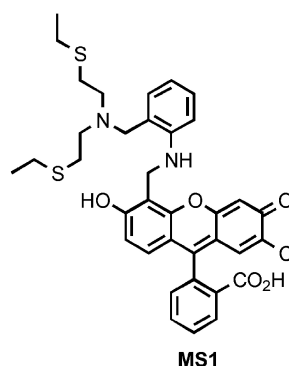
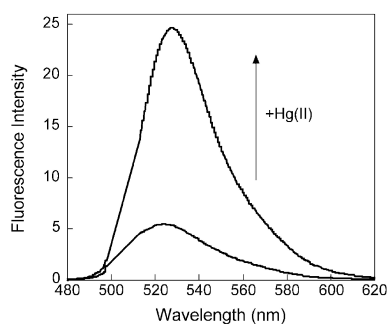


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A "Turn-On" Fluorescent Sensor for the Selective Detection of Mercuric Ion in Aqueous Media

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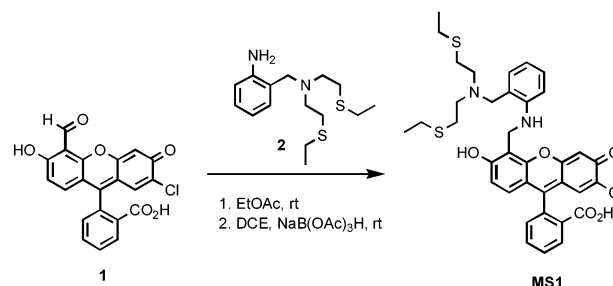
Heavy metal ion pollution poses severe risks for human health and the environment. Mercury contamination is widespread and occurs through a variety of natural and anthropogenic sources including oceanic and volcanic emission,¹ gold mining,² solid waste incineration, and the combustion of fossil fuels.³ Once introduced into the marine environment, bacteria convert inorganic mercury into methylmercury, which enters the food chain and accumulates in higher organisms, especially in large edible fish.^{1,3-7} Methylmercury is neurotoxic and has been implicated as a cause of prenatal brain damage,⁸⁻¹⁰ various cognitive and motion disorders,^{11,12} and Minamata disease.¹³

Our increased understanding of the deleterious effects of mercury exposure has sparked interest in the development of new tools for detecting Hg(II) in the environment. One major challenge involves creating Hg(II) sensors that function in water and are highly selective for Hg(II) against a background of competing analytes. Small synthetic molecules offer one approach to such probes. To date, a number of small-molecule Hg(II) detection methods have been examined and include colorimetric strategies,¹⁴⁻¹⁷ fluoroionophores,¹⁸⁻²⁷ and a dithioamide-functionalized lipid bilayer.²⁸ Most of these systems have limitations, which include interference from other metal ions, delayed response to Hg(II), and/or a lack of water solubility, requiring the use of organic or aqueous organic solvent mixtures. Although a fluorescent probe based on the indoaniline chromophore exhibiting selectivity for Hg(II) in water was recently described,²⁶ Hg(II) binding results in a decrease of quantum yield (ϕ) and brightness ($\phi\epsilon$).

Here, we report the synthesis and metal-binding properties of MS1 (Mercury Sensor 1), a water-soluble, turn-on fluorescein-based sensor that exhibits high selectivity and sensitivity for Hg(II). We selected fluorescein as the reporting group due to its superior brightness ($\phi \approx 1$, high ϵ) and water solubility. Since Hg(II) ion has a high affinity for soft donors such as sulfur, we incorporated a 3,9-dithia-6-azaundecane moiety²⁹ into an aniline-derived fluorescein-based ligand framework previously developed in our laboratory.³⁰ *N*-(2-Aminobenzyl)-3,9-dithia-6-azaundecane (**2**) was prepared in two steps starting from 3,9-dithia-6-azaundecane and commercially available 2-nitrobenzyl bromide. Condensation of 7'-chloro-4'-fluoresceincarboxaldehyde (**1**)³¹ with **2** in EtOAc, followed by reduction of the resulting imine using NaB(OAc)₃H in 1,2-dichloroethane and purification on silica gel (50:1 CHCl₃:MeOH), afforded MS1 as a magenta solid (Schemes 1 and S1, Supporting Information).

