ORGANIC LETTERS

2005
Vol. 7, No. 26
5881−5883

# Solid-Phase Synthesis of Callipeltin D. Stereochemical Confirmation of the Unnatural Amino Acid AGDHE

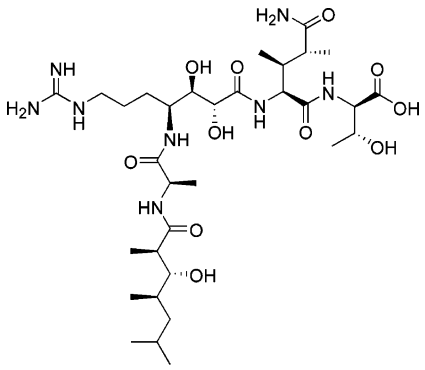
**David C. Cranfill, Ángel I. Morales-Ramos, and Mark A. Lipton***

*Department of Chemistry and Cancer Center, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907-2084*

*lipton@purdue.edu*

**Received October 8, 2005**

## ABSTRACT



**1**

**The lipopeptide callipeltin D (1) was synthesized using an Fmoc-based solid-phase strategy in seven steps and 35% overall yield. The $^1$H NMR of synthetic 1 correlated closely with that of the natural product, confirming the configurational assignment of the novel amino acid constituent (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid.**

Callipeltin D (**1**, Figure 1), an acyclic peptide isolated from the marine sponge *Latrunculia*,[1] was shown to be a truncated open-chain derivative of callipeltin A (**2**),[2] a previously isolated cyclic depsidecapeptide that possesses anti-HIV and antifungal activity and cytotoxicity against several human carcinoma cell lines.[2] Comparison of the anti-HIV activity of **2** with the closely related cyclic depsipeptide callipeltin B indicates that the N-terminal side chain of **2** is important for its antiviral activity.[1] Callipeltin D corresponds to the N-terminal five residues of **2**, which include two nonproteinogenic amino acids, (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE) and (2*S*,3*S*,4*R*)-3,4-dimethylglutamine (DiMeGln), and the fatty acid (2*R*,3*R*,4*R*)-3-hydroxy-2,4,6-trimethylheptanoic acid (TMHEA). The syntheses of various protected versions of AGDHE,[3] DiMeGln,[4] and TMHEA[5] have been reported by our group and others.

The original configurational assignment of AGDHE by Minale and co-workers was based on $^1$H NMR vicinal coupling constants and molecular mechanics calculations and required the assumption that the $\beta$-hydroxyamide exists in a

(1) Zampella, A.; Randazzo, A.; Borbone, N.; Luciani, S.; Trevisi, L.; Debitus, C.; D'Auria, M. V. *Tetrahedron Lett.* **2002**, *43*, 6163.

(2) (a) Zampella, A.; D'Auria, V.; Paloma, L.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202. (b) D'Auria, M. V.; Zampella, A.; Paloma, L. G.; Minale, L. *Tetrahedron* **1996**, *52*, 9589.

(3) (a) Chandraasekar, S.; Ramachandar, T.; Venkateswara Rao, B. *Tetrahedron: Asymmetry* **2001**, *12*, 2315. (b) Thoen, J. C.; Morales-Ramos, Á. I.; Lipton, M. A. *Org. Lett.* **2002**, *4*, 4455.

(4) (a) Liang, B.; Carrol, P. J.; Joullié, M. M. *Org. Lett.* **2000**, *2*, 4157. (b) Okamoto, N.; Hara, O.; Makino, K.; Hamada, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 1353. (c) Acevedo, C. M.; Kogut, E. F.; Lipton, M. A. *Tetrahedron* **2001**, *57*, 6353. (d) Çalimsiz, S.; Lipton, M. A. *J. Org. Chem.* **2005**, *70*, 6218.

(5) (a) Joullie, M. M.; Carroll, P. J.; Guerlavais, V. *Tetrahedron: Asymmetry* **2002**, *13*, 675. (b) D'Auria, M. V.; Zampella, A.; Sorgente, M. *Tetrahedron*: *Asymmetry* **2002**, *13*, 681. (c) D'Auria, M. V.; Zampella, A. *Tetrahedron*: *Asymmetry* **2002**, *13*, 1237. (d) Turk, J. A.; Visbal, G. S.; Lipton, M. A. *J. Org. Chem.* **2003**, *68*, 7841.

