BODIPY Appended Crown Ethers: Selective Fluorescence Changes for Hg²⁺ Binding

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Recently, the development of fluorescent chemosensors capable of selective recognition and sensing of metal ions is one of the most challenging fields from the vantage of organic and supramolecular chemistry.[1,2] The best effective fluorescence chemosensor must convert the event of metal ion recognition by the ionophore into light signals of the fluorophore with high sensitivity and ease of monitoring.[3,4] In designing sensors, the ionophore linked to the fluorophore should be preliminarily considered because they are responsible for the selectivity and binding efficiency of the whole chemosensors.

There is a growing interest in synthesizing macromolecule appended fluorescence materials showing marked changes upon cation, anion, and neutral molecule complexation, aiming to develop ion-selective and sensitive material. The "hard" ether-oxygen containing macrocycles show a binding preference toward "hard" alkali and alkaline earth metal cation, but the incorporation of "soft" sulfide or amine linkages shifts its preference toward "soft" heavy metal cations.[5] In addition, it has been demonstrated that macrocyclic ligands containing nitrogen-sulfur donor atoms can behave as highly selective complexing agents for transition metal cations.[6,7] In this regards, sulfur containing macrocycles such as thiacrown ethers have been prepared and their complexation properties have been investigated with various metal cations.[8-10]

BODIPY (boradiazaindacenes) is well known fluorescent dye in that it gives high quantum yields, large extinction coefficients and narrow emission bands. These properties facilitated their application in many fields, such as fluorescent labeling of biomolecules, ion sensing and signaling, energy transfer cassettes, light harvesting systems and fluorescent stains.[11]

Currently, considerable attention has been focused on fluorescent chemosensors for the selective and rapid determination of the toxic heavy metal ions, such as the Pb²⁺, Cd²⁺, and Hg²⁺ ions.[12] Especially, in this regard, the Hg²⁺ ion is considered highly dangerous because both elemental and ionic mercury can be converted into methyl mercury by bacteria in the environment, which subsequently bioaccumulates through the food chain.[13] Therefore, there is a high demand for the detection of the Hg²⁺ ion both in environmental analysis and in industrial waste treatment.

In this paper, we report the synthesis and the fluorometric properties of BODIPY appended thiacrown 1 and crown ethers 2 and 3. Compound 1 displayed a highly selective chelation enhanced fluorescence (CHEF) effect only with Hg²⁺. Similar CHEF effect was observed upon the addition of Ag⁻ to 1, suggesting that silver ions also bind to the thiacrown, but less strongly than does Hg²⁺.

