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# Visible Near-Infrared Chemosensor for Mercury Ion

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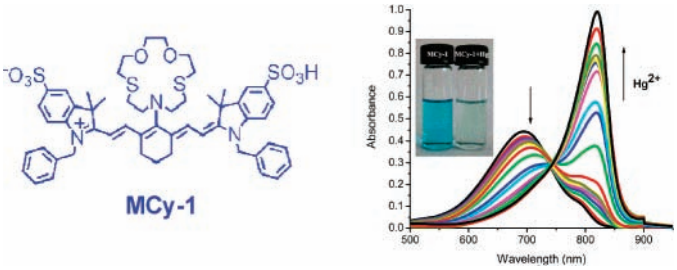
## ABSTRACT




**A visible near-infrared chemosensor (MCy-1) for mercury ions was successfully devised and characterized. A large red-shift (122 nm) of the absorption maximum of MCy-1 was observed. An important feature for the new chemosensor is its high selectivity towards mercury ions over the other competitive species, making the "naked-eye" detection of mercury ions possible.**

The development of selective chemosensors for the detection of transition- and heavy-metal ions draws particular interest, as these ions play important roles in living systems and have an extremely toxic impact on the environment.[1] Among them, mercury(II) ($Hg^{2+}$) is considered as one of the most toxic cations for the environment because it is widely distributed in air, water, and soil.[2] Mercury can accumulate in the human body and affects a wide variety of diseases even in a low concentration, such as digestive, kidney, and especially neurological diseases.[3] Despites their toxicity, mercury and mercuric salts are widely used in industrial processes, and a high percentage of mercury contamination can be attributed to anthropogenic sources. Recently, considerable efforts have been made to develop a colorimetric or fluorescent molecular probe for mercury ions.[4] Several chemosensors based on tricarbocyanine dyes have been reported.[5] However, most of the them are fluorometric sensors and the fluorescent

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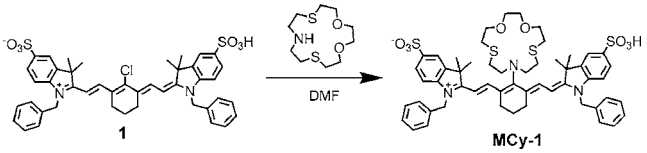
signals must be detected by spectroscopy or under UV light. Compared to fluorometric sensors, colorimetric sensors have attracted much attention for allowing so-called "naked-eye" detection in a straightforward and inexpensive manner, offering qualitative and quantitative information without using expensive equipment. Nagano et al. have reported a zinc ion probe based on tricarbocyanine dyes, which showed a 44 nm red-shift of the absorption maximum.[6] This assay makes the detection easier and more convenient.

As one of the important kinds of near-infrared (NIR) dyes, heptamethine cyanine dyes[7] have been widely used in various fields and have been employed as fluorescent labels in fluorescence imaging studies of biological mechanisms. As we know, the NIR region offers several advantages over the visible spectral range: (a) it is poorly absorbed by biomolecules, so it can penetrate deeply into tissues; (b) there is also less auto-fluorescence in this region, and so the characteristics of the NIR dyes are favorable for in vivo imaging;[8] and (c) there is an intense interest on the application of NIR probes to detect metal cations and biological compounds.[9]

Our work aims to design and construct a new class of NIR probes with colorimetric assay to specifically detect the presence of $Hg^{2+}$ over a wide range of other interfering cations. To achieve this goal, we report here the design and synthesis of a novel dye containg dithia-dioxa-monoaza crown ether moiety[10] (MCy-1) that can perform "naked-eye" detection of $Hg^{2+}$ ion in the NIR region.

The synthetic route of MCy-1 is shown in Scheme 1.

**Scheme 1.** Synthesis of Mcy-1




For designing a probe with better photostability, we chose tricarbocyanine dyes with benzyl groups on the nitrogen atoms of 3H-indo rings. The rigid chlorocyclohexenyl ring in the methine chain also plays an important role in maintaining the dyes' stability.[7a] To obtain an optimum response towards $Hg^{2+}$, we avoided the use of polythia or polyaza crowns known to bind most thio- or aminophilic metal ions. The vinyl chlorine on the cyclohexane bridgehead of compound 1 is reactive and can be replaced by a crown ether ligand which is a very strong nucleophile.

The ionophoric properties of MCy-1 were investigated by UV−vis and fluorescent measurements.

