marine drugs

MDPI

Perylenequione Derivatives with Anticancer Activities Isolated from the Marine Sponge-Derived Fungus, *Alternaria* sp. SCSIO41014

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Original Data of Antibacterial Activity Assay

Compounds **1–28** were test for antibacterial activities against *Staphylococcus aureus* (ATCC 29213) using the agar filter paper diffusion [1]. *S. aureus* stored in glycerinum were activated in LB medium (10 g tryptone, 5 g yeast extract, 10 g NaCl, distilled water added up to 1,000 mL, pH 7.2–7.4) in a shaker-incubator at 37 °C and 180 r.p.m for 24 h. Then dilution-plate method was used to get effective concentration of 10^6–10^7 CFU ml^{-1}. The plate of LB medium was painted with 100 μL of *S. aureus* with effective concentration. The sterile filter paper (a diameter of 5 mm) was painted with each 10 μL of DMSO as negative control, ampicillin (0.5 mg/mL) as positive control and compounds **1–28** (5 mg/mL), respectively. Compounds **10** and **25** with 50 μg/disc displayed an inhibition zone with a diameter of about 21 and 15 mm, and ampicillin with 5 μg/disc showed an inhibition zone with a diameter of about 30 mm (Figure S1), respectively. Further, their minimum inhibitory concentrations (MIC) were evaluated in 96-well microtiter plates using a modification of the broth microdilution method [1]. Each well was added 100 μL of *S. aureus* with effective concentration, 90 μL of sterile LB medium and 10 μL of tested compounds, respectively. Compounds **10**,**25** and ampicillin with different concentration were added with final concentration as showed in Table S1 and replicated three times. The MIC value of compound **25** was 31.25 μg/mL, while compound **10** showed more than 500 μg/mL. Ampicillin was used as positive control with the MIC value of 6.25 μg/mL.

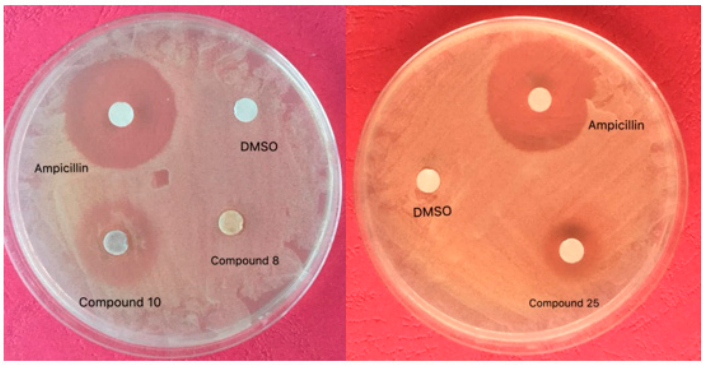

Figure S1 Pictures of inhibition zones in the filter paper diffusion test.

Table S1 The growth conditions of the *Staphylococcus aureus* after add different dilution concentration of compounds **10**, **25** and ampicillin.

Concentration μg/mL	500	250	125	62.5	31.25	15.63	7.82	3.91	1.96	0.98	0.49	DMSO
10	+	+	+	+	+	+	+	+	+	+	+	+
25	−	−	−	−	−	+	+	+	+	+	+	+
Concentration μg/mL	25	12.5	6.25	3.13	1.57	0.79	0.40	0.20	0.10	0.05	0.03	DMSO
Ampicillin	−	−	−	+	+	+	+	+	+	+	+	+

"+" indicated some *S. aureus* had grown. "−" indicated no *S. aureus* grew.

Detailed Process of Antitumor Activity Assay

Cytotoxicity was assayed with the CCK-8 (Dojindo, Kumamoto Prefecture, Japan) method [2]. Cell lines K562, SGC-7901 and BEL-7402 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were routinely grown and maintained in DMEM or RPMI media with 10% fetal bovine serum and with 1% streptomycin/penicillin. All cell lines were incubated in a Thermo/ Forma Scientific CO_2 water-jacketed incubator with 5% CO_2 in air at 37 °C. A cell viability assay was

determined with the CCK-8 assay. Cells were seeded at a density of 400–800 cells/well in 384-well plates and treated with various concentrations of compounds or solvent control. After 72 h incubation, CCK-8 reagent was added, and absorbance was measured at 450 nm using an Envision 2104 multilabel reader (PerkinElmer, Waltham, MA, USA). Dose−response curves were plotted to determine the IC_{50} values using Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Taxol was used as the positive control, with IC_{50} values of 0.18 ± 0.20, 0.89 ± 0.15 and 0.54 ± 0.20 μg/mL, respectively.

The 16S rRNA Gene Sequences Data of *Alternaria* sp. SCSIO41014

CTGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTT
TCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTC
AGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
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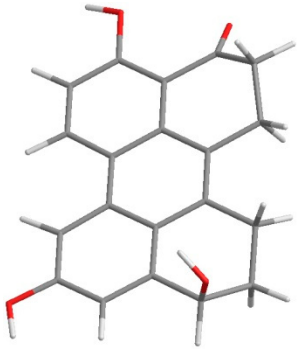
CAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAAT
TGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTC
TAGCATCCATTAAGCCTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC
TTAAGCATATCA

Theory and Calculation Details of Compound 1

To determine the absolute configuration of **1**, a computational modeling study was conducted using the Gaussian 03 program package [3,4]. The ECD of the lowest energy conformer was then calculated by the TDDFT method at the B3LYP/6-31G(d) level in methanol solution.



B3LYP/6-31G(d) optimized lowest energy 3D conformer of **1**.

References

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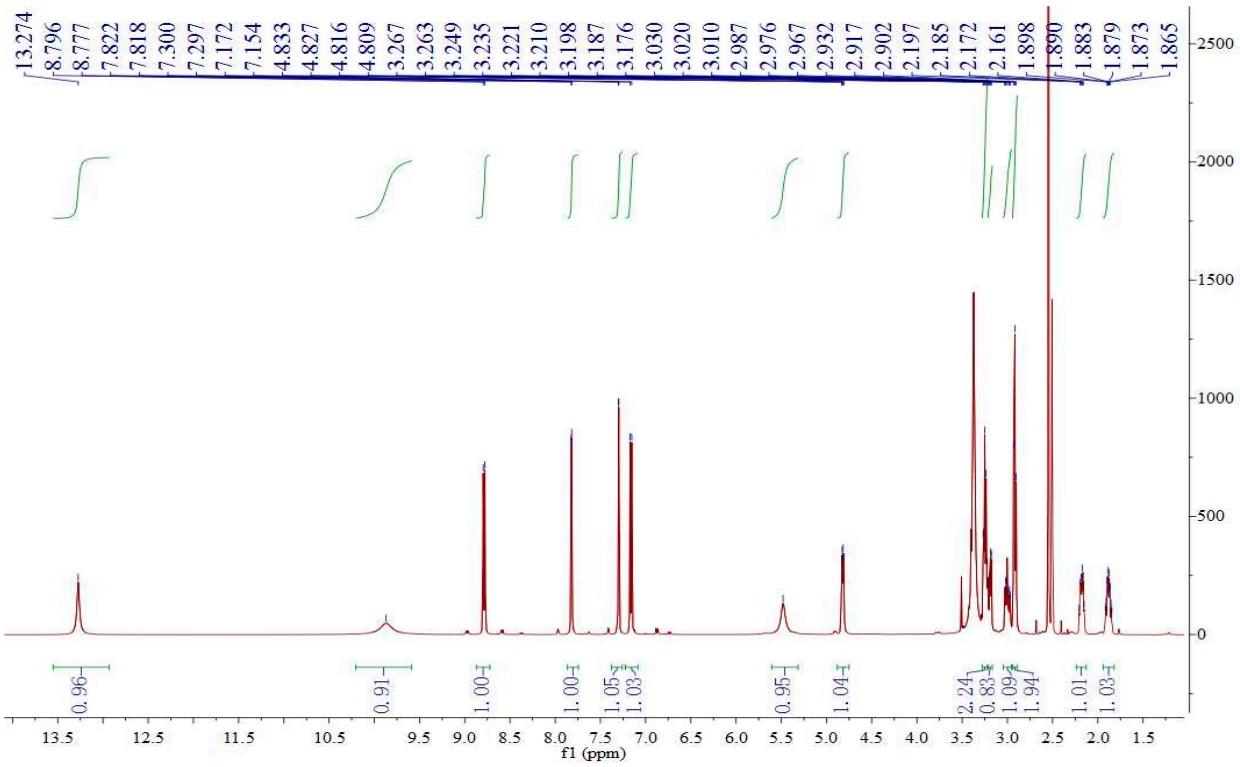

Figure S2. [1]H NMR spectrum of **1** in DMSO-*d*6.

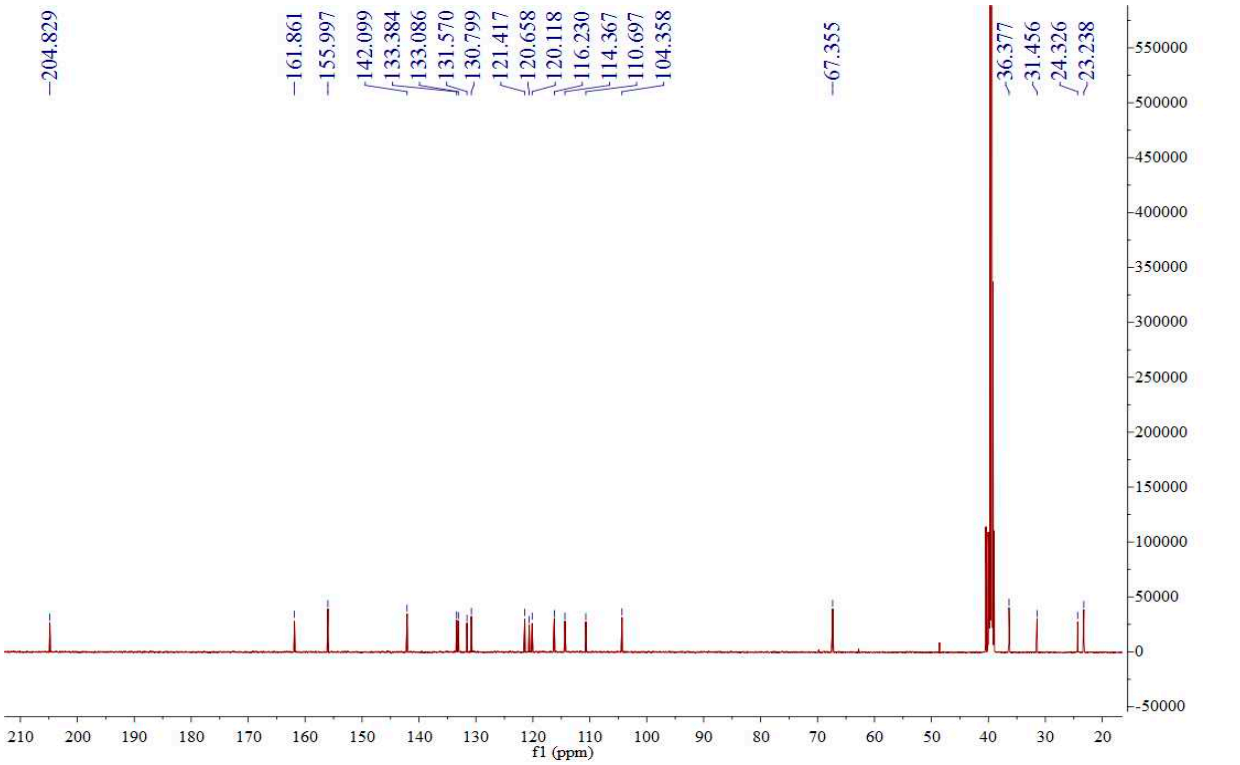

Figure S3. [13]C NMR spectrum of **1** in DMSO-*d*6.

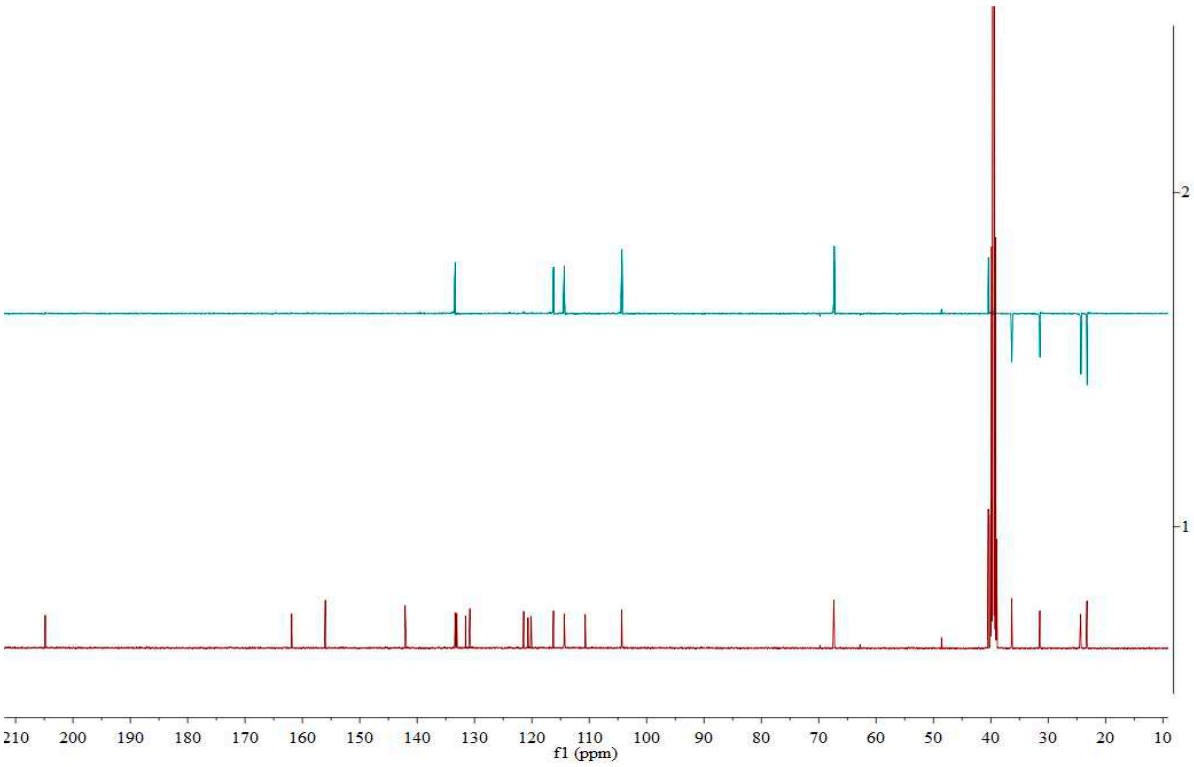

Figure S4. DEPT NMR spectrum of **1** in DMSO-d_6.

