ORGANIC LETTERS

2010
Vol. 12, No. 5
940−943

Efficient Discovery of Fluorescent Chemosensors Based on a Biarylpyridine Scaffold

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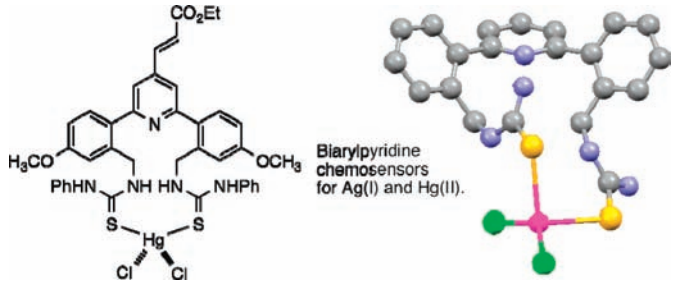
Received December 17, 2009

ABSTRACT



The discovery of several fluorescent chemosensors for Hg(II) and Ag(I) in mixed aqueous solution is reported. The ease with which these fluorionophores were prepared from a common core underscores the utility of conformational restriction as a signaling mechanism. In addition, for the first time, significant changes were observed in biarylpyridine emission wavelength, allowing ratiometric detection of Hg(II) and Ag(I). Finally, on the basis of computational analyses, beneficial structural modifications were predicted for the next generation of chemosensors.

Fluorionophores are powerful tools for the measurement of ion concentration in environmental or biological samples.[1] A key concern in designing such sensors is the connection between substrate recognition and photophysical changes in the reporting fluorophore. The most common signaling mechanisms remain photoinduced electron transfer (PET) and intramolecular charge transfer (ICT).[1] The ubiquity of these mechanisms derives from their robustness; for instance, addition of a benzylic amine to a fluorophore almost invariably leads to PET quenching, fluorescence being recovered upon protonation or metal ion coordination by the amine. However, every signaling mechanism places limitations on potential receptor structure, and no single mechanism will be universally suitable. There is thus continued need for the development of new ways to turn recognition events into changes in fluorescence.

We have pursued signaling based on conformational restriction, in which substrate binding restricts excited-state biaryl torsion and leads to fluorescence enhancement, with the hypothesis that this might minimize structural limitations on potential binding domains.[2,3] 2,6-Biarylpyridines have emerged as our preferred fluorophore core (Figure 1). On the basis of this scaffold, we first developed fluorionophores

(1) For representative reviews of fluorescent chemosensors, see: (a) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588. (b) Bell, T. W.; Hext, N. M. *Chem. Soc. Rev.* **2004**, *33*, 589–598. (c) Rurack, K.; Resch-Genger, U. *Chem. Soc. Rev.* **2002**, *31*, 116–127. (d) Bren, V. A. *Russ. Chem. Rev.* **2001**, *70*, 1017–1036. (e) de Silva, A. P.; Gunarathe, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 515. (f) *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993.

(2) (a) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2001**, *123*, 1260–1261. (b) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2001**, *124*, 1178–1179. (c) Mello, J. V.; Finney, N. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 1536–1539. (d) Mello, J. V.; Finney, N. S. *Org. Lett.* **2001**, *3*, 4263–4265. (e) McFarland, S. A.; Finney, N. S. *Chem. Commun.* **2003**, 388–389. (f) Fang, A. G.; Mello, J. V.; Finney, N. S. *Org. Lett.* **2003**, *5*, 967–970. (g) Fang, A. G.; Mello, J. V.; Finney, N. S. *Tetrahedron* **2004**, *60*, 11075–11087. (h) McFarland, S. A.; Finney, N. S. *Inorg. Chem.* **2005**, *44*, 4066–4076. (i) Mello, J. V.; Finney, N. S. *J. Am. Chem. Soc.* **2005**, *127*, 10124–10125.