Figure 1a shows the absorption spectral changes of MCy-1 as a function of the $Hg^{2+}$ concentration in methanol at room

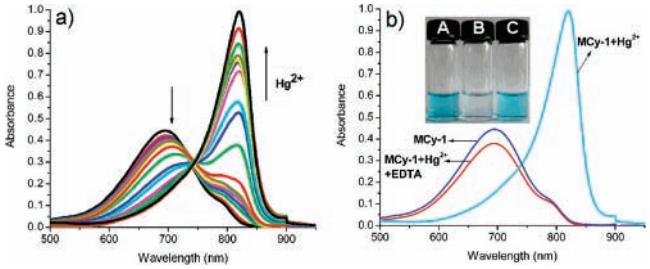



**Figure 1.** (a) Changes in the UV−vis spectra of MCy-1 (5.16 $\mu$M in methanol) upon titration by $Hg(ClO_4)_2$ from 12.4 to 60 $\mu$M. (b) Changes in the UV−vis spectra of MCy-1 (5.16 $\mu$M in methanol) upon addition of mercury ions and subsequent of excess EDTA. Inset: color change of MCy-1 in the visible region. (A) MCy-1 solution in methanol; (B) and (A) + $Hg^{2+}$; (C) and (B) + excess of EDTA.

temperature. The UV−vis spectrum of MCy-1 in methanol is characterized by a very intense band centered at 695 nm ($\epsilon$ = 86 000 $M^{-1}\cdot cm^{-1}$), which is responsible for the blue color of the solution. The absorption maximum of MCy-1 has about a 88 nm blue-shift in comparison to that of the parent dye 1.[11] This blue-shift was assigned to an efficient excited-state intramolecular charge transfer (ICT)[11] process from the donor nitrogen atom on the dithia-dioxa-monoaza macrocycles to the acceptor tricarbocyanine group. The absorption at 695 nm decreased sharply with the gradual addition of $Hg^{2+}$ to the solution of MCy-1. At the same time a new band centered at 817 nm ($\epsilon$ = 190 000 $M^{-1}\cdot cm^{-1}$) increased prominently with one isosbestic point at 740 nm. Such a large red-shift (122 nm) makes the color of the solution change from blue to almost colorless, and subsequent addition of an excess of EDTA resulted in recovery of the original color (Figure 1b).

According to the linear Benesi−Hildebrand expression,[12] the measured absorbance $[1/(A - A_0)]$ at 695 nm varied as a function of $1/[Hg^{2+}]$ in a linear relationship ($R$ = 0.99639),

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indicating the 1:1 stoichiometry between the $Hg^{2+}$ ion and MCy-1 (Figure 2). To confirm the stoichiometry between the $Hg^{2+}$ ion and MCy-1, electrospray ionization mass

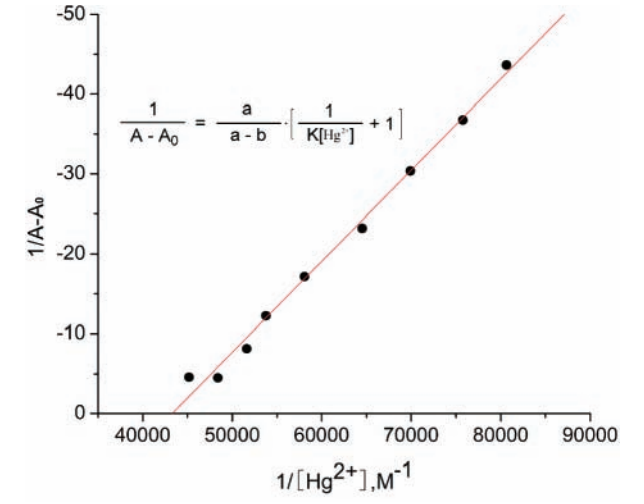


**Figure 2.** Benesi−Hilderbrand plot of MCy-1 with $Hg^{2+}$.

spectrometry (ESI-MS) spectra analysis was conducted (see Supporting Information). Mass peaks at *m*/*z* 1010.3 and 1210.2 corresponding to $[MCy\text{-}1 + H]^+$ and $[MCy\text{-}1 + Hg - H]^+$ are clearly observed, which gave solid evidence for the formation of a 1:1 complex. On the basis of 1:1 stoichiometry and UV−vis titration data in Figure 1, the association constant of MCy-1 with $Hg^{2+}$ ion in MeOH was found to be $4.335 \times 10^4$ $M^{-1}$ (Figure 2).

