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Lobocyclamide B from *Lyngbya confervoides*. Configuration and Asymmetric Synthesis of β-Hydroxy-α-amino Acids by (−)-Sparteine-Mediated Aldol Addition

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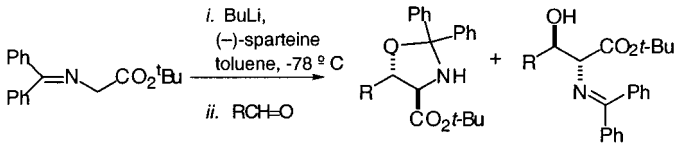
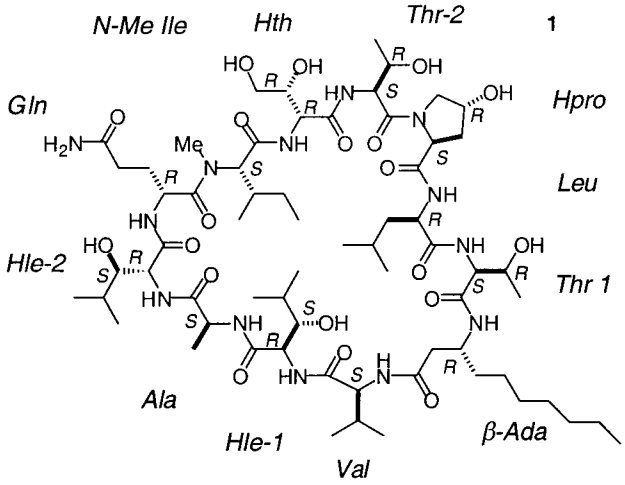
ABSTRACT



Lobocyclamide B, a cyclododecapeptide containing five β-hydroxy-α-amino acid residues, was isolated from *Lyngbya confervoides*. This is the first reported occurrence of γ-hydroxythreonine in a natural peptide. Optically active β-hydroxy-α-amino acids required for configurational analysis of the title compound were prepared using a novel (−)-sparteine-mediated asymmetric aldol addition of *N*-(diphenylmethylene)glycine *tert*-butyl ester to aldehydes. The method is general for aliphatic and aryl aldehydes and notable for operational simplicity.

Marine cyanobacterium of the genus *Lyngbya* produce a wide variety of novel cyclopeptides with potent biological activity.[1] Related genera of cyanobacteria are often associated as symbiotic assemblages with marine sponges and other invertebrates. In this report we disclose the structure and configurational assignment of a novel antifungal cyclododecapeptide, lobocyclamide B (**1**), isolated from a benthic mat of *Lyngbya confervoides* collected in the Bahamas.[2] The structure of **1** incorporates five β-hydroxy-α-amino acids, including 2 equiv of β-hydroxyleucine (β-Hle) and γ-hydroxythreonine (Hth). Although Hth has been reported as an intermediate in the biosynthesis of viamin B_6,[3] to the best of our knowledge this is the first report of its occurrence in a natural peptide. Lobocyclamide B exhibits antifungal activity against fluconazole-resistant *Candida albicans*.



Configurational assignment of **1** must meet the challenge of determining 10 asymmetric centers embodied in the

(1) For recent examples, see: (a) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*(1), 29−31. (b) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*(1), 16−20. For a recent review, see: (c) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. In *The Alkaloids*, Vol. 57; Cordell, G. A., Ed.; Academic Press: San Diego, 2001; pp 75−184.

(2) Partly presented: Ernst-Russell, M. A.; Molinski, T. F. 36th Western Regional Meeting of the American Chemical Society, San Francisco, CA, October 2000.

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β-hydroxy amino acid residues. For the purpose of determination of the absolute stereochemistry of **1**, we required a general method for preparation of several enantioenriched diastereomers of β-hydroxy-α-amino acids, in particular, β-hydroxyleucine (**2**) and γ-hydroxythreonine (**3**), as standards for chiral amino acid analysis. Recent strategies for β-hydroxy amino acid synthesis include chiral catalytic asymmetric aldol additions of benzophenone glycinates,[4] Sharpless epoxidation of allylic alcohols followed by a benzyl isocyanate addition−ring opening,[5] and Strecker synthesis of protected glyceraldehydes.[6]

