J. Am. Chem. Soc. **1996,** *118,* 8983−8984 8983

Combinatorial Approach to the Discovery of Novel Coordination Complexes

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Received May 31, 1996

The relationship of ligand structure to the chemical and physical properties of derived metal complexes is a central theme in such vital and disparate fields as selective catalysis,[1] sensor discovery,[2] and bioinorganic chemistry.[3] The numerous advances made in these fields highlight the utility of complexes with well-designed structural, electronic, and/or stereochemical features. However, the rational design of such complexes remains extremely challenging, especially if novel physical and chemical properties are sought. In this context, a systematic method for the expedient generation of new classes of coordination complexes would clearly be of great value.

The synthesis and screening of combinatorial libraries is a validated strategy for the identification and study of ligand−receptor interactions.[4,5] Combinatorial systems allow many structural changes to be examined simultaneously, thus allowing an evaluation of synergistic effects in ligand binding. Since the stability and activity of metal complexes are similarly dependent on numerous interrelated variables, such as the coordination geometry required by the metal and the steric and electronic characteristics of the ligand, combinatorial chemistry could provide a powerful approach for discovering new types of coordination compounds as well.[6] We outline herein our preliminary results in selective transition metal binding by ligands prepared through solid-phase combinatorial synthesis.

There are two fundamentally different strategies that may be adopted for the design of libraries of potential metal ion binders. One involves attaching a variety of possible binding elements to a known ligand structure. In this context, Still and co-workers recently described the preparation of solid-phase libraries containing cyclen units with short peptidic appendages.[7] Although cyclen is a known tetradentate ligand for metal ions such as Cu(II) and Co(II), these ions were found to bind with selectivity imparted by the variable library elements. This approach may prove particularly useful for the tuning and optimization of properties of known ligands.

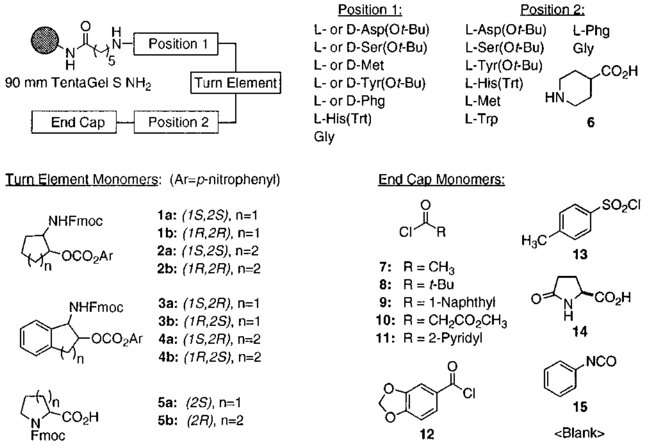

Figure 1. Composition of a turn-element-containing library for the binding of transition metals.

An alternative approach, and the one adopted in the present study, involves the design of libraries of potential ligands without predefined binding sites, but rather with a diverse set of functional groups and conformational restrictions that result in a range of potential coordination environments.

The ligand library comprises four variable components: two amino acids (positions 1 and 2), linked by a "turn element" (**1a**,**b**−**5a**,**b**) and terminated by various capping reagents (**7**−**15**) (Figure 1).[8] The turn elements are cyclic 1,2-amino alcohol or α-amino acid derivatives with defined relative and absolute stereochemistry and were introduced with the notion that this conformational restriction would encourage the formation of a potential binding site in which both amino acid side chains might interact with the metal. The ligand library was synthesized on poly(ethylene glycol)-grafted polystyrene (90 mm TentaGel S NH$_2$ resin) using standard splitting/pooling techniques[9] such that each polymer bead displayed a unique ligand structure. The library, which theoretically consists of 12 000 different ligands, was encoded using established tagging methods.[10]

The potential for members of this library to form coordination complexes was evaluated by exposing samples of the beads to homogeneous solutions of selected metal ions. In a representative experiment, a 10 mg portion of the library (ca. 24 000 beads) was subjected to 0.05 M Ni(OAc)$_2$ in MeOH for 30 min, rinsed with additional MeOH, and air dried. The resulting sample was then treated with a solution of dimethylglyoxime (DMG) in MeOH, a known qualitative test for Ni(II), and the beads were examined under a light microscope. A reddish-pink precipitate was observed to rapidly form and remain trapped in the polymer matrix of about 20% of the beads, confirming the selective incorporation of Ni(II) into some of these ligands (Figure 2A).