10.1021/ol902902m
Published on Web 02/04/2010

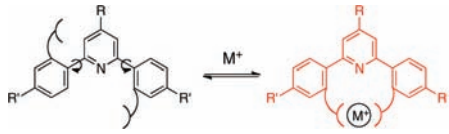


Figure 1. Binding-induced conformational restriction of biarylpyridines leads to increased fluorescence.

with simple polyether ligands.[2c,d,f,g] We then extended this approach to the construction of a solid-phase combinatorial library of potential chemosensors with amino acid/acyl end-cap binding domains, leading to the discovery of new fluorescent chemosensors for aqueous Hg(II).[2i]

Missing between these two extremes is an evaluation of smaller sets of conjugates of the biarylpyridine core. Given the task of pursuing the lead structures identified in our library, we expanded our efforts to include the concurrent synthesis of other simple biarylpyridines with chelating groups known to have high affinity for cations and anions, such as catechols and guanidines. In the course of these efforts, we took the opportunity to improve the previous synthesis of our core fluorophore in order to make it more accessible.

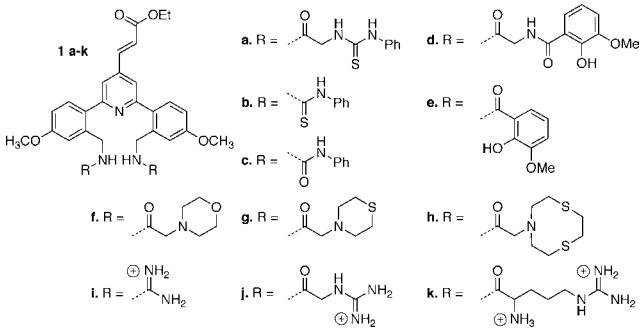


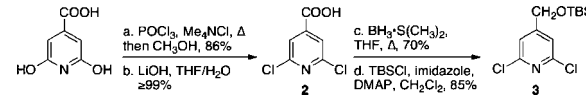
Figure 2. Collection of potential fluorescent chemosensors.

We present here a collection of 10 compounds that we expected might be ion-responsive chemosensors. All are based on fluorophore **1** (Figure 2, R = H; cations as Cl^- salts), appended in two or three steps with two identical receptor arms. Compounds **1a**−**h** were anticipated to display affinity for metal cations, while it was envisioned that **1a**, **1b**, and **1i**−**k** would interact with mono- or dianionic analytes.

The first class of compounds (**1a**−**c**) consists of a phenylthiourea moiety, with or without glycine as a linker, and a urea analogue.[4] (Compound **1a** is closely related to the chemosensors discovered from the solid-phase combinatorial library.)[2i] The second set of fluorophores contains 2-hydroxy-3-methoxy-benzoic acid as a binding domain (**1d,e**).[5] The third group comprises simple azacrown ethers (**1f**−**h**),[6] and in the last guanidine derivatives are appended to the fluorophore (**1i**−**k**).[7]

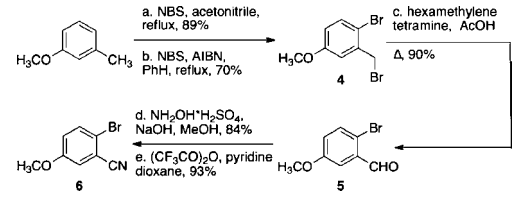
The preparation of **1a**−**k** began with the synthesis of an advanced intermediate that could then be converted to the desired potential chemosensors.[8] The previous synthetic approach to **1** has been significantly improved, reducing the number of total steps and enhancing the overall yield. (We can now routinely prepare 1−2 g quantities of the Boc-protected precursor to **1**.)

Scheme 1



The synthesis of the first key intermediate, **3**, begins with the inexpensive pyridine derivative citrazinic acid (Scheme 1). Treatment with $POCl_3$, quenching with methanol, and subsequent saponification provided **2** in good yield. Reduction with $BH_3{\cdot}SMe_2$ and protection of the alcohol efficiently provided **3** in an overall yield of 51%.

