Supporting Information

A Highly Sensitive Hemicyanine-Based Fluorescent Chemodosimeter for Mercury Ions in Aqueous Solution and Living Cells

Yu Chen, Chengyu Yang, Zhenni Yu, Bo Chen, Yifeng Han*

Department of Chemistry, The Key Laboratory of Advanced Textile Materials and Manufacturing Technology, Zhejiang Sci-Tech University, Hangzhou, 310018, China.

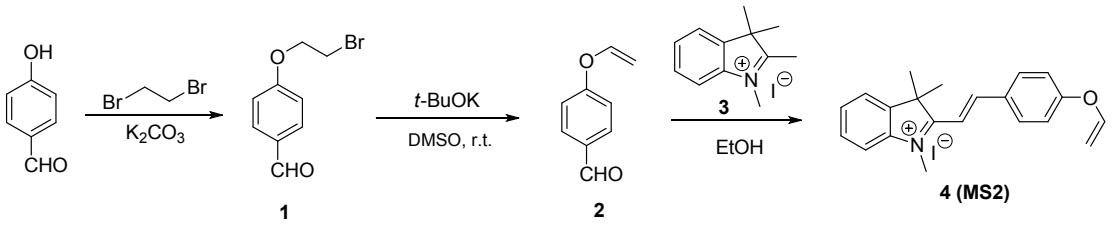
zstuchem@gmail.com

Contents

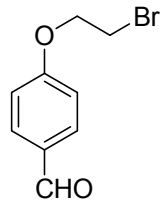
Materials and methods

All the solvents were of analytic grade. NMR experiments were carried out on a Bruker AV-400 NMR spectrometer with chemical shifts reported in ppm (in $CDCl_3$, D_2O, CD_3OD, or TMS as an internal standard). Mass spectra were measured on an Agilent 1290 LC-MS spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-10. Fluorescence spectra were determined on a PerkinElmer LS55 Fluorescence spectrophotometer. Absorption spectra were collected on a Shimadzu UV 2501(PC)S UV-Visible spectrophotometer. The excitation and emission widths for measurements were all 5 nm. All the cation solutions were prepared from $AgNO_3$, $CaCl_2$, $CdCl_2$, $CoCl_2$, $CrCl_3$, $CuCl_2$, $FeCl_2$, $FeCl_3$, KCl, LiCl, $MgCl_2$, $MnCl_2$, NaCl, $NiCl_2$, $Pb(OAc)_2$, $ZnCl_2$, and $HgCl_2$ in distilled water, with a concentration of 1 mM, respectively. The excitation and emission widths for **MS2** were all 10/5.

Synthesis and characterization



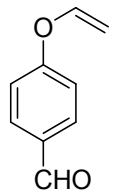
Scheme S1 Synthesis of **MS2**.



1

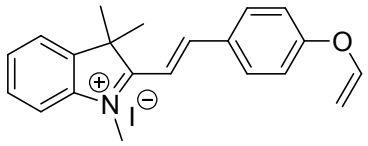
4-(2-bromoethoxy)benzaldehyde (1): 4-hydroxybenzaldehyde (10 g, 0.082 mol) and potassium carbonate (19 g, 0.138 mol) were dissolved in acetone (120 mL) under nitrogen atmosphere, then 1, 2-dibromoethane (41 mL, 0.475 mol) was added and the solution was refluxed for 10 h until all starting material got consumed which was monitored by TLC analysis. The reaction mixture was

washed with water (500 mL), extracted with ether acetate (3 × 150 mL). The extract was dried over sodium sulfate and then concentrated under vacuum. The product was purified by flash chromatography using petroleum ether/ethyl acetate (8:1, v/v) as eluant to give **1** (11.6 g, 62%) as a pale yellow gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.78 (d, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.2 Hz, 2H), 4.30 (t, *J* = 5.9 Hz, 2H), 3.61 (t, *J* = 5.8 Hz, 2H).



2

4-(vinyloxy)benzaldehyde (2): Potassium *tert*-butoxide (6.5 g, 57.8 mmol) was dissolved in anhydrous DMSO (75 mL) and then added dropwise into a 200 mL bottom flask containing a DMSO solution (50 mL) of **2** (11.0 g, 48.2 mmol) under nitrogen atmosphere and the mixture was stirred overnight at room temperature. Water (500 mL) was then added to the reaction solution. The mixture was extracted with ether acetate (3 × 150 mL), dried over sodium sulfate and then concentrated under vacuum. The product was purified by flash chromatography using petroleum ether/ethyl acetate (10:1, v/v) as eluant to give **2** (2.6 g, 37%) as a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.63 (dd, *J* = 13.6, 5.6 Hz, 1H), 4.89 (d, *J* = 13.6 Hz, 1H), 4.57 (d, *J* = 5.6 Hz, 1H).



