Supporting information for

Spiroseoflosterol, a Rearranged Ergostane-Steroid from the Fruiting Bodies of *Butyriboletus roseoflavus*

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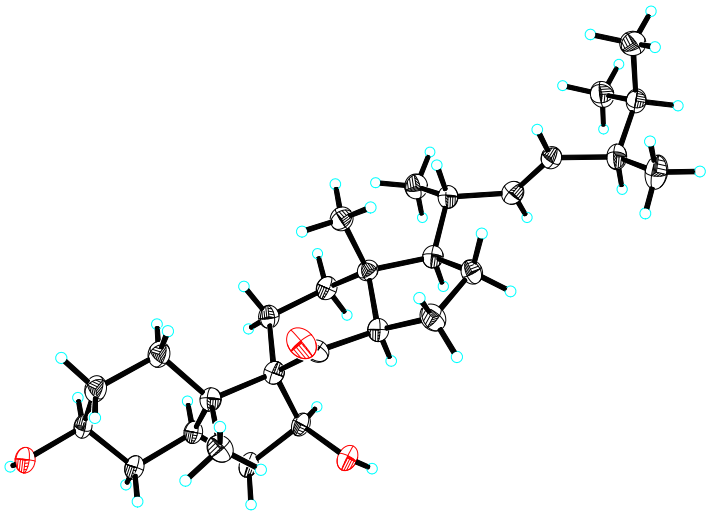
Contents

1. Crystallographic data of compound 1

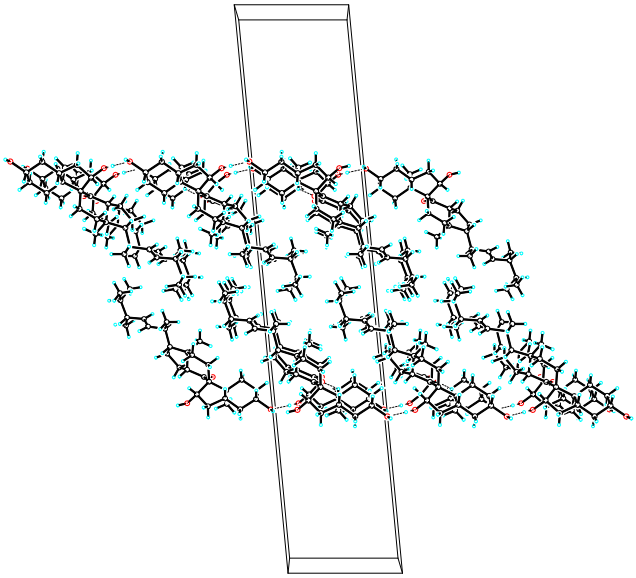
The crystal data of compound **1** have been deposited in CCDC with number 1967417.

Crystal data for spiroseoflosterol (**1**): $C_{28}H_{48}O_4$, M = 448.66, a = 45.216(2) Å, b = 6.5755(3) Å, c = 9.0610(4) Å, α = 90°, β = 95.407(3)°, γ = 90°, V = 2682.0(2) Å^3, T = 100.(2) K, space group $C121$, Z = 4, μ(Cu Kα) = 0.561 mm^{-1}, 33984 reflections measured, 5115 independent reflections (R_{int} = 0.1856). The final R_1 values were 0.0680 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1451 ($I > 2\sigma(I)$). The final R_1 values were 0.1239 (all data). The final $wR(F^2)$ values were 0.1767 (all data). The goodness of fit on F^2 was 1.040. Flack parameter = 0.9 (3).



View of a molecule of **1** with the atom-labelling scheme.

Displacement ellipsoids are drawn at the 30% probability level.



View of the pack drawing of **1**.

Hydrogen-bonds are shown as dashed lines.

2. Cytotoxicity assays

2.1 Cell lines and cell culture

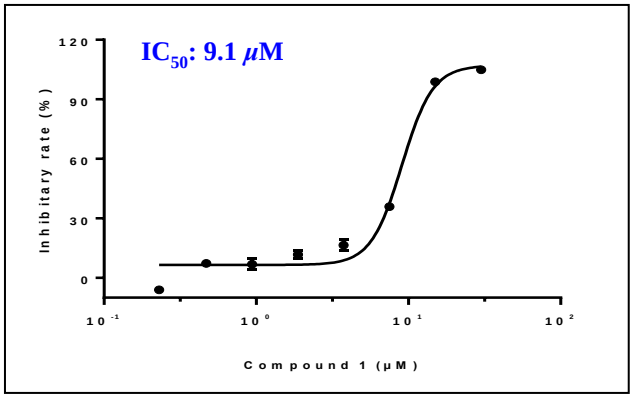
Human liver cancer cell line (HepG2) and normal liver cell line (L02) were obtained from the Jining Company (Shanghai, China), and human liver cancer cell line (Huh7/S) that resistant to sorafenib was acquired from Yuduo Biology Company (Shanghai, China). HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco). While the L02 and Huh7/S cells were cultured in RPMI medium 1640 (Gibco) with 10% heat-inactivated fetal bovine serum (FBS, Gibco), at 37 °C in an atmosphere of 5% CO_2, 95% air and > 95% humidity.

2.2 MTT assay

The cytotoxicity of the compounds **1** and **2** was tested by the MTT assay.[1,2] Briefly, cells in a density of 3×10^4 cells/well were seeded into 96-well plates and incubated at 37°C with 5% CO_2 for 24h. The culture medium was replaced with fresh medium

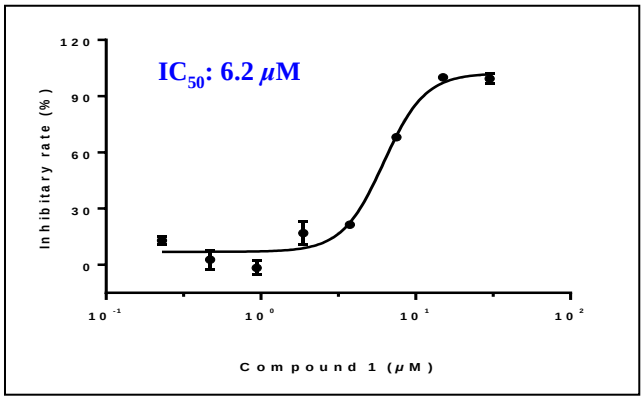
containing tested samples, and cells were incubated for additional 48 h. After removing the medium, 100 μL of MTT reagent (1mg/mL) was added into each well, and the plates were kept in incubator for 4 h. After that, 100 μL of dimethyl sulfoxide (DMSO) was added into each well, and the plates were measured at 490 nm using microplate reader (BIO-RAD, USA). The inhibition rate was calculated as [(A_{490} control - A_{490} treated)/A_{490} control] ×100%.

The cytotoxicity of compounds was expressed as IC_{50} values calculated by GraphPad Prism 5 (GraphPad Software, California, USA). The results are as follows:



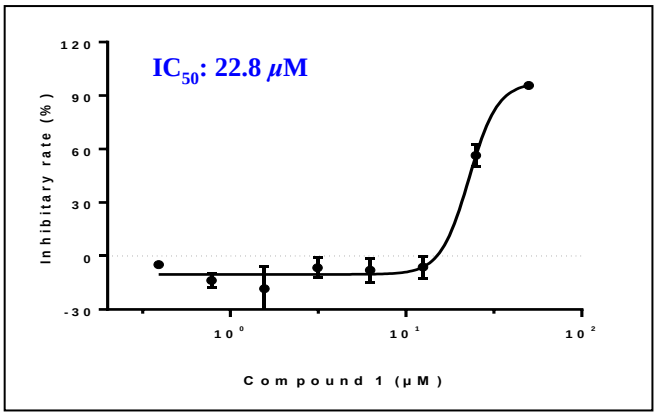
Note: Sorafenib was used as positive control. IC_{50}: 5.5 μM

Figure S1. IC_{50} curve of compound **1** inhibit HepG2 proliferation.



