Phenanthrenequinone enantiomers with cytotoxic activities from the tubers of *Pleione bulbocodioides*

Si-Yuan Shao, Chao Wang, Shao-Wei Han, Mo-Han Sun, Shuai Li*

Affiliation

State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Correspondence

Prof. Dr. Shuai Li, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 2 Nan Wei Road, Xicheng District, Beijing 100050, China.

E-mail: lishuai@imm.ac.cn

Phone: + 86 10 63164628

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Computational methods

Conformational analysis

Conformational analysis was initially performed using Confab [1] with systematic search at MMFF94 force field for the configurations of compounds **1** and **4** (**Figure S**). Room-temperature equilibrium populations were calculated according to Boltzmann distribution law (1). Conformers with population over 1% were chosen for subsequent Quantum Mechanics (QM) calculations. The energies and populations of dominative conformers were provided in **Table S1**.

$$\frac{N_i}{N} = \frac{g_i e^{-\frac{E_i}{k_B T}}}{\sum g_i e^{-\frac{E_i}{k_B T}}} \qquad \textbf{(1)}$$

where N_i is the number of conformer i with energy E_i and degeneracy g_i at temperature *T*, and k_B is Boltzmann constant.

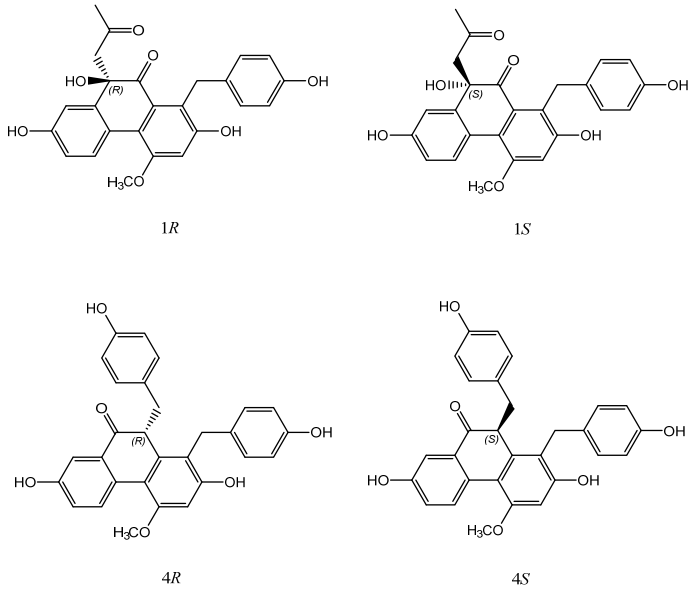


Figure S1. Chemical structure of configurations of compounds **1** and **4**.

ECD calculation

The theoretical calculations were carried out using Gaussian 09 [2]. At first, conformers were optimized at PM6 using semi-empirical theory method. The conformers with Boltzmann-population over 1% were chosen for further optimization at B3LYP/6-311G(d,p) in methanol using the

IEFPCM model. Vibrational frequency analysis confirmed the stable structures. Under the same condition, the ECD calculation was conducted using Time-dependent Density functional theory (TD-DFT). Rotatory strengths for a total of 30 excited states were calculated. The ECD spectrum was simulated in SpecDis [3] by overlapping Gaussian functions for each transition according to (2).

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

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.

Parameters of σ and UV-shift for compounds **1** and **4** were list as follows.

Configuration	σ (eV)	UV-shift (nm)
1R	0.37	5
1S	0.37	5
4R	0.40	-6
4S	0.40	-6

References

[1] N. M. OBoyle, T. V. ermeersch, C. J. Flynn, A. R. Maguire, and G. R. Hutchison, *J. Cheminformatics*, 2011, **3**, 3.

[2]M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian 09 Revision D.01. Gaussian Inc. Wallingford CT 2009.

[3] T. Bruhn, A. Schaumlffl, Y. Hemberger, and G. Bringmann, *Chirality*, 2013, **25**, 243.

Energies and Coordinates

Energies at MMFF94 force field

Systematic conformational search was performed by Confab program at MMFF94 force field. Conformers for each configuration were obtained with filtration by RMSD threshold of 0.5 Å.

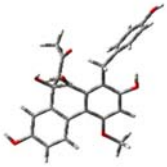
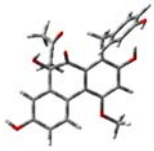
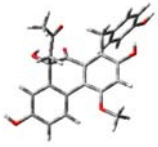
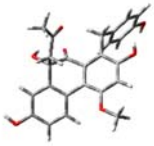
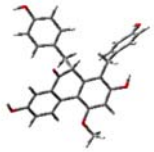
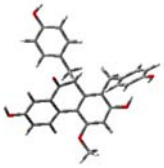
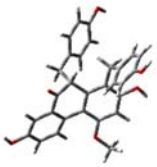
Table S1 Energies of compounds **1** and **4** at MMFF94 force field.

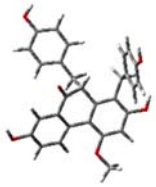
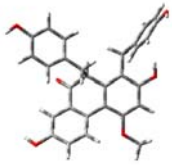
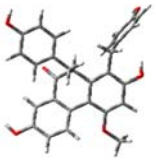
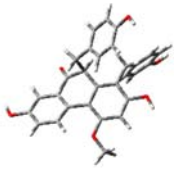
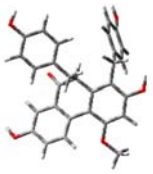
Configuration	Conformer	Energy (kcal/mol)	Population (%)
1R	1	75.75	40.90
1R	2	75.92	30.84
1R	3	76.25	17.47
1R	4	76.61	9.53
1S	1	76.96	69.20
1S	2	77.85	15.37
1S	3	77.98	12.24
1S	4	79.42	1.08
1S	5	79.44	1.04
4R	1	87.32	49.33
4R	2	88.02	15.22
4R	3	88.11	13.13
4R	4	88.45	7.32
4R	5	88.57	5.98
4R	6	89.02	2.81
4R	7	89.15	2.26
4R	8	89.41	1.46
4S	1	87.32	49.33
4S	2	88.02	15.22
4S	3	88.11	13.13
4S	4	88.45	7.32
4S	5	88.57	5.98
4S	6	89.02	2.81
4S	7	89.15	2.26
4S	8	89.41	1.46

Energies at B3LYP theory level

Structures for ECD calculations were optimized at B3LYP/6-311G(d,p) in methanol.

Table S2 Energies of compounds **1** and **4** at B3LYP/6-311G(d,p) in methanol.

Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
1R	1		-1492.87879709	-936795.58	18.34
1R	3		-1492.88020715	-936796.47	81.66
1S	1		-1492.88024126	-936796.49	50.90
1S	2		-1492.88020715	-936796.47	49.10
4R	1		-1571.30245880	-986007.17	23.57
4R	2		-1571.30320258	-986007.64	51.81
4R	3		-1571.30170837	-986006.70	10.64

4R	5		-1571.30196594	-986006.86	13.98
4S	1		-1571.30245880	-986007.17	23.57
4S	2		-1571.30320258	-986007.64	51.81
4S	3		-1571.30170837	-986006.70	10.64
4S	5		-1571.30196594	-986006.86	13.98

Racemic mixtures (1–4) separated by chiral HPLC

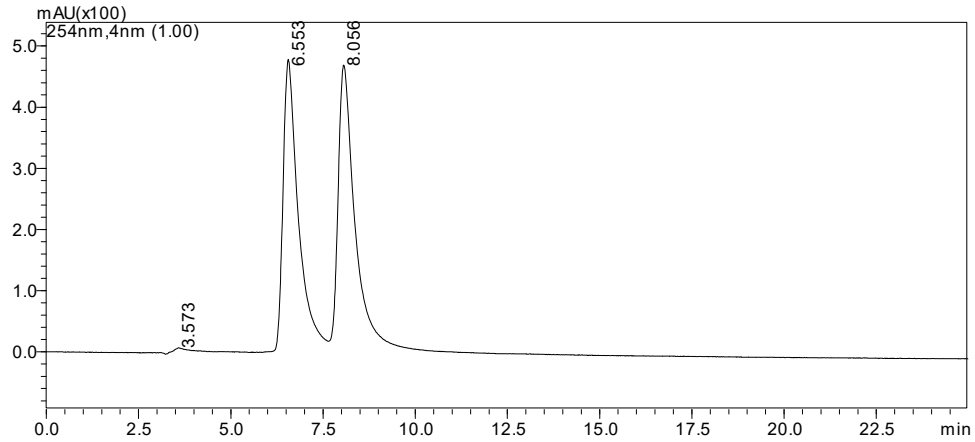


Figure S2. Chiral HPLC analysis of compounds **1a** and **1b** using *n*-hexane–isopropanol (70:30).

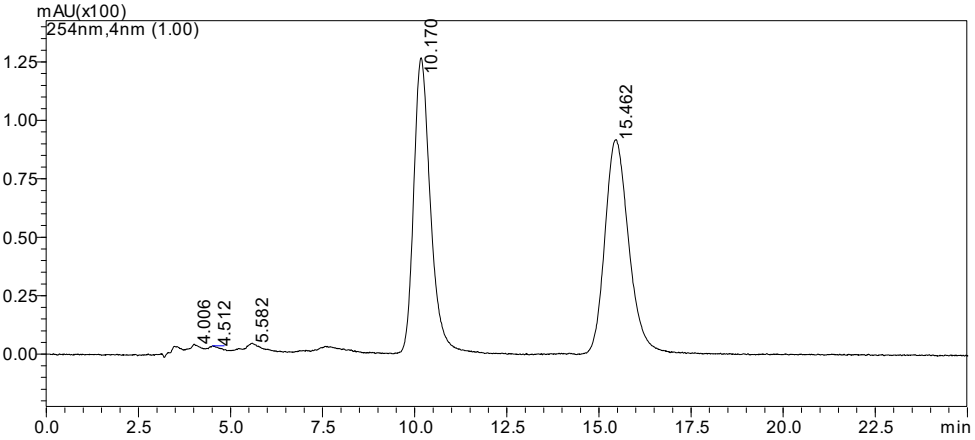


Figure S3. Chiral HPLC analysis of compounds **2a** and **2b** using *n*-hexane–isopropanol (60:40).

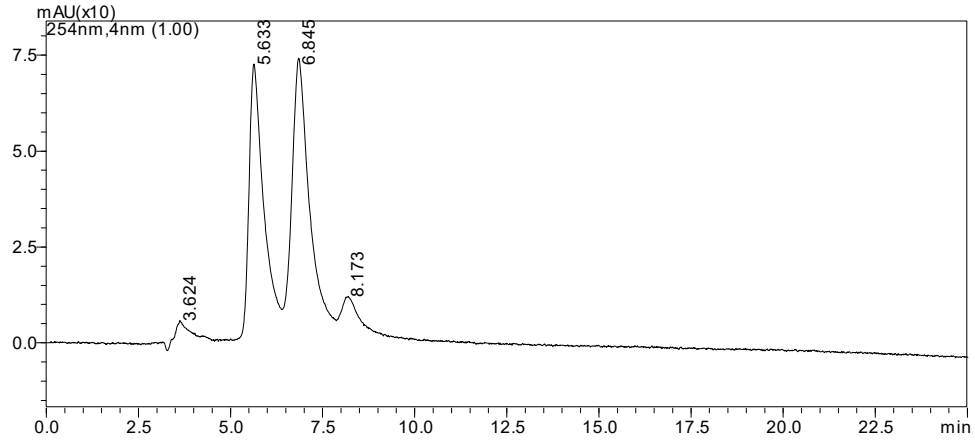


Figure S4. Chiral HPLC analysis of compounds **3a** and **3b** using *n*-hexane–isopropanol (70:30).

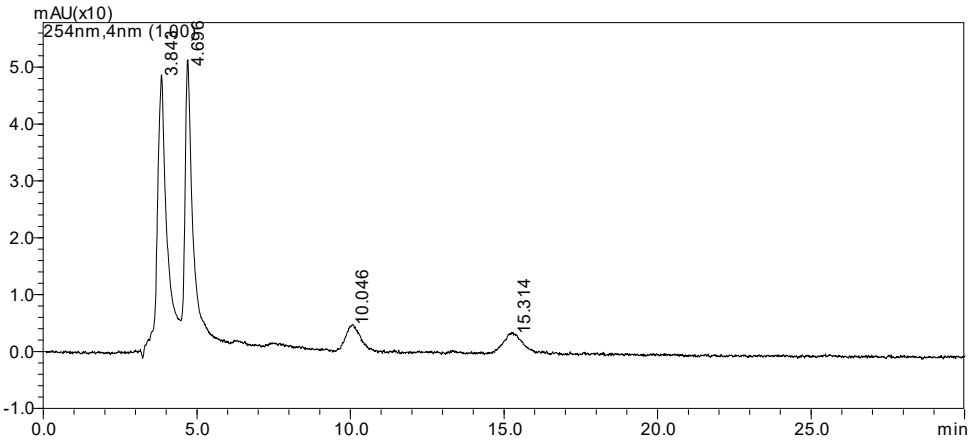


Figure S5. Chiral HPLC analysis of compounds **4a** and **4b** using *n*-hexane–isopropanol (60:40).

NMR spectra of compounds 1–4.

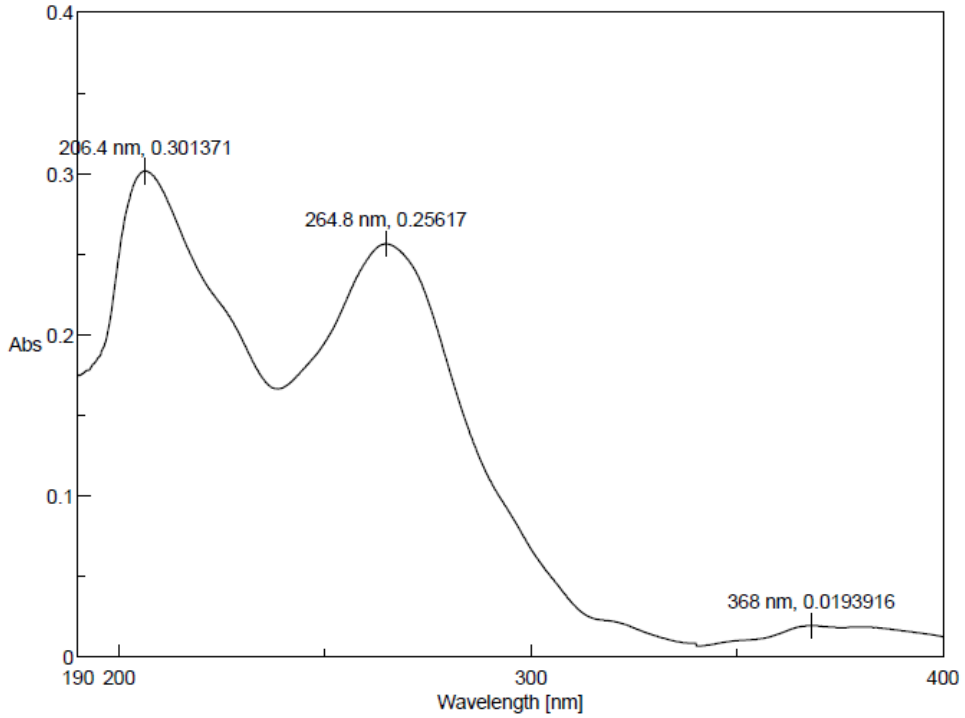


Figure S6. The UV spectrum of compound **1**.

