Syntheses and Therapeutic Potential of Hydroxamic Acid Based Siderophores and Analogues

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Received March 9, 1989 (Revised Manuscript Received June 15, 1989)

Contents

Marvin J. Miller was born on Jan 29, 1949, in Dickinson, ND. His interest in science was greatly stimulated at Assumption Abbey Prep, a Benedictine monastic high school in Richardton, ND. Undergraduate research with Professor S. Peter Pappas at North Dakota State University in Fargo, increased his fascination with science, especially organic chemistry. Further studies during his Ph.D. work with Professor G. Marc Loudon at Cornell and an NIH postdoctoral position with Professor Henry Rapoport at Berkeley solidified an interest in bioorganic chemistry. Since joining the faculty at the University of Notre Dame in 1977, Dr. Miller has been an A. P. Sloan Fellow, an NIH research career development awardee, and a Visiting Fellow at the Australian National University. Dr. Miller's current research interests involve the chemical and enzymatic synthesis and study of biologically important, heteroatom-containing systems including amino acids, peptides, antibiotics, siderophores, and others. He and his wife, Patty, are also the proud parents of four beautiful children, Chris, Katie, Joey, and Carl.

I. Introduction

Iron has played an essential role in the evolution of nearly every form of life on earth. Although iron is one of the most abundant elements on the planet, its pivotal role depended on the development of effective methods for its assimilation. Ionic forms of iron, especially iron(III), its most common state, are very insoluble under physiological conditions. To circumvent the solubility problem, many microbial, plant, and even higher organisms (i.e., octopi[1]) synthesize and utilize very specific low molecular weight iron chelators called siderophores.[2-4] When grown under iron-deficient conditions, many microbes will synthesize and excrete siderophores in excess of their own dry cell weight to sequester and solubilize iron.[5] This extreme focus on the need for iron is reflected by its requirement for the proper function of the enzymes that facilitate electron transport, oxygen transport, and other life-sustaining processes. In fact, competition for iron between a host and bacteria is one of the most important factors determining the course of a bacterial infection.[6] Because of their ubiquitous nature, microbial siderophores have been extensively studied, yet much still needs to be learned about their chemistry and biochemistry, as well as other important aspects of iron metabolism.

The chemical properties of ionic forms of iron have dictated the evolutionary design of siderophores. Thus, high-spin, octahedral ferric ion is most effectively chelated by three bidentate oxygen-containing ligands. In many cases, the required three bidentate ligands are incorporated into the same molecule. The result has been the evolution of compounds such as enterobactin,[7] agrobactin,[8] ferrichrome,[9] desferrioxamine,[10] mycobactin,[11] pseudobactin,[12] and others that are capable of binding ferric ion extremely well. For example, the formation constant for enterobactin is so high (log K_f = 52!)[13] that special reductive, proteolytic, and hydrolytic processes are apparently utilized by microbes to release the iron once it is in the cell.[2,14] On the basis of these structures and those of other siderophores, the types of ligands utilized by microbes have been divided into three general classes: catechols, hydroxamates, and miscellaneous (including oxazoline, α-hydroxy carboxylic acids, amides, and others) (Charts 1 and 2). Several other subclasses have also been defined.[15] Many volumes and reviews have been written about the inorganic and biological aspects of siderophores.[2-6,14c,15] The organic and inorganic chemical as well as biological aspects of multi-catechol-containing siderophores have also been recently described in detail.[16,17] This paper will focus on the chemical synthesis and therapeutic potential of hydroxamate-based siderophores, their analogues, and derivatives.

Hydroxamic acids, their derivatives, and often their iron complexes have long been implicated in a wide spectrum of biological activities.[18-20] A number are antibacterial[21,22] and antifungal agents,[23] anticancer agents,[5,23] and specific enzyme inhibitors.[24] Certain hydroxamic acids have been used to reactivate chymotrypsin and acetylcholinesterase after inactivation

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CHART 1. Catechol-Containing Siderophores

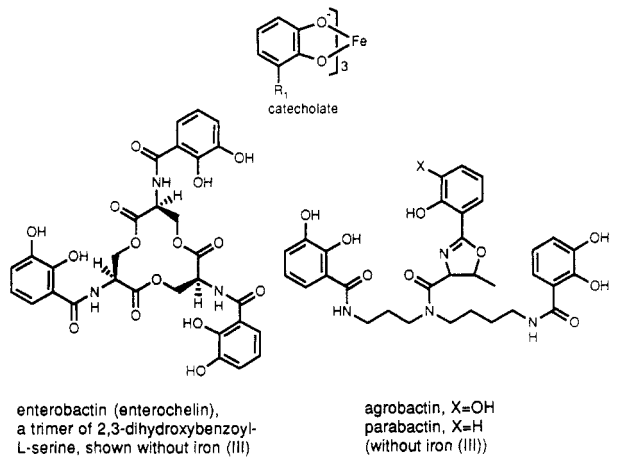
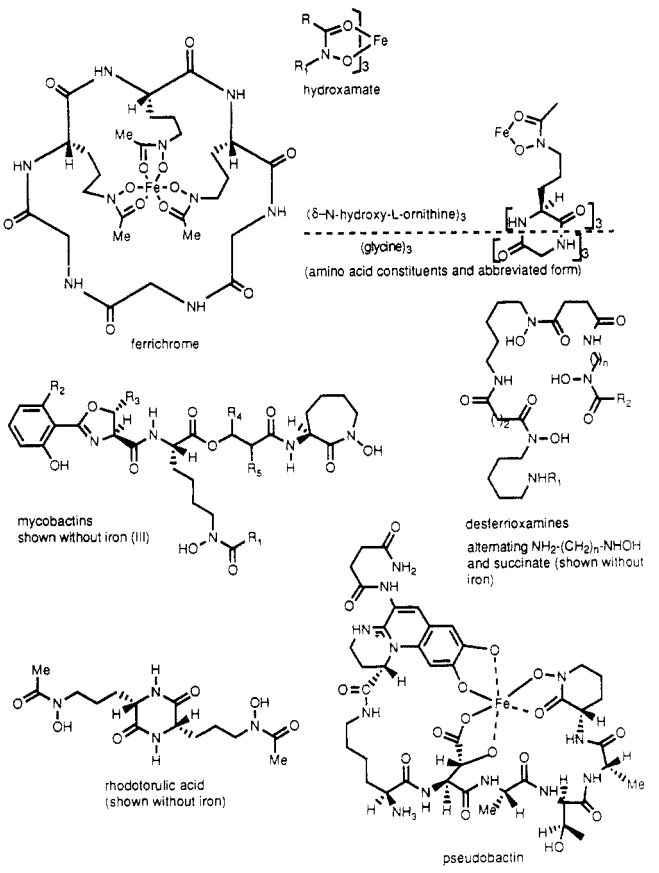


CHART 2. Hydroxamate-Containing Siderophores



by the potent nerve poison sarin.[25] While much needs to be learned about the application of hydroxamic acid derivatives to these and related biological and biochemical problems, current interest has emphasized the study of hydroxamic acids, and especially siderophores, for their metal ion transport properties and possible use for the development of either broad-spectrum or species-selective antibiotics.[22]

The potential use of siderophores and analogues for the treatment of iron metabolism disorders was recognized early on and has received considerable attention. One natural siderophore, desferrioxamine, is still the drug of choice for the treatment of iron overload associated with the transfusional treatment of β-thalassemia or Cooley's anemia.[26] Patients with this common genetic disease produce inadequate amounts of the β-chain of hemoglobin and therefore require frequent blood transfusions. Extensive iron overload induced by the multiple transfusions causes deposition of the metal in the heart, liver, endocrine glands, and other organs. The ultimate result is organic malfunction and early death. Since desferrioxamine must be administered by injection and large amounts are needed to mobilize iron faster than it is accumulated, the search for more effective drug candidates is extremely important until a practical genetic solution is found. Siderophores and their analogues are logical models from which effective iron chelating drugs might be developed. The same or similarly tailored compounds are potentially useful for removal of other toxic metals, including plutonium,[14c,27] from biological systems. Siderophore-producing microbes have also been found to promote crop growth by either inhibiting root colonization of pathogenic organisms by an iron starvation mechanism or providing the plant with a solubilized form of iron.[28]

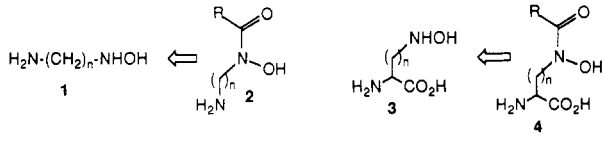
Several approaches have been suggested for the development of antibiotics based on siderophores and their analogues.[22] Very effective chelators may deprive pathogenic microbes of iron essential for growth. This could be accomplished by competitive chelation of iron or by blocking the iron–siderophore receptor site with a nonfunctional siderophore analogue or the natural siderophore substrate bound to another metabolically less useful metal.[29] Alternatively, direct attachment of lethal compounds to siderophores might lead to new forms of drug delivery that utilize the pathogenic organisms own iron transport system. As another mode of action, iron-mediated generation of hydroxyl radicals may induce microbial DNA damage.[22a]

Exploitation of the tremendous therapeutic potential of siderophores, their analogues, and derivatives requires access to sufficient quantities of materials for preliminary and long-term study. As indicated earlier, in iron-deficient media microbes can often be encouraged to produce significant quantities of their natural siderophores. Molecular cloning may eventually allow production of nearly unlimited supplies of siderophores.[3] In fact, the outer membrane receptor (the *tonA* protein) for ferrichromes,[30] the enterobactin gene complex of *E. coli*,[31] and other systems have already been cloned. Still, full exploration of desired structure–activity relationships and the development of novel analogues with favorable therapeutic properties that do not also promote growth of other pathogens require chemical modification (semisynthesis) of the natural siderophores or rational total synthesis.

II. Synthetic Design and Synthesis of Siderophore Components

At first glance, the impressively complex appearing structures of most siderophores of interest would suggest that total synthesis might not be a viable approach for exploring their therapeutic potential. Closer inspection reveals that, in many instances, nature has been quite conservative in the design of siderophores. As indicated earlier, most rely on the use of catechols and hydroxamic acids as the iron chelating ligands. Even within these classes minimal structural variation is observed. Catechol-containing siderophores are often constructed from simple dihydroxybenzoyl derivatives (i.e., enterobactin, agrobactin, and parabactin) or

SCHEME 1

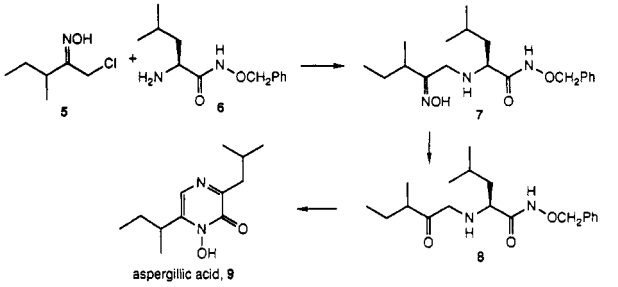


modified versions of dihydroxyphenylalanine (DOPA, as in pseudobactin). The hydroxamate-based siderophores usually derive the iron chelating ligands **2** and **4** (Scheme 1) from 1-amino-ω-(hydroxyamino)alkanes (**1**; also see desferrioxamine) or ω-*N*-hydroxy amino acids (**3**; also see ferrichrome). Most often, the ligands are connected through ester or amide linkages that, by current synthetic methodology, should be routinely constructed. Thus, synthetic approaches to the synthesis of the apparently complex hydroxamate-based siderophores was limited only by the need for efficient syntheses of the corresponding hydroxamic acid ligands themselves.

