 

# Synthetic Studies on Ceratospongamides, Cyclic Heptapeptides Containing Thiazole and Oxazoline Units: Total Synthesis of *cis,cis*-Ceratospongamide

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A total synthesis of *cis,cis*-ceratospongamide **1a**, a cycloheptapeptide isolated from the Indonesian red alga *Ceratodictyon spongiosum* and the symbiotic sponge *Sigmadocia symbiotica*, was accomplished. The heptapeptide chain **8** was produced by a stepwise connection of the following segments: (L-Phe-L-Pro)-thiazole residue, L-Phe-L-Pro-OMe, and L-Ile-L-*a*Thr-OMe. Macrolactamization of **8**, followed by cyclization to an oxazoline ring, provided *cis,cis*-ceratospongamide **1a** along with its diastereomer **1c**, which was different from the reported *trans,trans*-ceratospongamide **1b**. The structural determination of **1c** and its conversion to **1a** were successfully achieved.

Marine organisms are well known to be rich sources of novel secondary metabolites. Among them, a series of heterocycle-containing cyclopeptides are structurally unique, and exhibit a variety of biologically activities, on which many synthetic investigations have been reported.[1–5] According to a brine shrimp toxicity assay, *cis,cis*- and *trans,trans*-ceratospongamides **1a**, **1b** were isolated from the Indonesian red alga *Ceratodictyon spongiosum*, as well as the symbiotic sponge *Sigmadocia symbiotica* in 1999 by Gerwick et al.[6] *trans,trans*-Ceratospongamide **1b** was reported to possess potent inhibition against secreted phospholipase $A_2$ ($sPLA_2$) expression in a cell-based model for antiinflammation, whereas the *cis,cis*-isomer **1a** was inactive. Ceratospongamides **1a**, **1b** share a cyclic heptapeptide containing thiazole and oxazoline units; the difference between **1a** and **1b** is the stereochemistry of the two proline-amide bonds. Because these findings are related to isolatable conformers as well as bioactivities, we were prompted to initiate a synthetic study on ceratospongamides. We describe herein the total synthesis of *cis,cis*-ceratospongamide **1a** and its diastereomer **1c**. The structural elucidation of **1c**, which was different from that of the reported **1b**, and its chemical conversion into **1a** were accomplished (Fig. 1).[7,8]

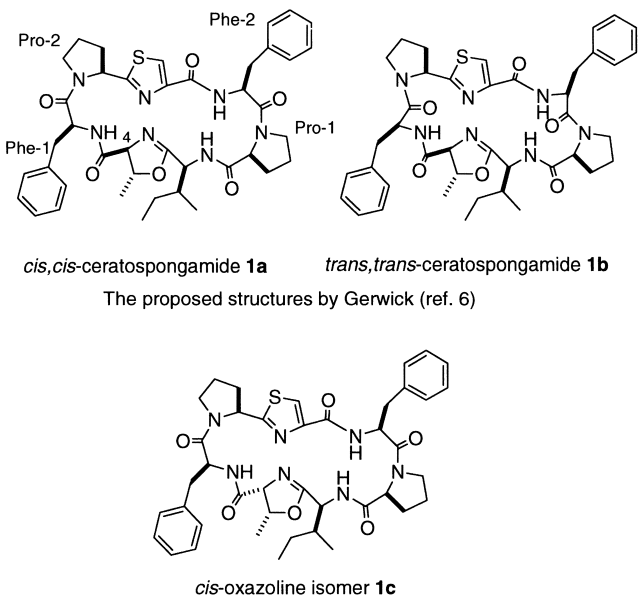


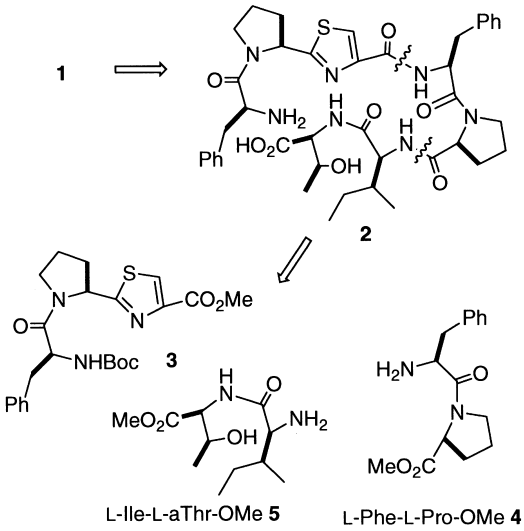

Fig. 1. Chemical Structures of ceratospongamides.

