Pergamon

Tetrahedron 58 (2002) 8127–8143

TETRAHEDRON

Total synthesis and conformational studies of ceratospongamide, a bioactive cyclic heptapeptide from marine origin

Fumiaki Yokokawa,[a,*] Hirofumi Sameshima,[a] Yasuko In,[b,*] Katsuhiko Minoura,[b] Toshimasa Ishida[b] and Takayuki Shioiri[a,†]

[a]*Graduate School of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan*
[b]*Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan*

Received 18 April 2002; revised 2 July 2002; accepted 25 July 2002

Abstract—The first total synthesis of *cis*,*cis*-ceratospongamide (cyclo[L-Pro-L-Ile-Me-oxazoline-L-Phe-L-Pro-thiazole-L-Phe-]) was accomplished and confirmed by X-ray crystal analysis. The heating of *cis*,*cis*-ceratospongamide in DMSO converted it not to the *trans*,*trans* isomer but to the *trans*,*trans*-[D-allo-Ile]-ceratospongamide, which was confirmed by total synthesis. Its solution conformation was constructed by the dynamic simulated annealing method using ROE cross peaks, revealing a rounded and flat ring structure which is in contrast with the slim and tight structure of *cis*,*cis* isomer. The results shows that the *trans*,*trans*-[D-allo-Ile] isomer is the main thermal product of *cis*,*cis*-ceratospongamide.

1. Introduction

Interest in marine organisms has been steadily increasing primarily due to the presence of metabolites capable of acting as potent bioactive agents for therapeutic use.[1–4] Marine cyclic peptides have, in particular, shown a broad range of bioactivities such as cytotoxic, tumor-promoting, anticancer, antiviral, and anti-inflammatory activities. Many of these cyclic peptides contain five-membered heterocycles (oxazolines, oxazoles, thiazolines and thiazoles). Therefore, the presence of heterocyclic building blocks as well as the challenges in macrocyclization has stimulated the interest of synthetic chemists in marine cyclic peptides.[5] On the other hand, upon the introduction of five-membered heterocycles into the cyclic peptide sequence, the conformational flexibility of the macrocycles is considerably reduced, and usually a single secondary structure is preferred in the solid state and in solution. Accordingly, a thorough understanding of structural and conformational properties can facilitate the studies concerning structure–activity relationships, which are very helpful in the rational design of new potent drugs.[6] Ceratospongamide is a bioactive oxazoline, thiazole-containing cyclic heptapeptide isolated by Gerwick and co-workers from the Indonesian red alga *Ceratodictyon spongiosum* and the symbiotic sponge *Sigmadocia symbiotica*.[7] This peptide consists of two L-phenylalanine residues, one (L-isoleucine)-methyloxazoline residue, one L-proline residue, and one (L-proline)-thiazole residue. Interestingly, Gerwick and co-workers reported that ceratospongamide can be isolated as two stable isomers: the major *cis*,*cis* isomer (**1**) and the minor *trans*,*trans* isomer (**2**) around the two proline–phenylalanine amide bonds. Although both isomers show moderate potency in the brine shrimp toxicity assay (LD_{50}=13–19 mM), considerably different activity is observed in terms of the inhibition of secreted phospholipase A_2 expression, a key enzyme in the inflammatory cascade: ED_{50}=32 nM for **2** and is inactive for **1**. We were intrigued by the highly functionalized macrocyclic ring structure as well as the difference in biological activity exhibited by the conformational isomers of ceratospongamide, and we therefore performed the total synthesis and conformational analysis of ceratospongamide.[8] Herein, we report the total synthesis of *cis*,*cis*-ceratospongamide (**1**) and its thermal product, *trans*,*trans*-[D-*a*Ile]-ceratospongamide (**3**), the X-ray single-crystal analysis of **1**, the solution conformation of **3** as determined by ^{1}H NMR spectroscopy and dynamic simulated annealing (SA) methods, and the conformational stabilities of **1**, **2** and **3** as determined by molecular mechanics/dynamics calculations (Fig. 1).

Keywords: *cis*,*cis*-ceratospongamide; *trans*,*trans*-[D-allo-Ile] ceratospongamide; thermodynamic isomerization; synthesis; conformation.

* Corresponding authors. Address: Novartis Pharma, Tsukuba Research Institute, Tsukuba 300-2611, Japan. Tel.: +81-298-65-2137; fax: +81-298-65-2308; e-mail: fumiaki.yokokawa@pharma.novartis.com; Tel.: +81-726-90-1069; fax: +81-726-90-1068; e-mail: in@gly.oups.ac.jp

† Present address: Graduate School of Environmental and Human Sciences, Meijo University, Nagoya, 468-8502, Japan.

2. Results and discussion

2.1. Synthesis

2.1.1. Retrosynthetic strategy. Our retrosynthetic analysis of ceratospongamide is shown in Scheme 1. Since the

0040–4020/02/$ - see front matter
PII: S0040-4020(02)00906-7

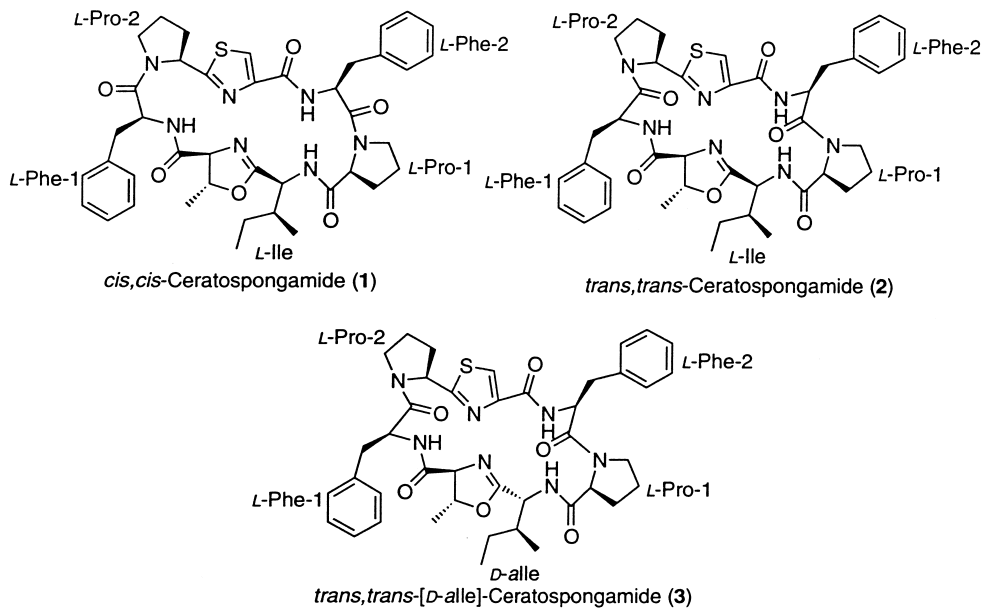
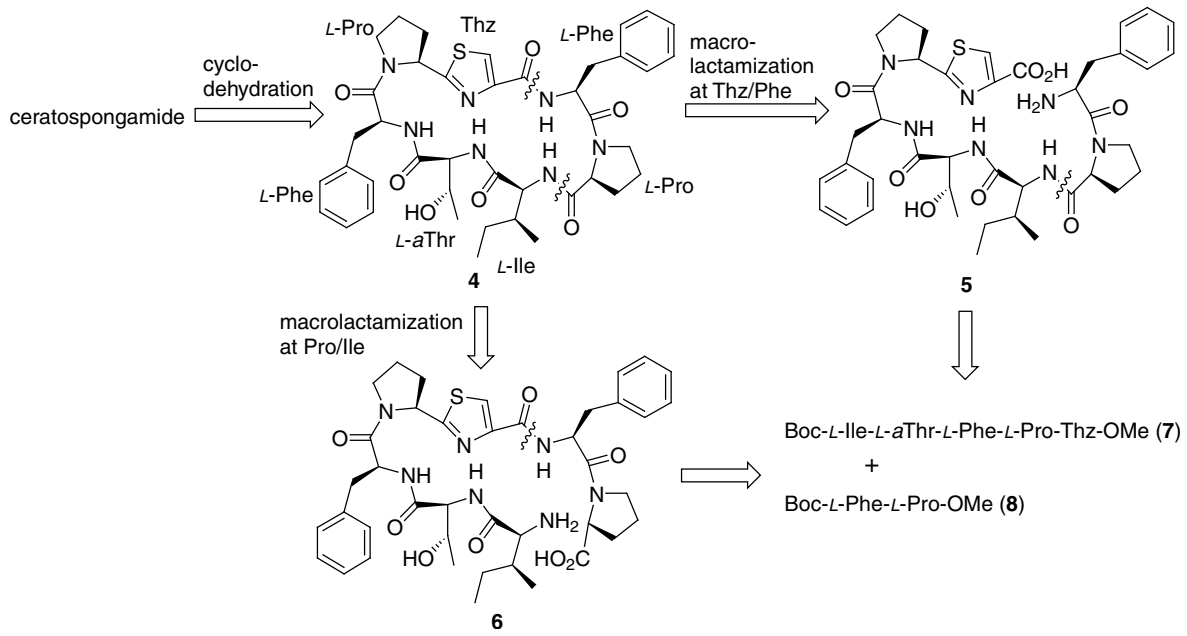
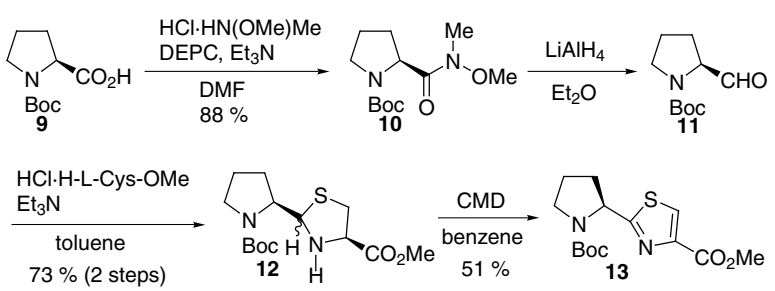


Figure 1. Structures of ceratospongamides.

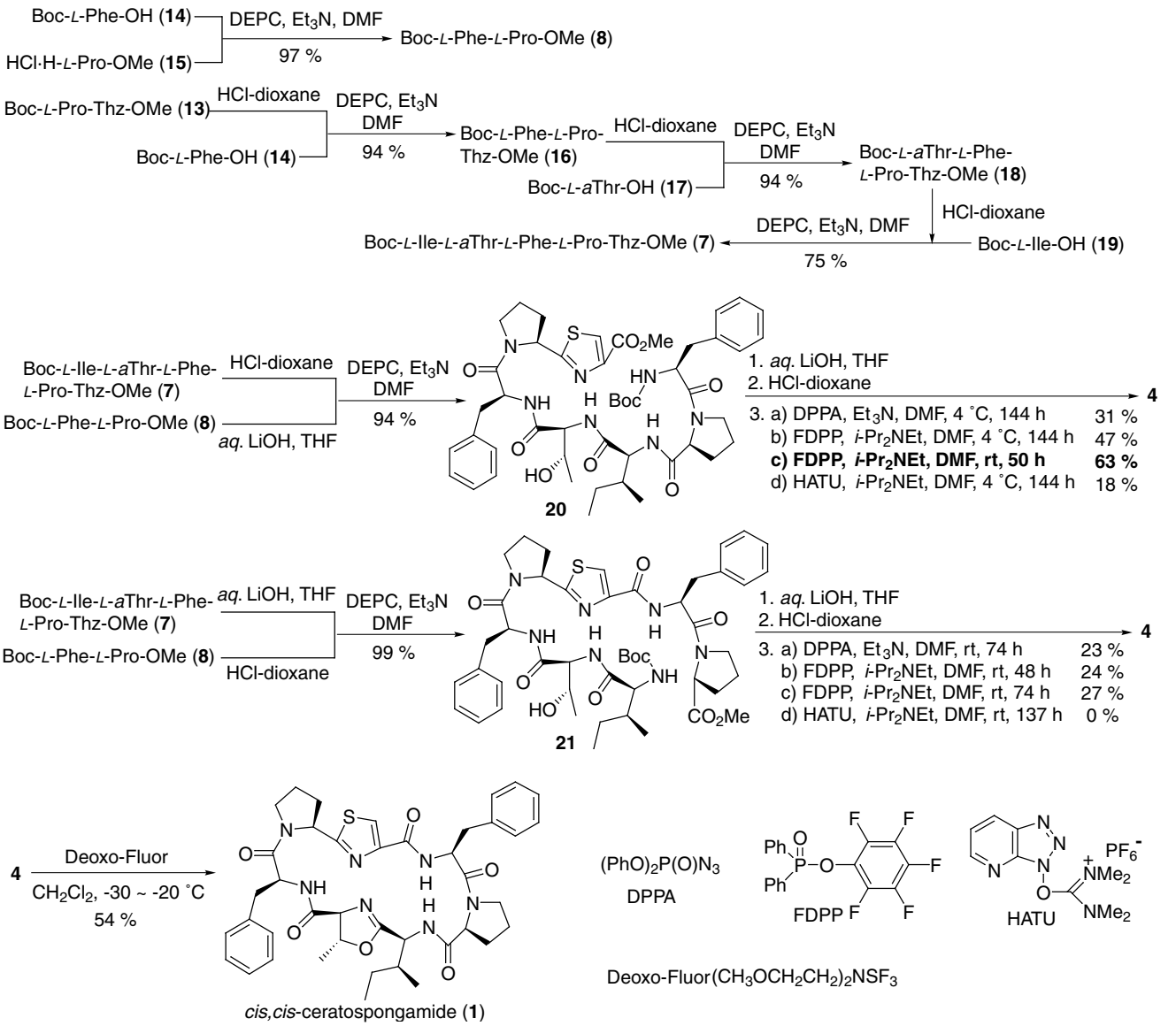


Scheme 1. Retrosynthetic analysis of ceratospongamide.

oxazoline ring is sensitive under acidic conditions and readily epimerized at the chiral centers attached to the heterocycle under mild acidic or basic conditions, the final formation of the oxazoline ring by cyclodehydration of the *allo*-L-threonine residue was employed to give the cyclic peptide **4**. Macrolactamization at the thiazole/L-phenylalanine or L-proline/L-isoleucine amide bond of **4** was chosen to avoid epimerization at the C-terminus to provide



Scheme 2. Synthesis of Boc-L-Pro-Thz-OMe (**13**).



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

the linear peptide **5** or **6**.[9] For the synthesis of the linear peptide **5** or **6**, a [5+2] convergent strategy was adopted to give the pentapeptide **7** and dipeptide **8**.

