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Synthesis of the Siderophore Coelichelin and Its Utility as a Probe in the Study of Bacterial Metal Sensing and Response

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Abstract

A convergent total synthesis of the siderophore coelichelin is described. The synthetic route also provided access to acetyl coelichelin and other congeners of the parent siderophore. The synthetic products were evaluated for their ability to bind ferric iron and promote growth of a siderophore-deficient strain of the Gram-negative bacterium *Pseudomonas aeruginosa* under iron restriction conditions. The results of these studies indicate coelichelin and several derivatives serve as ferric iron delivery vehicles for *P. aeruginosa*.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.orglett.8b03857](https://doi.org/10.1021/acs.orglett.8b03857).
Experimental procedures, characterization data, biological activity assays, and NMR spectra for new compounds (PDF)

The emergence of antibiotic-resistant strains of bacteria is a significant public health concern necessitating the discovery of novel antibiotics, and as a result, the study of host-pathogen interactions has garnered increasing attention.¹ It has been well-cited that bacteria require metals, such as iron, for metabolism and pathogenesis, rendering metal acquisition pathways as potential targets for the development of new antimicrobial agents.² In an effort to combat bacterial pathogenesis, host organisms employ several mechanisms to maintain low free-iron concentrations.³ Bacterial pathogens respond to these iron-deficient conditions through the secretion of high-affinity, small molecule iron chelators termed siderophores.⁴ Coelichelin (Figure 1) is one such siderophore produced by the bacterium *Streptomyces coelicolor* under iron-deficient conditions.⁵

The genesis of coelichelin's discovery dates back to 2000 when Challis and co-workers identified a unique gene cluster in *S. coelicolor* encoding a nonribosomal peptide synthetase (NRPS).^{5a} Coelichelin, a ferric-iron chelating peptide, was later isolated from *S. coelicolor* and characterized as its gallium(III) complex (**4**) by mass spectrometry and NMR spectroscopy.^{5b} Coelichelin was assigned the structure of a trishydroxamate tetrapeptide (**1**), enabling the formation of a proposed hexacoordinate, octahedral complex with ferric iron (**3**).

The study of siderophores and their analogues have gained attention for their potential use as novel therapeutics to combat antibiotic resistance and treat iron metabolism disorders.⁶ Notably, it has been demonstrated that siderophores can be employed as "Trojan horses" by conjugation to either small- or large-molecule antimicrobial agents, effectively harnessing the microbe's own cellular machinery to improve cellular uptake and consequently delivery of the antimicrobial payload.⁷ In this regard, coelichelin has the potential to serve as a probe molecule in the study of metal acquisition pathways in select microbes as well as an antimicrobial delivery vehicle.

Herein, we describe the first reported total synthesis of coelichelin (**1**) as well as an *N*-acetyl analogue (**2**, acetyl coelichelin). The parent siderophore, *N*-acetyl analogue, and derivatives were assayed for their ability to bind ferric iron and support growth of *Pseudomonas aeruginosa* under iron-deficient conditions. These studies revealed utilization of iron complexes of coelichelin and several congeners, including *N*-Boc-protected derivatives, by *P. aeruginosa*. The latter result is significant as it reveals a site of structural modification without loss of microbial uptake, implicating it as a candidate for conjugation to an antimicrobial agent payload.

Coelichelin (**1**) is a tetrapeptide belonging to the hydroxamate class of siderophores, which we envision could be assembled through two key peptide couplings leading to *N,O*-protected derivatives of coelichelin (**5**, Figure 2). Taking advantage of the pseudosymmetry of **5**, first coupling of *D*-*allo*-threonine (**7**) and *N*-benzyloxy-L-ornithine (**8**) would be followed by removal of Boc protecting groups to reveal peripheral amino groups. A second amide coupling of the diamino intermediate with 2 equiv of D-ornithine **6** would deliver protected coelichelins (**5**) and following global deprotection yield coelichelin (**1**) and acetyl coelichelin (**2**). A synthesis of *N*-Boc-*D*-*allo*-threonine (**7**) from D-threonine in five steps has been previously reported.⁸ *N*-Benzyloxy-D- and -L-ornithines (**6**) and (**8**) were derived from D- and L- pyroglutamic acid, respectively.

