

Supporting Information

Discovery and Characterization of a PKS-NRPS Hybrid in *Aspergillus terreus* by Genome Mining

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Table S1. The strains used in this study

Strain	Genotype	Reference
MEFC01- Δ ku80	<i>A. terreus</i> MEFC01, Δ ku80::ptrA	This study
MEFC01- Δ ku80- Δ pyrG	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, pyrG ⁻	This study
MEFC01-pr00903	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, pr00903	This study
MEFC01-pr00919	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, pr00919	This study
MEFC01-prptytR- Δ ptytA	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytA::hph	This study
MEFC01-prptytR- Δ ptytB	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytB::hph	This study
MEFC01-prptytR- Δ ptytC	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytC::hph	This study
MEFC01-prptytR- Δ ptytD	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytD::hph	This study
MEFC01-prptytR- Δ ptytE	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytE::hph	This study
MEFC01-prptytR- Δ ptytF	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytF::hph	This study
MEFC01-prptytR- Δ ptytG	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytG::hph	This study
MEFC01-prptytR- Δ ptytH	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytH::hph	This study
MEFC01-prptytR- Δ ptytI	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytI::hph	This study
MEFC01-prptytR- Δ ptytR	<i>A. terreus</i> MEFC01, Δ ku80::ptrA, prptytR, Δ ptytR::hph	This study

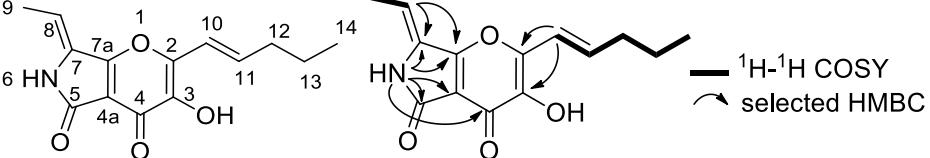
Table S2. The oligonucleotides used in this study

#	name	5' -> 3' sequence	template	function
1	pyrGAn-F	TAAGGGAGATGGTATTGAAC TAG	cDNA of <i>A. niger</i>	selectable marker gene for activation
2	pyrGAn-R(PgdAt)	GGATCCTCCCAGAGTGGTAA geatcaaategtcgtaacgca	cDNA of <i>A. niger</i>	selectable marker gene for activation
3	PgdAt-F	TTACACTCTGGGAGGGATCCAG	gDNA of <i>A. terreus</i>	strong promoter
4	PgdAt-R	GTGATGATTGATGAGTTGT	gDNA of <i>A. terreus</i>	strong promoter
5	U-00919-F	GATATTCCAACCGGATTCATAC	gDNA of <i>A. terreus</i>	flanking regions for activation
6	U-00919-R(pyrG)	TCAATCACCATCTCCCTTA GCCAAGCCACGGATCTGCTTAT	gDNA of <i>A. terreus</i>	flanking regions for activation
7	D-00919-F(PgdAt)	ACAACCTCATCAATCATCAC ATGGCTCACTTCAGCCGAGTCG	gDNA of <i>A. terreus</i>	flanking regions for activation
8	D-00919-R	CGTTCAACTTCATCCTCTCTCG	gDNA of <i>A. terreus</i>	flanking regions for activation
9	C-00919-F	ACAGTTCGGAGATATGTCAGTC	gDNA of <i>A. terreus</i>	flanking regions for activation
10	C-00919-R	CGACGTTAGAGCATGGACGATG	gDNA of <i>A. terreus</i>	flanking regions for activation
11	hph-F	tccgggatecgaagcgtaaag	hph-Plasmid	selectable marker gene for deletion
12	hph-R	caattatcttgcgaaacctcg	hph-Plasmid	selectable marker gene for deletion
13	U-g7163-F	cegtccgcaggatgcataate	gDNA of <i>A. terreus</i>	flanking regions for deletion
14	U-g7163-R(hph2)	CTTTACGCTTGCATCCCGAA ctccggttcagctatcaggcagg	gDNA of <i>A. terreus</i>	flanking regions for deletion
15	D-g7163-F(hph2)	CTGGGTTCGCAAAGATAATTG CACAAACGACCAGCAGCGAACAAAC	gDNA of <i>A. terreus</i>	flanking regions for deletion
16	D-g7163-R	gtaggctaccgtcggttctc	gDNA of <i>A. terreus</i>	flanking regions for deletion
17	C-g7163-F	ccgcaggatcgtagatgactc	gDNA of <i>A. terreus</i>	flanking regions for deletion
18	C-g7163-R	gtgttctcgccgtaaacgttg	gDNA of <i>A. terreus</i>	flanking regions for deletion
19	U-g7171-F	agaagtcacccacttgc	gDNA of <i>A. terreus</i>	flanking regions for deletion
20	U-g7171-R(hph2)	CTTTACGCTTGCATCCCGAA CATttgtgttgtggatgtgc	gDNA of <i>A. terreus</i>	flanking regions for deletion
21	D-g7171-F(hph2)	CTGGGTTCGCAAAGATAATTG CAGCTCTGGGTCTGACTCCAAG	gDNA of <i>A. terreus</i>	flanking regions for deletion
22	D-g7171-R	caaggcgtaccccggatgtcg	gDNA of <i>A. terreus</i>	flanking regions for deletion
23	C-g7171-F	cactgggttcaggcaagtctcg	gDNA of <i>A. terreus</i>	flanking regions for deletion
24	C-g7171-R	gatgagatcagtcccatggg	gDNA of <i>A. terreus</i>	flanking regions for deletion
25	U-g7170-F	gaaggatgggtcgatcatatc	gDNA of <i>A. terreus</i>	flanking regions for deletion
26	U-g7170-R(hph2)	CTTTACGCTTGCATCCCGAA ggtgagggtgaatgggtcaacc	gDNA of <i>A. terreus</i>	flanking regions for deletion
27	D-g7170-F(hph2)	CTGGGTTCGCAAAGATAATTG CTTCATCCACTGCCGCTACATG	gDNA of <i>A. terreus</i>	flanking regions for deletion

28	D-g7170-R	caacggtatccggcgttcagag	gDNA of <i>A. terreus</i>	flanking regions for deletion
29	C-g7170-F	caggcgcgttaatacgagg	gDNA of <i>A. terreus</i>	flanking regions for deletion
30	C-g7170-R	gaaatatctccgtcgagacg	gDNA of <i>A. terreus</i>	flanking regions for deletion
31	U-00911-F3	gactctcccaacctgacctc	gDNA of <i>A. terreus</i>	flanking regions for deletion
32	U-00911-R(hph2)3	CTTTACGCTTGCATCCCGAA caeggcacegagtcgtgtcttc	gDNA of <i>A. terreus</i>	flanking regions for deletion
33	D-00911-F(hph2)3	CTGGGTTCGCAAAGATAATTG catagacagcgggggtcacac	gDNA of <i>A. terreus</i>	flanking regions for deletion
34	D-00911-R3	catgaacagtcgttccctgea	gDNA of <i>A. terreus</i>	flanking regions for deletion
35	C-00911-F	GAGTTTCAGCGGGTGGGTCAACC	gDNA of <i>A. terreus</i>	flanking regions for deletion
36	C-00911-R	caccgcaggcgtcgaaatcc	gDNA of <i>A. terreus</i>	flanking regions for deletion
37	U-00912-F	cctcggtttcacgggggtctg	gDNA of <i>A. terreus</i>	flanking regions for deletion
38	U-00912-R(hph2)	CTTTACGCTTGCATCCCGAA gtcggcgagagggggttgc	gDNA of <i>A. terreus</i>	flanking regions for deletion
39	D-00912-F(hph2)	CTGGGTTCGCAAAGATAATTG ccatgttacactggatgtca	gDNA of <i>A. terreus</i>	flanking regions for deletion
40	D-00912-R	cctcagcatgttacaggcaaag	gDNA of <i>A. terreus</i>	flanking regions for deletion
41	C-00912-F	gegaggaaagccatggaaatg	gDNA of <i>A. terreus</i>	flanking regions for deletion
42	C-00912-R	gcacgcgtggcgatgtcgagac	gDNA of <i>A. terreus</i>	flanking regions for deletion
43	U-00913-F	atgccccaggatcgatcaatg	gDNA of <i>A. terreus</i>	flanking regions for deletion
44	U-00913-R(hph2)	CTTTACGCTTGCATCCCGAA ttttcttccaatgcattgtc CTGGGTTCGCAAAGATAATTG	gDNA of <i>A. terreus</i>	flanking regions for deletion
45	D-00913-F(hph2)	cgctatgaacaggccgtcaag	gDNA of <i>A. terreus</i>	flanking regions for deletion
46	D-00913-R	ccacagcaggggccagtcac	gDNA of <i>A. terreus</i>	flanking regions for deletion
47	C-00913-F	ctccatgtggggcgccacaac	gDNA of <i>A. terreus</i>	flanking regions for deletion
48	C-00913-R	ctcacggatacgccacagc	gDNA of <i>A. terreus</i>	flanking regions for deletion
49	U-00914-F	atgcgttttttgtatcgcc	gDNA of <i>A. terreus</i>	flanking regions for deletion
50	U-00914-R(hph2)	CTTTACGCTTGCATCCCGAA ctttccatgttgcagacact	gDNA of <i>A. terreus</i>	flanking regions for deletion
51	D-00914-F(hph2)3	CTGGGTTCGCAAAGATAATTG Gagggttacactggatgtc	gDNA of <i>A. terreus</i>	flanking regions for deletion
52	D-00914-R2	cgccatgtttggggaaag	gDNA of <i>A. terreus</i>	flanking regions for deletion
53	C-00914-F	gcaccgcaacggattgtcagc	gDNA of <i>A. terreus</i>	flanking regions for deletion
54	C-00914-R	caaaaatgcattgacatgtctg	gDNA of <i>A. terreus</i>	flanking regions for deletion
55	U-00915-F	ggtgccggatgtatcgatcg	gDNA of <i>A. terreus</i>	flanking regions for deletion
56	U-00915-R(hph2)	CTTTACGCTTGCATCCCGAA cggtccatcttttttttggg	gDNA of <i>A. terreus</i>	flanking regions for deletion
57	D-00915-F(hph2)	CTGGGTTCGCAAAGATAATTG ctttttactacatcccattg	gDNA of <i>A. terreus</i>	flanking regions for deletion
58	D-00915-R	gactctgttgcgttcggagg	gDNA of <i>A. terreus</i>	flanking regions for deletion
59	C-00915-F	ggacgagaaacggatgttc	gDNA of <i>A. terreus</i>	flanking regions for deletion
60	C-00915-R	ggcggtgacaaatccgttgcgt	gDNA of <i>A. terreus</i>	flanking regions for deletion
61	U-00916-F	ctgcaggggccgtccatcg	gDNA of <i>A. terreus</i>	flanking regions for deletion
63	U-00916-R(hph2)	CTTTACGCTTGCATCCCGAA gctgtcgcccttggatattcc	gDNA of <i>A. terreus</i>	flanking regions for deletion
64	D-00916-F(hph)2	CTGGGTTCGCAAAGATAATTG ccttccggagaggaactagcaac	gDNA of <i>A. terreus</i>	flanking regions for deletion
65	D-00916-R2	ccgtcgctgttgcgttc	gDNA of <i>A. terreus</i>	flanking regions for deletion

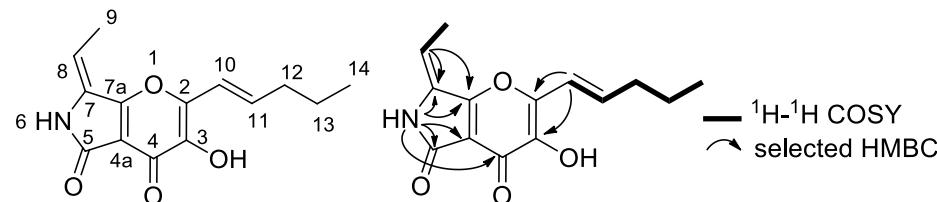
101	E-00912-R2	ttagccatccagggttacatgg	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
102	E-00913-F	gcgcgtatcgccagacacgc	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
103	E-00913-R	tggttcggttggatctecag	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
104	E-00914-F3	gtctggggcagtggcatcg	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
105	E-00914-R3	ctggccctcaaccgtggc	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
106	E-00915-F2	cagtttctgttcatcg	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
107	E-00915-R2	gtgggttagaagagcatgcac	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
108	E-00916-F3	ccaccatgtcaggggactgtac	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
109	E-00916-R3	ccagcttttttccacttcc	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
110	E-00917-F	aggcgaggtgacccttcage	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
111	E-00917-R	gtcgaaatgtgttgtatggcag	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
112	E-00918-F	gacgatccgcacaaacgcac	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
113	E-00918-R	gggcacataccaccccaactg	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
114	E-00919-F3	cagctgaaggaaacatcgac	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
115	E-00919-R3	gacatggataccggctggacg	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
116	E-00920-F2	ctggatcagagccacttggaa	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
117	E-00920-R2	gttcaccccggttcatgg	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
118	E-00921-F2	cggaatgtggagacggagtgg	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
119	E-00921-R2	gatacttcgggggttgcc	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
120	E-actin-F	gtgtttccccctccatgtc	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR
121	E-actin-R	tgttagaacgtgtatggcaga	gDNA, cDNA of <i>A. terreus</i>	semiquantitative RT-PCR

Table S3. NMR data for pyranterrone A (**1**) in DMSO-*d*₆ (600 and 150 MHz)



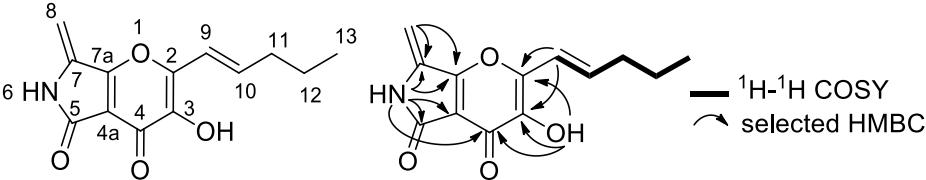
Position	δ_H (<i>J</i> in Hz)	δ_C	HMBC
2	—	145.2 (C)	
3	—	141.8 (C)	
4	—	168.9 (C)	
4a	—	109.6 (C)	
5	—	163.6 (C)	
6 (NH)	10.33 (1H, s)	—	4, 4a, 5, 7, 7a
7	—	129.5 (C)	
7a	—	162.6 (C)	
8	5.98 (1H, q, 7.2)	109.9 (CH)	7, 7a, 9
9	1.92 (3H, d, 7.2)	12.9 (CH ₃)	7, 7a, 8
10	6.53 (1H, d, 15.6)	117.7 (CH)	2, 3, 11, 12
11	6.58 (1H, dt, 15.6, 6.6)	136.2 (CH)	2, 10, 12, 13
12	2.25 (2H, m)	34.7 (CH ₂)	10, 11, 13, 14
13	1.49 (2H, m)	21.5 (CH ₂)	11, 12, 14
14	0.92 (3H, t, 7.2)	13.6 (CH ₃)	12, 13
3-OH	9.65 (1H, br s)	—	

Table S4. Data for pyranterrone B (**2**) in DMSO-*d*₆ (600 and 150 MHz)



Position	δ_{H} (<i>J</i> in Hz)	δ_{C}	HMBC
2	—	145.6 (C)	
3	—	142.2 (C)	
4	—	168.9 (C)	
4a	—	111.6 (C)	
5	—	163.3 (C)	
6 (NH)	10.07 (1H, s)	—	4, 4a, 5, 7, 7a
7	—	129.1 (C)	
7a	—	162.5 (C)	
8	5.79 (1H, q, 7.2)	114.0 (CH)	7, 7a, 9
9	2.17 (3H, d, 7.2)	13.1 (CH ₃)	7, 7a, 8
10	6.60 (1H, d, 16.2)	117.9 (CH)	2, 3, 11, 12
11	6.41 (1H, dt, 16.2, 6.6)	135.8 (CH)	2, 10, 12, 13
12	2.27 (2H, m)	34.5 (CH ₂)	10, 11, 13, 14
13	1.48 (2H, m)	21.6 (CH ₂)	11, 12, 14
14	0.92 (3H, t, 7.2)	13.5 (CH ₃)	12, 13
3-OH	9.78 (1H, br s)	—	

Table S5. NMR data for pyranterrone C (**3**) in DMSO-*d*₆ (600 and 150 MHz)

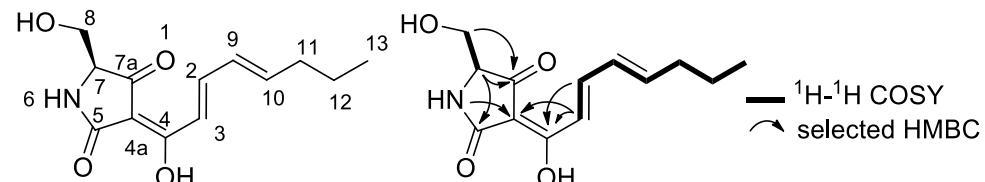


Position	δ_{H} (<i>J</i> in Hz)	δ_{C}	HMBC
2	—	145.5 (C)	
3	—	142.3 (C)	
4	—	168.8 (C)	
4a	—	110.5(C)	
5	—	163.5 (C)	
6 (NH)	10.29 (1H, s)	—	4, 4a, 5, 7, 7a
7	—	135.1 (C)	
7a	—	162.8 (C)	
8	5.10 (1H, d, 1.8), 5.47 (1H, d, 1.8)	95.7 (CH ₂)	7, 7a
9	6.55 (1H, d, 15.6)	117.7 (CH)	2, 3, 10, 11
10	6.63 (1H, dt, 15.6, 6.6)	136.5 (CH)	2, 9, 11, 12
11	2.25 (2H, m)	34.7 (CH ₂)	9, 10, 12, 13
12	1.49 (2H, m)	21.5 (CH ₂)	10, 11, 13
13	0.93 (3H, t, 7.2)	13.6 (CH ₃)	11, 12
3-OH	9.77 (1H, br s)	—	2, 3, 4

Table S6. NMR data for cordylactam (**4**) in DMSO-*d*₆ (600 and 150 MHz)

Position	δ_H (<i>J</i> in Hz)	δ_C
2	—	145.7 (C)
3	—	141.7 (C)
4	—	169.3 (C)
4a	—	112.0 (C)
5	—	165.4 (C)
6 (NH)	8.30 (1H, s)	—
7	4.57 (1H, t, 6.6)	50.0 (CH)
7a	—	177.5 (C)
8	1.36 (3H, d, 6.6)	17.4 (CH ₃)
9	6.53 (1H, d, 16.2)	117.8 (CH)
10	6.47 (1H, dt, 16.2, 6.6)	135.9 (CH)
11	2.24 (2H, m)	34.7 (CH ₂)
12	1.46 (2H, m)	21.6 (CH ₂)
13	0.91 (3H, t, 7.2)	13.7 (CH ₃)
3-OH	9.58 (1H, br s)	—

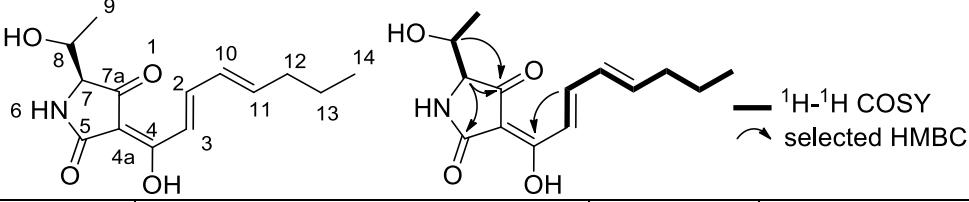
Table S7. NMR data for pyranterrone D (**5**) in $\text{DMSO}-d_6$ (600 and 150 MHz)



Position	δ_{H} (<i>J</i> in Hz)	δ_{C}	HMBC
2	7.42 (1H, dd, 15.6, 8.4)	144.1 (CH)	3, 4, 9, 10
3	6.96 (1H, d, 15.6)	119.1 (CH)	4, 4a, 9
4	—	172.4 (C)	
4a	—	100.8 (C)	
5	—	175.5 (C)	
6 (NH)	8.83 (1H, s)	—	4a, 7, 7a
7	3.85 (1H, br s)	64.1 (CH)	5, 7a, 8
7a	—	194.3 (C)	
8	3.64 (1H, dd, 11.4, 4.2) 3.60 (1H, dd, 11.4, 3.0)	60.4 (CH ₂)	4a, 7, 7a
9	6.41 (1H, m) 6.44 (1H, dd, 15.0, 10.2)*	129.5 (CH)	2, 3, 10, 11
10	6.41 (1H, m) 6.39 (1H, dt, 15.0, 6.0)*	147.7 (CH)	2, 9, 11, 12
11	2.18 (2H, m)	34.7 (CH ₂)	9, 10, 12, 13
12	1.43 (2H, m)	21.4 (CH ₂)	10, 11, 13
13	0.89 (3H, t, 6.6)	13.6 (CH ₃)	11, 12

* Measured in acetone-*d*₆

Table S8. NMR data for pyranterrone E (**6**) in DMSO-*d*₆ (600 and 150 MHz)



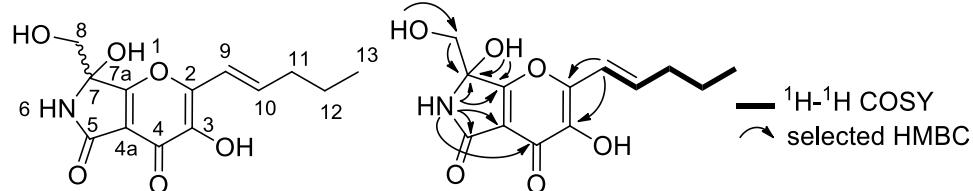
Position	δ_{H} (<i>J</i> in Hz)	δ_{C}	HMBC
2	7.37 (1H, dd, 13.8, 9.0)	143.8 (CH)	4, 10, 11
3	7.05 (1H, d, 13.8)	119.4 (CH)	
4	—	172.4 (C)*	
4a	—	100.8 (C)*	
5	—	175.5 (C)*	
6 (NH)	8.73 (1H, s)	—	
7	3.65 (1H, br s)	67.3 (CH)	5, 7a
7a	—	194.3 (C)*	
8	3.92 (1H, m)	65.6 (CH)	7a, 9
9	1.16 (3H, d, 6.6)	20.6 (CH ₃)	7, 8
10	6.38 (1H, m)	129.5 (CH)	2, 3, 11, 12
11	6.40 (1H, m)	147.3 (CH)	2, 10, 12, 13
12	2.18 (2H, m)	34.7 (CH ₂)	10, 11, 13, 14
13	1.43 (2H, m)	21.4 (CH ₂)	11, 12, 14
14	0.89 (3H, t, 6.6)	13.6 (CH ₃)	12, 13

* Chemical shifts are read from the HMBC spectrum

Table S9. NMR data for pyranterrone F (**7**) in DMSO-*d*₆ (600 and 150 MHz)

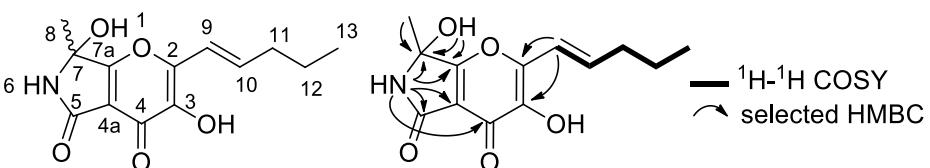
Position	δ_H (J in Hz)	δ_C	HMBC
2	—	145.7 (C)	
3	—	141.7 (C)	
4	—	169.1 (C)	
4a	—	113.6 (C)	
5	—	166.1 (C)	
6 (NH)	8.18 (1H, s)	—	4, 4a, 5, 7, 7a
7	4.55 (1H, t, 3.0)	56.8 (CH)	4a, 5, 7a, 8
7a	—	175.1 (C)	
8	3.72 (1H, dd, 11.4, 2.4), 3.77 (1H, dd, 11.4, 2.4)	60.0 (CH ₂)	7, 7a
9	6.54 (1H, d, 15.6)	117.9 (CH)	2, 3, 10, 11
10	6.48 (1H, dt, 15.6, 6.6)	136.8 (CH)	2, 9, 11, 12
11	2.24 (2H, m)	34.6 (CH ₂)	9, 10, 12, 13
12	1.48 (2H, m)	21.6 (CH ₂)	10, 11, 13
13	0.92 (3H, t, 7.8)	13.6 (CH ₃)	11, 12
3-OH	9.55 (1H, br s)	—	
8-OH	5.10 (1H, br s)	—	

Table S10. NMR data for pyranterrone G (**8**) in DMSO-*d*₆ (600 and 150 MHz)



Position	δ_H (<i>J</i> in Hz)	δ_C	HMBC
2	—	145.6 (C)	
3	—	142.3 (C)	
4	—	168.9 (C)	
4a	—	112.4 (C)	
5	—	164.8 (C)	
6 (NH)	8.41 (1H, s)	—	4, 4a, 5, 7, 7a, 8
7	—	85.2 (C)	
7a	—	174.4 (C)	
8	3.55 (1H, d, 10.8), 3.79 (1H, d, 10.8)	62.5 (CH ₂)	7, 7a
9	6.55 (1H, d, 16.2)	117.9 (CH)	2, 3, 10, 11
10	6.47 (1H, dt, 16.2, 6.6)	136.0 (CH)	2, 9, 11, 12
11	2.26 (2H, m)	34.6 (CH ₂)	9, 10, 12, 13
12	1.48 (2H, m)	21.6 (CH ₂)	10, 11, 13
13	0.92 (3H, t, 7.8)	13.6 (CH ₃)	11, 12
3-OH	9.67 (1H, br s)	—	
7-OH	6.81 (1H, br s)	—	7, 7a, 8
8-OH	5.20 (1H, br s)	—	7, 8

Table S11. NMR data for 7-hydroxycordylactam (**9**) in DMSO-*d*₆ (600 and 150 MHz)



Position	δ_H (<i>J</i> in Hz)	δ_C	HMBC
2	—	145.5 (C)	
3	—	142.3 (C)	
4	—	169.2 (C)	
4a	—	110.2 (C)	
5	—	163.9 (C)	
6 (NH)	8.63 (1H, s)	—	4, 4a, 5, 7, 7a
7	—	81.9 (C)	
7a	—	175.6 (C)	
8	1.59 (3H, s)	23.5 (CH ₃)	7, 7a
9	6.55 (1H, d, 15.6)	117.8 (CH)	2, 3, 10, 11
10	6.49 (1H, dt, 15.6, 6.6)	135.0 (CH)	2, 9, 11, 12
11	2.24 (2H, m)	34.6 (CH ₂)	9, 10, 12, 13
12	1.48 (2H, m)	21.6 (CH ₂)	10, 11, 13
13	0.91 (3H, t, 7.8)	13.6 (CH ₃)	11, 12
3-OH	9.68 (1H, br s)	—	
7-OH	6.60 (1H, br s)	—	7, 7a, 8

Table S12. NMR data for pyranterrone H (**10**) in DMSO-*d*₆ (600 and 150 MHz)

