Supporting Information

Photeroids A and B, Unique Phenol-Sesquiterpene Meroterpenoids from the Deep-Sea-Derived Fungus *Phomopsis tersa*

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1. Experimental Section

1.1 General experimental procedures

IR data were obtained by a Shimadzu IR Affinity-1 spectrometer (Shimadzu, Kyoto, Japan). UV spectra were collected using a Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotations were done with an Anton Paar MCP-500 spectropolarimeter (Anton Paar, Graz, Austria). Circular dichroism (CD) spectra were afforded under N_2 gas by a Jasco 820 spectropolarimeter (Jasco Corporation, Kyoto, Japan). HRESIMS were acquired on a Thermo MAT95XP (Thermo Fisher Scientific, Bremen, Germany). NMR spectra were acquired by a Bruker Avance-600 spectrometer (Bruker, Fällanden, Switzerland). Preparative HPLC was performed using a YMC-pack ODS-A column (250 × 20 mm, 5 μm, 12 nm, YMC Co., Ltd, Kyoto, Japan). A YMC-pack ODS-A/AQ column (250 × 10 mm, 5 μm, 12 nm, YMC CO., Ltd, Kyoto, Japan) was used for semipreparative HPLC separation. Silica gel (100-200 mesh and 200-300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 gel (Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden) were used in the chromatography processes. Fractions were monitored by TLC and spots were detected on heated TLC plates (silica gel GF_{254} plates, Qingdao Marine Chemical Inc., Qingdao, China) with 10% H_2SO_4 in EtOH under UV light.

1.2 Fungal material and identification

The strain FS441 used in this work was isolated from a sediment sample, which was collected at the depth of 3000 m in the Indian Ocean (88°58.640′ E, 0°00.307′ S) in April 2016. The sequence data for this strain have been submitted to the GenBank under accession No. MK592793. By using BLAST (nucleotide sequence comparison program) to search the GenBank database, FS441 has 98.9% similarity to Phomopsis tersa SYJM09 (Accession No. JF923840). And the strain was preserved at the Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology.

1.3 Fermentation, extraction and isolation

The marine fungus *P. tersa* FS441 was cultured on potato dextrose agar (PDA) at 28℃ for 7 days to prepare the seed culture, and then inoculated into flasks (3 L) containing 9 g sea salts, 250 g of rices and 300 mL of waters. After that, all flasks were incubated at 28 ℃ for one month and the fermented rice substrate was extracted repeatedly with EtOAc. After the evaporation of the solvent, a dark brown solid (177.9 g) was obtained. The crude extract was fractionated by silica

gel column chromatography (100-200 mesh) with two gradient systems of increasing polarity (petroleum ether/EtOAc, 10:1→1:1; CH_2Cl_2/CH_3OH, 10:1→0:1) to furnish seven fractions (A-G).

Fraction B (15.1 g) was subjected to silica gel CC (petroleum ether/EtOAc, 30:1→1:1) to afford seven subfractions (B1-B7). B4 was further divided into two parts (B4.1-B4.2) by Sephadex LH-20 CC (CH_2Cl_2-MeOH, 1:1). B4.2 was purified by preparative HPLC (MeOH-H_2O, 100:0, 5 mL/min) to offer seven subfractions (B4.2.1-B4.2.7). Semi-preparative HPLC (MeCN-H_2O, 70:30, 2 mL/min) analysis of B4.2.5 afforded **2** (20.9 mg, t_R = 20.6 min).

Fraction C (3.9 g) was divided into seven subfractions (C1-C7) by silica gel CC (petroleum ether/EtOAc, 10:1→1:1). C5 was separated by Sephadex LH-20 CC (CH_2Cl_2-MeOH, 1:1) to yield three subfractions (C5.1-C5.3). C5.3 was further purified by semi-preparative HPLC (MeCN-H_2O, 70:30, 2 mL/min) to yield **1** (11.4 mg, t_R = 30.9 min).

Photeroid A (**1**): yellow powders; $[\alpha]_D^{25}$ +23.2 (*c* 0.1, MeOH). CD (0.07 mg/mL, MeOH): 204 (−30.0) nm. UV (MeOH) λ_{max} (log ε): 204 (4.89), 283 (3.55) nm. IR ν_{max}: 2922, 1616, 1595, 1456, 1140, 1042, 841 cm^{-1}. ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectral data, see Table 1. HRESIMS: *m*/*z* 357.2432 $[M + H]^+$ (calcd for $C_{23}H_{33}O_3$, 357.2424).

Photeroid B (**2**): colorless powders; $[\alpha]_D^{25}$ +21.0 (*c* 0.09, MeOH). CD (0.11 mg/mL, MeOH): 204 (−33.6) nm. UV (MeOH) λ_{max} (log ε): 204 (4.56), 283 (3.09) nm. IR ν_{max}: 2920, 1614, 1597, 1456, 1138, 1039, 839 cm^{-1}. ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectral data, see Table 2. HRESIMS: *m*/*z* 339.2330 $[M + H]^+$ (calcd for $C_{23}H_{31}O_2$, 339.2319).

1.4 Computational details

Methods.

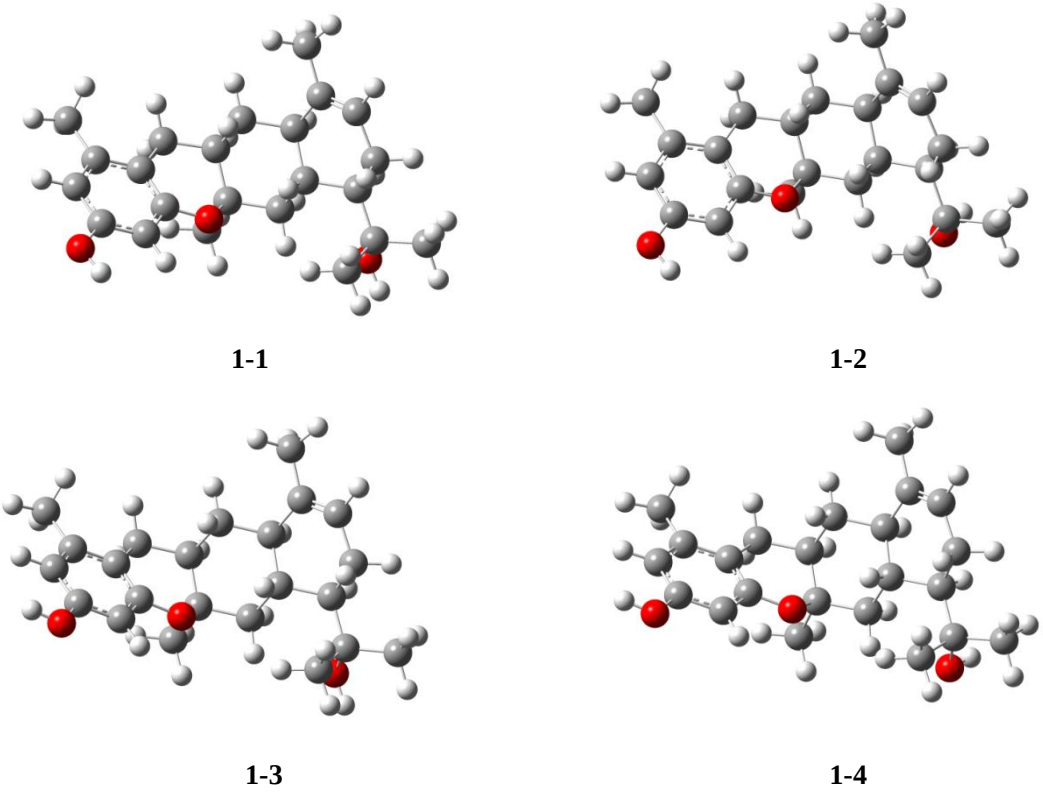
Merck molecular force field (MMFF) and DFT/TD-DFT calculations were carried out with the Spartan'14 software (Wavefunction Inc., Irvine, CA, USA) and the Gaussian 09 program, respectively[1]. Conformers within the 10 kcal mol^{-1} energy window were generated and optimized using DFT calculations at the B3LYP/6-31+G(d,p) level. Frequency calculations were performed at the same level to confirm that each optimized conformer was true minimum and to estimate their relative thermal free energy (ΔG) at 298.15 K. Conformers with the Boltzmann distribution over 5% were chosen for ECD calculations in methanol at the B3LYP/6-311+G(d,p) level. Solvent effects were taken into consideration using the self-consistent reaction field (SCRF) method with the polarizable continuum model (PCM)[2].The ECD spectrum was generated by the SpecDis

program[3] using a Gaussian band shape with 0.26 eV exponential half-width from dipole-length dipolar and rotational strengths.

Results.

Table S1. Energy analysis for the Conformers of **1**.

Compound	Conformation	E (Hartree)	E (Kcal/mol)	ΔE (Kcal/mol)	Boltzmann Dist (%)
1	**1-1**	-1121.49372314	-703740.5636	0.131079081	22.23%
	1-2	-1121.49393203	-703740.6947	0	27.73%
	1-3	-1121.49372729	-703740.5662	0.128474944	22.32%
	1-4	-1121.49393155	-703740.6944	0.000301201	27.72%



1-1 **1-2**

1-3 **1-4**

Figure S1. B3LYP/6-31+G(d,p) optimized low-energy conformers of **1**

Table S2. Energy analysis for the Conformers of **2**.

