Organic LETTERS
Cite This: Org. Lett. XXXX, XXX, XXX−XXX
Letter
pubs.acs.org/OrgLett

Total Synthesis of Ecumicin

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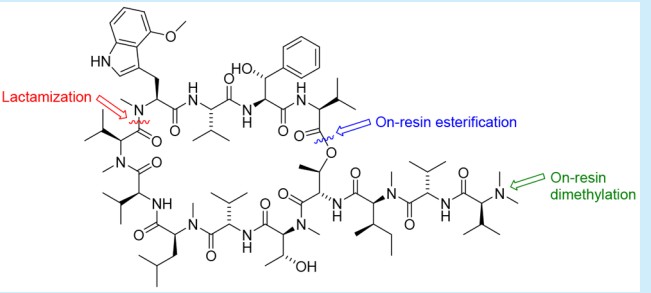
Supporting Information

ABSTRACT: The first total synthesis of the potent antimycobacterial cyclic depsipeptide natural product ecumicin is described. Synthesis was achieved via a solid-phase strategy, incorporating the synthetic non-proteinogenic amino acids *N*-methyl-4-methoxy-L-tryptophan and *threo*-β-hydroxy-L-phenylalanine into the growing linear peptide chain. The synthesis employed key on-resin esterification and dimethylation steps as well as a final macrolactamization between the unusual *N*-methyl-4-methoxy-L-tryptophan unit and a bulky *N*-methyl-L-valine residue. The synthetic natural product possessed potent antimycobacterial activity against the virulent H37Rv strain of *Mycobacterium tuberculosis* (MIC_{90} = 312 nM).



Tuberculosis (TB) is caused by infection with the bacterium *Mycobacterium tuberculosis* (*Mtb*) and was responsible for 1.3 million deaths in 2016, making it the most deadly infectious disease globally.[1] While effective treatment options are available for TB, this is dependent on the infection being drug-sensitive.[2] Alarmingly, there has been a steady increase in the number of cases of multiple-drug-resistant (MDR) and extensively drug-resistant (XDR) TB over the past decade, where treatment relies on the use of second-line antibiotics with increased toxicity.[1,2] The emerging TB drug resistance crisis has been fueled by poor compliance to the long and complex TB treatment regimens (6−24 months of a cocktail of drugs) as well as the narrow modes of action of the limited pool of approved antibiotics for TB treatment. As such, there is an urgent need for new antibiotics which operate via novel modes of action and that may shorten TB therapy.[3,4]

Ecumicin (**1**) is a cyclic depsipeptide natural product from an actinobacteria *Nonomuraea* sp. MJM5123 which was discovered following a high-throughput screening campaign by Gao et al. (Figure 1).[5] The natural product was shown to exhibit potent antibacterial activity against the virulent H37Rv strain of *Mtb* (MIC_{90} = 160 nM) and crucially retained activity against MDR and XDR strains of *Mtb*. Of particular interest is that ecumicin has been shown to possess *in vivo* activity with essentially complete inhibition of *Mtb* growth in mice after 12 doses at 20 mg kg^{-1}. Structurally, ecumicin is a tridecapeptide consisting of all L-amino acids, extensive backbone *N*-methylation, a dimethylated N-terminus, and two non-proteinogenic amino acids: *N*-methyl-4-methoxy-L-tryptophan and *threo*-β-hydroxy-L-phenylalanine.

The molecular target of ecumicin has also been elucidated through the spontaneous generation of ecumicin-resistant *Mtb* strains followed by gene sequencing.[6] Specifically, ecumicin is thought to stimulate ATPase activity of the chaperone protein ClpC1 while also invoking a marked decrease in proteolysis by the ClpC1-mediated ClpP1P2 protease complex. This results in the uncoupling of ATP hydrolysis from proteolysis. It is proposed that the accumulation of toxic undegraded proteins is responsible for the antimycobacterial effects of the natural product. Importantly, the Clp protease is essential in mycobacteria but not in other bacteria[7,8] which has significant benefits for ecumicin's potential use as a TB drug lead.[9] Interestingly, a similar mechanism of action has been described for lassomycin and cyclomarin A, cyclic peptide antibiotics