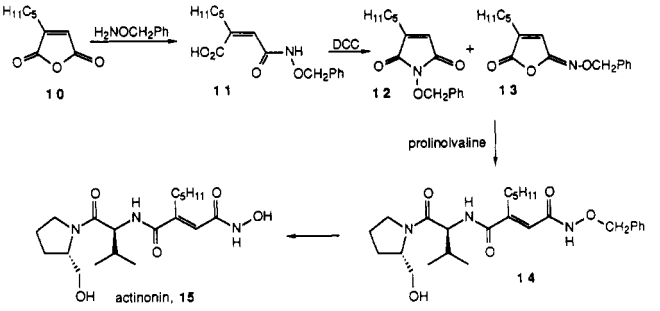
Many hydroxamic acids can be easily prepared by reaction of esters or active esters with hydroxylamine itself or various N- or O-alkylated derivatives. Unfortunately, most of the natural hydroxamate components of siderophores are secondary (probably to avoid nucleophilic reactions of primary hydroxamates or oxidation to toxic acyl nitroso compounds under physiological conditions). Moreover, the secondary hydroxamates are designed such that the *N*-alkyl substituent is involved in the linkage of the hydroxamate groups to the core of the siderophore. This arrangement has impeded the synthesis of many siderophores by requiring first the synthesis of unusual or sometimes complex hydroxylamine derivatives, including the ω-*N*-hydroxy α-amino acids.

Designing chemical syntheses of natural products and their components is often greatly facilitated by information obtained about the corresponding biosynthetic pathways. The key question concerning the biosynthesis of the hydroxamate-based siderophores has been the mechanism and timing involved for introduction of the hydroxyl group of the hydroxamic acid. Detailed studies have shown that α,ω-diamino acids are first oxidized to the ω-hydroxylamine (forms of **1** or **3**) by a process requiring molecular oxygen and then acylated by an acyl coenzyme A to give the hydroxamic acid.[32] This impressive direct molecular oxygen mediated amine oxidation has no equal in chemical synthesis. Most direct oxidations of primary amines to hydroxylamines have utilized peroxide derivatives, including dibenzoyl peroxide,[33] *tert*-butyl peroxybenzoate,[34] pivaloyl peroxide,[35] arylsulfonyl peroxides,[36] and bis(diphenylphosphinyl) peroxide.[37] Many of these oxidations are low yielding, primarily because of competitive acylation of the amines before oxidation, or are not compatible with other sensitive substituents. Amide oxidation has also been reported.[38] Most other attempts to chemically oxidize primary amines to hydroxylamines result in overoxidation. Consequently, syntheses of hydroxamate siderophore components have usually required introduction of preoxidized nitrogen in the form of other hydroxylamine derivatives, nitrones, or nitro compounds. Several of these methods will be discussed followed by a summary emphasizing the utility of direct incorporation of preformed hydroxylamine derivatives.

SCHEME 2



SCHEME 3



A. Simple Hydroxamates

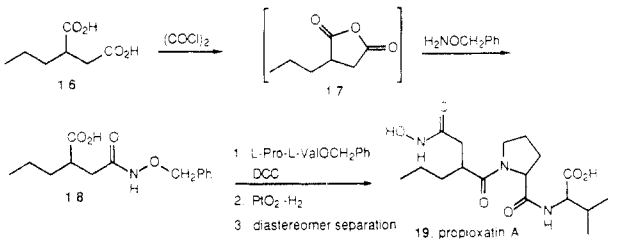
Not all hydroxamate-based siderophores contain three hydroxamic acids within the same molecule. In an early review,[15] Maehr subclassified the hydroxamates into mono-, di-, and trihydroxamic acids. Because of their less complex appearance, early synthetic efforts focused on the synthesis of some of the known monohydroxamic acids. The syntheses of aspergillic acid and actinonin are representative.

Aspergillic acid (**9**), an N-hydroxylated pyrazine isolated from *Aspergillus flavus*,[39] was one of the first naturally occurring hydroxamic acids isolated. Its synthesis, first reported in 1966,[40] relied on the direct preparation of the required hydroxamic acid from leucine (Scheme 2). *N*-Leucyl-*O*-benzylhydroxylamine (**6**) was alkylated with 1-chloro-2-(hydroxyimino)-3-methylpentane (**5**) to give the extended oxime **7**. Hydrolysis of the oxime to the ketone **8** followed by reductive debenzylation and treatment with ammonia induced an intramolecular condensation which, followed by air oxidation, provided racemic aspergillic acid (**9**). Several natural derivatives of aspergillic acid have subsequently been isolated and synthesized.

Actinonin (**15**), one of the few natural N-unsubstituted hydroxamic acids, is a rather broad-spectrum antibiotic isolated from *Streptomyces roseopallidus*. Like aspergillic acid, the synthesis of actinonin utilized an *O*-benzylhydroxylamine derivative to introduce the hydroxamic acid (Scheme 3).[41] Thus, reaction of *O*-benzylhydroxylamine with anhydride **10** gave hydroxamate **11**, which upon treatment with dicyclohexylcarbodiimide (DCC) gave a mixture of the N- and O-acylated products **12** and **13**. Treatment of this mixture with prolinolvaline followed by hydrogenation produced actinonin.

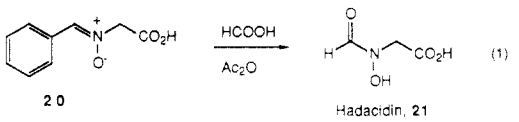
Propioxatin A (**19**),[42] a potent enkephalinase B inhibitor produced by *Kitasatosporia setae* SANK 60684, is another N-unsubstituted hydroxamic acid structurally related to actinonin. The synthesis of propioxatin A also utilized *O*-benzylhydroxylamine and closely re-

SCHEME 4



sembled the synthesis of actinonin (Scheme 4).

The simplest of the more common secondary hydroxamic acid derived siderophores is hadacidin (*N*-hydroxy-*N*-formylglycine (**21**), eq 1). This simple compound has antitumor activity and retards plant growth.[15] It has been formed by formylation of *N*-hydroxyglycine[43] and from the benzaldehyde-derived nitrone **20**.[44]



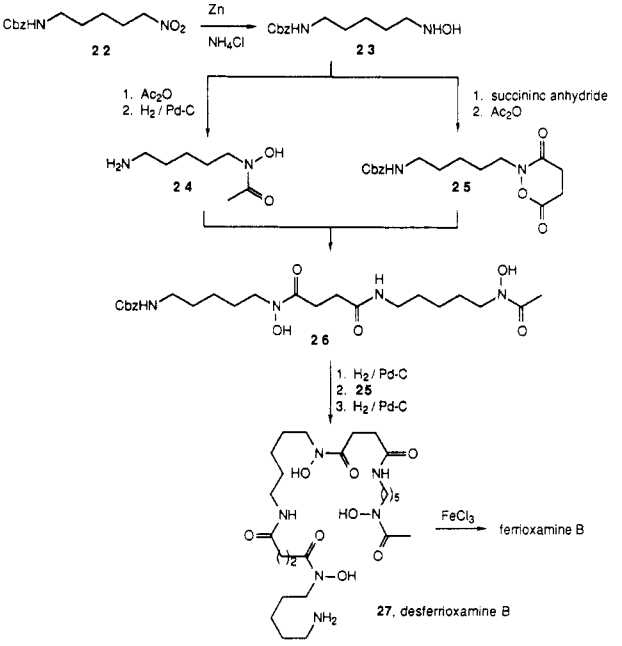
B. Siderophores Containing 1-Amino-ω-(hydroxyamino)alkanes

Synthesis of the dozens of more common and apparently complex siderophores required the development of efficient methods for their constituent 1-amino-ω-(hydroxyamino)alkanes or ω-*N*-hydroxyamino α-amino acids since simple acylations would provide the hydroxamate iron ligands (**1** to **2** and **3** to **4**, Scheme 1). Early approaches to the synthesis of these hydroxylamines relied on generation and reduction of the corresponding nitro compounds, generation and hydrolysis of aldehyde-derived nitrones, generation and hydrolysis of oxaziridines, and alkylation of *N*-alkoxysulfonamides. Several applications of these processes are summarized in the following schemes and discussions.

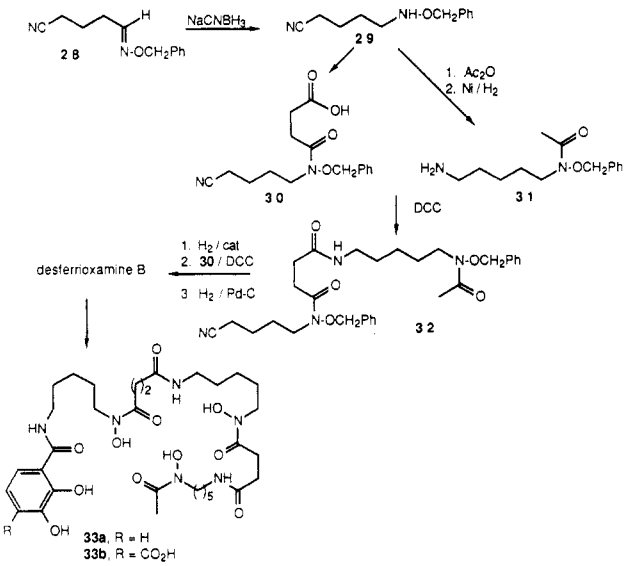
The 1-amino-5-(hydroxyamino)pentane component (**23**) of ferrioxamine B (the iron complex of desferrioxamine B) was first prepared[45] by reduction of the corresponding nitro derivative **22** with zinc (Scheme 5). Acylation followed by hydrogenolytic removal of the Cbz protecting group produced the parent hydroxamic acid **24**. Reaction of the same hydroxylamine **23** with succinic anhydride provided **25**, which served the dual role of N-acylating the hydroxylamine nitrogen with the required succinate and activating the other succinate carbonyl group. Thus, direct reaction of amine **24** and active ester **25** resulted in formation of the dihydroxamate **26**. Deprotection of **26** followed by a second reaction with **25** and subsequent removal of the Cbz group and addition of ferric chloride gave ferrioxamine B.

A more efficient synthesis of desferrioxamine B has been recently reported by Bergeron (Scheme 6).[46] In this synthesis, the amine backbone was constructed by condensing 4-cyanobutanal with *O*-benzylhydroxylamine and reduction of the resulting oxime **28** to the protected hydroxylamine **29** with sodium cyanoborohydride. Acetylation of **29** with acetic anhydride followed by hydrogenation of the nitrile provided the required 1-amino-5-(*N*-acetyl-*N*-(benzyloxy)amino)pentane (**31**). Alternatively, reaction of **29** with succinic

SCHEME 5



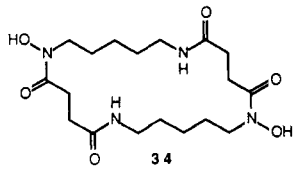
SCHEME 6



anhydride gave the acid amide **30**. DCC-mediated condensation of succinate **30** and amine **31** produced dihydroxamate **32** in 88% yield. Hydrogenation of the nitrile of **32** to the amino group was followed by a second condensation with succinate **30** to give the corresponding trihydroxamate. Finally, reduction of the terminal nitrile and reductive removal of all of the benzyl protecting groups to provide desferrioxamine B were accomplished with hydrogen and 10% Pd–C in 0.1 N HCl in methanol. This promises to be a versatile route for the synthesis of a number of important analogues of desferrioxamine B. An indication of the types of analogues that may be of interest was provided by Bergeron in the same paper in which he alluded to the extension of the ferrioxamine chelation system by continuing the sequential synthetic process to include additional chelation sites on a single molecule. Related concepts were also described by Raymond.[47] He found that acylation of the N-terminus of ferrioxamine B with dihydroxybenzoic acid derivatives, followed by deprotection, gave the catechoyl-modified siderophores **33**.

