Supplementary Information

Lithocaldehydes A and B, polyketones from the deep-sea-derived fungus *Phomopsis lithocarpus* FS508

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1. Experimental Section

1.1 General Experimental Procedures

UV spectra were taken on a Shimadzu UV-2600 spectrophotometer (Shimadzu, kyoto, Japan). IR data were recorded on a Shimadzu IR Affinity-1 spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were measured on an Anton Paar MCP-500 spectroplarimeter (Anton Paar, Graz, Austria) at 25 ℃. Circular dichroism (CD) spectra were obtained under N_2 gas on a Jasco 820 spectropolarimeter (Jasco Corporation, Kyoto, Japan). The NMR spectra were acquired using a Bruker Avance 600 MHz NMR spectrometer with TMS as an internal standard (Bruker, Fallanden, Switzerland). ESIMS data were collected on an Agilent Technologies 1290-6430A Triple Quad LC/MS (Agilent Technologies, Palo Alto, CA, USA). HERESIMS were done with a Thermo MAT95XP high resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Semi-preparative HPLC separations were performed utilizing a YMC-pack ODS-A/AQ column (250 × 10 mm, 5 μm, 12 nm, YMC Co., Ltd, Kyoto, Japan), a S-Chiral A column (250 × 10 mm, 5 μm, 12 nm, Acchrom Technologies Co., Ltd, Beijing, China). Column chromatography were performed with silica gel (200-300mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), respectively. Thin-Layer Chromatography (TLC) was conducted with precoated glass plates GF-254 (Merck KGaA, Darmastadt, Germany).

1.2 Fungal Material

The fungus *P. lithocarous* FS508 was isolated in 2016 from a deep-sea sediment sample collected in the Indian Ocean (111°53.335' E, 16°50.508' N; depth 3606 m). The sequence of amplified ITS region of the strain FS508 has been submitted to GenBank (Accession No. MG686131). A BLAST search of ITS region revealed that FS508 has 99% homology with *Phomopsis lithocarpus* CZ105B (Accession No. FJ755236). The strain is preserved at the Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology.

1.3 Fermentation, Extraction and Isolation

The fermentation was carried out in 3L Erlenmeyer flasks, which contained 250 g of rice and 300 ml of 0.5% saline water. Each flask was aseptically inoculated with the seed inoculums and statically fermented for a month at 28℃. The fermented rice substrate (40 flasks) was extracted three times with EtOAc, and the solvent was evaporated to dryness under vacuum to obtain a crude extract. The crude extract was subjected to silica gel chromatography (200-300 mesh) by step gradient elution with petroleum ether/EtOAc (10:1→0:1) and followed by CH_2Cl_2/MeOH in linear gradient (5:1→1:1) to yield 8 fractions (Frs. 1-8).

Fr.3 was separated by column chromatography over C-18 reversed-phase (RP) silica gel eluting with a MeOH/H_2O gradient (60:40→100:0) to produce 4 fractions Fr.3-1~Fr.3-4. Fr.3-3 was fractionated by column chromatography on silica gel eluting with *n*-hexane/EtOAc (5:1 and 3:1) to produce 2 fractions Fr.3-3-1~Fr.3-3-2. Fr.3-3-1 was purified by HPLC on a semipreparative YMC-pack ODS-A column (MeCN/H_2O, 80:20, 2 mL/min) to produce 17 fractions Fr.3-3-1-1~Fr.3-3-1-17. Fr.3-3-1-15 was further purified by HPLC on a semipreparative YMC-pack ODS-A column (MeCN/H_2O, 70:30, 2 mL/min) to produce 2 fractions Fr.3-3-1-15-1~Fr.3-3-1-15-2, and **1** (3 mg). Fr.3-3-1-15-2 was re-purified by HPLC on a S-Chiral A column (*n*-hexane/2-propanol, 70:30, 2 mL/min) to afford **2** (5 mg).

Lithocaldehyde A (**1**): white powder, $[\alpha]_D^{25}$-45.98 (*c* 0.0015, MeOH); UV(MeOH) λ_{max} (log ε) 208 (3.32), 226 (2.95), 265 (2.67), 353 (2.20) nm; IR (KBr) ν_{max} 3354, 2945, 2835, 1653, 1018, 667 cm^{-1}; ^1H and ^{13}C NMR data, table 1; (+)-ESIMS *m/z* 409 $[M + H]^+$; HRESIMS *m/z* 409.2005 $[M + H]^+$ (calcd. for $C_{25}H_{29}O_5$, 409.2010).

Lithocaldehyde B (**2**): white powder, $[\alpha]_D^{25}$+21.1 (*c* 0.0015, MeOH); UV(MeOH) λ_{max} (log ε) 208 (2.94), 225 (2.48), 267 (2.16), 353 (1.67) nm; IR (KBr) ν_{max} 3313, 2945, 2835, 1633, 1018, 667 cm^{-1}; ^1H and ^{13}C NMR data, table 1; (+)-ESIMS *m/z* 409 $[M + H]^+$; HRESIMS *m/z* 409.2009 $[M + H]^+$ (calcd. for $C_{25}H_{29}O_5$, 409.2010).

1.4 Quantum chemical calculation of ECD spectra

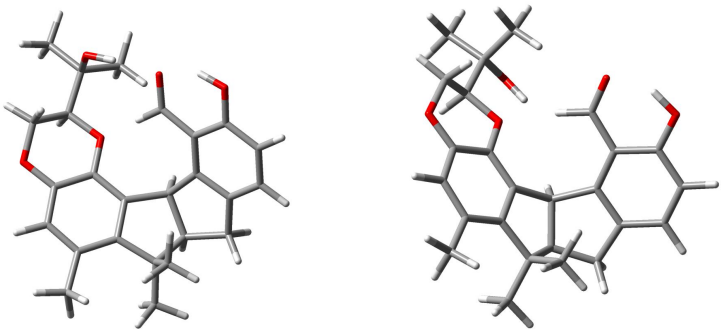
Methods.

MMFF and DFT/TD-DFT calculations were carried out using the Spartan'14 software (Wavefunction Inc., Irvine, CA, USA) and the Gaussian 09 program, respectively[1]. Conformers that had an energy window lower the 5 kcal • mol^{-1} were generated and optimized using DFT calculations at the b3lyp/def2svp 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. Additionally, solvent effects were considered based on the self-consistent reaction field (SCRF)method with the polarizable continuum model (PCM). The ECD spectrum was generated by the SpecDis program[2] using a Gaussian band shape with 0.22 eV exponential half-width from dipole-length dipolar and rotational strengths.

Results.

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

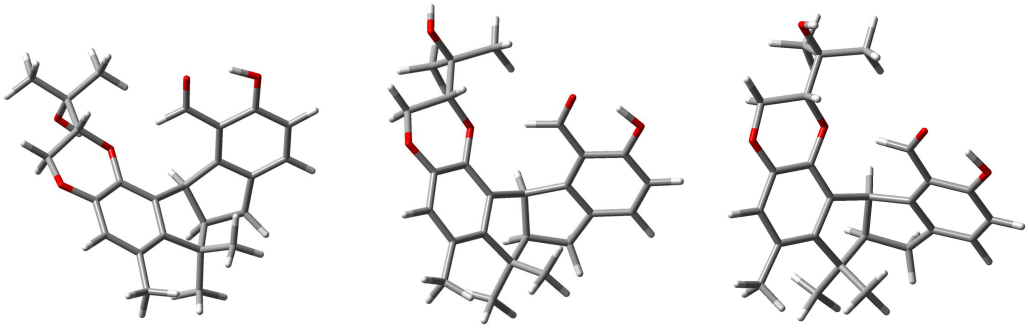
Compd.	Conformation	G (Hartree)	G (Kcal/mol)	ΔG (Kcal/mol)	Boltzmann Dist (%)
1	**1a**	-1345.70057172	-843386.7075	0	97.56%
	1b	-1345.70040770	-843384.5227	2.18	2.44%



1a **1b**

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

Compd.	Conformation	G (Hartree)	G (Kcal/mol)	ΔG (Kcal/mol)	Boltzmann Dist (%)
2	**2a**	-1345.70088107	-844643.4386	0	78.65%
	2b	-1345.69852866	-844642.1086	1.33	8.32%
	2c	-1345.69912631	-844642.3743	1.06	13.03%



