SUPPLEMENTARY MATERIAL

Neo-debromoaplysiatoxin C, with new structural rearrangement, derived from debromoaplysiatoxin

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Abstract

Neo-debromoaplysiatoxin C (**1**), a new member of the aplysiatoxin family, was isolated from the marine cyanobacterium *Lyngbya* sp.. The structure of **1** was elucidated based on spectroscopic data, and its stereochemistry was determined from NOESY spectrum and biosynthetic considerations. This new compound presents an intriguing 10-membered lactone ring skeleton derived from debromoaplysiatoxin by structural rearrangement, which is the first example in the aplysiatoxin family. Its biological properties were evaluated for cytotoxicity, PKCδ activation and inhibitory effects on potassium channel.

Keywords: neo-debromoaplysiatoxin C; debromoaplysiatoxin; newly discovered structural frame; *Lyngbya* sp.

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[f] These authors equally contributed to this work.

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Figure S1. Key COSY, HMBC and NOESY correlations of **1**

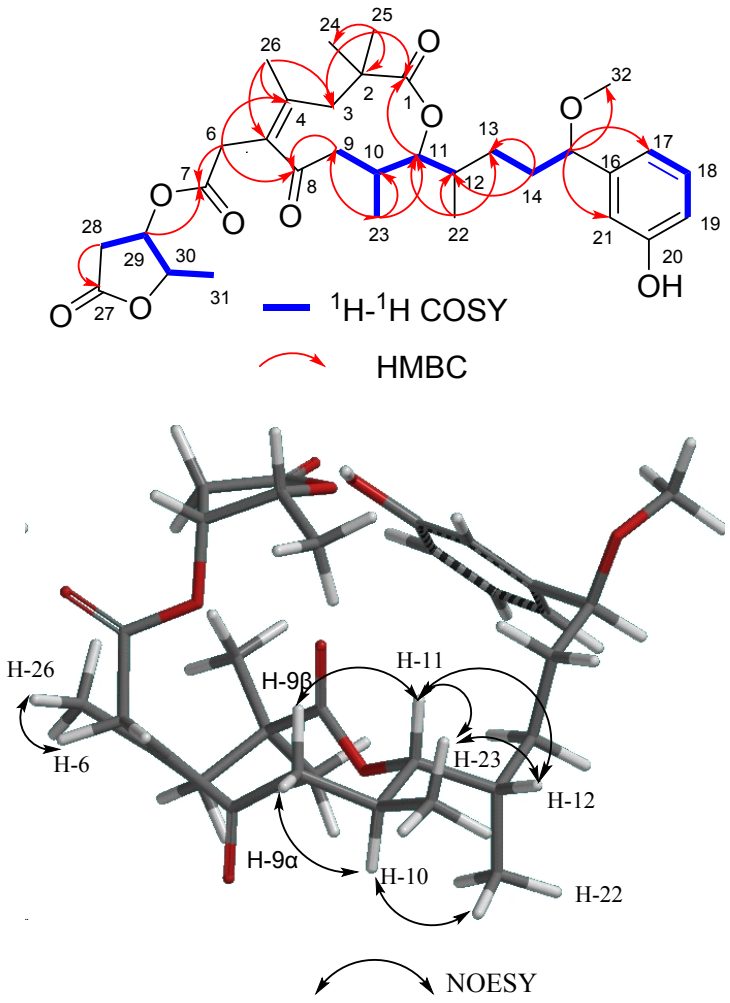


Figure S2. CD spectrum of **1** in MeOH

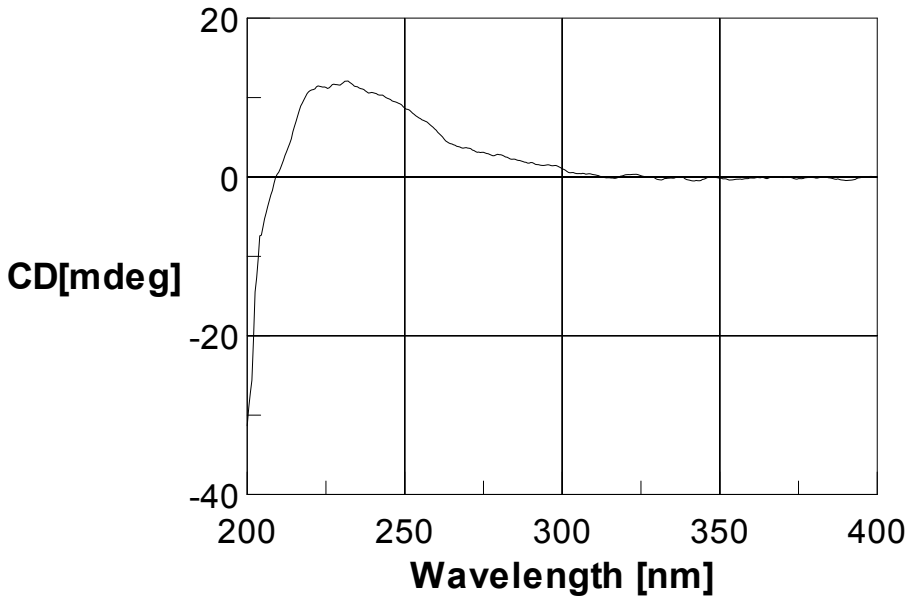


Table S1

NMR data for Neo-debromoaplysiatoxin C (**1**) in $CDCl_3$.