Unlike desferrioxamine B itself, **33b** was able to kinetically remove transferrin-bound iron.

The hydroxylamine and succinoyl components of ferrioxamine are present in a number of other natural siderophores. One of these, the recently discovered bishydroxamate bisucaberine (**34**), isolated from the



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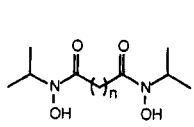
marine bacteria *Alteromonas haloplanktis* SB-1123, effectively sensitizes tumor cells to macrophage-mediated cytolysis.[48] Although no synthesis of bisucaberine has been reported,[49] the methods used for the synthesis of ferrioxamine should be directly applicable.

A number of analogues of the ω-(hydroxyamino)-1-aminoalkane-based siderophores have also been synthesized. Interestingly, the crystal structure of aliphatic dimeric *N*-isopropylhydroxamic acids **35** revealed that



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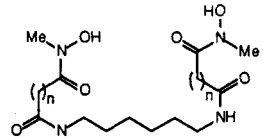


hexamates

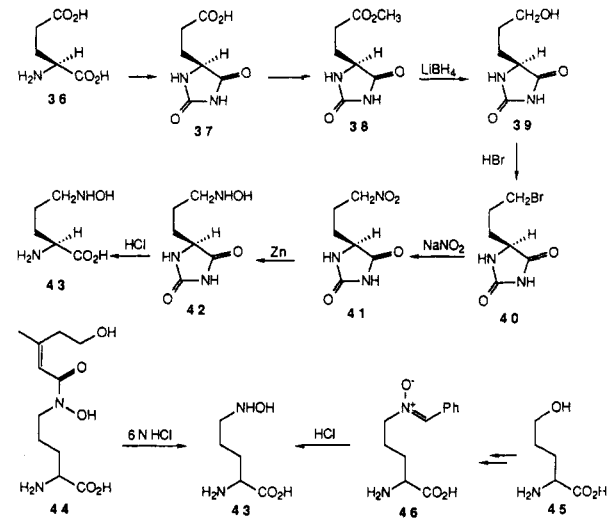
in the absence of metal ion coordination, the hydroxamic acid groups existed in the trans rather than the usual cis conformation.[50] Another series of synthetic bishydroxamates called hexamates have been reported.[51] These molecules consist of a 1,6-hexanediamine backbone acylated at both ends with malonyl- (n = 1), succinoyl- (n = 2), or glutaroyl-*N*-methylhydroxamates. None of the hexamates were able to stimulate the growth of *E. coli* or *Vibrio cholerae* and several mutants, suggesting a preference for hexadentate or amino acid based ligands for more effective iron transport.

C. More Complex Amino Acid Containing Hydroxamate-Based Siderophores and Analogues

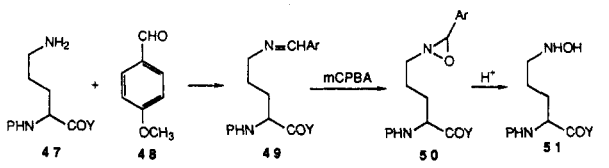
The most common hydroxamic acid components of siderophores are derivatives of δ-*N*-hydroxyornithine. This amino acid is the principal component of fusarinine,[52] fusarinine B and C,[53] rhodotorulic acid,[54] dimerumic acid,[55] coprogen,[56] ferribactin,[57] ferrichrome,[58,59] ferrichrome A,[60,61] ferrichrome C,[62] neurosporin,[63] albomycins,[64,65] ferrichrochin,[66] ferrichrysin,[58,67] ferrirubin,[68] ferrirhodin,[68] pseudobactin,[69] pseudobactin A,[70] the pyoverdines,[71] and other siderophores. Because of its prevalence, synthesis of δ-*N*-hydroxyornithine has received considerable attention.

As in the synthesis of desferrioxamine, a nitro group was used as the hydroxylamine precursor in the first synthesis of δ-*N*-hydroxy-L-ornithine reported by Neilands (Scheme 7).[72] This synthesis, though low yielding, served as a model for many related syntheses. Neilands recognized that L-glutamic acid (**36**) contained the appropriate carbon framework for the synthesis of δ-*N*-hydroxy-L-ornithine. He first protected the α-amino and carboxyl groups as the hydantoin **37** and then esterified the γ-carboxyl to produce ester **38**. Reduction

SCHEME 7



SCHEME 8

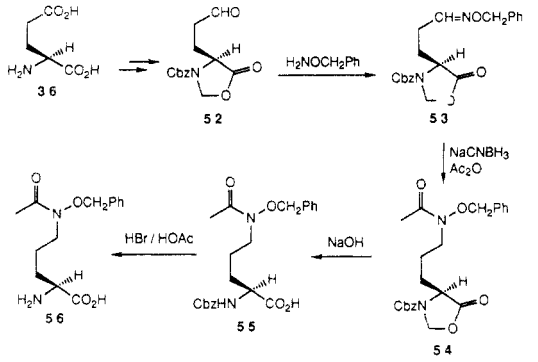


with lithium borohydride gave alcohol **39**, which upon treatment with HBr gave the corresponding bromide **40**. The required nitrogen was introduced by reaction with sodium nitrite to give **41**. Zinc reduction provided the hydroxylamine **42**, which was hydrolyzed with 6 N HCl to give the synthetic sample of δ-*N*-hydroxy-L-ornithine (**43**) in about 7% overall yield. Prior to that, the only source of this unusual amino acid was hydrolysis of siderophores, such as *cis*-fusarinine (**44**),[73] which contained it. Keller-Schierlein subsequently reported an alternate route to the δ-nitro-L-norvaline precursor of δ-*N*-hydroxy-L-ornithine.[74] In his review, Maehr[15,75] reported an improved synthesis of δ-*N*-hydroxyornithine from 5-hydroxy-2-aminovaleric acid (**45**), which, in turn, can be prepared by reduction of the γ-carboxyl group of glutamic acid. The nitrone **46** was the key intermediate. Hydrolysis of **46** provided the desired amino acid **43** directly.

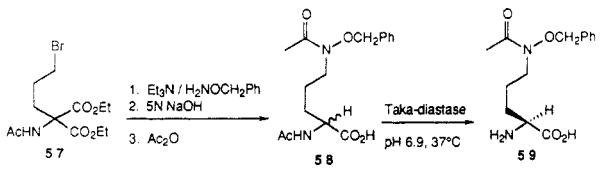
The first syntheses of ε-*N*-hydroxy-L-lysine also relied on the use of a nitro group[65] and nitrone[76] as the hydroxlyamine precursors. This amino acid, a homologue of δ-*N*-hydroxy-L-ornithine, is the functional constituent of aerobactin[77] and the mycobactins.[11a]

Ornithine is also a logical choice of starting material for the synthesis of δ-*N*-hydroxyornithine. As indicated, direct chemical oxidation of the δ-amino group is problematic. However, Keller-Schierlein's group was able to perform the oxidation indirectly (Scheme 8).[78] Reaction of α-amino- and carboxyl-protected ornithine or ornithinyl peptides with *p*-methoxybenzaldehyde formed the imine derivatives **49**. Epoxidation with *m*-chloroperbenzoic acid gave the oxaziridine **50**, which upon hydrolysis produced the desired *N*-hydroxyornithine derivatives **51**. This became a very attractive route to hydroxamate constituents of siderophores. However, the yields are often variable and the final hydrolysis often gives mixtures of products, including regeneration of the starting ornithine and aldehyde.

SCHEME 9



SCHEME 10

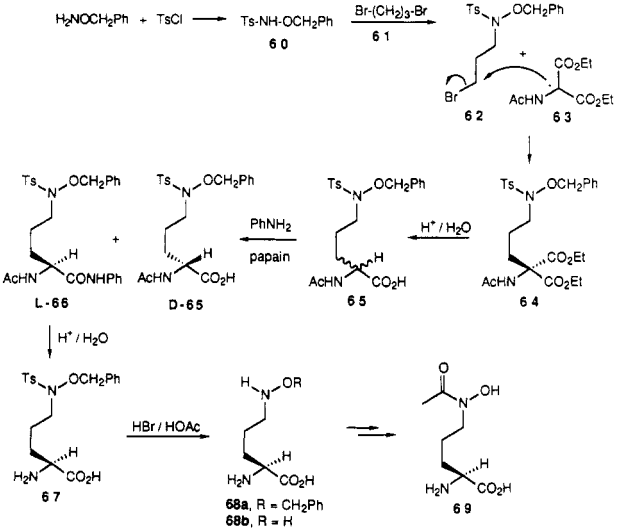


Several attempts have been made to synthesize ω-*N*-hydroxy amino acids by incorporating intact hydroxylamines and hydroxamic acids into preformed or existing amino acid frameworks. The keys to these processes have been to circumvent the ambident nucleophilcity of hydroxylamine and to incorporate appropriate functionality at the ω-terminus of the amino acids. In one approach (Scheme 9),[79] the hydroxylamino group was initially introduced by formation of an oxime **53** from *O*-benzylhydroxylamine and the glutamic acid derived aldehyde **52**. Subsequent reduction with sodium cyanoborohydride in the presence of acetic anhydride produced the O-protected hydroxamic acid **54** directly. Further deprotection of the amino acid proceeded normally to ultimately give δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine (**56**). Complete protection of the α-amino group of the amino acid was necessary to avoid intramolecular cyclization of the amino group onto the aldehyde as it was formed.[80] A very similar route, in which the α-amino group was protected as the diallyl derivative, was used by Benz[81] in his studies related to the synthesis and structure determination of albomycin.

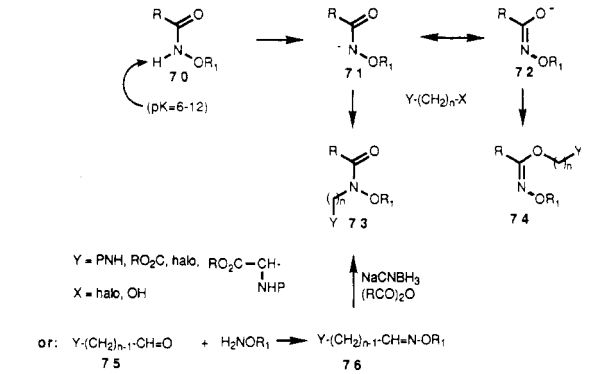
Fujii also utilized *O*-benzylhydroxylamine as the source of the hydroxamic acid (Scheme 10).[82] Alkylation of *O*-benzylhydroxylamine with bromide **57**, itself derived from diethyl acetamidomalonate by a standard alkylation, followed by saponification, decarboxylation, and acetylation produced the racemic hydroxamic acid **58**. Enzymatic resolution (Taka-diastase) gave the optically active amino acid **59**. Problems with poly-N-alkylation of the *O*-benzylhydroxylamine might be expected with this approach. However, enough material was prepared to enable a total synthesis of rhodotorulic acid to be accomplished.

