ORGANIC LETTERS

2005
Vol. 7, No. 19
4237−4240

# Total Synthesis and Cytotoxicity Studies of a Cyclic Depsipeptide with Proposed Structure of Palau'amide

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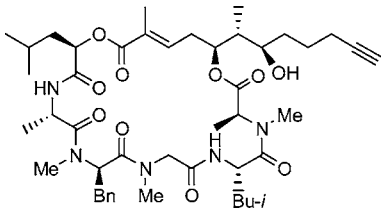
**Received July 18, 2005**

## ABSTRACT




**Total synthesis of a cyclic depsipeptide with proposed structure of palau'amide is achieved, which features a stereoselective elaboration of its 5,7-dihydroxy-2,6-dimethyldodec-2-en-11-ynoic acid unit. The synthetic compound has potent cytotoxicity against three tumor cell lines but different rotation and NMR data compared to those reported for palau'amide, which implies that its conformation is close to that of palau'amide but that some stereochemistry in palau'amide was misassigned.**

Palau'amide is a cyclic depsipeptide that was isolated by Moore and co-workers from a species of the marine cyanobacterium *Lyngbya*, which showed potent cytotoxicity to KB cells ($IC_{50}$ = 13 nM).[1] From this source, several potent antitumor agents such as lyngbyabellins[2] and apratoxins[3] have been discovered. These compounds have become the focus of recent synthetic endeavors.[4,5]

By combination of NMR studies and chiral HPLC analysis of the degradation of palau'amide, its structure was established as depicted in Figure 1. However, the stereochemistry of C-37 awaited further confirmation since it could not be rigorously established by chemical means. More interestingly, Moore and co-workers found that there was a structurally related minor component accompanying with palau'amide, which showed even more potent cytotoxicity ($IC_{50}$ = 1 nM against KB cells), but its structure was unclear because insufficient material was available for adequate NMR characterization.[1] These unsolved structural problems, together with the remarkable biological activity displayed by these two compounds, make palau'amide an attractive target for total synthesis.

During investigations on the total synthesis and structure−activity relationship (SAR) of some biologically important cyclopeptides,[5c,6] we became interested in development of a flexible synthetic protocol to palau'amide. Our retrosynthetic analysis for this molecule is shown in Figure 1. Macrocy-

(1) Williams, P. G.; Yoshida, W. Y. Quon, M. K.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 1545.

(2) (a) Luesch, H.; Yioshida, W. Y.; Moore, R. E.; Paul, V. J.; Moobery, S. L. *J. Nat. Prod.* **2000**, *63*, 611. (b) Luesch, H.; Yioshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2000**, *63*, 1437.

(3) (a) Leusch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418. (b) Leusch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *Bioorg. Med. Chem.* **2002**, *10*, 1973.

(4) (a) Yokokawa, F.; Sameshima, H.; Shioiri, T. *Tetrahedron Lett.* **2001**, *42*, 4171. (b) Yokokawa, F.; Sameshima, H.; Katagiri, D.; Aoyama, T.; Shioiri, T. *Tetrahedron* **2002**, *58*, 9445.

(5) (a) Chen, J.; Forsyth, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 8734. (b) Chen, J.; Forsyth, C. *Proc. Natl. Acad. Sci. U.S.A.*, **2004**, *101*, 12067. (c) Zou, B.; Wei, J.; Cai, G.; Ma, D. *Org. Lett.* **2003**, *5*, 3503. (d) Xu, Z.; Chen, Z.; Ye, T. *Tetrahedron: Asymmetry* **2004**, *15*, 355.

(6) (a) Xie, W.; Zou, B.; Pei, D.; Ma, D. *Org. Lett.* **2005**, *7*, 2775. (b) Yu, S.; Pan, X.; Lin, X.; Ma, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 135. (c) Zhu, J.; Ma, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 5348. (d) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2001**, *42*, 5279. (e) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2000**, *41*, 9089.

