Spiroaspertrione A, a Bridged Spirocyclic Meroterpenoid as a Potent Potentiator of Oxacillin against Methicillin-Resistant *Staphylococcus aureus* from *Aspergillus* sp. TJ23

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Contents of Supporting Information

Biological Assay Supplement Data

Combination Susceptibility Test against MRSA

Table S1. Combination susceptibility test of compounds **1** and **2** with various antibacterial antibiotics against MRSA in Checkerboard assay.

Antibiotics	MIC (μg/mL)[a]			$\sum FIC_{1+A}$[c]	$\sum FIC_{2+A}$[c]
	Antibiotic alone (A)	A+ Cmpd **1**	A+ Cmpd **2**		
Oxacillin	32	1[b]	8	0.28*	0.75
Piperacillin	64	4	16	1.06	1.25
Chloramphenicol	16	4	16	1.25	2.0
Vancomycin	0.5	0.5	0.5	1.12	1.03

[a]The MIC of compounds **1** and **2** alone against MRSA were 4 μg/mL and 16 μg/mL, respectively.
[b]The MIC of oxacillin combined with compound **1** against MRSA was 1 μg/mL (oxacillin and **1** were co-dosed at sub-MIC levels, both 1 μg/mL).
*Compound **1** was determined to work synergistically with oxacillin, with a $\sum$FIC value of 0.28. However, it did not show any synergistic effect on other antibiotics. In addition, combination effect of piperacillin, chloramphenicol, and vancomycin with **1** against MRSA seem to be accumulative.
[c]$\sum FIC = FIC_{Cmpd} + FIC_{Antibiotic}$; FIC_{Cmpd} = [MIC_{Cmpd} in combination]/[MIC_{Cmpd} alone]; $FIC_{Antibiotic}$ = [$MIC_{Antibiotic}$ in combination]/[$MIC_{Antibiotic}$ alone]. The combination is considered synergistic when the $\sum$FIC value is ≤ 0.5, accumulative or indifferent when the $\sum$FIC value is > 0.5 but < 2, and antagonistic when the $\sum$FIC is ≥ 2.

Time kill curves of compound 1 and/or oxacillin against MRSA

Table S2. Time kill curves of compound **1** and/or oxacillin against MRSA

Time (h)	Control	1/32MIC_{Ox} + 1/4MIC_1	1/32 MIC_{Ox}	1/4 MIC_1	1/2 MIC_1	MIC_1	2 MIC_1	4 MIC_1	MIC_{Ox}
0	5.50 ± 0.09	5.52 ± 0.25	5.50 ± 0.22	5.50 ± 0.15	5.50 ± 0.10	5.52 ± 0.10	5.50 ± 0.17	5.55 ± 0.11	5.50 ± 0.32
2	6.05 ± 0.25	5.26 ± 0.54	5.59 ± 0.10	5.65 ± 0.27	5.60 ± 0.30	5.34 ± 0.24	5.31 ± 0.25	5.27 ± 0.13	5.34 ± 0.20
4	7.10 ± 0.21	5.15 ± 0.17	6.15 ± 0.37	5.81 ± 0.21	5.51 ± 0.27	5.15 ± 0.26	4.98 ± 0.11	4.81 ± 0.25	5.22 ± 0.15
6	7.98 ± 0.17	4.74 ± 0.23	6.72 ± 0.39	5.92 ± 0.25	5.32 ± 0.41	4.92 ± 0.44	4.91 ± 0.23	4.70 ± 0.20	4.78 ± 0.21
8	8.41 ± 0.20	4.22 ± 0.21	7.51 ± 0.18	6.27 ± 0.13	5.50 ± 0.25	4.80 ± 0.27	5.10 ± 0.17	4.45 ± 0.15	5.15 ± 0.19
12	9.07 ± 0.16	5.02 ± 0.15	8.16 ± 0.15	6.50 ± 0.15	5.72 ± 0.18	5.13 ± 0.23	5.14 ± 0.22	5.05 ± 0.25	5.90 ± 0.09
24	10.10±0.19	5.36±0.31*	9.06±0.25*	7.47±0.11*	7.25 ± 0.17	5.55 ± 0.17	5.41 ± 0.12	5.11 ± 0.18	8.19 ± 0.22

*At 24h time point, the combination of compound **1** (1/4 MIC_1, 1 μg/mL) and oxacillin (1/32 MIC_{Ox}, 1 μg/mL) resulted in log CFU reductions of 3.70 and 2.11 for using oxacillin (1 μg/mL) or 1 (1 μg/mL) alone.

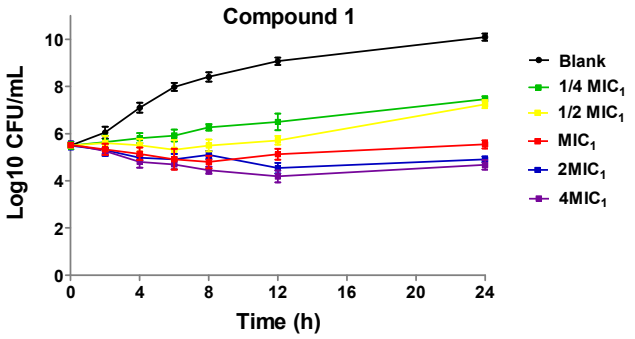


Figure S1 Time kill curves of compound **1** against MRSA. Time kill curves of compound **1**($\geq MIC_1$) inhibited the growth of bacteria entering to the exponential phase resulting nearly constant bacterial counts, which indicate that **1** is bacteriostatic in nature.

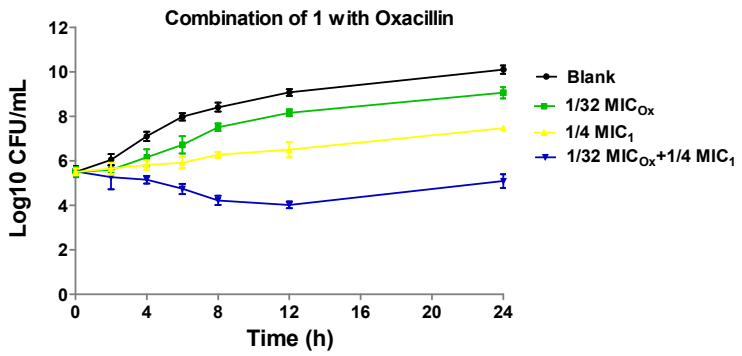


Figure S2 Time kill curves of combination of **1** and oxacillin against MRSA. Combination of **1** (1 μg/mL) with oxacillin (**1** μg/mL) showed bacteriostatic effects, with a slight reduction of colony counts after 2 h.

Molecular Docking

Table S3 Predicted binding free energies of compounds **1** and **2** with five PBPs of MRSA, which considered to be important or possible media for staphylococcal β-lactam resistance. (Surflx-Dock scores)[a]

PDBID	Protein name	Compounds	
		1	**2**
5TRO	PBP1	4.473	3.352
2ZC3	PBP2	1.094	1.579
4CJN	PBP2a[§]	5.516*	4.435
3PBR	PBP3	4.419	4.258
3HUM	PBP4	3.416	2.135

[a]Docking score/interaction potential of compounds with targets (kcal/mol).
*The docking scores predicted that PBP2a showed significantly higher binding affinity for **1** than other PBPs.
[§]The predicted docking score of the reported PBP2a inhibitors Ceftaroline and Meropenem were 6.017 and 5.602.

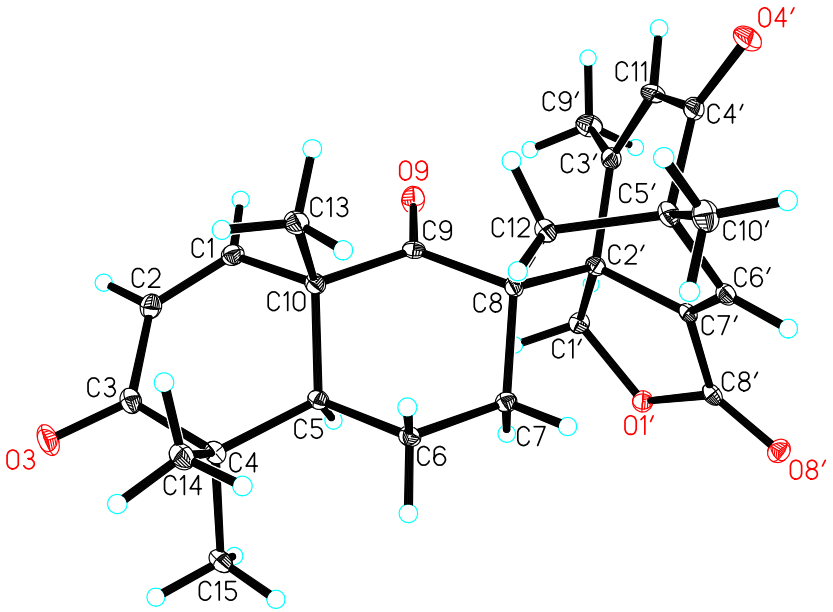


Figure S3. View of the molecule of **1** with the atom-labelling scheme (displacement ellipsoids are drawn at the 30% probability level).

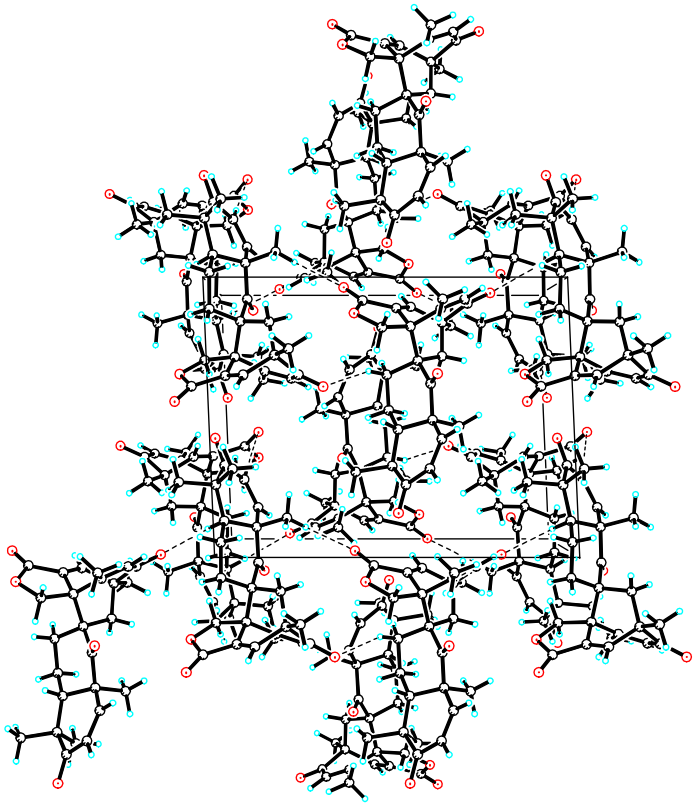


Figure S4. View of the hydrogen-bonded motif of **1** (hydrogen-bonds are shown as dashed lines).

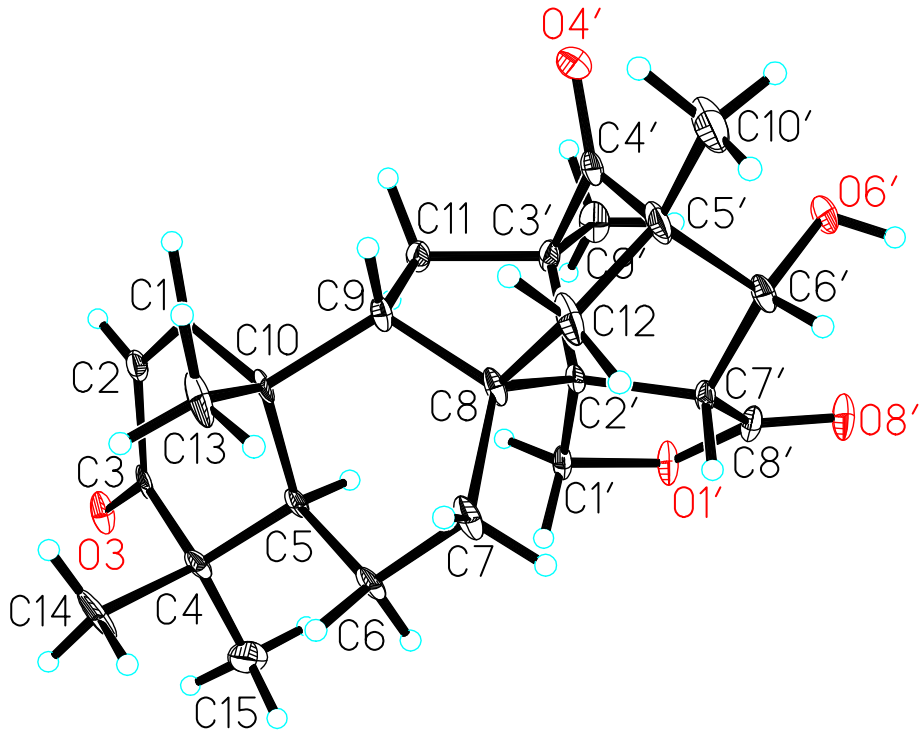


Figure S5. View of the molecule of **2** with the atom-labelling scheme (displacement ellipsoids are drawn at the 30% probability level).

