

SUPPLEMENTARY MATERIAL

Cytochalasins from Endophytic *Diaporthe* sp. GDG-118

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ABSTRACT

The plant *Sophora tonkinensis*, possessed a range of active compounds, was traditionally used in the medicine of Chinese minorities. Endophytic fungi were isolated from this plant, of which the fungus *Diaporthe* sp. GDG-118 was fermented and extracted with methanol. The extract was screened by antifungal and antibacterial assays leading to the discovery of two new 21-acetoxycytochalasins (**1-2**) and five known cytochalasins (**3-7**). These two new compounds were elucidated by spectroscopic analyses, and further their absolute configurations were determined by the X-ray of compound **3** and comparing their experimental CD spectra. The antibacterial and antifungal effects of these compounds were evaluated. Compound **2** showed moderate inhibitory activity against *Bacillus anthracis* and *Escherichia coli* with MIC value of 12.5 $\mu\text{g/mL}$, and **7** showed strong antifungal activity against *Alternaria oleracea*, *Pestalotiopsis theae* and *Colletotrichum capsici* with MIC values of 3.125 $\mu\text{g/mL}$, 1.56 $\mu\text{g/mL}$ and 1.56 $\mu\text{g/mL}$, respectively.

KEYWORDS

Sophora tonkinensis; *Diaporthe* sp.; 21-acetoxycytochalasins; antibacterial effects; antifungal effects

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Table S1. ^1H and ^{13}C NMR data of compounds **1** (in Acetone- d_6 , J in Hz) and **2** (in CDCl_3 , J in Hz)

position	1		2	
	δ (H)	δ (C)	δ (H)	δ (C)
1		175.0		174.4
2			5.59, s	
3	3.30 (<i>m</i>)	54.1	3.28, <i>m</i>	53.7
4	2.20 (<i>dd</i> , $J=5.5, 2.7$)	50.1	2.18, <i>t</i> (4.6)	48.1
5	2.70, <i>m</i>	33.0	2.94, <i>m</i>	36.6
6		150.9		148.9
7	3.68, <i>d</i> (10.2)	71.5	4.07, <i>dd</i> (12.9, 1.9)	76.7
8	3.02, <i>t</i> (9.9)	48.5	2.42, <i>t</i> (12.8)	45.4
9		49.5		47.3
10	2.93, <i>dd</i> (13.1, 6.3)	45.5	2.90, <i>dd</i> (13.6, 3.6)	45.8
	2.78, <i>m</i>		2.53, <i>dd</i> (13.6, 9.6)	
11	0.62, <i>d</i> (6.8)	13.4	1.15, <i>d</i> (6.7)	15.2
12	5.08, <i>m</i> ,	112.6	5.39, <i>m</i>	114.3
	4.90, <i>m</i>		5.21, <i>t</i> (2.2)	
13	5.98, <i>dd</i> (15.4, 9.6)	132.2	1.88, <i>m</i>	44.9
14	5.65, <i>m</i>	136.0	3.71, <i>m</i>	88.2
15	2.12, <i>m</i>	44.2	2.00, <i>m</i>	40.0
	1.95, <i>dd</i> (12.9, 4.8)		1.40, <i>m</i>	
16	2.74, <i>m</i>	32.4	2.13, <i>m</i>	30.5
17	5.23, <i>d</i> (7.3)	136.4	5.24, <i>s</i>	133.6
18		133.3		138.2
19	6.70, <i>d</i> (16.5)	135.9	2.27, <i>m</i>	36.2
20	5.58, <i>dd</i> (16.5, 3.5)	122.8	2.61, <i>m</i>	31.7
			2.08, <i>m</i>	
21	5.44, <i>d</i> (3.2)	78.3	5.18, <i>t</i> (2.7)	73.6
22	1.00, <i>d</i> (6.9)	24.3	1.11, <i>d</i> (7.1)	24.8
23	1.77, <i>s</i>	21.0	1.72, <i>s</i>	23.8
24		170.5		170.4
25	2.30, <i>s</i>	21.0	2.12, <i>s</i>	21.4
1'		138.7		137.3
2'/6'	7.25, <i>m</i>	130.6	7.13, <i>m</i>	129.2
3'/5'	7.30, <i>m</i>	129.3	7.32, <i>m</i>	129.1
4'	7.23, <i>m</i>	127.4	7.26, <i>m</i>	127.3

Table S2. Antifungal activities of compounds 1–7

Compounds	Strains (MIC, $\mu\text{g/mL}$)			
	<i>Alternaria</i>	<i>Pestalotiopsis</i>	<i>Colletotrichum</i>	<i>Ceratocystis</i>
	<i>oleracea</i>	<i>theae</i>	<i>capsici</i>	<i>paradoxa</i>
1	50	50	100	100
2	50	50	100	100
3	6.25	12.5	6.25	25
4	6.25	6.25	100	100
5	100	25	100	100
6	100	25	100	100
7	3.125	1.56	1.56	100
Carbendazim	1.56	1.56	1.56	100

Table S3. Antibacterial activities of compounds 1–7

Compounds	Strains (MIC, $\mu\text{g/mL}$)					
	<i>Bacillus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Proteus</i>	<i>Escherichia</i>	<i>Salmonella</i>
	<i>subtilis</i>	<i>megaterium</i>	<i>anthraci</i>	<i>vuigaris</i>	<i>coli</i>	<i>paratyphi B</i>
1	25	50	50	100	25	50
2	25	50	12.5	100	12.5	50
3	50	25	50	-	50	100
4	50	25	25	-	50	100
5	100	50	25	100	100	50
6	50	100	100	50	25	50
7	100	50	100	50	50	50
Ampicillin	3.125	3.125	3.125	1.56	3.125	12.5

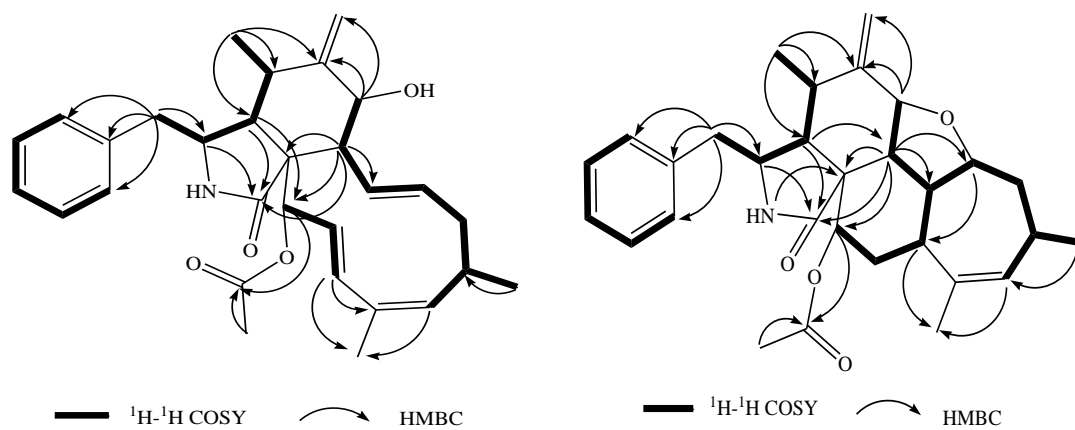


Figure S1. Selected HMBC and ^1H - ^1H COSY correlations of compounds **1** and **2**

