Total Synthesis of *cis,cis*-Ceratospongamide, a Bioactive Thiazole-Containing Cyclic Peptide from Marine Origin

Fumiaki Yokokawa,* Hirofumi Sameshima, Takayuki Shioiri*

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan
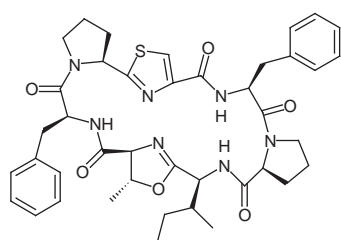Fax +81 (52) 8344172; E-mail: shioiri@phar.nagoya-cu.ac.jp

Dedicated to Professor Ryoji Noyori in recognition of his remarkable contributions to modern asymmetric synthesis
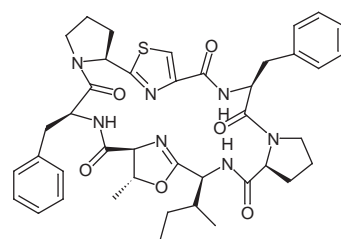
Abstract: The first total synthesis of *cis,cis*-ceratospongamide (**1a**), isolated from marine source, was accomplished via thiazole synthesis using CMD methodology, DEPC-mediated peptide coupling, macrolactamization, and cyclodehydration. Comparison of the cyclization sites and coupling reagents in the macrolactamization step was also investigated.

Key words: ceratospongamide, chemical manganese dioxide (CMD), macrolactamization, cyclic peptide, total synthesis

Ceratospongamide is a bioactive thiazole-containing cyclic heptapeptide isolated by Gerwick and co-workers from the Indonesian red alga *Ceratodictyon spongiosum* and symbiotic sponge *Sigmadocia symbioti*ca.[1] This peptide consists of two *L*-phenylalanine residues, one (*L*-isoleucine)-*L*-methyloxazoline residue, one *L*-proline residue, and one (*L*-proline)thiazole residue. Interestingly, ceratospongamide is able to be isolated as two stable isomers, which are assigned as *cis,cis*-and *trans,trans*-isomers (**1a**, **1b**) of the two proline amide bonds by Gerwick and co-workers (Figure 1). Both compounds show moderate potency in the brine shrimp toxicity assay ($LD_{50} = 13$-$19\ \mu M$). In addition, *trans,trans*-ceratospongamide (**1b**) exhibits potent inhibition of the expression of a key enzyme in the inflammatory cascade, secreted phospholi-
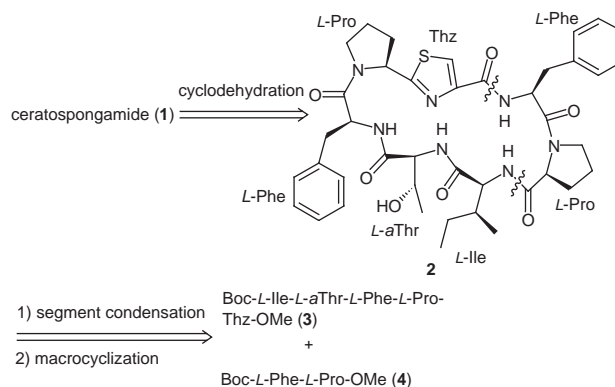

Scheme 1 Retrosynthetic analysis of ceratospongamide

pase A_2 ($sPLA_2$) with an ED_{50} of 32 nM, whereas the *cis,cis*-conformer (**1a**) is inactive. As a part of our program toward the total synthesis of marine natural products,[2] we have embarked on the total synthesis of ceratospongamide.[3]

Our retrosynthetic analysis of ceratospongamide is shown in Scheme 1. As the oxazoline ring is sensitive under acidic conditions, we used *allo*-*L*-threonine as a precursor of the oxazoline and postponed the cyclodehydration of the threonine residue to the final stage of the total synthesis for reducing the risk of racemization at the chiral center attached to the heterocycle.[4] For the synthesis of the macrocycle **2**, a [5+2] convergent strategy was adopted to give the pentapeptide segment **3** and dipeptide segment **4**. Because the thiazole and *L*-proline residues located on the C-terminus in peptide coupling would be tolerant of racemization, the segment condensation and macrocyclization at the thiazole/*L*-phenylalanine and *L*-proline/*L*-isoleucine amide bonds were chosen among the six amide bonds.

For the preparation of the thiazole amino acid fragment **8**, we applied our CMD (chemical manganese dioxide) oxidation for the conversion of thiazolidine to thiazole (Scheme 2).[3b,5] Coupling of Boc-protected *L*-proline with *N,O*-dimethylhydroxylamine using DEPC (diethyl phosphorocyanidate)[6] afforded the amide **5** in 88% yield. After reduction of the amide **5** with lithium aluminum hydride,[7] the resulting aldehyde **6** was condensed with *L*-cysteine methyl ester to give the thiazolidine **7** as a diastereomeric

cis,cis-Ceratospongamide (**1a**)

trans,trans-Ceratospongamide (**1b**)