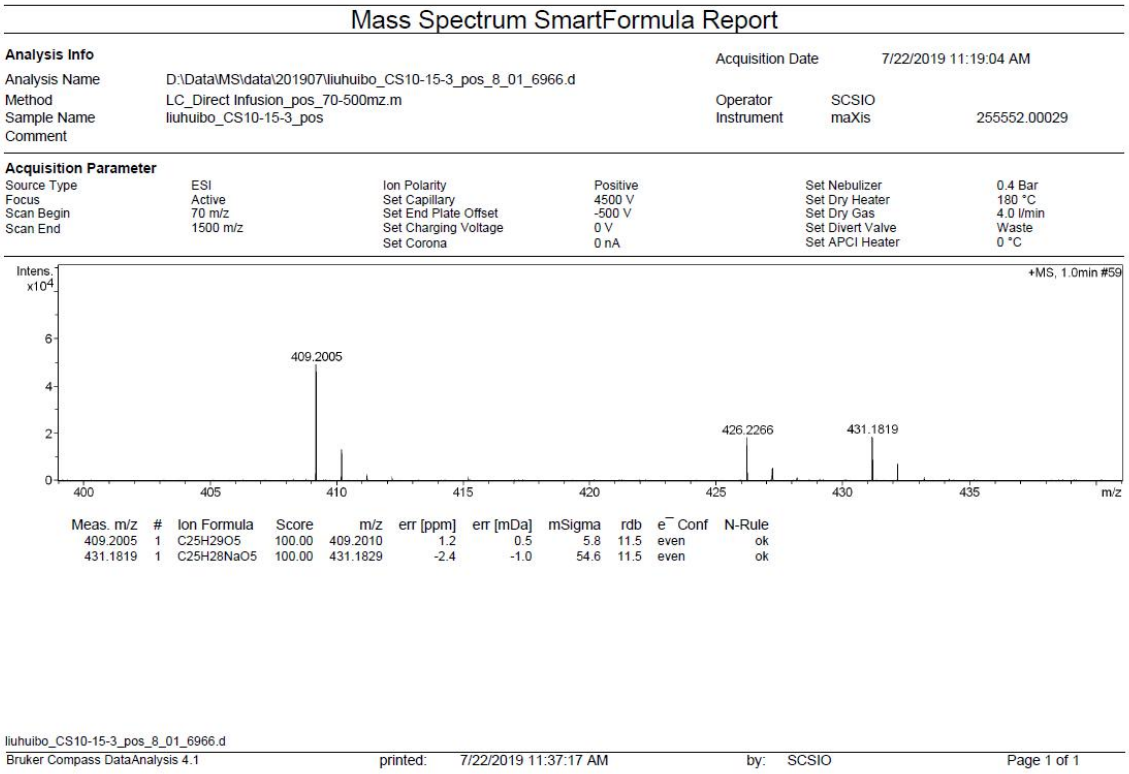
2a **2b** **2c**

1. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; et al. Gaussian 09, revision D.01, Gaussian, Inc., Wallingford, CT, **2013**.
2. Bruhn, T., Schaumlöffel, A., Hemberger, Y., Bringmann, G. SpecDis: Quantifying the comparison of calculated and experimental electronic circular dichroism spectra. Chirality. 2013, 25, 243−249.

1.5 Antimicrobial activity assay

Antimicrobial activity was evaluated against two bacterial strains *Staphylococcus aureus* and *Bacillus subtilis* with ampicillin as the positive control as well as antifungal activity against three fungal strains *Candida albicans*, *Aspergillus niger*, and *A. flavus* with nystatin as the positive control by using the resazurin staining method. The mid-logarithmic-phase tested bacteria and fungus were diluted to 2.0×10^6 cfu/mL by LB broth and potato dextrose broth (PDB), meanwhile, compounds to be tested and positive controls (ampicillin and nystatin) were diluted by DMSO to obtain the solution with concentration ranging from 1000-15.6 μM. Then, 20 μL of the tested samples in the DMSO solution was added to 96-well flat-bottom microtiter plate and followed by 180 μL microbials solution per hole. The 96-well plates were incubated at 37 ℃ for 24 h for bacteria and 28 ℃ for 72 h for fungus, respectively. After that, 20 μL resazurin in DMSO solution with the concentration of 0.2 mg/mL were added per hole. The lowest concentration of the tested compound that did not observe the blue-to-pink color changed, was determined as MIC. All assays were done in triplicate, and the results presented as mean values of the three measurements. Ampicillin and nystatin, DMSO (1%), and culture medium were used as a positive control, negative control, and blank control, respectively.

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



Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	liuhuibo_CS10-15-3_pos	Instrument	maXis 255552.00029
Comment			

Acquisition Parameter

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Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	70 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e¯ Conf	N-Rule
409.2005	1	C25H29O5	100.00	409.2010	1.2	0.5	5.8	11.5	even	ok
431.1819	1	C25H28NaO5	100.00	431.1829	-2.4	-1.0	54.6	11.5	even	ok

liuhuibo_CS10-15-3_pos_8_01_6966.d

Bruker Compass DataAnalysis 4.1 printed: 7/22/2019 11:37:17 AM by: SCSIO Page 1 of 1

Figure S1. HRESIMS spectrum of **1**.

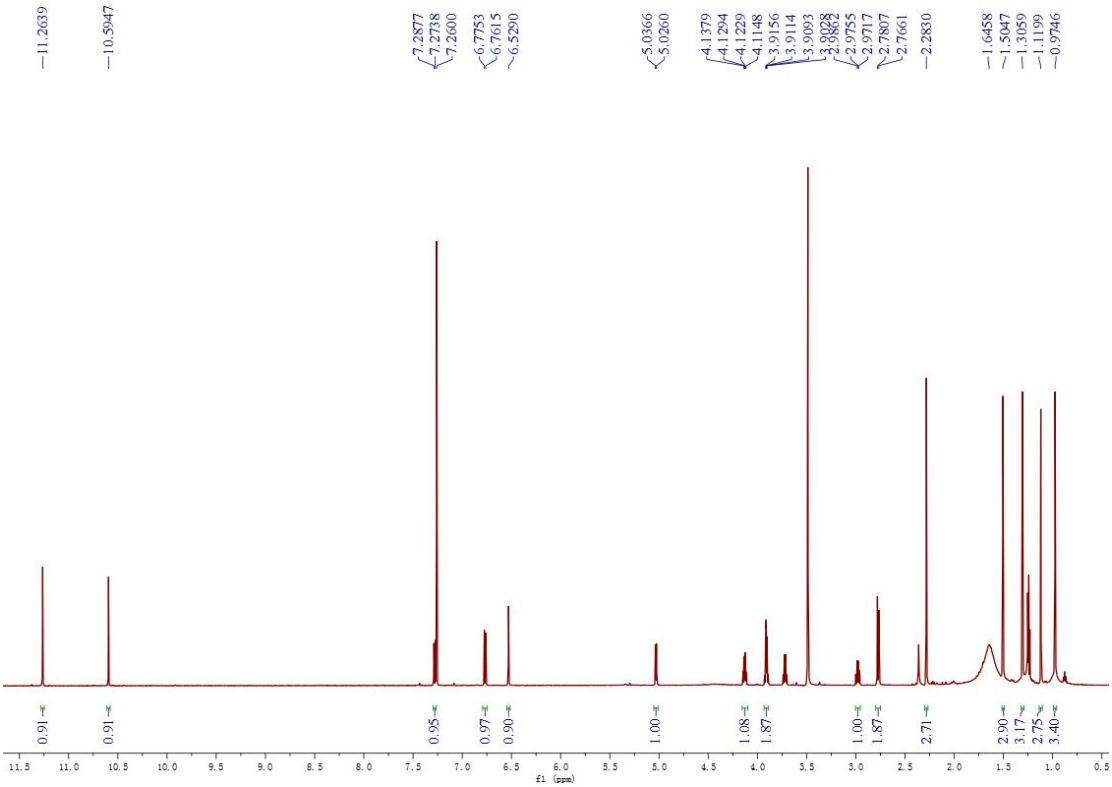

Figure S2. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **1**.

