



## Dual optical detection of a novel selective mercury sensor based on 7-nitrobenzo-2-oxa-1,3-diazolyl subunits

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### ABSTRACT

A novel macromolecule based on 2-[3-(2-aminoethylthio)propylthio]ethanamine covalently bound to two 7-nitrobenzo-2-oxa-1,3-diazolyl moieties was prepared as a fluoroionophore and a chromophore for the selective optical detection of Hg<sup>2+</sup>. The sensor was prepared in two steps and its fluoroionophoric and chromophoric properties toward various transition metal, alkali, and alkali earth cations were investigated. Compound **4** selectively binds Hg<sup>2+</sup>, and the binding is indicated by both fluorescence quenching and a chromogenic change which can be detected by the naked eye. In an 80:20 acetonitrile/water solvent mixture, **4** acts as an ON–OFF fluorescence switch upon Hg<sup>2+</sup> binding, exhibiting efficient quenching and a detection limit of 10<sup>−7</sup> M or 20 ppb.

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Mercury is a highly toxic and hazardous environmental contaminant, even at low levels. Bacteria living in the sediment of aqueous and marine environments can transform inorganic mercury (Hg<sup>2+</sup>) into methylmercury, which can easily enter the food chain and accumulate in the upper levels of the chain, particularly in the tissues of large edible fishes and other marine mammals.<sup>1</sup> Excessive exposure of the human body to mercury leads to DNA damage,<sup>2</sup> brain damage,<sup>3</sup> and nervous system defects,<sup>2</sup> including Minamata disease.<sup>4</sup> Considering the extreme toxicity of mercury, the United States Environmental Protection Agency (EPA) provides the standard for the maximum allowed level of mercury in dietary and environmental sources, such as edible fishes, to be 0.55 ppm.<sup>1,5</sup>

The design and synthesis of fluorescent sensors for mercury ions has recently become attractive for developing chemical detectors that offer high selectivity and sensitivity.<sup>6</sup> Recently, a number of ligands have been investigated as mercury ionophores including hydroxyquinolines,<sup>7</sup> cyclams,<sup>8</sup> dioxocyclams,<sup>9</sup> diazatetraethia crown ethers,<sup>10</sup> azines,<sup>11</sup> and diaza-crown ethers.<sup>12</sup> These studies have illustrated that the nitrogen, oxygen, and sulfur atoms present in the ionophores promote the coordination of mercury ions.

Although many fluorescent mercury sensors have been designed for Hg<sup>2+</sup> sensing, they are often inefficient in the presence of other ions, particularly copper (Cu<sup>2+</sup>) and lead (Pb<sup>2+</sup>) due to their close chemical behavior to Hg<sup>2+</sup>,<sup>13</sup> as well as their abilities to complex to sulfur- and nitrogen-containing ionophores.<sup>8,9,11</sup> In this study, a major challenge involves the development of a mercury

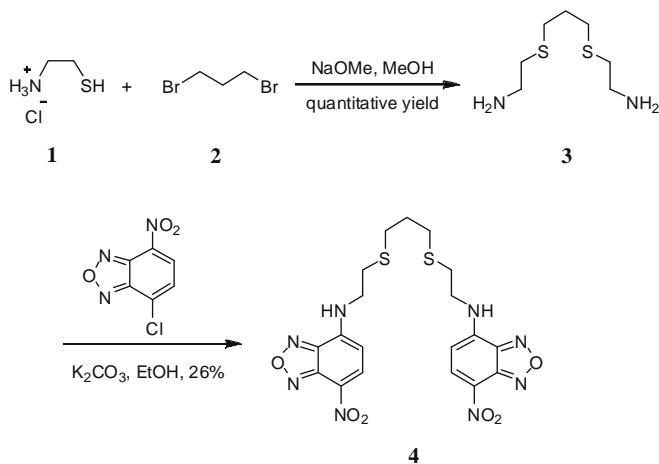
sensor that offers a low detection limit and is highly selective for mercury ions in the presence of other ions including transition metal, alkali, and alkali earth cations in aqueous solution. The sensor **4** is fabricated from a sulfur- and nitrogen-containing ionophore based on a 2-[3-(2-aminoethylthio)propylthio]ethanamine ligand covalently bound to two 7-nitrobenzo-2-oxa-1,3-diazolyl (NBD) moieties. Compound **4**, bearing two NBD fluorophore units, is expected to increase the sensitivity of the fluorescence sensor system.

