Terpene Isocyanides, Isocyanates, and Isothiocyanates from the Okinawan Marine Sponge *Stylissa* sp.

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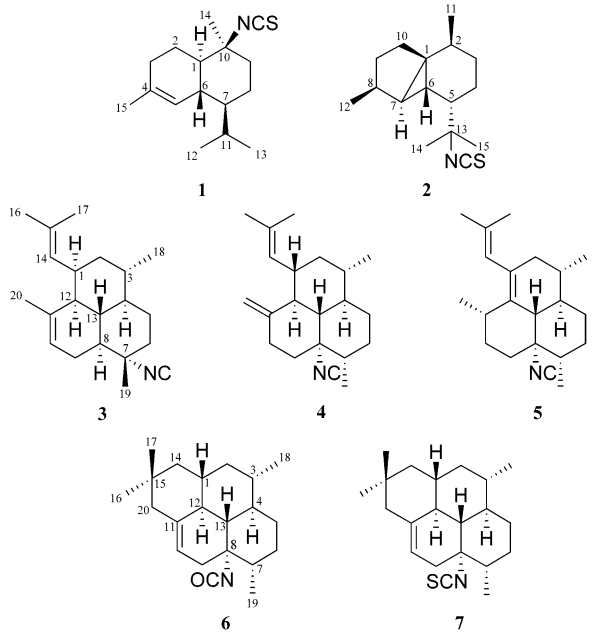
Received October 22, 2003

Seven new nitrogenous terpenoids, (1*R**,6*R**,7*S**,10*S**)-10-isothiocyanatocadin-4-ene (**1**), (1*S**,2*S**,5*S**,6*S**,7*R**,8*S**)-13-isothiocyanatocubebane (**2**), (1*R**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-10,14-diene (**3**), (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-8-isocyanoamphilecta-11(20),14-diene (**4**), (3*S**,4*R**,7*S**,8*S**,11*S**,13*S**)-8-isocyanoamphilecta-1(12),14-diene (**5**), 8-isocyanatocycloamphilect-10-ene (**6**), and 8-isothiocyanatocycloamphilect-10-ene (**7**), were isolated from the Okinawan sponge *Stylissa* sp., along with 12 known related compounds. Structural determinations of these compounds were made by spectroscopic analysis, and assessment was made of their cytotoxicity toward HeLa cells.

Terpene isocyanides, isocyanates, and isothiocyanates are frequently isolated from marine sponges.[1,2] Many such compounds express antimicrobial,[3] antifungal,[4] cytotoxic,[5] antifouling,[6] and antimalarial activity.[7] These terpenoids are also interesting from the perspective of the origin of their nitrogenous functionalities.[8] During the course of our investigation on the chemical constituents of Okinawan marine invertebrates,[9] isolation was made of seven new nitrogenous terpenoids, (1*R**,6*R**,7*S**,10*S**)-10-isothiocyanatocadin-4-ene (**1**), (1*S**,2*S**,5*S**,6*S**,7*R**,8*S**)-13-isothiocyanatocubebane (**2**), (1*R**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-10,14-diene (**3**), (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-8-isocyanoamphilecta-11(20),14-diene (**4**), (3*S**,4*R**,7*S**,8*S**,11*S**,13*S**)-8-isocyanoamphilecta-1(12),14-diene (**5**), 8-isocyanatocycloamphilect-10-ene (**6**), and 8-isothiocyanatocycloamphilect-10-ene (**7**) from the Okinawan sponge *Stylissa* sp., along with several known compounds of a related nature. Their structural elucidation and assessment for cytotoxicity toward HeLa cells were conducted in the present study.



1 **2** **3** **4** **5** **6** **7**

Results and Discussion

Sponge specimens of *Stylissa* sp. (wet wt 2.4 kg) were extracted with MeOH and then acetone. The combined extracts were partitioned between H_2O and EtOAc. The EtOAc-soluble portion was partitioned between 80% aqueous MeOH and *n*-hexane. The *n*-hexane-soluble portion was purified to give new nitrogenous terpenoids **1**−**7** along with 12 known related compounds.[10−16]

Compounds **1** and **2** were found to have the same molecular formula $C_{16}H_{25}NS$ based on high-resolution mass measurement data. From IR (2086 and 2102 cm^{-1}, respectively), UV (246 nm, both), and ^{13}C NMR (δ_C 129.5 and 128.7, respectively), both compounds appeared to be sesquiterpene isothiocyanates. Compound **1** was found to possess a trisubstituted C−C double bond [δ_H 5.47 (1H, d, $J = 4.5$), δ_C 123.5 (CH), 134.6 (C)] according to ^1H and ^{13}C NMR (Table 1). Determination was made of the carbon sequences in **1**, C-1 to C-3, C-1 to C-6, C-5 to C9, C-7 to C-11, and C-12 to C-13 through C-11 based on data from HMQC and $^1H-^1H$ COSY. The HMBC spectrum indicated Me-15 to be correlated with C-3, C-4, and C-5, while Me-14 correlated with C-1, C-9, and C-10. On the basis of these findings, the planar structure of **1** was clarified. The planar structure of **1** was identical to that of 10-isothiocyanatocadin-4-ene,[12] but these two compounds were found to be stereoisomers from a direct comparison of their NMR spectra. The relative configuration of **1** was determined on the basis of the following NOESY correlations: those of H-1 with H-7 and Me-14, and that of H-6 with Me-13. The structure of **1** was confirmed to be (1*R**,6*R**,7*S**,10*S**)-10-isothiocyanatocadin-4-ene. In compound **2** were found present the sequences C-2 to C-10, C-8 to C-12, and C-2 to C-11, on the basis of HMQC and $^1H-^1H$ COSY data. The planar structure was derived on the basis of the following correlations in the HMBC spectrum: those of C-1, C-6, C-7, and C-10 with H-2; those of C-1, C-2, C-6, and C-7 with H-10; those of C-5 and C-13 with Me-14; and those of C-5 and C-13 with Me-15. From comparison of the NMR spectra of **2** and (1*S**,2*R**,5*S**,6*S**,7*R**,8*S**)-13-isothiocyanatocube-

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10.1021/np030458p CCC: $27.50
Published on Web 04/21/2004

