(±)-Corysaxicolaine A, a pair of antitumor enantiomeric alkaloid dimers from *Corydalis saxicola* †

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† Electronic supplementary information (ESI) available.

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Contents

1. General Experimental Procedures.

UV spectra measured with a PerkinElmer 650 spectrophotometer. IR spectra (KBr disks) were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer. Optical rotations were acquired based on a JASCO P-2000 polarimeter. Nu-clear magnetic resonance (NMR) spectra were recorded on Bruker AV-400 NMR instruments (Bruker, Karlsruhe, Germany). Chemical shifts are expressed in δ (ppm) and referenced to the residual solvent signals. HRESIMS data were obtained with a Thermo-Fisher Scientific Exactive high-resolution mass spectrometer. Silica gel (200-300 mesh, Qingdao Marine Chemical Factory, China), Sephadex LH-20 gel (Pharmacia Biotech, Sweden), ODS (50 μm, YMC, Japan), and MCI gel (CHP20, 75-150 μm, Mitsubishi Chemical Corporation, Japan) were used for column chromatography (CC). Preparative high-performance liquid chromatography (HPLC) was carried out using a Shimadzu LC-6AD (Shimadzu, Japan) instrument with a YMC-Pack ODS-A column (20 mm I.D. ×250 mm, S-5 μm, 12 nm) and a SPD-20A wavelength detector at 210 nm. TLC was carried out with GF254 plates (Qingdao Marine Chemical Factory).

For HPLC-ECD analysis, a YMC-Amylose-C Neo column (250 × 4.6 mm, 5 μm) was used to perform chiral-phase separation on a JASCO LC-4000 HPLC system equipped with a JASCO CD-4095 detector. On a JASCO J-1500 ECD spectropolarimeter equipped with a 10 mm HPLC flow cell, the online HPLC-ECD spectra were recorded via stopping the flow at the UV absorption maximum of each peak. ECD ellipticity (Φ) values were not corrected based on concentration. An HPLC-ECD spectrum was continuously scanned three times and averaged with 2 s response, 2 nm bandwidth, and standard sensitivity.

2. Plant Material.

The aerial parts of *C. saxicola* were collected from Jingxi County, Guangxi Zhuang Autonomous Region in August 2013. The plant was identified by Professor Shao-Qing Tang (Guangxi Normal University), and a voucher specimen (No. 20130189) was deposited at the School of Life Sciences, Guangxi Normal University.

3. Extraction and isolation.

Air-dried aerial parts of *C. saxicola* (10 kg) were extracted three times with 95% EtOH (75 L) under reflux for 3 h for each extraction. The obtained crude extract (0.9 kg) was suspended in 3% tartaric acid-H_2O; the water-insoluble material (425.0 g) was suspended in H_2O and partitioned successively with petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2-soluble fraction (100.0 g) was subjected to column chromatography (CC) on silica gel, eluting with petroleum ether-acetone-diethylamine (10:1:0.1~1:1:0.1), to yield eight pooled fractions (A-H). Fraction E was subjected to column chromatography (CC) on silica gel (10:1:0.1~1:1:0.1) and was further purified by Sephadex LH-20 CC (MeOH, 2.6 ×120 cm), and then purified by semi-preparative HPLC (MeOH: H_2O: diethylamine, v/v = 60:40:0.1, 8 ml/min) to obtain **1** (4.5 mg, t_R=28.25 min). Compound **1** was further isolated by HPLC-ECD with a YMC-Amylose-C Neo column (*n*-hexane/IPA 62:38, 0.7 mL/min) to afford two peaks (+)-**1**(0.06 mg, t_R=15.34 min) and (-)-**1**(0.06 mg, t_R=23.89 min).

4. Cytotoxicity assay

All of compounds was evaluated for cytotoxicity against five human cancer cell lines (T24, A549, HepG2, MGC-803 and SKOV3) by means of the MTT method. Cell lines were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences. All were individually cultured in 96-well microtitre plates at a cell density of 1×10^5 cells/well in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum. Plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2 overnight. Cells were treated in triplicate with five concentrations (2.5, 5, 10, 20, and 50 μM) of the tested compounds for 48 h, with doxorubicin as positive control. Cells were stained with 10 mL (10 mg/mL) of MTT in the incubator at 37 °C for about 4 h. After removal of the supernatant, DMSO (100 μL) was added to dissolve the formazan crystals. The absorbance was read by a microplate reader at 570/630 nm, with the data processed by Student's *t*-test with a significance level of $P < 0.05$ using SPSS software (17.0; SPSS, Inc., Chicago, IL, USA). All the tests were repeated in three independent experiments.

Figure S1. ^{1}H NMR (400 MHz) spectrum of compound **1** in DMSO-d_6.

