

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/49648896>

Yaku'amides A and B, Cytotoxic Linear Peptides Rich in Dehydroamino Acids from the Marine Sponge *Ceratopsion* sp. °

ARTICLE in JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · DECEMBER 2010

Impact Factor: 12.11 · DOI: 10.1021/ja109275z · Source: PubMed

CITATIONS

23

READS

28

6 AUTHORS, INCLUDING:



[Susumu Ohtsuka](#)

Hiroshima University

146 PUBLICATIONS 1,192 CITATIONS

SEE PROFILE



[Shigeru Okada](#)

The University of Tokyo

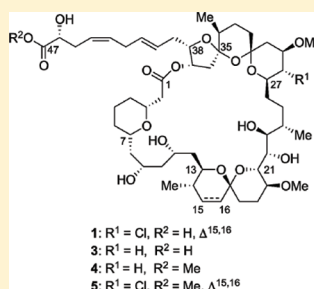
74 PUBLICATIONS 1,109 CITATIONS

SEE PROFILE

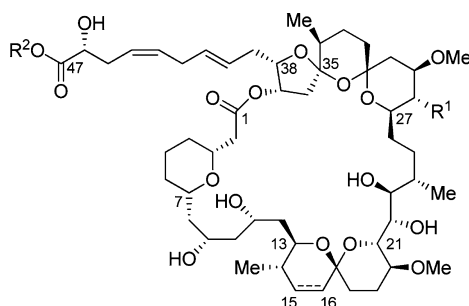
Isolation of Spirastrellolides A and B from a Marine Sponge *Epipolasis* sp. and Their Cytotoxic ActivitiesMasashi Suzuki,[†] Reiko Ueoka,[†] Kentaro Takada,[†] Shigeru Okada,[†] Susumu Ohtsuka,[‡] Yuji Ise,[§] and Shigeki Matsunaga^{*,†}[†]Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, 113-8657, Japan[‡]Takehara Marine Science Station, Hiroshima University, Takehara Hiroshima 725-0024, Japan[§]Misaki Marine Biological Station, The University of Tokyo, Miura, Kanagawa, 238-0225, Japan

S Supporting Information

ABSTRACT: Spirastrellolides A (1) and B (3) have been isolated as free acids from a marine sponge *Epipolasis* sp. collected in the East China Sea. These compounds had been isolated from the Caribbean marine sponge *Spirastrella coccinea* after conversion to the methyl ester. We examined the cytotoxic activities of 1 and 3 and found that the activities of the free acids are comparable to those of the corresponding methyl esters.

*Epipolasis* sp.

Spirastrellolides are antimitotic macrolides isolated from the Caribbean marine sponge *Spirastrella coccinea*.^{1–4} Their isolation was accomplished only after conversion to methyl esters. Even though potent inhibitory activity of spirastrellolide A (1) against protein phosphatase 2A was reported,³ neither the cytotoxic activities nor spectroscopic data of the free acids for this class of metabolites have been reported. Intensive synthetic efforts have culminated in the total syntheses of the methyl esters of spirastrellolides A (1) and F (2),^{5–8} but not the free acids. In the course of our search for cytotoxic agents from marine sponges, we found activity in a marine sponge *Epipolasis* sp. collected in the East China Sea. From this sponge we have isolated spirastrellolides A (1) and B (3) as the predominant cytotoxic constituents. The isolation, structural assignment, and cytotoxic activities of 1 and 3 are reported below.



- 1: R¹ = Cl, R² = H, Δ^{15,16}
2: R¹ = Cl, R² = H
3: R¹ = H, R² = H
4: R¹ = H, R² = Me
5: R¹ = Cl, R² = Me, Δ^{15,16}

The MeOH extract of the marine sponge *Epipolasis* sp. was subjected to a solvent partitioning scheme, from which the active CHCl₃ fraction was subjected to ODS flash chromatography, followed by RP-HPLC purification with C₁₈ (Cosmosil AR-II), Phenyl-Hexyl (Luna 5u), and C₁₈ (Cosmosil MS-II) stationary phases to afford spirastrellolides A (1, 3.9 × 10^{−8} % yield, wet weight) and B (3, 5.4 × 10^{−8} % yield, wet weight).

