



## New Fluorescent Chemosensors for Cationic Guests: 1,8-Bis(azacrown)anthracenes

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### Abstract

New macrocyclic ditopic anthracenyl receptors display large chelation-enhanced fluorescence (CHEF) effects upon the addition of linear dications  $^+\text{NH}_3(\text{CH}_2)_n\text{NH}_3^+$ . The amplitude of the effect, which is dependent on the chain length, was at a maximum for propylenediammonium cation. This 1,8-bisazacrown anthracene displays different binding affinities toward metal ions compared to those of 9-azacrown anthracene.

### Introduction

Fluorescence offers an analytical tool with unique and useful properties. The sensitivity of a fluorescence assay can be exquisite; in the extreme, single atoms can be visualized. In particular, anthracene derivatives containing a benzylic amine group have been used extensively as photoinduced electron transfer (PET) sensors for cations, anions or even neutral organic guests [1, 2]. The report by Fages and his co-workers in which a ditopic bisanthracenyl compound was used as a receptor for alkyldiammonium ions was particularly noteworthy [3]. Also, Gawley *et al.* reported that 9-(azacrownmethyl)anthracenes showed up to a 30-fold increase in fluorescence in the presence of saxitoxin [4].

Recently, 1,8-anthrylpolyamines have been utilized as fluorescence chemosensors for pyrophosphate or as binuclear dephosphorylation reagents [5]. These compounds possess two binding sites and generate a rigid binding pocket, due to the distance between the 1- and 8-positions of the anthracene moiety. Furthermore, the rigid immobilization of polyamine or azacrown ligands to the 1,8-position of anthracene is reported to change the binding properties of these ligands for metal ions [6].

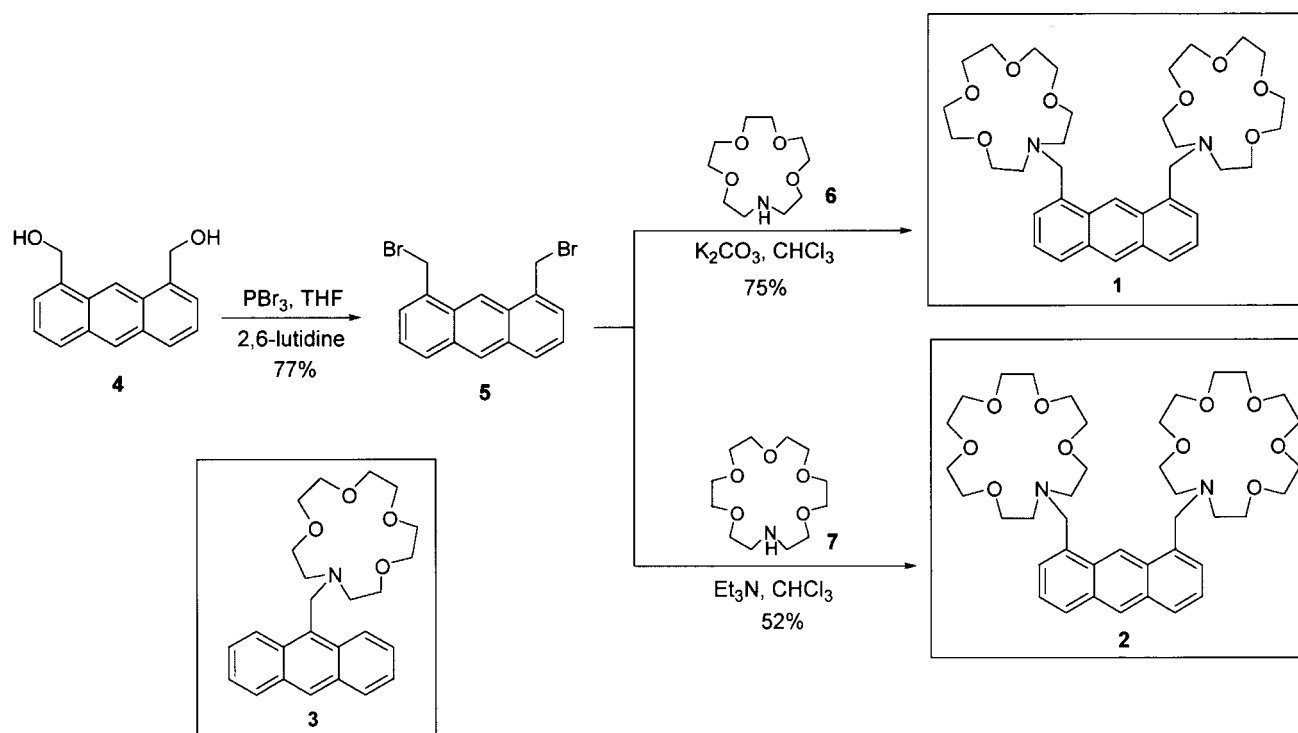
Herein, we report two new 1,8-bisazacrown anthracene derivatives as potential PET sensors for alkyldiammonium ions. These compounds contain two azacrown ligands at the 1- and 8-position of anthracene, which act as two binding sites for ammonium ions. Also, the binding properties of compound **1** toward metal ions are quite different from those of 9-azacrownmethylantracene (**3**).

