New Reagents for the Syntheses of Fluorescent Chemosensors. Anthrylogous Ethylene Dibromides[1]

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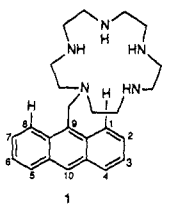
Ethylene dibromide is an important building block used in the construction of crown ethers, aza macrocycles, and cryptands. We describe herein the syntheses of two new compounds that serve as anthrylogous derivatives of ethylene dibromide. Each compound can be utilized in the syntheses of fluorescent chemosensors retaining the essential binding properties of the parent ligands.

As an analytical tool, fluorescence proffers unique and useful properties. The sensitivity of a fluorescence assay can be exquisite; in the extreme, single atoms can be visualized. With appropriate fluorophores, fluorescence emission can often be viewed at relatively long wavelengths that minimize background signals. Furthermore, there are many ways in which a fluorescence signal can be modulated, including intensity increase or decrease at a single wavelength, simultaneous intensity increase at one wavelength and decrease at another (ratiometry), fluorescence lifetime change, and fluorescence depolarization. The intensitometric methods suffer from certain limitations, as do all of the above. However, this mode of signal transduction has found increasing utility in recent years in the construction of fluorescent chemosensors for engendering real-time sensing of cationic, anionic, and neutral analytes.[2]

The design of any fluorescent chemosensor requires knowledge of three topics: (1) how can one bind a species with selectivity, (2) how can one generate signals from such binding events that are easy to measure, and (3) what mechanisms for binding and signal transduction intersect. With regard to selective binding, the classes of compounds known as crown ethers and their nitrogen analogues have found extensive application.[3] Most syntheses of these species involve at some stage the use of two-carbon building blocks, typically ethylene dibromide or ditosylate, to prepare the crown derivative. We have been involved in developing general methods in which known syntheses for the preparation of selective, abiotic receptors might be used in the syntheses of fluorescent chemosensors with intact binding selectivities. While the preparation of 9-substituted anthryl aza macrocycles such as 1 are



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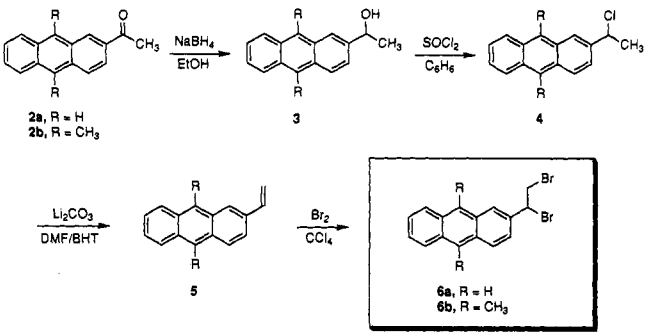
accomplished conveniently, peri interactions with the 1- and 8-hydrogens will tend to change the binding spectrum of ligands as compared to that of the parent aza macrocycles. Furthermore, precursors such as 9-(chloromethyl)anthracene virtually preclude the construction of cryptand or sepulcrate aza-crowns. In one approach toward the solution of this problem, we now report the syntheses of two new compounds, **6a** and **6b**, which serve as anthrylogous ethylene dibromides in the syntheses of fluorescent aza-crown ether and cryptand chemosensors. Each starting material permits the incorporation of chelating ligands unaffected by peri interactions and retaining the benzylic amine essential for signaling.

Scheme I



Results

Syntheses. 2-Vinylanthracene (**5a**), prepared according to the method of Pearson,[4] was brominated at −25°C to afford dibromide **6a**, crystallizable from chloroform (Scheme I). This compound could be stored in the freezer for several months without decomposition but experienced dehydrobromination at room temperature that thwarted efforts at microanalysis; spectroscopic characterization confirmed the structure assignment. The synthesis of the corresponding 9,10-dimethyl derivative **6b** was accomplished from 9,10-dimethylanthracene[5] by acetylation at the 2-position[6] to afford **2b**; reduction and conversion of alcohol **3b** to chloride **4b** were carried out as for compound **4a**. Dehydrochlorination of **4b** required the addition of 10 mol % added BHT to inhibit polymerization of the product alkene **5b**, after which bromination at reduced temperature gave dibromide **6b** in good yield. By comparison to **6a**, dibromide **6b** appears stable at room

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(1) Taken in part from the Ph.D. Thesis of Mi-Young Chae, The Ohio State University, 1993.

