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Total Synthesis and Structure Revision of Mirubactin, and Its Iron Binding Activity

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Dechloro-chlorocatechelin A (**3**), a deschloro-derivative of a microbial siderophore chlorocatechelin A (**2**), was synthesized from 2,3-dihydroxybenzoic acid, D-arginine, and 1-benzyl D-glutamate. The spectral data were unambiguously identical with those of mirubactin (**1**), revising its chemical structure. Mirubactin showed potent iron binding affinity, which was comparable to that of chlorocatechelin A (**2**).

Siderophores are low-molecular-weight metabolites that microbes and plants excrete to acquire iron from the environment.[1,2] Siderophores usually contain three bidentate ligands in a molecule to form a stable octahedral Fe(III)-siderophore complex. Bidentate groups in siderophores can be classified into three types:[3] catecholate type (e.g. 2,3-dihydroxybenzoic acid (DHB) unit in enterobactin[4]), hydroxamate type (e.g. *N*-δ-hydroxy-*N*-δ-formyl ornithine (hfOrn) unit in amychelin[5]), and α-hydroxycarboxylate type (e.g. citrate unit in rhizobactin 1021[6]). Some siderophores contain a single species of the bidentate group, while others have two or three species.

In 2012, Marahiel and co-workers reported the isolation, structure characterization, and biosynthesis of a siderophore named mirubactin (**1**), from *Actinosynnema mirum*.[7] This metabolite consists of two units of DHB, one unit each of D-arginine (D-Arg) and D-hfOrn (Figure 1). The most characteristic feature of this compound was that one unit of DHB was linked to D-hfOrn by forming an unusual *O*-acyl hydroxamic acid ester. However, this linkage was not determined with certainty by chemical methods. Additionally, the *O*-acyl hydroxamic acid ester disables the chelation ability of hydroxamate, which should significantly decrease the affinity of the metabolite to Fe(III).

Recently, we reported the discovery and total synthesis of chlorocatechelin A (**2**), a novel siderophore from *Streptomyces* sp. ML93-86F2 (Figure 1).[8,9] The substructures of **2** are similar to those of **1**, except for the fact that **2** contains 4-chloro-2,3-dihydroxybenzoic acid (CDB) instead of DHB. The major structural difference between **1** and **2** is the location of the catecholate group: **2** has an acylguanidine structure composed of CDB and D-Arg, not an *O*-acyl hydroxamic acid ester. However, the NMR and MS/MS spectra of **1** closely resembled those of **2**. These observations led us to reinvestigate the chemical structure of mirubactin (**1**). Here we report total synthesis of dechloro-chlorocatechelin A (**3**) and structure revision of mirubactin (**1**) (Figure 1).

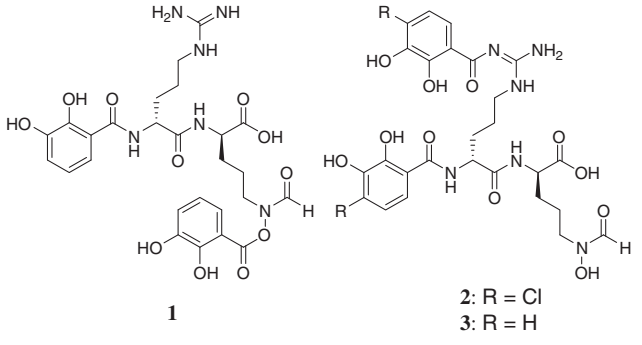
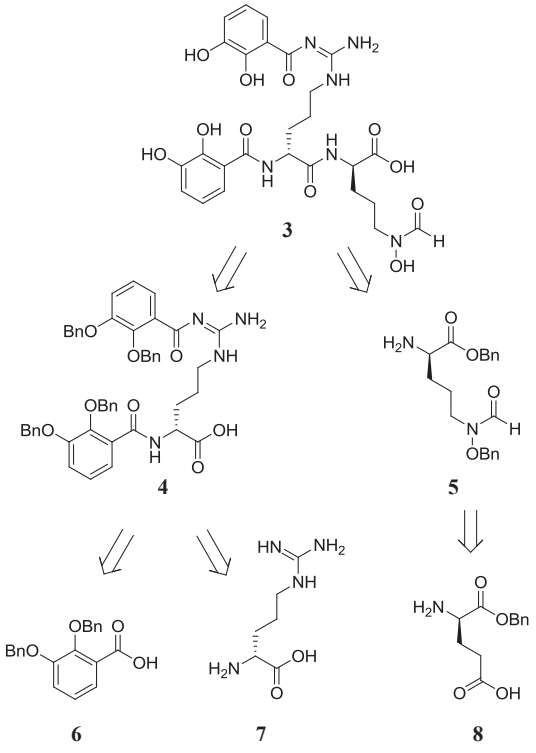


