Electronic Supplementary Information

Fluorescent Detection of Methylmercury by Desulfurization Reaction of Rhodamine Hydrazide Derivatives

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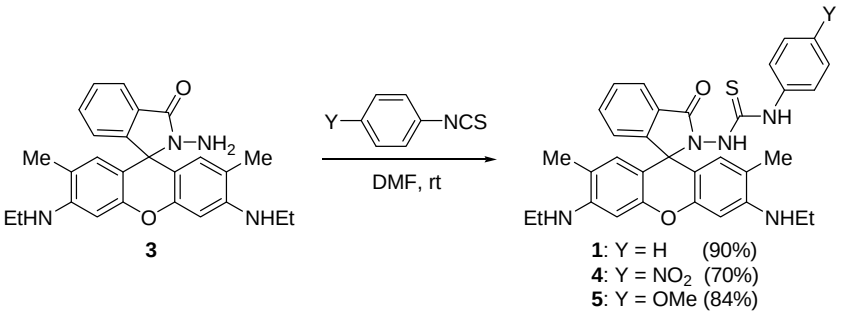
General synthetic materials and spectroscopic methods.

Silica gel 60 (230–400 mesh, Merck) was used for column chromatography. Analytical thin layer chromatography was performed using Merck 60 F_{254} silica gel (precoated sheets, 0.25 mm thick). All reagents and solvents for reactions were used as received with the following exceptions. All other chemicals used were purchased from Sigma-Aldrich and were used as received. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ unless otherwise stated, with tetramethylsilane (TMS) as internal reference at ambient temperature, mainly on a Bruker Avance II-400 Fourier Transform Spectrometer operating at 400 MHz for ^1H and at 100.6 MHz for ^{13}C. Mass spectra were recorded on a ZQ-4000 LC-MS and QUATTRO LC Triple Quadrupole Tandem mass spectrometer for both low resolution and high resolution mass spectra. The pH was recorded by HI-8014 instrument (HANNA). Melting points were measured on a Z289078 (Sigma-Aldrich) microscope and were uncorrected. Infrared absorption spectra were recorded as a solution in CH_2Cl_2 on an Avatar 360 FT-IR spectrophotometer. Fluorescence emission spectra were obtained using a Hitachi F-4500 spectrofluorimeter linked to a Pentium PC running SpectraCalc software package. The slit width was 2.5 nm for both excitation and emission. The photon multiplier voltage was 400 V. A circulating PBS buffer/DMSO bath was used during all experiments to regulate the temperature at 25.0 ± 0.1 ^oC. Samples were contained in 10.0 nm path length quartz cuvettes (3.5 mL volume). Upon excitation at 500 nm, the emission spectra were integrated over the range 510–650 nm. All measurements were conducted at least in triplicate. We used a TE2000 fluorescence microscope for the fluorescence imaging experiments and a dissecting microscope (Stemi 2000-C, ZAISS) for the microscopic imaging experiments.

Synthesis of rhodamine thiosemicarbazides.



The rhodamine 6G hydrazide **3** (200 mg, 0.47 mmol) in DMF (1.5 mL) was added to a solution of phenyl isothiocyanate (0.1 mL, 0.65 mmol) in DMF (1.5 mL). The reaction mixture was stirred for 6 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was column chromatographed on silica-gel (elution with hexanes/EtOAc/CH_2Cl_2 = 4:1:1) to give the 236 mg (90%) of **1**: R_f = 0.5 (silica gel, hexanes/EtOAc = 1:1); mp 150-152 ^oC; ^1H NMR (250 MHz, $CDCl_3$) δ = 8.06-8.03 (m, 1H), 7.66-7.57 (m, 3H), 7.26-7.22 (m, 3H), 3.26-3.17 (m, 4H), 1.84 (s, 6H), 1.32 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 182.2, 166.9, 152.5, 150.8, 148.4, 138.1, 134.1, 129.5, 129.0, 128.2, 128.1, 125.8, 125.2, 124.4, 123.2, 118.9, 104.4, 95.9, 67.0, 37.9, 16.1, 13.7; IR (film, cm^{-1}) 3326, 2964, 2960, 2950, 2356, 2351, 1713, 1620, 1517, 1430, 1424, 1347, 1274, 1212, 1089, 1016; HRMS (FAB) *m/z* calcd for $C_{33}H_{33}N_5O_2S$ $[(M+H)^+]$ 564.2423; found 564.2433.