At pH 7 and 100 mM ionic strength (50 mM PIPES buffer, 100 mM KCl), and in the presence of EDTA to scavenge any adventitious metal ions, MS1 exhibits an emission maximum at 524 nm and a quantum yield of 0.04. The low quantum yield of the unbound sensor results from photoinduced electron transfer (PET) quenching of the fluorescein emission by the lone pair of the aniline nitrogen atom.³² This nitrogen atom has a pK_a of 7.1 (Figure S1), which indicates that the deprotonation equilibrium

Scheme 1



allows efficient PET quenching at neutral pH. Upon disruption of this quenching pathway by Hg(II) coordination, the emission maximum red-shifts slightly to 528 nm and the quantum yield increases ~2.75-fold to 0.11. The absorption spectrum exhibits a blue shift from 505 nm ($\epsilon = 61\,300\text{ M}^{-1}\text{ cm}^{-1}$) to 501 nm ($\epsilon = 73\,200\text{ M}^{-1}\text{ cm}^{-1}$) upon Hg(II) binding, resulting in a ~3.3-fold increase in brightness. A ~5-fold increase in integrated emission is observed upon addition of Hg(II) (Figure 1). The magnitude of this response depends on the chloride ion concentration (Figure S2, Supporting Information). Metal-binding titrations indicate that MS1 forms a 1:1 complex with Hg(II) in solution, which is responsible for the fluorescence enhancement, with an EC_{50} ³³ of 410 nM.

The fluorescence response of MS1 to various cations and its selectivity for Hg(II) are illustrated in Figure 2. The Hg(II) response of MS1 is unaffected in a background of environmentally relevant³⁴ alkali and alkaline earth metals including Li(I), Na(I), Rb(I), Mg(II), Ca(II), Sr(II), and Ba(II). The Group 12 metals Zn(II) and Cd(II), in addition to Cr(III) and Pb(II), do not inhibit the fluorescence response of MS1 to Hg(II). Of the first-row transition metal ions considered, only Cu(II) interferes with the Hg(II)-induced

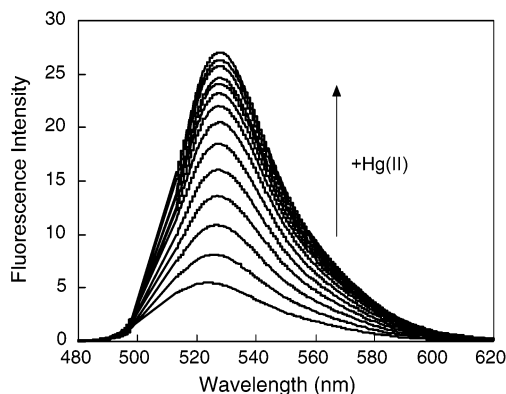


Figure 1. Fluorescence response of MS1 to addition of Hg(II) in water at pH 7 with 50 mM PIPES, 100 mM KCl buffer. [MS1] = 1 μM . Aliquots of 0.1 and 1 mM HgCl₂ were added to yield final Hg(II) concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.3, 1.5, and 3 μM . Excitation was at 500 nm.

