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2-[3-(2-Aminoethylsulfanyl)propylsulfanyl]ethanamine Bearing Dansyl Subunits: An Efficient, Simple, and Rapid Fluorometric Sensor for the Detection of Mercury(II) Ions

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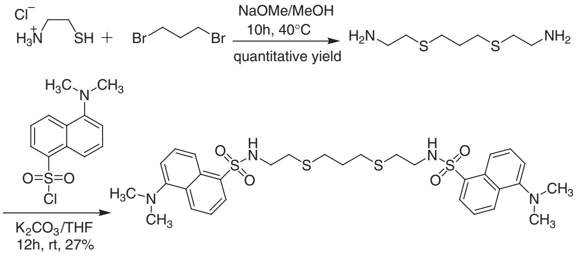
A novel fluorometric sensor possessing two dansyl moieties based on 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine **1** was prepared by a ready synthetic approach using a conventional two-step synthesis. **1** exhibits highly sensitive and selective on–off Hg^{2+} fluorescence quenching behavior in aqueous acetonitrile solutions and is shown to discriminate various competing metal ions such as Cu^{2+}, Ag^{+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Na^{+}, and K^{+} exhibiting a detection limit of 7 nM or 1.4 ppb with a working range of 1.8–15 ppb.



Scheme 1. Synthesis of **1**.

Pollution from the release of heavy metals into the environment causes severe problems to human health. Mercury is one of the most highly toxic contaminants with a recognized accumulative character in the environment and biota.[1] Recently, the design and synthesis of fluorescent sensors for Hg^{2+} has become attractive since they offer simple and rapid tracking of Hg^{2+} for environmental monitoring. Although many fluorescent mercury sensors have been designed for Hg^{2+}-sensing, only a few showed high sensitivity and selectivity to detect Hg^{2+} in aqueous solutions and mixed aqueous/organic solvents.[2] A number of fluorescent mercury ionophores have been reported for Hg^{2+}-sensing such as hydroxyquinolines, azines, cyclams, dioxocyclams, diazatetrathia crown ethers, and calixarene.[3–5] However, most of these sensors have serious drawbacks in term of synthetic difficulty, high cost of starting materials or lack of selectivity, particularly in the presence of copper (Cu^{2+}) and silver (Ag^{+}) due to their close chemical behavior to Hg^{2+}.[3,4]

In this study, the design concept for the sensor is based on the fundamental requirements for selective host–guest interactions in supramolecular chemistry. We have focused on utilizing the 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine ligand as a highly flexible ligand which offers synthetic simplicity and provides appropriately located nitrogen and sulfur atoms as donor atoms that can self assemble around the guest molecule due to the favorable electrostatic interactions.

In most fluoroionophore systems, the ionophore is responsible for the selectivity of ion binding while the sensitivity is usually governed by the chosen fluorophore. In this study, we have therefore focused on the effect of utilizing a dansyl fluorophore aiming to increase the sensitivity of the sensor system due to its strong fluorescence, relatively long emission wavelength in the visible region and a very large Stokes shift which can prevent self absorption.[5] In addition, our molecular design involves the increase of the number of dansyl fluorophore moieties into the sensor system in order to elevate the sensitivity. We would like to report herein the unique properties of this ligand connected to the dansyl fluorophore in selective mercury recognition. The novel fluoroionophore system is expected to be superior to many fluoroionophores based on multidentate thioether-containing ligand, cyclam or cyclen in terms of selectivity, sensitivity, economy of starting materials and synthetic simplicity compared to several previous reports.[4]

Compound **1** is fabricated from a sulfur- and nitrogen-containing ionophore based on a 2-[3-(2-aminoethylsulfanyl)-propylsulfanyl]ethanamine ionophore bearing two dansyl subunits. Scheme 1 outlines the synthesis of **1**. In this study, 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine was prepared by the alkylation of cysteamine hydrochloride with 1,3-dibromopropane in quantitative yield.[6] Nucleophilic substitution of the resulting compound with 5-(dimethylamino)naphthalene-1-sulfonyl chloride afforded **1** in 27% yield.[7]

