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FULL PAPER

Total Synthesis and Antimycobacterial Activity of Ohmyungsamycin A, Deoxyecumicin, and Ecumicin

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Abstract: The ohmyungsamycin and ecumicin natural product families are structurally related cyclic depsipeptides that display potent antimycobacterial activity. Herein the total synthesis of ohmyungsamycin A, deoxyecumicin, and ecumicin are reported, together with the direct biological comparison of members of these natural product families against *Mycobacterium tuberculosis (Mtb)*, the etiological agent of tuberculosis (TB). The synthesis of each of the natural products employed a solid-phase strategy to assemble the linear peptide precursor, involving a key on-resin esterification and an optional on-resin dimethylation step, before a final solution-phase macrolactamization between the non-proteinogenic *N*-methyl-4-methoxy-L-tryptophan amino acid and a bulky *N*-methyl-L-valine residue. The synthetic natural products possessed potent antimycobacterial activity against *Mtb* with MIC_{90}'s ranging from 110-360 nM and retained activity against *Mtb* in *Mtb*-infected macrophages. Deoxyecumicin also exhibited rapid bactericidal killing against *Mtb*, sterilizing cultures after 21 days.

Introduction

Tuberculosis (TB) is caused by infection with the bacterium *Mycobacterium tuberculosis* (*Mtb*) and was responsible for 1.5 million deaths in 2018, making it the deadliest infectious disease globally. It is particularly striking that a quarter of the global population are latently infected with *Mtb*, with 10 million new cases diagnosed in 2018.[1] TB treatment involves intensive daily administration of various combinations of the four first-line antibiotics: rifampicin, isoniazid, pyrazinamide, and ethambutol for a period of six months.[2] While this is effective for drug-sensitive *Mtb*, 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.[1, 3] Treatment of drug-resistant TB is long (up to two years) and complex, relying on the use of second-line antibiotics that are more expensive and toxic than their first-line counterparts.[1, 3a] MDR-TB is considered both a public health crisis and a threat to health security with only 56% of global cases treated successfully.[1] Due to this emerging crisis there has been increased focus on the development of new antibiotics that target *Mtb*, i.e. antimycobacterials. Testament to this, in the last decade the U.S. Food and Drug Administration fast-tracked the approval of two novel antibiotics: bedaquiline and pretomanid (the first new TB drugs to be approved in 40 years).[3a, 4] While this progress is welcoming, owing to poor treatment compliance during long drug regimens and the ongoing development of resistance to current and novel drugs, there still remains an urgent need for new antibiotics which operate *via* novel modes of action and that show rapid bactericidal activity with a view to shortening TB treatment regimens.[3b, 5]

Ohmyungsamycin A (**1**) is a cyclic depsipeptide natural product that was isolated from a marine actinobacteria, *Streptomyces sp*. SNJ042 from Jeju Island, South Korea, by Um *et al.* (Figure 1).[6] Ohmyungsamycin A demonstrated anti-prolific effects against a range of human cancer cell lines, which were not replicated against normal cells.[6] More recently, Kim and co-workers have shown that ohmyungsamycin A (**1**) is also active against *Mtb* (MIC_{50} = 57 nM).[7] Moreover, the authors also validated the activity in an *in vivo* efficacy model of *Mtb*, using flies (*D. melanogaster*) infected with *Mycobacterium marinum*.[7] Structurally, ohmyungsamycin A is a dodecapeptide consisting of all L-amino acids, extensive backbone *N*-methylation, a monomethylated N-terminus, and two non-proteinogenic amino acids: *N*-methyl-4-methoxy-L-tryptophan and *threo*-β-hydroxy-L-phenylalanine.[6]

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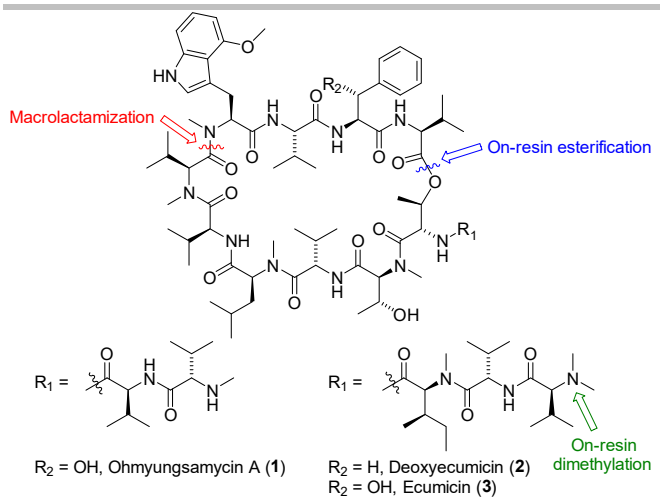

Figure 1. Structure of the natural products ohmyungsamycin A (**1**), deoxyecumicin (**2**), and ecumicin (**3**), showing key synthetic transformations.

Deoxyecumicin (**2**) was also isolated from a soil actinobacteria *Nonomuraea sp.* MJM5123, from Jeju Island, South Korea, alongside the natural product ecumicin (**3**) by Gao *et al.* (Figure 1).[8] Ecumicin has been well studied for its antimycobacterial activity, displaying promising *in vitro* activity (MIC_{90} = 160 nM against *Mtb* strain H37Rv) and *in vivo* activity, inhibiting *Mtb* growth in mice after 12 doses at 20 mg kg^{-1}.[9] For this reason, ecumicin was our first natural product target and in 2018 we reported the first total synthesis of this molecule and confirmed its structure (including absolute configuration) and antimycobacterial activity.[10] Deoxyecumicin has been less well studied although, like ecumicin, it was shown to exhibit potent activity against *Mtb* strain H37Rv.[8b] Deoxyecumicin and ecumicin are tridecapeptides structurally related to ohmyungsamycin A (**1**), with notable distinctions being a dimethylated N-terminus and the presence of an additional *N*-methyl-*allo*-L-isoleucine on its exocyclic tail component. Additionally, unlike ecumicin and ohmyungsamycin A, deoxyecumicin lacks the β-hydroxy functionality on L-phenylalanine.[8b]

The molecular target of ecumicin (**3**) is the chaperone protein ClpC1, which is involved in protein degradation in mycobacteria. Very recently, Wolf *et al.* reported a co-crystal structure of ecumicin bound to the ClpC1 N-terminal domain.[11] Interestingly, the complex showed a 1:2 target:ligand stoichiometry, with binding triggering significant conformational changes in the N-terminus of the protein.[11] Mechanistically, ecumicin is thought to stimulate ATPase activity of ClpC1, while also invoking a marked decrease in proteolysis by the ClpC1-mediated ClpP1P2 protease complex, ultimately leading to 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.[9] Given the structural similarities between the ecumicin and ohmyungsamycin natural product families, it is likely that both ohmyungsamycin A (**1**) and deoxyecumicin (**2**) also target ClpC1. Importantly, ClpC1 has been recognized as a promising antimycobacterial target, because it is only essential in mycobacteria.[12] Three other natural products have also been shown to target the N-terminal domain of ClpC1: lassomycin, cyclomarin A, and rufomycin. Like ecumicin, lassomycin and rufomycin inhibit proteolysis, meanwhile cyclomarin A has been shown to increase proteolysis, triggering cell death through unregulated protein degradation.[13]

Given the potent *in vitro* and *in vivo* antimycobacterial activity of ohmyungsamycin A (**1**), this molecule also recently succumbed to a total synthesis.[14] Suh and co-workers employed a convergent, solution-phase fragment condensation strategy to produce ohmyungsamycin A in a 4% overall yield (longest linear sequence).[14] Herein, we describe the use of a unified solid-phase synthetic strategy with a late-stage macrolactamization for the assembly of ohmyungsamycin A (**1**), ecumicin (**3**), and the first total synthesis of deoxyecumin (**2**). This enabled the direct comparsion of the structural features that are important for biological activity, to guide future analogue generation for the development of potential new TB drug leads that target the ClpC1 chaperone.

