New Lysophosphatidylcholines and Monoglycerides from the Marine Sponge *Stelletta* sp.

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Two new lysophosphatidylcholines (**1**, **2**) and four new monoglycerides (**5**−**8**) were isolated from the marine sponge *Stelletta* sp. by bioactivity-guided fractionation. The planar structures of the new compounds were established on the basis of NMR and MS analyses. The stereochemistry was defined by comparison of the optical rotation. The compounds were evaluated for cytotoxicity against a small panel of five human tumor cell lines.

Marine sponges of the genus *Stelletta* are reported to contain various sterols,[1−3] terpenoids,[4−6] alkaloids,[7−9] and fatty acids.[10] In the course of screening for cytotoxic constituents from marine sponges, we have noticed significant activity in the crude extract of the sponge *Stelletta* sp. collected from Korean waters. Bioactivity-guided fractionation of the MeOH extract has afforded four new acetylenic acids.[11] In our continuing study on the cytotoxic compounds of the same sponge, two new lysophosphatidylcholines (**1**, **2**) and four new monoglycerides (**5**−**8**), together with two known monoglycerides (**3**, **4**)[12,13] and two known ω-hydroxy fatty acid methyl esters,[14,15] were isolated. The isolation, structure elucidation, and cytotoxicity evaluation of these compounds are described herein.

The MeOH extract of the sponge displayed toxicity to brine shrimp larvae (LD_{50}, 296 μg/mL). Guided by the brine shrimp lethality assay, the MeOH extract was further partitioned between water and CH_2Cl_2, followed by partitioning of the CH_2Cl_2 solubles between aqueous MeOH and *n*-hexane. The aqueous MeOH layer was subjected to reversed-phase flash column chromatography, Sephadex LH-20 column chromatography, and HPLC to afford compounds **1**−**10**.

Stellettacholine A (**1**) was isolated as a pale yellow oil. The molecular formula of **1** was established as $C_{28}H_{50}NO_8P$ on the basis of MS and NMR spectral analyses. The FABMS of **1** showed the $[M + H]^+$ peak at *m*/*z* 560 accompanied by the $[M + Na]^+$ peak at *m*/*z* 582. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 582.3180) matched well with the expected molecular formula $C_{28}H_{50}NO_8PNa$ (Δ +0.8 mmu). The ^1H NMR spectrum showed methylene and methine proton signals integrated to nine protons at δ 4.28−3.63 (Table 1) and a signal integrated to nine protons at δ 3.21 (three *N*-methyl groups). Seven carbon signals attached to heteroatoms, including a triplet *N*-methyl carbon signal, were observed at δ_C 69.9−54.9 (Table 1) in the proton-decoupled ^{13}C NMR spectrum. Characteristic couplings ($^2J_{CP}$, $^3J_{CP}$) between phosphorus and relevant carbons (C-2, C-3, C-1″, and C-2″) were also observed (Table 1). The carbon attached to the quaternary ammonium nitrogen (C-2″) was observed as a broad peak due to additional coupling with the quadrupolar ^{14}N. A doublet of doublets of triplets at δ 5.82 and two doublets of doublets at δ 4.98 and 4.91 were attributed to a monosubstituted olefin. The additional olefinic protons at δ 5.81 and 5.43 were correlated with the acetylenic carbons C-5′ and C-6′ in the HMBC spectrum, indicating that the double bond was conjugated with the triple bond. The geometry of the double bond was deduced to be *cis* on the basis of the coupling constant of the olefinic protons (J = 10.5 Hz). The

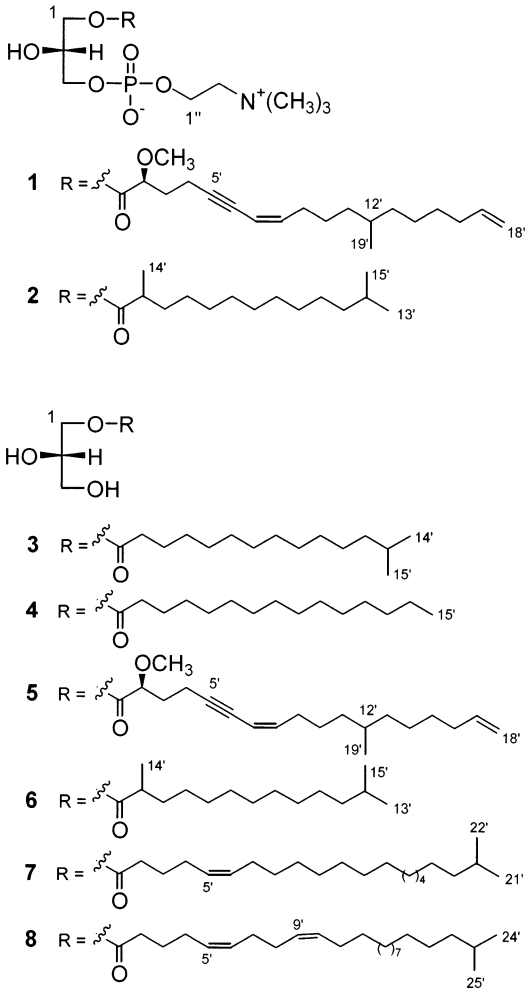

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **5**[a]

