# Total Synthesis, Structural Revision, and Biological Evaluation of Calafianin, a Marine Spiroisoxazoline from the Sponge, *Aplysina gerardogreeni*

## Takahisa Ogamino,<sup>1</sup> Rika Obata,<sup>1</sup> Hiroshi Tomoda,<sup>2</sup> and Shigeru Nishiyama<sup>\*1</sup>

<sup>1</sup>Department of Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522

<sup>2</sup>School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641

Received August 4, 2005; E-mail: nisiyama@chem.keio.ac.jp

The reported structure of the natural calafianin (1) and its isomer in the epoxide region 13 were successfully synthesized through the *cis*- and *trans*-spiroisoxazoline compounds, which were produced by electrochemical oxidation of the hydroxyimino-phenol derivative 7, followed by  $Zn[BH_4]_2$  reduction. Comparison of the spectroscopic data of the synthetic (±)-1 and (±)-13 resulted in structural revision of the natural calafianin to 13, possessing the *trans*-relationship between an epoxide and an oxygen of the isoxazoline. In addition, a significant difference of antimicrobial activity between 1 and 13 is discussed.

A series of the spiroisoxazoline natural products, such as aerothionin (2),<sup>1</sup> homoaerothionin (3), and aerophobin-1 (4), have been isolated from marine sponges since the early 1970's (Fig. 1).<sup>2</sup>

These spiroisoxazolines share a spiro skeleton, which consists of an isoxazoline ring and a cyclohexadiene moiety **A**. Biosynthetic condensation of **A** with alkyl amines or diamines produces a variety of structural diversities of these class natural products, which display a wide range of biological activities, such as antimicrobial, cytotoxic, and antiinflammatory activities. Accordingly, spiroisoxazoline natural products have been recognized as attractive synthetic targets. Indeed, many research groups have endeavored in their total synthesis.<sup>3–5</sup> In this context, we constructed the natural *trans*-form **5** and unnatural *cis*-form **6** of spiroisoxazolines by thallium(III) nitrate (TTN)<sup>3</sup> or anodic oxidation<sup>4</sup> of the corresponding hydroxyimino-phenol **7**, followed by reduction of the spirodienone **8** with Zn[BH<sub>4</sub>]<sub>2</sub> (Scheme 1). The relative stereochemistries of **5** and **6** were determined by conversion into natural products, such as aerothionin (**2**),<sup>3</sup> the stereochemistry of which was unambiguously determined by X-ray crystallographic analysis.<sup>1b</sup> In addition, exposure of the unnatural **6** to bases caused  $\beta$ -elimination to provide **7**.

From among the spiroisoxazoline natural products, calafianin (1), isolated from the sponge *Aplysina gerardogreeni* n. sp (aplysinidae), possesses a dimeric structure of the typical spiroisoxazoline fused with the cyclohexenone carrying epoxide rings.<sup>6</sup> From a stereochemical viewpoint, it was reported that the *cis*-relationship of two oxygen atoms between the epoxide and the isoxazoline was deduced by the NOE correlation



Fig. 1. Proposed structure of calafianin (1) and related natural products.

Published on the web January 13, 2006; DOI 10.1246/bcsj.79.134



Scheme 1. Synthesis of the spiroisoxazoline structure.



Scheme 2. Synthesis of proposed structure of calafianin (1).

between H-1 and H-4' protons,<sup>6</sup> as well as that no significant biological activity against the multidrug-resistant clinical isolate *M. tuberculosis* H37Rv, occurred in contrast to that of 2.<sup>7</sup> In spite of closely related structures, the clear biological difference between 1 and 2 prompted us to synthesize 1 by utilizing the spiroisoxazoline derivatives mentioned above and to examine its biological activity. We describe herein synthesis of calafianin in ( $\pm$ )-form and its antimicrobial activity.<sup>8</sup>