Synthesis of 1-3 was achieved by adaptation of procedures reported earlier.[14] 4-6 were treated with 2,4-dimethylpyrrole in the presence of TFA, which were subsequently oxidized (*p*-chloranil), neutralized (Et₃N), and treated with BF₃·Et₂O
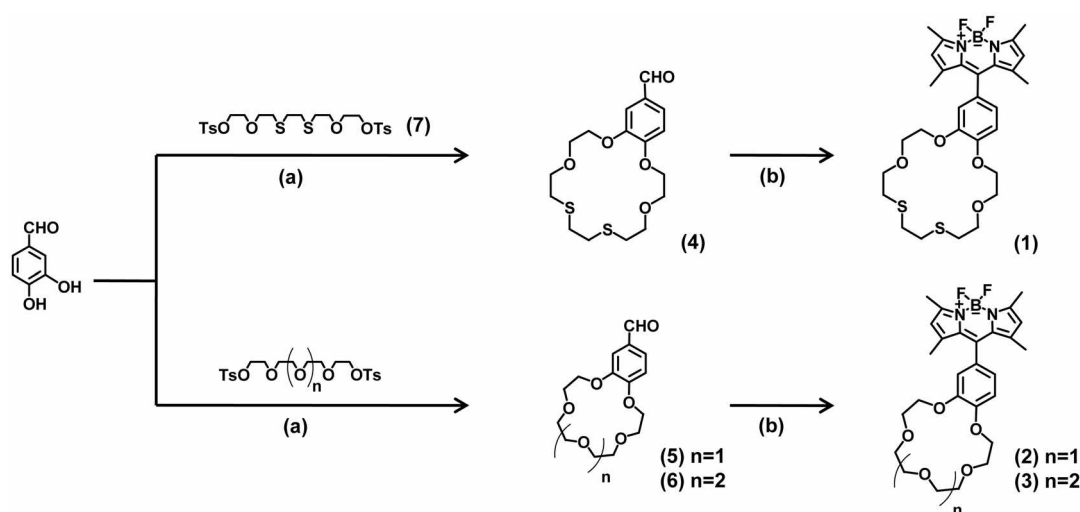
Scheme 1. Synthetic route to fluorogenic ligands **1-3**. Reagents: (a) K₂CO₃/CH₃CN; (b) 2,4-dimethylpyrrole/TFA/*p*-chloranil/Et₃N/BF₃·Et₂O/CH₂Cl₂.
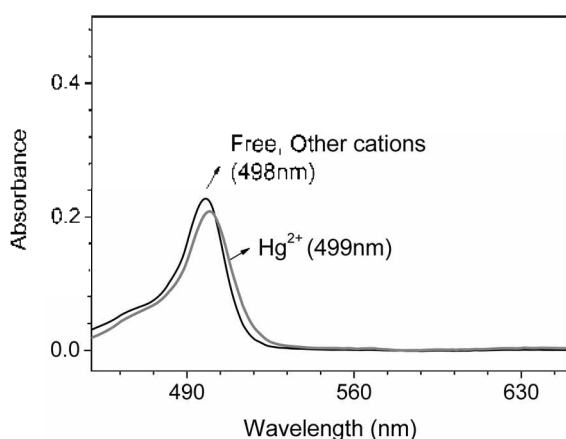
Figure 1. Absorption spectra of **1** (15.0 μM) with addition of ClO$_4$ salts of Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, Ag$^+$, Cd^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Co^{2+}, Cu^{2+}, and Al^{3+} (50 equiv, respectively) in H$_2$O/CH$_3$CN (6:4, v/v).

to produce the desired BODIPY derivatives **1-3**, respectively.

Metal ion binding properties of **1** were investigated by monitoring fluorescence and UV/vis changes upon the addition of Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, Ag$^+$, Cd^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Co^{2+}, Cu^{2+}, and Al^{3+} ions in H$_2$O/CH$_3$CN (6:4, v/v). The aqueous media (H$_2$O/CH$_3$CN (6:4 = v/v)) we have used in this experiment reasoned that water media are all required when fluorescence molecules can be utilized in a biological system as a fluorescent chemosensor. Free **1** showed a sharp and strong absorption band at 498.0 nm (Figure 1). When Hg^{2+} is bound to **1**, the absorption band of **1** at 498 nm was slightly red-shifted to 499.5 nm ($\Delta\lambda$ = 1.5 nm). However, other ions did not cause any detectable changes with **1**. To get insight into the binding mode of **1** especially two sulfur atoms for the Hg^{2+} binding, corresponding crowns **2** and **3** have been prepared, too. Although the crown-5 (**2**) and crown-6 (**3**) loops are well known to adopt Na$^+$ and K$^+$ ion, respectively, the UV/Vis band shift could not be observed in this experiment. This is because the BODIPY is positioned in perpendicular to benzocrown unit, resulting in that the ICT change is no longer influenced upon metal ion binding.

