Supporting Information

Fluorescent Probe for Biological Signaling Molecule H_2S Based on a Specific H_2S Trap Group

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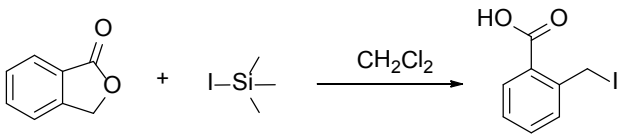
1. General information and methods. All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Deionized water was used throughout all experiments. All reactions were magnetically stirred and monitored by thin layer chromatography (TLC). Column chromatography was conducted over silica gel (mesh 200–300). Fluorescence spectra were taken on Varian Cary Eclipse fluorescence spectrometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer.

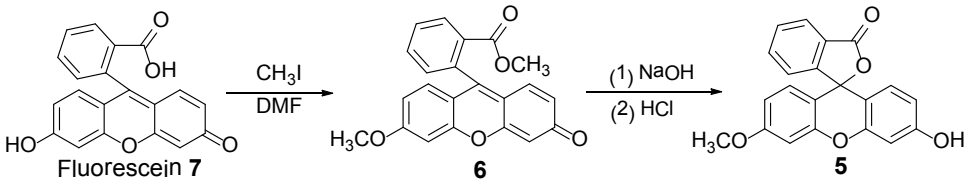
2. Synthesis and Characterization of Compounds

2.1 2-iodomethylbenzoic acid



A solution of phthalide (1.34 g, 10 mmol) and iodotrimethylsilane (3.00 g, 15 mmol) in CH_2Cl_2 (15 mL) was refluxed for 3 h, cooled to room temperature, quenched with water (10 mL) and the precipitate was filtered off, washed with water to give a white solid (2.4 g, 90 %). The crude product was used directly without further purification.

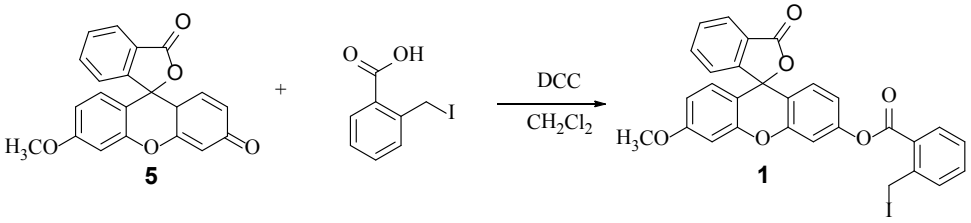
2.2 Synthesis of Compound **2**



CH_3I (2.84 g, 20 mmol) was added to the mixture of fluorescein **7** (3.32 g, 10 mmol) and K_2CO_3 (2.77 g, 20 mmol) in DMF (10 mL) at room temperature. After stirring for 24 hours, the reaction mixture was diluted with H_2O (150 mL), the resulting precipitate was filtered, washed with water, the filtrate was extracted with ethyl acetate, the organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The combined residue was subjected to silica gel chromatography with CH_2Cl_2/CH_3OH (50:1) to give a yellow solid **6** (91 %).

10% aqueous solution of NaOH (10 mL, 25 mmol) was added to the solution of **3** (3.60 g, 10 mmol) in CH_3OH (36 mL) at 30 °C. After stirring for 5 hours, CH_3OH was evaporated and the reaction mixture was diluted with H_2O (100 mL). The solution was acidified to pH 5 with 1 M HCl, the resulting precipitate was filtered, washed with water and dried under vacumm to give a pale yellow solid **5** (69 %) which was used for next synthesis without further purification.

