Practical Synthesis of Hydroxamate-Derived Siderophore Components by an Indirect Oxidation Method and Syntheses of a DIG−Siderophore Conjugate and a Biotin−Siderophore Conjugate

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A practical large-scale synthesis of hydroxamate-derived siderophore components (**30** and **40**) that utilizes an efficient indirect oxidation method is described and applied to the syntheses of nonradioactive labeled siderophores. Oxidation of imines derived from L-ornithine (**17**) and its tripeptide (**19**) afforded oxaziridines that were isomerized to stable nitrones (**16** and **18**). Acid-catalyzed hydrolysis of nitrones provided hydroxylamines that were converted to the desired hydroxamic acids (**30** and **40**) suitable for constructing siderophore−drug conjugates (**2**). The entire synthetic sequence required no chromatographic separation. DIG- and biotin-labeled ferrichrome analogues designed to detect and isolate ferrichrome receptors in various microbes were also synthesized.

Introduction

Siderophores are secreted by organisms as microbial iron transport agents to solubilize iron(III) under iron-deficient conditions.[1] Hydroxamate-based siderophores, such as albomycins (**1**, Figure 1), contain ligands derived from the tripeptide of N^5-hydroxy-N^5-acetyl-L-ornithine. These compounds form strong, selective complexes with Fe(III). The siderophore−iron complex is recognized by outer membrane receptors and actively transported into the cell. Because iron is a limiting nutrient at the onset of microbial infection, siderophores are essential for microbial pathogenicity.[2]

Effective drug delivery is becoming increasingly important because of the emergence of multidrug-resistant pathogens. Bacteria have developed the ability to limit the permeability of their cell walls, to make proteins that pump drugs rapidly out of their cells, to acquire enzymes to deactivate drugs, and to alter the drugs' targets.[3] Lack of effective drug delivery to the target cell due to cell wall permeability is partially responsible for current drug resistance.

A unique way to combat the cell permeability problem is to use the cell's natural mechanism for nutrient assimilation to smuggle drugs into cells. This "Trojan Horse" approach in drug delivery is being rigorously studied in our laboratory.[4] Siderophore−drug conjugates (**2**, Figure 1) are composed of iron chelators, chemical linkers, and biologically active components ("drug"). Albomycins, antibiotics produced by *Streptomyces griseus* and *Streptomyces substropicus*, are examples of natural siderophore−drug conjugates. They all contain the siderophore component derived from the tripeptide of N^5-hydroxy-N^5-acetyl-L-ornithine, the common iron chelator in most hydroxamate-derived siderophores. The cytotoxic unit is connected to the iron chelator via a serine spacer.

We have been engaged in the synthesis of siderophore−drug conjugates (**2**) for biological evaluation.[5] In a previous study, we found that a siderophore component derived from the tripeptide of N^5-hydroxy-N^5-acetyl-L-ornithine was recognized by certain microbes and actively transported inside the cell. This was determined by the growth-promoting ability of the siderophore for *S. flexneri* SA 240.[6] Our current strategy for active drug transport focuses on the connection of drugs to siderophores. These siderophore−drug conjugates (**2**) could be recognized by microbes and transported inside the cell by the iron uptake system. Once inside the cell, the drug could be released and deliver an effective dosage to the microbes. This strategy would overcome the cell permeability problem and thus has great potential for the development of a new generation of antibacterial and antifungal agents.

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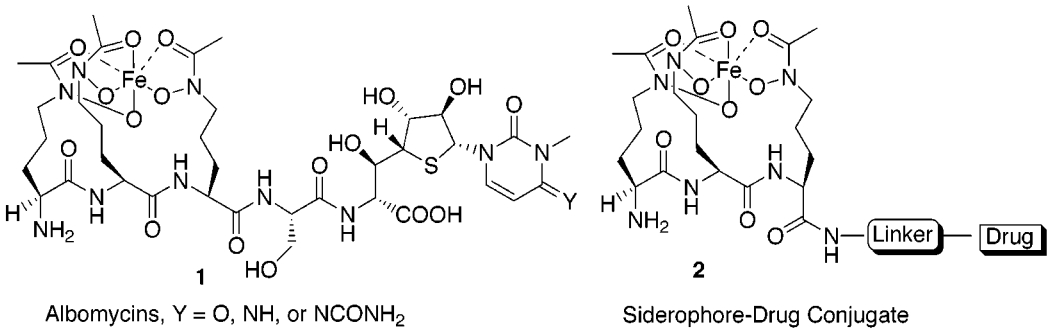
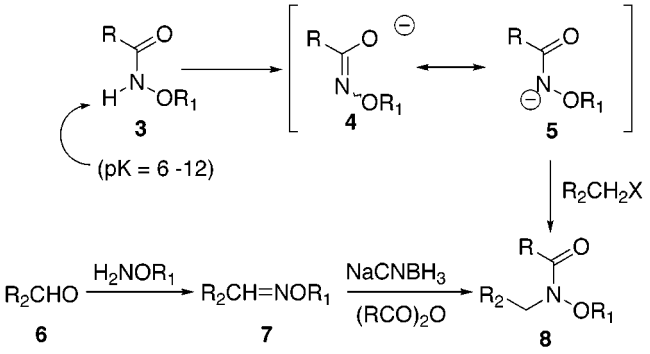


Figure 1. Albomycins and siderophore−drug conjugates.

Scheme 1

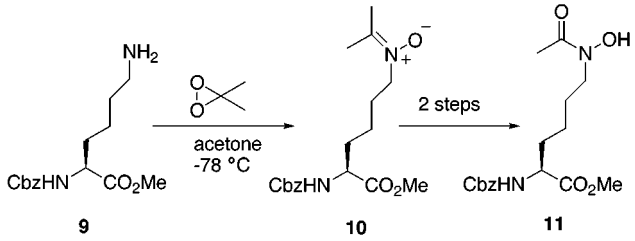


Hydroxamic acids are also essential iron(III) chelators for hydroxamate-derived siderophores. Thus, efficient and rapid access to large quantities of hydroxamates are highly desirable not only for the syntheses of a siderophore−drug conjugates library, but also for the syntheses of natural siderophore analogues such as mycobactin analogues.[7] Our goal was to develop a practical synthesis in which primary amines would be converted to the corresponding hydroxylamines and hydroxamic acids.

Hydroxamates were synthesized previously in this laboratory by N-alkylation of simple O-substituted hydroxamic acid **3** with a variety of alkylating agents. Alternatively, formation of oxime **7** from aldehyde **6** and O-substituted hydroxylamine followed by subsequent reduction and in situ acylation produced the suitably protected hydroxamic acid derivatives **8** (Scheme 1).[8] These methods allowed us to synthesize several siderophores and many analogues.[9]

Most recently, hydroxylamines and hydroxamic acids were synthesized by direct oxidation of lysine and ornithine using dimethyldioxarine (DMD) (Scheme 2) that led to the efficient total synthesis of mycobactins and analogues.[10] Oxidation of lysine methyl ester (**9**) with DMD provided nitrone **10** that was converted to hydroxamic acid (**11**) in two steps. Practical large-scale synthesis of hydroxamate-derived siderophore components such as

Scheme 2



11 was therefore limited by the generation and utilization of DMD solution in higher concentration. Benzoyl peroxide-mediated oxidation of ω amino group of protected lysine was employed in Bergeron's total synthesis of nannochelin A.[11] This oxidation protocol was recently improved by Phanstiel.[12]

In 1978, Naegeli and Keller-Schierlein reported an elegant total synthesis of D-ferrichrome by an indirect oxidation method (Scheme 3).[13] Their indirect oxidation featured the conversion of primary amines to imines followed by oxidation of imines to oxaziridines. Acid-catalyzed hydrolysis of oxaziridines afforded hydroxylamines which led to the successful synthesis of D-ferrichrome. This indirect oxidation is an attractive approach for the synthesis of hydroxamate-derived siderophore components from amino acids and peptides since more than one amino group could be oxidized simultaneously. Furthermore, it should be possible to scale-up this oxidation process. We therefore adopted this indirect oxidation strategy and have found that alternate generation of intermediate nitrone derivatives provides distinct advantages for large scale, practical syntheses of essential hydroxylamine and hydroxamate components of siderophores.