10.1021/ol052438f CCC: $30.25 © 2005 American Chemical Society
**Published on Web 11/24/2005**

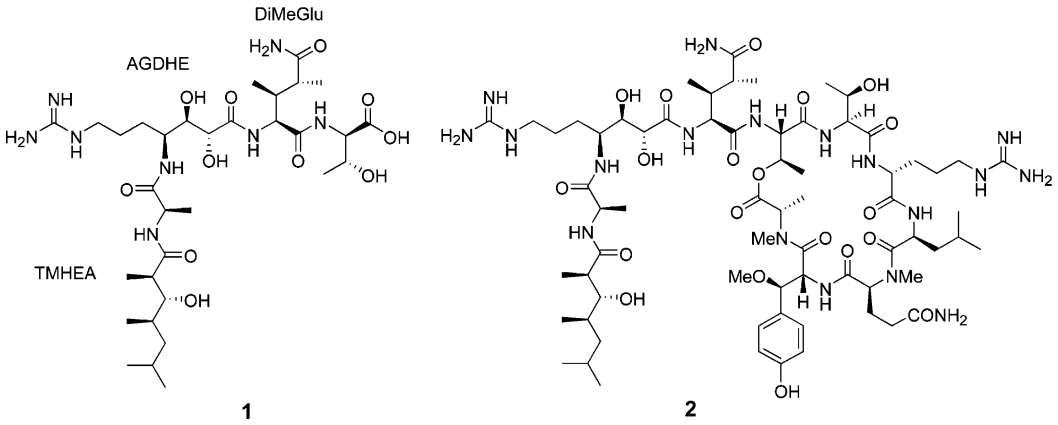


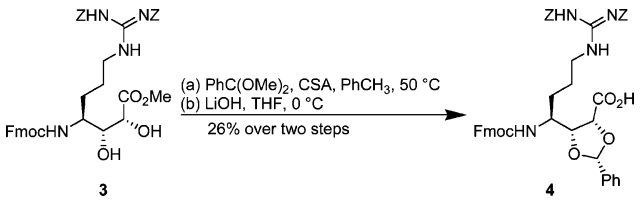

**Figure 1.**

single, six-membered, cyclic hydrogen-bonded conformation.[2a] This same method was originally used to assign the configuration of TMHEA as (2*R*,3*R*,4*S*) and was later revised,[5c] raising concerns about the reliability of the methodology used. During our synthesis of AGDHE, both the (2*R*,3*R*,4*S*) and (2*S*,3*S*,4*S*) diastereomers were made and their $^1$H NMR spectra were compared to those of AGDHE in callipeltins A and D. Although those studies supported the original configurational assignment, it was felt that a complete and unambiguous verification of the configurational assignment of AGDHE would require the synthesis of callipeltin D for spectral comparison to the natural product. Herein we report the efficient solid-phase synthesis of **1** and its spectral correlation with the natural product.

Our approach to the synthesis of callipeltin D uses an Fmoc-based solid-phase strategy. It is envisaged that the coupling conditions used in the synthesis of callipeltin D will also be employed in the synthesis of callipeltin A. Due to the acid labile (2*R*,3*R*)-*β*-methoxytyrosine present in callipeltin A, the synthesis, cleavage from resin, and removal of protecting groups must be performed under either mild or nonacidic conditions. For these reasons, the 2-chlorotrityl chloride resin[6] was chosen for use in the synthesis. It is also known that this resin discourages the formation of diketopiperazines during the attachment of the third residue owing to the bulky nature of the linker.[7] The protecting groups used in the synthesis were chosen for their facile removal by hydrogenolysis.

The component residues of **1** were either purchased as their Fmoc-protected derivatives or synthesized in our laboratories.

