Mercury Detection

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A Bright and Specific Fluorescent Sensor for Mercury in Water, Cells, and Tissue**

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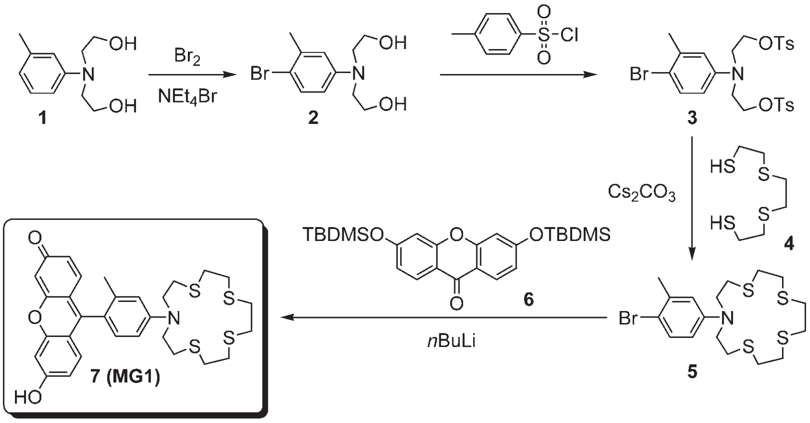
Mercury pollution pervades the globe and remains a danger to human health and the environment. The US Environmental Protection Agency (EPA)'s estimates of annual total global mercury emissions from all sources—both natural and human-generated—reach nearly 7500 tons per year.[1] Atmospheric oxidation of mercury vapor to water-soluble Hg^{2+} ions and its subsequent metabolism by aquatic microbes produces methylmercury, a potent neurotoxin linked to many cognitive and motion disorders.[2] Relevant cellular targets of methylmercury include glutathione and other thiol-containing antioxidants, but mechanistic details of mercury toxicity at the molecular level are still insufficiently understood.

Fluorescent small-molecule sensors[3] and dosimeters,[4] materials,[5] and biomolecules[6] offer an attractive approach to tracing neurotoxic mercury. However, despite widespread interest and recent advances in this area, there are only a few examples of fluorescent probes for successful detection of Hg^{2+} ions in biological samples,[7] as creating new synthetic molecules that meet the criteria of appropriate selectivity, water solubility, and optical sensitivity in natural settings remains an outstanding challenge. In particular, a common limitation for heavy-metal detection in the environment is the low quantum efficiency of metal-bound dyes in water compared to that in organic or in mixed aqueous-organic solvents. We now report the synthesis and applications of Mercury Green 1 (MG1), a new Hg^{2+}-ion-specific fluorescent chemosensor that has the highest quantum efficiency to date in water at physiological pH ($\Phi = 0.72$), excellent selectivity for Hg^{2+} ions over relevant competing metal ions, a 44-fold turn-on response, and sensitivity to ppm–ppb levels of Hg^{2+} in complex aqueous solutions, cells, and tissue. Furthermore, we show that because of these features, MG1 is capable of tracking changes in mercury levels within living cells and distinguishing safe and toxic amounts of mercury in edible fish.

In a previous study we described Mercuryfluor-1 (MF1), a fluorescein-like sensor for selective detection of aqueous Hg^{2+} ions to ppm levels.[7b] This initial design is limited by the relatively modest quantum yield for MF1 in its Hg^{2+}-ion-bound form ($\Phi = 0.16$), which is in part due to the propensity of heavy metals, such as mercury, to quench fluorescent dyes in aqueous media. Seeking to maintain high Hg^{2+}-ion specificity while increasing optical brightness values in water, we reasoned that introducing an *ortho*-methyl group on the phenylene linker would restrict its rotation with respect to the xanthene reporter unit and potentially enhance quantum efficiencies, an approach inspired by Tokyo Green reporters of β-galactosidase activity.[8] Based on this restricted rotation design, the new Hg^{2+}-ion-responsive dye MG1 was prepared as shown in Scheme 1.



Scheme 1. Synthesis of MG1. Ts = tosyl, TBDMS = *tert*-butyldimethylsilyl.