Herein, we would like to report an efficient scalable synthesis of hydroxylamines and hydroxamic acids starting from N^2-protected L-ornithine (**17**) and its tripeptide (**19**) through nitrone intermediates (**16** and **18**) by an indirect oxidation method (Scheme 4). The syntheses of biotin−siderophore conjugate (**20**, Figure 2) and DIG−siderophore conjugate (**21**, Figure 2) suitable for detecting receptor proteins (vida infra) are also described.

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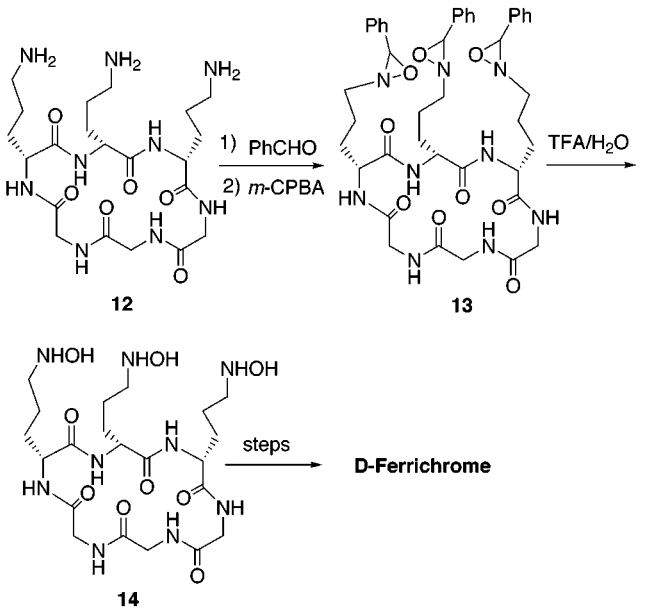
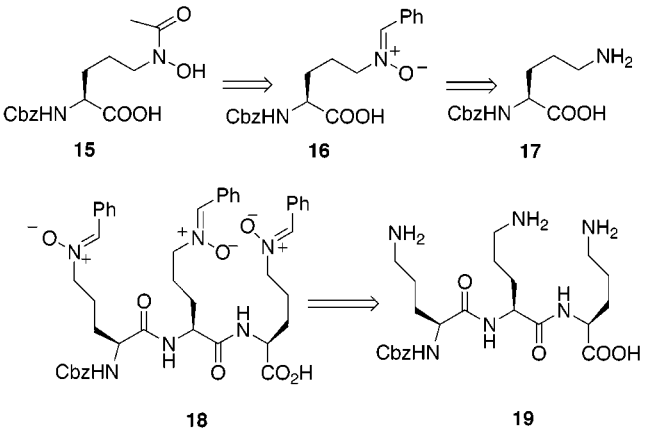
Scheme 3



Scheme 4



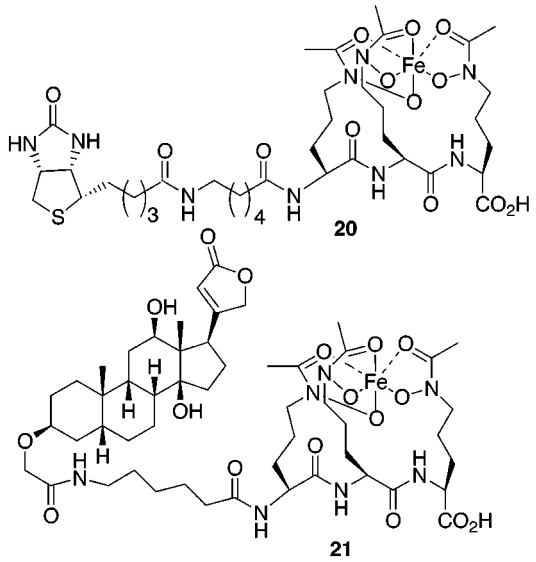
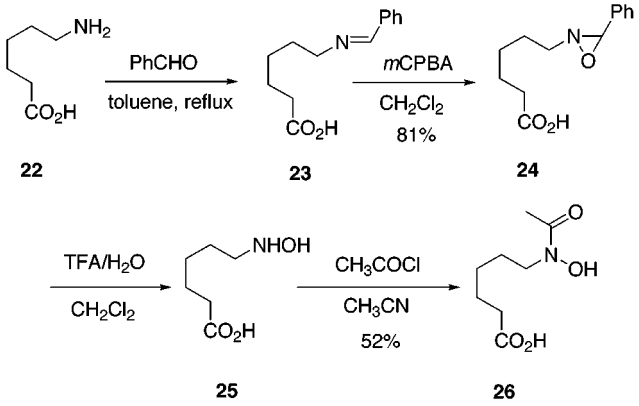


Figure 2. DIG−siderophore conjugate and biotin−ferrichrome analogue.

Scheme 5



Results and Discussion

To examine the feasibility and practical aspects of this indirect oxidation approach, we conducted model studies on 6-aminocaproic acid. As shown in Scheme 5, imine formation of 6-aminocaproic acid (**22**) with benzaldehyde was effective when refluxed in toluene with removal of water. The resulting imine **23** was oxidized with *m*-chloroperoxybenzoic acid (*m*-CPBA) to give oxaziridine **24** in 81% overall yield.

Oxaziridine **24** was hydrolyzed to hydroxylamine **25** under acid-catalyzed conditions. Benzaldehyde byproduct and other organic impurities were readily removed by extraction. Concentration of the aqueous solution thus provided pure hydroxylamine **25**. Subsequent acetylation of **25** with acetyl chloride afforded hydroxamic acid **26** in 52% overall yield from oxaziridine **24**. It is noteworthy that the entire process required only one column chromatographic purification in the last step and it did not require the protection of the carboxyl group.

Indirect oxidation of N^2-Cbz-L-ornithine **17** was then attempted. Although the benzaldehyde-derived imine was not formed in refluxing toluene, it was obtained in quantitative yield under basic conditions. As shown in Scheme 6, imine formation with benzaldehyde was effective when **17** was treated with 1 equiv of potassium hydroxide in the presence of 3 Å molecular sieves.[14] Oxidation of the resulting imine with *m*-CPBA under basic conditions proceeded rapidly and gave oxaziridine **27**. Oxaziridine **27** was hydrolyzed to provide the corresponding hydroxylamine **28** under conditions similar to those described in the model study.

