Notes

Renierosides, Cerebrosides from a Marine Sponge *Haliclona* (*Reniera*) sp.

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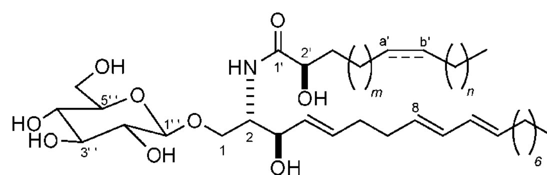
Guided by the brine shrimp lethality assay, eight new cerebrosides (**1**–**8**) have been isolated from an extract of the marine sponge *Haliclona* (*Reniera*) sp. A novel feature of these cerebrosides was the presence of unprecedented amide-linked long-chain fatty acid moieties. The planar structures of the cerebrosides (**1**–**8**) were established by 1D and 2D NMR spectroscopic techniques, mass spectrometric analyses, and chemical degradation methods. The isolated compounds did not display cytotoxicity to a panel of five human solid tumor cell lines.

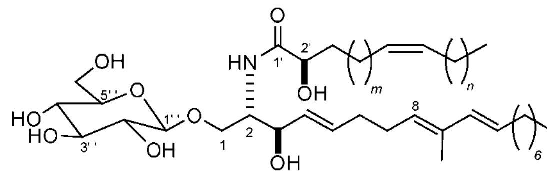
Cerebrosides are glycosphingolipids, consisting of a ceramide and a single sugar residue (glucose or galactose) at C-1. The hydrophobic ceramide portion involves a sphingoid base and an amide-linked fatty acyl chain. These amphipathic molecules have been reported to exhibit antitumor/cytotoxic,[1,2] anti-HIV-1,[3] neuritogenic,[4] antihepatotoxic,[5] immunosuppressive,[6] immunomodulatory,[7] cyclooxygenase-2 inhibitory,[8] antifungal,[2] antimicrobial,[9] and antifouling activities.[10]

Sponges of the genus *Haliclona* have been studied extensively and afforded around 200 compounds belonging to different classes. These compounds include the cytotoxic tertiary alkaloids haliclonacyclamines,[11] antifungal pentacyclic alkaloid papuamine,[12] antitumor alkaloid manzamine,[13] and anti-inflammatory cyclic depsipeptides halipeptins,[14] to name a few. A bioactivity-guided chemical study of a marine sponge *Haliclona* (*Reniera*) sp. collected from the Korean waters (see the Supporting Information), has led to the isolation of eight new cerebrosides (**1**–**8**). The MeOH extract of the sponge, active in the brine shrimp assay (LD_{50} 126 μg/mL), was partitioned between CH_2Cl_2 and H_2O, and the CH_2Cl_2 layer was further partitioned between aqueous MeOH (LD_{50} 27 μg/mL) and *n*-hexane (LD_{50} 45 μg/mL). The *n*-hexane layer was chromatographed on a normal-phase column, followed by reverse-phase high-performance liquid chromatography (RP-HPLC) to yield eight new cerebrosides (**1**–**8**). Herein, we describe the isolation, structure elucidation, and biological evaluation of these new compounds (**1**–**8**).

Renieroside A_1 (**1**) was isolated as a white amorphous solid. Its molecular formula was assigned as $C_{52}H_{95}NO_9$ on the basis of high-resolution fast-atom-bombardment mass spectrometry (HRFABMS; $[M - H]^-$ at *m/z* 876.6946, Δ + 1.7 mmu) and ^{1}H and ^{13}C NMR spectroscopic analyses. The characteristic signals of a sugar (an



1 $C_{52}H_{95}NO_9$, *m* = 17, *n* = 5, $\Delta^{21'}$
2 $C_{50}H_{91}NO_9$, *m* = 13, *n* = 7, $\Delta^{17'}$
3 $C_{49}H_{89}NO_9$, *m* = 12, *n* = 7, $\Delta^{16'}$
4 $C_{46}H_{85}NO_9$, *m* + *n* = 16; a',b' = dihydro
5 $C_{48}H_{89}NO_9$, *m* + *n* = 18; a',b' = dihydro



6 $C_{53}H_{97}NO_9$, *m* = 17, *n* = 5, $\Delta^{21'}$
7 $C_{51}H_{93}NO_9$, *m* = 13, *n* = 7, $\Delta^{17'}$
8 $C_{52}H_{95}NO_9$, *m* = 16, *n* = 5, $\Delta^{20'}$

anomeric proton at δ_H 4.26), an amide linkage (a nitrogenated methine proton at δ_H 3.97 and a carbonyl carbon at δ_C 177.2), and a long chain (terminal methyl protons at δ_H 0.89 and methylene protons at δ_H 1.24–1.38) were observed, indicating its nature as a glycosphingolipid. In the ^{13}C NMR spectrum, the carbon resonances appeared at δ 62.7 (CH_2), 71.6 (CH), 75.0 (CH), 77.9 (CH), 78.0 (CH), and 104.7 (CH), revealing the presence of a β-glucopyranoside.[15] The coupling constant of the anomeric proton at δ 4.26 (d, J_{HH} = 7.5 Hz; δ_C 104.7) further confirmed the β configuration of the glucose unit (in the case of α-glucopyranoside: J_{HH} = 3.7 Hz; δ_C 98.5).[16] The absolute configuration of the glucose moiety was deduced to be D (*vide infra*). The ^{1}H NMR spectrum showed two olefinic proton signals at δ 5.73 (1H, dt, *J* = 15.0, 5.5 Hz, H-5) and 5.48 (1H, dd, *J* = 15.0, 7.5 Hz, H-4), attributed to the typical Δ^4 double bond of a sphingosine.[1] The large vicinal coupling