The fluorescence change results are represented in Figure 2. **1** shows a pronounced selectivity and sensitivity for Hg^{2+} when it is irradiated at 499 nm, whereas **2** and **3** does less selective towards most metal cations. Similar fluorescence change was observed upon the addition of Ag$^+$ to **1**, suggesting that the silver ions also bind to the sulfur atoms, but less strongly than does Hg^{2+} ion. The fluorescence enhancement phenomena of **1** upon Hg^{2+} binding are ascribable to the CHEF (chelation-induced enhanced fluorescence) mechanism.[15] When the Hg^{2+} ion interacts with sulfur atoms of the thiacrown part, the PET (photo-induced electron transfer) from sulfur atoms to fluorescence BODIPY group is inhibited. With Cu^{2+} ions, however, a marked quenching effect was observed. Coordination of **1** to Cu (II) having d^9-electronic configuration is likely to induce the photo-induced electron transfer to give a considerable fluorescence quenching.
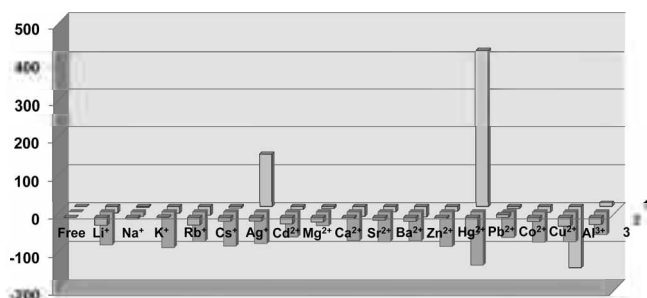

Figure 2. CHEF: fluorescence emission changes (I-I_0) of 0.50 μM solutions of **1-3** in H$_2$O/CH$_3$CN (6:4, v/v) upon addition of 50 equiv of various metal ions. Excitation at 498 nm; I_0: fluorescence emission intensity of free **1-3**. I: fluorescence emission intensity of metal complexes of **1-3**. (+) and (-) denote fluorescence increase and decrease, respectively.
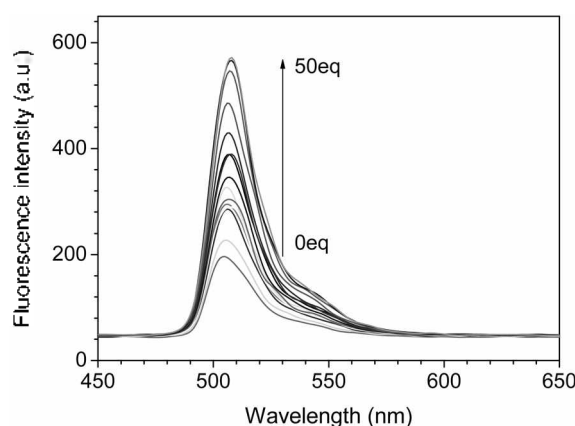

Figure 3. Fluorescence emission spectra of **1** (0.50 μM) for Hg^{2+} ion titration in H$_2$O/CH$_3$CN (6:4, v/v). (λ_{ex} = 499 nm).
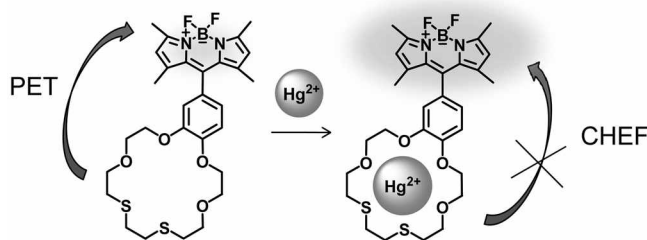

Figure 4. Suggested complexation mechanism of **1** for Hg^{2+} ion.

Free **1** showed an intense greenish fluorescence at 507 nm, in H$_2$O/CH$_3$CN (6:4, v/v) solution (λ_{ex} = 498 nm). With an excitation at 498 nm, the fluorescence intensity of **1** markedly increased in a function of [Hg^{2+}] (Figure 3). According to the extent of the fluorescence emission changes, we could obtain the association constants[16] of **1** (K_a = 1.59 × 10^5 M^{-1}) for Hg^{2+} ion.

We also carried out Job's plot experiment by varying the concentration of both **1** and Hg^{2+}. The maximum point at the mole fraction of 0.5 indicates typical ligand mole fraction for 1:1 (ligand : metal) complexes (Figure 5).