4 (MS2)

MS2 (4): The mixture of **2** (1.0 g, 6.8 mmol) and 1,2,3,3-tetramethyl-3H-indol-1-ium iodide **3**[1] (1.8 g, 6.1 mmol) of dry ethanol (100 mL) solution was refluxed under nitrogen atmosphere for 12 h. The solvent was then evaporated in vacuo. The crude product was purified by flash chromatography using dichloromethane/methanol (20:1, v/v) as eluant to give **4** (2.3 g, 87%) as a

white solid;

R_f = 0.48 (DCM/MeOH 10:1);

M.p. = 70-72 ^{o}C. (Decomposed);

^{1}H NMR (400 MHz, MeOD) δ 8.52 (m, 1H), 8.30 (d, *J* = 8.6 Hz, 2H), 7.92 (m, 2H), 7.75 (m, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.05 (dd, *J* = 13.6, 6.0 Hz, 1H), 5.05 (d, *J* = 13.6 Hz, 1H), 4.77 (d, *J* = 6.0 Hz, 1H), 4.34 (s, 3H), 2.00 (s, 6H);

^{13}C NMR (100 MHz, MeOD) δ 184.0, 162.7, 154.9, 147.9, 144.9, 143.4, 134.3, 131.0, 130.7, 124.1, 118.3, 116.2, 112.5, 98.5, 54.0, 26.8;

HR-MS (TOF-ESI): *Calcd*. for $([M-I])^{+}$, 304.1696; Found, 304.1744.

Photophysical properties of MS2

Table S1 Photophysical properties of the probe.

entry	λ_{ab} (nm)	λ_{em} (nm)	Φ^a	ε / M^{-1} cm^{-1}
MS2	400	551	0.004	31007
MS2+Hg^{2+}	520	551	0.018[b]	42067

(a) The quantum yield (Φ) of **MS2** and **MS2**-Hg^{2+} system were determined according to the literature.[2] (b) Φ was determined in the present of 3.0 equiv. of Hg^{2+}.

$$\Phi_{Sample} = \frac{\Phi_{QS} \cdot A_{QS} \cdot F_{Sample} \cdot \lambda_{exQS} \cdot \eta^2_{Sample}}{A_{Sample} \cdot F_{QS} \cdot \lambda_{exSample} \cdot \eta^2_{QS}}$$

Where Φ is quantum yield; A is absorbance at the excitation wavelength; F is integrated area under the corrected emission spectra; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the Sample and QS refer to the sample and the standard, respectively. We chose Rhodamine 6G in EtOH as standard, which has the quantum yield of 0.95.[3]

Additional spectroscopic data

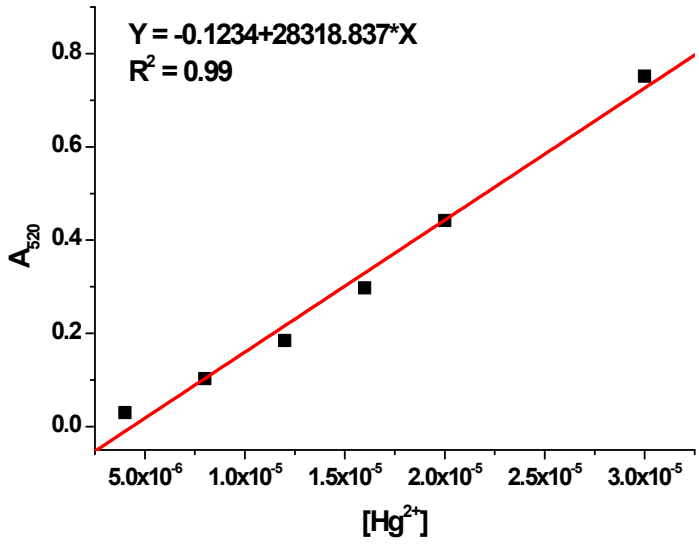


Fig. S1 UV-vis absorption of **MS2** (20.0 μM) at 520 nm as a function of Hg^{2+} concentration (0-30.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% CH_3CN).

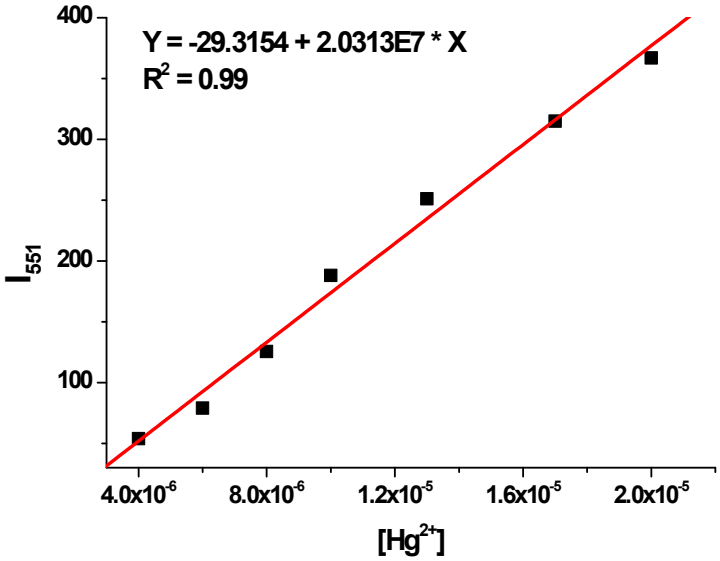


Fig. S2 Fluorescent intensity of **MS2** at 551 nm as a function of Hg^{2+} concentration (0-20.0 μM) under the same condition as the Hg^{2+} titration.

