Electronic Supplementary Information

Jatrocurcadiones A and B: two novel diterpenoids with an unusual 10,11-*seco*-premyrsinane skeleton from *Jatropha curcas*

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S1. General Experimental Procedures

Optical rotations were measured on a Rudolph Autopol I automatic polarimeter, and ECD spectra were obtained on an Applied Photophysics Chirascan spectrometer. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. IR spectra were determined on FT-IR Equinox 55 and Bruker Tensor37 infrared spectrophotometers. NMR spectra were measured on Bruker AM-400 and Avance III-600 spectrometers at 25°C. ESIMS was measured on a Finnigan LCQ Decainstrument, and HRESIMS was performed on a Waters Micromass Q-TOF spectrometer. A Shimadzu LC-20 AT equipped with a SPD-M20A PDA detector was used for HPLC. A YMC-pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm) was used for semi-preparative HPLC separation.Silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd.), C_{18} reversed-phase silica gel (12 nm, S-50 μm, YMC Co., Ltd.), Sephadex LH-20 gel (Amersham Biosciences), and MCI gel (CHP20P, 75−150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography (CC). All solvents used were of analytical grade (Guangzhou Chemical Reagents Company, Ltd.). TrxR was purchased from Sigma-Aldrich (St. Louis, USA).

S2. Plant Material

The twigs of *J. curcas* were collected in July 2014 in the Yunnan Province, P. R. China, and were authenticated by Prof. You-Kai Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (accession number: MFS201407) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

S3. Extraction and Isolation

The air-dried powder of the twigs of *J. curcas* (5 kg) was extracted with 95% EtOH (3 × 8 L) at room temperature to give 150 g of crude extract. The extract was suspended in H_2O (1.5 L) and successively partitioned with petroleum ether (PE, 3 × 2 L), EtOAc (3 × 2 L), and *n*-BuOH (3 × 2 L). The EtOA cextract (39 g) was subjected to MCI gel (CC) eluted with a MeOH/H_2O gradient (3:7 → 10:0) to afford five fractions (I−V). Fraction III (6 g) was chromatographed over C_{18} reversed-phase (RP-18) silica gel CC eluted with MeOH/H_2O (4:6 → 10:0) to afford five fractions (IIIa–IIIe). Fraction IIIc was subjected to Sephadex LH-20 gel to give three fractions (IIIc1–IIIc3). Fraction IIIc1 was further purified on a semi-preparative reversed-phase (RP) HPLC system equipped with a YMC column (MeOH/H_2O, 7:3, 3 mL/min), to give **1** (10.6 mg, t_R 16.5 min). Fraction IIIe was subjected to silica gel CC (PE/CH_2Cl_2, 2:1 → 1:2) to give four fractions (IIIe1–IIIe4). Fraction IIIe2 was purified using RP-HPLC (CH_3OH/H_2O, 8:2, 3 mL/min), to give **2** (0.6 mg, t_R 15.6 min).

S4. Chemical Correlation of 1 to 2

Acetic anhydride (10 μL) was added to a stirred solution of compound **1** (1 mg) in freshly distilled pyridine (1 mL). After 12 h at rt, water (1 mL) was added and the mixture was extracted with ethyl acetate (3 × 1 mL). The combined organic solvents were evaporated and the residue was purified by RP-HPLC (CH_3OH/H_2O, 8:2, 3 mL/min) to yield **2** (0.52 mg), which was identified by the ^{1}H NMR spectrum, MS data and specific rotation.

S5. Evaluation of the TrxR inhibitory activities

For determining the TrxR inhibitory activity of the compounds, the DTNB reduction assay was employed. All assays were conducted at 25 °C in a total volume of 40 μL. In each measurement, 0.3 μL of TrxR (0.04 μM) was added to an assay buffer containing 1 M potassium phosphate (pH 7.0), 500 mM EDTA (pH 7.4), NADPH (0.48 mM) and 1 μL of inhibitor at various concentrations. After 5 min pre-incubation, the reaction was initiated with the addition of 3.2 μL of DTNB (final concentration of 5.0 mM). The control was incubated with the same amount of DMSO (2.5%, v/v). The increase in absorbance at 412 nm ($\Delta\varepsilon$ 412 = 13.6 mM^{-1} cm^{-1}) was monitored in the initial 120 s. The IC_{50} values were calculated to represent the TrxR inhibitory effect of compounds.

Table S1. The TrxR inhibitory activity of some compounds (IC_{50}, μM)

compound	IC_{50} (μM)[a]
1	10.0 ± 2.6
curcumin[b]	25.0 ± 2.2

[a]Values are represented as means ± SD based on three independent experiments.

[b]Positive control.

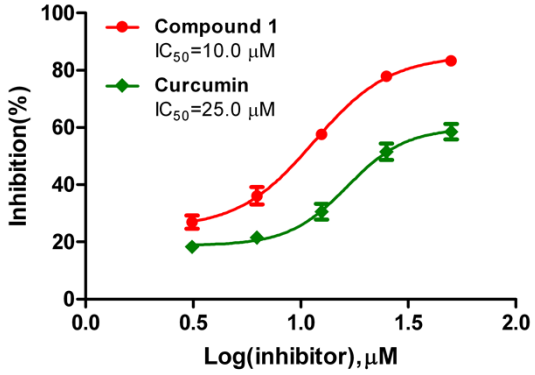


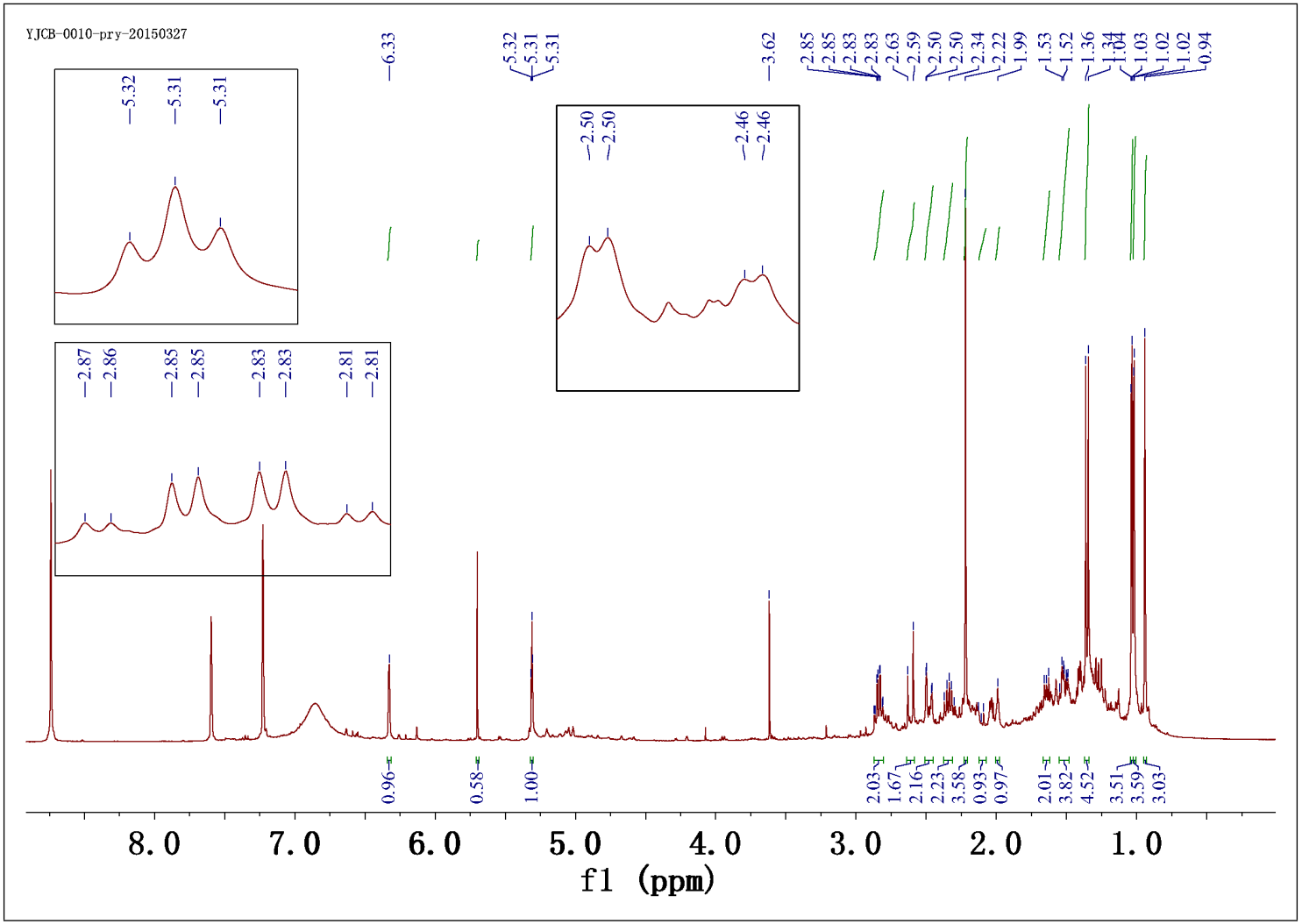
Fig. S1 Inhibition curves of compounds **1** and curcumin (positive control) against TrxR.