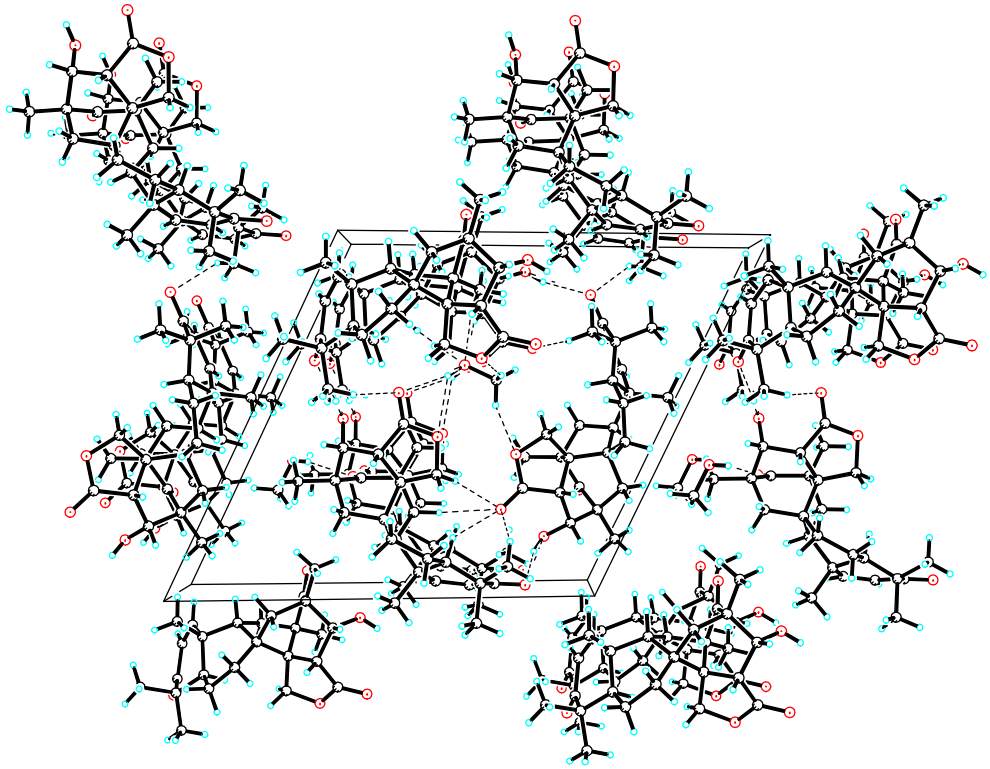


Figure S6. View of the hydrogen-bonded motif of **2** (hydrogen-bonds are shown as dashed lines).

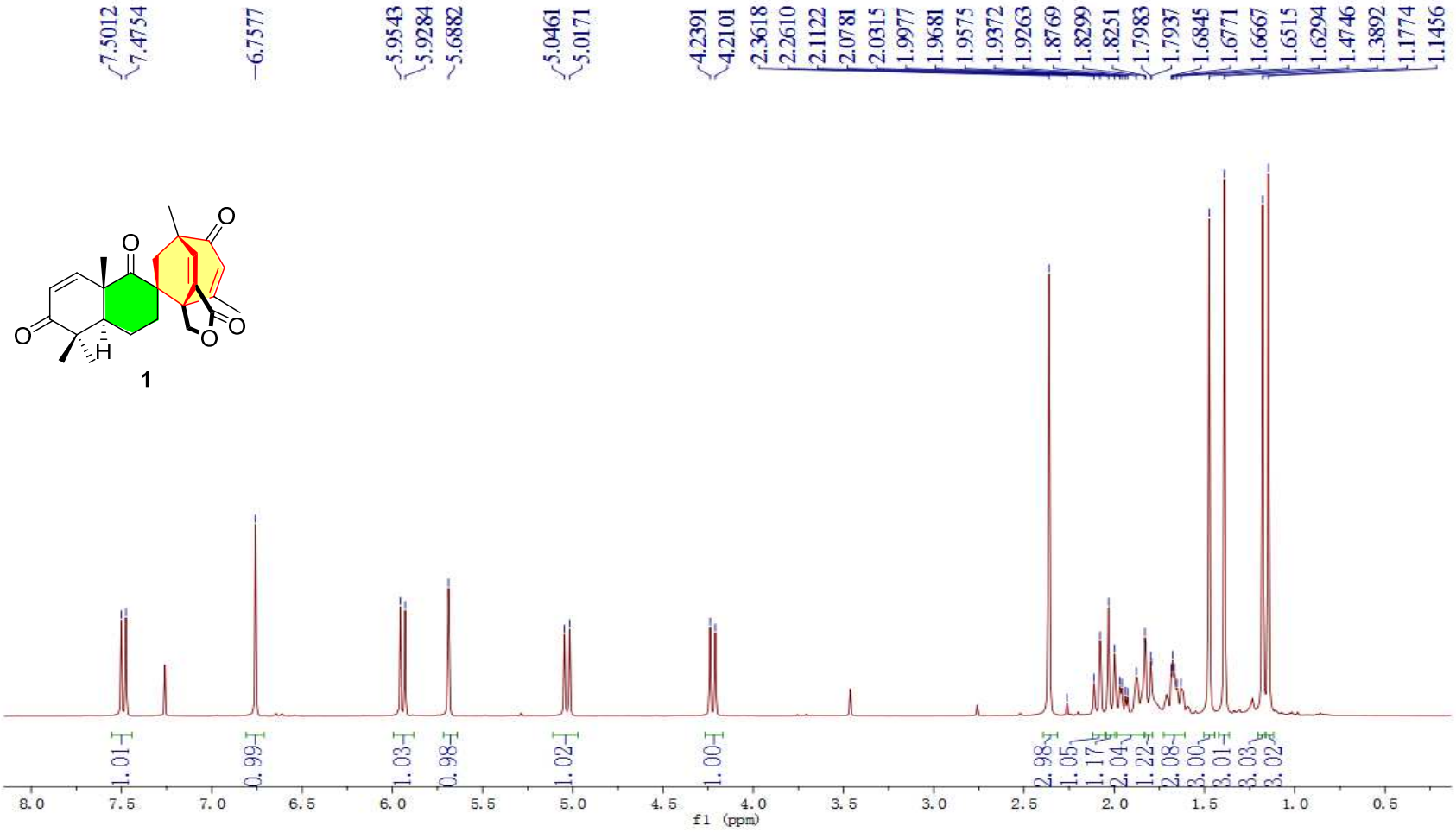


Figure S7. ^{1}H NMR spectrum of spiroaspertrione A (**1**) in $CDCl_3$.

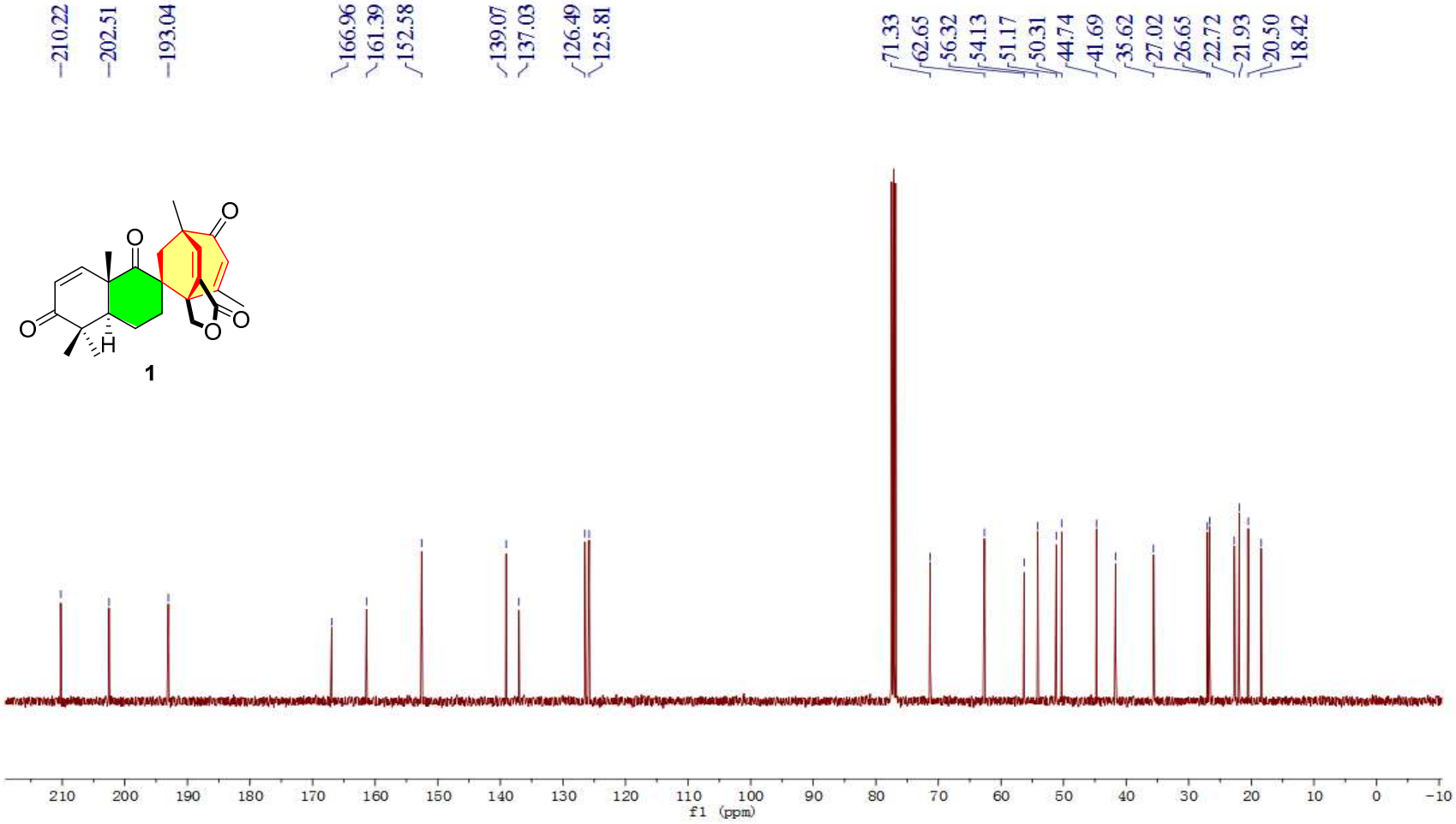


Figure S8. ^{13}C NMR spectrum of spiroaspertrione A (**1**) in $CDCl_3$.

