Supplementary Materials

Chemical and biological study of aplysiatoxin derivatives showing inhibition of potassium channel Kv 1.5

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Table of Contents

1. Experimental details

1.1 Molecular docking

Homology Modelling: Homology modelling was performed using the Prime 3.3 module implemented in the modelling software Schrodinger suite 2015.The homology modelling program was used to generate a 3D homology model of the open state of the Kv1.5 channel, using the 2.9A˚ crystal structure of the Kv1.2 channel (PDB ID: 2A79) as a template. The hybrid modelled structure was validated using PROCHECK (http://nihserver.mbi.ucla.edu/SAVES) for stereochemical property. The final predicted model was submitted to the Amber 12.0 molecular dynamic simulation program package for structural refinement. Flexible-ligand rigid-protein docking was performed using the Glide program from Schrödinger. The applied scoring function was Glide XP (extra-precision). The ligand structures were prepared using LigPrep. Protonation states were generated at pH 7.0 ± 2.0 A.

Molecular docking and Prime MM-GBSA calculations: Kv1.5 channel was prepared using protein preparation wizard (Schrodinger LLC, 2010, New York, NY) (Maestro 11.0 version) for energy minimisation of the resulting protein. Using OPLS, force field hydrogens were added, and bond orders were assigned. Automated docking of vernakalant (positive control), compounds **2** and **3** was performed using the Glide program. In the work described here, MM-GBSA was used to calculate the binding free energy between ligand and protein. By using the binding energy estimation program, we calculated binding free energy (MM-GBSA) for each ligand. We also used the ligand interaction program to show protein-ligand interaction. Three-dimensional interactome network figure was made by PyMOL software (pymol.org). In our study, all representational structures were displayed with PyMOL.

Results:

Table S1.1 Docking studies of Kv1.5 channel with binding affinity values

Compound	MM-GBSA(Kcal/mol)
Vernakalant	-37.374
2	-37.645
3	-32.217

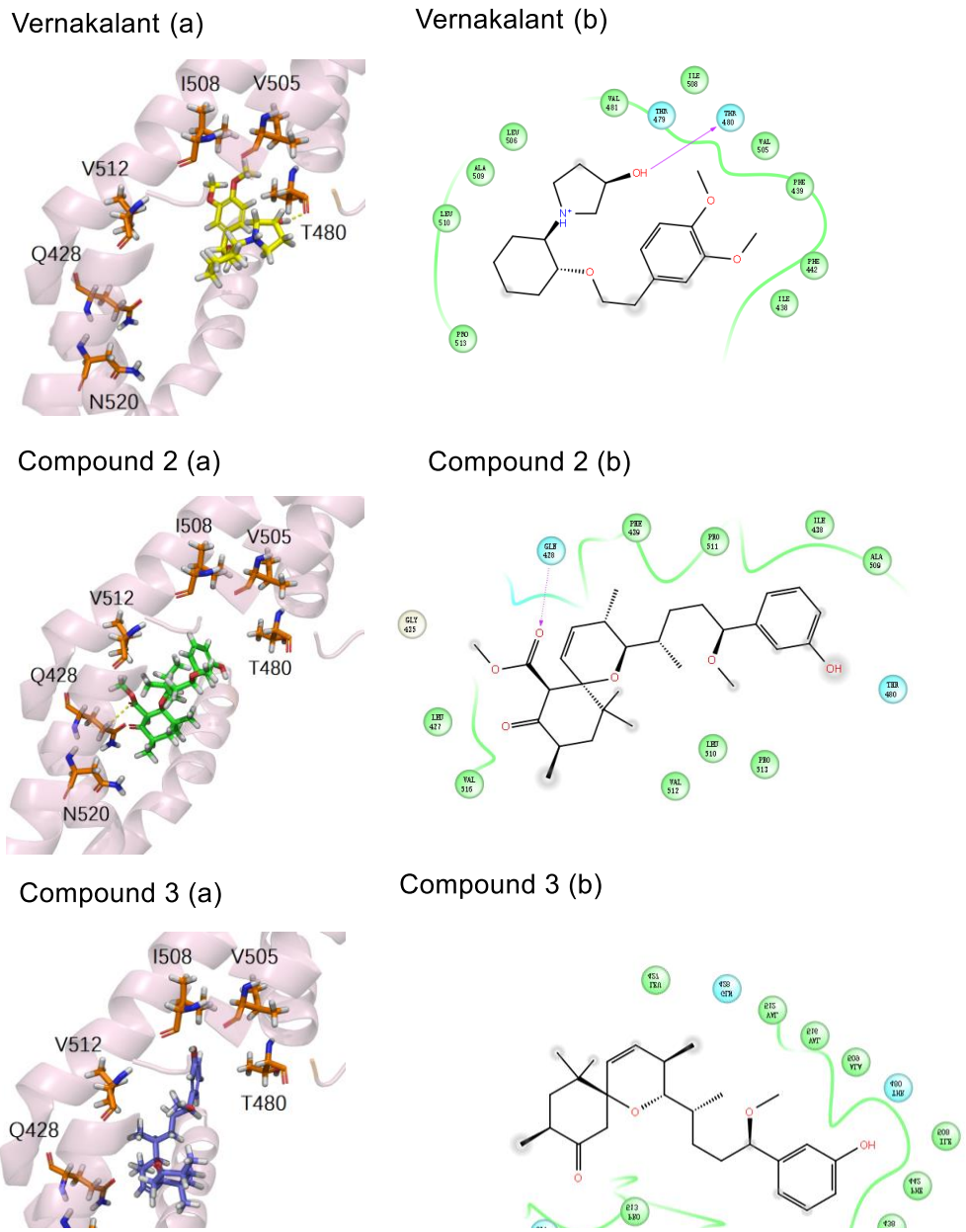

Figure S1.1 Ligand interaction map of vernakalant, compound **2** and compound **3.** The proposed interaction modes of all three reference compounds have been shown in the stick format. Hydrogen bonding interactions are represented as *violat arrow lines,* interacting proteins are shown in *green and cyan*.

References:

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1.2 Bioassays

1.2.1 PKC activation

Protein kinase C (PKC) is a growing family of isozymes that mediate a wide range of cellular signal transduction processes,[1] and PKC has been considered a potential target of anticancer chemotherapy.[2] To date, 11 different PKC isozymes have been identified,[3] it is activated to both trigger a tumor-promoting response and promote apoptosis. Certain tumor promoters such as phorbol ester (PMA) activate PKC by phosphorylation leading to a certain tumorigenic effect.[4] Others have a strong affinity for PKCδ isoenzymes, and the tumor-promoting activity is significantly reduced.[5] We tested whether our compounds over-express phosphorylation of PKCδ by western blotting analysis and PMA was used as a positive control. [6-10]

Experimental method: HepG2 cells were seeded in 6-well-plates at a density of 2.0×10^5 per well and appropriately treated. The total cell proteins were extracted. After adherent, compounds **1-7** (10 μM) and PMA (1 μM) were added into 6-well plates containing cells to obtain different final concentration, and then incubating for 1 h in a 37 ℃ humidified incubator. Cells were collected and lysed with cell lysis buffer [20 mM Tris (pH 7.5), 150 mM NaCl, 1 % Triton X-100 sodium pyrophosphate, β-glycerophosphate, EDTA, Na_2CO_3, 1 mM PMSF, leupeptin] and then incubated on shake cultivation containing ice for 30 min before centrifuging at maximum speed 14,800 rpm at 4 °C for 10 min and then took the supernatant, determined the protein concentration using BCA Protein Assay Kit (Beyotime Institute of Biotechnology, China). Equal amounts of protein (25 μg per sample) were resolved on a 10 % SDS-polyacrylamide gel. After electrophoresis, transmembrane, blocking, proteins were respectively incubated with primary anti-bodies of β-actin, PKCδ, phospho-PKCδ at 4 °C shake cultivation overnight followed by horseradish peroxidase-conjugated anti-rabbit

secondary antibodies for 1 h at room temperature. Bands were then recorded by a digital camera.

Results: Compounds **1-7** (10 μM) and PMA (1 μM) were applied to HepG2 cells for 1 h, and the results showed that compounds **1** and **4-6** remarkably up-regulated the expression of phosphor-PKCδ at 10 μM, and the expression was comparable to PMA at 1 μM. However, compounds **2**, **3** and **7** didn't increase the expression of phosphor-PKCδ.

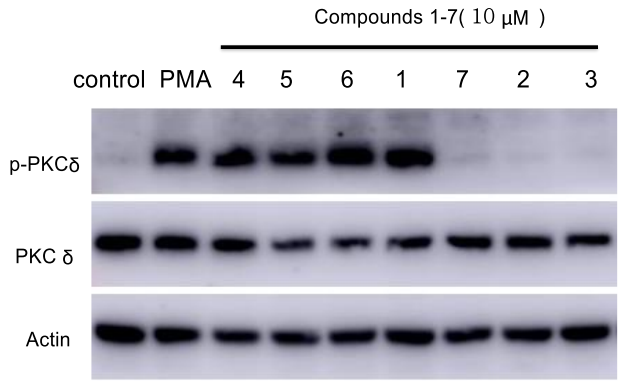


Figure S1.2.1 Effect of compounds **1-7** on phosphor-PKCδ expression in HepG2 cells. Cells were treated with indicated concentration (10 μM) of compounds **1-7**, PMA (1 μM) for 1 h and the expression of p-PKCδ protein was determined to use cell lysates by western blotting.

1.2.2 Ion channel experiment

Debromoaplysiatoxin (**5**), 3-methoxydebromoaplysiatoxin (**6**) and 30-methyloscillatoxin D (**7**) were selected to screen for the voltage-gated channel (Kv1.1, Kv1.2, Kv1.3, Kv1.4 and Kv1.5), these compounds all have significant inhibitory effects on Kv1.5 (**Figure S1.2.2.1**). The Kv1.5 inhibition activities of the metabolites **1-3** and **5-7** were subsequently investigated and the results showed that compounds **2** and **7** which did not have phosphor-PKCδ activity in western blotting also showed Kv1.5 inhibitory activity at 1 μM (**Table S1.2.2**). Thus, we selected compounds **2**, **5** and **7** for further Kv1.5 electrophysiological investigation to collect IC_{50} with acacetin as a positive control.

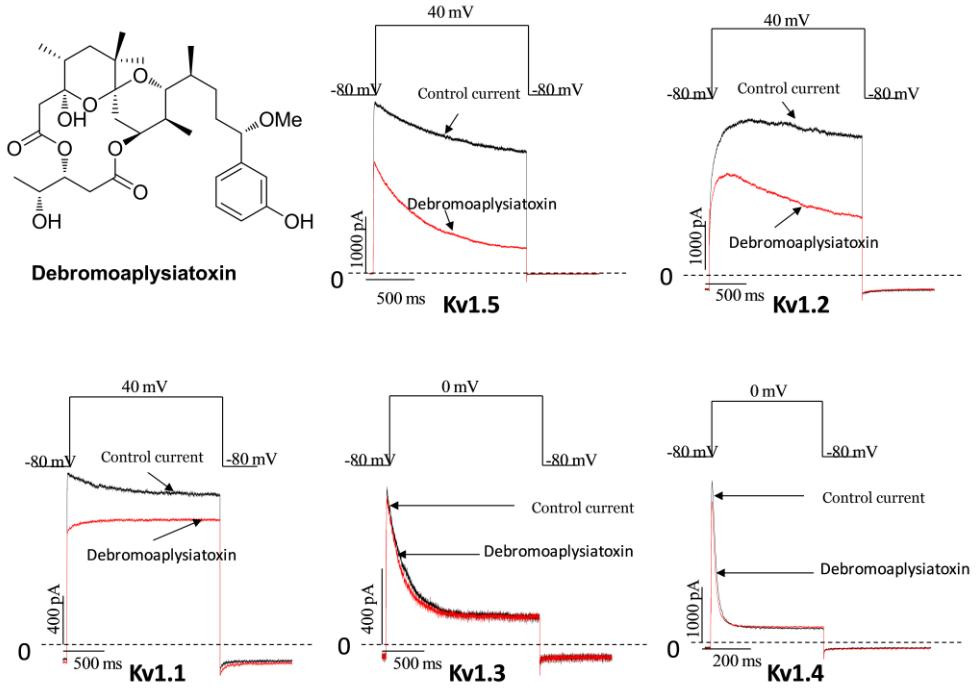
Cell preparation: The day before the experiment, digestion of CHO cells (Sigma Chemical Co., St. Louis, MO, USA) with density of 60%-80% by trypsin, and split into some small glass plates, which placed in 35 mm petri dish, then added 10% FBS, and DMEM culture medium without P/S was cultured overnight in incubator.

Electrophysiology: All experiments were performed at 25 °C. Whole-oocyte recordings were

conducted by electrode patch clamp. Electrodes were filled with intracellular fluid and had resistances of 2-3 MΩ. The intracellular fluid contained Aspartate (130 mM), $MgCl_2$ (5 mM), EGTA (5 mM), Hepes (10 mM), Tris-ATP (4 mM). PH was adjusted to 7.2 with NaOH and the solution was filtered. The extracellular fluid used to record currents contained NaCl (137 mM), KCl (4 mM), $MgCl_2$ (1 mM), Hepes (10 mM), Glucose (10 mM). PH was adjusted to 7.4 with NaOH and the solution was filtered. Kv1.5 voltage stimulation program first to a -80 mV holding voltage, and then give 20 mV depolarization voltage, again back to -80 mV holding voltage.

Data analysis and statistics: Data acquisition and analysis were carried out using PC2C. Data fitting and statistical analyses were performed using ORIGIN 8.0 (GraphPadSoftware Inc., San Diego, CA). IC_{50} value was determined by fitting the data points to the equation. Where IC_{50} is the concentration at which half-maximal currents were inhibited, all the data were presented as mean ±SEM.

Results: Compounds **2**, **5** and **7** were chosen to undertake further investigation to gather their IC_{50} with acacetin as a positive control, the results showed that inhibitory effect of **2**, **5** and **7** exhibited IC_{50} value of 0.79±0.032 μM, 1.28±0.08 μM, 1.47±0.138 μM, and acacetin of 5.96±0.564 μM (**Figure S1.2.2.2, Figure S1.2.2.3, Figure S1.2.2.4** and **Figure S1.2.2.5)**.