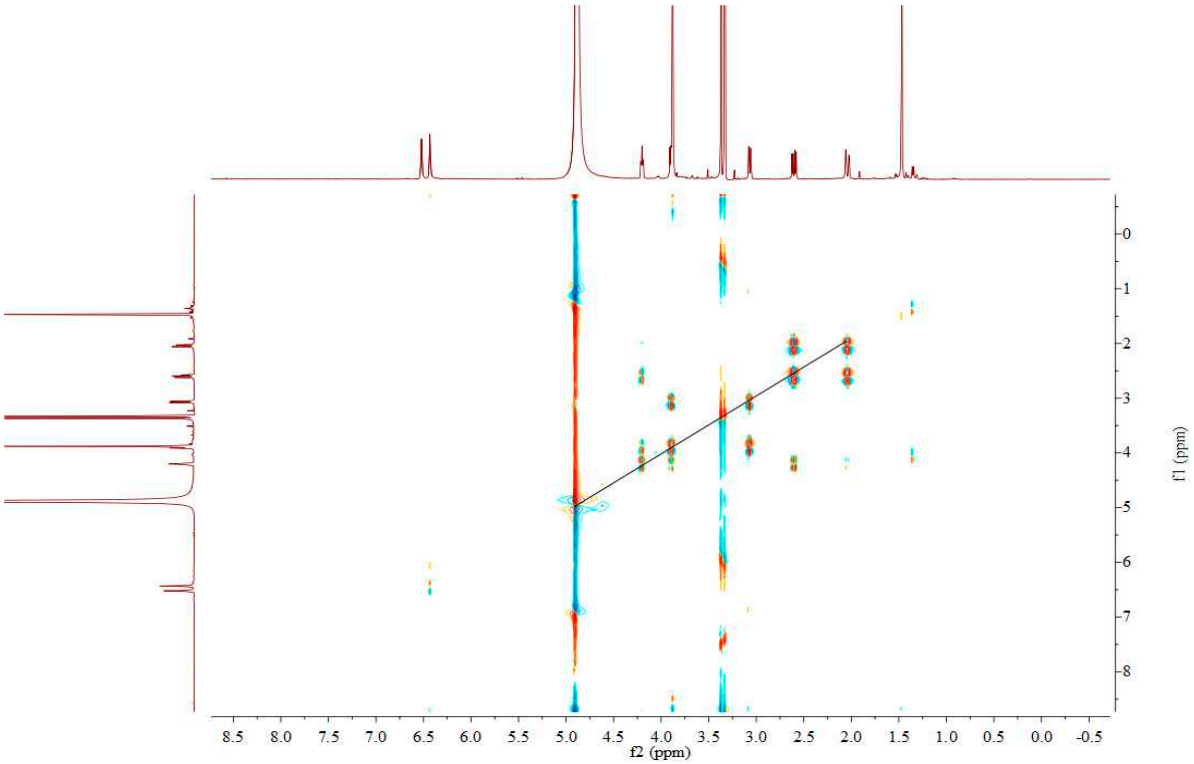

Figure S5. ^{1}H-^{1}H COSY spectrum of **1** in DMSO-d_6.

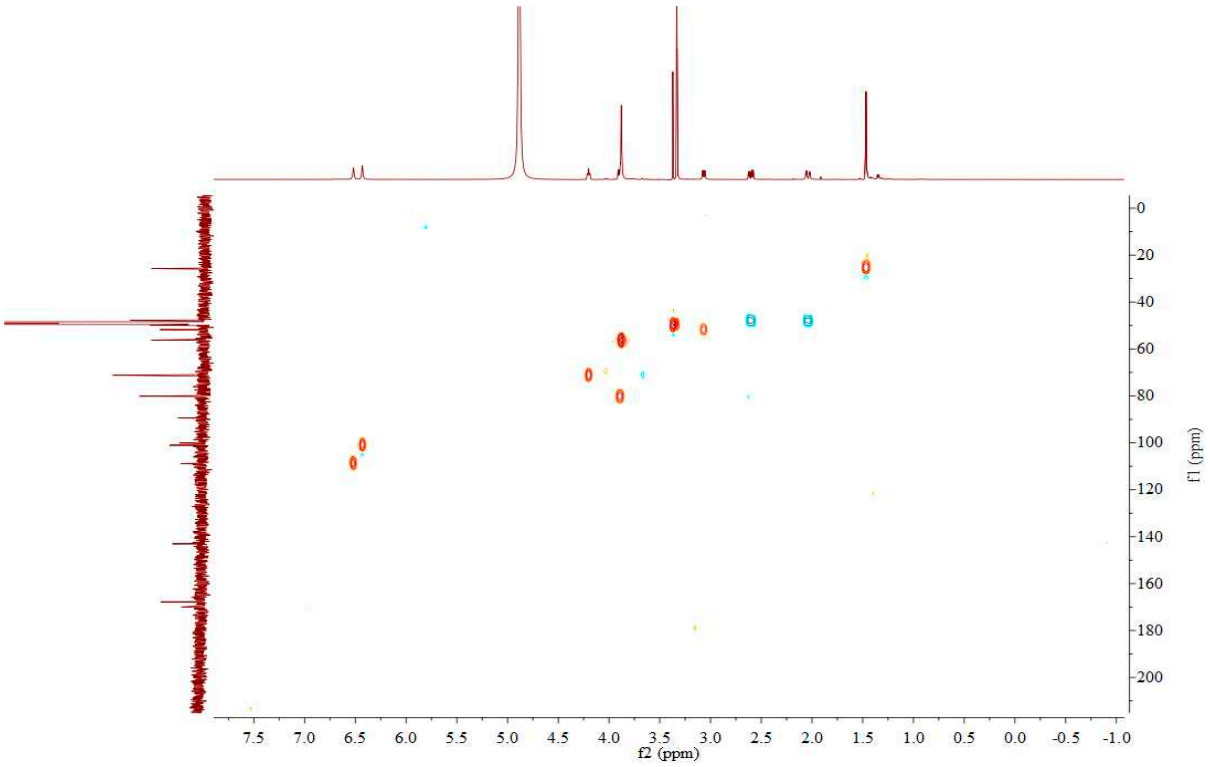

Figure S6. HSQC spectrum of **1** in DMSO-d_6.

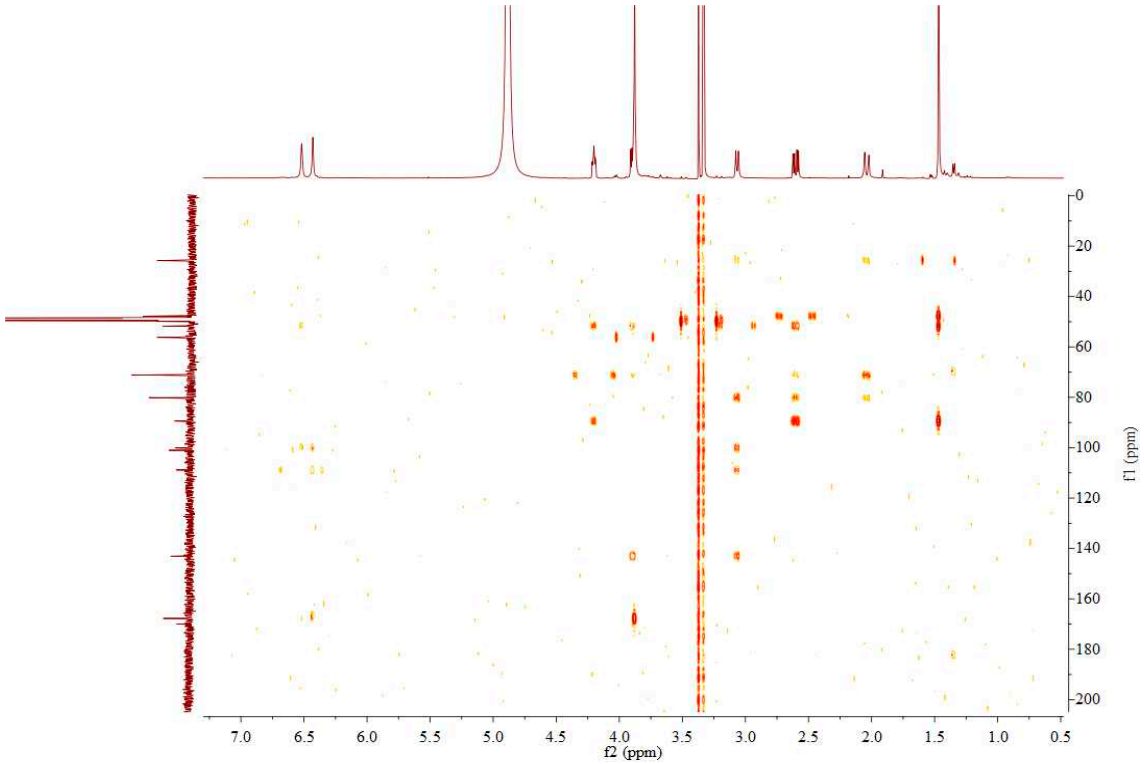

Figure S7. HMBC spectrum of **1** in DMSO-d_6.

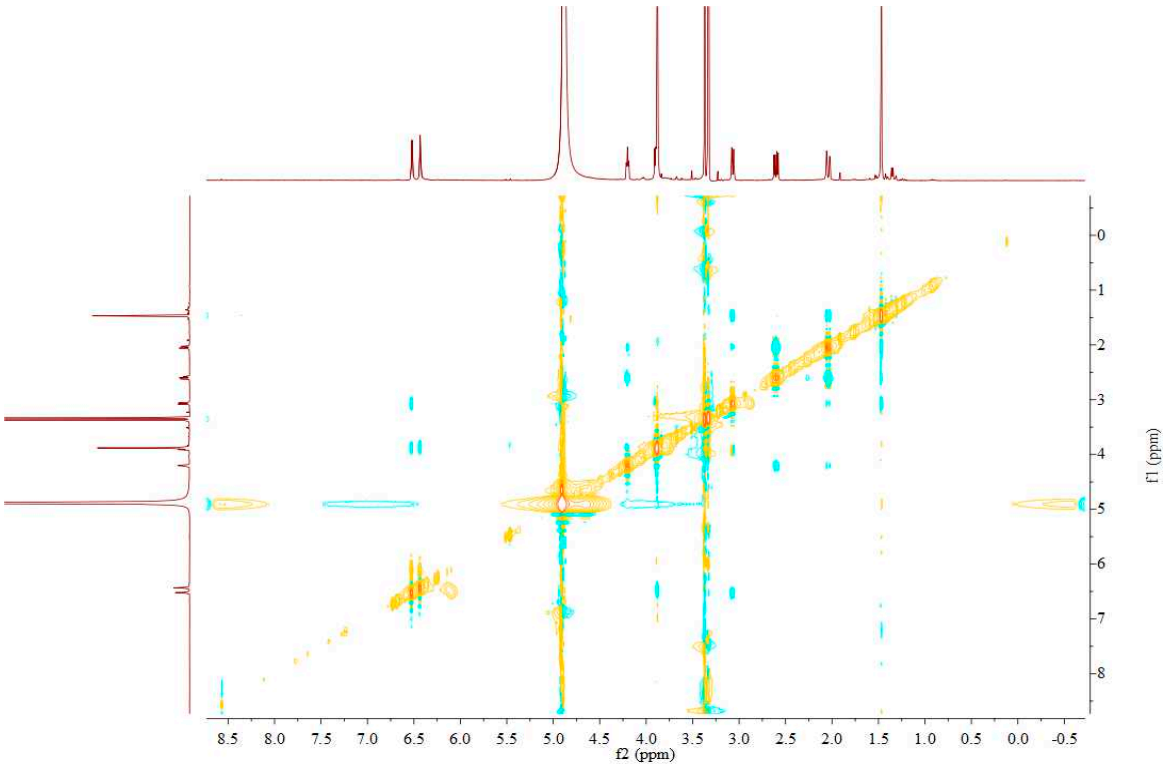

Figure S8. NOESY spectrum of **1** in DMSO-d_6.

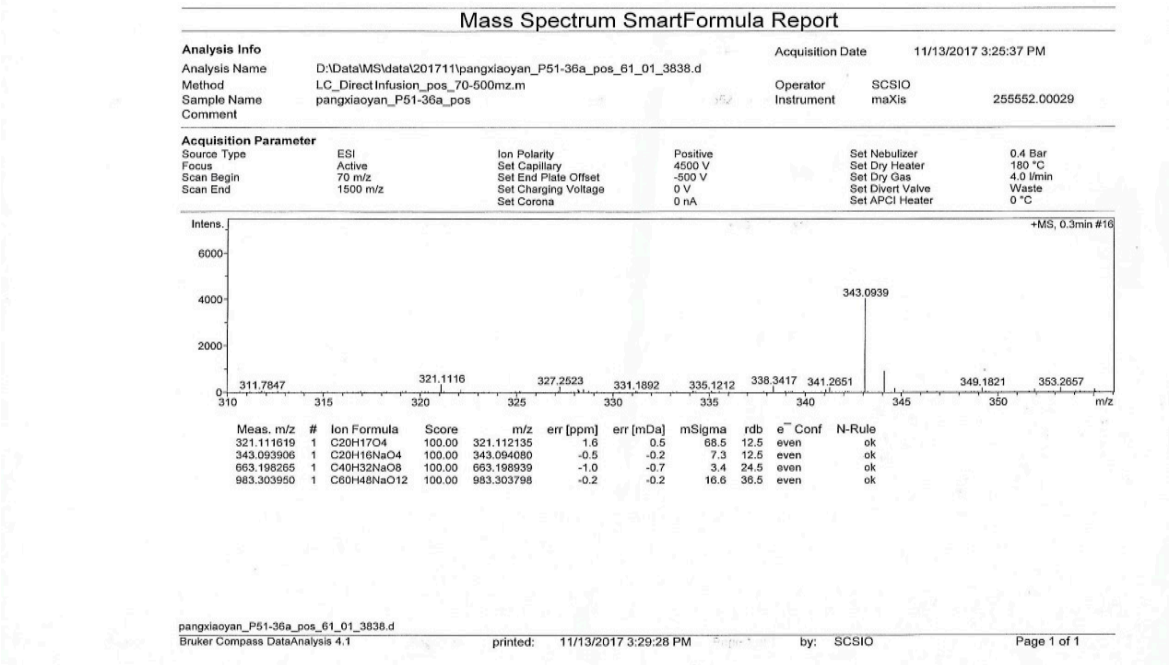

Figure S9. HRESIMS spectrum of **1** in CD_3OD.