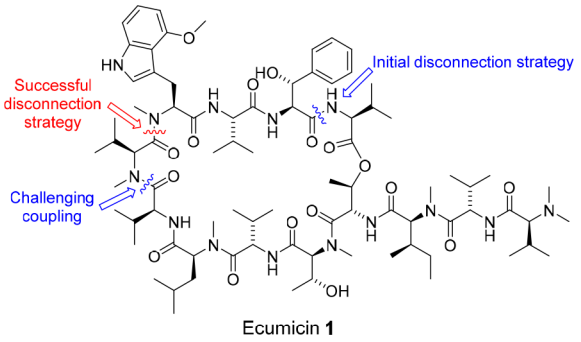


Figure 1. Structure of ecumicin (**1**) showing synthetic disconnection strategies; successful strategy (red) and unsuccessful strategy with the challenging peptide bond formation (blue).

Received: December 21, 2017

DOI: 10.1021/acs.orglett.7b03967
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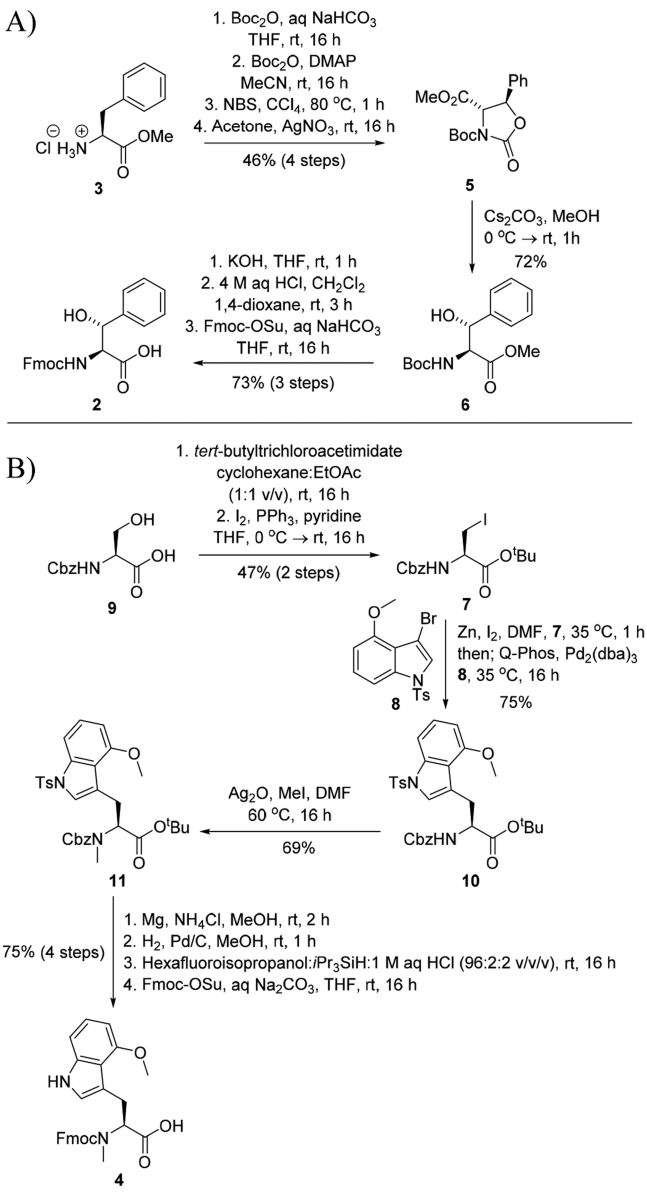
targeting *Mtb*; however, the significant structural differences between the cyclic peptides suggests that the three natural products target different binding sites on the ClpC1 subunit.[10−12]

Given the potent *in vitro* and *in vivo* antimycobacterial activity of ecumicin, together with its novel mechanism of action, we were interested in the natural product as a target for total synthesis. Ultimately, we envisaged that a robust synthetic route to ecumicin would facilitate future analogue generation which would allow SAR to be generated en route to the development of potential new leads for the treatment of drug-resistant TB.