Compound	Conformation	E (Hartree)	E (Kcal/mol)	ΔE (Kcal/mol)	Boltzmann Dist (%)
2	**2-1**	-1045.24357464	-655893.3743	0	44.05%
	2-2	-1045.24171368	-655892.2065	1.167757797	6.13%
	2-3	-1045.24356970	-655893.3712	0.003099864	43.82%
	2-4	-1045.24169370	-655892.194	1.180295305	6.00%

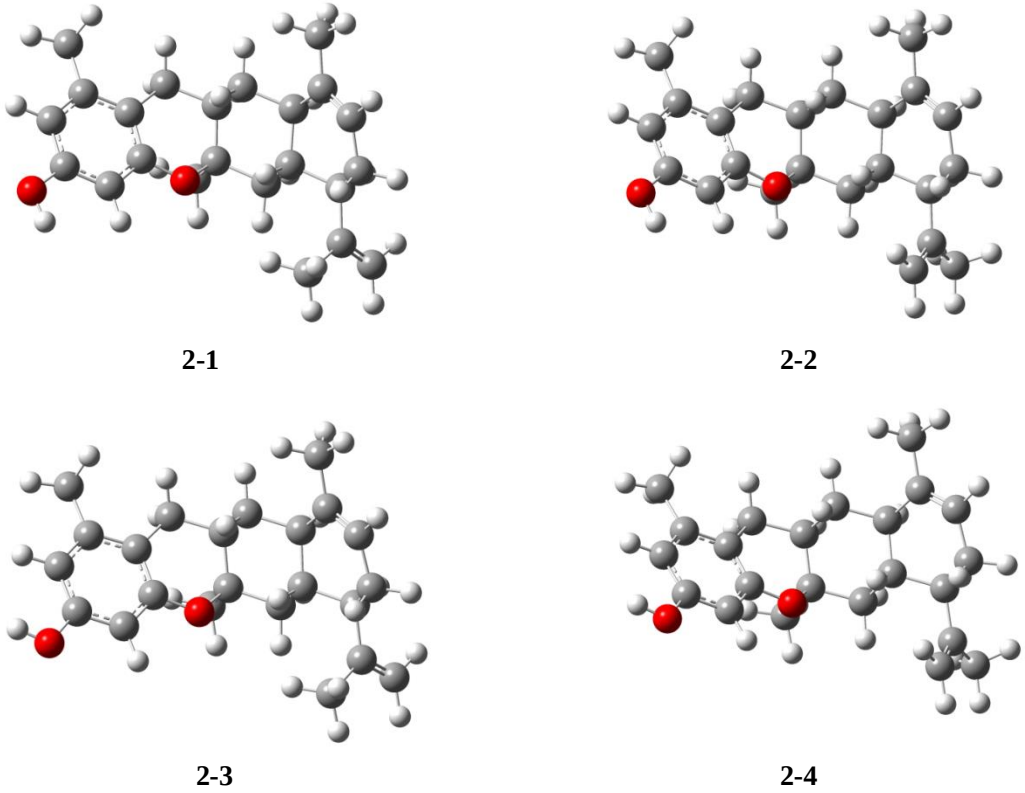


Figure S2. B3LYP/6-31+G(d,p) optimized low-energy conformers of **2**

1.5 Cytotoxic activity assay

Compounds **1** and **2** were evaluated for their cytotoxic activity against SF-268, MCF-7, HepG-2, A549 cell lines by using the SRB method[4]. The cells (180 μL) with a density of 3×10^4 cells/mL of media on 96-well plate were put under 37 ℃ at 5% CO_2 condition and incubated for 24 h. Then, 20 μL of various concentrations of compounds were added and further incubated for 72 h. After that, the cell monolayers were fixed by 50% (wt/v) trichloroacetic acid (50 μL) and stained for 30 min by 0.4% (wt/v) SRB, which was dissolved in 1% acetic acid. The unbound dye was removed by washing repeatedly with 1% acetic acid, and then dissolved into the protein-bound dye in 10 mM Tris base solution (200 μL) for OD determination at 570 nm using a microplate reader. Adriamycin was used as a positive control possessing potent cytotoxic activity. All data were obtained in triplicate, and the IC_{50} values were calculated by the SigmaPlot 10.0 software (Systat Software Inc., San Jose, California, America) with the use of a non-linear curve-fitting method. The SF-268, MCF-7, HepG-2, A549 cell lines were provided by the Chinese Academy of Sciences Cell Bank.

References

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2. NMR, HRESIMS, CD, UV and IR spectrum of compounds 1 and 2