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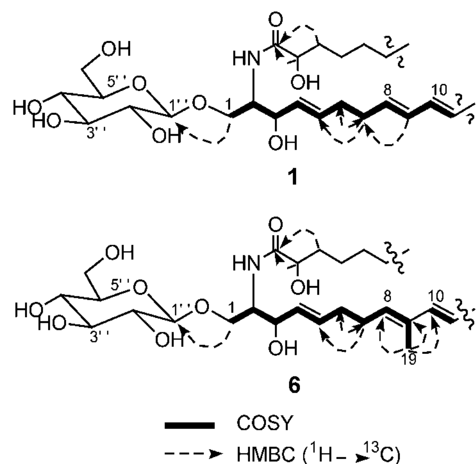

Figure 1. Key COSY and HMBC correlations of compounds **1** and **6**.

constants of these olefinic protons ($J = 15.0$ Hz) clearly indicated an *E* geometry for the double bond.[9] The correlation spectroscopy (COSY) correlations between the H-2 signal (δ 3.97, 1H, m) and both the H-3 (δ 4.12, 1H, m) and the H-1 (δ 4.13, 1H, m; 3.68, 1H, m) signals defined the 2-amino-1,3-dioxygenated fragment. Furthermore, in the ^{1}H NMR spectrum, the characteristic signals for a conjugated diene were observed at δ 5.99 (2H, m, H-9, H-10), 5.56 (1H, m, H-8), and 5.54 (1H, m, H-11).[1] The ^{13}C NMR chemical shifts of allylic carbons C-7 and C-12 at δ 33.0 indicated the *E* geometry of the diene moiety.[1] The key heteronuclear multiple-bond correlation (HMBC) correlations from H-7 to C-5, C-6, and C-9 and from H-6 to C-7, and COSY correlations from H-6 to H-8, were useful to locate the position of double bonds in the sphingosine base (Figure 1). In addition, the HMBC spectrum also revealed the correlation from H-1 at δ 4.13 (1H, m) and 3.68 (1H, m) to the anomeric carbon at δ 104.7. The chemical shifts of C-2 at δ 54.6 and C-3 at δ 73.1 supported the D-*erythro* stereochemistry at C-2 and C-3, which was in agreement with those of synthetic D-*erythro*-ceramide (δ 55.2, 73.6) and D-glucosyl-D-*erythro*-ceramide (δ 53.8, 72.6).[17] In case of *threo* isomers of these model compounds, C-2/C-3 were reported to appear at δ 55.2/71.2 and δ 53.7/70.7, respectively.[17] Methanolysis of **1** yielded fatty acid methyl ester (FAME), and its molecular formula was established as $C_{29}H_{56}O_3$ ([M + H]$^+$ at *m/z* 453, [M + Na]$^+$ at *m/z* 475). The location of the double bond was determined by collision-induced-dissociation fast-atom-bombardment tandem mass spectrometry (FAB-CID-MS/MS) analysis of the [M + Na]$^+$ ion at *m/z* 475. Allylic cleavages were observed as enhanced peaks at *m/z* 403 and 349, indicating the location of the double bond at C-21′ (Figure 2). The geometry of $\Delta^{21'}$ was assigned as *Z* on the basis of chemical shifts of allylic carbons C-20′ and C-23′, which appeared at δ 27.2.[6] The optical rotation of the ester ($[\alpha]^{22}_{D}$ –4, MeOH) identified it as the *R* isomer.[1] On the basis of these data, the fatty acid moiety was deduced to be (2*R*,21*Z*)-2-hydroxyoctacos-21-enoic acid. To the best of our knowledge, this appears to be the first report on the isolation of 2-hydroxyoctacos-21-enoic acid from any natural or synthetic source. However, (2*R*,21*Z*)-2-methoxyoctacos-21-enoic acid has been reported as a naturally occurring α-methoxy fatty acid from a marine sponge *Higginsia tethyoides*.[18] On the basis of the above mentioned data, the structure of renieroside A_1 (**1**) was defined as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,21′*Z*)-2′-hydroxyoctacos-21′-enoylamino]-octadeca-4,8,10-triene-1,3-diol.

The molecular formula of renieroside A_2 (**2**), isolated as a white amorphous solid, was assigned as $C_{50}H_{91}NO_9$ on the basis of high-resolution FAB mass spectroscopy ([M + Na]$^+$ at *m/z* 872.6594, Δ +0.2 mmu) and the results of ^{1}H and ^{13}C NMR spectroscopic interpretations (Tables 1 and 2). The NMR results were found to

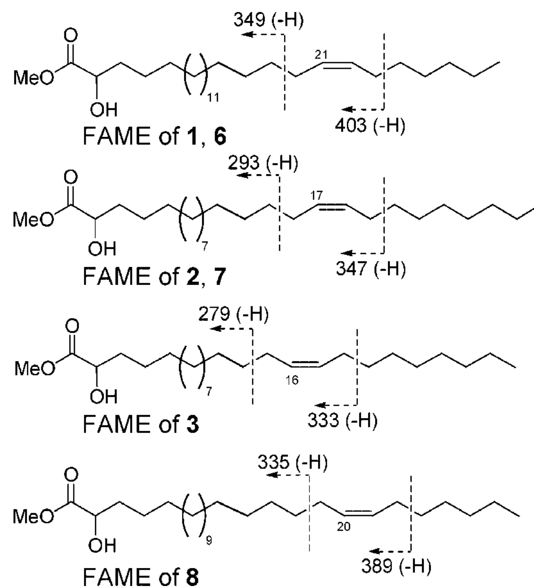

Figure 2. Key FAB-CID tandem mass fragmentations of the [M + Na]$^+$ ions of the FAMEs derived from **1**–**3** and **6**–**8**.

