

Supporting Information

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Two new compounds from the leaves of *Forsythia suspensa*

(Thunb.) Vahl

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Experimental

General experimental procedures

The HR-ESI-MS spectra were recorded on an Agilent Technologies 6550 Q-TOF (Santa Clara, CA, USA); 1 D and 2 D NMR spectra were recorded on Bruker-AVANCE 400 instrument (Bruker, Rheinstetten, Germany) with TMS as an internal standard; Semipreparative HPLC was performed on a system comprising an NP7000 SERIALS pump (Hanbon Sci. Tech., China) equipped with NU3000 serials UV/VIS detector and Capcell Pak C₁₈ column, (10 mm × 250 mm, 5 µm particles); Reversed-phase C₁₈ silica gel (5 µm, YMC Co., Ltd. Japan); Silica gel (100-200 and 200-300 mesh, Qingdao Haiyang Chemical, China); All solvents used in CC were of analytical grade (Sinopharm Chemical Reagent Co., Ltd. China). Mouse mononuclear macrophage leukaemia cells (RAW264.7, Chinese Academy of Sciences Cell Bank).

Plant material

The *Forsythia suspensa* was collected from Xunyi Xianyang, Shaanxi Province of China, and identified as *P. terminalis* by Professor Wei Wang (School of Pharmacy, Shaanxi University of Chinese Medicine). A voucher specimen (Herbarium No. 20190911LQ) has been deposited in the Medicinal Plants Herbarium, School of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang, China.

Extraction and isolation

The leaves of *Forsythia suspensa* (6.0 kg) were air-dried and ground into powder. The powder was extracted three times with 80% ethanol. After removing the ethanol under reduced pressure, the extract was separated by RP-C18 column chromatography using a gradient solvent system (methanol/water, volume ratio, 20:80–100:0), yielding three fractions (Fs.1-Fs.3). Fs.1 (18.0 g) was separated by RP-C18 column chromatography and eluted with a solvent system (methanol/water, volume ratio, 40:60–90:0), yielding four fractions (Fs.1.1-Fs.1.4). Fr.1.1 (3.5 g) was purified by semi-preparative HPLC (methanol/water, volume ratio 65:35, flow rate 2 mL·min⁻¹), yielding compound **1** (5.8 mg, *t_R* = 24.2 min). Fraction 1.2 (1.3 g) was purified by semi-preparative HPLC (methanol/water, volume ratio 70:30, flow rate 2 mL·min⁻¹), yielding compound **2** (6.3 mg, *t_R* = 27.3 min).

Anti-inflammatory activity

A LPS-induced inflammatory model of RAW264.7 cells was used to screen the activity of the isolated pure compounds. Logarithmically growing RAW264.7 cells were seeded at a density of 5.0×10^5 cells/mL in a 96-well plate and cultured at 37 °C in a 5% CO₂ incubator for 24 hours. Control group: 100 μ L of complete medium containing 0.1% DMSO (without cells) was added; Model group: 100 μ L of complete medium containing 0.1% DMSO and LPS (1 μ g/mL) was added; AG positive control group: 100 μ L of complete medium containing AG (12 μ mol/L) and LPS (1 μ g/mL) was added; Drug treatment group: Add 100 μ L of complete medium containing the test compound (3.125, 6.25, 12.5, 25, 50, 100 μ mol/L) and LPS (1 μ g/mL). Add the medium to different groups by replacing the medium. Induce for 24 hours, then add 50 μ L of cell supernatant and 100 μ L of Griess reagent to a 96-well plate for reaction. Protect from light, measure the A value of each well at a wavelength of 550 nm, and calculate the inhibition rate of the compound on LPS-induced NO production in RAW 264.7 cells according to the formula (1). Repeat the experiment three times. The IC₅₀ values of compounds **1-2** were calculated.

Statistical analysis

All cytotoxicity results were expressed as means \pm OD for three independent experiments. The IC₅₀ values were calculated based on inhibition rates at different concentrations by SPSS 22.0 statistical software.

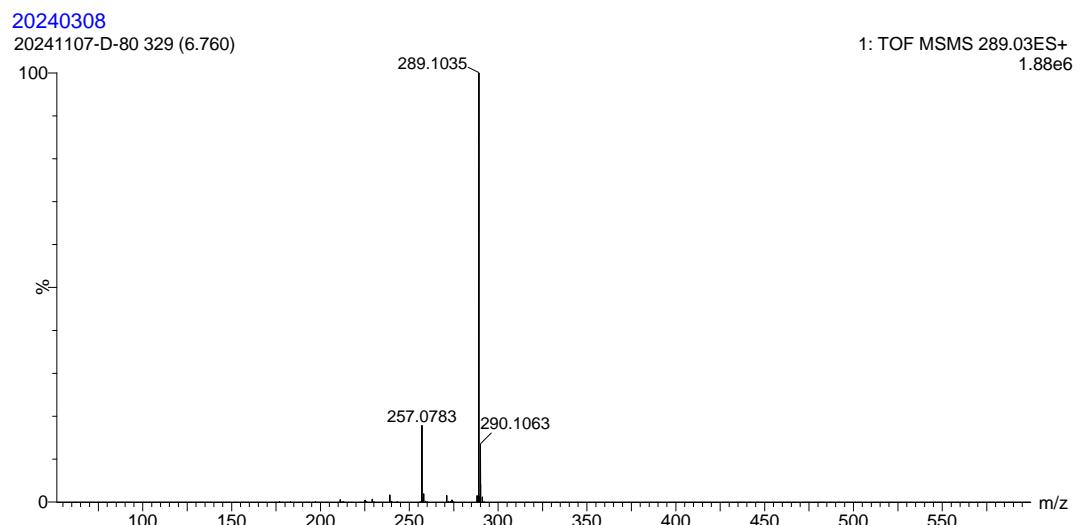


Figure S1. The HR-ESI-MS spectrum of **1** (in MeOH)

