Supporting Information

A "Turn-On" Fluorescent Sensor for the Selective Detection of Mercuric Ion in Aqueous Media

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Experimental.

Reagents. Ethyl acetate (EtOAc) was dried over 3 Å molecular sieves. 1,2-Dichloroethane (DCE) was distilled from calcium hydride under nitrogen and stored over molecular sieves. Acetonitrile was either distilled over CaH_2 under nitrogen or was saturated with Ar and dried by passing through an activated Al_2O_3 column. 3,9-Dithia-6-azaundecane was synthesized as previously described.[S1] All other reagents were used as received.

Methods. Silica gel-60 (230-400 mesh) was used as the solid phase for flash chromatography and thin layer chromatography (TLC) was performed by using Merck F254 silica gel-60 plates. TLC plates were visualized with UV light or after developing with ninhydrin stain. ^{1}H and ^{13}C NMR spectra were obtained either on a Varian 300 MHz or a Varian 500 MHz spectrometer operating at ambient probe temperature, 283 K, and referenced to internal probe standards. IR spectra were recorded by using an Avatar 360 FTIR instrument. Electrospray ionization (ESI) spectroscopy was performed in the MIT Department of Chemistry Instrumentation Facility.

Syntheses.

***N*-(2-Nitrobenzyl)-3,9-dithia-6-azaundecane (1).** 2-Nitrobenzylbromide (560 mg, 2.59 mmol), K_2CO_3 (400 mg, 2.89 mmol) and molecular sieves were combined in 25 mL of CH_3CN and stirred. A 10 mL solution of 3,9-dithia-6-azaundecane (501 mg, 2.59 mmol) in CH_3CN was added dropwise. The reaction was stirred for 8 h, filtered through Celite and the solvent was evaporated to afford a yellow oil. The oil was flushed through a short silica plug (7:1 hexanes:EtOAc) and dried to yield the product (726 mg, 85%). TLC R_f = 0.38 (silica, 7:1 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.11 (6H, t), 2.35 (4H, q), 2.51 (4H, m), 2.71 (4H, m), 3.85 (2H, s), 7.28 (1H, t), 7.7.45 (1H, t), 7.70 (2H, m). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.86, 26.03, 29.06, 54.03, 55.55, 124.10, 127.65, 130.68, 132.35, 134.68, 149.23. HRMS (ESI) Calcd for MH^+, 329.1352; Found, 329.1359.

***N*-(2-Aminobenzyl)-3,9-dithia-6-azaundecane (2).** A portion (512 mg) of Pd black was placed in a flask purged with Ar after which 10 mL of MeOH was added. *N*-(2-Nitro-benzyl)-3,9-dithia-6-azaundecane (**1**, 301 mg, 920 μmol) dissolved in MeOH (20 mL) was transferred to the reaction flask with a syringe. Hydrogen was introduced to the reaction with vigorous stirring for 7 h. After purging with Ar, the solution was filtered through Celite and the solvent was removed in vacuo to yield a brown oil. The crude material was purified by flash chromatography on silica (7:1 hexanes:EtOAc), which afforded a yellow oil (84 mg, 31%). TLC R_f = 0.33 (silica, 7:1 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.21 (6H, t), 2.47 (4H, q), 2.71 (8H, m), 3.64 (2H, s), 4.75 (2H of NH_2, s), 6.64 (2H, m), 6.97 (1H, d), 7.08 (1H, td). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.63. 25.71, 28.86, 52.93, 58.00, 115.12, 117.09, 121.93, 128.09, 129.98, 146.59. HRMS (ESI) Calcd for MNa^+, 321.1430; Found, 321.1427.