NO.	Neo-debromoaplysiatoxin C (**1**)				
	δ_C, type [a]	δ_H, mult (*J* in Hz)[b]	COSY	HMBC	NOESY
1	175.6, qC				
2	46.0, qC				
3α	44.0, CH_2	3.38, d (13.2)		C-1, 2, 4, 5, 24, 25, 26	H-24
3β		1.66, d (13.2)		C-1, 2, 4, 5, 24, 25, 26	H-25
4	138.5, qC				
5	134.2, qC				
6	34.9, CH_2	3.18, overlap		C-4, 5, 7, 8	H-26
7	169.2, qC				
8	205.2, qC				
9α	46.1, CH_2	2.12, dd (15.0, 1.0)	H-9β	C-8, 10, 11, 23	H-10
9β		2.51, dd (15.0, 11.2)	H-9α, 10	C-8, 10, 11	H-11
10	33.1, CH	2.76, overlap	H-9β, 11, 23	C-23	H-9α, 22
11	80.1, CH	4.64, dd (10.8, 1.0)	H-10, 12	C-1, 9, 10, 12, 13, 22, 23	H-9β, 12, 23
12	34.4, CH	1.64, m	H-11	C-13, 22	H-11, 23
13α	30.3, CH_2	1.15, m	H-13β, 14,		
13β		0.92, overlap	H-13α, 14		
14	35.7, CH_2	1.74, m	H-13α, 13β, 15	C-12, 13, 15, 16	
15	84.3, CH	3.94, t (6.7)	H-14	C-13, 14, 16, 17, 21, 32	
16	143.8, qC				
17	119.4, CH	6.76, overlap	H-18	C-15	
18	129.4, CH	7.16, t (7.7)	H-17, 19		
19	114.9, CH	6.74, overlap	H-18		
20	156.4, qC				
21	113.5, CH	6.79, m (2.0)		C-15	
22	13.7, CH_3	0.87, d (6.8)		C-11, 12, 13	
23	17.9, CH_3	0.93, d (6.9)	H-10	C-9, 10,11	
24	28.6, CH_3	1.01, s		C-1, 2, 3, 25	
25	24.3, CH_3	1.13, s		C-1, 2, 3, 24	
26	22.3, CH_3	1.80, s		C-3, C-4, C-5	
27	174.7, qC				
28α	36.5, CH_2	2.74, overlap	H-29, 28β	C-27, 29, 30	
28β		2.91, dd (18.5, 6.4)	H-29, 28α	C-27, 30	H-29, 30
29	72.0, CH	5.47, ddd (6.2, 4.3, 1.6)	H-28α, 28β, 30	C-7, 27, 30	H-28β
30	79.1, CH	4.72, ddd (13.0, 6.5, 4.3)	H-29, 31	C-29, 31	H-28β
31	14.4, CH_3	1.37, d (6.5)	H-30	C-29, 30	
-OCH_3	56.7, CH_3	3.18, overlap		C-15	

[a]Data recorded at 600 MHz. [b]Data recorded at 150 MHz.

