Supporting Information

Rhodomollanol A, a Highly Oxygenated Diterpenoid with a 5/7/5/5 Tetracyclic Carbon Skeleton from the Leaves of *Rhododendron molle*

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1. Experimental Section

1.1. General Experimental Procedures.

Optical rotations were measured using a Perkin-Elmer 341 polarimeter. Melting points were recorded on a Beijing Tech X-5 microscopic melting point apparatus. The NMR spectra were acquired on a Bruker AM-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C). HRESIMS were detected on a Thermo Fisher LC-LTQ-Orbitrap XL and a Bruker micrOTOF II spectrometer by the positive-ion mode. Samples were purified by preparative HPLC using an Agilent 1200 quaternary system with a UV detector and a C_{18} column (5 μM, 10 × 250 mm, YMC-pack ODS-A). Silica gel of 100–200, 200–300, and 300–400 mesh (Qingdao Marine Chemical Factory in China), ODS with 50 μm (ODS-A-HG, YMC Co. Ltd., Japan) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) were applied to isolate and purify samples. The glass precoated silica gel GF254 plates (Yantai Chemical Industry Research Institute in China) were tested under UV light and wetting with 10% H_2SO_4 in EtOH–H_2O before heating.

1.2. Plant Material.

The leaves of *Rhododendron molle* G. Don were collected at the Qichun, Hubei province, P. R. China, in May 2014, and identified by Prof. Jianping Wang at Tongji Medical College, Huazhong University of Science and Technology. A voucher specimen (No. 20140520) has been deposited at the Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology.

1.3. Extraction and Isolation.

Dried leaves (25 kg) of *Rhododendron molle* were powered and extracted with EtOH−H_2O (95:5, v/v) (5 × 120 L) at room temperature (7 days each). Concentration of the combined percolates under reduced pressure afforded a dark brown crude extract (3.7 kg). The corresponding extract (3.7 kg) was suspended in 30 L H_2O (30 °C). After cooling to room temperature (15 °C), the extract was successively partitioned with

petroleum ether (PE), $CHCl_3$, EtOAc, and *n*-BuOH (seven times with 15 L each, respectively), yielding PE (335 g), $CHCl_3$ (1190 g), EtOAc (160 g), and *n*-BuOH (1155 g) fractions. The $CHCl_3$ fraction (1190 g) was separated by a silica gel column (100−200 mesh) with an eluent of CH_2Cl_2−Acetone (20:1−0:1, v/v) to get six fractions (A−F) on the basis of TLC analysis. Fraction D (eluted with CH_2Cl_2−Acetone 3:1, 102.5 g) was further resolved on a silica gel column (200−300 mesh) and eluted in a gradient of PE−Acetone (8:1−0:1, v/v) to obtain six fractions (D1−D6). Fraction D3 (eluted by PE−Acetone 3:1, 8.9 g) was chromatographed on a Sephadex LH-20 column eluted with methanol to yield four subfractions D3A−D3D. Subfraction D3A (1.9 g) was loaded onto an ODS C_{18} column and eluted in a gradient of MeOH−H_2O (1:9−10:0, v/v) to afford four parts (D3Aa−D3Dd). Subfraction D3Ab (eluted by 20% MeOH, 160 mg) was further purified by RP C_{18} HPLC, with MeCN−H_2O (30:70, v/v) 1.5 mL/min, to afford compound **1** (t_R 28.2 min, 6.0 mg, 0.000024 %). Subfraction D3Aa (eluted by 10% MeOH, 720 mg) was subjected to chromatography on a silica gel column (300−400 mesh) using PE−Acetone (5:1 to 0:1) to yield four subfractions D1Aa1−D1Aa4. Finally, subfraction D1Aa2 (eluted by PE−Acetone 3:1, 110 mg) was further purified by RP C_{18} HPLC, with MeOH−H_2O (60:40, v/v) 1.5 mL/min, to yield compounds **2** (t_R 36.5 min, 7.9 mg, 0.0000316 %) and **3** (t_R 29.2 min, 12.9 mg, 0.0000516 %).

1.4. Physical and chemical properties of 1 and 2.

*Rhodomollanol A (**1**):* a colorless prism; mp 187−188 °C; $[\alpha]^{20}_D$ −44.9 (*c* 0.1, MeOH); HRESIMS *m/z* 371.1825 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 371.1834).

*Rhodomollein XXXI (**2**):* a colorless prism; mp 235−236 °C; $[\alpha]^{25}_D$ −24.7, (*c* 0.1, MeOH); HRESIMS *m/z* 373.1990 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

1.5. Single-crystal X-ray diffraction analysis for 1 and 2.

Crystal X-ray diffraction data was measured on a Bruker APEX-II CCD diffractometer through a graphite-monochromatized Cu Kα radiation (λ = 1.54178 Å) at 298(2) K. Data was collected by Bruker APEX2 software. Bruker SAINT was applied to data reduction. Structure solution and refinement were

performed with the SHELXTL program package. All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms.

Crystallographic Data for Rhodomollanol A (1). Empirical formula: $C_{20}H_{32}O_{7.50}$. Formula weight: 392.46. Temperature: 297(2) K. Wavelength: 1.54178 Å. Monoclinic, C 2y, crystal size: 0.15 × 0.1 × 0.1 mm^3. Unit cell dimensions: a = 39.283(5) Å, b = 7.0114(9) Å, c = 7.3524(9) Å, α = 90°, β = 94.433°(2), γ = 90°. V = 2019.0(4) $Å^3$, Z = 4, D_{calcd} = 1.291 mg/cm^3. Absorption coefficient: 0.812 mm^{-1}. F(000) = 848, theta range for data collection: 6.04 to 68.95°, index ranges: −43<=h<=45, −8<=k<=7, −8<=l<=8. Reflections collected 10122, independent reflections: 3421 [R(int) = 0.1058]. Completeness to theta = 68.95°, 97.3%. Largest diff. peak and hole: 0.707 and −0.346 e.$Å^{-3}$. Refinement method was Full-matrix least-squares on F^2, with Goodness-of-fit on F^2 = 1.013. Data / restraints / parameters: 3421 / 7 / 258. Final R indices [I > 2sigma(I)]: R_1 = 0.0602, wR_2 = 0.1627, R indices (all data): R_1 = 0.0639, wR_2 = 01640. Flack parameter −0.04(19). Extinction coefficient 0.0040(6).

Crystallographic Data for Rhodomollein XXXI (2). Empirical formula: $C_{20}H_{30}O_5$. Formula weight: 350.44. Temperature: 298(2) K. Wavelength: 1.54178 Å. Monoclinic, crystal size: 0.11 × 0.15 × 0.18 mm^3, P 21. Unit cell dimensions: a = 7.8050(3) Å, b = 11.0195(4) Å, c = 21.5680(7) Å, α = 90°, β = 93.9070°(10), γ = 90°. V = 1850.69(17) $Å^3$, Z = 4, D_{calcd} = 1.258 mg/cm^3. Absorption coefficient: 0.721 mm^{-1}. F(000) = 760, theta range for data collection: 4.51 to 68.43°, index ranges: −9<=h<=8, −13<=k<=13, −25<=l<=26. Reflections collected 19917, independent reflections: 6671 [R(int) = 0.0335]. Completeness to theta = 68.43°, 99.1%. Largest diff. peak and hole: 0.259 and −0.144 e.$Å^{-3}$. Refinement method was Full-matrix least-squares on F^2, with Goodness-of-fit on F^2 = 1.046. Data / restraints / parameters: 6671 / 3 / 469. Final R indices [I > 2sigma(I)]: R_1 = 0.0349, wR_2 = 0.0976, R indices (all data): R_1 = 0.0363, wR_2 = 0.0990. Flack parameter –0.0(1).

Crystallographic data for **1** and **2** have been deposited at the Cambridge Crystallographic Data Centre (Deposition numbers CCDC 1556790 and 1556791 for **1** and **2**, respectively). Copies of these data can be

obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK. [Fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk].

1.6. Bioassay procedures.

Recombinant human PTP1B was purchased from SIGMA-ALDRICH. And the reagent 4-nitrophenyl phosphate disodium salt (*p*NPP) was applied as a substrate for the assessment of PTP1B activity. PTP1B protein was dissolved in enzyme dilution buffer (50 mmol/L 3-[*N*-morpholino] propanesulfonic acid (Mops), 1 mM EDTA, and 1 mM dithiothreitol, pH 6.5), and inhibitors were pre-incubated with the recombinant human PTP1B at 30 °C for 10 min. The bioassay was performed in a reaction mixture containing 30 nmol/L recombinant PTP1B, 2 mM *p*NPP, 1 mM EDTA, and 1 mM dithiothreitol in 50 mmol/L Mops, pH 6.5, incubated at 30 °C for 30 min in a 96-well plate (100 uL per well), and the reaction was terminated by addition of 50 μL 3 M NaOH each well. Oleanolic acid was used as the positive control, a similar system without samples was applied as the negative control, and parallel experiments without PTP1B protein and samples were utilized as the blank control. Dephosphorylation of *p*NPP could generate product *p*-nitrophenol (*p*NP), which could be monitored at an absorbance of 405 nm by microplate reader (read every 5 min for 30 min) and the slope of the initial rate for the kinetic curve in each well was applied to determine the activity of PTP1B. Samples with good inhibition rate (>50% at 200 μM) were tested for further determination of IC_{50} values. Oleanolic acid, rhodomollanol A (**1**), rhodomollein XXXI (**2**), and rhodojaponin III (**3**) exhibited 101.3%, 87.4%, 31.5%, and 13.4% inhibitory ratio against PTP1B protein at final concentration of 200 μM, respectively. Then, PTP1B inhibitory activity of rhodomollanol A (**1**) and the positive control, oleanolic acid, were tested under different dosages. The IC_{50} value (50% percentage inhibition concentration) was calculated with Origin 8.0 software from the non-linear curve fitting of the percentage of inhibition (% inhibition) versus logarithm of the inhibitor concentration [I] by using the following equation: % Inhibition = $100/(1+[IC_{50}/10^{[I]}]^{k})$, where k is the Hill coefficient.

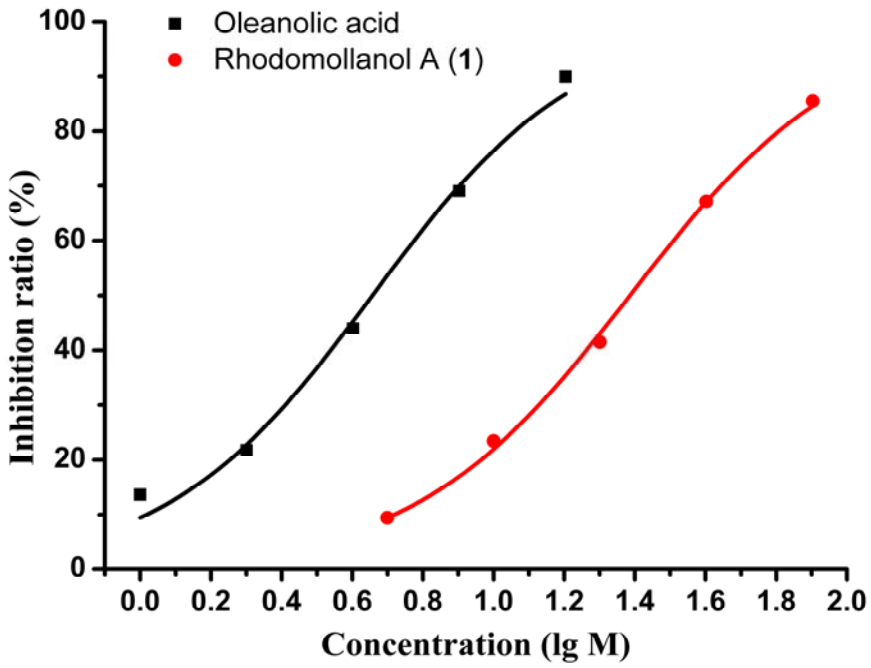

Figure S1. Concentration−inhibition ratio curves of oleanolic acid and rhodomollanol A (**1**)

The final concentration of oleanolic acid were 1, 2, 4, 8, and 16 μM. The final concentration of rhodomollanol A (**1**) were 5, 10, 20, 40, and 80 μM.

