

### Peptide Bond Modification for Metal Coordination: 3. Metal-Binding Properties of Phosphorus-Based Pseudo-peptides

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#### Introduction

Phosphorus-based peptidomimetics have been attractive target compounds during research and development of metalloproteinase inhibitors [1]. Their inhibiting activities are thought to be associated with mimicry of the metal-mediated transition state or tetrahedral-intermediate of the amide hydrolysis [2]. Because of the important roles of metal ions in the structures and functions of metalloproteinases, we envision that systematic investigation of the metal binding properties of phosphorus-based peptidomimetics should provide better understanding of their mechanism of action and assist the rational design of potent and specific inhibitors.

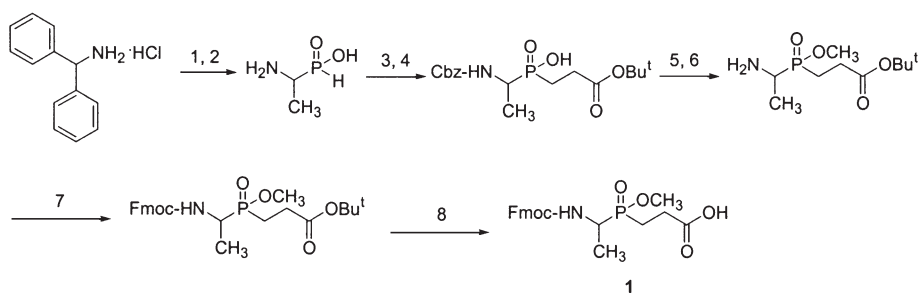
#### Results and Discussion

Based on our previous studies on metal binding hydroxamate-based pseudo-peptides, the following four novel linear and cyclic diphosphinic acid-based pseudo-peptides with a general structure of {AlaΨ[P(O)(OH)CH<sub>2</sub>]Gly---Linker---AlaΨ[P(O)(OH)CH<sub>2</sub>]Gly} were designed as models to explore the potential of phosphorus-containing moieties as metal binding sites in the pseudopeptides.

1. Ac-Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-Phe-Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-NH<sub>2</sub>
2. Ac-Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-Phe-Phe-Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-NH<sub>2</sub>
3. Cyclo{Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-Phe-Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-Phe}
4. Cyclo{Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-Phe-Phe-Ala-Ψ[P(O)(OH)CH<sub>2</sub>]Gly-Phe-Phe}

A Fmoc-protecting *pseudo*-dipeptide (Fmoc-(*R,S*)-AlaΨ[P(O)(OCH<sub>3</sub>)CH<sub>2</sub>]Gly, **1**) was first synthesized [3] as a module for solid phase assembly of the four designed compounds (Figure 1).

Compounds **1** and **2** were similarly assembled on Fmoc-Amide MBHA resin using conventional Fmoc chemistry. The linear peptidic precursors for compound **3** and **4**



1. CH<sub>3</sub>CHO, H<sub>2</sub>O, reflux, 1.5 h; 2. HCl solution (18%); 3. Cbz-Cl, Na<sub>2</sub>CO<sub>3</sub>, dioxane, H<sub>2</sub>O;
4. CH<sub>2</sub>=CHCOOBu<sup>t</sup>, CH<sub>3</sub>C[=NSi(CH<sub>3</sub>)<sub>3</sub>]OSi(CH<sub>3</sub>)<sub>3</sub>, CH<sub>3</sub>CN; 5. CH<sub>3</sub>OH, EDCl, DMAP;
6. Pd/C (10%), HCOONH<sub>4</sub>, CH<sub>3</sub>OH; 7. Fmoc-Cl, Na<sub>2</sub>CO<sub>3</sub>, dioxane, H<sub>2</sub>O; 8. TFA/DCM.

Fig. 1. Synthesis of Fmoc-protecting *pseudo*-dipeptide (Fmoc-(*R,S*)-AlaΨ[P(O)(OCH<sub>3</sub>)CH<sub>2</sub>]Gly, **1**).

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were assembled starting from Fmoc-Phe Wang resin, cleaved with TFA, and cyclized in solution in the presence of PyBOP/HOBT/DIEA. Finally hydrolysis of the methyl esters using LiOH in dioxane/H<sub>2</sub>O afforded the desired products. All the crude products were purified with HPLC. As estimated, the products were in the diastereoisomeric forms and showed a cluster of peaks in HPLC profiles.

Some results of the metal binding study using ESI-MS are tabulated in Table 1. It was found that all the di-phosphinic based pseudo peptides exhibited high binding affinity and selectivity for Fe<sup>3+</sup> and Fe<sup>2+</sup> among the metal ions Cu<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, Fe<sup>2+</sup> and Mn<sup>2+</sup>, indicating that the phosphinic moiety may be a highly selective ligand for iron ion coordination. Competition binding assays revealed that their iron(III) binding affinities were in the following order: 1. **1** ≈ **2** and **4** > **3**, suggesting that the 8-atom length between two phosphinic groups in a linear ligand may provide enough conformational flexibility for iron coordination but a longer length between two phosphinic groups in a cyclic ligand may be essential to maintain high metal binding affinity; 2. **2** ≈ **4**, indicating that cyclization may not make significant contributions to the binding affinity.

Table 1. The relative binding  $\{[Ligand+M^{n+}]/([Ligand+M^{n+}] + [Ligand])\}$ , % from positive-ion ESI-MS for solutions of Ligand 1 and 2 with different metal ions at a ligand-to-metal molar ratio of 1 : 2 in CH<sub>3</sub>OH.

Ligand	Cu <sup>2+</sup>	Mn <sup>2+</sup>	Zn <sup>2+</sup>	Ni <sup>2+</sup>	Co <sup>2+</sup>	Fe <sup>2+</sup>	Cd <sup>2+</sup>	Fe <sup>3+</sup>
1	58.6	66.5	53.1	34.6	57.6	94.3	27.0	99
2	54.5	77.3	45.6	51.8	60.5	94.9	31.3	99.0

The capacity of the phosphinic acid moiety as chelation sites in peptides has been demonstrated and its further application to the development of metal-binding peptides is in progress.

### Acknowledgments

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### References

1. Buchardt, J., Ferreras, M., Krog-Jensen, C., Delaisse, J.-M., Foged, N.T., Meldal, M. *Chem. Eur. J.* **5**, 2877–2884 (1999).
2. Morgan, B.P., Scholtz, J.M., Ballinger, M.D., Zipkin, I.D., Bartlett, P.A. *J. Am. Chem. Soc.* **113**, 297–307 (1991).
3. Baylis, E.K., Campbell, C.D., Dingwall, J.G. *J. Chem. Soc., Perkin Trans. 1* 2845–2853 (1984).