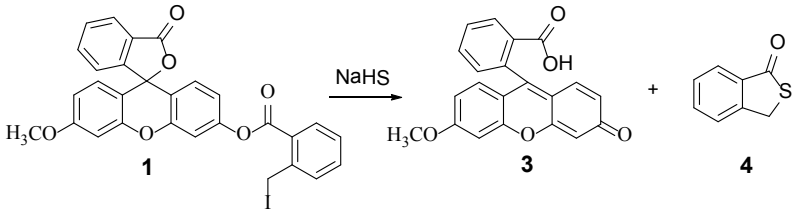
2.3 Synthesis of probe **1**



To a solution of DCC (0.206 g, 1 mmol) in CH_2Cl_2 (10 mL) cooled at 0 °C was added 2-iodomethylbenzoic acid (0.262 g, 1 mmol) followed by DMAP (0.012 g, 0.1 mmol) and **5** (0.346 g, 1 mmol). The mixture was stirred at room temperature for 24 hours and then filtered, washed with CH_2Cl_2. The filtrate was concentrated to afford the crude product, then purified by silica gel column chromatography (PE : CH_2Cl_2 = 1 : 1) to afford a white solid **1** (0.24 g, 40 %). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) : 8.18 (d, J = 7.8 Hz, 1H), 8.04 (d, J = 7.2 Hz, 1H), 7.69 (t, J = 7.2 Hz, 1H), 7.64 (t, J =

7.2 Hz, 1H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.26 (t, *J* = 5.4 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 6.97 (m, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 6.80 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.68 (m, 1H), 4.96 (s, 2H), 3.85 (s, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 172.1, 167.4, 164.4, 156.0, 155.2, 154.9, 154.8, 145.2, 137.9, 136.4, 134.9, 134.4, 132.7, 132.0, 131.8, 131.1, 130.0, 129.4, 128.0, 126.9, 120.5, 120.0, 114.9, 113.9, 113.5, 103.8, 85.2, 58.4, 6.1. Anal. Calcd for $C_{29}H_{19}IO_6$: C, 59.00; H, 3.24. Found C, 59.12; H, 3.33. HRMS: calcd for $[M+H]^+$ 591.0299, found 591.0306.

2.5 Synthesis of compound **4**



To a solution of **1** (0.118 g, 0.2 mmol) in CH_3CN (5 mL) and PBS buffer (5 mL, 20 mM, pH = 7.4) was added NaHS (0.112 g, 2 mmol), the reaction mixture was stirred for 1 h at room temperature. The color of solution turned to yellow. CH_3CN was evaporated under reduced pressure and water (25 mL) was added to the resulting residue, extracted with ethyl acetate, dried over anhydrous Na_2SO_4, evaporated under reduced pressure to give the crude product, which was purified by column chromatography (CH_2Cl_2 : PE = 1 : 3) to afford compound **4** as a white solid (27 mg, 90 %). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.82 (d, *J* = 3.6 Hz, 1H), 7.62 (m, 1 H), 7.54 (d, *J* = 7.4 Hz, 1H), 7.47 (d, *J* = 6.9 Hz, 1H), 4.47 (s, 2H). ^{13}C NMR ($CDCl_3$, 600 MHz) δ (ppm) : 200.5, 149.8, 138.7, 136.2, 130.8, 129.2, 126.7, 37.4. HRMS: calcd for $[M+H]^+$ 151.0212, found 151.0212.

3. Preparation of the test solution

All solutions of the anions were prepared from their sodium salts in deionized water. All solutions of the cations were prepared from their chloride salts in deionized water. NO was generated form SNP (Sodium Nitroferricyanide (III) dihydrate). SNP was added into degassed deionized water under Ar atmosphere then stirred for 30 min at

room temperature. Superoxide solution was prepared by adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min. The stock solution of probe **1** (2 mM) was prepared in CH_3CN, then diluted to 5 μM for testing with the solution of PBS (20 mM, pH = 7.4, containing 20 mM CTAB). The stock solution of NaHS (20 mM) was prepared in deionized water, which was freshly prepared each time before use.

4. Quantum Yields.

Fluorescence quantum yields of **1** and methylfluorescein **3** were determined in PBS buffer (10 mM, pH 7.4, containing 20 mM CTAB) with fluorescein (Φ = 0.95, in 0.1 M NaOH) as a reference. Methylfluorescein **3** was obtained in the experiment by addition of 8 equiv of NaSH to the solution of probe **1**. The quantum yields were calculated using Eq.1:

$$\Phi_u = [(A_s F A_u \eta^2)/ (A_u F A_s \eta_0^2)]\Phi s. \qquad \text{Eq.1}$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η_0 are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

Quantum yield of **1**: Φ = 0.0140

Quantum yield of **3**: Φ = 0.3786

5. Cell culture and fluorescence imaging: The COS-7 cell line was provided by Institute of Biotechnology of Shanxi University. Cells were grown in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO_2. Cells were plated on 6-well plate at 5×106 cells per well and allowed to adhere for 12 hours. Before the experiments, cells were washed with PBS and then incubated with **1** (5 μM) in CMEM medium for 30 min at 37 °C and washed 3 times with PBS. Experiments to assess H_2S uptake were performed in the same media supplemented with 100 μM NaHS for 30 min at 37 °C. Cell imaging was then carried out after washing cells with

PBS for 3 times. Fluorescence imaging was performed with by a DeltaVision Microscope.