2-{5-[(2-{[Bis-(2-ethylsulfanyl-ethyl)-amino]-methyl}-phenylamino)-methyl]-2-chloro-6-hydroxy-3-oxo-3H-xanthen-9-yl}-benzoic acid (MS1). A portion (73 mg, 245 μmol) of N-(2-aminobenzyl)-3,9-dithia-6-azaundecane, **2**, was dissolved in 3 mL of

EtOAc and 7'-chloro-4'-fluoresceincarboxaldehyde[S2] (97 mg, 245 μmol) was added. The reaction became orange-pink and cloudy. An additional 1 mL of EtOAc was added and the mixture was stirred at room temperature for 18 h. During this time, the solution clarified and turned red. The EtOAc was removed to yield a magenta foam, which was dried in vacuo. The dried foam was dissolved in 3 mL of 1,2-dichloroethane, $NaB(OAc)_3H$ (65 mg, 307 μmol) was added, and the reaction was left to stir overnight at room temperature. The solution was diluted with 5 mL of CH_2Cl_2, extracted (3 x 8 mL) with saturated $NaHCO_3$, and washed (2 x 8 mL) with deionized water. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the crude product as a red solid. Chromatography on silica gel (50:1 $CHCl_3$:MeOH) followed by preparative TLC using the same solvent system yielded the purified product as a deep magenta solid (88 mg, 52 %). TLC R_f = 0.41 (silica, 9:1 $CHCl_3$:MeOH); mp = 58-61 ^{0}C. ^{1}H NMR (CD_3OD, 300 MHz) δ 1.02 (6H, t), 2.24 (4H, q), 2.2.38 (4H, m), 2.57 (4H, m), 3.59 (2H, s), 4.46 (1H, d), 4.4.60 (1H, d), 6.58 (2H, m), 6.68 (1H, s), 6.95-7.11 (4H, m), 7.20 (1H, m), 7.29 (1H, d), 7.57 (2H, m), 8.03 (1H, d). ^{13}C NMR (DMF-d_7, 125 MHz) δ 14.81, 25.53, 28.28, 40.63, 53.95, 58.03, 103.64, 108.83, 110.31, 110.81, 112.69, 115.17, 122.69, 122.87, 126.48, 128.07, 128.21, 128.61, 128.69, 129.68, 129.89, 130.08, 130.75, 135.18, 170.66, 173.15. FTIR (KBr, cm^{-1}) 3423, 3049, 2963, 2919, 1647, 1607, 1571, 1509, 1458, 1374, 1342, 1302, 1220, 1149, 1044, 1007, 937, 883, 828, 746, 714, 689, 621, 598, 547, 469. HRMS (ESI) Calcd for MNa+, 699.1725; Found, 699.1720.

General Spectroscopic Procedures. Ultrol grade PIPES (piperazine-*N,N'*-bis(2-ethanesulfonic acid) from Calbiochem, KCl (99.997%) and anhydrous $HgCl_2$ (99.998%) were purchased and used as received. Millipore filtered water was used to prepare all aqueous solutions. With the exception of the pK_a determination, all spectroscopic measurements were conducted at neutral pH with 50 mM PIPES, 100 mM KCl buffer adjusted to pH 7. An Orion glass electrode, calibrated prior to use, was

employed to record solution pH. Mercury solutions were prepared from 10 mM stock solutions of $HgCl_2$. Stock solutions of MS1 (1 mM in DMSO) were prepared, stored at –4 ^{o}C, and thawed in the dark immediately prior to use. After addition of this stock solution to aqueous buffers, the resulting solution contained 0.1% DMSO for fluorescence and 1% DMSO for absorption measurements. The KaleidaGraph software package was used to manipulate all spectral data.

Optical Absorption Spectroscopy. UV-visible spectra were obtained by using either a Cary IE scanning spectrophotometer or a Hewlet Packard diode array spectrophotometer. Both instruments were controlled by Pentium PCs and were run using the manufacturer supplied software packages. A circulating water bath was used during acquisition to maintain the temperature at 25.0 ^{o}C $\pm$ 1.0 ^{o}C. Samples were contained in 1-cm path length quartz cuvettes (3.5 mL volume). All manipulations were performed at least three times.

Hg(II) Binding Studies by Absorption Spectroscopy. Metal binding titrations and Job plots were obtained for MS1 in order to determine the stoichiometry of the metal-bound complex in solution. In a typical titration, 3 μL aliquots of a 1 mM $HgCl_2$ solution in water were added to a solution of 10 μM MS1 and the absorbance changes at 498 and 520 nm were plotted against equivalents of Hg(II) added.