We first studied the structure of compound 3, which was more abundant. The molecular formula of 3 was established as C₅₂H₈₄O₁₇ on the basis of HRESIMS data. The ¹H NMR spectrum in CD₃OD exhibited three methyl doublets (δ 0.94, 1.00, and 1.04) and two methoxy signals (δ 3.29 and 3.38). Additionally 20 aliphatic methylene carbons, 16 aliphatic methine carbons including 13 oxymethines, and four olefinic carbons were observed in the HSQC spectrum. The olefinic protons were conspicuously broad. Even though the molecular formula of 3 matched that of spirastrellolide B, one aliphatic methylene and one oxymethine carbon were lacking in our assignment. Interpretation of the COSY and TOCSY data in conjunction with the HSQC data revealed the presence of four partial structures (C-2 to C-16, C-18 to C-30, C-31 to C-34, and C-36 to C-43). The HMBC spectrum demonstrated the following correlations: H₂-18, H-21/C-17; H₂-30, H₂-32/C-31; H-33a, 34-CH₃, H-36b, and H-37/C-35. These correlations suggested that the carbon skeleton from C-2 to C-43 of 3 was identical with that of spirastrellolide B. Because we envisioned that the lack of correlation between H-43 and H-44 and the disappearance of H₂-45 and H-46 signals were due to signal

Received: December 9, 2011

Published: June 4, 2012

Table 1. ^1H and ^{13}C NMR Data for Compounds 1 and 3 (600 MHz, CD_3OD)

| position | spirastrellolide A (1) | | | spirastrellolide B (3) | | |
|----------|------------------------------|-------------------------------|---------------|------------------------------|-------------------------------|-------------------|
| | $\delta_{\text{C}}, ^a$ type | δ_{H} (J in Hz) | HMBC | $\delta_{\text{C}}, ^a$ type | δ_{H} (J in Hz) | HMBC |
| 1 | 171.5, C | | | 171.2, C | | |
| 2 | 43.3, CH_2 | 2.43, m | 1, 3 | 42.1, CH_2 | 2.36, m | 1, 3 |
| | | 2.59, m | 1 | | 2.63, m | 1 |
| 3 | 75.0, CH | 3.85, m | 1 | 75.2, CH | 3.71, m | |
| 4 | 32.2, CH_2 | 1.34, m | | 30.9, CH_2 | 1.51, m | 3 |
| | | 1.52, m | | | 1.51, m | 3 |
| 5 | 24.9, CH_2 | 1.53, m | | 24.4, CH_2 | 1.51, m | 7 |
| | | 1.84, m | | | 1.82, m | |
| 6 | 33.5, CH_2 | 1.21, m | | 32.4, CH_2 | 1.18, m | |
| | | 1.53, m | | | 1.50, m | |
| 7 | 76.4, CH | 3.66, m | | 76.0, CH | 3.57, m | 3 |
| 8 | 45.8, CH_2 | 1.39, m | | 44.8, CH_2 | 1.40, m | 7 |
| | | 1.55, m | 7 | | 1.54, m | |
| 9 | 65.9, CH | 4.07, t (10.5) | | 65.4, CH | 4.10, m | |
| 10 | 47.8, CH | 1.32, m | | 46.9, CH_2 | 1.38, m | 11 |
| | | 1.38, m | | | 1.38, m | 11 |
| 11 | 64.8, CH | 41.8, m | | 65.4, CH | 4.10, m | |
| 12 | 42.5, CH_2 | 1.39, m | | 41.6, CH_2 | 1.29, m | |
| | | 1.73, m | | | 1.67, m | 11, 13 |
| 13 | 71.9, CH | 3.68, m | | 72.7, CH | 3.59, m | 11, 12, 15 |
| 14 | 35.4, CH | 1.97, m | 15 | 35.5, CH | 1.23, m | 13 |
| 15 | 135.6, CH | 5.69, dd (9.7, 1.3) | 13, 14, 17 | 29.4, CH_2 | 1.49, m | 13, 14 |
| | | | | | 1.49, m | 13, 14 |
| 16 | 130.0, CH | 5.59, dd (9.7, 2.7) | 14, 15, 17 | 35.3, CH_2 | 1.49, m | 15, 17 |
| | | | | | 1.66, m | 17 |
| 17 | 95.1, C | | | 96.1, C | | |
| 18 | 35.2, CH_2 | 1.72, m | | 35.4, CH_2 | 1.52, m | 17 |
| | | 1.72, m | | | 1.68, m | 17, 20 |
| 19 | 24.9, CH_2 | 1.76, m | | 24.8, CH_2 | 1.71, m | 20 |
| | | 2.09, m | | | 2.00, m | |
| 20 | 75.4, CH | 3.39, m | | 75.2, CH | 3.34, m | |
| 21 | 72.5, CH | 4.03, d (9.8) | 19, 20 | 70.9, CH | 3.85, d (9.4) | 17, 19, 22, 23 |
| 22 | 69.3, CH | 3.83, d (10.1) | 23 | 68.8, CH | 3.88, d (9.4) | 20, 21, 23 |
| 23 | 75.3, CH | 3.57, d (10.1) | 22, 25, 24-Me | 75.4, CH | 3.63, m | 21, 22, 25, 24-Me |
| 24 | 34.5, CH | 1.85, m | | 34.0, CH | 1.86, m | 25 |
| 25 | 26.5, CH_2 | 1.10, m | | 26.6, CH_2 | 1.10, m | |
| | | 1.90, m | | | 1.96, m | |
| 26 | 31.7, CH_2 | 1.28, m | | 35.3, CH_2 | 1.30, m | |
| | | 2.16, m | | | 1.57, m | |
| 27 | 76.4, CH | 3.66, m | | 70.8, CH | 3.67, m | 25 |
| 28 | 66.3, CH | 3.34, m | 26, 27, 29 | 38.8, CH_2 | 0.96, m | 29 |
| | | | | | 2.03, m | |
| 29 | 79.8, CH | 3.63, m | | 74.1, CH | 3.67, m | |
| 30 | 44.3, CH_2 | 1.34, m | 28, 29, 31 | 43.8, CH_2 | 1.08, m | 29, 31, 32 |
| | | 2.18, m | 28, 29, 31 | | 1.96, m | 28, 29, 31 |
| 31 | 98.7, C | | | 98.8, C | | |
| 32 | 37.1, CH_2 | 1.66, m | | 37.5, CH_2 | 1.60, m | 31, 33, 34 |
| | | 1.79, m | | | 1.72, m | 31 |
| 33 | 24.6, CH_2 | 1.39, m | | 24.2, CH_2 | 1.34, m | 31, 35 |
| | | 1.92, m | | | 1.94, m | 31, 34 |
| 34 | 39.4, CH | 1.75, m | | 38.8, CH | 1.68, m | |
| 35 | 110.1, C | | | 109.2, C | | |
| 36 | 47.8, CH_2 | 2.19, m | 34, 37 | 47.5, CH_2 | 2.07, m | 34, 37, 38 |
| | | 2.44, m | 35 | | 2.33, m | 35, 38 |
| 37 | 74.2, CH | 5.50, m | | 73.5, CH | 5.33, m | 35 |
| 38 | 84.2, CH | 4.16, m | 37 | 83.4, CH | 4.07, m | |
| 39 | 31.2, CH_2 | 2.33, m | | 30.5, CH | 2.33, m | 38, 40, 41 |
| | | 2.79, m | | | 2.67, m | 38 |
| 40 | 126.4, CH | 5.35, m | | 126.4, CH | 5.33, m | 42 |

