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Synthesis of the Peptaibol Framework of the Anticancer Agent Culicinin D: Stereochemical Assignment of the AHMOD Moiety

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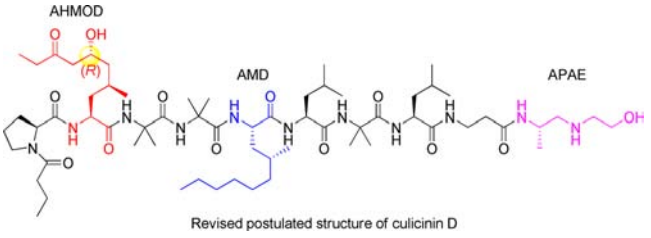
ABSTRACT



The postulated structure of the potent anticancer peptaibol culicinin D has been synthesized using Fmoc-based solid-phase peptide synthesis (SPPS). Comparison of the ^{1}H NMR data for the reported structure of culicinin D with the data obtained for the two synthetic polypeptides epimeric at C-6 in the AHMOD unit established the C-6 stereochemistry of the AHMOD residue in the natural product to be (*R*).

Culicinin D (**1**) (Figure 1) is a member of the linear peptaibol family of peptides isolated from the cultures of the fungus *Culicinomyces clavisporus*.[1] Not only has culicinin D (**1**) been found to suppress proliferation of PTEN-negative MDA468 breast tumor cells with an impressive IC_{50} value of <6 nM,[1] this intriguing compound also contains 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD) **2** which is an unnatural amino acid found in many members of the peptaibol family.[2] In addition to the unique AHMOD **2** unit, structural analysis of culicinin D (**1**) revealed the presence of 2-amino-4-methyldecanoic acid (AMD) **3** and 2-(2-aminopropylamino)ethanol (APAE) **4**.

While experimental evidence established that both the leucine and proline residues in natural culicinin D (**1**) were L-amino acids[1a] and that the absolute stereochemistry of the AMD **3** unit was (2*S*,4*R*),[3] the chiral centers in the AHMOD **2** and APAE **4** building blocks were only assumed to exhibit the (*S*)-configuration.[1a,4] With the overarching aim of developing a synthetic program to enable future investigations of the biological activity of culicinin D and related members of the peptaibol family, we herein report the first synthesis of the postulated structure of culicinin D (**1**). Importantly, the synthesis established the absolute stereochemistry of C-6 in the AHMOD moiety of the natural product to be (*R*).

Lengthy syntheses of fully protected AHMOD **2**, AMD **3**, and APAE **4** residues with Boc-protected *N*-termini have been reported.[4,5] Moreover, the one reported synthesis of

(1) (a) He, H.; Janso, J. E.; Yang, H. Y.; Bernan, V. S.; Lin, S. L.; Yu, K. *J. Nat. Prod.* **2006**, *69*, 736–741. (b) Baker, D. D.; Chu, M.; Oza, U.; Rajgarhia, V. *Nat. Prod. Rep.* **2007**, *24*, 1225–1244.

(2) See, for example: (a) Fukushima, K.; Arai, T.; Mori, Y.; Tsuboi, M.; Suzuki, M. *J. Antibiot.* **1983**, *36*, 1613–1630. (b) Grafe, U.; Ihn, W.; Ritzau, M.; Schade, W.; Stengel, C.; Schlegel, B.; Fleck, W. F.; Kunkel, W.; Hartl, A.; Gutsche, W. *J. Antibiot.* **1995**, *48*, 126–133. (c) Degenkolb, T.; Heinze, S.; Schlegel, B.; Dornberger, K.; Mollmann, U.; Dahse, H. M.; Grafe, U. *J. Antibiot.* **2000**, *53*, 184–190. (d) Pruksakorn, P.; Arai, M.; Kotoku, N.; Vilchèze, C.; Baughn, A. D.; Moodley, P.; Jacobs, W. R., Jr.; Kobayashi, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3658–3663.

(3) Zhang, W.; Ding, N.; Li, Y. *J. Pept. Sci.* **2011**, *17*, 576–580.

(4) (a) Hadrami, M. E.; Lavergne, J. -P.; Viallefont, P.; Itto, M. Y. A.; Hasnaoui, A. *Tetrahedron Lett.* **1991**, *32*, 3985–3988. (b) Degenkolb, T.; Berg, A.; Gams, W.; Schlegel, B.; Gräfe, U. *J. Pept. Sci.* **2003**, *9*, 666–678.