Fluorescence Spectroscopy. Emission spectra were obtained with a Hitachi F-3010 spectrofluorimeter linked to a Pentium PC running the SpectraCalc software package. A rhodamine quantum counter was used to normalize the spectra for excitation intensity, and manufacturer-supplied correction curves were used to normalize the emission spectra. Manufacturer supplied photomultiplier curves were used to correct for emission intensity. A circulating water bath was used during all experiments to regulate the temperature at 25.0 ^{o}C $\pm$ 0.1 ^{o}C. Spectra were obtained with 3 nm slit widths and either a 240 nm/min or 600 nm/min scan speed. All measurements were conducted at least in triplicate.

Quantum Yield Measurements. The quantum yield of MS1 was determined by comparison to fluorescein in 0.1 N NaOH (ϕ = 0.95)[S3] as a reference. In a typical experiment, a 6 mL solution of ~1 μM MS1 was prepared. For metal-free studies, 10 μL of 100 mM K_4EDTA was added to chelate any adventitious metal ions. To determine the quantum efficiencies of the metal-bound dye, 10 μL of a 10 mM $HgCl_2$ solution was added to a 1 μM MS1 solution. In order to avoid inner filter effects, the solution A_{max} – values were typically ~0.05 to ~0.08. The concentration of the reference solution was adjusted such that A_{max} (490 nm) equaled A_{max} of MS1 (505 nm) or A_{max} of the Hg(II) complex (501 nm), and the excitation wavelength was chosen as the wavelength determined from where the reference and probe excitation spectra intersect. Excitation was at 497 nm for MS1 and at 496 nm for the Hg(II) complex. Emission spectra were integrated from 510-650 nm and the quantum yields were calculated standard methods.[S4]

Determination of Protonation Constants. The pK_a values for MS1 affect fluorescence were determined by plotting the integrated emission intensity versus pH from ~12 to ~4 (Figure S1). In a typical experiment, a 30 mL solution of 1 μM MS1 in 100 mM KCl, 10 mM KOH was prepared (pH ~12). Aliquots of 6 N, 2 N, 1 N, 0.5 N, and 0.5 N HCl were added to achieve pH changes of approximately 2.5, and the emission spectrum was recorded after each addition. The overall volume change for each experiment did not exceed ~2%. Upon excitation at 500 nm, the emission spectra were integrated over the range 510 nm to 650 nm, normalized and plotted against pH. The data were fit to the non-linear expression previously described.[S4]

Selectivity of Mercury-Induced Fluorescence in the Presence of Other Metal Ions. The selectivity of MS1 for Hg(II) against a background of various alkali, alkaline earth, transition metal ions, and Zn(II), Cd(II) or Pb(II) was investigated by using fluorescence spectroscopy. Aqueous metal ion solutions of Li(I), Na(I), Rb(I), Mg(II), Ca(II), Sr(II), Ba(II), Mn(II), Co(II), Ni(II), Cd(II), and Hg(II) were prepared from the

chloride salts. The Cu(II) solution was prepared from copper sulfate and the Pb(II) was prepared from lead nitrate. A solution of Cr(III) was prepared from chromium acetate and stored at pH 1. Solutions of Fe(II) were prepared immediately before use with ferrous ammonium sulfate and water that was thoroughly purged with Ar. All stock solutions were ~10 mM, with the exception of 100 mM NaCl. In a typical experiment, the emission spectrum of the free dye was recorded. A 20 μL aliquot of a ~10 mM metal solution was then added to a 1 μM L solution of MS1 (3 mL) and the emission spectrum was recorded with excitation at 500 nm. Subsequently, a 20 μL portion of 10 mM $HgCl_2$ was added and the emission spectrum was obtained. In order to ascertain the affect of mM concentrations of alkali and alkaline earth metals on the fluorescence of MS1, solutions of 100 mM Li(I), Na(I), Ca(II) and Mg(II), 10 mM Rb(II) and Sr(II), and 1 mM Ba(II) were prepared (50 mM PIPES, 100 mM KCl, pH 7). A solution of 1 μM MS1 in the buffer containing the cation of interest was prepared and the emission spectrum was recorded. A 20 μL portion of 10 mM $HgCl_2$ was then added and the emission spectrum was obtained. For all experiments, the spectra were integrated from 510-650 nm and normalized with respect to the free dye.