Table 1. continued

| position | spirastrellolide A (1) | | | spirastrellolide B (3) | | |
|----------|--------------------------------|----------------------|------------|--------------------------------|----------------------|------------|
| | δ_C , ^a type | δ_H (J in Hz) | HMBC | δ_C , ^a type | δ_H (J in Hz) | HMBC |
| 41 | 132.7, CH | 5.65, m | | 132.2, CH | 5.63, m | |
| 42 | 31.7, CH ₂ | 2.78, m | | 31.2, CH ₂ | 2.72, m | |
| | | 2.78, m | | | 2.82, m | |
| 43 | 131.2, CH | 5.50, m | | 130.7, CH | 5.45, m | |
| 44 | 126.5, CH | 5.51, m | | 126.5, CH | 5.48, m | |
| 45 | ND ^b | ND | | ND | ND | |
| 46 | ND | ND | | ND | ND | |
| 47 | ND | ND | | ND | ND | |
| 14-Me | 17.0, CH ₃ | 0.94, d (6.9) | 13, 14, 15 | 17.6, CH ₃ | 0.81, d (5.5) | 13, 14, 15 |
| 24-Me | 18.5, CH ₃ | 1.31 | 23, 24, 25 | 18.4, CH ₃ | 1.04, d (6.8) | 23, 24, 25 |
| 34-Me | 17.3, CH ₃ | 1.16 | 33, 34, 35 | 16.6, CH ₃ | 1.00, d (6.2) | 33, 34, 35 |
| 20-OMe | 56.8, CH ₃ | 3.49, s | 20 | 56.4, CH ₃ | 3.38, s | 20 |
| 29-OMe | 55.3, CH ₃ | 3.41, s | 29 | 54.8, CH ₃ | 3.29, s | 29 |