Results and Discussion

The synthetic logic for the assembly of the natural products **1** and **2** was inspired by the solid-phase strategy developed in our total synthesis of ecumicin (**3**).[10] The peptide cyclization junction was chosen between the *N*-Me-L-Val-OH and *N*-methyl-4-methoxy-L-tryptophan residues (Figure 2).[10] Although this cyclization involves an *N*-methylated N-terminus and possesses significant steric hindrance at the putative cyclization site, it was envisioned that the termini could react through pre-organization of the fully assembled linear peptide chain. Inspection of the intramolecular hydrogen bonds present in the crystal structure of ecumicin (**3**) reported by Gao *et al*. revealed a β-sheet stabilized by four intramolecular hydrogen bonds (blue) and two possible n→π* interactions (green) (Figure 2).[8a] Based on these interactions, it would appear that the amino sequence between Val-2-Val-3 is highly turn-inducing and predominantly responsible for the twisted β-sheet structure of ecumicin. For ecumicin, we had previously hypothesized that disconnection of the cyclic core in the middle of this turn-inducing sequence (*N*-Me-L-Val-OH and *N*-Me-4-OMe-L-Trp) could be beneficial for macrolactamization and may pre-organize the peptide for macrolactamization.[10] Given that ohmyungsamycin A (**1**) and deoxyecumicin (**2**) share a near identical cyclic core, we envisaged that the same strategy would be effective to generate these natural products.

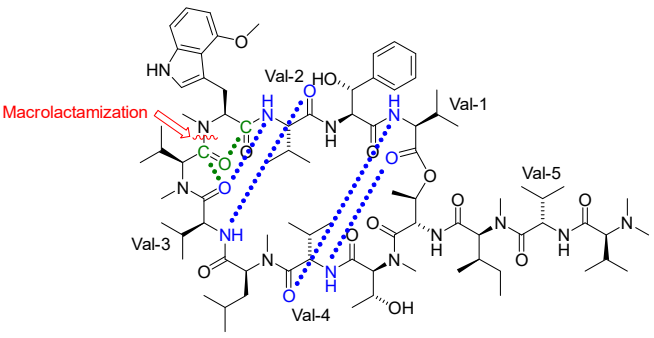

Figure 2. Structure of ecumicin (**3**), showing intramolecular hydrogen bonds (blue), n→π* interactions (green), and macrolactamization site (red).

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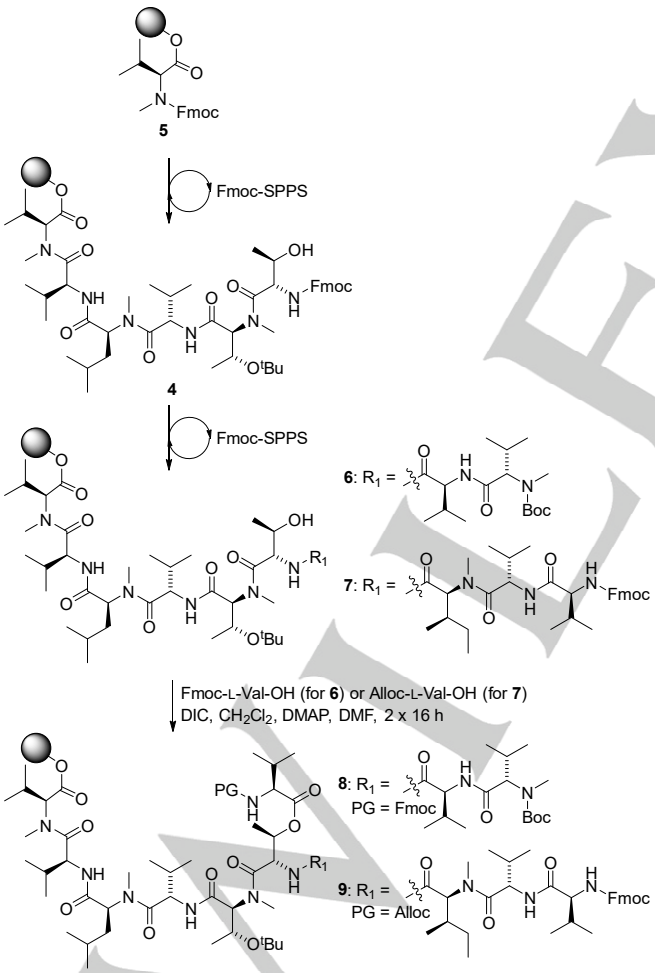
We proposed that natural products **1**, **2**, and **3** could all be obtained and diverged from a single common resin-bound peptide precursor **4**. Towards this end, Fmoc-*N*-Me-L-Val-OH was loaded onto activated 4-(diphenylhydroxymethyl)benzoic acid (Trityl-OH)-functionalized polyethylene glycol-based ChemMatrix® resin to generate resin-bound amino acid **5** (Scheme 1). 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-oxid hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) as the coupling reagents and *i*Pr$_2$NEt as the base proceeded readily to afford resin-bound hexapeptide **4**. 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). In particular, the coupling of Fmoc-L-Thr-OH to the hindered *N*-Me-L-Thr(O^{t}Bu) required careful monitoring to ensure reaction completion.



Scheme 1. Synthesis of resin-bound depsipeptides **8** and **9**.

At this point, the solid-phase synthesis diverged and to one pool of resin was coupled Fmoc-L-Val-OH and Boc-*N*-Me-L-Val-OH sequentially using standard Fmoc-SPPS conditions to provide **6**. A separate pool of resin was elongated by three residues, specifically, Fmoc-*N*-Me-*allo*-L-Ile-OH, Fmoc-L-Val-OH, and Fmoc-L-Val-OH to provide resin-bound **7**.

From here, the key on-resin esterification between the L-threonine sidechain and the symmetric anhydride of L-Val-OH [generated using *N,N'*-diisopropylcarbodiimide (DIC) with catalytic 4-dimethylamino)pyridine (DMAP)] was carried out twice, to provide resin-bound depsipeptide **8** and **9**. It should be noted, that while an Fmoc-protecting group could be used for depsipeptide **8**, an Alloc-protecting group was required for depsipeptide **9** to provide differentiation between the N-terminal valine tail and esterified sidechain valine.

With resin-bound depsipeptide **8** in hand, the Fmoc group was next removed *via* treatment with piperidine in DMF to afford **10** (Scheme 2). Depsipeptide **9** was also Fmoc-deprotected before the resulting amine was dimethylated on-resin through a modified Eschweiler-Clarke reaction using formaldehyde, dilute acetic acid, and sodium cyanoborohydride. Having successfully generated the tertiary amine en bloc, the Alloc-protecting group on the branched valine was removed *via* treatment with catalytic palladium(0) tetrakistriphenylphosphine (Pd(PPh$_3$)$_4$), using phenylsilane as an allyl scavenger to generate resin-bound **11**.

Standard Fmoc-SPPS was continued on peptide **10** to afford **12**. This included the incorporation of two synthetic amino acid building blocks, Fmoc-β-OH-L-Phe-OH and Fmoc-*N*-Me-4-OMe-L-Trp-OH, the latter prepared *via* Negishi cross coupling chemistry (see Supporting Information for details).[10] Two separate pools of **11** were also elongated by Fmoc-SPPS to afford the resin-bound linear peptides, **13** (incorporating Fmoc-L-Phe-OH) and **14** (incorporating Fmoc-β-OH-L-Phe-OH).

Having assembled the target linear peptides **12**, **13**, and **14**, these were next cleaved from resin using 30% hexafluoroisopropanol in CH_2Cl_2, purified using RP-HPLC and lyophilized to provide the linear peptides as trifluoroacetate salts in good yield (average yield of 90-93% per step).

With the linear peptides in hand, macrolactamization was next attempted. A range of conditions were trialed for the cyclization of HFIP-cleaved linear peptide **12** and **14** in an attempt to minimize epimerization; however, 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 proved optimal.[10] These conditions were then replicated for the cyclization of the HFIP-cleaved linear peptide **13**.