	1		**5**	
position	^{1}H	^{13}C	^{1}H	^{13}C
1	4.26 (1H, dd, 11.5, 4.5)	66.9	4.27 (1H, dd, 11.0, 6.0)	65.9
	4.21 (1H, dd, 11.5, 6.5)		4.16 (1H, dd, 11.0, 4.5)	
2	4.01 (1H, m)	69.9 (d, 8.6)[b]	3.84 (1H, quint, 6.5)	69.7
3	3.91 (2H, m)	67.8 (d, 5.9)[b]	3.56 (2H, m)	62.4
1′		174.1		172.8
2′	3.98 (1H, dd, 8.5, 4.5)	80.2	3.99 (1H, dd, 8.5, 3.5)	78.6
3′	1.98 (1H, m)	31.4	1.97 (1H, m)	31.6
	1.86 (1H, m)		1.87 (1H, m)	
4′	2.47 (2H, m)	16.3	2.47 (1H, m)	15.2
5′		93.6[d]		92.2[d]
6′		79.3[d]		78.0[d]
7′	5.43 (1H, br d, 10.5)	110.5	5.46 (1H, dt, 11.0, 1.5)	109.2
8′	5.81 (1H, dt, 10.5, 7.0)	143.8	5.87 (1H, dt, 11.0, 7.0)	142.4
9′	2.25 (2H, q, 7.0)	31.4	2.26 (2H, q, 7.0)	31.2
10′	1.26−1.37 (2H, m)	27.5	1.28−1.38 (2H, m)	26.3
11′	1.26−1.37 (1H, m)	38.1	1.28−1.38 (1H, m)	36.1
	1.10 (1H, m)		1.10 (1H, m)	
12′	1.37 (1H, m)	33.3	1.37 (1H, m)	32.2
13′	1.26−1.37 (1H, m)	37.7	1.28−1.38 (1H, m)	36.1
	1.10 (1H, m)		1.10 (1H, m)	
14′	1.26−1.37 (2H, m)	27.7	1.28−1.38 (2H, m)	26.1
15′	1.26−1.37 (2H, m)	30.5	1.28−1.38 (2H, m)	30.1
16′	2.04 (2H, q, 7.0)	33.8	2.04 (2H, q, 7.0)	34.3
17′	5.82 (1H, ddt, 17.0, 11.5, 7.0)	140.3	5.82 (1H, ddt, 17.0, 11.5, 7.0)	139.2
18′	4.98 (1H, dd, 17.0, 2.0)	114.8	4.99 (1H, dd, 17.0, 2.0)	114.2
	4.91 (1H, dd, 11.5, 2.0)		4.91 (1H, dd, 11.5, 2.0)	
19′	0.85 (3H, d, 6.5)	20.2	0.86 (3H, d, 7.0)	19.6
1″	4.28 (2H, m)	60.6 (d, 5.5)[b]		
2″	3.63 (2H, m)	67.6 (br s)		
OCH_3	3.34 (3H, s)	58.9	3.40 (3H, s)	57.8
N-CH_3	3.21 (9H, s)	54.8 (t, 3.9)[c]		

[a] Measured at 500 MHz in CD_3OD. [b] Multiplicities represent $^{13}C-^{31}P$ couplings. [c] Multiplicity represent $^{13}C-^{14}N$ coupling. [d] Assignment with the same superscript in the same column may be interchanged.