Compound 4: R_f = 0.7 (silica gel, hexanes/EtOAc = 1:1); mp 222–224 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 8.02–7.98 (m, 4H), 7.69 (t, *J* = 7.2 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 5.2 Hz, 2H), 7.25 (s, 1H), 6.37 (s, 2H), 6.30 (s, 2H), 3.59 (bs, 2H), 3.18–3.16 (m, 4H), 1.87 (s, 6H), 1.29 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 181.9, 167.8, 152.9, 150.3, 148.3, 144.1, 144.0, 134.8, 129.3, 128.9, 127.4, 125.0, 124.2, 124.0, 122.8, 118.7, 104.3, 97.2, 68.0, 38.3, 16.9, 14.8; IR (film, cm^{-1}) 3421, 3317, 2967, 2924, 2863, 1714, 1619, 1593, 1515, 1467, 1420, 1329, 1269, 1212, 1165, 1113, 1083, 1014; ESI-MS *m/z* calcd for $C_{33}H_{32}N_6O_4S$ $[(M + Na)^+]$ 631; found 631.

Compound 5: R_f = 0.5 (silica gel, hexanes/EtOAc = 1:1); mp 210–212 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 8.04 (d, *J* = 8.4 Hz, 1H), 7.67–7.60 (m, 2H), 7.42 (s, 1H), 7.27–7.23 (m, 1H), 6.89 (s, 1H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.71 (d, *J* = 8.8 Hz, 2H), 6.41 (s, 2H), 6.25 (s, 2H), 3.75 (s, 3H), 3.58 (bs, 2H), 1.79 (s, 6H), 1.34 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 183.0, 167.3, 157.8, 152.8, 150.6, 148.2, 134.5, 130.6, 129.2, 129.1, 126.8, 124.9, 123.9, 118.7, 113.7, 104.5, 97.3,

67.5, 55.5, 38.4, 16.9, 14.8; IR (film, cm^{-1}) 3326, 2958, 2924, 2863, 1722, 1679, 1614, 1519, 1467, 1420, 1355, 1273, 1243, 1217, 1161, 1178, 1087; ESI-MS *m/z* calcd for $C_{34}H_{35}N_5O_3S$ $[(M + Na)^+]$ 616; found 616.

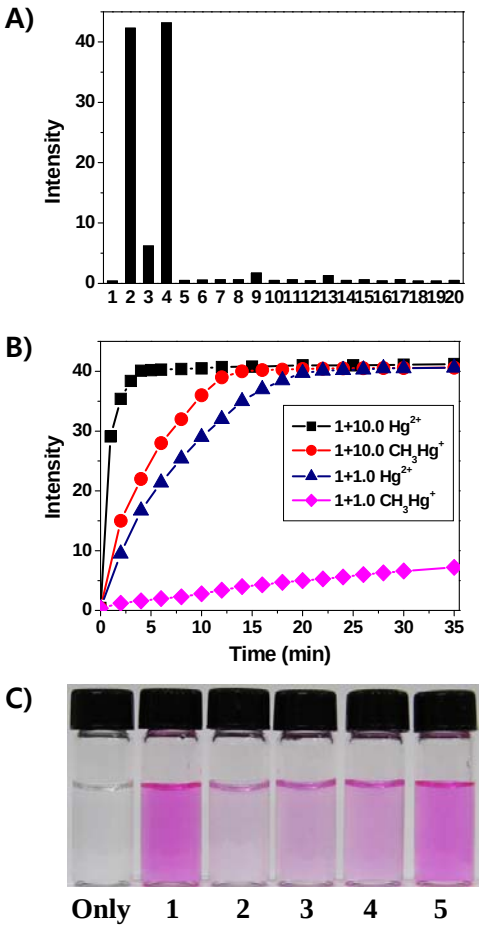
Imaging of HeLa cells incubated with methylmercury and 1: HeLa cells were seeded in a 6-well plate at a density of 1×10^5 cells per well in culture media. After 24 h, the cells were incubated with **1** (20 μM) in culture media for 30 min at 37 ^oC. After washing with PBS to remove the remaining probe, the cells were further treated with CH_3Hg^+ (5–20 μM) in culture media for 10 min. The cells incubated under these conditions were imaged by fluorescence microscope (TE2000, Nikon).