6. Supplemental spectra

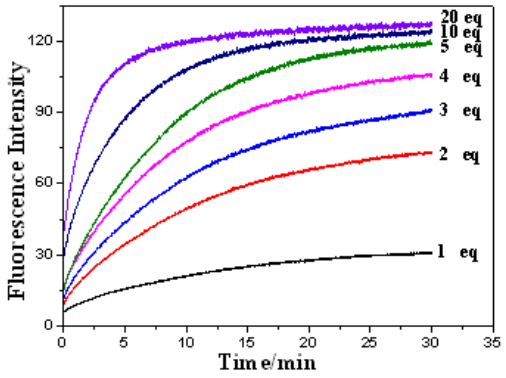


Fig. S1 Time-dependent fluorescence intensity changes of **1** (5 μM) upon addition of varied concentrations of NaHS in PBS buffer (10 mM, pH = 7.4, containing 20 mM CTAB).

7. ^{1}H NMR, ^{13}C NMR and HRMS chart of compounds 1 and 4

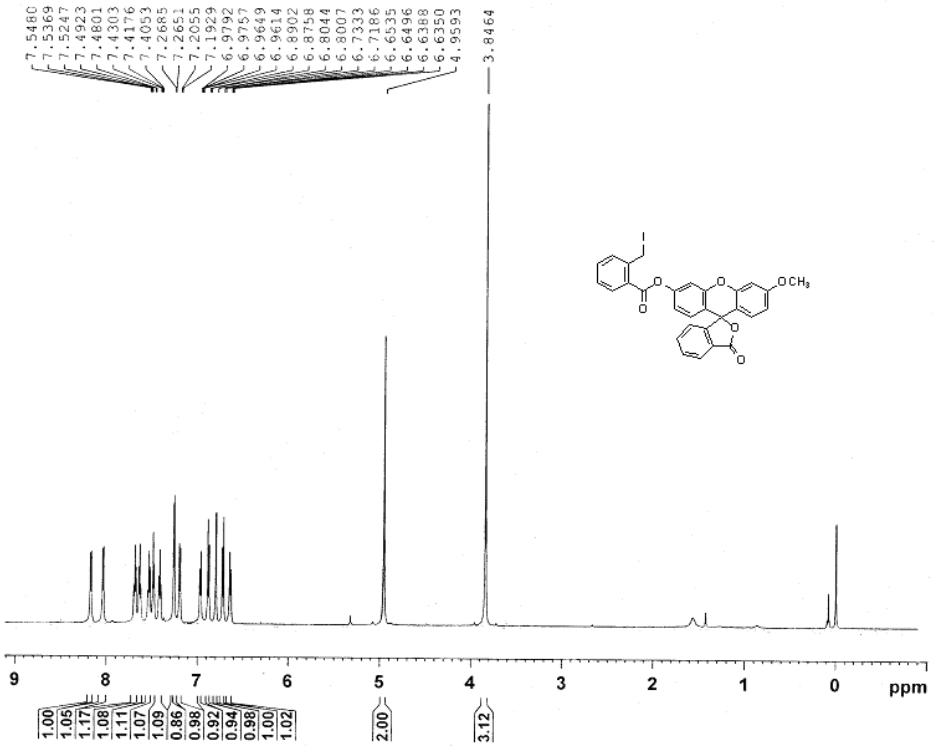


Fig. S2. ^{1}H NMR chart of **1** ($CDCl_3$, 600 MHz).