In order to ascertain which library members had the highest binding affinity for Ni(II), 10 mg library samples were exposed to solutions of decreasing Ni(OAc)$_2$ concentration in methanol buffered with 0.10 M NaOAc and 0.10 M HOAc.[11] As the concentration was lowered, fewer Ni(II) binders were observed,

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[1] (a) *Catalytic Asymmetric Synthesis*; Ojima, I., Ed.; VCH: New York, 1993. (b) *Comprehensive Organometallic Chemistry II*; Wilkinson, G., Stone, F. G. A., Abel, E. W., Hegedus, L. S., Eds.; Pergamon: New York, 1995; Vol. 12.

[2] *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; ACS Symposium Series 538; American Chemical Society: Washington, DC, 1992.

[3] Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

[4] For recent reviews on strategies for the synthesis of small-molecule libraries, see: (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555. (b) Armstrong, R. W.; Combs, A. P.; Tempest, P. A.; Brown, S. D.; Keating, T. A. *Acc. Chem. Res.* **1996**, *29*, 123. (c) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.

[5] For combinatorial approaches to the study of ligand−receptor interactions, see: (a) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155 and references therein. (b) Yu, H.; Chen, J. K.; Feng, S.; Dalgarno, D. C.; Brauer, A. W.; Schreiber, S. L. *Cell* **1994**, *76*, 933. (c) Combs, A. P.; Kapoor, T. M.; Feng, S.; Chen, J. K.; Daudé-Snow, L. F.; Schreiber, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 287. (d) Zuckermann, R. N.; et al. *J. Med. Chem.* **1994**, *37*, 2678. (e) Wang, G. T.; Li, S.; Wideburg, N.; Krafft, G. A.; Kempf, D. J. *J. Med. Chem.* **1995**, *38*, 2995. (f) Campbell, D. A.; Bermak, J. C.; Burkoth, T. S.; Patel, D. V. *J. Am. Chem. Soc.* **1995**, *117*, 5381.

[6] Spatially addressed synthetic libraries have been applied with success for the identification of metal-containing solid-state materials (Briceño, G.; Chang, H.; Sun, X.; Schultz, P. G.; Xiang, X.-D. *Science* **1995**, *270*, 273), Tc-binding peptides (Malin, R.; Schneider-Mergenet, J.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 11821), and selective catalysts (Burgess, K.; Lim, H.-J.; Porte, A. M.; Sulikowski, G. A. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 220).

[7] Burger, M. T.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 7382.

[8] Experimental procedures for the synthesis of the library and its components are provided as supporting information.

[9] Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487.

[10] Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922.

[11] Equilibration experiments have shown the binding of Ni(II) to be reversible under these conditions.

S0002-7863(96)01842-2 CCC: $12.00

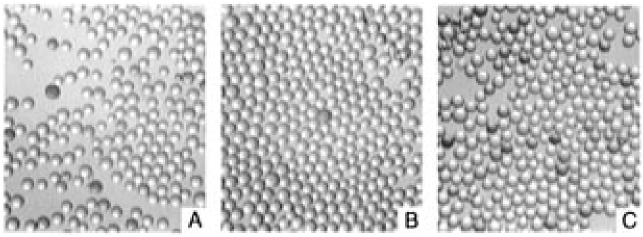


Figure 2. Photographs of metal-containing library samples. (A) Sample containing Ni(II) (red-pink), stained with dimethylglyoxime in MeOH. (B) Sample containing Fe(III) (orange), stained with KSCN in 2% AcOH−MeOH. (C) Sample containing both Ni(II) (blue) and Fe(III) (red-orange), stained with dithiooxamide in THF followed by KSCN in MeOH.

Table 1. Structures of Highest Affinity Ni(II) Binders

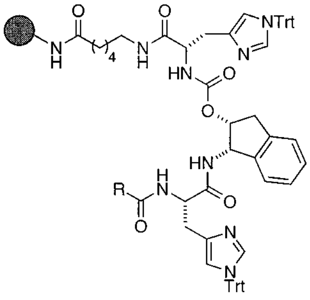
structure	position 1	turn element	position 2	end cap
1, 2	L-His(Trt)	**3a**	L-His(Trt)	**7**
3, 4	L-His(Trt)	**3a**	L-His(Trt)	**9**
5	L-His(Trt)	**2a**	L-His(Trt)	**7**
6	L-His(Trt)	**2a**	L-His(Trt)	**9**

until at a $Ni(OAc)_2$ concentration of 2.5×10^{-4} M only 6 binders out of ∼24 000 beads[12] were identified.