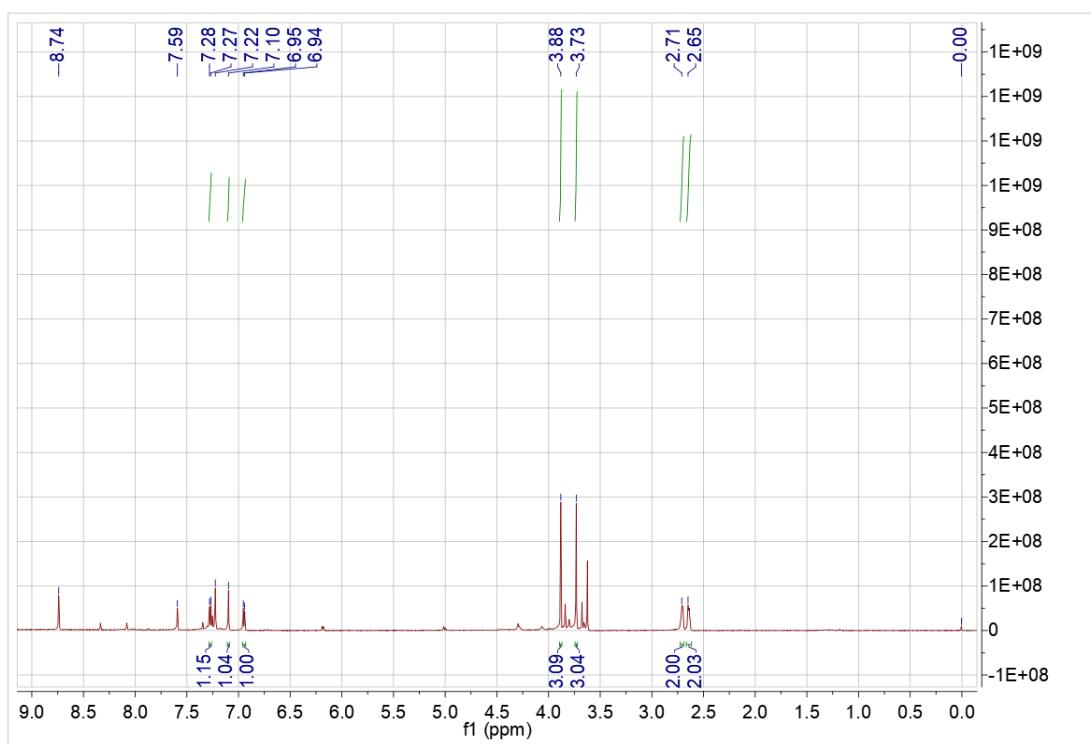


Figure S2. The ^1H NMR spectrum of **1** (in Pyridine- d_5)

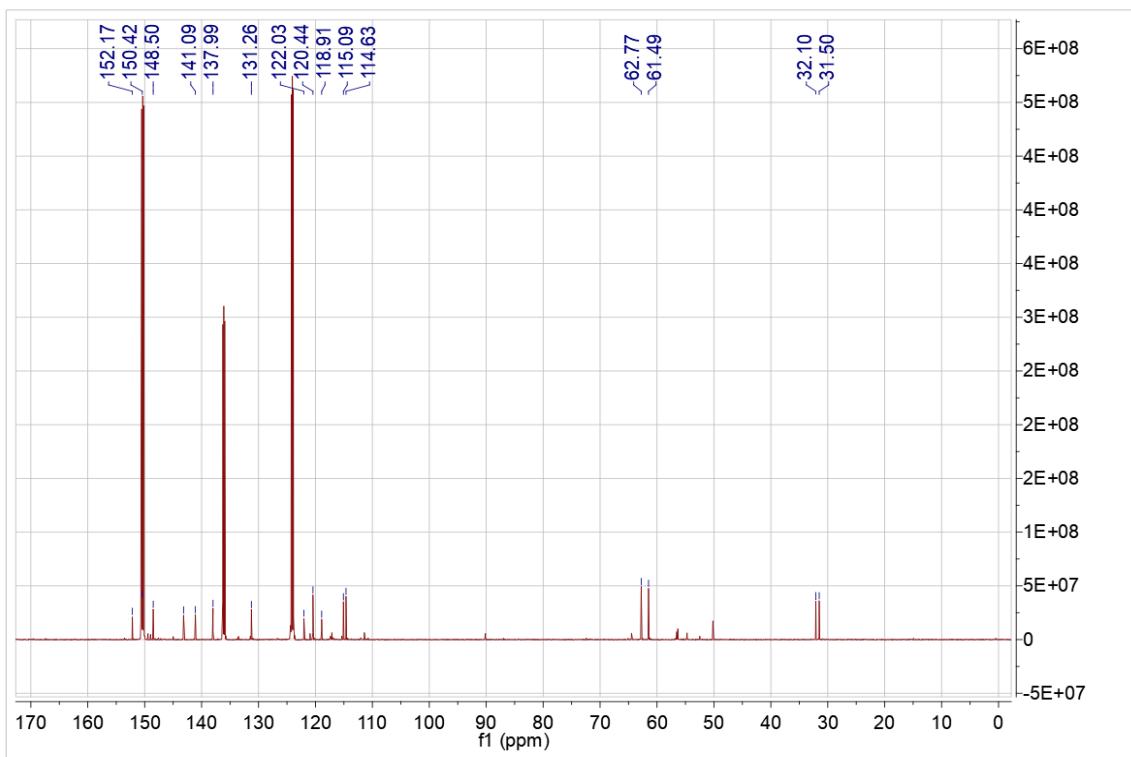


Figure S3. The ^{13}C NMR spectrum of **1** (in Pyridine- d_5)

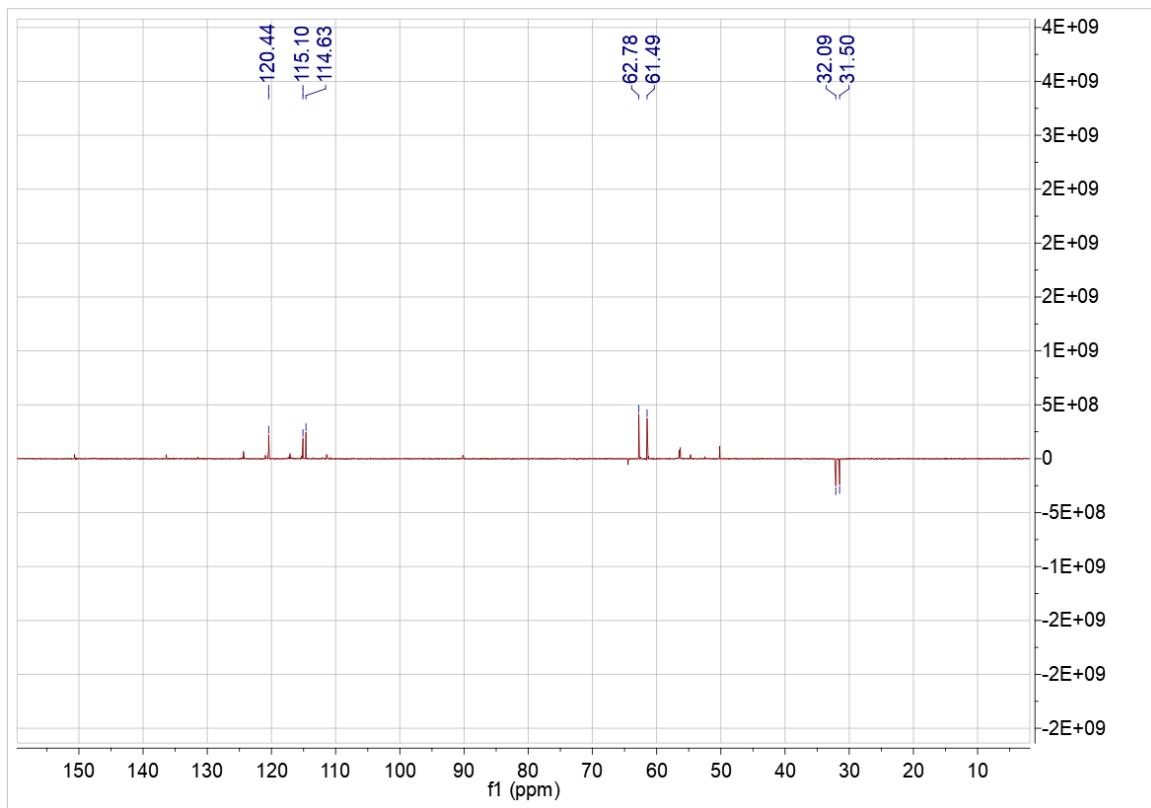


Figure S4. The DEPT 135 spectrum of **1** (in Pyridine- d_5)