**Scheme 1**



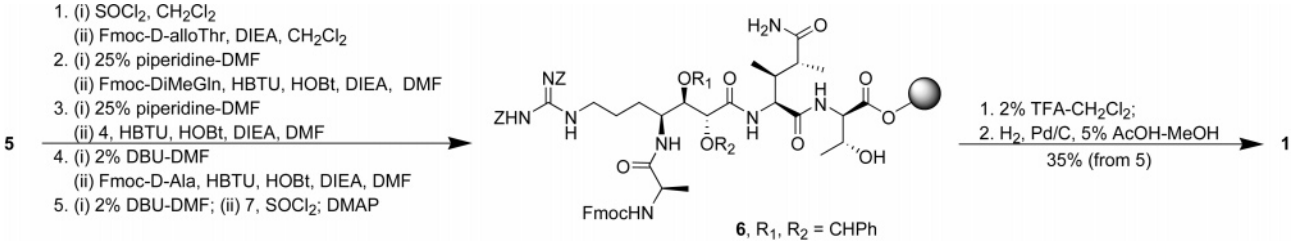

Early model studies of the coupling conditions revealed that it was necessary to have the diol of AGDHE protected during peptide coupling to reduce the formation of byproducts, and thus the diol of a previously reported intermediate[3b] (**3**) was protected as a benzylidene acetal (Scheme 1), and the methyl ester was saponified to afford **4** in 26% yield over two steps. The saponification reaction led to an incomplete consumption of starting material and partial Fmoc and benzyl carbamate deprotection. The single diastereomer of **4** shown in Scheme 1 was the only diastereomer that could be isolated in pure form and was used in the synthesis of **1**. It was also shown in our model studies that the hydroxy group on D-allothreonine did not need to be protected, because it did not interfere in subsequent peptide coupling reactions.

The synthesis of **1** (Scheme 2) started with the activation of the 2-chlorotrityl chloride resin (**5**) by treatment with thionyl chloride before use.[8] Following activation, commercially available Fmoc-D-allothreonine was attached to the resin followed by the remaining residues. It was found in model studies that piperidine can lead to partial loss of the benzyl carbamates of the guanidine residue of **4**, and therefore DBU was used in Fmoc deprotection reactions of the tri- and tetrapeptide intermediates. Peptide couplings were accomplished using 1.7−4.0 equiv of HBTU/HOBt and 1.7−4.0 equiv of the Fmoc-protected amino acid. All reactions were monitored by deprotection of small (∼1 mg) quantities of resin and analysis of the crude cleavage product by reverse-phase HPLC and MALDI-TOF mass spectrometry. Coupling **5** with D-allothreonine, DiMeGlu, AGDHE, and D-alanine, respectively, afforded the resin-bound tetrapeptide **6** in 81% purity as judged by HPLC analysis.

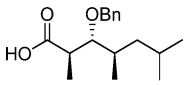
The Fmoc group was removed from tetrapeptide **6**, followed by treatment with the acyl chloride of benzyl-protected TMHEA (**7**, Figure 2) and DMAP, yielding the fully protected, resin-bound version of **1**. It was found

(6) Barlos, K.; Chatzi, O.; Dimitrios, G.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *38*, 513.
(7) Barlos, K.; Gatos, D.; Kapolos, S.; Poulos, C.; Schafer, W.; Wenqing, Y. *Int. J. Pept. Protein Res.* **1991**, *38*, 555.
(8) Harre, M.; Nickisch, K.; Tilstam, U. *React. Funct. Polym.* **1999**, *41*, 111.

**Scheme 2**




that addition of DMAP was required to acylate **6**, but the use of DMAP raised questions about possibile epimerization of **7** during the acylation reaction. To test this possibility, the acyl chloride of **7** was treated with DMAP and monitored for epimerization by $^1$H NMR. No epimerization was detected.



**7**

**Figure 2.**

Cleavage of the fully protected **1** from the resin was accomplished with 2% TFA/$CH_2Cl_2$. Removal of the remaining protecting groups by hydrogenolysis and purification by reverse-phase HPLC afforded **1** in 35% overall yield. Comparison of the $^1$H and $^{13}$CNMR spectra of **1** to those of callipeltin D showed no significant differences,[9] thereby providing additional support that the configurational assignment of AGDHE in **1** and **2** is correct.

In summary, a reliable synthesis of callipeltin D was developed using a solid-phase strategy. Callipeltin D was synthesized in seven steps and 35% overall yield. In addition, the configuration of 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid was confirmed to be (2*R*,3*R*,4*S*) on the basis of a comparison of the NMR spectra of the synthesized material with those of the natural product.

**Acknowledgment.** We thank the National Institutes of Health (AI-50888) for support of this work.

**Supporting Information Available:** Full experimental details and tabulated NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052438F

(9) Tabulated $^1$H and $^{13}$C NMR resonances are compared in Table 1 in the Supporting Information.