J|A|C|S
COMMUNICATIONS

Published on Web 06/30/2005

Reversing the Discovery Paradigm: A New Approach to the Combinatorial Discovery of Fluorescent Chemosensors

Jesse V. Mello and Nathaniel S. Finney*,†

Department of Chemistry and Biochemistry, University of California, San Diego 9500 Gilman Drive, La Jolla, California 92093-0358

Received October 18, 2004; E-mail: finney@oci.unizh.ch

Fluorescent chemosensors (small molecules whose fluorescence emission changes in response to a binding event) have become widely appreciated as tools for monitoring biological, biomedical, and environmental processes.[1] The reigning paradigm for chemosensor discovery is to identify a binding event of interest and derivatize the receptor component with an appropriate fluorophore. This makes the development of new chemosensors extremely challenging as chemists' collective ability to design new binding events remains limited. While the power of combinatorial methods in the discovery of new molecular recognition phenomena is now established,[2] very little progress has been made in the combinatorial discovery of fluorescent chemosensors despite the acknowledged appeal of this approach.[3,4] This reflects the limitations of the common mechanisms for turning a binding event into a fluorescent response, which either restrict the potential diversity of the binding domains or require molecular architecture too complex for ready combinatorial variation.[5]

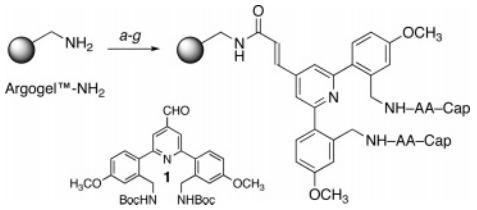
We have found that reversal of the discovery paradigm, using fluorescence to search for new binding events, and the construction of combinatorial chemosensor libraries can be simultaneously effected by exploiting a new signaling mechanism, binding-induced restriction of fluorophore biaryl torsion.[6,7] This mechanism allows the ready construction of solid-phase libraries of potential fluorescent chemosensors, as it does not restrict binding domain diversity and relies on comparatively simple molecular architecture. Here we describe the synthesis of a proof-of-principle library based on a core biarylpyridine fluorophore, and the discovery of novel visibly emissive Hg^{2+}-responsive chemosensors from this library.

We previously found polyarylpyridines to be versatile scaffolds for chemosensor development and selected a 2,6-biaryl-4-vinylpyridine as the core fluorophore for our library.[6b,f] Following preparation of an appropriately functionalized, 2,6-biarylpyridine-4-carboxaldehyde (**1**, Scheme 1), this fluorophore precursor was attached to PEG-derivatized aminomethyl polystyrene via coupling with an immobilized phosphonate.[8,9] Subsequent removal of the Boc groups provided resin derivatized with the free diaminofluorophore.

We then appended the fluorophore with two identical arms consisting of an amino acid followed by an acylating end cap. This provided both synthetic simplicity and a high likelihood that library elements would exhibit metal binding,[10] in turn allowing us to quickly test the central hypothesis that our fluorophore could be used to search for new binding events. The amino acid and acyl end cap components were selected to allow for direct interaction with metal ions as well as additional hydrophobic interactions induced by metal binding (Chart 1, **a**−**r**, **s**−**cc**).

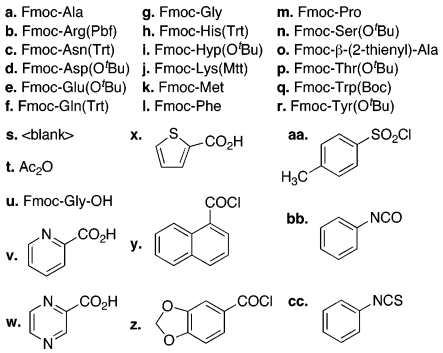
Suitably protected Fmoc amino acids were coupled by manual parallel synthesis to the fluorophore with DEPBT and $^{i}Pr_2NEt$ in CH_2Cl_2.[11] Acyl end caps were appended after removal of the Fmoc protecting groups and division of the resin (Scheme 1). Subsequent removal of the trityl (Tr), methoxytrityl (Mtt), and Boc protecting groups from all library members except those capped with phenylthiourea (**cc**) provided the library of 198 spatially arrayed potential fluorescent chemosensors.[12]

Scheme 1. Synthesis of the Chemosensor Library[a]



[a] Reagents and conditions: a.$(EtO)_2P(O)CH_2CO_2H$, DEPBt, DIEA, THF; b. LiBr, NEt_3, **1**. CH_3CN; c. TFA/CH_2Cl; d. Fmoc-AA (Chart 1), DEPBT, $^{i}PrNEt$, CH_2Cl_2; e. piperidine/DMF; f. [t]: Ac_2O, DMAP, CH_2Cl_2; [u−x]: $ArCO_2H$, as *d*; [y−z,aa]: ArCOCl/SO_2Cl, NEt_3, DMAP, CH_2Cl_2; [bb-cc]: PhNCS/PhNCO, CH_2Cl_2 (Chart 1); g. TFA, $^{i}Pr_3SiH$, CH_2Cl_2.

