

Immobilization of Azacrown Ligand onto a Fluorophore

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Abstract

Geometric immobilization of azacrown ligand onto a fluorophore is expected to change their binding properties toward ion discrimination. An immobilized azacrown ligand **1** onto anthracene fluorophore senses Al(III), Cu(II) and Ga(III) in ethanol among the metal ions examined. In 100% aqueous solution, ligand **1** shows large CHEF effects with Al(III) and Ga(III) and large CHEQ effect with Hg(II). By comparison, non-immobilized azacrown ligand **2** showed large CHEF effects with Al(III), Ce(III), Ga(III), La(III) and Zn(II) in ethanol.

Introduction

Selective binding of chemical species upon molecular recognition can lead to large perturbations of the host environment, especially when the guest is ionic. Fluoroionophores chemically communicate ion concentrations and have been the subject of substantial investigation in metal ion analysis [1]. The advent of ligand engineering has introduced a more systematic approach to the design of chemosensors with new selectivity and signal transduction schemes.

In 1977, Sousa described the synthesis of naphthalene-crown ether probes in which the fluorophore π -system was insulated from the donor atoms by at least one methylene group ('conjugate' fluoroionophores) [2]. These compounds demonstrated fluorescence changes of up to 6-fold upon the binding of alkali-metal salts in 95% ethanol glass at 77 K. Subsequent reports by Bouas-Laurent [3], de Silva [4], Czarnik [5], Fabbrizzi [6], Shinkai [7] and other groups [1] have built on this original premise, in which binding of ions or even neutral organic guests to the appropriate ligands has been coupled to emission changes of covalently attached fluorophore.

As a consequence of the rapid development of supramolecular chemistry, supramolecular technology has developed the synthesis of fluorescent probes for metal cations. In addition, the general high affinity of azacrown ligands or polyamine ligands for metal ions has provided for the formulation of metal ion chemosensors. However, the same high affinity makes ion discrimination based on complexation affinities problematical. Recently, Czarnik and his coworkers reported that the immobilization of polyamine ligands to the fluorophore changed the binding properties of these ligands

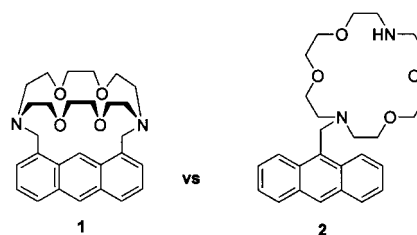


Figure 1. Immobilized ligand (**1**) and non-immobilized ligand (**2**).

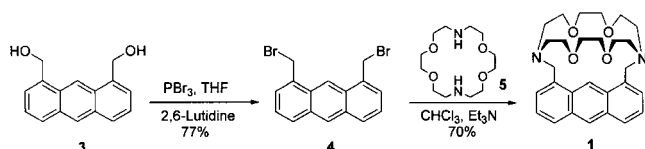
toward transition metal ions [8]. In this paper, the immobilized tris(3-aminopropyl)amine ligand showed a sufficient selectivity for Hg(II).

We report here an approach to the attainment of binding discrimination in chemosensor, based upon the rigid immobilization of azacrown ligand onto a fluorophore framework (Figure 1).

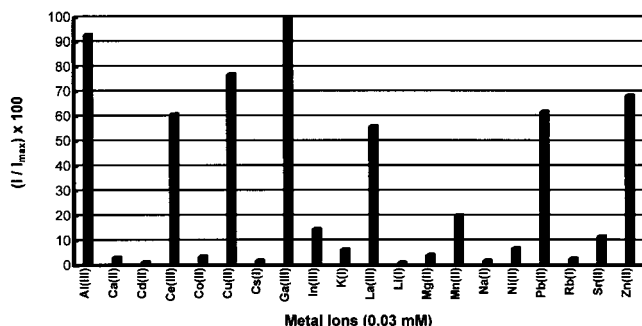
Results and discussion

We prepared immobilized ligand **1** by reaction of the azacrown ligand (**5**) with 1,8-bis(bromomethyl)anthracene (**4**) as shown in Scheme 1. Our synthesis began with 1,8-bis(hydroxymethyl)anthracene (**3**), which was then transformed to 1,8-bis(bromomethyl)anthracene (**4**) using the procedures of Nakagawa and co-workers [9]. Compound **1** was synthesized by the addition of **4** to a mixture of azacrown (**5**; 1 eq.), K₂CO₃ and CHCl₃ at room temperature. After the purification by silica gel column chromatography using 10% MeOH/CHCl₃, compound **1** was obtained in 70% yield. Compound **2** was synthesized following the literature procedure [10].

