Supplementary data

New Transformation Pathway and Cytotoxic Derivatives from the Acid Hydrolysis of Timosaponin B III

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Abstract

Timosaponin B III is a major bioactive steroidal saponin isolated from *Anemarrhena asphodeloides* Bge. To potentially discover derivatives with better biological activity, timosaponin B III was structurally modified via acid hydrolysis to yield one new (**2**, timopregnane A I) C_{21} steroidal glycoside and seven known compounds. Their structures were elucidated on the basis of NMR spectroscopy and mass spectrometry. All eight compounds were evaluated for cytotoxic activity against MCF7, SW480, HepG2, and SGC7901 cell lines *in vitro*. As a result, compounds **6** and **7** showed significant activity (IC_{50}2.94–12.2μM) against all tested cell lines. Structure–activity relationships of these compounds were investigated and the preliminary conclusions were provided. Moreover, a new transformation pathway was discovered in the acid hydrolysis of timosaponin B III for the first time.

Keywords: Timosaponin B III, Acid hydrolysis, Steroidal saponins, C_{21} steroidal glycoside, Cytotoxic activity.

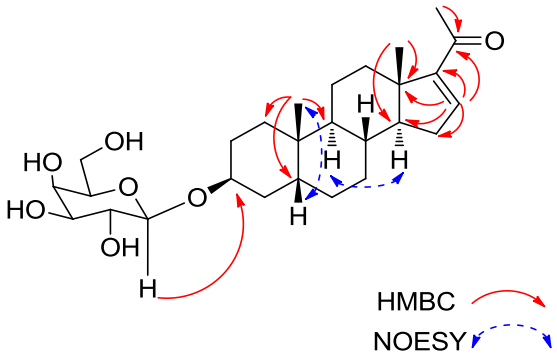


Figure S1 Key NOESY and HMBC correlations of compound **2**

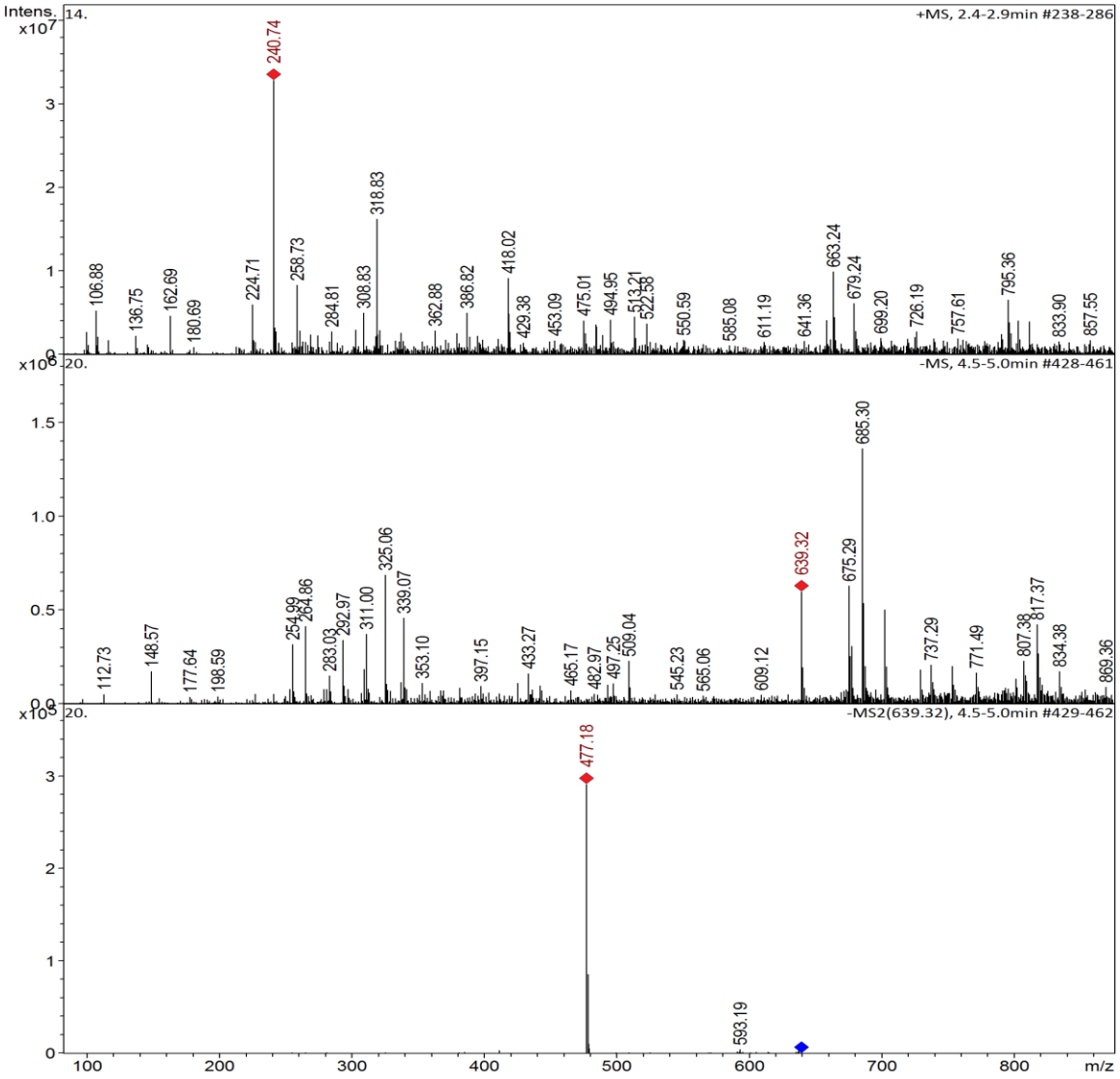


Figure S2 ESI-MS spectrum of compound **1**

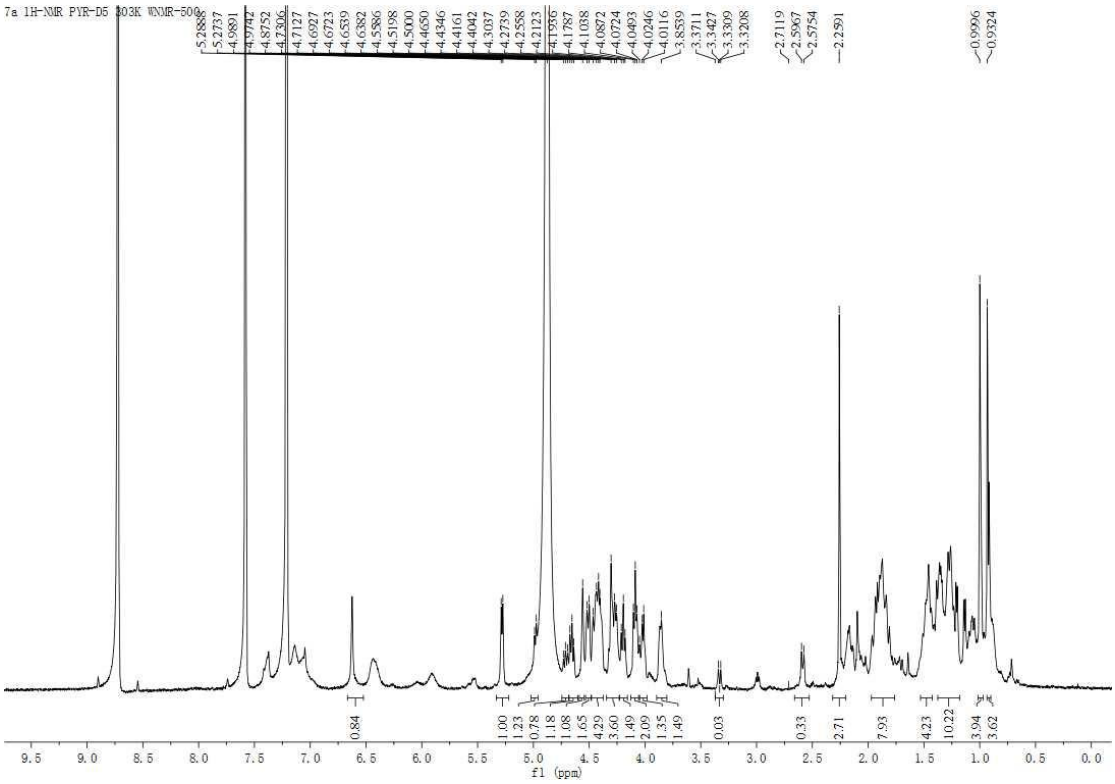


Figure S3 ^{1}H-NMR spectrum of compound **1** (500 MHz, C_5D_5N)

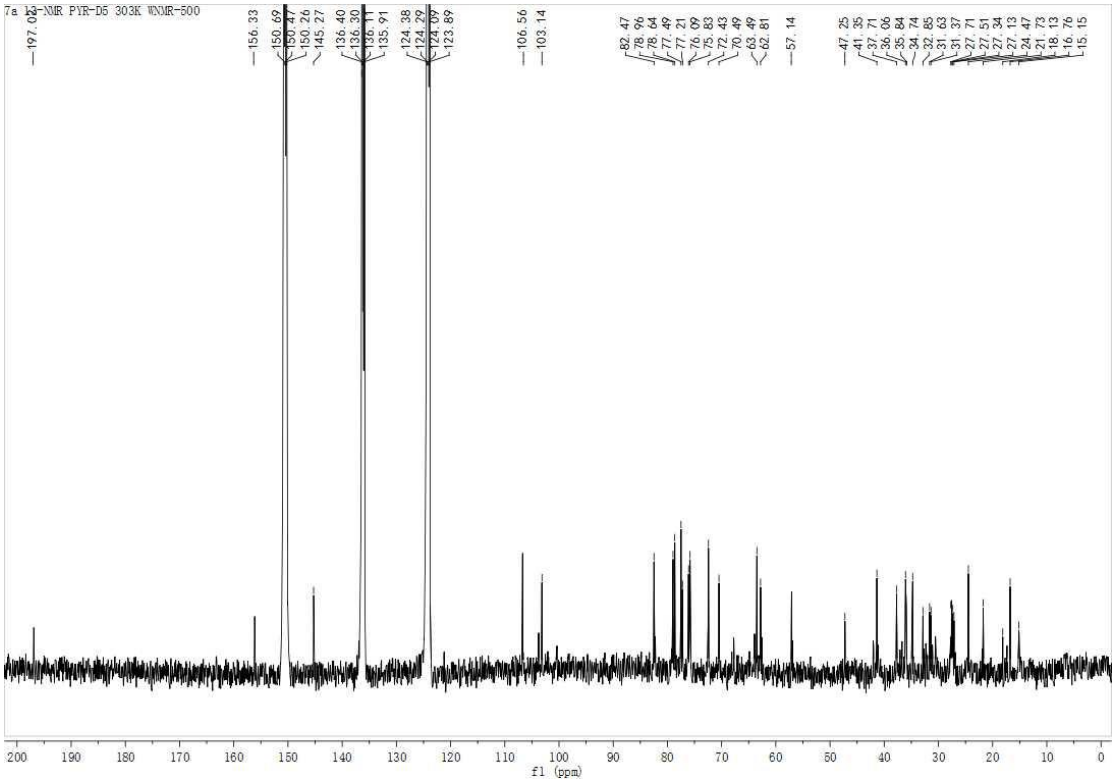
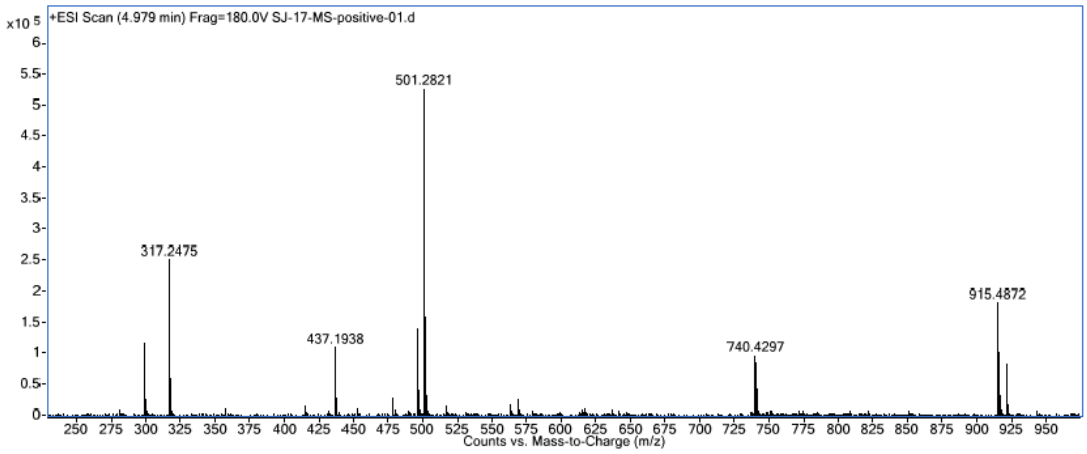