Position	δ_H (<i>J</i> in Hz)	δ_C	HMBC
2	—	145.9 (C)	
3	—	142.4 (C)	
4	—	168.7 (C)	
4a	—	112.6 (C)	
5	—	164.2 (C)	
6 (NH)	8.61 (1H, s)	—	4, 4a, 5, 7, 7a
7	—	86.2 (C)	
7a	—	172.6 (C)	
8	1.63 (3H, s)	22.5 (CH ₃)	7, 7a
9	6.55 (1H, d, 15.6)	117.8 (CH)	2, 3, 10, 11
10	6.49 (1H, dt, 15.6, 6.6)	136.3 (CH)	2, 9, 11, 12
11	2.24 (2H, m)	34.6 (CH ₂)	9, 10, 12, 13
12	1.48 (2H, m)	21.5 (CH ₂)	10, 11, 13
13	0.92 (3H, t, 7.8)	13.6 (CH ₃)	11, 12
3-OH	9.82 (1H, br s)	—	2, 3, 4
7-OCH ₃	2.99 (3H, s)	50.1 (CH ₃)	7

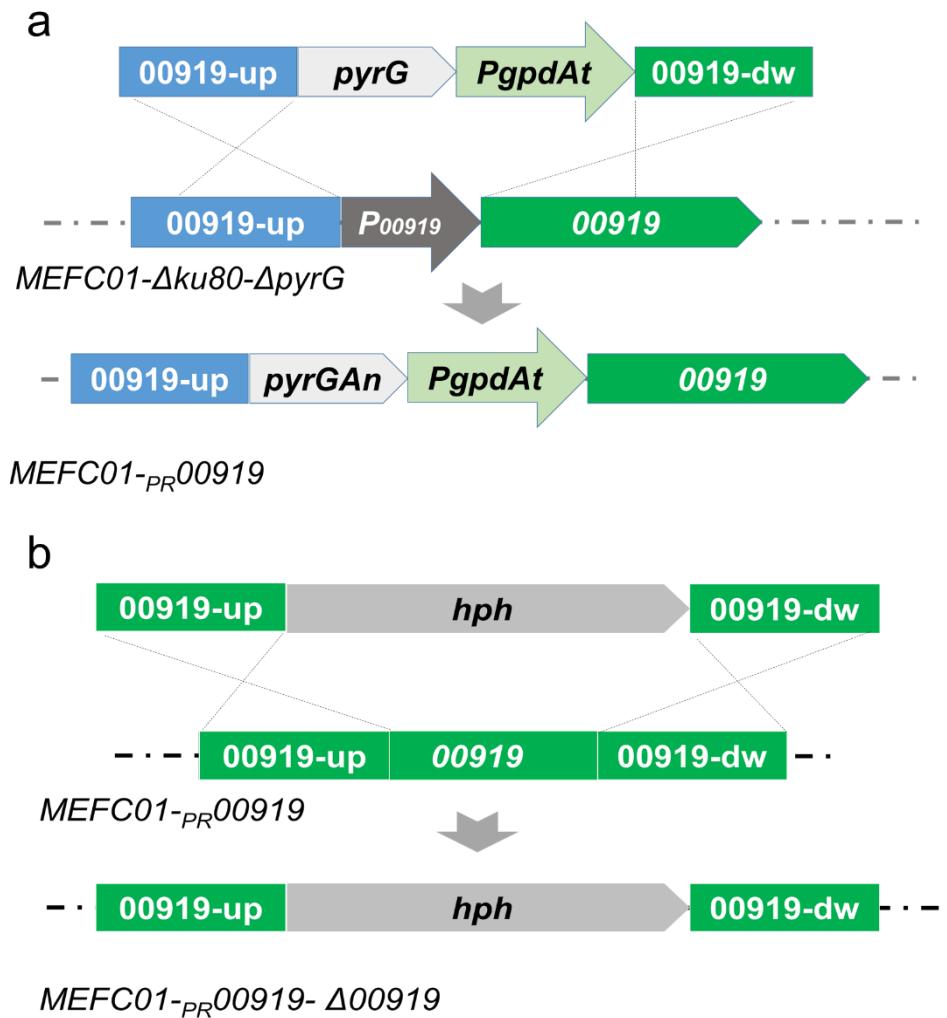


Figure S1. The strategies of promoter replacement (a) and gene deletion (b)

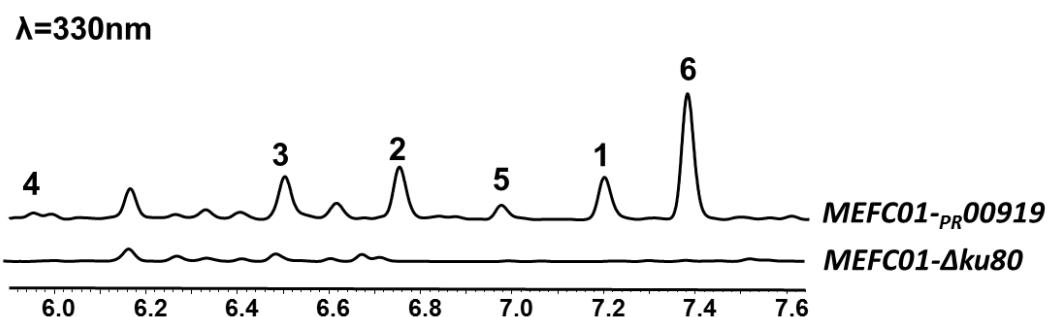


Figure S2. HPLC profiles of the crude extracts of the solid cultures of both MEFC01-Δku80 and MEFC01-_{PR}00919

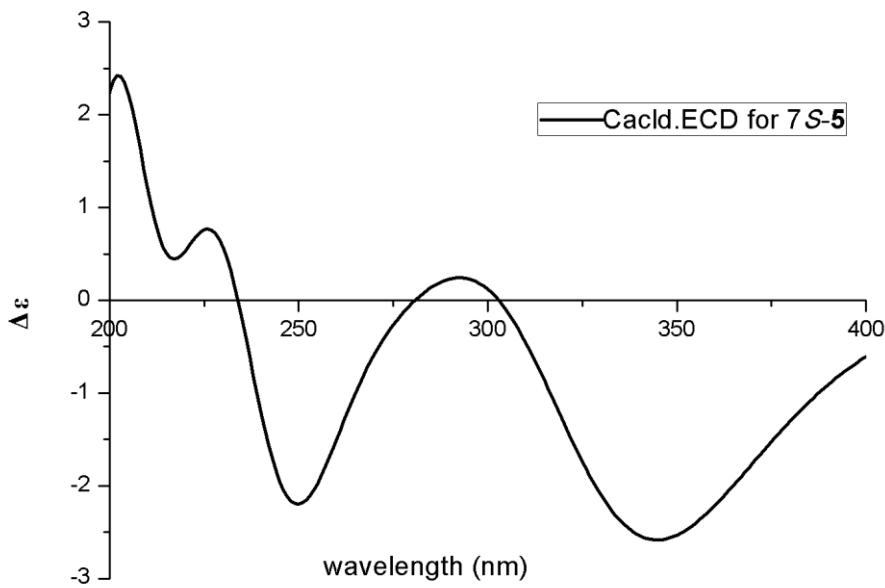


Figure S3. Calculated ECD spectra for pyranterrone D (**5**)

The absolute configuration of **5** was investigated by quantum chemical TDDFT calculations of its ECD spectrum. Conformational searches were performed using MMFF94S force field for **7S-5**. All geometries (37 lowest energy conformers) with relative energy from 0-10 kcal/mol used in optimizations at the B3LYP/6-31G (d) level using Gaussian09 package. The B3LYP/6-31G (d)-optimized conformers (27 lowest energy conformers) with relative energy from 0 to 2.5 kcal/mol were then re-optimized at the B3LYP/6-311+G (d) level. ECD computations for all conformers were carried out at the B3LYP/6-311++G (2d, p) level in the gas phase. Boltzmann statistics were performed for ECD simulations with standard deviation of σ 0.3 eV. The predicted ECD for **7S-5** look similar to the experimental result of **5**. Thus, the absolute configuration of **5** was assigned as **7S**.

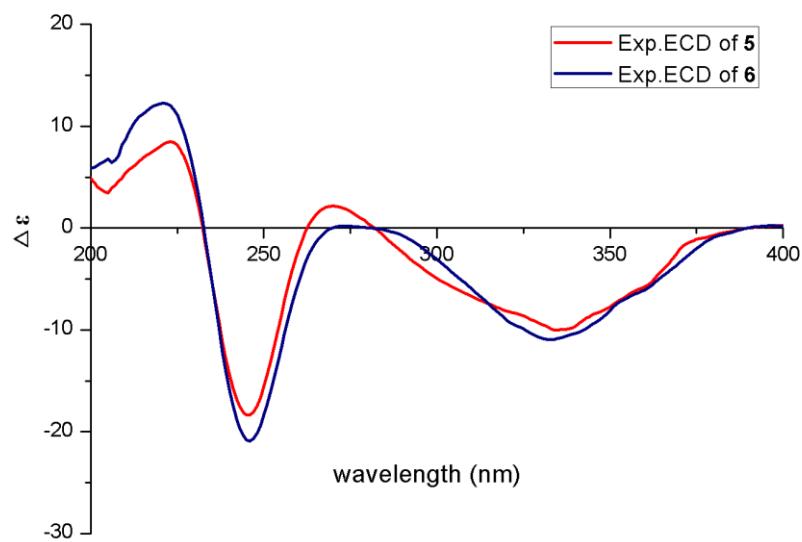


Figure S4a. Experimental ECD spectra of pyranterrone D (**5**) and pyranterrone E (**6**)

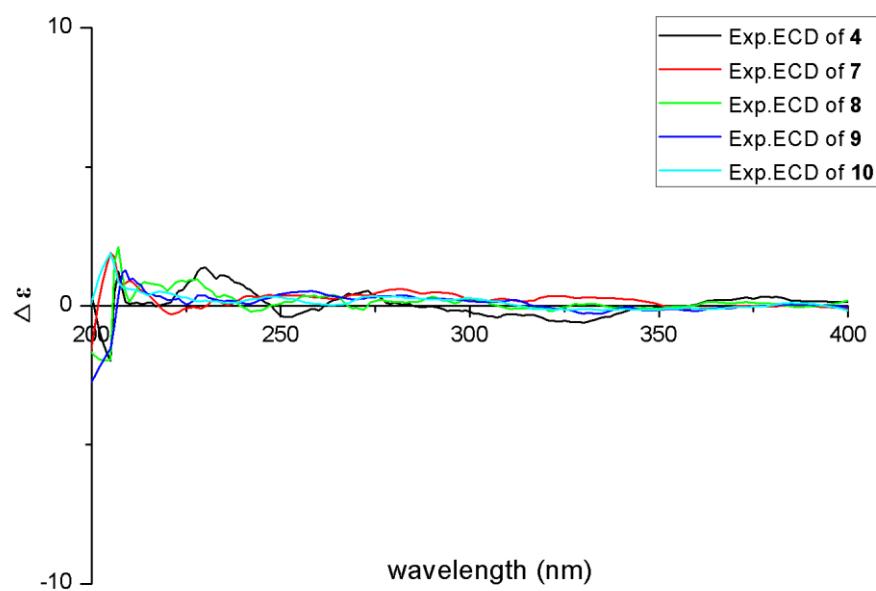


Figure S4b. Experimental ECD spectra of compounds **4**, **7**, **8**, **9**, and **10**.

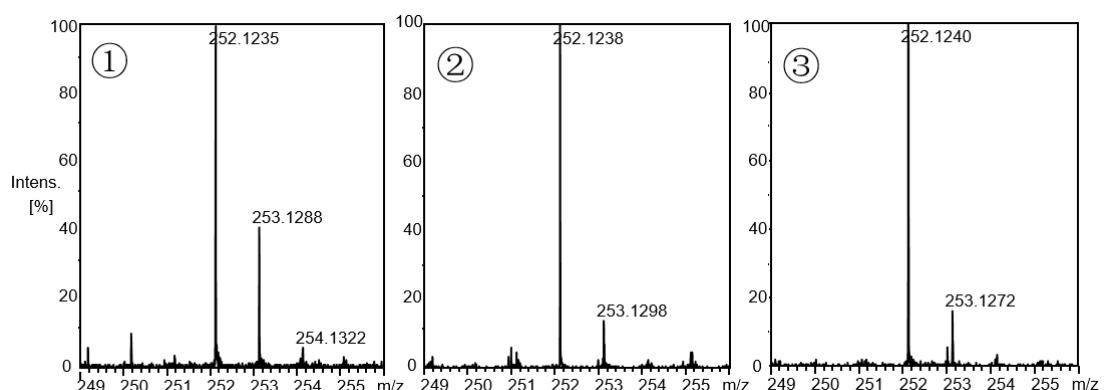


Figure S5a. The abundance of isotopic peaks of **5**. ① About 40% enrichment isotopic peak of **5** isolated from $^{13}\text{C}_1$ -L-serine fed MEFC01-PR $_{\text{pytR}}$. ② About 15% enrichment isotopic peak of **5** isolated from regular L-serine fed MEFC01-PR $_{\text{pytR}}$. ③ About 15% enrichment isotopic peak of **5** isolated from MEFC01-PR $_{\text{pytR}}$ without feeding L-serine.

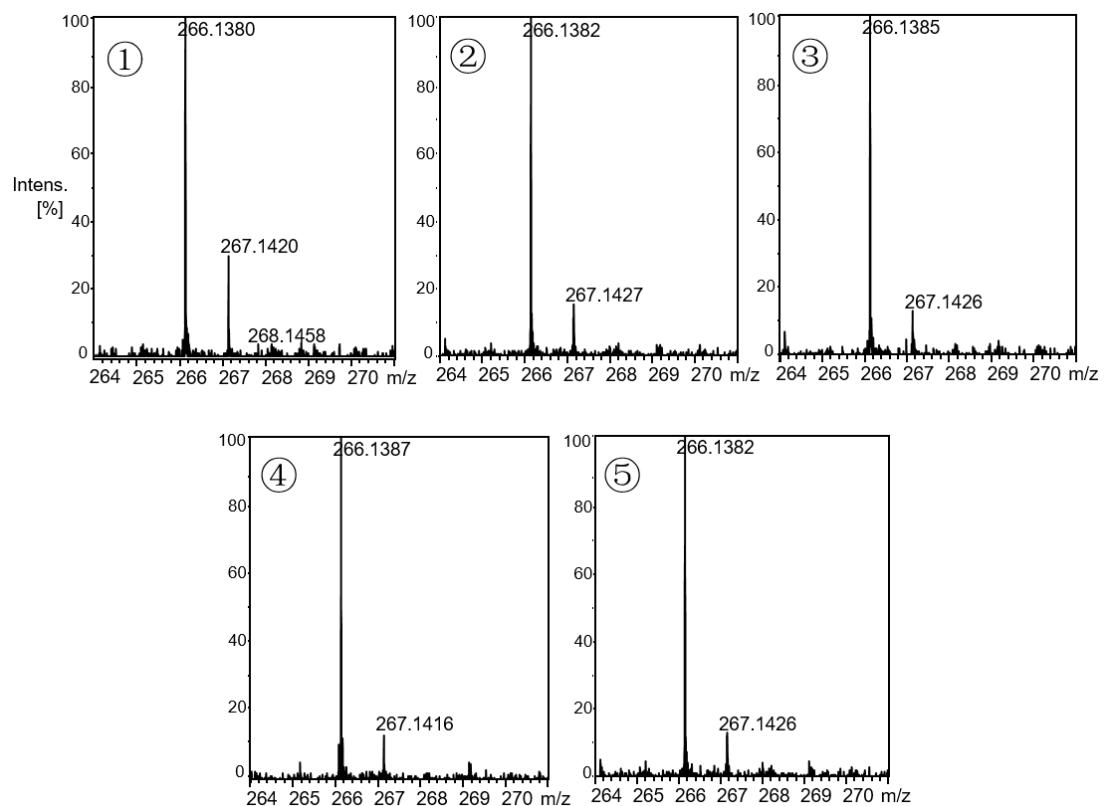


Figure S5b. The abundance of isotopic peaks of **6**. ① About 30% enrichment isotopic peak of **6** isolated from $^{13}\text{C}_1$ -L-serine fed MEFC01-PR $_{\text{pytR}}$. ② About 16% enrichment isotopic peak of **6** isolated from regular L-serine fed MEFC01-PR $_{\text{pytR}}$. ③ About 16% enrichment isotopic peak of **6** isolated from MEFC01-PR $_{\text{pytR}}$ without feeding L-serine. ④ About 16% enrichment isotopic peak of **6** isolated from $^{13}\text{C}_1$ -L-threonine fed MEFC01-PR $_{\text{pytR}}$. ⑤ About 16% enrichment isotopic peak of **6** isolated from regular L-threonine fed MEFC01-PR $_{\text{pytR}}$.

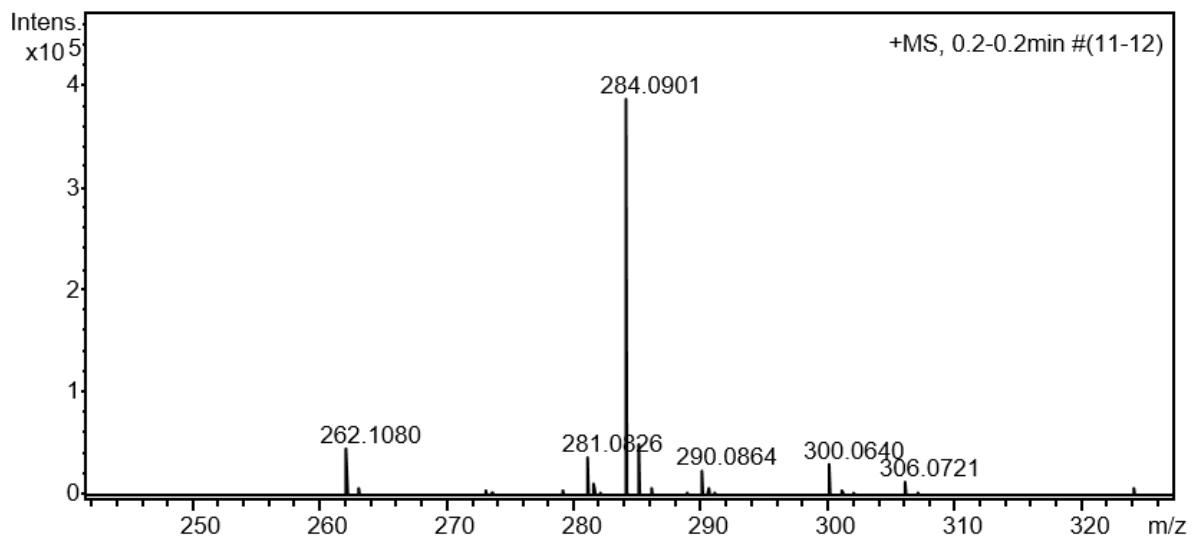


Figure S6. HRESIMS spectrum of pyranterrone A (**1**)

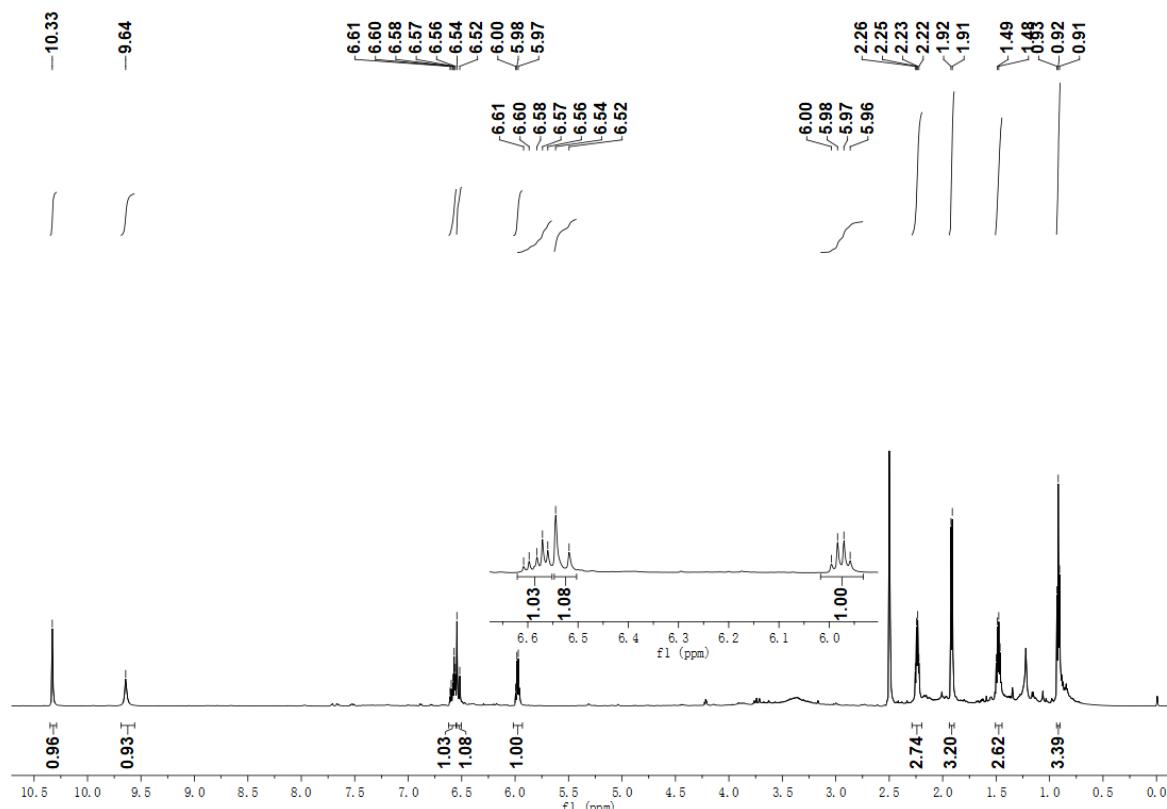


Figure S7. ^1H NMR spectrum of pyranterrone A (**1**)

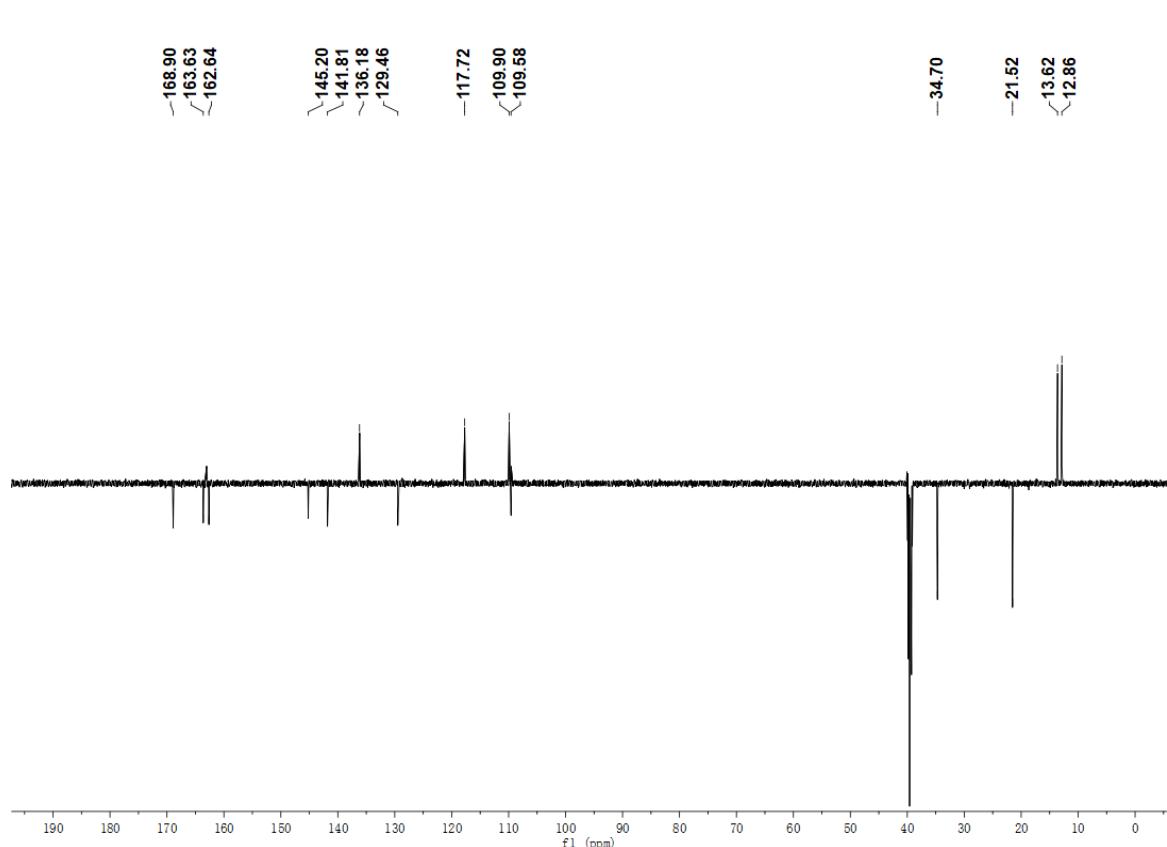


Figure S8. DEPTQ¹³C NMR spectrum of pyranterrone A (**1**)

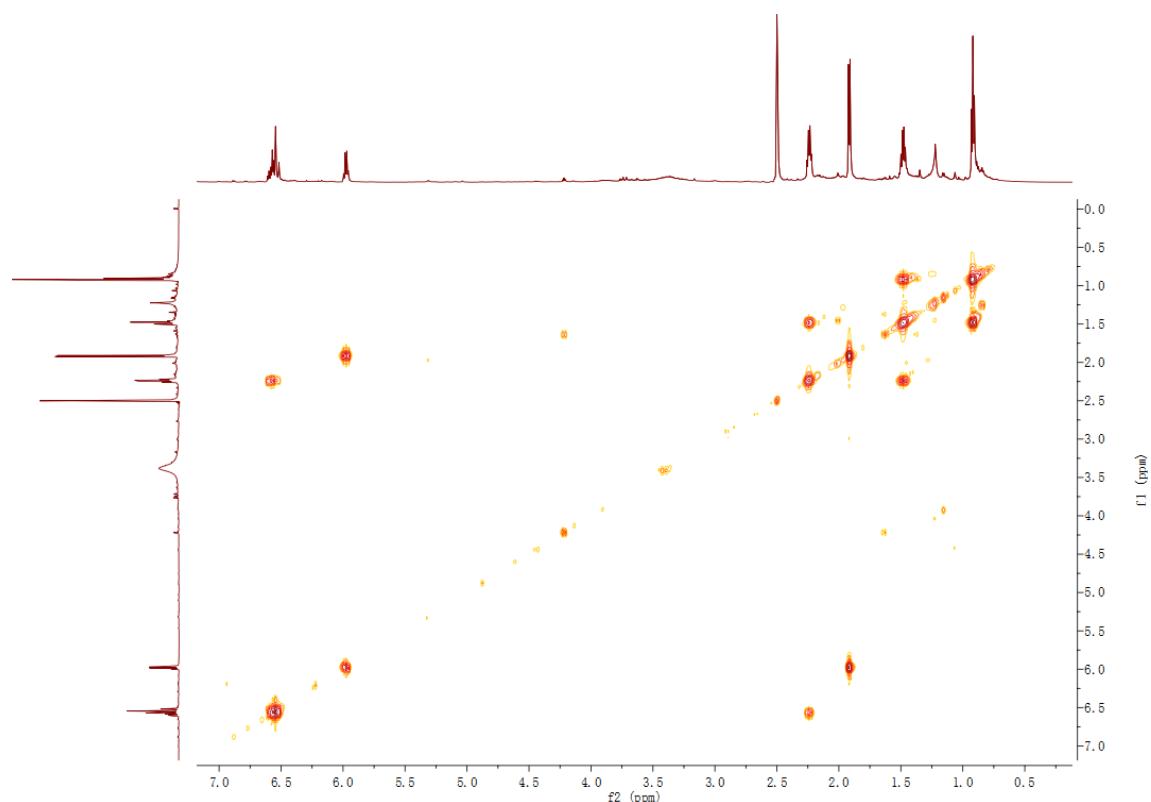


Figure S9. ¹H-¹H COSY spectrum of pyranterrone A (**1**)

