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Articles

Cooperative Ratiometric Chemosensors: Pinwheel Receptors with an Integrated Fluorescence System

Joseph Raker and Timothy E. Glass*

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

teg@chem.psu.edu

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The synthesis and fluorescent properties of a second generation cooperative chemical sensor are described. The sensor has two interacting binding pockets for cooperative recognition of two analytes. Cooperative binding activates a ratiometric fluorescent response via formation of an excimer. Binding was characterized by NMR, absorption, and fluorescence spectroscopy. The advantages of separating the recognition elements from the fluorescent response elements are discussed.

Introduction

Fluorescent chemical sensing of biologically important analytes continues to be an essential tool in biochemistry.[1] Expanding the scope of analytes which can be quantified with fluorescent probes is therefore an area of active interest. We have recently reported a cooperative "pinwheel" chemical sensor based on a bistritylacetylene backbone (Figure 1).[2] The sensor was designed to bind three analytes in a cooperative fashion giving the sensor a higher overall affinity for the analyte than a similar noncooperative sensor. It is anticipated that this effect will be general for different types of recognition elements providing a common platform from which sensors for various analytes can be generated. The major conceptual drawback to the overall design of this sensor is the lack of a convenient fluorescent read-out. The original sensor

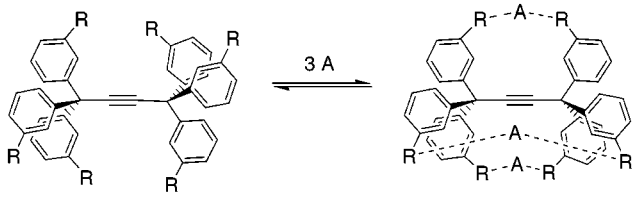

Figure 1. Three-site cooperative sensor. R = Recognition Element; A = Analyte.

design required that the fluorescent response be incorporated into the recognition elements. Therefore, to create a sensor for a particular analyte, both the recognition and the fluorescent read-out need to be optimized. To prepare a truly general sensor platform, it would clearly be desirable to separate entirely the fluorescent read-out from the recognition elements such that any binding event would produce a fluorescent signal regardless of the recognition element employed or the analyte targeted.[3] Thus, the fluorescent groups should ideally be appended to the sensor framework in a position separate from the actual recognition elements. Further, it would

(1) For reviews of chemical sensors, see: (a) *Chemosensors of Ion and Molecule Recognition*. Desvergne, J. P., Czarnik, A. W., Eds.; NATO ASI Series C, 492; Kluwer: New York, 1997. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Funnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. (c) *Applied Fluorescence in Chemistry, Biology and Medicine*; Rettig, W., Strehmel, B., Schrader, S., Seifert, H., Eds.; Springer-Verlag: Berlin, 1999.

(2) Glass, T. E. *J. Am. Chem. Soc.* **2000**, *122*, 4522−4523.

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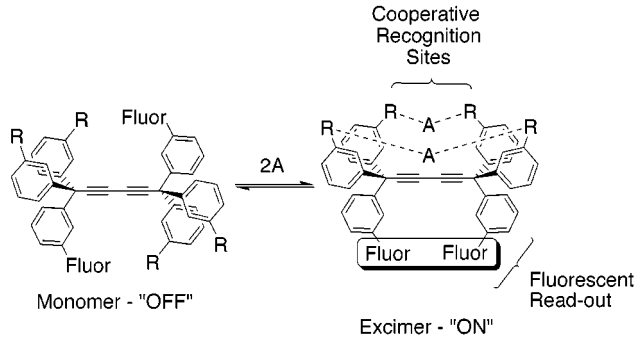

Figure 2. Sensor mechanism of activation. R = Recognition Element; A = Analyte; Fluor = Fluorophore.

be advantageous to utilize a shift in the wavelength of emission upon analyte binding in order to produce a ratiometric response.[4] This type of response provides internal calibration of the sensor, a useful attribute in cases where the concentration of the sensor varies or is not exactly known. Herein is described a second generation cooperative sensor possessing a ratiometric fluorescent read-out which is integrated directly into the sensor framework, separate from the recognition elements.

Sensor Design. The current sensor design utilizes a bis-trityl butadiyne core (Figure 2). The longer butadiyne spacer is warranted due to the steric congestion observed with the sensor based on a single acetylene spacer (Figure 1).[2] On each trityl group is appended two recognition elements (R) and one fluorophore (Fluor). The recognition elements are spatially convergent such that a pair can bind an analyte across the butadiyne axis. The fluorophores are similarly designed to interact across the butadiyne axis. In the unbound state of the sensor, the trityl groups have free rotation relative to each other which results in little interaction between the fluorescent groups (left in Figure 2). The fluorescence will be dominated by the emission of the monomeric fluorophore which will be read as the "OFF" state. Binding of the first analyte restricts the natural rotation of the trityl groups, aligning the second pair of recognition elements for stronger binding of the second analyte and thus, creating the cooperative response (right in Figure 2).[5] The loss of rotational freedom will similarly force the two fluorescent groups into spatial proximity. This interaction could produce an excimer[6] between the fluorophores. The excimer emission would be read as the "ON" state. Excimer emission is substantially red-shifted relative to the monomer emission and can be used for a ratiometric response.[7]

This design utilizes the same mechanism of cooperativity as the original sensor. The primary advantage of this design is that the cooperative recognition domain is entirely separated from the read-out domain. On the basis of this mechanism, it is anticipated that any recognition event which restricts the rotational freedom of the sensor framework will elicit a fluorescent response. Therefore, once the appropriate fluorescent groups are identified, little or no modification of the fluorescence system will be required regardless of the chosen recognition element or target analyte.

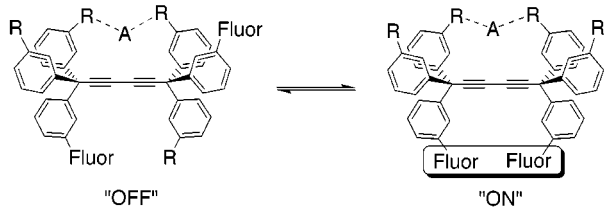

Figure 3. Single analyte binding modes. R = Recognition Element; A = Analyte; Fluor = Fluorophore.

Alternate Binding Modes. Positive cooperative recognition implies that the second binding event is stronger (higher K_a) than the first binding event. Consequently, a singly bound sensor is disfavored relative to the doubly bound sensor such that the major fluorescent species in solution will be the unbound and the doubly bound sensor as shown Figure 2. However, to the extent that it exists in solution, a singly occupied sensor might contribute to the observed fluorescence. There are two distinct singly occupied isomers (Figure 3), one of which precludes fluorescent response (left in Figure 3) and one of which should give a fluorescent response which is similar to the doubly occupied sensor (right in Figure 3). Assuming that these isomers are in rapid equilibrium and have similar energies, it follows that a singly bound sensor will give rise to a 50% response since only one of the two possible isomers can produce a signal. Thus, the small concentration of singly occupied sensor should give rise to the same amount of signal per bound analyte and should not interfere with the analysis of the system.

