Triterpene Galactosides of the Pouoside Class and Corresponding Aglycones from the Sponge *Lipastrotethya* sp.

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S *Supporting Information*
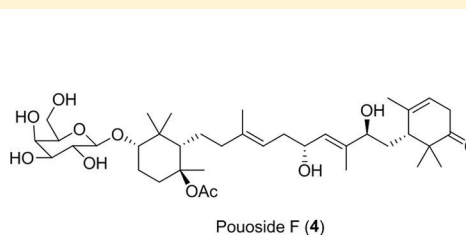
Pouoside F (4)

ABSTRACT: Nine new triterpene galactosides and aglycones, along with three known compounds from the rare pouoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Micronesia. The structures of these new compounds were determined by combined spectroscopic methods and designated as pouosides F–I (**4**, **8**, **10**, and **12**) and pouogenins A–E (**5–7**, **9**, and **11**). The absolute configurations of the asymmetric centers and the cyclohexenone moiety, which had been previously undetermined, were assigned by NOESY analyses and Mosher's methods. Several of these compounds exhibited weak cytotoxicity against the K562 cell line.

Marine sponges produce a wide variety of terpenes and related metabolites possessing diverse carbon skeletons and bioactivities.[1] However, triterpenes and triterpene saponins are relatively rare and represent only a minor fraction of the known sponge-derived terpenoids. Of these compounds, pouosides are a class of cytotoxic triterpene galactosides that have been isolated from the tropical sponge *Asteropus* sp. collected from the Truk Lagoon.[2] The symmetric carbon skeletons of these compounds are reminiscent of carotenoids and are unique among sponge-derived terpenoids. Despite the numerous works on marine sponges, compounds of this structural class have not been investigated since the first report on pouosides A–E by Schmitz[2] several decades ago.

During the course of our search for bioactive metabolites from tropical marine organisms, we encountered the sponge *Lipastrotethya* sp. (order Halichondrida, family Dictyonellidae), the organic extract of which exhibited weak cytotoxicity (LC$_{50}$ 146 µg/mL) against the K562 cell line. Bioassay-guided separation of the extract yielded sarasinosides, widely recognized nortriterpene pentaglycosides, as the major constituents.[3–9] However, moderately polar vacuum flash

chromatographic fractions yielded several compounds of a different structural class. Here we report the structures of nine new triterpenes of the pouoside class along with three other known compounds of the same structural class (**1–3**).[2] The new compounds were designated as the new triterpene galactosides pouosides F–I (**4**, **8**, **10**, and **12**) and their aglycones, pouogenins A–E (**5–7**, **9**, and **11**), respectively. In addition, the absolute configurations of the asymmetric carbon centers and the cyclohexenone moiety were determined for the first time using a series of Mosher's esterification methods and NOESY analyses. Several of these compounds showed weak cytotoxicity against the K562 cell line.