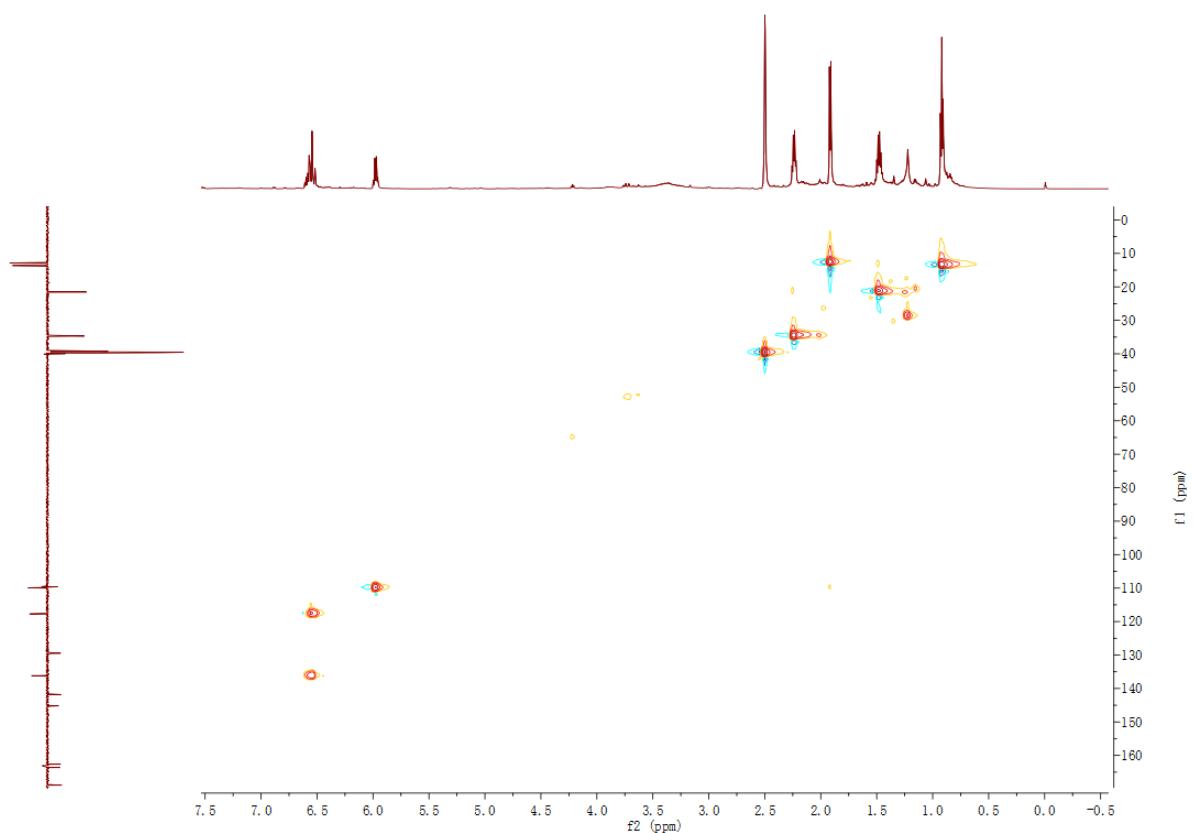


Figure S10. HSQC spectrum of pyranterrone A (**1**)

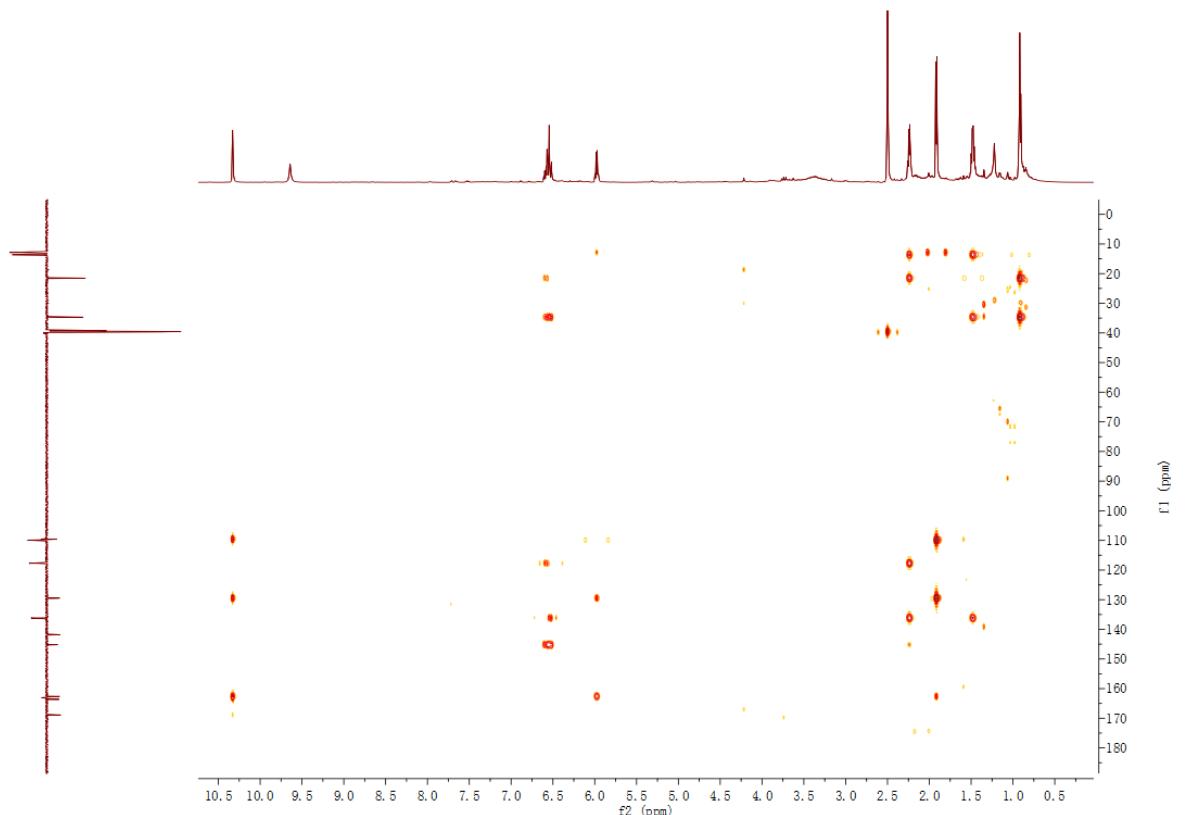


Figure S11. HMBC spectrum of pyranterrone A (**1**)

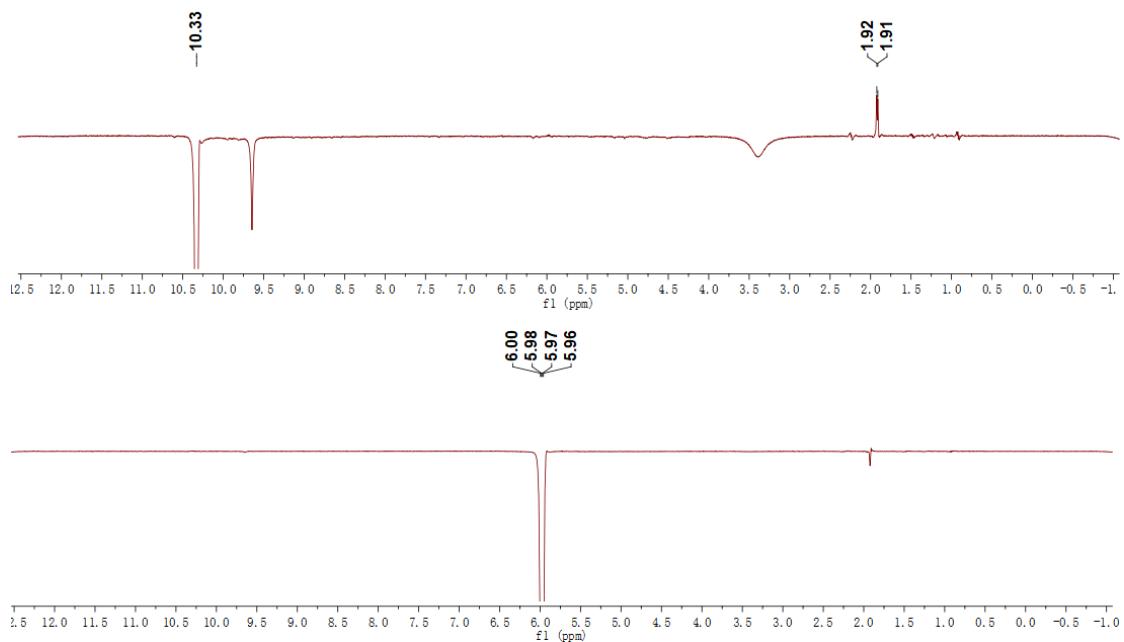


Figure S12. NOE spectra of pyranterrone A (1)

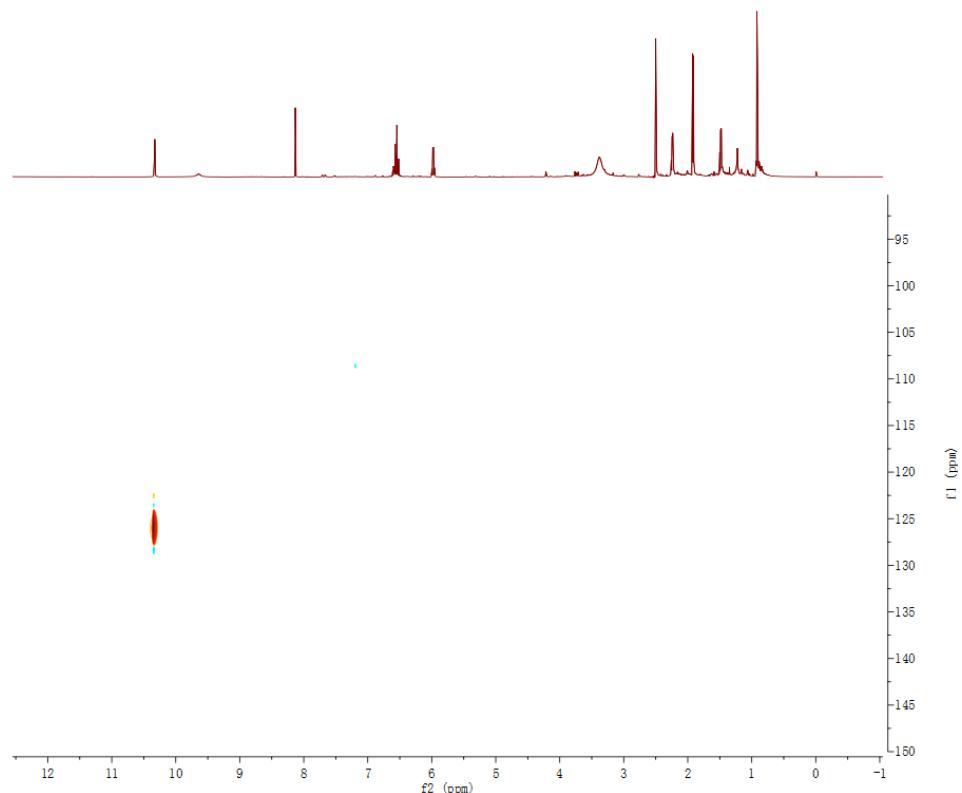


Figure S13. ¹H-¹⁵N HSQC spectrum of pyranterrone A (1)

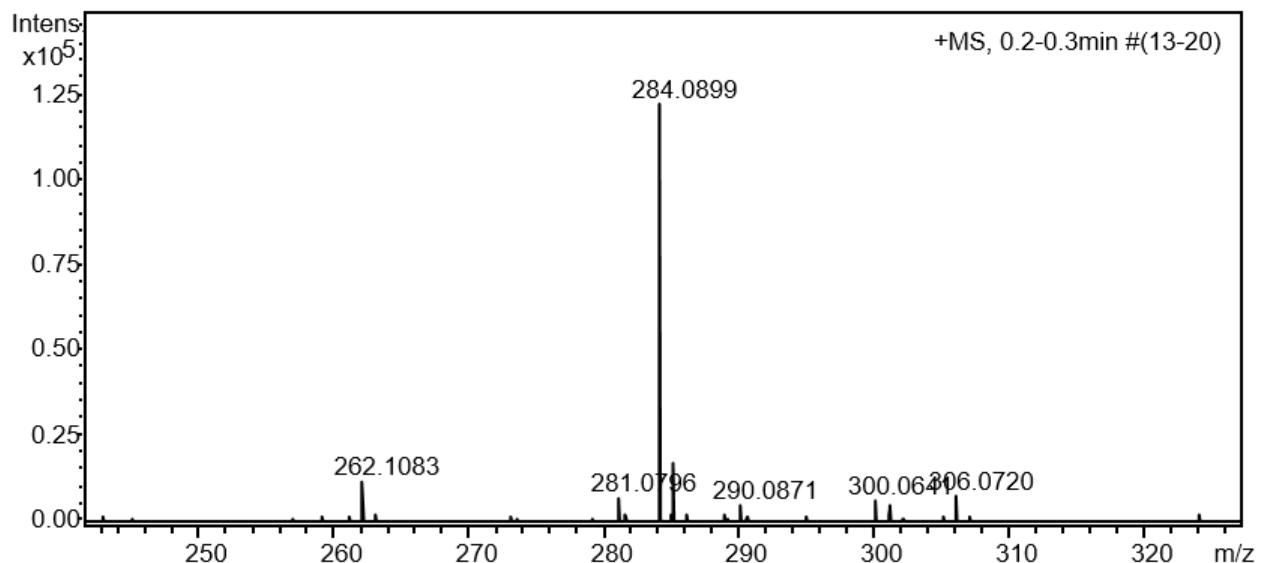


Figure S14. HRESIMS spectrum of pyranterrone B (**2**)

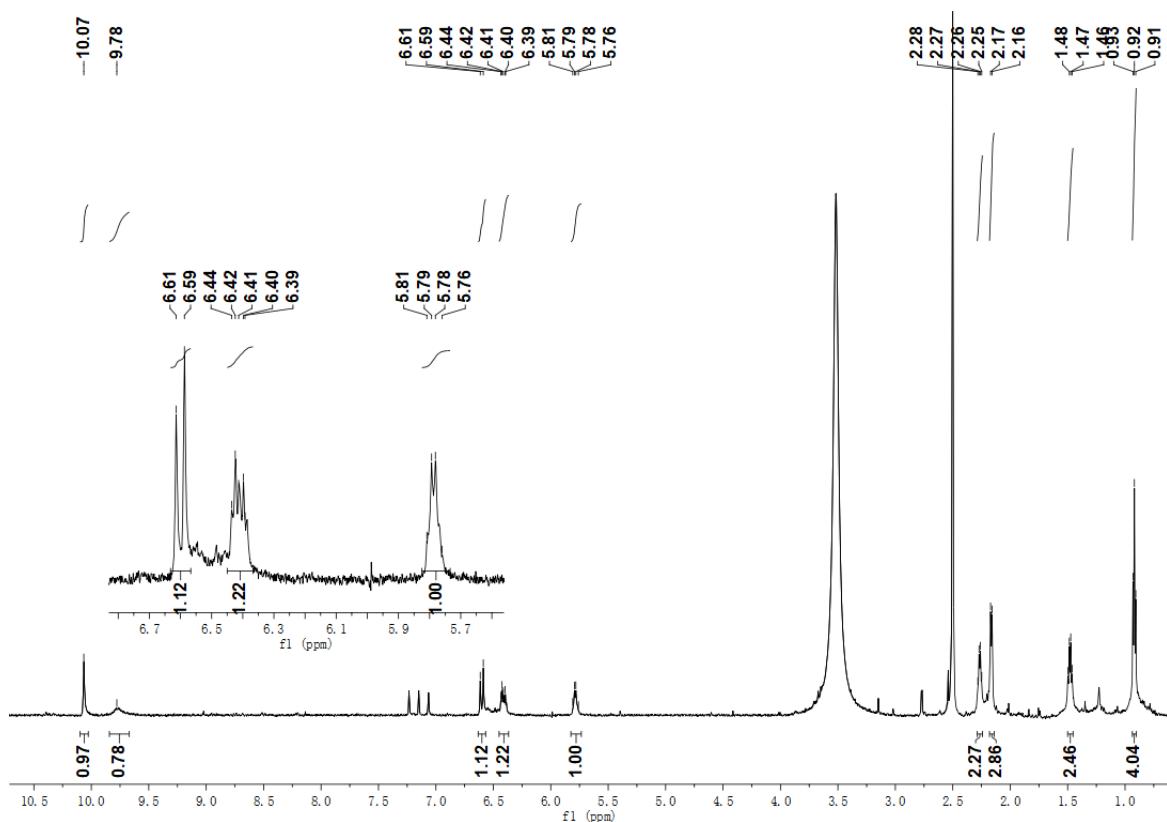


Figure S15. ^1H NMR spectrum of pyranterrone B (**2**)

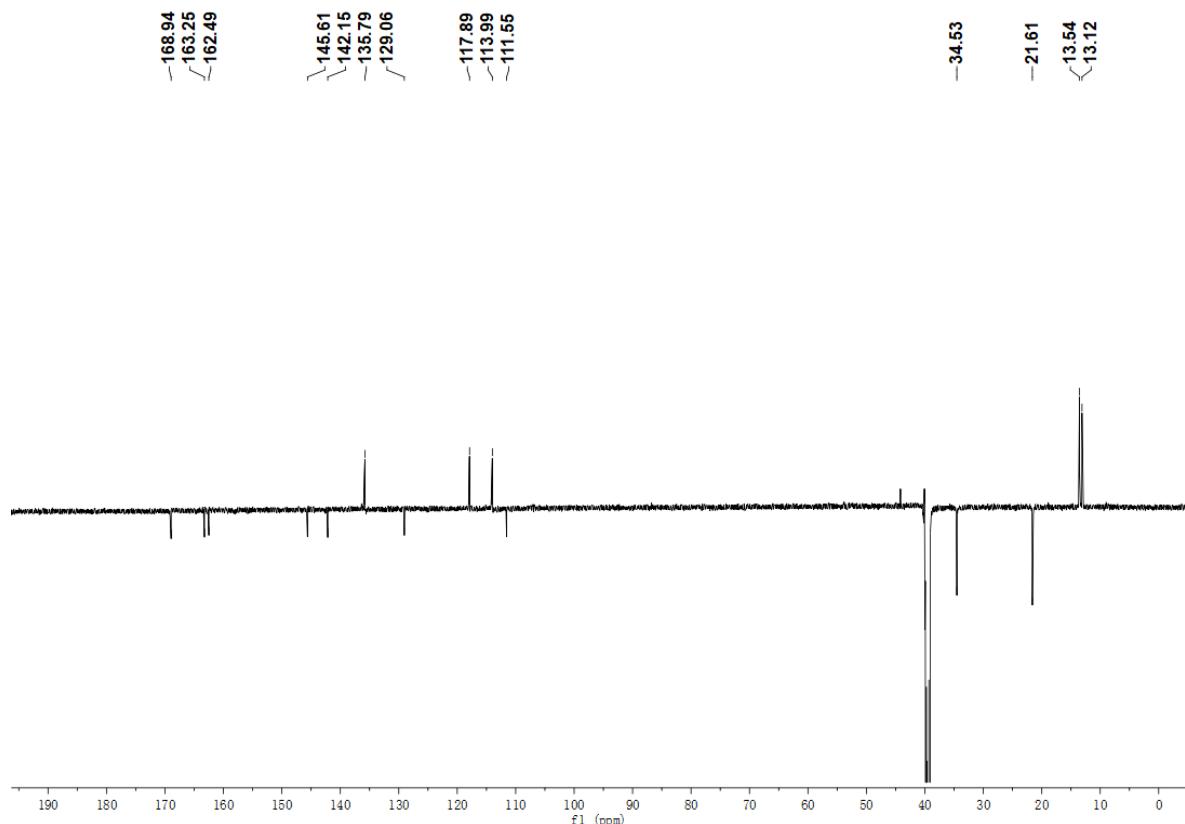


Figure S16. DEPTQ ^{13}C NMR spectrum of pyranterrone B (2)

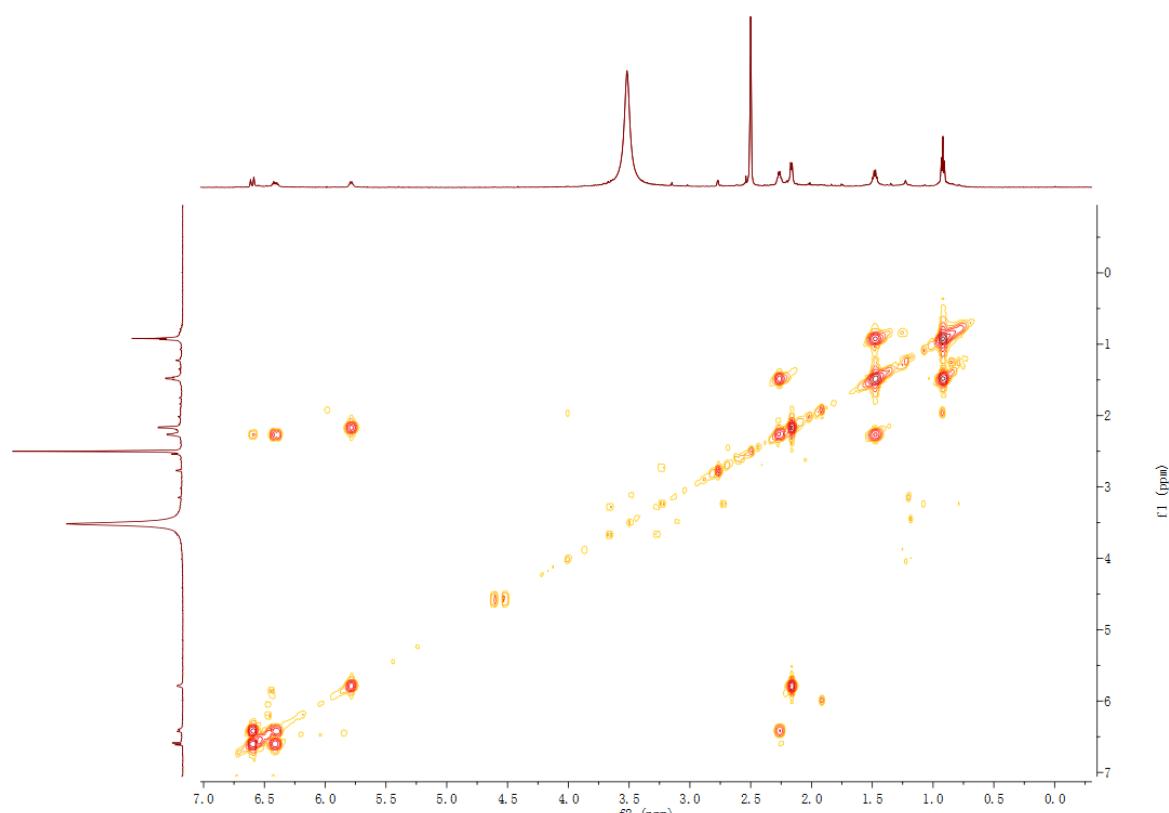


Figure S17. ^1H - ^1H COSY spectrum of pyranterrone B (2)

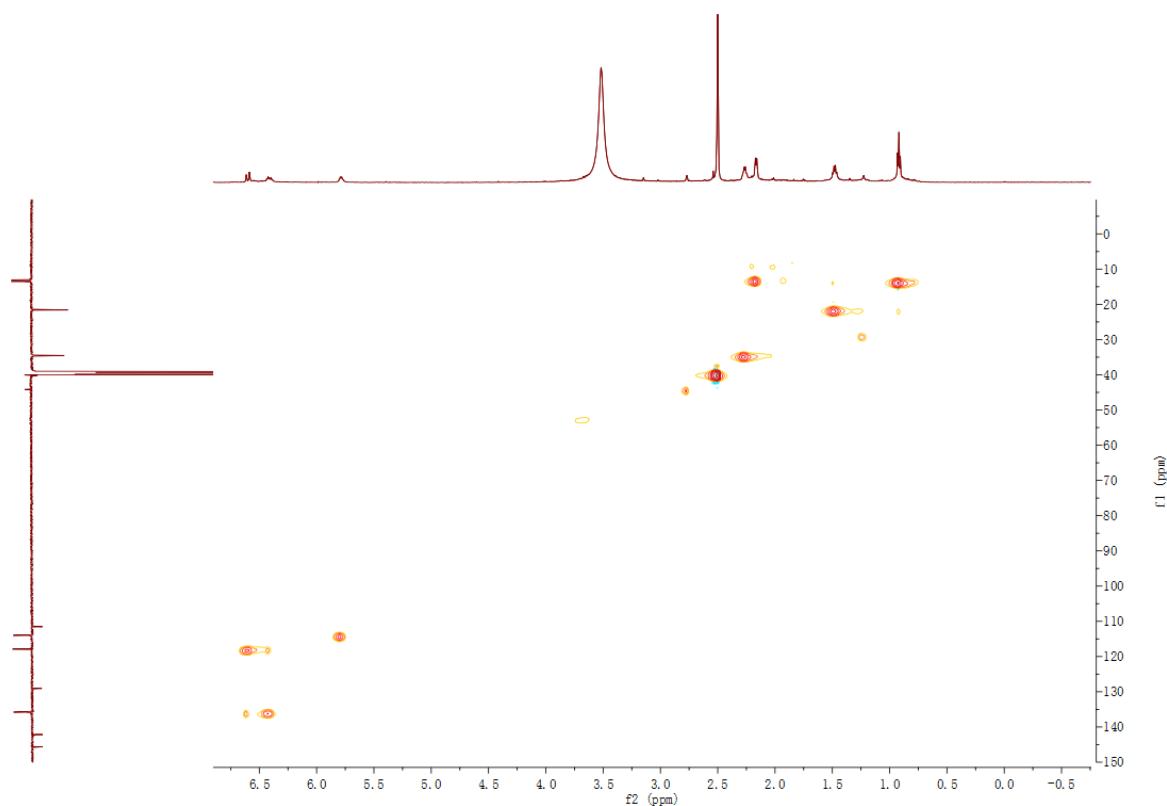


Figure S18. HSQC spectrum of pyranterrone B (2)