Table 1. NMR Data for **1** and **2**

	1		**2**	
no.	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1	44.4 (CH)	1.77 (1H, m)	30.8 (C)	
2	20.2 (CH_2)	1.88 (1H, m)	30.9 (CH)	1.81 (1H, dq, J = 6.6, 6.9)
		1.72 (1H, m)		
3	31.0 (CH_2)	2.03 (1H, m)	33.1 (CH_2)	1.62 (1H, m)
		2.02 (1H, m)		0.90 (1H, m)
4	134.6 (C)		20.8 (CH_2)	1.52 (1H, m)
				0.92 (1H, m)
5	123.5 (CH)	5.47 (1H, d, J = 4.5)	44.9 (CH)	1.92 (1H, m)
6	35.3 (CH)	1.98 (1H, m)	16.7 (CH)	0.83 (1H, d, J = 4.2)
7	43.5 (CH)	1.29 (1H, m)	31.8 (CH)	1.06 (1H, d, J = 4.2)
8	20.0 (CH_2)	1.48 (1H, m)	34.7 (CH)	2.26 (1H, m)
		1.17 (1H, m)		
9	34.4 (CH_2)	1.83 (1H, m)	29.2 (CH_2)	1.54 (1H, m)
		1.77 (1H, m)		0.72 (1H, m)
10	64.9 (C)		31.6 (CH_2)	1.72 (1H, m)
				1.71 (1H, m)
11	26.3 (CH)	1.88 (1H, m)	20.3 (CH_3)	1.01 (3H, d, J = 6.9)
12	21.4 (CH_3)	0.88 (3H, d, J = 7.0)	18.3 (CH_3)	1.00 (3H, d, J = 6.6)
13	15.1 (CH_3)	0.80 (3H, d, J = 7.0)	65.1 (C)	
14	26.9 (CH_3)	1.50 (3H, s)	27.5 (CH_3)	1.41 (3H, s)
15	23.4 (CH_3)	1.66 (3H, s)	26.8 (CH_3)	1.41 (3H, s)
NCS	129.5 (C)		128.7 (C)	

[a] 125 MHz, $CDCl_3$. [b] 500 MHz, $CDCl_3$.

bane,[17] **2** was shown to likely be a stereoisomer at the C-2 position. The relative configuration of **2** was confirmed to be 1*S**,2*S**,5*S**,6*S**,7*R**,8*S** from the following NOESY correlations: Me-11 and Me-12 with H-10β (δ_H 1.71); H-5 and Me-12 with H-6; H-2 and Me-15 with H-7.

The common molecular formula, $C_{21}H_{31}N$, was established on the basis of high-resolution mass measurement data for each of **3**, **4**, and **5**. All these compounds were found to be diterpene isocyanides from IR (2130, 2123, and 2127 cm^{-1}, respectively) and ^{13}C NMR (δ_C 152.9, 155.4, and 155.4, respectively). Compound **3** possessed the carbon sequences C-1 to C-6, C-1 to C-12, C-1 to C-14, C-3 to C-18, C-4 to C-13, C-8 to C-10, C-8 to C-13, and C-12 to C-13, from HMQC and $^1H-^1H$ COSY data. The planar structure of **3** was identical to that of the known metabolite (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-10,14-diene[14] as indicated by the following HMBC correlations: C-7 with H-5; C-6 with H-8; C-14, C-15, and Me-17 with Me-16; C-6 with Me-19; C-10, C-11, and C-12 with Me-20. The ^1H and ^{13}C NMR spectra of **3** (Table 2), however, differed from that of (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-10,14-diene as given in the literature. The following NOESY correlations clearly demonstrated **3** to be a C-1 epimer of (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-10,14-diene: H-13 and H-14 with H-3; H-4 and H-12 with H-8; Me-19 with H-13.

Compound **4** was noted to possess four methyls, five sp^3 methylenes, one sp^2 methylene, six sp^3 methines, one sp^2 methine, one sp^3 quaternary carbon, two sp^2 quaternary carbons, and one sp quaternary carbon from ^{13}C NMR and DEPT spectra (Table 3). HMQC and $^1H-^1H$ COSY correlations indicated the connections from C-1 to C-7, C-1 to C-12, C-1 to C-14, C-3 to C-18, C-4 to C-13, C-7 to C-19, C-9 to C-10, and C-12 to C-13 in **4**. HMBC correlations between C-8/C-9 and H-13; C-14/C-15/Me-17 and Me-16; C-4 and Me-18; C-8 and Me-19; and C-10/C-11/C-12 and H-20 confirmed the planar structure of **4** to be 8-isocyanoamphilecta-11(20),14-diene. The relative stereochemistry of **4** was clearly shown to be 1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S** on the basis of the following NOESY correlations: H-4/H-12, Me-18; H-9β (δ_H 1.27)/H-7; H-13/H-1, H-3, H-7; H-14/Me-16, H-12.

From ^{13}C NMR and DEPT, all 21 carbons of **5** were observed as five methyls, five sp^3 methylenes, five sp^3

Table 2. NMR Data for **3**

no.	δ_C[a]	δ_H[b]
1	33.0 (CH)	2.92 (1H, m)
2	35.5 (CH_2)	1.29 (1H, m)
		1.05 (1H, dt, J = 9.2, 4.0)
3	31.1 (CH)	1.26 (1H, m)
4	37.4 (CH)	0.95 (1H, m)
5	26.8 (CH_2)	1.88 (1H, m)
		0.93 (1H, m)
6	34.0 (CH_2)	2.04 (1H, m)
		1.80 (1H, m)
7	60.6 (C)	-
8	42.7 (CH)	1.82 (1H, m)
9	25.0 (CH_2)	2.26 (1H, m)
		2.13 (1H, m)
10	121.8 (CH)	5.44 (1H, m)
11	133.8 (C)	
12	44.5 (CH)	2.00 (1H, m)
13	34.6 (CH)	1.70 (1H, m)
14	127.4 (CH)	5.46 (1H, m)
15	131.3 (C)	
16	26.0 (CH_3)	1.72 (3H, s)
17	17.8 (CH_3)	1.64 (3H, s)
18	19.8 (CH_3)	0.80 (3H, d, J = 6.0)
19	26.5 (CH_3)	1.57 (3H, s)
20	21.1 (CH_3)	1.72 (3H, s)
NC	152.9 (C)	

[a] 125 MHz, $CDCl_3$. [b] 500 MHz, $CDCl_3$.