be essentially identical to those of **1**, which confirmed that renieroside A_2 (**2**) was also a glycosphingolipid and differed only in the length of the lipid base or the lipid amide units. The difference was in the fatty acid portion because methyl (2*R*,17*Z*)-2-hydroxyhexacos-17-enoic acid ($[\alpha]^{23}_{D}$ –4, MeOH) was obtained by the methanolysis of **2**. The location of the double bond was determined by FAB-CID-MS/MS analysis of the [M + Na]$^+$ ion at *m/z* 447. The enhanced peaks of allylic cleavages at *m/z* 347 and 293 indicated the location of the double bond at C-17 (Figure 2). This unsaturated fatty acid has been reported in chill-resistant rye, oats, and wheat,[19] and in the pig brain.[20] In reports from the terrestrial sources, however, the stereochemistry of the hydroxyl group at C-2 of the fatty acid has not been defined. The methanolysis of **2** also yielded an α- and β-glucopyranosyl mixture. The optical rotation of this mixture ($[\alpha]^{26}_{D}$ +70.8, MeOH) identified glucose as the D isomer. On the basis of these data, the structure of renieroside A_2 (**2**) was established as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,17′*Z*)-2′-hydroxyhexacos-17′-enoylamino]-octadeca-4,8,10-triene-1,3-diol.

The molecular formula of renieroside A_3 (**3**) was found to be $C_{49}H_{89}NO_9$ on the basis of high-resolution FAB mass spectroscopy ([M + Na]$^+$ at *m/z* 858.6436, Δ +0.1 mmu) and ^{1}H and ^{13}C NMR spectroscopic results. Again, the ^{1}H and ^{13}C NMR spectroscopic data were found to be nearly identical to those of renierosides A_1 and A_2. The methanolysis of **3** gave rise to a different FAME. Its molecular formula was established as $C_{26}H_{50}O_3$ on the basis of FABMS data ([M + Na]$^+$ at *m/z* 433). Together with the optical rotation value ($[\alpha]^{23}_{D}$ –1, MeOH), the fatty acid portion in renieroside A_3 was defined as a (2*R*,16*Z*)-2-hydroxypentacos-16-enoic acid. The location of the double bond was determined by FAB-CID-MS/MS analysis of the [M + Na]$^+$ ion at *m/z* 433. Allylic cleavages were observed as enhanced peaks at *m/z* 333 and 279, indicating the location of the double bond at C-16 (Figure 2). Similarly, as for the fatty acid of **2**, it has also been reported in chill-resistant rye, oats, and wheat,[19] and in the pig brain,[20] without information about stereochemistry at C-2. All these data were in agreement with the proposed structure of renieroside A_3 (**3**) as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,16′*Z*)-2′-hydroxypentacos-16′-enoylamino]-octadeca-4,8,10-triene-1,3-diol.

Renieroside A_4 (**4**) showed a molecular formula of $C_{46}H_{85}NO_9$, as deduced from high-resolution FAB mass spectroscopy ([M + Na]$^+$ at *m/z* 818.6123, Δ +0.1 mmu) as well as ^{1}H and ^{13}C NMR spectroscopic results. The acid hydrolysis of **4** gave rise to a

Table 1. ^{1}H[a] and ^{13}C[b] NMR Data of Renieroside A$_1$ (**1**)[c] (CD$_3$OD)

position	δ_H	δ_C	position	δ_H	δ_C
lipid base unit			*N*-acyl unit		
1	4.13 (m)	69.8	1′		177.2
	3.68 (m)		2′	4.12 (dd, *J* = 8.0, 4.8 Hz)	72.8
2	3.97 (m)	54.6	3′	1.71 (m)	35.6
3	4.12 (m)	73.1		1.56 (m)	
4	5.48 (dd, *J* = 15.0, 7.5 Hz)	131.2	4′–19′ or 24′–26′	1.38–1.24 (m)	29–33
5	5.73 (dt, *J* = 15.0, 5.5 Hz)	134.3	20′ or 23′	2.02 (q, *J* = 6.0 Hz)	27.2
6	2.14 (m)	33.1	21′ or 22′	5.34 (m)	131.1
7	2.09 (m)	33.0	27′	1.38–1.24 (m)	22.9
8	5.56 (m)	131.7	28′	0.89 (t, *J* = 7.0 Hz)	14.4
9	5.99 (m)[d]	132.3	glucose unit		
10	5.99 (m)[d]	132.3	1″	4.26 (d, *J* = 7.5 Hz)	104.7
11	5.54 (m)	133.5	2″	3.18 (m)	75.0
12	2.06 (m)	33.0	3″	3.35 (m)	78.0
13–16	1.38–1.24 (m)	29–33	4″	3.28 (m)[e]	71.6
17	1.38–1.24 (m)	22.9	5″	3.28 (m)[e]	77.9
18	0.89 (t, *J* = 7.0 Hz)	14.4	6″	3.87 (dd, *J* = 12.0, 1.0 Hz)	62.7
				3.71 (m)	

[a] Multiplicities and coupling constants are in parentheses (measured at 500 MHz). [b] Measured at 100 MHz. [c] The ^{1}H and ^{13}C NMR data of **2**–**5** were identical to those of **1** for the lipid base and glucose units (for differences in NMR data of *N*-acyl units of **2**–**5**, please refer to their respective FAMEs in Experimental Section). [d] Values with same superscript within same column may be interchanged. [e] Overlapped with solvent peak (assignments were secured by HSQC experiment).

