Supporting Information

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A New Isoflavone Glucoside from the Roots of *Astragalus membranaceus* var. *mongholicus*

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1. General experimental procedures

X-ray data were collected using an Agilent Xcalibur Nova X-ray diffractometer. Optical rotations were measured on a Rodolph Research Analytical Autopol I Automatic Polarimeter. UV data were obtained using Shimadzu UV-2450 spectrophotometry, and IR spectra were obtained using a Bruker Tensor 27 spectrometer. 1D and 2D NMR spectra were measured on a Bruker AM-500 spectrometer. HRESIMS were performed on a Waters Micromass Q-TOF spectrometer. Semipreparative HPLC separations were carried out by photodiode array (PDA) analysis using a Wufeng LC-100 apparatus with a Kromasil 100-5 C_{18} column (250 × 10 mm, 5 μm). TLC analysis was carried out on silica gel plates (Marine Chemical Ltd., Qingdao, China). Silica gel (300−400 mesh, Qingdao Haiyang Chemical Co., Ltd.), reversed-phase C_{18} (RP-C_{18}) silica gel (Fuji, 40−75 μm), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) were used for column chromatography (CC).

2. Plant material

The roots of *A. membranaceus var. mongholicus* were collected from Gansu Province, China, in October 2019. The identification of the plant was done by one of the author (Y. Wang), and a voucher specimen (201910-HQ) was deposited at School of Environmental Science and Engineering, Southern University of Science and Technology.

3. Extraction and isolation

The dried roots of *A. membranaceus var. mongholicus.* (15 kg) were powdered and extracted with 80% EtOH (45 L) at room temperature three times. After the evaporation of solvent under reduced pressure, the crude extract was suspended in water partitioned with EtOAc. The EtOAc portion (165 g) was subjected to silica gel CC with a a gradient of CH_2Cl_2/MeOH (100:1 → 5:1, v/v) to yield five fractions (Fr. A–Fr. E). Fr. B (29 g) was applied to RP-C_{18} silica gel column with MeOH/H_2O (30:70 → 100:0) as eluents to afford six subfractions (Fr. B1–Fr. B6). Fr. B2 (210 mg) further subjected to semipreparative HPLC separation (MeCN/H_2O, 30:70) to afford compound **7** (24 mg, t_R 16 min). Fr. B3 (800 mg) was purified using semipreparative HPLC (MeCN/H_2O, 45:55) to afford compounds **4** (60 mg, t_R 14.5 min), **6** (88 mg, t_R 15.3 min), **5** (75 mg, t_R 16.5 min). Fr. B5 (250 mg) was loaded onto a Sephadex LH-20 column using MeOH as eluent to give compound **1** (15 mg). Fr. C (8 g) was subjected to separation over a Sephadex LH-20 column (MeOH) to give one subfraction, which was further purified by semipreparative HPLC (MeCN/H_2O, 50:50) to yield **2** (160 mg, t_R 12.5 min) and **3** (85 mg, t_R 13 min).

4. Crystallographic data of compound 1a

monoclinic, space group I2 (no. 5), a = 5.03499(5) Å, b = 10.24226(11) Å, c = 46.0558(4) Å, β = 92.4946(8)°, V = 2372.83(4) Å^3, Z = 4, T = 100.00(10) K, μ (Cu Kα) = 0.864 mm^{-1}, *Dcalc* = 1.300 g/cm^3, 24799 reflections measured (7.686° ≤ 2Θ ≤ 158.27°), 5026 unique (R_{int} = 0.0485, R_{sigma} = 0.0310) which were used in all calculations. The final R_1 was 0.0536 (I > 2σ (I)) and wR_2 was 0.1495 (all data). Crystallographic data for **1** have been deposited in the Cambridge Crystallographic Data Center (CCDC number: 2108374).

5. Cell culture and viability assay

RAW 264.7 cells were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultivated in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin at 37 °C in a humidified incubator containing 5% CO_2. The cell viability assay was detected by using MTT method.

6. Measurement of NO production

The NO concentration was measured by the Griess reagent. Briefly, RAW264.7 cells were treated with LPS (1.0 μg/mL) and compounds for 24 h. After that, the 50 μL of culture supernatant was collected to react with the same volume of Griess reagent for 10 min at room temperature in the dark. Then, the absorbance was measured at 540 nm using a microplate reader. Inhibition (%) = $(1 - (A_{LPS + sample} - A_{untreated})/(A_{LPS} - A_{untreated}) \times 100$. The experiments were performed in triplicates. Quercetin was used as a positive control.