Table 3. NMR Data for **4** and **5**

	4		**5**	
no.	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1	37.4 (CH)	2.37 (1H, m)	132.2 (C)	
2	41.1 (CH_2)	1.53 (1H, m)	41.4 (CH_2)	1.85 (1H, m)
		0.94 (1H, m)		1.76 (1H, m)
3	36.2 (CH)	1.13 (1H, m)	32.5 (CH)	1.29 (1H, m)
4	42.7 (CH)	1.15 (1H, m)	41.2 (CH)	1.32 (1H, m)
5	29.8 (CH_2)	2.00 (1H, m)	28.9 (CH_2)	1.92 (1H, m)
		0.85 (1H, m)		0.93 (1H, m)
6	29.9 (CH_2)	1.56 (1H, m)	30.7 (CH_2)	1.62 (1H, m)
		1.46 (1H, m)		1.47 (1H, m)
7	40.7 (CH)	1.38 (1H, m)	41.9 (CH)	1.50 (1H, m)
8	66.8 (C)		66.3 (C)	
9	38.6 (CH_2)	2.24 (1H, m)	35.2 (CH_2)	2.22 (1H, m)
		1.27 (1H, m)		1.57 (1H, m)
10	32.8 (CH_2)	2.38 (1H, m)	28.6 (CH_2)	1.57 (1H, m)
		2.23 (1H, m)		1.56 (1H, m)
11	149.4 (C)		36.3 (CH)	2.17 (1H, m)
12	45.9 (CH)	1.95 (1H, dd, J = 11.0, 10.5)	131.5 (C)	
13	54.5 (CH)	0.95 (1H, m)	52.2 (CH)	1.83 (1H, m)
14	131.0 (CH)	4.86 (1H, d, J = 8.7)	126.8 (CH)	5.67 (1H, s)
15	129.1 (C)		131.6 (C)	
16	25.6 (CH_3)	1.64 (3H, s)	25.1 (CH_3)	1.70 (3H, s)
17	17.8 (CH_3)	1.65 (3H, s)	19.1 (CH_3)	1.63 (3H, s)
18	19.6 (CH_3)	0.89 (3H, d, J = 6.5)	18.4 (CH_3)	0.88 (3H, d, J = 6.5)
19	15.7 (CH_3)	0.99 (3H, d, J = 6.3)	15.5 (CH_3)	1.01 (3H, d, J = 6.5)
20	107.4 (CH_2)	4.74 (1H, s)	20.8 (CH_3)	1.10 (3H, d, J = 6.5)
		4.52 (1H, s)		(3H, d, J = 6.5)
NC	155.4 (C)		155.4 (C)	

[a] 125 MHz, $CDCl_3$. [b] 500 MHz, $CDCl_3$.

Table 4. NMR Data for **6** and **7**

	6		**7**	
no.	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1	37.8 (CH)	1.36 (1H, m)	37.9 (CH)	1.31 (1H, m)
2	38.2 (CH_2)	1.20 (1H, m)	43.1 (CH_2)	1.56 (1H, m)
		1.20 (1H, m)		0.90 (1H, m)
3	43.2 (CH)	1.55 (1H, m)	38.1 (CH)	1.20 (1H, m)
4	43.2 (CH)	1.05 (1H, m)	43.2 (CH)	1.04 (1H, m)
5	30.0 (CH_2)	1.96 (1H, m)	29.9 (CH_2)	2.02 (1H, m)
		1.48 (1H, m)		0.89 (1H, m)
6	30.0 (CH_2)	1.36 (1H, m)	30.1 (CH_2)	1.54 (1H, m)
		1.28 (1H, m)		1.38 (1H, m)
7	41.7 (CH)	1.33 (1H, m)	42.0 (CH)	1.41 (1H, m)
8	62.2 (C)		66.0 (C)	
9	38.6 (CH_2)	2.32 (1H, dd, J = 16.4, 5.6)	37.3 (CH_2)	2.42 (1H, dd, J = 17.6, 5.5)
		2.07 (1H, m)		2.06 (1H, m)
10	116.0 (CH)	5.21 (1H, m)	115.4 (CH)	5.20 (1H, m)
11	137.6 (C)		137.6 (C)	
12	44.5 (CH)	1.40 (1H, m)	44.4 (CH)	1.48 (1H, m)
13	50.8 (CH)	1.07 (1H, m)	50.7 (CH)	1.11 (1H, t, J = 10.0)
14	46.3 (CH_2)	1.32 (1H, m)	46.2 (CH_2)	1.32 (1H, m)
		1.03 (1H, m)		1.07 (1H, m)
15	32.2 (CH_3)		31.7 (CH_3)	
16	31.7 (CH_3)	0.92 (3H, s)	32.2 (CH_3)	0.93 (3H, s)
17	25.1 (CH_3)	0.80 (3H, s)	25.1 (CH_3)	0.79 (3H, s)
18	19.6 (CH_3)	0.94 (3H, d, J = 6.0)	19.5 (CH_3)	0.93 (3H, d, J = 6.7)
19	15.5 (CH_3)	0.90 (3H, d, J = 6.4)	15.6 (CH_3)	0.99 (3H, d, J = 6.3)
20	47.6 (CH_2)	1.88 (1H, m)	47.5 (CH_2)	1.92 (1H, m)
		1.87 (1H, m)		1.88 (1H, m)

[a] 125 MHz, $CDCl_3$. [b] 500 MHz, $CDCl_3$.

methines, one sp^2 methine, one sp^3 quaternary carbon, three sp^2 quaternary carbons, and one sp quaternary carbon (Table 3). The sequences C-2 to C-7, C-3 to C-18, C-4 to C-13, C-9 to C-11, and C-11 to C-20 were noted in the HMQC and $^1H-^1H$ COSY spectra of **5**. The planar structure was clarified from the following HMBC correlations: C-1/C-8/C-9/C-12 and H-13; C-2 and H-14; C-14/C-15/C-17 and Me-16; C-8 and Me-19; C-12 and Me-20. NOESY correlations between H-3/H-7/H-11 and H-13 and between H-4 and Me-18 indicated **5** to be (3*S**,4*R**,7*S**,8*S**,-11*S**,13*S**)-8-isocyanoamphilecta-1(12),14-diene.