Table 2. ^{1}H[a] and ^{13}C[b] NMR Data of Renieroside B$_1$ (**6**)[c] (CD$_3$OD)

position	δ_H	δ_C	position	δ_H	δ_C
lipid base unit			*N*-acyl unit		
1	4.13 (m)	69.8	1′		177.2
	3.67 (m)		2′	4.12 (dd, *J* = 8.0, 4.8 Hz)	72.8
2	3.98 (m)	54.6	3′	1.69 (m)	35.5
3	4.13 (m)	73.1		1.54 (m)	
4	5.48 (dd, *J* = 15.0, 8.0 Hz)	131.2[d]	4′–19′ or 24′–26′	1.38–1.24 (m)	29–33
5	5.73 (dt, *J* = 15.0, 6.5 Hz)	134.3	20′ or 23′	2.02 (q, *J* = 6.0 Hz)	27.2
6	2.09 (m)	33.1	21′ or 22′	5.34 (m)	131.1
7	2.21 (m)	36.1	27′	1.38–1.24 (m)	22.9
8	5.33 (m)	130.8	28′	0.89 (t, *J* = 7.0 Hz)	14.6
9		134.8	glucose unit		
10	6.02 (d, *J* = 15.5 Hz)	135.9	1″	4.26 (d, *J* = 7.5 Hz)	104.7
11	5.52 (dt, *J* = 15.0, 7.0 Hz)	131.8	2″	3.18 (m)	75.0
12	2.07 (q, *J* = 7.0 Hz)	33.2	3″	3.35 (m)	78.0
13–16	1.38–1.28 (m)	29–33	4″	3.28 (m)[e]	71.8
17	1.38–1.28 (m)	22.9	5″	3.28 (m)[e]	77.9
18	0.89 (t, *J* = 7.0 Hz)	14.6	6″	3.87 (dd, *J* = 12.0, 1.0 Hz)	62.7
19	1.71 (s)	12.4		3.71 (m)	

[a] Multiplicities and coupling constants are in parentheses (measured at 500 MHz). [b] Assignments were based on HSQC and HMBC experiments (500 MHz). [c] The ^{1}H and ^{13}C NMR data of **7** and **8** were identical to those of **6** for the lipid base and glucose units (for differences in NMR data of *N*-acyl units of **7** and **8**, please refer to their respective FAMEs in Experimental Section). [d] Values with same superscript within same column may be interchanged. [e] Overlapped with solvent peak (assignments were secured by HSQC experiment).

saturated FAME, which was characterized as (2*R*)-hydroxydocosanoic acid on the basis of its ^{1}H NMR, FABMS, and optical rotation ($[\alpha]^{22}_D$ –19, MeOH) data. This fatty acid has been reported from several marine sponges including *Verongula gigantea* and *Aplysina archeri*.[21] All these data were in agreement with the proposed structure for renieroside A$_4$ (**4**) as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*)-hydroxydocosanoylamino]-octadeca-4,8,10-triene-1,3-diol.

Renieroside A$_5$ (**5**) was isolated as a white amorphous solid. Its molecular formula was found to be $C_{48}H_{89}NO_9$ on the basis of high-resolution FAB mass spectroscopy ($[M + Na]^+$ at *m/z* 846.6433, Δ –0.2 mmu) and the results of ^{1}H and ^{13}C NMR spectroscopic interpretations. The ^{1}H and ^{13}C NMR and mass spectroscopic data of **5** were found to be nearly identical to those of renieroside A$_4$ (**4**). Its methanolysis gave rise to a different FAME, which was identified as (2*R*)-hydroxytetracosanoic acid on the basis of its low-resolution FABMS, NMR, and optical rotation ($[\alpha]^{22}_D$ –4, MeOH) data. This fatty acid has been reported in several marine sponges including *Verongula gigantea* and *Aplysina archeri*.[21] All these data were in agreement with the structure for renieroside A$_5$ (**5**) as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*)-hydroxytetracosanoylamino]-octadeca-4,8,10-triene-1,3-diol.

Renieroside B$_1$ (**6**) was isolated as a white amorphous solid, and its molecular formula was established as $C_{53}H_{97}NO_9$ on the basis of high-resolution FABMS and ^{13}C NMR spectroscopic data. The exact mass of the $[M - H]^-$ ion at *m/z* 890.7089 matched well with the expected molecular formula (890.7085, Δ +0.4 mmu). Its ^{1}H and ^{13}C NMR spectroscopic data were clearly different from those of reniero-sides A$_1$–A$_5$ (**1**–**5**), indicating the difference was in the sphingosine base. The ^{1}H NMR spectrum of **6** revealed the presence of an additional methyl group (δ_H 1.71, δ_C 12.4). Furthermore, a lone downfield doublet at δ 6.02 (1H, d, J = 15.5 Hz), rather than a multiplet as in the case of renierosides A$_1$–A$_5$, suggested the possible substitution was either at C-9 or C-10 of the conjugated diene. Two different sets of COSY correlations were useful to solve this issue. The H-6 showed COSY correlation to H-7, which was in turn correlated with H-8. Another partial structure was established according to the COSY correlations from H-10 through H-13, suggesting the presence of a methyl group at C-9 (Figure 1). In addition, the HMBC correlations from H$_3$-19 to C-8, -9, and -10, and a weak long-range COSY correlation between H$_3$-19 and H-8, further confirmed that the methyl group was located at C-9. These 2D NMR spectroscopic interpretations were also helpful in defining the location of double bonds in the sphingosine base. The comparison of spectroscopic data of **6** with those reported[1] supported

the theory that the long-chain base of **6** had the same skeleton and stereochemistry. The acid hydrolysis of **6** yielded the same FAME as that of **1**, as confirmed by the comparison of NMR, low-resolution FABMS, FAB-CID-MS/MS, and optical rotation data (see the Experimental Section). On the basis of all these data, the structure of renieroside B_1 (**6**) was defined as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,21′*Z*)-2′-hydroxyoctacos-21′-enoylamino]-9-methyloctadeca-4,8,10-triene-1,3-diol.

