Total Syntheses and Evaluation of the Siderophore Functions of Fimsbactin B and Its Analogs

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Iron is an essential element for the survival of all forms of life, in which it functions as a cofactor during key metabolic processes. However, due to the low aqueous solubility of Fe(III), most organisms possess dedicated systems for active assimilation of iron from the aerobic environment. Siderophores are small-molecule, high-affinity Fe(III) chelators produced and utilized by most bacteria for this purpose. Currently, more than 500 siderophores have been identified, and some have been extensively studied to elucidate their physiological functions and mechanisms.[1,2] Because of the essentiality of iron for bacterial homeostasis and infection, siderophore-related mechanisms have received considerable attention as promising, novel targets for the discovery of antibiotics.[3] Among the various approaches in this regard, development of siderophore–drug conjugates (SDCs) has attracted particular interest,[3–6] in which the siderophores are utilized as carrier vectors to deliver the conjugated drugs intracellularly. Because the uptake of SDCs mediates designated transport machineries, they have been considered to be effective in treating Gram-negative pathogens, particularly of which the drug resistance is associated with the restricted outer membrane (OM) permeability.[7–9]

Acinetobacter baumannii is a Gram-negative bacterium and one of the ESKAPE pathogens, referring to highly problematic drug-resistant strains.[10,11] Indeed, nosocomial and community infection of *A. baumannii* has been an increasing cause of concern due to its rapid acquisition of resistance to clinically employed antibiotics including carbapenems.[12–14] Notably, it has been shown that *A. baumannii* features intrinsically high OM permeability barrier compared to other Gram-negative pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa*.[15] Thus, the employment of the SDC strategy seems perfectly suited to overcome this recalcitrant bacterium, and indeed a number of promising early results in this direction have been reported.[16,17]

For a while, acinetobactin has been known as the sole siderophore produced and utilized by *A. baumannii*,[18,19] but recently an alternative set of siderophores called fimsbactins were isolated from *A. baumannii* ATCC 17978 as well as nonpathogenic *Acinetobacter baylyi* ADP1 strains by Proschak *et al.*[20] In their report, structural characterization of six fimsbactins (A–F) (Figure 1(a)), as well as a proposal for their biosynthesis, was described. However, the biological and physicochemical properties of fimsbactins have yet to be established. In this regard, we have devised an effective synthetic route to fimsbactins to study fimsbactin-based SDCs effective for *A. baumannii*. Here we describe the successful syntheses of fimsbactin B and its analogs, and also the *in vivo* evaluation of their siderophore functions.

Our retrosynthetic analysis for fimsbactin B (**2**) is outlined in Figure 1(b), in which the backbone construction is accomplished through an assembly of two fragments, oxazoline carboxylate **7** and the putrescine derivative **8**. We opted to employ Mo(VI) oxide-catalyzed dehydrative cyclization, recently developed by Ishihara *et al.*,[21–23] for synthesis of an oxazoline moiety, because this biomimetic approach would be advantageous over other methods, since it would allow the establishment of the correct stereochemistry from natural amino acid derivatives with L-configuration.

The synthesis of amine fragment **8** was accomplished by adopting Bergeron's reaction sequence[24] with some modifications, initiated by the substitution of 4-chlorobutyronitrile (**9**) with *N*-Boc-*O*-benzyloxyamine (Scheme 1(a)). The Boc protecting group of **10** was removed with trifluoroacetic acid, and the following *N*-acetylation established the hydroxamate moiety in an *O*-benzyl protected form. Then, chemoselective hydrogenation of **11** using Raney-Ni as a catalyst allowed reduction of a nitrile group to afford amine **12**. The primary amine **12** was then coupled with *N*,*O*-protected L-serine **13**[25] with EDC, and subsequent brief exposure to trifluoroacetic acid led to chemoselective Boc deprotection to afford the desired fragment **8**.