Spectroscopic measurements for MG1 were performed in aqueous solution buffered to pH 7 (20 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, HEPES) and they establish the marked benefits of restricting rotation between receptor and reporter units. Figure 1a displays the fluorescence turn-on response of the sensor to Hg^{2+} ions. Metal-free (apo) MG1 has a single visible absorption maximum at 485 nm ($\varepsilon = 2.9 \times 10^4\,\text{M}^{-1}\,\text{cm}^{-1}$) and is weakly fluorescent ($\lambda_{em} = 514$ nm, $\Phi = 0.02$). Addition of Hg^{2+} ions triggers a

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

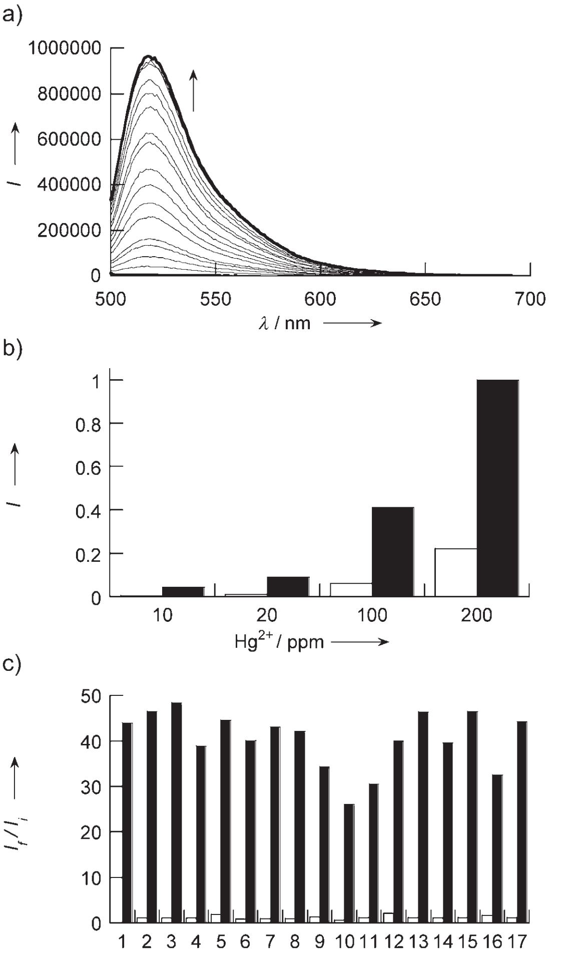

Figure 1. a) Fluorescence response of 1 μм MG1 to Hg^{2+} ions in aqueous solution. Spectra shown are for Hg^{2+}-ion concentrations of 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.8, 2.0, 3.2, 4.0, and 6.7 μм. The arrow indicates change with increasing Hg^{2+}-ion concentration. Spectra were acquired in 20 mм HEPES (pH 7) with excitation at 480 nm. b) Relative responses of MG1 (black bars) and its unrestricted analogue MF1 (white bars) to ppb levels of Hg^{2+} ions. Data were acquired under similar conditions to obtain an accurate reflection of the relative brightness of the dyes in response to Hg^{2+} ions (1 μм dye, 20 mм HEPES, pH 7). Excitation was provided at 495 nm, and the emission was integrated over 505 to 700 nm. c) Fluorescence responses of MG1 to various metal ions. Bars represent the final integrated fluorescence response (I_f) over the initial integrated emission (I_i). Initial spectra were acquired in 20 mм HEPES, pH 7. White bars represent the addition of an excess of the appropriate metal ion (5 mм for Li^+, Na^+, K^+, Mg^{2+}, and Ca^{2+}, 300 μм for Fe^{2+}, Fe^{3+}, and Cu^+, and 500 μм for all other cations) to a 1 μм solution of MG1. Black bars represent the addition of 5 or 50 μм Hg^{2+} to solutions containing MG1. Excitation was provided at 495 nm, and the emission was integrated over 505 to 700 nm. 1) Hg^{2+}; 2) Li^+; 3) Na^+; 4) K^+; 5) Mg^{2+}; 6) Ca^{2+}; 7) Sr^{2+}; 8) Mn^{2+}; 9) Fe^{2+}; 10) Fe^{3+}; 11) Co^{2+}; 12) Ni^{2+}; 13) Cu^+; 14) Cu^{2+}; 15) Zn^{2+}; 16) Cd^{2+}; 17) Pb^{2+}.