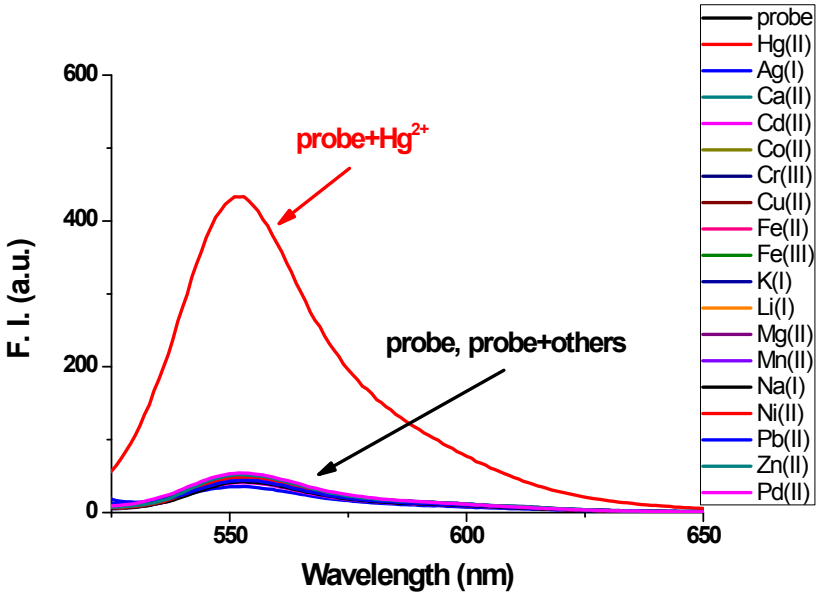


Fig. S3 Fluorescence responses of **MS2** (10.0 μM) with 3.0 equiv. of metal ions in PBS buffer solution (10 mM, pH 7.4, containing 1% CH_3CN). Metal ions include Hg^{2+}, Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, and Pd^{2+}, (λ_{ex} = 510 nm).

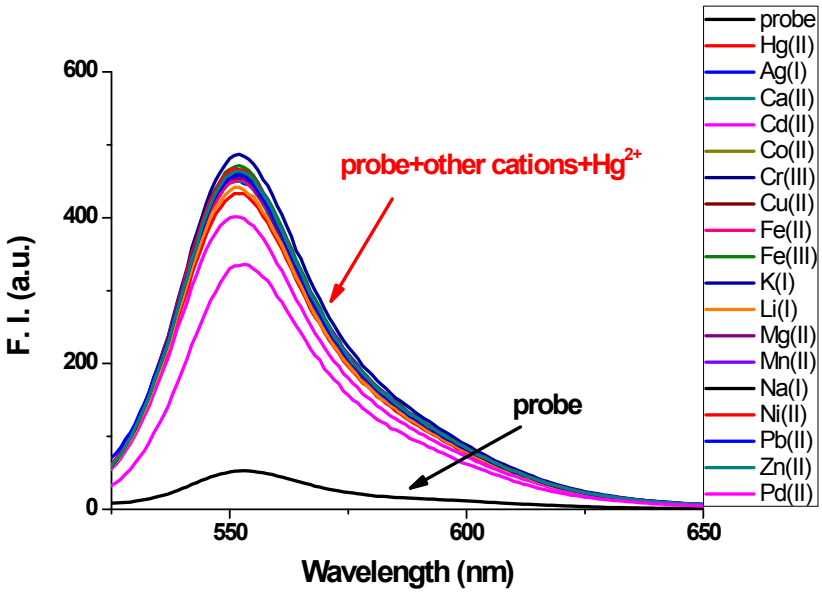


Fig. S4 Fluorescence responses of **MS2** (10.0 μM) in the presence of 3.0 equiv. of metal ions (Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, and Pd^{2+}) in PBS buffer solution (10 mM, pH 7.4, containing 1% CH_3CN), followed by 3.0 equiv. of Hg^{2+} (λ_{ex} = 510 nm).

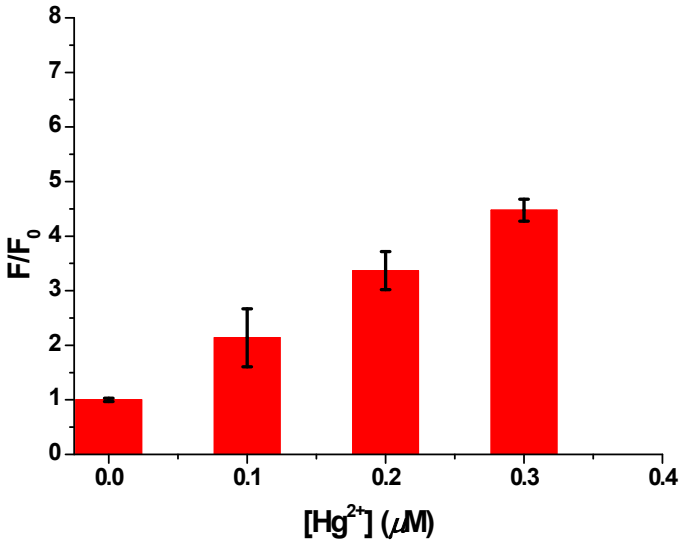


Fig. S5 Detection limit of **MS2** determination. **MS2** (0.5 μM) in PBS (10 mM, pH 7.4) was treated with various concentrations of Hg^{2+} and the fluorescence intensity at emission maxima was recorded and the detection limit was determined to be the concentration of Hg^{2+} that induced 3-fold fluorescence increase. Experiments were done in triplate.

