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Total Synthesis of Teixobactin

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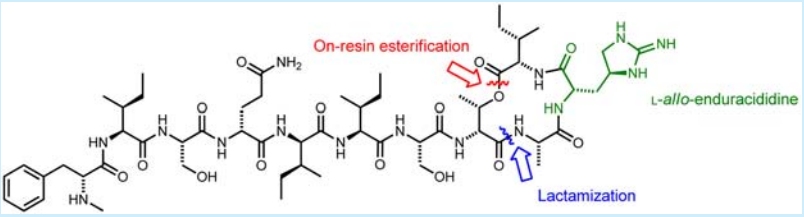
Supporting Information



ABSTRACT: The first total synthesis of the cyclic depsipeptide natural product teixobactin is described. Synthesis was achieved by solid-phase peptide synthesis, incorporating the unusual L-*allo*-enduracididine as a suitably protected synthetic cassette and employing a key on-resin esterification and solution-phase macrolactamization. The synthetic natural product was shown to possess potent antibacterial activity against a range of Gram-positive pathogenic bacteria, including a virulent strain of *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus* (MRSA).

The emergence of drug resistant strains of pathogenic bacteria has compromised the effectiveness of a growing number of clinically employed antibiotics.[1] *Mycobacterium tuberculosis* (*Mtb*), the etiological agent of tuberculosis (TB), is an example of a pathogen to which widespread resistance to frontline antibiotic treatments has developed.[2−4] *Mtb* is estimated to latently infect one-third of the global population, and of the 9.6 million new cases of TB in 2014, a significant proportion were infected with drug-resistant strains of *Mtb*, thus complicating treatment and compromising global efforts to eradicate the disease.[4] Unfortunately, the growing burden of antibiotic resistance is coupled with decreased effort in the development of new antibiotics.[5] Indeed, of the antibiotics currently in clinical trials, the majority are variations on current drug architectures, e.g., rifapentine and delamanid for TB.[6,7] It is well established that nature provides a rich source of diverse molecules with privileged antibacterial activity, highlighted by the fact that numerous clinically approved antibiotics are natural products or derivatives thereof.[8,9] However, very few genuine antibiotic leads have been discovered from natural sources over the past two decades.

In early 2015, Ling et al.[10] reported the isolation and characterization of a novel peptidic natural product called teixobactin (**1**, Figure 1) from a previously "uncultivable" soil bacterium *Eleftheria terrae*. Teixobactin was discovered using iChip, a new technology that enabled the bacterium to be cultured for the production of sufficient material for isolation and structural and functional characterization. The natural product was shown to exhibit potent antibacterial activity against a wide range of Gram-positive bacteria including virulent (H37Rv) and drug-resistant clinical isolates of *Mtb* (MIC = 0.125 μg mL^{-1}) and methicillin-resistant *Staphylococcus aureus* (MRSA, MIC = 0.25 μg mL^{-1}). Structurally, teixobactin is an undecadepsipeptide with a cyclized *C*-terminus and a methylated *N*-terminus. The natural product possesses four D-amino acids, the unusual amino acid L-*allo*-enduracididine, and has some structural similarities to other natural products, e.g. hypeptin and mannopeptimycin.[11,12]

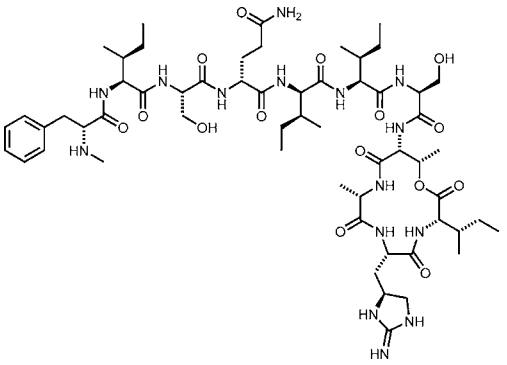


Figure 1. Structure of teixobactin (**1**).