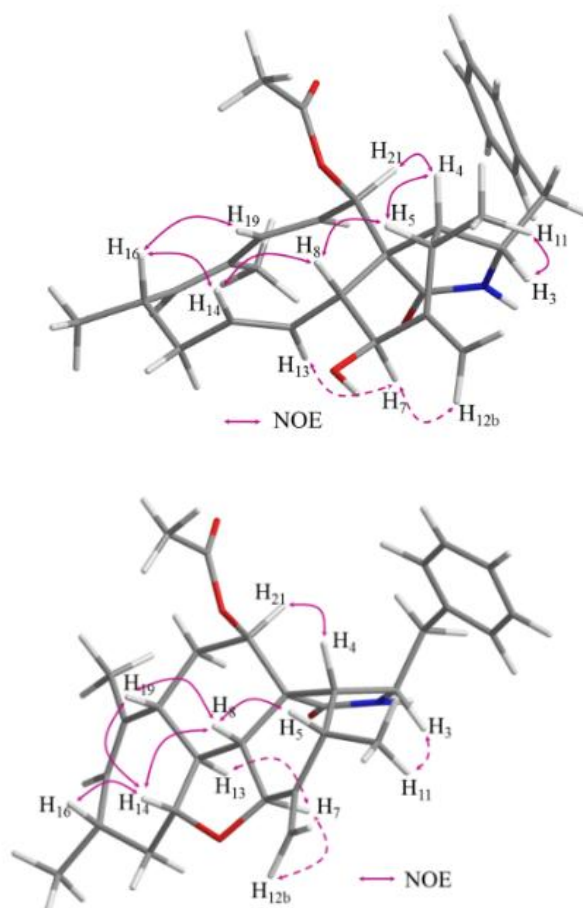


Figure S2. The NOESY correlations of compounds **1** and **2**

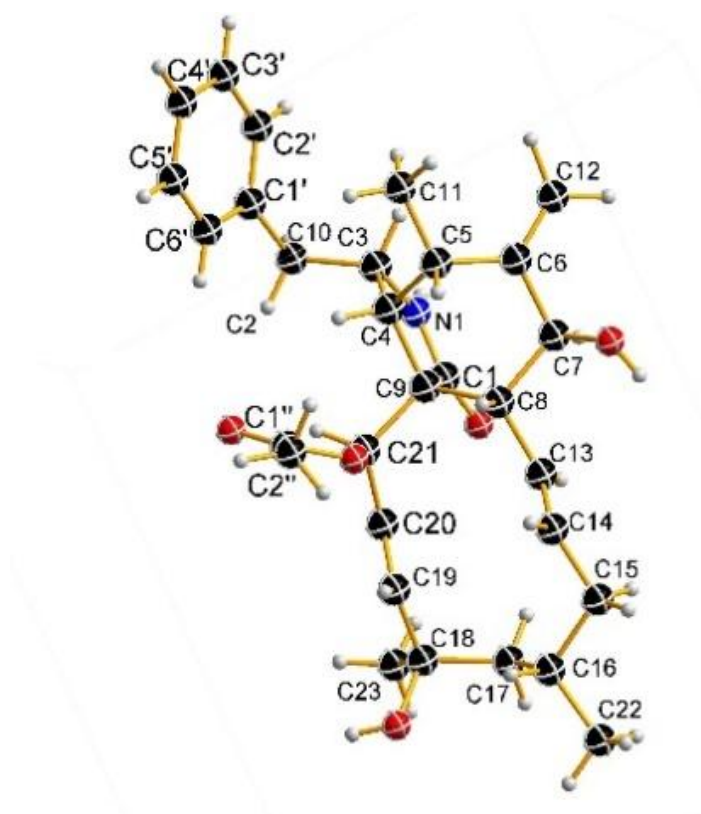


Figure S3. X-ray structure of compound **3**

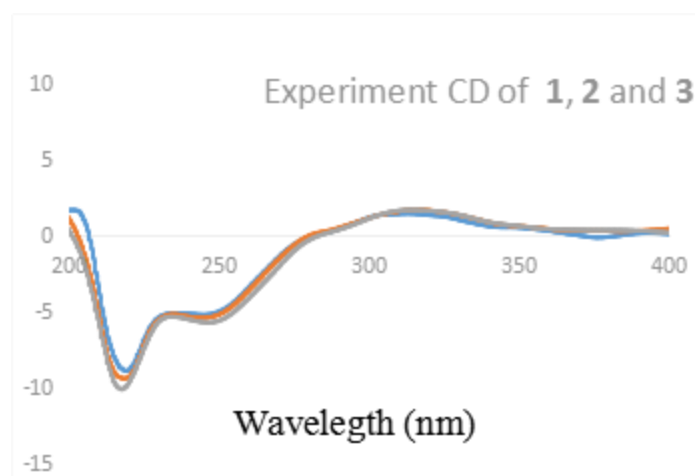
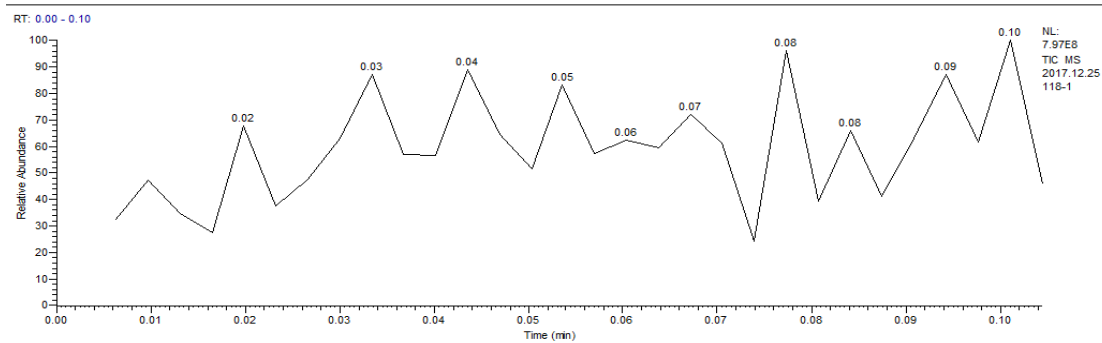


Figure S4. Experimental and calculated ECD spectra of compounds **1** and **2**



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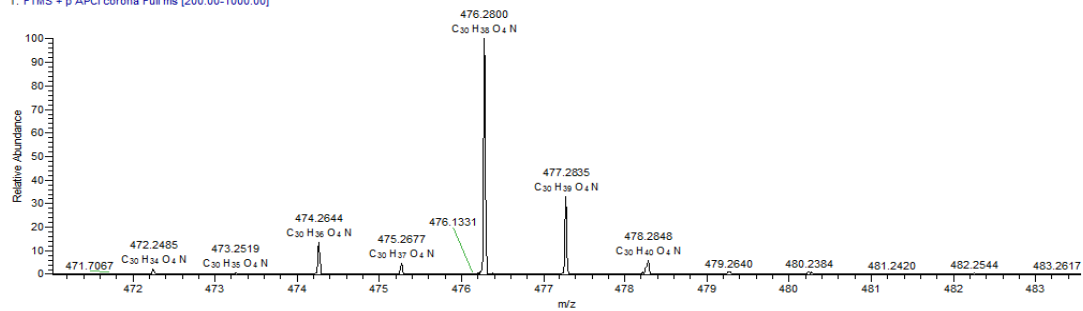