## Results and Discussion

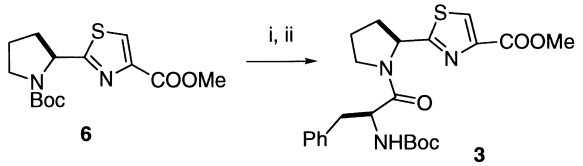
In a retrosynthetic analysis (Scheme 1), the ceratospongamides would be produced by macrolactamization of the linear heptapeptide **2**, followed by dehydrocyclization to construct the oxazoline moiety.[9] The intermediate **2** was divided into a tripeptide unit, including the thiazole **3**, and the two dipeptide units **4**, **5**.

According to the above-mentioned analysis, thiazole **3** was synthesized from **6**[10] (Scheme 2). After deprotection of **6** with TFA, BOP (benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate)-mediated coupling with Boc-L-Phe-OH afforded the thiazole segment **3** in 78% yield.

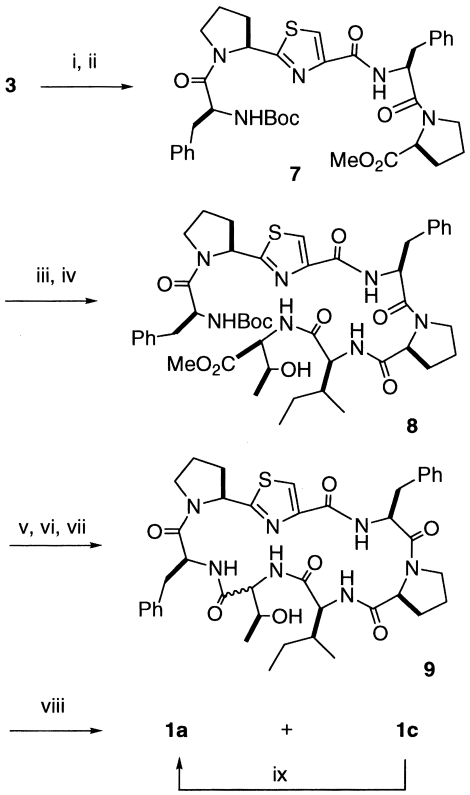
Saponification of **3** with aq NaOH, and then condensation of the resulting acid with L-Phe-L-Pro-OMe **4** by the same procedure as described above, provided the pentapeptide **7** in 97% yield in two steps (Scheme 3). Subsequently, **7** was deprotected under basic conditions, and the corresponding carboxylic acid was further coupled with L-Ile-L-*a*Thr-OMe **5**[11] to afford the linear heptapeptide **8** in 51% yield from **7**. After successive deprotection of the C- and N-termini in **8**, the corresponding amino-acid was treated with DPPA (diphenylphosphoryl azide)–DIPEA (diisopropylethylamine) under high-dilution conditions to give the desired cyclic heptapeptide **9**, as a mixture of two diastereomers. Dehydrocyclization of **9** with




Scheme 1.




Scheme 2. Reagents: i. TFA, $CH_2Cl_2$, 0 °C; ii. Boc-L-Phe-OH, BOP, $Et_3N$, DMF (78% in 2 steps).




Scheme 3. Reagents: i. 1 M aq NaOH, MeOH; ii. **4**, BOP, $Et_3N$, DMF (97% in 2 steps); iii. 1 M aq NaOH, MeOH; iv. **5**, BOP, $Et_3N$, DMF (51% in 2 steps); v. 1 M aq NaOH, MeOH; vi. TMSOTf, $CH_2Cl_2$; vii. DPPA, DIPEA, DMF (18% in 3 steps); viii. Deoxo-Fluor, $CH_2Cl_2$ (**1a**: 12%, **1c**: 46%); ix. NaOMe, MeOH (38%).