Teixobactin was shown to exhibit its antibacterial action by binding to lipid II and lipid III, two key enzymatic substrates in the biosynthesis of peptidoglycan and teichoic acid, respectively.[10,13] Ling et al. attempted to generate teixobactin-resistant

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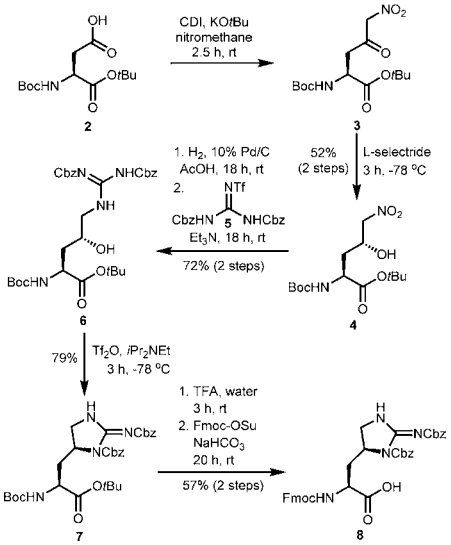
mutants in *S. aureus* and *Mtb* by treating the organisms with sublethal doses of the natural product; however, no resistant mutants could be generated.[10] This striking result is thought to be due to teixobactin binding to multiple enzymatic substrates rather than to an enzyme; ultimately, gaining resistance by mutating the substrate for an enzyme is inherently more difficult for an organism than mutating amino acids within an enzyme. This mechanism of action coupled with the potent antibacterial activity against a range of clinically relevant pathogens has made teixobactin a realistic antibiotic candidate for Gram-positive and *Mtb* infections and an attractive target for total synthesis.

While no total synthesis of teixobactin has been reported, an efficient synthesis of the unnatural amino acid L-*allo*-enduracididine was published by Craig et al.[14] In addition, Jad et al.[15] and Parmar et al.[16] both reported the synthesis of an analogue of the natural product in which the synthetically challenging L-*allo*-enduracididine residue was replaced by a simplified L-arginine residue. A second analogue was also reported by Parmar et al.,[16] whereby the D-configured amino acids, except threonine, were replaced by L-configured residues. Both analogues exhibited less potent inhibition of *S. aureus*, revealing the importance of both the D-configured amino acids and the L-*allo*-enduracididine residue for antibacterial activity of the natural product. In this study, we sought to develop a robust synthetic route to teixobactin (**1**) which would be efficient, amenable to rapid analogue generation, and would ultimately facilitate thorough profiling of the antibiotic activity.

We began by preparing a suitably protected L-*allo*-enduracididine building block that could be installed directly into Fmoc-SPPS. The early steps in our synthesis took inspiration from Rudolph et al.[17] Specifically, nitromethane addition to the free carboxylate side chain of Boc-L-Asp-O*t*Bu (**2**) provided nitroketone **3** (Scheme 1). Stereoselective reduction of

Scheme 1. Synthesis of Suitably Protected L-*allo*-Enduracididine Building Block 8



the ketone in **3** with L-Selectride provided a diastereomeric mixture (dr: 5:1 (2*S*,4*R*):(2*S*,4*S*)) which was readily separable by flash column chromatography to afford alcohol **4** as a single diastereoisomer in 52% yield over two steps. From here, hydrogenation of the δ-nitro moiety,[18] followed by guanidinylation of the resulting amine with a bis-Cbz-protected variant of Goodman's guanidinylating reagent **5**, gave **6** in 72% yield over the two steps. Triflation of the γ-alcohol in **6** under basic conditions then afforded cyclic guanidine **7** in good yield. Finally, acidic deprotection of the α-amine and α-carboxylate followed by Fmoc protection furnished the target building block **8** [Fmoc-End(Cbz)$_2$-OH] in 57% yield over two steps.

