Research Article

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Synthesis of the *C*-terminal pentapeptide of the peptaibol culicinins

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The synthesis of the *C*-terminal pentapeptide of culicinins has been achieved using [4 + 1] protocol and reduction-coupling strategy.

Supporting information may be found in the online version of this article

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Peptaibols are a growing family of polypeptides, which amount to more than 800 members recently [1]. Generally speaking, these α-aminoisobutyric acid (Aib)-rich linear peptides show interesting physicochemical and biological activities such as the formation of pores in bilayer lipid membranes as well as antibacterial, antifungal, occasionally antiviral and antitumor activities [2].

Culicinins [3] (Figure 1) are novel ten-membered linear peptaibols isolated from the fungus *Culicinomyces clavisporus* in the year 2006. These natural products have received significant attention because of their unique structures and remarkable biological activities [1,4]. Very recently, we have reported the preparation of the novel non-standard amino acid (2*S*, 4*R*)-2-amino-4-methyldecanoic acid (AMD) [5], a key residue of culicinins. As a continuation of our efforts, we now describe the synthesis of the *C*-terminal pentapeptide fragments of these peptaibols.

As shown in Figure 1, the target *C*-terminal pentapeptide could be constructed from a tetrapeptide and a modified Ala residue, 2-(2′-aminopropyl)-aminoethanol (AAE). Commercial reagent β-alanine (**5**) was converted to methyl ester **6** and subsequently coupled under standard conditions with Boc-Leu-OH, giving the dipeptide **7** in 92% yield (Scheme 1). As one of the most hindered amino acids in nature, Aib unit was smoothly introduced into tripeptide **9** using PyAOP as coupling reagent, which was much better than other coupling methods (PyAOP, 96%; EDCI/HOBt, 0%; EDCI/HOAt, $<$10%; mixed anhydride $\sim$60%). Exposure of tripeptide **9** to TFA afforded the free amine, which was coupled with Boc-Leu-OH to give the fully protected tetrapeptide **10** in high yield.

AAE and 2-[(2′-aminopropyl)-methylamino]-ethanol (AMAE) are not uncommon fragments in peptaibols. However, the chemical synthesis of these units was seldom reported. Generally, these fragments are regarded as the reduced forms of the *C*-terminal dipeptide units – Ala-Gly-OH and – Ala-MeGly-OH, respectively [6]. Therefore, initially we tried to reduce the dipeptide to the corresponding amino alcohol (Scheme 2). Coupling of H-Gly-OMe to Boc-Ala-OH with EDCI/HOBt resulted in the formation of the corresponding dipeptide (Boc-Ala-Gly-OMe) in 90% yield. Unfortunately, reduction of the protected dipeptide ester with Red-Al® or $LiAlH_4$ resulted in very poor yields [7]. We then turned to another coupling-reduction protocol (Scheme 2). However, we found the benzyl ether was destroyed simultaneously with the removal of Boc group.

Since the coupling-reduction strategies above were not successful, we therefore adopted an alternative approach for the synthesis of the AAE unit, following a 'reduction-coupling' protocol, as shown in Scheme 3. Boc-Ala-OH was esterified and then reduced to corresponding amino alcohol **12** in satisfactory yields. This alcohol was first tosylated, and then treated with 2-aminoethanol, giving amino alcohol **14** in satisfactory yield. Initially, we planed to protect the imino group of **14** with Cbz group employing Cbz-Cl. However, the di-Cbz compound **15** was also formed ($\sim$10% yield when 1.2 equiv. Cbz-Cl was used). This result promoted us to mask the free imino and hydroxyl groups simultaneously. Exposure of compound **14** to 3 equivalent of Cbz-Cl gave the di-Cbz product in moderate yield, which could be removed in a single hydrogenation step whenever needed.

Deprotection of **15** with 4-M HCl in dioxane and of the tetrapeptide **10** with LiOH liberated the amine and acid, respectively, which were coupled to give the target pentapeptide **16** in high yield.

In conclusion, we have achieved the synthesis of the *C*-terminal pentapeptide of culicinins following a [4 + 1] strategy that included a reduction-coupling step. Further studies toward*** the preparation of other fragments and the total synthesis of culicinins are currently underway.

