Supplementary Materials

Isolation and Biomimetic Synthesis of Acylphloroglucinol Meroterpenoids as Anti-breast cancer agents from *Dryopteris crassirhizoma*

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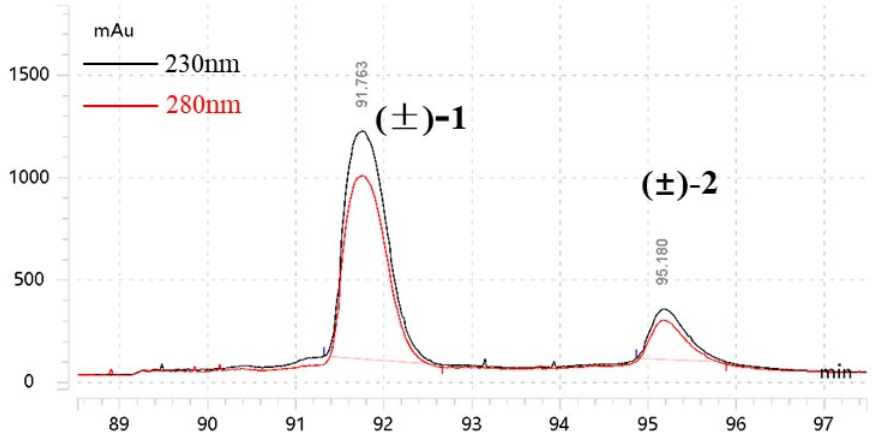
Contents

1. General experimental procedures

Optical rotations were measured with a Rudolph AUTOPOL VI polarimeter. UV spectra were obtained using an Agilent Cary60 spectrophotometer. CD spectra were obtained using a Chirascan instrument (Applied Photophysics Limited, England). IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with a Bruker Avance III 500 or 600 instrument at room temperature. ESI-MS and HR-ESI-MS were performed on an Agilent G6230 time-of-flight mass spectrometer. Silica gel (200–300 mesh) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Semi-prep HPLC was performed on an AS20005 series (Hanbon, China) using a 5C18-AR-II column (5 μm, 10 × 250 mm, 3.0 mL/min, Nacalai Tesque, Japan), a Chiralpak IG chiral column (5 μm, 10 mm × 250 mm, 3.0 mL/min, Daicel Chiral Technologies Co. Ltd., Tokyo, Japan), or a P3500 series (Dalian Elite, China) using a Sinochrom ODS-BP column (10 μm, 30 × 250 mm, 15 mL/min, Dalian Elite, China).

2. Figure S1 The chiral HPLC separation chromatograms of compounds (±)-1, (±)-2 and synthetic compounds (1~8).



Injection time: 83.0 min.

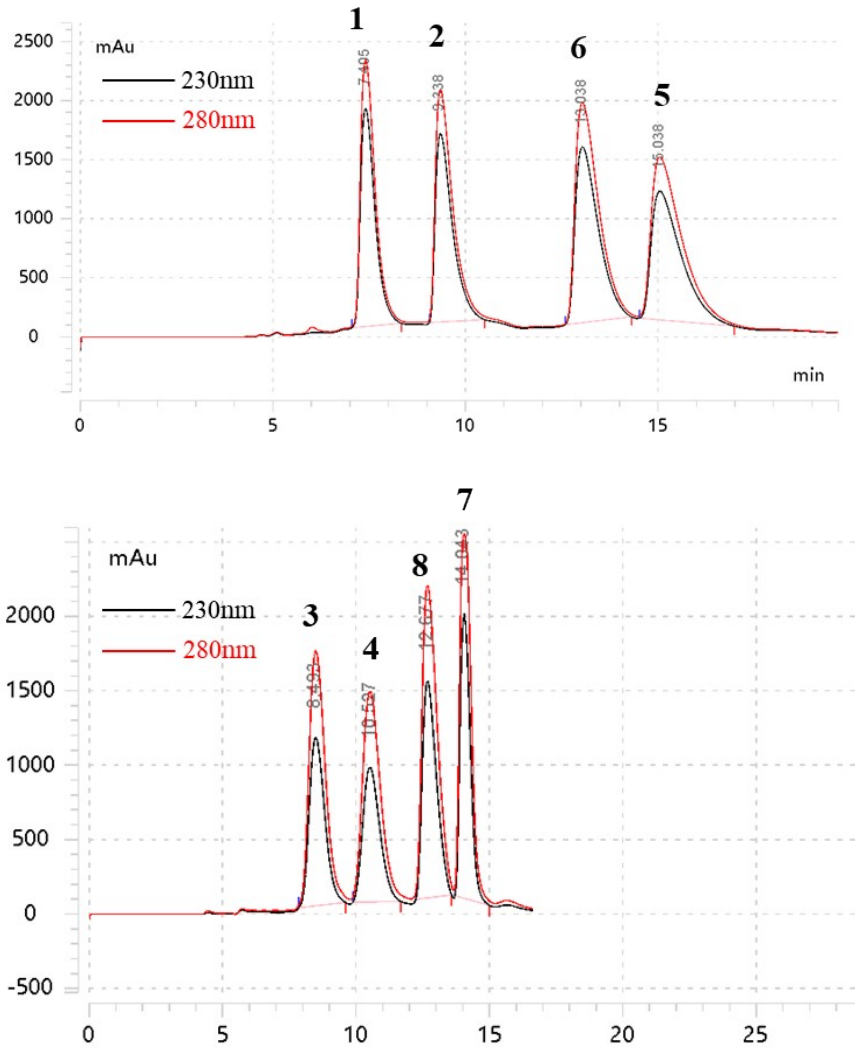


Figure S1 Semipreparative of the mixture of (±)**1**/(±)**2** by chiral HPLC (Chiralpak IG, 5 μm, 10 × 150 mm), (±)-**1**/(±)-**2** [n-hexane/EtOH = 88/20(V/V), 3.0 mL/min]; **1**, **2**, **5**, and **6** [n-hexane/isopropanol = 85/15(V/V)], 3.0 mL/min); **3**, **4**, **7**, and **8** [n-hexane/isopropanol = 92/8(V/V)], 3.0 mL/min).

2. Computational Details

2.1 Conformation searches based on molecular mechanics with MMFF94s force field were performed for **1~8** gave 4, 3, 16, 13, 7, 6, 5, and 4 conformers with populations higher than 1%[1], respectively. All these conformers were further optimized by the density functional theory method at the B3LYP/6-31G(d) level in Gaussian 16 program package[2].

Table S1. Energy analysis for conformers of **1~8** at B3LYP/6-31G(d) level in the gas phase.

Species	Gibbs free energy	Δ*E*(kcal/mol)	P_E%
1u	-2116.345732	0.000	41.39%
1w	-2116.345327	0.649	26.87%
1x	-2116.345327	0.649	26.87%
1v	-2116.343011	0.055	2.27%
2d	-2116.348581	0.000	74.84%
2c	-2116.347360	0.766	20.35%
2e	-2116.346003	1.617	4.79%
3h	-1729.261122	0.000	16.18%
3a	-1729.261118	0.002	16.11%
3g	-1729.261117	0.003	16.09%
3f	-1729.260535	0.368	8.65%
3c	-1729.26041	0.446	7.57%
3b	-1729.260408	0.448	7.56%
3o	-1729.259907	0.762	4.43%
3s	-1729.259859	0.792	4.21%
3m	-1729.259854	0.795	4.19%
3p	-1729.259751	0.860	3.75%
3r	-1729.259198	1.207	2.08%
3j	-1729.259026	1.315	1.73%
3k	-1729.258903	1.392	1.52%
3l	-1729.258903	1.392	1.52%
3n	-1729.258775	1.472	1.32%
3e	-1729.258654	1.548	1.16%
4b	-1729.264735	0.000	20.28%
4d	-1729.264734	0.001	20.25%
4a	-1729.264725	0.006	20.06%
4c	-1729.263981	0.473	9.07%
4g	-1729.263978	0.475	9.05%
4e	-1729.263959	0.487	8.86%
4h	-1729.262791	1.220	2.55%
4q	-1729.262312	1.520	1.53%
4i	-1729.262311	1.521	1.53%
4j	-1729.262159	1.616	1.30%
4l	-1729.26211	1.647	1.23%
4o	-1729.261977	1.731	1.07%
4p	-1729.261976	1.731	1.07%
5h	-2116.344897	0.000	43.07%
5c	-2116.343818	0.677	13.63%
5d	-2116.343818	0.677	13.63%
5f	-2116.343818	0.677	13.63%
5g	-2116.343232	1.044	7.30%
5b	-2116.343141	1.101	6.62%
5e	-2116.341964	1.840	1.89%

Continued table 1

6a	-2116.347973	0.000	49.48%
6d	-2116.347256	0.450	23.04%
6e	-2116.346821	0.723	14.49%
6f	-2116.346094	1.179	6.67%
6c	-2116.345571	1.507	3.82%
6g	-2116.345134	1.782	2.40%
7a	-2116.351014	0.000	48.89%
7c	-2116.350389	0.392	25.11%
7b	-2116.349884	0.709	14.65%
7g	-2116.349403	1.010	8.77%
7f	-2116.348061	1.853	2.10%
8l	-2116.35265	0.000	53.09%
8h	-2116.352247	0.252	34.55%
8c	-2116.350972	1.052	8.87%
8b	-2116.349815	1.778	2.58%

2.2 ECD calculation details of compounds 1~8

The optimized conformers were further subjected to theoretical calculation of ECD at B3LYP/6-311+G (2d, p) level in methanol with IEFPCM model by using time-dependent density functional theory (TDDFT)[3]. The calculated ECD curves for were generated using SpecDis 1.71[4].

3. NMR and MS spectra of compound (±)-1

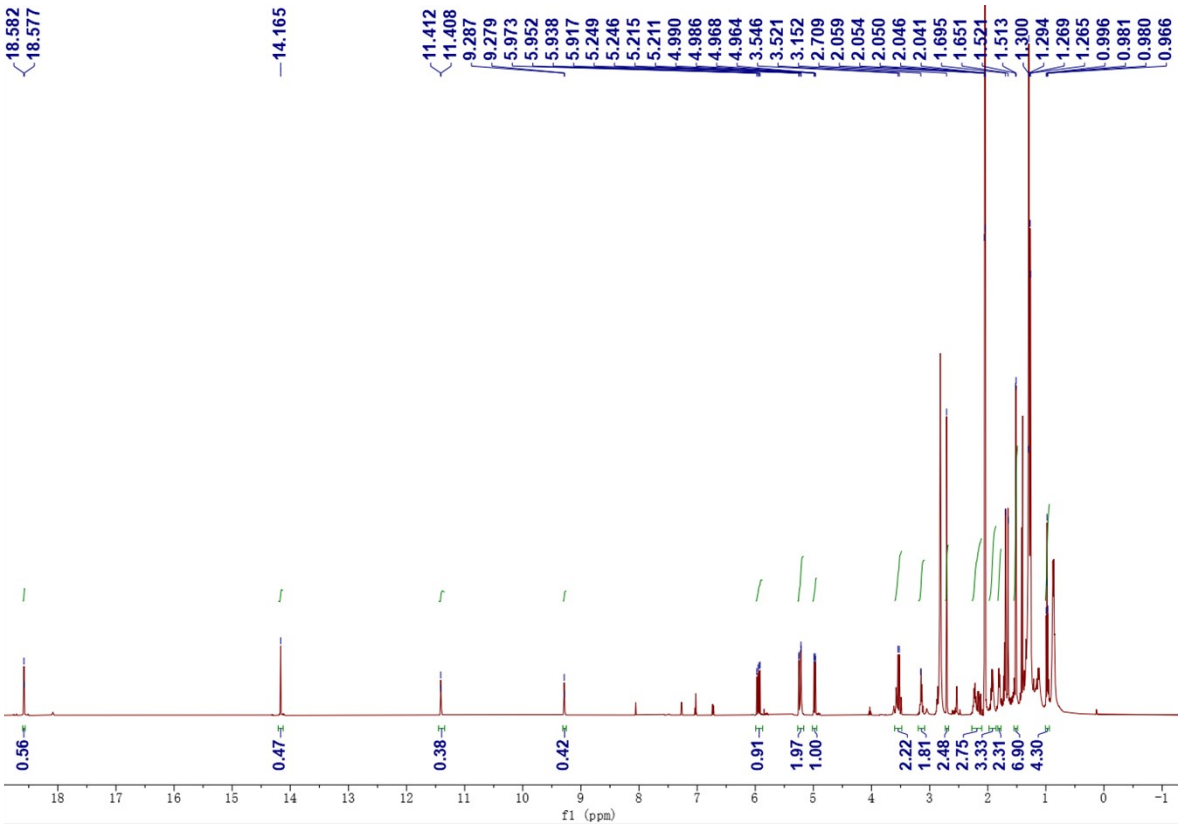


Figure S1 ^{1}H-NMR spectrum (500 MHz) of (±)-1 in acetone-d_6.

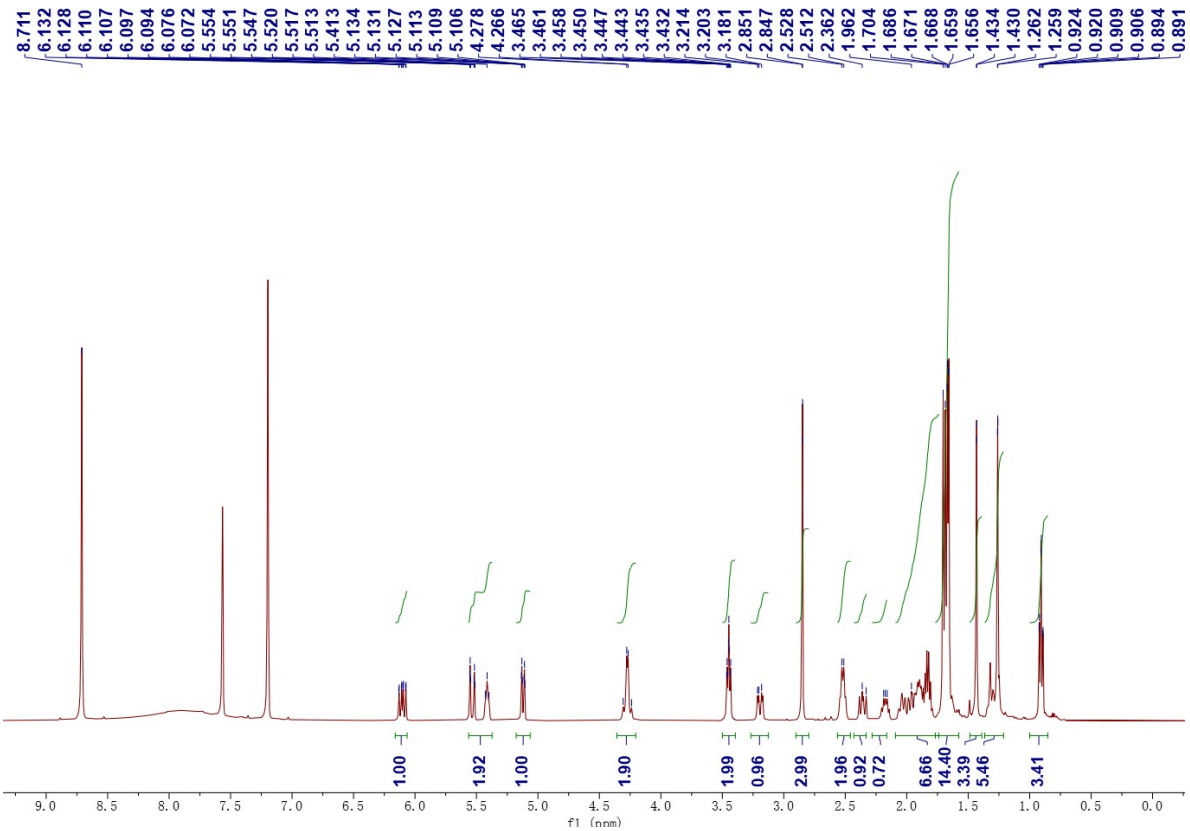


Figure S2 ^{1}H-NMR spectrum (500 MHz) of (±)-1 in pyridine-d_5.

