An Easily Prepared Hypersensitive Water-Soluble Fluorescent Probe for Mercury (II) Ions

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(Supporting Information)

Experimental Section

All chemicals were obtained from commercial suppliers and used without further purification. ^{1}H NMR spectra were measured using a Bruker Varian Unity-500MHz spectrometer using TMS as an internal standard. Mass spectra were measured on a QTRAP mass spectrometer (Applied Biosystems Sciex Foster city, USA). Fluorescence spectra were measured on a Shimadzu RF-5301PC fluorescence spectrophotometer. Infrared (IR) spectra were recorded with a Bruker Vertex 80V FTIR spectrophotometer.

(1) Synthesis and characterization of dansyl-L-tryptophan methyl ester (1).

In an ice bath, a solution of dansyl chloride (1.46 g; 5.427 mmol) in anhydrous acetone (10 ml) was added slowly to a mixture of L- tryptophan methyl ester (1.43 g; 6.562 mmol) and 0.2 ml triethylamine in anhydrous acetone (20 ml) and then the mixture was stirred for 0.5 h. The reaction was then continued for 12 h at room temperature, and the progress of the reaction was monitored by TLC (30:1, chloroform to methanol; R_f = 0.5). After the solvent was removed under reduced pressure, the solid was dissolved in chloroform firstly and then was washed with the saturated $NaHCO_3$ to alkalescence (pH~7.5), further washed with hydrochloric acid to weak acidity (pH~6.2), and finally with saturated NaCl solution to neutrality. The organic solution was filtered after drying over anhydrous $MgSO_4$. After evaporation of solvent, purification of 1 was performed by using column chromatography on silica gel (elution with chloroform) resulting in an 81.7% yield. 1H NMR (500 MHz, CD_3OD : D_2O = 2 : 1): δ = 8.380-6.831 (m, 11 H, dansyl and indole(H)), δ = 4.012 (t, 1 H, -CH), δ = 3.339(s, 3 H, $-OCH_3$), δ =3.106-2.877 (m, 2H, $-CH_2-$), δ = 2.859 (s, 6 H, $-N(CH_3)_2$). IR (KBr) 3414, 3280, 2947, 2867, 1739, 1333, 1310, 1162, 1145, 1096 cm-1. Mass spectral data (ESI-MS): for $C_{24}H_{25}N_3SO_4$ calcd. 451.0; found 452.1.

(2) Mild hydrolysis of 1 in 1 M NaOH solution (45 °C, 12 h) resulted in about 40% yield of dansyl-L-tryptophan (2). The coupling form of it with cyclohexylammonium salt is also commercially available (Sigma-Aldrich Corporation), both show identical fluorescent properties.

(3) Measurements of Fluorescence spectra

Fluorescence emission spectra were recorded on a Shimadzu (Japan) RF-5301PC fluorescence spectrophotometer. To reduce the fluctuation in the excitation intensity during measurement, the lamp was kept on for 1 h and the samples were stock-still for 3 min prior to the experiment. Samples for emission measurement were contained in 1

cm × 1 cm quartz cuvettes (4 mL volume). All spectroscopic measurements of **1** were performed in 10.0 mM HEPES buffer solution (pH 7.5) using distilled water, and the concentrations of **1** in all the fluorescent experiments are 5.0 μM in 10.0 mM HEPES buffer solution (pH 7.5). A fixed excitation wavelength at 355 nm was used. Fluorescence titration was performed in aqueous solution using respective chloride salt of metal ion (except for $AgNO_3$). For the pH titration of **1**, the spectra were monitored in solutions of various pH containing 0.1 M NaCl to keep the ionic strength.

(4) Detection of Hg^{2+} in live cells

4.1 Hela Cells Culture. Hela cells were cultured according to the reported protocol:[1] Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigima) containing 10% fetal bovine serum (FBS, Gibco) and 1% Antibiotic-Antimycotic (Gibco) at 37 °C in a 5% CO_2/95% air incubator. Cells were plated 24 h before study into 6 well plates and at the time of study, the medium was changed with a fresh 2% serum one.

4.2 Fluorescence Imaging Experiments. Imaging experiments were performed with a FV1000 confocal laser-scanning fluorescent microscope (Olympus, Japan) with a 10× objective lens. Excitation of **1**-loaded cells at 405 nm was carried out with an argon ion laser, and emission was collected in a window from 475 to 505 nm. **1** (from a 1.0 mM stock solution in DMSO) was added to each well except control of cultured Hela cells and a 5.0 μM of **1** was obtained in final. The cells were incubated for 15 min at 37 °C, at which point the medium was removed and the cells were washed thoroughly twice by PBS (pH 7.0). After that, a confocal fluorescent microscope was used to record them.

