Mycaperoxide H, a New Cytotoxic Norsesterterpene Peroxide from a Thai Marine Sponge *Mycale* sp.[1]

Preecha Phuwapraisirisan,[†] Shigeki Matsunaga,[†] Nobuhiro Fusetani,*[,†] Nilnaj Chaitanawisuti,[‡] Sirusa Kritsanapuntu,[⊥] and Piamsak Menasveta[§]

Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, Aquatic Resources Research Institute, Chulalongkorn University, Bangkok 10330, Thailand, Prince of Songkla University, Suratthani Campus, Suratthani 84000, Thailand, and Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

Received August 30, 2002

Mycaperoxide H, a new cyclic norsesterterpene peroxide, was isolated from a Thai marine sponge *Mycale* sp. The structure of mycaperoxide H was deduced by spectroscopic and chemical analysis. Mycaperoxide H was cytotoxic against HeLa cells with an IC_{50} value of 0.8 μg/mL.

Two types of stable cyclic peroxides have been reported from marine sponges: fatty acid-derived peroxides from *Chondrilla*, *Chondrosia* (order Hadromerida), *Callyspongia*, *Xestospongia* (order Haplosclerida), *Plakinastrella*, and *Plakortis* (order Homosclerophorida), and norterpenoid peroxides from *Latrunculia* (order Hadromerida), *Mycale* (order Poecilosclerida), and *Prianos* (order Haplosclerida).[2] These peroxides show a variety of biological activities, including cytotoxicity,[3a,b] antimalarial,[3c] and antileishmania.[3d] During our search for bioactive compounds from Thai sponges, we found considerable cytotoxicity in the lipophilic extract of a *Mycale* sponge collected in the Gulf of Thailand.

Bioassay-guided isolation afforded a new norsesterterpene peroxide, mycaperoxide H (**1**). This paper describes the isolation and structure elucidation of this new metabolite.

The MeOH extract was partitioned between $CHCl_3$ and H_2O, and the organic layer was successively separated by ODS flash chromatography followed by ODS HPLC to furnish mycaperoxide H (**1**) in a yield of 0.00064% based on wet weight, together with the known metabolite mycaperoxide B (**2**).

The molecular formula of **1** was established as $C_{24}H_{40}O_4$ by HRFABMS. The ^{1}H NMR spectrum indicated the presence of four singlet methyls (δ 0.85, 0.89, 0.98, and 1.25), two doublet methyls (δ 0.86 and 1.12), one oxymethine (δ 4.11), and one olefinic proton (δ 5.34), together with unfunctionalized methylenes and methines (δ 1.05−2.49). In addition to these functionalities, an oxygenated quaternary carbon (δ 81.4) and a carboxylic acid (δ 178.2) were observed in the ^{13}C NMR spectrum. Interpretation of COSY, HMQC, and HMBC data led to the gross structure of **1**, which had a bicyclic framework found in the halimane diterpenoids to which was attached a C_{10} side chain encompassing a 1,2-dioxane ring.

The relative stereochemistry of the cyclic peroxide moiety was established by application of Capon's empirical rule;[4] H-3 was axial on the basis of a coupling constant of 8.4 Hz, while the ^{1}H chemical shift of CH_3-2 at 1.12 ppm indicated an *erythro* relationship for C-2 and C-3. CH_3-6 was axially disposed as judged from the ^{13}C chemical shift

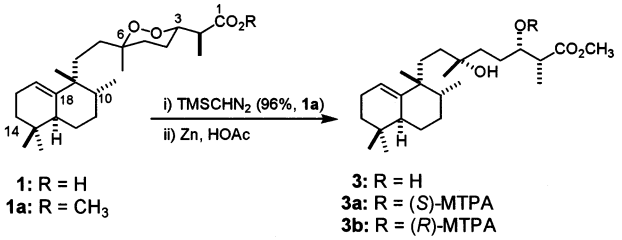


Figure 1. Conversion of mycaperoxide H (**1**) to diol **3** and its MTPA derivatives.