Fig. S6 The photos of the corresponding solutions in Fig. 6 (from left to right including **MS2** only, and **MS2** with Hg^{2+}, Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, and Pd^{2+}, respectively).

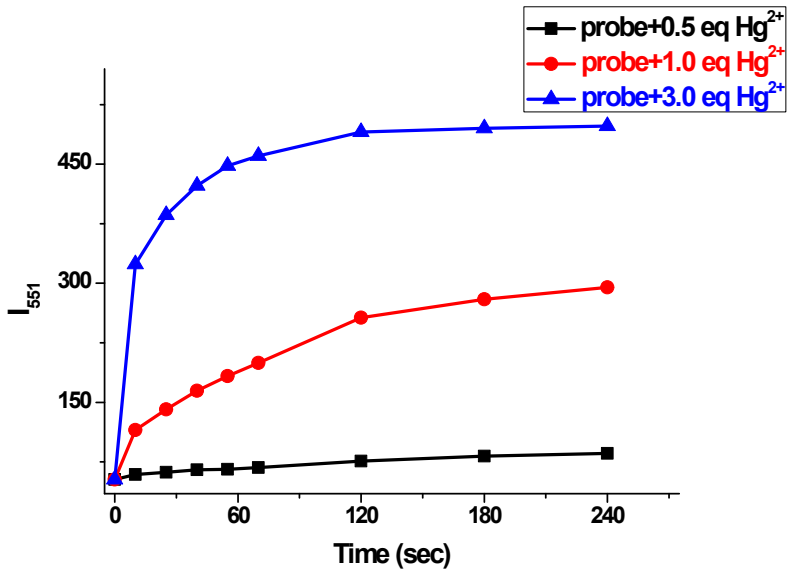


Fig. S7 Time-dependent fluorescence intensity changes of **MS2** (10.0 μM) upon addition of Hg^{2+} (0.5, 1.0, and 3.0 equiv., respectively) in PBS buffer solution (10 mM, pH = 7.4, containing 1% CH_3CN) (λ_{ex} = 510 nm).

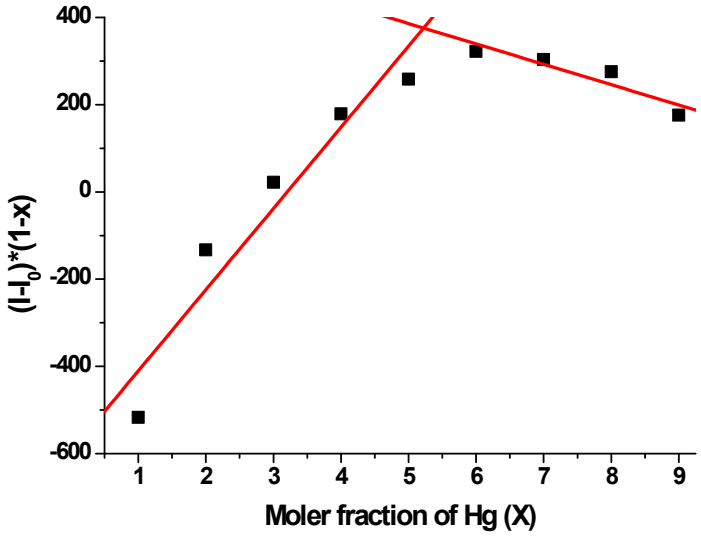


Fig. S8 The Job's plot of **MS2**, the total concentration of the sensor and Hg^{2+} is 20.0 μM (10 mM PBS buffer solution, pH 7.4, containing 1% CH_3CN, λ_{ex} = 510 nm).

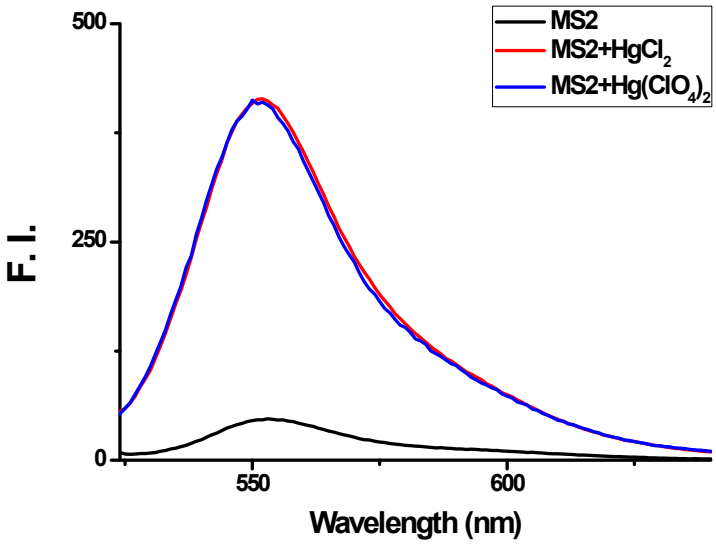


Fig. S9 The experiments between **MS2** (10.0 μM) with different source of Hg^{2+} (3.0 equiv. $HgCl_2$ and $Hg(ClO_4)_2$, respectively) in 10 mM PBS buffer solution, pH 7.4, containing 1% CH_3CN, λ_{ex} = 510 nm.