Imaging of zebrafish incubated with methylmercury and 1: Zebrafish was kept at 28 ^oC and maintained at optimal breeding conditions. For mating, male and female zebrafish was maintained in one tank at 28 ^oC on a 12 h light/12 h dark cycle and then the spawning of eggs were triggered by giving light stimulation in the morning. Almost all the eggs were fertilized immediately. The 4-day old zebrafish was maintained in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10–5% methylene blue; pH 7.5).[19] The 4-day old zebrafish was incubated with chemosensor **1** (20 μM) in E3 embryo media for 30 min at 28 ^oC. After washing with PBS to remove the remaining chemosensors, the zebrafish was further treated with CH_3Hg^+ (20 μM) for 10 min at 28 ^oC. The zebrafish was imaged by a fluorescence microscope (TE2000, Nikon).

Real-time monitoring of methylmercury uptake in HeLa cells and A549 cells: HeLa cells and A549 cells were seeded in a 96-well plate at a density of 10^4 cells per well in culture media. After 24 h, the culture media were replaced with fresh media and the cells were incubated with **1** (20 μM) in culture media for 30 min. After washing with PBS to remove the remaining sensors, various concentrations of CH_3Hg^+ (0–200 μM) were added to the cells in culture media, and fluorescence intensity was determined using a fluorescent microplate reader (SpectraMax GeminiEM, Molecular Devices).

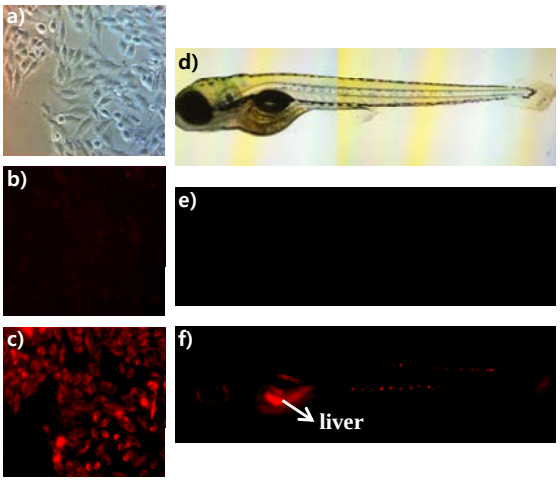
Fluorescence intensity and color changes after additions of Hg^{2+} and CH_3Hg^+ .

A solution of **1** (10 μM) was prepared in spectroscopic PBS-buffer (DMSO 1%) at pH 7.4. A solution of **1** (2.0 mL) was placed in a quartz cell (10.0 mm width) and the fluorescence spectrum was recorded (excitation at 500 nm, emission at 560 nm, 25 ^oC).