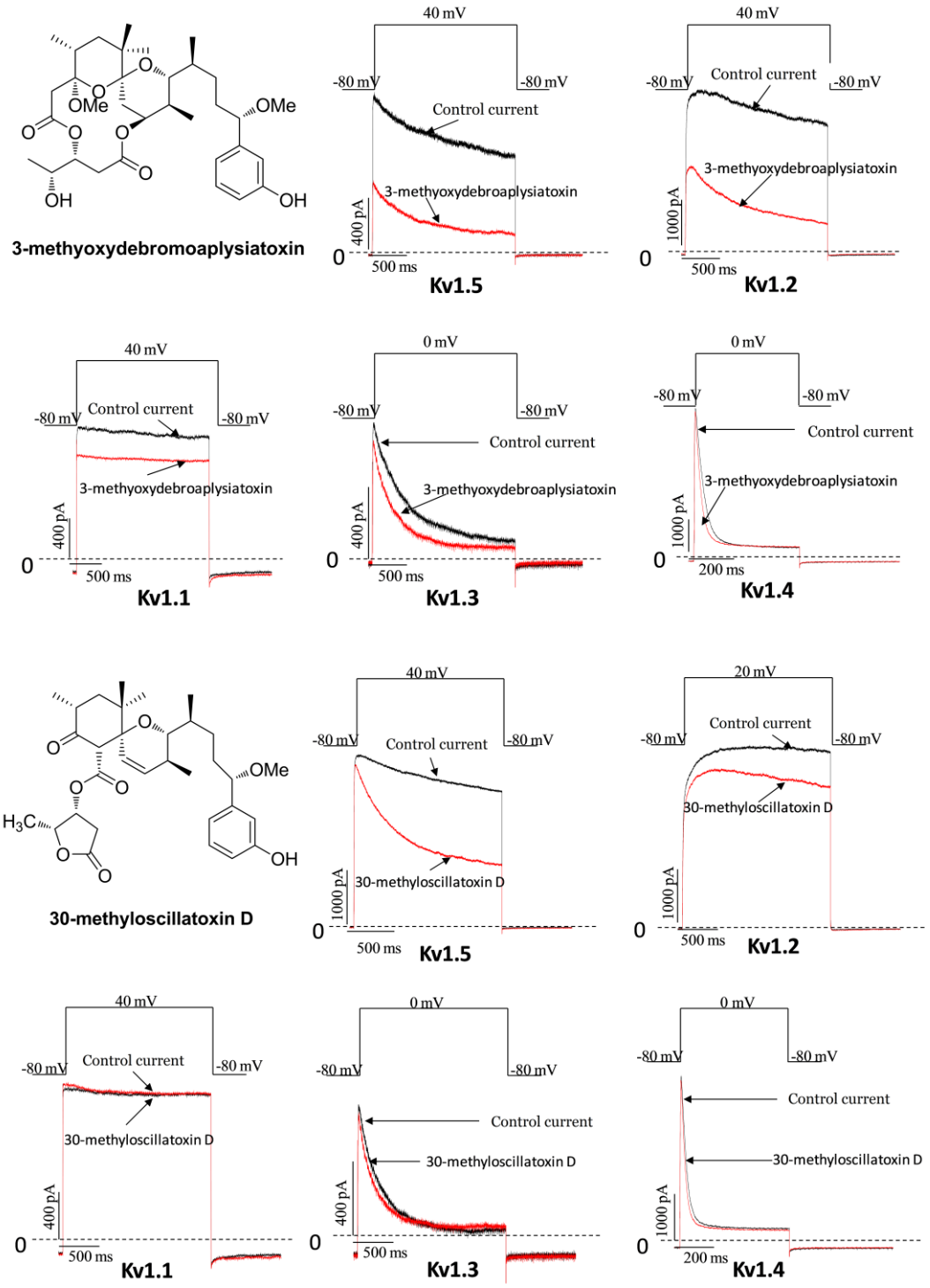


Figure S1.2.2.1 Kv1.1-Kv1.5 traces elicited by 5s pulses from -80 to 0/+20/+40 mV and tail currents recorded at -80 mV in the absence and presence of debromoaplysiatoxin (**5**), 3-methoxydebromoaplysiatoxin (**6**) and 30-methyloscillatoxin D (**7**).

Table S1.2.2 Kv1.5 inhibition activities of the metabolites **2-7** at 1 μM

compounds	**1**	**2**	**3**	**5**	**6**	**7**
Inhibition ration(%)	22.4	65.40	29.1	48.68	48.81	43.36

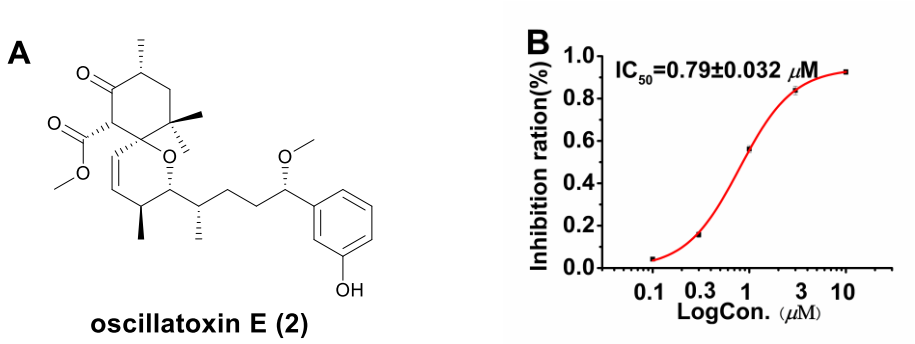


Figure S1.2.2.2 (A) The structure of compound **2**. (B) Percent blocked-Concentration curves. The abscissa represents the concentration, and the ordinate represents the percentage of Kv1.5 current that is blocked at different concentrations of compound **2**. Data points represent mean ±SEM of 3 to 5 measurements, and inhibitory effect showed IC_{50} value of 0.79±0.032 μM.

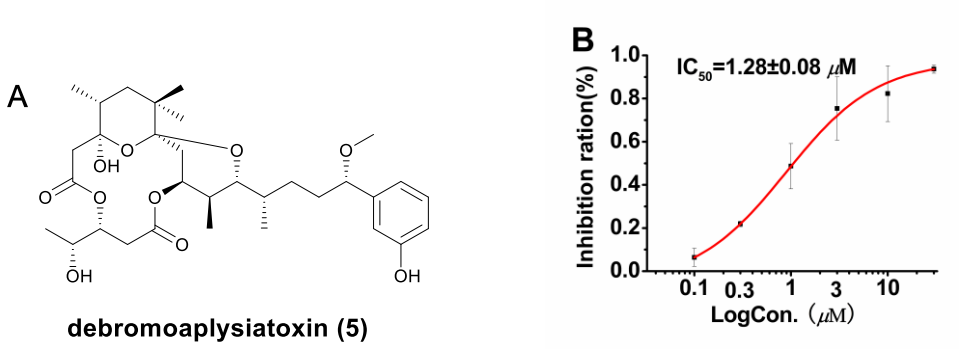


Figure S1.2.2.3 (A) The structure of compound **5**. (B) Percent blocked-Concentration curves. The abscissa represents the concentration, and the ordinate represents the percentage of Kv1.5 current that is blocked at different concentrations of compound **5**. Data points represent mean ±SEM of 3 to 5 measurements, and inhibitory effect showed IC_{50} value of 1.28±0.08 μM.

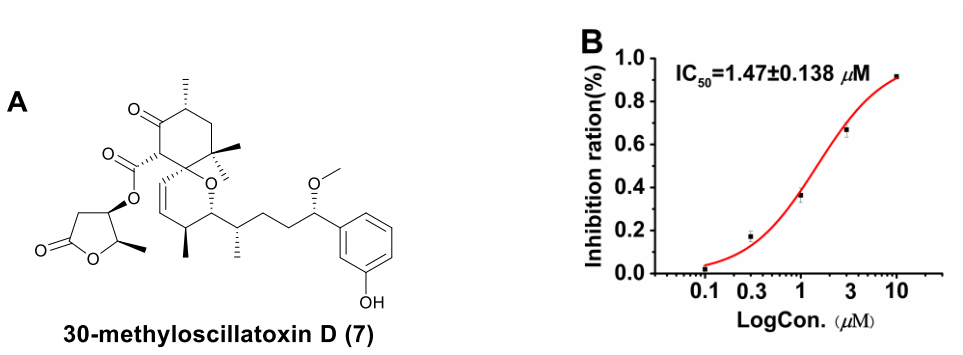


Figure S1.2.2.4 (A) The structure of compound **7**. (B) Percent blocked-Concentration curves. The abscissa represents the concentration, and the ordinate represents the percentage of Kv1.5 current that is blocked at different concentrations of compound **7**. Data points represent mean ±SEM of 3 to 5 measurements, and inhibitory effect showed IC_{50} value of 1.47 ± 0.138 μM.

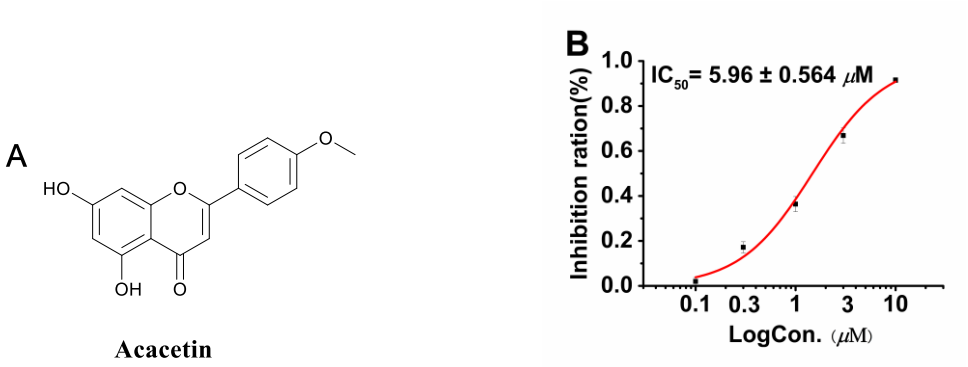


Figure S1.2.2.5 (A) The structure of acacetin. (B) Percent blocked-Concentration curves. The abscissa represents the concentration, and the ordinate represents the percentage of Kv1.5 current that is blocked at different concentrations of acacetin. Data points represent mean ±SEM of 3 to 5 measurements, and inhibitory effect showed IC_{50} value of 5.96 ± 0.564 μM.

2. Tables

Table S1. Details NMR data of compound **1**

NO.	**1**				
	δ_C, type	δ_H (J in Hz)	COSY	HMBC	NOESY
1	170.9, qC				
2	62.8, CH	3.24, s		C-1, 3, 6, 7, 8	H-8, 24
3	211.6, qC				
4	40.4, CH	3.29, m	H-5α, 5β, 26	C-3, 5, 26	
5β	43.8, CH_2	2.33, dd (13.4, 10.6)		C-3, 6, 24	H_3-25, 26
5α		1.13, m		C-6, 7, 24, 26	H-4
6	40.8, qC				
7	78.4, qC				
8	33.3, CH_2	2.03, d (3.2)		C-2, 6, 7, 9, 10	H-2, 24
9	73.0, CH	5.01, dd (m)	H-8,10	C-7	
10	33.1, CH	1.63, m	H-11, 23	C-11	H-9, 22
11	71.7, CH	4.00, dd (10.8, 1.6)		C-9, 12, 13, 22	H-12, 23, 29
12	34.4, CH	1.48, m		C-13, 14	
13	31.6, CH_2	1.57, overlap	H-14α	C-14	
14α	37.3, CH_2	1.85, m	H-15		
14β		1.57, overlap	H-15		
15	85.4, CH	4.08, m		C-32	
16	144.9, qC				
17	116.9, CH	6.88, d (7.6)		C-15	
18	129.6, CH	7.20, t (7.8)	H-17, 19		
19	114.5, CH	6.75, dd (8.1, 2.5)			
20	156.6, qC				
21	114.3, CH	6.99, m		C-15	
22	13.5, CH_3	0.89, d		C-11, 12, 13	
23	13.9, CH_3	0.75, d		C-9, 10, 11	
24	25.4, CH_3	0.79, s		C-5, 6, 7, 25	
25	25.1, CH_3	1.02, s		C-5, 6, 7, 24	
26	15.3, CH_3	1.10, d		C-3, 4, 5	
27	169.8, qC				
28β	37.3, CH_2	2.80, dd (15.6, 5.9)		C-27	
28α		2.64, dd (15.6, 10.3)		C-27, 29, 30	
29	76.8, CH	5.14, ddd(10.3, 6.9, 5.8)	H-28α,28β,30	C-1, 28, 30	
30	69.8, CH	3.92, m	H-29, 31	C-29, 31	
31	18.3, CH_3	1.25, d		C-29, 30	H-28α,28β,29
32	57.4, CH_3	3.27, s		C-15	

Table S2. Details NMR data of compound **2**

NO.	**2**				
	δ_C, type	δ_H (J in Hz)	COSY	HMBC	NOESY
1	169.6, qC				
2	64.4, CH	3.84, s		C-1, 3, 6, 7,8	
3	205.9, qC				
4	41.2, CH	2.60, m	H-5α, 26	C-3, 5, 26	H-2
5β	43.7, CH_2	1.65, dd (14.0, 6.8)		C-3, 4, 7, 24, 25, 26	H-4
5α		1.35, dd (14.0, 13.4)		C-3, 4, 24, 25, 26	H-8, 25, 26
6	40.6, qC				
7	81.5, qC				
8	125.4, CH	5.45, dd (10.4, 2.9)		C-7, 10	
9	134.2, CH	5.74, dd (10.4, 1.7)	H-8, H-10	C-7, 10, 11, 23	
10	30.1, CH	2.09, m	H-11, 23	C-8, 9, 11, 23	H-22
11	77.9, CH	2.96, dd (9.5, 1.8)		C-12, 13, 22, 23	H-12, 23, 2
12	34.1, CH	1.55, overlap		C-13, 14, 22	
13α	30.7, CH_2	1.43, m		C-11, 12, 14, 15, 22	
13β		1.23, m	H-12,14α	C-11, 12, 14, 15, 22	
14α	35.9, CH_2	1.78, m		C-12, 13, 15, 16	
14β		1.53, overlap		C-12, 13, 15, 16	
15	84.8, CH	4.0, t (6.6)	H_2-14	C-13,14,16,17,21,28	
16	144.3, qC				
17	119.3, CH	6.84, d (7.6, 1.2)			
18	129.6, CH	7.20, t (7.8)	H-17, 19		
19	114.7, CH	6.76, dd (8.0, 2.6)			
20	156.1, qC				
21	113.8, CH	6.81, brs			
22	13.0, CH_3	0.85, d		C-11, 12, 13	
23	17.0, CH_3	0.82, d		C-9, 10, 11	
24	22.5, CH_3	1.21, s		C-5, 6, 7, 25	H-2, 4
25	24.9, CH_3	0.88, s		C-5, 6, 7, 24	
26	14.4, CH_3	1.04, d		C-3, 4, 5	
27	51.8, CH_3	3.56, s		C-1	
28	56.8, CH_3	3,21, s		C-15	

Table S3. Details NMR data of compound **3**

NO.	**3**				
	δ_C, type	δ_H (J in Hz)	COSY	HMBC	NOESY
1α	47.8, CH_2	2.50, d (13.5)		C-2, 3, 5, 6, 7	
1β		2.38, d (13.5)		C-2, 5, 6, 7	
2	212.4, qC				
3	41.4, CH	2.56, m	H_3-25, H_2-4	C-2, 4, 25	
4α	44.3, CH_2	1.31, dd (14.2, 11.5)		C-2, 3, 5, 6, 23, 24, 25	H-7, 23, 25
4β		1.71, dd (14.0, 6.8)		C-2, 3, 5, 6, 23, 24, 25	
5	38.6, qC				
6	79.7, qC				
7	128.1, CH	5.39, dd (10.3, 2.8)		C-6, 9	
8	133.6, CH	5.58, dd (10.3,1.7)	H-7	C-6, 9, 10, 22	
9	30.3, CH	2.12, m	H-7, 8, 10, 22	C-7, 8, 10, 22	H-21
10	75.5, CH	3.05, dd (9.5, 1.8)	H-11	C-6, 8, 9, 11, 21, 22	H-1α,1β, 11, 22
11	33.7, CH	1.61, overlap	H-12, 21	C-12, 13, 21	
12	30.2, CH_2	1.33, m	H-13α, 13β	C-10, 11, 13, 14, 21	
13α	36.1, CH_2	1.78, m	14	C-11, 12, 14, 15	
13β		1.61, m	14	C-11, 12, 14, 15	
14	84.4, CH	3.99, t (6.6)		C-12, 13, 15, 16, 20, 26	
15	144.3, qC				
16	119.5, CH	6.79, overlap			
17	129.5, CH	7.17, t (8.0)	H-16, 18		
18	114.9, CH	6.74,dd (8.1, 2.5)			
19	156.4, qC				
20	113.5, CH	6.79, overlap			
21	13.4, CH_3	0.84, d (6.8)		C-10, 11, 12	
22	16.7, CH_3	0.80, d (7.2)		C-8, 9, 10	
23	24.9, CH_3	0.87, s		C-4, 5, 6, 24	
24	22.6, CH_3	1.07, s		C-4, 5, 6, 23	H-1β, 3, 4β
25	14.9, CH_3	1.03, d (6.6)		C-2, 3, 4	
26	56.7, CH_3	3.20, s		C-14	

Figure S1. ^{1}H NMR spectrum of Compound **1** (600 MHz, $CDCl_3$)

Figure S2. ^{13}C NMR spectrum of Compound **1** (150 MHz, $CDCl_3$)