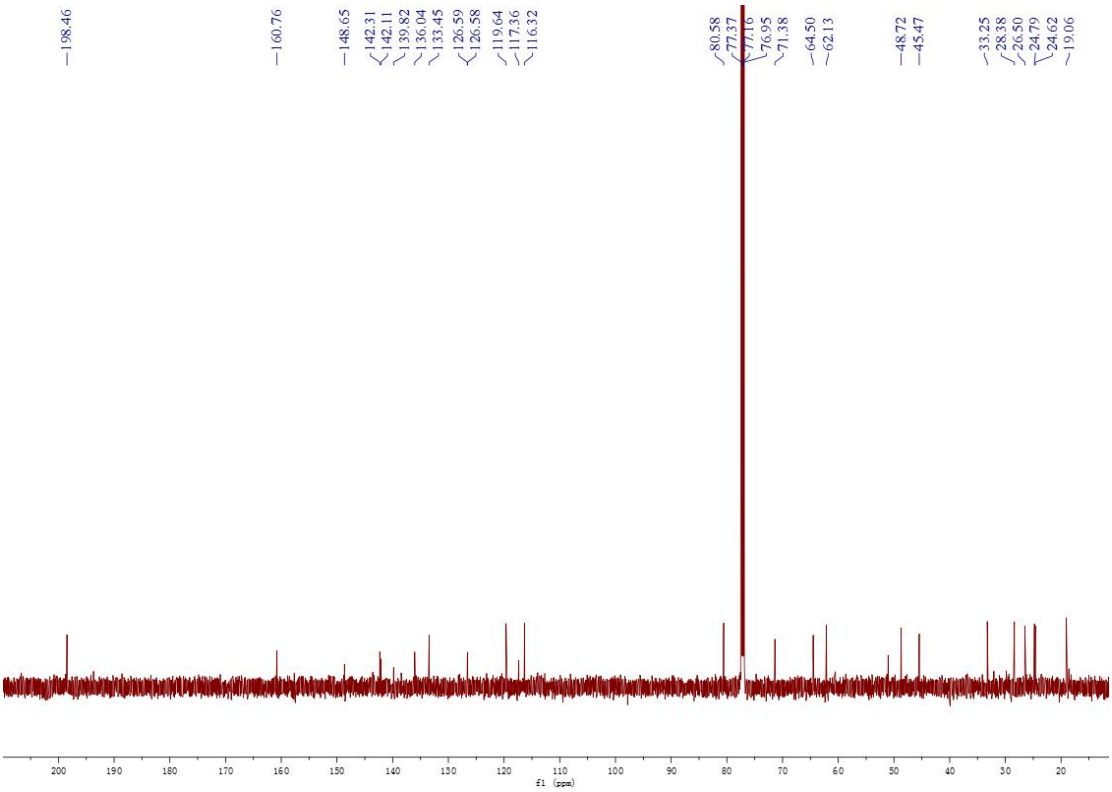

Figure S3. ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of **1**.

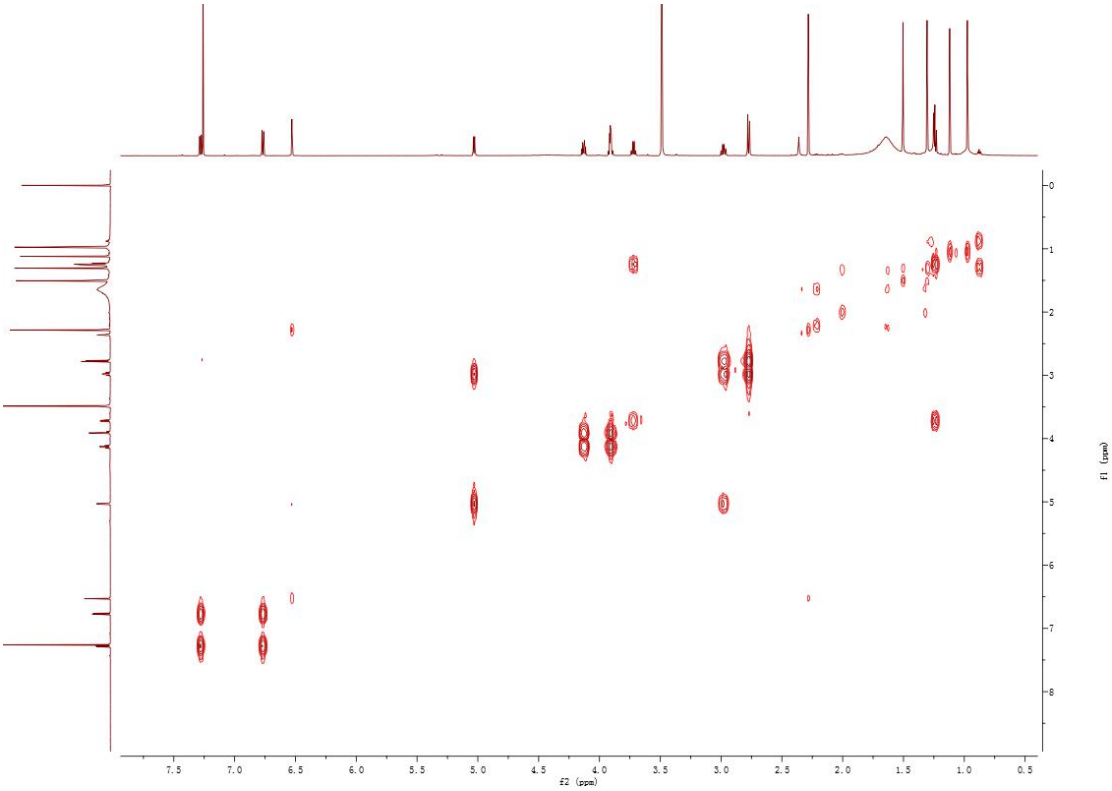

Figure S4. ^{1}H-^{1}H COSY spectrum (600 MHz, $CDCl_3$) of **1**.

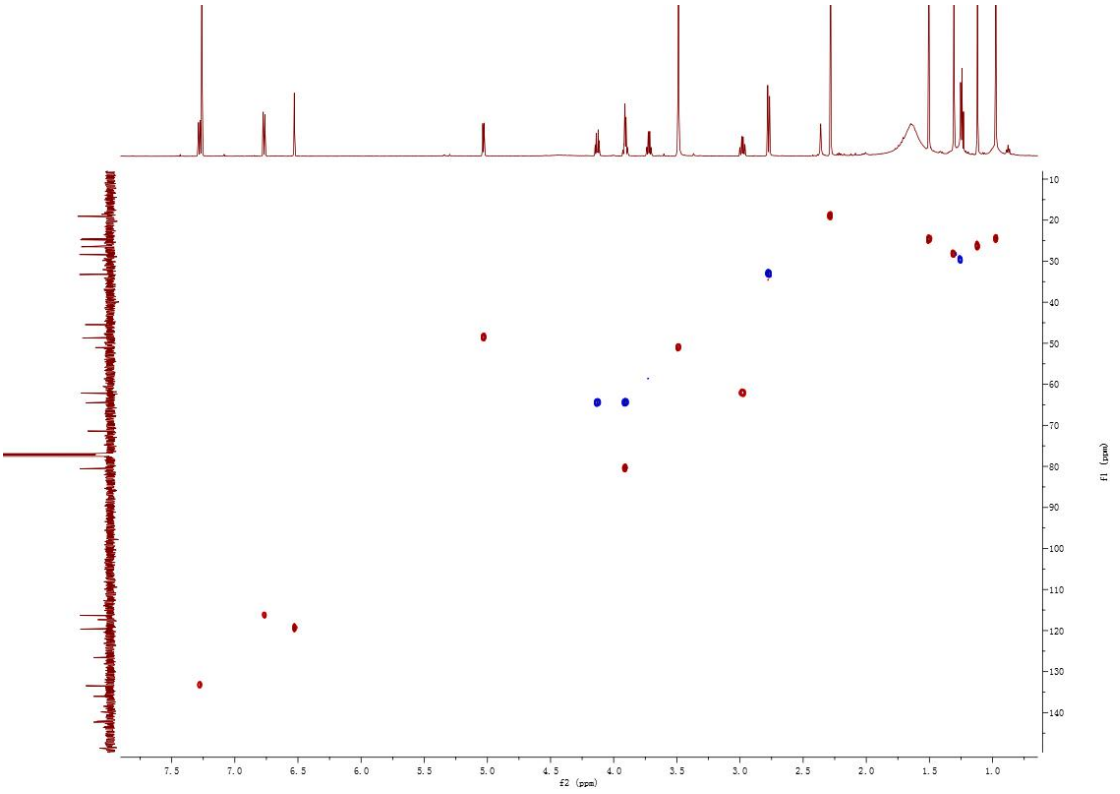

Figure S5. HSQC spectrum of **1**.

