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Fluorescent Chemosensors Based on Conformational Restriction of a Biaryl Fluorophore

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Fluorescent chemosensors—molecules that change their fluorescence in response to substrate binding—offer an extremely sensitive optical method for the real-time monitoring of molecular interactions.[1] Such chemosensors are finding increased use in fields as diverse as biology, medical analysis, and environmental monitoring.[1a,6] The majority of fluorescent chemosensors operate by one of three mechanisms: (1) suppression of photoinduced electron transfer or enhancement of heavy-atom quenching; (2) variation of the distance between two fluorophores, modulating the efficiency of interchromophore energy transfer; and (3) alteration of the microenvironment of a solvatochromic fluorophore (e.g., by displacement from a cyclodextrin cavity).[3] We describe here an alternative mechanism for fluorescent chemosensor action, in which substrate binding leads to conformational restriction of a biaryl fluorophore, in turn producing fluorescence enhancement. Even simple systems based on this approach exhibit strong fluorescence enhancement and high binding selectivity.

While it is well established that more rigid fluorophores are more fluorescent,[4] binding-induced conformational restriction of fluorophores has received little attention.[5] The observation that the quantum yield (ϕ) of dihydrophenanthrene is approximately 50-fold greater than that of 2,2′-dimethylbiphenyl (Figure 1)[4a] led us to select biphenyl ethers **1**−**4** (Figure 2) as the simplest potential embodiment of this signal transduction pathway.[6,7]

Titration of **1**−**4** with group I and II metal cations leads to fluorescence enhancement at the emission maxima (λ_{em}, Figure 3).[8,9] The emission from **1**, **3**, and **4** intensifies only slightly upon titration with any of the five metals, although **1** exhibits a significant hypsochromic shift with Li$^+$ and Ca^{2+}. In contrast, **2** responds strongly and selectively to the presence of Ca^{2+}.

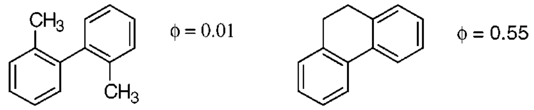

Figure 1. Quantum yield of reference biaryls.

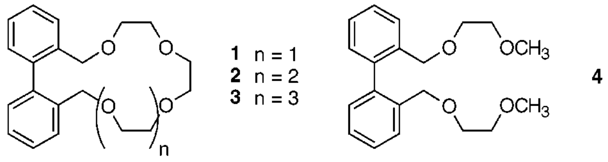

Figure 2. Biaryl ethers investigated.

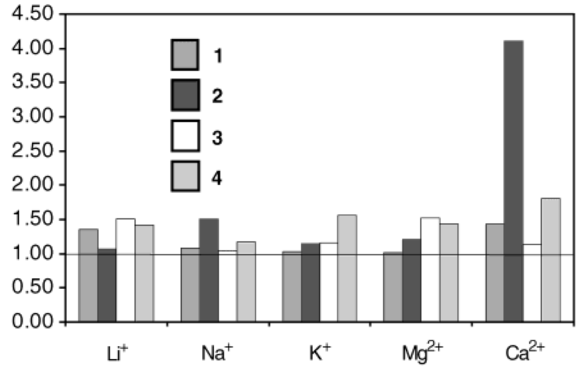

Figure 3. Fluorescence emission response profiles of **1**−**4** at λ_{max}.

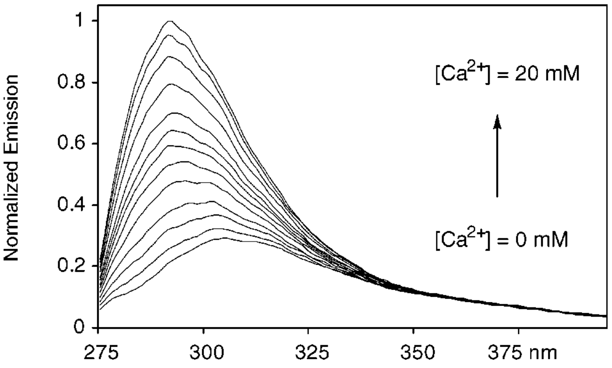

Figure 4. Titration of **2** in CH$_3$CN with Ca(ClO$_4$)$_2$.