The sensing properties of **1** were investigated by fluorescence measurements in common organic solvents and aqueous-miscible organic solvent systems. After a preliminary survey with various solvent systems, it was found that fluoroionophore **1** provided good sensitivity to Hg^{2+} in dichloromethane, 1,4-dioxane, and acetonitrile and offered high sensitivity in aqueous-acetonitrile solutions.[7] Based on this observation, we therefore focused on the fluorescence behavior of **1** in response to various metal ions in 95:5 acetonitrile:water.

To elucidate the quantitative binding affinity of **1**, fluorescence titrations of **1** with Hg^{2+} were carried out. Figure 1 shows the fluorescence spectra obtained from **1** in the absence and presence of Hg^{2+}. When ion-complexation was operative, on–off switching occurred as indicated by the fluorescence emission. In the absence of Hg^{2+}, the fluorescence response was at a maximum and the response decreased as Hg^{2+} concentration increased. When the added mercury acetate attained a concentration 2.2 times higher than that of **1**, the fluorescence response reached a minimum point and more than 95% quenching of initial fluorescence of **1** was observed. The effective fluorescence quenching of **1** might be due to the inherent quenching nature of Hg^{2+} ions, and a similar quenching behavior was

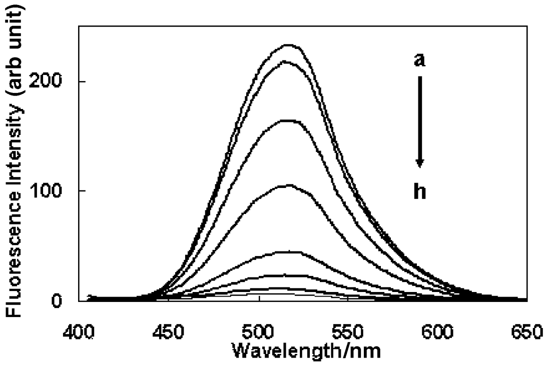


Figure 1. Fluorescence emission spectra ($\lambda_{ex} = 342\,nm$) of **1** ($0.54\,\mu M$) in 95:5 CH_3CN:H_2O as a function of [Hg^{2+}]. a: 0, b: 0.01, c: 0.03, d: 0.05, e: 0.07, f: 0.09, g: 0.27, and h: $1.20\,\mu M$.

previously observed in many mercury fluorescence sensors.[4c,4d,8] The fluorescence quantum yield (ϕ_f) of **1** in acetonitrile was found to be 0.57, using quinine sulfate with a ϕ_f of 0.56 in 0.5 M H_2SO_4 as a reference.[9]

The absorption and emission spectra of **1** illustrated a very large Stokes shift, approximately 177 nm.[7] The data collected in Figure 1 provides a good linear correlation between the emission response and mercury concentration over a range of 1.8–15 ppb, which is sufficient for the detection of sub-micromolar concentrations of Hg^{2+} ions found in environmental and many biological systems such as edible fish.[3,10] The detection limit of **1** as a Hg^{2+}-fluorescent sensor was determined from the plot of the fluorescence intensity as a function of the Hg^{2+} concentrations[11] and was found to be 7.0 nM or 1.4 ppb.