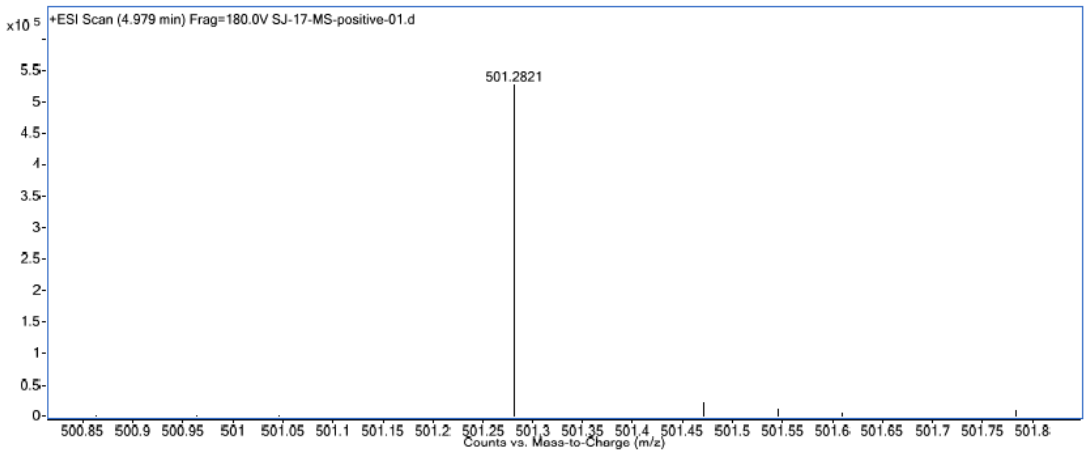
Figure S4 ^{13}C-NMR spectrum of compound **1** (125 MHz, C_5D_5N)

TCM-CPU HR-ESI-MS Display Report

Sample Name: SJ-17 **Instrument:** Agilent 6520B Q-TOF

Acq. Date: 12/30 /2016 **Operator:** Administrator





emental Composition Calculator

Target m/z:	501.2821	**Result type:**	Positive ions	**Species:**	$[M+Na]^+$
Elements:		C (0-80); H (0-120); O (0-30); Na(0-10);			
Ion Formula		**Calcalated m/z**		**PPM Error**	
C27H42 Na O7		501.2823		0.33	

Figure S5 HR-ESI-MS spectrum of compound **2**

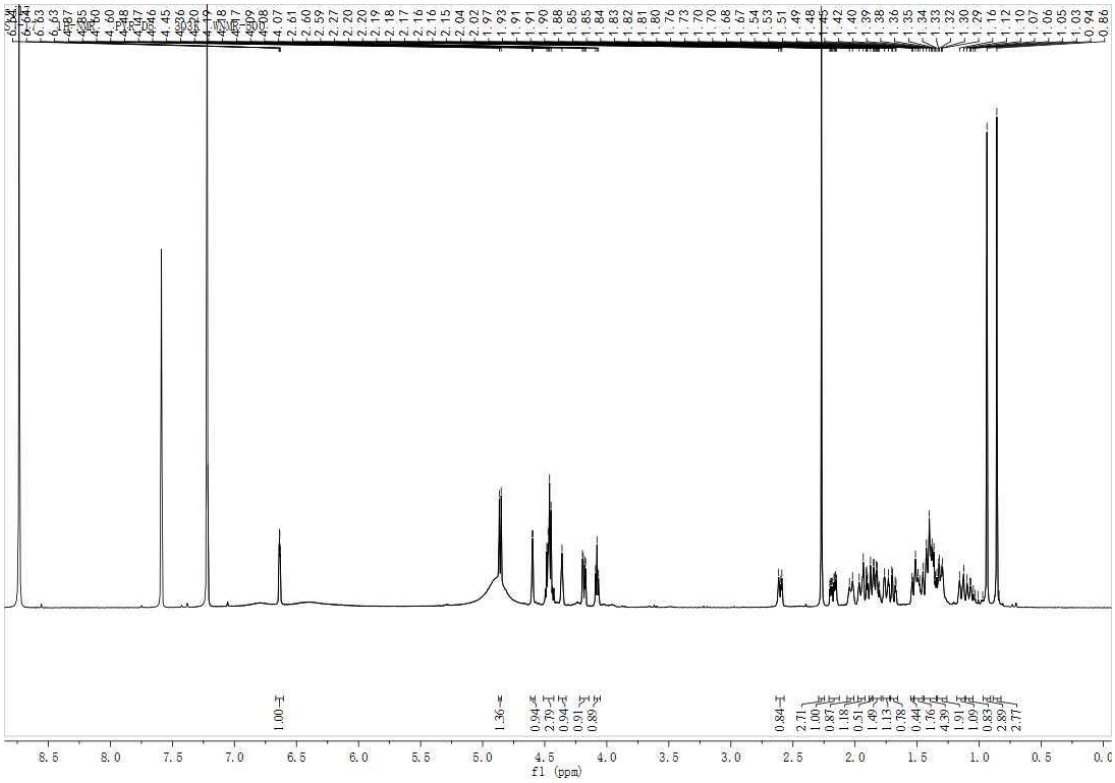


Figure S6 ^{1}H-NMR spectrum of compound **2** (500 MHz, C_5D_5N)

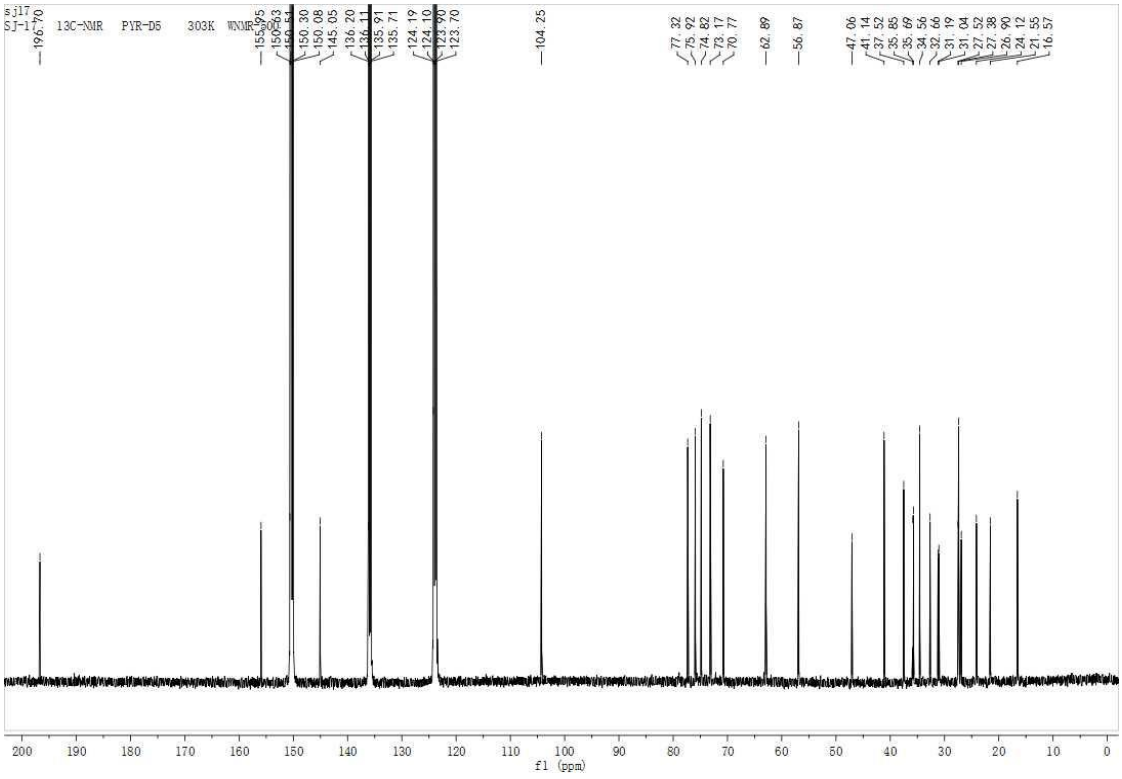


Figure S7 ^{13}C-NMR spectrum of compound **2** (125 MHz, C_5D_5N)

