

Ratiometric Fluorescent Chemosensor for Hg^{2+} Based on Heptamethine Cyanine Containing a Thymine Moiety

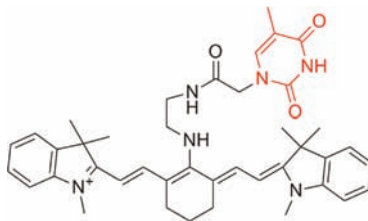
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ABSTRACT



Based on a T– Hg^{2+} –T binding mode, a sensitive ratiometric fluorescent chemosensor for aqueous Hg^{2+} was developed with a heptamethine cyanine chromophore containing a thymine moiety.

The release of heavy metals into the environment originates from a variety of man-made and natural sources, including industrial processes for agriculture, the paper industry, pharmaceutical uses, fossil fuel combustion, the electronics industry, and the burning of coal.¹ Thus, the detection of the toxic metal ions in various chemical systems is an important topic. Among other heavy metal ions, Hg^{2+} has attracted great interest from chemists in recent years because of its special chemical properties and its lethal effects on the environment and living organisms.² As a consequence, a number of new chemosensors for Hg^{2+} with desirable properties have been designed and synthesized and have recently been discussed in excellent review articles.³ Monitoring of Hg^{2+} by most of these chemosensors is involved in only changes in the emissive

intensities; however, in most practical applications, changes in fluorescence intensity can also be caused by other variable factors such as photobleaching, variation of concentration of the probe molecule, different microenvironments around the probe molecule, or the stability of the light source. Therefore, it is generally preferable for a fluorescent sensory signal to involve new emission at a different wavelength rather than modulation of an existing signal. This new approach should take the ratiometric signal measurements at two emissive wavelengths where the intensity responses are generally reversed. Therefore, this ratiometric method can overcome the limitations of intensity-based measurements due to a built-in correction for environmental effects. Up to now, only a few ratiometric fluorescent probes for Hg^{2+} have been reported, and the ratiometric fluorescent signal of these probes, however, are clearly based on mechanisms namely

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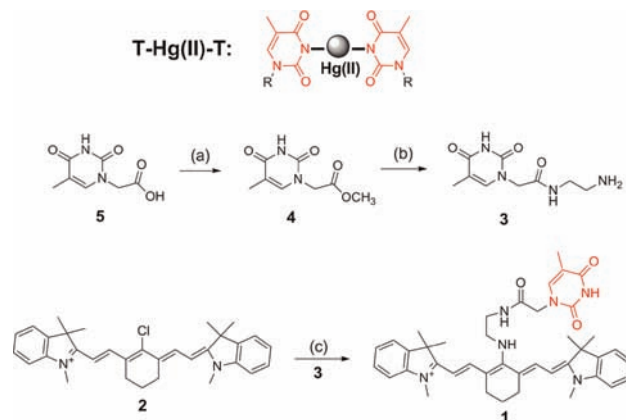
intramolecular charge transfer (ICT),⁴ excimer–monomer transfer,⁵ the excited-state intramolecular proton transfer (ESIPT), or energy transfer.^{6,7}

On the other hand, the self-association of dyes is a frequently encountered phenomenon, especially for many classes of dyes when used in aqueous solution. The aggregation effect of dyes also exerts a strong influence on their spectroscopic characteristics, and these spectral changes can be attributed to the aggregation of the dye molecules in water to form dimers and higher order aggregates in the “J-” or “H-” type aggregation state. Though polymethine cyanines are among the best known self-associating dyes in aqueous solutions, and this self-association is clearly reflected by changes in the absorption spectra,⁸ chemosensor studies based on this behavior have seldom been reported, except for a colorimetric and ratiometric fluorescent chemosensor for Ag⁺.⁹ On the basis of these facts, we herein report a new polymethine cyanine-based probe for the efficient chromogenic and ratiometric fluorescent recognition of Hg²⁺ as a new advance in this field.

Based on the fact that thymine (T) has proven to be one of the most selective ligand binding to Hg²⁺ in the form of T–Hg²⁺–T,¹⁰ we envisioned that this binding mode would contribute to fulfill the Hg²⁺-modulation of the aggregation state of a heptamethine cyanine-based chromophore in aqueous medium. Therefore, we introduced a thymine group into the cyclohexene bridgehead of the heptamethine cyanine chromophore, leading to a new

thymine-modified heptamethine cyanine probe **1**. The synthetic procedures of **1** are given in Scheme 1, and its structural identification was confirmed by ¹H NMR, ¹³C NMR, and ESI-MS spectroscopy (Supporting Information).