### Results and discussion

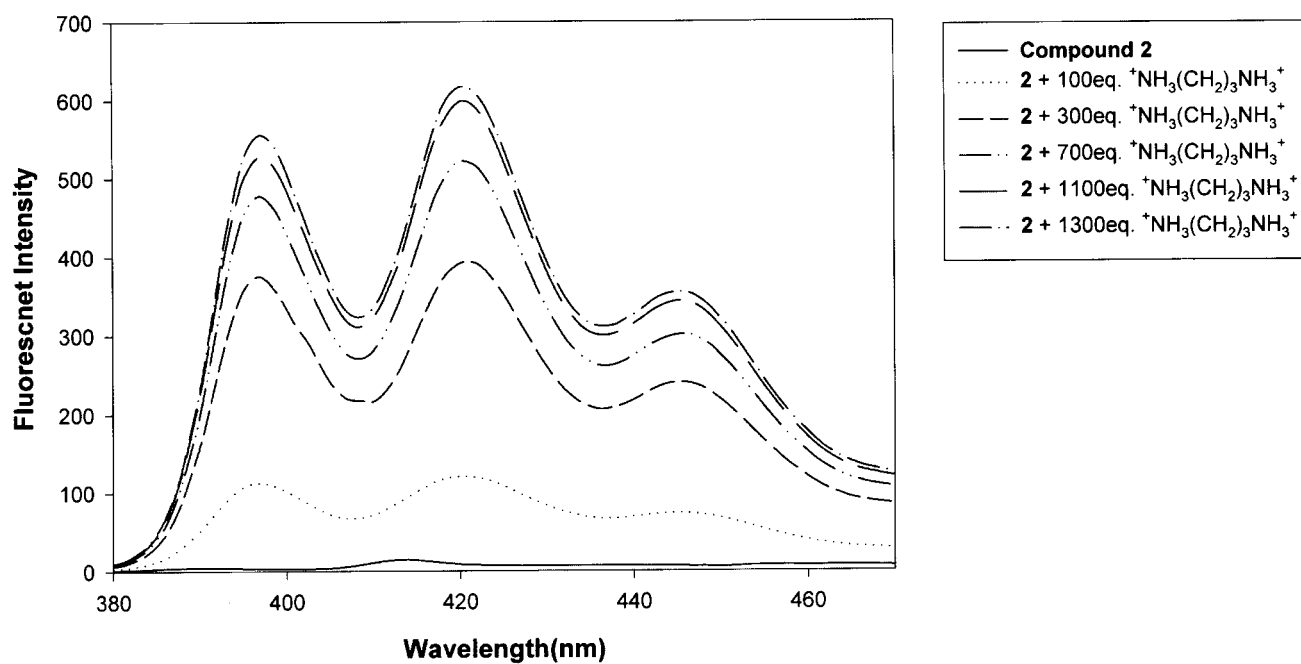
Compounds **1** and **2** were prepared by reacting the appropriate azacrown ligand with 1,8-bis(bromomethyl)anthracene (**5**) as shown in Scheme 1. The synthesis began with 1,8-bis(hydroxymethyl)anthracene (**4**), which was then transformed to 1,8-bis(bromomethyl)anthracene (**5**) using the procedures of Nakagawa and co-workers [7]. Compound **1** was synthesized by adding **5** to a mixture of 1-aza-15-crown-5 (**6**; 3 eq.),  $\text{K}_2\text{CO}_3$  and  $\text{CHCl}_3$  at room temperature. After purification by silica gel column chromatography, using 10% MeOH/ $\text{CHCl}_3$  as eluent, compound **1** was obtained in 75% yield. Application of the above method to 1-aza-18-crown-6 (**7**; 3 eq) and triethylamine instead of  $\text{K}_2\text{CO}_3$  gave compound **2** in 52% yield, after the column chromatography ( $\text{CHCl}_3$ :MeOH, 9:1). The structures of these compounds were confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and exact mass EI mass spectroscopy. Compound **3** was synthesized following the literature procedure [8].