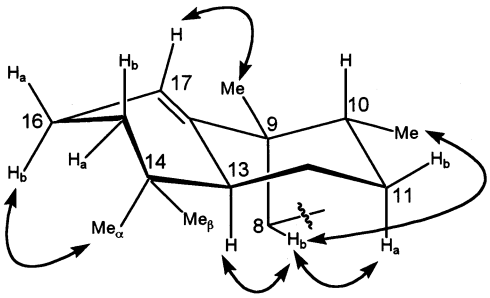


Figure 2. Key NOESY correlations observed for the bicyclic moiety of **1**.

of 21.0 ppm for CH_3-6. To assign the absolute stereochemistry, compound **1** was converted to the methyl ester, followed by reductive cleavage of the peroxide ring to afford diol **3** (Figure 1). Mosher analysis[5] of **3** resulted in the determination of a 3*S* configuration. Therefore, the absolute configuration of the cyclic peroxide moiety was 2*S*, 3*S*, 6*R*.

The relative stereochemistry of the bicarbocyclic portion was assigned on the basis of NOESY data and ^{1}H,^{1}H-coupling constant values. Large coupling constants between H-11a and H-12a ($J_{H11a,H12a}$ = 12 Hz), and H-12a and H-13 ($J_{H12a,H13}$ = 13 Hz), and NOESY cross-peaks between H-11a and H-13, and H-13 and H-8b, demonstrated the conformation of the saturated cyclohexane ring to be chair form with both methyls in equatorial positions. Additional NOESY cross-peaks [H-13/CH_3-14α (intense), H-13/CH_3-14β (medium), H-12b/CH_3-14β (intense), H-15b/CH_3-14β (medium), H-16b/CH_3-14α (intense), H-17/CH_3-9 (intense), H-16a/H-17 (intense), H-16b/H17 (medium), H-7b/CH_3-14α (medium), and H-15b/H-16a] allow us to assign the conformation of the other six-membered ring[6,7] as shown in Figure 2.

* To whom correspondence should be addressed. Tel: +81-3-5841-5299. Fax: +81-3-5841-8166. E-mail: anobu@mail.ecc.u-tokyo.ac.jp.
[†] The University of Tokyo.
[‡] Aquatic Resources Research Institute.
[⊥] Prince of Songkla University.
[§] Department of Marine Science.

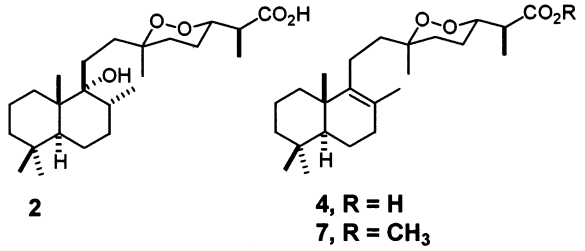
10.1021/np020417d CCC: $25.00 © 2003 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/04/2003

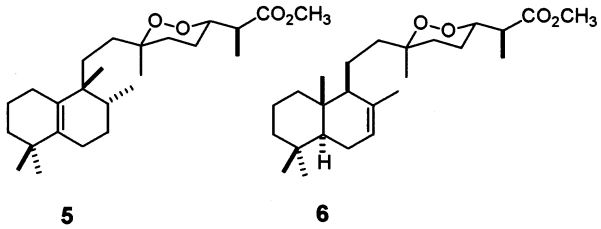
Table 1. ^{1}H and ^{13}C NMR Data of Mycaperoxide H (600 MHz, CD_3OD)