(2) For an overview of work by many laboratories, see: *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993.

(3) (a) Pedersen, C. L. *J. Inclusion Phenom.* **1988**, *6*, 337. (b) Lehn, J.-M. *Ibid.* **1988**, *6*, 351. (c) Cram, D. J. *Ibid.* **1988**, *6*, 397.

(4) Stolk, M.; Yanus, J. F.; Pearson, J. M. *Macromolecules* **1976**, *9*, 710.

(5) Duerr, B. F.; Chung, Y.-S.; Czarnik, A. W. *J. Org. Chem.* **1988**, *53*, 2120.

(6) Van Hove, L. *Bull. Soc. Chim. Belg.* **1957**, *66*, 413.

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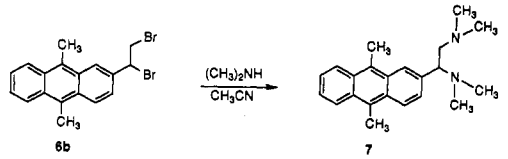
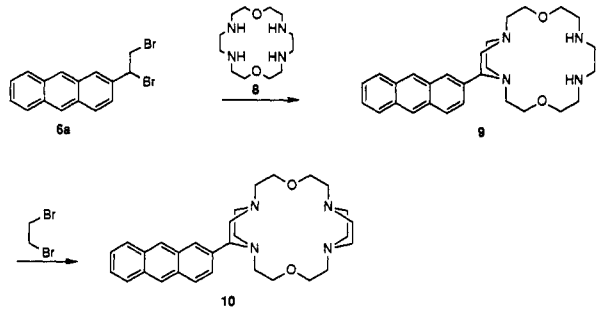
Scheme II



Scheme III



temperature for years when stored in the dark; the reason is not obvious.

The double displacement reaction of dibromide **6b** with dimethylamine yielded anthryl-TMEDA derivative **7** as an oil after acid–base extraction with chloroform (Scheme II). The low yield of this reaction (16%) is directly attributable to the facile amine-induced elimination to afford the corresponding alkenyl bromide, which is a more pronounced side product in the reactions of **6a** and **6b** than in the reactions of ethylene dibromide itself for obvious reasons. If this were a total synthesis of a natural product, such a step would be unacceptable. However, the syntheses of fluorescent chemosensors is somewhat unique in that a milligram of compound can easily provide sufficient material for hundreds of experiments. Of much greater importance is the product purity, which was acceptable in all of the compounds prepared using **6a** and **6b**.

More challenging was the reaction of dibromide **6a** with aza macrocycle **8**[7] leading to hemicryptand **9** (Scheme III). The analogous reaction of **8** with ethylene dibromide has been reported by Hancock[8] to make the nonanthrylogous ligands, and our yield using **6a** is again predictably lower. However, the presence of the anthracene chromophore greatly facilitated purification by cation exchange chromatography.[9] The ^{1}H NMR spectrum of **9** from $CDCl_3$ (Figure 1, top spectrum) demonstrates the presence of only one benzylic proton (dd at 4.1 ppm), which argues for the formation of only one of two possible diastereomeric products. Space-filling models clearly indicate a steric preference for an *endo*-anthracene on the ethylene bridge. Closure to give anthryl cryptand **10** was accomplished by reaction of **9** with ethylene dibromide. It was not apparent that **10** could be separated readily from the inevitable mixture with **9**, given their very similar polarities and an inability to use silica gel chromatography. We were fortunate to discover that the two compounds showed baseline separation using cation-exchange chromatography with a linear gradient of eluting buffer. Under these conditions, compound **9** elutes first, indicating that cryptand **10** is more highly charged at the pH of the column eluant (about 8.5). The samples of both **9** and **10** as obtained directly by lyophilization of column fractions contain inorganic impurities that were readily removed by extraction from basic aqueous solution into chloroform, drying, and evaporation. The UV extinction coefficients (ϵ_{254}) thus determined were found to be within 2% of that of 1-(2-anthryl)ethanol, a reference anthracene with similar substitution at the 2-position. The ^{1}H NMR spectrum of **10** obtained in this way (Figure 1, bottom spectrum) also indicates the presence of a single diastereomer.