S6. OR, UV, ECD, IR, and MS data of 1 and 2

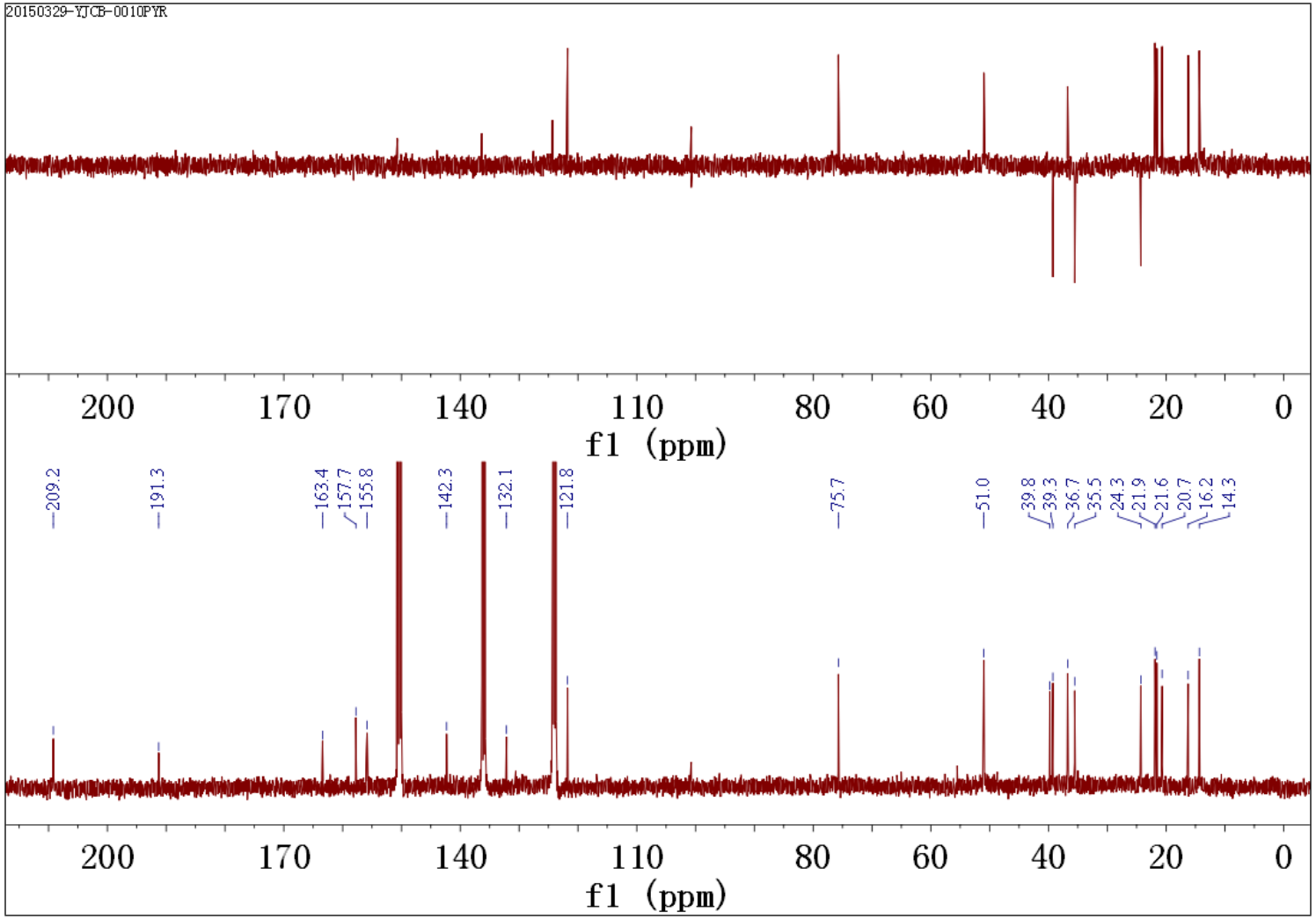
Jatrocurcadione A (**1**): yellow oil; $[\alpha]^{20}_{D}$ +268.8 (*c* 0.54, CH_2Cl_2); UV (CH_3CN) λ_{max} (log ε) 355 (2.21), 248 (3.60), 197 (3.67); ECD (*c* 3.31 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 341 (1.27), 278 (0.21), 251 (–0.59), 229 (0.73), 204 (–1.13) nm; IR (microscope) ν_{max} 3441, 2963, 1713, 1587, 1456, 1374, 1259, 1209, 1040, 889, and 738 cm^{-1}; ^1H and ^{13}C NMR data recorded in Pyridine-d_5, see **Table 1**; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 6.36 (1H, s, H-11), 4.81 (1H, dd, *J* = 2.6, 2.6 Hz, H-1), 2.52 (1H, m, H-5a), 2.45 (1H, overlap, H-2), 2.45 (1H, overlap, H-5b), 2.45 (1H, overlap, H-10), 2,25 (1H, m, H-8a), 2.17 (1H, m, H-8b), 2.05 (3H, s, H_3-20), 1.69 (2H, m, H_2-7), 1.29 (3H, d, *J* = 7.5 Hz, H_3-16), 1.10 (6H, br d, *J* = 6.3 Hz, H_3-18 and H_3-19), 0.97 (3H, s, H_3-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 208.6 (C, C-3), 191.9 (C, C-14), 158.9 (C, C-9), 158.6 (C, C-15), 155.0 (C, C-12), 143.6 (C, C-4), 131.8 (C, C-13), 120.9 (CH, C-11), 76.4 (CH, C-1), 48.7 (CH, C-2), 39.4 (CH_2, C-7), 38.4 (C, C-6), 36.6 (CH, C-10), 35.6 (CH_2, C-5), 23.9 (CH_2, C-8), 21.6 (CH_3, C-17), 21.5 (CH_3, C-18), 21.0 (CH_3, C-19), 15.9 (CH_3, C-20), 13.4 (CH_3, C-16); positive ESIMS *m*/*z* 315.2 $[M + H]^+$; HRESIMS *m*/*z* 315.1964 $[M + H]^+$ (calcd 315.1955).

Jatrocurcadione B (**2**): light yellow oil; $[\alpha]^{20}_{D}$ +143.3 (*c* 0.06, CH_2Cl_2); UV (CH_3CN) λ_{max} (log ε) 356 (3.45), 252 (3.69), 198 (4.01); ECD (*c* 2.92 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 348 (2.99), 249 (–2.42), 225 (2.25), 193 (–13.35) nm; IR (KBr) ν_{max} 3123, 2921, 1796, 1600, 1374, 1229, 1142, and 878 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 1**; HRESIMS *m*/*z* 357.2050 $[M + H]^+$ (calcd 357.2060).

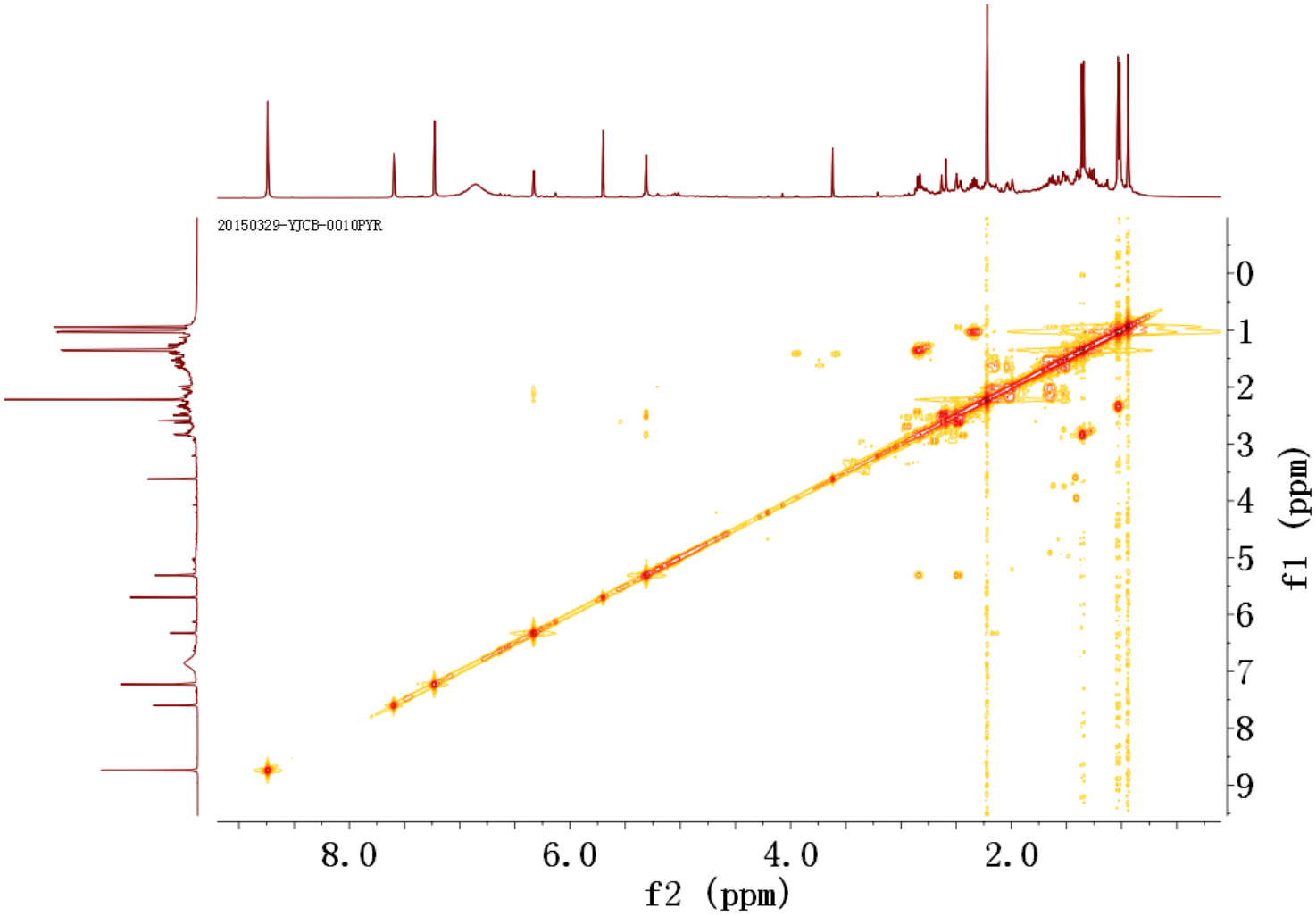
S7. ^{1}H NMR spectrum of 1 in Pyridine-d_5