Synthesis of *N*-benzyloxy-L-ornithine (**8**) began with conversion of commercially available L-pyroglutamic acid to the corresponding benzyl ester, followed by *N*-Boc protection and reduction of the derived *N*-Boc amide to afford alcohol **9** (Scheme 1).⁹ Parikh-Doering oxidation of **9** yielded an intermediate aldehyde that was condensed in situ with *O*-benzylhydroxylamine hydrochloride, leading to a mixture of oximes (**10**). Following chromatographic purification, reduction of **10** with sodium cyanoborohydride at low pH provided *N*-benzyloxy-L-ornithine (**8**).¹⁰ Similarly, *N*-benzyloxy-D-ornithine (**6a**) was prepared from D-pyroglutamic acid starting with esterification using allyl alcohol¹¹. Once again, *N*-Boc protection of the derived ester was followed by reduction to afford alcohol **12**.¹² The latter was subjected to the previously described one-pot oxidation-oxime formation to yield oxime **13** in 71–82% yield. In this case, reductive amination of benzyloxime **13** was followed by *N*-formylation and allyl ester group removal to afford *N*-benzyloxy-D-ornithine (**6a**). In anticipation of preparing acetyl coelichelin (**2**), *N*-acetylation replaced *N*-formylation to ultimately yield acetamide **6b**.

The remaining central amino acid, *D*-*allo*-threonine (**7**, Figure 2), was prepared employing a known synthetic route starting from D-threonine in five steps⁸ and the amino protected as its *tert*-butyl carbamate to give **7**. Coupling of *N*-benzyloxy-L-ornithine (**8**) and *D*-*allo*-threonine (**7**) using HATU reagent followed by TFA-mediated removal of the Boc protecting groups afforded the bis-ammonium salt **11**. Gratifyingly, the latter was coupled with 2 equiv of ornithine **6a** or **6b** to complete protected tetrapeptide **5a** or **5b**, respectively. Hydrogenolysis of **5a** in methanol resulted in removal of benzyl ether and ester groups to provide *N*-Boc- protected coelichelin (**12a**). Likewise, hydrogenolysis of **5b** yielded *N*-Boc-protected acetyl coelichelin (**12b**). Upon treatment with TFA in dichloromethane, carbamates **12a** and **12b** afforded coelichelin (**1**) and acetyl coelichelin (**2**), respectively. For

the purpose of structural correlation, the gallium(III) complex of synthetic coelichelin was prepared, and its mass spectral and ^1H NMR data compared favorably to data reported for the gallium(III) complex of natural coelichelin as reported by Challis.⁵

Affinity testing for ferric iron using CAS assays was performed on coelichelin, acetyl coelichelin, and various protected derivatives (**12a**, *N*-Boc; **12b**, acetyl *N*-Boc, *N*,*O*-protected **5a**, and acetyl *N*,*O*-protected **5b**). The commercial siderophore desferrioxamine (DFO) served as a positive control and doubly distilled water (dd- H_2O) as a negative control. The results of the CAS assays are presented in Figure 3 and reveal that desferrioxamine (DFO) is a slightly superior iron chelator in comparison to coelichelin (**1**) and *N*,*O*-protected coelichelin (**5a**) and *N*,*O*-protected acetyl coelichelin (**5b**) are effectively inactive. *N*-Boc-protected coelichelin (**12a**), *N*-Boc-protected acetyl coelichelin (**12b**), and acetyl coelichelin (**2**) were also capable of chelating ferric iron. This result has positive implications for the potential development of coelichelin probes and/or antibiotic conjugates, and siderophore activity of coelichelin and its congeners was further investigated in the following assays.

Coelichelin and its various synthetic congeners were then assayed for their ability to serve as the sole iron source for a bacterial reporter strain that is devoid of endogenous siderophore production. Under iron restriction, many bacteria express not only the biosynthetic and uptake machinery for endogenously produced siderophores but also receptor proteins for pirating siderophores produced by heterologous microorganisms.¹³ To induce expression of both endogenous and “xenosiderophore” uptake systems, *P. aeruginosa* was grown under iron restriction. In the absence of endogenously synthesized siderophores, growth of *P. aeruginosa* is wholly dependent upon the uptake and utilization of the siderophore(s) provided (i.e., coelichelin). To this end, a siderophore-deficient strain of *P. aeruginosa*¹⁴ was grown under iron restriction, and apo- and holo-forms of coelichelin, acetyl coelichelin, and its synthetic congeners were provided as the sole iron source and evaluated for their ability to support growth of the bacteria. The results of these studies are summarized in Figure 4. Apo- (iron loaded) forms were superior in promoting bacterial growth across all forms of natural (**1**, coelichelin) and unnatural (**2**, acetyl coelichelin; *N*-Boc derivatives **12a/12b**). Notably, acetyl coelichelin (**2**) promoted *P. aeruginosa* growth to the greatest extent relative to all chelators assayed. Interestingly, *N*-Boc-protected congeners (**12a/12b**) promoted *P. aeruginosa* growth, suggesting that the peripheral amino groups present in coelichelin (**1**) can tolerate additional functionality without perturbing siderophore activity. This flexibility in uptake of structurally modified siderophores is likely facilitated by the siderophore receptor FoxA, which in other pseudomonads has been linked to piracy of ferrioxamine and coelichelin.¹⁵ To confirm the bioactivity of coelichelin (**1**), a fluorescence assay was employed to assess iron-restriction of siderophore-proficient *P. aeruginosa* PAO1 through detection of its fluorescent siderophores, pyochelin and pyoverdine (Supporting Figure S1).¹⁶ In the presence of coelichelin (**1**), a dose-dependent decrease in pyochelin and pyoverdine fluorescence is observed, indicating that this siderophore is being utilized by *P. aeruginosa* as an iron source. In contrast, no dose-dependent changes to fluorescence were observed when *P. aeruginosa* was provided with the fully protected tetrapeptide (**5a**), indicating that is not utilized as an iron source by the bacterium. The results of these studies together have