Compound **4** exhibits Hg<sup>2+</sup> selective ON–OFF fluorescence signaling behavior in aqueous acetonitrile solutions and is shown to discriminate various cations such as Cu<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ba<sup>2+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup>. The detection limit of **4** is low enough for the detection of sub-micromolar concentration ranges of Hg<sup>2+</sup> ions as found in the environment and many biological systems.<sup>1</sup> Additionally, the binding of Hg<sup>2+</sup> ions causes a color change of the sensor solution, providing a second means of ion detection and further utility of **4** as a selective mercury sensor.

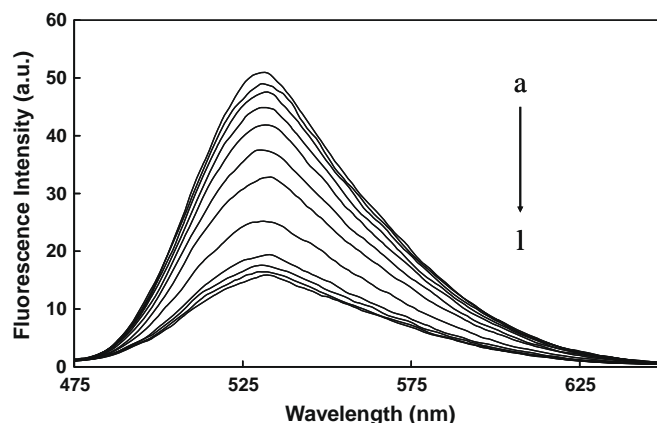
The major motivation for this work is the design and synthesis of a mercury fluoroionophore with high sensitivity and selectivity, but with a significantly reduced synthetic effort. In this study, fluoroionophore **4** was synthesized using a conventional two-step synthesis, *Scheme 1*. 2-[3-(2-Aminoethylthio)propylthio]ethanamine **1** was prepared by the alkylation of cystamine hydrochloride **1** with 1,3-dibromopropane **2**.<sup>14</sup> Compound **4** was obtained by nucleophilic aromatic substitution of **3** with 4-chloro-7-nitrobenzofurazan.<sup>15</sup> Compound **4** is a podant, acyclic host with pendant binding sites,<sup>16</sup> containing two sulfur atoms and two nitrogen atoms which are covalently bound to two NBD subunits. We propose that **4** will exhibit high sensitivity and selectivity to

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Scheme 1.



**Figure 2.** Fluorescence emission spectra ( $\lambda_{\text{ex}}$  458 nm) of **4** (0.5  $\mu\text{M}$ ) in 80:20  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  as a function of  $[\text{Hg}^{2+}]$ . (a) 0  $\mu\text{M}$ , (b) 0.1  $\mu\text{M}$ , (c) 0.2  $\mu\text{M}$ , (d) 0.3  $\mu\text{M}$ , (e) 0.5  $\mu\text{M}$ , (f) 0.7  $\mu\text{M}$ , (g) 1.0  $\mu\text{M}$ , (h) 1.7  $\mu\text{M}$ , (i) 2.7  $\mu\text{M}$ , (j) 4.7  $\mu\text{M}$ , (k) 5.3  $\mu\text{M}$ , (l) 6.0  $\mu\text{M}$ .

mercury ions over other cations due to the well-known interaction of mercury ions with sulfur and nitrogen atoms.<sup>6,8,9</sup>

The fluorescent spectral properties of **4** were investigated in both organic solvent systems (e.g., dichloromethane and acetonitrile) and aqueous-acetonitrile solutions. It was found that fluoroionophore **4** is sensitive to mercury ions in several solvents (S1–S2 Supplementary data). In order to optimize the conditions for practical applications in environmental and biological samples, the effects of water on the fluorescence emission of **4** in the absence and presence of mercury ions were investigated in acetonitrile solutions.