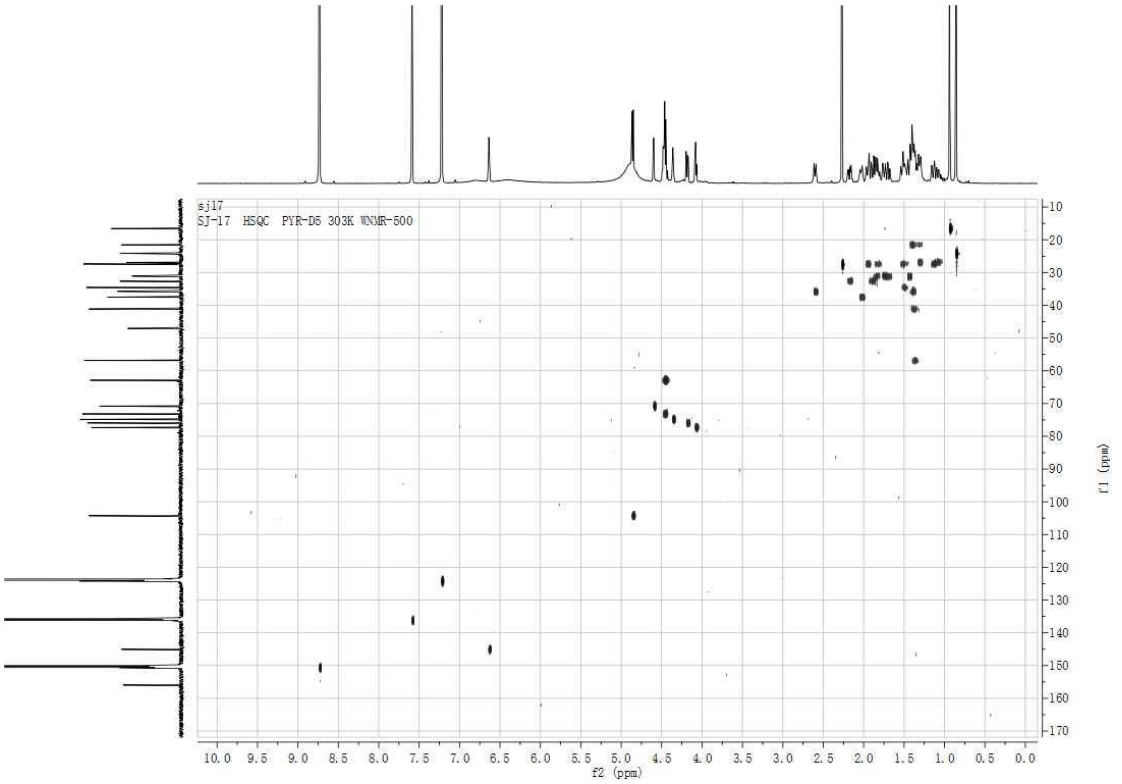


Figure S8 HSQC spectrum of compound **2** (C_5D_5N)

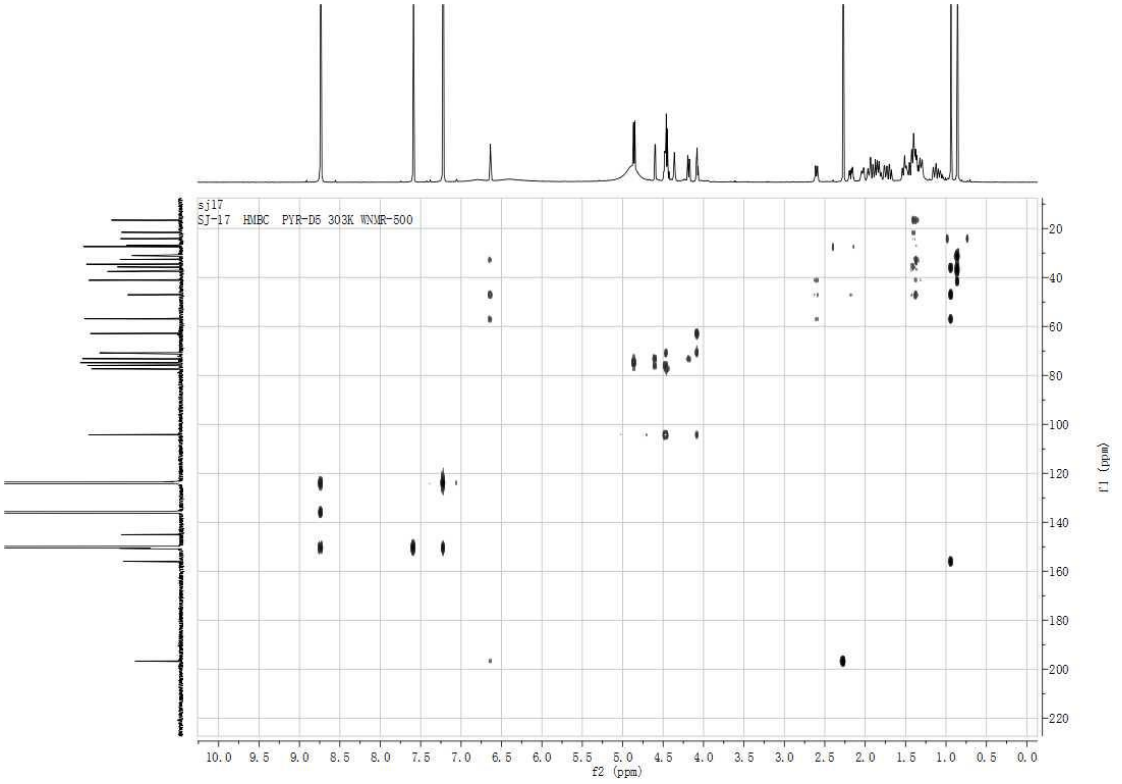


Figure S9 HMBC spectrum of compound **2** (C_5D_5N)

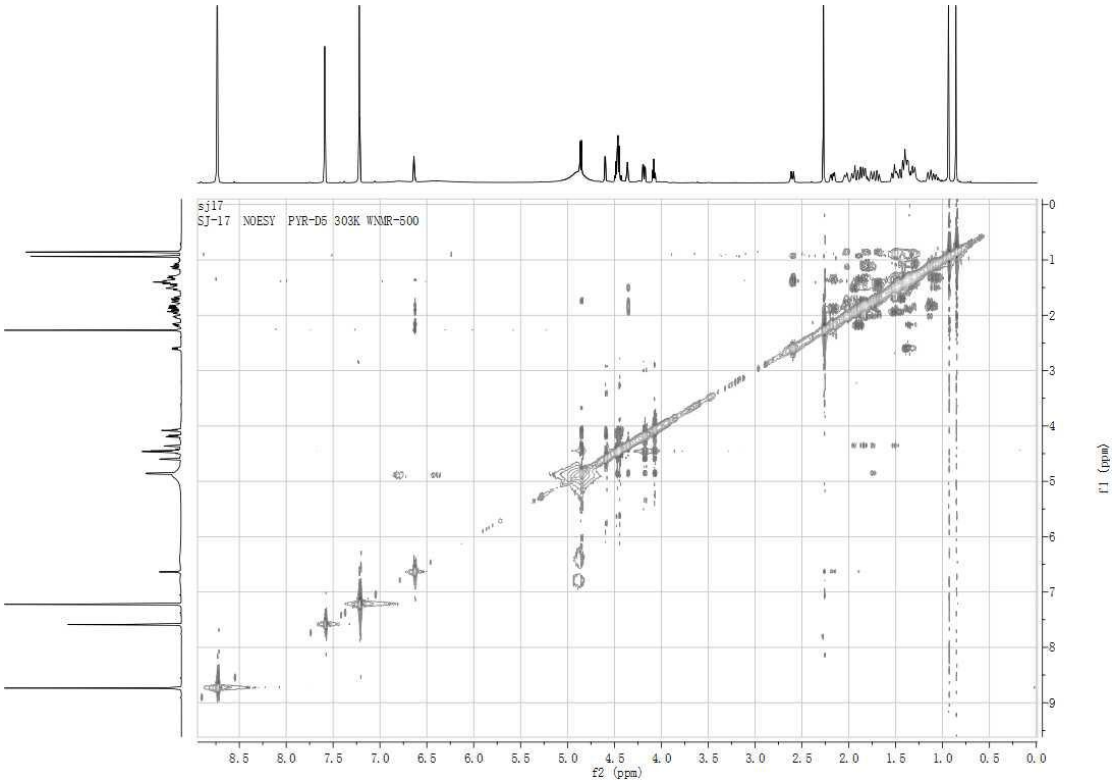


Figure S10 NOESY spectrum of compound **2** (C_5D_5N)

