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Nortriterpene Glycosides of the Sarasinoside Class from the Sponge *Lipastrotethya* sp.

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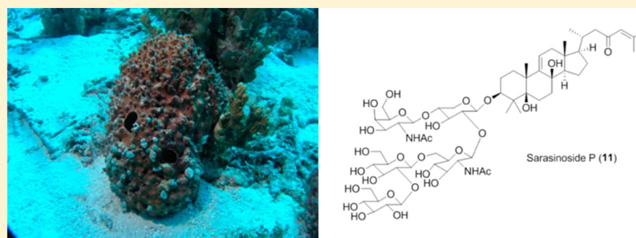
Supporting Information

ABSTRACT: Five new nortriterpene glycosides, along with eight known compounds of the sarasinoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Chuuk, Micronesia. The structures of these new compounds, designated as sarasinosides N−R (**9**−**13**), were determined by combined spectroscopic and chemical methods. The aglycone portions of **10**−**13** were found to be unprecedented among nortriterpeneoids on the basis of extensive NMR analyses. Several of these compounds exhibited cytotoxicity against A549 and K562 cell lines as well as weak inhibitory activity against Na^+/K^+-ATPase.



Sponges are widely recognized as prolific sources of marine natural products with diverse biogenetic origins and bioactivities.[1] However, saponins possessing triterpene or steroidal aglycones are relatively rare and occupy only a minor fraction of the known sponge-derived metabolites. The most notable examples of sponge-derived saponins include erylosides, pouosides, and sarasinosides.[2−13] Of these compounds, the sarasinosides are norlanostane glycosides isolated from tropical sponges of the genera *Asteropus* and *Melophlus*.[5−12] These compounds are reported to possess antimicrobial,[10,11] cytotoxic,[7,8] and piscicidal[8] activities.

Recently, we reported the isolation and structural determination of cytotoxic triterpene galactosides, the pouosides, and their corresponding aglycone metabolites from the sponge *Lipastrotethya* sp. (order Halichondrida, family Dictyonellidae), collected from Chuuk, Micronesia.[13] As noted in that study, however, the extract of the sponge contained sarasinoside A_1 (**1**) as the major metabolite. Subsequent chromatographic analyses of the polar fractions of the extract yielded several saponins of the same structural class, including known compounds (**2**−**8**). Herein, we report the structures of sarasinosides N−R (**9**−**13**), new constituents of the sarasinoside class. Sarasinoside N is structurally analogous to sarasinoside A_1, possessing a triglycoside moiety, while sarasinosides O−R possess nortriterpene aglycones with distinct oxidation patterns. In addition, a previously reported semisynthetic derivative (**2**) was isolated as a natural product. Several of these compounds exhibited cytotoxicity against K562 and A549 cell lines as well as weak inhibition of Na^+/K^+-ATPase. Structure−activity characterizations of these compounds provided important information about the role of the aglycone and glycoside portions in cytotoxicity.

RESULTS AND DISCUSSION

The sponge samples were lyophilized, macerated, and repeatedly extracted with CH_2Cl_2 and MeOH. The combined extracts were separated by solvent partitioning followed by ODS vacuum flash chromatography and then repeated ODS-HPLC. The major constituent, sarasinoside A_1 (**1**), and the minor components sarasinosides A_2 (**3**), A_3 (**4**), H_1 (**5**), H_2 (**6**), J (**7**), and M (**8**) were identified by combined spectroscopic methods and by comparisons of ^1H and ^{13}C NMR data to authentic samples and to literature citations.[7,8,10,11] Also isolated was a sarasinoside derivative (**2**), which was originally reported as a glycolysis product obtained from sarasinoside A_1.[8]

Sarasinoside N (**9**) was isolated as a white, amorphous solid that analyzed for $C_{50}H_{80}N_2O_{16}$ by HRFABMS analysis and was supported by ^{13}C NMR data. The spectroscopic data of this compound were reminiscent of those from **1**, suggesting a nortriterpene saponin nature. Detailed examination of the ^{13}C and ^1H NMR data revealed that these compounds have the

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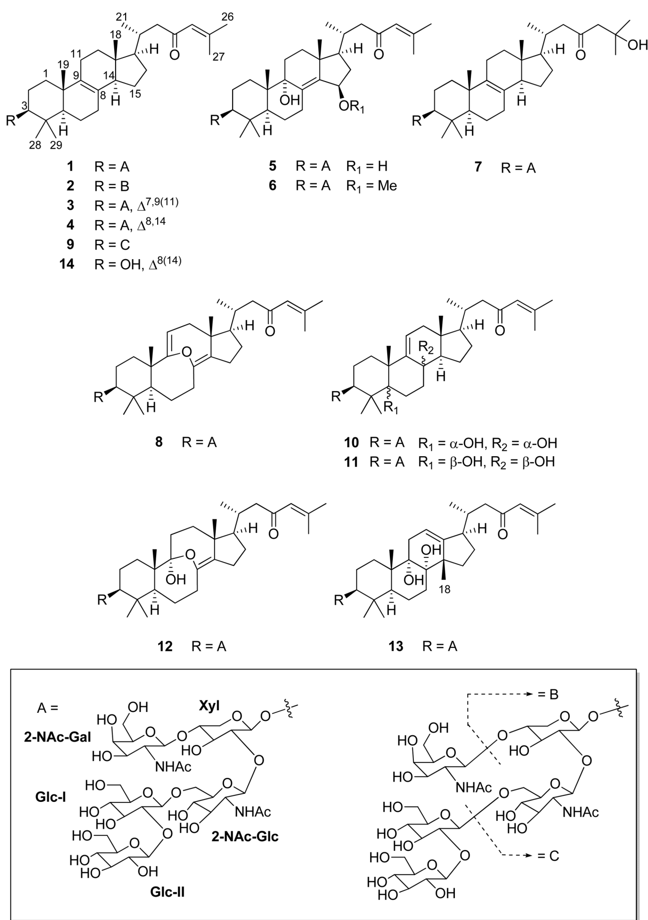

same aglycone moiety, which has the relative configurations 3*S**, 5*R**, 10*S**, 13*R**, 14*R**, 17*R**, and 20*R**; thus, structural differences occurred only in their glycoside portion (Tables 1 and 2). The number of carbon signals in the ^{13}C NMR spectrum of **9** suggested the presence of a triglycoside moiety that was related to the pentaglycoside of **1**. This interpretation was further supported by the number of characteristic anomeric signals in the NMR data (δ_H 4.88−4.37, δ_C 106.0−102.5) (Table 3). These sugar units were identified by an acid-catalyzed hydrolysis of **9** followed by GC analysis that clearly showed the presence of a xylose, a 2-*N*-acetylglucosamine, and a 2-*N*-acetylgalactosamine. The ring sizes of these sugar residues were determined by 2D NMR experiments. After assigning the individual NMR signals for these sugar units by extensive COSY, TOCSY, and gHSQC analysis (Table 3), gHMBC experiments showed long-range correlations at H-1′/C-5′, H-5′/C-1′, H-5″/C-1″, H-1‴/C-5‴, and H-5‴/C-1‴, thus revealing the pyranose nature of all of these sugars (Figure 1).