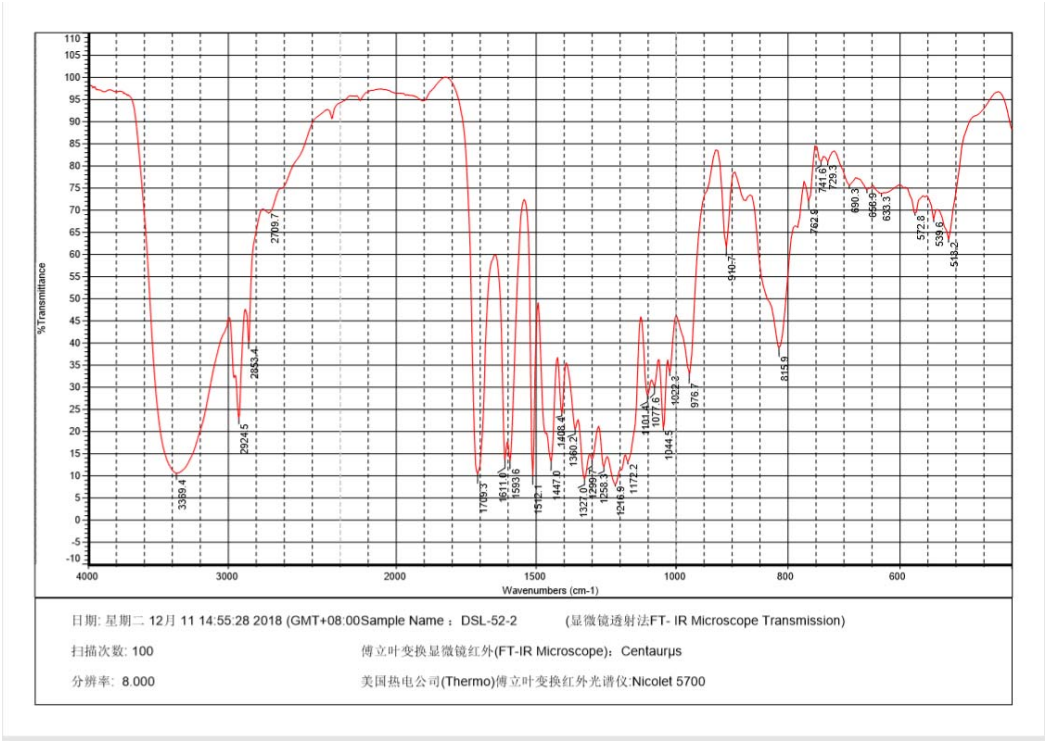


Figure S7. The IR spectrum of compound **1**.

MS Formula Results: - Scan (5.867 min) Sub (2014051403.d)

m/z	Ion	Formula	Abundance
433.1296	(M-H)-	C25 H21 O7	1627958.8

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross S	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Mat	Mass Match	m/z	DBE
☐	C26 H18 N4 O3	C26 H17 N4 O3	433.1306	98.66		434.137	434.1379	2.07	2.07	95.9	99.58	99.86	433.1296	20
☑	C25 H22 O7	C25 H21 O7	433.1293	97.96		434.137	434.1366	-0.98	0.98	93.16	99.73	99.97	433.1296	15

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

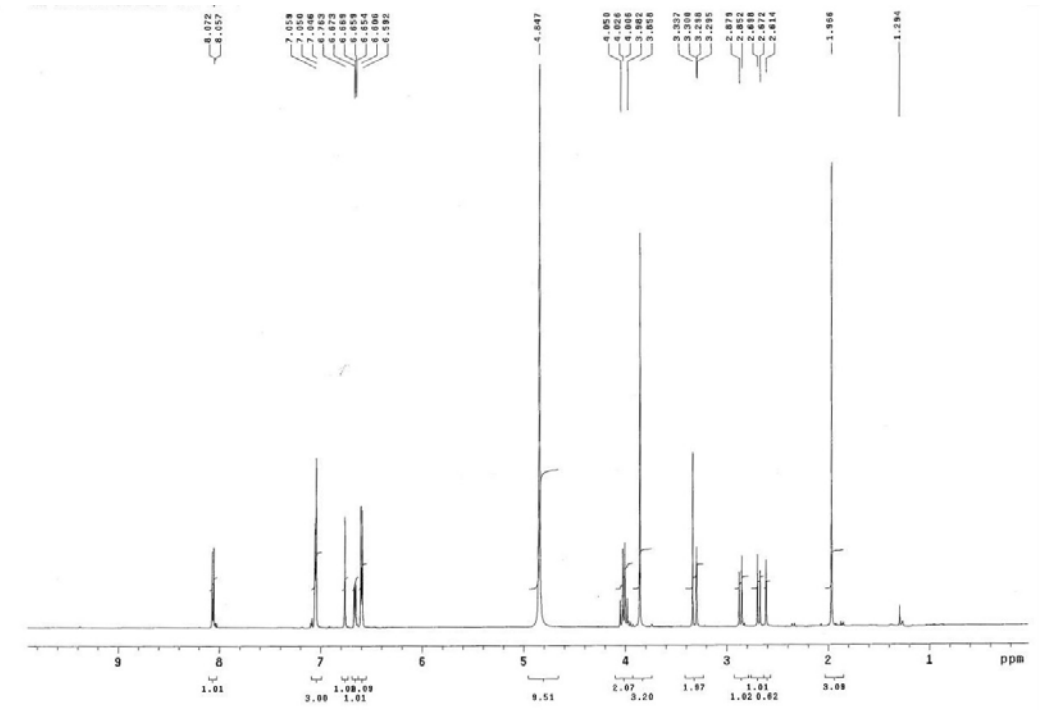


Figure S9. The ^{1}H NMR spectrum of compound **1** methanol-d_4 (600 MHz).

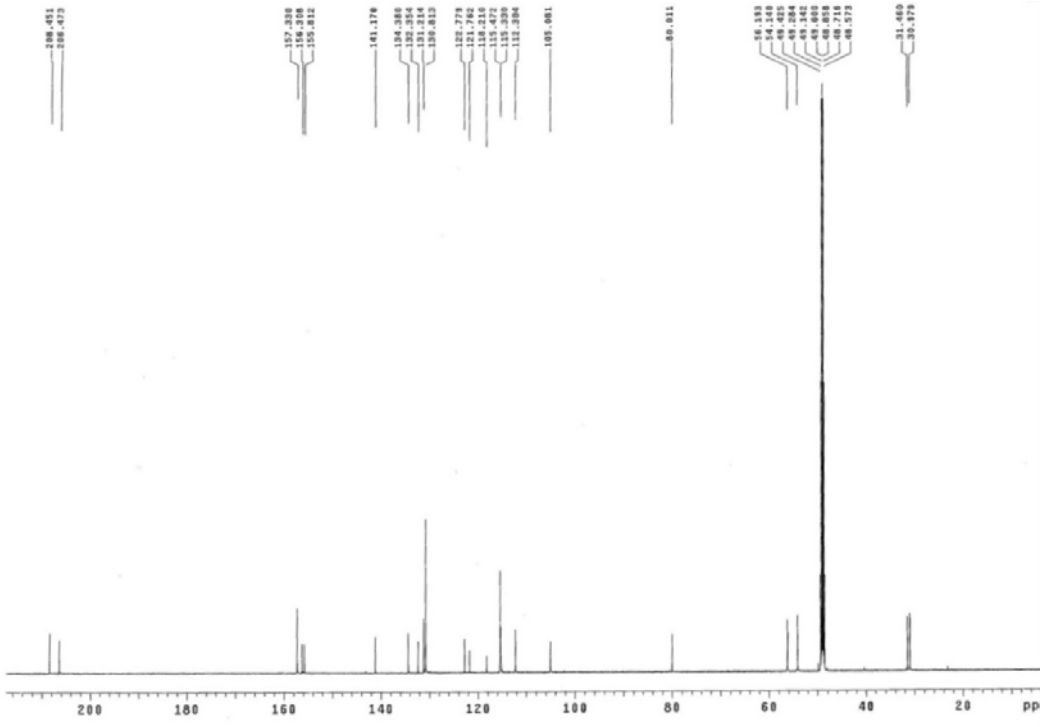


Figure S10. The ^{13}C NMR spectrum of compound **1** methanol-d_4 (150 MHz).

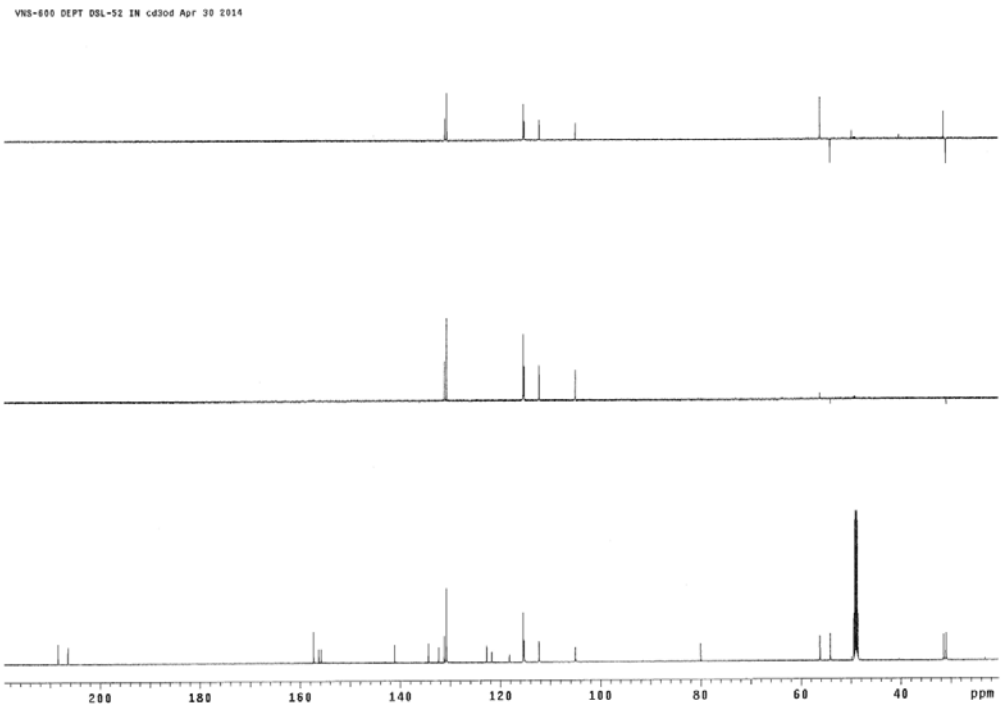


Figure S11. The DEPT spectrum of compound **1** methanol-d_4 (150 MHz).

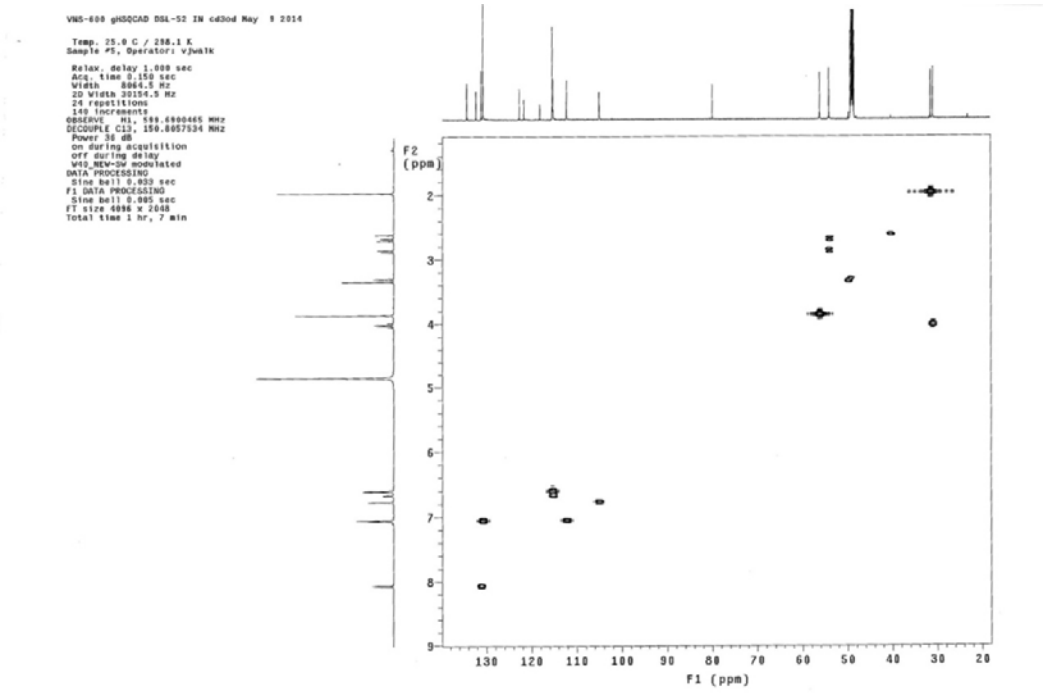


Figure S12. The HSQC spectrum of compound **1** methanol-d_4 (600 MHz).

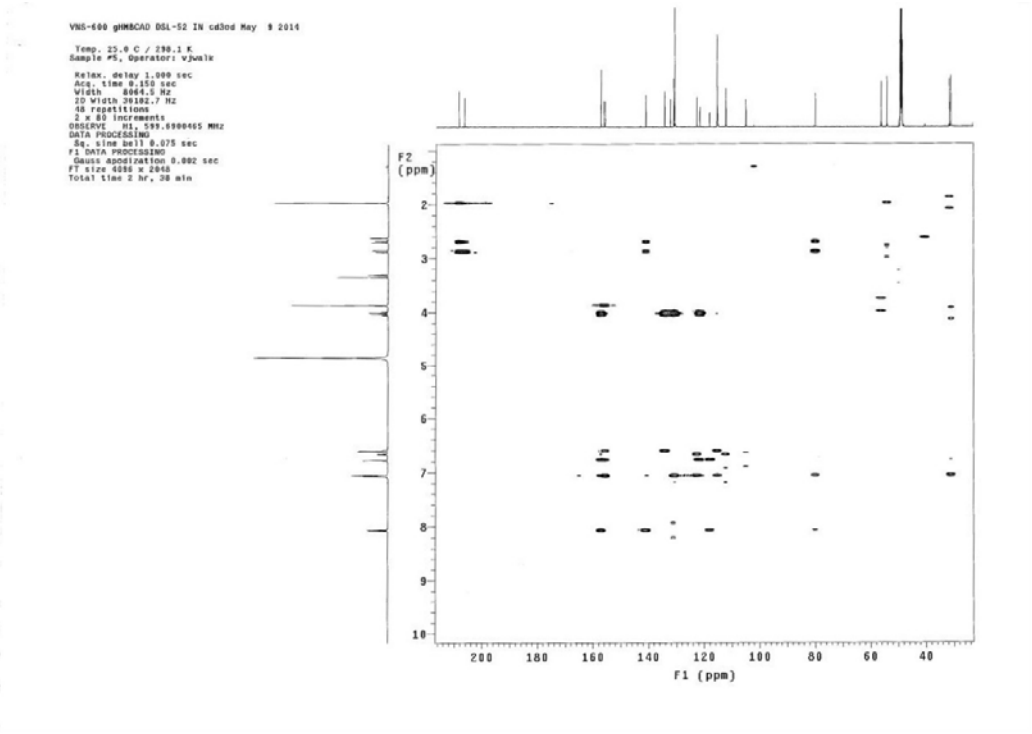


Figure S13. The HMBC spectrum of compound **1** methanol-d_4 (600 MHz).