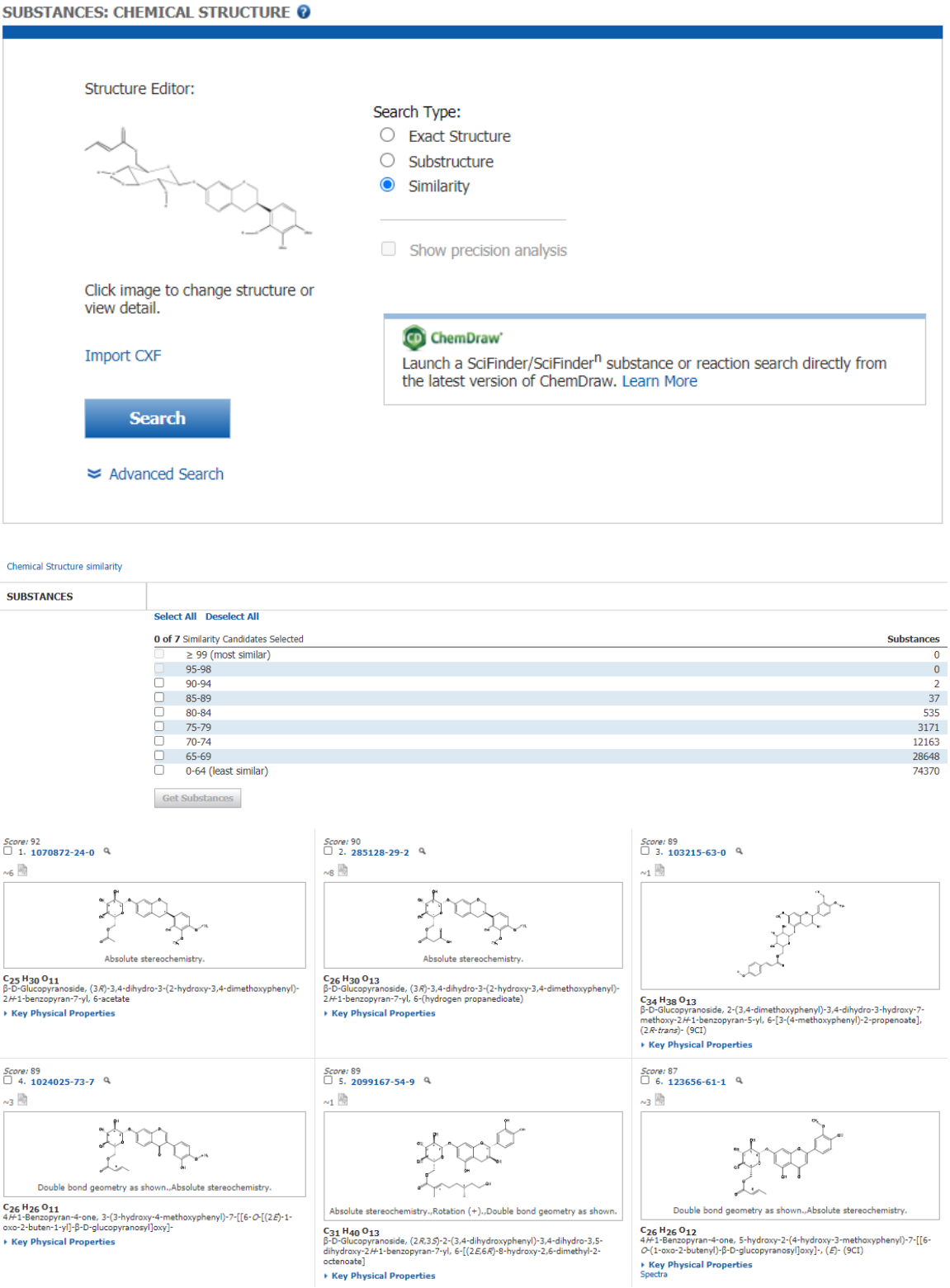
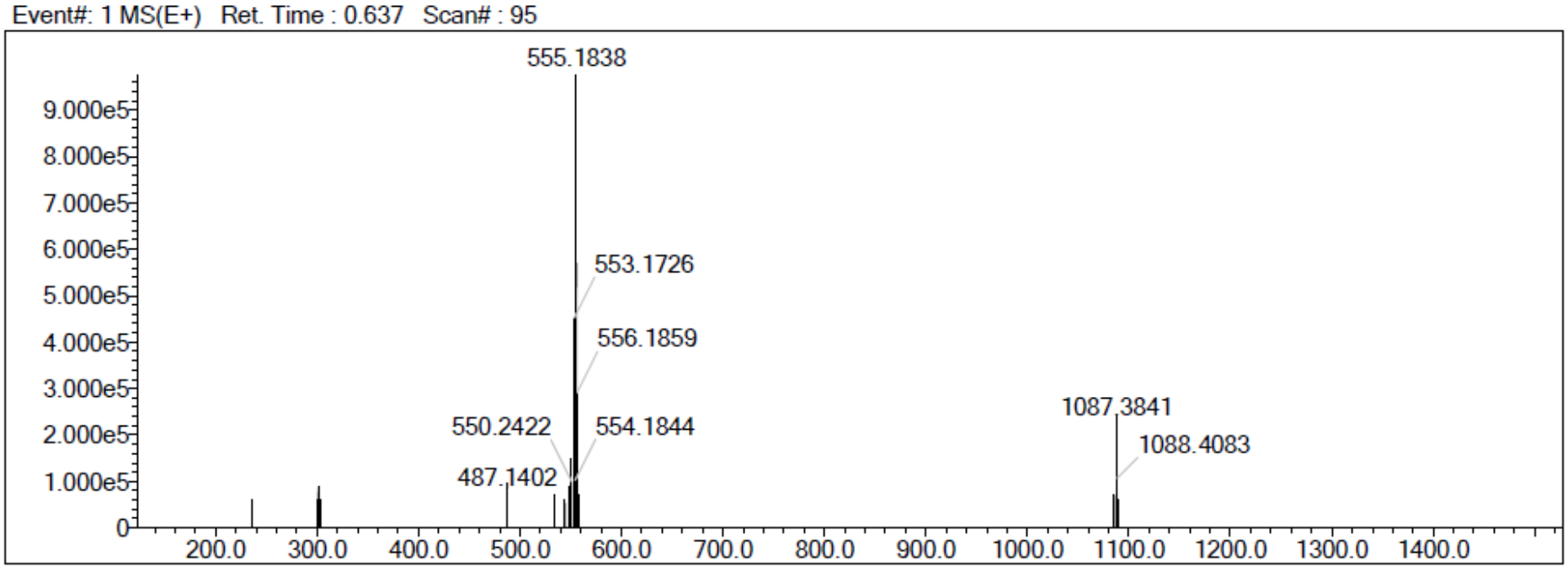