^aChemical shifts were assigned from the HSQC and HMBC data. ^bND means “not detected”.

broadening arising from unfavorable equilibrium at ambient temperature, we heated the sample to 40 °C or cooled it to 0 °C, both of which did not extend TOCSY correlations from H-43. Next we anticipated that neutralization of the carboxylic acid would alter the equilibrium and measured the TOCSY spectrum in pyridine-*d*₅. Unexpectedly the ¹H NMR spectrum gave two sets of cross-peaks in the C-12 to C-34 region, indicating the presence of an unidentified equilibrium in the corresponding portion of the molecule. However, no additional cross-peak was observed from H-43. The compound did not decompose under these conditions. From the above-mentioned experiments we considered it highly likely that **3** was spirastrellolide B itself. In order to confirm this idea, we converted **3** to the methyl ester **4** and measured the ¹H NMR, COSY, and TOCSY spectra in benzene-*d*₆. Even though the ¹H signals of H-44, H₂-45, and H-46 were clearly observed in the methyl ester **4**, chemical shifts of ¹H signals in the region between C-2 and C-12 did not perfectly coincide with those in the literature.² It was reported that the ¹H NMR spectrum of spirastrellolide F methyl ester exhibited a strikingly dynamic behavior due to the presence of an intricate hydrogen-bonding network, whereas the ¹³C chemical shifts did not fluctuate.⁶ We therefore considered that the ¹H NMR chemical shift discrepancies observed in our case arose from the different degree of hydrogen-bond formation, which should be concentration-dependent. We changed the concentration of **4** from 700 µg/mL to 1.3 mg/mL and observed the ¹H NMR spectrum. The data at the higher concentration matched better with those in the literature.² Additionally, and importantly, the ¹³C chemical shifts determined by the HSQC spectrum of **4** coincided well with those in the literature, permitting us to conclude that **3** was the free acid of spirastrellolide B (Supporting Information).

The molecular formula of **1**, C₅₂H₈₁ClO₁₇, as deduced from the HRESIMS data, was identical with that of spirastrellolide A. The structure of **1** was studied by interpretation of 2D NMR data, revealing the C-2 to C-43 partial structure identical with that of spirastrellolide A. However, no further cross-peaks were observed from H-43, as observed for **3**. However, because of the coincidence of the molecular formula and the partial structure, we considered **1** to be spirastrellolide A itself. Therefore, we converted **1** to the methyl ester **5** and measured the ¹H NMR, COSY, and TOCSY spectra at a concentration of 300 µg/mL. Significant ¹H NMR chemical shift discrepancies

with those in the literature were observed for **5**.³ Then we measured the NMR spectrum at a concentration of 900 µg/mL and found that the chemical shifts became closer to those reported for spirastrellolide A methyl ester in the literature; ¹³C chemical shifts of **5** coincided well with those in the literature.¹ Therefore, we concluded that **1** was the free acid of spirastrellolide A.³

We evaluated the cytotoxicities of **1** and **3**–**5** against HeLa cells. The acids, **1** and **3**, exhibited IC₅₀ values of 20 and 40 nM, respectively, whereas the methyl esters, **4** and **5**, exhibited comparable activities: IC₅₀ values of 70 and 30 nM, respectively.

In this study, we successfully isolated spirastrellolides A and B as the free acids from a marine sponge for the first time. Even though the amounts of the isolated spirastrellolides were small, we were able to acquire their spectroscopic data, confirm their identity, and conduct cytotoxicity tests.

Unexpectedly, NMR signals for H-45, H-46, and C-45 to C-47 were not detectable with our samples. Also unexpectedly, the cytotoxicities of spirastrellolides A and B were comparable to those of the corresponding methyl esters.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were measured on a Shimadzu Biospec 1600 DNA/protein/enzyme analyzer. NMR spectra were recorded on a JEOL alpha 600 NMR spectrometer at 300 and 273 K. Chemical shifts were referenced to solvent peaks: δ_H 3.31 and δ_C 49.15 for MeOH-*d*₄; δ_H 8.74 and δ_C 150.35 for pyridine-*d*₅; δ_H 7.16 and δ_C 128.39 for benzene-*d*₆. ESI mass spectra were measured on a JEOL JMS-T100LC mass spectrometer. HPLC were carried out on a Shimadzu LC 20 AT with a SCL-10Avp controller and a SPD-Avp detector.

