

Highly sensitive and selective fluorescent chemosensors for Hg(II) in an aqueous environment based on carbamodithioate

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Received 22 May 2006; revised 29 August 2006; accepted 31 August 2006

Available online 25 September 2006

Abstract—Two highly sensitive (detection limits ~ 20 nM) and selective (selectivity >30) fluorescent chemosensors were developed for detecting Hg^{2+} in a fully aqueous environment by using the rarely-studied carbamodithioate to create an Hg^{2+} binding site. © 2006 Elsevier Ltd. All rights reserved.

Mercury is a widespread and highly toxic pollutant that poses a significant threat to the environment. Despite the recent reduction of its industrial use due to more stringent regulations, mercury contamination still occurs through a variety of natural and anthropogenic sources including oceanic and volcanic emission, gasoline combustion and solid waste incineration.¹ Once introduced into the aqueous environment, inorganic mercury (mainly in the form of Hg^{2+}) may be converted by bacteria into methylmercury, which then accumulates through the food chains in the tissues of higher organisms including edible fish.² Subsequent ingestion of neurotoxic methylmercury by humans can cause various mercury-pollution-related diseases symptomized by serious sensory, motor and cognitive disorders.³

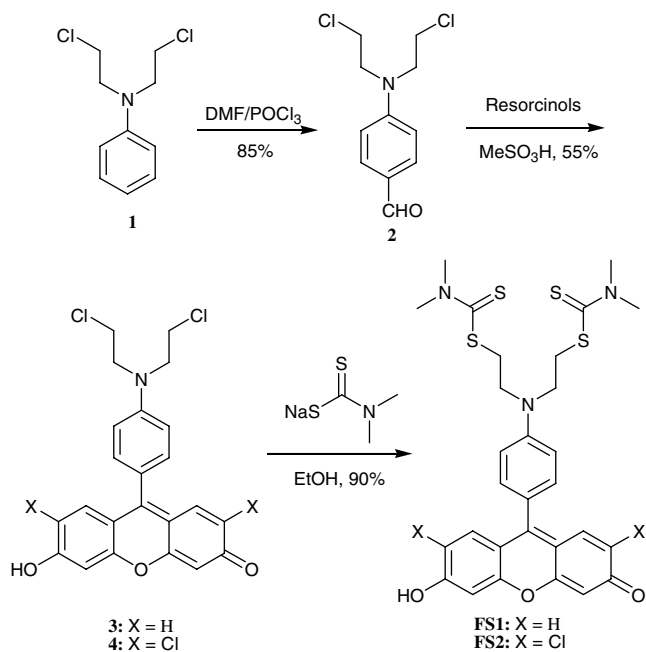
To monitor and prevent mercury pollution, considerable efforts have been made worldwide to develop efficient analytic methods for the determination of mercury. In this regard, a number of redox, colorimetric and fluorescent Hg^{2+} sensors have been devised by using proteins,⁴ nucleic acids⁵ and several types of small molecules⁶ as Hg^{2+} receptors. Although some degree of success has been achieved, two major challenges remain to be overcome before any Hg^{2+} sensor can be utilized for real applications. First, the sensor must be sensitive enough to detect Hg^{2+} at ppb concentrations easily. (Note: the maximum level of Hg in drinking water permitted by the United States Environmental Protection Agency is 2 ppb = 10 nM.) Second, the sensor must be very selec-

tive so that interference from other competing metal ions (in particular, Cu^{2+} , Cd^{2+} and Pb^{2+}) is negligible.⁷

Among the many Hg^{2+} sensors that have been reported to date, only the very recent one (MF-1) developed by Wong and Chang has successfully met both of the above two challenges.⁸ In this sensor an N1S4 crown ether was utilized as the Hg^{2+} receptor. The detection limit of MF-1 was found to be about 60 nM. Using MF-1, Wong and Chang have successfully measured the mercury levels in fish within the safe edible limit. Herein, we wish to report that by using carbamodithioate to build the Hg^{2+} receptor, we have successfully obtained novel fluorescent sensors for Hg^{2+} with a better sensitivity than MF-1. Moreover, due to their easier syntheses, our new Hg^{2+} sensors are probably more economically competitive than MF-1.