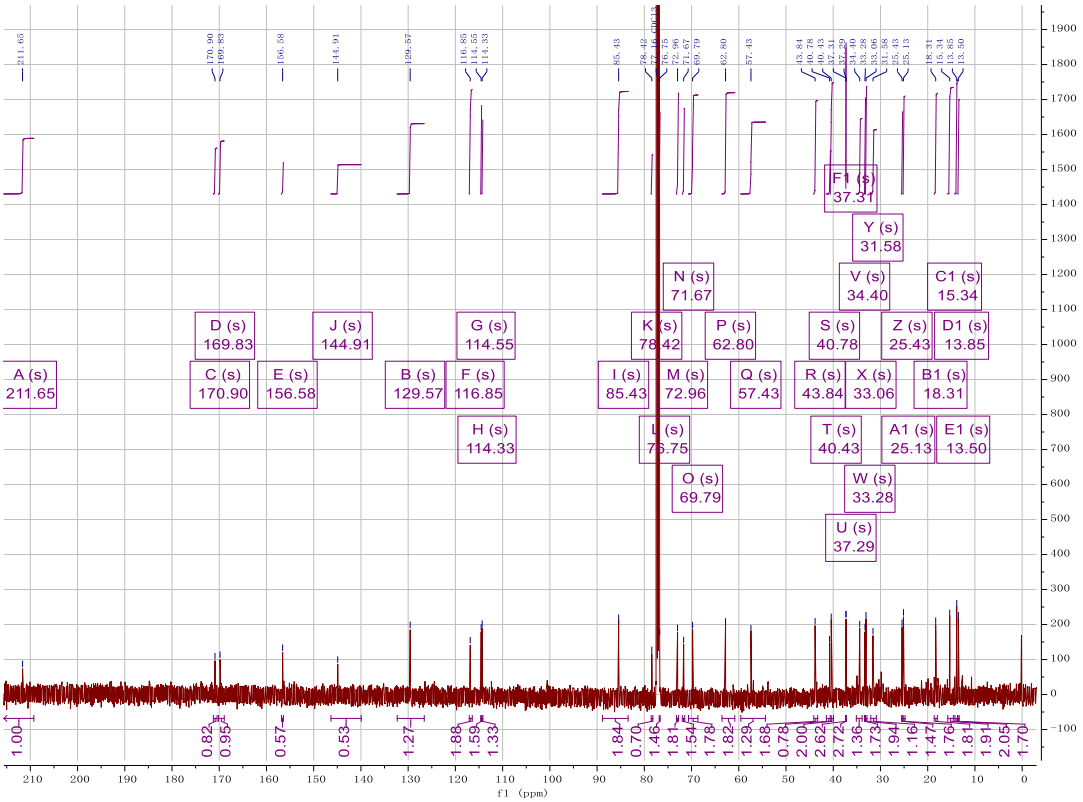


Figure S3. DEPT spectrum of Compound **1** (150 MHz, CDCl3)

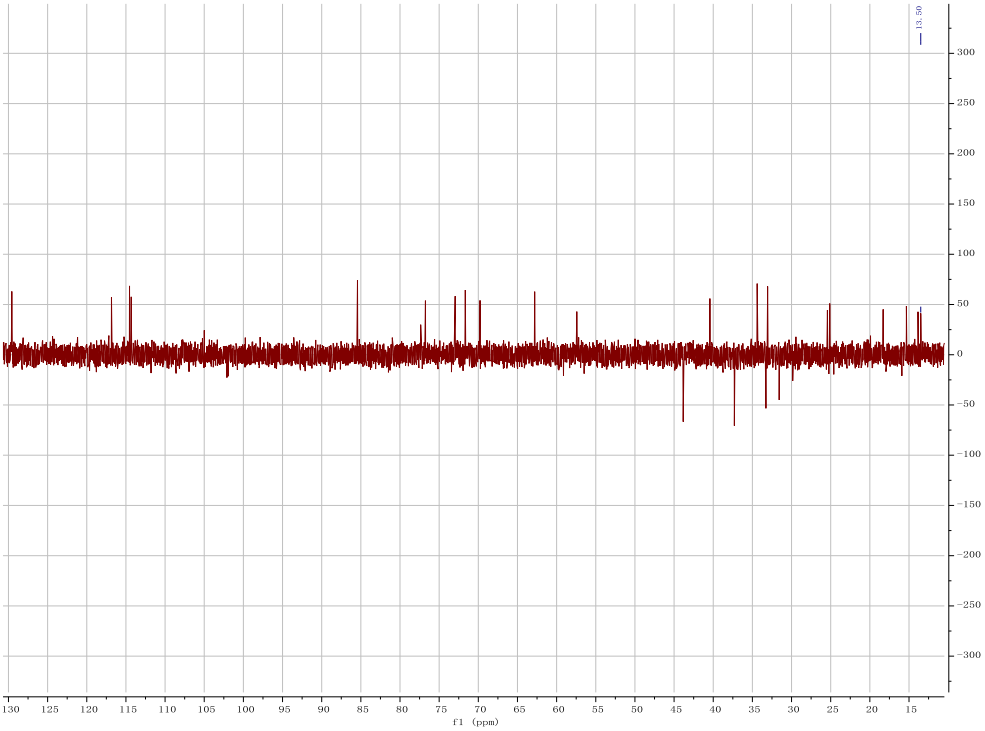


Figure S4.HSQC spectrum of Compound **1** (600 MHz, CDCl3)

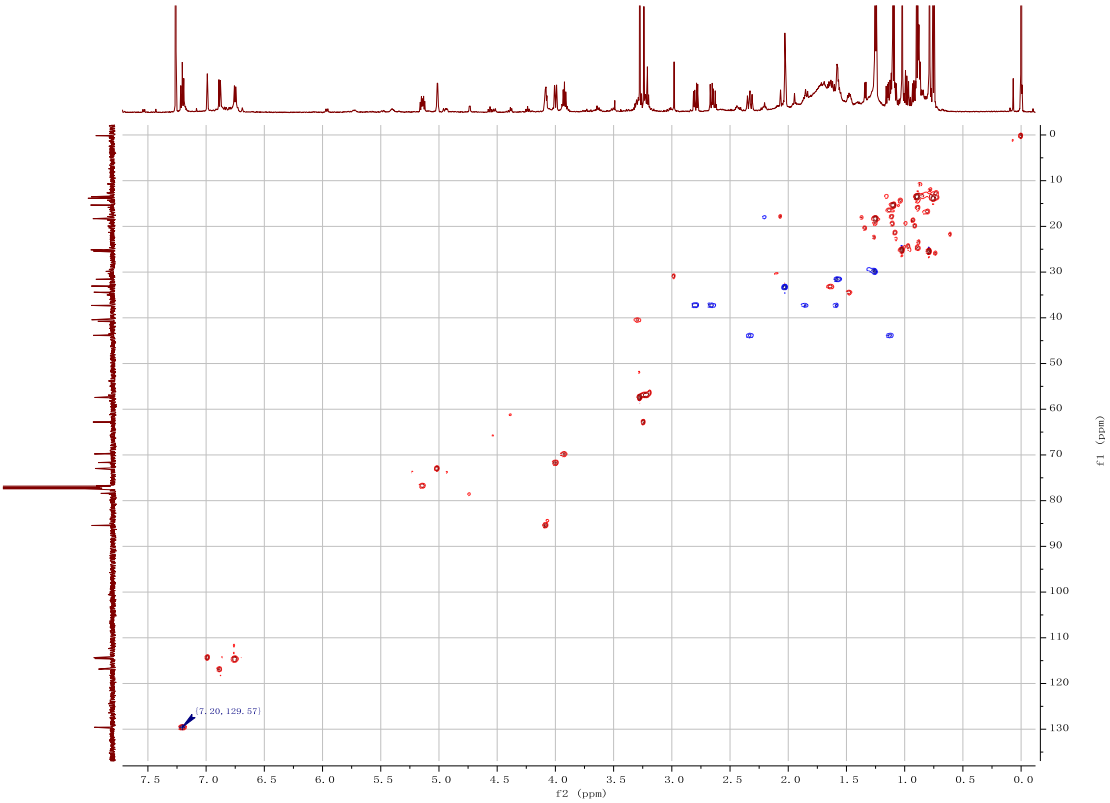


Figure S5. ^{1}H-^{1}H COSY spectrum of Compound **1** (600 MHz, CDCl3)

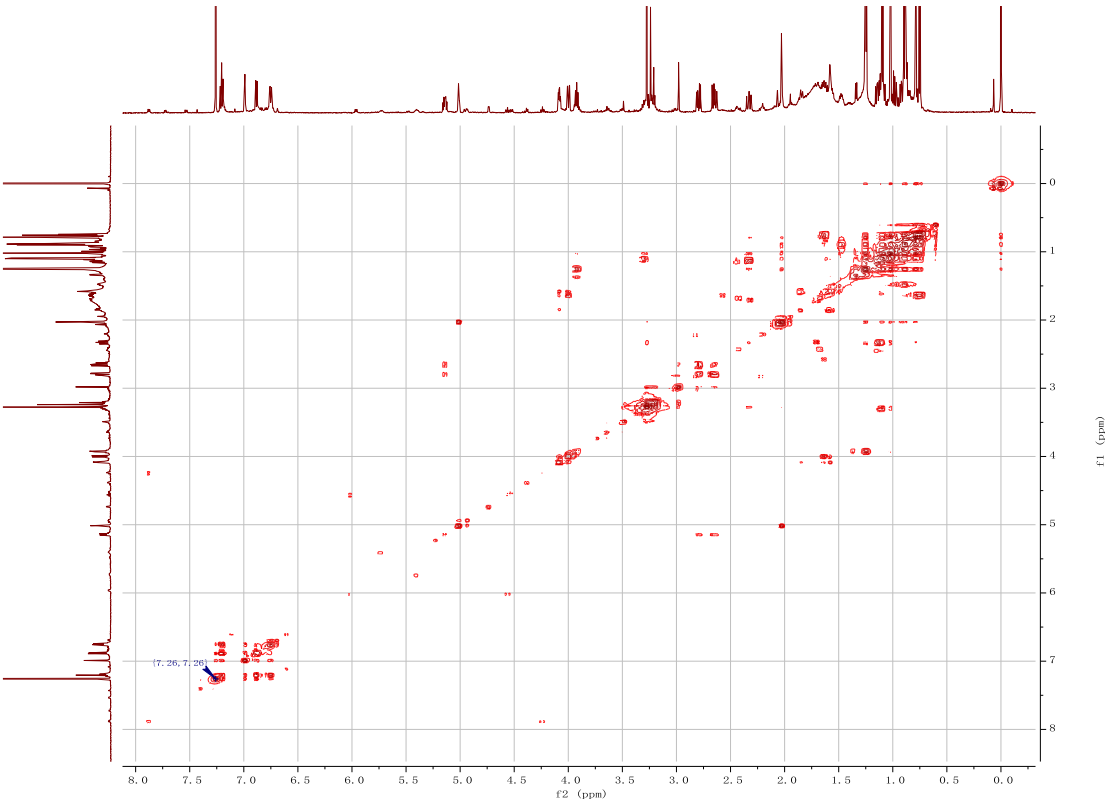


Figure S6. HMBC spectrum of Compound 4 (600 MHz, $CDCl_3$)

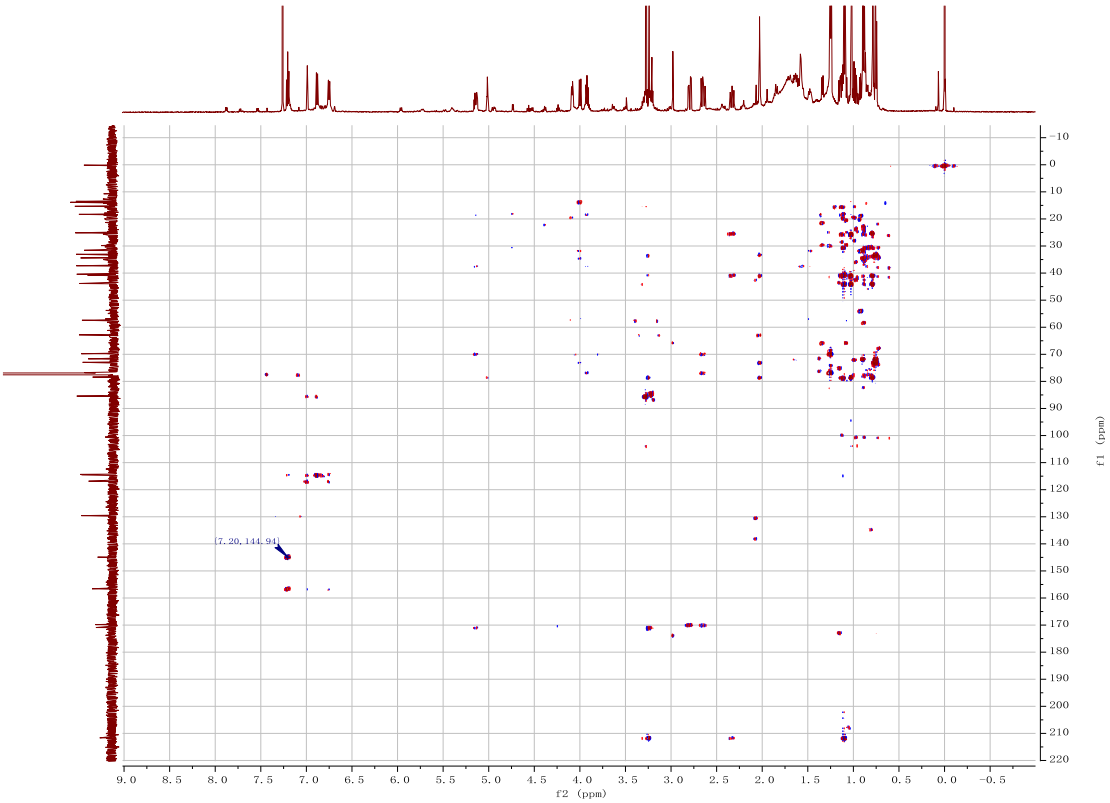


Figure S7. NOESY spectrum of Compound **1** (600 MHz, $CDCl_3$)