From the absorption spectra of MCy-1/$Hg^{2+}$, we can see that not only was the absorption band at 695 nm red-shifted but also the developed new band at 817 nm had a larger molar absorption coefficient than the original one. A possible reason is that the coordination of the $Hg^{2+}$ to the ligand reduces the electron-donating ability of the nitrogen atom at the dithia-dioxa-aza macrocycle which was linked to the tricarbocyanine core; thus, the ICT process is not possible and the blue-shift in absorption spectra is suppressed. In other words, the red-shift in absorption spectra is observed upon $Hg^{2+}$ binding. More importantly, the $Hg^{2+}$ sensing and the concomitant absorption changes were clearly visible to the naked eye, as can be seen in the photograph, where the blue solution of MCy-1 became almost colorless upon titration with $Hg^{2+}$ ions. $^1H$ NMR studies provide further evidence for the interaction between MCy-1 and $Hg^{2+}$. Upon the addition of 10 equiv of $Hg^{2+}$, chemical shift of protons on the crown ether especially those near the sulfur atoms broadened and shifted downfield as shown in Figure 3.

An important feature of a new chemosensor is its high selectivity towards the $Hg^{2+}$ over the other competitive species such as $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$,

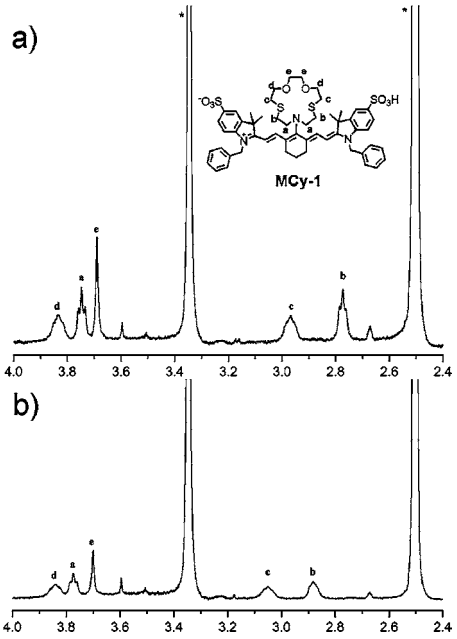


**Figure 3.** Partial $^1H$ NMR spectra (400 MHz, DMSO-$d_6$) of MCy-1 before (a) and after (b) addition of 10 equiv of mercury ions.

$Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$. As shown in Figure 4, under identical conditions to the $Hg^{2+}$ ion, no significant changes

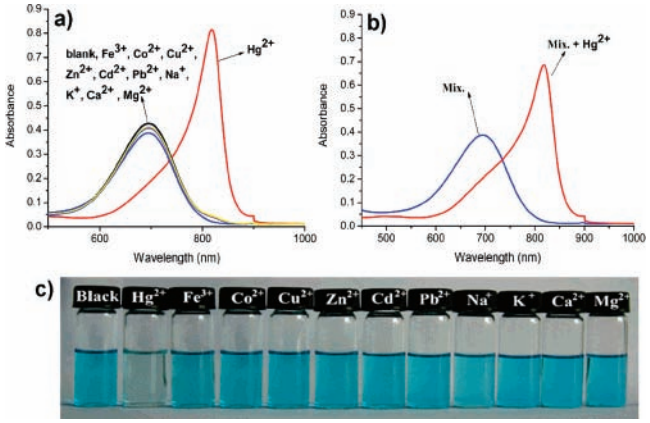


**Figure 4.** (a) Absorbance spectra change of MCy-1 (5.16 $\mu$M in methanol) upon addition of different metal cations (10 equiv). (b) Absorbance spectra change of MCy-1 (5.16 $\mu$M) upon addition of mixed cations (mix = $Fe^{3+}$ + $Co^{2+}$ + $Cu^{2+}$ + $Zn^{2+}$ + $Cd^{2+}$ + $Pb^{2+}$ + $Na^+$ + $K^+$ + $Ca^{2+}$ + $Mg^{2+}$); each one is 10 equiv of metal cations and subsequent addition of 50 equiv of $Hg^{2+}$ in methanol. (c) Color change of MCy-1 in the presence of different metal cations. From left to right: blank, $Hg^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$.

were observed in the UV−vis spectra of MCy-1 upon addition of $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Li^+$, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$, respectively. This could probably be attributed to their low affinity with the receptor MCy-1. The

miscellaneous competitive cations also did not induce any significant color change of MCy-1. Therefore, MCy-1 can be considered as an effective colorimetric probe for $Hg^{2+}$. Thus, naked-eye detection of $Hg^{2+}$ becomes possible.

