Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Electronic Supplementary Information

Spiroetherones A and B, Sesquiterpene Naphthoquinones as Angiogenesis Inhibitors from the Marine Sponge *Dysidea etheria*

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Contents

Figure S43. The Experimental ECD Spectrum of Spiroetherone B (**2**) in MeOH.

Experimental Section

^{13}C NMR calculation details

(1) Methods

In general, conformational analyses were carried out via random searching in the Sybyl-X 2.0 using the MMFF94S force field with an energy cutoff of 3.0 kcal/mol.[1] The results showed lowest energy conformers for compounds **1** and **2**. Subsequently, the conformers were re-optimized using DFT at the b3lyp/6-311+g(2d,p) level by the GAUSSIAN 09 program.[2] The ^{13}C shielding constants were calculated using the Gauge-Independent Atomic Orbital (GIAO) method at the b3lyp/6-311+g(2d,p) level with SMD in $CDCl_3$.[4] To get the final ^{13}C NMR chemical shifts, the ^{13}C NMR chemical shifts of the conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG).

(2) Results

Table S1. The experimental and calculated ^{13}C NMR data for compounds **1** and **2** in $CDCl_3$.

	1		**2**	
Position	δ_C (experimental)	δ_C (calculated)	δ_C (experimental)	δ_C (calculated)
1	38.5551	48.385	34.5962	43.435
2	29.3894	35.164	26.8964	31.519
3	32.6063	40.449	119.4124	131.846
4	158.5914	179.612	142.7172	158.044
5	39.8859	52.481	38.0693	49.544
6	37.3969	44.215	36.8325	45.667
7	28.3743	34.398	28.3694	35.166
8	44.7986	55.21	44.8627	53.83
9	43.7337	53.668	43.3106	51.345
10	59.9353	66.194	58.2062	64.152
11	103.7203	108.243	16.8482	21.768

12	19.9291	23.455	18.6141	22.375
13	16.8281	22.164	16.7561	20.588
14	16.6177	20.334	16.816	21.304
15	54.1447	63.149	54.2583	63.039
16	54.9882	64.791	55.8694	63.638
17	200.1071	219.251	200.4208	218.48
18	135.4127	144.362	135.8092	144.529
19	127.7612	137.232	127.7219	137.445
20	134.3375	144.072	134.6932	144.278
21	133.6975	144.023	133.539	143.59
22	126.0165	135.022	126.0217	135.287
23	134.875	144.185	134.3865	143.909
24	196.631	216.758	196.5742	216.127
25	48.6076	58.487	49.2126	58.461

(3) Picture of ^{13}C NMR calculation

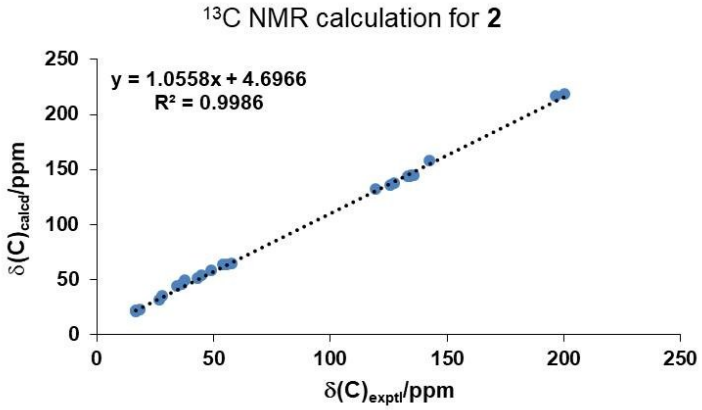


Figure S1. Correlation graphs for the calculated and experimental ^{13}C chemical shifts of the 1*S**,5*S**,8*S**,9*R**,10*S**,16*S** stereoisomer of spiroetherone B (**2**).

ECD calculation details

(1) Methods

In general, conformational analyses were carried out via random searching in the Sybyl-X 2.0 using the MMFF94S force field with an energy cutoff of 2.5 kcal/mol.[1] The results showed eight

lowest energy conformer for both compounds. Subsequently, the conformers were re-optimized using DFT at the b3lyp/6-311+g(d, p) level in MeOH using the polarizable conductor calculation model (CPCM) by the GAUSSIAN 09 program.[2] The energies, oscillator strengths, and rotational strengths (velocity) of the first 50 electronic excitations were calculated using the TDDFT methodology at the b3lyp/6-311+g(d,p) level in MeOH. The ECD spectra were simulated by the overlapping Gaussian function (half the bandwidth at 1/e peak height, sigma = 0.30 for all).[3] To get the final spectra, the simulated spectra of the conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). By comparing the experiment spectra with the calculated model molecules, the absolute configuration of the only chiral center was determined to be.

(2) Results

Cartesian coordinates for the low-energy optimized conformers with Boltzmann distribution of (1*S*,5*S*,8*S*,9*R*,10*S*,16*S*)-**1** at B3LYP/6-311+G (d, p) level of theory in MeOH.

Table S2. Gibbs free energies[a] and equilibrium populations[b] of low-energy conformers of ECGT.

Conformers	ΔG	P(%)/100
1a_tddft	0.0	100.0
1b_tddft	0.01541	0.0

[a]B3LYP/6-311+G(d,p), in kcal/mol. [b]From ΔG values at 298.15K.

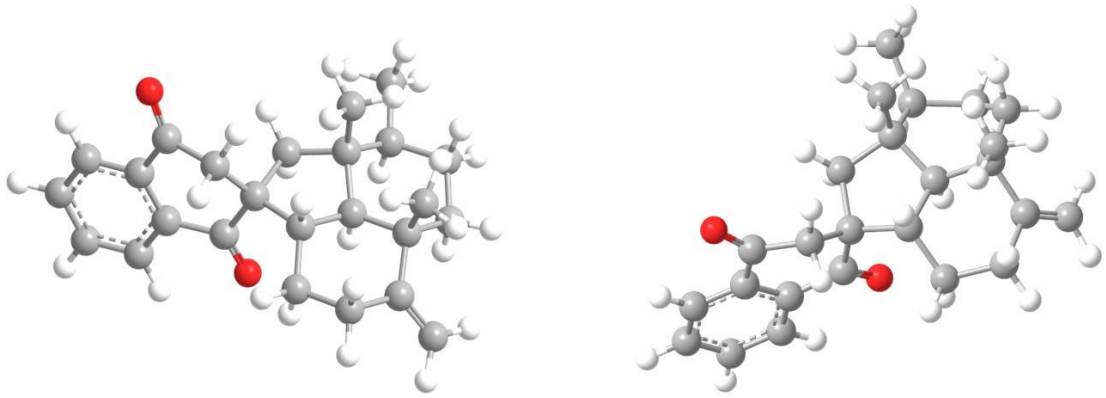


Figure S2. Most stable conformers of **1** (the relative populations are in parentheses)

Table S3. Cartesian coordinates for the low-energy reoptimized random search conformers of ECGT at B3LYP/6-311+G(d,p) level of theory in MeOH.

1a_tddft		Standard Orientation (Ångstroms)			
Center number	Atomic number	Atomic Type	X	Y	Z
0	6	0	-0.889784	-3.110419	2.994721
1	6	0	1.082978	-5.225939	3.127085
2	6	0	2.119784	-5.900209	0.562462
3	6	0	3.353392	-3.718815	-0.853961
4	6	0	1.385167	-1.597452	-0.863656
5	6	0	0.228752	-0.847783	1.631761
6	6	0	3.881776	-4.269316	-3.652588
7	6	0	4.480046	-1.901082	-5.196793
8	6	0	2.489009	0.196646	-5.011806
9	6	0	2.022615	0.876531	-2.224111
10	6	0	-0.444722	2.343116	-1.735181
11	6	0	-1.46263	1.462135	0.928639
12	6	0	-1.150995	3.521351	2.932822
13	6	0	-2.813453	5.769143	2.582849

14	6	0	-5.461359	5.222755	1.919973
15	6	0	-6.140224	2.809173	1.043168
16	6	0	-4.217077	0.840878	0.487601
17	6	0	-7.306437	7.075667	2.226167
18	6	0	-9.812217	6.537348	1.717554
19	6	0	-10.495886	4.141938	0.871214
20	6	0	-8.672824	2.302814	0.520895
21	8	0	-2.049699	7.937929	2.92001
22	6	0	1.934945	-8.239712	-0.341572
23	6	0	5.837415	-3.113344	0.522108
24	6	0	4.225562	2.378797	-1.112654
25	6	0	3.161549	2.430089	-6.684763
26	1	0	-0.183995	-2.389766	-1.958807
27	1	0	1.708591	-0.0972	2.857611
28	8	0	-4.869036	-1.15395	-0.503979
29	1	0	-1.504169	-2.606101	4.898824
30	1	0	-2.536156	-3.812356	1.988367
31	1	0	0.256641	-6.894824	4.005235
32	1	0	2.629821	-4.622675	4.356481
33	1	0	5.431761	-5.618055	-3.842129
34	1	0	2.215126	-5.172028	-4.467976
35	1	0	6.321522	-1.145749	-4.654131
36	1	0	4.677963	-2.447468	-7.177115
37	1	0	0.706987	-0.590656	-5.712259
38	1	0	-1.81451	1.86984	-3.196345
39	1	0	-0.199839	4.383581	-1.782619
40	1	0	0.800792	4.147343	3.040139
41	1	0	-1.635823	2.727314	4.780659
42	1	0	-6.757466	8.922904	2.901714
43	1	0	-11.240086	7.97426	1.983965

44	1	0	-12.455066	3.721306	0.471905
45	1	0	-9.188525	0.456534	-0.180483
46	1	0	2.659195	-8.783824	-2.173308
47	1	0	1.04903	-9.727951	0.752087
48	1	0	6.86317	-4.861206	0.890603
49	1	0	7.070576	-1.917846	-0.596628
50	1	0	5.547162	-2.178467	2.327767
51	1	0	6.057544	1.668104	-1.697447
52	1	0	4.12144	4.342526	-1.717892
53	1	0	4.211152	2.386871	0.941463
54	1	0	1.758589	3.933065	-6.546628
55	1	0	3.255835	1.855856	-8.662117
56	1	0	4.992753	3.235728	-6.195609

1b_tddft		Standard Orientation (Ångstroms)			
Center number	Atomic number	Atomic Type	X	Y	Z
0	6	0	-0.845975	-3.090713	2.978969
1	6	0	0.972359	-5.358889	2.927091
2	6	0	2.181578	-5.795018	0.376919
3	6	0	3.584414	-3.49806	-0.644691
4	6	0	1.493701	-1.491269	-0.750613
5	6	0	0.32366	-0.771424	1.734575
6	6	0	4.6597	-3.701182	-3.34924
7	6	0	2.886133	-2.706801	-5.392354
8	6	0	2.031782	0.089325	-5.076624
9	6	0	2.02435	0.910324	-2.250814
10	6	0	-0.257296	2.583015	-1.501256
11	6	0	-1.373349	1.536156	1.0643
12	6	0	-1.203698	3.488605	3.186339

13	6	0	-2.883087	5.728711	2.873978
14	6	0	-5.477949	5.185926	2.023358
15	6	0	-6.067484	2.81641	0.977521
16	6	0	-4.087212	0.900144	0.434826
17	6	0	-7.366133	6.996696	2.318828
18	6	0	-9.828516	6.458496	1.631156
19	6	0	-10.424424	4.105728	0.616029
20	6	0	-8.55636	2.310434	0.277097
21	8	0	-2.178945	7.883956	3.384456
22	6	0	1.90498	-7.976342	-0.841227
23	6	0	5.777079	-2.947661	1.180645
24	6	0	4.427695	2.298066	-1.469602
25	6	0	3.46911	1.855488	-6.825193
26	1	0	-0.044106	-2.400888	-1.786084
27	1	0	1.780334	-0.037932	2.996063
28	8	0	-4.654412	-1.048538	-0.691601
29	1	0	-1.398803	-2.699527	4.927297
30	1	0	-2.547056	-3.624897	1.963274
31	1	0	-0.050041	-7.049679	3.505634
32	1	0	2.449841	-5.072464	4.33715
33	1	0	6.41052	-2.619089	-3.423381
34	1	0	5.21347	-5.636509	-3.783328
35	1	0	3.810296	-2.919096	-7.223661
36	1	0	1.21932	-3.91739	-5.474441
37	1	0	0.057972	0.14239	-5.676592
38	1	0	-1.690057	2.537292	-2.975625
39	1	0	0.273537	4.556498	-1.273776
40	1	0	-1.768608	2.591574	4.963082
41	1	0	0.732917	4.129557	3.425203
42	1	0	-6.885252	8.809409	3.126542

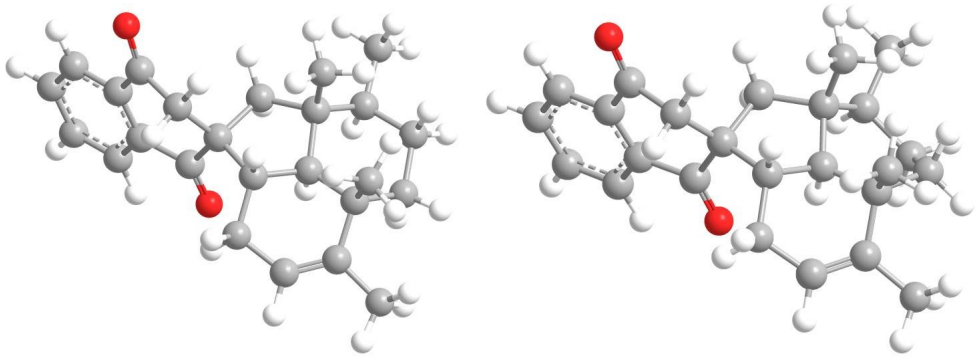
43	1	0	-11.290658	7.862098	1.888522
44	1	0	-12.349606	3.684572	0.076741
45	1	0	-9.001135	0.499638	-0.553181
46	1	0	2.727956	-8.338712	-2.676248
47	1	0	0.823913	-9.512621	-0.02508
48	1	0	6.960597	-4.627536	1.344605
49	1	0	6.973674	-1.427358	0.509187
50	1	0	5.164734	-2.448166	3.074045
51	1	0	6.14043	1.3327	-2.051303
52	1	0	4.478563	4.174222	-2.311771
53	1	0	4.516253	2.555462	0.570092
54	1	0	2.847656	3.809472	-6.616012
55	1	0	3.161875	1.324133	-8.792236
56	1	0	5.500855	1.805766	-6.490731

Cartesian coordinates for the low-energy optimized conformers with bolzmann distribution of (1*S*,5*S*,8*S*,9*R*,10*S*,16*S*)-**2** at B3LYP/6-311+G (d, p) level of theory in MeOH.

Table S4. Gibbs free energies[a] and equilibrium populations[b] of low-energy conformers of **2**.

Conformers	ΔG	P(%)/100
2a_tddft	0.0	100.0
2b_tddft	0.01495	0.0

[a]B3LYP/6-311+G(d,p), in kcal/mol. [b]From ΔG values at 298.15K.



2a 100% **2b** 0%

Figure S3. Most stable conformers of **2** (the relative populations are in parentheses)

Table S5. Cartesian coordinates for the low-energy reoptimized random reseach conformers of **2** at B3LYP/6-311+G(d,p) level of theory in MeOH.

2a_tddft		Standard Orientation (Ångstroms)			
Center number	Atomic number	Atomic Type	X	Y	Z
0	6	0	-1.086009	-3.680906	3.064642
1	6	0	-0.058586	-6.032643	1.86612
2	6	0	1.744209	-6.19569	0.104706
3	6	0	3.137312	-3.84845	-0.819143
4	6	0	1.355205	-1.621574	-0.459446
5	6	0	0.208249	-1.282916	2.121474
6	6	0	3.688248	-3.911032	-3.676751
7	6	0	4.497333	-1.347987	-4.740412
8	6	0	2.685751	0.840642	-4.16493
9	6	0	2.218294	1.009871	-1.299196
10	6	0	-0.125646	2.562865	-0.540226
11	6	0	-1.327087	1.212406	1.841819
12	6	0	-1.060759	2.806528	4.233983
13	6	0	-2.537851	5.207242	4.233392
14	6	0	-5.107381	5.067808	3.169959
15	6	0	-5.863418	2.935034	1.777275

16	6	0	-4.069256	0.876528	1.120633
17	6	0	-6.816824	7.023065	3.602919
18	6	0	-9.263225	6.863595	2.69903
19	6	0	-10.022913	4.747427	1.333749
20	6	0	-8.332806	2.808749	0.86892
21	8	0	-1.690962	7.169695	5.147064
22	6	0	5.608178	-3.715108	0.690768
23	6	0	4.510132	2.093218	0.08701
24	6	0	3.583198	3.284925	-5.373796
25	1	0	-0.237145	-2.055596	-1.706831
26	1	0	1.721621	-0.852468	3.450266
27	8	0	-4.795418	-0.923474	-0.151936
28	6	0	2.534525	-8.704832	-0.934478
29	1	0	-0.898776	-3.834578	5.117388
30	1	0	-3.11384	-3.640238	2.7178
31	1	0	-0.934065	-7.774546	2.513978
32	1	0	5.142009	-5.308943	-4.104905
33	1	0	1.977007	-4.522577	-4.657033
34	1	0	4.691688	-1.520461	-6.787979
35	1	0	6.382461	-0.868559	-4.052171
36	1	0	0.85932	0.362173	-5.013542
37	1	0	0.306899	4.526613	-0.113103
38	1	0	-1.472105	2.580333	-2.097528
39	1	0	-1.747419	1.707911	5.847281
40	1	0	0.911522	3.242053	4.603555
41	1	0	-6.213488	8.648419	4.681775
42	1	0	-10.58554	8.378902	3.059089
43	1	0	-11.93441	4.622269	0.623028
44	1	0	-8.903076	1.181048	-0.22285
45	1	0	6.534786	-5.55347	0.698201

46	1	0	6.952953	-2.381123	-0.094894
47	1	0	5.268815	-3.1963	2.651697
48	1	0	6.292222	1.345166	-0.596893
49	1	0	4.583231	4.134452	-0.159469
50	1	0	4.442497	1.742742	2.110937
51	1	0	2.323376	4.85631	-4.937697
52	1	0	3.650434	3.100665	-7.425735
53	1	0	5.474242	3.80651	-4.746382
54	1	0	4.566103	-9.012726	-0.752565
55	1	0	2.103268	-8.868866	-2.944572
56	1	0	1.579939	-10.249185	0.033011