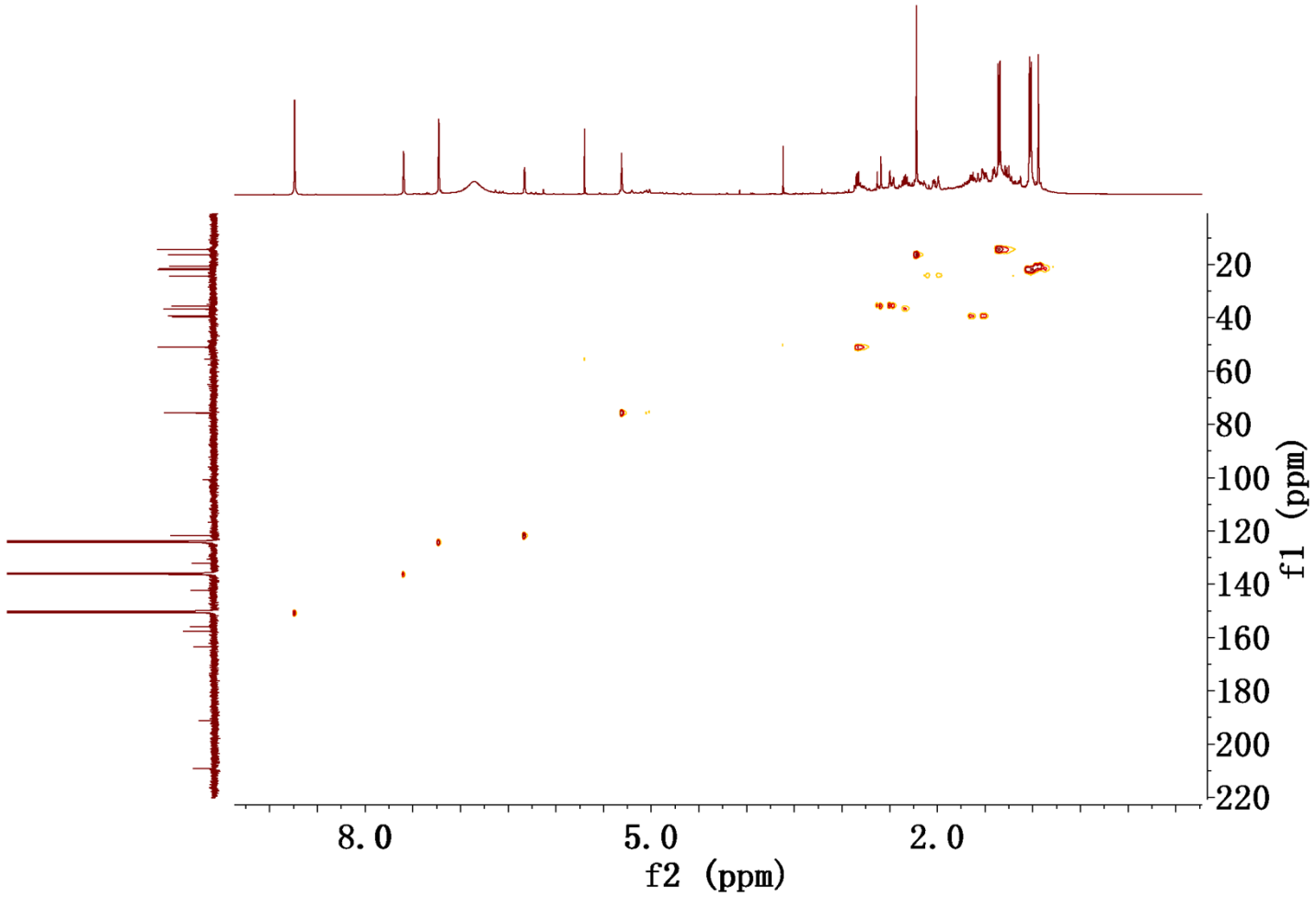
S8. ^{13}C NMR spectrum of 1 in Pyridine-d_5



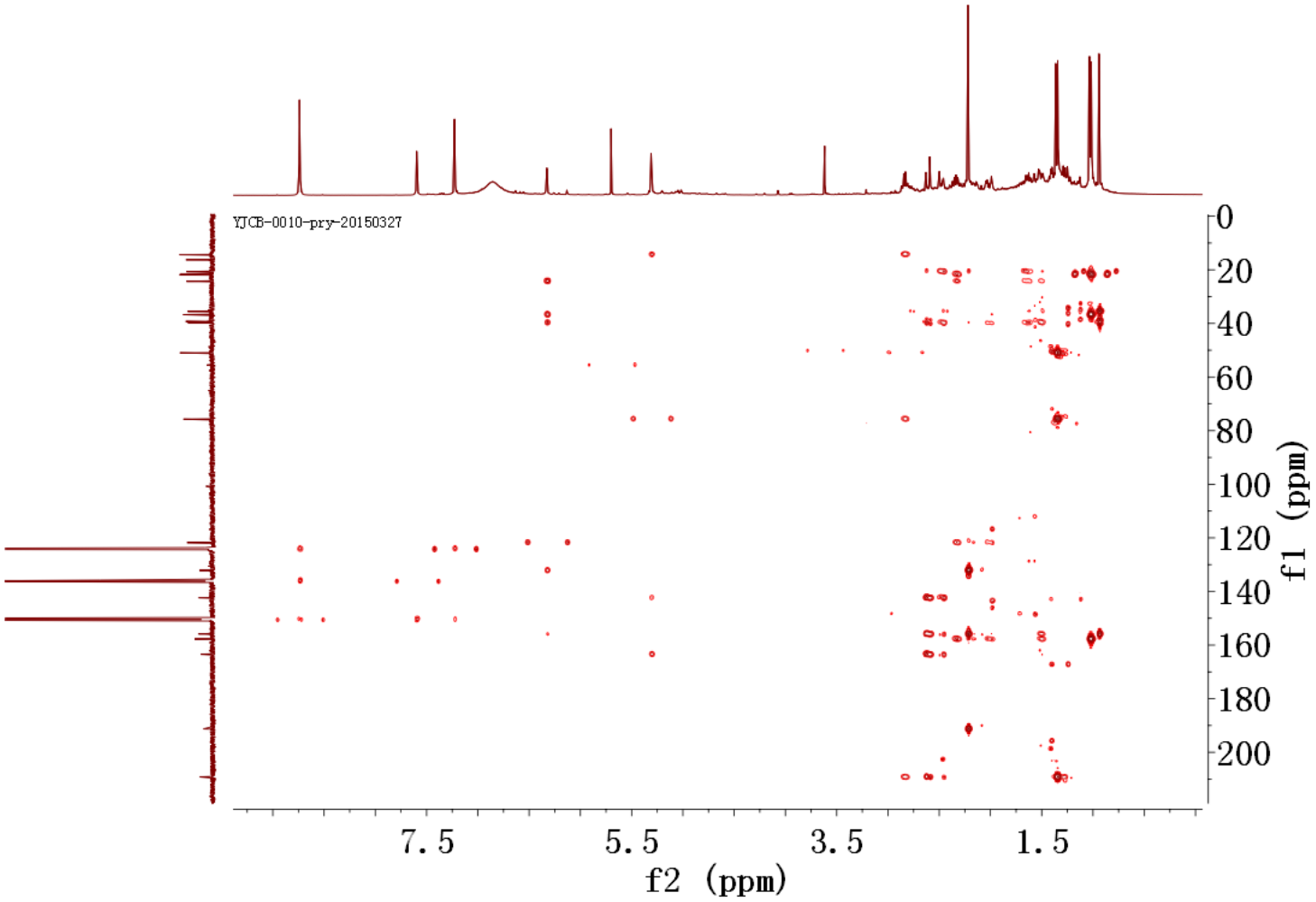
S9. ^{1}H–^{1}H COSY spectrum of 1 in Pyridine-d_5