Figure 1 shows the effect of water concentration on the fluorescence behavior of **4** in acetonitrile solution. When the concentration of water increases, the fluorescence emission intensity of **4** decreases progressively. In the low water concentration range, similar decreases in the response of **4** in the presence of 20 equiv of  $\text{Hg}^{2+}$  were observed, but with much larger changes compared to the high water concentration region. Based on this observation, we focused on the fluorescence behavior of **4** in response to various metal ions in aqueous 80% acetonitrile solution.

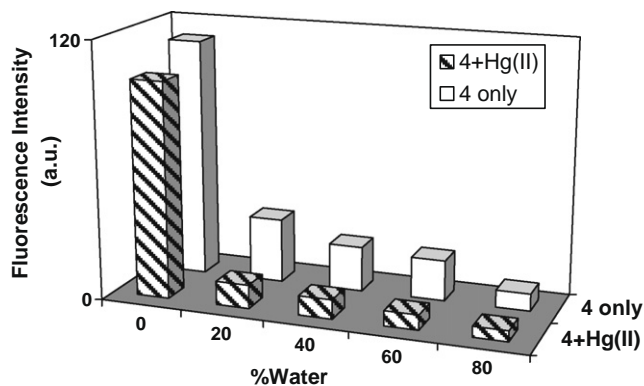
Figure 2 shows the fluorescence spectra obtained for **4** in the absence and presence of mercury(II) ions in 80:20 acetonitrile/water. In the absence of mercury(II) ions, the fluorescence response is at a maximum and this response decreases as the mercury concentration is increased.

The fluorescence behavior of **4** clearly demonstrates that an ON–OFF switching mechanism occurs in response to ion complex-

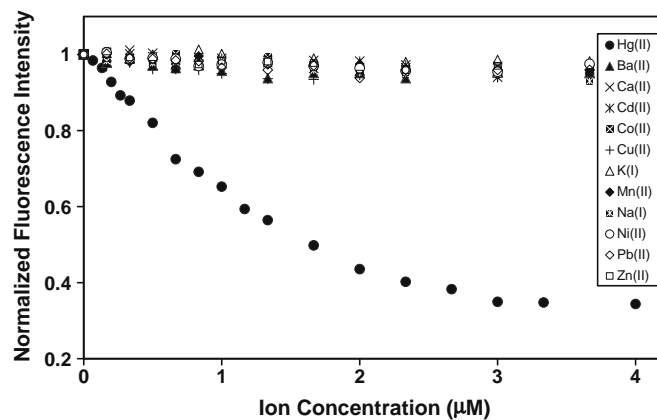
ation. The addition of mercury ions to **4** causes a decrease in the fluorescence emission intensity up to a concentration of mercuric acetate equal to 12 times the concentration of **4**, where it begins to reach the minimum point and more than 66% quenching of the initial fluorescence of **4** was observed. The effective fluorescence quenching of **4** might be due to the inherent quenching nature of  $\text{Hg}^{2+}$ , and similar quenching behavior was observed in many mercury fluorescence sensors.<sup>7,9,10</sup> The fluorescence quantum yield ( $\phi_f$ ) of **4** in acetonitrile was measured to be 0.90, using coumarin 153 with a  $\phi_f$  of 0.87 in acetonitrile as a reference.<sup>17</sup> The data collected in Figure 2 show a good linear correlation between emission response and mercury concentration over a range of 26–267 ppb. The detection limit of **4** as a fluorescent sensor for the analysis of  $\text{Hg}^{2+}$  was determined from the plot of the fluorescence intensity as a function of the  $\text{Hg}^{2+}$  concentration.<sup>18</sup> It was found that **4** has a detection limit of approximately  $10^{-7}$  M or 20 ppb for  $\text{Hg}^{2+}$  ions, which is sufficiently low for the detection of sub-micromolar concentrations of  $\text{Hg}^{2+}$  ions as found in many chemical and biological systems.<sup>1</sup>

Sensitivity studies of **4** were performed in 80:20 acetonitrile/water solution by a method similar to the Separate Solution Method (SSM) used in ion-selective electrode applications.<sup>19</sup> SSM involves the measurement of a series of separate solutions where each solution contains a salt of the determined ion only.<sup>19</sup>

Figure 3 shows the dependence of the fluorescence intensity of **4** as a function of cation concentrations for  $\text{Hg}^{2+}$ , various transition



**Figure 1.** Fluorescence intensity changes (345 nm) of **4** (0.5  $\mu\text{M}$ ) as a function of water content in aqueous acetonitrile solution at 534 nm in the absence and presence of mercury(II) ions (20 equiv),  $\lambda_{\text{ex}}$  458 nm.