The preparation of oxazoline carboxylate **7** started from the *o*-xylyl-protected 2,3-dihydroxybenzoic acid **15** (Scheme 1(b)). As proposed by Sakakura *et al.*,[22] the *o*-xylyl protection was crucial for the success of fimsbactin synthesis, because the employment of other protective groups such as dibenzyl had detrimental effects on the downstream modifications due to the bulkiness.[26–28] After amide coupling of acid **15** with L-Thr-OCH_3, a dehydrative oxazoline formation to provide **17** was successfully accomplished by treatment of **16** with a catalytic amount of $MoO_2(TMHD)_2$ in toluene under azeotropic reflux conditions. Interestingly, when the same precursor (**16**) was treated with Burgess' reagent, production of

an epimer of **17** with 9(*S*),10(*S*)-*syn*-configuration was observed, clearly demonstrating the mechanistic difference between these two oxazoline formation conditions.[21] The ester moiety in **17** was then hydrolyzed with KOTMS,[29] and the subsequent amide formation with **8** proceeded seamlessly with no hint of epimerization at C-10. The introduction of the second catechol group involved the removal of the silyl protection of **18** using HF•pyridine complex followed by carbodiimide-promoted esterification with **15,** giving rise to the penultimate intermediate **19**. Finally, completion of total synthesis of fimsbactin B (**2**) was realized by global deprotection under palladium-catalyzed hydrogenolysis conditions.

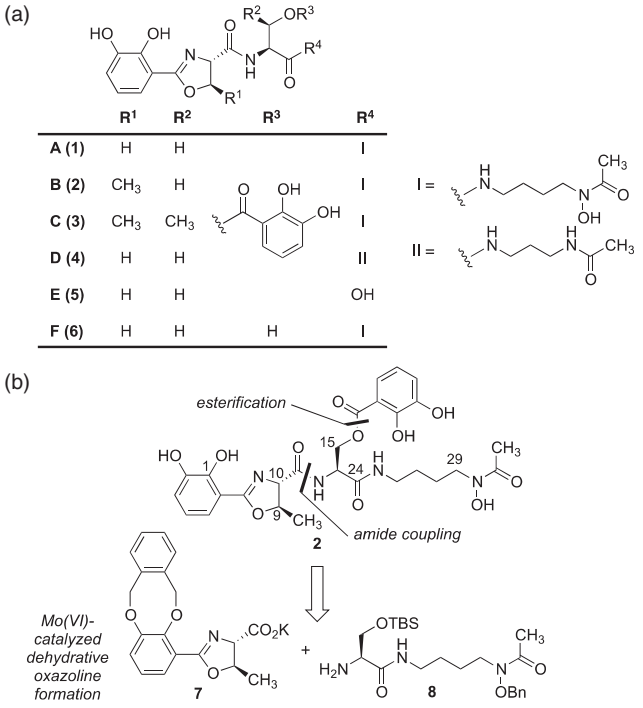
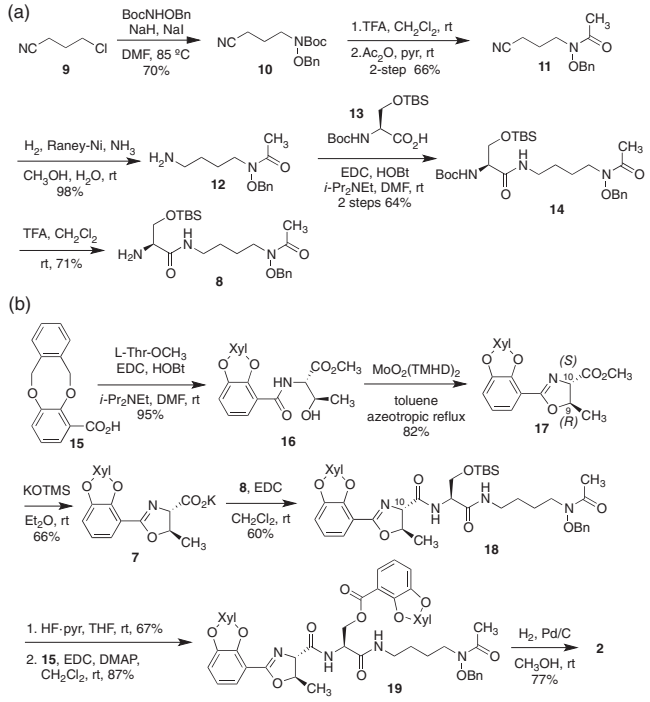
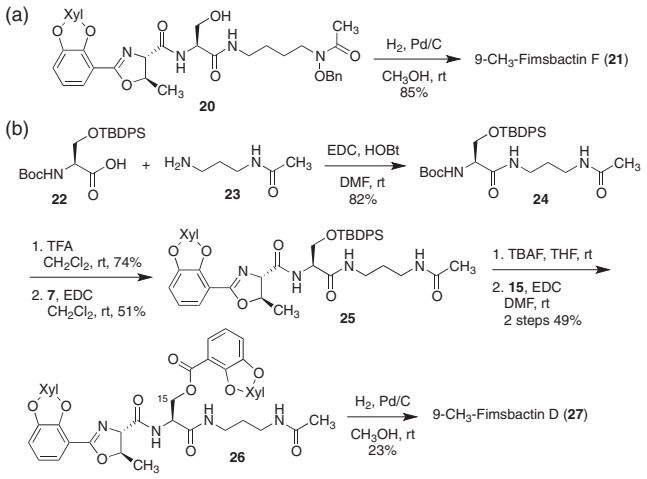