The connectivity of the sugar residues was also determined by gHMBC experiments in which the characteristic three-bond correlations were found for all of the glycosidic linkages among these sugars and aglycone. This HMBC-based linkage was further supported by the ROESY experiments, in which cross-peaks were found at H-3/H-1′, H-2′/H-1″, and H-4′/H-1‴. Overall, the NMR spectra showed that **9** had the same branched sugar assembly as **1**. Thus, the structure of sarasinoside N (**9**) was unambiguously determined to be a triglycoside derivative of sarasinoside A_1 (**1**).

The molecular formula of sarasinoside O (**10**) was deduced as $C_{62}H_{100}N_2O_{28}$ by combined HRMS and ^{13}C NMR analyses. The NMR data of this compound were very similar to those of sarasinoside A_1 (**1**). Detailed examination of the NMR data showed that the pentaglycoside portion of **1** was intact in **10**, which was confirmed by acidic hydrolysis followed by GC analysis of the sugar hydrolysates. However, the ^{13}C NMR spectrum of this compound showed the replacement of the C-8 double bond (δ_C 137.4 C, 128.8 C) of **1** with a trisubstituted bond (δ_C 156.2 C, 112.6 CH), which corresponded to a newly apparent signal (δ_H 5.03, 1 H) in the ^{1}H NMR spectrum (Tables 1 and 2). The ^{13}C NMR data also showed the appearance of two oxy-quaternary carbons at δ_C 93.8 and 87.3.

These changes at the aglycone portion were determined by a combination of 2D NMR analyses. After adequately assigning all of the proton-bearing carbons and their protons by COSY and gHSQC data, the connectivity of the partial structures was defined by gHMBC experiments (Figure 1). That is, the placement of a hydroxy group at C-5 was determined by long-range couplings of a quaternary carbon at δ_C 93.8 with singlet methyl protons at δ_H 1.26 (H-19), 1.05 (H-28), and 1.18 (H-29). The trisubstituted double bond was placed at C-9(11) on the basis of correlations of the olefinic proton and carbon with neighboring ones, specifically H-19/C-9 and H-11/C-13. Finally, the remaining hydroxy group was placed at C-8 by the key HMBC correlation, H-11/C-8. In addition, the pyranose nature of the sugar units and their assembly pattern were defined to be the same as in **1** by these 2D NMR experiments (Table 3).

The configurations at the asymmetric centers were assigned by ROESY analysis (Figure 2). The *trans* A/B ring junction with the 5α-OH was secured by the NOE cross-peaks at H-2β/H-19, H-2β/H-28, H-6α/H-29, H-6β/H-19, H-6β/H-28, and H-19/H-28. Similarly, the *trans* B/C ring junction with the 8α-OH was assigned by those at H-7α/H-15β, H-7β/H-18, and H-7β/H-19. Thus, the structure of sarasinoside O (**10**) was determined to be a $\Delta^{9(11)}$-5α,8α-dihydroxy derivative of sarasinoside A_1 (**1**).

The molecular formula of sarasinoside P (**11**) was established as $C_{62}H_{100}N_2O_{28}$, the same as **10**, by HRFABMS analysis. The spectroscopic data of this compound were very similar to those of **10**, thereby suggesting the nortriterpene pentaglycoside nature of this compound. Careful examination of the ^{13}C and ^{1}H NMR data revealed that signals at the ring portion of the aglycone shifted noticeably, while those at the side chain and sugar moieties were intact (Tables 1 and 2). The most significant differences occurred for the signals of carbons and protons near the A/B and B/C ring junctions, implying the structural differences at these junctions. This interpretation was supported by the combined 2D NMR analyses, which defined an identical planar structure for **10** and **11**, despite the significant NMR chemical shifts between these compounds (Figure 1).

The configurational differences were determined by ROESY experiments. The NOE cross-peaks at H-1α/H-6α, H-3/H-6α, H-6α/H-29, H-6β/H-29, and H-19/H-28 suggested a 5β-OH, thus a *cis* A/B ring junction. Similarly, the 8β-OH and the accompanying *cis* B/C ring junction were also assigned by the H-7α/H-14 and H-18/H-19 cross-peaks. This interpretation was further supported by the lack of an H-7β/H-18 cross-peak that was crucial for the 8α-OH assignment in **10**. Thus, the structure of sarasinoside P (**11**) was determined to be the 5,8-bisepimer of sarasinoside O (**10**). This is the first reported example of a sarasinoside with a *cis* A/B ring junction.

Another minor constituent, sarasinoside Q (**12**), was isolated as a white, amorphous solid. The molecular formula of **12** was

Table 1. ^{13}C NMR (ppm, mult) Data of the Aglycone Moieties of Compounds **9**–**13** in CD_3OD^a

position	9	10	11	12	13
1	37.1, CH_2	39.2, CH_2	32.3, CH_2	36.4, CH_2	34.5, CH_2
2	27.8, CH_2	28.5, CH_2	28.4, CH_2	26.7, CH_2	27.8, CH_2
3	91.8, CH	88.2, CH	87.7, CH	91.8, CH	91.5, CH
4	40.7, C	41.8, C	45.0, C	42.3, C	40.3, C
5	52.0, CH	93.8, C	84.2, C	46.3, CH	44.8, CH
6	19.4, CH_2	27.8, CH_2	24.6, CH_2	26.7, CH_2	24.8, CH_2
7	29.5, CH_2	29.0, CH_2	33.0, CH_2	32.0, CH_2	18.2, CH_2
8	129.0, C	87.3, C	76.6, C	142.3, C	70.0, C
9	137.2, C	156.2, C	150.6, C	107.6, C	72.2, C
10	37.9, C	45.9, C	43.7, C	48.3, C	37.4, C
11	23.0, CH_2	112.6, CH	118.7, CH	31.3, CH_2	25.2, CH_2
12	38.2, CH_2	43.0, CH_2	42.0, CH_2	34.0, CH_2	115.1, CH
13	43.4, C	43.9, C	42.9, C	49.7, C	148.4, C
14	53.2, CH	52.4, CH	57.8, CH	129.4, C	49.2, C
15	24.8, CH_2	21.6, CH_2	19.8, CH_2	24.5, CH_2	33.7, CH_2
16	30.0, CH_2	29.5, CH_2	29.3, CH_2	28.9, CH_2	25.5, CH_2
17	56.3, CH	58.1, CH	56.4, CH	54.9, CH	50.3, CH
18	11.7, CH_3	15.4, CH_3	12.7, CH_3	17.8, CH_3	22.1, CH_3
19	20.3, CH_3	23.4, CH_3	30.3, CH_3	25.3, CH_3	18.2, CH_3
20	35.1, CH	34.3, CH	34.4, CH	33.3, CH	33.2, CH
21	20.1, CH_3	19.7, CH_3	19.9, CH_3	20.85, CH_3	18.8, CH_3
22	52.4, CH_2	52.3, CH_2	52.1, CH_2	51.8, CH_2	48.4, CH_2
23	204.1, C	203.9, C	203.9, C	203.9, C	203.9, C
24	125.3, CH	125.3, CH	125.3, CH	125.3, CH	125.1, CH
25	157.1, C	157.0, C	157.0, C	157.0, C	157.1, C
26	20.9, CH_3	20.8, CH_3	20.8, CH_3	20.89, CH_3	20.9, CH_3
27	27.7, CH_3	27.6, CH_3	27.6, CH_3	27.7, CH_3	27.7, CH_3
28	16.6, CH_3	19.0, CH_3	19.1, CH_3	17.1, CH_3	17.2, CH_3
29	28.3, CH_3	21.8, CH_3	25.9, CH_3	29.3, CH_3	29.0, CH_3