Fluorescent Read-Out. The key factor for successful implementation of this strategy is incorporation of the appropriate fluorescent groups which will interact to give an excimer upon binding. Equally important is the requirement that the fluorophores do not interact in the unbound "OFF" state. Many fluorophores are known to aggregate in solution[8] and the free rotation of the trityl groups in the unbound "OFF" state might permit such an association. Should the fluorophores be prone to association in the unbound state of the sensor, excimer emission[9] may result even in the "OFF" state, frustrating the intended fluorescent response mechanism. Thus, only a narrow range of fluorescent groups are expected to function properly in these sensors.

Results and Discussion

Target Selection. For the purpose of testing the fluorescent read-out, simple ethylenediamine binding groups were chosen as recognition elements (R_1) since similar recognition elements were shown to freeze rotation of these pinwheel receptors upon metal binding.[2] An initial screen of several different fluorophores and con-

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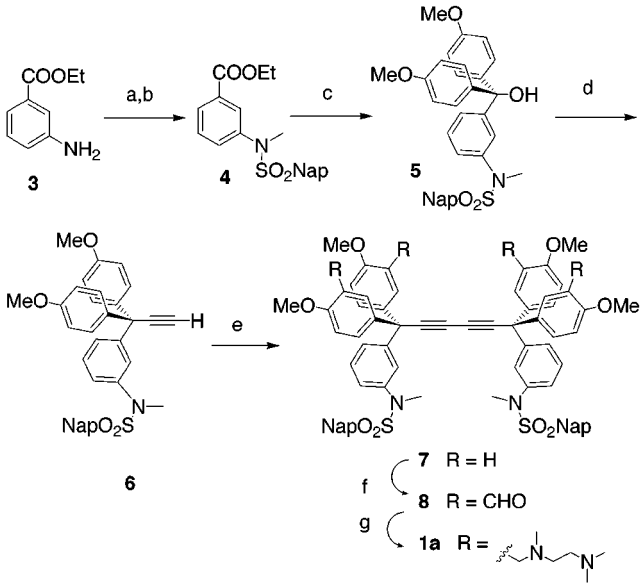
(6) Birks, J. B. *Photophysics of Aromatic Molecules*; John Wiley: New York, 1970.

(7) (a) Lewis, F. D.; Zhang, Y.; Liu, X.; Xu, N.; Letsinger, R. L. *J. Phys. Chem. B* **1999**, *103*, 2570−2578. (b) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463−9464. (c) Lewis, F. D.; Zhangt, Y.; Letsinger, R. L. *J. Org. Chem.* **1997**, *62*, 8565−8568. (d) Ueno, A.; Suzuki, I.; Osa, T. *J. Am. Chem. Soc.* **1989**, *111*, 6391−6397. (e) Hamada, F.; Minato, S.; Osa, T.; Ueno, A. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1339−1346.

(8) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587−614.

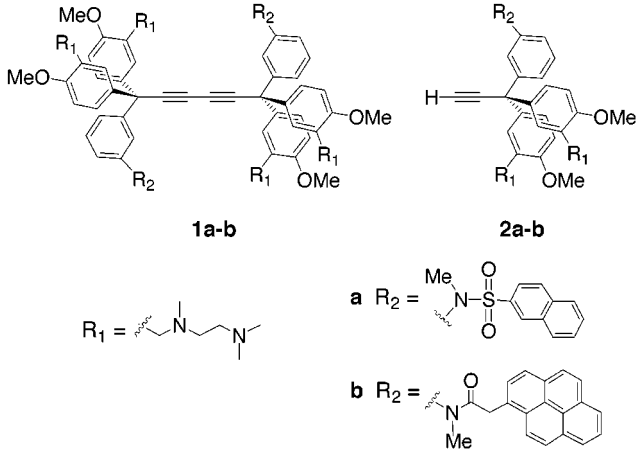
(9) The term "excimer" is actually inappropriate in the context of a ground-state association, although the observed emission is often similar. "Excimer" is defined as an association between two fluorophores which occurs after one of them has been locally excited. See ref 6.

Scheme 1[a]



[a] Key: (a) 2-Naphthalenesulfonyl chloride, DMAP, Pyr (100%). (b) MeI, NaOH, EtOH (75%). (c) 4-Bromoanisole/*n*-BuLi, THF (94%). (d) (1) AcCl (2) ethynyl magnesium bromide, PhH (85%). (e) CuCl, *N*-methyl-pyrrolidine, O_2, DCM (74%). (f) Cl_2CHOCH_3, $TiCl_4$, DCM (87%). (g) Trimethyl-ethylenediamine, AcOH, EtOH, 4 Å M.S., $NaBH_3CN$ (48%).

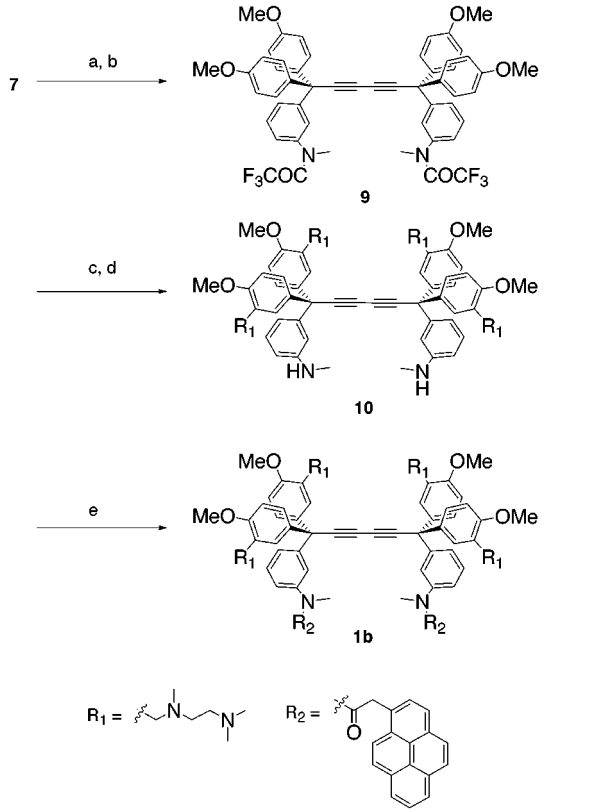
necting tethers (R_2) revealed that only two of the fluorophores tested could form an excimer in this pinwheel system: naphthyl sulfonanilide **1a** and pyrenyl acetanilide **1b**. The fluorescence spectra of sensors containing



groups such as dansyl, anthracene, and *N*-methyl-acridone did not indicate a ratiometric response. In fact, pyrene or naphthalene groups connected via alternate tethers (e.g., pyrenyl sulfonanilide) failed to demonstrate excimer fluorescence. Thus, proper orientation of the two fluorophores is essential for formation of an excimer.[10] On the basis of this initial screening of fluorophores, compounds **1a** and **1b** were explored in detail. To probe the mechanism of this class of sensors, the mono-trityl compounds **2a** and **2b** were also analyzed as control compounds.