Figure S5. HRMS spectrum of compound 1

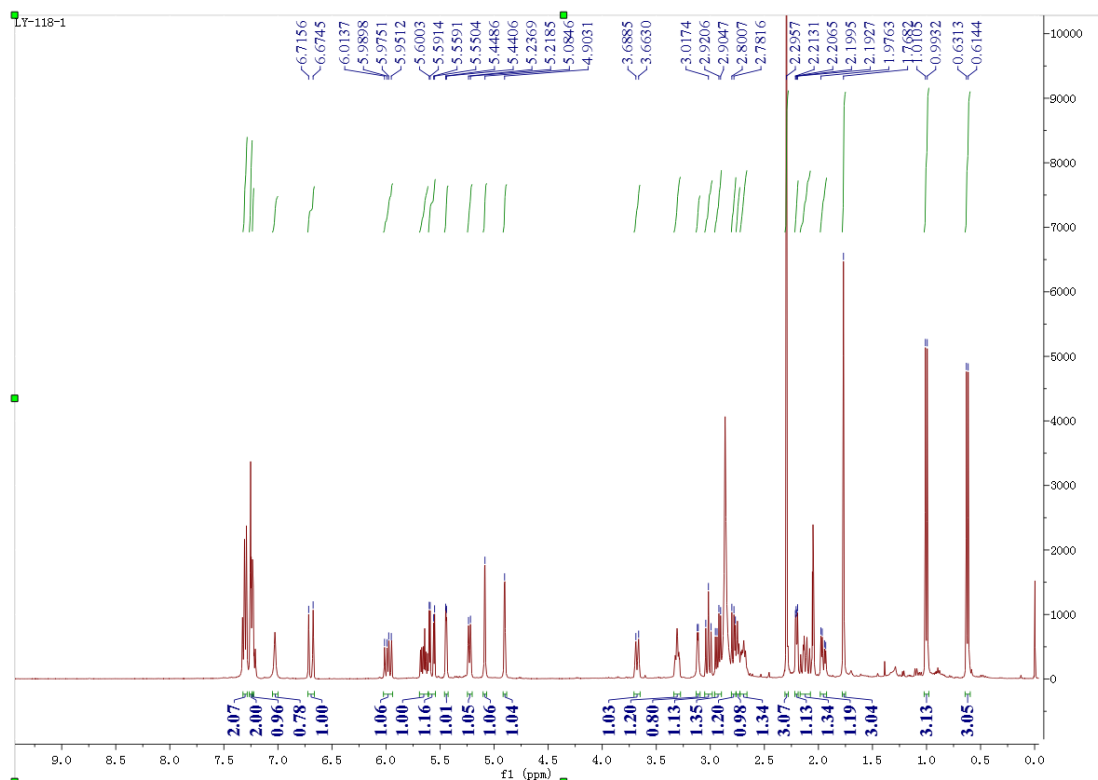


Figure S6. ¹H NMR spectrum (400 MHz, Methanol-*d*₄) of compound 1

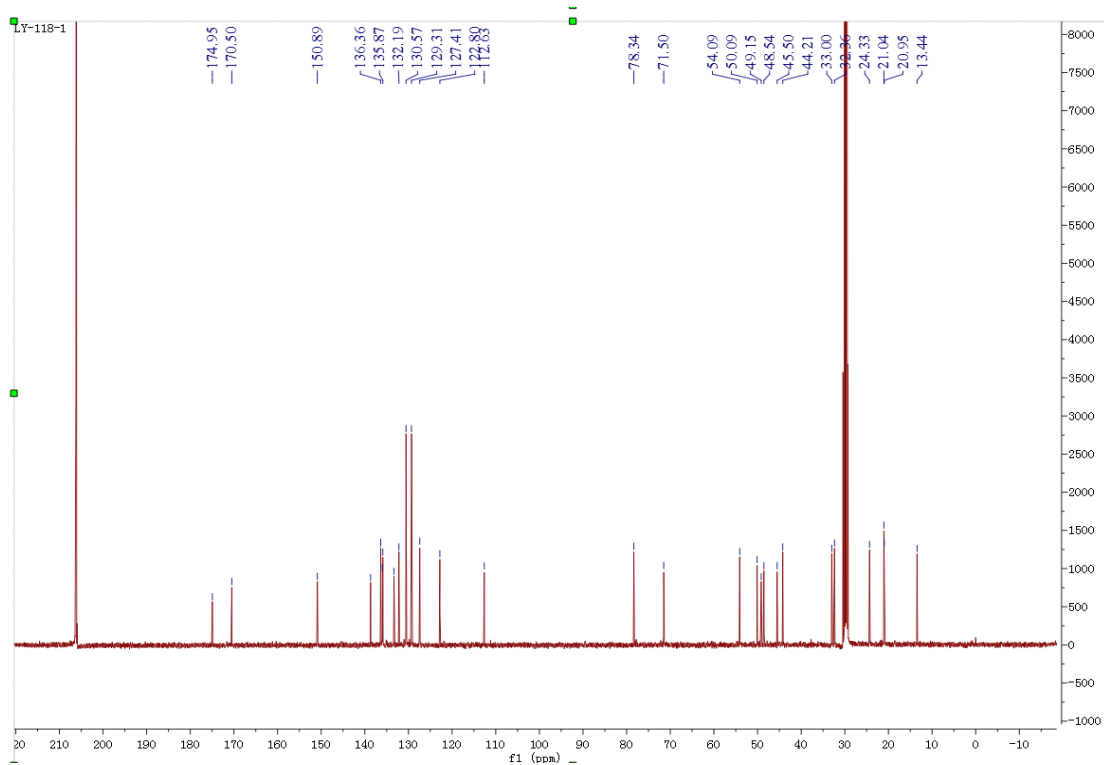


Figure S7. ^{13}C NMR spectrum (100 MHz, Methanol- d_4) of compound 1

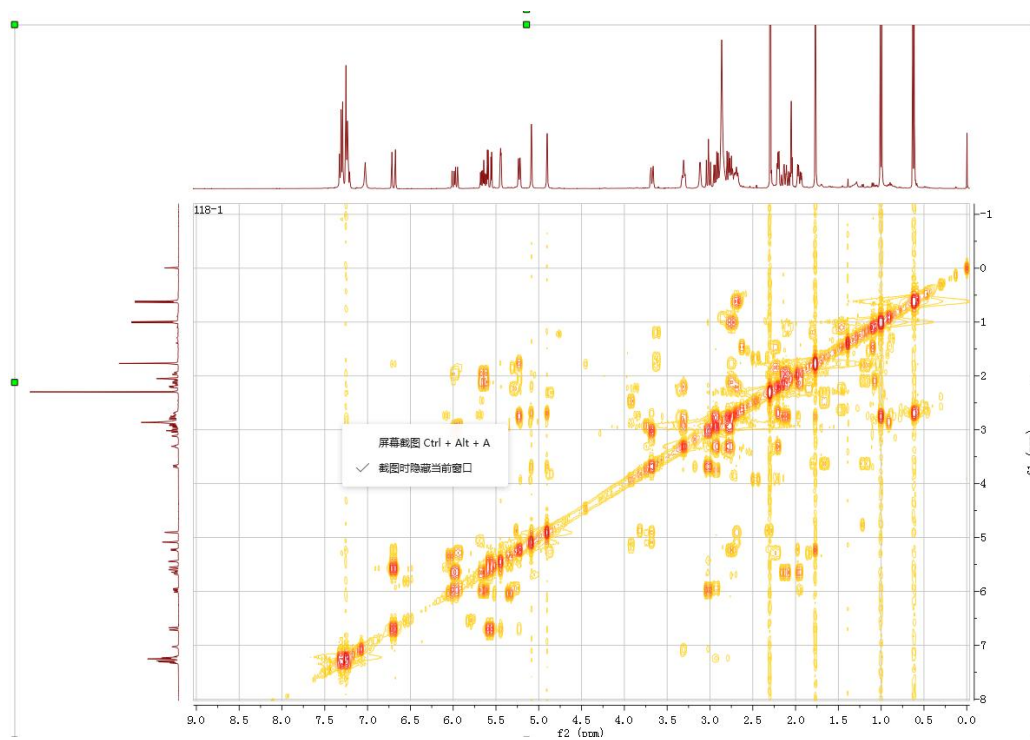


Figure S8. ^1H - ^1H COSY spectrum (400 MHz, Methanol- d_4) of compound 1

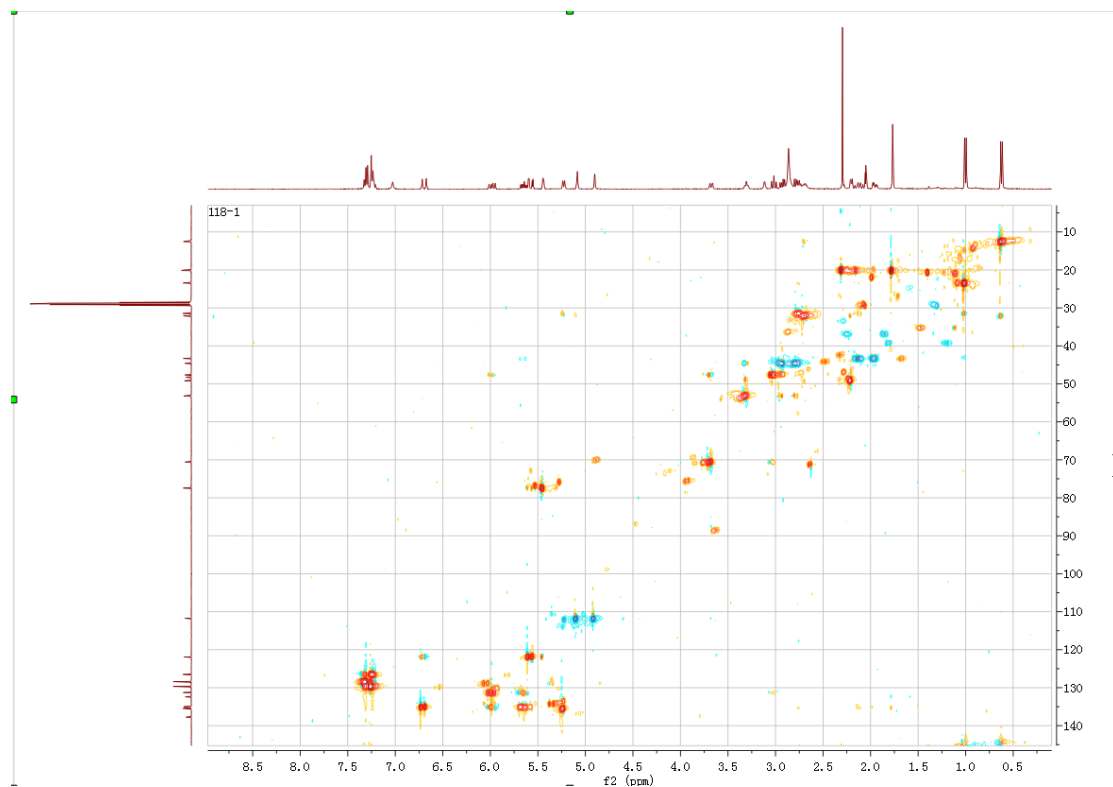


Figure S9. HSQC spectrum (400 MHz, Methanol- d_4) of compound **1**