[a]Data were measured at 150 MHz for **9** and **13** and 125 MHz for **10**–**12**.

found to be $C_{62}H_{100}N_2O_{28}$ by HRFABMS and ^{13}C NMR analyses. The NMR data of this compound showed the typical characteristics of sarasinosides, namely, 29 carbons for the aglycone including seven methyls and a terminal unsaturated ketone, and a pentaglycoside. The most conspicuous features of the ^{13}C NMR data of this compound were signals that corresponded to a tetrasubstituted double bond at δ_C 142.3 and 129.4 and an oxy-quaternary carbon at δ_C 107.6 (Table 1). The placements of these groups were determined by the gHMBC experiments. Long-range correlations of the olefinic carbons with the neighboring H-6, H-7, H-12, H-15, H-16, and H-18 placed this double bond at C-8(14). Similarly, the oxy-quaternary carbon was placed at C-9 by its long-range correlations with H-12 and H-19. This interpretation and the identification of the functionality to be a hemiacetal group was further supported by the long-range correlations of the hydroxy proton (δ_H 7.05 in pyridine-d_5, Supporting Information) with C-9, C-10, and C-11 (Figure 1).

The configurations of **12** were assigned by ROESY experiments. Several cross-peaks among the protons in the A and B rings, coupled with chemical shifts of the NMR signals for the A ring, suggested a *trans* junction. The newly apparent C-9 hydroxy group was determined to be α-oriented to the B/C junction by its NOE cross-peaks with H-1α, H-5, H-11α, and H-12α. Thus, the structure of sarasinoside Q (**12**) was determined to be the 9,11-hydrated derivative of the congener sarasinoside M (**8**).

Finally, the molecular formula of sarasinoside R (**13**) was determined to be $C_{62}H_{100}N_2O_{28}$, identical to several congeners such as **5**, **10**, and **11**, by combined HRFABMS and ^{13}C NMR analyses. Although the NMR data of this compound were highly reminiscent of other pentaglycosidic sarasinosides, the ^1H and ^{13}C NMR signals for the ring portion of the aglycone showed significant shifts (Tables 1 and 2). On the basis of the matching of protons and their corresponding carbons by the COSY and gHSQC experiments, the planar structure of the ring portion was determined by gHMBC experiments. Long-range couplings with the H-19 methyl and H-5 methine protons placed a hydroxy group at C-9 (δ_C 72.2). Similarly, couplings with methyl protons (δ_H 1.02) placed four carbons (δ_C 148.4, 70.0, 49.2, and 33.7) nearby. However, the lack of long-range coupling of these methyl protons with C-17 (δ_C 50.3), as was observed in other compounds, suggested the migration of this methyl group from C-18. The placement of the oxy-quaternary carbon (δ_C 70.0) at C-8 by its long-range correlation with H-6 helped to confirm the attachment of the methyl group at C-14. The remaining trisubstituted double bond was placed at C-12 by several correlations of the olefinic carbons and protons with neighboring ones. Thus, the planar structure of the aglycone portion of **13** was determined to be a C-18 methyl-rearranged nortriterpene, which is unprecedented among the sarasinosides.

The configurations at the C-8, C-9, and C-14 asymmetric centers at the ring junctions were determined by ROESY experiments. First, the *trans* A/B ring junction was defined by a series of cross-peaks: H-1α/H-3, H-1α/H-5, H-3/H-5, H-6β/H-19, H-6β/H-28, and H-19/H-28. Then, additional cross-peaks at H-6β/H-18, H-11β/H-18, H-11β/H-19, H-15α/H-17,

Table 2. ^{1}H MMR (δ, mult (J in Hz)) Data of the Aglycone Moieties of Compounds **9**–**13** in CD_3OD^a