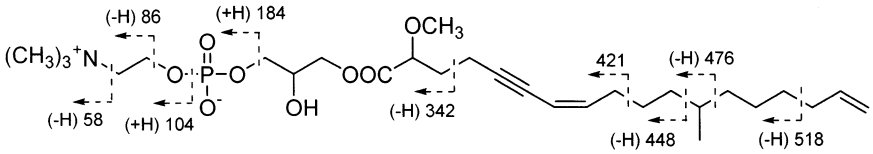

Figure 1. Key FAB-CID tandem mass fragmentations of the $[M + H]^+$ ion of **1**.

position of the methyl branching was clearly recongnized from the 28-mass gap between the fragment ions at *m/z* 476 and 448 in the FAB-CID tandem mass spectrum of the $[M + H]^+$ ion (Figure 1). The above data revealed a phosphatidylcholine skeleton with (*Z*)-stellettic acid B[11] as the acyl moiety. The absolute configuration at C-2′ of the acyl moiety was presumed to be *S*, which is the same as that of (*Z*)-stellettic acid B co-occuring in the same sponge.[11] The stereochemistry of the glycerol moiety was presumed to be the same as those of the phospholipids from sponges.[16] Mosher's MTPA esterification of the C-2 hydroxyl group was unsuccessful, presumably due to steric hindrance. Thus, the structure of **1** was determined to be 1-*O*-[(*Z*)-(2*S*)-2-methoxy-12-methyloctadeca-7,17-dien-5-ynoyl]-*sn*-glycero-3-phosphocholine.

Stellettacholine B (**2**) was isolated as a white amorphous solid. The molecular formula of **2** was established as $C_{23}H_{48}NO_7P$ on the basis of MS and NMR spectral analyses. The FABMS of **2** showed the $[M + H]^+$ peak at *m/z* 482 accompanied by the $[M + Na]^+$ peak at *m/z* 504. The exact mass of the $[M + Na]^+$ ion (*m/z* 504.3076) matched well with the expected molecular formula $C_{23}H_{48}NO_7PNa$ (Δ +1.0 mmu). The ^{1}H NMR spectrum featured a characteristic pattern of a phosphatidylcholine skeleton. The ^{1}H NMR data of the acyl moiety featured a three-proton doublet at δ 1.14, which was correlated with the methine proton sextet at δ 2.47 in the COSY spectrum. In the HMBC spectrum these methyl and methine proton signals were correlated with that of the carbonyl carbon at δ 178.1. This revealed that the methyl group was present at C-2′. The configuration of the C-2′ methyl branch remains to be determined. Although some α-methyl fatty acids have been synthesized for industrial applications,[17] the only report from natural source is the fatty acid of a halophilic *Bacillus* sp.[18] with one less carbon than the acyl moiety of **2**. The stereochemistry of the glycerol moiety was presumed to be identical to that of **1**. Thus, the structure of **2** was determined to be 1-*O*-(2,12-dimethyltridecanoyl)-*sn*-glycero-3-phosphocholine.

The known compounds 1-*O*-(13-methyltetradecanoyl)-*sn*-glycerol (**3**) and 1-*O*-pentadecanoyl-*sn*-glycerol (**4**) were identified by comparing the physical properties, ^{1}H NMR, and MS data with those reported.[12,13] The configuration at C-2 was established from the positive optical rotation, which is a general feature of the long-chain 1-*O*-acyl-*sn*-glycerols.[19]

Compound **5** was isolated as a pale yellow oil. The molecular formula of **5** was established as $C_{23}H_{38}O_5$ on the basis of MS and NMR spectral analyses. The FABMS of **5** showed the $[M + H]^+$ peak at *m/z* 395 accompanied by the $[M + Na]^+$ peak at *m/z* 417. The exact mass of the $[M + Na]^+$ ion (*m/z* 417.2616) matched well with the expected molecular formula $C_{23}H_{38}O_5Na$ (Δ −0.1 mmu). The ^{1}H and ^{13}C NMR spectra featured a characteristic pattern of the monoacyl glyeride skeleton. Analysis of the ^{1}H and ^{13}C NMR spectral data indicated that **5** contains the same acyl

moiety as that of **1** (Table 1). The position of the methyl branching was again clear from the 28-mass gap between the fragment ions at *m*/*z* 333 and 305 in the FAB-CID tandem mass spectrum of the $[M + Na]^+$ ion. Thus, the structure of **5** was determined to be 1-*O*-[(*Z*)-(2*S*)-2-methoxy-12-methyloctadeca-7,17-dien-5-ynoyl]-*sn*-glycerol.

Compound **6** was isolated as a white amorphous solid. The molecular formula of **6** was established as $C_{18}H_{36}O_4$ on the basis of MS and NMR spectral analyses. The FABMS of **6** showed the $[M + H]^+$ peak at *m*/*z* 317 accompanied by the $[M + Na]^+$ peak at *m*/*z* 339. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 339.2522) matched well with the expected molecular formula $C_{18}H_{36}O_4Na$ (Δ +1.1 mmu). The ^1H and ^{13}C NMR spectra featured the presence of the same acyl moiety as that of **2**. Thus, the structure of **6** was determined to be 1-*O*-(2,12-dimethyltridecanoyl)-*sn*-glycerol.