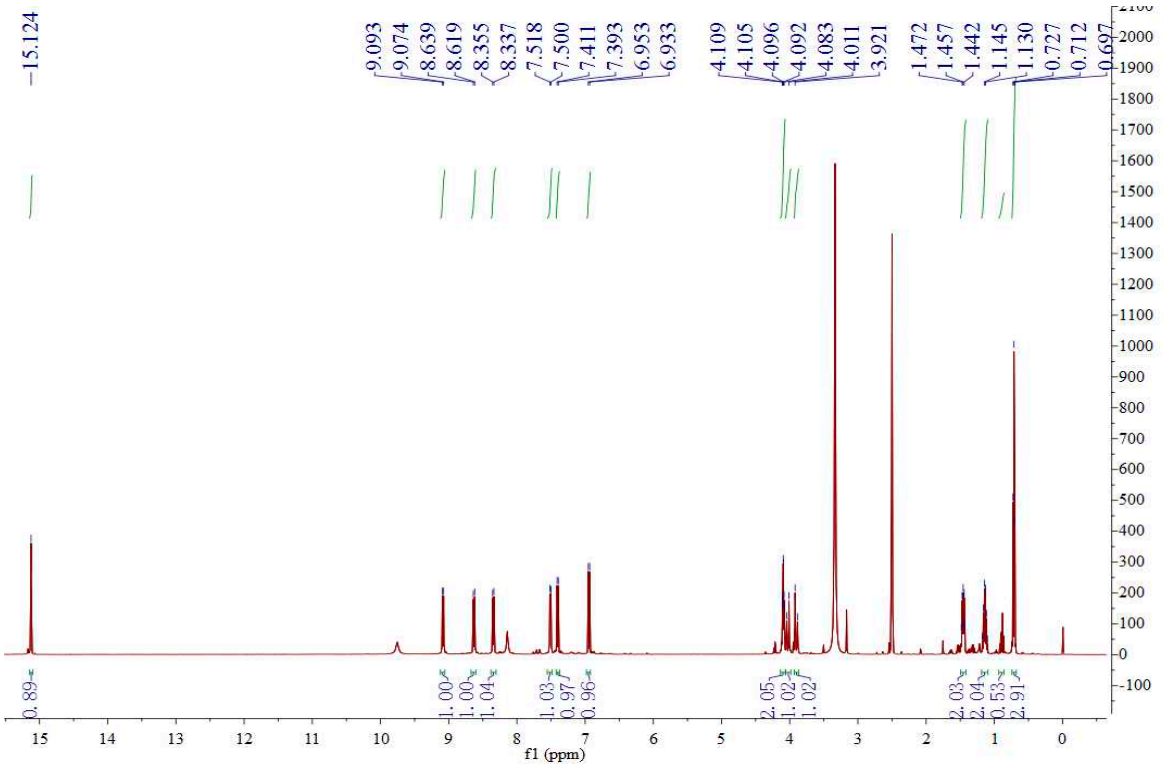

Figure S10. ^{1}H NMR spectrum of **2** in DMSO-*d*$_6$.

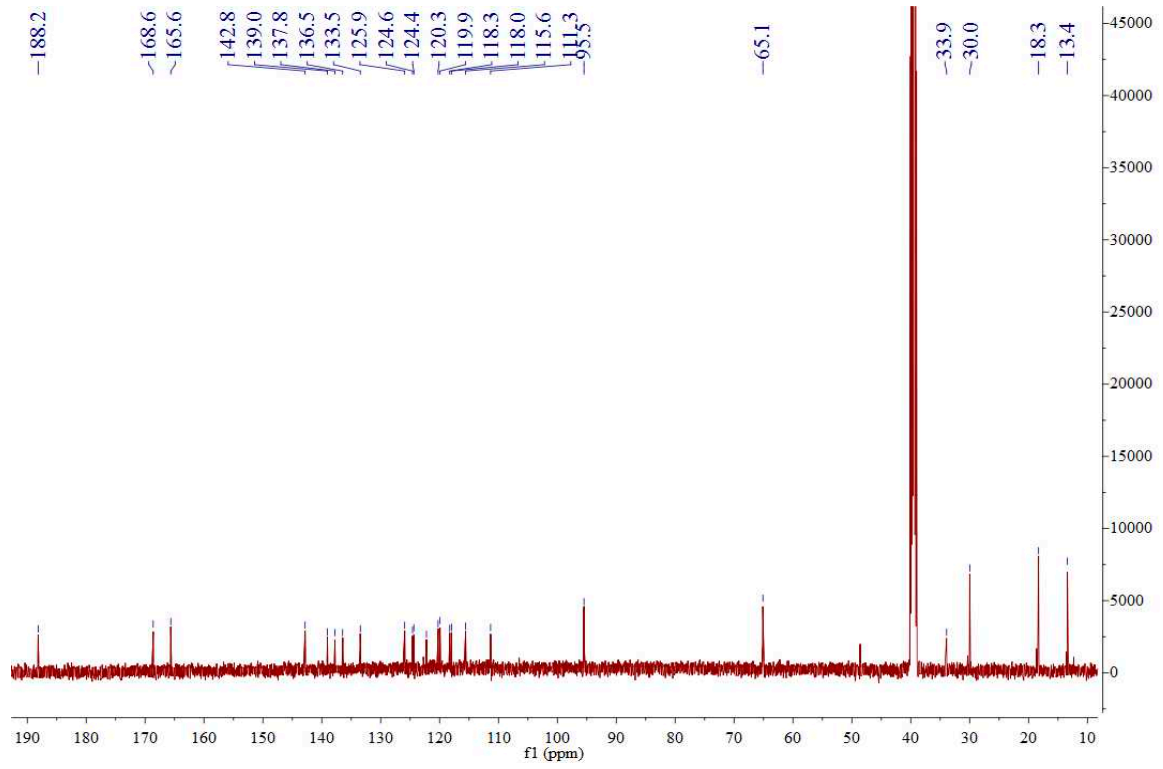

Figure S11. ^{13}C NMR spectrum of **2** in DMSO-*d*$_6$.

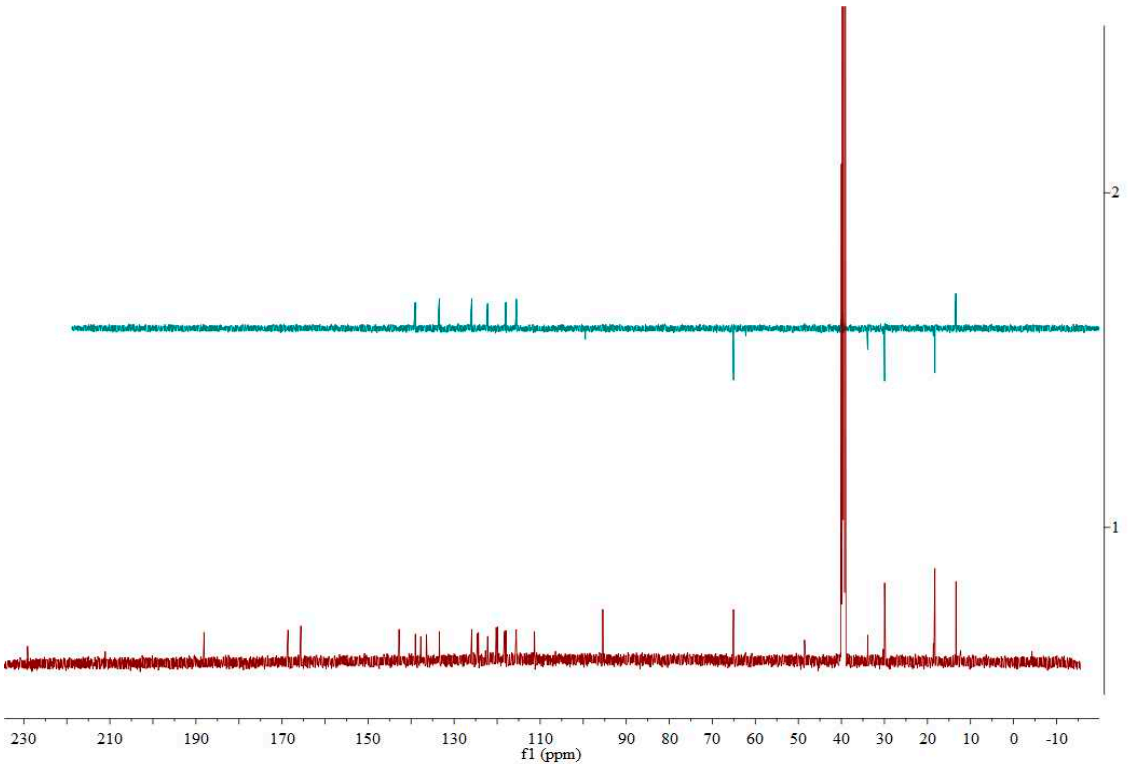

Figure S12. DEPT NMR spectrum of **2** in DMSO-d_6.

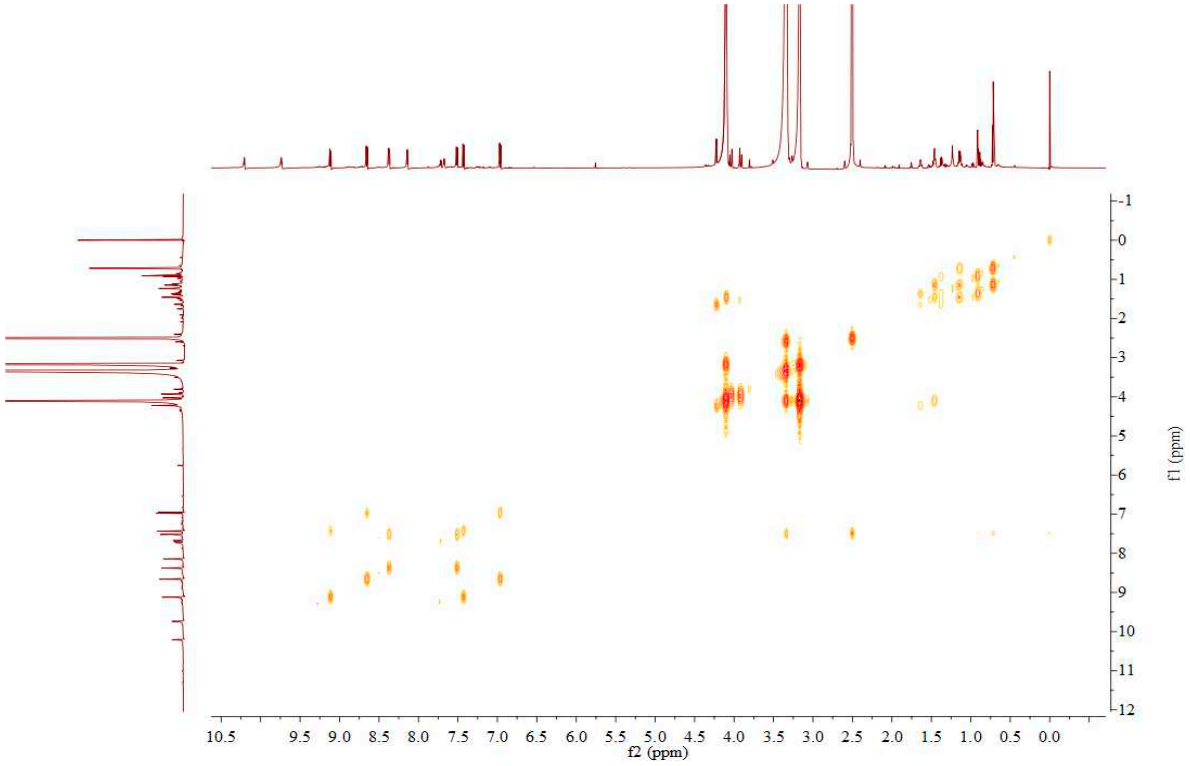

Figure S13. ^{1}H-^{1}H COSY spectrum of **2** in DMSO-d_6.

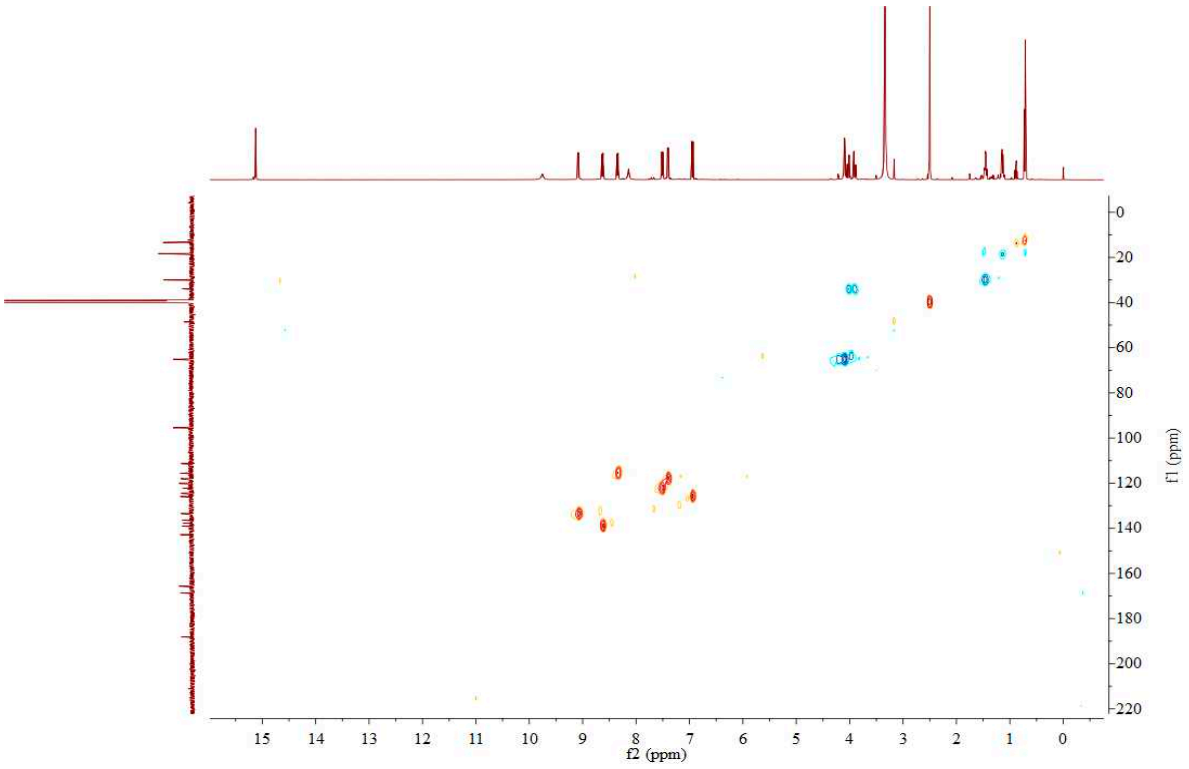

Figure S14. HSQC spectrum of **2** in DMSO-d_6.