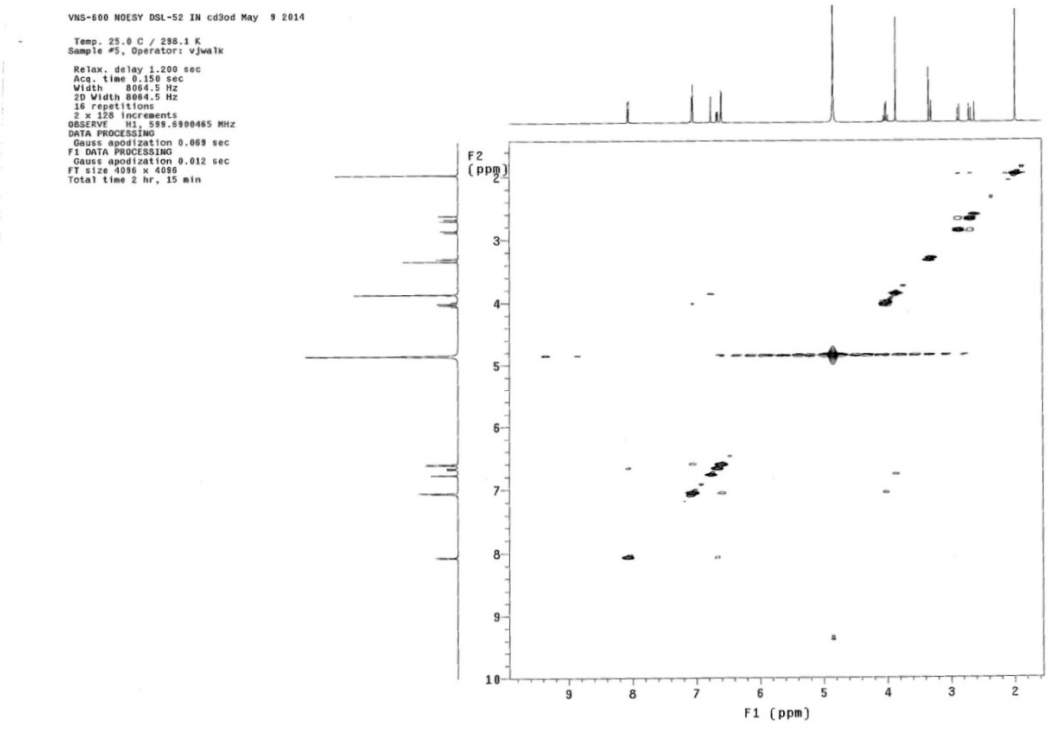


Figure S14. The NOESY spectrum of compound **1** methanol-d_4 (600 MHz).

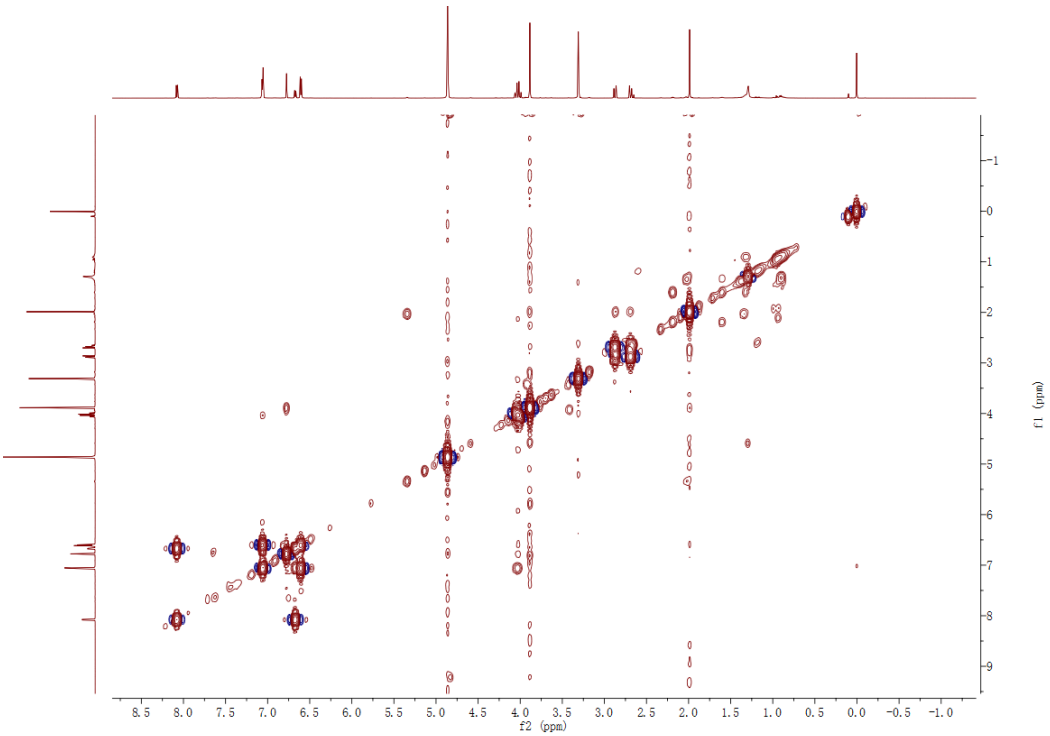


Figure S15. The ^{1}H-^{1}H COSY spectrum of compound **1** methanol-d_4 (600 MHz).

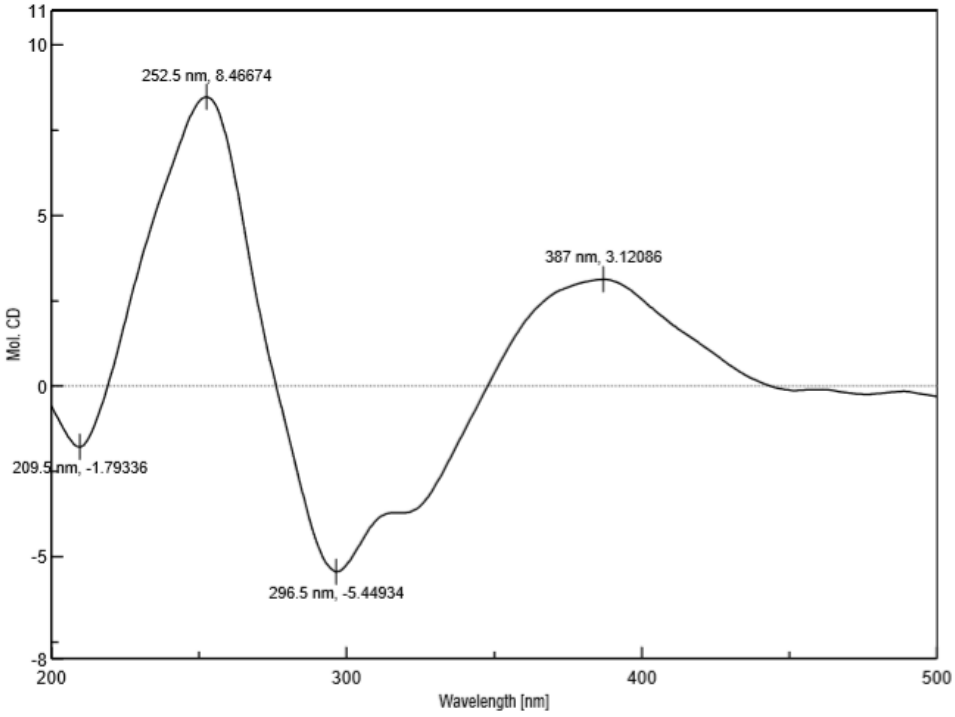


Figure S16. The ECD spectrum of compound **1a**.

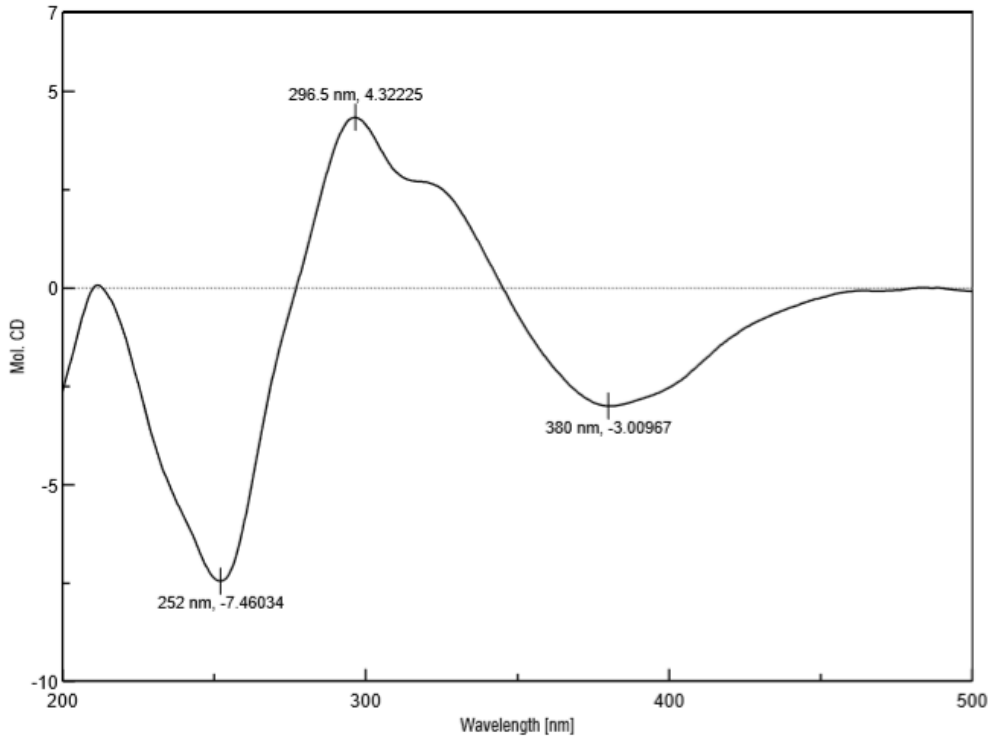


Figure S17. The ECD spectrum of compound **1b**.

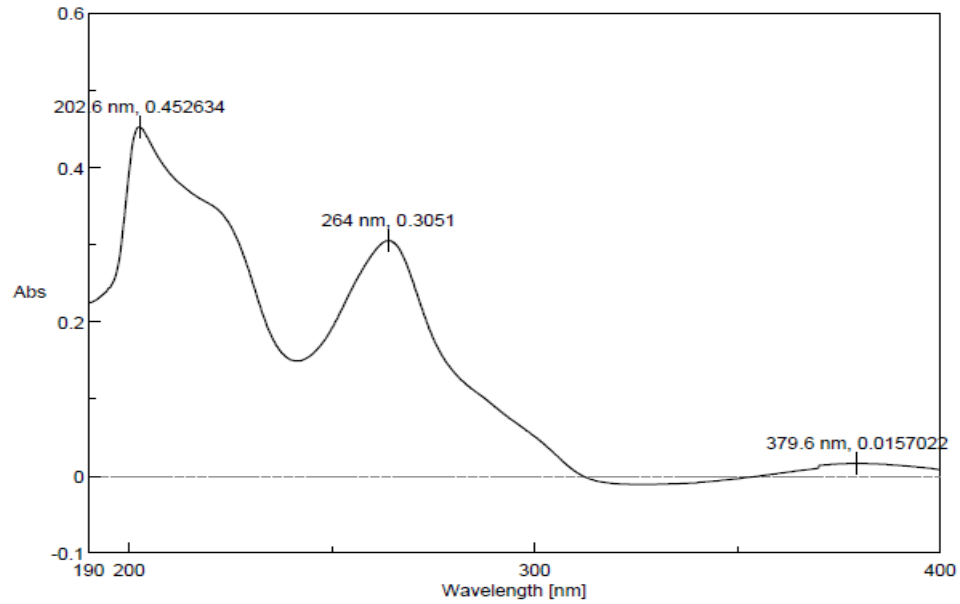


Figure S18. The UV spectrum of compound **2**.

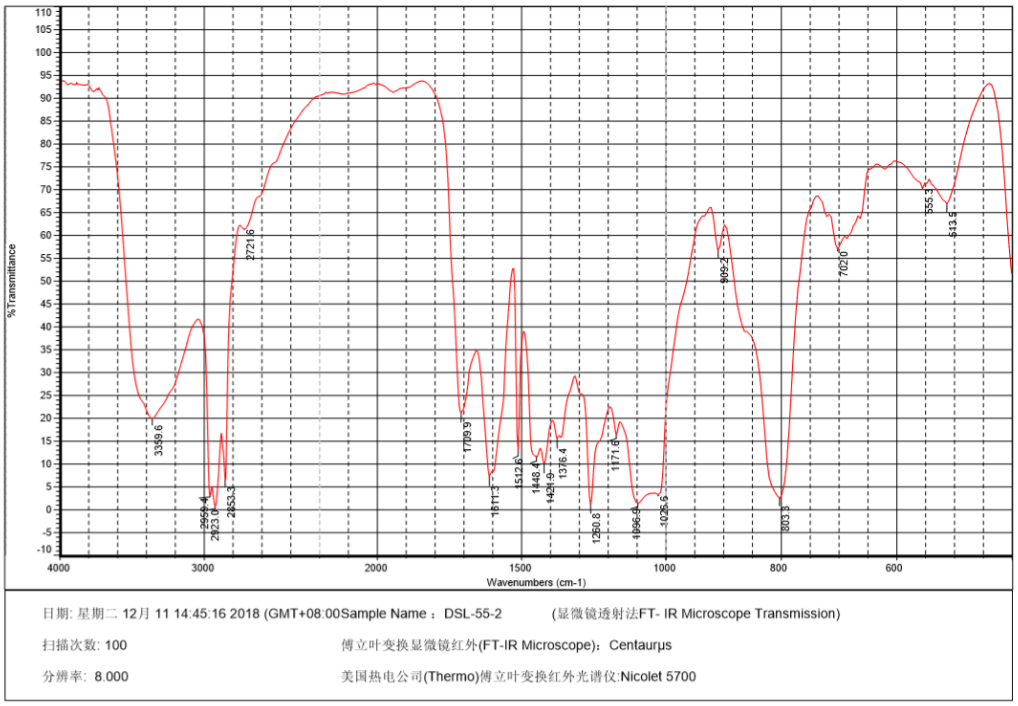


Figure S19 The IR spectrum of compound **2**.

MS Formula Results: - Scan (6.598 min) Sub (2014051601.d)

m/z	Ion	Formula	Abundance
539.1718	(M-H)-	C32 H27 O8	2481207

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross S	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Mat	Mass Match	m/z	DBE
	C33 H24 N4 O4	C33 H23 N4 O4	539.1725	99.94		540.1791	540.1798	1.19	1.19	99.9	99.95	99.98	539.1718	24
✓	C32 H28 O8	C32 H27 O8	539.1711	99.87		540.1791	540.1784	-1.26	1.26	99.64	99.97	99.94	539.1718	19

Figure S20. The HRESIMS of compound **2**.

