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Total Synthesis and Stereochemical Revision of the Anti-Tuberculosis Peptaibol Trichoderin A

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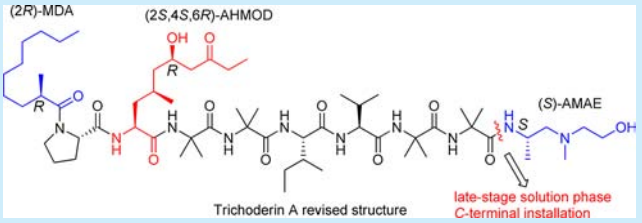
Supporting Information

ABSTRACT: The first total synthesis of the postulated structure of the aminolipopeptide trichoderin A and its epimer are reported. A late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety. This methodology provides a foundation to prepare analogues of trichoderin A to establish a structure−activity relationship. NMR spectroscopic analysis established that the C-6 position of the 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD) residue in trichoderin A possesses an (*R*)-configuration as opposed to the originally proposed (*S*)-configuration.



Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (*Mtb*), which mainly affects the respiratory system.[1] TB now ranks alongside human immunodeficiency virus (HIV) as a leading cause of death worldwide.[2] Although several anti-TB drugs have been identified and developed over the years, TB continues to cause considerable morbidity and mortality worldwide. Globally in 2014, an estimated 480,000 people developed multidrug-resistant TB (MDR-TB).[3] Naturally occurring antimicrobial peptides are a promising source for the development of a new class of drugs to prevent and treat systemic and topical infections,[4−6] and recent work in our group has focused on the synthesis of antimicrobial peptides.[7−9]

Recently, Kobayashi et al.[10] isolated a novel family of aminolipopeptide antibiotics, the trichoderins A **1**, A1 and B, from a marine sponge-derived fungus *trichoderma sp*. Notably, trichoderin A **1** (Figure 1) was found to exhibit a more potent minimum inhibitory concentration activity (MIC, 0.12 μg/mL) than the first-line anti-TB drug isoniazid (MIC, >100 μg/mL) against *Mtb* H37Rv under hypoxic conditions. The anti-mycobacterial activity of the trichoderins has been suggested to be attributed to the inhibition of ATP synthesis in the mycobacteria,[11] but further mode of action studies are required to validate this hypothesis.

Since *Mtb* can lie dormant for years before being activated leading to TB, the intriguing activity of trichoderin A against *Mtb* in its dormant states suggests that trichoderin A can potentially serve as a new peptide-based lead compound for the treatment of TB. Thus, an efficient synthesis of trichoderin A **1**

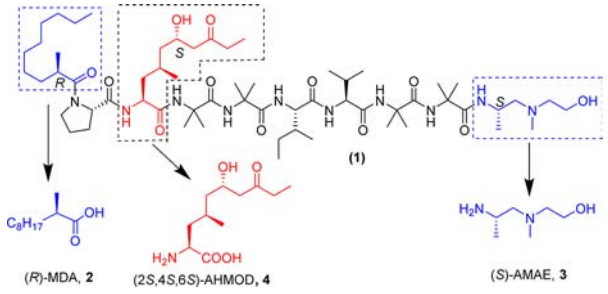


Figure 1. Originally proposed structure of trichoderin A **1** and its nonproteinogenic residues MDA **2**, AMAE **3**, and AHMOD **4**.

and confirmation of its reported structure will enable further structure−activity relationship (SAR) studies.

Trichoderin A **1** is composed of eight amino acids with an *N*-terminal lipid chain, (*R*)-2-methyldecanoic acid (MDA) (**2**), and a *C*-terminus comprising an amino alcohol moiety, (*S*)-2-((2-aminopropyl(methyl)amino)ethanol (AMAE) (**3**). Structural analysis revealed that trichoderin A **1** also possesses several unnatural amino acids, namely, α-aminoisobutyric acid (Aib) and the unusual β-hydroxyketone (AHMOD) **4**. All chiral proteinogenic amino acids were reported to be L-amino acids.[10] The C-6 position of the AHMOD residue was also assumed to exhibit the (*S*)-configuration by comparison with the NMR data for structurally similar peptaibol trichopolyn I.[10] However, in order to determine the absolute stereochemistry of

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C-6 in trichoderin A, we herein report the first synthesis of the postulated structure of trichoderin A **1** and its epimer at the C-6 position of the AHMOD unit.