2b_tddft			Standard Orientation (Ångstroms)		
Center number	Atomic number	Atomic Type	X	Y	Z
0	6	0	-0.795687	-3.648414	3.241031
1	6	0	-0.059532	-5.929365	1.709031
2	6	0	1.714543	-6.045604	-0.089226
3	6	0	3.301294	-3.69451	-0.638488
4	6	0	1.472131	-1.500754	-0.299453
5	6	0	0.41814	-1.184019	2.309609
6	6	0	4.395253	-3.430557	-3.336504
7	6	0	2.759945	-1.85139	-5.112552
8	6	0	2.148581	0.881012	-4.207781
9	6	0	2.232998	1.097662	-1.275818
10	6	0	0.122548	2.782529	-0.133722
11	6	0	-1.188468	1.24904	2.072755
12	6	0	-1.176365	2.711992	4.560964
13	6	0	-2.691593	5.089713	4.535593
14	6	0	-5.131188	4.980471	3.194878

15	6	0	-5.722875	2.893504	1.660704
16	6	0	-3.864366	0.845125	1.156632
17	6	0	-6.888385	6.916599	3.506096
18	6	0	-9.219232	6.786732	2.332387
19	6	0	-9.814671	4.717638	0.821656
20	6	0	-8.077383	2.79354	0.48576
21	8	0	-1.977807	7.011345	5.631189
22	6	0	5.49432	-3.80586	1.261593
23	6	0	4.763759	2.062022	-0.289252
24	6	0	3.715653	2.846728	-5.593911
25	1	0	-0.155576	-2.011547	-1.454721
26	1	0	1.957193	-0.69826	3.590109
27	8	0	-4.487372	-0.9931	-0.112714
28	6	0	2.288022	-8.45553	-1.441074
29	1	0	-0.286079	-3.982831	5.216515
30	1	0	-2.85038	-3.524025	3.244541
31	1	0	-1.095848	-7.642125	2.167645
32	1	0	6.248684	-2.542413	-3.186973
33	1	0	4.747312	-5.273201	-4.183377
34	1	0	0.991721	-2.856758	-5.453942
35	1	0	3.696056	-1.752831	-6.947788
36	1	0	0.180701	1.216189	-4.731899
37	1	0	0.865898	4.551651	0.606375
38	1	0	-1.252449	3.299496	-1.575007
39	1	0	-1.989199	1.520638	6.04502
40	1	0	0.747946	3.151709	5.13108
41	1	0	-6.413843	8.503533	4.700302
42	1	0	-10.579346	8.288739	2.593687
43	1	0	-11.634876	4.616687	-0.100957
44	1	0	-8.518154	1.199421	-0.710664

45	1	0	6.602555	-5.513482	0.93756
46	1	0	6.775258	-2.213772	1.085725
47	1	0	4.82482	-3.879309	3.204182
48	1	0	6.368726	1.052019	-1.072257
49	1	0	5.011725	4.048814	-0.762859
50	1	0	4.870773	1.921696	1.761352
51	1	0	3.266099	4.759976	-4.971963
52	1	0	3.348379	2.760573	-7.619868
53	1	0	5.738113	2.561657	-5.328853
54	1	0	4.291151	-8.940037	-1.326362
55	1	0	1.836699	-8.337194	-3.451321
56	1	0	1.216297	-10.027747	-0.658181

(3) Picture of ECD

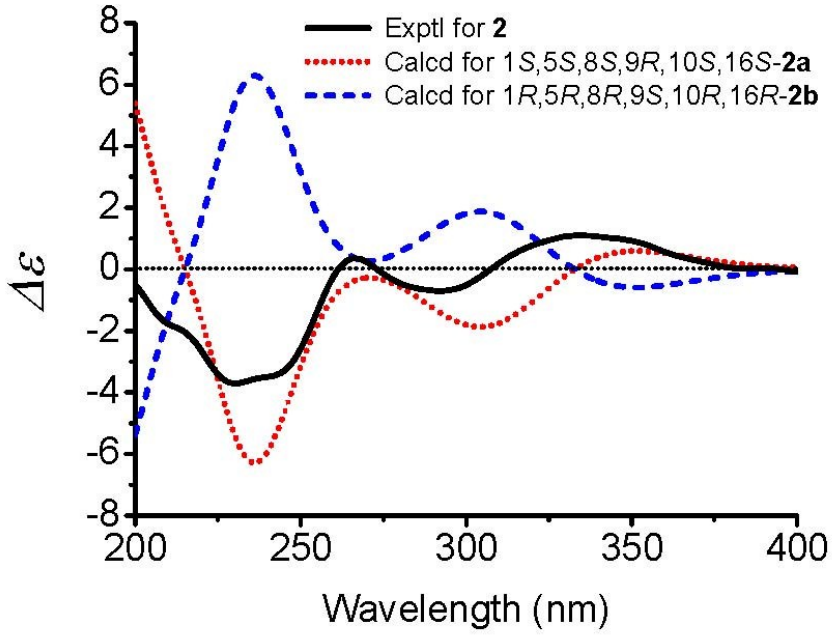


Figure S4. Experimental ECD spectra (200-400 nm) of compound **2** in MeOH and the calculated ECD spectra of the model molecules of **2** at the B3LYP/6-311+G(d, p) level.

Antiproliferative Bioassay

Cytotoxicity was evaluated as IC_{50} values by using the MTT assay as described previously.[5]

Four human cancer cell lines, including myeloma NCI-H929, lung carcinoma A549, hepatoma HepG2, and ovarian SK-OV-3 cell lines, were cultured in RPMI 1640 medium at 37 °C with 5% CO_2 and 95% air, supplemented with 10% (V/V) bovine calf serum and 80 U·mL^{-1} penicillin/streptomycin. The cells were seeded into 96-well plates and treated with compounds at different concertations for 48 h, respectively. The light absorption was measured at 490 nm on a Spectra MAX 340 microplate spectrophotometer (GMI Co., Belmont, USA). Inhibition rate was calculated by the formula:

$$\text{Inhibition (\%)} = 100\% - (OD_{\text{treatment}} - OD_{\text{blank}})/(OD_{\text{control}} - OD_{\text{blank}}) \times 100\%$$

The cells were incubated with compounds (100 μg·mL^{-1}) for 48 h, and stained with 0.1 mg·mL^{-1} of acridine orange (AO) at room temperature for 5 min. Then the cells were observed and photographed using the fluorescent stereo microscope (Olympus, Tokyo, Japan).

Zebrafish maintenance and embryo handling

Healthy transgenic zebrofish Tg(VEGFR2:GFP) at 24 hpf (hours post fertilization) was used as animal model. Adult zebrafish were maintained at 28 °C under a 14 h light/10 h dark cycle and supplied with freshwater, aeration, and food. Embryos were obtained from natural spawning; they were collected within 30 min and cultured in an aquarium. The embryos were used within 24 h. The experiments were performed in accordance with standard ethical guidelines. The procedures were approved by the Ethics Committee of the Biology Institute of Shandong Academy of Science.

Anti-angiogenesis Bioassay

Anti-angiogenesis activity was evaluated as described previously.[6] Stock solutions (10 mg/mL) of all samples were prepared by dissolving the test compounds in DMSO. These solutions were diluted in sterile salt water (5 mmol/L NaCl, 0.17 mmol/L KCl, 0.4 mmol/L $CaCl_2$, and 0.16 mmol/L $MgSO_4$) to obtain solutions with the test compounds dissolved in 0.1% DMSO. These

solutions with different concentrations were aliquoted into 96-well plates, and embryos at 24 hpf were also transferred tandomly into the above wells. After 24 h of treatment, the intersegment vessels (ISV) of embryos were visualized with green fluorescent protein labeling and endogenous alkaline phosphatase staining. The anti-angiogenic activities of test compounds were calculated from the inhibition ratio angiogenesis. Vatalanib (PTK787) was used as the positive control.

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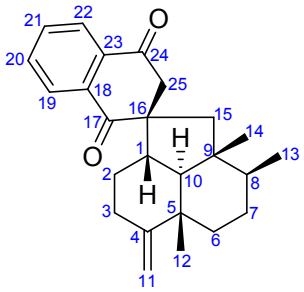
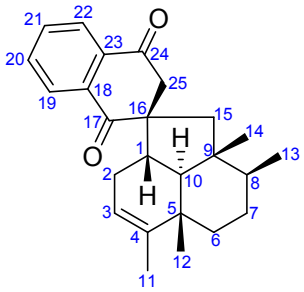


Spiroetherone A (**1**)

Table S6. The ^{1}H and ^{13}C NMR Data for Spiroetherone A (**1**) in $CDCl_3$

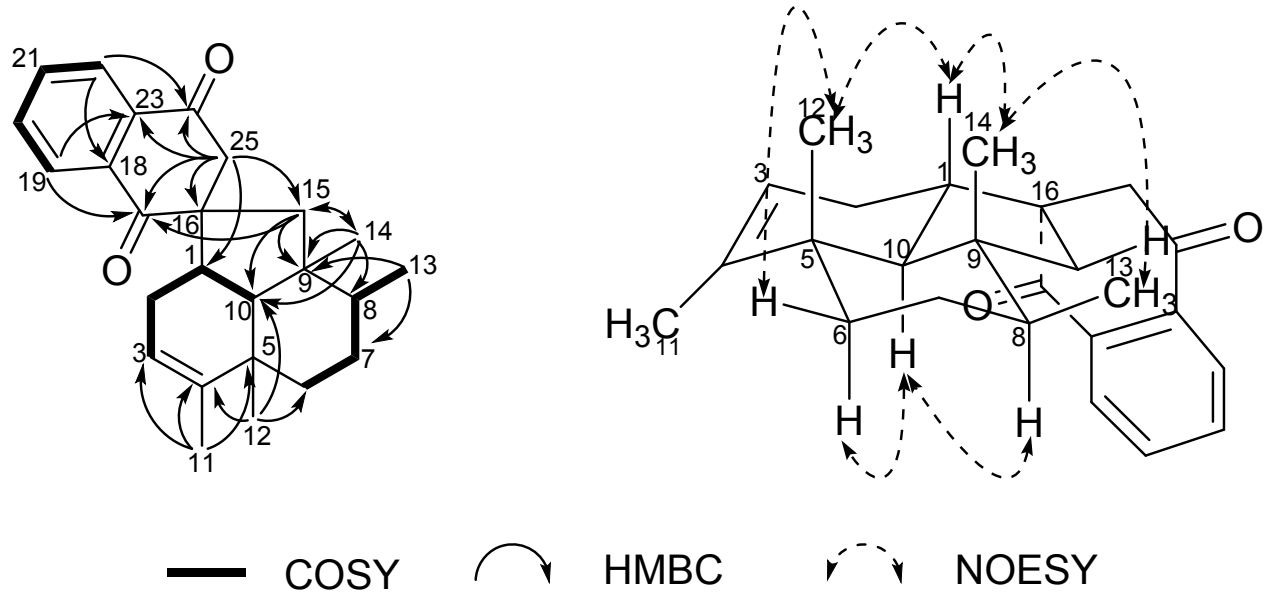
Position	δ_C	δ_H (J in Hz)	COSY	HMBC (H→C)	NOESY
1	38.6, CH	2.89, ddd (14.4, 13.2, 4.2)	H-2β, 10		H-3β, 12, 14, 25β
2α		1.28, dd (13.2, 7.2)			
2β	29.4, CH_2	1.71, m	H-3α, 3β		
3α		2.16, ddd (13.8, 7.2, 2.4)	H-2β		H-11a
3β	32.6, CH_2	2.32, td (13.8, 4.2)	H-2β, 3α		H-1, 12
4	158.6, C				
5	39.9, C				
6α		1.49, m			H-10
6β	37.4, CH_2	1.68, m	H-6β, 7		H-12
7	28.4, CH_2	1.47, m	H-6β		
8	44.8, CH	1.20, m	H_3-13		H-10
9	43.7, C				
10	59.9, CH	1.01, d (14.4)			H-2α, 6α, 8, 15α
11a	103.7, CH_2	4.57, s		C-3, 4, 5	H-3α
11b		4.55, s		C-3, 4, 5	H-6α, 6β
12	19.9, CH_3	1.04, s		C-4, 5, 6, 10	H-1, 3β, 6β
13	16.8, CH_3	0.76, d (7.2)	H-8	C-7, 8, 9	
14	16.6, CH_3	0.81, s		C-8, 9, 10, 15	H-1
15α		1.23, d (12.6)	H-15β	C-17	H-10
15β	54.1, CH_2	2.06, d (12.6)	H-15α	C-9, 10, 14, 17	H_3-14
16	55.0, C				
17	200.1, C				
18	135.4, C				
19	127.8, CH	8.10, dd (7.8, 1.2)	H-20	C-17, 21	
20	134.3, CH	7.74, td (7.8, 1.2)	H-19	C-18, 22	
21	133.7, CH	7.72, td (7.8, 1.2)	H-22	C-19, 23	
22	126.0, CH	8.02, dd (7.8, 1.2)	H-21	C-18, 24	
23	134.9, C				
24	196.6, C				
25α	48.6, CH_2	2.95, d (15.6)	H-25β	C-1, 15, 16, 17, 23, 24	H-15α
25β		2.76, d (15.6)	H-25α	C-1, 15, 16, 17, 23, 24	H-1



Spiroetherone B (**2**)

Table S7. The ^{1}H and ^{13}C NMR Data for Spiroetherone B (**2**) in $CDCl_3$

Position	δ_C	δ_H (J in Hz)	COSY	HMBC (H→C)	NOESY
1	34.6, CH	2.89, td (14.4, 10.2, 7.8)	H-2a, 10		H_3-12, 14
2a	29.7, CH_2	1.91, m	H-3		
2b		1.32, m			
3	119.4, CH	5.11, s	H-2a, 3		H_3-12
4	142.7, C				
5	38.1, C				
6α		1.15, m			
6β	36.8, CH_2	1.66, m	H-6β, 7		
7	28.4, CH_2	1.42, m	H-6β		
8	44.9, CH	1.23, m	H_3-13		
9	43.3, C				
10	58.2, CH	1.28, m			
11	16.9, CH_2	1.58, d (1.2)		C-3, 4, 5	H-3
12	18.6, CH_3	0.92, s		C-4, 5, 6, 10	H-1
13	16.76, CH_3	0.78, d (7.2)	H-8	C-7, 8, 9	
14	16.82, CH_3	0.79, s		C-8, 9, 10, 15	H-1
15α		1.27, d (13.2)	H-15β	C-17	
15β	54.3, CH_2	2.19, d (13.2)	H-15α	C-9, 10, 14, 17	H_3-14
16	55.9, C				
17	200.4, C				
18	135.8, C				
19	127.7, CH	8.07, dd (7.2, 1.8)	H-20	C-17, 21	
20	134.7, CH	7.74, td (7.2, 1.8)	H-19	C-18, 22	
21	133.5, CH	7.72, td (7.2, 1.8)	H-22	C-19, 23	
22	126.0, CH	8.02, dd (7.2, 1.8)	H-21	C-18, 24	
23	134.4, C				
24	196.6, C				
25α	49.2, CH_2	2.98, d (15.6)	H-25β	C-15, 16, 17, 23, 24	H-15α
25β		2.80, d (15.6)	H-25α	C-1, 16, 17, 23, 24	



2

Figure S5. Key COSY, HMBC, and NOESY Correlations of Spiroetherone B (**2**).

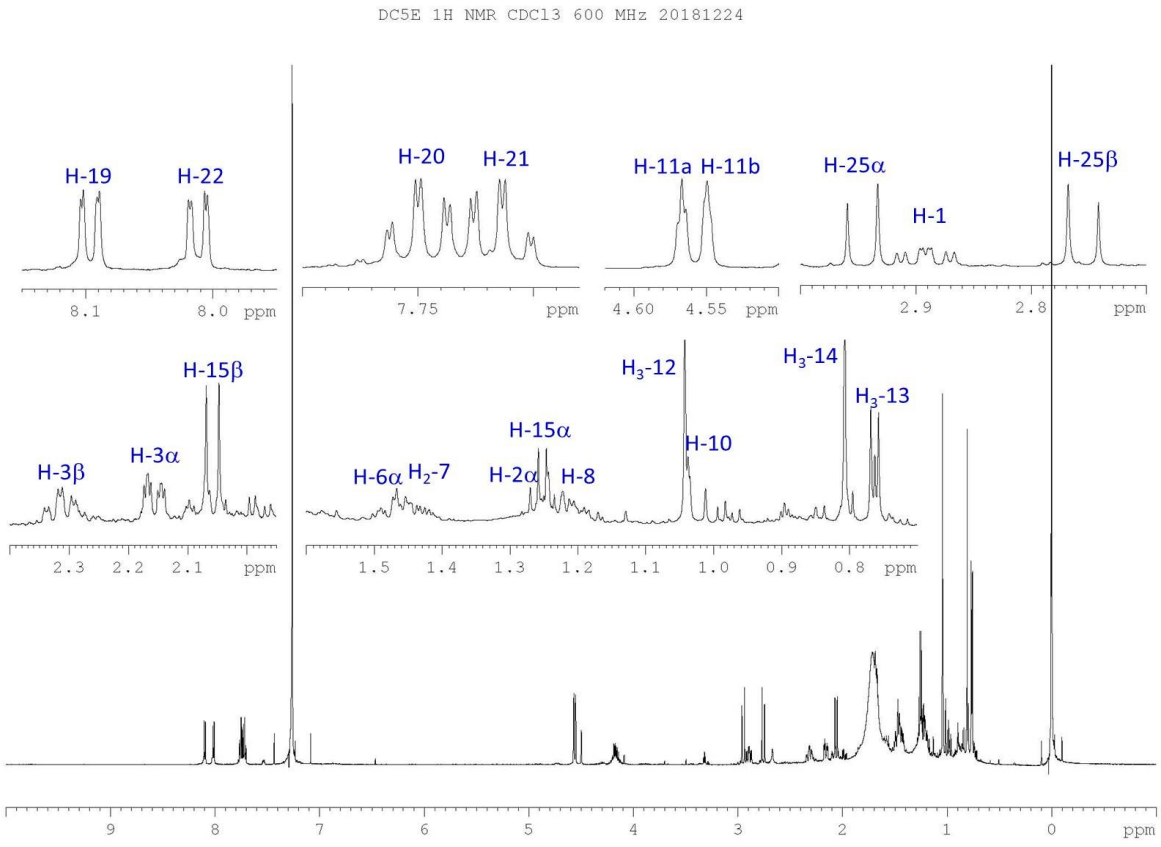


Figure S6. The ^{1}H NMR Spectrum (600 MHz) of Spiroetherone A (**1**) in $CDCl_3$.

