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# Synthesis and structure confirmation of fuscachelins A and B, structurally unique natural product siderophores from *Thermobifida fusca*†

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**The fuscachelin siderophores have been prepared synthetically as have their metal chelation complexes. The heterodimeric nature of the fuscachelin decamer lends itself to a convergent synthetic strategy. Synthetic access to the natural products and intermediates will provide readily adaptable tools in future studies examining iron-sequestration and the biosynthetic machinery.**

The acquisition of the essential nutrient iron constitutes a serious challenge to microorganisms due to the virtual insolubility of ferric hydroxide complexes that form under aerobic growth conditions.[1] Siderophores are natural product secondary metabolites that are capable of solubilizing iron(III) *via* the formation of high-affinity chelation complexes.[2]

Several classes of siderophores are known with structurally diverse scaffolds that evolved to sequester iron. The natural products are assembled by both nonribosomal peptide synthetase (NRPS)-dependent and independent biosynthetic pathways.[3] Genome mining for orphan biosynthetic gene clusters has recently led to the discovery of novel natural products, including siderophores.[4,5] We recently described the structure elucidation of the fuscachelins (**1–3**, Fig. 1) using a genome mining approach.[6] The fuscachelins are produced by the moderately thermophilic actinomycete *Thermobifida fusca* and represent some of the only known secondary metabolites isolated from thermophilic bacteria.

The work revealed a novel structural scaffold for iron chelation along with the description of a unique biosynthetic gene cluster. The predicted parent natural product is the macrocyclic lactone, fuscachelin A (**1**). Two additional isolated products, fuscachelins B and C (**2**, **3**), were believed to be the result of hydrolysis and aminolysis, respectively, of **1**. Described here is the first total synthesis of this family of natural products.[7] The synthesis confirms the structure assignment of the fuscachelins in addition to providing a source of small molecule tools for use in future experiments aimed at probing the biosynthetic machinery and the chemistry and biology of iron acquisition.

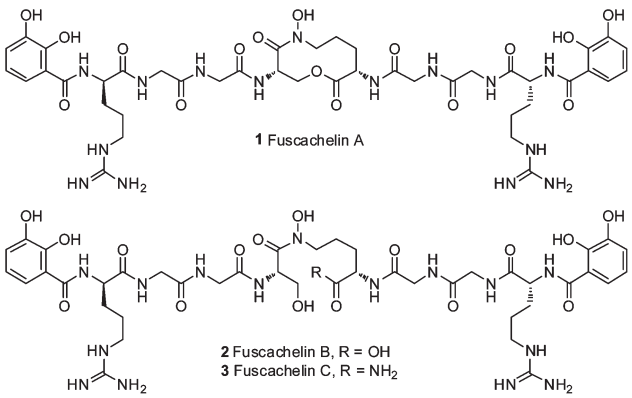


**Fig. 1** The fuscachelins.

The fuscachelins are decameric peptide siderophores with a scaffold consisting of eight peptide bonds, one hydroxamic acid linkage and one depsipeptide bond that serves to form the 10-membered lactone of fuscachelin A (**1**). The *N*-δ-hydroxyornithine/serine macrocycle provides a framework for the *cis*-hydroxamate necessary for metal binding and has no direct precedent.

The core dipeptide **6** contains hydroxamate functionality that is utilized in the natural product as a bidentate ligand in the chelation of ferric iron. The synthesis begins with activation of *N*-Boc-L-Ser(*O*-Bn) using DCC–HOBt, followed by addition of *O*-benzylhydroxylamine to afford the benzyl protected hydroxamate **4**. *N*-Boc-δ-bromo-L-norvaline benzyl ester **5** is prepared in two steps from *N*-Boc-L-Glu-OBn, serving as the precursor to the *N*-δ-hydroxyornithine (*HO*Orn) residue.[8] *N*-Alkylation of **4** is achieved *via* nucleophilic displacement of bromide **5** under basic conditions using potassium iodide as a catalyst to give a protected version of the Ser-*N*-δ-hydroxyornithine core dipeptide. Alkylation of protected hydroxamates frequently results in a mixture of *N*- and *O*-alkylation products. We screened various conditions and electrophiles (tosyl, iodo) and found that the use of KI with an alkyl bromide gave the desired N-alkylated product as the major regioisomer (~1.2 : 1), consistent with previous observations.[7d,9] Brief treatment of this intermediate with

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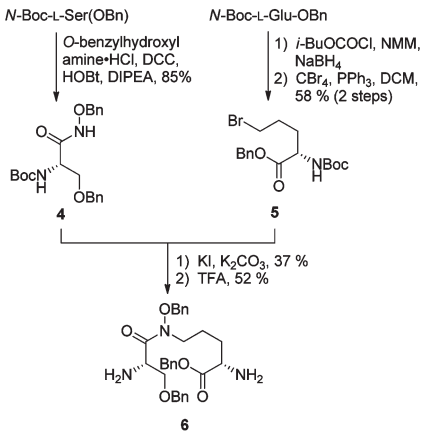
neat TFA affords the *O*-benzyl protected Ser-*HO*Orn building block (**6**, Scheme 1).