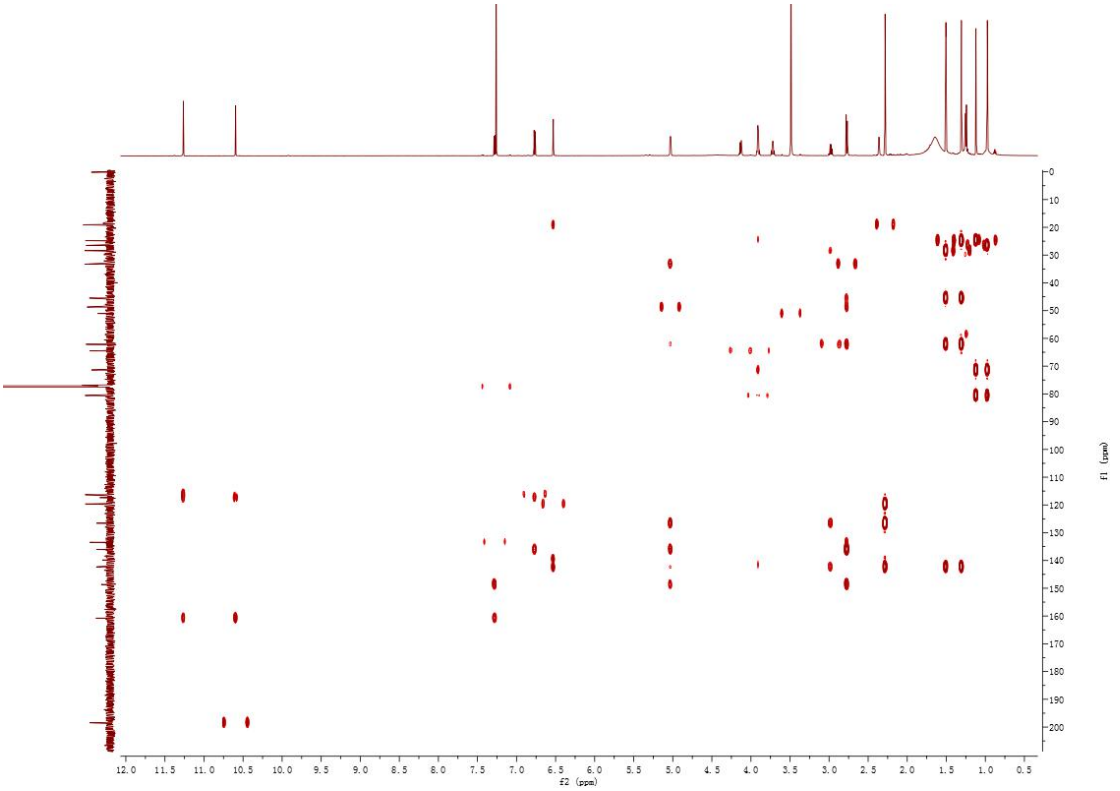

Figure S6. HMBC spectrum of **1**.

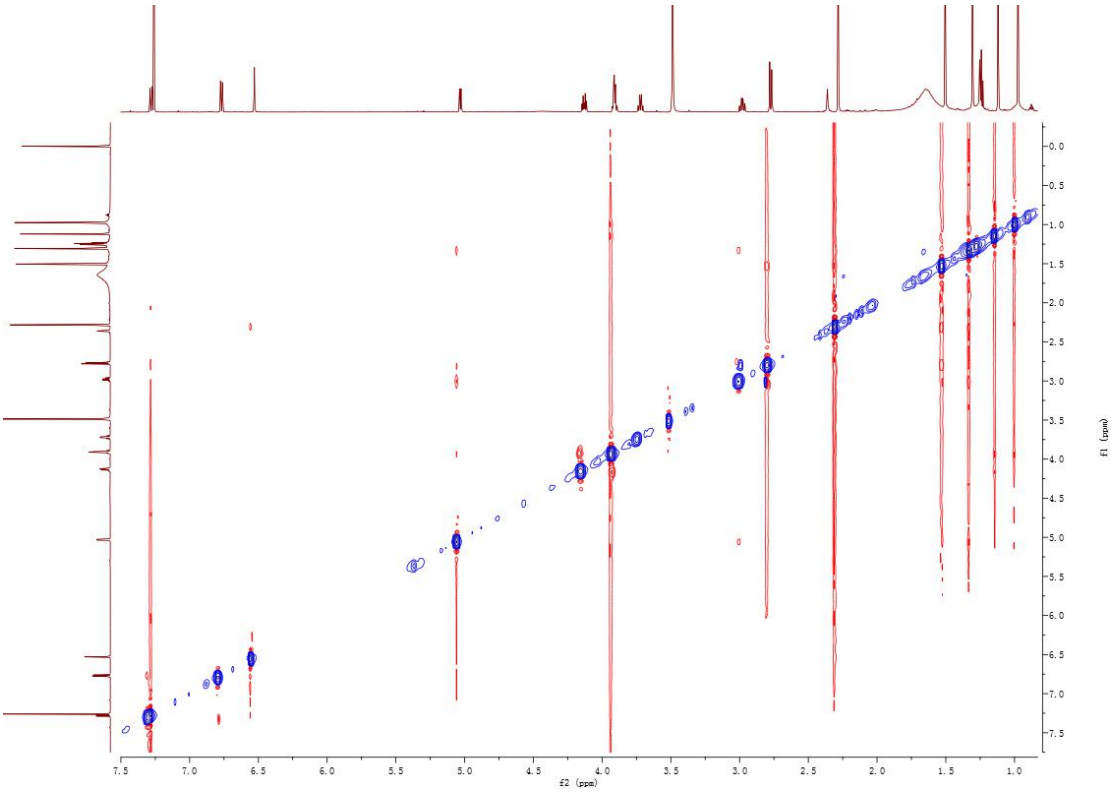

Figure S7. NOESY spectrum of **1**.

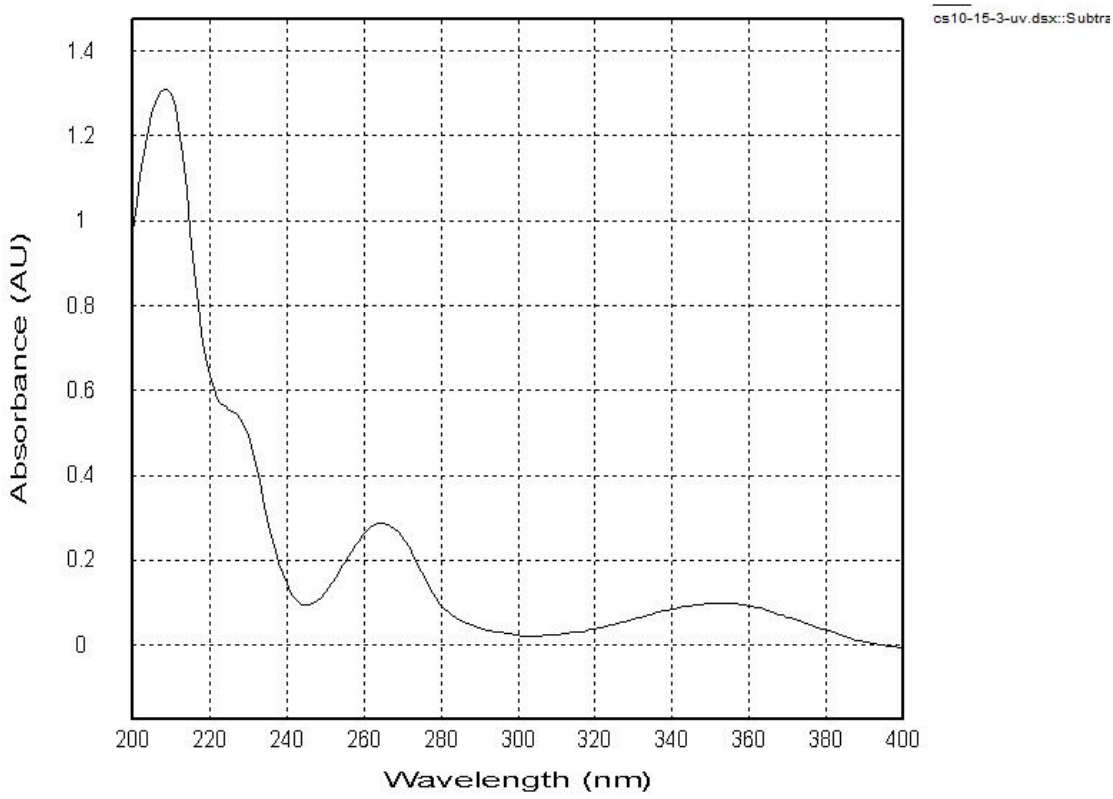

Figure S8. UV spectrum of **1**.