Figure S3. ^{1}H NMR (600 MHz) spectrum of **1** in $CDCl_3$

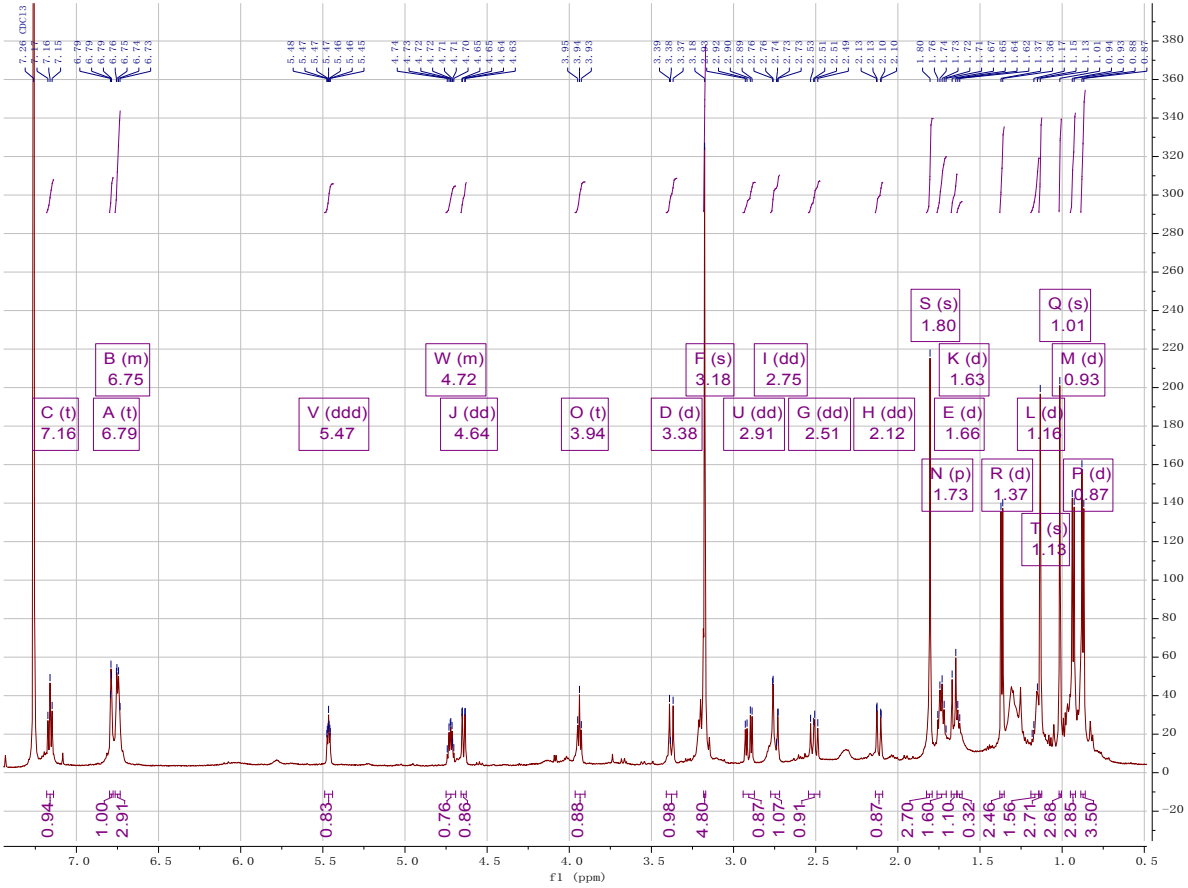


Figure S4. ^{13}C NMR (150 MHz) spectrum of **1** in $CDCl_3$

