## NATURAL PRODUCTS

# Revised Structure of Cyclolithistide A, a Cyclic Depsipeptide from the Marine Sponge *Discodermia japonica*

Hiroki Tajima,<sup>†</sup> Toshiyuki Wakimoto,<sup>\*,†</sup> Kentaro Takada,<sup>‡</sup> Yuji Ise,<sup>§</sup> and Ikuro Abe<sup>\*,†</sup>

<sup>†</sup>Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan <sup>‡</sup>Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan <sup>§</sup>Misaki Marine Biological Station, Graduate School of Science, The University of Tokyo, Miura, Kanagawa, 238-0225, Japan

**Supporting Information** 

**ABSTRACT:** A cyclic peptide was isolated from the deep-sea marine sponge *Discodermia japonica*, and its NMR spectroscopic data were identical to those reported for cyclolithistide A, a known antifungal depsipeptide. However, the interresidue HMBC correlations suggested that the amino acid sequence was different from that of the original structure. Moreover, chiral-phase GC-MS, combined with Marfey's analysis, indicated that the absolute configurations of three amino acids were also antipodal. Here, we propose the revised structure of cyclolithistide A and address the configuration of the previously unassigned 4-amino-3,5-dihydroxyhexanoic acid (Adha) moiety.

igcap tructurally complicated secondary metabolites having a Variety of biological activities are often isolated from the lithistid sponges, particularly from the Theonella and Discodermia genera. These compounds include polyketides such as the swinholides<sup>1</sup> from *Theonella* sp. and discodermolide<sup>2</sup> from Discodermia dissoluta and polypeptides such as the theonellamides<sup>3</sup> from Theonella sp. and the discodermins<sup>4</sup> from Discodermia kiiensis. We recently reported two cytotoxic cyclic peptides, calyxamides A and B,<sup>5</sup> from Discodermia calyx. Their structures were closely related to those of the keramamides isolated from the *Theonella* genus.<sup>6</sup> The structural resemblance between the secondary metabolites from Theonella and Discodermia sponges has been reported for cyclic peptides and polyketides.<sup>7</sup> Likewise, in this study, the chemical investigation of Japanese Discodermia japonica revealed that the major metabolite of the sponge was a depsipeptide, which was originally isolated from the Indonesian Theonella swinhoei. However, we discovered inconsistencies between the NMR data and the original structure reported in 1998.8 We now present a reinvestigation of the structure of cyclolithistide A.

The sponge *D. japonica* was collected at a depth of 200 m in Sagami Bay, Japan. The MeOH extract of the sponge (200 g, wet weight) was partitioned between EtOAc and  $H_2O$ , and the organic layer was subjected to chromatography on a silica gel column to yield 1 as an amorphous, white solid (160.1 mg, 0.080% wet weight).

The molecular formula was established as  $C_{54}H_{86}ClN_{11}O_{15}$  by positive ion HRFABMS. The <sup>1</sup>H NMR spectrum in CD<sub>3</sub>OD showed  $\alpha$ -protons between 3.5 and 4.5 ppm, as well as amide protons between 7.0 and 9.0 ppm, which were detectable for several hours even in CD<sub>3</sub>OD. Interpretation of the 1D and 2D NMR data including COSY, HMQC, and HMBC in CD<sub>3</sub>OD



