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Design and synthesis of a novel protected mixed ligand siderophore

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Abstract

A novel siderophore analog (**4**) has been designed to facilitate iron transport-mediated drug delivery and drug release. This mixed ligand siderophore analog includes three bidentate ligands intended to octahedrally coordinate iron (III). The ligands include a 2,3-dihydroxy benzoic acid moiety, N^5-acetyl-N^5-hydroxy-L-ornithine, and a β-*N*-hydroxy-α,β-diaminopropionic acid derivative. The total synthesis of **15**, a form of **4** that is suitably protected, yet contains a free carboxylic acid for subsequent drug conjugation, is described.

The evolution of antibiotic resistance is inevitable as bacteria compete for limited resources for survival. Bacteria exhibit antibiotic resistance by several mechanisms. Examples include the (1) production of enzymes, such as β-lactamases, to destroy antibiotics, (2) adaptation of an efflux mechanism to shuttle drugs out of the cell, (3) chemical alteration of the target of the antibiotic, and (4) altered cell wall permeability to prevent diffusion of the drugs into the cells.[1] The growing antibacterial resistance observed in the world today translates into staggering consequences for the medical community and pharmaceutical industry.[2] In addition to searching for new antibiotics, attention must also be paid to make wiser use of the effective medicines available today. The utilization of specific drug delivery agents coupled with known and new antibiotics is under development in our group, among others, as a potentially new avenue for antimicrobial drug therapy.[3]

Due to the low solubility of iron at physiological pH, bacteria biosynthesize low molecular weight iron chelators called siderophores to sequester and deliver the Fe^{3+} essential to their survival.[4] The most effective siderophores consist of three bidentate ligands, most commonly catechol, hydroxamic acid, or α-hydroxycarboxylic acid iron-binding moieties. These structures reflect the high affinity of oxygen for ferric ions and allow for complete octahedral coordination of Fe^{3+}. By the conjugation of a drug to a siderophore, the iron transport process can be exploited to smuggle antibiotics into microorganisms. The concept of utilizing a siderophore as a transport agent parallels the mode of action of several naturally occurring antibiotics, such as the albomycins, ferrimycin, and salmycins (Fig. 1).[5] These antibiotics consist of a siderophore component and an antimicrobial component, and are meant to provide the parent organism with a selective growth advantage. When other microbes try to utilize the iron from these siderophores, they also assimilate the antibiotic and are killed.

Several biological studies have proven the utility of siderophore drug conjugates for use as antibacterial, antitumor, and antiviral agents. This method of disguising a drug has been called the 'Trojan Horse' microbe selective drug delivery concept.[3] The conjugates usually consist of a synthetic siderophore for binding the iron, a linker for attaching the drug to the siderophore, and a drug for conveying the antibacterial activity (Fig. 2). Once the microorganism absorbs the siderophore–drug conjugate, the complex may kill the cell in a variety of ways: by releasing the drug, by acting as an intact antibacterial agent, or by blocking further iron assimilation. The linker plays a vital role in the conjugate because it can dictate if the drug is released (chemically or enzymatically) within the cell and could potentially allow controlled release of drugs.[3]

