Supporting Information

Two Furanharzianones with 4/7/5/6/5 Ring System from Microbial Transformation of Harzianone

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1. Experimental section

1.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer Model-343 digital polarimeter (PerkinElmer Inc., Waltham, Massachusetts, USA). IR spectra were acquired on a Nicolet 5700 FT-IR microscope spectrometer (FTIR Microscope Transmission, Thermo Electron Scientific Instrument Corp., Madison, Wisconsin, USA). 1D and 2D NMR spectra were obtained at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR on a VNOVA SYSTEM-600 spectrometer (Varian Inc., Palo Alto, California, USA). Chemical shifts (δ) are given in ppm, and coupling constants (*J*) are given in hertz (Hz). HR-ESI-MS data were measured using an Agilent Technologies 6520 Accurate Mass Q-TOF LC/MS spectrometer (Agilent Technologies, Santa Clara, USA). Column chromatography (CC) was carried out with silica gel (200-300 mesh, Qingdao Marine Chemical Inc. Qingdao, China). Semi-preparative HPLC was performed on a Shimadzu HPLC instrument equipped with a Shimadzu RID-10A detector (Shimadzu Corp., Kyoto, Japan) and a Grace Allsphere silica column (250 mm × 10 mm, i.d., 5 μm, W.R. Grace Corp., Columbia, Maryland, USA) by eluting with mixtures of *n*-hexane and EtOAc or a Grace Adsorbosphere C_{18} column (250 mm × 10 mm, i.d., 5 μm, W.R. Grace Corp., Columbia, Maryland, USA) by eluting with mixtures of CH_3OH and H_2O or CH_3CN and H_2O. Analytical TLC was carried out on pre-coated silica gel GF254 plates (Qingdao Marine Chemical Industry, Qingdao, China), and spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH followed by heating at 120 °C.

1.2 Substrates, bacterium, media and cultivation conditions

Harzianone identified by NMR analysis was isolated from the fungal strain *Trichoderma* sp. Xy24 as reported[1], and dissolved in DMSO at a concentration of 40 mg/mL before use. The bacterium identified as *Bacillus* sp. IMM-006 according to the molecular analysis (16s rDNA sequence, 27F and 1492R primer pair for amplification) was kept in 10% glycerol at −80 °C. The seed cultures were prepared in 50 mL flask with 20 mL LB liquid medium containing yeast extract (5 g/L), tryptone (10 g/L) and NaCl (10 g/L) for 2 days of incubation. The media were autoclaved at 121 °C for 25 min before use. A volume of 1 mL of the seed cultures was added to each 250 mL flask containing 100 mL LB liquid medium

and shaken at 200 r/min and 30 °C in the dark.

1.3 Microbial transformation test by HPLC-MS analysis

Analytical scale fermentation was performed using a 250 mL flask containing 100 mL of medium. After two days of cultivation, 2.0 mg of the substrate was added. The culture control consisted of a fermentation blank, in which the organisms were grown under identical conditions without a substrate. After four days of incubation, the cultures were centrifuged at 6,000×g to get the bacteria liquid. Then the liquid was evaporated under reduced pressure and the resulting residue was dissolved in MeOH before analysis by HPLC-MS.

1.4 Chemical and physical data of harzianone and its metabolites

*Harzianone (**1**).* Colorless oil; $[\alpha]^{20}_{D}$ +42.9 (*c* 0.14, MeOH); HR-ESI-MS (positive) *m/z* 287.2361 $[M+H]^+$ (calcd 287.2369 for $C_{20}H_{31}O$). ^1H and ^{13}C NMR data, see **Table S1**.

*3α-Hydroxy-3β,17-epoxy-harzianone (**2**).* Colorless needle crystals (MeOH-H_2O); $[\alpha]^{20}_{D}$ +16.0 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 254.0 (3.79) nm; IR (ν_{max}): 3319, 2955, 2878, 1739, 1666, 1443, 1359, 1259, 1154, and 1026 cm^{-1}; HR-ESI-MS (positive) *m/z* 317.2105 $[M+H]^+$ (calcd 317.2111 for $C_{20}H_{29}O_3$). ^1H and ^{13}C NMR data, see **Table 1**.

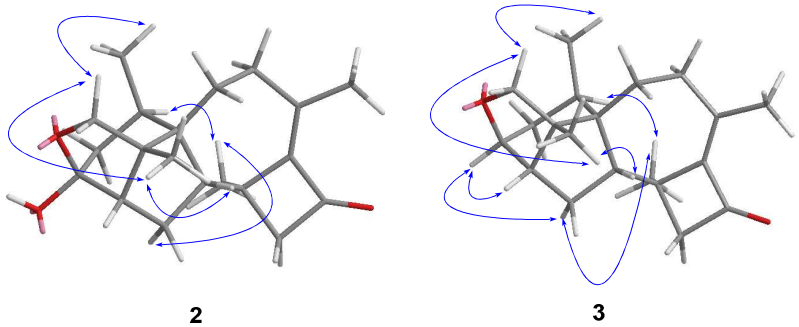
*3β,17-Epoxy-harzianone (**3**).* Colorless oil; $[\alpha]^{20}_{D}$ +83.3 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 255.0 (4.02) nm; IR (ν_{max}): 2929, 2879, 1734, 1663, 1451, 1374, 1118, 1023 cm^{-1}; HR-ESI-MS (positive) *m/z* 301.2153 $[M+H]^+$ (calcd 301.2162 for $C_{20}H_{29}O_2$). ^1H and ^{13}C NMR data, see **Table 1**.

*Harziandione (**4**).* Colorless oil; $[\alpha]^{20}_{D}$ +22.9 (*c* 0.05, MeOH); HR-ESI-MS (positive) *m/z* 301.2153 $[M+H]^+$ (calcd 301.2162 for $C_{20}H_{29}O_2$). ^1H and ^{13}C NMR data, see **Table S1**. All the data were in good agreement with those of harziandione [2].

Table S1. ^{1}H and ^{13}C NMR data of compounds **1** and **4**

no.	**1**		**4**	
	δ_C [a]	δ_H [b]	δ_C [c]	δ_H [d]
1	47.1		49.7	
2	44.3	1.68 (1H, m)	59.4	2.27 (1H, d, 7.8)
3	26.7	2.02 (1H, m)	214.4	
		1.33 (1H, m)		
4	26.4	2.18 (1H, m)	42.8	2.90 (1H, m)
		1.34 (1H, m)		2.05 (1H, m)
5	30.6	2.52 (1H, m)	30.0	2.93 (1H, m)
6	52.0		51.7	
7	31.3	1.92 (1H, m)	30.1	1.91 (1H, ddd, 13.5, 6.4, 1.5)
		1.36 (1H, m)		1.42 (1H, t, 13.5)
8	30.2	2.48 (1H, m)	29.7	2.45 (1H, m)
		1.98 (1H, m)		2.02 (1H, m)
9	148.5		146.5	
10	151.0		149.6	
11	201.2		198.0	
12	60.5	2.59 (1H, d, 16.2)	60.1	2.57 (1H, d, 16.2)
		2.36 (1H, d, 16.2)		2.45 (1H, d, 16.2)
13	41.9		40.1	
14	53.6	2.26 (1H, dd, 11.9, 8.8)	53.2	2.49 (1H, m)
15	28.4	1.94 (1H, m)	26.8	2.05 (1H, m)
		1.46 (1H, dd, 13.3, 8.8)		1.53 (1H, m)
16	26.4	0.91 (3H, s)	25.2	1.00 (3H, s)
17	23.0	1.10 (3H, s)	23.4	0.98 (3H, s)
18	21.1	1.09 (3H, d, 7.3)	21.1	1.12 (3H, d, 7.3)
19	21.9	1.52 (3H, s)	20.9	1.52 (3H, s)
20	22.5	2.09 (3H, s)	22.7	2.13 (3H, s)

[a]150 MHz in MeOD; [b]600 MHz in MeOD; [c]150 MHz in $CDCl_3$; [d]600 MHz in $CDCl_3$.



2 **3**

Scheme S1. Key NOESY (↔) correlations of **2** and **3**.

1.5 Cytotoxicity bioassay [3]

The HCT-8 human colorectal adenocarcinoma cell line, the Bel-7402 human liver

cancer cell line, and the BGC-823 human gastric cancer cell line were purchased from the Institute of Cell Biology (Shanghai, P.R. China). The A549 human lung carcinoma cell line and the A2780 human ovarian cancer cell line were obtained from ATCC (Manassas, VA, USA). All five tumor cell lines were maintained in RRMI 1640 medium supplemented with 10% (*v/v*) fetal bovine serum (FBS), 100 units/mL penicillin, and 100 g/mL streptomycin. Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Tumor cells were seeded in 96-well microtiter plates at 1,200 cells/well. After 24 h, compounds were added to the wells. After incubation for 96 h, cell viability was determined by measuring the metabolic conversion of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) into purple formazan crystals by viable cells. The MTT assay results were read using an MK3 Wellscan (Labsystem Dragon, Helsinki, Finland) plate reader at 570 nm. All compounds were tested at five concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} M) and were dissolved in 100% DMSO with a final concentration of DMSO of 0.1% (*v/v*) in each well. Paclitaxel was used as a positive control. Each concentration of the compounds was tested in three parallels. IC_{50} values were calculated using Microsoft Excel software.

1.6 Anti-inflammatory activity assay [4]

The BV2 microglia cell line was obtained from the Cell Culture Center at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. LPS (Lipopolysaccharides) (from *Escherichia coli* 055:B5) was obtained from Sigma-Aldrich (Saint Louis, USA). After pre-incubation for 24 h in a 96-well plate (at 37 °C with 5% CO_2), the cells were treated with various concentrations of the test compounds (10^{-5}, 10^{-6}, and 10^{-7} M), followed by stimulation with LPS for 24 h. The production of NO was determined by measuring the concentration of nitrite in the culture supernatant. $NaNO_2$ was utilized to generate a standard curve. The absorbances at 550 nm were measured. Curcumin was used as a positive control. Each concentration of the compounds was tested in three parallels and the inhibitory rate was calculated by the average of the 3 parallels.