Figure S1: Scifinder search of new compound **1**



Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)
C27 H32 O11	[M+Na]+	555.1838	555.1837	0.1	0.18

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

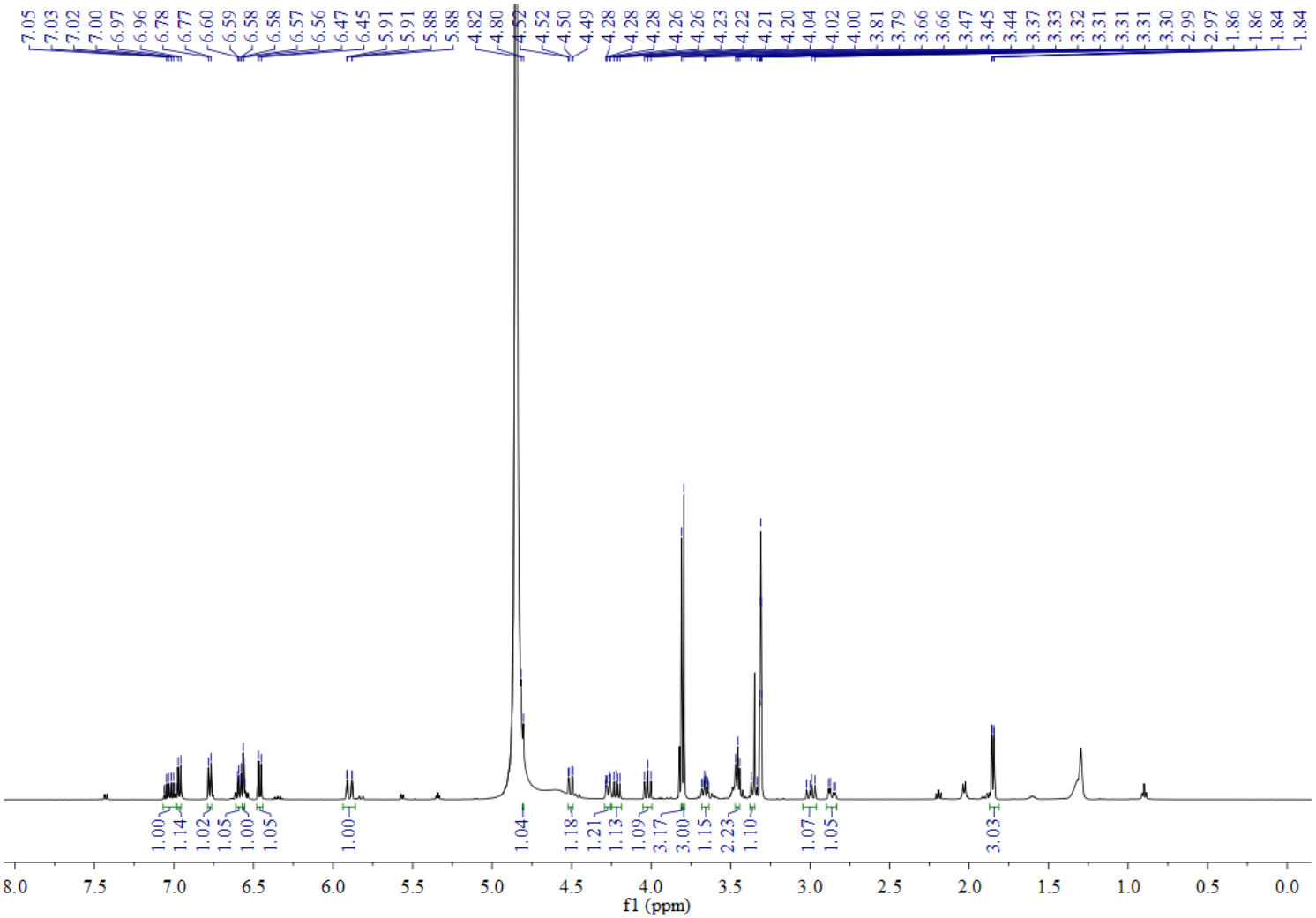


Figure S3: ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **1**

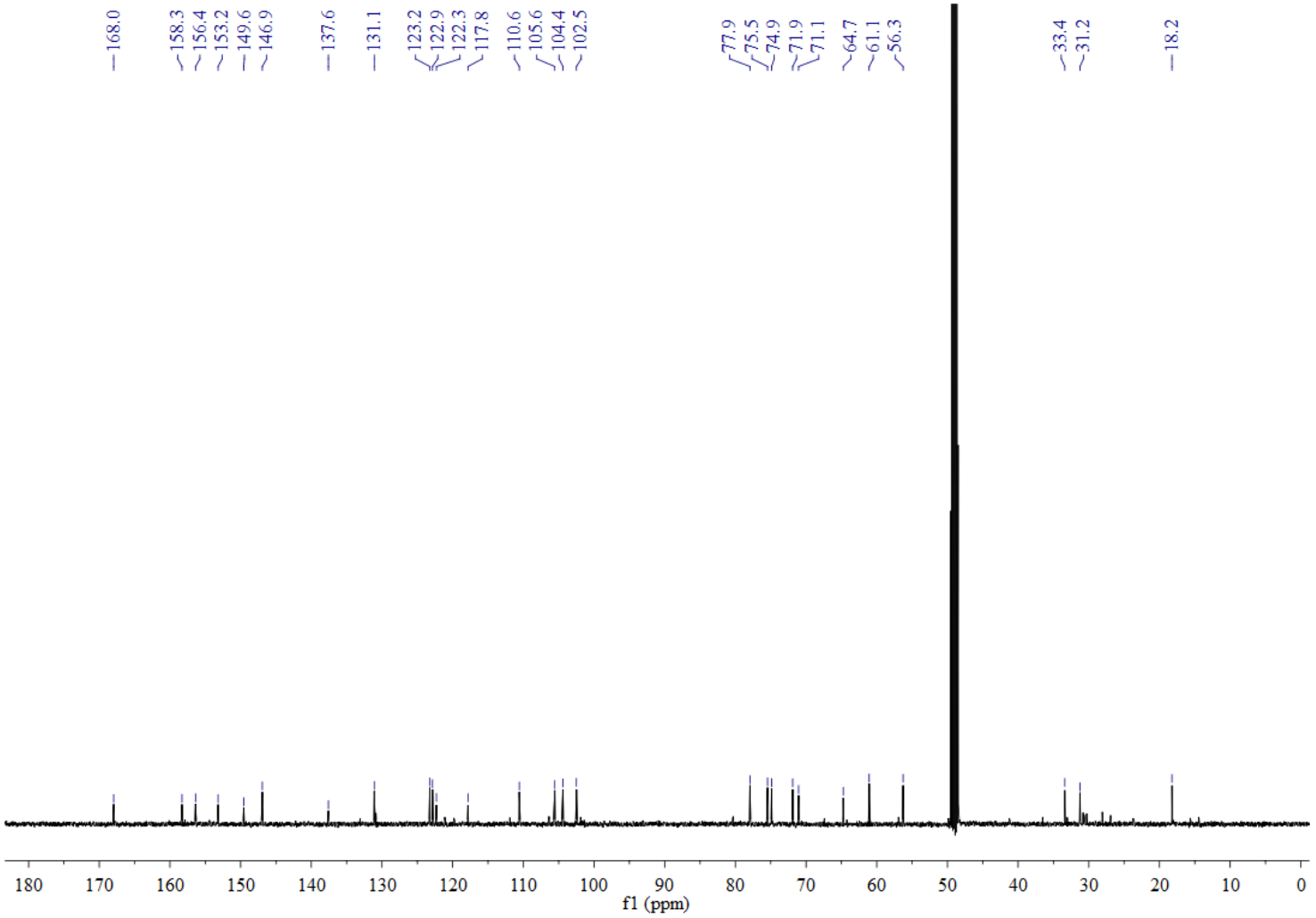