The fluorescence spectra for titration of **2** with Ca^{2+} are shown in Figure 4. Upon addition of Ca^{2+}, a hypsochromic shift (∼15 nm) is observed, accompanied by a 4-fold fluorescence enhancement. Only small changes in the UV spectrum occur during the titration, indicating that the increase in emission results primarily from an increase in the effective quantum yield.[10] The magnitude and selectivity of the enhancement in emission from **2** is remarkable, given its structural simplicity, and the upper limit set by covalent restriction suggests that even greater enhancement should be possible in related systems.

On the basis of the fluorescence titrations of **2**, we have determined K_a for Ca^{2+} to be 2.0×10^3 M^{-1},[11] and analysis by Job's method indicates the formation of a 1:1 complex.[12] For the association of **2** with Li$^+$, Na$^+$, K$^+$, and Mg^{2+}, we measure K_a's

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(1) For leading reviews, see: (a) de Silva, A. P.; Wilers, J.; Zlokarnik, G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8336−8337. (b) deSilva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. (c) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Sacchi, D.; Taglietti, A. *Analyst* **1996**, *121*, 1763−1768. (d) *Fluorosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1994; Vol. 538.

(2) For representiative overviews, see: (a) Wolfbeis, O. S. In *Molecular Luminescence Spectroscopy: Methods and Applications - Part 2*; Schulman, S. G., Ed.; Chemical Analysis, Vol. 77; Wiley & Sons: New York, 1987; Chapter 3. (b) Schenk, G. H. In *Molecular Luminescence Spectroscopy: Methods and Applications - Part 3*; Schulman, S. G., Ed.; Chemical Analysis, Vol. 77; Wiley & Sons: New York, 1993; Chapter 8.

(3) Other mechanistic bases for fluorescent chemosensors, are known (induced charge transfer, excited-state proton transfer, e.g.). See ref 1.

(4) For representative fluorescence properties of covalently tethered biaryls, see: (a) Berlman, I. *Handbook of Fluorescence Spectra of Aromatic Molecules*; Academic Press: New York; 1971. (b) Nizhegorodov, N. I.; Downey, W. S. *J. Phys. Chem.* **1994**, *98*, 5639−5643 and references therein.

(5) For the elegant use of covalent sugar binding to induce fluorescence enhancement in flexible fluorophores, see: (a) Sandanayake, K.; Nakashima, K.; Shinkai, S. *Chem. Commun.* **1994**, 1621−1622. (b) Takeuchi, M.; Mizuno, T.; Shinmori, H.; Nakashima, M.; Shinkai, S. *Tetrahedron* **1996**, *52*, 1195−1204. (c) Takeuchi, M.; Yoda, S.; Imada, T.; Shinkai, S. *Tetrahedron* **1997**, *53*, 8335−8348.

(6) Compounds **1**−**4** were prepared from 2,2′-bis(bromomethyl)-biphenyl. See Supporting Information.

(7) For previous studies on cation binding by **2** and **3**, see: (a) Rebek, J., Jr.; Wattley, R. V. *J. Am. Chem. Soc.* **1980**, *102*, 4853−4. (b) Rebek, J., Jr.; Marshall, L. *J. Am. Chem. Soc.* **1983**, *105*, 6668−70. (c) Gavina, F. L.; S. V.; Costero, A. M.; Burguete, M. I.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 7140−3 and references therein.

(8) Partial quenching is observed on exposure of **1**−**4** to Hg^{2+} or Pb^{2+}.

(9) All fluorescence spectra were acquired at 4×10^{-4} M in CH$_3$CN; no effort was made to exclude air or water; metals were added as ClO$_4^-$ salts in CH$_3$CN; $\lambda_{exc} = 256$ nm, $\lambda_{max} = 292$−312 nm.