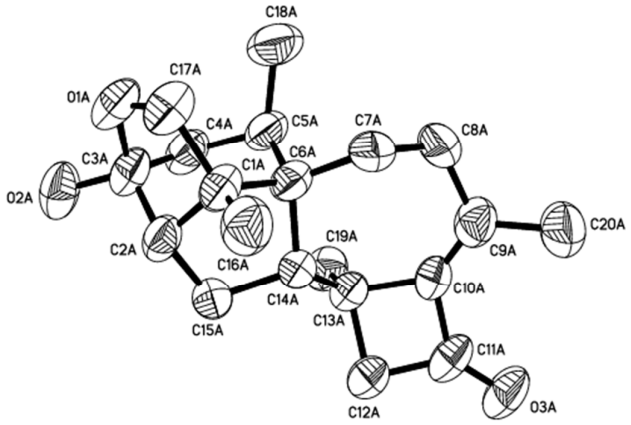
1.7 HIV-inhibitory bioassay [5]

293T cells (2×10^5) were co-transfected with 0.6 μg of pNL-Luv-E^--Vpu^- and 0.4 μg of pHIT/G. After 48h, the VSV-G pseudotyped viral supernatant (HIV-1) was harvested by filtration through a 0.45 μm filter and the concentration of viral capsid protein was determined by p24 antigen capture ELISA (Biomerieux). SupT1 cells were exposed to VSV-G pseudo typed HIV-1 (MOI =1) at 37 °C for 48 h in the absence or presence of test compounds (Efavirenz was used as positive control). The inhibition rate was determined by using a firefly Luciferase Assay System (Promega).

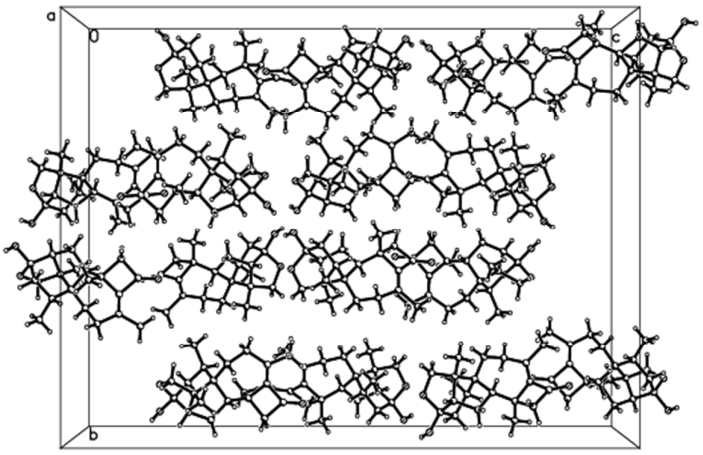
1.8 X-ray crystallographic analyses of 3α-hydroxy-3β,17-epoxy-harzianone (2)

Crystal data for 3α-hydroxy-3β,17-epoxy-harzianone (**2**): Colorless needle crystals of **2** were obtained in$CH_3OH–H_2O$ (4:1). Crystal data were collected on a Rigaku MicroMax 002+ diffractometer with Cu Kα radiation using the ω and κ scan technique to a maximum 2θ value of 145.8°. The crystal structures were solved by direct methods with SHELXS-97, and all non-hydrogen atoms were refined anisotropically using the least-squares method. All hydrogen atoms were positioned by geometric calculations and difference Fourier overlapping calculations. The absolute configuration was not determined attributing to small crystals with poor diffraction. The relative configuration was determined based on the Flack parameter, 0.1(3). Crystallographic data for the structure of **2** have been submitted to the Cambridge Crystallographic Data Centre as supplementary publication CCDC 1511323. Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

$C_{20}H_{28}O_3$, M=316.56, orthorhombic crystal system, space group $P2_12_12_1$, crystal dimensions 0.67×0.25×0.04 mm, Cu Kα radiation, a=8.502(2), b=25.020(5), c=32.864(7)Å, V=6991(3)$Å^3$, Z=16, D_{calcd}=1.203g.cm^{-3}. The total number of independent reflections measured was 13317, of which 7644 were observed ($|F|^2\geq2\sigma|F|^2$). The final indices were R_1=0.0955, wR_2=0.2228, S=0.997.



Scheme S2. Single-crystal X-ray structure of **2**.



Scheme S3. Crystal cell diagram for **2.**

References

1. Zhang, M.; Li, N.; Chen, R.; Zou, J.; Wang, C.; Dai, J. *J Chin Pharm Sci*, **2014**, *23*, 424−424.

2. Ghisalberti, E. L.; Hockless, D. C. R.; Rowland, C.; White, A. H. *J. Nat. Prod.* **1992**, *55*, 1690−1694.

3. (a) Mosumann, T. J. *Immunol. Methods.* **1983**, *65*, 55–63. (b) Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936–943.

4. (a) Kim, H. Y.; Park, E. J.; Joe, E.; Jou, I. *J. Immunol.* **2003**, *171*, 6072−6079. (b) Yang, S.; Zhang, D.; Yang, Z.; Hu, X.; Qian, S.; Liu, J.; Wilson, B.; Block, M.; Hong, J. S. *Neurochem. Res.* **2008**, *33*, 2044−2053. (c) Pang, H. Y.; Liu, G.; Liu, G. T. *Acta Pharmacol. Sin.* **2009**, *30*, 209−218.

5. Zhang, Q.; Liu, Z.; Mi, Z.; Li, X.; Jia, P.; Zhou, J.; Yin, X.; You, X.; Yu, L.; Guo, F.;

Ma, J.; Liang, C.; Cen, S. *Antiviral Res.***2011**, *91*, 321–329.

