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Synthesis of a Conformationally Restricted Substrate Analogue of Siderophore Biosynthetases

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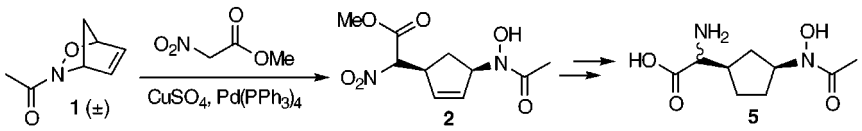
ABSTRACT



A conformationally restricted analogue (5) of N^{ω}-acetyl-N^{ω}-hydroxyornithine and -lysine was synthesized. The synthesis features an efficient acylnitroso hetero-Diels–Alder cycloadduct (1) ring opening with palladium(0) and methylnitroacetate.

Over the past several decades, the rise in antibiotic drug resistance has created an increasing need for new antibiotics with new modes of action. When designing new antibiotics, targeting metabolic processes specific to the pathogenic microorganisms is highly desirable. One such process that is unique to microbes is the acquisition of metabolically essential iron(III) through the use of siderophores.[1] Siderophores are relatively low molecular weight iron chelators that are secreted by microbes and used to obtain iron(III) from the environment.[2−5] Siderophores generally employ two or three iron-chelating moieties such as catechols, α-hydroxy acids, or hydroxamic acids. Because siderophores are vital to a microorganism's acquisition of iron, fully understanding their biosynthetic pathway could lead to the development of new antibiotics with novel modes of action.

The biosynthesis of many amino acid based siderophores such as ferrichrome, rhodotorulic acid, and aerobactin begins with the corresponding amino acids (Scheme 1).[6−13] N^{ω}-Hydroxylation of the amino acids, followed by N^{ω}-acetylation, gives the N^{ω}-acetyl-N^{ω}-hydroxyamino acid precursors necessary for the construction of the siderophores. The final synthetic step is carried out by the corresponding siderophore synthetases. To gain further insight into this final enzymatic process, and thus the entire biosynthetic pathway, a conformationally restricted substrate analogue (**5**) of N^{ω}-acetyl-N^{ω}-hydroxylysine and -ornithine was constructed.

The synthesis of the conformationally restricted substrate analogues began with *N*-acetyl hetero-Diels−Alder derived cycloadduct **1**.[14] We[15] and others[16,17] have shown that cycloadducts such as **1** are prone to reaction with Pd(0),

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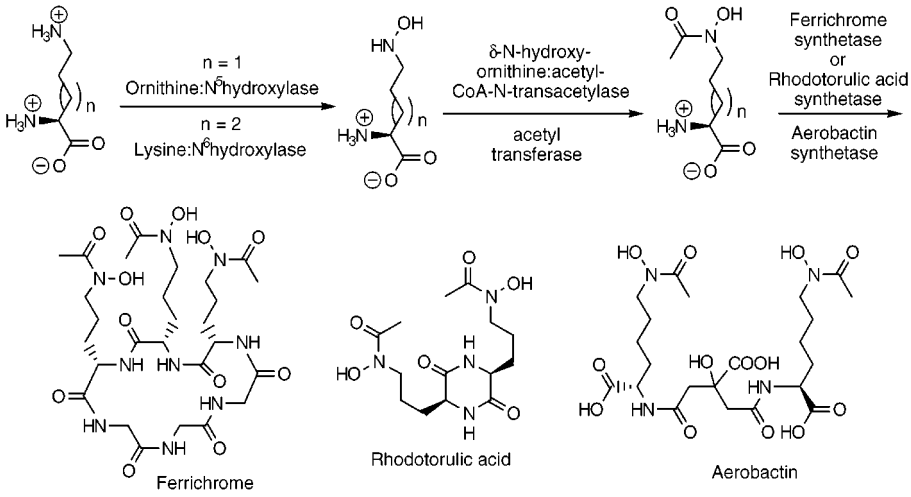
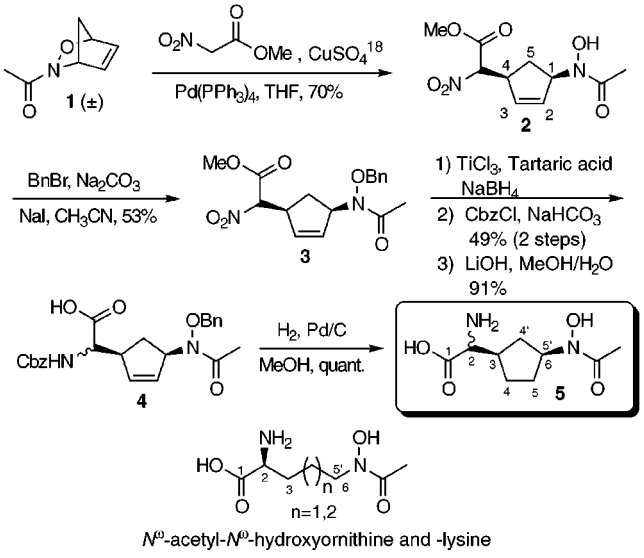
Scheme 1