Our synthesis commenced with the preparation of two suitably protected non-proteinogenic amino acid building blocks: Fmoc-*threo*-β-hydroxy-L-phenylalanine **2** (obtained in nine steps from L-phenylalanine methyl ester **3** using a synthetic sequence similar to that previously reported by Crich and Banerjee,[13] see Scheme 1A) and Fmoc-*N*-methyl-4-methoxy-L-tryptophan **4** (Scheme 1B). Synthesis of **4** took inspiration from the work of Tanaka et al.[14] and employed a key Negishi cross-coupling reaction between β-iodo-L-alanine **7** and substituted 3-bromoindole **8** (Scheme 1B). β-Iodo-L-alanine **7** was synthesized from Cbz-L-Ser-OH (**9**) by *tert*-butyl protection of the α-carboxylate followed by iodination. The critical Negishi cross coupling was achieved by reaction of bromoindole **8** (synthesized in two steps from 4-methoxyindole; see the Supporting Information (SI) for details) with the in situ generated organozinc iodide of **7**, catalytic $Pd_2(dba)_3$, and the Q-Phos ligand. This provided the protected 4-methoxy-L-tryptophan **10** in 75% yield following flash column chromatography. Next, *N*-methylation of **10** was achieved by treatment with methyl iodide in the presence of silver oxide to provide **11** in 69% yield. From here, only protecting group manipulations remained to generate target building block **4**: reduction of the tosylated indole with magnesium, hydrogenation of the Cbz protecting group, acidic *tert*-butyl ester deprotection,[15] and finally Fmoc protection of the resulting amine provided Fmoc-*N*-methyl-4-methoxy-L-tryptophan **4** in 75% yield over the four steps. It should be noted that the protecting groups were carefully chosen to preserve the chiral integrity of the α-center of the amino acid, as it was found that even mildly basic reagents used to remove base-labile protecting groups resulted in racemization (details not shown).

With the suitably protected amino acids **2** and **4** in hand, we focused our efforts on the synthesis of the linear peptide. It was envisaged that the linear peptide could be synthesized on-resin using Fmoc-SPPS including key on-resin esterification and N-terminal dimethylation steps. We proposed that the polypeptide could be grown on a hyper-acid-labile resin such that the side-chain-protected polypeptide could be released from the resin, enabling a solution-phase macrolactamization and final acidic deprotection to furnish ecumicin. Due to the hindered nature of the peptide bonds within the natural product, there were no obvious cyclization junctions as all sites possess bulky β-branched amino acids.[16] However, disconnection between *threo*-β-OH-L-Phe-OH and L-Val (Figure 1, blue) was initially chosen as the site for macrolactamization as it used an N-terminal amino acid without *N*-methylation, and the C-terminal *threo*-β-OH-L-Phe-OH could be masked as an oxazolidine (through synthetic manipulation of building block **2** to prevent epimerization; see the SI for synthetic details).[17] While oxazolidine-protected Fmoc-*threo*-β-OH-L-Phe-OH was successfully loaded onto the resin and elongated to a resin-

Scheme 1. (A) Synthesis of suitably protected *threo*-β-Hydroxy-L-phenylalanine 2 and (B) Synthesis of Suitably Protected *N*-Methyl-4-methoxy-L-tryptophan Building Block 4



bound tetrapeptide, which included the precious *N*-methyl-4-methoxy-L-tryptophan amino acid, the peptide chain could not be further extended due to steric hindrance in the coupling between resin-immobilized *N*-Me-L-Val and Fmoc-L-Val-OH (Figure 1, blue, see the SI for the synthetic scheme). Unfortunately, we were unable to forge this amide bond despite trialing an exhaustive set of conditions including microwave irradiation, *N*-silylation,[18] acid fluorides,[19] and even solution-phase condensation.