**Figure 3.** Normalized emission intensity of **4** (0.5  $\mu\text{M}$ ) in 80:20  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  versus the concentration of various metal ions:  $\text{Hg}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Zn}^{2+}$ .

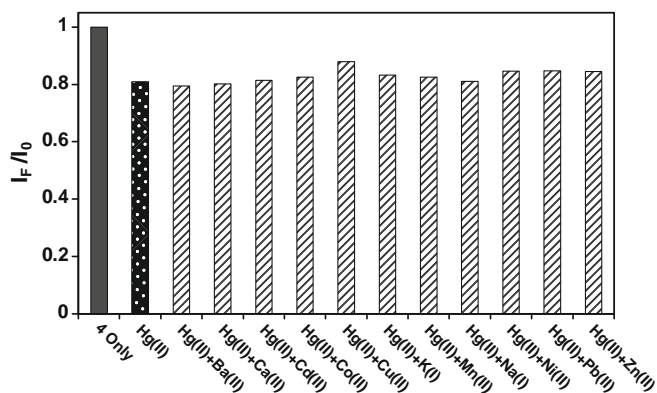
metal, heavy metal, alkali earth, and alkali cations including  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ , and  $\text{Zn}^{2+}$ . The values in the plot are normalized to the fluorescence intensity in the absence of any metal ions. The results illustrate the high selectivity of **4** for  $\text{Hg}^{2+}$  in comparison with the other ions. The results show that the fluorescence intensity decreases as a function of added mercury ions until it reaches the minimum point, beyond which it is constant up to the maximum concentration tested ( $4 \mu\text{M}$ ). In contrast, the fluorescence response of **4** shows negligible change (<5%) after adding the other ions as detailed above. In particular, **4** shows selectivity for  $\text{Hg}^{2+}$  over  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  which are potential competitors since  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  can also bind to sulfur and nitrogen atoms.<sup>8,9,11</sup> The association constant,  $K_{\text{assoc}}$ , was determined by nonlinear curve fitting of the changes in the fluorescence titration results<sup>20</sup> and was found to be  $4.36 \times 10^{-12} \text{ M}^{-2}$  for 1:2 complex formation of **4**- $\text{Hg}^{2+}$  in 80:20 acetonitrile/water.

To further explore the utility of **4** as an ion-selective fluorescence sensor for  $\text{Hg}^{2+}$ , competition experiments were conducted in the presence of  $\text{Hg}^{2+}$  ( $0.5 \mu\text{M}$ ) with 10 equiv of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and 100 equiv of  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$  as background (Fig. 4).

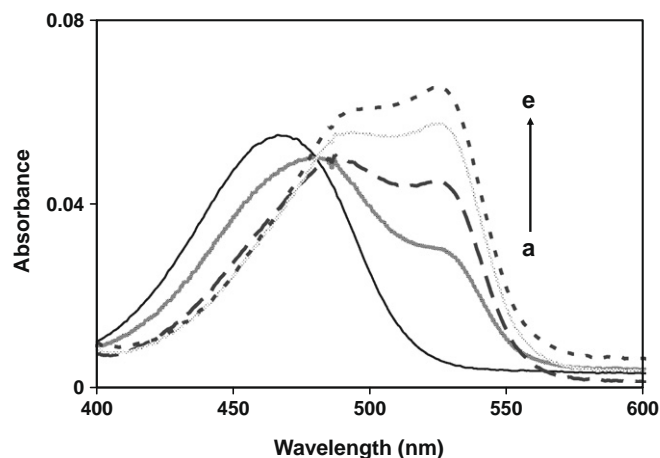
The bars represent the final fluorescence emission response ( $I_F$ ) over the initial fluorescence emission response ( $I_0$ ) at 534 nm.  $I_F$  is the fluorescence response of **4** in the presence of an appropriate background metal ion ( $50 \mu\text{M}$   $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $5 \mu\text{M}$   $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ) and  $\text{Hg}^{2+}$  ( $0.5 \mu\text{M}$ ). The  $I_F/I_0$  values were found to lie between 0.79 and 0.88, which indicates that a relatively consistent  $\text{Hg}^{2+}$ -induced fluorescence quenching is observed in the background foreign ions.  $I_F/I_0$  where  $I_F$  is the fluorescence emission intensity of **4** in the presence of  $\text{Hg}^{2+}$  ( $0.5 \mu\text{M}$ ) was used as a reference.