Scheme 2



The synthesis of the second key intermediate, **6** (Scheme 2), began with electrophilic bromination of 3-methylanisole

(3) For conceptually related but mechanistically distinct work on "molecular ridigification" in carbohydrate sensing, see: (a) Samankumara, K. R.; Nakashima, K.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1621. (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.

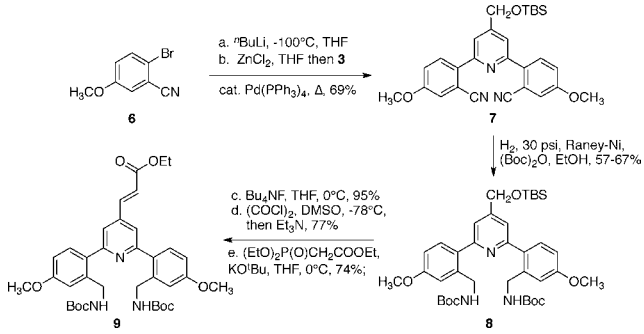
(4) For an overview of metal ion coordination by ureas/thioureas, see: (a) Koch, K. R. *Coord. Chem. Rev.* **2001**, *216*−*217*, 473–488, and references therein. For reviews of anion coordination by ureas/thioureas, see: (b) Gunnlaugsson, T.; Ali, H. D. P.; Glynn, M.; Kruger, P. E.; Hussey, G. M.; Pfeffer, F. M.; Santos, C. M. G.; Tierney, J. *J. Fluor.* **2005**, *15*, 287–299. (c) Amendola, V.; Bonizzoni, M.; Esteban-Gomez, D.; Fabbrizzi, L.; Licchelli, M.; Sancenon, F.; Taglietti, A. *Coord. Chem. Rev.* **2006**, *250*, 1451–1470.

(5) For reviews of metal chelation by catechols, see: (a) Albrecht, M.; Froehlich, R. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 797–808. (b) McMurry, T. J.; Raymond, K. N.; Smith, P. H. *Science* **1989**, *244*, 938–43.

(6) For an overview of metal ion coordination by crown ether and cryptand species, see: (a) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S. *Chem. Rev.* **1991**, *91*, 1721–2085. For a recent example of Ag(I)-responsive fluorescent chemosensors based on morpholine and thiomorpholine ligands, see: (b) Swamy, K. M. K.; Kim, H. N.; Soh, J. H.; Kim, Y.; Kim, S.-J.; Yoon, J. *Chem. Commun.* **2009**, 1234–1236. For metal ion coordination by 7-aza-1,4-dithiacyclononane, see: (c) McAuley, A.; Subramanian, S. *Inorg. Chem.* **1990**, *29*, 2830–2837. (d) Blake, A. J.; Danks, J. P.; Fallis, I. A.; Harrison, A.; Li, W.-S.; Parsons, S.; Ross, S. A.; Whittaker, G.; Schröder, M. *J. Chem. Soc., Dalton Trans.* **1998**, 3969–3976. (e) van de Water, L. G. A.; ten Hoonte, F.; Driessen, W. L.; Reedijk, J.; Sherrington, D. C. *Inorg. Chem. Acta* **2000**, *303*, 77–85.

followed by radical bromination. Transformation of benzyl bromide **4** to aldehyde **5** via Sommelet reaction, followed by conversion to the oxime and dehydration with TFAA,[9] provided **6** in an overall yield of 44%.

Scheme 3



The aromatic components **3** and **6** were combined via Negishi coupling (Scheme 3).[10] The hydrogenation of the CN groups in **7** proved more reliable than our previous $NiCl_2$/$NaBH_4$ reduction protocol,[11] and in the presence of Boc anhydride consistently provided reasonable yields of **8**. Removal of the TBS group and subsequent Swern oxidation, followed by Wittig−Horner coupling with ethyl phosphonoacetate, provided Boc protected diamine **9**, which is a stable and convenient precursor for **1**. This represents an overall net reduction of 3 steps relative to our original route.