2. HR-ESI-MS of compounds 1 and 2, NMR Spectra of Compounds 1−3.

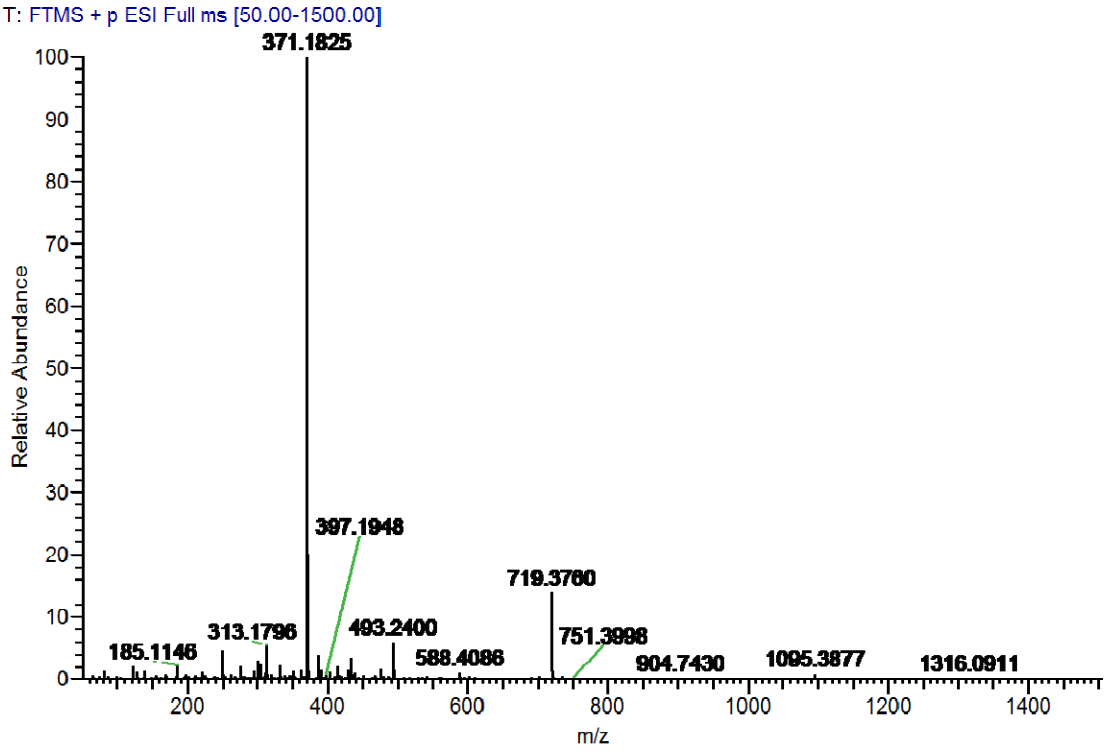

Figure S2. HR-ESI-MS spectrum of **1**

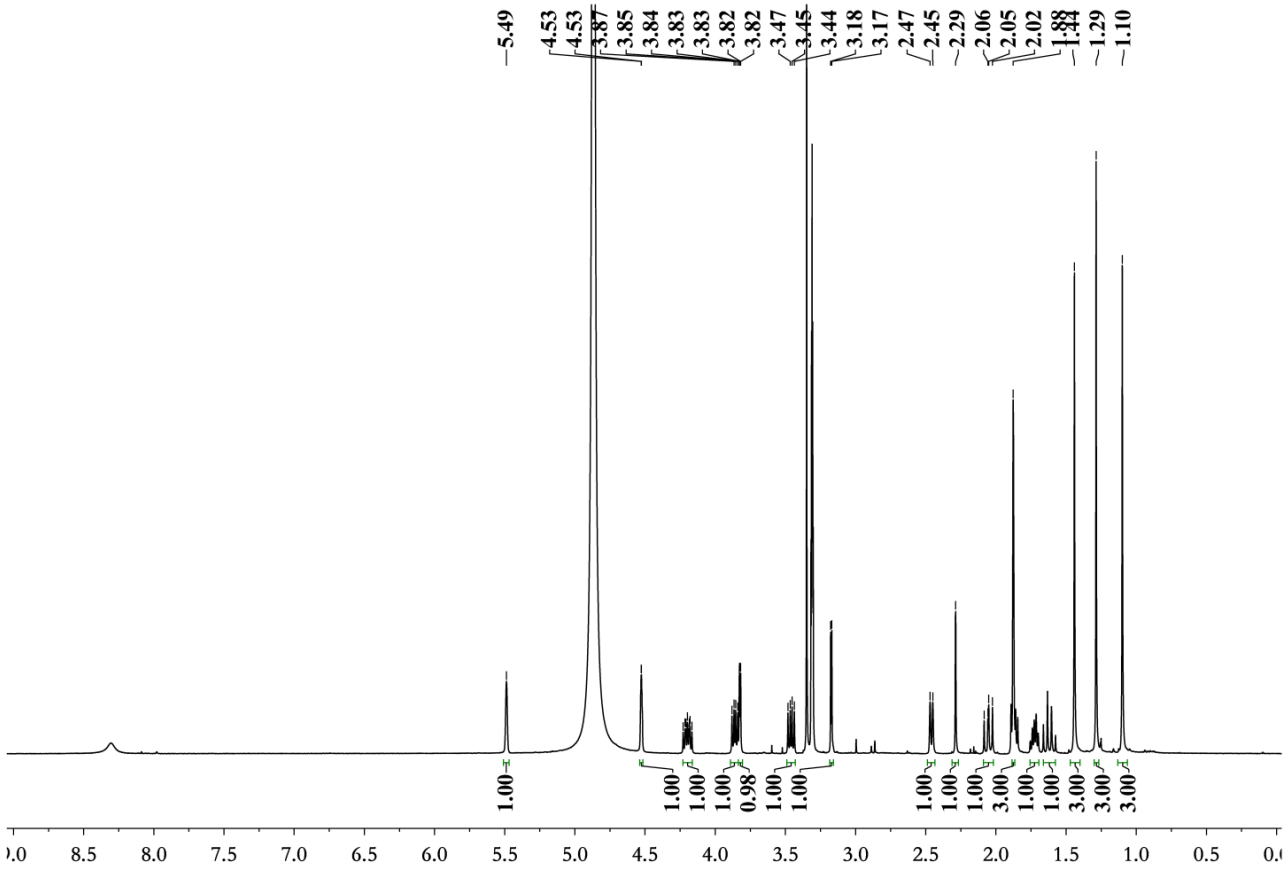

Figure S3. ^{1}H NMR spectrum of **1** (400 MHz, methanol-d_4)

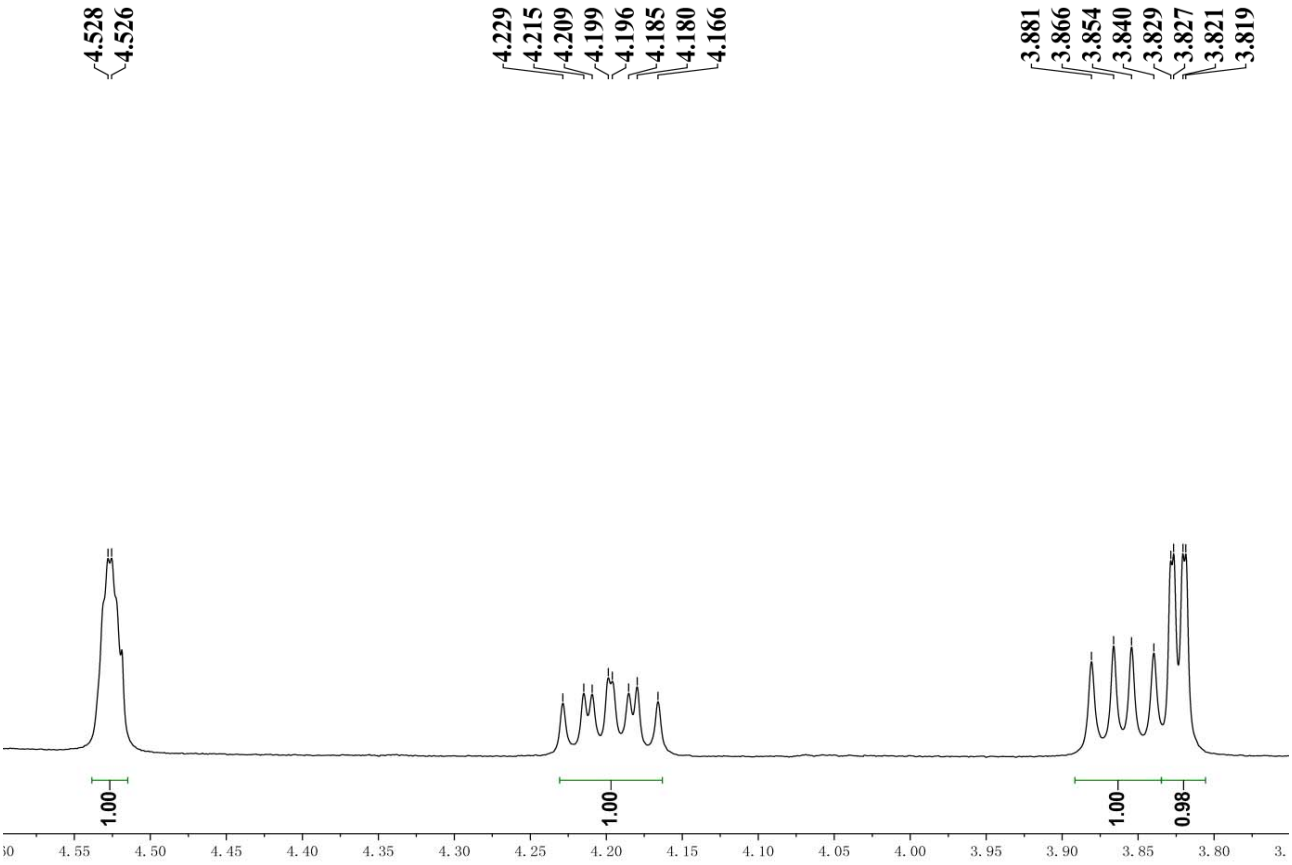

Figure S4. ^{1}H NMR spectrum (amplified) of **1** (400 MHz, methanol-d_4)

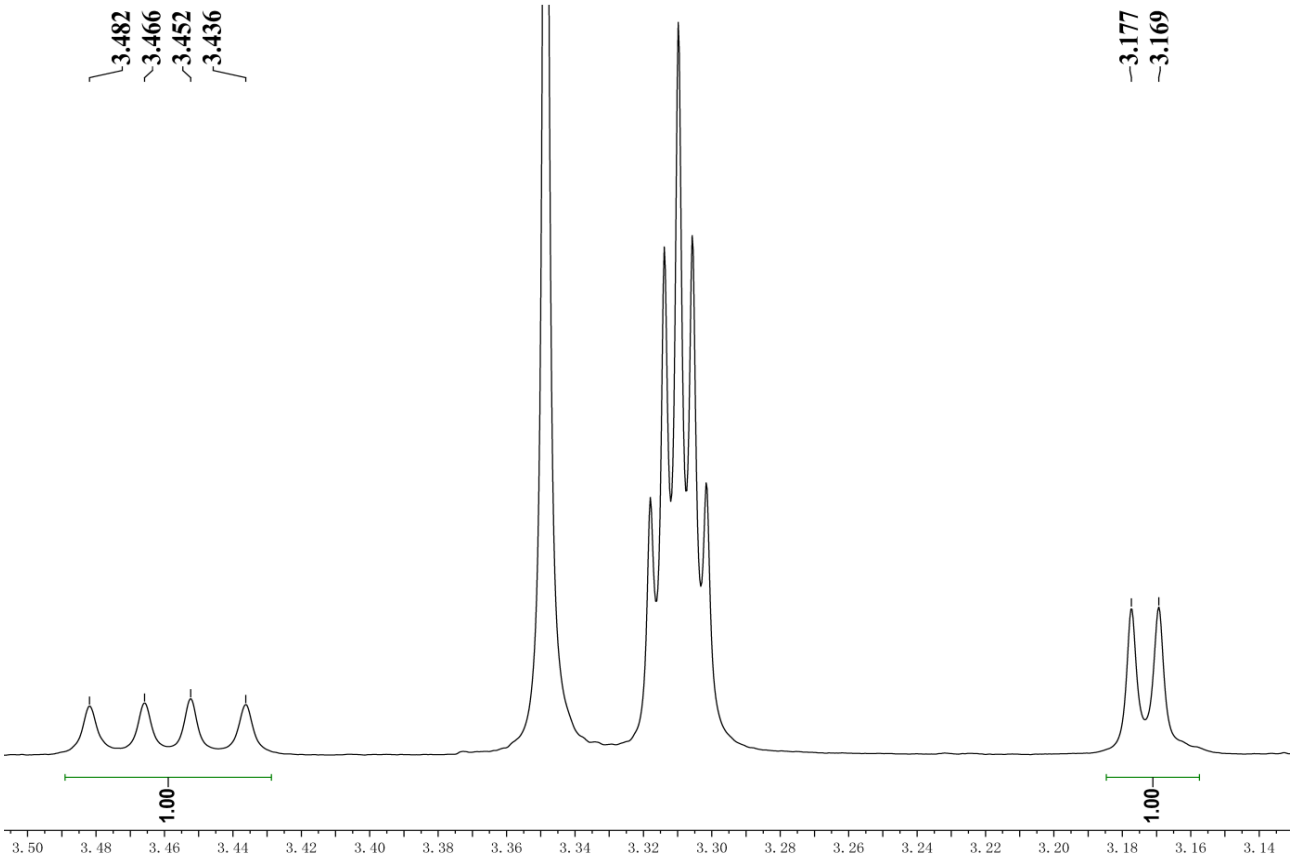

Figure S5. ^{1}H NMR spectrum (amplified) of **1** (400 MHz, methanol-d_4)

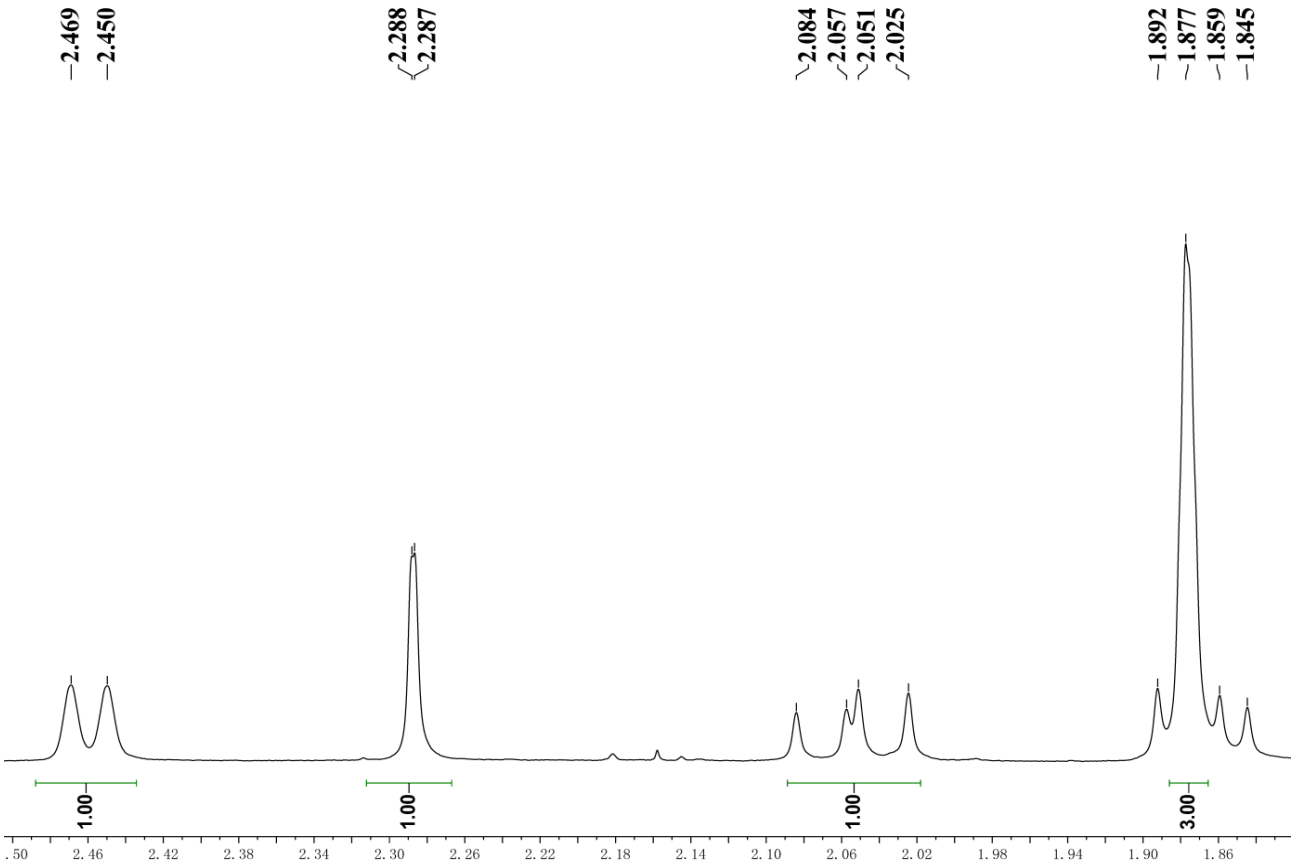

Figure S6. ^{1}H NMR spectrum (amplified) of **1** (400 MHz, methanol-d_4)

