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Solid-phase synthesis of dehydropeptide, AM-toxin II, using a novel selenyl linker by side-chain tethered strategy

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Abstract—The cyclic dehydrodepsipeptide, AM-toxin II, was efficiently synthesized by a solid-phase method using a novel selenyl linker. The cleavage from the resin with formation of a double bond is successfully achieved under mild oxidative conditions with TBHP. © 2001 Elsevier Science Ltd. All rights reserved.

A number of dehydroamino acids are found from natural sources as a component of bioactive peptides.[1] It is reported that dehydroamino acid residues possess the activities of a Michael-acceptor, and it is presumed that various biological activities are due to such reactivity. In addition, based on structural considerations, they are strong inducers of folded conformations in peptides.[2]

AM-toxins are known as members of this class of molecules (Fig. 1), as well as host-specific phytotoxins produced by *Alternaria alternata*, the fungus causing leafspot disease of apple trees.[3–5] The structure of AM-toxins is that of cyclic depsipeptides containing α,β-dehydroalanine (ΔAla). Synthetic studies employing solution-phase cyclization are already reported.[6–9]

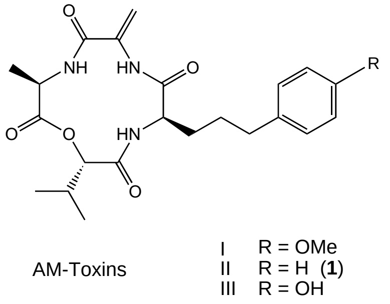

Figure 1. Structure of AM-toxins.

Recently, we presented a side-chain tethered strategy for a solid-phase synthesis using a novel linker, and the synthesis of glycopeptides and Ser/Thr containing peptide, a silyl ether-type linker, was used to bind a side-chain hydroxyl group onto the solid support.[10–12] This approach has several advantages due to the orthogonal protection of the N- and C-terminal of amino acids to allow bi-directional peptide chain elongation after selective deprotection.

Based on this study, we designed a selenated alanine as an anchoring residue of peptide for the synthesis of dehydropeptides.[13] A key feature of this approach, an oxidative cleavage from the solid support, will give the corresponding dehydroalanine residue after peptide elongation and cyclization. The solid-phase synthesis of cyclic compounds may provide a possible solution to these problems by anchoring molecules to prevent oligomerization,[14] along with the feasibility of providing libraries for effective screening of new bioactive lead compounds.

Our synthesis started with the preparation of a selenated alanine. *N*-Fmoc-L-serine allyl ester was reacted with *o*-nitro phenyl selenocyanide with Bu_3P in pyridine[15] to give the corresponding selenoether[16] in 83% yield (Scheme 1). The resulting compound **2** was successively reduced with Zn–AcOH to give the aniline derivative **3** (82%), which was treated with succinic

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Keywords: AM-toxin; solid-phase synthesis; dehydropeptide; cyclic peptide; selenyl linker.