Finally, removal of the protecting groups with TFA and tri*iso*propylsilane in CH_2Cl_2 and subsequent RP-HPLC purification and lyophilization afforded ohmyungsamycin A (**1**), deoxyecumicin (**2**), and ecumicin (**3**) in 22-41% yield over the cyclization and deprotection steps following HPLC purification (2-5% overall yields over the 24-28 steps).

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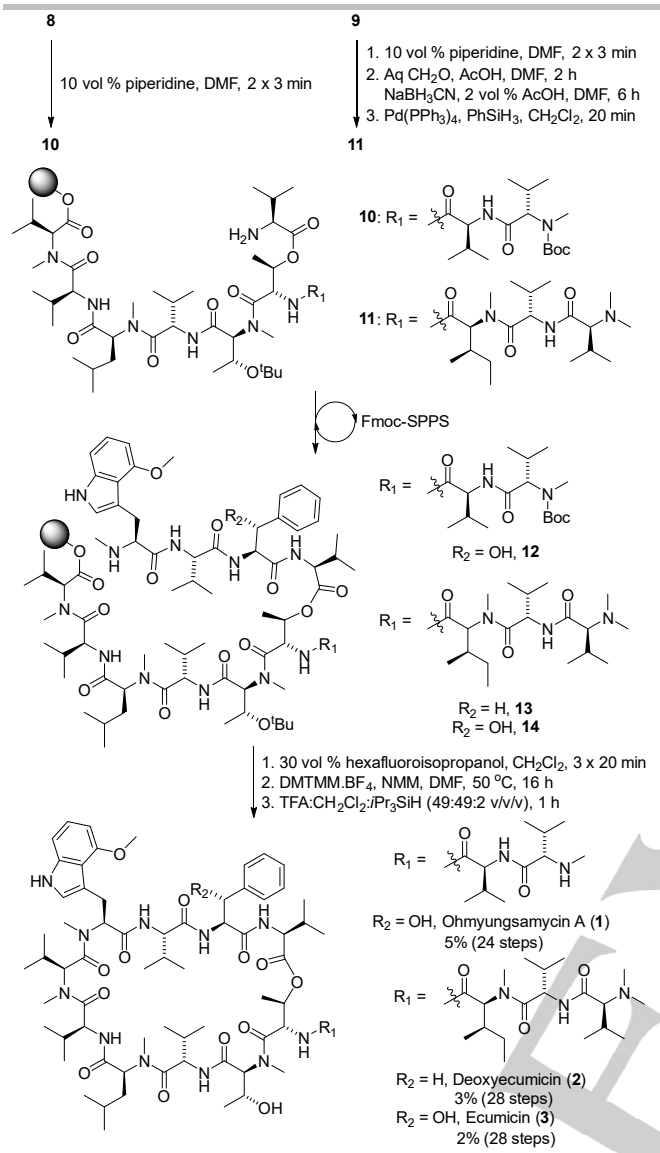

Scheme 2. Total synthesis of ohmyungsamycin A (**1**), deoxyecumicin (**2**), and ecumicin (**3**).

Gratifyingly, spectroscopic data for synthetic natural products **1**-**3** was consistent with that reported for the isolated natural products by Um *et al.* and *Gao et al.*[6, 8] (see Supporting Information for comparison data). Having successfully completed the total synthesis of the three natural products, we next assessed the antimycobacterial activity by screening against *Mtb* strain H37Rv using a resazurin-based assay.[15] The activity of each of the three synthetic natural products (**1**-**3**) against *Mtb* strain H37Rv was consistent with those reported for the isolated natural products (Table 1).[7, 8b, 9] It was interesting to find that ohmyungsamycin A (**1**) (MIC_{90} of 110 nM) displayed slightly better antimycobacterial activity than the other natural products (MIC_{90}'s ranging from 280-360 nM). These findings indicate that the shorter Val-Val mono-methylated N-terminal tail of ohmyungsamycin A provides an improvement in activity against *Mtb*, in contrast to the findings recently reported by Wolf *et al.*[11] Additionally, our study has shown that the presence of the non-proteinogenic β-hydroxy functionality on L-Phe is not critical for biological activity. Importantly, each of the natural products exhibited activity that was only 1-1.5 orders of magnitude less potent compared with the frontline TB drug rifampicin which was used as a positive control in the assay.

Table 1. Activity of synthetic natural products against *Mtb* strain H37Rv.

	MIC_{90} (nM)
Ohmyungsamycin A (**1**)	110 ± 5
Deoxyecumicin (**2**)	280 ± 7
Ecumicin (**3**)	360 ± 30
Rifampicin	7 ± 0.3

Mtb resides in lung macrophages and as such, the synthetic natural products were also assessed in an intracellular killing assay. Pleasingly they all inhibited the growth of *Mtb* in infected human THP-1 macrophages (Figure 3). Of these compounds, deoxyecumicin (**2**) and ecumicin (**3**) were most effective, with 1-2 log_{10} reduction in *Mtb* growth at 10 μM, providing similar activities to the frontline TB drug rifampicin. The apparent contrast in the order of activity compared with those observed against virulent H37Rv *Mtb* (Table 1) may reflect differences in the ability of the natural products to penetrate macrophages.

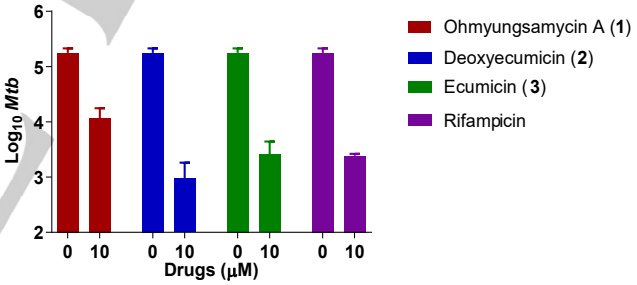

Figure 3. Activity of natural products against *Mtb* in infected human THP-1 macrophages. All experiments were performed in biological triplicate and the standard error of the mean is shown.

Finally, synthetic deoxyecumicin (**2**) and ecumicin (**3**) were screened in a time-kill kinetic assay to better understand the killing kinetics of the natural products (Figure 4). For these cell killing experiments we used *Mtb* strain mc^{2}6230 (Δ*RD1* Δ*panCD*).[16] Strain mc^{2}6230 (Δ*RD1* Δ*panCD*) is an avirulent auxotrophic *Mtb* mutant that behaves identically to wild-type *Mtb* strain H37Rv when grown in pantothenate-supplemented medium, but is incapable of causing disease even in severe combined immunodeficiency (SCID) mice.[16] The MIC values for deoxyecumicin and ecumicin of *Mtb* strain mc^{2}6230 (Δ*RD1* Δ*panCD*) were comparable to *Mtb* strain H37Rv. When *Mtb* strain mc^{2}6230 was challenged with the test compounds at 10× the MIC (Table 1), deoxyecumicin exhibited rapid bactericidal activity, with complete sterilization of the *Mtb* culture after 21 days (Figure 4A). Isoniazid (INH, 2 μM) was used as a positive control and showed early bactericidal activity but tolerance to isoniazid emerged after

7 days (Figure 4B), a result that has been observed previously for INH-treated cultures.[17]

Ecumicin demonstrated strong early bactericidal activity with a three $\log_{10}$ reduction in cells (CFU/mL) at day seven, but this was followed by the emergence of cells that became resistant to the compound. INH-treated cells showed a similar pattern of cell killing followed by the emergence of resistant cells (Figure 4B).

The rapid bactericidal activity exhibited by deoxyecumicin and the lack of tolerant or resistant mutants arising as observed with isoniazid, a front line drug for TB treatment, now provides the impetus to generate analogues of this natural product for the development of novel TB drug leads.