An important feature of chemosensor has to show high selectivity toward specific analyte over other competitive
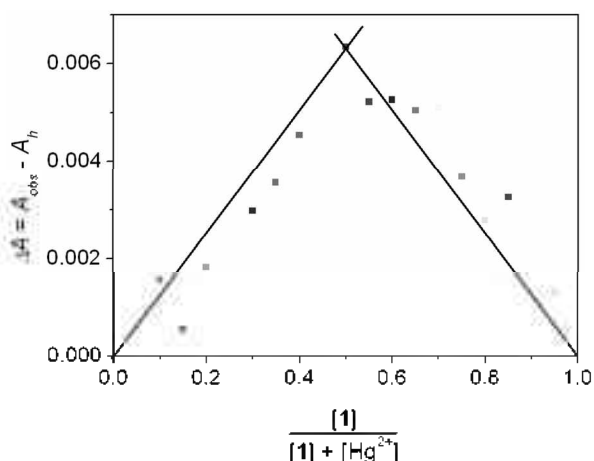
Figure 5. Job plot of a 1:1 complex of 1 and Hg^{2+} ion.

species. For the Hg^{2+} ion selectivity of **1**, competition experiments using miscellaneous cations including Li^-, Na^+, K^+, Rb^+, Cs^-, Ag^-, Cd^{2+}, Mg^{2-}, Ca^{2+}, Sr^{2+}, Ba^{2-}, Zn^{2+}, Pb^{2+}, Co^{2+}, Cu^{2-}, and Al^{3-} in H_2O/CH_3CN (6:4, v/v) were carried out and their results are recorded in Figure 6. The miscellaneous competitive cations did not lead to any significant fluorescence changes. Moreover, in the presence of miscellaneous competitive cations, the Hg^{2-} ion still resulted in the similar fluorescence increase. In addition, the increases of fluorescence intensity resulting from the addition of the Hg^{2-} ion was not influenced by the subsequent addition of miscellaneous cations.

Experimental Section

Precursors **5** and **6** were prepared by adaptation of reported procedures.[17]

Preparation of 1. This compound was prepared from **4** (300 mg, 1.1 mmol) according to a similar procedure outlined above for **3**. The compound was purified by flash column chromatography on silica gel using ethylacetate as eluent. Evaporation of the solvent yielded pale yellow vis-

cous oil which upon refrigeration gave the desired product as a pale yellow solid. Yield: 250 mg (53%). ^{1}H NMR (400 MHz, CDCl₃): δ 6.94 (d, 2H, Ar*H*), 6.77 (d, 1H, Ar*H*), 5.97 (s, 2H, Ar*H*), 4.20 (m, 4H, -O*CH₂*CH₂O-), 3.92 (m, 4H, -OCH₂*CH₂*O-), 3.80 (m, 4H, -O*CH₂*CH₂O-), 2.88 (s, 4H, -S*CH₂CH₂*S-), 2.80 (q, 4H, -S*CH₂*CH₂O-), 2.54 (s, 6H, -Ar*H*), 1.46 (s, 6H, -Ar*H*). ^{13}C NMR (100 MHz, CDCl₃): δ 146.9, 143.5, 143.2, 138.2, 129.0, 126.1, 125.6, 121.3, 119.4, 119.2, 72.6, 69.7, 46.4, 32.8, 29.9, 14.7, 8.8 ppm. FAB MS *m/z* (M⁻): calcd. 590.23 Found, 590.0.

Preparation of 2. This compound was prepared from **5** (300 mg, 1 mmol) according to a similar procedure outlined above for **3**. The compound was purified by flash column chromatography on silica gel using ethylacetate as eluent. Evaporation of the solvent yielded pale yellow viscous oil which upon refrigeration gave the desired product as a pale yellow solid. Yield: 300 mg (57%). ^{1}H NMR (400 MHz, CDCl₃): δ 6.95 (d, 2H, Ar*H*), 6.79 (d, 1H, Ar*H*), 5.97 (s, 2H, Ar*H*), 4.20 (m, 4H, -O*CH₂*CH₂O-), 3.96 (m, 4H, -OCH₂*CH₂*O-), 3.79 (m, 4H, -O*CH₂*CH₂O-, 4H, -OCH₂*CH₂*O-), 2.54 (s, 6H, -Ar*H*), 1.46 (s, 6H, -Ar*H*). ^{13}C NMR (100 MHz, CDCl₃): δ 155.7, 143.4, 130.0, 128.2, 121.3, 113.6, 70.9, 70.7, 70.0, 69.5, 68.9, 14.7 ppm. FAB MS *m/z* (M⁻): calcd. 514.25 Found, 514.0.