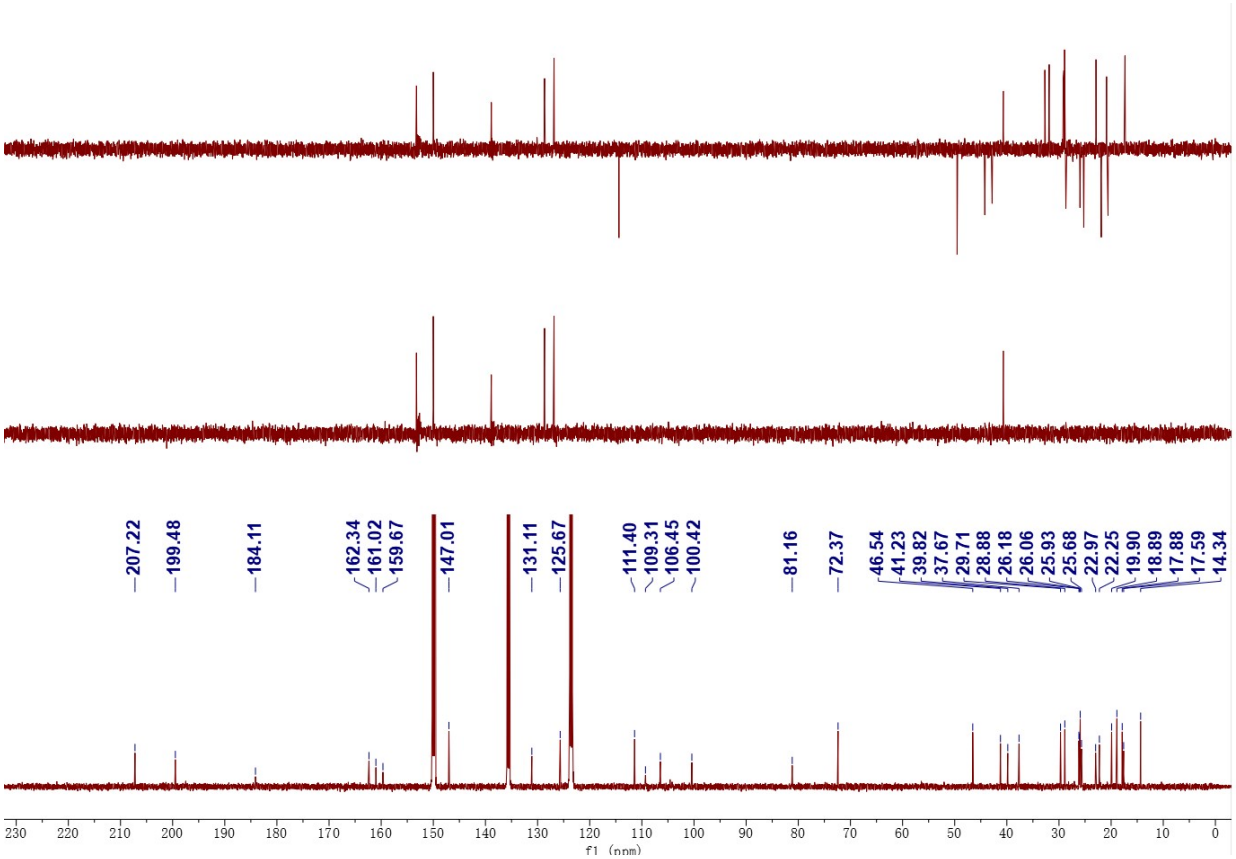


Figure S3 ^{13}C-NMR spectrum (125 MHz) of (±)-1 in pyridine-d_5.

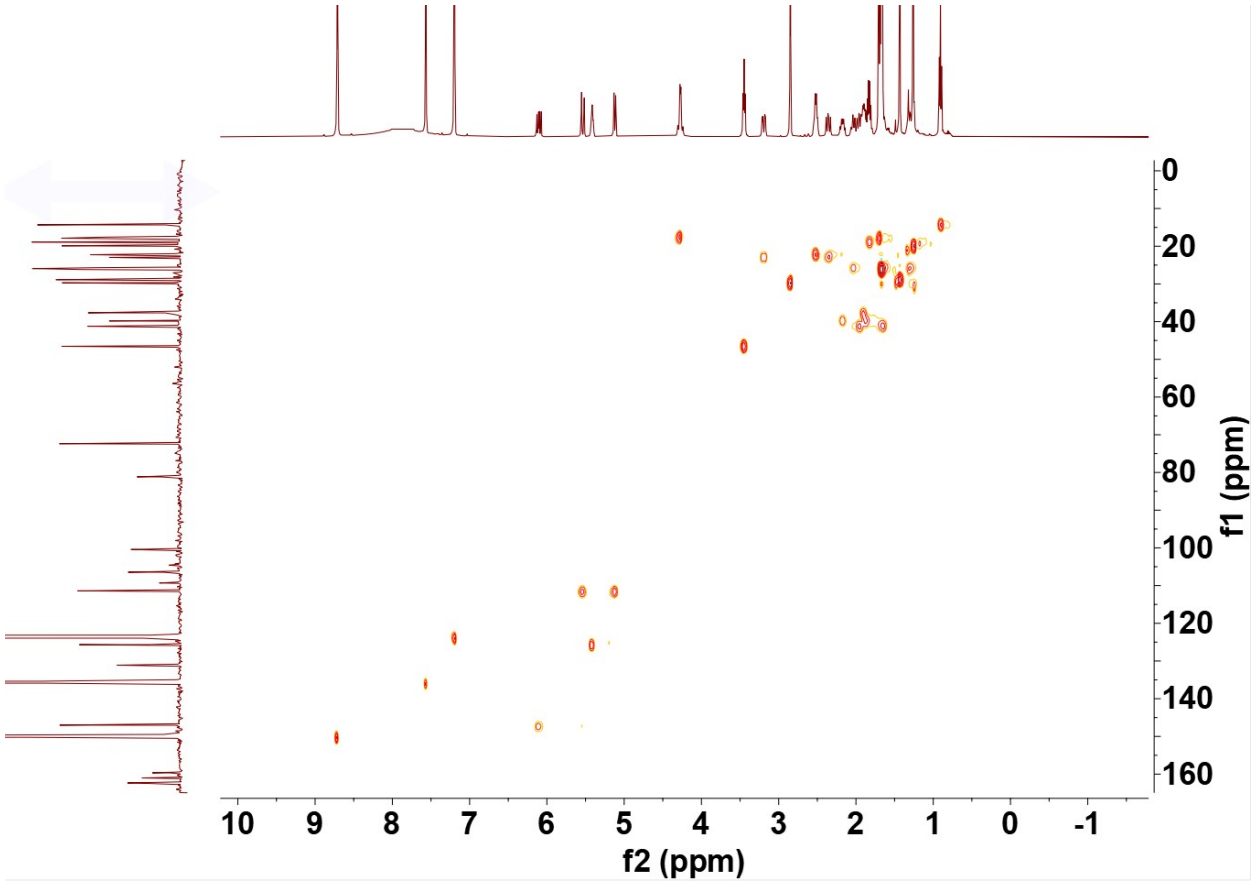


Figure S4 HSQC spectrum (500 MHz) of (±)-1 in pyridine-d_5.

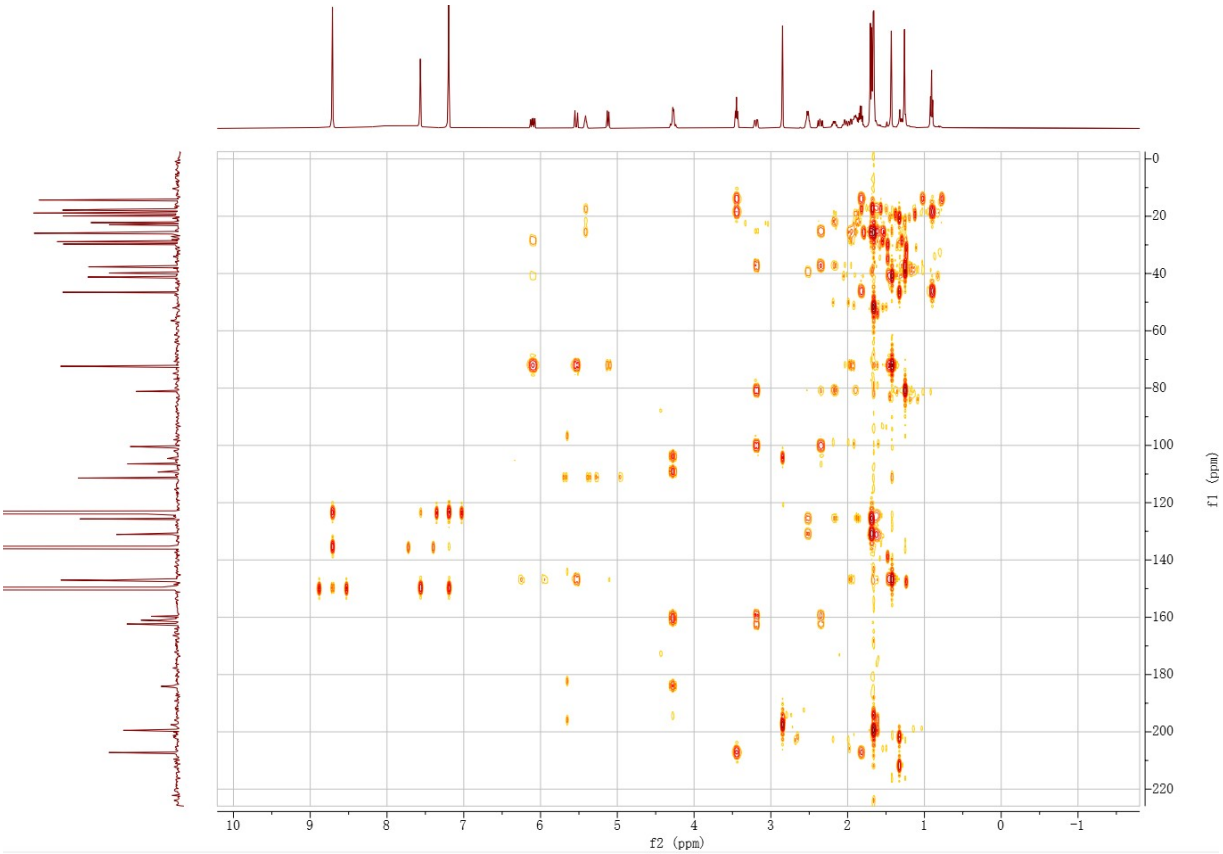


Figure S5 HMBC spectrum (500 MHz) of 1 in pyridine-d_5.

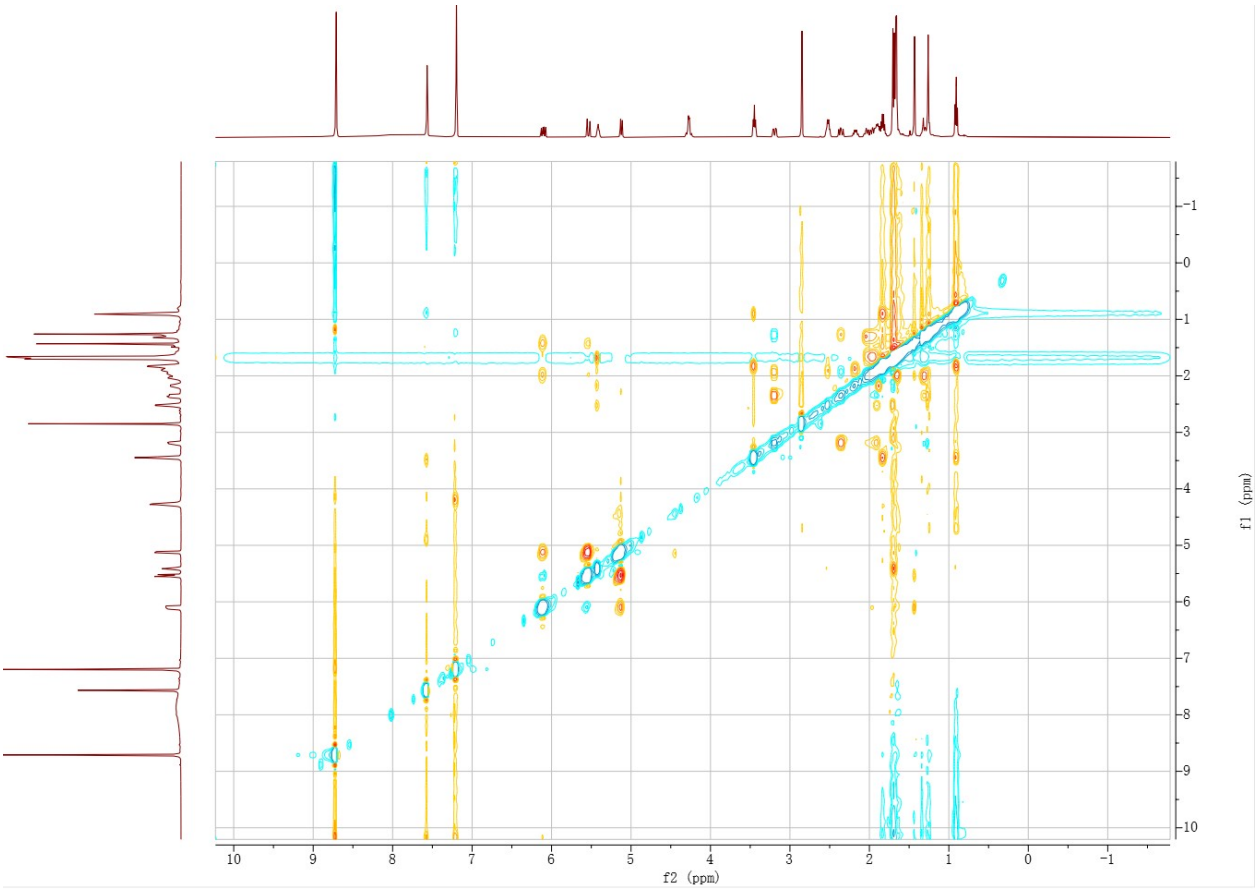


Figure S6 ROESY spectrum (500 MHz) of (±)-1 in pyridine-d_5.