Figure
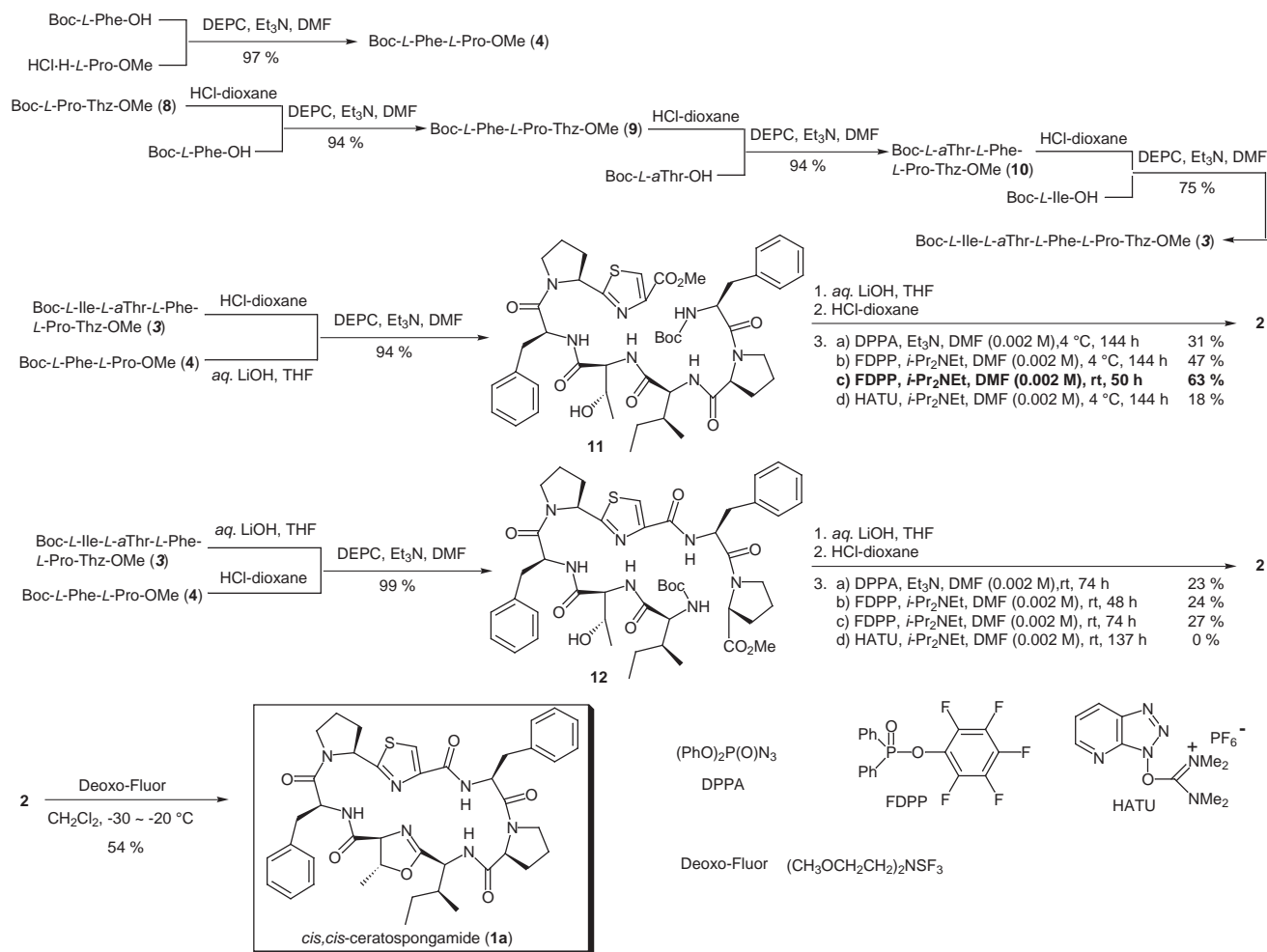
Scheme 3   Total synthesis of *cis,cis*-ceratospongamide (**1a**)

mixture in 73% yield. Subsequent CMD oxidation of the thiazolidine **7** provided the thiazole fragment **8** in 51% yield.
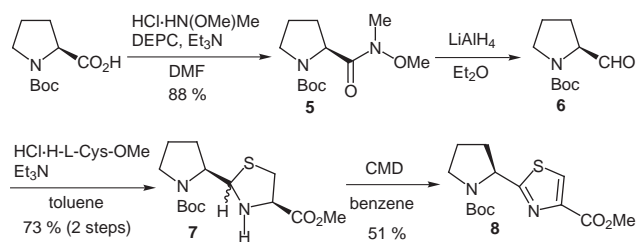
Scheme 2   Synthesis of Boc-*L*-Pro-Trz-OMe