Note: Cisplatin was used as positive control. IC_{50}: 1.6 μM

Figure S2. IC_{50} curve of compound **1** inhibit Huh7/S proliferation.



Note: Sorafenib was used as positive control. IC_{50}: 9.1 μM

Figure S3. IC_{50} curve of compound **1** inhibit L02 proliferation.

References

(1) Liu,Y. Y; Wang, W.; Fang, B.; Ma, F. Y.; Zheng, Q.; Deng, P. Y.; Zhao, S. S.; Chen, M. J.; Yang, G. X.; He, G. Y. *Eur. J. Pharmacol.* **2013**, *698*, 95-102.

(2) Chai, F. N.; Ma,W. Y.; Zhang, J.; Xu, H. S.; Li,Y. F.; Zhou, Q. D.; Li, X. G.; Ye, X. L. *Biomed. Pharmacother.* **2018**, *103*, 1002-1011.

3. Computational data of 1

The 3D structure of **1** was obtained from X-ray data, which was optimized by DFT calculation at b3lyp/6-31G(d,p) level in the gas phase, which was further checked by frequency calculation and resulted in no imaginary frequencies. ECD calculation were performed on the Gaussian 09 program using TD-DFT-b3lyp/6-31G(d,p) level (in methanol).[3,4]

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	5.451022	-0.239243	-1.276167
2	1	0	4.456724	-0.106152	1.612903
3	8	0	-7.969911	-1.383488	-0.591190
4	1	0	-8.416201	-1.700014	0.205452
5	8	0	-1.865778	1.547279	-1.713205
6	8	0	-2.356575	2.182387	1.757400
7	1	0	-2.494654	2.494500	2.661372
8	6	0	-6.568850	-1.360284	-0.322923
9	1	0	-6.212613	-2.383640	-0.103650

10	6	0	-5.876179	-0.881506	-1.603745
11	1	0	-6.092796	-1.596508	-2.404870
12	1	0	-6.334122	0.066827	-1.904443
13	6	0	-4.350231	-0.724835	-1.432246
14	1	0	-3.907569	-0.336274	-2.355185
15	1	0	-3.924239	-1.722257	-1.268882
16	6	0	-4.011708	0.200925	-0.244765
17	6	0	-2.509557	0.250563	0.248693
18	6	0	-1.558230	1.108586	-0.618543
19	6	0	-0.141972	1.173280	-0.093939
20	1	0	-0.199216	1.395664	0.976965
21	6	0	0.518632	-0.242650	-0.210573
22	6	0	1.961241	0.115681	0.279245
23	1	0	1.889201	0.260741	1.368545
24	6	0	3.117839	-0.888352	0.032051
25	1	0	3.218749	-1.033084	-1.051868
26	6	0	4.419713	-0.309053	0.539267
27	6	0	5.498030	-0.042593	-0.203216
28	6	0	6.788996	0.552886	0.306586
29	1	0	6.659365	0.753235	1.379659
30	6	0	8.006553	-0.415266	0.190518
31	1	0	8.879261	0.160256	0.533460
32	6	0	7.851249	-1.621308	1.127171
33	1	0	8.737993	-2.263529	1.092318
34	1	0	7.709755	-1.303257	2.166257
35	1	0	6.984375	-2.229327	0.846662
36	6	0	-4.508097	1.639882	-0.560350
37	1	0	-5.598255	1.702171	-0.549377
38	1	0	-4.124934	2.368448	0.155956
39	1	0	-4.161768	1.944517	-1.547136
40	6	0	-1.823095	-1.163946	0.296818
41	1	0	-1.930588	-1.635384	-0.683749
42	1	0	-2.361173	-1.808589	0.999812
43	6	0	-0.323465	-1.178802	0.678428
44	1	0	0.021671	-2.216028	0.614013
45	1	0	-0.194595	-0.879853	1.727263
46	6	0	0.858696	2.129287	-0.739457
47	1	0	0.697344	2.170676	-1.820012
48	1	0	0.743094	3.149323	-0.362370
49	6	0	2.237596	1.508439	-0.378401
50	1	0	2.862453	1.388623	-1.269783
51	1	0	2.807313	2.146918	0.302300
52	6	0	7.060656	1.904606	-0.383070
53	1	0	6.253888	2.613770	-0.174410
54	1	0	8.000561	2.344272	-0.030597
55	1	0	7.128514	1.798632	-1.471046
56	6	0	8.299562	-0.879274	-1.245141
57	1	0	7.490650	-1.505511	-1.637129
58	1	0	8.437900	-0.038802	-1.932246
59	1	0	9.216160	-1.478053	-1.272322
60	6	0	2.887458	-2.267217	0.682074
61	1	0	2.714651	-2.172715	1.760714
62	1	0	2.027679	-2.785220	0.249608
63	1	0	3.765629	-2.904954	0.541897
64	6	0	0.552482	-0.753090	-1.667316

65	1	0	-0.439239	-0.776411	-2.124720
66	1	0	1.177863	-0.122408	-2.304602
67	1	0	0.960760	-1.768180	-1.706051
68	6	0	-2.653065	0.781116	1.726837
69	1	0	-1.942399	0.256358	2.379086
70	6	0	-4.114819	0.472248	2.168195
71	1	0	-4.654174	1.419874	2.276104
72	1	0	-4.155658	-0.041816	3.134729
73	6	0	-4.708569	-0.361757	1.025810
74	1	0	-4.347508	-1.391937	1.154225
75	6	0	-6.227894	-0.473800	0.889956
76	1	0	-6.699619	0.506053	0.759247
77	1	0	-6.660979	-0.913721	1.800263

References

(3) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, Jr. J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V.N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J.C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision C.01; Gaussian, Inc., Wallingford CT: **2010**.

(4) Willoughby, P. H.; Jansma, M. J.; Hoye, T. R. *Nat. Protoc.* **2014**, *9*, 643-660.

