Synthetic Ionophores. Encoded Combinatorial Libraries of Cyclen-based Receptors for Cu^{2+} and Co^{2+}

Matthew T. Burger and W. Clark Still*
Department of Chemistry, Columbia University, New York, New York 10027

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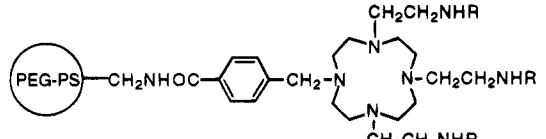
A common approach to new receptor molecules involves beginning with a core receptor having established binding properties and modifying its substitution to induce changes in binding strength or selectivity. Typically, such studies involve synthesizing a small number of derivatives and analyzing them individually for their binding properties. In this paper, we use such an approach in conjunction with encoded combinatorial chemistry to prepare 10^5-member ionophore libraries in a search for new molecules that tightly bind Cu^{2+} and Co^{2+}. The core we chose is cyclen (**5**)—a macrocyclic tetraamine known to tightly bind many divalent transition metals[1] —and the substituents are oligopeptides. One concern was that cyclen complexes can be exceptionally stable (*e.g.*, $\log K_{eq} = 23$ for Cu^{2+}) and that binding might be so dominated by the cyclen core that the substituents would exert little effect. As we will show, however, the metal ion-binding properties of peptide-substituted cyclens do indeed vary with the nature of the peptide sequences and the preferred sequences differ for copper and cobalt.

The strategy for the construction of cyclen libraries **1**–**4** was to use one of the ring nitrogens for attachment to the resin and to use the remaining three nitrogens for substituent elaboration using encoded split synthesis[2] with Fmoc amino acids. A two-carbon spacer between the ring nitrogen and the first amino acid for each of the three arms was included to retain the amine nature of the core. Nineteen amino acids were used in each step of the syntheses of **1**–**4**: AA_n = D- and L-Ala, -Val, -Pro, -Ser, -Gln, -Asn, -Lys, -Glu, -Asp, and -Gly.[3]



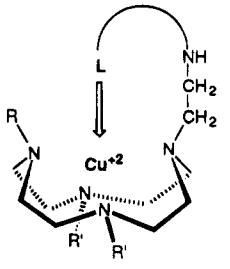
1 R = AA_1NH_2 (19 members)
2 R = $AA_1AA_2NH_2$ (361 members)
3 R = $AA_1AA_2AA_3NH_2$ (6859 members)
4 R = $AA_1AA_2AA_3AA_4NH_2$ (130321 members)
5 R = H

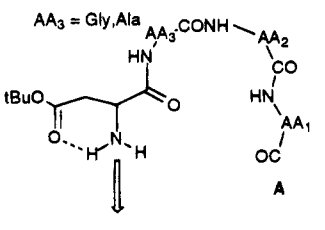
Library synthesis was preceded by preparation of cyclen derivative **5**. Protected libraries **1-4** were synthesized from **5** using standard solid phase methodology and encoded as described previously.[2,3] Libraries **1-4** differed solely in the length of the peptidic arms, with **1** having a single amino acid on each arm and **4** having a tetrapeptide on each arm. Deprotected libraries were obtained from protected **1-4** by side chain deprotection (trifluoroacetic acid) and neutralization (Et_3N).

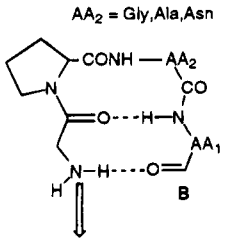
The protected and deprotected cyclen libraries **1-4** (eight libraries in total) were assayed separately for Cu^{2+} binding by agitating them (≥4 days) with aqueous solutions of Cu^{2+} at a concentration (initially 100 μM) that gave ~1% binding to the library beads. All aqueous solutions were prepared using 18 MΩ deionized water and buffered to pH 7.4 using HEPES. Binding was readily detectable because the originally pale yellow library beads turned bright blue upon significant binding to Cu^{2+}. Interestingly, only a small percentage of the beads in each library picked up the intense blue color while the others showed no color change, at least to the naked eye. In each assay, 40 or more of the blue beads were isolated, washed, and decoded as previously described.[2]