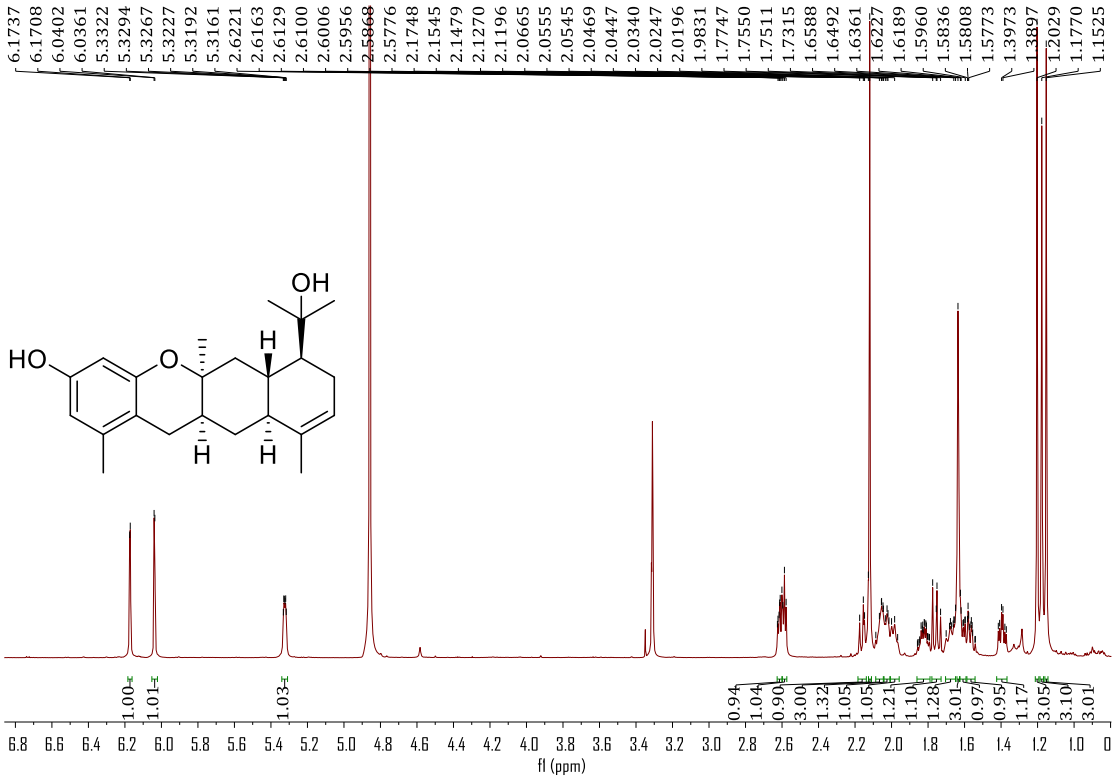

Figure S3. ^{1}H NMR spectrum (600 MHz, CD_3OD) of **1**

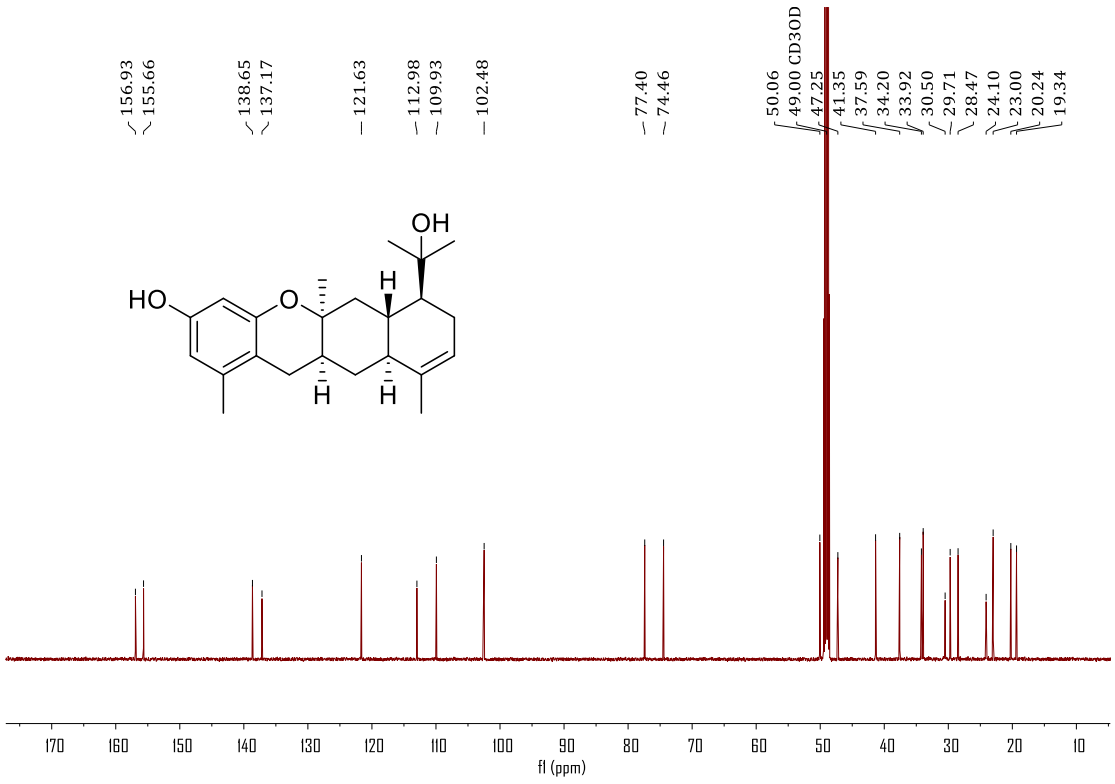

Figure 4. ^{13}C NMR spectrum (150 MHz, CD_3OD) of **1**

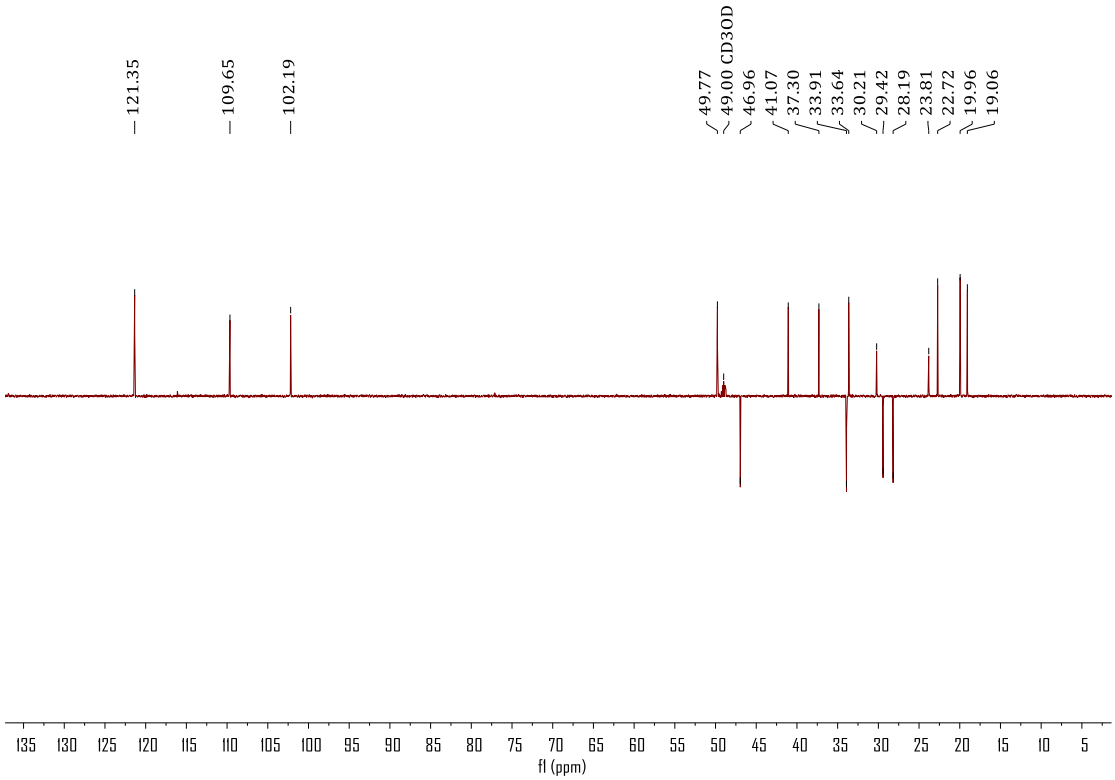

Figure S5. DEPT-135 spectrum of **1** in CD_3OD

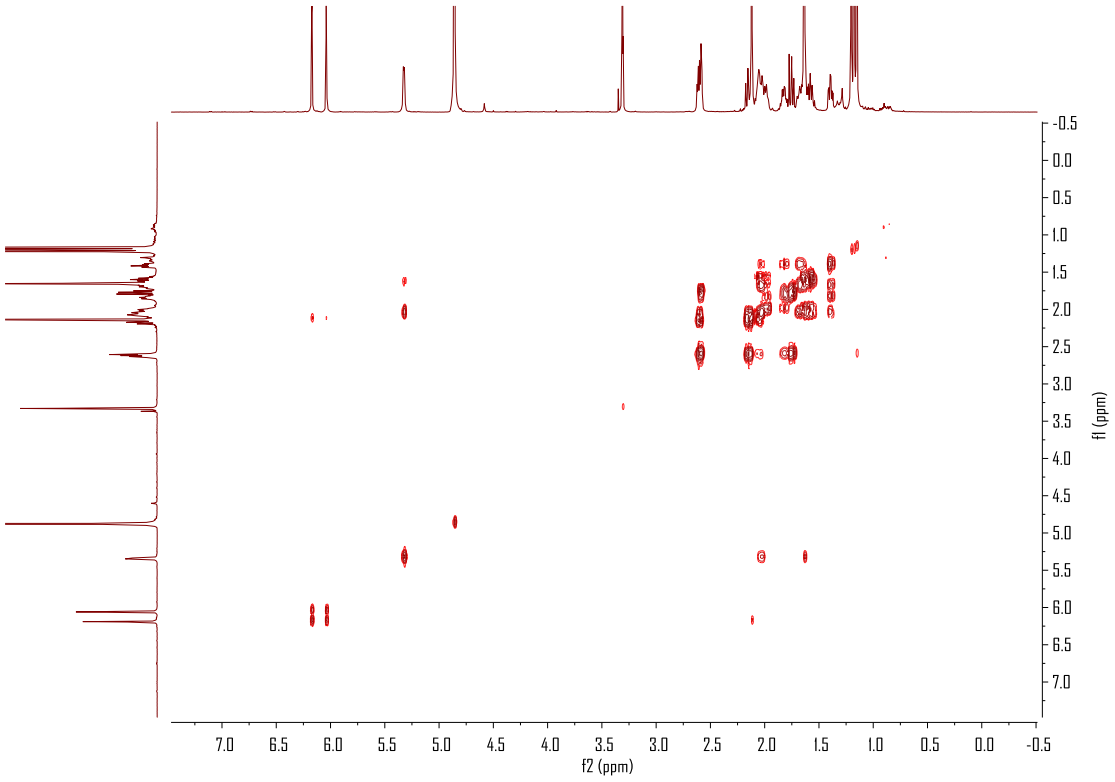