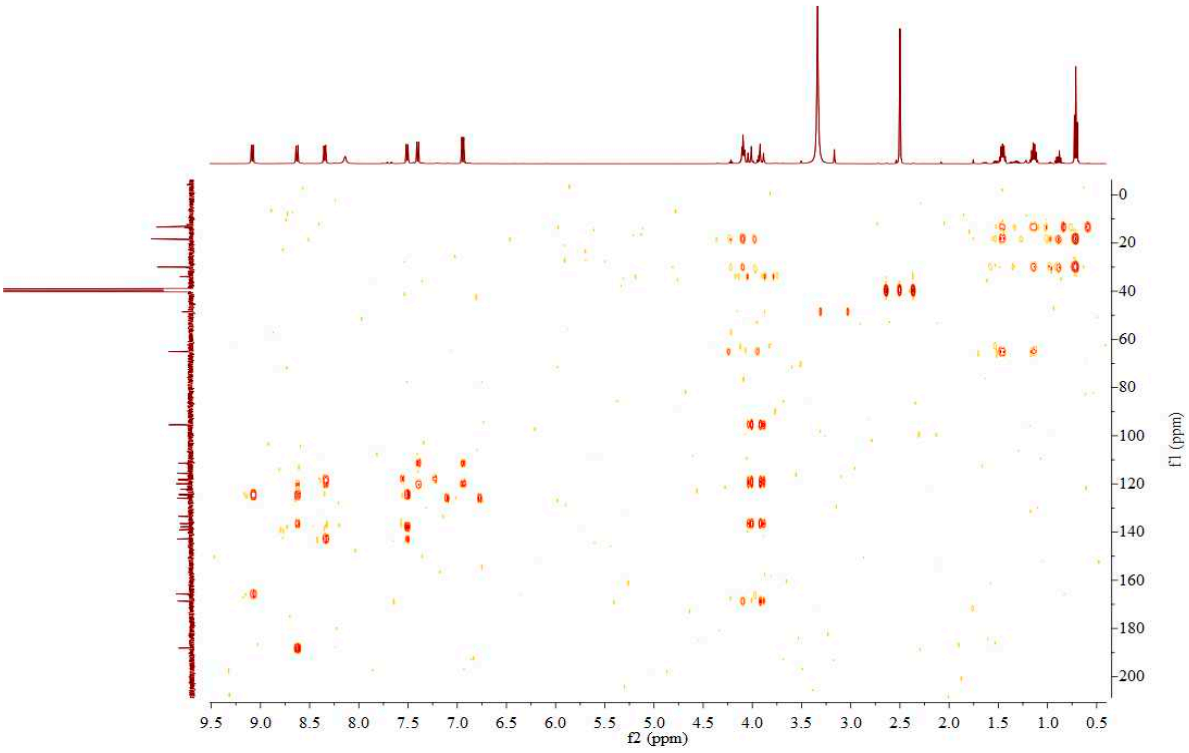

Figure S15. HMBC spectrum of **2** in DMSO-d_6.

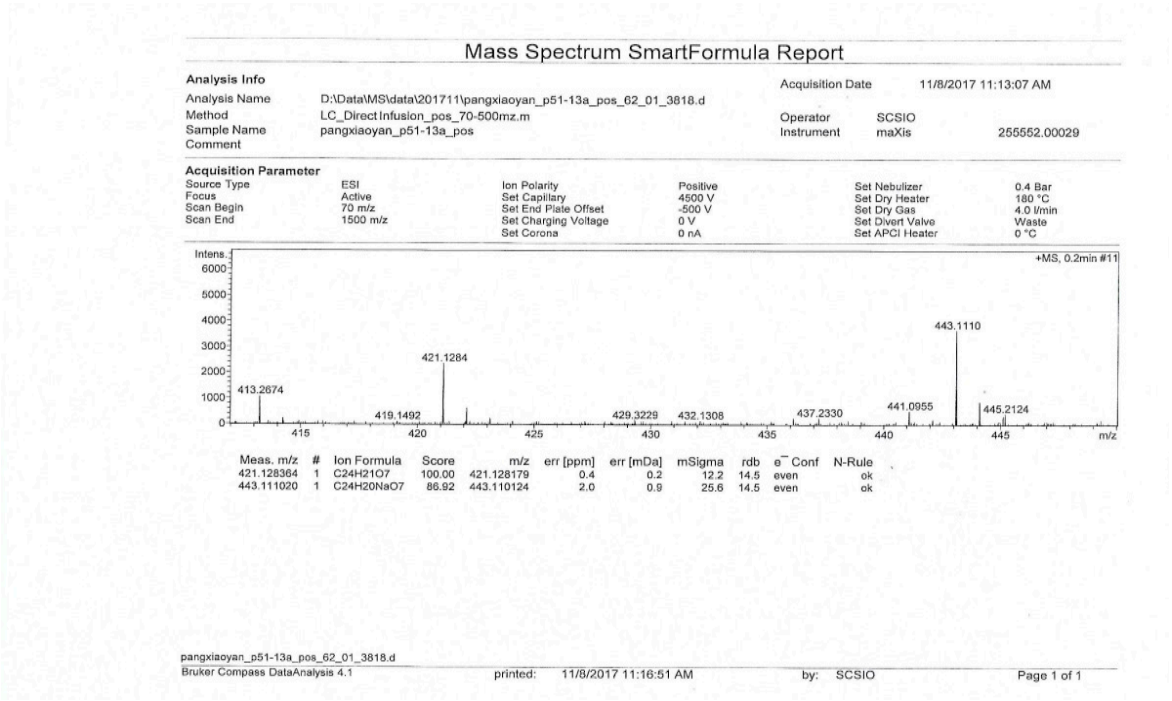

Figure S16. HRESIMS spectrum of **2** in CD_3OD.

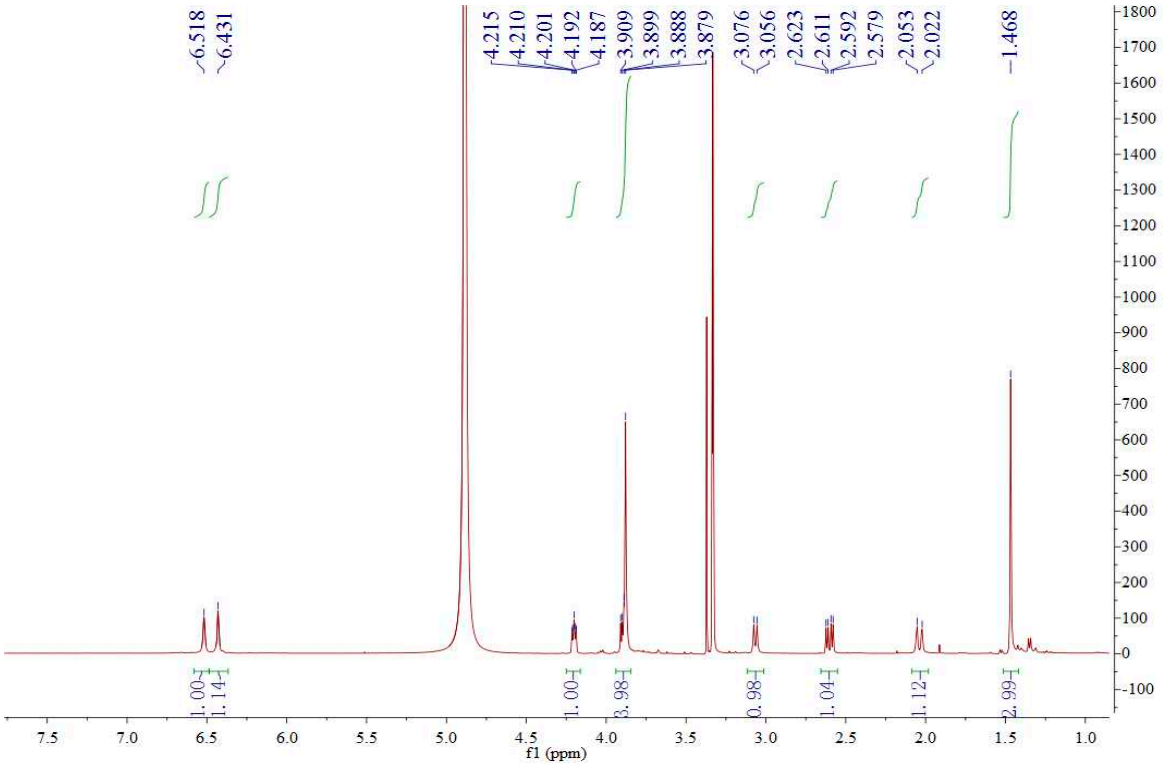

Figure S17. ^1H NMR spectrum of **3** in CD_3OD.

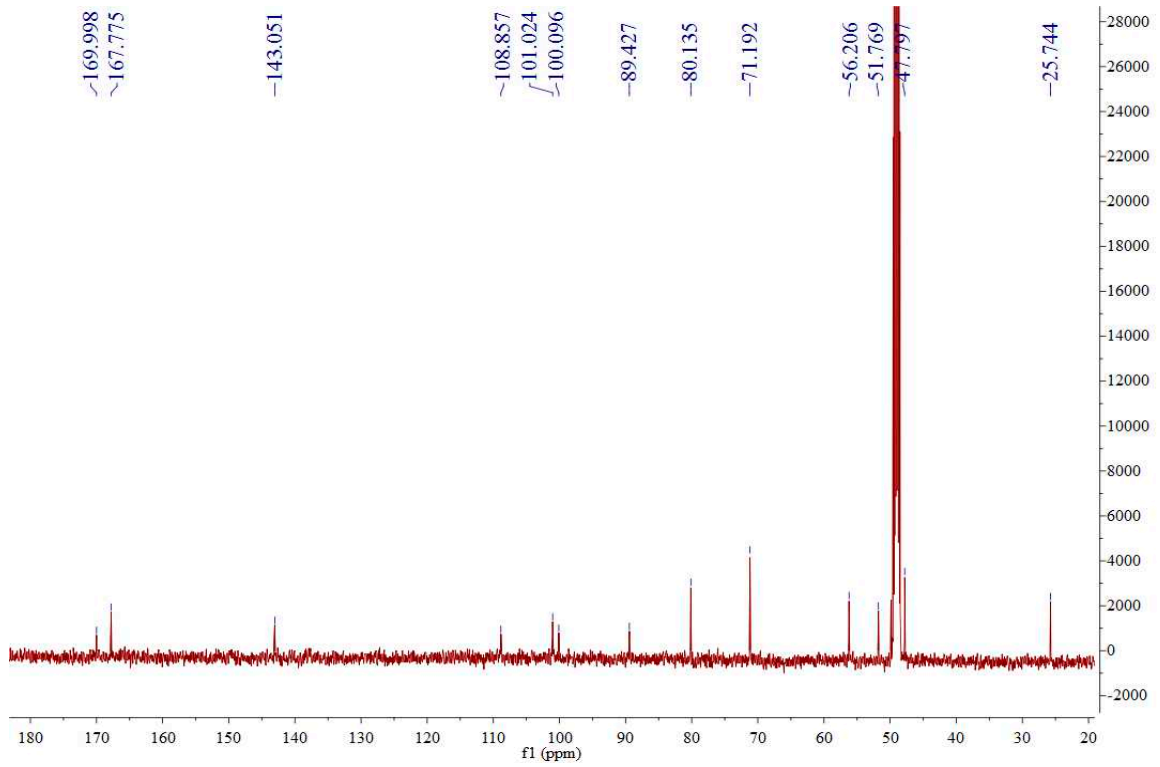

Figure S18. ^{13}C NMR spectrum of **3** in CD_3OD.

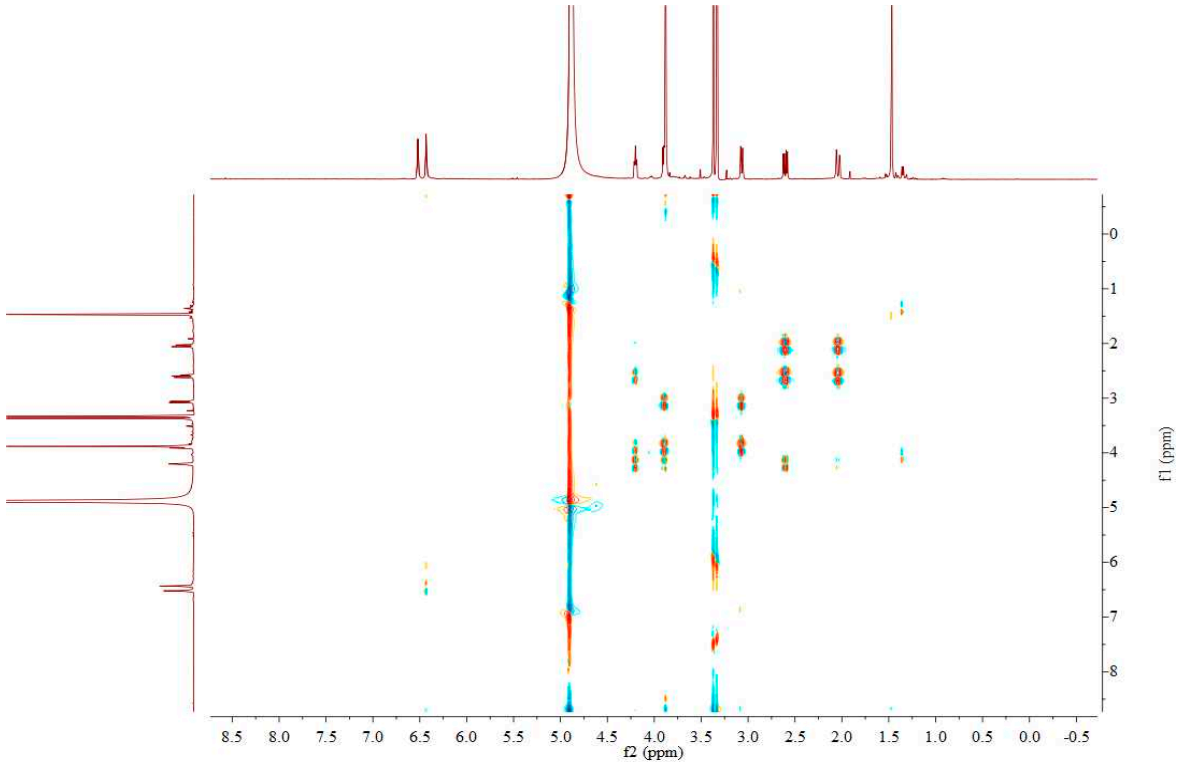

Figure S19. ^{1}H-^{1}H COSY spectrum of **3** in CD_3OD.