Figure S4: ^{13}C-NMR (125 MHz, CD_3OD) spectrum of **1**

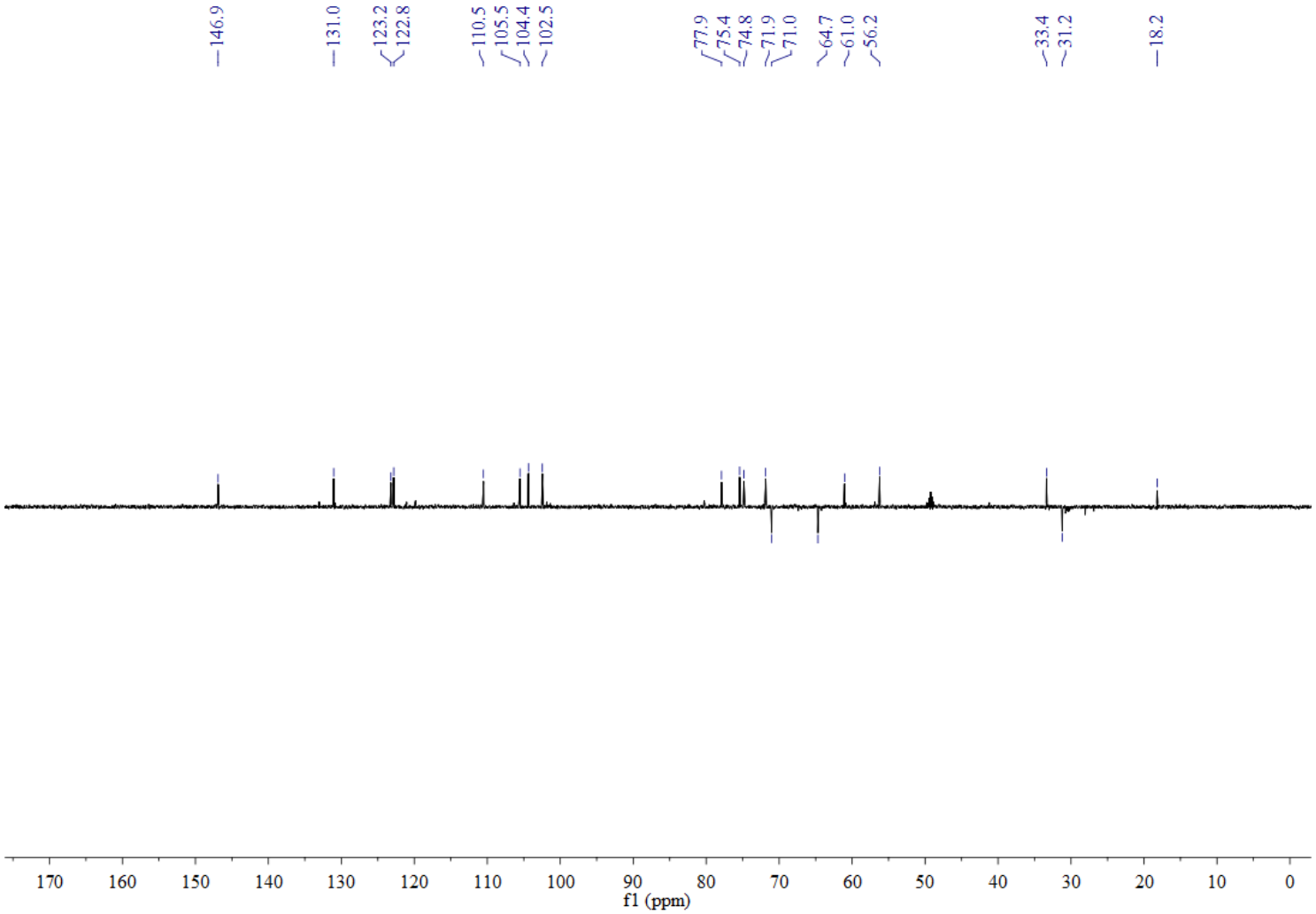


Figure S5: DEPT135 (125 MHz, CD_3OD) spectrum of **1**