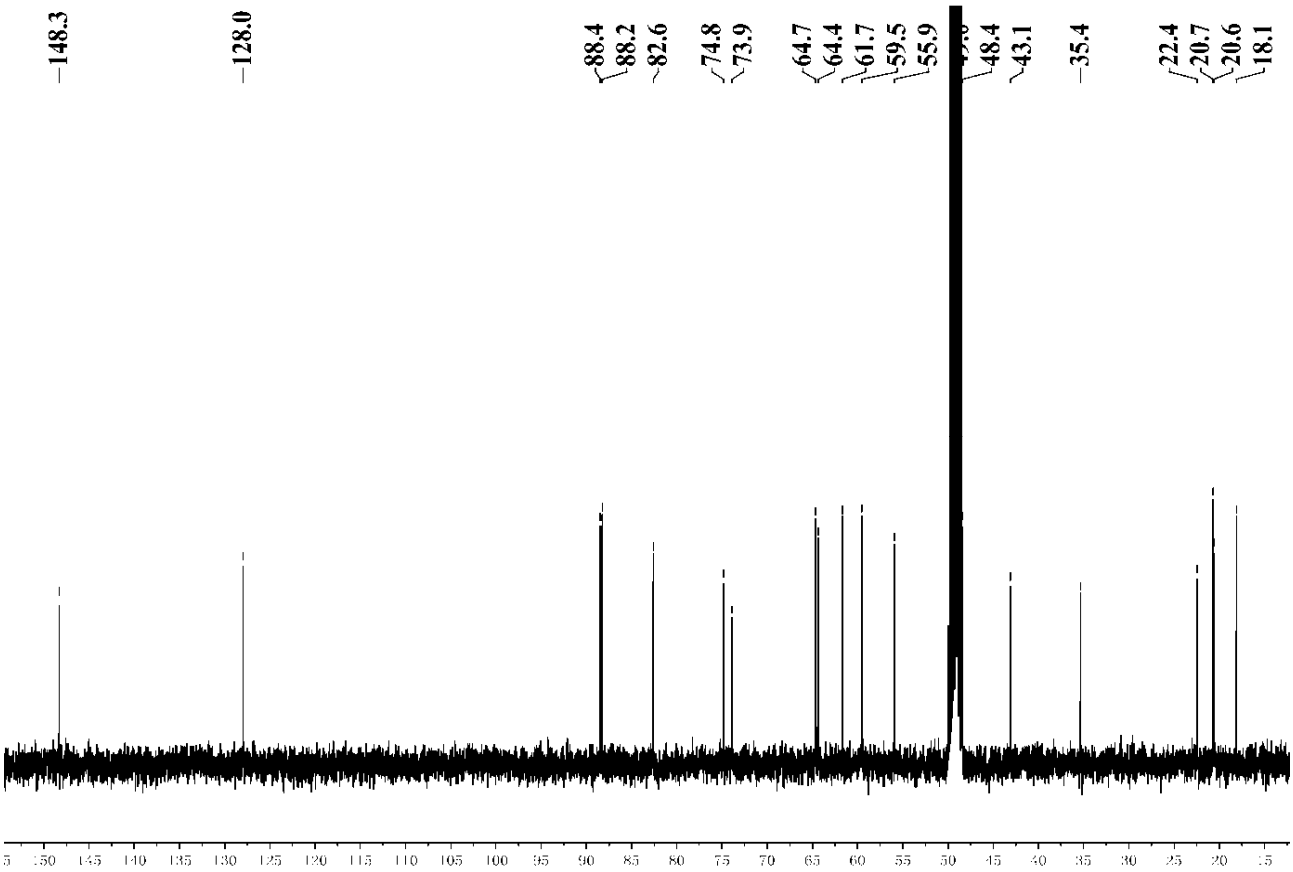

Figure S7. ^{13}C NMR spectrum of **1** (100 MHz, methanol-d_4)

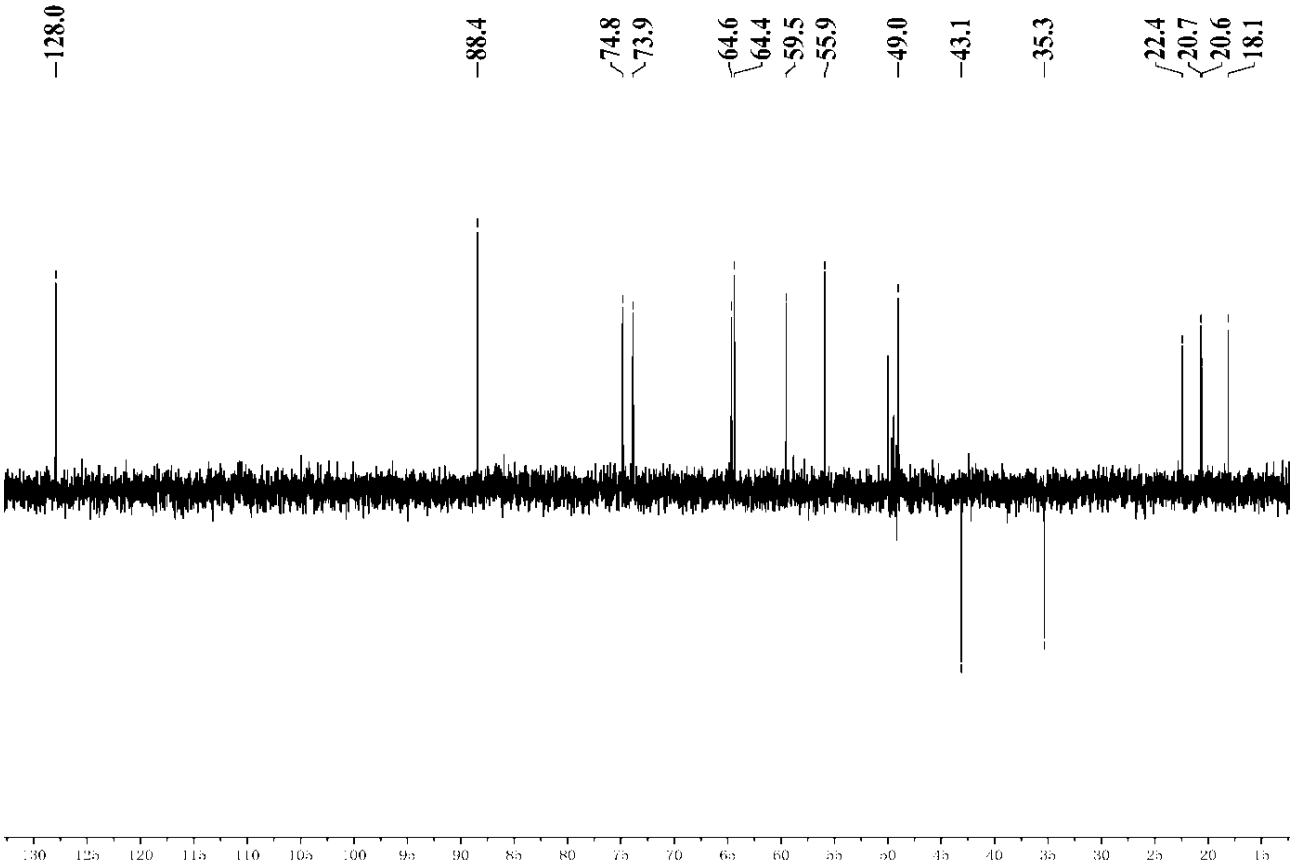

Figure S8. DEPT spectrum of **1** (100 MHz, methanol-d_4)