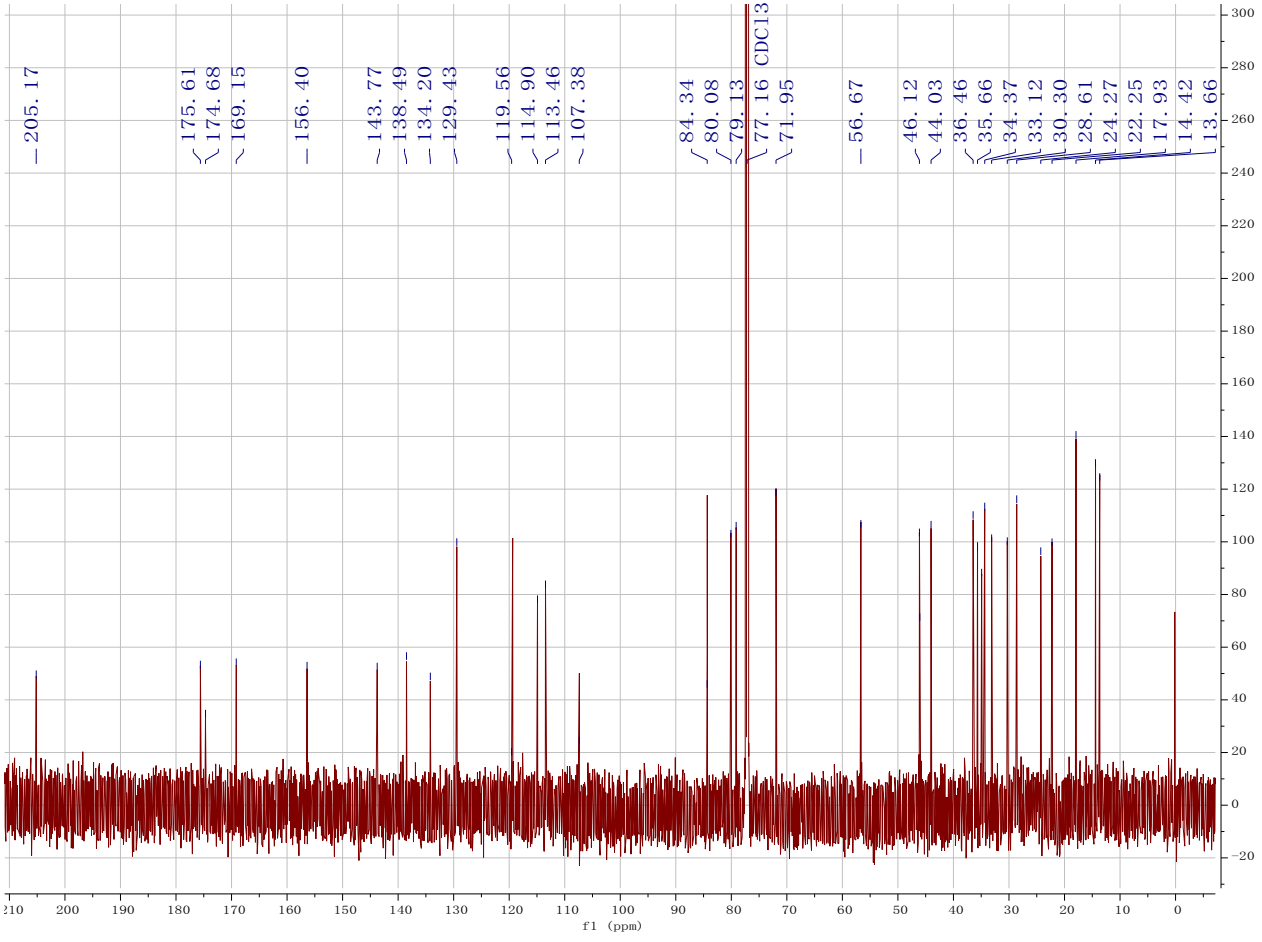


Figure S5. DEPT NMR spectrum (150 MHz) of **1** in $CDCl_3$

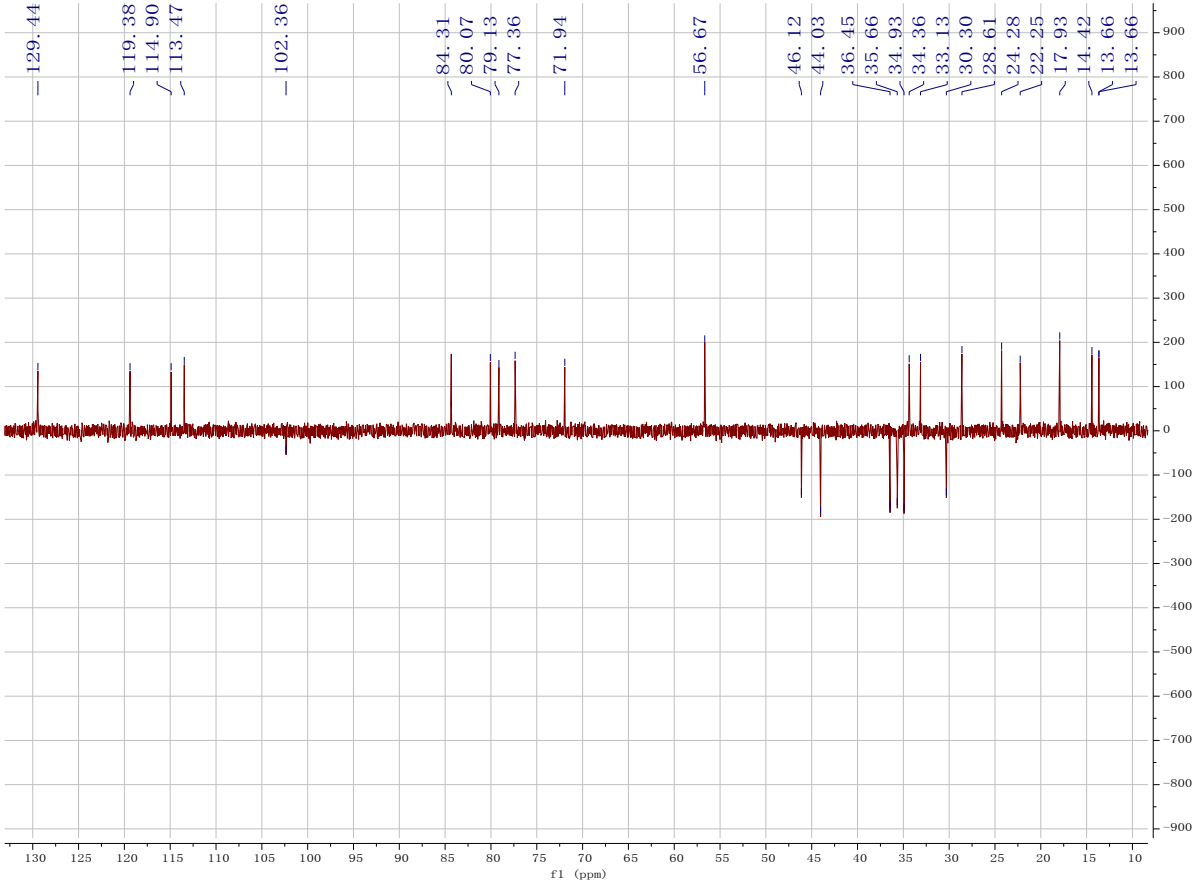


Figure S6. HSQC spectrum of **1** in $CDCl_3$