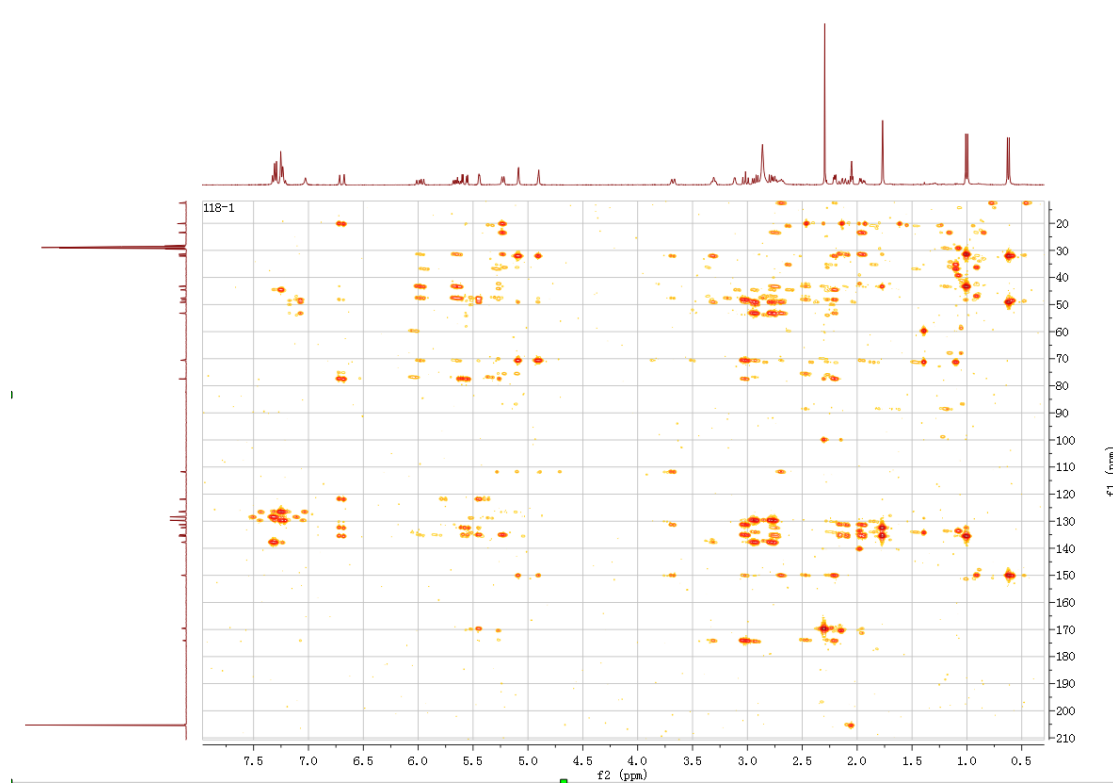


Figure S10. HMBC spectrum (400 MHz, Methanol- d_4) of compound **1**

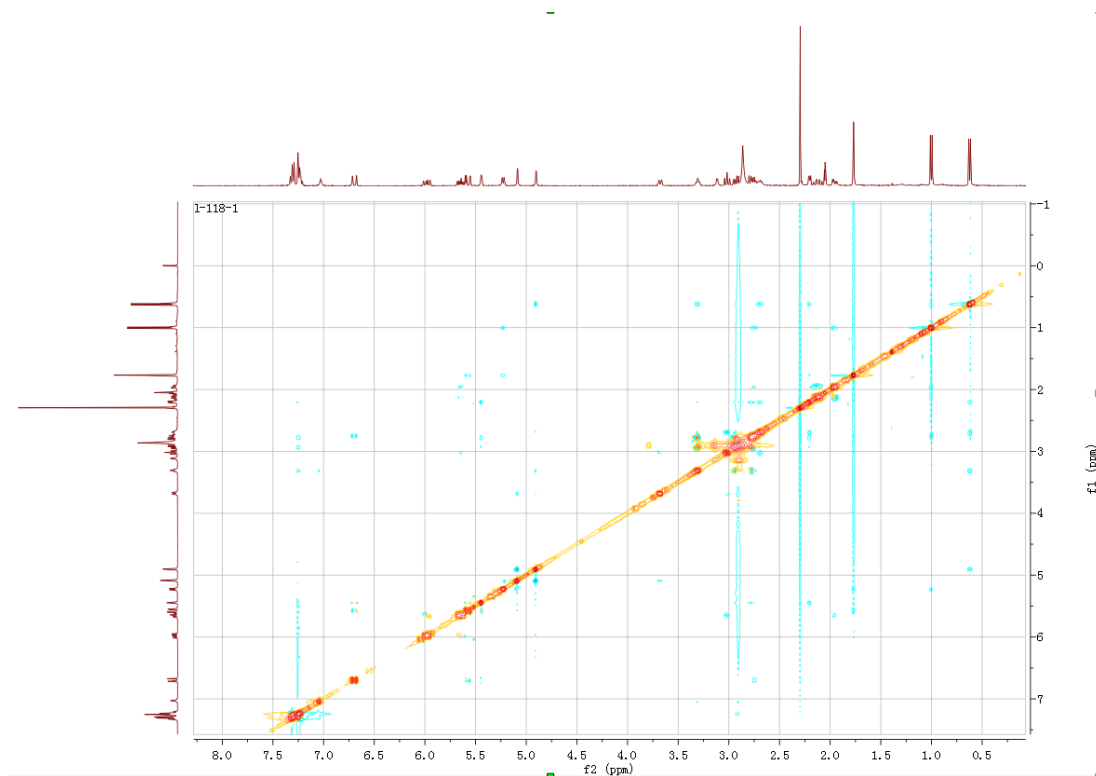
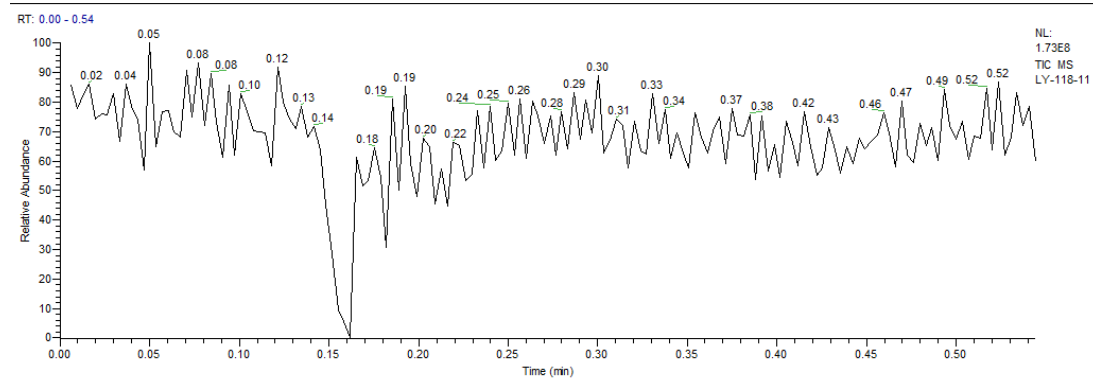


Figure S11. NOESY spectrum (400 MHz, Methanol- d_4) of compound **1**

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03/14/18 10:21:11



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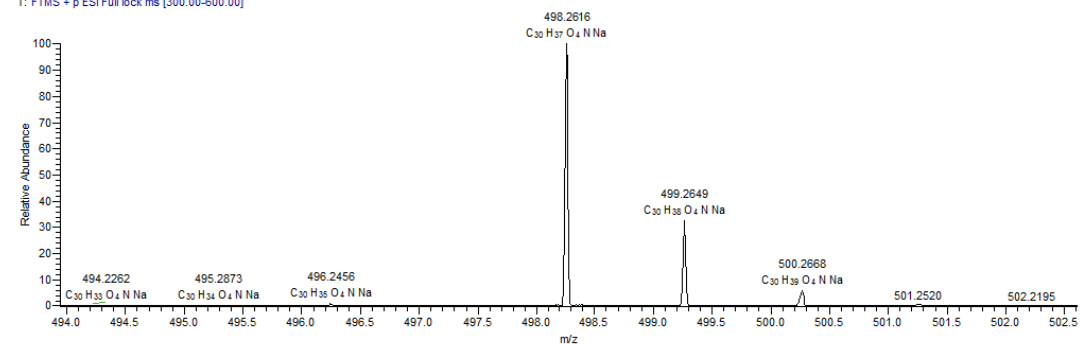