From NMR (Table 4), MS, IR, and UV spectra, **6** ($C_{21}H_{31}$-NO) and **7** ($C_{21}H_{31}NS$) appeared to be cycloamphilectane diterpenoids, with an isocyanato and isothiocyanato group at C-8, respectively. The structures of **6** and **7** were determined by synthesis from 8-isocyanocycloamphilect-10-ene.[16] On treating 8-isocyanocycloamphilect-10-ene with $Pb(OAc)_4$ in benzene,[18] isocyanate **6** was obtained (Figure 1). Isothiocyanate **7** was afforded from 8-isocyanocycloamphilect-10-ene with use of sulfur, tellurium, and Et_3N in THF.[19] For X-ray crystallography, 8-isocyanocycloamphilect-10-ene was converted to *p*-bromobenzamide **9** by hydrolysis of the isonitrile group to give amine **8**, followed by amidation (Figure 2). The absolute configuration of 8-isocyanocycloamphilect-10-ene was confirmed to be 1*S*,3*S*,4*R*,7*S*,8*S*,12*S*,13*S* on the basis of the Flack parameter[20] [0.023(5)] in the X-ray analysis of *p*-bromobenzamide **9** (Figure 3).[21] These results clearly indicate **6** and **7** to each have the 1*S*,3*S*,4*R*,7*S*,8*S*,12*S*,13*S* configuration.

The new nitrogenous terpenoids (**1**, **2**, **3**, **5**, **6**, and **7**) were found to display weak cytotoxicity (IC_{50} 73.7, 57.8, 20.0, 11.2, 38.3, and 88.7 μM, respectively) toward HeLa cells in vitro.[22,23]

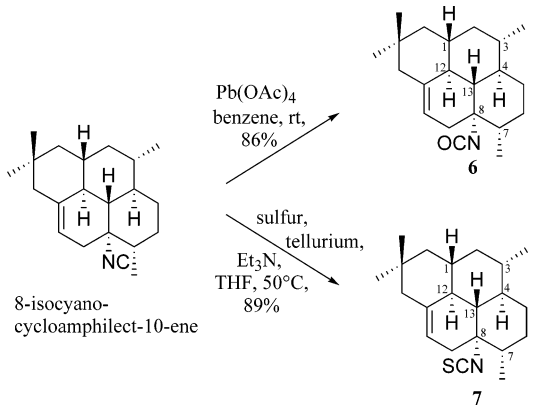

Figure 1. Synthesis of **6** and **7** from 8-isocyanocycloamphilect-10-ene.

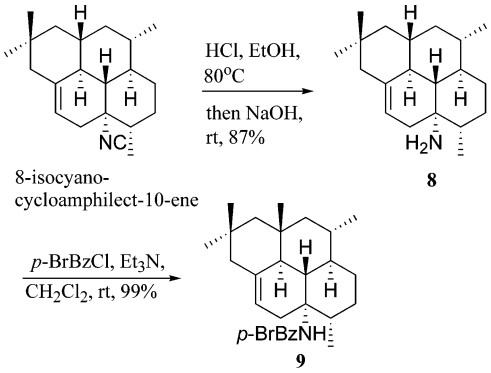

Figure 2. Synthesis of **9** from 8-isocyanocycloamphilect-10-ene.

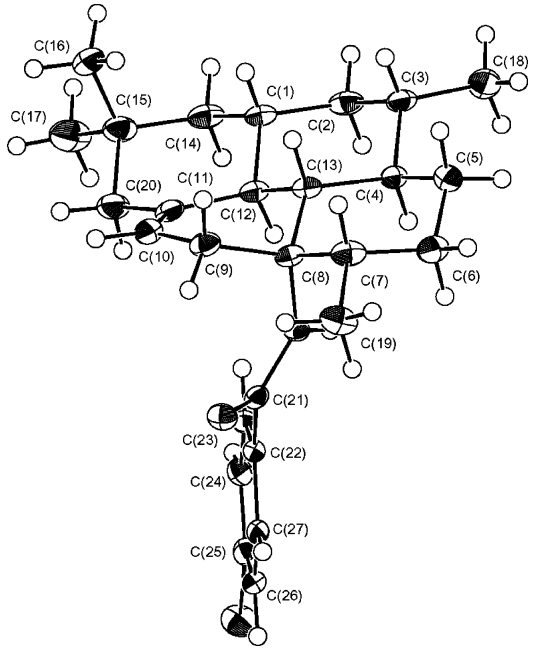

Figure 3. ORTEP drawing of *p*-bromobenzamide **9**.

Experimental Section

General Experimental Procedures. Optical rotation was measured with a JASCO DIP-360 polarimeter, IR spectra were taken with a JASCO FT-IR/620 spectrometer, and UV spectra were taken with a JASCO V-550 spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker DPX-400 and DRX-500 spectrometers. Chemical shifts were expressed on a δ (ppm) scale with tetramethylsilane (TMS) as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). EIMS were obtained with a Thermo Quest TSQ 700 spectrometer, and the high-resolution EIMS (HREIMS) spectrum was ob-

tained using a VG Auto Spec E spectrometer. Electorospray ionization (ESI) MS was obtained with a Micromass LCT spectrometer. X-ray diffraction was measured on a Bruker MXC18 KHF22 diffractometer. Flash column chromatography was carried out on Kanto Chemical silica gel 60N (spherical, neutral) 40−50 μm or ODS Wakogel LP-40 C-18. HPLC separation was conducted using a YMC-Pack R&D ODS (250 × 20 mm) column and UV detector (254 nm).

Animal Material. Sponge specimens (*Stylissa* sp. (family Axinellidae)) were taken from the coral reef of Iriomote Island, Okinawa, Japan, at a depth of 5 m by hand using scuba, in June 2001. A voucher specimen has been deposited at University of Amsterdam (ZMA POR 17253), and another may be found at Tokyo University of Pharmacy and Life Science (S-01-2).

Extraction and Isolation. Wet specimens (2.4 kg) were cut into small pieces and extracted with MeOH (8.0 L × 4) and then acetone (8.0 L × 1). The combined extract (146 g) was concentrated and partitioned between EtOAc (2.0 L × 3) and water (1.0 L) to give an EtOAc-soluble portion (18.6 g), which was then dissolved in 80% aqueous MeOH (700 mL) and extracted with *n*-hexane (400 mL × 4) to afford an *n*-hexane-soluble portion (14.8 g).