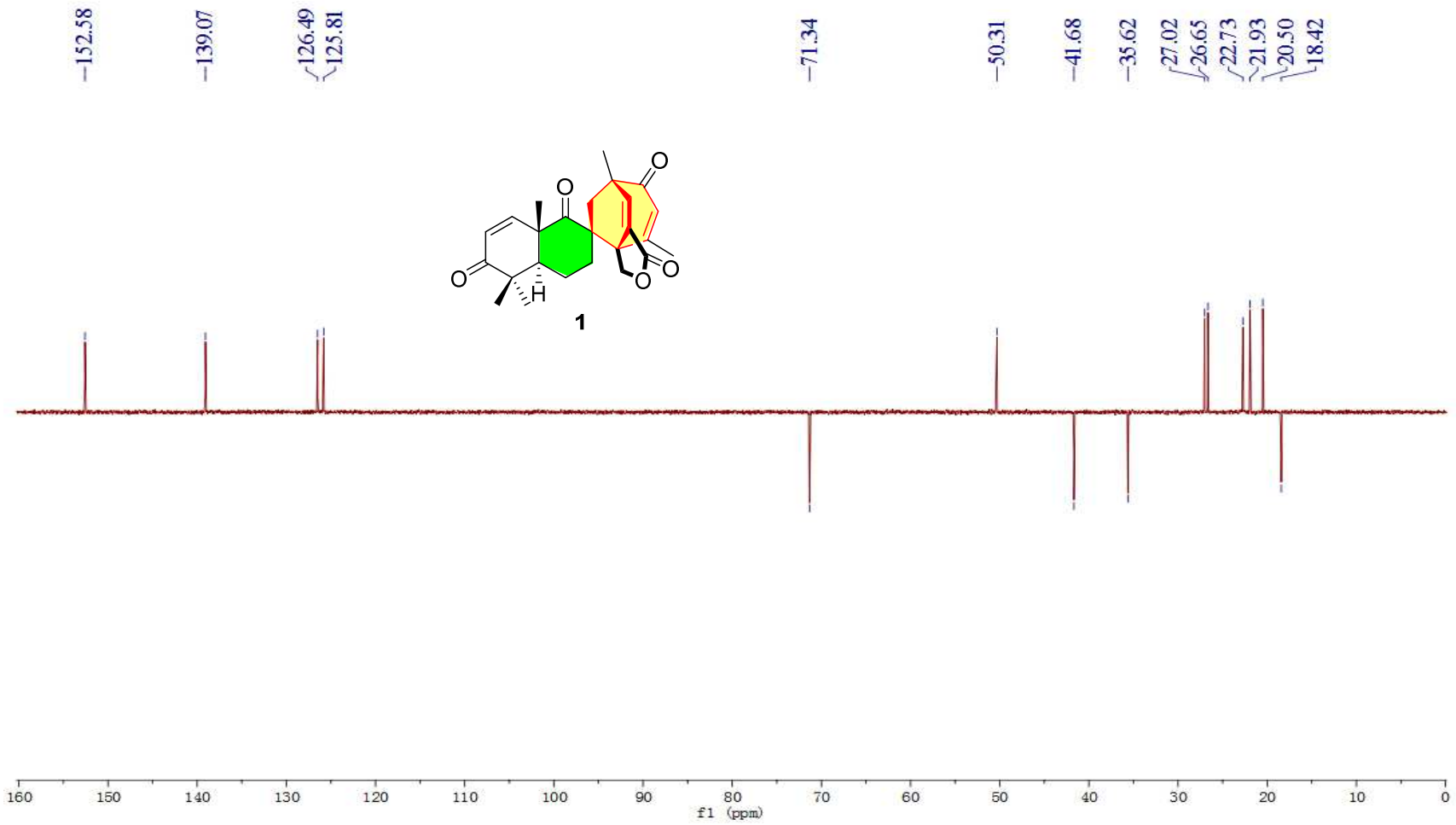


Figure S9. DEPT NMR spectrum of spiroaspertrione A (**1**) in $CDCl_3$.

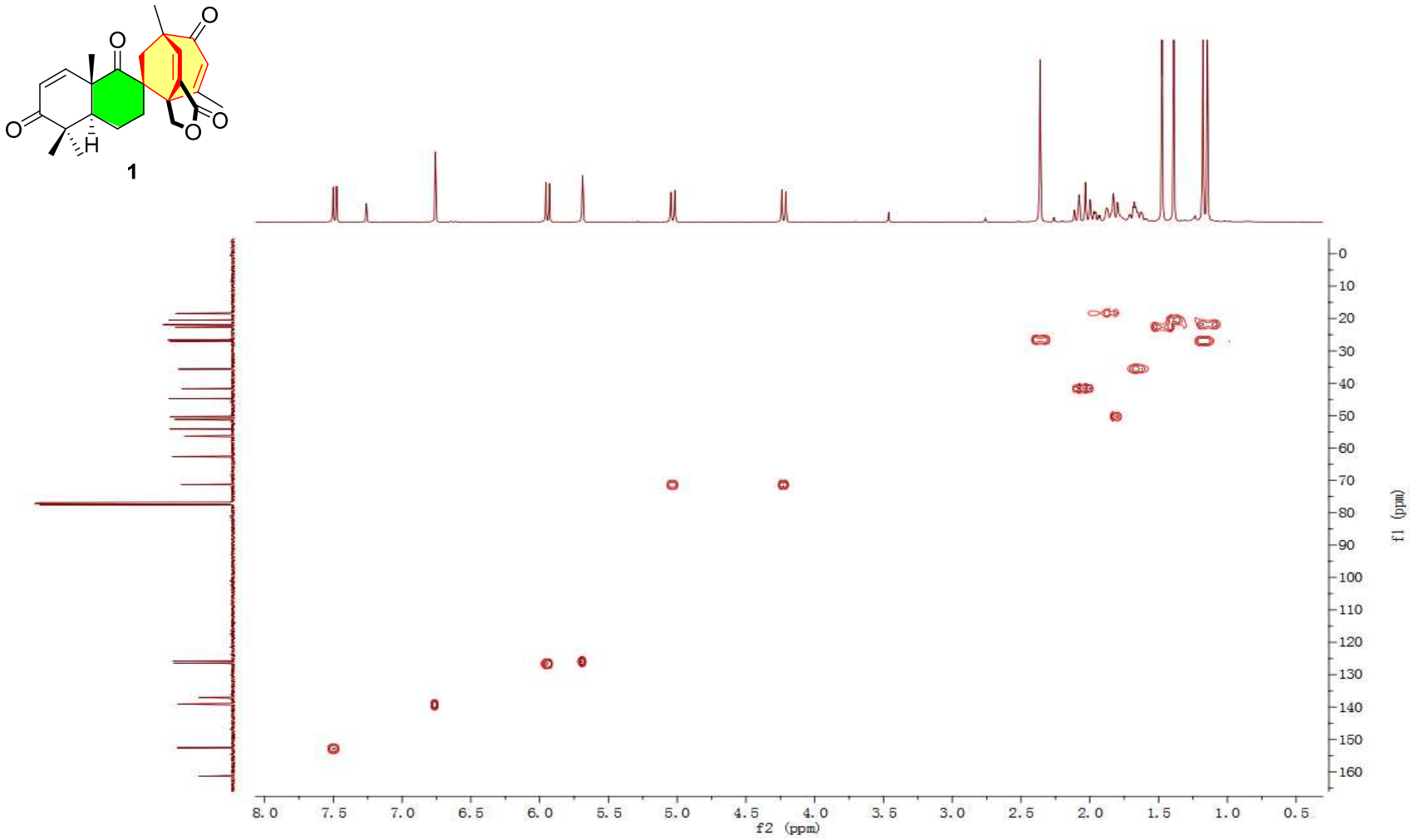


Figure S10. HSQC spectrum of spiroaspertrione A (**1**) in $CDCl_3$.

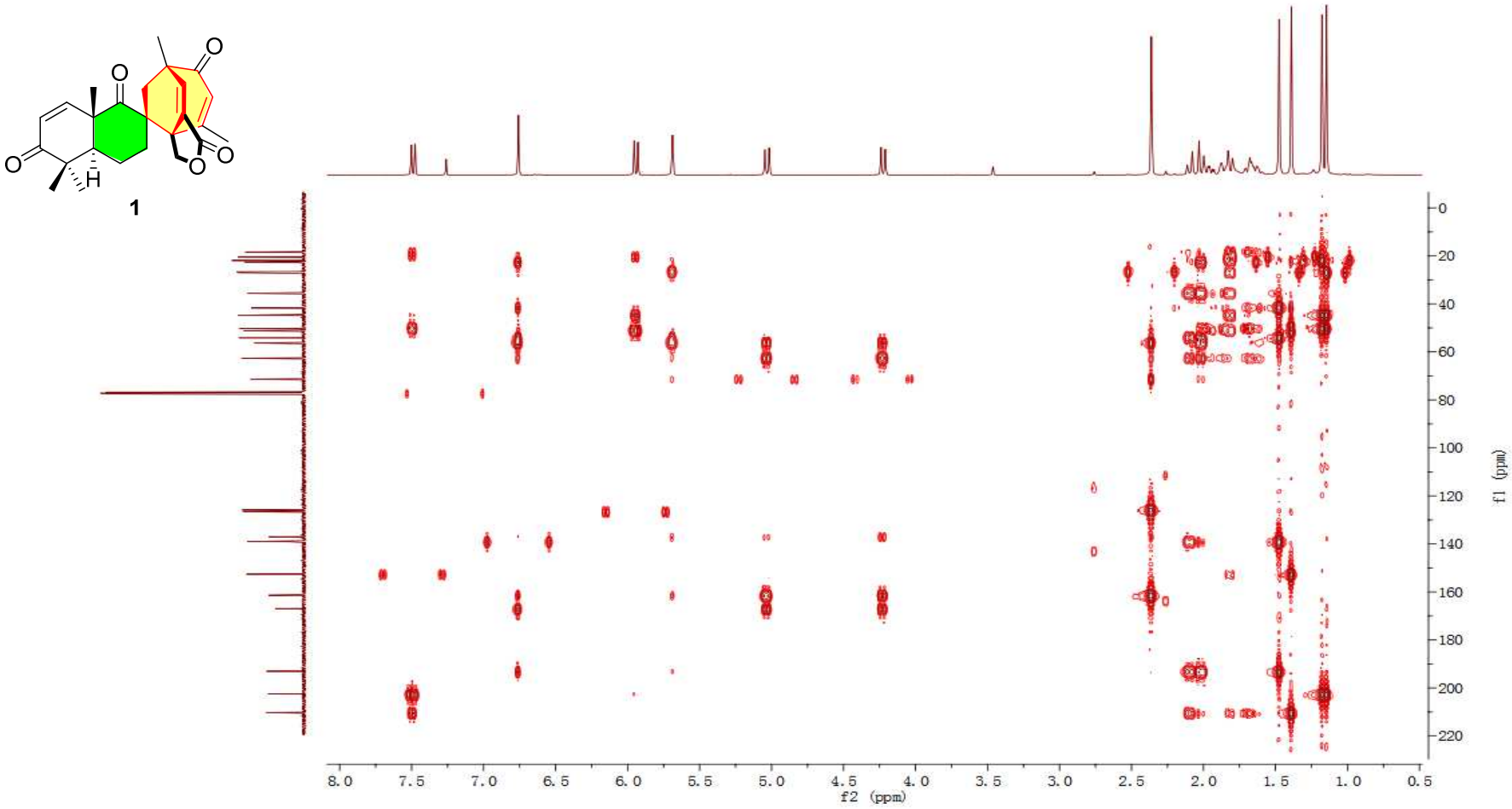


Figure S11. HMBC spectrum of spiroaspertrione A (**1**) in $CDCl_3$.