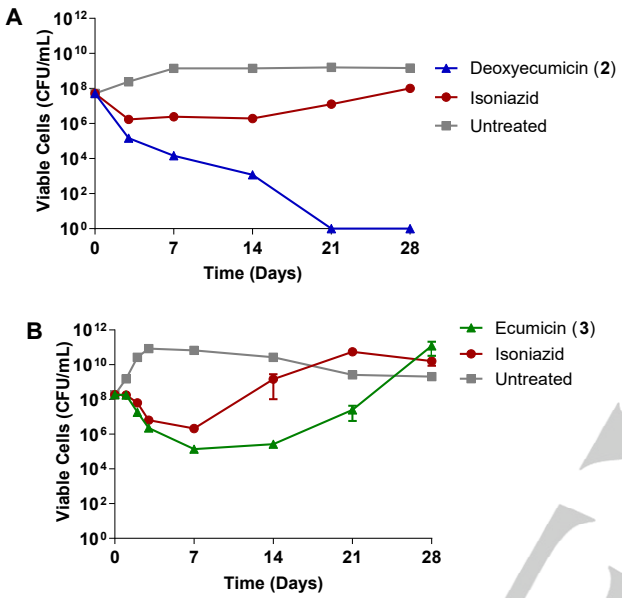

Figure 4. Effect of test compounds on the cell viability of *Mtb* strain mc²6230. Test compounds were added to *Mtb* strain mc²6230 cultures grown to an OD_{600} of 0.5 (time zero, approximately 1×10^8 CFU/mL). The culture was challenged with 10× the MIC of the test compound and cell viability monitored for 28 days by plating onto 7H11 media, and colony forming units determined after four weeks of incubation at 37°C. All experiments were performed in biological triplicate and the standard error of the mean is shown.

Conclusion

In summary, we have described the total synthesis of the three marine actinobacterially-derived cyclic depsipeptide natural products ohmyungsamycin A (**1**), deoxyecumicin (**2**), and ecumicin (**3**) using an efficient solid-phase synthetic strategy with a late stage macrolactamization step. Access to all three natural products enabled direct comparison of the antimycobacterial activities of the family of natural products. Ohmyungsamycin A was found to be the more active congener against *Mtb* with an MIC_{90} of 110 nM, however, ecumicin and deoxyecumicin displayed superior activity against intracellular *Mtb* (within infected THP-1 cells). Deoxyecumicin also displayed rapid bactericidal activity against *Mtb* where it was capable of sterilizing a bacterial culture after 21 days at a concentration 10× the MIC. This is a particularly encouraging feature that could be harnessed for the development of potential new TB drug leads based on the sructure of this molecule. Overall, this preliminary work provides the basis for further structure-activity relationship studies on synthetic analogues which can be rapidly assembled using the robust solid-phase route that we have reported here.

Experimental Section

In vitro inhibition assay against *Mtb*

The compounds were originally stored as 10 mM stock solutions in 100% DMSO. Two-fold serial dilutions of the compounds were made in a 96 well plate using Middlebrook 7H9 medium supplemented with ADC (Difco Laboratories, Detroit, MI, USA), 0.5% v/v glycerol and 0.05% v/v Tween-80. *Mtb* strain H37Rv was grown to mid-exponential phase to an OD_{600} of 0.6-0.8 in 7H9 media at 37 °C. On the day of the assay, the culture was diluted to an OD_{600} of 0.002 in 7H9 medium and 100 μL of bacterial suspension was added to the 96 well plate containing 100 μL of the diluted compounds. The plate was incubated for 5 days at 37 °C in a humidified incubator and 30 μL of Resazurin (0.02% w/v) and 12.5 μL of Tween-80 was added to each well and incubated for a further 24 h. On day 6, the fluorescence was read using a BMG Labtech Polarstar plate reader (excitation 530 nm and emission 590 nm). The results are presented as *Mtb* survival as a percentage of negative control (no drug controls).

Mtb inhibition using infected macrophage culture

Mtb strain H37Rv (NR-123, BEI Resources) was grown in Middlebrook 7H9 broth medium supplemented with ADC (Difco Laboratories), 0.05% glycerol and 0.05% Tween-80. Bacterial cultures were grown at 37 °C, to mid-exponential phase (OD_{600} 0.4–0.8) and then used to infect a human macrophage-like cell line (THP-1; ATCC TIB-202). THP-1 cells were cultured in RPMI-1640 medium (with phenol red, 25 mM HEPES and 2 mM L-glutamine) supplemented with 10% FBS (FBS-500) and 0.05 mM β-mercaptoethanol. 1×10^5 cells/well of THP-1 cells were plated in 96-well tissue culture plates and differentiated with phorbol myristic acetate (PMA; 100 nM) for 48 h at 37 °C at 5% CO_2. *Mtb* strain H37Rv was sonicated to achieve single cell suspension in RPMI-1640 medium and used to infect differentiated THP-1 cells at a multiplicity of infection of 1 for 4 h at 37 °C at 5% CO_2. Supernatant was then removed from all wells, THP-1 cells were washed with 200 μL phosphate buffered saline (PBS) three times and were subsequently replenished with fresh RPMI-1640 cell culture medium and incubated for a further 24 h at 37 °C and 5% CO_2. The compounds were diluted in fresh RPMI-1640 cell culture medium and added to corresponding wells. Positive controls were dissolved in 100% dimethyl sulfoxide (DMSO) and after 72 h of incubation at 37 °C at 5% CO_2, tissue culture medium containing the test compound was removed from the wells and cells lysed with sterile water containing 0.1% Triton X-100. Cell lysates were serially diluted, 1:10, and plated on Middlebrook 7H11/OADC (283010; Difco) agar through to 1:10,000 dilution. Agar plates were then incubated at 37 °C for 3–4 weeks, after which the bacteria colonies were counted and CFU mL^{-1} of cell lysates were determined.

Kinetics of cell killing by deoxyecumicin (2) and ecumicin (3)

The avirulent auxotrophic *Mtb* strain mc²6230 (Δ*RD1* Δ*panCD*) was used to monitor the kinetics of cell killing by deoxyecumicin (**2**) and ecumicin (**3**). Isoniazid were used as a positive control. Strain mc²6230 (Δ*RD1* Δ*panCD*) is an avirulent auxotrophic *Mtb* mutant that behaves identically to wild-type

Mtb when grown in pantothenate-supplemented medium, but is incapable of causing disease even in severe combined immunodeficiency (SCID) mice.[16] Growth of *M. tuberculosis* strain mc^{2}6230 was grown in Middlebrook 7H9 broth supplemented with OADC (0.005% oleic acid, 0.5% bovine serum albumin, 0.2% dextrose, 0.085% catalase), 0.05% tyloxapol (Sigma) and 25 µg/mL pantothenic acid (PAN). Cultures were maintained in 10 mL volumes in 30 mL inkwells at 37°C with agitation (140 rpm). When required mc^{2}6230 was grown on Middlebrook 7H11 agar supplemented with OADC and PAN.

The MIC values for deoxyecumicin (**2**), ecumicin (**3**), and isoniazid were determined by inoculating *Mtb* into 96-well plates containing supplemented Middlebrook 7H9 broth, at a starting OD600 of 0.05, in a total starting volume of 200 µL. Test compounds were dispensed from a 9-point, 3-fold dilution gradient to each well, with a maximum of 2% dimethyl sulfoxide (DMSO). 96-well plates were incubated without shaking at 37°C for 10 days. On day 7, OD_{600} values were measured in a Varioskan LUX microplate reader, and MIC values were determined in biological triplicate.

To determine the effects of test compounds on the cell viability of *Mtb,* cells were grown in 10 mL volumes in 30 mL inkwells at 37°C with agitation (140 rpm). When cells reached an OD_{600} of 0.5 (time zero, approximately 1×10^8 CFU/mL), the culture was challenged with 10× the MIC of the test compound and cell viability monitored for 28 days. At various time points (as indicated), culture was removed from the desired inkwell and diluted along a four-point ten-fold dilution curve. Five µL was spotted onto 7H11 media, and colony forming units determined after four weeks of incubation at 37°C. All experiments were performed in biological triplicate and the standard error of the mean is shown.