Figure S6. ^{1}H-^{1}H COSY spectrum of **1** in CD_3OD

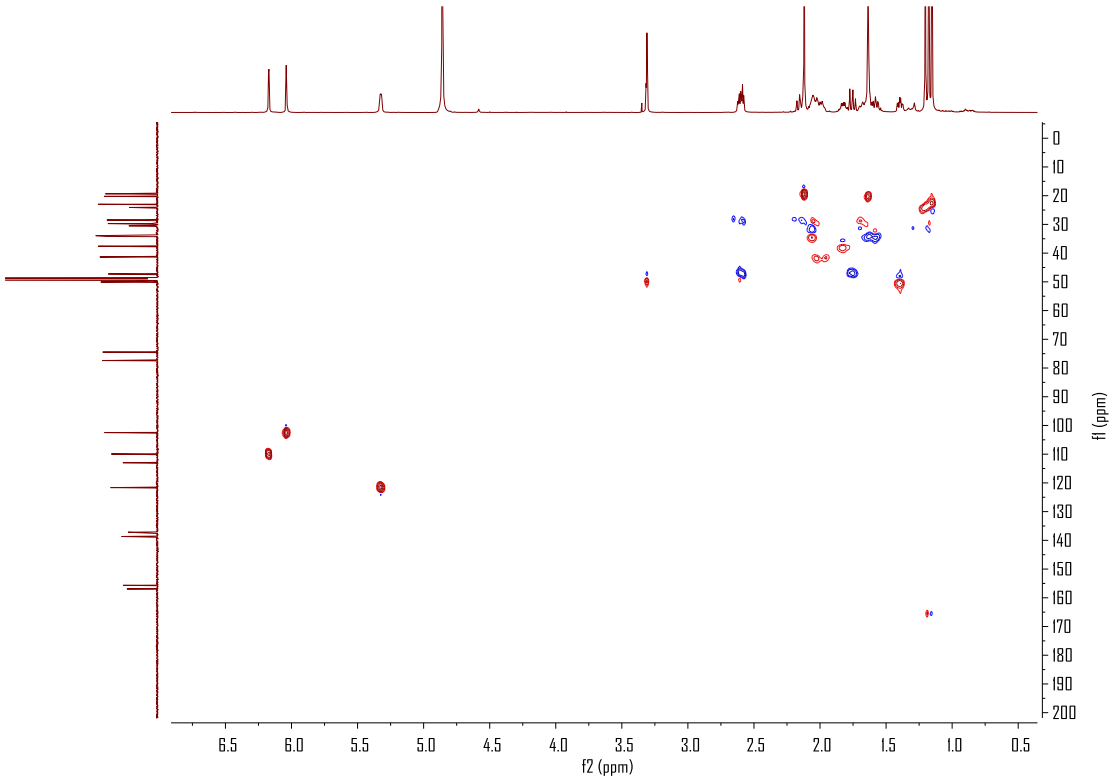

Figure S7. HSQC spectrum of **1** in CD_3OD

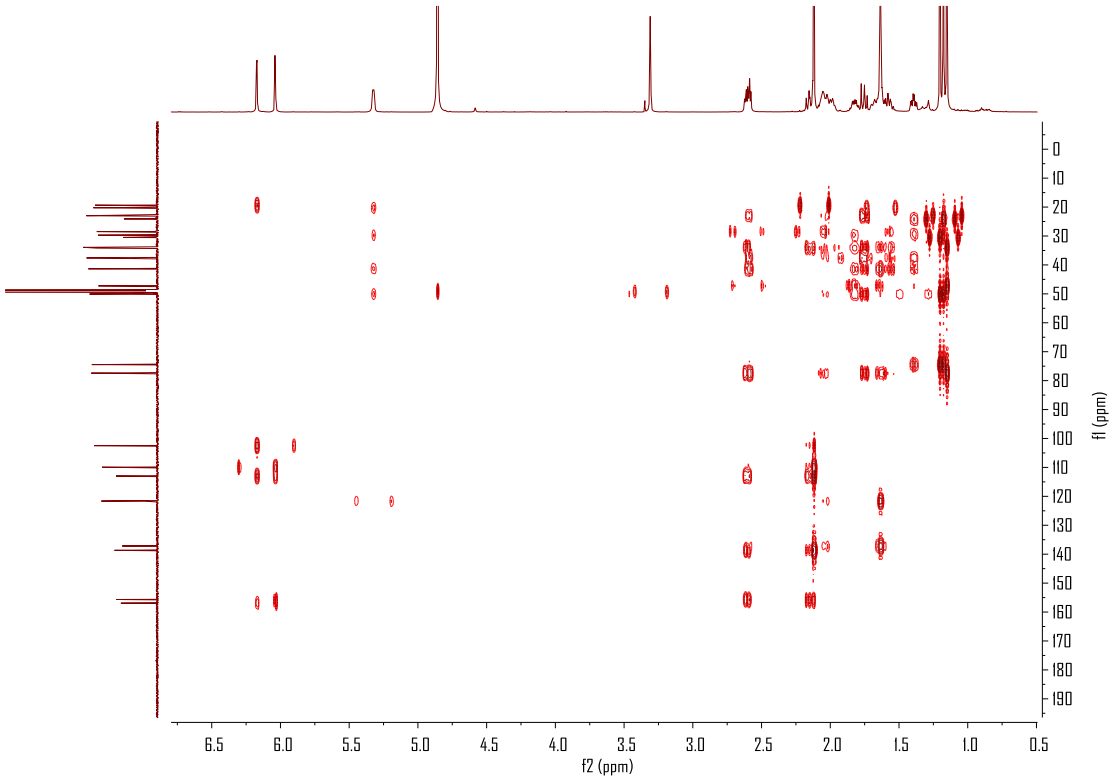

Figure S8. HMBC spectrum of **1** in CD_3OD

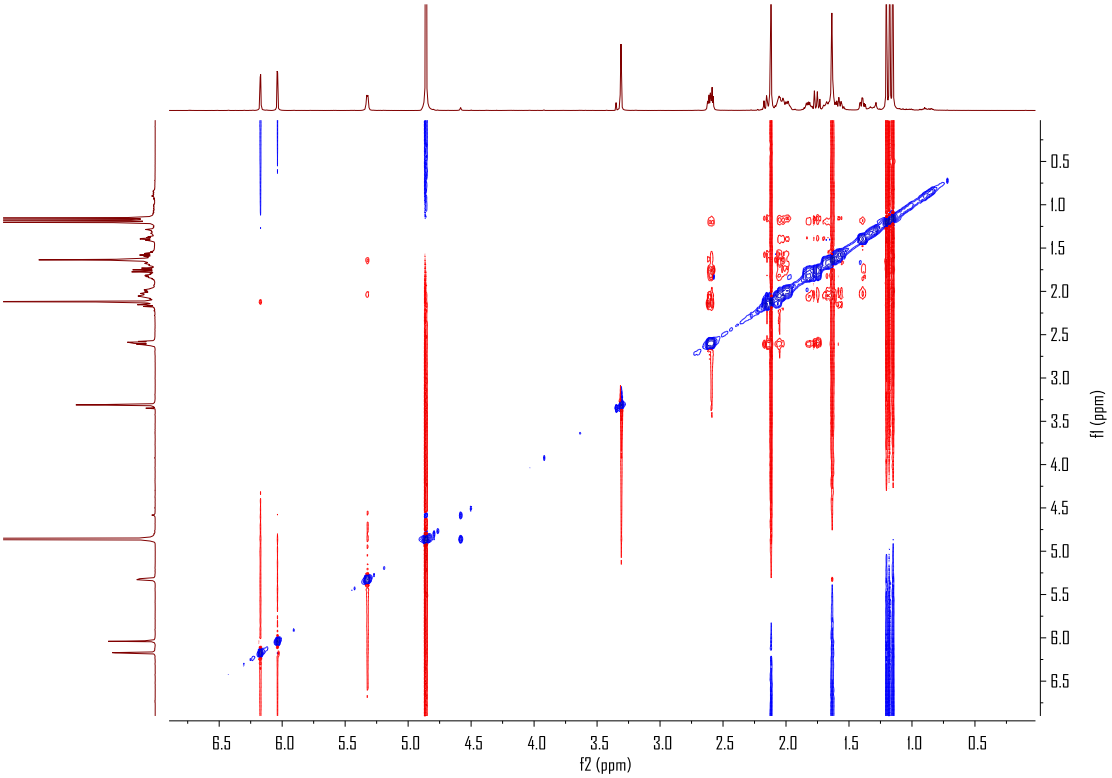

Figure S9. NOESY spectrum of **1** in CD_3OD