revealed the amino acid composition, including Ala, Phe, Thr, Gln, norvaline (norVal), Gly, and two Leu residues. An intense HMBC correlation between an N-methyl singlet ( $\delta_{\rm H}$  3.05) and an  $\alpha$ -carbon of Leu ( $\delta_{\rm C}$  65.2) suggested that one of the two leucines was N-methylated (N-MeLeu). The other Leu turned out to be N-formylated (fyl-Leu), because of mutual HMBC correlations between the formyl proton ( $\delta_{\rm H}$  8.16) and the second  $\alpha$ -carbon of Leu ( $\delta_{\rm C}$  52.7) and between the corresponding lpha-proton of Leu ( $\delta_{
m H}$  4.62) and the formyl carbon ( $\delta_{\rm C}$  163.6). The 4-amino-3,5-dihydroxyhexanoic acid (Adha) moiety was established by simultaneous analysis of the COSY and HMBC spectra. A downfield quartet (H-45,  $\delta_{\rm H}$ 5.31) showed COSY and HMBC correlations with the adjacent CH<sub>3</sub>-46 ( $\delta_{\rm H}$  1.27,  $\delta_{\rm C}$  19.4) and COSY correlations with H-44 ( $\delta_{\rm H}$  3.98), which in turn was COSY-correlated with an amide proton ( $\delta_{\rm H}$  8.52). The H-45 quartet also showed an HMBC correlation to the deshielded <sup>13</sup>C resonance of oxymethine CH-43 ( $\delta_{\rm C}$  72.4,  $\delta_{\rm H}$  4.48). This proton showed COSY correlations with the methylene protons (H<sub>2</sub>-42,  $\delta_{\rm H}$  2.58/2.15), which had HMBC correlations to a carbonyl carbon (C-41,  $\delta_{\rm C}$  175.6). As the presence of a chlorine atom in 1 was inferred from the isotopic pattern of the pseudomolecular ion in the FABMS spectrum, the remaining amino acid residue therefore contains a chlorine atom. On the basis of the COSY and HMBC correlations of  $\delta_{\rm H}$  0.99/ $\delta_{\rm C}$  10.2 and  $\delta_{\rm H}$  1.51/ $\delta_{\rm C}$  23.4, this amino acid possessed an isoleucine-like structure, in which the  $\gamma$ position ( $\delta_{\rm H}$  4.53/ $\delta_{\rm C}$  59.2) is substituted with a chlorine atom. Here, the rare amino acid chloroisoleucine (Cl-Ile) was established.

Received: August 16, 2013 Published: December 13, 2013

ACS Publications

© 2013 American Chemical Society and American Society of Pharmacognosy



L-norVal, L-allo-Thr, n=2 (L-GIn), R=Me, L-Ala Phoriospongin A (4) *D-nor*Val, *D-allo*-Thr, n=1 (D-Asn), R=H, D-Ala Phoriospongin B (5) *D-nor*Val, *D-allo*-Thr, n=1 (L-Asn), R=Me, D-Ala

Eventually, the molecular weight together with 1D and 2D NMR spectroscopy revealed that the isolated compound was identical to cyclolithistide A (1'), a known antifungal cyclodepsipeptide isolated from the sponge Theonella swinhoei, reported by Crews and co-workers in 1998.8 While their assignment was mainly accomplished by the interpretation of 2D NMR spectroscopic data in DMSO- $d_{6}^{8}$  our assignment of the data in DMSO- $d_6$  was hampered by the presence of the surplus signals observed in the COSY spectrum, probably due to rotamers (Figure S6).

The amino acid sequence and the position of the macrocyclic ring junction were confirmed by the interresidue HMBC and NOESY cross-peaks. First, the tetrapeptide sequence norVal-Gly-Adha-fylLeu was confirmed on the basis of HMBC correlations,  $\delta_{\rm H}$  8.06 (norVal NH)/ $\delta_{\rm C}$  173.3 (Gly),  $\delta_{\rm H}$  3.87, 3.67 (Gly)/ $\delta_{\rm C}$  175.6 (Adha), and  $\delta_{\rm H}$  8.52 (Adha NH)/ $\delta_{\rm C}$  175.2 (fylLeu). The ester linkage between the  $\delta$ -hydroxy group of Adha and the C-terminus of Cl-Ile, in place of norVal in the original structure, was deduced from the HMBC correlation between  $\delta_{\rm H}$  5.31 (Adha) and  $\delta_{\rm C}$  173.4 (Cl-Ile). The amide proton of Cl-Ile ( $\delta_{\rm H}$  7.76) was correlated to a carbonyl carbon at  $\delta_{\rm C}$  174.6 (Ala), which overlaps with that of Phe. In spite of this overlap, the presence of a NOESY cross-peak between the  $\alpha$ -proton of Phe and N-Me protons corroborated that the assignment originally reported to be  $\delta_{\rm H}$  3.05 (N-MeLeu)/ $\delta_{\rm C}$ 174.6 (Ala) should be revised to  $\delta_{\rm H}$  3.05 (*N*-MeLeu)/ $\delta_{\rm C}$  174.6 (Phe). Along with these correlations, the HMBC cross-peak between  $\delta_{\rm H}$  7.32 (Ala NH) and  $\delta_{\rm C}$  172.7 (*N*-MeLeu) provided evidence that the tetrapeptide sequence Phe-N-MeLeu-Ala-Cl-Ile should be present in the revised structure. At this point, it became apparent that the original <sup>13</sup>C chemical-shift assignments of four carbonyl carbons of Gly, Adha, N-MeLeu, and