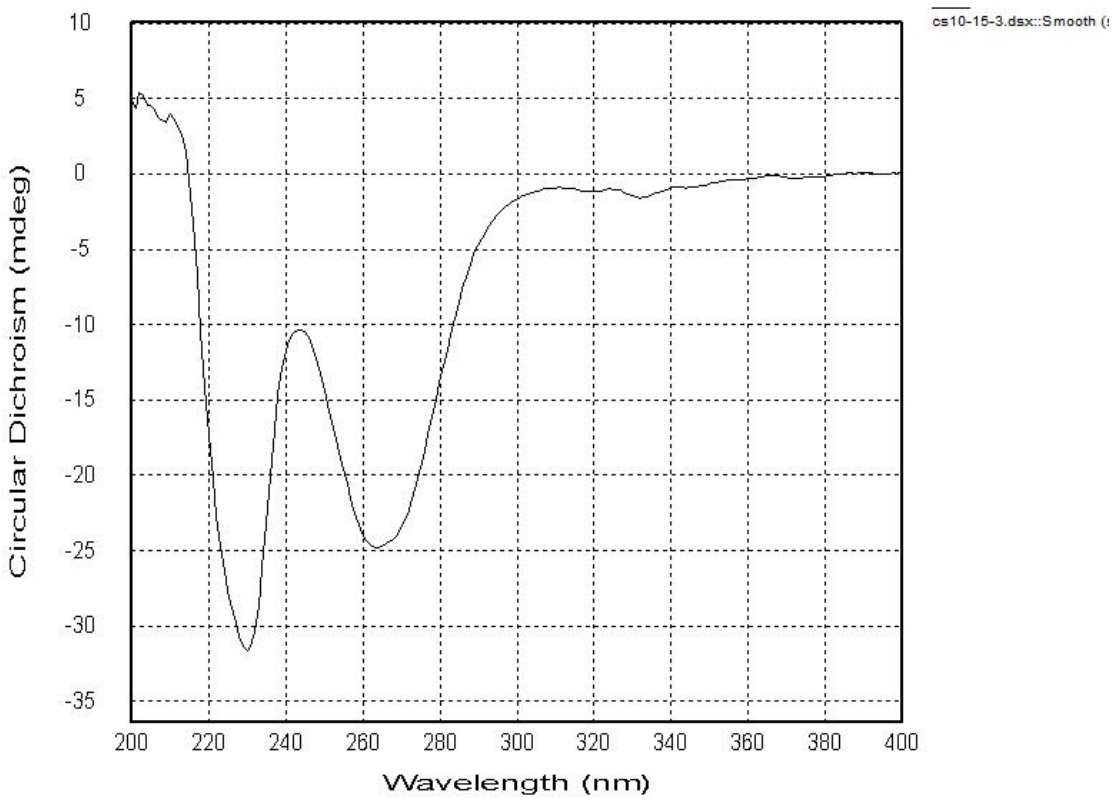

Figure S9. CD spectrum of **1**.

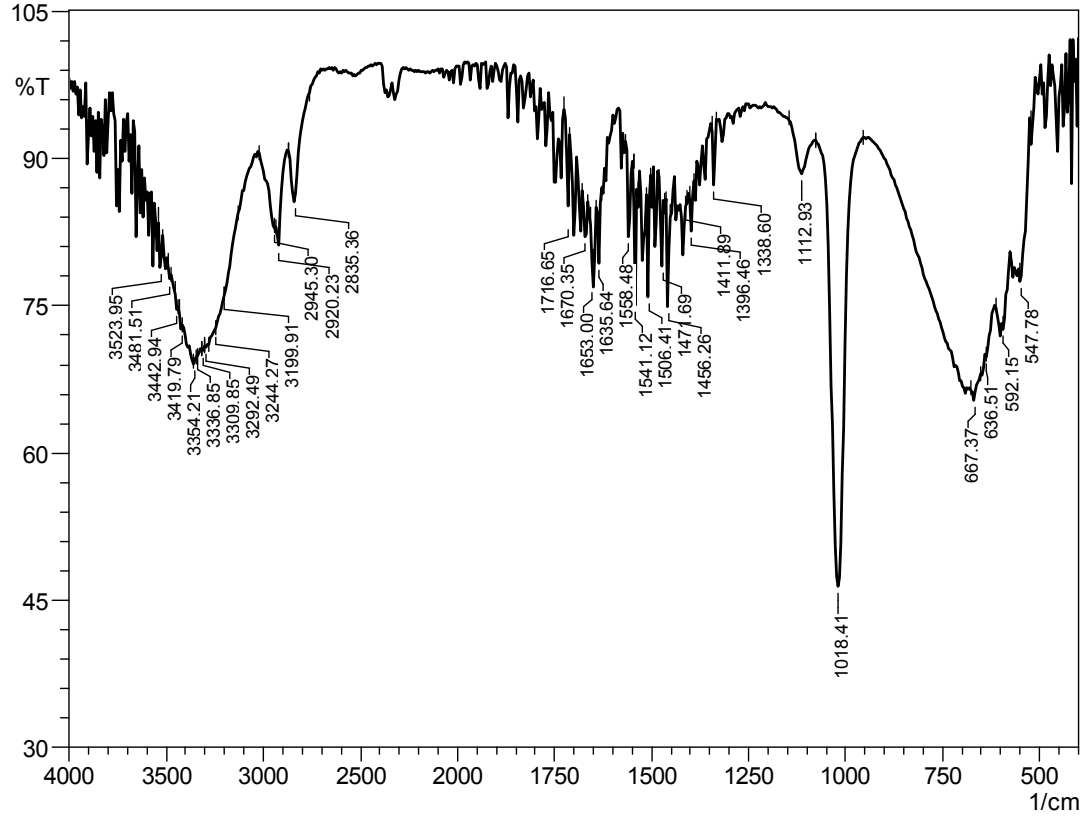

Figure S10. IR spectrum of **1**.

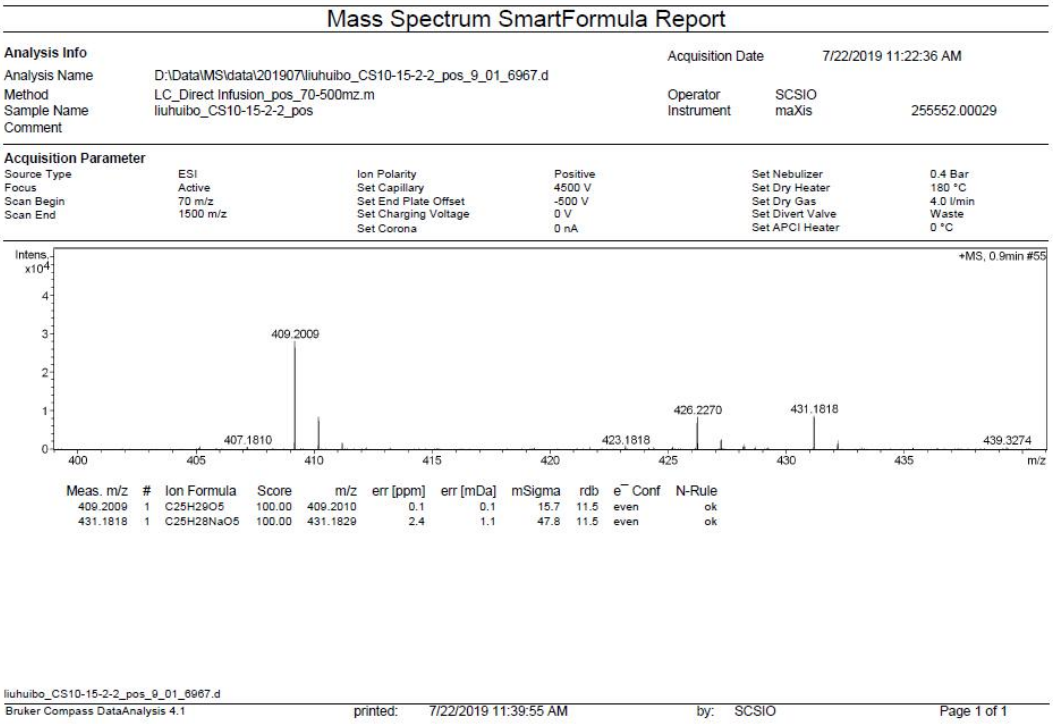

Figure S11. HRESIMS spectrum of **2**.

