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Note

Facile Syntheses of AM-toxins and Analogs as Cyclic Depsipeptides by the Solid-phase Method

Masahiro Miyashita, Tomoko Nakamori, Takahiro Murai, Hisashi Miyagawa, Miki Akamatsu, and Tamio Ueno

Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

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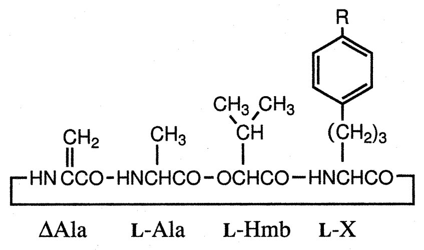
The cyclic depsipeptides, AM-toxins I and II and AM-toxin I analogs, were efficiently and rapidly prepared by the Fmoc-based solid-phase method for the synthesis of linear depsipeptides, with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) being used for their subsequent cyclization.

Key words: AM-toxin; host-specific toxin; cyclic depsipeptide; solid-phase synthesis; HATU



	X	R
I	Amp	OCH_3
II	App	H
III	Ahp	OH

Fig. 1. Structures of AM-toxins I-III.

AM-toxins I-III are host-specific phytotoxins of the *Alternaria alternata* apple pathotype which cause spot disease on apple leaves of susceptible cultivars such as Indo and Starking Delicious.[1,2] The structure of AM-toxin I is that of a cyclic tetradepsipeptide containing an L-2-hydroxy-3-methylbutanoic acid (L-Hmb) residue, along with two unusual amino acids, α,β-dehydroalanine (ΔAla) and L-2-amino-5-(*p*-methoxyphenyl)pentanoic acid (L-Amp).[3] In AM-toxins II and III, L-Amp of AM-toxin I is replaced by L-2-amino-5-phenylpentanoic acid (L-App) and L-2-amino-5-(*p*-hydroxyphenyl)pentanoic acid (L-Ahp), respectively (Fig. 1). AM-toxins act on the chloroplasts and plasma membranes,[4] and have been reported to bind to putative receptors on apple leaves.[5] However, the mechanism for action of these toxins is not completely understood.

An analysis of the structure-activity relationship of AM-toxin analogs would be useful for a characterization of the putative receptors[6] and for the construction of labeled probes[7,8] designed to locate these receptors. Thus, a rapid and facile procedure for the preparation of AM-toxin analogs would be useful. Current methodology involves the total synthesis of these peptides *via* the solution-phase method to obtain linear depsipeptides, this being followed by cyclization using a succinimide ester method.[9] However, this procedure involves many steps and is time-consuming.

Compared to a peptide synthesis in solution, the solid-phase method is more facile and can also be carried out more rapidly. Therefore, solid-phase synthesis plays an important role in structure-activity studies, since it allows the rapid generation of a large number of diverse compounds. However, there are only a few examples of the solid-phase synthesis of depsipeptides, and AM-toxins themselves have not yet been synthesized by the solid-phase method. In addition, a variety of novel coupling reagents have been developed for the cyclization of linear peptides. Among these, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) appears to be one of the most effective in terms of the cyclization reaction.[10]

This study reports the development of a new method for the synthesis of AM-toxin I and its analogs. The 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide synthesis method and HATU were adopted for synthesizing the linear depsipeptides and for their subsequent cyclization, respectively.

The scheme for the synthesis of AM-toxin I (**1**) is shown in Fig. 2. It is important to prepare a reasonable linear precursor which can be preferentially cyclized to a monomeric form among four possible linear precursors of the AM-toxins. Based on investigations by Shimohigashi *et al.*, a linear depsipeptide which contains an aromatic amino acid such as L-Amp, at the *C*-terminal end, is preferable for obtaining cyclic monomers.[11] They also showed that the use of a D-amino acid, which is the ΔAla precursor at the *N*-terminal end, was effective in this reaction.[12]

Therefore, L-Amp and D-2,3-diaminopropanoic acid (D-Dap) were selected as the amino acids at the *C*- and *N*-termini of the linear depsipeptide precursor of AM-

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Abbreviations: L-Hmb, L-2-hydroxy-3-methylbutanoic acid; ΔAla, α,β-dehydroalanine; L-Amp, L-2-amino-5-(*p*-methoxyphenyl)pentanoic acid; L-App, L-2-amino-5-phenylpentanoic acid; L-Ahp, L-2-amino-5-(*p*-hydroxyphenyl)pentanoic acid; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, previously known as *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Fmoc, 9-fluorenylmethoxycarbonyl; D-Dap, D-2,3-diaminopropanoic acid; DIPCDI, *N*,*N*-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; HOBt, *N*-hydroxybenzotriazole; Boc-D-Dap(Z), N^2-*tert*-butoxycarbonyl-N^3-benzyloxycarbonyl-D-2,3-diaminopropanoic acid; TFA, trifluoroacetic acid; DIEA, diisopropylethylamine.