Isowa recognized the need for further protection of the hydroxylamine precursors (Scheme 11).[83] His group found that *O*-benzyl-*N*-(*p*-tolylsulfonyl)-hydroxylamine (**60**) could be cleanly N alkylated. Thus, reaction of **60** with 1,3-dibromopropane (**61**) gave the substituted hydroxylamine **62**, which upon reaction with diethyl acetamidomalonate anion (**63**) followed by hydrolysis gave the racemic δ-*N*-hydroxyornithine derivative **65**. Enzymatic resolution of the corresponding

SCHEME 11



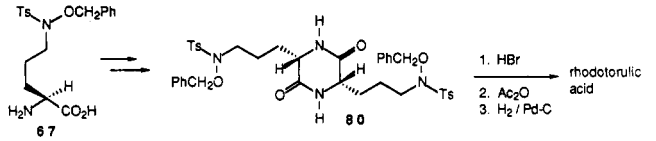
SCHEME 12



anilide with papain produced the L-amino acid L-**66**. Subsequent reactions produced δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine derivatives suitable for elaboration to the parent amino acid **69** and its dimer, the diketopiperazine rhodotorulic acid.

Direct N-alkylation of an intact hydroxamic acid conceptually provides the most efficient method for preparing a number of hydroxamate constituents of siderophores. The acidity and reactivity of the NH bond of O-protected hydroxamic acids are well documented, and N-alkylation has proven to be synthetically useful.[21] However, intermolecular alkylation of *O*-alkyl- or *O*-acylhydroxamic acids [**70**, R_1 = alkyl (pK ~ 9–12), R_1 = acyl (pK ~ 6–8)] often provides mixtures of N-alkylation and carbonyl O-alkylation products [hydroxamates (**73**) and hydroximates (**74**), Scheme 12].[81,84,85] Slight modification of the hydroxamate allows direct N-alkylation. Thus, replacement of the acyl group of the hydroxamate with an alkoxycarbonyl group (**70**, R = OR) promoted clean N-alkylation with a number of alkyl halides.[85,86] This approach has been used to prepare a number of *N*-alkylhydroxylamines and hydroxamic acids, including δ-*N*-hydroxy-L-ornithine and ε-*N*-hydroxy-L-lysine. This route and oxime formation followed by reduction and acylation (**75** → **76** → **73**) are now the most frequently used methods for the preparation of the hydroxamate constituents of siderophores. Applications of these methods to the total synthesis of a number of amino acid based siderophores and siderophore analogues will be described in the next section.

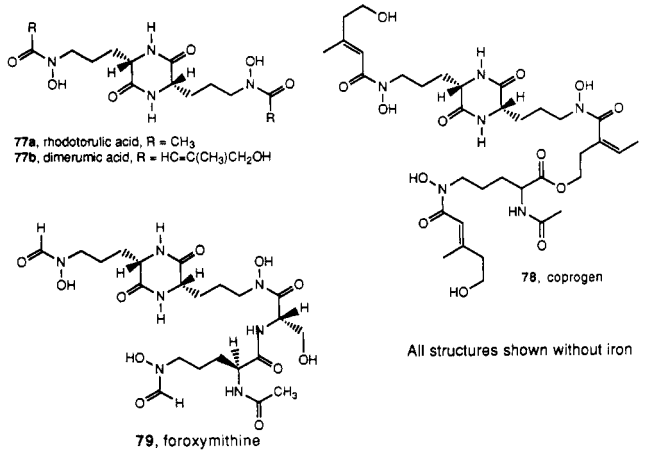
SCHEME 13



D. Total Syntheses of Hydroxamate Amino Acid Based Siderophores and Analogues

Many of the methods described for the syntheses of hydroxamic acids were developed with the intent of incorporating them into total syntheses of siderophores and therapeutically useful analogues. Several of these syntheses will be summarized in this section. Since syntheses of ferrioxamine B have already been described, emphasis will be on, but not limited to, the siderophores containing the amino acid based hydroxamic acids.

The δ-*N*-hydroxyornithine-containing siderophores ferrichrome and rhodotorulic acid have received the most synthetic attention. Rhodotorulic acid (**77a**) is a

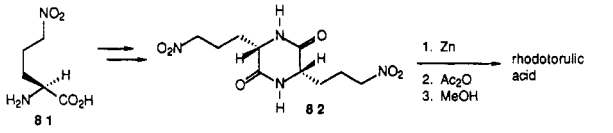


diketopiperazine derived from dimerization of two δ-*N*-hydroxy-L-ornithine residues.[55] Several natural analogues of rhodotorulic acid have also been found. Most of these, including dimerumic acid (**77b**),[58] differ only in the type of their δ-*N*-acyl substituents. Others, such as coprogen (**78**)[57] and foroxymithine (**79**),[24] contain more extensive δ-*N*-acyl groups that also incorporate additional iron chelating ligands. As a dihydroxamate, rhodotorulic acid is quadridentate and cannot effectively serve as a hexadentate ligand for ferric ion. Consequently, the rhodotorulate–iron complex must have a minimum of a 3:2 stoichiometry.[87] Despite this, early studies indicated that rhodotorulic acid was more effective than desferrioxamine in promoting iron excretion from iron-overloaded animal models. Unfortunately, the painful local reaction to the molecule when administered by injection diminished its use.[88]

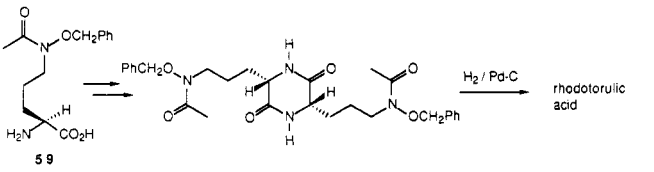
Isowa's group first synthesized rhodotorulic acid by converting the previously described δ-*N*-tosyl-δ-*N*-benzyloxy-L-ornithine (**67**) to diketopiperazine **80** using conventional peptide synthetic techniques. Detosylation with HBr, followed by δ-*N*-acetylation and catalytic hydrogenation to remove the benzyl protecting groups gave rhodotorulic acid (Scheme 13).[89]

Keller-Schierlein's group synthesized rhodotorulic acid by first assembling the diketopiperazine **82** from

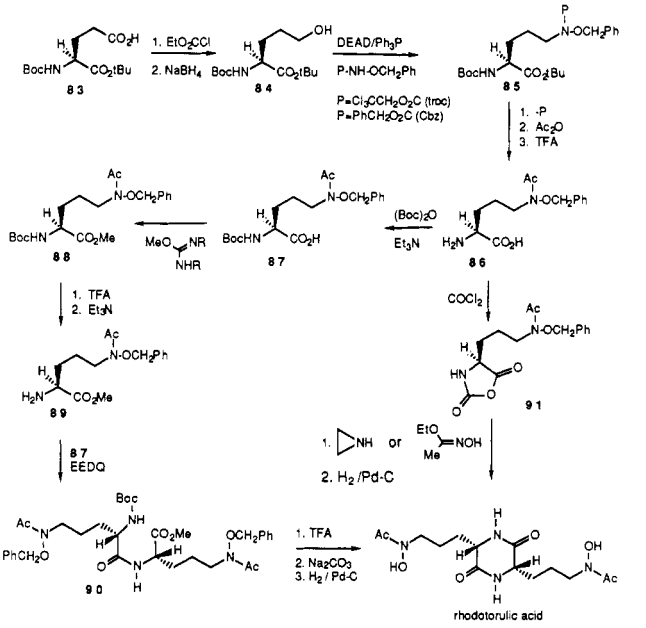
SCHEME 14



SCHEME 15



SCHEME 16

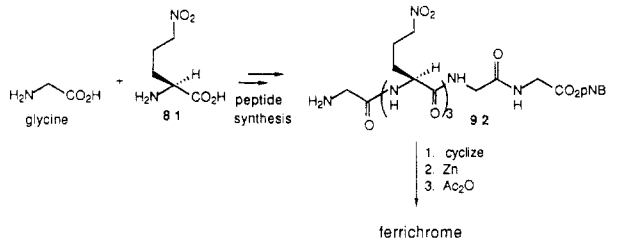


δ-nitro-L-norvaline (**81**), reducing the two nitro groups with zinc and acetylating the bis(hydroxylamine) (Scheme 14).[90] As expected, acetylation occurred at both the nitrogen and oxygen of the hydroxylamines. The *O*-acetyl groups were readily removed by solvolysis.

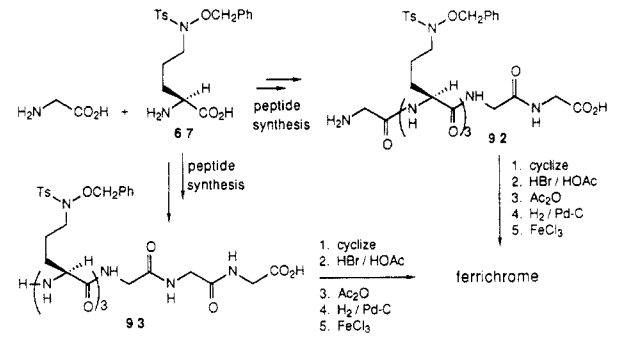
Fujii first prepared the monomeric *O*-benzyl-δ-*N*-hydroxy-δ-*N*-acetyl-L-ornithine (**59**) as previously described and then converted it to the diketopiperazine by stepwise peptide chemistry. Catalytic hydrogenation removed the benzyl groups without overreduction of the hydroxamate N–O bonds (Scheme 15).[82]

In a similar approach, Lee, Gerfen, and Miller[91] first prepared the α-N- and O-protected forms (**87** and **89**) respectively of δ-*N*-acetyl-δ-*N*-benzyloxy-L-ornithine, using the hydroxamate N-alkylation approach (Scheme 12). Thus, as shown in Scheme 16, protected glutamic acid **83** was converted to the mixed anhydride and reduced with sodium borohydride to give δ-hydroxy-L-norvaline derivative **84**, which upon treatment with protected *O*-benzylhydroxylamines under the Mitsunobu conditions produced the fully protected δ-*N*-hydroxy-L-ornithines **85**. Deprotections and δ-N-acetylation gave the free amino acid **86**. Protection of **86** with the Boc group gave **87**, which was also esterified with an *O*-methyldiisopropylisourea (R = *i*-Pr). Removal of the Boc group from the resulting ester **88** gave the free amine **89**. Conversion to the diketopiperazines was accomplished by both conventional peptide tech-

SCHEME 17



SCHEME 18



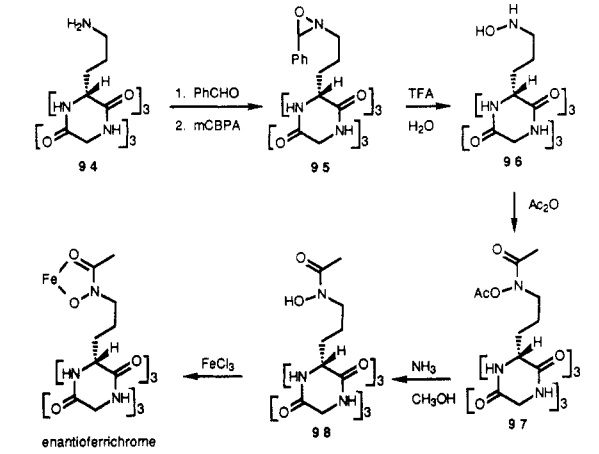
niques (**87** + **89** → **90** → rhodotorulic acid) and direct dimerization of the Leuch anhydride (**91**) derived from **86** and phosgene. Subsequent removal of the *O*-benzyl protecting groups gave rhodotorulic acid in good overall yields.