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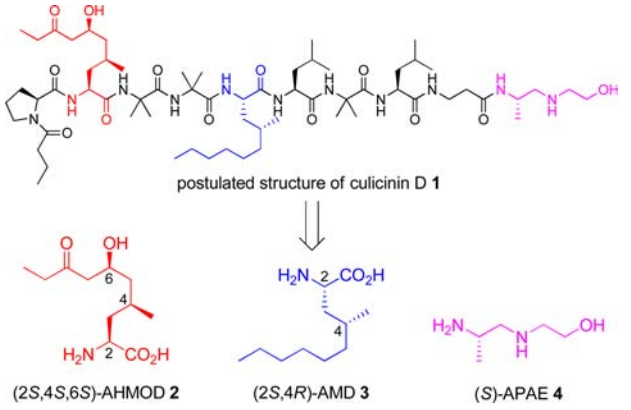
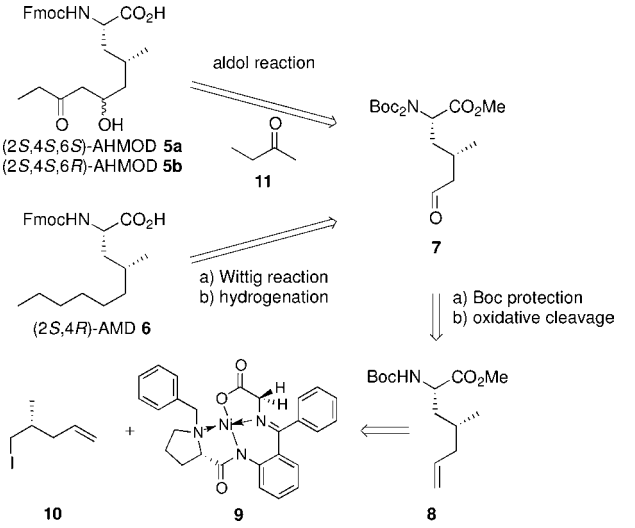


Figure 1. Postulated structure of culicinin D (**1**) showing the three unnatural building blocks abbreviated as (2*S*,4*S*,6*S*)-AHMOD **2**, (2*S*,4*R*)-AMD **3**, and (*S*)-APAE **4**.

AHMOD has been identified as difficult to reproduce on a large scale.[6] In order to adopt Fmoc solid-phase peptide synthesis (SPPS) to form the linear peptide backbone of culicinin D (**1**), the three key building blocks were prepared using alternative synthetic routes that afford the more desirable Fmoc-protected amino acids. It was decided to use 2-chlorotrityl resin as the solid support for SPPS, thus enabling successful anchoring of the hydroxyl group[7] in the *C*-terminal APAE residue and also allowing facile cleavage of the polypeptide using 1% TFA in order to minimize degradation of the sensitive β-hydroxy ketone motif in AHMOD **2**.

Focusing initially on the synthesis of Fmoc-protected AHMOD **5a** and Fmoc-protected AMD **6**, it was realized that both building blocks were accessible from the common aldehyde **7** that in turn is derived from olefin **8** (Scheme 1). Olefin **8** is available from asymmetric alkylation of Belokon complex **9**[8] with iodide **10**.[9] Subsequent asymmetric aldol reaction of aldehyde **7** with butan-2-one **11** should allow access to Fmoc-protected AHMOD **5**. Bis-boc protection of aldehyde **7** was required to prevent intramolecular cyclization of the amino group onto the incumbent aldehyde group.[10] Importantly, the new route proposed for the synthesis of AHMOD **5** and AMD **6** enables access to both (2*S*,4*S*,6*S*)-AHMOD **5a** and (2*S*,4*S*,6*R*)-AHMOD **5b** that can be readily incorporated into the culicinin D polypeptide skeleton thereby enabling the assignment of the absolute chirality at C-6 in the AHMOD residue of the natural product.