(10) Using biphenyl ($\phi = 0.18$) as a reference, we determined the quantum yield of **2** to be 0.01, identical to that of 2,2′-dimethylbiphenyl.

(11) K_a values were determined according to: Fery-Forgues, S.; Le Bris, M. T.; Guette, J.-P.; Valeur, B. *J. Phys. Chem.* **1988**, *92*, 6233−6237.

10.1021/ja005701a CCC: $20.00

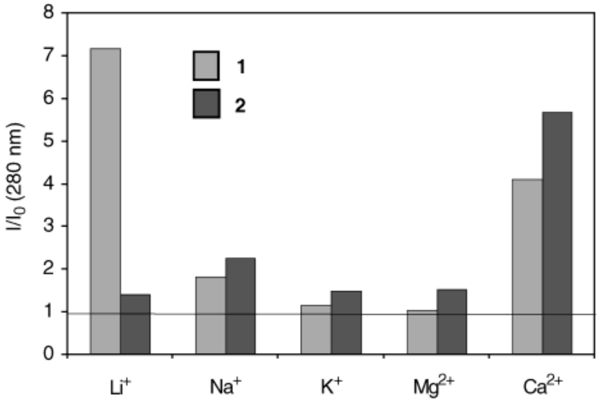

Figure 5. Blue-edge fluorescence response profiles of **1** and **2**.

of 44, 6.0 × 10^2, 28, and 59 M^{-1}, respectively.[13,14] Competition experiments in which **2** was exposed to excess Ca^{2+}, then to an equal amount of each of the other metal ions, lead to a slight reduction in emission intensity, in the order $Na^+ > Mg^{2+} > Li^+ \approx K^+$. This observation is consistent with the relative affinities that would be predicted on inspection of the fluorescence enhancements shown in Figure 3. There is no linear correspondence between I/I_0 and ln K_a, so that while the fluorescence response of **2** correlates with binding, the maximum possible value of I/I_0 varies from metal to metal. The observed selectivity does not follow the widely accepted "size-fit" model in which a crown binds most tightly to a cation whose diameter closely matches that of the crown cavity. This emphasizes the importance of effects such as solvation and chelate geometry, and highlights the difficulty of predicting even simple binding affinities.[15]

While fluorescent intensity ratios taken at λ_{max} provide the most structurally relevant information about the complexation of cations by **1**−**4**, the emissive blue-shift observed for **1** and **2** can be exploited to provide enhanced signaling sensitivity and selectivity. Figure 5 shows the response profile for **1** and **2** under the same conditions as Figure 3, but with the emission ratio taken at 280 nm.[16] Analyzed in this manner, **1** exhibits a 7-fold fluorescence enhancement on binding Li^+ and a 4-fold enhancement on binding Ca^{2+}, and both the intensity and selectivity of the response of **2** to Ca^{2+} is intensified.

Regarding the origin of fluorescence enhancement, two possibilities must be considered. The first is that the increased fluorescence is caused by suppression of intramolecular photoinduced electron transfer (PET) from the oxygen lone pairs. The second is that metal binding alters the rate of one or more relaxation processes from the excited state: radiative decay, internal conversion (IC), or intersystem crossing (ISC). As the addition of excess trifluoroacetic or sulfuric acid has no effect on the fluorescence emission of **1**−**4**, the first possibility may reasonably be excluded.[17] Therefore, metal complexation must be altering the rate of one or more relaxation pathways.

Assuming that the radiative rate is constant,[18] it might be anticipated that conformational restriction would reduce the rate of vibrationally coupled internal conversion (IC). However, the large (>100 kcal/mol) energy gap between S_1 and S_0 makes it very unlikely that IC contributes significantly to nonradiative decay.[19] Thus, metal complexation must be reducing the efficiency of ISC.