Fluorescence Titrations. Anthryl-TMEDA **7** in acetonitrile displays fluorescence emission with vibrational structure and λ_{max} at 434 nm. Protonation of **7** in aqueous solution leads to increased fluorescence with a 12 nm red-shift. At pH 4, both amines are protonated and fluorescence emission is 3.6-fold greater than at pH 10 (Figure 2). A solution of compound **7** (25 μM) in acetonitrile likewise displays chelation-enhanced fluorescence upon introduction of $Zn(ClO_4)_2$ (Figure 3). Complete chelation of Zn(II) results in a net 14-fold increase in fluorescence, with saturation occurring at approximately 1 equiv.

The pH–fluorescence profile of hemicryptand **9** (not shown) displays the expected enhancement at acidic pHs, showing a net 6-fold increase in emission in going from pH 8 to pH 3. Fluorescence titrations of cryptand **10** with various metal ions were carried out in buffered methanol solution, as shown in Figure 4. Because the chelation-enhanced quenching effect observed with Pb(II) is small, background noise in the other titrations is prominent.

Discussion

9-Substituted anthryl aza macrocycles have proven a mainstay in fluorescent chemosensor studies to date, due largely to their synthetic accessibility. The reaction of 9-(chloromethyl)anthracene with aza-crowns yields compounds such as **1** with the benzylic amine essential for large signal ranges but also limiting the types of receptor molecules that might be conjugated to anthracene because: (a) ligands at the 9-position experience an unwanted interaction with the anthracene H-1 and H-8 and (b) tertiary amines cannot be conjugated in this way, ruling out the syntheses of anthryl cryptands. To widen the range of ligands useful for incorporation into fluorescent chemosensors, reagents are required that permit attachment at the anthracene 2-position with a resulting benzylic amine at a bridgehead position. The successful syntheses of fluorescent chemosensors **7** and **10** demonstrate that dibromides **6a** and **6b** can be used as fluorescent analogues of ethylene dibromide, fulfilling these requirements. The fact that 9,10-dimethyl derivative **6b** is more stable, is obtained in microanalytically pure form, and incorporates a fluorophore with higher inherent quantum yield[10] suggests that it may prove the more useful of these two new reagents.

The reactions of **6a** and **6b** with amines give substitution as minor products; in both cases, elimination to the 2-bromovinyl derivative dominates. Facile elimination in each anthracene derivative is anticipated, given the enhanced acidity of the benzylic protons as compared to that in ethylene dibromide itself. While undesirable, this

(7) Biernat, J. F.; Luboch, E. *Tetrahedron* **1984**, *40*, 1927.

(8) (a) Damu, K. V.; Shaikjee, M. S.; Michael, J. P.; Howard, A. S.; Hancock, R. D. *Inorg. Chem.* **1986**, *25*, 3879. (b) Hancock, R. D.; Evers, A.; Ngwenya, M. P.; Wade, P. W. *J. Chem. Soc., Chem. Commun.* **1987**, 1129.

(9) An Abridged Outline Dealing with the Practical Aspects of Ion Exchange Chromatography. Ace Glass Company: Vineland, NJ.

(10) Quantum yields in ethanol: anthracene, 0.30; 9,10-dimethylanthracene, 0.89 (Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: London, 1970; pp 127–128.)