44-fold increase in integrated emission for MG1 with slight shifts in excitation (494 nm, $\varepsilon = 4.8\times10^4\,\text{M}^{-1}\text{cm}^{-1}$) and emission (513 nm) maxima. The quantum yield $\Phi = 0.72$ for Hg^{2+}-bound MG1 is the highest reported value to date for a Hg^{2+}-ion sensor in water. For comparison, the unrestricted analogue MF1 has a Hg^{2+}-bound quantum yield of 0.16. Figure 1 b gives the relative responses of MG1 and MF1 to various aqueous Hg^{2+}-ion levels in the ppm–ppb range and shows that restricting rotation between reporter and receptor units dramatically increases the absolute change in fluorescence turn-on upon Hg^{2+}-ion detection. For example, at 10 ppb Hg^{2+} ion, MG1 is over 11 times more responsive than its unrestricted congener MF1. Owing to its improved optical brightness, MG1 is sensitive enough to detect environmentally relevant concentrations of Hg^{2+} ions in aqueous solution. Addition of 2 ppb of Hg^{2+} ions, the maximum US EPA limit for allowable levels of Hg^{2+} ions in drinking water, to a 1-μм solution of MG1 affords a (72 ± 10) % emission increase. In contrast, the unrestrained analogue MF1 is not capable of detecting 2 ppb levels of Hg^{2+} ions under similar conditions. Finally, binding studies, using the method of continuous variations, point to a 1:1 Hg^{2+}/MG1 complex being responsible for the observed fluorescence turn-on with $K_d = (895 \pm 85)$ nм.

Because of its thioether-rich receptor,[9] MG1 retains excellent selectivity for Hg^{2+} ions in the presence of a range of competing metal ions found in environmental and biological settings (Figure 1 c). MG1 exhibits excellent selectivity for Hg^{2+} ions over alkali and alkaline-earth cations, including millimolar concentrations of Li^+, Na^+, K^+, Mg^{2+}, and Ca^{2+}, first-row transition-metal ions Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^+, and Cu^{2+} added at 300- to 500-fold excess, Group 12 metal ions Zn^{2+} and Cd^{2+}, and the common heavy-metal contaminant Pb^{2+}. Of note are the observed selectivities for Hg^{2+} over Cu^{2+} and Pb^{2+}, which are major competitors in real-life field samples.

We explored opportunities for MG1 in environmental and biological applications. Initial studies tested the ability of this bright probe to track changes in mercury levels in living cells. Live-cell imaging experiments utilized the acetoxymethyl ester protected form of MG1 (MG1-AM) to enhance membrane permeability. Confocal microscopy images of live HEK 293T cells loaded with 1 μм of MG1-AM for up to 60 min at 37 °C show relatively low levels of background intracellular fluorescence (Figure 2 a), consistent with the photoinduced-electron-transfer-quenched form of the apo dye. In contrast, MG1-labeled cells exposed to 4 ppm of Hg^{2+} for 30 min at 37 °C show increased intracellular fluorescence compared to cells not treated with mercuric salts (Figure 2 b). Treatment with the heavy-metal chelator TPEN (*N*,*N*,*N*′,*N*′-tetrakis(2-picolinyl)ethylenediamine) for 1 min at 25 °C reduces the observed fluorescence enhancements (Figure 2 c). Brightfield measurements and Hoescht-33342 staining confirm that the cells are viable throughout the imaging studies (Figure 2 d and Supporting Information), and control experiments on cells without dye give no fluorescence over background levels. These data establish that MG1 can respond reversibly to increases or decreases in intracellular mercury levels within living cells at ppm levels.

We also investigated MG1 for Hg^{2+}-ion detection in complex tissue. Owing to widespread interest in determining mercury contamination in edible fish, we applied MG1 to analyze fish mercury levels. Experiments utilized a series of fish collected from freshwater sources in greater Northern California. Small tissue samples (< 100 mg) with mercury