During a scale-up reaction, however, a substantial amount (ca. 50%) of nitrone **16** was isolated as a white solid from the reaction mixture. We, therefore, decided to intentionally isomerize the unstable oxaziridine **27** to nitrone **16**. Thus, isomerization of crude oxaziridine **27** with TFA in methylene chloride afforded nitrone **16** in 80% overall yield from **17** after recrystallization. Although this isomerization added one step to the synthesis, it offered the important advantage of a stable protected hydroxylamine equivalent that was easily crystallized. This indirect oxidation process has been carried out on 30 g of N^2-Cbz-L-ornithine **17** to afford 33 g of nitrone **16**. In contrast to hydroxylamine **28**, which is susceptible to decomposition, oxidation, and disproportionation reactions,[15] nitrone **16** can be stored at room temperature indefinitely without any decomposition.

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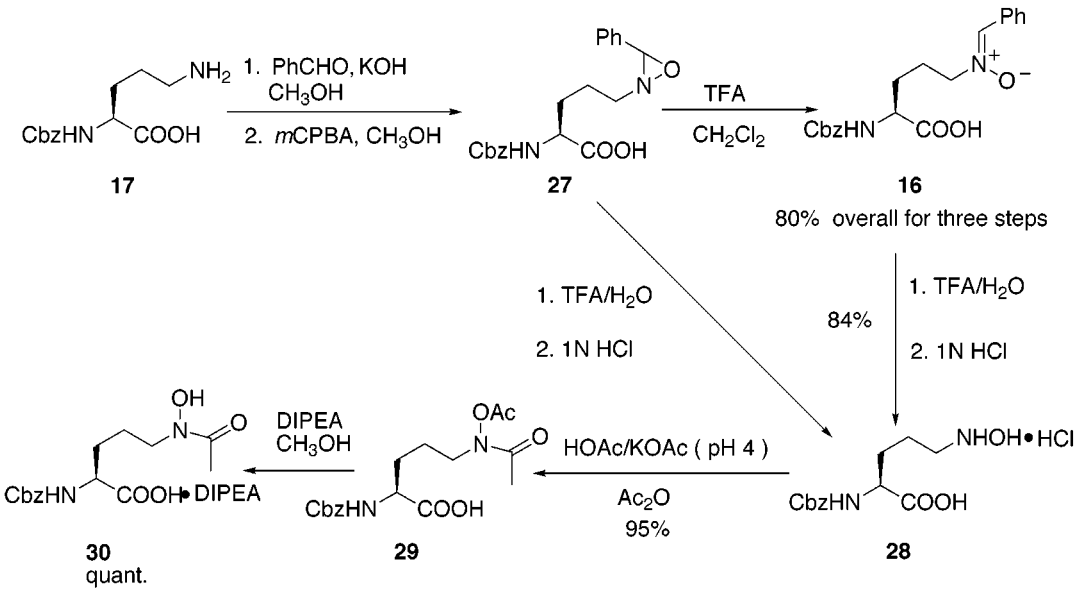
Scheme 6



Nitrone **16** is an advanced intermediate in preparing exochelin MN[16] and analogues.

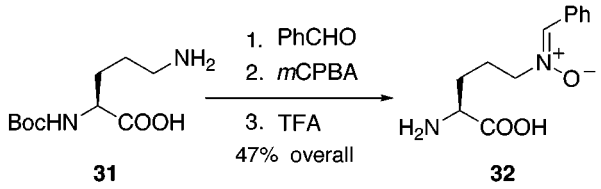
Acid-catalyzed hydrolysis of nitrone **16** afforded hydroxylamine **28** in 84% yield after removal of benzaldehyde by extraction. The acetylation of hydroxylamine **28** was then attempted. Under standard acetylation conditions (e.g, acetyl chloride and base in acetonitrile), the free carboxylic group was readily activated and led to a mixture of products due to an intramolecular cyclization. Thus, other reaction conditions were examined in order to effect the selective acetylation of the hydroxylamine functional group without the need for protection of the carboxylic acid. At pH 4, the hydroxylamine functional group is nucleophilic and acetic anhydride is activated by acid catalysis. This would lead to acceleration of the acetylation reaction of hydroxylamine. The activation of the carboxyl functionality of **28** would be minimized. Treatment of hydroxylamine **28** with acetic anhydride at pH 4 resulted in selective N,O-diacetylation and provided hydroxamate **29** in 95% yield.

Hydroxamate **29** is hydrophobic and, therefore, easily extracted from the reaction mixture in pure form. The new acetylation conditions avoided the last-step column chromatograhic separation that was required in the model system. Finally, treatment of hydroxamate **29** with Hunig's base in methanol afforded desired hydroxamic acid **30** as the corresponding salt in quantitative yield. It is noteworthy that this indirect oxidation process does not require any chromatographic purification or carboxyl protection.

A fully unprotected nitrone (**32**) was obtained through this indirect oxidation−isomerization protocol in 47% total yield from N^2-Boc-L-ornithine (**31**) (Scheme 7). This should allow the preparation of fully unprotected amino acid-derived hydroxylamines that are of significant biological interest.[17]

We next focused our attention on the corresponding ornithine tripeptide. To synthesize the desired siderophore components of albomycins by this indirect oxidation procedure, an appropriately protected ornithine tripeptide was required. A partially protected tripeptide **35** was synthesized under active ester-mediated coupling conditions[18] from N^2-Cbz-N^5-Boc-L-ornithine (**33**) and N^5-Boc-L-ornithine in an iterative approach. Thus, the *N*-hydroxysuccinimide (NHS) active ester of **33** was synthesized by treating **33** and NHS with dicyclohexylcarbodiimide (DCC) in THF. The resulting active ester was aminolyzed with N^5-Boc-L-ornithine to give dipeptide **34** in quantitative yield. Under the same coupling conditions, dipeptide **34** was converted to the partially protected tripeptide **35** in quantitative yield. The side-chain protecting groups were cleanly removed using 70% TFA in acetic acid[19] to give **36** in 82% yield (Scheme 8).

Scheme 7



When treated with 3 equiv of benzaldehyde and 4 equiv of KOH, triamine **36** was converted to the corresponding triimine, which was oxidized to afford trioxaziridine **37** (Scheme 9). Crude oxaziridine **37** was isomerized to nitrone **18** in 58% overall yield from tripeptide **36**. Nitrone **18** was readily purified by recrystallization.