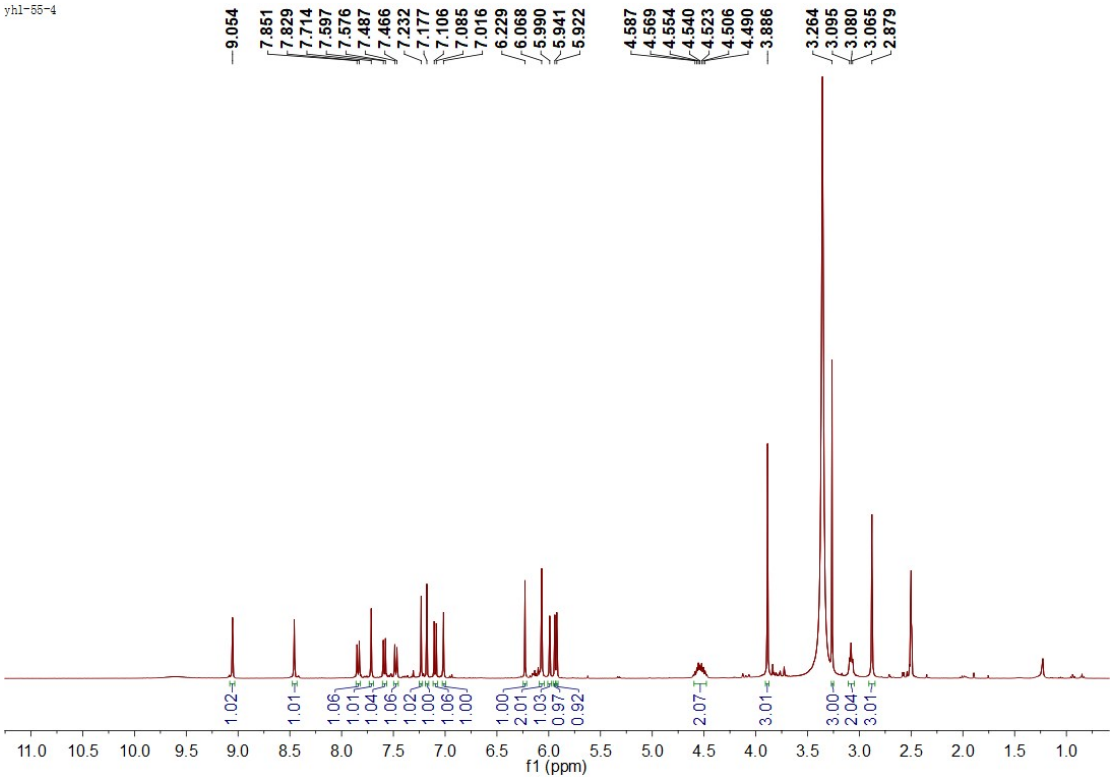


Figure S2. ^{13}C NMR (100 MHz) spectrum of compound **1** in DMSO-d_6.

