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Suvanine Sesterterpenes and Deacyl Irciniasulfonic Acids from a Tropical *Coscinoderma* sp. Sponge

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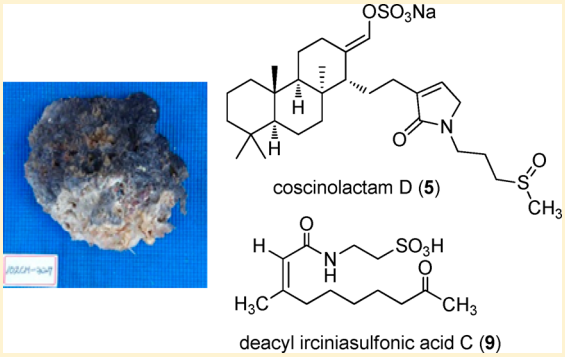
Supporting Information

ABSTRACT: The suvanines, a new suvanine salt, five new (**2**, **4**–**8**) and two known sesterterpenes from the same structural class, and two new modified lipids (**9** and **10**) were isolated from a *Coscinoderma* sp. sponge collected from Chuuk Island, Micronesia. On the basis of the results of combined spectroscopic and chemical analyses, a new suvanine salt was determined to be the suvanine *N*,*N*-dimethyl-1,3-dimethylherbipoline salt (**2**) and suvanine-lactam derivatives (**4**–**8**) formed by condensations between an oxidized furan moiety and amino acids. The lipid metabolites were found to be new derivatives of the taurine-containing deacyl irciniasulfonic acid class. The suvanines exhibited moderate cytotoxicities against the K562 and A549 cell lines, while the new suvanine salt (**2**) had significant antibacterial activity.



The structural variety and wide phyletic distribution of sesterterpenes is one of the most distinctive features of sponge-derived terpenoids, and their presence distinguishes sponges from other marine or terrestrial organisms.[1−3] Sulfate-containing sesterterpenes, represented by halisulfates[4a] and suvanines,[4b] are frequently found in tropical sponges, and they exhibit diverse bioactivities, including cytotoxic, antimicrobial, and anti-inflammatory activities and inhibitory effects on isocitrate lyase and CDC25 phosphatase.[5−8] In addition, according to a recent report, suvanine is an antagonist of the mammalian bile acid sensor farnesoid-X-receptor.[9] During our search for bioactive compounds from tropical sponges, we have also reported several compounds of these structural classes.[10]

In our continuing search for bioactive compounds, we collected a *Coscinoderma* sp. sponge from Chuuk Island, Micronesia, whose organic extract exhibited moderate cytotoxicity (LC_{50} = 320 μg/mL) against the K562 leukemia cell line. Bioassay-guided separation of the organic extract yielded several sesterterpenes. Here, we report the structures of five new derivatives of suvanine, designated as coscinolactams C−G (**4**–**8**), and of a new suvanine salt, the *N*,*N*-dimethyl-1,3-dimethylherbipoline salt (**2**), as well as the previously reported suvanine *N*,*N*-dimethylguanidium salt (**1**)[4b] and coscinolactam A (**3**).[6] The new lactam derivatives are biosynthetically derived through condensation of the oxidized furans of suvanine and diverse amino acids. These compounds exhibit moderate cytotoxicities against the K562 and A549 cell lines, whereas significant antibacterial activity was observed only for the new suvanine salt (**2**).

In addition to the sesterterpenes, taurine-containing modified fatty acids deacyl irciniasulfonic acid C (**9**) and sodium deacyl irciniasulfonate D (**10**) were isolated from this *Coscinoderma* sp. and structurally defined by combined spectroscopic and chemical methods. Despite the distinct structural features, these compounds failed to exhibit significant activities in common antibacterial, cytotoxic, and enzyme inhibition bioassays.

RESULTS AND DISCUSSION

The sponge was lyophilized, macerated, and repeatedly extracted with CH_2Cl_2 and MeOH. The combined extracts were separated by solvent partitioning followed by reversed-phase vacuum flash chromatography. On the basis of the results of bioassays and ^{1}H NMR analyses, the polar and moderately polar fractions were separated by reversed-phase HPLC to afford 10 compounds as amorphous solids or colorless gums. Among the three major constituents (**1**−**3**), compounds **1** and

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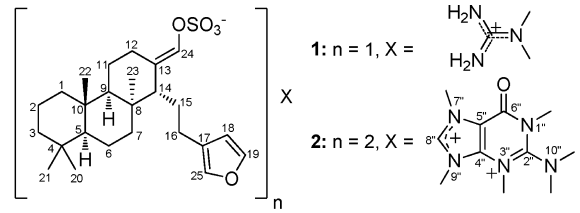

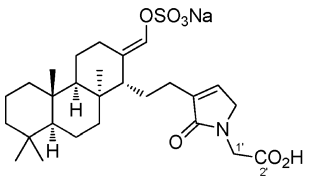

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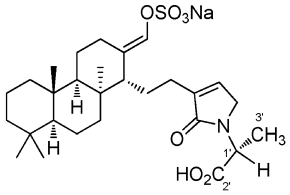
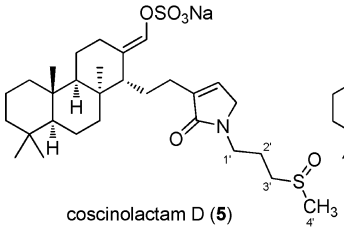
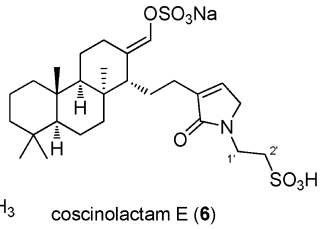


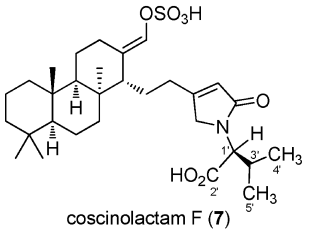
coscinolactam C (**4**)



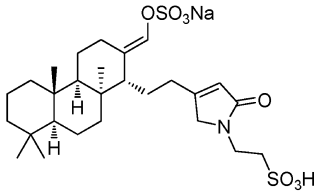
coscinolactam D (**5**)



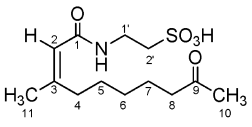
coscinolactam E (**6**)



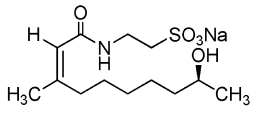
coscinolactam F (**7**)



coscinolactam G (**8**)



deacyl irciniasulfonic acid C (**9**)



sodium deacyl irciniasulfonate D (**10**)

3 were readily identified as suvanine *N,N*-dimethylguanidium salt and coscinolactam A, respectively, on the basis of combined spectroscopic analyses and comparison of the spectroscopic data with those in the literature.[4b,6]

The molecular formula of another major constituent, **2**, was deduced to be $C_{61}H_{92}N_5O_{11}S_2$ from the HRFABMS analysis. Detailed examination of the ^{1}H and ^{13}C NMR data, aided by 2-D NMR experiments, revealed that this compound possessed the same sulfated tricyclic sesterterpene moiety as suvanine, which indicated the two anion moieties of this compound as well as the association with a counterion having the mass formula $C_{11}H_{19}N_5O$. The ^{13}C NMR data of this counterion showed signals from five downfield quaternary carbons and six methyl carbons, suggesting the presence of a highly methylated aromatic moiety (Tables 1 and 2). The corresponding ^{1}H NMR signals at 4.1−2.8 for the methyl groups revealed that these were indeed *N*-methyls. Other important features in the ^{13}C NMR data were the doublet splitting patterns of two carbons at δ_C 158.3 and 28.2, which indicated the presence of quaternary ammoniums at these carbons.[11a,b]