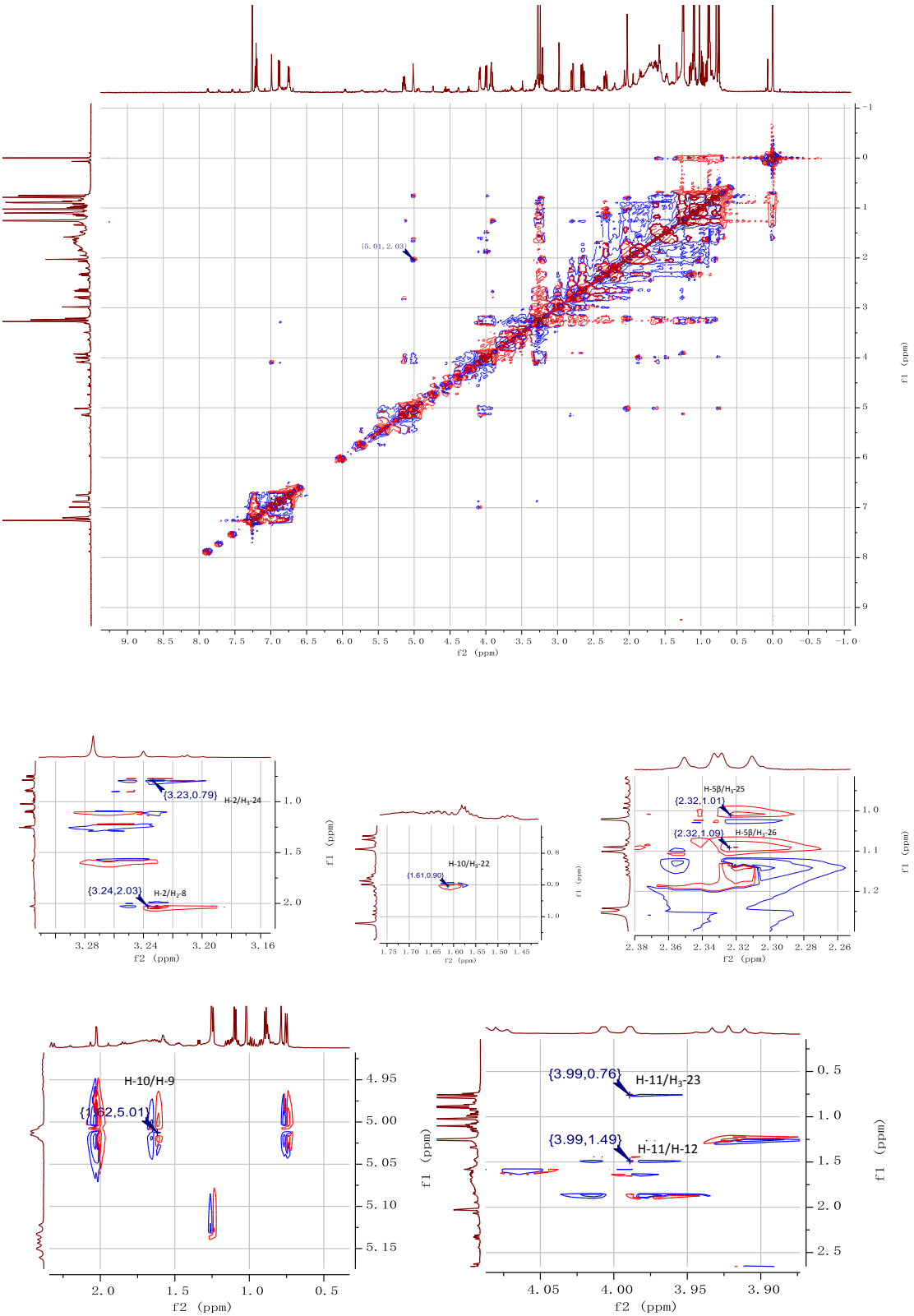
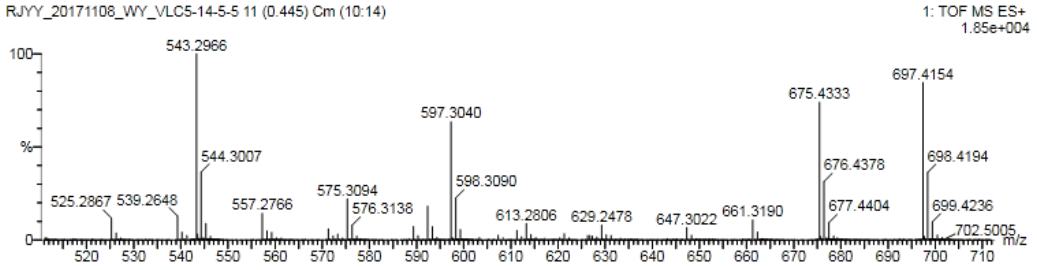


Figure S8. HRESIMS spectrum of **1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
709 formula(e) evaluated with 15 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-50 H: 1-80 N: 0-2 O: 0-20 Na: 0-1
RJYY_20171108_WY_VLC5-14-5-5 11 (0.445) Cm (10:14)



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		10.0	10.0	50.0			
597.3040	597.2941	9.9	16.6	14.5	773.7	3.6	C34 H42 N2 O6 Na
	597.3040	0.0	0.0	9.5	772.1	2.0	C32 H46 O9 Na
	597.2981	5.9	9.9	18.5	775.1	4.9	C39 H42 O4 Na
	597.3093	-5.3	-8.9	18.5	774.1	4.0	C38 H42 N2 O3 Na
	597.2999	4.1	6.9	5.5	771.5	1.3	C27 H46 N2 O11 Na
	597.3098	-5.8	-9.7	0.5	772.3	2.1	C25 H50 O14 Na
	597.3133	-9.3	-15.6	22.5	777.3	7.2	C43 H42 O Na
	597.3082	-4.2	-7.0	-0.5	772.6	2.4	C22 H49 N2 O16
	597.2965	7.5	12.6	17.5	774.5	4.3	C36 H41 N2 O6
	597.3005	3.5	5.9	21.5	776.1	5.9	C41 H41 O4
	597.3064	-2.4	-4.0	12.5	773.4	3.2	C34 H45 O9
	597.3023	1.7	2.8	8.5	771.9	1.7	C29 H45 N2 O11
	597.2970	7.0	11.7	-0.5	773.4	3.3	C23 H49 O17
	597.3117	-7.7	-12.9	21.5	775.9	5.7	C40 H41 N2 O3
	597.3122	-8.2	-13.7	3.5	773.1	2.9	C27 H49 O14

Figure S9. ^{1}H NMR spectrum of Compound **2** (600 MHz, $CDCl_3$)

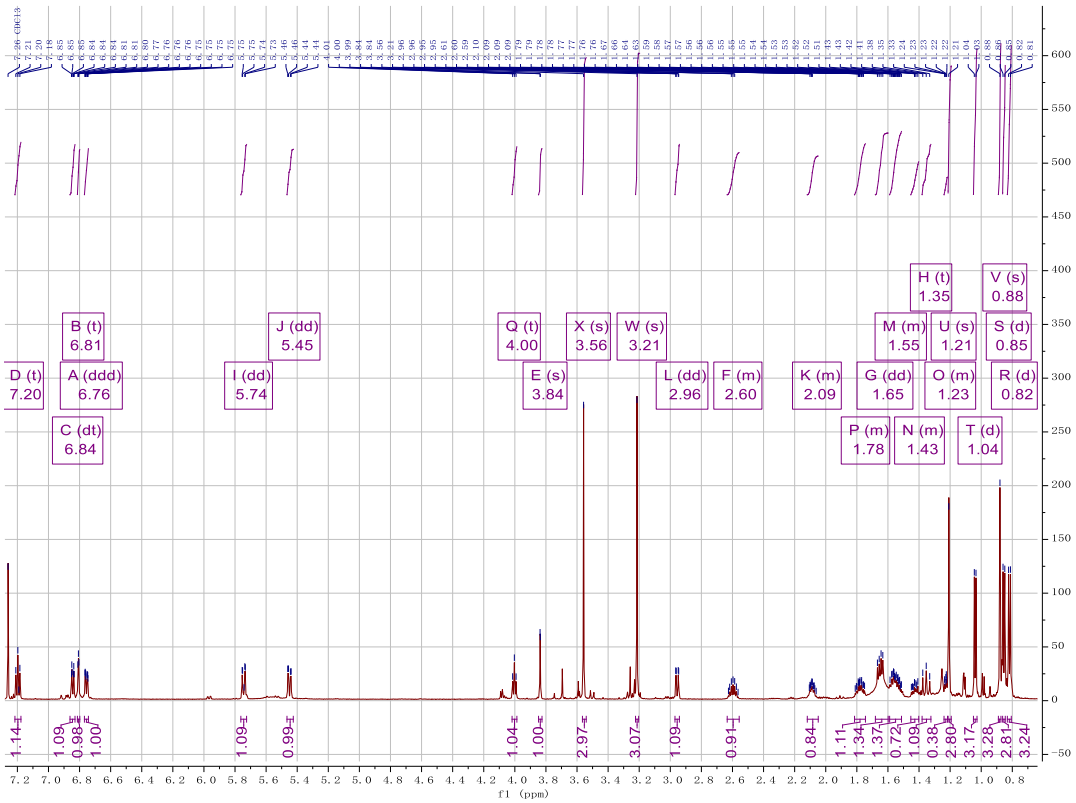


Figure S10. ^{13}C NMR spectrum of Compound **2** (150 MHz, $CDCl_3$)

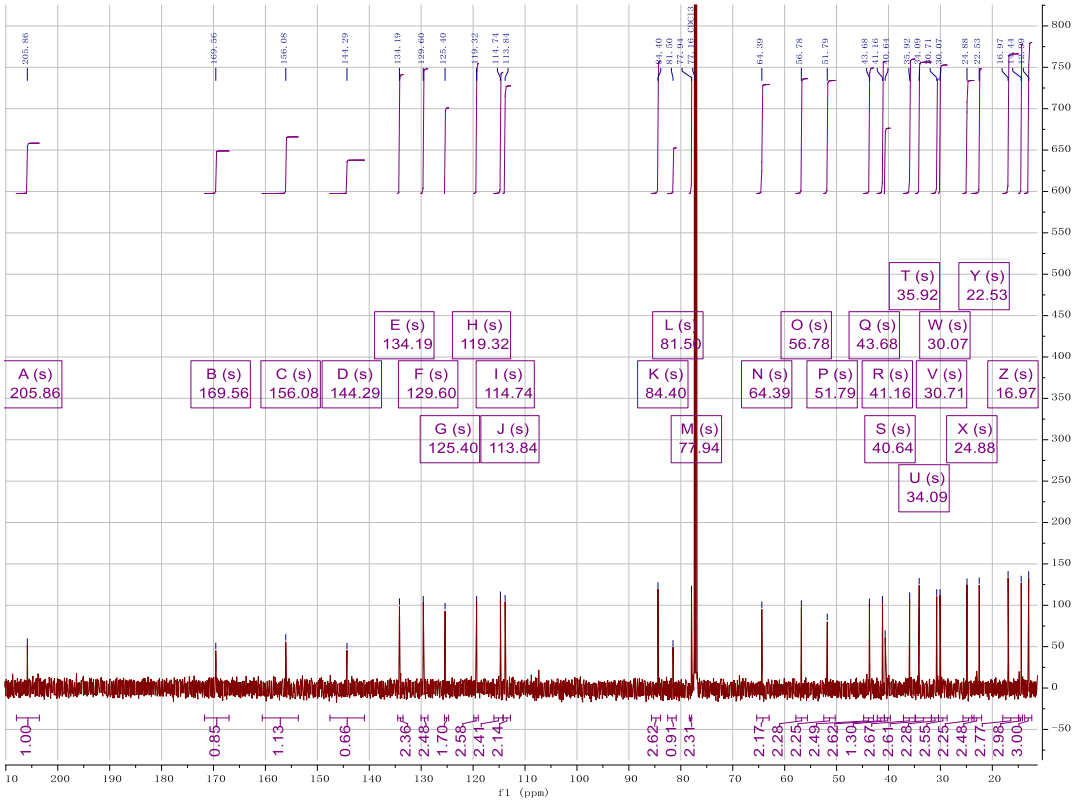


Figure S11. DEPT spectrum of Compound **2** (150 MHz, $CDCl_3$)

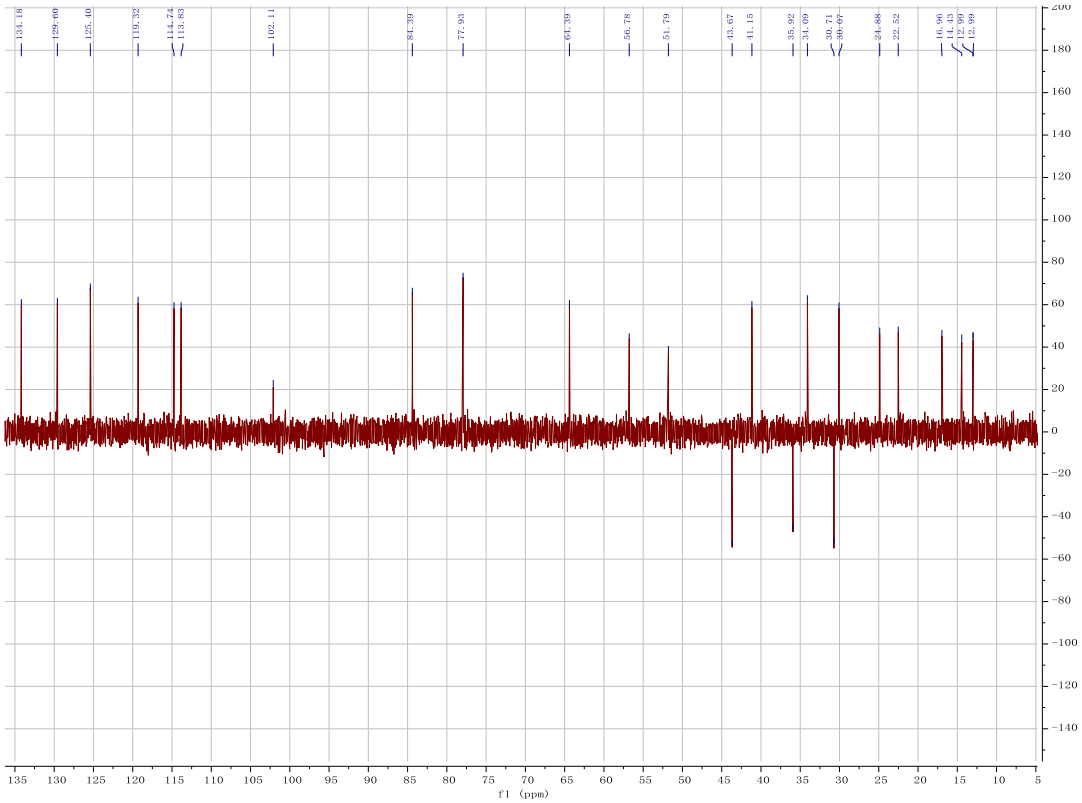


Figure S12. HSQC spectrum of Compound **2** (600 MHz, $CDCl_3$)

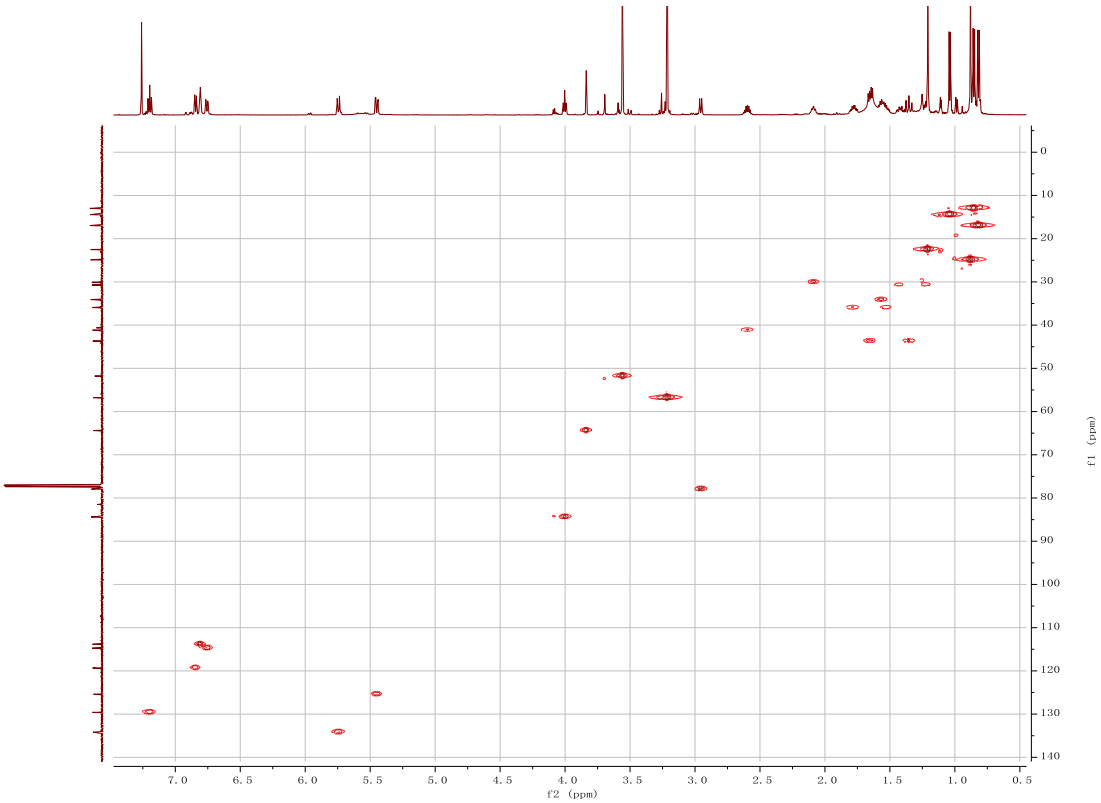


Figure S13. ^{1}H-^{1}H COSY spectrum of Compound **2** (600 MHz, $CDCl_3$)