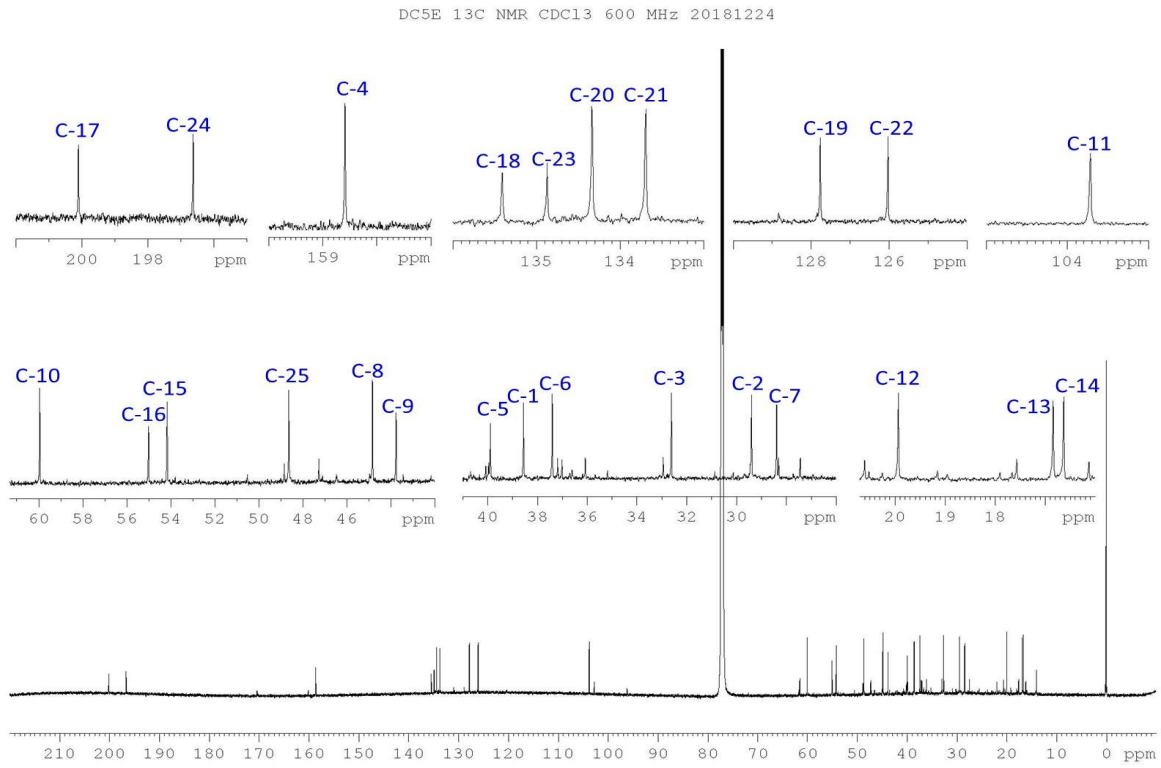


Figure S7. The ^{13}C NMR Spectrum (150 MHz) of Spiroetherone A (**1**) in $CDCl_3$.

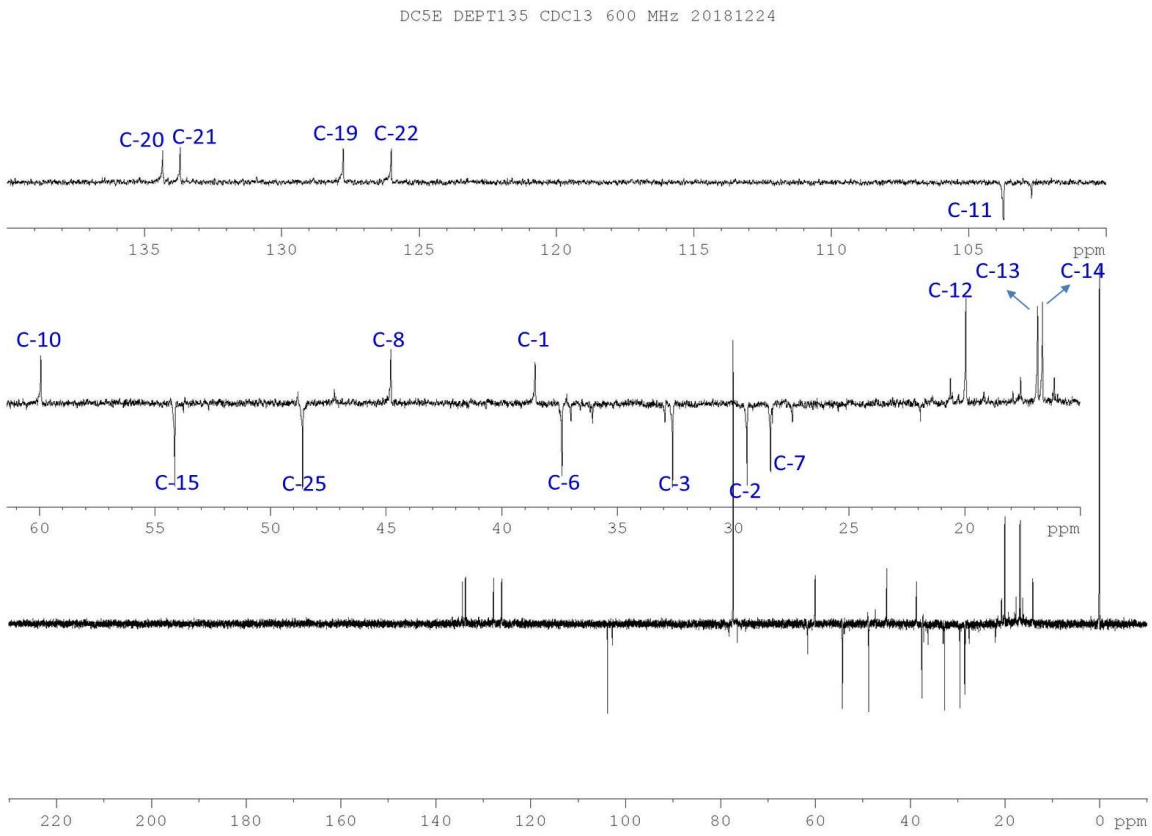


Figure S8. The DEPT135 Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

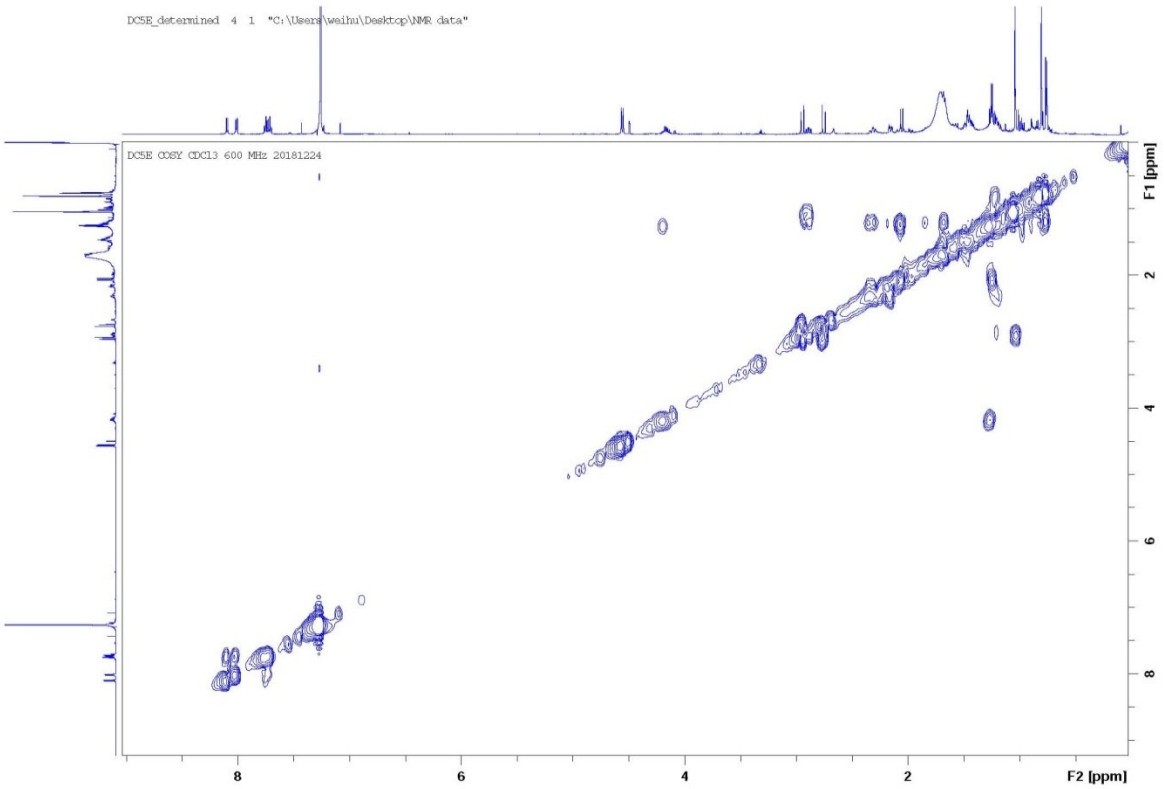


Figure S9. The ^{1}H-^{1}H COSY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

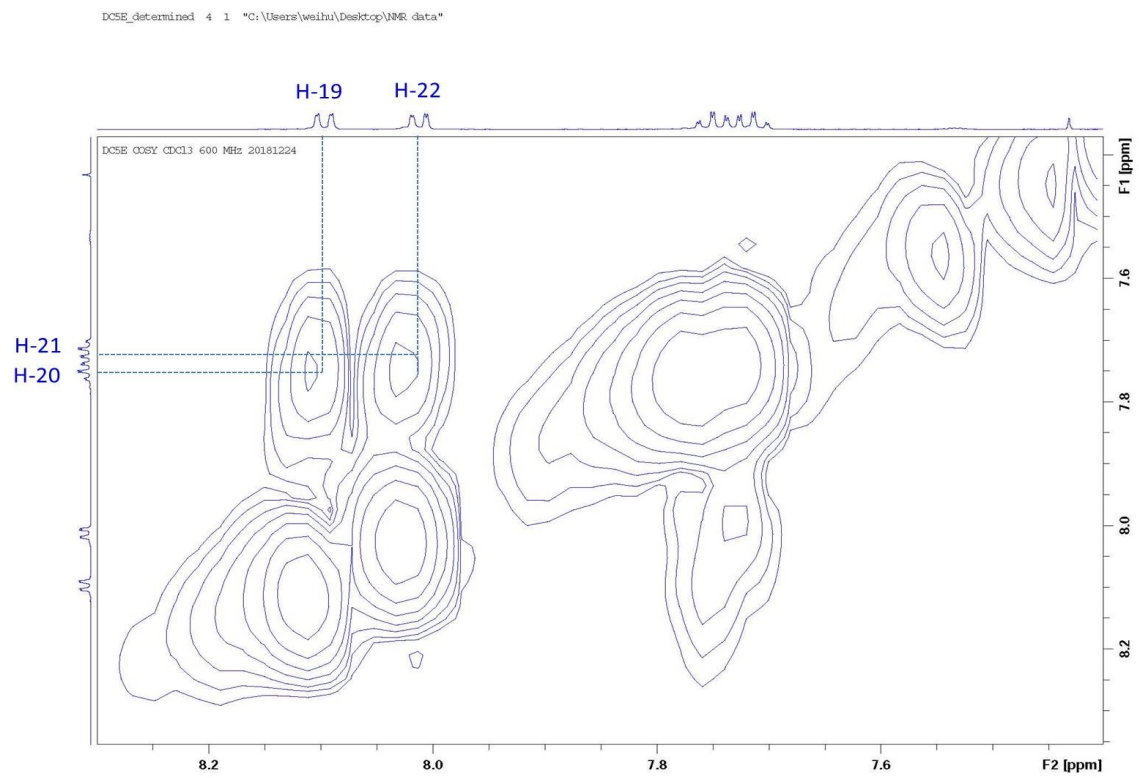


Figure S10. The ^{1}H-^{1}H COSY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

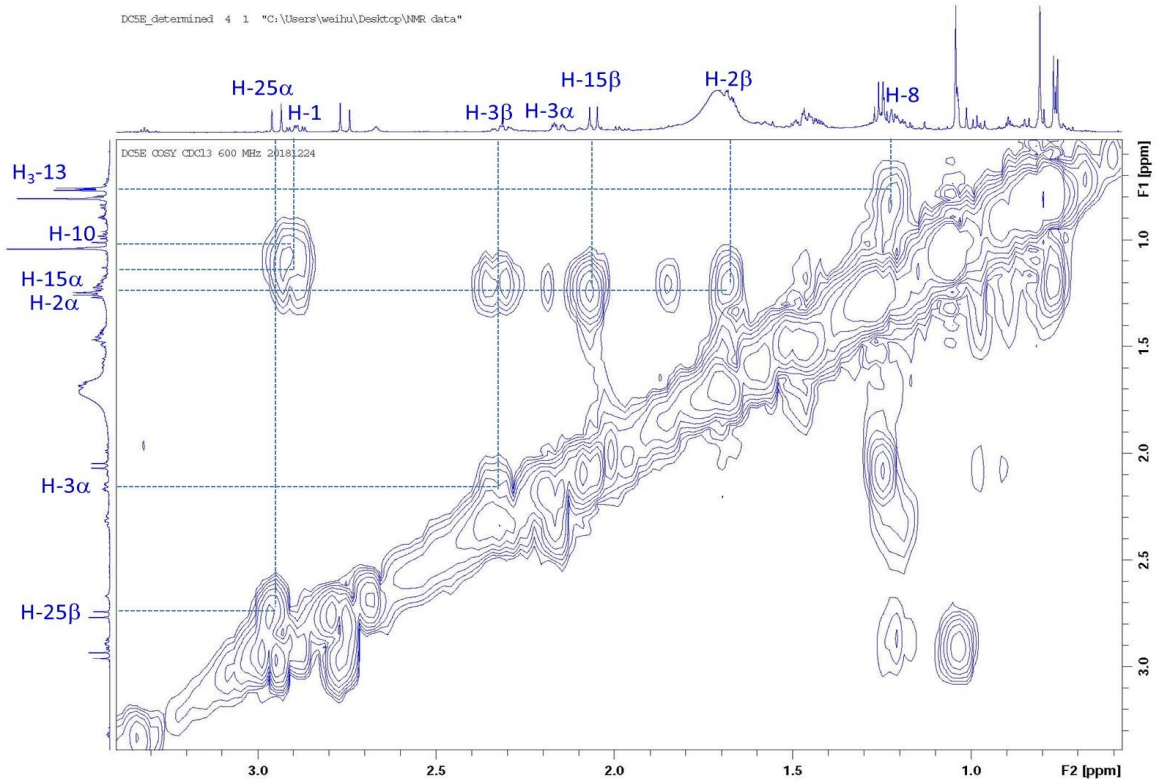


Figure S11. The ^{1}H-^{1}H COSY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

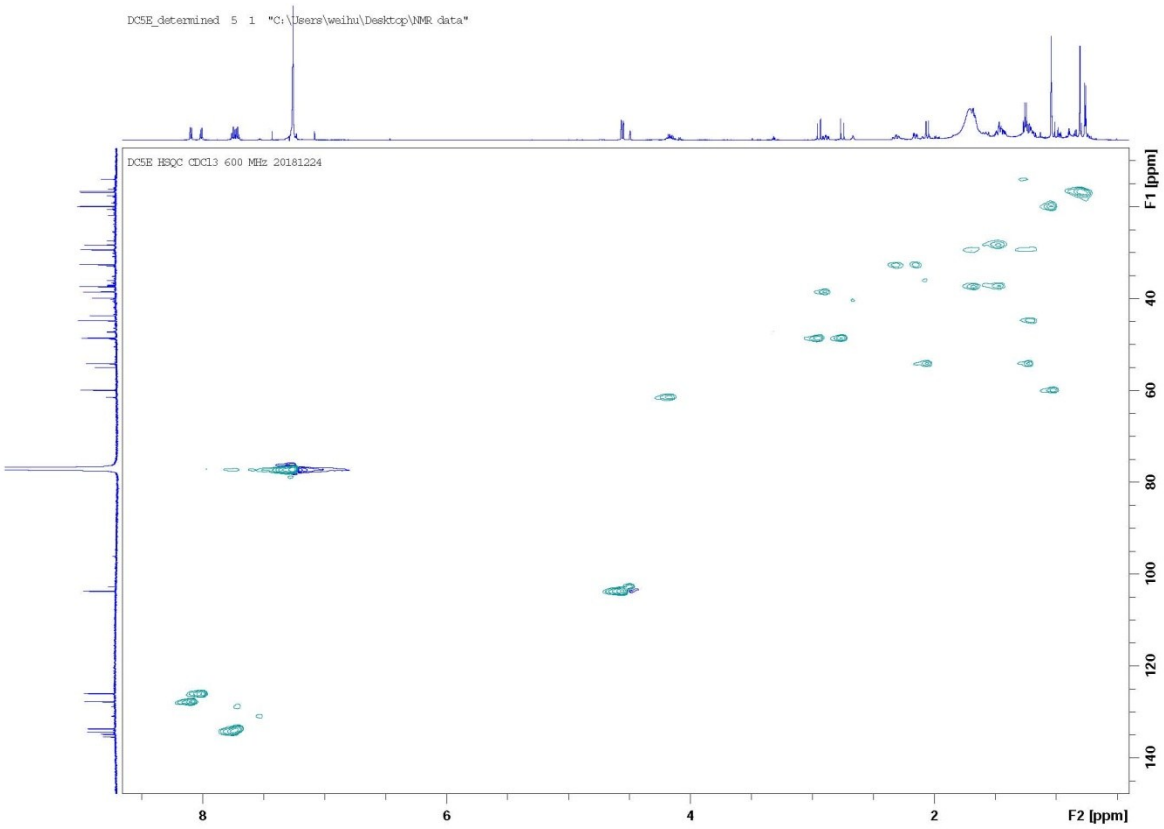


Figure S12. The HSQC spectrum of Spiroetherone A (**1**) in $CDCl_3$.