The syntheses of **1a,d,e,j,k** consist of two or three steps: deprotection of **9** with HCl/dioxane and coupling with the corresponding carboxylic acid, followed by deprotection as needed. Compounds **1b** and **1c** were prepared from phenyl isothiocyanate and isocyanate, respectively. For **1f**−**h**, deprotected **9** was coupled with α-bromoacetic acid and then alkylated with morpholine, thiomorpholine, or 7-aza-1,4-dithiacyclononane.[6c−e] Compound **1i** was prepared by deprotection of **9**, coupling with *N*,*N*′-di-Boc-*N*″-triflylguanidine.[12]

This collection of potential fluorescent chemosensors was evaluated in the presence of ions chosen on the basis of physiological or environmental relevance. The cations chosen for titration were Li^+, Na^+, K^+, Cs^+, Ca^{2+}, Ba^{2+}, Sr^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, $Fe^{2+/3+}$, Ag^+, and Hg^{2+}. Anions used were F^-, Cl^-, Br^-, I^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, ClO_4^-, acetate, malonate, and oxalate.[8,13]

(7) For a review of guanidine coordination chemistry, see: Bailey, P. J.; Pace, S. *Coord. Chem. Rev.* **2001**, *214*, 91–141.

(8) See Supporting Information for complete experimental details and compound characterization.

(9) Carotti, A.; Campagna, F. *Synthesis* **1979**, 56.

(10) Negishi, E.-i.; Zeng, X.; Tan, Z.; Qian, M.; Hu, Q.; Huang, Z. *Metal-Catalyzed Cross Coupling Reactions*, 2nd ed.; de Meijere, A., Diederich, F., Eds.; Wiley-VCH: New York, 1998; Chapter 15.

(11) Vergne, F.; Aitken, D. J.; Husson, H.-P. *J. Org. Chem.* **1992**, *57*, 6071.

(12) Feichtinger, F.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804–3805.

(13) Li^+, Na^+, and K^+ were added as perchlorate salts, and Ag^+ as its tosylate salt. All other metal ions were added as their chlorides. Anions were added as Li^+ salts except acetate, malonate and oxalate, which were added as their Na^+ salts.

Table 1. Optical Properties and Association Constants for **1a,b,h**

1x	ε^a	ϕ^b	K_{eq} **1x**·Hg^{2+} (I/I_0)^c	K_{eq} **1x**·Ag^+ (I/I_0)^c
1a	5.91	0.01	1.97 (4.2)	8.05 (10.1)
1b	5.83	0.01	5.42 (3.0)	1.10 (3.0)
1h	5.79	0.01	17.50 (2.7)	2.45 (2.8)

[a] For longest-wavelength λ_{max}; ε, 10^3 cm^{-1} M^{-1}. [b] Relative to pyrene ($\phi = 0.32$). [c] K_{eq}, 10^4 M^{-1}; I/I_0 ratiometric increase of emission at λ_{max}(em); 1:1 DMSO/pH 7.4 $MOPS_{aq}$ solution.

Compounds **1a**−**k** are only modestly water-soluble, and thus titrations were carried out with 5×10^{-5} M solutions of fluorophore in 1:1 DMSO/pH 7.4 MOPS buffer. Salts were added as 10^{-3} M solutions in the same solvent mixture. Somewhat surprisingly, the majority of titrations led to little or no change in emission intensity. (This will be discussed below.) However, three compounds, **1a**, **1b**, and **1h**, showed strong responses to the addition of Ag(I) and Hg(II) salts (Table 1).[14−17]

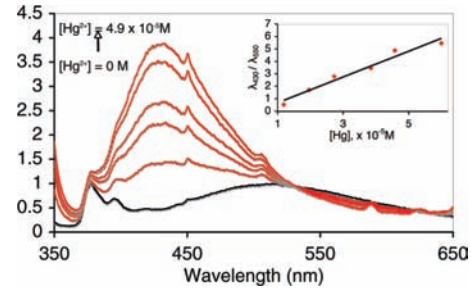


Figure 3. Titration of **1a** (5×10^{-5} M) in DMSO/pH 7.4 $MOPS_{aq}$ solution with $HgCl_2$. Inset: change of I_{430}/I_{550}.