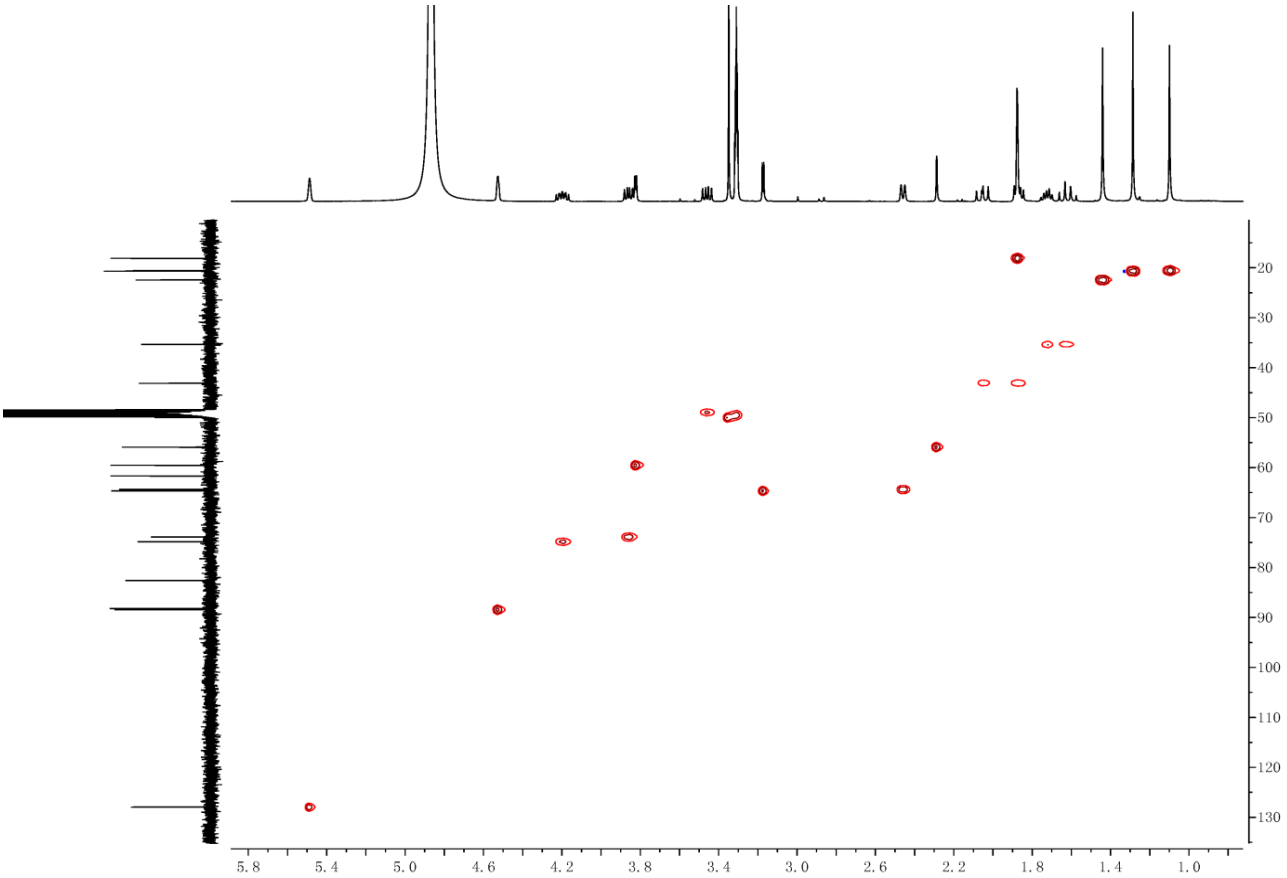

Figure S9. HSQC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

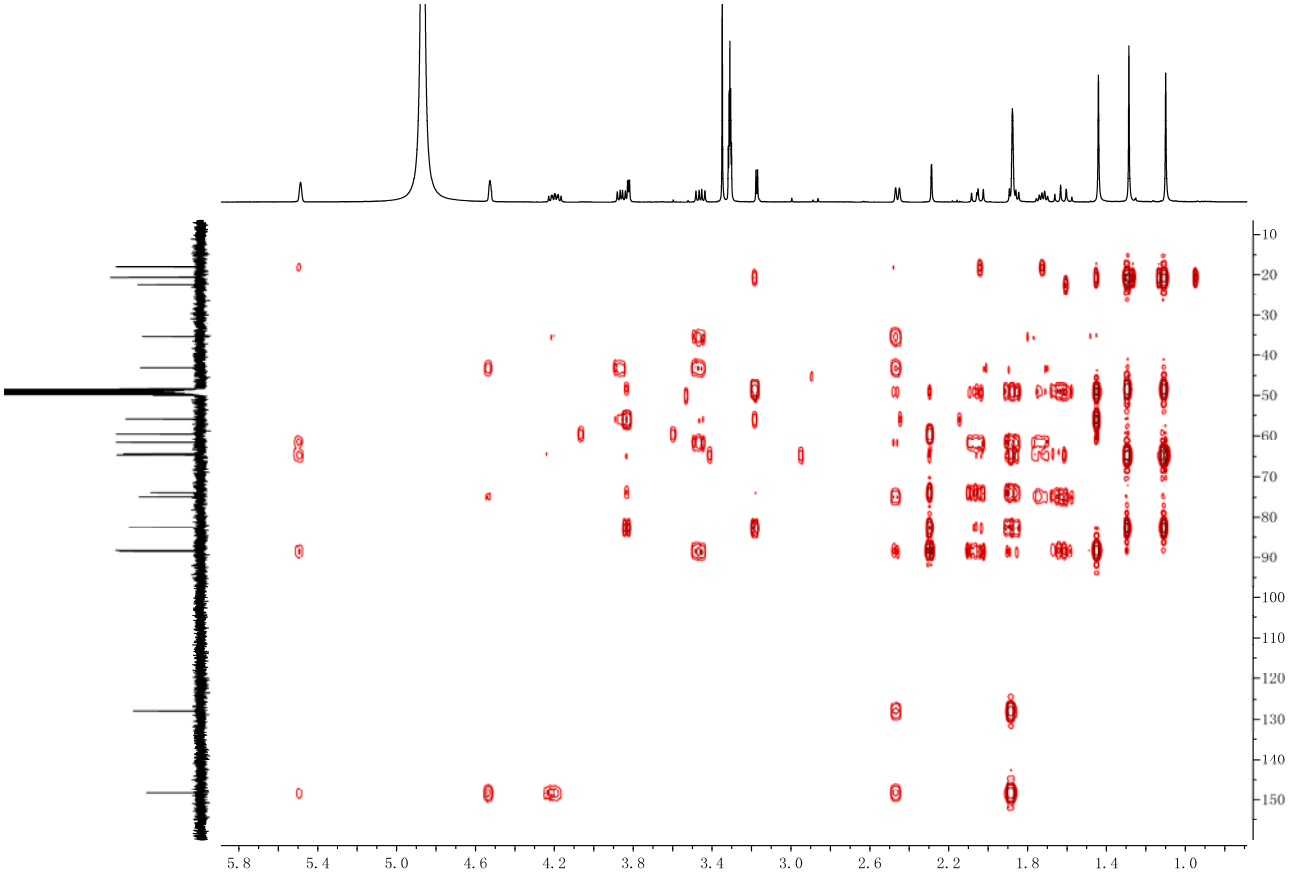

Figure S10. HMBC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

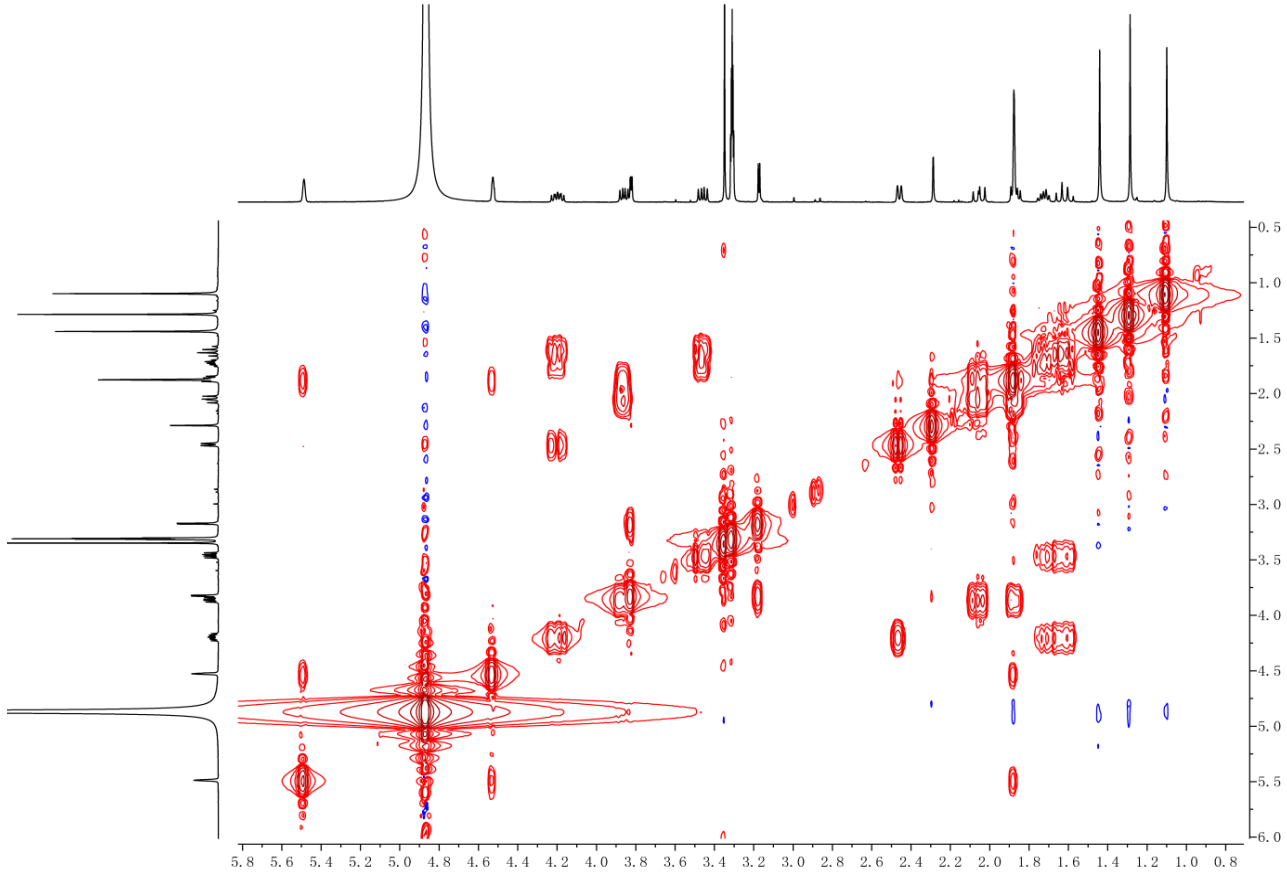

Figure S11. ^{1}H–^{1}H COSY spectrum of **1** (400 MHz, methanol-d_4)

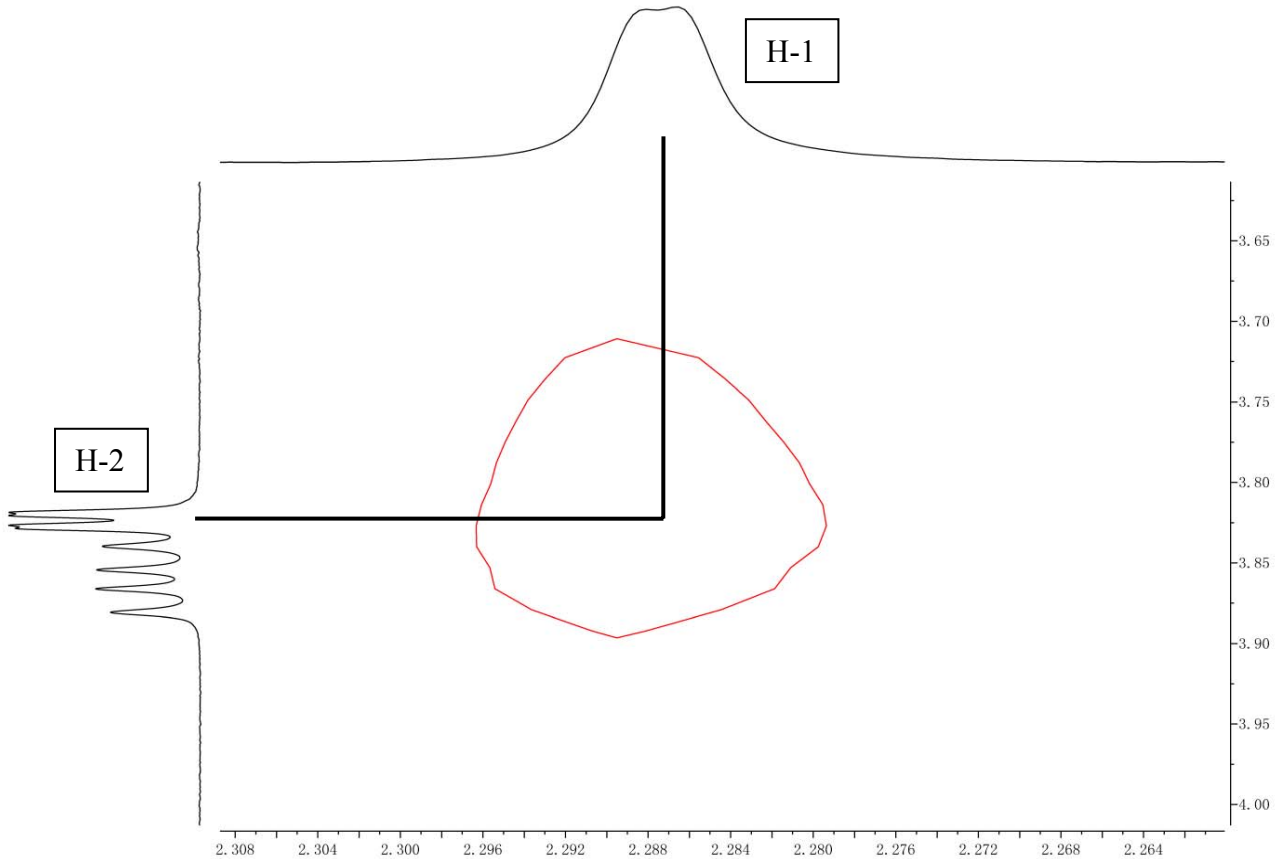

Figure S12. ^{1}H–^{1}H COSY spectrum (amplified) of **1** (400 MHz, methanol-d_4)

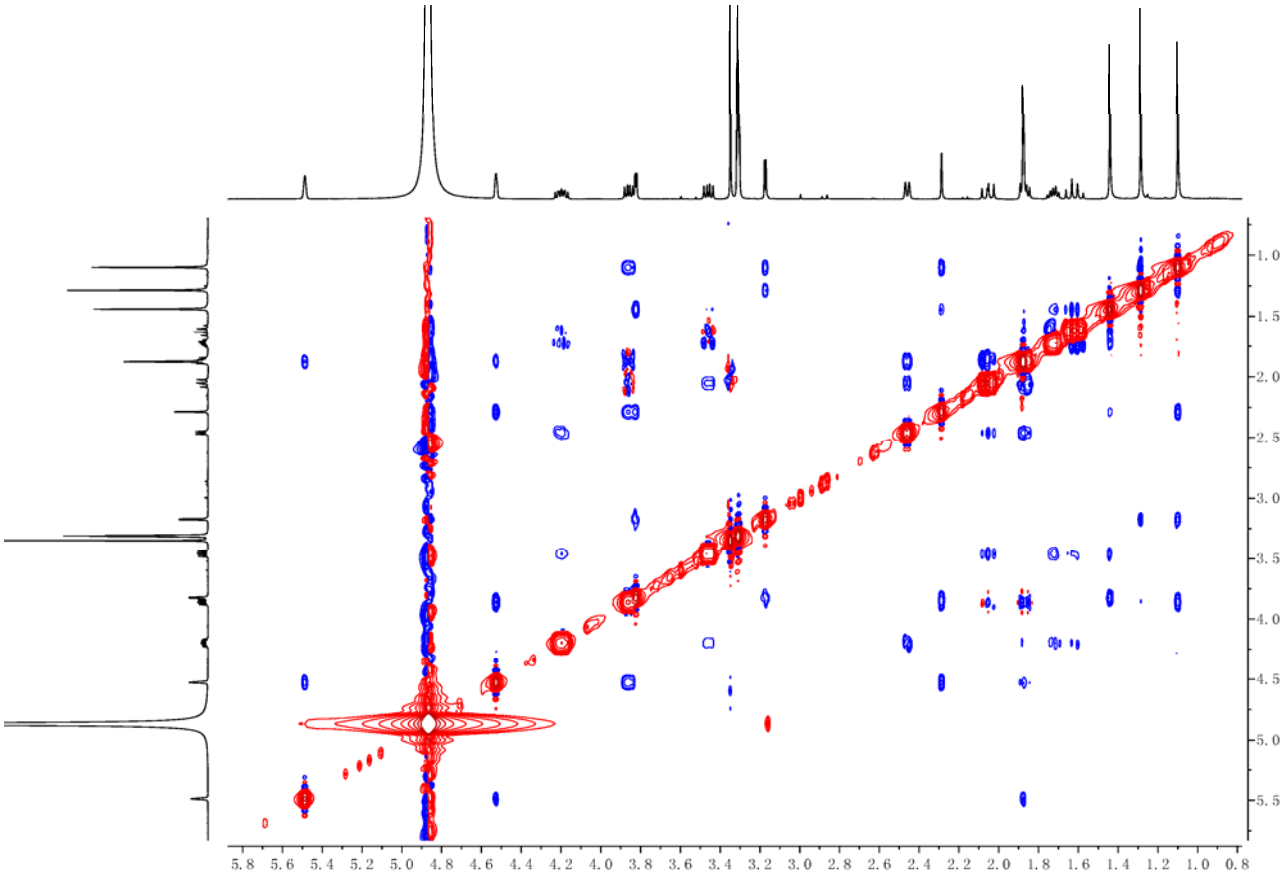

Figure S13. NOESY spectrum of **1** (400 MHz, methanol-d_4)