The *n*-hexane-soluble portion was chromatographed on Si gel with *n*-hexane, EtOAc, and then MeOH as eluent to produce fractions FH-1 (0.44 g), -2 (0.81 g), -3 (3.32 g), -4 (3.17 g), -5 (1.37 g), and -6 (5.44 g).

Flash ODS-Si gel column chromatography (elution with acetone−water (9:1)) was conducted on FH-2 to give fractions FH-21 (718 mg) and -22 (89.8 mg). The former underwent flash ODS-Si gel column chromatography with acetonitrile to provide *ent*-epipolasin[10] (20.8 mg) and **2** (284 mg). The ODS-HPLC of FH-22 (elution with acetonitrile then MeOH−acetone (4:1)) provided **6** (24.3 mg) and **7** (29.0 mg).

The flash Si gel column chromatography of FH-3 (elution with *n*-hexane) produced fractions FH-31 (3.21 g) and FH-32 (84.6 mg). FH-31 flash ODS-Si gel column chromatography (elution with acetone−water (9:1)) and ODS-HPLC (elution with acetonitrile then MeOH−water (4:1)) gave 10-isothiocyanatoamorph-4-ene[13] (2.05 g) and 10α-isothiocyanoalloaromadendrane[11] (171 mg). FH-32 ODS-HPLC (elution with acetonitrile−MeOH (1:1)) afforded **1** (24.5 mg) and 10-isothiocyanatocadin-4-ene[12] (2.7 mg).

FH-4 underwent flash ODS-Si gel column chromatography (elution with acetonitrile) to give fractions FH-41 (2.45 g) and -42 (714 mg). ODS-HPLC of the former fraction (elution with MeOH then acetonitrile−acetone (49:1)) provided 8-isocyanocycloamphilect-10-ene[16] (2.00 g), 8-isocyanocycloamphilect-11-ene[16] (418 mg), **4** (4.1 mg), and **5** (29.8 mg).

FH-5 ODS-HPLC (elution with acetonitrile then acetonitrile−MeOH (7:3)) produced 10-isothiocyanatoamorph-5-en-4-ol[13] (23.6 mg), (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-10,14-diene[14] (98.4 mg), (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-11(20),14-diene[14] (247 mg), (3*S**,4*R**,7*S**,8*R**,13*R**,14*R**)-7-isocyanoneoamphilecta-11,15-diene[15] (173 mg), (1(14)-*E*,3*S**,4*R**,7*S**,8*S**,11*R**,12*R**,13*R**)-7-isocyanoneoamphilecta-1(14),15-diene[14] (42,7 mg), **3** (101 mg), and (1*R**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-11(20),14-diene[14] (135 mg).

(1*R,6*R**,7*S**,10*S**)-10-Isothiocyanatocadin-4-ene (1):** colorless powder; mp 54−56 °C; $[\alpha]^{26}_D$ +75.7 (*c* 2.4, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 246 (1085) nm; IR (KBr) ν_{max} 2086 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; $^1H-^1H$ COSY correlation (H/H) H-1/H-2α (δ_H 1.88), H-6; H-2α (δ_H 1.88)/H-3α (δ_H 2.02); H-2β (δ_H 1.72)/H-3β (δ_H 2.02); H-5/H-6; H-6/H-7; H-7/H-8α (δ_H 1.17), H-8β (δ_H 1.48), H-11; H-8β/H-9α (δ_H 1.83), H-9β (δ_H 1.77); H-11/Me-12, Me-13; HMBC correlation (H/C) H-1/C-2, C-5; H-2/C-4, C-6; H-3/C-2, C-4, C-5, Me-15; H-5/C-6, Me-15; H-6/C-2, C-7; H-7/C-9, C-11, Me-12, Me-13; H-8/C-7, C-9; H-9/C-1, C-8, Me-14; H-11/Me-12, Me-13; Me-12/C-7, Me-13; Me-13/C-7, C-11, Me-12; Me-14/C-1, C-9; Me-15/C-4, C-5; NOESY correlation (H/H) H-1/H-2α (δ_H 1.88), H-7, Me-14; H-6/H-2β (δ_H 1.72), Me-12; H-5/H-11, Me-12, Me-15; H-8β (δ_H 1.48)/Me-13; Me-14/H-9α (δ_H 1.83), H-2α; EIMS *m/z* 263 [M^+] (100), 205 (16), 161 (32); HREIMS *m/z* 263.1696 (calcd for $C_{16}H_{25}NS$, 263.1708).

(1*S,2*S**,5*S**,6*S**,7*R**,8*S**)-13-Isothiocyanatocubebane (2):** colorless oil; $[\alpha]^{26}_D$ −15.0 (*c* 1.2, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 246 (1339) nm; IR (neat) ν_{max} 2102 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; $^1H-^1H$ COSY correlation (H/H) H-2/H-3α (δ_H 0.90), H-3β (δ_H 1.62), Me-11; H-3α/H-4α (δ_H 1.52); H-3β/H-4α, H-5; H-4β (δ_H 0.92)/H-5; H-5/H-6; H-6/H-7; H-7/H-8; H-8/H-9α (δ_H 1.54), H-9β (δ_H 0.72), Me-12 (δ_H 1.00); H-9α/H-10α (δ_H 1.71); HMBC correlation (H/C) H-2/C-1, C-3, C-6, C-7; H-3/C-1, C-2, C-11; H-4/C-2, C-3,C-6,C-13; H-5/C-3, C-6, C-7, C-13, C-14, C-15; H-6/C-1, C-4, C-5, C-8, C-10; H-7/C-2, C-5, C-6, C-8, C-9, C-10; H-8/C-6, C-7, C-9; H-9/C-1, C-7, C-8, C-10; H-10/C-1, C-2, C-6, C-7, C-8, C-9; Me-11/C-1, C-2, C-3; Me-12/C-7, C-8, C-9; Me-14/C-5, C-13; Me-15/C-5, C-13; NOESY correlation (H/H) H-4α (δ_H 1.52)/Me-14 or Me-15; H-4β (δ_H 0.92)/H-5; H-6/H-5, H-9β (δ_H 0.72), Me-12; H-7/H-2, H-8, Me-14 or Me-15; H-10β (δ_H 1.71)/Me-11, Me-12; EIMS *m/z* 263 [M^+] (26), 205 (50), 163 (100), 107 (32); HREIMS *m/z* 263.1694 (calcd for $C_{16}H_{25}NS$, 263.1708).