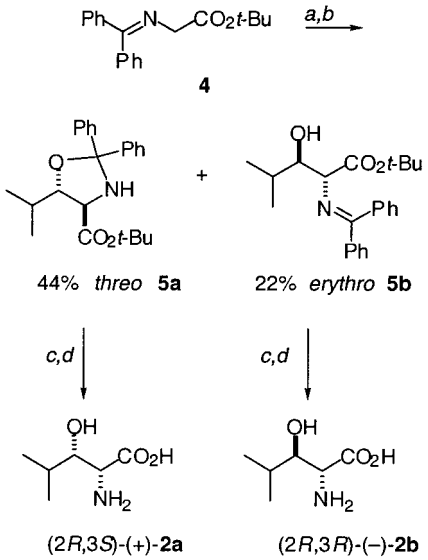
We found aldol that addition of the lithium enolate of *N*-(diphenylmethylene)glycine *tert*-butyl ester (**4**) to aldehydes, in the presence of (−)-sparteine, produced *erythro* and *threo* diastereomers of β-hydroxy-α-amino esters in good yield and each with good levels of asymmetric induction. The method is general for aliphatic and aryl aldehydes and is notable for its operational simplicity and easy transformation into optically active β-hydroxy-α-amino acid standards.

Aldol addition of the lithium enolate of **4** (−78 °C LDA, THF) to isobutyraldehyde gave a mixture of racemic *threo* oxazolidine (±)-**5a** and *erythro* imine (±)-**5b** (78%, 1:1.8 ratio, respectively).[7] When the reaction was carried out in toluene using *n*-BuLi/(−)-sparteine as base (−78 °C, 3 h, Scheme 1), compounds **5a** and **5b** were obtained in comparable yield (67%) but reversed diastereoselectivity (2:1). It is notable that deprotonation of **4** was efficient under these conditions with no detectable byproducts (NMR, <2%) arising from addition of *n*-BuLi.

Chiral HPLC analysis of purified isomers **5a** and **5b**, which were easily separated by silica chromatography, showed enantioselectivities of 60% and 56% ee, respectively.[8] The individual compounds were readily converted by hydrolysis−hydrogenolysis to (+)-(2*R*,3*S*)-β-hydroxyleucine (**2a**) and (−)-(2*R*,3*R*)-β-hydroxyleucine (**2b**), respectively (Scheme 1).[9,10]

The absolute configuration of each amino acid, and by correlation, the aldol products **5a** and **5b**, was determined by comparison of the measured optical rotations with literature values ((+)-**2a**, [α_D] +2.3°; lit.[4a] +3.5°; (−)-**2b**, [α_D] −18.5°, lit.[4a] −37°) and showed a preference for the D-amino acid configuration in each diastereomer.

Scheme 1[a]



[a] Reagents and conditions: (a) *n*-BuLi, (−)-sparteine, −78 °C, toluene; (b) isobutyraldehyde, −78 °C, 3 h; (c) TFA, H_2O, CH_2Cl_2, 25 °C; (d) 6 M HCl aq, 1 h.

The method was applied to the synthesis of γ-hydroxythreonine isomers **3a** and **3b** (Scheme 2). Aldol addition of **4** to *O*-benzylglyoxal (**6**), in the presence of (−)-sparteine/*n*-BuLi, gave oxazolidine **7a** and imine **7b** (62%, dr 2.3:1). The enantioselectivity of the latter reaction was similar to the above-described aldol addition and, again, gave amino esters of predominantly D-configuration (58% ee and 56% ee for **7a** and **7b**, respectively). Esters **7a** and **7b** were converted to the γ-hydroxythreonine diastereomers **3a** and **3b**, employing the procedure described above, and their configurations were secured by comparison of optical rotations with samples prepared using an independent route.[11]

The scope of the asymmetric aldol addition was briefly surveyed with selected alkyl and aryl aldehydes (Table 1).

(3) Hill, R. E.; Himmeldirk, K.; Kennedy, I. A.; Pauloski, R. M.; Sayer, B. G.; Wolf, E.; Spenser, I. D. *J. Biol. Chem.* **1996**, *271(48)*, 30426−30435.