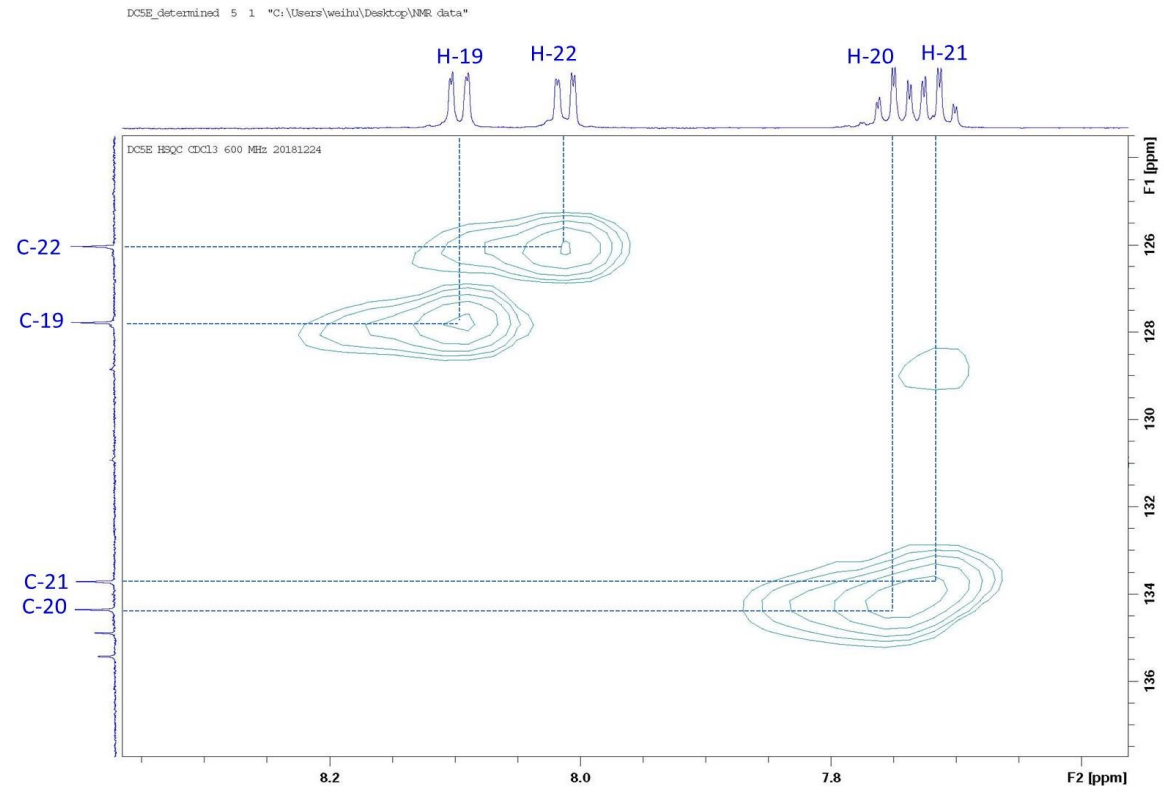


Figure S13. The HSQC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

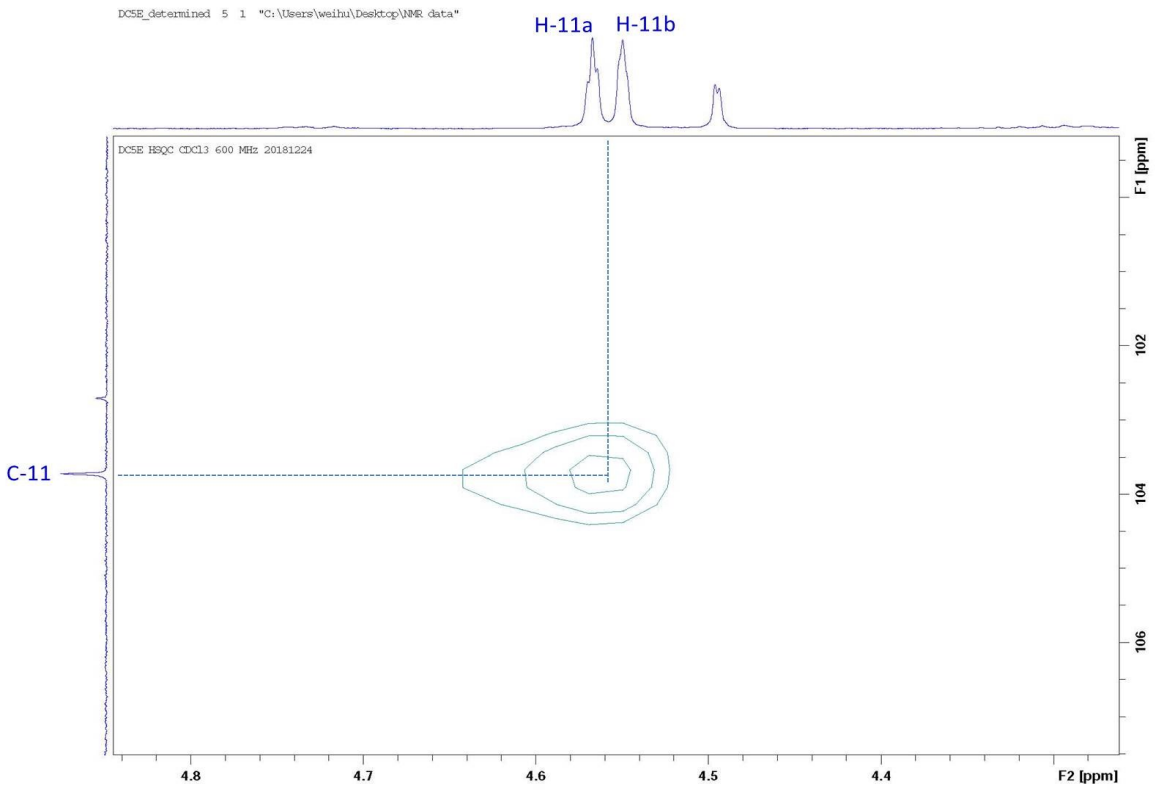


Figure S14. The HSQC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

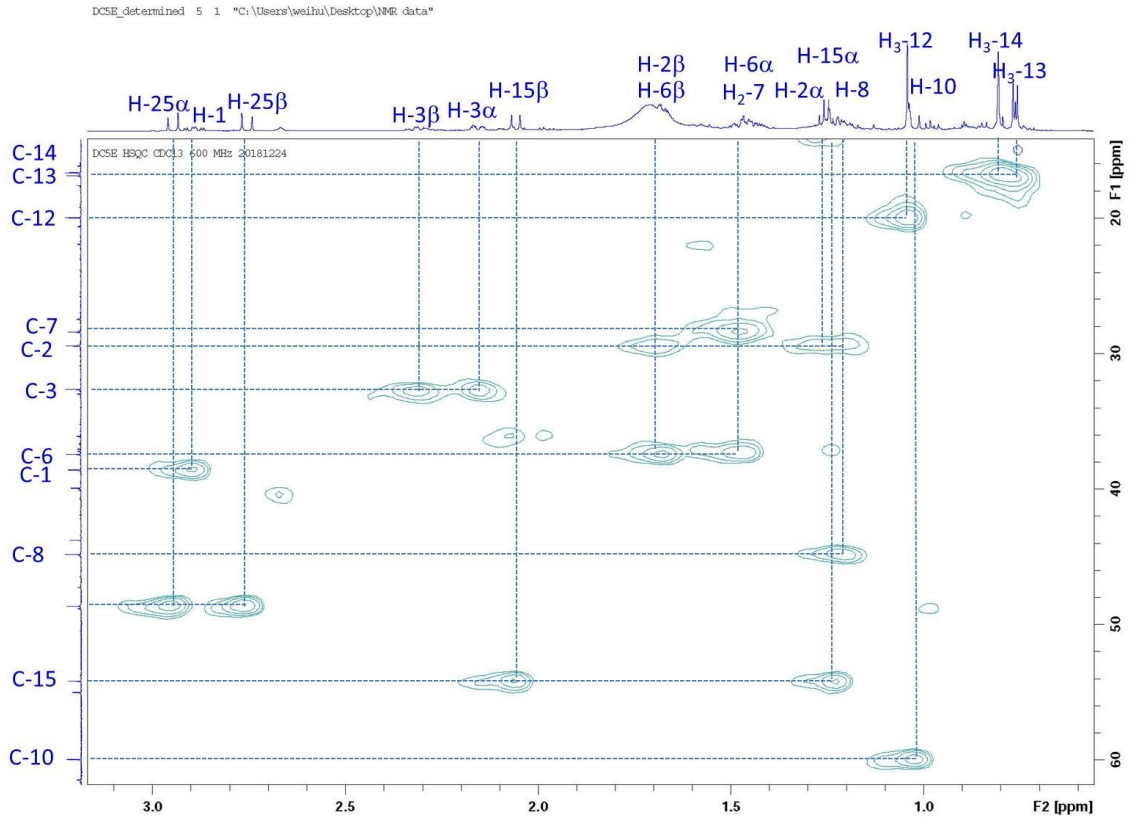


Figure S15. The HSQC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

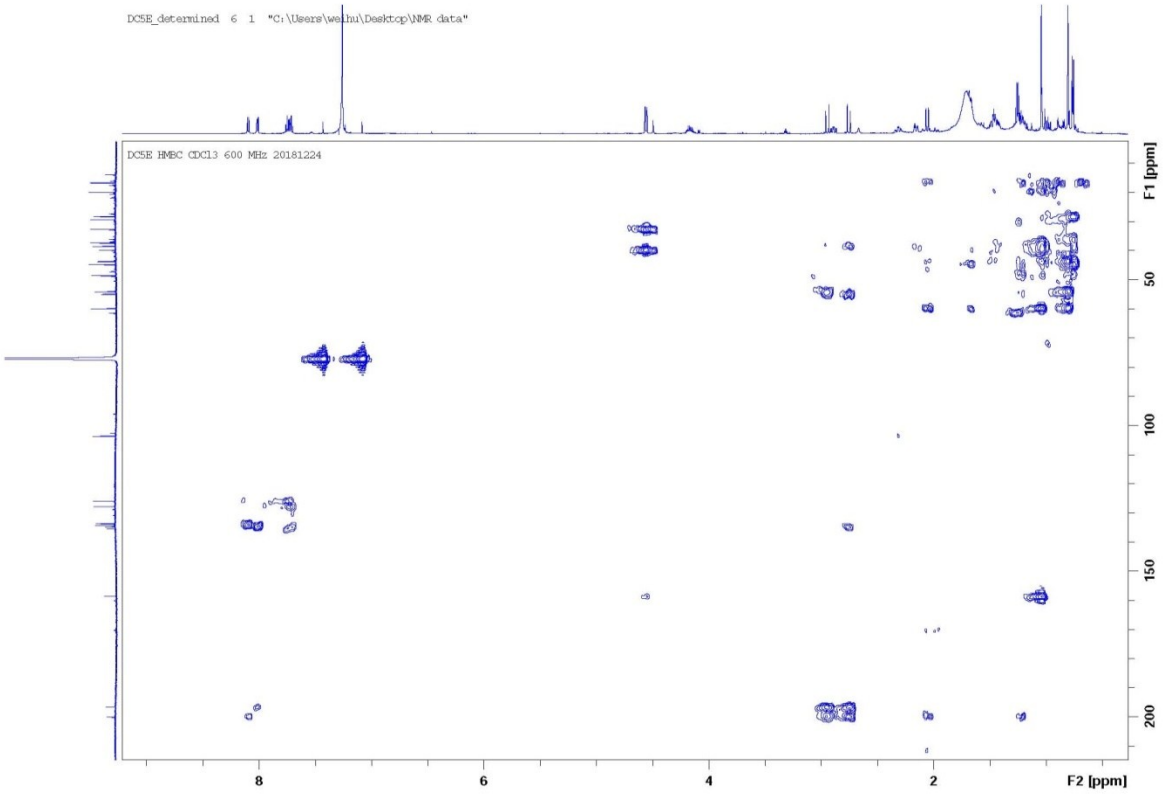


Figure S16. The HMBC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

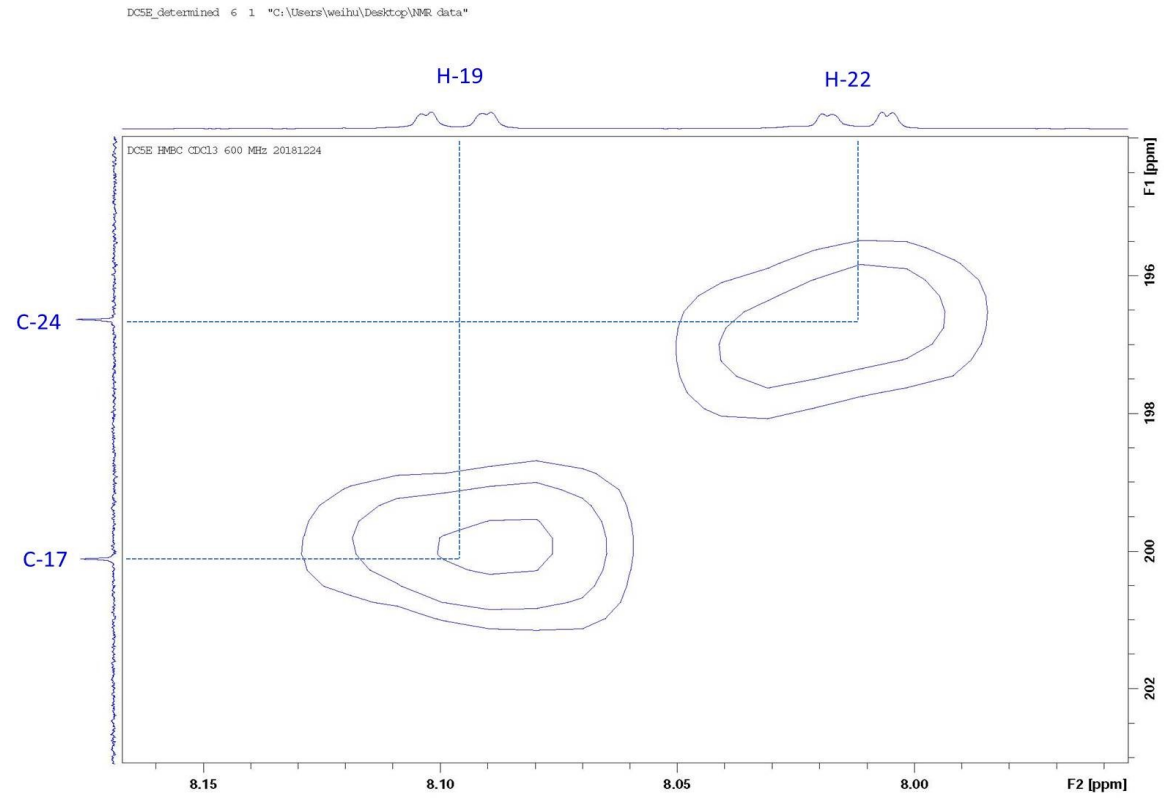


Figure S17. The HMBC spectrum of Spiroetherone A (**1**) in $CDCl_3$.

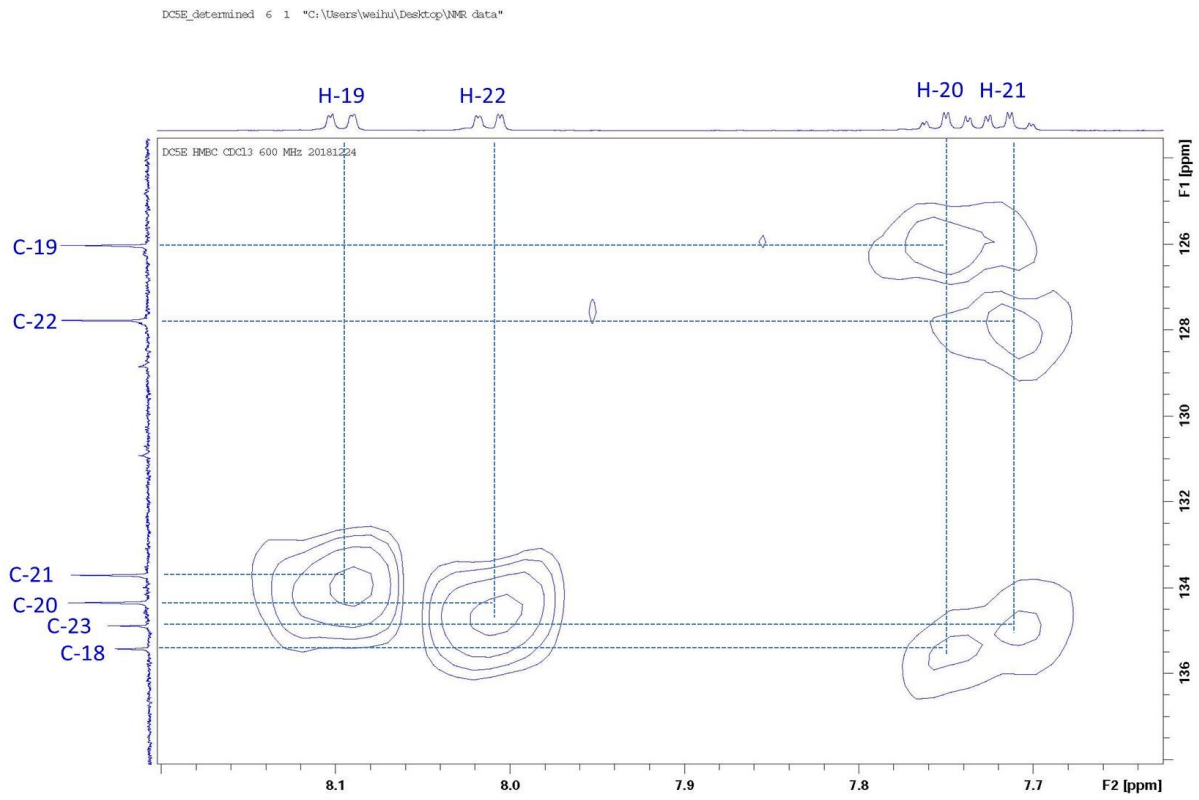


Figure S18. The HMBC spectrum of Spiroetherone A (**1**) in $CDCl_3$.