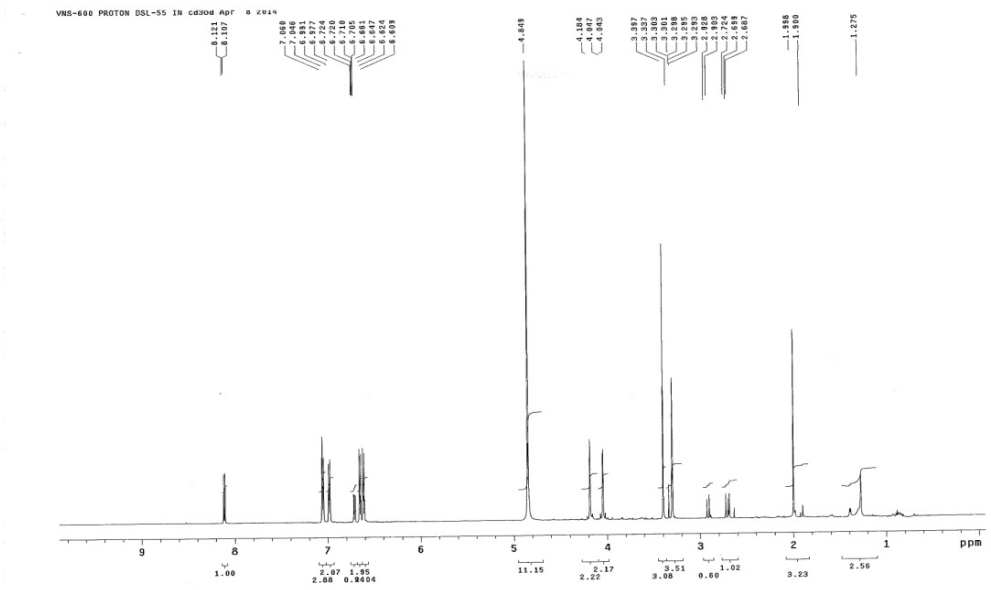


Figure S21. The ^{1}H NMR spectrum of compound **2** in methanol-d_4 (600 MHz).

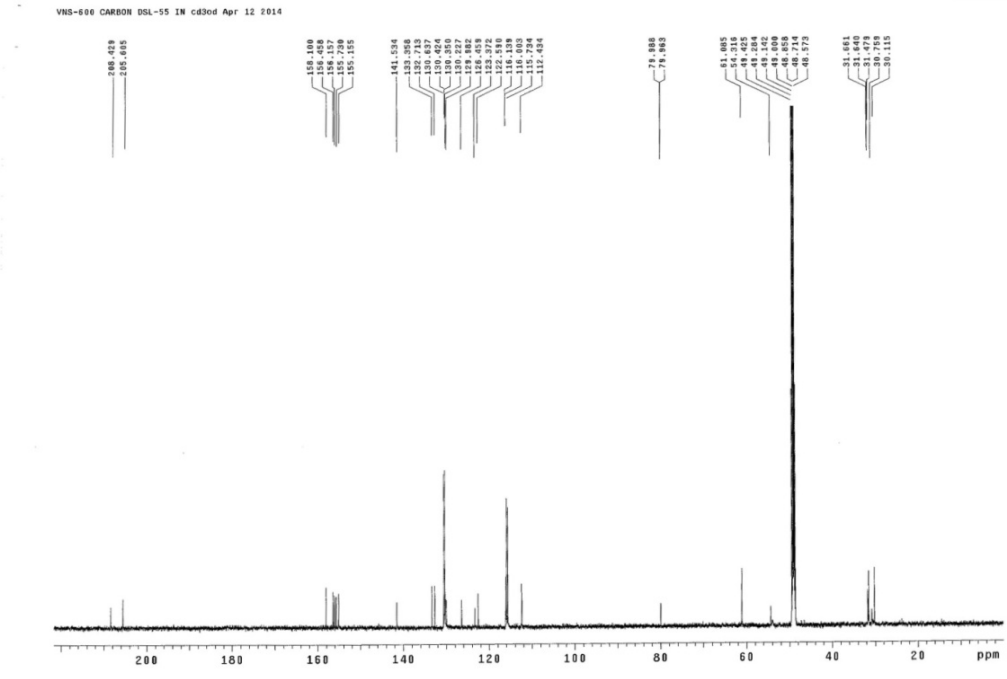


Figure S22. The ^{13}C NMR spectrum of compound **2** in methanol-d_4 (150 MHz).

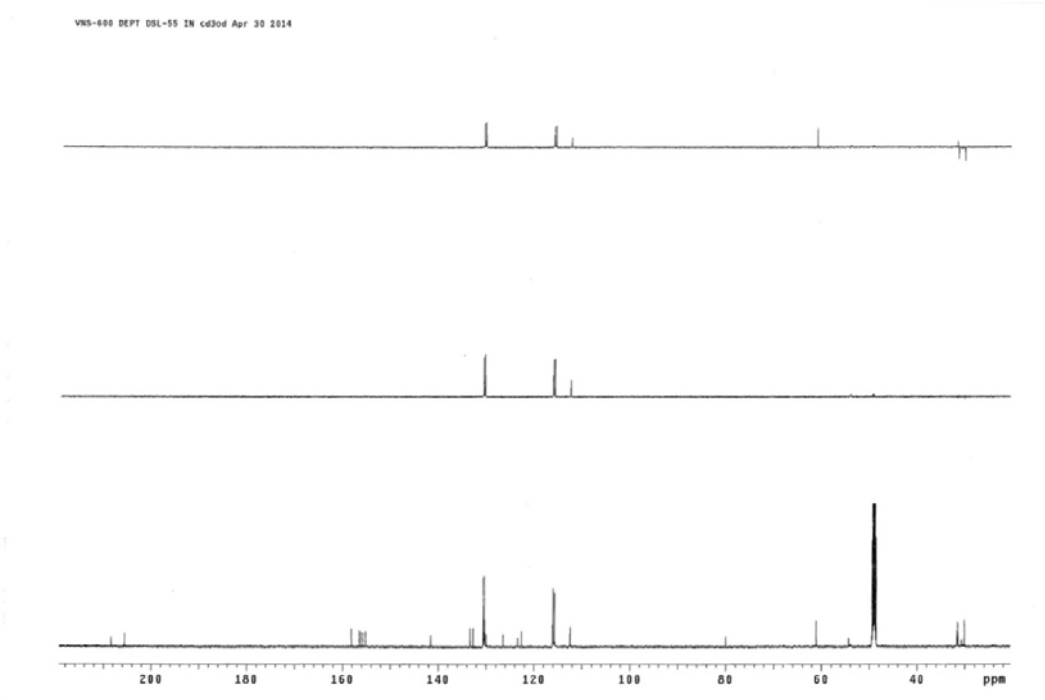


Figure S23. The DEPT spectrum of compound **2** in methanol-d_4 (150 MHz).

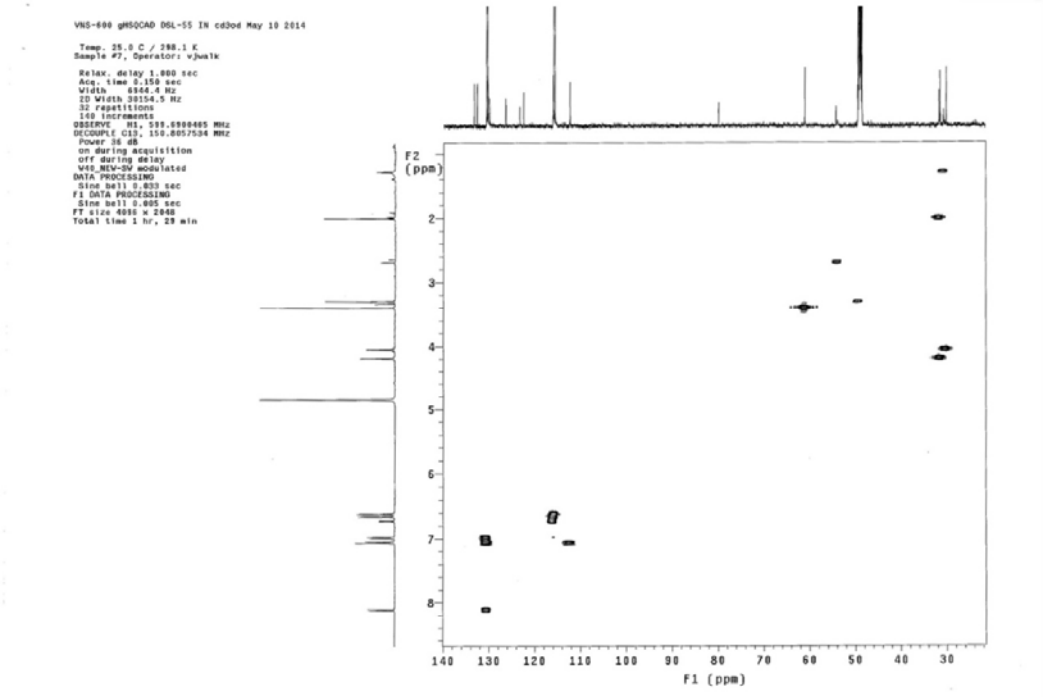


Figure S24. The HSQC spectrum of compound **2** in methanol-d_4 (600 MHz).

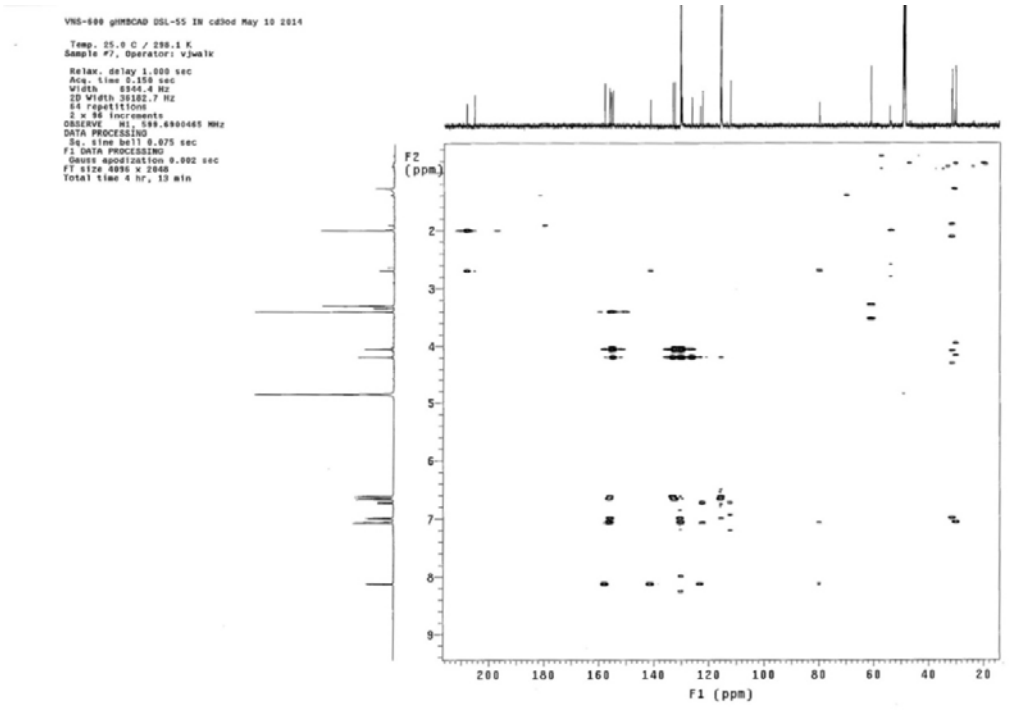


Figure S25. The HMBC spectrum of compound **2** in methanol-d_4 (600 MHz).

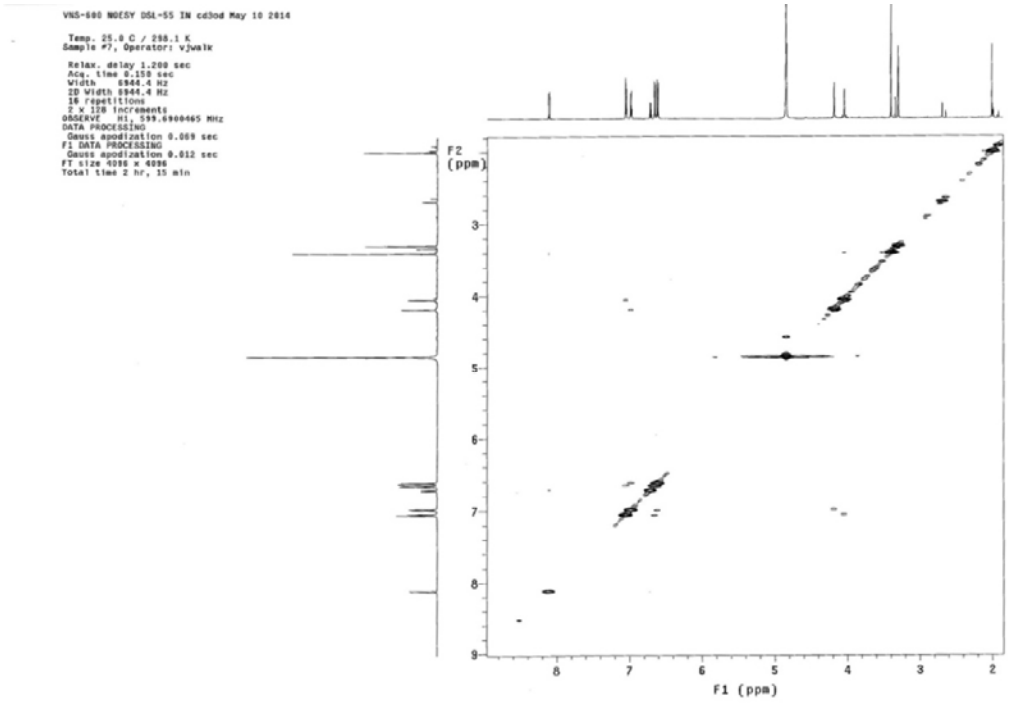


Figure S26. The NOESY spectrum of compound **2** in methanol-d_4 (600 MHz).