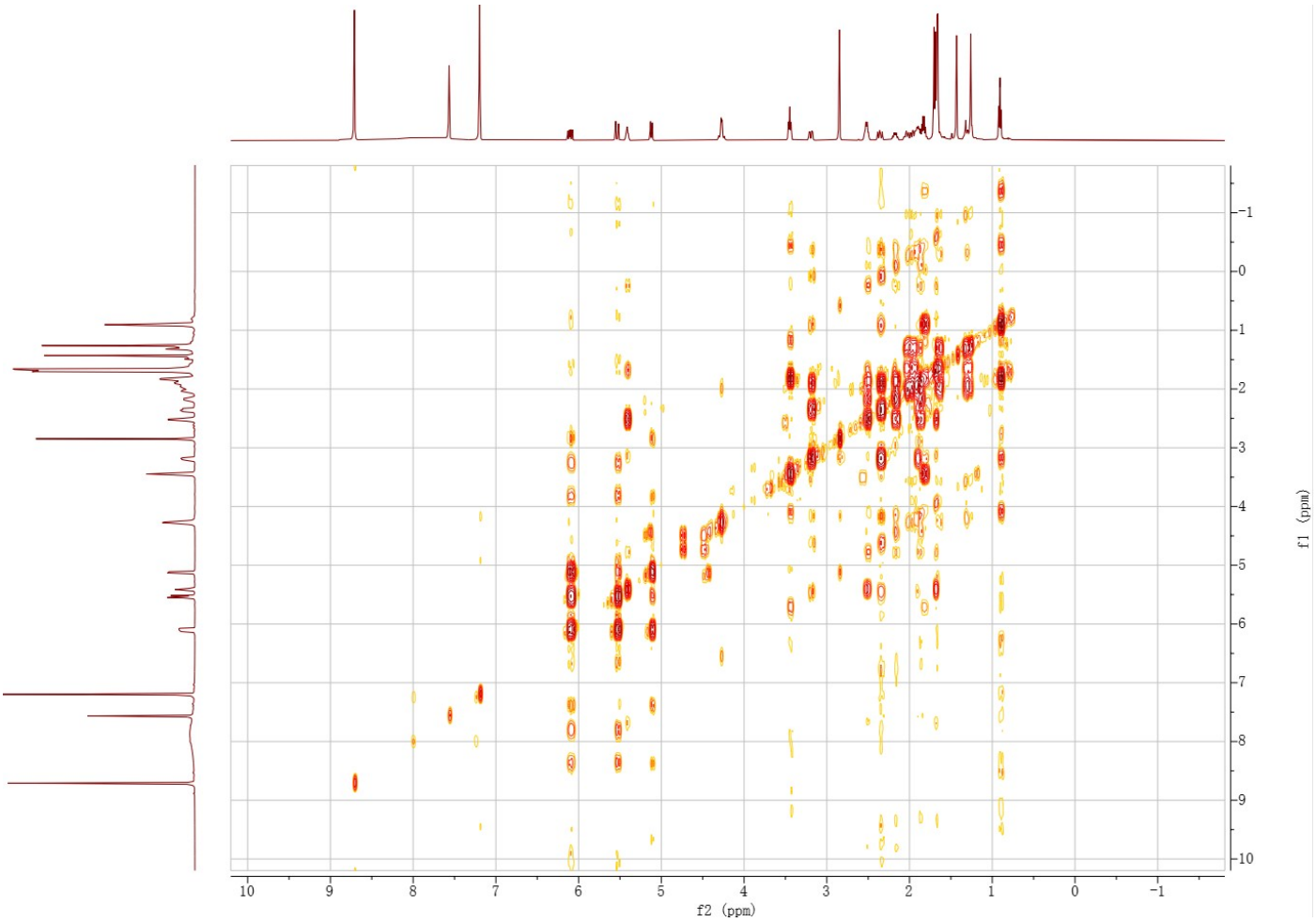


Figure S7 COSY spectrum (500 MHz) of (±)-1 in pyridine-d_5.

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C	4	10	50	Mg	2	0	0	Co	2	0	0	Ag	1	0	0	
N	3	0	10	Si	4	0	0	Cu	2	0	0	I	3	0	0	
O	2	0	30	P	3	0	0	Se	2	0	0					

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Use MSn Info: yes
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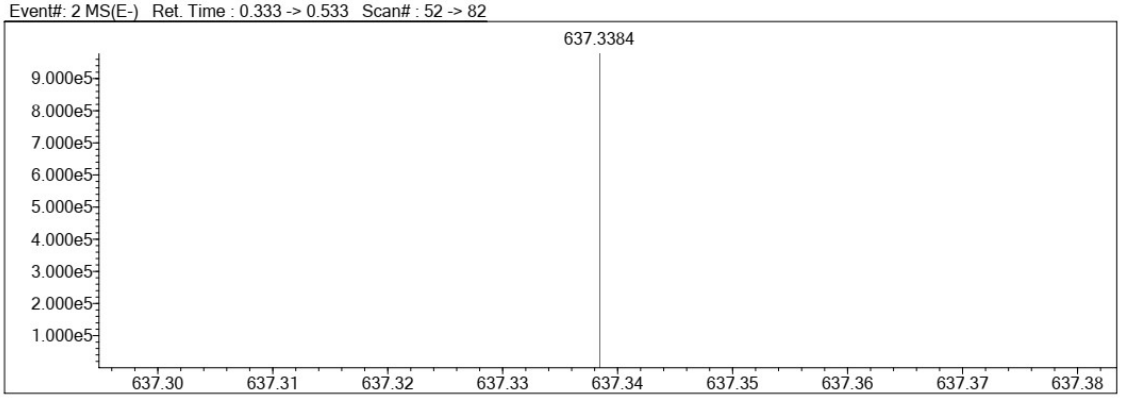
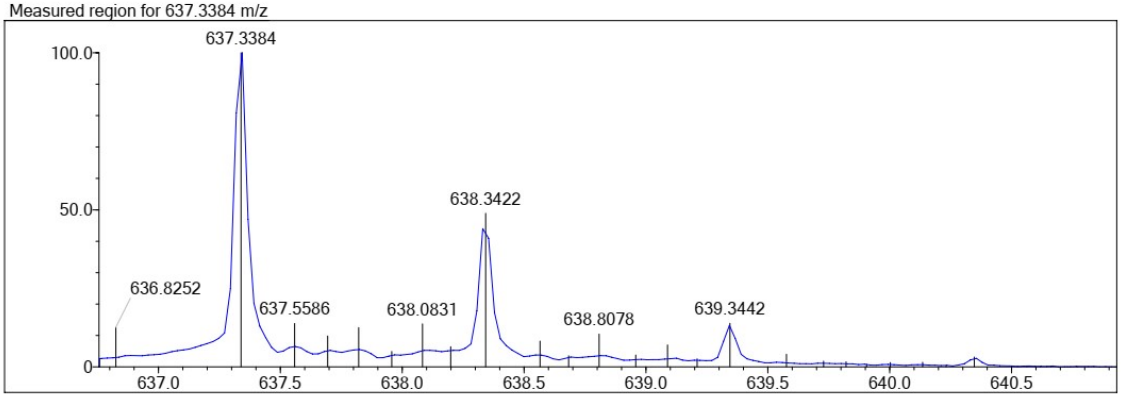
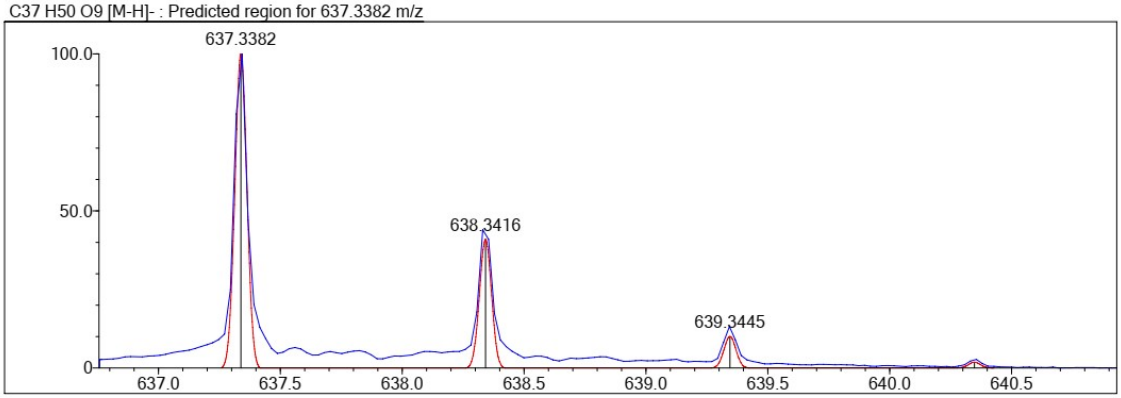


Measured region for 637.3384 m/z



C37 H50 O9 [M-H]- : Predicted region for 637.3382 m/z



Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	DBE
C37 H50 O9	[M-H]-	637.3384	637.3382	0.2	0.31	13.0

Figure S8 HRESIMS spectrum of (±)-1.

4. NMR and MS spectra of compound (±)-2

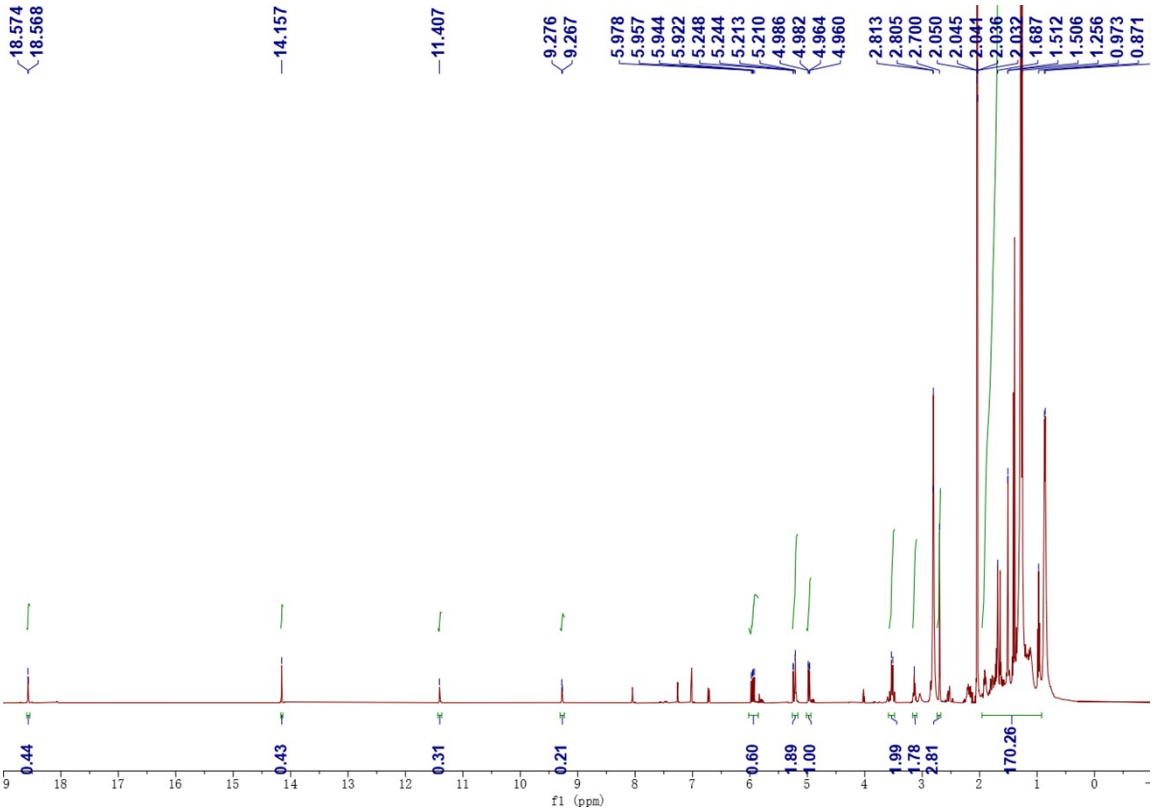


Figure S9 ^{1}H-NMR spectrum (500 MHz) of (±)-2 in acetone-d_6.

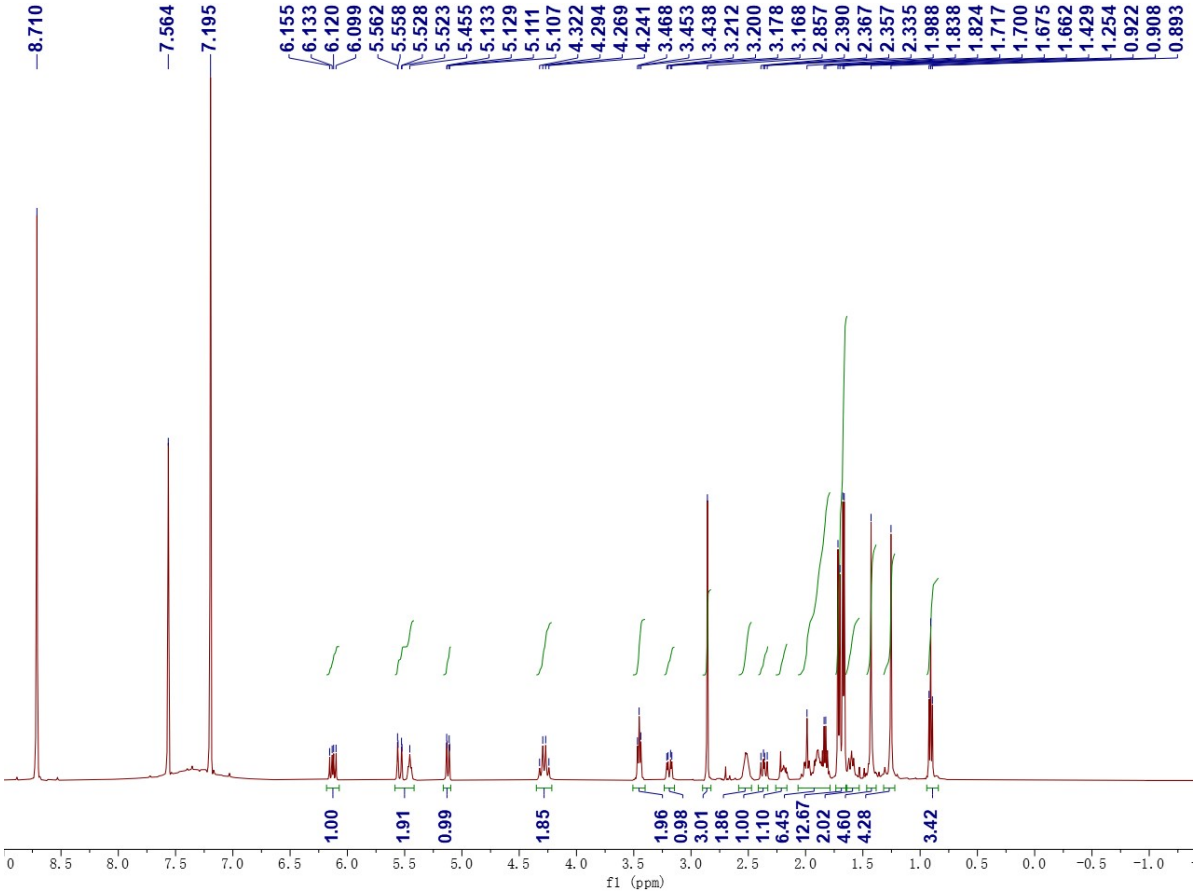


Figure S10 ^{1}H-NMR spectrum (500 MHz) of (±)-2 in pyridine-d_5.

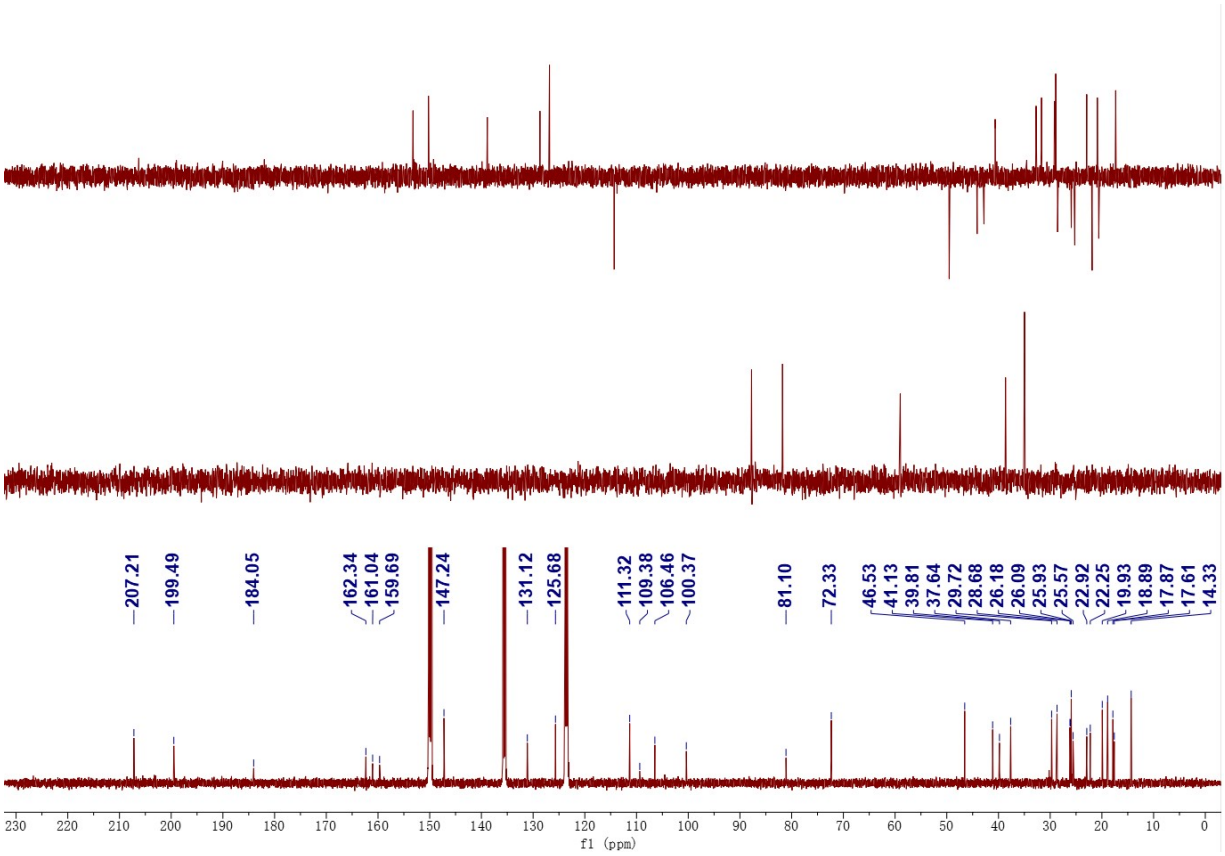


Figure S11 ^{13}C-NMR spectrum (125 MHz) of (±)-2 in pyridine-d_5.