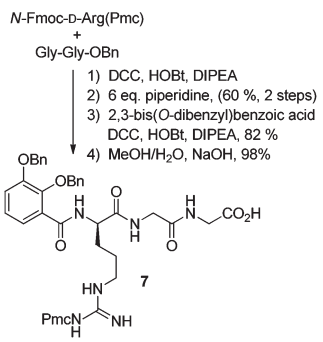
The core-flanking tetramer (**7**) is comprised of an D-Arg-Gly-Gly sequence N-capped with 2,3-dihydroxybenzoic acid (DHB). The four catecholate oxygen atoms serve as ligands for iron(III) in the natural product, and taken with the core-hydroxamate, provide the hexadentate coordination sphere for metal chelation. The synthesis of **7** utilizes solution phase peptide coupling methodology. A two step coupling–deprotection sequence provides the N-terminal deprotected D-Arg(Pmc)-Gly-Gly(OBn) tripeptide from *N*-Fmoc-D-Arg(Pmc) and Gly-Gly(OBn) starting materials. In an additional DCC mediated coupling reaction, 2,3-bis(dibenzyloxy)benzoic acid[10] is appended to the N-terminus to afford the fully protected tetrapeptide benzyl ester. Saponification with NaOH gives **7**, the liberated C-terminus of which is now available for coupling to core dipeptide **6** (Scheme 2).

With **6** and **7** in hand, the stage was set for the convergent assembly of the fully protected fuscachelin B decapeptide. This was achieved in one step *via* a DCC–HOBt mediated peptide coupling reaction, which gave the full length, fully protected peptide in 49% yield for the two couplings. The Pmc protecting groups were quantitatively removed from the arginine side chains *via* treatment with a solution of 95 : 5 : 0.1 of TFA : $H_2O$ : thioanisole (Scheme 3).

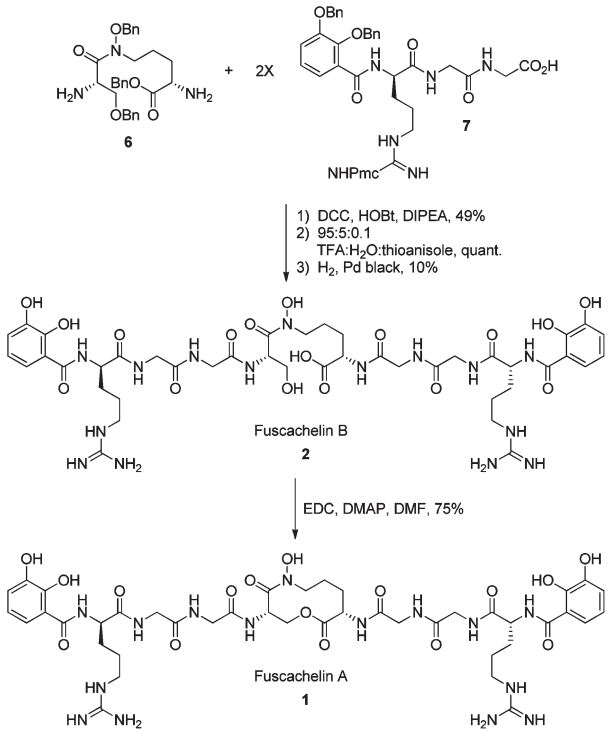
Removal of the seven benzyl groups posed an unexpected challenge. A one step, global deprotection of the peptide was envisioned, based on well established precedent, employing




