Electronic Supplementary Information

A Rhodamine-based Fluorescent Sensor for the Highly Selective and Sensitive Detection of Mercury in Aqueous Solution and Living Cells

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1. Materials, Measurement and Methods

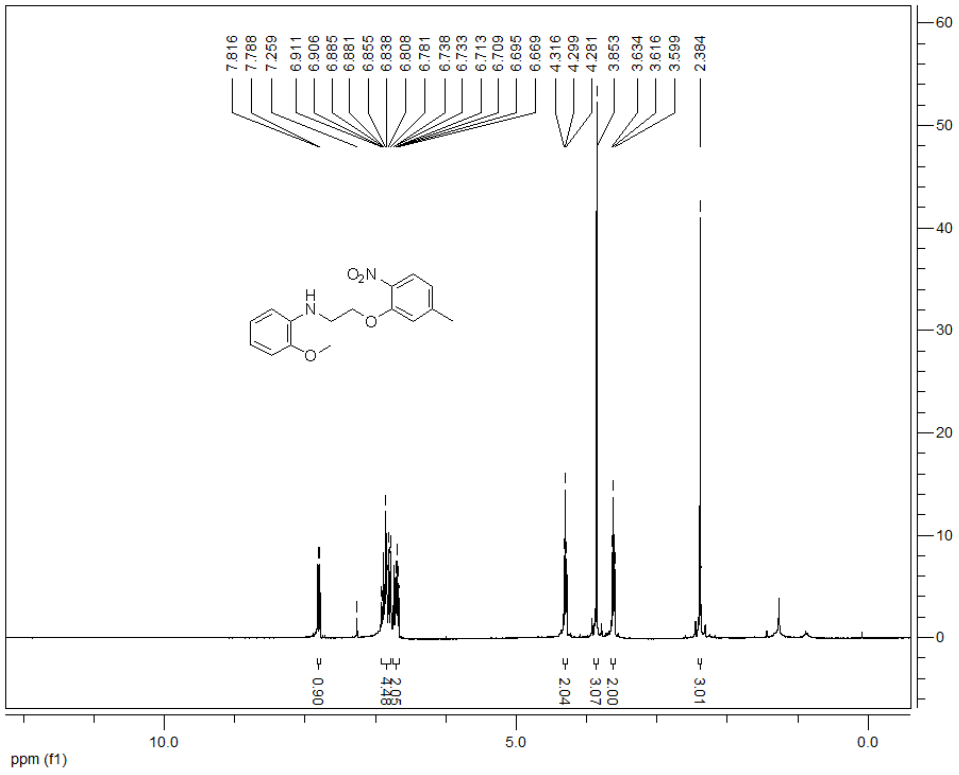
Unless otherwise noted, all materials were obtained from Heowns Biochem Technologies LLC and were used without further purification. Flash chromatography was carried out on silica gel (300-400 mesh). ^{1}H NMR spectra were recorded using Varian 300 MHz. Fluorescence spectra were measured on the F-280 fluorometer. The mineral water was taken from Kangshihfu mineralized water and the tap water was taken from the tap. No other pretreatment was required for these water samples. The fluorescent images of the cells were captured by the Olympus IX81 inverted research microscope.