(4) (a) O'Donnell, M. J. *Aldrichimica Acta* **2001**, *34*, 3−15 and references cited within. (b) Horikawa, M.; Busch-Petersen, J.; Corey, E. J. *Tetrahedron Lett.* **1999**, *40*, 3843−3846. (c) Belokon, Y. N.; Kochetkov, K. A.; Ikonnikov, N. S.; Strelkova, T. V.; Harutyunyan, S. R.; Saghiyan, A. S. *Tetrahedron: Asymmetry* **2001**, *12*, 481−485

(5) (a) Nagamitsu, T.; Sunazuka, T.; Tanaka, H.; Omura, S.; Sprengeler, P. A.; Smith, A. B., III. *J. Am. Chem. Soc.* **1996**, *118*, 3584−3590. (b) Nagamitsu, T.; Sunazuka, T.; Tanaka, H.; Omura, S.; Sprengeler, P. A.; Smith, A. B., III. *Tetrahedron Lett.* **1993**, *34*, 4447−4448.

(6) Cataviela, C.; Diaz-de-Villegas, M. D.; Galvez, J. A.; Garcia, J. I. *Tetrahedron* **1996**, *52*, 9563−9574.

(7) The *threo* aldol products were isolated consistently in the form of the cyclized oxazolidines. *Cf.* Corey, E. J.; et al.[4]

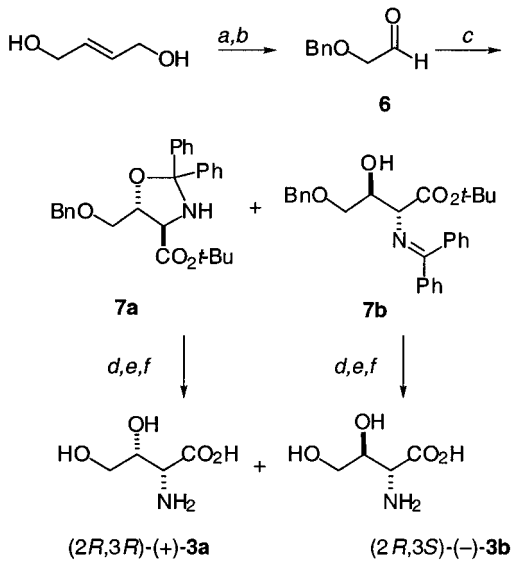
(8) Optical purities of all purified *threo* and *erythro* diastereomers were determined by chiral HPLC (Chiral Pak OD column, 1−2.5% *i*-PrOH: hexanes, UV and evaporative light scattering detectors).

(9) Jung, M. E.; Jung, Y. H. *Tetrahedron Lett* **1989**, *48*, 6637−6640.

(10) For recent syntheses of β-hydroxyleucine stereoisomers, see: (a) Cardillo, G.; Gentilucci, L.; Gianotti, M.; Tolomelli, A. *Tetrahedron: Asymmetry* **2001**, *12*, 563−569. (b) Davis, F. A.; Srirajan, V.; Fanelli, D. L.; Portonovo, P. *J. Org. Chem.* **2000**, *65*, 7663−7666. (c) Laib, T.; Chastanet, J.; Zhu, J. P. *J. Org. Chem.* **1998**, *63*, 1709−1713. (d) Laib, T.; Chastanet, J.; Zhu, J. P. *Tetrahedron Lett.* **1997**, *38*, 1771−1772. (e) Williams, L.; Zhang, Z. D.; Ding, X. B.; Joullie, M. M. *Tetrahedron Lett.* **1995**, *36*, 7031−7034. (f) Yadav, J. S.; Chandrasekhar, S.; Reddy, Y. R.; Rao, A. V. R. *Tetrahedron* **1995**, *51*, 2749−2754. (g) Hale, K. J.; Manaviazar, S.; Delisser, V. M. *Tetrahedron* **1994**, *50*, 9181−9188. (h) Corey, E. J.; Lee, D. H.; Choi, S. Y. *Tetrahedron Lett.* **1992**, *33*, 6735−6738. (i) Caldwell, C. G.; Bondy, S. S. *Synthesis* **1990**, 34−36. (j) Jung, M. E.; Jung, Y. H. *Tetrahedron Lett.* **1989**, *30*, 6637−6640.