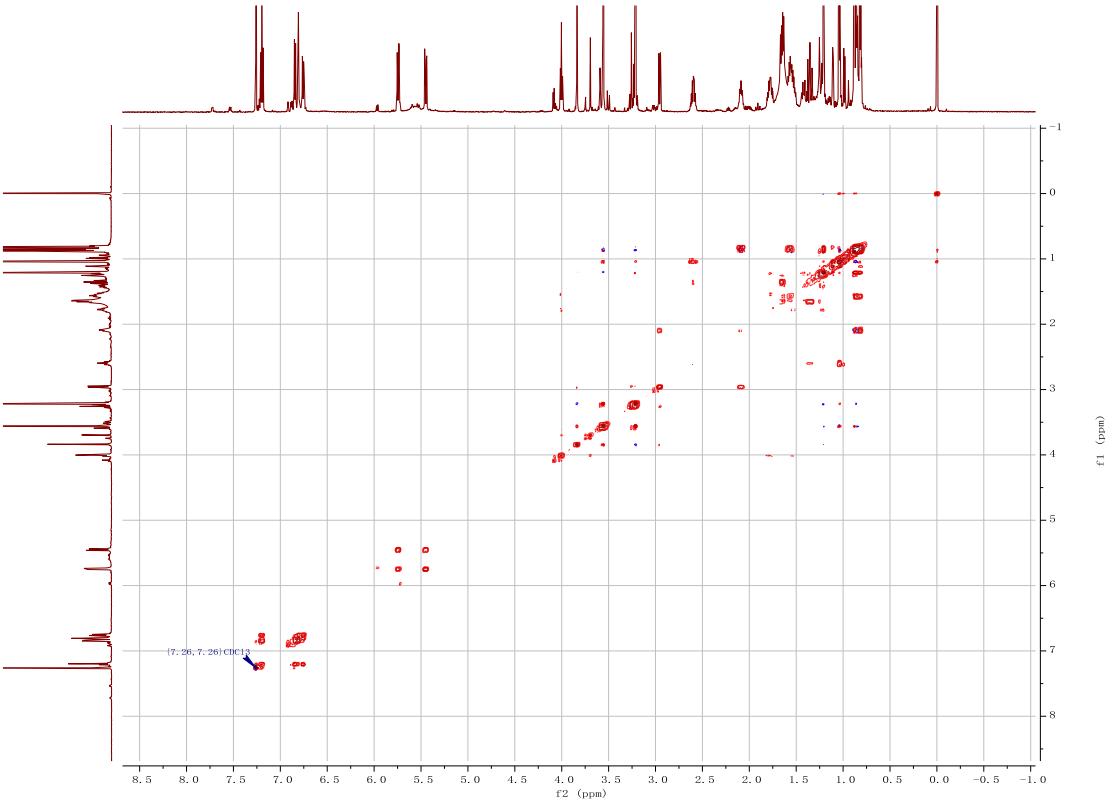


Figure S14. HMBC spectrum of Compound **2** (600 MHz, $CDCl_3$)

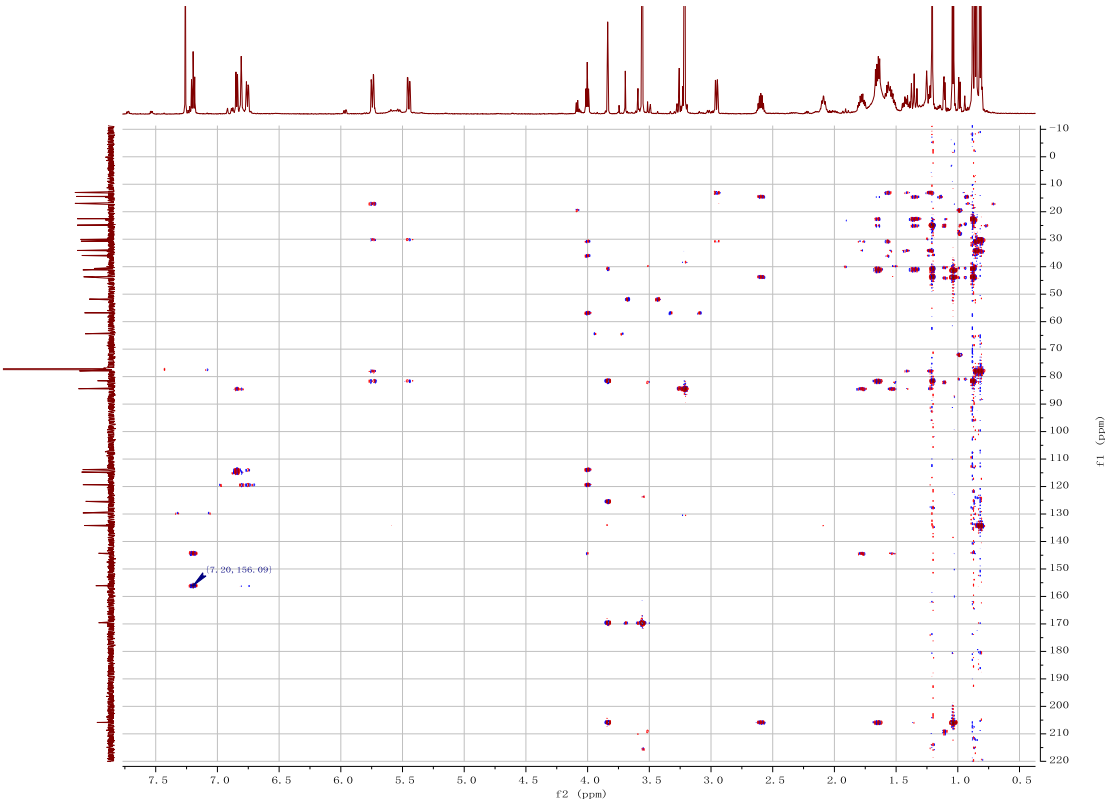


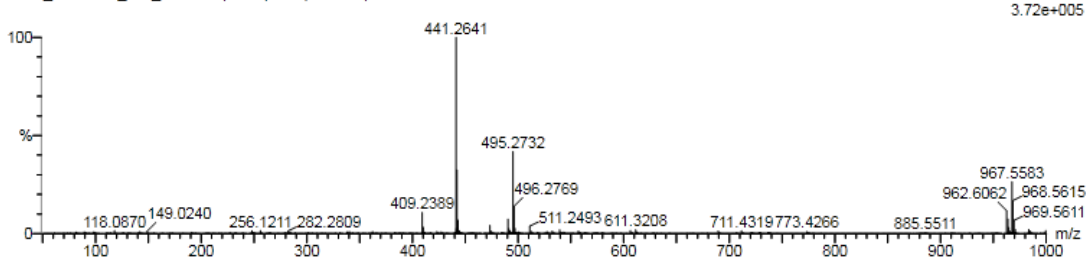
Figure S15. HRESIMS spectrum of **2**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

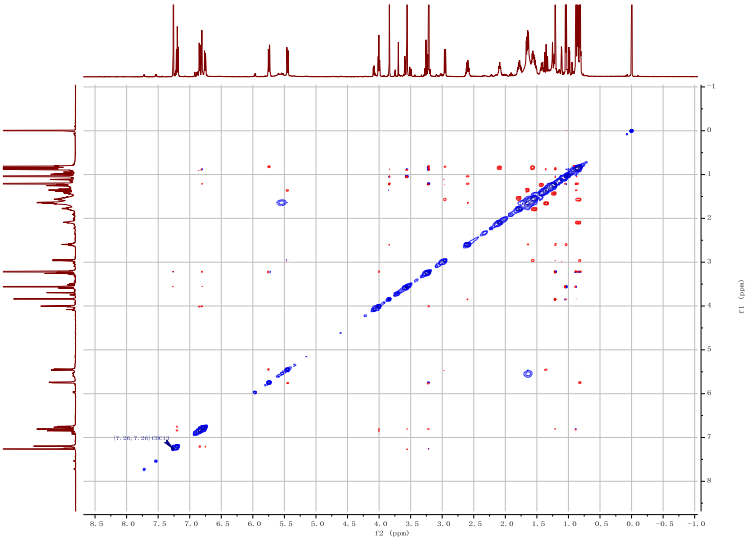
Monoisotopic Mass, Even Electron Ions
63 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-80 O: 0-10 Na: 1-1
RJYY_20171115_WY_9-3-2 11 (0.445) Cm (9:13-1:7)

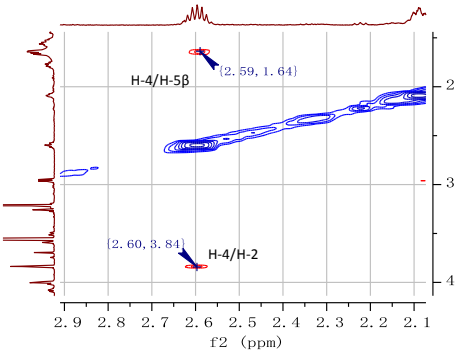
1: TOF MS ES+
3.72e+005

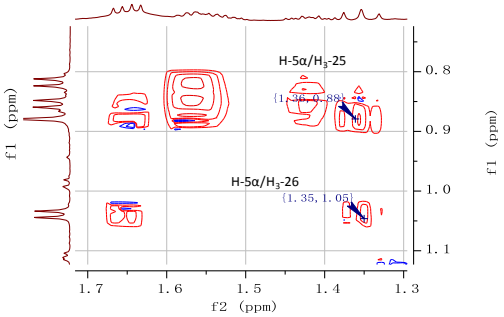


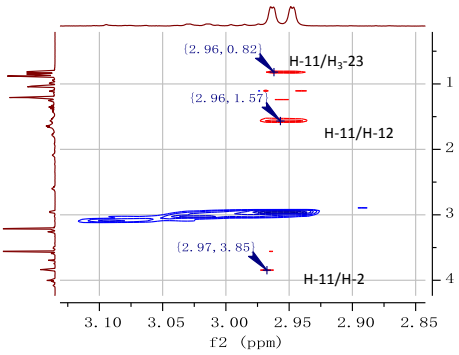
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
495.2732	495.2723	0.9	1.8	8.5	1336.0	0.0	C28 H40 O6 Na

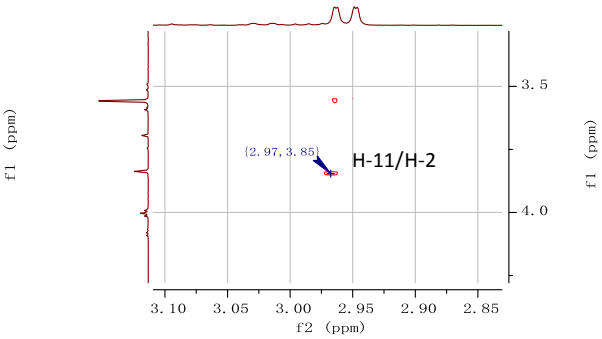
Figure S16. NOESY spectrum of Compound **2** (600 MHz, $CDCl_3$)

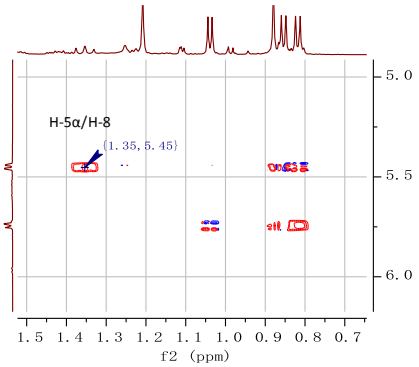












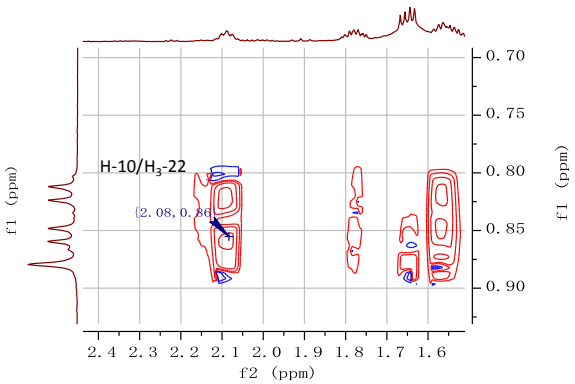


Figure S17. ^{1}H NMR spectrum of Compound **3** (600 MHz, $CDCl_3$)

Figure S18. ^{13}C NMR spectrum of Compound **3** (150 MHz, $CDCl_3$)

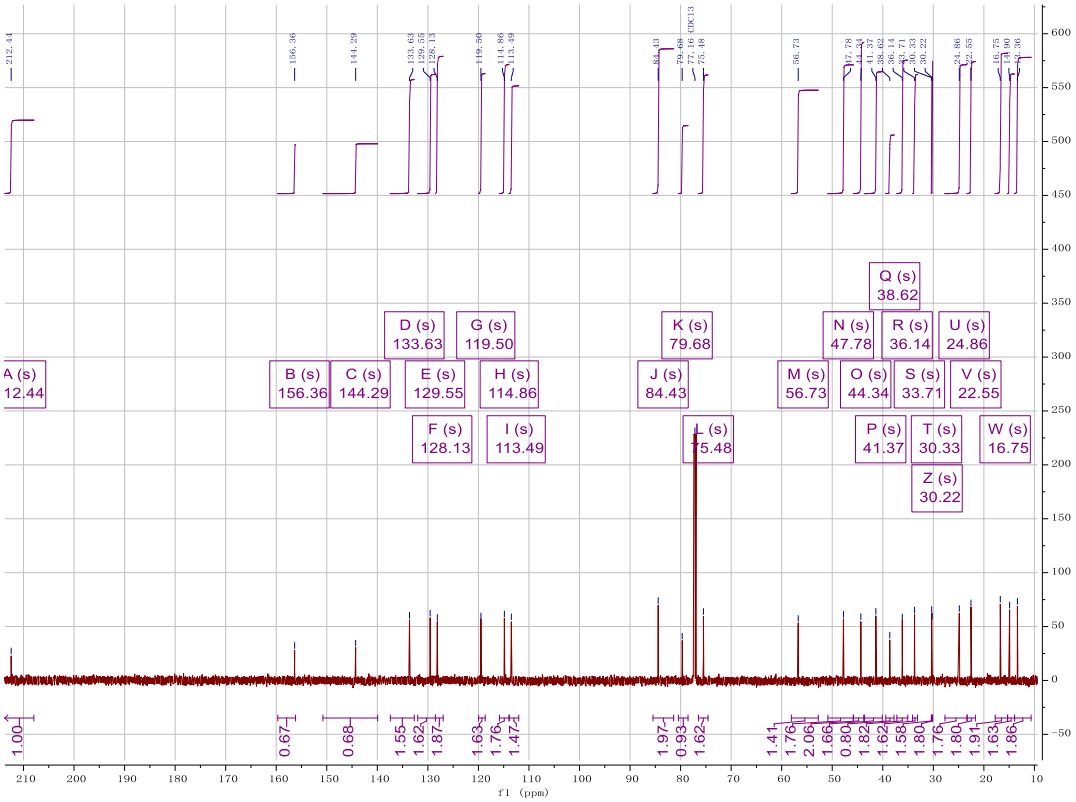


Figure S19. DEPT spectrum of Compound **3** (150 MHz, $CDCl_3$)

