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A Fluorescent Chemosensor Signalling Only Hg(II) and Cu(II) in Water

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Abstract: Geometric immobilization of polyamine ligands is expected to change their binding properties toward practical ion discrimination. Chemosensor **5** senses only two transition metal ions in water- Hg(II) ($K_d \leq 1$ μM) and Cu(II) (K_d 56 μM)- which can be compared with bindings of a non-immobilized reference compound (9-(trpnmethyl)-anthracene; **3**) with Hg(II) (K_d 14 μM) and Cu(II) (K_d 39 μM). A related bridged cyclen derivative (**7**) showed no effect on fluorescence by any metal ion examined. © 1997 Elsevier Science Ltd.

Chemosensors are abiotic devices, molecule-sized or larger, that signal interactions with analytes reversibly and in real-time.[1] Of the many signal types available, fluorescence signaling has the potential to afford high sensitivity and direct application to fiber optic-based (and other[2]) remote sensing schemes.[3] The generally high affinity of polyamine ligands for transition metal ions has provided for the formulation of metal ion chemosensors that function in water. However, the same high affinity makes ion discrimination based on complexation affinities problematical. Thus, there are as yet no fluorescent chemosensors reported for any transition metal ion that combine real-time reversibility with sufficient selectivity for the monitoring of complex ionic mixtures. Engendering such selectivity based on ligand engineering is a primary goal of chemosensor research. We now report a novel approach to the attainment of binding discrimination in chemosensors, based upon the rigid immobilization of polyamine ligands onto a fluorophore framework. This approach has yielded a fluorescent chemosensor for the Hg(II) and Cu(II) ions.[4]

Our synthesis began with 1,8-bis(bromomethyl)anthracene (**4**), available using the procedures of Nakagawa.[5] Compound **5** was synthesized by addition of **4** to a warm (50-60°C) mixture of trpn (tris(3-aminopropyl)amine; **2**; 1 eq.), K_2CO_3 and $CHCl_3$ (Scheme 1). After complete addition, the solution was washed with 50 mM pH 7.5 phosphate (2 x 200 mL). The aqueous washes were adjusted to pH>11 with NaOH and extracted with $CHCl_3$ to give **5**, and the HCl salt[6] was precipitated from EtOH in 19 % overall yield. Bridged cyclen **7** was synthesized by the reaction of **4** with cyclen (**6**) at room temperature. Compound **7**[7] could be purified by silica gel column chromatography using 30% MeOH/$CHCl_3$. Anthrylpolyamine **3** was made using the literature procedure.[8]

The perchlorate salts of Al(III), Ca(II), Cd(II), Co(II), Cr(III), Cu(II), Eu(III), Fe(III), Ga(III), Gd(III), Hg(II), In(III), Mg(II), Mn(II), Ni(II), Pb(II), Rb(I), Sr(II), Yb(III), and Zn(II) were used to evaluate metal ion binding. All titration studies were conducted at pH 7 (0.1 M HEPES) and using a 4 μM concentration of chemosensor. Using these metal ions (100 μM), compound **5** displayed chelation-enhanced quenching (CHEQ) effects *only* with Hg(II) and Cu(II).[9] Titrations of **5** with Hg(II) ($K_d \leq 1$ μM) and Cu(II) (K_d 56 μM),[10] with overall emission changes of 18-fold and 4-fold, are shown in Figure 1. All K_d values for Hg(II)