2.1.2. Total synthesis of *cis,cis*-ceratospongamide (1). Since we have shown that optically pure thiazole amino acids can be prepared by oxidation of thiazolidines with chemical manganese dioxide (CMD) that is manufactured industrially for batteries,[10] we applied this methodology to the synthesis of thiazole fragment **13**. Coupling of Boc-protected L-proline (**9**) with *N,O*-dimethylhydroxylamine using diethyl phosphorocyanidate (DEPC, $(EtO)_2$-P(O)CN)[11] afforded amide **10**[12] in 88% yield. After reduction of amide **10** with lithium aluminum hydride,[13] the resulting aldehyde **11** was condensed with L-cysteine methyl ester to give thiazolidine **12** as a diastereomeric mixture in 73% yield. Subsequent CMD oxidation of thiazolidine **12** provided thiazole fragment **13** in 51% yield (Scheme 2).

Dipeptide segment **8** was obtained by coupling of Boc-protected L-phenylalanine (**14**) with L-proline methyl ester (**15**) using DEPC in 97% yield. Preparation of pentapeptide segment **7** was initiated by removing the Boc protective group of thiazole fragment **13** with hydrogen chloride in dioxane followed by condensation with Boc-protected L-phenylalanine (**14**) using DEPC in 94% yield. Iterative removal of the Boc protective group followed by peptide coupling with Boc-protected L-*allo*-threonine (**17**) and Boc-protected L-isoleucine (**19**) gave pentapeptide segment **7**. After acidic deprotection of the Boc group of **7**, segment condensation with the acid derived from dipeptide **8** smoothly proceeded to give fully protected heptapeptide **20** in 94% yield. Similarly, deprotection of the Boc group of **8** and segment condensation with the acid derived from **7** also afforded linear peptide **21** in 99% yield. Then, the comparison of the macrolactamization step at two sites (Thz/Phe vs. Pro/Ile) was carried out. After saponification of the methyl ester in **20** and **21** with aqueous lithium hydroxide and acidic cleavage of the Boc group, macrolactamizations of the resulting two free linear peptides were performed in DMF solution (0.002 M) by

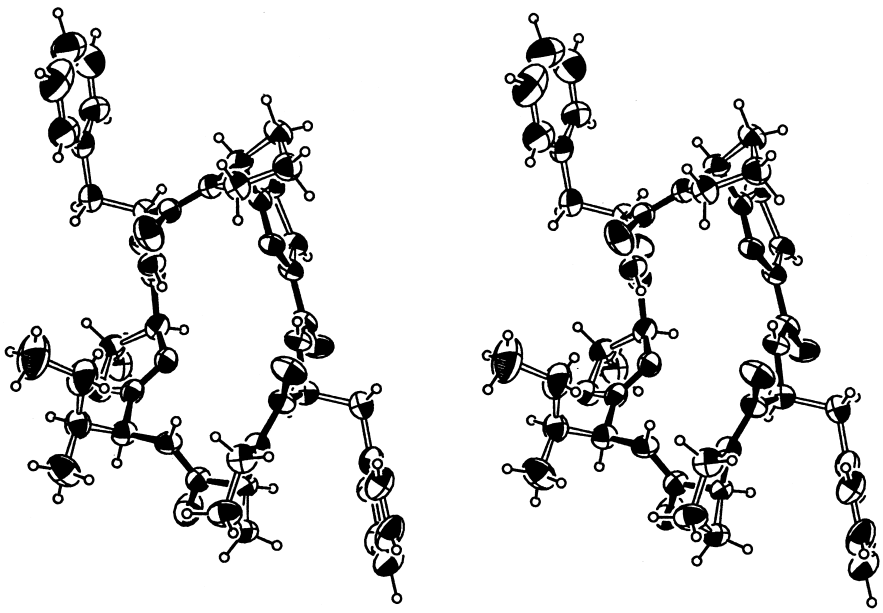


Figure 2. Stereoscopic molecular conformation of **1**. The ω torsion angles of two Pro amide bonds have *cis* orientation. No intramolecular hydrogen bonds are formed.

using diphenyl phosphorazidate (DPPA),[11,14] pentafluorophenyl diphenylphosphinate (FDPP),[15] or *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU).[16] The cyclization at Thz/Phe site smoothly proceeded and the yield by FDPP was higher than those by DPPA and HATU. In contrast, the cyclization at Pro/Ile site was sluggish, and no cyclized product was obtained by using HATU. Accordingly, we carried out the macrolactamization at Thz/Phe site with 1.5 equiv. of FDPP and 4 equiv. of *i*-Pr_2NEt at room temperature to give cyclic peptide **4** in 63% yield from corresponding linear peptide **20**. Finally, dehydrative cyclization of the *allo*-threonine residue using bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-fluor) to the oxazoline[17] provided a single isomer of ceratospongamide, which was completely identical with natural *cis*,*cis*-ceratospongamide (**1**) as judged from ^{1}H and ^{13}C NMR spectra, HPLC, and TLC R_f values. Furthermore, crystallization of synthetic *cis*,*cis*-ceratospongamide (**1**) from chloroform–heptane mixture followed by X-ray analysis allowed us to confirm that both Phe-Pro amide bonds have *cis* orientation as described below. Accordingly, our total synthesis and X-ray analysis have demonstrated that the proposed structure of *cis*,*cis*-ceratospongamide (**1**) by Gerwick and co-workers is correct (Scheme 3).

2.2. X-Ray crystal structure of 1

A stereoscopic view of *cis*,*cis*-isomer of the ceratospongamide is shown in Fig. 2.[18] Conformational torsion angles are listed in Table 1. Because of the relatively high thermal motion of each residue, the estimated standard deviation (esd) values for bond lengths (~0.1 Å) and angles (0.5–1°) are somewhat larger than usual. However, these geometric parameters are all within the range of those of similar-sized depsipeptides, such as ascidiacyclamide[19] and didemnin B.[20] Both Phe-Pro amide bonds take *cis* orientation of ω torsion angle (−2.5 and −3.4°), whereas the other peptide bonds are all in the *trans* region. No notable anomaly was observed for the conformational torsion angles. The thiazole and oxazoline rings are almost planar and are nearly orthogonal to each other (dihedral angle=80.2°). The two Pro cyclic rings exhibit typical C^{β}-*exo*-C^{γ}-*endo* ring puckering (C2-conformation).

The cyclic backbone chain of the molecule assumes a distorted saddle-shaped rectangular structure. The backbone structure of the thiazole-Phe-2-Pro-1 sequence forms a warped plane; the distance between the N(Pro-1)-Cα(Pro-2) line and the C(4)–C(6) bond is about 2.5 Å. This plane forms one side of the rectangular long axes. The other side is formed by the Ile-oxazoline-Phe-1 sequence, although the backbone structure is considerably distorted from the planarity because the oxazoline ring at the central position forms a dihedral angle of 64.8° with respect to the amide plane of Phe-1. Residues of Pro-1, Ile, Phe-1 and Pro-2 are located at each corner of the ring structure, with the Ile side chain and the oxazoline methyl group protruding above and below the ring chain, and two Phe aromatic rings are oriented antiparallel to each other along the ring structure. One conformational feature of **1** is the absence of intramolecular hydrogen bonds, which contribute to the conformational stabilization of many cyclic peptides. All peptide amide groups are approximately on the belt-plane of cyclic backbone ring, thereby hindering the formation of the intramolecular NH···O=C hydrogen bonds. This indicates that several restraints are imposed on the cyclic ring structure, meaning the absence of conformational flexibility.

No intermolecular hydrogen bonds or short contacts less than 3.5 Å are observed between neighboring molecules, although three water solvents molecules are included. These solvents occupy the cavities created by the molecular packing of **1**, and hydrogen bonds are formed amidst them. On the other hand, because of the tetragonal crystal symmetry, the respective molecular packings along the *a*- and *b*-axes are completely the same. Thus, such

Table 1. Conformational torsion angles (degrees) of **1**, **2**, and **3**

		1 (X-ray[a])	**2** (MD)	**3** (NMR/SA)
Pro-1	ϕ	−90.3 (8)	−74.5	−66.7
	ψ	−5.5 (7)	44.4	85.9
	ω	175 (1)	178.8	176.0
	$\chi 1$	33.0 (7)	−22.2	−18.1
	$\chi 2$	−31.3 (7)	40.5	33.7
	$\chi 3$	16.6 (7)	−40.1	−34.9
	$\chi 4$	4.4 (6)	26.2	25.0
Ile/D-*allo*-Ile	ϕ	−100.3 (8)	−103.5	138.0
	ψ	−17.5 (8)	30.1	54.7
	ω	−177.7 (8)	−170.8	155.4
	$\chi 1,1$	−66.0 (8)	−72.2	−35.1
	$\chi 1,2$	65.3 (8)	56.5	92.7
	$\chi 2$	163 (1)	164.1	76.8
Oxazoline	ϕ[b]	124.0 (8)	138.7	135.4
	ψ[c]	−7.6 (7)	−68.7	−66.8
	ω	177.1 (8)	−168.3	−173.7
Phe-1	ϕ	−155.0 (8)	−154.3	−98.6
	ψ	108.8 (8)	−70.3	136.1
	ω	−2.5 (6)	−171.7	−173.2
	$\chi 1$	−175.8 (9)	−41.9	−176.0
	$\chi 1,2$	70 (1)	106.0	85.6
	$\chi 2,1$	−108 (1)	−84.0	−92.7
Pro-2	ϕ	−67.1 (7)	−63.2	−40.7
	ψ	−31.5 (7)	−52.8	93.4
	ω[d]	−177.5 (7)	−179.5	−158.8
	$\chi 1$	33.1 (6)	−19.7	−37.6
	$\chi 2$	−40.3 (7)	36.8	41.3
	$\chi 3$	30.8 (7)	−37.7	−28.0
	$\chi 4$	−9.2 (6)	27.7	3.8
Thiazole	ϕ[e]	−176.5 (8)	−172.9	158.4
	ψ[f]	−2.0 (6)	−7.0	−13.8
	ω	174.6 (9)	164.4	−169.4
Phe-2	ϕ	−165.8 (8)	−117.6	−172.2
	ψ	141.9 (8)	153.9	163.8
	ω	−3.4 (6)	−156.0	169.7
	$\chi 1$	−170.9 (8)	176.4	62.4
	$\chi 1,2$	85.1 (9)	102.5	87.8
	$\chi 2,1$	−94.1 (9)	−87.7	−93.8

The X-ray, MD and NMR/SA results are given for **1**, **2**, and **3**, respectively.
[a] The esd values of X-ray torsion angles in **1** are given in parentheses.
[b] C2–N–C4–C6.
[c] N–C4–C6–N(Phe-1).
[d] Cα–C2–N(thiazole)–C4.
[e] C2–N–C4–C6.
[f] N–C4–C6–N(Phe-2).

crystallographic restraints may significantly affect the molecular conformation of **1**. The partial crystal packing that shows the most crowded molecular association is shown in Fig. 3. Two different interfaces could be observed: one is between the right and central molecular layers, and the other, between the central and left ones. The former interface is formed by van der Waals forces such that the uneven structures of the respective molecules are oriented in such a way that they complement each other. In the latter interface, the partial insertion of an L-Phe aromatic ring into the concave ring structure of **1** is observed. However, such interfaces are relatively weak, and each space created by the molecular packing is occupied by three water molecules.

2.3. Thermal product of 1 and structural analysis

We next investigated the synthesis of *trans*,*trans*-ceratospongamide (**2**). Gerwick and co-workers reported that *cis*,*cis*-ceratospongamide (**1**) could be converted into the *trans*,*trans*-isomer (**2**) by heating at 175°C in DMSO according to HPLC monitoring.[7] Therefore, we tried the thermodynamic isomerization from our synthetic *cis*,*cis*-ceratospongamide (**1**) to the *trans*,*trans*-isomer (**2**). As was reported in their paper,[7] thermal treatment of synthetic *cis*,*cis*-ceratospongamide (**1**) at 175°C for 30 min in DMSO gave a compound different from **1** in the isolated yield of 10–30% (entry 1). This thermal conversion proceeded slowly even below 100°C and was almost completed after 8 days (entry 2). Interestingly, we also found that slightly acidic conditions (in the presence of pyridinium *p*-toluenesulfonate) facilitated the thermal conversion (100°C for 40 min, 75% isolated yield, entry 3) as shown in Scheme 4.

Since MS measurements showed the same molecular weight as **1**, this thermal product could be a conformational and/or structural isomer of **1**. Therefore, we performed the total synthesis of [D-*allo*-Ile]-ceratospongamide (**3**) using D-*allo*-Ile instead of L-Ile. As shown in Scheme 5, the successive attachment of Boc-D-*a*Ile-OH (**22**) and Boc-L-Phe-L-Pro-OMe (**8**) to Boc-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**18**) afforded the protected linear precursor **24**, which, after deprotection at C- and N-termini, underwent macrolactamization by using FDPP to give macrocycle **25**. Final construction of the oxazoline ring using Deoxo-fluor afforded [D-*allo*-Ile]-ceratospongamide (**3**).

Surprisingly, synthetic peptide **3** was completely identical with the thermal product of *cis*,*cis*-isomer (**1**) as judged from ^{1}H and ^{13}C NMR spectra and TLC R_f values. The direct comparison of ^{1}H NMR spectra for the synthetic **3** with natural *trans*,*trans*-ceratospongamide (**2**) showed a good spectroscopic match, although there were subtle differences around 5 ppm. Moreover, there were no differences ($\Delta\delta<0.4$ ppm) in the reported data of ^{13}C NMR spectra between natural **2** and synthetic **3**. In addition, the optical rotation of synthetic **3** ($[\alpha]_D=-38.8$ (*c* 0.5, $CHCl_3$) was also the same to that of the natural **2** ($[\alpha]_D=-39.2$ (*c* 0.5, $CHCl_3$)). During the preparation of this manuscript, Deng and Taunton published the total synthesis of *trans*,*trans*-ceratospongamide (**2**) together with *cis*,*cis*-ceratospongamide (**1**).[21] Interestingly, their synthetic *trans*,*trans* isomer (**2**) was completely identical with our synthetic **3** in ^{1}H and ^{13}C NMR spectra. On the other hand, Deng and Taunton reported[21] that their synthetic *trans*,*trans*-ceratospongamide was identical to naturally derived one, and **1** was thermally transformed into an equilibrium mixture of **1** and **2** in a ratio of 1:5.