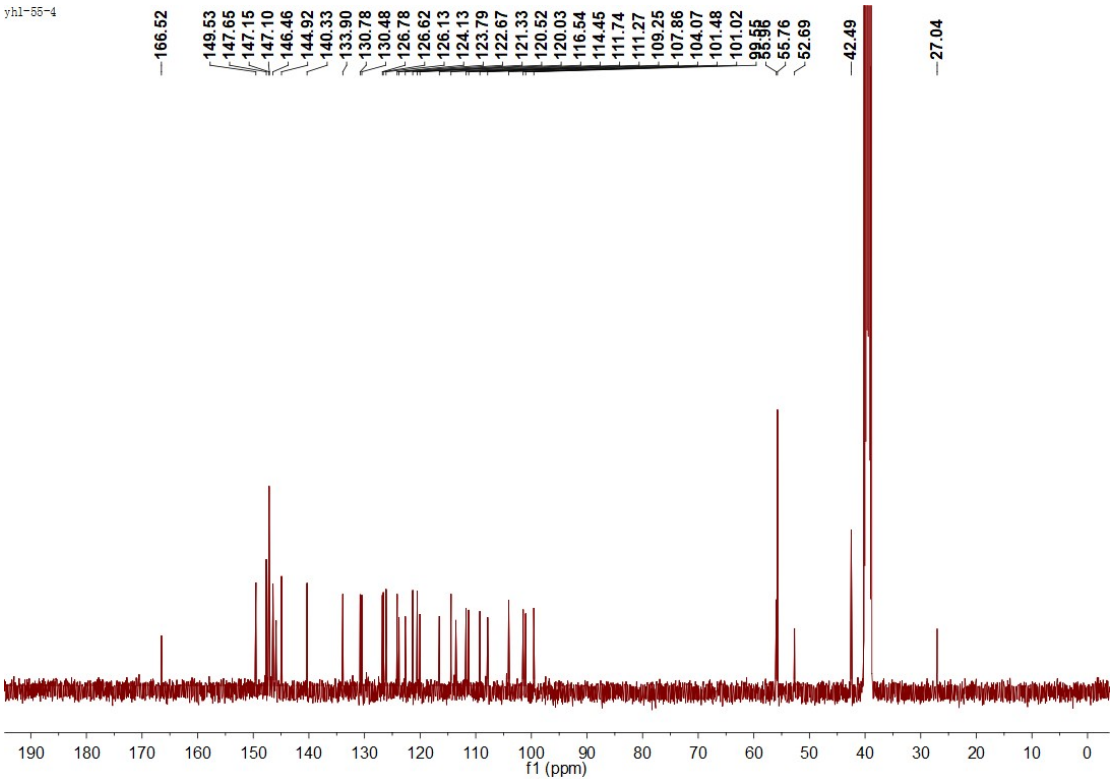


Figure S3. DEPT NMR (100 MHz) spectrum of compound **1** in DMSO-d_6.

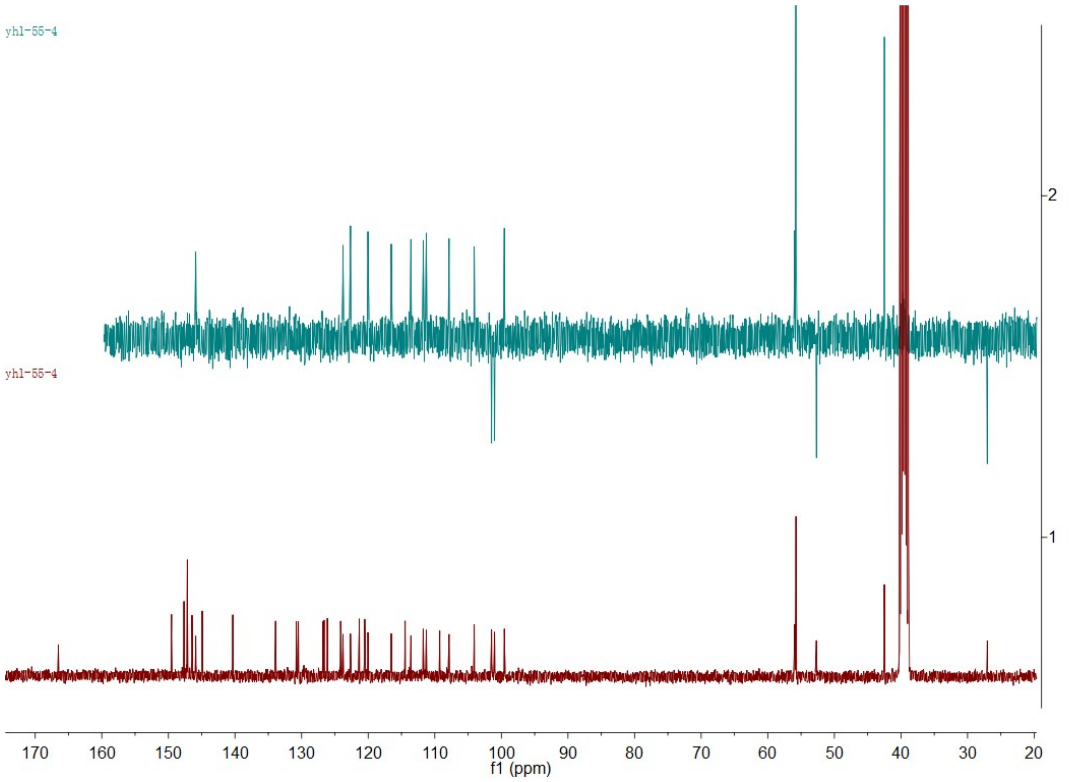


Figure S4. HSQC spectrum of compound **1** in DMSO-d_6.