4. NMR, MS, IR and ECD spectra of compound 1

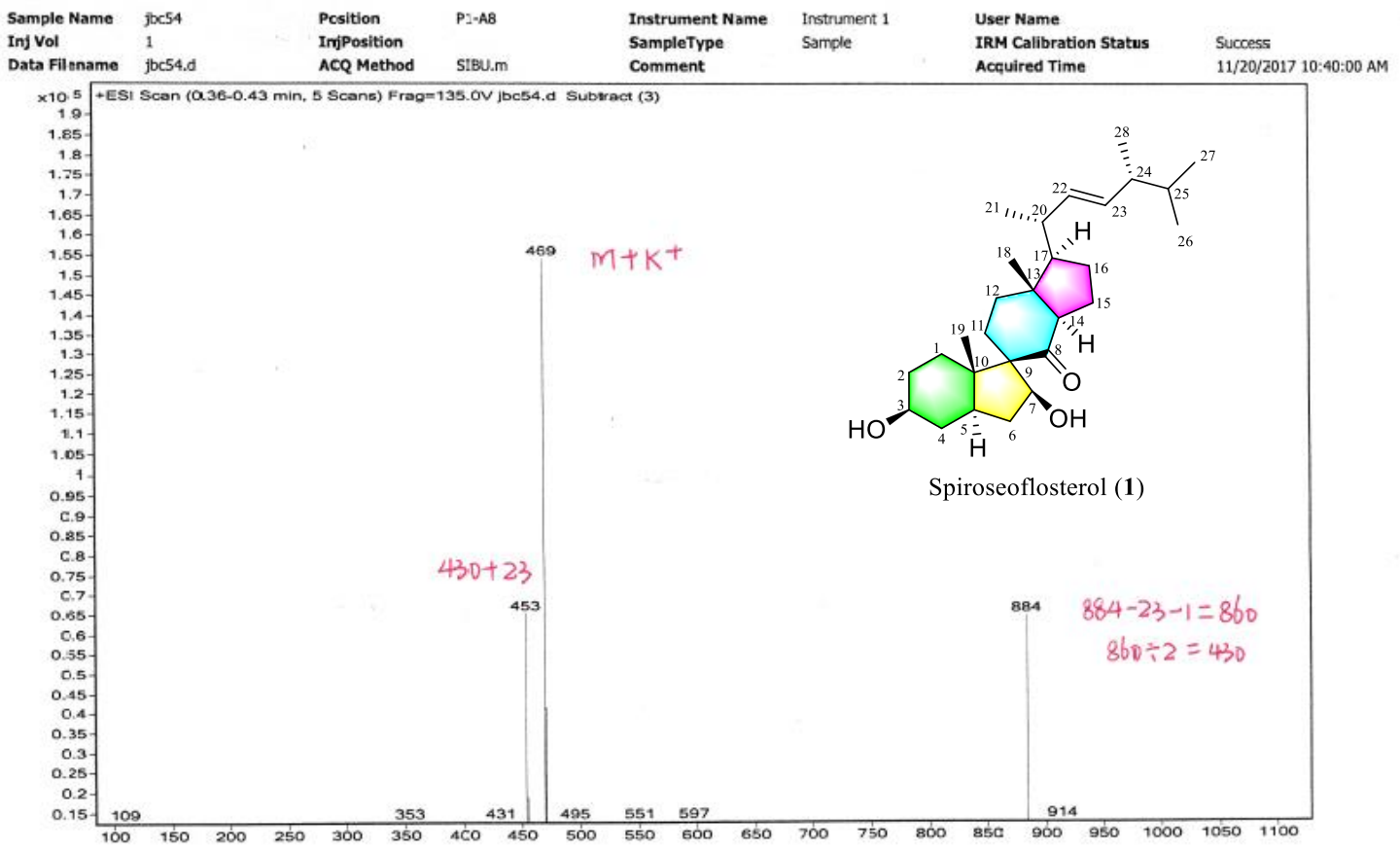


Figure S4. ESIMS spectrum of Spiroseoflosterol (**1**).

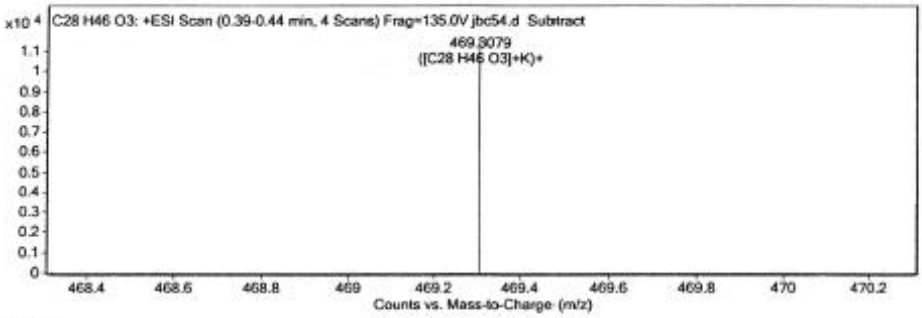
Qualitative Analysis Report

Data Filename	jbc54.d	**Sample Name**	jbc54
Sample Type	Sample	**Position**	P2-A4
Instrument Name	Instrument 1	**User Name**	
Acq Method	SIBU.m	**Acquired Time**	11/24/2017 9:48:11 AM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.2)

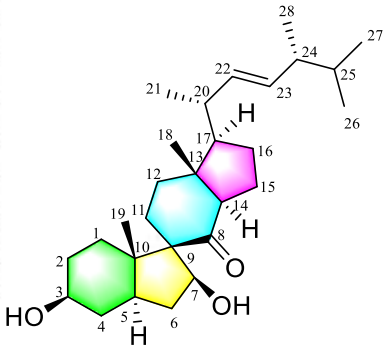
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
107.0408		1777.57		
453.1698	1	903.53		
453.3344	1	3183.25		
454.3378	1	915.18		
463.2614	1	874.41		
469.3079	1	11759.01	C28 H46 O3	(M+K)+
470.3109	1	3546.43	C28 H46 O3	(M+K)+
471.3099	1	1184.89	C28 H46 O3	(M+K)+
883.6777	1	1235.53		
884.6831	1	777.39		



Spiroseoflosterol (**1**)

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C28 H46 O3	430.3447	469.3079	469.3079	0.0	-0.1	6.0000

--- End Of Report ---

Figure S5. HRESIMS spectrum of Spiroseoflosterol (**1**).