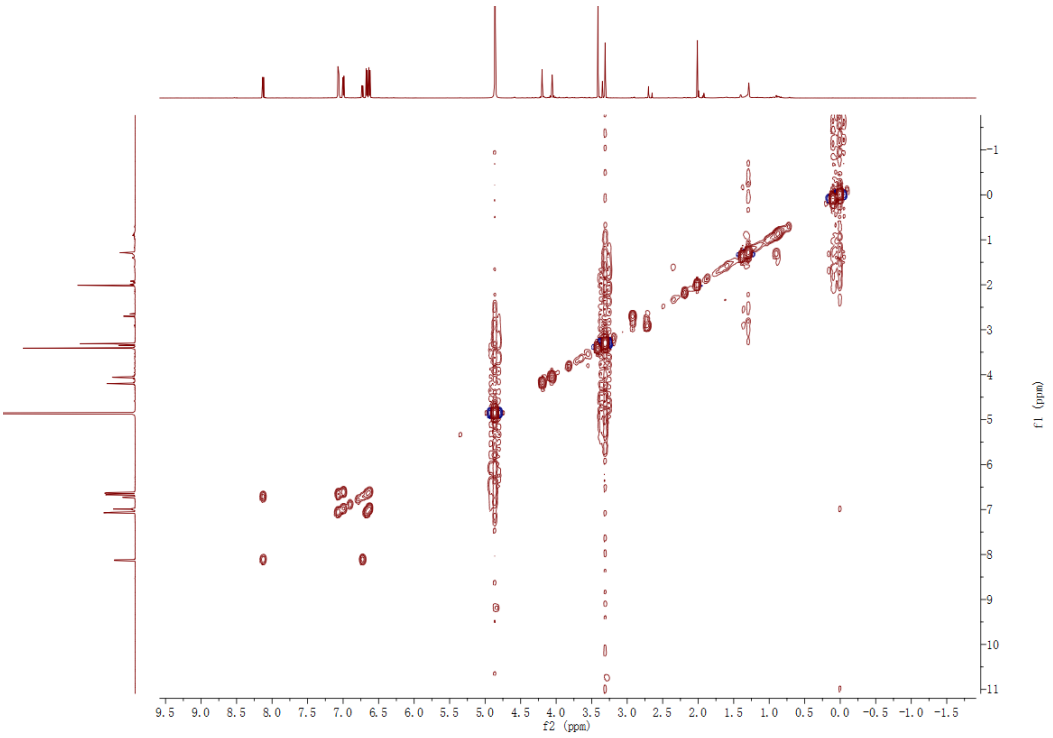


Figure S27. The ^{1}H-^{1}H COSY spectrum of compound **2** methanol-d_4 (600 MHz).

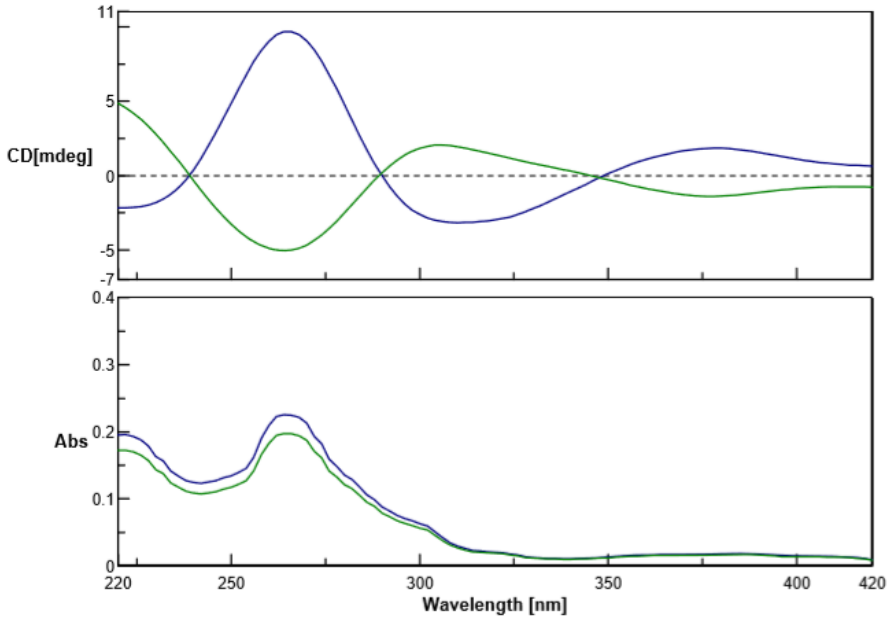


Figure S28. The online mirror-like ECD spectrum of compounds **2a** (blue) and **2b** (green).

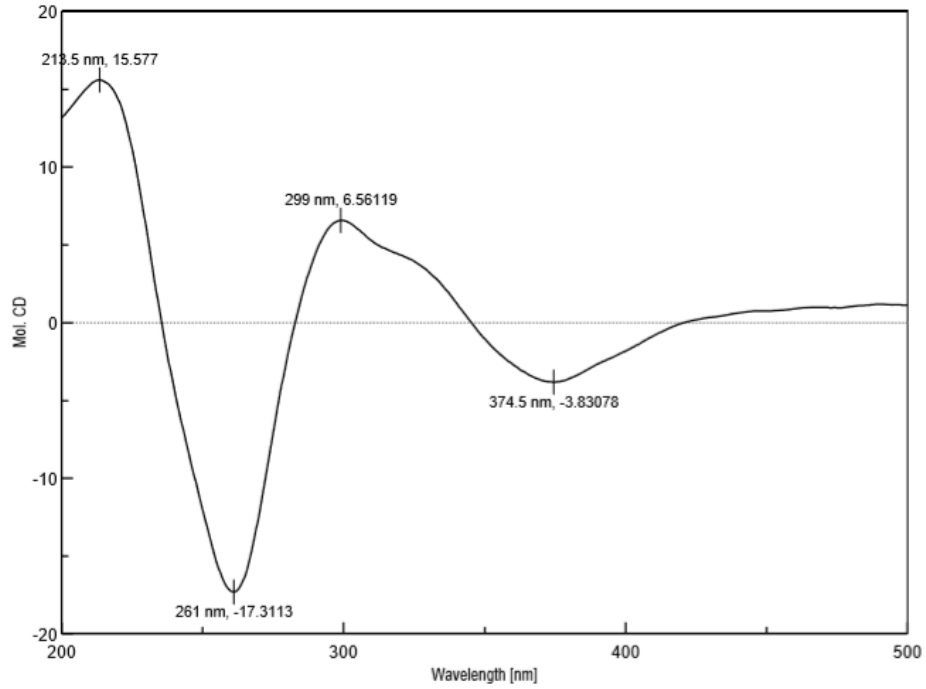


Figure S29. The ECD spectrum of compound **2b**.

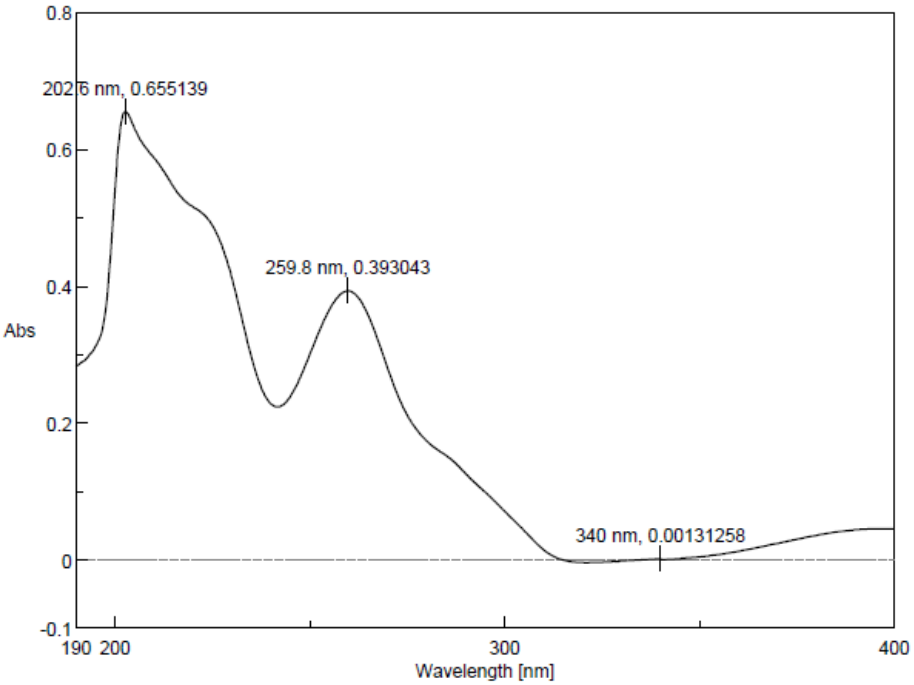


Figure S30. The UV spectrum of compound **3**.

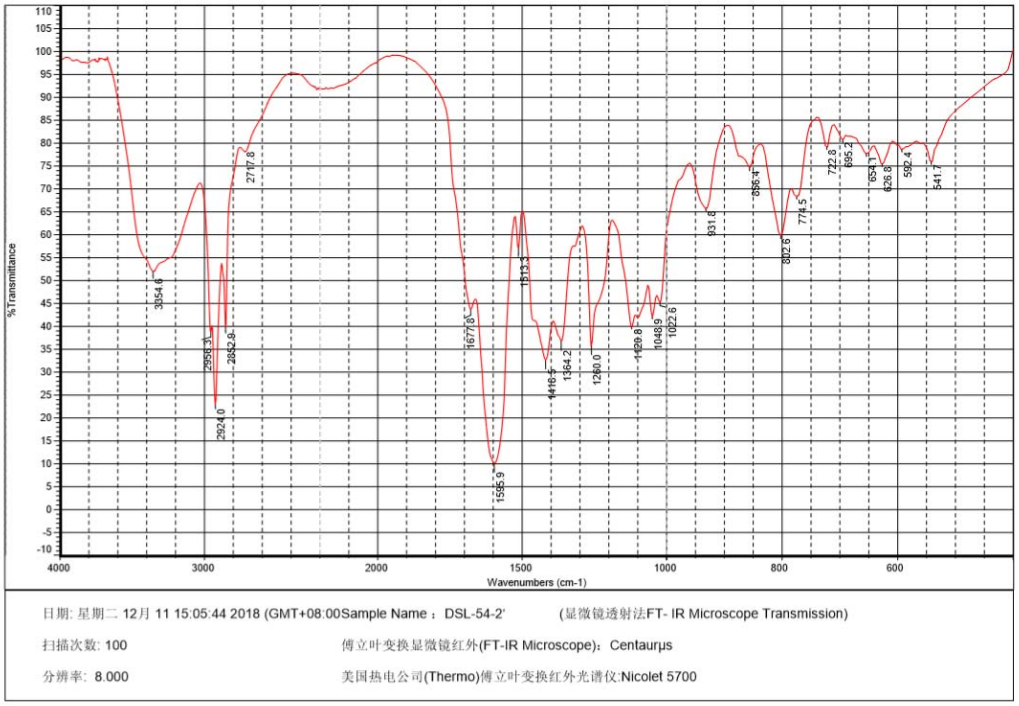


Figure S31. The IR spectrum of compound **3**.

MS Formula Results: - Scan (6.245 min) Sub (2014051404.d)

m/z	Ion	Formula	Abundance
483.1455	(M-H)-	C29 H23 O7	213069.3

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross S	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Mat	Mass Match	m/z	DBE
☑	C29 H24 O7	C29 H23 O7	483.1449	99.88		484.1527	484.1522	-1.05	1.05	99.72	99.9	99.96	483.1455	18
☐	C30 H20 N4 O3	C30 H19 N4 O3	483.1463	99.55		484.1527	484.1535	1.7	1.7	98.82	99.95	99.9	483.1455	23
☐	C26 H28 O7 S	C26 H27 O7 S	483.1483	98.73		484.1527	484.1556	5.91	5.91	97.89	99.56	98.81	483.1455	13
☐	C21 H28 N2 O9 S	C21 H27 N2 O9 S	483.1443	98.49		484.1527	484.1516	-2.41	2.41	95.49	99.47	99.8	483.1455	9
☐	C30 H28 O2 S2	C30 H27 O2 S2	483.1458	97.67		484.1527	484.1531	0.73	0.73	92.47	99.3	99.98	483.1455	17
☐	C17 H28 N2 O14	C17 H27 N2 O14	483.1468	97.4		484.1527	484.1541	2.76	2.76	91.4	99.93	99.74	483.1455	5

Figure S32. The HRESIMS of compound **3**.

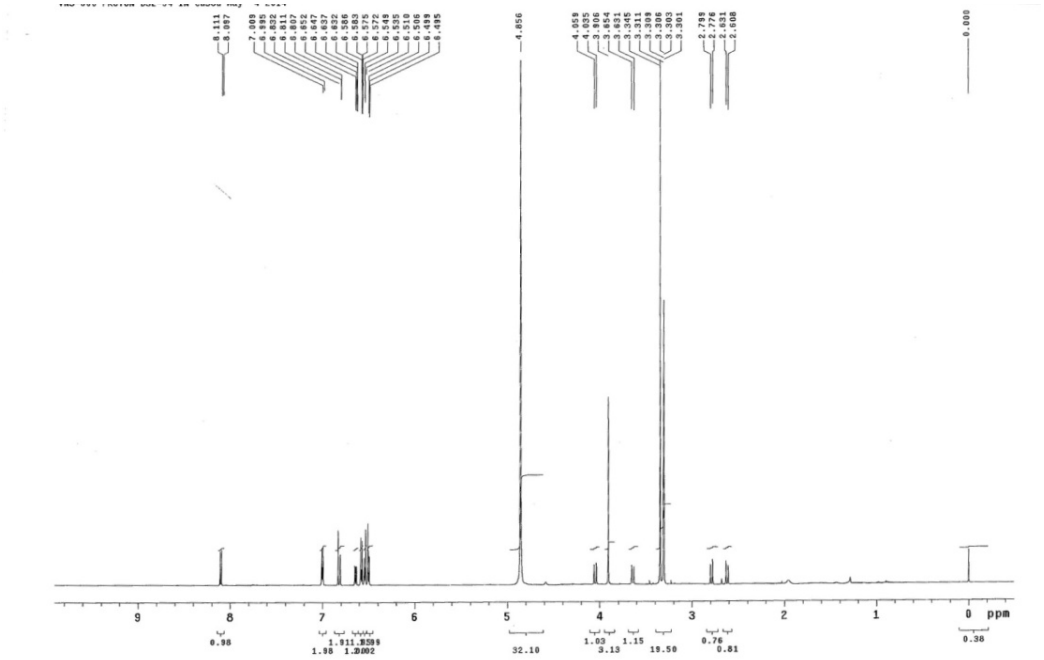


Figure S33. The ^{1}H NMR spectrum of compound **3** in methanol-d_4 (600 MHz).

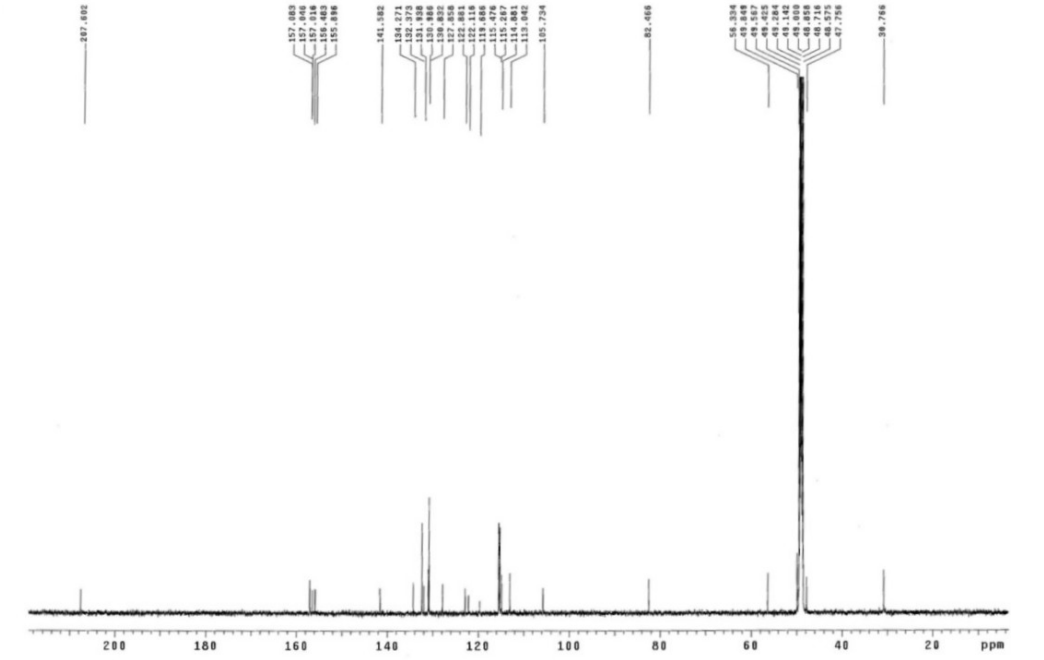


Figure S34. The ^{13}C NMR spectrum of compound **3** in methanol-d_4 (150 MHz).