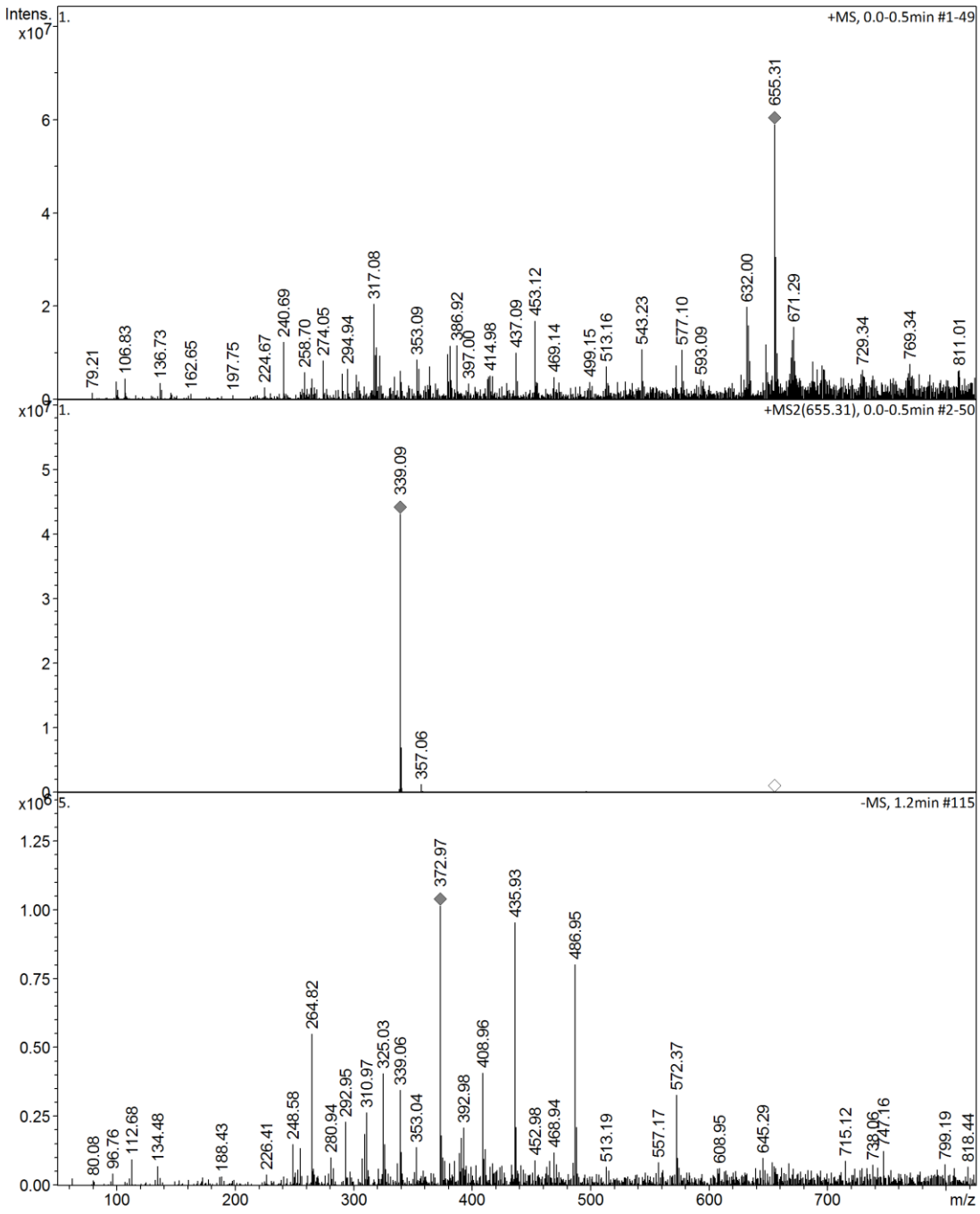


Figure S11 ESI-MS spectrum of compound **3**

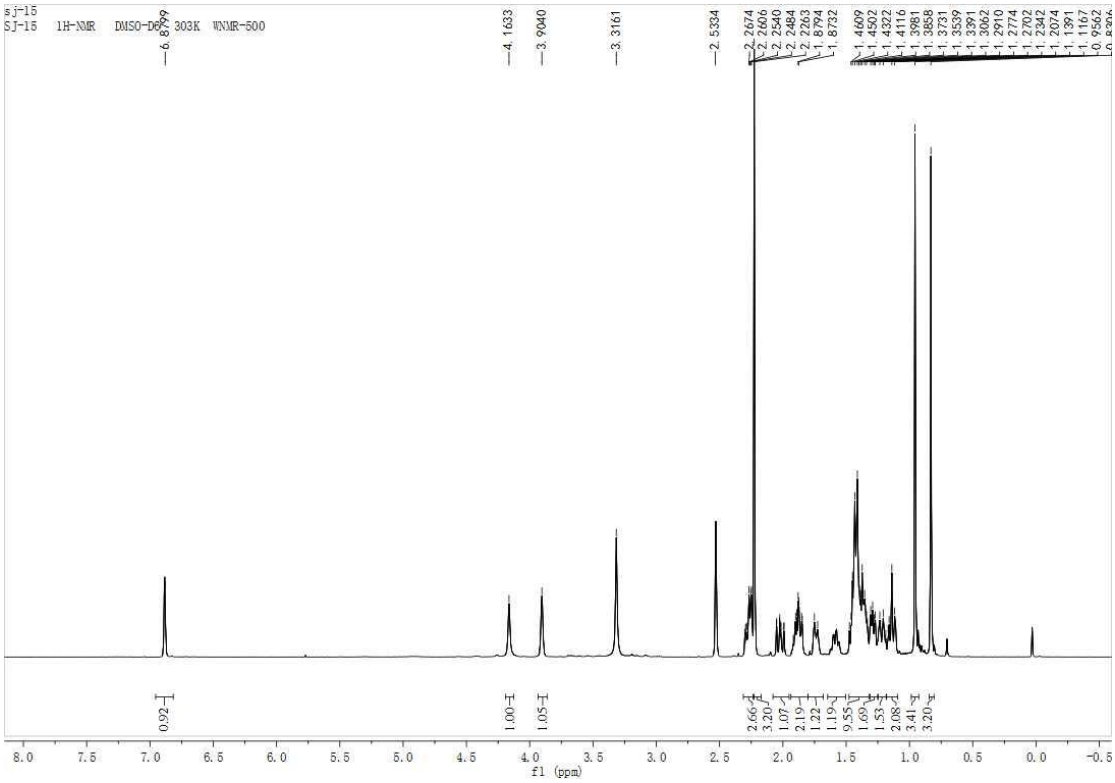


Figure S12 ^{1}H-NMR spectrum of compound **3** (500 MHz, C_5D_5N)

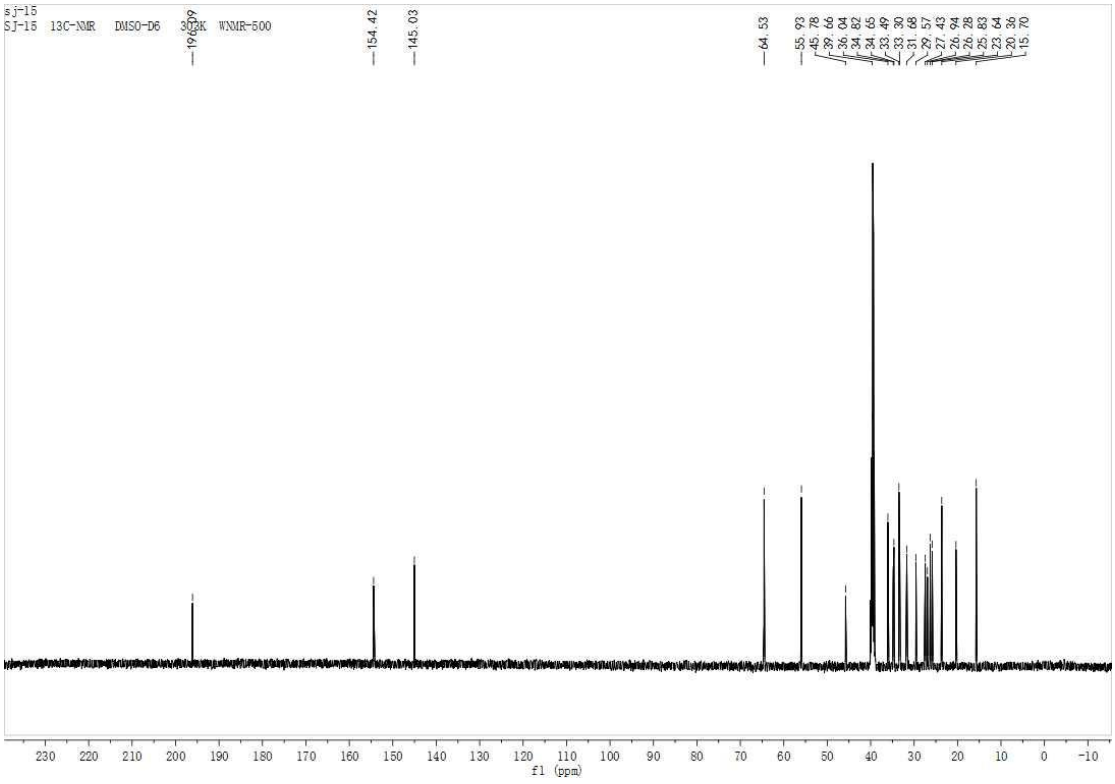


Figure S13 ^{13}C-NMR spectrum of compound **3** (125 MHz, C_5D_5N)

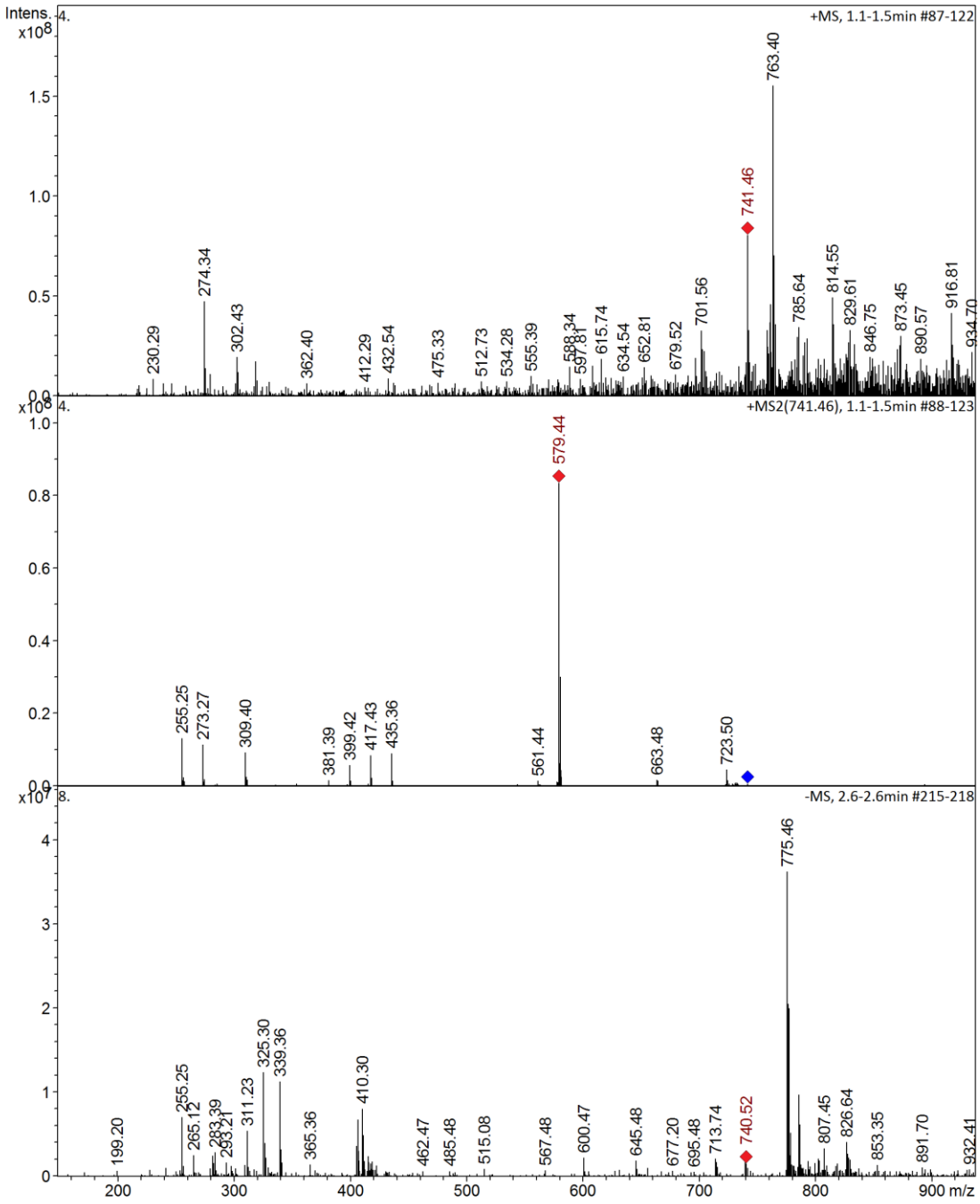


Figure S14 ESI-MS spectrum of compound **4**

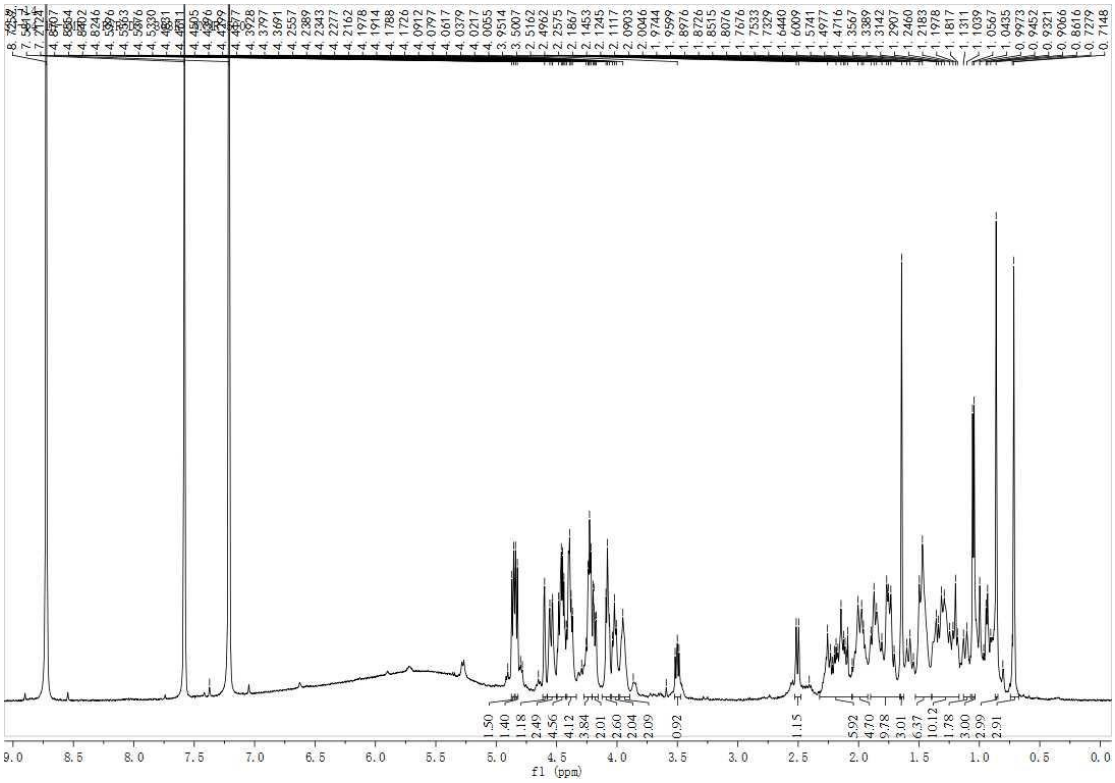


Figure S15 ^{1}H-NMR spectrum of compound **4** (500 MHz, C_5D_5N)