#### **Results and Discussion**

At the outset, synthesis of  $(\pm)$ -1 was attempted by chemical modification of  $(\pm)$ -cis-aerothionin (2');<sup>3,9</sup> however, an appropriate method could not be found under the several reaction conditions examined, owing to the labile character of the same cis-spiroisoxazoline structure as that of the  $\beta$ -eliminationprone 6. Accordingly, as an alternative method,  $(\pm)$ -6 prepared from selective reduction of 8 with Na[BH<sub>4</sub>]<sup>10</sup> was converted into the epoxy ketone 10 carrying the partial-structure of 1 (Scheme 2). Thus, cleavage of a methyl enol ether of 6 under acidic conditions gave 9 as a diastereomeric mixture, which upon treatment with DBU afforded the epoxy derivative 10 in 2 steps. Upon using refluxing AcOH, the epoxy compound 10 was obtained in 32% yield, along with the dienone  $11^9$  (8%) and phenol 7 (32%), whereas no desired reaction proceeded in MsOH or TFA. The main reason of the low yield might be the labile property of the *cis*-spiroisoxazoline **6** under basic or even acidic conditions, which gave the spirodienone 11 by  $\beta$ elimination, along with 7 by the ring-opening reaction of isoxazoline. Despite the accompanying problems, no better method was available than this approach using **6** as an intermediate. Alkaline hydrolysis of **10** in aq 1,4-dioxane afforded the carboxylic acid **12** in high yield, while reaction in MeOH was unsuccessful owing to undesired decarboxylation.<sup>4b</sup> Accordingly, compound **12** should be submitted to the next step without purification for its instability. In the final step, upon using such reagents as carbonyl diimidazole (CDI), 1-ethyl-3-(3-dimethyl-aminopropyl)carbodimide (EDCI), and 1*H*-benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), coupling of **12** with 1,4-diaminobutane gave calafianin (**1**) at the most in 12% yield. After extensive evaluation to improve the yield, the final condensation could be achieved in 41% yield by using pivaloyl chloride (PivCI) as a coupling reagent.

As shown in Table 1, comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data of the synthetic sample with the reported data<sup>6</sup> exhibited clear differences in the regions of H-3, H-6, and H-4' and C-1, C-4, C-6, and C-1". This observation indicated that the reported configuration of the epoxide residue should be revised.

As mentioned above, the reported calafianin has the *cis* vicinal relationship of two oxygen atoms. However, other spiroisoxazoline natural products, such as aerothionin (**2**), homoaerothionin (**3**), and aerophobin-1 (**4**), have the *trans* vicinal relationship (Fig. 1). In hypothesis of their biosynthesis, the fundamental framework **A** is constructed by oxidation of the *p*-methoxyphenyl derivative **B**, followed by an intramolecular nucleophilic attack on the epoxide part of the arene oxide **C** (Scheme 3).<sup>11</sup>

Based on the assumption that natural calafianin was assem-

	Synthetic 1		Synthetic 13		Natural data (Ref. 6)	
No.	<sup>1</sup> H (mult, J in Hz)	<sup>13</sup> C	<sup>1</sup> H (mult, J in Hz)	<sup>13</sup> C	<sup>1</sup> H (mult, J in Hz)	<sup>13</sup> C
1	4.12 (dd, 2.8, 4.0)	55.0	4.12 (dd, 2.4, 4.0)	56.8	4.12 (dd, 2.6, 3.7)	56.9
2		85.4		83.9		84.0
3	7.29 (d, 2.8)	144.2	7.48 (d, 2.4)	143.7	7.49 (d, 2.6)	143.7
4		119.7		122.7		122.8
5		185.8		185.9		186.0
6	3.81 (d, 4.0)	51.8	3.92 (d, 3.6)	52.9	3.94 (d, 3.5)	53.0
3'		154.6		154.8		154.9
4′	3.56 (d, 18.4)	43.3	3.67 (d, 18.0)	43.3	3.68 (d, 17.8)	43.6
	3.41 (d, 18.4)		3.60 (d, 18.0)		3.61 (d, 17.9)	
1″		159.7		158.2		158.3
2″	8.61 (t, 6.0)		8.64 (t, 6.0)		8.63 (t, 5.7)	
3″	3.19 (br)	38.5	3.20 (br)	38.5	3.19 (br)	38.6
4″	1.52 (br)	26.3	1.48 (br)	26.3	1.50 (br)	26.4

Table 1. Comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data ( $\delta$ , DMSO- $d_6$ ) of the Synthetic 1 and 13 with the Reported Data (Ref. 6)



Scheme 3. Biosynthetic hypothesis of the natural spiroisoxazolines.

bled by a similar biosynthetic pathway to those of other spiroisoxazoline derivatives, we expected the natural product should possess the relative stereochemistry of **13**, which has the *trans* relationship. Accordingly, synthesis of **13** was carried out starting from the *trans*-alcohol **5** by employing essentially the same approach as that of **1** (Scheme 4).