Note that a fluorometric detection of $Hg^{2+}$ is also possible for MCy-1. A strong decrease of the fluorescence is observed upon mercury complexation, which can be explained in terms of the prevention of the excited-state ICT process from the donor to the acceptor. To determine the amount of $Hg^{2+}$ ion required to induce the complete quenching of fluorescence from MCy-1, titration experiments were carried out as shown in Figure 5; when 8 equiv of $Hg^{2+}$ ion was added, the

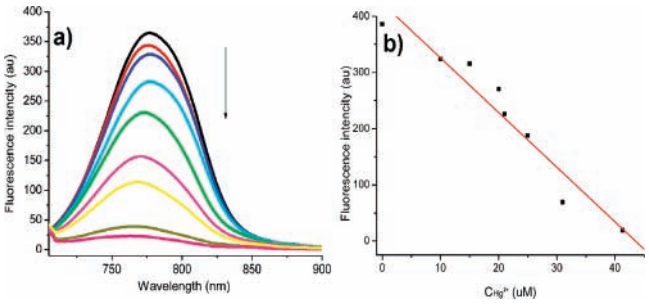


**Figure 5.** (a) Emission spectra of compound MCy-1 in the presence of increasing $Hg^{2+}$ concentration (0, 10, 15, 20, 21, 25, 30, 40 $\mu$M) in methanol. Excitation wavelength was 695 nm with 5 nm slit widths. The concentration of the chemosensor was 5.16 $\mu$M. (b) Fluorescence intensity of MCy-1 versus $Hg^{2+}$ concentration.

emission of MCy-1 was almost completely quenched. The detection limit of the sensor for $Hg^{2+}$ is about $1.1 \times 10^{-6}$ M (Figure 5b).

The changes in the fluorescence properties of MCy-1 caused by different metal ions, including $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Li^{+}$, $Na^{+}$, $K^{+}$, $Ca^{2+}$, and $Mg^{2+}$ were measured. The fluorescence of MCy-1 quenched markedly with the addition of $Hg^{2+}$. Cations such as $Li^{+}$, $Na^{+}$, $K^{+}$, $Ca^{2+}$, and $Mg^{2+}$, which exist at high concentrations under physiological conditions, have slight influence on fluorescence intensity. Transition-metal ions of $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, and heavy-metal ions of $Cd^{2+}$ and $Pb^{2+}$ also did not induce significant fluorescene change of MCy-1 (Figure 6).

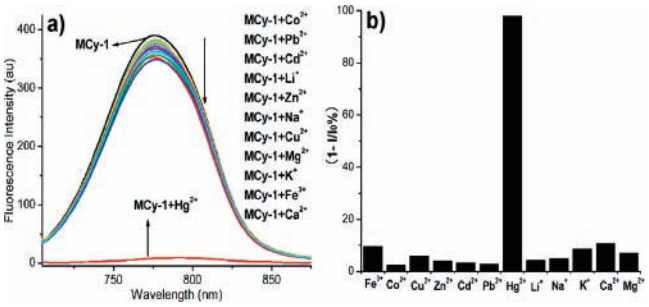


**Figure 6.** Fluorescence responses of MCy-1 to various metal ions (including $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Li^{+}$, $Na^{+}$, $K^{+}$, $Ca^{2+}$, and $Mg^{2+}$). Excitation was provided at 695 nm, and the emission was integrated over 705−900 nm. The bars represent the percentage of fluorescence quenched ($1 - I/I_0$).

In conclusion, we have successfully devised a novel NIR probe (MCy-1) towards $Hg^{2+}$ ions. A large red-shift (122 nm) of the absorption maximum of MCy-1 was observed upon titration with $Hg^{2+}$ ions followed by a solution color change from blue to colorless, making the "naked-eye" detection of $Hg^{2+}$ ions possible. Importantly, the selectivity of this system for $Hg^{2+}$ over other metal ions is extremely high. This assay offers a method for the detection of $Hg^{2+}$ that is easier and more convenient for application.

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**Supporting Information Available:** Synthesis, characterization, binding analysis of MCy-1. This material is available free of charge via the Internet at http://pubs.acs.org.

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