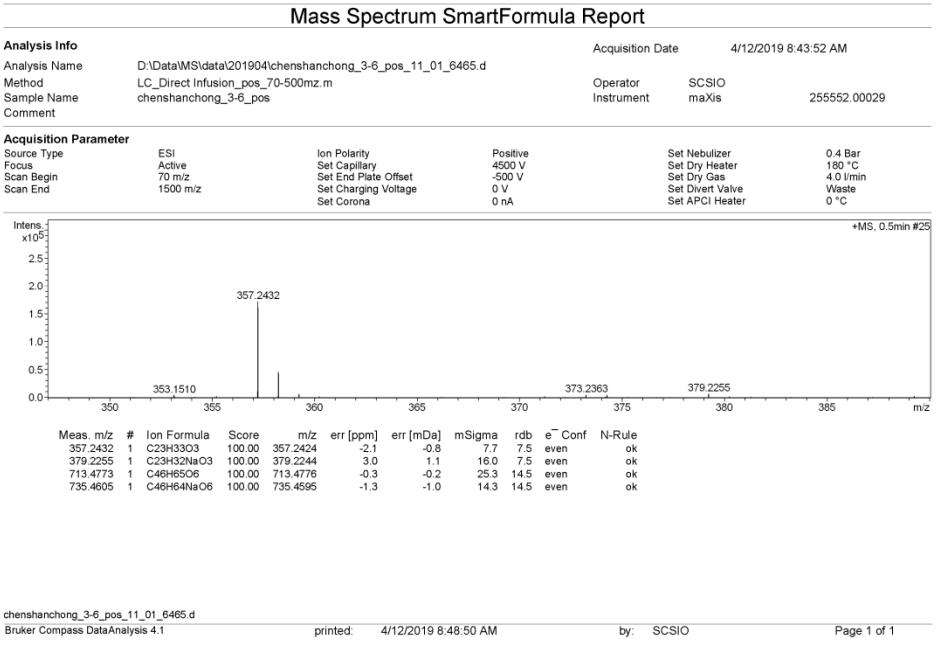

Figure S10. HRESIMS spectrum of **1**

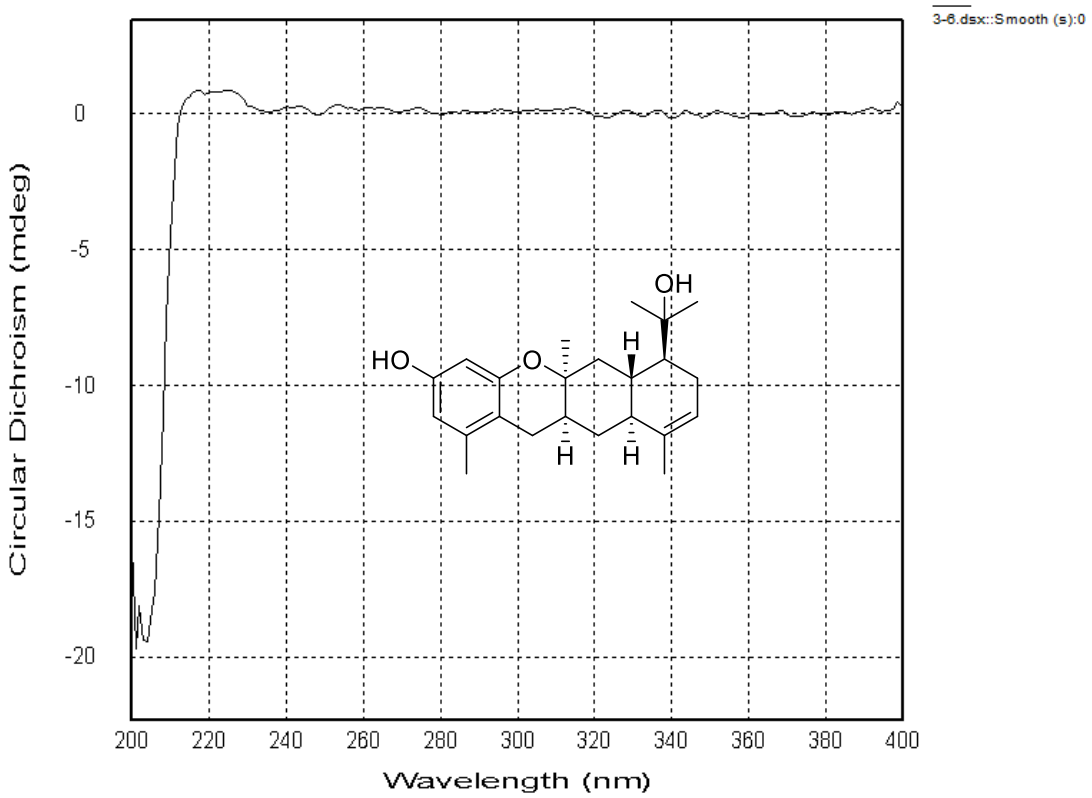

Figure S11. CD spectrum of **1**

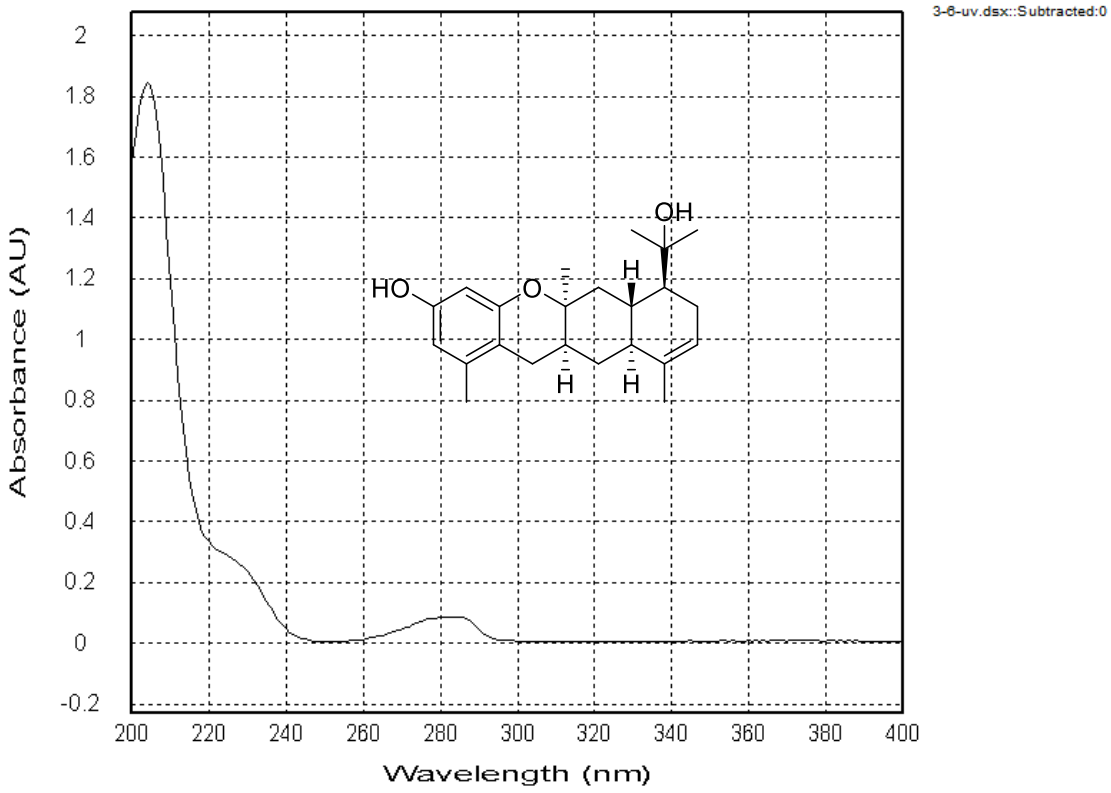

Figure S12. UV spectrum of **1**

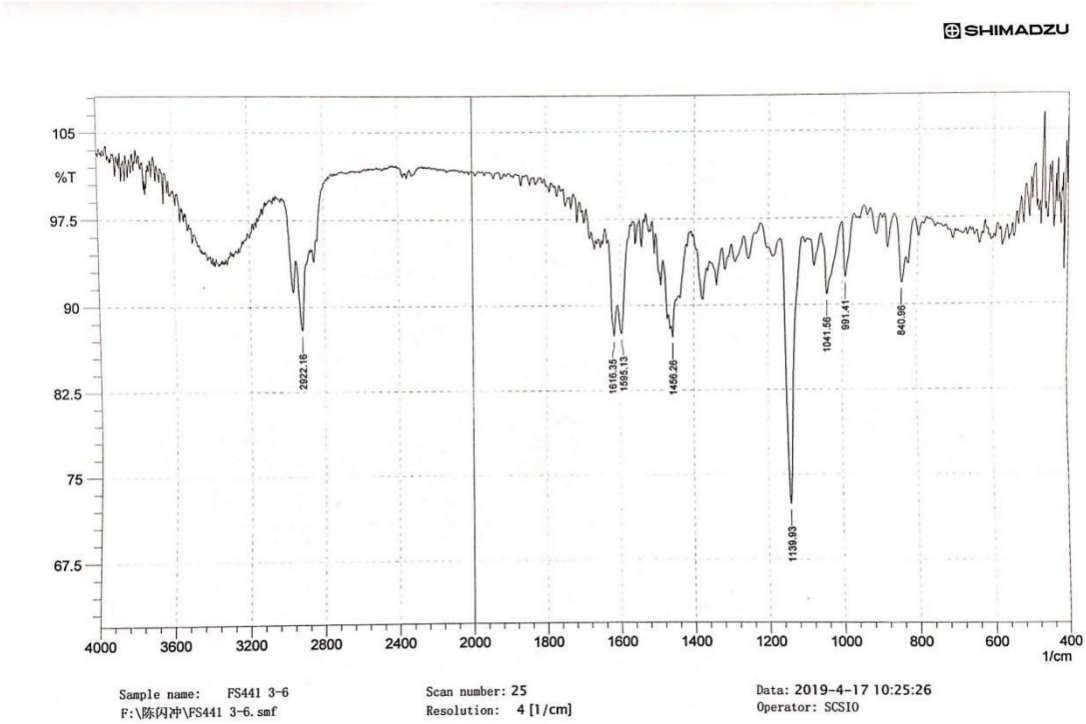

Figure S13. IR spectrum of **1**