10.1021/ol051685g CCC: $30.25 
**Published on Web 08/11/2005**

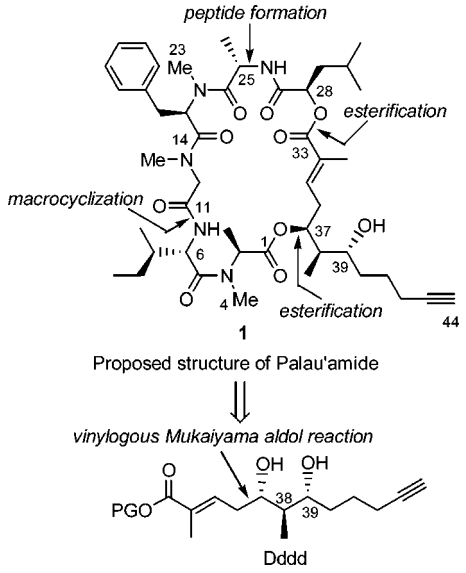


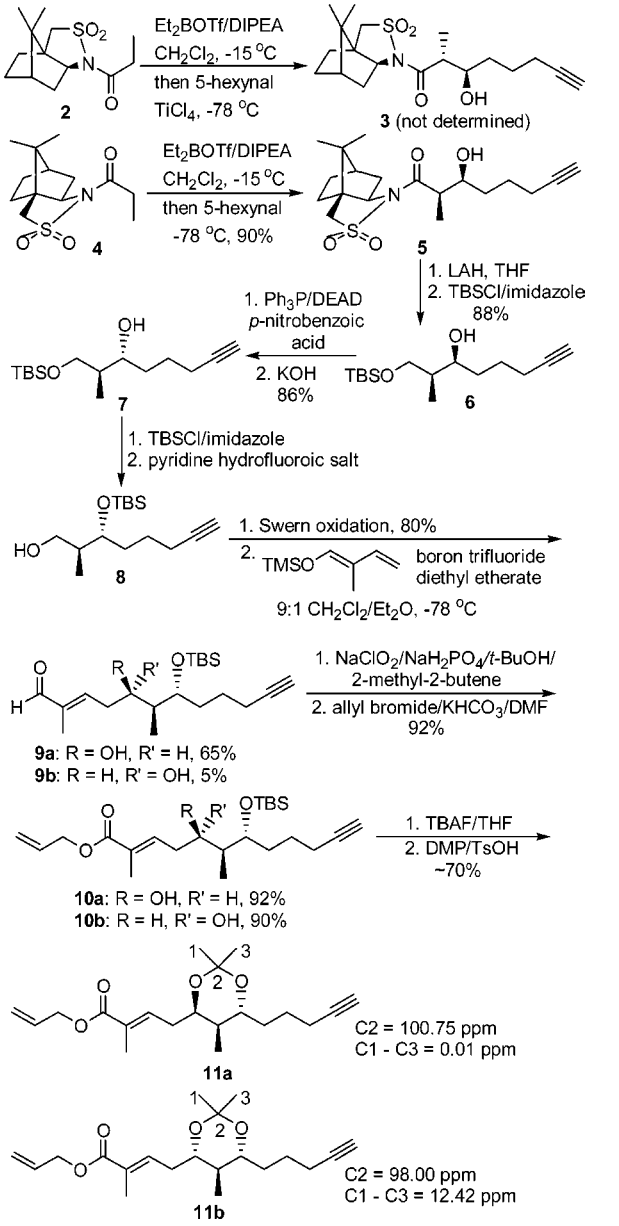

**Figure 1.** Proposedstructure of palau'amide and its retrosynthetic analysis.

clization was chosen at the Ile-Gly site because it is sufficiently unhindered to allow a smooth peptide formation reaction. Further bond disconnection revealed that a stereoselective elaboration of the 5,7-dihydroxy-2,6-dimethyldodec-2-en-11-ynoic acid (Dddd) part was required. We decided to assemble it using a vinylogous Mukaiyama aldol reaction[7] as the key step.