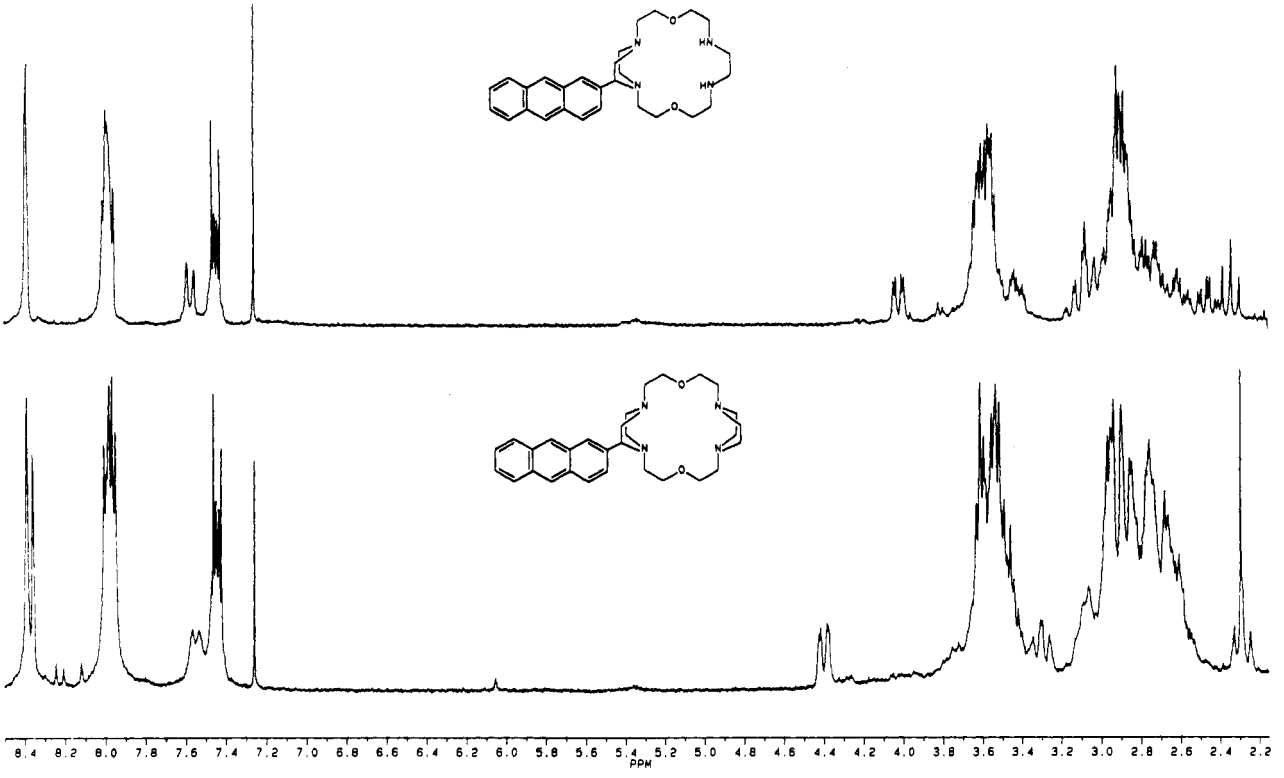


Figure 1.

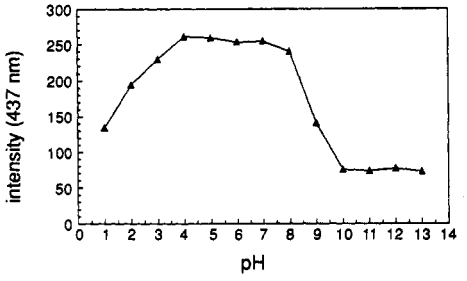


Figure 2. pH-Fluorescence profile of compound **7**.

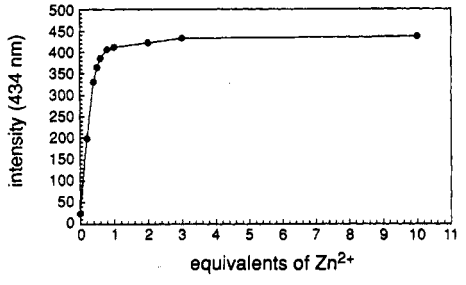


Figure 3. Fluorescence titration of compound **7** with Zn^{2+}.

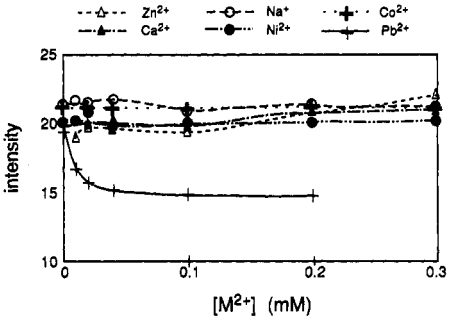


Figure 4. Fluorescence titrations of compound **10** with various metal ions.

extensive side reaction does not lessen the utility of **6a** or **6b** for three reasons. First, because the desired product is an amine while the undesired product is not, the isolation of even small amounts of desired product is made easy *via* acid–base extraction. Second, the anthracene fluorophore greatly facilitates column chromatography in that even small amounts are visualized with a hand-held, long-wavelength UV lamp as they elute down the column. And third, the production of even a few milligrams of product affords sufficient material for many fluorescence experiments. The ability to make any of the desired product is much more important than doing so in high yield.