From the pH-fluorescence profile,  $\text{p}K_a$ 's of benzylic amines in compounds **1** and **2** were calculated as 6.6 and 7.4, respectively. In ethanol or methanol/chloroform (9:1), fluorescence was quenched by PET from the fairly reducing amine group to the excited singlet state of anthracene (Figure 1). Upon the addition of linear dications such as alkyldiammonium ions, these compounds (6  $\mu\text{M}$ ) displayed large chelation-enhanced fluorescence (CHEF) effects (up to 60 fold) since hydrogen bonding between benzylic amine and ammonium ion can cause inhibition of the PET process. Figure 1 explains the CHEF effect of compound **2** upon adding  $^+\text{NH}_3(\text{CH}_2)_3\text{NH}_3^+$ . Also, Figure 2 proposes the possible binding mode of compound **1** with  $^+\text{NH}_3(\text{CH}_2)_4\text{NH}_3^+$ .

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Scheme 1. Syntheses of compounds 1 and 2.

Figure 1. Fluorescence emission spectra of **2** ( $6 \mu\text{M}$ ) upon the addition of various amounts of 1,3-propane diammonium ion in ethanol (excitation: 367 nm).

The structure of compound **1** complexed with  $^+\text{NH}_3(\text{CH}_2)_4\text{NH}_3^+$  was fully optimised with *ab initio* MO calculations at the RHF/3-21G level using Gaussian94 [9].

The azacrown ligands of these compounds are immobilized onto the 1- and 8-positions of the anthracene moiety. The stability constants of the fluorescence titrations of compound **1** with alkyldiammonium ions, were calculated as follows:  $^+\text{NH}_3(\text{CH}_2)_3\text{NH}_3^+$  ( $K_s = 4412 \text{ M}^{-1}$ ),  $^+\text{NH}_3(\text{CH}_2)_4\text{NH}_3^+$  ( $K_s = 272 \text{ M}^{-1}$ ),  $^+\text{NH}_3(\text{CH}_2)_6\text{NH}_3^+$  ( $K_s = 98 \text{ M}^{-1}$ ),  $^+\text{NH}_3(\text{CH}_2)_5\text{NH}_3^+$  ( $K_s = 35 \text{ M}^{-1}$ ). Figure 3 explains the fluorescence emission changes of **1** upon the addition of 300 equivalents of alkyl diammonium ions in ethanol. We expect this selectivity is the result of the distance between the two ligands, which are located at the 1- and 8-positions of anthracene. In our case, the anthracene moiety does not only act as a signal source, but also serves as a template for such selectivity. From the fluorescent titration experiments, compound **2** also displayed similar binding selectivity.

The CHEF effects of compound **1**, caused by metal ion binding are quite different from those of compound **3**, in which an azacrown ligand is attached at the 9-position of anthracene.

Ca(II), Cd(II), Co(II), Cu(II), Mg(II), Mn(II), Na(I), Ni(II), Sr(II) and Zn(II) were used to evaluate metal ion binding in ethanol. Using these metal ions (0.3 mmol, 50 eq.), compound **3** (6  $\mu\text{M}$ ) displayed large chelation-enhanced fluorescence (CHEF) effects with Ca(II), Co(II), Cu(II), Mg(II), Mn(II) and Ni(II) (Figure 4). Compound **3** also showed relatively small CHEF effects with Sr(II) and Zn(II). By comparison, a large CHEF effect was observed for compound **1** (6  $\mu\text{M}$ ) only with Cu(II), even though compound **1** also displayed relatively small CHEF effects with Cd(II), Co(II), Mg(II), Mn(II) and Ni(II) (Figure 5). The CHEF effects of compound **1** with Ca(II), Co(II), Mg(II), Mn(II), Ni(II), Sr(II) and Zn(II) were significantly suppressed compared to those of compound **3**.