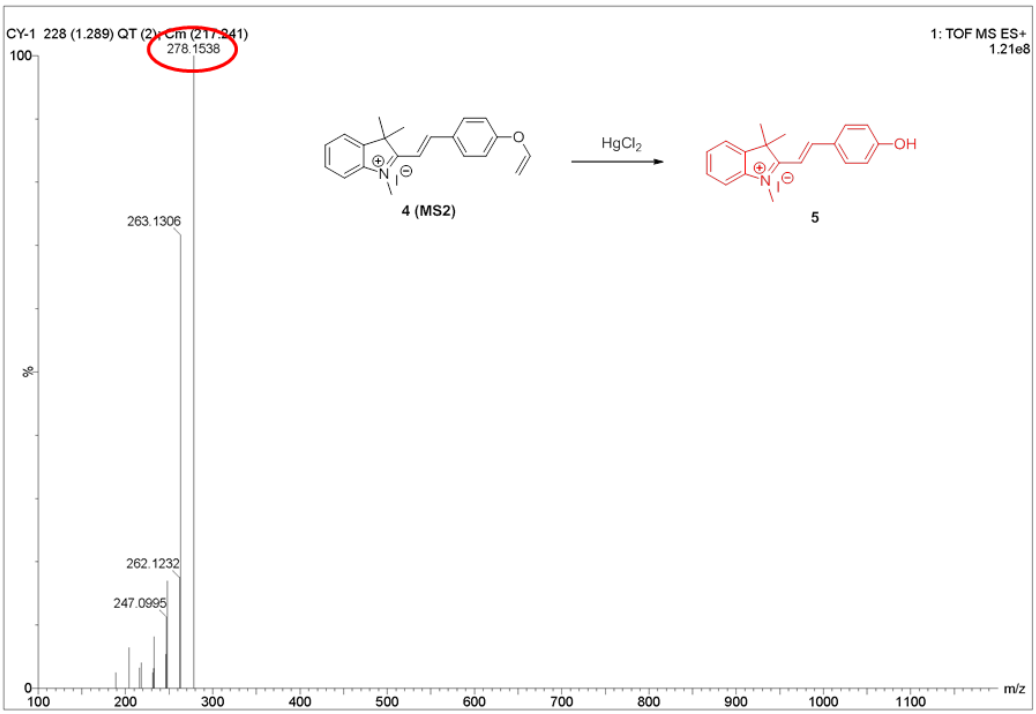


Fig. S10 HPLC-MS (TOF-ESI) spectrum of **MS2**-Hg^{2+} system shows the free hemicyanine moiety (**5**). [**5**-I]$^+$ = 278.1538 (*calcd*. For 278.1539) (t = 1.289 min).

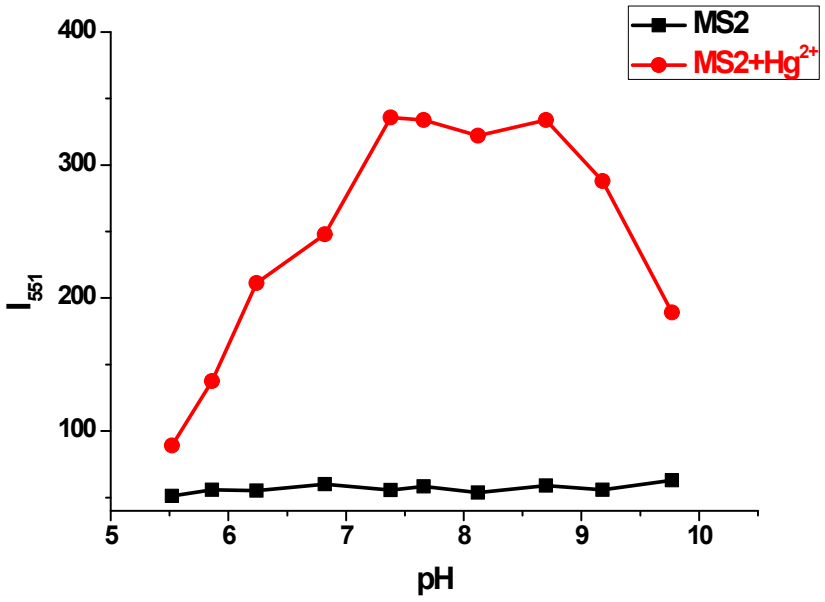


Fig. S11 Effect of the pH on the fluorescence emission of **MS2** (10.0 μM) alone and **MS2** (10.0 μM) reacted with Hg^{2+} (2.0 equiv.) (λ_{ex} = 510 nm).