Both pH and metal ion titrations of **7**, **9**, and **10** indicate clearly that fluorescence quenching by a benzylic amine at the anthracene 2-position is not as efficient as that at the 9-position. For example, while amine protonation results in a 20-fold fluorescence increase using 9-(cyclenylmethyl)anthracene **1** or even 9-[(dimethylamino)-methyl]anthracene,[11] protonation of 2-anthryl-TMEDA **7** affords only a 3.6-fold increase. Given that the anthracene central ring is the likely locus of excited-state reduction, this trend is not too surprising. In all cases we examined, the change in fluorescence intensity was easily measurable and large enough for analytical purposes.

The Zn(II) titration of **7** demonstrates chelation-enhanced fluorescence and saturation behavior, as observed previously with the 9,10-bis(TMEDA)anthracene. However, unlike the 9,10-compound, the titration in Figure 3 gives no indication of sigmoidal behavior. While this might have resulted from positive binding cooperativity in the case of the 9,10-bis(TMEDA)anthracene, we believe it more likely arises from the fact that complexation of the 9,10-bis(TMEDA)anthracene to the first metal leaves one benzylic amine uncomplexed. Thus, speciation with

(11) Beeson, J. Unpublished results, this laboratory.

removal of both quenching amines does not occur until more than 1 equiv of Zn(II) has been added.

Hancock has reported previously both the synthesis of the parent cryptand we conjugated (i.e., compound **10** minus the anthracene) and its selectivity for the complexation of the Pb(II) ion.[8] The binding specificity in this cryptand comes at the expense of the binding affinity ($K_a^{Pb(II)}$ is 10^5 M^{-1}), such that at the micromolar chemosensor concentrations employed, **10** does not bind Pb(II) from aqueous solution. Addition of $PbClO_4$ to an acetonitrile solution of **10** gave rise to a 10-fold enhancement in fluorescence, but this was quickly determined to result from specific acid protonation (arising from contaminating water) rather than metal ion complexation. It is possible, however, to observe ion discrimination in methanol solution buffered with lithium succinate/succinic acid[12] as shown in Figure 4. Pb(II) is a weakly quenching metal, and its binding by anthryl cryptand **10** is manifest by chelation-enhanced quenching. Under these conditions, saturation occurs by 100 μM Pb(II), contrasting with the lack of signal change using Ca(II), Ni(II), Co(II), or, most notably, Zn(II). Indeed, Pb(II)/Zn(II) size selectivity is a dominant property of the parent cryptand as reported by Hancock. We have observed previously that 9-anthryl aza macrocycles bind both Pb(II) and Zn(II) almost stoichiometrically in water at these concentrations without discrimination. Because Pb(II) affords a net weak quenching of fluorescence, the signal range is too small to be of utility in routine analysis. However, it does appears that our goal of retaining the binding profile by location at the anthracene 2-position has been realized in this instance.

Conclusion

The availability of anthrylogous ethylene dibromides **6a** and **6b** permits the syntheses of fluorescent chemosensors using ligands not previously amenable to conjugation. Syntheses, while of low overall yield, provide useful quantities of products easily isolable by extraction and ion-exchange chromatography. These derivatives demonstrate chelation-enhanced fluorescence and chelation-enhanced quenching with metal ions, depending on the inherent quenching ability of the ion, with usefully large signal changes. The synthesis of an anthryl cryptand demonstrates that novel ligands with bridgehead amines can be prepared retaining the ion discrimination of the parent ligand.