The first total synthesis of ferrichrome, a cyclic hexapeptide containing three sequential residues of δ-*N*-hydroxy-L-ornithine and three sequential residues of glycine, was described by Keller-Schierlein's group (Scheme 17).[92] On the basis of Neilands' and his own precedent, Keller-Schierlein used δ-nitro-L-norvaline as the precursor to the δ-*N*-hydroxy-L-ornithine residues. Using conventional peptide synthetic methods of the time, he constructed hexapeptide **92**. Glycine residues were judiciously placed at both the N- and C-termini of the peptide to avoid racemization during the *p*-dinitrophenyl ester mediated activation required for forming the corresponding cyclic hexapeptide. Subsequent reduction of all three of the nitro groups to the hydroxylamines with zinc followed by acetylation with acetic anhydride and extensive purification provided a 10.5% yield of ferrichrome from the trinitro cyclohexapeptide.

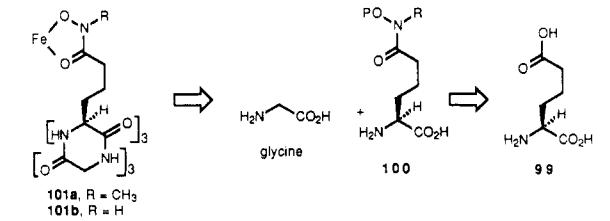
Using the previously described δ-*N*-tosyl-δ-*N*-benzyloxy-L-ornithine (**67**), Isowa's group prepared two different sequence hexapeptides **92** and **93** by conventional means. Both of these precursors were cyclized with DCC in dilute solution to the desired *cyclo*-triglycyl-tri-(δ-*N*-hydroxy-δ-*N*-benzyloxy-L-ornithinyl) peptide. Subsequent removal of the tosyl groups followed by N-acetylation and hydrogenolytic removal of the benzyl protecting groups and addition of ferric ion gave ferrichrome (Scheme 18).[93]

Keller-Schierlein's group later published a synthesis of enantioferrichrome (Scheme 19).[78] In this case, the cyclohexapeptide (D-Orn)$_3$-Gly$_3$ (**94**) was first prepared by conventional methods. Reaction of the δ-amino groups with benzaldehyde followed by oxidation with *m*-chloroperbenzoic acid gave the trioxaziridine **95**. Subsequent hydrolysis with aqueous trifluoroacetic acid produced the tri-(δ-*N*-hydroxy-D-ornithinyl) peptide **96**, which upon acetylation with acetic anhydride produced

SCHEME 19



SCHEME 20

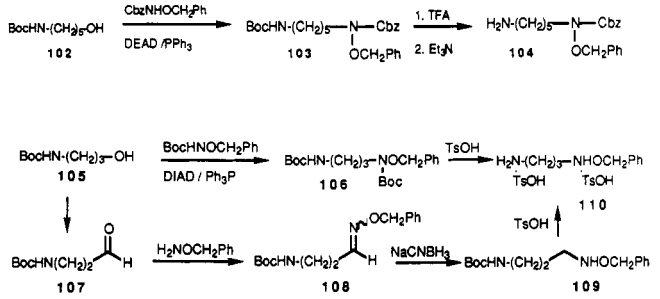


the N- and O-peracetylated peptide **97**. Simple treatment with methanolic ammonia removed the *O*-acetyl groups, and reaction with ferric chloride in methanol/water produced the enantioferrichrome. The optical rotation and CD curves were the opposite of natural ferrichrome. Quite interestingly, the ability of the enantioferrichrome to support microbial growth was comparable to that of ferrichrome itself.

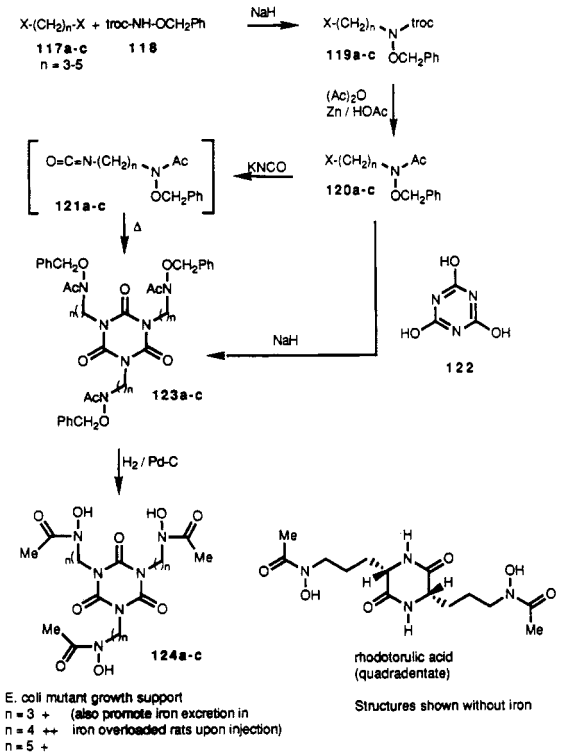
These syntheses have prompted the preparation of a number of analogues of ferrichrome containing δ-*N*-hydroxyornithine or its "retro" analogues **101**. Akiyama described the synthesis and properties of a number of sequence isomers of linear and cyclic hexapeptides containing three δ-*N*-hydroxy-L-ornithine and three glycine residues.[94] Unfortunately, no microbial transport studies or other biological tests were described in the original report. Olsen described the synthesis of two ferrichrome analogues [**101a** (R = CH_3) and **101b** (R = H)] in which the side-chain *N*-hydroxyl and acyl functions were transposed relative to natural ferrichrome; hence the term "retro ferrichrome" (Scheme 20).[95] The peptides were constructed from glycine and hydroxamic acids **100** derived from α-L-aminoadipic acid (**99**). The *N*-methyl compound **101a** was as effective as natural ferrichrome as a microbial transport agent, while the demethyl analogue **101b** was completely inactive.[96]

Many other non amino acid based "rhodotorulic acid and ferrichrome analogues" (di- and trihydroxamates) have also been prepared. The general hydroxamate alkylation and O-protected oxime reduction processes (Scheme 12) were especially useful for the syntheses of most of these. In many cases,[97] the constituent hydroxylamines and hydroxamic acids were prepared from α-amino ω-alcohols by first N-protection and then elaboration either by reaction with N- and O-diprotected hydroxylamines under Mitsunobu[98] conditions or by oxidation to the corresponding aldehydes, conversion to the oximes, and sodium cyanoborohydride reduction

SCHEME 21



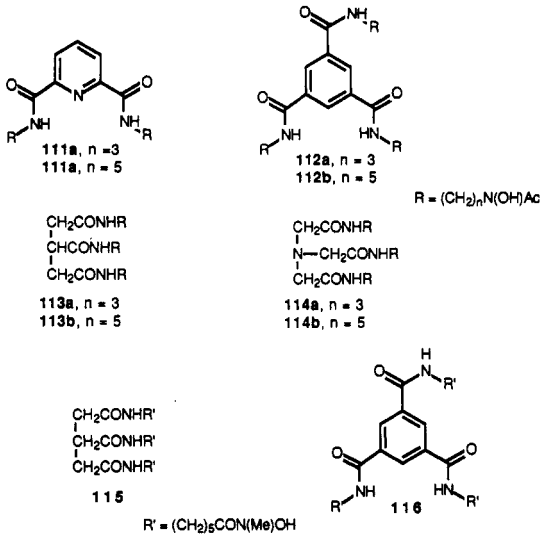
SCHEME 22



to the required *O*-benzylhydroxylamine. Specific examples are shown for the preparation of diaminopentane (**104**) and diaminopropane (**110**) derivatives (Scheme 21).

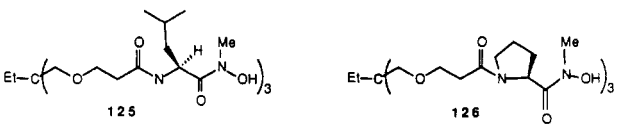
Reactions of these α-amino-ω-hydroxyamino derivatives (**104** and **110**) with a variety of multiacyl-containing substrates and minor elaboration provided a number of siderophore analogues (**111–116**).[97] Essentially all of these analogues, which were water soluble, behaved nutritionally like ferrichrome when tested for their ability to support microbial growth.

Extensions of the analogue studies led to the synthesis of several tris[(acetylhydroxyamino)alkyl] isocyanurates (**124a–c**, Scheme 22).[99] The biological activity of these compounds was quite dependent on the length of the alkyl chains. *E. coli* strains not mutated in any of the functions required for iron hydroxamate uptake were able to use all of these trihydroxamates for iron transport. Interestingly, tris[(acetylhydroxyamino)butyl] isocyanurate **124b** (n = 4) and to some extent its pentyl analogue **124c** (n = 5) were able to support the growth of *fhuB* mutants, which are unresponsive to other analogues and all natural siderophores tested. Compound **124b** was also found to stimulate excretion of iron from hypertransfused mice in a model study for the development of iron chelators

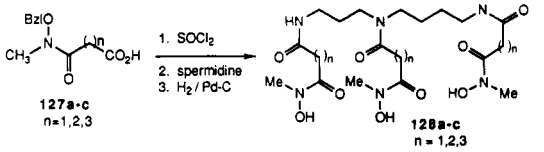


for the treatment of Cooley's anemia.

Chiral ferrichrome analogues **125** and **126** have also been recently synthesized by Shanzer's group.[100] Both of these compounds are tripodal structures assembled from L-amino acid hydroxamates linked by α-N-acylation to a tricarboxylate core. They are "retro" hydroxamates and like "retro ferrichrome", they function effectively as microbial growth promoters.[101]



Several trihydroxamate (**128a–c**, spermexatins) and mixed hydroxamate–catechol (**133a–c**, spermexatols) analogues of the catechol-containing spermidine-based siderophores agrobactin and parabactin have also been synthesized and screened for microbial transport ability (eq 2 and Scheme 23).[51] Although no known natural



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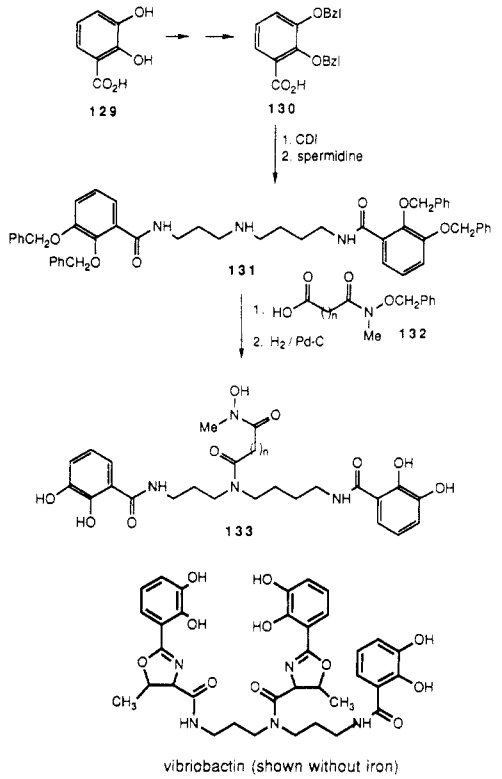
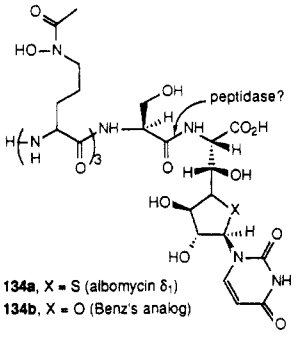
hydroxamate-containing siderophores are spermidine or norspermidine based, these synthetic compounds were found to substitute for the natural catechol-containing siderophore vibriobactin in iron transport in mutants of *Vibrio cholerae* and *E. coli*. Compound **128c** stimulated growth of mutant organisms lacking a functional catechol receptor for transport, implying that this spermidine-based iron chelator was recognized by other receptors, such as the hydroxamate ferrichrome receptor.

