

Combinatorial Syntheses of Polyhydroxamate Siderophores: Desferrioxamine, Exochelin, Mycobactin, and Aerobactin Libraries

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Introduction

Siderophores are natural products, often containing amino acid derivatives, that are produced by microorganisms to chelate ferric ion as part of an iron-uptake system essential for survival. In order to explore several therapeutic opportunities, we have developed a combinatorial synthetic strategy to prepare libraries of analogs of a number of different siderophores, including desferrioxamine (DFO), for potential treatment of iron overload [1]; and exochelin, mycobactin and aerobactin for potential antibiotic applications. These siderophores all contain hydroxamate groups as primary coordination sites for the ferric ion (Figure 1). Their typical stability constants (K_s) for ferric ion are from 10^{23} to 10^{32} .

Results and Discussion

To facilitate synthesis of various analogs of the parent siderophore, we have developed novel synthetic approaches in which the hydroxamate groups were constructed *in*

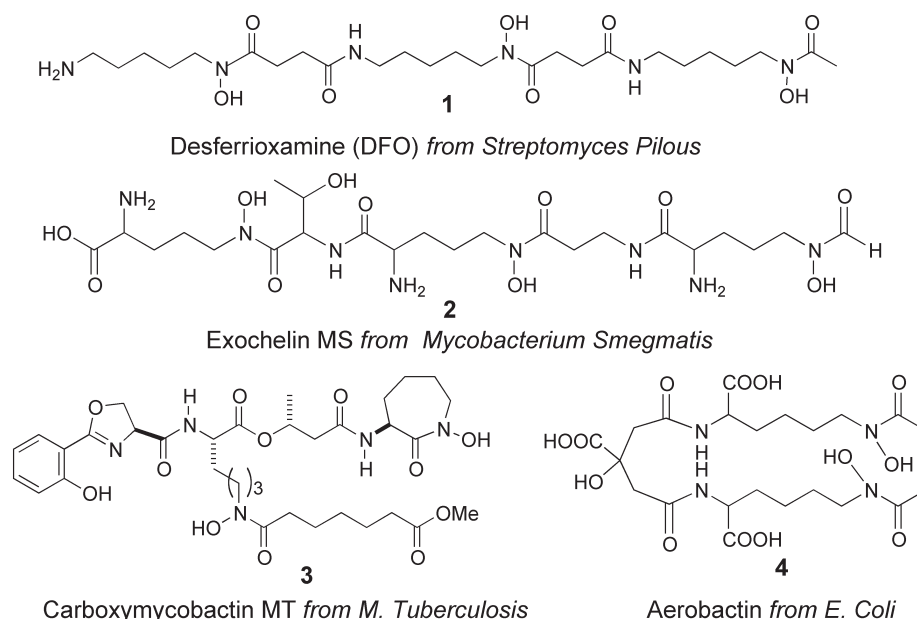
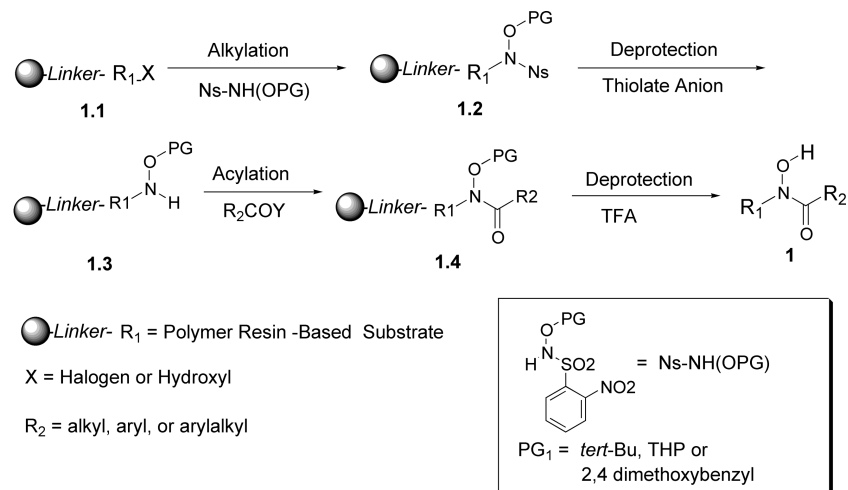


Fig. 1. Polyhydroxamate siderophores.

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situ during the solid-phase procedure (Scheme 1). The key to this approach was choice of *N*-nosyl (2-nitrophenylsulfonyl = Ns) protection [2] of the hydroxylamine group and an appropriate protecting group PG. The presence of the temporary *N*-protecting 2-nosyl group activates the nitrogen for alkylation with alkyl halides by conventional methods or alcohols under Mitsunobu reaction conditions to give **1.2**. Selective removal of the nosyl group with thiolate anion then leads to **1.3** that facilitates further elaboration of the molecule by *N*-acylation to afford the intermediate **1.4**. The choice



Scheme 1. Solid-phase synthesis of hydroxamate.

of acid-labile *O*-protecting groups (*e.g.* *tert*-Bu, THP, 2,4-dimethoxybenzyl) for the hydroxylamine moiety makes our automated high-throughput synthesis a robust process, as the protecting group is also removed during the final cleavage from the solid support. Also, some of these protecting groups (*e.g.* THP) offer the convenience of on-resin deprotection leading to libraries on solid support for further evaluation. Removal of the permanent *O*-protecting group (PG) leads to the desired hydroxamate **1**. When this approach is combined with an orthogonal protecting scheme for other building blocks (*e.g.* acid-sensitive groups for permanent protection and base, thiolate or mild acid-sensitive groups for temporary protection) such as amino acids, amino alcohols or carboxylic acids, the strategy is very versatile and suitable for generation of a large number of polyhydroxamate analogs. Another advantage of this approach is the ease of synthesis through the use of readily available, simple building blocks. A wide variety of structurally diverse analogs of DFO [3], aerobactin, carboxymycobactin, and exochelin (more than 200 compounds) were prepared via solid-phase combinatorial synthesis to explore metabolism, bioavailability, metal affinity and selectivity, and iron transport.

High-throughput screening to determine metal binding was developed based on competitive colorimetric and mass spectrometric assays. The spectrophotometric methods have proven to be more suitable for high-throughput screening, with sulfoxine (8-hydroxyquinoline 5-sulfonic acid) being the most robust. The measurement reflects relative affinities of ligands, expressed as a percentage of iron stripped