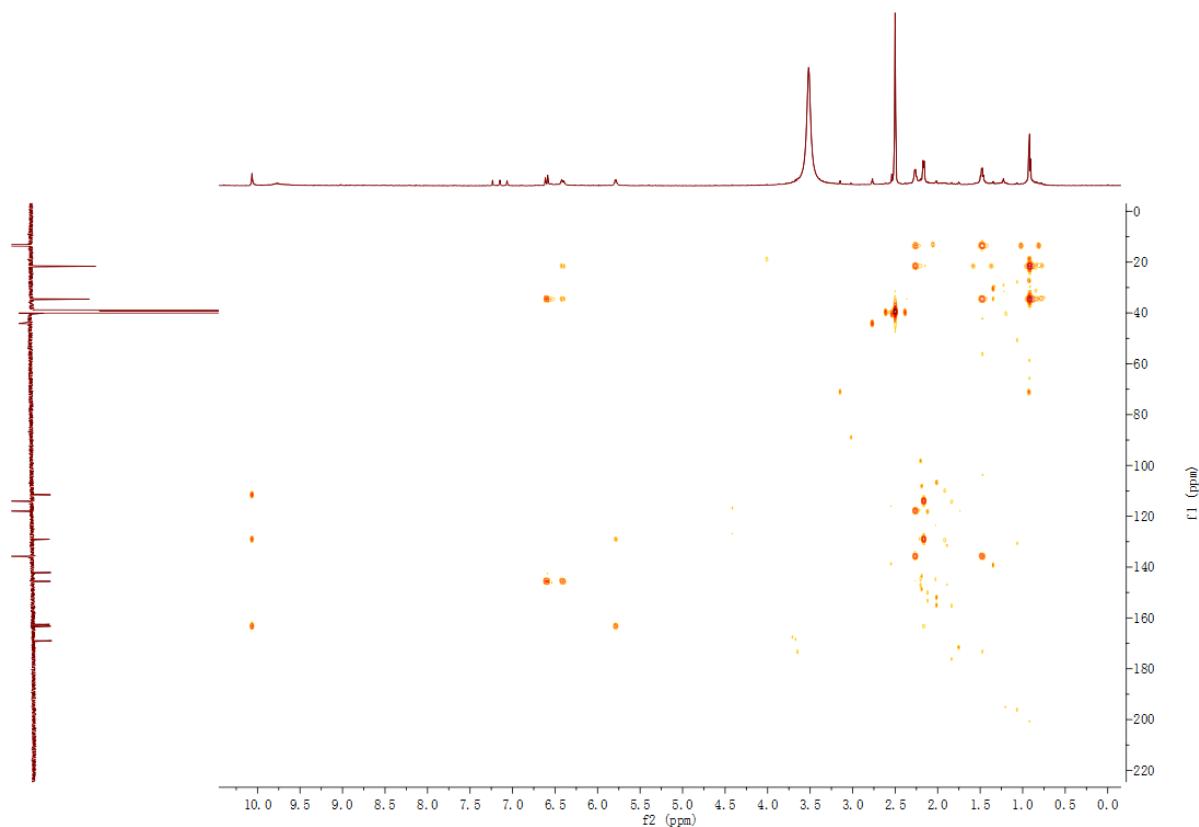


Figure S19. HMBC spectrum of pyranterrone B (2)

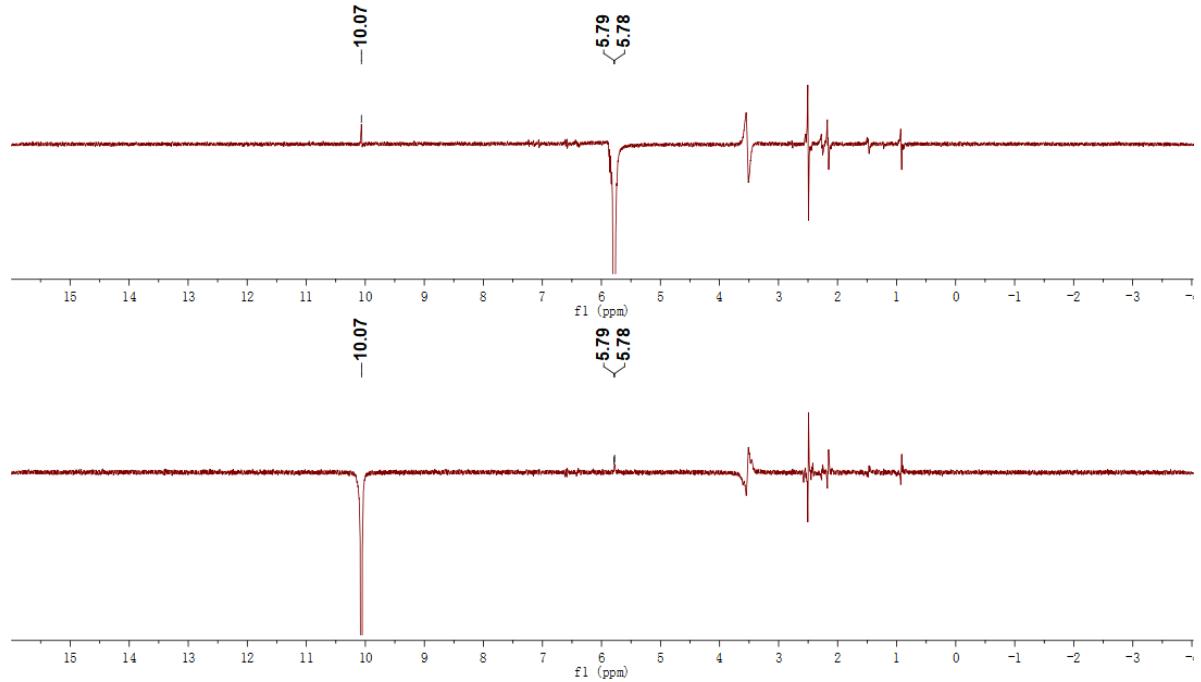


Figure S20. NOE spectra of pyranterrone B (2)

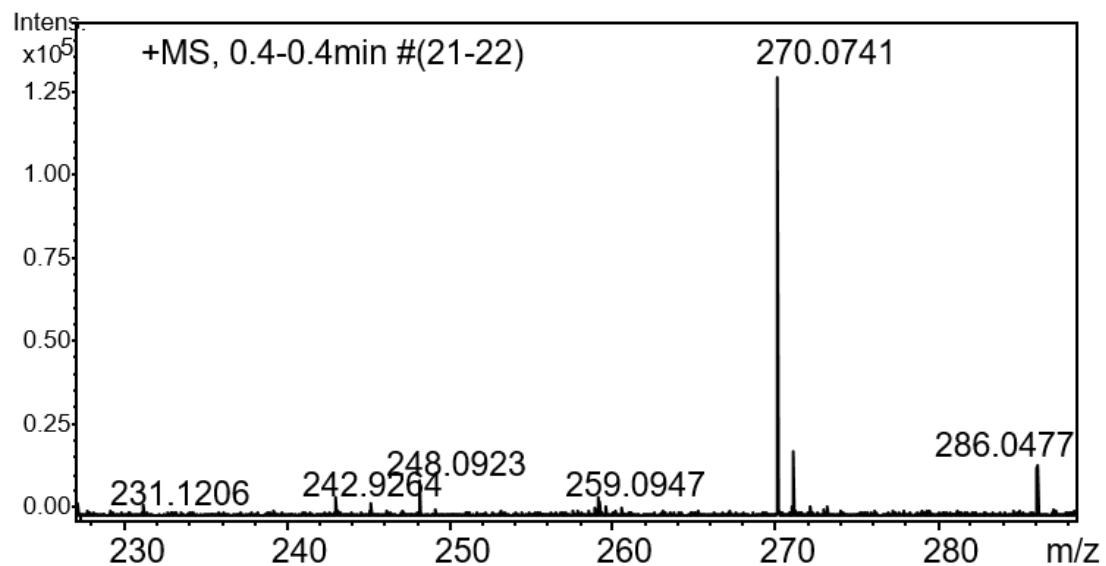


Figure S21. HRESIMS spectrum of pyranterrone C (3)

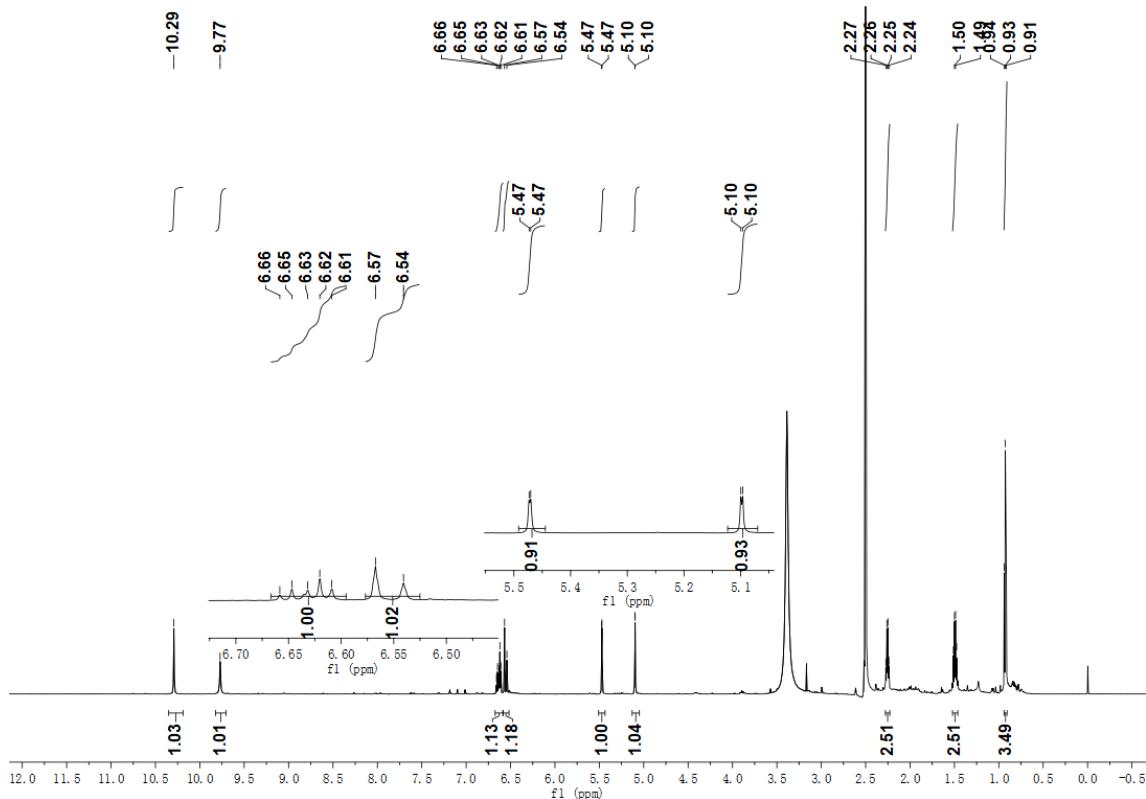


Figure S22. ^1H NMR spectrum of pyranterrone C (3)

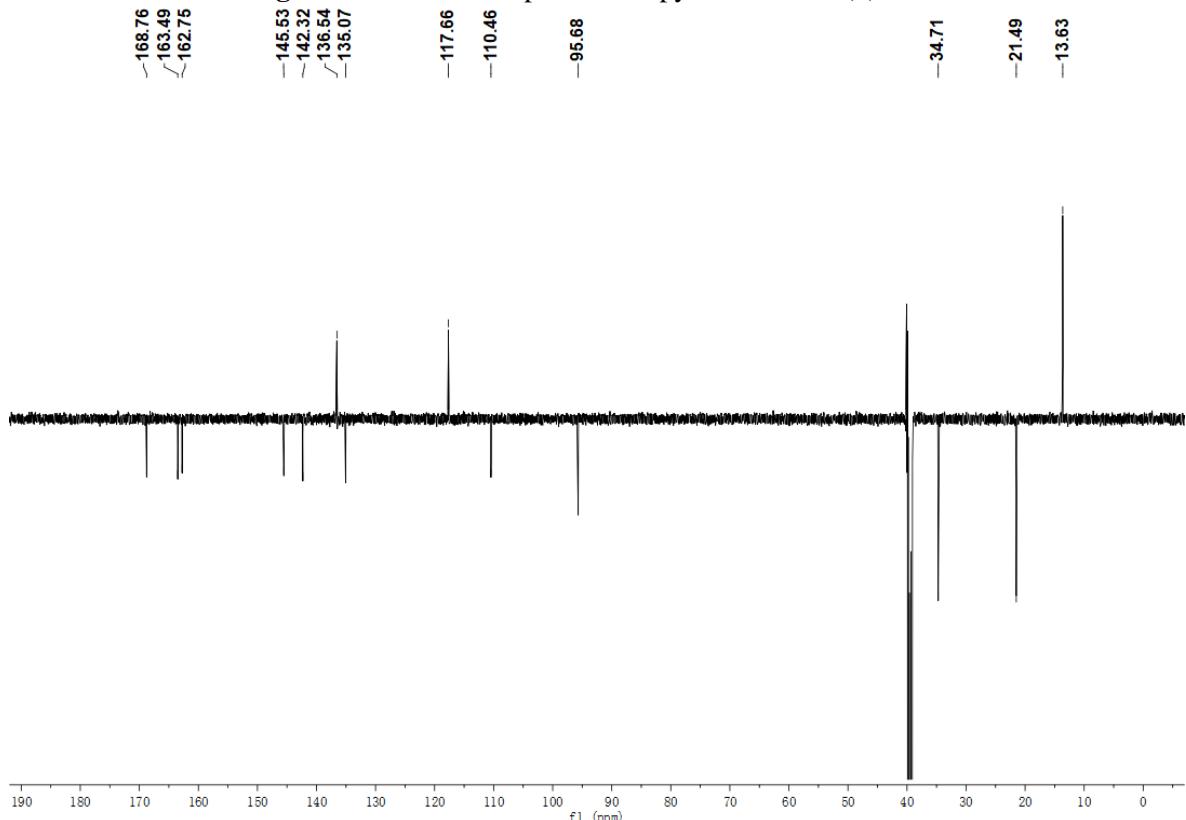


Figure S23. DEPTQ¹³C NMR spectrum of pyranterrone C (3)

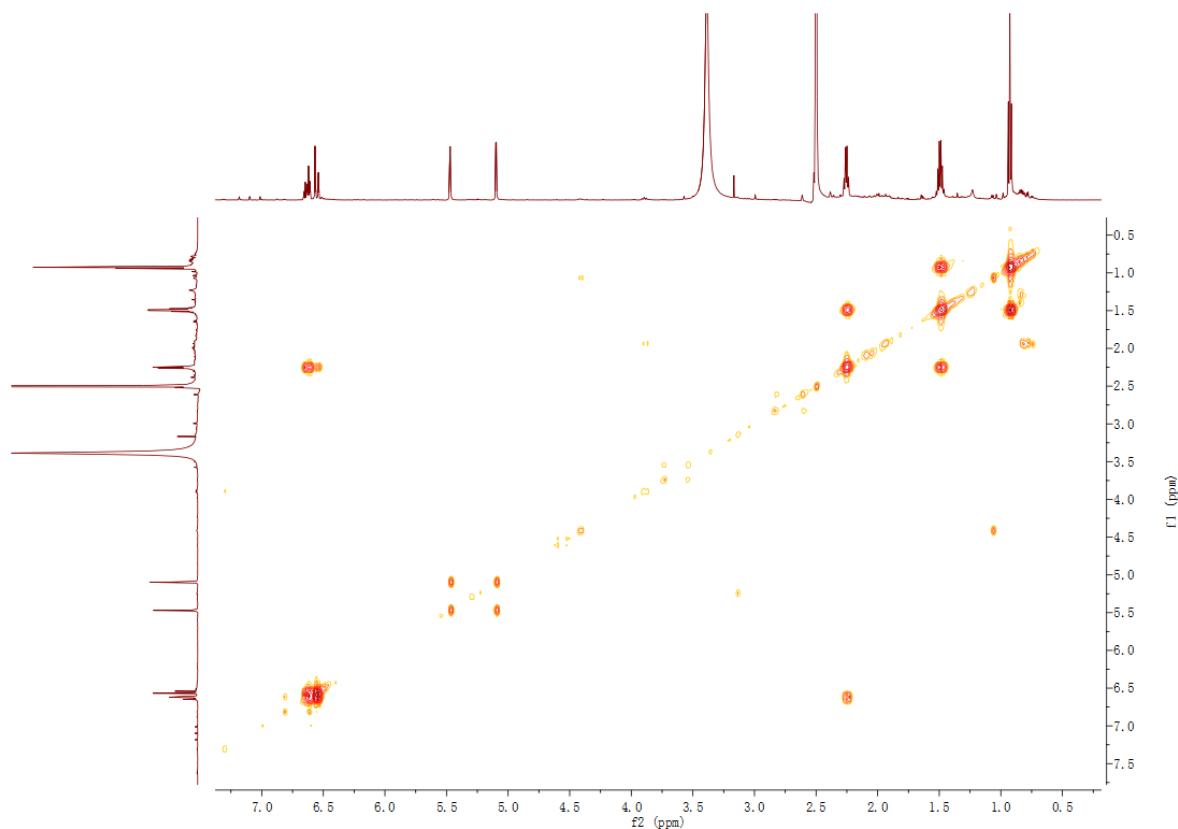


Figure S24. ^1H - ^1H COSY spectrum of pyranterrone C (3)

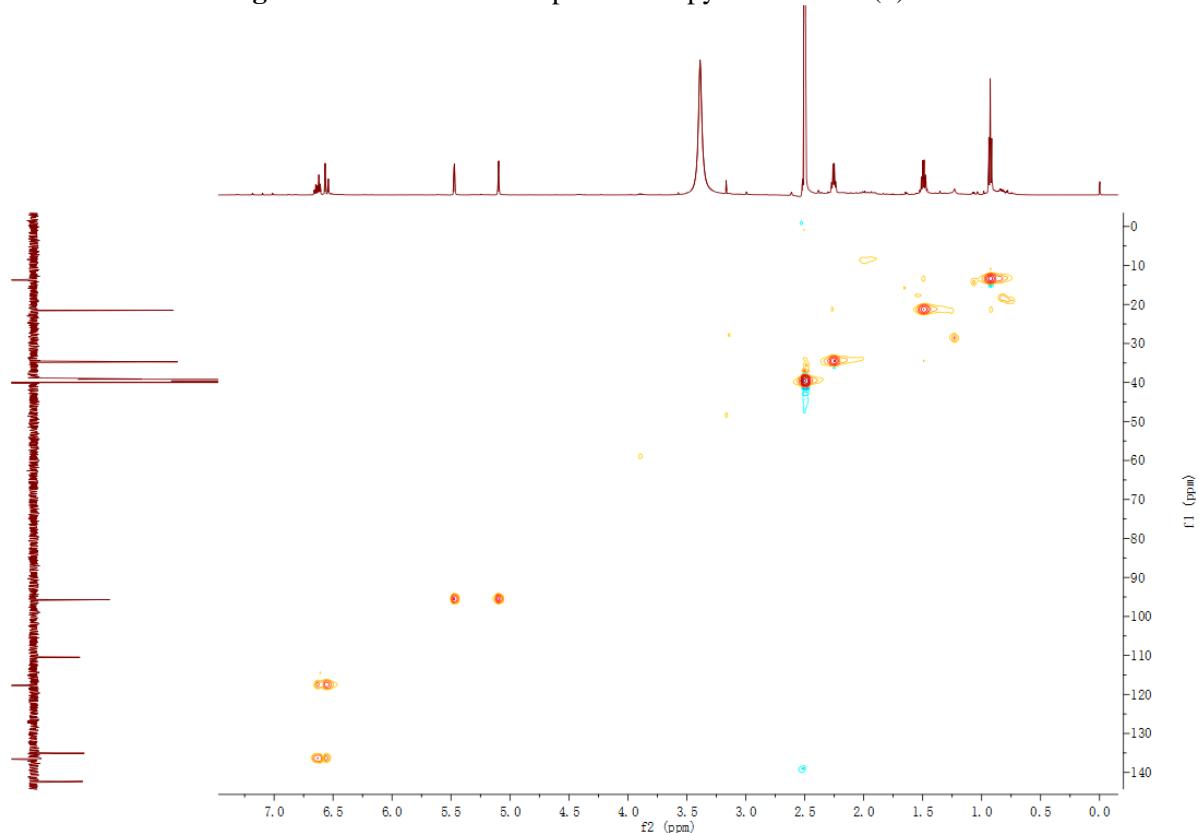


Figure S25. HSQC spectrum of pyranterrone C (3)

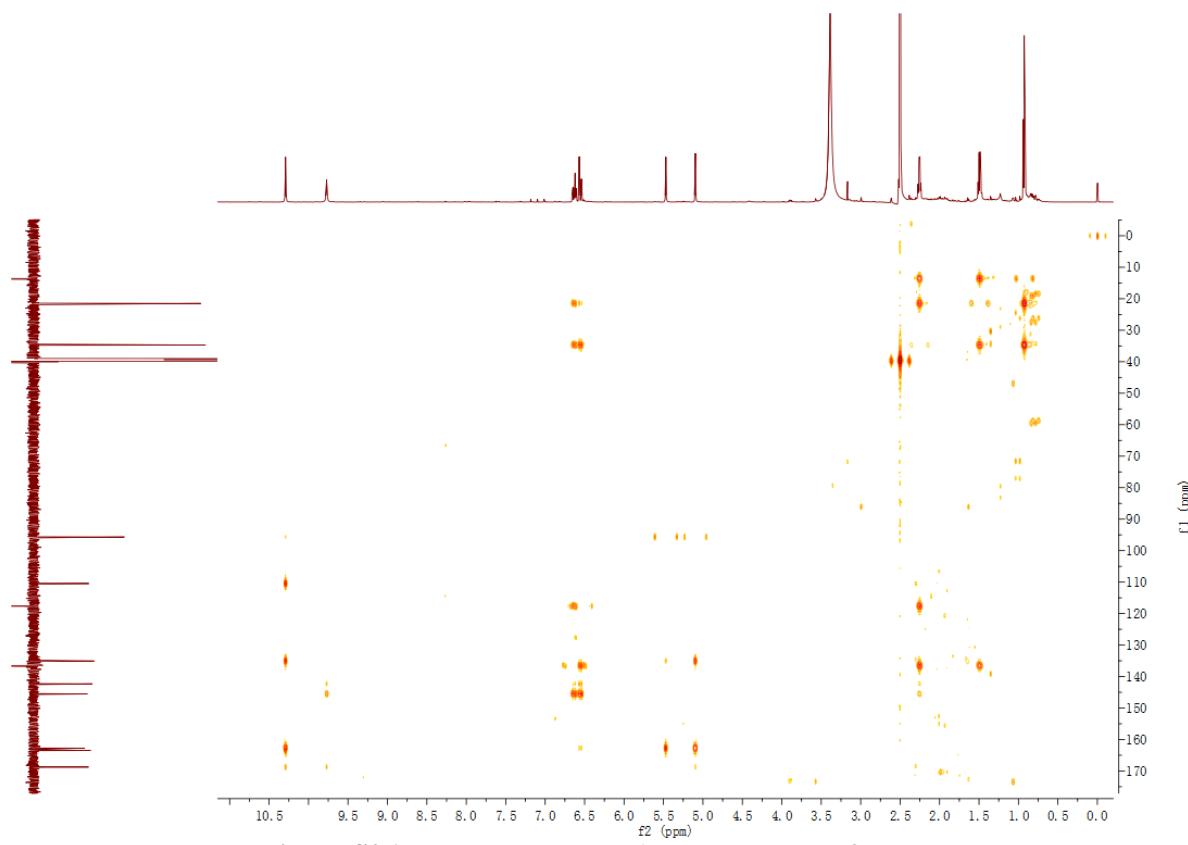


Figure S26. HMBC spectrum of pyranterrone C (**3**)

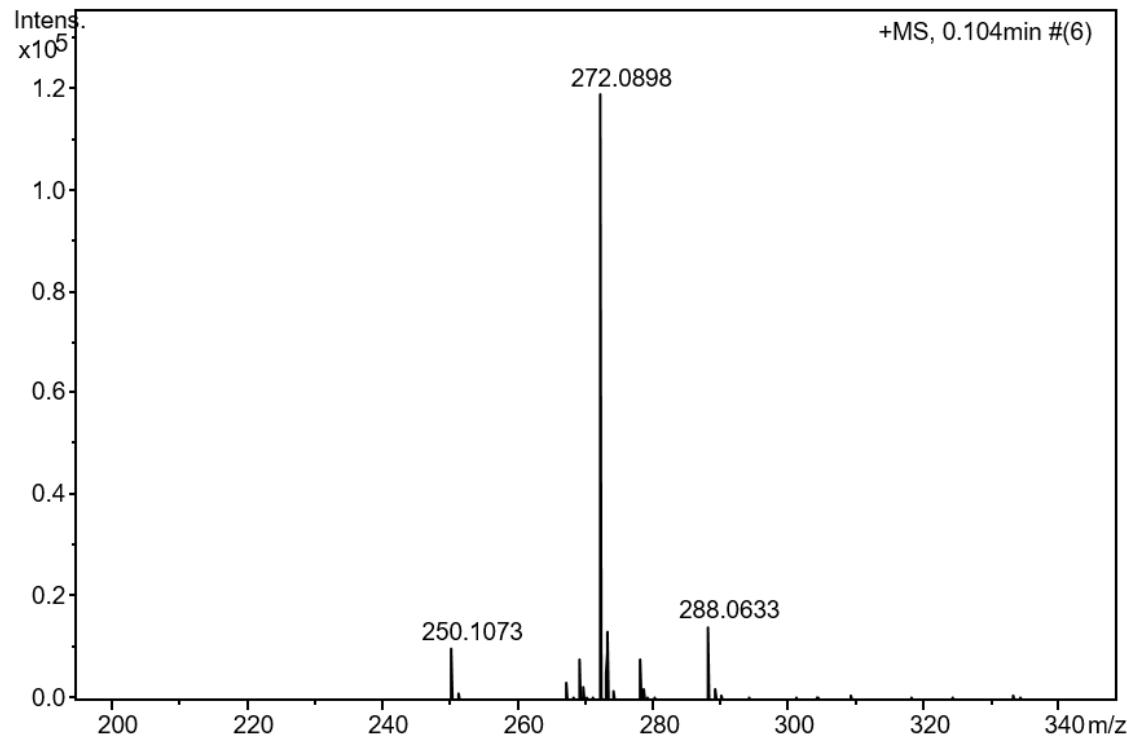


Figure S27. HRESIMS spectrum of cordylactam (**4**)