Thus, a combination of HSQC and HMBC analyses in conjunction with a literature survey determined that this counterion was *N,N*-dimethyl-1,3-dimethylherbipoline (**2a**) (Figure 1).[12] In the guanidium cation, the positive charge was not dispersed around C-2″ and the neighboring three nitrogens, but instead was localized at C-2″/N-3″ based on the significant C−N couplings at these atoms: $^1J_{C\text{-}2'',N\text{-}3''}$ = 48.5 Hz and $^1J_{N\text{-}3''\text{-}Me,N\text{-}3''}$ = 6.0 Hz. These coupling constants agreed with the data for aromatic iminiums in the literature.[11b] The identity of this counterion was further supported by an ion exchange reaction with TFA in which *N,N*-dimethyl-1,3-dimethylherbipoline was isolated as the TFA salt of **2a**. Thus, **2** was determined to be a new salt of suvanine. Herbipoline salts of sponge metabolites have been previously reported from *Jaspis* sp.[12] and *Coscinoderma mathewsi*.[13] However, the herbipoline salt of **2** is unique not only because it is the first example of an *N,N*-dimethyl derivative but also because of its divalent cation form.

The molecular formula of coscinolactam C (**4**) was established as $C_{28}H_{42}NO_7SNa$ by HRFABMS analysis. The NMR data of this compound were similar to those of **3**, indicating that both the tricyclic enol-sulfate and lactam moieties were intact in these compounds. The identity of the 17-ene-25-oxo butyrolactam moiety was further supported by combined 2-D NMR analyses, including HMBC data showing important long-range correlations of H-16/C-17, H-16/C-18, H-18/C-16, H-18/C-17, H-18/C-25, H-19/C-17, and H-19/C-25. Accordingly, the structural variation occurred in the auxiliary chain of **4**, where the glycine-derived moiety of **3** was replaced with a methylated moiety: δ_C 179.1 (C), 53.6 (CH), and 17.3 (CH_3) and δ_H 4.62 (1 H) and 1.43 (3 H) (Tables 1 and 2). A combination of 2-D NMR analyses readily determined this to be an alanine-derived moiety attached to the lactam nitrogen from the long-range correlations at H-19/C-1′, H-1′/C-19, and H-1′/C-25 in the HMBC data. Based on the NOESY data, the configuration of **4** was found to be the same as that of other suvanines through the cross-peaks between the bridgehead methyl protons and spatially close ring protons. For the absolute configuration at the new asymmetric center C-1′, a PGME method assigned the D configuration to the alanine-derived unit (Figure 2).[14] Thus, the structure of coscinolactam C (**4**) was determined to be a new sesterterpene lactam of the suvanine class.

A related metabolite, coscinolactam D (**5**), was isolated as an amorphous solid and was determined to have a molecular formula of $C_{29}H_{46}NO_6S_2Na$ by HRFABMS analysis. The NMR data of this compound showed the presence of a suvanine butyrolactam moiety similar to **3** and **4**. The ^{13}C NMR data showed four additional carbons at δ_C 52.1 (CH_2), 42.4 (CH_2), 38.3 (CH_3), and 23.0 (CH_2), whose corresponding proton signals were observed at δ_H 2.77 (2 H), 3.61 (2 H), 2.63 (3 H), and 2.05 (2 H), respectively, in the ^{1}H NMR spectra. Proton COSY experiments showed that these signals represent a linear array of three methylenes and an isolated methyl group (Figure 3). The placement of a sulfoxide between these groups was revealed from the downfield shifts of the carbons at δ_C 52.1 and 38.3 and their attached protons as well as the HMBC correlations of H-3′/C-4′ and H-4′/C-3′ and the molecular formula.[15] This interpretation was also supported by the long-range HMBC correlations at H-3′/C-4′ and H-4′/C-3′ as well as a characteristic absorption band at 1060 cm^{-1} in the IR spectrum. The linkage between the alkylsulfoxide chain and butyrolactam moiety was also confirmed by the long-range correlations at H-19/C-1′, H-1′/C-19, and H-1′/C-25 in the HMBC data (Figure 3). Thus, coscinolactam D (**5**) was determined to be a new suvanine lactam.

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Table 1. ^{13}C NMR (ppm, mult) Assignments for Compounds 2 and 4−8 in CD_3OD solutions

position	2	4	5	6	7	8
1	43.3, CH_2	43.4, CH_2	43.3, CH_2	43.3, CH_2	43.4, CH_2	43.4, CH_2
2	20.0, CH_2	19.9, CH_2	19.9, CH_2	19.9, CH_2	20.3, CH_2	19.9, CH_2
3	43.2, CH_2	43.2, CH_2	43.2, CH_2	43.2, CH_2	43.2, CH_2	43.2, CH_2
4	34.3, C	34.3, C	34.0, C	34.0, C	34.3, C	34.3, C
5	54.6, CH	53.9, CH	54.5, CH	54.5, CH	53.7, CH	54.1, CH
6	19.5, CH_2	19.6, CH_2	19.5, CH_2	19.5, CH_2	19.8, CH_2	19.7, CH_2
7	36.7, CH_2	35.9, CH_2	36.5, CH_2	36.5, CH_2	35.9, CH_2	35.9, CH_2
8	39.7, C	39.6, C	39.8, C	39.8, C	39.6, C	39.6, C
9	58.6, CH	58.8, CH	58.6, CH	58.6, CH	58.7, CH	58.6, CH
10	40.2, C	40.0, C	40.2, C	40.2, C	40.0, C	40.3, C
11	21.2, CH_2	20.3, CH_2	21.2, CH_2	21.2, CH_2	21.1, CH_2	21.1, CH_2
12	25.4, CH_2	25.4, CH_2	25.9, CH_2	26.0, CH_2	25.4, CH_2	25.4, CH_2
13	125.5, C	125.4, C	125.3, C	125.3, C	125.0, C	125.1, C
14	43.2, CH	44.9, CH	43.9, CH	43.9, CH	44.2, CH	44.2, CH
15	26.3, CH_2	24.7, CH_2	23.7, CH_2	23.9, CH_2	23.9, CH_2	24.1, CH_2
16	24.3, CH_2	26.0, CH_2	25.5, CH_2	25.4, CH_2	29.2, CH_2	29.4, CH_2
17	126.5, C	140.7, C	140.4, C	140.5, C	163.9, C	164.3, C
18	111.9, CH	137.8, CH	138.5, CH	138.3, CH	121.5, CH	121.6, CH
19	143.9, CH	49.8, CH_2	52.3, CH_2	52.7, CH_2	174.9, C	174.2, C
20	34.0, CH_3	33.9, CH_3	34.3, CH_3	34.3, CH_3	33.9, CH_3	34.0, CH_3
21	22.3, CH_3	22.3, CH_3	22.4, CH_3	22.4, CH_3	22.3, CH_3	22.3, CH_3
22	18.7, CH_3	18.6, CH_3	18.8, CH_3	18.8, CH_3	18.6, CH_3	18.7, CH_3
23	26.8, CH_3	26.7, CH_3	26.8, CH_3	26.7, CH_3	26.6, CH_3	26.6, CH_3
24	133.6, CH	133.8, CH	133.8, CH	133.8, CH	133.8, CH	133.6, CH
25	140.4, CH	173.7, C	173.9, C	173.6, C	53.6, CH_2	56.6, CH_2
1′		53.6, CH	42.4, CH_2	39.8, CH_2	65.0, CH	39.6, CH_2
2′		179.1, C	23.0, CH_2	50.6, CH_2	177.7, C	50.7, CH_2
3′		17.3, CH_3	52.1, CH_2		30.6, CH	
4′			38.3, CH_3		26.6, CH_3	
5′					19.6, CH_3	
2″	158.3, C					
4″	150.0, C					
5″	107.9, C					
6″	154.9, C					
8″	140.2, C					
N-1″-Me	28.8, CH_3					
N-3″-Me	28.2, CH_3					
N-7″-Me	36.0, CH_3					
N-9″-Me	31.4, CH_3					
N-10″-Me_2	38.3, CH_3					