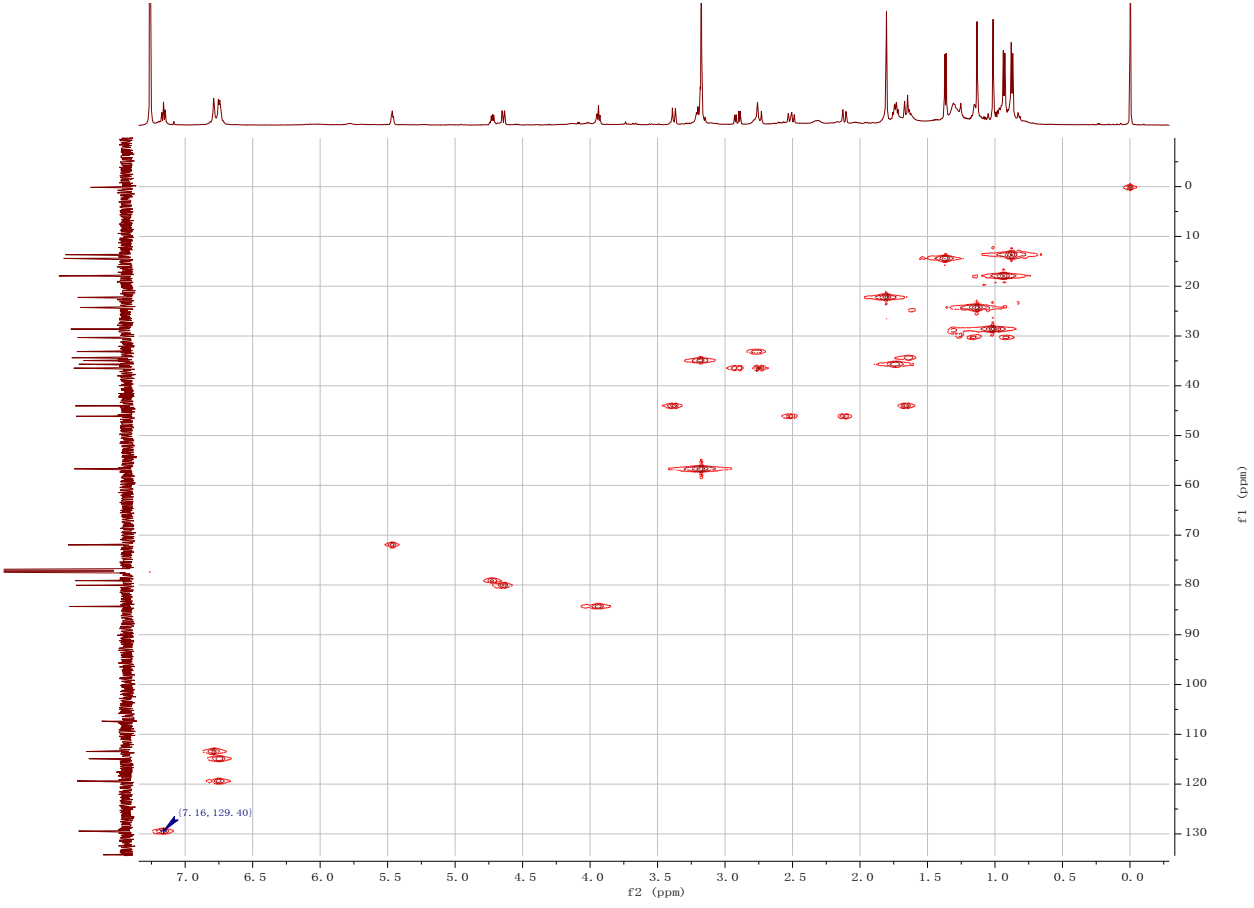


Figure S7. COSY spectrum of **1** in $CDCl_3$

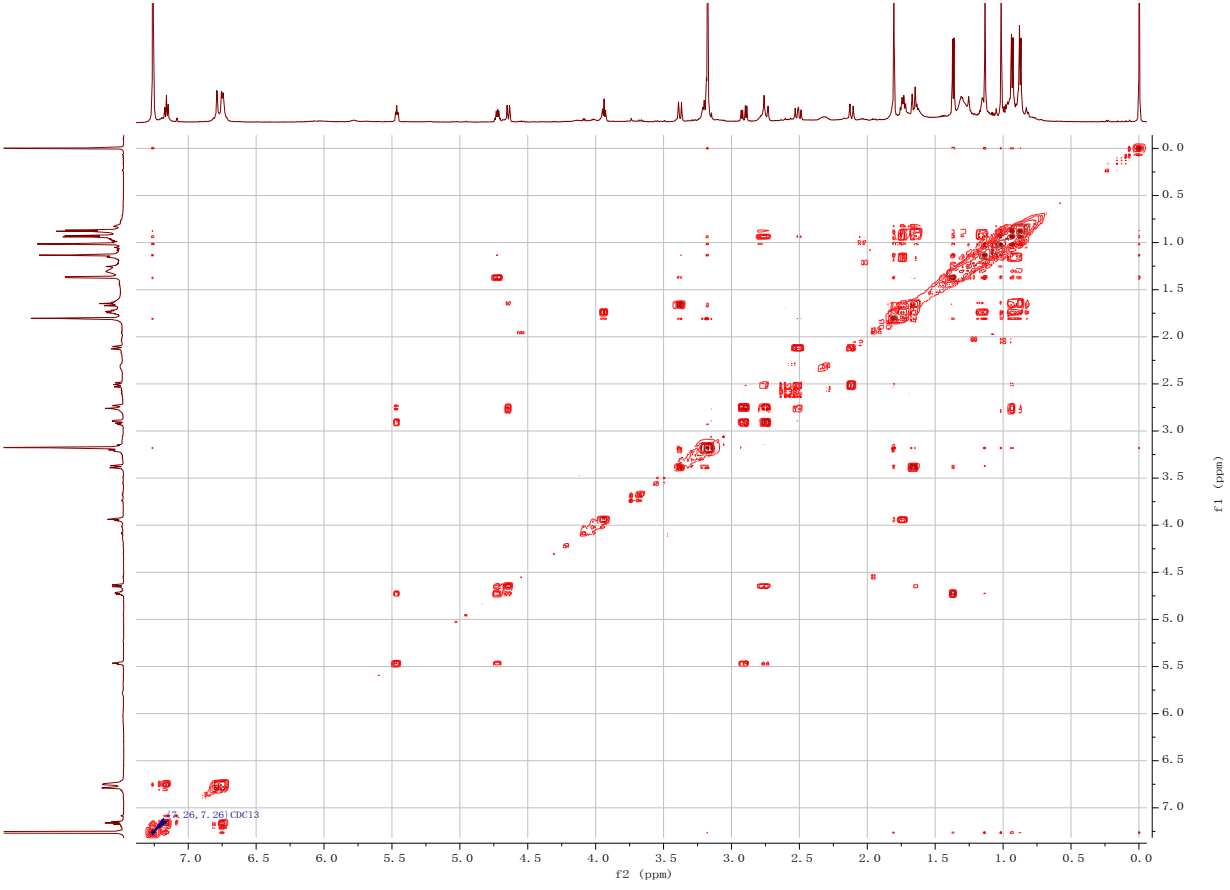


Figure S8. HMBC spectrum of **1** in $CDCl_3$