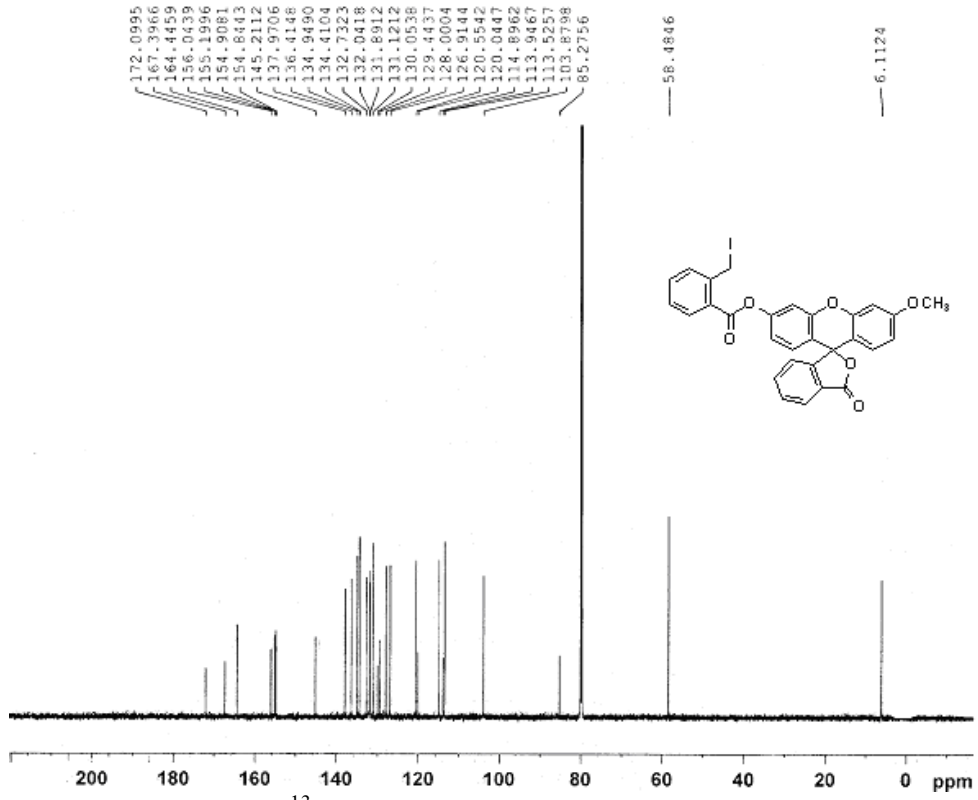


Fig. S3. ^{13}C NMR chart of **1** ($CDCl_3$, 150 MHz).

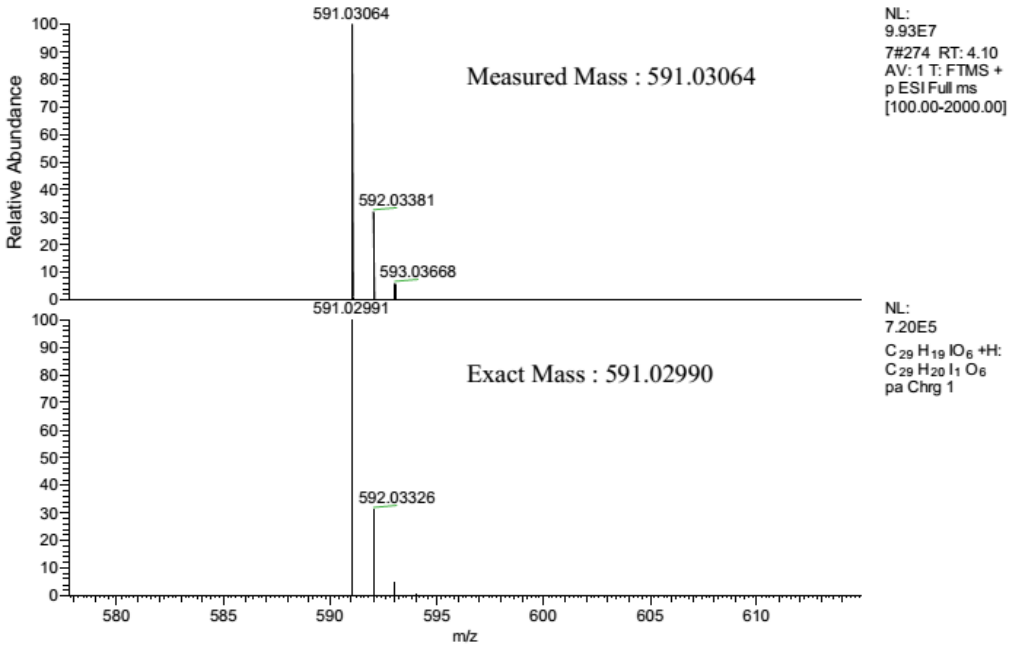


Fig. S4. HRMS chart of **1**.