(1*R,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-Isocyanoamphilecta-10,14-diene (3):** colorless oil; $[\alpha]^{24}_D$ −19.1 (*c* 1.52, $CHCl_3$); IR (neat) ν_{max} 2130 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; $^1H-^1H$ COSY correlation (H/H) H-1/H-2α (δ_H 1.29), H-2β (δ_H 1.05), H-12, H-14; H-2β/H-3; H-3/H-4, Me-18; H-4/H-5α (δ_H 1.88), H-13; H-5α/H-6α (δ_H 2.04), H-6β (δ_H 1.80); H-5β (δ_H 0.93)/H-6α, H-6β; H-8/H-9α (δ_H 2.26), H-9β (δ_H 2.13), H-13; H-9α/H-10; H-9α/H-10; H-12/H-13; HMBC correlation (H/C) H-1/C-3, C-12, C-13, C-15; H-2/C-14, Me-18; H-3/C-1, C-4, Me-18; H-4/C-3, C-5, C-6, C-13; H-5/C-3, C-4, C-7; H-6/C-4, C-8, Me-19; H-8/C-6, C-9, C-10, C-12, C-13, Me-19; H-9/C-8, C-13; H-10/C-9, Me-20; H-12/C-1, C-8, C-14, Me-20; H-13/C-5, C-8; H-14/C-1, C-2, Me-16, Me-17; Me-16/C-14. C-15, Me-17; Me-17/Me-16; Me-18/C-2, C-3; Me-19/C-6; Me-20/C-10, C-11, C-12; NOESY correlation (H/H) H-2α (δ_H 1.29)/H-1, Me-18; H-3/H-2β (δ_H 1.05), H-14; H-4/Me-18; H-12/H-1, H-8; H-13/Me-19; Me-16/H-14; EIMS *m/z* 297 [M^+] (100), 282 (40), 254 (28), 240 (32), 215 (24), 158 (24), 108 (32); HREIMS *m/z* 297.2469 (calcd for $C_{21}H_{31}N$, 297.2457).

(1*S,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-8-Isocyanoamphilecta-11(20),14-diene (4):** colorless oil; $[\alpha]^{26}_D$ −34.1 (*c* 0.4, $CHCl_3$); IR (neat) ν_{max} 2123 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; $^1H-^1H$ COSY correlation (H/H) H-1/H-2α (δ_H 0.94), H-2β (δ_H 1.53), H-12, H-14; H-2β/H-3; H-3/Me-18; H-4/H-5α (δ_H 0.85), H-13; H-5β (δ_H 2.00)/H-6α (δ_H 1.56), H-6β (δ_H 1.46); H-6α/H-7; H-7/Me-19; H-9α (δ_H 2.24)/H-10α (δ_H 2.38); H-9β (δ_H 1.27)/H-10β (δ_H 2.23); H-12/H-13; HMBC correlation (H/C) H-1/C-2, C-11, C-12, C-13, C-14; H-2/C-1, Me-18; H-3/C-2, C-4, C-5, Me-18; H-4/C-2, C-5, C-6, Me-18; H-5/C-6; H-6/Me-19; H-7/C-5, C-6, Me-19; H-9/C-10; H-10/C-9, C-20; H-12/C-2, C-10, C-13, C-14, C-20; H-13/C-5, C-8, C-9, C-12; H-14/C-12, Me-16, Me-17; Me-16/C-14, C-15, Me-17; Me-17/C-14, C-15, Me-16; Me-18/C-2; Me-19/C-6; H-20/C-10, C-11, C-12; NOESY correlation (H/H) H-4/H-12, Me-18; H-7/H-9β (δ_H 1.27); H-13/H-1, H-3, H-7; H-14/Me-16, H-12; EIMS *m/z* 297 [M^+] (12), 270 (100), 255 (60), 227 (68); HREIMS *m/z* 297.2466 (calcd for $C_{21}H_{31}N$, 297.2457).

(3*S,4*R**,7*S**,8*S**,11*S**,13*S**)-8-Isocyanoamphilecta-1(12),14-diene (5):** colorless powder; mp 58−60 °C; $[\alpha]^{24}_D$ +239.6 (*c* 3.0, $CHCl_3$); IR (KBr) ν_{max} 2127 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; $^1H-^1H$ COSY correlation (H/H) H-2α (δ_H 1.76)/H-3; H-2β (δ_H 1.85)/H-3; H-3/H-4, Me-18; H-4/H-5β (δ_H 0.93), H-13; H-5α (δ_H 1.92)/H-6α (δ_H 1.47); H-5β/H-6α; H-6α/H-7, Me-19; H-9α (δ_H 2.22)/H-10α (δ_H 1.56), H-10β (δ_H 1.56); H-10β/H-11; H-11/Me-20; HMBC correlation (H/C) H-2/C-1, C-4, C-12, C-14, Me-18; H-3/C-2, C-4, Me-18; H-4/Me-18; H-5/C-4; H-6/C-5, C-8, Me-19; H-7/Me-19; H-9/C-8; H-10/C-11, C-12, Me-20; H-11/C-12, Me-20; H-13/C-1, C-3, C-4, C-5, C-8, C-9, C-12; H-14/C-12, Me-16, Me-17; Me-16/C-15, Me-17; Me-17/C-15, Me-16; Me-18/C-2, C-3, C-4; Me-19/C-6, C-7, C-8; Me-20/C-10, C-11, C-12; NOESY correlation (H/H) H-13/H-3, H-5β (δ_H 0.93), H-7, H-9β (δ_H 1.57), H-11; H-14/Me-16; Me-18/H-4, H-5; Me-19/H-9α (δ_H 2.22); EIMS *m/z* 297 [M^+] (100), 282 (40), 255 (60), 215 (48), 159 (40); HREIMS *m/z* 297.2429 (calcd for $C_{21}H_{31}N$, 297.2457).