2. Synthesis

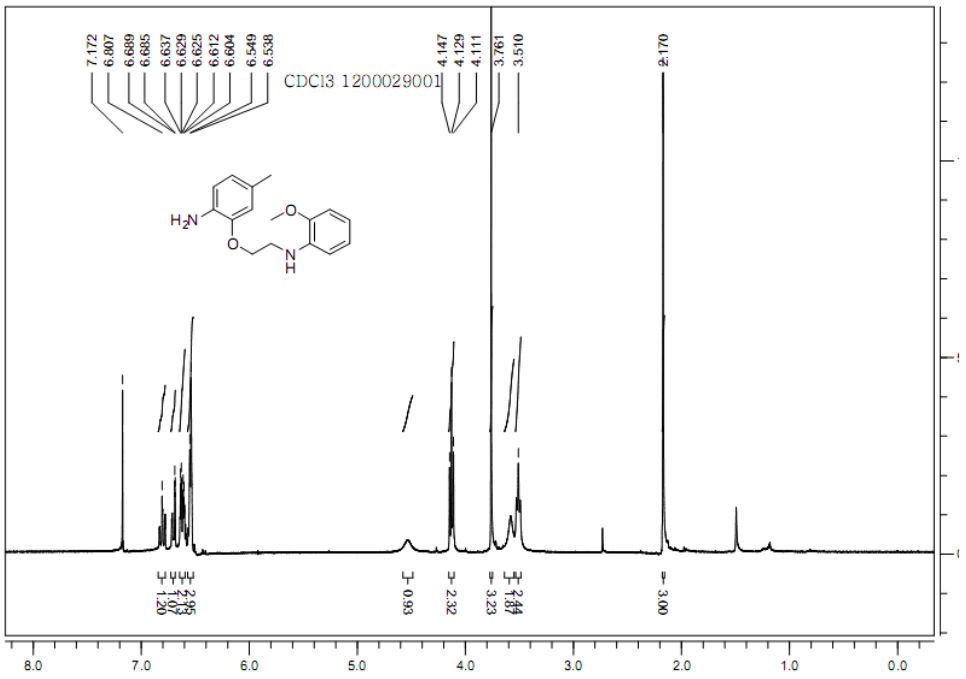
The compounds **1**, **3** and **4** were synthesized according to a previously reported procedure. [1]

Synthesis of 3 A suspension of 80 g (307.59 mmol) compound **1**, 25 g (205.06 mmol) compound **2**, 56 g (410.12 mol) K_2CO_3 and 33.5 g (205.06 mol) KI in 300 ml acetonitrile was heated under reflux for 20 h. The progress was monitored by TLC

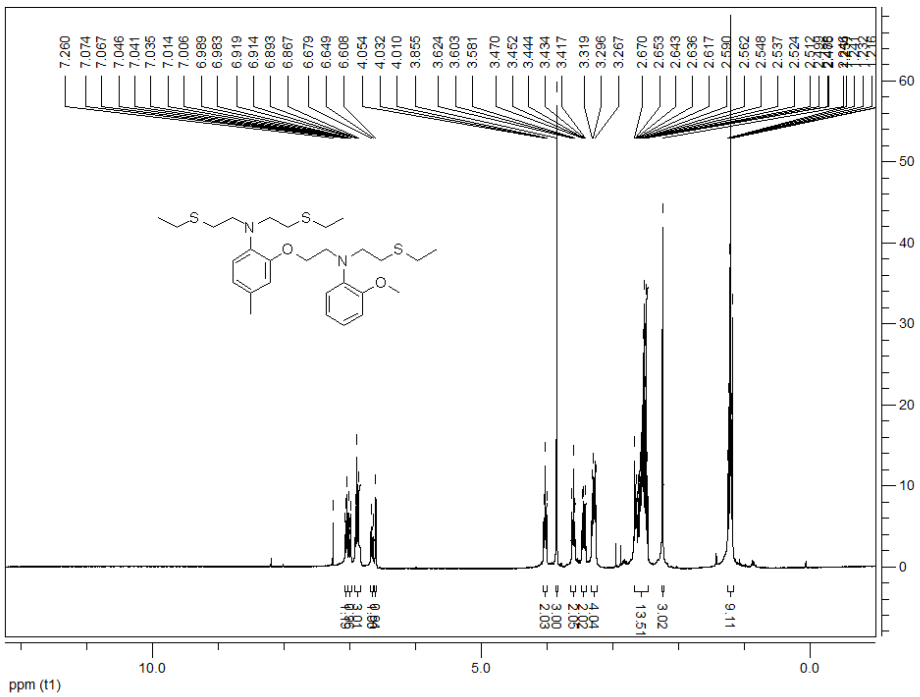
(PE: EA=5:1). After the reaction was completed, the mixture was cooled and solvent was evaporated. The residue was purified by flash column chromatography, to obtain product 44g. ^{1}HNMR ($CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 1H), 6.86 (ddd, *J* = 22.3, 12.4, 4.9 Hz, 4H), 6.75 – 6.66 (m, 2H), 4.30 (t, *J* = 5.3 Hz, 2H), 3.85 (s, 3H), 3.62 (t, *J* = 5.3 Hz. 2H), 2.38 (s, 3H).