The association constant was determined by nonlinear curve fitting of the changes in fluorescence titration results[12] and was found to be $1.02 \times 10^{15}\,M^{-2}$ for 1:2 complex formation of **1**–Hg^{2+} in 95:5 acetonitrile:water. The 1:2 complex formation is also confirmed by ESI-MS analysis: aqueous acetonitrile solution containing **1** and 10 equiv of $Hg(CF_3SO_3)_2$ shows a peak at *m*/*z* 1209.1, assigned to $[2Hg^{2+} + \mathbf{1}(-2H) + CF_3SO_3^{-}]^{+}$, and by molecular modeling experiments.[7] The lowest complexation energy of 1:2 complex formation of **1**–Hg^{2+} (-128.621 kcal mol^{-1}) showed the stability of this complex whereas the 1:1 complex formation provided a less stable complex. The dynamic molecular modeling observations suggested that ions-recognition of the sensor originated from self-assembly of compound **1** and $2Hg^{2+}$ from the favorable electrostatic interactions of Hg^{2+} coordinated with S and O atoms as well as aromatic moiety to form a helical wrapping-like structure.[7] The distances to indicate the binding sites of Hg^{2+} bound to compound **1** are illustrated in Figure 2. From the optimization using DFT, one Hg^{2+} was bound between a S atom and a dansyl ring with the distances of 2.87 and 3.01 Å while the other Hg^{2+} was bound with the S and O atoms with the distances of 2.85 and 2.61 Å, respectively. The steric effect from two dansyl rings could lead to a less stable 1:1 complex formation compare to a 1:2 complex formation.

Selectivity studies of **1** were performed in 95:5 acetonitrile:water solutions by recording the fluorescence spectra of the solution of **1** before and after the addition of each representative metal ion. Herein, the selectivity studies were obtained by a similar method to the separate solution method (SSM) used in ion-selective electrode applications.[13] This involves the measurement of a series of separate solutions, each containing only a salt of the determined ion.[13] Figure 3 shows the dependence of the fluorescence intensity of **1** as a function of cation concentrations for Hg^{2+}, transition-metal, heavy metal, alkali earth, and alkali ions including Cu^{2+}, Ca^{2+}, Ag^{+}, Mn^{2+}, Zn^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Na^{+}, and K^{+}.

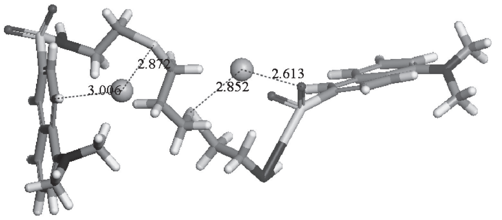


Figure 2. Optimized structure of 1:2 **1**–Hg^{2+} complex from molecular dynamics with local density approximation (LDA) of local functional PWC.

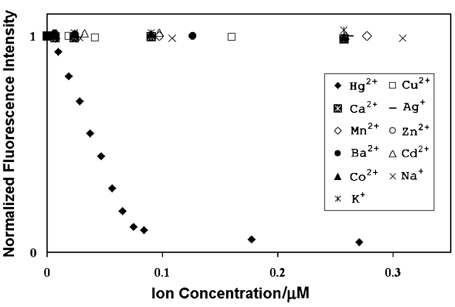


Figure 3. Normalized emission intensity (512 nm) of **1** ($0.54\,\mu M$) in 95:5 CH_3CN:H_2O versus the concentration of various metal ions.

The values in the plot are normalized to the fluorescence intensity (512 nm) in the absence of any cations. The results from Figure 3 demonstrate the high selectivity of **1** to Hg^{2+} in comparison with the other cations. The results showed that the fluorescence intensity decreased as a function of added Hg^{2+} until it reached the minimum point (95% quenching). In contrast, the fluorescence response of **1** did not cause any significant changes after the addition of Cu^{2+}, Ca^{2+}, Ag^{+}, Mn^{2+}, Zn^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Na^{+}, and K^{+} up to $70\,\mu M$. In particular, **1** illustrated the high selectivity for Hg^{2+} over Cu^{2+} and Ag^{+} which are potential competitors and revealed a greater affinity of several previous reported of Hg^{2+} sensors.[4] The selectivity of **1** presented here was due to the favorable electrostatic interactions of Hg^{2+} to the sensor. The appropriate locations of the S donor atom of the 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine ligand, the aromatic ring, and the O donor atom of the dansyl moiety to Hg^{2+} ions can provide the cation–dipole interaction and cation–π interaction, causing the selective self-assembly of the sensor molecule around the Hg^{2+} ions.