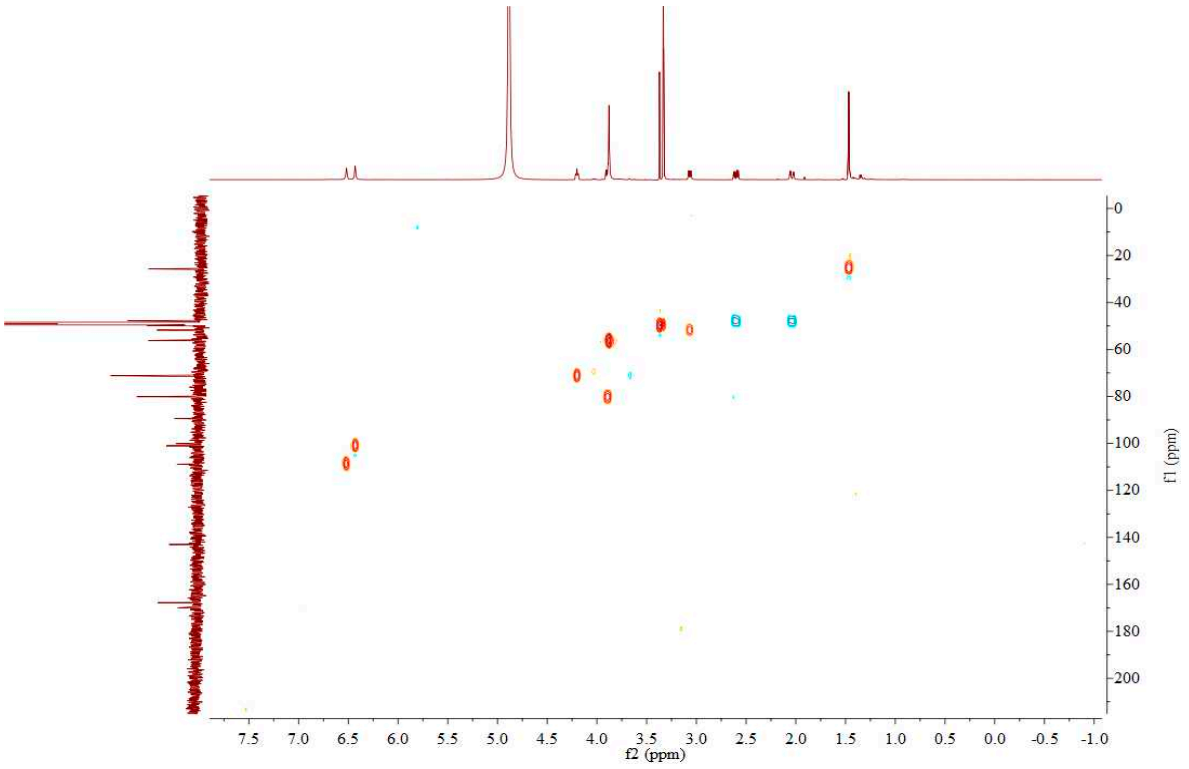

Figure S20. HSQC spectrum of **3** in CD_3OD.

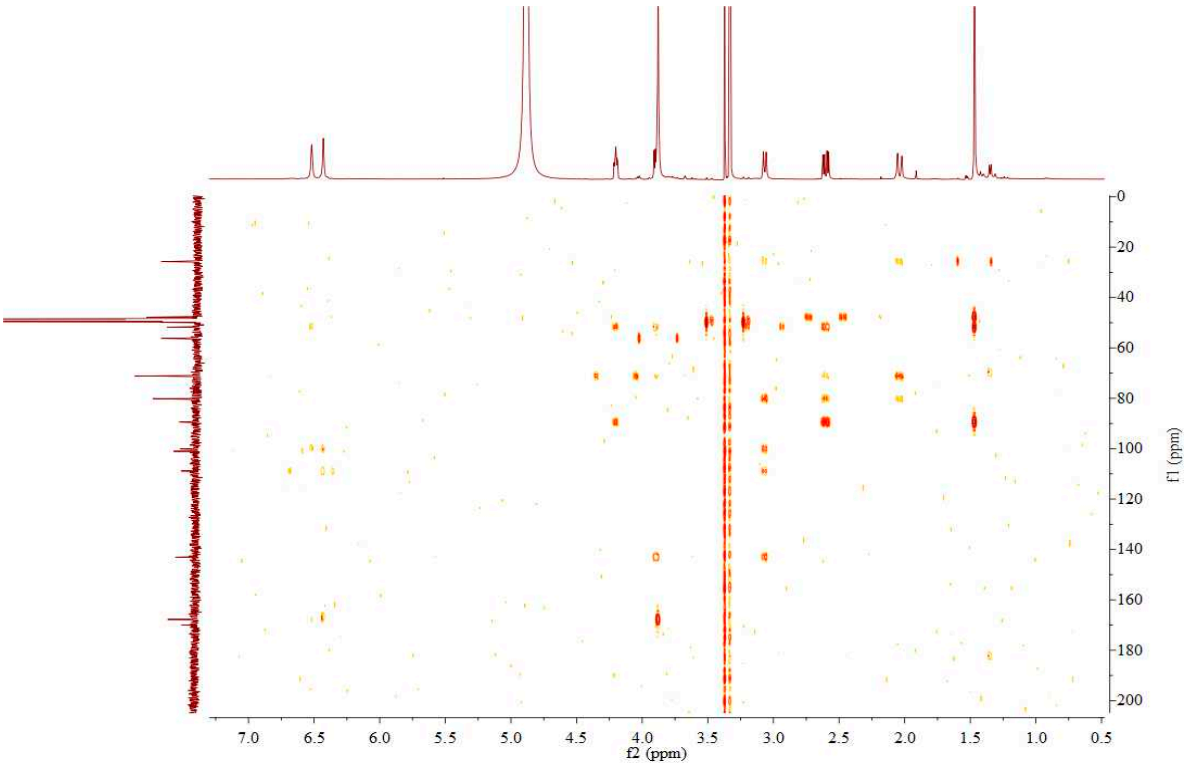

Figure S21. HMBC spectrum of **3** in CD_3OD.

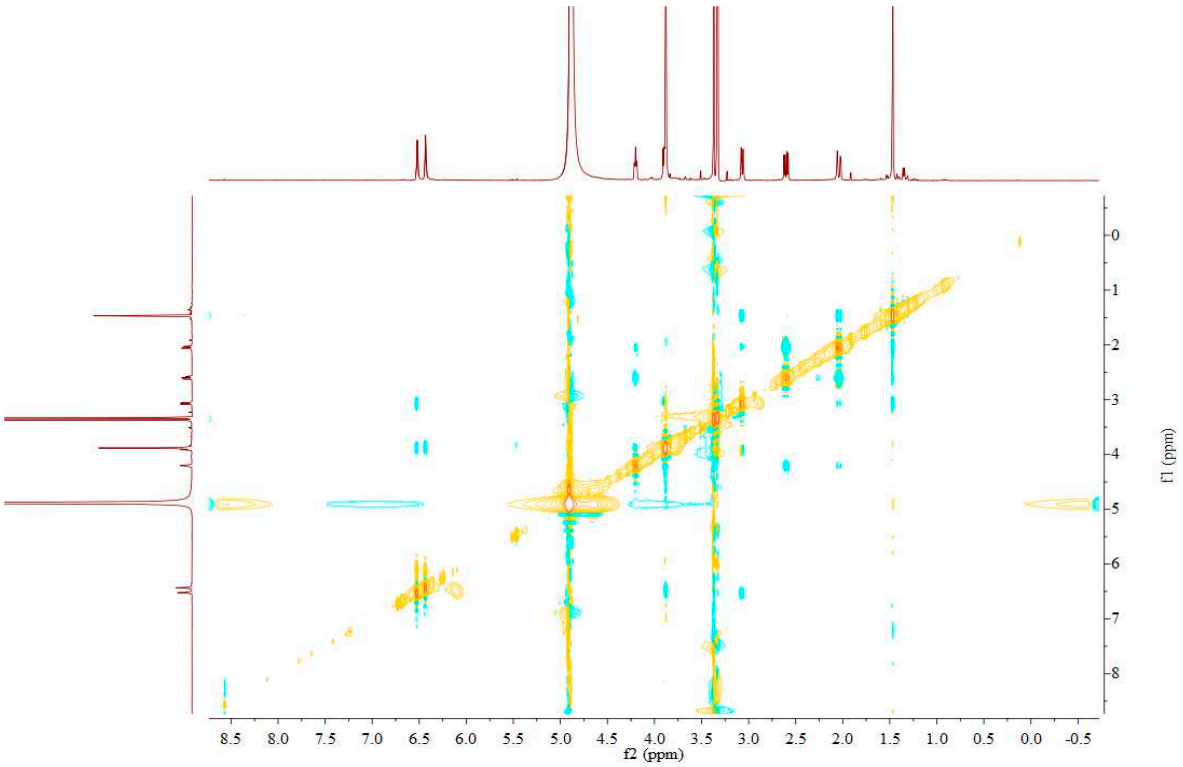

Figure S22. NOESY spectrum of **2** in CD_3OD.

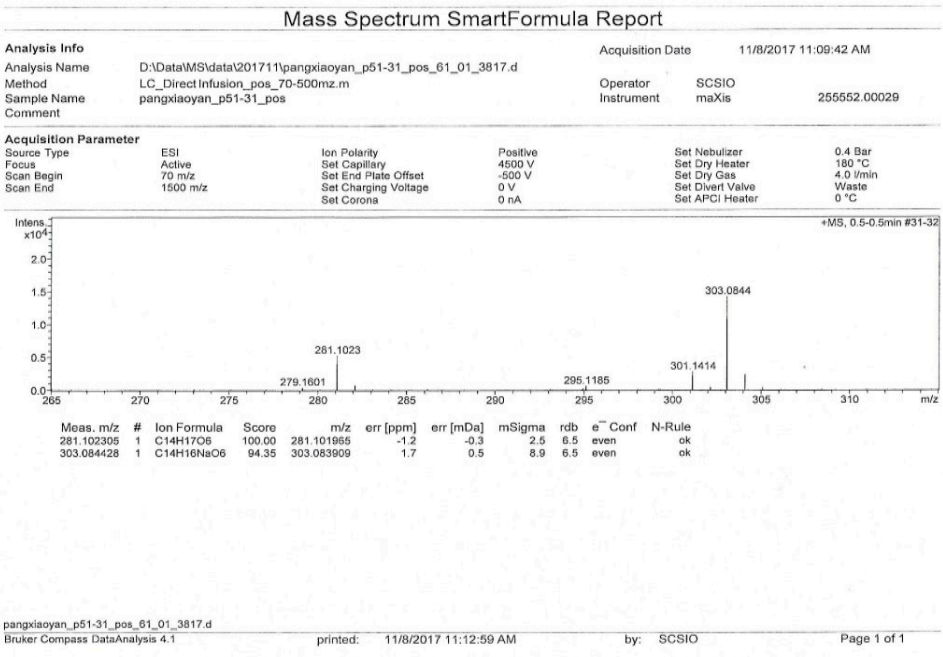

Figure S23. HRESIMS spectrum of **3** in CD_3OD.

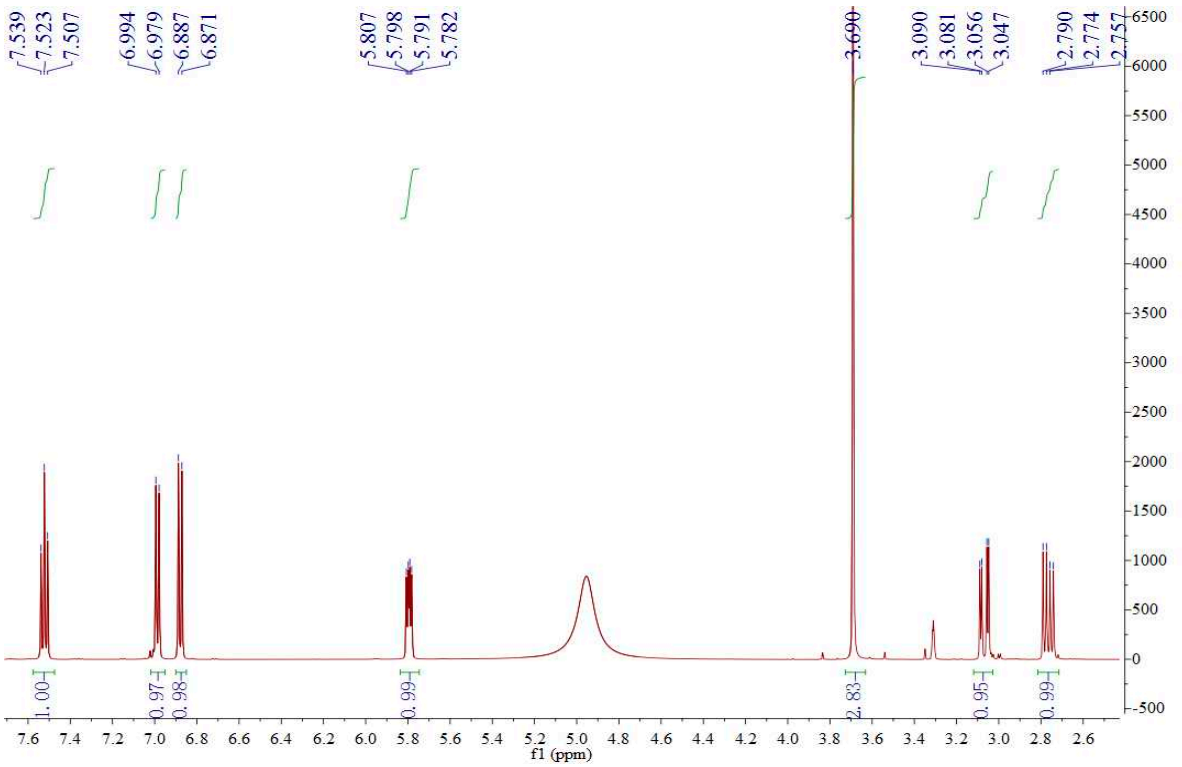

Figure S24. ^{1}H NMR spectrum of **4/5** in CD_3OD.