The detailed synthesis for Dddd unit is outlined in Scheme 1. Initially, we planned to employ Oppolzer's "*anti*" aldolization strategy to build the C37−C44 unit in the target molecule.[8] Unfortunately, reaction of 5-hexynal with (1*S*)-2,10-camphorsultam-derived *N*-propionylsultam **2** under standard conditions did not afford any condensation products. However, we were pleased to find that following the Oppolzer's procedure for "*syn*" aldolization,[8] 5-hexynal could react with (1*R*)-2,10-camphorsultam-derived *N*-propionylsultam **4**, providing aldol adduct **5** in 90% yield. This difference might be caused by some side reactions between $TiCl_4$ and 5-hexynal since the *N*-propionylsultam was recovered and 5-hexynal disappeared completely in the former case.

Reduction of **5** with LAH followed by selective protection with TBSCl produced alcohol **6**, which was subjected to Mitsunobu inversion[9] to afford alcohol **7** with the desired stereochemistry. Treatment of **7** with TBSCl to protect the secondary hydroxyl group and subsequent selective cleavage of silyl ether of the primary hydroxyl group with pyridine hydrofluoric acid salt delivered alcohol **8**. Next, Swern

**Scheme 1**




oxidation of **8** provided an aldehyde, which was reacted with (*E*)-(2-methylbuta-1,3-dienyloxy)-trimethylsilane[10] mediated with boron trifluoride diethyl etherate to furnish **9a** in 65% yield, together with its diastereomer **9b** in 5% yield.[7c] Oxidation of aldehydes **9** with $NaClO_2$ provided the corresponding acids, which were masked with an allyl group to give esters **10**. The stereochemistry at the newly created sterogenic center in **9a** was assumed to be *R*, which resulted from a 1,3-asymmetric induction based on a model proposed by Evans and co-workers.[11] The assumption was further confirmed using a method reported by Rychnovsky and co-workers.[12] Accordingly, cleavage of the silyl ether in **10**

(7) (a) Mukaiyama, T.; Banno, K.; Narasaka, K. *J. Am. Chem. Soc.* **1974**, *96*, 7503. (b) Paterson, I.; Smith, J. D. *J. Org. Chem.* **1992**, *57*, 3261. (c) Paterson, I.; Smith, J. D.; Ward, R. A. *Tetrahedron* **1995**, *51*, 9413. (d) Mutou, T.; Suenaga, K.; Fujita, T.; Itoh, T.; Takada, N.; Hayamizu, K.; Kigoshi, H.; Yamada, K. *Synlett*. **1997**, 199. (e) Grieco, P. A.; Speake, J. D. *Tetrahedron Lett.* **1998**, *39*, 1275.

(8) Oppolzer, W.; Lienard, P. *Tetrahedron Lett.* **1993**, *34*, 4321.

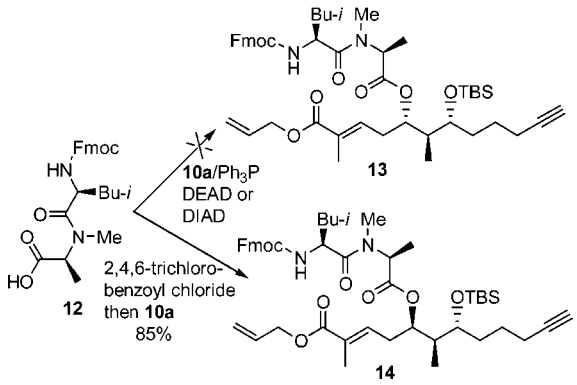
(9) Mitsunobu, O. *Synthesis* **1981**, 1.

(10) Krohn, K.; Tolkiehn, K.; Lehne, V.; Schmalle, H. W.; Grützmacher, H.-F. *Liebigs Ann. Chem.* **1985**, 1311.