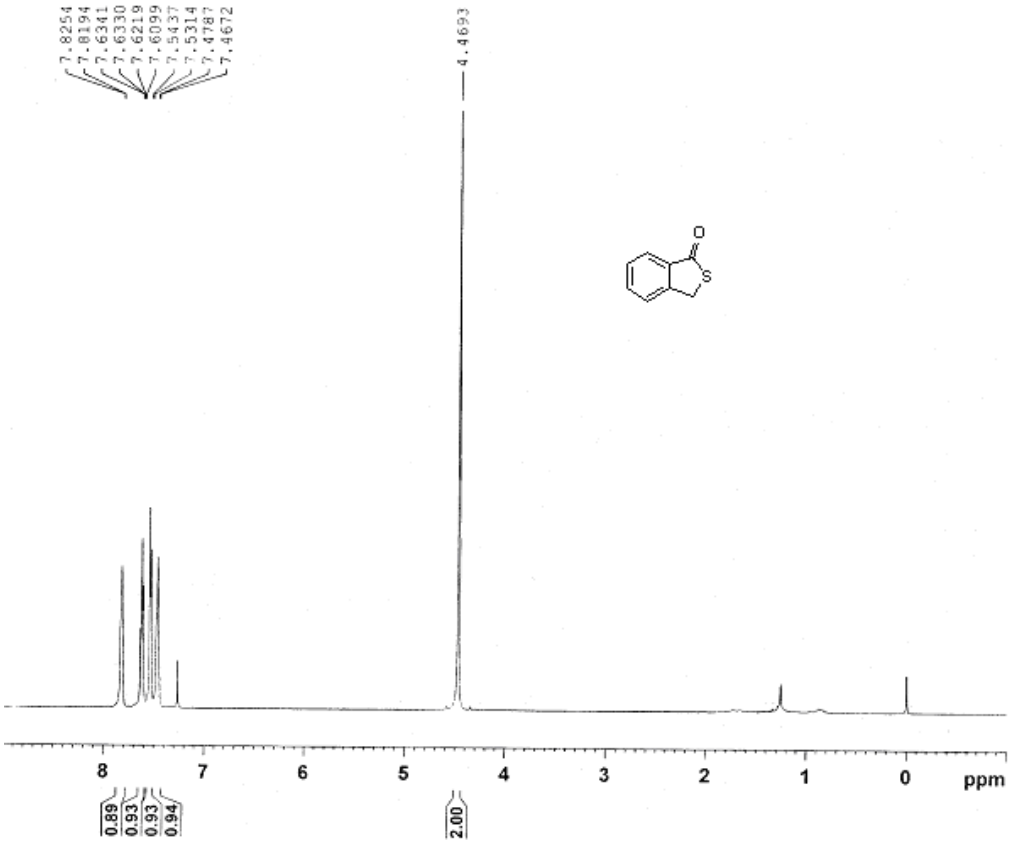


Fig. S5. ^{1}H NMR chart of **4** ($CDCl_3$, 600 MHz).