To thoroughly understand the structure of our [D-*allo*-Ile]-ceratospongamide (**3**), determination of the sequence and the connection of amino acid residues and other units (thiazole and methyloxazoline) was performed by a combination of both one- and two-dimensional ^{1}H NMR spectral measurements (GCOSY, TOCSY and ROESY). The results are given in Table 2, where the extensive overlapping of the Phe-1 and Phe-2 aromatic protons made complete peak assignment impossible.

The *trans* orientations of the two proline amide bonds were shown by (i) the absence of ROEs of Hα(Pro-1)-Hα(Phe-2)/

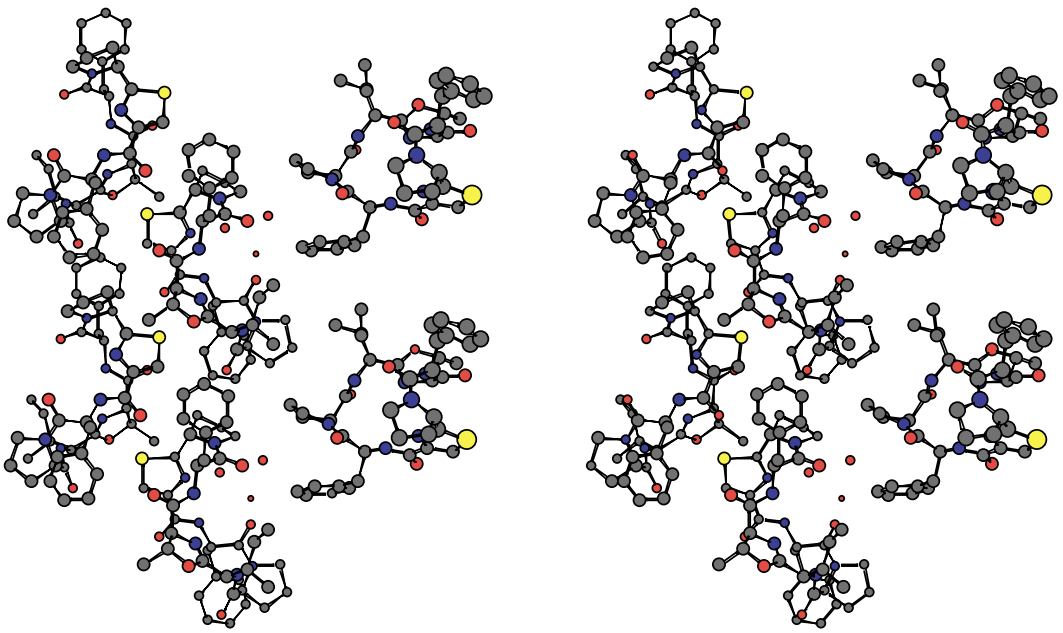
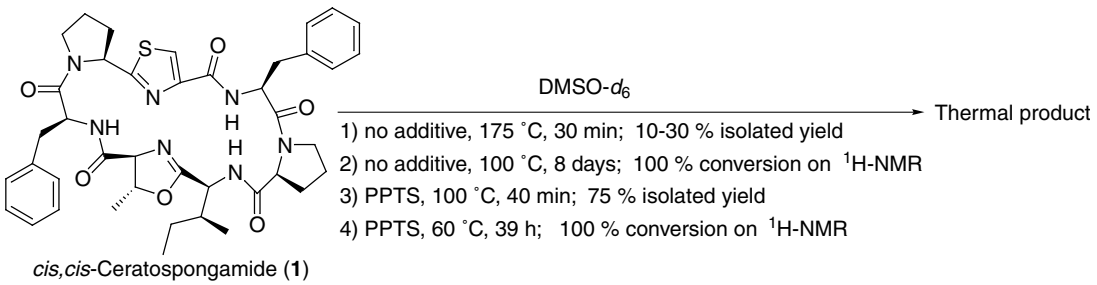
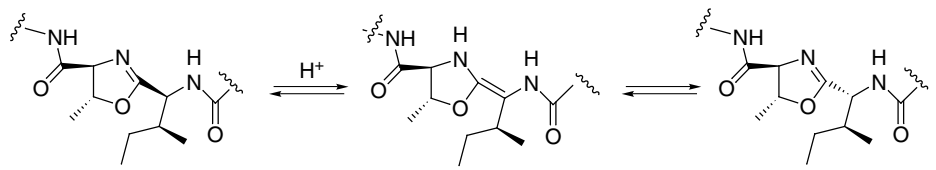
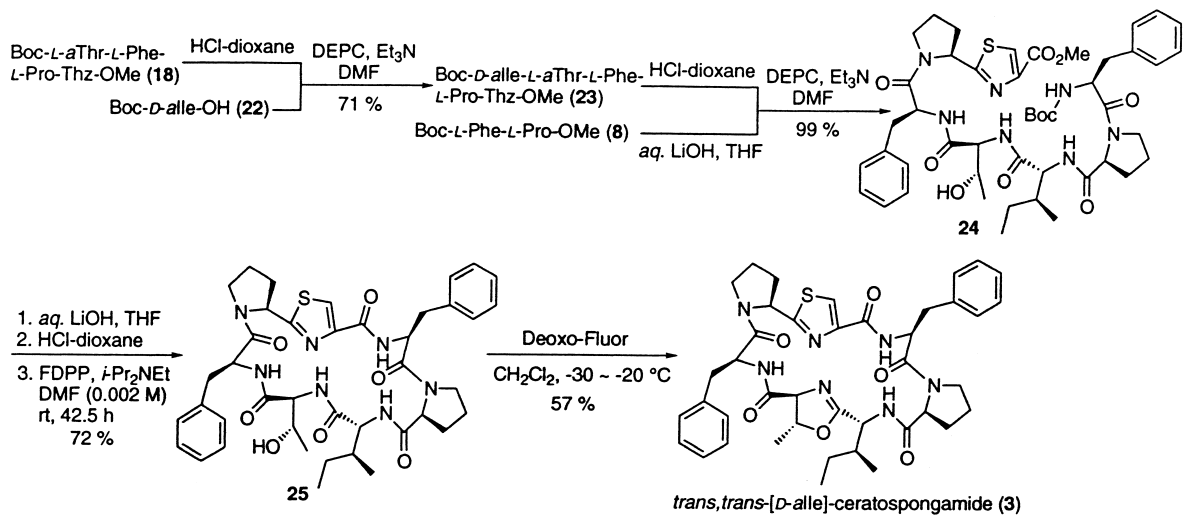


Figure 3. Stereoscopic view of molecular interaction modes formed in the crystal structure. The left and central molecular layers running from the bottom to the top are related to each other by a diad screw symmetry, and the central and right ones, by a diad symmetry. Open circles (red color) represent water molecules (W). These water molecules are hydrogen bonded to one another: O(1)W···O(2)W=2.930 (6) Å, O(1)W···O(3)W=2.63 (2) Å, O(2)W···O(3)W=2.32 (2) Å.





Scheme 4. Thermodynamic isomerization of *cis,cis*-ceratospongamide (**1**).



Scheme 5. Total synthesis of *trans,trans*-[D-*a*lle]-ceratospongamide (**3**).

Table 2. ^{1}H- and ^{13}C NMR spectral data (in ppm) for **3** with HMBC and ROESY correlations

	Position	^{1}H	m	*J* (Hz)	^{13}C	ROESY[a]
Pro-1	Hα	4.54	t	7.5	60.01	7.00–7.36 (w), 8.49 (s)
	Hβ	1.71	m		29.62	0.86 (w), 0.93 (w)
	Hβ	2.16	m			0.86 (w)
	Hγ	1.82	m		25.07	
	Hγ	1.93	m			
	Hδ	3.12	dt	9.4, 7.4	46.69	2.92 (m), 4.89 (m), 7.00–7.36 (m)
	Hδ	3.65	m			2.92 (m), 4.89 (s)
Ile	NH	8.49	d	10.1		4.54 (s)
	Hα	4.97	dd	10.1, 3.7	48.31	1.18 (m), 7.00–7.36 (m)
	Hβ	1.83	m		36.85	1.18 (m), 3.99 (w)
	Hγ1	1.21	m		25.71	
	Hγ1	1.30	m			1.18 (s)
	$(H\gamma 2)_3$	0.86	d	7.0	14.06	1.18 (m), 1.71 (w), 2.16 (w), 3.68 (w), 3.99 (w)
	$(H\delta 1)_3$	0.93	t	7.4	11.57	1.18 (m), 1.71 (w), 3.99 (w)
Me-oxazoline	2			167.79		
	4	3.99	d	6.5	73.18	0.86 (w), 0.93 (w), 1.83 (w), 7.06 (m), 7.00–7.36 (w)
	5	3.68	quint	6.5	79.35	0.86 (w), 7.00–7.36 (m)
	6			169.72		
	7	1.18	d	6.5	21.53	0.86 (m), 0.93 (m), 1.30 (s), 1.83 (m), 4.97 (m)
Phe-1[b]	NH	7.06	d	8.5		3.99 (m)
	Hα	4.87	ddd	12.8, 8.5, 2.0	52.81	3.87 (s)
	Hβ	3.22	dd	12.8, 2.0	37.14	3.87 (m)
	Hβ	3.38	t	12.8		3.87 (m)
	Cγ				137.45	
	Hδ–Hζ	7.00–7.36				
	Cδ–Cζ				125.85	
	C═O				168.48[c]	
Pro-2	Hα	5.42	dd	8.2, 4.0	57.77	
	Hβ	2.05	m		31.82	
	Hβ	2.48	m			
	Hγ	1.97	m		24.69	
	Hγ	2.08	m			
	Hδ	3.87	m		46.29	3.22 (m), 3.38 (w), 4.87 (s), 7.00–7.36 (m)
Thiazole	2				169.89	
	4				147.69	
	5	8.15	s		123.66	
	6				159.13	
Phe-2[b]	NH	8.26	d	8.9		7.00–7.36 (w)
	Hα	4.89	ddd	8.9, 7.8, 5.7	51.21	3.12 (m), 3.65 (s), 7.00–7.36 (m)
	Hβ	2.55	dd	13.7, 7.8	38.54	7.00–7.36 (w)
	Hβ	2.92	dd	13.7, 5.7		3.12 (m), 3.65 (w), 7.00–7.36 (w)
	Cγ				136.20	
	Hδ–Hζ	7.00–7.36				
	C═O				168.36[c]	

ROESY correlation is listed only for the inter-residual ROE intensities observed in DMSO-d_6 solution of **3**. The letters s, m, and w in parentheses represent strong, medium and weak ROE intensities, respectively.

[a] Since the respective aromatic protons of Phe-1 and Phe-2 were not completely assigned on the NMR spectra, their ROE constraints were not included in the model consideration.

[b] The aromatic carbons (Cδ–Cζ) of Phe-1 and Phe-2 could not be separately assigned, although they correspond to one of 125.85, 126.21, 127.50, 127.96, 129.15, and 129.82 ppm.

[c] These data are exchangeable.

Hα(Pro-2)-Hα(Phe-1) proton pairs and (ii) the strong ROEs of Hδ(Pro-1)-Hα(Phe-2)/Hδ(Pro-2)-Hα(Phe-1) proton pairs (Fig. 4). These *trans* assignments were also suggested by the lack of any notable difference between the β and γ carbon chemical shifts ($\Delta\delta_{\beta\gamma}$) of Pro-1 and Pro-2 (4.6 and 7.1 ppm, respectively), because the *cis*/*trans* conformational differences correlate well with differential values of $\Delta\delta_{\beta\gamma}$[22] and the present values are significantly small as compared with those of **1** (9.1 and 13.6 ppm, respectively).[7] The ROEs of (H7)3 (Me-oxazoline) with Hβ/Hγ/Hδ (Ile) and H4 (Me-oxazoline) indicate their orientations on the same side of the oxazoline plane. These data suggest the D-configuration of the Ile residue and the *S*-configuration of the oxazoline C4 atom. Based on these NMR and synthetic studies, it is concluded that the thermal product of **1** in DMSO solution results in the α-epimerization of the Ile residue and the *cis*→*trans* conversion at the two Phe-Pro amide bonds, consequently leading to the *trans*,*trans*-[D-*allo*-Ile] isomer of **3**. Furthermore, these results support that the proposed structure of natural *trans*,*trans*-cerato-spongamide (**2**) should be revised to *trans*,*trans*-[D-*allo*-Ile] isomer (**3**).

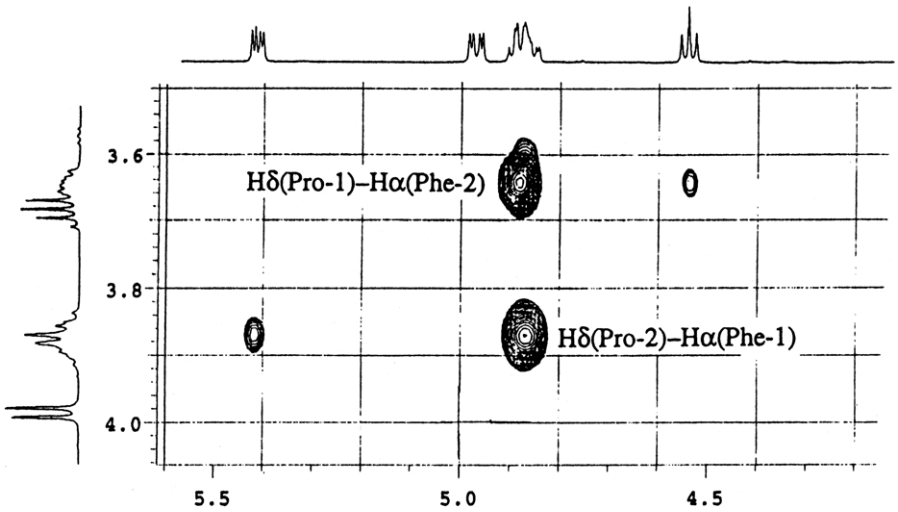


Figure 4. Cross peaks of Hδ(Pro-1)-Hα(Phe-2) and Hδ(Pro-2)-Hα(Phe-1) proton pairs in partial ROESY spectrum of **3** in DMSO-d_6 solution.