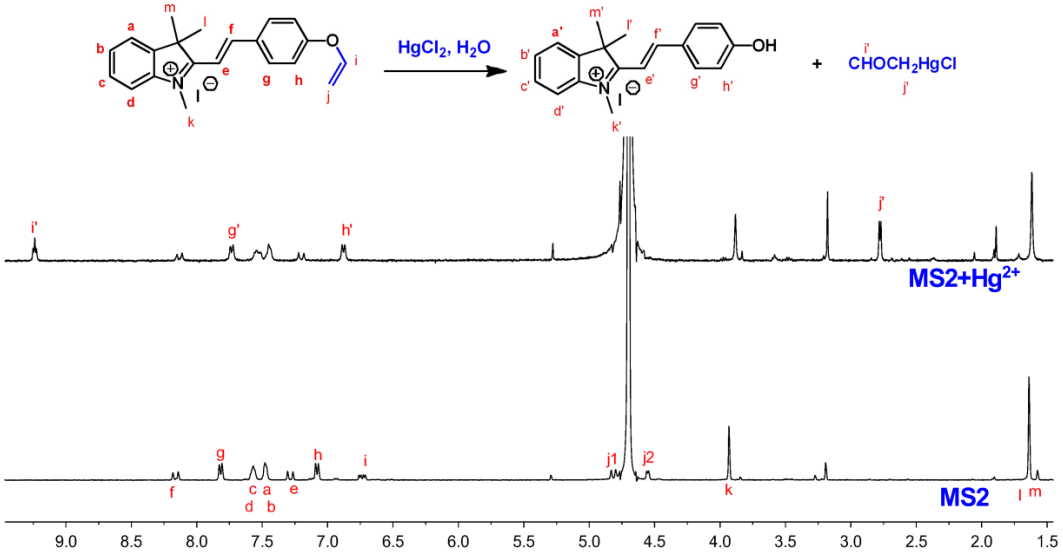
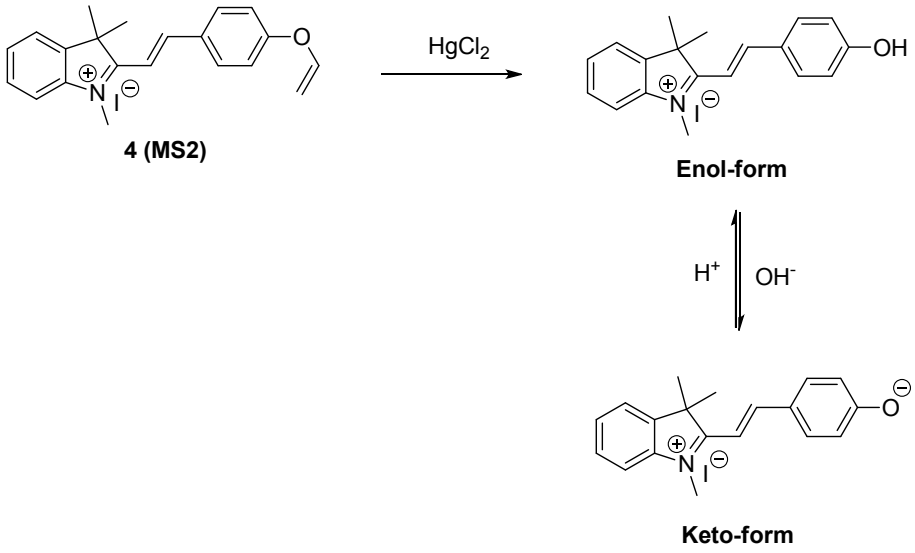


Fig. S12 ^{1}H NMR spectra of **MS2** in the absence and presence of Hg^{2+} (1.0 equiv. in D_2O).



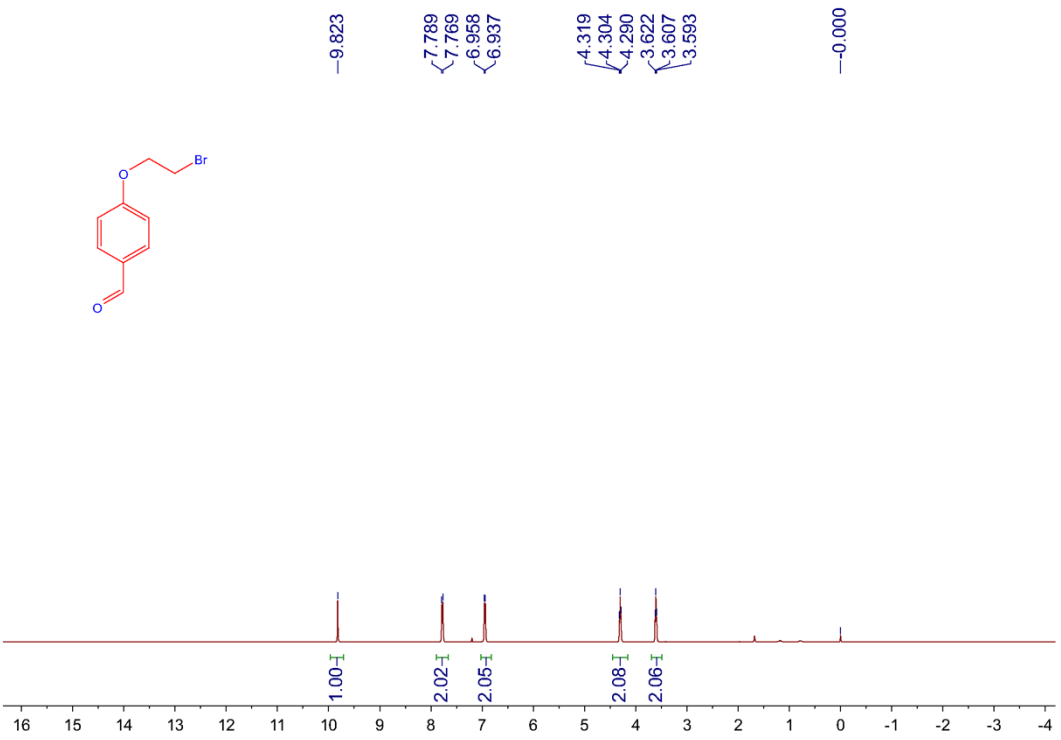
Scheme S2 Enol-form and Keto-from of free hemicyanine fluorophore.