Dependence of Hg(II)-Induced Fluorescence on Chloride Ion Concentration. The magnitude of the Hg(II)-induced fluorescence increase of MS1 is anion-dependent. A ~5-fold increase in integrated emission occurs upon addition of Hg(II) to MS1 in 50 mM PIPES, 100 mM KCl, pH 7. When KNO_3 is employed instead of KCl (50 mM PIPES, 100 mM KNO_3, pH 7), the fluorescence increase is ~1.6-fold. A ~1.6-fold increase is also observed upon addition of Hg(II) to MS1 in water (no buffer). If a portion of KCl is added to an aqueous (no buffer) solution of MS1 and Hg(II), the fluorescence increase is restored to ~5-fold. This behavior is illustrated in Figure S2. The selectivity of the mercury-induced fluorescence enhancement in the presence of other metal ions was determined in 50 mM PIPES, 100 mM KNO_3 at pH 7 (data not shown). The substitution of KCl for KNO_3 does not alter the metal ion selectivity. Only Cu(II)

interferes with the Hg(II)-induced fluorescence increase. The Cd(II) complex shows slightly greater fluorescence under these conditions (~1.9-fold fluorescence increase), but addition of Hg(II) causes displacement of the Cd(II). The addition of KBr or KI to an aqueous solution of MS1 and Hg(II) does not cause any additional fluorescence increase (data not shown).

Reversibility of Hg(II)-Induced Fluorescence. Figure S3 displays the reversible binding of Hg(II) to MS1 upon addition of the chelating agent TPEN.

References.

(S1) Tanaka, M.; Nakamura, M.; Ikeda, T.; Ikeda, K.; Ando, H.; Shibutani, Y.; Yajima, S.; Kimura, K. *J. Org. Chem.* **2001**, *66*, 7008-7012.

(S2) Nolan, E. M.; Burdette, S. C.; Harvey, J.; Hilderbrand, S. A.; Lippard, S. J. *Unpublished results.*

(S3) Brannon, J. H.; Magde, D. *J. Phys. Chem.* **1978**, *82*, 705-709.

(S4) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831-7841.

