

Peptide Bond Modification for Metal Coordination: 2. Metal-Binding Properties of Peptide-Derived Pentaaza-Macrocyclic Templates

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Introduction

Conformation templates have been applied to elucidation of receptor-bound conformation, design of peptidomimetics, construction of combinatorial libraries, and *de novo* peptide and protein design. We are exploring pentaaza-macrocyclic templates (Figure 1) in conjunction with metal coordination as a means to preorganize peptides for probing molecular recognition. One consideration is that the peptide backbone of cyclic pentapeptides, used by the Kessler group and others as receptor probes, can be readily transformed to chiral pentaaza-macrocycles *via* selective reductions of the amide bonds [1] with side chains displayed around the macrocyclic scaffold in a defined geometrical manner. In addition, the pentaazamacrocycle is an excellent ligand system for coordination of various metals [2] that can preorganize the molecular structures even further to help define the conformation responsible for biological activity. We are also exploring the effects of the chiral pendant side chains on the metal-binding properties and related molecular recognition of the azacrown scaffold.

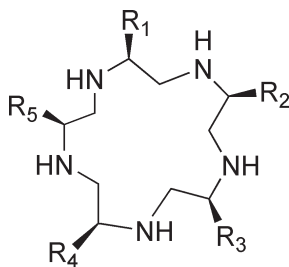


Fig. 1. The general structure of peptide-derived pentaazamacrocyclic template.

Results and Discussion

As an initial part of our project, a series of cyclic pentapeptide precursors containing Ala, Leu, Phe, His, Tyr, Lys, Met, Cys, β -Ala, D-Phe, D-Ala, and (D)-Leu residues were prepared from their corresponding linear analogs using PyBOP as a coupling reagent in presence of HOBT and DIEA in DMF/DCM solution. After purified by flash column chromatography, the cyclic peptides were reduced by refluxing in a solution of $\text{LiAlH}_4/\text{THF}$ (1 M) overnight to afford the desired products that were purified using HPLC and identified using ESI-MS. Some representative compounds of the synthesized library are listed in Table 1.

As some pentaaza-crown-Mn(II) complexes have been reported to exhibit very significant SOD mimetic activities, we focused on the Mn(II) binding properties. All

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Table 1. Positive-ion ESI-MS data for some synthesized peptide-derived pentaaza-macrocyclic templates and their Mn²⁺ complexes.

Entry	Structure	ESI-MS	
		[L + H] ⁺	[L + Mn-Cl] ⁺
1	Pentaaza-cyclo [Ala-Cys(4MBZ)-Leu-Leu-Phe] ^a	582.3	671.5
2	Pentaaza-cyclo [Ala-Met-Leu-Leu-Phe]	506.3	595.5
3	Pentaaza-cyclo [Cys(4MBZ)-Cys(4MBZ)-Leu-Leu-Phe]	718.3	807.6
4	Pentaaza-cyclo [Ala-Tyr(Bz)-Leu-Leu-Phe]	628.4	717.6
5	Pentaaza-cyclo [Ala-Tyr-Leu-Leu-Phe]	538.3	627.3
6	Pentaaza-cyclo [Ala-His(Bom)-Leu-Leu-Phe]	632.4	721.6
7	Pentaaza-cyclo [(β)-Ala-Tyr(Bz)-Leu-Leu-Phe]	628.4	717.6
8	Pentaaza-cyclo [(β)-Ala-Tyr-Leu-Leu-Phe]	538.3	717.6
9	Pentaaza-cyclo [Ala-Lys-Leu-Leu-Phe]	503.6	637.7

^a Pentaaza-cyclo [Ala-Cys(4MBZ)-Leu-Leu-Phe] refers to the pentaazamacrocyclic product from reducing the corresponding cyclo [Ala-Cys(4MBZ)-Leu-Leu-Phe]. All compounds are designated similarly.

the synthesized compounds coordinated with MnCl₂ in methanol to form the 1 : 1 Mn(II) complexes with very high ion abundances in ESI-MS spectra (Table 1). Competition studies were carried out to compare their binding properties and revealed that the relative binding affinities of the ligands are in the following order: L2 ≈ L1, L1 > L3, L5 > L4, and L5 > L2, indicating that the side chains may have significant influences on the metal coordination. The ligands can also bind with other metal ions such as Cu(II), Zn(II), Ni(II), and Cd(II) strongly to form very stable and identifiable coordination species with the characteristic isotope clusters in ESI-MS spectra.

The effects of side chains, especially metal binding phenol, imidazole, methylthio, and amino groups which originate from the corresponding amino acid residues such as tyrosine, histidine, methionine, and lysine should be taken into account in further molecular design.

Acknowledgments

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References

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