With the suitably protected L-*allo*-enduracididine building block **8** in hand, we next began assembly of the depsipeptide chain of teixobactin. It was envisaged that the depsipeptidic core of the natural product could be assembled using Fmoc-SPPS including a key on-resin esterification step. It was proposed that, following cleavage of the resin under weakly acidic conditions, a solution-phase cyclization followed by global side-chain deprotection (including of the Cbz protection on the enduracididine residue) under strongly acidic conditions would afford the natural product.

Initial efforts involved the loading of Fmoc-D-Thr-OH (with an unprotected side chain) to 2-chlorotrityl chloride (2-CTC) functionalized polystyrene resin followed by coupling of Fmoc-L-Ser(*t*Bu)-OH. At this point, the key on-resin esterification step with protected L-Ile was attempted using a number of esterification conditions. Surprisingly, we could not find an effective set of conditions to facilitate this on-resin transformation, with starting material remaining even after multiple treatments. We reasoned that the steric bulk of the 2-CTC linker adjacent to the side chain hydroxyl of the D-Thr side chain was impeding esterification, and as such, we sought a less sterically encumbered resin linker as an alternative. Toward this end, we selected (4-(hydroxymethyl)-3-methoxyphenoxy)acetic acid (HMPB) functionalized polyethylene glycol-based NovaPEG resin, taking advantage of the decreased steric bulk surrounding the loaded amino acid as well as the increased swelling properties provided by the polyethylene glycol-based support.[19] Fmoc-D-Thr(TES)-OH (**9**) was loaded to the resin via the symmetrical anhydride (generated by treatment with *N*,*N*′-diisopropylcarbodiimide (DIC) and 4-(dimethylamino)pyridine (DMAP) to afford **10** (Scheme 2). Next, the Fmoc group was removed via treatment with piperidine in DMF, followed by removal of the triethylsilyl (TES) protecting group by double treatment with acetic acid buffered tetrabutylammonium fluoride (TBAF). Fmoc L-Ser(*t*Bu)-OH was next coupled using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as the coupling reagent and 4-methylmorpholine (NMM) as the base in DMF to afford **11**. The key on-resin esterification to the D-Thr side chain was then attempted with Alloc-L-Ile-OH using DIC and catalytic DMAP as the esterification conditions. Gratifyingly, complete esterification was achieved in one 16 h treatment to provide depsipeptide **12** as judged by LC−MS analysis. Having successfully branched the peptide chain, the remaining linear portion of the target peptide was extended using conventional Fmoc-SPPS, including incorporation of the L- and D-configured amino acids within the natural product and the *N*-terminal *N*-methyl-Boc-D-Phe-OH to afford resin-bound **13**.

Following the successful assembly of resin-bound **13**, the synthesis continued on the branched L-Ile residue (Scheme 3). Specifically, on-resin Alloc deprotection was achieved by treatment with Pd(PPh$_3$)$_4$ and PhSiH$_3$. The next step involved coupling of the suitably protected L-*allo*-enduracididine building block Fmoc-End(Cbz)$_2$-OH **8**, which was smoothly effected through the use of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) as the coupling reagent in combination with 1-hydroxy-7-azabenzotriazole (HOAt) as an additive and Hünig's