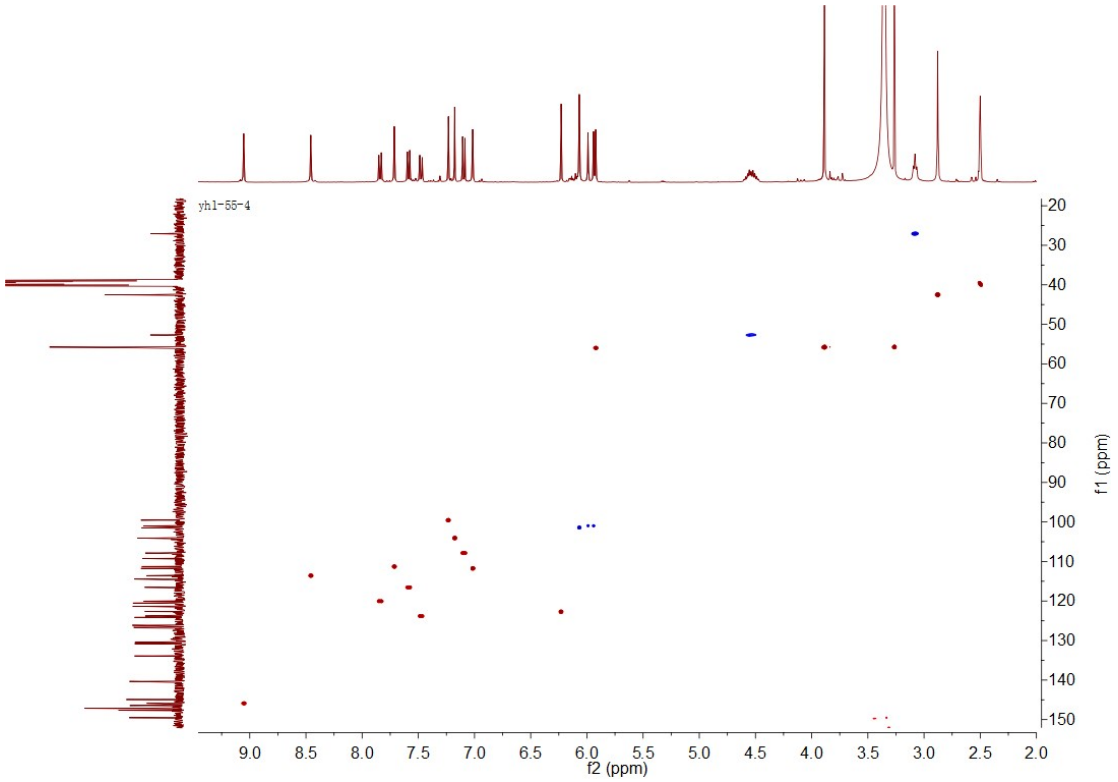


Figure S5. ^{1}H-^{1}H COSY spectrum of compound **1** in DMSO-d_6.

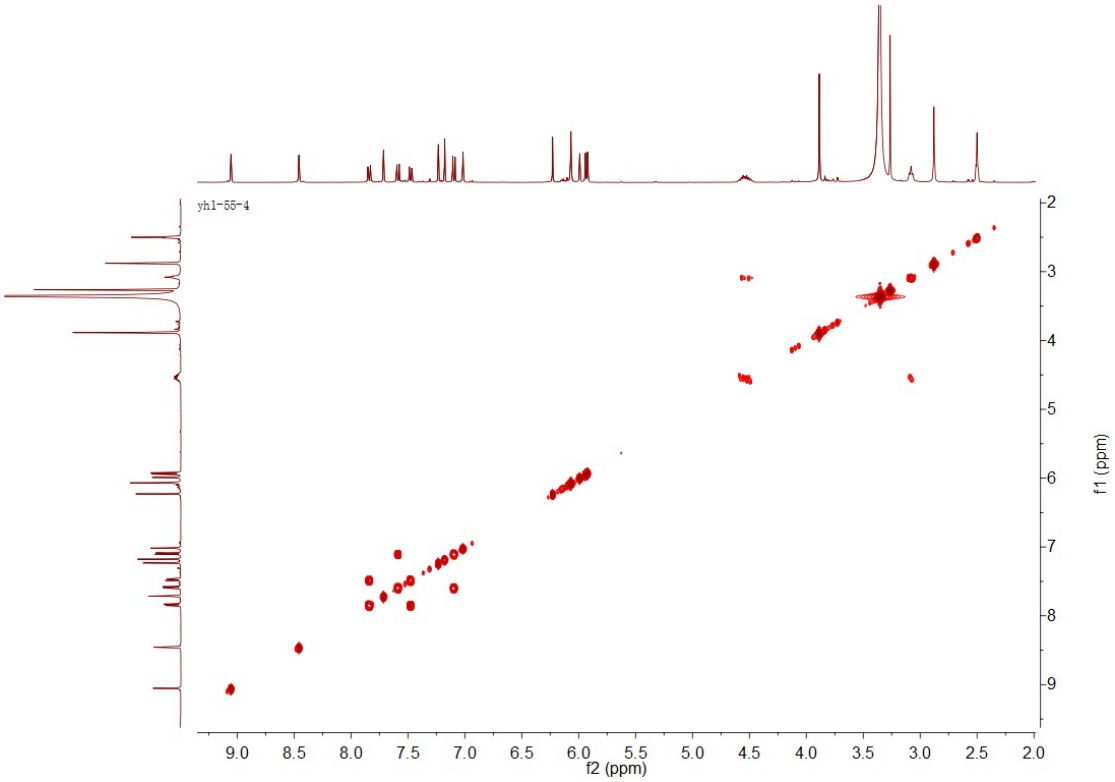


Figure S6. HMBC spectrum of compound **1** in DMSO-d_6.