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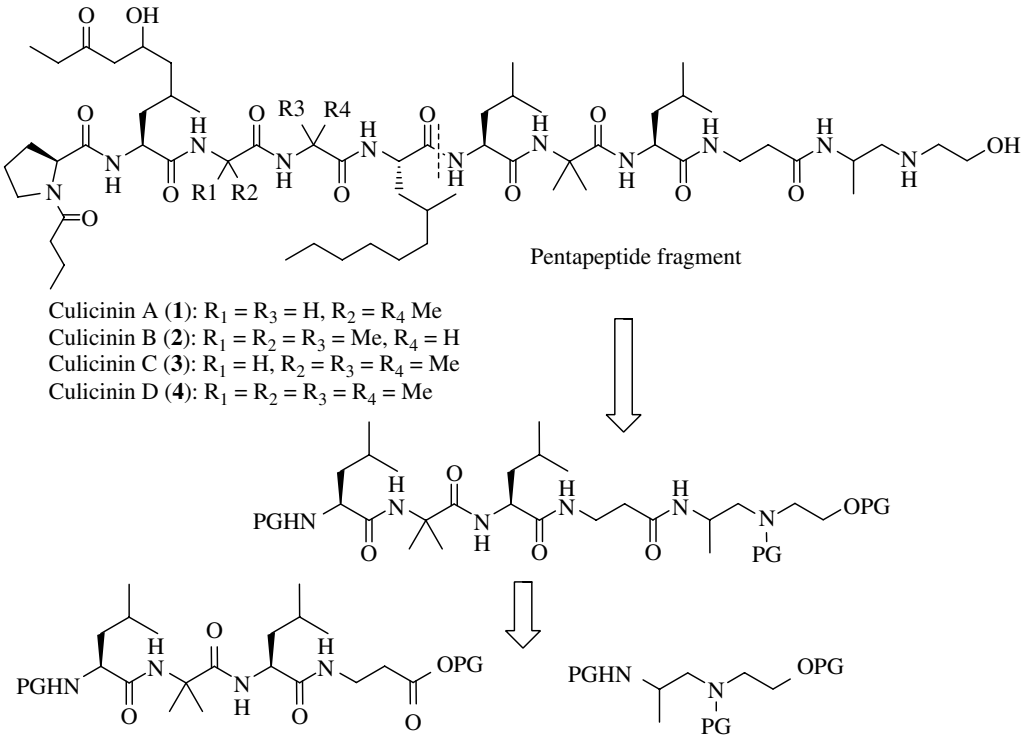
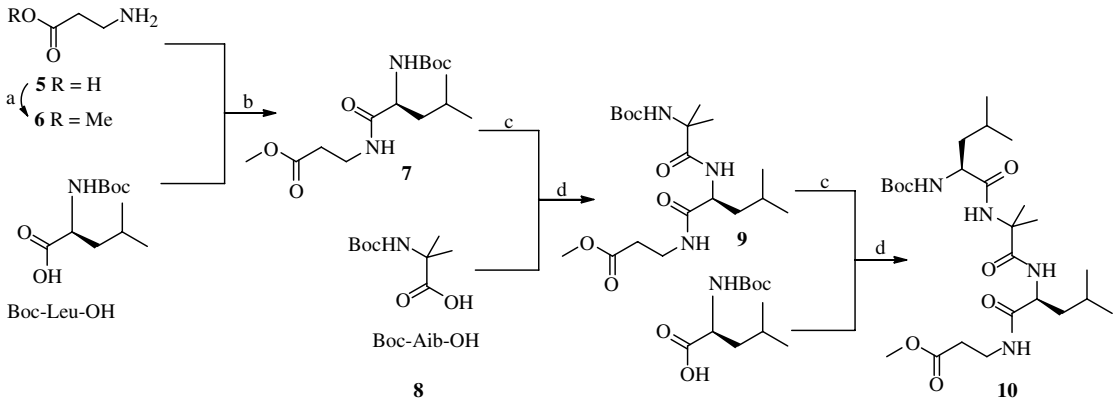
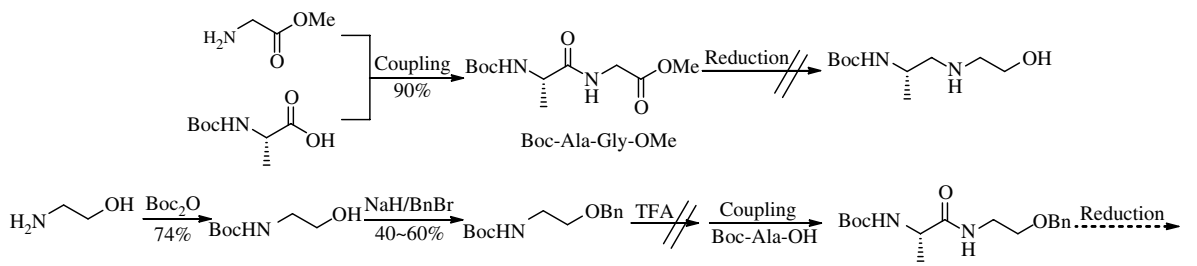