The major trends for the amino acid sequences in the best Cu^{2+}-binding side chain-protected receptors **1-4** are summarized in Table 1. The most striking result is that only a few of the amino acid sequences in the libraries bind Cu^{2+} significantly under the assay conditions, and the strongest trends occur at the residue positions most distant from the cyclen cores. These consensus sequences are of two different types. The most prevalent one is an N-terminal *tert*-butyl aspartate connected to (in **2-4**) a small amino acid (glycine or alanine). Interestingly, sequences terminating in the closely related *tert*-butyl glutamate were found only once among the ~150 blue beads we decoded. Among the Ala-Asp(tBu) receptors, we found little evidence of diastereoselection except in the case of **2** where the homochiral dipeptide sequence was found four times as frequently as the heterochiral one. The other sequence that was common to the Cu^{2+}-binding receptors was a N-terminal glycine connected to proline.

Perhaps the most interesting feature of the receptors defined in Table 1 is that their strongest consensus sequences are found at the positions most remote from the cyclen core. This result suggests that the terminal amino group of one or more of the peptide substituents is turned back on the cyclen binding site and may interact with bound Cu^{2+} as indicated generally below. Amine complexes of Cu^{2+} in water are known to incorporate four ligands tightly bound to copper and one or two additional ligands that are more weakly bound.[5] While the details of the turn will vary from receptor to receptor, the two consensus sequences in **4** might involve geometries generally indicated by **A** and **B** below.







(1) Review of cyclens and related ionophores: (a) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S. *Chem. Rev.* **1991**, *91*, 1721. (b) Kimura, E. In *Crown Ethers and Analogous Compounds, Studies in Organic Chemistry*; Elsevier Science Publishers B. V.: New York, 1992; Vol. 45, pp 381–478. (c) Tsukube, H. *Talanta* **1993**, *40*, 1313.

(2) 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 and references therein. See also: Yoon, S. S.; Still, W. C. *Tetrahedron* **1995**, *51*, 567.

(3) Side chain protection: Ser(*O*-tBu), Asp(*O*-tBu), Glu(*O*-tBu), Asn(*N*-trityl), Gln(*N*-trityl), Lys (*N*-Boc).

(4) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; Wiley: New York, 1980; p 815.

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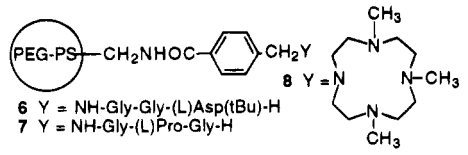
Table 1. Binding of Side Chain-Protected 1–4 to Cu^{2+}

AA_1[a]	AA_2[a]	AA_3[a]	AA_4[a]	found[b] (%)	expected[c] (%)
			Receptor Library 1		
Asp(tBu)	–	–	–	100	11
			Receptor Library 2		
Gly	D/L-Asp(tBu)	–	–	37	0.6
D/L-Ala	D/L-Asp(tBu)	–	–	43	1
D/L-Ala	L/D-Asp(tBu)	–	–	9	1
Pro	Gly	–	–	9	0.6
			Receptor Library 3		
X	Gly	Asp(tBu)	–	24	0.6
X	D/L-Ala	D/L-Asp(tBu)	–	20	1
X	D/L-Ala	L/D-Asp(tBu)	–	15	1
X	Pro	Gly	–	8	0.6
			Receptor Library 4		
X	X	Gly	Asp(tBu)	81	0.6
X	D/L-Ala	Gly	D/L-Asp(tBu)	28	0.06
X	D/L-Ala	Gly	L/D-Asp(tBu)	13	0.06
X	Gly,Ala,Asn	Pro	Gly	19	0.6

[a] X indicates any amino acid, – indicates a residue absent in the receptor, AA_1 is the C-terminal residue and is connected via an aminoethyl linker to cyclen. [b] Percentage of blue beads having the indicated amino acid sequence. [c] Percentage of beads having the indicated amino acid sequence expected from random bead picking.