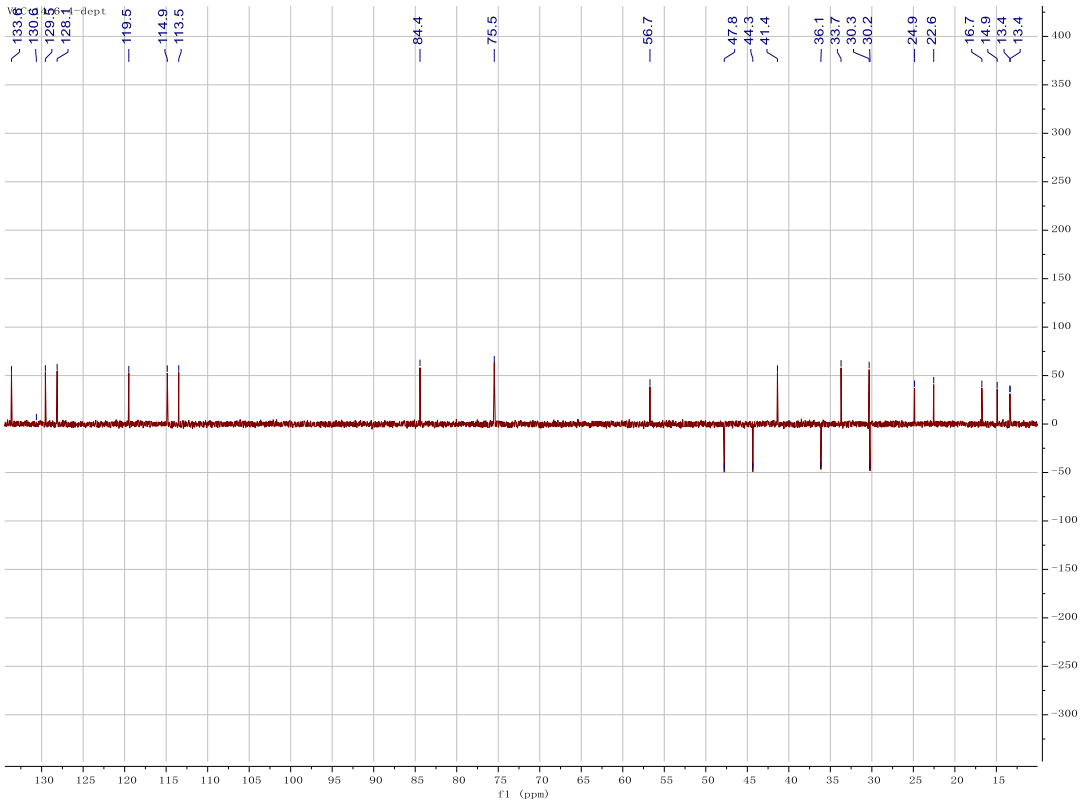


Figure S20. HSQC spectrum of Compound **3** (600 MHz, $CDCl_3$)

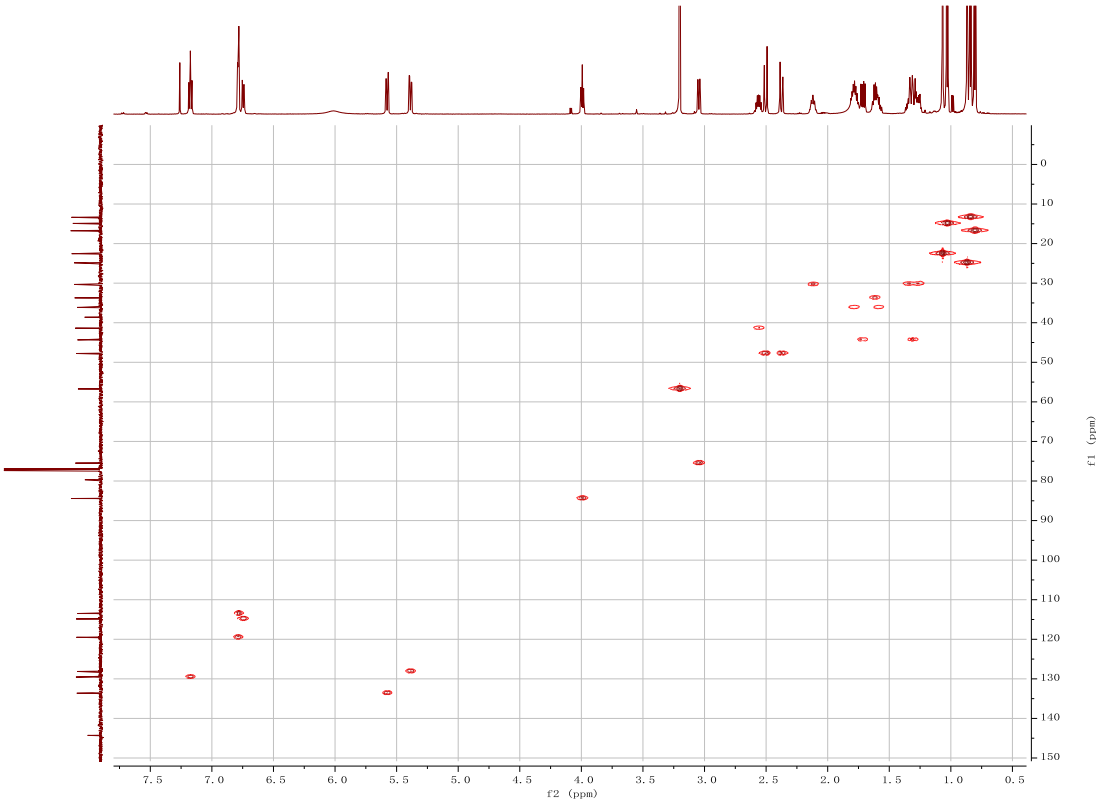


Figure S21. ^{1}H-^{1}H COSY spectrum of Compound **3** (600 MHz, $CDCl_3$)

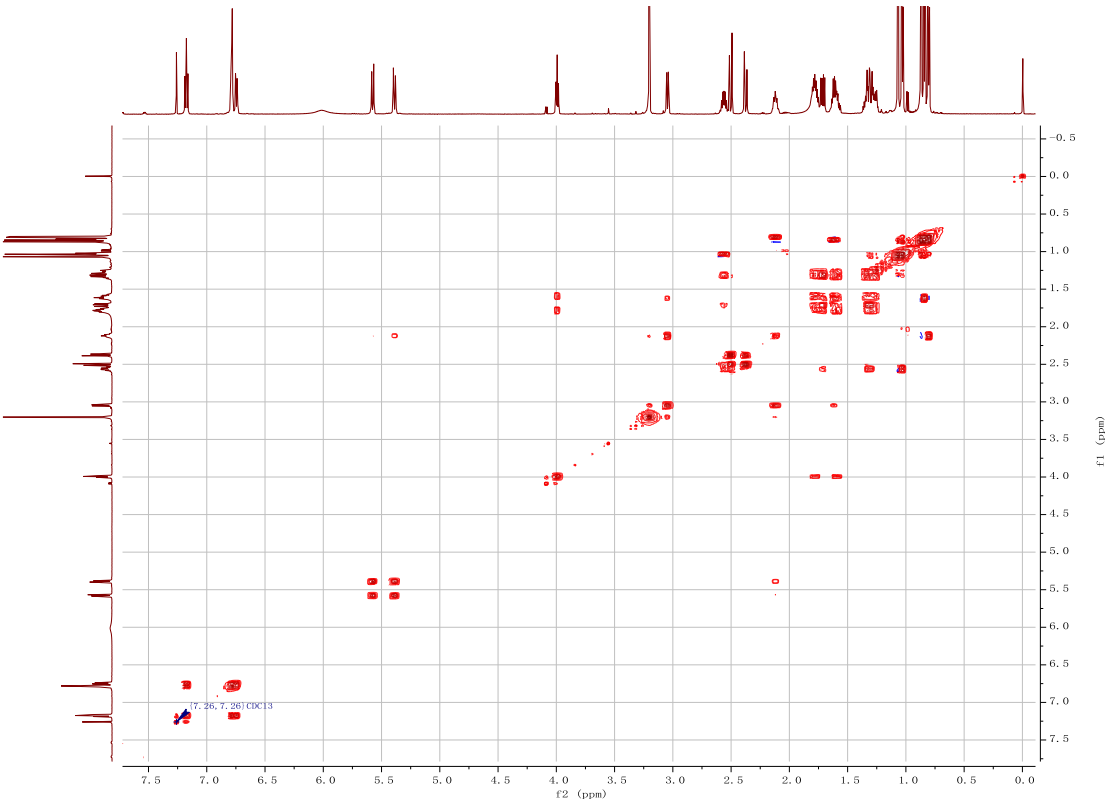


Figure S22. HMBC spectrum of Compound **3** (600 MHz, $CDCl_3$)

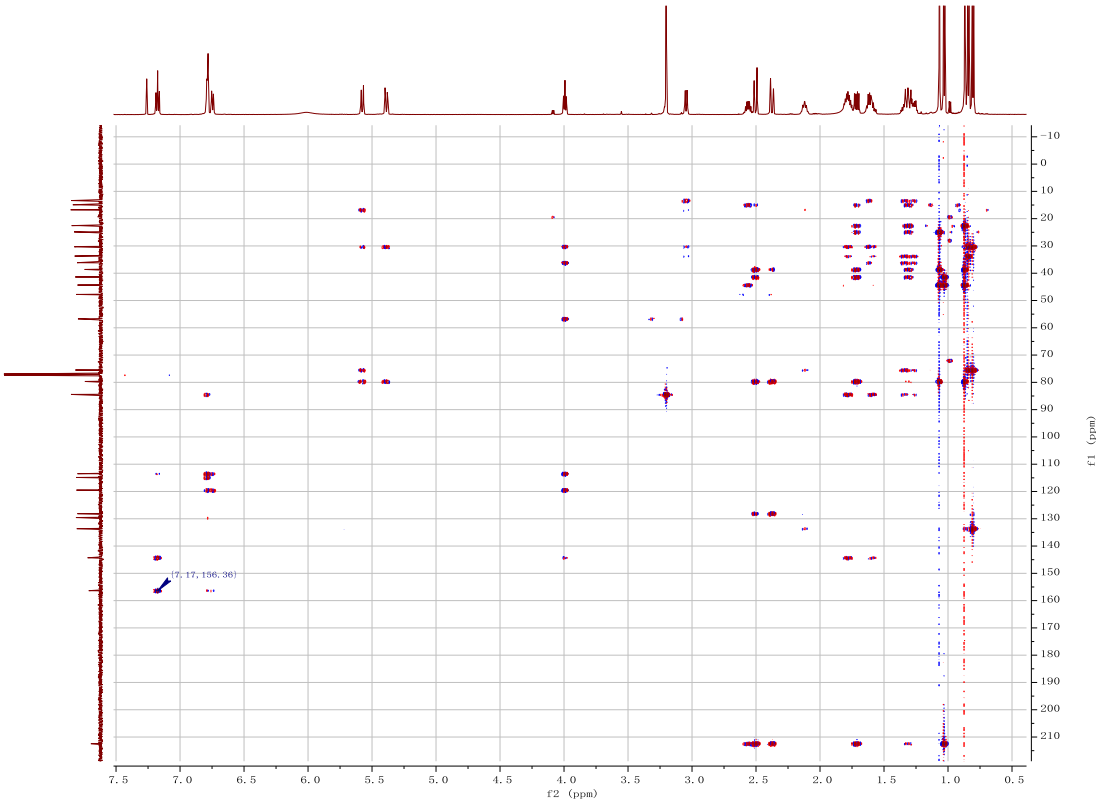


Figure S23. NOESY spectrum of Compound **3** (600 MHz, $CDCl_3$)

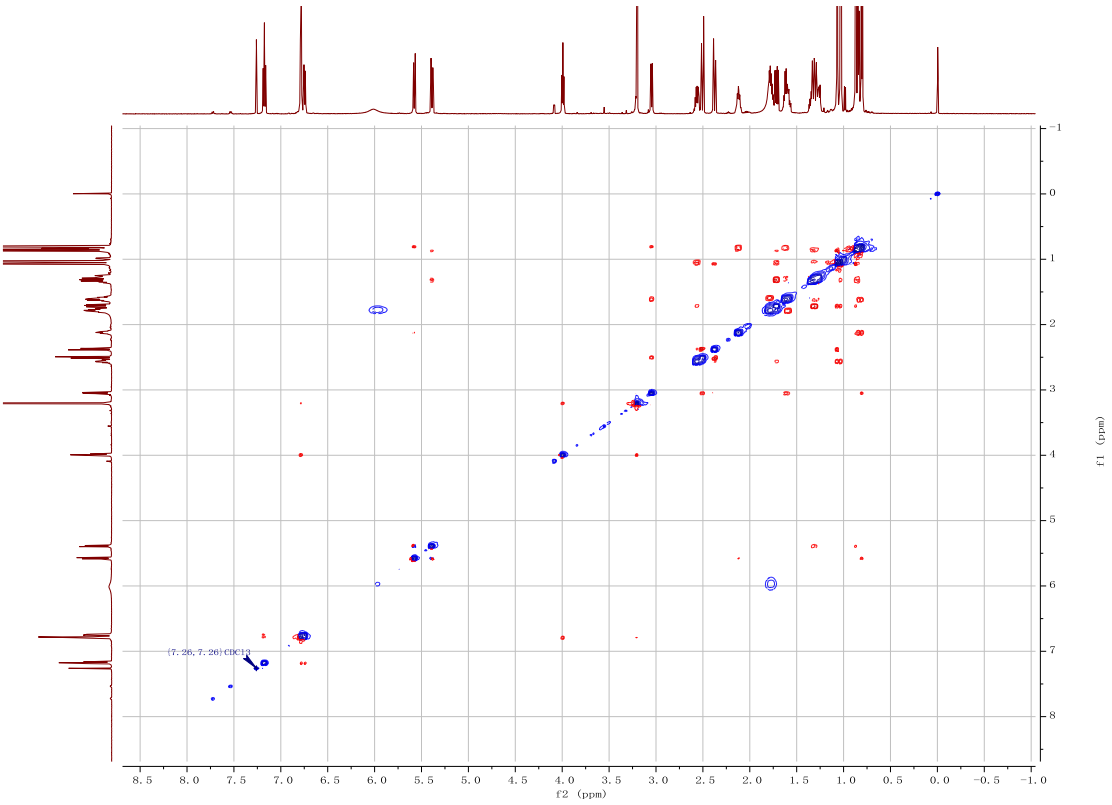
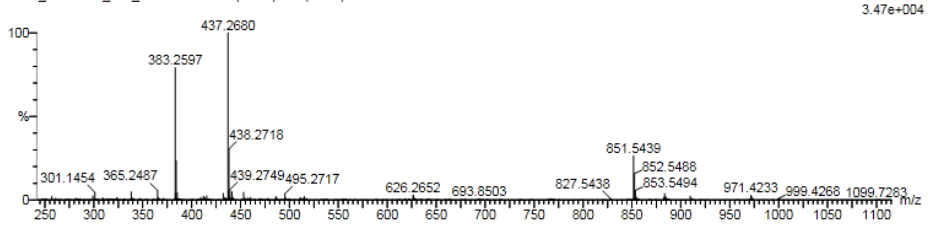


Figure S24. HRESIMS spectrum of **3**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
169 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-50 H: 1-80 O: 0-20 Na: 0-1
RJYY_20171108_WY_VLC5-14-6-4 11 (0.445) Cm (11:15) 1: TOF MS ES+ 3.47e+004



Minimum:				-1.5				
Maximum:		10.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
437.2680	437.2692	-1.2	-2.7	10.5	1196.2	1.1	C28 H37 O4	
	437.2668	1.2	2.7	7.5	1196.3	1.2	C26 H38 O4	Na
	437.2727	-4.7	-10.7	-1.5	1197.3	2.1	C19 H42 O9	Na
	437.2751	-7.1	-16.2	1.5	1196.6	1.5	C21 H41 O9	

Figure S25. ^{1}H NMR spectrum of Compound **4** (600 MHz, $CDCl_3$)