position	9	10	11	12	13
1α	1.23, ddd (13.0, 13.0, 3.5)	1.74, ddd (12.5, 12.5, 3.5)	1.57, ddd (12.5, 12.5, 4.0)	2.19, ddd (13.5, 13.5, 3.0)	1.32, m
1β	1.70, m	1.46, ddd (12.5, 3.5, 3.5)	1.84, m	1.23, ddd (13.5, 3.0, 3.0)	1.73, m
2α	1.87, dddd (13.0, 3.5, 3.5, 3.5)	1.69, dddd (12.5, 3.5, 3.5, 3.5)	1.92, m	1.70, m	1.89, m
2β	1.73, dddd (13.0, 13.0, 12.0, 3.5)	1.74, dddd (12.5, 12.5, 12.5, 3.5)	1.84, m	1.60, dddd (13.5, 13.5, 11.5, 3.0)	1.72, m
3	3.16, dd (12.0, 3.5)	3.43, m	3.33, m	3.11, dd (11.5, 3.5)	3.03, dd (11.8, 4.2)
5	1.11, dd (12.5, 1.4)			1.95, br d (12.5)	1.48, dd (12.5, 1.8)
6α	1.71, m	1.90, m	1.80, m	1.50, dddd (12.5, 12.5, 12.5, 4.7)	2.17, m
6β	1.51, dddd (12.5, 12.5, 12.5, 6.7)	1.88, ddd (10.5, 10.5, 4.0)	2.28, m	1.70, m	1.69, m
7α	1.87, m	1.70, m	1.40, ddd (12.5, 12.5, 1.9)	1.87, m	1.49, m
7β	1.98, m	1.49, ddd (10.5, 10.5, 3.5)	2.21, ddd (12.5, 12.5, 7.0)	2.34, m	1.34, m
11α	1.97, m	5.03, dd (3.9, 3.0)	5.46, dd (7.4, 1.6)	1.59, m	2.37, dd (17.0, 7.1)
11β	2.07, m			2.44, br dd (13.5, 13.5)	2.48, dd (17.0, 1.2)
12α	1.39, ddd (11.9, 10.3, 2.3)	2.22, dd (17.0, 3.9)	2.29, dd (16.4, 7.4)	2.36, m	5.18, br d (7.1)
12β	1.97, m	1.95, m	1.81, dd (16.4, 1.6)	1.67, m	
14	2.06, m	1.98, m	1.62, m		
15α	1.62, m	1.67, m	1.51, m	2.00, m	1.54, m
15β	1.35, m	1.61, m	1.61, m	2.07, m	1.54, m
16α	1.86, m	1.92, m	1.88, m	1.86, m	1.69, m
16β	1.31, m	1.34, m	1.30, m	1.40, m	1.43, ddd (11.0, 11.0, 3.5)
17	1.19, m	1.32, m	1.17, m	1.39, m	2.49, m
18	0.67, s	0.75, s	0.77, s	0.97, s	1.02, s
19	1.01, s	1.26, s	1.52, s	1.29, s	1.13, s
20	1.97, m	1.97, m	1.93, m	2.02, m	2.24, m
21	0.91, d (6.4)	0.91, d (6.5)	0.89, d (6.5)	0.94, d (6.4)	0.90, d (6.6)
22	2.52, dd (14.9, 2.9)	2.54, dd (15.0, 3.0)	2.52, dd (15.0, 3.1)	2.54, dd (15.0, 2.7)	2.44, dd (14.5, 2.3)
	2.09, m	2.12, dd (15.0, 9.8)	2.08, dd (15.0, 9.9)	2.12, dd (15.0, 10.1)	2.16, dd (14.5, 10.7)
24	6.16, br s	6.17, br s	6.15, br s	6.15, br s	6.14, br s
26	2.11, d (1.0)	2.11, d (1.0)	2.10, d (1.0)	2.11, d (1.0)	2.10, d (1.0)
27	1.90, d (1.0)	1.90, d (1.0)	1.90, d (1.0)	1.90, d (1.0)	1.89, d (1.0)
28	0.89, s	1.05, s	1.15, s	0.97, s	0.95, s
29	1.08, s	1.18, s	1.18, s	1.15, s	1.07, s

^{a}Data were measured at 600 MHz for **9** and **13** and 500 MHz for **10**–**12**.

H-15β/H-18, H-16β/H-18, and H-18/H-19 unambiguously defined the *cis* B/C ring junction with both 8-OH and 9-OH in the α-orientation. In addition, the C-18 methyl group was placed in a β-orientation at the C/D junction, which is consistent with the 1,2-migration of this methyl group from the original norlanostane skeleton (Figure 2). Thus, sarasinoside R (**13**) was determined to be a nortriterpene pentaglycoside possessing a rearranged norlanostane aglycone.

Previous studies of the bioactivities of sarasinosides reported antimicrobial, cytotoxic, and piscicidal activities for these compounds. However, the antimicrobial activity is controversial, possibly due to the selection of different microbial strains. In our study, all of these compounds were inactive (MIC > 100 μg/mL) against selected strains of Gram-positive (*Bacillus subtilis* ATCC 6633, *Micrococcus luteus* IFO 12708, and *Staphylococcus aureus* ATCC 6538p) and Gram-negative (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 3851, and *Salmonella typhimurium* ATCC 14028) bacteria and pathogenic fungi (*Aspergillus fumigatus* HIC 6094, *Candida albicans* ATCC 10231, *Trichophyton mentagrophytes* IFO 40996, and *Trichophyton rubrum* IFO 9185). None of these compounds were noticeably active (IC_{50} > 50 μg/mL) against microbial-derived sortase A and isocitrate lyase, key enzymes of microbial metabolism. Therefore, the antimicrobial activity of sarasinosides either is strain specific or should be reinvestigated more thoroughly.

Several of these compounds showed cytotoxicity against K562 leukemia and A549 lung carcinoma strains (Table 4). Although the degree of inhibition differed among the strains, a few significant structure–activity relationships were found for the sarasinosides. Comparison of the activity among **1**, **2**, **9**, and **14**, a glycolysis product from **1**, showed that the cytotoxicity significantly depends on the presence of the glycoside portion. However, the size or units of the sugar moieties only weakly influenced the activity. As shown from **1** and **7**, the loss of the terminal double bond at C-24 weakly decreased the cytotoxicity. This result suggested that the presence of hydroxy groups at the aglycones negatively contributed to the cytotoxicity (**1**, **5**, **6**, **10**, **11**, and **13**; **8** and **12**). It is also noteworthy that configurations at the ring junction significantly affected the bioactivity (**10** and **11**). In addition, compounds **1**, **2**, and **9** weakly inhibited Na^+/K^+-ATPase (IC_{50} 60.0, 59.4, and 54.1 μg/mL for **1**, **2**, and **9**, respectively), while the remaining ones were inactive (IC_{50} > 100 μg/mL).[14]

Table 3. ^1H and ^{13}C NMR Data of the Sugar Moieties of Compounds 9 and 10 in CD_3OD^a