Scheme 1. Retrosynthesis of (2*S*,4*S*,6*S*)-AHMOD **5a**, (2*S*,4*S*,6*R*)-AHMOD **5b**, and (2*S*,4*R*)-AMD **6**



The synthesis of both AHMOD **5a** and **5b** commenced with asymmetric alkylation of Belokon complex **9** with iodide **10** affording olefin **8** after Boc protection and methyl ester formation (Scheme 2). Iodide **10** in turn was readily prepared by asymmetric alkylation of *N*-propionyl pseudoephedrine with allyl iodide.[9] Exhaustive Boc protection of olefin **8** by refluxing with $(Boc)_2O$, Et_3N and DMAP in CH_2Cl_2 for 3 days, delivered olefin **12** in 97% yield that was converted into aldehyde **7** in 89% yield via dihydroxylation followed by oxidative cleavage using OsO_4, 2,6-lutidine and $NaIO_4$.[11] Treatment of butan-2-one **11** with (+)-Ipc_2BCl and Et_3N followed by addition of aldehyde **7** effected the desired aldol reaction in 72% yield forming an inseparable mixture of AHMOD **13a** and **13b** (dr 2.6:1) with the facial selectivity favoring formation of AHMOD **13a**.[12]

After removal of both Boc groups in AHMOD **13a** and **13b** using 10% TFA in CH_2Cl_2, Fmoc protection of the resultant amines afforded a chromatographically separable mixture of Fmoc-protected AHMOD **14a** (48%) and **14b** (18%). The absolute configuration of C-6 in the major epimer AHMOD **14a** was confirmed to be (*S*) by Mosher ester analysis[13] as predicted from the use of (+)-Ipc_2BCl to effect the key asymmetric aldol reaction.[12] Treatment of both AHMOD **14a** and **14b** with powdered NaOH in 0.8 M $CaCl_2$ solution (*i*-PrOH/H_2O v/v 7:3)[14] allowed hydrolysis of the methyl ester in the presence of the base-labile

(6) Private communication from ALMAC Sciences.

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(8) Belokon', Y. N.; Zeltzer, I. E.; Bakhmutov, V. I.; Saporovskaya, M. B.; Ryzhov, M. G.; Yanovsky, A. I.; Struchkov, Y. T.; Belikov, V. M. *J. Am. Chem. Soc.* **1983**, *105*, 2010–2017.

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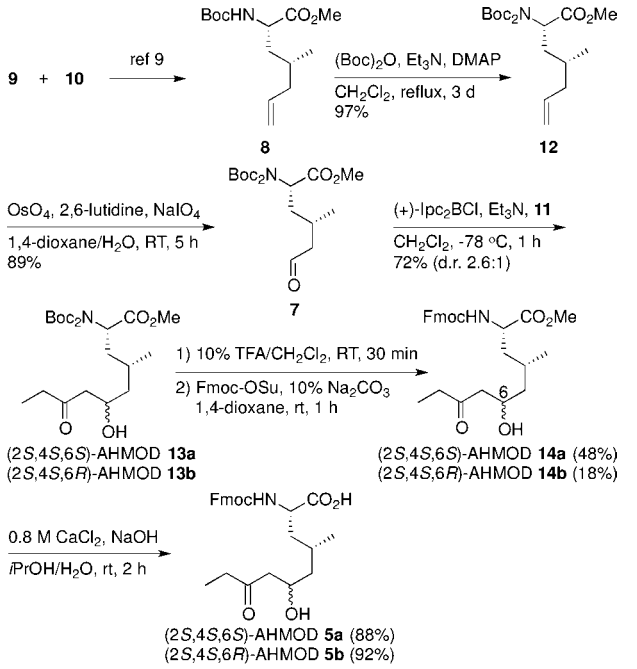
(11) Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217–3219.

(12) Goodman, J. M.; Paterson, I. *Tetrahedron Lett.* **1992**, *33*, 7223–7226.

(13) (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519. (b) Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451–2458.

(14) Pascal, R.; Sola, R. *Tetrahedron Lett.* **1998**, *39*, 5031–5034.

Scheme 2. Synthesis of (2*S*,4*S*,6*S*)-AHMOD **5a** and (2*S*,4*S*,6*R*)-AHMOD **5b**



Fmoc group thus affording Fmoc-protected AHMOD **5a** and **5b** in 88% and 92% yield, respectively, ready for incorporation into SPPS.

For the synthesis of AMD **6**, the hydrophobic side chain was introduced by Wittig reaction of the ylide generated from phosphonium salt **15** with aldehyde **7** in toluene at −78 °C, affording *cis*-olefin **16** (*cis*/*trans* > 95%) in 86% yield (Scheme 3). Hydrogenation of the olefin over 10% Pd/C in MeOH at 20 °C and 30 psi gave Boc-protected methyl ester **17** in 88% yield. Both Boc groups in methyl ester **17** were removed by stirring in 50% TFA in CH_2Cl_2 and treatment of the crude product with Fmoc-OSu and 10% Na_2CO_3 in 1,4-dioxane furnished Fmoc-protected methyl ester **18** in 90% yield over 2 steps. Finally, saponification of methyl ester **18** with powdered NaOH in 0.8 M $CaCl_2$ solution (*i*-PrOH/H_2O v/v 7:3) delivered the desired building block AMD **6** in 84% yield after purification by flash chromatography.