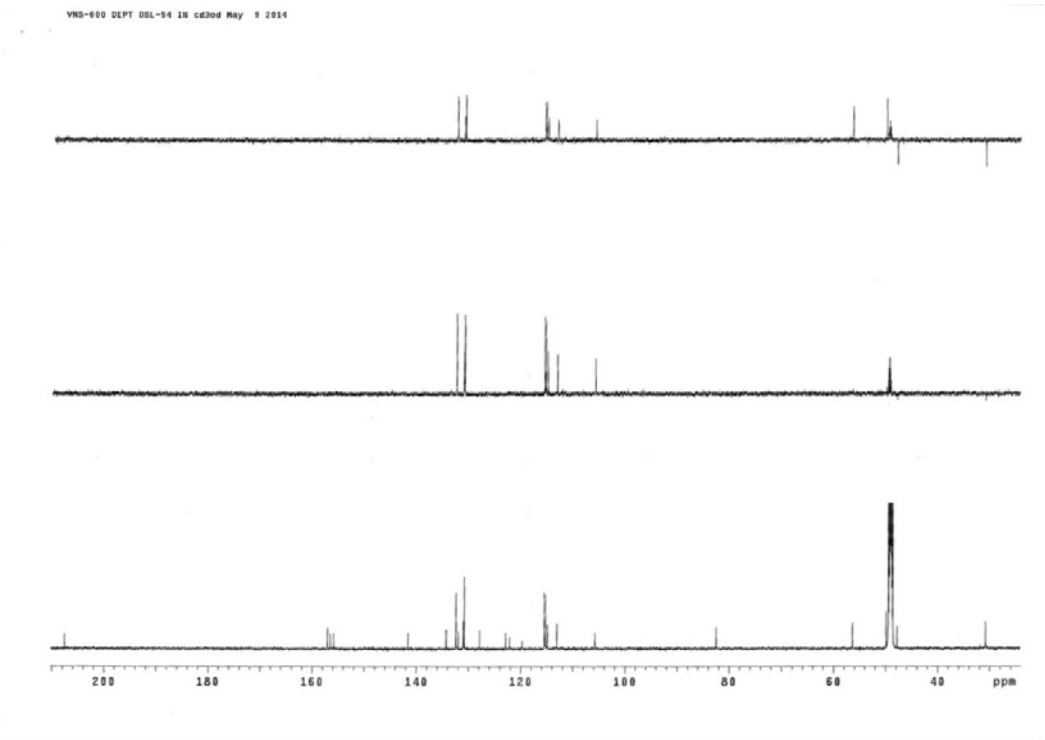


Figure S35. The DEPT spectrum of compound **3** in methanol-d_4 (150 MHz).

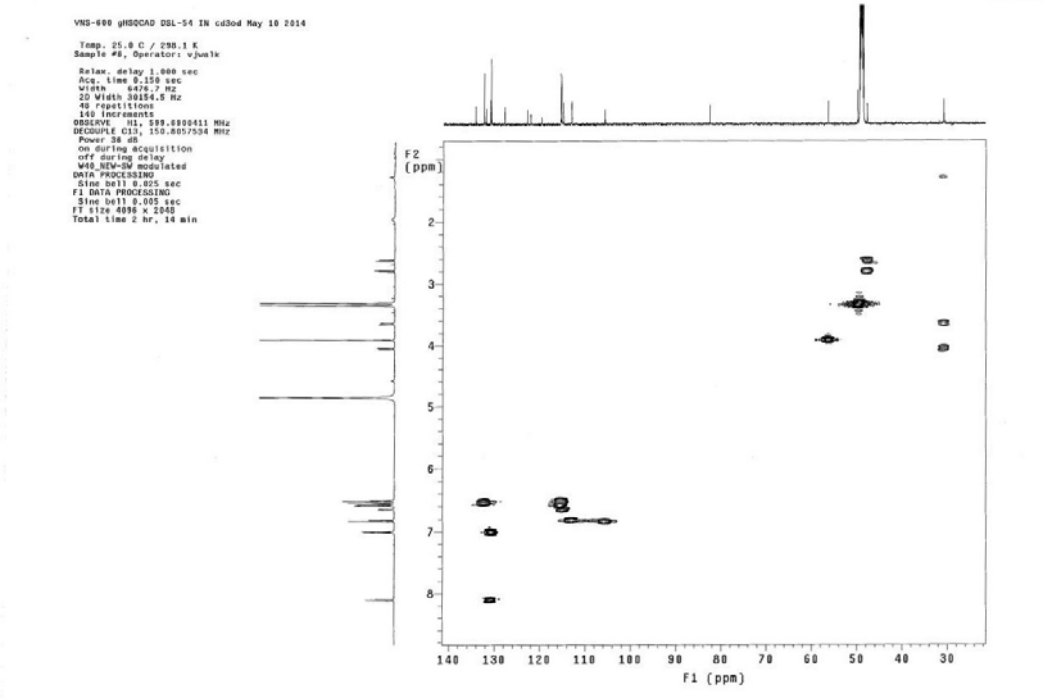


Figure S36. The HSQC spectrum of compound **3** in methanol-d_4 (600 MHz).

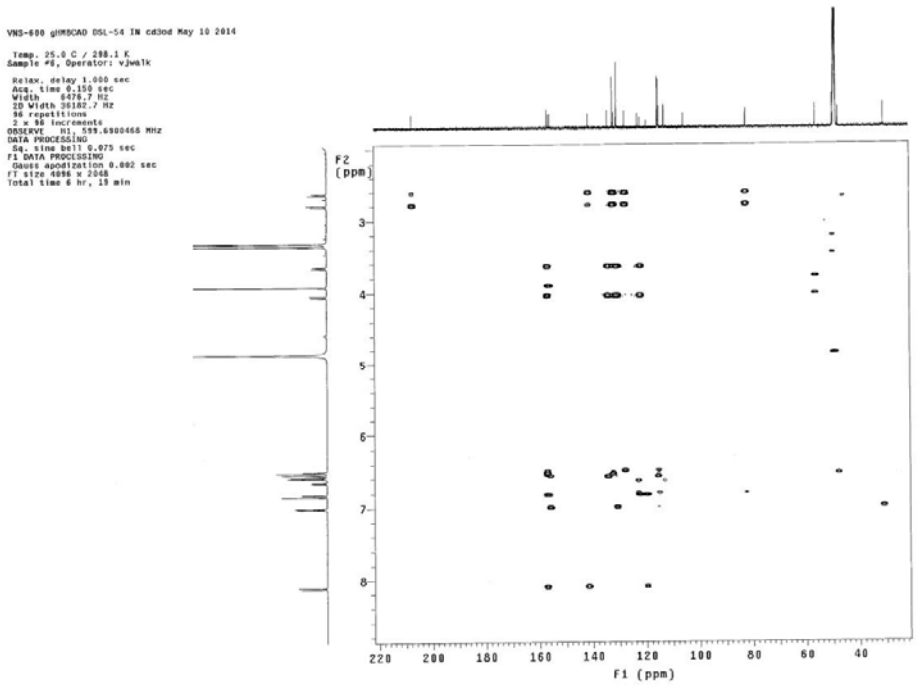


Figure S37. The HMBC spectrum of compound **3** in methanol-d_4 (600 MHz).

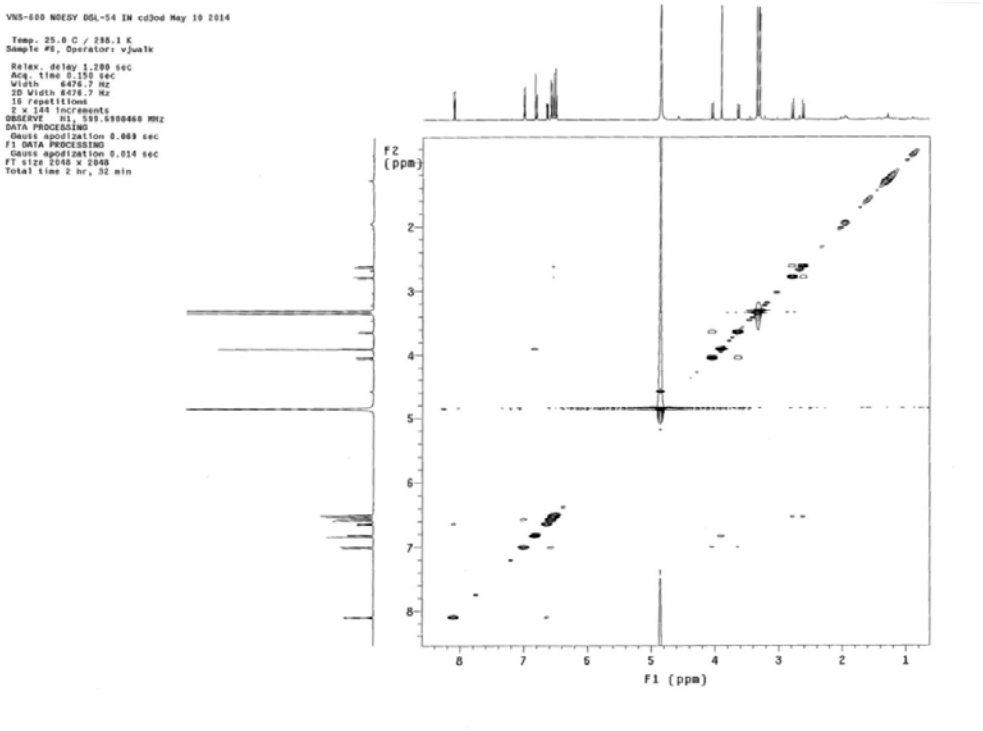


Figure S38. The NOESY spectrum of compound **3** in methanol-d_4 (600 MHz).

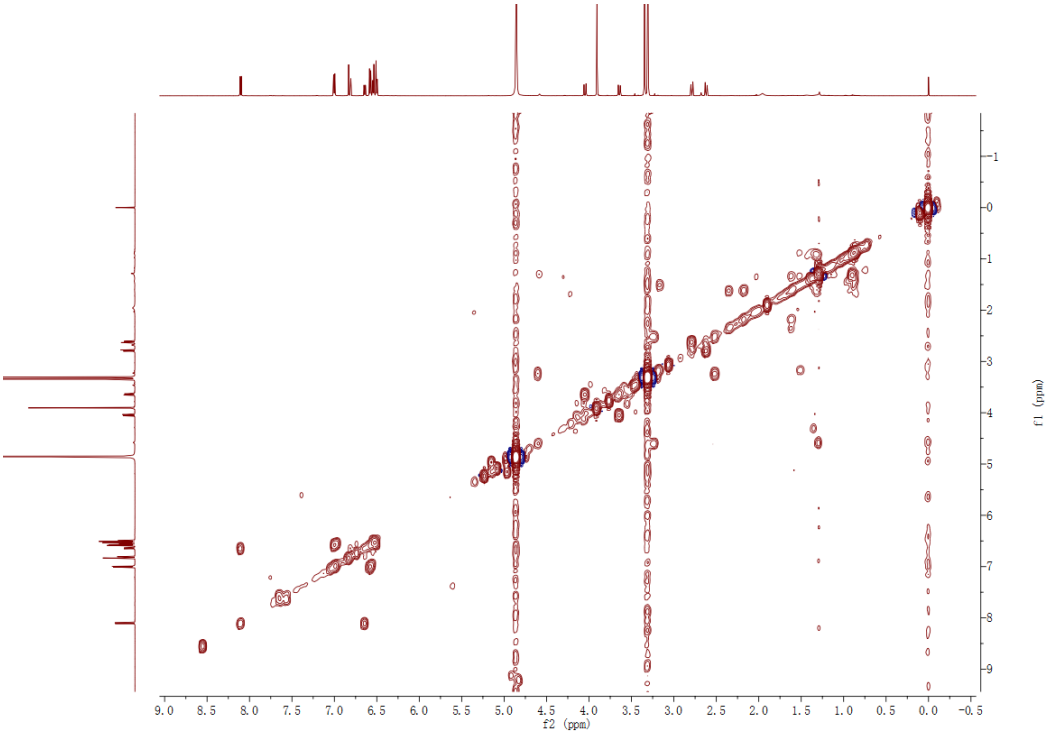


Figure S39. The ^{1}H-^{1}H COSY spectrum of compound **3** methanol-d_4 (600 MHz).

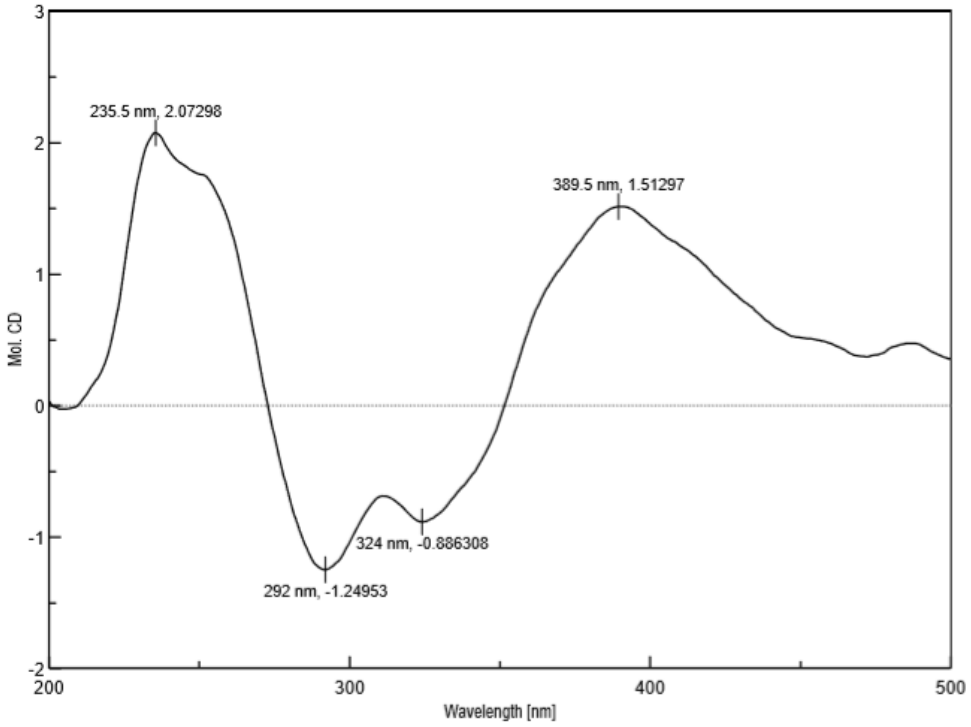


Figure S40. The ECD spectrum of compound **3a**.