Scheme 1

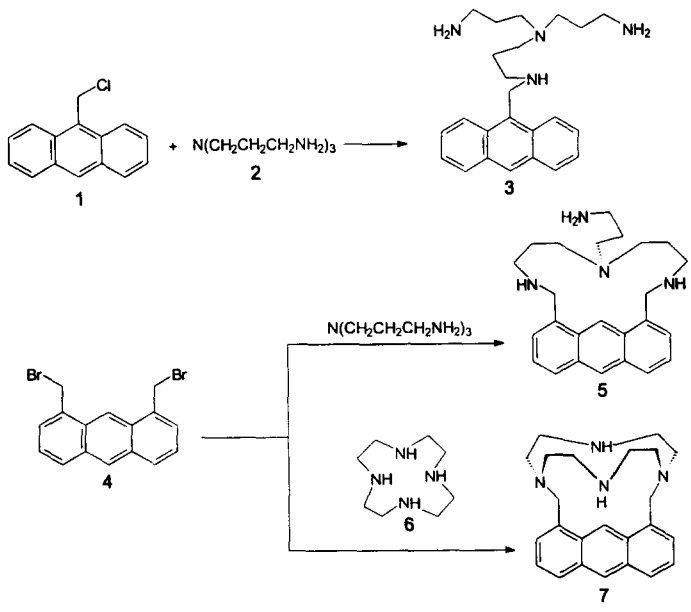
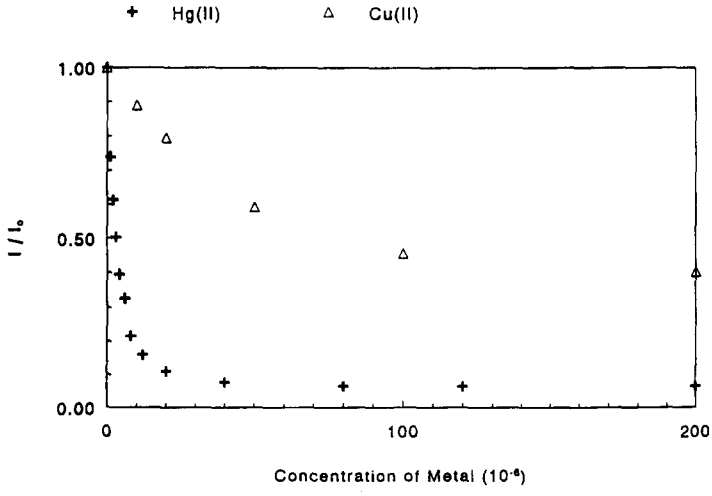
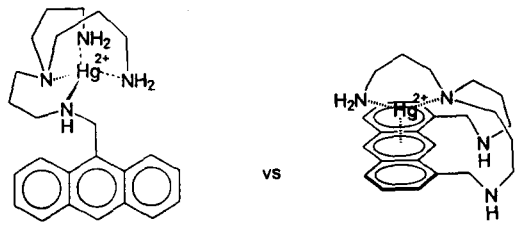


Figure 1



titrations are reported as upper limits, as the sensitivity of the fluorescence readout is insufficiently sensitive to distinguish the measured K_d's from much lower ones with such low ratios of bound/free Hg(II) under these conditions. Emission intensities of **5** with 8 μM Hg(II) in the presence of 1 mM of Al(III), Ca(II), Cd(II), Cr(III), Cu(II), Eu(III), Ga(III), Gd(III), In(III), Mg(II), Mn(II), Ni(II), Pb(II), Rb(I), Sr(II), Yb(III), and Zn(II) were as same as that using 8 μM Hg(II) alone (±5%). To confirm that selectivity occurs at the level of metal ion binding, the K_d's for Hg(II) were redetermined in the presence of 1 mM Zn(II) (K_d^{obsd} ≤1.1 μM), Ni(II) (K_d^{obsd} ≤1.0 μM), Co(II) (K_d^{obsd} ≤1.2 μM), and Gd(III) (K_d^{obsd} ≤1.1 μM); no increases in observed dissociation constants resulted. By contrast, while compound **7** demonstrates a typical pH-fluorescence profile (not shown, pK_a(app) 7.25), we observe **no** effect on its fluorescence by any metal ion examined up to 100 μM. We conclude that this cyclen immobilization serves to prohibit chelative complexation and thus supress metal ion (but not proton) binding. The binding data of chemosensor **5** can be compared productively with those of 9-(trpnmethyl)anthracene **3**. Anthrylpolyamine **3** likewise senses Hg(II) and Cu(II) among the metal ions examined. However, there is less discrimination between Hg(II) (K_d 14 μM) and Cu(II) (K_d 39 μM). Furthermore, the fluorescence quenching mechanism with Hg(II) may be different as well. The absorbance peak of **5** (12 μM) at 368 nm changed as the concentration of Hg(II) was increased, while there was no corresponding UV change in the titration of compound **3** with up to 800 μM Hg(II). After the addition of 8 μM Hg(II), the shape of the absorbance peak of **5** at 368 nm began to change with complete loss of the charateristic absorbance peak of anthracene after addition of 40 μM Hg(II). We propose different modes for the binding of **3** and **5** with Hg(II) as shown in Scheme 2. The additional aromatic interaction proposed for **5**/Hg(II) is reminescent of a previous observation from this lab,[11] and could be used to rationalize the similar (1.4-fold) affinities of Cu(II) to **5** and to **3**, but enhanced (13-fold) affinity of Hg(II) for chemosensor **5**.