The structures of the two new Hg^{2+} sensors (**FS1** and **FS2**) are shown in **Scheme 1**. In designing these sensors, we focused on using an acyclic receptor for the metal ion because the synthesis of a cyclic structure is usually expensive. After many unsuccessful attempts, we were very pleased to find out that carbamodithioate had an exceptionally strong affinity towards Hg^{2+} . A further literature search showed that although carbamodithioate had rarely been utilized for metal binding purposes, it was once used by Roundhill and co-workers to create upper-rim-substituted calix[4]arenes as selective extractants.⁹ Inspired by these findings, we decided to attach two carbamodithioate moieties to a fluorescein reporter that has been reported to have desirable optical properties and water solubility.¹⁰

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Scheme 1. The synthesis of sensor **FS1** and **FS2**.

The syntheses of sensor **FS1** and **FS2** started from readily available *N,N*-bis-(chloroethyl)aniline (see **Scheme 1**), which was formylated at the 4-position using the standard DMF/ POCl_3 procedure (yield = 85%). The resulting aldehyde **2** was then reacted with resorcinol or 4-chlororesorcinol in MeSO_3H to afford the fluorescein derivative **3** or **4** (yield = 55%). Finally, **3** and **4** were reacted with sodium dimethylcarbamodithioate in ethanol to give the target products in good yields (90%). Thus, sensors **FS1** and **FS2** were successfully synthesized through three simple steps with an overall yield of about 42%. Preliminary tests indicated that **FS1** and **FS2** had sufficient water solubility and that they were hydrolytically stable in water under common conditions.

Further experiments showed that the absorption bands of **FS1** and **FS2** were centered at 488 nm and 495 nm, respectively. The maximum emission wavelengths of **FS1** and **FS2** were found to be 515 nm and 530 nm. The fluorescence quantum yields of free **FS1** and **FS2** were 0.0039 and 0.0045 under physiological conditions (pH 7.2, 50 mM KCl).¹¹ These low quantum yields were probably caused by the intramolecular photo-induced electron transfer from the anilinic nitrogen atom to the fluorophore.¹² Upon addition of Hg^{2+} , the fluorescence intensities of the two sensors were found to increase dramatically by about 90-fold, without any detectable time lag. The corresponding quantum yields rose to 0.293 and 0.352, respectively (See **Fig. 1**). The mechanism for the fluorescence enhancement was presumably that the coordination to Hg^{2+} inactivated the anilinic nitrogen atom in the electron transfer.

The Hill plot analysis revealed that the maximum fluorescence response could be obtained at a 1:1 ratio between the sensor and Hg^{2+} . This suggested that both **FS1** and **FS2** should form a 1:1 complex with Hg^{2+} . The dissociation constants, K_d , of the Hg^{2+} complexes