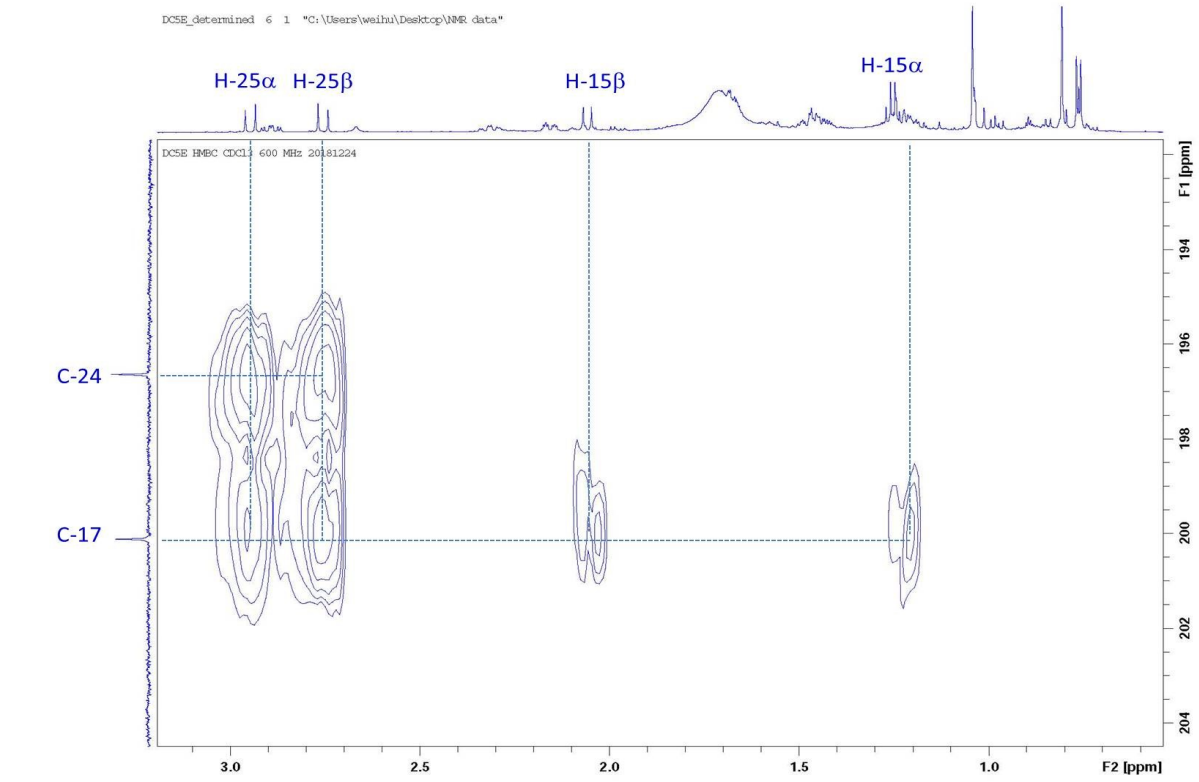


Figure S19. The HMBC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

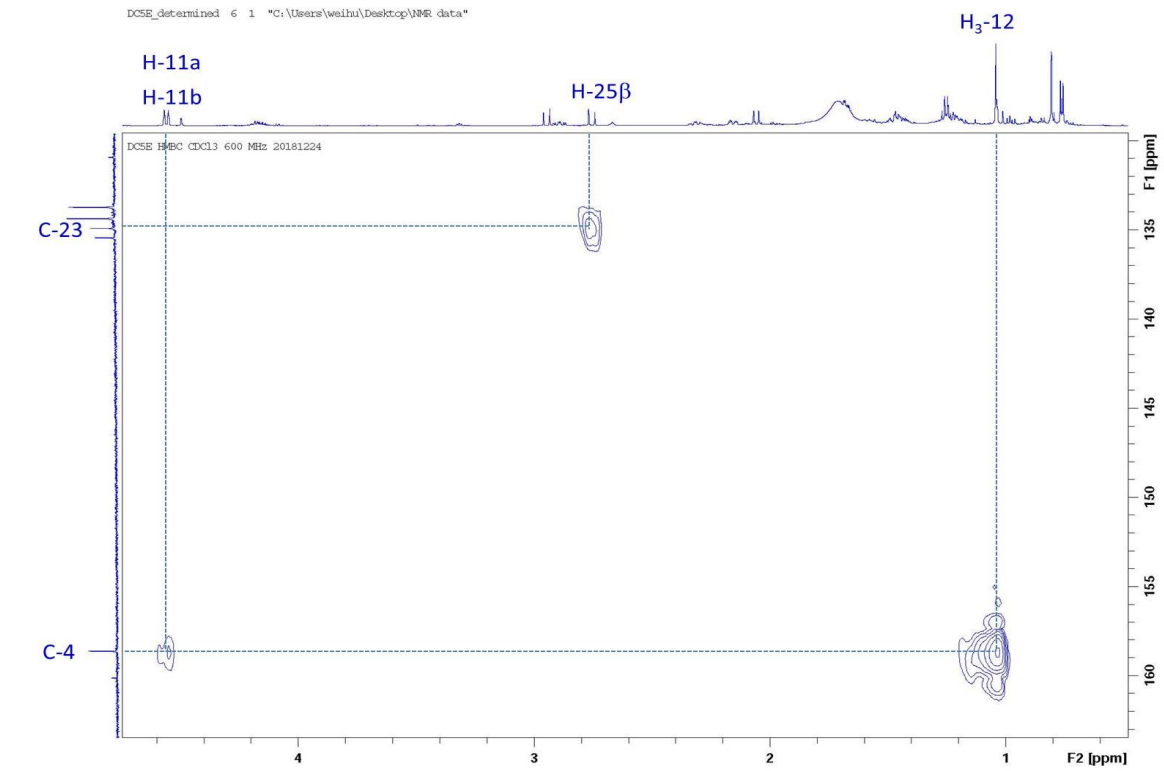


Figure S20. The HMBC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

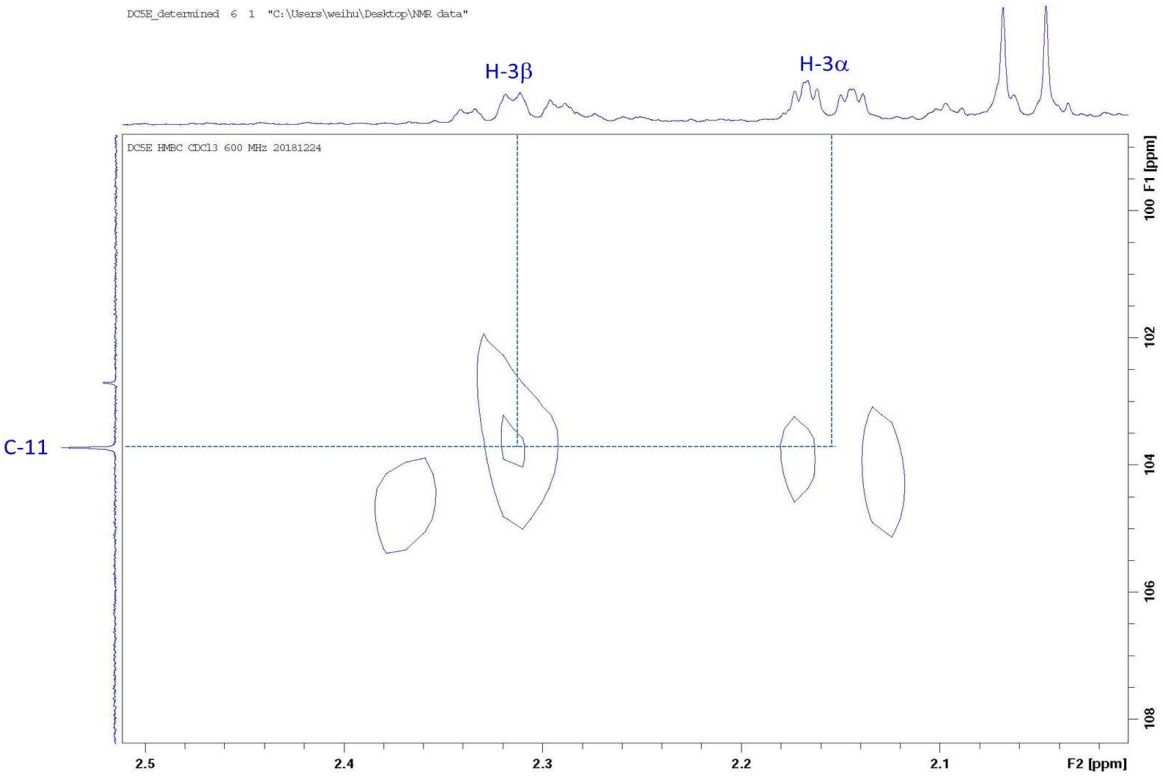


Figure S21. The HMBC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

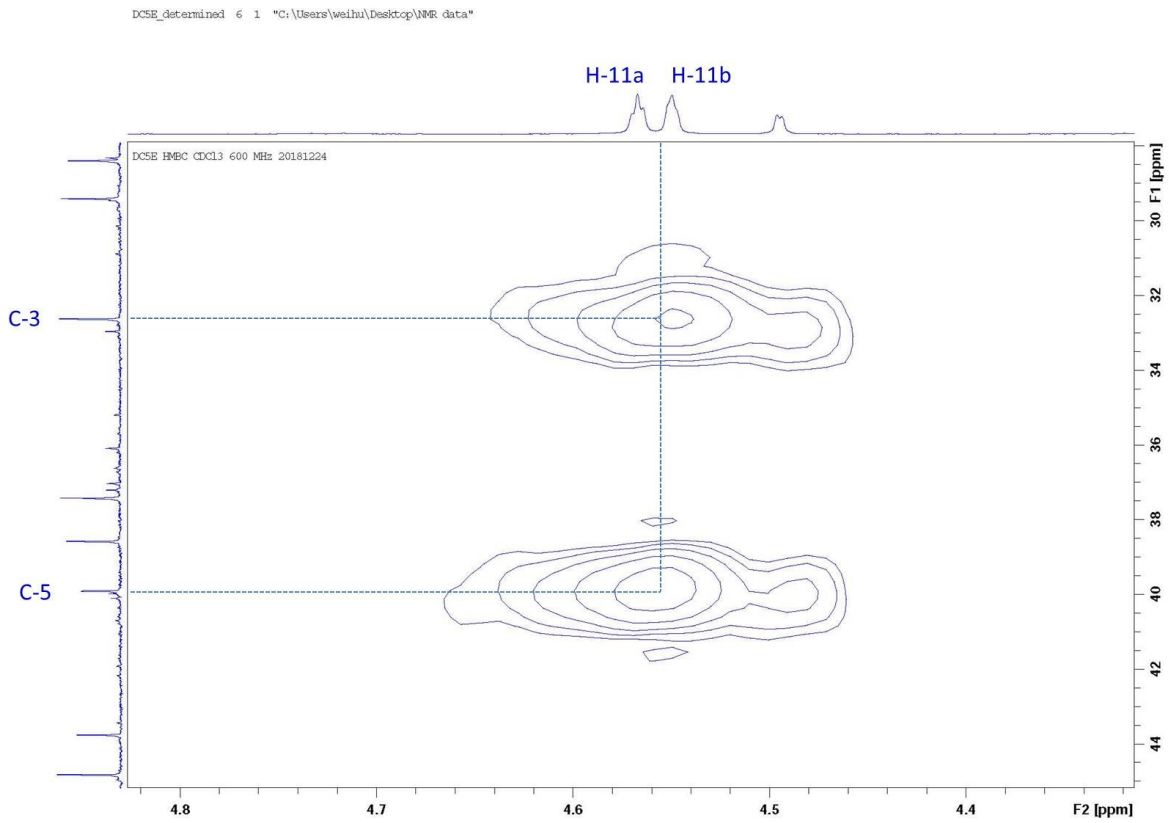


Figure S22. The HMBC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

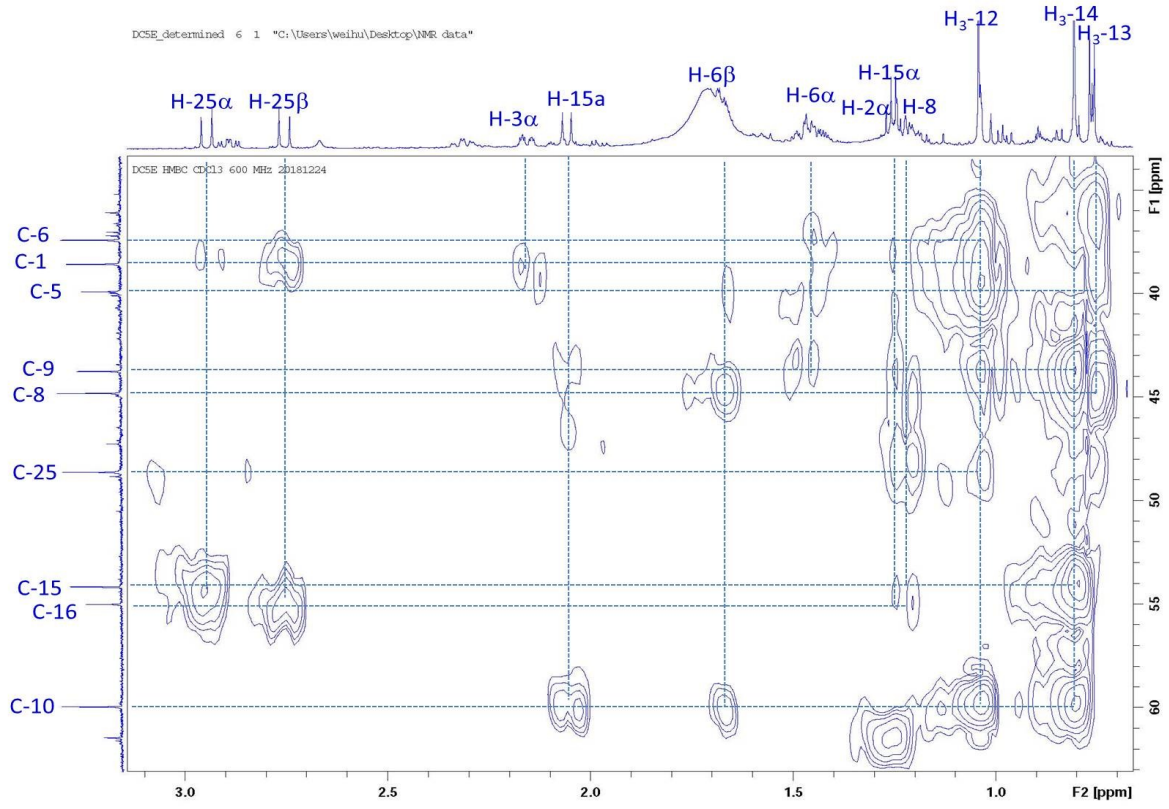


Figure S23. The HMBC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

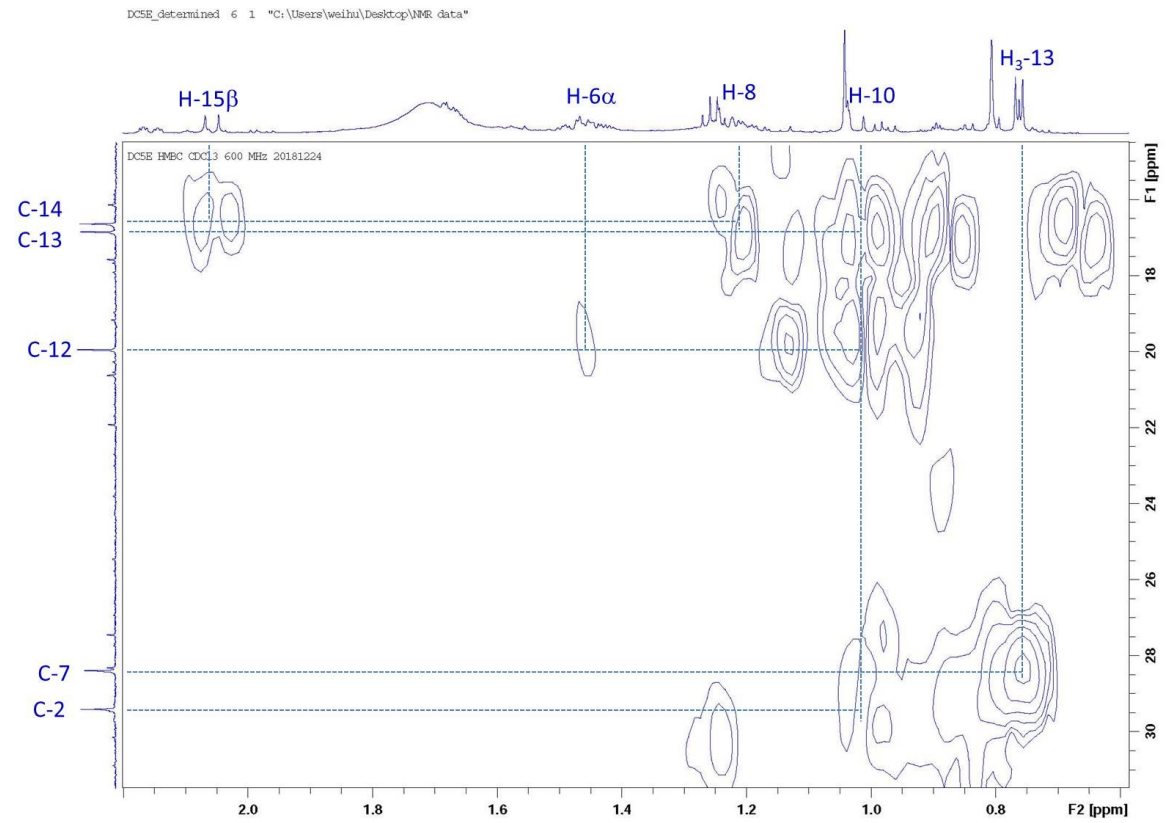


Figure S24. The HMBC Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

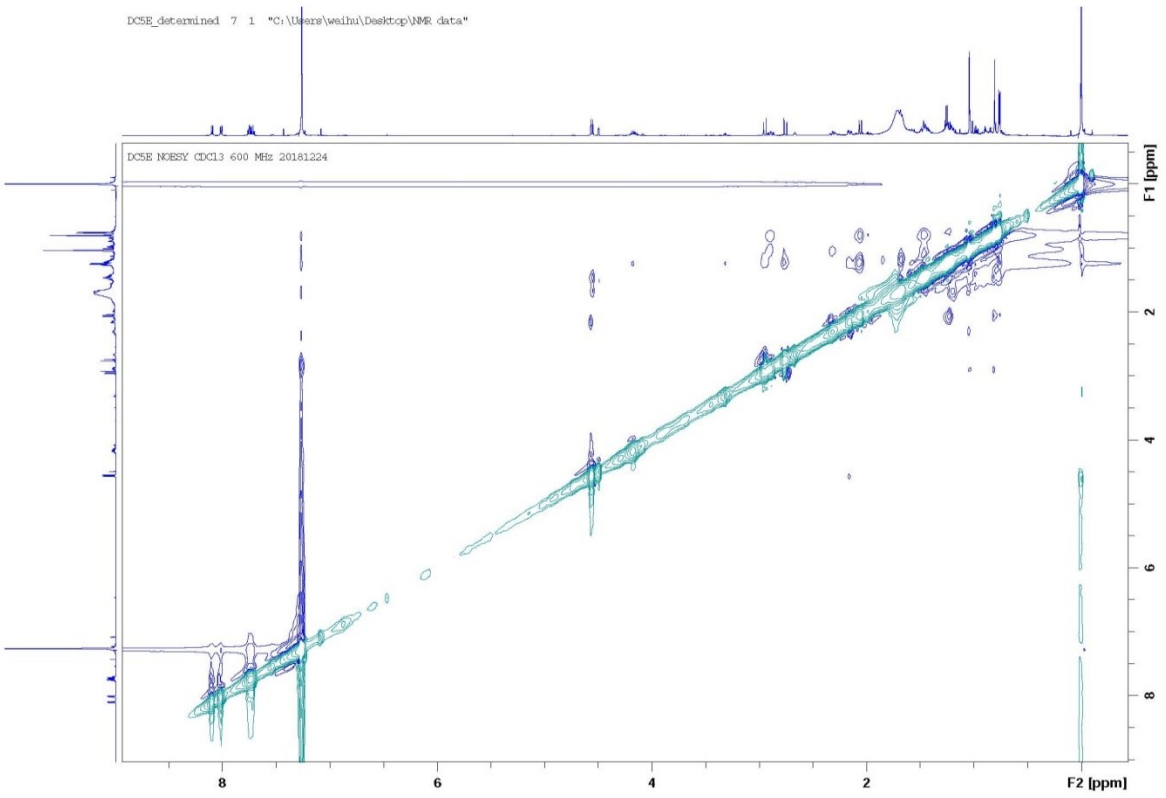


Figure S25. The NOESY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

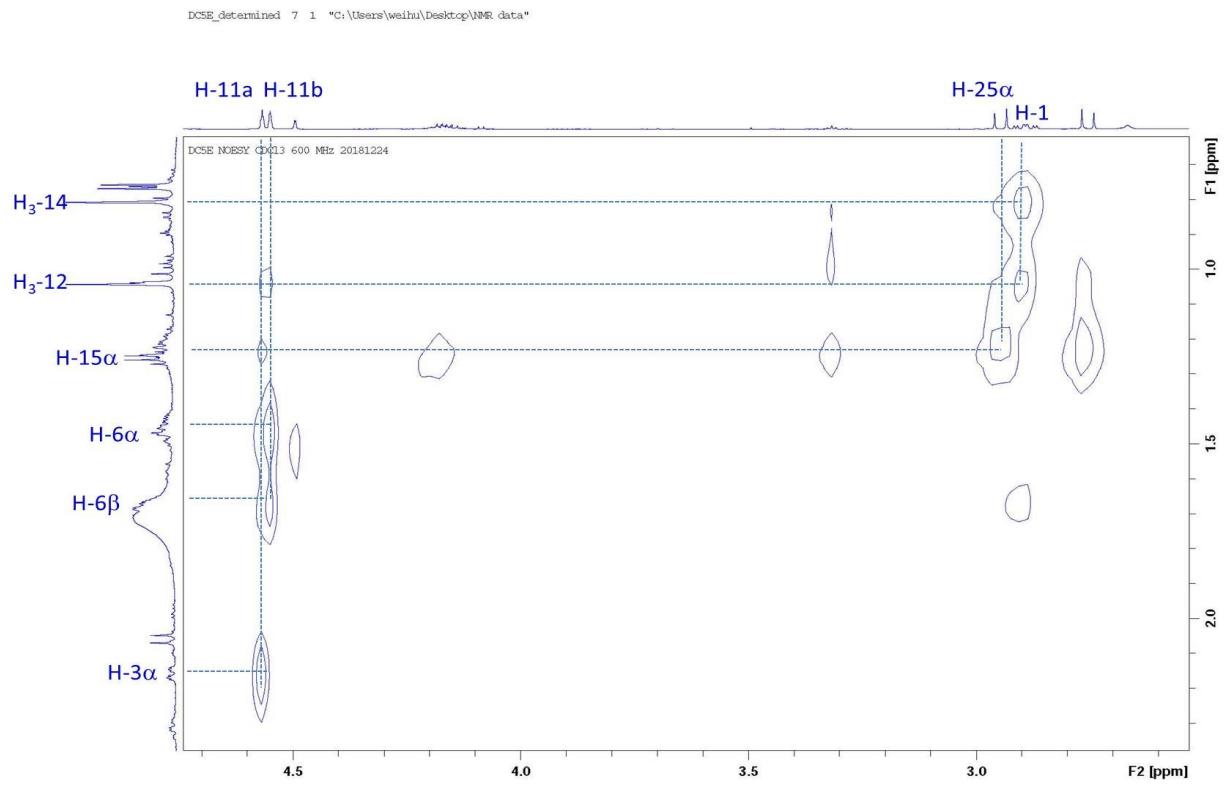


Figure S26. The NOESY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

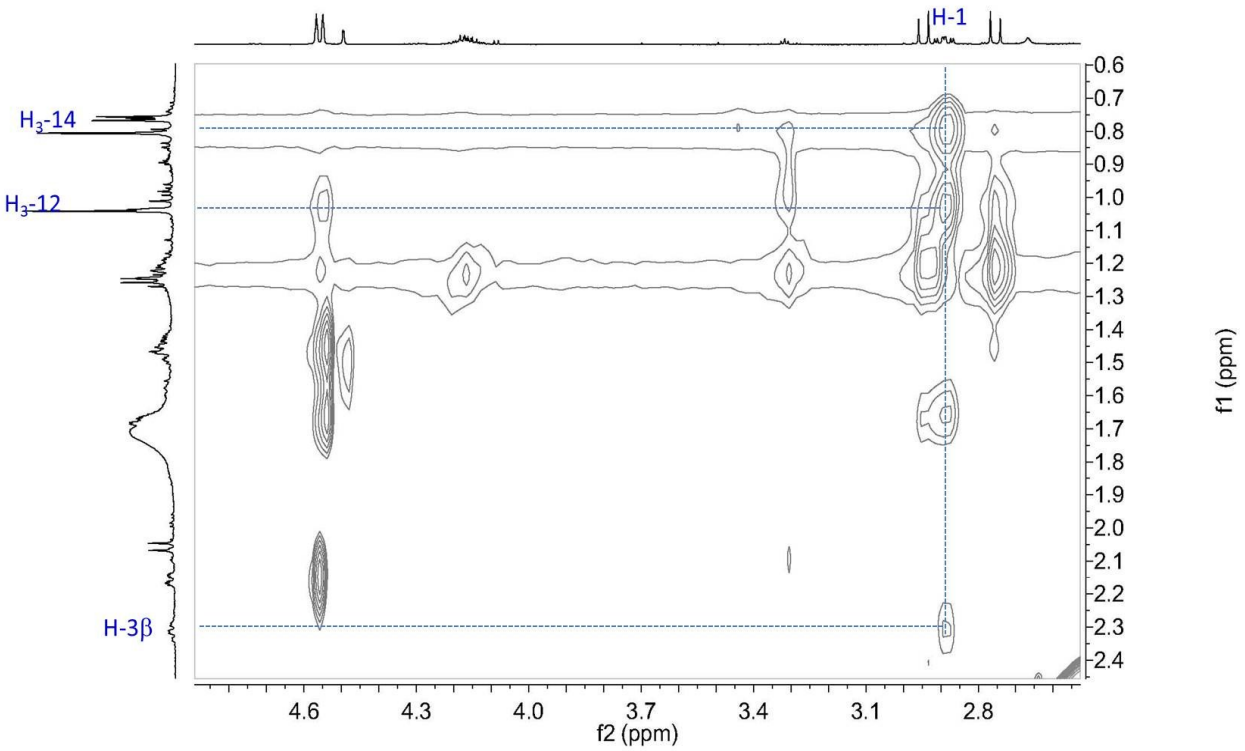


Figure S27. The NOESY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

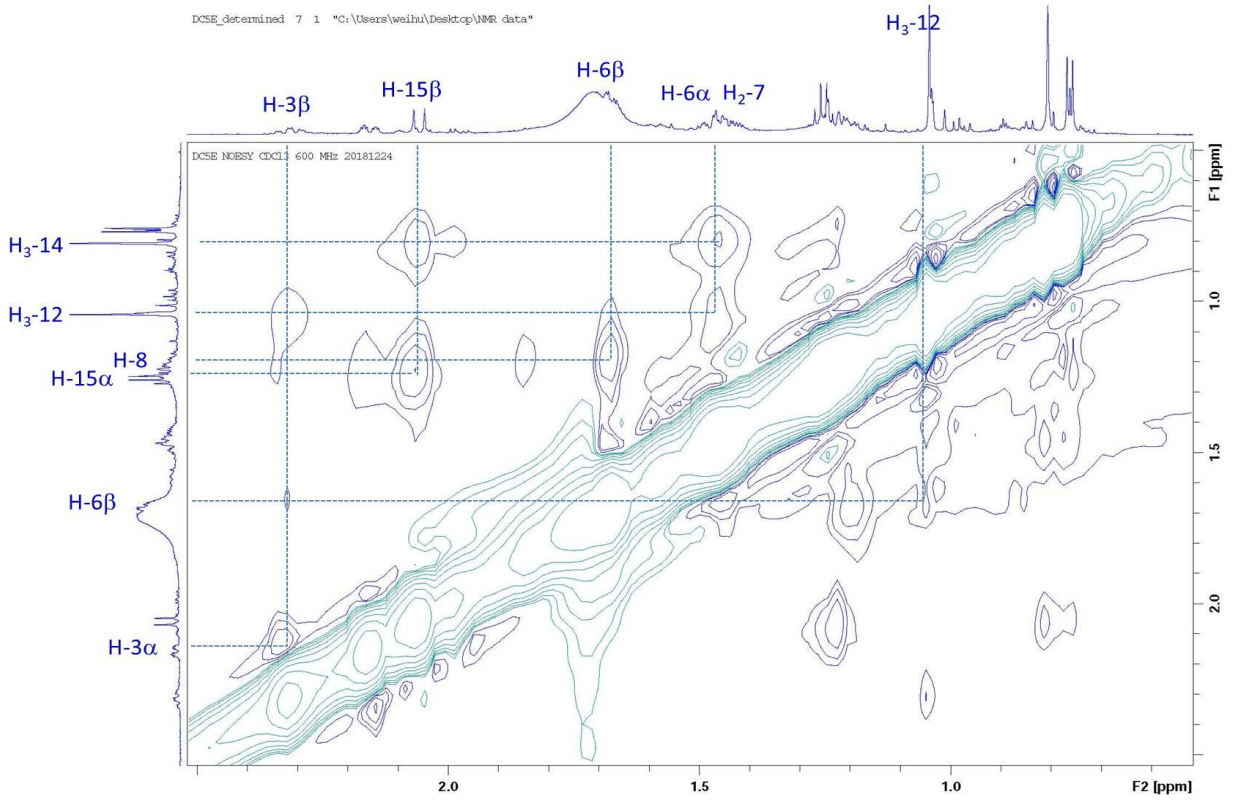