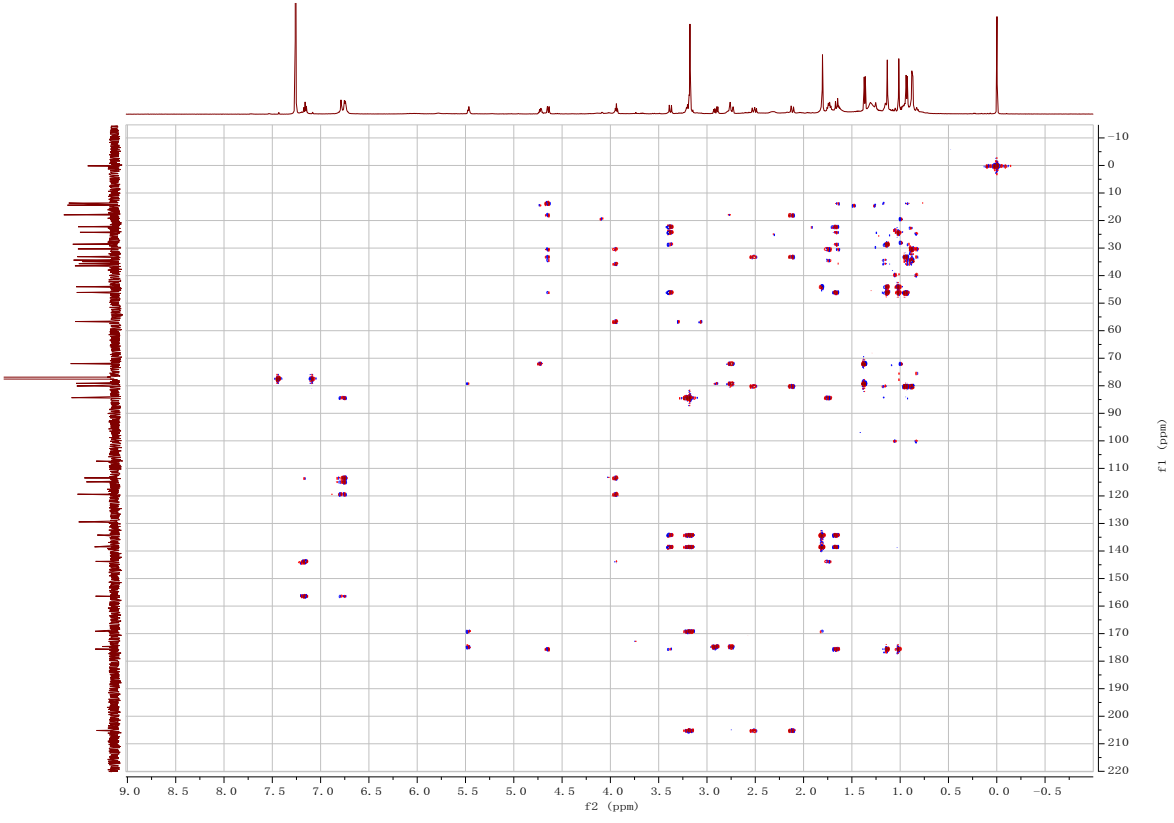


Figure S9. NOESY spectrum of **1** in $CDCl_3$

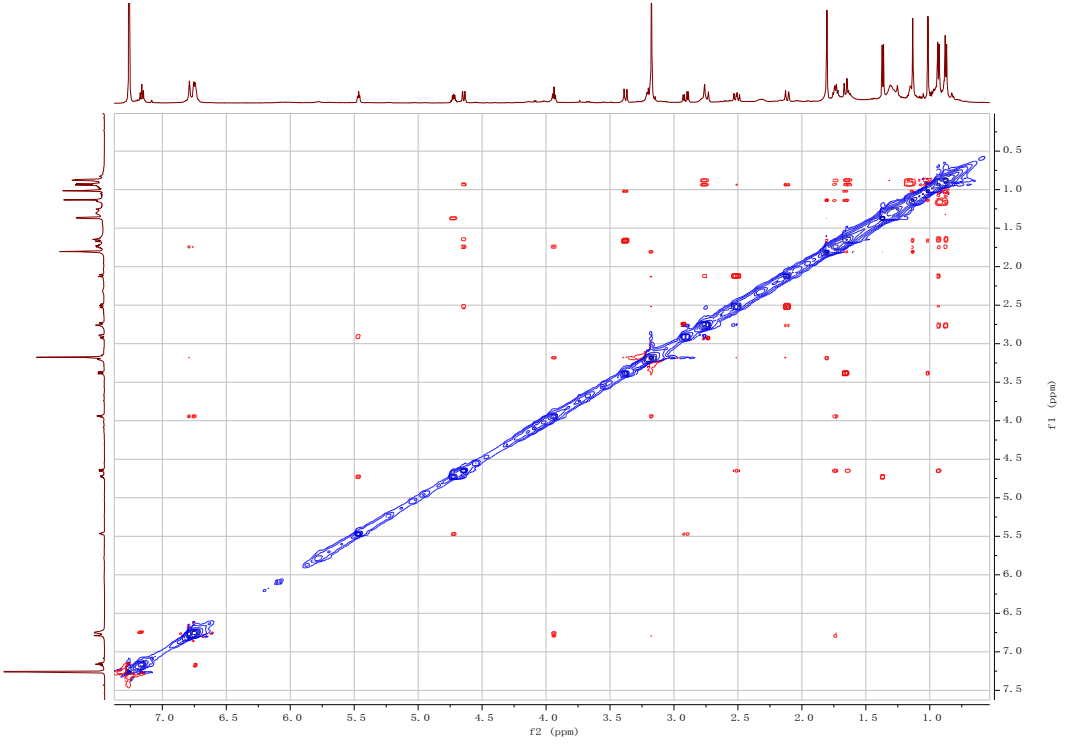


Figure S10. HRESIMS of **1**