Figure S12. HRMS spectrum of compound **2**

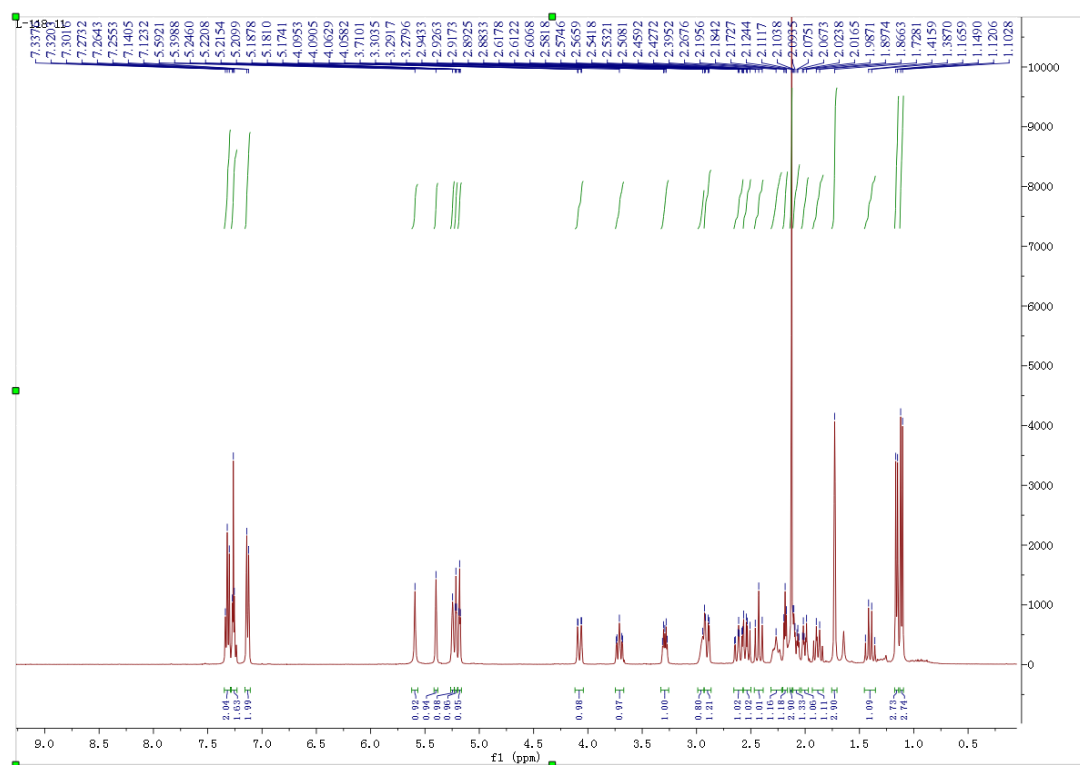


Figure S13. ^1H NMR spectrum (400 MHz, Methanol- d_4) of compound 2

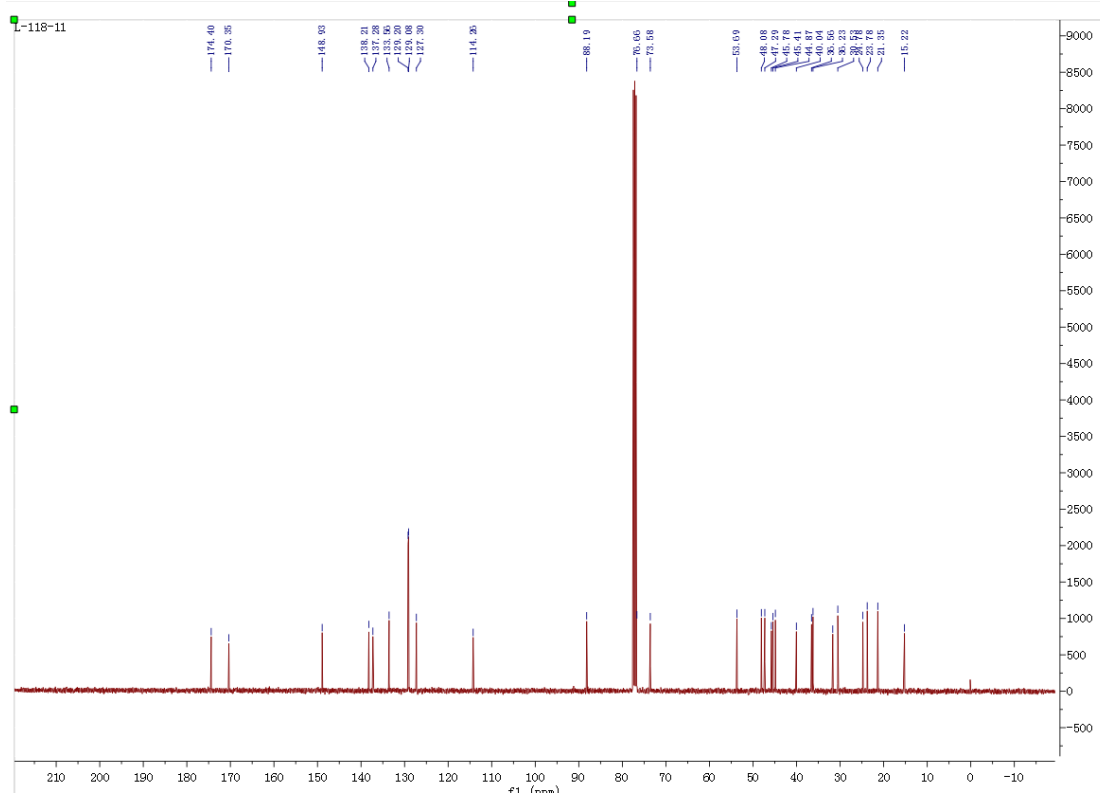


Figure S14. ^{13}C NMR spectrum (100 MHz, Methanol- d_4) of compound 2

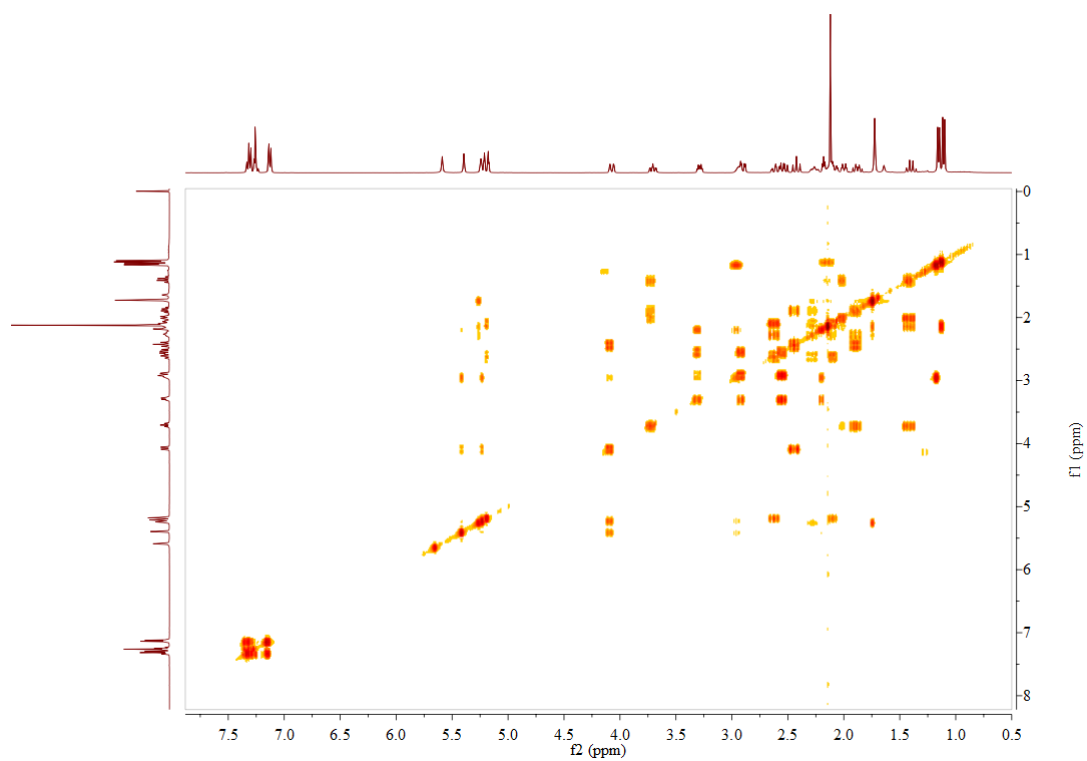


Figure S15. ¹H-¹H COSY spectrum (400 MHz, Methanol-*d*₄) of compound **2**

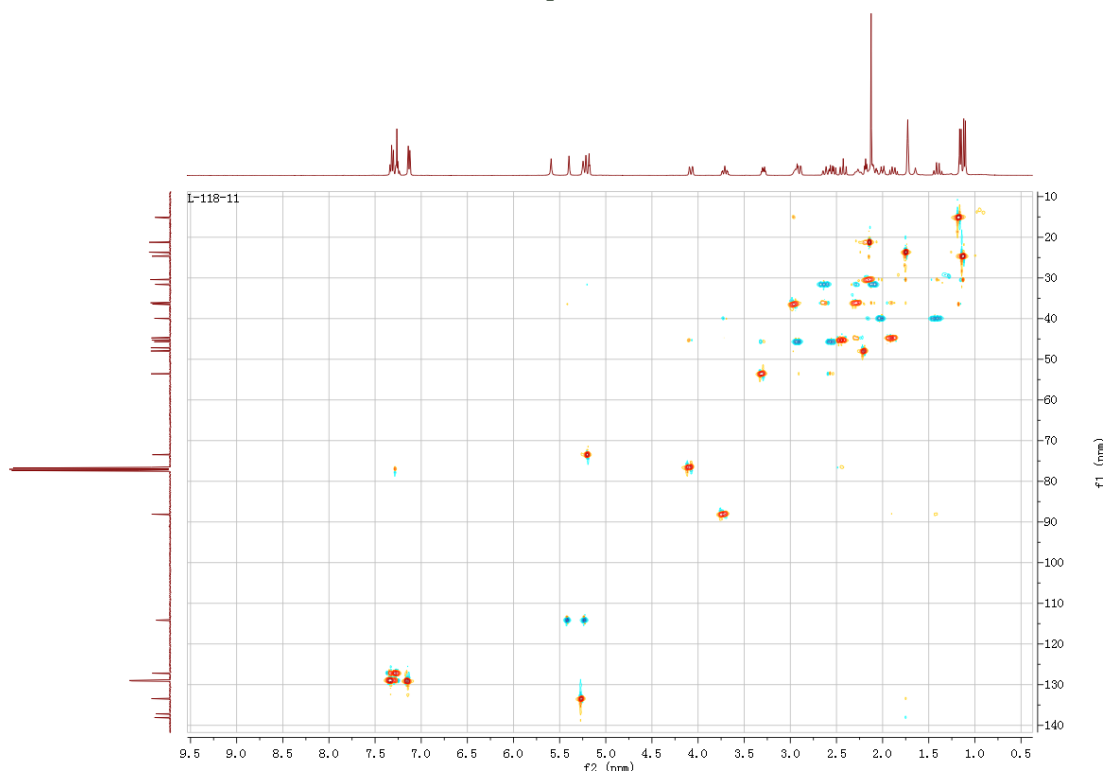


Figure S16. HSQC spectrum (400 MHz, Methanol-*d*₄) of compound **2**