	position	9		10	
		δ_H	δ_C	δ_H	δ_C
Xyl	1′	4.37, d (7.6)	106.0	4.26, d (7.8)	106.5
	2′	3.61	79.0	3.90, dd (7.8, 9.1)	78.3
	3′	3.52, dd (8.8, 8.8)	77.5	3.50, dd (9.1, 9.1)	77.6
	4′	3.59, ddd (10.7, 8.8, 5.5)	79.3	3.66	79.8
	5′	3.88, dd (12.0, 5.5); 3.18, dd (12.0, 10.7)	64.3	3.86; 3.12, dd (10.8, 10.8)	64.2
2-NAc-Glc	1″	4.88, d (8.4)	102.5	4.82	102.0
	2″	3.65, dd (10.3, 8.4)	58.1	3.67	57.8
	3″	3.41, dd (10.3, 8.8)	76.7	3.41	77.0
	4″	3.11, dd (8.8, 9.8)	72.9	3.14, dd (9.8, 9.1)	72.4
	5″	3.22, ddd (9.8, 7.6, 2.2)	78.6	3.67	78.1
	6″	3.84, dd (12.0, 2.2); 3.56, dd (12.0, 7.6)	63.8	4.10, br d (12.0); 3.76	70.1
	Ac	1.98, s	23.2	1.98, s	23.0
			174.1		174.2
Glc-I	1‴			5.07, d (7.9)	102.7
	2‴			3.38, dd (7.9, 9.1)	83.5
	3‴			3.69	78.0
	4‴			3.31	71.7
	5‴			3.45, ddd (9.8, 5.7, 2.5)	77.2
	6‴			3.83, dd (11.5, 2.5); 3.70	62.7
Glc-II	1‴′			4.61, d (7.4)	105.8
	2‴′			3.40	75.9
	3‴′			3.40	77.9
	4‴′			3.31	71.5
	5‴′			3.27, ddd (9.5, 6.0, 2.0)	78.3
	6‴′			3.87, br d (11.7); 3.72, dd (11.7, 6.0)	62.7
2-NAc-Gal	1‴″	4.44, d (8.7)	102.5	4.49, d (8.4)	102.5
	2‴″	3.89, dd (10.6, 8.7)	54.5	3.85	54.6
	3‴″	3.60	73.0	3.63	72.8
	4‴″	3.81	69.8	3.79	69.9
	5‴″	3.52, ddd (7.4, 4.3, 0.8)	77.4	3.63	77.4
	6‴″	3.80, dd (11.6, 7.4); 3.70, dd (11.6, 4.3)	62.9	3.87; 3.71	63.1
	Ac	1.97, s	23.2	1.99, s	23.0
			174.3		173.9

[a]Data were measured at 600 MHz for ^1H and 150 MHz for ^{13}C for **9** and 500 MHz for ^1H and 125 MHz for ^{13}C for **10**.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Spectroscopic data were recorded on the same equipment as described previously.[13] Mass spectrometric data were obtained at the Korea Basic Science Institute (Daegu, Korea) and were acquired using a JEOL JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol as a matrix for the FABMS. HPLC was performed on a SpectraSystem p2000 equipped with a SpectraSystem RI-150 refractive index detector. All solvents were spectroscopic grade or distilled from glass prior to use.

Animal Material. Specimens of *Lipastrotethya* sp. (voucher collection number 102CH-232) were collected and identified as described previously.[13]

Extraction and Isolation. Collected specimens were treated as described previously.[13] An aliquot of the H_2O−MeOH (15:85) layer (9.22 g) was separated by C_{18} reversed-phase vacuum flash chromatography using sequential mixtures of MeOH and H_2O as eluents (six fractions in gradient, H_2O−MeOH, from 50:50 to 0:100), acetone, and finally EtOAc.

On the basis of the results of ^1H NMR and cytotoxicity analyses, the fractions eluted with H_2O−MeOH (30:70) (0.33 g), H_2O−MeOH (20:80) (0.74 g), and H_2O−MeOH (10:90) (1.10 g) were chosen for separation. The fraction eluted with H_2O−MeOH (30:70) was separated by semipreparative reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O−MeOH, 35:65), yielding two peaks rich with secondary metabolites. Further purification of the first peak by reversed-phase HPLC (YMC-Pack CN column, 10 mm × 250 mm; H_2O−MeOH, 65:35) isolated compound **6** as an amorphous solid, while purification of the second peak yielded compound **5**.

The H_2O−MeOH (20:80) fraction was separated by reversed-phase HPLC (YMC-ODS column; H_2O−MeOH, 30:70) to yield, in order of elution, compounds **12**, **7**, **13**, and **11**. These metabolites were then purified by reversed-phase HPLC (YMC-Pack CN column; H_2O−MeOH, 60:40).

The H_2O−MeOH (10:90) fraction was separated and purified by reversed-phase HPLC (YMC-ODS column; H_2O−MeOH, 20:80), yielding, in order of elution, compounds **8**, **10**, **4**, **3**, **1**, **2**, and **9**. These metabolites were then purified by HPLC (YMC-Pack CN column; H_2O−MeCN, 65:35, for **8**, **10**, **4**, and **3** and YMC-Pack ODS column; H_2O−MeOH, 25:75, for **1**, **2**, and **9**, respectively). The purified metabolites were isolated in the following amounts: 721.8, 9.2, 18.2, 16.4, 53.6, 18.2, 15.0, 39.6, 4.9, 4.5, 3.6, 18.2, and 6.5 mg of **1**−**13**, respectively.

Sarasinoside N (9): white, amorphous solid; $[\alpha]^{25}_D$ −10.0 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 236 (2.01) nm; IR (ZnSe) ν_{max} 3400 (br), 2940, 1650 (br), 1565, 1380 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1, 2, and 3; HRFABMS *m/z* 987.5409 [M + Na]$^+$ (calcd for $C_{50}H_{80}N_2O_{16}Na$, 987.5406).

Sarasinoside O (10): white, amorphous solid; $[\alpha]^{25}_D$ −2.6 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ε) 238 (2.05) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1650 (br), 1570, 1450, 1375 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1, 2, and 3; HRFABMS *m/z* 1325.6221 [M − H_2O + Na]$^+$ (calcd for $C_{62}H_{98}N_2O_{27}Na$, 1325.6255).