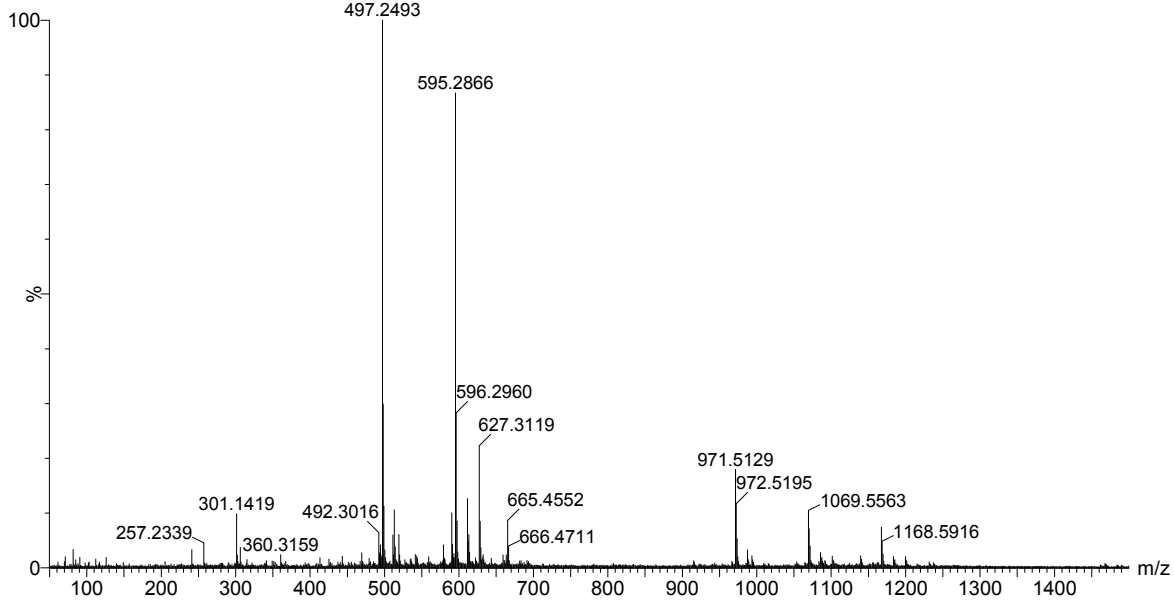


Figure S11.UV spectrum of **1**

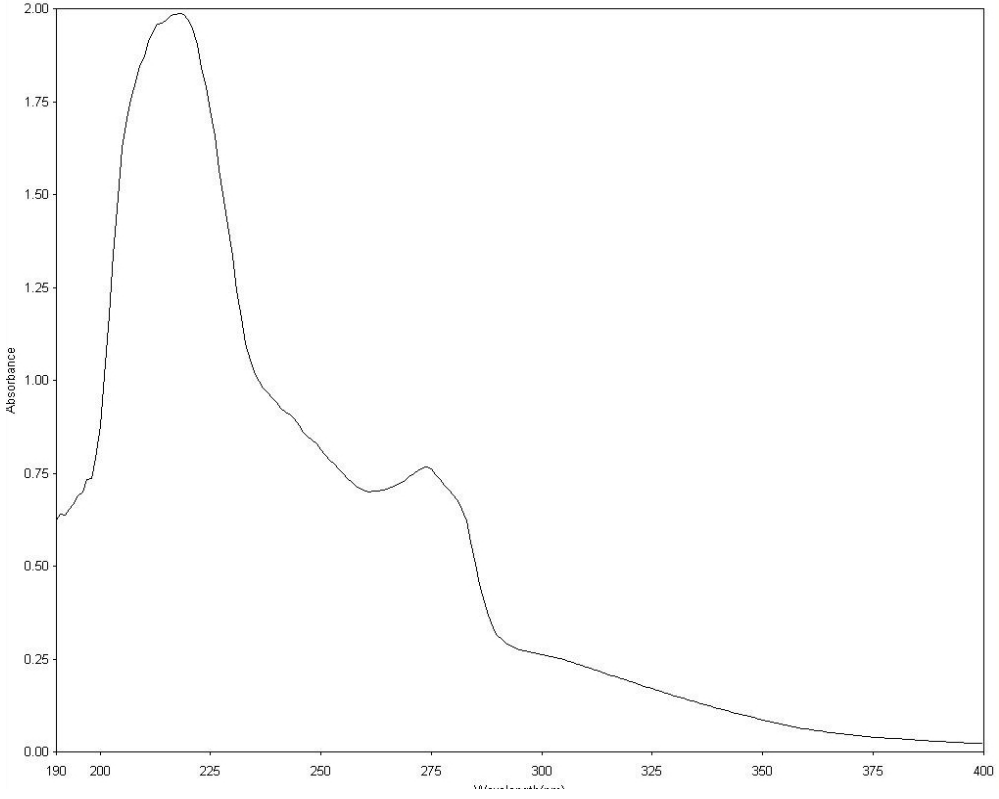
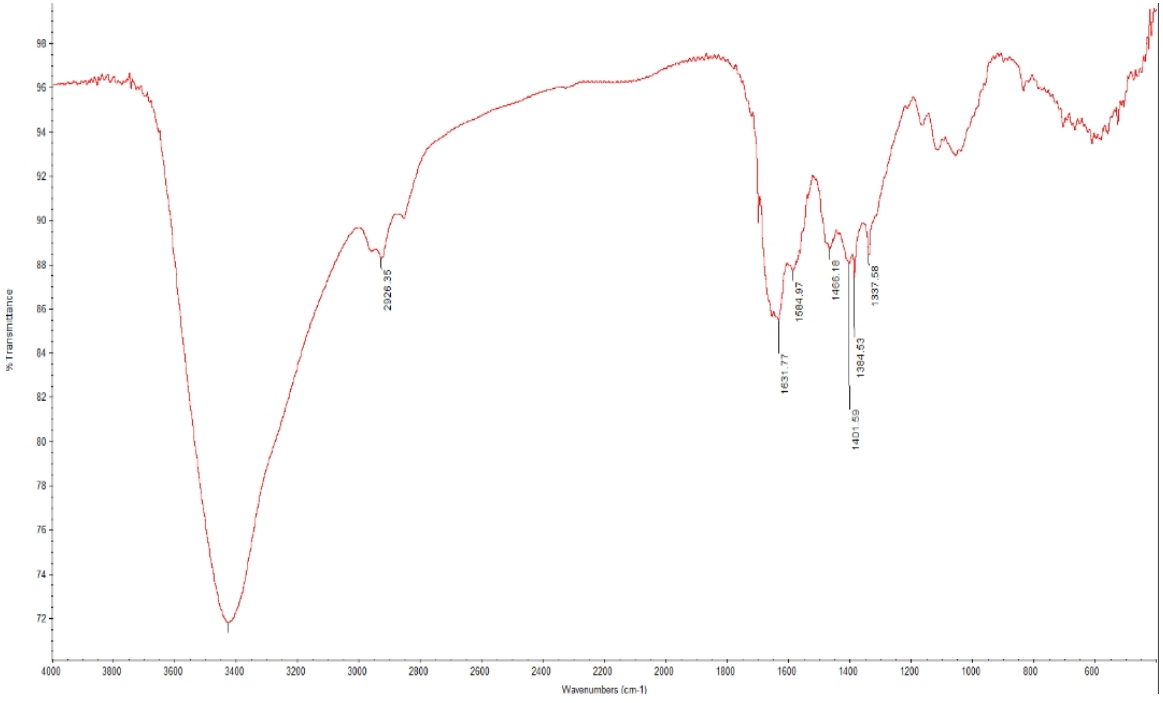