Scheme S1. Nolan & Lippard

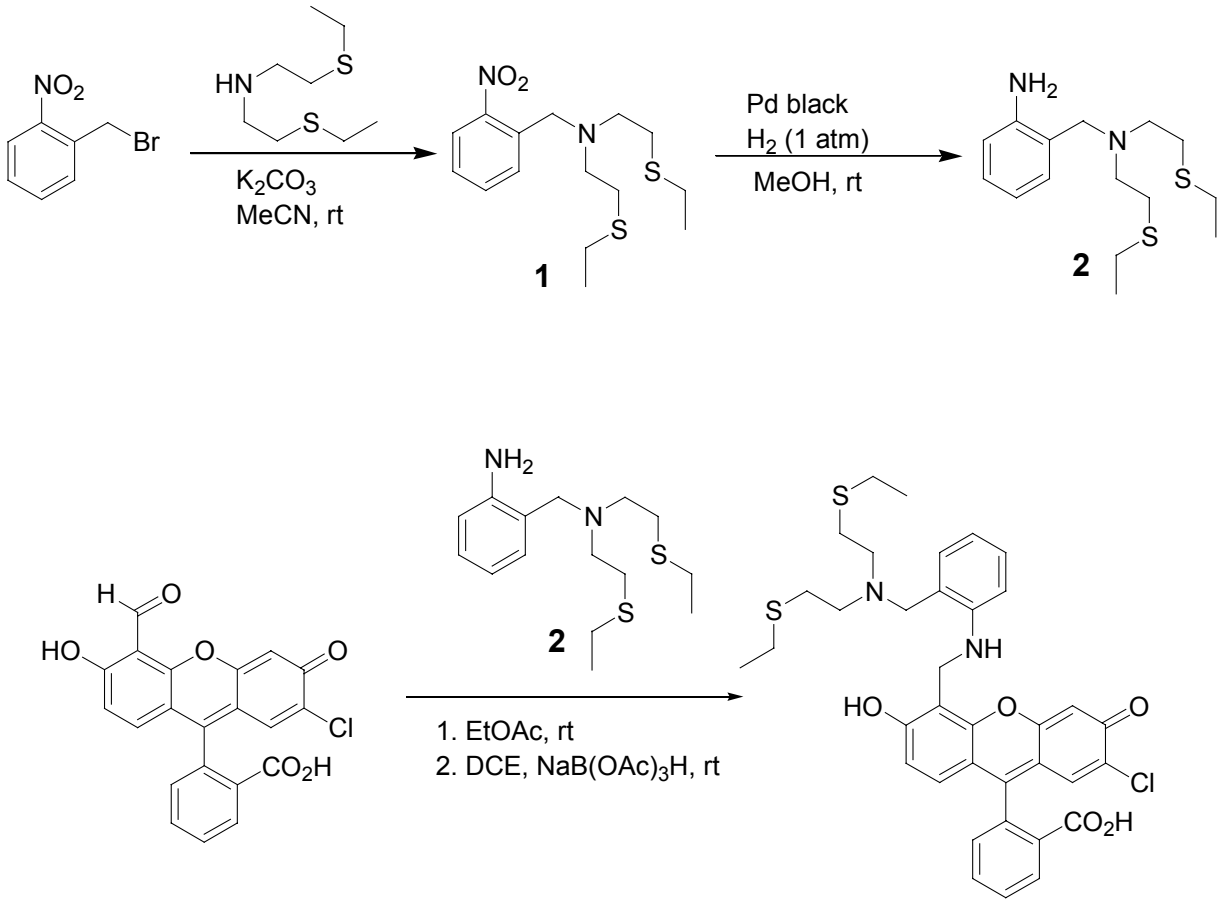

Figure S1. Nolan & Lippard

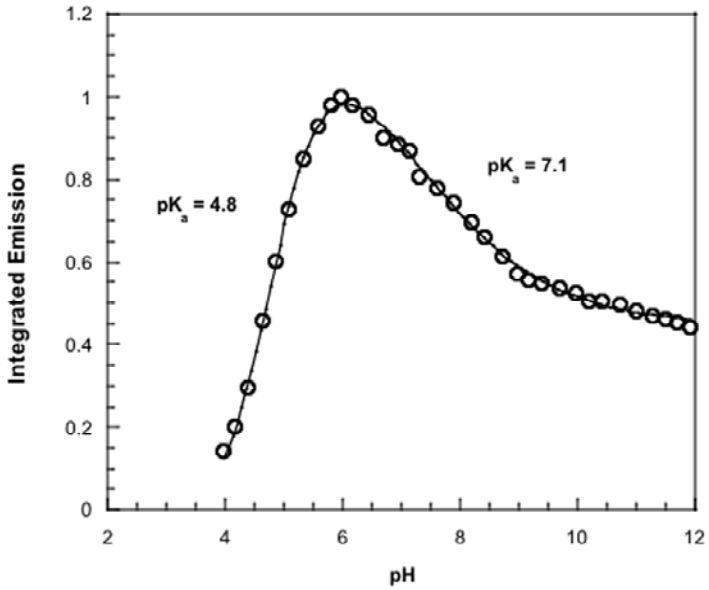

Figure S1. Fluorescence response of MS1 to pH. [MS1] = 1 μM. λ_{ex} = 500 nm and the emission was integrated from 510-650 nm.