After cells were treated with 5.0 μM **1** for 15 min and then washed twice with PBS, fresh medium with 5.0 μM $HgCl_2$ was added. After further incubation of 15 min, cells were washed several times with PBS until neutral condition (pH~7.0) and confocal fluorescent microscopy was recorded for them. Measured conditions of the microscope were the same for a) and b).

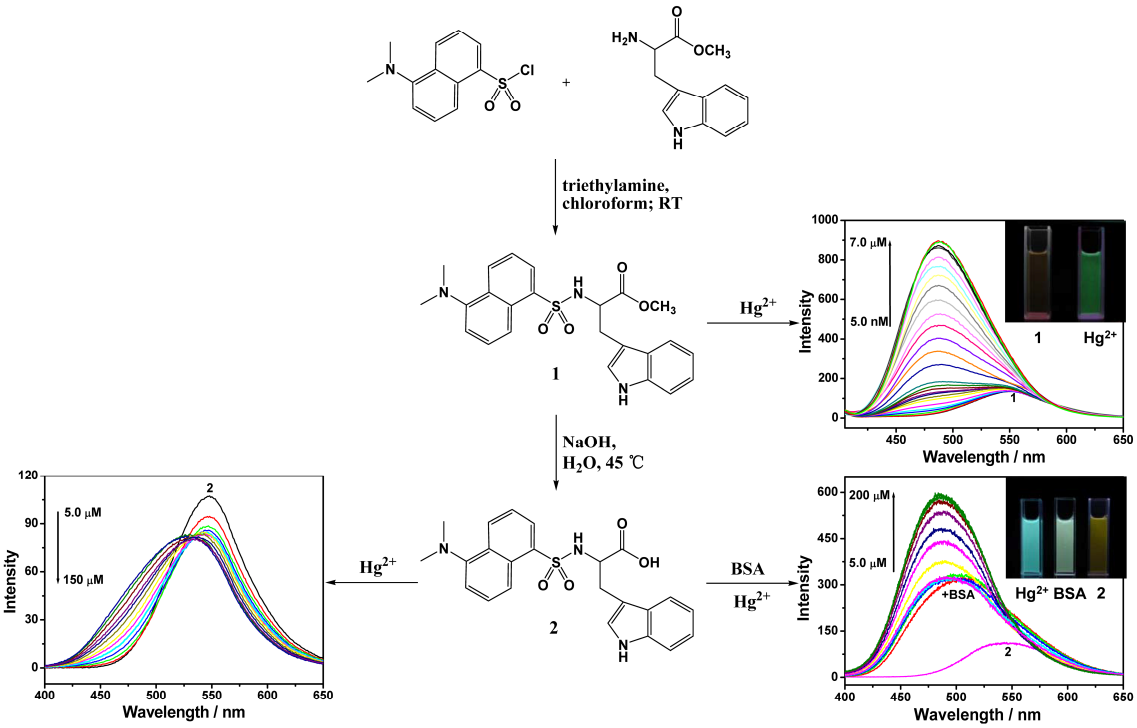


Fig. S1 Synthesis route of probes **1** and **2**, and the enhancements of their fluorescence intensity upon addition of Hg^{2+} (for **1**) or Hg^{2+} and BSA (for **2**) with visual fluorescence color changes upon irradiation at 365 nm.

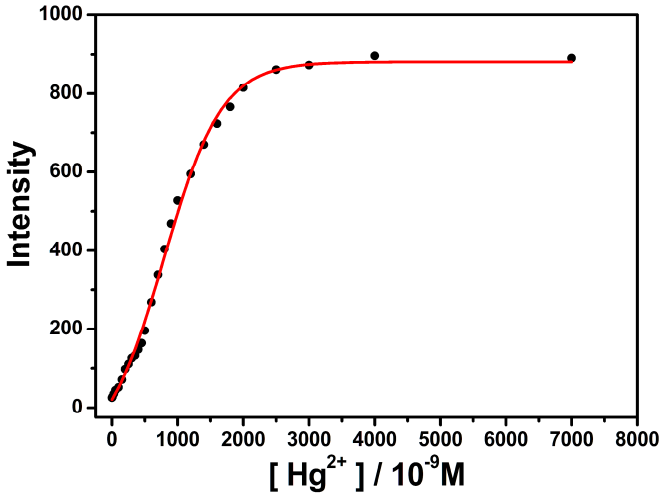


Fig. S2 Fitting to the fluorescence intensity changes of **1** at 487 nm (R = 0.9977) upon gradual addition of Hg^{2+} from 5.0 nM to 7.0 μM (corresponds to Figure 1, λ_{ex} = 355 nm, λ_{em} =487 nm)