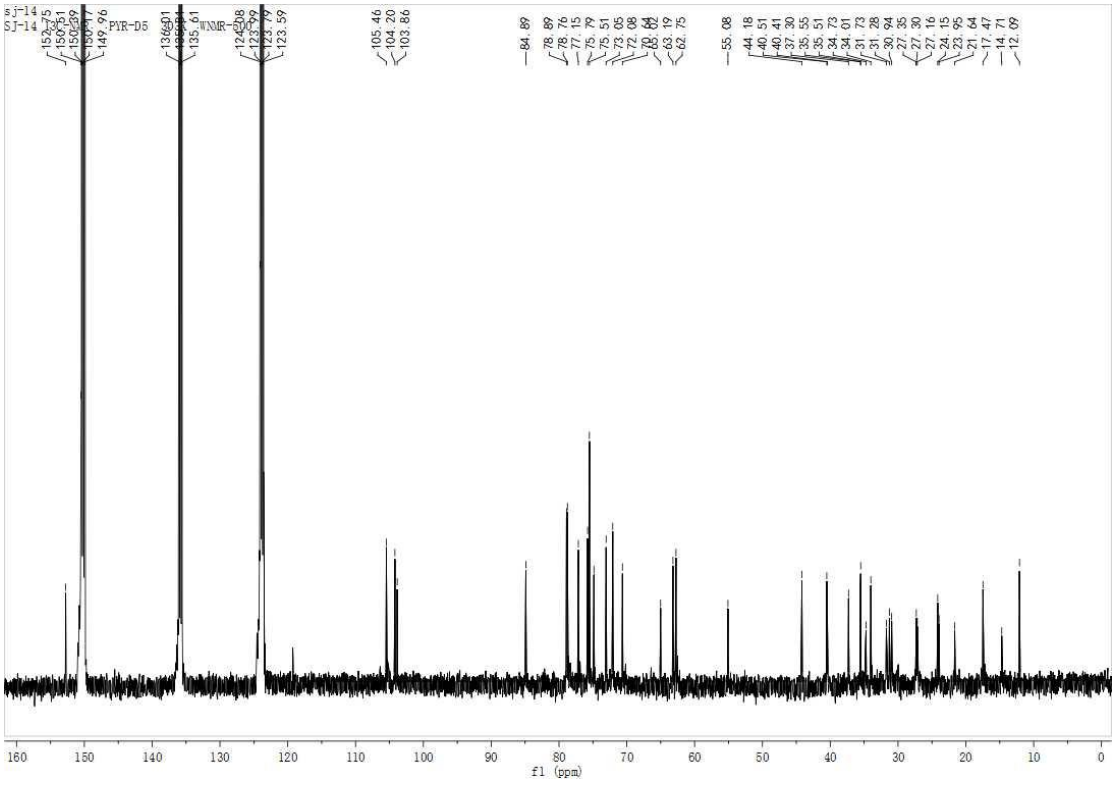


Figure S16 ^{13}C-NMR spectrum of compound **4** (125 MHz, C_5D_5N)

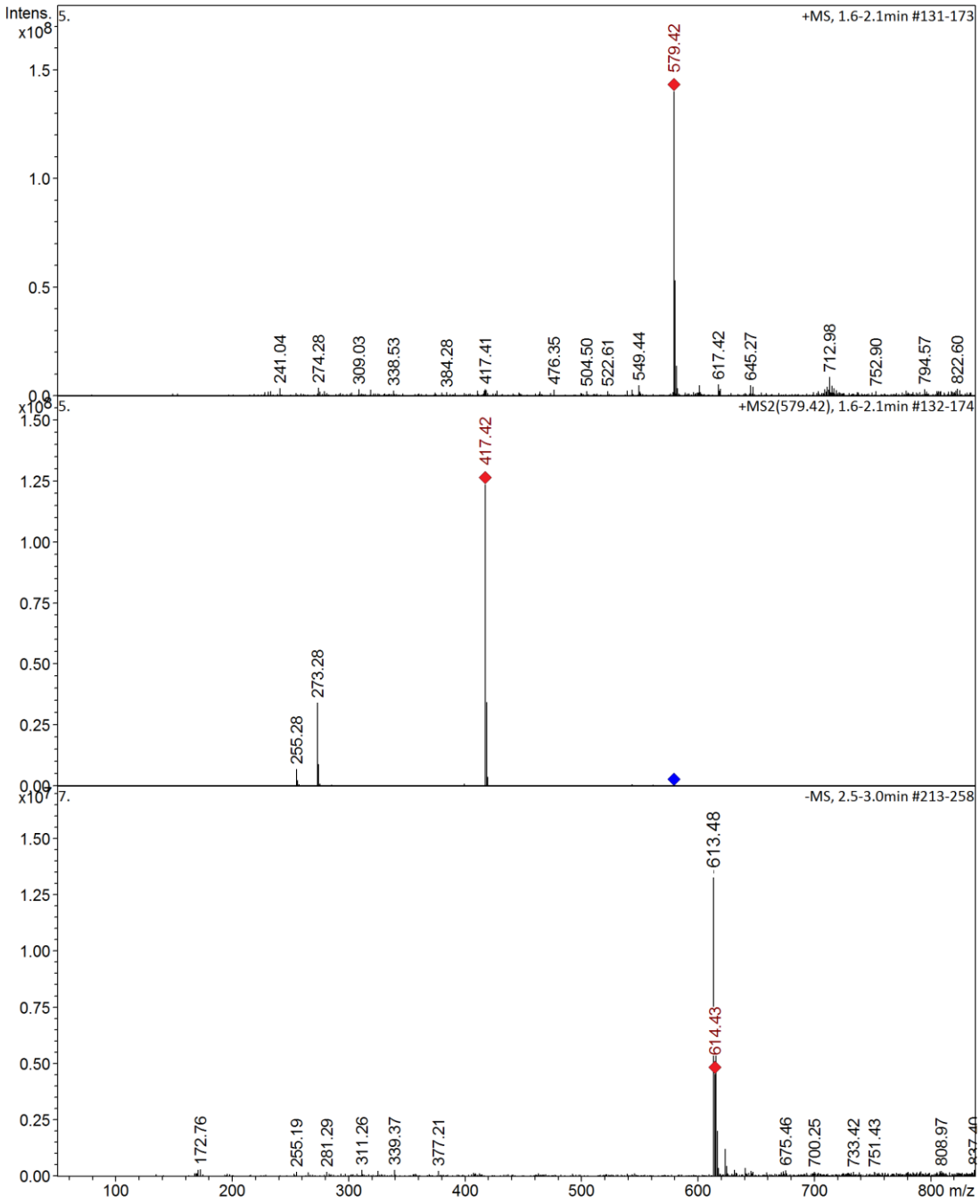


Figure S17 ESI-MS spectrum of compound **5**

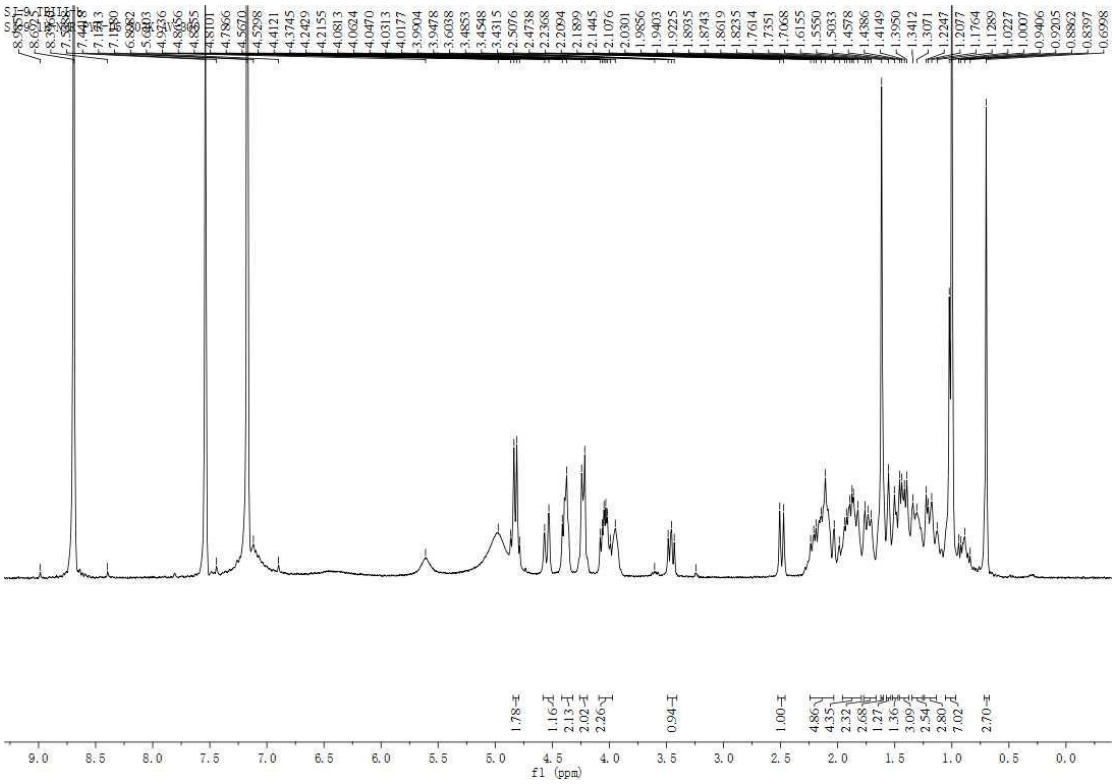


Figure S18 ^{1}H-NMR spectrum of compound **5** (500 MHz, C_5D_5N)