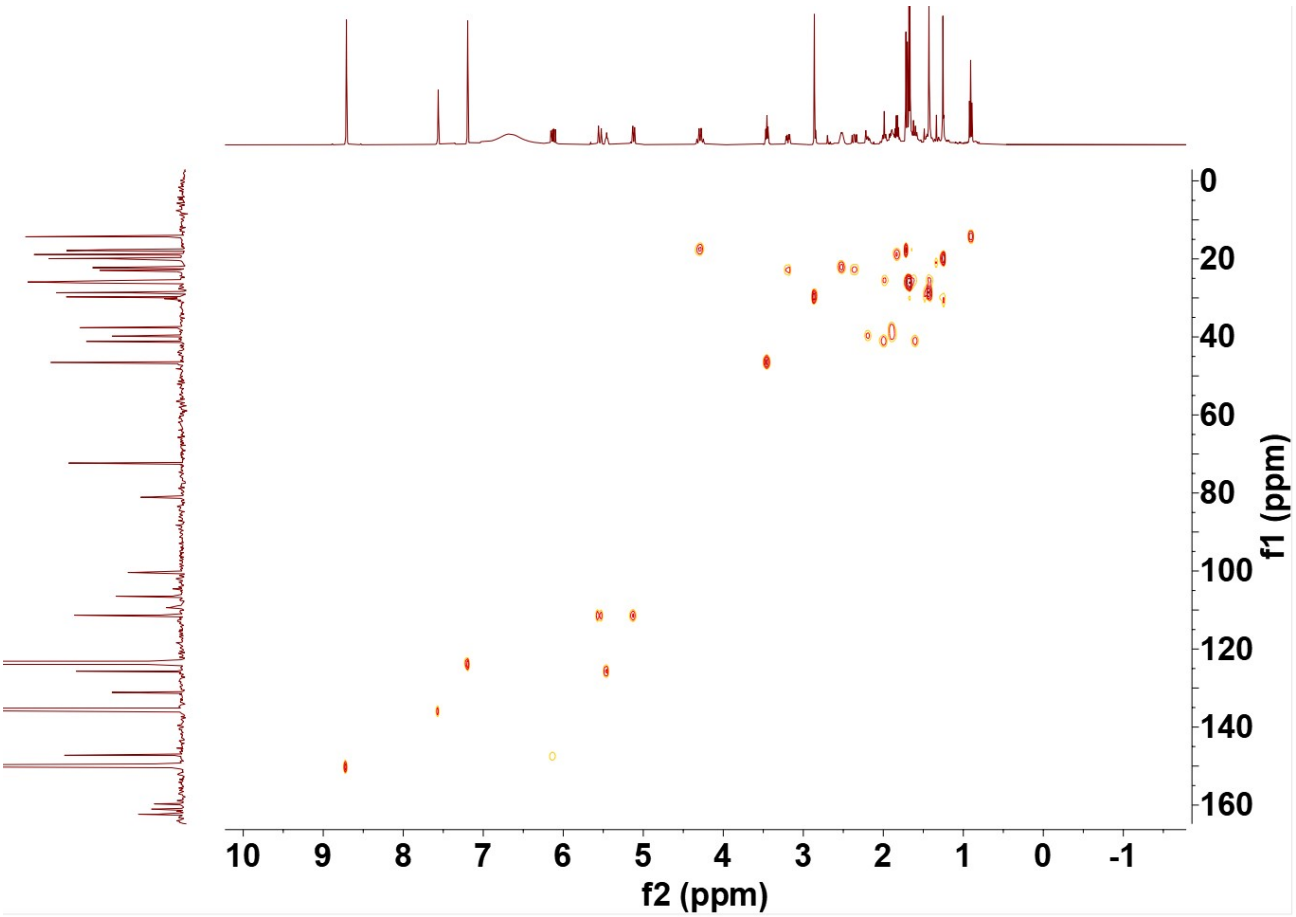


Figure S12 HSQC spectrum (500 MHz) of (±)-2 in pyridine-d_5.

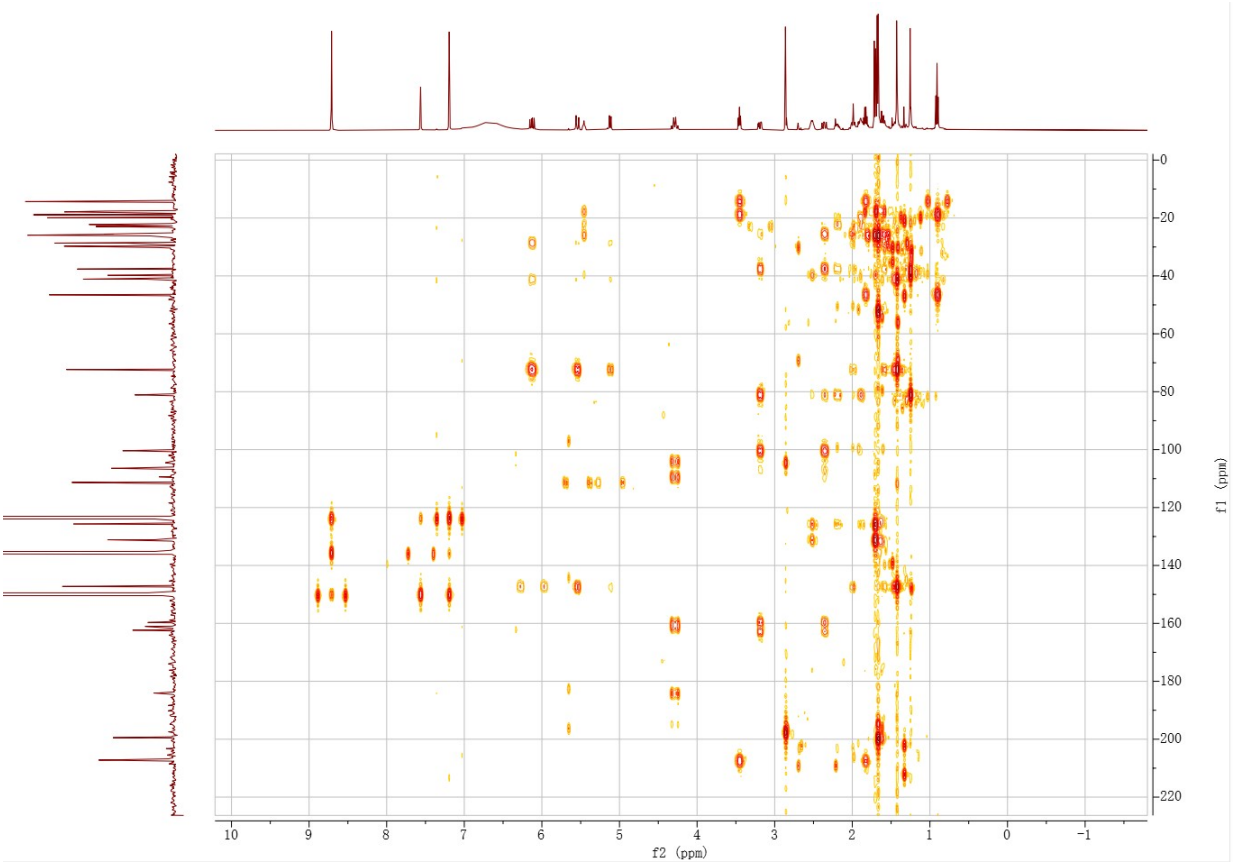


Figure S13 HMBC spectrum (500 MHz) of (±)-2 in pyridine-d_5.

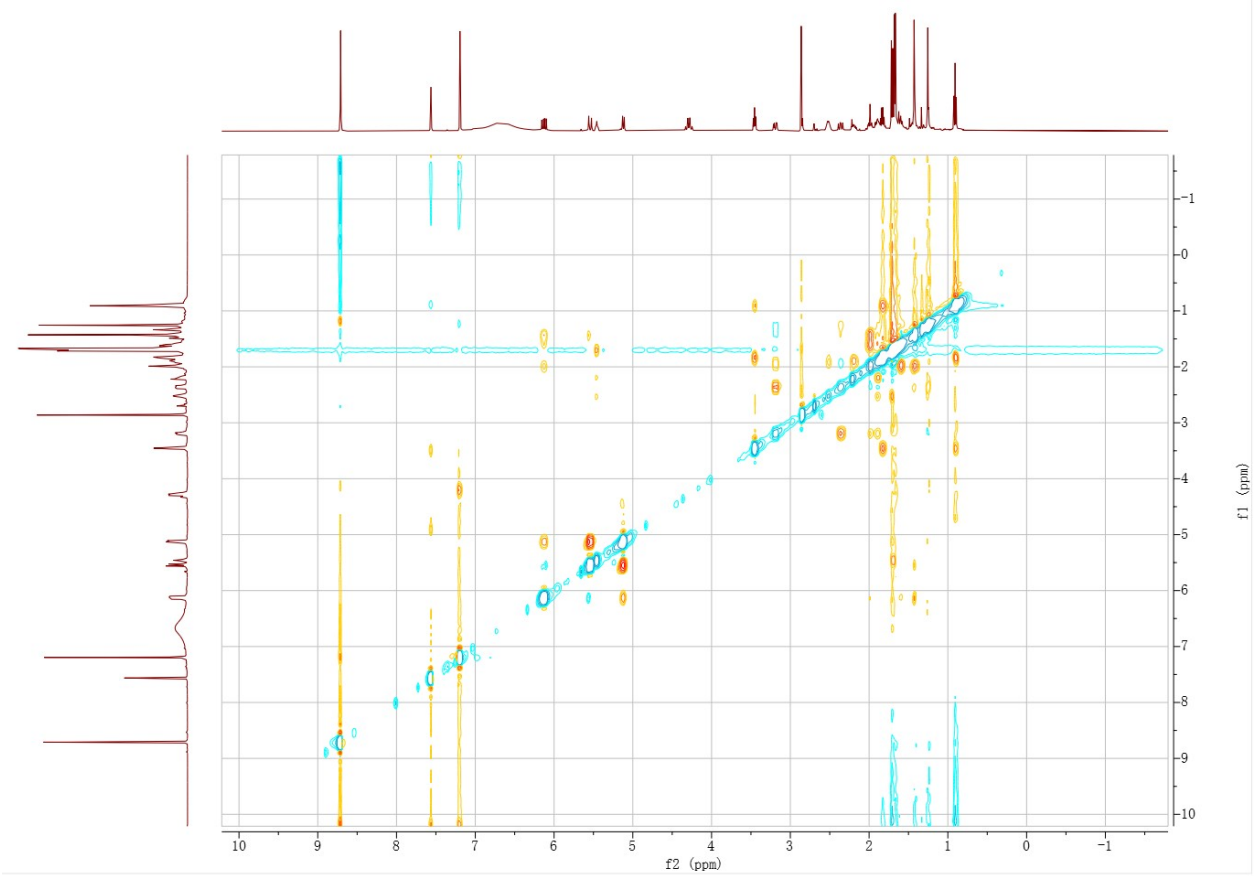


Figure S14 ROESY spectrum (500 MHz) of (±)-2 in pyridine-d_5.

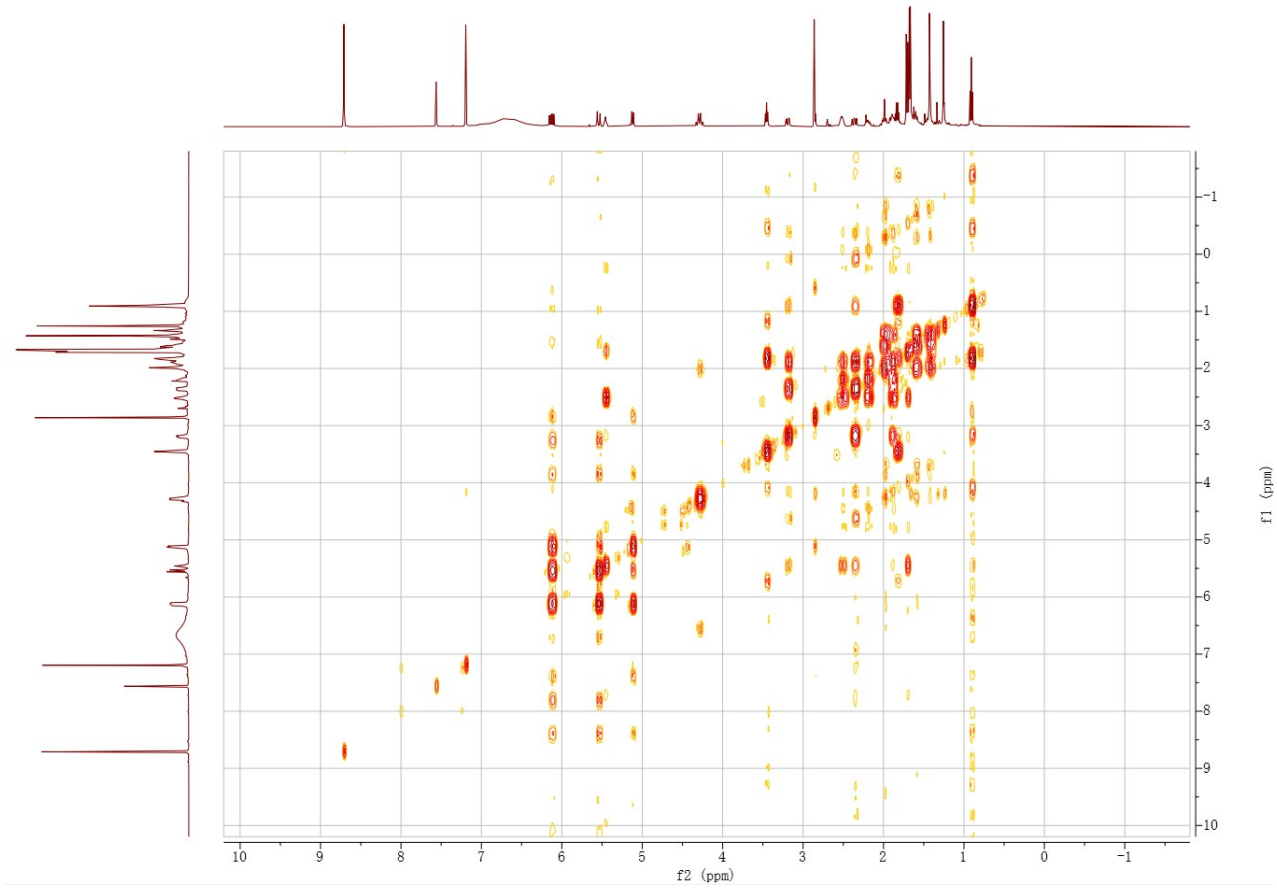


Figure S15 COSY spectrum (500 MHz) of (±)-2 in pyridine-d_5.