(11) The (2*S*,3*S*)-L-*threo* and (2*R*,3*S*)-D-*erythro* β-hydroxythreonines were synthesised as follows. Strecker synthesis with 2,3-isopropylidene-D-glyceraldehyde and 9-aminofluorene (KCN, $NaHSO_3$, MeOH aq) under thermodynamic conditions (Inaba, T.; Fujita, M.; Ogura, K. *J. Org. Chem.* **1991**, *56*, 1274−1279) gave a mixture of (2*S*,3*R*)-(−)-2-*N*-(9′-aminofluorenyl)-3,4-*O*-isopropylidene-3,4-dihydroxybutyronitrile and the corresponding (2*R*,3*R*)-isomer (81%, 3:1 ratio, respectively) which was separated by normal phase HPLC (2.5% *i*-PrOH:hexane). Separate acid hydrolysis of the aminonitriles (1:1 HCl:AcOH, rt) followed by hydrogenolysis (H_2,1 atm, $Pd(OH)_2$, MeOH) gave the γ-hydroxythreonine diastereomers (2*S*,3*S*)-(−)-2-amino-3,4-dihydroxybutyric acid and (2*R*,3*S*)-(+)-2-amino-3,4-dihydroxybutyric acid, respectively. For a synthesis of the enantiomer of the latter compound, see: Pirrung, M. C.; Nunn, D. S.; McPhail, A. T.; Mitchell, R. E. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2095−2098. For the definitive configurational assignments of β-hydroxythreonine stereoisomers, see: Hamel, E. E.; Painter, E. P. *J. Am. Chem. Soc.* **1953**, *75*, 1362−1368. Niemann, C.; Nichols, P. L. *J Biol. Chem.* **1942**, *143*, 191−199.

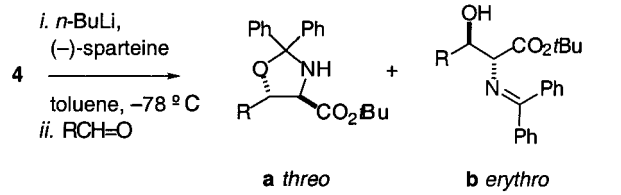
Scheme 2[a]



[a] Reagents and conditions: (a) NaH, BnBr, DMF, 85%; (b) O_3, MeOH, −78 °C, 82% yield; (c) *n*-BuLi, (−)-sparteine, **4**, toluene, −78 °C; (d) TFA, H_2O, CH_2Cl_2:THF (1:1), 1 h, 94%; (e) 6M HCl, 106 °C, 1 h, 87%; (f) H_2, Pd(C) 10%, MeOH, 85%.

The yields of products **8**−**12** derived from aryl aldehydes (52−75%) were comparable to those of **5** and **7**. Stereochemical analysis revealed a consistent preference for the *threo* diastereomer with a selectivity of ∼2:1 with the exceptions of cyclohexanecarboxaldehyde (entry 3) and *n*-heptaldehyde (entry 4) which lacked diastereoselectivity (dr 1:1). Enantiomeric excesses of aldol products **10**−**12** derived from aryl aldehydes were consistently in the range of 50−56% ee and somewhat lower for other alkanals (entries 3 and 4; 39−49% ee). No significant differences in % ee's were observed between paired *threo*−*erythro* isomers.

Table 1. (−)-Sparteine−*n*-BuLi-Mediated Aldol Additions of **4**[a]



entry	R	product	% yield[b]	time (h)	**a**:**b**	% ee of **a**	% ee of **b**
1	*i*-Pr	**5**	68	3	2:1	60	56
2	$BnOCH_2$	**7**	62	2.5	2.3:1	58	56
3	*c*-C_6H_{11}	**8**	63	5	1:1	42	39
4	*n*-C_6H_{13}	**9**	52	5	1:1	45	49
5	Ph	**10**	71	2.5	1.9:1	57	56
6	2-naphthyl	**11**	69	6	3:1	52	55
7	2-furyl	**12**	75	4	2.5:1	53	50

[a] **General Procedure:** Freshly distilled (−)-sparteine was stirred with solution of *n*-BuLi (1.2 equiv) in toluene at −78 °C (45 min) followed by addition of a solution of **4** (1.0 equiv) in toluene. After stirring for 30 min at −78 °C, the heterogeneous mixture was treated with a solution of aldehyde (2.0 equiv) in toluene and further stirred for the requisite time before work up in the usual manner. See Supporting Information for full details and stereochemical analysis. [b] Yields are for combined diastereomers, **a** + **b**. For typical reactions, unreacted **4** (∼10−20%, NMR) was observed.