Animal Material. The sponge *Epipolasis* sp. was collected by dredging off Nagannu Island (26°14.06' N, 127°33.12' E), East China Sea, at a depth of 42 m, during a cruise of R/V *Toyoshio-maru*, on May 25, 2007. The sponge was a large mass of 11 kg wet weight. Sponge description: subspherical mass; surface covered by sand except oscular region; color ocher in ethanol; consistency compressible. The precise observation of the ectosomal region could not be conducted because the surface area of the sponge was used for extraction of chemical compounds. Choanosome cavernous. Choanosomal skeleton confused, with a few vague tracts of oxeas. Spicules, oxeas in a large size range and raphidae. Oxeas fusiform, slightly arched at middle part; 565.5 ± 63.5 (450–720) µm in length, 17.9 ± 4.8 (10.0–27.5) µm in width (*N* = 30). Trichodragmas straight or slightly arched, 84.8 ± 6.1 (75.0–92.5) µm in length, less than 2 µm in width (*N* = 20). Two

species, *E. suluensis* (Wilson, 1925) from the Philippines and *E. maraensis* Kang & Sim, 2008 from Korea, have a geographical distribution close to the present species. Of these, the large category of oxea of *E. maraensis* is much larger than those of the present species. The raphidae length of the present species falls within the range of the longest raphidae of *E. maraensis* and the shortest raphidae of *E. suluensis*, and the external morphology of *E. suluensis* is lamellated, but the present species is subspherical. Furthermore the present species is distinct by lacking the small category of oxea present in the two species. Trichodragmas straight or slightly arched. The specimen used for identification (NSMT-Po-1976) was deposited at the National Museum of Nature and Science, Tokyo.

Extraction and Isolation. The frozen specimens (11 kg) were cut into pieces and extracted with MeOH (25 L \times 3) at room temperature (rt). The combined MeOH extracts were evaporated to dryness and partitioned between CHCl₃ (2 L \times 3) and H₂O (2 L). The CHCl₃ layer was partitioned between 90% MeOH (1 L) and *n*-hexane (1 L \times 3). The active 90% MeOH fraction was further partitioned between 60% MeOH and CHCl₃ (1 L \times 4). The CHCl₃ layer was subjected to ODS flash column chromatography using 50%, 70%, 90%, and 100% MeOH and CHCl₃/MeOH/H₂O (7:3:0.5) as eluents. The fraction eluted with 90% MeOH was purified by ODS HPLC (Cosmosil AR-II; 20 \times 250 mm) with 70–100% MeOH containing 0.2% acetic acid to afford 15 fractions. The seventh fraction was separated by RP-HPLC (Phenomenex Phenyl-Hexyl; 21.2 \times 250 mm) with 55–75% MeCN to afford 22 fractions. Fractions 11 to 15 were combined and purified by ODS HPLC (Cosmosil MS-II; 10 \times 250 mm) with 70–85% MeOH containing 0.2% acetic acid to afford **1** (0.43 mg). The ninth fraction from the Cosmosil AR-II column HPLC was separated by HPLC (Phenomenex Phenyl-Hexyl; 21.2 \times 250 mm) with 55–75% MeCN to afford 20 fractions. Fractions 8 to 11 were combined and purified by ODS HPLC (Cosmosil MS-II; 10 \times 250 mm) with 70–85% MeOH containing 0.2% acetic acid to afford **3** (0.59 mg).

Spirastrellolide A (1): [α]_D²⁵ +14 (c 0.03, CH₂Cl₂); UV (MeOH) λ_{\max} (log ϵ) 210 nm (3.67); ¹H NMR data (CD₃OD), see Table 1; ¹³C NMR data (CD₃OD, 600 MHz), see Table 1; HRESIMS *m/z* 1035.5097 [M + Na]⁺ (calcd for C₅₂H₈₁ClO₁₇Na, 1035.5060).

Spirastrellolide B (3): [α]_D²⁵ +31 (c 0.04, MeOH); UV (MeOH) λ_{\max} (log ϵ) 213 nm (3.54); ¹H NMR data (CD₃OD, 600 MHz), see Table 1; ¹³C NMR data (CD₃OD), see Table 1; HRESIMS *m/z* 1003.5628 [M + Na]⁺ (calcd for C₅₂H₈₄O₁₇Na, 1003.5606).