Figure S28. The NOESY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.

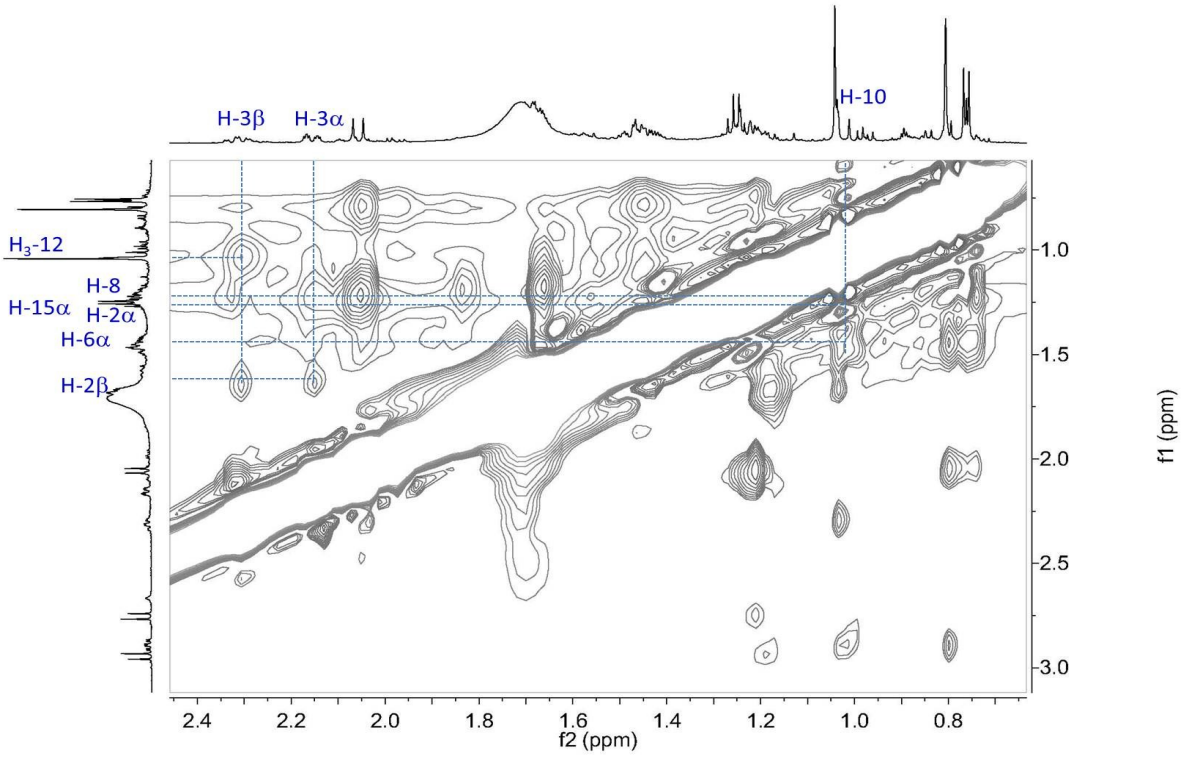
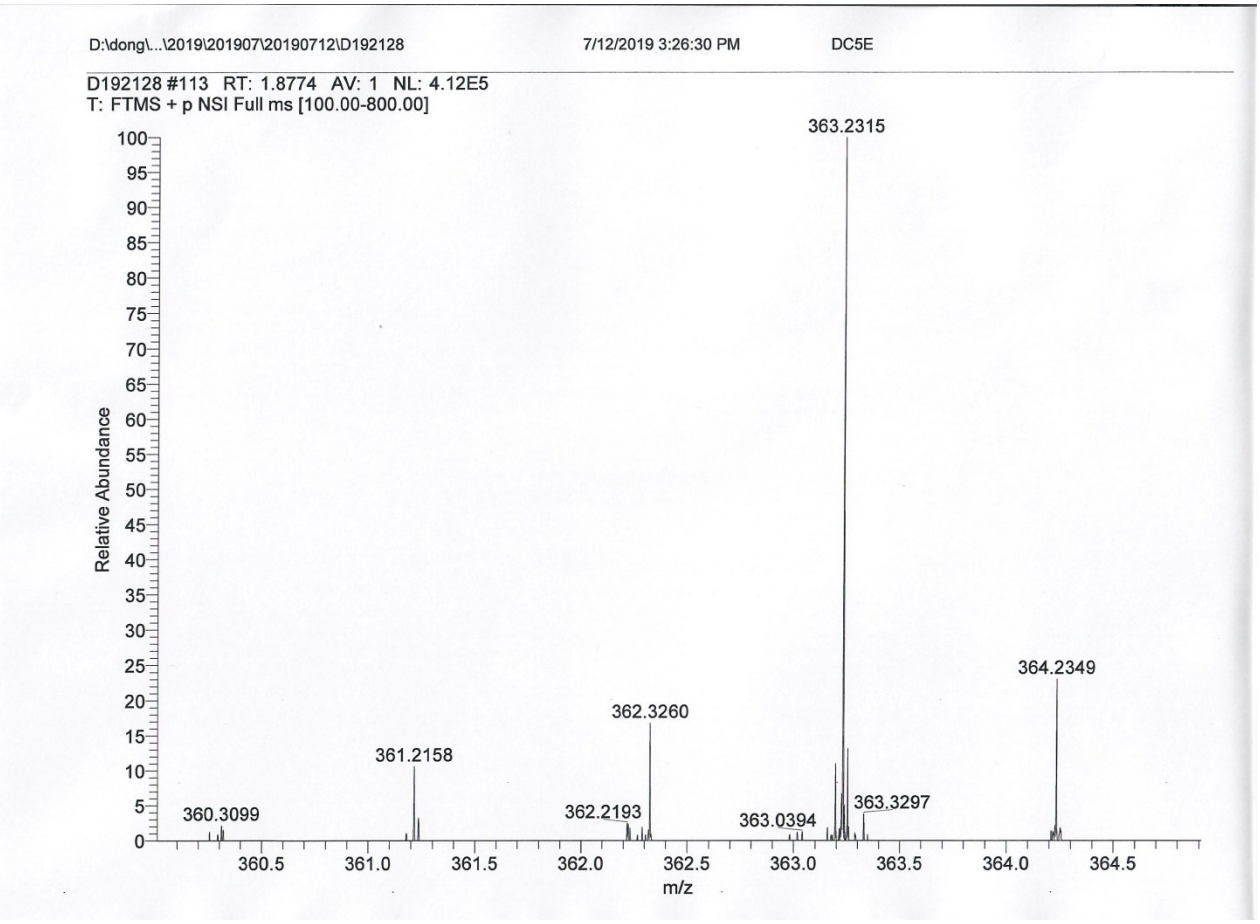


Figure S29. The NOESY Spectrum of Spiroetherone A (**1**) in $CDCl_3$.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National Center for Organic Mass Spectrometry in Shanghai
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: Thermo Fisher Scientific LTQ FT Ultra

Card Serial Number : D192128

Sample Serial Number: DC5E

Operator : DONG Date: 2019/07/12

Operation Mode: DART POSITIVE

Elemental composition search on mass 363.23

m/z= 358.23-368.23

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	363.2319	-0.84	10.5	$C_{25}H_{31}O_2$
	363.2312	0.95	1.5	$C_{17}H_{35}O_4N_2S$
	363.2310	1.39	-9.5	$C_9H_{36}O_4N_2F_5S$
	363.2310	1.55	-2.5	$C_{15}H_{37}ON_2F_2S_2$
	363.2321	-1.60	-6.5	$C_{12}H_{38}O_2N_2F_3S_2$
	363.2323	-2.20	-2.5	$C_{14}H_{36}O_5N_2FS$
	363.2306	2.74	3.5	$C_{20}H_{31}OF_4$
	363.2328	-3.39	2.5	$C_{20}H_{34}F_3S$
	363.2330	-3.99	6.5	$C_{22}H_{32}O_3F$

Figure S30. The DART-HRMS Spectrum of Spiroetherone A (**1**).