Compound **7** was isolated as a pale yellow oil. The molecular formula of **7** was established as $C_{25}H_{48}O_4$ on the basis of MS and NMR spectral analyses. The FABMS of **7** showed the $[M + Na]^+$ peak at *m*/*z* 435. A two-proton multiplet at δ 5.38, a two-proton mutiplet at δ 2.10, and a two-proton broad quartet at δ 2.02 suggested the presence of a double bond in **7**. The COSY spectrum was helpful in assigning the position of the double bond. The two-proton signal at δ 2.37 (H-2′) was correlated with the two-proton signal at δ 1.72 (H-3′), which in turn was correlated with an allylic proton resonance at δ 2.10 (H-4′). The correlation between the allylic proton signals at δ 2.10 with the olefinic proton at δ 5.38 was also observed. Thus, the double bond was shown to be located at C-5′, and the geometry of the double bond was deduced to be *cis*, as the allylic carbons resonated at δ_C 26.5 and 27.4.[20] Accordingly, the structure of **7** was determined to be 1-*O*-[(*Z*)-20-methylhenicos-5-enoyl]-*sn*-glycerol.

Compound **8** was isolated as a pale yellow oil. The molecular formula of **8** was established as $C_{28}H_{52}O_4$ on the basis of MS and NMR spectral analyses. The FABMS of **8** showed the $[M + Na]^+$ peak at *m*/*z* 475. The ^1H NMR spectrum was similar to that of **7**. A four-proton multiplet at δ 5.30−5.40, a six-proton multiplet at δ 2.09, and a two-proton broad quartet at δ 2.02 were observed. In the COSY spectrum, the correlations from H-2′ to H-11′ showed that the double bonds were located at C-5′ and C-9′. It was further confirmed by the 54-mass gap between the fragment ions at *m*/*z* 277/223 and 223/169 in the FAB-CID tandem mass spectrum of the $[M + Na]^+$ ion. The geometry of the double bonds was deduced to be *cis*, as the allylic carbons resonated at δ_C 26.5, 27.3, 27.3, and 27.4.[20] Thus the structure of **8** was determined to be 1-*O*-[(5*Z*,9*Z*)-23-methyltetracosa-5,9-dienoyl]-*sn*-glycerol. The configuration of the acyl moiety of compounds **5**−**8** was assumed to be the same as that of **1**−**4**.

Together with compounds **1**−**8**, two known compounds, methyl ω-hydroxyheptadecanoate (**9**)[14] and methyl ω-hydroxypentadecanoate (**10**),[15] were also isolated. The record of ω-hydroxy fatty acid methyl esters from sponge was unprecedented.

These compounds were evaluated for cytotoxicity against a small panel of five human tumor cell lines (Table 2). Only the lysophosphatidylcholines (**1**, **2**) showed moderate cytotoxicity, and the monoglycerides (**5**−**8**) were virtually inactive.

Table 2. Cytotoxicities (ED_{50}, μg/mL) of Compounds **1**, **2**, and **5**−**10** against Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	11.8	14.5	9.1	13.6	15.1
2	7.4	4.8	6.3	24.1	26.9
5	31.3	>30	>30	42.5	>30
6	20.0	38.9	21.8	30.7	18.6
7	>30	>30	>30	>30	>30
8	>30	>30	>30	>30	>30
9	>30	>30	>30	>30	>30
10	25.9	35.7	>30	>30	16.5
doxorubicin	0.03	0.13	0.06	0.19	0.29

[a] A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT 15: human colon cancer.

Experimental Section

General Experimental Procedures. Optical rotations were obtained using a JASCO DIP-370 digital polarimeter. IR spectra were measured using a JASCO FT/IR-410 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian Inova 500 instrument. Chemical shifts were reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD; δ_H 7.27 and δ_C 77.0 for $CDCl_3$). FAB-CID tandem MS data were obtained using a JEOL JMS SX-102A. HPLC was performed with YMC ODS-H80 (250 × 10 mm i.d., 4 μm, 80 Å) and YMC-Pack CN (250 × 10 mm i.d., 5 μm, 120 Å) column using a Shodex RI-71 detector. Gel filtration chromatography was performed with Sephadex LH-20 (Pharmacia Biotech AB, 350 × 20 mm).