Materials and Methods for the Synthesis of 1-3

All reactions were carried out under an argon atmosphere and at room temperature (22 °C) unless the reaction was performed under aqueous conditions or unless otherwise specified. The reactions carried out at 0 °C employed a bath of water and ice and reactions carried at -40 °C employed a bath of dry ice and MeCN. Anhydrous THF, CH_2Cl_2, MeOH, MeCN, DMF, and toluene were obtained using a PureSolv® solvent purification system. The reactions were monitored by thin layer chromatography (TLC) on aluminium backed silica plates (Merck Silica Gel 60 F254). Visualisation of TLC plates was undertaken with an ultraviolet (UV) light at λ = 254 nm and staining with solutions of vanillin, ninhydrin, and potassium permanganate followed by exposure of the stained plates to heat. Silica flash column chromatography (Merck Silica Gel 60 40 – 63 µm) was undertaken to purify crude reaction mixtures using solvents as specified. Fractions were collected manually or with a Biotage Isolera Spektra automated flash purification system.

All commercially available reagents were used as obtained from Sigma-Aldrich, Merck, Acros Organics or AK Scientific. Amino acids, coupling reagents and Trityl-OH ChemMatrix® resins were obtained from NovaBiochem, PCAS, GL Biochem, or Mimotopes and peptide synthesis grade DMF was obtained from Merck or Labscan. All non-commercially available reagents were synthesized according to literature procedures as referenced.

^{1}H NMR spectra were obtained using a Bruker DRX 300, DRX 400 or DRX 500 at frequencies of 300 MHz, 400 MHz or 500 MHz respectively in $CDCl_3$, MeOD, or pyridine-d_5. Chemical shifts are reported in parts per million (ppm) and coupling constants in Hertz (Hz). ^{1}H NMR data is reported as follows: chemical shift values (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant(s) and relative integral. ^{13}C NMR spectra were obtained using a Bruker DRX 400 or DRX 500 at 100 MHz or 125 MHz in $CDCl_3$, MeOD, CD_3CN, D_2O, pyridine-d^5, DMSO-d^6, and acetone-d^6 unless otherwise specified. ^{13}C NMR data is reported as chemical shift values (ppm). Any rotamers were confirmed *via* variable temperature NMR experiments or saturation transfer NMR experiments. Low resolution mass spectra for novel compounds were recorded on a Bruker amaZon SL mass spectrometer (ESI) operating in positive/negative mode or on a Shimadzu 2020 (ESI) mass spectrometer operating in positive/negative mode. High resolution mass spectra were recorded on a Bruker-Daltronics Apex Ultra 7.0T Fourier transform (FTICR) mass spectrometer.

LC-MS was performed on a Shimadzu UPLC-MS instrument with an LC-M20A pump, SPD-M30A diode array detector and a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode. Separations on the UPLC system were performed on a Waters Acquity 1.7 µm, 2.1 x 50 mm (C18) column. These separations were performed using a mobile phase of 0.1 vol % trifluoroacetic acid in water (Solvent A) and 0.1 vol % trifluoroacetic acid in MeCN (Solvent B) using linear gradients. Preparative reverse-phase HPLC was performed using a Waters 500 pump with a 2996 photodiode array detector and a Waters 600 Multisolvent Delivery System.

Fmoc-SPPS General Protocols

Fmoc-strategy solid-phase peptide synthesis (Fmoc-SPPS) procedures were employed on acid-labile 4-(diphenylhydroxymethyl)benzoic acid (Trityl-OH)-functionalized polyethylene glycol resin (Chem-Matrix) within fritted syringes. All reagent equivalents are with respect to the amount of the initial amino acid loaded to resin.

Resin Loading

Trityl-OH Chem-Matrix® resin (0.30 mmol/g) was swelled in CH_2Cl_2 (0.015 M) and shaken for 20 min. The solvent was discharged and the resin washed with CH_2Cl_2 (x 5). Resin was treated with 5% (v/v) thionyl chloride/CH_2Cl_2 and shaken for 4 h. The activating solution was discharged and resin washed with CH_2Cl_2 (x 5) and 5% (v/v) *i*Pr$_2$NEt/CH_2Cl_2 (x 2). The resin was treated with Fmoc-*N*-Me-L-Val-OH (4 eq. in regards to predicted resin loading) and *i*Pr$_2$NEt (8 eq.) in CH_2Cl_2 (0.05 M) and shaken at room temperature for 16 h. The coupling solution was discharged and washed with CH_2Cl_2 (x 5), DMF (x 5) and CH_2Cl_2 (x 5). The resin was treated with a capping solution of 17:2:1 (v/v/v) CH_2Cl_2/*i*Pr$_2$NEt/MeOH and shaken at room temperature for 30 min. The capping solution was discharged and the resin washed with CH_2Cl_2 (x 5) and DMF (x 5).

Resin Loading Determination

The resin-bound peptide was treated with a solution of 10 vol% piperidine/DMF (2 x 3 min). The deprotection solution was discharged and collected and the resin washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5). The combined deprotection solutions were made up to 25 mL using 10 vol% piperidine/DMF and diluted 1:100 with 10 vol% piperidine/DMF. The amount of peptide loaded to resin was determined by measurement of the UV absorbance at λ = 301 nm of the diluted deprotection solution.

Fmoc Deprotection

The resin-bound peptide was treated with a solution of 10 vol% piperidine/DMF (2 x 3 min). The deprotection solution was discharged and the resin washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5).

HATU Coupling Conditions

The resin-bound peptide was treated with a solution of a given amino acid (1.2-4 eq.), HATU (1.2-4 eq.), HOAt (2.4-8 eq.) and *i*Pr$_2$NEt (2.4-8 eq.) in DMF (0.05 M in regards to Chem-Matrix or NovaPEG loaded peptide or 0.1 M in regards to polystyrene loaded peptide) and shaken for 8 h at room

temperature. The coupling solution was discharged and the resin washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5).

On-resin Esterification Conditions

To a solution of Alloc-L-Val-OH or Fmoc-L-Val-OH (10 eq.) in CH_2Cl_2 (0.06 M) was added DIC (5 eq.). The solution was stirred for 30 min at room temperature before being concentrated under a stream of N_2. The resultant slurry was dissolved in DMF (0.05 M in regards to Chem-Matrix or NovaPEG loaded peptide or 0.1 M in regards to polystyrene loaded peptide) and sucked into the fritted syringe containing the resin-bound peptide. Subsequently, a solution of DMAP (catalytic, ~6 small crystals) in DMF (0.1 mL) was sucked up and the resin was shaken at room temperature for 8 h. The coupling solution was discharged and the resin washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5). The procedure was repeated 1-3 times until all starting material had converted into product as judged by UPLC-MS monitoring.

On-resin Dimethylation Conditions

The resin-bound peptide was treated with a solution of 37% aqueous formaldehyde (10 eq.), acetic acid (10 eq.) in DMF (0.05 M in regards to loaded peptide). The resin was shaken at room temperature for 2 h at which point an additional solution of sodium cyanoborohydride (4 eq.) in 2% (v/v) acetic acid/DMF (2.2 mL) was sucked into the fritted syringe. The resin was shaken for 6 h, the solution was discharged and the resin washed with DMF (x 5), CH_2Cl_2 (x 5) and DMF (x 5). The procedure was repeated 1-2 times until all starting material had converted into product as judged by UPLC-MS monitoring.

Alloc Deprotection

The resin-bound peptide was washed with CH_2Cl_2 (x 10) and treated with a solution of $Pd(PPh_3)_4$ (0.2 eq.), phenylsilane (20 eq.) in CH_2Cl_2 (0.018 M in regards to loaded peptide) and shaken at room temperature for 20 min. The deprotection solution was discharged and the resin washed with CH_2Cl_2 (x 5) and DMF (x 5).

Resin-cleavage Conditions

The resin-bound peptide was washed with CH_2Cl_2 (x 10) and then treated with a solution of 30 vol% HFIP/ CH_2Cl_2 (3 x 20 min). The cleavage solution was discharged, collected and concentrated under a stream of N_2 and dried *in vacuo*.

Cyclization Conditions

The protected linear peptide (1 eq.) was dissolved in DMF (0.005 M) and to this solution was added DMTMM.BF_4 (2 eq.) followed by NMM (2 eq.). The resulting reaction mixture was heated to 50 °C and stirred for 16 h. The cyclization solution was concentrated under a stream of N_2 and to this residue was added *i*Pr_3SiH:TFA:CH_2Cl_2 (2:49:49, v/v/v, 5 mL) and stirred at room temperature for 1 h. The deprotection solution was concentrated under a stream of N_2 and *in vacuo* to afford the cyclic peptide as a crude solid which was subsequently purified by reversed-phase HPLC.