The molecular formula of coscinolactam E (**6**) was established as $C_{27}H_{42}NO_8S_2Na$ by HRFABMS analysis. The NMR data of this compound were similar to those of the other suvanine lactams, and the only notable changes were in the auxiliary chain: δ_C 50.6 (CH_2) and 39.8 (CH_2) and δ_H 3.89 (1 H, dt, J = 14.8, 7.0 Hz), 3.84 (1 H, dt, J = 14.8, 7.0 Hz), and 3.06 (2 H, t, J = 7.0 Hz). A combination of 2-D NMR analyses readily established the presence of an ethylene chain and its linkage to the lactam nitrogen. The presence of a sulfonic acid moiety at the terminus of the ethylene group to form a taurine moiety was confirmed by the downfield shift of C-2′ at δ_C 50.6, IR absorption bands at 1345 and 1155 cm^{-1}, and the mass data, showing coscinolactam E (**6**) to be a suvanine taurine-lactam.

The molecular formula of coscinolactam F (**7**) was deduced as $C_{30}H_{47}NO_7S$ by HRFABMS data. Although the ^1H and ^{13}C NMR data of this compound were similar to those of **4**, detailed examination revealed noticeable differences at the butyrolactam moiety: δ_C 174.9 (C), 163.9 (C), 121.5 (CH), and 53.6 (CH_2) and δ_H 5.83 (1 H, s), 4.44 (1 H, d, J = 20.4 Hz), and 4.05 (1 H, d, J = 20.4 Hz) (Tables 1 and 2). The moiety was determined to be a 19-oxo-17-ene butyrolactam by the long-range correlations at H-16/C-18, H-16/C-25, H-18/C-16, H-18/C-19, H-18/C-25, H-25/C-16, H-25/C-17, H-25/C-18, and H-25/C-19 in the HMBC data.

In addition, significant NMR changes were observed regarding the auxiliary amino acid at δ_C 177.7 (C), 65.0 (CH), 30.6 (CH), 26.6 (CH_3), and 19.6 (CH_3) and δ_H 4.29 (1 H, d, J = 9.9 Hz), 2.18 (1 H, dheptet, J = 9.9, 6.5 Hz), 1.02 (3 H, d, J = 6.5 Hz), and 0.83 (3 H, d, J = 6.5 Hz) (Tables 1 and 2). Aided by the combined 2-D NMR experiments, these changes were readily explained by replacement of the alanine-derived unit of **4** with a valine-derived moiety in **7**. Similar to compound **4**, the absolute configuration at C-1′ in **7** was determined using the PGME method. Interestingly, the results showed that the auxiliary chain of **7** was derived from L-valine, in contrast to D-alanine in **4** (Figure 2). Comparison of spatial models provided more insight into the stereochemistry of these compounds. That is, the carbonyl group of the lactam moiety

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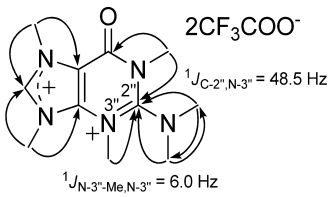
Table 2. ^{1}H NMR (δ, mult (J in Hz)) Assignments for Compounds 2 and 4−8 in CD_3OD solutions

position	2	4	5	6	7	8
1	1.83, m	1.84, m	1.86, m	1.86, m	1.84, m	1.84, m
	0.84, m	0.86, m	0.85, m	0.85, m	0.85, m	0.86, m
2	1.61, m	1.61, m	1.61, m	1.61, m	1.59, m	1.61, m
	1.38, br d (12.3)	1.38, m	1.40, br d (11.0)	1.40, br d (11.0)	1.39, br d (11.6)	1.40, br d (11.5)
3	1.41, m	1.40, m	1.40, m	1.40, m	1.40, m	1.40, m
	1.13, dt (13.5, 3.6)	1.16, dt (12.8, 2.8)	1.15, dt (12.9, 3.1)	1.15, dt (12.9, 3.2)	1.14, dt (12.4, 3.1)	1.14, dt (13.0, 3.6)
5	1.01, dd (11.5, 4.6)	1.09, dd (11.7, 4.8)	1.09, dd (11.3, 4.9)	1.09, dd (11.5, 4.9)	1.09, dd (11.0, 5.1)	1.08, dd (10.9, 5.1)
6	1.60, m	1.60, m	1.62, m	1.63, m	1.64, m	1.60, m
	1.38, m	1.38, m	1.39, m	1.38, m	1.41, m	1.41, m
7	1.82, m	1.84, m	1.89, m	1.89, m	1.84, m	1.89, m
	1.34, m	1.37, m	1.37, m	1.32, m	1.38, m	1.37, m
9	0.88, t (5.6)	0.91, m	0.91, t (6.0)	0.91, m	0.91, t (6.0)	0.91, t (6.0)
11	1.69, dq (14.7, 5.9)	1.67, dq (13.9, 6.8)	1.67, dq (13.9, 6.1)	1.67, dq (13.7, 5.8)	1.67, dq (14.3, 7.7)	1.70, dq (14.2, 5.7)
	1.61, m	1.58, m	1.58, m	1.54, m	1.56, m	1.58, m
12	2.59, dt (15.9, 6.2)	2.53, dt (16.2, 6.4)	2.46, m	2.50, m	2.53, dt (15.7, 6.2)	2.59, dt (15.9, 6.0)
	2.14, m	2.16, m	2.25, dd (14.5, 7.2)	2.22, m	2.16, m	2.18, m
14	2.34, br d (10.7)	2.31, d (10.2)	2.32, d (10.9)	2.32, d (10.4)	2.30, d (10.6)	2.33, d (10.7)
15	1.81, m	1.83, m	1.90, m	1.85, m	1.85, m	1.84, m
	1.53, m	1.59, m	1.44, m	1.48, m	1.60, m	1.59, m
16	2.61, ddd (16.4, 8.1, 4.0)	2.47, m	2.56, dt (15.5, 5.7)	2.59, dt (16.2, 6.2)	2.64, dt (15.5, 6.0)	2.66, dt (15.5, 4.5)
	2.39, dt (16.4, 8.1)	2.18, dt (16.1, 7.5)	2.18, m	2.20, m	2.42, dt (15.5, 8.2)	2.37, dt (15.5, 7.9)
18	6.32, br s	6.89, s	6.92, s	6.88, s	5.83, s	5.83, s
19	7.37, t (1.8)	4.25, d (19.3)	4.00, br s	4.10, d (20.4)		
		3.95, d (19.3)		4.02, d (20.4)		
20	0.84, s	0.85, s	0.82, s	0.85, s	0.85, s	0.85, s
21	0.84, s	0.88, s	0.88, s	0.88, s	0.86, s	0.88, s
22	0.96, s	1.01, s	1.04, s	1.04, s	1.01, s	1.04, s
23	0.82, s	0.85, s	0.85, s	0.81, s	0.86, s	0.85, s
24	6.32, s	6.41, s	6.31, s	6.41, s	6.28, s	6.26, s
25	7.27, s				4.44, d (20.4)	4.17, br s
					4.05, d (20.4)	
1′		4.62, q (7.3)	3.61, td (7.2, 2.5)	3.89, dt (14.8, 7.0)	4.29, d (9.9)	3.83, td (7.2, 2.5)
				3.84, dt (14.8, 7.0)		
2′			2.05, quint (7.2)	3.06, t (7.0)		3.06, t (7.2)
3′		1.43, d (7.3)	2.77, m		2.18, dhep (9.9, 6.5)	
4′			2.63, d (1.1)		0.83, d (6.5)	
5′					1.02, d (6.5)	
N-1″-Me	3.45, s					
N-3″-Me	2.82, s					
N-7″-Me	4.10, s					
8″	8.91, s					
N-9″-Me	3.78, s					
N-10″-Me_2	3.03, s					