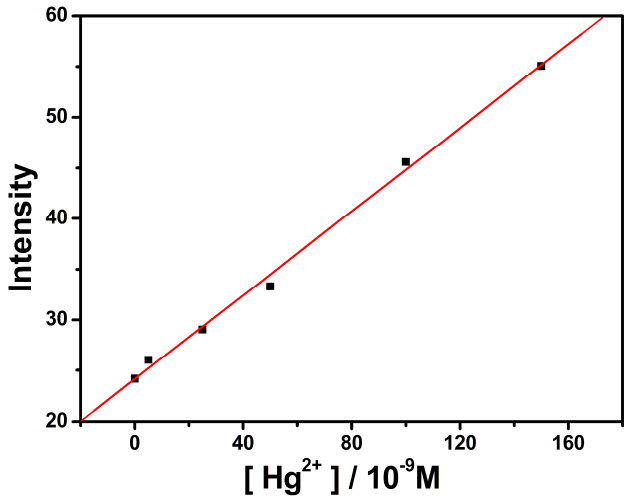


Fig. S3 Fluorescence intensity of **1** (5.0 μM) at 487 nm as a function of low concentration range of Hg^{2+} (0~150 nM) in 10.0 mM HEPES buffer solution (pH 7.5), a linear dependence was obtained with a correlation coefficient of 0.9982 (λ_{ex} = 355 nm, λ_{em} =487 nm).

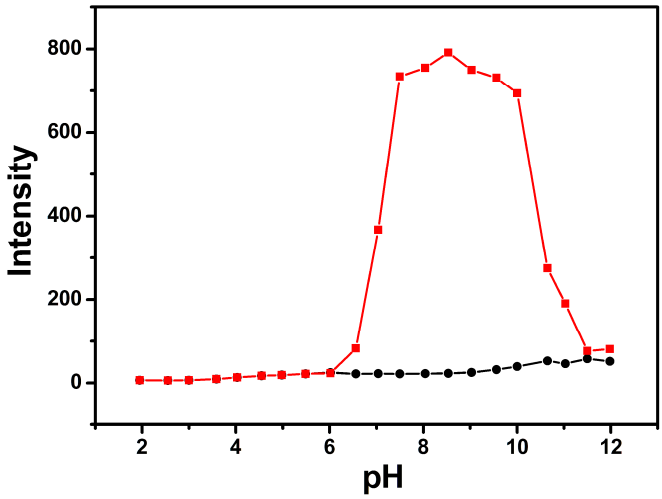


Fig. S4 Variation of fluorescence intensity at 487 nm of **1** (5.0 μM) in aqueous solution in the presence (red) and absence (black) of Hg^{2+} ion (1.0 equiv.) as a function of pH (λ_{ex} = 355 nm, λ_{em} =487 nm).

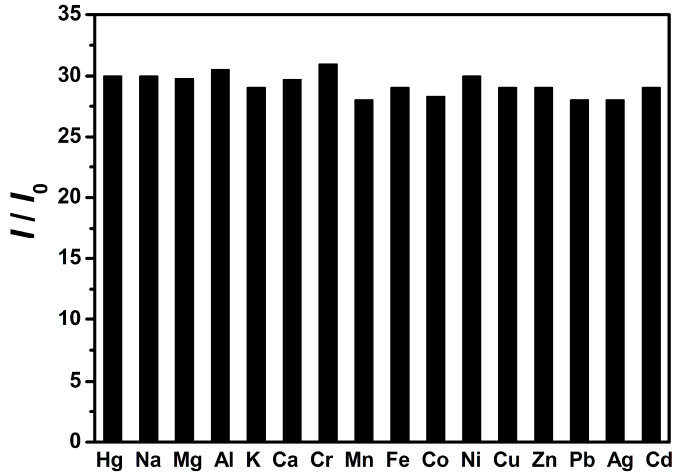


Fig. S5 Fluorescence intensity ratios of **1** (5.0 μM) in 10.0 mM HEPES solution (pH 7.5) in the presence of 2.0 μM Hg^{2+} in mixing with 10.0 μM Na^{+}, Mg^{2+}, Al^{3+}, K^{+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Ag^{+}, Cd^{2+}, respectively. (λ_{ex} = 355 nm, λ_{em} =487 nm).