The epoxy derivative **15**,<sup>12</sup> possessing the *trans*-relationship between two oxygen atoms, was synthesized from  $(\pm)$ -**5** by acidic hydrolysis followed by epoxidation of the ketone **14** without production of by-products such as **11**. Finally, **13** was produced in 39% yield by condensation of the carboxylic acid form (**16**) of **15** with 1,4-diaminobutane. As expected, the spectral data of the synthetic  $(\pm)$ -**13** was superimposable to those reported (Table 1).<sup>6,13</sup> Against the report by Encarnación et al., the clear NOE correlation between H-1 and H-4' (2%) was observed even in the *trans*-relationship. Accordingly, the relative stereochemisty of calafianin should be revised to **13**, as shown in Scheme 4. Also, **13** was obtained from aerothionin  $(\pm)$ -(**2**),<sup>1</sup> which was prepared from **5**<sup>3</sup> in 2 steps as an alternative access (Scheme 5).

**Bioactivity.** Despite of the weak activity of calafianin reported,<sup>6</sup> the synthetic samples were submitted for biological assessment. Consequently, **13** showed antimicrobial activity against Gram-positive bacteria including MRSA, while **1** showed no activity (Table 2). Although **1** and **13** possess closely similar structures, a large difference in biological activity against several Gram-positive bacteria between them was observed. Further biological investigation is in progress.

In conclusion,  $(\pm)$ -1 carrying the proposed structure of calafianin and the isomeric  $(\pm)$ -13 were successfully synthesized by using the spiroisoxazolines 5 and 6 as an efficient demonstration of chemical modification of a spiroisoxazoline structure. This investigation enabled the structural revision of 1 to 13, which has the *trans*-relationship between the epoxy ring and the oxygen atom in the spiroisoxazoline residue. In addition, biological assessment of the synthetic samples showed a significant difference in antimicrobial activity between 1 and 13 involving MRSA.

#### Experimental

**General.** Melting points were measured on a Yanaco MP-S3 and are uncorrected. IR spectra were recorded on a JASCO Model A-202 spectrophotometer. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were obtained on JEOL JNM EX-270 and JEOL JNM GX-400 spectrometers in deuteriochloroform using tetramethylsilane as an internal standard, unless otherwise stated. High-resolution mass spectra were obtained on JEOL JMS-700 (FAB) and Hitachi M-80 B GC-MS spectrometers. Preparative and analytical TLC were carried out on silica-gel plates (Kieselgel 60 F254, E. Merck AG., Germany) using UV light and/or 5% molybdophosphoric acid in ethanol for detection. Kanto Chemical silica 60N (spherical, neutral, 63–210 µm) was used for column chromatography.

Synthesis of Methyl (1*R*,2*S*,6*S*)-4-Bromo-5-oxo-7-oxaspiro-[bicyclo[4.1.0]hept-3-ene-2,5'(4'*H*)isoxazole]-3'-carboxylate (10). A solution of 6 (680 mg, 1.7 mmol) in AcOH (20 mL) was stirred at refluxing temperature for 6 h. After evaporation, the mixture



Scheme 4. Synthesis of 13, revised structure of calafianin (1).



Scheme 5. Synthesis of 13, prepared through aerothionin (2).

was submitted to the next step without further purification.

A mixture of the crude and DBU (0.4 mL) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was stirred for 1 h. The mixture was diluted with H<sub>2</sub>O, and extracted with CHCl<sub>3</sub>. The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. Purification by silica-gel column chromatography (hexane/Et<sub>2</sub>O = 1/1) gave **10** (166 mg, 32%), **7** (224 mg, 32%), and **11** (52 mg, 8%).

**10:** mp 159–160 °C (needles, EtOAc–hexane); IR (KBr) 1705, 1603 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  3.41 (1H, d, J = 17.5 Hz), 3.45 (1H, d, J = 17.5 Hz), 3.74 (1H, d, J = 3.6 Hz), 3.83 (1H, dd, J = 2.6, 3.6 Hz), 3.93 (3H, s), 6.99 (1H, d, J = 2.6 Hz); <sup>13</sup>C NMR  $\delta$  43.0, 51.5, 53.2, 54.9, 86.6, 121.0, 141.9, 150.5, 159.5, 185.2; HRMS found m/z 300.9580, calcd for C<sub>10</sub>H<sub>8</sub><sup>79</sup>BrNO<sub>5</sub>: M, 300.9585. Found: C, 39.47; H, 2.71; N, 4.50%. Calcd for C<sub>10</sub>H<sub>8</sub>BrNO<sub>5</sub>: C, 39.76; H, 2.67; N, 4.64%.