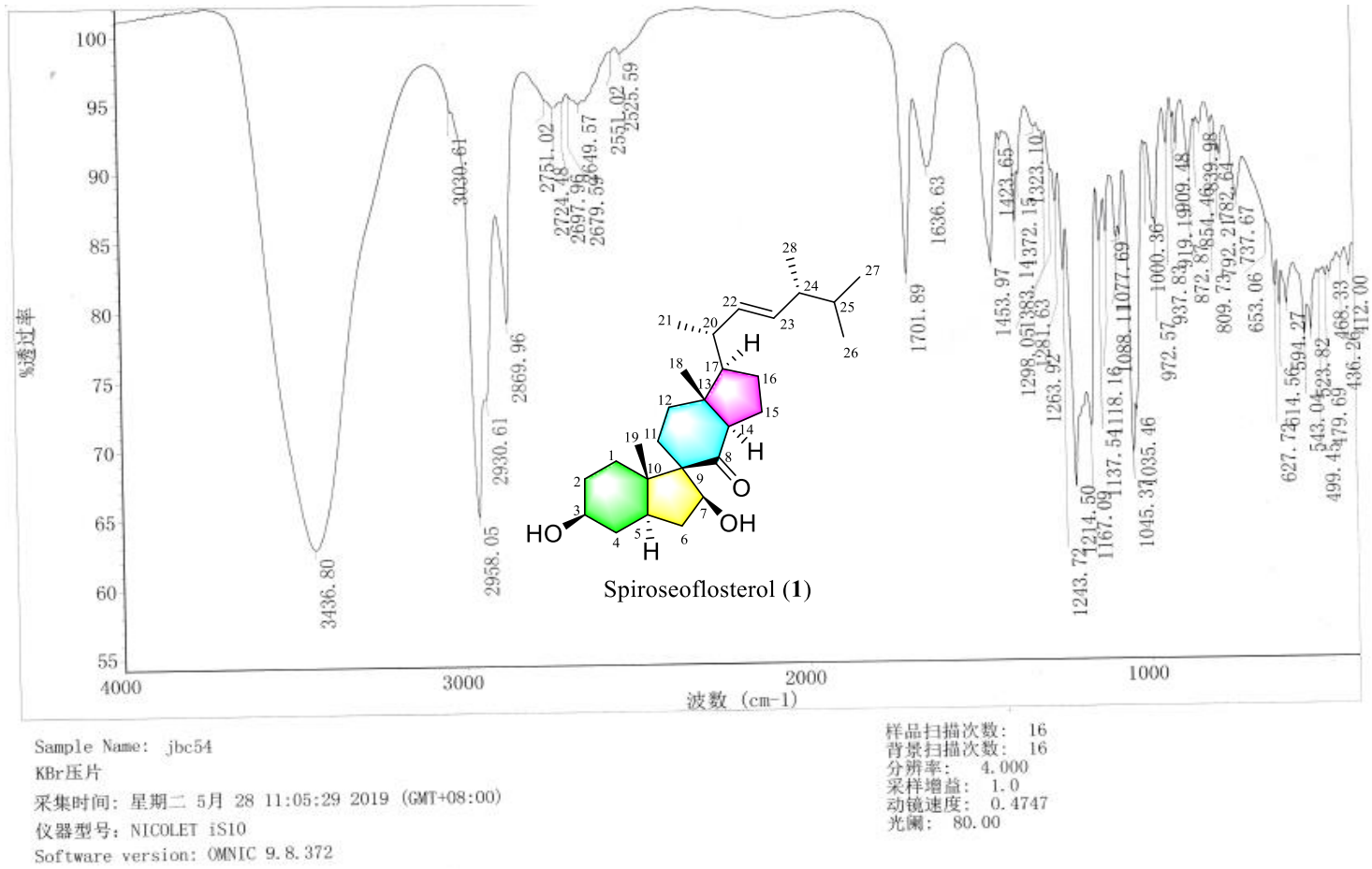


Figure S6. IR spectrum of Spiroseoflosterol (**1**).

Rudolph Research Analytical

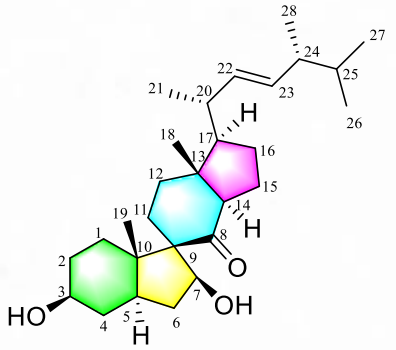
This sample was measured on an Autopol VI, Serial #91058
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : Friday, 24-MAY-2019

Set Temperature : OFF

Time Delay : Disabled

Delay between Measurement : Disabled



Spiroseoflosterol (**1**)

n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	-131.48	1.09	-0.82	-129.57	-132.17

S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	jbc54	01:12:38 PM	-131.74	SR	-0.0303	589	100.00	0.023	26.9
2	jbc54	01:12:46 PM	-131.74	SR	-0.0303	589	100.00	0.023	26.9
3	jbc54	01:12:54 PM	-132.17	SR	-0.0304	589	100.00	0.023	27.0
4	jbc54	01:13:02 PM	-132.17	SR	-0.0304	589	100.00	0.023	27.0
5	jbc54	01:13:10 PM	-129.57	SR	-0.0298	589	100.00	0.023	27.0

Figure S7. Optical rotation spectrum of Spiroseoflosterol (**1**).

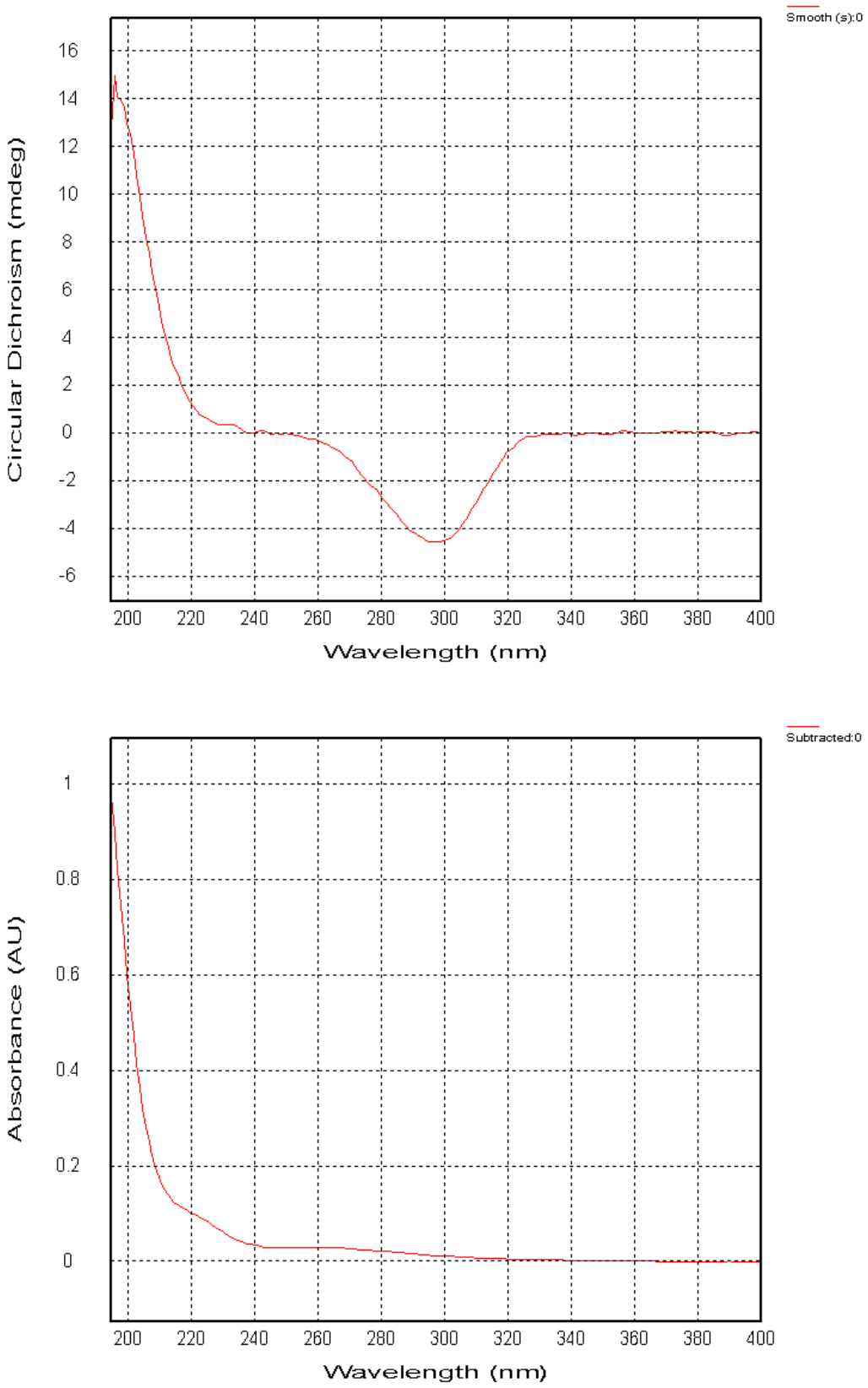


Figure S8. ECD (top) and UV (bottom) spectrum of Spiroseoflosterol (**1**).