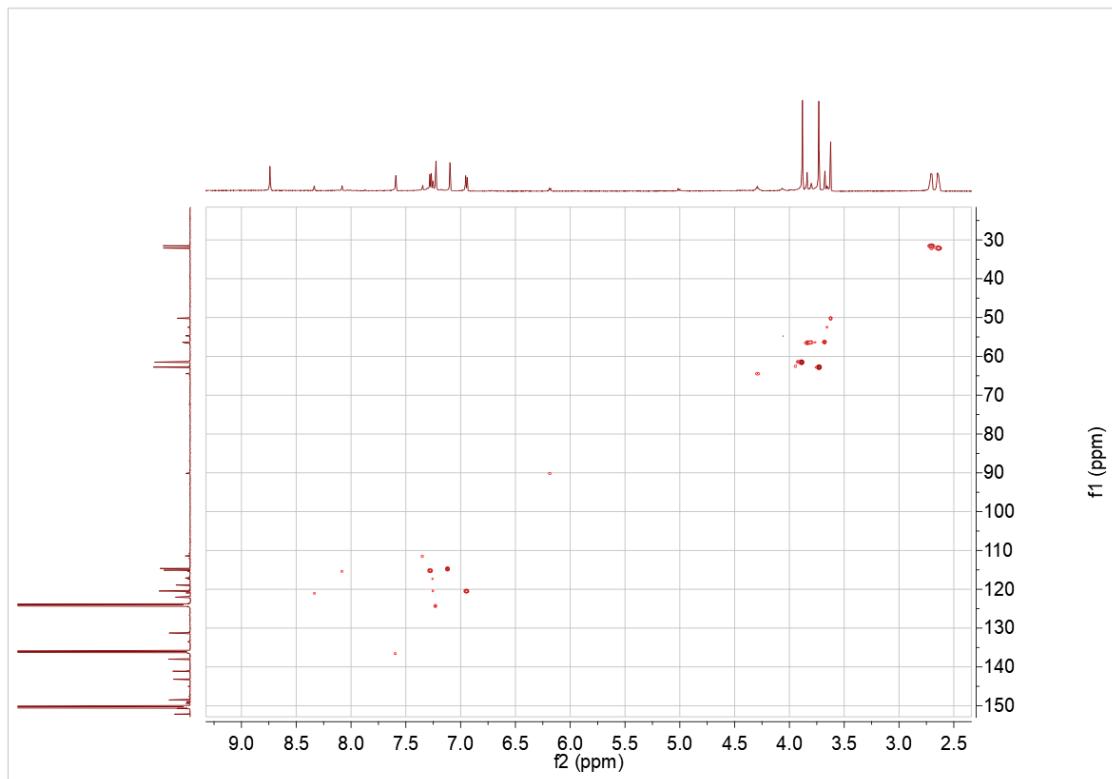


Figure S5. The HSQC spectrum of **1** (in Pyridine-*d*₅)

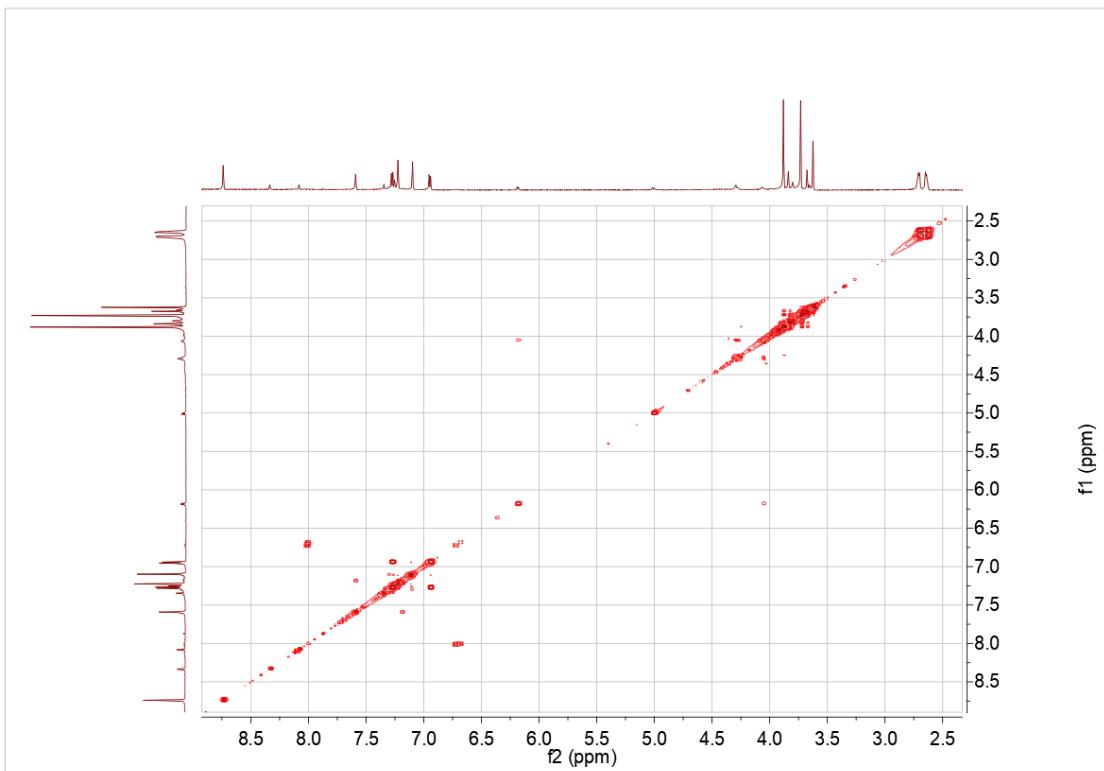


Figure S6. The ¹H-¹H COSY spectrum of **1** (in Pyridine-*d*₅)