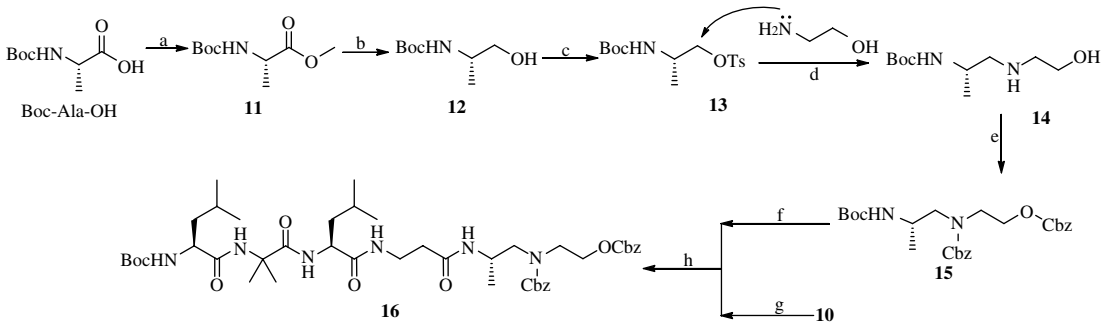
Figure 1. Structures of culicinins and retrosynthetic analysis of the *C*-terminal pentapeptide. PG, protecting group.



Scheme 1. Reagents and conditions: (a) $SOCl_2$/MeOH, −10 °C to rt, 2 d, 67%; (b) EDCI (1.2 equiv.), HOBt (1.2 equiv.), DIPEA (2.0 equiv.), 0 °C to rt, overnight, 92%; (c) TFA/DCM, 0 °C to rt, 2 h; (d) PyAOP (1.2 equiv.), HOAt (1.2 equiv.), DIPEA (2.0 equiv.), 0 °C to rt, 20 h, 96% for **9** and 90% for **10**. EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide chloride; PyAOP, (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.



Scheme 2. Unsuccessful attempts for the preparation of AAE termini.



Scheme 3. Reagents and conditions: (a) MeI (1.5 equiv.), $KHCO_3$ (2.0 equiv.), rt, 6 h, 99%; (b) $LiAlH_4$ (1.1 equiv.), Et_2O, 0 °C to rt, 30 min, 93%; (c) TsCl (1.5 equiv.), DMAP (0.1 equiv.), Et_3N (3.0 equiv.), DCM, 0 °C to rt, 8 h, 86%; (d) DMF 50 °C, 1 h, 76%; (e) Cbz-Cl (3.0 equiv.), DMAP(0.1 equiv.), DCM, 0 °C to rt, 8 h, 51%; (f) 4 M HCl/dioxane, 0 °C to rt, 1 h; (g) LiOH monohydrate (2.0 equiv.), THF-MeOH-H_2O, 0 °C to rt, 1 h; then H_3O^+; (h) PyAOP (1.5 equiv.), HOAt (1.5 equiv.), DIPEA (3.0 equiv.), THF-DMF, 0 °C to rt, 24 h, 95% in three steps. TsCl, *p*-toluenesulfonyl chloride; Cbz-Cl, benzyl chloroformate.

Supporting Information

Supporting information may be found in the online version of this article.

Acknowledgements

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