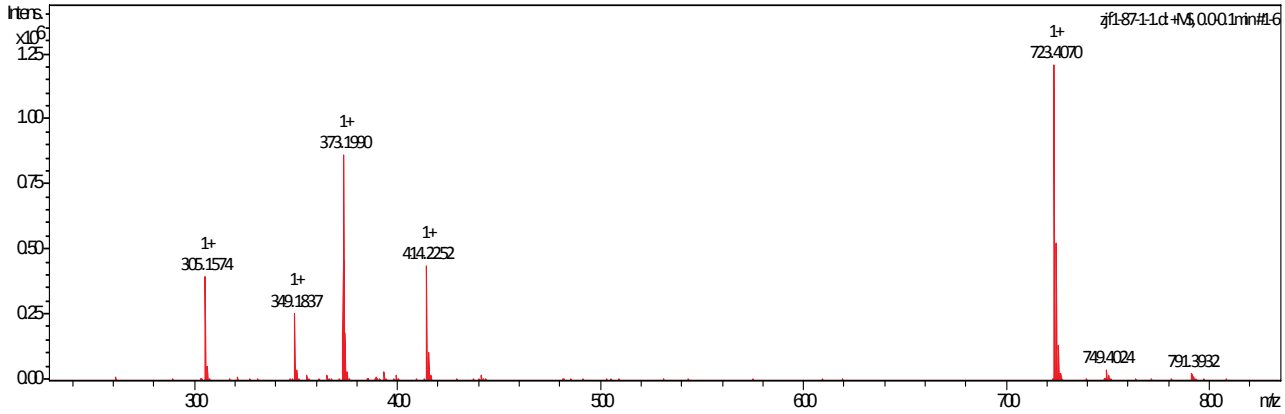

Figure S14. The HRESIMS spectrum of **2**

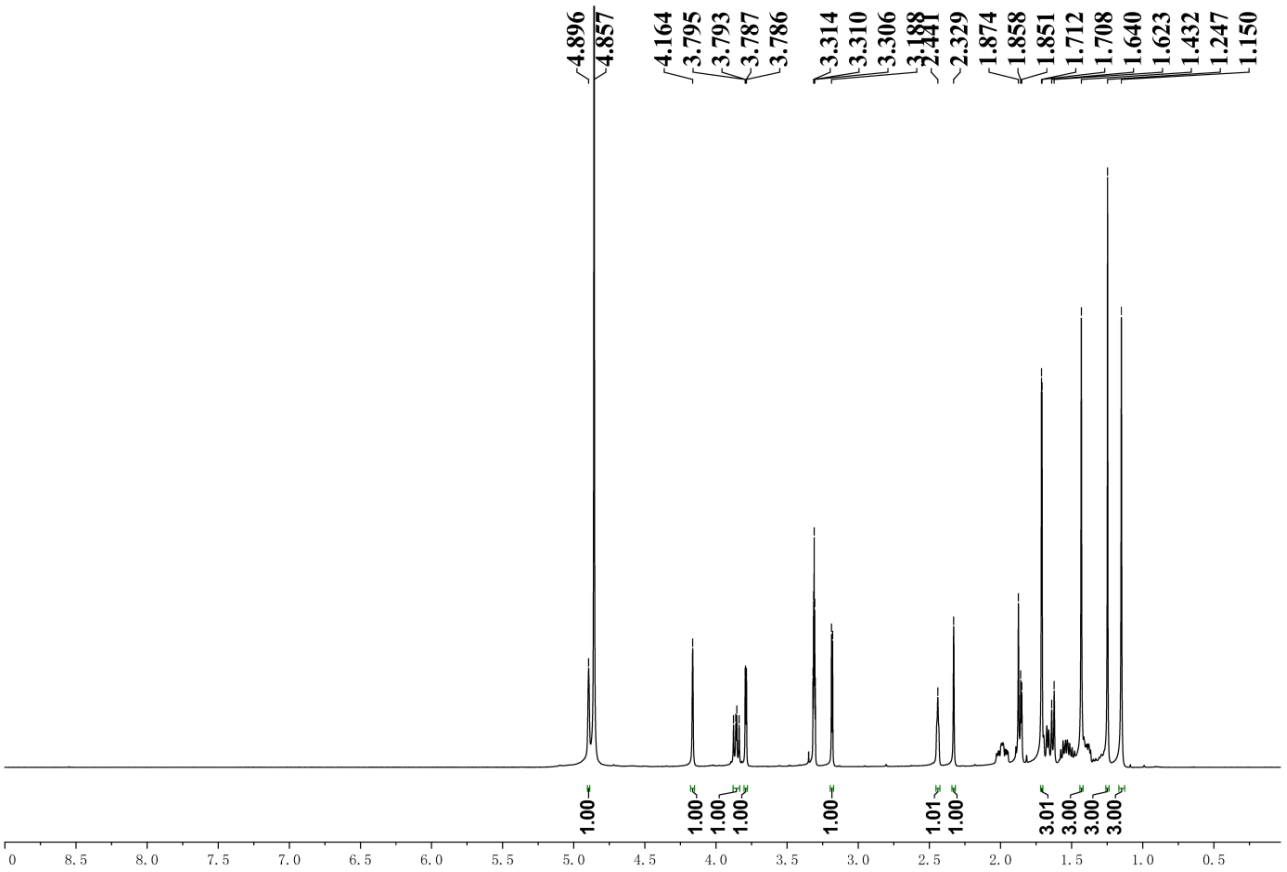

Figure S15. ^{1}H NMR spectrum of **2** (400 MHz, methanol-d_4)

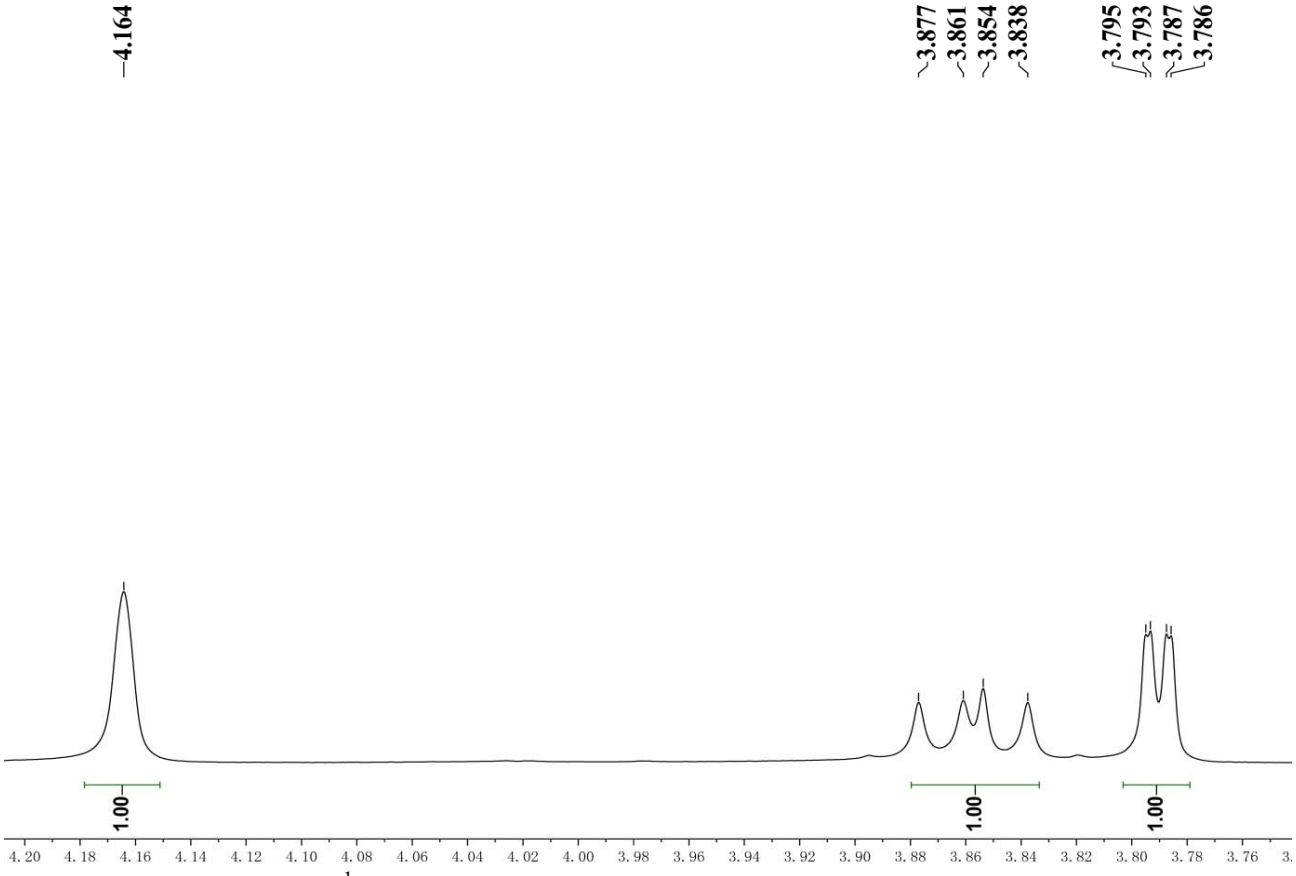

Figure S16. ^{1}H NMR spectrum (amplified) of **2** (400 MHz, methanol-d_4)

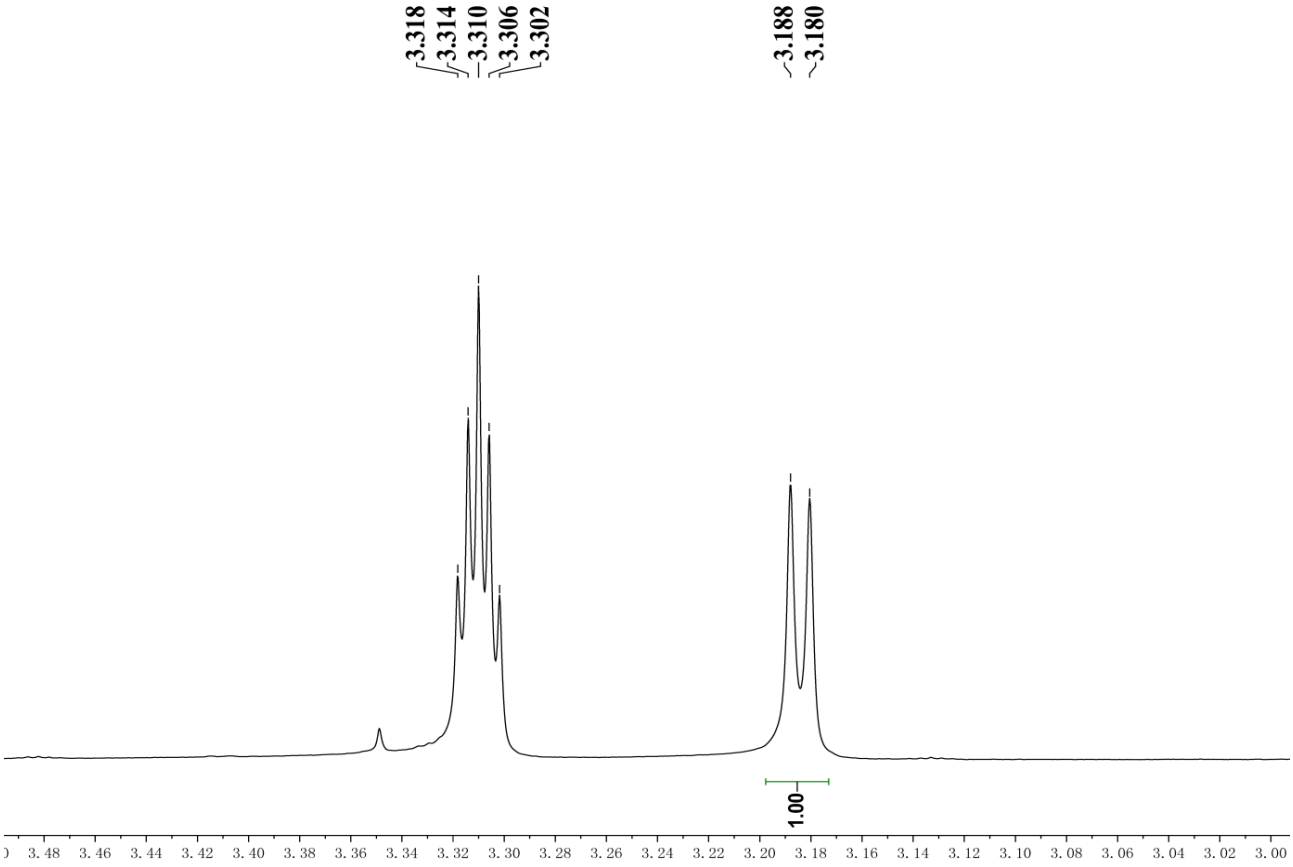

Figure S17. ^{1}H NMR spectrum (amplified) of **2** (400 MHz, methanol-d_4)

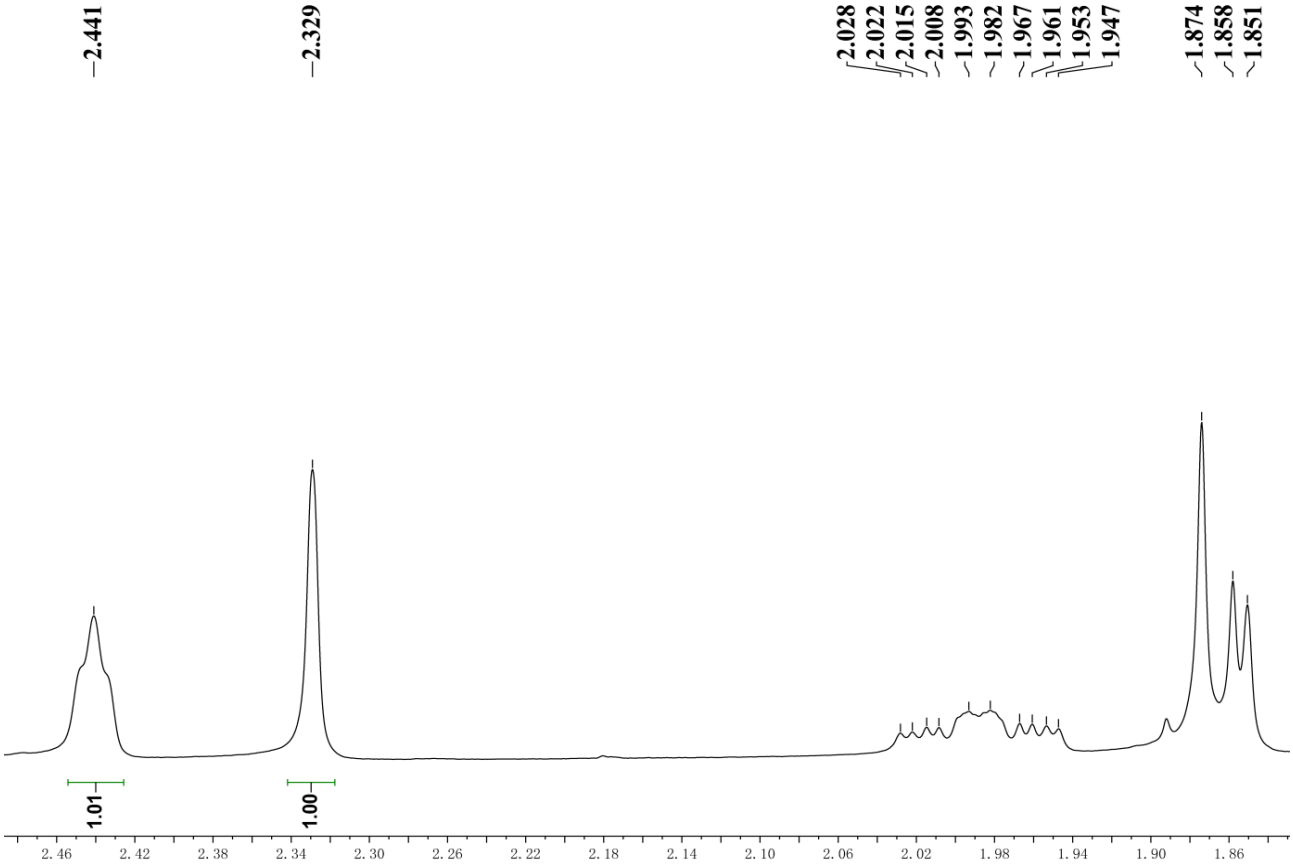

Figure S18. ^{1}H NMR spectrum (amplified) of **2** (400 MHz, methanol-d_4)