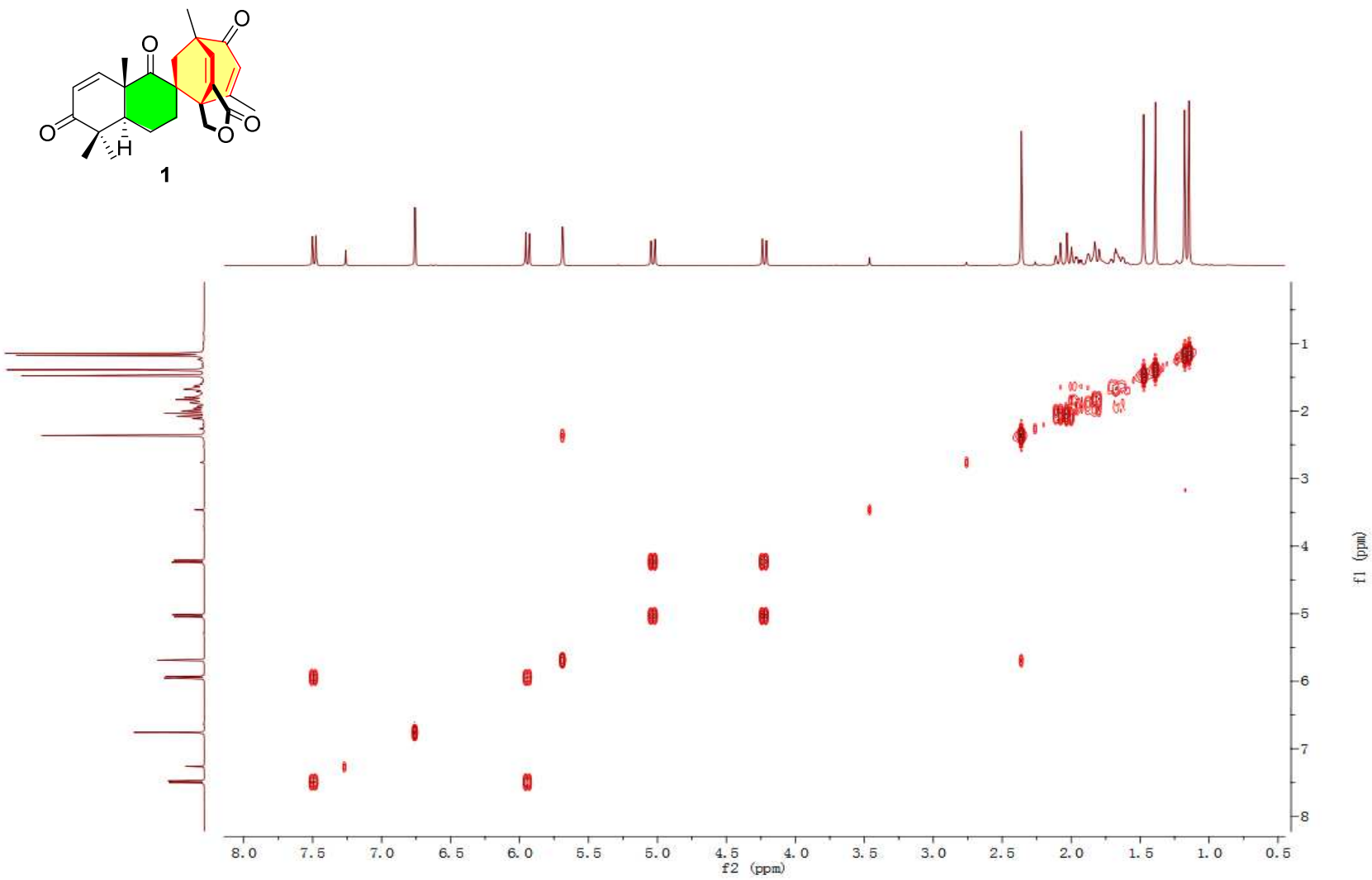


Figure S12. ^{1}H–^{1}H COSY spectrum of spiroaspertrione A (**1**) in $CDCl_3$.

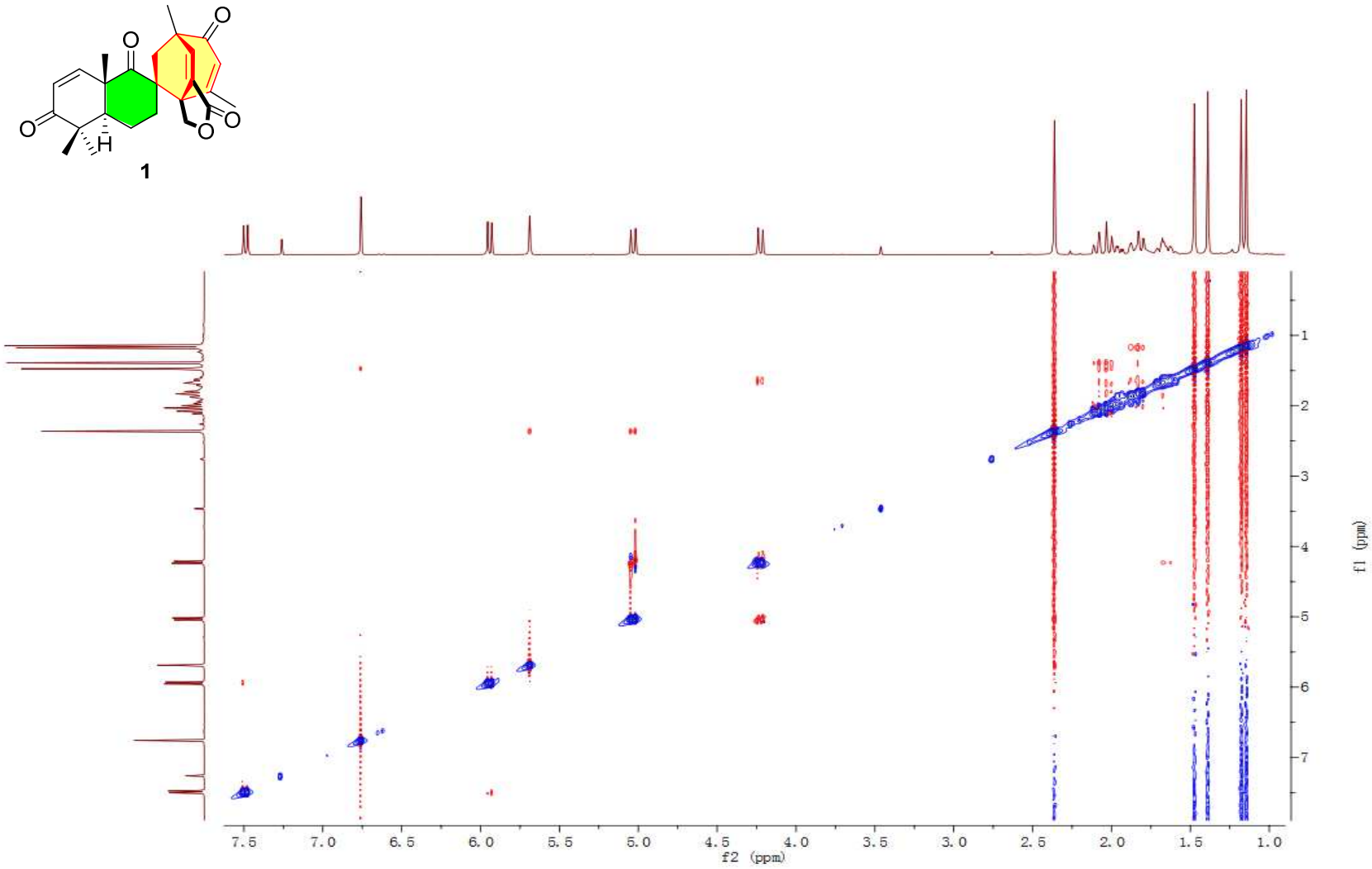


Figure S13. NOESY spectrum of spiroaspertrione A (**1**) in $CDCl_3$.

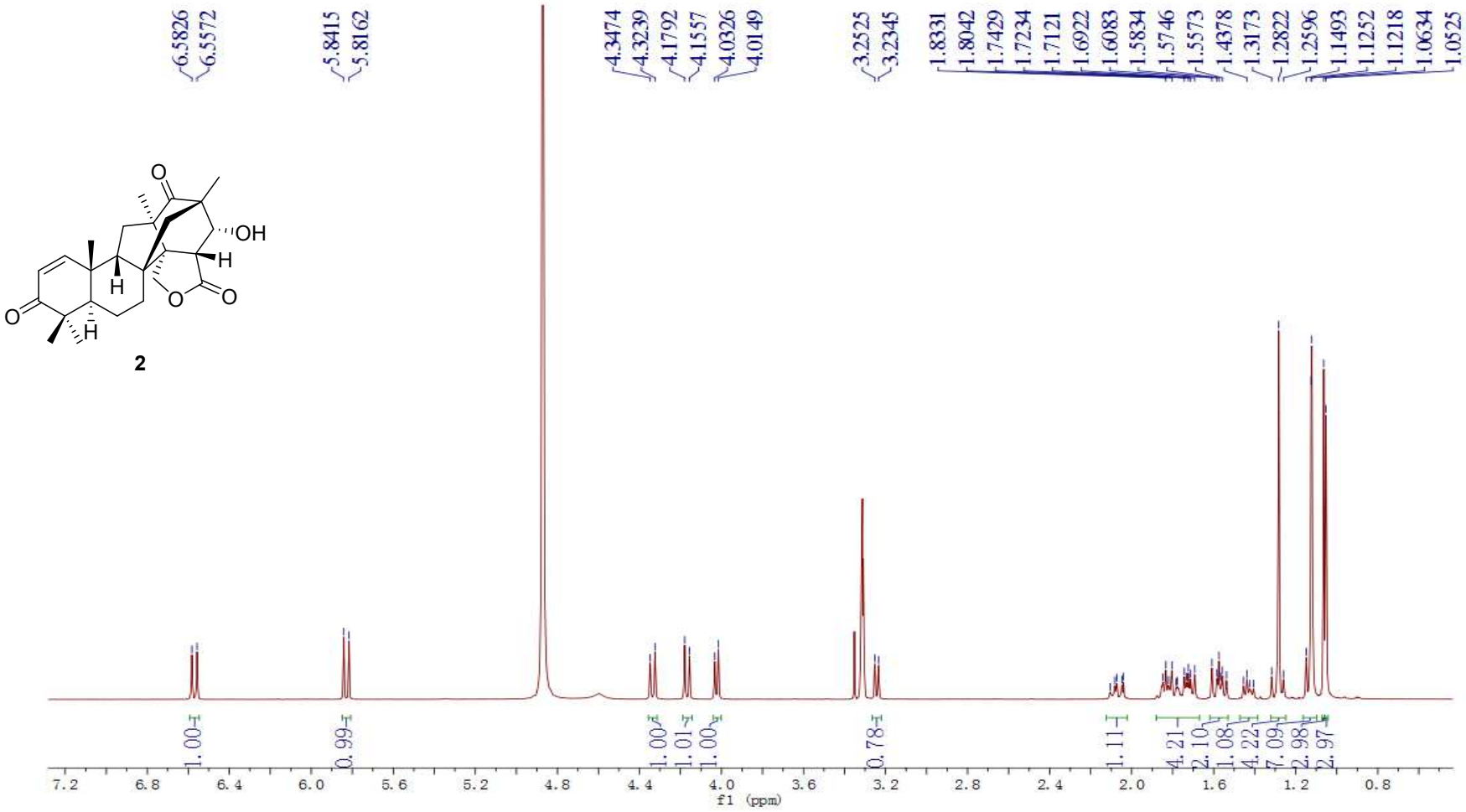


Figure S14. ^{1}H NMR spectrum of andiconin B (**2**) in CD_3OD.