Deoxo-Fluor (bis(2-methoxyethyl)aminosulfur trifluoride)[12] provided two chromatographically separable diastereomers **1a** and **1c** in 12% and 46% yields, respectively. The $^1$H NMR spectrum of synthetic **1a** was identical to that of the natural *cis,cis*-ceratospongamide **1a**,[6] although the spectrum of **1c** was not identical to that of natural *trans,trans*-ceratospongamide **1b**. A detailed analysis of the 2D NMR data indicated that **1c** has the same peptide sequence as that of **1a**. In addition, the stereochemistry of the proline-amide of **1c** was assigned to be the same *cis,cis*-conformation as that of **1a** from NOESY data, which showed a strong correlation between the $\alpha$-protons of Pro-2/Phe-1 and Pro-1/Phe-2, as well as the $^{13}$C NMR chemical shifts: *cis* and *trans* conformational differences of the proline amide bonds correlates with differential values between proline $\beta$ and $\gamma$ carbons.[13] However, several chemical shifts around the methyl oxazoline residue of **1c** were different from those of **1a** in the $^1$H NMR spectrum. From among chemical conversions attempted under acidic and basic conditions, it was fortunately found that **1c** could be converted to **1a** with NaOMe in refluxing MeOH.[14] This observation suggested that racemization at the C4 position of the oxazoline residue took place. To obtain further evidence, both **1a** and **1c** were submitted to acid-hydrolysis, followed by GC analysis using a chiral capillary column. It appeared that both of them possessed the same optically active amino acid, with the exception of threonine. Accordingly, **1c** is an isomer of **1a** at the oxazoline part: the stereochemistry of threonine was the L-configuration in synthetic *cis,cis*-ceratospongamide **1a**, whereas the D-*allo* isomer was found in **1c**.[15]

In conclusion, total syntheses of *cis,cis*-ceratospongamide **1a** and its *cis*-oxazoline isomer **1c** were accomplished. A further investigation related to the chemical structure of the unknown *trans,trans*-ceratospongamide **1b** is in progress.

## Experimental

IR spectra were recorded on a JASCO Model A-202 spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were obtained on a JEOL JNM EX-270, a JEOL JNM GX-400 NMR, or a JEOL JNM ALPHA-400 spectrometer in a deuteriochloroform ($CDCl_3$) solution using tetramethylsilane as an internal standard, unless otherwise stated. High-resolution mass spectra were obtained on a Hitachi M-80 B GC-MS spectrometer operating at an ionization energy of 70 eV or a JEOL JMS-700 spectrometer. Gas chromatography was obtained on a GL Science GC 353 apparatus with a Chromatocorder 21 (System Instruments Ltd). Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. All of the melting points were obtained on a Yanaco MP-S3 and are uncorrected. Preparative and analytical TLC were carried out on silica-gel plates (Kieselgel 60 $F_{254}$, E. Merck A. G., Germany) using UV light and/or 5% phosphomolybdic acid in ethanol for detection. Katayama silica-gel (K 070) was used for column chromatography.

**Methyl 2-{(*S*)-1-[(*S*)-2-*tert*-butoxycarbonylamino-3-phenylpropanoyl]pyrrolidin-2-yl}-thiazole-4-carboxylate (3).** To an ice-cooled solution of **6** (1.0 g, 3.2 mmol) in $CH_2Cl_2$ (20 mL) was

gradually added TFA (19 mL); the mixture was stirred at the same temperature for 3 h. Evaporation of the mixture provided the corresponding TFA salt (1.5 g, quant.).

To a mixture of the salt (349 mg, 1.1 mmol) and Boc-L-Phe-OH (384 mg, 1.5 mmol) in DMF (20 mL) were added the BOP reagent (710 mg, 1.6 mmol) and $Et_3N$ (0.3 mL, 2.1 mmol) at 0 °C; the reaction mixture was stirred at the same temperature for 2 h, and then at ambient temperature overnight. The resulting mixture was diluted with EtOAc, washed successively with sat. aq $NaHCO_3$, 5% aq $KHSO_4$, then brine. After being dried ($Na_2SO_4$), evaporation of the mixture afforded a crude product, which upon purification by silica-gel column chromatography (hexane/EtOAc = 5/1) gave **3** (381 mg, 78%): $[\alpha]_D^{18}$ −64.8° (*c* 1.00, $CHCl_3$); IR (film) 3316, 1710, and 1646 $cm^{-1}$; $^1H$ NMR $\delta$ 1.38 (9H, complex), 2.00 (2H, complex), 2.25 (1H, m), 2.41 (1H, m), 2.91 (1H, m), 3.04 (1H, m), 3.35 (1H, m), 3.73 (1H, m), 3.94 (3H, s), 4.42 (1H, m), 4.75 (1H, dd, *J* = 16, 7 Hz), 7.23 (5H, complex), and 8.06 (1H, m) $^{13}C$ NMR $\delta$ 24.6, 28.4, 31.5, 39.4, 47.2, 52.4, 53.1, 58.6, 59.5, 79.8, 126.8, 127.0, 128.4, 129.2, 136.0, 146.3, 171.0, and 172.4. Found (FAB): *m/z* 459.1799. Calcd for $C_{23}H_{29}N_3O_5S$: M, 459.1826.