The chromosensor behavior of **4** with other ions was investigated in both dichloromethane and aqueous-acetonitrile solutions. The UV-visible spectrum of **4** showed a broad absorption band around 460 nm which is typical of NBD subunit.<sup>20</sup>

The addition of  $\text{Hg}^{2+}$  to a solution of **4** leads to a red shift of the absorption maximum by approximately 70 nm (Fig. 5). This new absorption band is responsible for the change of color in the solution from yellow to pink, which can be detected by the naked eye. The absorption spectral change can indicate an interaction between  $\text{Hg}^{2+}$  and the fluorophore **4** in the ground state. The bathochromic shift of the absorption maximum of **4** in the presence of  $\text{Hg}^{2+}$  presumably arises due to the involvement of the nitrogen atom(s) at the 4-position of the NBD fluorophore(s) in coordination. The coordination of the nitrogen atom at the 4-position can reduce the charge separation within the NBD-fluorophore, leading



**Figure 4.** Competitive experiments in the **4**- $\text{Hg}^{2+}$  system with common foreign metal ions: [**4**] =  $0.5 \mu\text{M}$ ,  $\text{Hg}^{2+}$  =  $0.5 \mu\text{M}$  and  $[\text{M}^{n+}]$  =  $5 \mu\text{M}$  or  $50 \mu\text{M}$  in 80:20  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  solution, ( $\lambda_{\text{ex}}$  458 nm).



**Figure 5.** UV-visible spectra obtained during the titration of **4** ( $1.3 \mu\text{M}$ ) in 80:20  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  with mercuric acetate. (a)  $0 \mu\text{M}$  (solid line), (b)  $1.0 \mu\text{M}$ , (c)  $2.3 \mu\text{M}$ , (d)  $5.0 \mu\text{M}$ , (e)  $11.6 \mu\text{M}$ .

to destabilization of the excited state energy.<sup>21</sup> In contrast, titration of the other ions (20 equiv of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$ ) induced negligible spectral changes in both aqueous-acetonitrile and dichloromethane solutions.

In summary, we have described a two-step synthesis of a new member of mercury fluoroionophores that exhibits high sensitivity and selectivity for mercury ions over other cations, but with a significantly reduced synthetic effort. Compound **4**, based on 2-[3-(2-aminoethylthio)propylthio]ethanamine covalently bound to two NBD moieties, shows selective fluorescence quenching behavior toward  $\text{Hg}^{2+}$  in aqueous acetonitrile solutions. The remarkable feature of **4** is the presence of a red shift (approximately 70 nm) in the absorbance spectra toward  $\text{Hg}^{2+}$  ions which changes the color of the solution from yellow to pink. The high degree of selectivity and dual signaling behavior of compound **4** for  $\text{Hg}^{2+}$  ions in aqueous solution make it attractive for potential use in molecular level sensor devices for the analysis of sub-micromolar concentration ranges of mercury contamination in actual environmental samples. In addition, we are currently synthesizing other mercury fluoroionophores based on 2-[3-(2-aminoethylthio)propylthio]ethanamine and are studying their ion sensing ability. These results will be reported shortly.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.01.118.