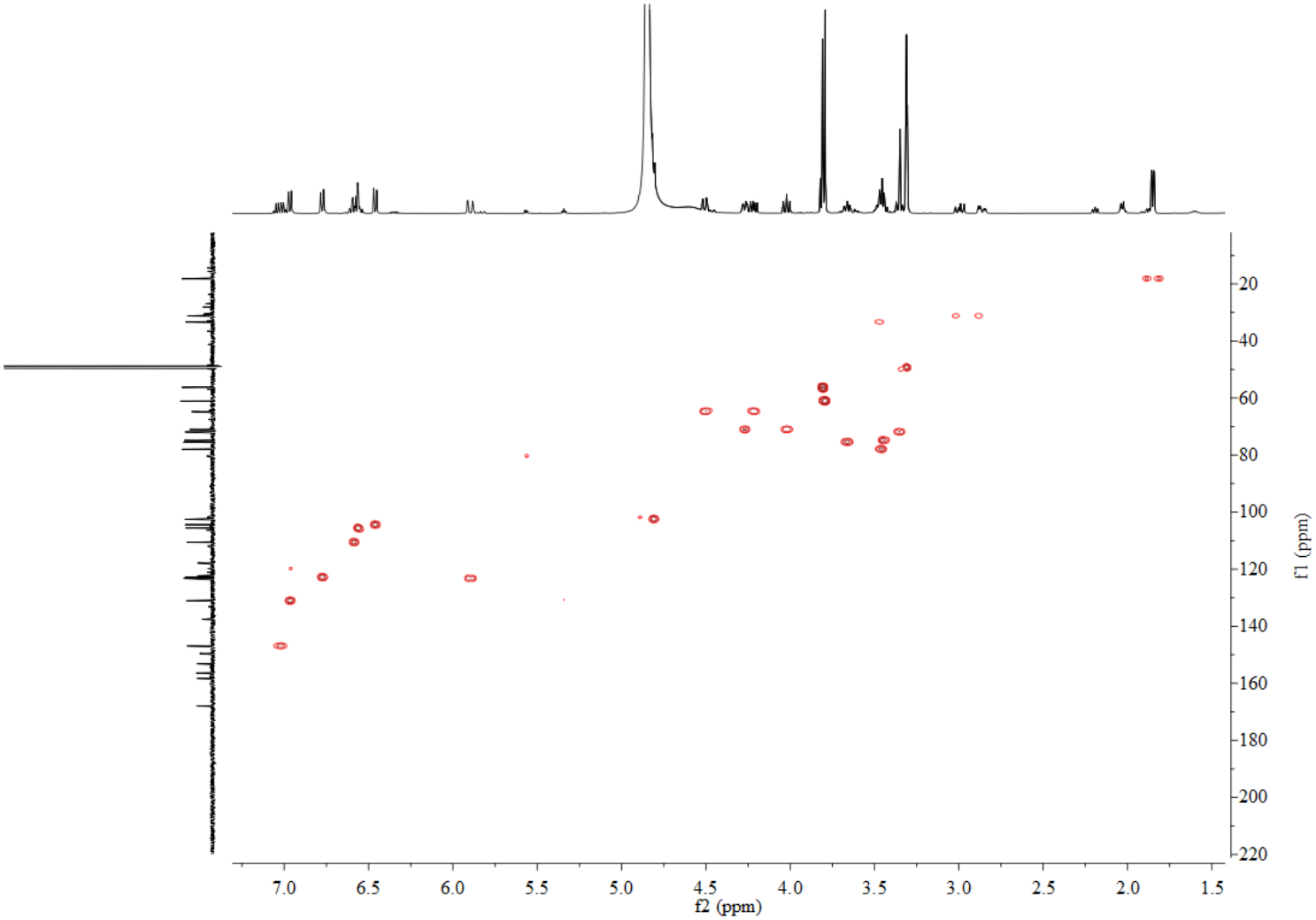


Figure S6: HSQC spectrum of **1**

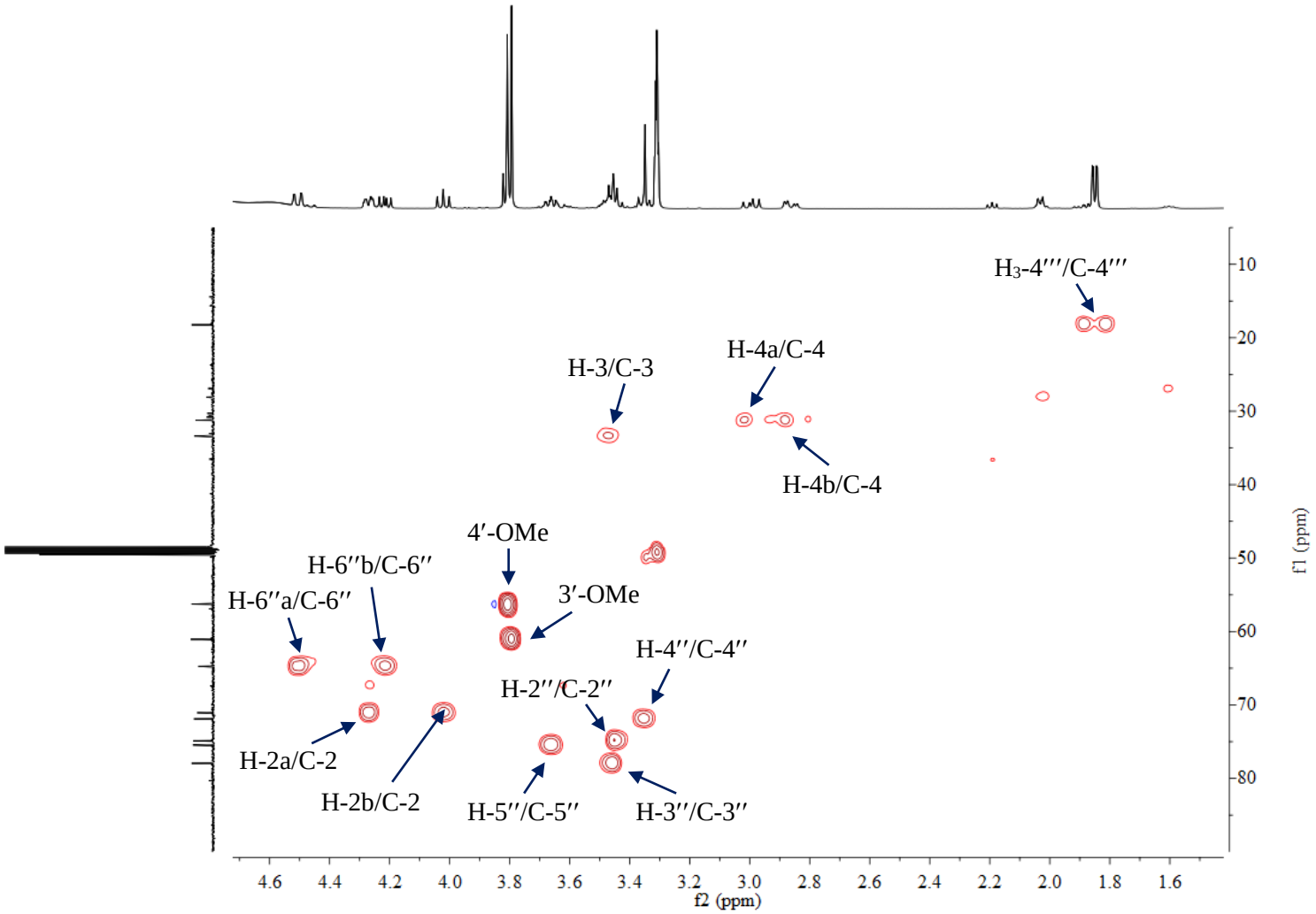


Figure S7: HSQC spectrum of **1** (From δ_C 5 ppm to δ_C 90 ppm)