The response of **1a** was anticipated, as this is a close analogue of the Hg(II)-responsive chemosensors discovered from the previous combinatorial library.[2i] Fluorescence emission from **1a** increased ca. 4-fold upon titration with Hg(II) (Figure 3).[18] In addition, it exhibited a significant (80 nm) blue-shift in emission. This allows for ratiometric Hg(II)

(14) For a review of Hg(II)-responsive fluorescent chemosensors, see: Nolan, E. M.; Lippard, S. J. *Chem. Rev.* **2008**, *108*, 3443.

(15) For recent examples of fluorescent chemosensors for Ag(I) recognition in aqueous solution, see ref 6b and. (a) Park, C. S.; Lee, Y.; Kang, E.-J.; Lee, J.-E.; Lee, S. S. *Tetrahedron Lett.* **2009**, *50*, 671. (b) Iyoshi, S.; Taki, M.; Yamamoto, Y. *Inorg. Chem.* **2008**, *47*, 3946.

(16) Binding constants were determined by non-linear least-squares fitting of plots of emission intensity versus log[M] using the program Prism3 (Graphpad, Inc., San Diego, CA).

(17) All ligand−Ag(I) complexes were of 1:1 stoichiometry, as determined by the method of continuous variation. We were not able to accurately evaluate the stoichiometry of Hg(II) complex formation because the increases in emission are almost completely offset by dilution. For purposes of binding constant determination, we have treated them as 1:1 complexes, although we suspect that multiple species are present.

(18) Three minor features in the emission spectra warrant comment. Two invariant peaks at ca. 380 and 400 nm arise from impurities in the DMSO/buffer solution that we were not able to remove despite repeated purification. The small spike at ca. 450 nm is a Wood's anomaly characteristic of our fluorimeter.

analysis, which can provide more accurate and quantitative measurements of metal ion concentration in biological or other heterogeneous media. Ratiometric detection is comparatively rare with single-fluorophore chemosensors and has not been observed with previous biarylpyridine fluorophores. (No changes are observed in the absorbance spectra upon metal ion titration, and the blue shift is thus an excited state effect.)

Using the intensity at $\lambda = 550$ nm as a measure of [**1a**] and at $\lambda = 430$ for [**1a**·Hg(II)], we see an excellent linear correlation up to 1:1 **1a**:Hg(II) (Figure 3, inset).[8] Binding to Ag(I) occurs with higher affinity and leads to a larger (ca. 10-fold) enhancement of emission, accompanied by a smaller hypsochromic shift (35 nm), which in turn suggests the possibility of discriminating Hg(II) and Ag(I) with a common chromophore. In both cases, we attribute the blue-shift to formation of a more twisted (i.e., less conjugated) yet still conformationally restricted excited state from the **1a**·metal complexes.[19] Removal of the glycine linker (**1a** → **1b**) doubles the affinity for mercury and decreases the affinity to silver ions (Table 1). No significant blue shift was observed for either binding event.

While the simple morpholine and thiomorpholine derivatives (**1f,g**) did not respond to titration with metal ions, dithioazacrown **1h** exhibits the highest affinity for Hg(II) of any of **1a,b,h**, as well as responding to Ag(I). Like **1a** and **1b**, **1h** does not respond to the addition of other thiophilic metal ions.

Despite prolonged effort, we have not yet obtained crystals of any biarylpyridine fluorophore−metal complex suitable for crystallographic analysis. As an alternative, we have undertaken computational study of simple analogues of the $HgCl_2$ complexes of **1a** and **1b**, in which the OMe, Ph, and vinylogous amide have been removed to simplify calculation.[8,20,21] We find two low-energy conformations for each complex, inspection of which is highly instructive.