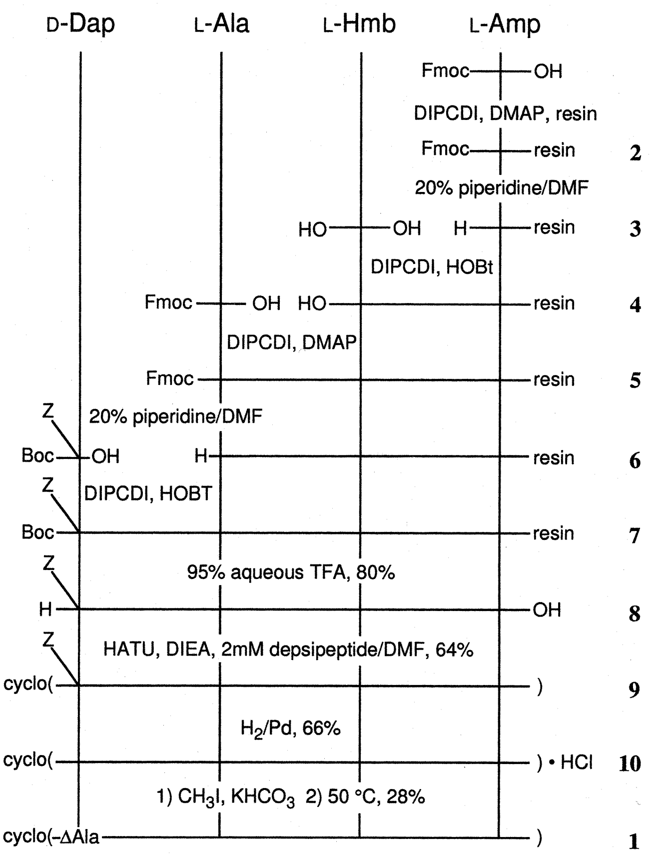

Fig. 2. Synthesis of AM-toxin I.

toxin I, respectively. D-Dap has been utilized as the ΔAla precursor, which can be converted into ΔAla *via* a Hofmann degradation, in the synthesis of AM-toxins.[13] β-Phenylselenoalanine has also been recently reported to be an effective ΔAla precursor for the synthesis of AM-toxins.[14] However, the phenylselenyl group of this amino acid is very unstable and sensitive to oxidation. To achieve a facile synthesis of AM-toxins, D-Dap as a ΔAla precursor is preferable because of its stability and its ease of conversion into ΔAla.

The introduction of L-Hmb and L-Ala was achieved stepwise by amide- and ester-forming reactions, respectively, while in the solution-phase synthesis of AM-toxins reported by Izumiya's group, a unit of didepsipeptide Boc-L-Ala-L-Hmb was separately prepared and then coupled with the Amp residue.[9] This stepwise elongation is useful for efficiently synthesizing AM-toxin analogs in which the L-Ala or L-Hmb residue is replaced.

Fmoc-L-Amp[15] was coupled with a *p*-alkoxybenzyl alcohol resin by using *N*,*N*-diisopropylcarbodiimide (DIPCDI)/4-dimethylaminopyridine (DMAP) to give **2** (Fig. 2). After removing the Fmoc group of L-Amp in **2** with 20% piperidine in *N*,*N*-dimethylformamide (DMF), L-Hmb could be introduced into **3** by using DIPCDI/*N*-hydroxybenzotriazole (HOBt) without protecting its hydroxyl group in this solid-phase method. The ester bond between Fmoc-L-Ala and L-Hmb of **4** was efficiently formed by using DIPCDI/DMAP. Removing the Fmoc group of L-Ala was achieved by treating resin-bound peptide **5** with 20% piperidine in DMF for a short time (5 min) to avoid hydrolysis of the ester bond. After introducing N^2-*tert*-butoxycarbonyl-N^3-benzyloxycarbonyl-D-2,3-diaminopropanoic acid (Boc-D-Dap(Z))[16] into **6** by using DIPCDI/HOBt, depsipeptide **8** was cleaved from resin-bound peptide **7** with 95% aqueous trifluoroacetic acid (TFA) at room temperature (1 h). The ester bond was stable during deprotection of the Fmoc group and during cleavage from the resin.

In previous cyclization studies that applied the succinimide ester method, the carboxyl group of the linear precursor was activated by *N*-hydroxysuccinimide prior to cyclization, and 3–6 days were required for this activation and the subsequent cyclization reactions.[9,13] In this study, HATU was used for the cyclization reaction by simply mixing, in the presence of diisopropylethylamine (DIEA), with the linear depsipeptide in which both termini are free, with minimal racemization. After 24 h, no starting material **8** was detectable and only cyclic monomer **9** was obtained in a high yield (64%). No cyclic dimer formation, which was a troublesome problem in the previous studies on AM-toxin synthesis, was apparent.

The total yield for the formation of the linear depsipeptide and subsequent cyclization to AM-toxin I in the present study was 51% which is significantly higher than the 36% yield reported by Izumiya's group.[9] In the final step after removing the Z group of **9** with H_2/Pd-black, the Dap residue of cyclic depsipeptide **10** was converted into ΔAla by using CH_3I/$KHCO_3$ in anhydrous ethyl acetate. Purification of the product (AM-toxin I, **1**) was carried out by reversed-phase HPLC.

The same procedure as that used for the synthesis of AM-toxin I enabled AM-toxin II and several AM-toxin I analogs, in which the L-Ala residue is replaced by L-2-aminobutanoic acid, L-norvaline, L-norleucine and L-Leu, to also be successfully synthesized. The apple-leaf necrotic activity and spectral data for AM-toxins I and II synthesized in this study were identical with those of natural samples. The structure-activity relationship of the analogs will be discussed in a future paper.

In conclusion, AM-toxins I and II and AM-toxin I analogs were rapidly obtained in a satisfactory yield by using the Fmoc-based solid-phase method for synthesizing the linear depsipeptides and then HATU for their subsequent cyclization. The facile and efficient synthetic method described here may also be applicable to other cyclic depsipeptides such as ionophores and antibiotics.

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