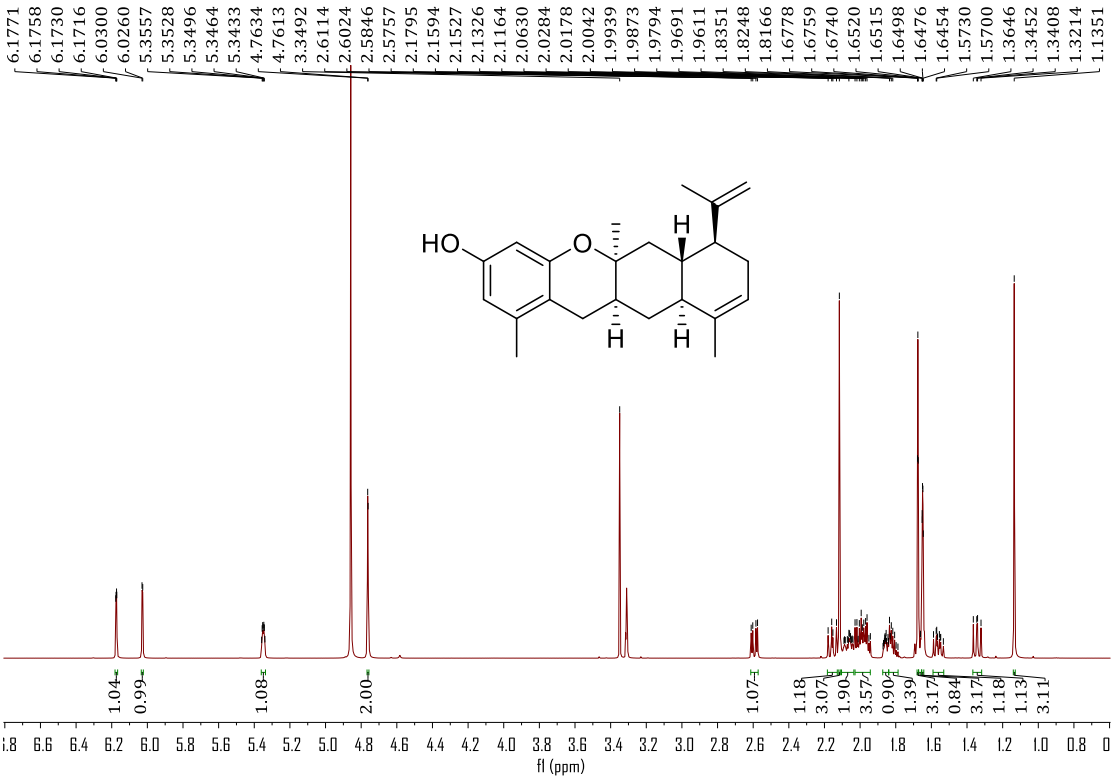

Figure S14. ^{1}H NMR spectrum (600 MHz, CD_3OD) of **2**

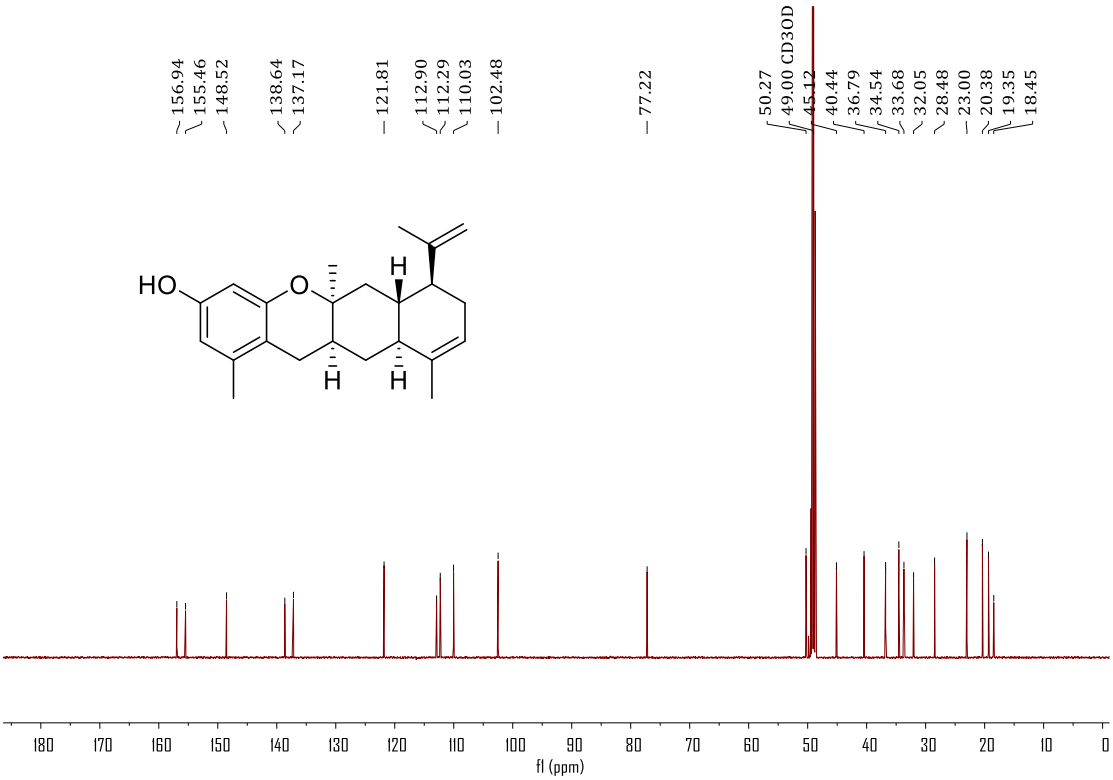

Figure S15. ^{13}C NMR spectrum (150 MHz, CD_3OD) of **2**

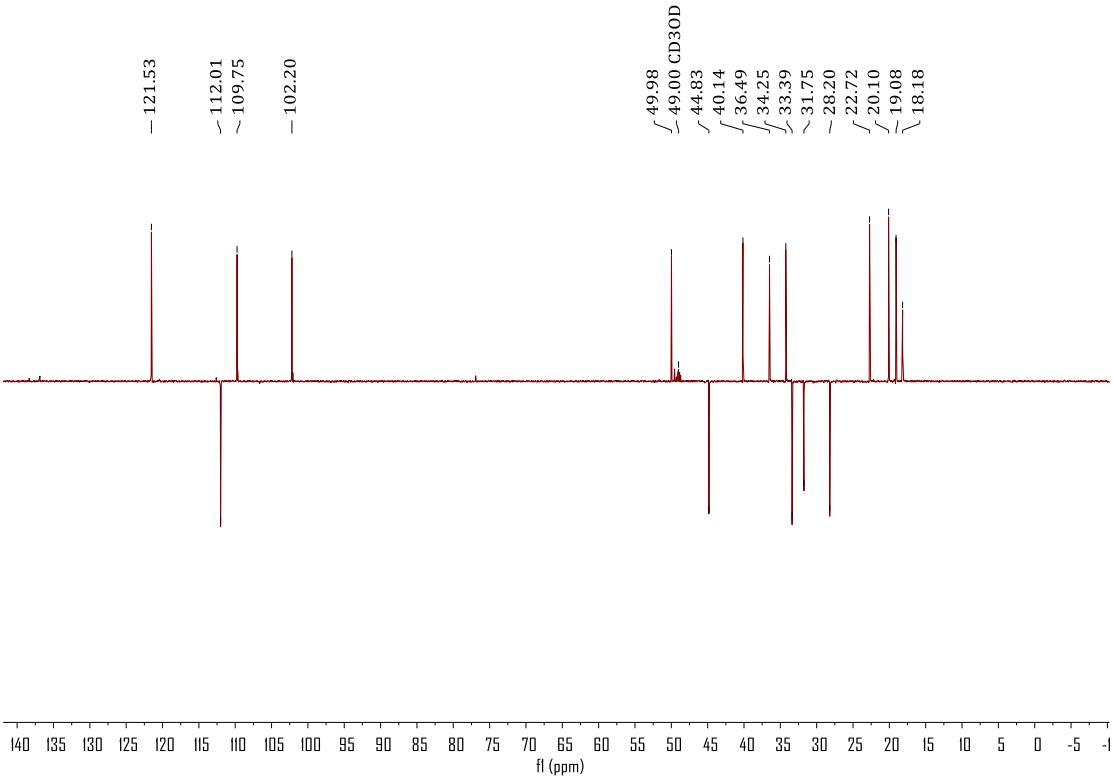

Figure S16. DEPT-135 spectrum of **2** in CD_3OD

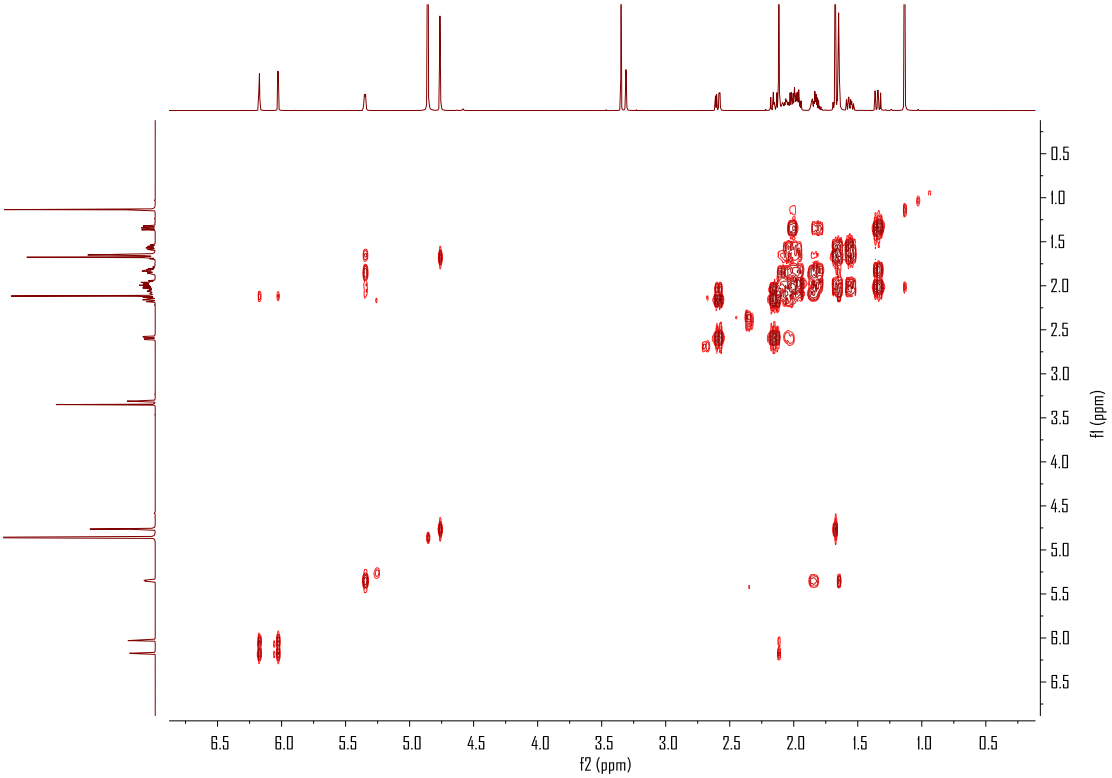