Figure 1. Structures of mirubactin (**1**; originally reported), chlorocatechelin A (**2**) and dechloro-chlorocatechelin A (**3**).

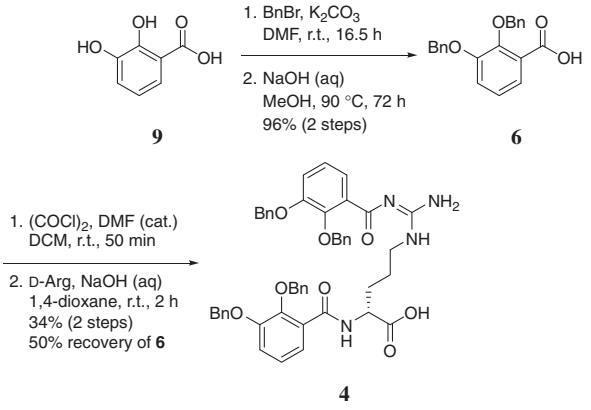
We planned to synthesize **3** in a similar way to **2**.[9] Scheme 1 shows the retrosynthesis of **3**, which was fragmented into two segments **4** and **5**. The left segment **4** would be obtained by coupling benzylated DHB **6** and D-Arg **7**, while the right segment **5** can be synthesized from 1-benzyl D-glutamate **8** as reported previously.[9]

Synthesis of the left segment **4** commenced from benzylation of DHB **9**. Compound **9** was reacted with BnBr, and then hydrolyzed in alkaline conditions to give a carboxylic acid **6**. Protected DHB **6** was converted to acid chloride with $(COCl)_2$ and subsequently reacted with D-Arg under Schotten–Baumann conditions to yield the left segment **4** (34%) and the recovered material **6** (50%) (Scheme 2).

The protected right segment **10** was prepared from 1-benzyl D-glutamate in 6 steps (totally 70% yield) as described.[9] The right segment **5**, which was obtained by removal of the Boc group of



Scheme 1. Retrosynthesis of dechloro-chlorocatechelin A (**3**).



Scheme 2. Synthesis of the left segment **4**.

10 with TFA, was condensed with the left segment **4** using HATU and HOAt to give compound **11**. Compound **11** was subjected to hydrolysis followed by hydrogenolysis for removal of the benzyl groups, yielding dechloro-chlorocatechelin A (**3**) (Scheme 3). The NMR data of synthesized **3** were unambiguously identical with those reported for mirubactin (Supporting Information, Table S1),[7] revealing that the chemical structure of mirubactin should be **3**.

We finally investigated the iron binding properties of synthesized mirubactin (**3**). We examined a spectrophotometric titration experiment of compound **3** with $FeCl_3$ in 50 mM BisTris buffer (pH 7.0, $I = 0.1$ M, Figures 2a and 2b). An increasing absorbance at 516 nm was observed until 1 equiv of $FeCl_3$ was added, indicating that **3** formed a complex with Fe(III) at a 1:1 ratio. Notably, the UV spectra of the synthesized compound **3** in the absence and presence of Fe(III) resembled those reported for natural mirubactin.[7] Next, the redox potential of the Fe(III)-**3** complex was measured in cyclic voltammetry (CV) experiments.[10] The mixture of **3** (200 μM) and $FeCl_3$ (140 μM) in 50 mM BisTris buffer (pH 7.0, $I = 0.1$ M, Figure 2c) gave a reversible voltammogram with a half wave potential ($E_{1/2}$) of −550 mV (vs. NHE), which was slightly higher than that of chlorocatechelin A (**2**, $E_{1/2} = -578$ mV)[8] indicating that the iron binding affinity of **3** is slightly lower than that of **2**.[10] These results suggest that chloro substituents partly contribute to the potency of the iron binding affinity of these metabolites.