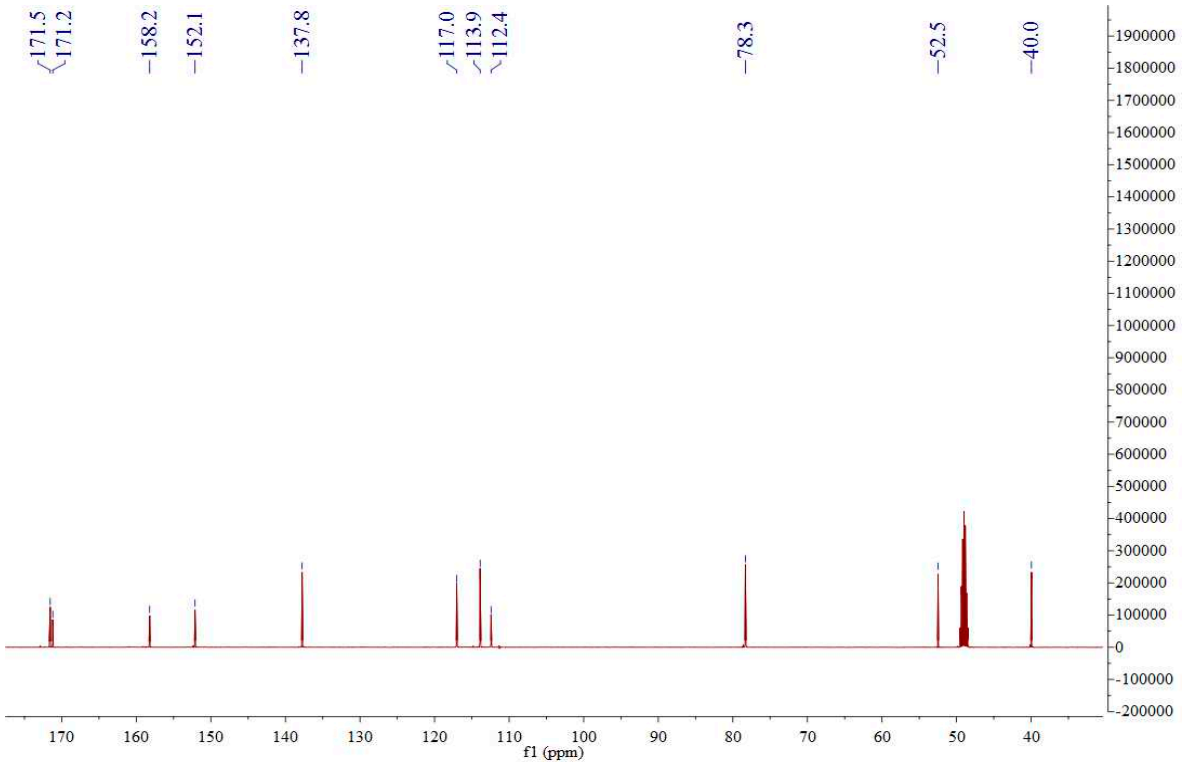

Figure S25. ^{13}C NMR spectrum of **4/5** in CD_3OD.

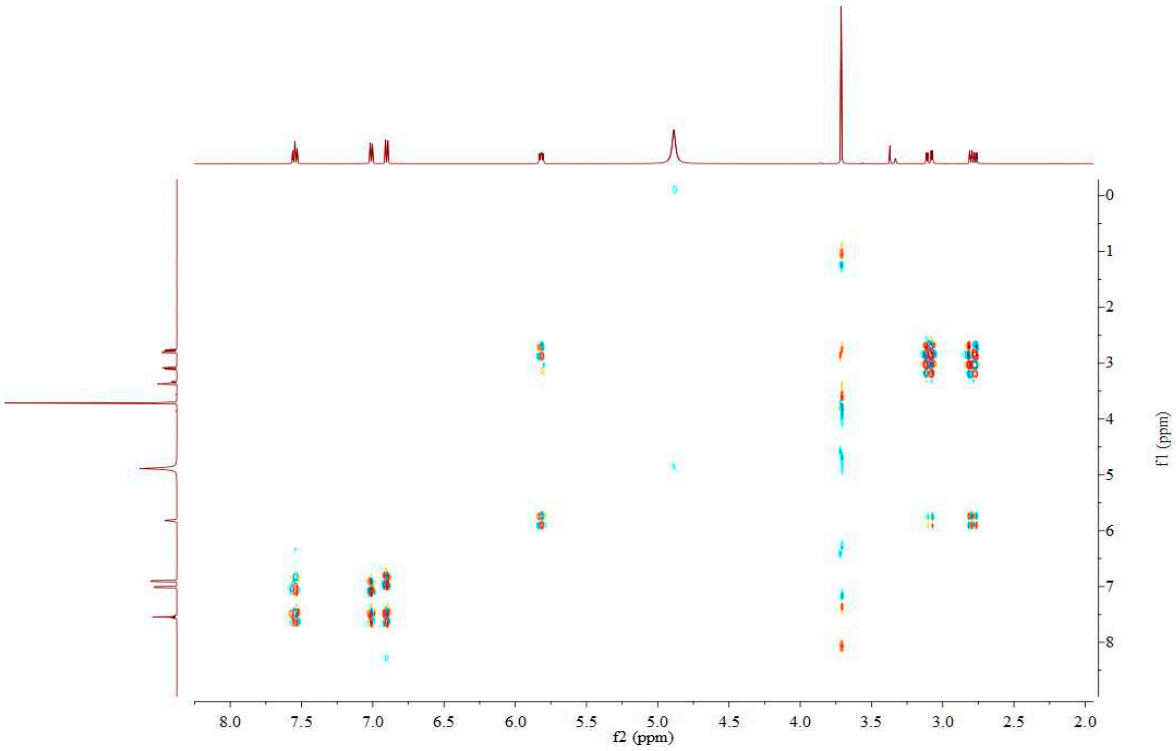

Figure S26. ^{1}H-^{1}H COSY spectrum of **4/5** in CD_3OD.

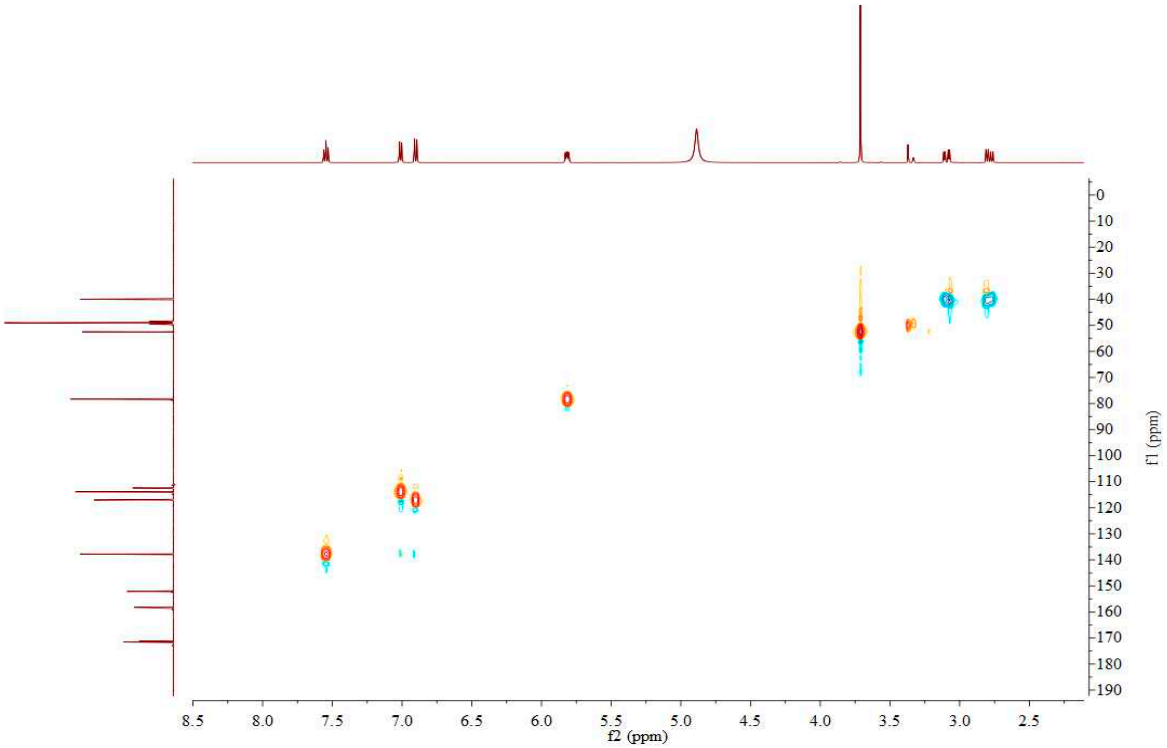

Figure S27. HSQC spectrum of **4/5** in CD_3OD.

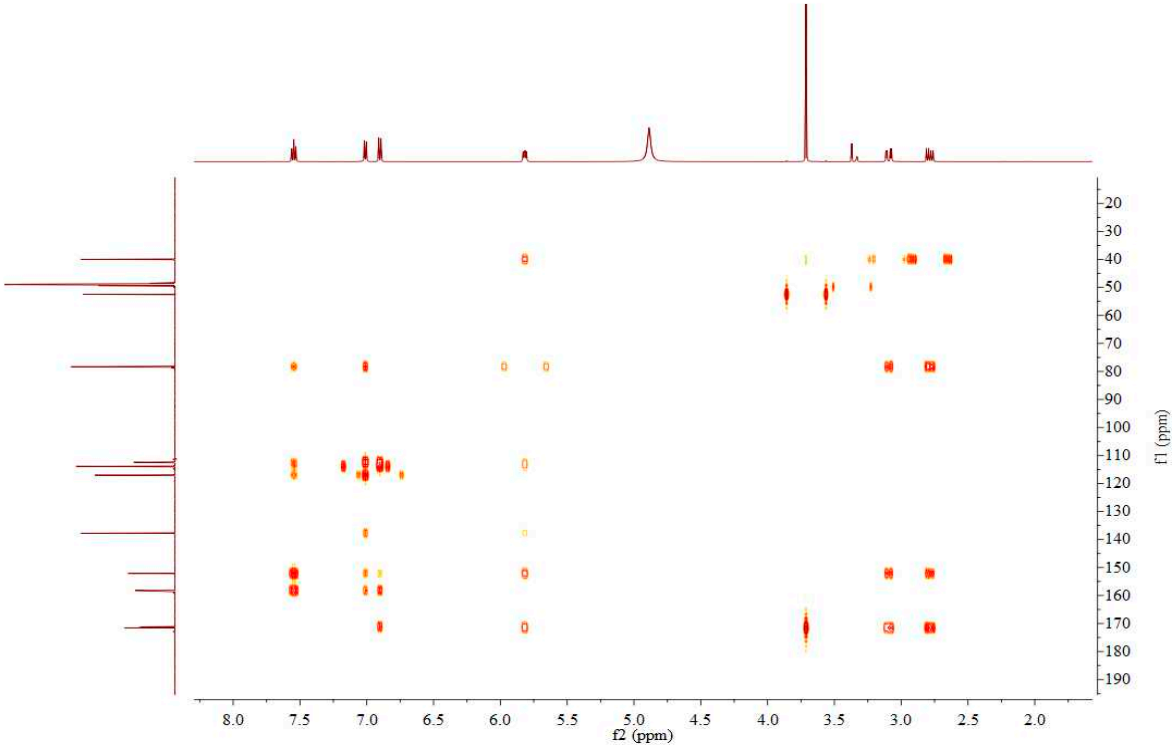

Figure S28. HMBC spectrum of **4/5** in CD_3OD.

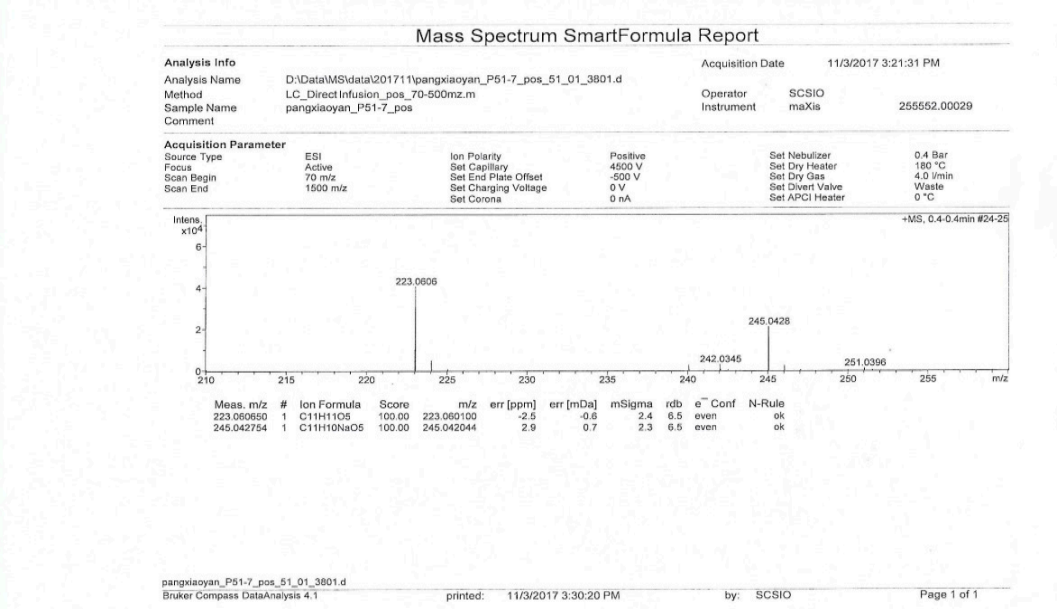

Figure S29. HRESIMS spectrum of **4/5** in CD_3OD.