Synthesis of Ohmyungsamycin A (1)

Fmoc-*N*-Me-L-Val-OH (0.60 mmol) was loaded to Trityl-OH as per the general procedures. Resin loading was determined to be 117 µmol.

The resin-bound linear peptide (117 µmol) was elongated using HATU coupling conditions and Fmoc-deprotection conditions, incorporating the following amino acids in turn: Fmoc-L-Val-OH, Fmoc-*N*-Me-L-Leu-OH, Fmoc-L-Val-OH, Fmoc-*N*-Me-L-Thr-(O^{t}Bu)-OH, Fmoc-L-Thr-OH, Fmoc-L-Val-OH and Boc-*N*-Me-L-Val-OH. Amino acid couplings following *N*-methylated residues required two treatments with fresh coupling solution.

The resin-bound peptide (47 µmol) was esterified with Fmoc-L-Val-OH using the on-resin esterification conditions. The procedure was repeated 2-3 times until all starting material had converted into product as judged by UPLC-MS monitoring.

The resin-bound peptide (47 µmol) was then elongated using HATU coupling conditions and Fmoc-deprotection conditions incorporating the following amino acids in turn: Fmoc-*threo*-β-OH-L-Phe-OH (1.2 eq. of amino acid used, 16 h coupling), Fmoc-L-Val-OH and Fmoc-*N*-Me-4-OMe-L-Trp-OH (1.2 eq. of amino acid used, 16 h coupling).

The resin-bound peptide was cleaved using the resin-cleavage conditions. The crude protected linear peptide was purified by preparative RP-HPLC using a Waters X-Bridge 5 µm 19 x 150 mm (C18) column using a gradient of 60 – 70% MeCN in H_2O (0.1% TFA) over 30 min at a flow rate of 7 mL min^{-1}. The peptide was lyophilized to yield the protected linear peptide as a trifluoroacetate salt (11.1 mg, 21% over 22 steps from resin loading, average of 93% per step).

The protected linear peptide (11.1 mg, 6.37 µmol) was cyclized according to the general cyclization conditions described above. The crude cyclic peptide was purified by preparative RP-HPLC using a Waters X-Bridge 5 µm 19 x 150 mm (C18) column using a gradient of 70 – 100% MeOH in H_2O (0.1% TFA) over 40 min at a flow rate of 7 mL min^{-1}. The peptide was lyophilized to give a white fluffy powder of pure ohmyungsamycin A (**1**) as a trifluoroacetate salt (2.2 mg, 5% over 24 steps from resin loading, 22% for the cyclization and deprotection).

HR-MS: (+ESI) Calc. for $C_{75}H_{119}N_{13}O_{16}$: 751.9341 $[M+2Na]^{2+}$, Found: 751.9342 $[M+2Na]^{2+}$; **IR (ATR)**: ν_{max} = 3290, 2964, 2929, 1671, 1634, 1542, 1469, 1204, 1140, 723 cm^{-1}; **$[\alpha]_D^{20}$** = -47° (c = 0.1 in MeOH); **^{1}H NMR** (500 MHz, pyridine-d_5): 0.66 (d, 5.8 Hz, 1.5H), 0.67 (d, 5.6 Hz, 1.5H), 0.95 (d, 6.5 Hz, 1.5H), 0.95 (d, 6.5 Hz, 1.5H), 0.97 (d, 6.5 Hz, 1.5H), 1.03 (d, 6.2 Hz, 1.5H), 1.04 (d, 6.2 Hz, 1.5H), 1.12 (d, 6.9 Hz, 1.5H), 1.14 (d, 6.9 Hz, 1.5H), 1.16 (d, 6.5 Hz, 1.5H), 1.20 (d, 6.5 Hz, 1.5H), 1.21 (m, 1.5H), 1.22 (m, 1H), 1.23 (d, 5.8 Hz, 1.5H), 1.27 (d, 6.5 Hz, 1.5H), 1.33 (d, 6.4 Hz, 3H), 1.35 (d, 6.7 Hz, 1.5H), 1.40 (m, 1H), 1.57 (d, 6.1 Hz, 3H), 1.58 (m, 1H), 1.74 (m, 1H), 2.23 (m, 1H), 2.29 (m, 1H), 2.39 (m, 1H), 2.49 (s, 3H), 2.56 (m, 1H), 2.64 (m, 1H), 2.66 (m, 1H), 2.98 (s, 3H), 3.03 (m, 1H), 3.19 (s, 3H), 3.20 (m, 1H), 3.52 (s, 3H), 3.84 (s, 3H), 3.71 (s, 3H), 4.31 (app. dd, 11.2, -13.2 Hz, 1H), 4.37 (d, 5.7 Hz, 1H), 4.44 (app. dd, 4.6, -13.5 Hz, 1H), 4.58 (dd, 4.6, 10.6 Hz, 1H), 4.67 (app. t, 8.6 Hz, 1H), 4.98 (app. t, 9.2 Hz, 1H), 5.06 (m, 1H), 5.20 (app. t, 8.0 Hz, 1H), 5.31 (app. t, 8.0 Hz, 1H), 5.38 (m, 1H), 5.49 (m, 1H), 5.59 (m, 1H), 5.65 (m, 1H), 5.90 (app. d, 10.8 Hz, 1H), 5.93 (app. s, 1H), 6.08 (m, 1H), 6.67 (d, 7.4 Hz, 1H), 7.19 (s, 1H), 7.27 (m, 2H), 7.32 (d, 8.1 Hz, 1H), 7.41 (app. t, 7.4 Hz, 2H), 7.67 (d, 8.1 Hz, 2H), 8.06 (d, 12.4 Hz, 1H), 8.08 (d, 8.7 Hz, 1H), 9.28 (d, 8.7 Hz, 1H), 9.38 (d, 9.9 Hz, 1H), 9.76 (d, 7.4 Hz, 1H), 9.87 (d, 8.7 Hz, 1H), 10.18 (d, 8.7 Hz, 1H), 11.84 (br s, 1H); **^{13}C NMR** (125 MHz, pyridine-d_5, shifts were extracted from HSQC and HMBC data): 17.2, 17.6, 18.6, 18.9, 19.1, 19.1, 19.4, 19.5, 19.5, 20.0, 20.1, 20.1, 20.1, 20.1, 20.8, 22.1, 22.2, 23.5, 25.4, 27.5, 29.5, 31.0, 31.3, 31.5, 31.8, 32.3, 32.9, 32.9, 32.9, 35.0, 38.9, 40.4, 41.1, 53.1, 54.7, 54.9, 55.8, 55.9, 58.6, 58.7, 59.6, 60.4, 62.9, 66.8, 68.3, 69.9, 71.0, 71.2, 73.5, 99.9, 106.3, 112.9, 118.8, 122.9, 124.2, 127.3, 127.8, 128.8, 139.5, 143.0, 155.1, 168.6, 169.9, 171.0, 171.7, 173.2, 173.3, 173.3, 173.9, 174.6, 176.0. ^{1}H NMR and ^{13}C NMR data are compared to the isolated natural product in Table S1. These data is in agreement with that previously reported by Um *et al*.[6]

Synthesis of Deoxyecumicin (2)

Fmoc-*N*-Me-L-Val-OH (0.95 mmol) was loaded to Trityl-OH as per the general procedures. Resin loading was determined to be 190 µmol.

A portion of the resin-bound linear peptide (109 µmol) was elongated using HATU coupling conditions and Fmoc-deprotection conditions, incorporating the following amino acids in turn: Fmoc-L-Val-OH, Fmoc-*N*-Me-L-Leu-OH, Fmoc-L-Val-OH, Fmoc-*N*-Me-L-Thr-(O^{t}Bu)-OH, Fmoc-L-Thr-OH, Fmoc-*N*-Me-*allo*-L-Ile-OH, Fmoc-L-Val-OH and Fmoc-L-Val-OH. Amino acid couplings following *N*-methylated residues required two treatments with fresh coupling solution.