2. NMR, MS, IR and UV spectra of compounds 2–3

Figure S1. ^{1}H NMR spectrum of compound **2** in methanol-d_4 at 600 MHz

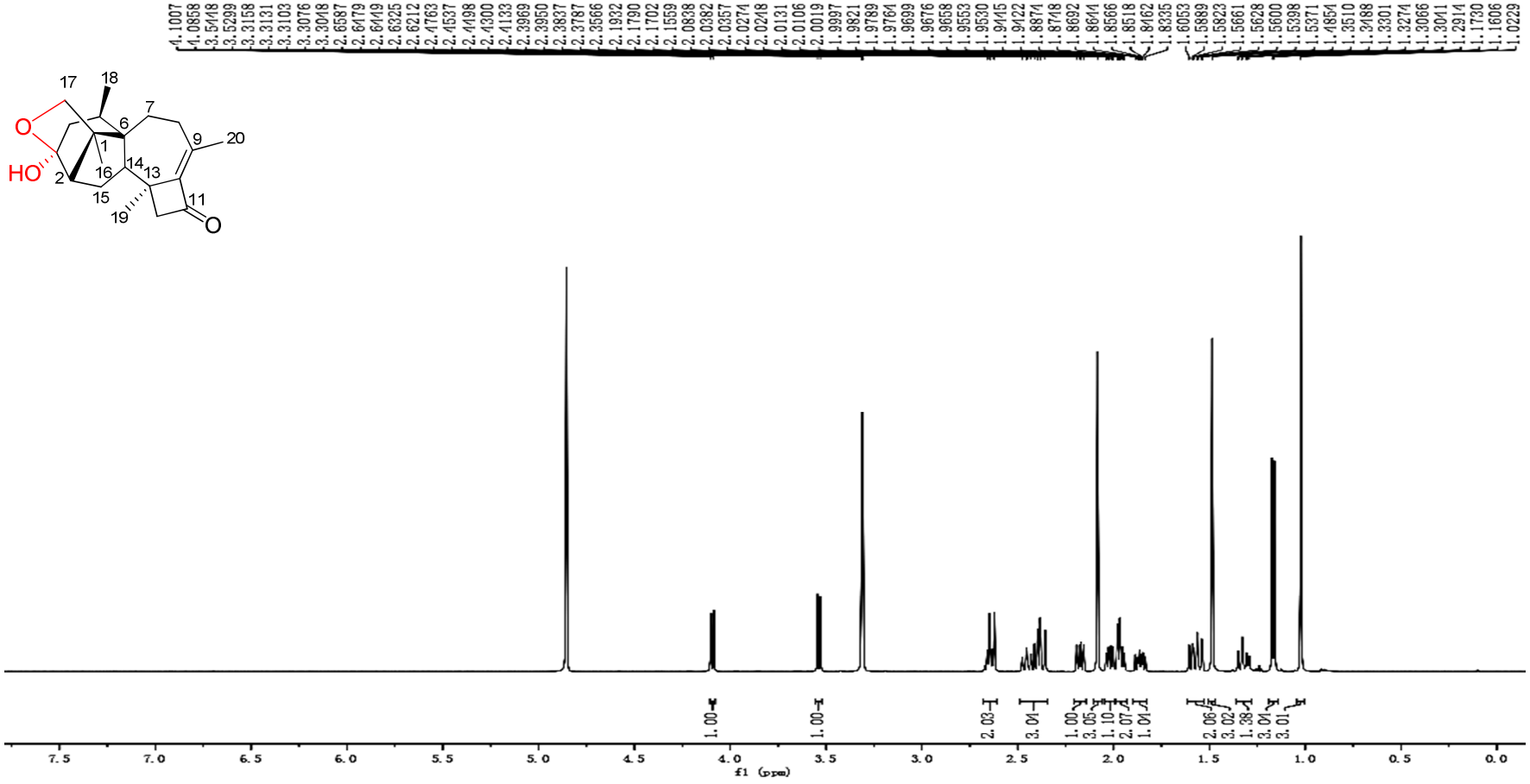


Figure S2. ^{13}C NMR spectrum of compound **2** in methanol-d_4 at 150 MHz

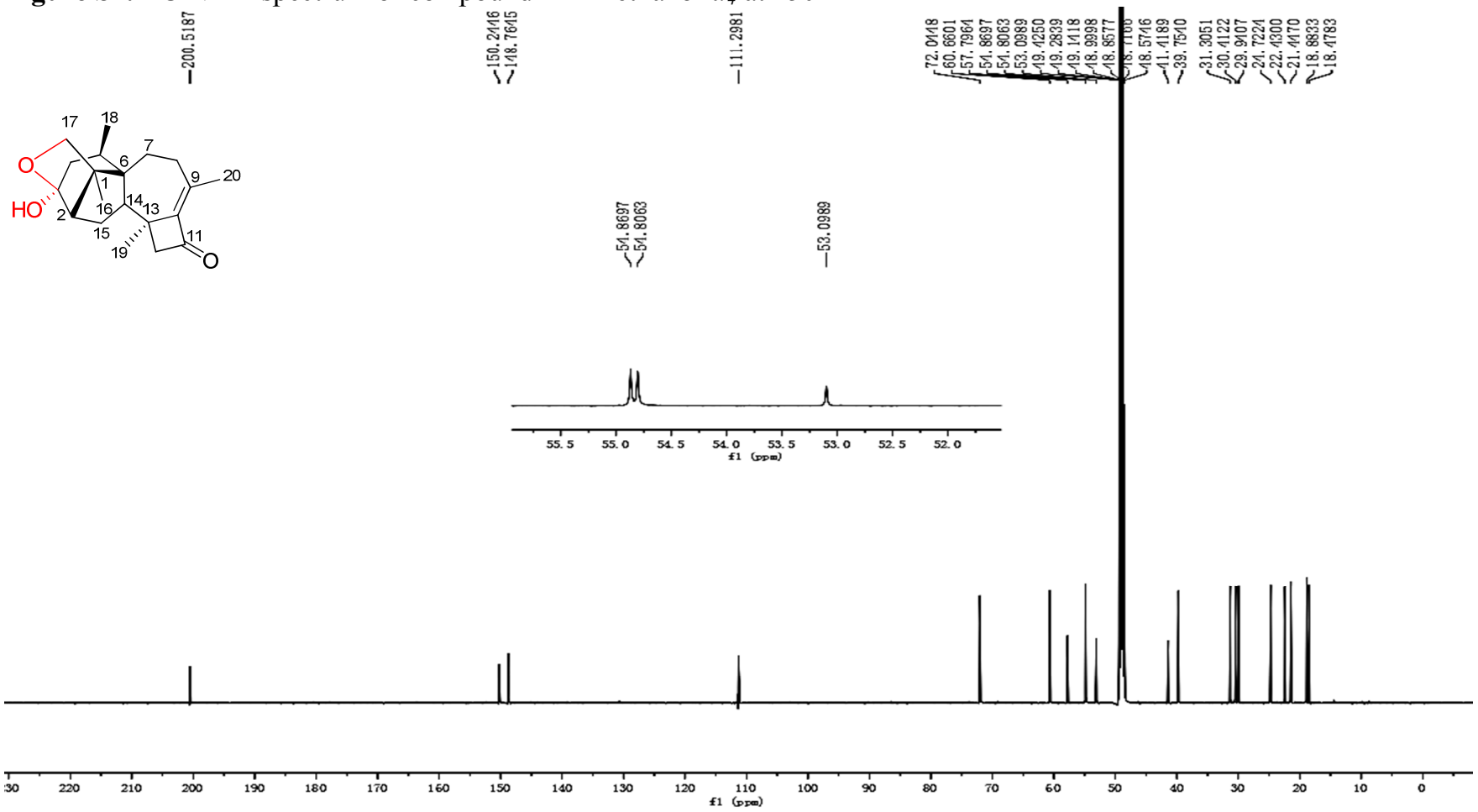


Figure S3. DEPT spectrum of compound **2** in methanol-d_4

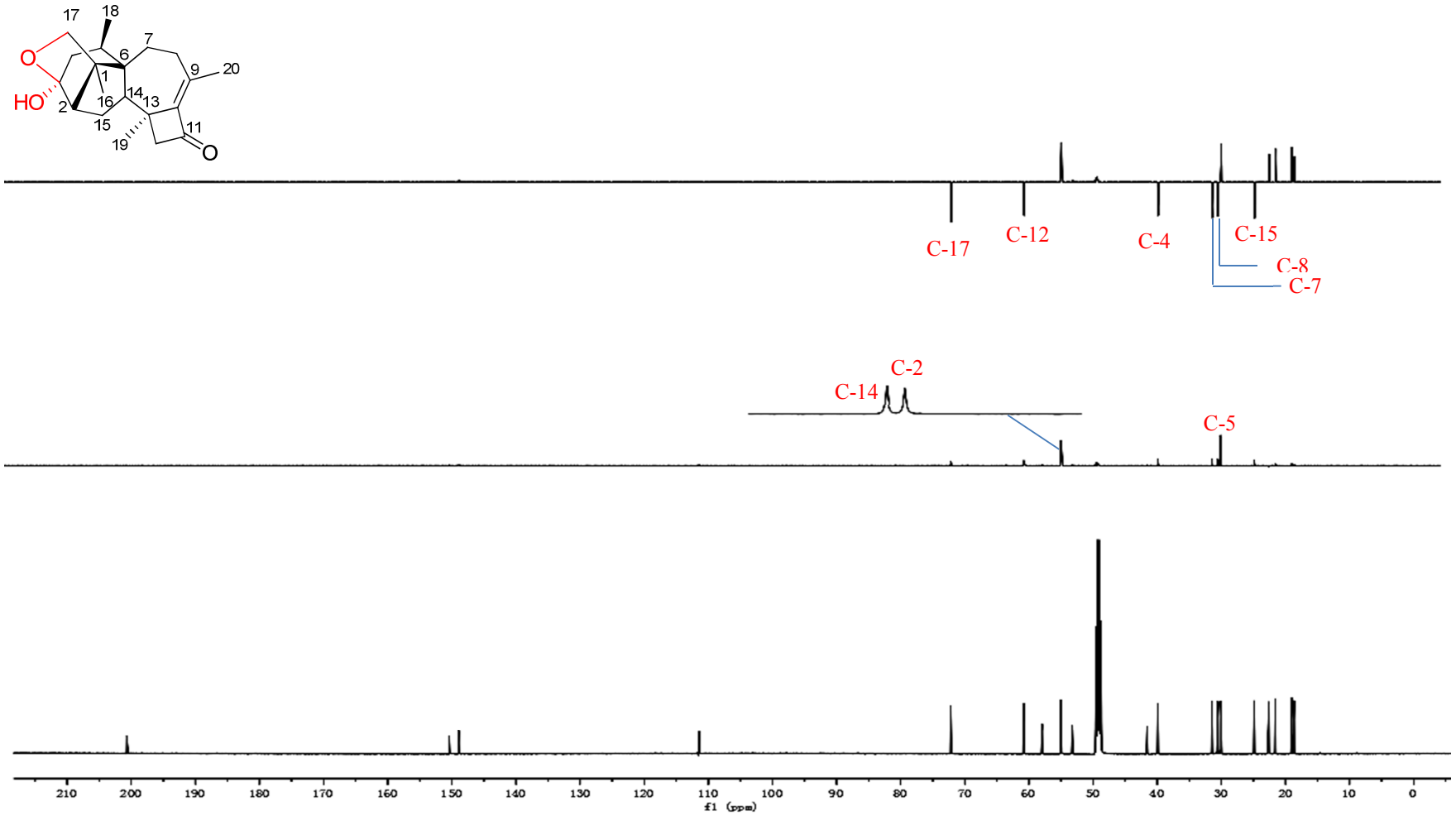