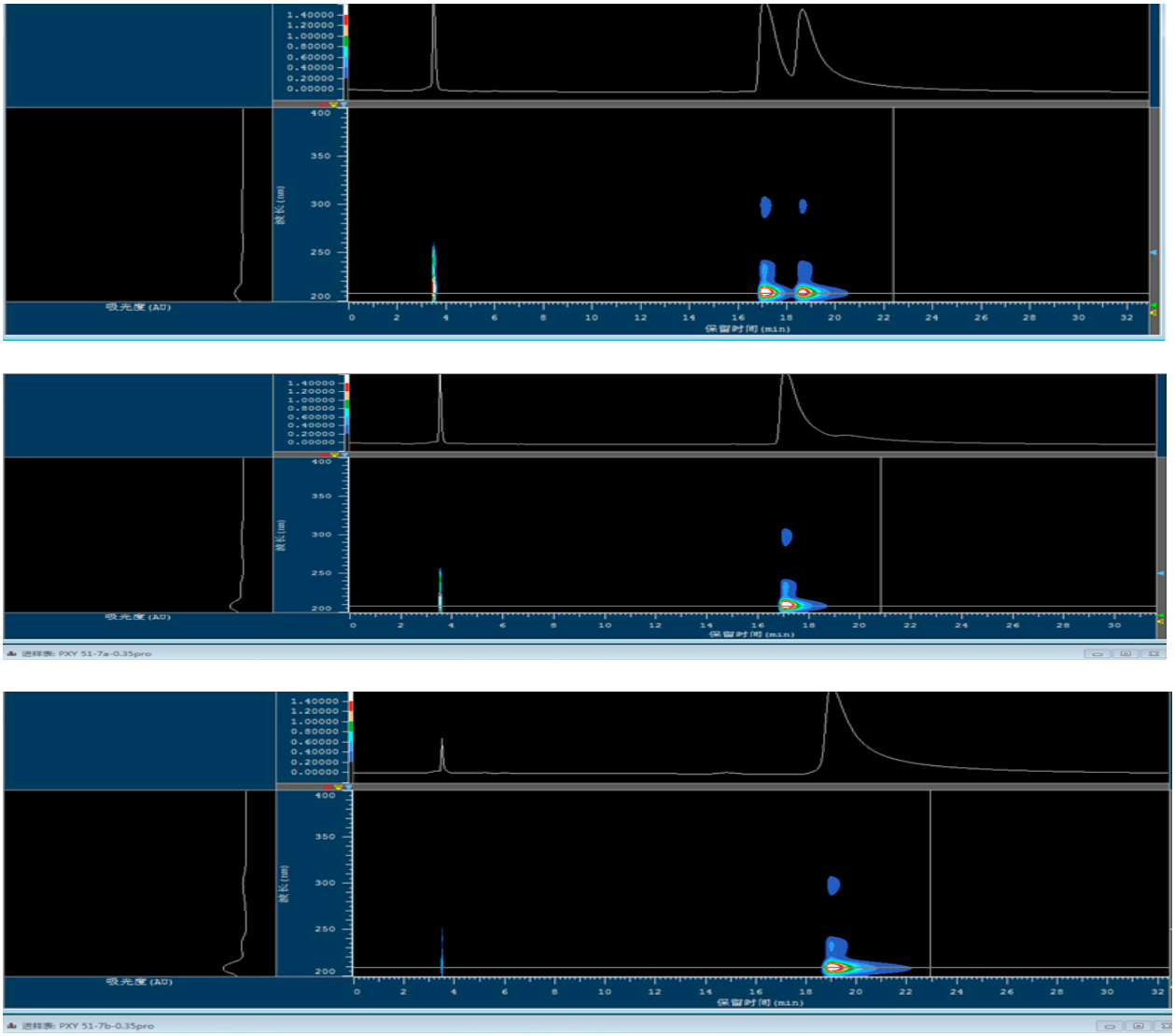

Figure S30. Chiral HPLC analyses of compounds **4** and **5** (Daicel Chiraloak IC-3 column, 4.6 mm × 25 mm, eluent n-hexane–iso-propanol, 65:35 *v*/*v*, 1 mL/min).

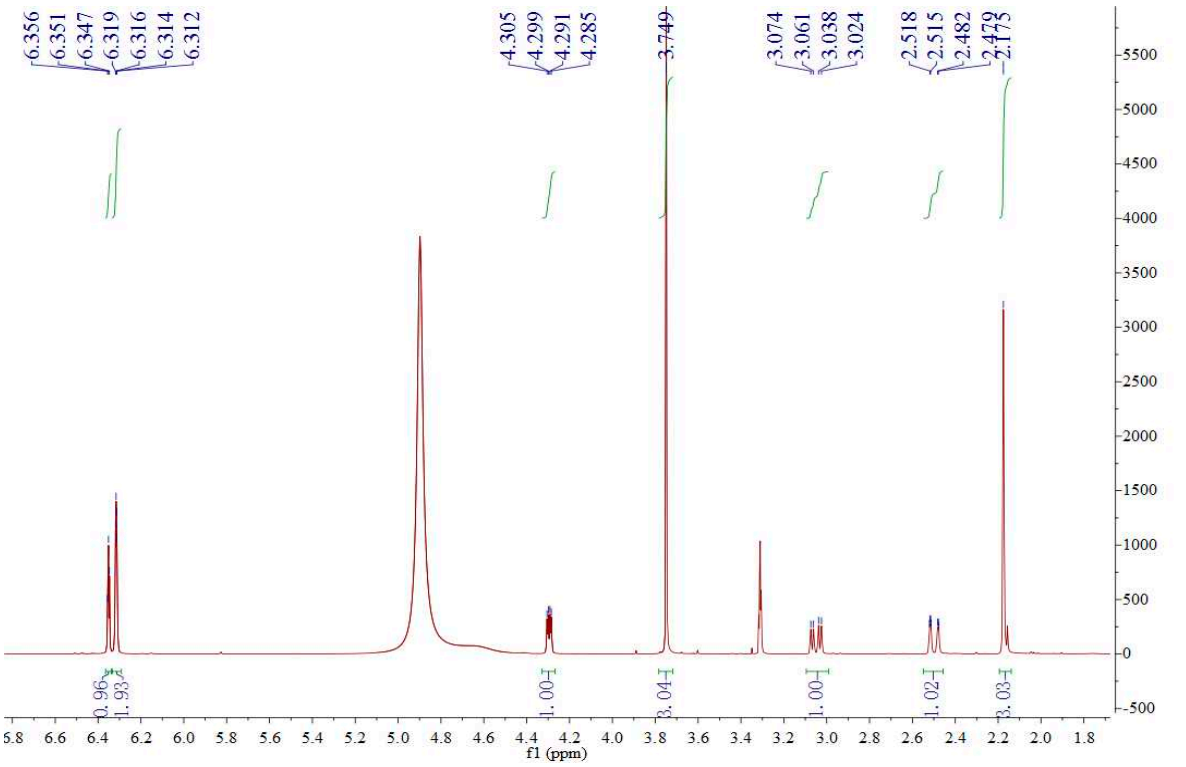

Figure S31. ^{1}H NMR spectrum of **6/7** in CD_3OD.

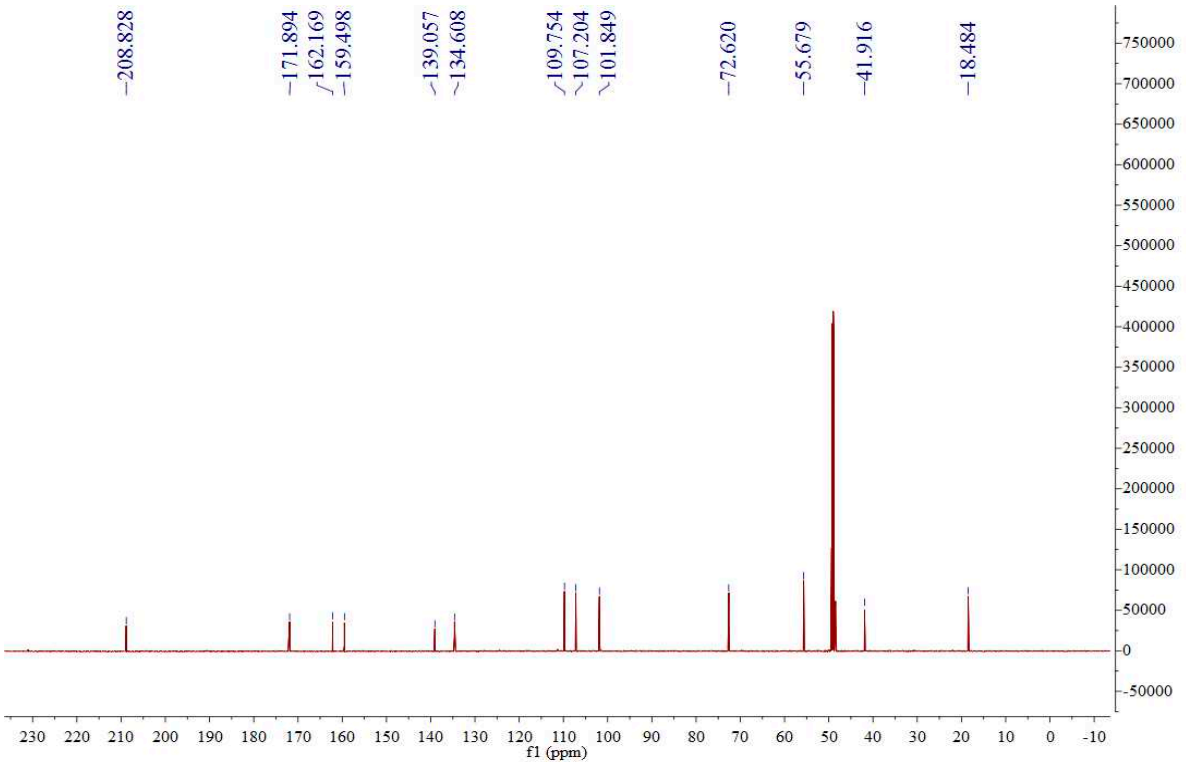

Figure S32. ^{13}C NMR spectrum of **6/7** in CD_3OD.

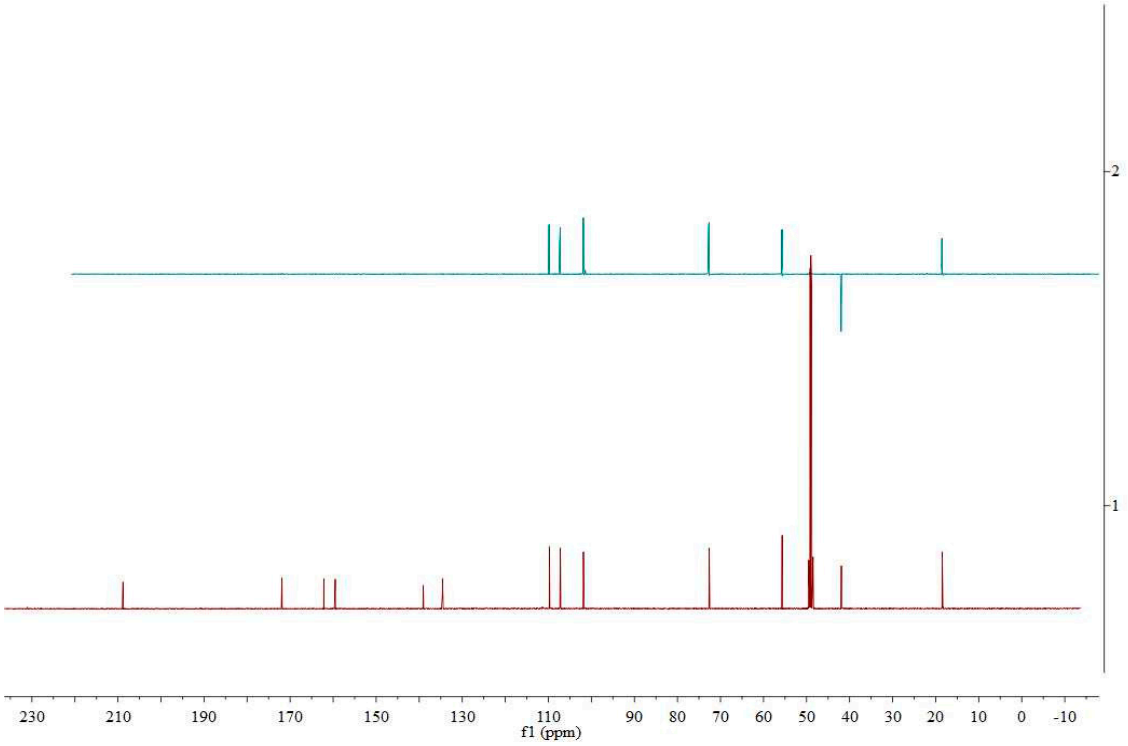

Figure S33. DEPT NMR spectrum of **6/7** in CD_3OD.

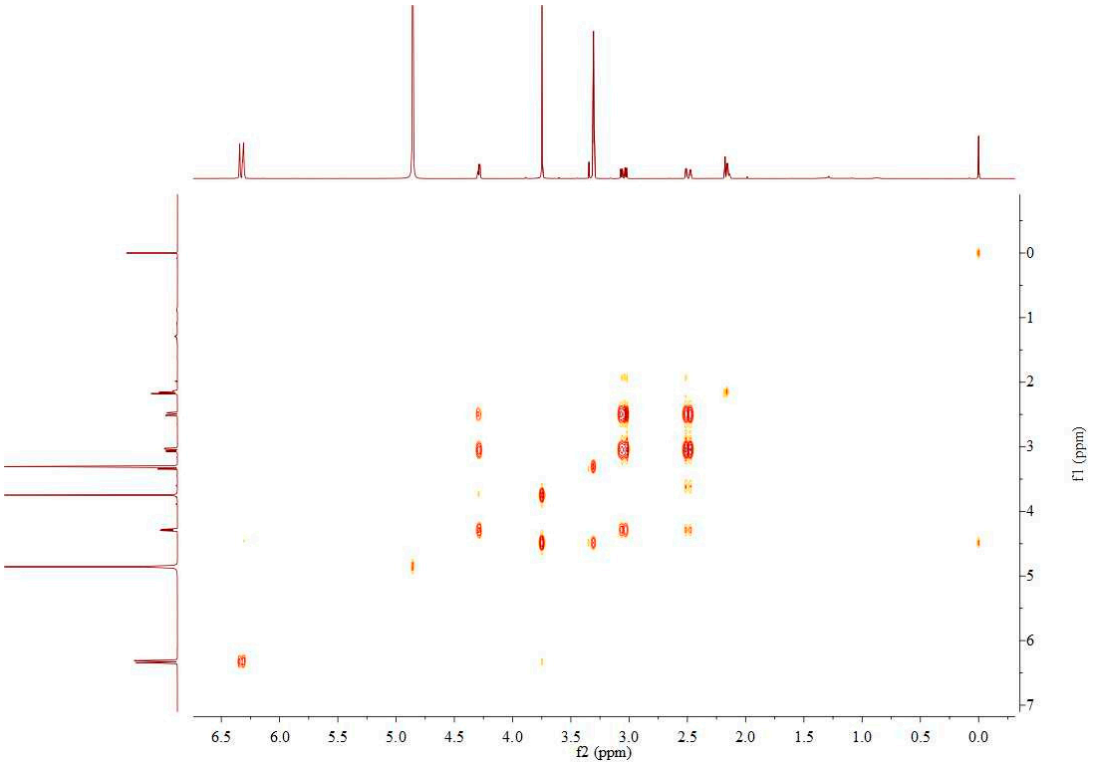

Figure S34. COSY spectrum of **6/7** in CD_3OD.