The molecular formula of renieroside B_2 (**7**), isolated as a white amorphous solid, was assigned as $C_{51}H_{93}NO_9$ on the basis of high-resolution FAB mass spectroscopy ($[M + Na]^+$ at *m/z* 886.6746, Δ –0.2 mmu) and the results of ^{1}H and ^{13}C NMR spectroscopic interpretations (Tables 1 and 2). The NMR results were found to be essentially identical to those of **6**, which confirmed that renieroside B_2 (**7**) was also a C-9 methylated cerebroside and differed only in the length of lipid base or lipid amide units. In addition to the pseudo-molecular ion peak at *m/z* 862 $[M - H]^-$, the FABMS of **7** also exhibited an intense fragment peak at *m/z* 700, which was produced by elimination of the glucosyl unit from the molecular ion. The difference in the side chain was similarly located in the fatty acid portion because of the isolation of methyl (2*R*,17*Z*)-2-hydroxyhexacos-17-enoic acid in the methanolysis of **7**, which was same as the FAME of **2** (see the Experimental Section). Therefore, the structure of renieroside B_2 (**7**) was proposed as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,17′*Z*)-2′-hydroxyhexacos-17′-enoylamino]-9-methyloctadeca-4,8,10-triene-1,3-diol.

The molecular formula of renieroside B_3 (**8**) was found to be $C_{52}H_{95}NO_9$ on the basis of high-resolution FAB mass spectroscopy ($[M + Na]^+$ at *m/z* 900.6903, Δ –0.2 mmu) as well as ^{1}H and ^{13}C NMR spectroscopic results. Again, the ^{1}H and ^{13}C NMR spectroscopic data were found to be nearly identical to those of renierosides B_1 and B_2. The acid hydrolysis of **8** gave rise to (2*R*,20*Z*)-2-hydroxyheptacos-20-enoic acid (see the Experimental Section). The location of the double bond was determined by FAB-CID-MS/MS analysis of the $[M + Na]^+$ ion at *m/z* 461. Allylic cleavages were observed as enhanced peaks at *m/z* 389 and 335, indicating the location of the double bond at C-20 (Figure 2). As far as the authors are aware, the 2-hydroxyheptacos-20-enoic acid, isolated as a part of cerebroside, has previously been reported from neither natural nor synthetic sources. All these data were in agreement with the proposed structure for renieroside B_3 (**8**) as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,20′*Z*)-2′-hydroxyheptacos-20′-enoylamino]-9-methyloctadeca-4,8,10-triene-1,3-diol.

The prominent feature of six of these cerebrosides (**1**–**3** and **6**–**8**) was the presence of very long chain fatty acid moieties. Some of these fatty acids were unprecedented (fatty acids of **1**, **6**, and **8**), while others have been reported for the first time from a marine source (fatty acids of **2**, **3**, and **7**). The very long chain fatty acids have been reported to be biosynthesized in marine primary producers by the condensation of precursor fatty acids with malonyl-CoA, using different enzymes (desaturases and elongases).[22] The desaturases insert a double bond at a specific carbon atom in the fatty acid chain, and the elongases elongate the precursors in two-carbon increments. It might be speculated that similar elongation and desaturation from precursor fatty acids, followed by hydroxylation, led to the formation of unsaturated hydroxylated fatty acids in these cerebrosides. Further investigation on the metabolic pathways and participation of these large molecules in cellular functions of the lower sessile animals is of profound interest.

The cerebrosides (**1**–**8**) were evaluated for cytotoxicity against a panel of five human solid tumor cell lines including human lung cancer (A549), human ovarian cancer (SK-OV-3), human skin cancer (SK-MEL-2), human CNS cancer (XF498), and human colon cancer (HCT15) cell line, but were found inactive (ED_{50} > 10 *μ*g/mL) to all cell lines in the panel.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Varian UNITY 400 and Varian INOVA 500 spectrometers. Chemical shifts are reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS SX-102A. HRFABMS data were obtained on a JEOL JMS SX-101A. HPLC was performed with a C_{18}-5E Shodex packed column (250 × 10 mm, 5 *μ*m, 100 Å) using a Gilson 133-RI detector.

Animal Material. The sponge, *Haliclona* (*Reniera*), was collected by hand using scuba gear (20 m depth) in October of 2001, off Ulleung Island, Korea. The collected sample was frozen immediately. The specimen (sample No. J01U-6) was identified as *Haliclona* (*Reniera*) species by C.J.S. It was thickly encrusting on the shell surface. The surface was smooth. The color was purple in life and beige in alcohol. The texture was soft and fragile. The megascleres were thick oxea (200–260 *μ*m × 8–10 *μ*m) and thin oxea (100–180 *μ*m × 1–5 *μ*m). A voucher specimen of the sponge (registry No. Spo. 45) was deposited at the Natural History Museum, Hannam University, Daejon, Korea.

Extraction and Isolation. The frozen sponge (7 kg) was extracted with MeOH at room temperature. The MeOH extract (29.2 g) showed toxicity against brine shrimp larvae (LD_{50} 126 *μ*g/mL). The MeOH extract was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer (6.6 g, LD_{50} 203 *μ*g/mL) was further partitioned between aqueous MeOH (1.9 g, LD_{50} 27 *μ*g/mL) and *n*-hexane (3.9 g, LD_{50} 45 *μ*g/mL). A portion of the *n*-hexane fraction (400 mg) was subjected to Si gel 60 (15–40 *μ*m) column chromatography with a solvent system of 100% CH_2Cl_2 to 80% CH_2Cl_2 with MeOH, to afford 10 fractions. Fraction 7 (70 mg) was subjected to RP-HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 *μ*m, 100 Å) eluted with 100% MeOH to afford cerebrosides **4** (1.1 mg), **3** (2.1 mg), **2** (2.6 mg), **5** (1.2 mg), **7** (2.4 mg), **8** (0.8 mg), **1** (1.2 mg), and then **6** (0.8 mg).