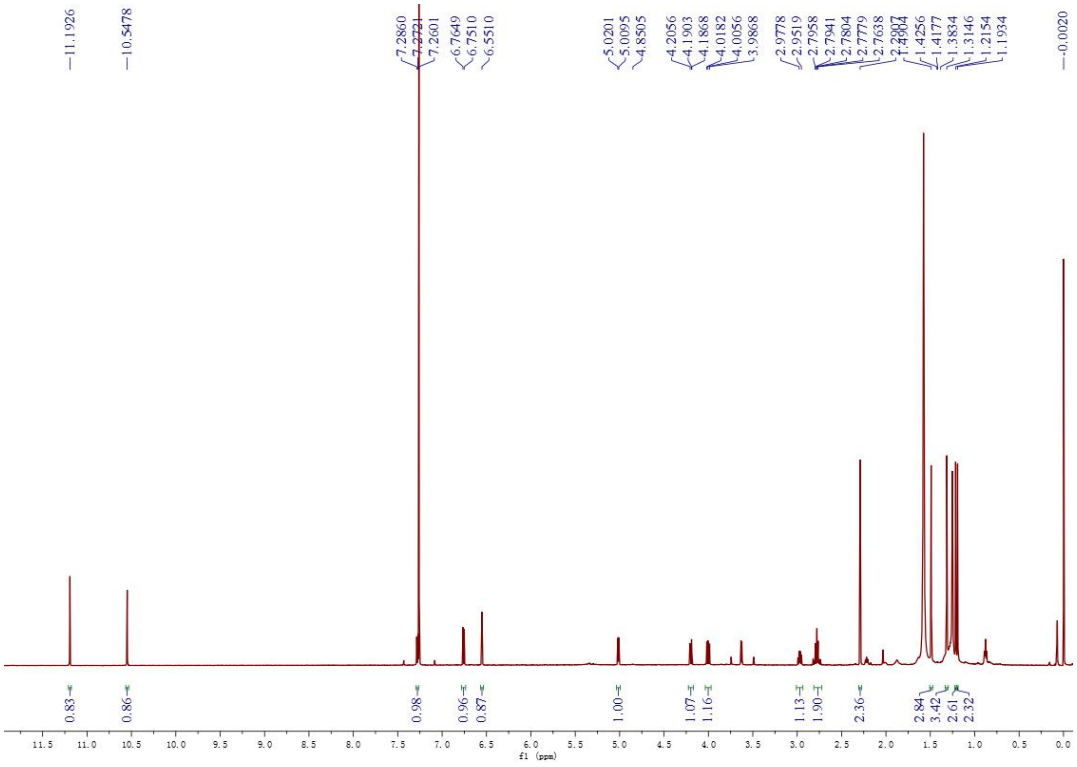

Figure S12. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **2**.

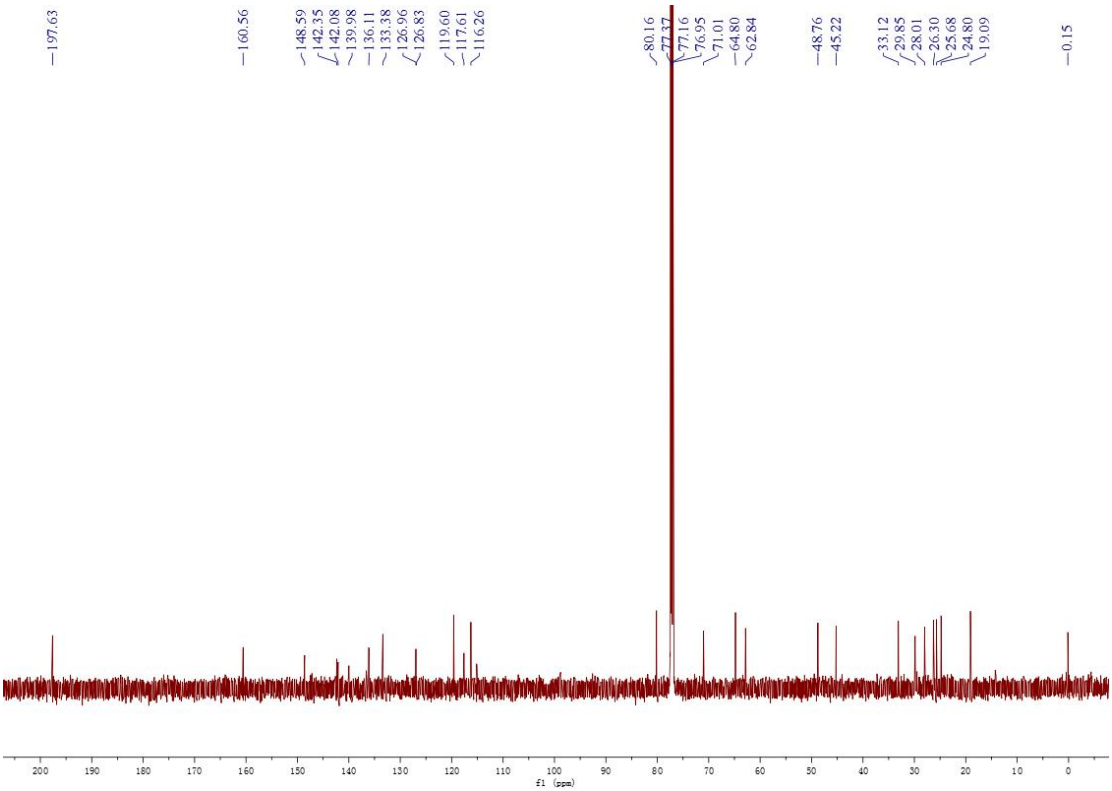

Figure S13. ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of **2**.

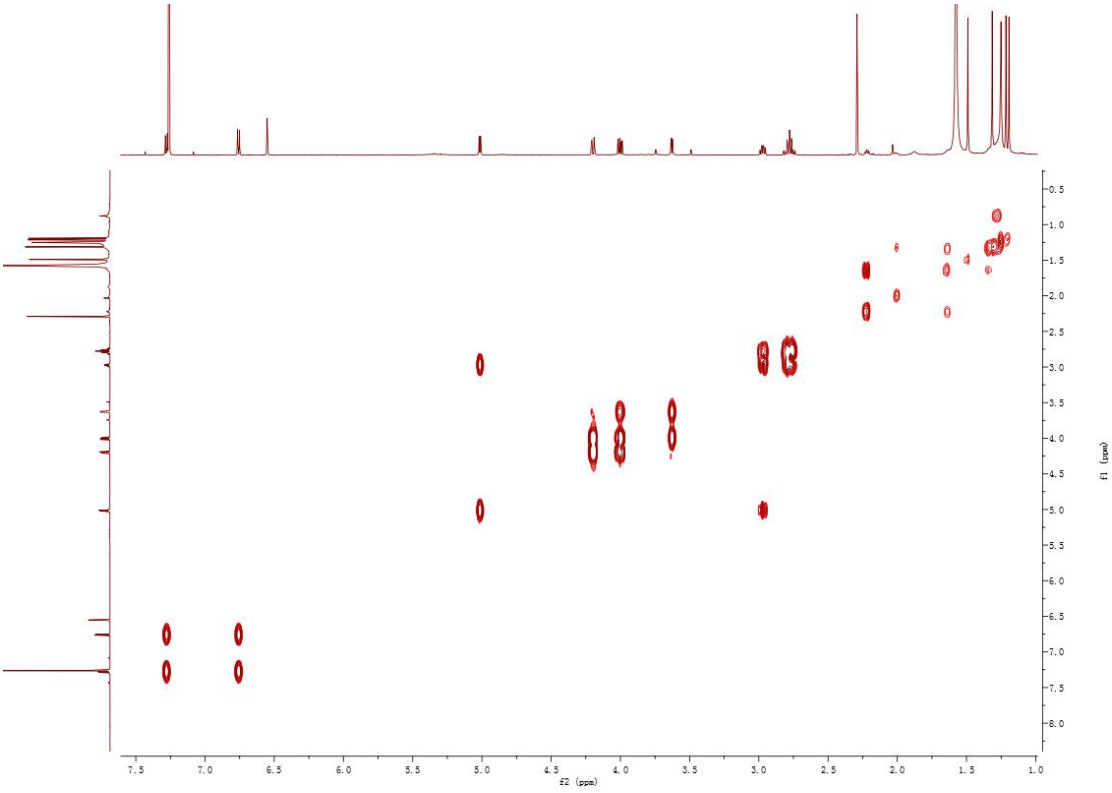

Figure S14. ^{1}H-^{1}H COSY spectrum (600 MHz, $CDCl_3$) of **2**.