## References and notes

- (a) Yoon, S.; Albers, A. E.; Wong, A. P.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16030; (b) Renzoni, A.; Zino, F.; Franchi, E. *Environ. Res.* **1998**, *77*, 68; (c) Hardy, S.; Jones, P. J. *Chromatogr., A* **1997**, *791*, 333; (d) Harris, H. H.; Pickering, I. J.; George, G. N. *Science* **2003**, *301*, 1203.
- Tchounwou, P. B.; Ayensu, W. K.; Ninashvili, N.; Sutton, D. *Environ. Toxicol.* **2003**, *18*, 149.
- (a) Davidson, P. W.; Myers, G. J.; Cox, C.; Shamlaye, C. F.; Marsh, D. O.; Tanner, M. A.; Berlin, M.; Sloane-Reeves, J.; Cernichiari, E.; Choisy, O.; Choi, A.; Clarkson,

- T. W. *Neurotoxicology* **1995**, *16*, 677; (b) Grandjean, P.; Weihe, P.; White, R. F.; Debes, F. *Environ. Res.* **1998**, *77*, 165.
4. Harada, M. *Crit. Rev. Toxicol.* **1995**, *25*, 1.
5. US EPA, Regulatory Impact Analysis of the Clean Air Mercury Rule: EPA-452/R-05-003, 2005.
6. (a) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968; (b) Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272; (c) Yu, J. C. C.; Lai, E. P. C.; Sadeghi, S. *Sens. Actuators, B* **2004**, *101*, 236; (d) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270; (e) Ha-Thi, M.-H.; Penhoat, M.; Michelet, V.; Leray, I. *Org. Lett.* **2007**, *9*, 1133; (f) Shiraiishi, Y.; Maehara, H.; Ishizumi, K.; Hirai, T. *Org. Lett.* **2007**, *9*, 3125; (g) Lee, M. H.; Wu, J.-S.; Lee, J. W.; Jung, J. H.; Kim, J. S. *Org. Lett.* **2007**, *9*, 2501; (h) Yang, H.; Zhou, Z.; Huang, K.; Yu, M.; Li, F.; Yi, T.; Huang, C. *Org. Lett.* **2007**, *9*, 4729; (i) Wu, D.; Huang, W.; Duan, C.; Lin, Z.; Meng, Q. *Inorg. Chem.* **2007**, *46*, 1538; (j) Soh, J. H.; Swamy, K. M. K.; Kim, S. K.; Kim, S.; Lee, S.-H.; Yoon, J. *Tetrahedron Lett.* **2007**, *48*, 5966.
7. (a) Moon, S. Y.; Cha, N. R.; Kim, Y. H.; Chang, S. K. *J. Org. Chem.* **2004**, *69*, 181; (b) Zhang, H.; Han, L.-F.; Zachariasse, K. A.; Jiang, Y.-B. *Org. Lett.* **2005**, *7*, 4217.
8. (a) Kim, S. H.; Kim, J. S.; Park, S. M.; Chang, S. K. *Org. Lett.* **2006**, *8*, 371; (b) Park, S. M.; Kim, M. H.; Choe, J. I.; No, K. T.; Chang, S. K. *J. Org. Chem.* **2007**, *72*, 3550; (c) Moon, S. Y.; Youn, N. J.; Park, S. M.; Chang, S. K. *J. Org. Chem.* **2005**, *70*, 2394.
9. Song, K.-C.; Kim, M. H.; Kim, H. J.; Chang, S.-K. *Tetrahedron Lett.* **2007**, *48*, 7464.
10. Kim, S. H.; Song, K. C.; Ahn, S.; Kang, Y. S.; Chang, S.-K. *Tetrahedron Lett.* **2006**, *47*, 497.
11. Martinez, R.; Espinosa, A.; Tarraga, A.; Molina, P. *Org. Lett.* **2005**, *7*, 5869.
12. Kim, J. H.; Hwang, A.-R.; Chang, S.-K. *Tetrahedron Lett.* **2004**, *45*, 7557.
13. Wang, J.; Qian, X. *Chem. Commun.* **2006**, 109.