In the case of **A**, a small AA_3 amino acid might facilitate close approach of side chain-chelated amine to the bound metal. In the case of **B**, the small N-terminal glycine might be delivered to the metal binding site via a peptidic β-turn, a structural feature commonly associated with Pro(AA_3 in **4**)–Gly/Asn(AA_2 in **4**) sequences in proteins.[6] Whatever the origin of the Cu^{2+}-binding abilities of these peptide-substituted cyclens, it is not due to the conserved peptide sequences alone because neither consensus sequence on the same solid support (**6**, **7**) detectably binds Cu^{2+} at even millimolar concentrations.



An encoded resin-bound tri-*N*-methylcyclen derivative (**8**) was also prepared as a standard (log K_{eq} = 18.4 for Cu^{2+} binding tetra-*N*-methylcyclen) for Cu^{2+}-binding strength comparisons with libraries **1-4**. In all such comparisons, we found that the strongest binders of Cu^{2+} were the beads of **1-4** (both side chain-protected and -deprotected forms)—no blue **8** beads were ever found in any these competitions. Thus, addition of peptidic appendages to the cyclen core significantly improves the Cu^{2+} binding ability relative to the unfunctionalized tetraalkylated cyclen **8**.

With the side chain-deprotected receptors **1-4**, strong trends for Cu^{2+} binding were also observed. In particular, all of the copper-binding receptors had their peptidic substituents made up largely of aspartic and glutamic acids. Thus, the best copper-binding, deprotected receptors were all structurally related to well-known metal chelators such as EDTA.

To assess the relative strengths for Cu^{2+} binding of the various libraries, additional competition experiments were carried out. These experiments involved mixing different types of receptors on solid supports together and allowing them to compete for a deficiency of Cu^{2+}. These experiments showed that, among the protected receptor libraries, the best members of **4** bound Cu^{2+} more effectively than did the best members of **1-3**. We also found that the EDTA-like deprotected **4** bound Cu^{2+} better than did protected **4**.

A similar series of binding experiments was carried out with **1-4** and aqueous Co^{2+}. Here, metal ion complexation was indicated when library beads developed a red coloration, and the results of decoding such red beads of **1-4** are summarized in the supporting information. While there are similarities between some of the Co^{2+} sequences and the Gly/Ala-Asp(tBu) Cu^{2+} consensus sequence, the sequences selected by Co^{2+} clearly represent a different population from those selected by Cu^{2+}. For example, Co^{2+} primarily selects receptors for libraries **3** and **4** that have Asp(tBu) one residue removed from the N-terminal residue. Furthermore, there is significant selectivity for Asp(tBu) at the AA_2 position of both libraries **2** and **3**. With library **2**, Co^{2+} selected Gly/Ala-Asp(tBu) in only 4% of the beads whereas Cu^{2+} selected it in 89% of the beads. No member of library **1** bound Co^{2+} detectably at any concentration.

These results show how encoded combinatorial libraries of ionophores can be prepared and screened to find those members that best bind certain metals in water. While the specific assay methodology we describe here is limited to metals whose complexes are colored, it should be extendable to colorless ions by using chromophoric or fluorophoric receptors or by employing colored counterions. One problem with our solid phase assay and very tight binding ligand/metal combinations (*e.g.*, cyclens + Cu^{2+}) is that it may not effectively distinguish kinetic from thermodyamic binding events. Though our equilibrations have been carried out for as long 6 months with no apparent change in receptor selectivity, there is no guarantee that our systems have reached equilibrium.

Though different populations of **1-4** bind Cu^{2+} and Co^{2+}, no library member could be found that bound Co^{2+} more tightly than Cu^{2+}. This finding is consistent with the large (~10^8-fold), intrinsic selectivity of the cyclen core for Cu^{2+} over Co^{2+}. It further suggests that ionophore libraries having members capable of selectively binding many different ions are best constructed around cores that have relatively poor intrinsic ion-binding selectivities.

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Supporting Information Available: Experimental procedures and characterization data for **1-8**. Binding data for protected **1-4** with Co^{2+} deprotected **1-4** with Cu^{2+} and Co^{2+} (8 pages).

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(6) Hancock, R. D.; Wade, P. W.; Ngwenya, M. P., De Sousa, A. S.; Damu, K. V. *Inorg. Chem.* **1990**, *29*, 1968.