2a

Figure 1. HMBC (arrows) correlations and C−N coupling constants of 2a.

selects the amino acid residues to minimize spatial crowding. Thus, the C-25 carbonyl group of 4 and the C-19 carbonyl group of 7 favor the D- and L-amino acid, respectively, to avoid steric hindrance between the carbonyl group and the amino acid side chain.

In addition to the presence of an L-valine-derived lactam, another unique structural feature of 7 was the presence of an enol-sulfuric acid group at C-13 that was deduced from the (FAB, ESI) HRMS data and by much higher hydrophilicity of this compound compared to the other coscinolactams containing the sodium salt of the enol-sulfate.

Another metabolite, coscinolactam G (8), was isolated as an amorphous solid and was determined to be $C_{27}H_{42}NO_8S_2Na$ by HRFABMS analysis. The ^{1}H and ^{13}C NMR data of this compound were very similar to those of 7, indicating the presence of the identical suvanine lactam moiety in both these compounds. Using the COSY and HMBC data, the remaining signals were found to be due to an ethylene attached at the lactam nitrogen: δ_C 50.7 (CH_2) and 39.6 (CH_2) and δ_H 3.83 (2 H, td, J = 7.2, 2.5 Hz) and 3.06 (2 H, t, J = 7.2 Hz). The attachment of a sulfonic acid group at the terminus was

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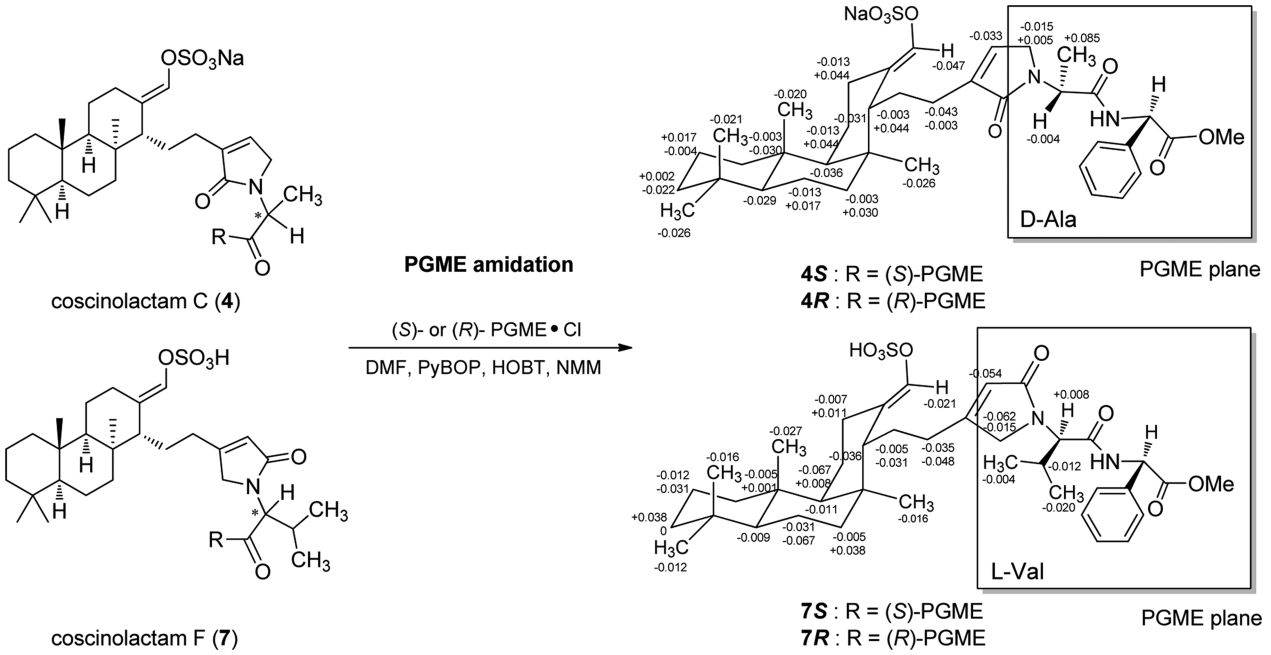

Figure 2. $\Delta\delta_H$(**4***S* − **4***R*) and $\Delta\delta_H$(**7***S* − **7***R*) values (ppm) obtained for the (*S*)- and (*R*)-PGME amides of **4** and **7**.

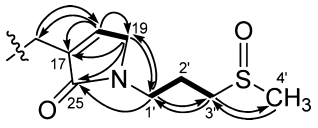

Figure 3. Selected HMBC (arrows) and COSY (bold lines) correlations in the α,β-unsaturated-γ-lactam moiety of coscinolactam D (**5**).

determined by the downfield shift of the carbon at δ_C 50.7 as well as the HRFABMS data. Thus, the structure of coscinolactam G (**8**) was determined to be a new suvanine lactam-taurine and an isomer of **6**.