position	δ_C	δ_H mult (J in Hz)[a]	^{1}H−^{1}H COSY	HMBC[b]
1	178.2			
2	44.1	2.49 t (6.6)	CH_3-2, H-3	
CH_3-2	13.4	1.12 d (7.2)	H-2	C-1, C-2, C-3
3	83.3	4.11 dt (4.2, 8.4)	H-2, H-4	C-1, C-2
4	23.8	1.71 (2H, m)	H-3, H-5	ND[c]
5a	33.6	1.55 m	H-4	C-4, C-6, CH_3-6, C-7
5b		1.65 m	H-4	C-3, C-4
6	81.4			
CH_3-6	21.0	1.25 s		C-5, C-7
7a	35.2	1.08 m	H-8ab	C-6, C-8
7b		1.17 dt (3.9, 13.1)	H-8ab	C-6, C-8
8a	24.7	1.10 m	H-7ab	ND
8b		1.70 m	H-7ab	ND
9	43.3			
CH_3-9	23.6	0.98 s		C-8, C-9, C-10, C-18
10	46.2	1.27 m	CH_3-10, H-11ab	C-8, C-9, CH_3-10, C-11
CH_3-10	16.7	0.86 d (7.2)	H-10	C-9, C-10, C-11
11a	32.2	1.48 dq (4.2, 13)	H-10, H-11b, H-12ab	C-9, C-10, CH_3-10, C-12, C-13
11b		1.52 m	H-10, H-11a, H-12ab	C-9, C-10, C-12, C-13
12a	31.4	1.07 m	H-11ab, H-12b, H-13	ND
12b		1.82 ddt (2.7, 12.2, 4.2)	H-11ab, H-12a, H-13	C-10, C-11
13	44.6	1.61 brdd (3.8, 12.7)	H-12ab	C-11, C-14, CH_3-14
14	32.2			
CH_3-14a	28.5	0.85 s		C-13, C-14, CH_3-14b, C-15
CH_3-14b	28.1	0.89 s		C-13, C-14, CH_3-14a, C-15
15a	32.5	1.08 m	H-15b, H-16	ND
15b		1.38 ddd (6.2, 11.2, 13.2)	H-15a, H-16	C-13, C-14, CH_3-14ab, C-16
16a	24.2	1.98 ddt (2.7, 17.4, 5.8,)	H-15ab, H-16b, H-17	C-15, C-17, C-18
16b		2.06 dddt (6.2, 9.2, 17.4, 2.4)	H-15ab, H-16a, H-17	C-15, C-17, C-18
17	118.3	5.34 dd (2.3, 5.0)	H-16ab	C-9, C-13, C-15, C-16
18	147.4			

[a] Assignment based on HMQC, HMBC, and COSY. [b] Correlation from protons to indicated carbons. [c] Not determined due to overlapping.

With mycaperoxide B (**2**) in hand, we envisaged assigning the absolute stereochemistry of mycaperoxide H (**1**) by chemical conversion.[8] Acid treatment of mycaperoxide B (**2**) under mild conditions[9] furnished a dehydration product **4** and its methyl ester **7**, a Wagner-Meerwein rearrangement product **5**, and **6**, in addition to mycaperoxide H methyl ester (**1a**).





^{1}H and ^{13}C NMR data of **7** coincided well with those reported for mycaperoxide G methyl ester[10] and deoxydiacarnoate B benzyl ester.[11] Compound **5** had the same planar structure as that of a dehydration product of mycaperoxide E[12] on the basis of ^{1}H NMR data. Since the absolute stereochemistry of mycaperoxide B (**2**) was proved, successful conversion of mycaperoxide B (**2**) to mycaperoxide H methyl ester (**1a**) established the assignment of the absolute stereochemistry of **1**.

Mycaperoxide H (**1**) was cytotoxic against HeLa cells with an IC_{50} value of 0.8 μg/mL.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on either a JEOL A600 or a JEOL A500 NMR spectrometer. Chemical shifts were referenced to solvent peaks. FAB mass spectra were measured on a JEOL JMX-SX102/SX102 mass spectrometer. Optical rotations were carried out on a Jasco DIP-1000 digital polarimeter.

Animal Material. The sponge *Mycale* sp. (family Mycalidae) was collected off Sichang Island, Thailand, in July 2000, at depths of 7−10 m and was kept frozen until extracted. It has encrusting, arborescent, bushy, irregular branches and a blue color. The surface is opaque and hispid with a mucousy and soft texture. The specimen was primarily identified by N.C. and S.K. and confirmed by Dr. John N. A. Hooper. A voucher specimen (SROT 177) was deposited at Aquatic Resources Research Institute, Chulalongkorn University.