The minimum-energy conformations are C_s-symmetry structures, in contrast to what might be intuited by inspection of simple molecular models. The lowest-energy conformation of **1b**·$HgCl_2$ is illustrative (Figure 4). In addition to S−Hg coordination, there are hydrogen-bonding interactions between the thiourea N−H groups and the chlorine atoms, and the thioureas exist exclusively in the *s-trans* conformation. In addition, the complexes are highly sterically congested, with the thioureas held very close to the biarylpyridine core. Notably, the presumed location of one of the N-Ph groups would lead to significant steric repulsion between the phenyl group and the fluorophore. Finally, even the inclusion of a glycine spacer (**1a**·$HgCl_2$, not shown) does little to relieve the steric congestion.

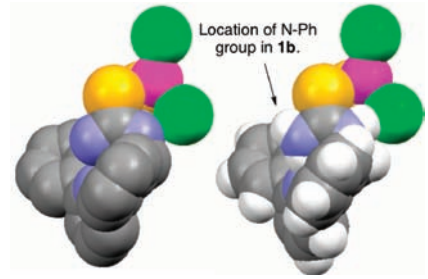


Figure 4. B98/LANL2DZ optimized lowest-energy structure for a minimal analogue of **1b**·$HgCl_2$, without (left) and with (right) hydrogen atoms.

These calculations suggest that the majority of **1a**−**k** are not effective chemosensors because they are too sterically congested to allow cooperative substrate binding, a prerequisite for conformational restriction. As important, the calculations lead to two significant structural predictions. First, it should be possible to increase the affinity of **1a** and **1b** for $HgCl_2$ by removing the N−Ph group of the thiourea or replacing it with a smaller substituent. Second, and more generally, insertion of a longer linker between the fluorophore and the binding domains should relieve steric congestion and broaden the range of effective binding elements.

In summary, we have validated binding-induced conformational restriction as a general signaling mechanism with wide tolerance for structural variation of recognition domains, discovered three new fluorescent chemosensors for aqueous Ag(I) and Hg(II), and made specific, computation-based structural predictions regarding next-generation chemosensors with improved performance. Experimental verification of these predictions is in progress.

Supporting Information Available: Synthetic procedures. Complete experimental details for optical measurements, titrations, and computational modeling. Full characterization of relevant compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgment. We thank the University of Zurich and the SNF for financial support.

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(19) This blue shift is not observed in CH_3CN, raising the possibility that the blue shift arises from changes in polarization of the excited state rather than distortion from planarity.

(20) The conformational analyses of the molecular systems described in this study, including structural and orbital arrangements as well as property calculations, were carried out using the GAMESS[a] software package. The B98 density functional[b] was used together with the LANL2DZ basis set.[c] Full geometry optimizations were performed and uniquely characterized via second derivatives (Hessian) analysis to determine the number of imaginary frequencies (0=minima; 1=transition state). Molecular orbital contour plots, used as an aid in the analysis of results, were generated and depicted using the programs WEBMO and QMView.[d] (a) Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Elbert, S. T. *J. Comput. Chem.* **1993**, *14*, 1347. (b) Schmider, H. L.; Becke, A. D. *J. Chem. Phys.* **1998**, *108*, 9624. (c) Wadt, W. R.; Hay, P. J. *J. Chem. Phys.* **1985**, *82*, 284. (d) Baldridge, K. K.; Greenberg, J. P. *J. Mol. Graphics* **1995**, *13*, 63.

(21) There are numerous examples of the formation of $HgCl_2$ complexes with thioureas in polar protic media, including water. (As opposed to complexes in which the chlorides are dissociated.) For a representative case, see Aucken, I. *Inorg. Syn.* **1960**, *6*, 27–30.