In addition to suvanine sesterterpenes, two modified lipid congeners were isolated. The molecular formula of deacyl irciniasulfonic acid C (**9**) was determined to be $C_{13}H_{23}NO_5S$ by HRFABMS analysis. The ^{13}C NMR data of this compound showed signals from two carbonyls at δ_C 212.3 and 169.5 and two olefinic carbons at δ_C 155.9 and 119.6 (Table 3). The remaining signals included seven methylenes in the region of δ_C 51.5−24.6 and two methyls at δ_C 29.8 and 24.8. Aided by these analyses, the structure of **9** was defined using a combination of 2-D NMR analyses (Figure 4). As found for compounds **6** and **8**, attachment of the sulfonic acid at C-2′ was confirmed by the characteristic chemical shift of this carbon at δ_C 51.5. In addition, the C-2 double bond was assigned the *Z* configuration from the downfield shift of C-11 at δ_C 24.8 as well as the NOESY cross-peak at H-2/H_3-11. Thus, deacyl irciniasulfonic acid C (**9**) was determined to be a taurine-containing fatty acid amide structurally related to deacyl irciniasulfonic acid B with oxidation at C-9 (ketone) in **9** instead of a C-8 hydroxy group.[16a] Compared to deacyl irciniasulfonic acid A, structural differences are in both the amide linkage and C-9 ketone group, which correspond to an ester and hydroxy group, respectively, of deacyl irciniasulfonic acid A.[16b]

The molecular formula of sodium deacyl irciniasulfonate D (**10**) was established as $C_{13}H_{24}NO_5SNa$ by HRFABMS analysis. The ^1H and ^{13}C NMR data of this compound were very similar to those of **9**, with the most noticeable difference

Table 3. ^{13}C and ^1H NMR Assignments for Compounds 9 and 10 in CD_3OD Solutions

	9		**10**	
position	^{13}C (ppm, mult)	^1H δ, mult (*J* in Hz)	^{13}C (ppm, mult)	^1H δ, mult (*J* in Hz)
1	169.5, C		169.0, C	
2	119.6, CH	5.64, s	119.5, CH	5.64, s
3	155.9, C		155.8, C	
4	33.6, CH_2	2.59, t (7.5)	33.8, CH_2	2.60, t (7.5)
5	29.6, CH_2	1.46, dt (15.6, 7.5)	29.3, CH_2	1.47, m
6	30.2, CH_2	1.31, ddt (15.6, 7.5, 2.5)	30.8, CH_2	1.38, m
7	24.6, CH_2	1.56, dt (15.2, 7.3)	26.7, CH_2	1.34, m
8	44.2, CH_2	2.47, t (7.3)	40.2, CH_2	1.40, m
9	212.3, C		68.5, CH	3.70, m
10	29.8, CH_3	2.12, s	23.4, CH_3	1.13, d (6.3)
11	24.8, CH_3	1.82, d (1.3)	24.8, CH_3	1.82, d (1.3)
1′	36.2, CH_2	3.60, t (6.8)	36.2, CH_2	3.60, t (6.8)
2′	51.5, CH_2	2.97, t (6.8)	51.6, CH_2	2.97, t (6.8)

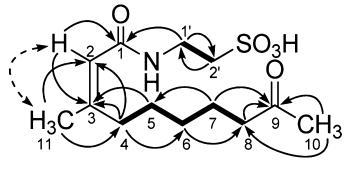

Figure 4. HMBC (arrows), COSY (bold lines), and NOESY (dashed arrows) correlations of deacyl irciniasulfonic acid C (**9**).

being the replacement of carbonyl signals with oxymethine signals at δ_C 68.5 and δ_H 3.70 (1 H, m) (Table 3). On the basis of the results of combined 2-D NMR analyses, the main structural difference was an oxymethine in place of the C-9 carbonyl. In addition, the replacement of the sulfonic acid with a sodium sulfonate group was secured by HRMS analysis. This interpretation was further supported by the HPLC analysis, in which compounds **9** and **10** showed very similar retention times in the given condition (YMC-ODS column, 4.6 mm ×

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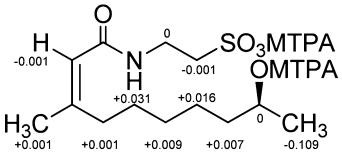
250 mm; H_2O–MeOH, 65:35; t_R = 11.43 and 11.41 min for **9** and **10**, respectively) despite the presence of a ketone and hydroxy group at C-9, respectively. The absolute configuration of the new asymmetric center was determined to be 9*S* by Mosher's method (Figure 5). Thus, sodium deacyl irciniasulfonate D (**10**) was determined to be a new deacyl irciniasulfonic acid possessing a sodium sulfonate group.



10*S* : (*S*)- MTPA
10*R* : (*R*)- MTPA

Figure 5. $\Delta\delta_H$(**10*S*** − **10*R***) values (ppm) obtained for the (*S*)- and (*R*)-MTPA ester (**10*S***, **10*R***) of sodium deacyl irciniasulfonate D (**10**).

Sponge-derived sesterterpene sulfates, including suvanines and halisulfates, exhibit diverse bioactivities such as cytotoxicity, antimicrobial activity, and inhibitory effects on isocitrate lyase and phosphatase.[5−8] In our measurements against the cancer cell lines K562 and A549, compounds **1**–**8** displayed moderate cytotoxicities (LC_{50} = 0.9–5.5 μM) that were comparable to those of doxorubicin (Table 4). These data suggest that there is no significant trend in the structure–cytotoxicity relationship in these compounds. Surprisingly, only compound **2**, the suvanine *N*,*N*-dimethyl-1,3-dimethylherbipoline salt, showed significant activity against several strains of Gram-positive and Gram-negative bacteria. All of the suvanine derivatives were weakly active (isocitrate lyase and Na^+/K^+-ATPase) or inactive (sortase A, IC_{50} > 100 μM) against enzymes. The new modified fatty acids **9** and **10** were inactive in all of these bioassays, which agreed with the literature.[16a]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. UV spectra were acquired with a Hitachi U-3010 spectrophotometer. IR spectra were recorded on a JASCO 300E FT-IR spectrometer using a ZnSe cell. NMR spectra were recorded in CD_3OD with solvent peaks as the δ_H 3.30 and δ_C 49.0 internal standard on Bruker Avance 400, 500, and 600 spectrometers. Proton and carbon NMR spectra were measured at 400 and 100 MHz (**1**, **3**, **8**, **9**, and **10**), 500 and 125 MHz (**2**, **5**, and **6**), and 600 and 150 MHz (**4** and **7**), respectively. High-resolution FAB mass spectrometric data were obtained at the Korea Basic Science Institute (Daegu, Korea) and acquired using a JEOL JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol (NBA) as the matrix for the FABMS. High-resolution electrospray ionization (ESI) mass spectrometric data were obtained at the National Instrumentation Center for Environmental Management (Seoul, Korea) using a Thermo-Finnigan LTQ-Orbitrap instrument equipped with a Dionex U-3000 HPLC system. Low-resolution ESIMS data were recorded on an Agilent Technologies 6130 Quadrupole mass spectrometer with an Agilent Technologies 1200 series HPLC. HPLC was performed on a Spectrasystem p2000 equipped with a refractive index detector (Spectrasystem RI-150). All solvents used were of spectroscopic grade or were distilled from glass prior to use.

Animal Material. Specimens of a *Coscinoderma* sp. sponge (sample number 102CH-327) were collected by hand with scuba equipment at a depth of 15 m off the coast of Weno Island, Chuuk state, Federated States of Micronesia, on February 10, 2010. The sponge was small and mass shaped and had dimensions of 13 × 9 × 3 cm. The surface was smooth with low conules, the texture was tough and compressible, and in life, the sponge was black outside and brown inside. The skeleton was composed of cored primary fibers and uncored secondary fibers with diameters of 80–100 μm and 10–25 μm, respectively. Comparison of their overall morphological features showed that these specimens had skeletons similar to those of another *Coscinoderma* sp. previously collected at nearby Weno Island and had secondary fiber sizes noticeably different from those of *C. mathewsi* (Lendenfeld, 1886).[10] However, the small size and mass shape of these specimens also differed remarkably from the size of those from Weno Island. A voucher specimen (registry no. spo. 70) was deposited at the Natural History Museum, Hannam University, Daejeon, Korea, under the curatorship of C.J.S.