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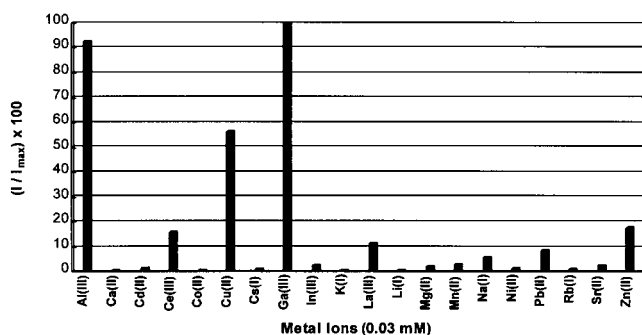
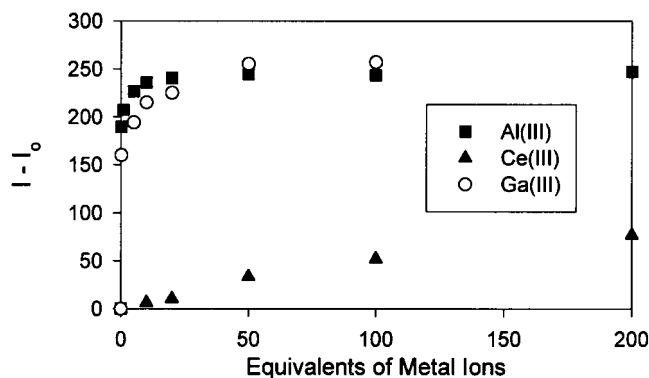


Scheme 1. Synthesis of compound 1.

Figure 2. CHEF effects of compound 2 (6 μM) with metal ions (50 eq.) in ethanol at 420 nm.

If the metal ion binds to the azacrown, a large CHEF effect is observed due to the inhibition of photoinduced electron transfer. Al(III), Ca(II), Cd(II), Ce(III), Cs(I), Cu(II), Co(II), Ga(III), In(III), K(I), La(III), Mg(II), Mn(II), Na(I), Ni(II), Pb(II), Rb(I), Sr(II), and Zn(II) ions were used to evaluate metal ion binding in ethanol [11]. Using these metal ions (0.3 mmol, 50 eq.), compound 2 (6 μM) displayed large chelation-enhanced fluorescence (CHEF) effects with Al(III), Ce(III), Cu(II), Ga(III), La(III) and Zn(II) (Figure 2). Compound 2 also showed relatively small CHEF effects with In(III), Mn(III) and Sr(II). By comparison, large CHEF effects were obtained for compound 1 (6 μM) only with Al(III), Cu(II), and Ga(III), even though compound 1 also displayed relatively small CHEF effects with Ce(III), La(III) and Zn(II) (Figure 3). The CHEF effects of compound 1 with Ce(III), La(III) and Zn(II) were suppressed quite significantly compared to those of compound 2.

The fluorescence titrations of immobilized ligand 1 with Al(III) ($K_d \leq 1 \mu\text{M}$), Ga(III) ($K_d \leq 1 \mu\text{M}$) and Ce(III) ($K_d 1.72 \text{ mM}$) [12] are shown in Figure 4. The K_d values for Al(III) and Ga(III) 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 metal ion under these conditions. For compound 2, the apparent dissociation

Figure 3. CHEF effects of compound 1 (6 μM) with metal ions (50 eq.) in ethanol at 420 nm.Figure 4. Fluorescent titrations of compound 1 (6 μM) with Al(III), Ce(III) and Ga(III) in ethanol at 420 nm.

constants were calculated as follows; Al(III) ($K_d \leq 1 \mu\text{M}$), Ga(III) ($K_d 19.6 \mu\text{M}$) and Ce(III) ($K_d 370 \mu\text{M}$).

We expect that this immobilization of azacrown ligand to the fluorophore changed the binding properties of this ligand toward metal ions. Azacrown immobilization serves to prohibit chelative complexations with Ce(III), La(III) and Zn(III) ions. From the pH-fluorescence profile, pK_a of compound 1 was calculated as 6.5. An observed fluorescence dependence on pH is in keeping with the intramolecular amine quenching mechanism that has been described previously. Protonation of an amine group in fluorophore-amine conjugates results in the elimination of photoinduced electron transfer. Therefore, fluorescence is expected to be a function of pH, and pH measurement using anthrylamines has been described by de Silva [13]. To evaluate metal ion binding in 100% aqueous solution, we checked the fluorescence change of compound 1 at pH 7 since both CHEF and CLEQ (chelation-enhanced quenching) effects can be observed at this pH. If a quenching metal ion (e.g., open-shell, paramagnetic, large or easily reducible cation [14]) binds tightly to the immobilized ligand 1, intracomplex quenching takes place.

50 Equivalents of Al(III), Ca(II), Cd(II), Ce(II), Cs(I), Cu(II), Co(II), Ga(III), Hg(II), In(III), K(I), La(III), Li(I), Mg(II), Mn(II), Na(I), Ni(II), Pb(II), Rb(I), Sr(II), and Zn(II) ions were used to evaluate metal ion binding of compound 1 (6 μM) at pH 7 (0.1 M HEPES) [11]. Among the metal ions examined, Al(III) and Ga(III) showed large CHEF effects, on the other hand, Hg(II), an inherent quenching metal ion, showed a CHEQ effect (Figure 5).