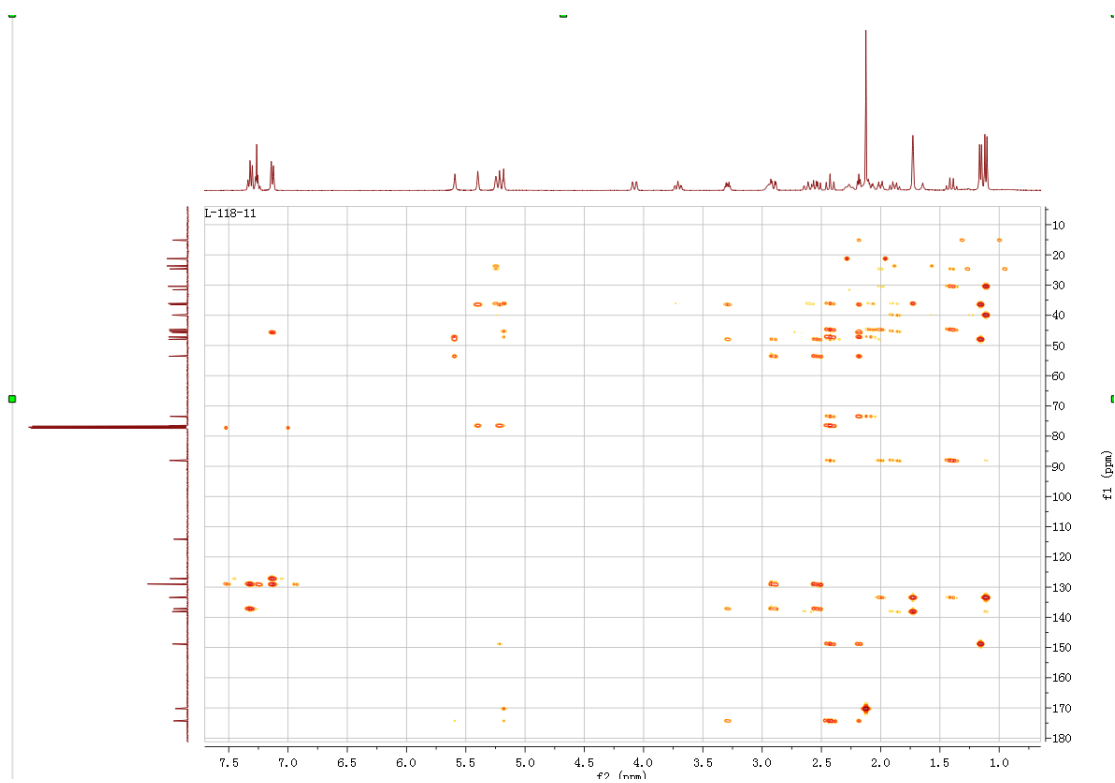


Figure S17. HMBC spectrum (400 MHz, Methanol- d_4) of compound **2**

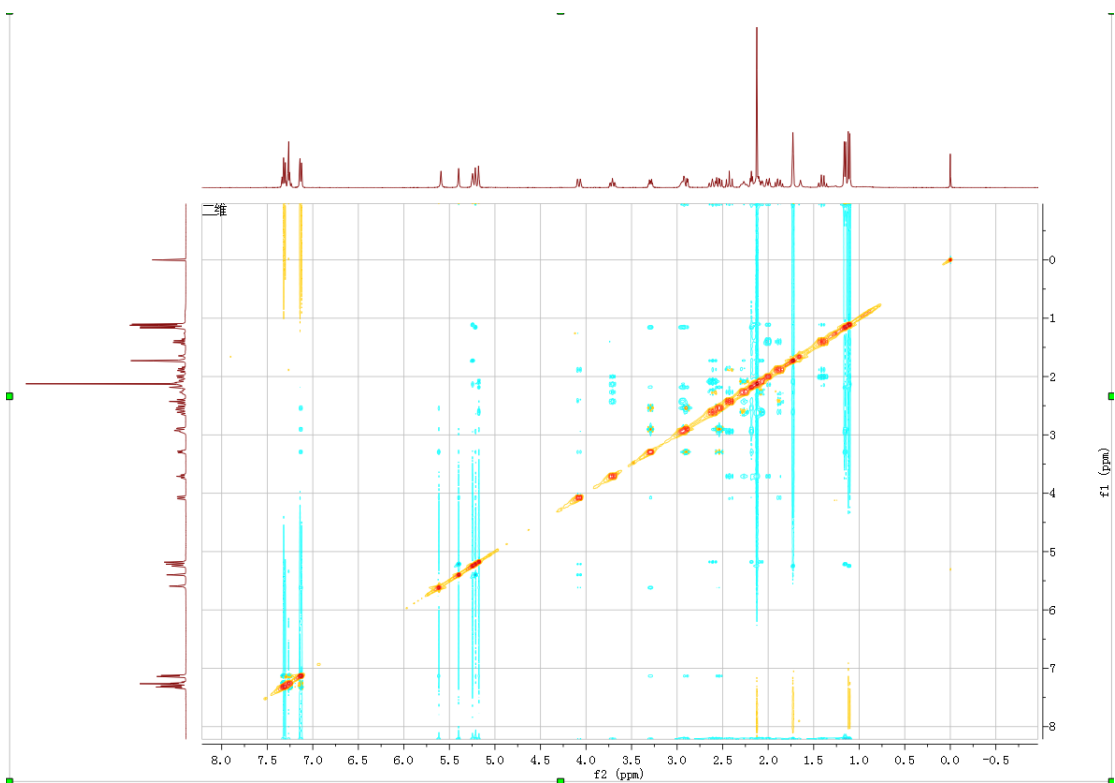


Figure S18. NOESY spectrum (400 MHz, Methanol- d_4) of compound **2**

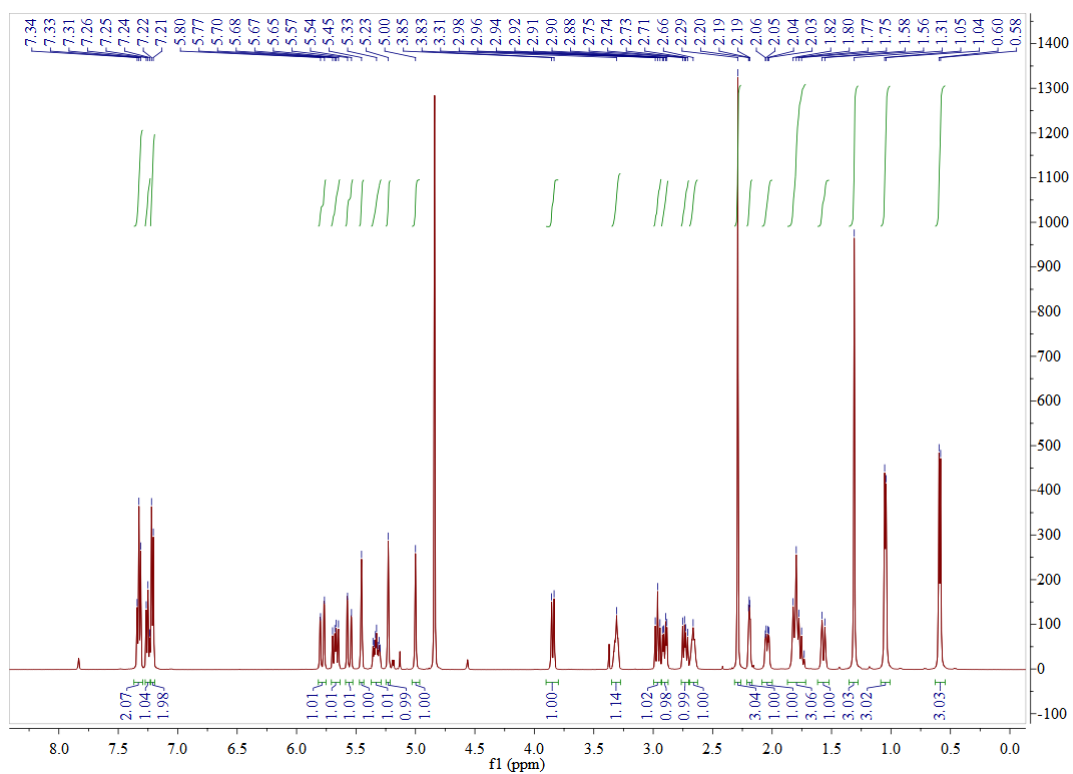


Figure S19. ^1H NMR spectrum (400 MHz, Methanol- d_4) of compound **3**

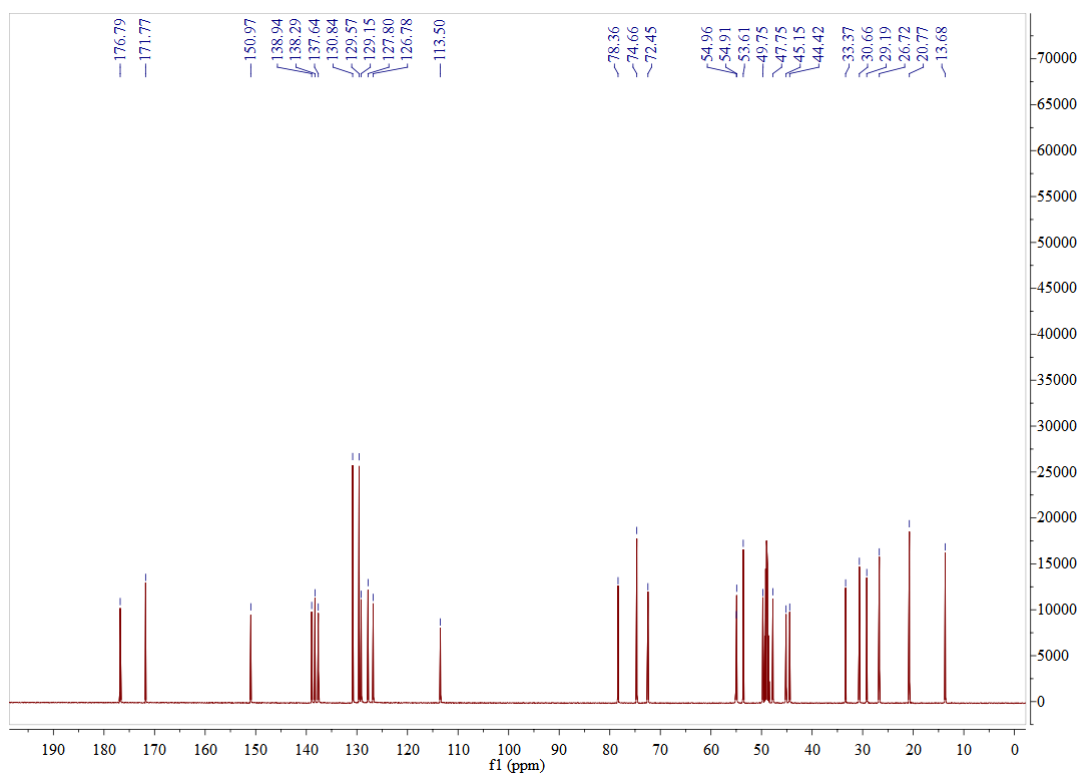


Figure S20. ^{13}C NMR spectrum (100 MHz, Methanol- d_4) of compound **3**