Albomycin (**134a**) is another natural siderophore that can be considered an analogue of ferrichrome. This very interesting compound has been "discovered" several times.[22] It was first isolated from *Streptomyces griseus* in 1947 and given the name grisein.[102] Four years later, the same compound was isolated from *Streptomyces subtropicus* and given the name albomycin.[103] Several years later, careful comparisons demonstrated that the two compounds were identical.[104] Extensive structural studies indicated that, like ferrichrome, the iron chelating component was based on

SCHEME 23



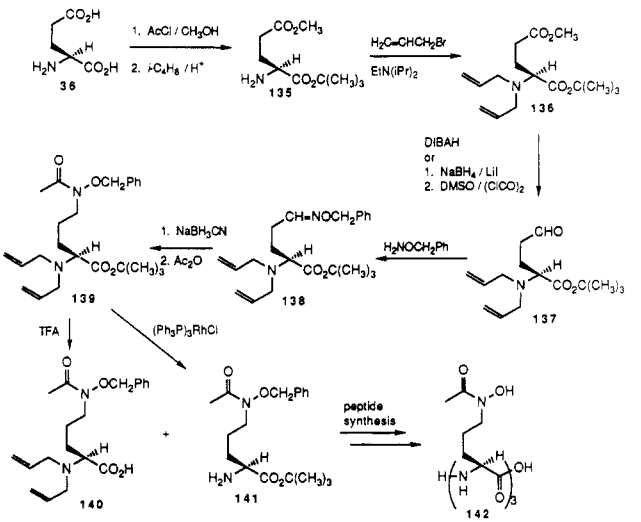
tri-(δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine).[105] In fact, until recently, albomycin was also thought to be a cyclic hexapeptide as is ferrichrome. However, in 1982, Benz provided conclusive evidence that the structure is comprised of a pentapeptide with a thionucleoside attached to the C-terminal amino acid residue (**134a**).[106] The extensive studies of albomycin have been well justified since it is a rather broad-spectrum antibiotic. A description of the implications of its biological activity will be deferred until the last section of this paper.



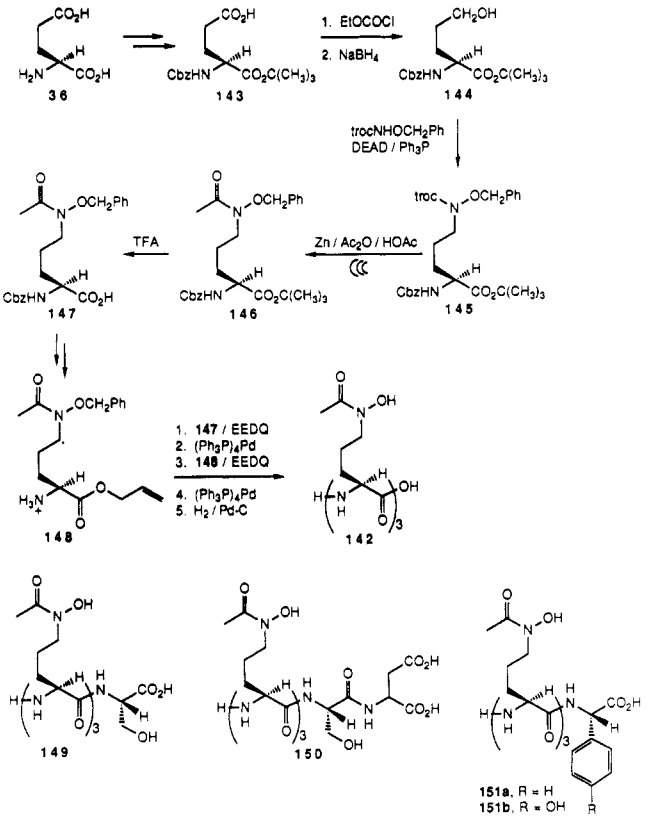
In an elegant sequence of papers, Benz also described chemical and enzymatic methods for the cleavage of the hydroxamate components from the seryl nucleoside[107] which also allowed him to determine that the absolute configuration of the constituent amino acids were of the natural L form.[108] He followed this with syntheses of δ-*N*-acetyl-δ-*N*-benzyloxy-L-ornithine derivatives **139–141** from glutamic acid,[81] the tripeptide **142** (tri-(δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine)),[109] and the oxygen analogue (**134b**) of the deferriform of δ_1-albomycin (Scheme 24).[110] Quite interestingly, the simple substitution of the oxygen for the sulfur resulted in loss of antibiotic activity.

An alternate synthesis of δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine from glutamic acid has also been elaborated

SCHEME 24



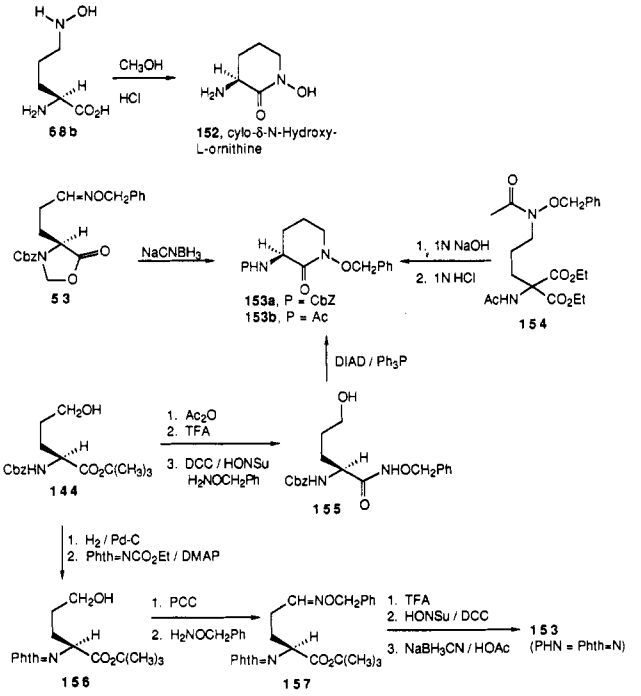
SCHEME 25



to the synthesis of the triornithinyl peptide **142** and extended tetra- and pentapeptides (**149, 150,** and **151,** Scheme 25).[111] In limited assays, several of these peptides stimulated microbial growth, but neither δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine itself nor the corresponding dipeptide displayed any growth-promoting activity. The failure of the diornithinyl peptide to serve as a microbial growth promoter is especially interesting since the related diketopiperazine (rhodotorulic acid) is a very effective siderophore. As described later, several of these peptides have also been attached to antibiotics for antimicrobial studies.

Other natural siderophores containing three residues of δ-*N*-hydroxy-L-ornithine include the coprogens (**78**) and foroxymithine (**79**). These interesting compounds resemble rhodotorulic acid by incorporating two of the

SCHEME 26

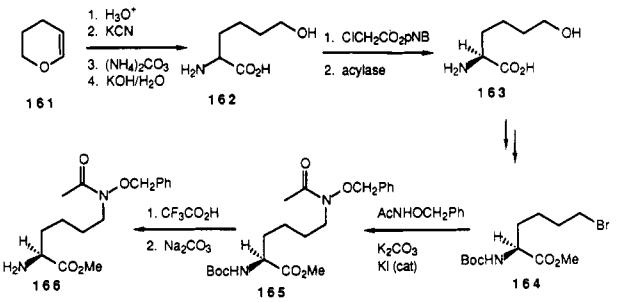


hydroxamates in a diketopiperazine, but, as described earlier, they also contain a third peripheral hydroxamate. Although foroxymithine is also an effective ACE (angiotensin converting enzyme) inhibitor, no efforts related to its synthesis have been reported.

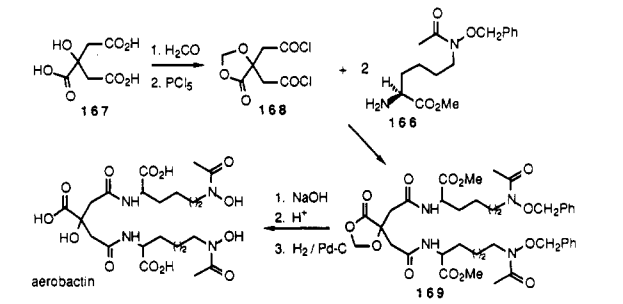
The pseudobactins are the most complex δ-*N*-hydroxyornithine-containing siderophores. The parent compound, pseudobactin,[12] itself contains the δ-*N*-hydroxyornithine in its cyclic form (**152**). A hydroxyl group and a carboxyl group from a β-hydroxyaspartic acid component and a catechol, apparently derived from DOPA, provide the additional iron chelation sites. Pseudobactin A[12] contains both *cyclo*-δ-*N*-hydroxy-L-ornithine and the more common linear form as well as the same catechol derivative as the iron ligands. Several syntheses of *cyclo*-δ-*N*-hydroxyornithine have been reported (Scheme 26). Simple treatment of δ-*N*-hydroxyornithine (**68b**) itself with methanolic HCl produces the cyclic form **152**.[112] Sodium cyanoborohydride reduction of the oxime precursor (**53**) in the absence of acetic anhydride also produced the doubly protected form of *cyclo*-δ-*N*-hydroxyornithine **153a**.[79] Fujii noted that saponification of the malonate **154** followed by acidification gave racemic *cyclo*-δ-*N*-hydroxyornithine **153b**. Alternate syntheses from glutamic acid derivative **144** have also been effective. Conversion of **144** to the α-hydroxamate **155** followed by a Mitsunobu cyclization produced **153a**. Protecting group exchange of **144** gave the phthalimide **156**, which upon oxidation and reaction with *O*-benzylhydroxylamine gave oxime **157**. α-Carboxyl deprotection and activation as the *N*-hydroxysuccinimide ester with subsequent oxime reduction allowed direct cyclization to the desired *cyclo*-δ-*N*-hydroxy-L-ornithine.[113]

Synthesis of the remaining peptide component of pseudobactin has also been completed.[114] Protected forms of the characteristically fluorescent dihydroxyquinoline portion (**158**) have been prepared from DOPA.[114] Slight modification of the reaction sequence[114] also provided the analogous tetracycle **159**,

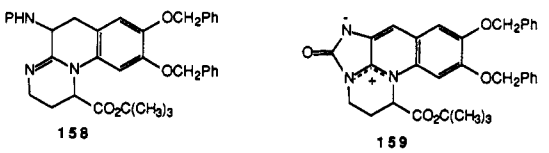
SCHEME 27



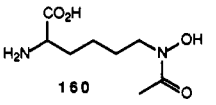
SCHEME 28



which is the protected fluorescent portion of azotobactin.[115]