**Methyl (*S*)-1-[(*S*)-2-(2-{(*S*)-1-[(*S*)-2-*tert*-butoxycarbonylamino-3-phenylpropanoyl]pyrrolodin-2-yl}thiazol-4-ylcarbonylamino)-3-phenylpropanoyl]pyrrolidine-3-carboxylate (7).** A solution of **3** (781 mg, 1.7 mmol) in 1 M (= 1 mol $dm^{-3}$) aq NaOH (14 mL, 14 mmol) and MeOH (10 mL) was stirred at 0 °C for 2 h. After the addition of 5% aq $KHSO_4$, the mixture was partitioned between EtOAc and brine. The organic extracts were combined, dried ($Na_2SO_4$), then evaporated to give the corresponding carboxylic acid (758 mg, quant.): IR (film) 2977, 1708, and 1645 $cm^{-1}$; $^1H$ NMR $\delta$ 1.38 (9H, complex), 2.98 (2H, complex), 3.28 (1H, m), 7.19 (5H, complex), and 8.15 (1H, m).

To a mixture of the carboxylic acid (0.758 g, 1.7 mmol) and **4** (1.17 g, 3.0 mmol) in DMF (30 mL) were added the BOP reagent (1.13 mg, 2.6 mmol) and $Et_3N$ (1.65 mL, 12 mmol). After being stirred at 0 °C for 2 h and at ambient temperature overnight, the reaction mixture was diluted with EtOAc, washed successively with sat. aq $NaHCO_3$, 5% aq $KHSO_4$, and brine. After being dried ($Na_2SO_4$), evaporation of the solution gave a crude product, which upon purification by silica-gel column chromatography (hexane/EtOAc = 1/3) afforded **7** as an oil (1.17 g, 97%): $[\alpha]_D^{18}$ −74.7°(*c* 1.00, $CHCl_3$); IR (film) 3404, 1743, 1703, and 1644 $cm^{-1}$; $^1H$ NMR $\delta$ 1.38 (9H, complex), 2.38 (1H, m), 2.91–3.26 (8H, complex), 4.42 (1H, m), 4.70 (1H, m), 5.10 (1H, m), 5.25 (1H, m), 5.40 (1H, m), 7.20 (10H, complex), and 7.95 (1H, m); $^{13}C$ NMR $\delta$ 14.2, 21.0, 22.2, 24.9, 28.3, 29.0, 30.7, 39.1, 47.0, 52.1, 52.4, 58.0, 58.9, 79.6, 123.6, 126.8, 127.1, 128.2, 128.3, 128.5, 128.7, 129.2, 129.3, 129.6, 136.0, 148.6, 150.3, 155.0, 160.2, 169.8, 170.9, and 172.0. Found (FAB): *m/z* 704.3102. Calcd for $C_{37}H_{46}N_5O_7S$: M + H, 704.3115.

**Methyl (2*S*,3*S*)-2-[(2*S*,3*S*)-{(*S*)-1-[(*S*)-2-(2-{(*S*)-1-[(*S*)-*tert*-butoxycarbonylamino-3-phenylpropanoyl]pyrrolidin-2-yl}thiazol-4-ylcarbonylamino)-3-phenylpropanoyl]pyrrolidin-2-ylcarbonylamino}-3-methylpentanoylamino]-3-hydroxybutanoate (8).** A solution of **7** (1.17 g, 1.7 mmol) in 1M aq NaOH (10 mL)–MeOH (12 mL) was stirred at 0 °C for 90 min. After the reaction was quenched by the addition of 5% aq $KHSO_4$, the mixture was partitioned between EtOAc and brine. The organic extracts were dried ($Na_2SO_4$), then evaporated to give a carboxylic acid (1.07 g, 94%): $[\alpha]_D^{24}$ –60.4° (*c* 1.00, $CHCl_3$); IR (film) 3390, 3062, 1709, and 1644 $cm^{-1}$; $^1H$ NMR ($CD_3OD$) $\delta$ 1.38 (9H, complex), 2.22 (2H, complex), 4.40 (1H, m), 4.50 (1H, m), 5.05 (1H, m), and 5.40 (1H, m); $^{13}C$ NMR $\delta$ 14.2, 21.1, 24.7, 27.8, 28.3, 31.0, 39.2, 47.1, 47.5, 52.3, 58.1, 59.6, 60.4, 79.8, 124.0, 126.8, 127.1, 128.3, 128.6, 128.7, 129.3, 129.5, 135.4, 135.9, 148.3, 160.5, 171.2, 171.7, and 172.4.