Data File: E:\DATA\2022\0610\1\Efuhp222.lcd

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2H	1	0	0	Na	1	0	0	Co	2	0	0	I	3	0	0	
B	3	0	0	Mg	2	0	0	Cu	2	0	0	Ir	3	0	0	
C	4	5	50	Si	4	0	0	Se	2	0	0					
N	3	0	10	P	3	0	0	Br	1	0	0					
O	2	0	30	S	2	0	0	Pd	2	0	0					

Error Margin (ppm): 5
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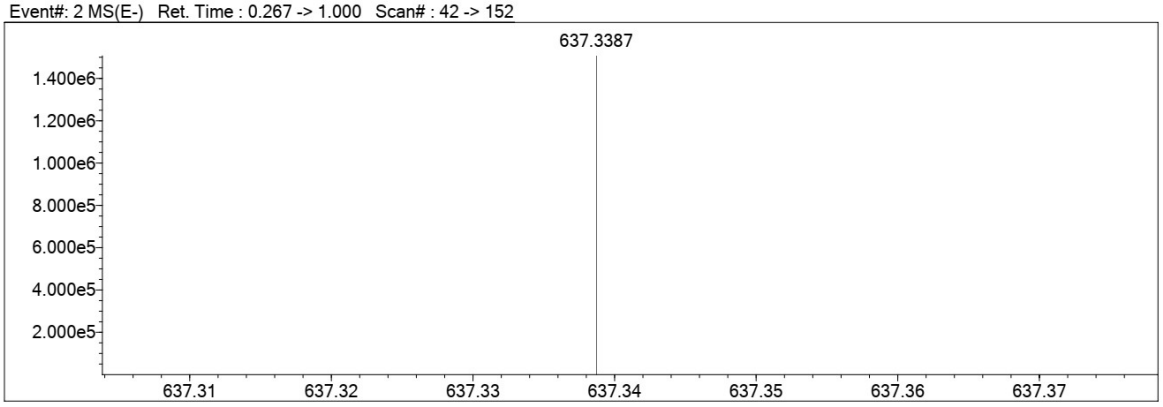
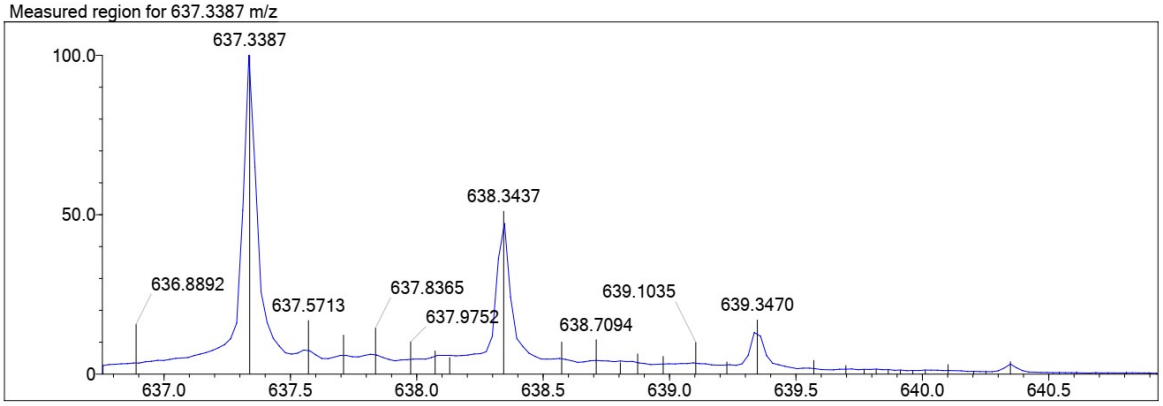
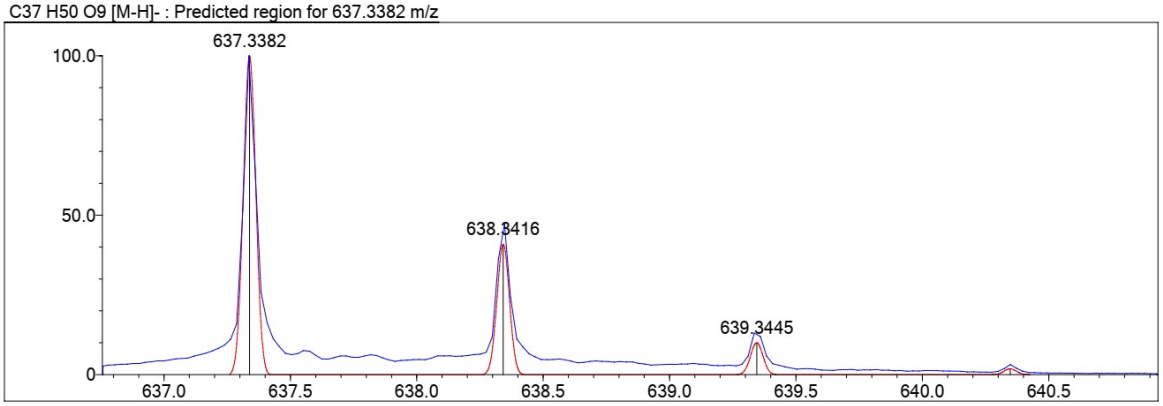


Measured region for 637.3387 m/z



C37 H50 O9 [M-H]- : Predicted region for 637.3382 m/z



Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	DBE
C37 H50 O9	[M-H]-	637.3387	637.3382	0.5	0.78	13.0

Figure S16 HRESIMS spectrum of (±)-2.

5. NMR and MS spectra of compound 1~8.

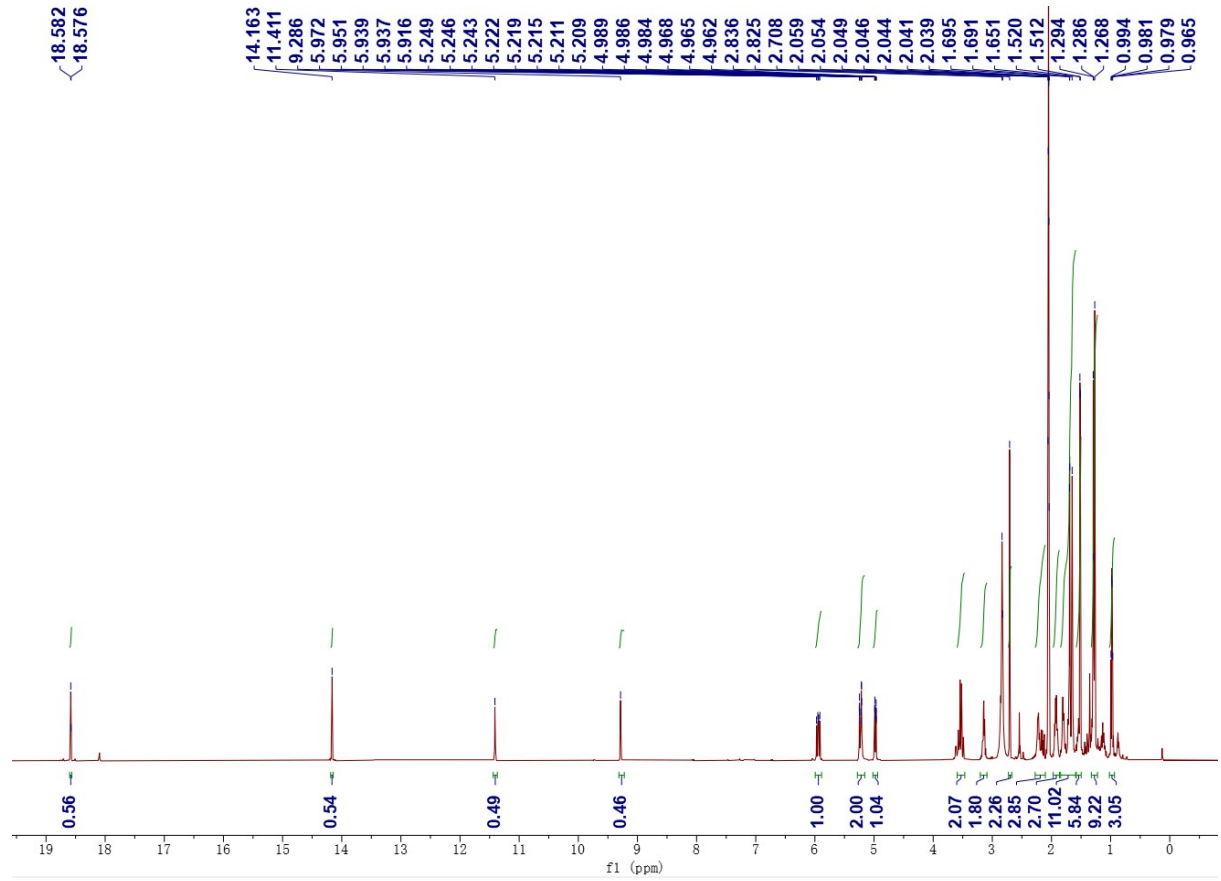


Figure S17 ^{1}H-NMR spectrum (500 MHz) of 1 in acetone-d_6.

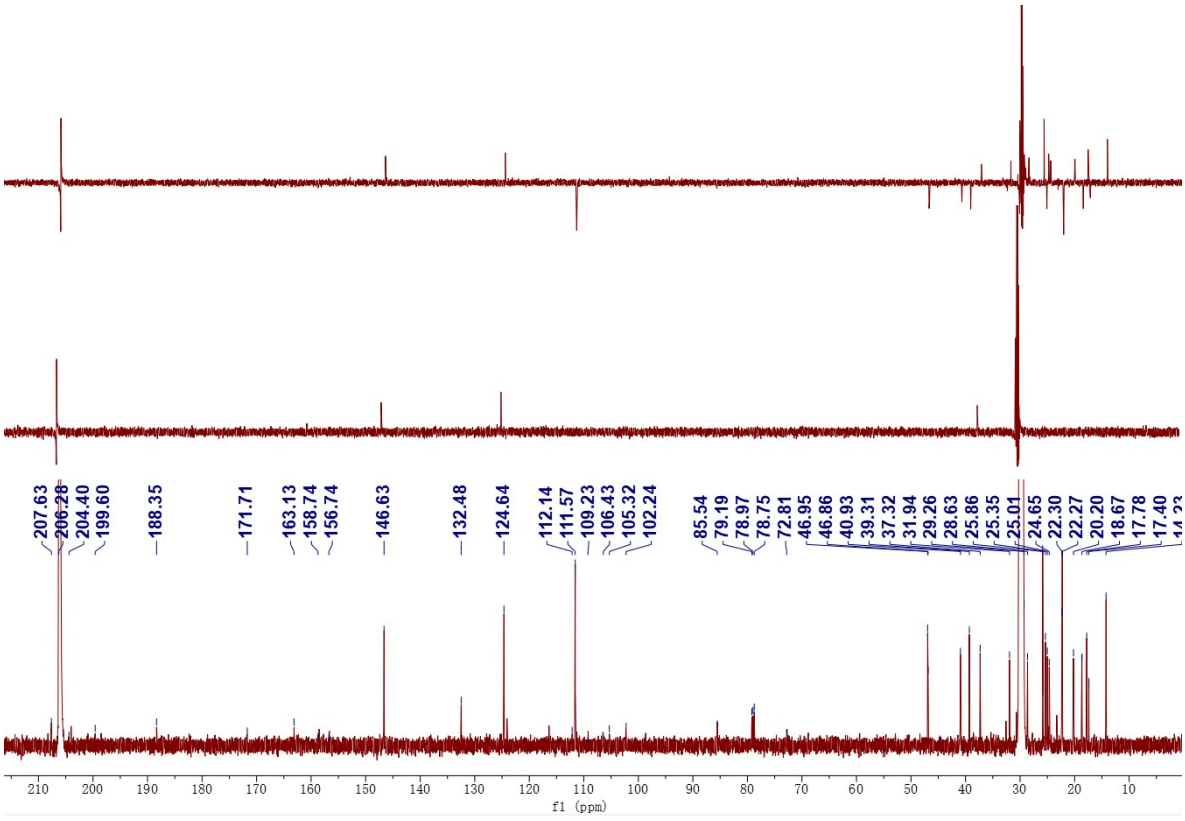


Figure S18 ^{13}C-NMR spectrum (125 MHz) of 1 in acetone-d_6.

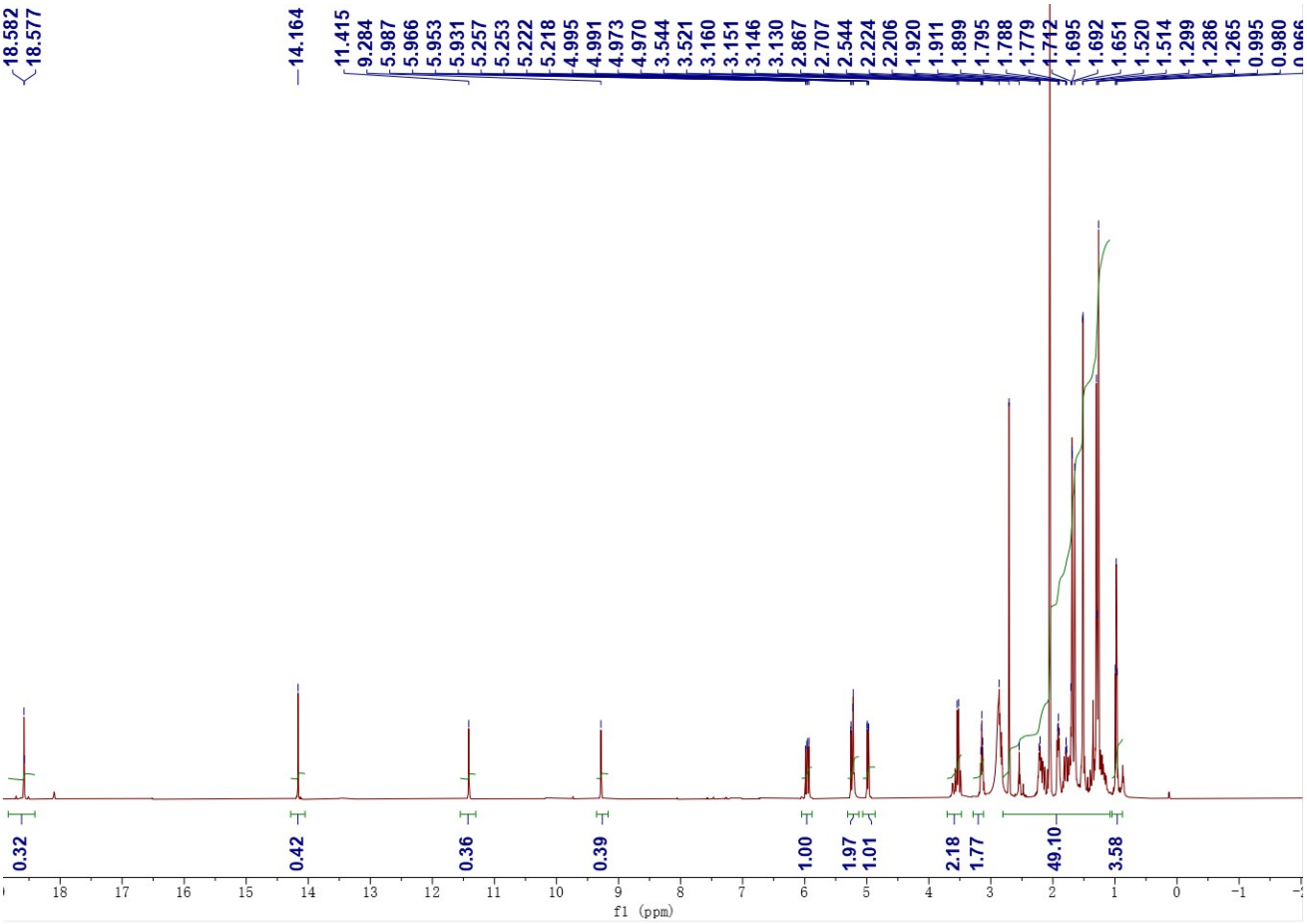


Figure S19 ^{1}H-NMR spectrum (500 MHz) of 2 in acetone-d_6.