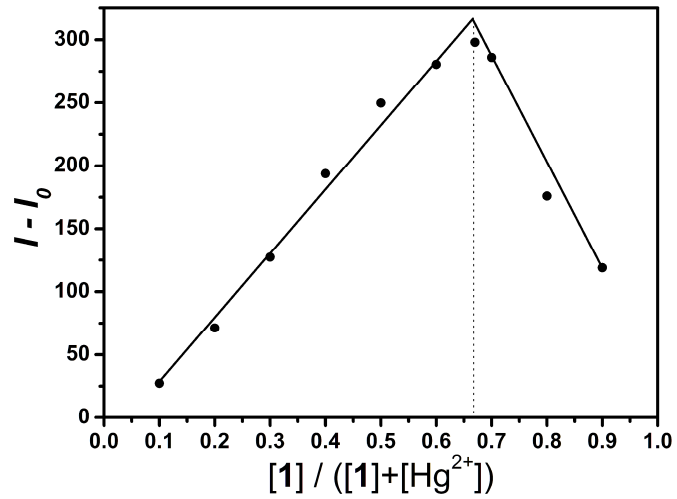


Fig. S6 Fluorescence intensity changes of band at 487 nm of **1** and Hg^{2+} with a total concentration of 5.0 μM in 10.0 mM HEPES solution (pH 7.5), indicating a 2:1 stoichiometric ratio of **1**:Hg^{2+} (λ_{ex} = 355 nm, λ_{em} =487 nm).

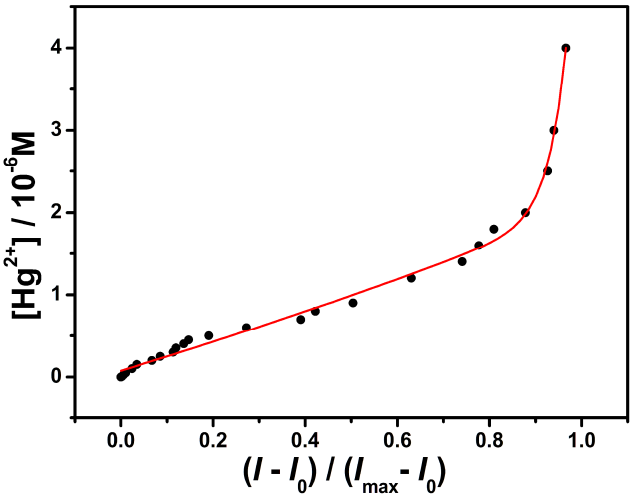


Fig. S7 Estimation of binding constant for **1** and Hg^{2+}, the plot was calculated based on the equation and a 2 : 1 binding model (R = 0.9866, λ_{ex} = 355 nm, λ_{em} =487 nm).[2]

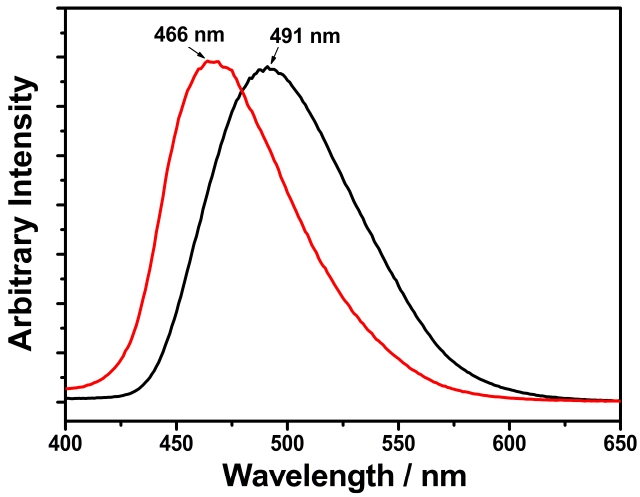


Fig. S8 The fluorescence spectra of **1** in solid (black line) and the crystal complex of **1**/Hg^{2+} (red line). And *I* is the arbitrary intensity of fluorescence emission (λ_{ex} = 355 nm).