Acid-catalyzed hydrolysis of nitrone **18** gave trihydroxylamine **38** in 97% yield. Acetylation of **38** with acetic anhydride under the previously described buffered conditions gave fully acetylated product **39** in 93% yield. The *O*-acetyl groups were removed to afford the desired siderophore component **40** as the diisopropylethylamine salt in quantitative yield. Again, column chromatographic separation was not required in the entire indirect oxidation process. Large-scale indirect oxidation of ornithine tripeptide **36** (ca. 12 g) provided gram quantities of **18**

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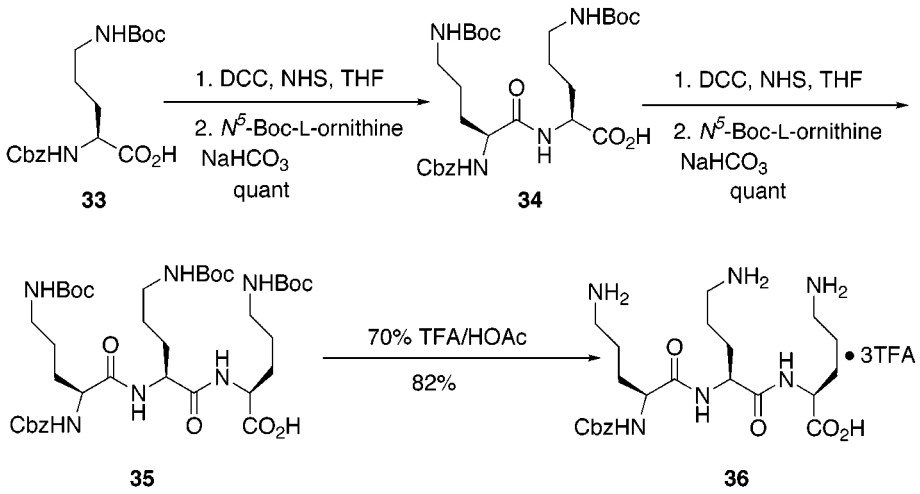
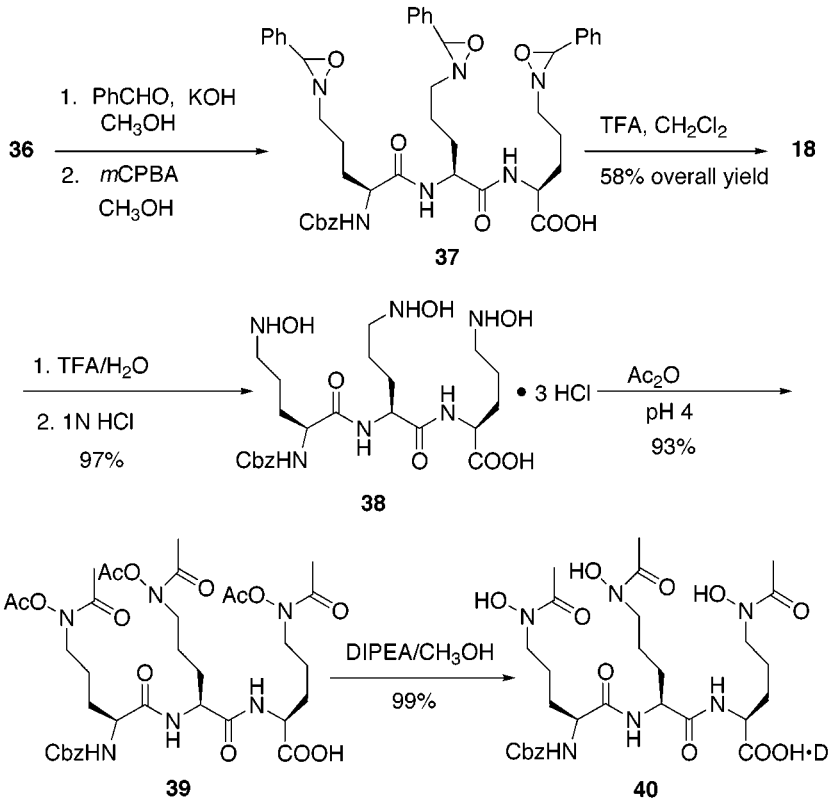
Scheme 8



Scheme 9



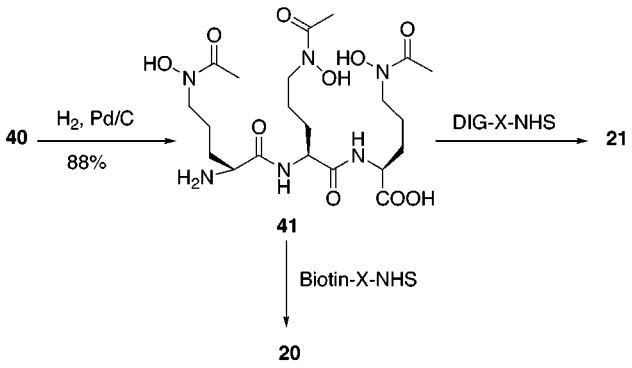
(ca. 6 g) suitable for further elaboration and construction of siderophore−drug conjugate libraries that will be reported in due course.

Hydrogenolysis of **40** provided fully deprotected siderophore component **41** in 88% yield (Scheme 10). Treatment of **41** with commercially available biotin active ester and digoxigenin (DIG) active ester afforded the desired biotin−ferrichrome analogue (**20**) and DIG−siderophore conjugate (**21**) without the need for protection of the constituent hydroxamic and carboxylic acid.

These two siderophore conjugates were designed to detect the presence of receptor proteins in new microbial strains in order to target specific microbial species in the "Trojan Horse" active drug delivery approach. Binding of the siderophore−iron complex in **20** or in **21** to receptor proteins should immobilize the receptors on an avidin or a DIG antibody coated stationary phase. Affinity column chromatography is anticipated to separate ligand bound protein from other proteins. The utilization and biological testing of these two conjugates is currently underway.

In conclusion, a practical and efficient synthesis of hydroxylamines and hydroxamate siderophore components derived from amino acids has been achieved through an indirect oxidation method. It provides a large laboratory-scale synthetic protocol for preparation of hydroxylamines and hydroxamates through stable nitrone intermediates with no need of column chromatographic purification. In addition, a pH 4 buffered solution was found to facilitate the acetylation of hydroxylamines selectively in the presence of free carboxylates. Large quantities of siderophore component **40** are now readily available for the preparation of siderophore−drug conjugate libraries for biological evaluation.

Scheme 10



Experimental Section

General Methods. All reactions were carried out under N_2 unless otherwise stated. Instruments and general methods used have been described earlier.[20] Benzaldehyde was freshly distilled before use. Technical-grade *m*-CPBA (ACROS) was dehydrated over P_2O_5 in a desiccator under vaccum until mp > 90 °C before use.

2-(5′-Carboxyl)-3-phenyloxaziridine (24). To a suspension of 6-aminocaproic acid **22** (2.6 g, 20 mmol) in toluene (40 mL), was added benzaldehyde (2.0 mL, 20 mmol). The mixture was refluxed under N_2, using a Dean−Stark trap (3 Å molecular sieves) for 12 h. The volatiles were removed to give 4.0 g of imine **23** as a bright yellow syrup, which was used without further purification.

To a solution of imine **23** (4.0 g) in CH_2Cl_2 (20 mL) at 0 °C was added a solution of *m*-CPBA (4.1 g, 20.7 mmol) in CH_2Cl_2 (50 mL) dropwise. The reaction mixture was stirred at 0 °C for 5 h. The resulting *m*-CPBA precipitate was filtered off and washed with CH_2Cl_2. The filtrate was washed with $Na_2S_2O_3$ and brine, dried (Na_2SO_4), filtered, and concentrated to give 3.5 g (81% in two steps) of **24** as a yellow solid, which was used without further purification. An analytical sample was obtained by flash column chromatographic separation on silica gel (2:1 hexanes/EtOAc) as a white solid: mp 71−72 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 5H), 4.49 (s, 1H), 3.03 (dt, J = 12.3, 4.5 Hz, 1H), 2.73 (dt, J = 12.3, 6.9 Hz, 1H), 2.37 (t, J = 7.5 Hz, 2H), 1.75−1.68 (m, 4H), 1.51−1.49 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.9, 134.6, 130.0, 128.4, 127.5, 80.4, 61.8, 33.8, 27.6, 26.7, 24.4; IR (neat) 3050, 1690, 1465, 1410 cm^{-1}; HRFABMS calcd for $C_{13}H_{18}NO_3$ (MH^+) 236.1287, found 236.1263.