Extraction and Isolation. The frozen sponge *Mycale* sp. (313 g, wet wt) collected off Sichang Island (July 2000) was extracted with MeOH. The crude extract was partitioned between $CHCl_3$ and H_2O, and the organic layer was chromatographed on an ODS flash column. The fractions eluted with MeOH−H_2O (7:3) and MeCN−H_2O (9:1) were combined and subjected to Sephadex LH-20 [MeOH−$CHCl_3$ (1:3 to 1:1)]. Final purification was performed by ODS HPLC with MeOH−H_2O (9:1) to yield mycaperoxide H (**1**, 2 mg) and mycaperoxide B (**2**, 45 mg).

Mycaperoxide H (1): $[\alpha]^{25}_D$ −142.9° (*c* 0.10, acetone); ^{1}H and ^{13}C NMR data in CD_3OD, see Table 1; HRFABMS *m*/*z* 415.2824 (calcd for $C_{24}H_{40}O_4Na$ 415.2824).

Preparation of Diol 3. To a solution of **1** (1.0 mg) in MeOH (200 μL) was added 2.0 M $TMSCHN_2$ in hexane dropwise until a permanent yellow solution persisted. After standing at room temperature for 1 h, the reaction mixture was evaporated to give mycaperoxide H methyl ester (**1a**). A mixture of **1a**, EtOAc (2 mL), HOAc (100 μL), and zinc (60 mg) was stirred at room temperature overnight. The solution was filtered, evaporated,

and separated on a short silica gel column with CH_2Cl_2−EtOAc (1:4) to afford **3**.

Mycaperoxide H methyl ester (1a): ^{1}H NMR (CD_3OD) δ 5.34 (1H, brs, H-17), 4.08 (1H, m, H-3), 3.68 (3H, s, OCH_3), 2.55 (1H, m, H-2), 1.23 (3H, s, CH_3-6), 1.10 (3H, d, J = 7.2 Hz, CH_3-2), 0.98 (3H, s, CH_3-9), 0.88, 0.85 (3H each, s, CH_3-14a, CH_3-14b), 0.86 (3H, d, J = 5.4 Hz, CH_3-10); FABMS m/z 429 (M + Na).

Diol 3: ^{1}H NMR (CD_3OD) δ 5.34 (1H, brs, H-17), 3.67 (3H, s, OCH_3), 3.63 (1H, m, H-3), 2.54 (1H, t, J = 7.8 Hz, H-2), 1.28 (3H, s, CH_3-6), 1.11 (3H, d, J = 7.2 Hz, CH_3-2), 1.02 (3H, s, CH_3-9), 0.91, 0.86 (3H each, s, CH_3-14a, CH_3-14b), 0.87 (3H, d, J = 6.6 Hz, CH_3-10); FABMS m/z 431 (M + Na).

Modified Mosher Analysis of 3. To a solution of **3** (250 μg) in 200 μL of pyridine was added (-)-MTPA chloride (7 μL), and the mixture was left to stand at room temperature overnight. After dilution with 1 M $NaHCO_3$, the reaction mixture was extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The residue was purified on a short silica gel column to afford the tris-(*S*)-MTPA derivative (**3a**). The tris-(*R*)-MTPA derivative (**3b**) was prepared by the same procedure. Selected $\Delta\delta$ values are as follows: CO_2CH_3 = +0.018; H-2 = +0.031; 2-CH_3 = +0.087; 6-CH_3 = 0.000; 9-CH_3 = −0.099.

Acid-Catalyzed Rearrangement of 2. To a solution of **2** (20 mg) in MeOH (500 μL) was added HCl−HOAc−MeOH (1:1:1, 200 μL), and the mixture was heated at 50 °C for 2 h. The reaction mixture was evaporated and partitioned between EtOAc and brine. The organic layer was purified by ODS HPLC (MeOH−H_2O, 9:1) to afford **4** (1 mg), **5** (0.9 mg), **6** (1.4 mg), **7** (14 mg), and mycaperoxide H methyl ester (**1a**, 0.5 mg).