Synthesis. The synthesis of sensor **1a** is outlined in Scheme 1. Trityl alcohol **5** was produced in three steps from ethyl-3-aminobenzoate (**3**) in good yield. Chlorination of **5** followed by addition of acetylene Grignard[11] afforded alkyne **6**. Glaser coupling[12] of **6** produced diyne **7** in good yield. Formylation[13] of **7** and reductive amination of the resulting tetra-aldehyde **8** with trimethyl ethylenediamine yielded sensor **1a**.

Scheme 2[a]



[a] Key: (a) Mg(0) MeOH, DCM (100%). (b) TFAA, THF (98%). (c) Cl_2CHOCH_3, $TiCl_4$, DCM (91%). (d) (1) Trimethyl-ethylenediamine, AcOH, EtOH, 4 Å M.S., $NaBH_3CN$ (2) NH_3, MeOH (93%). (e) 1-Pyreneacetic acid, DIC, THF (33%).

To append alternate fluorophores such as the pyrene of **1b**, we found that the naphthyl sulfonamide must be removed prior to installing the recognition elements (Scheme 2). Thus, compound **7** was deprotected under mild reductive conditions.[14] The resulting anilines were reprotected as the trifluoroacetanilides to produce compound **9** in good overall yield. Reprotection was necessary in order to electronically deactivate the aniline rings prior to the electrophilic substitution that follows. The recognition elements were installed as before and the trifluoroacetanilides deprotected to give compound **10**. Pyrene acetic acid was then coupled to the free aniline groups to yield sensor **1b**. Control compounds **2a** and **2b** were prepared in a similar fashion.

NMR. The binding mode of sensor **1a** was first examined by ^1H NMR spectroscopy (2.4 mM in CD_3CN).

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(11) Oyler, R. E.; Ketz, B. E.; Glass, T. E. *Tetrahedron Lett.* **2000**, *41*, 8247−8250.

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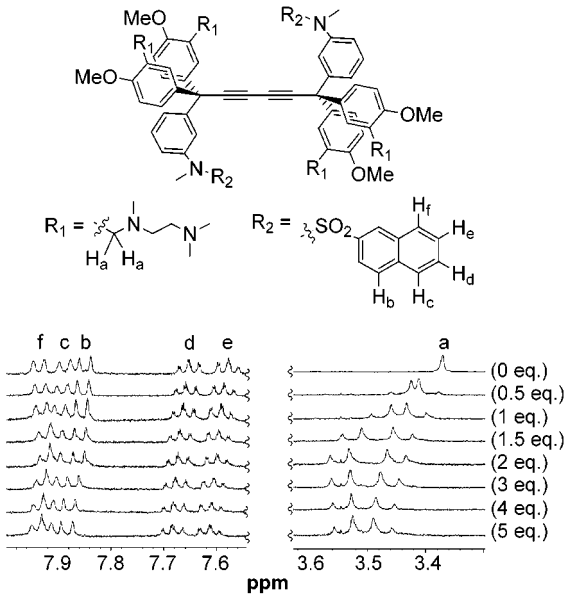

Figure 4. Naphthyl and benzylic region of the ^{1}H NMR of sensor **1a** (2.4 mM in CD_3CN) with the indicated equivalents of $AgClO_4$ added.

Relevant portions of the spectra are shown in Figure 4 with varying amounts of added $AgClO_4$. Nearly all resonances in the spectrum shift slightly downfield upon addition of $AgClO_4$, presumably due to solvent effects from the change in ionic strength. The singlet assigned to the benzylic protons of the recognition elements shifts substantially downfield and splits into a pair of doublets. This splitting is consistent with tetrahedral binding of a metal ion which constrains the dihedral angles in the recognition element and puts the two benzylic protons in nonequivalent positions. The shift reaches saturation after the addition of 2 equiv of metal ion, a result consistent with 2:1 binding, however the various metal bound species (i.e., singly and doubly bound sensor) are rapidly interconverting and cannot be observed directly.

Only subtle shifts in the naphthyl protons were observed upon addition of Ag(I). Intramolecular aromatic interactions are often accompanied by dramatic (0.1−0.5 ppm) upfield shifts of all aromatic resonances.[15] Only one naphthyl proton (H_f in Figure 3) shifts upfield which may indicate some fluorophore−fluorophore interaction upon binding. The relatively small shifts in the naphthyl region of compound **1a** indicate that metal binding is accompanied by only a small change in the interaction of the naphthyl groups.

In contrast to the bis-trityl sensor **1a**, the monotrityl control compound **2a** (2.4 mM in CD_3CN) gave a broad and complex ^{1}H NMR spectrum upon addition of $AgClO_4$. This behavior is consistent with intermolecular aggregation at these high concentrations in which one metal ion is bound between recognition elements on two separate molecules. These data support the proposed mode of binding for sensor **1a** (Figure 2) where the metal is bound across the acetylene axis, rather than between two recognition elements on the same trityl group.[16] If two recognition elements on the same trityl group were interacting to bind the metal ion, one would expect the control compound **2a** to demonstrate clean 1:1 binding of metal ions. The NMR spectra suggest that simple 1:1 binding does not exist for this control compound.

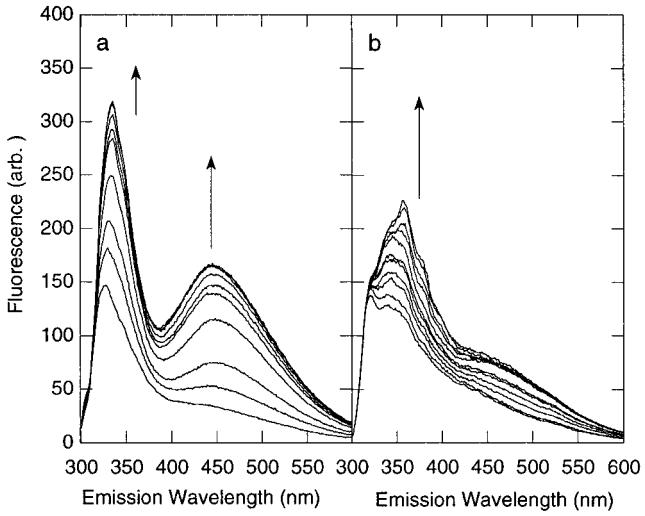

Figure 5. Fluorescence emission spectra (λ_{ex} = 290 nm) with increasing amounts of $AgClO_4$ for (a) compound **1a** (1 μM in acetonitrile with 5 mM NMe_4ClO_4). The spectra resulting from addition of 0, 1, 2, 4, 6, 8, 10, 14, 18, and 25 equiv of Ag(I) are shown. (b) compound **2a** (1 μM in acetonitrile with 5 mM NMe_4ClO_4). The spectra resulting from addition of 0, 1, 2, 4, 6, 8, 10, 15, and 25 equiv of Ag(I) are shown.