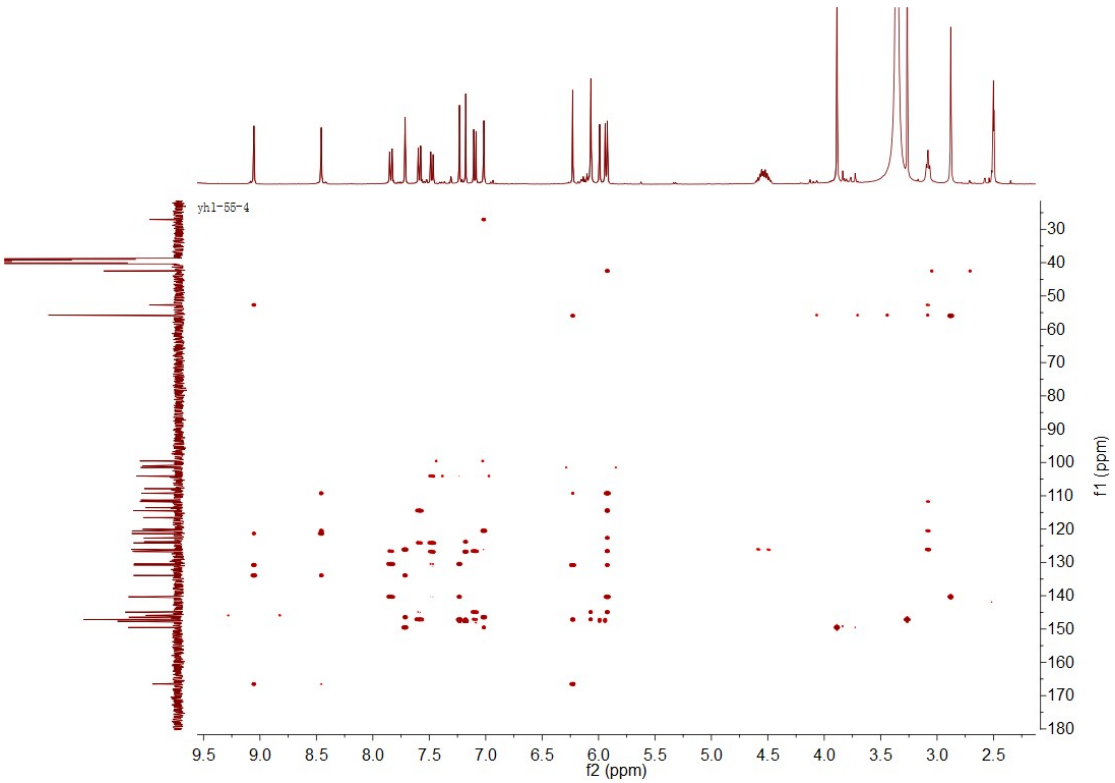


Figure S7. NOESY spectrum of compound **1** in DMSO-d_6.