Although several asymmetric (−)-sparteine-mediated reactions have been rationalized by mechanisms involving enantioselective removal of enantiotopic protons and alkylation of the resultant "chiral" sp^3 hybridized anions,[12−14]this mechanism seems unlikely in the present case as a stabilized enolate is generated from **4** and expected to adopt planar sp^2 enolate geometry. The low polarity of the solvent and ability of C_2 symmetric (−)-sparteine to coordinate the lithium counterion suggests another mechanism based on π-face selection mediated by a complex chiral amine-coordinated lithium enolate rather than a chiral base mechanism.[15] Such an alternate reaction pathway is consistent with the observed reversal of diastereoselectivity in the aldol addition of lithium enolate of **4** in the presence of (−)-sparteine, together with induction of a consistent level of asymmetry in the product that is only weakly dependent upon the structure of the aldehyde.[16] A structure for such a putative complex chiral enolate is speculative at this point, and a detailed analysis of the reaction mechanism is beyond the scope of this work.

Procurement of optically pure enantiomers of β-aminodecanoic acid (β-Ada, **13**) allowed completion of configurational analysis of **1** (Scheme 3). Michael addition of phthalimide to the chiral 2-decenoate menthyl ester **14** (DBU, DMF, 110 °C) gave β-phthalimido esters (+)-**15a** and (−)-**15b** (65% combined yield, dr 1.2), which were separated by HPLC.[17] Separate hydrazinolysis of (+)-**15a** and (−)-**15b** followed by saponification (2 M NaOH aq) gave *S*-(+)-**13a** and *R*-(−)-**13b**, respectively (∼80% yield, two steps).[18]

The absolute stereochemistry of **1** was determined by a combination of chiral HPLC and Marfey's methods.[19] The

(12) Derdau, V.; Snieckus, V. *J. Org. Chem.* **2001**, *66*, 1992−1998.

(13) Kim, B. J.; Park, Y. S.; Beak, P. *J. Org. Chem.* **1999**, *64*, 1705−1708 and references cited within. For a review of enantioselective deprotonations using chiral bases, see: O'Brien, P. *J. Chem. Soc., Perkin Trans.* **1998**, 1439.

(14) Deiters, A.; Hoppe, D. *J. Org. Chem.* **2001**, *66*, 2842−2849. The use of (−)-sparteine in $TiCl_4$-promoted aldol reactions of chiral *N*-acyloxazolidinethiones has been reported (Crimmins, M. T.; King, B. W.; Tabet, E. A.; Chaudhary, K. *J. Org. Chem.* **2001**, *66*, 894−902). In view of the fact that similar, very high diastereoselectivities were obtained under these highly coordinating conditions, even when achiral *tert*-amines were substituted for (−)-sparteine, the role of the chiral diamine in enantiodifferentiation is somewhat masked.

(15) For enantioselective additions of aggregates of organolithiums/chiral aminoalkoxides to aldehydes and ketones, see: Briggs, T. F.; Winemiller, M. D.; Xiang, B.; Collum, D. B. *J. Org. Chem.* **2001**, *66*(19), 6291−6298 and references cited within.

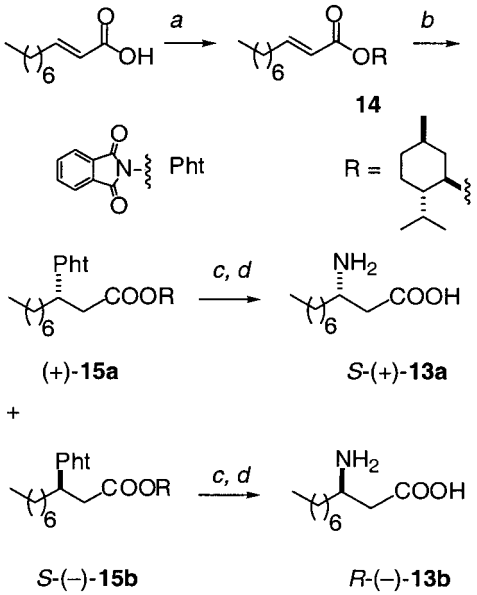
(16) Since the aldol reaction mixtures were observed to be heterogeneous and contained sparingly soluble white precipitates, we cannot discount the possibility that reactions occur at the surface of heterogeneous chiral aggregates which influence the stereochemical outcome.