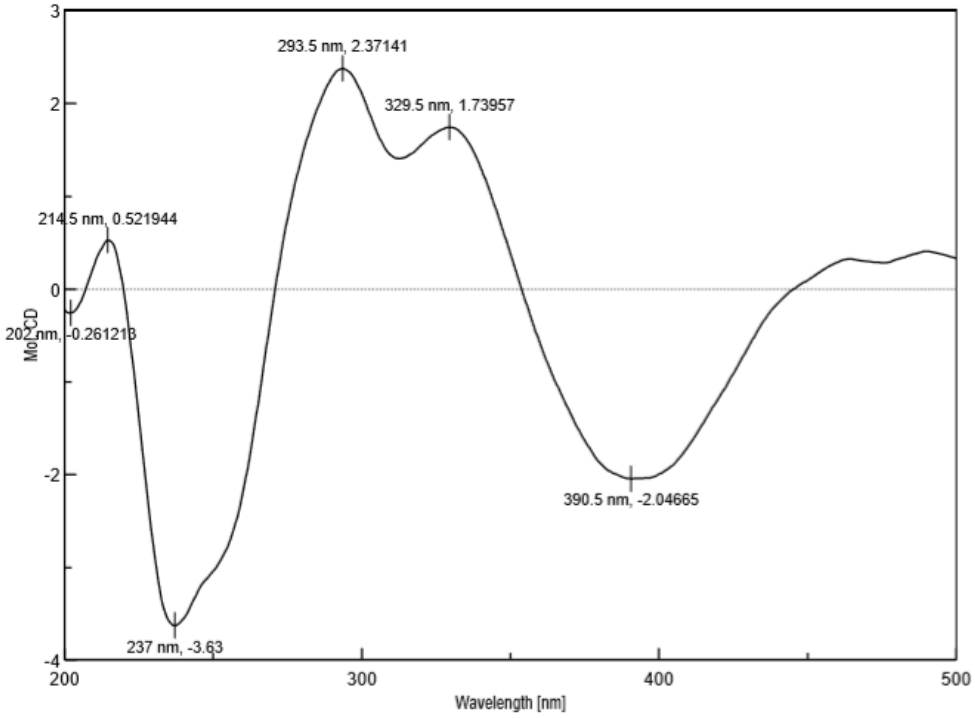


Figure S41. The ECD spectrum of compound **3b**.

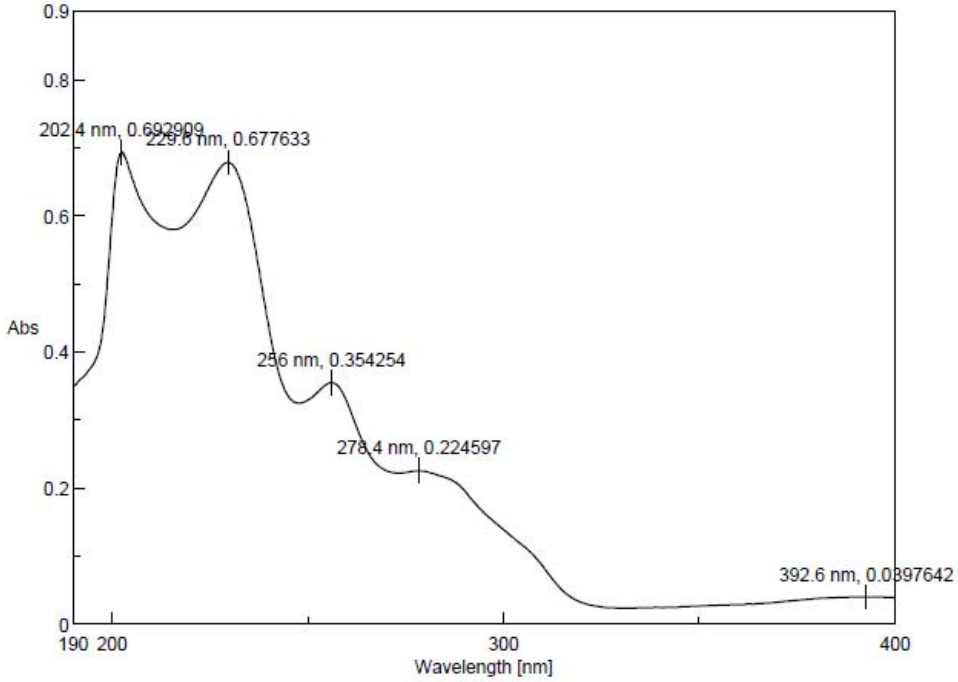


Figure S42. The UV spectrum of compound **4**.

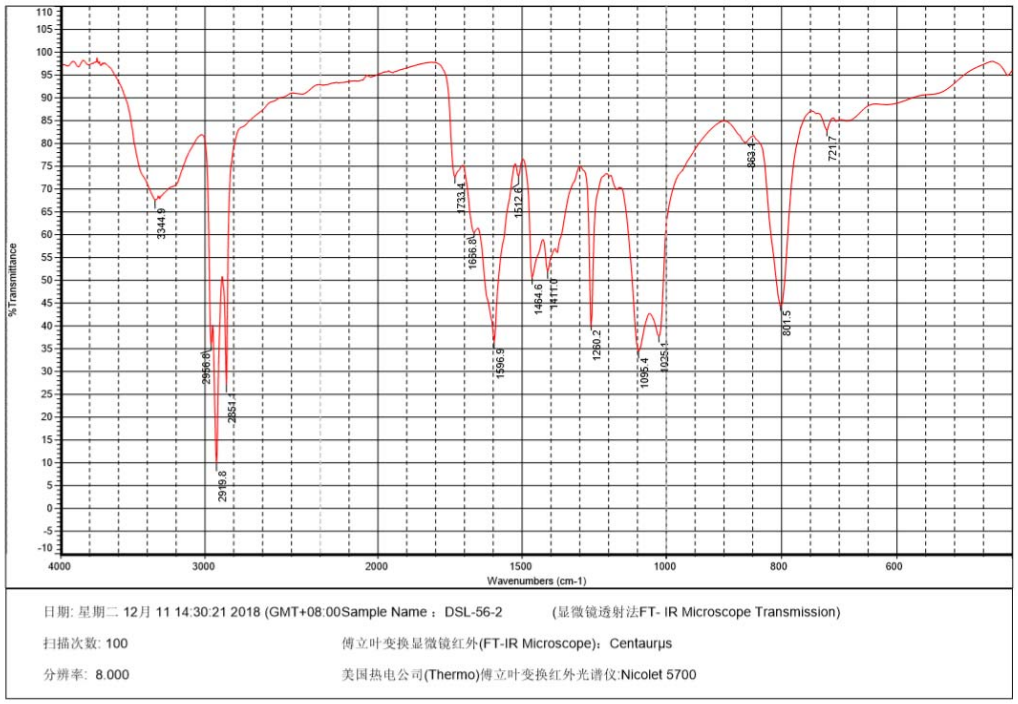


Figure S43. The IR spectrum of compound **4**.

MS Formula Results: - Scan (6.804 min) Sub (2014051406.d)

m/z	Ion	Formula	Abundance
467.1486	(M-H)-	C29 H23 O6	31105.7

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross S	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Mat	Mass Match	m/z	DBE
✓	C29 H24 O6	C29 H23 O6	467.15	99.8		468.1558	468.1573	3.13	3.13	99.93	99.92	99.67	467.1486	18
	C30 H20 N4 O2	C30 H19 N4 O2	467.1513	99.33		468.1558	468.1586	5.96	5.96	99.73	99.93	98.8	467.1486	23
	C33 H24 O 5	C33 H23 O 5	467.1475	99.23		468.1558	468.1548	-2.23	2.23	97.68	99.88	99.83	467.1486	22
	C24 H24 N2 O8	C24 H23 N2 O8	467.146	98.87		468.1638	468.1533	-5.48	5.48	97.78	99.96	98.98	467.1486	14
	C21 H28 N2 O8 S	C21 H27 N2 O8 S	467.1494	97.84		468.1558	468.1566	1.71	1.71	92.9	99.65	99.9	467.1486	9
	C30 H28 O S2	C30 H27 O S2	467.1609	97.53		468.1558	468.1582	4.97	4.97	93.14	99.5	99.16	467.1486	17
	C29 H28 N2 O3 S2	C29 H27 N2 O3 S2	467.1469	97.32		468.1558	468.1541	-3.64	3.64	91.92	99.34	99.55	467.1486	13

Figure S44. The HRESIMS of compound **4**.

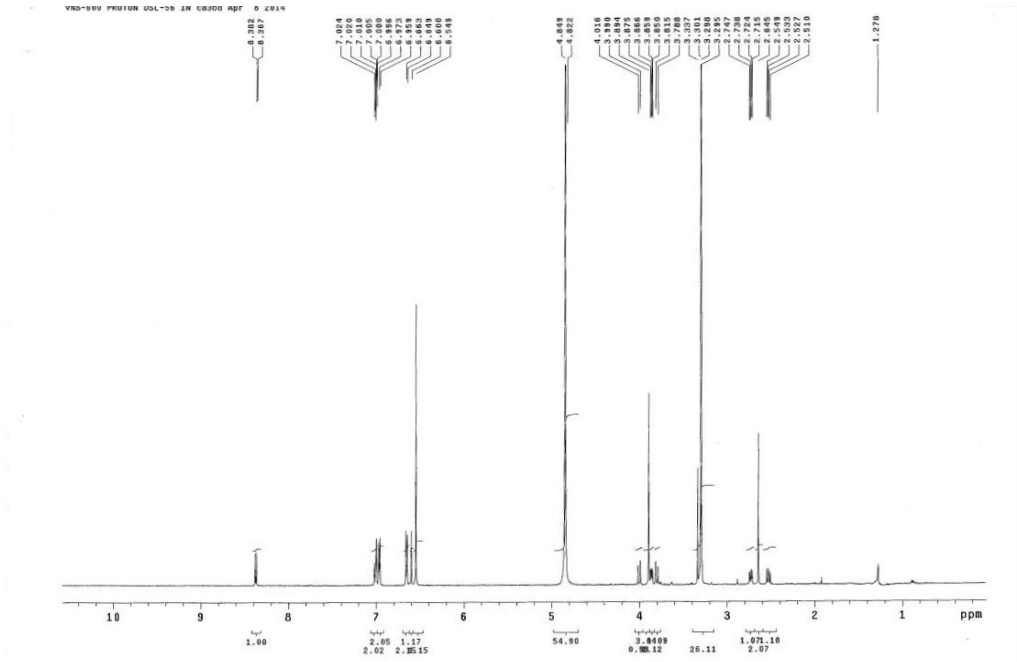


Figure S45. The ^{1}H NMR spectrum of compound **4** in methanol-d_4 (600 MHz).

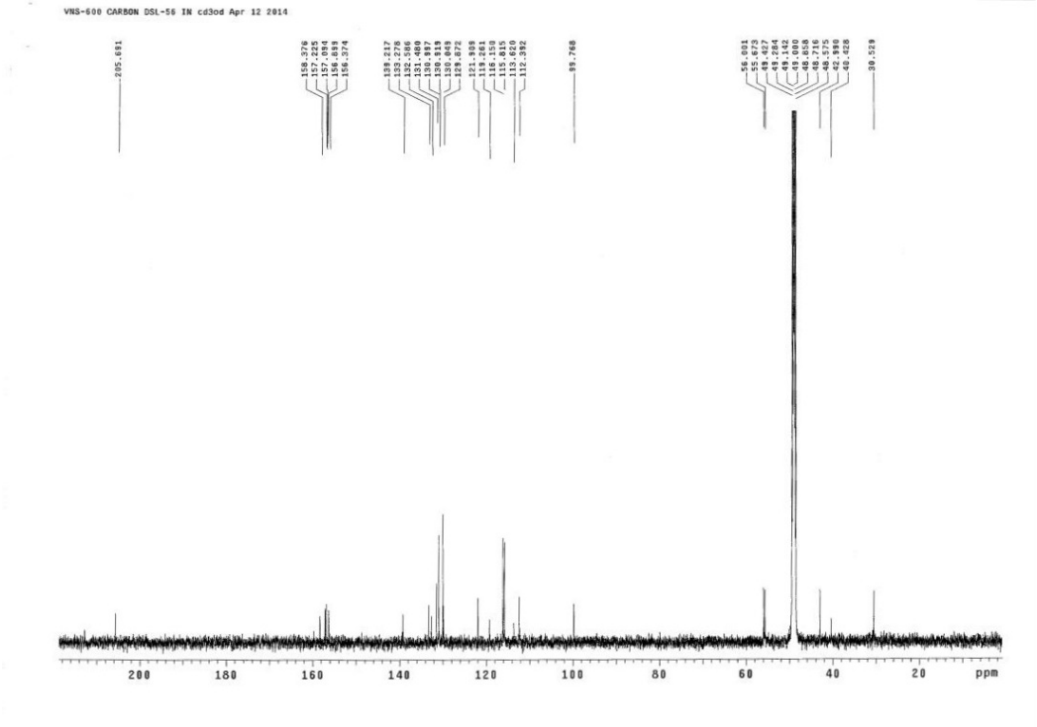


Figure S46. The ^{13}C NMR spectrum of compound **4** in methanol-d_4 (150 MHz).

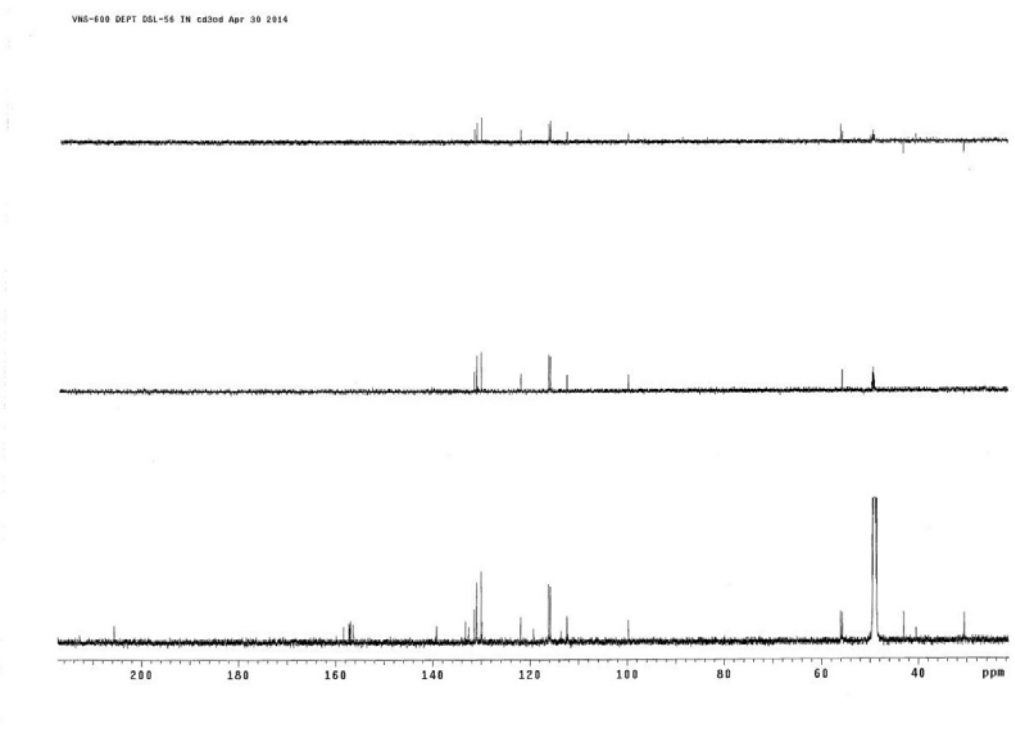


Figure S47. The DEPT spectrum of compound **4** in methanol-d_4 (150 MHz).