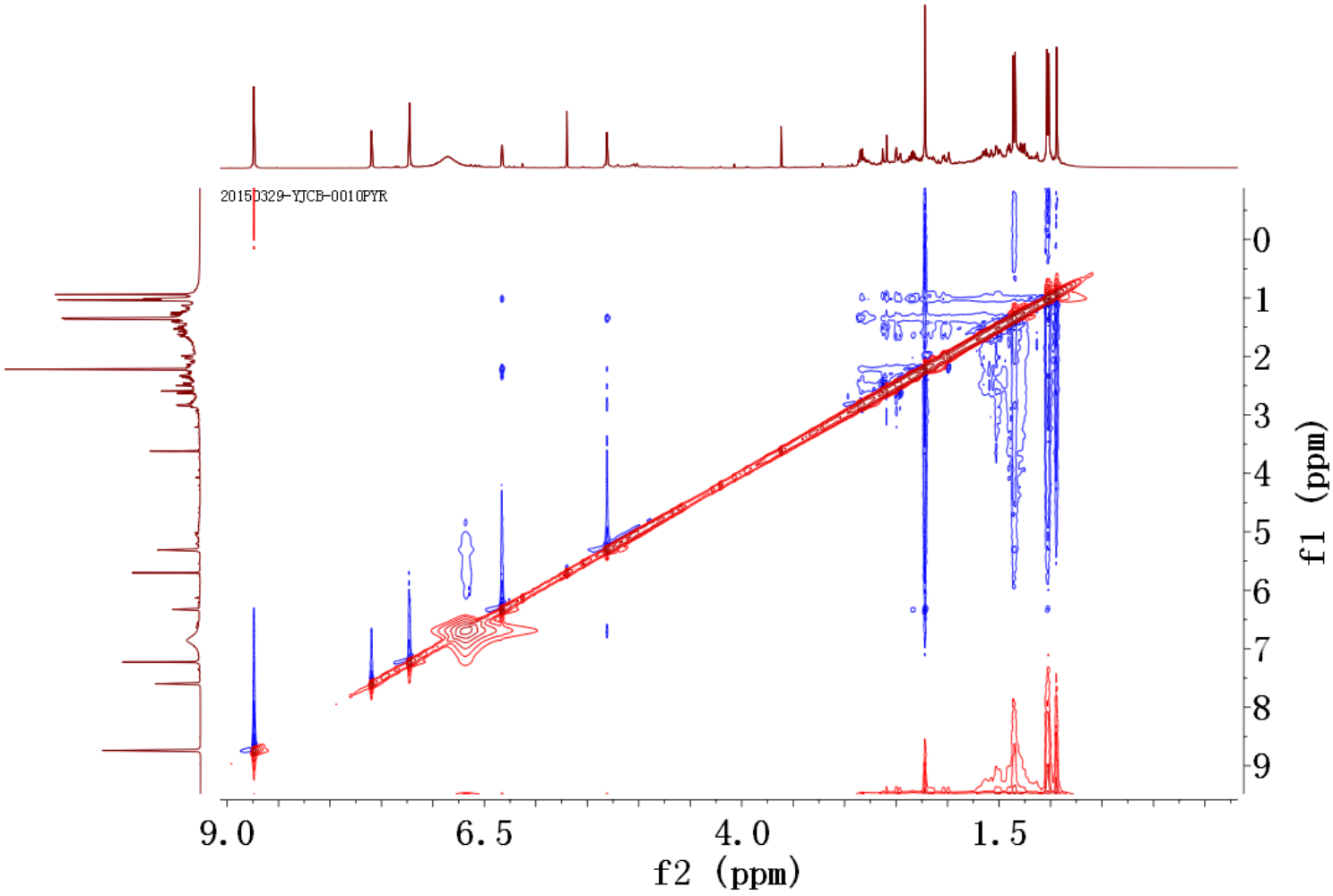
S10. HSQC spectrum of 1 in Pyridine-d_5



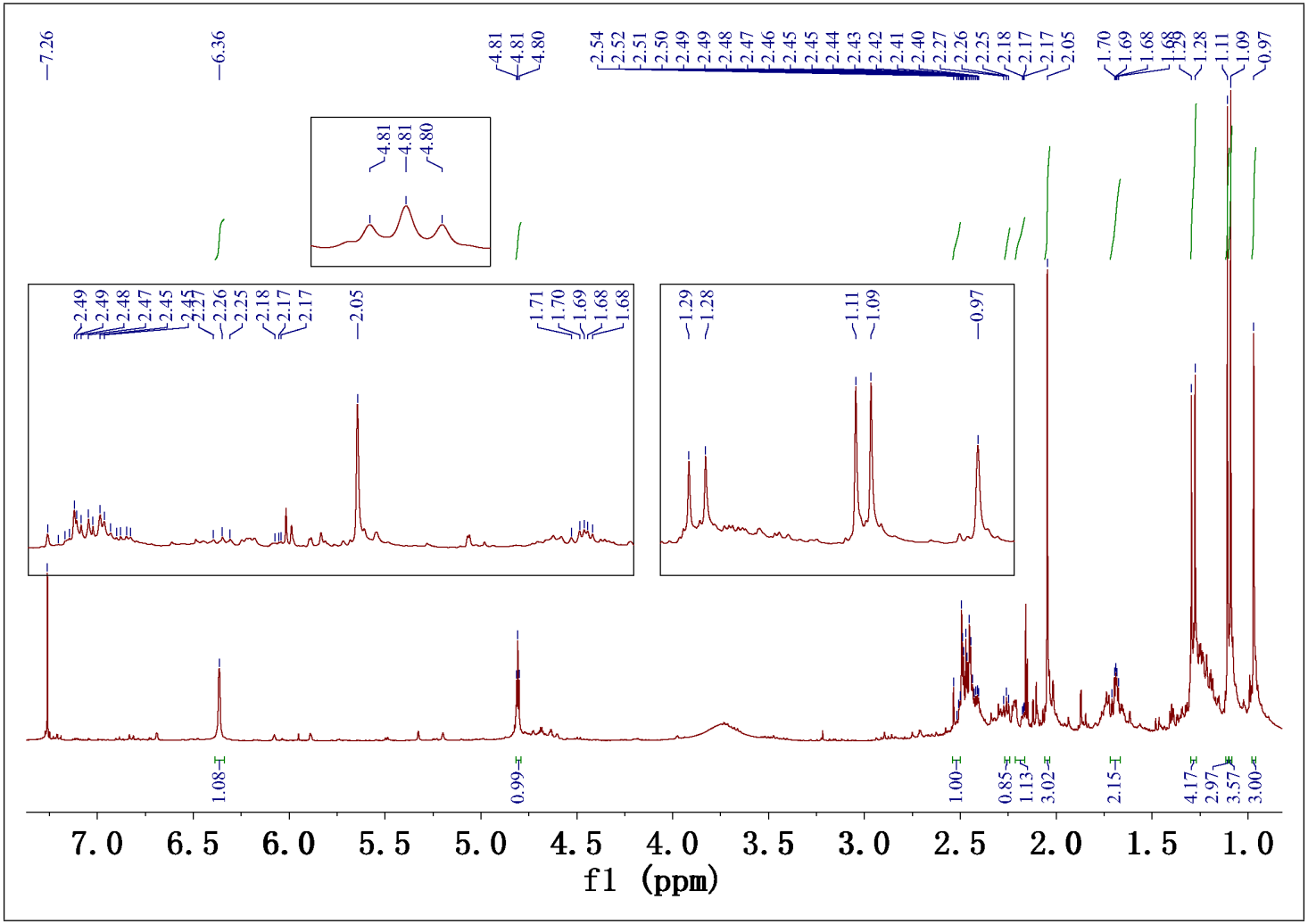
S11. HMBC spectrum of 1 in Pyridine-d_5



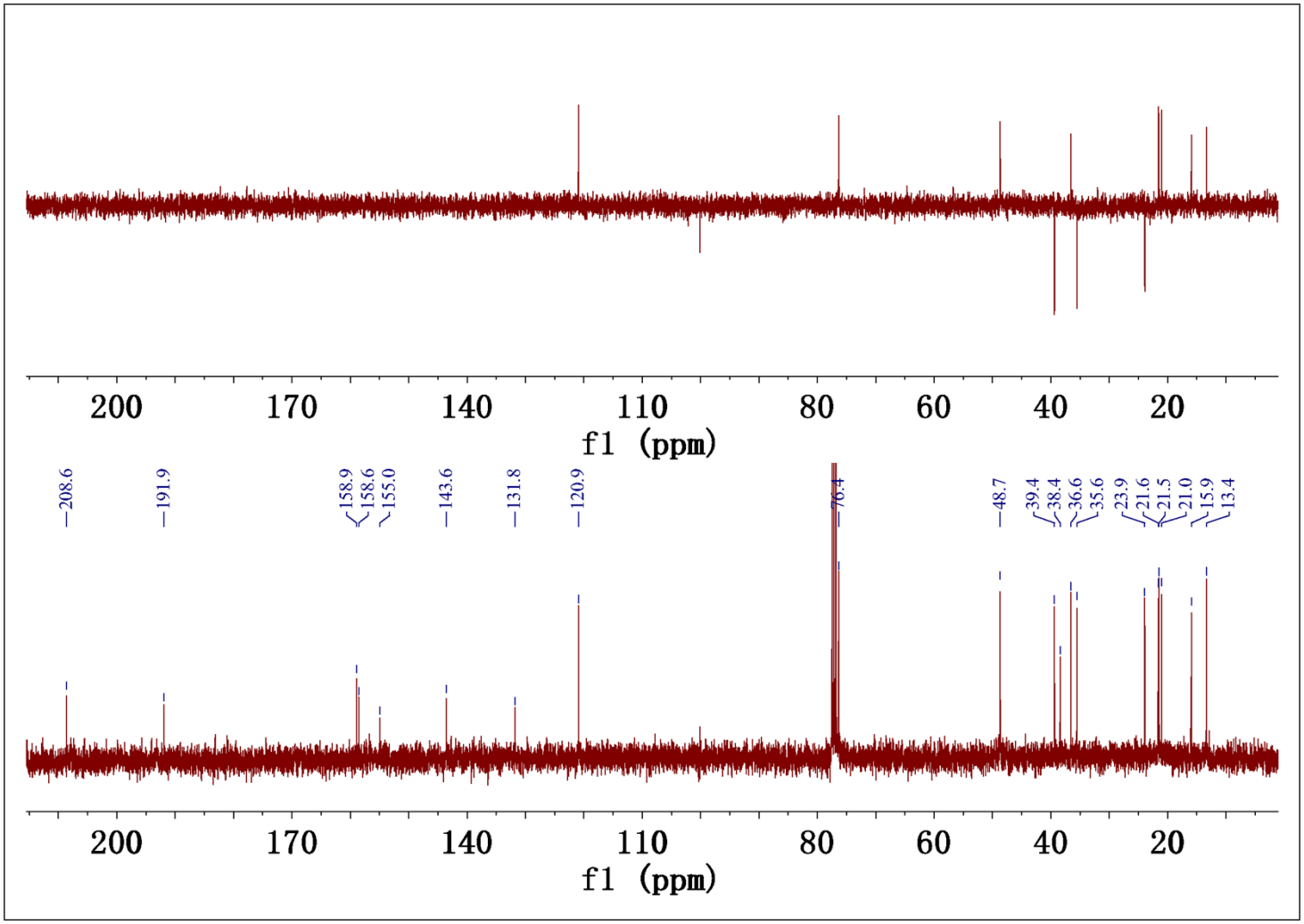
S12. NOESY spectrum of 1 in Pyridine-d_5



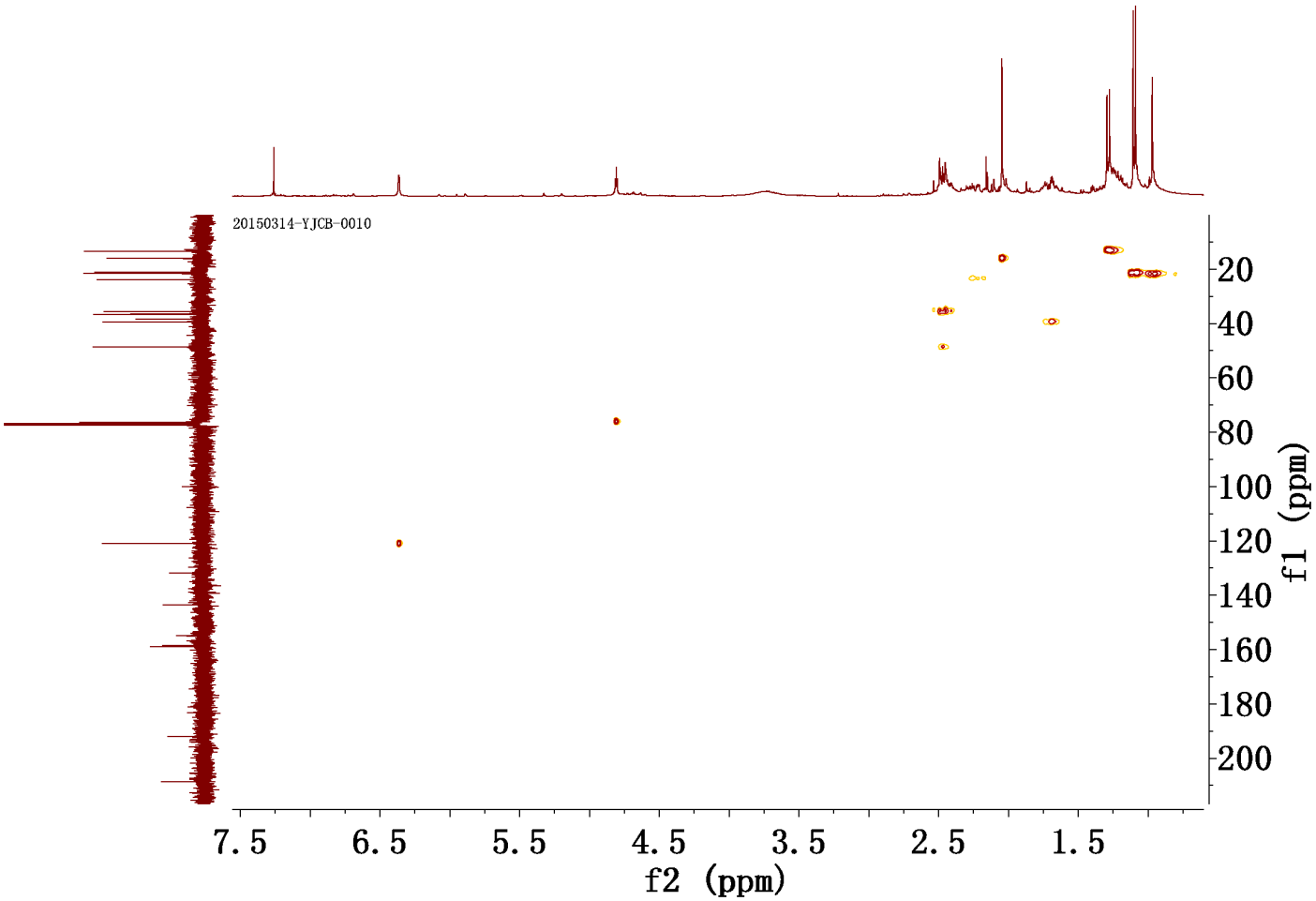
S13. ^{1}H NMR spectrum of 1 in $CDCl_3$