Experimental Section

General Procedures. Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Microanalyses were carried out at Atlantic Microlab Inc., Norcross, GA. Mass spectra were obtained by use of a Kratos-30 mass spectrometer. FT-NMR spectra were obtained at 7.0 T (300 MHz) or 5.87 T (250 MHz). UV spectra were obtained on a Hewlett-Packard 8451A Diode Array spectrophotometer; all wavelength data reported are ±1 nm. The pH of solutions used in the pH–fluorescence profile experiments were adjusted using concentrated HCl and NaOH and determined using a Fisher ACCUMET Model 810 pH meter. Fluorescence measurements were made on a Perkin-Elmer LS-5 luminescence spectrometer with excitation at 345 nm; both emission and excitation slit widths varied from 3 to 20 nm, depending on the measurement. Anthracene was purchased from J. T. Baker Inc., Phillipsburg, NJ. *n*-Butyllithium (2.4 M, hexane), methyl iodide, sodium borohydride, thionyl chloride, anhyrdous DMF, BHT, and bromine (1.0 M, carbon tetrachloride) were purchased from the Aldrich Chemical Company, Milwaukee, WI. Zinc chloride was obtained from GFS Chemical, Columbus, OH.

1-(9,10-Dimethyl-2-anthryl)ethanol (3b). 2-Acetyl-9,10-dimethylanthracene (1.77 g, 7.1 mmol) and ethanol (75 mL) were placed in a 250-mL round-bottomed flask fitted with a reflux condenser and a dropping funnel. To the refluxing solution was added dropwise sodium borohydride (0.885 g, 23 mmol) dissolved in water (15 mL). The resulting mixture was refluxed for an additional 3 h. After half of the solvent was evaporated under reduced pressure, the product was precipitated with water and washed with dilute hydrochloric acid and then with water. After treatment with decolorizing carbon, the crude product was recrystallized from benzene to give 1.57 g (88%) of a bright crystalline solid: mp 135.5–136 °C; ^{1}H NMR ($CDCl_3$) δ 1.65 (d, 3, CH_3), 1.95 (d, 1, OH), 3.1 (d, 6, CH_3), 5.15 (q, 1, CH), 7.5–7.6 (m, 3, Ar-H), 8.25–8.4 (m, 4, Ar-H); EI mass spectrum *m/e* 250.1381 (M^+, calcd 250.1358).

Anal. Calcd for $C_{18}H_{18}O \cdot 0.1\ H_2O$: C, 85.74; H, 7.27; O, 6.99. Found: C, 85.68; H, 7.21.

2-(1-Chloroethyl)-9,10-dimethylanthracene (4b). Thionyl chloride (0.9 mL, 12.3 mmol) was added to a boiling solution of 1-(9,10-dimethyl-2-anthryl)ethanol (1.5 g, 6 mmol) dissolved in anhydrous benzene (40 mL). The resulting mixture was refluxed for an additional 3 h. Half of the solvent was evaporated under reduced pressure and added to petroleum ether (35–60 °C, 40 mL). The precipitate was filtered, and the filtrate was evaporated under reduced pressure to give a greenish-yellow solid (82%). After treatment with decolorizing carbon in benzene, the crude product was recrystallized from benzene/petroleum ether: mp 111–113 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.0 (d, 3, CH_3), 3.1 (s, 6, CH_3), 5.4 (q, 1, CH), 7.5–7.6 (m, 3, Ar-H), 8.25–8.4 (m, 4, Ar-H); EI mass spectrum *m/e* 268.1003 (M^+, calcd 268.1019).

9,10-Dimethyl-2-vinylanthracene (5b). 2-(1-Chloroethyl)-9,10-dimethylanthracene (2.77 g, 10 mmol), lithium carbonate (1.61 g, 22 mmol), BHT (0.23 g, 1.0 mmol), and DMF (100 mL) were placed in a 250-mL round-bottomed flask fitted with a reflux condenser. The mixture was heated to 135 °C (oil bath temperature) for 4.5 h. The mixture was cooled and poured into an excess of water, and the precipitate was collected by filtration and washed with water until the washings were neutral. The crude product was purified by silica-gel column chromatography (eluent: hexane/chloroform) and recrystallized from chloroform to give a yellow crystalline solid (58%): mp 124.5–125 °C; ^{1}H NMR ($CDCl_3$) δ 3.1 (s, 6, CH_3), 5.4 (d, 1, $=CH_2$), 5.95 (d, 1, $=CH_2$), 7.0 (d of d, 1, CH=), 7.5 (m, 2, Ar-H), 7.7 (d, 1, Ar-H), 8.2 (s, 1, Ar-H), 8.3 (m, 3, Ar-H); EI mass spectrum *m/e* 232.1249 (M^+, calcd 232.1252).

Anal. Calcd for $C_{18}H_{16}$: C, 92.34; H, 6.97. Found: C, 92.32; H, 6.61.