Scheme 1. Synthetic Route of **1**^a



^a Key: (a) CH₃OH, SOCl₂, reflux 3 h; (b) ethylenediamine, CH₃OH, reflux 3 h, N₂ atmosphere; (c) 1,8-bis(dimethylamino)naphthalene, CH₃OH, reflux 6 h, N₂ atmosphere.

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Next, the spectroscopic characteristics of probe **1** in aqueous solution was studied. The absorption spectral traces of **1** upon coordination with Hg²⁺ in an optimized buffer solution of 3,3-dimethylglutaric acid–NaOH (10 mM in MeOH/H₂O = 2/98, v/v) at pH 6.6 were monitored first. As shown in Figure 1, the solution of **1** alone (2.0 × 10^{−5} M) exhibits an absorption maximum at 628 nm, which is responsible for the blue color of the solution. With increasing Hg²⁺ concentrations, the absorbance at 628 nm decreased, while the absorbance at 510 nm increased accordingly. This pronounced hypsochromic shift of the maximum absorption wavelength can be ascribed to the H-aggregation state of the cyanine dye resulted from the coordination of Hg²⁺.⁸ Meanwhile, an isosbestic point was clearly observed around 538 nm, indicating the conversion of the free molecules into aggregation molecules. In addition, such a large blue-shift of 118 nm in the absorption behavior changes the color of the resultant solution from blue into pink, allowing “naked-eye” detection (Figure S3, Supporting Information). Furthermore, the experiments of ratiometric absorbance (A₅₁₀/A₆₂₈) response of **1** toward other metal ions, including Ag⁺, Zn²⁺, Cd²⁺, Mn²⁺, Fe³⁺, Cu²⁺, Ni²⁺, Pb²⁺, Cr³⁺, Al³⁺, Co²⁺, Na⁺, Ba²⁺, Mg²⁺, Ca²⁺, and K⁺, suggested the high absorption selectivity to Hg²⁺ (Figures S4 and S5, Supporting Information).

We also noticed that the reaction of **1** with Hg²⁺ produced obvious fluorescence emission changes (Figure 2). A solution of **1** displayed two emission peaks, strong at 537 nm and weak at 714 nm, when excited at 500 nm. When Hg²⁺ was added to the solution of **1**, a distinct decrease in the 714 nm emission and an increase in the fluorescence at 537 nm were observed, with a clear isoemission point at

662 nm. Both the absorbance ratio of A_{510}/A_{628} and emission ratio of F_{537}/F_{714} increase with the increasing in Hg^{2+} concentrations, allowing the Hg^{2+} concentration to be determined ratiometrically (Figure S6, Supporting Information).

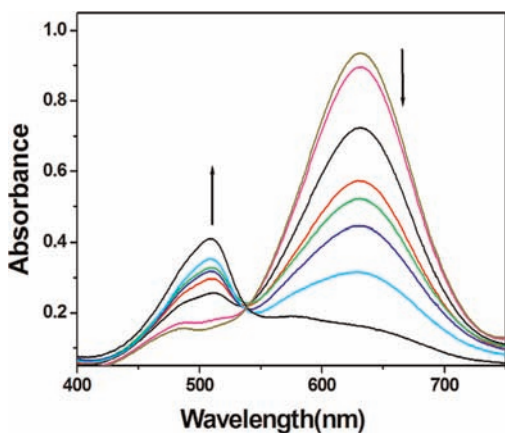


Figure 1. Absorption spectra of **1** with Hg^{2+} at pH 6.60 of 0.01 M 3,3-dimethylglutaric acid–NaOH buffer solution (MeOH/ H_2O = 2/98, v/v). [**1**] = 20.0 $\mu\text{mol/L}$, the concentration of Hg^{2+} values ranging from 0 to 10.0 $\mu\text{mol/L}$. $\lambda_{\text{ex}}/\lambda_{\text{em}}$ = 500 nm/537 nm, 714 nm; slit: ex/em = 10.0/20.0 nm.