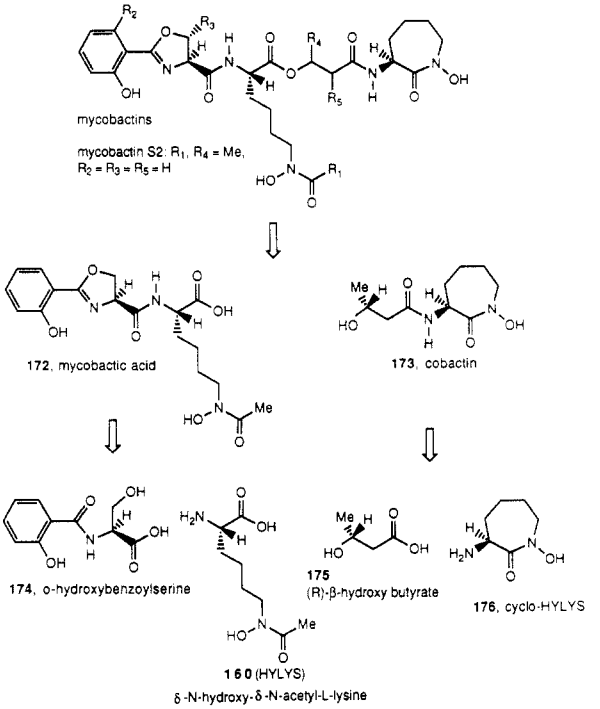


As indicated earlier, fewer of the known siderophores contain ε-*N*-acetyl-ε-*N*-hydroxylysine (**160**), the homologue of δ-*N*-acetyl-δ-*N*-hydroxyornithine. Both aerobactin and mycobactin, which contain ε-*N*-hydroxylysine, have been synthesized.[116,117] As with most siderophore syntheses, the key was the preparation of hydroxamate-containing amino acid constituents. In these cases, incorporation of the hydroxamate group was accomplished by directly alkylating *O*-benzyl acetohydroxamate (Scheme 27) rather than utilizing the previous nitro or oxime approaches. The amino acid backbone was ultimately derived from dihydropyran (**161**). Thus, hydrolysis of dihydropyran to the open-chain hydroxy aldehyde followed by a Strecker reaction gave the racemic amino acid **162**. The amino acid was resolved by chloroacetylation and subsequent hydrolysis with acylase. α-Amino and carboxyl protection followed by conversion of the ω-hydroxyl group to the bromide **164** provided the substrate for the hydroxamate alkylation. Treatment of **164** with *O*-benzyl acetohydroxamate in the presence of potassium carbonate and potassium iodide produced the N-alkylated hydroxamate **165** surprisingly well. Removal of the amino protecting group (Boc) produced free amine **166** suitable for elaboration to aerobactin.



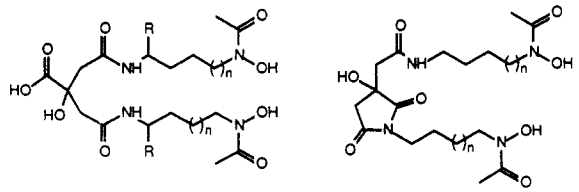
The two ε-*N*-hydroxylysine residues of aerobactin are attached to the terminal carboxyl groups of citric acid (**167**). To avoid reaction of **166** with citric acid's internal carboxyl, it and the hydroxyl group were protected by

SCHEME 29



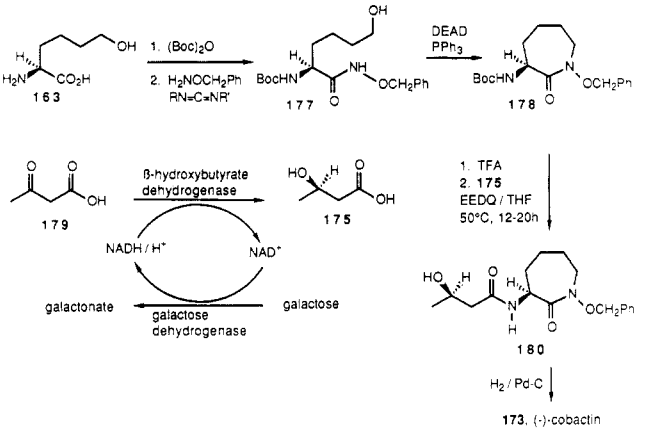
reaction with formaldehyde to form the anhydromethylenecitrate, which was then converted to the diacid chloride **168** (Scheme 28). Direct coupling of **168** with **166** produced the fully protected aerobactin **169**. Saponification and hydrogenation produced the first synthetic sample of aerobactin.

Although it has not yet been isolated from natural sources, the citric acid based δ-*N*-hydroxy-L-ornithine-containing siderophore awaitin A (**170a**) has been synthesized as a process directly related to that used for aerobactin (**170d**).[118] Similar approaches were used for the syntheses[119,120] of other citrate-based siderophores, schizokinen (**170e**),[121] schizokinen A (**171**), and arthrobactin (**170f**).[122] Formation of imide during the synthesis of schizokinen led to the assignment of the unknown structure of schizokinen A as **171**.

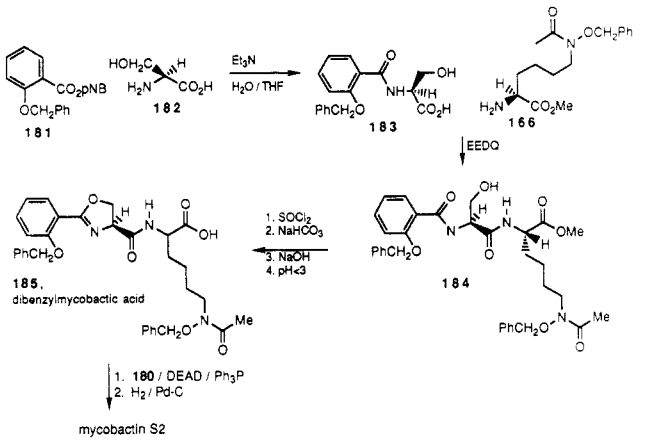


The mycobactins (Scheme 29, R groups are variable)[11a] contain two residues of ε-*N*-hydroxy-L-lysine, one in the usual linear form and one cyclic. This group of siderophores was isolated from *Mycobacterium tuberculoci*, the organism that causes tuberculosis. To simplify its synthesis, an analogue, mycobactin S2 (R_1 = R_4 = Me, R_2 = R_3 = R_5 = H), was chosen as the first target.[117] Since ε-*N*-hydroxy-L-lysine derivatives were made available during the synthesis of aerobactin, preparations of the *cyclo*-ε-*N*-hydroxy-L-lysine (cobactin (**173**)) and 2-(*o*-hydroxyphenyl)oxazoline (the third iron chelating group) residues were of primary concern.[112] Dissection of the molecule at the ester linkage allowed

SCHEME 30



SCHEME 31



attention to be focused on the synthesis of the known mycobactin hydrolysis products, mycobactic acid (**172**) and cobactin (**173**). Each of these fragments could be broken down to two additional components (Scheme 29).

The synthesis of cobactin required the preparation of *cyclo*-ε-*N*-hydroxylysine (**176**) and D-(*R*)-β-hydroxybutyric acid (**175**). As shown in Scheme 30, the cyclic hydroxamic acid was prepared from the alcohol **163** used previously for the synthesis of the linear form of ε-*N*-hydroxy-L-lysine. Thus, the α-amino group of **163** was first protected with a Boc group. The carboxyl group was then coupled with *O*-benzylhydroxylamine to give hydroxamate **177**. An intramolecular Mitsunobu reaction gave the seven-membered-ring cyclic hydroxamic acid plus considerable amounts of the corresponding carbonyl O-alkylated (hydroximate) products. The β-hydroxybutyrate was prepared by enzymatic reduction of lithio acetoacetate with β-hydroxybutyrate dehydrogenase.[123] Since this enzyme requires NADH for the reduction, the reaction was coupled with the galactose dehydrogenase mediated conversion of galactose to galactonate. Thus, the NADH was recycled and the ultimate reducing agent was galactose. Removal of the Boc group from **178** followed by EEDQ-mediated coupling of the β-hydroxybutyrate (**175**) and the α-amino group of resulting *O*-benzyl-protected *cyclo*-ε-*N*-hydroxy-L-lysine generated **180**, and subsequent hydrogenation produced (−)-cobactin T (**173**).

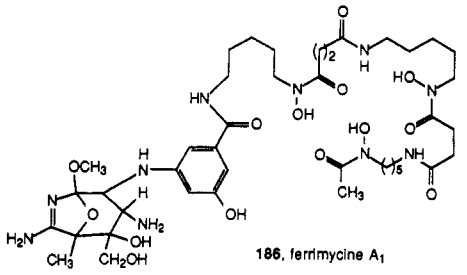
The synthesis of the mycobactic acid fragment was initiated by coupling 2-benzyloxy benzoate active ester **181** with L-serine to give **183** (Scheme 31). Reaction

of 183 with 166, the ε-*N*-hydroxy-L-lysine derivative used in the aerobactin synthesis, in the presence of EEDQ produced peptide 184. Cyclization to the desired oxazoline was accomplished by reaction with thionyl chloride under carefully controlled conditions. Saponification generated dibenzylmycobactic acid S2 (185). Interestingly, attempts to first prepare the oxazoline from 183 and then couple it to amino acid derivative 166 failed. A Mitsunobu-mediated coupling of protected mycobactic acid and (−)-cobactin fragments 185 and 180, respectively, resulted in formation of the final ester linkage with the desired and required inversion at the carbinol center of mycobactin S2. Hydrogenation removed all of the benzyl groups to complete the synthesis of mycobactin S2.

In summary, it appears that the fundamentally simple reactions of N-alkylation of hydroxamic acid derivatives or oxime formation, followed by reduction and acylation, provide direct and versatile access to important iron-binding constituents. Commonly employed protecting groups and routine coupling reactions now allow these constituents to be combined to chemically prepare many of the known siderophores and analogues. These studies have evolved so well that attention can now be shifted to specific therapeutic applications of synthetic siderophores and their analogues.

III. Siderophores and Siderophore Derivatives as Antibiotics

An excellent review on iron-containing antibiotics has been published by Neilands and Valenta.[22a] The review focuses on natural siderophores with antimicrobial activity. Thus, this rather brief section will review only attempts to couple siderophores or analogues with antimicrobial agents to take advantage of iron transport mechanisms for antibiotic delivery. Albomycin and the ferrioxamine B derivative ferrimycin A_1 (186)[22a,124] are

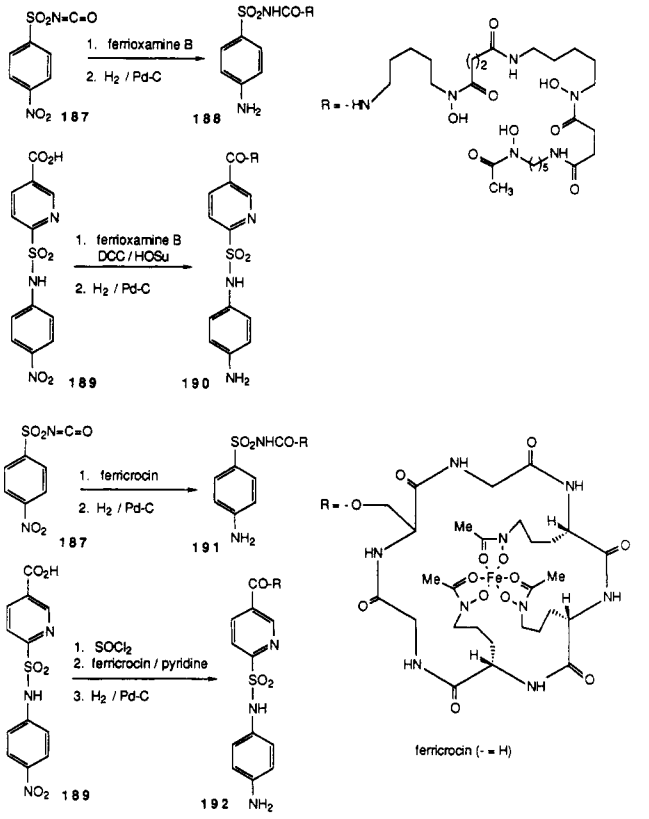


186, ferrimycine A_1

natural examples of the use of an iron transport system to deliver a toxic substance to a microbe. Evidence now indicates that albomycin is actively carried into microbial cells by normal iron transport processes and, once in the cells, the toxic thioribosyl moiety is enzymatically released, perhaps by a peptidase-mediated cleavage.[125] Again, it is worth recalling that Benz's oxygen analogue of the thioribosyl group was apparently devoid of antimicrobial activity.