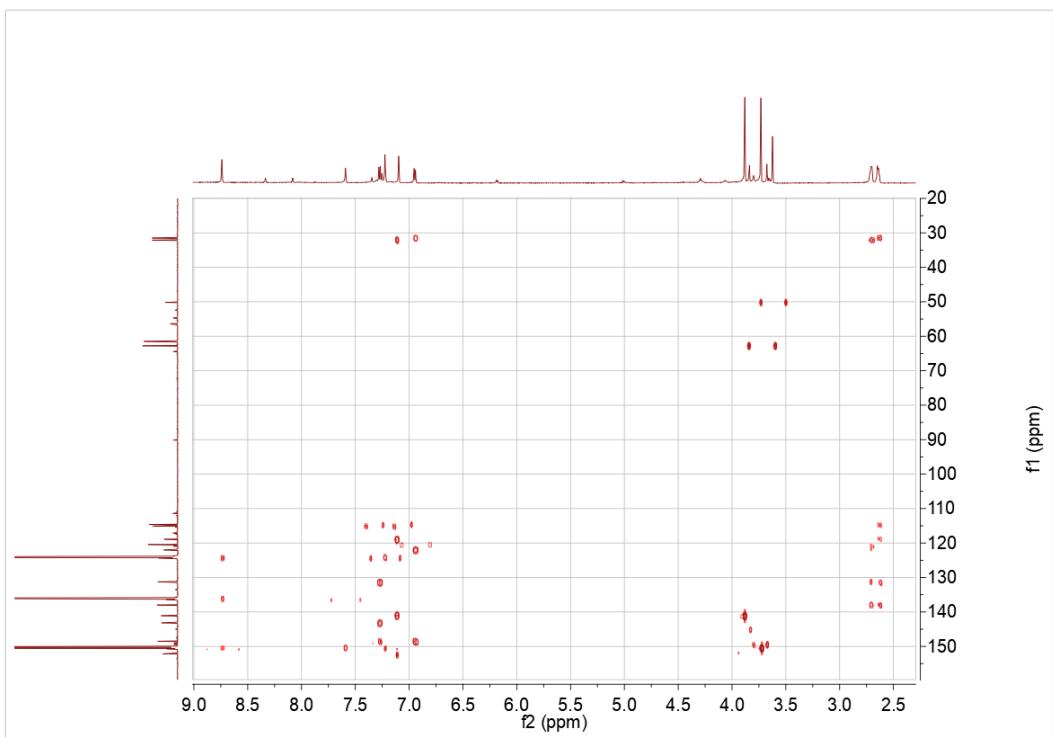


Figure S7. The HMBC spectrum of **1** (in Pyridine-*d*₅)

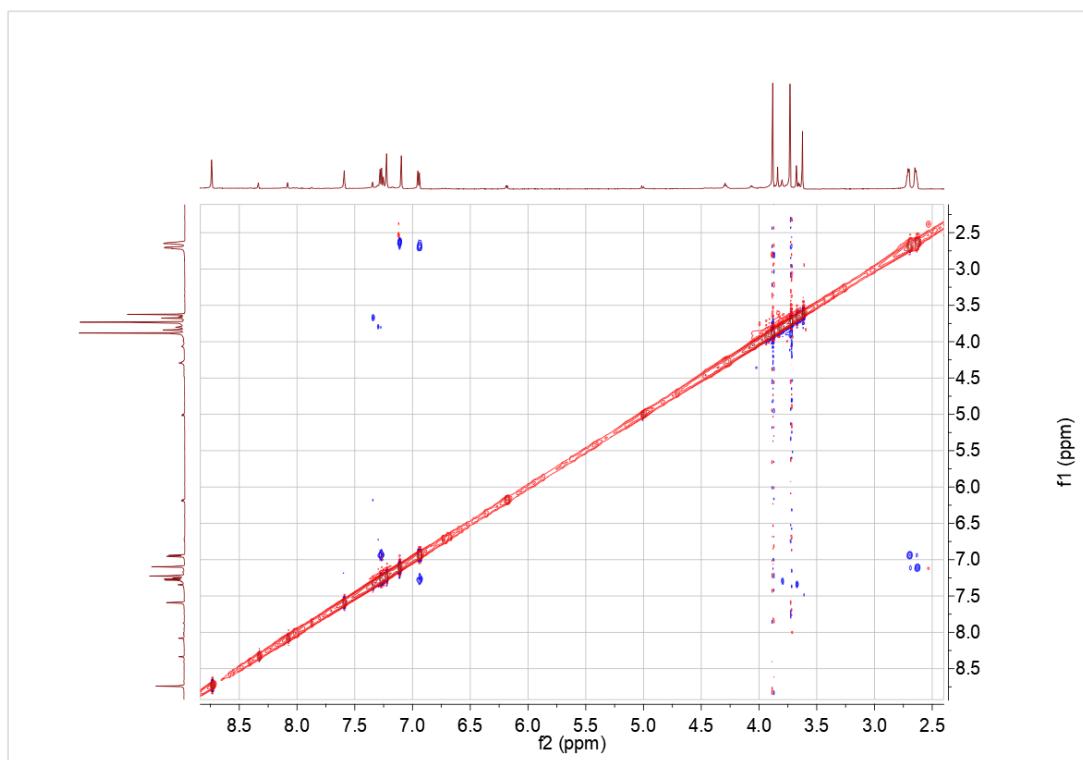


Figure S8. The NOESY spectrum of **1** (in Pyridine-*d*₅)

Substances (30)

[View in CAS SciFinder](#)

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Figure S9. Scifinder similarity report for compound **1**