To explore the utility of **1** as a Hg^{2+}-selective sensor, competitive studies of **1** were performed. The competitive studies of **1** were performed in 95:5 acetonitrile:water solutions by recording the fluorescence spectra of the solution of **1** before and after the addition of Hg^{2+} in the absence and presence of 10 equivalents of environmentally important metal ions (Ca^{2+},

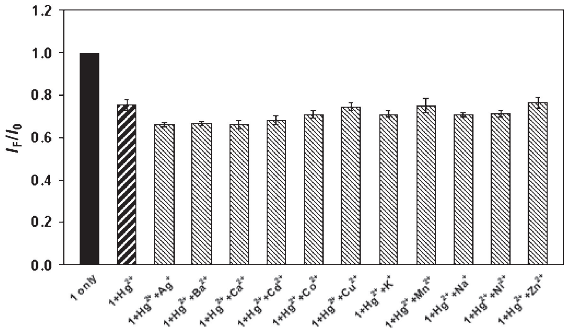


Figure 4. Competitive experiments in the **1**–Hg^{2+} system with common foreign metal ions: [**1**] = 0.54 µM, [Hg^{2+}] = 0.02 µM, and [M^{n+}] = 0.2 µM in 95:5 CH_3CN:H_2O solution. (λ_{ex} = 342 nm). Small bars represent the standard deviation.

Ba^{2+}, K^+, Cu^{2+}, Ag^+, Co^{2+}, Na^+, Cd^{2+}, Zn^{2+}, Mn^{2+}, and Ni^{2+}). (Figure 4)

The bars represent the final fluorescence emission response (I_F) over the initial fluorescence emission response (I_0) at 512 nm. I_F is the fluorescence response of **1** in the presence of a competitive background cation (0.2 µM each of Ca^{2+}, Ba^{2+}, K^+, Cu^{2+}, Ag^+, Co^{2+}, Na^+, Cd^{2+}, Zn^{2+}, Mn^{2+}, and Ni^{2+}) and Hg^{2+} (0.02 µM). I_F/I_0 (where I_F is the fluorescence emission intensity of **1** in the presence of Hg^{2+} only) was used as a reference and the I_F/I_0 reference value is equal to 0.75. The I_F/I_0 values were found to lie between 0.66–0.76, which indicated that a relatively consistent Hg^{2+}-induced fluorescence quenching was observed in the background competing ions. It should be noted that the sensing ability of **1** showed the sensitivity for Hg^{2+} in the background Cu^{2+} and Ag^+ which are potential competitors. The observed selectivity for Hg^{2+} is notable compared to many multidentate sulfide-containing ligands, cyclams and cyclen in previous reports.[4] In addition, it should be noted here that the operation of the sensor is suitable for samples having matrices of less than 10 equivalents of competitive background cations compared to the Hg^{2+}.

In summary, we have successfully prepared a novel mercury sensor that exhibits high sensitivity and high selectivity for Hg^{2+} over a wide range of competing ions, but with a significantly reduced synthetic effort. The readily accessible synthetic sensor was prepared by a conventional two-step synthesis. The sensor presented here is superior to many previous mercury sensors in terms of 1) synthetic simplicity, 2) low detection limit for the detection of Hg^{2+}, 3) high selectivity with particular discrimination of Cu^{2+} and Ag^+, and 4) the low cost of starting materials which make it possible for commercial use. In addition, the strong fluorescence and long emission wavelength in the visible region of **1** make it an attractive and inexpensive on-site tool for the determination of Hg^{2+}. The molecular design presented here could serve as an alternative mercury fluorometric sensor and may contribute to the further development of more potentially efficient chemosensors.

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