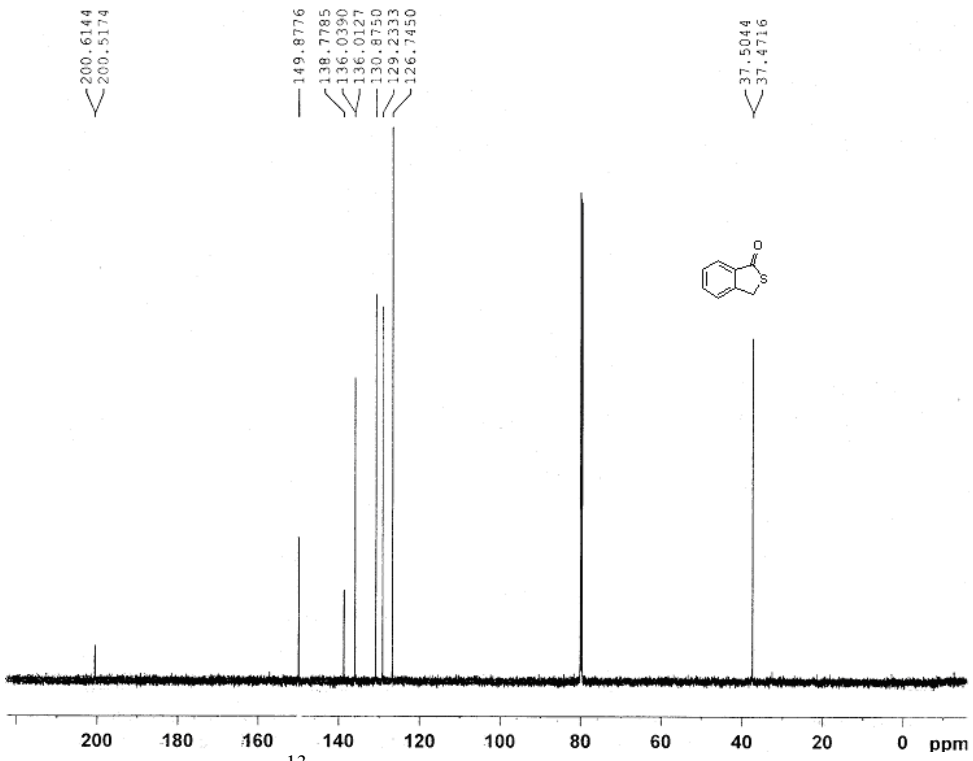


Fig. S6. ^{13}C NMR chart of **4** ($CDCl_3$, 150 MHz).

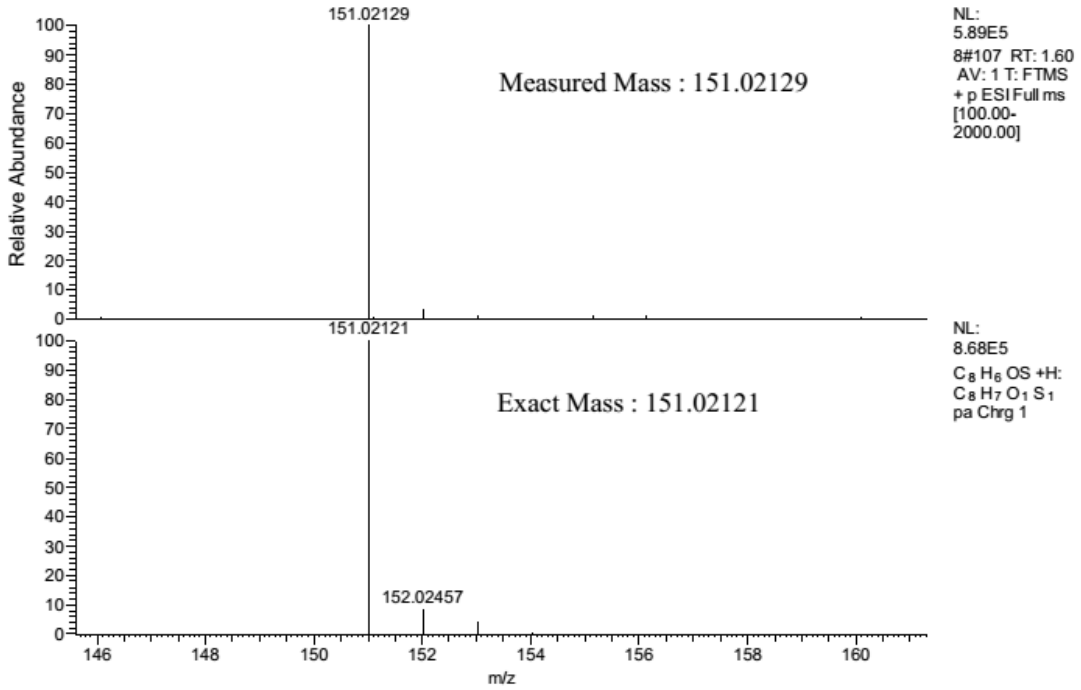


Fig. S7. HRMS chart of **4**.