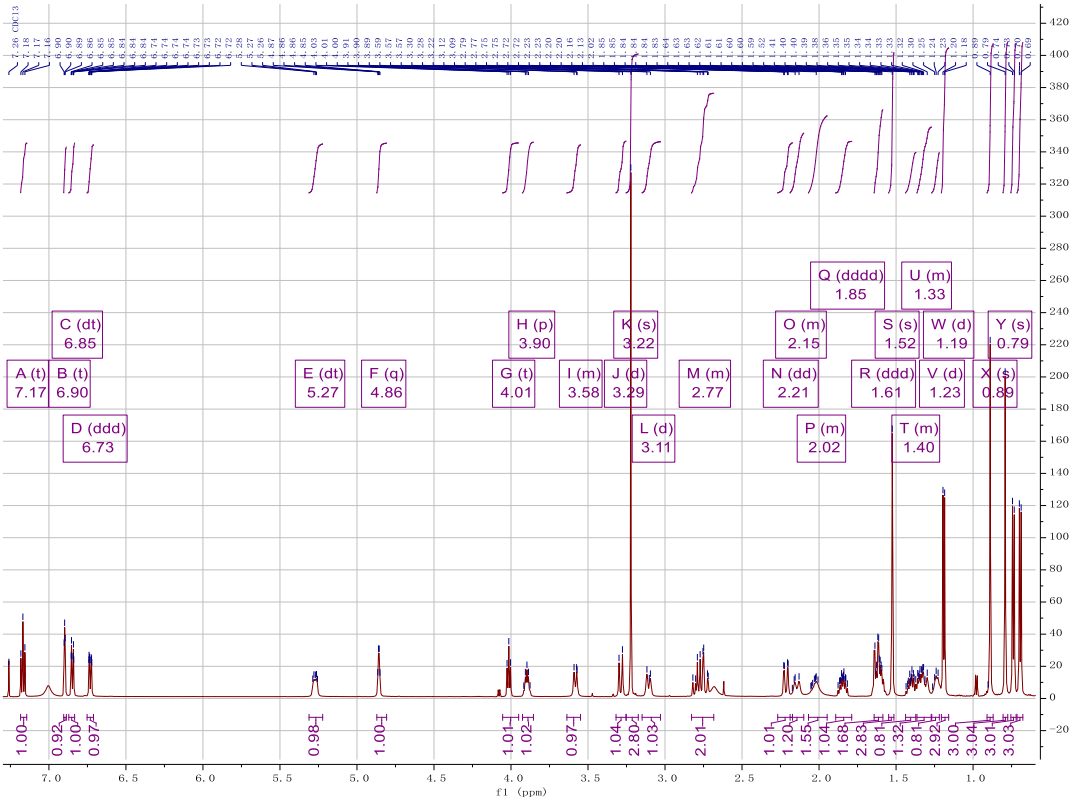


Figure S26. ^{13}C NMR spectrum of Compound **4** (150 MHz, $CDCl_3$)

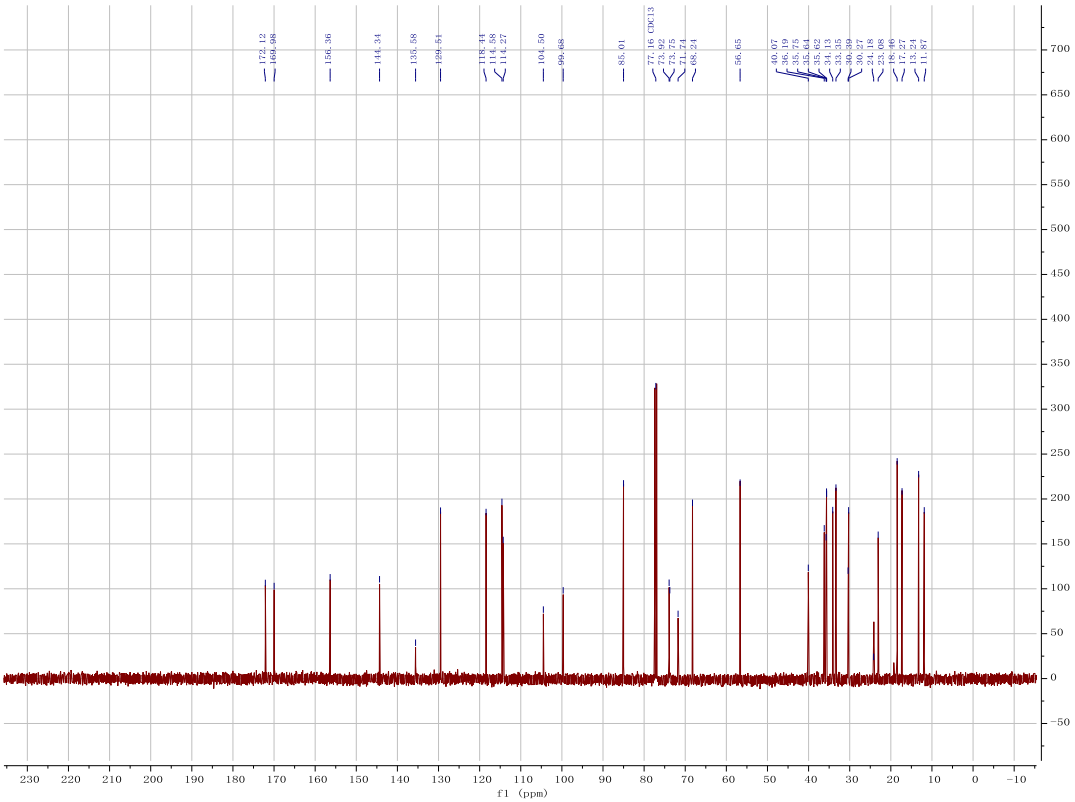


Figure S27. HRESIMS spectrum of **4**

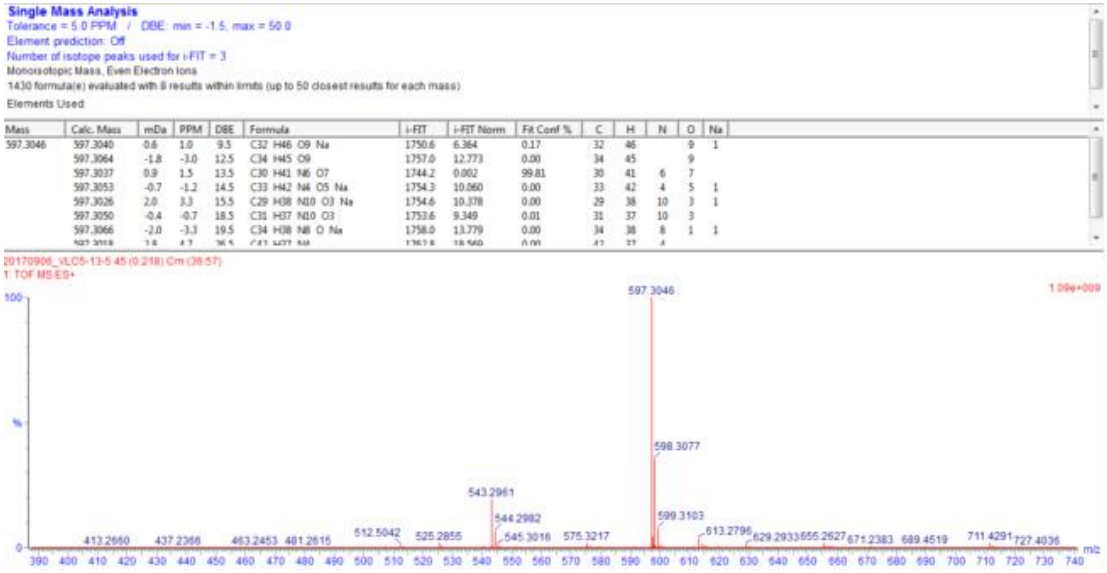


Figure S28. ^{1}H NMR spectrum of Compound **5** (600 MHz, $CDCl_3$)

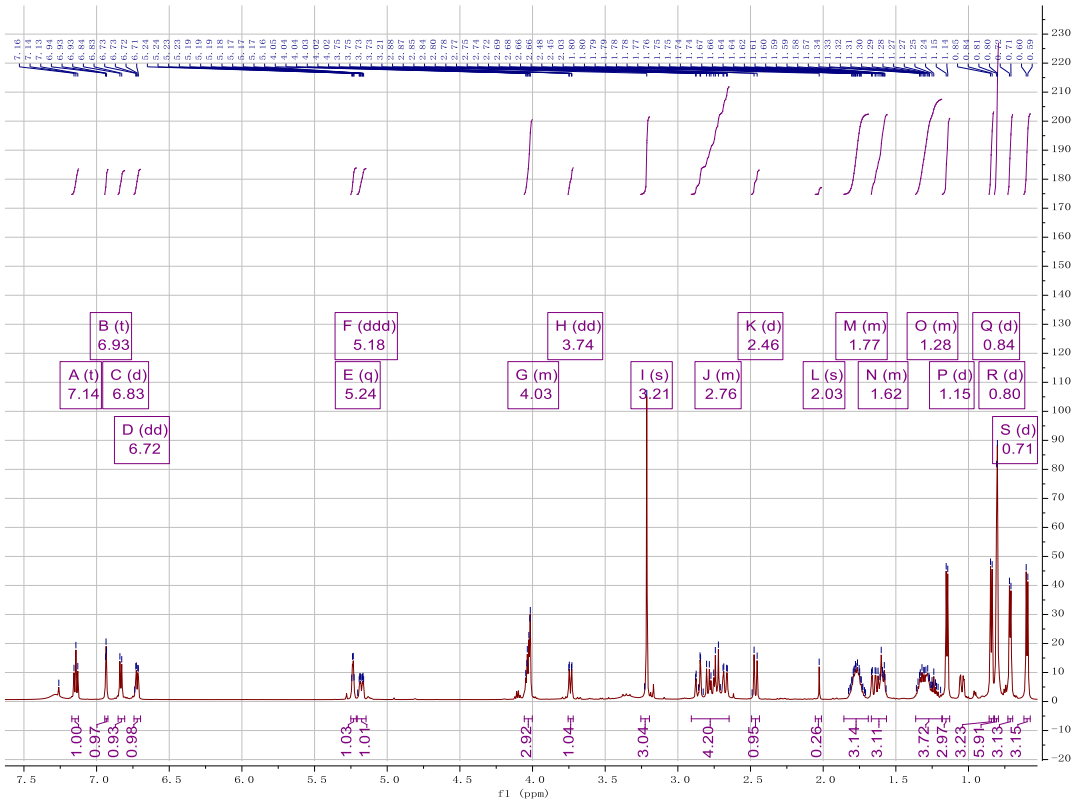


Figure S29. ^{13}C NMR spectrum of Compound **5** (150 MHz, $CDCl_3$)

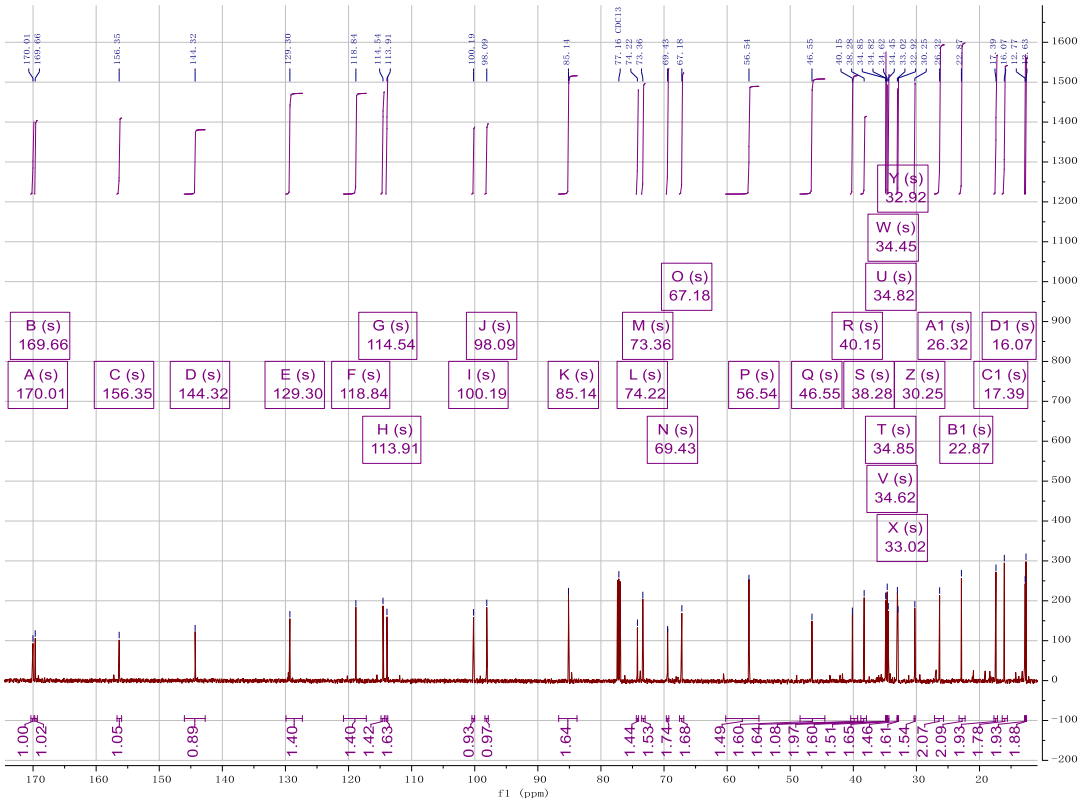


Figure S30. HRESIMS spectrum of **5**

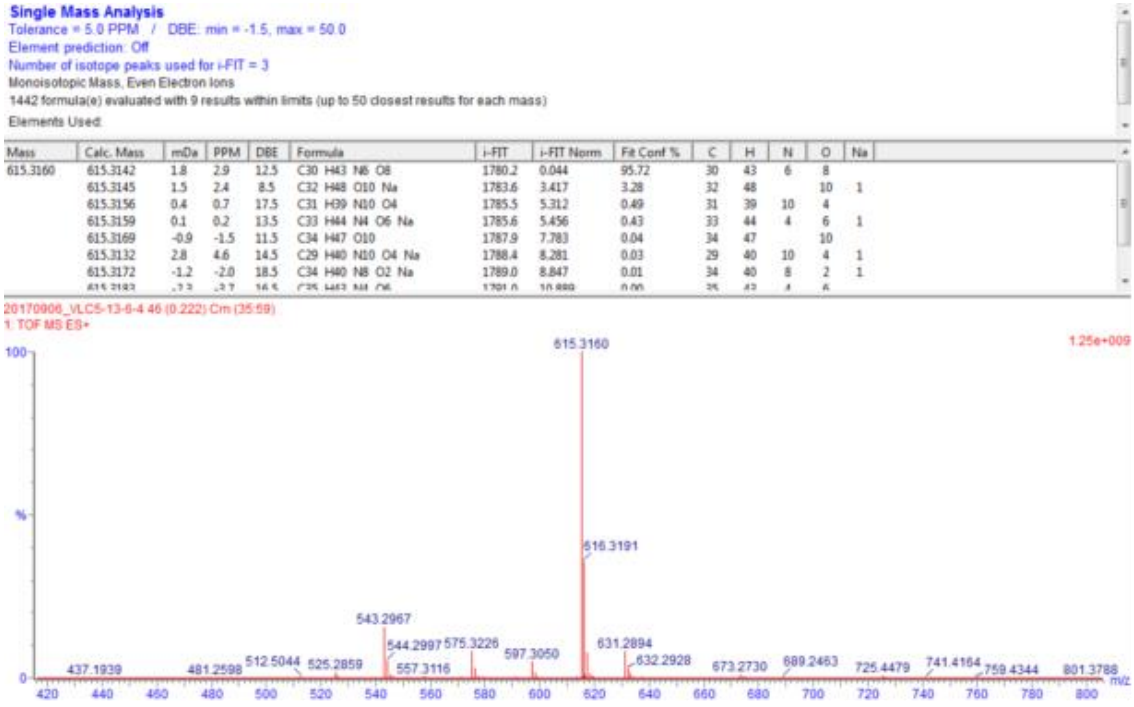


Figure S31. ^{1}H NMR spectrum of Compound **6** (600 MHz, $CDCl_3$)