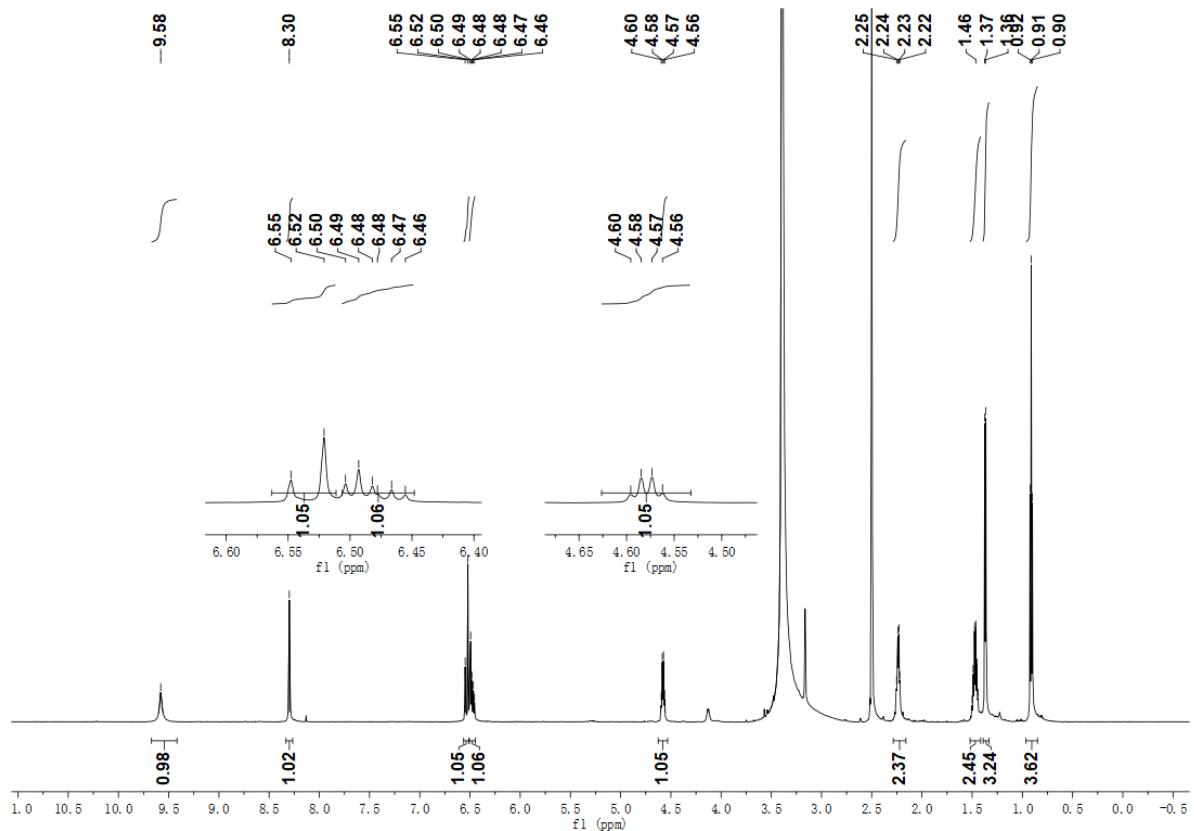


Figure S28. ^1H NMR spectrum of cordylactam (**4**)

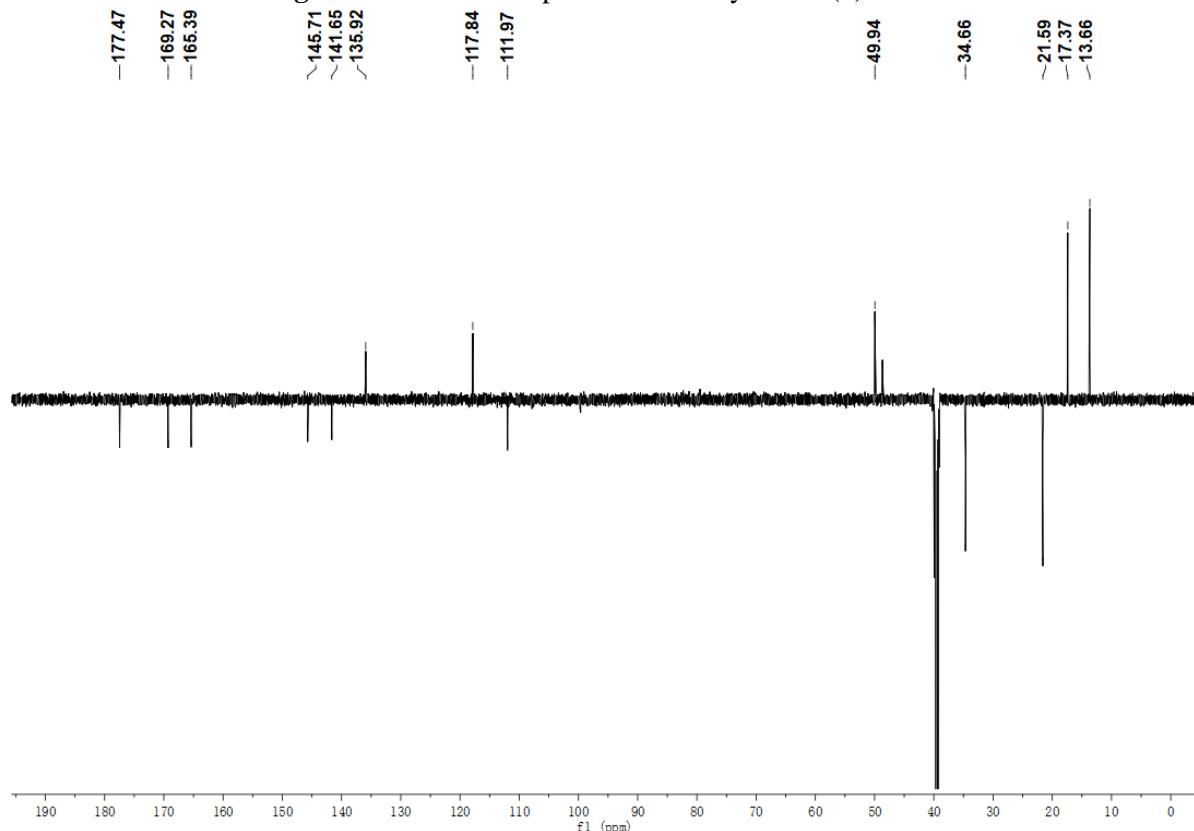


Figure S29. DEPTQ ^{13}C NMR spectrum of cordylactam (**4**)

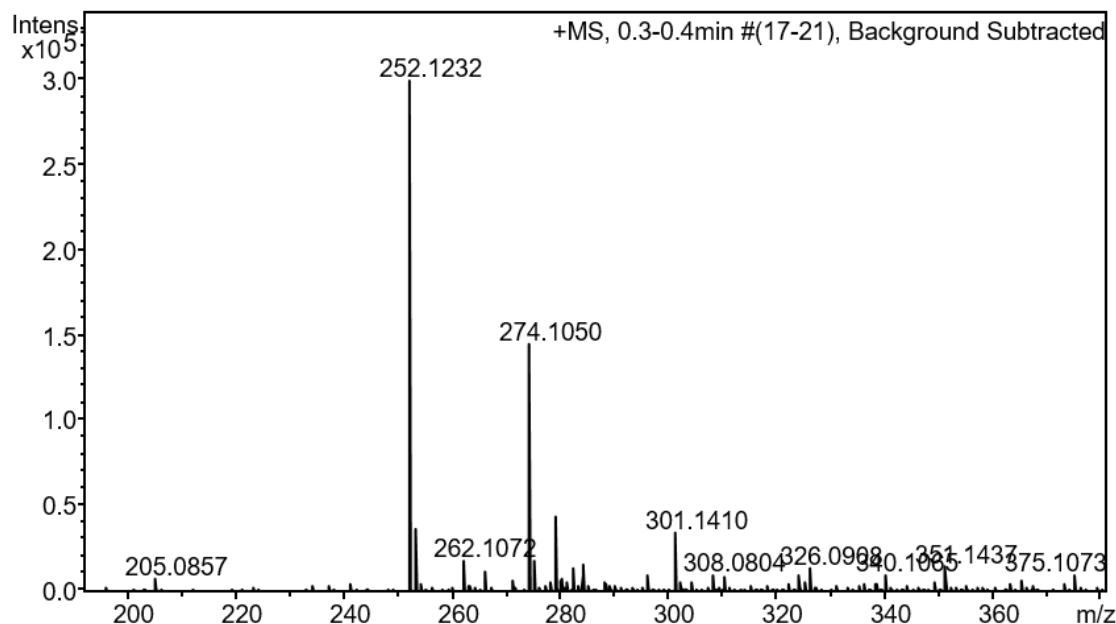


Figure S30. HRESIMS spectrum of pyranterrone D (**5**)

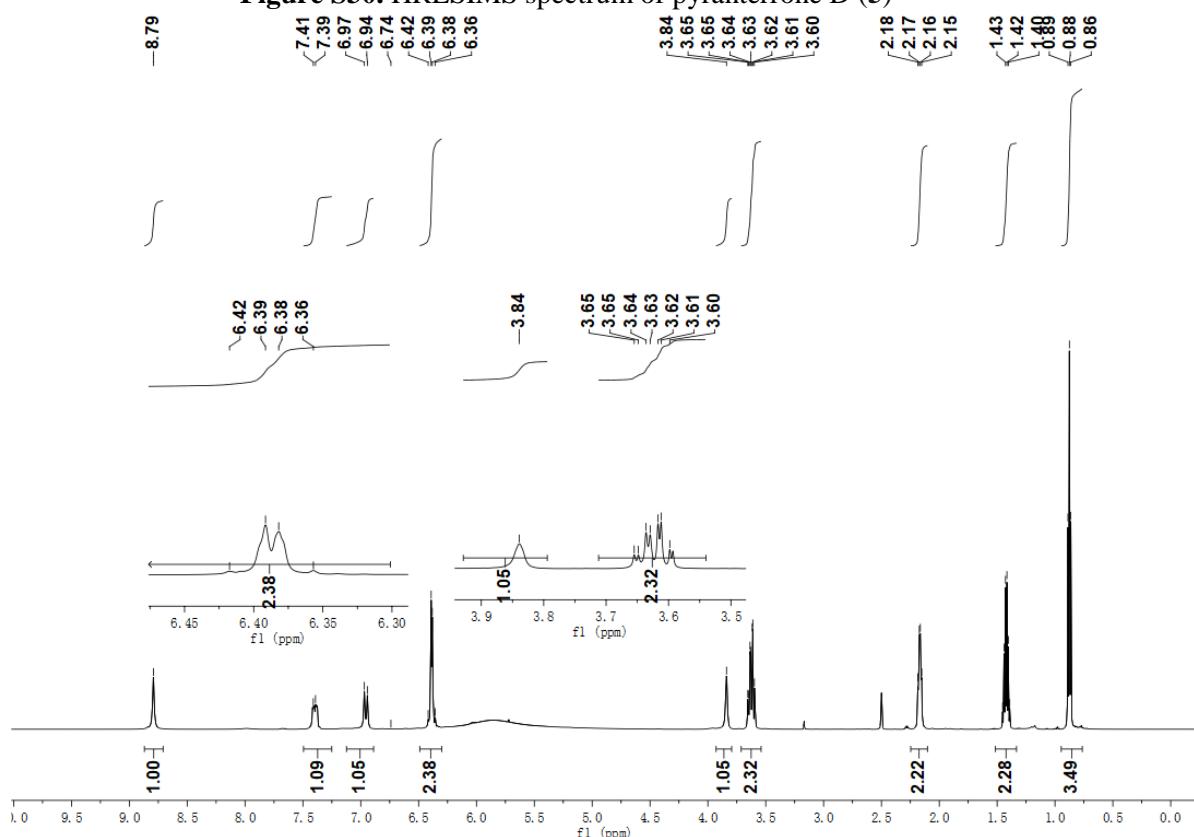


Figure S31. ^1H NMR spectrum (DMSO- d_6) of pyranterrone D (**5**)

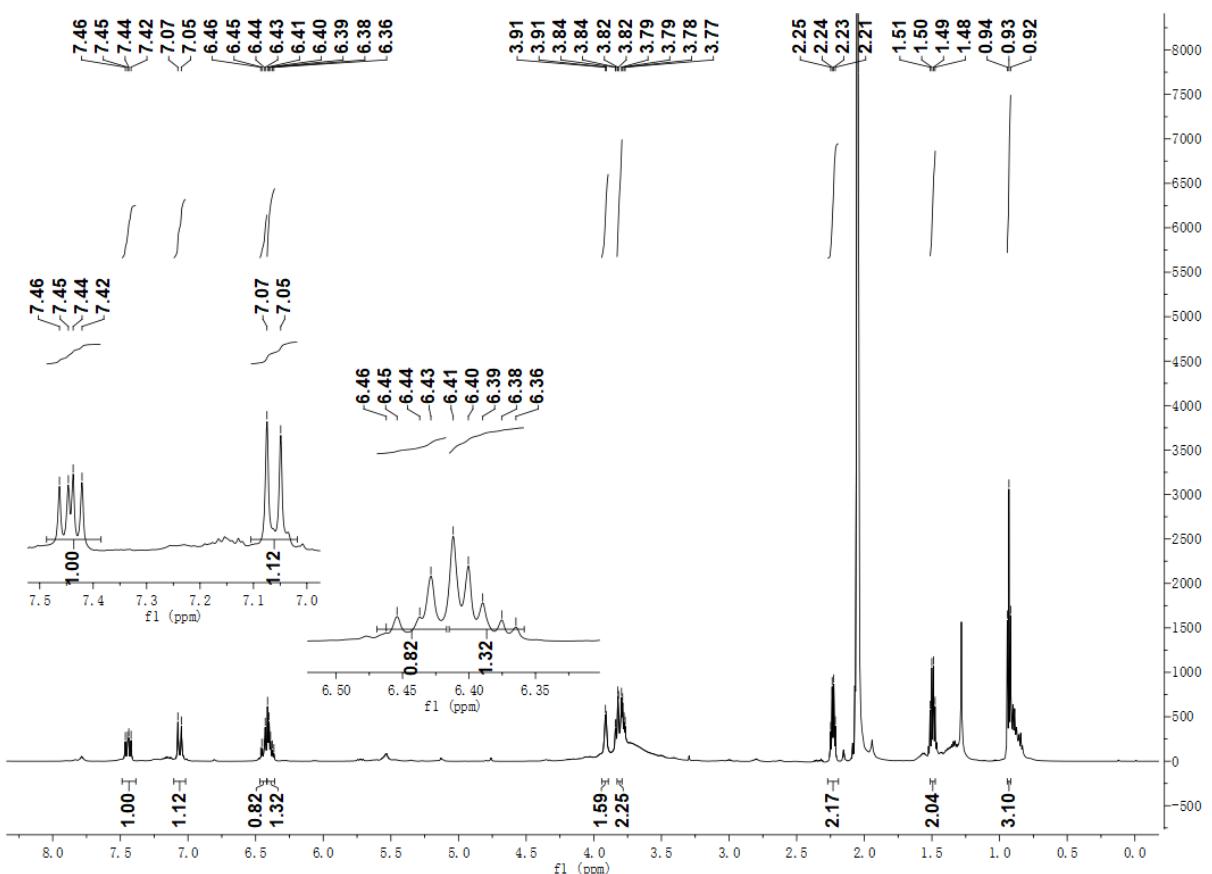


Figure S32. ^1H NMR spectrum (acetone- d_6) of pyranterrone D (**5**)

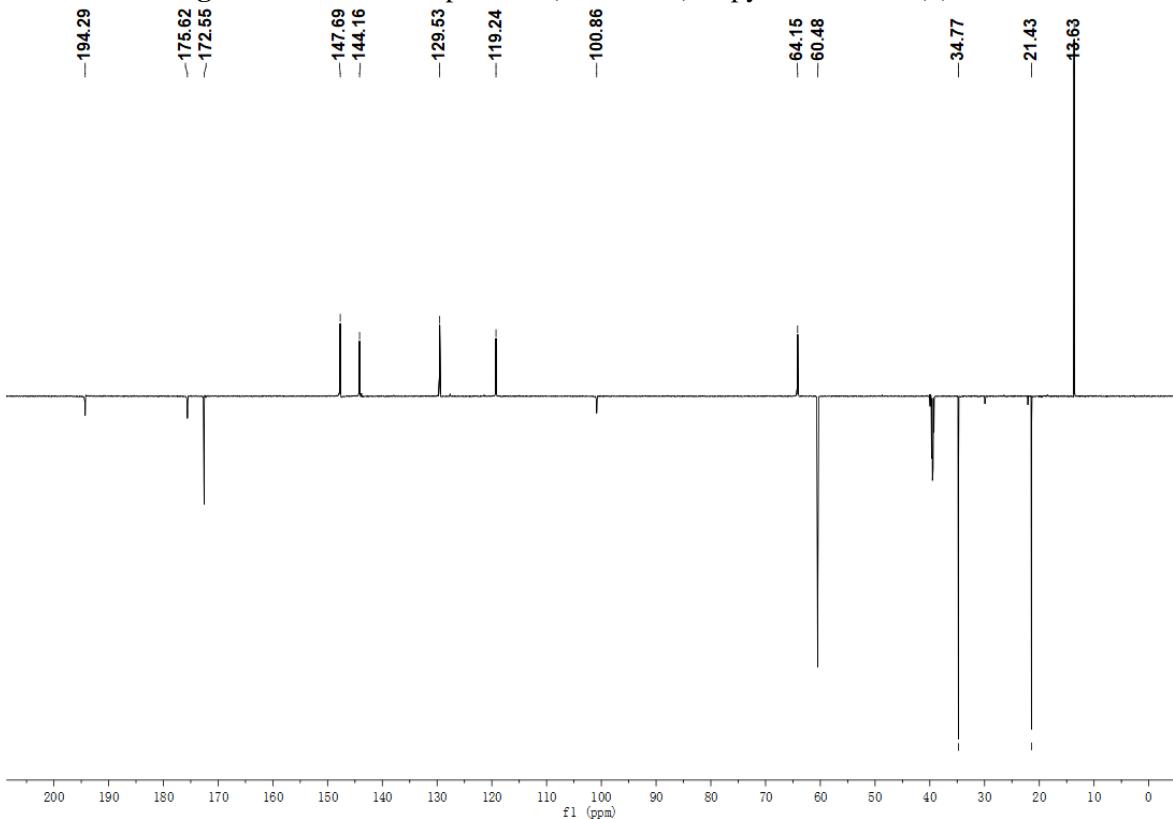


Figure S33. DEPTQ ^{13}C NMR spectrum of pyranterrone D (**5**)

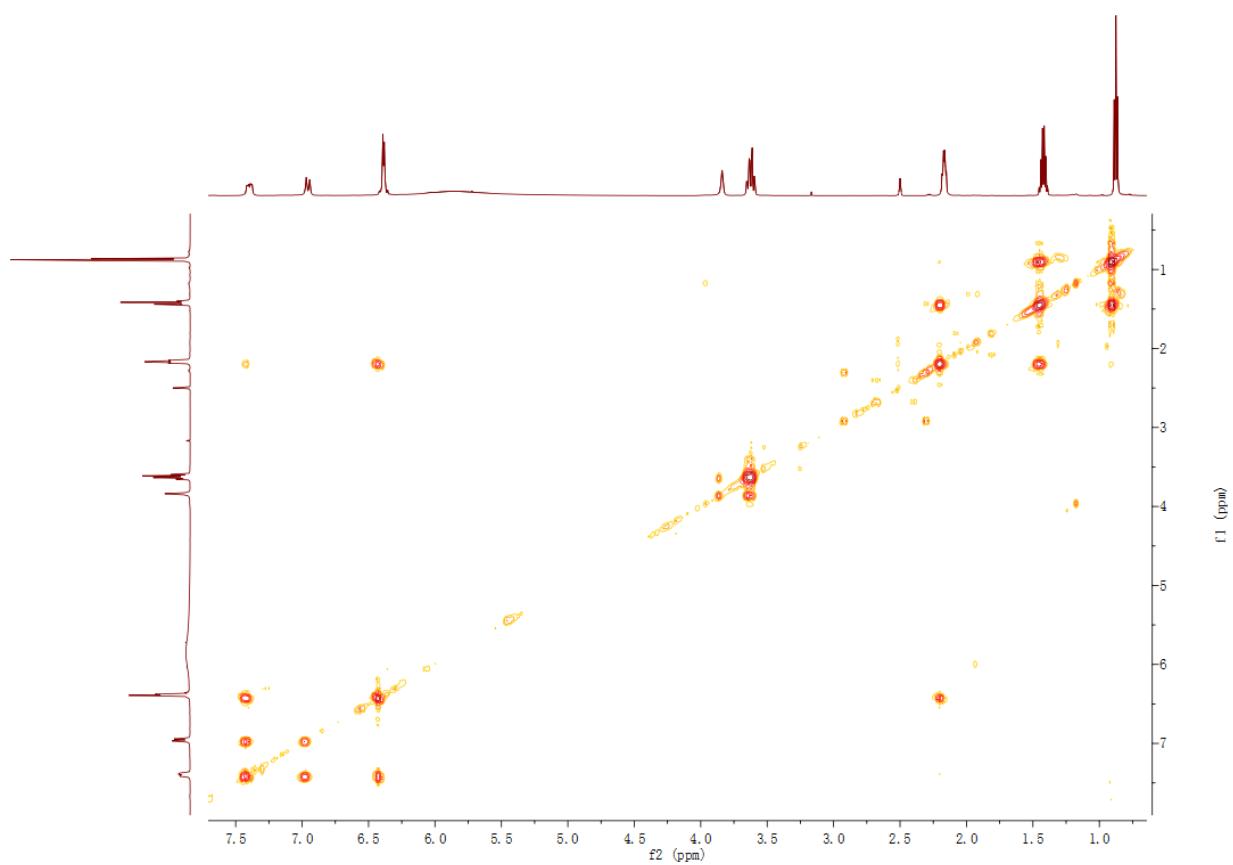


Figure S34. ^1H - ^1H COSY spectrum of pyranterrone D (5)

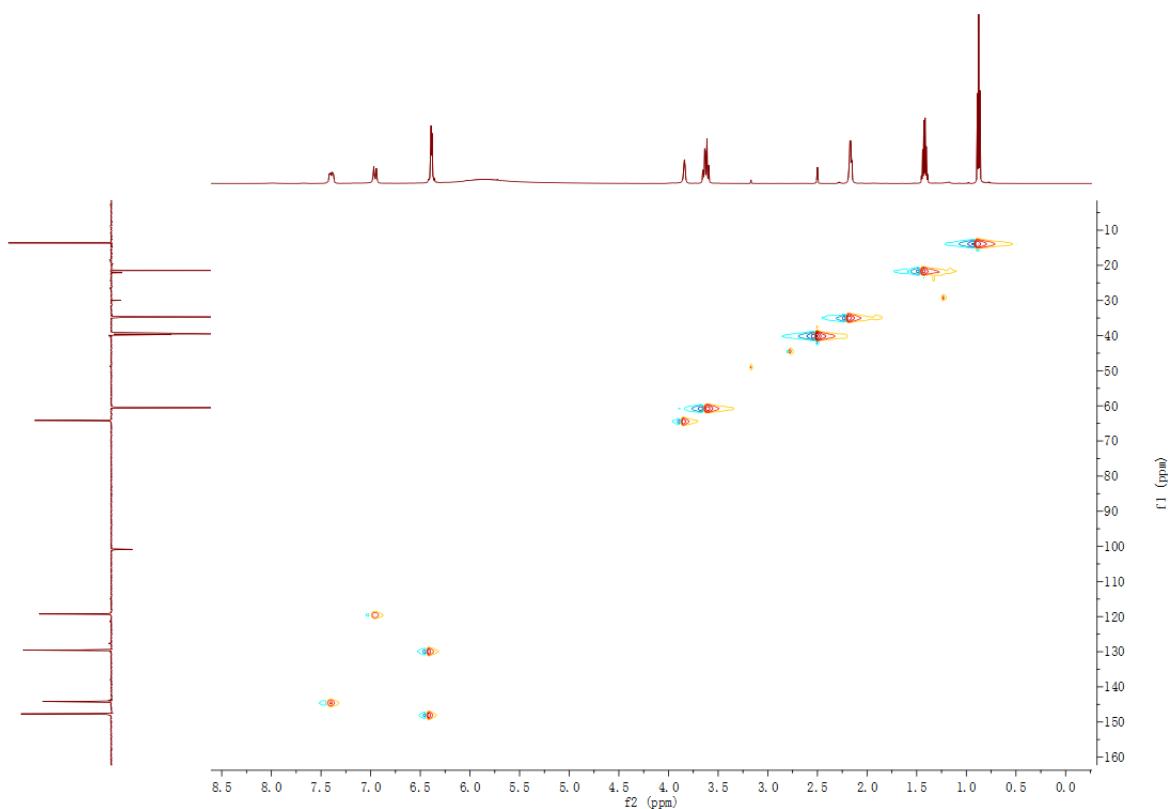


Figure S35. HSQC spectrum of pyranterrone D (5)

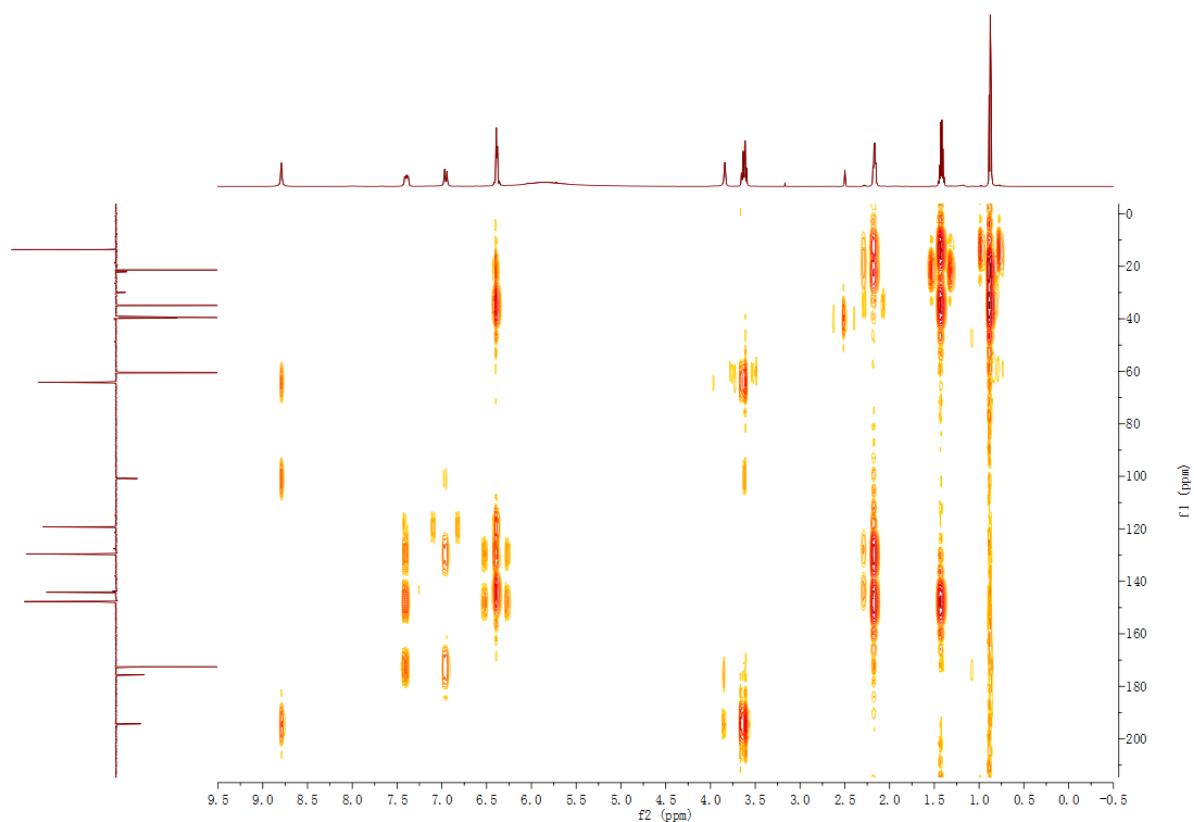


Figure S36. HMBC spectrum of pyranterrone D (**5**)