Cl-Ile should be reassigned to those of N-MeLeu, Gly, Cl-Ile, and Adha, respectively. Furthermore, the amide proton of Phe  $(\delta_{\rm H}, 7.96)$  showed an HMBC correlation to the carbonyl carbon at  $\delta_{\rm C}$  172.7 (allo-Thr), which is also overlapped with that of N-MeLeu. Considering that the dipeptide fragment Phe-N-MeLeu has already been established, allo-Thr should precede Phe. Finally, the ring closure by placing the remaining Gln residue between the N-terminus of allo-Thr and the C-terminus of norVal satisfied the molecular formula. This was supported by the ROESY correlation between  $\delta_{\rm H}$  8.54 (allo-Thr NH) and  $\delta_{\rm H}$ 4.59 (Gln). Thus, the new structure 1 was established as shown in Figure 1.



Figure 1. Key 2D NMR correlations for 1.

With the revised structure of cyclolithistide A (1) in place, we turned our attention to the MS/MS data of 1' reported in the original paper.<sup>8</sup> Surprisingly, all of the MS/MS fragmentation patterns were in full agreement with the revised sequence (Figure S14). The fragment ions are as follows: loss of Phe-Thr (m/z 916), loss of Phe-Thr-Gln (788), loss of Phe-Thr-Gln-norVal-Gly (632), loss of Phe-Thr-Gln-norVal-Gly and the formyl group (604), loss of Phe-Thr-Gln-norVal-Gly and fyl-Leu (490), and loss of Phe-Thr-Gln-norVal-Gly/fyl-Leu-Adha (346). All of these results corroborated our proposed structure, but were still insufficient to preclude the reported one.

For this reason, 1 was subjected to partial hydrolysis with 4 M HCl, which yielded a tripeptide 2, Thr-Phe-N-MeLeu (m/z)394, see Figures 2 and S15 and Table S2). This sequence was not present in the original structure, but composed a segment of the revised structure (Figure 2). Furthermore, we measured the ESIMS/MS data of the linearized peptide 3, which was prepared by methanolysis. As a result, four diagnostic MS/MS fragments were detected, which validated the revised sequence without exception (Figures 2 and S16).

Phoriospongins A (4) and B (5), analogues of cyclolithistide A, were reported by Capon and co-workers in 2002.9 Phoriospongins possess the same amino acid composition as 1', except that Asn is substituted for Gln. However, the absolute configurations of three amino acids (norVal, allo-Thr, Ala) were reportedly antipodal to those of 1'. To clarify the discrepancies in the stereochemical assignments between cyclolithistide A and the phoriospongins, we reinvestigated the absolute configurations of the amino acids of 1.

The absolute configurations of most of the component amino acids were established by a chiral-phase GC analysis of the N-trifluoroacetyl/methyl ester derivatives of the hydrolysate of 1 (Figures S17 and S18). This approach successfully identified the existence of D-Leu, D-norVal, L-Gln, D-allo-Thr, L-



Figure 2. Summary of the partial hydrolysis and MS/MS experiments.

Phe, and D-Ala (Table 1). On the other hand, although authentic standards of *N*-MeLeu were synthesized from Leu<sup>10</sup>

Table 1. Absolute Configurations of Amino Acids of 1, 1', and 5

	cyclolithistide A		phoriospongin B
amino acid	revised (1)	original $(1')^8$	5 <sup>9</sup>
(fyl-)Leu	D	D	D
<i>nor</i> Val	D	L	D
Gln	L	L	l (Asn)
allo-Thr	D	L	D
Phe	L	L	L
Ala	D	L	D
N-MeLeu	L	L	L

and analyzed by the same procedure, the configuration could not be elucidated, due to the overlapped retention times of the enantiomers. Instead, Marfey's analysis of the acid hydrolysate and standards revealed that it had the L-configuration.<sup>11</sup> Overall, the absolute configurations of Leu, *N*-Me Leu, Gln, and Phe matched those of 1', but those of *nor*Val, *allo*-Thr, and Ala were in full accordance with 5, rather than 1'. Given that the absolute configuration of 1' was originally elucidated by chiral-phase TLC methods,<sup>8</sup> our result is more reliable because of the higher resolution of GC and HPLC.