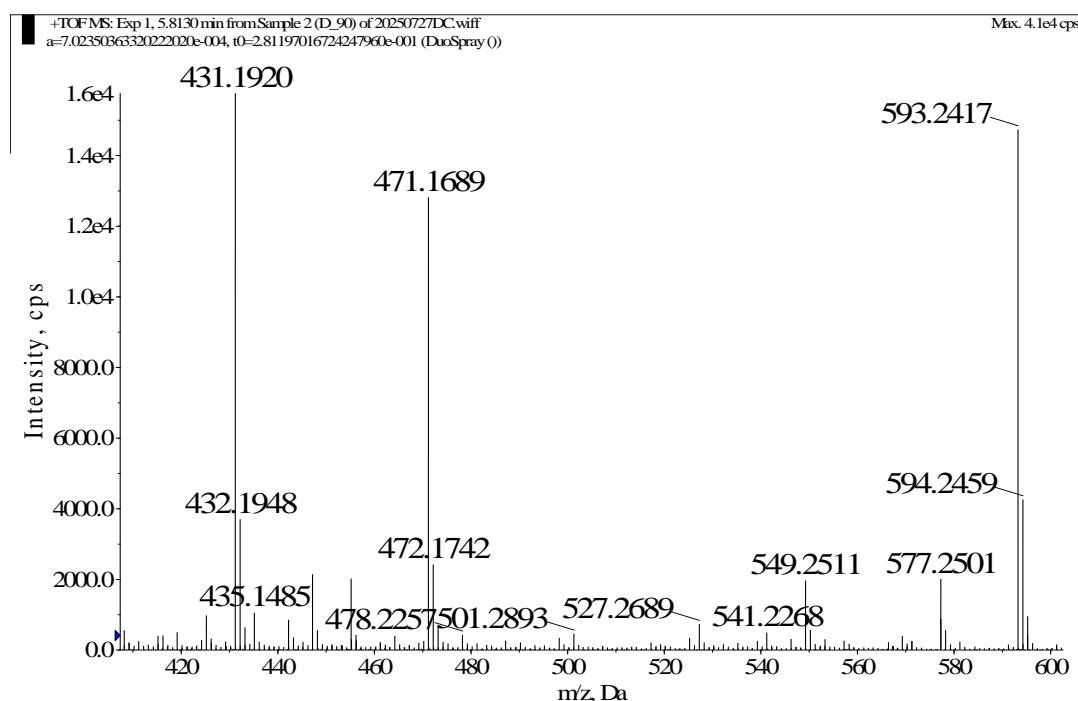


Figure S10. The HR-ESI-MS spectrum of **2** (in MeOH)

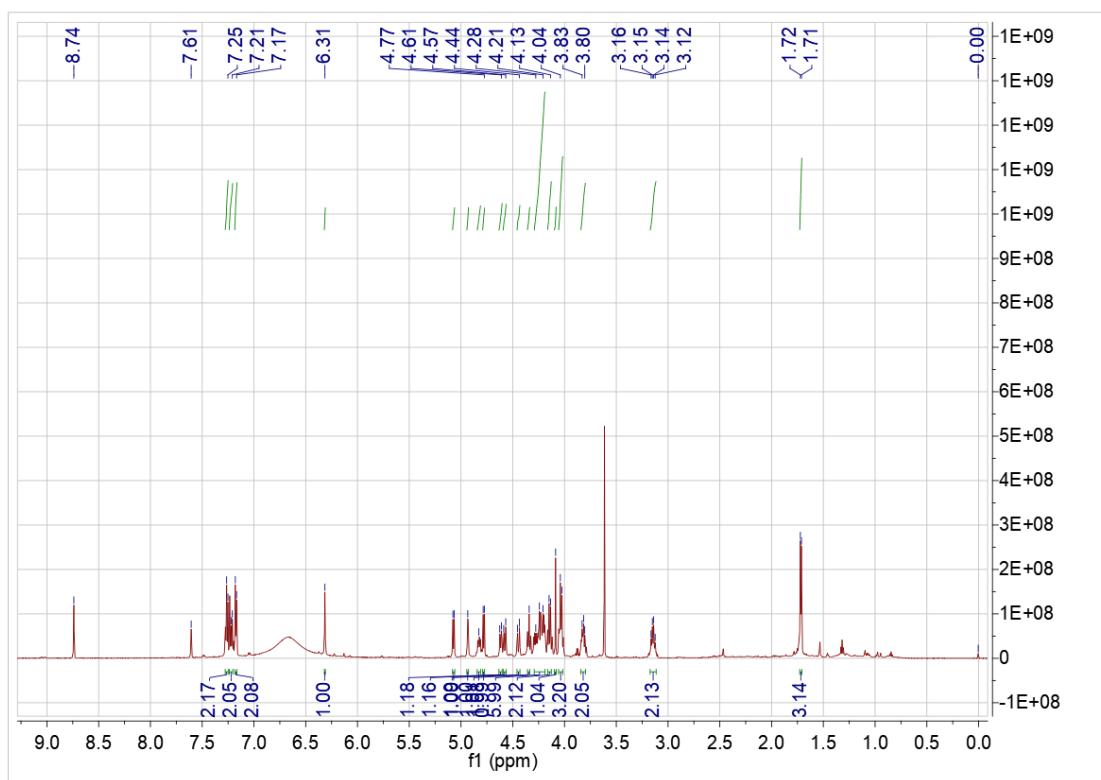


Figure S11. The ^1H NMR spectrum of **2** (in Pyridine- d_5)

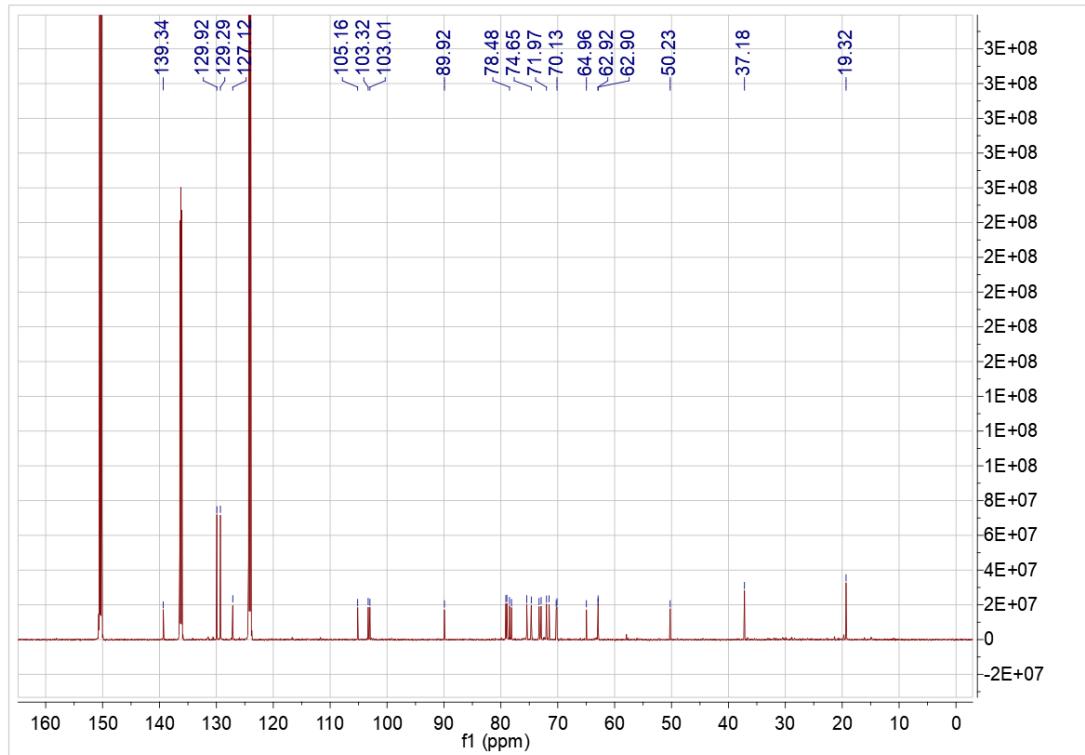


Figure S12. The ^{13}C NMR spectrum of **2** (in Pyridine- d_5)