14. **Synthesis of 2-[3-(2-aminoethylthio)propylthio]ethanamine 3:** Sodium methoxide (1.50 g, 27.8 mmol) was dissolved in dry methanol (7 mL). Cystamine hydrochloride (1.00 g, 8.80 mmol) was added to the solution. The solution was stirred for 30 min whereupon 1,3-dibromopropane (0.36 mL, 3.52 mmol) was added. This solution was then stirred for an additional 10 h at 40 °C under an argon atmosphere. The solvent was removed on a rotary evaporator. Aqueous sodium hydroxide solution (30% w/v, 15 mL) was added to the residue and the resulting solution was stirred slowly overnight. The solution was extracted three times with 20 mL of dichloromethane. The dichloromethane phase was collected and washed once with 60 mL of distilled water and then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The dichloromethane was removed under vacuum to give a quantitative yield of **3** as a yellow oil. The product was used without further purification. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.62 (br s, 4H), 1.87 (quintet, *J* = 6.9 Hz, 2H), 2.60–2.65 (m, 8H), 2.88 (t, *J* = 6.3 Hz, 4H), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 29.4 (CH<sub>2</sub>), 30.6 (2CH<sub>2</sub>), 36.1 (2CH<sub>2</sub>), 40.9 (2CH<sub>2</sub>). HR-ESI MS calcd for C<sub>7</sub>H<sub>19</sub>N<sub>2</sub>S<sub>2</sub> (M+H)<sup>+</sup> 195.0990, found 195.1066.
15. **Synthesis of compound 4.** In a 10 mL round-bottomed flask, 2-[3-(2-aminoethylthio)propylthio]ethanamine (0.10 g, 0.51 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.84 g, 5.60 mmol) were stirred in 5 mL of dry ethanol for 30 min under an argon atmosphere. 4-Chloro-7-nitrobenzofurazan (0.21 g, 1.02 mmol) was added and the mixture was refluxed overnight. The resulting insoluble precipitate was removed by filtration, and the filtrate was concentrated under vacuum. The crude product was purified by preparative thin layer chromatography, run in the dark (1% methanol in hexane/ethyl acetate 60:40 eluent) to yield 69 mg of an orange solid, yield 26%. *R*<sub>f</sub> = 0.5 (1% methanol in hexane:ethyl acetate 60:40); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.86 (quintet, *J* = 7.2 Hz, 2H), 2.66 (t, *J* = 7.2 Hz, 4H), 2.89 (t, *J* = 6.3 Hz, 4H), 3.65 (t, *J* = 6.0 Hz, 4H), 6.14 (d, *J* = 8.4 Hz, 2H), 8.42 (d, *J* = 8.4 Hz, 2H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 21.7 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 29.7 (CH<sub>2</sub>), 41.3 (2CH<sub>2</sub>), 97.8 (2CH), 100.6 (2C), 135.2 (2CH), 142.3 (2C), 142.8 (2C), 143.3 (2C). HR-ESI MS calcd for C<sub>19</sub>H<sub>20</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>Na (M+Na)<sup>+</sup> 543.0845 *m/z*, found 543.0712.
16. Steed, J. W.; Atwood, J. L. *Supramolecular Chemistry*; John Wiley and Sons: New York, 2000.
17. Rechthaler, K.; Köhler, G. *Chem. Phys. Lett.* **1994**, *189*, 99.
18. Shortreed, M.; Kopelman, R.; Kuhn, M.; Hoyland, B. *Anal. Chem.* **1996**, *68*, 1414.
19. (a) Bakker, E.; Buhlmann, P.; Pretsch, E. *Chem. Rev.* **1997**, *97*, 3083; (b) Benco, J. S.; Nienaber, H. A.; Dennen, K.; McGimpsey, W. G. *J. Photochem. Photobiol., A* **2002**, *152*, 33; (c) Wanichacheva, N.; Soto, E. R.; Lambert, C. R.; McGimpsey, W. G. *Anal. Chem.* **2006**, *78*, 7132.
20. (a) Valeur, B.; Pouget, J.; Bourson, J.; Kaschke, M.; Ernstring, N. P. *J. Phys. Chem.* **1992**, *96*, 6545; (b) Bourson, J.; Pouget, J.; Valeur, B. *J. Phys. Chem.* **1993**, *97*, 4552; (c) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. *Org. Lett.* **2006**, *8*, 859.
21. Ramachandram, B.; Samanta, A. *Chem. Phys. Lett.* **1998**, *290*, 9.