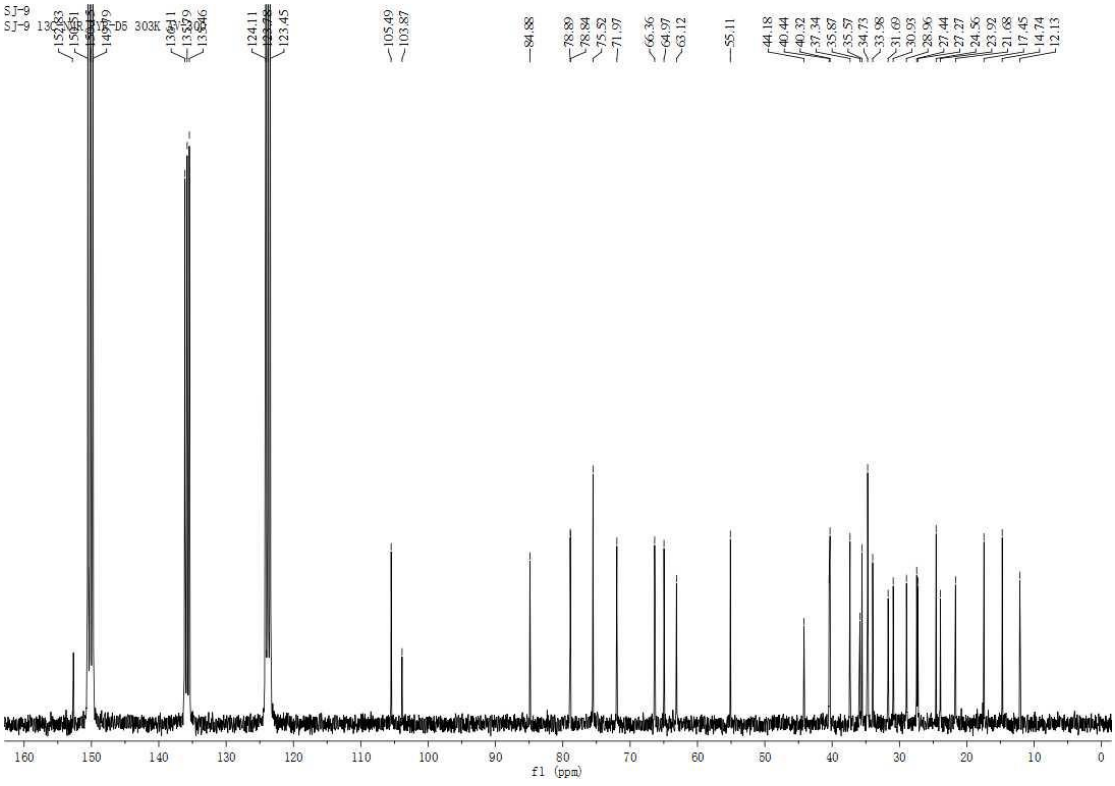


Figure S19 ^{13}C-NMR spectrum of compound **5** (125 MHz, C_5D_5N)

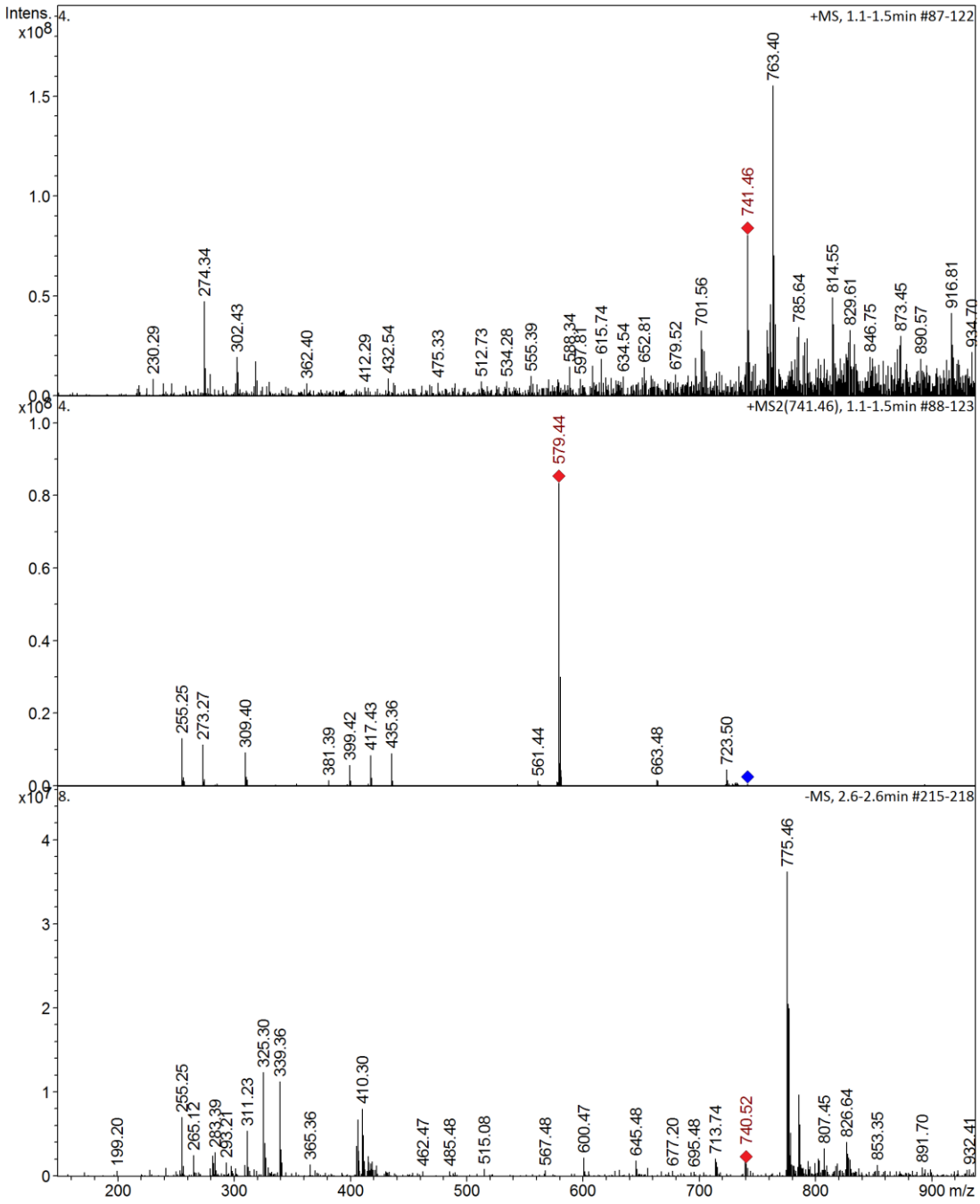


Figure S20 ESI-MS spectrum of compound **6**

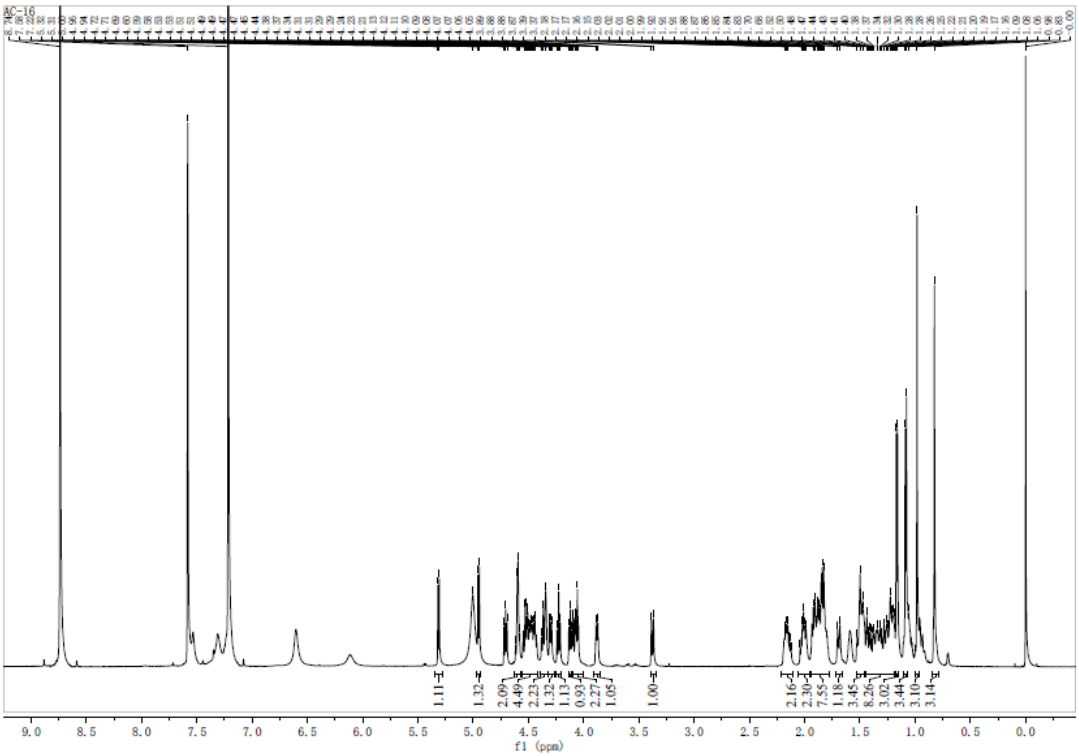


Figure S21 ^{1}H-NMR spectrum of compound **6** (500 MHz, C_5D_5N)

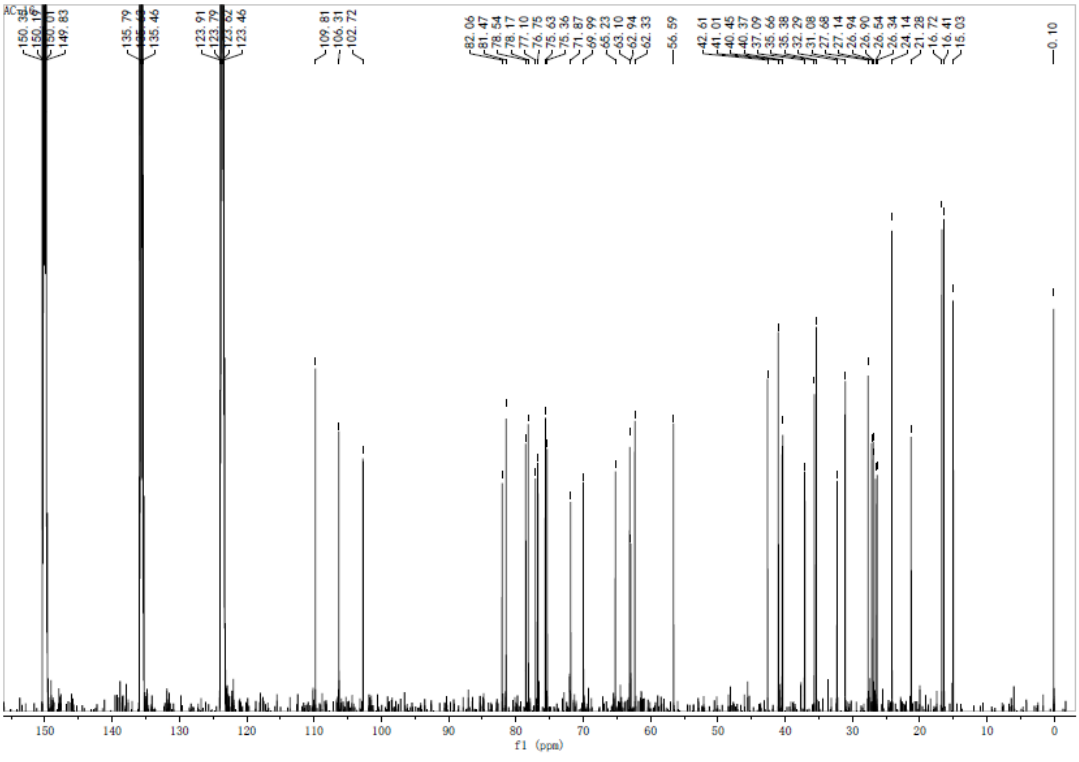


Figure S22 ^{13}C-NMR spectrum of compound **6** (125 MHz, C_5D_5N)