Cyclic peroxide 4: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.27 (1H, m, H-3), 2.66 (1H, m, H-2), 1.58 (3H, s, CH_3-10), 1.37 (3H, s, CH_3-6), 1.18 (3H, d, J = 7.2 Hz, CH_3-2), 1.03 (3H, s, CH_3-18), 0.90, 0.85 (3H each, s, CH_3-14a, CH_3-14b); FABMS m/z 415 (M + Na).

Cyclic peroxide 5: $[\alpha]^{22}_D$ −49.1° (c 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.25 (1H, m, H-3), 3.73 (3H, s, OCH_3), 2.59 (1H, m, H-2), 1.28 (3H, s, CH_3-6), 1.18 (3H, d, J = 6.0 Hz, CH_3-2), 0.99, 0.97, 0.83 (3H each, s, CH_3-9, CH_3-14a, CH_3-14b), 0.84 (3H, d, J = 7.2 Hz, CH_3-10); FABMS m/z 429 (M + Na).

Cyclic peroxide 6: $[\alpha]^{22}_D$ −25.6° (c 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.40 (1H, brs, H-11), 4.26 (1H, d, J = 4.2 Hz, H-3), 3.73 (3H, s, OCH_3), 2.61 (1H, t, J = 7.8 Hz, H-2), 1.98 (1H, brd, J = 18.0 Hz, H-12a), 1.87 (1H, m, H-12b), 1.86 (1H, brd, J = 13.2 Hz, H-17a), 1.73 (2H, m, H-5), 1,72 (2H, m, H-4), 1.71 (3H, s, CH_3-10), 1.57 (1H, m, H-16a), 1.55 (1H, m, H-9), 1.51 (2H, m, H-8), 1.47 (1H, m, H-16b), 1.43 (2H, m, H-15), 1.35 (3H, s, CH_3-6), 1.19 (1H, m, H-13), 1.18 (3H, d, J = 7.2 Hz, CH_3-2), 1.18 (2H, m, H-7), 0.96 (1H, dt, J = 2.4, 12.0 Hz, H-17), 0.89, 0.87, 0.77 (3H each, s, CH_3-14a, CH_3-14b, CH_3-18); ^{13}C NMR ($CDCl_3$, 150 MHz)[13] δ 174.5 (C-1), 135.1 (C-10), 122.3 (C-11), 81.8 (C-3), 80.6 (C-6), 55.3 (C-9), 51.9 (CO_2CH_3), 50.2 (C-13), 42.6 (C-2), 42.2 (C-7, C-15), 39.3 (C-17), 36.9 (C-18), 33.3 (14-CH_3), 33.1 (C-14), 32.0 (C-5), 23.7 (C-12), 23.0 (C-4), 22.2 (CH_3-10), 22.0 (CH_3-14), 20.3 (C-8, CH_3-6), 18.8 (C-16), 13.7 (CH_3-18), 12.9 (CH_3-2); HRFABMS m/z 407.3160 (calcd for $C_{25}H_{43}O_4$ 407.3159, M + H).

Cyclic peroxide 7: $[\alpha]^{23}_D$ −44.1° (c 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.26 (1H, m, H-3), 3.73 (3H, s, OCH_3), 2.62 (1H, m, H-2), 1.57 (3H, s, 10-CH_3), 1.35 (3H, s, CH_3-6), 1.18 (3H, d, J = 7.2 Hz, CH_3-2), 1.00 (3H, s, CH_3-18), 0.90, 0.85 (3H each, s, CH_3-14); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.5 (C-1), 139.6 (C-9), 126.2 (C-10), 81.7 (C-3), 51.9 (C-8, C-13, CO_2CH_3), 42.7 (C-2), 41.8 (C-15), 40.4 (C-7), 39.1 (C-18), 37.0 (C-17), 33.6 (C-11), 33.3 (C-14, 14-CH_3), 32.1 (C-5), 22.8 (C-4), 21.7 (CH_3-14), 21.1 (CH_3-18), 20.1 (CH_3-6), 19.4 (CH_3-10), 19.0 (C-12, C-16), 12.8 (CH_3-2), C-6 not detected; FABMS m/z 429 (M + Na).