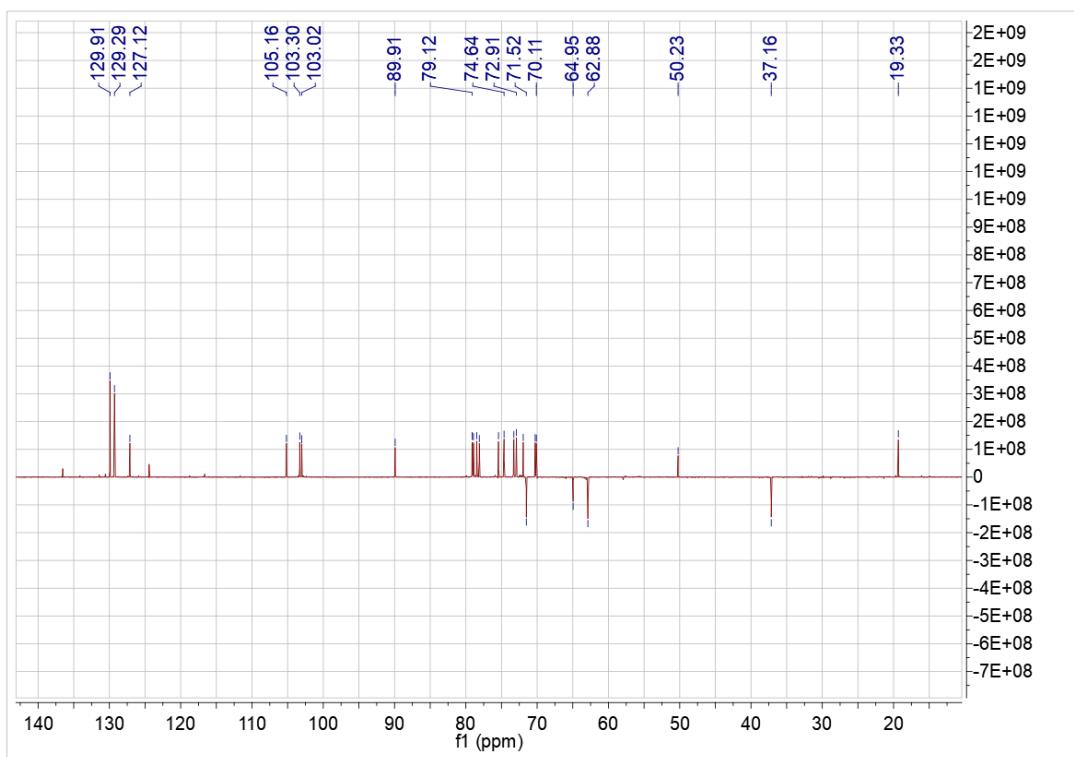


Figure S13. The DEPT 135 spectrum of **2** (in Pyridine-*d*₅)

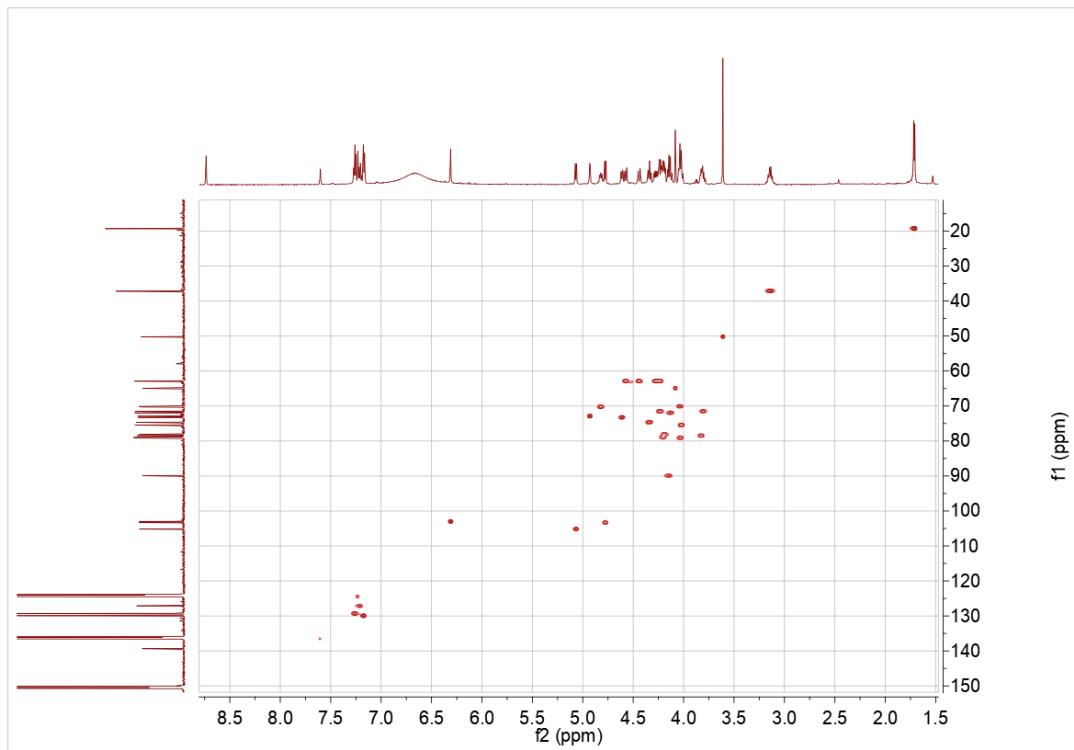


Figure S14. The HSQC spectrum of **2** (in Pyridine-*d*₅)