UV Absorption. To further probe the fluorophore−fluorophore interactions in these systems, the behavior of their absorption spectra was investigated. The absorption spectra of compounds **1a** and **1b** were recorded in CH_3CN (10 μM in sensor). Addition of excess $AgClO_4$ gave no change in the absorption spectra of either sensor. Given that the concentration used for these titrations is well within the range in which fluorescent changes are observed (vide infra), it appears that binding is taking place, but not giving rise to a significant ground-state interaction of the two fluorescent groups in compounds **1a** or **1b**. Furthermore, the absorption spectra of compounds **1a** and **2a** were identical, indicating that there is little interaction of the fluorophores even in the free sensor **1a**.[17] This situation is also mirrored in the case of the pyrene containing compounds **1b** and **2b** in that no difference is observed between the absorption spectra of the two compounds. These results, combined with the NMR data, indicate that there is little ground-state interaction of either fluorophore in the bound or unbound states of the sensor.

Fluorescence. The fluorescence spectra of compounds **1a** and **2a** are shown in Figure 5. In the absence of metal ion, both compounds have an emission at 335 nm assigned to the fluorescence of the naphthyl sulfonanilide. Compound **1a** also has a weak emission centered at 450 nm assigned to an excimer between the two naphthalene groups. Upon addition of Ag(I) to sensor **1a**, a substantial increase in excimer emission is observed along with a

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(16) A trityl based bis-crown ether has been recently reported to bind one metal between two recognition elements on the trityl unit; however, the binding geometry in this system is not clear: Kimura, K.; Mizutani, R.; Yokoyama, M.; Arakawa, R.; Sakurai, Y. *J. Am. Chem. Soc.* **2000**, *122*, 5448−5454.

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smaller increase in the monomer emission (Figure 5a). The ratio of the two peaks varies 2-fold between the free sensor and the saturated sensor. Because the NMR and absorption data do not support the formation of a ground-state fluorophore complex upon binding, the emission at 450 nm is due to a true excimer.[6]

The control compound **2a** shows an increase in monomer emission upon titration with Ag(I) (Figure 5b). This change is due to photoinduced electron transfer (PET) processes[18] whereby the amines present in the recognition elements quench the fluorescence of the naphthalenes in the unbound state.[19] Binding of the metal sequesters the lone pairs on nitrogen which stops the PET quenching and produces a rise in the monomer emission. By analogy, the fluorescent response of sensor **1a** is a combination of PET which produces a rise in the monomer emission along with excimer formation.

A small excimer emission arises in compound **2a** upon titration with Ag(I). The excimer emission here is due to a small amount of intermolecular aggregation (in line with the NMR data) which gives rise to a weak excimer band. As expected, this effect is strongly concentration dependent and the excimer emission of **2a** upon titration with Ag(I) is more pronounced at higher sensor concentrations (10 μM in **2a**, data not shown). The ratiometric variation of sensor **1a** is concentration independent, an observation which argues for intramolecularity of the excimer in the bis-trityl system. Thus, the change in ratio between excimer and monomer in sensor **1a** upon metal ion binding is substantial and consistent with the mechanism proposed in Figure 2.

In the case of the pyrene fluorophore (**1b**), a significant excimer emission is evident at 477 nm even in the unbound state[20] along with a typical monomer emission (Figure 6a). Upon addition of Ag(I), the excimer emission remains relatively constant but the monomer emission increases substantially, giving a 2-fold change in ratio between the monomer and excimer peaks. The control compound **2b** has no excimer emission but shows a strong increase in its monomer emission upon titration with Ag(I) (Figure 6b). Thus, the rise in the monomer emission of compounds **1b** and **2b** can be explained solely by the PET quenching mechanism.

Clearly, sensor **1b** is binding the metal ion as indicated by the change in monomer emission, yet the excimer emission remains constant. The excimer emission observed with sensor **1b** may be derived from a ground-state dimer of the pyrene fluorophores which is unperturbed by metal binding. However, there is no evidence for such a dimer in the absorption data. Furthermore, the excitation spectra of compound **1b** monitored for the monomer and excimer emission have the same shape and wavelength of band maxima. Ground-state dimers often show substantial differences between the two excitation spectra.[8] Alternatively, the pyrene fluorophores may have such a strong propensity toward excimer formation upon excitation that the excimer easily forms even in the conformationally unrestricted "OFF" state (Figure 2).[21]

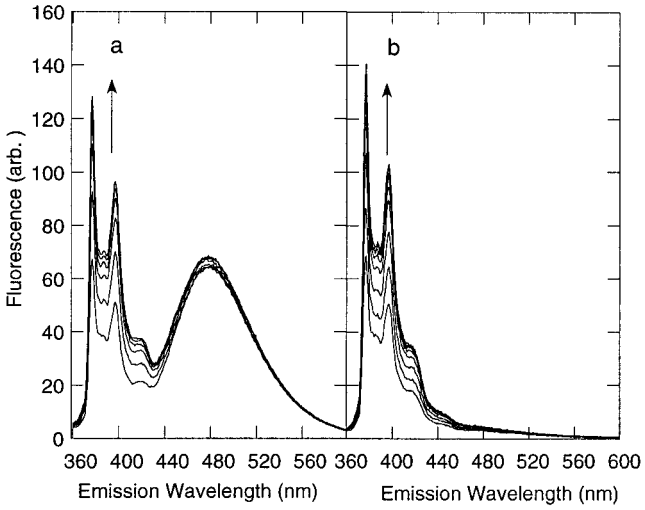

Figure 6. Fluorescence emission spectra (λ_{ex} = 345 nm) with increasing amounts of $AgClO_4$ for (a) compound **1b** (1 μM in acetonitrile). The spectra resulting from addition of 0, 2, 4, 6, 8, and 10 equiv of Ag(I) are shown. (b) compound **2b** (1 μM in acetonitrile). The spectra resulting from addition of 0, 1, 2, 4, 6, 8, 10, 15, and 20 equiv of Ag(I) are shown.

The conformational restriction caused by the binding event does not alter the amount of excimer observed. Thus, although compound **1b** gives a ratiometric response to Ag(I) binding, the mechanism is attributable to PET quenching rather than the proposed mechanism (Figure 2). This result indicates that the pyrene fluorophore will not function as a generally useful fluorophore in this system.

Analysis. Importantly, the new fluorophore-containing sensors retain the cooperative binding properties of the original design. A plot of the ratio of the monomer and excimer peaks for both sensors **1a** and **1b** is presented in Figure 7. The Hill coefficients[22] for sensors **1a** and **1b** are 1.98 and 1.97, respectively (inset in Figure 7a and 7b), consistent with a two site cooperative binding event. Moreover, the binding constants of the two sensors are similar (1.3×10^{11} M^{-2} and 2.0×10^{11} M^{-2} for sensors **1a** and **1b**, respectively). Thus, the binding of metal ions by the ethylenediamine recognition elements is not significantly perturbed by variations in the fluorescent groups. Job analysis of the excimer emission of compound **1a** confirms the stoichiometry of 2:1 (Figure 8).