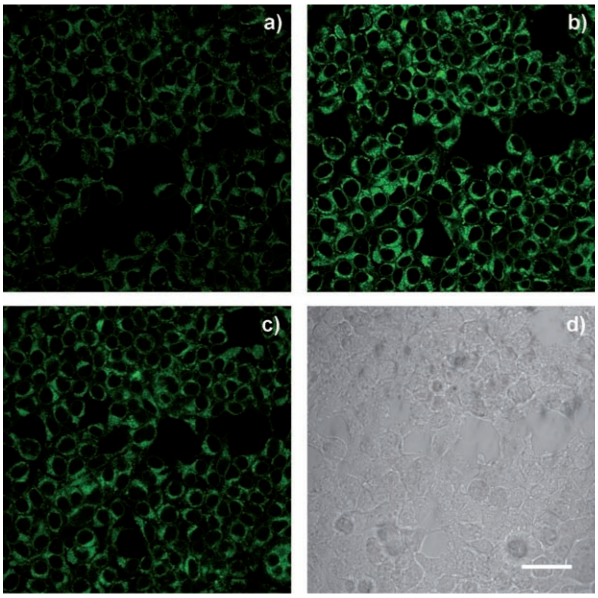

Figure 2. Confocal fluorescence imaging of Hg^{2+}-ion levels using MG1 in live HEK 293T cells. a) Fluorescence image of cells incubated with 1 μM MG1-AM for 60 min at 37 °C. b) Fluorescence image of MG1-loaded cells exposed to 4 ppm of $HgCl_2$ for 30 min at 37 °C. c) Fluorescence image of cells in panel (b) treated with 2 mM of the competing heavy-metal chelator TPEN for an additional 1 min at 25 °C. d) Bright-field image of cells in panel (c), confirming their viability. Scale bar = 40 μm.

concentrations ranging from 0.03 to 13 ppm as determined by atomic absorption spectrometry (AAS) were digested in nitric acid under microwave irradiation and then made basic, brought to pH 7 in 20-mM HEPES buffer, and assayed with MG1. Figure 3 shows a linear relationship between fluorescence response and mercury content in this series of fish, establishing that MG1 can distinguish safe and toxic levels of mercury in edible fish samples according to the 0.55 ppm US EPA standard by an emission response.[10]

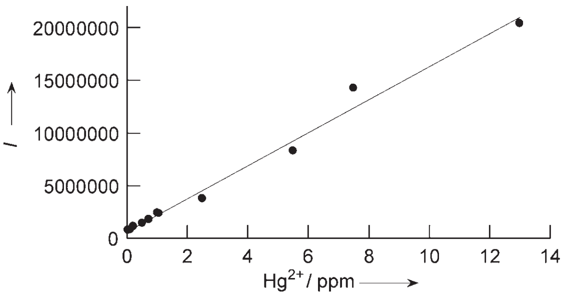

Figure 3. Fluorometric detection of Hg^{2+} ions in fish with MG1: North Thermolito Afterbay (carp, 0.03 ppm Hg), Lime Saddle Marina (bluegill, 0.10 ppm Hg), North Thermolito Afterbay (red ear sunfish, 0.21 ppm Hg), Lake Oroville Upper MF (spotted bass, 0.52 ppm Hg), Lake Oroville Bidwell Arm (spotted bass, 0.72 ppm Hg), Lake Oroville SF Arm, McCabe Cove (large mouth bass, 1.06 ppm Hg), Calero Reservoir (bass, 1.0 ppm Hg), Lake Almaden (bass, 2.5 ppm Hg), Almaden Reservoir (bass, 5.5 ppm Hg), Guadelupe Reservoir (bass, 7.5 ppm Hg), Guadelupe Reservoir (bass, 13 ppm Hg). Excitation was provided at 485 nm, and the emission was integrated over 495 to 700 nm.

In conclusion, we have described the synthesis and properties of MG1, a bright and specific fluorescent sensor for Hg^{2+} ions in water, cells, and tissue. MG1 features the highest quantum efficiency to date for a Hg^{2+}-bound sensor in water ($\Phi = 0.72$), excellent Hg^{2+}-ion specificity, a 44-fold turn-on response, and sensitivity to environmentally and biologically relevant mercury levels in the ppm to ppb range in complex natural samples. Additional experiments establish the utility of this chemosensor for tracking mercuric ions in living cells as well as assaying safe and toxic levels of Hg^{2+} ions in fish according to US EPA standards. Because a common limitation of applying small-molecule fluorophores to natural samples in the laboratory and the field is low optical brightness in aqueous media, we anticipate that this restricted-rotation approach should find utility in the future design of metal-ion sensors with high quantum efficiencies in water for a variety of chemical and biological applications.

Experimental Section

Experimental Details are provided in the Supporting Information

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