8-Isocyanatocycloamphilect-10-ene (6): colorless oil; $[\alpha]^{26}_D$ −20.1 (*c* 2.0, $CHCl_3$); IR (neat) ν_{max} 2256 cm^{-1}; ^1H and ^{13}C NMR, see Table 4; $^1H-^1H$ COSY correlation (H/H) H-1/H-2β (δ_H 1.20), H-14α (δ_H 1.03); H-2α (δ_H 1.20)/H-3; H-3/H-4; H-4/H-5α (δ_H 1.48), H-13; H-5β /H-6β (δ_H 1.36); H-6α (δ_H 1.28)/H-7; H-7/Me-19; H-12/H-13; HMBC correlation (H/C) H-1/C-1, C-3, C-12; H-2/C-1, C-3; H-2/C-3, Me-18; H-3/C-5, Me-18; H-4/C-5, C-13, Me-18; H-5/C-4, C-7, C-8; H-6/Me-19; H-7/C-5, Me-19; H-9/C-10, C-11, C-13; H-10/C-9, C-18, C-12, C-20; H-12/C-1, C-2, C-10, C-14, C-20; H-13/C-4, C-5, C-12; H-14/C-2, C-20, Me-16, Me-17; Me-16/C-15, Me-17; Me-17/C-15, Me-16; Me-18/C-4; H-20/C-10, C-11, C-12, C-14, Me-16, Me-17; NOESY correlation (H/H) H-1/H-3, H-13; H-4/H-12, Me-18; H-7/H-9β (δ_H 2.32), H-13; EIMS *m/z* 313 $[M^+]$ (88), 270 (100), 255 (44), 213 (24); HREIMS *m/z* 313.2405 (calcd for $C_{21}H_{31}NO$, 313.2406).

8-Isothiocyanatocycloamphilect-10-ene (7): colorless powder; mp 73−75 °C; $[\alpha]^{22}_D$ −46.9 (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 2093 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 245 (1268) nm; ^1H and ^{13}C NMR, see Table 4; $^1H-^1H$ COSY correlation (H/H) H-1/H-2β (δ_H 1.52), H-12, H-14β (δ_H 1.07); H-2β/H-3; H-3/H-4, Me-18; H-4/H-5α (δ_H 2.02), H-13; H-5α/H-6α (δ_H 1.54), H-6β (δ_H 1.38); H-5β/H-6α; H-6α/H-7; H-7/Me-19; H-12/H-13; HMBC correlation (H/C) H-1/C-2, C-13; H-2/C-4, C-14; H-3/C-1, Me-18; H-4/C-2, Me-18; H-5/C-6, C-13; H-6/C-5, Me-19; H-7/C-5, C-6, C-9, Me-19; H-9/C-10; H-10/C-9, C-20; H-12/C-2, C-10, C-20; H-13/C-1, C-4, C-9; H-14/C-2, C-15, C-20, Me-16, Me-17; Me-16/C-14, C-15, C-20, Me-17; Me-17/Me-16; Me-18/C-2, C-3, C-4; Me-19/C-6, C-7, C-8; H-20/C-10, C-14, Me-16, Me-17; NOESY correlation (H/H) H-1/H-2β (δ_H 1.52), H-13, Me-17; H-2β/H-3; H-4/Me-18; H-7/H-13; Me-19/H-9α (δ_H 2.42), H-12; EIMS *m/z* 329 $[M^+]$ (100), 314 (8), 271 (40), 255 (28); HREIMS *m/z* 329.2153 (calcd for $C_{21}H_{31}NS$, 329.2177).

Synthesis of 6 from 8-Isocyanocycloamphilect-10-ene. To a benzene (1.00 mL) solution of 8-isocyanocycloamphilect-10-ene (19.8 mg, 66.6 μmol) was added lead tetraacetate (35.5 mg, 79.9 μmol) with stirring at room temperature for 1 h. The reaction mixture was diluted with diethyl ether (5.00 mL) and then washed with water and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The mixture thus obtained underwent Si gel column chromatography (elution with *n*-hexane−EtOAc (4:1)) to produce **6** (17.9 mg, 57.2 μmol, 86% yield): colorless oil; $[\alpha]^{25}_D$ −20.1 (*c* 1.8, $CHCl_3$); spectral data were identical with that of natural **6**.

Synthesis of 7 from 8-Isocyanocycloamphilect-10-ene. To a tetrahydrofuran (0.1 mL) solution of 8-isocyanocycloamphilect-10-ene (9.5 mg, 32.0 μmol) were added sulfur (1.2 mg, 38.4 μmol), tellurium (1.0 mg, 8.0 μmol), and triethylamine (11.0 μL, 76.8 μmol) with stirring at room temperature for 10 h. The reaction mixture was diluted with diethyl ether (3.00 mL), filtered through Si gel, and then concentrated under reduced pressure. The mixture then underwent Si gel column chromatography (elution with *n*-hexane) to give **7** (9.4 mg, 28.6 μmol, 89% yield): colorless oil; $[\alpha]^{23}_D$ −56.3 (*c* 0.9, $CHCl_3$); spectral data were identical with that of natural **7**.

8-Aminocycloamphilect-10-ene (8) from 8-Isocyanocycloamphilect-10-ene. To an ethanol (0.1 mL) solution of 8-isocyanocycloamphilect-10-ene (64.3 mg, 216 μmol) was added aqueous HCl (5 M, 0.2 mL) with stirring at 80 °C for 30 h. To the reaction mixture was added aqueousNaOH (5 M, 0.3 mL), and then the reaction mixture was stirred for 2 h. The system was concentrated under reduced pressure, and its subsequent Si gel column chromatography (elution with *n*-hexane−EtOAc (1:1)) provided **8** (53.8 mg, 187 μmol, 87% yield): colorless powder; mp 78−80 °C; $[\alpha]^{24}_D$ +47.3 (*c* 2.2, $CHCl_3$); IR (KBr) ν_{max} 3440, 3380 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, *J* in Hz) δ ppm 5.21 (1H, t, *J* = 4.4), 1.94 (3H, m), 1.84 (2H, m), 1.54 (1H, m), 1.41−1.24 (8H, m), 1.01−0.81 (4H, m), 0.90 (3H, s), 0.88 (3H, d, *J* = 6.8), 0.87 (3H, d, *J* = 6.8), 0.79 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 136.8, 117.9, 50.7, 50.6, 47.9, 46.6, 43.8, 43.5, 42.6, 42.1, 40.0, 38.5, 38.1, 32.3, 31.8, 30.7, 29.7, 25.1, 19.8, 14.7; EIMS *m/z* 287 $[M^+]$ (52), 270 (100), 255 (32), 213 (24), 110 (24); HREIMS *m/z* 287.2624 (calcd for $C_{20}H_{33}N$, 287.2613).