Renieroside A_1 (1), 1-*O*-*β*-D-Glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,21′*Z*)-2′-hydroxyoctacos-21′-enoylamino]-octadeca-4,8,10-triene-1,3-diol. White amorphous solid. $[\alpha]^{22}_D$ +10 (*c* 0.1, MeOH). ^{1}H and ^{13}C NMR data, see Table 1. FABMS (+ ve mode) *m/z*: 860 $[(M + H) - H_2O]^+$, 900 $[M + Na]^+$, 698 $[(M + H) - H_2O - 162]^+$; (− ve mode) *m/z* 876 $[M - H]^-$, 714 $[(M - H) - 162]^-$, 696 $[(M - H) - 162 - H_2O]^-$. HRFABMS (− ve mode) *m/z*: 876.6946 (calcd for $C_{52}H_{94}NO_9$, 876.6929).

Methanolysis of 1. Cerebroside (0.70 mg) was dissolved in 5% 1 M HCl–MeOH (700 *μ*L), and the mixture was refluxed on a magnetic stirrer for 18 h at 80 °C. The reaction mixture was then cooled and extracted with *n*-hexane (3 mL × 3). The *n*-hexane layer was evaporated under N_2 to yield the FAME (methyl 2-hydroxyoctacos-21-enoic acid; 0.2 mg). $[\alpha]^{22}_D$ –4 (*c* 0.1, MeOH). ^{1}H NMR data (CD_3OD, 400 MHz): *δ* 5.34 (2H, m, H-21, H-22), 4.12 (1H, dd, *J* = 8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 2.02 (4H, q, *J* = 6.0 Hz, H-20, H-23), 1.61 (2H, m, H-3), 1.28 (40H, m, H-4–H-19, H-24–H-27), 0.89 (3H, t, *J* = 7.0 Hz, H-28). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): *δ* 177.2 (C-1), 131.1 (C-21, C-22), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0–33.0 (C-4–C-19, C-24–C-26), 27.2 (C-20, C-23), 22.9 (C-27), 14.6 (C-28). FABMS: *m/z* 453 $[M + H]^+$, 475 $[M + Na]^+$. FAB-CID-MS/MS: *m/z* 475 $[M + Na]^+$ (100), 459 (0.08), 445 (0.07), 431 (0.06), 417 (0.22), 403 (0.26), 389 (0.07), 375 (0.04), 363 (0.03), 349 (0.24), 335 (0.08), 321 (0.07), 307 (0.12), 293 (0.11), 279 (0.09), 265 (0.08), 251 (0.07), 237 (0.08), 223 (0.08), 209 (0.06), 195 (0.06), 181 (0.03), 167 (0.05), 153 (0.02), 139 (0.03), 125 (0.08), 111 (0.13).

Renieroside A_2 (2), 1-*O*-*β*-D-Glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,17′*Z*)-2′-hydroxyhexacos-17′-enoylamino]-octadeca-4,8,10-triene-1,3-diol. White amorphous solid. $[\alpha]^{25}_D$ +4 (*c* 0.18, MeOH). ^{1}H and ^{13}C NMR data, see Table 1. FABMS: *m/z* 872 $[M + Na]^+$. HRFABMS (+ ve mode): *m/z* 872.6594 (calcd for $C_{50}H_{91}NO_9Na$, 872.6592).

Methanolysis of 2. Cerebroside (2.32 mg) was subjected to methanolysis similarly to **1** to yield a FAME (methyl-2-hydroxyhexacos-17-enoic acid; 1.1 mg). $[\alpha]^{23}_D$ –4 (*c* 0.1, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): *δ* 5.34 (2H, m, H-17, H-18), 4.12 (1H, dd, *J* = 8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 2.02 (4H, q, *J* = 6.0 Hz, H-16, H-19), 1.63 (2H, m, H-3), 1.28 (36H, m, H-4–H-15, H-20–H-25), 0.90 (3H, t, *J* = 7.0 Hz, H-26). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): *δ* 177.2 (C-1), 131.1

(C-17, C-18), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0–33.0 (C-4–C-15, C-20–C-24), 27.2 (C-16, C-19), 22.9 (C-25), 14.6 (C-26). FABMS: *m/z* 447 [M + Na]$^+$. FAB-CID-MS/MS: *m/z* 447 [M + Na]$^+$ (100), 431 (0.13), 417 (0.16), 403 (0.22), 389 (0.24), 375 (0.19), 361 (0.25), 347 (0.24), 333 (0.11), 321 (0.18), 307 (0.15), 293 (0.32), 279 (0.21), 265 (0.20), 251 (0.17), 237 (0.14), 223 (0.15), 209 (0.13), 195 (0.10), 181 (0.07), 167 (0.08), 153 (0.04), 139 (0.07), 125 (0.18), 111 (0.29).

The aqueous MeOH layer of the hydrolysate was evaporated under a vacuum to remove HCl. The resulting residue was eluted with MeOH on a Sephadex LH-20 column to afford methyl glucopyranoside (mixture of two anomers, 0.4 mg). $[\alpha]^{26}_D$ +70.8 (*c* 0.05, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 4.64 (1H, d, J = 3.8 Hz, H-1, α-anomer), 4.16 (1H, d, J = 7.8 Hz, H-1, β-anomer), 3.52 (3H, s, OCH_3, β-anomer), 3.39 (3H, s, OCH_3, α-anomer).

Renieroside A$_3$ (3), 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,16′*Z*)-2′-hydroxypentacos-16′-enoylamino]-octadeca-4,8,10-triene-1,3-diol. White amorphous solid. $[\alpha]^{25}_D$ +3 (*c* 0.14, MeOH). ^{1}H and ^{13}C NMR data, see Table 1. FABMS: *m/z* 858 [M + Na]$^+$. HRFABMS (+ ve mode): *m/z* 858.6436 (calcd for $C_{49}H_{89}NO_9Na$, 858.6435).