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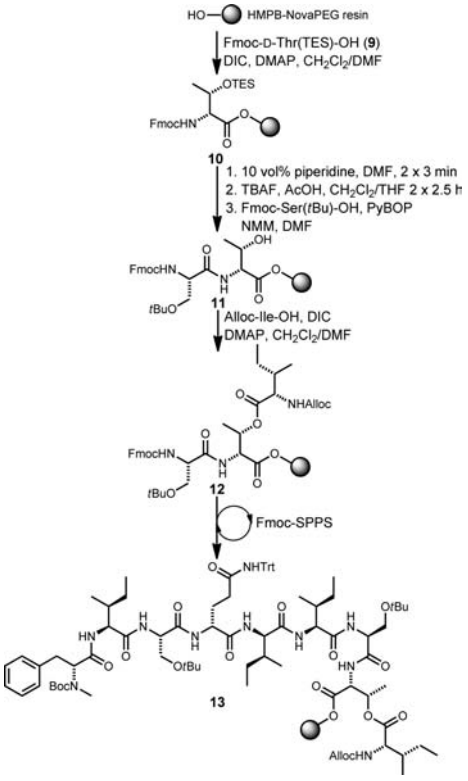
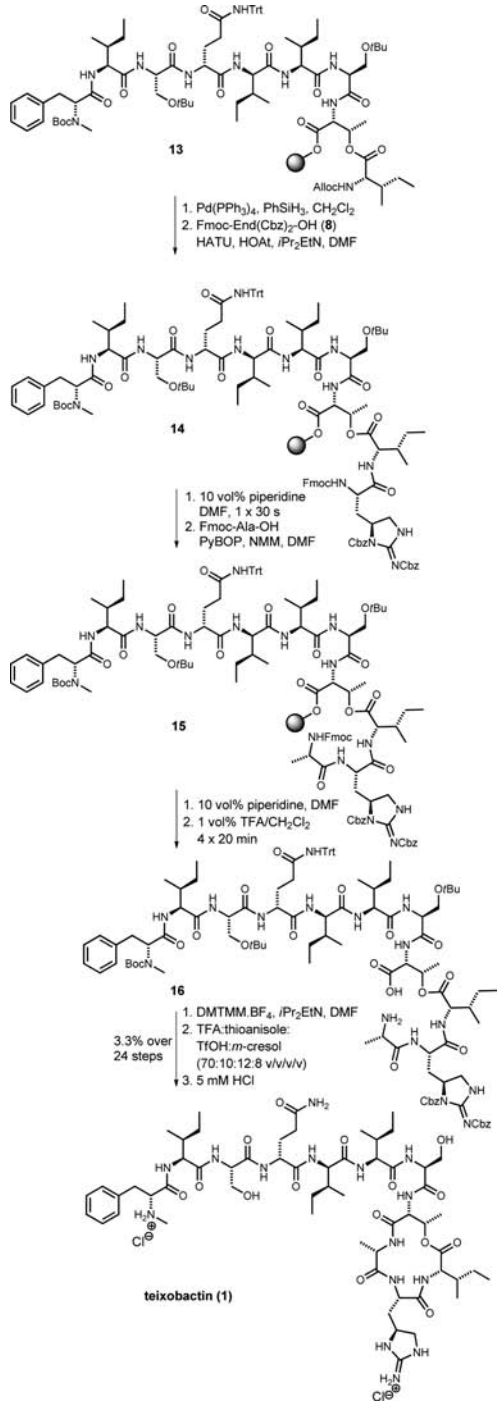
Scheme 2. Synthesis of Resin-Bound Depsipeptide Teixobactin Precursor 13



Scheme 3. Synthesis of Teixobactin (1)



base to afford resin-bound **14**. At this point, **14** was subjected to conventional Fmoc deprotection conditions (10 vol % piperidine in DMF, 2 × 3 min). Unfortunately, this treatment led to the formation of a de-esterified resin-bound peptide, presumably due to unwanted diketopiperazine formation caused by the nucleophilic cyclization of the α-amine of the deprotected L-*allo*-enduracididine residue onto the α-carboxyl of L-isoleucine.[20,21] To overcome diketopiperazine formation, the resin was treated with 10 vol % of piperidine in DMF for a shorter duration (30 s) and washed rapidly with DMF and DCM before immediately treating the resin with a preactivated solution of Fmoc-L-Ala-OH, PyBOP, and NMM in DMF. This led to the formation of resin-bound **15** with minimal diketopiperazine formation. The Fmoc protecting group from the coupled L-Ala residue was next removed under standard conditions followed by cleavage of the linear side-chain-protected depsipeptide **16** from the resin using 1% TFA in CH_2Cl_2.