In fluorescence titrations of compound **3** with metal ions in ethanol, the apparent dissociation constants were calculated as follows; Cu(II) ( $K_d \leq 1 \mu\text{M}$ ), Mg(II) ( $K_d = 4.8 \mu\text{M}$ ) and Co(II) ( $K_d = 6.6 \mu\text{M}$ ) [10]. The  $K_d$  value for Cu(II) titration is reported as the upper limit, 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 **1** also in ethanol, the apparent dissociation constants were calculated as follows; Cu(II) ( $K_d = 3.2 \mu\text{M}$ ), Mg(II) ( $K_d = 2.4 \text{ mM}$ ) and Co(II) ( $K_d = 26 \text{ mM}$ ) [10]. As shown in Job's Plot (Figure 6), compound **1** shows 1:1 binding with Mg(II) in ethanol. Compound **1** was about 1000 times more selective for binding Cu(II) over Mg(II) and about 10,000 times more selective for binding Cu(II) over Co(II). These results can be productively compared with those of compound **3**.

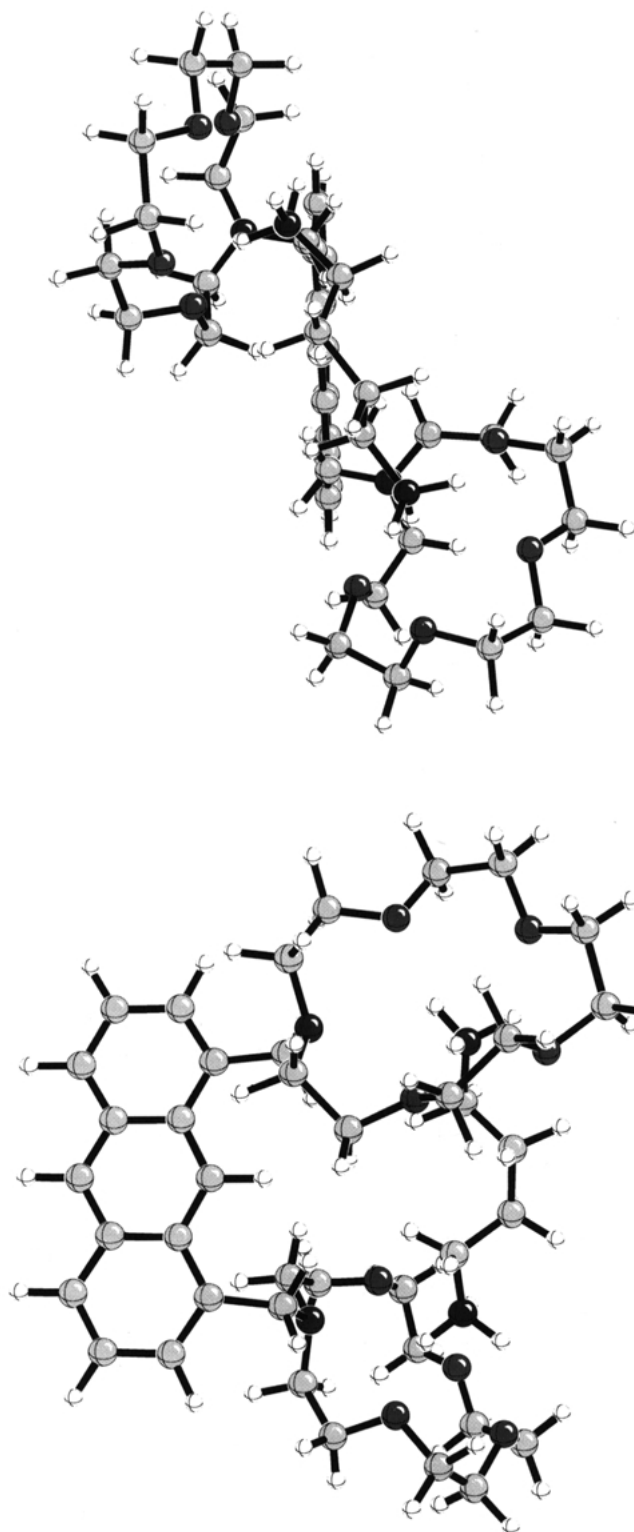


Figure 2. Proposed binding mode of compound **1** with  $^+\text{NH}_3(\text{CH}_2)_4\text{NH}_3^+$  using *ab initio* molecular orbital calculations.