2-(1,2-Dibromoethyl)anthracene (6a). A solution of 2-vinylanthracene **5b** (2.0 g, 9.8 mmol) in chloroform (80 mL) was cooled to −25 °C with stirring. To this solution was added very slowly bromine (0.5 mL, 9.8 mmol) in chloroform (10 mL) using a double-headed needle. The reaction temperature was maintained around −25 °C during the addition. The resulting solution was allowed to warm slowly to room temperature. The progress of the reaction was monitered by TLC (R_f 0.35, silica gel, hexane/chloroform (4:1)). After the solvent was evaporated, the crude reaction products were chromatographed through a column of silica gel eluted with hexane and then with hexane containing chloroform (10–50%). The fractions were pooled and crystallized from chloroform (5 mL) to afford **6a** (650 mg, 18%) as yellow crystals: mp 167–168 °C; ^{1}H NMR ($CDCl_3$) δ 4.16–4.19 (d, J = 8.4 Hz, 1H), 5.35–5.41 (t, J = 8.0 Hz, 2H), 7.47–7.51 (m, 3, Ar-H), 8.00–8.07 (m, 4, Ar-H), 8.42–8.50 (d, 2, Ar-H); ^{13}C NMR ($CDCl_3$) δ 34.5, 51.8, 123.5, 125.7, 125.9, 126.3, 126.9, 127.7, 128.2, 129.6, 130.7, 131.3, 132.3, 134.8; mass spectrum *m/e* 364 (M^+).

Anal. Calcd for $C_{16}H_{12}Br_2$: C, 52.78; H, 3.32; Br, 43.89. Found: C, 53.59; H, 3.35; Br, 41.86. (As noted in the text, room temperature elimination results in lower Br and higher C values than calculated.)

2-(1,2-Dibromoethyl)-9,10-dimethylanthracene (6b). 9,10-Dimethyl-2-vinylanthracene (493 mg, 2.1 mmol) was dissolved in carbon tetrachloride (50 mL), and the solution was cooled to

(12) Alfenaar, M.; de Ligny, C. L. *Recl. Trav. Chim. Pays-Bas* **1967**, *86*, 1185.

-20 °C. To the stirred, cooled solution was added dropwise a 1 M solution of bromine in carbon tetrachloride (2.2 mL, 2.2 mmol). The resulting mixture was warmed to room temperature, and the solvent was removed under reduced pressure to give a greenish-yellow solid (82%): mp 167–168 °C dec; ^{1}H NMR ($CDCl_3$) δ 3.1 (s, 6, CH_3), 4.2 (d, 2, CH_2), 5.4 (t, 1, CH), 7.5–7.65 (m, 3, Ar-H), 8.3–8.5 (m, 4, Ar-H); EI mass spectrum *m/e* 389.9622 (M^+, calcd 389.9616).

Anal. Calcd for $C_{18}H_{16}Br_2$: C, 55.13; H, 4.11; Br, 40.75. Found: C, 55.03; H, 4.06; Br, 40.82.

1-(9,10-Dimethyl-2-anthryl)-*N,N,N,N*-tetramethyl-1,2-ethylenediamine (7). 2-(1,2-Dibromoethyl)-9,10-dimethylanthracene (0.68 g, 1.52 mmol) was dissolved in acetonitrile (200 mL). Dimethylamine (1.46 g, 40% in water, 13.1 mmol) was added to the stirred solution at room temperature; the resulting mixture was stirred overnight. After the solvent was evaporated completely under reduced pressure, the yellow solid was dissolved in chloroform (30 mL) and extracted with 5% aqueous HCl (7 × 70 mL). The aqueous layer was basified with sodium hydroxide and extracted with chloroform (3 × 50 mL). The chloroform layer was dried over anhydrous sodium sulfate and evaporated to give **7** as an oil (80 mg, 16%): ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 12, NCH_3), 2.80 (q, 1, CH_2), 3.05 (q, 1, CH_2), 3.12 (d, 6, CH_3), 3.80 (t, 1, CH), 7.50 (m, 3, Ar-H), 8.15 (s, 1, Ar-H), 8.35 (m, 3, Ar-H); EI mass spectrum *m/e* 320.2301 (M^+, calcd 320.2252).