**Scheme 1** Synthesis of core dipeptide **6**.




**Scheme 2** Synthesis of core-flanking tetramer **7**.



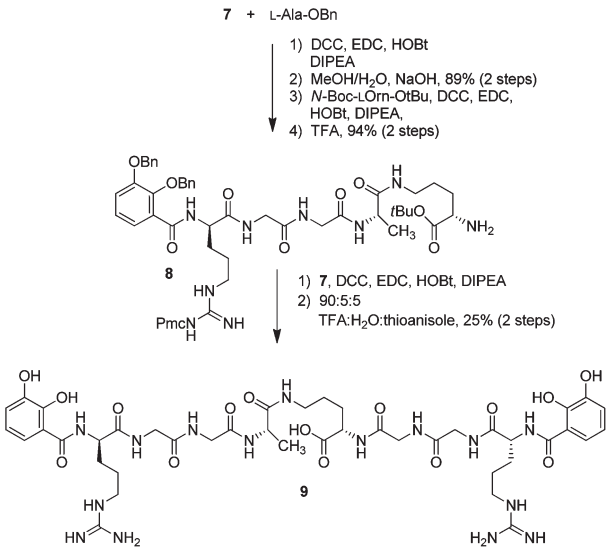

**Scheme 3** Convergent assembly of fuscachelin A.

commonly utilized hydrogenolysis conditions.[7a,c,d,11] Unfortunately, the benzyl protected peptide proved to be insoluble in common organic solvents. In addition, a screen of typical carbon supported metal hydrogenolysis catalysts failed to provided any product at both atmospheric and increased hydrogen pressures. Use of catalytic amounts of perchloric acid provided a small amount of the desired product, but resulted primarily in product decomposition.[7b] Ultimately, hydrogenolytic conditions (1 atm) employing Pd black as a catalyst in a 1 : 3 methanol : acetic acid solution yielded the fully deprotected fuscachelin B (**2**) (Scheme 3). The observed yield was not a result of turnover, but a consequence of high affinity of the product for the metal catalyst and low recovery from reverse phase preparative HPLC in the final purification.

Macrolactonization of **2** provides the parent compound, fuscachelin A (**1**). The acyl coupling reagents PyBOP, DCC, DMAP and HOBt were screened in varying combinations, as were the Yamaguchi–Yonemitsu macrolactonization conditions.[12] However, a combination of the reagents EDC and DMAP in DMF proved to be the highest yielding, giving the lactone in 75% yield after HPLC purification (Scheme 3). The synthetic samples of fuscachelins A and B match the natural samples isolated from *T. fusca* (see ESI†). Formation of a seven-membered lactone is a possible competing reaction in the condensation. However, a single product is observed and the final product is identical to the natural product, extensively characterized as the ten-membered seryl lactone.[6]

As a demonstration of the utility of the synthetic scheme, we undertook the preparation of an analog of the natural product for use as a probe into siderophore biosynthesis. We have a longstanding interest in the biosynthetic mechanisms of macrocyclization catalyzed by thioesterase domains of nonribosomal

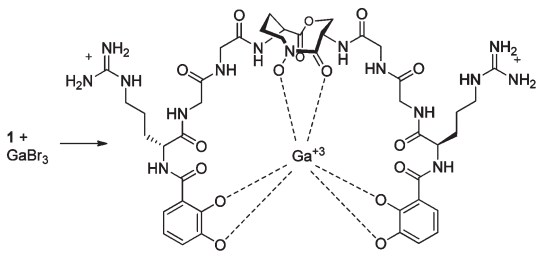



**Scheme 4** Synthesis of a fuscachelin analog for use as a probe into the biosynthetic pathway.

peptide synthetases and have recently demonstrated the utility of synthetic probes to provide a structural basis of enzyme function.[13] To probe the mechanism of enzyme catalyzed cyclization of fuscachelin A, a non-cyclizable analog was designed lacking the nucleophilic serine hydroxyl. In addition, the hydroxamate functionality was removed to decrease metal binding and ease handling. The common intermediate **7** is coupled to L-Ala-*O*Bn then *N*-Boc-L-Orn-O*t*Bu using standard conditions. A second equivalent of **7** is then added to the free amine and global deprotection provides the desired compound. For this analog, iterative coupling of **7** was favored as standard peptide coupling can be used for each building block (Scheme 4).

Siderophores have evolved to selectively bind, solubilize and transport ferric iron into iron-starved cells. Once charged with metal, highly conserved protein pathways are employed in the processes of ferri-siderophore recognition, uptake, iron release and metal trafficking.[2,14] Several well characterized systems have been studied using $Ga^{3+}$-siderophore complexes as ferric-siderophore mimicks.[14] Unlike their paramagnetic, ferric counterparts, these complexes offer the possibility of examination using NMR spectroscopy.[15a,b,e,16] In addition, gallic-siderophore complexes have proven their worth in the crystallographic analysis of protein-siderophore co-complexes.[14c] Due to the versatility of these complexes in numerous studies and the importance of confirming the metal-chelating ability of the synthetic molecules, we prepared gallic complexes of the fuscachelins. This can be done in a straightforward manner by treating fuscachelin A or B with an excess of aqueous $GaBr_3$ (Scheme 5).[15a] The complexes were purified using reverse phase HPLC and characterized by mass spectrometry (see ESI†). Synthetic access to pure $Ga^{3+}$-fuscachelin complexes will facilitate the study of proteins implicated in iron metabolism in actinomycetes, an ongoing effort in our laboratory.

Here we have outlined a facile synthetic route to the fuscachelins, novel siderophore natural products isolated from the actinomycete *T. fusca*. The fuscachelins represent an unprecedented scaffold for siderophore-based acquisition of iron.




**Scheme 5** Preparation of $Ga^{+3}$-fuscachelin A.

Completion of their total synthesis confirms the recently determined structures of the isolated natural products. The biomimetic route provides useful small molecule tools in our ongoing studies of peptide biosynthesis. In addition, the synthetic route provides facile access to the siderophores and analogues for the future study of high affinity metal chelation and biological iron acquisition.

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