Summary and Future Prospectives

In conclusion, we have shown that integrating fluorescent groups directly onto our pinwheel receptor produces sensors which are capable of binding an analyte cooperatively and eliciting a ratiometric response from reporter moieties that are isolated spatially from the binding groups. The choice of fluorescent groups is restricted to those which have the correct orientation to form an excimer. Furthermore, a useful fluorophore must form the excimer only in the bound state of the sensor. Thus, of those fluorophores tested, both pyrene and naphthalene give a ratiometric response to binding of silver ion. However, only the naphthalene fluorophore

(18) Control studies indicate that there is no direct interaction of the metal with the fluorophores. Thus, all of the observed effects on fluorescence are controlled by metal binding to the recognition elements alone.

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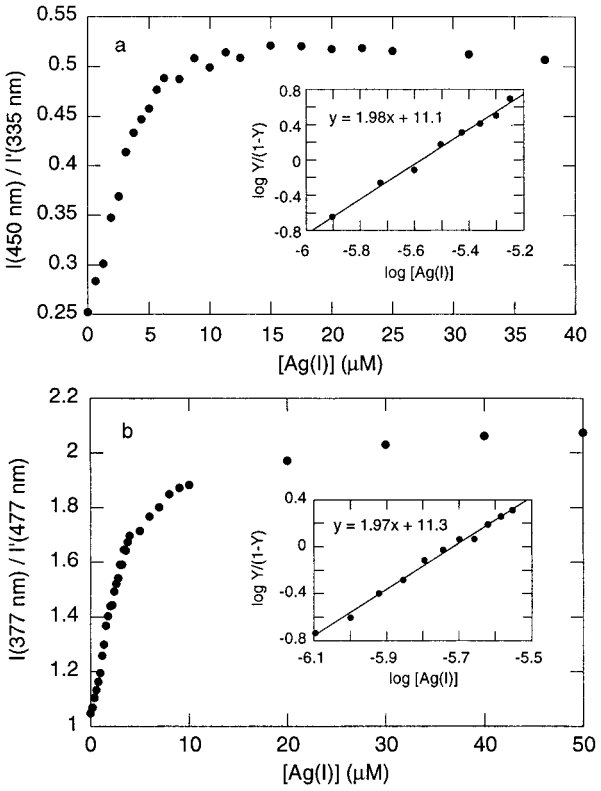

Figure 7. Plot of the ratio of fluorescence emission as a function of added $AgClO_4$ for (a) compound **1a** (1 μM in acetonitrile with 5 mM NMe_4ClO_4) at 450 nm (excimer) and 335 nm (monomer). (b) compound **1b** (1 μM in acetonitrile) at 377 nm (monomer) and 477 nm (excimer). Inset in both plots are Hill plots of the data using the equation $\log(Y/1 - Y) = n^*\log[\mathrm{Ag(I)}] + \log K_a$ where n is the Hill coefficient, Y is the fractional saturation, and [Ag(I)] is the equilibrium concentration of free Ag(I) in solution.

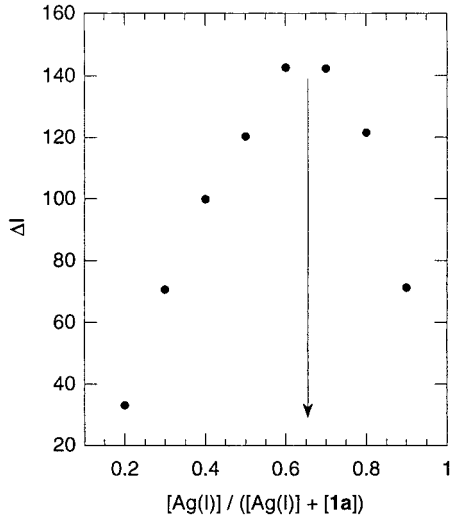

Figure 8. Job plot of sensor **1a** following the change in fluorescence at 450 nm in CH_3CN. The value of ([Ag(I)] + [**1a**]) was maintained at 10^{-5} M. A maximum at 66% Ag(I) indicates 2:1 binding stoichiometry.

follows the proposed mechanism (Figure 2) and would be useful in a general sense. It is anticipated that cooperative sensors for different analytes can be prepared by variation of the recognition elements of the sensors presented herein.

Currently, alternative recognition elements are being explored for ratiometric sensing of biologically relevant analytes. Furthermore, alternative fluorescent read-out methods are also being tested. For example, a pair of fluorophores which can undergo fluorescence resonance energy transfer (FRET) could similarly produce a ratiometric response from this pinwheel system. Several well-characterized FRET pairs have longer wavelengths of excitation and emission than the fluorophores used in this study, a property which is often desirable for biological sensing applications.

Experimental Section

General Methods. All reactions were carried out in dried glassware under argon atmosphere unless otherwise noted. Tetrahydrofuran (THF) and benzene were distilled from sodium benzophenone ketyl under argon immediately before use. Methylene chloride (CH_2Cl_2) and triethylamine (Et_3N) were distilled from CaH_2 under argon immediately before use. Flash chromatography[23] was performed with 32−63 μm silica gel. All melting points are uncorrected. NMR spectra were recorded on a Bruker WP-200, AC-200, DPX-300, AMX-360, or DRX-400 in $CDCl_3$ using TMS as a reference.

Compound 4. A solution of pyridine (1 L), (dimethylamino)-pyridine (1.22 g, 10 mmol), ethyl-3-aminobenzoate (14.9 mL, 100 mmol), and 2-naphthalenesulfonyl chloride (23.8 g, 105 mmol) was stirred at 75 °C for 18 h. The solvent was removed in vacuo, and the resulting residue was purified using flash chromatography (EtOAc/CH_2Cl_2, 10:90). *N*-(2-naphthalene-sulfonyl) ethyl-3-aminobenzoate was isolated as a white crystalline solid (35.5 g, 99.9 mmol, 100% yield). Mp 122−124 °C; ^{1}H NMR (300 MHz) δ 1.32 (t, J = 7.1 Hz, 3H), 4.33 (q, J = 7.1 Hz, 2H), 7.28 (t, J = 7.9 Hz, 1H), 7.46−7.61 (m, 3H), 7.73 (dt, J = 1.2, 7.8 Hz, 1H), 7.71−7.86 (m, 6H), 8.41 (d, J = 1.3 Hz, 1H); ^{13}C NMR (100 MHz) δ 14.2, 61.5, 122.1, 122.1, 125.2, 126.0, 127.6, 127.9, 129.0, 129.3, 129.4, 129.6, 131.5, 132.0, 134.9, 135.8, 137.1, 166.1; IR (neat) 3248, 3058, 2982, 2904, 1719, 1696, 1590, 1471, 1404, 1214, 1020, 961, 882, 755 cm^{-1}; HRMS calcd for $C_{19}H_{18}NO_4S$ (M + H$^+$): 356.0957. Found: 356.0953.