Anthryl Macrocycle 9. To a solution of tetraza-crown 8[7] (20 mg, 0.076 mmol) in dry benzene (10 mL) was added anhydrous K_2CO_3 (20 mg). After the solution was stirred at room temperature for 30 min, a solution of 2-(1,2-dibromoethyl)anthacene (**6a**; 26 mg, 0.076 mmol) in dry benzene (5 mL) was added dropwise. The reaction temperature was increased slowly to gentle reflux. The disappearence of dibromide **6a** was monitored by TLC (R_f 0.35, silica gel, hexane/chloroform (4:1)). Reflux was continued for 7 days. After evaporation of the solvent, the residue was suspended in water and the solution was made acidic. The unreacted starting material was removed by extracting with chloroform. The aqueous layer's pH was adjusted to 7, and the aqueous layer was then extracted with chloroform and dried over anhydrous Na_2SO_4. The crude product was purified by an ion-exchange column (CM-25, gradient 0.05–0.8 M ammonium bicarbonate). The appropriate fractions were pooled and lyophilized to afford **9** plus some inorganic impurities from the buffer. This sample was dissolved into aqueous NaOH (2 M) and extracted with chloroform to obtain **9** after removal of the solvent (3 mg, 9%): ^{1}H NMR ($CDCl_3$) δ 2.22–36 (m, 26H), 3.92–3.97 (dd, 1H), 7.35–8.31 (m, 9, Ar-H); FAB mass spectrum *m/e* 463 (M^+); high resolution FAB mass spectrum calcd for $C_{28}H_{39}N_4O_2$ 463.3064, measured 463.3098.

Anthryl Cryptand 10. 1,2-Dibromoethane (4 μL, 0.04 mmol) was added to a solution of anthryl macrocycle **9** (10 mg, 0.02 mmol) in ethanol (1 mL). The reaction mixture was stirred under argon, and then the reaction temperature was increased to reflux for 7 days. The brown residue obtained after evaporating the solvent was dissolved in water and purified by ion-exchange chromatography (CM-25, gradient 0.05–1.0 M ammonium bicarbonate containing 5% MeOH) to obtain **10** (2 mg, 10% based on unconverted starting material), which eluted immediately after unreacted **9**. The appropriate fractions were pooled and lyophilized to afford **10** plus some inorganic impurities from the buffer. This sample was dissolved into aqueous NaOH (2 M) and extracted with chloroform to obtain **10** after removal of the solvent: ^{1}H NMR ($CDCl_3$) δ 2.21–3.65 (m, 30H), 4.35–4.45 (dd, 1H), 7.42–8.38 (m, 9, Ar-H); FAB mass spectrum *m/e* 489 (M^+); high resolution FAB mass spectrum calcd for $C_{30}H_{41}N_4O_2$ 489.3216, measured 489.3210.

Preparation of pH–Fluorescence Profile Solutions. A stock solution of **7** (2.5 mM) was prepared in acetonitrile. Test solutions were prepared by placing 40 μL of the **7** stock solution into a polypropylene test tube and adding 3.96 mL of the appropriate buffer (0.1 M): trichloroacetate (pH 1), dichloroacetate (pH 2), chloroacetate (pH 3), acetate (pH 4 and 5), MES (pH 6), HEPES (pH 7 and 8), CHES (pH 9), CAPS (pH 10 and 11), or NaOH (pH 12 and 13).

Preparation of Fluorimetric Zn(II) Titration Solutions. A stock solution of $Zn(ClO_4)_2$ (20 mM) was prepared in acetonitrile. A stock solution of **7** (250 μ*M*) in acetonitrile was prepared and protected from light and then diluted to 25 μM using acetonitrile. After the fluorescence spectrum of **7** (100 mL) alone was recorded, the Zn(II) solution was added portionwise to the solution of **7** (final volume 101.25 mL).

Fluorimetric Titrations of 10 in Buffered Methanol. A solution of **10** (10 μM) in methanol was buffered with succinic acid/lithium succinate (5 mM) as described by Alfenaar and de Ligny.[12] Aqueous stock solutions of the corresponding metal perchlorates were prepared, and the total amount of water (8 μL) in each titration (4.000 mL) was kept constant by addition of water as needed. Excitation was at 340 nm; emission was measured at the maximum at 403 nm.

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