We are investigating the structure and binding affinity of these metal ions with azacrown ligands (1, 2) using DFT calculations. The results will be reported elsewhere.

Conclusion

In conclusion, we report an observation of binding discrimination based upon the rigid immobilization of azacrown ligand onto a fluorophore framework. The binding data of immobilized ligand 1 can be compared productively with those of non-immobilized ligand 2. In addition, immobilized ligand 1 senses Al(III), Ga(III), and Hg(II) ions in

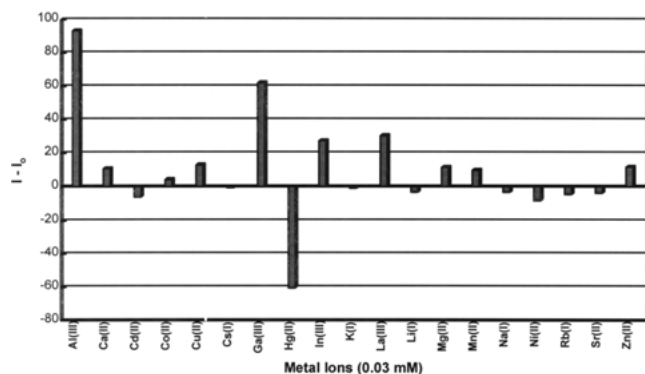


Figure 5. CHEF effects of compound **1** (6 μ M) with metal ions (50 eq.) at pH 7 (0.1 M HEPES) at 420 nm.

100% aqueous solution. These results suggest that the rigid immobilization of azacrown ligand onto a fluorophore framework may be employed successfully in the creation of selective chemosensors. Such selectivity is not yet predictable, but readily established by screening techniques.

Experimental

Instruments and chemicals

NMR spectra were recorded at 300 MHz (for ^1H -NMR) and at 75 MHz (for ^{13}C -NMR) using a Varian FT-NMR Spectrometer in the Korea Basic Science Institute in Pusan, Korea. Mass spectra were obtained using a Micromass spectrometer in the Korea Basic Science Institute in Pusan, Korea. Melting point was determined in open capillaries, and are uncorrected. UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence measurements were made on a RF-5301 PC Spectrofluorophotometer with excitation at 367 nm; both emission and excitation slit widths were 5 nm. pH measurements were determined using a HI 9321 Microprocessor pH meter. Flash chromatography was carried out using Merck silica gel 60 (230 to 400 mesh). Thin layer chromatography was carried out using Merck 60 F₂₅₄ plates with a 0.25-mm thickness.

Most of the chemicals including metal salts and HEPES were obtained from Sigma-Aldrich Chemical Company, Milwaukee, WI. 1,8-bis(hydroxymethyl)anthracene and 4,13-diazacrown were purchased from TCI (Tokyo Kasei Organic Chemicals) The CHCl_3 , CH_2Cl_2 , and MeOH were distilled from CaH_2 . Compound **2** was prepared as described in the literature [10].

Synthesis

1,8-(4,13-Diazacrown)bismethylantracene (**1**)

To a solution of 4,13-diazacrown (0.71 g, 2.7 mmol), triethylamine (0.75 ml, 5.4 mmol) and anhydrous CHCl_3 (200 ml) was added 1,8-bis(bromomethyl)anthracene (1.0 g 2.7 mmol). The reaction mixture was stirred at room temperature for 4 hrs. The solution was concentrated *in vacuo* to yield a tan solid, which was loaded onto a silica gel column and

eluted with 10% MeOH/ CHCl_3 . The appropriate fractions were combined, and concentrated *in vacuo* to yield **1** as a yellow solid (0.83 g, 70%): mp 163 $^\circ\text{C}$; UV (λ_{max} , ethanol) 350 nm, 367 nm, 388 nm; ^1H NMR (CDCl_3 , 300 MHz) δ 2.92 (br s, 8H), 3.53–3.75 (m, 16H), 4.48 (s, 4H), 7.30 (t, J = 8.1 Hz, 2H), 7.45 (br s, 2H), 7.80 (d, J = 8.6 Hz, 2H), 8.31 (s, 1H), 9.72 (s, 1H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 54.47, 58.09, 70.13, 70.98, 121.17, 124.90, 125.02, 126.46, 127.29, 129.99, 131.65, 136.62; exact mass EI mass spectrum m/e 464.2684 (M^+), calcd for $\text{C}_{28}\text{H}_{36}\text{N}_2\text{O}_4$ 464.2675.

General fluorescence solution preparation

For metal titrations, stock solutions of **1** and **2** (0.06 mM) were prepared in ethanol. A given titration solution was prepared by placing 0–3000 μl of the appropriate metal (1 mM or 3 mM) in ethanol solution followed by addition of the probe solution (100 μl), resulting in a total volume of 4.000 ml.

For all measurements, excitation was at 367 nm; emission was measured at 420 nm. Both excitation and emission slit widths were 5 nm.

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