With depsipeptide **16** in hand, and without purification, we next performed the key macrolactamization step by treating **16** with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM·BF_4) and Hünig's base in a dilute (10 mM) solution of DMF, which provided the cyclized side-chain protected depsipeptide after 16 h. All that remained for the completion of the target was removal of the side-chain protecting groups and purification. Using deprotection conditions reported by Koide et al.[22] for the 4-methoxybenzyl protecting group, and later adopted by Hondal et al.,[23] we were able to remove all the protecting groups, including the Cbz moieties, in one step using a mixture of 70:12:10:8 v/v/v/v TFA, trifluoromethanesulfonic acid (TfOH), thioanisole, and *m*-cresol. Subsequent purification by RP-HPLC and lyophylization yielded teixobactin as a TFA salt. This was then lyophilized multiple times in the presence of 5 mM HCl[24] to yield teixobactin as the bis-HCl salt in 3.3% yield (over 24 steps from original resin loaded amino acid **10**, average of 87% per step). This conversion to the bis-HCl salt was carried out to enable direct comparison with the isolated natural product, which was characterized in this salt form. Gratifyingly, all spectroscopic data were consistent with that reported for the isolated natural product reported by Ling et al.[10]

We next assessed the antibacterial activity of **1** to further characterize the synthetic natural product. Specifically, synthetic teixobactin **1** was screened against the virulent H37Rv strain of *Mtb* using a resazurin-based assay[25] and against a range of Gram-negative and Gram-positive strains including *Bacillus subtilis* 168,

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Staphylococcus aureus, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli, Providencia alcalifaciens, Ochrobactrum anthropi, Enterobacter aerogenes, Acinetobacter baumannii, Vibrio cholerae, Salmonella typhimurium, Pseudomonas aeruginosa,* and *Yersinia pseudotuberculosis* using standard methods.[26] The activity of synthetic **1** against these organisms was consistent with that for the natural product reported by Ling et al.,[10] despite some differences in the strains of the organisms used in this study (Table 1). Specifically, **1** exhibited potent activity against *Mtb*

Table 1. Activity of Synthetic Teixobactin (1) against a Panel of Pathogenic Bacteria[a]

organism	teix MIC (μM)	vanc MIC (μM)	line MIC (μM)	cipro MIC (μM)
S. aureus (MSSA)	1.1	0.69	1.4	0.69
S. aureus (MRSA)	1.1	0.87	1.2	>66
E. coli	>27	>66	>66	0.013
B. subtilis	0.21	0.17	0.22	0.13
P. alcalifaciens	>27	>66	>66	0.027
O. anthropi	>27	>66	>66	0.85
E. aerogenes	>27	>66	>66	0.022
A. baumanii	>27	>66	>66	2.4
V. cholerae	>27	>66	>66	0.016
S. typhimurium	>27	>66	>66	0.027
P. aeruginosa	>27	>66	>66	1.4
Y. pseudotuberculosis	>27	>66	>66	0.0081

[a]teix = teixobactin. Controls: vanc = vancomycin, line = linezolid, cipro = ciprofloxacin. MIC against *Mtb* H37Rv = 1.5 ± 0.03 μM. Control: rifampicin MIC = 10.7 ± 0.33 nM.

with an MIC of 1.5 μM, *S. aureus* and MRSA (MIC = 1.1 μM), and *B. subtilis* (MIC = 0.21 μM). Unsurprisingly, teixobactin **1** showed no activity up to the highest tested concentration against the tested Gram-negative pathogens.

In summary, we have developed a solid-phase synthetic route to access teixobactin (**1**), a potent antibacterial natural product. The synthetic natural product was shown to possess potent antibacterial activity against *Mtb* and a number of pathogenic Gram-positive organisms. The work described here lays the foundation for generating analogues of teixobactin with a view to developing potential candidates for the treatment of TB and Gram-positive infections.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01324.

Experimental procedures for synthesis, antibacterial screening and characterization data, and NMR spectra of teixobactin (**1**) and all novel intermediates (PDF)

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Author Contributions

⊥A.M.G. and L.J.D. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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