Finally, the absolute configuration of the 4-amino-3,5dihydroxyhexanoic acid moiety, which remained unassigned in the original report, was elucidated by chemical transformation in conjunction with the modified Mosher's method. At first, the 1,3-diol portion of the methyl ester 3 was converted to the acetonide, thus forming 43,45-O-isopropylidene-3. The geminal dimethyl groups resonated at diagnostic <sup>13</sup>C chemical shifts ( $\delta_{\rm C}$  18.8 and 29.0) for the chair conformation of a syn-1,3-diol acetonide.<sup>12</sup> This was confirmed by the NOESY correlations between the axial methyl protons ( $\delta_{\rm H}$  1.49) and the two downfield oxymethines at H-43 ( $\delta_{\rm H}$  4.53) and H-45 ( $\delta_{\rm H}$  4.23). Thus, these two protons are axial (Figure 3).<sup>13</sup> Furthermore, the coupling constant between oxymethine H-43 ( $\delta_{\rm H}$  4.53) and amidomethine H-44 ( $\delta_{\rm H}$  3.71) was 2.0 Hz, indicating that the amide substituent is also axial (Figure 3). Thus, the relative configuration of this moiety was considered to be either (43S,44R,45R) or (43R,44S,45S). The absolute configuration was conclusively determined to be the former, by



Figure 3. Assignment of the absolute configuration of the Adha residue.  $\Delta \delta = \delta(R$ -bis-MPA ester) -  $\delta(S$ -bis-MPA ester).

the modified Mosher's method (Figure 3 and Table S4) with the bis-MPA (methoxyphenylacetic acid) ester of  $\mathbf{1}$ ,<sup>14</sup> which implied that this PKS/NRPS hybrid portion could be derived from L-Thr.

In summary, the revised structure (1) differs from the original (1') in the amino acid sequence and the absolute configurations of *nor*Val, *allo*-Thr, and Ala. Compound 1 and the phoriospongins are unique macrocyclic depsipeptides containing the nonproteinogenic amino acids norvaline (*nor*Val) and chloroisoleucine (Cl-Ile) as well as a 4-amino-3,5-dihydroxyhexanoic acid in their structures. We successfully determined the absolute configuration of Adha, while that of Cl-Ile is still unassigned. Given that the component amino acids of the phoriospongins were almost the same as 1, their structures are also worthy of reconsideration.

## EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. The UV

spectrum was obtained by a Gene Spec III UV/vis spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a JEOL ECX-500 spectrometer in CD<sub>3</sub>OD and CD<sub>3</sub>OH. <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts were reported in parts per million and referenced to solvent peaks:  $\delta_{\rm H}$  = 3.29 and  $\delta_{\rm C}$  = 47.8 for both CD<sub>3</sub>OD and CD<sub>3</sub>OH. HRFABMS data were obtained from a JEOL JMS-700T spectrometer, using *m*-nitrobenzyl alcohol as the matrix. MS/MS experiments were performed on a Bruker amaZon SL spectrometer. GC/MS experiments were performed on a Shimadzu GCMS-QP2010 spectrometer.

Animal Material. The sponge Discodermia japonica was collected off Jogashima, Sagami Bay, Japan, 35°6.172' N, 139°34.257' E, 364-158 m depth, by dredging of R/V Rinkai-maru of Misaki Marine Biological Station, the University of Tokyo, on January 13, 2012. Sponge description: two thick branches ramified on the base; each trunk having a hollow on its summit with several small oscules; color beige; consistency stony hard. Ectosomal skeleton made of phyllotriaene and a few discotriaene, with long diactinal spicules traversing the choanosome. Choanosomal skeleton consisted of dense articulation of tetraclone desma. Microscleres acanthoxea and microstrongyle. Phyllotriaene spatulate with short rhabd, 781 (630-890)  $\mu$ m in longest diameter. Discotriaene oval, some with slightly incised margins, 372 (260-470)  $\mu$ m in longest diameter. Tetraclone desmas, smooth on shaft, 118 (113–130)  $\mu$ m in thickness. Acanthoxea almost uniform in shape and size, fusiform, straight or slightly bent at midpoint of the spicule, sharply pointed at both extremities, 70 (55.0-77.5)  $\mu$ m in length, 3.6 (3.0–3.8) in width. Micostrongyle almost uniform in shape and size, straight, with rough surface, 13.1 (11.3-16.3)  $\mu$ m in length, 3.7 (3.0–5.0)  $\mu$ m in width. The sponge is most likely *D. japonica* (Döderline, 1884) according to Tanita and Hoshino (1989),<sup>15</sup> because external morphology and spicule composition are similar and the type locality of D. japonica is very close to the sampling locality. The sample was identified by Y.I. The specimen used for identification (NSMT-Po2451) is deposited at the National Museum of Nature and Science, Tokyo.