Methylation of Spirastrellolides. To a solution of **1** (0.05 mg) in MeOH (1.0 mL) was added 100 μ L of 2.0 M trimethylsilyldiazomethane in hexane. The solution was left at rt for 30 min, and the excess trimethylsilyldiazomethane was removed by a stream of N₂. The reaction mixture was purified by ODS HPLC to afford spirastrellolide A methyl ester (**5**, 0.05 mg). A 0.1 mg portion of **3** was treated in the same manner to afford spirastrellolide B methyl ester (**4**, 0.1 mg).

Spirastrellolide A methyl ester (5): UV (MeOH) λ_{\max} (log ϵ) 203 nm (3.67); ¹H NMR (C₆D₆, 600 MHz), see Table S2; HRESIMS *m/z* 1049.5263 [M + Na]⁺ (calcd for C₅₃H₈₃ClO₁₇Na, 1049.5217).

Spirastrellolide B methyl ester (4): UV (MeOH) λ_{\max} (log ϵ) 205 nm (3.77); ¹H NMR (C₆D₆, 600 MHz), see Table S1; HRESIMS *m/z* 1017.5800 [M + Na]⁺ (calcd for C₅₃H₈₆O₁₇Na, 1017.5763).

MTT Assay against HeLa Cells. The cytotoxicities of **1** and **3–5** against HeLa cells were evaluated by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HeLa human cervical cancer cells (Cell Resource Center for Biomedical Research, Tohoku University) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 2 μ g/mL antibiotic-antimycotic (Gibco) at 37 °C under an atmosphere of 5% CO₂. After 24 h preincubation, to each well of a 96-well microplate containing 200 μ L of tumor cell suspension (1 \times 10⁴ cells/mL) was added the sample in duplicate, and the plate was incubated for 72 h. After addition of (MTT) saline solution (1 mg/mL, 50 μ L) to each well, the plate was incubated for 3 h. After the incubation, the supernatant was discarded and DMSO (150 μ L) was added. The absorbance was measured to determine IC₅₀ values. In this assay, adriamycin was used as a positive control (IC₅₀ value of 0.05 μ M).

■ ASSOCIATED CONTENT

Supporting Information

NMR data for **1** and **3–5**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +81-3-5841-5297. Fax: +81-3-5841-8166. E-mail: assmats@mail.ecc.u-tokyo.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the captain and crew of *T&R/V Toyoshio-maru* of Hiroshima University for their assistance in collecting the sponge specimens. We are indebted to Prof. I. Paterson, University of Cambridge, and Prof. R. J. Andersen, University of British Columbia, for valuable discussions and copies of ¹H NMR spectra. This work was partly supported by a Grant-in-Aid for Scientific Research on Innovative Area (23102007) from MEXT.

■ REFERENCES

- (1) Williams, D. E.; Roberge, M.; Van Soest, R.; Andersen, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 5296–5297.
- (2) Warabi, K.; Williams, D. E.; Patrick, B. O.; Roberge, M.; Andersen, R. J. *J. Am. Chem. Soc.* **2007**, *129*, 508–509.
- (3) Williams, D. E.; Lapawa, M.; Feng, X.; Tarling, T.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2004**, *6*, 2607–2610.
- (4) Williams, D. E.; Keyzers, R. A.; Warabi, K.; Desjardine, K.; Riffell, J. L.; Roberge, M.; Andersen, R. J. *J. Org. Chem.* **2007**, *72*, 9842–9845.
- (5) Paterson, I.; Anderson, E. A.; Dalby, S. M.; Lim, J. H.; Genovino, J.; Maltas, P.; Moessner, C. *Angew. Chem.* **2008**, *47*, 3021–3025.
- (6) Benson, S.; Collin, M. P.; O'Neil, G. W.; Ceccon, J.; Fasching, B.; Fenster, M. D.; Godbout, C.; Radkowski, K.; Goddard, R.; Fürstner, A. *Angew. Chem.* **2009**, *48*, 9946–9950.
- (7) Benson, S.; Collin, M. P.; Arlt, A.; Gabor, B.; Goddard, R.; Fürstner, A. *Angew. Chem.* **2011**, *50*, 8739–8744.
- (8) Paterson, I.; Maltas, P.; Dalby, S. M.; Lim, J. H.; Anderson, E. A. *Angew. Chem.* **2012**, *51*, 2749–2753.