Figure S4. ^{1}H-^{1}H COSY spectrum of compound **2** in methanol-d_4

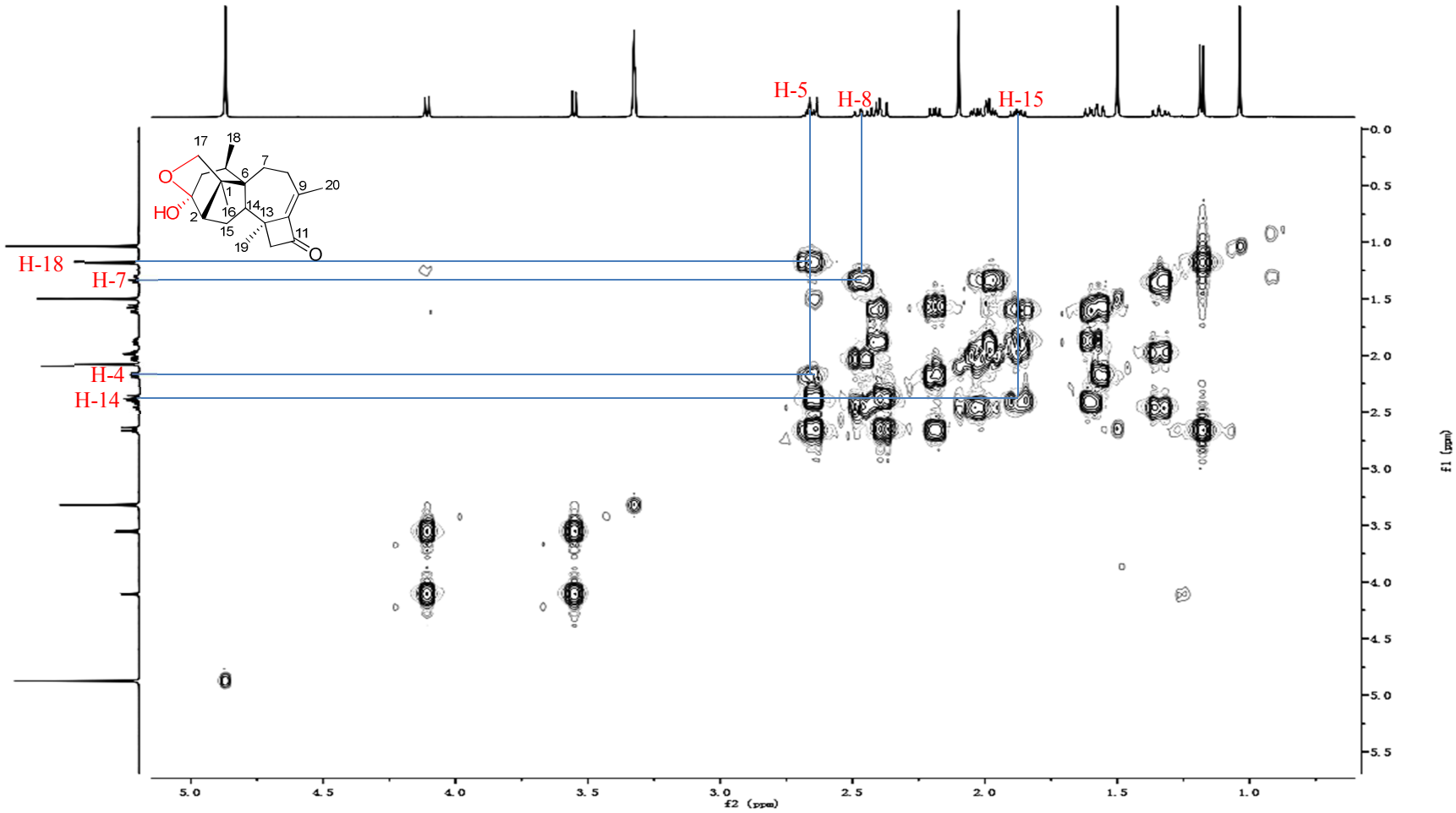


Figure S5. HSQC spectrum of compound **2** in methanol-d_4

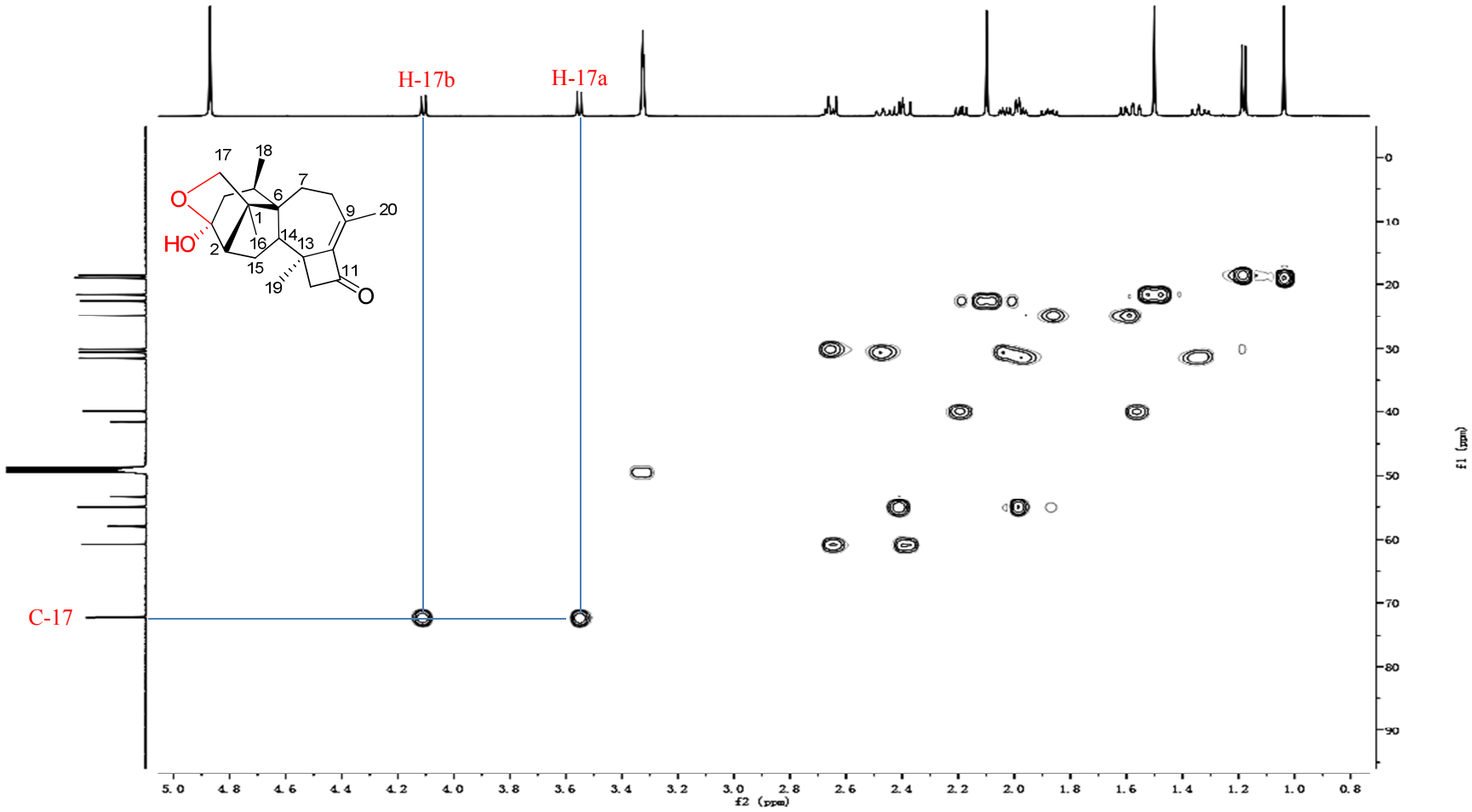


Figure S6. HMBC spectrum of compound **2** in methanol-d_4

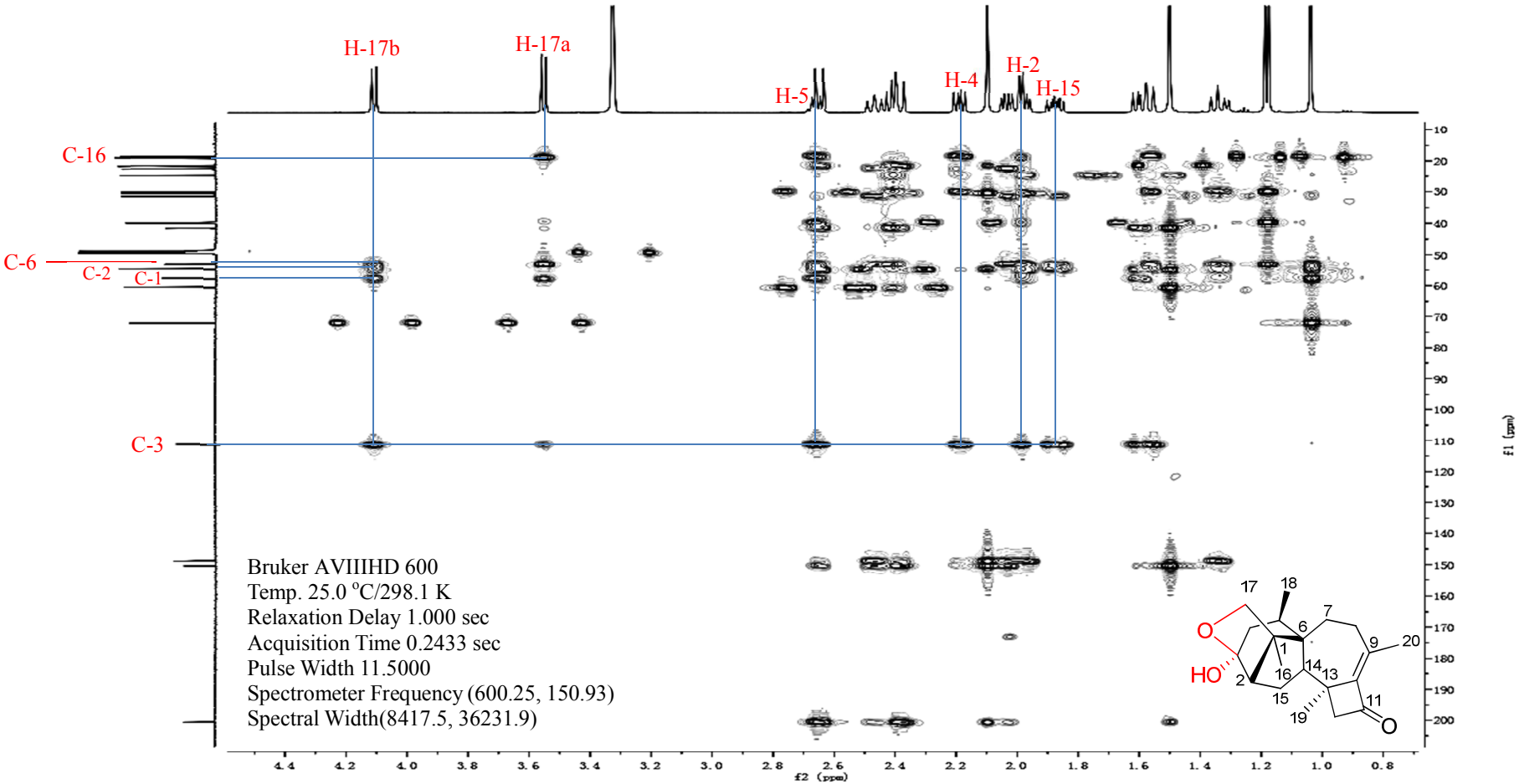


Figure S7 1D-NOE spectrum of compound **2** in methanol-d_4

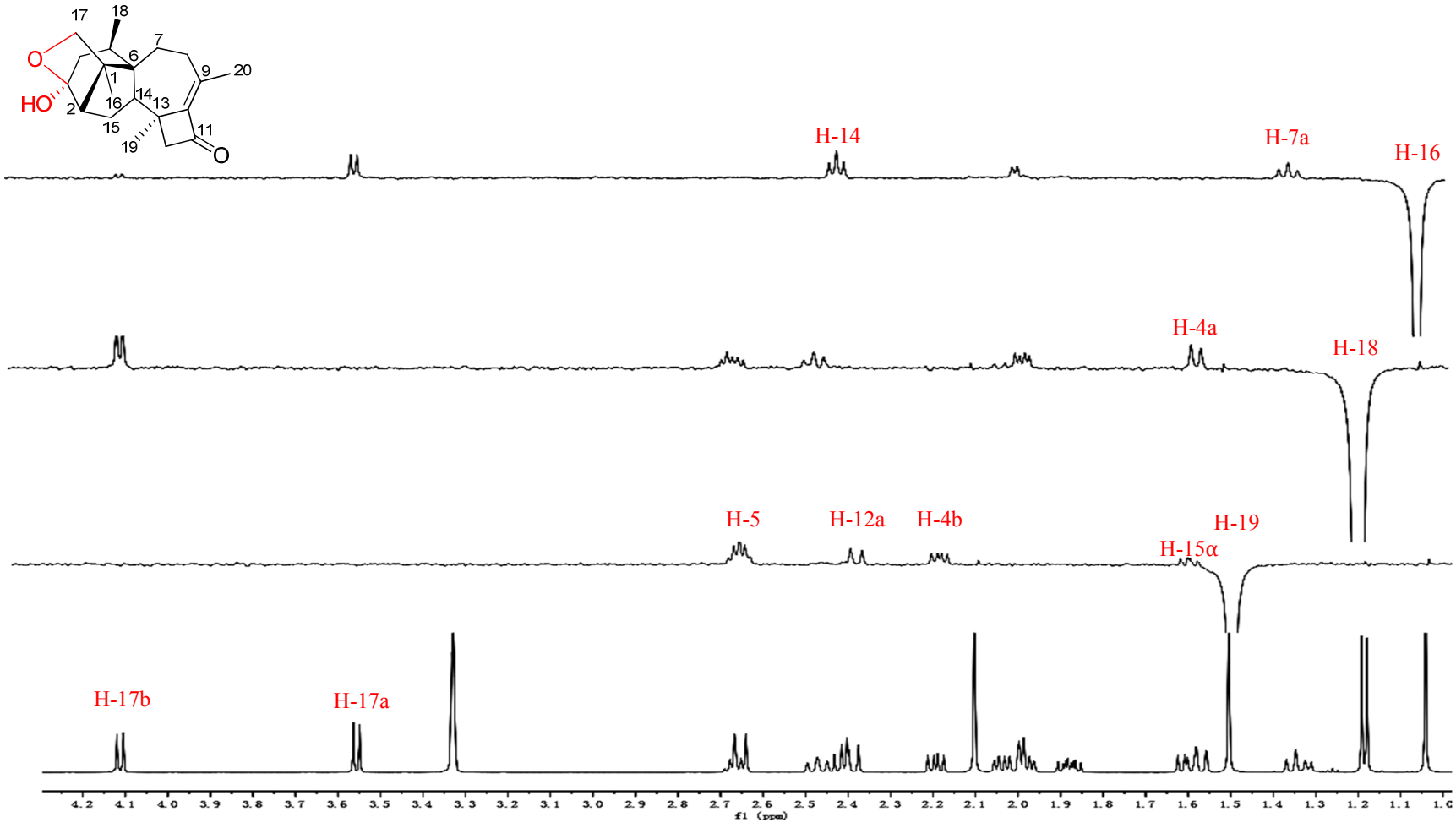
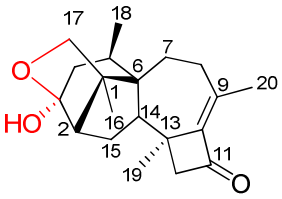