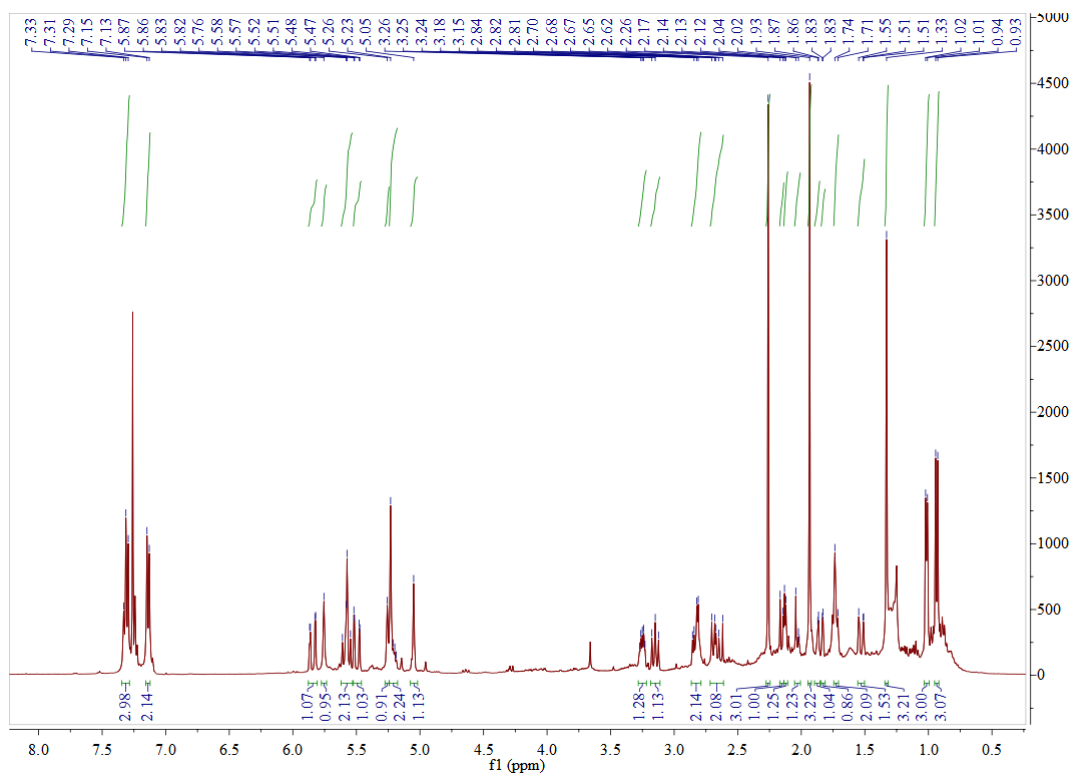


Figure S21. ^1H NMR spectrum (400 MHz, CDCl_3) of compound 4

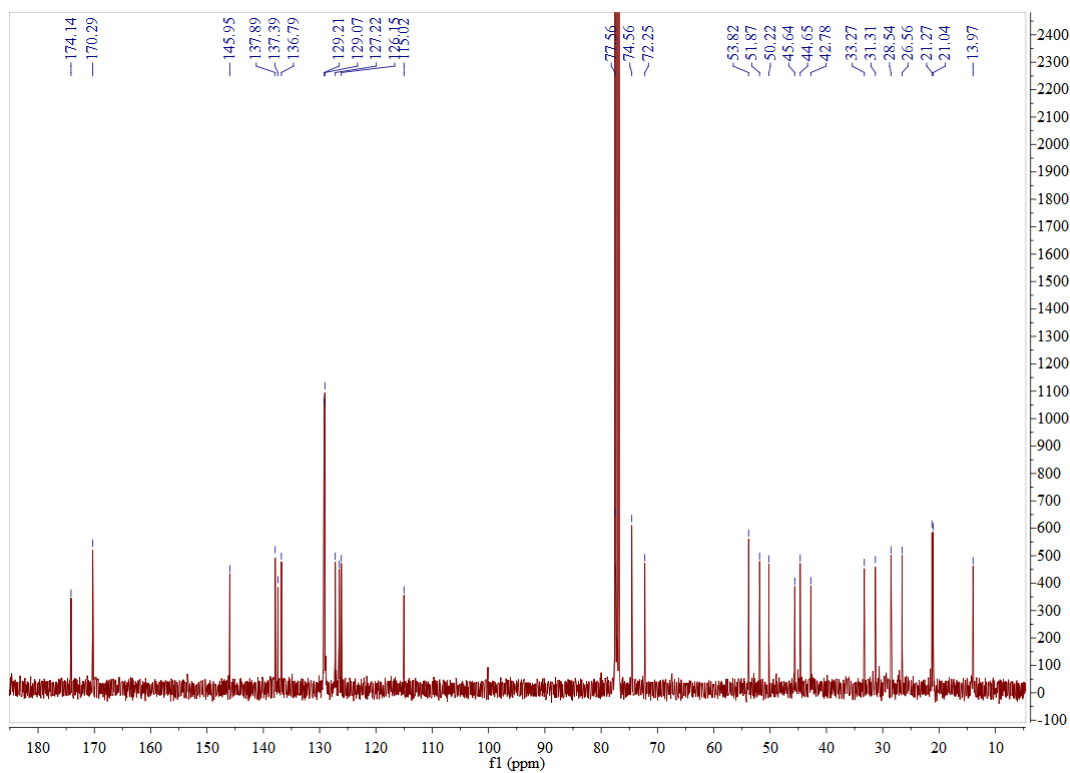


Figure S22. ^{13}C NMR spectrum (100 MHz, CDCl_3) of compound 4

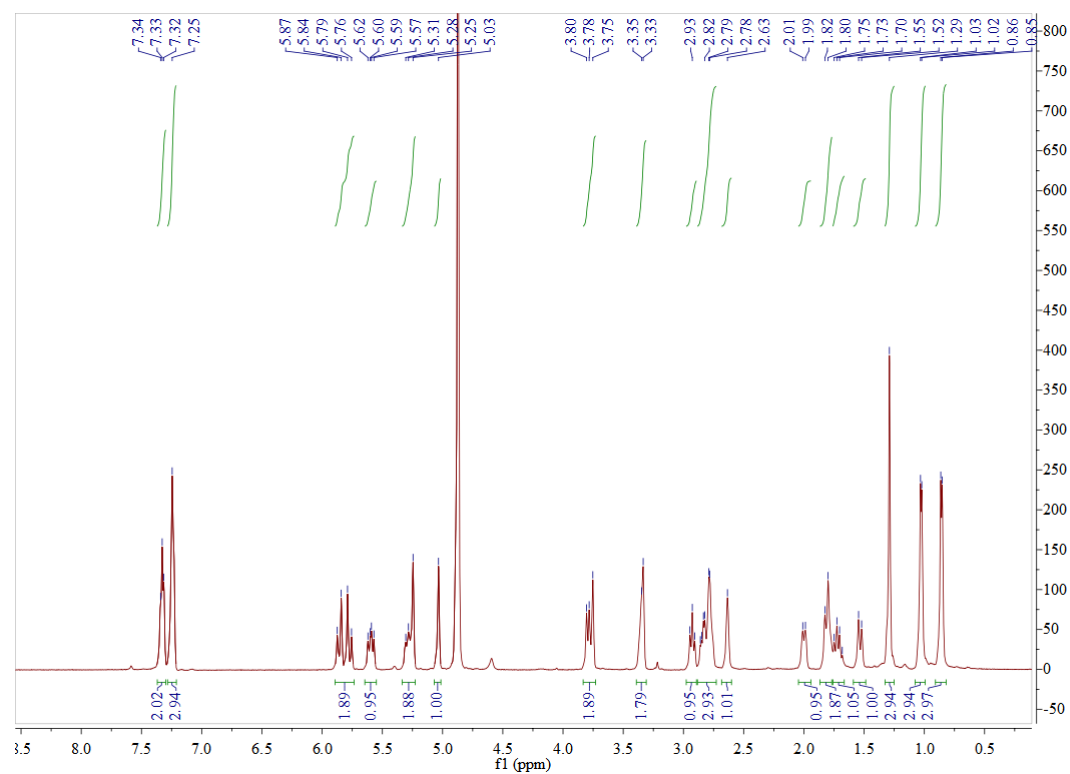


Figure S23. ^1H NMR spectrum (400 MHz, $\text{Methanol-}d_4$) of compound **5**

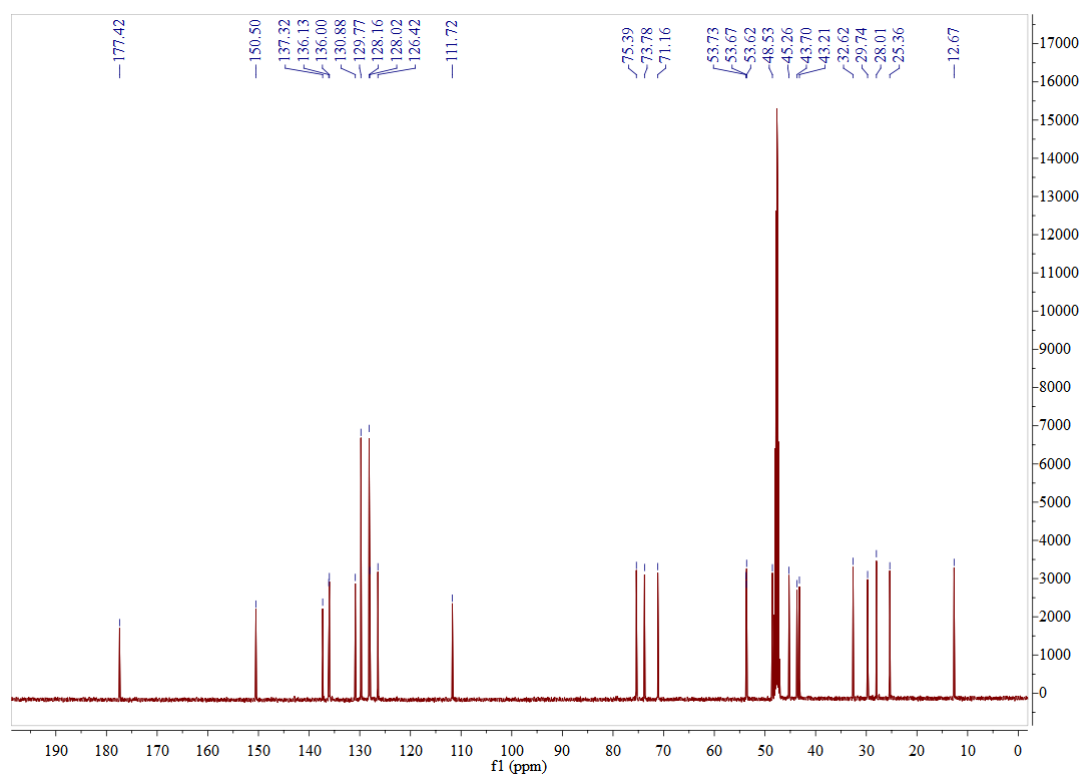


Figure S24. ^{13}C NMR spectrum (100 MHz, $\text{Methanol-}d_4$) of compound **5**