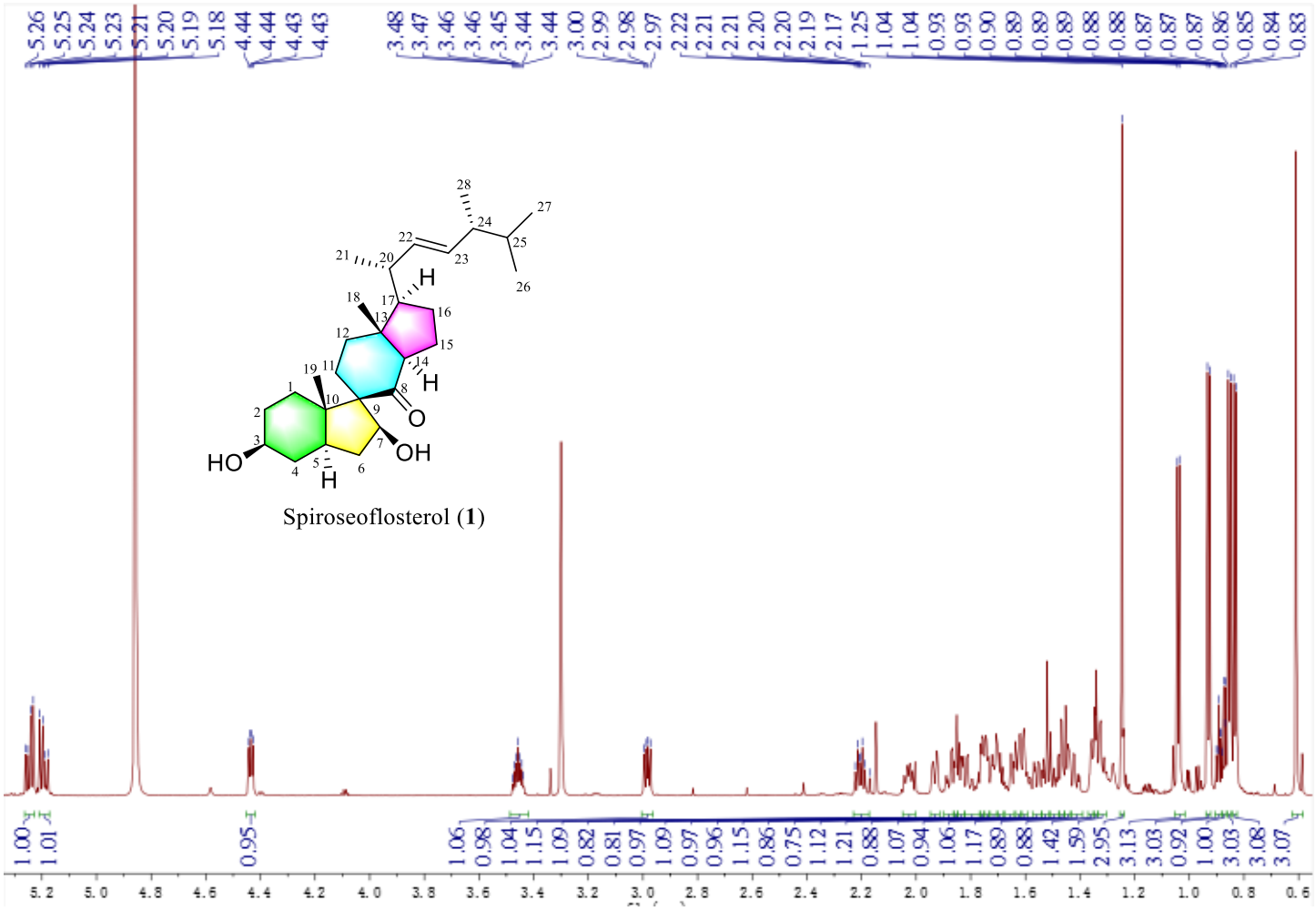


Figure S9. ^{1}H spectrum of Spiroseoflosterol (**1**) in MeOD.

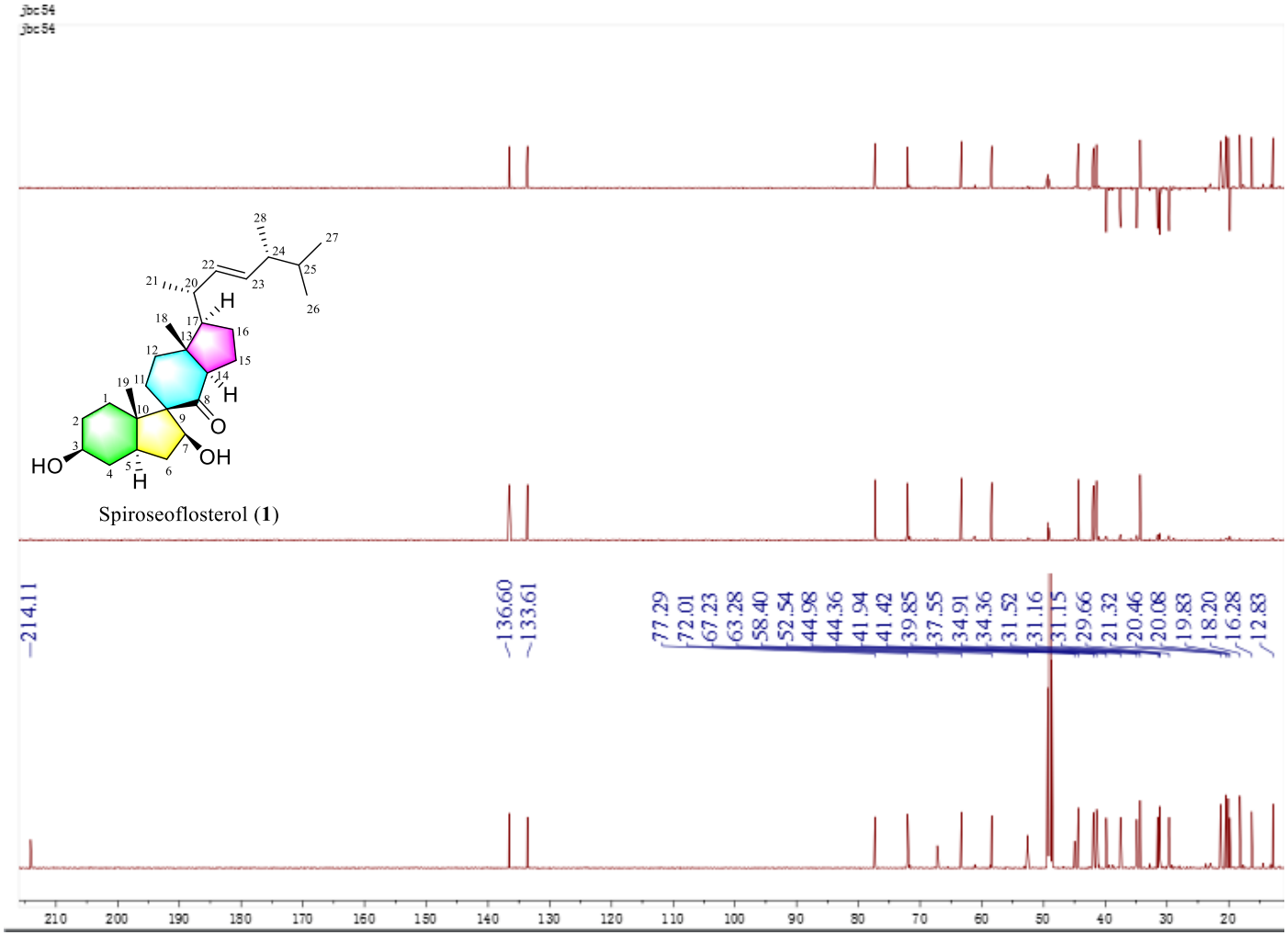


Figure S10. ^{13}C and DEPT spectra of Spiroseoflosterol (**1**) in MeOD.