Figure S17. ^{1}H-^{1}H COSY spectrum of **2** in CD_3OD

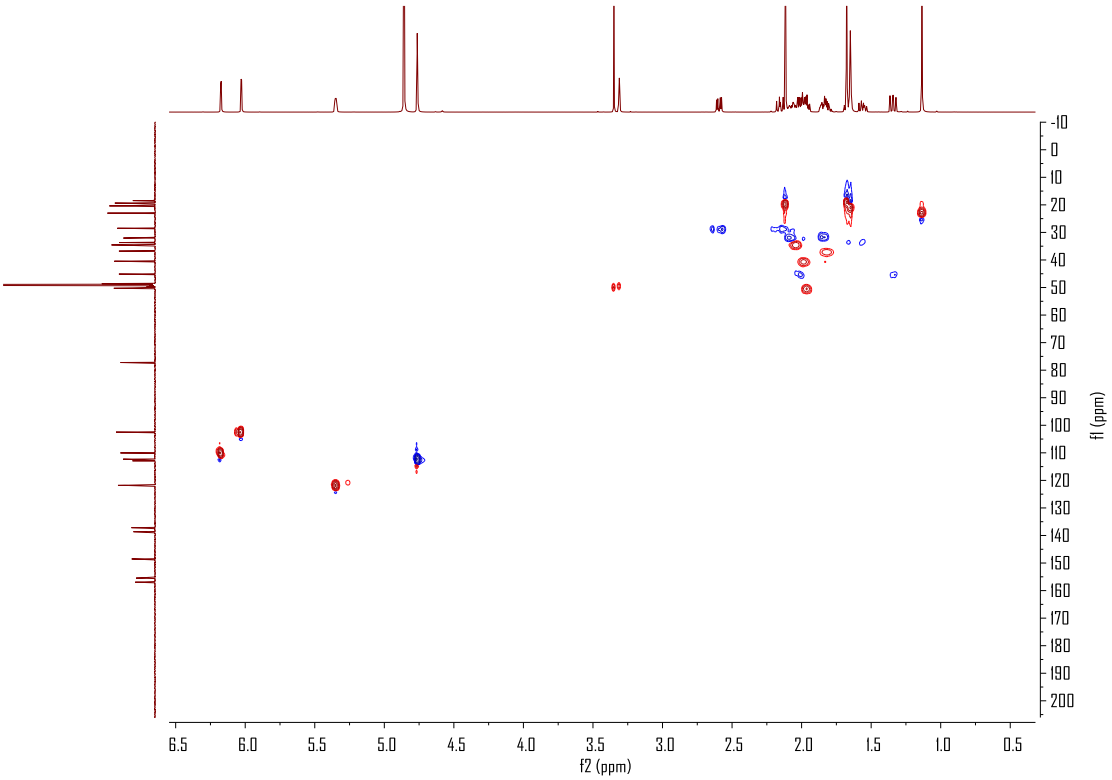

Figure S18. HSQC spectrum of **2** in CD_3OD

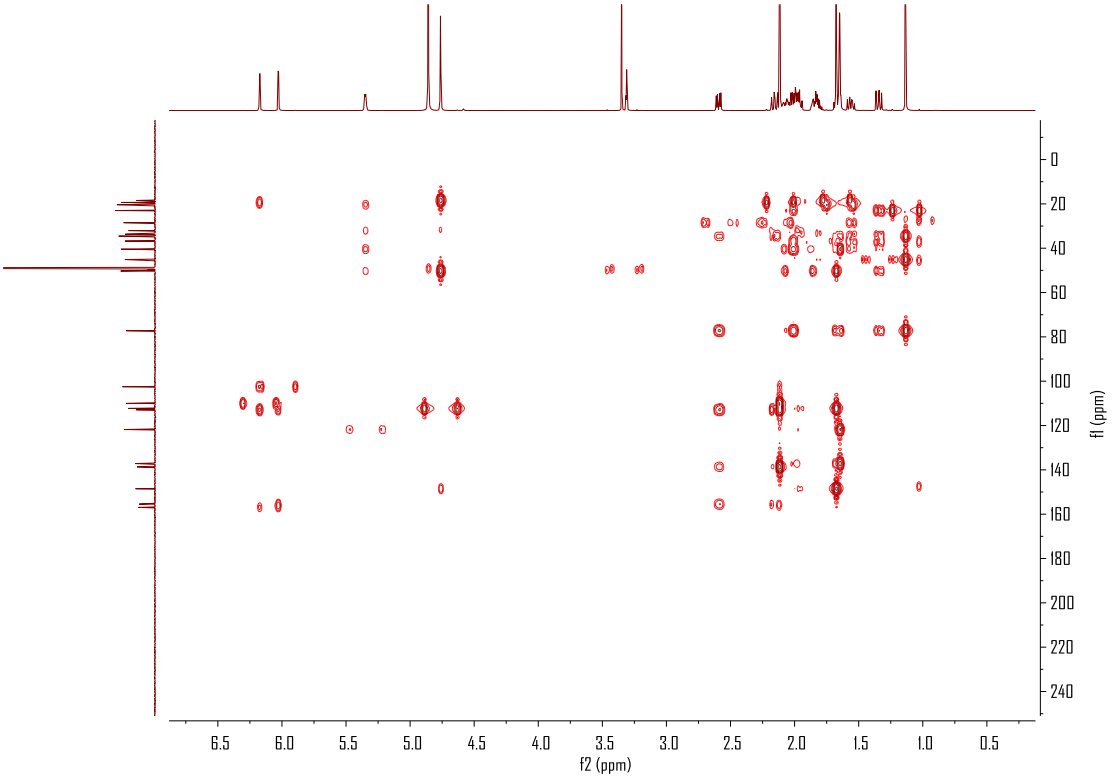

Figure S19. HMBC spectrum of **2** in CD_3OD

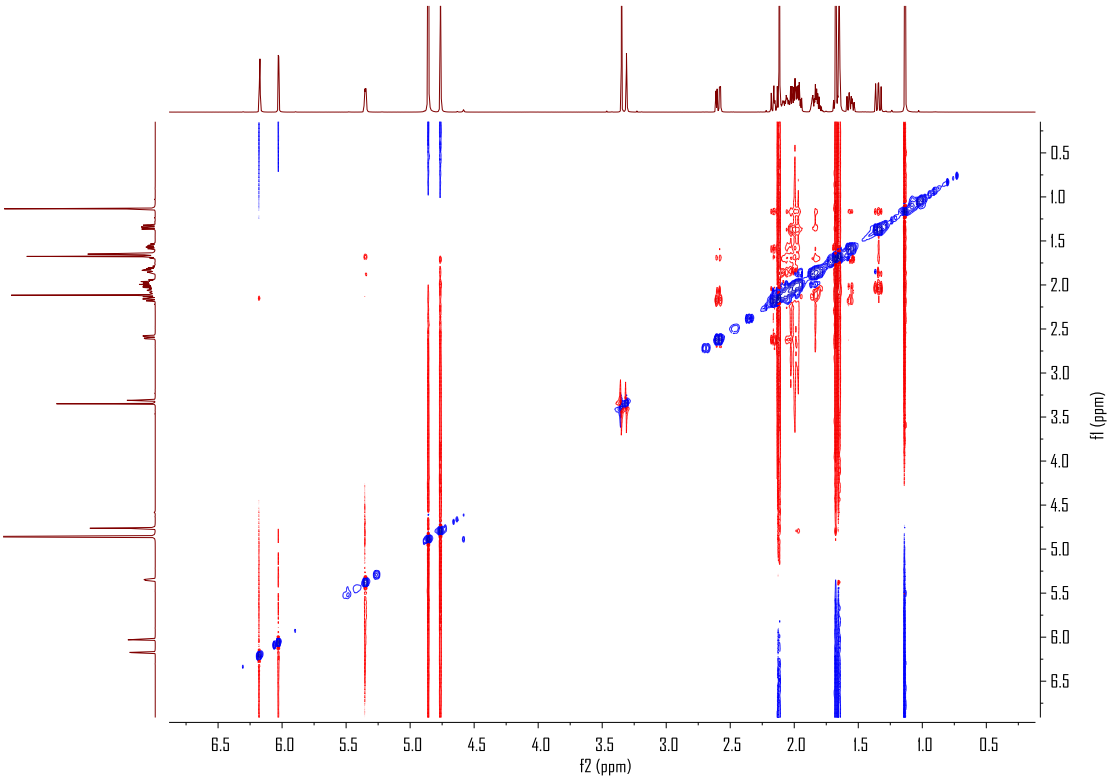

Figure S20. NOESY spectrum of **2** in CD_3OD