Extraction and Isolation. Freshly collected specimens were immediately frozen and stored at −25 °C until use. Lyophilized specimens were macerated and repeatedly extracted with MeOH (3 × 3 L) and CH_2Cl_2 (2 × 3 L). The combined extracts (96.34 g) were successively partitioned between H_2O (71.45 g) and *n*-BuOH (26.97 g); the latter fraction was repartitioned between H_2O–MeOH (15:85,

Table 4. Results of Bioactivity Tests

			MIC (μg/mL)							
	LC_{50} (μM)		Gram(+) bacteria			Gram(−) bacteria			IC_{50} (μM)	
compound	K562	A549	A[a]	B[b]	C[c]	D[d]	E[e]	F[f]	ICL	Na^+/K^+–ATPase
1	0.9	1.7	>100	12.5	>100	25	100	>100	51	80
2	2.2	1.9	6.25	0.78	6.25	0.78	6.25	>100	28	33
3	1.3	1.4	>100	>100	>100	>100	>100	>100	62	50
4	1.9	1.1	>100	>100	>100	50	>100	>100	48	34
5	3.9	5.5	>100	ND[h]	ND	50	ND	ND	24	19
6	4.6	2.0	>100	>100	>100	>100	>100	>100	90	73
7	3.9	1.9	>100	>100	>100	100	>100	>100	64	27
8	3.6	3.5	>100	>100	>100	>100	>100	>100	50	60
9	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	ND	ND	>100	ND	ND	>100	>100
doxorubicin	0.7	0.8								
ampicillin			0.39	0.39	0.39	0.39	0.78	3.12		
3-NP[g]									2.5	
ouabain										3.3

[a]A: *Staphylococcus aureus* (ATCC 6538p). [b]B: *Bacillus subtilis* (ATCC 6633). [c]C: *Kocuria rhizophila* (NBRC 12708). [d]D: *Salmonella enterica* (ATCC 14028). [e]E: *Proteus hauseri* (NBRC 3851). [f]F: *Escherichia coli* (ATCC 35270). [g]3-Nitropropionic acid. [h]Not detected.

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20.09 g) and *n*-hexane (3.87 g). An aliquot of the former layer (10.4 g) was separated by C_{18} reversed-phase flash chromatography using sequential mixtures of MeOH and H_2O as the eluents (six fractions in H_2O−MeOH gradient, from 50:50 to 0:100), followed by acetone and finally EtOAc.

On the basis of the results of ^{1}H NMR and cytotoxicity analyses, fractions eluted with 50:50 H_2O−MeOH (2.49 g), 40:60 H_2O−MeOH (0.36 g), and 20:80 H_2O−MeOH (1.96 g) were chosen for separation. The 20:80 H_2O−MeOH fraction was separated by semipreparative reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O−MeOH, 30:70), yielding compounds **1** and **2**. The 40:60 H_2O−MeOH fraction was separated by semipreparative reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O−MeOH, 45:55), yielding five peaks rich in secondary metabolites. Further purification of these peaks by reversed-phase HPLC (YMC-ODS column, 4.6 mm × 250 mm; H_2O−MeOH, 65:35) afforded as white, amorphous solids, in order of elution, compounds **7**, **6**, **8**, **3**, **4**, and **5**, with retention times (t_R) of 22.34, 34.41, 36.17, 40.32, 48.14, and 72.51 min, respectively.

The 50:50 H_2O−MeOH fraction was separated by Sephadex LH-20 gel permeation chromatography eluting with 100% MeOH. Guided by the results of the ^{1}H NMR analyses, the fractions containing secondary metabolites were combined (24.1 mg) and separated by reversed-phase HPLC (YMC-ODS column, 4.6 mm × 250 mm; H_2O−MeOH, 55:45; t_R = 10.53 min) to afford compound **9**. Finally, compound **10** was separated by semipreparative reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O−MeOH, 80:20; t_R = 28.32 min) from the 50:50 H_2O−MeOH fraction. The purified metabolites were isolated in the following amounts: 85.1, 68.1, 33.4, 5.4, 1.7, 24.5, 4.0, 2.5, 8.1, and 3.9 mg of **1**−**10**, respectively.

*Suvanine N,N-dimethyl-1,3-dimethylherbipoline salt (**2**):* white, amorphous solid; $[\alpha]_D^{25}$ +11 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 207 (5.32), 259 (4.67) nm; IR (ZnSe) ν_{max} 3273, 2938, 1658, 1458, 1218 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 1134.6216 $[M]^+$ (calcd for $C_{61}H_{92}N_5O_{11}S_2$, 1134.6235).

*Coscinolactam C (**4**):* white, amorphous solid; $[\alpha]_D^{25}$ +2 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 206 (5.22), 248 (4.58) nm; IR (ZnSe) ν_{max} 3456, 2934, 1654, 1454, 1254 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 560.2653 $[M + H]^+$ (calcd for $C_{28}H_{43}NO_7SNa$, 560.2658), *m*/*z* 582.2480 $[M + Na]^+$ (calcd for $C_{28}H_{42}NO_7SNa_2$, 582.2477).

*Coscinolactam D (**5**):* white, amorphous solid; $[\alpha]_D^{25}$ +8 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 206 (5.39), 250 (4.73) nm; IR (ZnSe) ν_{max} 3461, 2931, 1665, 1462, 1248, 1060 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 592.2717 $[M + H]^+$ (calcd for $C_{29}H_{47}NO_6S_2Na$, 592.2743), *m*/*z* 614.2576 $[M + Na]^+$ (calcd for $C_{29}H_{46}NO_6S_2Na_2$, 614.2562).

*Coscinolactam E (**6**):* white, amorphous solid; $[\alpha]_D^{25}$ +5 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 206 (5.27), 248 (4.67) nm; IR (ZnSe) ν_{max} 3482, 2941, 1652, 1593, 1345, 1250, 1155 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 596.2322 $[M + H]^+$ (calcd for $C_{27}H_{43}NO_8S_2Na$, 596.2328).

*Coscinolactam F (**7**):* white, amorphous solid; $[\alpha]_D^{25}$ +4 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 206 (5.23), 250 (4.64) nm; IR (ZnSe) ν_{max} 3458, 2934, 1679, 1405, 1248 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 566.3154 $[M + H]^+$ (calcd for $C_{30}H_{48}NO_7S$, 566.3152); HRESIMS *m*/*z* 566.3131 $[M + H]^+$ (calcd for $C_{30}H_{48}NO_7S$, 566.3128), *m*/*z* 564.2997 $[M - H]^-$ (calcd for $C_{30}H_{46}NO_7S$, 564.2995), *m*/*z* 486.2630 $[M - SO_3 + H]^+$ (calcd for $C_{30}H_{48}NO_4$, 486.2632).

*Coscinolactam G (**8**):* white, amorphous solid; $[\alpha]_D^{25}$ +13 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 206 (5.27), 248 (4.67) nm; IR (ZnSe) ν_{max} 3472, 2923, 1662, 1452, 1350, 1249, 1170 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 596.2324 $[M + H]^+$ (calcd for $C_{27}H_{43}NO_8S_2Na$, 596.2328).