Table 3. Averaged RMS violation, RMS deviation and calculated energy values of 50 converged structures

RMS violation (Å)	0.041±0.009
RMS deviation (backbone) (Å)	0.710±0.298
Energies (kcal/mol)	
Overall	249.90±1.21
Forcing potential	3.12±1.23
Bond	31.52±0.32
van der Waals	84.00±0.96

2.4. Solution conformation of 3

Three-dimensional (3D) models of **3** were constructed by the dynamic SA method using the proton–proton distance restraints derived from the ROE cross peaks. The ROE constraints were not imposed on Phe-1 and -2 aromatic protons, because of the lack of complete peak assignments. Starting with 50 sets of conformations with random arrays of atoms, energy-minimization trials were performed to eliminate any possible source of initial bias in the folding pathway, where the actual error function was minimized by changing the respective ϕ, Ψ, ω and χ torsion angles. The constructed NMR structures satisfied the distance constraints within the allowable range and one of four possible ϕ torsion angles within ±30°. The structural statistics are given in Table 3. Among them, 10 structures, which converged to less than 0.4 Å for the averaged root mean square (RMS) distance deviation of the backbone chains, were selected to discuss the solution conformation of **3**. Their superposition is shown in Fig. 5. The most stable conformer is shown in Fig. 6 and its conformational torsion angles are given in Table 1.

Based on these well-converged structures, it could be said that **3** takes a solution conformation, where the amide bonds of the two proline residues have the *trans* orientation. As a whole, the ring structure of **3** may be very round and flat, as compared with the slim and tight structure of **1**. This is obviously due to the difference in orientation between the two Phe-Pro peptide bonds. Since the temperature dependence of the NH protons of Ile, Phe-1 and Phe-2 is −5.45, −0.06 and −0.84 ppb/K, respectively, the amide protons of Phe-1 and Phe-2 could form intramolecular hydrogen bonds with the Phe-2 and Phe-1 carbonyl oxygens, respectively.

2.4.1. Molecular dynamics simulations of 1, 2, and 3. In order to compare the relative stability among the conformational energies of **1**, **2**, and **3**, these isomers were subjected to molecular dynamics (MD) calculations of 100 ps duration. The X-ray and NMR/SA structures listed in

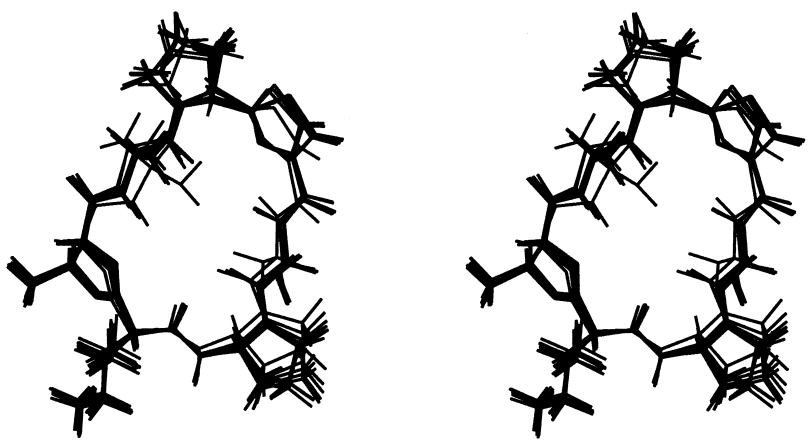


Figure 5. Stereoscopic best-fit superposition of the 10 energetically stable converged structures. The aromatic rings of Phe-1 and Phe-2 were omitted for clarity, because of the large variation of their orientations.

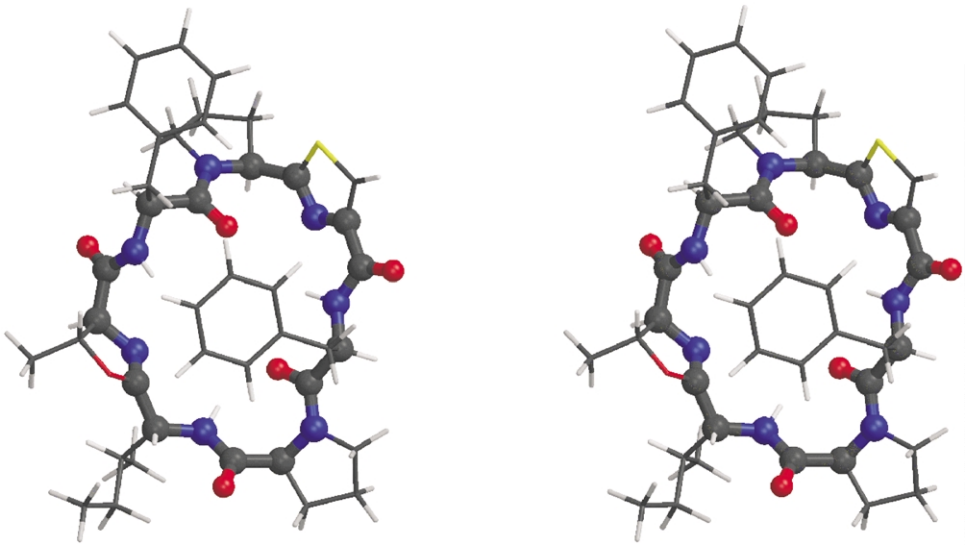


Figure 6. Stereoscopic view of the most stable conformers of **3**, derived from the NMR/SA method. Dotted lines represent possible intramolecular hydrogen bonds.

Table 4. Averaged total energies, torsion angles, and distances between Cα atoms of Phe-1 and Phe2 and between N atoms of thiazole and oxazoline rings, together with their estimated standard deviations in parentheses

	1		**2**	**3**
	298 K	473 K	298 K	298 K
Total energy (kcal/mol)	367 (9)	474 (13)	377 (9)	379 (8)
ω torsion angles (°)				
Pro-1 amide bond	−2 (16)	−2 (15)	−171 (13)	179 (15)
Pro-2 amide bond	−1 (13)	−6 (17)	−180 (11)	178 (12)
Distances (Å)				
Cα(Phe-1)–Cα(Phe-2)	7.5 (5)	7.5 (5)	7.9 (5)	7.8 (5)
N(thiazole)–*N*(Me-oxazoline)	4.5 (6)	4.4 (5)	6.6 (5)	6.6 (5)

Table 1 were used as starting conformers of **1** and **3**, and that of **2** was constructed by the dynamic SA method in reference to the former two conformers. All MD calculations were performed at 298 K using Insight/Discover software. No distance/torsional constraints were imposed to enable the conformational space of the molecule to be fully explored.

The time profiles of total energies, the ω torsion angles of the two Pro amide bonds, and the Cα(Phe-1)-Cα(Phe-2) and *N*(thiazole)-*N*(Me-oxazoline) distances were monitored to estimate their conformational fluctuations. The results are summarized in Table 4. The respective systems were observed to fluctuate within their stationary states throughout the MD simulation for 100 ps, because the estimated standard deviations of the total energies were all within ±5%. No conformational preference was observed among the energies of **1**, **2**, and **3**, and the conformational flexibility was nearly the same, as determined from the respective standard deviations of the torsion angles and the intra-residual atomic distances. Also, the *cis*⇔*trans* conversions of the ω torsion angles were not observed for all conformers during the MD simulation. Most conformers of **1** and **3** show the same backbone conformations as the X-ray and NMR/SA conformers, respectively. The most stable conformer of **2** in the MD simulation is given in Fig. 7, and the torsion angles are given in Table 1. From these MD results, it could be considered that each isomer assumes the conformation with the energy minimum and there is no conformational preference among them.

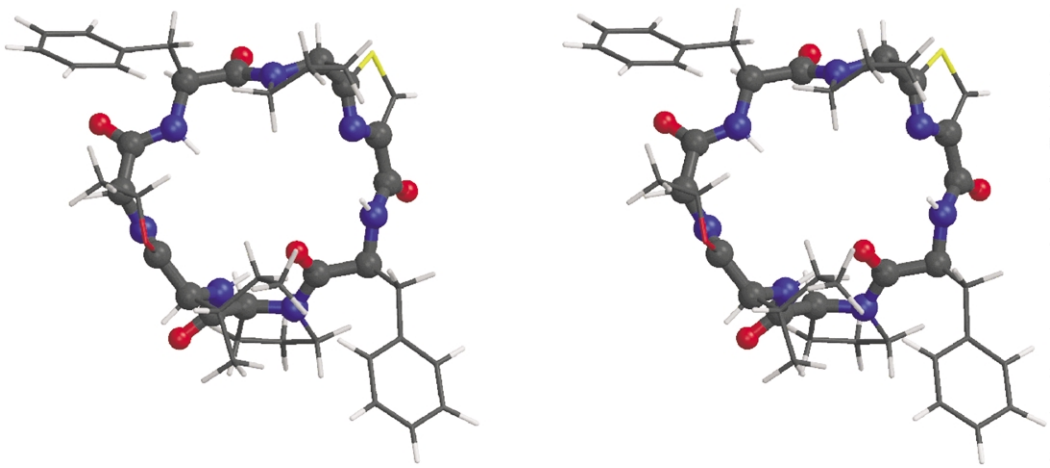
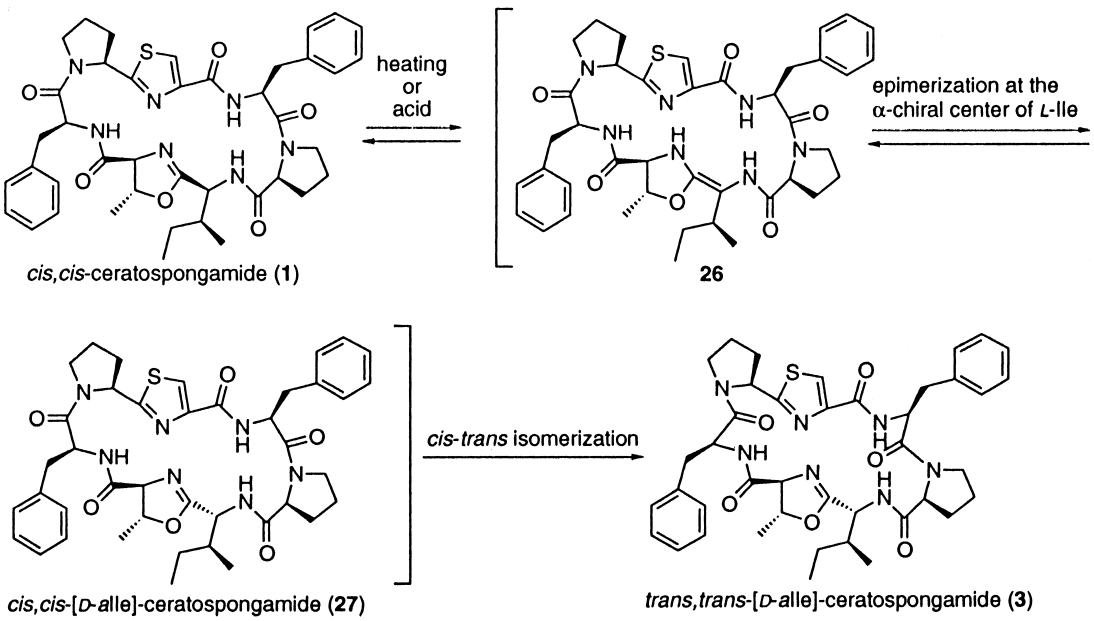


Figure 7. Stereoscopic view of the most stable conformer of **2**, derived from MD simulations for 100 ps.



Scheme 6. Proposed mechanism of the thermal isomerization.

Since the conformational stability of **2** could be nearly the same as that of **1**, it is interesting to examine the possibility of **1**→**2** conversion, because Gerwick et al.[7] reported the thermal conversion of **1** to **2**, whereas the thermodynamic isomerization of **1** by the same condition afforded **3**. Thus, the MD simulation of **1** was performed at a high temperature (473 K). Consequently, no *cis*→*trans* conversion was observed during the MD simulation for 100 ps (Table 4). Although this simulation system as well as the simulation time may be insufficient for deriving a conclusion, a high energy barrier for their conformational conversion could be considered, if this is even possible.

Based on this conformational analysis, we have proposed that the thermodynamic isomerization of *cis*,*cis*-ceratospongamide (**1**) was initiated by the Cα epimerization of Ile residue to provide the intermediate *cis*,*cis*-[D-*allo*-Ile] isomer (**27**), which was immediately isomerized at the two Phe-Pro amide bonds to produce the *trans*,*trans*-[D-*allo*-Ile] isomer (**3**) as shown in Scheme 6. Since the acidic condition facilitates the initial Cα epimerization of Ile residue via **26**, this thermodynamic isomerization in the presence of PPTs smoothly proceeded at even 60°C for 39 h (Scheme 4, entry 4). Deng and Taunton[21] reported that *trans*,*trans*-ceratospongamide (**2**) could be obtained by macrolactamization after forming the oxazoline. However, these studies indicate that Cα epimerization of Ile residue should be happened during the alkaline deprotection of both termini or macrolactamization to produce not *trans*,*trans* isomer (**2**), but *trans*,*trans*-[D-*allo*-Ile] isomer (**3**) as a minor product.

The conformational analysis of ceratospongamide, a new bioactive cyclic heptapeptide, is important for considering its biologically active conformation. The present studies established the total syntheses of **1** and its thermal product **3**, and made clear their molecular conformations and energy stabilities, together with nearly the same conformational stabilities of **2** and **3**. The important issue that should be solved is the structural analysis of thermal product of **1** in DMSO solution, because this is conflicting between Gerwick–Taunton's and ours. However, based on our present studies, we were able to revise the originally proposed structure of natural *trans*,*trans*-ceratospongamide (**2**) to *trans*,*trans*-[D-*allo*-Ile]-isomer (**3**). In any event, the present study indicated that a conformational similarity was observed between **2** and **3**. Because the conformation of **2** is important for its highly potent inhibition of the expression of secreted PLA2 in the inflammatory cascade,[7] biological activity for **3** would also be expected.