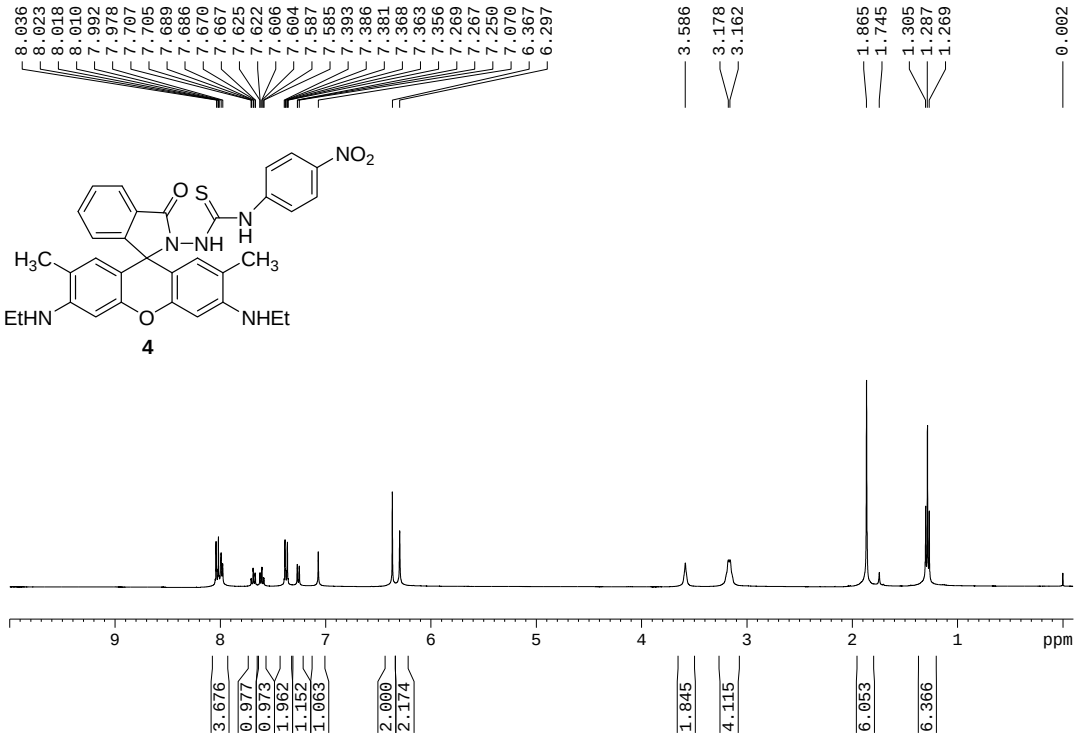
A) Fluorescence spectra of **1** (10 μM) upon addition of CH_3Hg^+ and Hg^{2+} in PBS-buffer (DMSO 1%) at pH 7.4 (excitation at 500 nm, emission at 560 nm, at 25 ^oC): in the presence of the following metal ions: 1, only; 2, Hg^{2+} 1.0 equiv; 3, CH_3Hg^+ 1.0 equiv; 4, CH_3Hg^+ 10.0 equiv; 5, Fe^{2+}; 6, Pb^{2+}; 7, Cu^{2+}; 8, Mg^{2+}; 9, Ag^+; 10, Ca^{2+}; 11, Ni^{2+}; 12, Co^{2+}; 13, Zn^{2+}; 14, Cd^{2+}; 15, Mn^{2+}; 16, Cr^{2+}; 17, Ba^{2+}; 18, Na^+; 19, K^+; 20, Li^+ (addition of 20.0 equiv, respectively). B) Time dependent fluorescence intensity changes of **1** (10 μM) with CH_3Hg^+ and Hg^{2+} in PBS-buffer (DMSO 1%) at pH 7.4 (excitation at 500 nm, emission at 560 nm). C) Color changes of **1** (10 μM) upon additions of 1-Hg^{2+} (1.0 equiv), 2-CH_3Hg^+ (1.0 equiv), 3-CH_3Hg^+ (2.0 equiv), 4-CH_3Hg^+ (3.0 equiv), and 5-CH_3Hg^+ (10.0 equiv) in water (DMSO 1%) at 25 ^oC (from left to right, respectively).

Nanomolar detection of methylmercury in HeLa cells and zebrafish.