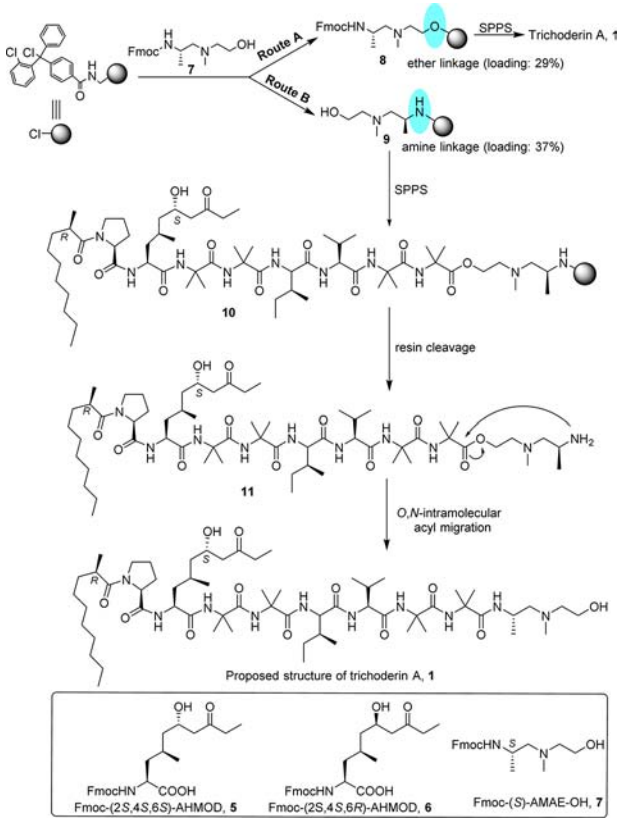
An Fmoc-based SPPS strategy was envisaged for the synthesis of the peptaibol framework of trichoderin A due to the presence of the highly acid-sensitive *β*-hydroxyketone AHMOD residue. 2-Chlorotrityl-functionalized aminomethyl polystyrene resin (2-ClTrt) was also selected for Fmoc-SPPS, as it is cleavable under mild conditions using hexafluoropropan-2-ol (HFIP) thus preventing undesired elimination of the *β*-hydroxy ketone moiety in the AHMOD residue.[12]

Incorporation of a (2*S*,4*S*,6*S*)-AHMOD **5** or (2*S*,4*S*,6*R*)-AHMOD **6** residue into the peptaibol framework of trichoderin A is required to determine the absolute configuration of C-6 in the AHMOD residue. Our group recently reported the total synthesis of the related anticancer peptaibol culicinin D and confirmed the (6*R*)-stereochemistry of the hydroxyl group in its AHMOD unit.[12] The synthesis of the two Fmoc-protected AHMOD residues that are epimeric at the C-6 position were carried out following our earlier work.[13] The synthesis of Fmoc-protected (*S*)-AMAE **7** was also achieved using a similar strategy to that employed for our synthesis of 2-(2-aminopropylamino)ethanol (APAE) present in culicinin D (see Supporting Information).[12] The *N*-terminal lipid residue (*R*)-MDA **2** was obtained in a three-step procedure using an Evans chiral auxiliary mediated asymmetric alkylation (see Supporting Information).[14] Having prepared the requisite building blocks, we proceeded to incorporate these into Fmoc-SPPS to access trichoderin A **1** and its C-6 AHMOD epimer **22**.