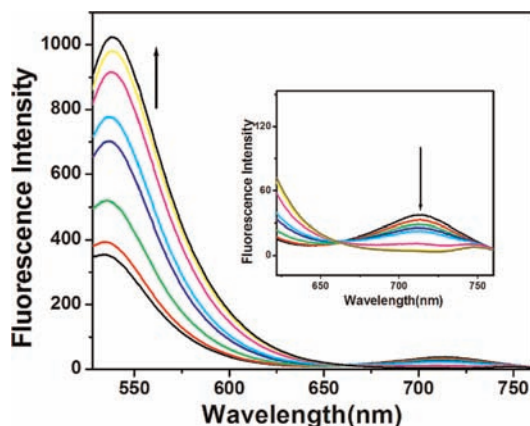


Figure 2. Fluorescence emission spectra of **1** with Hg^{2+} at pH 6.60 of 0.01 M 3,3-dimethylglutaric acid–NaOH buffer solution (MeOH/ H_2O = 2/98, v/v). [**1**] = 20.0 $\mu\text{mol/L}$, the concentration of Hg^{2+} values ranging from 0 to 10.0 $\mu\text{mol/L}$. $\lambda_{\text{ex}}/\lambda_{\text{em}}$ = 500 nm/537 nm, 714 nm; slit: ex/em = 10.0/20.0 nm.

Meanwhile, the limits of detection were calculated to be 1.6×10^{-8} M for spectrophotometry and 4.8×10^{-9} M

(11) According to the definition by IUPAC (CDL = 3Sb/m). See: *IUPAC compendium of analytical nomenclature, Definitive Rules*; Irving, H. M. N. H., Freiser, H., West, T. S., Eds.; Pergamon Press, Oxford, 1981.

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for fluorimetry.¹¹ The association constant between **1** and Hg^{2+} was estimated as $2.2 \times 10^{10} \text{ M}^{-2}$.¹² In addition, the fluorescence quantum efficiency (Φ) of probe **1** in the absence and presence of Hg^{2+} were determined to be 0.012 and 0.042, respectively.¹³

Then, the ratiometric fluorescent response of **1** was analyzed to determine its selectivity in the presence of metal ions under identical conditions. As shown in Figure 3, the solution of **1** alone exhibits a low emission ratio of F_{537}/F_{714} , but upon the addition of 1.0 equiv of Hg^{2+} there is a prominent enhancement of this value, and the remainder of the tested metal ions did not greatly alter the ratiometric value relative to **1** alone. In addition, a competitive experiment revealed that all of the other tested metal ions had minor or no interference with the ratiometric signal response to Hg^{2+} ; therefore, these results suggest that **1** has a high fluorescent selectivity for Hg^{2+} in the presence of these tested foreign metal ions (Figure S7, Supporting Information).

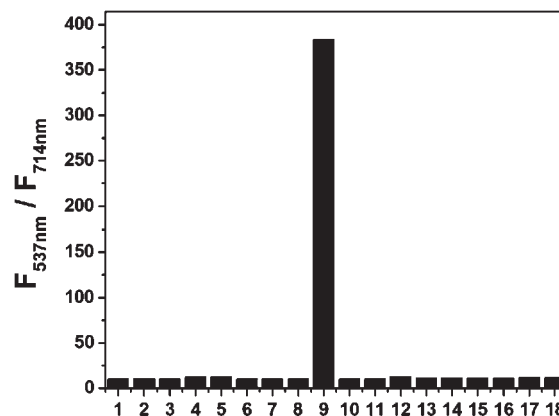


Figure 3. Fluorimetric responses of **1** to various cations at pH 6.60 of 0.01 M 3,3-dimethylglutaric acid–NaOH buffer solution (MeOH/ H_2O = 2/98, v/v). [**1**] = 20.0 $\mu\text{mol/L}$, [M^{n+}] = 10.0 $\mu\text{mol/L}$. From left to right: no cation (1), Ag^+ (2), Zn^{2+} (3), Mn^{2+} (4), Fe^{3+} (5), Cu^{2+} (6), Ni^{2+} (7), Pb^{2+} (8), Hg^{2+} (9), Cr^{3+} (10), Al^{3+} (11), Co^{2+} (12), Na^+ (13), Ba^{2+} (14), Mg^{2+} (15), Ca^{2+} (16), K^+ (17), Cd^{2+} (18). $\lambda_{\text{ex}}/\lambda_{\text{em}}$ = 500/537 nm, 714 nm; slit: ex/em = 10.0/20.0 nm.