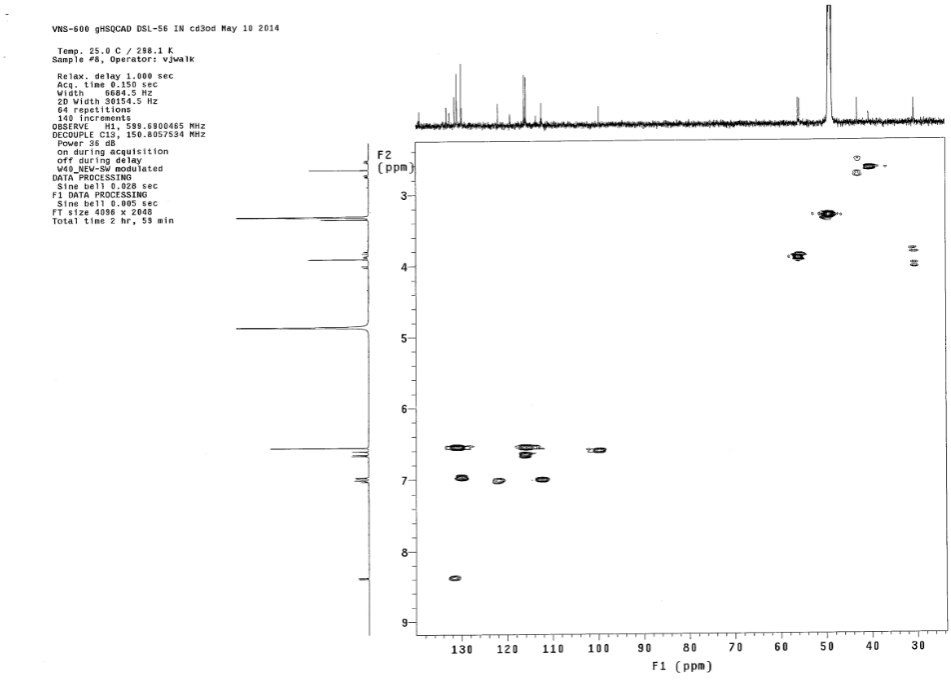


Figure S48. The HSQC spectrum of compound **4** in methanol-d_4 (600 MHz).

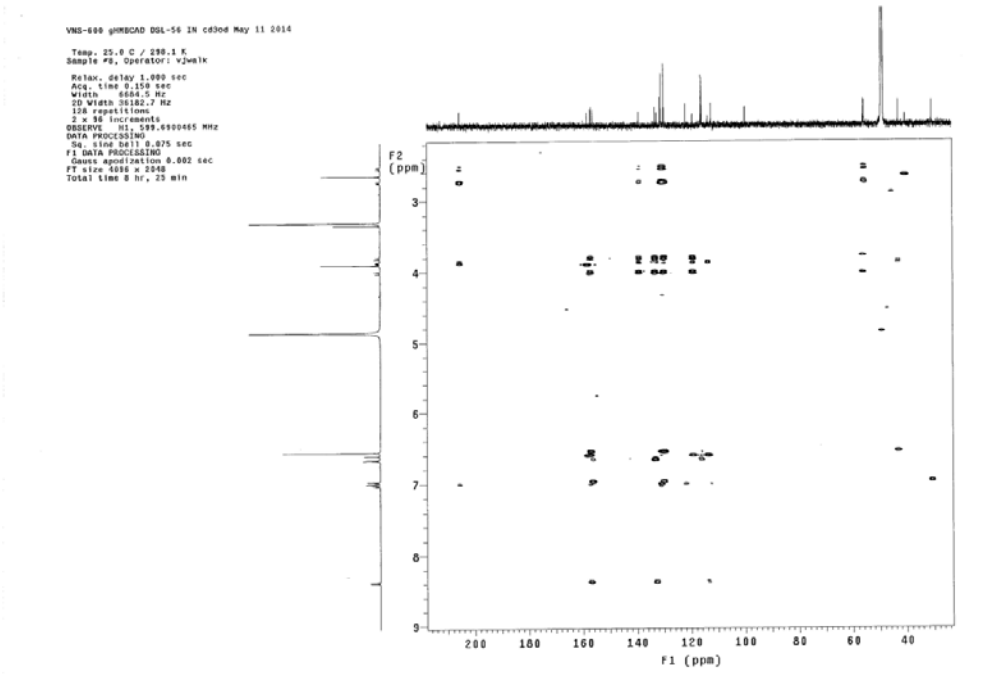


Figure S49. The HMBC spectrum of compound **4** in methanol-d_4 (600 MHz).

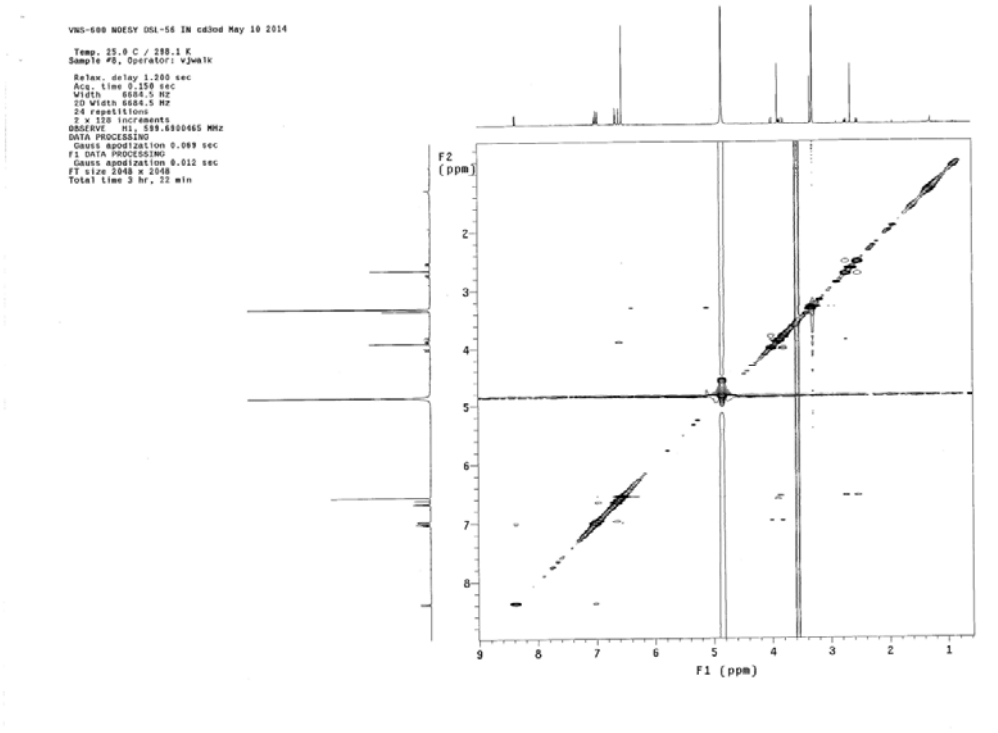


Figure S50. The NOESY spectrum of compound **4** in methanol-d_4 (600 MHz).

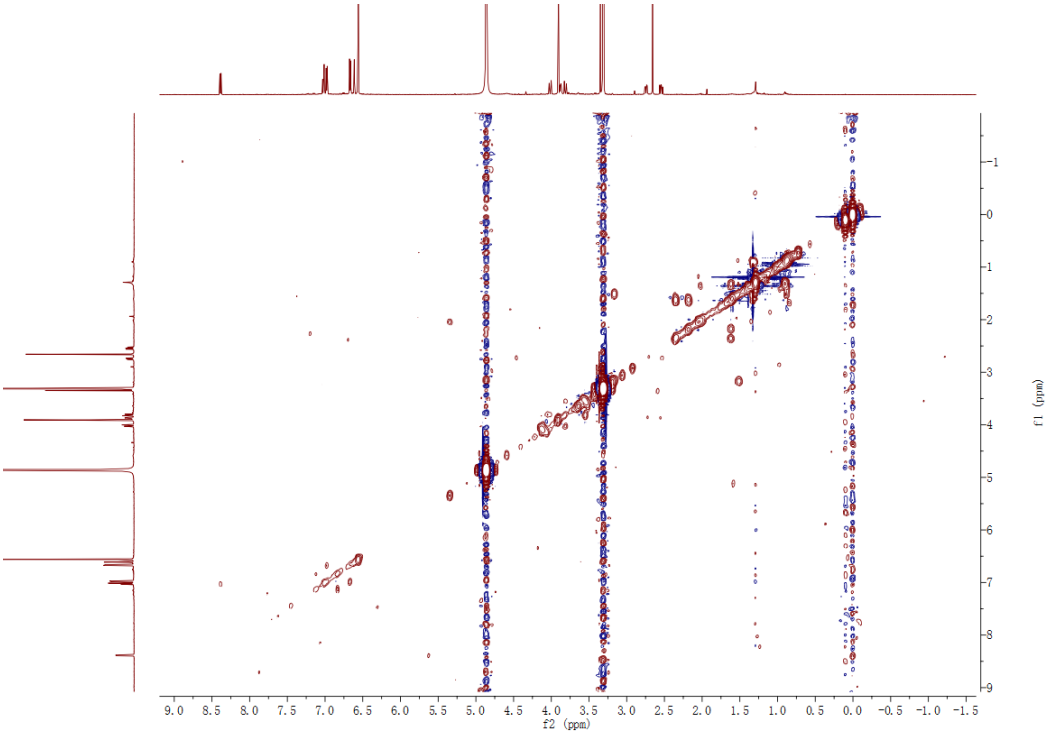


Figure S51. The ^{1}H-^{1}H COSY spectrum of compound **4** methanol-d_4 (600 MHz).

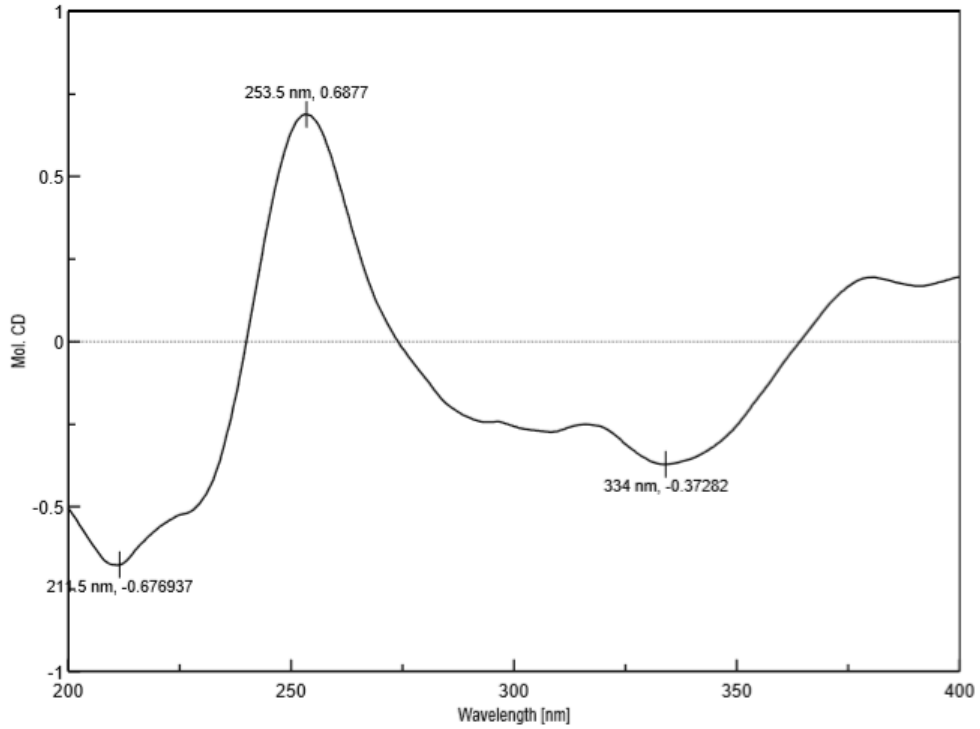


Figure S52. The ECD spectrum of compound **4a**.

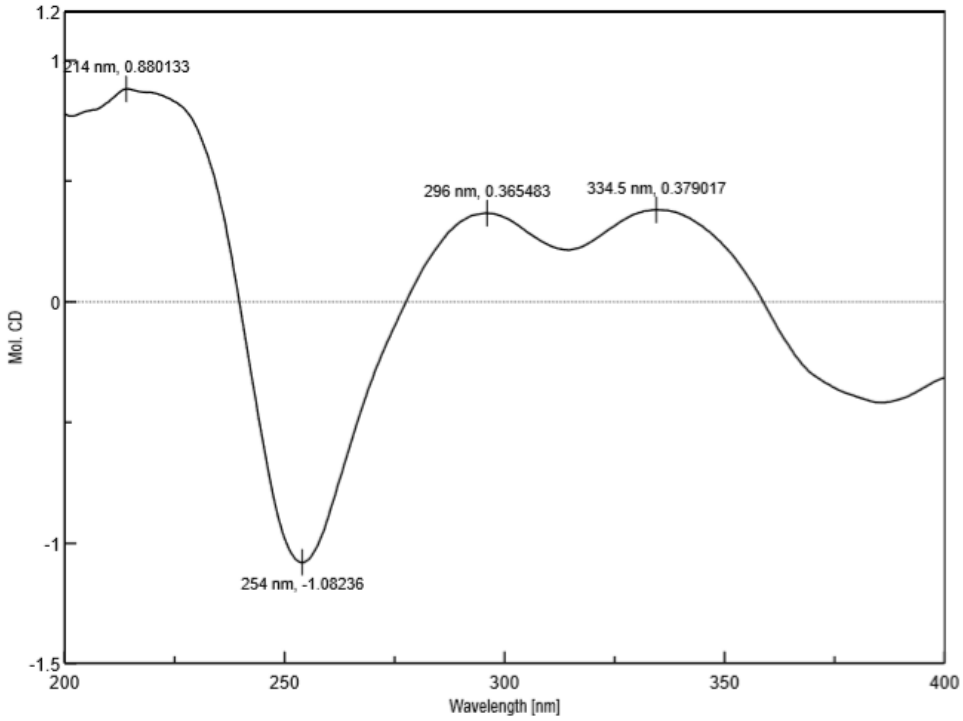


Figure S53. The ECD spectrum of compound **4b**.