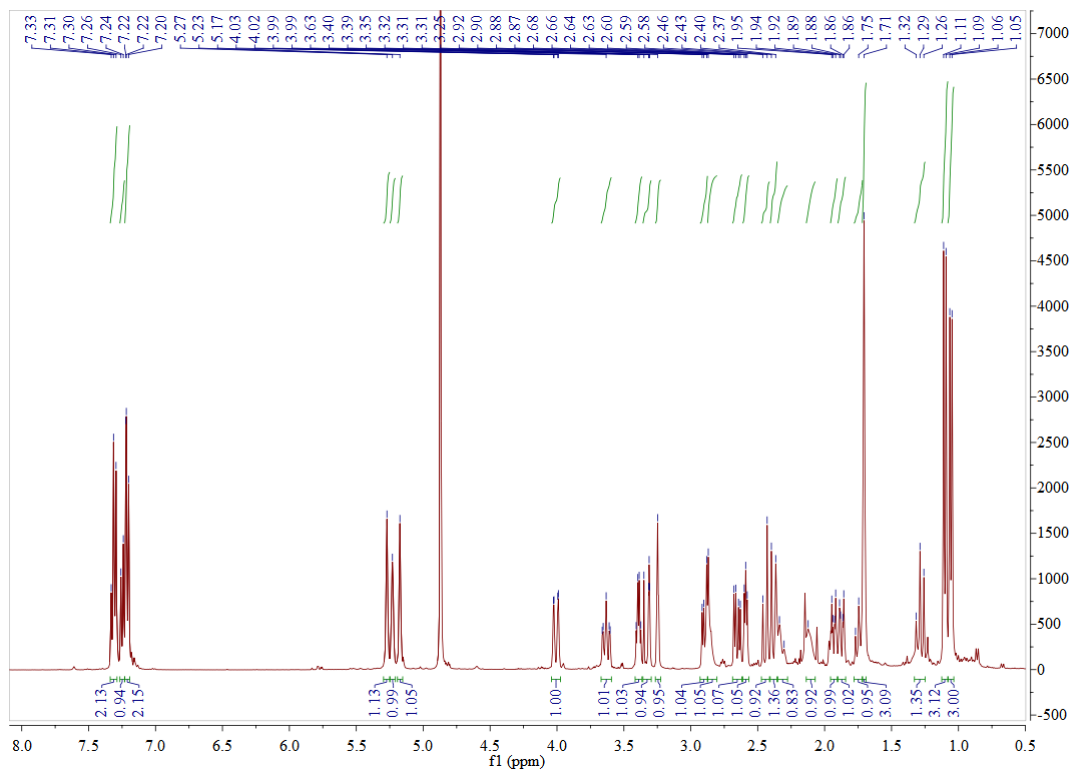


Figure S25. ^1H NMR spectrum (400 MHz, Methanol- d_4) of compound **6**

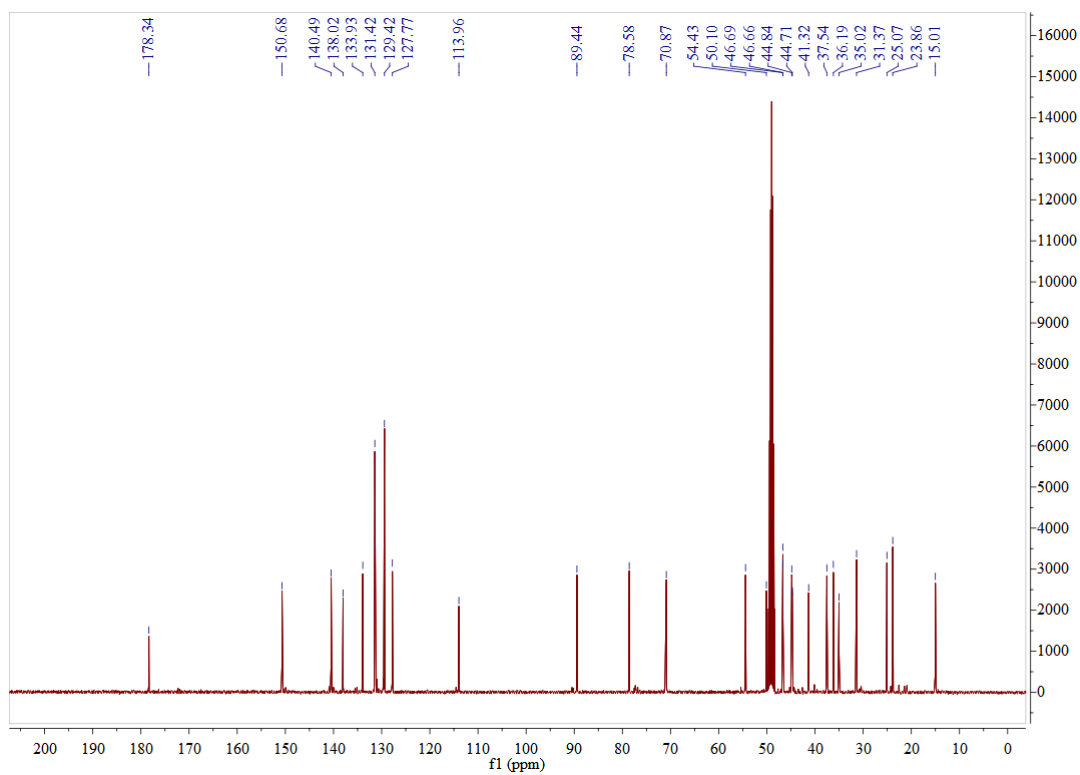


Figure S26. ^{13}C NMR spectrum (100 MHz, Methanol- d_4) of compound **6**

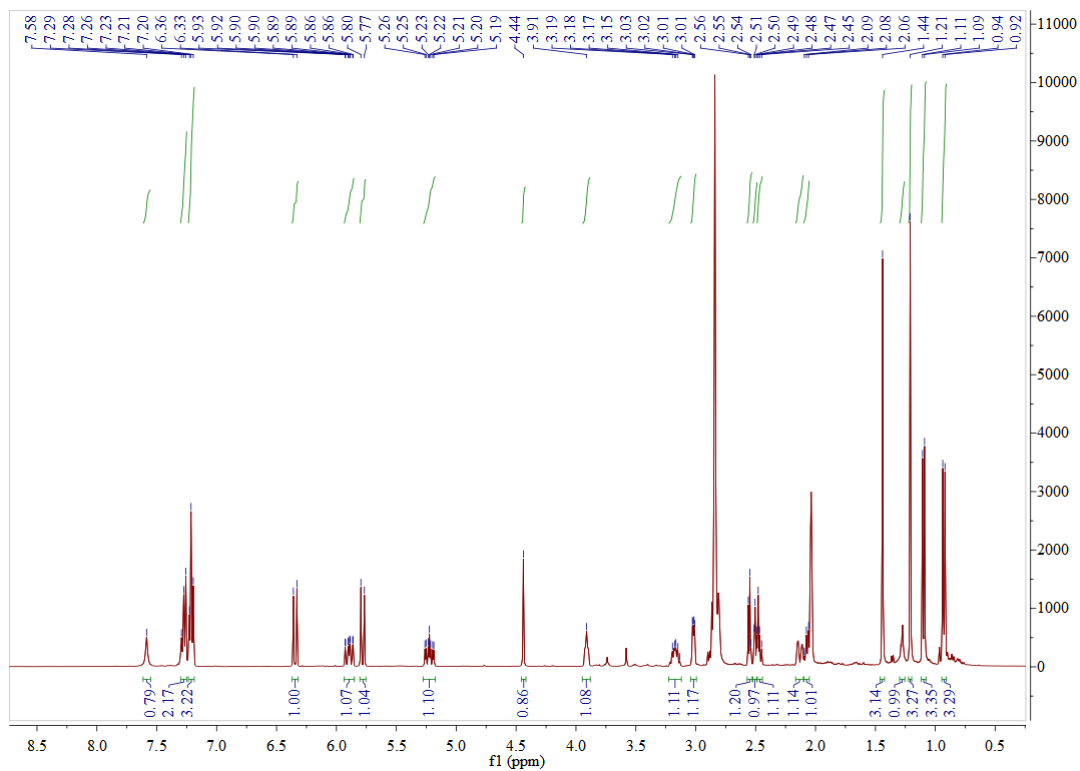


Figure S27. ^1H NMR spectrum (400 MHz, Acetone- d_6) of compound 7

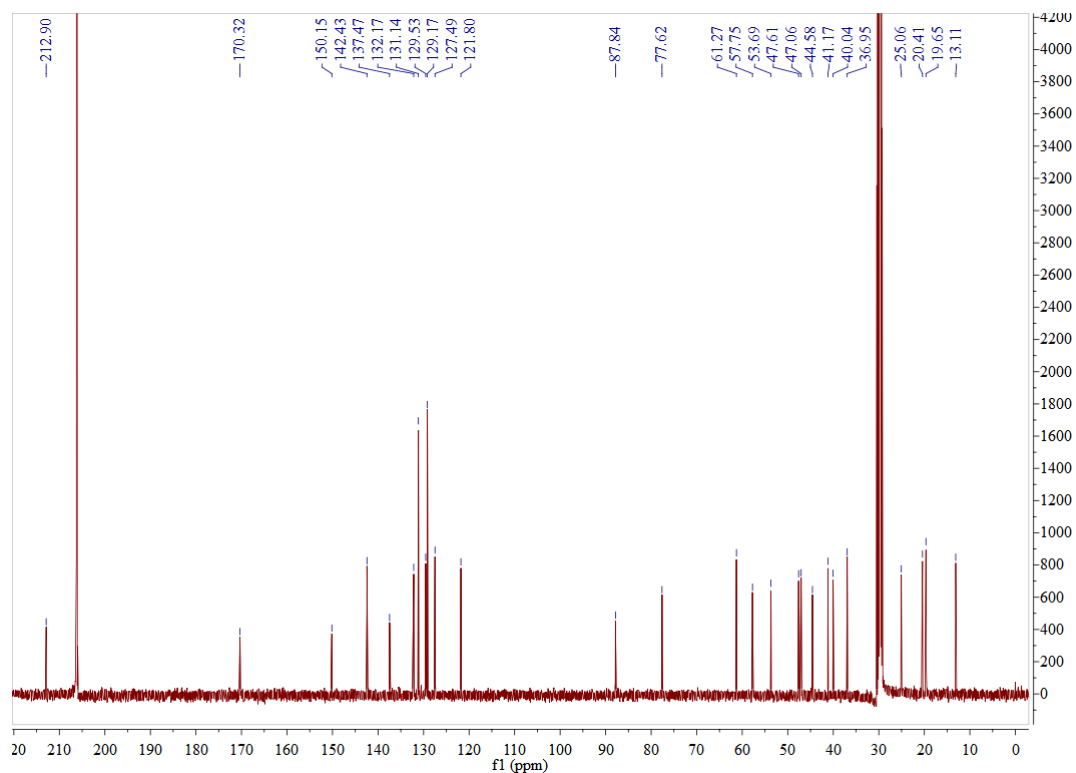


Figure S27. ^{13}C NMR spectrum (400 MHz, Acetone- d_6) of compound 7