Because of the difficulty in achieving the above coupling, a new retrosynthetic strategy was developed. We theorized that the bulky amino acids were sterically occluding the reaction site, and so preorganizing this peptide may allow the reactive centers to react by proximity. As such, a new cyclization junction was chosen between the *N*-Me-L-Val-OH and *N*-methyl-4-methoxy-L-tryptophan residues. While this cyclization used an *N*-methylated N-terminus and possessed significant

DOI: 10.1021/acs.orglett.7b03967
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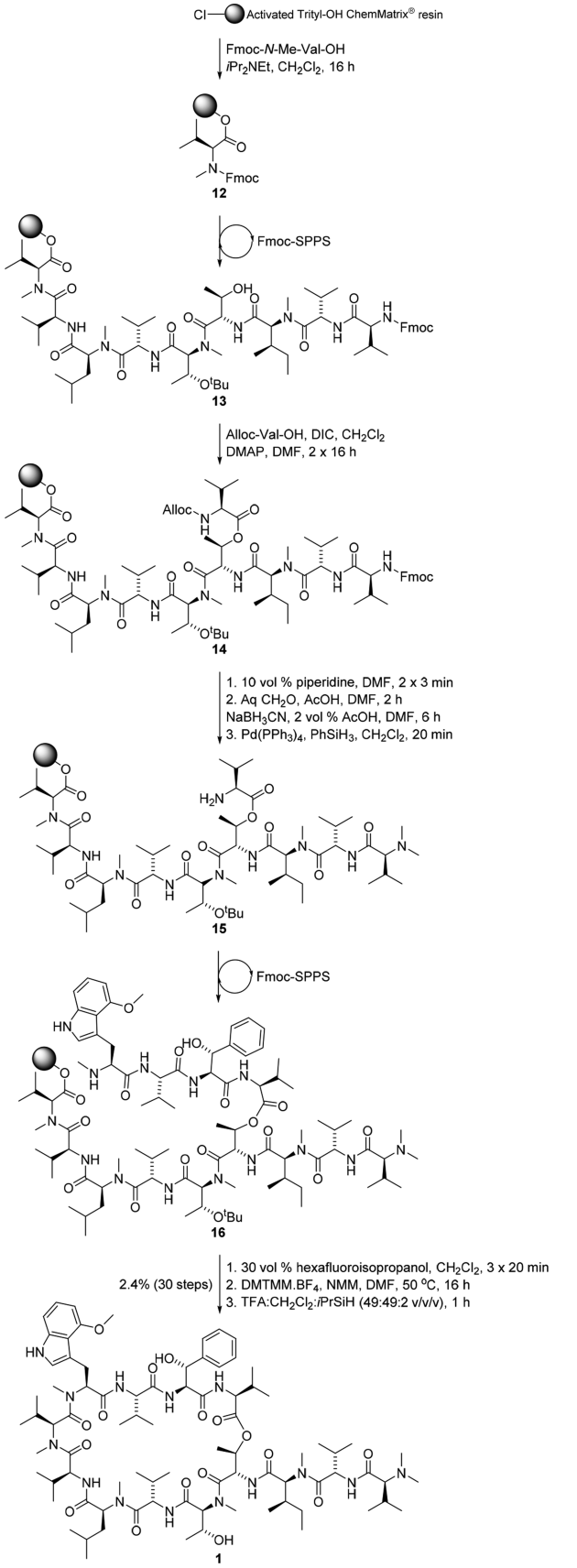
steric hindrance at the putative cyclization site, it was envisioned that the termini could react through preorganization of the fully assembled linear peptide chain. Toward this end, Fmoc-*N*-Me-L-Val-OH was loaded onto activated 4-(diphenylhydroxymethyl)benzoic acid (trityl-OH) functionalized polyethylene glycol-based ChemMatrix resin to provide resin-bound amino acid **12** (Scheme 2). A PEG-based resin was chosen for the improved swelling properties that were deemed important given the highly lipophilic nature of the peptide. Pleasingly, extension of the peptide chain using standard Fmoc-SPPS conditions with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5b]pyridinium 3-oxide hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) as the coupling reagents and iPr$_2$NEt as the base proceeded readily to afford resin-bound nonapeptide **13**. It should be noted that couplings to *N*-methylated residues required two treatments with the coupling solution to ensure complete conversion (as judged by UPLC–MS analysis of a small amount of cleaved peptide).