***N*^{6}-Hydroxy-6-aminocaproic Acid (25).** A solution of oxaziridine **24** (470 mg, 2.0 mmol) in CH_2Cl_2 (30.0 mL) was treated with TFA/H_2O (10.0 mL, v/v, 4/1) at room temperature. The reaction mixture was stirred for 4 h, H_2O (20 mL) was added, and the layers were separated. The aqueous layer was washed with CH_2Cl_2 and concentrated to yield 300 mg of **25** as a yellow oil: ^{1}H NMR (300 MHz, CD_3CN) δ 5.65 (br, 3H), 3.16 (t, J = 7.5 Hz, 2H), 2.28 (t, J = 7.2 Hz, 2H), 1.68−1.56 (m, 4H), 1.41−1.39 (m, 2H); ^{13}C NMR (75 MHz, CD_3CN) δ 175.2, 52.0, 33.8, 26.4, 24.9, 23.8.

***N*^{6}-Acetyl-*N*^{6}-hydroxy-6-aminocaproic Acid (26).** To a mixture of hydroxylamine **25** (300 mg, 2.0 mmol) and $NaHCO_3$ (420 mg, 5.0 mmol) in CH_3CN (20.0 mL) at room temperature was added CH_3COCl (0.16 mL, 2.26 mmol). The reaction mixture was stirred at room temperature for 12 h. The solid was filtered off and washed with methanol. The filtrate was concentrated and purified by flash column chromatography on silica gel (EtOAc to 1:2 MeOH/EtOAc), yielding 200 mg (52% from **24**) of **26** as a white foam: ^{1}H NMR (300 MHz, CD_3OD) δ 3.59 (t, J = 6.9 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 2.09 (s, 3H), 1.63 (m, 4H), 1.34 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 177.7, 173.5, 48.6, 34.8, 27.4, 27.2, 25.7, 20.2; IR (neat) 3189, 1683, 1624 cm^{-1}; HRFABMS calcd for $C_8H_{16}NO_4$ (MH^+) 190.1079, found 190.1082.

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***N*^{2}-Benzyloxycarbonyl-*N*^{5}-(3-phenyloxaziridinyl)-L-ornithine (27).** To a solution of KOH (6.6 g, 117.8 mmol) in methanol (250 mL) at room temperature was added **17** (30.0 g, 112.8 mmol), followed by benzaldehyde (12 mL, 118.2 mmol) and 3 Å molecular sieves. The reaction mixture was stirred at room temperature for 16 h. The molecular sieves were filtered off and washed with methanol. The filtrate was concentrated to give 43.3 g (98%) of imine as a white foam: ^{1}H NMR (300 MHz, CD_3OD) δ 8.35 (s, 1H), 7.74 (m, 2H), 7.48−7.30 (m, 8H), 5.10 (m, 2H), 4.12 (m, 1H), 3.65 (m, 2H), 1.94−1.70 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 179.4, 164.4, 158.2, 138.4, 137.1, 132.0, 129.7, 129.4, 129.3, 128.9, 128.8, 67.3, 61.9, 57.5, 31.9, 28.0; IR (neat) 3285, 1708, 1692 cm^{-1}.

To a solution of imine (43.3 g, 110.4 mmol) in CH_3OH (200 mL) at 0 °C was added a solution of *m*-CPBA (25.1 g, 124.0 mmol) in CH_3OH (80 mL) over 1 h. The reaction was stirred at 0 °C for an additional h. The resulting precipitate was filtered off and washed with CH_3OH. The filtrate was concentrated at room temperature to give a white solid, which was partitioned between H_2O (250 mL) and EtOAc (250 mL). The pH of the mixture was adjusted to 2 with 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give crude oxaziridine **27** as a white solid, which was used without further purification. An analytical sample was obtained as a mixture of diastereomers by trituration from diethyl ether and hexanes: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.33 (m, 10H), 6.75 (br, 1H), 5.57 (d, J = 8.1 Hz, 1H), 5.10 (s, 2H), 4.51 (s, 1H), 4.41 (m, 1H), 3.16 (m, 1H), 2.62 (m, 1H), 2.06−1.84 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 156.2, 136.1, 134.3, 130.1, 128.52, 128.48, 128.2, 128.1, 127.6, 80.5, 80.3, 67.1, 61.2, 53.7, 30.1, 29.9, 24.0. IR (neat) 3206, 1713, 1683, 1413 cm^{-1}; HRFABMS Calcd for $C_{20}H_{23}N_2O_5$ (MH^+) 371.1607, found 371.1610.

Nitrone 16. To crude oxaziridine **27** at room temperature was added TFA (50 mL), followed by CH_2Cl_2 (50 mL). The reaction mixture was stirred at room temperature for 1 h. The volatiles were removed, and the resulting slurry was dissolved in EtOAc (200 mL). Hexanes (400 mL) and benzaldehyde (6 mL) were added, and the reaction mixture was stirred at room temperature for 12 h. The mixture was cooled to 0 °C, and the resulting solid was filtered off, washed with EtOAc, and dried to give 37.1 g of nitrone **16** as an off-white solid. The crude product was recrystallized from ^{i}PrOH/EtOAc/hexanes to give 33.4 g (80% yield from **17**) of **16** as a fluffy white solid: mp 150.5−151 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.24 (m, 2H), 7.85 (s, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.42−7.29 (m, 8H), 5.03 (s, 2H), 4.03 (m, 1H), 3.91 (t, J = 7.0 Hz, 2H), 1.91 (m, 2H), 1.76 (m, 1H), 1.65 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.8, 156.4, 137.1, 133.8, 131.1, 130.0, 128.5, 128.1, 128.0, 127.8, 65.6, 53.6, 27.9, 24.2; IR (KBr) 3340, 1717, 1696 cm^{-1}; HRFABMS calcd for $C_{20}H_{23}N_2O_5$ (MH^+) 371.1607, found 371.1615.

***N*^{2}-Benzyloxycarbonyl-*N*^{5}-hydroxy-L-ornithine Hydrochloride (28).** A mixture of nitrone **16** (3.7 g, 0.01 mol), hexanes (20 mL), 0.5 N HCl (40 mL), and TFA (10 mL) was heated at 60 °C for 15 min. The volatiles were removed to give a light yellow oil. To this residue were added CH_2Cl_2 (40 mL) and 1 N HCl (60 mL). The mixture was heated at 40 °C briefly to dissolve the residue and allowed to stir at room temperature for 40 min. The organic layer was separated, and the aqueous layer was washed with CH_2Cl_2 and hexanes. The aqueous layer was concentrated to give 2.68 g (84%) of **28** as a white foam. No further recrystallization was attempted due to the instability of hydroxylamine: ^{1}H NMR (300 MHz, CD_3CN) δ 7.34 (m, 5H), 6.14 (br, 1H), 5.08 (AB, J = 12.6 Hz, 2H), 4.27 (m, 1H), 3.54 (m, 2H), 2.07−1.71 (m, 4H); ^{13}C NMR (75 MHz, CD_3CN) δ 166.7, 157.9, 137.8, 129.4, 129.0, 128.8, 67.5, 52.0, 51.3, 28.1, 20.9; IR (KBr) 3331, 1739, 1687 cm^{-1}; HRFABMS calcd for $C_{13}H_{19}N_2O_5$ (MH^+) 283.1294, found 283.1311.