Scheme 2



In summary, we report an observation of binding discrimination based upon the rigid immobilization of polyamine ligands onto a fluorophore framework, and a fluorescence chemosensor for Hg(II) (K_d ≤1 μM) that combines real-time reversibility with sufficient selectivity for the monitoring of an ionic mixture in 100% aqueous solution.

References and Notes

1. For an overview of work by many laboratories, see: (a) *Fluorescent Chemosensors for Ion and Molecule*

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2. For a novel, non-fiber optic based sensor platform, see: Colvin, A. E.; Phillips, T. E.; Miragliotta, J. A.; Givens, R. B.; Bargeron, C. B. *Johns Hopkins Applied Phys. Lab. Tech. Digest* **1996**, *17*, 377-385 (also viewable at: http://www.s4ms.com/.
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4. Additional work aimed at the creation of a fluorescent chemosensor for Hg(II) has been reported: Vaidya, B.; Zak, Y.; Bastiaans, G. J.; Porter, M. D.; Hallman, J. L.; Nabulsi, N. A. R.; Utterback, M. D.; Strzelbricka, B.; Bartsch, R. A. *Anyl. Chem.* **1995**, *67*, 4101-4111.
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6. **5:** ^{1}H NMR (250 MHz, D_2O) δ 1.88-2.00 (m, 2H, 1xCH_2), 2.05-2.24 (m, 4H, 2xCH_2), 2.88 (t, 2H, 1xCH_2), 3.10-3.24 (m, 10H, 5xCH_2), 4.57 (s, 4H, NH's and HOD), 7.27-7.35 (m, 5H, ArH), 7.78 (d, 2H, ArH), 7.89 (s, 1H, ArH [9- or 10-]), 8.16 (s, 1H, ArH [10- or 9-]); ^{13}C NMR (62.89 MHz, D_2O) δ 37.7, 38.87, 53.27, 59.96, 62.83, 66.64, 69.42, 131.86, 142.16, 142.87, 145.81, 146.03, 146.12, 146.90, 147.44, 148.00; Exact Mass EI mass spectrum, *m/e* calcd for $C_{25}H_{34}N_4$ $(M\text{-}4HCl)^+$, 390.278, found 390.278 Anal. Calcd for $C_{25}H_{34}N_4 \cdot 4HCl \cdot 4H_2O$: C, 49.35; H, 7.62; N, 9.21; Cl, 23.31. Found: C, 49.48; H, 7.54; N, 9.26; Cl, 23.37.
7. **7:** ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.59 (br s, 16H, 8xCH_2), 4.30 (br s, 4H, NH's and HOD), 7.32-7.39 (m, 4H, ArH), 7.93 (d, 2H, ArH), 8.44 (s, 1H, ArH [9- or 10-]), 9.44 (s, 1H, ArH [10- or 9-]); ^{13}C NMR (62.89 MHz, $CDCl_3$) 47.69, 53.55, 61.58, 121.73, 124.67, 128.39, 129.20, 129.26, 130.19, 132.23, 134.50; Exact Mass EI mass spectrum, *m/e* calcd for $C_{24}H_{33}N_4$ 374.247, found 374.247.
8. Huston, M. E.; Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1989**, *111*, 8735-8737.
9. There was also a fluorescence intensity decrease with Fe(III). However, we conclude that this decrease comes not from the CHEQ, but instead from the absorbance of Fe(III). Indeed, Fe(III) solution has a significant absorbance at 368 nm, which we used as the excitation wavelength. Furthermore, adding EDTA did not change the fluorescent intensity. We confirmed this does not come from Fe(III)-catalyzed decomposition of compound **5** as follows. First, the NMR spectra of compound **5** and **7** taken after extraction with $CHCl_3$ showed no sign of decomposion. Second, after the solution was basified with NaOH, $Fe(OH)_3$ was filtered and the pH of the resulting solution was readjusted to pH 7; the emission intensity was the same (±5%) as that of the solution that does not contain any Fe(III). Another piece of supporting evidence is that 1,8-(dihydroxymethyl)anthracene (not shown) and 9-(trpnmethyl)anthracene (**3**) showed almost same fluorescence effects upon Fe(III) addition as did compounds **5** and **7**.
10. Dissociation constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom.
11. Huston, M. E.; Engleman, C.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 7054-7056.

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