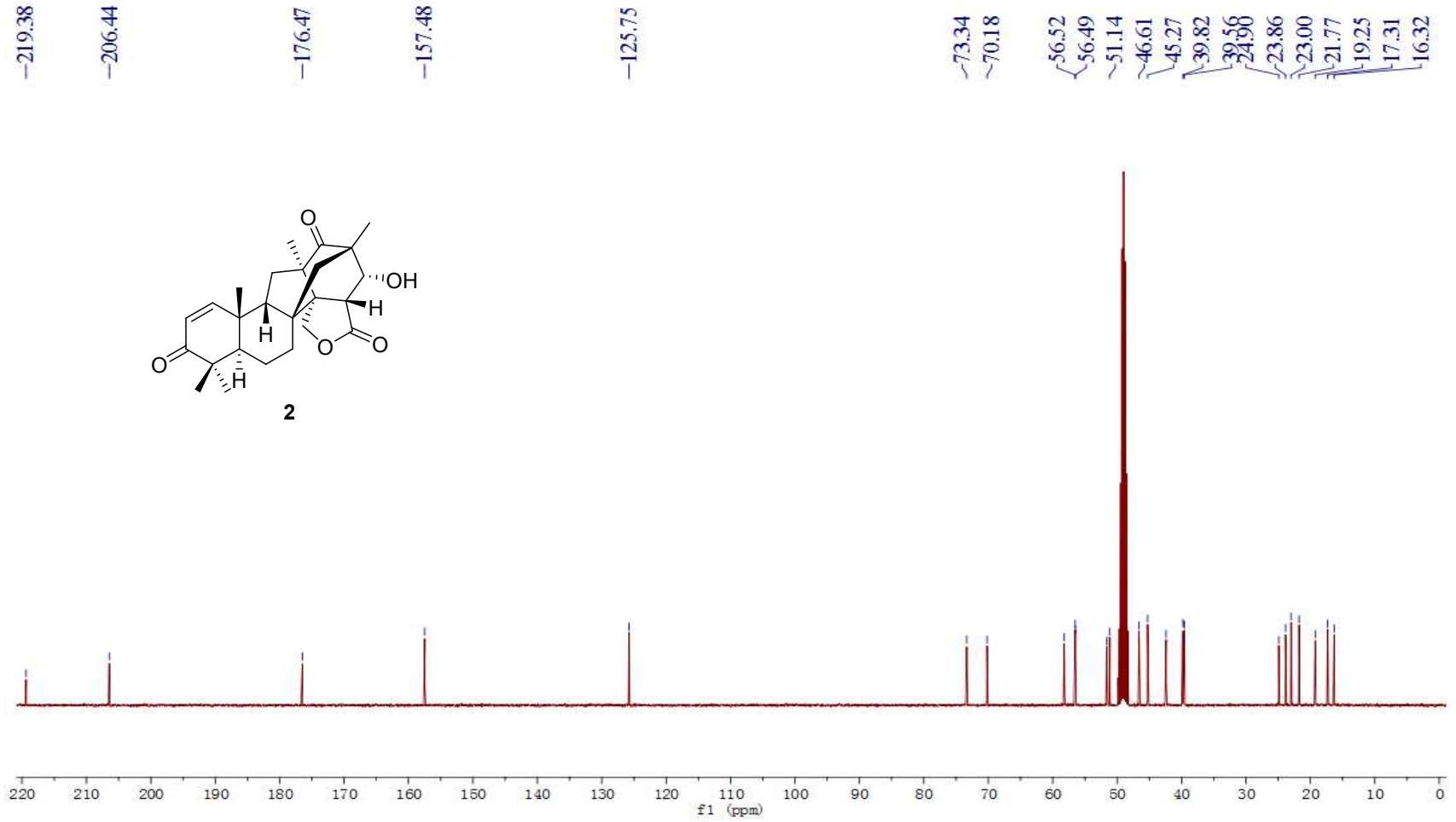


Figure S15. ^{13}C NMR spectrum of andiconin B (**2**) in CD_3OD.

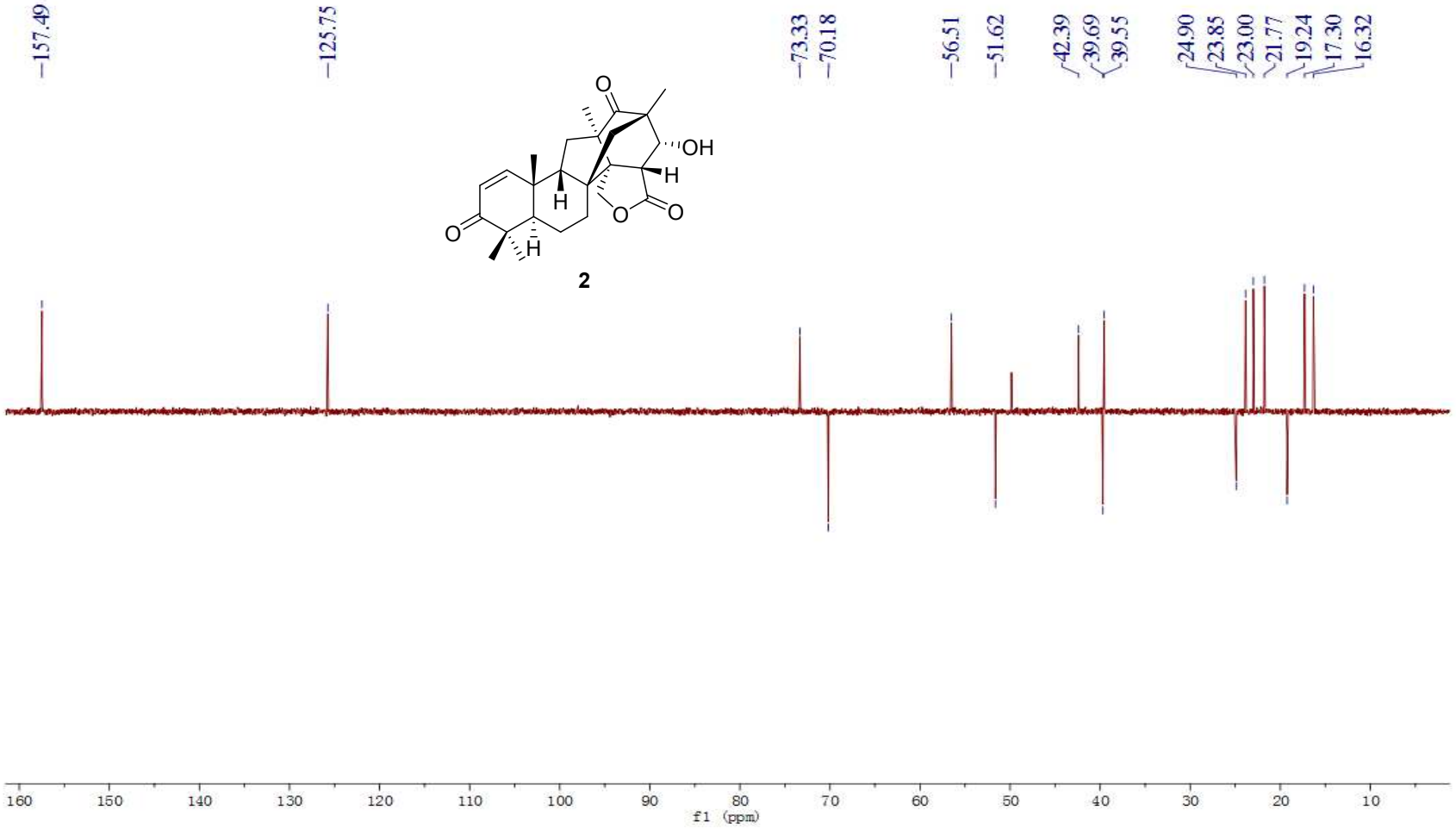


Figure S16. DEPT NMR spectrum of andiconin B (**2**) in CD_3OD.

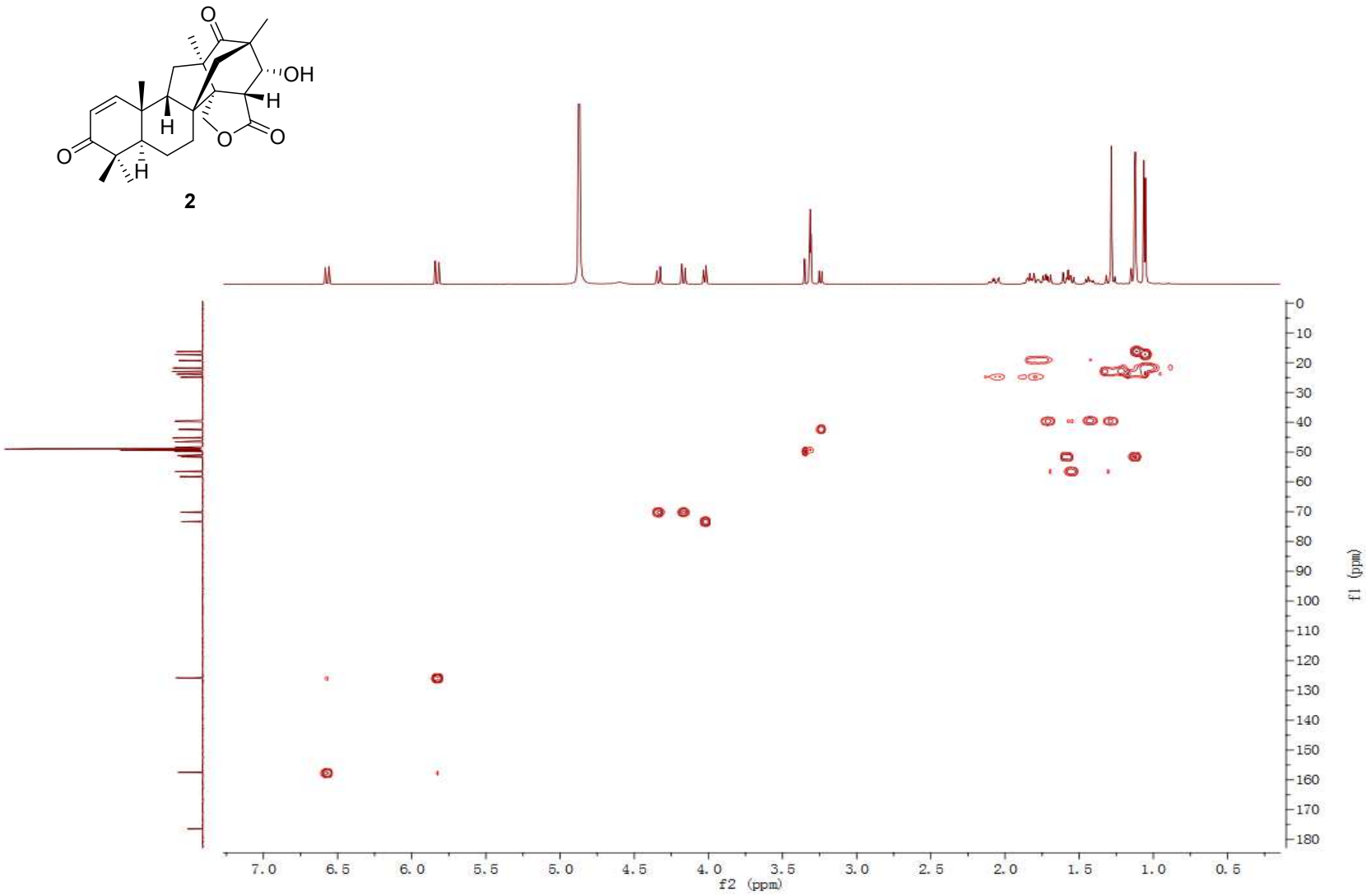


Figure S17. HSQC spectrum of andiconin B (**2**) in CD_3OD.