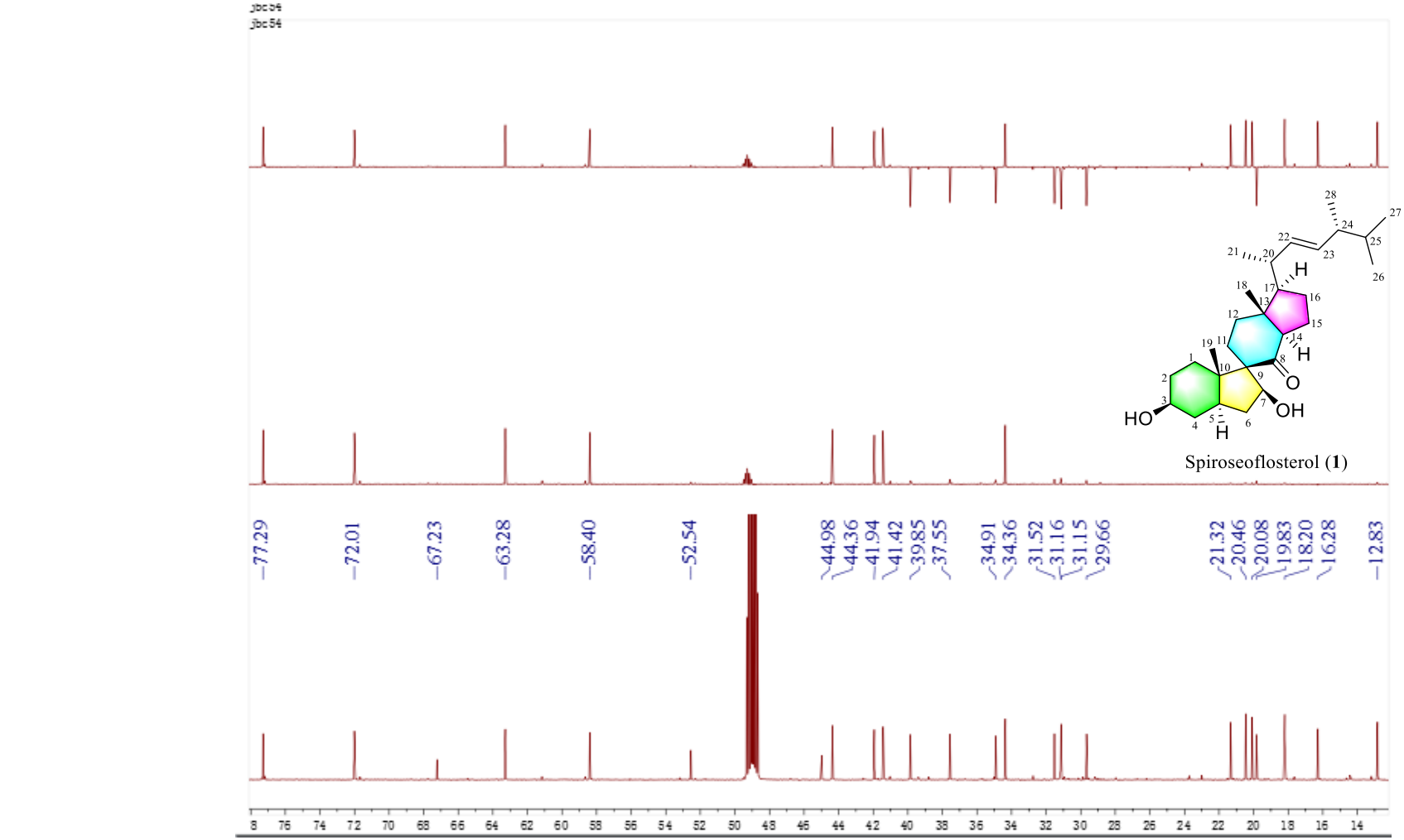


Figure S11. Enlarged ^{13}C spectrum of Spiroseoflosterol (**1**) in MeOD.

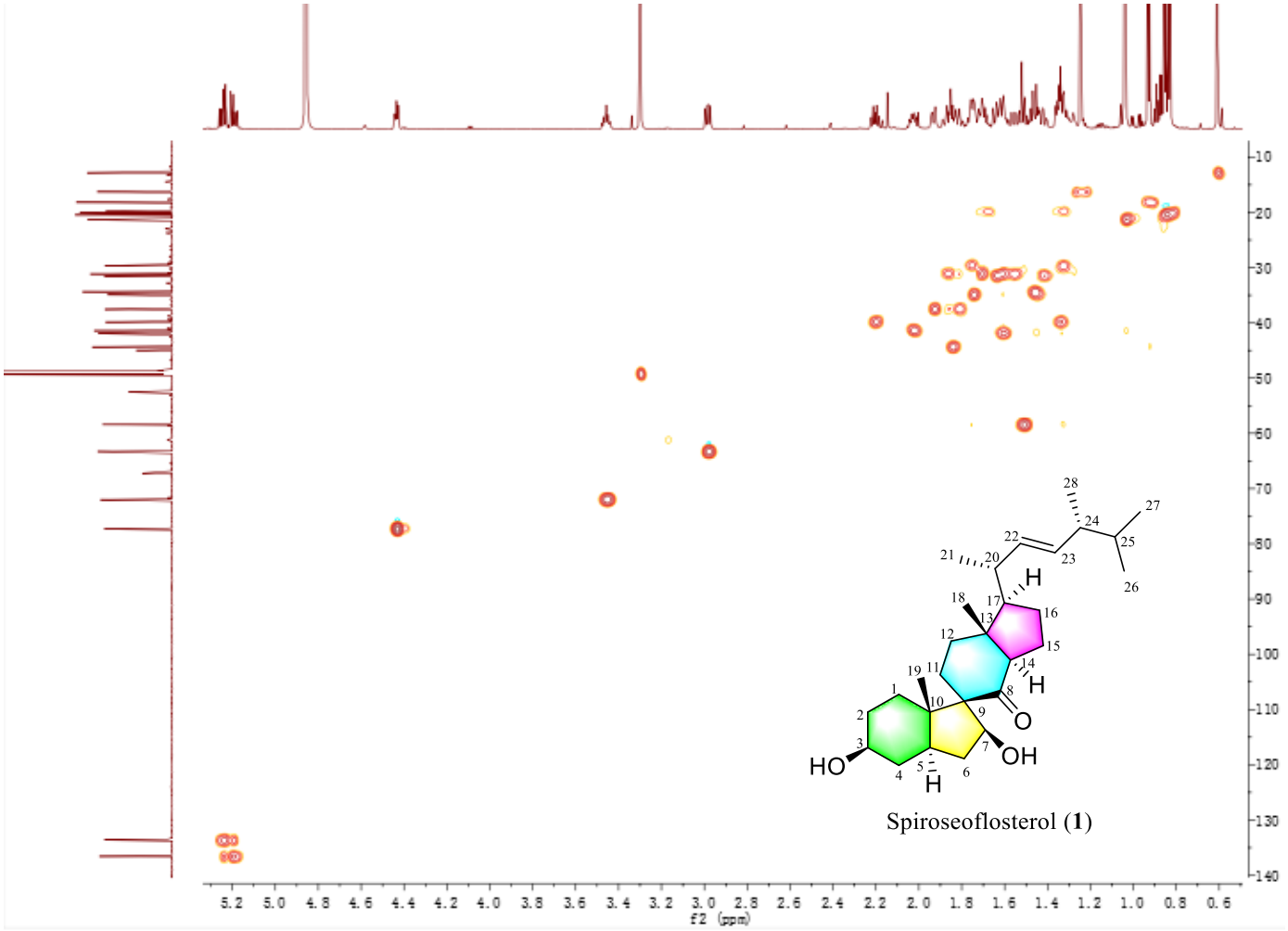


Figure S12. HSQC spectrum of Spiroseoflosterol (**1**) in MeOD.