Cell lines and imaging experiments

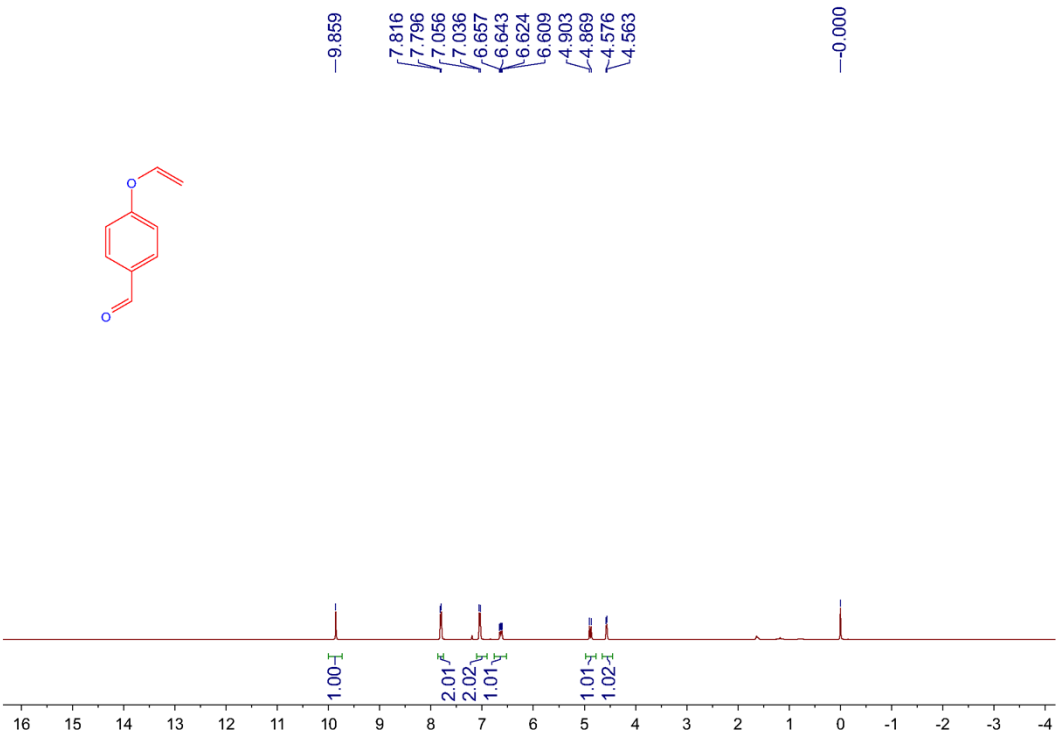
HeLa cells were cultured in DMEM (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37 ^oC. For imaging experiments, **e**xponentially growing cells (at a density of 20000-40000 cells per well, respectively) were seeded in 24-well plate. Cells were cultured at 37 ^oC in a 5% CO_2 atmosphere for 24 h before they were exposed to reagents. After the staining steps as described in figure captions, the images were collected upon excitation using the corresponding filters for DAPI (blue). Furthermore, MTT assay was employed to evaluate the cytotoxicity of MS2 and it turned out that this probe is virtually nontoxic to HeLa cells even at 20 μM after 24 h incubation.