Synthesis of Calafianin (Proposed Structure) (1). A solution of 10 (78 mg, 0.26 mmol) in 1,4-dioxane (4 mL), H<sub>2</sub>O (1.6 mL), and 1 M (1 M = 1 mol dm<sup>-3</sup>) KOH (0.5 mL) was stirred at 0 °C for 1 h. After treatment with Amberlite IR-120B (H<sup>+</sup>), the mixture was filtered and the filtrate was evaporated to give 12 (77 mg, quant.) as a colorless solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  3.38 (1H, d, *J* = 18.0 Hz), 3.49 (1H, d, *J* = 18.0 Hz), 3.68 (1H, d, *J* = 4.0 Hz), 3.93 (1H, dd, *J* = 2.4, 4.0 Hz), 7.16 (1H, d, *J* = 2.4 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$ 45.3, 53.1, 56.6, 86.9, 121.1, 145.4, 157.3, 164.8, 187.2.

A mixture of **12** (77 mg, 0.26 mmol) and PivCl ( $32 \mu L$ , 0.26 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and pyridine (1.6 mL) was stirred at -10 °C for 10 min, and then 1,4-diaminobutane ( $13 \mu L$ , 0.13 mmol) was added. After further stirring for 1 h, the mixture was diluted with

H<sub>2</sub>O and extracted with EtOAc. The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was purified by preparative TLC (hexane/acetone = 1/1) to give **1** (33 mg, 41%) as a colorless solid: IR (KBr) 3375, 1703, 1666 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.52 (4H, br), 3.19 (4H, br), 3.41 (2H, d, J = 18.4 Hz), 3.56 (2H, d, J = 18.4 Hz), 3.81 (2H, d, J = 4.0 Hz), 4.12 (2H, dd, J = 2.8, 4.0 Hz), 7.29 (2H, d, J = 2.8 Hz), 8.61 (2H, t, J = 6.0 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 26.3, 38.5, 43.3, 51.8, 55.0, 85.4, 119.7, 144.2, 154.6, 159.7, 185.8; HRMS *m*/*z* 629.9620, calcd for C<sub>22</sub>H<sub>20</sub><sup>81</sup>Br<sub>2</sub>N<sub>4</sub>O<sub>8</sub>: M, 629.9614.

Synthesis of Methyl (1S,2S,6R)-4-Bromo-5-oxo-7-oxaspiro-[bicyclo[4.1.0]hept-3-ene-2,5'(4'H)isoxazole]-3'-carboxylate (15). A solution of 5 (331 mg, 0.83 mmol) in AcOH (10 mL) was stirred at refluxing temperature for 4 h. After concentration of the mixture, the residue was diluted with CH2Cl2 (10mL) and then DBU (0.4 mL) was added at room temperature. After being stirred for 1 h, the mixture was diluted with H<sub>2</sub>O and extracted with  $CHCl_3$ . The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was purified by silica-gel column chromatography (hexane/EtOAc = 4/1) to give 15 (165 mg, 66%): mp 156-158 °C (needles, EtOAc-hexane); IR (KBr) 1708, 1608 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  3.43 (1H, d, J = 17.6 Hz), 3.74 (1H, d, J = 17.6Hz), 3.78 (1H, d, J = 3.4 Hz), 3.80 (1H, dd, J = 2.4, 3.4 Hz), 3.95 (3H, s), 6.93 (1H, d, J = 2.4 Hz); <sup>13</sup>C NMR  $\delta$  43.7, 53.28, 53.29, 57.0, 85.2, 125.1, 140.8, 151.2, 159.6, 184.3; HRMS: m/z 300.9575, calcd for C<sub>10</sub>H<sub>8</sub><sup>79</sup>BrNO<sub>5</sub>: M, 300.9585. Anal. Found: C, 39.57; H, 2.90; N, 4.42%. Calcd for C<sub>10</sub>H<sub>8</sub>BrNO<sub>5</sub>: C, 39.76; H, 2.67; N, 4.64%.