S14. ^{13}C NMR spectrum of 1 in $CDCl_3$



S15. HSQC spectrum of 1 in $CDCl_3$



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C	4	10	30	P	3	0	0	Zn	2	0	0					
N	3	0	0	S	2	0	0	Se	2	0	0					

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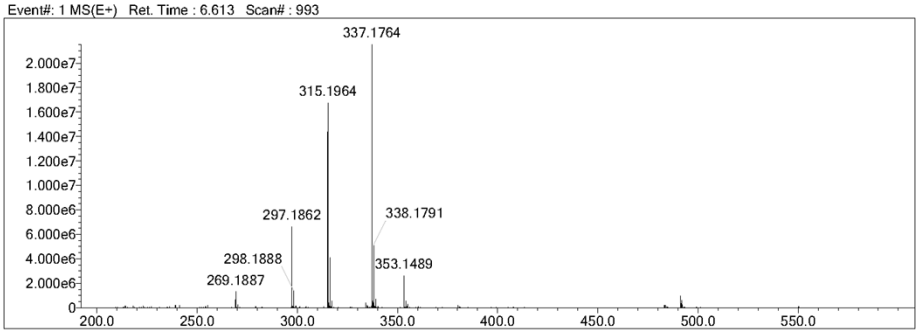
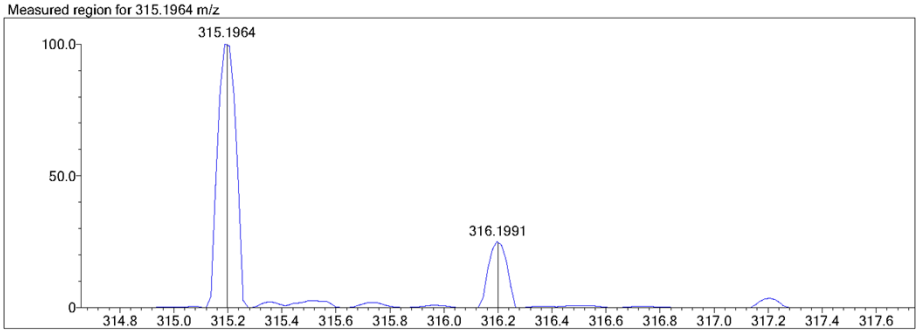
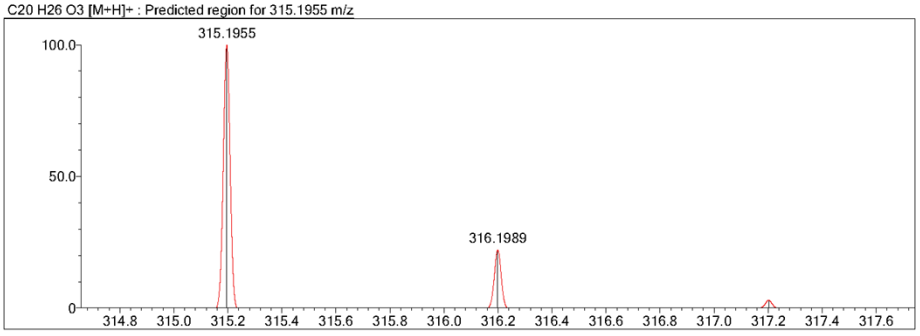