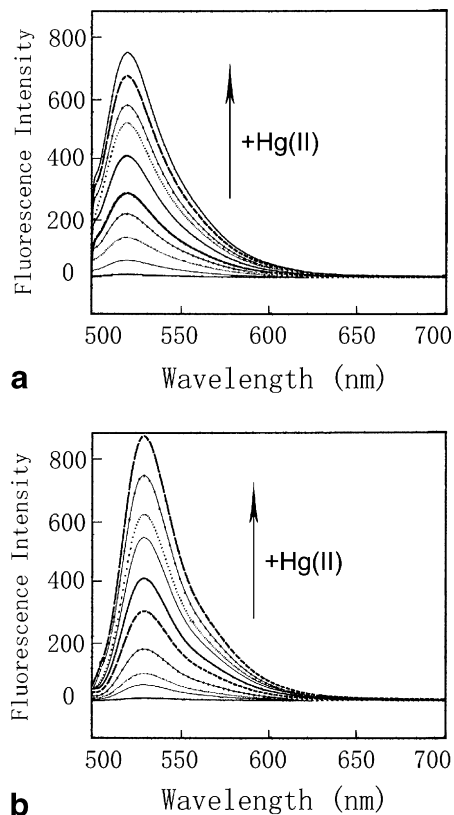


Figure 1. Fluorescence responses of **FS1** (a) and **FS2** (b) upon the addition of Hg^{2+} in water (experimental conditions: 10 μM sensor, 50 mM HEPES buffer, 50 mM KCl, pH = 7.2, 0–10 μM Hg^{2+} , excitation wavelength: 488 nm (**FS1**), 505 nm (**FS2**)).

were determined to be 2.6×10^{-9} M and 1.1×10^{-9} M for **FS1** and **FS2**, respectively. Moreover, the binding event was found reversible, because when 1 equiv of a competing chelator, *N,N,N',N'*-tetra-(2-picolyl)-ethylenediamine, was added to a solution of sensor and Hg^{2+} (1:1), the fluorescence intensity of the solution decreased to 8.7% (**FS1**) or 6.1% (**FS2**) of the original value (see **Supplementary data**). Due to this property, **FS1** and **FS2** represent reusable fluorescence sensors for Hg^{2+} . Finally, the detection limits of the two new sensors for Hg^{2+} in water were found to be 20 ± 5 nM (when the lowest fluorescence increase equals to three-fold of the instrument noise). It is worth mentioning that the detection limit of another promising Hg^{2+} sensor (MF-1) was recently determined to be 60 nM.⁸ Compared to MF-1, **FS1** and **FS2** exhibited even lower detection limits and, therefore, might find more success in laboratory and field applications.

Having confirmed that the new compounds were instant, reusable and highly sensitive fluorescent sensors for Hg^{2+} , we next paid attention to the selectivity of **FS1** and **FS2**. This is an important issue for the success of a mercury chemosensor, because many other heavy metal ions (in particular, Cu^{2+} , Cd^{2+} and Pb^{2+}) often coexist with Hg^{2+} in the environment. Thus we measured the fluorescence responses of **FS1** and **FS2** to other commonly encountered metal cations including Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Hg^{2+} , Fe^{2+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Zn^{2+} , Ag^+ , Cd^{2+} , Ni^{2+} , Pb^{2+} and Al^{3+} (See **Fig. 2**). It was

found that **FS1** and **FS2** showed almost zero fluorescence enhancement in the presence of most of these metal ions, except for Cu^{2+} , Ag^{+} and Cd^{2+} (Note: Cd^{2+} showed the strongest interference). Nonetheless, comparing the fluorescence enhancements of Hg^{2+} (90-fold) and Cd^{2+} (3-fold), we concluded that the selectivity of **FS1** and **FS2** was higher than 30 favouring Hg^{2+} over all the other common metal ions (See Fig. 3).

With the new Hg^{2+} sensors in hand, we next decided to test their effectiveness in solving a real problem, namely, how to rapidly monitor Hg^{2+} concentrations in drinking water without using expensive instruments. Towards this end we collected water from four different sources: (1) Chaohu lake, (2) tap water in Hefei city, (3) waste water from our chemistry department, and (4) waste water from Hefei Steel Company. By using the standard atomic absorption/emission spectroscopy method we measured their Hg^{2+} concentrations to be 0.3, 0.3, 0.9 and 1.6 nM, respectively.¹³ In comparison, by using our sensors on the samples that were concentrated by 100 times (using a rotavapor), we obtained values of 0.5, 0.8, 2.6 and 2.4 nM, respectively. It is clear that the values measured by the new sensors are in modest agreement with the authoritative data. A possible reason for the inaccuracy is that other interfering metal ions may exist in much higher concentrations than Hg^{2+} , so that the present selectivity is still not sufficient.