Sarasinoside P (11): white, amorphous solid; $[\alpha]^{25}_D$ −6.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 238 (1.75) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1650 (br), 1580, 1450, 1375 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ^1H NMR data of the sugar portion (CD_3OD) Xyl δ_H 4.26 (1H, d, *J* = 7.8 Hz, H-1′), 3.88 (H-2′), 3.85 (H-5′), 3.65 (H-4′), 3.49 (1H, dd, *J* = 9.1, 9.1 Hz, H-3′), 3.14 (1H, dd, *J* = 11.3, 10.2 Hz, H-5′); 2-NAc-Glc δ_H 4.81 (1H, d, *J* = 8.6 Hz, H-1″), 4.10 (1H, br d, *J* = 12.6 Hz, H-6″), 3.74 (1H, dd, *J* = 12.6, 7.6 Hz, H-6″), 3.66 (H-2″, H-5″), 3.37 (H-3″), 3.15 (1H, dd, *J* = 9.8, 8.7 Hz, H-4″), 1.97 (3H, s, NHAc); Glc-I δ_H 5.03 (1H, d, *J* = 7.9 Hz, H-1‴), 3.82 (1H, dd, *J* = 11.8, 2.5 Hz, H-6‴), 3.70 (H-6‴), 3.67 (H-3‴), 3.43 (1H, dd, *J* = 9.8, 5.2, 2.5 Hz, H-5‴), 3.37 (H-2‴), 3.31 (H-4‴); Glc-II δ_H 4.61 (1H, d, *J* = 7.4 Hz, H-1‴′), 3.87 (H-6‴′), 3.72 (1H, dd, *J* = 12.5, 6.0 Hz, H-6‴′), 3.40 (H-2‴′, H-3‴′), 3.32 (H-4‴′), 3.27 (1H, ddd, *J* = 9.5, 5.5, 2.3 Hz, H-5‴′); 2-NAc-Gal δ_H 4.48 (1H, d, *J* = 8.4 Hz, H-1‴″), 3.88 (H-6‴″), 3.85 (H-2‴″), 3.78 (H-4‴″), 3.71 (H-6‴″), 3.62 (H-3‴″, H-5‴″), 1.98 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ_C 106.4 (C-1′), 79.9 (C-4′), 78.0 (C-2′), 77.5 (C-3′), 64.3 (C-5′); 2-NAc-Glc δ_C 174.3 (NHAc), 102.1 (C-1″), 78.0 (C-5″), 77.0 (C-3″), 72.4 (C-4″), 70.1 (C-6″), 57.9 (C-2″), 23.0 (NHAc); Glc-I δ_C 102.6 (C-1‴), 83.6 (C-2‴), 77.9 (C-3‴), 77.0 (C-5‴), 71.6 (C-4‴), 62.8 (C-6‴); Glc-II δ_C 105.8 (C-1‴′), 78.3 (C-5‴′), 77.7 (C-3‴′), 75.8 (C-2‴′), 71.5 (C-4‴′), 62.6 (C-6‴′); 2-NAc-Gal δ_C 173.9 (NHAc), 102.7 (C-1‴″), 77.2 (C-5‴″), 72.8 (C-3‴″), 69.9 (C-4‴″), 63.1 (C-6‴″), 54.6 (C-2‴″), 23.0 (NHAc); HRFABMS *m/z* 1341.6031 [M − H_2O + K]$^+$ (calcd for $C_{62}H_{98}N_2O_{27}K$, 1341.5994).

Sarasinoside Q (12): white, amorphous solid; $[\alpha]^{25}_D$ −29.1 (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ε) 240 (1.96) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1645 (br), 1565, 1375 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ^1H NMR data of the sugar portion (CD_3OD) Xyl δ_H 4.29 (1H, d, *J* = 7.8 Hz, H-1′), 3.90 (1H, dd, *J* = 9.1, 7.8 Hz, H-2′), 3.86 (1H, dd, *J* = 11.4, 5.5 Hz, H-5′), 3.67 (H-4′), 3.50 (1H, dd, *J* =

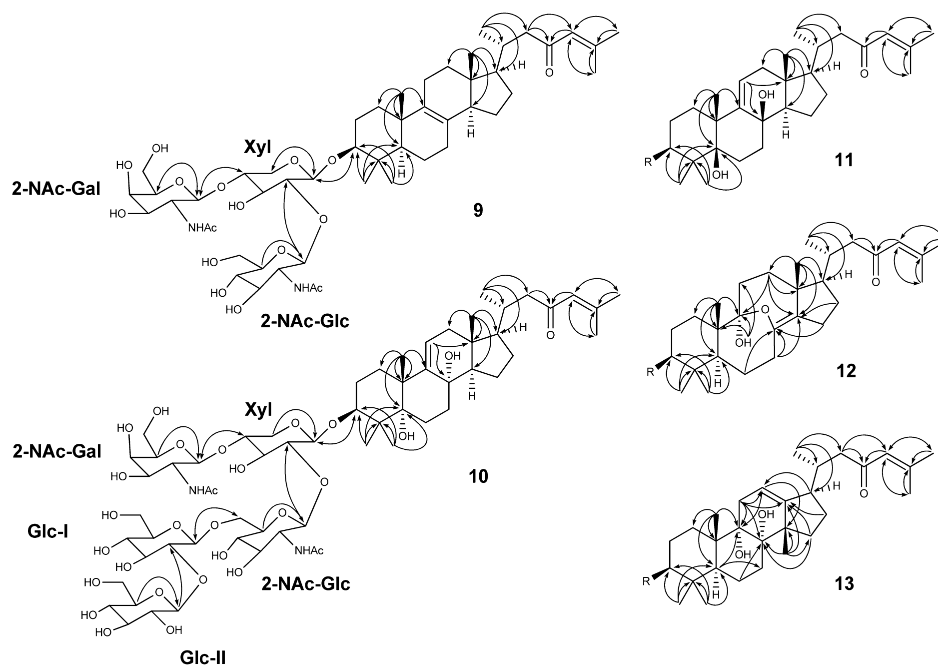

Figure 1. gHMBC correlations for compounds **9**−**13**.