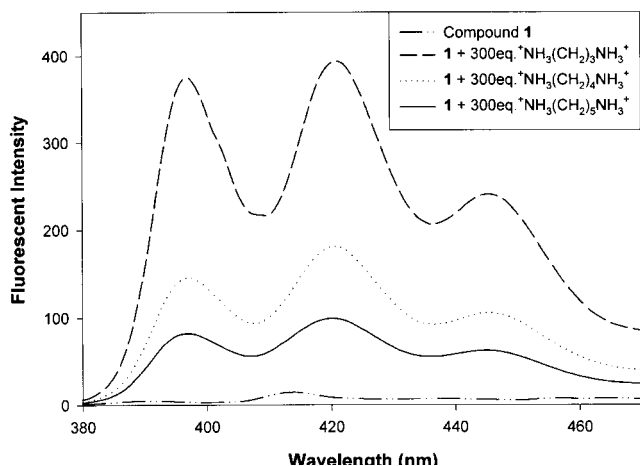


Figure 3. Fluorescence emission spectra of **1** ( $6 \mu\text{M}$ ) upon the addition of 300 equivalents of alkyl diammonium ion in ethanol (excitation: 367 nm).

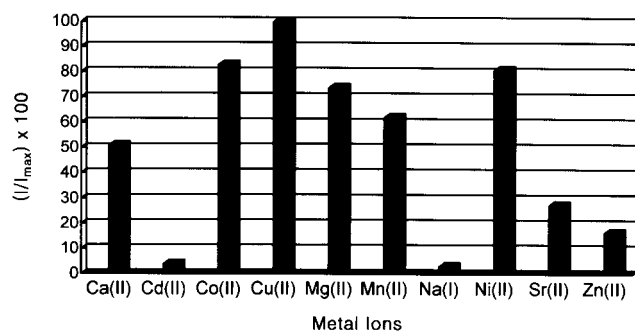


Figure 4. CHEF effects of compound **3** ( $6 \mu\text{M}$ ) with various metal ions (50 eq.) in ethanol at 420 nm.

## Conclusion

In conclusion, we report large CHEF effects of 1,8-bis(azacrownmethyl)anthracenes with alkyldiammonium ions. Also, of the metal ions examined, only Cu(II) showed a large CHEF effect with compound **1**. These results suggest that the immobilization of azacrown ligand onto a fluorophore framework may be employed successfully in the creation of selective chemosensors.

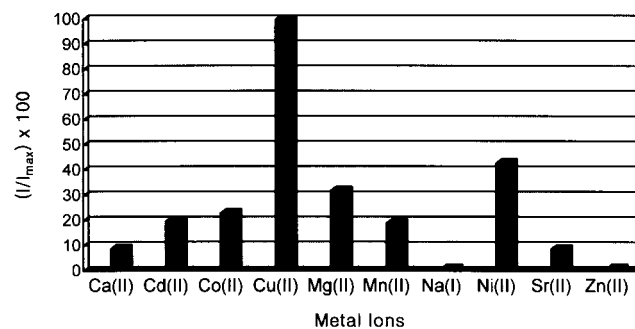


Figure 5. CHEF effects of compound **1** ( $6 \mu\text{M}$ ) with various metal ions (50 eq.) in ethanol at 420 nm.