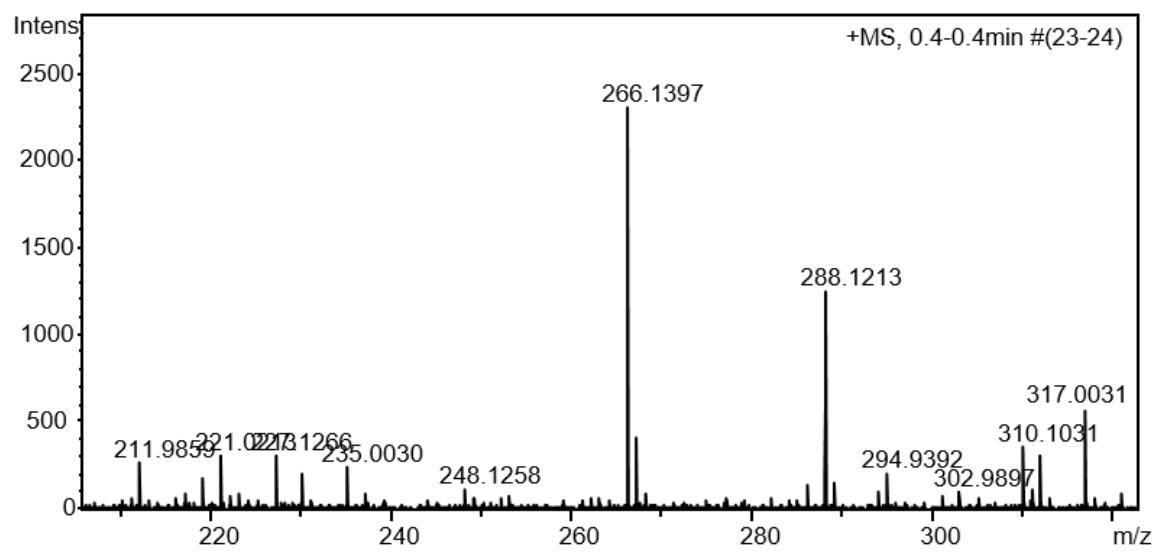


Figure S37. HRESIMS spectrum of pyranterrone E (**6**)

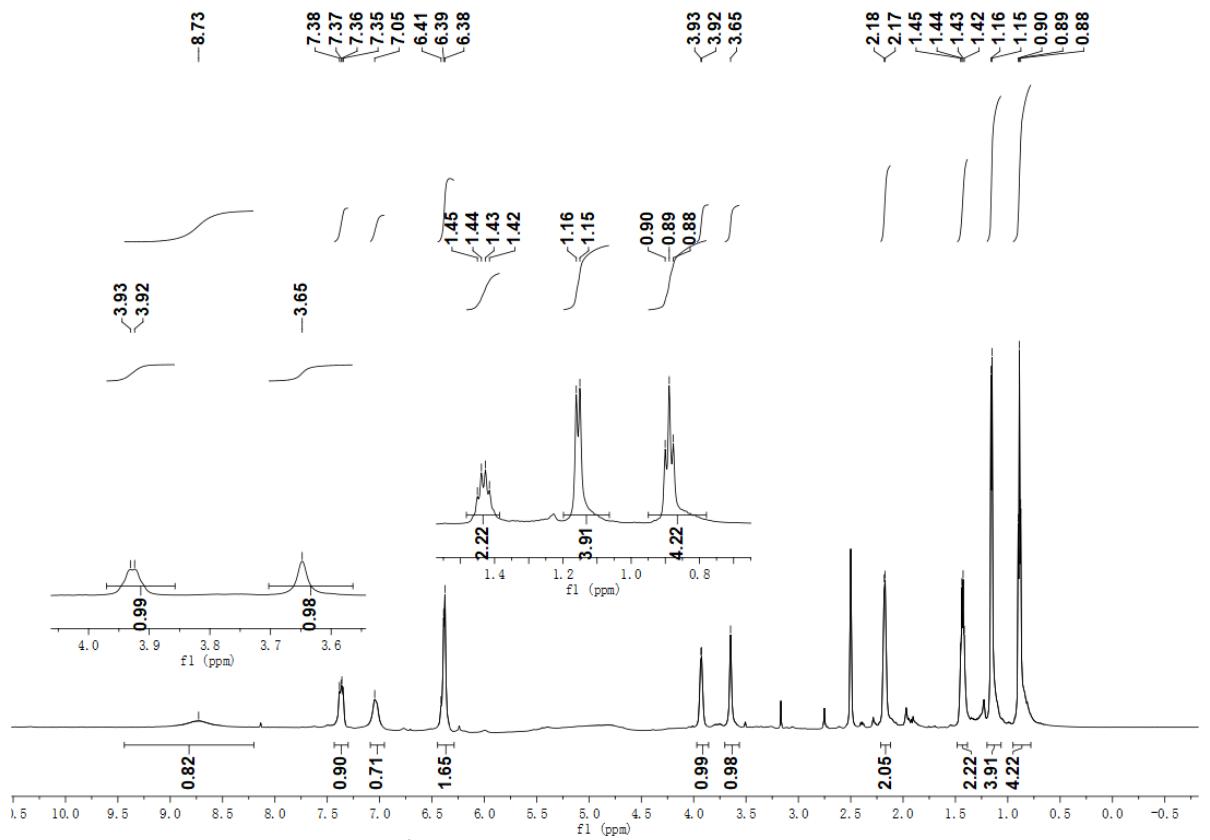


Figure S38. ¹H NMR spectrum of pyranterrone E (6)

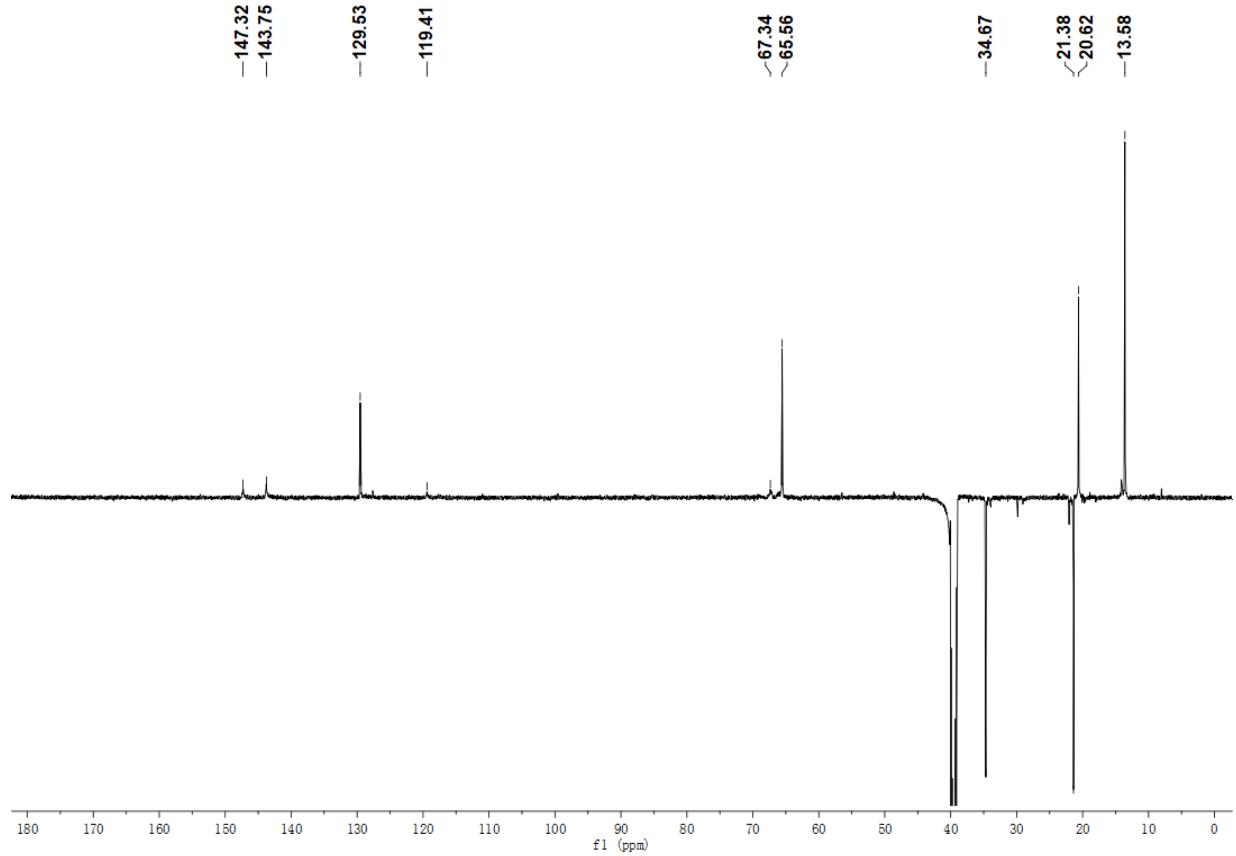


Figure S39. DEPTQ¹³C NMR spectrum of pyranterrone E (6)

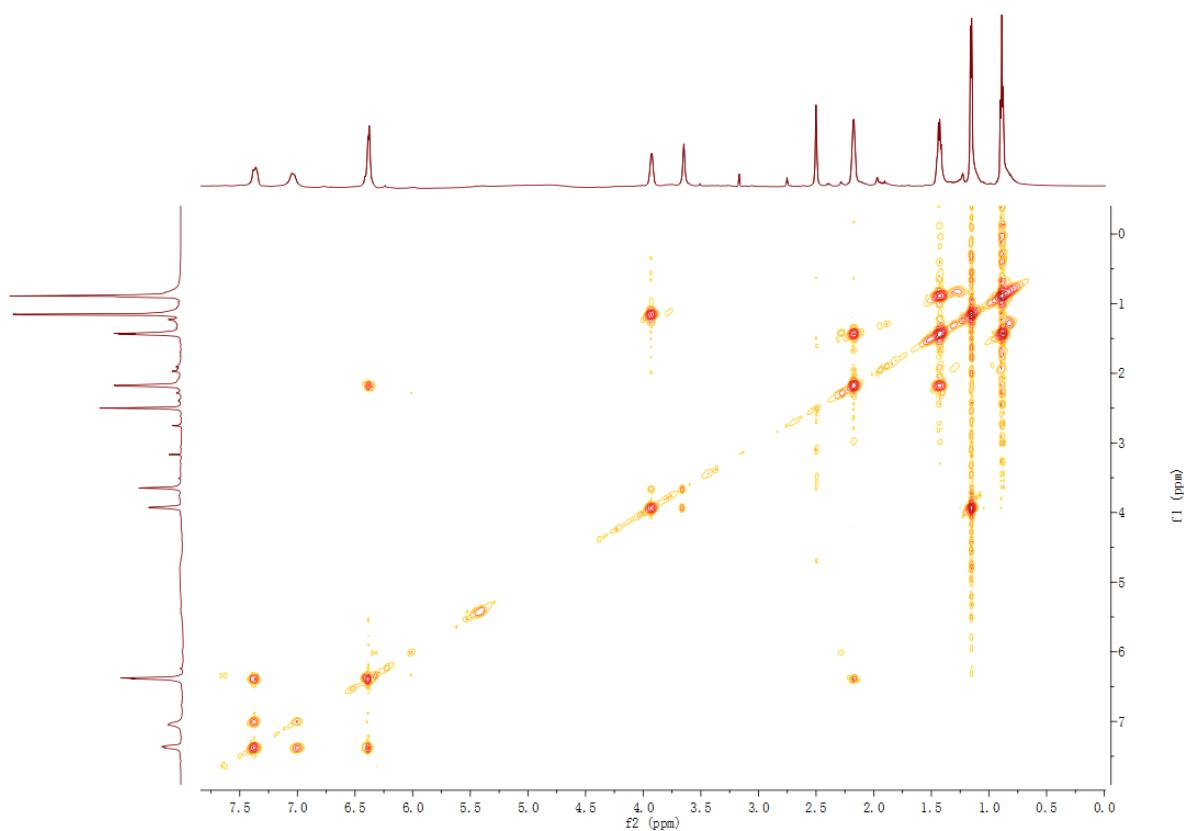


Figure S40. ^1H - ^1H COSY spectrum of pyranterrone E (6)

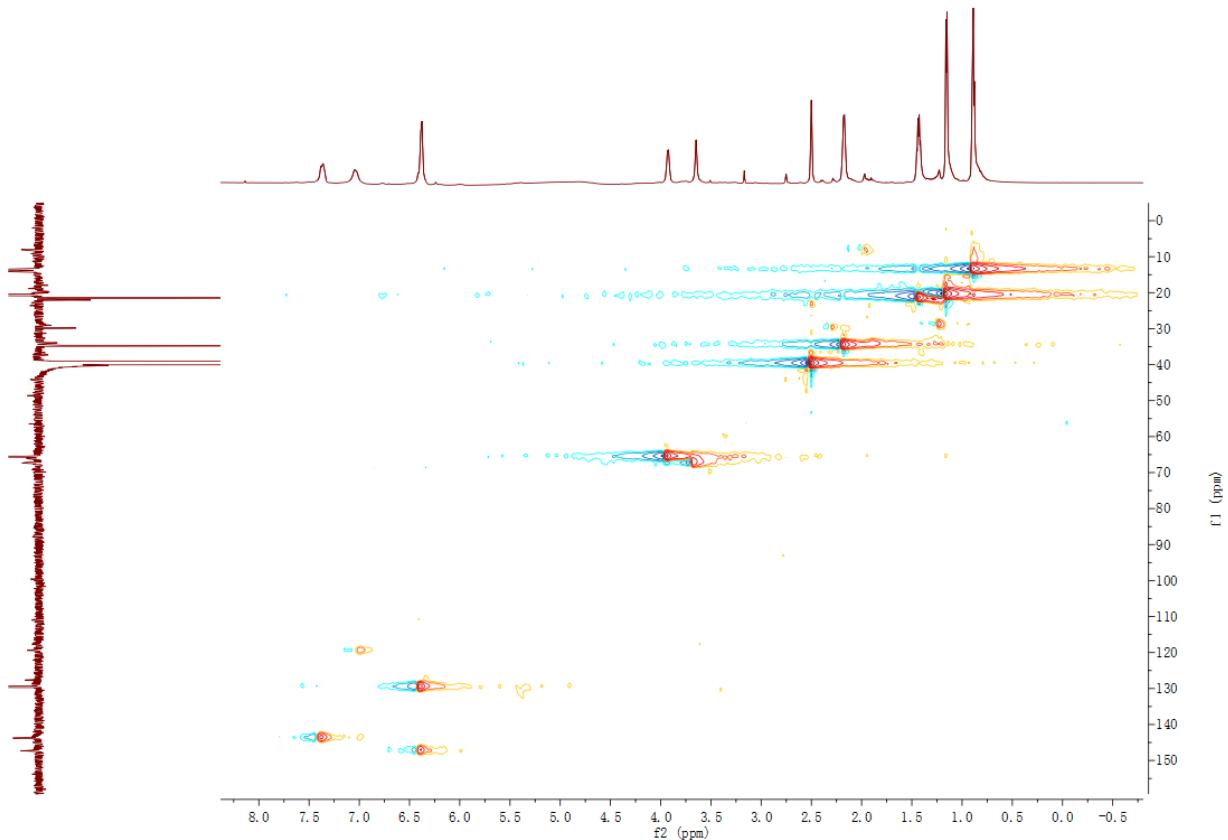


Figure S41. HSQC spectrum of pyranterrone E (6)

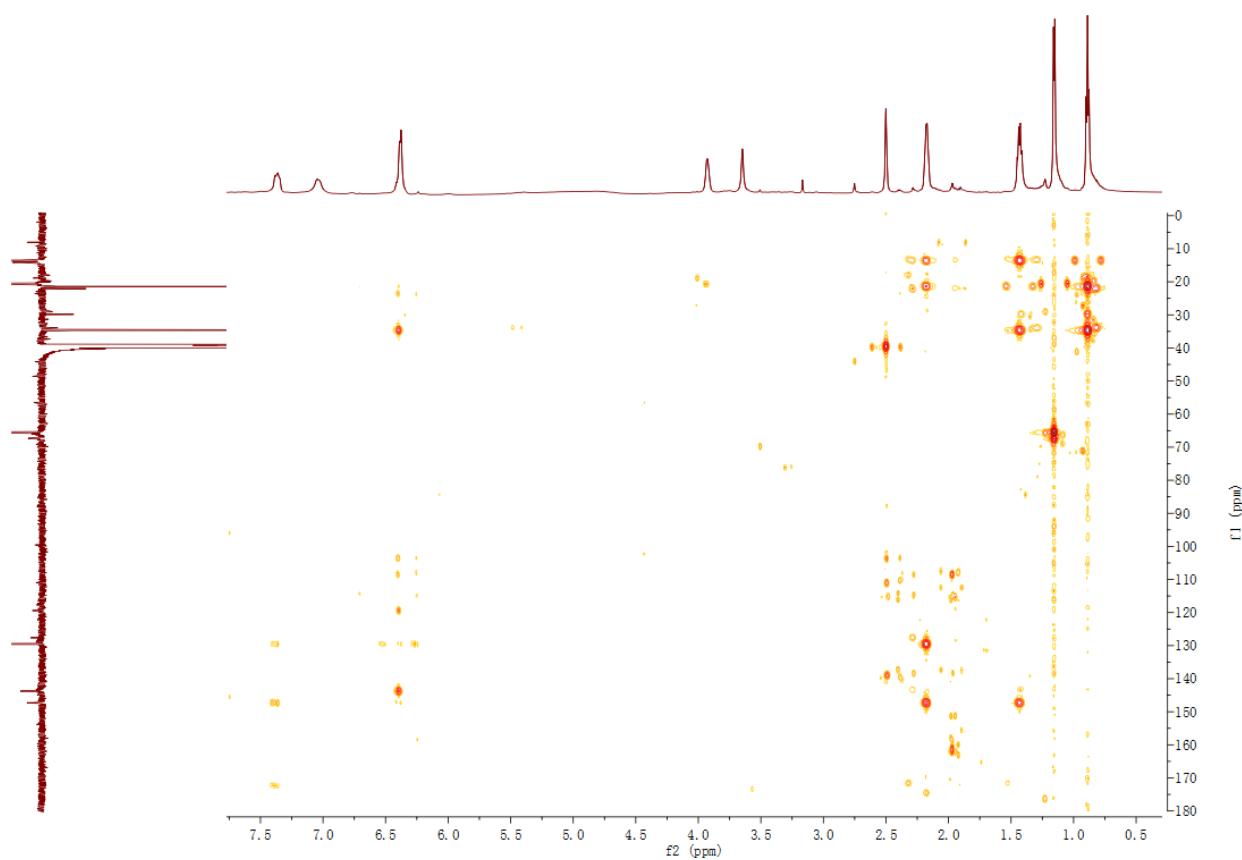


Figure S42. HMBC spectrum of pyranterrone E (**6**)

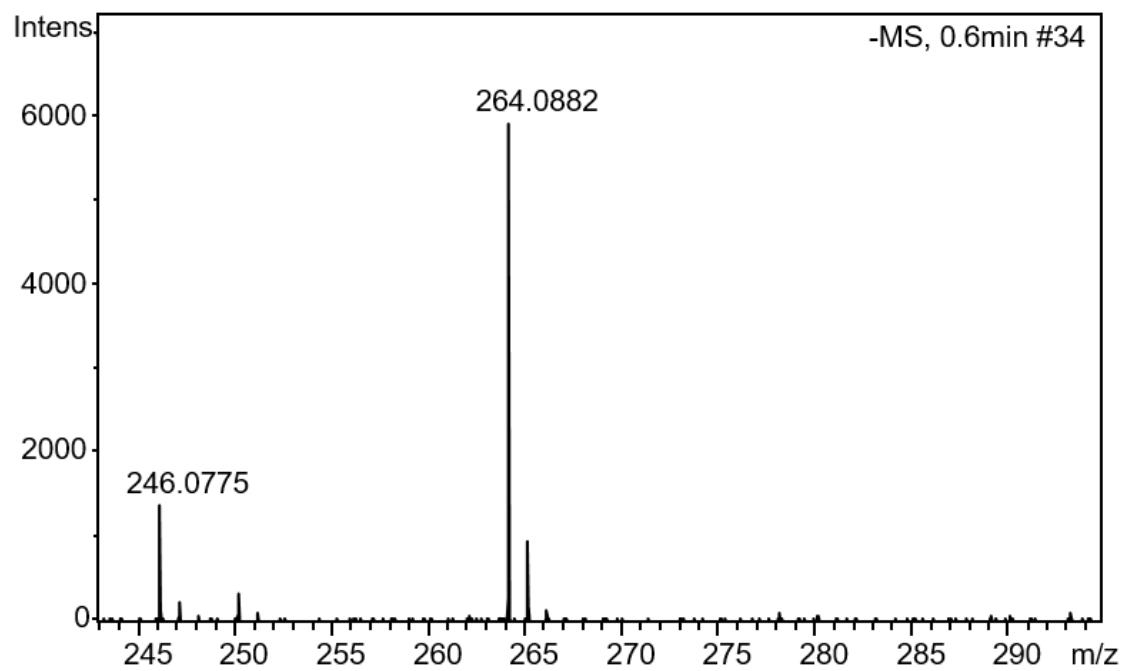


Figure S43. HRESIMS spectrum of pyranterrone F (**7**)

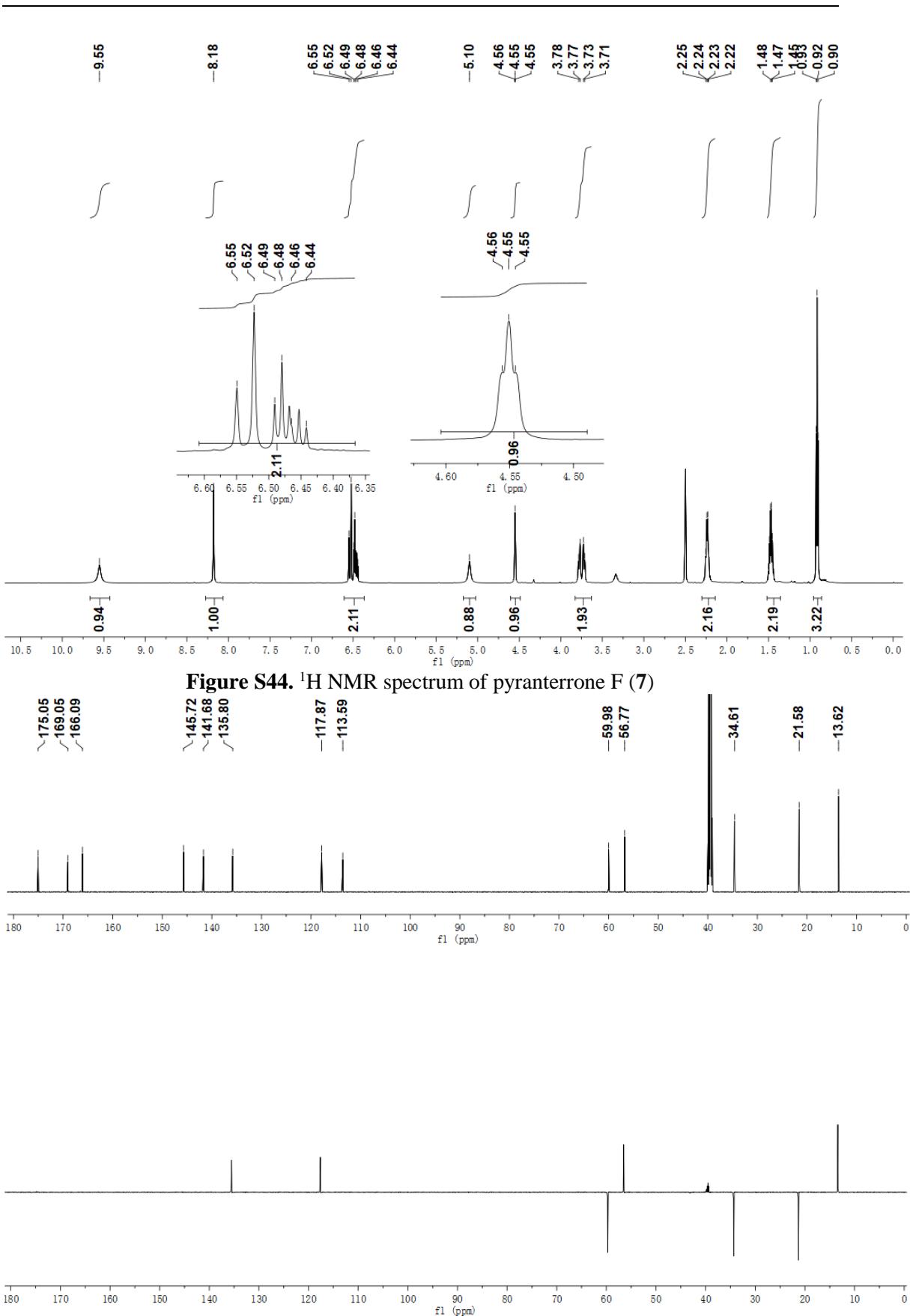


Figure S44. ¹H NMR spectrum of pyranterrone F (7)

Figure S45. ¹³C NMR and DEPT spectra of pyranterrone F (7)