Prior to the synthesis of culicinin D (**1**) itself, optimal reaction conditions for SPPS were investigated by synthesis of an analogue of culicinin D **19** in which the valuable AHMOD **5** and AMD **6** building blocks were substituted by L-leucine (Figure 2). Using HATU as the coupling reagent with DIPEA in DMF, the synthesis of analogue **19** proceeded smoothly until the point where peptide bond formation between two Aib residues proved problematic affording an unknown byproduct in 50% yield. Gratifyingly, replacement of HATU with tetramethylfluoroformamidinium hexafluorophosphate (TFFH) at this point in

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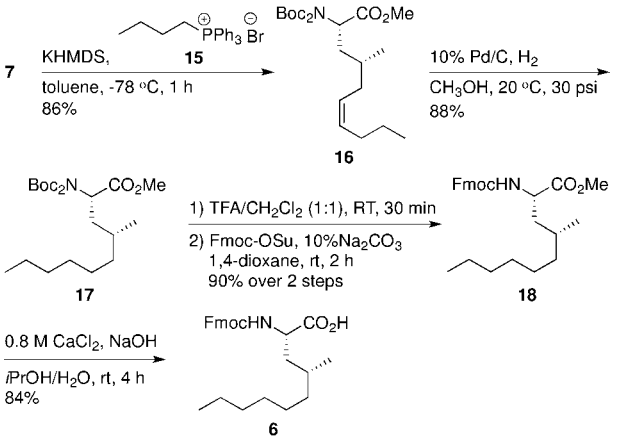
Scheme 3. Synthesis of (2*S*,4*R*)-AMD **6**



the synthesis led to clean formation of the desired peptide via in situ generation of the Fmoc-Aib acid fluoride.[15]

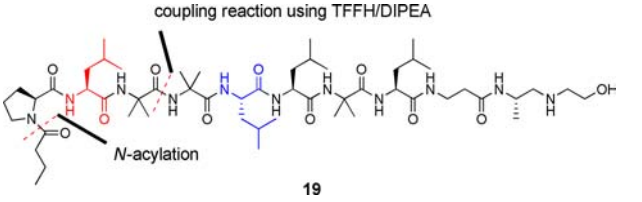


Figure 2. Analogue of culicinin D **19** where AHMOD **5** and AMD **6** are both substituted by L-leucine.

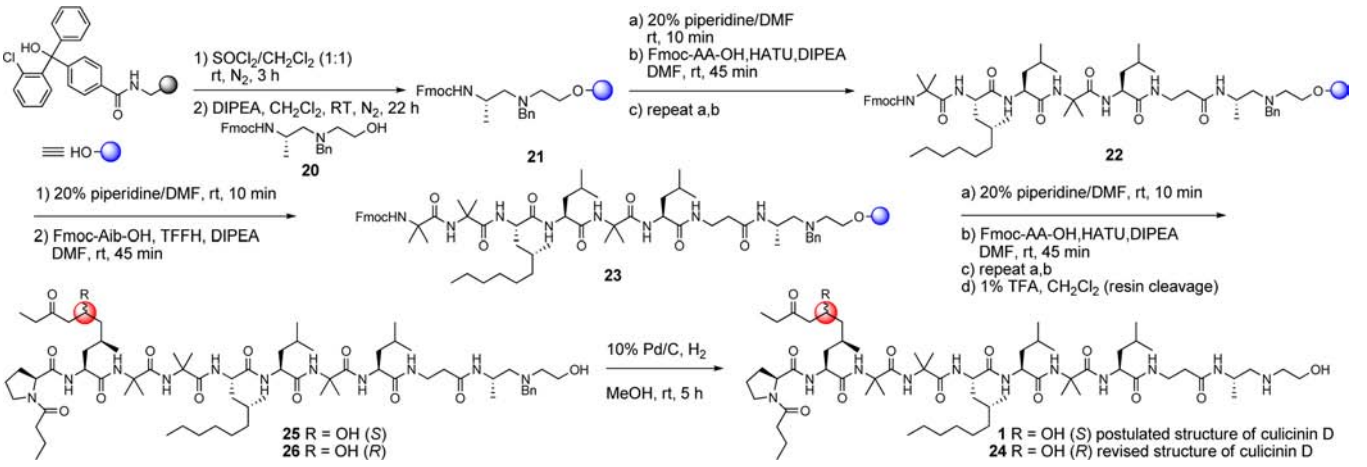
SPPS of culicinin D (**1**) itself began with attachment of Fmoc-protected (*S*)-APAE **20** (see the Supporting Information) onto 2-chlorotrityl-functionalized aminomethyl polystyrene resin[16] (Scheme 4). Although APAE-loaded resin **21** was only obtained with a loading yield of 23%,[17] SPPS of culicinin D (**1**) from resin **21** proceeded uneventfully initially using HATU/DIPEA to effect peptide bond formation between the appropriate building blocks including AHMOD **5a** and AMD **6**. TFFH/DIPEA was used to effect the difficult coupling between peptidyl resin **22** and sterically hindered Fmoc-Aib-OH forming peptidyl resin **23**. SPPS was continued using HATU/DIPEA to effect coupling of subsequent amino acids. The final L-proline residue was precoupled to butyric acid before incorporation into the solid-phase synthesis of culicinin D (**1**).[18]