In this work, with the aid of the T– Hg^{2+} –T binding mode, we carried out the ratiometric fluorescent sensing of Hg^{2+} in aqueous solution by modulating the aggregation state of the heptamethine cyanine fluorophore. Therefore, the binding stoichiometry of **1** to Hg^{2+} was further determined using a Job's plot,¹⁴ and the results showed that the ratiometric values of both the absorbance (A_{510}/A_{628}) and the fluorescence (F_{537}/F_{714}) of the system (**1** + Hg^{2+}) reached a maximum value when the molecular fraction of Hg^{2+} was close to 0.3 (Figure S8, Supporting

(13) In reference to rhodamine B (Φ) of 0.69 in methanol. See: Velapoldi, R. A.; Tonnesen, H. H. *J. Fluoresc.* **2004**, *14*, 465–472.

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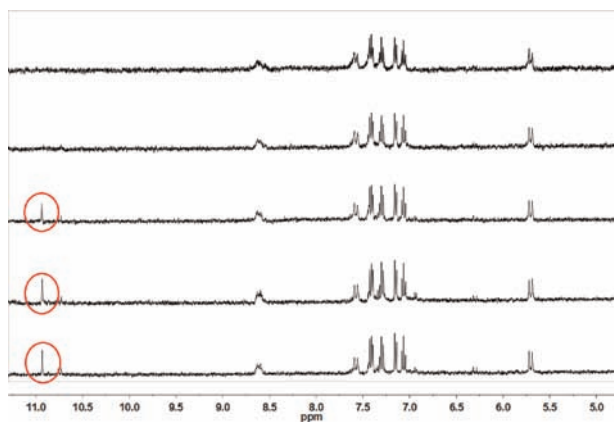


Figure 4. Partial spectra of ^1H NMR titration of **1** with Hg^{2+} . Equivalents of Hg^{2+} relative to **1** (from bottom to top): 0, 0.1, 0.3, 0.5, 0.7.

Information). These data indicate the formation of a 2:1 (**1**: Hg^{2+}) complex, and the binding stoichiometry agrees well with the known T- Hg^{2+} -T mode.

The ^1H NMR titration in deuterated DMSO solution was also carried out (Figure 4). The data revealed that the proton signal of imide in thymine moiety at the lowest field (marked in red circle) in the NMR spectra disappeared in the presence of Hg^{2+} , strongly suggesting that Hg^{2+} bound to the thymine moiety at the nitrogen atom of imide group. In addition, the chemical shifts (δ 5.0–8.0) of the unsaturated protons in the heptamethine cyanine moiety and the chemical shifts (δ 8.6, overlap, s + s, 2H) of two protons of -NH (one at the bridgehead of cyclohexenyl ring and one at amide group) did not show any significant changes, which can exclude the interaction of the heptamethine cyanine chromophore with Hg^{2+} . From the above experimental evidence of both the binding stoichiometry and the ^1H NMR analysis of the Hg^{2+} titrations, we can conclude

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that, in aqueous solutions, the binding of Hg^{2+} with the thymine moiety in probe **1** results in the formation of the **1**- Hg^{2+} -**1** complex, bringing the two inner heptamethine cyanine chromophores of this complex into proximity, and this conformation is similar to that of “H”-aggregates, leading to a change in the solution color and to changes in the ratiometric fluorescence behavior. In addition, as the formation of a tightly bound dimer aggregate may suppress the short-lived decay channel and favor of the long-lived decay component,¹⁵ fluorescent lifetime induced by a tightly bound aggregation would be much (always several folds) longer than that by monomer.^{15,16} Therefore, the lifetimes of probe **1** before and after the addition of Hg^{2+} , were determined to be 0.29 and 0.36 ns, respectively, and this moderate increasing in lifetime may suggest the formation of loosely bound H-dimer aggregate of **1** in the presence of Hg^{2+} .

In summary, compound **1** was designed as new fluorescent Hg^{2+} sensor by making use of the binding of thymine with Hg^{2+} to induce cyanine aggregation in aqueous solution. The fluorescent spectral results clearly indicate that compound **1** can be used as a ratiometric fluorescent sensor for Hg^{2+} with excellent selectivity and sensitivity. Further studies including design of more fluorescent chemosensors toward various analytes based on same strategy are underway in this laboratory.

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Supporting Information Available. Synthetic details and ^1H and ^{13}C NMR characterizations of **3**, **4**, and **1**, the pH and time course effect, details of Job’s plots, and some related absorption and fluorescence spectral responses of **1** for Hg^{2+} . This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.