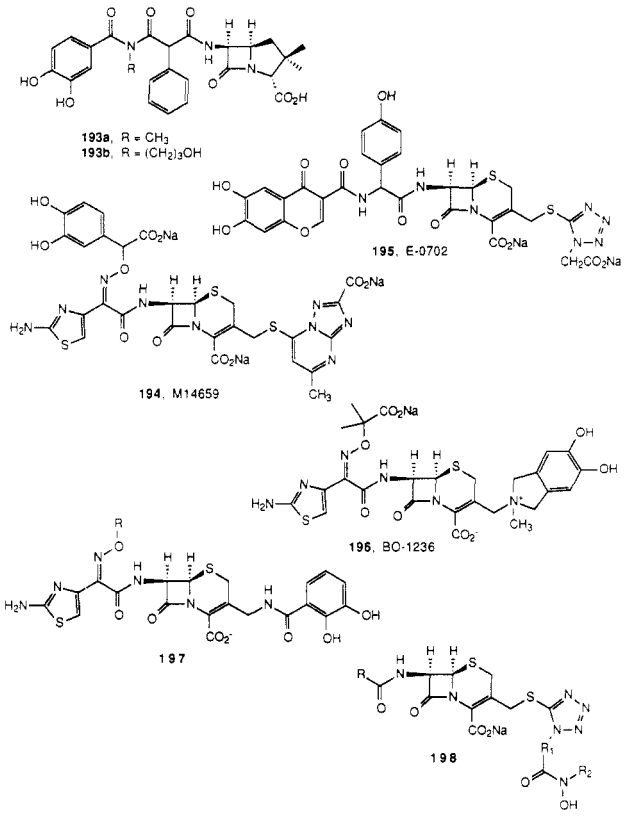
Attempts to mimic albomycin's antimicrobial activity with other less labile siderophore–antimicrobial agent conjugates have been of considerable interest. The earliest tests of this possibility utilized a semisynthetic approach in which various sulfonamides were covalently bound to ferrioxamine B and ferricrocin to produce the adducts 188, 190, 191, and 192 (Scheme 32).[126] Ferrioxamine B contains a terminal amino group to which

SCHEME 32



the sulfonamides were attached by reaction with isocyanate 187 or the *N*-hydroxysuccinimide derived from 189. Ferricrocin differs from ferrichrome only by the replacement of the central glycine residue with a serine. The primary hydroxyl group of the serine residue provided a convenient point for attaching the sulfonamides. Of the four compounds made, only the two sulfanilamidonicotinic acid derivatives 190 and 192 displayed any antimicrobial activity and it was limited to *Staphylococuss aureus*.

Recently, many groups have attempted to attach catechol or hydroxamate groups to β-lactam antibiotics with the anticipation that the corresponding iron complexes may be carried into microbial cells by iron transport mechanisms. This approach has met with considerable success. Ureidopenicillins (193a,b) having a terminal catechol moiety were synthesized and found to have 30–60-fold increase in activity relative to piperacillin against various microbes, including *Pseudomonas aeruginosa* strains, which are generally less sensitive to β-lactam antibiotics.[127,128] Related catechol derivatives of ureidocephalosporins and ureidocephamycins also displayed enhanced activity against *Pseudomonas aeruginosa*.[129] It was speculated that all of these compounds owed their enhanced antimicrobial activity to their ability to bind iron and be actively carried into cells by the iron transport mechanisms. Similarly, the antimicrobial activity of the catechol-containing cephalosporin M14659 (194) was found to be enhanced in low-iron environments, suggesting that it may be assimilated by microbial iron transport systems.[130] E-0702 (195) is a new semisynthetic iron chelating cephalosporin derivative which has been shown to be incorporated into *E. coli* cells by the *tonB*-dependent iron transport system.[131,132] All of these cephalosporin derivatives have the catechol moiety



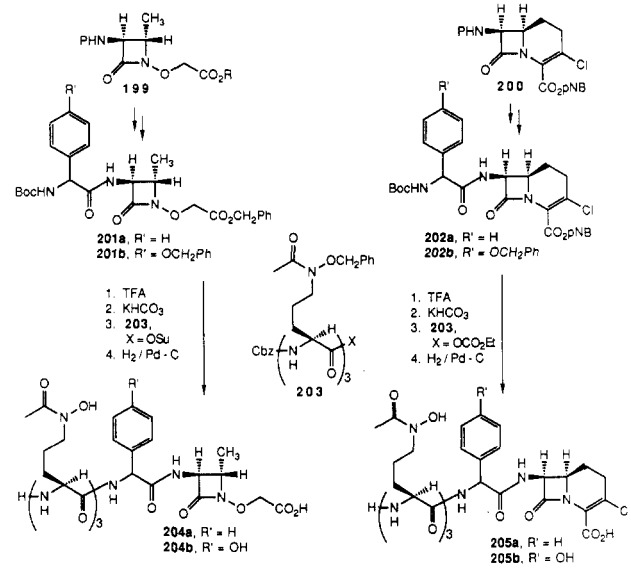
attached to the α-acyl group of the β-lactam.

Several compounds have also been prepared in which catechols are incorporated at other positions around the periphery of the cephalosporin nucleus. Most of these are C-3 methyl substituted derivatives. BO-1236 (**196**) is a new anti *Pseudomonal* cephalosporin which apparently owes its enhanced activity to the iron-binding capability of the pendant catechol.[133] The activity of dihydroxybenzoyl cephalosporin derivatives **197** has also been correlated with *tonB*-dependent illicit transport across the outer membranes of microbes.[134] As expected, the patent literature contains numerous examples of related catechol-containing cephalosporin derivatives.[135] One patent also disclosed a cephalosporin derivative (**198**) that incorporated a hydroxamic acid, presumably for promoting transport by iron shuttle processes.[136] The compound exhibited significant activity against both Gram negative and Gram positive bacteria.

The apparent ability of many of these iron chelating β-lactam antibiotics to utilize iron transport processes for cellular delivery is quite impressive since most of the compounds would not be considered efficient hexadentate iron ligands. Presumably the ligand:iron stoichiometry is not crucial for transport. This was previously demonstrated with rhodotorulic acid, which forms a 3:2 complex with ferric ion, yet is a very effective siderophore. On the other hand, and as indicated earlier, δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine and the corresponding linear dipeptide did not promote microbial growth in select studies and, if covalently bound to antibiotics, they presumably would not be able to utilize iron transport mechanisms for drug delivery. Thus, some attention has been given to the synthesis and study of antibiotics containing hexadentate iron ligands.

Albomycin mimics were chosen as logical siderophore–antibiotic synthetic targets. Since tri(δ-*N*-acetyl-δ-*N*-hydroxy-L-ornithine) (**142**) had been shown to be an effective microbial growth promoter, covalent linkage of β-lactam antibiotics was expected to provide an interesting test of the conceptual use of siderophores as drug delivery agents. To avoid problems that might be associated with the eventual hydrogenolytic removal of several benzyl protecting groups, non-sulfur-containing β-lactams were first considered. Two such types of β-lactams are the oxamazins (**199**)[137] and the carbacephalosporons (**200**).[138] Both of these types of antibiotics were acylated with protected D-phenylglycine or D-*p*-hydroxyphenylglycine to give derivatives **201** and **202**, which were then covalently attached to the C-terminal end of the protected triornithinyl peptide (**203**, Scheme 33). The phenylglycyl residues were added since they are common side chains for many β-lactam antibiotics (but not the oxamazins). The penultimate peptide–antibiotic conjugates were completely protected with hydrogenolytically labile groups. Thus, in each case, careful catalytic hydrogenation released the final products. Neither of the oxamazin derivatives (**204a** nor **204b**) displayed antibiotic activity against a variety of organisms. This should not have been too disappointing since the activity of the oxamazins is significantly affected by the side chain. Incorporation of the ATMO (aminothiazolone methoxime) side chain induces considerable activity against Gram negative bacteria, whereas the phenylglycyl side chains are nearly devoid of activity. Still it would have been quite interesting if the oxamazin derivatives would have had demonstrable activity because of more effective iron-mediated transport into the microbial cells.

SCHEME 33



In sharp contrast, the carbacephalosporin–siderophore conjugates **205a** and **205b** displayed significant, but very species-selective, antimicrobial activity. Incubation with β-lactam hypersensitive *E. coli* X580 (Lilly) resulted in delayed growth (Figure 1). The bacteria that did eventually grow were isolated and separately incubated again in the presence of each of the carbacephalosporin derivatives. In both cases, no delay of bacterial growth was observed. Among other possibilities, these results suggest that in the first incubation, a mutant of the parent *E. coli* strain had been selected that was deficient in some form of iron trans-

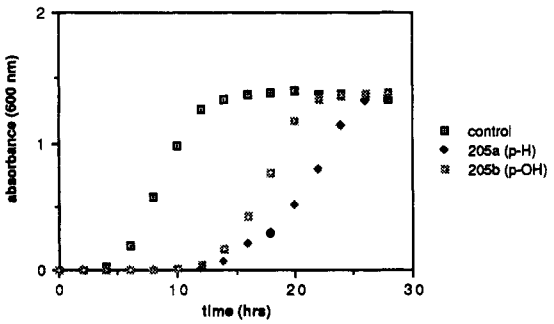


Figure 1. Effects of preformed Fe(III) complexes of compounds **205a** and **205b** (10 μM) in Mueller–Hinton broth on the growth rate of *E. coli* X580.

port since its growth was unaffected by the presence of either **205a** or **205b**. Iron transport deficient mutants might not be expected to be virulent pathogens. The same compounds (**205a** and **205b**) displayed moderate activity against a number of bacteria and significant activity against *Salmonella* X514 (MIC = 1.0 and 0.06 μg/L, respectively). The normal broad-spectrum activity of the carbacephalosporins was apparently diminished because either they were not carried into other microbial cells by the iron transport system and also no longer recognized by the normal β-lactam transport processes or they were actively transported by an iron shuttle system, but the antibiotic group was not intracellularly released. These preliminary exciting results indicate that albomycin analogues and perhaps many other siderophores and analogues may effectively carry unnatural antibiotics into cells, perhaps selectively, and inhibit microbial growth.

While it may be easy to predict that the concept of iron transport mediated illicit drug delivery has considerable potential, much work needs to be done. Further synthetic and biological studies are certain to reveal extremely interesting aspects of iron metabolism as well as new modes of chemotherapy. This review has intended to illustrate that synthetic organic chemistry has emerged and matured so well that even apparently complex synthetic and biochemical problems can be addressed effectively by what must now be considered rather practical organic chemistry.

Acknowledgments. Support from the National Institutes of Health and Eli Lilly and Company is gratefully acknowledged. The author wishes to sincerely extend his appreciation to his co-workers who have contributed the siderophore studies in his laboratory. These include M. King-Morris, J. M. Pachta, P. J. Mauer, B. H. Lee, G. Gerfen, R. T. Ronau, B. A. Steinbaugh, S. K. Sharma, C.-E. Lin, C. A. Gigilio, T. Kolasa, E. K. Dolence, J. A. McKee, and A. A. Minnick. Sincere thanks are also due to Professors J. B. Neilands, Shelley Payne, and T. Viswanatha as well as Dr. C. A. Prody, the E.E.&G. Mason Research Institute, and many interested scientists at Eli Lilly and Company for helpful discussions and biological tests.

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