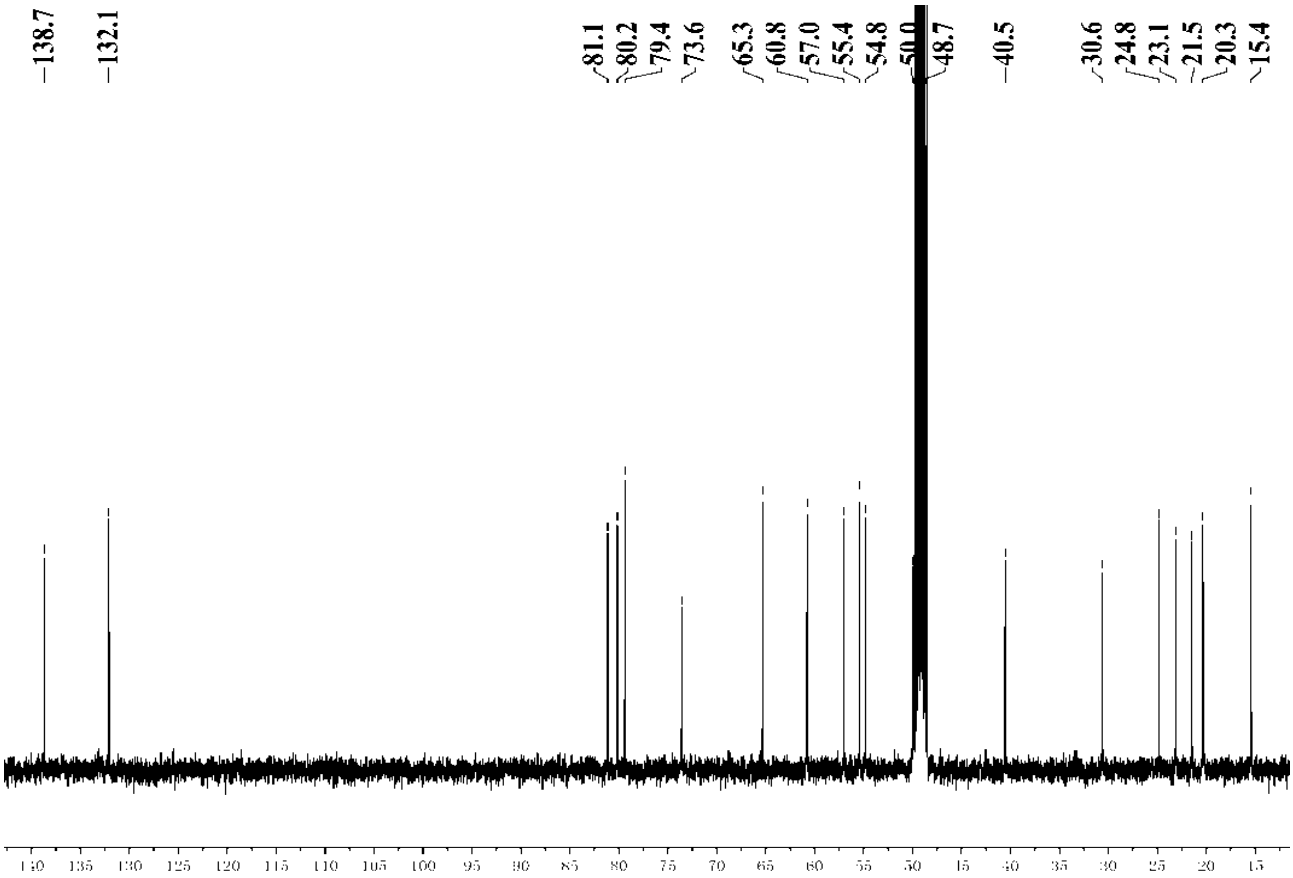

Figure S19. ^{13}C NMR spectrum of **2** (100 MHz, methanol-d_4)

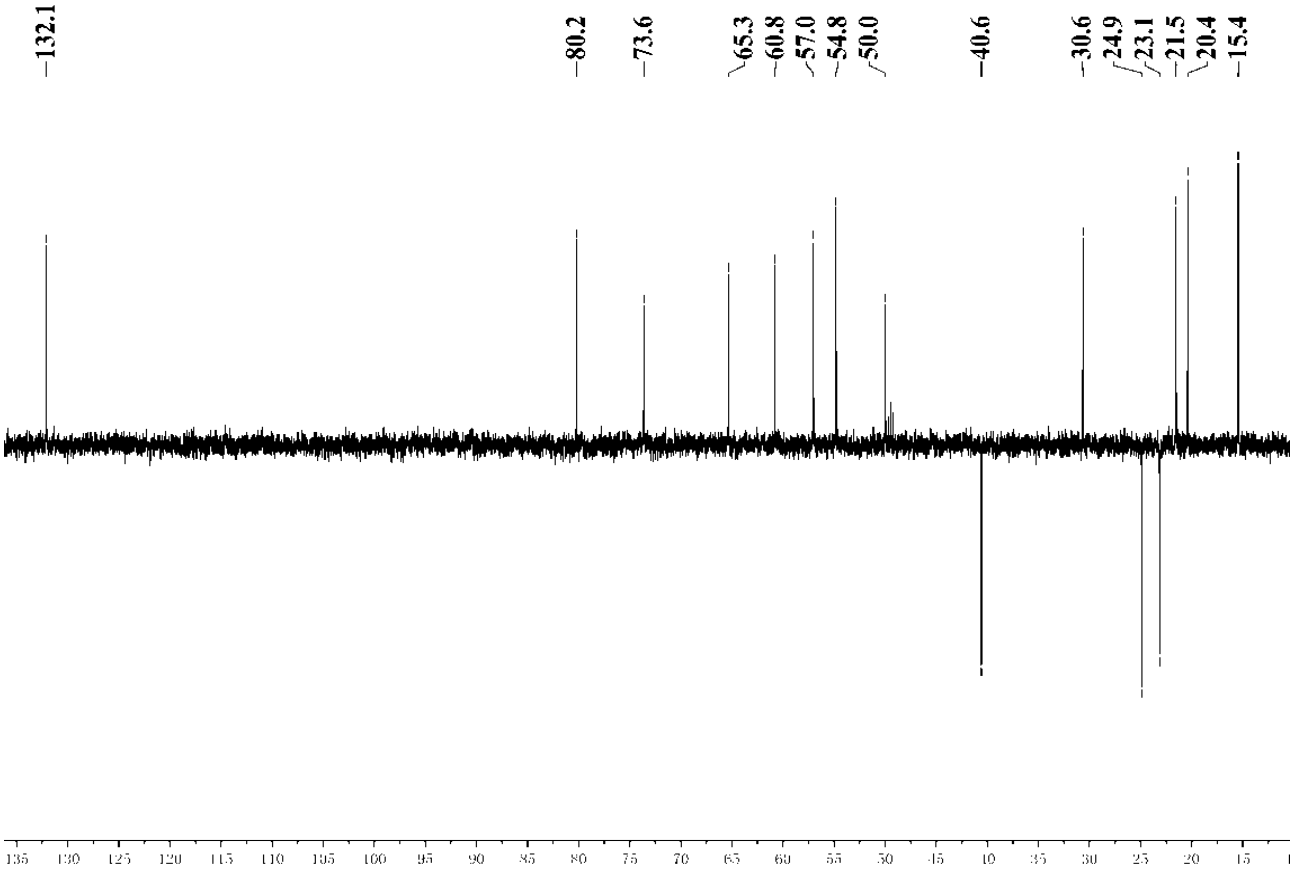

Figure S20. DEPT spectrum of **2** (100 MHz, methanol-d_4)

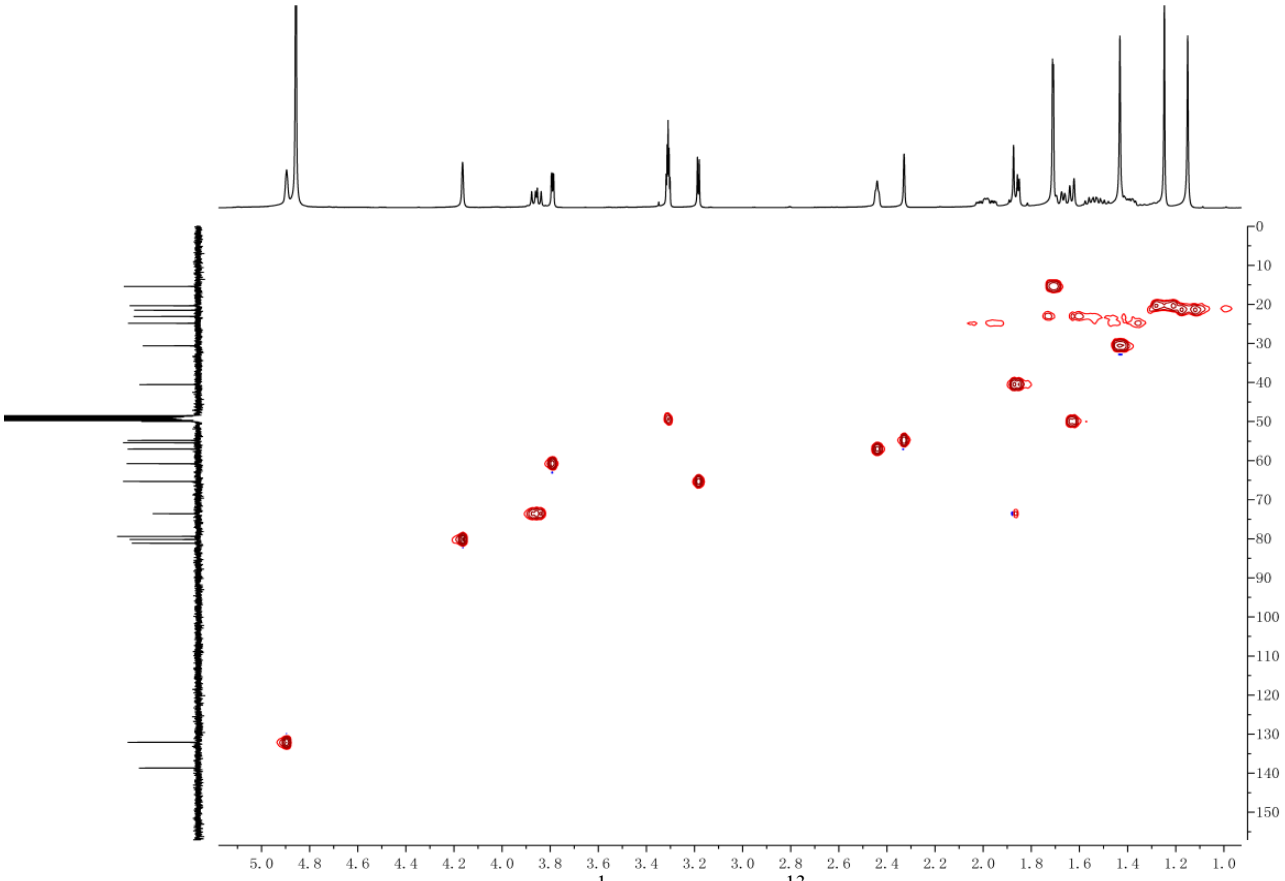

Figure S21. HSQC spectrum of **2** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

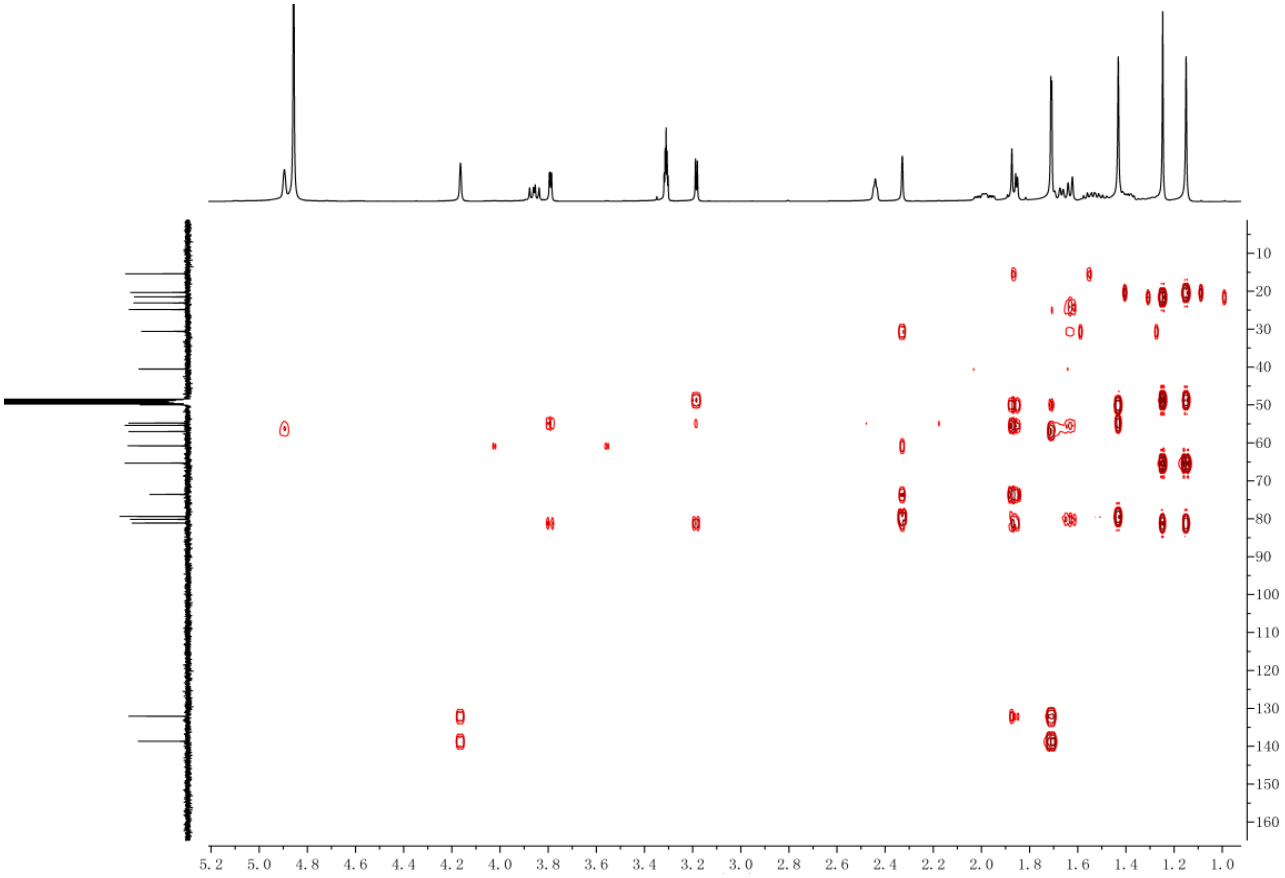

Figure S22. HMBC spectrum of **2** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