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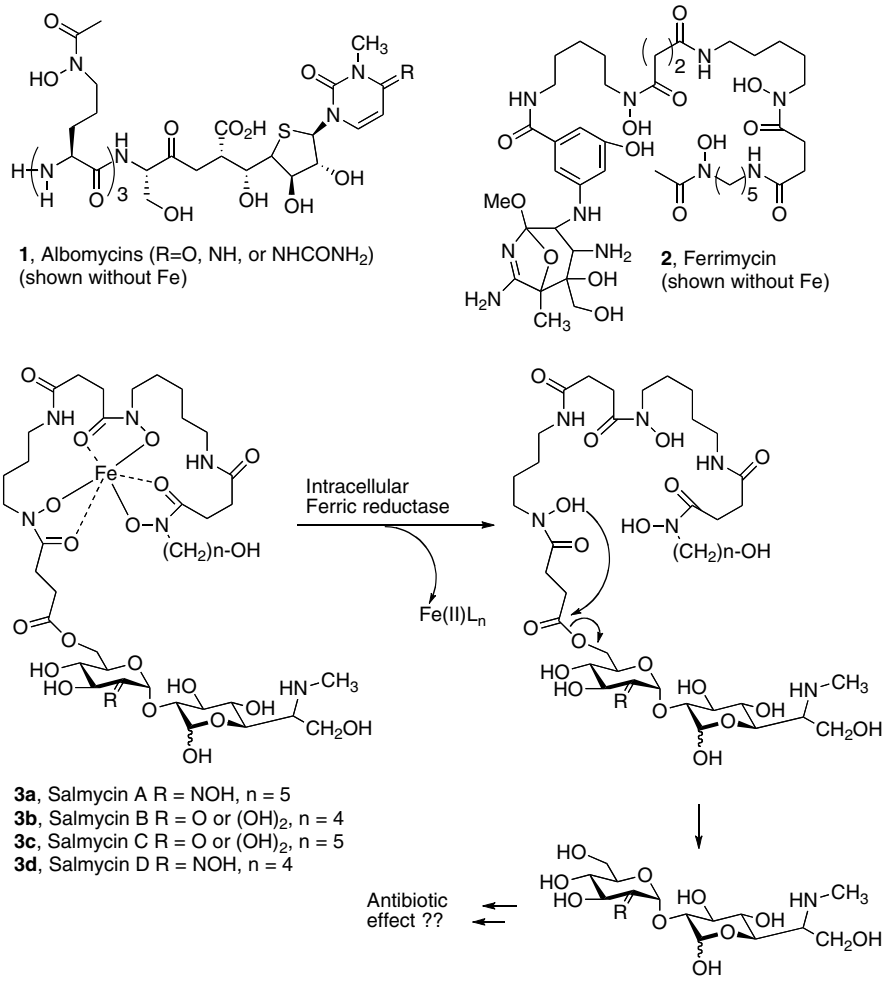


Fig. 1. Naturally occurring siderophore–antibiotic conjugates and a potential 'drug release' process.

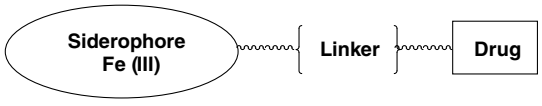


Fig. 2. Schematic representation of a siderophore–drug conjugate.

The most appealing aspect of the 'Trojan Horse' principle is the wide array of opportunities for specific drug delivery. Because bacteria have specific siderophore-binding receptor proteins, harmful bacteria can potentially be selectively targeted with the most effective and appropriate drugs. The arena is also open for the introduction of more toxic drugs, and the reinstitution of drugs that currently meet with resistance. If toxicity can be diminished by conjugation to a siderophore, perhaps some of the most deadly compounds could be added to the arsenal in the fight against virulent bacteria. Additionally, this strategy may be particularly useful for combating common resistance mechanisms, especially those associated with efflux of the drug or blocked diffusion.[3b] Several biological studies have shown that some siderophore–drug conjugates have been able to overcome previous permeability problems for certain drugs.[3] Studies have also shown that mixed ligand siderophores (containing more than one type of iron-binding moiety) can gain access to the cell by more than one transport pathway, or type of receptor, possibly lending them an advantage as drug delivery agents.[6] One additional benefit of siderophore–drug conjugates is that because the membrane no longer serves as a barrier to the drug, active transport of the complexes can increase the rate of entry into the cell beyond the passive diffusion rate. This phenomenon is reflected in lower MIC values by more than two orders of magnitude.[7]

Thus far, many natural and artificial siderophores have been synthesized and shown to exhibit good microbial growth promoting activity. Several recent papers review many of the chemical syntheses of these siderophores, highlighting much of the previous work that has been done in our laboratory and others.[3,4a] The original report on the isolation[3] of the salmycins and our subsequent total syntheses of salmycins and analogs[8] indicated that they were less stable in the deferri (iron free) form. We hypothesize that this might be due to an intramolecular nucleophilic reaction of a released hydroxamate to induce cleavage of the linker to the aminoglycoside antibiotic component of the salmycins (Fig. 1). If so, the result would be another