Figure S8. UV spectrum of compound **2** in methanol



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Results Table - scan044

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All calculations have been performed to double precision as defined by ANSI/IEEE STD 754-1985 but have been rounded for display purposes.

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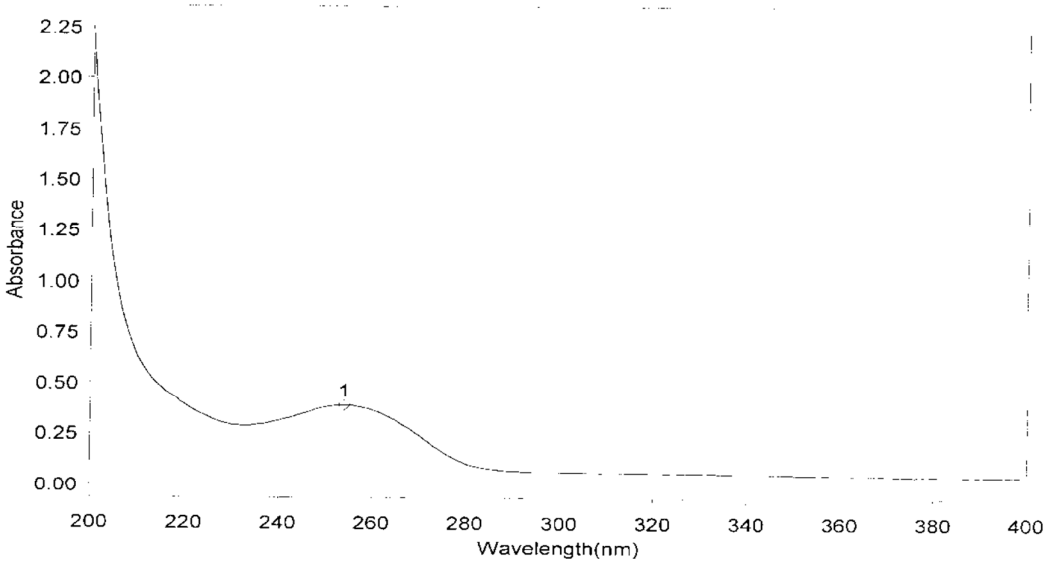