positive implications for the potential development of coelichelin probes and/or antibiotic conjugates.

In conclusion, we describe a highly convergent synthesis to coelichelin and various congeners and demonstrate the ability of several to deliver iron(III) to a siderophore-deficient strain of *P. aeruginosa*, a known opportunistic Gram-negative human pathogen. Also revealed are sites tolerant of modification, implying the potential for development of siderophore– antibiotic conjugates. Preliminary results indicate that coelichelin can chelate other physiologically relevant metals of interest, and efforts are underway to probe this metal selectivity as we develop siderophore conjugates to enable advanced study of these metal acquisition pathways.^{1–4}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- (1). Clatworthy AE; Pierson E; Hung DT Nat. Chem. Biol. 2007, 3, 541–548. [PubMed: 17710100]
- (2). (a) Skaar EP PLoS Pathog. 2010, 6, No. e1000949. [PubMed: 20711357] (b) Nairz M; Schroll A; Sonnweber T; Weiss G Cell. Microbiol. 2010, 12, 1691–1702. [PubMed: 20964797] (b1) Nairz M; Haschka D; Demetz E; Weiss G Front. Pharmacol. 2014, 5, 1–10. [PubMed: 24478702] (c) Bullen JJ Clin. Infect. Dis. 1981, 3, 1127–1138.(d) Ratledge C; Dover L Annu. Rev. Microbiol. 2000, 54, 881–941. [PubMed: 11018148] (e) Wandersman C; Delepelaire P Annu. Rev. Microbiol. 2004, 58, 611–647. [PubMed: 15487950]
- (3). Ganz T; Nemeth E Biochim. Biophys. Acta, Mol. Cell Res. 2006, 1763, 690–699.
- (4). (a) Wilson BR; Bogdan AR; Miyazawa M; Hashimoto K; Tsuji Y Trends Mol. Med. 2016, 22, 1077–1090. [PubMed: 27825668] (b) Chu BC; Garcia-Herrero A; Johanson TH; Krewulak KD; Lau CK; Peacock R; Slavinskaya Z; Vogel HJ. Siderophore Uptake in Bacteria and the Battle for Iron with the Host; a bird's eye view. BioMetals 2010, 23, 601–611. [PubMed: 20596754] (c) Behnsen J; Raffatellu M mBio 2016, 7 (6), No. e01906–16.(d) Saha R; Saha N; Donofrio RS; Bestervelt LL. J. Basic Microbiol. 2013, 53, 303–317. [PubMed: 22733623] (e) Miethke M; Marahiel MA Microbiol. Mol. Biol. Rev. 2007, 71, 413–451. [PubMed: 17804665] (f) Neilands JB J. Biol. Chem. 1995, 270, 26723–26726. [PubMed: 7592901]
- (5). (a) Challis GL; Ravel J FEMS Microbiol. Lett. 2000, 187, 111–114. [PubMed: 10856642] (b) Lautru S; Deeth RJ; Bailey M; Challis GL. Nat. Chem. Biol. 2005, 1, 265–269. [PubMed: 16408055]
- (6). (a) Wilson BR; Bogdan AR; Miyazawa M; Hashimoto K; Tsuji Y Trends Mol. Med. 2016, 22, 1077–1090. [PubMed: 27825668] (b) Braun V; Pramanik A; Gwinner T; KÖberle M; Bohn E BioMetals 2009, 22, 3–13. [PubMed: 19130258]
- (7). (a) Hatcher HC; Singh RN; Torti FM; Torti SV. Future Med. Chem. 2009, 1, 1643–1670. [PubMed: 21425984] (b) Miller MJ Acc. Chem. Res. 1993, 26, 241–249.(c) Wenciewicz TA; Miller MJ In Sideromycins as Pathogen-Targeted Antibiotics; Fisher JF, Mobashery S, Miller MJ, Eds.; Springer: Berlin, 2017; Antibacterials Vol. 2; pp 151–183.
- (8). (a) Liang X; Lee C-J; Chen X; Chung HS; Zeng D; Raetz CRH; Li Y; Zhou P; Toone EJ. Bioorg. Med. Chem. 2011, 19, 852–860. [PubMed: 21194954] (b) Duranti A; Tontini A; Antonietti F;