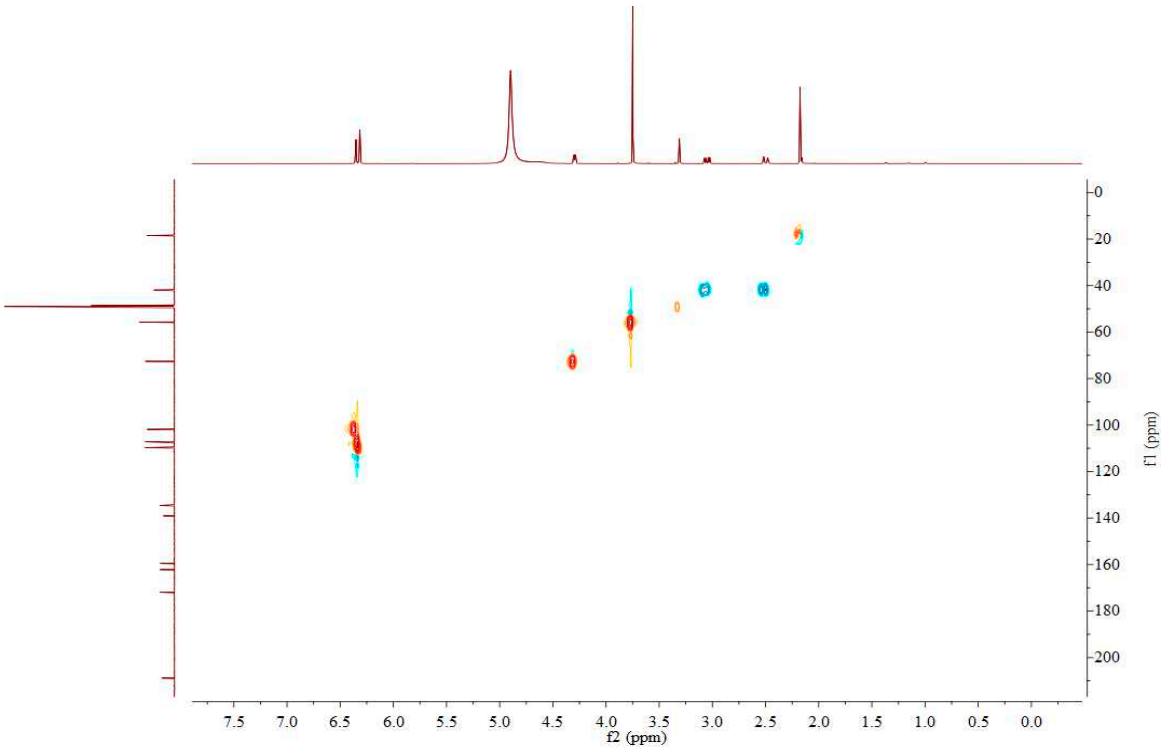

Figure S35. HSQC spectrum of **6/7** in CD_3OD.

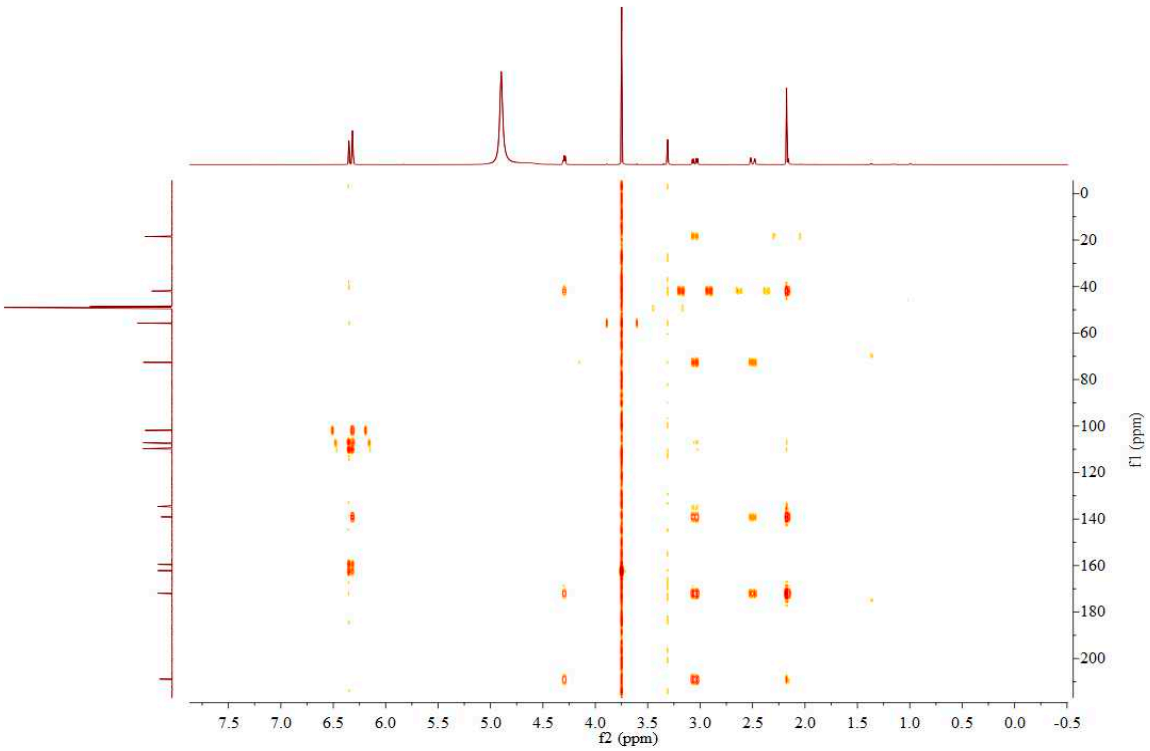

Figure S36. HMBC spectrum of **6/7** in CD_3OD.

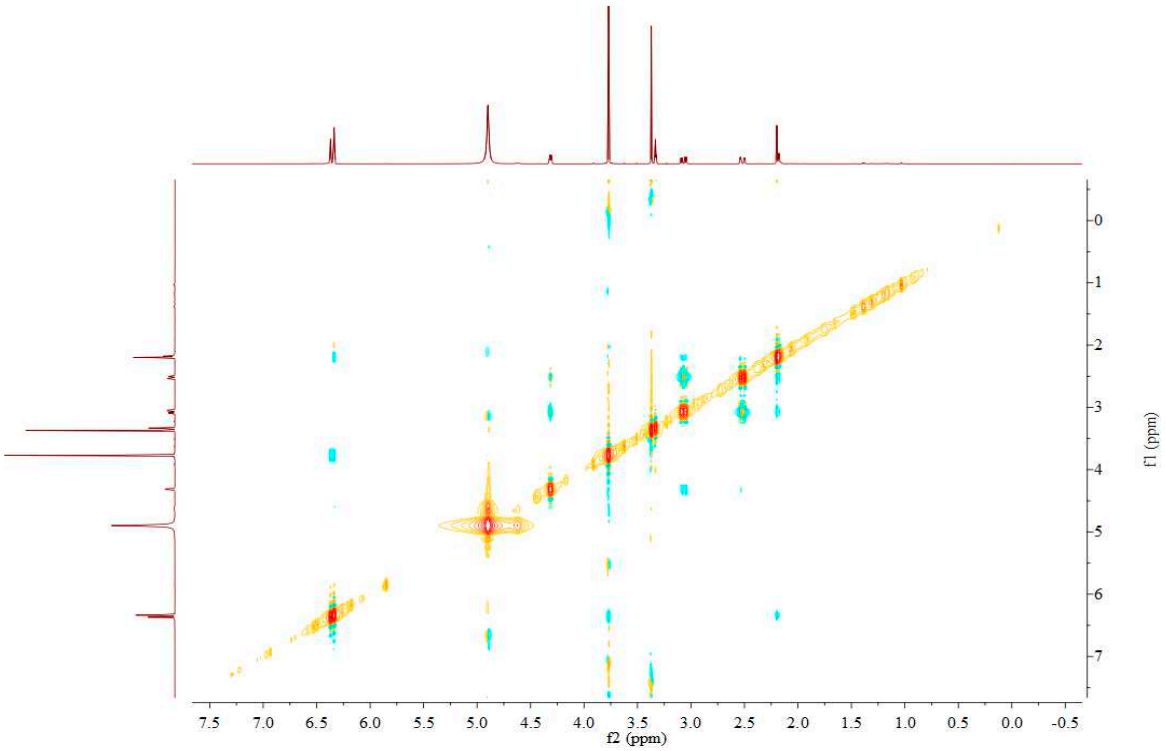

Figure S37. NOESY spectrum of **6/7** in CD_3OD.

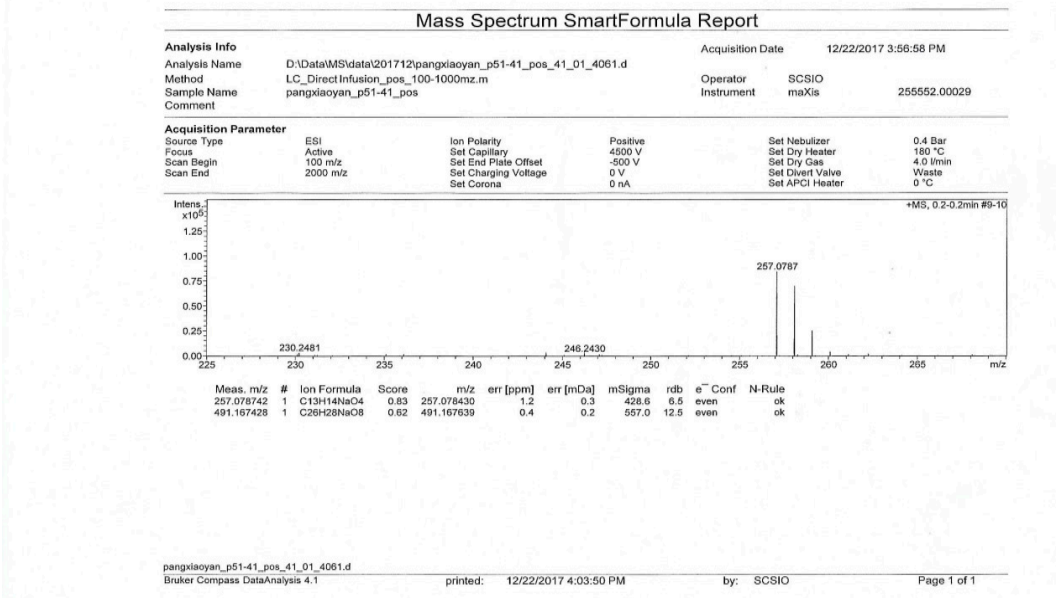

Figure S38. HRESIMS spectrum of **6/7** in CD_3OD.

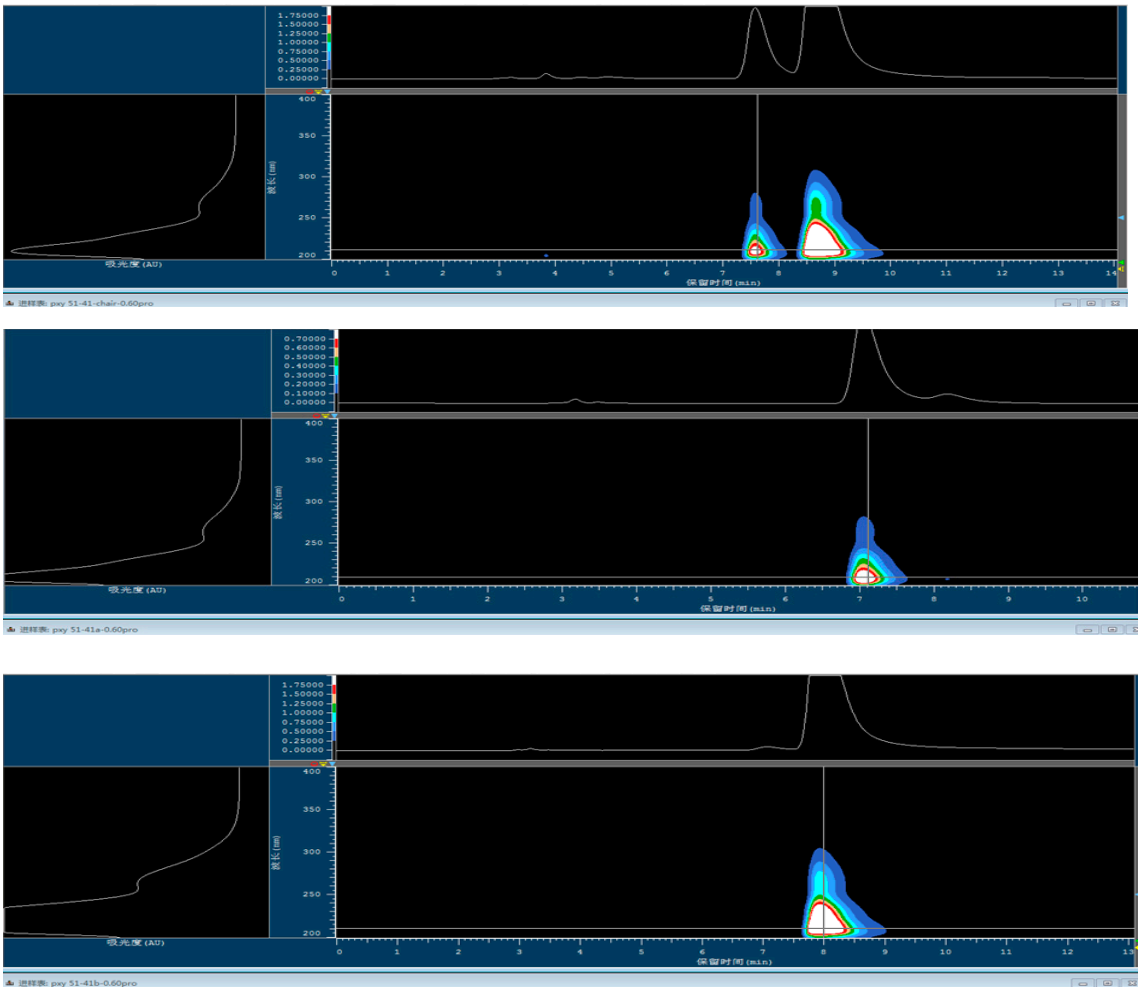

Figure S39. Chiral HPLC analyses of compounds **6** and **7** (Phenomenex Lux Cellulose-2, 4.6 mm × 25 mm, eluent n-hexane–iso-propanol, 40:60 *v*/*v*, 1 mL/min).