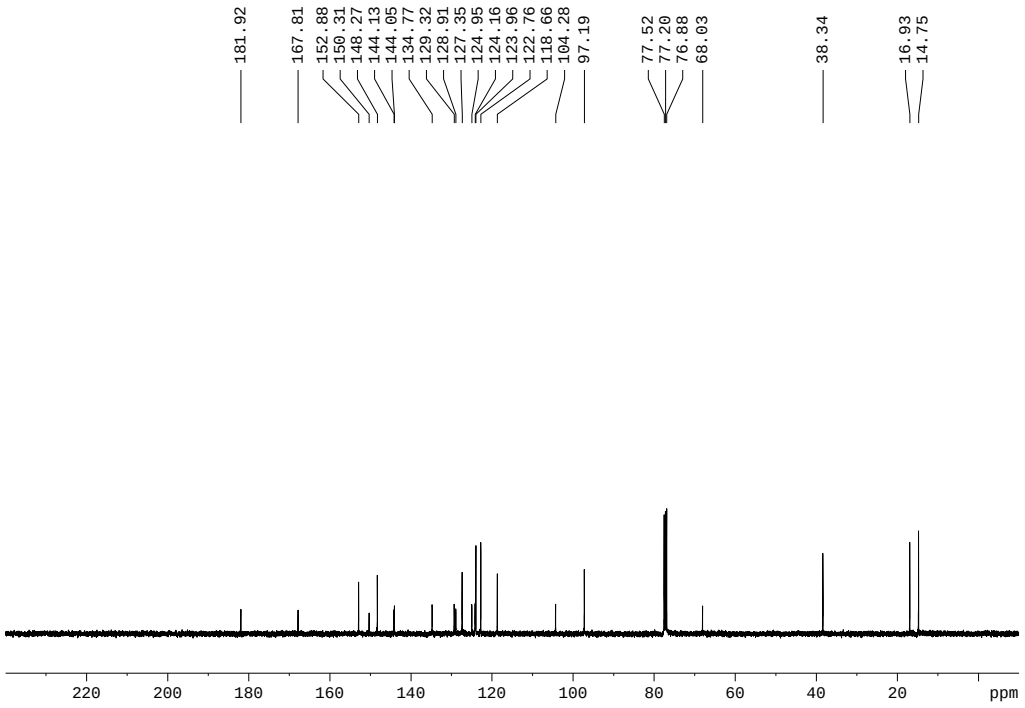


a), d) Microscopic images of HeLa cells and zebrafish treated with **1** (20 μM) in the absence of CH_3Hg^+ (control). b), e) Fluorescence images of HeLa cells and zebrafish treated with **1** (20 μM) in the absence of CH_3Hg^+ (control). c), f) Fluorescence images of HeLa cells and zebrafish treated with both CH_3Hg^+ (300 nM, 100 nM) and **1** (20 μM).

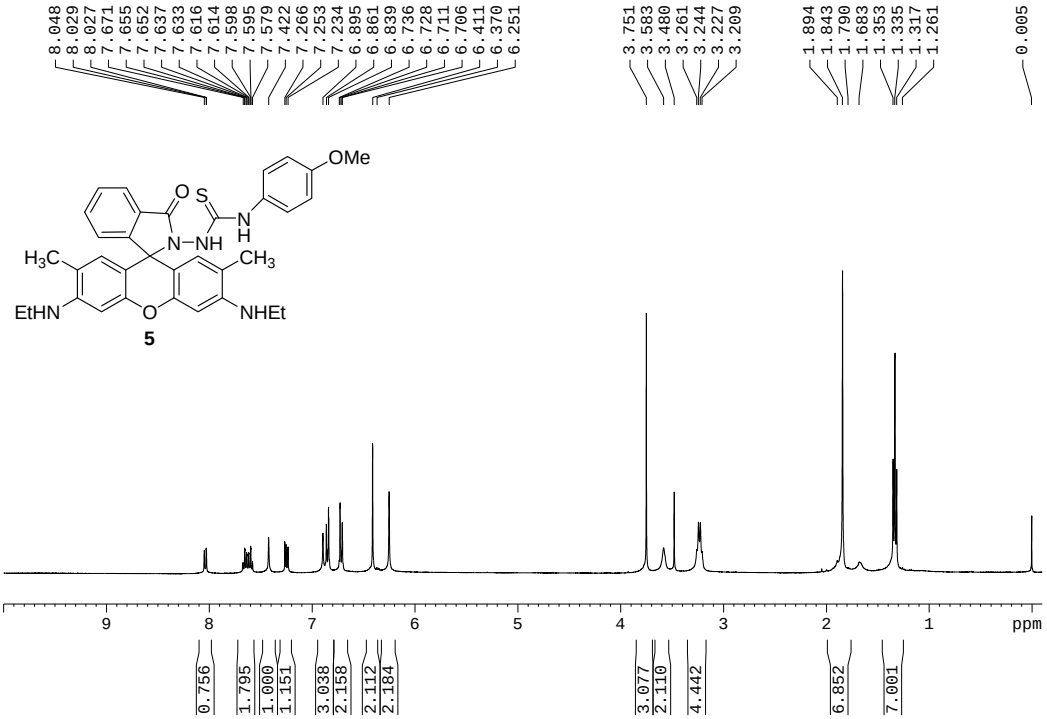
Compound 4 ^{1}H-NMR data (in $CDCl_3$).



Compound 4 ^{13}C-NMR data (in $CDCl_3$).



Compound 5 ^{1}H-NMR data (in $CDCl_3$).



Compound 5 ^{13}C-NMR data (in $CDCl_3$).