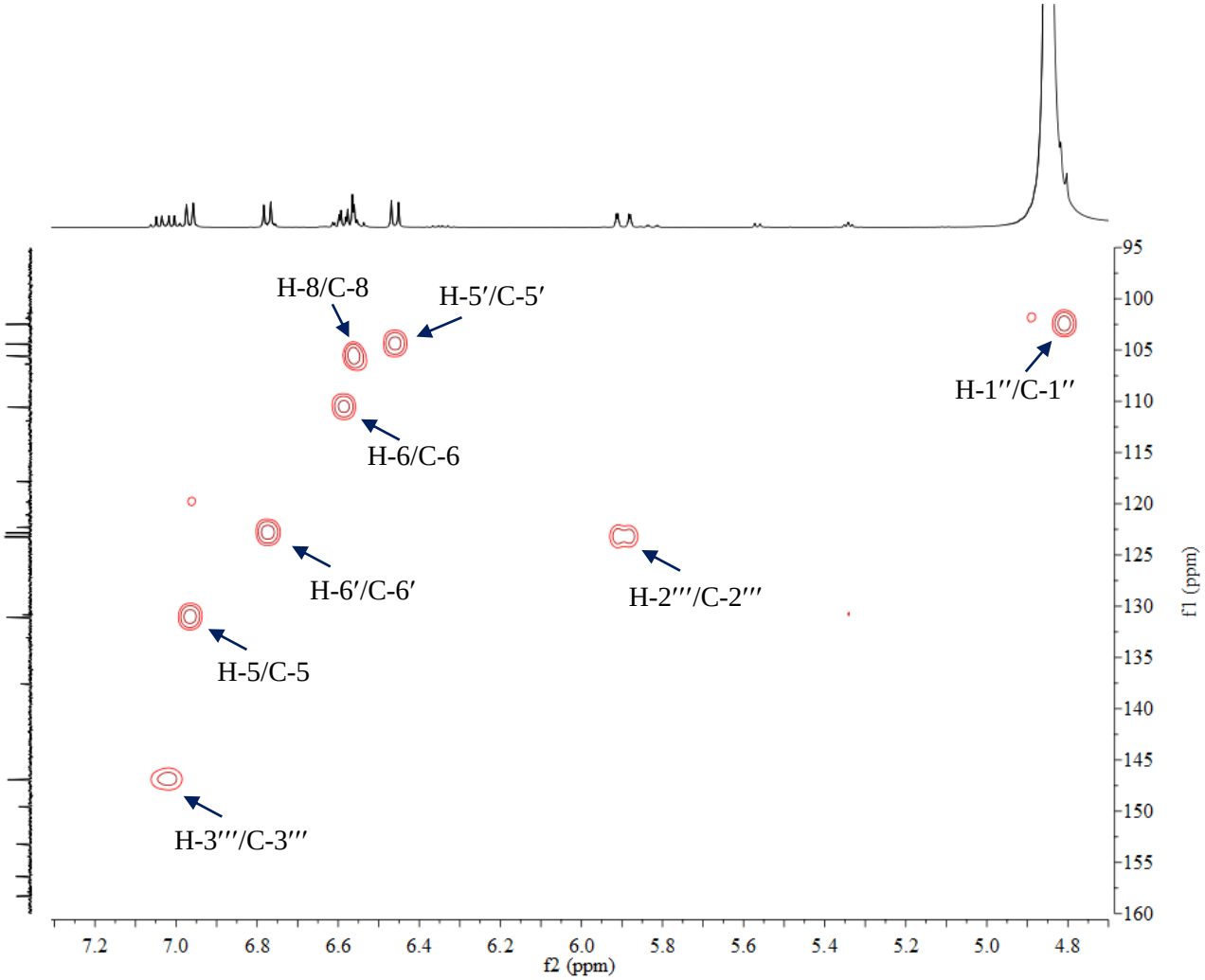


Figure S8: HSQC spectrum of **1** (From δ_C 95 ppm to δ_C 160 ppm)