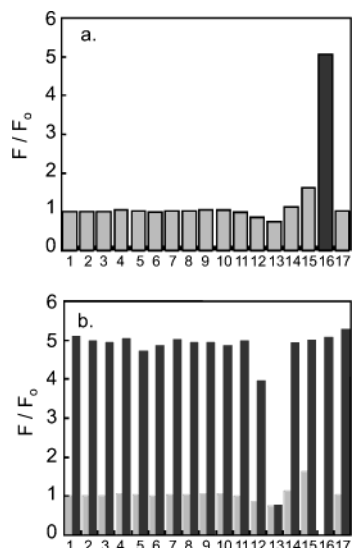


Figure 2. (a) Fluorescence response of MS1 to various cations in water at pH 7 (50 mM PIPES, 100 mM KCl buffer). The response is normalized with respect to the free dye (F_0). The bars represent the emission of MS1 in the presence of 67 equiv the cation of interest: 1, Li(I); 2, Na(I); 3, Rb(I); 4, Mg(II); 5, Ca(II); 6, Sr(II); 7, Ba(II); 8, Cr(III); 9, Mn(II); 10, Fe(II); 11, Co(II); 12, Ni(II); 13, Cu(II); 14, Zn(II); 15, Cd(II); 16, Hg(II); 17, Pb(II). (b) The selectivity of MS1 for Hg(II) in the presence of other cations. The light bars represent the emission of MS1 in the presence of 67 equiv of the cation of interest. The dark bars represent the change in integrated emission that occurs upon subsequent addition of 67 equiv of Hg(II) to solution containing MS1 and the cation of interest. The fluorescence of MS1 is also unaffected by millimolar concentrations of Li(I), Na(I), Rb(I), Mg(II), Ca(II), Sr(II), and Ba(II) (data not shown). [MS1] = 1 μ M; λ_{ex} = 500 nm. The emission was integrated from 510 to 650 nm.

fluorescence increase. This behavior is similar to that encountered with several multidentate thioether-containing ligands that exhibit selectivity for metal ions in the “copper triangle” of the periodic table.³⁵ MS1 binds Hg(II) reversibly. Addition of 1 equiv of the heavy metal ion chelator N,N',N'',N'' -tetra(2-picolyl)ethylenediamine (TPEN) to a stoichiometric mixture of MS1 and Hg(II) results in an immediate fluorescence decrease to within $\sim 20\%$ of the background value. This on/off behavior can be reversed by introduction of another equivalent of Hg(II), restoring the fluorescence attributed to Hg(II) complexation (Figure S3, Supporting Information).

The EPA standard for the maximum allowable level of inorganic Hg(II) in drinking water is 2 ppb.³ When MS1 is added to an aqueous solution (50 mM PIPES, 100 mM KCl, pH 7) containing 2 ppb of Hg(II), a fluorescence increase of $11.3 \pm 3.1\%$ is observed,³⁶ indicating that MS1 can detect environmentally relevant concentrations of Hg(II). A highly sensitive and selective fluorescence-based probe for Hg(II) that is water-soluble and gives a positive response upon analyte binding may be of some practical utility.

