

Peptide Bond Modification for Metal Coordination: 1. Metal-Binding Properties of Hydroxamate-Based Pseudo-Peptides

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Introduction

Most reports in the literatures focus on metal-peptide complexes utilizing sites on the peptide side chains, *i.e.* the amino group, imidazole ring, carboxylic acid group, phenol group, *etc.* In our efforts to developing peptides with high metal-binding affinity and selectivity, we have targeted synthesis of siderophore-like peptides with backbone metal chelation through modification of the peptide amide bond. This would leave the side chains free to interact with receptors while the backbone would be preorganized during metal complexation. In this work we focused on the design, synthesis, and metal-binding properties of a hydroxamate-based pseudo-peptide library.

Results and Discussion

Design and synthesis: We initially chose to design the following novel dihydroxamate-containing pseudo-oligopeptides: $X_{AA1}-\Psi[\text{CON}(\text{OH})]-X_{AA2}\cdots X_{AA_n}-\text{NH}(\text{OH})$. Several strategies for synthesis of the designed pseudo-peptides have been developed based on *N*-hydroxylamino acid derivatives as building blocks. *N*-benzyloxy-L- α -phenylalanine synthesized from the corresponding hydroxyl analog [1] was incorporated into peptides by reacting with Fmoc-amino acid chloride/AgCN in toluene [2]. The resulting pseudo-tripeptide Fmoc-AA₁- $\Psi\{\text{CON}(\text{OBz})\}$ -Phe-AA₃-OBu^t were further deblocked with piperidine/DMF or TFA/DCM to afford two modules which were used to assemble the target compounds in solution or on a solid support. The protecting *O*-benzyl groups were removed with HCOONH₄ in the presence of Pd/C (5%) in CH₃OH [3]. A library of mono-, di-, and trihydroxamate-containing linear and cyclic pseudo-peptides have been constructed in order to establish the relationship between the structure and metal binding properties of hydroxamate-based pseudopeptides. Table 1 showed some of the synthesized compounds.

Metal-Binding Properties: ESI-MS was used to screen the metal-binding properties of all the compounds and the relative ion abundances in a metal-competition assay were measured. All ligands exhibited a clear preference for binding with iron. The results revealed some significant information about the structure-metal-binding relationship. 1. **9>8**: cyclization can significantly improve the binding affinities of this type of ligand; 2. **5>4**, **4>1**, and **9>7**: the results indicate the importance of the distance between the two hydroxamate groups as a suitable distance allows adjustment of the bidentate hydroxamate groups to the geometry required for metal coordination; 3. **1>11**: the introduced two hydroxamate groups cooperate in coordination to efficiently improve the binding affinity; 4. **10>5**, **3>2**, and **3>4**: the steric hindrance of -(CONOH)-neighboring groups could exert an influence on the metal-binding properties; 5. **2>4**: this suggested the phenyl group may cooperate in the metal coordination. 6. **9>12** and **9>13**: high metal-binding selectivity and affinity are attainable with the dihydroxamate-containing oligopeptide system.

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Table 1. Example of some synthesized peptides from the library.

Entry	Sequence
1	H-Leu-Ψ[CON(OH)]-Phe-Ala-NHOH
2	H-Val-Ψ[CON(OH)]-Phe-Ala-Leu-NHOH
3	H-Val-Ψ[CON(OH)]-Gly-Ala-Leu-NHOH
4	H-Val-Ψ[CON(OH)]-Ala-Ala-Leu-NHOH
5	H-Val-Ψ[CON(OH)]-Phe-Ala-Pro-Leu-NHOH
6	H-{Leu-Ψ[CON(OH)]-Phe-Ala} ₂ -OH
7	Cyclo-{Leu-Ψ[CON(OH)]-Phe-Ala} ₂
8	H-{Leu-Ψ[CON(OH)]-Phe-Ala-Pro} ₂ -OH
9	Cyclo-{Leu-Ψ[CON(OH)]-Phe-Ala-Pro} ₂
10	H-Leu-Ψ[CON(OH)]-Phe-Ala-Pro-Leu-NHOH
11	H-Leu-Ψ[CON(OH)]-Phe-Ala-OH
12	H-{Leu-Ψ[CON(OH)]-Phe-Ala-Pro} ₃ -OH
13	H-{Leu-Ψ[CON(OH)]-Phe-Ala-Pro} ₂ -Leu-NHOH

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