Measured region for 315.1964 m/z

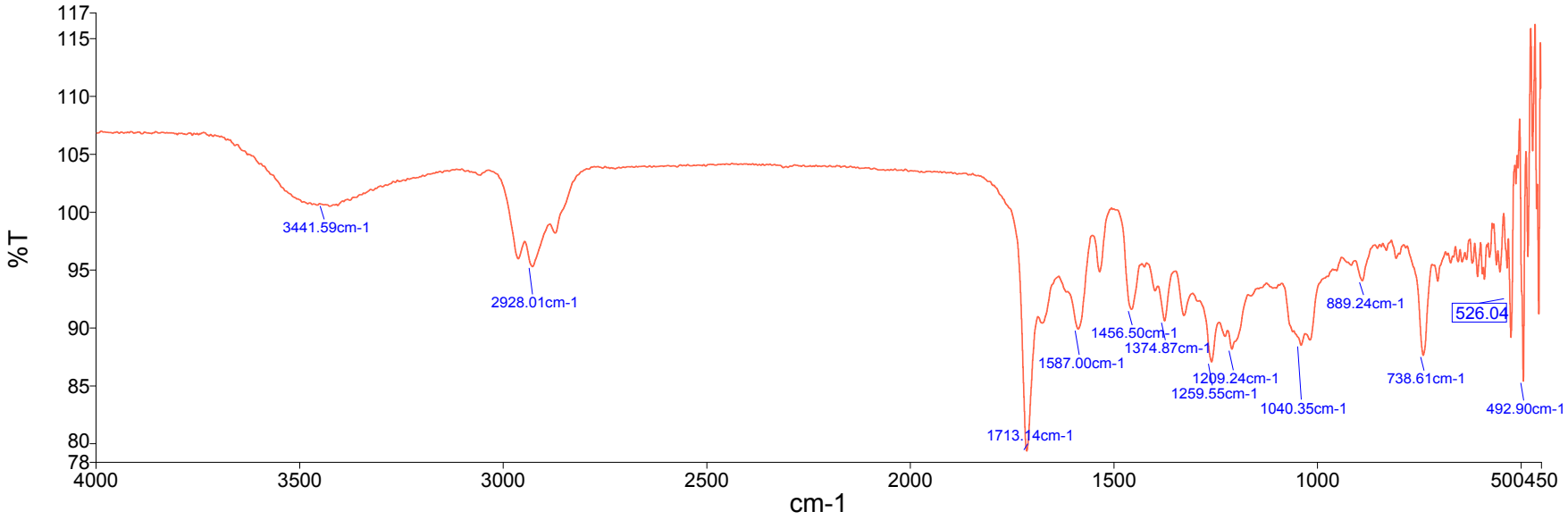


C20 H26 O3 [M+H]+ : Predicted region for 315.1955 m/z

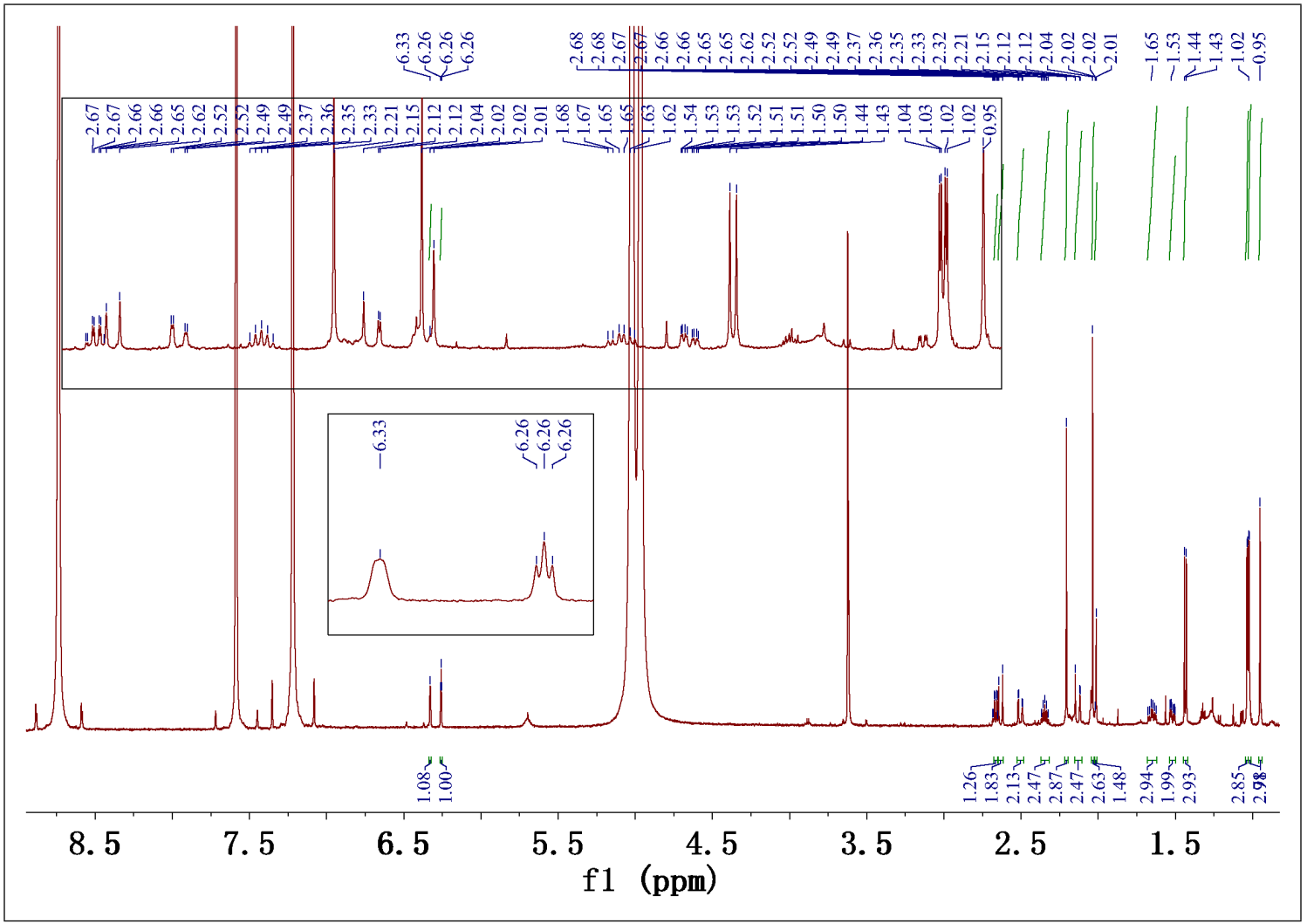


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	82.95	C20 H26 O3	[M+H]+	315.1964	315.1955	0.9	2.86	87.00	8.0