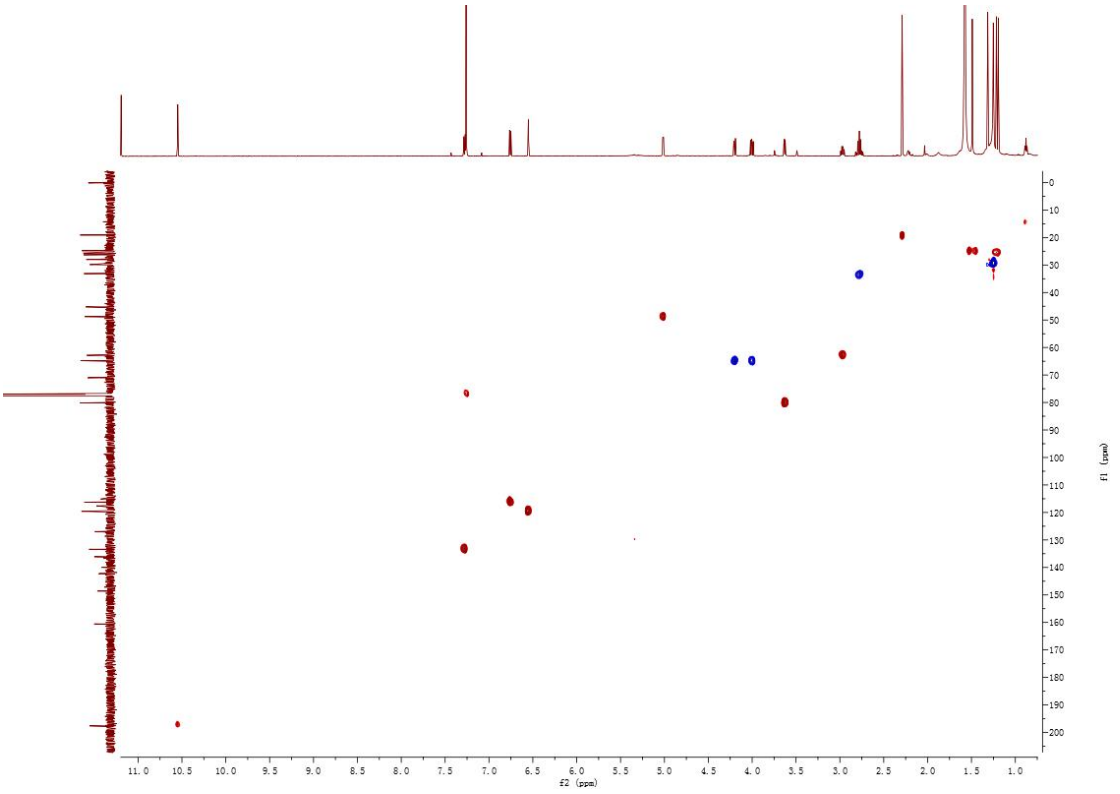

Figure S15. HSQC spectrum of **2**.

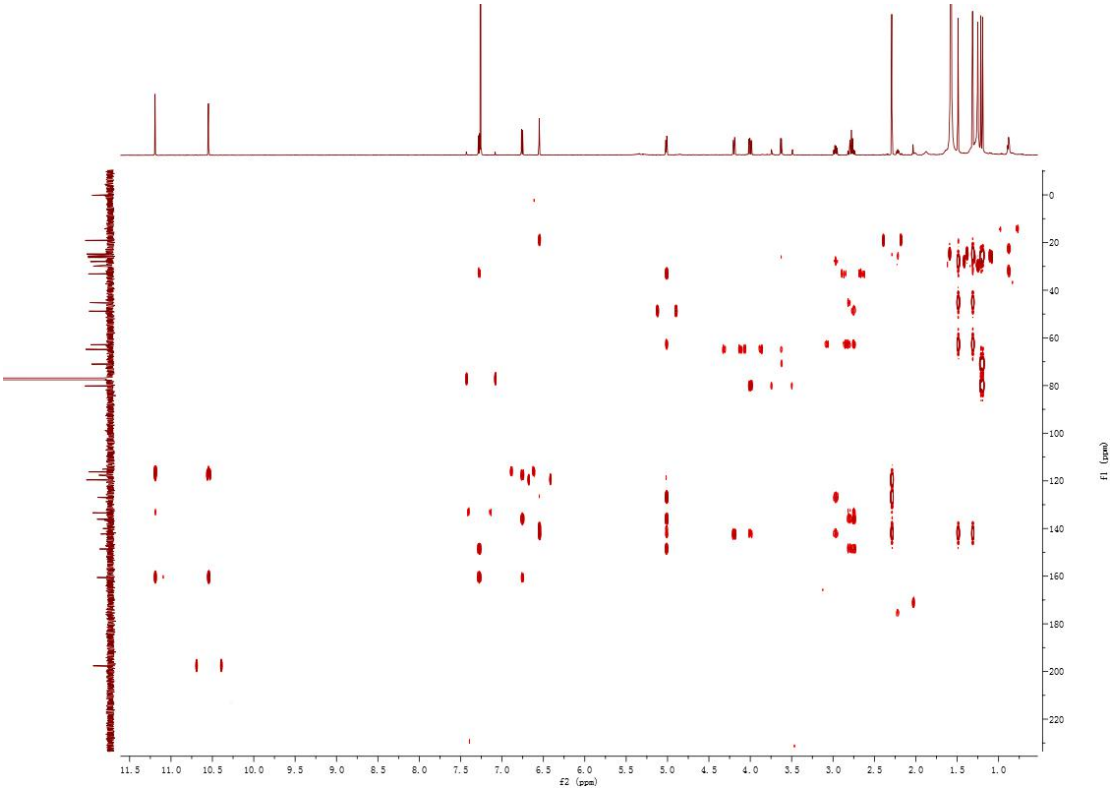

Figure S16. HMBC spectrum of **2**.

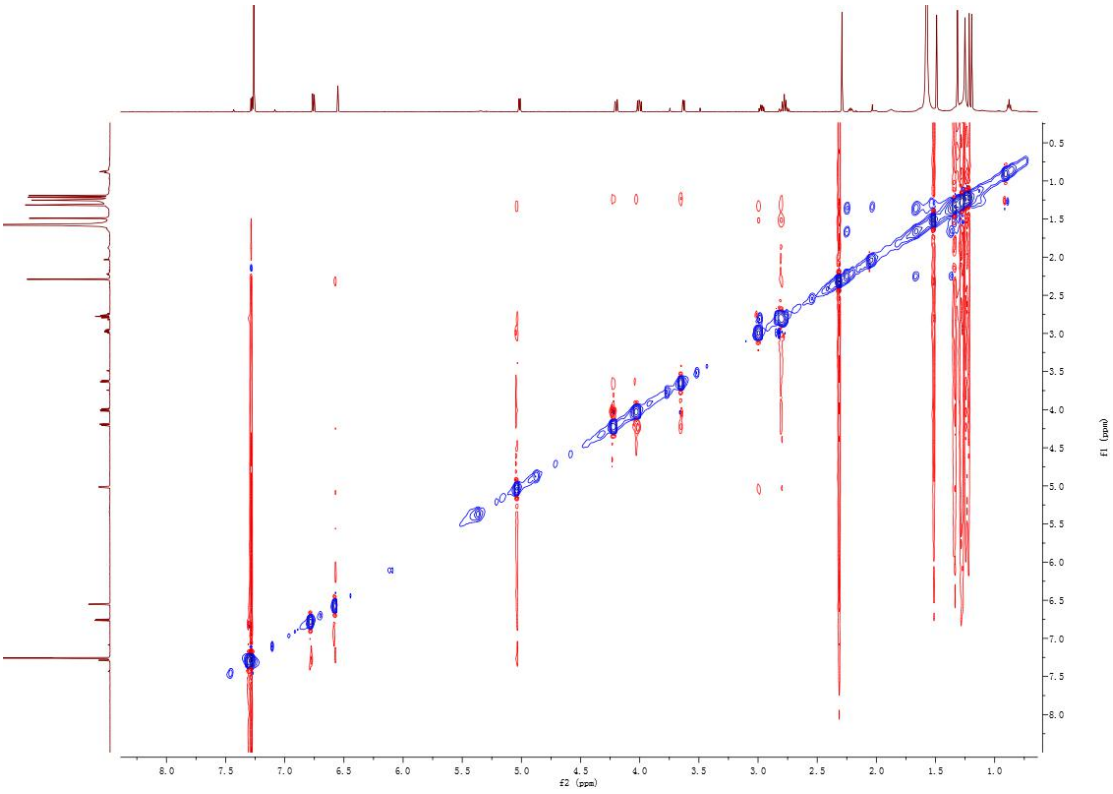

Figure S17. NOESY spectrum of **2**.