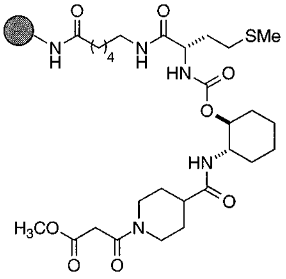
Tag photolysis and GC-ECD analysis (Table 1) allowed the identification of these hits and revealed a strong structural consensus among the nickel binders. Four different ligands and two duplicates were found, each bearing L-His(Trt) in both amino acid positions. Furthermore, only two turn elements (**3a** and **2a**) and two end caps (**7** and **9**) were incorporated, suggesting that high-affinity binding does not simply result from the presence of the two histidine residues. The identified structures were confirmed to be Ni(II) binders by independent synthesis of two of the ligands, **16** and **17**, both on solid phase and in solution.[13,14]

The same ligand library is selective in its complexation of other metal ions. Using conditions similar to those described above, Fe(III) complexes were indentified by staining with KSCN in MeOH (Figure 2B). Slightly stronger and less discriminate complexation was observed than with Ni(II), with approximately 1% of the ligands incorporating Fe(III) upon exposure to 5×10^{-4} M $FeCl_3$ and solutions as dilute as 5×10^{-6} M still producing identifiable binders. The structures revealed by tag analysis were found to be very different from those of the Ni(II) binders and displayed a perfect consensus (64/64 structures) for the unexpected combination of isonipecotic acid (**6**) in position 2 followed by end cap **10**. Half of the high-affinity structures were found to contain Met in position 1 (e.g. **18**), but no preference for a particular turn element was apparent.

The fact that there is no structural overlap between binders of Ni(II) and of Fe(III) suggested that the library might selectively bind one metal in the presence of the other. A 10 mg sample of the library was exposed to an equimolar solution of $Ni(OAc)_2$ and $FeCl_3$. After 2 h, the sample was rinsed with MeOH and stained sequentially with dithiooxamide in THF (a stain that forms a blue complex with Ni(II)) and KSCN in MeOH (a stain that forms an orange-red complex with Fe(III)). As can be seen in Figure 2C, selective binding to each metal can be identified.



16 R = CH_3
17 R = 1-naphthyl



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Binding of other ions to the library was also observed, albeit with varying degrees of selectivity. Cu(II) bound to ∼30% of the beads, with a marked preference for members containing L-His(Trt). Pt(IV) was also found to complex with up to 30% of the ligands, the majority of which contained a Met residue. Sn(IV) and Pd(II) were observed to bind with little-to-no selectivity, suggesting that complexation of these ions might occur solely through the peptide backbone.[15]

In conclusion, we have demonstrated that combinatorial libraries can be successfully applied to the discovery of novel metal−ligand complexes. The structural information thus obtained has revealed unanticipated and nonintuitive structural effects in binding selectivity and affinity. The investigation of the properties and applications of the coordination complexes uncovered by this approach is being actively pursued in our laboratories and will be reported in due course.

Acknowledgment. This work was supported by a generous gift from Versicor, Inc. We thank Prof. S. L. Schreiber and his research group for helpful discussions and valuable experimental advice. Predoctoral fellowship support to M.B.F. (NSF) and postdoctoral fellowship support to N.S.F. (NIGMS) are gratefully acknowledged.

Supporting Information Available: Experimental details and full characterization of intermediates pertaining to both the solid and solution phase synthesis of metal binders; consensus binding data for Pt(IV), Sn(IV), Cu(II), and Pd(II) (8 pages). See any current masthead page for ordering and Internet access instructions.

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[12] A sample of 24 000 beads, or 2.0 copies of the library, assures that 95% of the ligands are represented with 99% confidence. Burgess, K.; Liaw, A. I.; Wang, N. *J. Med. Chem.* **1994**, *37*, 2985.

[13] Binding experiments carried out three times using $[Ni(OAc)_2] = 2.5 \times 10^{-4}$ M and at a variety of higher Ni concentrations led to the identification of **16** and **17** as ligands in every case.

[14] For both **16**·Ni and **17**·Ni, the predominant species corresponds to a 1:1 complex of Ni(II) and doubly-deprotonated ligand ($Ni \cdot LH_{n-2}$). Several other hits from this binding screen have been resynthesized on solid phase for binding confirmation. Experiments to determine the association constants of these complexes are underway and will be reported separately.

[15] In all cases, control experiments revealed no detectable metal ion binding to the poly(ethylene glycol) support. The structures of representative ligands for each of these metals are provided in the supporting information.