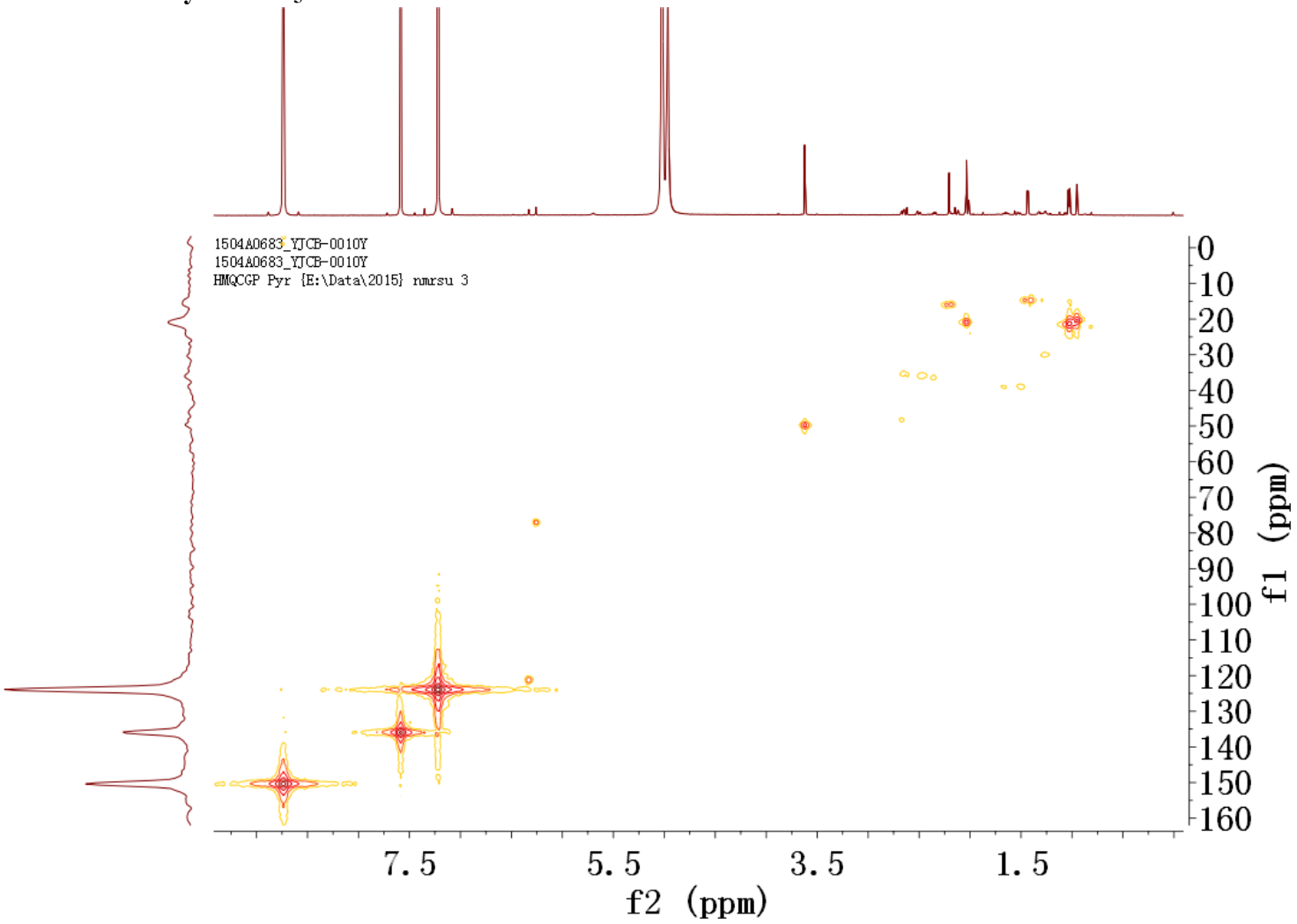
S17. IR spectrum of 1



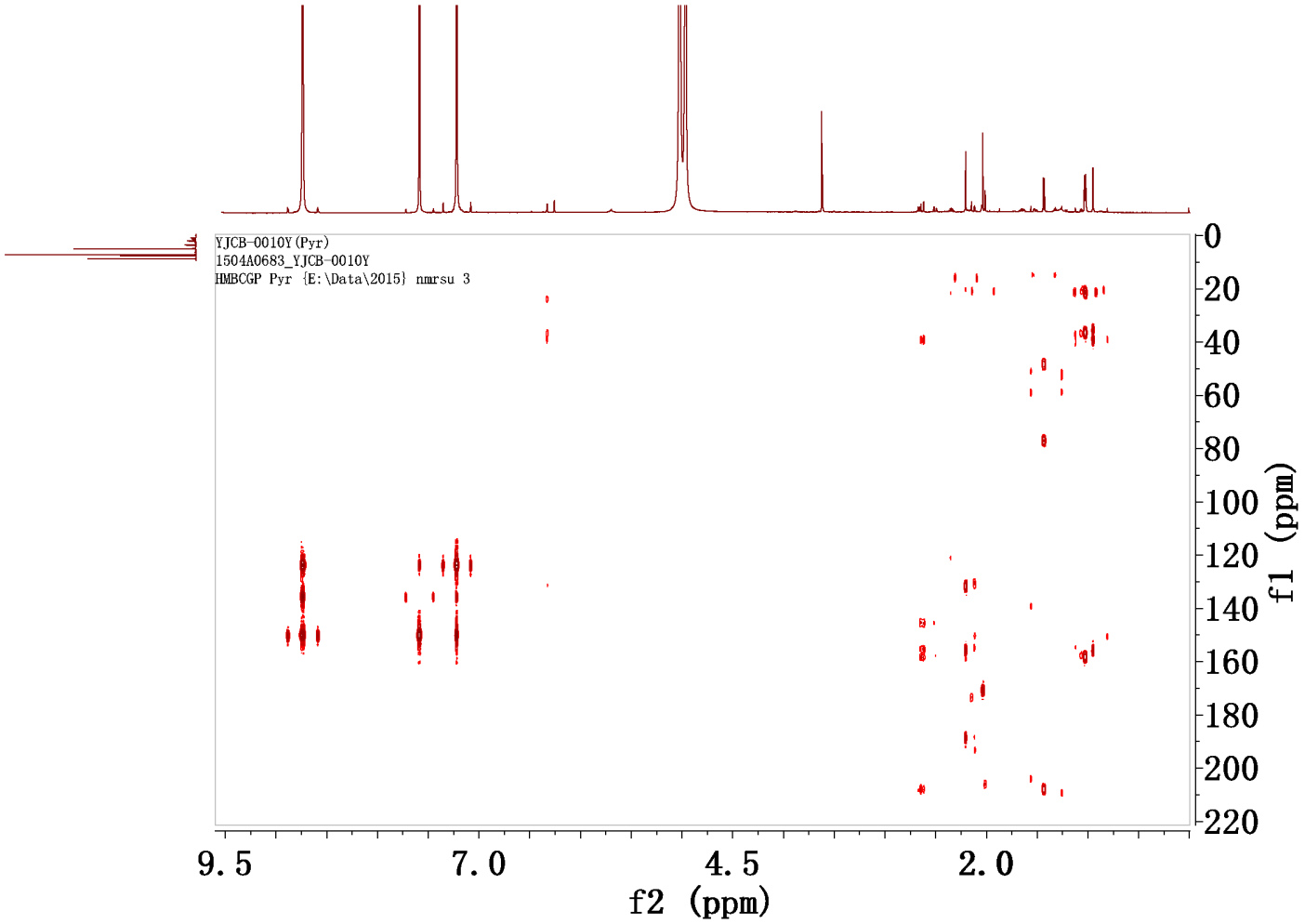
S18. ^{1}H NMR spectrum of 2 in Pyridine-d_5



S19. HSQC spectrum of 2 in Pyridine-d_5



S20. HMBC spectrum of 2 in Pyridine-d_5



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B	3	0	0	F	1	0	0	Cl	1	0	2	Br	1	0	0	
C	4	0	35	Si	4	0	0	Cu	2	0	0	I	3	0	0	
N	3	0	0	P	3	0	0	Zn	2	0	0					

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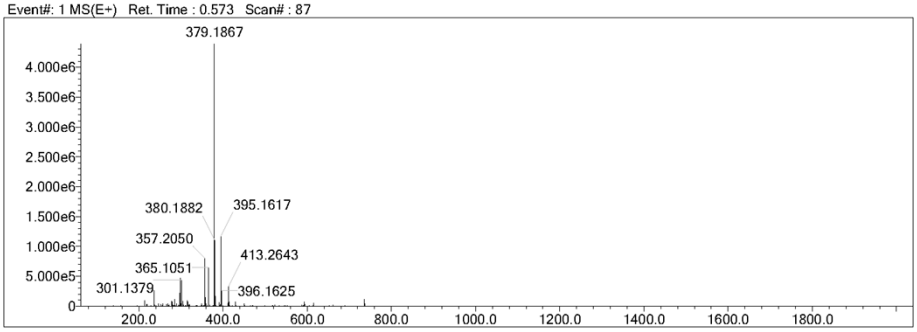

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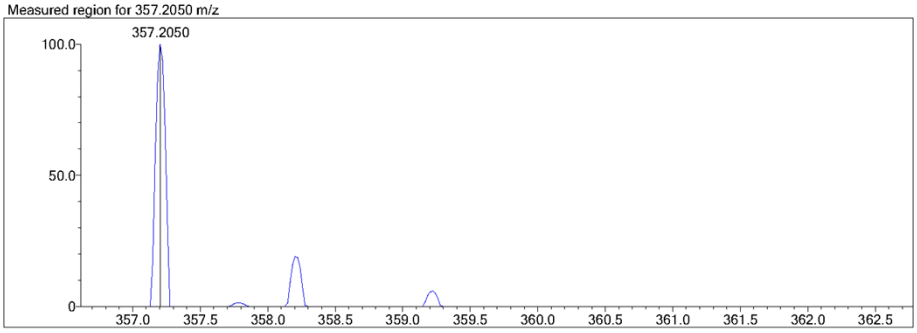
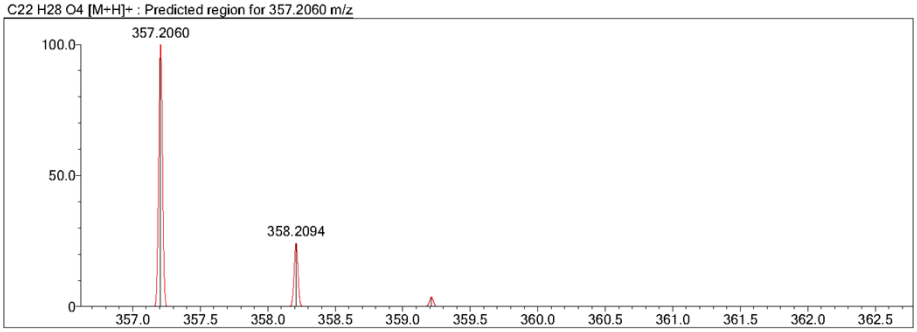
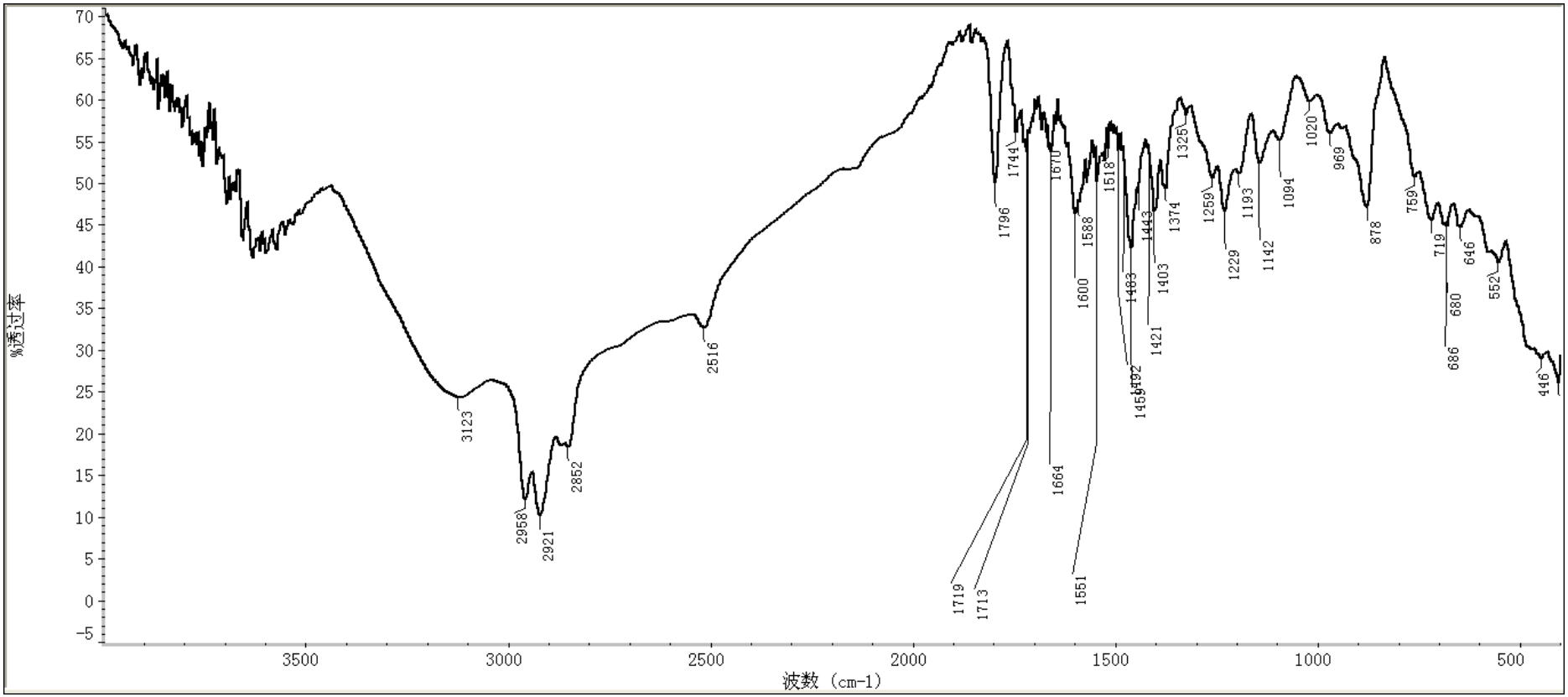