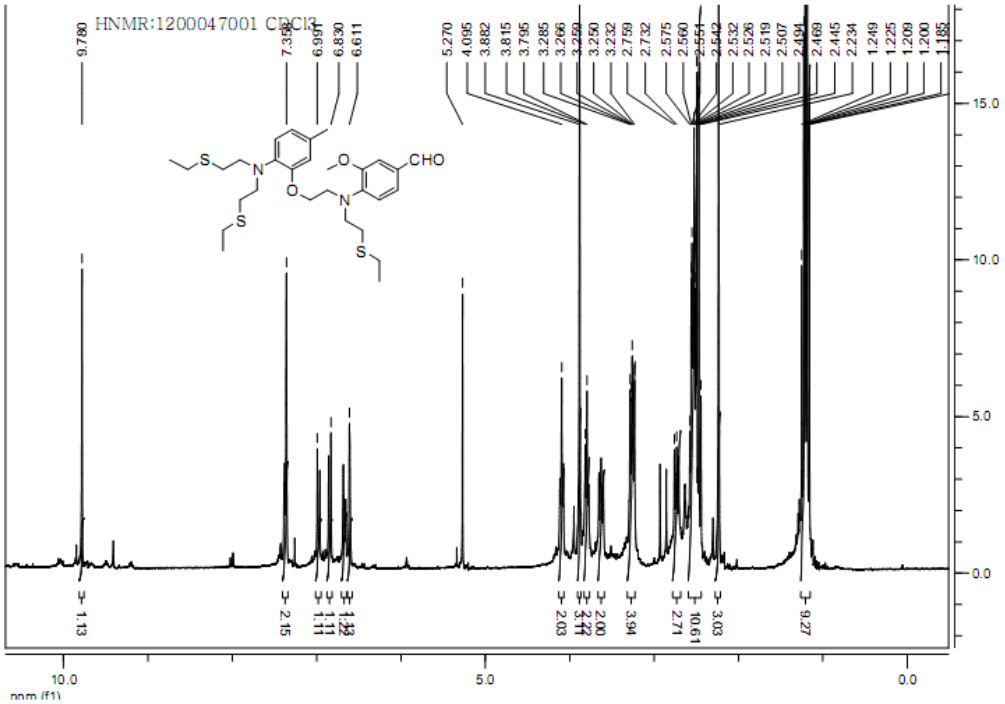
Synthesis of 4： 10.0 g (33.08 mmol) compound **3** was dissolved in 50 ml mixed solvent (DCM: MeOH=1:5), 1 g 10% palladium on activated carbon was added. This suspension was hydrogenated at 2.2 atm. for 18 h, till no more hydrogen uptake was observed. The progress was monitored by TLC (PE: EA=4:1).The catalyst was filtered off and the solvent was evaporated, to obtain product 7.7 g. ^{1}HNMR ($CDCl_3$) δ 2.17(s, 3H), 3.51(m, 2H), 3.54(s, 2H), 3.76(s, 3H), 4.12(t, 2H), 4.24(s, 1H), 6.5-7.17(m, 7H).



Synthesis of 5: A suspension of 0.5 g (1.84 mmol) of compound **4**, 3.2 mL (27.54 mmol) of 2-chloroethyl ethyl sulfide, 9.1 mL (55.08 mmol) N, N-diisopropylethyl - amine and 4.57 g (27.54 mmol) of KI in DMF (10 mL) was heated at 110°C for 20h under nitrogen atmosphere. The progress was monitored by TLC (PE: EA=4:1). After the reaction was complete, the mixture was cooled and poured into water. The resultant precipitate was filtered, dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4, filtered and evaporated to get 0.75 g crude product, which was purified by flash column chromatography, to afford product 295 mg. ^{1}HNMR ($CDCl_3$) δ 7.06 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.00 (dd, *J* = 7.3, 2.1 Hz, 1H), 6.90 (dd, *J* = 10.7, 4.6 Hz, 3H), 6.66 (d, *J* = 8.9 Hz, 1H), 6.61 (s, 1H), 4.03 (t, *J* = 6.5 Hz, 2H), 3.86 (s, 3H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.48 – 3.41 (m, 2H), 3.34 – 3.25 (m, 4H), 2.68 – 2.47 (m, 12H), 2.25 (s, 3H), 1.22 (ddd, *J* = 10.0, 7.0, 3.3 Hz, 9H).

