtiter plate by row containing 78 µL of RPMI 1640 media and 2 µL diluted compounds (The final concentrations were 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625 µg/mL, respectively.), while the positive control was diluted 2-fold by column in a 96-well microtiter plate (The final concentrations were 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 µg/mL, respectively). The MIC was defined as the lowest concentrations of samples with no visible growth of fungus after incubation at 35°C for 24 hours. All MIC assays were performed in two independent experiments.