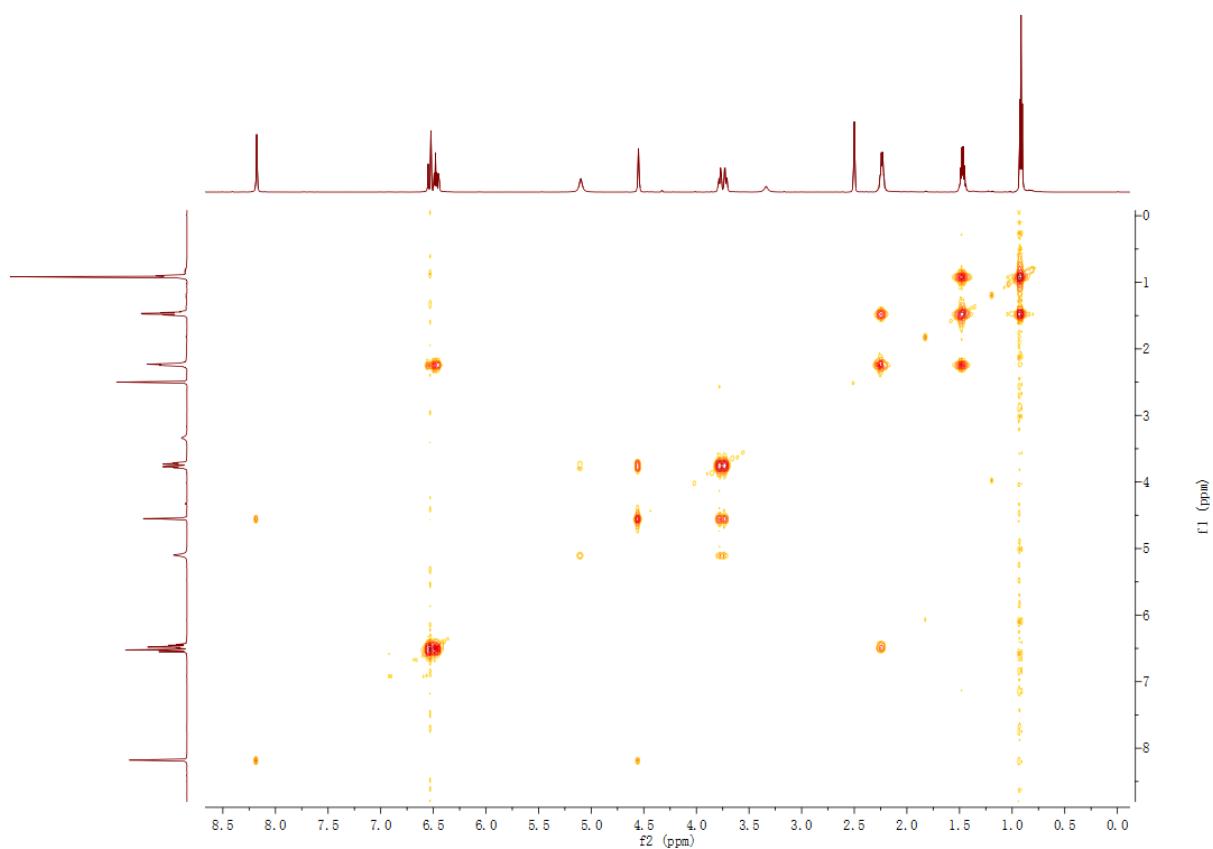


Figure S46. ¹H-¹H COSY spectrum of pyranterrone F (7)

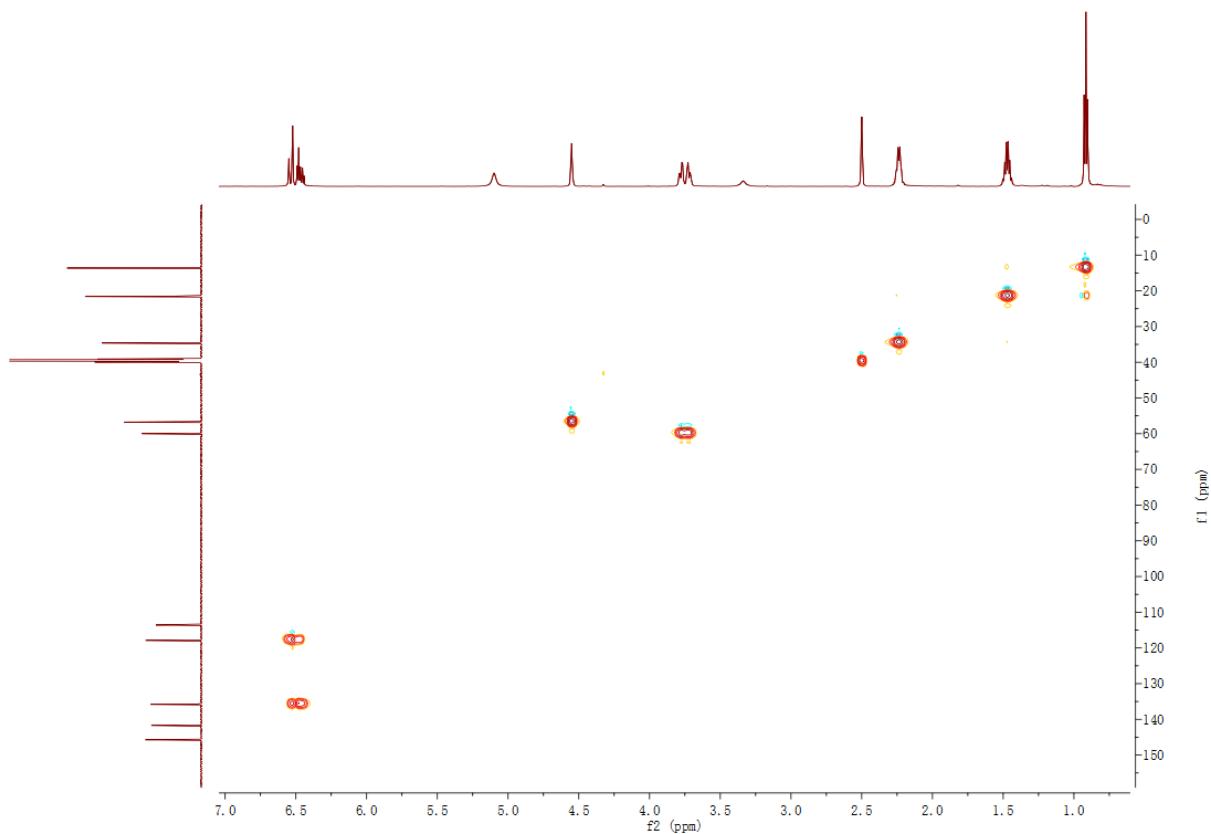


Figure S47. HSQC spectrum of pyranterrone F (7)

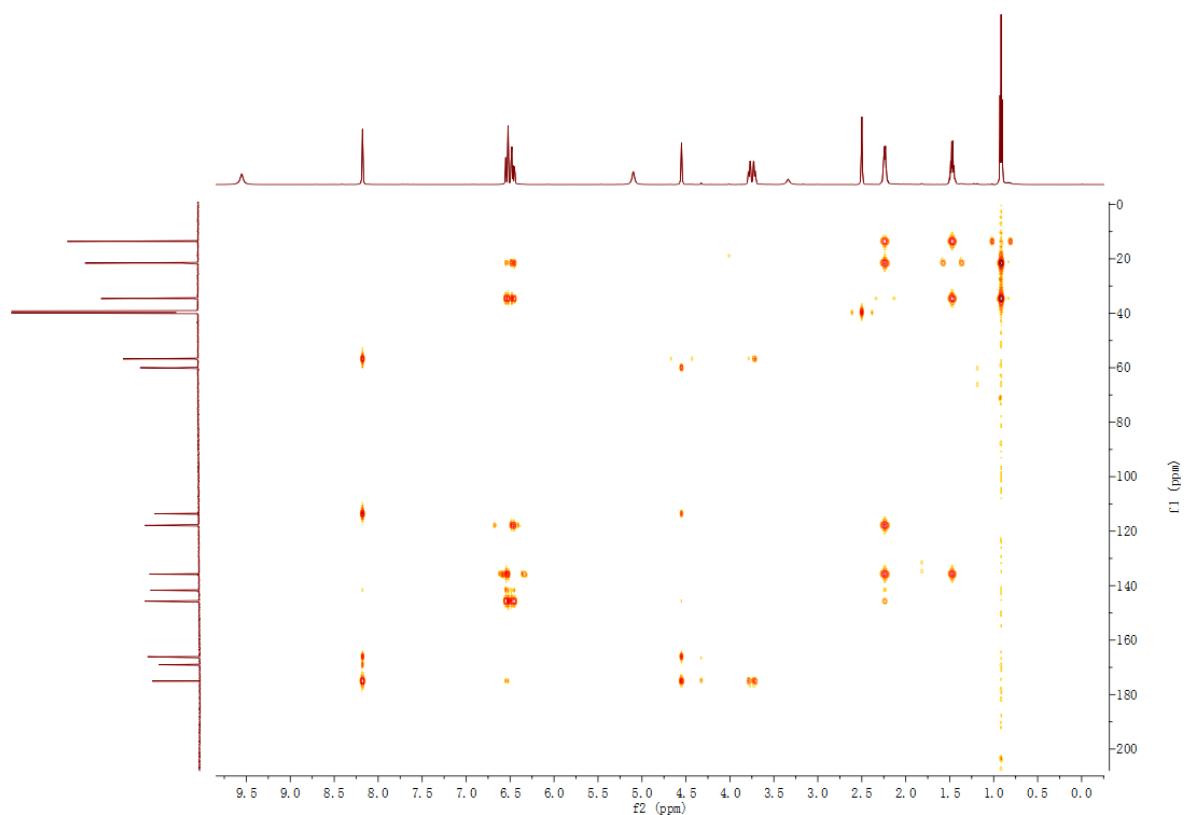


Figure S48. HMBC spectrum of pyranterrone F (**7**)

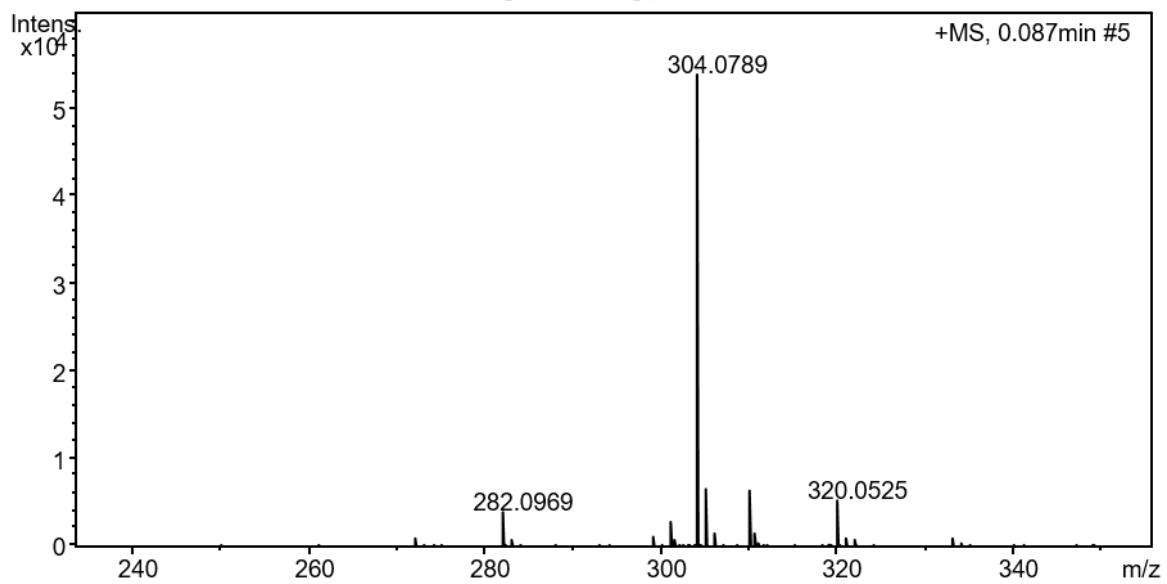


Figure S49. HRESIMS spectrum of pyranterrone G (**8**)

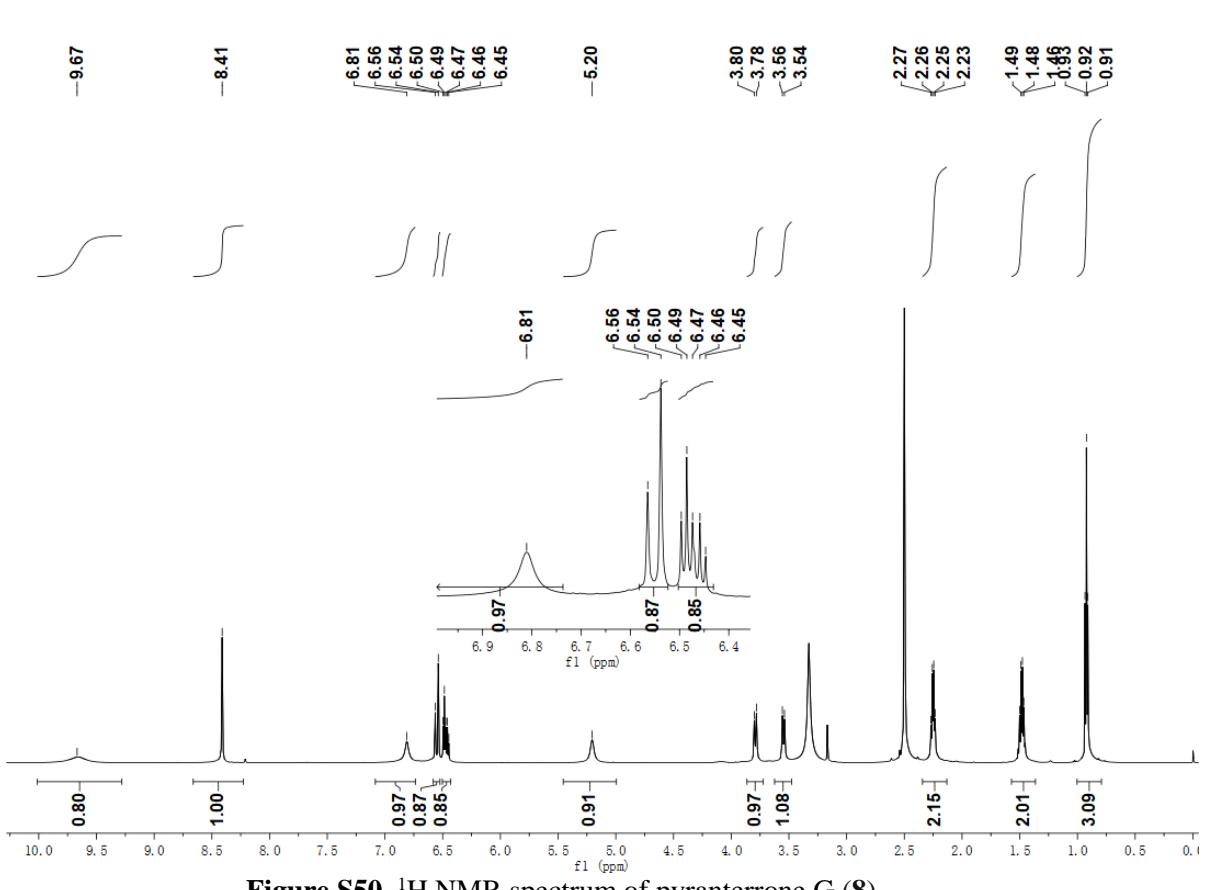


Figure S50. ^1H NMR spectrum of pyranterrone G (8)

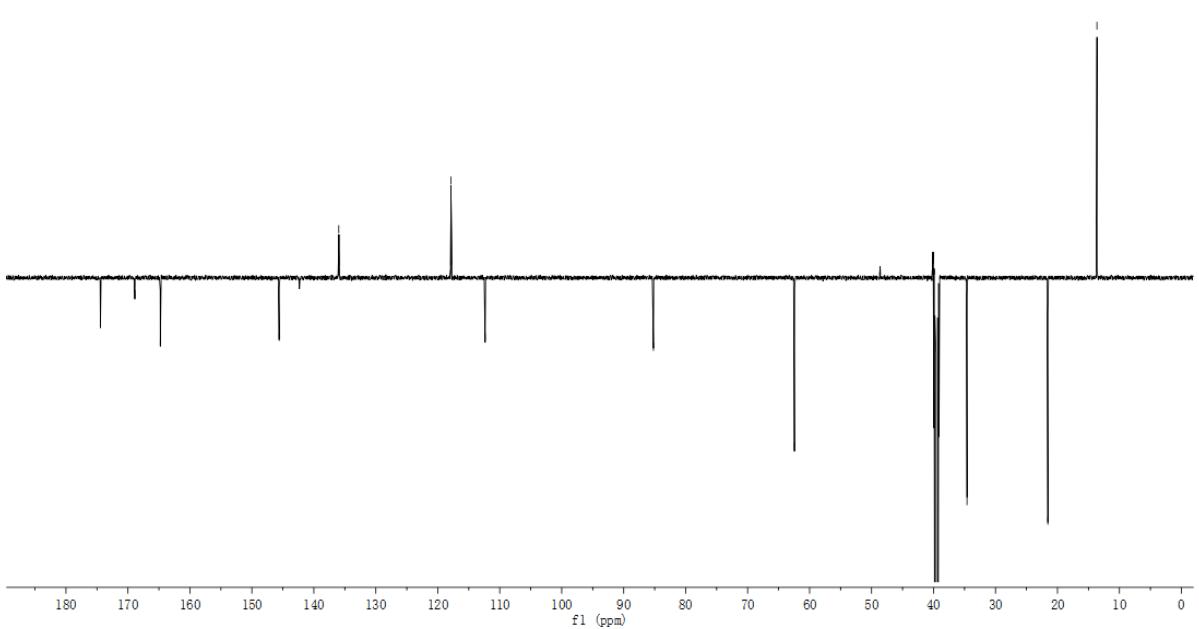


Figure S51. DEPTQ ^{13}C NMR spectrum of pyranterrone G (8)

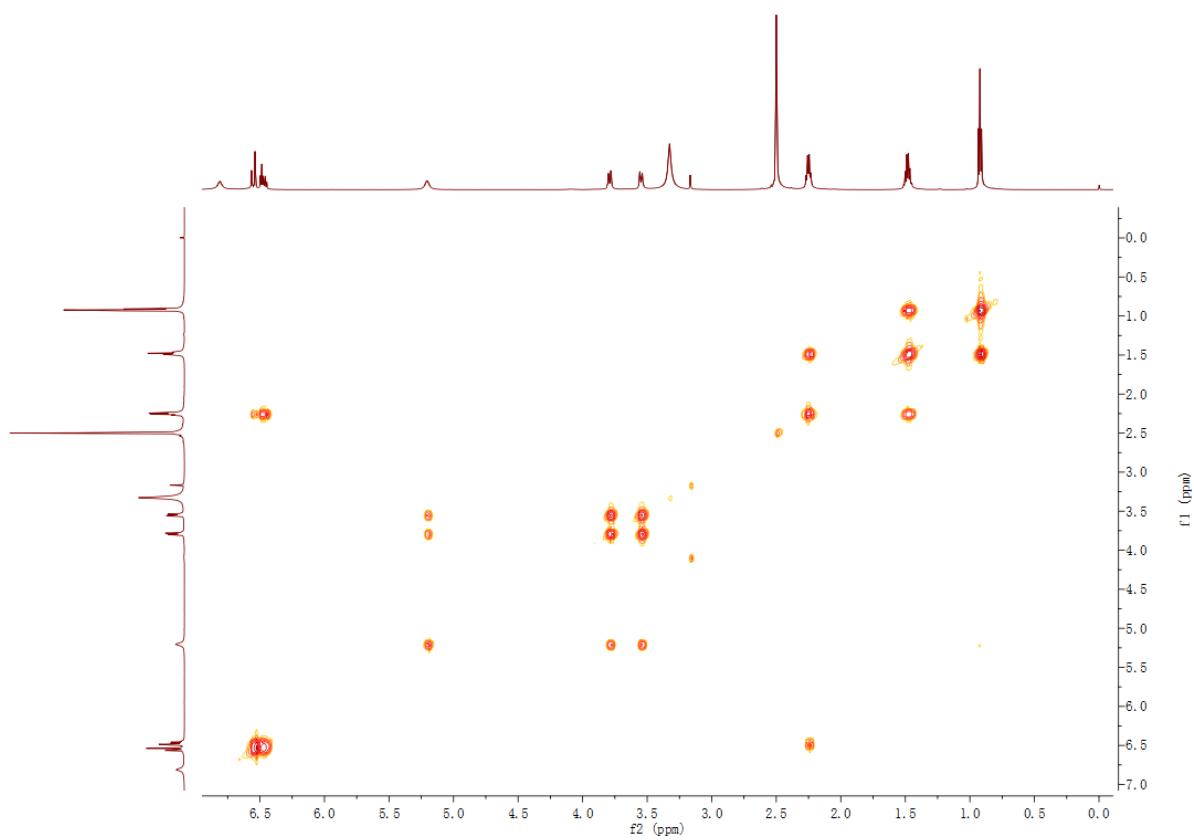


Figure S52. ^1H - ^1H COSY spectrum of pyranterrone G (8)

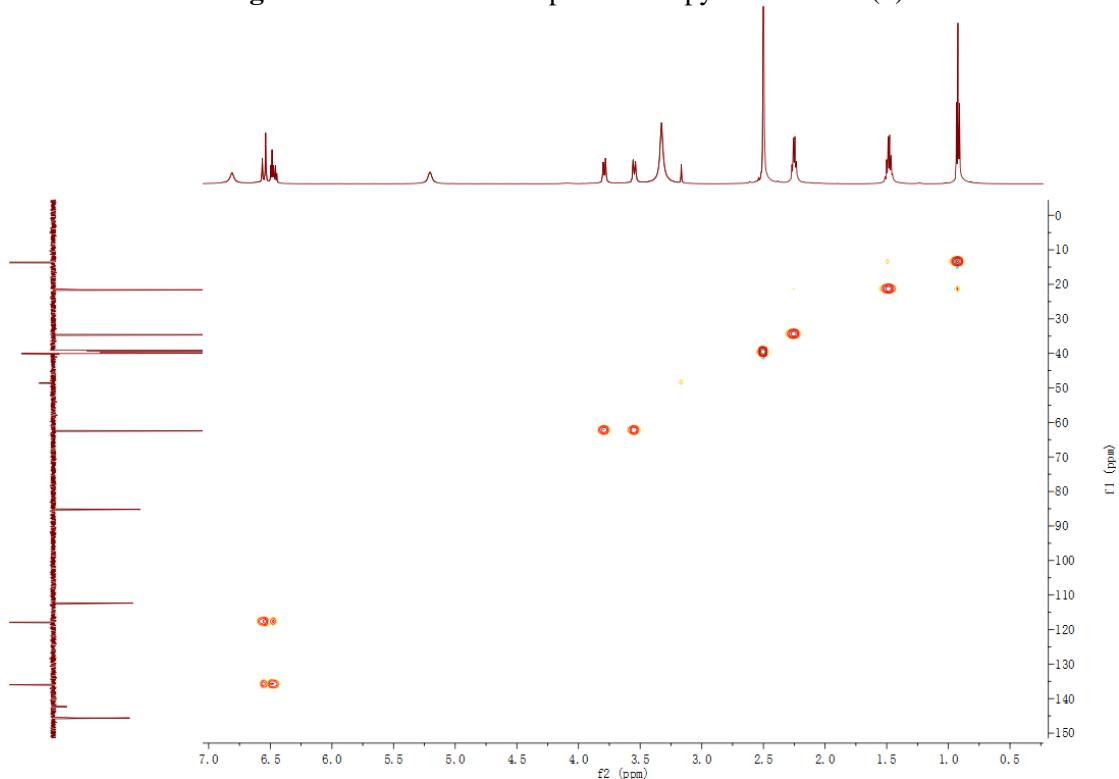


Figure S53. HSQC spectrum of pyranterrone G (8)

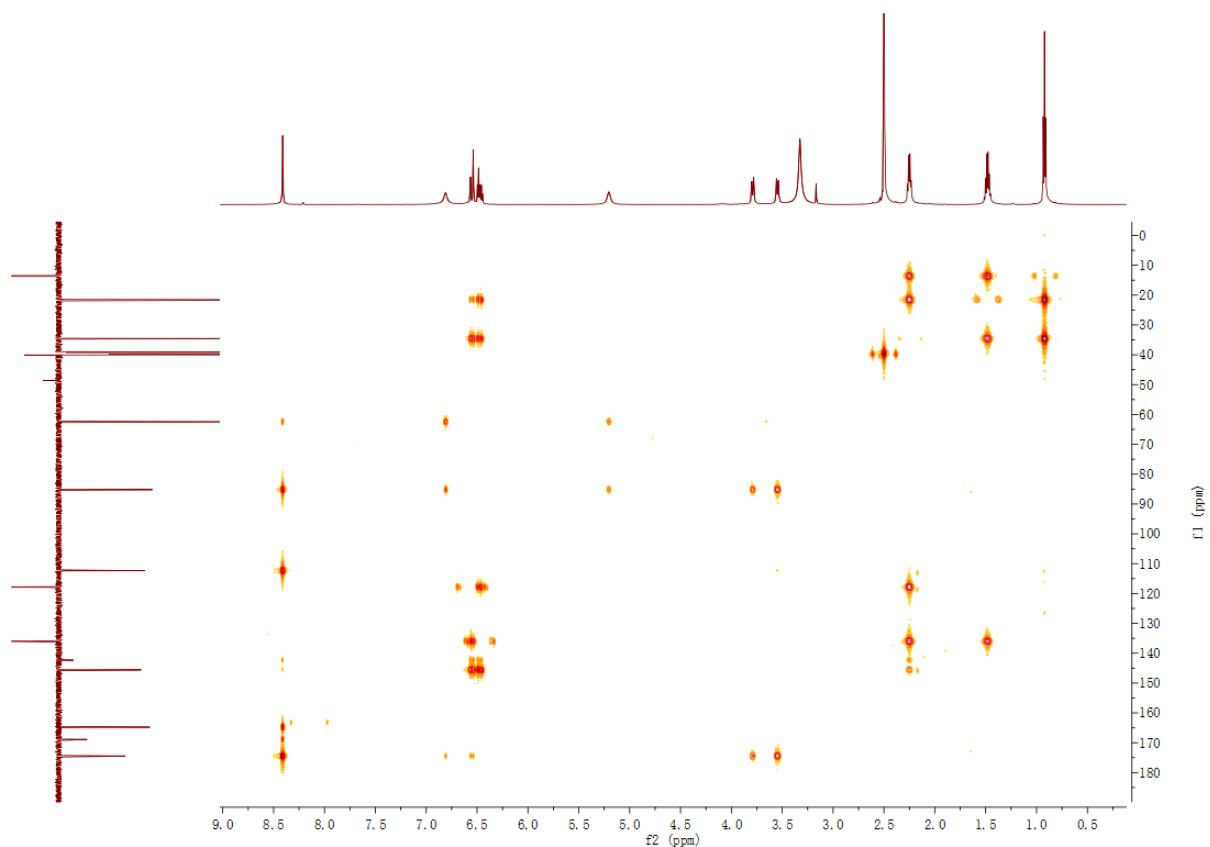


Figure S54. HMBC spectrum of pyranterrone G (**8**)