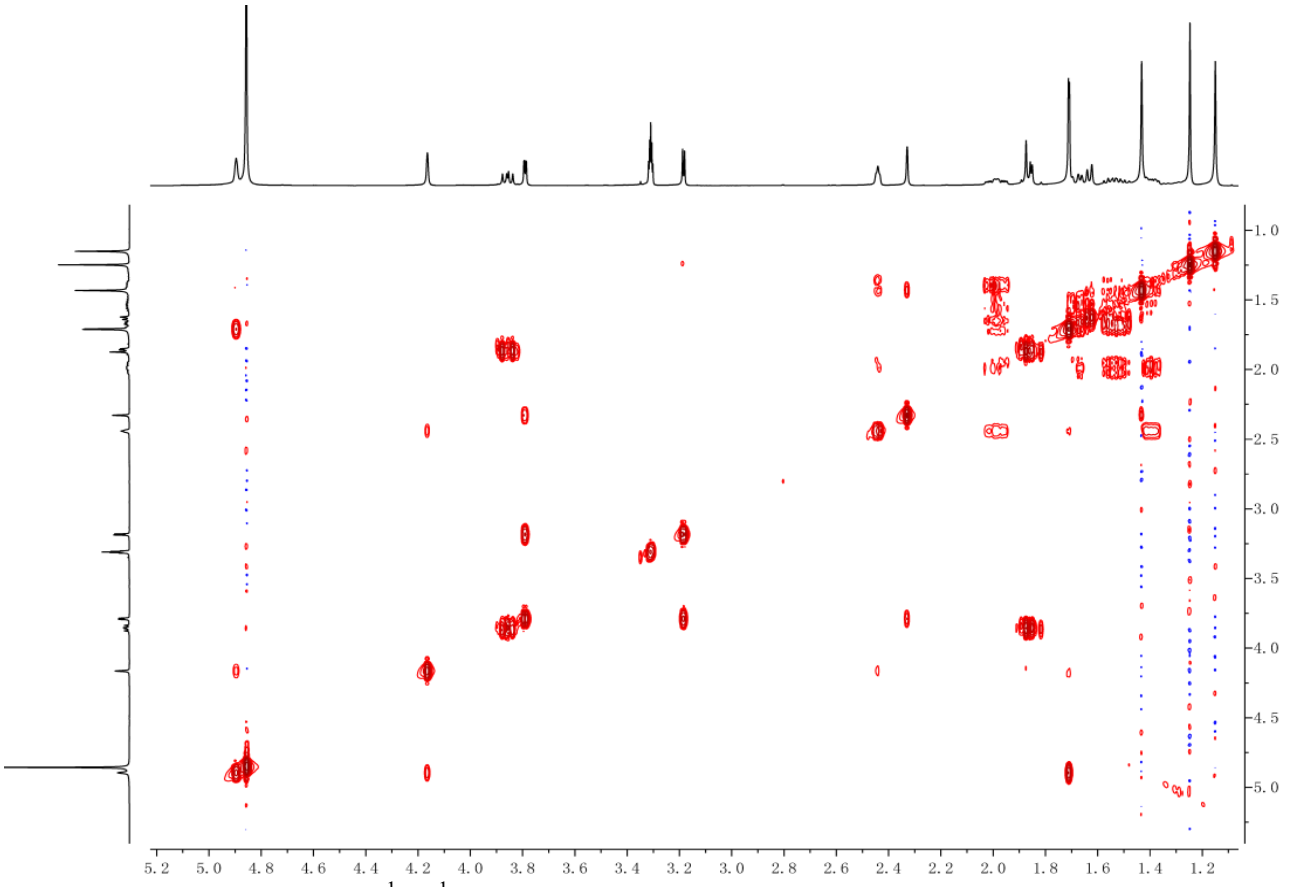

Figure S23. $^{1}H-^{1}H$ COSY spectrum of **2** (400 MHz, methanol-d_4)

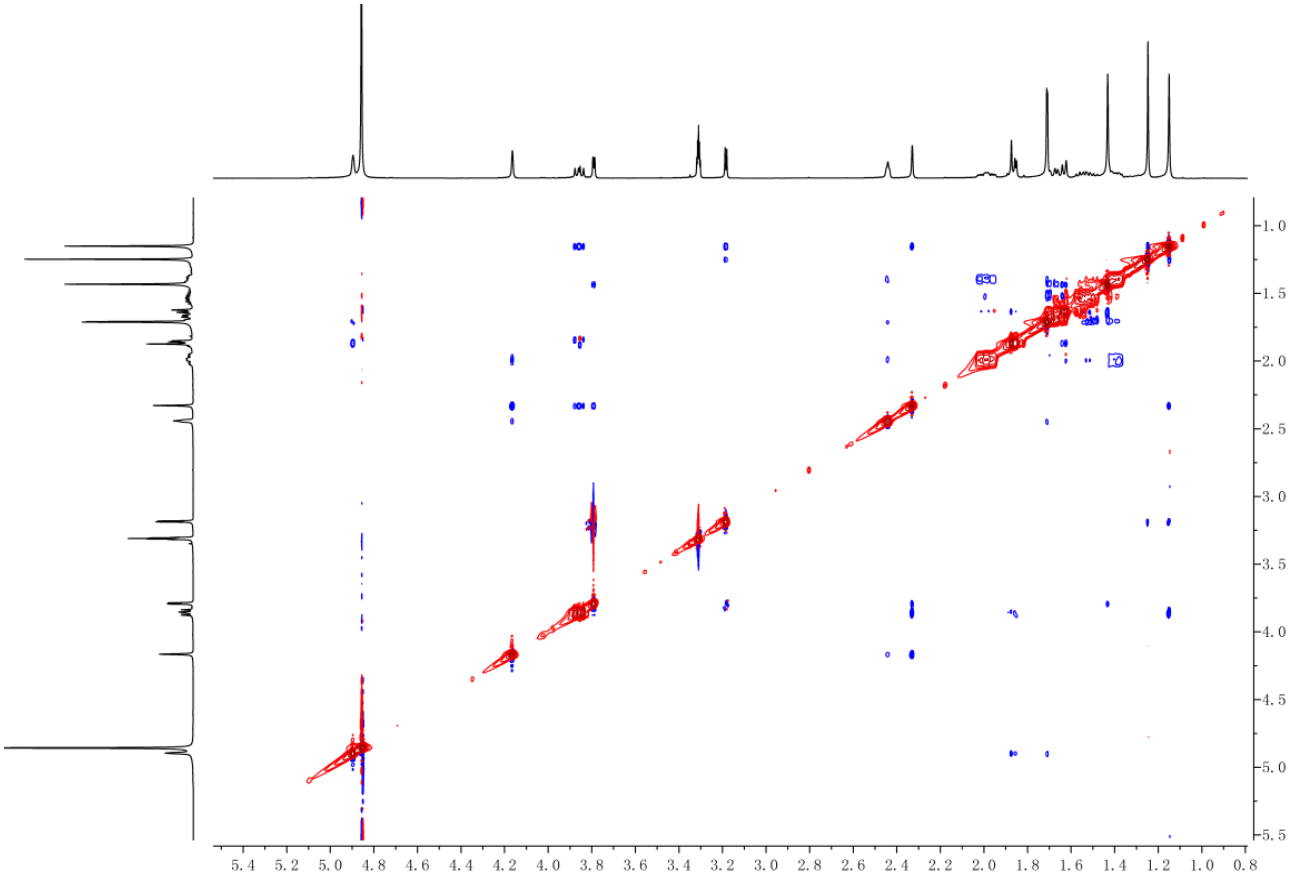

Figure S24. NOESY spectrum of **2** (400 MHz, methanol-d_4)

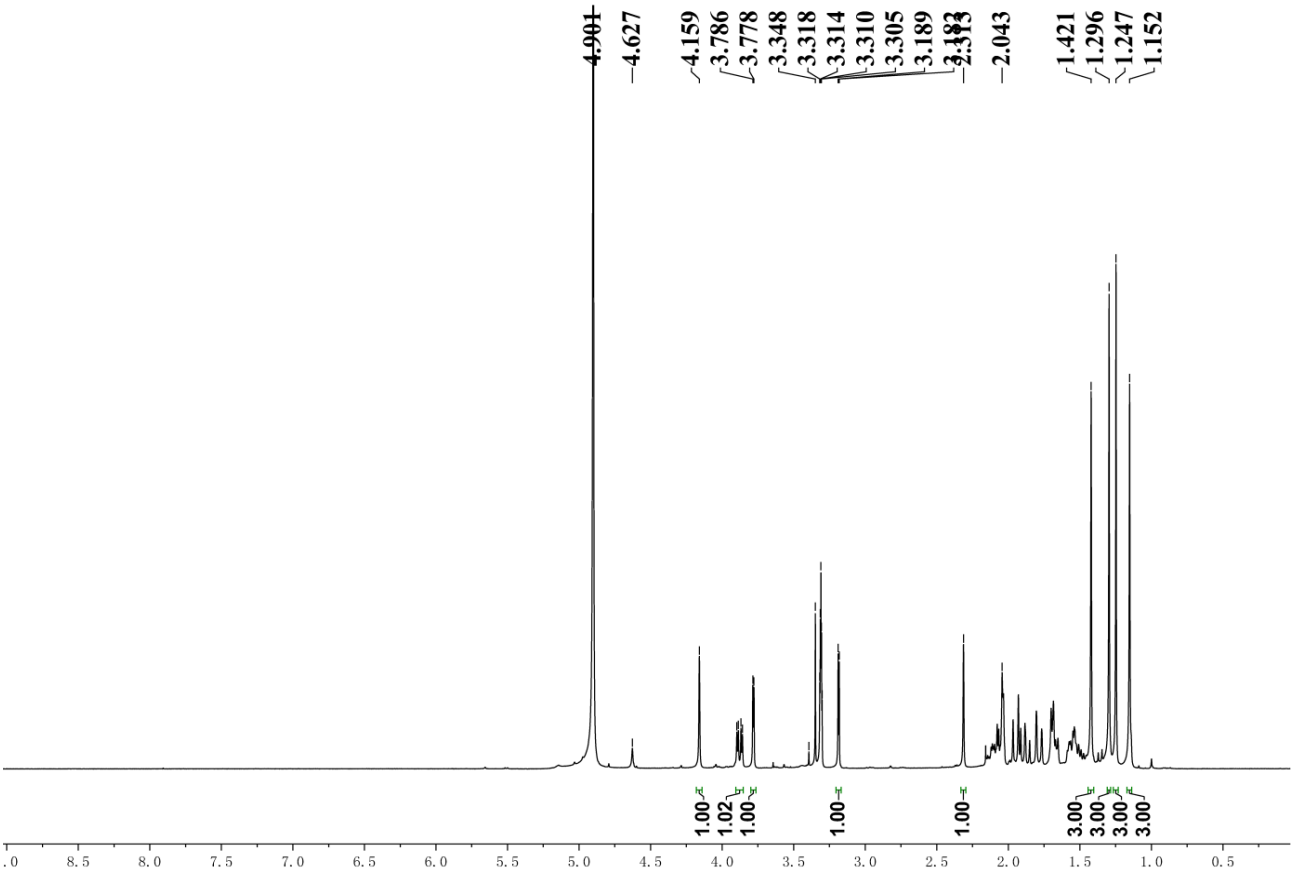

Figure S25. ^{1}H NMR spectrum of **3** (400 MHz, methanol-d_4)

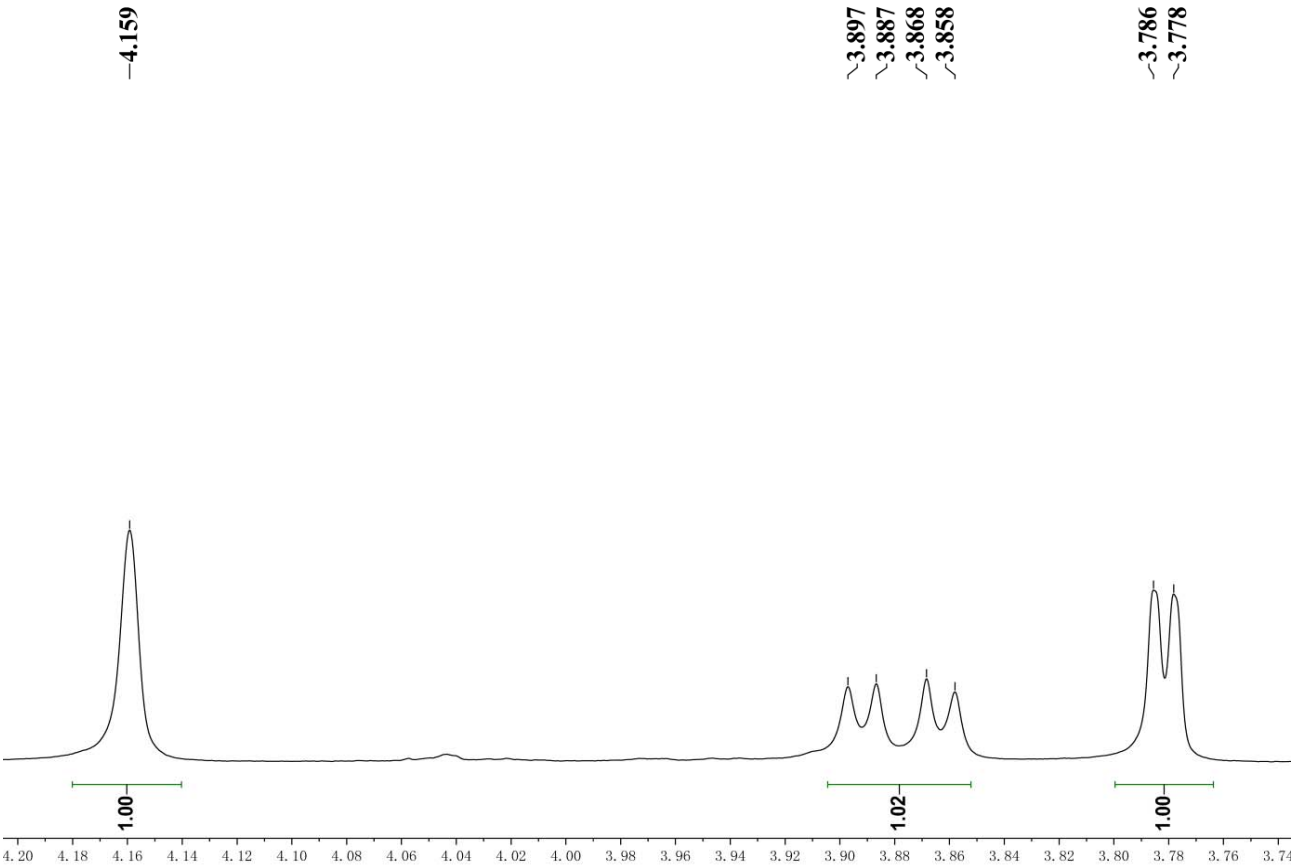

Figure S26. ^{1}H NMR spectrum (amplified) of **3** (400 MHz, methanol-d_4)