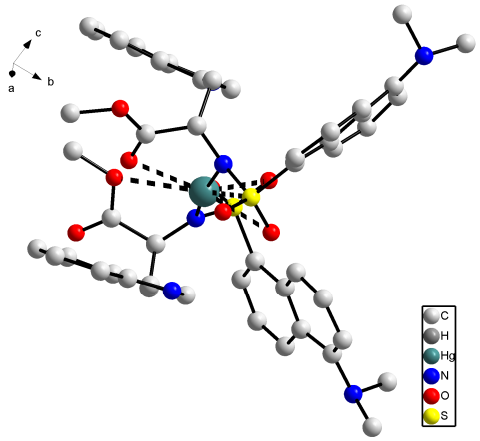
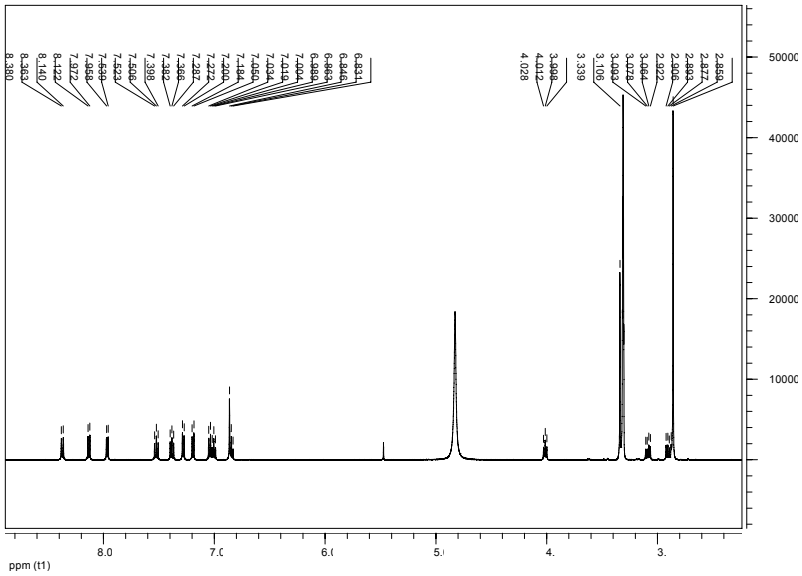


Fig. S9 View of the X-ray crystal structure of complex **1**/Hg^{2+} (H-atoms were omitted for clarity) in another visual angle.

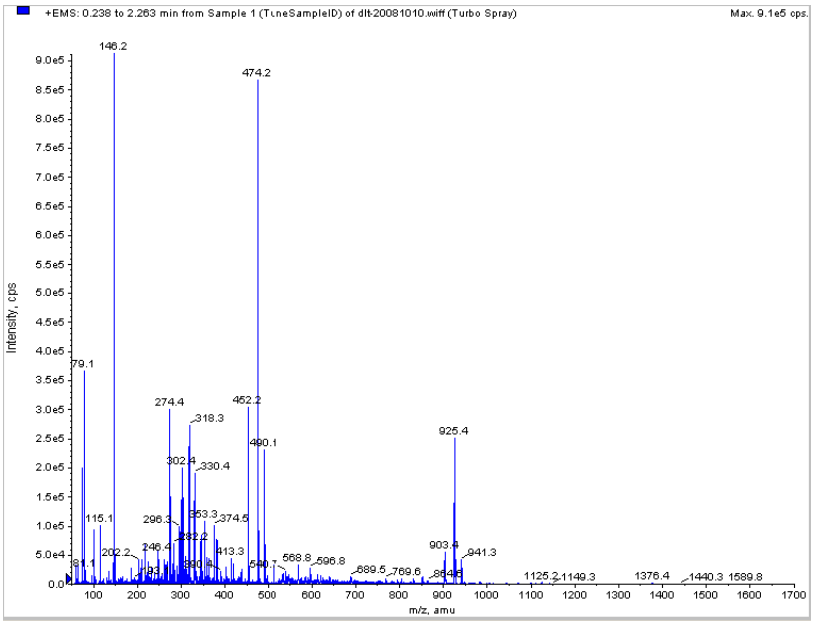
Table S1. Proton chemical shifts (δ, in ppm) of 1 before and after associated with Hg^{2+}.

	H(4)	H(2)	H(8)	H(3)	H(7)	H(6)	H(12)
δ_a	8.234	7.951	7.951	7.480	7.364	7.339	7.116
δ_b	8.205	8.002	8.044	7.514	7.426	7.497	7.095
$\Delta\delta_H$	-0.029	0.051	0.093	0.034	0.062	0.158	-0.021
	H(15)	H(13)	H(10)	H(14)	H(18)		H(21)
δ_a	7.060	6.995	6.855	6.822	4.059		3.537
δ_b	7.055	6.984	6.868	6.800	4.239	4.095	3.595
$\Delta\delta_H$	-0.005	-0.011	0.013	-0.022	0.180	0.036	0.058

^{1}H NMR spectrum of **1** in CD_3OD: D_2O = 2 : 1



ESI-MS spectrum of **1**



References:

1 M. Taki, M. Desaki, A. Ojida, S. Iyoshi, T. Hirayama, I. Hamachi and Y. Yamamoto， *J. Am. Chem. Soc.*, **2008**, *130*, 12564.

2 Y. Kubo, M. Kato, Y. Misawa and S. Tokita, *Tetrahedron Lett.*, **2004**, *45*, 3769.