	Diameter of inhibition zone <sup>a)</sup> /mm		
Microorganism	1	13	
Bacillus subtilis		20.9	
Staphylococcus aureus	_	11.8	
Micrococcus luteus		8.1	
Esherichia coli	_	7.9	
Pseudomonas aeruginosa	_	—	
Xanthomonas campestris pv. oryzae	14.6	18.0	
Bacteroides fragilis		_	
Acholeplasma laidlawii	_	7.4	
Pyricularia oryzae		_	
Aspergillus niger		—	
Mucor racemosus		_	
Candida albicans	_	—	
Saccharomyces cerevisiae		_	
Mycobacterium smegmatis		_	
nethicillin-resistant Staphyloccocus aureus (MRSA)	7.9	17.5	

Table 2. Antimicrobial Activities of Calat	fianin	Derivat	ive
--	--------	---------	-----

a) —: No inhibition zone.

Synthesis of Calafianin (Revised Structure) (13). Method A: Hydrolysis of 15 (98 mg, 0.33 mmol) in 1,4-dioxane (5 mL), H<sub>2</sub>O (2 mL), and 1 M KOH (0.6 mL) was undertaken by essentially the same procedure as in the case of 10 to give 16 (97 mg, quant.) as a colorless solid: <sup>1</sup>HNMR (CD<sub>3</sub>OD)  $\delta$  3.48 (1H, d, *J* = 18.0 Hz), 3.66 (1H, d, *J* = 18.0 Hz), 3.74 (1H, d, *J* = 3.6 Hz), 3.93 (1H, dd, *J* = 2.4, 3.6 Hz), 7.22 (1H, d, *J* = 2.4 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$  46.0, 54.5, 57.4, 85.3, 124.4, 144.6, 157.1, 165.0, 187.1.

Condensation of **16** (97 mg, 0.33 mmol) with 1,4-diaminobutane (16 µL, 0.17 mmol) as in the case of **1** afforded **13** (40 mg, 39%) as a colorless solid: IR (KBr) 3410, 1701, 1670 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.48 (4H, br), 3.20 (4H, br), 3.60 (2H, d, *J* = 18.0 Hz), 3.67 (2H, d, *J* = 18.0 Hz), 3.92 (2H, d, *J* = 3.6 Hz), 4.12 (2H, dd, *J* = 2.4, 4.0 Hz), 7.48 (2H, d, *J* = 2.4 Hz), 8.64 (2H, t, *J* = 6.0 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  26.3, 38.5, 43.3, 52.9, 56.8, 83.9, 122.7, 143.7, 154.8, 158.2, 185.9; HRMS *m*/*z* 650.9520, calcd for C<sub>22</sub>H<sub>20</sub><sup>79</sup>Br<sup>80</sup>BrN<sub>4</sub>O<sub>8</sub>Na: M + Na, 650.9527.

**Method B:** To a solution of aerothionin (2) (55 mg, 0.068 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was added MsOH (0.5 mL) at 0 °C. After being stirred for 1 h, the reaction mixture was diluted with H<sub>2</sub>O and extracted with CHCl<sub>3</sub>. The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was diluted with CH<sub>2</sub>Cl<sub>2</sub> (1 mL) and then DBU (0.1 mL) was added at room temperature. After being stirred for 1 h, the mixture was diluted with H<sub>2</sub>O and extracted with CHCl<sub>3</sub>. The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was diluted with H<sub>2</sub>O and extracted with CHCl<sub>3</sub>. The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was purified by silica-gel column chromatography (toluene/acetone = 2/1) to give **13** (4 mg, 11%).

**Bioassay.**<sup>14</sup> Antimicrobial activity against 15 species of microorganisms was measured by the agar diffusion method using paper disks (i.d. 6 mm, ADVANTEC). The microorganisms were as follows; *Bacillus subtilis* PCI 219, *Staphylococcus aureus* FDA 209P, methicillin-resistant *S. aureus* K-24 (a clinica isolate, MRSA), *Micrococcus luteus* PCI 1001, *Mycobacterium smegmatis* ATCC 607, *Escherichia coli* NIHJ, *Pseudomonas aeruginosa* P-3, *Xanthomonas campestris* pv. oryzae KB 88, *Bacteroides fragilis* ATCC 23745, *Acholeplasma laidlawii* PG 8, *Pyricularia oryzae* KF 180, *Aspergillus niger* ATCC 6275, *Mucor racemosus* IFO 4581, *Candida albicans* ATCC 64548, and *Saccharomyces cerevisiae*. Media for the microorganisms were as follows: GAM agar (Nissui Seiyaku Co.) for *B. fragilis*; Bacto PPLO agar (Difco) supplemented with 15% horse serum, 0.1% glucose, 0.25% phenol red (5 mg mL<sup>-1</sup>), and 1.5% agar for *A. laidlawii*; Mueller-Hinton broth (Difico) and 1.5% agar (Shimizu Shokuhin Co.) for MRSA; Taiyo agar (Shimizu Syokuhin Kaisya Ltd.) for the other bacteria; a medium composed of 1.0% yeast extract and 0.8% agar for fungi and yeasts. A paper disk containing 10 µg of a sample was placed on an agar plate. Bacteria, except for *X. oryzae*, were incubated at 37 °C for 24 h. Yeasts and *X. oryzae* were incubated at 27 °C for 24 h. Fungi were incubated at 27 °C for 48 h. Antimicrobial activity was expressed as diameter (mm) of the inhibitory zone.