Acknowledgment. We thank J. N. A. Hooper (Queensland Museum) for sponge identification and U. Kokpol for his initial encouragement in this project. P.P. is grateful to Chulalongkorn University for a degree fellowship under Thailand-Japan Technology Transfer Project (TJTTP). This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: 1D and 2D NMR spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Part 115 of Bioactive Marine Metabolites Series. Part 114: Okada, Y.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *Org. Lett.* **2002**, *4*, 3039−3042.
(2) (a) Casteel, D. A. *Nat. Prod. Rep.* **1992**, *9*, 289−312. (b) Casteel, D. A. *Nat. Prod. Rep.* **1999**, *16*, 55−73.
(3) For recent reports, see: (a) Campagnuolo, C.; Fattorusso, E.; Taglialatela-Scafati, O.; Ianaro, A.; Pisano, B. *Eur. J. Org. Chem.* **2002**, *1*, 61−69. (b) Williams, D. E.; Allen, T. M.; Van Soest, R.; Behrisch, H. W.; Andersen, R. J. *J. Nat. Prod.* **2001**, *64*, 281−285. (c) Gochfeld, D. J.; Hamann, M. T. *J. Nat. Prod.* **2001**, *64*, 1477−1479. (d) Compagnone, R. S.; Pina, I. C.; Rangel, H. R.; Dagger, F.; Suarez, A. I.; Reddy, M. V. R.; Faulkner, D. J. *Tetrahedron* **1998**, *54*, 3057−3068.
(4) Capon, R. J.; Macleod, J. K. *Tetrahedron* **1985**, *41*, 3391−3404.
(5) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(6) The assignment of relative stereochemistry of the bicyclic portion of mycaperoxide H was supported by carbon chemical shifts. Analysis of the ^{13}C NMR data of six previously reported compounds showed that they consist of two distinctive patterns. In austrodorin,[7a] 5-epi-agalasine C,[7b] toxiusol,[7c] and halisulfate 7,[7d] C-8, C-10, and C-12 resonated at 24−29, 45, and 30 ppm, respectively; in agelasine C[7e] and a plant-derived diterpene,[7f] these carbons resonated at 38, 40, and 24 ppm, respectively. It is highly likely that the carbon chemical shift values reflect the relative stereochemistry in the bicyclic portion.
(7) (a) Gavagnin, M.; Trivellone, E.; Castelluccio, F.; Cimino, G.; Cattaneo-Vietti, R. *Tetrahedron Lett.* **1995**, *36*, 7319−7322. (b) Hattori, T.; Adachi, K.; Shizuri, Y. *J. Nat. Prod.* **1997**, *60*, 411−413. (c) Isaacs, S.; Hizi, A.; Kashman, Y. *Tetrahedron* **1993**, *49*, 4275−4282. (d) Kernan, M. R.; Faulkner, D. J. *J. Org. Chem.* **1988**, *53*, 4574−4578. (e) Nakamura, H.; Wu, H.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1984**, *28*, 2989−2992. (f) Hara, N.; Asaki, H.; Fujimoto, Y.; Gupta, Y. K.; Singh, A. K.; Sahai, M. *Phytochemistry* **1995**, *38*, 189−194.
(8) The structure of mycaperoxide B was proposed on the basis of spectral data and a biogenetic argument. See: Tanaka, J.; Higa, T.; Suwanborirux, K.; Kokpol, U.; Bernardinelli, G.; Jefford, C. W. *J. Org. Chem.* **1993**, *58*, 2999−3002.
(9) Capon, R. J.; Macleod, J. K. *J. Nat. Prod.* **1987**, *50*, 225−229.
(10) Capon, R. J.; Rochfort, S. J.; Oveden, S. P. B.; Metzger, R. P. *J. Nat. Prod.* **1998**, *61*, 525−528.
(11) D'Ambrosio, M.; Guerriero, A.; Deharo, E.; Debitus, C.; Munoz, V.; Pietra, F. *Helv. Chim. Acta* **1998**, 1285−1292.
(12) Capon, R. J. *J. Nat. Prod.* **1991**, *54*, 190−195.
(13) Carbon chemical shifts of **6** were deduced from HMBC and HMQC spectra.

NP020417D