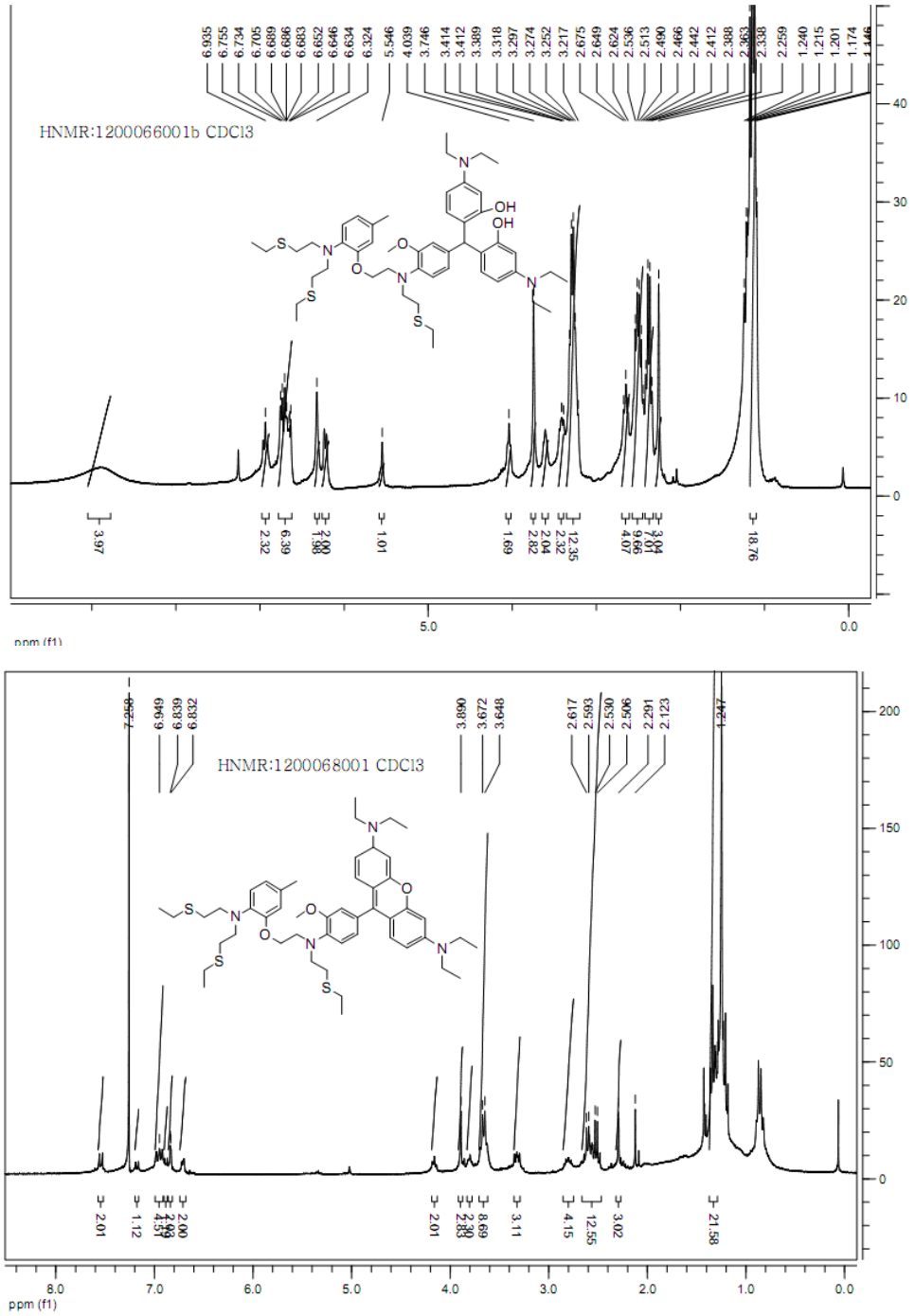


Synthesis of 6: The Vilsmeier reagent was prepared by adding $POCl_3$ (1 mL) dropwise to ice-cold dry DMF (2 mL) under stirring. After 30 min, to the above Vilsmeier reagent was added compound **5** (250 mg, 0.465 mmol) as a solution in DMF (1.0 mL). Then the mixture was further heated at 65°C. The progress was monitored by TLC (PE: EA=4:1). After the reaction was complete, the mixture was cooled to room temperature, then poured into ice-water and the pH of the aqueous mixture was adjusted to 8~9 with Na_2CO_3 saturated solution. The product was extracted into twice. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered. The organic layer was removed under reduced pressure, and then the residue was purified by flash column chromatography, to obtain product 200 mg. ^{1}HNMR ($CDCl_3$) δ 9.780 (s, 1H), 7.06 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.00 (dd, *J* = 7.3, 2.1 Hz, 1H), 6.90 (dd, *J* = 10.7, 4.6 Hz, 3H), 6.66 (d, *J* = 8.9 Hz, 1H), 6.61 (s, 1H), 4.03 (t, *J* = 6.5 Hz, 2H), 3.86 (s, 3H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.48 – 3.41 (m, 2H), 3.34 – 3.25 (m, 4H), 2.68 – 2.47 (m, 12H), 2.25 (s, 3H), 1.22 (ddd, *J* = 10.0, 7.0, 3.3 Hz, 9H).



Synthesis of Sensor Hg 8: A solution of compound **6** (90 mg, 0.159 mmol), 3-dimethylaminophenol (66 mg, 0.398 mmol), and p-TsOH•H_2O (20 mg, 0.1 mmol) in propionic acid (5 mL) was stirred at 65°C overnight. After cooling to room temperature, the mixture was poured into 3 M NaOAc (70 mL). The resulting suspension was extracted with CH_2Cl_2 (20 mL×3). The combined organic extracts were dried over $MgSO_4$ and evaporated, and purification by preparative thin-layer chromatography gave pure compound **7** as a white solid. A solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 159 mg, 0.70 mmol) in 1:1 AcOH/benzene (10 mL) was added dropwise into a solution of compound **7** (219 mg, 0.35 mmol) in 10 mL of 1:1 AcOH/benzene at room temperature. The resulting purple-colored mixture was stirred 2h at room temperature. The progress was monitored by TLC. After the reaction was complete, the reaction was concentrated in vacuo, and purification by preparative thin-layer chromatography gave pure **8** as a dark purple solid. ^{1}HNMR

($CDCl_3$) δ 1.21(m，9H), 2.25(s, 3H), 2.55(m, 12H), 3.27(m, 4H), 3.53(t, 2H), 3.78(t, 2H), 3.84(s, 3H), 4.18(t, 2H), 6.68-7.43(m, 12H)



Reference:

(1) Huarui He, Mark A. Mortellaro, Marc J. P. Leiner, Robert J. Fraatz, James K. Tusa *J. AM. CHEM. SOC.* 2003, **125**, 1468-1469

3. Procedures for Hg^{2+} detection in real samples

First, 25 μM Sensor-Hg solution is made by mixed uniformly 350 μL of water sample (mineral and tap waters), 150 μL of 500 μM Sensor-Hg, and 2500 μL of 20 mM HEPES buffer. The fluorescence emission intensities of Sensor-Hg were monitored. Then, different concentrations of Hg^{2+} were added to the mixture solution. Finally, the fluorescence emission intensities were monitored. For the sensitivity experiment, the concentrations of Hg^{2+} were of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0 μM, respectively. For the analysis of real samples experiment, the spiked concentrations of Hg^{2+} were of 100, 150, 200 $ng \cdot mL^{-1}$, respectively.

4. Cell imaging experiment

The cell imaging experiment was carried out as follows:

(a): The dye Sensor Hg was dissolved in DMSO.

(b): HeLa cells incubated with 10 μM Sensor Hg for 24h, then washed three times by PBS buffer solution.

(c): Then further incubated with 10 μM Hg^{2+} for 4h, then washed three times by PBS buffer solution.

(d): The fluorescent images of the cells were captured by the Olympus IX81 inverted research microscope.