Vacondio F; Fioni A; Silva C; Lodola A; Rivara S; Solorzano C; Piomelli D; Tarzia G; Mor MJ Med. Chem. 2012, 55, 4824–4836.

- (9). (a) Gloanec P; Herve Y; Bremand N; Lecouve J-P; Breard F; De Nanteuil G Tetrahedron Lett. 2002, 43, 3499–3501. (b) Aggarwal VK; Astle CJ; Rogers-Evans M Org. Lett. 2004, 6, 1469–1471. [PubMed: 15101769] (c) Cordova A; Reed NN; Ashley JA; Janda KD Bioorg. Med. Chem. Lett. 1999, 9, 3119–3122. [PubMed: 10560736] (d) Hyun Lee B; Miller MJ Tetrahedron Lett. 1984, 25, 927–930.
- (10). Kishimoto S; Nishimura S; Hatano M; Igarashi M; Kakeya HJ Org. Chem. 2015, 80, 6076–6082.
- (11). Rigo B; Lespagnol C; Pauly MJ J. Heterocycl. Chem. 1988, 25, 49–57.
- (12). Qu S; Chen Y; Wang X; Chen S; Xu Z; Ye T Chem. Commun. 2015, 51, 2510–2513.
- (13). Sheldon JR; Laasko HA; Heinrichs DE Iron Acquisition Strategies for Bacterial Pathogens In Virulence Mechanisms of Bacterial Pathogens, 5th ed.; Cornick NA, Ed.; ASM Press: Washington, DC, 2016; p 43.
- (14). Ankenbauer RG; Cox CD. J. Bacteriol. 1988, 170, 5364–5367. [PubMed: 3141387]
- (15). (a) Galet J; Deveau A; Hôtel L; Frey-Klett P; Leblond P; Aigle B Appl. Environ. Microbiol. 2015, 81, 3132–3141. [PubMed: 25724953] (b) Llamas MA; Sparrius M; Kloet R; Jiménez CR; Vandenbroucke-Grauls C; Bitter WJ Bacteriol. 2006, 188, 1882–1891.
- (16). Dumas Z; Ross-Gillespie A; Kümmerli R Proc. R Soc. London, Ser. B 2013, 280, 20131055.

Figure 1.
Structures of coelichelin, corresponding ferric and galliumion chelate complexes, and acetyl coelichelin.

Figure 2.
Overview of synthetic strategy toward coelichelin and acetyl coelichelin.

Figure 3.

Chrome azurol S (CAS) assay for the detection of iron-binding capabilities. (A) CAS activity of coelichelin (1), acetyl coelichelin (2), **5a**, **5b**, **12a**, **12b**, dd-water (ddH₂O; negative control), and desferrioxamine (DFO; positive control) at the concentrations indicated, where a decrease in absorbance at 630 nm is reflective of an increase in iron-binding activity. (B) Visual comparison at 100 μM concentration of each chelator. Data are reflective of three independent experiments.

Figure 4.

Coelichelin and congeners can facilitate growth of *P. aeruginosa* under iron restriction. Agar plate bioassays were performed to assess the ability of coelichelin (**1**, red bars) and its various synthetic congeners (white bars) to promote the iron-dependent growth of siderophore-deficient *P. aeruginosa*. Apo- (clear bars) and holo- (checkered bars) forms of the substrates were assayed. 2,5- Dihydroxybenzoic acid (DHBA; green) and ddH₂O (blue) served as positive and negative controls, respectively. Free ferric chloride was provided at the same concentrations as used to iron-load the substrates (black). Statistical differences relative to apo-coelichelin (apo-1) were determined by one-way ANOVA with Dunnett's multiple comparison test where $*p < 0.05$ and $****p < 0.0001$.

Scheme 1.

Total Synthesis of Coelichelin (1) and Acetyl Coelichelin (2)