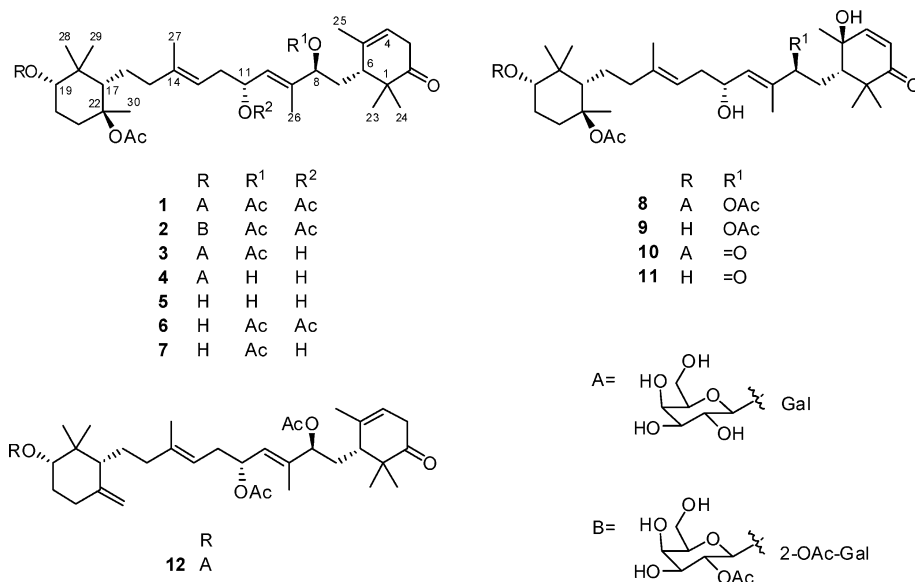## ■ RESULTS AND DISCUSSION

The sponge samples were lyophilized, macerated, and repeatedly extracted with CH$_2$Cl$_2$ and MeOH. The combined extracts were separated by solvent-partitioning followed by ODS vacuum flash chromatography and then repeated ODS-

Chart 1

	R	R¹	R²
1	A	Ac	Ac
2	B	Ac	Ac
3	A	Ac	H
4	A	H	H
5	H	H	H
6	H	Ac	Ac
7	H	Ac	H

	R	R¹
8	A	OAc
9	H	OAc
10	A	=O
11	H	=O

	R
12	A

HPLC. The major constituents were identified as sarasinosides A$_1$ and A$_3$ by combined spectroscopic methods and a comparison of ^{1}H and ^{13}C NMR data with authentic samples.[5,7]

In addition to the sarasinosides, 12 other compounds were isolated by the same HPLC separation methods. The structures of compounds **1**−**3** were identified as pouosides A, D, and B, respectively, on the basis of spectroscopic analyses and a comparison of NMR data with the literature.[2]

The molecular formula of pouoside F (**4**) was C$_{38}$H$_{62}$O$_{11}$ as determined by HRFABMS analysis. The ^{13}C and ^{1}H NMR data of this compound were similar to those of **1** and implied a monoacetylated triterpene glycoside. The triterpene nature of this compound was revealed by the presence of eight upfield methyl signals in the NMR data (Tables 1 and 2). Also, the ^{13}C NMR spectrum contained signals corresponding to a ketone (δ_C 217.4), an acetoxy group (δ_C 172.2 and 23.0), and three double bonds (six signals at δ_C 141.0−119.0), which were precisely matched with their corresponding signals in the ^{1}H NMR spectrum and absorption bands at 1730 and 1710 cm^{-1} in the IR spectrum. Similarly, the presence of a sugar unit was revealed by characteristic signals at of δ_C 107.3 and 77−62 and δ_H 4.3−3.4. Overall, these NMR features clearly indicated that **4** was an analogue of pouoside A (**1**).

Given this information, the structure of **4** was determined by a combination of 2-D NMR experiments. First, the presence of a sugar moiety was secured by a combination of COSY and gHSQC data for the signals in the aforementioned NMR regions. Similarities in the chemical shifts and splitting patterns of these signals with those of **1** suggested the presence of a D-galactose group that was confirmed by hydrolysis of **4** followed by GC analysis with authentic sugars. The pyranose nature of this galactose group was supported by long-range correlations at H-1′/C-5′ and H-5′/C-1′ in the gHMBC data. In addition, the β-configuration at the anomeric center was also assigned by its carbon chemical shift (δ_C 107.3) and large ^{1}H/^{1}H coupling constant ($J_{1′,2′}$ = 7.5 Hz). Taken together, these data indicate the presence of a β-D-galactopyranose unit on compound **4**.

The structure of the aglycone was also determined by combined NMR analyses. In particular, several long-range correlations between upfield methyl protons and neighboring

ring carbons in the gHMBC data revealed the presence of 4,6,6-trimethyl-β,γ-cyclohexenone (C-1−C-6) and 1,1,4-trimethylcyclohexane (C-17−C-22) moieties. The downfield shift of C-19 at δ_C 89.0, the HMBC correlation H-19/C-1′, and the NOESY cross-peak at H-19/H-1′ indicated a galactose moiety at this location via a glycosidic linkage.