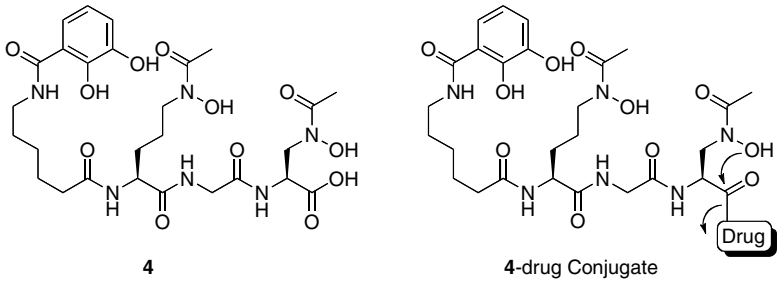


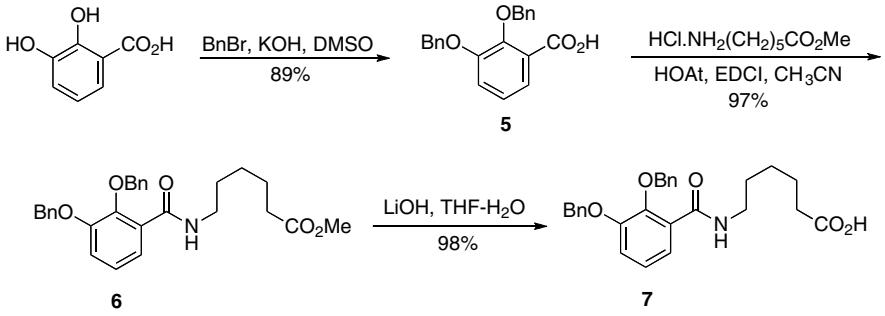
Fig. 3. Targeted mixed ligand siderophore.

clever extension of natural siderophore antibiotics that are not only recognized and actively transported by microbial iron assimilation systems, but also contain an iron reductase that would trigger intracellular release of the antibiotic. While studies are in progress to demonstrate this concept with derivatives of danoxamine, the siderophore component of the salmycins,[8] we initiated studies to design artificial siderophores capable of similar anchimerically assisted drug release that is triggered by the reductive removal of the metal inside the cell. Herein we report the synthesis of a suitably protected form of a novel mixed ligand siderophore analog, **4**, for the subsequent preparation of drug conjugates to further test this hypothesis. As described previously,[3,4a] the use of mixed ligands is intended to facilitate broader microbial recognition and assimilation through multiple siderophore receptors while the existence of the short side chain on the C-terminal amino acid may induce intracellular release of the drug by an intramolecularly assisted cleavage as suggested for the salmycins (Fig. 3).

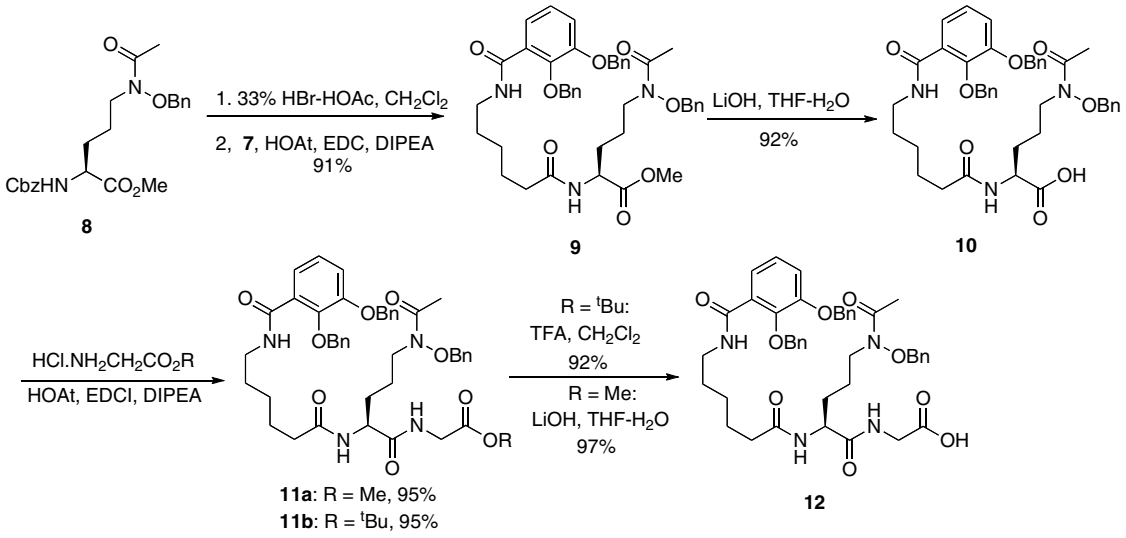
As shown in Scheme 1, the synthesis of the catechol ligand **7** began with the selective benzylation of 2,3-dihydroxybenzoic acid which was accomplished with excess benzyl bromide and KOH in DMSO to yield **5** in 89% yield.[9] Benzoic acid **5** was then coupled to methyl 6-aminocaproate hydrochloride, which was prepared in quantitative yield from 6-aminocaproic acid in HCl (g) MeOH solution. Several different coupling additives were experimented with and HOAt gave the highest and the most reproducible yield, thus the two fragments were coupled in the presence of HOAt, EDC, and DIPEA in CH_3CN to give **6** in 97% yield. The methyl ester of the coupled product **6** was then saponified in 98% yield with 2 equiv of LiOH in a 1:1 solution of THF and water. This completed the synthesis of the protected catechol-containing ligand **7**.