*Deacyl irciniasulfonic acid C (**9**):* colorless gum; $[\alpha]_D^{25}$ −1 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 219 (5.50) nm; IR (ZnSe) ν_{max} 3415, 2934, 1665, 1536, 1345, 1255, 1150 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRFABMS *m*/*z* 306.1373 $[M + H]^+$ (calcd for $C_{13}H_{24}NO_5S$, 306.1375), *m*/*z* 328.1086 $[M + Na]^+$ (calcd for $C_{13}H_{23}NO_5SNa$, 328.1088).

*Sodium deacyl irciniasulfonate D (**10**):* colorless gum; $[\alpha]_D^{25}$ −4 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 220 (5.50) nm; IR (ZnSe) ν_{max} 3390, 2931, 1665, 1540, 1348, 1253, 1168 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRFABMS *m*/*z* 330.1348 $[M + H]^+$ (calcd for $C_{13}H_{25}NO_5SNa$, 330.1351), *m*/*z* 352.1168 $[M + Na]^+$ (calcd for $C_{13}H_{24}NO_5SNa_2$, 352.1171).

Trifluoroacetate of *N,N*-Dimethyl-1,3-dimethylherbipoline (2a). A vial containing 4 mg of **2** with TFA (100 μL) was kept standing for 5 min at room temperature (rt), and then TFA was evaporated under vacuum. The residue was purified by reversed-phase HPLC (YMC-ODS column, 4.6 mm × 250 mm; H_2O−MeOH−TFA, 90:10:0.05) to give **2a** (0.4 mg); ^{1}H NMR (CD_3OD, 600 MHz) δ_H 8.91 (0.2 H, s, H-8″), 4.10 (3 H, s, N-7″-Me), 3.78 (3 H, s, N-9″-Me), 3.45 (3 H, s, N-1″-Me), 3.03 (6 H, s, N-10″-Me_2), 2.82 (3 H, s, N-3″-Me); LRESIMS *m*/*z* 485.4 $[M + Na]^+$ (calcd for $C_{15}H_{18}F_6N_5O_5Na$, 485.2).

Preparation of the (*S*)- and (*R*)-Phenylglycine Methyl Ester (PGME) Amides of Compounds 4 and 7. Reactions were carried out following the general procedure previously reported for the stereochemical assignment of α,α-disubstituted carboxylic acids. To a dry DMF solution (500 μL) of **4** (1.0 mg, l.7 μmol) and (*S*)-PGME (1.4 mg, 8.4 μmol) were successively added PyBOP (4.4 mg, 8.4 μmol), HOBT (1.3 mg, 8.4 μmol), and *N*-methylmorpholine (100 μL) at rt. After stirring for 1 h, 5% HCl solution (1 mL) and EtOAc (2 mL) were added to the reaction mixture. The EtOAc layer was successively washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous Na_2SO_4. The residue was purified by reversed-phase HPLC (YMC-ODS column, 4.6 mm × 250 mm; H_2O−MeOH, 40:60) to give (*S*)-PGME amide **4*S*** (0.7 mg). Compound **4*R***, the (*R*)-PGME amide of **4** (0.5 mg), was prepared from (*R*)-PGME in a similar fashion. Compounds **7*S*** and **7*R***, the (*S*)- and (*R*)-PGME amides of **7** (0.7 and 0.6 mg), respectively, were also prepared using this method.

*(S)-PGME amide of 4 (**4S**):* brown, amorphous solid; ^{1}H NMR (CD_3OD, 600 MHz) δ_H 7.379−7.323 (5 H, m, PGME-Ar), 6.930 (1 H, s, H-18), 6.319 (1 H, s, H-24), 5.421 (1 H, s, PGME-H-1), 4.553 (1 H, br s, H-1′), 4.177 (1 H, d, *J* = 19.3 Hz, H-19a), 4.036 (1 H, d, *J* = 19.3 Hz, H-19b), 3.698 (3 H, s, PGME-OMe), 2.555 (1 H, dt, *J* = 16.0, 5.9 Hz, H-12a), 2.454 (1 H, m, H-16a), 2.292 (1 H, d, *J* = 10.4 Hz, H-14), 2.206 (1 H, dt, *J* = 14.5, 7.2 Hz, H-16b), 2.165 (1 H, m, H-12b), 1.830 (3 H, m, H-1a, H-7a, H-15a), 1.677 (1 H, dq, *J* = 13.7, 6.3 Hz, H-11a), 1.620 (2 H, m, H-2a, H-6a), 1.584 (2 H, m, H-11b, H-15b), 1.485 (3 H, d, *J* = 7.3 Hz, H-3′), 1.455 (1 H, m, H-3a), 1.386 (3 H, m, H-2b, H-6b, H-7b), 1.149 (1 H, m, H-3b), 1.043 (1 H, m, H-5), 1.020 (3 H, s, H-22), 0.891 (1 H, m, H-9), 0.869 (3 H, s, H-21), 0.850 (1 H, m, H-1b), 0.827 (3 H, s, H-20), 0.801 (3 H, s, H-23); LRESIMS *m*/*z* 706.1 $[M]^+$ (calcd for $C_{37}H_{51}N_2O_8SNa$, 706.3).

*(R)-PGME amide of 4 (**4R**):* brown, amorphous solid; ^{1}H NMR (CD_3OD, 600 MHz) δ_H 7.366−7.327 (5 H, m, PGME-Ar), 6.963 (1 H, s, H-18), 6.366 (1 H, s, H-24), 5.446 (1 H, s, PGME-H-1), 4.557 (1 H, br s, H-1′), 4.192 (1 H, d, *J* = 19.3 Hz, H-19a), 4.031 (1 H, d, *J* = 19.3 Hz, H-19b), 3.675 (3 H, s, PGME-OMe), 2.558 (1 H, dt, *J* = 16.1, 6.2 Hz, H-12a), 2.497 (1 H, m, H-16a), 2.323 (1 H, d, *J* = 10.8 Hz, H-14), 2.209 (1 H, m, H-16b), 2.131 (1 H, m, H-12b), 1.833 (3 H, m, H-1a, H-7a, H-15a), 1.690 (1 H, dq, *J* = 14.1, 6.2 Hz, H-11a), 1.603 (2 H, m, H-2a, H-6a), 1.540 (2 H, m, H-11b, H-15b), 1.477 (1 H, m, H-3a), 1.400 (3 H, d, *J* = 7.3 Hz, H-3′), 1.333 (3 H, m, H-2b, H-6b, H-7b), 1.147 (1 H, dt, *J* = 13.9, 3.9 Hz, H-3b), 1.072 (1 H, m, H-5), 1.040 (3 H, s, H-22), 0.927 (1 H, t, *J* = 7.4 Hz, H-9), 0.890 (3 H, s, H-21), 0.880 (1 H, m, H-1b), 0.853 (3 H, s, H-20), 0.827 (3 H, s, H-23); LRESIMS *m*/*z* 706.1 $[M]^+$ (calcd for $C_{37}H_{51}N_2O_8SNa$, 706.3).