A solution of EtOH (abs., 1 L) and *N*-(2-naphthalenesulfonyl) ethyl-3-aminobenzoate (35.5 g, 99.9 mmol) was warmed to 60 °C. NaOH (6.0 g, 149.9 mmol) was added, and the reaction was stirred until all of the solid had dissolved. Methyl iodide (9.33 mL, 150 mmol) was added, and the resulting mixture was stirred at 60 °C for 22 h. The solvent was removed in vacuo. The resulting residue was dissolved in CH_2Cl_2 and washed with saturated NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 (3 × 250 mL), and the organic phase was dried over $MgSO_4$. After removal of the CH_2Cl_2, the resulting residue was purified using flash chromatography (CH_2Cl_2). Compound **4** was isolated as a white solid (27.5 g, 74.4 mmol, 75% yield). Mp 74−76 °C; ^{1}H NMR (400 MHz) δ 1.30 (t, J = 7.2 Hz, 3H), 3.24 (s, 3H), 4.30 (q, J = 7.1 Hz, 2H), 7.36−7.41, (m, 2H), 7.73 (dd, J = 1.8, 6.9 Hz, 1H), 7.56−7.66 (m, 2H), 7.72 (s, 1H), 7.87−7.90 (m, 3H), 7.94−7.97 (m, 1H), 8.18 (d, J = 1.2 Hz, 1H); ^{13}C NMR (100 MHz) δ 12.3, 36.3, 59.3, 121.1, 125.3, 125.7, 126.1, 126.6, 127.1, 127.1, 127.2, 127.4, 127.4, 129.5, 129.6, 130.1, 131.5, 133.1, 140.0, 163.8; IR (neat) 3057, 2980, 1719, 1586, 1443, 1349, 1286, 1240, 1072, 927, 759 cm^{-1}; HRMS calcd for $C_{20}H_{19}NO_4SNa$ (M + Na$^+$): 392.0932. Found: 392.0950.

Compound 5. *n*-BuLi (126 mL, 201 mmol, 1.6M in hexanes) was added to a stirred solution of THF (2.2 L) and 4-bromoanisole (27.9 mL, 223 mmol) at −78 °C. The reaction mixture was allowed to stir for 20 min., followed by addition of compound **4** (27.5 g, 74.4 mmol) in THF (250 mL, − 78 °C). The reaction mixture was allowed to warm to 0 °C and was quenched with NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 (3 × 250 mL). The organic layer was dried over $MgSO_4$ and the solvent

(23) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

removed in vacuo. Flash chromatography (EtOAc/Hex, 35:65) afforded compound **5** as a white solid (37.6 g, 69.7 mmol, 94% yield). Mp 61−62 °C; ^{1}H NMR (400 MHz) δ 2.61 (s, 1H), 3.14 (s, 3H), 3.76 (s, 6H), 6.71 (d, J = 8.9 Hz, 4H), 6.87 (t, J = 1.9 Hz, 1H), 7.01 (d, J = 8.8 Hz, 4H),7.17−7.28 (m, 3H), 7.39 (dd, J = 1.8, 6.0 Hz, 1H), 7.58−7.64 (m, 2H), 7.74 (d, J = 8.7 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 8.12 (s, 1H); ^{13}C NMR (100 MHz) δ 38.6, 55.7, 81.4, 113.6, 123.4, 125.4, 126.4, 127.1, 127.6, 128.1, 128.6, 129.0, 129.0, 129.1, 129.3, 129.5, 132.4, 134.0, 135.2, 139.2, 141.5, 148.7, 159.1; IR (neat) 3507, 3058, 3001, 2954, 2836, 1607, 1508, 1347, 1250, 1175, 1033, 828, 734 cm^{-1}; HRMS calcd for $C_{32}H_{29}NO_5SNa$ (M + Na^+): 562.1664. Found: 562.1676.

Compound 6. A solution of compound **5** (18 g, 33.4 mmol) and acetyl chloride (180 mL) was stirred at room temperature for 2 h. The acetyl chloride was removed in vacuo, and the resulting solid was carefully dried under high vacuum. The solid was dissolved in benzene (1 L). The solution was sparged with a steady stream of argon for 15 min. Ethynylmagnesium bromide (334 mL, 167 mmol, 0.5M in THF) was added to the reaction and stirred at room temperature for 1.5 h. The reaction was quenched with saturated NH_4Cl, and the aqueous layer was extracted with CH_2Cl_2 (3 × 125 mL). The organic layer was then dried over $MgSO_4$, and the solvent was removed in vacuo. Flash chromatography (EtOAc/Hex, 30:70) afforded compound **6** as an amorphous solid (15.5 g, 28.3 mmol, 85% yield). ^{1}H NMR (400 MHz) δ 2.39 (s, 1H), 3.12 (s, 3H), 3.75 (s, 6H), 6.68 (d, J = 8.9 Hz, 4H), 6.75 (t, J = 1.8 Hz, 1H), 6.98 (d, J = 8.8 Hz, 4H), 7.18 (dt, J = 1.8, 7.1 Hz, 1H), 7.22−7.28 (m, 2H), 7.43 (dd, J = 1.7, 8.6 Hz, 1H), 7.56−7.66 (m, 2H), 7.78 (d, J = 8.7 Hz, 1H), 7.87 (t, J = 8.8 Hz, 2H), 8.12 (s, 1H); ^{13}C NMR (100 MHz) δ 38.7, 54.3, 55.6, 73.6, 89.8, 113.7, 123.6, 126.5, 126.8, 127.8, 128.3, 128.5, 128.9, 129.2, 129.3, 129.5, 129.8, 130.3, 132.4, 134.0, 135.2, 136.9, 141.6, 146.8, 158.8; IR (neat) 3289, 3057, 2932, 2835, 1605, 1507, 1347, 1251, 1177, 1033, 826 cm^{-1}; HRMS calcd for $C_{34}H_{29}NO_4SNa$ (M + Na^+): 570.1715. Found: 570.1714.

Compound 7. Copper (I) chloride (28.0 g, 283 mmol) was added to a stirred solution of CH_2Cl_2 (290 mL) and compound **6** (15.5 g, 28.3 mmol). *N*-Methylpyrrolidine (59 mL, 566 mmol) was added in a dropwise fashion. The solution was stirred at ambient temperature for 2.5 h with a steady stream of bubbling O_2. The reaction mixture was filtered through a short silica column with EtOAc, and the solvent was removed in vacuo. The residue was purified via flash chromatography (EtOAc/Hex, 40:60), and compound **7** was isolated as a white amorphous solid (11.4 g, 20.9 mmol, 74% yield). ^{1}H NMR (400 MHz) δ 3.09 (s, 6H), 3.74 (s, 12H),6.63−6.68 (m, 10H), 6.94 (d, J = 8.8 Hz, 8H), 7.17−7.28 (m, 7H), 7.40 (dd, J = 2.0, 8.5 Hz, 2H), 7.53−7.62 (m, 4H), 7.74 (d, J = 8.5 Hz, 2H),7.79−7.84 (m, 4H), 8.10 (s, 2H); ^{13}C NMR (100 MHz) δ 36.2, 52.7, 53.3, 67.7, 82.3, 111.4, 121.1, 124.1, 124.2, 125.4, 125.9, 126.3, 126.7, 126.8, 126.9, 127.1, 127.3, 127.9, 130.0, 131.6, 132.8, 134.0, 139.2, 144.0, 156.5; IR (neat) 3010, 2932, 2836, 1605, 1057, 1348, 1177, 1072, 1033, 825 cm^{-1}; HRMS calcd for $C_{68}H_{56}N_2O_8S_2Na$ (M + Na^+): 1115.337. Found: 1115.3395.