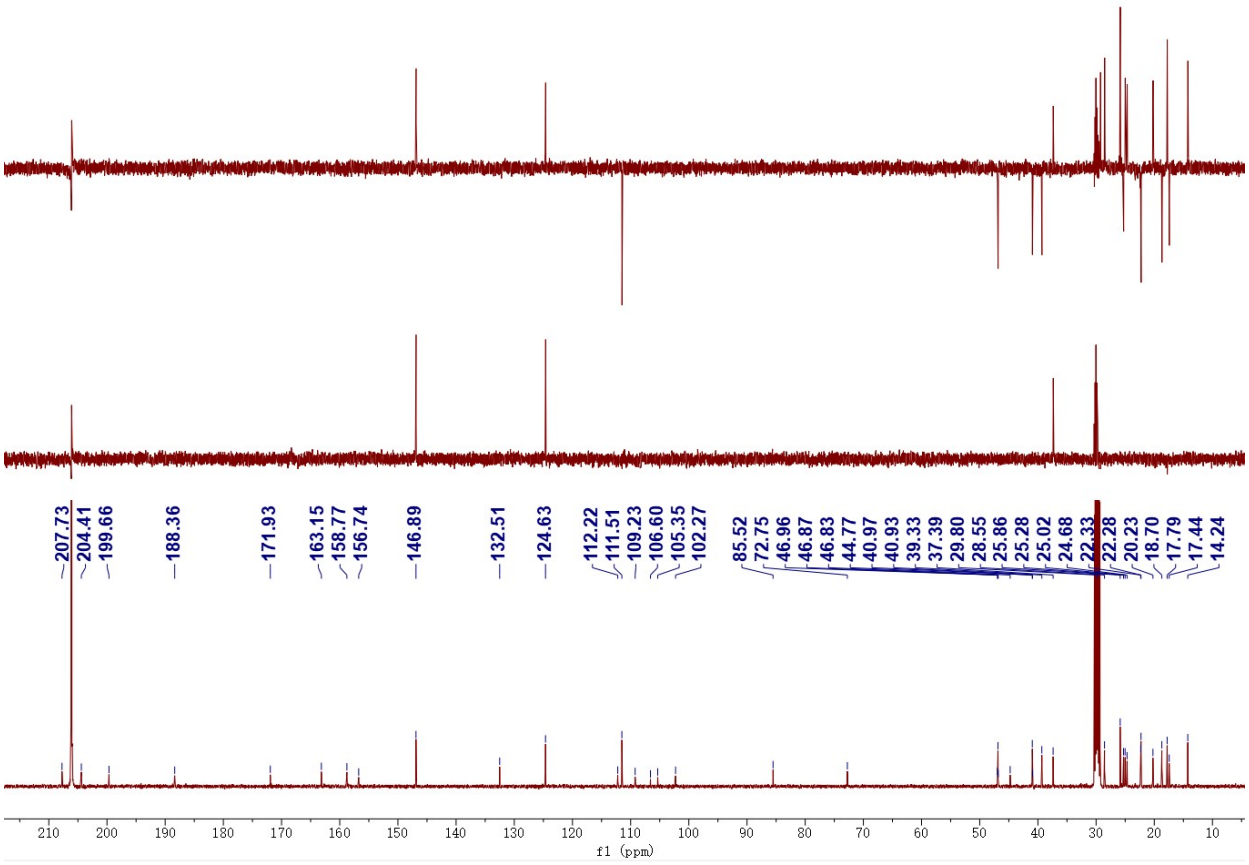


Figure S20 ^{13}C-NMR spectrum (125 MHz) of 2 in acetone-d_6.

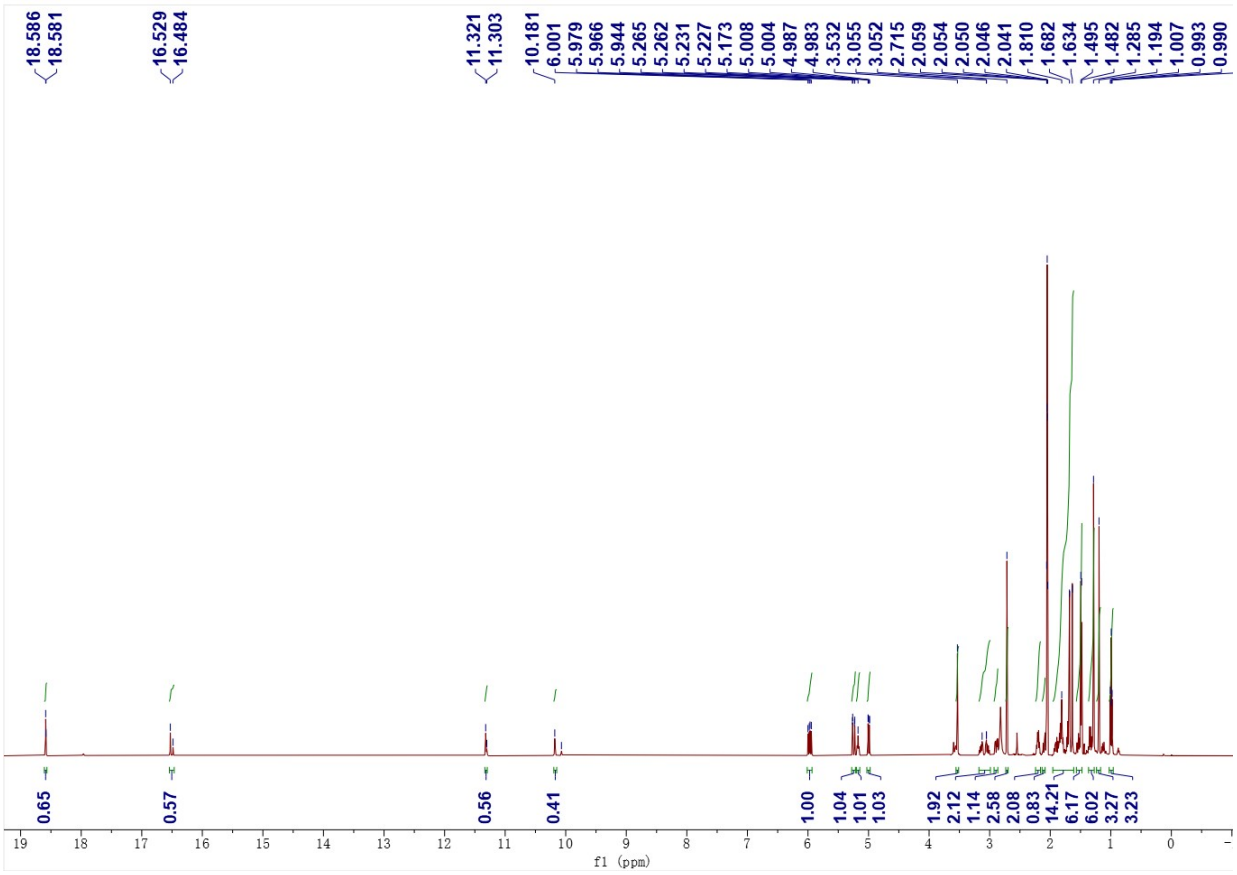


Figure S21 ^{1}H-NMR spectrum (500 MHz) of 3 in acetone-d_6.

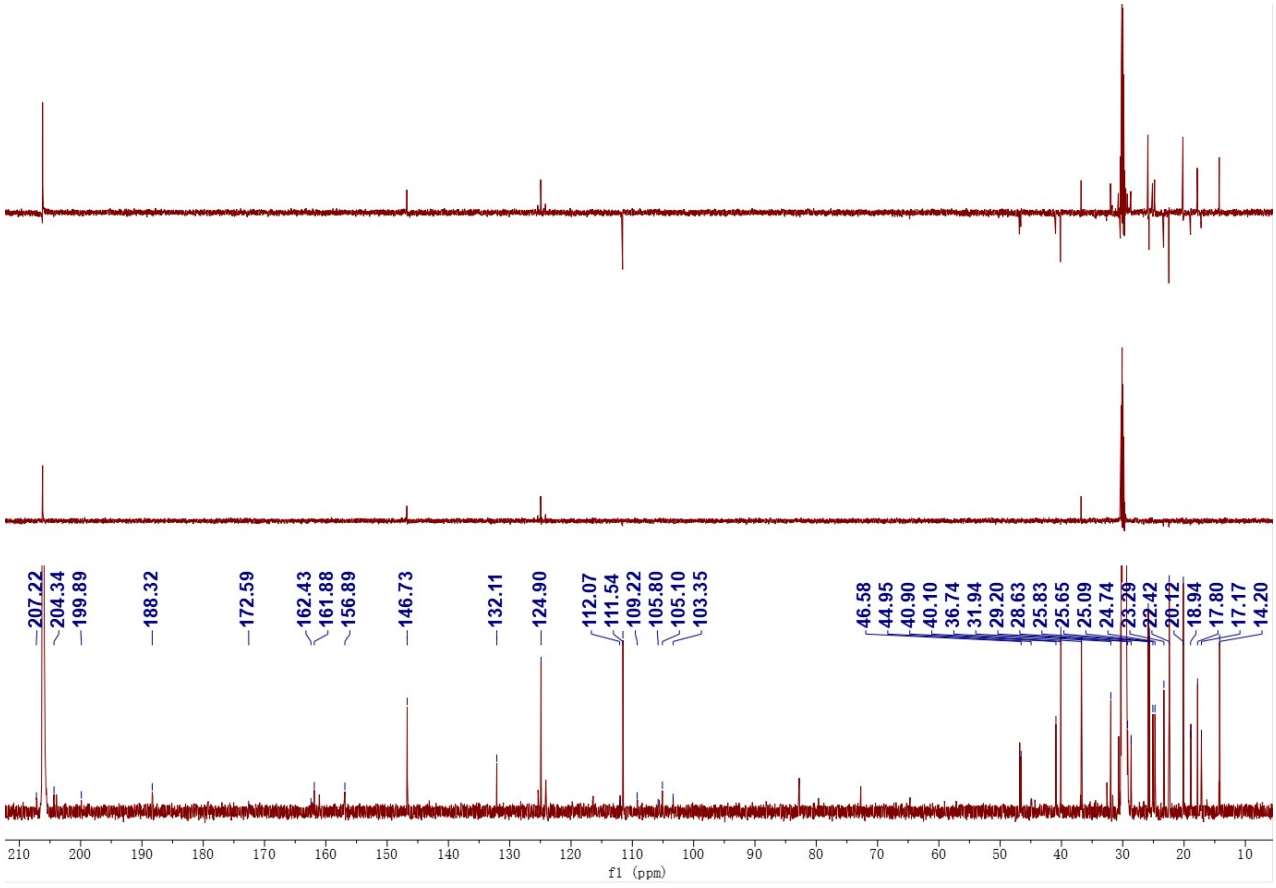


Figure S22 ^{13}C-NMR spectrum (125 MHz) of 3 in acetone-d_6.

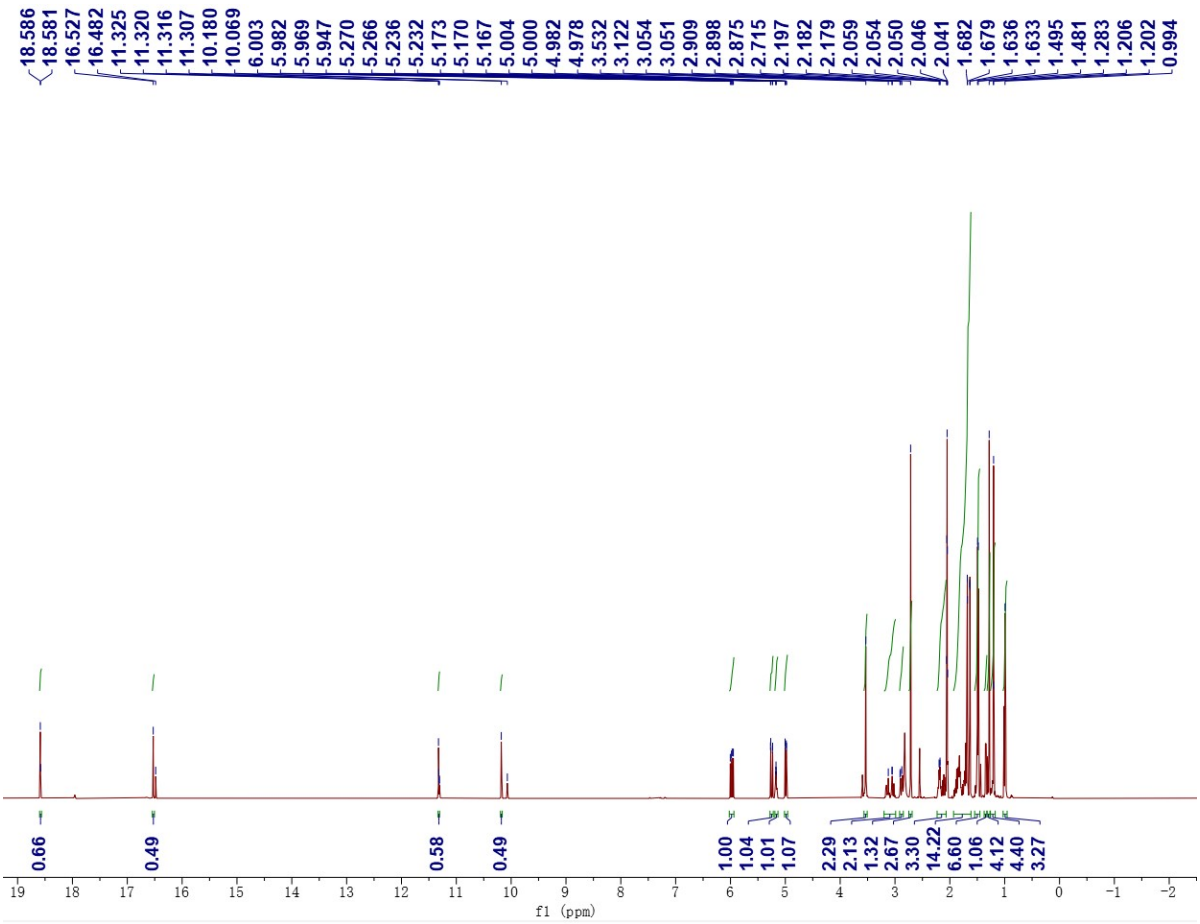


Figure S23 ^{1}H-NMR spectrum (500 MHz) of 4 in acetone-d_6.

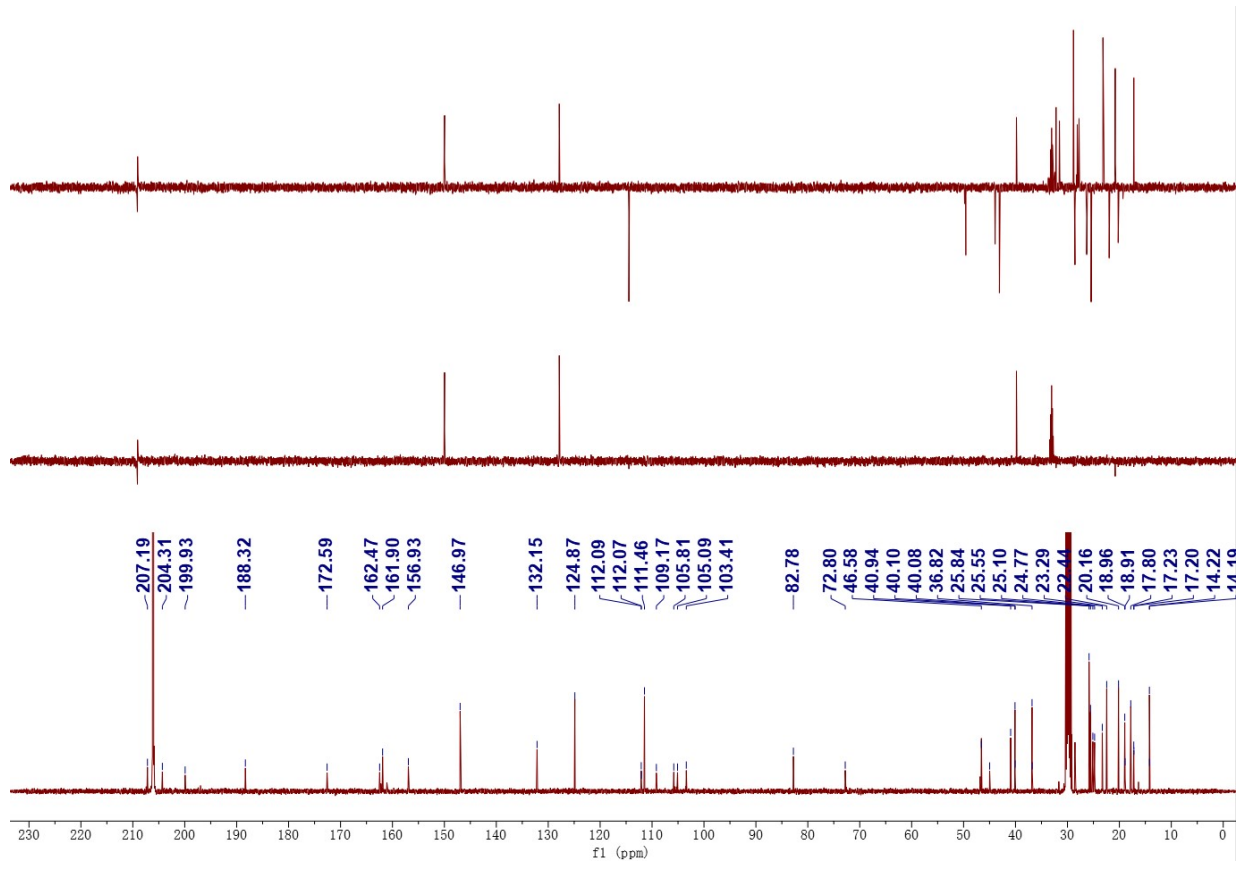


Figure S24 ^{13}C-NMR spectrum (125 MHz) of 4 in acetone-d_6.