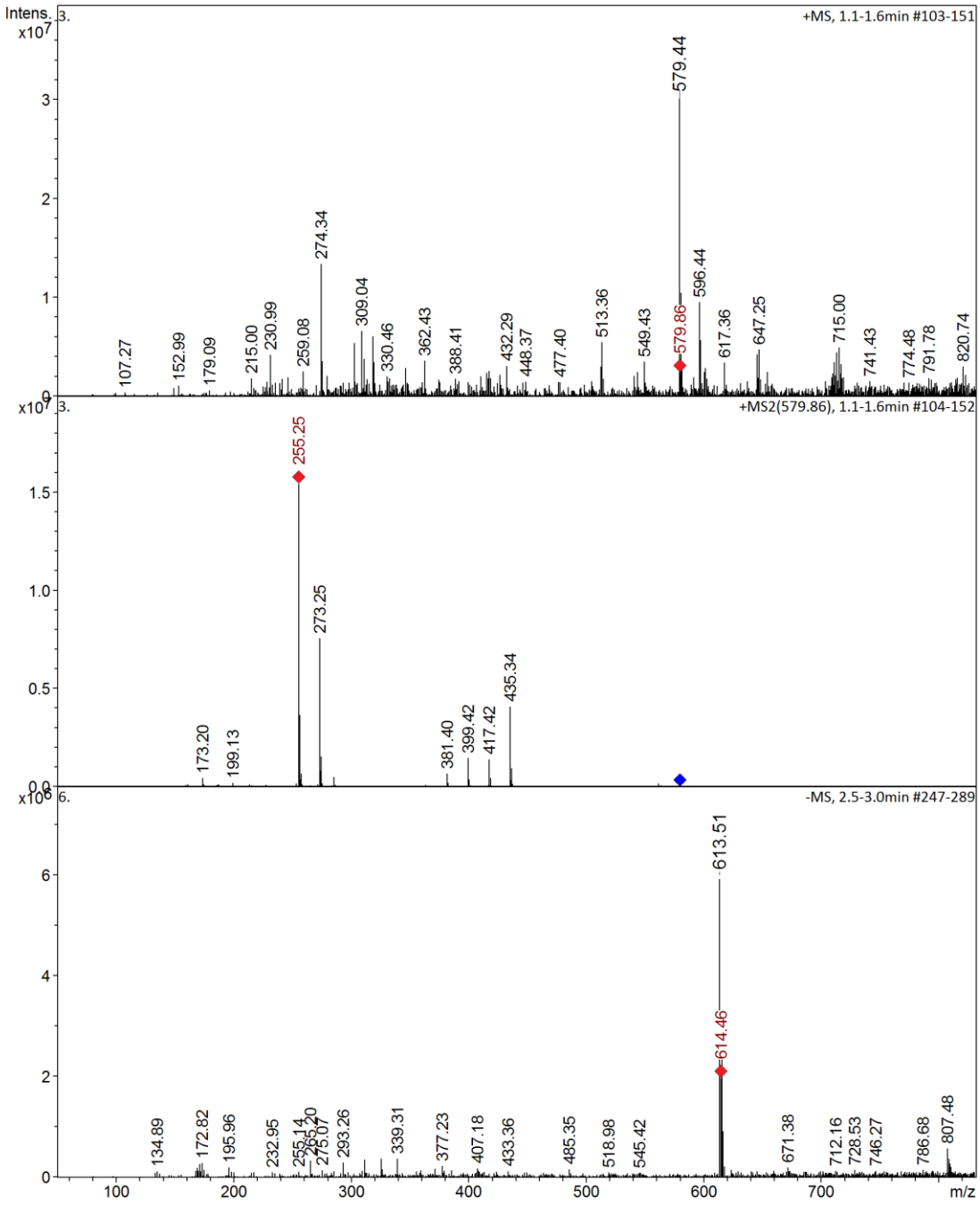


Figure S23 ESI-MS spectrum of compound **7**

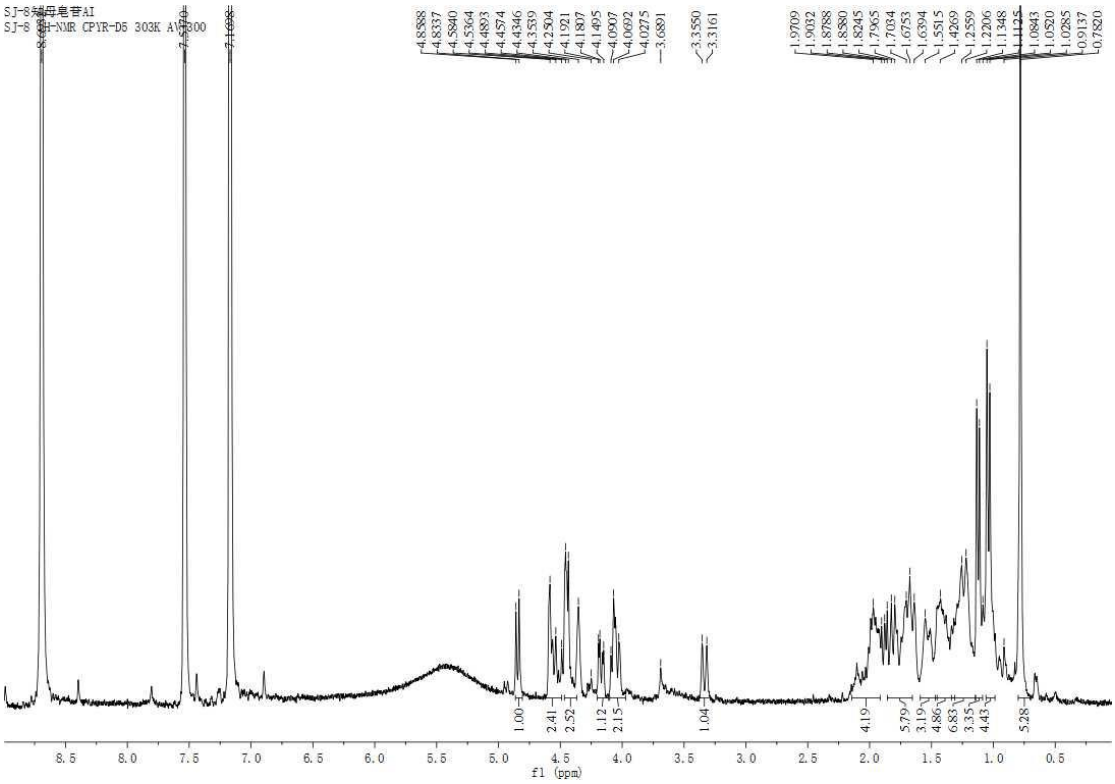


Figure S24 ^{1}H-NMR spectrum of compound **7** (500 MHz, C_5D_5N)

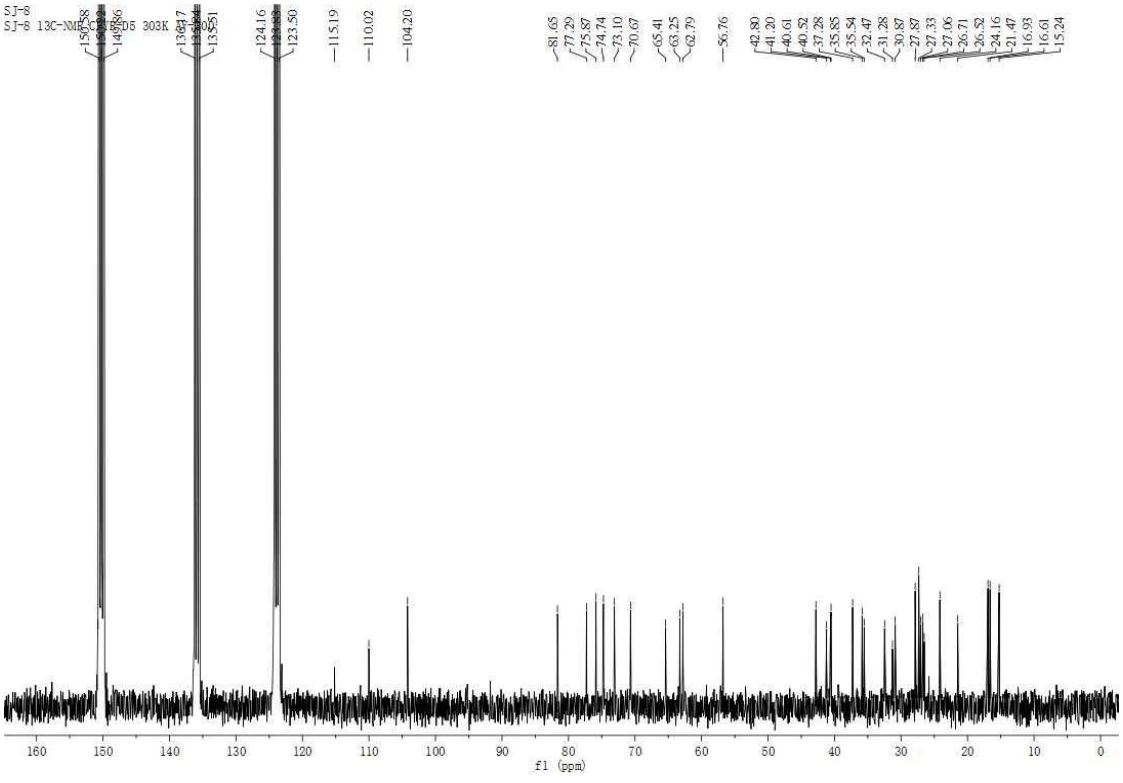


Figure S25 ^{13}C-NMR spectrum of compound **7** (125 MHz, C_5D_5N)