Animal Material. The sponge was collected by hand using scuba (20 m depth) in October 2001, off the coast of Ullung Island, Korea. The specimen was identified as *Stelletta* sp. by Prof. C. J. Sim, Hannam University. A voucher specimen (registry No. Spo. 37) was deposited at the Natural History Museum, Hannam University, Korea, and has been described elsewhere.[11]

Extraction and Isolation. The frozen sponge (15 kg) was extracted with MeOH at room temperature. The MeOH extract of the sponge displayed moderate toxicity against brine shrimp larvae (LD_{50}, 296 μg/mL). The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane to yield aqueous MeOH- (5.2 g) and *n*-hexane-soluble (19.1 g) fractions. The aqueous MeOH fraction was subjected to step gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh) eluting with a solvent system of 50 to 0% H_2O/MeOH, to afford 20 fractions. These fractions were evaluated for activity employing the brine shrimp assay, and fractions 8−16 were found active. Fraction 13 was further separated by Sephadex LH-20 column chromatography eluting with MeOH, to afford 18 fractions. The subfraction 13-8 was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm i.d., 4 μm, 80 Å) eluting with 15% H_2O/MeOH to yield compound **1** (3.8 mg). Subfraction 13-9 was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm i.d., 4 μm, 80 Å) eluting with 17% H_2O/MeOH, and by reversed-phase HPLC (YMC-Pack CN, 250 × 10 mm i.d., 5 μm, 120 Å) eluting with 48% H_2O/CH_3CN to yield compound **2** (1.4 mg). Subfraction 13-13 was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm i.d., 4 μm, 80 Å) eluting with 15% H_2O/MeOH to yield compounds **3** (1.5 mg), **5** (2.8 mg), **6** (2.1 mg), and **9** (7.6 mg). Subfraction 13-14 was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm i.d., 4 μm, 80 Å) eluting with 15% H_2O/MeOH to yield compounds **4** (1.6 mg) and **10** (1.2 mg). Fraction 16 was further separated by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm i.d., 4 μm, 80 Å) eluting with 10% H_2O/MeOH to yield compounds **7** (1.5 mg) and **8** (0.8 mg).

Stellettacholine A (1): pale yellow oil; $[\alpha]^{21}_D$ −0.1° (*c* 0.25, MeOH); IR (film) ν_{max} 3392, 2925, 2854, 2359, 1733, 1645, 1458, 1226, 1085 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; FAB-CID MS/MS *m*/*z* 582 $[M + Na]^+$ (43), 560 $[M + H]^+$ (100), 518 (0.2), 504 (0.1), 490 (0.2), 476 (0.1), 448 (0.1), 434 (0.3), 421 (0.3), 377 (0.2), 342 (0.2), 329 (0.6), 314 (0.7), 312 (0.5), 256 (0.2), 184 (1.0), 166 (1.5), 104 (4.0), 86 (1.3), 58 (0.2); HRFABMS *m*/*z* 582.3180 $[M + Na]^+$ (calcd for $C_{28}H_{50}O_8PNa$, 582.3172).

Stellettacholine B (2): white amorphous solid; $[\alpha]^{21}_D$ −3.2° (*c* 0.09, MeOH); IR (film) ν_{max} 2953, 2923, 2853, 1733, 1558, 1506, 1456, 1070 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 4.28 (2H, m, H-1″), 4.15 (1H, dd, J = 11.5, 4.5 Hz, H-1$_a$), 4.11 (1H, dd, J = 11.5, 6.5 Hz, H-1$_b$), 3.96 (1H, m, H-2), 3.89 (2H, m, H-3), 3.63 (2H, m, H-2″), 3.21 (9H, s, *N*-CH_3), 2.47 (1H, sextet, J = 6.5 Hz, H-2′), 1.63 (1H, m, H-3′$_a$), 1.52 (1H, m, H-12′), 1.42 (1H, m, H-3′$_b$), 1.22−1.32 (14H, m, H-4′−H-10′), 1.16 (2H, m, H-11′), 1.14 (3H, d, J = 6.5 Hz, H-14′), 0.87 (6H, d, J = 6.5 Hz, H-13′, H-15′), 0.87 (3H, d, J = 6.5 Hz, H-13); ^{13}C NMR (CD_3OD, 125 MHz) δ 178.1 (C-1′), 69.9 (d, $^3J_{CP}$ = 7.6 Hz, C-2), 67.8 (d, $^2J_{CP}$ = 5.8 Hz, C-3), 67.5 (br s, C-2″), 66.3 (C-1), 60.6 (d, $^2J_{CP}$ = 5.5 Hz, C-1″), 54.8 (t, $^1J_{CN}$ = 3.8 Hz, *N*-CH_3), 39.6 (C-2′), 33.6 (C-3′), 29.8−29.2 (C-4′-10′), 39.0 (C-11′), 24.8 (C-12′), 21.8 (C-13′, C-15′), 16.3 (C-14′); FAB-CID MS/MS *m*/*z* 504 $[M + Na]^+$ (100), 482 $[M + H]^+$ (9.6), 438 (0.5), 424 (0.3), 326 (1.5), 313 (0.9), 309 (0.7), 184 (10.7), 166 (1.2), 104 (2.1), 86 (0.7); HRFABMS *m*/*z* 504.3076 $[M + Na]^+$ (calcd for $C_{23}H_{48}NO_7PNa$, 504.3066).