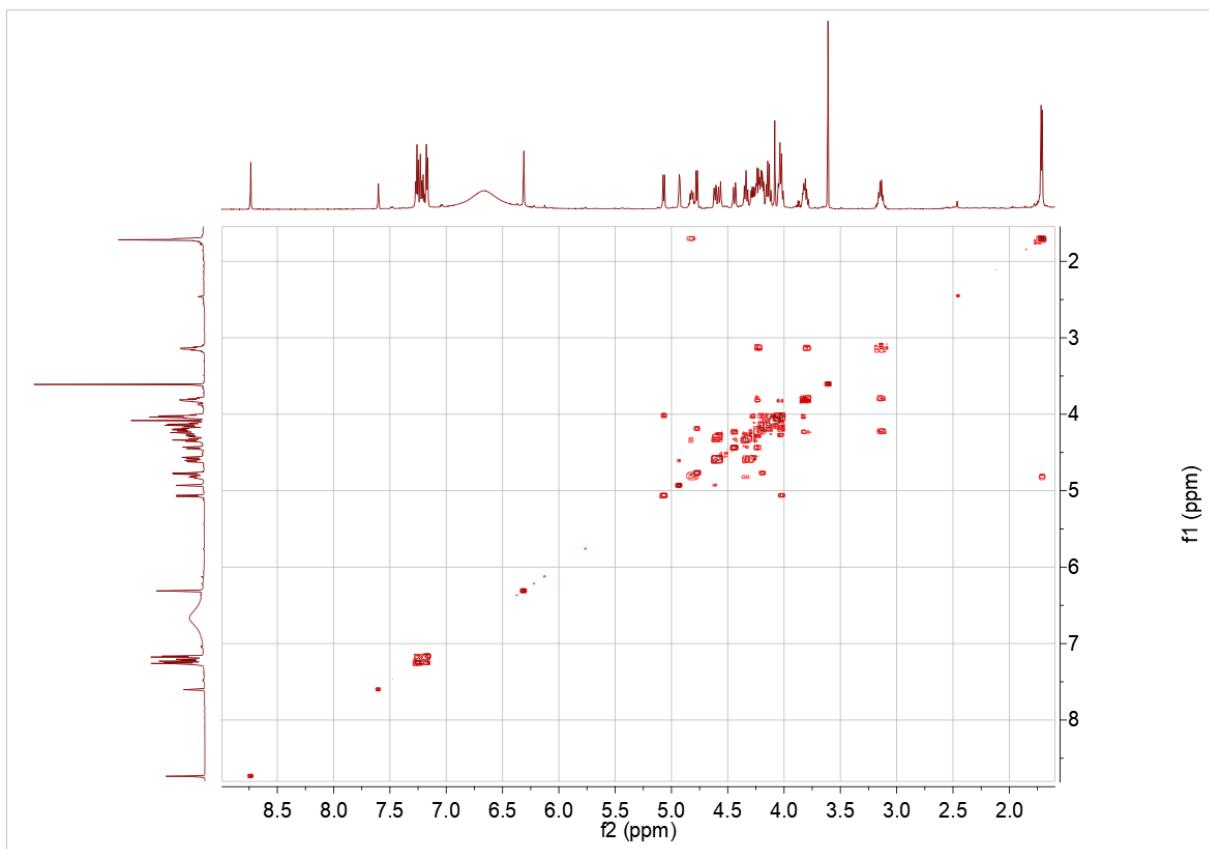


Figure S15. The ^1H - ^1H COSY spectrum of **2** (in Pyridine- d_5)

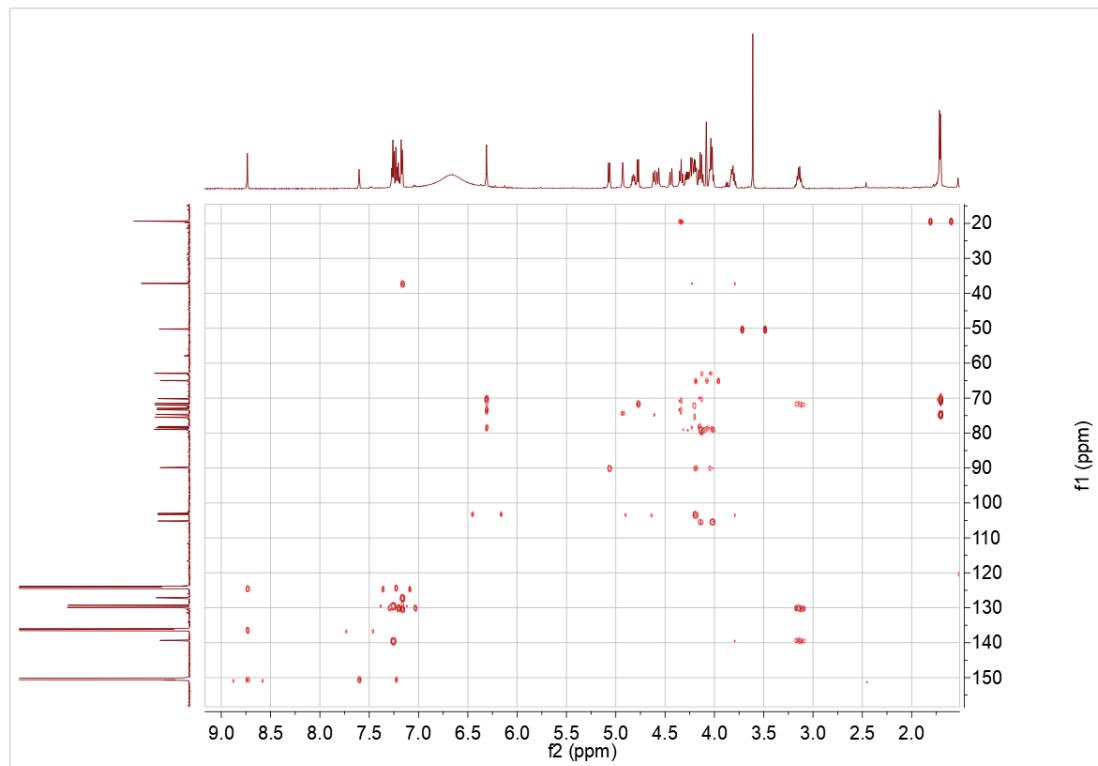


Figure S16. The HMBC spectrum of **2** (in Pyridine- d_5)

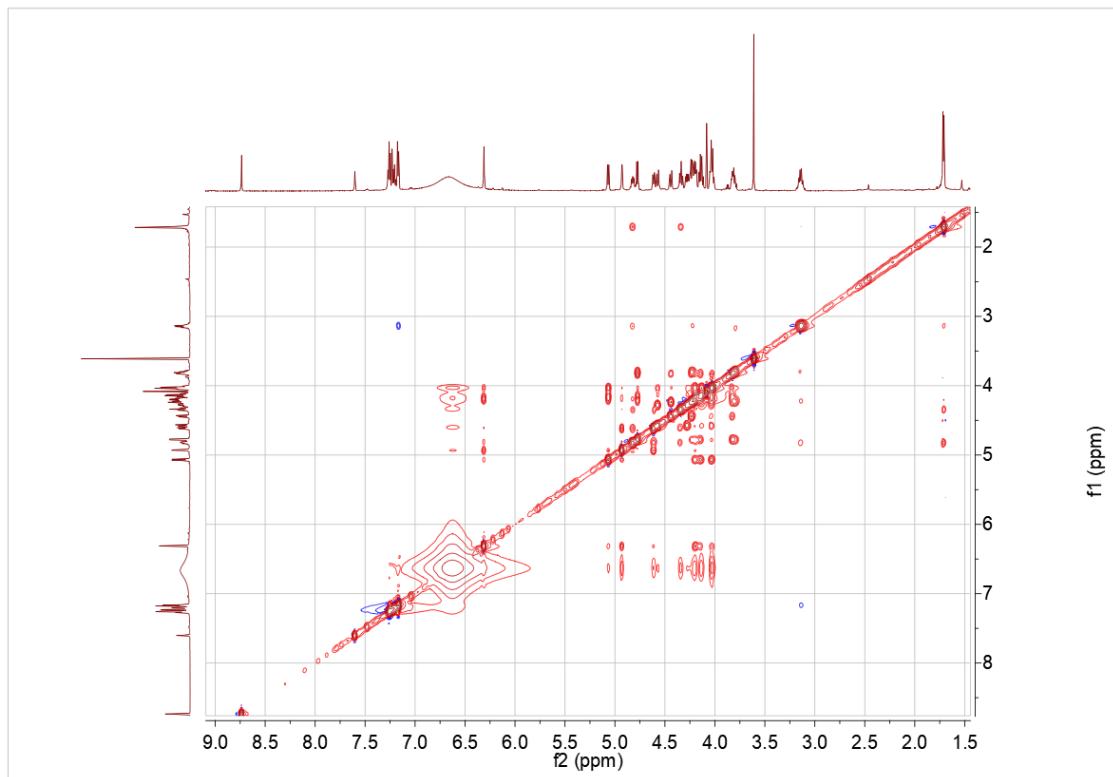


Figure S17. The NOESY spectrum of **2** (in Pyridine-*d*₅)