Figure S2. Nolan & Lippard

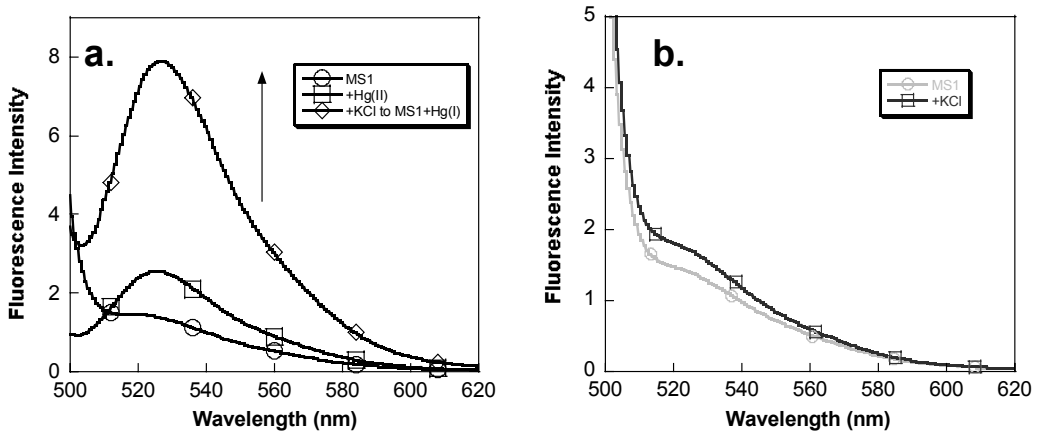

Figure S2. Chloride ion concentration dependence of the fluorescence of the Hg(II) complex of MS1. (a) Fluorescence response of MS1 to Hg(II) in unbuffered aqueous solution. Emission spectrum of MS1 (circles), MS1 in the presence of 67 equiv of Hg(II) (squares), and the MS1:Hg(II) complex upon addition of KCl to a final concentration of 100 mM (diamonds). (b) Control. Emission spectrum of MS1 in water (circles) and of MS1 upon addition of KCl. [MS1] = 1 μM; λ_{ex} = 492 nm.

Figure S3. Nolan & Lippard

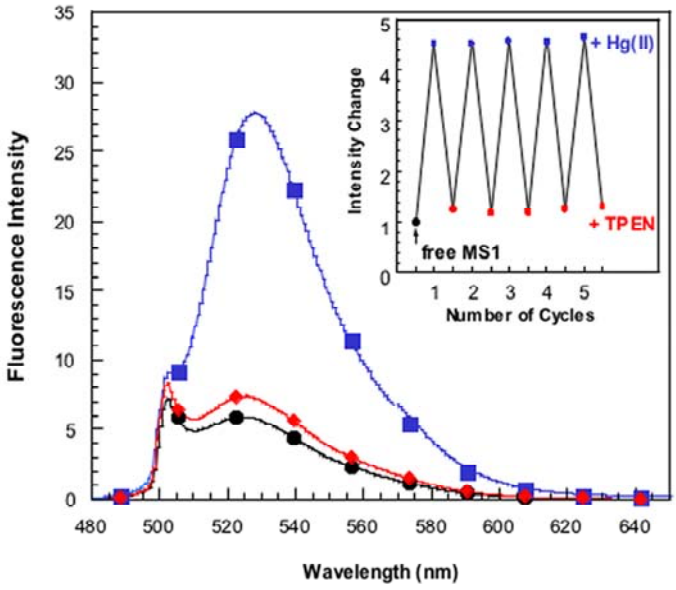

Figure S3. Reversibility of Hg(II) binding to MS1 upon addition of TPEN with excitation at 500 nm. Circles: free MS1, [MS1] = 1 μM; squares: fluorescence increase upon addition of 1 equiv Hg(II); diamonds: decrease in fluorescence resulting from addition of 1 equiv TPEN. Inset: normalized integrated emission versus cycle number showing the restoration and decrease of fluorescence upon addition of 1 equiv Hg(II) and 1 equiv TPEN, respectively, over the course of five cycles.