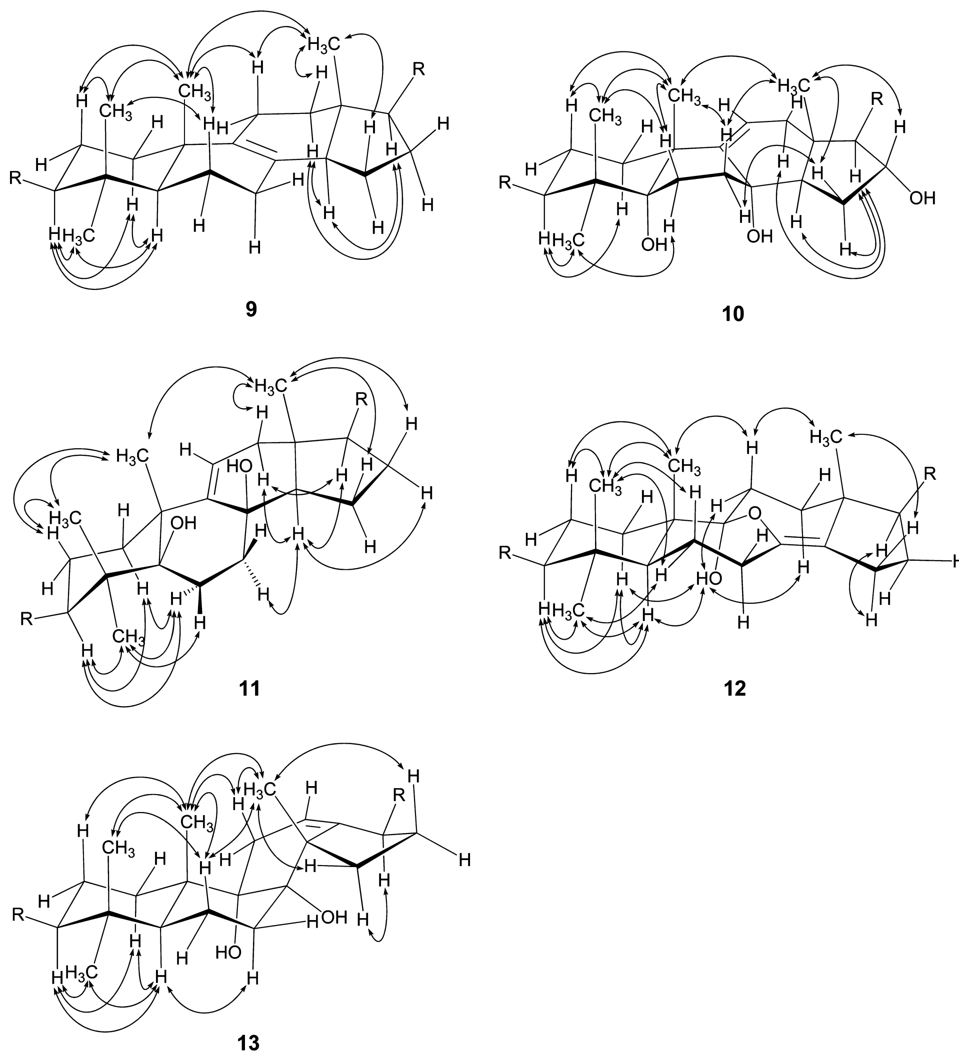

Figure 2. Selected NOE correlations for the aglycone portions of compounds **9**−**13**.

Table 4. Results of Cytotoxicity Tests

compound	K562 LC_{50} (μM)	A549 LC_{50} (μM)
1	7.4	7.6
2	18.7	9.1
3	8.5	7.6
4	24.8	10.1
5	37.4	14.2
6	42.7	>100
7	20.8	10.3
8	12.1	7.7
9	20.2	10.7
10	61.5	54.7
11	11.6	17.9
12	17.9	14.0
13	>100	14.7
14	69.4	41.6
doxorubicin	4.7	1.7

9.1, 9.1 Hz, H-3′), 3.14 (1H, dd, J = 11.4, 10.0 Hz, H-5′); 2-NAc-Glc δ_H 4.82 (H-1″), 4.08 (1H, br d, J = 12.5 Hz, H-6″), 3.77 (1H, dd, J = 12.5, 7.4 Hz, H-6″), 3.67 (H-2″, H-5″), 3.40 (H-3″), 3.14 (1H, dd, J = 8.9, 8.9 Hz, H-4″), 1.99 (3H, s, NHAc); Glc-I δ_H 5.08 (1H, d, J = 7.9 Hz, H-1‴), 3.81 (1H, dd, J = 11.8, 2.5 Hz, H-6‴), 3.69 (H-3‴, H-6‴), 3.45 (1H, ddd, J = 9.4, 5.4, 2.5 Hz, H-5‴), 3.37 (1H, dd, J = 9.2, 7.9 Hz, H-2‴), 3.31 (H-4‴); Glc-II δ_H 4.61 (1H, d, J = 7.5 Hz, H-1‴′), 3.88 (H-6‴′), 3.72 (1H, dd, J = 12.2, 6.0 Hz, H-6‴′), 3.40 (H-2‴′, H-3‴′), 3.32 (H-4‴′), 3.27 (1H, ddd, J = 9.6, 6.0, 2.4 Hz, H-5‴′); 2-NAc-Gal δ_H 4.48 (1H, d, J = 8.4 Hz, H-1‴″), 3.89 (H-6‴″), 3.87 (H-2‴″), 3.79 (H-4‴″), 3.71 (H-6‴″), 3.63 (H-3‴″, H-5‴″), 1.98 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ_C 106.6 (C-1′), 79.8 (C-4′), 78.0 (C-2′), 77.8 (C-3′), 64.2 (C-5′); 2-NAc-Glc δ_C 173.9 (NHAc), 102.0 (C-1″), 78.2 (C-5″), 77.0 (C-3″), 72.4 (C-4″), 70.1 (C-6″), 57.9 (C-2″), 23.0 (NHAc); Glc-I δ_C 102.6 (C-1‴), 83.4 (C-2‴), 78.0 (C-3‴), 77.2 (C-5‴), 71.6 (C-4‴), 62.6 (C-6‴); Glc-II δ_C 105.7 (C-1‴′), 78.2 (C-3‴′, C-5‴′), 75.9 (C-2‴′), 71.5 (C-4‴′), 62.7 (C-6‴′); 2-NAc-Gal δ_C 174.2 (NHAc), 102.6 (C-1‴″), 77.2 (C-5‴″), 72.9 (C-3‴″), 69.9 (C-4‴″), 63.1 (C-6‴″), 54.5 (C-2‴″), 23.0 (NHAc); HRFABMS m/z 1343.6366 $[M + Na]^+$ (calcd for $C_{62}H_{100}N_2O_{28}Na$, 1343.6360).

Sarasinoside R (13): white, amorphous solid; $[\alpha]^{25}_D$ −12.4 (c 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 238 (1.60) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1645 (br), 1570, 1450, 1380 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ^{1}H NMR data of the sugar portion (CD_3OD) Xyl δ_H 4.28 (1H, d, J = 7.8 Hz, H-1′), 3.90 (1H, dd, J = 9.2, 7.8 Hz, H-2′), 3.86 (H-5′), 3.68 (H-4′), 3.51 (1H, dd, J = 9.2, 9.2 Hz, H-3′), 3.16 (1H, dd, J = 10.7, 10.7 Hz, H-5′); 2-NAc-Glc δ_H 4.82 (H-1″), 4.09 (1H, br d, J = 12.6 Hz, H-6″), 3.76 (1H, dd, J = 12.6, 7.6 Hz, H-6″), 3.67 (H-2″, H-5″), 3.41 (H-3″), 3.14 (1H, dd, J = 8.9, 8.9 Hz, H-4″), 1.98 (3H, s, NHAc); Glc-I δ_H 5.07 (1H, d, J = 7.9 Hz, H-1‴), 3.82 (1H, dd, J = 11.8, 2.5 Hz, H-6‴), 3.70 (H-6‴), 3.69 (H-3‴), 3.45 (1H, ddd, J = 10.1, 5.2, 2.5 Hz, H-5‴), 3.38 (1H, dd, J = 9.2, 7.9 Hz, H-2‴), 3.32 (H-4‴); Glc-II δ_H 4.61 (1H, d, J = 7.3 Hz, H-1‴′), 3.88 (1H, br d, J = 12.4 Hz, H-6‴′), 3.72 (1H, dd, J = 12.4, 6.0 Hz, H-6‴′), 3.40 (H-2‴′, H-3‴′), 3.32 (H-4‴′), 3.27 (1H, ddd, J = 9.5, 6.0, 2.0 Hz, H-5‴′); 2-NAc-Gal δ_H 4.49 (1H, d, J = 7.8 Hz, H-1‴″), 3.88 (H-6‴″), 3.85 (H-2‴″), 3.79 (H-4‴″), 3.71 (H-6‴″), 3.63 (H-3‴″, H-5‴″), 1.98 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ_C 106.8 (C-1′), 79.7 (C-4′), 78.0 (C-2′), 77.4 (C-3′), 64.3 (C-5′); 2-NAc-Glc δ_C 174.3 (NHAc), 102.0 (C-1″), 77.9 (C-5″), 77.0 (C-3″), 72.3 (C-4″), 70.1 (C-6″), 57.9 (C-2″), 23.1 (NHAc); Glc-I δ_C 102.5 (C-1‴), 83.3 (C-2‴), 77.9 (C-3‴), 77.0 (C-5‴), 71.7 (C-4‴), 62.7 (C-6‴); Glc-II δ_C 105.8 (C-1‴′), 78.3 (C-5‴′), 77.7 (C-3‴′), 75.9 (C-2‴′), 71.5 (C-4‴′), 62.7 (C-6‴′); 2-NAc-Gal δ_C 173.9 (NHAc), 102.6 (C-1‴″), 77.3 (C-5‴″), 72.9 (C-3‴″), 69.9 (C-4‴″), 63.2 (C-6‴″), 54.6 (C-2‴″), 23.1 (NHAc); HRFABMS m/z 1325.6223 $[M - H_2O + Na]^+$ (calcd for $C_{62}H_{98}N_2O_{27}Na$, 1325.6255).