Figure S9. HR-ESI-MS spectrum of compound **2**

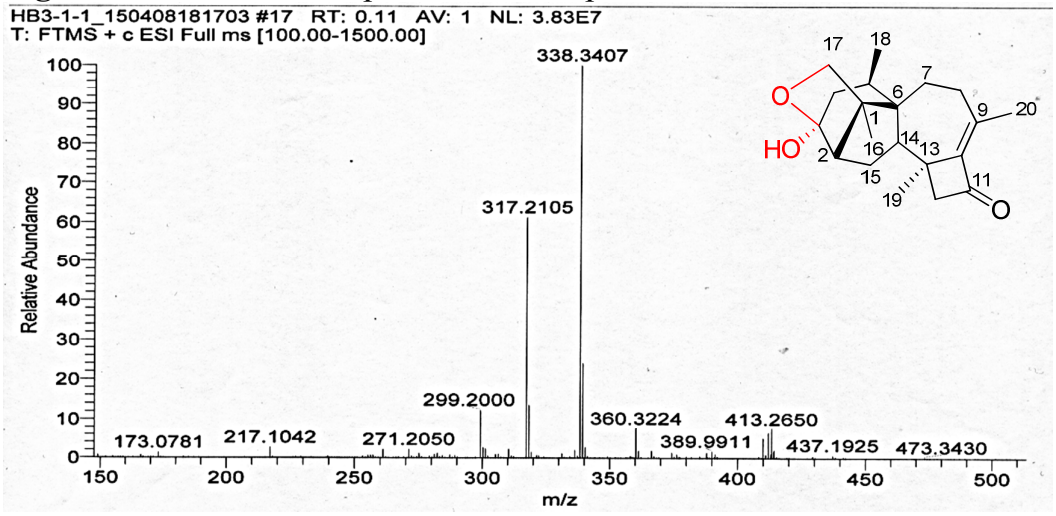


Figure S10. IR spectrum of compound **2**

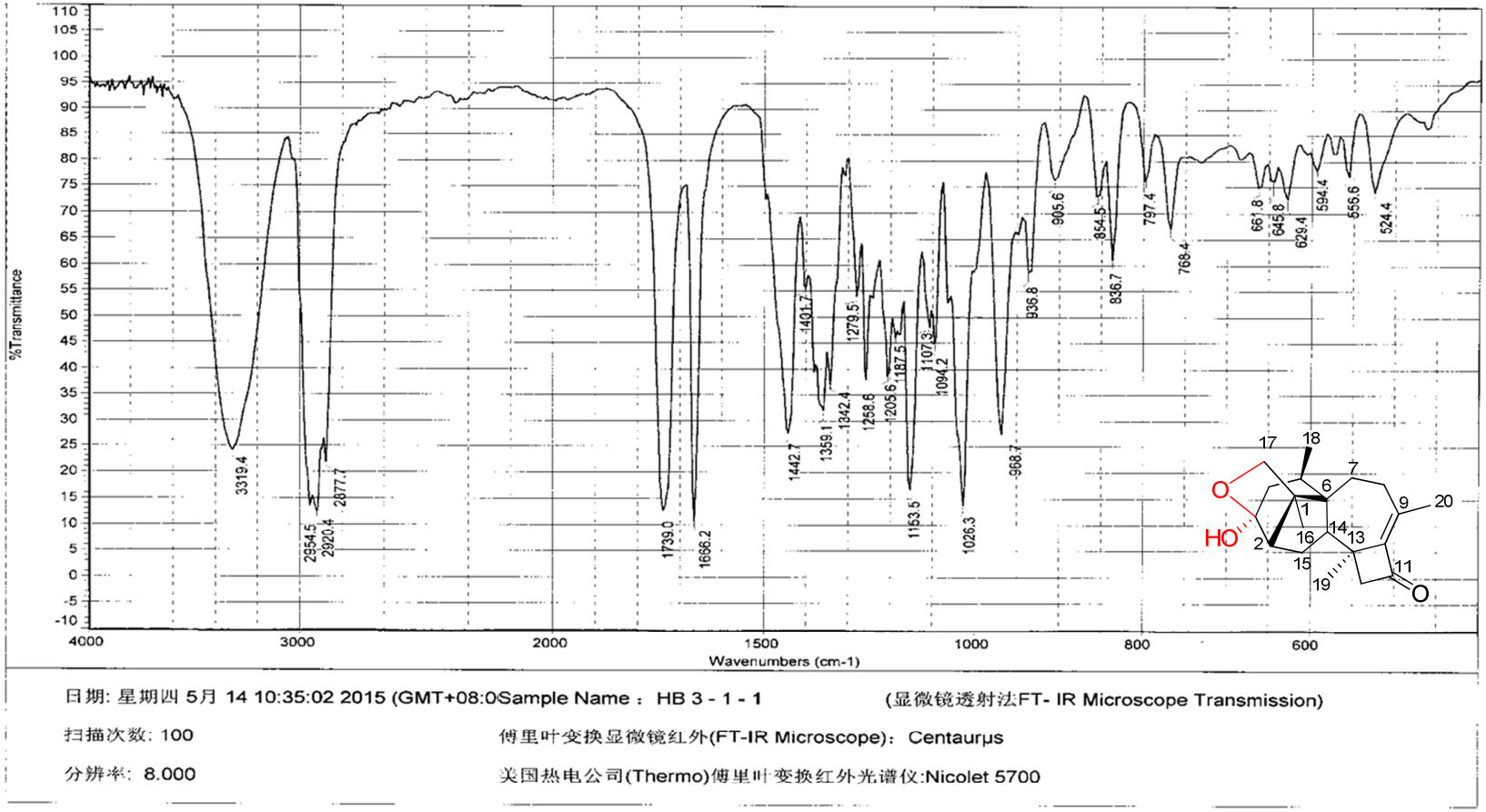


Figure S11. ^{1}H NMR spectrum of compound **3** in methanol-d_4 at 600 MHz

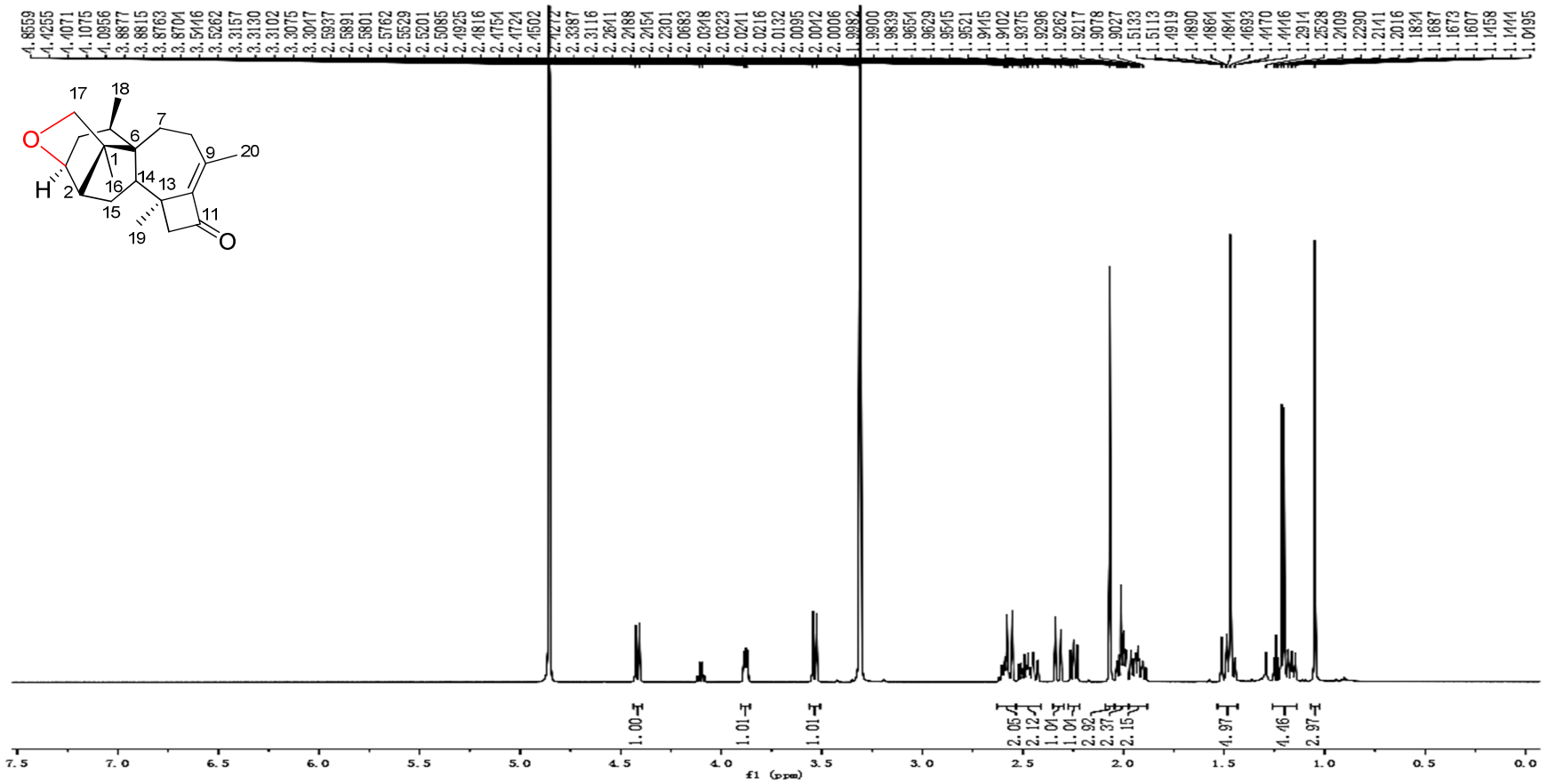


Figure S12. ^{13}C NMR spectrum of compound **3** in methanol-d_4 at 150 MHz

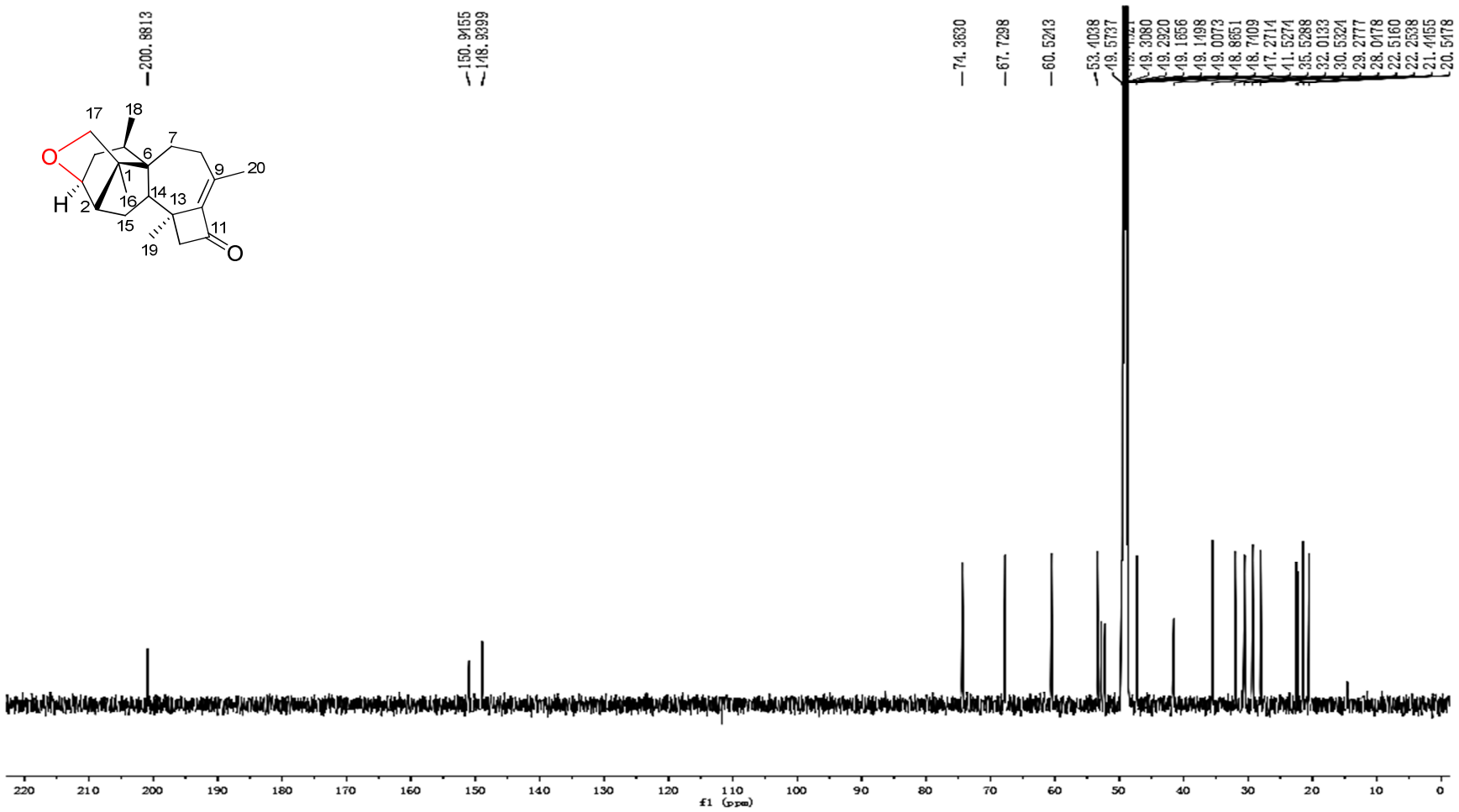


Figure S13. DEPT spectrum of compound **3** in methanol-d_4