Preparation of 3. To a solution of 2,4-dimethylpyrrole (0.18 g, 1.89 mmol) and **6** (0.25 mg, 0.73 mmol)in dried dichloromethane, 2 drops of CF_3CO_2H was added. The yellow solution was stirred for 3 h at room temperature under N_2. A solution of *p*-chloranil (0.47 g, 1.89 mmol) in CH_2Cl_2 (100 mL) was then added. After stirring for 30 min, Et_3N and $BF_3 \cdot OEt_2$ were subsequently added until a bright-green fluorescence was observed. The solution was washed with water, and the organic layer was dried over anhydrous $MgSO_4$. Removal of the organic solvent *in vacuo* afforded a reddish solid. Column chromatography on silica gel with EtOAc-hexane (2:1) as eluents gave 0.29 g (70%) of **3**. ^{1}H NMR (400 MHz, CDCl₃): δ 6.95 (d, 2H, Ar*H*), 6.76 (d, 1H, Ar*H*), 5.97 (s, 2H, Ar*H*), 4.20 (m, 4H, -O*CH₂*CH₂O-), 3.98 (m, 4H, -OCH₂*CH₂*O-), 3.80 (m, 4H, -O*CH₂*CH₂O-), 3.73
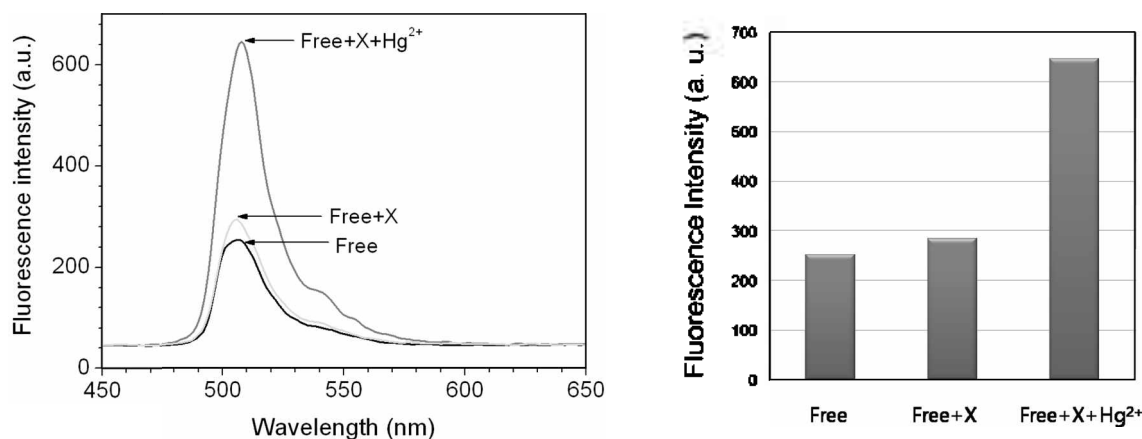
Figure 6. Fluorescence spectra of **1** (10 μM) in H_2O/CH_3CN (6:4, v/v) in the presence of the Hg^{2-} ion and miscellaneous cations including Li^-, Na^-, K^-, Rb^+, Cs^-, Ag^+, Cd^{2-}, Mg^{2-}, Ca^{2-}, Sr^{2-}, Ba^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Cu^{2-}, and Al^{3-} (10 equiv, respectively; excitation wavelength at 498 nm). All spectral data were recorded at 10 min after Hg^{2+} addition.