Compound 3: white amorphous solid; $[\alpha]^{21}_D$ +3.4° (*c* 0.10, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.14 (1H, dd, J = 11.0, 4.5 Hz, H-1$_a$), 4.05 (1H, dd, J = 11.0, 6.5 Hz, H-1$_b$), 3.81 (1H, quintet, J = 4.5 Hz, H-2), 3.54 (2H, m, H-3), 2.34 (2H, t, J = 7.5 Hz, H-2′), 1.61 (2H, m, H-3′), 1.51 (1H, m, H-13′), 1.22−1.32 (16H, m, H-4′-11′), 1.16 (2H, m, H-12′), 0.87 (6H, d, J = 6.5 Hz, H-14′, H-15′); FABMS *m*/*z* 339 $[M + Na]^+$ (100), 317 $[M + H]^+$ (29).

Compound 4: white amorphous solid; $[\alpha]^{21}_D$ +2.6° (*c* 0.11, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.14 (1H, dd, J = 11.0, 4.5 Hz, H-1$_a$), 4.05 (1H, dd, J = 11.0, 6.5 Hz, H-1$_b$), 3.81 (1H, m, H-2), 3.54 (2H, m, H-3), 2.34 (2H, t, J = 7.5 Hz, H-2′), 1.61 (2H, m, H-3′), 1.22−1.32 (20H, m, H-4′-13′), 1.13 (2H, m, H-14′), 0.87 (3H, t, J = 6.5 Hz, H-15′); FABMS *m*/*z* 339 $[M + Na]^+$ (100), 317 $[M + H]^+$ (48).

Compound 5: pale yellow oil $[\alpha]^{21}_D$ −1.0° (*c* 0.18, MeOH); IR (film) ν_{max} 3365, 2926, 2854, 1739, 1456, 1194, 1124, 1045, 910 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; FAB-CID MS/MS *m*/*z* 417 $[M + Na]^+$ (100), 395 $[M + H]^+$ (9.5), 375 (0.2), 347 (0.2), 333 (0.2), 305 (0.2), 291 (0.3), 278 (0.4), 199 (0.3), 186 (0.5), 169 (0.5); HRFABMS *m*/*z* 417.2616 $[M + Na]^+$ (calcd for $C_{23}H_{38}O_5Na$, 417.2617).

Compound 6: white amorphous solid; $[\alpha]^{21}_D$ −3.5° (*c* 0.14, MeOH); IR (film) ν_{max} 3365, 2924, 2853, 1733, 1558, 1457, 1172, 1116, 1054 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 4.13 (1H, dd, J = 11.0, 4.5 Hz, H-1$_a$), 4.06 (1H, dd, J = 11.0, 6.5 Hz, H-1$_b$), 3.81 (1H, quintet, J = 4.5 Hz, H-2), 3.54 (2H, m, H-3), 2.48 (1H, sextet, J = 6.5 Hz, H-2′), 1.63 (1H, m, H-3′$_a$), 1.51 (1H, m, H-12′), 1.41 (1H, m, H-3′$_b$), 1.22−1.32 (14H, m, H-4′-10′), 1.16 (2H, m, H-11′), 1.14 (3H, d, J = 6.5 Hz, H-14′), 0.87 (6H, d, J = 6.5 Hz, H-13′, H-15′); ^{13}C NMR (CD_3OD, 125 MHz) δ 177.5 (C-1′), 69.9 (C-2), 65.2 (C-1), 62.8 (C-3), 39.6 (C-2′), 39.0 (C-11′), 33.6 (C-3′), 29.8−29.2 (C-4′-10′), 24.8 (C-12′), 21.8 (C-13′, C-15′), 16.3 (C-14′); FAB-CID MS/MS *m*/*z* 339 $[M + Na]^+$ (100), 317 $[M + H]^+$ (42), 295 (0.3), 281 (0.2), 267 (0.1), 253 (0.1), 239 (0.1), 225 (0.2), 211 (0.1), 197 (0.2), 183 (2.2), 170 (1.0), 113 (0.5), 97 (0.3), 84 (0.2); HRFABMS *m*/*z* 339.2522 $[M + Na]^+$ (calcd for $C_{18}H_{36}O_4Na$, 339.2511).