To a mixture of the carboxylic acid (719 mg, 1.0 mmol) and the triflate salt of **5** (727 mg, 1.8 mmol) in DMF (30 mL) were added the BOP reagent (553 mg, 1.3 mmol) and $Et_3N$ (0.58 ml, 4.2 mmol). After being stirred at 0 °C for 2.5 h, the reaction mixture was diluted with EtOAc, washed successively with sat. aq $NaHCO_3$, 5% aq $KHSO_4$, and brine, then dried ($Na_2SO_4$). Removal of the solvent under reduced pressure provided a crude product, which upon purification by preparative TLC ($CHCl_3$/MeOH = 20/1) gave **8** as an oil (512 mg, 54%): $[\alpha]_D^{18}$ −52.8° (*c* 1.00, $CHCl_3$); IR (film) 3397, 1740, and 1644 $cm^{-1}$; $^1H$ NMR $\delta$ 0.82–0.95 (6H, complex), 1.40 (9H, s), 3.78 (3H, s), 5.05 (1H, m), 5.28 (1H, d, *J* = 9.3 Hz), 5.43 (1H, dd, *J* = 2.4, 7.8 Hz), 7.2 (10H, complex), and 7.9 (2H, complex). Found (FAB): *m/z* 940.4244. Calcd for $C_{47}H_{63}N_7O_{10}SNa$: M + Na, 940.4251.

**14-[(*S*)-1-Methylpropyl]-11-[(*S*)-1-hydroxyethyl]-(2*S*,8*S*,14*S*,17*S*,23*S*)-6,9,12,15,21,24,29-heptaaza-8,23-dibenzyl-28-thiatetracyclo[24.2.1.$0^{2,6}$.$0^{16,20}$]nonacosa-1(29),26-diene-7,10,12,15,21,24-hexone (9).** A triflate salt (470 mg, 0.51 mmol), obtained by a treatment of **8** with 1 M aq NaOH (20 mL)–MeOH (20 mL), followed by TMSOTf, was dissolved in DMF (500 mL). After the addition of DPPA (0.33 mL, 1.5 mmol) and DIPEA (0.53 mL, 3.0 mmol), the resulting mixture was stirred at ambient temperature for 3 d. The mixture was diluted with EtOAc, washed successively with 5% $KHSO_4$, sat. aq $NaHCO_3$, and brine, dried ($Na_2SO_4$), then evaporated. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 20/1) to give **9** as a mixture of the corresponding diastereomers (71 mg, 18% yield in three steps). Found (FAB): *m/z* 786.3612. Calcd for $C_{41}H_{52}N_7O_7S$: M + H, 786.3646.