**Isolation.** The MeOH (0.5 L × 3) extract of the sponge *D. japonica* (200 g, wet weight) was partitioned between EtOAc (350 mL × 2) and H<sub>2</sub>O (350 mL). The EtOAc-soluble material (1.2 g) was subjected to open chromatography on a silica gel column, eluted with a stepwise gradient of MeOH (0–40%) and H<sub>2</sub>O (0–10%) in CHCl<sub>3</sub>, to afford cyclolithistide A (1, 160.1 mg, 0.080% wet weight).

Cyclolithistide A (1): white, amorphous solid;  $[\alpha]^{24}_{D} - 51.5$  (c 0.12, MeOH);  $[\alpha] -29.3$  (c 0.015, MeOH, in the original report);<sup>8</sup> UV (MeOH)  $\lambda_{max}$  256 nm; <sup>1</sup>H and <sup>13</sup>C NMR (Table S2); HRFABMS m/z 1186.5875  $[M + Na]^{+}$  (calcd for C<sub>54</sub>H<sub>86</sub>ClN<sub>11</sub>NaO<sub>15</sub>, 1186. 5891).

**Partial Hydrolysis of Cyclolithistide A. 1** (5 mg) was hydrolyzed with 4 M HCl (2 mL) at 100 °C for 1 h. The reaction mixture was purified by reversed-phase HPLC on ODS (Cosmosil MS-II column  $\phi$  10 × 250 mm; flow rate 3 mL/min; 10–100% MeOH/H<sub>2</sub>O containing 0.05% TFA over 30 min; UV detection at 200 nm) to afford tripeptide **2** (Table S2 and Figure S15).

**MS/MS Fragment Analysis.** A solution of 1 (1 mg) and  $K_2CO_3$  (0.1 mg, 0.8 equiv) in MeOH (200  $\mu$ L) was stirred at room temperature (rt) for 4 h. The reaction mixture was purified by reversed-phase HPLC on ODS (Cosmosil MS-II column  $\phi$  10 × 250 mm; flow rate 3 mL/min; 80% MeOH/H<sub>2</sub>O containing 0.05% TFA; UV detection at 200 nm) to afford 3. The MS/MS fragments thus obtained are *m*/*z* 946.7, 801.6, 636.4, and 553.4 (Figure S16).

Amino Acid Analysis by Chiral-Phase GC. 1 (200  $\mu$ g) was hydrolyzed with 6 M HCl (500  $\mu$ L) at 110 °C for 24 h. The reaction mixture was treated with 5–10% HCl/MeOH (500  $\mu$ L) at 100 °C for 30 min, followed by a treatment with trifluoroacetic anhydride (TFAA)/CH<sub>2</sub>Cl<sub>2</sub> (1:1, 500  $\mu$ L) at 100 °C for 5 min. The chiral-phase GC analysis of the *N*-trifluoroacetyl (TFA)/methyl ester derivatives was performed using a CP-Chirasil-D-Val column (Alltech, 0.25 mm × 25 m; N<sub>2</sub> as the carrier gas; program rate 50–200 at 4 °C/min) and showed peaks at  $t_R$  = 5.5, 7.3, 7.5, 9.1, 11.3, 11.5, 17.6, and 18.7 min. Standard amino acids were also converted to the TFA/Me derivatives by the same procedure. Retention times (min) were as follows: L-Ala (4.3), D-Ala (5.2), Gly (6.9), N-Me-L-Leu (7.5), N-Me-D-Leu (7.5) LnorVal (8.1), D-norVal (9.1), L-Leu (10.0), D-Leu (11.3), L-Thr (6.9), D-Thr (7.8), L-allo-Thr (10.5), D-allo-Thr (11.5), L-Gln (17.6), D-Gln (18.6), L-Phe (18.9), and D-Phe (19.5) (Figures S17 and S18). Thus, the presence of D-Ala, Gly, D-norVal, D-Leu, D-allo-Thr, L-Gln, and L-Phe was confirmed. The remaining peaks that were not mentioned above should include those of the Cl-Ile and Adha moieties. The absolute configuration of N-Me-Leu could not be elucidated from this experiment.