The resin-bound peptide (109 µmol) was esterified with Alloc-L-Val-OH using the on-resin esterification conditions. The procedure was repeated 2-3 times until all starting material had converted into product as judged by UPLC-MS monitoring.

The resin-bound peptide was Fmoc-deprotected and then dimethylated using the on-resin dimethylation conditions. The procedure was repeated 1-2 times until all starting material had converted into product as judged by UPLC-MS monitoring.

The resin-bound peptide was Alloc-deprotected before 46 µmol of resin was elongated using HATU coupling conditions and Fmoc-deprotection conditions incorporating the following amino acids in turn: Fmoc-L-Phe-OH, Fmoc-L-Val-OH and Fmoc-*N*-Me-4-OMe-L-Trp-OH (1.2 eq. of amino acid used, 16 h coupling).

The resin-bound peptide was cleaved using the resin-cleavage conditions. The crude protected linear peptide was purified by preparative RP-HPLC using a Sunfire OBD 5 µm 19 x 150 mm (C18) column using a gradient of 50 – 60% MeCN in H_2O (0.1% TFA) over 40 min at a flow rate of 7 mL min^{-1}. The peptide was lyophilized to yield the protected linear peptide as a trifluoroacetate salt (6.0 mg, 7% over 26 steps from resin loading, average of 90% per step).

The protected linear peptide (6.0 mg, 3.20 µmol) was cyclized according to the general cyclization conditions described above. The crude cyclic peptide was purified by preparative RP-HPLC using a Waters X-Bridge 5 µm 19 x 150 mm (C18) column using a gradient of 70 – 100% MeOH in H_2O (0.1% TFA) over 40 min at a flow rate of 7 mL min^{-1}. The peptide was lyophilized to give a white fluffy powder of pure deoxyecumicin (**2**) as a trifluoroacetate salt (2.2 mg, 3% over 28 steps from resin loading, 41% for the cyclization and deprotection).

HR-MS: (+ESI) Calc. for $C_{83}H_{134}N_{14}O_{16}$: 803.5034 $[M+2Na]^{2+}$, Found: 803.5028 $[M+2Na]^{2+}$; **IR (ATR)**: ν_{max} = 3383, 2967, 2929, 1679, 1541, 1510, 1442, 1206, 1139 cm^{-1}; **^{1}H NMR** (500 MHz, MeOD): 0.21 (d, 6.6 Hz, 1.5H), 0.32 (d, 6.6 Hz, 1.5H), 0.72 (app. t, 7.4 Hz, 3H), 0.76 (d, 6.9 Hz, 3H), 0.87 (d, 7.2 Hz, 1.5H), 0.88 (dd, 6.7 Hz, 3H), 0.91 (d, 6.8 Hz, 1.5H), 0.92 (d, 6.9 Hz, 1.5H), 0.93 (d, 7.3 Hz, 1.5H), 0.98 (m, 3H), 0.98 (m, 2.5H), 1.00 (m, 2.5H),1.02 (d, 6.6 Hz, 1.5H), 1.06 (d, 6.5 Hz, 1.5H), 1.09 (d, 6.7 Hz, 4.5H), 1.27 (m, 1H), 1.29 (m, 1H), 1.31 (d, 6.6 Hz, 3H), 1.43 (m, 1H), 1.96 (m, 2H), 2.04 (m, 1H), 2.15 (m, 1H), 2.16 (s, 3H), 2.18 (m, 1H), 2.37 (m, 1H), 2.40 (m, 1H), 2.59 (m, 1H), 2.92 (s, 3H), 2.84 (app dd, 10.7, -14.2 Hz, 1H), 3.06 (d, 7.7 Hz, 1H), 3.15 (s, 3H), 3.23 (s, 3H), 3.26 (s, 3H), 3.32 (s, 3H), 3.37 (app. dd, 12.4, -14.4 Hz, 1H), 3.54 (app. dd, 10.9, -13.5 Hz, 1H), 3.68 (app. 11.1, -15.8 Hz, 1H), 3.71 (d, 5.7 Hz, 1H), 3.81 (s, 3H), 4.09 (dd, 4.4, 10.9 Hz, 1H), 4.36 (app. t, 9.4 Hz, 1H), 4.40 (app. t, 9.1 Hz, 1H), 4.44 (dd, 3.8, 6.3 Hz, 1H), 4.59 (app. t., 9.1 Hz, 1H), 4.72 (d, 8.8 Hz, 3H), 4.79 (m, 1H), 4.83 (m, 1H), 4.95 (d, 10.9 Hz, 1H), 5.03 (d, 3.6 Hz, 1H), 5.13 (dd, 2.1, 8.8 Hz, 1H), 5.16 (d, 7.6 Hz, 1H), 5.79 (m, 1H), 6.44 (d, 8.1 Hz, 1H), 6.70 (s, 1H), 6.92 (d, 8.1 Hz, 1H), 6.98 (app. t, 8.1 Hz, 1H), 7.09 (d, 7.2 Hz, 2H), 7.16 (d, 7.2 Hz, 1H), 7.20 (m, 2H), 7.70 (d, 8.7 Hz, 1H), 7.80 (d, 9.9 Hz, 1H), 8.53 (d, 9.1 Hz, 1H), 8.66 (d, 7.4 Hz, 1H), 9.02 (d, 9.6 Hz, 1H), 9.13 (d, 9.6 Hz, 1H); **^{13}C NMR** (125MHz, MeOD, shifts were extracted from HSQC and HMBC data): 11.3, 15.2, 16.4, 16.6, 18.9, 18.9, 19.2, 19.2, 19.2, 19.2, 19.3, 19.5, 19.5, 19.5, 19.5, 19.5, 19.8, 21.6, 21.8, 22.2, 23.2, 25.3, 26.2, 26.7, 28.4, 29.6, 31.3, 31.3, 31.6, 31.6, 32.7, 32.8, 33.4, 34.1, 34.0, 37.3, 39.2, 40.3, 40.7, 42.3, 53.3, 55.2, 55.3, 55.5, 56.2, 56.6, 57.1, 59.0, 59.0, 61.5, 62.7, 66.6, 69.3, 70.8, 71.3, 73.7, 99.6, 105.8, 112.2, 118.1, 123.0, 123.6, 127.2, 129.3, 129.9, 139.8, 139.8, 155.0, 166.5, 170.7, 170.7, 171.6, 171.6, 172.5, 173.2, 173.2, 173.8, 174.0, 174.0, 174.0, 174.6. ^{1}H NMR and ^{13}C NMR data are compared to the isolated natural product in Table S2. This data is in agreement with that previously reported by Gao *et al*.[8b]

Synthesis of Ecumicin (3)

Fmoc-*N*-Me-L-Val-OH (0.60 mmol) was loaded to Trityl-OH as per the general procedures. Resin loading was determined to be 117 µmol.

The resin-bound linear peptide (117 µmol) was elongated using HATU coupling conditions and Fmoc-deprotection conditions, incorporating the following amino acids in turn: Fmoc-L-Val-OH, Fmoc-*N*-Me-L-Leu-OH, Fmoc-L-Val-OH, Fmoc-*N*-Me-L-Thr-(O^{t}Bu)-OH, Fmoc-L-Thr-OH, Fmoc-*N*-Me-*allo*-L-Ile-OH, Fmoc-L-Val-OH and Fmoc-L-Val-OH. Amino acid couplings following *N*-methylated residues required two treatments with fresh coupling solution.

The resin-bound peptide (60 µmol) was esterified with Alloc-L-Val-OH using the on-resin esterification conditions. The procedure was repeated 2-3 times until all starting material had converted into product as judged by UPLC-MS monitoring.

The resin-bound peptide Fmoc-deprotected and then dimethylated using the on-resin dimethylation conditions. The procedure was repeated 1-2 times until all starting material had converted into product as judged by UPLC-MS monitoring.