Methanolysis of 3. Cerebroside (1.83 mg) was subjected to methanolysis similarly to **1** to yield a FAME (methyl 2-hydroxypentacos-16-enoic acid; 1.2 mg). $[\alpha]^{23}_D$ –1 (*c* 0.1, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 5.34 (2H, m, H-16, H-17), 4.12 (1H, dd, J = –8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 2.02 (4H, q, J = 6.0 Hz, H-15, H-18), 1.62 (2H, m, H-3), 1.28 (34H, m, H-4–H-14, H-19–H-24), 0.90 (3H, t, J = 7.0 Hz, H-25). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): δ 177.2 (C-1), 131.1 (C-16, C-17), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0–33.0 (C-4–C-14, C-19–C-23), 27.2 (C-15, C-18), 22.9 (C-24), 14.6 (C-25). FABMS: *m/z* 433 [M + Na]. FAB-CID-MS/MS: *m/z* 433 [M + Na]$^+$ (100), 417 (0.11), 403 (0.16), 389 (0.09), 375 (0.13), 361 (0.14), 347 (0.11), 333 (0.13), 319 (0.05), 307 (0.10), 293 (0.08), 279 (0.11), 265 (0.08), 251 (0.07), 237 (0.06), 223 (0.07), 209 (0.05), 195 (0.05), 181 (0.03), 167 (0.04), 153 (0.02), 139 (0.03), 125 (0.05), 111 (0.06).

Renieroside A$_4$ (4), 1-*O*-β-D-Glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*)-hydroxydocosanoylamino]-octadeca-4,8,10-triene-1,3-diol. White amorphous solid. ^{1}H and ^{13}C NMR data, see Table 1. FABMS: *m/z* 818 [M + Na]$^+$. HRFABMS (+ ve mode): *m/z* 818.6123 (calcd for $C_{46}H_{85}NO_9Na$, 818.6122).

Methanolysis of 4. Cerebroside (0.71 mg) was subjected to methanolysis similarly to **1** to yield a FAME (methyl-2-hydroxydocosanoic acid; 0.2 mg). $[\alpha]^{22}_D$ –19 (*c* 0.02, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 4.12 (1H, dd, J = 8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (36H, m, H-4–H-21), 0.89 (3H, t, J = 7.0 Hz, H-22). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): δ 177.2 (C-1), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0–33.0 (C-4–C-20), 22.9 (C-21), 14.6 (C-22). FABMS: *m/z* 393 [M + Na]$^+$.

Renieroside A$_5$ (5), 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*)-hydroxytetracosanoylamino]-octadeca-4,8,10-triene-1,3-diol. White amorphous solid. ^{1}H and ^{13}C NMR data, see Table 1. FABMS (+ ve mode): *m/z* 846 [M + Na]$^+$; (– ve mode): *m/z* 822 [M − H]$^-$, 660 [(M − H) – 162]$^-$. HRFABMS (+ ve mode): *m/z* 846.6433 (calcd for $C_{48}H_{89}NO_9Na$, 846.6435).

Methanolysis of 5. Cerebroside (0.76 mg) was subjected to methanolysis similarly to **1** to yield a FAME (methyl-2-hydroxytetracosanoic acid; 0.4 mg). $[\alpha]^{22}_D$ –4 (*c* 0.1, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 4.12 (1H, dd, J = 8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (40H, m, H-4–H-23), 0.89 (3H, t, J = 7.0 Hz, H-24). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): δ 177.2 (C-1), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0−33.0 (C-4–C-22), 22.9 (C-23), 14.6 (C-24). FABMS: *m/z* 421 [M + Na]$^+$.

Renieroside B$_1$ (6), 1-*O*-β-D-Glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,21′*Z*)-2′-hydroxyoctacos-21′-enoylamino]-9-methyloctadeca-4,8,10-triene-1,3-diol. White amorphous solid. $[\alpha]^{22}_D$ +10 (*c* 0.1, MeOH). ^{1}H and ^{13}C NMR data, see Table 2. FABMS (+ ve mode): *m/z* 914 [M + Na]$^+$, 874 [(M + H) – H_2O]$^+$, 712 [(M + H) – H_2O – 162]$^+$; (– ve mode): *m/z* 890 [M − H]$^-$, 728 [(M − H) – 162]$^-$, 711 [(M − H) – H_2O – 162]$^-$. HRFABMS (– ve mode): *m/z* 890.7089 (calcd for $C_{53}H_{96}NO_9$, 890.7085).

Methanolysis of 6. Cerebroside (0.42 mg) was subjected to methanolysis similarly to **1** to yield a FAME (methyl-2-hydroxyoctacos-21-enoic acid; 0.2 mg): $[\alpha]^{22}_D$ –18 (*c* 0.02, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 5.34 (2H, m, H-21, H-22), 4.12 (1H, dd, J = 8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 2.02 (4H, q, J = 6.0 Hz, H-20, H-23), 1.61 (2H, m, H-3), 1.28 (40H, m, H-4–H-19, H-24–H-27), 0.90 (3H, t, J = 7.0 Hz, H-28). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): δ 177.2 (C-1), 131.1 (C-21, C-22), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0−33.0 (C-4–C-19, C-24–C-26), 27.2 (C-20, C-23), 22.9 (C-27), 14.6 (C-28). FABMS: *m/z* 453 [M + H]$^+$, 475 [M + Na]$^+$. FAB-CID-MS/MS: *m/z* 475 [M + Na]$^+$ (100), 459 (0.08), 445 (0.07), 431 (0.06), 417 (0.22), 403 (0.26), 389 (0.07), 375 (0.04), 363 (0.03), 349 (1.45), 335 (0.08), 321 (0.07), 307 (0.12), 293 (0.11), 279 (0.18), 265 (0.08), 251 (0.07), 237 (0.08), 223 (0.08), 209 (0.06), 195 (0.06), 181 (0.03), 167 (0.05), 153 (0.02), 139 (0.03), 125 (0.08), 111 (0.13).