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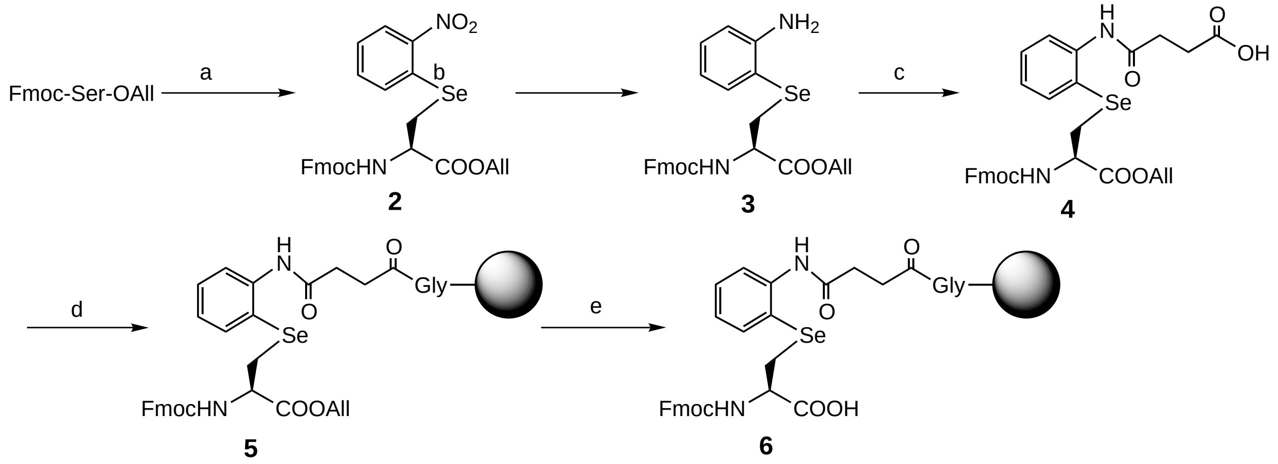
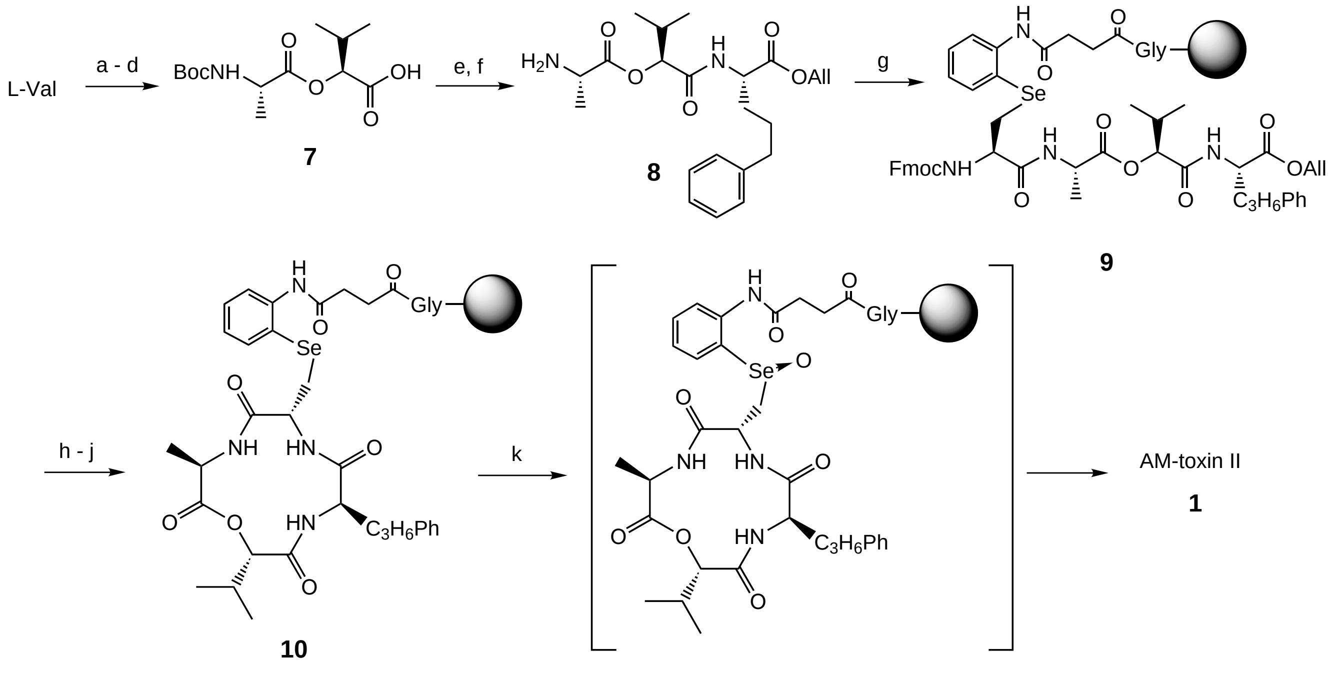