*(S)-PGME amide of 7 (**7S**):* brown, amorphous solid; ^{1}H NMR (CD_3OD, 600 MHz) δ_H 7.368−7.323 (5 H, m, PGME-Ar), 6.253 (1 H, s, H-24), 5.848 (1 H, s, H-18), 5.396 (1 H, s, PGME-H-1), 4.339 (1 H, d, *J* = 10.8 Hz, H-1′), 4.169 (1 H, d, *J* = 19.3 Hz, H-25a), 4.069 (1 H, d, *J* = 19.3 Hz, H-25b), 3.693 (3 H, s, PGME-OMe), 2.630 (1 H, m, H-16a), 2.549 (1 H, dt, *J* = 15.3, 6.1 Hz, H-12a), 2.379 (1 H, dt, *J* = 15.6, 8.0 Hz, H-16b), 2.285 (1 H, d, *J* = 10.6 Hz, H-14), 2.245 (1 H,

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m, H-3′), 2.197 (1 H, m, H-12b), 1.946 (3 H, m, H-1a, H-7a, H-15a), 1.692 (1 H, dq, J = 14.1, 7.2 Hz, H-11a), 1.625 (3 H, m, H-2a, H-6a, H-15b), 1.526 (2 H, m, H-11b, H-6b), 1.382 (1 H, br d, J = 12.6 Hz, H-2b), 1.379 (2 H, m, H-3a, H-7b), 1.141 (1 H, dt, J = 12.8, 4.6 Hz, H-3b), 1.075 (1 H, dd, J = 9.5, 4.0 Hz, H-5), 0.988 (3 H, s, H-22), 0.905 (1 H, m, H-9), 0.871 (1 H, m, H-1b), 0.850 (3 H, d, J = 6.3 Hz, H-5′), 0.846 (3 H, s, H-21), 0.835 (3 H, s, H-23), 0.825 (3 H, s, H-20), 0.806 (3 H, d, J = 6.3 Hz, H-4′); LRESIMS m/z 735.2 $[M + Na]^+$ (calcd for $C_{39}H_{56}N_2O_8SNa$, 735.4).

*(R)-PGME amide of **7** (**7R**):* brown, amorphous solid; ^1H NMR (CD_3OD, 600 MHz) δ_H 7.369−7.328 (5 H, m, PGME-Ar), 6.274 (1 H, s, H-24), 5.902 (1 H, s, H-18), 5.438 (1 H, s, PGME-H-1), 4.331 (1 H, d, J = 10.7 Hz, H-1′), 4.231 (1 H, d, J = 20.4 Hz, H-25a), 4.084 (1 H, d, J = 20.4 Hz, H-25b), 3.661 (3 H, s, PGME-OMe), 2.665 (1 H, m, H-16a), 2.556 (1 H, dt, J = 15.4, 6.0 Hz, H-12a), 2.427 (1 H, dt, J = 15.7, 8.9 Hz, H-16b), 2.321 (1 H, d, J = 10.7 Hz, H-14), 2.257 (1 H, m, H-3′), 2.189 (1 H, m, H-12b), 1.951 (3 H, m, H-1a, H-7a, H-15a), 1.684 (1 H, dq, J = 14.2, 7.0 Hz, H-11a), 1.656 (3 H, m, H-2a, H-6a, H-15b), 1.593 (2 H, m, H-11b, H-6b), 1.394 (1 H, br d, J = 12.9 Hz, H-2b), 1.341 (2 H, m, H-3a, H-7b), 1.141 (1 H, m, H-3b), 1.084 (1 H, dd, J = 10.7, 4.4 Hz, H-5), 1.015 (3 H, s, H-22), 0.916 (1 H, t, J = 5.8 Hz, H-9), 0.890 (1 H, m, H-1b), 0.870 (3 H, d, J = 6.3 Hz, H-5′), 0.862 (3 H, s, H-21), 0.851 (3 H, s, H-23), 0.837 (3 H, s, H-20), 0.810 (3 H, d, J = 6.3 Hz, H-4′); LRESIMS m/z 735.2 $[M + Na]^+$ (calcd for $C_{39}H_{56}N_2O_8SNa$, 735.4).

Preparation of the (*S*)- and (*R*)-MTPA Esters of Compound 10. To a solution of **10** (0.9 mg, 2 μmol) in dry pyridine (500 μL) were successively added (*S*)-MTPA chloride (10 μL, 5.2 μmol) and DMAP (0.5 mg). The mixture was allowed to stand under a N_2 stream at 40 °C. After stirring for 2 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by reversed-phase HPLC (YMC-ODS column, 4.6 mm × 250 mm; H_2O−MeOH, 65:35) to give **10*S***, the (*S*)-MTPA ester of **10** (0.5 mg). Compound **10*R***, the (*R*)-MTPA ester of **10** (0.6 mg), was prepared from (*R*)-MTPA in a similar fashion.

*(S)-MTPA ester of **10** (**10S**):* white, amorphous solid; ^1H NMR (CD_3OD, 600 MHz) δ_H 7.621−7.609 (2 H, m, MTPA-Ar), 7.348−7.330 (3 H, m, MTPA-Ar), 5.638 (1 H, s, H-2), 4.209 (1 H, m, H-9), 3.603 (2 H, t, J = 7.2 Hz, H-1′), 3.540 (3 H, s, MTPA-OMe), 2.959 (2 H, t, J = 7.2 Hz, H-2′), 2.596 (2 H, t, J = 7.8 Hz, H-4), 1.823 (3 H, d, J = 1.3 Hz, H-11), 1.674 (2 H, m, H-8), 1.478 (2 H, m, H-5), 1.379 (2 H, m, H-7), 1.351 (2 H, m, H-6), 1.128 (3 H, d, J = 6.2 Hz, H-10); LRESIMS m/z 740.2 $[M + H]^+$ (calcd for $C_{33}H_{40}F_6NO_9S$, 740.4).

*(R)-MTPA ester of **10** (**10R**):* white, amorphous solid; ^1H NMR (CD_3OD, 600 MHz) δ_H 7.617−7.605 (2 H, m, MTPA-Ar), 7.345−7.336 (3 H, m, MTPA-Ar), 5.639 (1 H, s, H-2), 4.209 (1 H, m, H-9), 3.604 (2 H, t, J = 7.2 Hz, H-1′), 3.540 (3 H, s, MTPA-OMe), 2.959 (2 H, t, J = 7.2 Hz, H-2′), 2.595 (2 H, t, J = 7.4 Hz, H-4), 1.822 (3 H, d, J = 1.3 Hz, H-11), 1.667 (2 H, m, H-8), 1.447 (2 H, m, H-5), 1.363 (2 H, m, H-7), 1.342 (2 H, m, H-6), 1.237 (3 H, d, J = 6.2 Hz, H-10); LRESIMS m/z 740.1 $[M + H]^+$ (calcd for $C_{33}H_{40}F_6NO_9S$, 740.4).

*HPLC Analysis of **9** and **10**.* Analytical HPLC injection of compound **9** provided a peak at t_R = 11.43 min in the given chromatographic condition (YMC-ODS column, 4.6 mm × 250 mm; H_2O−MeOH, 65:35, flow = 0.7 mL/min). Compound **10** gave its single peak at t_R = 11.41 min in the same condition. Co-injection of **9** and **10** gave a single peak at t_R = 11.41 min.

Biological Assays. Cytotoxicity assays were performed in accordance with literature protocols.[17] Isocitrate lyase,[7] sortase A,[18] Na^+/K^+−ATPase,[19a] and antimicrobial inhibition assays were performed according to previously described methods.[19b]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C and 2-D NMR spectra of compounds **2** and **4**−**10** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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