Chart 1. L-Amino Acid and Acyl End Cap Library Components



Library members were transferred to 96-well microtiter plates and evaluated in parallel for their response to the addition of solutions of 11 separate metal ions, as evaluated with a microscope in a dark room using a hand-held TLC lamp as the illumination source. An attractive feature of this library is that it is self-assaying: upon substrate binding, "hits" reveal themselves through an increase in visible emission.[13] The ions Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} were chosen on the basis of physiological or environmental relevance,[14] and were added as 1 mM solutions of metal perchlorate in 5 mM aqueous MOPS buffer (pH 7.5).

The majority of metal/library element combinations led to little or no change in fluorescence emission. However, all library elements bearing a phenylthiourea end cap (**cc**) gave a readily seen increase in visible green fluorescence emission upon addition of Hg^{2+} (Figures 1, 2).[15−18] While secondary to the apparent requirement for an Hg−thiourea interaction, the identity of the amino acid also influenced emission intensity; for instance, after the addition of Hg^{2+}, the emission from library element **k**−**cc** (Figures 1, 2A) was slightly brighter than that from **g**−**cc** and much brighter than

† Present address: Organisch-chemisches Institut, Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland.

10.1021/ja043682p CCC: $30.25

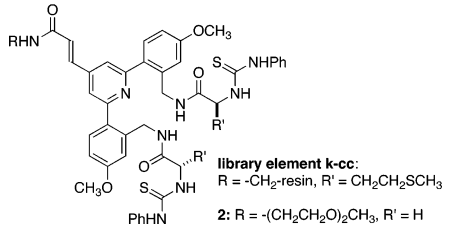


Figure 1. Representative solid- and solution-phase chemosensors for Hg^{2+}.

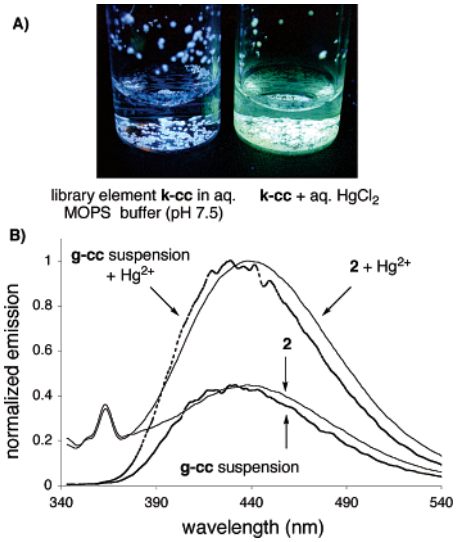


Figure 2. Solid- and solution-phase emission from **g**−**cc**, **k**−**cc**, and **8**.

that from **h**−**cc**. That this variation is seen with the side-chain protecting groups still in place bodes well for larger future libraries with more robust sulfur-containing capping groups.

The response of library elements such as **g**−**cc** to Hg^{2+} was confirmed by the synthesis of a solution-phase analogue, glycol amide **2**.[8] While **2** was not sufficiently soluble in aqueous MOPS buffer to allow an exact solution-phase repetition of the solid-phase titration, comparison of the normalized emission spectra for a 10^{-5} M CH_3CN solution of **2** and a suspension of resin bound **g**−**cc** in CH_3CN provides confirmation of the solid-phase results and shows that the solution- and solid-phase steady-state fluorescence properties are nearly identical (Figure 2B).[17,18] Titration of **2** in CH_3CN revealed $K_a = 1.8 \times 10^{-6}$ M^{-1} for the formation of **2**·Hg^{2+}.[8] This affinity is an order of magnitude greater than that of 18-crown-6 for K^+,[19] and represents both a remarkable first hit and a promising starting point for the development of more sensitive Hg^{2+} sensors.[20]

In conclusion, we have demonstrated a new and broadly applicable approach to the identification of new fluorescent chemosensors. This approach reverses the reigning discovery paradigm, which requires a preestablished binding event. The ease with which we found a new class of Hg^{2+}-responsive chemosensors demonstrates the power of this approach and confirms the hypothesis that our fluorophore and signaling mechanism are well suited to such explorations. Future work includes further study of **2** and its Hg(II) complex, the development of analogues with increased solubility, selectivity, and affinity, and the search for chemosensors for other metal ions.

Acknowledgment. We thank the National Science Foundation for support of this research (CHE-9876333) and the Departmental NMR facilities (CHE-9709183). We dedicate this manuscript to the fond memory of Prof. Murray Goodman (UCSD).

Supporting Information Available: Complete experimental details; spectral data for **1**, **2** and precursors thereof; K_a determination for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For representative reviews of fluorescent chemosensors, see: (a) Rurack, K.; Resch-Genger, U. *Chem. Soc. Rev.* **2002**, *31*, 116−127. (b) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3118−3130. (c) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3−40.

(2) Overviews of peptide-containing combinatorial libraries for the discovery of new molecular recognition phenomena: (a) Wennemers, H. *Chimia* **2003**, *57*, 237−240. (b) Tunnemann, R.; Jung, G. *Nachr. Chem.* **2000**, *48*, 453−457. (c) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155.