Optimal conditions for the solid phase synthesis of trichoderin A were investigated by carrying out the synthesis of an analogue of trichoderin A wherein L-alanine, L-leucine, and dodecanoic acid were used as substitutes for the synthetically challenging AMAE **7**, AHMOD **5**, and MDA **2** building blocks respectively (see Supporting Information). Our initial attempts using the powerful coupling reagent tetramethylfluoroformamidinium hexafluorophosphate (TFFH) to form acyl fluorides *in situ*, which we had successfully used for the total synthesis of culicinin D did not enable coupling of the two consecutive Aib residues in the simplified trichoderin analogues.[12] Several coupling conditions were therefore evaluated to investigate the difficult coupling of the sterically hindered Aib residues. A mixture of the uronium-type coupling reagent 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)dimethylaminomorpholinomethylene)]-methanaminium hexafluorophosphate (COMU)[15] and 2-cyano-2-(hydroxyimino)acetate (Oxyma) minimized Aib deletion (see Supporting Information). Having established conditions to enable formation of the problematic Aib-Aib unit, two different pathways were investigated for the synthesis of trichoderin A **1** (Scheme 1).

Our initial approach to trichoderin A **1** (Scheme 1, route A) began with anchoring the hydroxyl group of Fmoc protected (*S*)-AMAE **7** to 2-ClTrt resin resulting in a resin loading of 29% (see Supporting Information). The synthesis of trichoderin A was then continued with removal of the Fmoc group on resin **8** using 20% piperidine in DMF followed by the use of 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium hexafluorophosphate 3-oxide (HATU) and diisopropylethylamine (DIPEA) to effect coupling of all the natural amino acids. A combination of COMU/Oxyma/DIPEA was used for the difficult coupling of the Aib amino acids to the peptide chain. Fmoc-protected (2*S*,4*S*,6*S*)-AHMOD **5** was effectively coupled to the growing peptidyl resin using HATU/DIPEA without hydroxyl group protection (see Supporting Information). Coupling of the N-terminal MDA residue 2 was then achieved using COMU/Oxyma/DIPEA. After completion of the peptide sequence, a solution of HFIP/CH_2Cl_2 (v/v, 1:4) was used to release the peptide from the solid support. Analysis of the crude product by LC-MS only showed the presence of a trace amount of desired product (see Supporting Information); therefore this approach (Scheme 1, route A) was deemed to be inefficient. Thus, an alternative anchoring strategy wherein Fmoc-protected AMAE **7** was attached to the resin via the more reactive amino group was investigated (Scheme 1, route B).

Scheme 1. Initial Synthetic Strategies Towards Trichoderin A 1



It was anticipated that a final *O,N*-intramolecular acyl migration would afford the desired peptaibol.[16] Briefly, Fmoc-AMAE-OH **7** was attached to 2-ClTrt resin using 2% DBU in DMF to effect concomitant removal of the Fmoc group and attachment of the resultant amino group[17] to afford loaded resin **9** (see Supporting Information). Fmoc-Aib-OH was next coupled to the free hydroxyl group on resin using DIC/DMAP. The Fmoc protecting group was then removed, and the loading for the resin-bound dipeptide was 37%. It was unclear whether the low loading was due to the inefficient attachment of AMAE to 2-ClTrt resin or due to a low yielding esterification between the Aib and AMAE residue. Despite the low loading, peptide elongation proceeded in the same manner as route A. After complete assembly of the desired sequence, the resin-bound peptide **10** was released from the solid support using a solution of HFIP/CH_2Cl_2 (v/v, 1:4). Peptide **11** was next dissolved in DMF and treated with 10 equiv of DBU to give trichoderin A **1**.