(17) Normal phase HPLC(10 × 250 mm, 0.5% *i*-PrOH:hexane, 2.0 mL/min).

(18) For an alternate five-step synthesis of (*S*)-*N*-BOC-β-aminodecanoic acid from nonanoyl chloride, see: Expósito, A.; Fernández-Suárez, M.; Iglesias, T.; Muñoz, L.; Riguera, R. *J. Org. Chem.* **2001**, *66*, 4206−4213.

(19) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596. Briefly, amino acid standards and the crude hydrolyzate of **1** (6 M HCl, 110 °C, 16 h) were derivatized using (2,4-dinitro-5-fluorophenylamino)-L-alaninamide. HPLC retention times were measured under standard conditions (4.6 × 250

Scheme 3[a]



[a] Reagents and conditions: (a) (+)-menthol, DCC, DMAP, CH_2Cl_2. 72%; (b) phthalimide, DBU, DMF, 120 °C, 65%; (c) $NH_2NH_2 \cdot H_2O$, EtOH; (d) 2 M NaOH aq, 1 h, then H^+, ~80% for 2 steps.

results showed that both β-hydroxyleucine residues in **1** have the *threo*-(2*R*,3*S*) configuration while the γ-hydroxythreonine has the *threo*-(2*R*,3*R*) configuration. The rare β-amino-decanoic acid residue was found to have the 3*R* configuration, consistent with the configurations of β-aminooctanoic[20] and β-aminodecanoic acids found in related lipopeptides.[21,22]

In conclusion, a simple, practical method has been developed for synthesis of β-hydroxy-α-amino acids and applied to complete determination of configuration of the novel peptide, lobacyclamide B (**1**). The method provides facile access to both respective enantioenriched diastereomers each amino acid required as standards in chiral amino acid analysis.

Acknowledgment. We thank Michael Ernst-Russell for isolation of **1** and preparation of 2-decenoic acid, Karen Smith for the Strecker synthesis of Hth precursors, Valerie Paul, (University of Guam) for identification of *L. confervoides*, Joe Pawlik (UNC Wilmington), the captain and crew of the *R/V Seward Johnson* for ship support, and the Government of the Bahamas for permission to collect. The 400 MHz NMR instrument was partially funded by the NSF (CHE-9808183). Ship-time on the *R/V Seward Johnson* was funded by NSF (OCE-9711255 to J. Pawlik). This research was supported by the NIH (AI 39987 to T.F.M.).

Supporting Information Available: Experimental procedures for synthesis and full characterization of (±)-**2a**, (±)-**2b**, and optically active isomers, **a** and **b**, of **5**, **7**−**13**, and **15** and a chiral HPLC chromatogram of **5a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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mm C_{18} column; gradient of 20:40 → 60:40 CH_3CN:50 mM triethylammonium phosphate, aq, 1.0 mL/min). To obtain the retention times of *ent*-**2a**,**2b** and *ent*-**3a**,**3b** the amino acids **2a**,**2b** and **3a**,**3b** were reacted with the "*ent*-" Marfey's reagent [(2,4-dinitro-5-fluorophenylamino)-D-alaninamide], prepared from D-alaninamide according to Marfey. L-Valine and D-glutamine (as D-glutamate) were determined separately using chiral HPLC (D-penicillamine-based column).

(20) Gerwick, W. H.; Jiang, Z. D.; Agarwal, S. K.; Farmer, B. T. *Tetrahedron* **1992**, *48*, 2313−2324.

(21) (a) Bonnard, I.; Rolland, M.; Francisco, C.; Benaigs, B. *Lett. Pept. Sci.* **1997**, *4*, 289−292. (b) Frankmölle, W. P.; Larsen, L. K.; Caplan, F. R.; Patterson, G. M. L.; Knübel, G.; Levine, I. A.; Moore, R. E. *J. Antibiot.* **1992**, *45*, 1451−1457.

(22) The remainder of the amino acid residues in **1** were determined by Marfey's procedure as L-Ala, *N*-Me-L-Ile, *allo*-L-Thr (×2), and *trans*-L-Hpro using commercially available standards.