***N*^{2}-Benzyloxycarbonyl-*N*^{5}-acetyl-*N*^{5}-*O*-acetyl-L-ornithine (29).** To a solution of **28** (2.64 g, 8.30 mmol) in KOAc/HOAc buffer (pH 4, [KOAc] = 0.05 M, 80 mL), was added $KHCO_3$ (0.83 g). Acetic anhydride (14 mL, 150 mmol) was

added dropwise at room temperature. The pH of the solution was maintained at 4 by adding solid potassium bicarbonate during this process. The reaction mixture was stirred at room temperature for 4.5 h. The volatiles were removed to give an oily residue. To this residue were added 0.5 N HCl (200 mL) and EtOAc (150 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give 2.9 g (95%) of **29** as a colorless oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (br, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.35−7.28 (m, 5H), 5.02 (s, 2H), 3.93 (m, 1H), 3.58 (m, 2H), 2.18 (s, 3H), 1.90 (s, 3H), 1.70−1.55 (m, 4H); ^{13}C NMR (125 MHz,CD_3OD) δ 175.7, 173.4, 170.4, 158.8, 138.4, 129.6, 129.1, 128.9, 67.7, 55.0, 48.2, 29.9, 24.6, 20.3, 18.3; IR (neat) 3329, 1796, 1715, 1652 cm^{-1}; HRFABMS calcd for $C_{17}H_{23}N_2O_7$ (MH^+) 367.1505, found 367.1495.

***N*^{2}-Benzyloxycarbonyl-*N*^{5}-acetyl-*N*^{5}-hydroxy-L-ornithine (30).** A solution of hydroxamate **29** (2.60 g, 7.10 mmol) in a diisopropylethylamine methanol solution (6%, 30 mL) was stirred at room temperature for 12 h. The volatiles were removed to give 3.22 g (quant) of **30** as a light yellow oil: ^{1}H NMR(500 MHz, CD_3OD) δ 7.36−7.28 (m, 5H), 5.06 (s, 2H), 4.03 (m, 1H), 3.70 (hept, J = 6.5 Hz, 2H), 3.60 (m, 2H), 3.19 (q, J = 7.5 Hz, 2H), 2.08 (s, 3H), 1.82−1.66 (m, 4H) 1.35 (d, J = 6.5 Hz, 12H), 1.35 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 178.9, 173.7, 158.2, 138.6, 129.6, 129.0, 128.9, 67.5, 57.5, 55.8, 48.6, 43.8, 31.5, 24.3, 20.4, 18.1, 13.3; IR (neat) 3396, 3265, 1715, 1610 cm^{-1}.

Nitrone 32. A crude oxaziridine was prepared from *N*^{2}-Boc-L-ornithine **31** (10.0 g, 43.1 mmol) by the indirect oxidation procedure as described above. It was then isomerized by adding TFA (60.0 mL), followed by CH_2Cl_2 (60 mL) at room temperature. The resulting red solution was allowed to stir at room temperature for 1 h. The volatiles were removed, and the residue was dissolved in EtOAc (150 mL). Then a THF solution of Hunig's base was added until the pH of the mixture was 8−9. The mixture was cooled at 0 °C for 1 h, and the resulting precipitate was filtered off and dried to give 7.36 g (47% overall yield from **31**) of **32** as a yellow solid. An analytical sample was obtained by recrystallization from H_2O/CH_3CN: mp 207−207.5 °C dec; ^{1}H NMR (300 MHz, D_2O) δ 8.18 (m, 2H), 7.96 (s, 1H), 7.60−7.54 (m, 3H), 4.06 (m, 2H), 3.77 (m, 1H), 1.95 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6/D_2O (95%)) δ 175.3, 142.5, 133.9, 131.5, 130.5, 130.4, 66.6, 55.7, 28.8, 24.2; IR (KBr) 3436, 1626, 1591 cm^{-1}; HRFABMS calcd for $C_{12}H_{16}N_2O_3$ (MH^+) 237.1239, found 237.1231.

Dipeptide 34. To a solution of **33** (24.0 g, 65 mmol) and NHS (8.0 g, 69 mmol) in THF (150 mL) at 0 °C was added a solution of DCC (13.6 g, 66 mmol) in THF (100 mL). The reaction was stirred to room temperature for 10 h. The resulting DCU was filtered off and washed with THF. The THF filtrate was used as the active ester solution in the following reaction.

To a solution of *N*^{5}-Boc-L-ornithine (18.0 g, 77 mmol) and $NaHCO_3$ (14 g, 170 mmol) in H_2O/THF (400 mL/300 mL) at room temperature was added the active ester obtained from above. The reaction mixture was stirred at room temperature for 6 h. The volatiles were evaporated, and the aqueous residue was diluted with EtOAc (500 mL) and acidified to pH 2 with 10% citric acid. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give 38 g (quant) of **34** as a white foam, which was used without further purification. An analytical sample was obtained by flash column chromatographic separation on silica gel (2.5% HOAc/EtOAc) as a white solid: mp 58−60 °C; ^{1}H NMR (500 MHz, DMSO-d_6/D_2O, (95%) 60 °C) δ 7.29 (m, 5H), 4.98 (s, 2H), 4.15 (m, 1H), 3.99 (dd, J = 5.5, 9.0 Hz, 1H), 2.88 (t, J = 6.5 Hz, 4H), 1.69−1.35 (m, 8H), 1.32 (s, 18H); ^{13}C NMR (125 MHz, DMSO-d_6/D_2O (95%)) δ 173.5, 172.1, 156.0, 155.7, 137.1, 128.4, 127.8, 127.7, 77.5, 65.4, 54.2, 51.7, 39.8, 29.4, 28.5, 28.3, 26.1; IR (neat) 3320 (br), 1710, 1700, 1690, 1665, 1520 cm^{-1}; HRFABMS calcd for $C_{28}H_{44}N_4O_9Na$ (M + Na)$^+$ 603.3006, found 603.3005.

Tripeptide 35. Analogous active ester-mediated coupling (DCC, NHS) of dipeptide **34** (38 g, 65.5 mmol) with *N*^{5}-Boc-L-ornithine provided 53 g (quant) of **35** as a white foam, which was used without further purification. An analytical sample was obtained by flash column chromatographic separation on silica gel (2.5% HOAc/EtOAc) as a white solid: mp 88−90 °C; ^{1}H NMR (500 MHz, DMSO-d_6/D_2O, (95%) 60 °C) δ 7.29 (m, 5H), 4.98 (s, 2H), 4.23 (dd, J = 5.0, 8.5 Hz, 1H), 4.14 (dd, J = 5.0, 8.5 Hz, 1H), 3.95 (dd, J = 5.0, 8.5 Hz, 1H), 2.88 (m, 6H), 1.69−1.35 (m, 12H), 1.32 (s, 27H); ^{13}C NMR (125 MHz, DMSO-d_6/D_2O) δ 174.2, 173.1, 172.6, 157.0, 156.9, 137.5, 129.3, 128.8, 128.4, 79.0, 65.5, 55.2, 52.9, 52.5, 40.0, 29.8, 28.9, 26.6, 26.4; IR (neat) 3320 (br), 1710, 1695, 1665, 1660, 1525 cm^{-1}; HRFABMS calcd for $C_{38}H_{62}N_6O_{12}Na$ (M + Na)$^+$ 817.4323, found 817.4347.