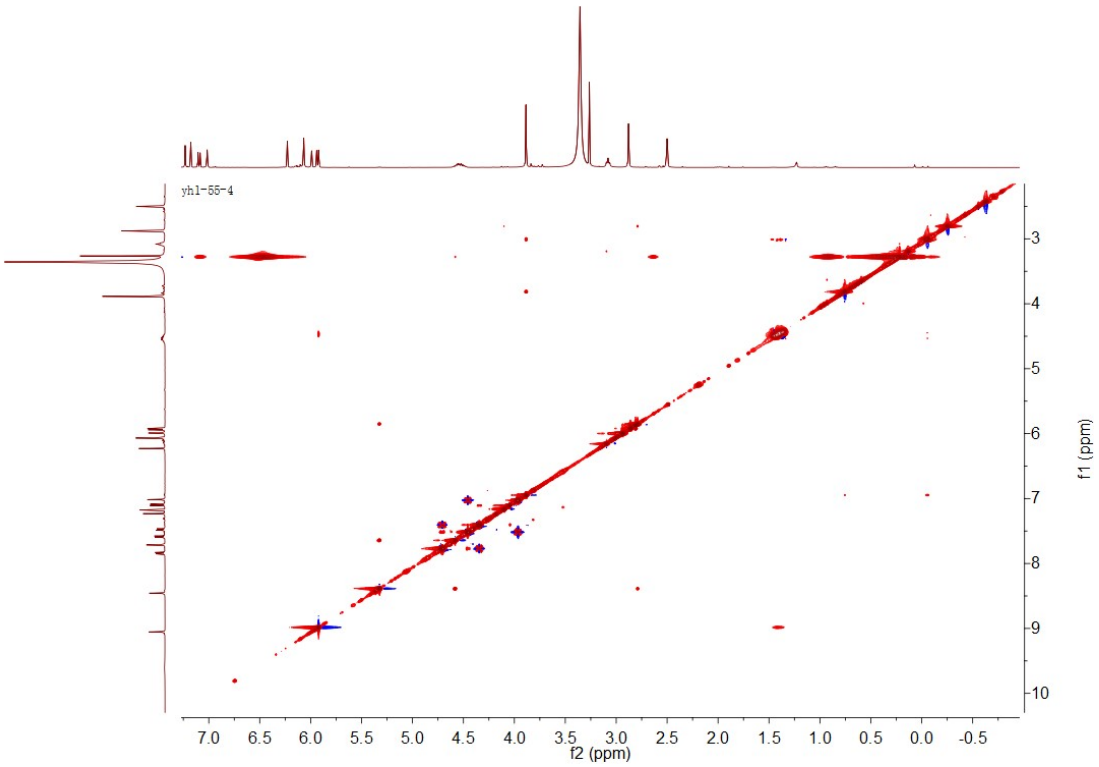


Figure S8. HRESIMS data of compound **1**.

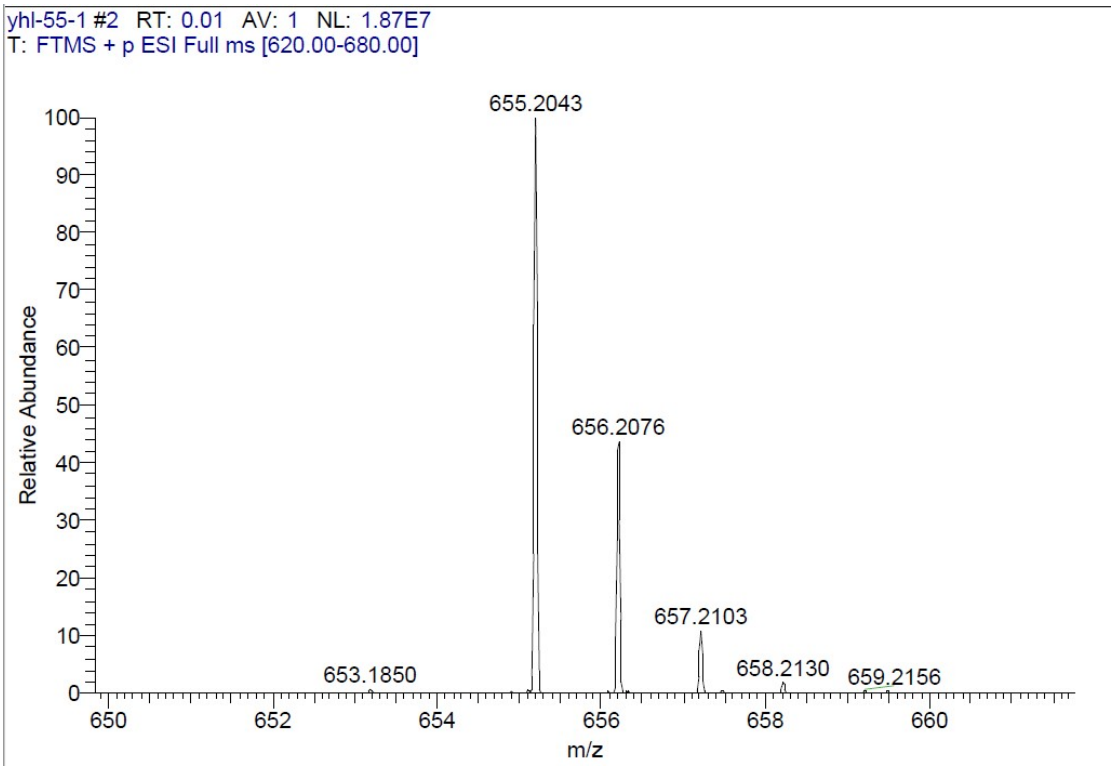


Figure S9. IR spectrum of compound **1**.