(11) Evans, D. A.; Duffy, J. L.; Dart, M. J. *Tetrahedron Lett.* **1994**, *35*, 8537.

produced diols, which were protected with DMP to afford 1,3-diol acetonides **11**. Since the C(2)-acetals in **11a** and **11b** had $^{13}C$ chemical shifts of 100.75 and 98.00 and differences between two $^{13}C$ methyl signals in **11a** and **11b** were 0.01 and 12.42, they should have *anti*-acetonide and *syn*-acetonide moieties, respectively.

**Scheme 2**

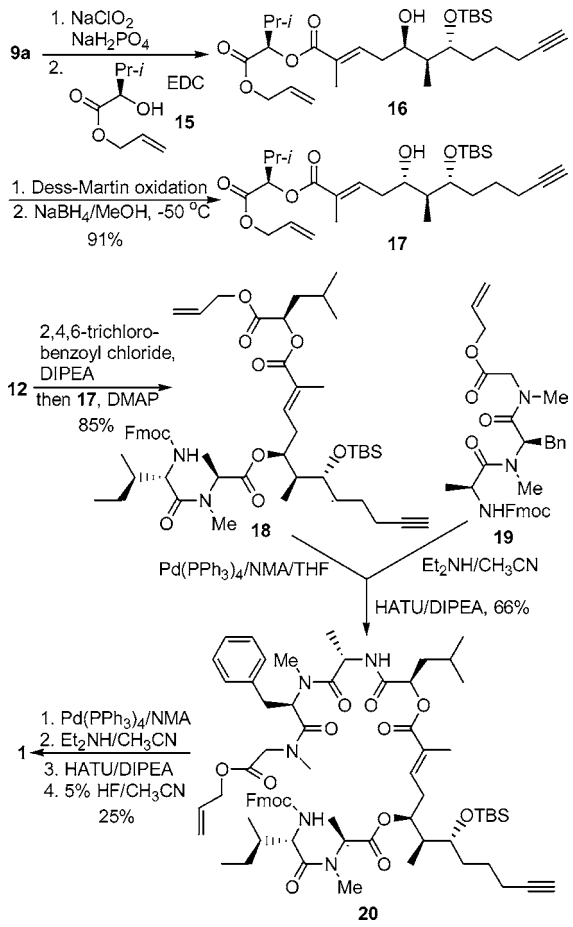



With alcohol **10a** in hand, we attempted an esterification reaction of **10a** and **12** accompanied by stereochemistry inversion using Mitsunobu's method.[9] However, under several standard conditions such as using $Ph_3P$/DEAD or $Ph_3P$/DIAD as activators and THF or toluene as solvent, no desired product **13** was isolated. In contrast, esterification of **10a** and **12** using Yamaguchi's method[13] provided ester **14** in good yield.

On the basis of the above observations, we adjusted our synthetic plan by running stereochemistry inversion prior to esterification. For this purpose the oxidation/reduction strategy was considered. As demonstrated in Scheme 3, after the aldehyde **9a** was oxidized to the corresponding acid, coupling with D-leucine-derived alcohol **15** was carried out to afford ester **16**. Dess−Martin oxidation of **16** followed by $NaBH_4$ reduction delivered protected *syn*-1,3-diol **17** as a single product. Next, coupling of **17** with the acid **12** mediated with 2,4,6-trichlorobenzoyl chloride and diisopropylethylamine in benzene resulted in ester **18**. The allyl protecting group in **18** was removed via palladium chemistry[14] to yield an acid, which was connected with a liberated amine from tripeptide **19** to deliver amide **20**. Finally, sequential liberation of allyl ester and Fmoc-protected amine moieties in **20** with $Pd(Ph_3P)_4$/NMA and diethylamine followed by macrocyclization with HATU produced a cyclic peptide in 30% yield, which was treated with 5% HF in acetonitrile to furnish **1**[15] in 83% yield.

Unfortunately, both rotation ($[\alpha]^{20}_D = +18.3$ (*c* 0.15, MeOH)) and NMR data for synthetic **1** were not in agreement with those reported for palau'amide ($[\alpha]^{23}_D = -22$ (*c* 0.4,

**Scheme 3**




MeOH)), which clearly ruled out the proposed structure for palau'amide. For $^1H$ NMR data, the major difference came from the proton signals of C-4. Compound **1** has a chemical shift of 3.16, whereas the reported one is 3.36, which implied that the stereochemistry of its surrounding amino acid residues were misassigned. Similar problems have often been observed.[16] In addition, the stereochemistry of C-37 might also cause the above difference if it was misassigned.