As shown in Scheme 2, the protected ornithine-derived hydroxamate **8**[10,11] was subjected to deprotection with HBr–HOAc. The resulting free amine HBr salt was coupled to acid **7** using HOAt and EDC as coupling reagents in the presence of DIPEA to afford dipeptide methyl ester **9** in 91% yield. Hydrolysis of **9** then provided dipeptide acid **10** in 92% yield. Dipeptide acid **10** was next coupled to glycine methyl ester or glycine *tert*-butyl ester in the presence of HOAt and EDC to give the corresponding tripeptide methyl ester **11a** or *tert*-butyl ester **11b**, both in 95% yield. Methyl ester **11a** was hydrolyzed by LiOH to provide tripeptide acid **12** in 97% yield; while the removal of the *tert*-butyl protecting group by the treatment of **11b** with TFA in CH_2Cl_2 also gave tripeptide acid **12** in 92% yield.

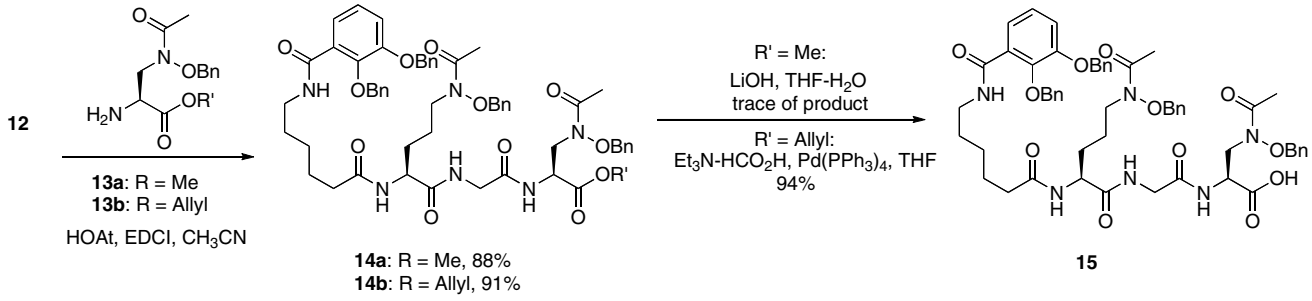
The final peptide-bond-forming reaction required to synthesize protected tetrapeptide acid **15** is shown in Scheme 3. Tripeptide acid **12** was first coupled to methyl L-3-(*N*-acetyl-*N*-benzyloxyamino)aminoalaninate **13a** using HOAt and EDC in CH_3CN to provide tetrapeptide methyl ester **14a** in 88% yield. Unfortunately, however, the saponification of **14a** was not always reproducible within an optimized reaction time. On several occasions, the saponification reaction took up to three days to complete. The lengthy reaction time led to racemization of the terminal α-center and the formation of other by-products, so the isolated yield was extremely low. In further studies, the problem was solved by using an allyl ester as an alternative to the methyl ester. Allyl ester **14b** was



Scheme 1.



Scheme 2.



Scheme 3.

readily prepared by coupling tripeptide acid **12** to allyl L-3-(*N*-acetyl-*N*-benzyloxyl)aminoalaninate **13b** in good yield. An allyl ester **14b** was initially deprotected with catalytic palladium(0) using *O*-benzylhydroxylamine as the allyl cation scavenger.[12] However, this reaction was only completed after 36 h and required the addition of up to 20% mmol palladium(0) and 3 equiv of *O*-benzylhydroxylamine. The long reaction time also resulted in some racemization. Alternatively, the allyl protecting group was readily cleaved to furnish the desired tetrapeptide acid **15** by the treatment of **14b** with triethylammonium formate (5 equiv) in the presence of a catalytic amount of $Pd(PPh_3)_4$.[13]

In conclusion, the total synthesis of a novel suitably protected mixed ligand siderophore **15** has been accomplished using fully protected hydroxamate **8** as the starting material and using appropriate protecting groups. Syntheses of antibacterial and antifungal siderophore drug conjugates are underway in our laboratory. Subsequent biological test results and the drug release assays of these siderophore drug conjugates along with their iron (III) complexes will provide us useful information to further understand the drug transportation and release mechanism. Those results will be reported in due course.

Acknowledgments

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Supplementary data

Experimental procedures, NMR spectra and analytic data for all new compounds are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.02.007.

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