Scheme 1. *Reagents and conditions*: (a) *o*-NO_2-C_6H_4-SeCN, Bu_3P/pyridine, rt, 2 h (83%); (b) Zn, AcOH/THF, rt, 2 h (82%); (c) succinic anhydride, DIEA/CH_2Cl_2, rt, 3 h (81%); (d) Gly-Wang resin (0.65 mmol/g), HATU, DIEA/DMF, voltex, rt, overnight, 98% in Keiser test; (e) $Pd(PPh_3)_4$, dimedone/THF, voltex, rt, 3 h.

anhydride to afford succinanilic acid[16] in 81% yield. The carboxylic acid was immobilized to the commercially available Gly-Wang resin (Novabiochem, Gly content 0.65 mmol/g) with HATU and DIEA. The final seleno amino acid content (0.17 mmol/g) was calculated by the weight of carboxylic acid after acid cleavage by the action of TFA. Then, the C-terminal protecting group was removed by palladium chemistry (cat. $Pd(PPh)_4$ and excess dimedone in THF) to give immobilized free acid **6**.

The C-terminal fragment was synthesized by the usual liquid-phase procedure. Boc-Ala and L-2-hydroxy-3-methylbutanoic acid (L-Hmb) allyl ester (prepared from L-valine by the van Slyke reaction in 76% yield; followed by allylation with Cs_2CO_3 and allyl bromide in 65%) were condensed with DCC–DMAP to give the corresponding ester (73%), which was deprotected by palladium chemistry (cat. $Pd(PPh)_4$ and excess piperidine in THF, 93%) to give carboxylic acid **7** (Scheme 2). The other unusual amino acid, L-2-amino-5-



Scheme 2. *Reagents and conditions*: (a) $NaNO_2$/AcOH aq., rt, 30 min (76%); (b) Cs_2CO_3 then allyl bromide, rt, overnight (65%); (c) Boc-Ala, DCC, DMAP/DMF, rt, overnight (73%); (d) cat. $Pd(PPh_3)_4$, piperidine/THF, rt, 2 h (93%); (e) L-App-OAll (see text), HATU, DIEA/DMF, rt, 6 h (88%); (f) 50% TFA/CH_2Cl_2, rt, 1 h (quant.); (g) **6**, HATU, DIEA/DMF, voltex, rt, overnight; (h) cat. $Pd(PPh_3)_4$, dimedone/THF, voltex, rt, 2 h; (i) 40% piperidine/DMF, voltex, rt, 1 h; (j) FDPP, DIEA/DMF, voltex, rt, 2 days; (k) TBHP/CH_2Cl_2–TFE, voltex, rt, 7 h.

phenylpentanoic acid (App), was synthesized by a similar protocol described by Shirahama and co-workers.[8] The known racemic acetyl amino acid was prepared from 3-phenyl propanol in four steps (1. PBr_3, 2. diethyl acetoamidomalonate–NaOEt/EtOH, 3. NaOH, 4. decarboxylation).[6] The enantiomerically pure[17] L-App was prepared by enzymatic hydrolysis with amino acylase (from *Aspergius genus*), which was converted into an allyl ester via an N^{α}-Boc derivative (1. Boc_2O, 2. Cs_2CO_3 then allyl bromide, 3. TFA) in 21% yield (in four steps including optical resolution). The coupling between Boc-Ala-Hmb (**7**) and App-OAll was carried out by treatment with HATU and DIEA in 88% yield, followed by deprotection with TFA to give free amine **8** (quant.).

The C-terminal condensation was achieved by the tripeptide allyl ester **8** using HATU–DIEA to give the protected dihydro *seco*-acid on the solid support (**9**). After deprotection with Pd(0) followed by piperidine treatment, cyclization was carried out just on the resin by using FDPP (pentafluorophenyl diphenylphosphinate)[8,18] and DIEA.