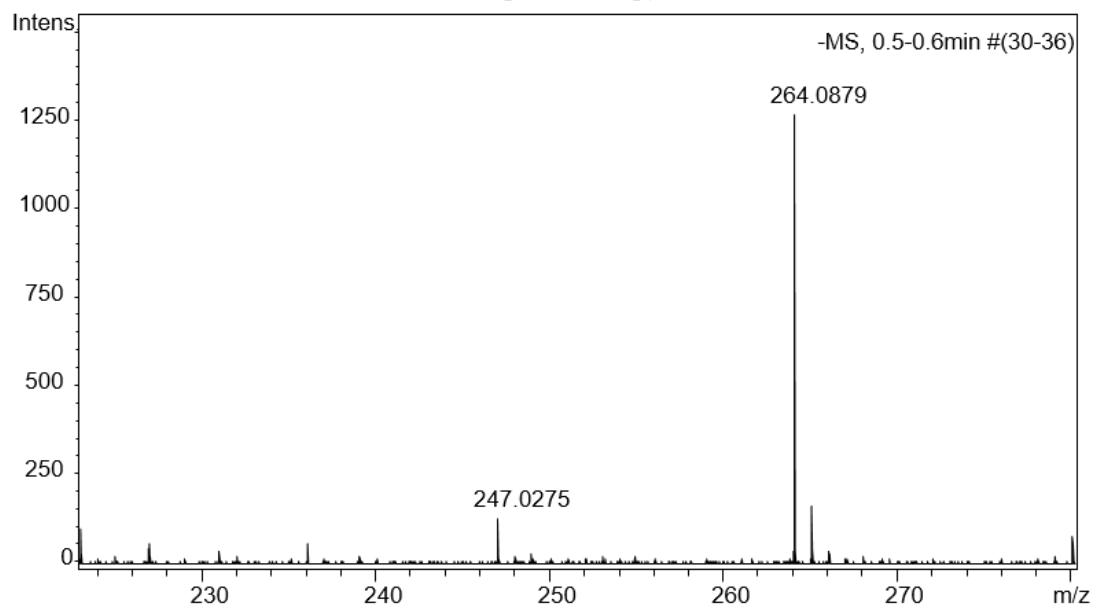


Figure S55. HRESIMS spectrum of 7-hydroxycordylactam (**9**)

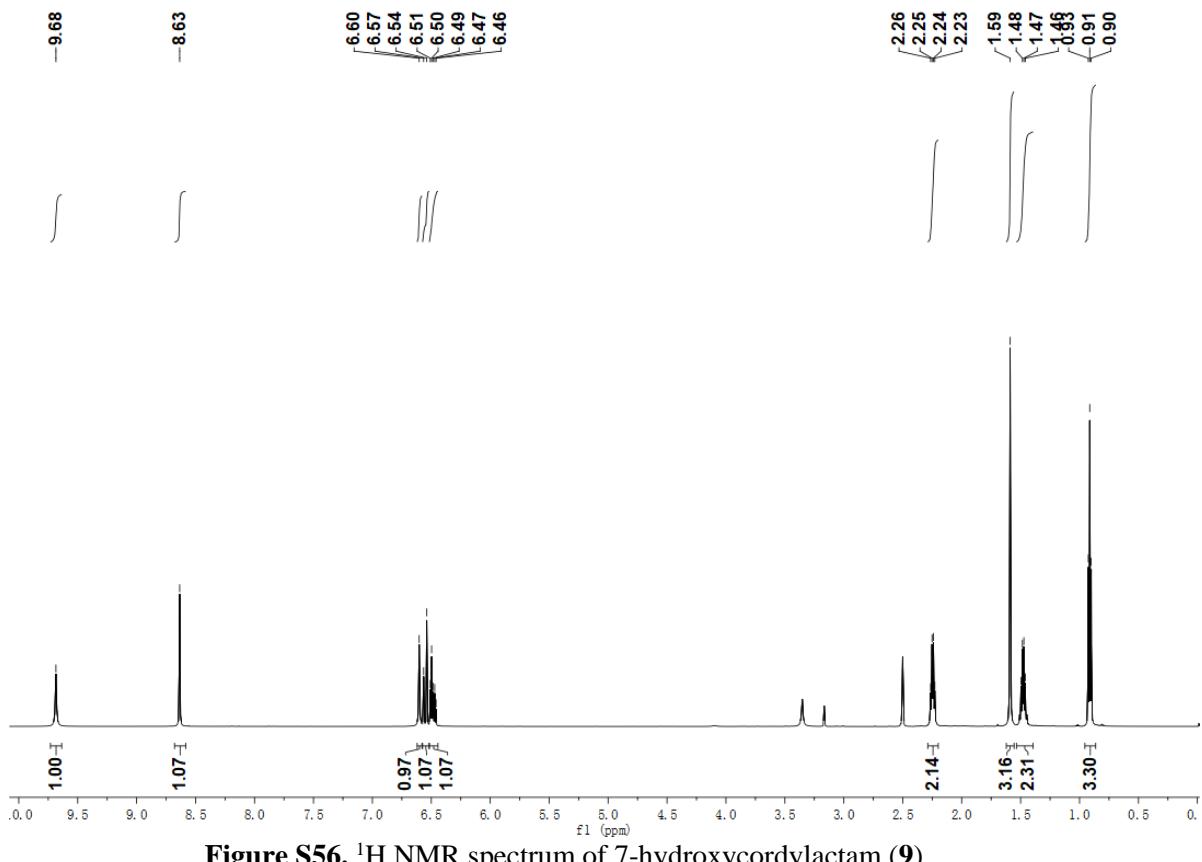


Figure S56. ^1H NMR spectrum of 7-hydroxycordylactam (**9**)

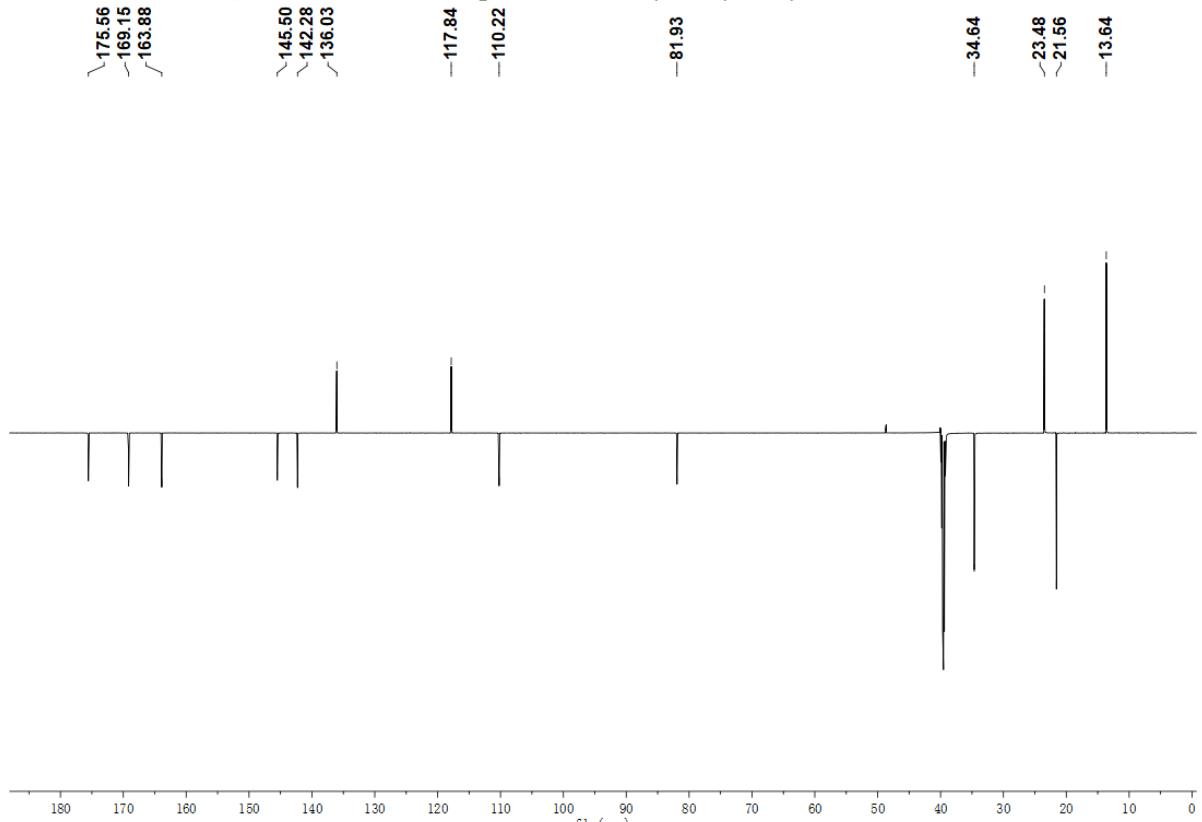


Figure S57. DEPTQ¹³C NMR spectrum of 7-hydroxycordylactam (**9**)

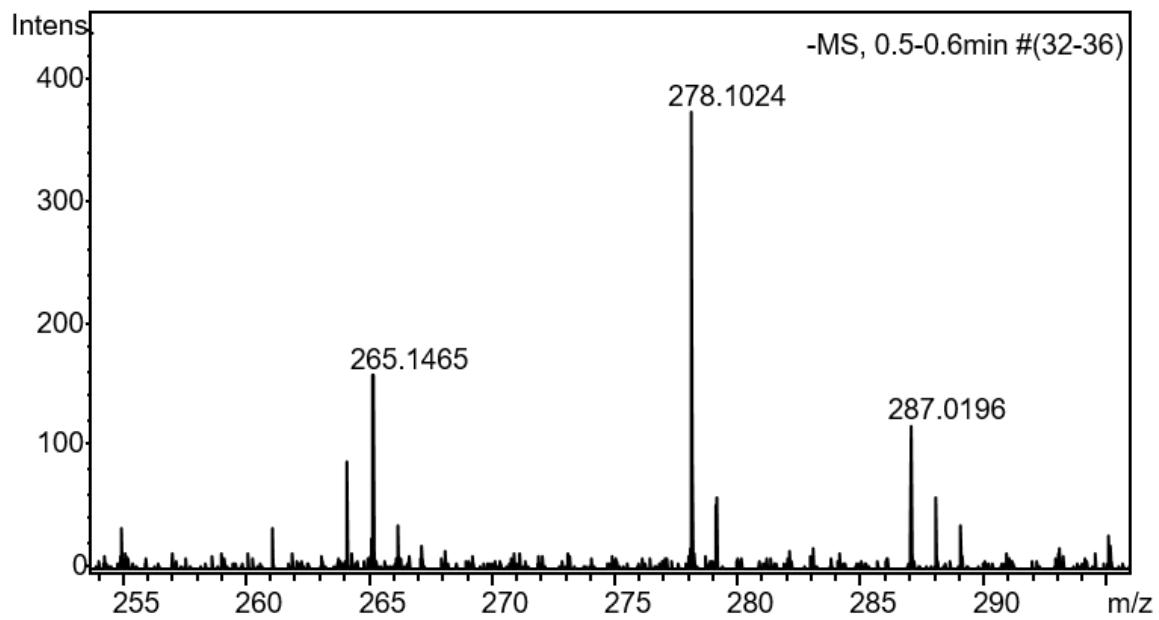


Figure S58. HRESIMS spectrum of pyranterrone H (**10**)

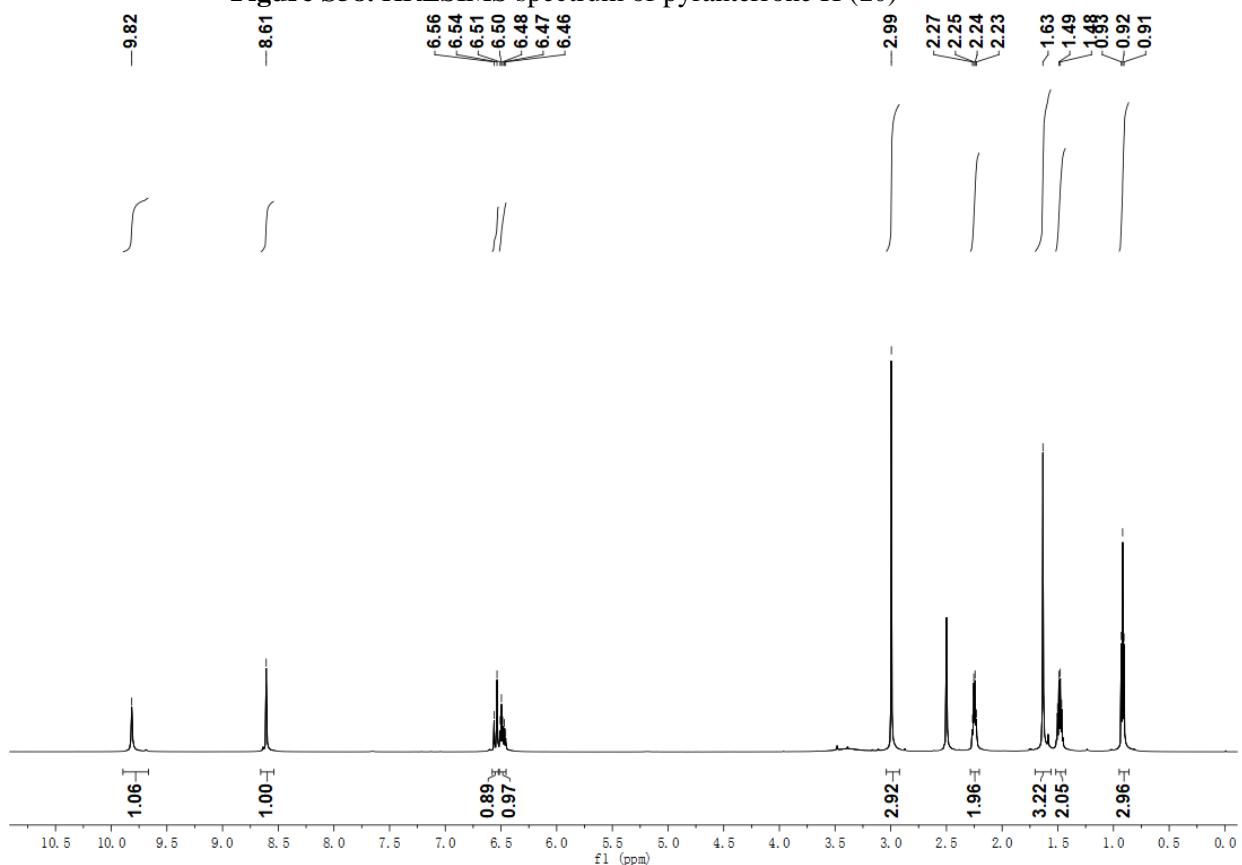


Figure S59. ^1H NMR spectrum of pyranterrone H (**10**)

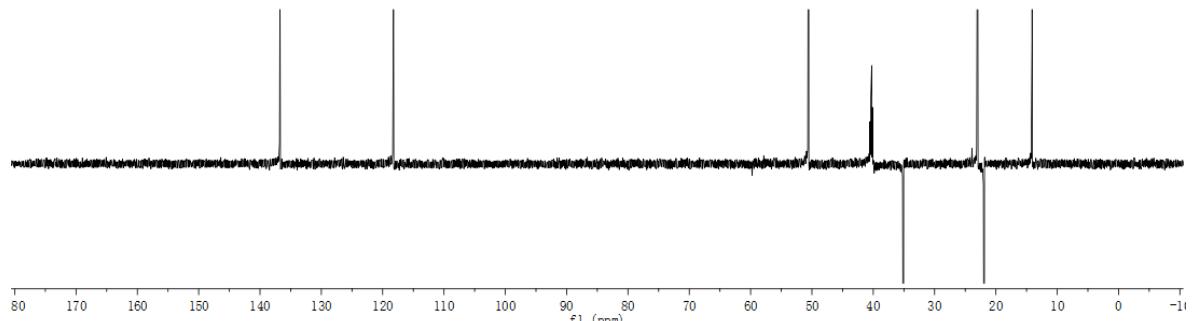
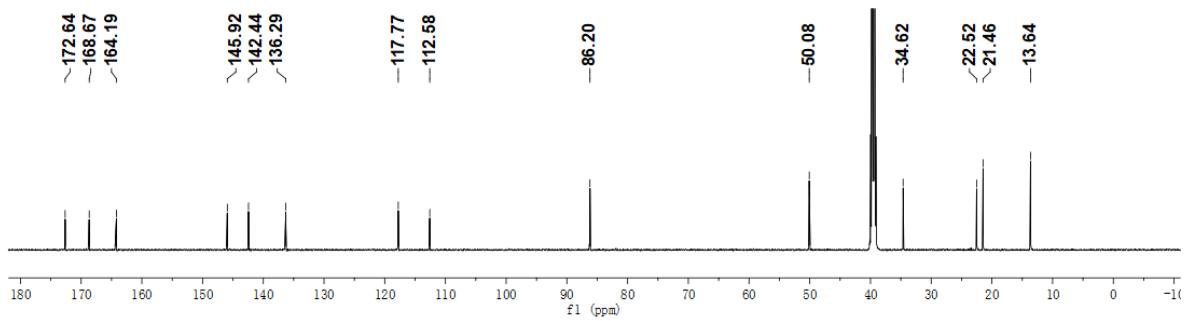


Figure S60. ¹³C NMR spectrum of pyranterrone H (**10**)

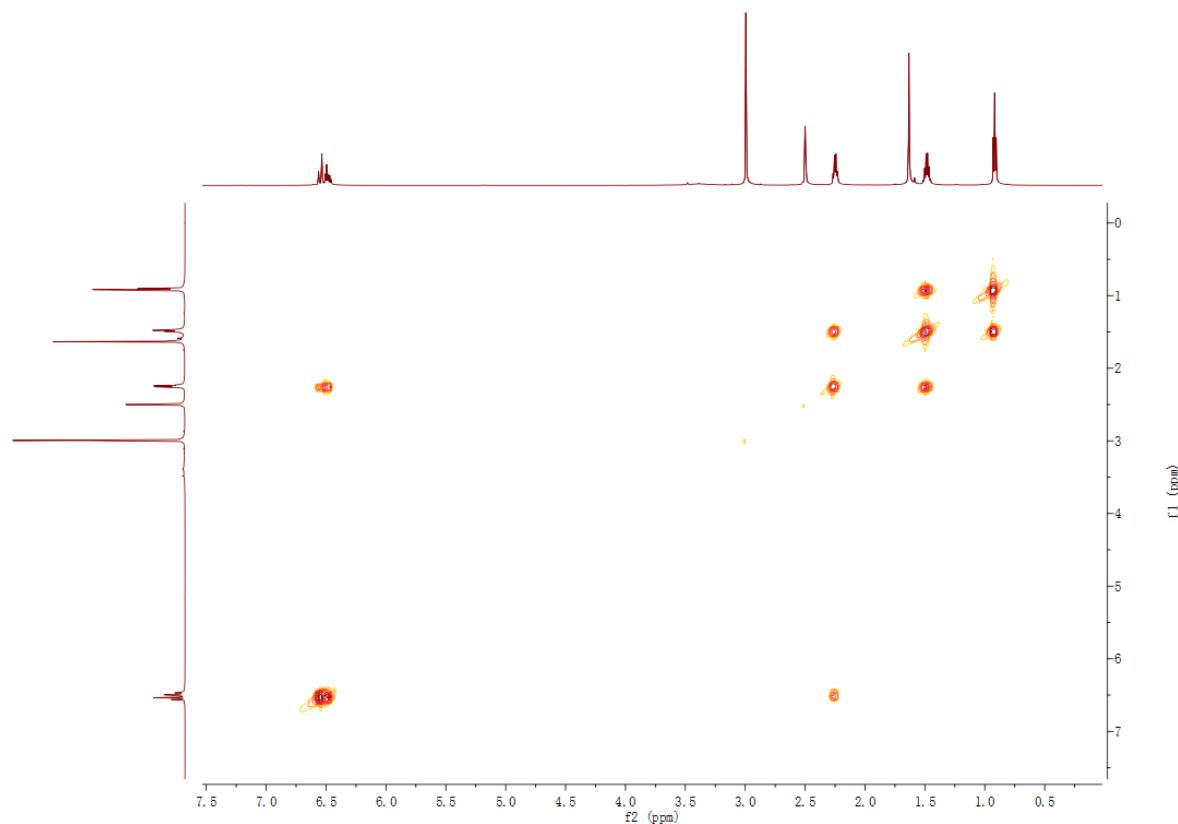


Figure S61. ¹H-¹H COSY spectrum of pyranterrone H (**10**)

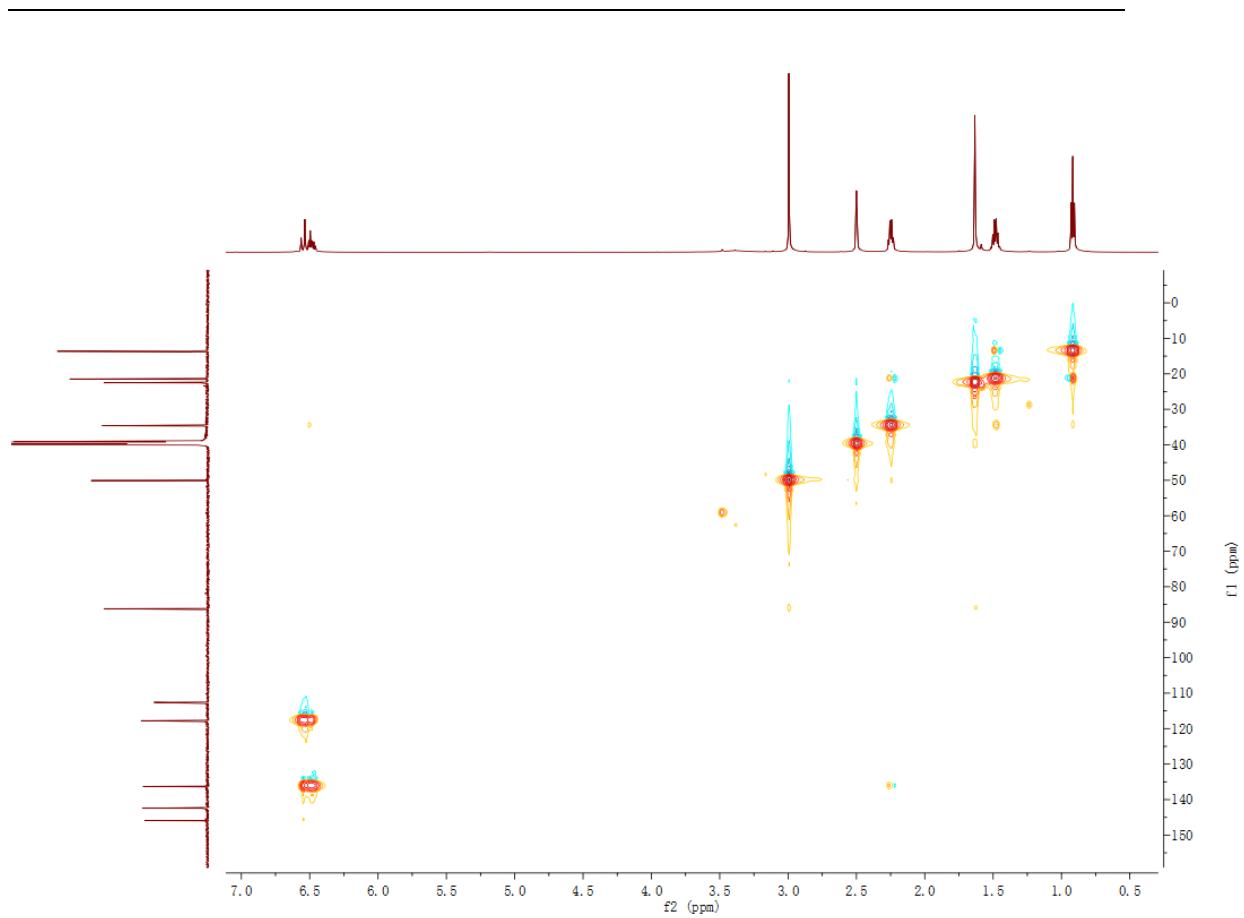


Figure S62. HSQC spectrum of pyranterrone H (**10**)

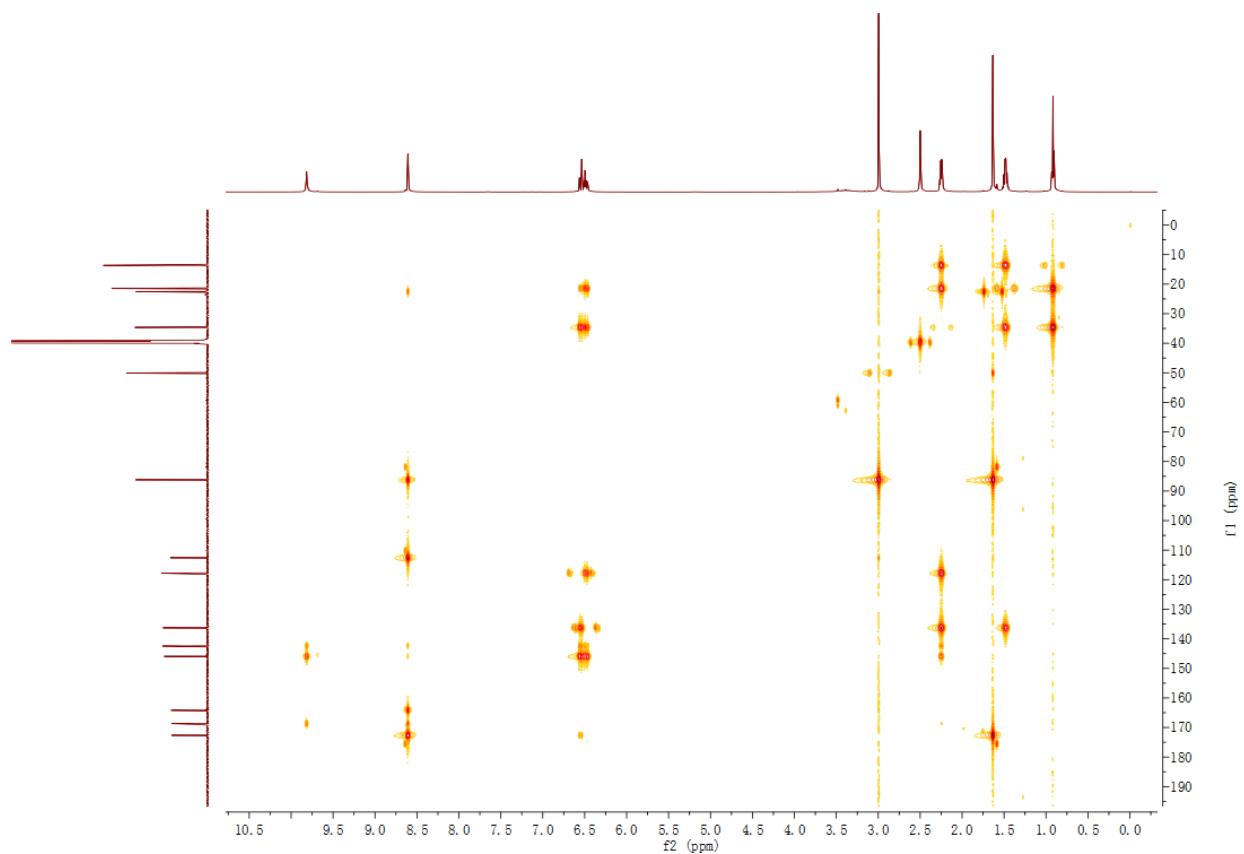


Figure S63. HMBC spectrum of pyranterrone H (**10**)