3. Experimental

3.1. Synthesis

General. Melting points were measured with a YANACO melting point apparatus and were uncorrected. Infrared spectra were recorded on a SHIMADZU FT IR-8100 spectrometer. Optical rotations were measured on a DIP-1000 digital polarimeter with a sodium lamp (λ=589 nm, D line) and are expressed as follows: $[\alpha]_D^T$ (*c* g/100 mL, solvent). ^{1}H NMR spectra were recorded on a JEOL EX-270 (270 MHz), ALPHA 500 (500 MHz) or LAMBDA (500 MHz) spectrometer. Chemical shifts are expressed in ppm with tetramethylsilane or solvent as the internal standard (CD_3OD: δ 4.78 ppm; DMSO-d_6: δ 2.49 ppm). Data are reported in terms of: chemical shift, integration, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants (Hz), and assignment. ^{13}C NMR spectra were recorded on a JEOL EX-270 (67.8 MHz), ALPHA 500 (500 MHz) or LAMBDA (500 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with the solvent as the internal standard (deuterochloroform: δ 77.0 ppm; DMSO-d_6: δ 39.5 ppm). Analytical thin layer chromatography was performed on Merck Art. 5715, Kieselgel 60F_{254}/0.25 mm thickness plates. Visualization was accomplished with UV

light, phosphomolybdic acid, or ninhydrin solution followed by heating. Preparative thin layer chromatography was performed on Merck Art. 5744, Kiselgel $60F_{254}$/0.5 mm thickness plates. High resolution mass spectra (HRMS) were measured at the Analytical Facility of Nagoya City University. Solvents for extraction and column chromatography were of reagent grade. Liquid column chromatography was performed with forced flow (flash chromatography of the indicated solvent mixture on silica gel BW-820MH or BW-200 (Fuji Davison Co.)). Tetrahydrofuran (THF) was distilled from sodium metal/benzophenone ketyl. Diethyl ether was distilled from lithium aluminum hydride. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. Toluene, dimethylsulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF) were dried over 4 Å molecular sieves. Triethylamine and *N*,*N*-diisopropylethylamine were dried over potassium hydroxide. All other commercially obtained reagents were used as received.

3.1.1. Total synthesis of *cis*,*cis*-ceratospongamide (1). (2S)-2-(Methoxy-methylcarbamoyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (10).[12] To a solution of Boc-L-Pro-OH (**9**) (10 g, 46.5 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (8.1 g, 83.0 mmol) in DMF (100 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (13 mL, 86 mmol) and triethylamine (17 mL, 122 mmol). The resulting solution was stirred at 0°C for 2 h and at room temperature for 12 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=2:1–1:1–0:1) to afford the desired product **10** as a yellow oil (10.6 g, 41.0 mmol, 88%): $[\alpha]_D^{26}=-13.1$ (*c* 1.1, $CHCl_3$); IR ν_{max}^{neat} (cm^{-1}) 1701, 1399, 1165, 1123; ^1H NMR (270 MHz, $CDCl_3$), minor rotamer in parentheses, δ 1.42 (1.46) (9H, s, ^{t}Bu), 1.86–2.23 (4H, m, Pro-$C_\beta H_2$, $C_\gamma H_2$), 3.20 (3H, s, OCH_3), 3.40–3.58 (2H, m, Pro-$C_\delta H_2$), 3.72 (3.78) (3H, s, *N*–CH_3), 4.61 (4.67) (1H, m, Pro-$C_\alpha H$); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 15.9 (16.0), 23.3 (23.9), 30.4 (29.5), 46.4 (46.7), 56.6 (56.3), 61.0 (61.1), 79.1 (79.3), 153.5 (154.1), 173.4 (172.9).

3.1.2. (2*S*)-2-(1-*tert*-Butoxycarbonyl-pyrrolidin-2-yl)-thiazolidine-4-carboxylic acid methyl ester (12). To a solution of amide **10** (852 mg, 3.3 mmol) in ether (10 mL) at 0°C was added $LiAlH_4$ (156 mg, 4.1 mmol). The resulting solution was stirred at 0°C for 15 min and quenched by dropwise addition of 1 M aq. $KHSO_4$. The mixture was extracted with ether (×3). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The residue and HCl–H-L-Cys-OMe (700 mg, 4.1 mmol) were dissolved in toluene (7.6 mL), and to this suspension was added triethylamine (0.71 mL, 5.1 mmol) at 0°C. The resulting suspension was stirred at room temperature, filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane/EtOAc=2:1) to afford the desired product **12** as a yellow oil (758 mg, 2.4 mmol, 73%): $[\alpha]_D^{27}=-86.3$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} (cm^{-1}) 3320, 1746, 1690, 1393, 1366, 1165; ^1H NMR (270 MHz, $CDCl_3$), minor rotamer in parentheses, δ 1.47 (1.50) (9H, s, ^{t}Bu), 1.71–1.99 (4H, m, Pro-$C_\beta H_2$, $C_\gamma H_2$), 3.15 (1H, br, thiazolidine-5), 3.23 (1H, m, thiazolidine-4), 3.39 (2H, m, Pro-$C_\delta H_2$), 3.77 (3.79) (3H, s, OCH_3), 4.14 (1H, dd, J=14.3, 7.1 Hz, NH), 4.47 (1H, br, Pro-$C_\alpha H$), 4.88 (1H, d, J=8.1 Hz, thiazolidine-2); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 22.7, 23.7 (23.6), 28.3 (28.5), 37.8 (38.7), 47.7 (47.2), 52.3, 58.1, 65.1, 68.0, 79.7. HRMS (EI) *m*/*z* calcd for $C_{14}H_{24}N_2O_4S$: 316.1457. Found: 316.1434.

3.1.3. Boc-L-Pro-Thz-OMe (13). A suspension of CMD (7.56 g, 87.0 mmol) in benzene (9 mL) was refluxed with stirring for 2 h using a Dean–stark apparatus (molecular sieves type 4A). To the resulting suspension was added a solution of the thiazolidine **12** (275 mg, 0.87 mmol) in benzene (1 mL), and then the mixture was refluxed for 2 h. The resulting mixture was filtered through a pad of celite and the filtrate was concentrated. The residue was purified by column chromatography (silica gel BW-820 MH, hexane–EtOAc=2:1) to afford thiazole **13** (139 mg, 0.44 mmol, 51%) as white crystals: mp 89–90°C (EtOAc–*n*-pentane); $[\alpha]_D^{24}=-107.8$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} (cm^{-1}) 3453, 1723, 1701, 1499, 1482, 1389, 1237; ^1H NMR (270 MHz, $CDCl_3$), minor rotamer in parentheses, δ 1.33 (1.48) (9H, s, ^{t}Bu), 1.90–1.98 (2H, m, Pro-$C_\beta H_2$), 2.05–2.35 (2H, br, $C_\gamma H_2$), 3.61 (2H, br, Pro-$C_\delta H_2$), 3.95 (3.98) (3H, s, OCH_3), 5.21 (1H, br, Pro-$C_\alpha H$), 8.01 (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 23.7 (24.4), 28.8, 34.8 (33.5), 47.2, 52.9, 60.1 (59.7), 80.8, 127.4 (127.7), 147.1, 153.8 (154.4), 162.2, 177.4. HRMS (EI) *m*/*z* calcd for $C_{14}H_{20}N_2O_4S$: 312.1144. Found: 312.1109.

3.1.4. Boc-L-Phe-L-Pro-OMe (8). To a solution of Boc-L-Phe-OH (**14**) (1.5 g, 5.65 mmol) and HCl–H-L-Pro-OMe (**15**) (1 g, 6.04 mmol) in DMF (18 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (0.94 mL, 6.20 mmol) and triethylamine (1.7 mL, 12.2 mmol). The resulting solution was stirred at 0°C for 1 h and at room temperature for 11 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=1:1) to afford the desired product **8** as a white solid (2.059 g, 5.47 mmol, 97%): mp 69–70°C; $[\alpha]_D^{26}=-35.8$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3343, 1750, 1736, 1707, 1694, 1653, 1644, 1536, 1518, 1437, 1368; ^1H NMR (270 MHz, DMSO-d_6, 100°C) δ 1.32 (9H, s, ^{t}Bu), 1.90 (3H, br, Pro-$C_\gamma H_2$, $C_\beta H_2$), 2.17 (1H, br, Pro-$C_\beta H_2$), 2.79 (1H, dd, J=14.0, 8.1 Hz, Phe-$C_\beta H_2$), 2.96 (1H, br, Phe-$C_\beta H_2$), 3.42 (1H, m, Pro-$C_\delta H_2$), 3.63 (4H, s, Pro-$C_\delta H_2$, OCH_3), 4.37 (2H, br, Pro-$C_\alpha H$, Phe-$C_\alpha H$), 6.36 (1H, br, NH), 7.25 (5H, m, PhH); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 24.9 (22.3), 28.4 (29.1), 39.2 (41.2), 46.8 (46.0), 52.2 (52.7), 53.2 (53.8), 58.9, 79.6 (77.2), 126.6 (126.8), 128.2 (128.4), 129.6 (129.2), 136.2, 155.0, 170.5, 172.1 (171.7). HRMS (EI) *m*/*z* calcd for $C_{20}H_{28}N_2O_5$: 376.1998. Found: 376.1992.

3.1.5. Boc-L-Phe-L-Pro-Thz-OMe (16). Boc-L-Pro-Thz-OMe (**13**) (1.806 g, 5.78 mmol) was treated with 4 M HCl–dioxane (7 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and

dried to afford the crude hydrochloride salt. To a solution of Boc-L-Phe-OH (**14**) (1.53 g, 5.65 mmol) and the above crude hydrochloride salt in DMF (19 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (1 mL, 6.59 mmol) and triethylamine (1.8 mL, 13.0 mmol). The resulting solution was stirred at 0°C for 1 h and at room temperature for 7 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=2:1–1:1–1:2) to afford the desired product **16** as an amorphous solid (2.492 g, 5.42 mmol, 94%): $[\alpha]_D^{26}=-76.7$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} (cm^{-1}) 3432, 3346, 1715, 1659, 1651, 1505, 1495, 1244; ^1H NMR (270 MHz, $CDCl_3$), minor rotamer in parentheses, δ 1.36 (1.40) (9H, s, ^{t}Bu), 1.58 (2H, s, Pro-$C_\gamma H_2$), 2.04 (2H, br, Pro-$C_\beta H_2$), 2.91–3.08 (2H, m, Phe-$C_\beta H_2$), 3.35 (1H, m, Pro-$C_\delta H_2$), 3.71–3.74 (1H, m, Pro-$C_\delta H_2$), 3.94 (3H, s, OCH_3), 4.42 (1H, d, *J*=7.9 Hz, Phe-NH), 4.73 (1H, br, Phe-$C_\alpha H$), 5.36 (1H, m, Pro-$C_\alpha H$), 7.17–7.26 (5H, m, PhH), 8.03 (8.06) (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 22.3, 27.6, 30.6, 36.9, 46.0, 51.1, 53.3, 57.8, 77.8, 125.6, 127.3, 127.7, 128.5, 136.7, 154.1, 160.5, 169.9, 171.2, 172.3. HRMS (EI) *m/z* calcd for $C_{23}H_{29}N_3O_5S$: 459.1828. Found: 459.1840.

3.1.6. Boc-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (18). Boc-L-Phe-L-Pro-Thz-OMe (**16**) (1.378 g, 3.00 mmol) was treated with 4 M HCl–dioxane (10 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude hydrochloride salt. To a solution of Boc-L-*a*Thr-OH (**17**) (672 mg, 3.07 mmol) and the above crude hydrochloride salt in DMF (10 mL) at 0°C was successively added dropwise diethyl phosphorocyanidate (0.5 mL, 3.30 mmol) and triethylamine (0.92 mL, 6.63 mmol). The resulting solution was stirred at 0°C for 2 h and at room temperature for 6 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=1:2–0:1, EtOAc–EtOH=20:1–10:1) to afford the desired product **18** as a colorless viscous oil (1.581 g, 2.82 mmol, 94%): $[\alpha]_D^{26}=-90.7$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} (cm^{-1}) 3325, 1723, 1715, 1646, 1634, 1244, 1217; ^1H NMR (270 MHz, DMSO-d_6) δ 0.95 (3H, d, *J*=6.1 Hz, *a*Thr-CH_3), 1.37 (9H, s, ^{t}Bu), 1.95–1.97 (3H, m, Pro-$C_\gamma H_2$, –OH), 2.13–2.18 (2H, m, Pro-$C_\beta H_2$), 2.79–3.03 (2H, m, Phe-$C_\beta H_2$), 3.52 (2H, br, Pro-$C_\delta H_2$), 3.71–3.74 (1H, m, *a*Thr-$C_\beta H$), 3.81 (3H, s, OCH_3), 3.88–3.91 (1H, m, *a*Thr-$C_\alpha H$), 4.73–4.79 (1H, m, Phe-$C_\alpha H$), 5.24–5.26 (1H, m, Pro-$C_\alpha H$), 6.61 (1H, d, *J*=8.7 Hz, BocNH), 7.19 (5H, br, PhH), 8.10 (1H, d, *J*=7.5 Hz, NH), 8.40 (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 19.7 (19.6), 21.5, 28.3, 31.4 (34.3), 38.1 (40.3), 47.3 (46.3), 52.0 (52.3), 58.8 (59.0), 60.3 (64.0), 65.2, 69.1, 127.0 (126.8), 127.4 (127.2), 128.3 (128.5), 129.2 (129.4), 135.5 (135.4), 155.5, 161.6 (161.3), 170.4 (169.9), 171.0 (170.7), 171.9 (173.4). HRMS (EI) *m/z* calcd for $C_{27}H_{36}N_4O_7S$: 560.2305. Found: 560.2311.

3.1.7. Boc-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (7). Boc-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**18**) (1.266 g, 2.75 mmol) was treated with MeOH (1 mL)–4N HCl–dioxane (8 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude hydrochloride salt. To a solution of Boc-L-Ile-OH (**19**) (637 mg, 2.75 mmol) and the above crude hydrochloride salt in DMF (10 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (0.45 mL, 2.97 mmol) and triethylamine (0.84 mL, 6.05 mmol). The resulting solution was stirred at 0°C for 1 h and at room temperature for 6 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=1:5–0:1, EtOAc–EtOH=10:1) to afford the desired product **7** as a white solid (1.399 g, 2.08 mmol, 75%): mp 111–112°C; $[\alpha]_D^{26}=-89.5$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3475, 3325, 1638, 1244; ^1H NMR (270 MHz, DMSO-d_6, 100°C) δ 0.80–0.85 (6H, m, Ile-$C_\gamma H_3$, Ile-$C_\delta H_3$), 1.05 (3H, d, *J*=6.1 Hz, *a*Thr-CH_3), 1.12–1.33 (2H, m, Ile-$C_\gamma H_2$), 1.39 (9H, s, ^{t}Bu), 1.74 (1H, br, –OH), 1.94–1.97 (2H, m, Pro-$C_\gamma H_2$), 2.17 (2H, br, Pro-$C_\beta H_2$), 2.83–2.97 (2H, m, Phe-$C_\beta H_2$), 3.44 (2H, br, Pro-$C_\delta H_2$), 3.83 (3H, s, OCH_3), 3.87–3.90 (2H, m, *a*Thr-$C_\beta H$, *a*Thr-$C_\alpha H$), 4.41 (1H, br, Ile-$C_\alpha H$), 4.79 (1H, br, Phe-$C_\alpha H$), 5.28 (1H, br, Pro-$C_\alpha H$), 6.31 (1H, d, *J*=8.0 Hz, BocNH), 7.17 (5H, br, PhH), 7.48 (1H, d, *J*=8.2 Hz, NH), 7.81 (1H, br, NH), 8.29 (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 11.4, 14.2, 15.6, 19.6, 24.75 (24.80), 28.3, 29.0, 37.8 (37.0), 39.1, 47.0 (47.1), 52.2 (51.0), 52.8, 57.7, 58.9 (59.1), 69.1, 80.1, 126.9 (127.2), 128.4 (128.6), 129.2 (129.3), 129.7 (129.4), 136.2 (135.8), 148.8, 155.8, 160.3 (160.2), 170.3 (170.6), 170.7 (171.8), 172.2 (172.0). HRMS (EI) *m/z* calcd for $C_{33}H_{47}N_5O_8S$: 673.3145. Found: 673.3124.