Finally, oxidative cleavage via selenoxide from resin was performed by treatment with TBHP in CH_2Cl_2–TFE to give successfully AM-toxin II (**1**) with concomitant formation of an unsaturated bond. The yield (ca. 10%)[19] was estimated on the basis of the calculated loading amount of selenated amino acid **5**. The synthetic compound was identical to a natural sample with spectrum aspects[4] and R_f values of TLC (four different solvent systems).[16]

In conclusion, we have demonstrated the potential of the newly developed synthetic strategy to dehydropeptide. AM-toxin has been successfully synthesized by C-terminal peptide elongation and cyclization, followed by oxidative cleavage by formation of the double bond. We have also demonstrated that this methodology is very useful to synthesize unsaturated compounds using solid-phase chemistry.

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16. Selected data of the key compounds. Compound **2**: $[\alpha]_D^{26}$ +6.1 (c 1, $CHCl_3$); δ_H ($CDCl_3$) 3.34 (1H, dd, $J=5.8$, 12.8 Hz), 3.48 (1H, dd, $J=5.8$, 12.8 Hz), 4.19 (1H, t, $J=7.0$ Hz), 4.38 (2H, d, $J=7.0$ Hz), 4.55 (2H, ddd, $J=6.3$, 6.3, 13.3 Hz), 4.80 (1H, br.q, $J=$ca. 6 Hz), 5.23 (1H, br.d, $J=10.4$ Hz), 5.35 (1H, br.d, $J=17.4$ Hz), 5.65 (1H, br.d, $J=7.6$ Hz, NH), 5.95 (1H, m), 7.20–8.22 (~12H, arom.). Compound **4**: $[\alpha]_D^{26}$ +16.1 (c 1, $CHCl_3$); δ_H ($CDCl_3$) 2.73 (2H, br.t, $J=$ca. 7 Hz), 2.77 (2H, dist.t), 3.14 (1H, dd, $J=5.0$, 12.8 Hz), 3.35 (1H, dd, $J=5.0$, 12.8 Hz), 4.20 (1H, t, $J=7.0$ Hz), 4.33 (2H, m), 4.35 (1H, m), 4.55 (1H, dd, $J=6.0$, 13.3 Hz), 4.67 (1H, br.dd, $J=$ca. 6, 6 Hz), 5.18 (1H, br.d, $J=$ca. 10 Hz), 5.24 (1H, br.d, $J=$ca. 17 Hz), 5.76 (1H, m), 5.87 (1H, d, $J=7.6$ Hz, NH), 7.0–7.7 (~12H, arom.), 8.26 (1H, d, $J=7.6$ Hz, NH). Synthetic AM-toxin II (**1**): $[\alpha]_D^{28}$ −18.7 (c 0.17, DMSO) (lit. $[\alpha]_D^{27}$ −14.2 (c 0.55, DMSO)); HRMS calcd for $C_{22}H_{29}N_3O_5$ m/z 415.2105, found m/z 415.2096; δ_H (DMSO-d_6, 45°C) 0.89 and 0.90 (each 3H, d, $J=$ca. 7 Hz, overlapped), 1.35 (3H, d, $J=7.5$ Hz), 1.53 (1H, m), 1.62 (2H, m), 1.86 (1H, m), 1.98 (1H, m), 2.56 (1H, m), 2.66 (1H, m), 4.32 (2×1H, m, overlapped), 4.68 (1H, d, $J=6.5$ Hz), 5.36 (1H, br.s), 5.40 (1H, s), 7.16–7.28 (5H, arom.), 7.99 (1H, br.s, NH), 8.11 (1H, br.d, $J=9.0$ Hz, NH), 9.05 (1H, br.s, NH); TLC (Merck silica gel 60) $R_f=0.56$ (PhH:acetone = 2:1; lit.[6] 0.57), $R_f=0.28$ (EtOAc:$CHCl_3$ = 1:1; lit.[7] 0.30), $R_f=0.28$ ($CHCl_3$:MeOH:AcOH = 95:5:1; lit.[7] 0.30), $R_f=0.84$ (n-BuOH:AcOH:H_2O = 4:1:1; lit.[7] 0.83).
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