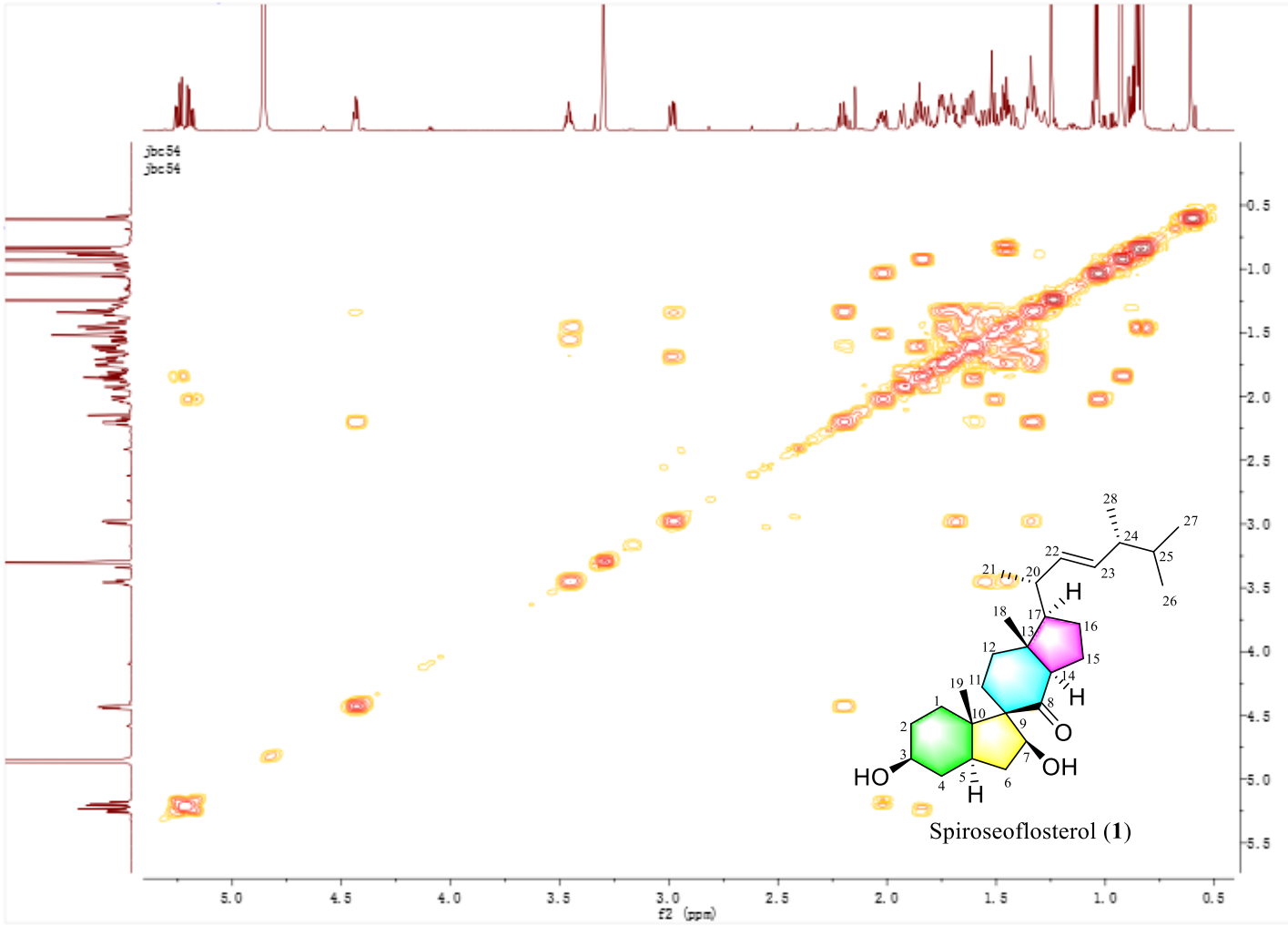


Figure S13. ^{1}H-^{1}H COSY spectrum of Spiroseoflosterol (**1**) in MeOD.

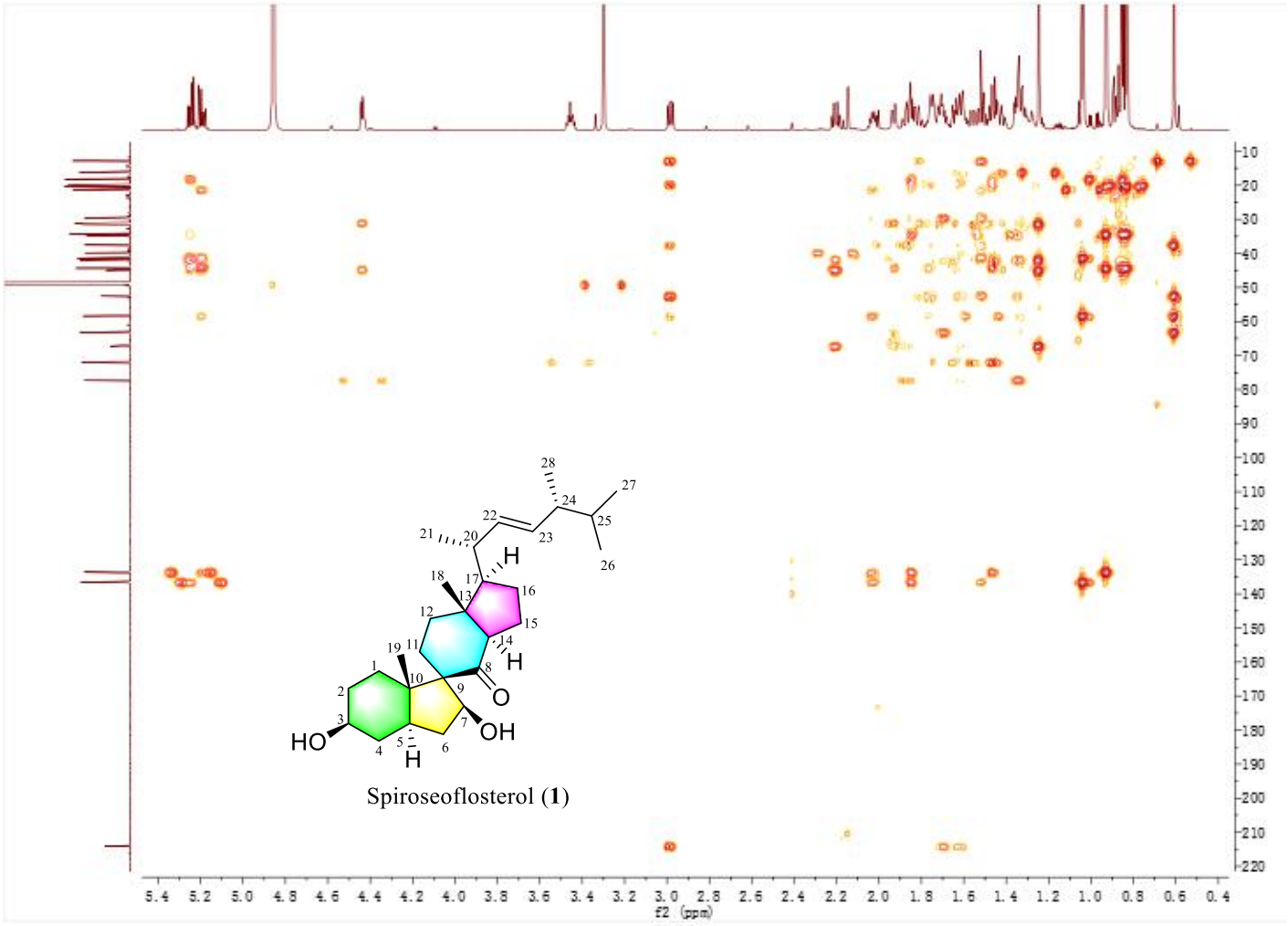


Figure S14. HMBC spectrum of Spiroseoflosterol (**1**) in MeOD.