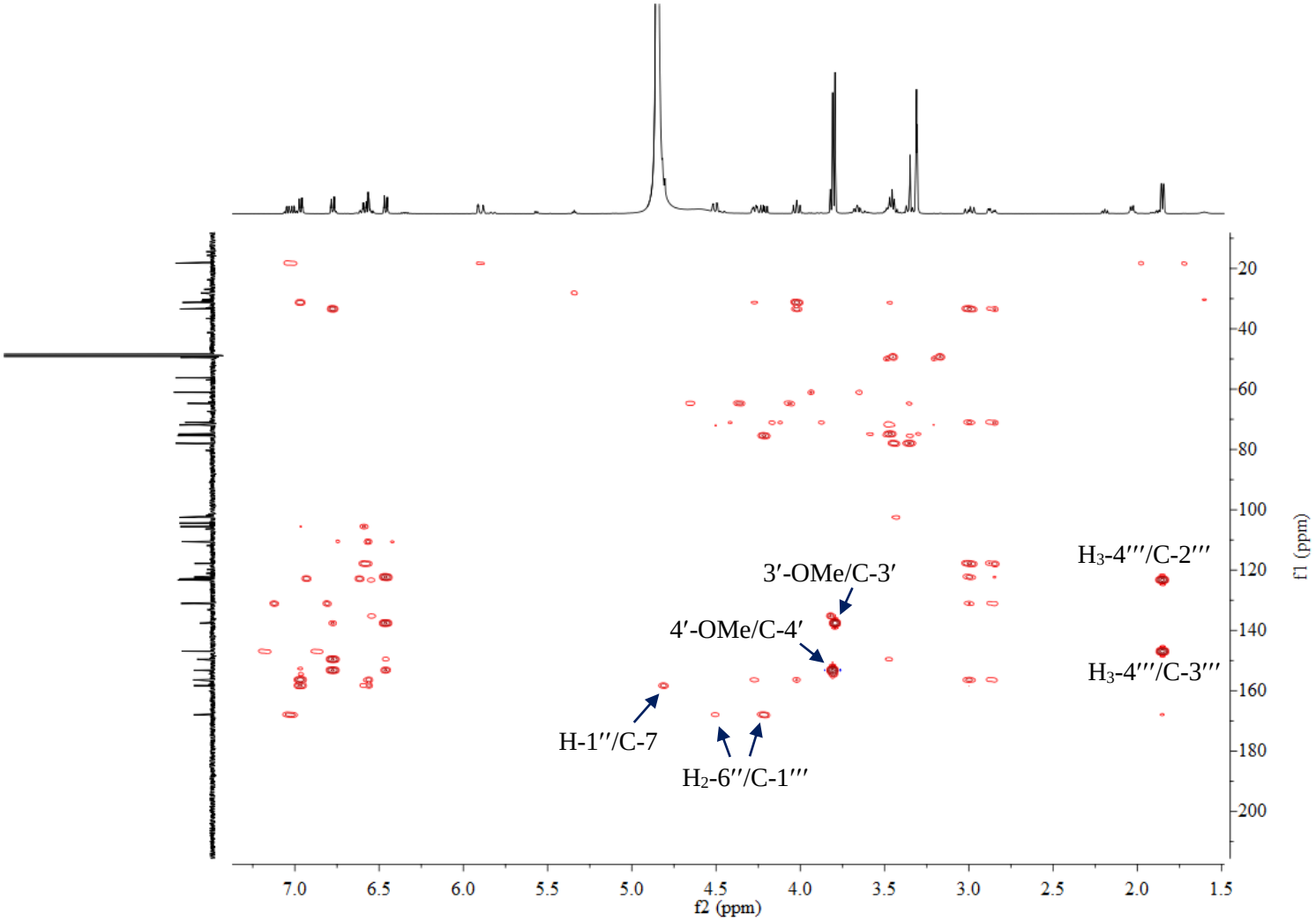


Figure S9: HMBC spectrum of **1**

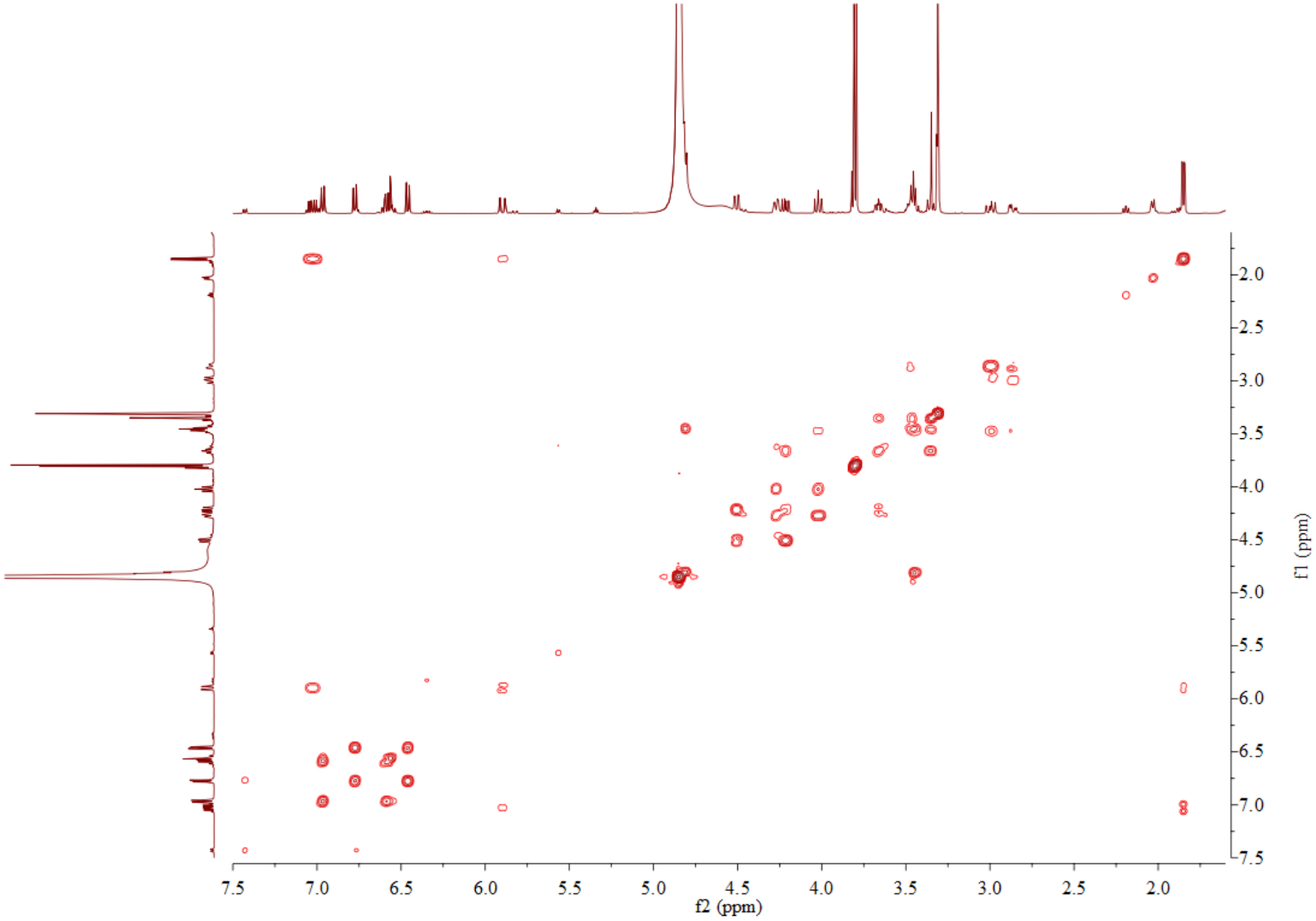


Figure S10: ^{1}H–^{1}H COSY spectrum of **1**