The characterization data of MS2

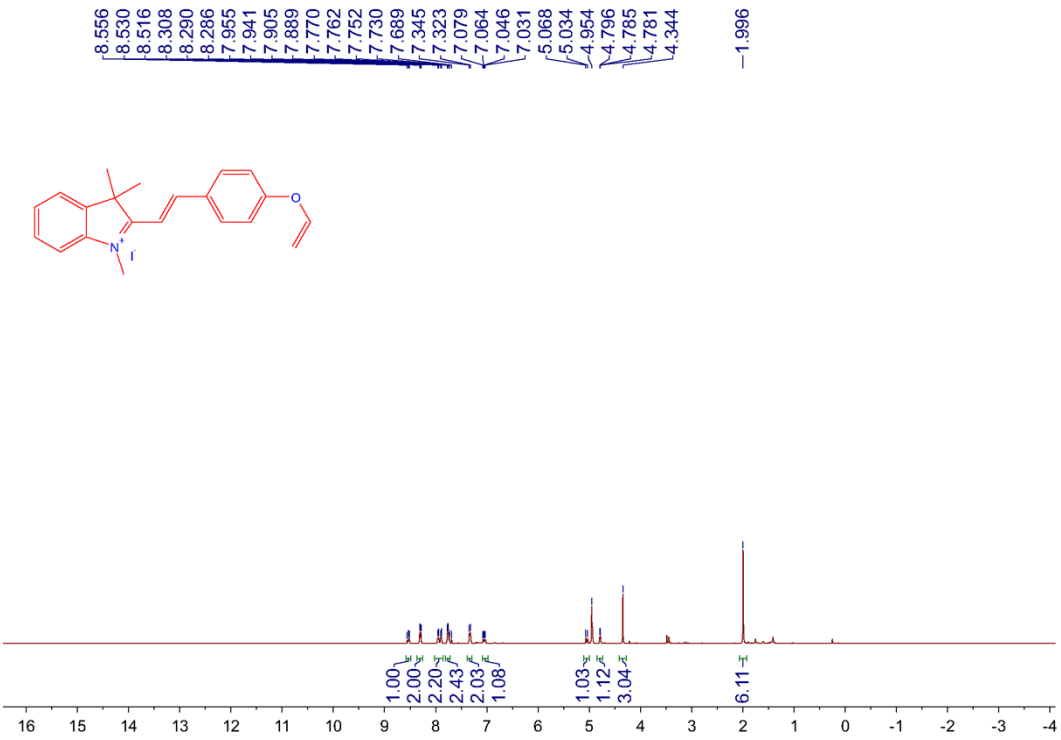
^{1}H NMR of **1**



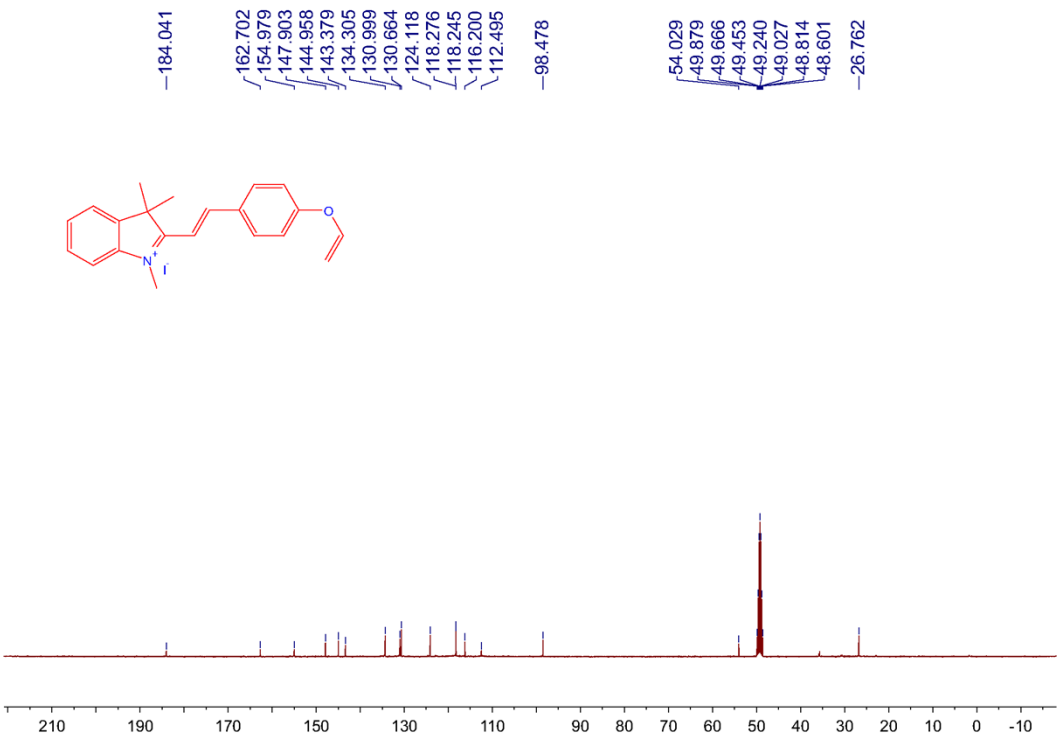
^{1}H NMR of **2**



^{1}H NMR of **4 (MS2)**

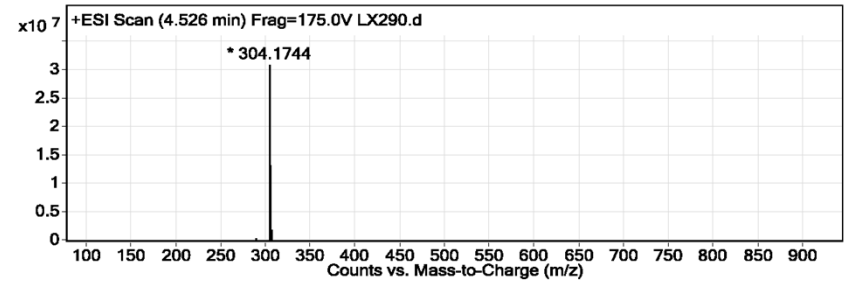


^{13}C NMR of **MS2**



HR-MS of **MS2**

Qualitative Analysis Report



Peak List

m/z	z	Abund
304.1744	1	31036770
304.3218		2708289.5
305.1742	1	13436354
306.1782	1	2048707.9

References

(1) J. Gu, U. R. Anumala, F. L. Monte, T. Kramer, R. H. Haussen, J. Holzer, V. Goetschy-Meyer, G. Mall, I. Hilger, C. Czech, and B. Schmidt, *Bioorg. Med. Chem. Lett*., 2012, **22**, 7667-7671.

(2) R. A. Velapoldi, and H. H. Tønnesen, *J. Fluoresc*., 2004, **14**, 465-472.

(3) (a) D. F. Eaton, *Pure Appl. Chem*., 1988, **60**, 1107-1114; (b) D. Magde, R. Wong, and P. G. Seybold, *Photochem. Photobiol*., 2002, **75**: 327-334.