The synthetic **1** displayed potent cytotoxicity against Hela, A549, and BGC cell lines with $IC_{50}$ values of 39, 19, and 26 nM, respectively. Although different tumor cells were used, these data indicated that compound **1** has a similar potency against tumor cells as palau'amide and implied that

(12) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511.

(13) (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367.

(14) Ciommer, M.; Kunz, H. *Synlett* **1991**, 593.

(15) Selected data for **1**: $[\alpha]^{20}_D = +18.3$ (*c* 0.15, MeOH); $^1H$ NMR (500 MHz, $CD_3OD$) $\delta$ 7.65 (m, 1H), 7.55 (m, 1H), 7.24 (t, $J = 7.3$ Hz, 1H), 7.15 (d, $J = 7.0$ Hz, 2H), 7.10 (t, $J = 6.8$ Hz, 2H), 6.76 (m, 1H), 5.47 (dd, $J = 5.7$, 9.8 Hz, 1H), 5.05 (m, 1H), 4.93 (dd, $J = 10.1$, 13.7 Hz, 1H), 4.90 (m, 1H), 4.51 (q, $J = 7.0$ Hz, 1H), 4.21 (d, $J = 18.7$ Hz, 1H), 3.80 (m, 1H), 3.48 (m, 1H), 3.16 (s, 3H), 3.11 (d, $J = 18.6$ Hz, 1H), 3.03 (s, 3H), 3.02 (dd, $J = 9.7$, 14.7 Hz, 1H), 2.95 (m, 1H), 2.91 (s, 3H), 2.82 (m, 1H), 2.58 (m, 1H), 2.20 (t, $J = 2.5$ Hz, 1H), 2.19 (m, 2H), 1.93 (s, 3H), 1.86 (m, 1H), 1.84 (m, 1H), 1.81 (m, 1H), 1.79 (m, 1H), 1.58 (m, 2H), 1.53 (m, 2H), 1.49 (d, $J = 7.5$ Hz, 3H), 1.39 (m, 1H), 1.37 (m, 1H), 1.33 (m, 1H), 0.99 (d, $J = 7.3$ Hz, 3H), 0.94 (t, $J = 7.4$ Hz, 3H), 0.91 (d, $J = 3.5$ Hz, 3H), 0.89 (d, $J = 6.7$ Hz, 3H), 0.85 (d, $J = 7.1$ Hz, 3H), 0.83 (d, $J = 7.2$ Hz, 3H); ESI-MS *m/z* 852 $(M + H)^+$, 874 $(M + Na)^+$; HRMS calcd for $C_{46}H_{69}N_5O_{10}SiNa$ $(M + Na)^+$ requires 874.4937, found 874.4938.

(16) For recent examples, see: (a) Xu, Z.; Peng, Y.; Ye, T. *Org. Lett.* **2003**, *5*, 2821. (b) Peng, Y.; Pang, H.; Ye, T. *Org. Lett.* **2004**, *6*, 3781.

it might share a similar conformation when interact with their target biomolecule.

In conclusion, we have developed a facile route to palau'amide analogues and found that the previous assignments for stereochemistry were improper. Further investigations on elaborating more analogues to establish the real structure of this natural product, as well as exploring their SAR, are in progress and will be reported in due course.

**Acknowledgment.** The authors are grateful to the Chinese Academy of Sciences (grant KGCX2-SW-209), National Natural Science Foundation of China (grants 20321202 and 20373074), and Science and Technology Commission of Shanghai Municipality (grant 04DZ14901) for their financial support.

**Supporting Information Available:** Experimental procedures and characterizations for compounds **5**−**9**, **11**, **12**, **16**−**20**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL051685G