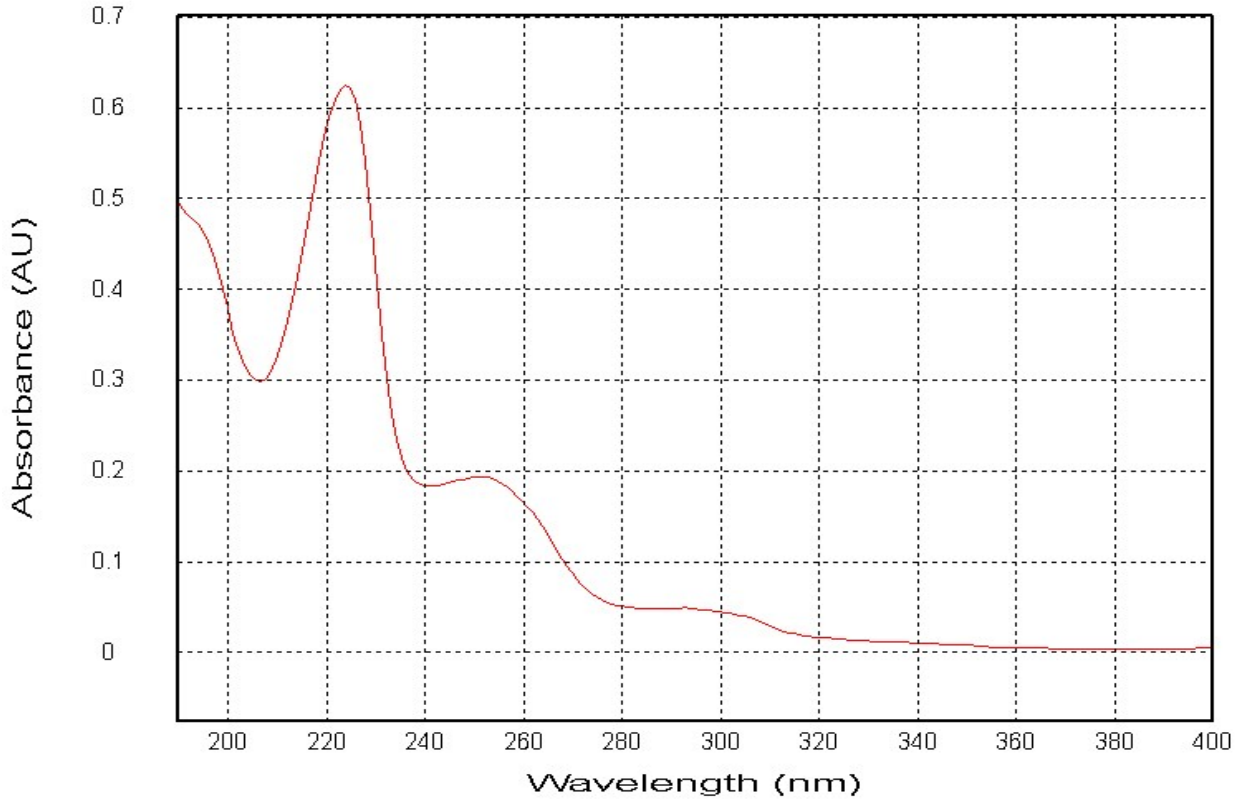


Figure S31. The UV Spectrum of Spiroetherone A (**1**) in MeOH.

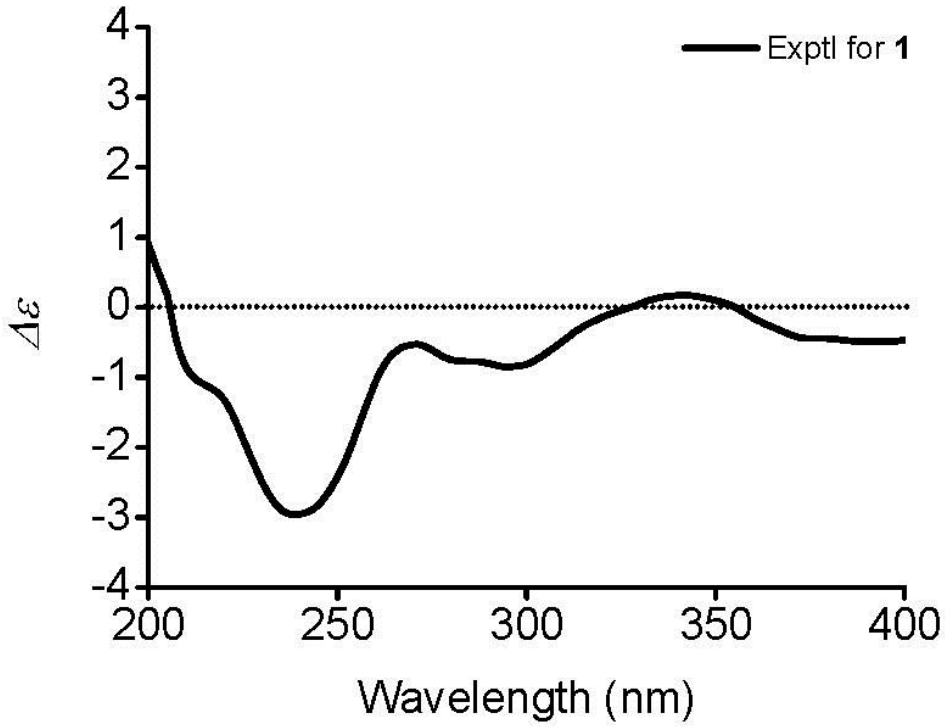


Figure S32. The Experimental ECD Spectrum of Spiroetherone A (**1**) in MeOH.

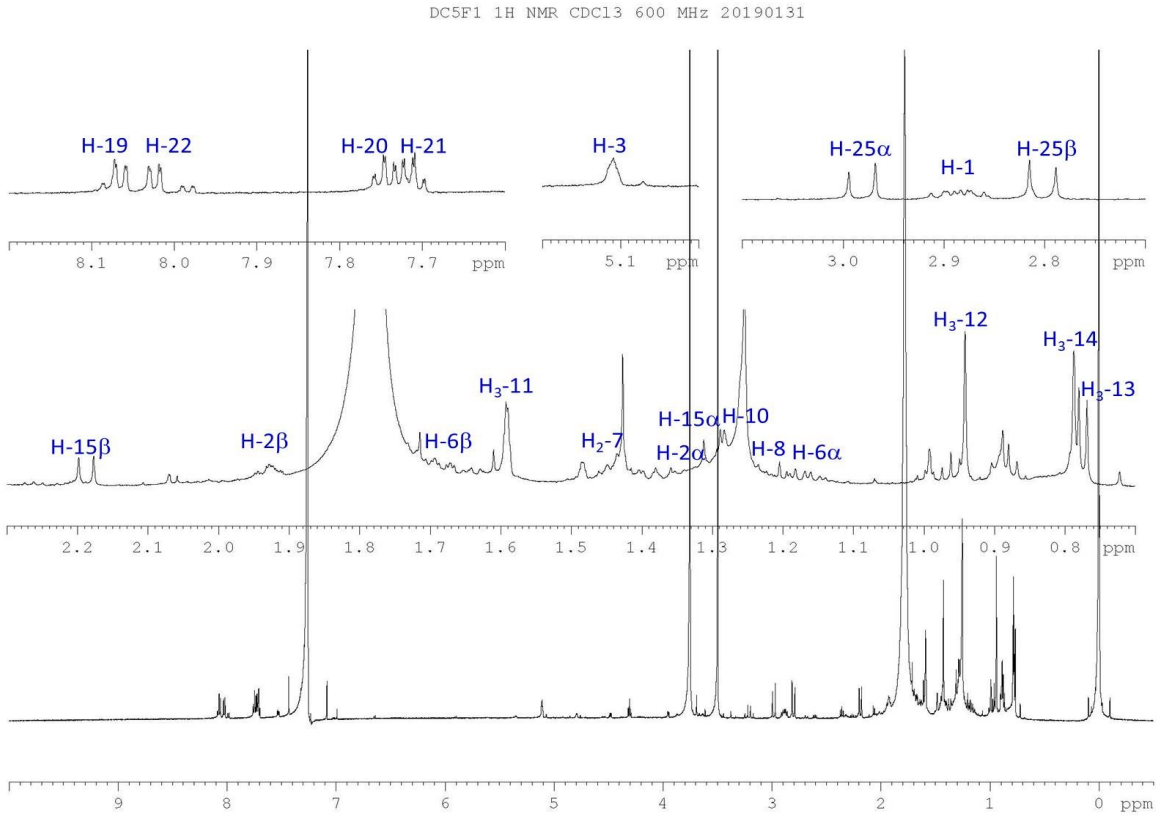


Figure S33. The ^{1}H NMR (600 MHz) Spectrum of Spiroetherone B (**2**) in $CDCl_3$.

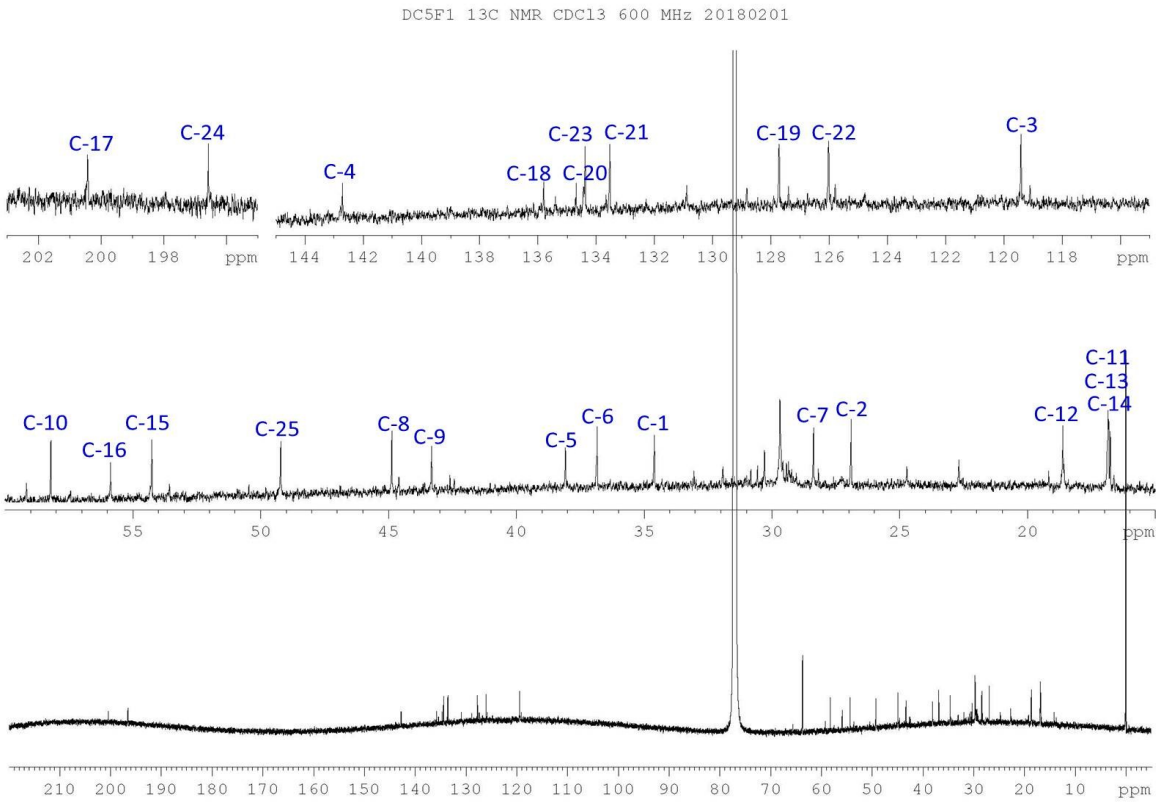


Figure S34. The ^{13}C NMR Spectrum of Spiroetherone B (**2**) in $CDCl_3$.

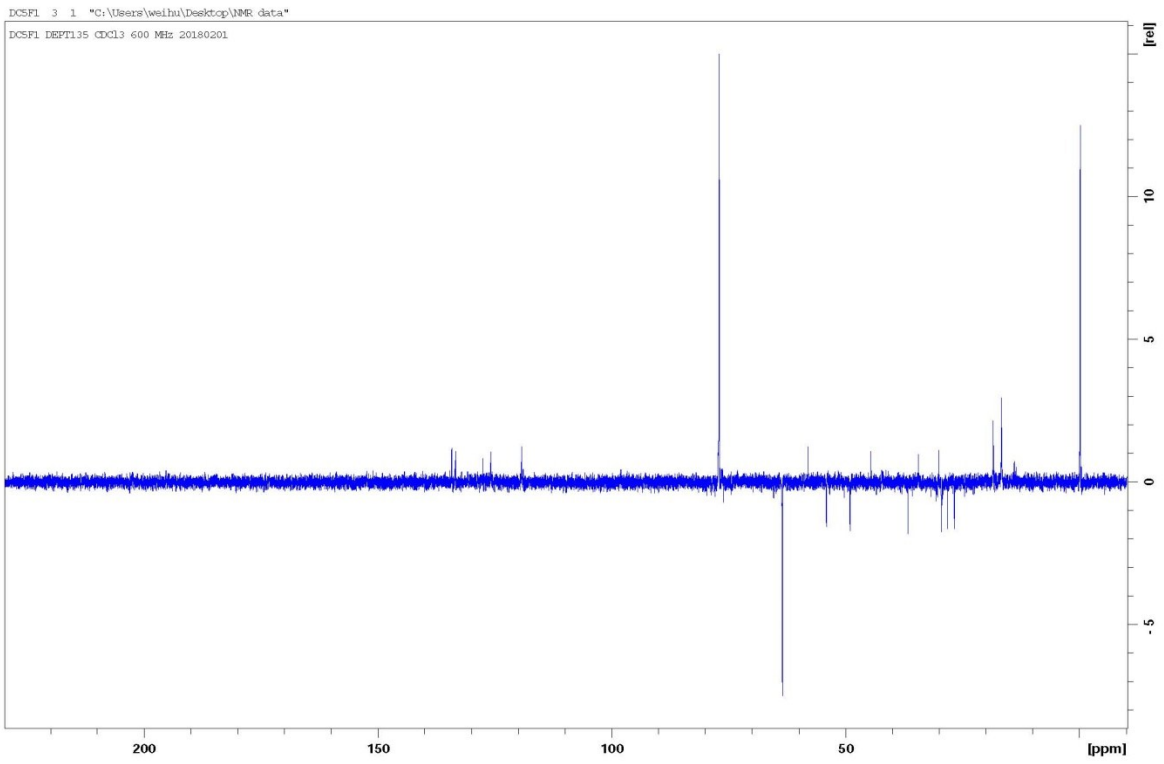


Figure S35. The DEPT135 Spectrum of Spiroetherone B (**2**) in $CDCl_3$.

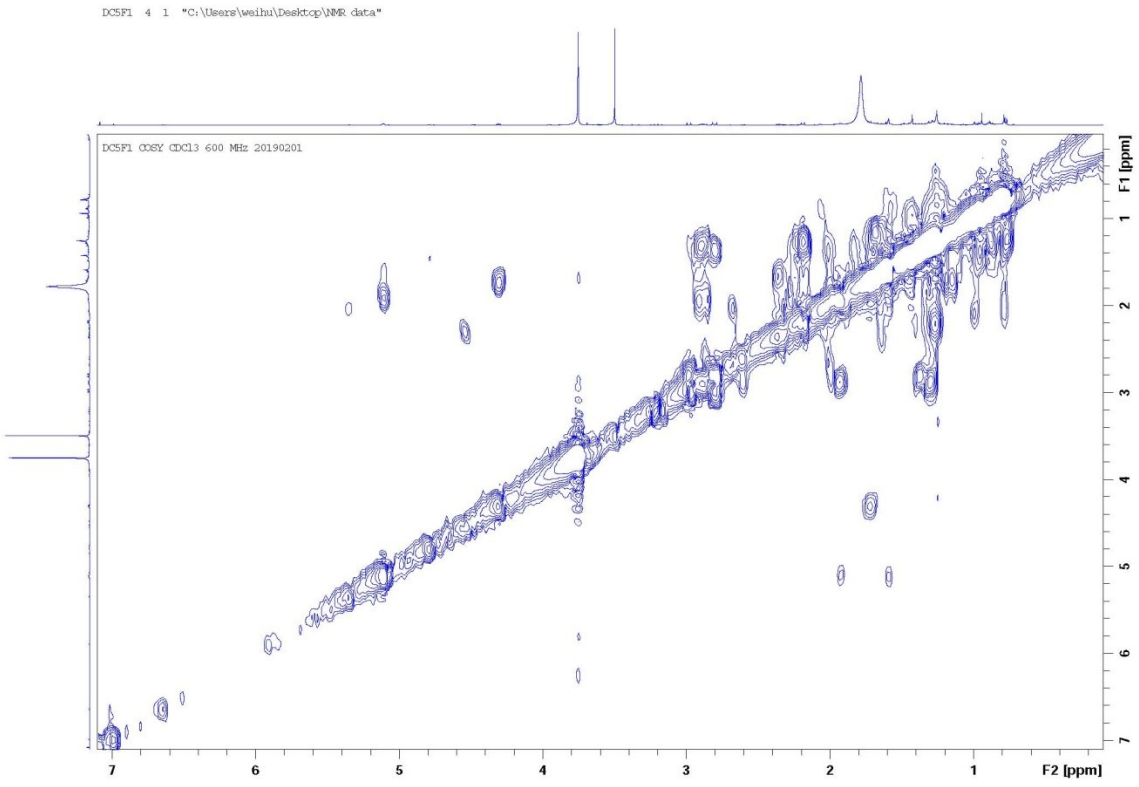


Figure S36. The ^{1}H-^{1}H COSY Spectrum of Spiroetherone B (**2**) in $CDCl_3$.

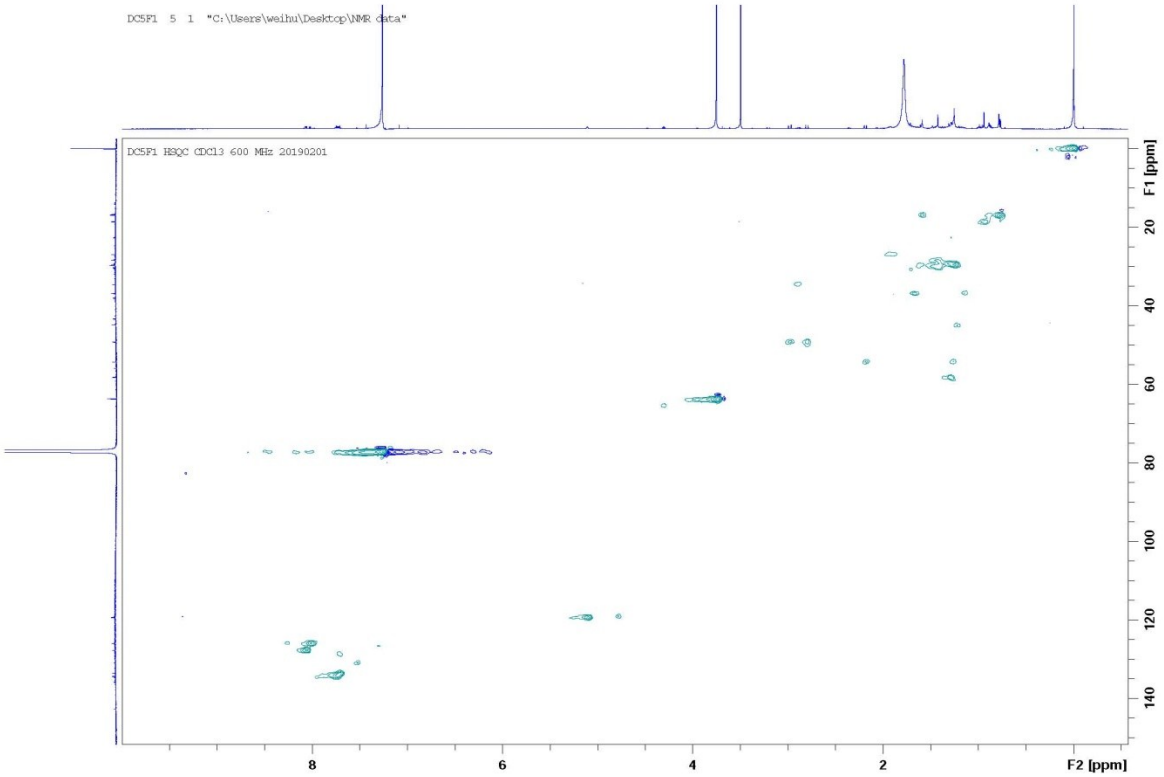


Figure S37. The HSQC Spectrum of Spiroetherone B (**2**) in $CDCl_3$.