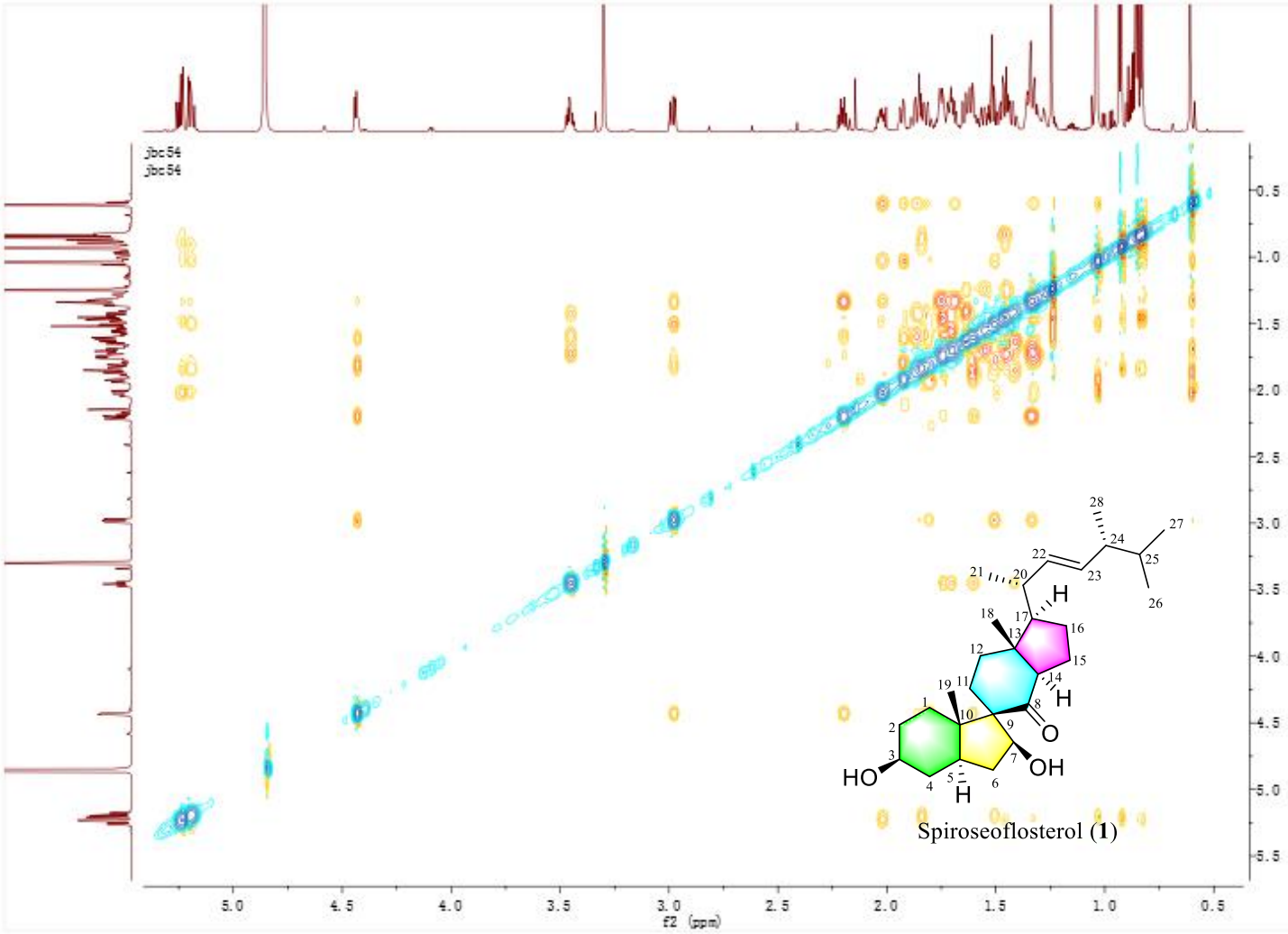
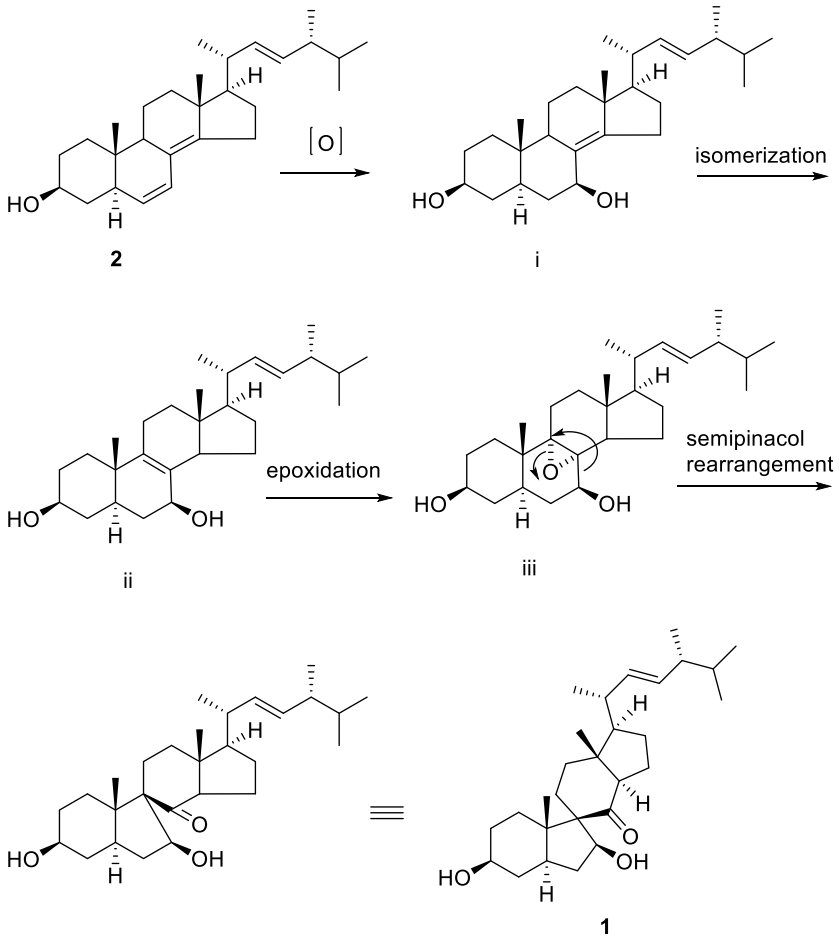


Figure S15. ROESY spectrum of Spiroseoflosterol (**1**) in MeOD.

5. Hypothetical biosynthetic pathways for compound 1



Scheme S1. Hypothetical biosynthetic pathways for compound 1