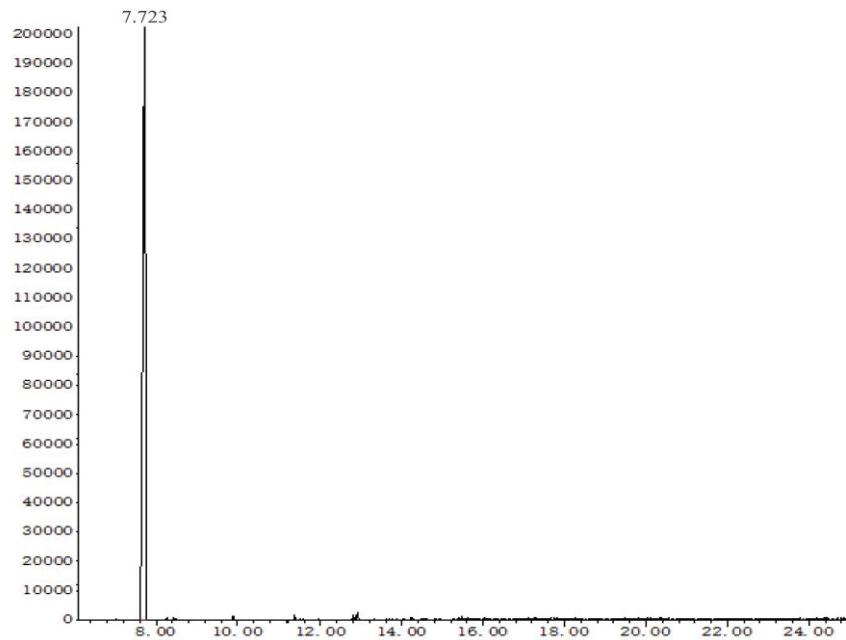


Figure S18. Gas chromatogram of D-glucose

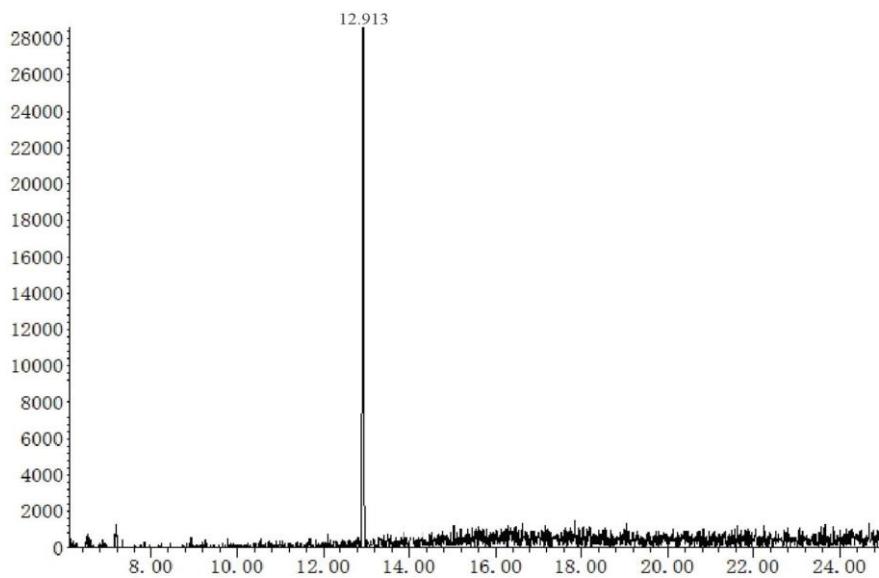


Figure S19. Gas chromatogram of L-rhamnose

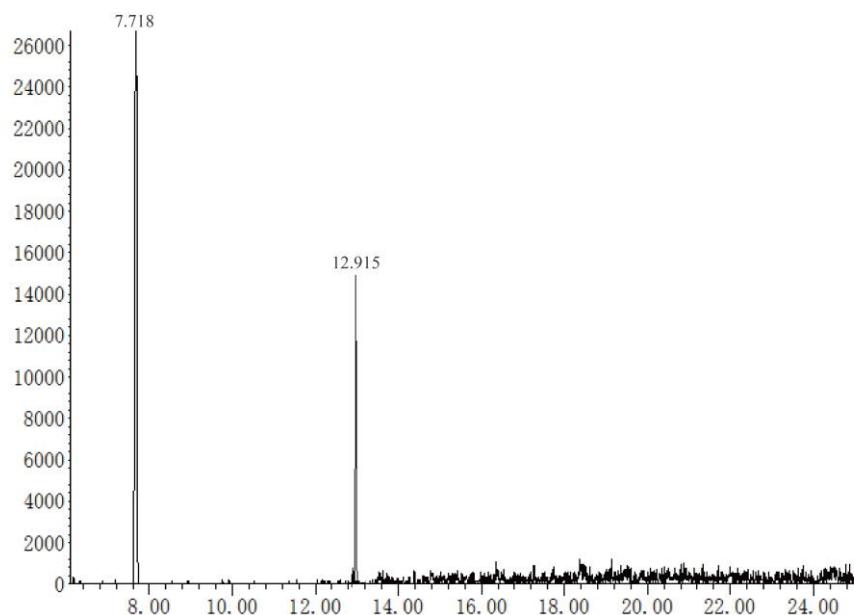


Figure S20. Gas chromatogram of compound 2

Substances (30)

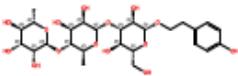
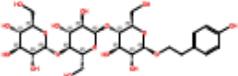
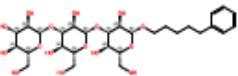
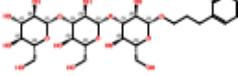
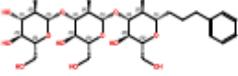
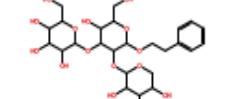
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 1  3  0		 1  5  0		 1  0  0	

Figure S21. Scifinder similarity report for compound 2