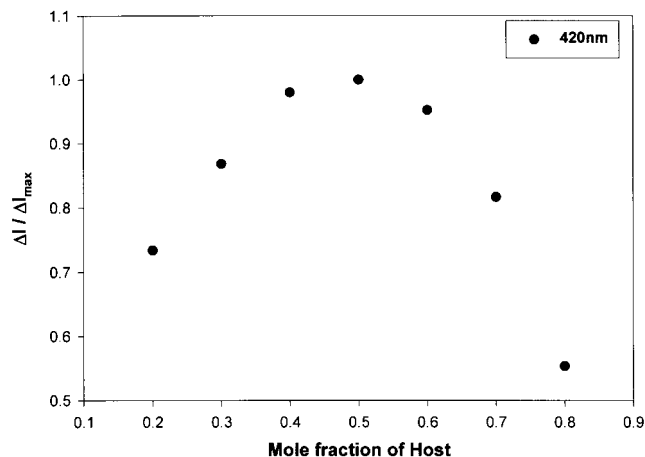


Figure 6. Job's plot between compound **1** and Mg(II) using fluorescence emission changes in ethanol. [**1**] + [Mg(II)] =  $10 \mu\text{M}$  (excitation: 367 nm, emission: 420 nm).

## Experimental

### Instruments and chemicals

NMR spectra were recorded with a Varian Inova Nuity spectrometer at 500 MHz ( $^1\text{H}$  NMR) and at 125 MHz ( $^{13}\text{C}$  NMR). Chemical shifts were given in ppm using TMS as internal standard. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). Fluorescence spectra were obtained using a RF-530/PC Spectrofluorophotometer (Shimadzu). Flash chromatography was carried out with Merck silica gel 60 (230–400 mesh). Thin layer chromatography was carried out with Merck 60 F<sub>254</sub> plates with 0.25 mm thickness.  $\text{CHCl}_3$ ,  $\text{CH}_2\text{Cl}_2$ , and MeOH were distilled from  $\text{CaH}_2$ , and THF was distilled from sodium-benzophenone ketyl. Most of the chemicals including metal salts and HEPES were obtained from Sigma-Aldrich Chemical Company, Milwaukee, WI. 1,8-Bis(hydroxymethyl)anthracene **4** was purchased from TCI, Tokyo, Japan.

### Synthesis

#### 1,8-Bis[(1-aza-15-crown-5)methyl]anthracene (**1**)

**Procedure A.** 1,8-Bis(bromomethyl)anthracene (364 mg, 1.00 mmol) was added to a stirred solution of 1-aza-15-crown-5 (657 mg, 3.00 mmol) and  $\text{K}_2\text{CO}_3$  (276 mg, 2.00 mmol) in  $\text{CHCl}_3$  (25 mL). After the reaction mixture was stirred at  $50^\circ\text{C}$  for 5 hours, the reaction mixture was passed through a celite washing with  $\text{CHCl}_3$ . The filtrate was concentrated under reduced pressure, and the crude product was purified by column chromatography ( $\text{CHCl}_3$ :MeOH = 9:1) to give **1** as a yellow oil in 75% yield (480 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  9.07 (s, 1H), 8.32 (s, 1H), 7.82 (d, 2H), 7.49 (d, 2H), 7.32 (t, 2H), 7.18 (s, 4H), 4.20 (s, 2H), 3.55 (m, 32H), 2.89 (t, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  135.61, 131.70, 130.30, 127.62, 127.11, 125.95, 124.88, 119.55, 77.42, 76.57, 59.40, 54.85; Exact Mass  $\text{C}_{36}\text{H}_{52}\text{N}_2\text{O}_8$  cal. 640.3723, found 640.3762.

### 1,8-Bis[(1-aza-18-crown-6)methyl]anthracene (**2**)

Application of procedure A to **5** (364 mg, 1.00 mmol), 1-aza-18-crown-6 (789 mg, 3.00 mmol), and K<sub>2</sub>CO<sub>3</sub> (276 mg, 2.00 mmol) in CHCl<sub>3</sub> (25 mL) gave **2** as a yellow oil in 52% yield (379 mg): <sup>1</sup>H NMR (CDCl<sub>2</sub>, 500 MHz) δ 9.12 (s, 1H), 8.44 (s, 1H), 7.94 (d, 2H), 7.59 (d, 2H), 7.44 (t, 2H), 4.28 (s, 2H), 3.57 (m, 48H); Exact Mass C<sub>40</sub>H<sub>60</sub>N<sub>2</sub>O<sub>10</sub> cal. 728.4247, found 728.4248.

### General fluorescence solution preparation

For metal titrations and alkyl diammonium 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 (perchlorate salts, 1 mM or 3 mM) or alkyl diammonium ions (1 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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