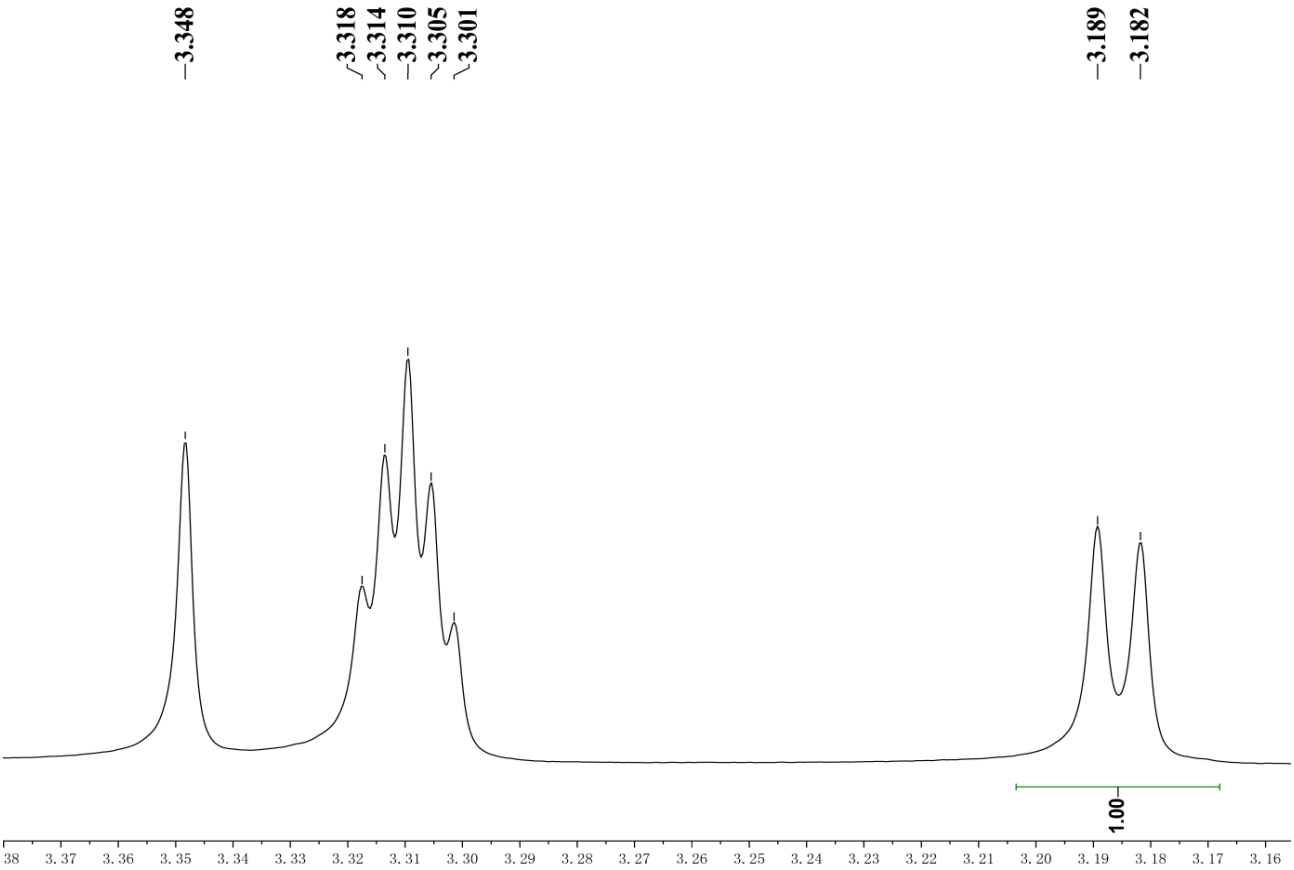

Figure S27. ^{1}H NMR spectrum (amplified) of **3** (400 MHz, methanol-d_4)

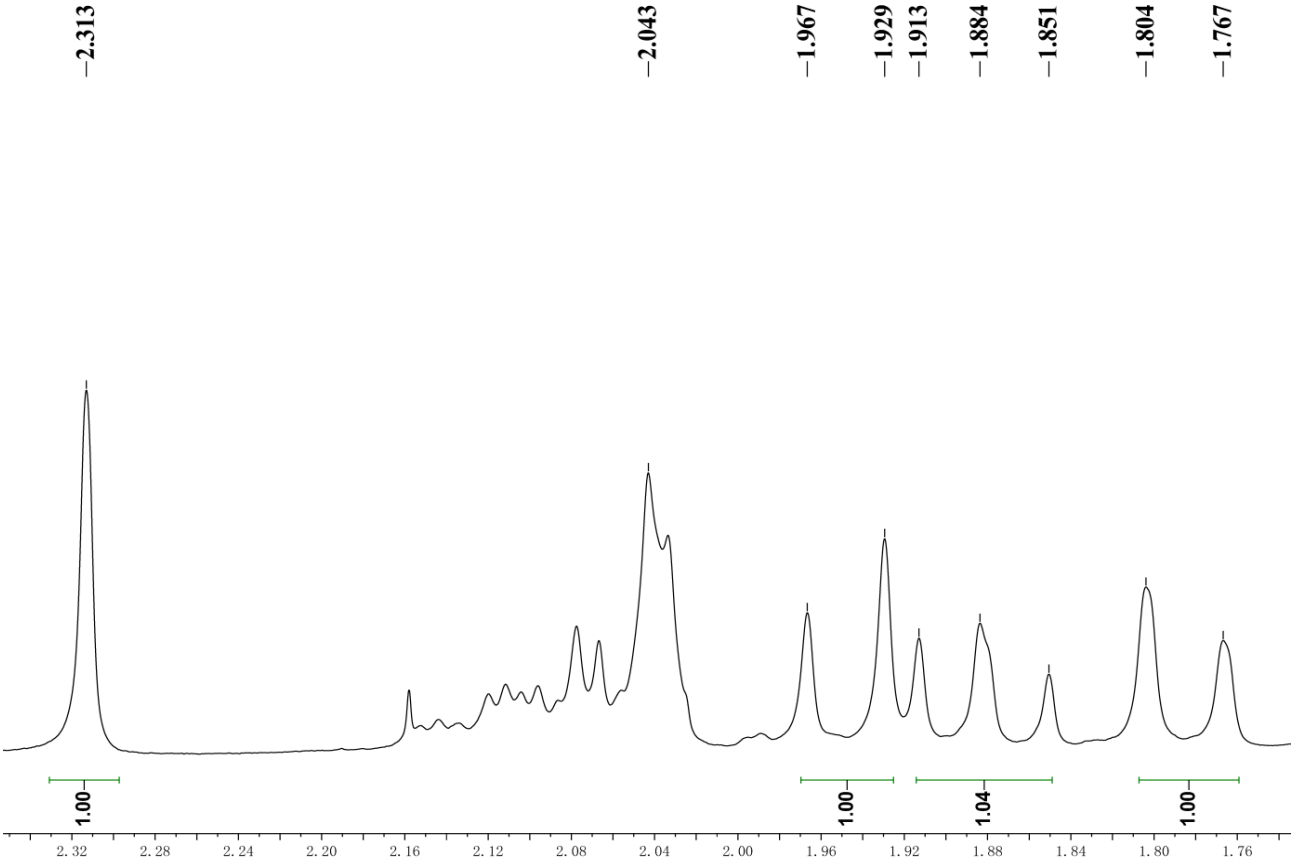

Figure S28. ^{1}H NMR spectrum (amplified) of **3** (400 MHz, methanol-d_4)

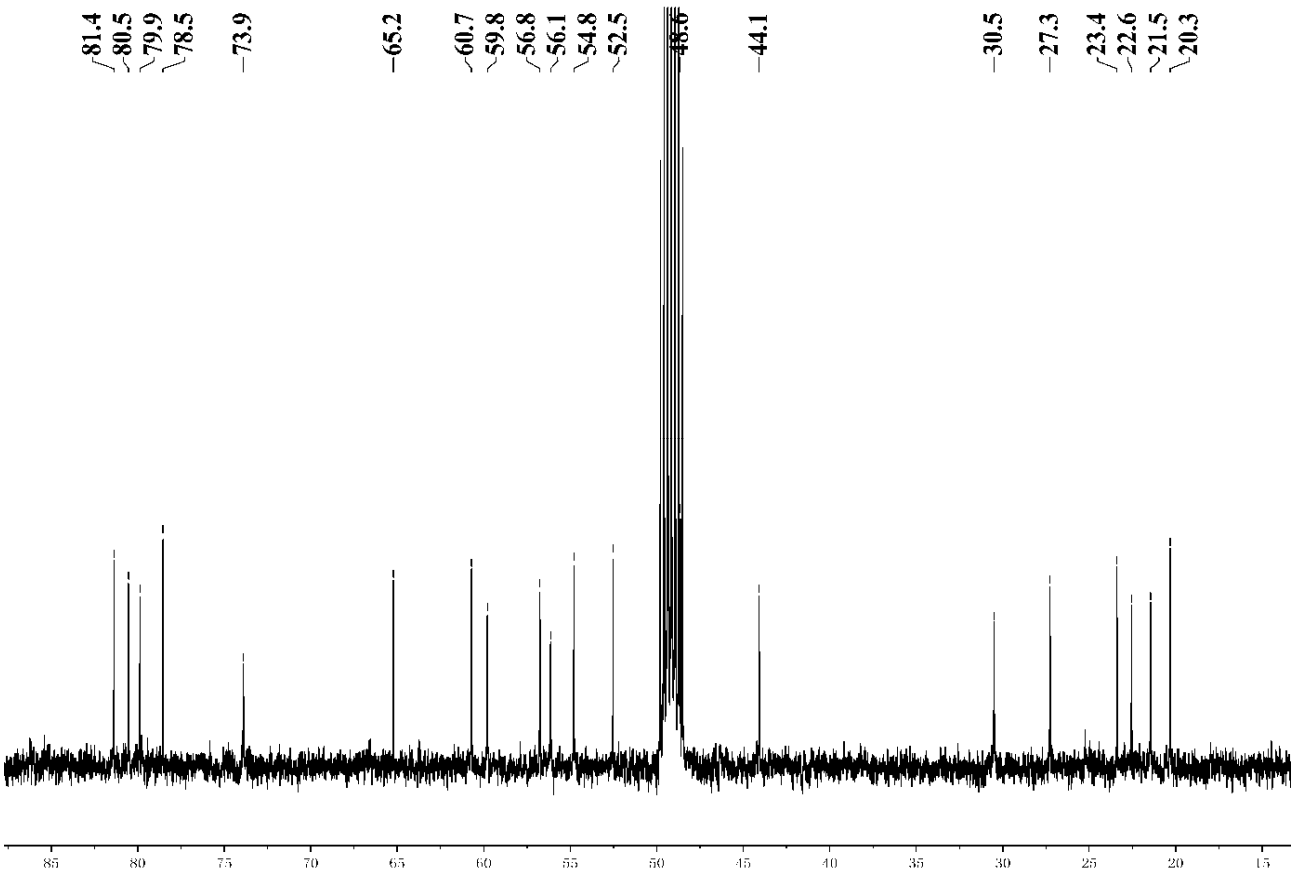

Figure S29. ^{13}C NMR spectrum of **3** (100 MHz, methanol-d_4)

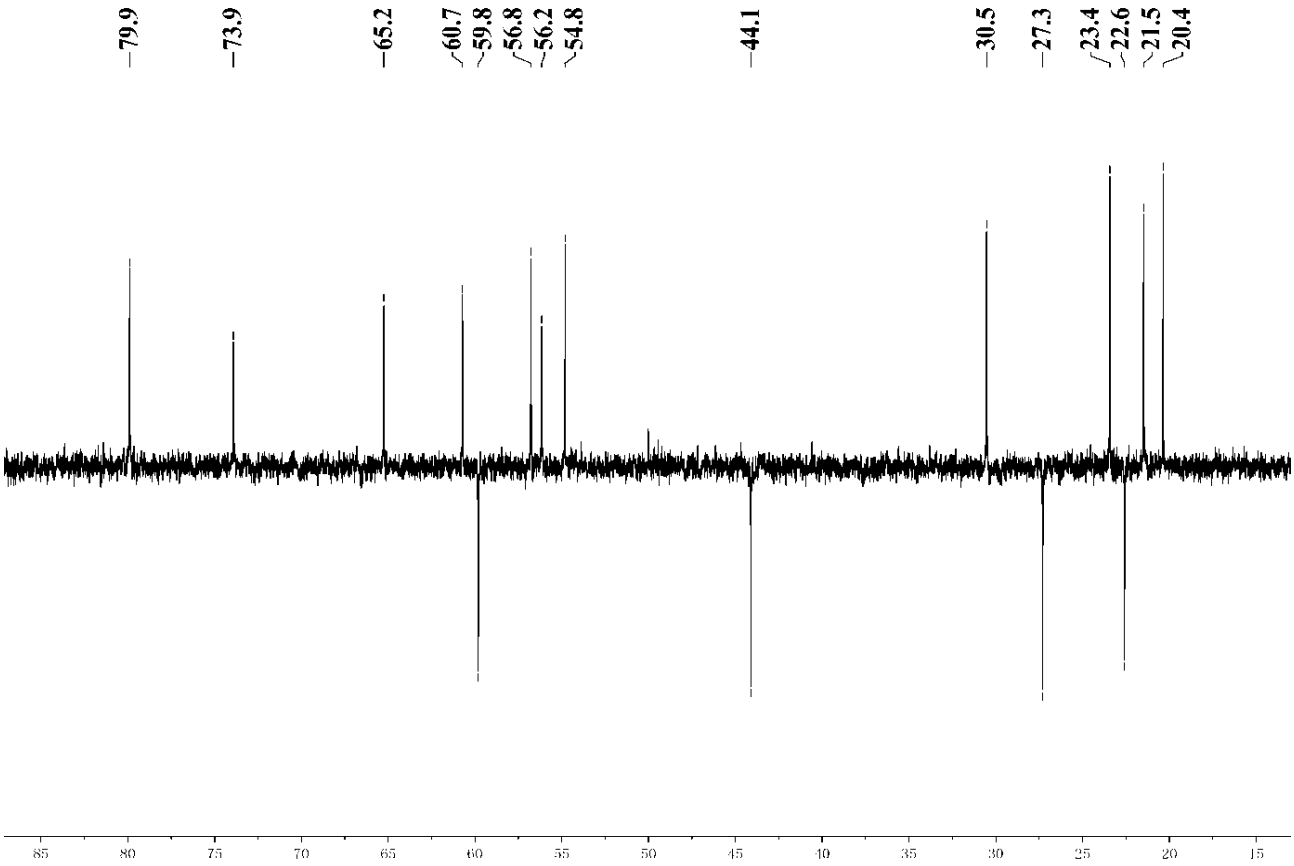

Figure S30. DEPT spectrum of **3** (100 MHz, methanol-d_4)