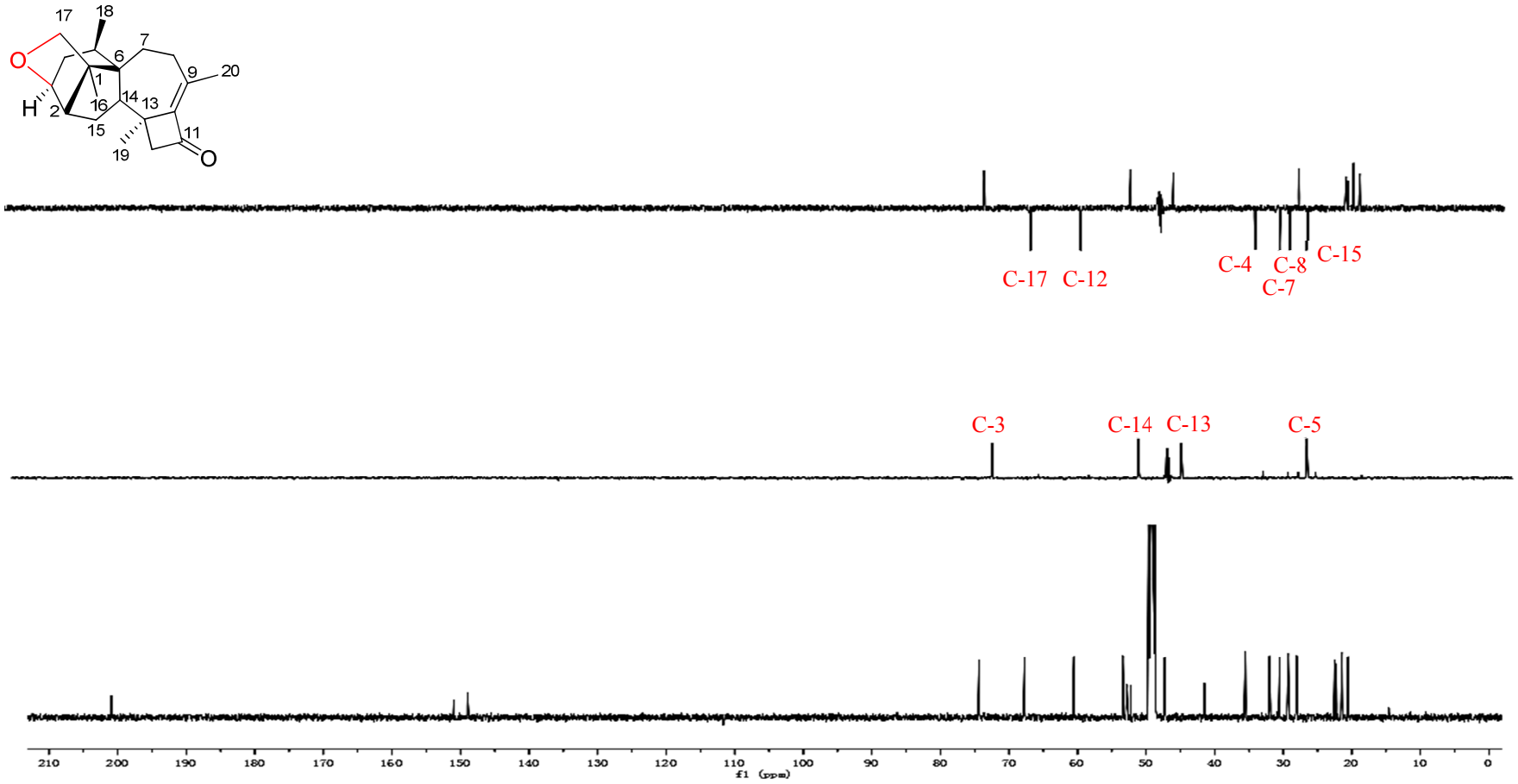


Figure S14. ^{1}H-^{1}H COSY spectrum of compound **3** in methanol-d_4

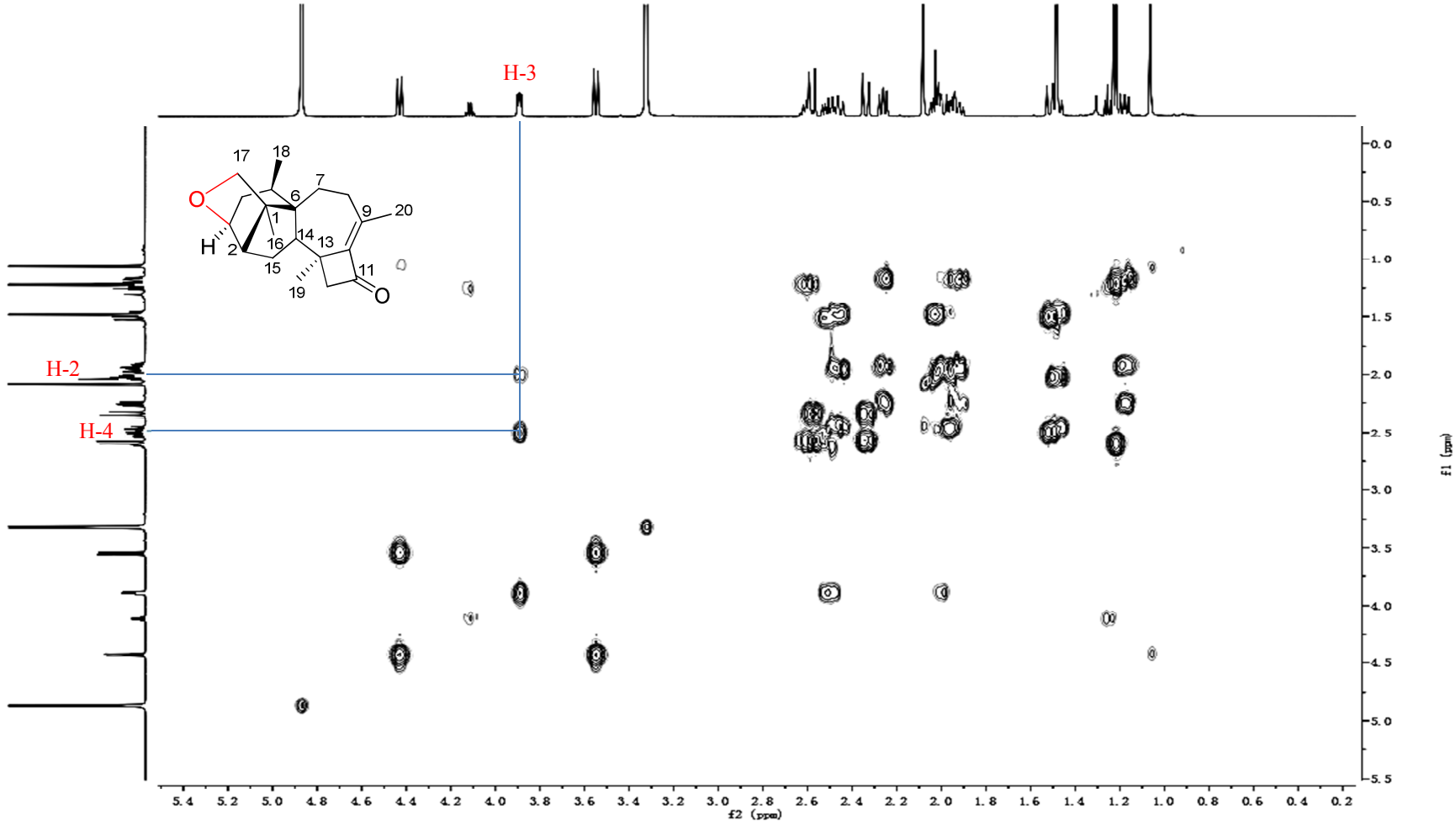


Figure S15. HSQC spectrum of compound **3** in methanol-d_4

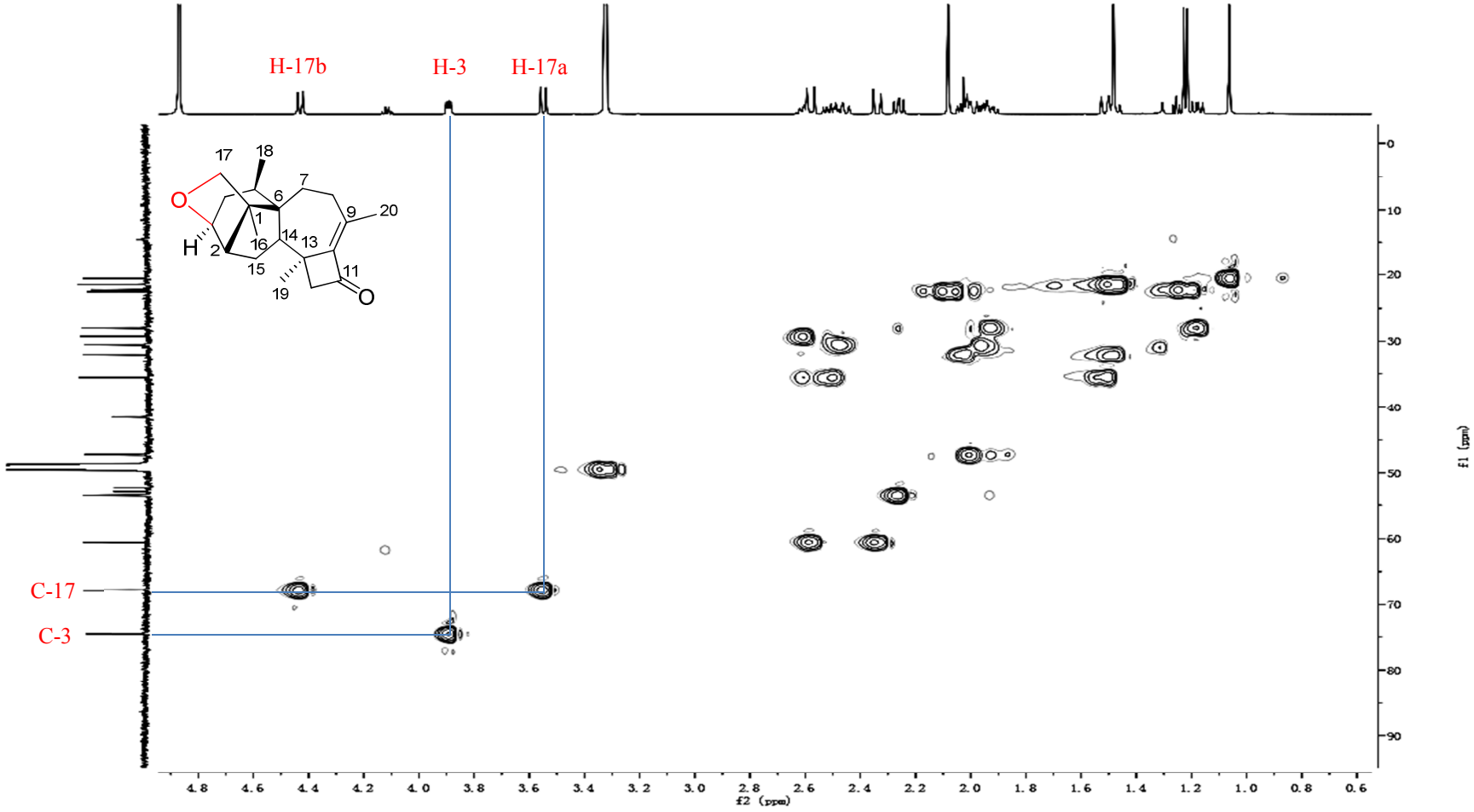


Figure S16. HMBC spectrum of compound **3** in methanol-d_4

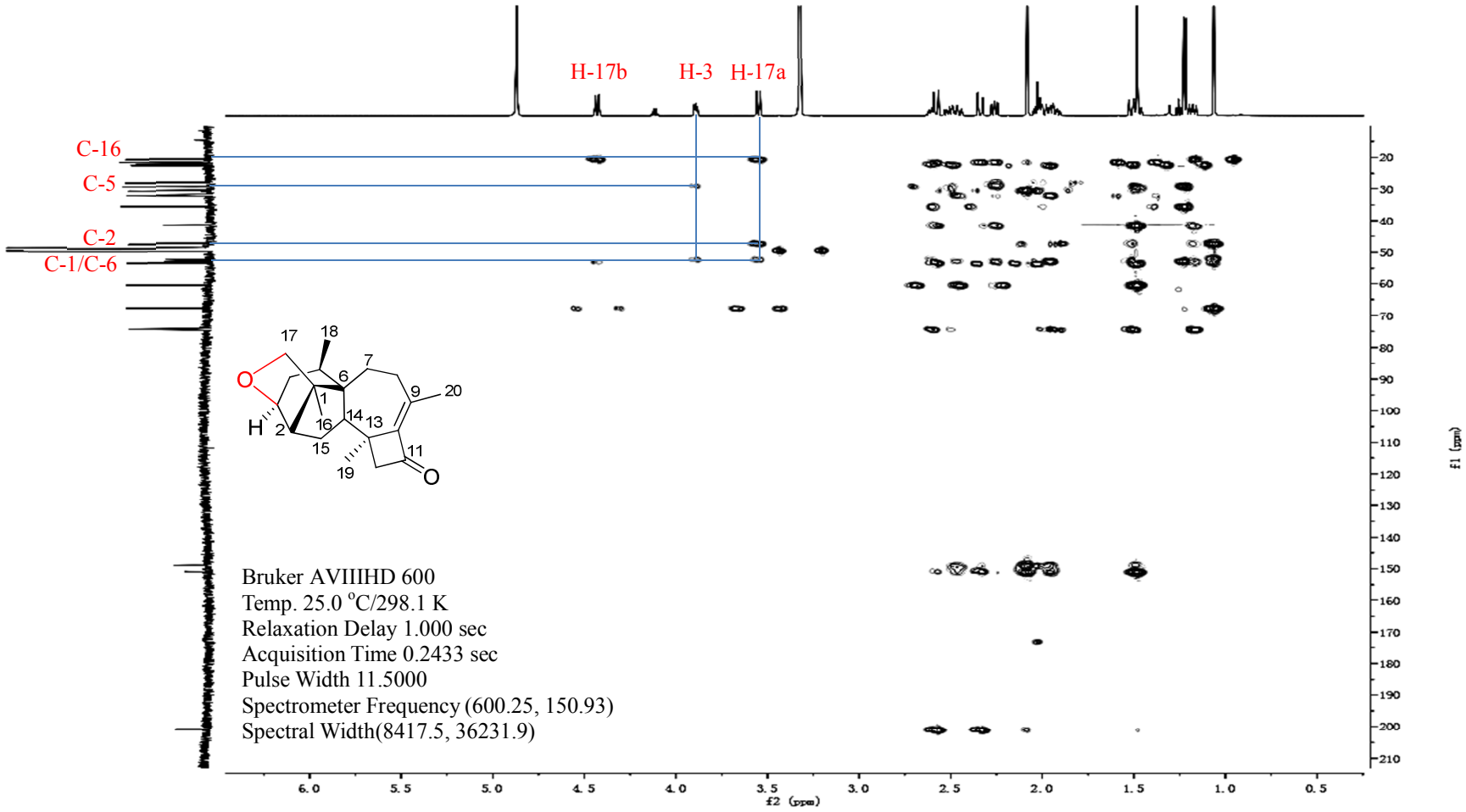


Figure S17. 1D-NOE spectrum of compound **3** in methanol-d_4

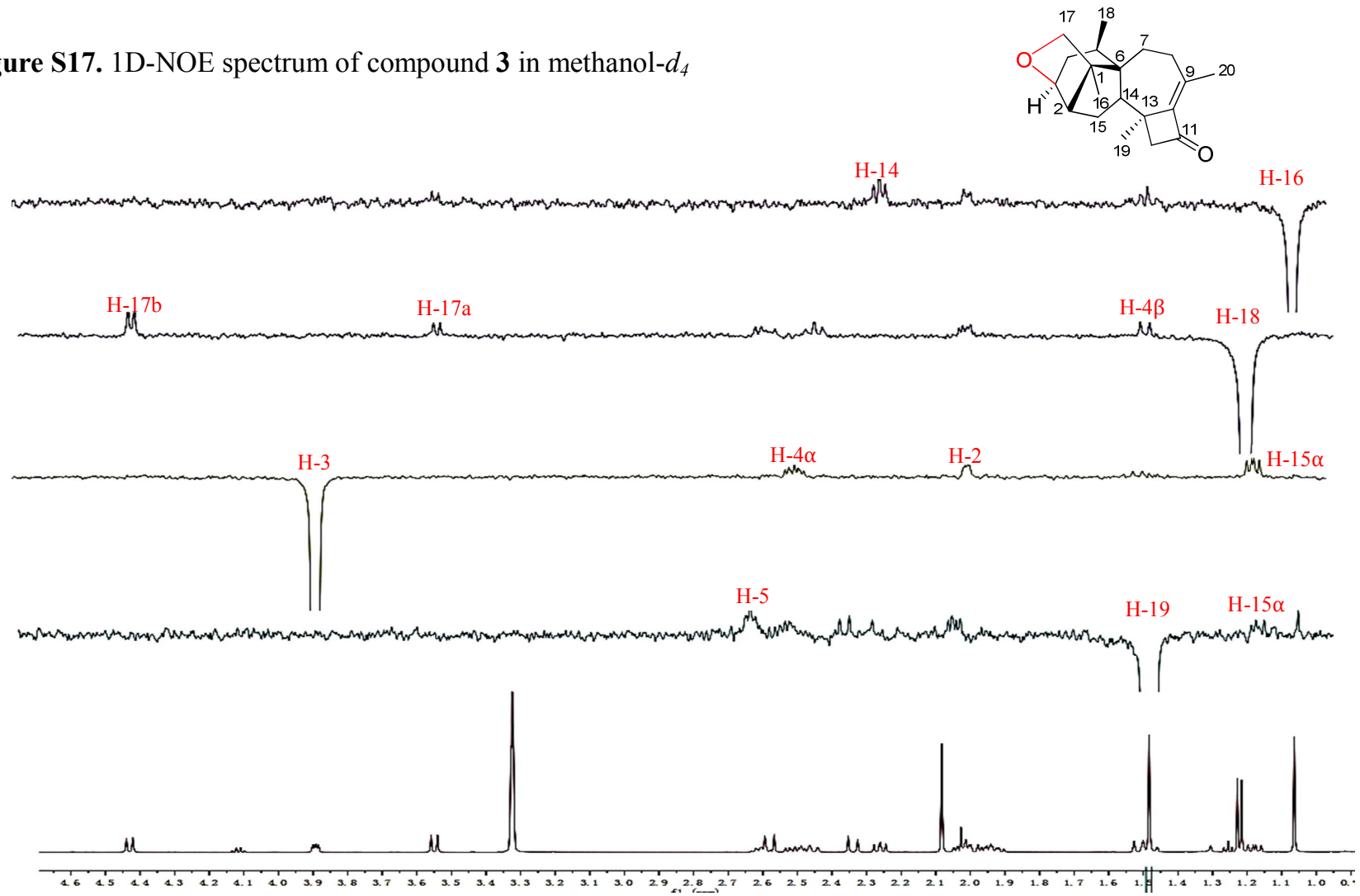