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Supporting Information Available: Figures S1 and S2, Scheme S1, and synthetic and experimental details (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Renzoni, A.; Zino, F.; Franchi, E. *Environ. Res.* **1998**, *77*, 68–72.
- (2) Malm, O. *Environ. Res.* **1998**, *77*, 73–78.
- (3) Mercury Update: Impact on Fish Advisories. EPA Fact Sheet EPA-823-F-01-011; EPA, Office of Water: Washington, DC, 2001.
- (4) Boening, D. W. *Chemosphere* **2000**, *40*, 1335–1351.
- (5) Nendatz, M.; Herbst, T.; Kussatz, C.; Gies, A. *Chemosphere* **1997**, *35*, 1875–1885.
- (6) Hardy, S.; Jones, P. J. *J. Chromatogr. A* **1997**, *791*, 333–338.
- (7) Harris, H. H.; Pickering, I. J.; George, G. N. *Science* **2003**, *301*, 1203.
- (8) McKeown-Eyssen, G. E.; Ruedy, J.; Neims, A. *Am. J. Epidemiol.* **1983**, *118*, 470–479.
- (9) Davidson, P. W.; Myers, G. J.; Cox, C.; Shamlaye, C. F.; Marsh, D. O.; Tanner, M. A.; Berlin, M.; Sloane-Reeves, J.; Cernichiaro, E.; Choisy, O.; Choi, A.; Clarkson, T. W. *Neurotoxicology* **1995**, *16*, 677–688.
- (10) Grandjean, P.; Weihe, P.; White, R. F.; Debes, F. *Environ. Res.* **1998**, *77*, 165–172.
- (11) Takeuchi, T.; Morikawa, N.; Matsumoto, H.; Shiraishi, Y. *Acta Neuropathol.* **1962**, *2*, 40–57.
- (12) Matsumoto, H.; Koya, G.; Takeuchi, T.; et al. *J. Neuropathol. Exp. Neurol.* **1965**, *24*, 563–574.
- (13) Harada, M. *Crit. Rev. Toxicol.* **1995**, *25*, 1–24.
- (14) Choi, M. J.; Kim, M. Y.; Chang, S.-K. *Chem. Commun.* **2001**, 1664–1665.
- (15) Brümmer, O.; La Clair, J. J.; Janda, K. D. *Org. Lett.* **1999**, *1*, 415–418.
- (16) Sancenón, F.; Martínez-Mañez, R.; Soto, J. *Chem. Commun.* **2001**, 2262–2263.
- (17) Sancenón, F.; Martínez-Mañez, R.; Soto, J. *Tetrahedron Lett.* **2001**, *42*, 4321–4323.
- (18) Prodi, L.; Bargossi, C.; Montalti, M.; Zaccaroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769–6770.
- (19) Rurack, K.; Resch-Genger, U.; Bricks, J. L.; Spieles, M. *Chem. Commun.* **2000**, 2103–2104.
- (20) Hennrich, G.; Sonnenschein, H.; Resch-Genger, U. *J. Am. Chem. Soc.* **1999**, *121*, 5073–5074.
- (21) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968–969.
- (22) Chae, M.-Y.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 9704–9705.
- (23) Yoon, J.; Ohler, N. E.; Vance, D. H.; Aumiller, W. D.; Czarnik, A. W. *Tetrahedron Lett.* **1997**, *38*, 3845–3848.
- (24) Winkler, J. D.; Bowen, C. M.; Michelet, V. *J. Am. Chem. Soc.* **1998**, *120*, 3237–3242.
- (25) Unterreitmaier, E.; Schuster, M. *Anal. Chim. Acta* **1995**, *309*, 339–344.
- (26) Descalzo, A. B.; Martínez-Mañez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 3418–3419.
- (27) Sakamoto, H.; Ishikawa, J.; Nakao, S.; Wada, H. *Chem. Commun.* **2000**, 2395–2396.
- (28) Sasaki, D. Y.; Padilla, B. E. *Chem. Commun.* **1998**, 1581–1582.
- (29) Tanaka, M.; Nakamura, M.; Ikeda, T.; Ikeda, K.; Ando, H.; Shibutani, Y.; Yajima, S.; Kimura, K. *J. Org. Chem.* **2001**, *66*, 7008–7012.
- (30) Burdette, S. C.; Frederickson, C. J.; Bu, W.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 1778–1787.
- (31) Nolan, E. M.; Burdette, S. C.; Harvey, J.; Hilderbrand, S. A.; Lippard, S. J. Unpublished results.
- (32) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302–308.
- (33) The concentration of Hg(II) required to achieve 50% of the total increase in integrated emission and [MS1] = 1 μ M.
- (34) Drever, J. I. *The Geochemistry of Natural Waters: Surface and Ground-water Environments*, 3rd ed.; Prentice Hall: Upper Saddle River, NJ, 1997.
- (35) Cooper, T. H.; Mayer, M. J.; Leung, K.-H.; Ochrymowycz, L. A.; Rorabacher, D. B. *Inorg. Chem.* **1992**, *31*, 3796–3804 and references therein.
- (36) Average of 28 independent trials with a range of 9.1–15.9%; [MS1] = 500 nM.

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