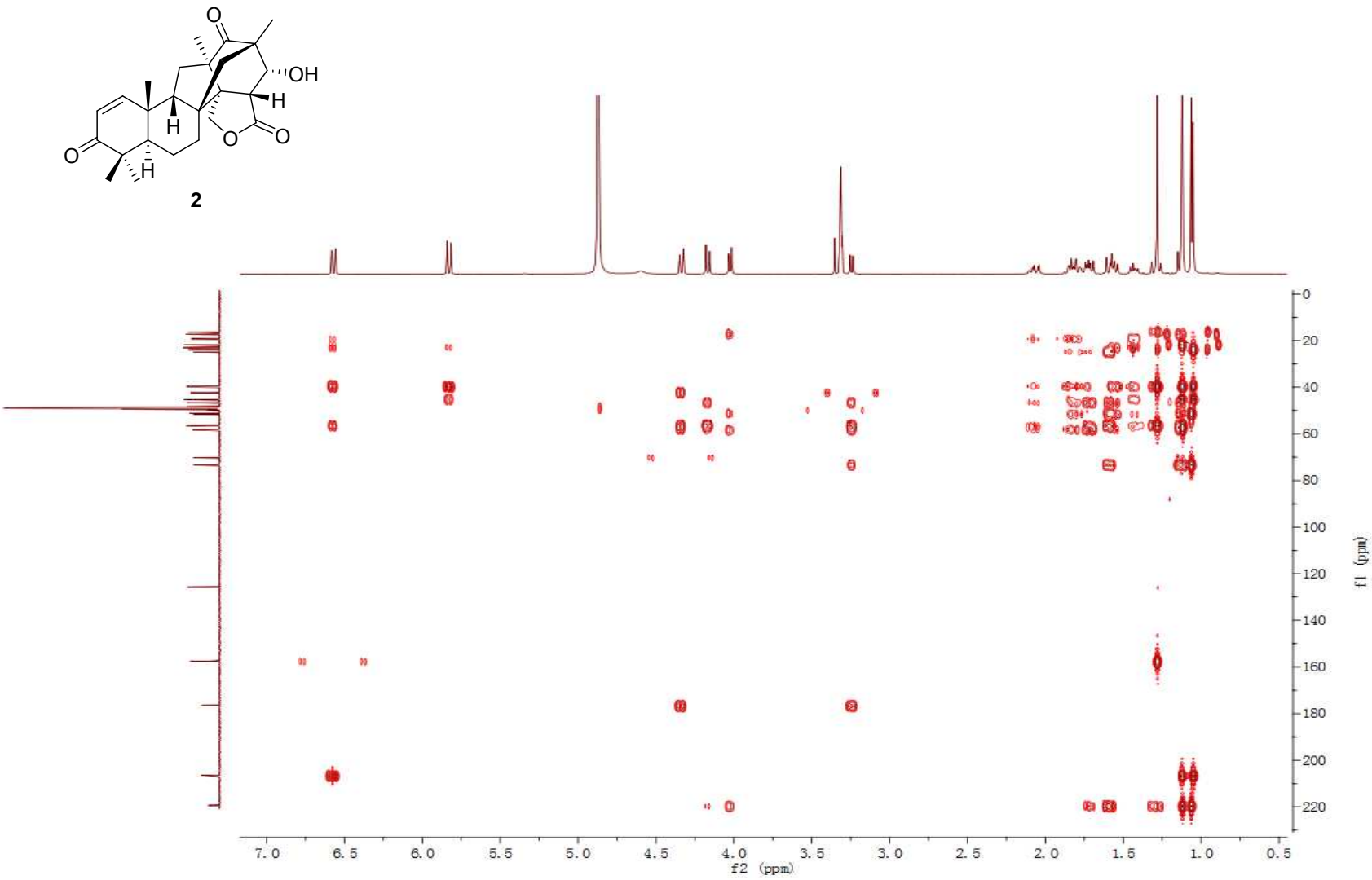


Figure S18. HMBC spectrum of andiconin B (**2**) in CD_3OD.

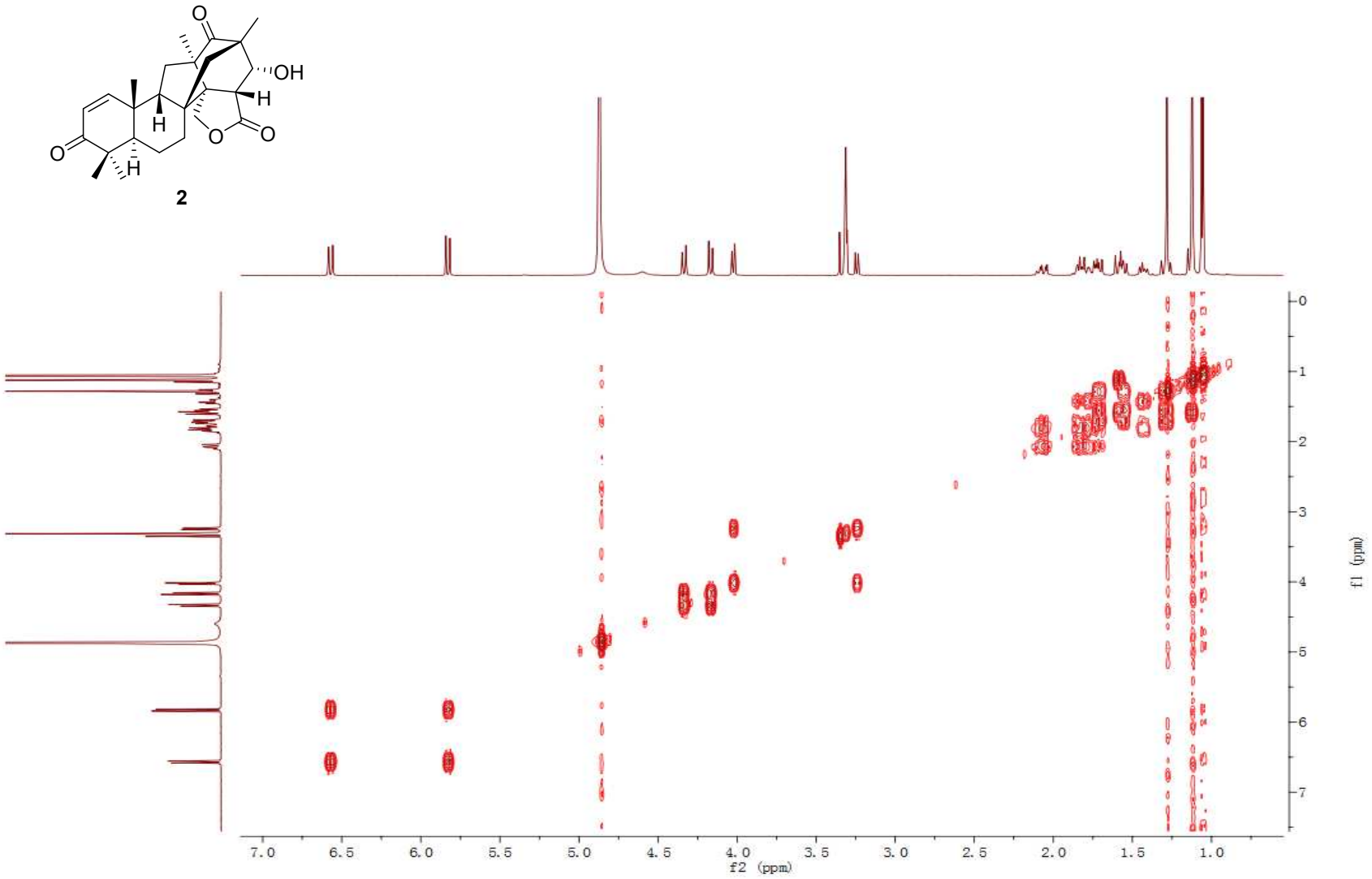


Figure S19. ^{1}H–^{1}H COSY spectrum of andiconin B (**2**) in CD_3OD.

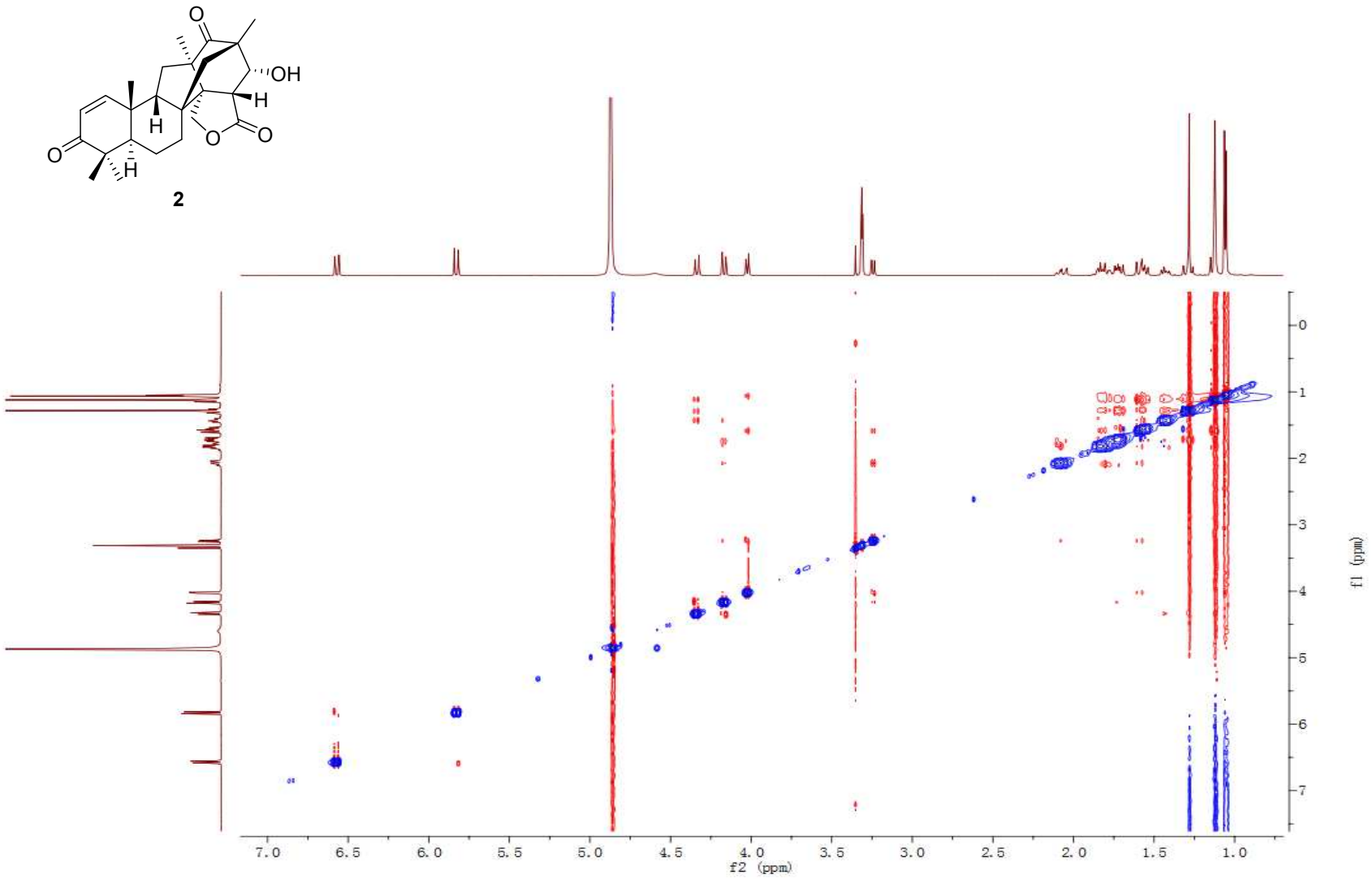


Figure S20. NOESY spectrum of andiconin B (**2**) in CD_3OD.

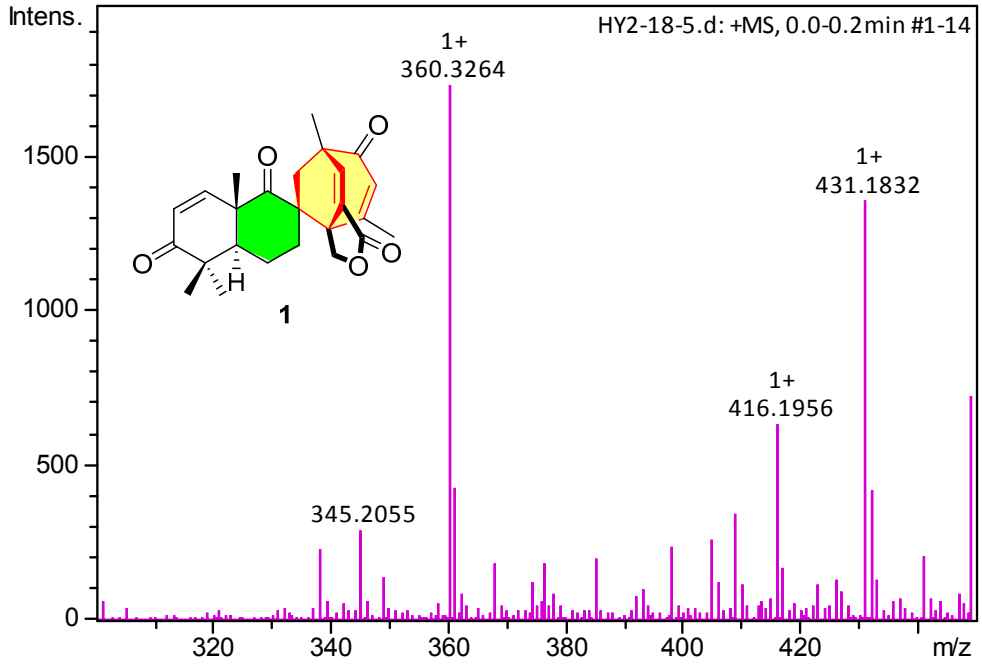


Figure S21. HRESIMS spectrum of spiroaspertrione A (**1**).