3.1.8. Boc-L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (20). To a solution of Boc-L-Phe-L-Pro-OMe (**8**) (222 mg, 0.59 mmol) in THF (2.4 mL) at 0°C was added 0.5N aq. LiOH (2.4 mL, 1.2 mmol). The resulting solution was stirred at 0°C for 30 min and at room temperature for 30 min. After the reaction mixture was acidified by the addition of 1 M aq. $KHSO_4$, the resulting mixture was extracted with $CHCl_3$ (×3). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to afford the crude Boc-L-Phe-L-Pro-OH. Boc-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**7**) (398 mg, 0.59 mmol) was treated with 4N HCl–dioxane (2 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude hydrochloride salt. To a solution of the above crude Boc-L-Phe-L-Pro-OH and the above crude hydrochloride salt in DMF (2 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (0.105 mL, 0.69 mmol) and triethylamine (0.18 mL, 1.30 mmol). The resulting solution was stirred at 0°C for 1.5 h and at room temperature for 13 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=1:5–0:1, EtOAc–EtOH=20:1–10:1) to afford the desired product **20** as a white solid (508 mg, 0.55 mmol, 94%): mp 124–125°C; $[\alpha]_D^{25}=-95.3$ (*c* 1.0,

$CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3496, 1645, 1559, 1455, 1246; ^1H NMR (270 MHz, DMSO-d_6) δ 0.78 (6H, br, Ile-$C_\gamma H_3$, Ile-$C_\delta H_3$), 1.00 (3H, d, J=6.3 Hz, *a*Thr-CH_3), 1.28 (9H, s, ^{t}Bu), 1.89 (7H, br, Ile-$C_\gamma H_2$, Pro-$C_\beta H_2$, Pro-$C_\delta H_2$, –OH), 2.12 (2H, br, Pro-$C_\gamma H_2$), 2.72–3.00 (4H, m, Phe-$C_\beta H_2$), 3.27–3.72 (4H, m, Pro-$C_\delta H_2$), 3.81 (3H, s, OCH_3), 4.16–4.27 (2H, m, *a*Thr-C_βH, Phe-C_αH), 4.45 (1H, br, *a*Thr-C_αH), 4.73 (1H, br, Ile-C_αH), 4.78 (1H, m, Phe-C_αH), 5.24 (1H, m, Pro-C_αH), 7.01 (1H, d, J=8.1 Hz, BocNH), 7.17–7.27 (10H, m, PhH), 7.74 (1H, d, J=8.4 Hz, NH), 7.86 (1H, d, J=8.6 Hz, NH), 8.18 (1H, d, J=7.4 Hz, NH), 8.29 (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 11.4, 15.7, 19.5 (19.2), 21.6, 24.6, 24.9, 25.2, 28.3, 31.5, 34.2, 36.7 (36.9), 38.2, 39.0, 40.5, 47.3 (47.5), 52.4 (52.5), 53.7 (53.5), 57.8 (57.4), 58.2 (58.1), 58.8, 59.4, 60.5 (60.3), 68.7, 80.8 (79.7), 126.8 (126.7), 126.9 (127.2), 127.5 (127.4), 128.4, 129.3 (128.8), 134.2, 135.5 (135.8), 146.1 (147.7), 155.1, 161.6, 168.8, 170.0, 170.4 (170.3), 171.2 (171.3), 171.6 (171.5), 172.2 (173.5). HRMS (FAB) *m/z* calcd for $C_{47}H_{67}N_7O_{10}S$ (M+H): 918.4435. Found: 918.4452.

3.1.9. Boc-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-L-Phe-L-Pro-OMe (21). To a solution of Boc-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**7**) (423 mg, 0.63 mmol) in THF (2.5 mL) at 0°C was added 0.5N aq. LiOH (2.5 mL, 1.3 mmol). The resulting solution was stirred at 0°C for 30 min and at room temperature for 30 min. After the reaction mixture was acidified by the addition of 1 M aq. $KHSO_4$, the resulting mixture was extracted with $CHCl_3$ (×3). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to afford the crude Boc-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH. Boc-L-Phe-L-Pro-OMe (**8**) (299 mg, 0.79 mmol) was treated with 4 M HCl–dioxane (2 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude hydrochloride salt. To a solution of the above crude Boc-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH and the above crude hydrochloride salt in DMF (2 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (0.11 mL, 0.72 mmol) and triethylamine (0.22 mL, 1.58 mmol). The resulting solution was stirred at 0°C for 1.5 h and at room temperature for 18 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=1:5–0:1, EtOAc–EtOH=20:1–10:1) to afford the desired product **21** as a white solid (574 mg, 0.63 mmol, 99%): mp 106–109°C; $[\alpha]_D^{24}=-87.2$ (c 1.0, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3410, 1752, 1638, 1541, 1453, 1171; ^1H NMR (270 MHz, $CDCl_3$), minor rotamer in parentheses, δ 0.90 (3H, d, J=6.3 Hz, Ile-$C_\gamma H_3$), 0.91 (3H, t, J=7.3 Hz, Ile-$C_\delta H_3$), 1.19 (1.12) (3H, d, J=6.1 Hz, *a*Thr-CH_3), 1.32–1.39 (3H, m, Ile-$C_\gamma H_2$, Ile-C_βH), 1.43 (9H, s, ^{t}Bu), 1.60–2.00 (5H, br, Pro-$C_\beta H_2$, OH), 2.12–2.26 (3H, m, Pro-$C_\gamma H_2$), 2.38 (1H, m, Pro-$C_\gamma H_2$), 2.93 (1H, d, J=7.3 Hz, Phe-$C_\beta H_2$), 3.02–3.12 (3H, m, Phe-$C_\beta H_2$), 3.17–3.25 (3H, m, Pro-$C_\delta H_2$), 3.39 (1H, m, Pro-$C_\delta H_2$), 3.75 (3.66) (3H, s, OCH_3), 3.96 (1H, m, Phe-C_αH), 4.12 (1H, q, J=7.1 Hz, *a*Thr-C_βH), 4.23–4.42 (2H, m, *a*Thr-C_αH, Ile-C_αH), 4.88 (1H, m, Phe-C_αH), 5.06–5.14 (2H, m, Pro-C_αH), 5.41 (1H, d, J=5.6 Hz, BocNH), 6.81 (1H, d, J=8.1 Hz, NH), 6.90 (6.95) (1H, d, J=7.7 Hz, NH), 7.05 (2H, m, PhH), 7.19 (2H, m, PhH), 7.29–7.35 (6H, m, PhH), 7.91 (1H, d, J=4.5 Hz, NH), 7.97 (8.01) (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 11.4, 15.7, 16.2, 19.6, 24.8, 24.9, 28.4, 29.1, 30.9, 37.1 (37.4), 37.9, 39.2, 47.0 (47.2), 52.2 (52.0), 52.9 (52.8), 58.2 (57.8), 59.0 (59.1), 59.5, 69.1, 77.2 (80.0), 123.7 (123.6), 126.8, 127.1, 128.3 (128.4), 128.5 (128.8), 129.1 (129.3), 129.6, 135.4, 136.0, 148.7, 155.7, 160.2, 164.5, 169.8, 170.1, 170.5 (170.4), 171.9 (171.7), 172.0 (172.2). HRMS (FAB) *m/z* calcd for $C_{47}H_{67}N_7O_{10}S$ (M+H): 918.4435. Found: 918.4456.

3.1.10. Cyclo[L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz] (4). To a solution of Boc-L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**18**) (93 mg, 0.10 mmol) in THF (0.4 mL) at 0°C was added 0.5 M aq. LiOH (0.4 mL, 0.2 mmol). The resulting solution was stirred at 0°C for 30 min and at room temperature for 30 min. After the reaction mixture was acidified by the addition of 1 M aq. $KHSO_4$, the resulting mixture was extracted with $CHCl_3$ (×3). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to afford the crude Boc-L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH. This crude heptapeptide was treated with 4 M HCl–dioxane (1 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude HCl-H-L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH. To a solution of the above crude HCl–H-L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH in DMF (51 mL) at 0°C were successively added dropwise pentafluorophenyl diphenylphosphinate (59 mg, 0.15 mmol) and *N*,*N*-diisopropylethylamine (0.071 mL, 0.41 mmol). The resulting solution was stirred at room temperature for 50 h, and concentrated. The residue was purified by flash chromatography (BW-820MH, EtOAc–EtOH=20:1–10:1) to afford the desired product **4** as a white solid (50 mg, 0.065 mmol, 63%): mp 168–169°C; $[\alpha]_D^{27}=-82.7$ (c 1.0, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3453, 1636, 1541, 1497, 1451; ^1H NMR (270 MHz, CD_3OD) δ 0.89 (3H, d, J=7.3 Hz, Ile-$C_\gamma H_3$), 0.96 (3H, t, J=6.6 Hz, Ile-$C_\delta H_3$), 1.14–1.23 (2H, m, Ile-$C_\gamma H_2$), 1.28 (3H, d, J=6.1 Hz, *a*Thr-CH_3), 1.46–1.70 (2H, m, Pro-$C_\gamma H_2$), 1.89–2.37 (7H, m, Ile-$C_\beta H_2$, Pro-$C_\beta H_2$, Pro-$C_\gamma H_2$), 3.13 (2H, d, J=7.1 Hz, Phe-$C_\beta H_2$), 3.46 (2H, brd, J=10.7 Hz, Phe-$C_\beta H_2$), 3.56 (2H, m, Pro-$C_\delta H_2$), 3.85–4.05 (2H, m, Pro-$C_\delta H_2$), 4.21–4.27 (1H, m, *a*Thr-C_βH), 4.38 (1H, d, J=5.1 Hz, *a*Thr-C_αH), 4.53 (1H, m, Pro-C_αH), 4.75 (1H, m, Ile-C_αH), 4.96–5.14 (3H, m, Phe-C_αH, Pro-C_αH), 7.01 (1H, d, J=8.1 Hz, BocNH), 7.20–7.29 (10H, m, PhH), 8.03 (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 11.8, 16.2, 20.1, 21.1, 24.3, 25.8, 29.7, 33.9, 36.8, 38.1, 38.3, 46.0, 47.4, 52.9, 53.8, 57.4, 58.1, 59.9, 62.8, 70.4, 123.9, 127.1, 127.7, 128.6, 129.1, 129.1, 131.1, 135.0, 136.5, 150.3, 160.9, 168.2, 169.7, 170.7, 170.9, 171.9. HRMS (FAB) *m/z* calcd for $C_{41}H_{52}N_7O_7S$ (M+H): 786.3649. Found: 786.3672.

3.1.11. *cis*,*cis*-Ceratospongamide (1). To a solution of cyclo[L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz] (**4**) (50 mg, 0.064 mmol) in CH_2Cl_2 (0.6 mL) at −30°C was added Deoxo-fluor (0.024 mL, 0.13 mL). The reaction mixture was stirred at −30 to −20°C for 20 min. The reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$, warmed to room temperature, and extracted with $CHCl_3$.

The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, EtOAc–EtOH=1:0–20:1) to afford the desired product **1** as colorless crystals (27 mg, 0.035 mmol, 55%): mp 236–239°C (benzene–*n*-pentane); $[\alpha]_D^{26}=-136.5$ (*c* 0.23, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3475, 1628, 1541, 1449; ^1H NMR (500 MHz, $CDCl_3$) δ 0.83 (3H, d, *J*=6.9 Hz, Ile-$C_\gamma H_3$), 0.90 (3H, t, *J*=7.5 Hz, Ile-$C_\delta H_3$), 1.01–1.14 (2H, m, Ile-$C_\gamma H_2$, Pro-$C_\beta H_2$), 1.31 (3H, d, *J*=6.0 Hz, Me-oxz-CH_3), 1.34 (1H, m, Ile-$C_\gamma H_2$), 1.58 (1H, m, Pro-$C_\gamma H_2$), 1.73–1.79 (2H, m, Ile-$C_\beta H_2$, Pro-$C_\gamma H_2$), 1.88–1.95 (3H, m, Pro-$C_\beta H_2$, Pro-$C_\gamma H_2$), 2.03–2.14 (1H, m, Pro-$C_\beta H_2$), 2.27–2.36 (1H, m, Pro-$C_\beta H_2$), 2.85 (1H, dd, *J*=12.9, 6.6 Hz, Phe-$C_\beta H_2$), 2.89 (1H, m, Phe-$C_\beta H_2$), 3.04 (1H, dd, *J*=13.2, 8.1 Hz, Phe-$C_\beta H_2$), 3.19 (1H, dd, *J*=9.3, 1.5 Hz, Me-oxz-C_4H), 3.42–3.59 (4H, m, Pro-$C_\alpha H$, Pro-$C_\delta H_2$, Phe-$C_\beta H_2$), 3.69–3.76 (1H, m, Pro-$C_\delta H_2$), 3.93–4.01 (2H, m, Me-oxz-C_5H, Pro-$C_\delta H_2$), 4.61–4.66 (1H, m, Ile-$C_\alpha H$), 4.71–4.80 (2H, m, Phe-$C_\alpha H$), 5.17 (1H, m, Pro-$C_\alpha H$), 6.55 (1H, d, *J*=9.6 Hz, Ile-NH), 6.61 (1H, d, *J*=8.7 Hz, Phe-NH), 7.16–7.36 (10H, m, PhH), 8.04 (1H, s, Thz-H), 8.14 (1H, d, *J*=6.3 Hz, Phe-NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.0, 16.0, 21.7, 21.9, 22.3, 25.1, 31.4, 35.5, 38.6, 39.5, 41.0, 46.9, 47.1, 51.8, 52.6, 54.2, 60.0, 61.6, 74.1, 81.9, 124.6, 127.0, 127.8, 128.6, 129.4, 129.9, 129.9, 136.5, 137.3, 149.1, 159.9, 169.2, 170.0, 170.0, 170.3, 170.9, 171.6. HRMS (FAB) *m/z* calcd for $C_{41}H_{50}N_7O_6S$ (M+H): 768.3543. Found: 768.3576.