Acidic Hydrolysis of Sarasinoside A_1 (1) (refs 7, 8). A mixture of **1** (25.1 mg) and 3 N HCl (1.5 mL) was stirred at 65 °C for 1 h. After cooling, the solution was concentrated under a stream of N_2 and then extracted with MeOH. The MeOH extract was purified by reversed-phase HPLC (YMC-Pack ODS column, 10 mm × 250 mm; H_2O−MeOH, 10:90) to furnish sapogenol **14** (3.6 mg) having a $\Delta^{8(14)}$ double bond that resulted from a double-bond migration; LR-FABMS m/z 427.50 $[M + H]^+$.

Acid Hydrolysis and GC Analysis of Compounds 9 and 10 (refs 10, 13). A solution of **9** (1.4 mg) in 3 N HCl (0.5 mL) was stirred at 80 °C for 5 h. After being cooled to room temperature, the solution was concentrated by evaporation under a stream of N_2. The residue was treated with hexamethyldisilazane (HMDS) and TMSCl (50 μL, v/v = 2:1) in pyridine (0.5 mL), and the solution was stirred at 60 °C for 30 min. After drying the solution with a stream of N_2, the residue was separated with H_2O and CH_2Cl_2 (1 mL, v/v = 1:1). The CH_2Cl_2 layer was analyzed by GC using an HP5 column (0.32 mm × 30 m). The injector and detector were both held at 200 °C. A temperature gradient was applied to the GC oven; the initial temperature was maintained at 60 °C for 3 min, then ramped to 200 at 4 °C/min, and maintained at 200 °C for 3 min. Peaks of the hydrolysate were detected at 27.02, 31.51, and 36.73 min. Retention times for authentic samples after being treated simultaneously with HMDS/TMSCl were 26.56 (L-xylose), 27.01 (D-xylose), 31.51 (2-*N*-acetyl-D-galactosamine), 32.53 (D-glucose), 32.62 (L-glucose), and 36.72 min (2-*N*-acetyl-D-glucosamine). Co-injection of the hydrolysate with the authentic silylated D-xylose, 2-*N*-acetyl-D-galactosamine, and 2-*N*-acetyl-D-glucosamine gave single peaks at 27.02, 31.49, and 36.73 min, respectively. A solution of **10** (3.1 mg) was prepared in a similar manner, and peaks of the hydrolysate were detected at 27.01, 31.51, 32.52, and 36.71 min. Co-injection of the hydrolysate with the authentic silylated D-xylose, 2-*N*-acetyl-D-glucosamine, D-glucose, and 2-*N*-acetyl-D-galactosamine gave single peaks at 27.01, 31.51, 32.52, and 36.73 min, respectively.

Biological Assays. Cytotoxicity assays were performed in accordance with the protocols in refs 15 and 16.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**, **9**−**13** 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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■ REFERENCES

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2012**, *29*, 144−222 , and earlier reports in the series.

(2) Ksebati, M. B.; Schmitz, F. J.; Gunasekera, S. P. *J. Org. Chem.* **1988**, *53*, 3917−3921.
(3) Carmely, S.; Roll, M.; Loya, Y.; Kashman, Y. *J. Nat. Prod.* **1989**, *52*, 167−170.
(4) Shin, J.; Lee, H.-S.; Woo, L.; Rho, J.-R.; Seo, Y.; Cho, K. W.; Sim, C. J. *J. Nat. Prod.* **2001**, *64*, 767−771.
(5) Kitagawa, I.; Kobayashi, M.; Okamoto, Y.; Yoshikawa, M.; Hamamoto, Y. *Chem. Pharm. Bull.* **1987**, *35*, 5036−5039.
(6) Kitagawa, I.; Kobayashi, M.; Okamoto, Y.; Yoshikawa, M.; Hamamoto, Y. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1987**, *29*, 568−575.
(7) Schmitz, F. J.; Ksebati, M. B.; Gunasekera, S. P.; Agarwal, S. *J. Org. Chem.* **1988**, *53*, 5941−5947.
(8) Kobayashi, M.; Okamoto, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1991**, *39*, 2867−2877.
(9) Espada, A.; Jiménez, C.; Rodríguez, J.; Crews, P.; Riguera, R. *Tetrahedron* **1992**, *48*, 8685−8696.
(10) Lee, H.-S.; Seo, Y.; Cho, K. W.; Rho, J.-R.; Shin, J.; Paul, V. J. *J. Nat. Prod.* **2000**, *63*, 915−919.
(11) Dai, H.-F.; Edrada, R. A.; Ebel, R.; Nimtz, M.; Wray, V.; Proksch, P. *J. Nat. Prod.* **2005**, *68*, 1231−1237.
(12) Santalova, E. A.; Denisenko, V. A.; Dmitrenok, P. S.; Berdyshev, D. V.; Stonik, V. A. *Nat. Prod. Commun.* **2006**, *1*, 265−271.
(13) Lee, J.-H.; Jang, K. H.; Lee, Y.-J.; Lee, H.-S.; Sim, C. J.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2011**, *74*, 2563−2570.
(14) Jeon, J.-e.; Bae, J.; Lee, K. J.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2011**, *74*, 847−851.
(15) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(16) Ulukaya, E.; Ozdikicioglu, F.; Oral, A. Y.; Demirci, M. *Toxicol. in Vitro* **2008**, *22*, 232−239.