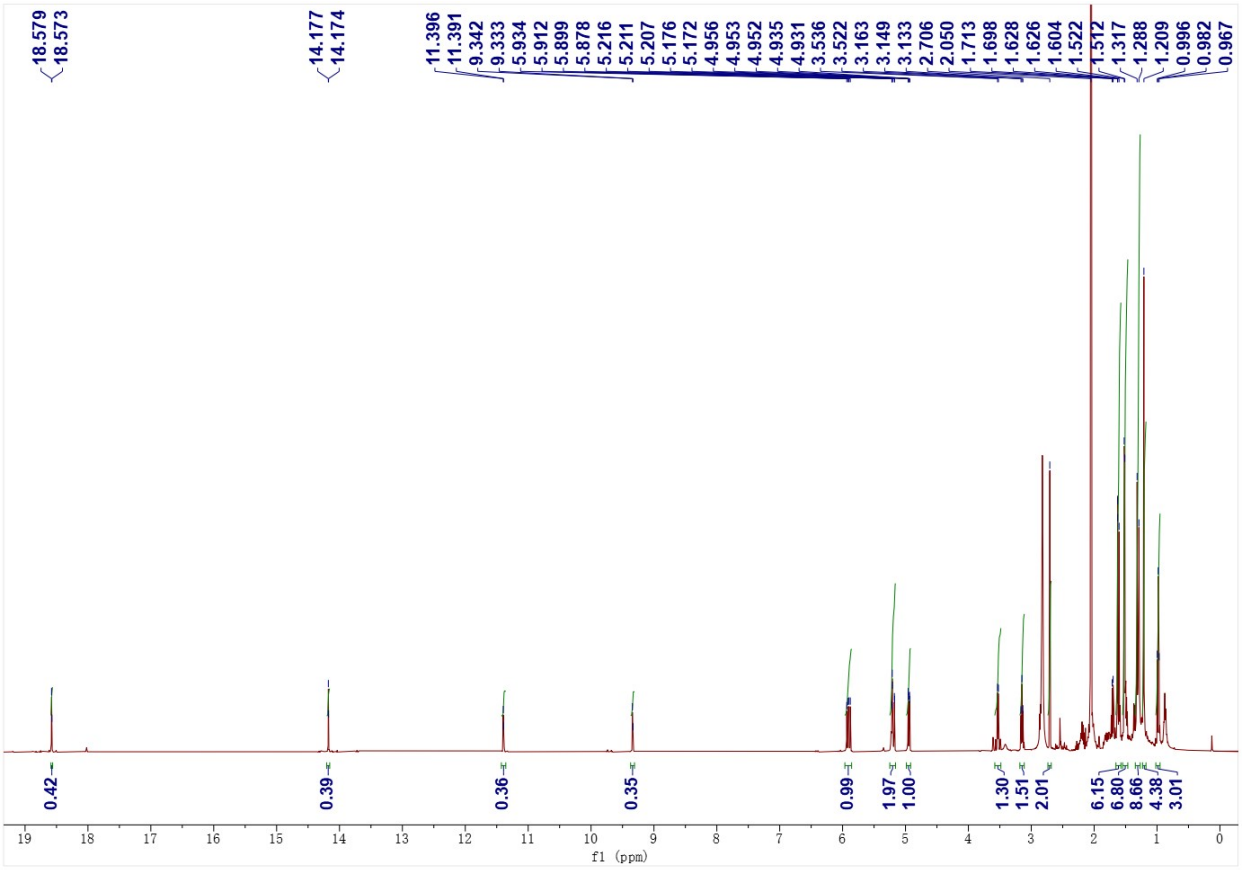


Figure S25 ^{1}H-NMR spectrum (500 MHz) of 5 in acetone-d_6.

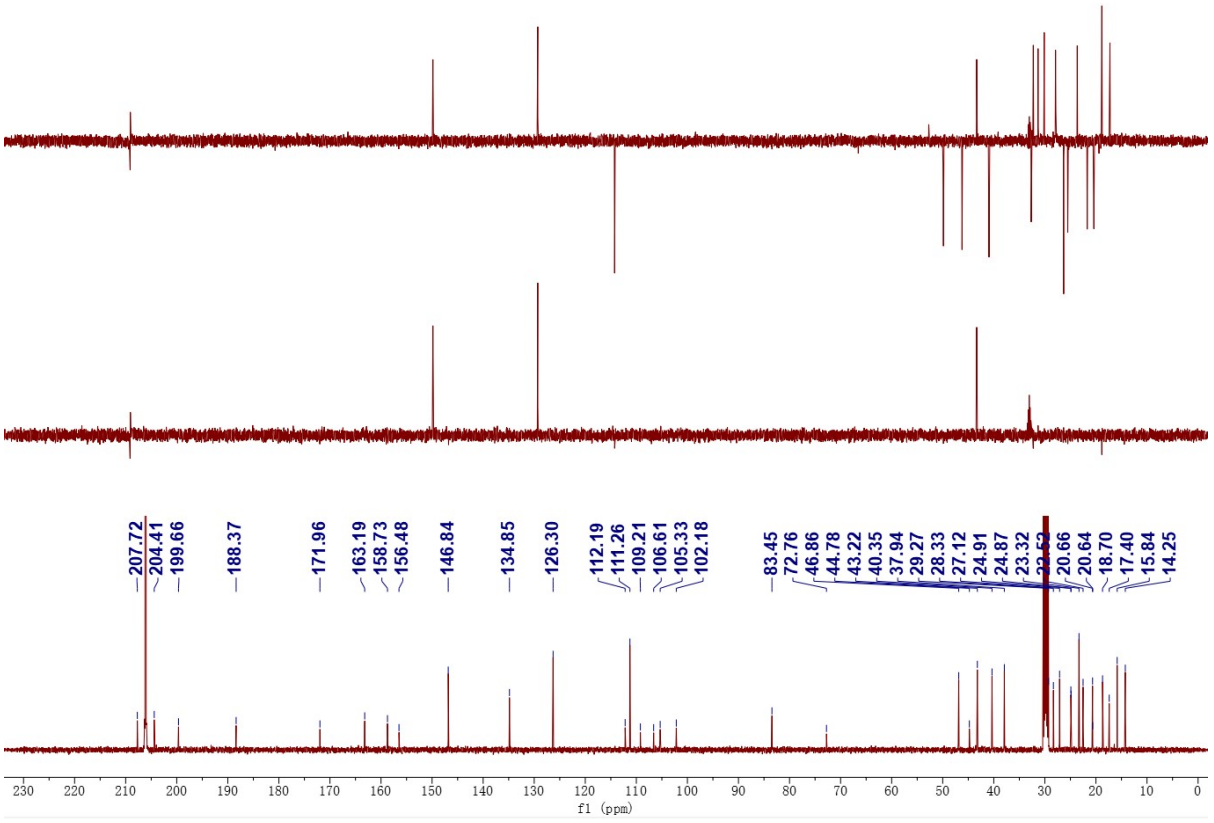


Figure S26 ^{13}C-NMR spectrum (125 MHz) of 5 in acetone-d_6.

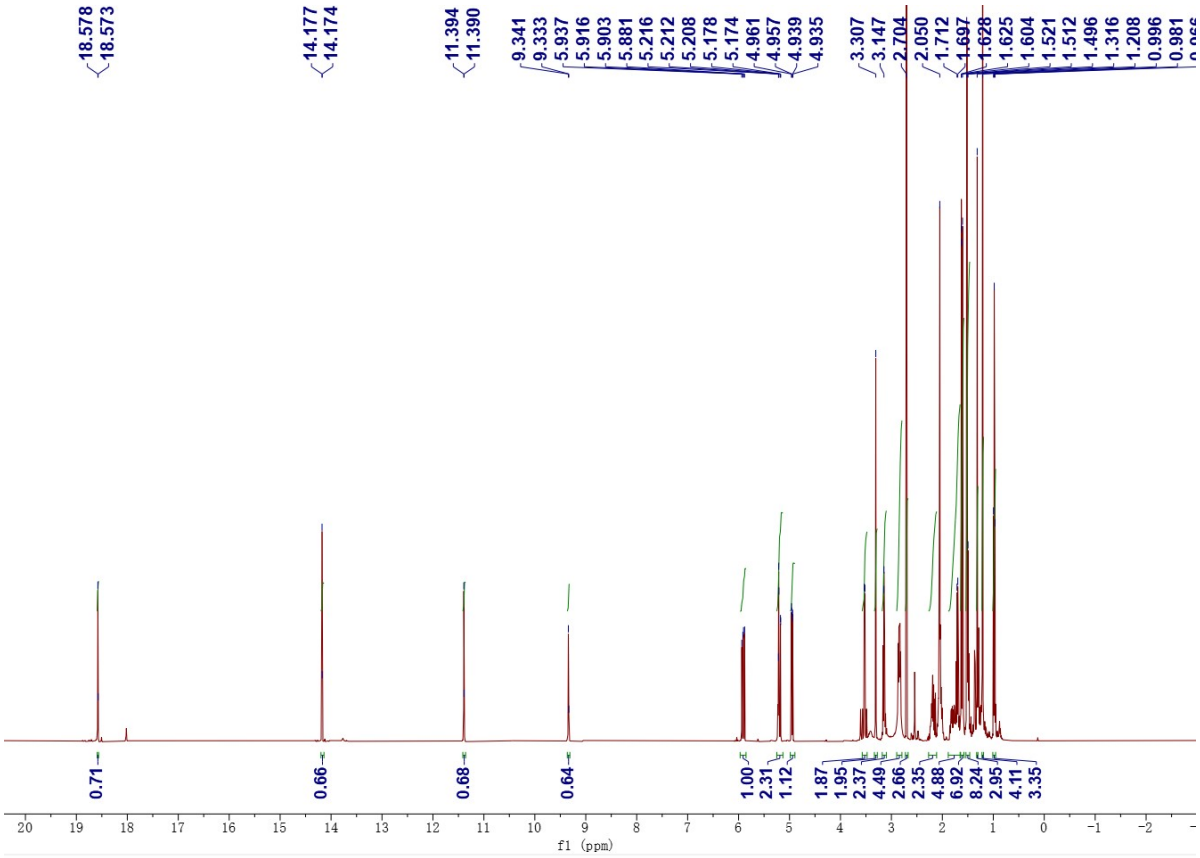


Figure S27 ^{1}H-NMR spectrum (500 MHz) of 6 in acetone-d_6.

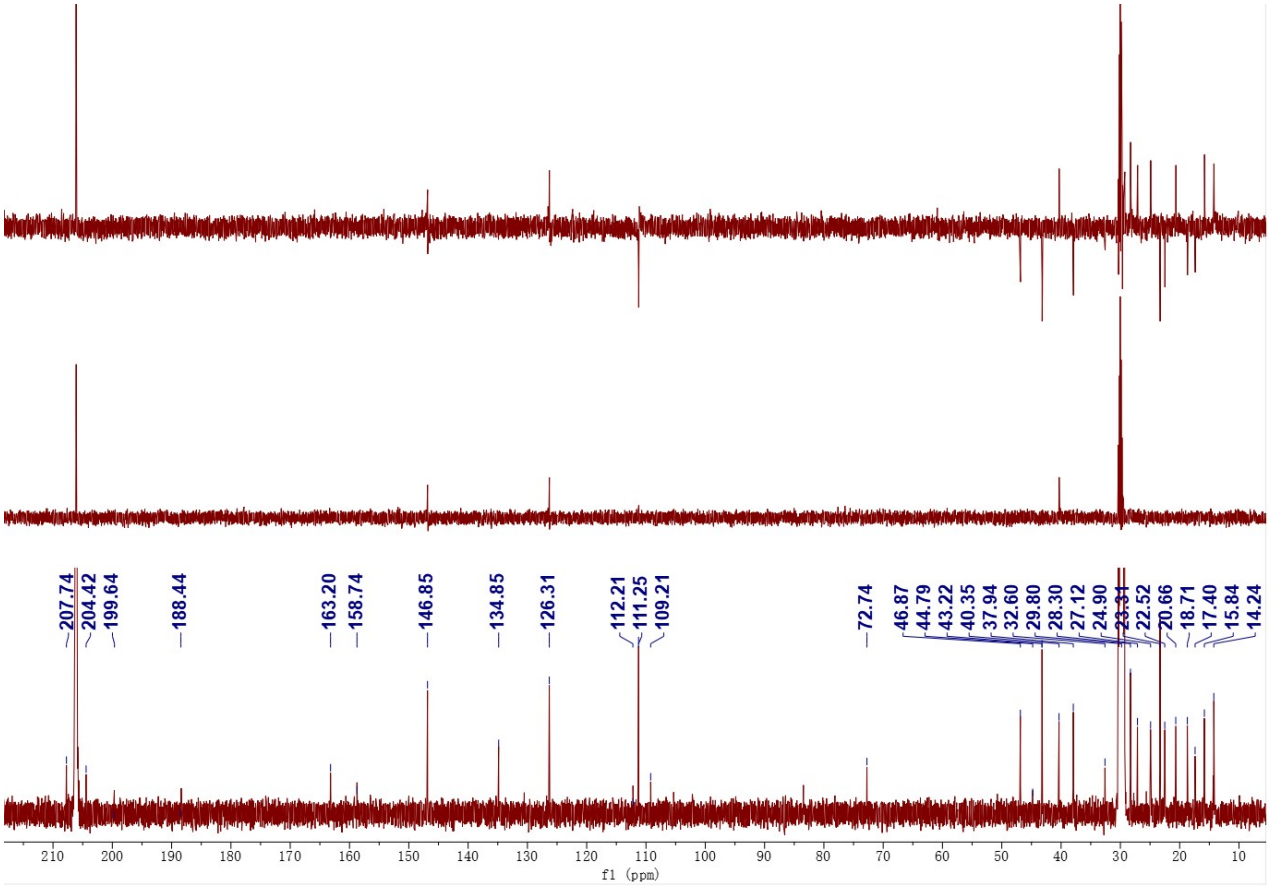


Figure S28 ^{13}C-NMR spectrum (125 MHz) of 6 in acetone-d_6.