(3) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423.

(4) For the few examples of combinatorial approaches to fluorescent chemosensor development, see: (a) Chen, C.-T.; Wagner, H.; Still, W. C. *Science* **1998**, *279*, 851−853. (b) Rothman, J. H.; Still, W. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 509−512. (c) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542−543. (d) Singh, A.; Yao, Q.; Tong, L.; Clark Still, W.; Sames, D. *Tetrahedron Lett.* **2000**, *41*, 9601−9605. (f) Iorio, E. J.; Shao, Y.; Chen, C.-T.; Wagner, H.; Still, W. C. *Bioorg Med. Chem. Lett.* **2001**, *11*, 1635−1638.

(5) Photoinduced electron transfer (PET), by far the most common signaling mechanism, requires only simple molecules but is restricted to binding domains containing anilinic or benzylic nitrogen atoms. Energy transfer signaling (FRET, e.g.) is permissive in terms of binding domain structure but requires large changes in interchromophore distance, thereby requiring complex architectures such as proteins or partial protein sequences. Displacement-based assays, in which substrate binding displaces a fluorophore fall between these two extremes. For examples, see ref 1. For an expanded discussion, see ref 6f.

(6) (a) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2001**, *123*, 1260−1261. (b) Mello, J. V.; Finney, N. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 1536−1438. (c) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2002**, *124*, 1178−1179. (d) McFarland, S. A.; Finney, N. S. *Chem. Commun.* **2003**, 388−389. (e) Fang, A. G.; Mello, J. V.; Finney, N. S. *Org. Lett.* **2003**, *5*, 967−970. (f) Fang, A. G.; Mello, J. V.; Finney, N. S. *Tetrahedron* **2004**, *60*, 11075−11087.

(7) For other work on biaryl conformational restriction as a signaling mechanism, see: (a) Takeuchi, M.; Yoda, S.; Imada, T.; Shinkai, S. *Tetrahedron* **1997**, *53*, 8335−8348. (b) Costero, A. M.; Andreu, R.; Monrabal, E.; Martinez-Manez, R.; Sancenon, F.; Soto, J. *J. Chem. Soc., Dalton Trans.* **2002**, 1769. (c) Lee, D. H.; Im, J. H.; Lee, J. H.; Hong, J. I. *Tetrahedron Lett.* **2002**, *43*, 9637. (d) Cody, J.; Fahrni, C. J. *Tetrahedron* **2004**, *60*, 11099−11107.

(8) See Supporting Information for complete details.

(9) (a) Johnson, C. R.; Zhang, B. *Tetrahedron Lett.* **1995**, *36*, 9253−9256. (b) Rathke, M. W.; Nowak, M. *J. Org. Chem.* **1985**, *50*, 2624−2626.

(10) For influential examples of seemingly simple combinatorial libraries of metal chelators, see: (a) Burger, M. T.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 7382. (b) Frances, M. B.; Finney, N. S.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1996**, *118*, 8983−8984.

(11) Li, H.; Jiang, X.; Ye, Y.-h.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91−94.

(12) Under the O^{t}Bu cleavage conditions we observed cleavage of the fluorophore from the resin; thus, the O^{t}Bu groups were left in place. Phenylthiourea-terminated library elements were left protected to avoid Edman degradation under acidic conditions.

(13) It is, of course, impossible to exclude the possibility that there are binding events that do not lead to increased emission. However, in every system we have studied,[6] we have observed perfect correlation between fluorescence enhancement and metal ion binding (as determined by ^{1}H NMR).

(14) Since MOPS buffer contains a Na^+ counterion, response to Na^+ was also evaluated in triethanolamine buffer. For a discussion of metals for which fluorescent detection would be valuable, see ref 1 and citations therein.

(15) The addition of excess ethanedithiol reverses the increase in emission, demonstrating reversibility of Hg(II) binding. This result was confirmed in solution with **2**, and **2** could be recovered unchanged from Hg(II) titrations by preparative TLC.

(16) Library elements bearing 2-pyridyl (**v**), 2-pyrazyl (**w**), or phenylthiourea (**cc**) end caps exhibited partial quenching upon addition of Cu^{2+}.

(17) Inspection of the emission spectra from suspended resin-bound fluorophore (Figure 2) indicates that the blue "background emission" seen in the absence of Hg^{2+} is scattered blue light from the illumination source. The quantum yield of **2** in the absence of Hg(II) is ~0.01 with $\epsilon \approx 12{,}000$.

(18) Other examples of "turn on" fluorescent chemosensors for aqueous Hg(II): (a) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300−4302. (b) Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272−2273. (c) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270−14271. (d) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968−969. (e) Prodi, L.; Bargossi, C.; Montalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769−6770.

(19) (a) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1991**, *91*, 1721−2085. (b) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1995**, *95*, 2529−2586.

(20) Fluorescence quenching by Hg^{2+} results from contact-mediated spin−orbit coupling (McClure, D. S. *J. Chem. Phys.* **1952**, *20*, 682−686), underscoring a key advantage of the spatial segregation of fluorophore and binding domain.

JA043682P