It has been observed that the rate of ISC in biphenyl increases with the dihedral angle of the biaryl bond.[20] However, the UV absorption spectra do not indicate a change in the degree of ground-state planarity, and the blue-shift in the emission spectra indicates a less-planar excited state in the presence of metal.[21] Our current hypothesis is that, despite an overall reduction in excited state planarity, binding-induced torsional restriction precludes access to the larger biaryl dihedral angles at which the rate of ISC is greater still.

We have presented proof of principle for a new fluorescent chemosensor mechanism based on the conformational restriction of fluorescent biaryls. The system is characterized by structural simplicity and strong, selective fluorescence response to metal binding. In addition to continued study of the structure and photophysics of **2**·Ca^{2+}, our efforts are focused on developing longer-wavelength and water-compatible polyaryl fluorescent chemosensors that signal metal binding by enhanced or red-shifted fluorescence.

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Supporting Information Available: Synthetic procedures, tabulated spectral data, UV and fluorescence spectra for **1**−**4**, UV and fluorescence spectra for all titrations of **2**, Job plot for **2**·Ca^{2+} (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Connors, K. A. *Binding Constants*; Wiley-Interscience: New York, 1987; pp 24−28.

(13) The value of K_a for Na^+ is significantly lower than that previously determined in $CHCl_3$, reflecting the large difference in solvent polarity. See ref 6.

(14) Mg^{2+} appears to form a 2:1 metal:ligand complex with **2**. The K_a was determined for coordination of the first equivalent of Mg^{2+}.

(15) Na^+ and Ca^{2+} have nearly identical ionic radii. For a discussion of the limitations of the size-fit model and the difficulties associated with predicting binding constants, see: (a) Martell, A. E.; Hancock, R. D.; Motekaitis, R. J. *Coord. Chem. Rev.* **1994**, *133*, 39−65. (b) Hancock, R. D. *Analyst* **1997**, *122*, R51−R58.

(16) At 280 nm, the emission intensities for **1**·Li^+, **1**·Ca^{2+}, and **2**·Ca^{2+} are still >50% of their maximum value.

(17) Acid-induced fluorescence enhancement is a convenient probe for PET. For early examples, see: (a) Cox, G. S.; Turro, N. J.; Yang, N. C.; Chen, M.-J. *J. Am. Chem. Soc.* **1984**, *106*, 422−424. (b) Konopelski, J. P.; Kotzyba-Hibert, F.; Lehn, J.-M.; Desvergne, J.-P.; Fagés, F.; Castellan, A.; Bouas-Laurent, H. *J. Chem. Soc., Chem. Commun.* **1985**, 433−436. (c) de Silva, A. P.; de Silva, S. *J. Chem. Soc., Chem. Commun.* **1986**, 1709−1711.

(18) The UV spectra suggest that the Einstein coefficient for absorption, and thus the rate of radiative decay, are unchanged in **2**·Ca^{2+}. See: Calvert, J. G.; Pitts, J. N., Jr. *Photochemistry*; Wiley & Sons: New York, 1966; pp 173−174.

(19) For leading references, see: (a) Freed, K. F. *Acc. Chem. Res.* **1978**, *11*, 74−80. (b) Klessinger, M.; Michl, J. *Excited States and Photochemistry of Organic Molecules*; VCH: New York, 1995; pp 252−260.

(20) See: a) Fujii, T.; Suzuki, S.; Komatsu, S. *Chem. Phys. Lett.* **1978**, *57*, 175−178. (b) Fujii, T.; Suzuki, S.; Komatsu, S. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2516−2520 and references therein.

(21) The S_0 state of biphenyl has a potential energy minimum at a dihedral angle of ~40°; the S_1 state is essentially planar. See: (a) Takei, Y.; Yamaguchi, T.; Osamura, Y.; Fuke, K.; Kaya, K. *J. Phys. Chem.* **1988**, *92*, 577−581. (b) Im.; H.-S.; Bernstein, E. R. *J. Chem. Phys.* **1988**, *88*, 7337−7347 and references therein.