Compound 8. 1,1-Dichloromethylmethyl ether (170 μL, 1.84 mmol) was added to a stirred solution of CH_2Cl_2 (10 mL), and compound **7** at 0 °C. $TiCl_4$ (2.53 mL, 2.53 mmol, 1 M in CH_2Cl_2) was added in a dropwise fashion to the reaction. The reaction was allowed to warm to ambient temperature over a period of 30 min. The mixture was slowly poured over ice, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed in vacuo. The resulting residue was purified via flash chromatography (EtOAc/Hex, 70:30) and compound **8** was isolated as a yellow amorphous solid (241 mg, 0.20 mmol, 87%). ^{1}H NMR δ 3.16 (s, 6H), 3.88 (s, 12H), 6.82 (d, J = 8.9 Hz, 4H), 6.96−7.00 (m, 4H), 7.16−7.26 (m, 4H), 7.39 (dd, J = 2.6, 8.8 Hz, 4H), 7.43 (dd, J = 1.8, 8.6 Hz, 2H), 7.54−7.62 (m, 8H), 7.81−7.84 (m, 4H),7.91 (d, J = 8.0 Hz, 2H), 8.16 (s, 2H), 10.38 (s, 4H). ^{13}C NMR δ 38.5, 55.1, 56.2, 70.9, 84.2, 112.3, 123.6, 124.6, 125.5, 127.6, 127.8, 128.2, 128.3, 128.8, 129.2, 129.4, 129.5, 129.7, 132.4, 133.7, 135.2, 136.2, 136.7, 141.9, 144.8, 161.4, 189.7. IR (neat) 3010, 2943, 2863, 1682, 1603, 1492, 1349, 1282, 1256, 1163, 1072, 911, 816, 650 cm^{-1}. HRMS for M + Na^+ calcd for $C_{72}H_{56}N_2O_{12}S_2Na$: 1227.1372. Found: 1227.3120.

Compound 1a. AcOH (1.5 mL, glacial) was added to a stirred solution of EtOH (13.5 mL), compound **8** (241 mg, 0.20 mmol), and *N*,*N*,*N*′-trimethylethylenediamine (0.76 mL, 6.0 mmol). THF was added dropwise to the solution until all reactants had dissolved. The reaction was stirred at ambient temperature for 10 h. $NaBH_3CN$ (251 mg, 4.0 mmol) was added to the solution, and the reaction was allowed to stir at ambient temperatures for 14 h. The reaction mixture was added to 10% HCl (25 mL) and allowed to stir for 15 min. The reaction was then made basic (pH ∼10) with 10 M NaOH. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and removed in vacuo. The resulting residue was purified via flash chromatography (NH_3 sat. MeOH/$CHCl_3$, 10:90), and compound **1a** was isolated as a yellow amorphous solid (149 mg, 0.096 mmol, 48%). ^{1}H NMR δ 2.07−2.13 (m, 36H), 2.33−2.44 (m, 16H), 3.06 (s, 6H), 3.42 (s, 8H), 3.75 (s, 12H), 6.54 (d, J = 8.7 Hz, 4H), 6.74 (ft, J = 1.6 Hz, 2H), 6.86 (dd, J = 2.5, 8.6 Hz, 4H), 7.06 (d, J = 2.4 Hz, 4H), 7.08−7.11 (m, 2H), 7.21−7.30 (m, 4H), 7.39 (dd, J = 1.6, 8.6 Hz, 2H), 7.52−7.61 (m, 4H), 7.73−7.79 (m, 4H),7.86 (d, J = 8.0 Hz, 2H), 8.15 (s, 2H). ^{13}C NMR δ 38.3, 42.6, 55.0, 55.6, 55.9, 56.4, 57.5, 70.0, 84.6, 109.9, 123.5, 125.9, 126.4, 126.9, 127.5, 128.1, 128.5, 128.7, 128.9, 129.1, 129.3, 129.5, 131.9, 132.2, 133.9, 135.0, 135.7, 141.4, 146.5, 157.1. IR (neat) 3054, 2942, 2817, 2414, 1601, 1496, 1463, 1349, 1251, 1162, 1131, 1031, 810, 737, 652 cm^{-1}. HRMS for M + H^+ calcd for $C_{92}H_{113}N_{10}O_8S_2$: 1549.8184. Found: 1549.8210.

Compound 9. Compound **7** (4.0 g, 3.7 mmol) and Mg turnings (17.8 g, 732 mmol) were added to a stirred solution of CH_2Cl_2 (500 mL) and MeOH (500 mL). After 30 min, the reaction began to reflux. The solution was then stirred at ambient temperature for 24 h. The solvent was removed in vacuo, and the residue was brought up in 50% AcOH (aq), extracted with CH_2Cl_2 (3 × 200 mL), and dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified via flash chromatography (EtOAc/Hex, 40:60) and the resulting bisaniline was isolated as a white solid (2.60 g, 3.7 mmol, 100% yield). Mp 123−125 °C; ^{1}H NMR (360 MHz) δ 2.77 (s, 6H), 3.79 (s, 12H),6.49−6.53 (m, 6H), 6.80 (d, J = 8.5 Hz, 8H), 7.10 (t, J = 7.8 Hz, 2H), 7.15 (d, J = 9.1 Hz, 8H); ^{13}C NMR (100 MHz) δ 31.1, 55.3, 55.7, 69.9, 84.9, 110.6, 113.6, 114.5, 118.8, 129.2, 130.6, 137.5, 146.3, 149.4, 158.7; IR (neat) 3416, 3000, 2953, 2835, 1605, 1507, 1298, 1251, 1177, 1033, 909, 828 cm^{-1}; HRMS calcd for $C_{48}H_{45}N_2O_4$ (M + H^+): 713.3379. Found: 713.3404.

Trifluoroacetic anhydride (1.29 mL, 9.1 mmol) was added to a stirred solution of THF (20 mL) and the bisaniline (649 mg, 0.91 mmol) at 0 °C. The solution was warmed to ambient temperature and quenched with water (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), and the collected organic layers were dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was filtered through a silica plug (EtOAc/Hex, 40:60), and compound **9** was isolated as an amorphous red solid (808 mg, 0.89 mmol, 98% yield). ^{1}H NMR (300 MHz) δ 3.30 (s, 6H), 3.80 (s, 12H), 6.84 (d, J = 8.8 Hz, 8H), 7.08−7.16 (m, 12H), 7.32−7.40 (m, 4H); ^{13}C NMR (75 MHz) δ 29.7, 54.8, 55.3, 69.7, 84.2, 113.6, 116.3 (q, J = 285 Hz), 125.8, 128.0, 129.3, 129.7, 130.0, 135.9, 140.3, 147.2, 157.0 (q, J = 35.8 Hz), 158.7; IR (neat) 3385, 3010, 2934, 1698, 1605, 1508, 1253, 1205, 1155, 1034, 910, 829 cm^{-1}; HRMS calcd for $C_{52}H_{42}N_2O_6F_6$ (M + Na^+): 927.2845. Found: 927.2874.