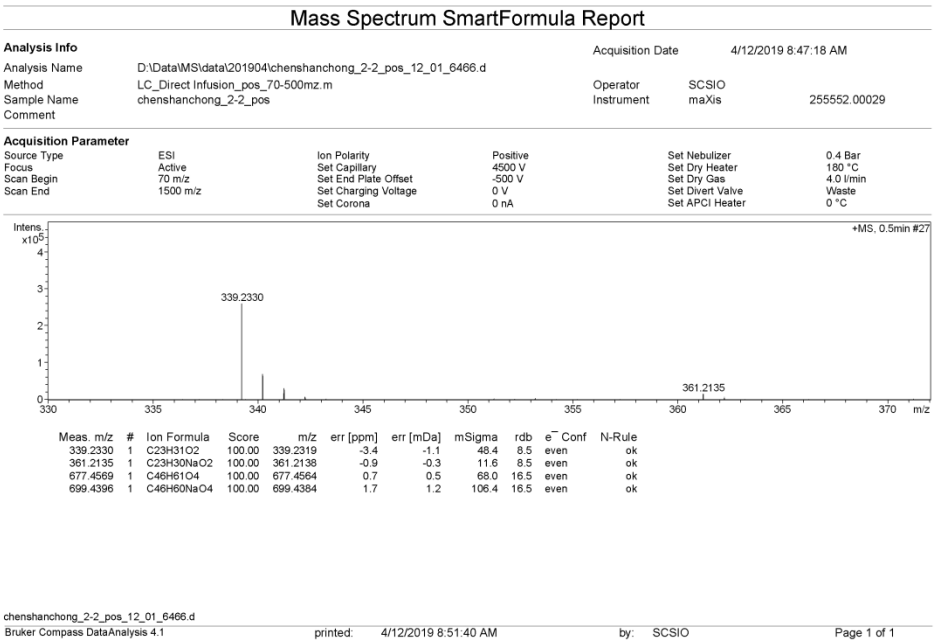

Figure S21. HRESIMS spectrum of **2**

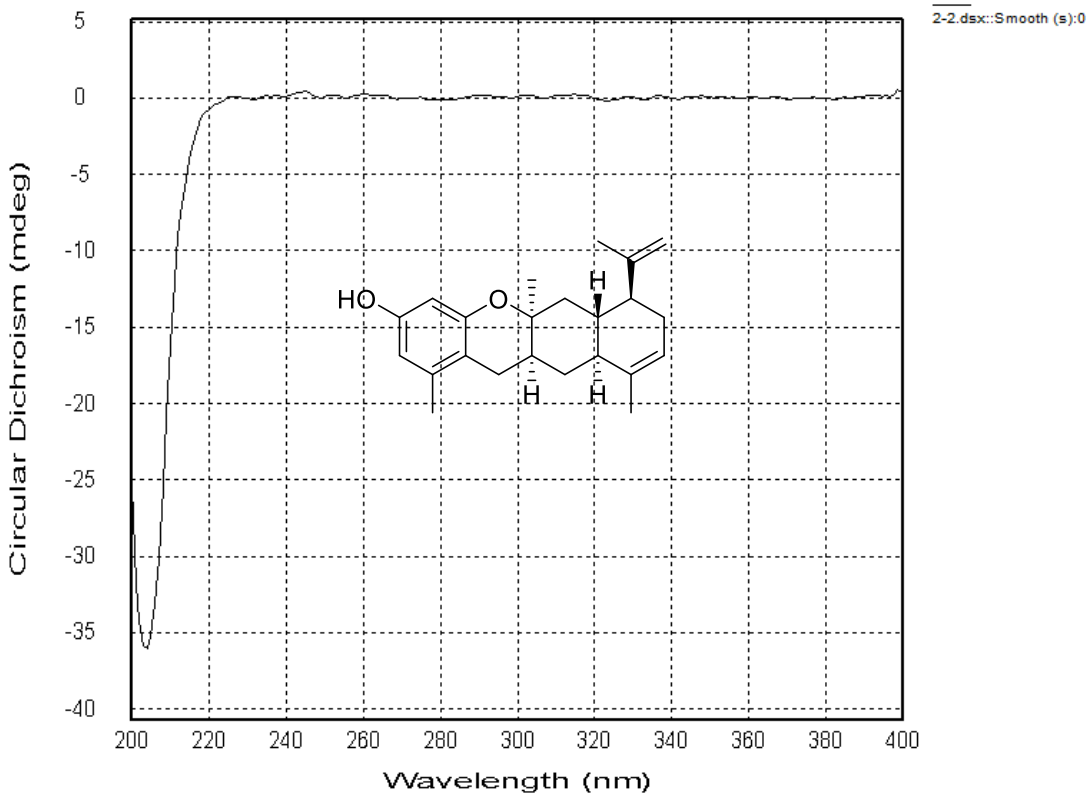

Figure S22. CD spectrum of **2**

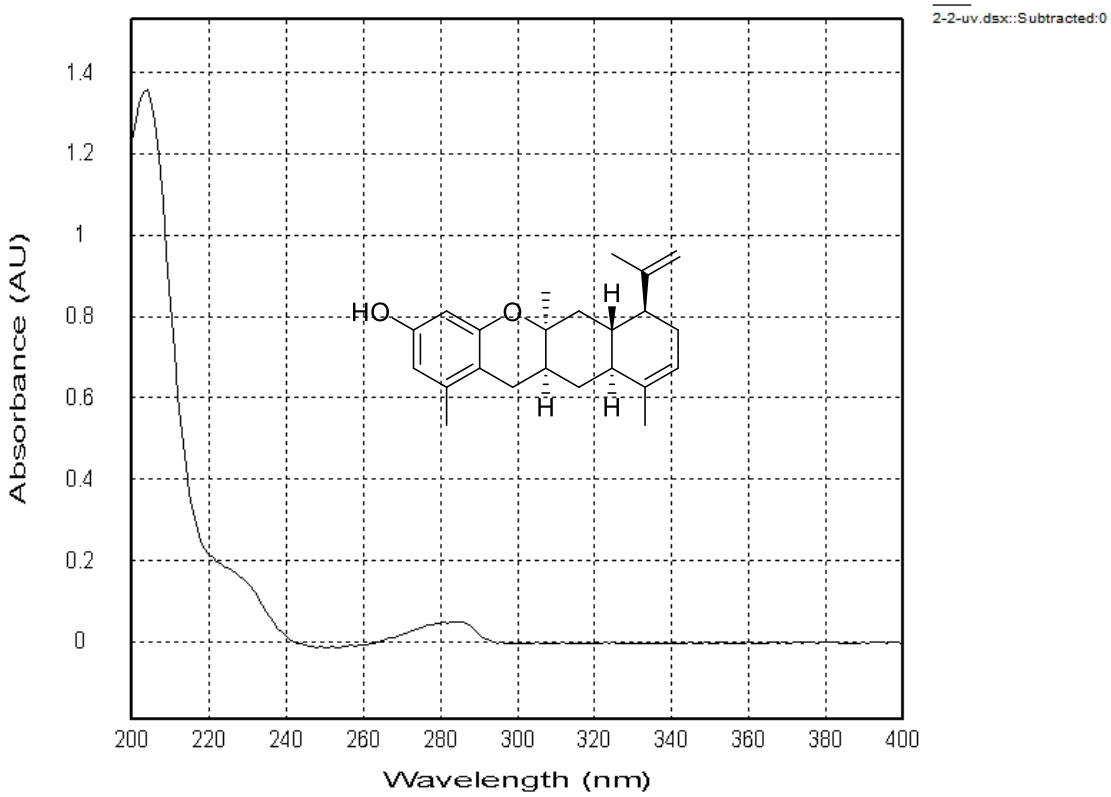

Figure S23. UV spectrum of **2**

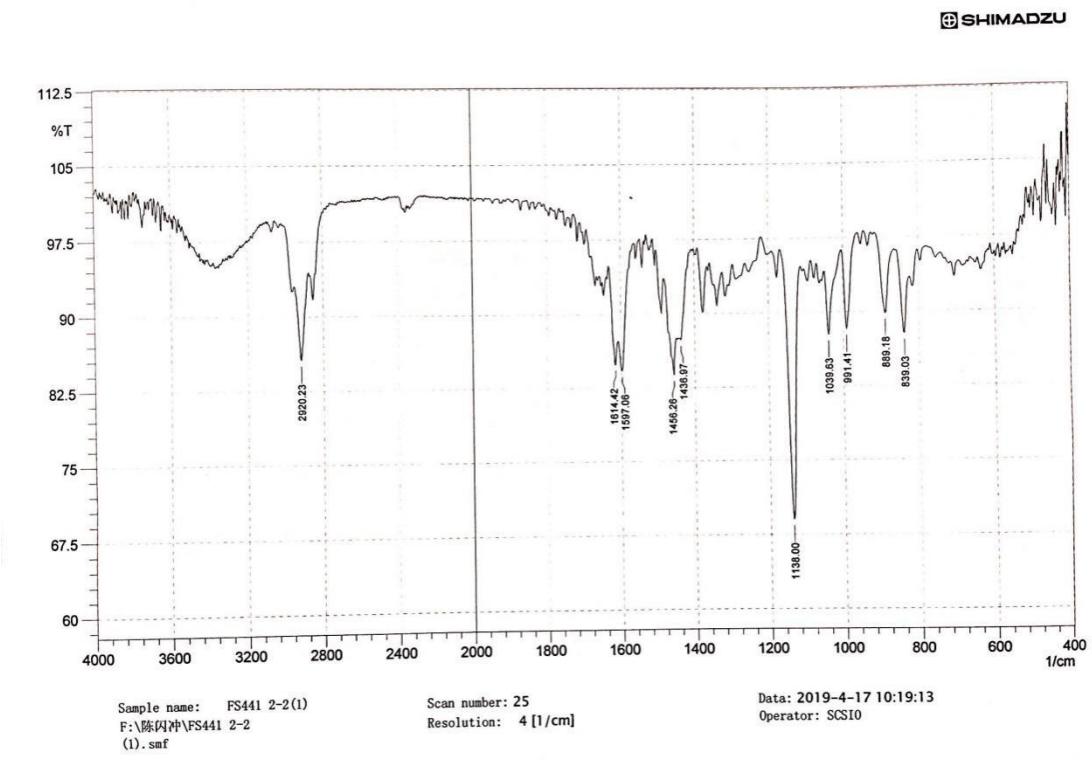

Figure S24. IR spectrum of **2**