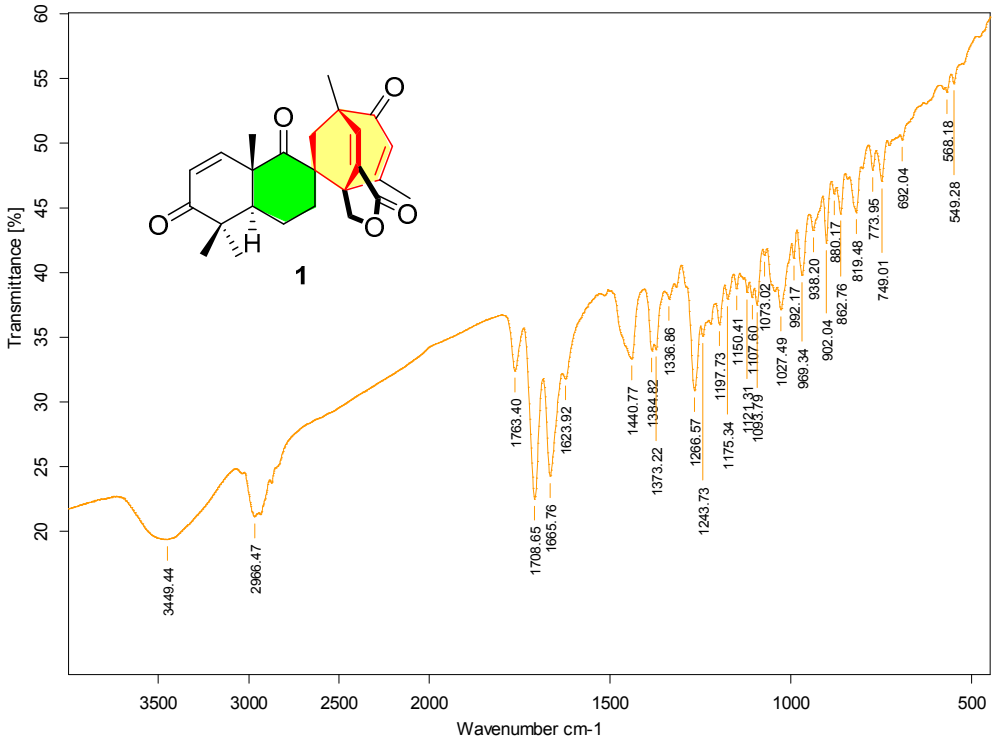


Figure S22. IR spectrum of spiroaspertrione A (**1**).

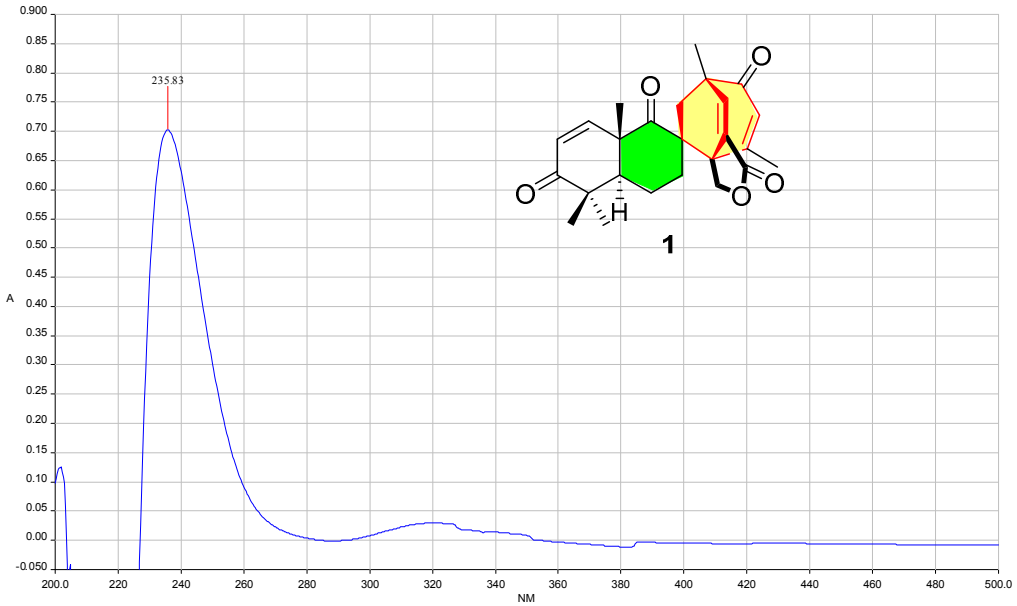


Figure S23. UV spectrum of spiroaspertrione A (**1**).

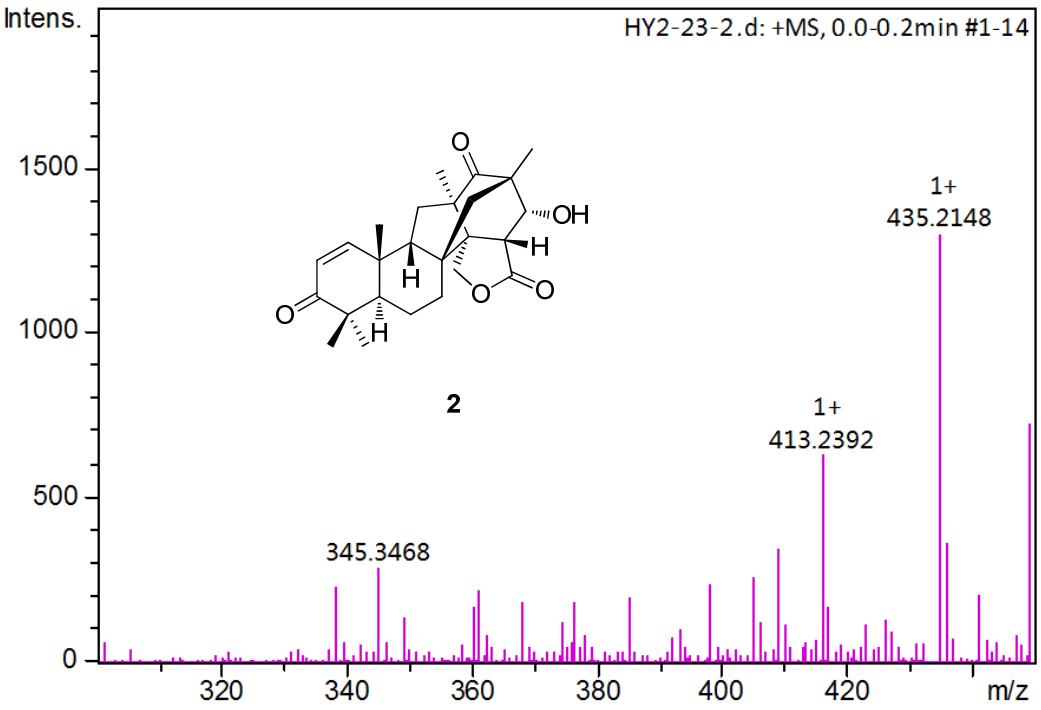


Figure S24. HRESIMS spectrum of andiconin B (**2**).

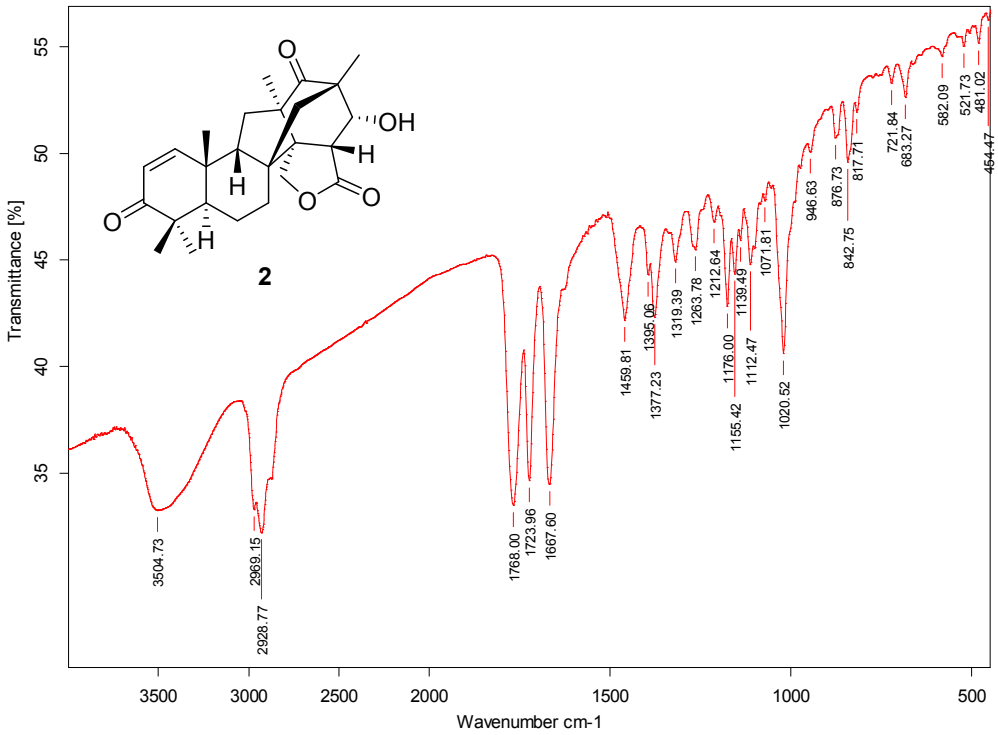


Figure S25. IR spectrum of andiconin B (**2**).

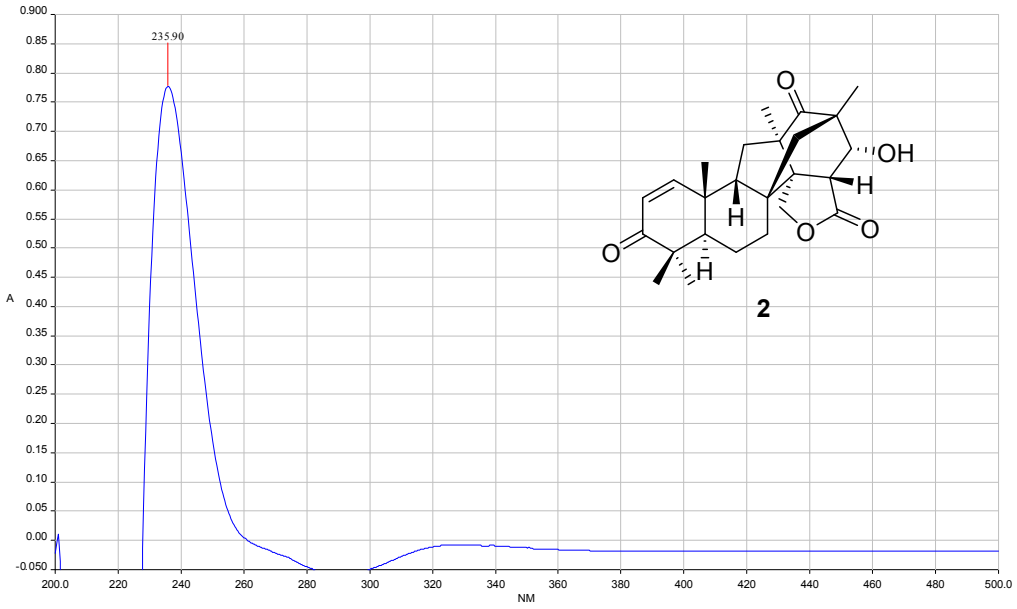


Figure S26. UV spectrum of andiconin B (**2**).

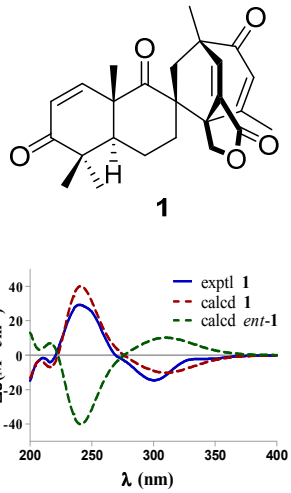


Figure S27. Comparison of the experimental ECD spectra of **1** with calculated ECD spectra of their respective enantiomers.

Table S4. Important thermodynamic parameters (a.u.) and Boltzmann distributions of the optimized **1** and ***ent*-1** at LC-wPBE/6-311++G**//B3LYP/6-311++G** level in the MeOH.