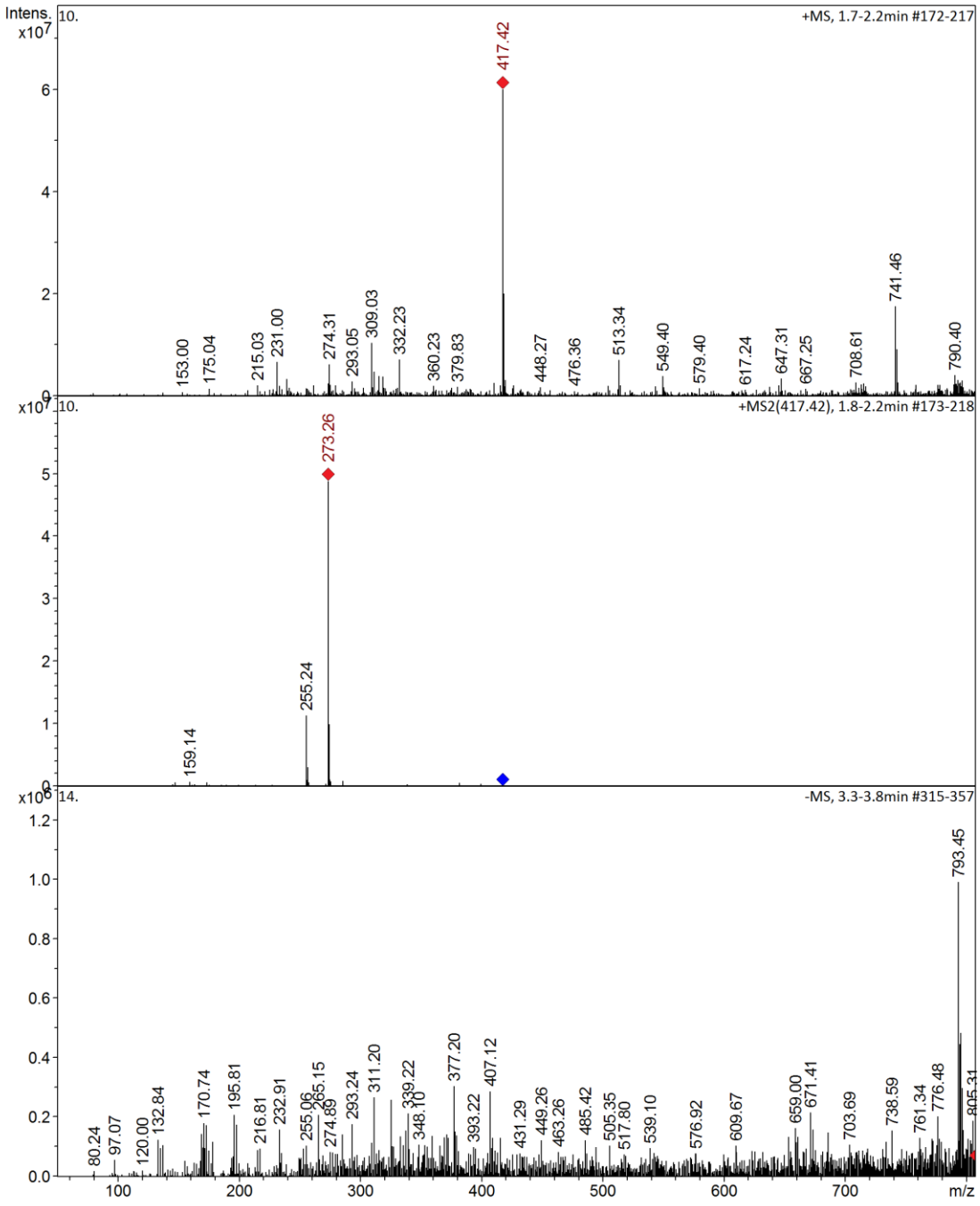


Figure S26 ESI-MS spectrum of compound **8**

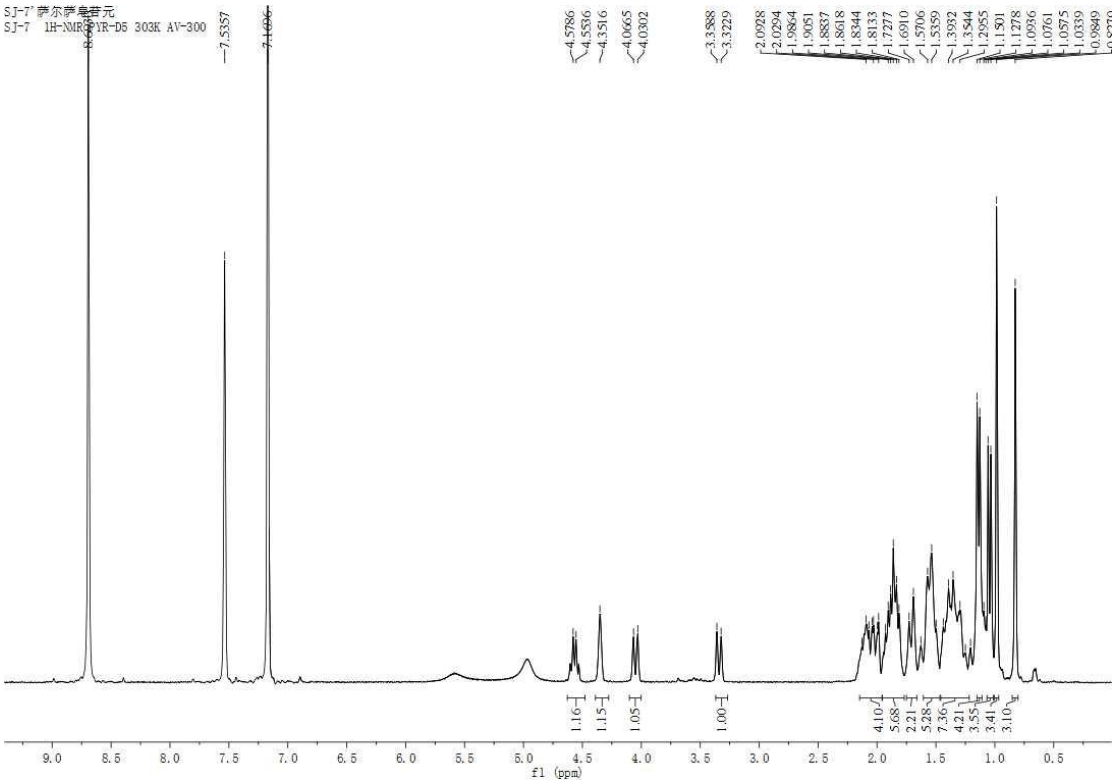


Figure S27 ^{1}H-NMR spectrum of compound **8** (500 MHz, C_5D_5N)

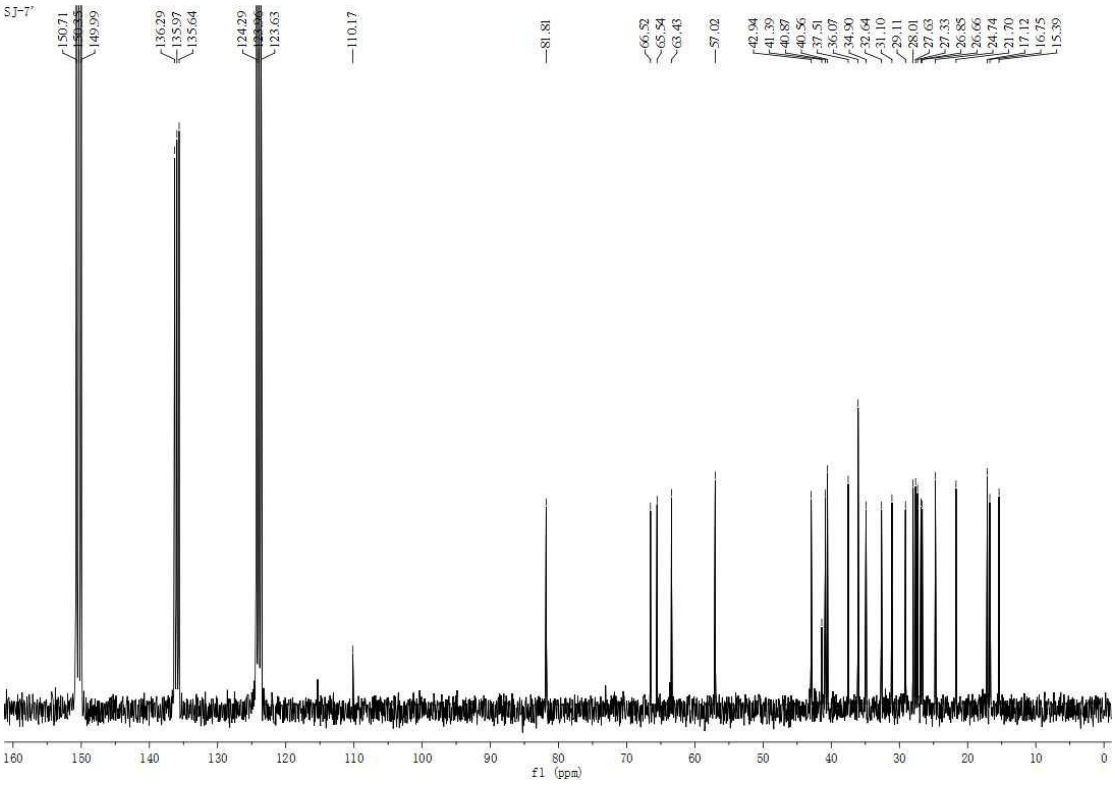


Figure S28 ^{13}C-NMR spectrum of compound **8** (125 MHz, C_5D_5N)

Table S1. NMR data of compound **2** in pyridine-d_5 (δ in ppm, J in Hz).

	2				
No.	δ_C	δ_H	No.	δ_C	δ_H
1	31.2	1.73 m, 1.43 m	Gal-1'	104.3	4.85 d (7.5)
2	27.4	1.90 m, 1.13 m	2'	73.2	4.66 m
3	74.8	4.36 m	3'	75.9	4.19 dd (3.0, 9.5)
4	31.0	1.83 m	4'	70.8	4.68 d (3.0)
5	37.5	2.02 m	5'	77.3	4.08 d (3.0)
6	27.4	1.94 m, 1.50 m	6'	62.9	4.48 m, 4.45 m
7	26.9	1.31 m, 1.08 m			
8	34.6	1.50 m			
9	41.1	1.38 m			
10	35.9	-			
11	21.6	1.40 m			
12	35.7	2.59 m, 1.38 m			
13	47.0	-			
14	56.9	1.36 m			
15	32.7	2.18 m, 1.90 m			
16	145.1	6.67 m			
17	156.1	-			
18	16.6	0.93 s			
19	24.1	0.86 s			
20	196.7	-			
21	27.5	2.25 s			

Table S2. The cytotoxic activity of TB-III and compounds **1–8** [a-b]

Compound	IC_{50}(μM) / Cell line			
	MCF7	SW480	HepG2	SGC7901
TB-III	>100	>100	>100	>100
1	>100	>100	>100	>100
2	>100	>100	>100	>100
3	49.5±4.89	78.2±8.79	71.0±4.36	>100
4	>100	>100	>100	>100
5	>100	>100	>100	>100
6	3.34±1.10	2.94±1.05	4.96±0.93	12.2±1.36
7	6.83±1.99	4.17±0.72	7.83±1.72	4.38±0.50
8	26.6±3.16	73.1±5.67	43.2±2.13	71.7±3.56

[a] MCF7 (human breast adenocarcinoma), SW480 (human colon adenocarcinoma), HepG2 (human hepatocellular carcinoma), SGC7901 (human gastric cancer).

[b] Compound **6** (timosaponin AIII) was used as positive control.