**Dehydrocyclization of 9 to 1a and 1c.** To an ice-cooled mixture of the diastereomer (**9**, 63 mg, 0.081 mmol) in $CH_2Cl_2$ (10 mL) was added the Deoxo-Fluoro reagent (89 μL, 0.48 mmol); the reaction mixture was stirred at the same temperature for 2 h. After the addition of sat. aq $NaHCO_3$, the mixture was diluted with $CHCl_3$, washed with brine, dried ($Na_2SO_4$), then evaporated. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 20/1) to give **1a** (7.3 mg, 12%) and **1c** (29 mg, 46%). **1c**: $[\alpha]_D^{24}$ −99.6° (*c* 1.00, $CHCl_3$); IR (film) 3385 and 1647 $cm^{-1}$; $^1H$ NMR $\delta$ 0.62 (3H, d, *J* = 6.4 Hz), 0.80 (3H, t, *J* = 7.3 Hz), 0.81 (1H, m), 0.86 (3H, d, *J* = 6.4 Hz), 1.00 (1H, m), 1.41 (1H, m), 1.53 (1H, m), 1.66 (1H, m), 1.70 (1H, m), 1.70 (2H, complex), 1.78 (1H, m), 1.84 (1H, m), 2.01 (1H, m), 2.71 (1H, dd, *J* = 10.3, 12.7 Hz), 2.78 (1H, dd, *J* = 11.7, 12.7 Hz), 3.11 (1H, dd, *J* = 4.4, 12.7 Hz), 3.32 (1H, m), 3.41 (1H, m), 3.52 (1H, br d, *J* = 9.8 Hz), 3.58 (1H, dd, *J* = 4.4, 12.7 Hz), 3.65 (1H, d, *J* = 7.8 Hz), 3.82 (1H, ddd, *J* = 2.4, 8.8, 11.2 Hz), 4.00 (1H, d, *J* = 5.9 Hz), 4.30 (1H, ddd, *J* = 4.4, 7.3, 11.7 Hz), 4.44 (1H, t, *J* = 9.3 Hz ), 4.48 (1H, *J* = d, 8.8 Hz), 4.61 (1H, ddd, *J* = 4.0, 4.4, 10.3 Hz), 4.74 (1H, dq, *J* = 8.8, 6.4 Hz), 7.05 (1H, d, *J* = 9.3 Hz), 7.18 (2H, complex), 7.21 (1H, m), 7.23 (2H, complex), 7.24 (1H, d, *J* = 7.3 Hz), 7.26 (2H, complex), 7.28 (2H, complex), 7.88 (1H, d, *J* = 4.0 Hz), and 7.89 (1H, s); $^{13}C$ NMR $\delta$ 10.5, 15.0, 16.0, 21.3, 21.5, 24.7, 30.8, 34.2, 35.5, 39.8, 40.8, 46.1, 46.4, 50.5, 53.6, 53.7, 58.8, 61.4, 71.2, 77.2, 77.8, 123.6, 127.1, 127.3, 128.5, 128.9, 129.3, 129.4, 135.8, 136.7, 149.0, 159.7, 167.9, 168.1, 170.2, 170.5, and 171.2. Found (FAB): *m/z* 768.3564. Calcd for $C_{41}H_{50}N_7O_6S$: M + H, 768.3543.

**Isomerization of 1c to 1a.** To a solution of **1c** (15 mg, 0.019 mmol) in MeOH (3 mL) was added NaOMe in MeOH (33 μL,

0.02 mmol). After being refluxed for 1h, the mixture was neutralized by the addition of Amberlyst 15E. The mixture was filtered, and the filtrate was evaporated. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 20/1) to give **1a** (5.6 mg, 38%) and the recovered **1c** (3.4 mg, 23%): $[\alpha]_D^{24}$ −52.2° (*c* 1.00, $CHCl_3$); IR (film) 3397, 1740, 1644, and 1537 $cm^{-1}$; $^1H$ NMR $\delta$ 0.82–0.95 (6H, complex), 1.00–1.55 (2H, complex), 1.21–1.24 (3H, d, $J$ = 6.4 Hz), 1.40 (9H, s), 1.71–1.73 (1H, m), 1.89–2.36 (9H, complex), 2.88–3.73 (8H, complex), 3.78 (3H, s), 4.09–4.72 (5H, complex), 7.00 (1H, d, $J$ = 7.3 Hz), 7.09–7.10 (1H, m), 7.14–7.39 (10H, complex), 7.84–7.90 (1H, m), and 7.95 (1H, s).

**Chiral GC analysis of 1a and 1c.** The cyclic peptide (1.3 mg) in 6 M aq HCl (2 mL) was refluxed for 24 h. The resulting mixture was evaporated, and the residue was dissolved in $CH_2Cl_2$ (0.5 mL). Ozone was bubbled into the solution at 0 °C for 15 min. The reaction mixture was concentrated in vacuo, and the residue in 10% HCl in MeOH (3 mL) was heated at 110 °C for 1 h; the mixture was then evaporated and the residue was reacted with trifluoroacetic anhydride in $CH_2Cl_2$ (0.5 mL–0.5 mL) at 110 °C for 15 min. The resulting solution was submitted to Chiral GC analysis using Chirasil-Val column (25 m×0.25 mm; the program rate: 50 °C (5 min), 50–70 °C at 1.5 °C/min, 70 °C (15 min), 70–80 °C at 1 °C/min, 80 °C (10 min), 80–100 °C at 2 °C/min, then 100–200 °C at 10 °C/min. The GC analysis for the *N*-trifluoroacetyl methyl ester derivatives of the amino acids established the presence of L-Thr (22.46 min) from **1a** and D-*a*Thr (44.20 min) from **1c** together with L-Pro (23.38 min), L-Ile (24.22 min), L-Phe (61.72 min).

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15 As heptapeptide **2** has the same L-allothreonine as that of **5** from GC analysis, the racemization at the C4 position of the oxazoline might proceed during the macrolactamization.