In summary, we synthesized dechloro-chlorocatechelin A (**3**) in 10 steps from 1-benzyl D-glutamate and revised the chemical structure of mirubactin. Mirubactin (**3**) showed potent iron binding properties, comparable to that of chlorocatechelin A (**2**).

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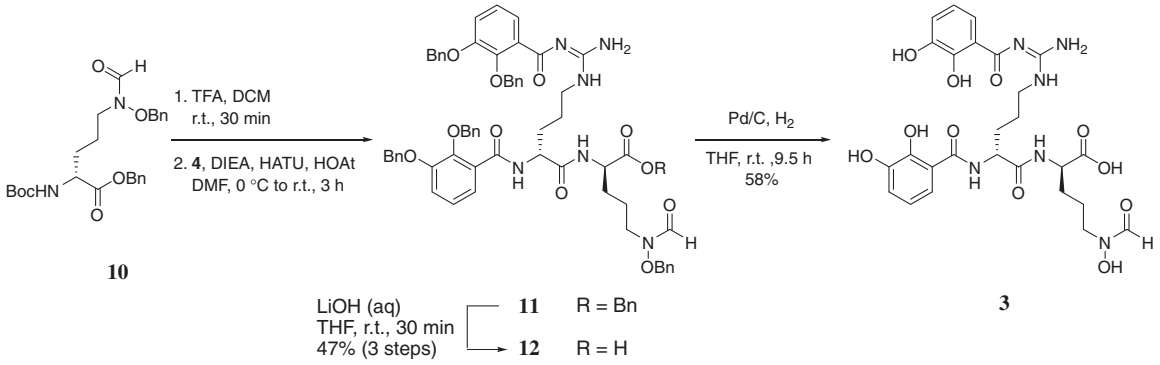
Supporting Information is available electronically on J-STAGE.



Scheme 3. Synthesis of dechloro-chlorocatechelin A (**3**).

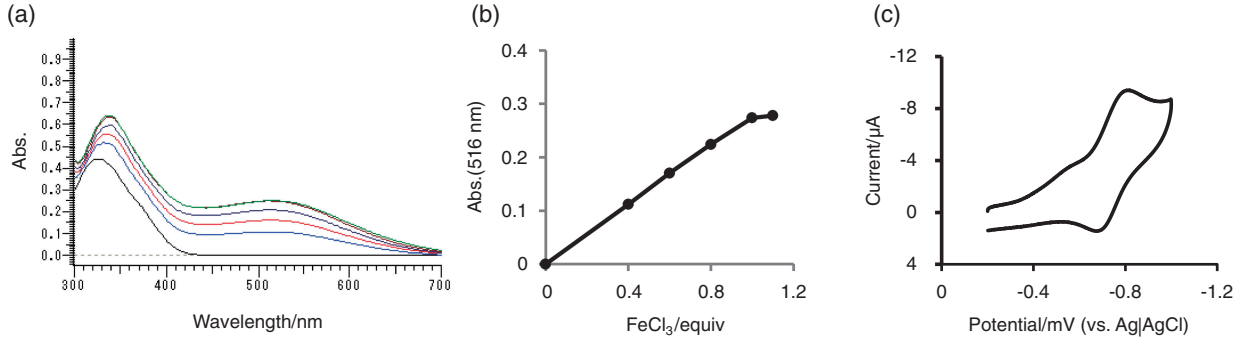


Figure 2. Iron binding properties of the synthesized compound **3**. (a, b) Spectrophotometric titration of **3** with $FeCl_3$ in 50 mM BisTris buffer (pH 7.0, $I = 0.1$ M with NaCl). UV absorption spectra are shown in (a); 0, 0.4, 0.6, 0.8, 1.0, and 1.1 equiv of $FeCl_3$ were added (black to green). The absorptions at 516 nm are plotted in (b). (c) Cyclic voltammogram of Fe(III)-**3** complex (100 μM of **3** and 60 μM of $FeCl_3$) in 50 mM BisTris buffer (pH 7.0, $I = 0.1$ M with NaCl).

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