C22 H28 O4 [M+H]+ : Predicted region for 357.2060 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	63.86	C22 H28 O4	[M+H]+	357.2050	357.2060	-1.0	-2.80	66.87	9.0

S22. IR spectrum of 2



S23. The quantum chemical calculations

ECD simulation:

ECD spectrum of each conformation is simulated according to the overlapping Gaussian functions expressed as:

$$\Delta\varepsilon(E)=\frac{1}{2.296\times10^{-39}\sqrt{\pi}\sigma}\sum_{i}^{\mathrm{A}}\Delta E_i \mathrm{R}_i e^{[-(E-\Delta Ei)^2/\sigma^2]}$$

Where σ is half the bandwidth at 1/e peak height and expressed in energy units. The parameters ΔE_i and R_i are the excitation energies and rotational strengths for the transition *i*, respectively.

The above function is converted to Δε, λ (wavelength) correlations as:

$$\Delta\varepsilon(\lambda)=\frac{1}{2.296\times10^{-39}\sqrt{\pi}\sigma}\sum_{i}^{\mathrm{A}}\Delta E_i \mathrm{R}_i e^{[-(1240/\lambda-\Delta Ei)^2/\sigma^2]}$$

and then simulation were accomplished by using the Excel 2003 and the Origin 7.0 software.

To get the final spectra, all the simulated spectra of conformations of each compound were averaged according to their energy and the Boltzmann distribution theory expressed as:

$$\frac{N_i^*}{N}=\frac{g_i e^{-\varepsilon_i/k_B T}}{\sum g_i e^{-\varepsilon_i/k_B T}}$$

Table S2. Energy analysis

conf.	Gibbs free energy (298.15 K)		
	G (Hartree)	ΔG (Kcal/mol)	Boltzmann Distribution
aC1	-1003.379779	0	0.760
aC2	-1003.378691	0.68273088	0.240
bC1	-1003.38314	0	0.759
bC2	-1003.382056	0.68022084	0.241

Table S3. Specific Optical Rotation

Conf.	Calc.	averaged	experimental
aC1	217.02	197.6	269
aC2	136.08		
bC1	421.80	412.6	
bC2	383.71		

Table S4. ECD Data

State	**aC1**		**aC2**	
	Excitation energies(eV)	Rotatory Strengths*	Excitation energies(eV)	Rotatory Strengths*
1	2.7879	13.3854	2.7848	12.5387
2	3.0326	3.9141	3.0344	4.0341
3	3.5135	-2.821	3.5163	-2.8246
4	4.0735	-1.3772	4.0768	-1.169
5	4.2311	13.5008	4.2307	13.9938

* R(velocity) 10**-40 erg-esu-cm

State	**bC1**		**bC2**	
	Excitation energies(eV)	Rotatory Strengths*	Excitation energies(eV)	Rotatory Strengths*
1	2.9324	21.3181	2.9253	26.2332
2	3.03	-16.1694	3.0303	-15.5859
3	3.4931	3.0784	3.4926	3.6438
4	4.0215	-4.1507	4.0238	-4.1525

* R(velocity) 10**-40 erg-esu-cm

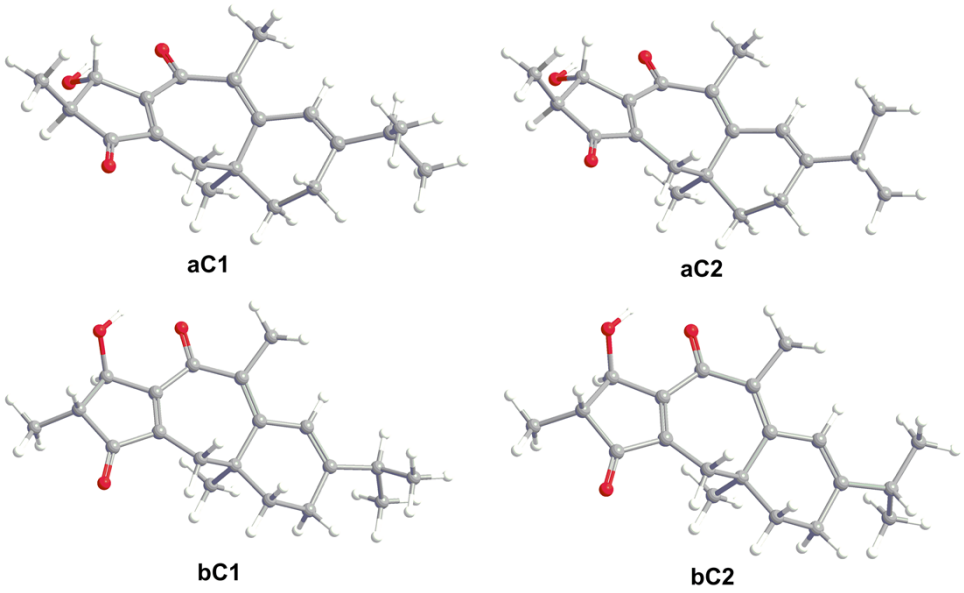


Fig. S2 B3LYP/6-311++G(2d,2p) optimized lowest energy 3D conformers of **1a** and **1b**.

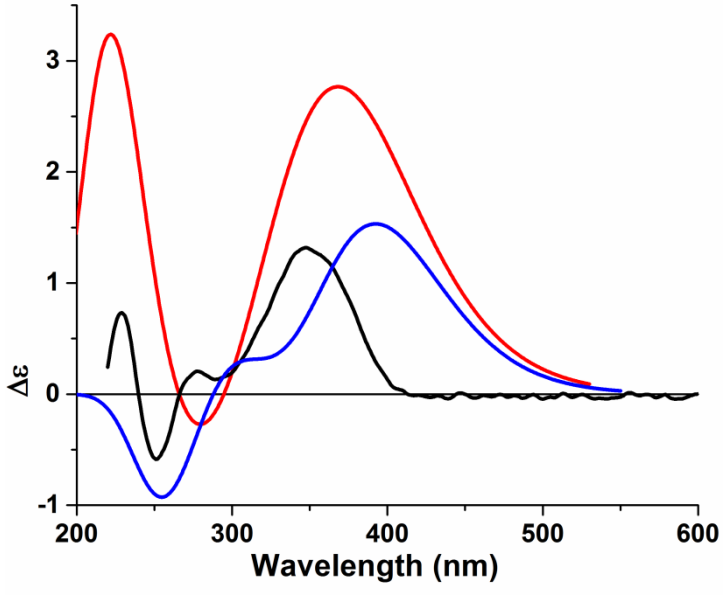


Fig. S3 Experimental (black line) and B3LYP-SCRF(PCM, acetonitrile)/aug-cc-pVDZ //B3LYP/6-311++G(2d,2p) calculated (σ = 0.4 eV) ECD spectra of **1a** (red line) and **1b** (blue line).