3.1.12. Total synthesis of *trans*,*trans*-[D-*a*Ile]-cerato-spongamide (3). Boc-D-aIle-L-aThr-L-Phe-L-Pro-Thz-OMe (23). Boc-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**18**) (343 mg, 0.746 mmol) was treated with MeOH (1 mL)–4 M HCl–dioxane (8 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude hydrochloride salt. To a solution of Boc-D-*a*Ile-OH (**22**) (228 mg, 0.762 mmol) and the above crude hydrochloride salt in DMF (2.5 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (0.14 mL, 0.922 mmol) and triethylamine (0.23 mL, 1.66 mmol). The resulting solution was stirred at 0°C for 1 h and at room temperature for 14 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=1:5–0:1, EtOAc–EtOH=10:1) to afford the desired product **23** as a white solid (355 mg, 0.527 mmol, 71%): mp 87–89°C; $[\alpha]_D^{26}=-66.9$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} (cm^{-1}) 3282, 1738, 1726, 1636, 1538, 1505, 1455, 1244; ^1H NMR (270 MHz, DMSO-d_6, 100°C) δ 0.81 (3H, d, *J*=6.8 Hz, D-*a*Ile-$C_\gamma H_3$), 0.88 (3H, t, *J*=7.3 Hz, D-*a*Ile-$C_\delta H_3$), 1.05 (3H, d, *J*=6.1 Hz, *a*Thr-CH_3), 1.10–1.36 (4H, m, Ile-$C_\gamma H_2$, Pro-$C_\beta H_2$), 1.41 (9H, s, ^{t}Bu), 1.82 (1H, m, D-*a*Ile-$C_\beta H_2$), 1.96 (2H, br, Pro-$C_\gamma H_2$), 2.18 (1H, br, –OH), 2.49 (2H, m, Phe-$C_\beta H_2$), 3.46 (1H, br, Pro-$C_\delta H_2$), 3.75 (1H, br, Pro-$C_\delta H_2$), 3.85 (3H, s, OCH_3), 4.00–4.05 (1H, m, *a*Thr-$C_\beta H$), 4.26 (1H, m, *a*Thr-$C_\alpha H$), 4.44 (1H, br, D-*a*Ile-$C_\alpha H$), 4.81 (1H, br, Phe-$C_\alpha H$), 5.21 (1H, br, Pro-$C_\alpha H$), 6.21 (1H, d, *J*=8.6 Hz, BocNH), 7.19 (5H, br, PhH), 7.52 (1H, d, *J*=8.4 Hz, NH), 7.82 (1H, br, NH), 8.30 (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 11.7, 14.2 (14.3), 19.5 (19.3), 24.6, 26.3, 28.3, 31.3, 37.4 (37.3), 47.2 (46.3), 52.3 (52.4), 52.8 (53.0), 57.7 (57.5), 58.7 (58.1), 69.0, 77.2 (79.9), 126.9 (127.2), 127.5 (127.4), 128.4 (128.3), 128.7, 129.2 (129.3), 135.6 (135.5), 146.2 (147.6), 155.6, 161.5 (161.3), 170.3 (169.0), 170.5, 171.9 (172.4), 172.1 (173.5). HRMS (EI) *m/z* calcd for $C_{33}H_{47}N_5O_8S$: 673.3145. Found: 673.3120.

3.1.13. Boc-L-Phe-L-Pro-D-aIle-L-aThr-L-Phe-L-Pro-Thz-OMe (24). To a solution of Boc-L-Phe-L-Pro-OMe (**8**) (182 mg, 0.48 mmol) in THF (1.2 mL) at 0°C was added 1 M aq. NaOH (0.58 mL, 0.58 mmol). The resulting solution was stirred at 0°C for 1 h and at room temperature for 1.5 h. After the reaction mixture was acidified by the addition of 1 M aq. $KHSO_4$, the resulting mixture was extracted with $CHCl_3$ (×3). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to afford the crude Boc-L-Phe-L-Pro-OH. Boc-D-*a*Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**23**) (303 mg, 0.45 mmol) was treated with 4 M HCl–dioxane (2 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude hydrochloride salt. To a solution of the above crude Boc-L-Phe-L-Pro-OH and the above crude hydrochloride salt in DMF (1.7 mL) at 0°C were successively added dropwise diethyl phosphorocyanidate (0.083 mL, 0.55 mmol) and triethylamine (0.15 mL, 1.04 mmol). The resulting solution was stirred at 0°C for 1 h and at room temperature for 12 h. After dilution with EtOAc, the mixture was washed with 1 M aq. $KHSO_4$, water, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, hexane–EtOAc=1:5–0:1, EtOAc–EtOH=20:1–10:1) to afford the desired product **24** as a white solid (408 mg, 0.44 mmol, 99%): mp 66–67°C; $[\alpha]_D^{26}=-23.7$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3346, 1717, 1638, 1522, 1453, 1246; ^1H NMR (270 MHz, DMSO-d_6) δ 0.75 (3H, d, *J*=6.6 Hz, D-*a*Ile-$C_\gamma H_3$), 0.83 (3H, t, *J*=7.4 Hz, D-*a*Ile-$C_\delta H_3$), 0.99 (3H, d, *J*=6.1 Hz, *a*Thr-CH_3), 1.16–1.30 (3H, m, D-*a*Ile-$C_\beta H$, D-*a*Ile-$C_\gamma H_2$), 1.27 (9H, s, ^{t}Bu), 1.90 (7H, br, Pro-$C_\beta H_2$, Pro-$C_\delta H_2$, –OH), 2.13 (2H, br, Pro-$C_\gamma H_2$), 2.73–2.95 (4H, m, Phe-$C_\beta H_2$), 3.26–3.73 (4H, m, Pro-$C_\delta H_2$), 3.81 (3H, s, OCH_3), 3.97–4.03 (1H, m, Pro-$C_\alpha H$), 4.11–4.17 (1H, m, Phe-$C_\alpha H$), 4.37 (1H, br, *a*Thr-$C_\beta H$), 4.49 (1H, br, *a*Thr-$C_\alpha H$), 4.74 (1H, m, D-*a*Ile-$C_\alpha H$), 4.78 (1H, m, Phe-$C_\alpha H$), 5.24 (1H, m, Pro-$C_\alpha H$), 6.98 (1H, d, *J*=7.9 Hz, BocNH), 7.17–7.27 (10H, m, PhH), 7.79 (2H, t, *J*=8.1 Hz, NH), 8.18 (1H, d, *J*=7.3 Hz, NH), 8.29 (1H, s, Thz-H); ^{13}C NMR (67.8 MHz, $CDCl_3$), minor rotamer in parentheses, δ 11.7, 14.5 (14.6), 16.0 (15.95), 19.7 (19.8), 24.5, 25.5, 26.7, 28.2 (28.1), 31.6, 35.2, 38.6 (37.8), 47.7, 47.3, 52.4 (51.9), 55.8, 58.8 (59.4), 61.4 (61.1), 65.1, 67.3, 77.1 (79.9), 126.7, 126.8, 127.3 (127.1), 128.3 (127.9), 128.4 (128.6), 129.2 (129.4), 129.6, 135.3, 135.8 (135.7), 146.3 (147.5), 155.0 (155.7), 161.5 (161.4), 168.6 (168.9), 169.3, 170.7 (170.8), 171.3, 171.8, 172.2 (173.1). HRMS (FAB) *m/z* calcd for $C_{47}H_{67}N_7O_{10}S$ (M+H): 918.4435. Found: 918.4464.

3.1.14. Cyclo[L-Phe-L-Pro-D-aIle-L-aThr-L-Phe-L-Pro-Thz] (25). To a solution of Boc-L-Phe-L-Pro-L-Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OMe (**24**) (189 mg, 0.21 mmol) in THF (0.82 mL) at 0°C was added 0.5 M aq. LiOH (0.82 mL, 0.41 mmol). The resulting solution was stirred at 0°C for

30 min and at room temperature for 1 h. After the reaction mixture was acidified by the addition of 1 M aq. $KHSO_4$, the resulting mixture was extracted with $CHCl_3$ (×3). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to afford the crude Boc-L-Phe-L-Pro-D-*a*Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH. This heptapeptide was treated with 4 M HCl–dioxane (1 mL)–THF (1 mL) at 0°C. The mixture was stirred at room temperature for 20 min. The mixture was concentrated and dried to afford the crude HCl–H-L-Phe-L-Pro-D-*a*Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH. To a solution of the above crude HCl–H-L-Phe-L-Pro-D-*a*Ile-L-*a*Thr-L-Phe-L-Pro-Thz-OH in DMF (103 mL) at 0°C were successively added dropwise pentafluorophenyl diphenylphosphinate (119 mg, 0.31 mmol) and *N*,*N*-diisopropylethylamine (0.15 mL, 0.83 mmol). The resulting solution was stirred at room temperature for 42.5 h, and concentrated. The residue was purified by flash chromatography (BW-820MH, EtOAc–EtOH=1:0–20:1–10:1) to afford the desired product **25** as a white powder (116 mg, 0.15 mmol, 72%): mp 188–191°C; $[\alpha]_D^{26}=-58.3$ (*c* 0.48, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3475, 1630, 1541, 1453; ^1H NMR (270 MHz, CD_3OD) δ 0.91 (3H, d, *J*=7.3 Hz, D-*a*Ile-$C_\gamma H_3$), 0.94 (3H, t, *J*=6.6 Hz, D-*a*Ile-$C_\delta H_3$), 1.25 (3H, d, *J*=6.1 Hz, *a*Thr-CH_3), 1.29–1.38 (2H, m, D-*a*Ile-$C_\gamma H_2$), 1.60 (2H, br, Pro-$C_\beta H_2$), 1.88–2.00 (6H, m, D-*a*Ile-$C_\beta H$, Pro-$C_\beta H_2$, Pro-$C_\gamma H_2$), 2.28 (1H, m, Pro-$C_\beta H_2$), 2.81–2.94 (3H, m, Pro-$C_\delta H_2$), 3.10 (1H, dd, *J*=13.5, 7.3 Hz, Phe-$C_\beta H_2$), 3.24 (1H, dd, *J*=12.9, 4.9 Hz, Phe-$C_\beta H_2$), 3.47 (2H, br, Phe-$C_\beta H_2$), 3.69 (1H, br, Pro-$C_\delta H_2$), 4.10 (1H, m, *a*Thr-$C_\beta H$), 4.35 (1H, d, *J*=5.1 Hz, *a*Thr-$C_\alpha H$), 4.46–4.57 (3H, m, Pro-$C_\alpha H$, D-*a*Ile-$C_\alpha H$, Phe-$C_\alpha H$), 4.97 (2H, m, Pro-$C_\alpha H$, Phe-$C_\alpha H$), 7.28–7.33 (10H, m, PhH), 7.75 (1H, d, *J*=8.7 Hz, NH), 8.12 (1H, s, Thz-H), 8.25 (1H, d, *J*=8.4 Hz, NH), 8.69 (1H, d, *J*=9.6 Hz), 9.41 (1H, d, *J*=7.1 Hz, NH); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 11.9, 14.6, 20.6, 21.4, 25.5, 26.8, 29.0, 33.2, 34.8, 39.7, 40.4, 45.3, 47.3, 52.4, 53.9, 56.4, 57.4, 58.1, 61.7, 70.4, 127.0, 127.1, 128.6, 128.7, 129.5, 129.5, 135.6, 136.3, 150.0, 160.1, 169.4, 170.3, 170.4, 170.5, 171.4, 171.7. HRMS (FAB) *m*/*z* calcd for $C_{41}H_{52}N_7O_7S$ (M+H): 786.3649. Found: 786.3661.

3.1.15. *trans*,*trans*-[D-*a*Ile]-Ceratospongamide (3). To a solution of cyclo[L-Phe-L-Pro-D-*a*Ile-L-*a*Thr-L-Phe-L-Pro-Thz] (**25**) (67 mg, 0.085 mmol) in CH_2Cl_2 (0.7 mL) at −30°C was added Deoxo-fluor (0.032 mL, 0.17 mmol). The reaction mixture was stirred at −30 to −20°C for 20 min, and then additional Deoxo-fluor (0.008 mL, 0.043 mmol) was added. After 10 min, the reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$, warmed to room temperature, and extracted with $CHCl_3$. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (BW-820MH, EtOAc–EtOH=1:0–20:1–8:1) to afford the desired product **3** as a colorless amorphous powder (37 mg, 0.047 mmol, 57%): mp 153–155°C; $[\alpha]_D^{25}=-38.8$ (*c* 0.47, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}) 3475, 1638, 1541, 1449; ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, d, *J*=6.7 Hz, D-*a*Ile-$C_\gamma H_3$), 1.01 (3H, t, *J*=7.3 Hz, D-*a*Ile-$C_\delta H_3$), 1.16 (1H, m, D-*a*Ile-$C_\gamma H_2$), 1.27 (3H, d, *J*=6.1 Hz, oxz-CH_3), 1.50 (1H, m, D-*a*Ile-$C_\gamma H_2$), 1.79 (1H, m, Pro-$C_\gamma H_2$), 1.92 (1H, m, Ile-$C_\beta H$), 1.98 (1H, m, Pro-$C_\beta H_2$), 2.04 (1H, m, Pro-$C_\gamma H_2$), 2.10 (1H, m, Pro-$C_\gamma H_2$), 2.19 (1H, m, Pro-$C_\beta H_2$), 2.22 (1H, m, Pro-$C_\gamma H_2$), 2.27 (1H, m, Pro-$C_\beta H_2$), 2.49 (1H, m, Pro-$C_\beta H_2$), 2.89 (1H, dd, *J*=13.7, 6.4 Hz, Phe-$C_\beta H_2$), 2.92 (1H, m, Pro-$C_\delta H_2$), 2.99 (1H, dd, *J*=13.7, 7.3 Hz, Phe-$C_\beta H_2$), 3.20 (1H, dd, *J*=13.4, 3.1 Hz, Phe-$C_\beta H_2$), 3.48 (1H, t, *J*=13.1 Hz, Phe-$C_\beta H_2$), 3.49 (1H, m, Pro-$C_\delta H_2$), 3.92 (2H, m, Pro-$C_\delta H_2$), 3.94 (1H, d, *J*=6.7 Hz, oxz-C_4H), 3.99 (1H, m, oxz-C_5H), 4.31 (1H, t, *J*=7.6 Hz, Pro-$C_\alpha H$), 4.95–5.00 (2H, m, Phe-$C_\alpha H$, D-*a*Ile-$C_\alpha H$), 5.09 (1H, dt, *J*=10.4, 3.1 Hz, Phe-$C_\alpha H$), 5.55 (1H, dd, *J*=7.9, 3.1 Hz, Pro-$C_\alpha H$), 6.80 (1H, d, *J*=8.6 Hz, D-*a*Ile-NH), 7.03 (1H, t, *J*=7.3 Hz, Phe-H), 7.13–7.36 (10 H, m, Phe-H, Phe-NH), 7.96 (1H, s, Thz-H), 8.48 (1H, d, *J*=8.6 Hz, Phe-NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.0, 13.8, 22.0, 25.0, 25.6, 26.3, 30.0, 32.0, 38.1, 38.3, 39.9, 46.5, 47.3, 50.2, 52.2, 52.9, 58.1, 61.6, 73.7, 80.8, 122.7, 126.5, 126.7, 128.1, 128.4, 129.7, 129.7, 135.8, 137.1, 149.1, 160.1, 168.4, 169.3, 170.1, 170.1, 170.4, 172.1. ^1H NMR and ^{13}C NMR spectral data in DMSO-d_6 are shown in Table 2. HRMS (FAB) *m*/*z* calcd for $C_{41}H_{50}N_7O_6S$ (M+H): 768.3543. Found: 768.3550.