Figure 1. (a) Structures of fimsbactin A–F. (b) Retrosynthesis for the total synthesis of fimsbactin B (**2**).



Scheme 1. (a) Synthesis of amine fragment **8**. (b) Completion of total synthesis of fimsbactin B (**2**).

Although the exact mode of the Fe(III) binding by fimsbactins is unknown, formation of the Fe(III) complex with an octahedral geometry for fimsbactin A (**1**), B (**2**), and C (**3**) is conceivable involving two catechol and one hydroxamate moieties, each as a bidentate ligand. However, other fimsbactins, D (**4**), E (**5**), and F (**6**), are deficient in one of those ligands, raising a question whether they would indeed function as siderophores. To address this issue, we pursued the syntheses of 9-methyl variants of fimsbactin D (**27**) and F (**21**) by slight modifications of the synthetic scheme for fimsbactin B (**2**). As delineated in Scheme 2(a), 9-CH_3-fimsbactin F (**21**) could be readily prepared by hydrogenolytic deprotection of the intermediate **20**. The synthesis of 9-CH_3-fimsbactin D (**27**) began with the amide formation between *N*-monoacetyl-1,3-propanediamine (**23**)[30] and *N*,*O*-protected L-serine derivative **22**[25] followed by chemoselective removal of the Boc group (Scheme 2(b)). The employment of the TBDPS moiety in **22** was advantageous over TBS in **13**, because the isolation of the resulting polar free amine intermediate was more facile due to the higher lipophilicity of TBDPS. In addition, gratifyingly, the bulkiness of TBDPS had no detrimental effects on the EDC-mediated coupling with **7** to afford **25**, and the TBDPS ether could be readily cleaved by treatment of TBAF. Finally, the attachment of the catechol ester at C-15 followed by global deprotection under hydrogenolysis conditions successfully led to the formation of 9-CH_3-fimsbactin D (**27**) albeit in a low yield of 23%, likely due to the low solubility of **27** in methanol.



Scheme 2. (a) Synthesis of 9-CH_3-fimsbactin F (**21**). (b) Synthesis of 9-CH_3-fimsbactin D (**27**).

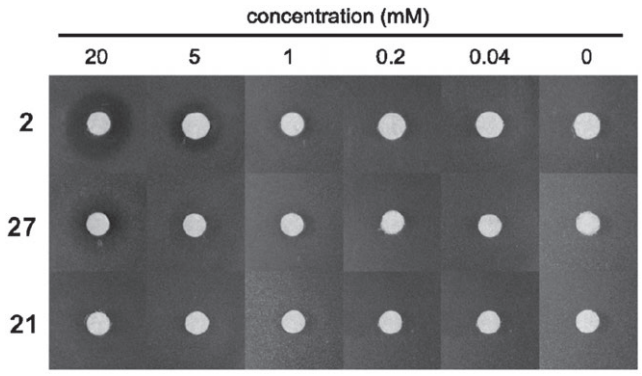


Figure 2. Evaluation of the siderophore functions of fimsbactin B (**2**) and its analogs (**21** and **27**) based on the agar diffusion assay. Paper disks were placed on iron-deficient agar plates (250 μM 2,2′-dipyridyl) overlaid with *A. baylyi* ADP1, and each was treated with 5 μL of the sample in DMSO of the indicated concentration. After 6 h of incubation at 37 °C, formation of a zone surrounding each disk was monitored.