Compound 10. 1,1-Dichloromethylmethyl ether (0.74 mL, 8.16 mmol) was added to a stirred solution of CH_2Cl_2 (40 mL) and compound **9** (923 mg, 0.33 mmol) at 0 °C. $TiCl_4$ (11.2 mL, 1.0 M in CH_2Cl_2, 11.2 mmol) was added in a dropwise fashion to the reaction. The reaction was allowed to warm to ambient temperature over a period of 45 min. The mixture was slowly poured over ice, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed in vacuo. The resulting residue was purified via flash chromatography (EtOAc/Hex, 70:30) to yield the tetraaldehyde as a white amorphous solid (941 mg, 0.928

mmol, 91% yield). ^{1}H NMR (360 MHz) δ 3.32 (s, 6H), 3.95 (s, 12H), 6.99 (d, J = 9.0 Hz, 4H), 7.09 (s, 2H), 7.20 (d, J = 7.9 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 7.38−7.47 (m, 6H), 7.58 (d, J = 2.7 Hz, 4H), 10.41 (s, 4H); ^{13}C NMR (90 MHz) δ 39.7, 54.7, 55.9, 70.5, 83.7, 112.1, 116.3 (q, J = 288 Hz), 124.4, 126.5, 128.0, 128.4, 129.3, 129.8, 135.5, 136.2, 140.7, 145.5, 156.8 (q, J = 35.6 Hz), 161.2, 189.3; IR (neat) 3020, 2944, 2867, 1690, 1686, 1604, 1492, 1283, 1257, 1204, 1155, 1024, 756 cm^{-1}; HRMS calcd for $C_{56}H_{42}N_2O_{10}F_6Na$ (M + Na$^+$): 1039.2641. Found: 1039.2677.

AcOH (2 mL, glacial) and 4 Å mol. sieves were added to a stirred solution of EtOH (abs., 2 mL,), the tetraaldehyde (500 mg, 0.49 mmol), and *N*,*N*,*N*′-trimethylethylenediamine (1.87 mL, 14.7 mmol). The reaction was stirred at ambient temperature for 4 h. $NaBH_3CN$ (616 mg, 9.8 mmol) was added to the solution, and the reaction was allowed to stir at ambient temperature for 14 h. The reaction mixture was added to 10% HCl (50 mL) and allowed to stir for 15 min. The reaction was then made basic (pH ∼10) with 10M NaOH. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and removed in vacuo. The resulting residue was placed in a sealed vessel with 10 mL of NH_3 in MeOH and stirred at RT for 3 h. The solvent was removed in vacuo, and the resulting residue was purified via flash chromatography (NH_3 sat. MeOH/$CHCl_3$, 10:90), and compound **10** was isolated as a yellow amorphous solid (533 mg, 0.46 mmol, 93% yield). ^{1}H NMR (400 MHz) δ 2.14 (s, 12H), 2.16 (s, 24H), 2.42−2.66 (m, 16H), 2.74 (s, 6H), 3.45 (d, J = 12.6 Hz, 4H), 3.50 (d, J = 13.1 Hz, 4H), 3.80 (s, 12H), 3.82 (s, 2H), 6.54 (d, J = 8.7 Hz, 4H), 6.74 (t, J = 1.6 Hz, 2H), 6.86 (dd, J = 2.5, 8.6 Hz, 4H), 6.45−6.54 (m, 6H), 6.73 (d, J = 9.1 Hz, 4H), 7.05−7.09 (m, 6H), 7.13 (d, J = 2.1 Hz, 4H); ^{13}C NMR (100 MHz) δ 31.1, 42.9, 46.2, 55.3, 55.8, 55.9, 56.7, 57.7, 69.9, 85.0, 110.1, 110.3, 114.6, 118.7, 126.2, 129.1, 132.5, 136.8, 146.4, 149.4, 157.1; IR (neat) 3266, 2942, 2814, 1604, 1496, 1463, 1251, 1112, 1031 cm^{-1}; HRMS calcd for $C_{72}H_{101}N_{10}O_4$ (M + H$^+$): 1169.8007. Found: 1169.8016.

Compound 1b. A mixture of 1-pyrene acetic acid (18 mg, 0.068 mmol), diisopropyl carbodiimide (11 μL, 0.068 mmol), and THF (1 mL) was stirred at ambient temperature for 3.5 h. The reaction was then added to a flask containing compound **10** (31 mg, 0.027 mmol). The resulting mixture was stirred at ambient temperature for 24 h followed by removal of the solvent in vacuo. The resulting residue was purified via flash chromatography (NH_3 sat. MeOH/$CHCl_3$, 10:90), and compound **1b** was isolated as a yellow amorphous solid (15 mg, 0.009 mmol, 33% yield). ^{1}H NMR (400 MHz) δ 2.03 (s, 12H), 2.08 (s, 24H), 2.25−2.39 (m, 16H), 3.22 (s, 6H), 3.38 (s, 8H), 3.68 (s, 12H), 4.10 (s, 4H), 6.54 (d, J = 8.6 Hz, 4H), 6.97 (dd, J = 2.3, 6.2 Hz, 4H), 7.03 (d, J = 7.7 Hz, 2H), 7.09 (d, J = 2.2 Hz, 6H), 7.23−7.27 (m, 2H), 7.32 (d, J = 7.7 Hz, 2H), 7.51 (d, J = 7.9 Hz, 2H), 7.89−7.97 (m, 12H), 8.05 (d, J = 7.5 Hz, 2H), 8.11 (d, J = 7.6 Hz, 2H); ^{13}C NMR (90 MHz) δ 37.8, 39.3, 42.4, 45.8, 55.0, 55.4, 55.7, 56.1, 57.3, 69.9, 84.8, 109.9, 123.4, 124.7, 124.9, 125.7, 126.5, 126.9, 127.5, 127.6, 128.1, 128.5, 129.3, 129.5, 130.3, 130.8, 131.3, 131.4, 135.3, 143.7, 147.7, 157.0, 171.0; IR (neat) 2940, 2766, 1661, 1598, 1496, 1462, 1365, 1250, 1112, 1031, 846, 711 cm^{-1}; HRMS calcd for $C_{96}H_{117}N_{10}O_6$ (M + H$^+$): 1505.9158. Found: 1505.9106.

Spectroscopic Analysis. Absorption spectra were recorded on a Cary 1E spectrophotometer at 25 °C. All solutions were prepared in acetonitrile. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectofluorimeter at ambient temperature. Excitation wavelengths were 290 and 345 nm for the compounds **1a** and **1b**, respectively, with an excitation slit width of 20 nm and an emission slit width of 5 nm. All solutions were prepared in acetonitrile with the indicated concentration of Me_4NClO_4 as an ionic strength buffer. Samples were mixed prior to each irradiation to ensure homogeneity.

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Supporting Information Available: Spectral Data for compounds **1a**, **1b**, **4**, **5**, **6**, **7**, **8**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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