3.2. Thermal isomerization

3.2.1. Thermal conversion of 1 to 3. Synthetic *cis*,*cis*-ceratospongamide (**1**, 3.2 mg, 0.0041 mmol) was dissolved in DMSO (3 mL) and heated to 175°C. After 30 min, the DMSO was removed under reduced pressure and the residue was purified by flash chromatography (BW-820MH, EtOAc–EtOH=20:1–5:1) to afford *trans*,*trans*-[D-*a*Ile]-ceratospongamide (**3**, 1 mg, 0.0013 mmol, 31%).

3.2.2. ^1H NMR monitoring of thermal conversion of 1 under neutral or acidic condition. A solution of *cis*,*cis*-ceratospongamide (**1**, 2 mg, 0.0026 mmol) in DMSO-d_6 (0.5 mL) was treated with PPTs (2 mg, 0.0080 mmol) and warmed to 100°C. The thermal conversion was monitored by 270 MHz ^1H NMR.

3.3. X-Ray crystal analysis of 1

Single crystals of ceratospongamide (**1**) were grown from a chloroform–heptane mixture as colorless plates by slow evaporation at room temperature. For the subsequent X-ray work, a single crystal of dimensions 0.2×0.3×0.5 mm^3 was used. A summary of crystallographic data is given in Table 5. Intensity data were collected in an ω–2θ scan mode by graphite-monochromated Cu Kα radiation (λ=1.5418 Å) on an automated AFC5R (Rigaku) diffractometer, where the backgrounds were counted for 5 s at both extremes of each reflection peak. The observed intensities were corrected for the Lorentz and polarization effects, but not for the absorption effect.

The crystal structures were solved by the direct method using the SHELXS97 program.[23] The reflections with $I>2\sigma(I)$ were used for the structure refinement. The refinements of non-H atoms were carried out by the full-matrix least-squares calculations on F_o^2 intensities with anisotropic thermal parameters using the program SHELXL97.[24] H atomic positions of methyl groups were found from the difference Fourier maps, and the others were geometrically located. These were treated as riding with fixed isotropic displacement parameters ($U_{iso}=1.2U_{eq}$ for the associated C or N atoms, or $U_{iso}=1.5U_{eq}$ for methyl C

Table 5. Summary of crystal data collections and structure refinement of **1**

Molecular formula	$C_{41}H_{49}N_7O_6S{\cdot}3H_2O$
Molecular weight	821.98
Crystal system	Hexagonal
Space group	$P4_12_12$
Unit cell dimensions	$a=b=12.815$ (2) Å, $c=54.358$ (9) Å, $\alpha=\beta=\gamma=90.00°$
Volume	8927 (2) Å^3
Z, molecules/unit cell	8
$F(000)$	3504
Density (calculated)	1.223 g cm^{-3}
Absorption coefficient	1.133 mm^{-1}
Index ranges	$0\leq h\leq 15$, $0\leq k\leq 10$, $0\leq l\leq 65$
No. of unique data measd	4687
No. of reflections with $I>2\sigma(I)$	2394
Refinement method	Full least-squares on F^2
Refinement weighting scheme	Calcd $w=1/[\sigma^2(F_o^2)+(0.1000P)^2]$ where $P=(F_o^2+2F_c^2)/3$
No. of variables refined	516
$R(Rw)$	0.0774 (0.2118)
$R(Rw)$ (all data)	0.1632 (0.2374)
Goodness-of-fit on F^2	1.313
Largest difference peak and hole	0.653 and −0.239 e Å^{-3}
Max and averaged shift/esd	0.041/0.002

atoms); their atomic positions were not included as variables for the refinements. The function of $\sum w(F_o^2-F_c^2)^2$ was minimized by using the weighting scheme of $w=1/[\sigma^2(F_o^2)+(0.1000P)^2]$, where $P=(F_o^2+2F_c^2)/3$. Final R [$=\sum(|F_o|-|F_c|)/\sum|F_o|$]], Rw [$=(\sum w(|F_o|-|F_c|)^2/\sum w|F_o|^2)^{1/2}$] and S [$=(\sum w(|F_o|-|F_c|)^2/(M-N))^{1/2}$, where M is the number of reflections and N is the number of variables used for the refinement] are also given in Table 5.

Supplementary data sets (tables of final atomic coordinates, anisotropic temperature factors, bond lengths, bond angles, torsion angles of non-H atoms, and the atomic coordinates of H atoms) have been deposited at the Cambridge Crystallographic Data Centre (**1**: CCDC 183755) and are available on request.

3.4. Solution conformation of 3

3.4.1. NMR spectroscopy. All NMR measurements (500 MHz for protons), except the temperature-variable experiment, were performed at 292 K. A 9 mM degassed solution of synthetic ceratospongamide (**3**) in DMSO-d_6 was used for all NMR experiments. Chemical shifts were measured relative to internal TMS at 0.0 ppm. Two-dimensional GCOSY, TOCSY and ROESY spectra were acquired in the phase-sensitive mode using standard pulse schemes. Mixing times for ROESY and TOCSY spectra were 400 and 80 ms, respectively. The time-domain matrix consisted of 128×1024 complex data points and was zero-filled to obtain a frequency domain matrix of 1024×1024 real data points. Thus, the spectra had digital resolutions of 0.4 Hz/point for the $\omega1$ and $\omega2$ directions. The $^1H-^{13}C$ shift correlations were measured by HMQC and HMBC spectroscopy using the standard pulse schemes. The respective spectra were processed on a Sun SPARC computer using Varian VNMRS software, applying normal processing functions.

3.4.2. ROE restraint molecular modeling. 3D structures of **3** that satisfy the ROE constraints of intramolecular proton pairs were constructed by dynamic SA calculations[25,26] using the Insight II/Discover 95 software.[27] This SA method consists of the following three steps. In the first step, an initial structure of the peptide is built with a completely random array of atoms. Then, a target function is determined in terms of the force constants of the covalent bond ($F_{covalent}$), repulsive van der Waals contact (F_{repul}), and interproton distance (F_{ROE}). The distance-restraint energy, F_{ROE}, is defined as the harmonic potential of

$$F_{ROE}=\begin{cases} K(r_i-r_{iu}/r_{il})^2 & \text{if } r_i>r_{iu} \text{ or } r_i<r_{il} \\ 0 & \text{if } r_{il}\leq r_i\leq r_{iu} \end{cases}$$

where r_i is the calculated distance of an inter-proton pair i, and r_{il} and r_{iu} are the lower (1.6 Å) and upper (5.0 Å) limits of its inter-proton distance, respectively. All proton–proton distances showing ROE peaks, in spite of their ROE intensities, were uniformly treated in the range of 1.6–5.0 Å. The energy term for intermolecular hydrogen bonds was not included in the model building. The force constant (K) was finally chosen to be 25.0 kcal/mol. After minimization by the steepest descents and successive conjugate gradients method, the system was simulated for 50 ps at 1000 K. The global minimum of the target function was initially investigated by substantially increasing the force constants until they regained their full values. In the second step, the temperature was decreased stepwise until 298 K. During this stage, the van der Waals repulsion terms were set such that they be predominant. In the third step, energy minimization was conducted again to refine the obtained structure. For the distance constraints that involve aromatic ring protons, that were not stereospecifically assigned, the pseudo-atom treatment was used. Calculations of other potential functions were performed according to the protocol in the Insight II/Discover software. As input data for constructing 3D structures, the restraints of ϕ torsion angles were not included in the calculations; instead, they were used as indicators to estimate the reliability of the constructed 3D structures.

3.5. Molecular dynamics simulations of 1–3

Molecular dynamics calculations were performed using the Insight II/Discover 95 program. The geometry optimization

was performed using the CVFF force field[28] for peptides and proteins. A target function was determined in terms of the force constants for the covalent bond ($F_{covalent}$) and repulsive van der Waals contact (F_{repul}). Calculations of these potential functions were performed according to the protocol in the Insight II/Discover software. The conformational energies in vacuo were minimized by the steepest descents and conjugate gradients methods. The respective systems were simulated for 100 ps by solving Newton's equation of motion. The simulation was conducted at 298 K, a time step of 1.0 fs, a temperature relaxation time of 0.02 ps, and a period of update of non-bonded atom list of 25 fs.

Acknowledgements

We thank Professor W. H. Gerwick and Mr L. T. Tan for comparison of our synthetic *cis*,*cis*-ceratospongamide and its thermal product with the natural sample. The high-resolution FAB mass spectra were measured by Dr Hideo Naoki and Mr Tsuyoshi Fujita (Suntory Institute for Bioorganic Research), to whom we are indebted. This work was financially supported in part by a Grant-in-Aid for Research in Nagoya City University (to F. Y.), the Uehara Memorial Foundation (to F. Y.), the Fujisawa Foundation (to F. Y.), and Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Kucuk, O.; Young, M. L.; Habermann, T. M.; Wolf, B. C.; Jimeno, J.; Cassileth, P. A. *Am. J. Clin. Oncol.* **2000**, *23*, 273–277.
2. Madden, T.; Tran, H. T.; Beck, D.; Huie, R.; Newman, R. A.; Pusztai, L.; Wright, J. J.; Abbruzzese, J. L. *Clin. Cancer Res.* **2000**, *6*, 1293–1301.
3. Hochster, H.; Oratz, R.; Ettinger, D. S.; Borden, E. *Invest. New Drugs* **1999**, *16*, 259–263.
4. Grabley, S.; Thiericke, R. *Adv. Biochem. Engng Biotechnol.* **1999**, *64*, 101–154.
5. (a) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115–2134. (b) Shioiri, T.; Hamada, Y. *Synlett* **2001**, 184–201.
6. Wipf, P.; Fritch, P. C.; Geib, S. J.; Sefler, A. M. *J. Am. Chem. Soc.* **1998**, *120*, 4105–4112.
7. Tan, L. T.; Williamson, R. T.; Gerwick, W. H.; Watts, K. S.; McGough, K.; Jacobs, R. *J. Org. Chem.* **2000**, *65*, 419–425.
8. A part of this work was preliminary reported. See Yokokawa, F.; Sameshima, H.; Shioiri, T. *Synlett* **2001**, *SI*, 986–988.
9. Shioiri, T.; Hamada, Y. *Studies in Natural Products Chemistry*, Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1989; Vol. 4, pp 83–110 Part C.
10. (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. *Pept. Sci.* **2000**, *1999*, 9–12. (d) Sugiyama, H.; Yokokawa, F.; Shioiri, T. *Org. Lett.* **2000**, *2*, 2149–2152.
11. Takuma, S.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147–3153, and references therein.
12. The Weinreb amide **10** is currently commercially available from Aldrich.
13. Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676–678.
14. Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203–6205.
15. (a) Chen, S.; Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711–6714. (b) Dudash, Jr. J.; 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.
16. Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831–8838.
17. Phillips, A. D.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165–1168.
18. (a) X-Ray and thermal isomerization works were presented at *36th Tennenbutsu Danwakai* (Nagoya, Japan), July 15–17, 2001 and at *18th International Congress of Heterocyclic Chemistry* (Yokohama, Japan), July 29–August 3, 2001; Abstracts; p 124. (b) Very recently, X-ray structure analysis of **1** was reported: Doi, M.; Yumiba, A.; Asano, A. *Acta Crystallogr.* **2002**, *E58*, o62–o64.
19. Ishida, T.; Tanaka, M.; Nabae, M.; Inoue, M.; Kato, S.; Hamada, Y.; Shioiri, T. *J. Org. Chem.* **1988**, *53*, 107–112.
20. Hossain, M. B.; van der Helm, D.; Antel, J.; Sheldrick, G. M.; Sanduja, S. K.; Weinheimer, A. J. *Proc. Natl Acad. Sci. USA* **1988**, *85*, 4118–4122.
21. Deng, S.; Taunton, J. *J. Am. Chem. Soc.* **2002**, *124*, 916–917.
22. Siemion, I. Z.; Wieland, T.; Pook, K. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 702–703.
23. Sheldrick, G. M. *SHELXS97. Program for the Solution of Crystal Structure*; University of Gottingen: Germany, 1997.
24. Sheldrick, G. M. *SHELXL97. Program for the Refinement of Crystal structure*; University of Gottingen: Germany, 1997.
25. Clore, G. M.; Nigles, M.; Sukumaran, D. K.; Bruenger, A. T.; Karplus, M.; Gronenborn, A. M. *EMBO J.* **1986**, *5*, 2729–2735.
26. Nilges, M.; Clore, G. M.; Gronenborn, A. M. *FEBS Lett.* **1988**, *229*, 317–324.
27. *INSIGHT/DISCOVER*; Biosym Technologies, Molecular Simulation Inc.: 9685 Scranton Road, San Diego, CA, USA.
28. Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. *Proteins Struct. Funct. Genet.* **1988**, *4*, 31–47.