Tripeptide 36. A solution of tripeptide **35** (30.0 g, 38 mmol) in TFA/HOAc (35 mL/15 mL) was stirred at room temperature for 1 h. The volatiles were removed, and the resulting residue was dissolved in H_2O (200 mL). The aqueous solution was washed with EtOAc, concentrated, and azeotroped with toluene to give 26 g (82%) of **36** as a glassy white foam: ^{1}H NMR (300 MHz, D_2O) δ 7.27 (m, 5H), 4.97 (s, 2H), 4.25 (m, 2H), 4.04 (br, 1H), 2.88 (m, br, 6H), 1.64 (m, br, 12H); ^{13}C NMR (75 MHz, D_2O) δ 173.7, 173.4, 172.4, 156.9, 135.5, 128.0, 127.6, 126.8, 66.3, 53.7, 52.5, 51.4, 38.1, 27.4, 27.1, 26.6, 22.5, 22.4; IR (neat) 3265, 1676 cm^{-1}; HRFABMS calcd for $C_{23}H_{39}N_6O_6$ (MH^+) 495.2931, found 495.2924.

Nitrone Tripeptide 18. To a solution of KOH (3.5 g, 62.5 mmol) in methanol (200 mL) at room temperature was added tripeptide **36** (12.0 g, 14.4 mmol), followed by benzaldehyde (4.8 mL, 47.3 mmol) and 3 Å molecular sieves. The reaction mixture was stirred at room temperature for 17 h. The molecular sieves were filtered off and washed with methanol. The filtrate was concentrated to give 17.7 g of imine as a white foam. To a solution of imine in methanol (200 mL) at 0 °C was added a solution of *m*-CPBA (9.6 g, 47.4 mmol) in methanol (50 mL) over 1 h. The reaction was stirred at 0 °C for an additional h. The reaction mixture was concentrated at room temperature to a small volume (ca. 50 mL). The resulting mixture was partitioned between H_2O (250 mL) and EtOAc (250 mL). The pH of this mixture was adjusted to 2 with 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give **37** as a white solid, which was used without further purification.To crude oxaziridine tripeptide **37** at room temperature was added TFA (30 mL), followed by CH_2Cl_2 (30 mL). The reaction mixture was stirred at room temperature for 1 h. The volatiles were removed, and the resulting residue was dissolved in EtOAc (100 mL). Hexanes (200 mL) and benzaldehyde (3 mL) were added. The mixture was then stirred at room temperature for 12 h. The reaction mixture was then concentrated to a small volume (ca 100 mL), and the resulting white solid was filtered off and rinsed with EtOAc. The filtrate was concentrated to give 12.65 g of crude nitrone **18** as a tan foam.The crude nitrone was recrystallized from CH_3CN/EtOAc /hexanes to give 6.72 g (58% total yield from **36**) of **18** as a tan solid: mp 83−85 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24 (m, 7H), 8.13 (d, J = 7.8 Hz, 1H), 7.84−7.83 (m, 3H), 7.52 (d, J = 8.1 Hz, 1H), 7.40−7.26 (m, 14H), 4.99 (m, 2H), 4.36 (m, 1H), 4.21 (m, 1H), 4.09 (m, 1H), 3.89 (m, 6H), 1.87−1.56 (m, 12H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.2, 171.5, 171.3, 156.0, 137.0, 133.4, 133.3, 133.28, 131.05, 131.03, 129.8, 128.3, 128.0, 127.8, 127.7, 79.2, 65.7, 65.68, 65.5, 65.46, 54.2, 51.9, 51.7, 29.1, 29.0, 28.0, 24.0, 23.9, 23.8; IR (KBr) 3412, 3294, 1710, 1676, 1661, 1637 cm^{-1}; HRFABMS calcd for $C_{44}H_{51}N_6O_9$ (MH^+) 807.3718, found 807.3737.

***N*^{2}-Z-*N*^{5}-Hydroxyl-L-ornithyl-*N*^{5}-hydroxyl-L-ornithyl-*N*^{5}-hydroxyl-L-ornithine Hydrochloride (38).** To a solution of nitrone **18** (644 mg, 0.80 mmol) in TFA/H_2O (9 mL/1 mL) at room temperature was added CH_2Cl_2 (10 mL). The solution was stirred at 40−50 °C for 15 min. The volatiles were removed to give a light yellow oily residue. To this residue were added CH_2Cl_2 (20 mL) and 1 N HCl (30 mL). The mixture was stirred at room temperature for 1 h, and the layers were separated. The aqueous layer was washed with CH_2Cl_2 and hexanes and concentrated to give 503 mg (97%) of **38** as a light yellow foam.

Further recrystallization was not attempted due to the instability of hydroxylamine: ^{1}H NMR (300 MHz, D_2O/DMSO-d_6) δ 7.24 (m, 5H), 4.95 (s, 2H), 4.18 (m 2H), 4.00 (m 1H), 3.12 (m 6H), 1.64 (m, 12H); ^{13}C NMR (75 MHz, D_2O/DMSO-d_6) δ 176.0, 175.5, 174.6, 159.2, 137.8, 130.4, 130.0, 129.2, 68.6, 55.9, 54.7, 53.7, 51.5, 29.6, 29.4, 29.0, 21.2, 21.1, 21.0; IR (KBr) 3441, 3265, 1705, 1661 cm^{-1}.

***N*^{2}-Z-*N*^{5}-Acetyl-*N*^{5}-*O*-acetyl-L-ornithyl-*N*^{5}-acetyl-*N*^{5}-*O*-acetyl-L-ornithyl-*N*^{5}-acetyl-*N*^{5}-*O*-acetyl-L-ornithine (39).** Acetylation of **38** (456 mg, 0.701 mmol) under the previously described pH 4 buffer conditions provided 520 mg (93%) of **39** as an oil. ^{1}H NMR (300 MHz,CD_3OD) δ 7.34 (m, 5H), 5.08 (m, 2H), 4.39 (m, 2H), 4.14 (m, 1H), 3.71−3.69 (m, 6H), 2.19 (s, br, 9H), 1.99 (br, 9H), 1.68 (m, 12H); ^{13}C NMR (75 MHz, CD_3-OD) δ 174.8, 174.6, 173.8, 170.4, 158.5, 138.3, 129.6, 129.2, 129.0, 67.8, 55.9, 54.0, 53.2, 48.6, 30.4, 29.8, 24.6, 20.4, 18.4; IR (KBr) 3328, 1796, 1718, 1655 cm^{-1}; HRFABMS calcd for $C_{35}H_{51}N_6O_{15}$ (MH^+): 795.3412, found: 795.3429.