**Synthesis of N-MeLeu.** Sodium hydride (15.6 mg, 10 equiv) was added to 15 mg (64.9  $\mu$ mol) of Boc-L-Leu dissolved in THF (200  $\mu$ L) at 0 °C. After stirring for 1 h, iodomethane (40  $\mu$ L, 10 equiv) was added at 0 °C. The reaction mixture was then stirred at 0 °C for 2 h and held at rt for 12 h before it was quenched with H<sub>2</sub>O. The reaction mixture was acidified by HCl (pH 1–2) and extracted with EtOAc twice. The combined organic layer was dried over sodium sulfate, concentrated under reduced pressure, and treated with TFA (500  $\mu$ L) at rt for 1 h. The reaction mixture was then freeze-dried. *N*-Me-L-Leu was obtained as a white solid (8.5 mg). *N*-Me-D-Leu was synthesized by the same scheme (7.7 mg).

**Determination of the Configuration of N-MeLeu.** Due to the overlapped retention times of the L and D isomers of N-MeLeu on GC-MS, the absolute configuration of this residue was determined by Marfey's analysis. To 150  $\mu$ g of the acid hydrolysate of the peptide and authentic amino acid standards of N-MeLeu was added 75  $\mu$ L of 1-FDAA (10 mg/mL) in acetone, and 150  $\mu$ L of 1 M NaHCO<sub>3</sub>(aq), and the mixture was incubated at 50 °C for 1 h. Subsequently, 75  $\mu$ L of 2 M HC1 and 300  $\mu$ L of MeOH were added. The solution was subjected to reversed-phase HPLC on ODS (Cosmosil MS-II column  $\phi$  4.6 × 250 mm; flow rate 0.8 mL/min; 10–50% CH<sub>3</sub>CN/H<sub>2</sub>O containing 0.05% TFA over 20 min; UV detection at 340 nm; oven 40 °C). The retention times (min) were as follows: hydrolysate (28.5), N-Me-L-Leu (28.5), and N-Me-D-Leu (29.7) (Figure S19). Thus, the configuration of N-MeLeu was established as the L form.

Determination of the Configuration of 4-Amino-3,5-dihydroxyhexanoic Acid. The relative configuration was elucidated as follows:  $K_2CO_3$  (1 mg, 0.8 equiv) was added to a solution of 1 (10 mg) in MeOH (1.5 mL). After stirring at rt for 4 h, the reaction mixture was extracted with EtOAc, dried with sodium sulfate, and concentrated under reduced pressure. The mixture, in 1,4-dioxane (1.5 mL), was treated with pyridinium *p*-toluenesulfonate (4.3 mg, 2 equiv) and 2,2-dimethoxypropane (20  $\mu$ L, 20 equiv) and was stirred at 40 °C for 2 h. The resulting solution was purified by reversed-phase HPLC on ODS (Cosmosil MS-II column  $\phi$  10 × 250 mm; flow rate 3 mL/ min; 75% MeOH/H<sub>2</sub>O; UV detection at 200 nm) to afford 43,45-Oisopropylidene-5 (Table S3, Figure S21). The coupling constants and the NOESY correlations of 43,45-O-isopropylidene-3 revealed the relative configuration to be either (43S,44R,45R) or (43R,44S,45S) (Figure S22). The absolute configuration was determined as follows: To a solution of 1 (1 mg) in  $CH_2Cl_2$  (200  $\mu$ L) were added DCC (1 mg, 5.7 equiv), DMAP (10 µg, 0.1 equiv), and either S- or R-MPA (800  $\mu$ g, 5.6 equiv). After stirring for 4 h, the reaction mixture was purified by reversed-phase HPLC on ODS (Cosmosil MS-II column  $\phi$ 10  $\times$  250 mm; flow rate 3.0 mL/min; 80–100% MeOH/H<sub>2</sub>O containing 0.05% TFA over 15 min; UV detection at 200 nm; oven 40 °C) to yield the S/R-MPA esters of 1 (FABMS m/z 1460 [M + H]<sup>+</sup>). According to the chemical shift difference ( $\delta^{R} - \delta^{S}$ ), the absolute configuration was determined to be (43S,44R,45R) (Table S4).