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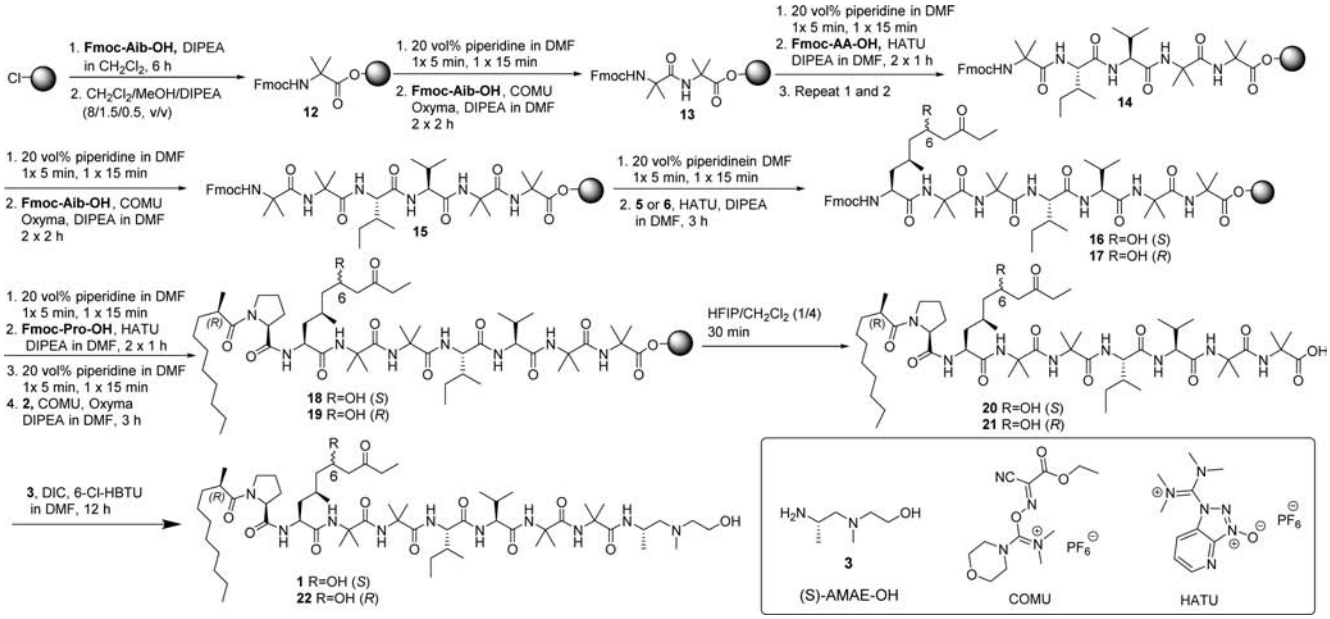
Scheme 2. Total Synthesis of Trichoderin A and Its C-6 AHMOD Epimer



LC-MS analysis of the mixture confirmed the presence of the desired product, but only in a trace amount similar to that observed for route A (see Supporting Information). The *O,N*-intramolecular acyl rearrangement strategy was therefore also abandoned.

We suspected that the low yield and poor recovery of the full length peptide chain were due to poor loading of the AMAE unit to the 2-ClTrt resin or the instability of the peptidyl resin linkage during the synthesis. In order to investigate the possibility of premature cleavage of peptide, Fmoc-AMAE-OH **7** was attached to 2-ClTrt resin via the hydroxyl group (route A) followed by acetylation after each coupling step. Analysis of the crude product by LC-MS after each coupling step showed *N*-acetylated AMAE ($[M + H]^+$: 175.2) which increased with each coupling step (see Supporting Information). This finding confirms the instability of the AMAE bond to the first Aib residue during peptide elongation.

We therefore developed a combined solid-phase and solution-phase strategy involving preparation of peptide **18** using SPPS, followed by cleavage of the peptide chain from the support and attachment of the fully unprotected AMAE **3** to the *C*-terminal carboxylic group of peptide **20** (Scheme 2). We envisaged that the amino group of **3** would react faster than the alcohol with the carboxylic acid of peptide **20**. Advantageously, given that the *C*-terminal amino acid in peptide **20** is achiral, racemization is not a concern.