Renieroside B$_2$ (7), 1-*O*-β-D-Glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,17′*Z*)-2′-hydroxyhexacos-17′-enoylamino]-9-methyloctadeca-4,8,10-triene-1,3-diol. White amorphous solid. $[\alpha]^{22}_D$ +3 (*c* 0.16, MeOH). ^{1}H and ^{13}C NMR data, see Table 2. FABMS (+ ve mode): *m/z* 886 [M + Na]$^+$; (– ve mode): *m/z* 862 [M − H]$^-$, 700 [(M − H) – 162]$^-$. HRFABMS (+ ve mode): *m/z* 886.6746 (calcd for $C_{51}H_{93}NO_9Na$, 886.6748).

Methanolysis of 7. Cerebroside (2.08 mg) was subjected to methanolysis similarly to **1** to yield a FAME (methyl-2-hydroxyhexacos-17-enoic acid; 0.9 mg). $[\alpha]^{23}_D$ –1 (*c* 0.07, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 5.34 (2H, m, H-17, H-18), 4.12 (1H, dd, J = 8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 2.02 (4H, q, J = 6.0 Hz, H-16, H-19), 1.61 (2H, m, H-3), 1.28 (36H, m, H-4–H15, H-20–H-25), 0.89 (3H, t, J = 7.5 Hz, H-26). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): δ 177.2 (C-1), 131.1 (C-17, C-18), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0–33.0 (C-4–C-15, C-20–C-24), 27.2 (C-16, C-19), 22.9 (C-25), 14.6 (C-26). FABMS: *m/z* 447 [M + Na]$^+$, FAB-CID-MS/MS: *m/z* 447 [M + Na]$^+$ (100), 431 (0.08), 417 (0.11), 403 (0.15), 389 (0.14), 375 (0.13), 361 (0.11), 347 (0.13), 333 (0.04), 321 (0.14), 307 (0.11), 293 (0.16), 279 (0.11), 265 (0.09), 251 (0.08), 237 (0.08), 223 (0.07), 209 (0.07), 195 (0.06), 181 (0.05), 167 (0.05), 153 (0.02), 139 (0.03), 125 (0.06), 111 (0.11).

The aqueous MeOH layer of the hydrolysate was evaporated under a vacuum to remove HCl. The resulting residue was eluted with MeOH on a Sephadex LH-20 column to afford methyl glucopyranoside (mixture of two anomers, 0.4 mg). $[\alpha]^{27}_D$ +62.4 (*c* 0.04, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 4.64 (1H, d, J = 3.8 Hz, H-1, α-anomer), 4.16 (1H, d, J = 7.8 Hz, H-1, β-anomer), 3.52 (3H, s, OCH_3, β-anomer), 3.39 (3H, s, OCH_3, α-anomer).

Renieroside B$_3$ (8), 1-*O*-β-D-Glucopyranosyl-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,20′*Z*)-2′-hydroxyheptacos-20′-enoylamino]-9-methyloctadeca-4,8,10-triene-1,3-diol. White amorphous solid. ^{1}H and ^{13}C NMR data, see Table 2. FABMS (+ ve mode): *m/z* 900 [M + Na]$^+$; (– ve mode): *m/z* 876 [M − H]$^-$, 714 [(M − H) – 162]$^-$. HRFABMS (+ ve mode): *m/z* 900.6903 (calcd for $C_{52}H_{95}NO_9Na$, 900.6905).

Methanolysis of 8. Cerebroside (0.37 mg) was subjected to methanolysis similarly to **1** to yield a FAME (methyl-2-hydroxyheptacos-20-enoic acid; 0.15 mg). $[\alpha]^{22}_D$ –61 (*c* 0.01, MeOH). ^{1}H NMR data (CD_3OD, 500 MHz): δ 5.34 (2H, m, H-20, H-21), 4.12 (1H, dd, J = 8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 2.02 (4H, q, J = 6.0 Hz, H-19, H-22), 1.61 (2H, m, H-3), 1.28 (38H, m, H-4–H-18, H-23–H-26), 0.89 (3H, t, J = 7.0 Hz, H-27). ^{13}C NMR data (CD_3OD, assignments based on HMBC and HSQC experiments, 500 MHz): δ 177.2 (C-1), 131.1 (C-20, C-21), 72.8 (C-2), 52.0 (OCH_3), 35.5 (C-3), 29.0–33.0 (C-4–C-18, C-23–C-25), 27.2 (C-19, C-22), 22.9 (C-26), 14.6 (C-27). FABMS: *m/z* 461 [M + Na]$^+$. FAB-CID-MS/MS: *m/z* 461 [M + Na]$^+$ (100), 445 (0.1), 431 (0.08), 417 (0.08), 403 (0.18), 389 (0.17), 375 (0.1), 361 (0.07), 349 (0.4), 335 (0.17), 321 (0.06), 307 (0.12), 293 (0.09), 279 (0.07), 265 (0.08), 251 (0.07), 237 (0.05), 223 (0.06), 209 (0.06), 195 (0.04), 181 (0.03), 167 (0.04), 153 (0.02), 139 (0.01), 125 (0.06), 111 (0.08).

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Supporting Information Available: ^{1}H NMR, HSQC, low-resolution FAB mass, and FAB-CID tandem mass spectra of compounds **1** and **6**, FAB-CID tandem mass spectra of compounds **2** and **8**, and a

color photograph of the sponge *Haliclona* (*Reniera*) sp. This material is available free of charge via the Internet at http://pubs.acs.org.

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