Figure S12. IR spectrum of **1**



Scheme S1. Plausible biosynthetic pathway of **1**

Neo-debromoaplysiatoxin C (**1**), contains a single 10-membered lactone ring core structure which, to the best our knowledge, is the first example in ATXs. The plausible biosynthetic pathway of **1** was represented in Scheme 1. We postulated that the structural rearrangement of debromoaplysiatoxin was caused by the instability of the hemiketal at C-3 and ketal at C-7 in the presence of weak acids or alkalis, such as in the case of 30-methyloscillatoxin D and neo-debromoaplysiatoxin A-B. We envisioned that the ester linkage at C-27 was attacked by 30-OH and resulted in the formation of γ-lactone ring and 9-OH. A nucleophilic reaction between anionic C-8 and cationic C-3 accompanied by dehydration of C-3 and C-4 result the appearance of intermediate III. III subsequently experienced dehydration, hydration and oxidation, and finally neo-debromoaplysiatoxin C (**1**) was produced.

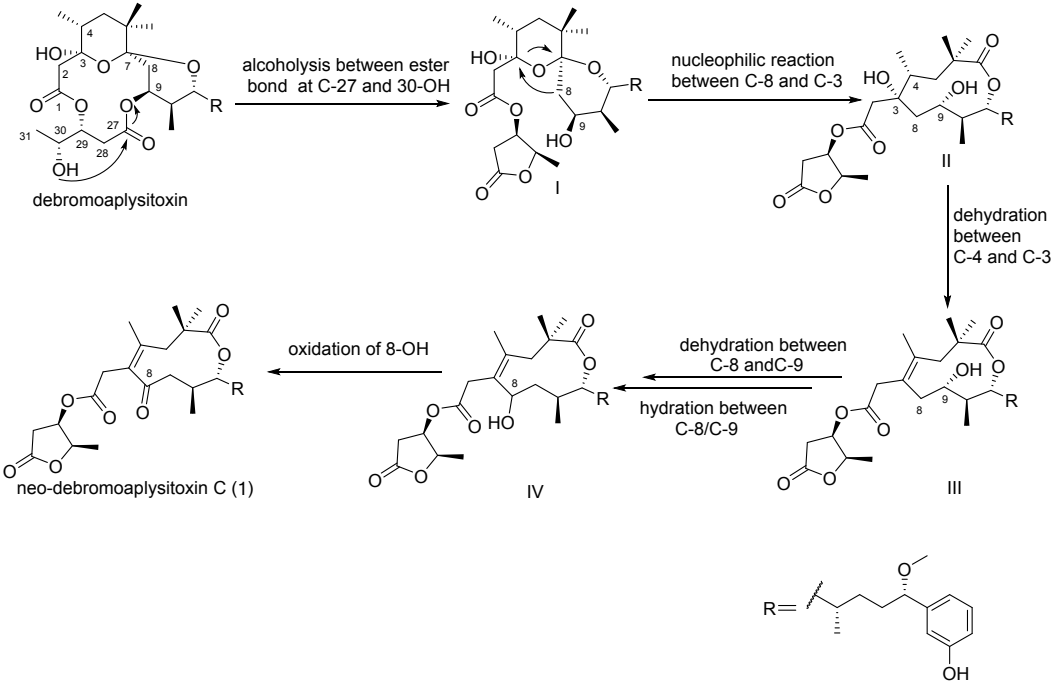


Figure 13. Diagram of all energetically reasonable rotamers (staggered) for the C-11/C-12 and C-10/C-11

The configuration of C-11 was assumed as *R,* the stereochemistry at C-12 and C-10 were defined using NOESY experiment and coupling constant in combination with a systematic analysis of all energetically reasonable (staggered) rotamers. The anti relationship of H-10 and H-11 and the gauch relationship of H-12 and H-11 were established from the coupling constants of H-11 (J=10.8, 1.0 Hz) and the presence of NOESY correlation of H-11/H-12 and the absence of correlation between H-10 and H-11, which indicated $J_{\text{H-10, H-11}}$=10.8 Hz and $J_{\text{H-11, H-12}}$=1.0 Hz. The NOESY cross-peaks of H-11/H-12 and the small coupling constant of H-11/H-12 ($J_{\text{H-11, H-12}}$=1.0) ruled out model A3 and B3, H-10/H_3-22 ruled out model A1 and B3, H-12/H_3-23 ruled out A2 and B2. Model B1 full all criteria for C-11/C-12. The large coupling constant of H-10 and H-11 ($J_{\text{H-10, H-11}}$=10.8) ruled out C1, C2, D1 and D2, the NOESY correlations from H-11 to H_3-23 ruled out C2 and D1, H-10/H_3-22 ruled out C1 and D1, H_3-23/H-12 ruled out model C3 and D2. Model D3 full all criteria for C-11/C-10. Thus, indicated a 10*S**, 11*R**, 12*S** configuration for neo-debromoaplysiatoxin C (**1**)