To summarize, we have developed two new fluorescent sensors that are highly sensitive and selective to Hg^{2+}

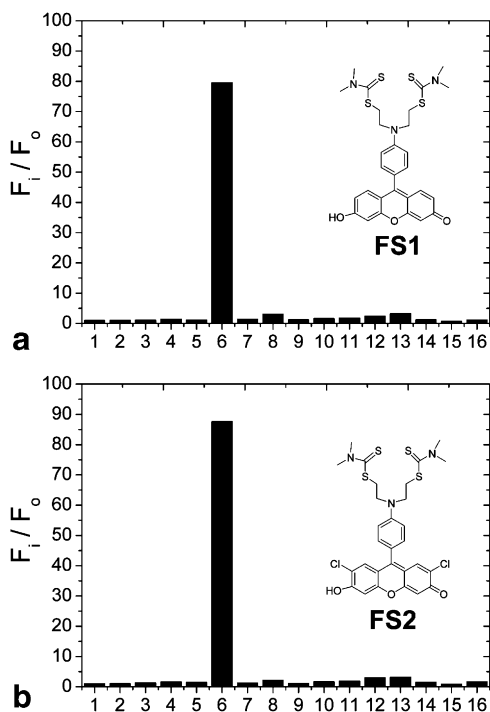


Figure 2. The fluorescence responses of **FS1** (a) and **FS2** (b) to different metal ions (experimental conditions: 5 μM sensor, 5 μM metal cation, 50 mM HEPES buffer, 50 mM KCl, pH = 7.2). The bars represent the fluorescence intensities (F_i) over the fluorescence intensity of the free sensor (F_0). 1. Sensor alone; 2. Na^+ ; 3. Ca^{2+} ; 4. Mg^{2+} ; 5. Ba^{2+} ; 6. Hg^{2+} ; 7. Fe^{2+} ; 8. Cu^{2+} ; 9. Mn^{2+} ; 10. Co^{2+} ; 11. Zn^{2+} ; 12. Ag^+ ; 13. Cd^{2+} ; 14. Ni^{2+} ; 15. Pb^{2+} ; 16. Al^{3+} .

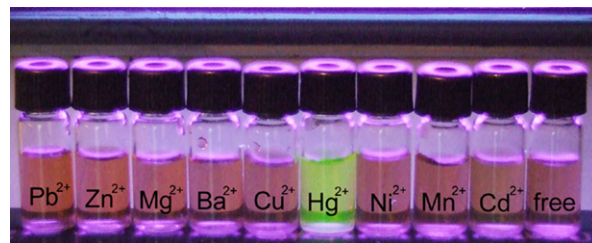


Figure 3. The fluorescence emissions of **FS2** (10 μM) in the presence of different heavy metal ions (10 μM) in water. The samples were excited at 365 nm using the UV lamp.

in fully aqueous environments. A key novelty of the work is the use of the rarely-known and rarely-utilized carbamodithioate to create the metal ion receptor that has a high affinity towards Hg^{2+} . The detection limit of the new sensors is 20 nM (or 4 ppb), which are lower than the best fluorescent Hg^{2+} sensor (i.e., MF-1) reported previously. Furthermore, the selectivity of the new sensors for Hg^{2+} over other metal ions is higher than 30. Preliminary field studies on the applications of the new sensors to the determination of Hg^{2+} in the environmental water samples showed some modest success. Thus, the two new sensors reported here add novel interesting tools to the arsenal for environmental and biological Hg^{2+} detection and imaging. Further studies about how to improve the sensitivity and selectivity of carbamodithioate-based sensors for Hg^{2+} and other thiophilic toxic metals (e.g., Cd^{2+} and Pb^{2+}) are now undergoing in our laboratory. It is also our hope that more attention could be paid to carbamodithioate and related structures in the future studies on the molecular recognition of heavy metals.¹⁴

Acknowledgments

This research was supported by the NSFC (Nos. 20332020 and 20472079).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.08.127.

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