***N*^{2}-Z-*N*^{5}-Acetyl-*N*^{5}-hydroxyl-L-ornithyl-*N*^{5}-acetyl-*N*^{5}-hydroxyl-L-ornithyl-*N*^{5}-acetyl-*N*^{5}-hydroxyl-L-ornithine, DIPEA salt (40).** Treatment of **39** (478 mg, 0.602 mmol) with 6% Hunig's base in methanol (20 mL) at room temperature for 12 h provided 474 mg (99%) of **40** as a light yellow oil. ^{1}H NMR (300 MHz, CD_3OD) δ 7.33 (m, 5H), 5.07 (s, 2H), 4.36 (m, 1H), 4.18 (m, 2H), 3.69 (hept, J = 6.6 Hz, 2H), 3.58 (m, 6H), 3.19 (q, J = 7.5 Hz, 2H), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 1.66 (m, 12H), 1.36−1.29 (m, 15H); ^{13}C NMR (75 MHz, CD_3OD) δ 178.1, 175.0, 174.0, 173.9, 173.7, 173.1, 158.5, 138.3, 129.6, 129.1, 129.0, 129.0, 67.8, 56.1, 56.0, 55.7, 54.8, 48.4, 43.8, 31.2, 30.6, 30.4, 24.5, 24.4, 24.2, 20.6, 20.4, 19.5, 18.2, 13.4; IR (KBr) 3403, 3278, 1715, 1630 cm^{-1}; HRFABMS calcd for $C_{29}H_{45}N_6O_{12}$ (MH^+): 669.3095, found: 669.3095. The free acid was obtained by passing **40** through a reverse phase column (C_{18}, 2:1 H_2O/CH_3OH, 10% of HOAc was added in the eluent). HPLC analysis indicated 99.5% purity of the free acid.[21]

Siderophore Component (41). A mixture of **40** (137 mg, 0.172 mmol) and Pd/C (10%, 15 mg) in methanol/water (20 mL/5 mL) was stirred under an atmosphere of hydrogen for 1 h. The catalyst was filtered off and washed with methanol. The filtrate was concentrated to give 100 mg (88%) of **41** as a colorless oil: ^{1}H NMR (300 MHz, CD_3OD) δ 4.21 (m, 1H), 3.97 (m, 1H), 3.74 (m, 1H), 3.56−3.47 (m, 8H), 3.00 (q, J = 7.5 Hz, 2H), 1.94−1.92 (m, 9H), 1.57−1.46 (m 12H), 1.15−1.08 (m, 15H); HRFABMS calcd for $C_{21}H_{39}N_6O_{10}$ (MH^+) 535.2728, found 535.2706.

DIG Conjugate (21). To a mixture of **41** (20.0 mg, 0.030 mmol) and $NaHCO_3$ (10.0 mg, 0.12 mmol) in DMF (1 mL) at room temperature was added a solution of digoxigenin-3-*O*-methylcarbonyl-ϵ-aminocaproic acid *N*-hydroxysuccinimide ester (Boehringer, 5 mg, 0.0076 mmol). The reaction mixture was stirred at room temperature for 12 h. CH_3OH was added, and the white solid was filtered off. The filtrate was concentrated and separated by reversed-phase column chromatography (C_{18}, 2:1 H_2O/CH_3OH to 1:1 H_2O/CH_3OH, 5% of HOAc was added in the eluent) to give 7 mg (85%) of **21** as an oil: ^{1}H NMR (600 MHz, CD_3OD) δ 7.69 (t, J = 6.0 Hz, 1H), 5.90 (s, 1H), 4.92 (m, 2H), 4.42 (m, 1H), 4.35 (m, 2H), 3.88 (AB, J = 15 Hz, 2H), 3.70 (m, 3H), 3.62−3.55 (m, 4H), 3.39 (dd, J = 11.4, 4.8 Hz, 1H), 3.33 (dd, J = 6.0, J = 9.6 Hz, 1H), 3.25 (m, 2H), 2.25 (t, J = 7.2 Hz, 2H), 2.14 (m, 1H), 2.10 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 1.90−1.87 (m, 4H), 1.75−1.60 (m, 26H), 1.44 (m, 1H), 1.34−1.26 (m, 5H), 0.97 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 179.0, 177.8, 176.8, 175.7, 174.9, 174.5, 174.3, 174.2, 173.6, 118.2, 87.3, 77.6, 76.1, 76.0, 68.8, 57.8, 54.8, 54.5, 54.1, 48.9, 48.8, 48.7, 47.5, 42.7, 40.3, 38.5, 37.1, 36.7, 34.1, 34.0, 31.8, 31.75, 31.3, 30.8, 30.7, 30.5, 30.4, 28.8, 28.2, 28.0, 27.0, 26.0, 24.8, 24.8, 24.7, 24.6, 23.2, 21.2, 20.8, 20.8, 10.4; IR (neat) 3292 (br), 1734, 1720, 1646, 1638, 1617 cm^{-1}; HRFABMS calcd for $C_{52}H_{83}N_7O_{17}Na$ $(M + Na)^+$ 1100.5743, found 1100.5732.

Biotin Conjugate (20). To a solution of **41** (30.0 mg, 0.056 mmol) and $NaHCO_3$ (5.0 mg, 0.059 mmol) in THF/H_2O (1:1, 4 mL) at room temperature was added D-biotinonyl-ϵ-aminocaproic acid *N*-hydroxysuccinimide ester (Calbiochem, 31.0 mg, 0.056 mmol) The reaction mixture was stirred at room temperature for 48 h. TFA was added, and the volatiles were removed. The residue was purified by reversed-phase column chromatography (C_{18}, 4:1 H_2O/CH_3OH to 2:1 H_2O/CH_3OH, eluent containing 10% HOAc) to give 8 mg (16%) of **20** as a colorless oil: ^{1}H NMR (300 MHz, D_2O) δ 4.61 (dd, J = 7.8, 4.8 Hz, 1H), 4.20 (dd, J = 7.8, 4.5 Hz, 1H), 4.36−4.30 (m, 3H), 3.65 (br, 6H), 3.33 (m, 1H), 3.17 (t, J = 6.6 Hz, 2H), 3.00 (dd, J = 12.9, 4.8 Hz, 1H), 2.30 (t, J = 8.1 Hz, 2H), 2.24 (t, J = 7.2 Hz, 2H), 2.14 (m, 6H), 2.08 (s, 3H), 1.70−1.32 (m, 24H); ^{13}C NMR (125 MHz, D_2O) δ 176. 9, 176.7, 176.5, 175.6, 173.8, 173.2, 169.2, 165.2, 62.0, 60.2, 55.3, 53.2, 52.8, 50.9, 47.1, 39.6, 39.0, 35.4, 35.1, 27.9, 27.8, 27.6, 25.5, 25.1, 24.8, 22.4. 22.2, 20.4, 19.2; IR (neat) 3281, 3089, 1685, 1642 (br) cm^{-1}; HRFABMS calcd for $C_{37}H_{63}N_9O_{13}SNa$ $(M + Na)^+$ 895.4086, found 895.4095.

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Supporting Information Available: Copies of ^{1}H NMR and ^{13}C NMR spectra for compounds **16**, **18**, **20**, **21**, **24**−**30**, **32**, **34**−**36**, and **38**−**41**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) HPLC analysis was carried out using a Nova-Pak C_{18} 3.9 × 150 mm column (WAT 086344). A CH_3CN/H_2O gradient (10% to 90%, with 0.1% TFA added to both solvents) was used as the mobile phase with a flow rate of 1.0 mL/min.