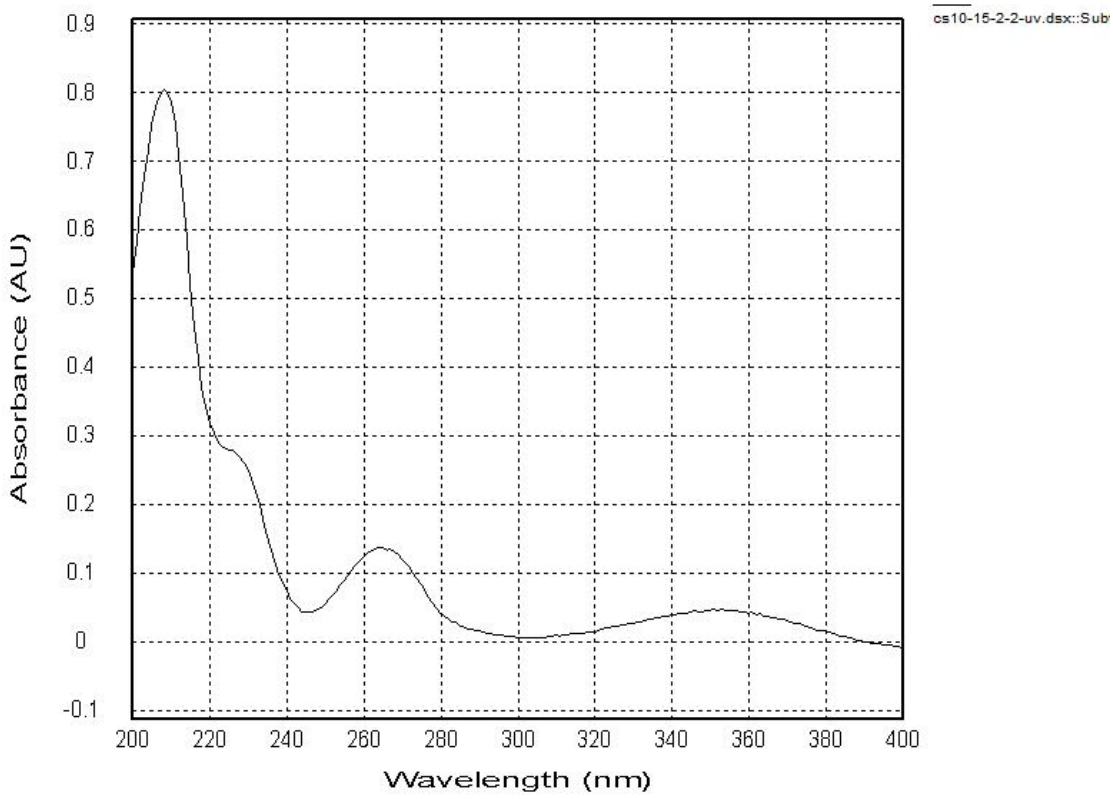

Figure S18. UV spectrum of **2**.

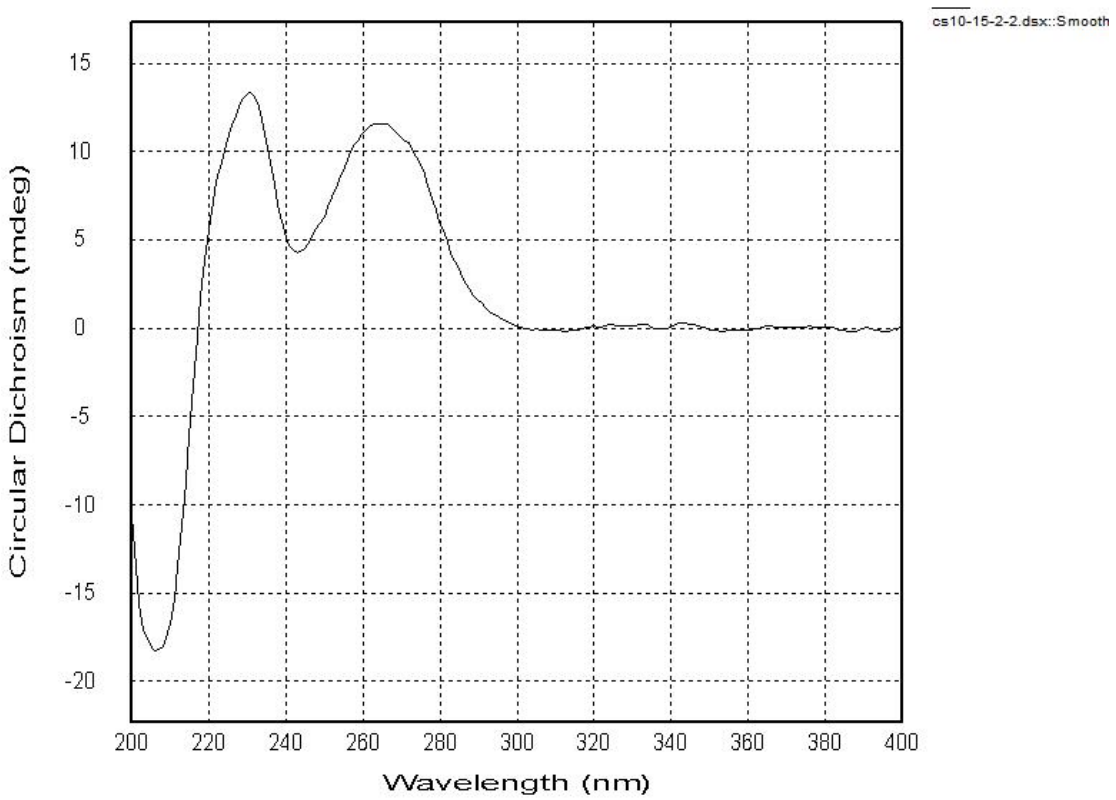

Figure S19. CD spectrum of **2**.

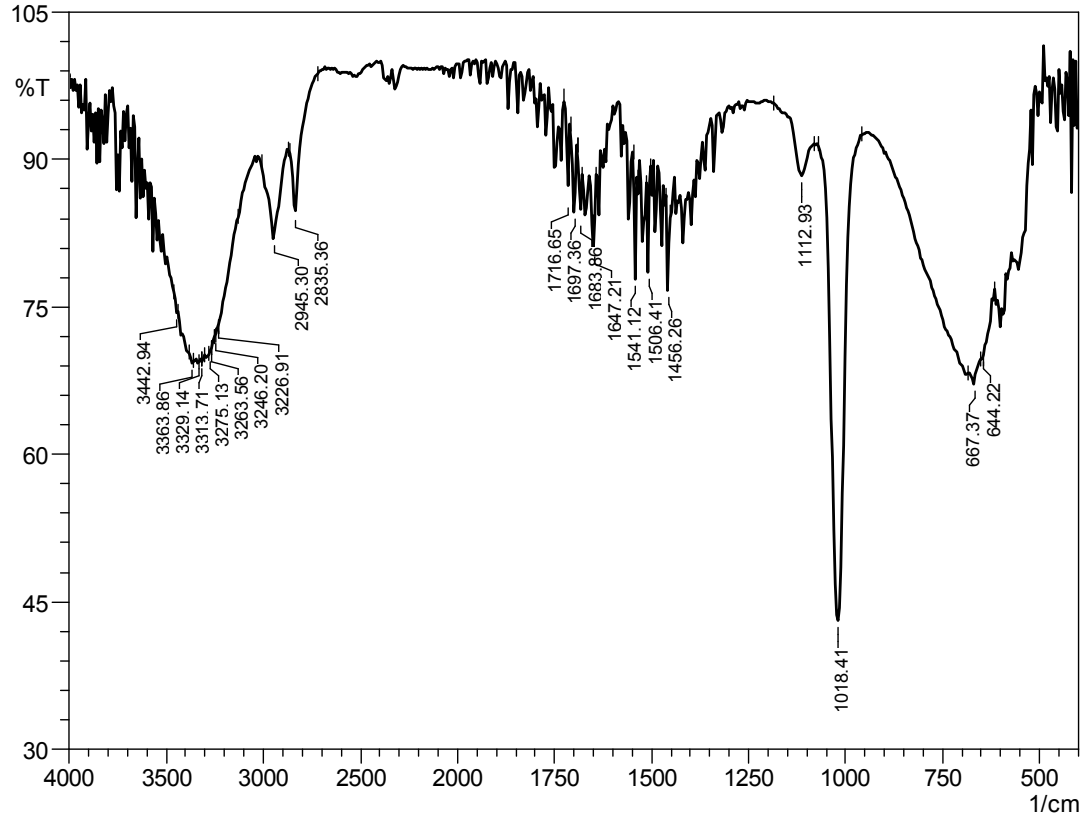

Figure S20. IR spectrum of **2**.