Compound 7: pale yellow oil; $[\alpha]^{21}_D$ +3.4° (*c* 0.10, MeOH); IR (film) ν_{max} 3397, 2924, 2853, 1733, 1558, 1506, 1456, 1173, 1120, 1054 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.38 (2H, m, H-5′, H-6′), 4.22 (1H, dd, J = 11.0, 5.0 Hz, H-1$_a$), 4.16 (1H, dd, J = 11.0, 6.5 Hz, H-1$_b$), 3.95 (1H, m, H-2), 3.70 (1H, dd, J = 11.0, 4.0 Hz, H-3$_a$), 3.60 (1H, dd, J = 11.0, 5.5 Hz, H-3$_b$), 2.37 (2H, t, J = 7.0 Hz, H-2′), 2.10 (2H, m, H-4′), 2.02 (2H, q, J = 7.0 Hz, H-7′), 1.72 (2H, quint, J = 7.0 Hz, H-3′), 1.52 (1H, m, H-20′), 1.22−1.32 (22H, m, H-8′-18′), 1.15 (2H, m, H-19′), 0.87 (6H, d, J = 7.0 Hz, H-21′, H-22′); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.5 (C-1′), 130.6 (C-6′), 128.5 (C-5′), 70.3 (C-2), 65.4 (C-1), 63.5 (C-3), 39.2 (C-19′), 33.6 (C-2′), 29.4−30.1 (C-8′−C-18′), 28.0 (C-20′), 27.4 (C-7′), 26.5 (C-4′), 24.6 (C-3′), 22.6 (C-21′, C-22′); FABMS *m*/*z* 435 $[M + Na]^+$ (100).

Compound 8: pale yellow oil; $[\alpha]^{21}_D$ +2.9° (*c* 0.05, MeOH); IR (film) ν_{max} 3419, 2924, 2853, 1733, 1653, 1558, 1539, 1506, 1456, 1206, 1177, 1054 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.30−5.40 (4H, m, H-5′, 6′, 9′, 10′), 4.22 (1H, dd, J = 11.0, 5.0 Hz, H-1$_a$), 4.16 (1H, dd, J = 11.0, 6.5 Hz, H-1$_b$), 3.95 (1H, m, H-2), 3.70 (1H, dd, J = 11.0, 4.0 Hz, H-3$_a$), 3.60 (1H, dd, J = 11.0, 5.5 Hz, H-3$_b$), 2.37 (2H, t, J = 7.0 Hz, H-2′), 2.09 (6H, m, H-4′, H-7′, H-8′), 1.72 (2H, quint, J = 7.0 Hz, H-3′), 2.02 (2H, q, J = 7.0 Hz, H-11′), 1.52 (1H, m, H-23′), 1.22−1.32 (20H, m H-12′−21′), 1.15 (2H, m, H-22′), 0.87 (6H, d, J = 7.0 Hz, H-24′, H-25′); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.4 (C-1′), 130.6 (C-6′), 130.5 (C-10′), 128.9 (C-9′), 128.5 (C-5′), 70.3 (C-2), 65.6 (C-1), 63.4 (C-3), 39.2 (C-22′), 33.4 (C-2′), 29.4−30.1 (C-12′−C-21′), 28.0 (C-23′), 27.4 (C-11′), 27.3 (C-7′ and C-8′), 26.5 (C-4′), 24.6 (C-3′), 22.6 (C-24′, C-25′); FAB-CID MS/MS *m*/*z* 475 $[M + Na]^+$ (36), 431 (0.4), 417 (0.2), 403 (0.2), 389 (0.1), 375 (0.1), 361 (0.2), 347 (0.1), 333 (0.1), 319 (0.1), 305 (0.2), 291 (0.2), 278 (0.4), 277 (0.4), 263 (0.1), 224 (1.4), 223 (0.5), 207 (0.1), 170 (0.4), 169 (1.0), 156 (0.5), 113 (0.2).

Compound 9: white amorphous solid; ^{1}H NMR (CD_3OD, 500 MHz) δ 3.64 (3H, s, OCH_3), 3.53 (2H, t, J = 7.0 Hz, H-17), 2.30 (2H, d, J = 7.5 Hz, H-2), 1.59 (2H, m, H-3), 1.52 (2H, m, H-16), 1.22−1.32 (24H, m, H-4−15); FABMS *m*/*z* 323 $[M + Na]^+$ (29), 301 $[M + H]^+$ (100).