(m, 4H, -OCH$_2$CH$_2$O-, 4H, -OCH$_2$CH$_2$O-). 2.54 (s, 6H, -Ar*H*), 1.46 (s, 6H, -Ar*H*). ^{13}C NMR (100 MHz, CDCl$_3$): δ 155.6. 143.4. 131.9. 131.8, 121.4. 121.3. 121.0, 70.4, 70.3. 70.2. 14.8, 14.7 ppm. FAB MS m/z (M$^+$): calcd. 558.27 Found. 558.0.

Preparation of 4. A mixture of 3.4-dihydroxybenz-aldehyde (1 g. 7.24 mmol) and K$_2$CO$_3$ (1 g. 7.24 mmol) was taken in CH$_3$CN (50 mL). Compound 7 (1.43 g, 7.23 mmol) was added to it with stirring and refluxed under nitrogen atmosphere for 18 hours. The cooled mixture was evaporated to dryness and the residue was extracted with CHCl$_3$ for 3 or 4 times. The extract was evaporated to yield a viscous oil which was purified by column chromatography on silica gel column using CHCl$_3$ (ethyl acetate/hexane, 1:2) as eluent. Yield: 2.1 g (77%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.83 (s, 1H, C*H*O). 7.46 (d, 2H, Ar*H*), 6.9 (d, 1H, Ar*H*). 4.36 (m, 4H, -OCH$_2$CH$_2$O-), 3.93 (m, 8H, -OCH$_2$CH$_2$O-), 3.86 (m, 4H, -OCH$_2$CH$_2$S-). 2.95 (s, 4H, -SCH$_2$CH$_2$S-). 1.22 (s, 4H, -OCH$_2$CH$_2$O-). ^{13}C NMR (100 MHz, CDCl$_3$): δ 190.9. 155.1. 150.2. 131.1. 127.4. 447.9. 72.8. 72.2. 71.9. 68.9. 32.9, 32.0. 29.9 ppm. FAB MS m/z (M$^+$): calcd, 372.11 Found, 372.0.

Preparation of 7. Under nitrogen, a solution of 1,2-ethanedithiol (0.5 g. 5.3 mmol), and NaOH (0.44 g. 10.6 mmol) in ethanol (100 mL) was stirred at room temperature. Then 2-(2-chloroethoxy)ethanol (1.38 g. 10.6 mmol) was added to it with stirring and refluxed under N$_2$ atmosphere for 2 hours. After stirring for 24 h, the solvent was evaporated *in vacuo*. The resulting brownwish oil was used directly for the next reaction. The resulting residue treated with tosyl chloride (1.47 g, 7.4 mmol) and NaOH (0.3 g, 7.7 mmol) in THF (100 mL). After stirring at room temperature for 24 h. the solvent was evaporated *in vacuo*, and the mixture was dissolved in CH$_2$Cl$_2$ (100 mL). The organic layer was washed with water (300 mL) and dried over anhydrous Na$_2$SO$_4$ and filtered. Purification by column chromatography on silica gel (ethyl acetate/hexane, 1:4) provided 1.5 g of yellowish oil 7 in 50% yield. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.74 (d, 4H, Ar*H*), 7.30 (d, 4H, Ar*H*). 4.06 (m, 4H, -OCH$_2$CH$_2$O-). 3.59 (m, 4H, -OCH$_2$CH$_2$O-). 3.51 (m, 4H, -OCH$_2$CH$_2$O-), 2.66 (s, 4H, -SCH$_2$CH$_2$S-). 2.58 (m, 4H, -SCH$_2$CH$_2$O-), 2.37 (s, 6H, -OCH$_2$CH$_2$O-). ^{13}C NMR (100 MHz, CDCl$_3$): δ 145.1, 133.0. 130.1. 128.1. 71.3, 69.4, 68.5. 38.7, 37.7, 32.8. 32.1. 31.6, 21.8 ppm. FAB MS m/z (M$^-$): calcd. 578.11 Found. 578.0.

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