The dipeptide segment **4** was obtained by coupling of Boc-protected *L*-phenylalanine with *L*-proline methyl ester using DEPC in 97% yield (Scheme 3). For the preparation of the pentapeptide segment **3**, removal of the Boc protective group of the thiazole fragment **8** with hydrogen chloride in dioxane followed by condensation with Boc-protected *L*-phenylalanine using DEPC in 94% yield. Iter-

ative removal of the Boc protective group followed by peptide coupling with the Boc derivatives of *L-allo*-threonine and *L*-isoleucine led to the pentapeptide segment **3**. After acidic deprotection of the Boc group of **3**, segment condensation with the acid derived from the dipeptide **4** proceeded smoothly to give the fully protected heptapeptide **11** in 94% yield (Scheme 3). Similarly, deprotection of the Boc group of **4** and segment condensation with the acid derived from **3** also afforded the other linear precursor **12** in 99% yield. The stage was now set for the comparison of macrolactamization step at two sites (Thz/Phe vs. Pro/Ile). After deprotection of the Boc group in **11** and **12** with hydrogen chloride in dioxane and saponification of the methyl ester with lithium hydroxide, macrolactamizations of the resulting two free linear precursors were performed in a DMF solution (0.002 M) by using DPPA (diphenyl phosphorazidate),[8] FDPP (pentafluorophenyl diphenylphosphinate),[9] and HATU [*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate],[10] respectively. The cyclization at the Thz/Phe site proceeded smoothly and the yield by FDPP was higher than those by DPPA and HATU. In contrast, the cyclization at Pro/Ile site was sluggish to proceed, and especially

no cyclized product was obtained by use of HATU. Accordingly, we carried out the macrolactamization at the Thz/Phe site with 1.5 equivalents of FDPP and 4 equivalents of *i*-Pr$_2$NEt at room temperature to give the cyclic peptide **2** in 63% yield from the corresponding linear precursor **11**. Finally, cyclodehydration of the *allo*-threonine residue with bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-fluor) to the oxazoline[11] produced the target compound, which was completely identical with natural *cis,cis*-ceratospongamide (**1a**) as judged by ^{1}H and ^{13}C NMR spectra, HPLC, and TLC R$_f$ values (Scheme 3).

In conclusion, we have developed an efficient strategy toward the total synthesis of *cis,cis*-ceratospongamide. The conversion of synthetic *cis,cis*-ceratospongamide (**1a**) to its *trans,trans*-isomer (**1b**) is currently underway.

Acknowledgement

References and Notes

(1) Tan, L. T., Williamson, R. T., Gerwick, W. H., Watts, K. S., McGough, K., Jacobs, R. *J. Org. Chem.* **2000**, *65*, 419-425.

(2) (a) Yokokawa, F.; Fujiwara, H.; Shioiri, T. *Tetrahedron* **2000**, *56*, 1759-1775. (b) Sugiyama, H.; Yokokawa, F.; Shioiri, T. *Org. Lett.* **2000**, *2*, 2149-2152. (c) Wu, M.; Okino, T.; Nogle, L. M.; Marquez, B. L.; Williamson, R. T.; Sitachitta, N.; Berman, F. W.; Murray, T. F.; McGough, K.; Jacobs, R.; Colsen, K.; Asano, T.; Yokokawa, F.; Shioiri, T.; Gerwick, W. H. *J. Am. Chem. Soc.* **2000**, *122*, 12041-12042. (d) Yokokawa, F.; Asano, T.; Shioiri, T. *Org. Lett.* **2000**, *2*, 4169-4172. (e) For a review, see Shioiri, T.; Hamada, Y. *Synlett* **2001**, 184-201.

(3) A part of this work was presented at 37th Japanese Peptide Symposium, Nagoya, Oct 18-20, 2000. See Yokokawa, F.; Sameshima, H.; Shioiri, T. *Peptide Science 2000*, **2001**, 55-58.

(4) See: Shioiri, T.; Hamada, Y. *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam **1989**; Vol. 4 (Part C), pp 83-110.

(5) (a) Hamada, Y., Shibata, M., Sugiura, T., Kato, S., Shioiri, T. *J. Org. Chem.* **1987**, *52*, 1252-1255. (b) Aoyama, T., Sonoda, N., Yamauchi, M., Toriyama, K., Anzai, A., Ando, A., Shioiri, T. *Synlett* **1998**, 35-36. (c) Fujiwara, H., Tojiki, K., Yokokawa, F., Shioiri, T. *Peptide Science 1999* **2000**, 9-12.

(6) Takuma, S., Hamada, Y., Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147-3153 and references therein.

(7) Fehrentz, J.-A., Castro, B. *Synthesis* **1983**, 676-678.

(8) Shioiri, T., Ninomiya, K., Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203-6205.

(9) (a) Chen, S., Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711-6714. (b) Dudash, J., Jr., Jiang, J., Mayer, S. C., Joullie, M. M. *Synth. Commun.* **1993**, *23*, 349-356. (c) Deng, J.; Hamada, Y.; Shioiri, T.; Matsunaga, S.; Fusetani, N. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1729-1731. (d) Deng, J.; Hamada, Y.; Shioiri, T. *Synthesis* **1998**, 627-638.

(10) Ehrlich, A., Heyne, H.-U., Winter, R., Beryermann, M., Haber, H., Carpino, L. A., Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831-8838.

(11) Phillips, A. D., Uto, Y., Wipf, P., Reno, M. J., Williams, D. R. *Org. Lett.* **2000**, *2*, 1165-1168.