Compound 10: white amorphous solid; ^{1}H NMR (CD_3OD, 500 MHz) δ 3.64 (3H, s, OCH_3), 3.53 (2H, t, J = 7.0 Hz, H-17), 2.30 (2H, d, J = 7.5 Hz, H-2), 1.59 (2H, m, H-3), 1.52 (2H, m, H-16), 1.22−1.32 (20H, m, H-4−15); FABMS *m*/*z* 295 $[M + Na]^+$ (29), 273 $[M + H]^+$ (100).

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References and Notes

(1) Li, H.; Matsunaga, S.; Fusetani, N. *Experientia* **1994**, *50*, 771−773.
(2) Yan, S. J.; Su, J. Y.; Zhang, G. W.; Wang, Y. H.; Li, H. *Zhongshan Daxue Xubao* **2001**, *40*, 54−57.
(3) Guerriero, A.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1991**, *74*, 487−494.
(4) Oku, N.; Matsunaga, S.; Wada, S. I.; Watabe, S.; Fusetani, N. *J. Nat. Prod.* **2000**, *63*, 205−209.
(5) McCormick, J. L.; McKee, T. C.; Cardellina, J. H.; Leid, M.; Boyd, M. R. *J. Nat. Prod.* **1996,** *59*, 1047−1050.
(6) Ryu, G.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 512−514.
(7) Tsukamoto, S.; Yamashita, T.; Matsunage, S.; Fusetani, N. *J. Org. Chem.* **1999**, *64*, 3794−3795.
(8) Nazawa, D.; Takikawa, H.; Mori, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1481−1483.
(9) Matsunaga, S.; Yamashita, T.; Tsukamoto, S.; Fusetani, N. *J. Nat. Prod.* **1999**, *62*, 1202−1204.
(10) Bergquist, P.; Lawson, M. P.; Lavis, A.; Cambie, R. C. *Biochem. Syst. Ecol.* **1984**, *12*, 63−84.
(11) Zhao, Q.; Lee, S.-Y.; Hong, J.; Lee, C.-O.; Im, K. S.; Sim, C. J.; Lee, D. S.; Jung, J. H. *J. Nat. Prod.* **2003**, in press.
(12) Ping, X.; Usuki, Y.; Akeda, Y.; Taniguchi, M. *J. Antibiot.* **1999**, *53*, 345−347.
(13) (a) Hu, W. Y.; Luo, S. D.; Cai, J. X. *Zhaocao Yao* **1994**, *25*, 59−60, 63. (b) Maruyama, T.; Niiya, I.; Imamura, M.; Okada, M.; Matsumoto, T. *Yukagaku* **1978**, *27*, 282−285.
(14) Ihara, F.; Kageyama, Y.; Hirata, M.; Nihira, T.; Yamada, Y. *J. Biol. Chem.* **1991**, *266*, 18135−18140.
(15) (a) Thomas G. B.; Scott, C.; Russell, G. K. *J. Org. Chem.* **1981**, *46*, 1564−1570. (b) Defretin, J.; Saez, J.; Franck, X.; Hocquemiller, R.; Figadere, B. *Tetrahedron Lett.* **1999**, *40*, 4041−4044.
(16) (a) Shin, B. A.; Kim, Y. R.; Lee, I. S.; Sung, C. K.; Hong, J. K.; Sim, C. J.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **1999**, *62*, 1554−1557. (b) Alam, N.; Bae, B. H.; Hong, J. K.; Lee, C. O.; Shin, B. A.; Im, K. S. Jung, J. H. *J. Nat. Prod.* **2001**, *64*, 533−535. (c) Fusetani, N.; Yasukawa, K.; Matsunaga, S.; Hashimoto, K. *Comp. Biochem. Physiol.* **1986**, *83B*, 511−513.
(17) Connor, D. S.; Scheibel, J. J.; Back, D. J.; Trinh, T.; Vinson, P. K.; Burckett-St. Laurent, J.; Sivik, M. R.; Wahl, E. H.; Frankenbach, G. M.; Demeyere, H.; Cripe, T. A.; Declercq, M. J.; Severson, R. G. PCT Int. Appl. WO9920722, 1999.
(18) Carballeira, N. M.; Miranda, C.; Lozano, C. M.; Nechev, J. T.; Ivanova, A.; Illieva, M.; Tzvetkova, I.; Stefanov, K. *J. Nat. Prod.* **2001**, *64*, 256−259.
(19) (a) Gaffney, P. R. J.; Reese, C. B. *Tetrahedron Lett.* **1997**, *38*, 2539−2542. (b) Burgos, C. E.; Ayer, D. E.; Johnson, R. A. *J. Org. Chem.* **1987**, *52*, 4973−4977.
(20) Rossi, R.; Carpita, A.; Quirici, M. G.; Veracini, C. A. *Tetrahedron* **1982**, *38*, 639−644.

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