## ASSOCIATED CONTENT

#### Supporting Information

Experimental Section, 1D and 2D NMR spectra, and chiralphase GC analytical data for 1. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

#### **Corresponding Authors**

\*E-mail: wakimoto@mol.f.u-tokyo.ac.jp; abei@mol.f.u-tokyo.ac. jp.

## Journal of Natural Products

\*Phone: +81-3-5841-4740. Fax: +81-3-5841-4744. E-mail: abei@mol.f.u-tokyo.ac.jp.

## Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

We thank Prof. P. Crews, University of California, Santa Cruz, for valuable discussions. This work was partly supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

### REFERENCES

(1) (a) Kitagawa, I.; Kobayashi, M.; Katori, T.; Yamashita, M.; Tanaka, J.; Doi, M.; Ishida, T. *J. Am. Chem. Soc.* **1990**, *112*, 3710– 3712. (b) Kobayashi, M.; Tanaka, J.; Katori, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2960–2966. (c) Kobayashi, J.; Tsukamoto, S.; Tanabe, A.; Sasaki, T.; Ishibashi, M. J. Chem. Soc., Perkin Trans. 1 **1991**, 2379–2383.

(2) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. J. Org. Chem. **1990**, 55, 4912.

(3) Matsunaga, S.; Fusetani, N. J. Org. Chem. 1995, 60, 1177–1181.
(4) Matsunaga, S.; Fusetani, N.; Konosu, S. Tetrahedron Lett. 1984,

(4) Matsunaga, S.; Fusetani, N.; Konosu, S. Terraneuron Lett. **1984**, 25, 5165–5168.

(5) Kimura, M.; Wakimoto, T.; Egami, Y.; Tan, K. C.; Ise, Y.; Abe, I. J. Nat. Prod. **2012**, 75, 290–294.

(6) Kobayashi, J.; Itagaki, F.; Shigemori, H.; Takao, T.; Shimonishi, Y. *Tetrahedron* **1995**, *S1*, 2525–2532.

(7) (a) Edrada, R. A.; Ebel, R.; Supriyono, A.; Wray, V.; Schupp, P.; Steube, K.; van Soest, R.; Proksch, P. *J. Nat. Prod.* **2002**, *65*, 1168– 1172. (b) Shinde, P. B.; Mansoor, T. A.; Luo, X.; Hong, J.; Lee, C. O.; Jung, J. H. *Bull. Korean Chem. Soc.* **2007**, *28*, 990–994.

(8) Clark, D. P.; Carroll, J.; Naylor, S.; Crews, P. J. Org. Chem. 1998, 63, 8757–8764.

(9) Capon, R. J.; Ford, J.; Lacey, E.; Gill, J. H.; Heiland, K.; Friedel, T. J. Nat. Prod. **2002**, 65, 358–363.

(10) Hu, T.; Panek, J. S. J. Org. Chem. 1999, 64, 3000-3001.

(11) (a) Marfey, P. Carlsberg Res. Commun. 1984, 49, 591–596.
(b) Bhushan, R.; Brückner, H. Amino Acids 2004, 27, 231–247.

(12) Rychnovsky, S. D.; Skalitzky, D. J. Tetrahedron Lett. 1990, 31, 945–948.

(13) (a) Evans, D. A.; Rieger, D. L.; Gage, J. R. *Tetrahedron Lett.* **1990**, 31, 7099–7100. (b) Boger, D. L.; Hikota, M.; Lewis, B. M. J. *Org. Chem.* **1997**, 62, 1748–1753.

(14) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. J. Org. Chem. 1969, 34, 2543–2549. (b) Hoye, T. R.; Jeffrey, C. S.; Shao, F. Nat. Protoc. 2007, 2, 2451–2458. (c) Seco, J. M.; Quinoa, E.; Riguera, R. Chem. Rev. 2004, 104, 17–117.

(15) Tanita, S.; Hoshino, T. The Demospongiae of Sagami Bay; Biological Laboratory of the Imperial Household of Japan, 1989; p 166. Note