The synthesis of trichoderin A **1** began with formation of resin **12** by loading of Fmoc-Aib-OH onto 2-ClTrt resin using DIPEA resulting in a higher loading of 49%. The second Fmoc-Aib-OH was then coupled to the resin using COMU/Oxyma/DIPEA effecting the difficult formation of the Aib-Aib unit to give **13**. Elongation of the peptide sequence was continued using the previously optimized conditions with HATU/DIPEA as a coupling reagent. The amino acid (2*S*,4*S*,6*S*)-AHMOD **5** was then effectively coupled to resin **15** using HATU/DIPEA to form **16**. Finally, L-proline and fatty acid (2*R*)-MDA **2** were sequentially coupled to complete the synthesis of linear peptidyl resin **18**. Treatment of resin-bound peptide **18** with HFIP/CH_2Cl_2 (v/v, 1:4), followed by analysis of the cleaved mixture by LC-MS, confirmed the presence of the desired peptide **20** as the major product (see Supporting Information). The lyophilized crude peptide was then purified by semi-preparative RP-HPLC using 0.1% formic acid to afford peptide **20** in 14% yield. Pleasingly, use of mild formic acid instead of 0.1% TFA avoided elimination of the β-hydroxy ketone to an enone byproduct.[12] The resulting peptide **20** was then subjected to solution phase *C*-terminal installation of unprotected AMAE. Use of HATU, COMU, or PyBOP in DMF or DIC in CH_2Cl_2 resulted in minimal *C*-terminal addition of AMAE. The coupling efficiency increased significantly using DIC/6-Cl-HOBt in DMF (see Supporting Information). Subsequent purification of the crude reaction mixture by RP-HPLC afforded 7 mg (6.0%) of the postulated structure of trichoderin A **1** (>97% purity). The synthetic route to the postulated structure of trichoderin A **1** outlined in Scheme 2 was adapted for the synthesis of the C-6 AHMOD epimer of trichoderin A **22** wherein (*S*)-AHMOD **5** residue was substituted for (*R*)-AHMOD **6**. Adopting this procedure, peptide **22** (4 mg, 3.4%) was synthesized from resin **12** (loading 47%) in >97% purity (see Supporting Information). The absolute stereochemistry at C-6 of the AHMOD residue in trichoderin A was determined by comparing the deviations of the ^1H and ^{13}C NMR chemical shifts between isolated trichoderin A and the two synthetic trichoderin A peptides **1** and **22** (see Supporting Information). The deviations of the ^1H and ^{13}C NMR chemical shifts between isolated trichoderin A and synthetic trichoderin A **1** were larger than the values obtained between isolated trichoderin A and **22**. It was therefore concluded that the natural product trichoderin A is the same structure as synthetic peptide **22** where the configuration at C-6 of the AHMOD residue is assigned as (*R*), rather than (*S*) as reported by Kobayashi et al.[10] This is identical to the stereochemistry exhibited by the AHMOD residue in culicinin D.[12]

The two C-6 AHMOD epimers of trichoderin A **1** and **22** were tested for their antimicrobial activity (MIC) against a range of bacteria (Table 1).[18] Though the anti-TB activity measured for both synthetic trichoderins were less potent than

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Table 1. Minimum Inhibitory Activity (MIC) of Synthetic Trichoderin A 1 and 22 against Selected Bacterial Pathogens

	MIC (μg/mL)	
bacterium	synthetic peptide, **1**	synthetic peptide, **22**
M. tuberculosis	9.3	9.3
M. smegmatis	9.3	9.3
S. aureus	2.3	2.3
E. coli	>595.4	>595.4
S. uberis	4.6	2.3

that reported for the natural product, this is possibly due to peptide **1** and **22** being assessed as the free base as compared to the natural product which was evaluated as a salt. Importantly, the anti-TB MIC values for synthetic trichoderins **1** and **22** (9.3 μg/mL) were found to be 10-fold more potent than the first-line anti-TB drug isoniazid (MIC, >100 μg/mL). Thus, these results highlight the importance of trichoderins **1** and **22** as promising new candidates for the treatment of TB.

Despite the biological importance of AHMOD-containing aminolipopeptides, only a limited number of synthetic studies have been reported due to the presence of the sensitive AHMOD residue and the sterically hindered Aib amino acids. As a consequence of our synthetic studies, stereochemical assignment of the C-6 position of the AHMOD moiety of trichoderin A was determined to be (*R*) as opposed to the originally proposed (*S*)-configuration.

Our successful first total synthesis of the postulated structure of the antituberculosis aminolipopeptide trichoderin A **1** and its C-6 AHMOD epimer **22** required use of a late-stage solution phase *C*-terminal coupling to effectively introduce the (*S*)-AMAE moiety **3**, and this proved to be the significant factor to enable an efficient synthesis. This methodology provides a foundation and framework to prepare further analogues of trichoderin A in order to construct a structure−activity relationship. Given the broad-spectrum activity of these compounds against Gram-positive bacterial pathogens and mycobacterial species, the mechanism of action of trichoderin A requires further investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01886.

Experimental procedures and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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