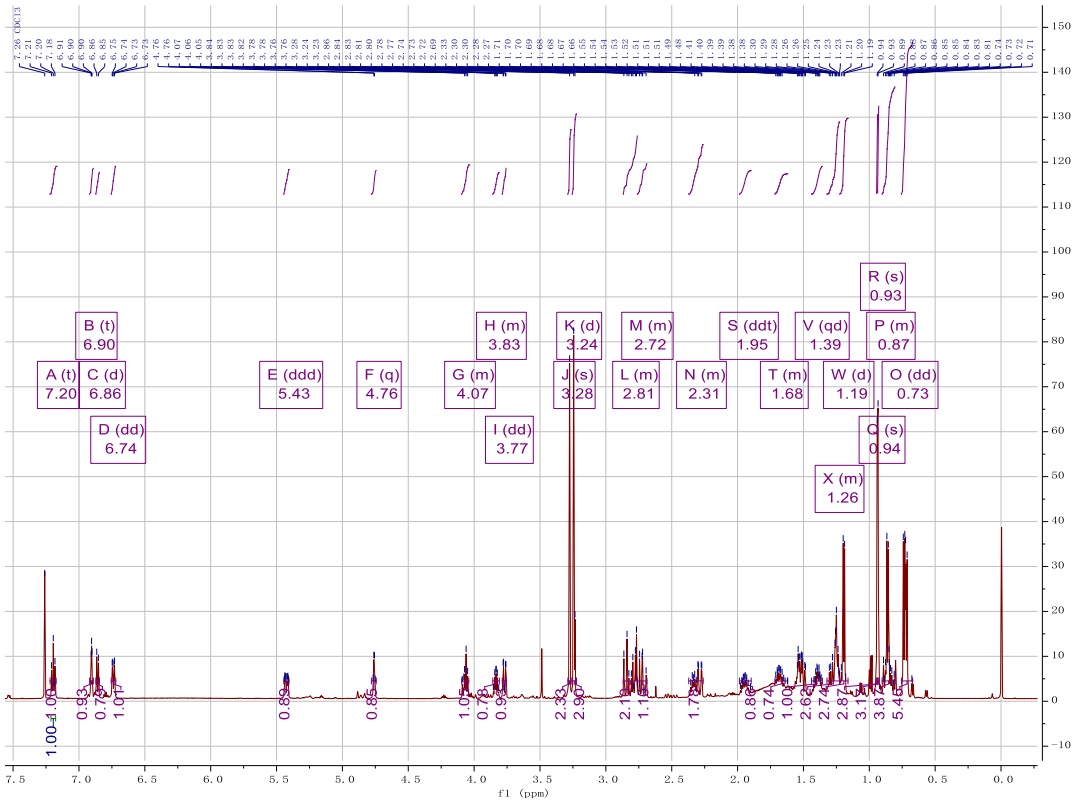


Figure S32. ^{13}C NMR spectrum of Compound **6** (150 MHz, $CDCl_3$)

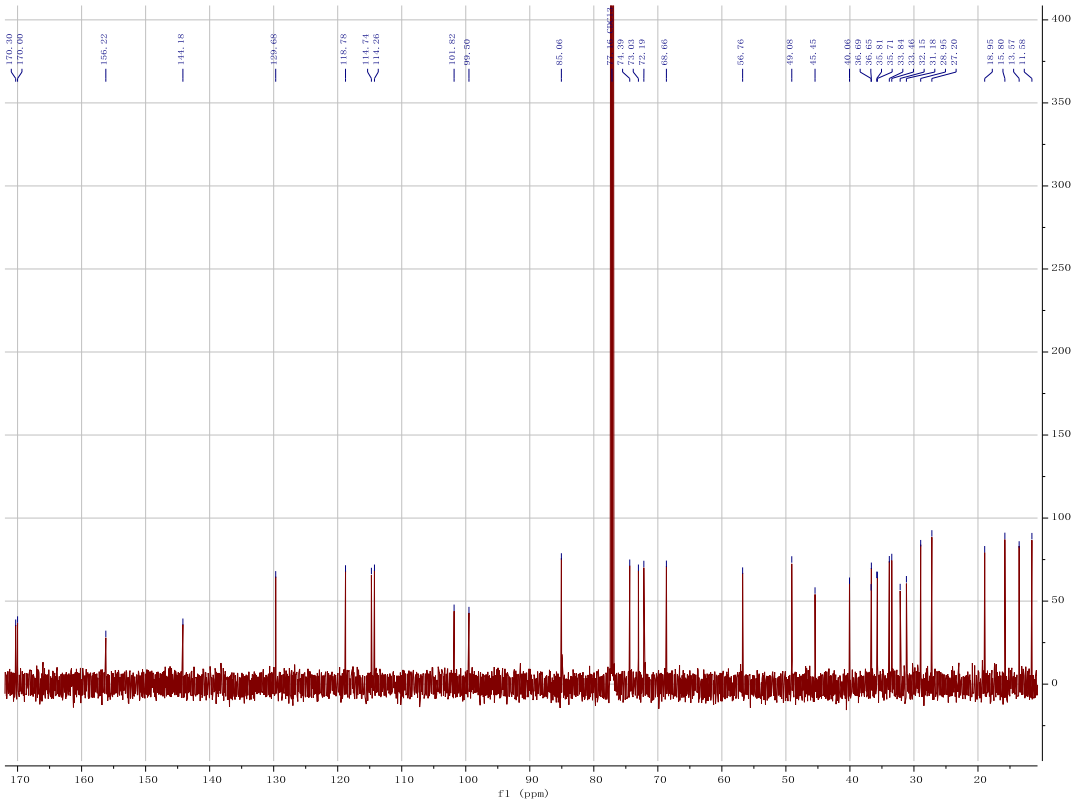


Figure S33. HRESIMS spectrum of **6**

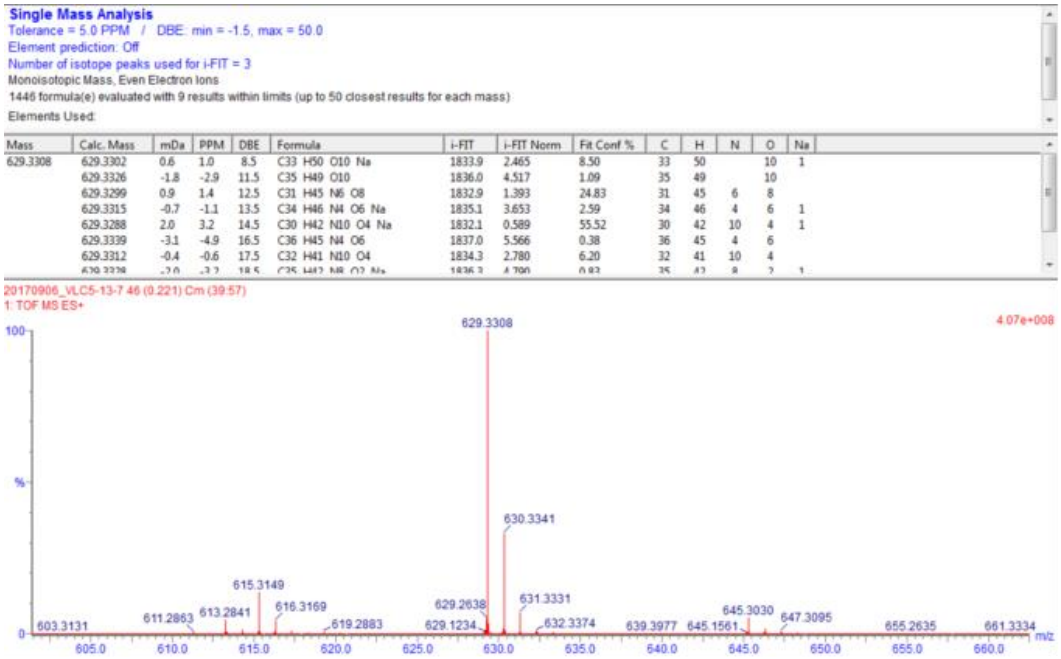


Figure S34. ^{1}H NMR spectrum of Compound **7** (600 MHz, $CDCl_3$)

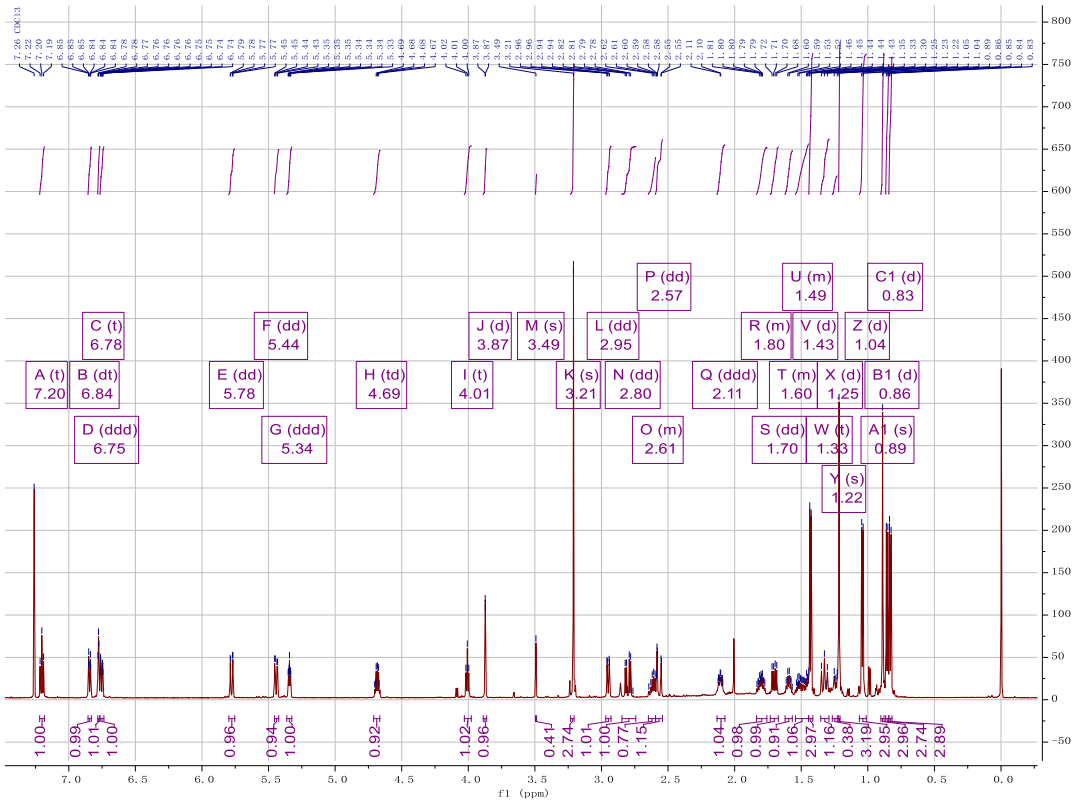


Figure S35. ^{13}C NMR spectrum of Compound **7** (150 MHz, $CDCl_3$)

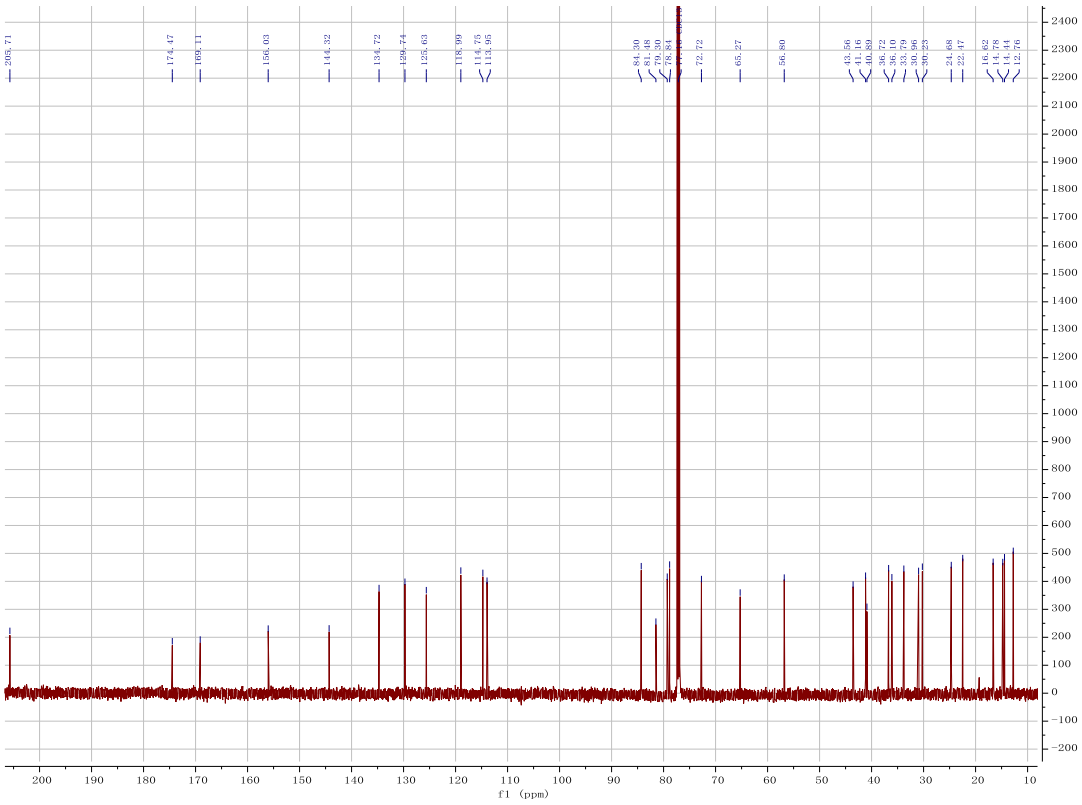


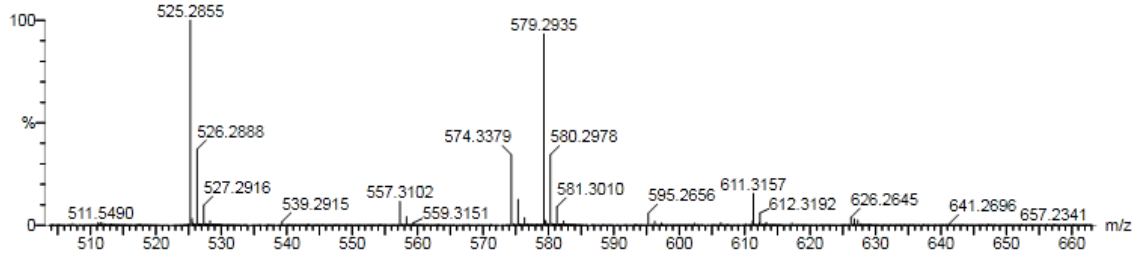
Figure S36. HRESIMS spectrum of **7**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

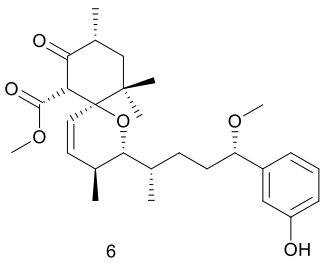
Monoisotopic Mass, Even Electron Ions
228 formula(e) evaluated with 8 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-50 H: 1-80 O: 0-20 Na: 0-1
RJYY_20171108_WY_VLC5-14-9-2 11 (0.445) Cm (10:15)

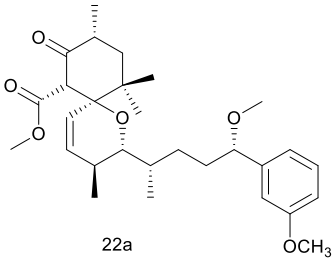
1: TOF MS ES+
2.36e+004



Minimum:				-1.5			
Maximum:		10.0	10.0	50.0			
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**i-FIT**	**i-FIT (Norm)**	**Formula**
579.2935	579.3028	-9.3	-16.1	23.5	912.1	7.4	C43 H40 Na
	579.2993	-5.8	-10.0	1.5	911.5	6.8	C25 H48 O13 Na
	579.2875	6.0	10.4	19.5	909.0	4.3	C39 H40 O3 Na
	579.2934	0.1	0.2	10.5	905.9	1.2	C32 H44 O8 Na
	579.3017	-8.2	-14.2	4.5	909.2	4.5	C27 H47 O13
	579.2864	7.1	12.3	0.5	916.7	12.0	C23 H47 O16
	579.2899	3.6	6.2	22.5	910.4	5.7	C41 H39 O3
	579.2958	-2.3	-4.0	13.5	905.1	0.4	C34 H43 O8

Figure S37. The structures and optical rotation values of compound **2** and **22a**





$[\alpha]^{25}_{D}$ -11.3(c 0.1, MeOH); $[\alpha]^{25}_{D}$ -18.2(c 1.02, $CHCl_3$);

Scheme S1. 1 was considered as a precursor to neo-debromoaplysiatoxin A in the plausible biosynthetic pathway of neo-debromoaplysiatoxin A