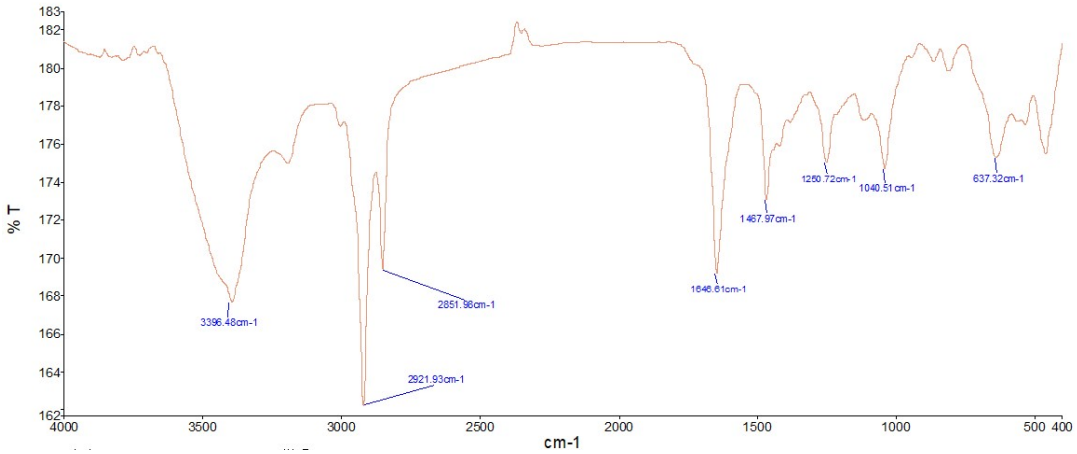


Figure S10. UV spectrum of compound **1**.

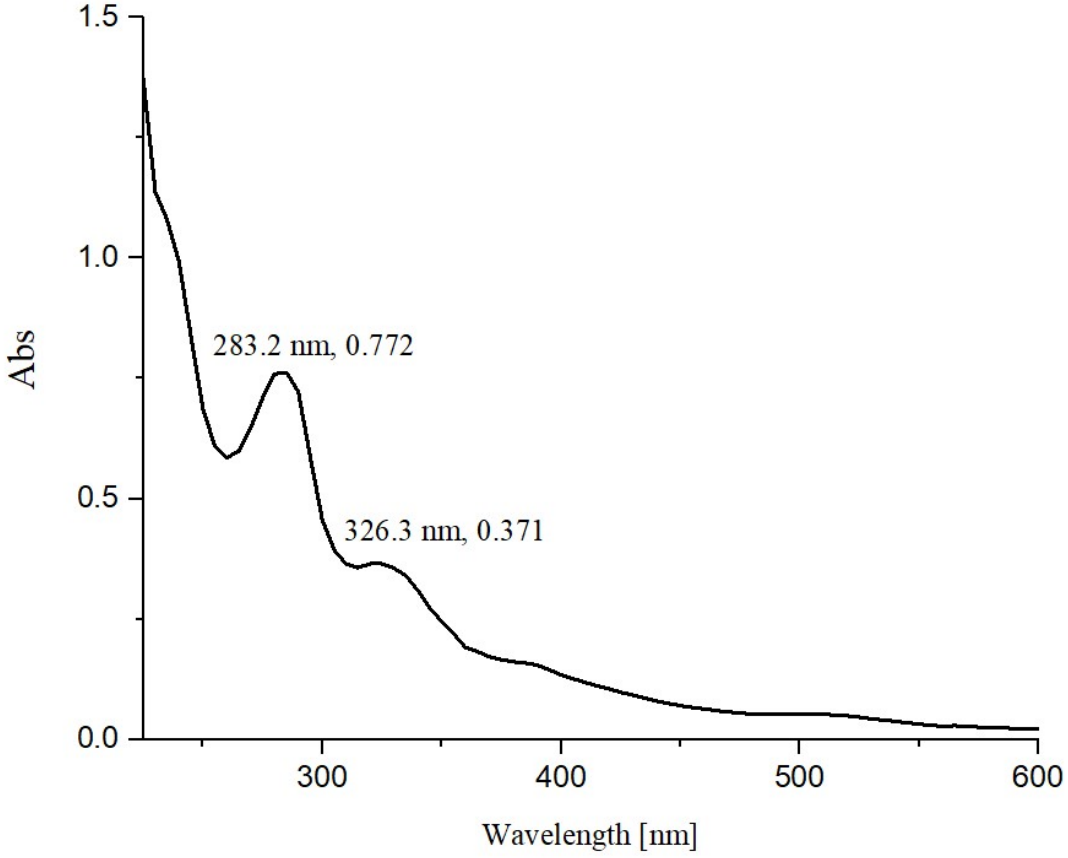
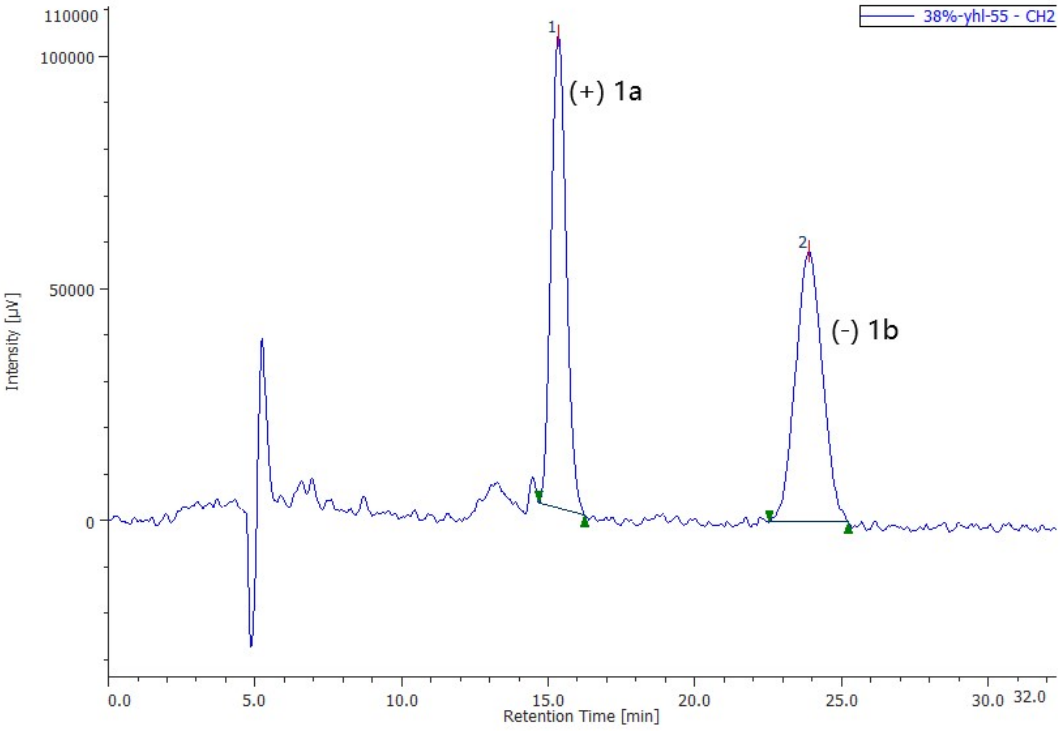