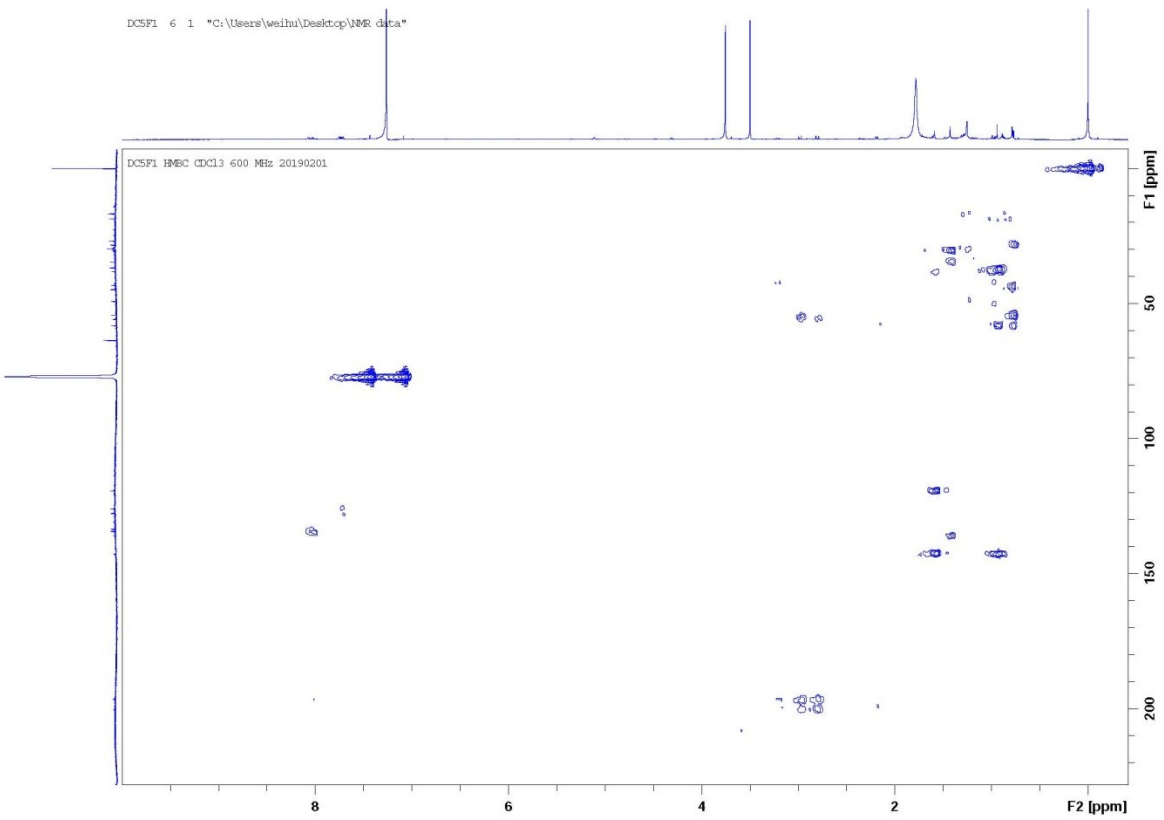


Figure S38. The HMBC Spectrum of Spiroetherone B (**2**) in $CDCl_3$.

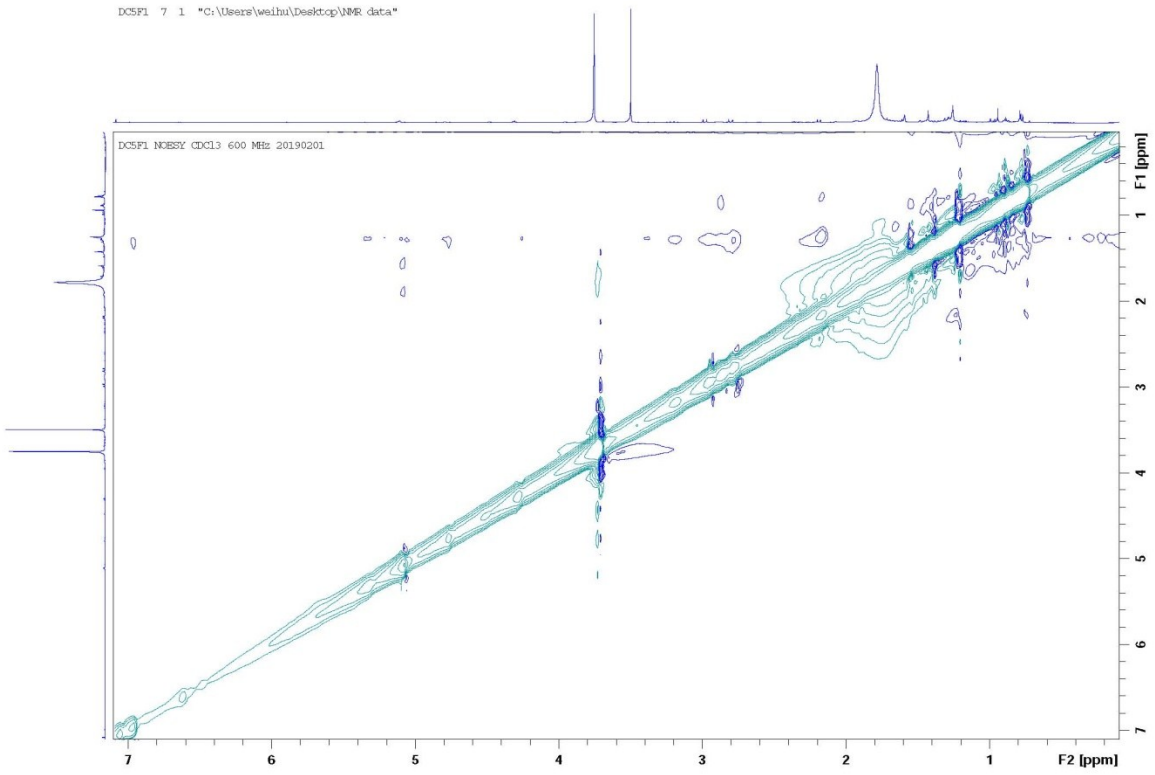
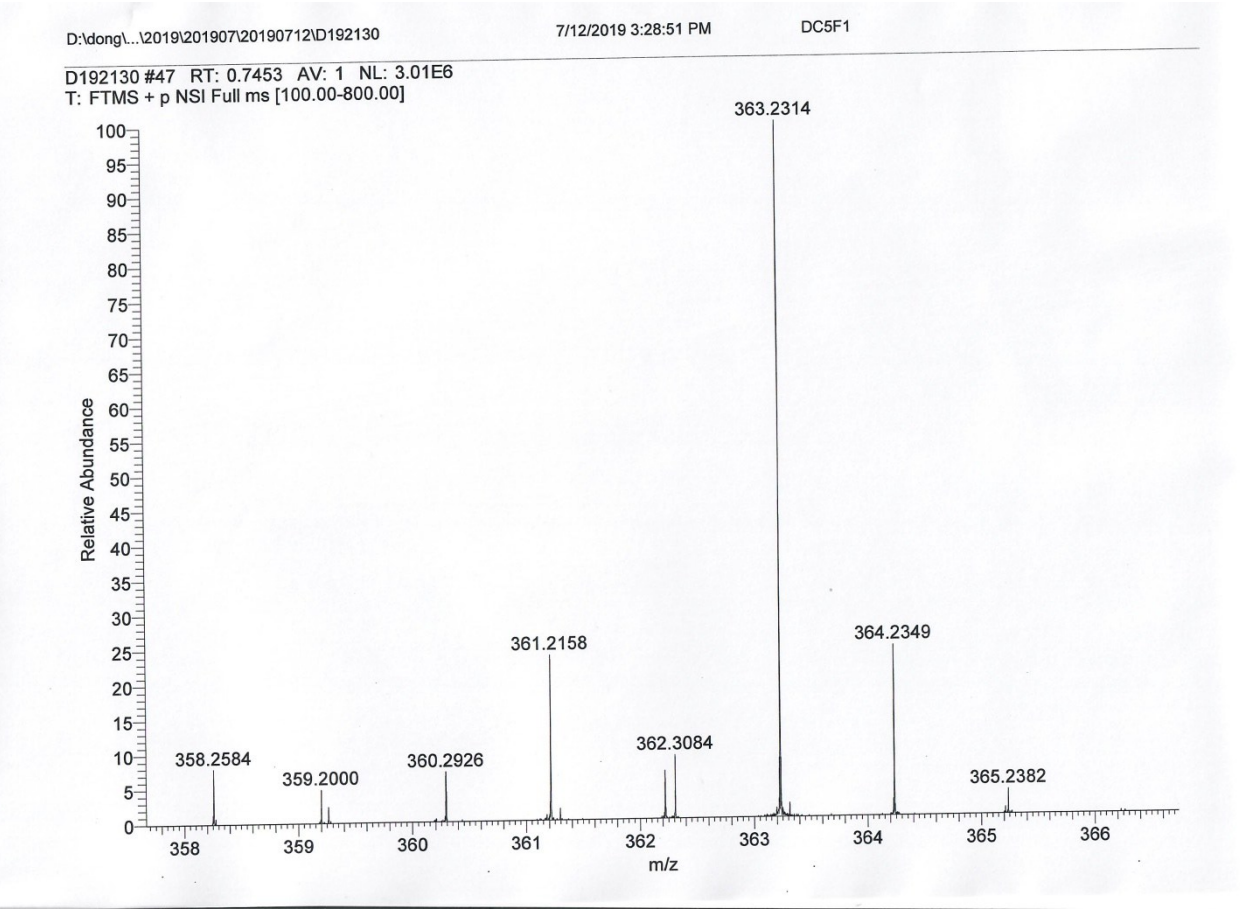


Figure S39. The NOESY Spectrum of Spiroetherone B (**2**) in $CDCl_3$.


D:\dong\...\2019\201907\20190712\D192130
7/12/2019 3:28:51 PM
DC5F1
D192130 #47 RT: 0.7453 AV: 1 NL: 3.01E6
T: FTMS + p NSI Full ms [100.00-800.00]
Relative Abundance
m/z
363.2314
364.2349
361.2158
362.3084
358.2584
359.2000
360.2926
365.2382

National Center for Organic Mass Spectrometry in Shanghai
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: Thermo Fisher Scientific LTQ FT Ultra

Card Serial Number : D192130

Sample Serial Number: DC5F1

Operator : DONG Date: 2019/07/12

Operation Mode: DART POSITIVE

Elemental composition search on mass 363.23

m/z= 358.23-368.23

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
363.2314	363.2312	0.43	1.5	$C_{17}H_{35}O_4N_2S$
	363.2310	0.87	-9.5	$C_9H_{36}O_4N_2F_5S$
	363.2317	-0.93	-0.5	$C_{17}H_{32}O_2F_5$
	363.2310	1.02	-2.5	$C_{15}H_{37}ON_2F_2S_2$
	363.2319	-1.37	10.5	$C_{25}H_{31}O_2$
	363.2321	-2.12	-6.5	$C_{12}H_{38}O_2N_2F_3S_2$
	363.2306	2.22	3.5	$C_{20}H_{31}OF_4$
	363.2323	-2.72	-2.5	$C_{14}H_{36}O_5N_2FS$
	363.2328	-3.92	2.5	$C_{20}H_{34}F_3S$
	363.2299	4.01	-5.5	$C_{12}H_{35}O_3N_2F_4S$

Figure S40. The DART-HRMS Spectrum of Spiroetherone B (**2**).

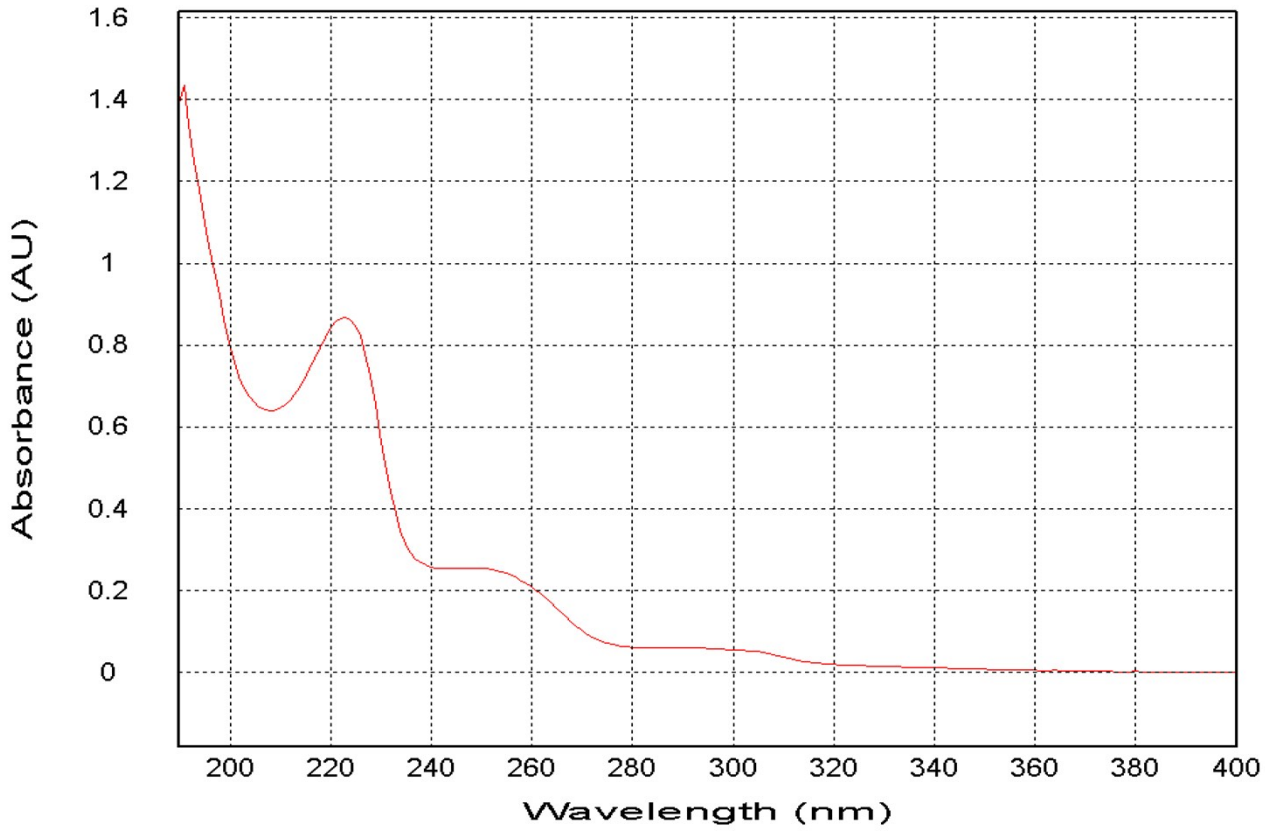


Figure S42. The UV Spectrum of Spiroetherone B (**2**) in MeOH.

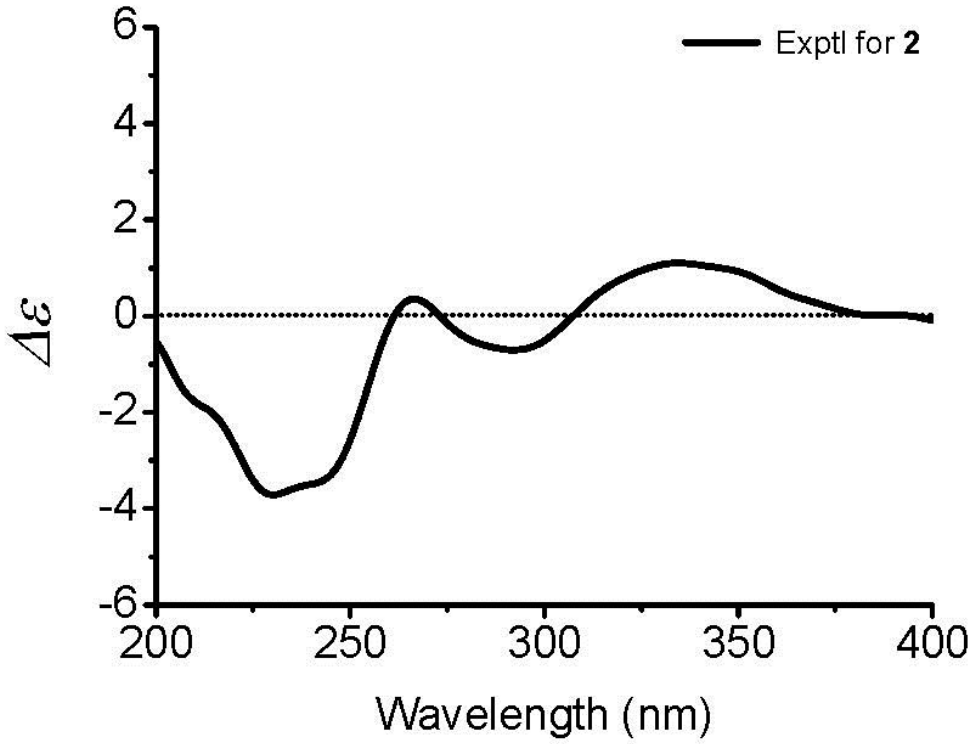


Figure S43. The Experimental ECD Spectrum of Spiroetherone B (**2**) in MeOH.