The resin-bound peptide was Alloc-deprotected before 48 µmol of resin was elongated using HATU coupling conditions and Fmoc-deprotection conditions incorporating the following amino acids in turn: Fmoc-*threo*-β-OH-L-Phe-OH (1.2 eq. of amino acid used, 16 h coupling), Fmoc-L-Val-OH and Fmoc-*N*-Me-4-OMe-L-Trp-OH (1.2 eq. of amino acid used, 16 h coupling).

The resin-bound peptide was cleaved using the resin-cleavage conditions. The crude protected linear peptide was purified by preparative RP-HPLC using a Sunfire OBD 5 µm 19 x 150 mm (C18) column using a gradient of 50 – 60% MeCN in H_2O (0.1% TFA) over 30 min at a flow rate of 7 mL min^{-1}. The peptide was lyophilized to yield the protected linear peptide as a trifluoroacetate salt (9.4 mg, 9% over 26 steps from resin loading, average of 91% per step).

The protected linear peptide (9.3 mg, 4.88 µmol) was cyclized according to the general cyclization conditions described above. The crude cyclic peptide was purified by preparative RP-HPLC using a Sunfire OBD 5 µm 19 x 150 mm (C18) column using a gradient of 50 – 60% MeCN in H_2O (0.1% TFA) over 20 min at a flow rate of 7 mL min^{-1}. The peptide was lyophilized to give a white fluffy powder of pure ecumicin (**3**) as a trifluoroacetate salt (2.3 mg, 2% over 28 steps from resin loading, 27% for the cyclization and deprotection).

HR-MS: (+ESI) Calc. for $C_{83}H_{134}N_{14}O_{17}$: 1600.0124 $[M+H]^+$, Found: 1600.0124 $[M+H]^+$; **IR (ATR):** ν_{max} = 3477, 3311, 2962, 2926, 1675, 1632, 1532, 1516, 1467, 1204, 1136, 822 cm^{-1}; **UV:** (7.3 µM, MeOH, 1 cm quartz cuvette, 25 °C, background subtracted): ε = 56420 (219 nm), 7339 (263 nm), 6433 (281 nm), 5540 (291 nm) M^{-1}cm^{-1}; **^{1}H NMR** (500 MHz, MeOD): 0.17 (d, 6.5 Hz, 1.5H), 0.33 (d, 6.5 Hz, 1.5H), 0.74 (app. t, 7.3 Hz, 3H), 0.76 (d, 6.7 Hz, 3H), 0.86 (d, 6.7 Hz, 1.5H), 0.91 (d, 6.5 Hz, 1.5H), 0.92 (d, 6.7 Hz, 4.5H), 0.93 (d, 6.9 Hz, 1.5H), 0.95 (m, 2.5H), 0.97 (d, 6.4 Hz, 1.5H), 0.98 (d, 6.9 Hz, 1.5H), 0.99 (m, 2.5H), 1.01 (d, 6.9 Hz, 1.5H), 1.06 (d, 6.7 Hz, 1.5H), 1.09 (d, 6.9 Hz, 1.5H), 1.03 (d, 6.8 Hz, 1.5H), 1.08 (d, 6.9 Hz, 1.5H), 1.09 (d, 6.6 Hz, 1.5H), 1.22 (m, 1H), 1.25 (m, 1H), 1.32 (d, 6.5 Hz, 3H), 1.45 (m, 1H), 1.99 (m, 1H), 1.99 (m, 2H), 2.15 (m, 1H), 2.15 (s, 3H), 2.18 (m, 1H), 2.33 (m, 1H), 2.34 (m, 1H), 2.58 (m, 1H), 2.81 (s, 3H), 3.06 (d, 7.6 Hz, 1H), 3.14 (s, 3H), 3.23 (s, 3H), 3.25 (s, 3H), 3.33 (s, 3H), 3.54

(app. dd, 11.6, -13.5 Hz, 1H), 3.54 (m, 1H), 3.68 (app. dd, 4.8, -13.7 Hz, 1H), 3.82 (s, 3H), 4.10 (dd, 4.7, 11.2 Hz, 1H), 4.40 (dd, 2.6, 8.9 Hz, 1H), 4.45 (dd, 3.9, 6.3 Hz, 1H), 4.52 (dd, 8.1, 9.0 Hz, 1H), 4.61 (app.t, 8.2 Hz, 1H), 4.72 (d, 8.7 Hz, 1H), 4.86 (m, 1H), 4.85 (m, 1H), 4.92 (d, 11.2 Hz, 1H), 5.01 (d, 3.8 Hz, 1H), 5.11 (app. t, 7.6 Hz, 1H), 5.16 (d, 2.3 Hz, 1H), 5.35 (d, 1.2 Hz, 1H), 5.78 (dd, 1.9, 6.6 Hz, 1H), 6.44 (d, 7.7 Hz, 1H), 6.70 (s, 1H), 6.92 (d, 8.4 Hz, 1H), 6.98 (app. t, 8.0 Hz, 1H), 7.20 (m, 1H), 7.24 (m, 2H), 7.26 (m, 2H), 7.72 (d, 8.6 Hz, 1H), 7.85 (d, 9.7 Hz, 1H), 8.21 (d, 7.5 Hz, 1H), 9.02 (d, 9.5 Hz, 2H); **^{13}C NMR** (125 MHz, MeOD, shifts were extracted from HSQC and HMBC data): 11.4, 15.1, 17.0, 17.0, 18.6, 18.9, 19.2, 19.2, 19.2, 19.2, 19.6, 19.6, 19.6, 19.6, 19.6, 19.6, 19.6, 19.6, 19.6, 21.9, 20.1, 20.1, 20.1, 20.1, 20.8, 21.9, 22.2, 23.5, 25.5, 26.5, 26.5, 28.6, 30.0, 31.4, 31.4, 31.8, 31.8, 33.0, 33.6, 33.6, 34.2, 34.2, 39.3, 40.3, 40.8, 41.1, 42.2, 53.3, 54.7, 54.9, 55.5, 55.5, 55.5, 56.3, 56.8, 59.1, 59.1, 59.5, 61.7, 62.9, 66.8, 68.3, 69.8, 71.2, 71.2, 72.8, 73.9, 99.8, 105.9, 112.4, 118.3, 123.2, 123.8, 124.2, 127.0, 128.3, 129.3, 139.9, 142.5, 155.2, 167.8, 169.9, 170.9, 170.9, 171.9, 171.9, 171.9, 173.4, 173.4, 173.4, 174.3, 174.3, 174.7, 174.7. ^{1}H NMR and ^{13}C NMR data are compared to the isolated natural product in Table S3. These data are in agreement with that previously reported by Gao *et al*.[8a]

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

- Synthesis of Fmoc-*threo*-β-hydroxy-L-phenylalanine and Fmoc-*N*-methyl-4-methoxy-L-tryptophan, NMR shifts and assignments for compounds **1**–**3**, NMR spectra, and raw biological data

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Keywords: natural products • peptides • solid-phase synthesis • total synthesis • tuberculosis

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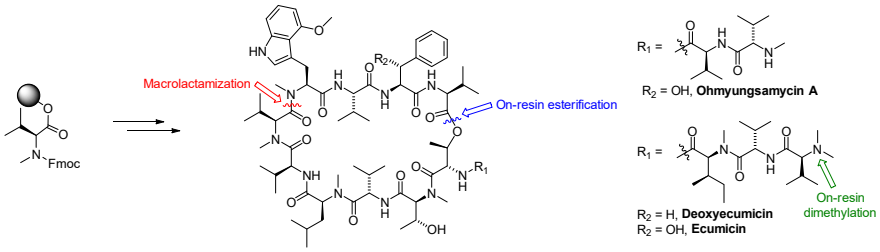

A unified strategy towards antimycobacterial natural products: The total synthesis of the cyclic depsipeptide natural products ohmyungsamycin A, deoxyecumicin, and ecumicin are described. Synthesis was achieved through a unified solid-phase synthetic strategy and a late-stage, pre-organized macrolactamization at an unusually hindered junction. Access to these natural products enabled a detailed comparison on their antimycobacterial activity.

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