Figure S11. The chiral HPLC of compound **1**.

Daicel YMC-Amylose-C Neo column (n-hexane/2-propanol, 62:38), 0.7 mL/min



Analysis Results

Compound	Area	t_R (min)	Height	Area%	Height%
(+)-**1**	3648311	15.342	101576	49.498	63.585
(-)-**1**	3722240	23.892	58172	50.502	36.415

Figure S12. Compound **1** and CH_2Cl_2 fraction by HPLC/HR-ESIMS analysis.

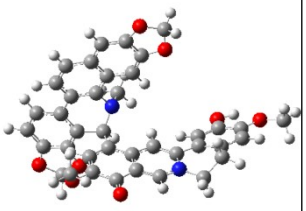
ECD Calculations

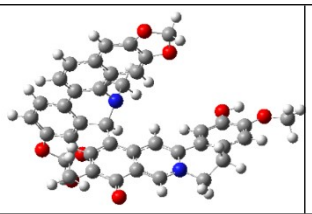
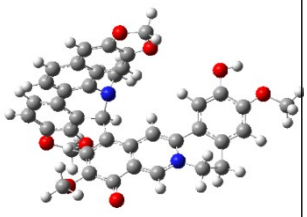
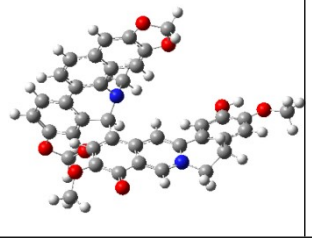
Conformational analysis was initially performed using Confab at MMFF94 force field for compound **1**. Room-temperature equilibrium populations were calculated according to Boltzmann distribution law. The conformers with Boltzmann-population from above 1% were chosen for ECD calculations. The chosen conformer was optimized at B3LYP/6-311G** using Density functional theory (DFT). Then, it was further optimized in MeOH using the CPCM polarizable conductor calculation model. The theoretical calculation of ECD was conducted using Time-dependent DFT (TD-DFT) method at B3LYP/6-311G*. Rotatory strengths for total excited states were calculated. The ECD spectrum is simulated in SpecDis by overlapping Gaussian functions for each transition according to:

$$\Delta\varepsilon(E) = \frac{1}{2.297\times10^{-39}} \times \frac{1}{\sqrt{2\pi\sigma}} \sum_{i}^{A} \Delta E_i R_i e^{-\left(\frac{E-E_i}{2\sigma}\right)^2}$$

where σ represents the width of the band at $1/e$ height, and ΔE_i and R_i are the excitation energies and rotatory strengths for transition i, respectively. σ = 0.25 eV and UV-Shift = 5 nm and R^{velocity} have been used in this work.

Table 1. Energies of the dominative conformers of compound **1**.

Compounds	No	Structure	E (Hartree)	E (kcal/mol)	Population (%)
(*S*)-**1**	1		-2215.93976034	-1390524.35901095	27.42

	2		-2215.93976133	-1390524.35963219	27.45
	3		-2215.93966099	-1390524.29666783	24.68
	4		-2215.93948321	-1390524.18510911	20.44