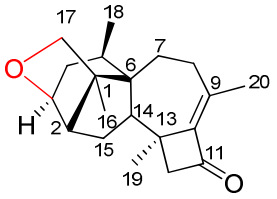


Figure S18. UV spectrum of compound **3** in methanol



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Results Table - scan036

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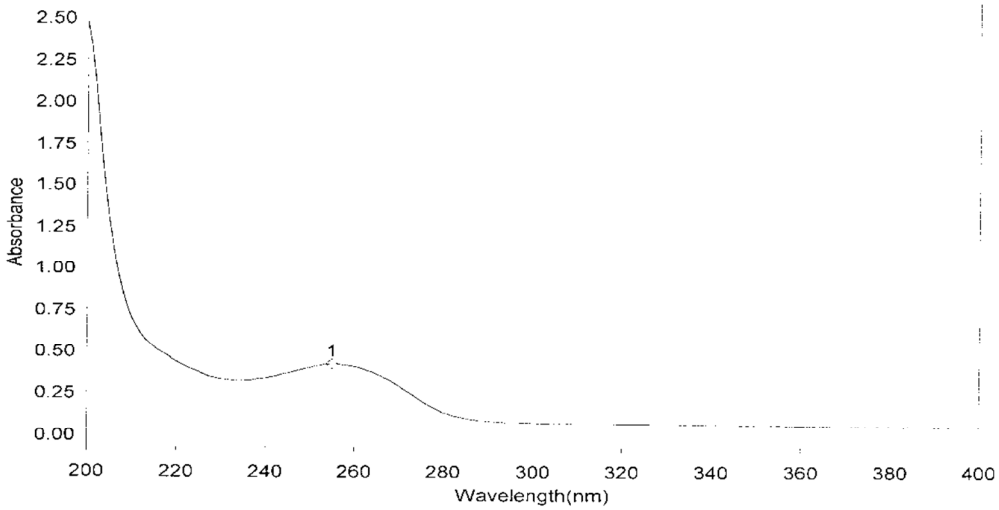

Figure S19. HR-ESI-MS spectrum of compound **3**

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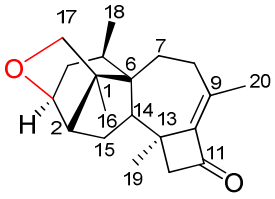

Figure S20. IR spectrum of compound **3**