At this point, the key on-resin esterification between the L-Thr side chain and the symmetric anhydride of Alloc-L-Val-OH [generated using *N*,*N*′-diisopropylcarbodiimide (DIC) with catalytic 4-(dimethylamino)pyridine (DMAP)] was carried out, affording resin-bound depsipeptide **14** following two treatments. Next, the Fmoc group was removed via treatment with piperidine in DMF, followed by an on-resin dimethylation of the uncovered amine through a modified Eschweiler–Clarke reaction. Having successfully dimethylated the N-terminus of the resin-bound peptide, the Alloc protecting group on the branched L-Val was removed via treatment with Pd(PPh$_3$)$_4$ and phenylsilane in CH_2Cl_2 to provide **15**. From here, standard Fmoc-SPPS was continued to afford the resin-bound linear peptide **16**. Notably, β-OH-L-Phe-OH building block **2** was coupled without the oxazolidine protecting group used in the initial synthetic strategy as at this late stage in the synthesis the secondary alcohol was no longer exposed to reaction conditions that could lead to the formation of side products. Subsequently, the linear peptide **16** was cleaved from resin using 30% hexafluoroisopropanol in CH_2Cl_2, purified using RP-HPLC, and lyophilized to provide the linear peptide as a trifluoroacetic acid (TFA) salt in 9% yield over 28 steps from resin-bound **12** (average of 92% per step).

With the linear peptide in hand, macrolactamization was next carried out. Pleasingly, the use of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM·BF$_4$) and *N*-methylmorpholine (NMM) in a dilute solution of DMF at 50 °C for 16 h effected the cyclization with neglible epimerization (as measured by HPLC). Removal of the protecting groups with TFA and triisopropylsilane in CH_2Cl_2 and subsequent RP-HPLC purification and lyophilization afforded ecumicin **1** as the TFA salt in 27% yield over the cyclization and deprotection steps following HPLC purification (2.4% overall yield over the 30 steps). It is important to note that monitoring of the macrolactamization via UPLC–MS showed complete conversion of the linear peptide to the cyclic peptide (with very few byproducts), and the lower than expected isolated yield was likely owing to the amphoteric nature of the natural product which had a broad elution profile during HPLC purification (see the SI). Gratifyingly, spectroscopic data for synthetic ecumicin **1** were consistent with those reported for the isolated natural product by Gao et al.[5] Having completed the total synthesis of ecumicin, we next assessed the antimycobacterial activity of the natural product by screening against the virulent H37Rv strain of *Mtb* using a resazurin-based assay.[20] The activity of the synthetic natural product **1**

Scheme 2. Total Synthesis of Ecumicin (1)



DOI: 10.1021/acs.orglett.7b03967
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against virulent H37Rv *Mtb* was consistent with the isolated natural product with **1** possessing a MIC_{90} against *Mtb* H37Rv of 312 ± 31 nM (control: rifampicin MIC_{90} = 7 ± 0.3 nM; see the SI for raw data).

In summary, we report here the first total synthesis of the potent antimycobacterial cyclic depsipeptide natural product ecumicin (**1**). This work now lays the foundation for the generation of synthetic analogues of ecumicin in order to generate SARs against the chaperone protein ClpC1, the putative target of the natural product that is essential for mycobacterial viability. This may ultimately stimulate the discovery of ecumicin analogues as new TB drug leads that operate through a novel mechanism of action to currently employed therapies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03967.

Experimental procedures for synthesis, antibacterial screening, and characterization data and NMR spectra of ecumicin (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Dr. Ian Luck (The University of Sydney) for technical support for NMR spectroscopy and Dr. Nick Proschogo (The University of Sydney) for technical support for mass spectrometry. We also acknowledge the Australian Postgraduate Award and the John A. Lamberton Research Scholarship for PhD funding (P.M.E.H. and A.M.G.).

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DOI: 10.1021/acs.orglett.7b03967
Org. Lett. XXXX, XXX, XXX−XXX