inducing cycloadduct ring opening at the C−O bond to afford cyclopentenyl hydroxamic acids. Indeed, treatment of cycloadduct **1** with methyl nitroacetate and Pd(0) in the presence of $CuSO_4$[18] gave the 1,4-*syn*[19] amino acid precursor **2** in 70% yield (Scheme 2). To our knowledge, this represents

Scheme 2



the most efficient example of ring opening of this type of cycloadduct with Pd(0) and a carbon nucleophile. Prior to unmasking the amino acid functionality through a titanium(III)-mediated nitro reduction, the hydroxamic acid required protection to avoid reduction of its N−O bond. Remarkably, even in the presence of several other reactive centers in **2**, protection of the hydroxamic acid was achieved. In addition to some minor alkylation and decarboxylation byproducts, protection of the hydroxamic acid was completed in 53% yield to give *O*-benzyl hydroxamate **3**.

Reduction of the nitro group with titanium(III) and sodium borohydride was followed by Cbz protection of the resulting amine in a combined yield of 49%.[20] Hydrolysis of the methyl ester with lithium hydroxide gave a 91% yield of carboxylic acid **4**. Removal of the benzyl and Cbz protecting groups and reduction of the cyclopentenyl olefin were carried out simultaneously under hydrogenation conditions to form the desired conformationally restricted siderophore component analogue **5** in quantitative yield as a mixture of diastereomers at the α-amino (C-2) position.

Analogue **5** will be a valuable tool in studying the biosynthesis of siderophores. The effects of some other substrate analogues on aerobactin synthetase from *Aerobacter aerogenes* 62-1 have been investigated, and all of the N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine analogues were found to be substrates for the synthetase enzyme.[21] However, all of the analogues were also linear and closely resembled the parent N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine. By creating a cyclic, conformationally restricted analogue (**5**), the specificity of the aerobactin synthetase enzyme can be tested further. Additionally, substrate analogue **5** mimics not only N^{ϵ}-acetyl-N^{ϵ}-hydroxylysine but also N^{δ}-acetyl-N^{δ}-hydroxyornithine, allowing for research into ornithine-based siderophore synthetases, such as ferrichrome and rhodotorulic acid synthetases. The use of substrate analogue **5** in the investigation of siderophore

(18) Ten mole percent of $CuSO_4$ was used in the reaction. When a stoichiometric amount of copper was used, the yield was somewhat lower at 62%. In both cases, the copper salt did not completely dissolve, indicating a catalytic role of the copper. Although the exact role of $CuSO_4$ in the ring opening reaction is unclear, in the absence of copper, lower yields of hydroxamic acid **2** were obtained.

(19) The *syn*-stereochemical assignment was based upon the ^{1}H NMR coupling pattern. The C(5) methylene protons of 1,4-disubstituted cyclopentene systems have a very characteristic coupling pattern. The C(5) protons of *syn*-1,4-aminocyclopentenols have a characteristic overlapping ddd pattern, with approximate J values of 3.9, 3.9, and 14.7 Hz and 7.8, 7.8, and 14.7 Hz for the *cis*- and *trans*-methylene protons, respectively. There is normally a difference in chemical shift ranging from 0.8 to 1.3 ppm between each respective C(5) proton. The C(5) protons of *anti*-1,4-aminocyclopentenols have a characteristic ddd pattern, with approximate J values of 3.9, 7.1, and 13.6 Hz with a smaller chemical shift, ranging from 0.2 to 0.35 ppm between the two C(5) protons. Hydroxamic acid **2** has the following ^{1}H NMR pattern: δ 1.66 (overlapping ddd, J = 5.7, 5.7, 13.5 Hz, 1H) and 2.35 (overlapping ddd, J = 8.4, 8.4, 13.5 Hz, 1H), clearly displaying *cis*-coupling.

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biosynthesis will be reported in due course. With a greater understanding of the biosynthesis of siderophores (obtained through the use of substrate analogues such as **5**) comes the possibility of finding new metabolic targets for much needed novel antibiotics.

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Supporting Information Available: Experimental procedures and characterization data for products **2**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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