(16) (a) Quibell, M.; Packman, L. C.; Johnson, T. *J. Am. Chem. Soc.* **1995**, *117*, 11656–11668. (b) Harris, P. W. R.; Yang, S. H.; Brimble, M. A. *Tetrahedron Lett.* **2011**, *52*, 6024–6026.

(17) (a) Tailhades, J.; Gidel, M. -A.; Grossi, B.; Lecaillon, J.; Brunel, L.; Subra, G.; Marinez, J.; Amblard, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 117–120. (b) Rizzi, L.; Cendic, K.; Vaiana, N.; Romeo, S. *Tetrahedron Lett.* **2011**, *52*, 2808–2811.

(18) Xiang, D. F.; Patskovsky, Y.; Xu, C.; Fedorov, A. A.; Fedorov, E. V.; Sisco, A. A.; Sauder, J. M.; Burley, S. K.; Almo, S. C.; Raushel, F. M. *Biochemistry* **2010**, *49*, 6791–6803.

Scheme 4. SPPS of the Postulated Structure of Culicinin D (**1**) and the Revised Structure of Culicinin D **24**



The epimer of culicinin D (**1**), namely culicinin D **24**, was also synthesized in a similar manner incorporating AHMOD **5b** into the SPPS to enable absolute stereochemistry assignment at C-6 of the AHMOD moiety in the natural product. The formation of both benzyl-protected culicinin D **25** (using AHMOD **5a**) and **26** (using AHMOD **5b**) was confirmed by MS analysis of a sample obtained after resin cleavage thus confirming that the β-hydroxyketone motif in the AHMOD unit was intact at this stage.

Pleasingly, brief exposure of both peptaibols to 1% TFA in CH_2Cl_2 during the resin cleavage step only caused negligible degradation of the sensitive β-hydroxy ketone motif in the AHMOD residues. Finally, the benzyl group in the APAE residues of **25** and **26** was removed by hydrogenolysis over 10% Pd/C in MeOH to successfully afford culicinin D peptaibols **1** and **24** in $>95\%$ purity after purification by semipreparative HPLC. Comparison of the ^{1}H NMR data of the synthetic postulated structure of culicinin D (**1**) and its epimer **24** with the data reported for the natural product (see the Supporting Information) confirmed that the absolute chirality at C-6 in the AHMOD moiety of the natural product agreed with the epimeric culicinin D structure **24** in which the stereochemistry at C-6 in the AHMOD residue was in fact (*R*).[19]

(19) An authentic sample of the natural product could not be obtained for comparison.

In summary, both Fmoc-protected AHMOD **5a** and **5b** and Fmoc-protected AMD **6** were efficiently synthesized from a common intermediate. The syntheses of these building blocks together with APAE **20** enabled the first SPPS of the postulated structure of the potent anticancer peptaibol culicinin D (**1**) and the polypeptide **24** epimeric at C-6 in the AHMOD unit. Comparison of the ^{1}H NMR data for the synthetic postulated structure of culicinin D (**1**) and its epimeric peptaibol **24** with the data reported for the natural product established that the C-6 stereochemistry in the AHMOD moiety in the natural product is in fact (*R*). The synthetic work reported herein establishes that the structure of culicinin D should be revised to structure **24** with (6*R*) stereochemistry in the AHMOD residue.

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Supporting Information Available. Experimental procedures and spectroscopic data for all compounds prepared. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.