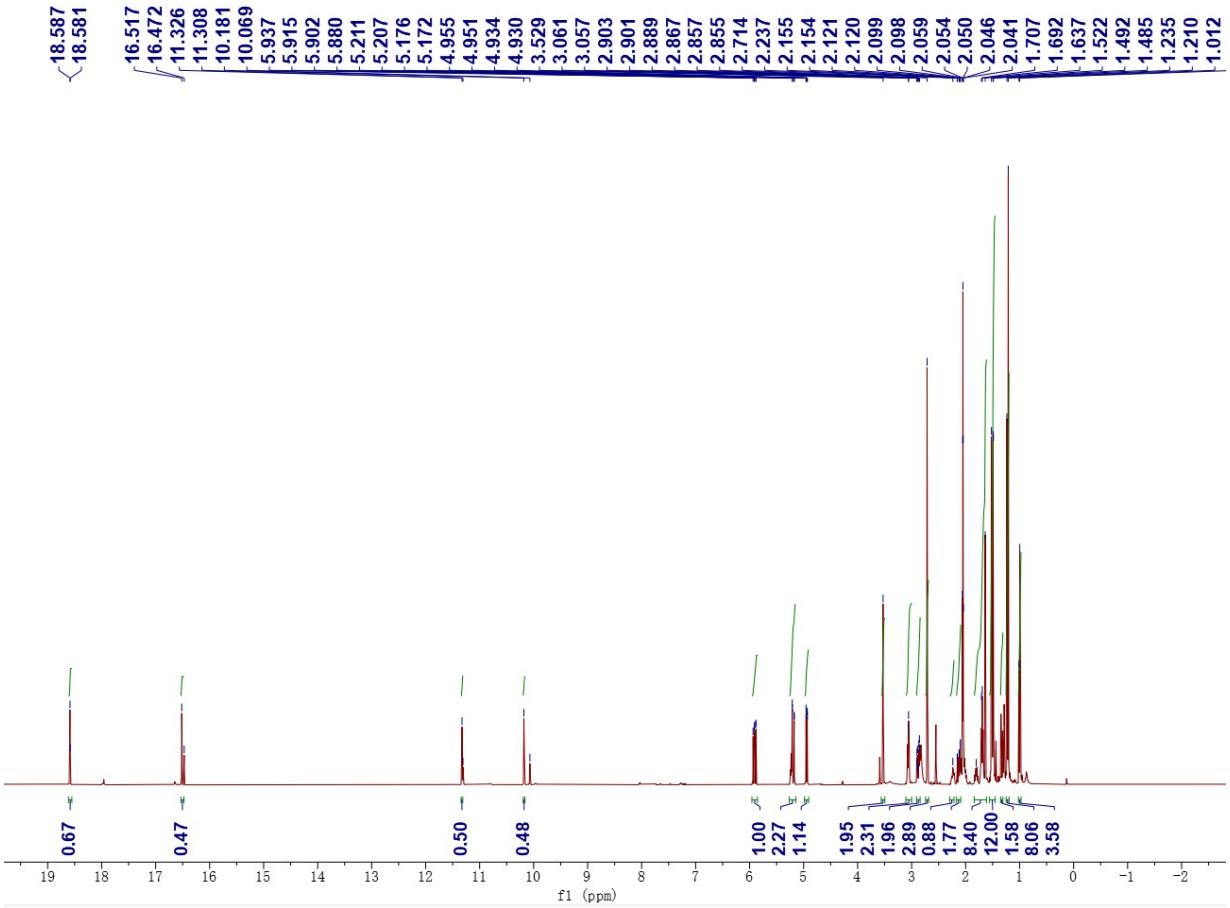


Figure S29 ^{1}H-NMR spectrum (500 MHz) of 7 in acetone-d_6.

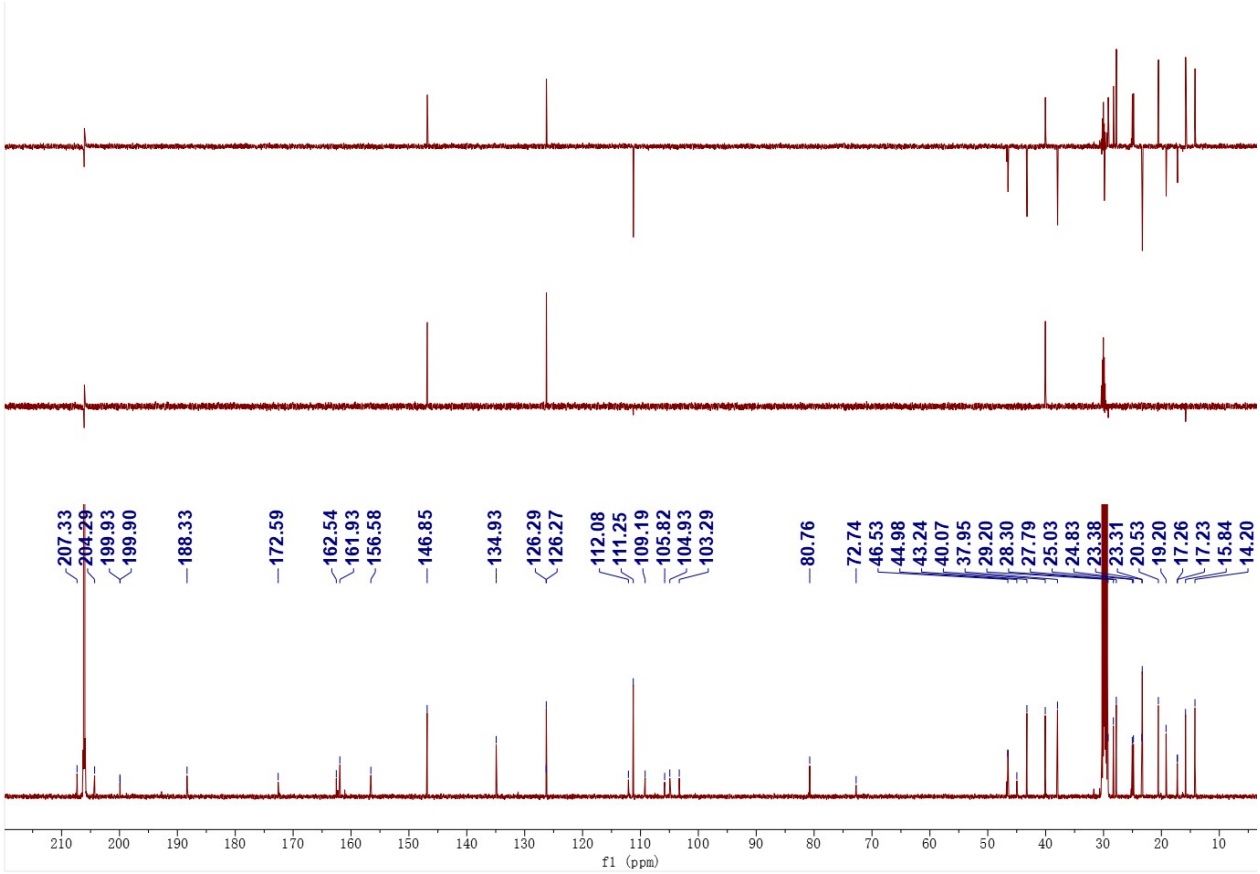


Figure S30 ^{13}C-NMR spectrum (125 MHz) of 7 in acetone-d_6.

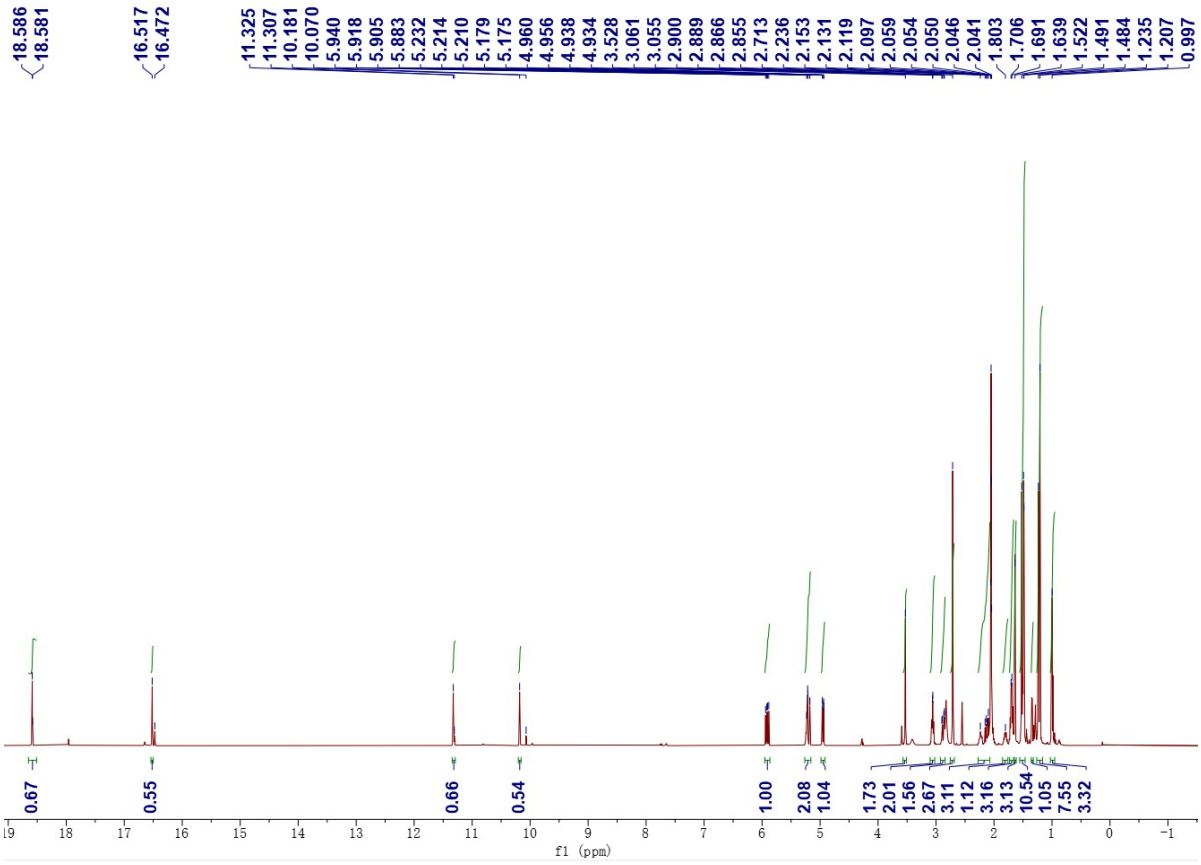


Figure S31 ^{1}H-NMR spectrum (500 MHz) of 8 in acetone-d_6.

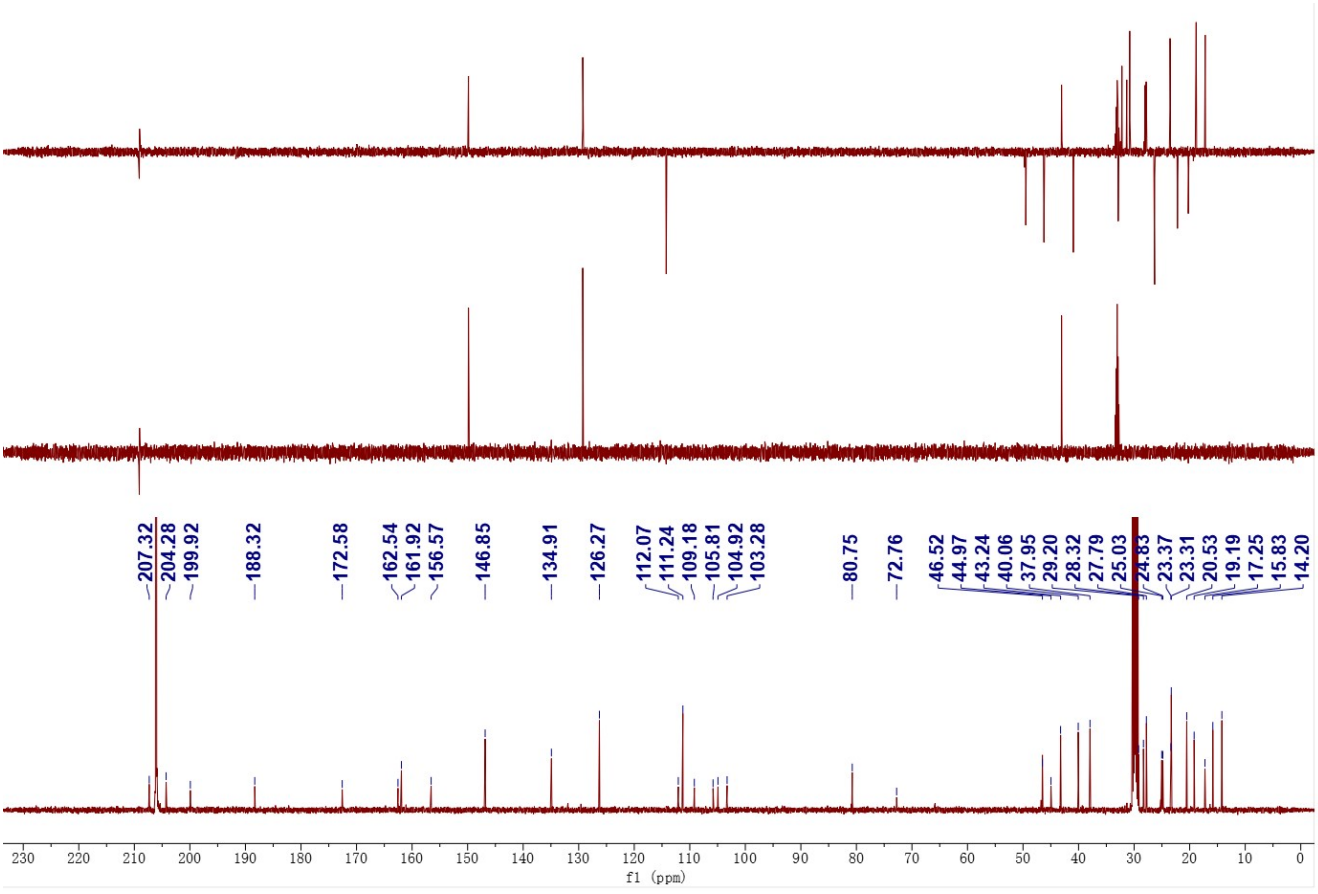


Figure S32 ^{13}C-NMR spectrum (125 MHz) of 8 in acetone-d_6.

12. Table S1 Antifungal activity of compounds 1~8 (MIC, μg/mL).

Fungal pathogen	Compound								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	ketoconazole
Colletotrichum acutatum Simmonds	>100	>100	>100	>100	>100	>100	>100	>100	0.78
Sclerotinia sclerotiorum	>100	>100	>100	>100	>100	>100	>100	>100	1.56
Verticillium dahlia Kleb	>100	>100	>100	>100	>100	>100	>100	>100	0.78
Rhizoctonia solani	>100	>100	>100	>100	>100	>100	>100	>100	6.25

13. Table S2 Antibacterial activity of compounds 1~8 (MIC, μg/mL).

Bacterial pathogen	Compound								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Ciprofloxacin
Staphylococcus aureus	100	50	50	50	50	50	50	100	0.78
Micrococcus lvsodeikticus	>100	>100	>100	>100	>100	>100	>100	>100	0.78
Bacillus magaterium	>100	>100	>100	>100	>100	>100	>100	>100	0.78
Micrococcus luteus	>100	100	>100	>100	>100	>100	>100	>100	0.78
Bacillus cereus	50	50	50	50	50	50	50	50	0.78

14. Cell viability assay

The cell viability was assessed using the CCK-8 assay (Meilunbio, Dalian, China). Briefly, 293T cells were seeded in 96-well plates at a density of 8×10^3 cells per well and treated with various concentrations of compounds **1**~**8** (0, 5, 10, 20, 40, 80 μM) for 48 hours. After the treatment period, the cells were incubated with the CCK-8 solution at 37°C for 1 to 4 hours. Absorbance was measured at 450 nm using a microplate reader (SpectraMax i3x, Molecular Devices, USA). Cell viability was calculated and analyzed using GraphPad Prism version 9 software.

MDA-MB-231 cells were plated in 96-well plates at a density of 5000 cells per well. On the subsequent day, the cells were treated with different concentrations of test compounds for 48 hours. Following the incubation period, 10 μL of CCK-8 solution was added to each well, and the plate was incubated at 37 °C for 2 hours. The absorbance values were then measured at 450 nm using a microplate reader (Molecular Devices, USA). The relative cell viability was calculated following normalization to untreated control cells and the IC_{50} values were calculated by GraphPad Prism 9.0.0.

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