

## **Synthesis of a Metal Binding Amino Acid- Solid Phase Peptide Synthesis- Compatible Hydroxamic Acid**

### Abstract

This study focuses on the practical usage of SPPS to synthesize N<sup>δ</sup>-acetyl-N<sup>δ</sup>-hydroxy-ornithine (Aho) containing peptides. Aho is a common hydroxamic acid that appears in molecules called siderophores. Siderophores are iron binding molecules produced by many microbes to obtain ferric iron. This type of molecule has been used for antimicrobials, anticancer drugs and in treatment of iron overload disease. Thus, it is an attractive synthetic target in many industries and scientific studies. Siderophores are sometimes peptides and could possibly be synthesized via solid phase peptide synthesis (SPPS). SPPS is the state of the art for peptide synthesis due to its speed and high yield, as well as its ability to generate libraries of related structures (combinatorial chemistry). However, to date hydroxamic acids have not been used in SPPS due to their difficulty of synthesis and protection needed for the side chain. Demonstration of SPPS compatibility would open the door to new hydroxamate peptide synthesis in a rapid yet inexpensive way. An SPPS compatible version of Aho was prepared by modifying an existing synthesis of Aho. N<sup>2</sup>-Cbz-N<sup>5</sup>-hydroxy-L-ornithine (hydroxylamine) was synthesized from a multi-step reaction of N-Cbz-L-ornithine (Lin, Y.-M.; Miller, M. J., *J. Org. Chem.* **1999**, 64, 7451-7458). The hydroxyl group in hydroxylamine was then benzylated with benzyl bromide in THF Potassium tert-butoxide solution to yield N<sup>2</sup>-Cbz-N<sup>5</sup>-(O-benzyl)-hydroxy-L-ornithine (O-benzyl hydroxylamine). O-benzyl hydroxylamine was acetylated, being the iron binding site after deprotection, to yield N<sup>2</sup>-Cbz- N<sup>5</sup>-acetyl-N<sup>5</sup>-(O-benzyl)-hydroxy-L-ornithine. The final step was the protecting group shuffle to yield SPPS compatible Aho. The N<sup>2</sup>-Cbz protecting group was replaced with Fmoc to yield N<sup>2</sup>-9-fluorenylmethyl- N<sup>5</sup>-acetyl-N<sup>5</sup>-(O-benzyl)-hydroxy-L-ornithine. The protected Aho will be combined with commercially available amino acids using SPPS and be exposed to all conditions associated with SPPS. The stability and characteristics of Aho during and after the removal of protecting groups will be tested using NMR. The resulting peptide will be characterized and studied using IR, NMR and GC-MS.

## References

1. Guyton, A. C.; *Textbook of Medical Physiology*. Fourth Edition ed.; W.B. Saunders Company: Philadelphia, 1971; p.859.
2. Butler, A., Acquisition and Utilization of Transition Metal Ions by Marine Organisms. *Science* **1998**, 281, 207-210.
3. Dertz, E. A.; Raymond, K. N., Siderophores and Transferrins. In *Comprehensive Coordination Chemistry II*, Que, L., Jr.; Tolman, W. B., Eds. Elsevier, Ltd.: 2003; Vol. 8, pp 141-168.
4. Miller, M. J., Syntheses and Therapeutic Potential of Hydroxamic Acid Based Siderophores and Analogues. *Chem. Rev.* **1989**, 89, 1563-1579.
5. Frazier, S. W.; Kretzschmar, R.; Kraemer, S. M., Bacterial Siderophores Promote Dissolution of UO<sub>2</sub> under Reducing Conditions. *Environ. Sci. Technol.* **2005**, 39, 5709-5715.
6. Kalinowski, D. S.; Richardson, D. R., Evolution of Iron Chelators For Treatment of Iron Overload Disease and Cancer. *Pharm* **2005**.
7. Lin, Y.-M.; Miller, M. J., Practical Synthesis of Hydroxamate-Derived Siderophore Components by an Indirect Oxidation Method and Syntheses of a DIG-Siderophore Conjugate and a Biotin-Siderophore Conjugate. *J. Org. Chem.* **1999**, 64, 7451-7458.
8. Poreddy, A. R.; Schall, O. F.; Osiek, T. A.; Wheatley, J. R.; Beusen, D. D.; Marshall, G. R.; Slomczynska, U., Hydroxamate-Based Iron Chelators: Combinatorial Syntheses of Desferrioxamine B Analogues and Evaluation of Binding Affinities. *J. Comb. Chem.* **2004**, 6, 239-254.