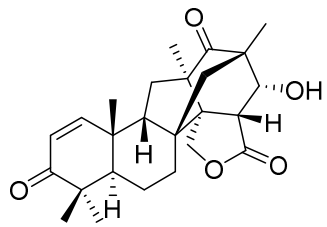
Conformations	E+ZPE	Gibbs	Ratio
1	-1271.940724	-1270.547484	100.0%
***ent*-1**	-1271.940724	-1270.488205	100.0%

E+ZPE, G: total energy (E) in the DMSO at LC-wPBE/6-311++G**//B3LYP/6-311++G** level with IEFPCM solvent continuum model, zero point energy (ZPE) and Gibbs free energy (G) in the MeOH at B3LYP/6-311++G** level with IEFPCM solvent continuum model. %: Boltzmann distributions, using the relative Gibbs free energies as weighting factors.

Table S5. Optimized coordinates of **1** and ***ent*-1** at B3LYP/6-31G(d) level in the MeOH.

1				***ent*-1**			
C1	-4.113	-0.574	1.758	C1	4.113	-0.574	1.758
C2	3.078	-1.424	1.372	C2	-3.078	-1.424	1.372
C3	-2.815	-0.918	1.768	C3	2.815	-0.918	1.768
C4	3.172	0.144	-1.295	C4	-3.172	0.144	-1.295
C5	2.801	1.034	-0.373	C5	-2.801	1.034	-0.373
C6	2.407	-0.309	1.739	C6	-2.407	-0.309	1.739
C7	-4.793	-0.056	0.557	C7	4.793	-0.056	0.557
C8	3.344	-1.928	0.015	C8	-3.344	-1.928	0.015
C9	3.034	2.487	-0.409	C9	-3.034	2.487	-0.409
C10	-1.486	0.614	-1.505	C10	1.486	0.614	-1.505
C11	-0.086	1.021	-1.012	C11	0.086	1.021	-1.012
C12	1.087	-1.224	-0.83	C12	-1.087	-1.224	-0.83
C13	-0.489	-0.408	1.055	C13	0.489	-0.408	1.055
C14	1.539	2.088	1.332	C14	-1.539	2.088	1.332
C15	-2.437	0.384	-0.32	C15	2.437	0.384	-0.32
C16	2.63	-1.258	-1.179	C16	-2.63	-1.258	-1.179
C17	-1.917	-0.792	0.559	C17	1.917	-0.792	0.559
C18	1.82	0.68	0.719	C18	-1.82	0.68	0.719
C19	-3.957	0.367	-0.673	C19	3.957	0.367	-0.673
C20	0.554	0.003	-0.023	C20	-0.554	0.003	-0.023
C21	2.235	-0.039	3.215	C21	-2.235	-0.039	3.215

C22	2.87	-2.064	-2.46	C22	-2.87	-2.064	-2.46
C23	-1.92	-2.193	-0.116	C23	1.92	-2.193	-0.116
C24	-4.336	-0.553	-1.857	C24	4.414	1.804	-1.03
C25	-4.414	1.804	-1.03	C25	4.336	-0.553	-1.857
O1	-6.019	0.034	0.536	O1	6.019	0.034	0.536
O2	4.077	-2.899	-0.146	O2	-4.077	-2.899	-0.146
O3	3.764	3.137	-1.128	O3	-3.764	3.137	-1.128
O4	2.224	3.07	0.516	O4	-2.224	3.07	0.516
H1	-4.749	-0.731	2.626	H1	4.749	-0.731	2.626
H2	3.49	-2.065	2.149	H2	-3.49	-2.065	2.149
H3	-2.383	-1.365	2.665	H3	2.383	-1.365	2.665
H4	3.823	0.413	-2.123	H4	-3.823	0.413	-2.123
H5	-1.43	-0.278	-2.14	H5	1.43	-0.278	-2.14
H6	-1.86	1.418	-2.148	H6	1.86	1.418	-2.148
H7	-0.185	2.003	-0.537	H7	0.185	2.003	-0.537
H8	0.575	1.159	-1.874	H8	-0.575	1.159	-1.874
H9	0.561	-1.268	-1.788	H9	-0.852	-2.156	-0.311
H10	0.852	-2.156	-0.311	H10	-0.561	-1.268	-1.788
H11	-0.09	-1.235	1.654	H11	0.09	-1.235	1.654
H12	-0.619	0.439	1.743	H12	0.619	0.439	1.743
H13	0.484	2.36	1.348	H13	-1.934	2.198	2.34
H14	1.934	2.198	2.34	H14	-0.484	2.36	1.348
H15	-2.323	1.265	0.331	H15	2.323	1.265	0.331
H16	1.208	0.251	3.461	H16	-1.208	0.251	3.461
H17	2.49	-0.925	3.801	H17	-2.49	-0.925	3.801
H18	2.888	0.777	3.552	H18	-2.888	0.777	3.552
H19	3.94	-2.17	-2.659	H19	-2.443	-3.068	-2.375
H20	2.443	-3.068	-2.375	H20	-2.403	-1.56	-3.313
H21	2.403	-1.56	-3.313	H21	-3.94	-2.17	-2.659
H22	-1.425	-2.196	-1.09	H22	1.425	-2.196	-1.09
H23	-1.4	-2.915	0.525	H23	1.4	-2.915	0.525
H24	-2.938	-2.562	-0.255	H24	2.938	-2.562	-0.255
H25	-4.061	-1.598	-1.694	H25	3.915	2.159	-1.937
H26	-5.417	-0.511	-2.017	H26	5.493	1.819	-1.204
H27	-3.843	-0.215	-2.775	H27	4.189	2.509	-0.221
H28	-4.189	2.509	-0.221	H28	3.843	-0.215	-2.775
H29	-3.915	2.159	-1.937	H29	4.061	-1.598	-1.694
H30	-5.493	1.819	-1.204	H30	5.417	-0.511	-2.017



2

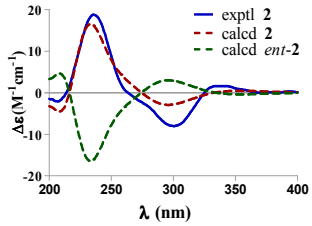


Figure S28. Comparison of the experimental ECD spectra of **2** with calculated ECD spectra of their respective enantiomers.

Table S6. Important thermodynamic parameters (a.u.) and Boltzmann distributions of the optimized **2** and ***ent*-2** at LC-wPBE/6-311++G**//B3LYP/6-311++G** level in the MeOH.

Conformations	E+ZPE	Gibbs	Ratio
2	-1348.423360	-1347.940047	100.0%
***ent*-2**	-1348.423360	-1347.940047	100.0%

E+ZPE, G: total energy (E) in the DMSO at LC-wPBE/6-311++G**//B3LYP/6-311++G** level with IEFPCM solvent continuum model, zero point energy (ZPE) and Gibbs free energy (G) in the MeOH at B3LYP/6-311++G** level with IEFPCM solvent continuum model. %: Boltzmann distributions, using the relative Gibbs free energies as weighting factors.

Table S7. Optimized coordinate of **2** and ***ent*-2** at B3LYP/6-31G(d) level in the MeOH.

2				***ent*-2**			
C1	-3.706	-0.961	1.714	C1	3.706	-0.961	1.714
C2	-2.679	-1.625	1.16	C2	2.679	-1.625	1.16
C3	-4.312	0.239	1.109	C3	4.312	0.239	1.109
C4	2.386	-1.327	0.913	C4	-2.386	-1.327	0.913
C5	2.818	2.388	-0.152	C5	-2.818	2.388	-0.152
C6	-1.781	0.606	-1.884	C6	1.781	0.606	-1.884
C7	-0.363	0.032	-2.152	C7	0.363	0.032	-2.152
C8	0.041	-0.754	1.499	C8	-0.041	-0.754	1.499
C9	1.598	-1.393	-1.453	C9	-1.598	-1.393	-1.453
C10	0.556	2.02	0.126	C10	-0.556	2.02	0.126
C11	2.493	1.063	-0.799	C11	-2.493	1.063	-0.799
C12	-2.366	0.227	-0.508	C12	2.366	0.227	-0.508
C13	-0.432	-1.272	0.12	C13	0.432	-1.272	0.12
C14	3.584	-0.027	-0.78	C14	-3.584	-0.027	-0.78
C15	-1.985	-1.228	-0.119	C15	1.985	-1.228	-0.119
C16	-3.863	0.64	-0.311	C16	3.863	0.64	-0.311
C17	1.438	-0.149	1.237	C17	-1.438	-0.149	1.237
C18	2.863	-1.381	-0.54	C18	-2.863	-1.381	-0.54
C19	0.44	-0.468	-0.932	C19	-0.44	-0.468	-0.932
C20	1.192	0.634	-0.095	C20	-1.192	0.634	-0.095
C21	-2.353	-2.315	-1.169	C21	2.353	-2.315	-1.169

C22	-4.007	2.168	-0.466	C22	4.007	2.168	-0.466
C23	-4.875	-0.039	-1.272	C23	4.875	-0.039	-1.272
C24	1.986	0.62	2.449	C24	-1.986	0.62	2.449
C25	3.783	-2.571	-0.804	C25	-3.783	-2.571	-0.804
O1	-5.201	0.848	1.699	O1	5.201	0.848	1.699
O2	2.688	-2.152	1.756	O2	-2.688	-2.152	1.756
O3	3.898	2.936	-0.05	O3	-3.898	2.936	-0.05
O4	1.682	2.93	0.334	O4	-1.682	2.93	0.334
O5	4.543	0.161	0.255	O5	-4.543	0.161	0.255
H1	-4.157	-1.296	2.645	H1	4.157	-1.296	2.645
H2	-2.312	-2.53	1.644	H2	2.312	-2.53	1.644
H3	-1.765	1.697	-1.969	H3	2.445	0.257	-2.682
H4	-2.445	0.257	-2.682	H4	1.765	1.697	-1.969
H5	-0.448	-0.812	-2.845	H5	-0.235	0.775	-2.695
H6	0.235	0.775	-2.695	H6	0.448	-0.812	-2.845
H7	-0.621	0.029	1.89	H7	-0.088	-1.541	2.256
H8	0.088	-1.541	2.256	H8	0.621	0.029	1.89
H9	1.909	-1.068	-2.455	H9	-1.231	-2.418	-1.563
H10	1.231	-2.418	-1.563	H10	-1.909	-1.068	-2.455
H11	0.007	2.376	-0.749	H11	0.076	2.111	1.009
H12	-0.076	2.111	1.009	H12	-0.007	2.376	-0.749
H13	2.257	1.307	-1.846	H13	-2.257	1.307	-1.846
H14	-1.843	0.846	0.236	H14	1.844	0.846	0.236
H15	-0.151	-2.33	0.056	H15	0.151	-2.329	0.056
H16	4.086	-0.066	-1.757	H16	-4.086	-0.066	-1.757
H17	-1.851	-2.154	-2.125	H17	1.851	-2.154	-2.125
H18	-3.427	-2.36	-1.356	H18	2.043	-3.299	-0.799
H19	-2.043	-3.299	-0.799	H19	3.427	-2.36	-1.356
H20	-3.892	2.47	-1.511	H20	3.892	2.47	-1.511
H21	-3.258	2.704	0.129	H21	4.996	2.483	-0.125
H22	-4.996	2.483	-0.125	H22	3.258	2.704	0.129
H23	-4.578	0.092	-2.318	H23	4.578	0.092	-2.318
H24	-5.859	0.423	-1.143	H24	4.989	-1.109	-1.081
H25	-4.989	-1.109	-1.081	H25	5.859	0.423	-1.143
H26	1.325	1.444	2.734	H26	-1.325	1.444	2.734
H27	2.986	1.018	2.26	H27	-2.059	-0.066	3.298
H28	2.059	-0.066	3.298	H28	-2.986	1.018	2.26
H29	4.052	-2.615	-1.865	H29	-4.052	-2.615	-1.865
H30	3.291	-3.511	-0.534	H30	-4.703	-2.49	-0.217
H31	4.703	-2.49	-0.217	H31	-3.291	-3.511	-0.534
H32	4.861	1.081	0.18	H32	-4.861	1.081	0.18