With three fimsbactins, **2**, **21**, and **27**, in hand, their siderophore functions were evaluated based on the agar diffusion assay, as shown in Figure 2.[31] At the outset, we had predicted that only fimsbactin B (**2**) would promote the cellular growth around the paper disc by functioning as a siderophore because of its possession of all three bidentate ligands, in contrast to the analogs of fimsbactin D (**27**) and F (**21**) lacking the hydroxamate or the catechol moiety, respectively. However, to our surprise, not only all of these three compounds failed to elicit any growth promotion under the iron-limiting conditions, but fimsbactin B (**2**) and 9-CH_3-fimsbactin D (**27**) were instead found to display inhibitory activity in a concentration-dependent manner indicated by the clear zone formation around the disc (also see Figure S1 in Supporting Information). Among them, the inhibitory activity of **2** appeared to be more pronounced than that of **27,** judged by comparing the sizes of the clear zones (diameters of ~19 mm vs. 12 mm, respectively, at each 20 mM). These unexpected antagonistic effects of **2** and **27** on the growth of *A. baylyi* is likely due to their metal chelating ability, further depleting the iron in the growth media, the observation of which is not unprecedented.[32]

Here we reported the first total synthesis of fimsbactin B (**2**) as well as its analogs 9-methyl variants of fimsbactin D (**27**) and F (**21**). Surprisingly, *in vivo* biological assays unveiled that none of them is capable of assimilating iron to promote the cellular growth under the iron-deficient conditions, presumably due to their failure to interact with the cellular fimsbactin uptake machineries (*e.g.*, FbsN, FbsO, and FbsP).[20] This unexpected finding, contrasted with their proposed physiological roles, suggests that fimsbactin A (**1**), the major metabolite (>90%) isolated from *A. baylyi*, would be the genuine siderophore for its producer. This implication is rather striking considering that the structural difference between **1** and **2** is merely the methyl substituent at C-9. Certainly, this claim still awaits experimental corroboration of the physiological function of fimsbactin A (**1**) as the siderophore, although this role of **1** has received indirect support from the observation that its biosynthesis is regulated in response to the environmental iron availability.[20] To address this issue, we attempted to synthesize fimsbactin A (**1**) by slight modifications of Scheme 1 utilized for the preparation of fimsbactin B (**2**). Unfortunately, however, the amide formation between **8** and 9-desmethyl-**7** led to epimerization at C-10 in contrast to the case in fimsbactin B synthesis. Thus, we are currently pursuing the optimization of this key step to resolve the stereoselectivity issue, of which establishment would be crucial not only for confirmation of the biological function of fimsbactin A (**1**) but also eventually for the design of effective fimsbactin-based SDCs overcoming the OM barrier of drug-resistant *A. baumannii*.

Experimental

Fimsbactin B (2). A catalytic amount of palladium on activated carbon (10 wt%) was suspended in a solution of compound **19** (43.0 mg, 0.0487 mmol) in methanol (2 mL) at room temperature, and this reaction mixture was charged with hydrogen gas in a balloon (1 atm). After 2 h, the reaction mixture was filtered through a pad of celite to remove the palladium catalyst, and the filtrate was concentrated under reduced pressure to afford the desired product fimsbactin B (**2**) (22.0 mg, 0.0374 mmol) in 77% yield without further purification.

^{1}H-NMR (500 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.63 (d, J = 8.1 Hz, 1H), 8.30 (s, 1H), 7.14 (d, J = 7.9 Hz, 1H), 7.06 (d, J = 7.7 Hz, 1H), 6.99 (*br* s, 2H), 6.73 (t, J = 7.7 Hz, 1H), 6.59 (t, J = 7.8 Hz, 1H), 4.87 (m, 1H), 4.74 (q, J = 6.6 Hz, 1H), 4.64–4.55 (m, 2H), 4.39 (dd, J = 10.9, 6.9 Hz, 1H), 3.46 (t, J = 6.7 Hz, 2H), 3.16–3.06 (m, 2H), 1.96 (s, 3H), 1.53–1.46 (m, 2H), 1.45 (d, J = 6.3 Hz, 3H), 1.41–1.36 (m, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.19, 169.82, 168.91, 167.85, 165.95, 149.49, 148.34, 146.03, 145.77, 120.84, 119.77, 119.46, 118.77, 118.65, 117.90, 112.77, 110.25, 78.65, 73.35, 64.46, 51.51, 46.47, 38.49, 26.11, 23.75, 20.50, 20.39. HR-MS (ESI-TOF) *m/z* for $[C_{27}H_{32}N_4NaO_{11}]^+$ ($[M + Na]^+$): calcd 611.1960, found 611.1960.

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Supporting Information. The detailed information including synthetic procedures, spectral data for all new compounds, and additional results of the *in vivo* cell-based assays is available free of charge via the Internet at http://kcsnet.or.kr

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