8-(4-Bromobenzamido)cycloamphilect-10-ene (9) from 8-Isocyanocycloamphilect-10-ene. To a dichloromethane (1.0 mL) solution of **8** (22.4 mg, 77.9 μmol) were added triethylamine (20 μL) and *p*-bromobenzoyl chloride (13.2 mg, 44.4 μmol) with stirring at room temperature for 1 h. The system was concentrated under reduced pressure, and Si gel column chromatography (elution with *n*-hexane−EtOAc (4:1)) gave **9** (36.2 mg, 77.0 μmol, 99% yield): colorless needles; mp 148−150 °C; $[\alpha]^{20}_D$ +63.6 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3439, 3352, and 1687 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 247 (9498) nm; ^1H NMR (400 MHz, $CDCl_3$, *J* in Hz) δ ppm 7.56 (1H, d, *J* = 8.4), 7.53 (1H, d, *J* = 8.4), 7.53 (1H, d, *J* = 8.4), 7.51 (1H, d, *J* = 8.4), 5.51 (1H, br s), 5.31 (1H, d, *J* = 4.6), 4.03 (1H, dd, *J* = 17.5, 6.0), 2.04 (1H, m), 1.87 (1H, br s), 1.83 (1H, m), 1.72 (1H, m), 1.61 (1H, dt, *J* = 12.8, 3.2), 1.53−1.42 (2H, m), 1.39−1.18 (7H, m), 1.04 (3H, d, *J* = 6.5), 1.02−0.91 (2H, m), 0.95 (3H, d, *J* = 6.5), 0.88 (3H, s), 0.89−0.81 (2H, m), 0.80 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.6, 136.1, 135.6, 131.8, 131.8, 128.1, 128.1, 125.3, 118.5, 57.8, 51.5, 47.7, 46.5, 45.2, 43.5, 43.3, 43.3, 38.8, 38.2, 32.6, 32.2, 31.8, 30.3, 29.8, 25.0, 19.7, 16.8; ESIMS *m/z* 470 $[MH^+]$ (100), 271 (10); HREIMS *m/z* 470.2074 (calcd for $C_{27}H_{37}NOBr$ $[MH^+]$, 470.2059).

Acknowledgment. The authors express appreciation to Taiho Pharmaceutical Co., Ltd. for measurement of the biological activity.

Supporting Information Available: Copies of ^1H and ^{13}C NMR spectra of new compounds (**1**−**9**). Lists of crystal data and data collection parameters, tables of fractional atomic coordinates and equivalent isotropic thermal parameters, anisotropic displacement parameters, and geometric parameters for **9**. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48, and previous reviews in the series.
(2) Scheuer, P. J. *Acc. Chem. Res.* **1992**, *25*, 433−439.
(3) Chang, C. W. J.; Patra, A.; Baker, J. A.; Scheuer, P. J. *J. Am. Chem. Soc.* **1987**, *109*, 6119−6123.
(4) Trimurtulu, G.; Faulkner, D. J. *J. Nat. Prod.* **1994**, *57*, 501−506.
(5) Fusetani, N.; Yasumuro, K.; Kawai, T.; Natori, T.; Brinen, L.; Clardy, J. *Tetrahedron Lett.* **1990**, *31*, 3599−3602.
(6) Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *95*, 2359−2368.
(7) (a) Miyaoka, H.; Shimomura, M.; Kimura, H.; Yamada, Y.; Kim, H.-S.; Wataya, Y. *Tetrahedron* **1998**, *54*, 13467−13474. (b) Wright, A. D.; König, G. M. *J. Nat. Prod.* **1996**, *59*, 710−716.
(8) Simpson, J. S.; Garson, M. J. *Tetrahedron Lett.* **2001**, *42*, 4267−4269, and references therein.
(9) Recent examples: (a) Mitome, H.; Nagasawa, T.; Miyaoka, H.; Yamada, Y.; Soest, R. W. M. *J. Nat. Prod.* **2003**, *66*, 46−50. (b) Mitome, H.; Nagasawa, T.; Miyaoka, H.; Yamada, Y.; Soest, R. W. M. *Tetrahedron* **2002**, *58*, 1693−1696. (c) Mitome, H.; Nagasawa, T.; Miyaoka, H.; Yamada, Y.; Soest, R. W. M. *J. Nat. Prod.* **2001**, *64*, 1506−1508.
(10) Capon, R. J.; Macleod, J. K. *Aust. J. Chem.* **1988**, *41*, 979−983.
(11) Ciminiello, P.; Fattorusso, E.; Magno, S.; Mayol, L. *Can. J. Chem.* **1987**, *65*, 518−522.
(12) Clark, R. J.; Stapleton, B. L.; Garson, M. J. *Tetrahedron* **2000**, *56*, 3071−3076.
(13) Alvi, K. A.; Tenenbaum, L.; Crews, P. *J. Nat. Prod.* **1991**, *54*, 71−78.
(14) König, G. M.; Wright, A. D.; Angerhofer, C. K. *J. Org. Chem.* **1996**, *61*, 3259−3267.
(15) Sharma, H. A.; Tanaka, J.; Higa, T.; Lithgow, A.; Bernardinelli, G.; Jefford, C. W. *Tetrahedron Lett.* **1992**, *33*, 1593−1596.
(16) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1980**, *21*, 315−318.
(17) He, H.; Salva, J.; Catalos, R. F.; Faulkner, D. J. *J. Org. Chem.* **1992**, *57*, 3191−3194.
(18) Stoelwinder, J.; Leusen, A. M. *J. Org. Chem.* **1993**, *58*, 3687−3691.
(19) Fujiwara, S.; Shin-Ike, T. *Tetrahedron Lett.* **1992**, *33*, 7021−7024.
(20) Flack, H. D.; Bernardinelli, G. *Acta Crystallogr.* **1999**, *A55*, 908−915, and references therein.
(21) The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 226525.
(22) Ishiyama, M.; Miyamoto, Y.; Sasamoto, K.; Ohkura, Y.; Ueno, K. *Talanta* **1997**, *44*, 1299−1305.
(23) Tominaga, H.; Ishiyama, M.; Ohseto, F.; Sasamoto, K.; Hamamoto, T.; Suzuki, K.; Watanabe, M. *Anal. Commun.* **1999**, *36*, 47−50.

NP030458P