This work was supported by Grant-in-Aid for the 21st Century COE program "Keio Life Conjugate Chemistry," as well as Scientific Research C from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors are grateful to the COE program and JSPS Research Fellowships for Young Scientests for financial support to TO.

### References

1 a) E. Fattorusso, L. Minale, G. Sodano, K. Moody, R. H. Thomson, J. Chem. Soc., Chem. Commun. **1970**, 752. b) J. A. McMillan, I. C. Paul, Y. M. Goo, K. L. Rinehart, Jr., W. C. Krueger, L. M. Pschigoda, *Tetrahedron Lett.* **1981**, 22, 39. c) G. Cimino, S. De Rosa, S. De Stefano, R. Self, G. Sodano, *Tetrahedron Lett.* **1983**, 24, 3029.

2 D. J. Faulkner, *Nat. Prod. Rep.* 2002, *19*, 1, many references are cited therein.

3 a) S. Nishiyama, S. Yamamura, *Tetrahedron Lett.* **1983**, 24, 3351. b) S. Nishiyama, S. Yamamura, *Bull. Chem. Soc. Jpn.* **1985**, 58, 3453.

4 a) T. Ogamino, Y. Ishikawa, S. Nishiyama, *Heterocycles* **2003**, *61*, 73. b) T. Ogamino, S. Nishiyama, *Tetrahedron* **2003**, *59*, 9419.

5 Related synthetic studies: a) M. Murakata, K. Yamada,
O. Hoshino, J. Chem. Soc., Chem. Commun. 1994, 443. b) M.
Murakata, K. Yamada, O. Hoshino, Tetrahedron 1996, 52, 14713.
c) M. Murakata, M. Tamura, O. Hoshino, J. Org. Chem. 1997, 62, 4428. d) M. Murakata, K. Yamada, O. Hoshino, Heterocycles

**1998**, *47*, 921. e) H. H. Wasserman, J. Wang, *J. Org. Chem.* **1998**, *63*, 5581. f) T. R. Boehlow, J. J. Harburn, C. D. Spilling, *J. Org. Chem.* **2001**, *66*, 3111.

6 R. D. Encarnación, E. Sandoval, J. Malmstrøm, C. Christophersen, J. Nat. Prod. 2000, 63, 874.

7 R. D. Encarnación, M. R. Ramirez, J. H. Luna-Herrera, *Pharm. Biol.* **2003**, *41*, 384.

8 A preliminary report of this investigation, see: T. Ogamino, S. Nishiyama, *Tetrahedron Lett.* **2005**, *46*, 1083.

9 a) A. R. Forrester, R. H. Thomson, S.-O. Woo, J. Chem. Soc., Perkin Trans. 1 **1975**, 2340. b) A. R. Forrester, R. H. Thomson, S.-O. Woo, *Liebigs Ann. Chem.* **1978**, 66.

10 Upon using NaBH<sub>4</sub>, *cis*-**6** was selectively produced, while  $Zn(BH_4)_2$  provided ca 1:1 mixture of **5** and **6**, see Ref. 3.

11 K. T. Okamoto, J. Clardy, *Tetrahedron Lett.* **1987**, *28*, 4969.

12 K. Goldenstein, T. Fendert, P. Proksch, E. Winterfeldt, *Tetrahedron* **2000**, *56*, 4173.

13 Synthetic product 13 might be a diastereomer mixture. Unfortunately, up to now, we have not been able to accomplish their separation. This problem was also encountered in the synthesis of aerothionin  $2.^3$ 

14 Y.-P. Kim, H. Tomoda, K. Iizima, T. Fukuda, A. Matsumoto, Y. Takahashi, S. Omura, *J. Antibiot.* **2003**, *56*, 448.