The remaining part of the molecule, including two trisubstituted double bonds, two oxymethine groups, and two vinyl methyl groups, made up a linear terpenoidal chain (C-7−C-16) based on COSY (H-7−H-8, H-10−H-13, H-15−H-16) and gHMBC (H-8/C-7, C-9, C-10, C-26; H-11/C-9, C-10, C-12, C-13; H-26/C-8, C-9, C-10; H-27/C-13, C-14, C-15) data. Connections of this chain with the cyclic moieties were also established with these data: COSY (H-6−H-8, H-15−H-17) and gHMBC (H-6/C-7, C-8; H-8/C-6; H-15/C-17). Of the oxygenated locations at C-8, C-11, and C-22, the remaining acetoxy group was placed at C-22 by the proton chemical shifts of H-8 (δ_H 3.95) and H-11 (δ_H 4.32). Thus, pouoside F (**4**) was identified as a triterpene galactoside.

Pouoside F possessed asymmetric double bonds at C-9 and C-13. The geometries at these bonds were both E on the basis of the upfield shifts of vinyl methyl carbons (δ_C 13.0 and 16.7 for C-26 and C-27, respectively) and cross-peaks at H-8/H-10, H-11/H-26, H-12/H-27, and H-13/H-15 in the NOESY data. This compound also possessed asymmetric carbon centers at C-6, C-8, C-11, C-17, C-19, and C-22, whose relative configurations had previously only been partially determined. Among these, the relative configurations of those in the six-membered rings and vicinity were assigned by proton coupling constants and NOESY analyses. First the anti-orientations were assigned for both H-6/H-7b (δ_H 1.14) and H-7a (δ_H 1.68)/H-8 by the large vicinal coupling constants ($J_{6,7b}$ = 9.5 Hz, $J_{7a,8}$ = 10.0 Hz). Given this information, the NOESY data showed cross-peaks at H-4/H-23, H-6/H23, H-24, H-25, and H-23/H-25, placing H-6 at the pseudoequatorial position in the cyclohexenone ring. Additional NOE cross-peaks were found at H-6/H-8, H-7a/H-24, H-8/H-25, H-26, H-10/H-7a, H-24, and H-25/H-26, supporting the 6R* and 8S* configurations. More crucial evidence was found from NOESY data obtained in pyridine-d_5 (Supporting Information). The 8-OH proton at δ

Table 1. ^{13}C NMR (ppm, mult) Assignments for Compounds 4−12 in CD$_3$OD

position	4	5	6	7	8	9	10	11	12
1	48.6, C	48.7, C	48.6, C	48.6, C	46.7, C	46.7, C	46.7, C	46.8, C	48.9, C
2	217.4, C	217.3, C	216.6, C	216.7, C	206.2, C	206.2, C	205.8, C	205.8, C	216.7, C
3	38.7, CH$_2$	39.0, CH$_2$	38.7, CH$_2$	38.9, CH$_2$	125.3, CH	125.3, CH	125.5, CH	125.6, CH	38.2, CH$_2$
4	119.0, CH	118.9, CH	119.6, CH	119.6, CH	158.0, CH	158.0, CH	157.7, CH	157.7, CH	119.5, CH
5	141.0, C	141.0, C	139.2, C	139.5, C	72.0, C	72.0, C	71.6, C	71.7, C	139.3, C
6	50.0, CH	50.0, CH	51.0, CH	51.1, CH	50.0, CH	50.0, CH	51.1, CH	51.1, CH	51.0, CH
7	38.8, CH$_2$	38.8, CH$_2$	35.6, CH$_2$	36.0, CH$_2$	31.0, CH$_2$	31.0, CH$_2$	35.1, CH$_2$	35.1, CH$_2$	35.6, CH$_2$
8	75.6, CH	75.6, CH	78.4, CH	78.4, CH	80.3, CH	80.3, CH	203.8, C	203.8, C	78.4, CH
9	141.0, C	141.0, C	139.2, C	139.5, C	137.1, C	137.1, C	137.7, C	137.8, C	139.1, C
10	129.1, CH	129.1, CH	127.6, CH	131.6, CH	131.5, CH	131.5, CH	144.0, CH	144.0, CH	127.6, CH
11	69.2, CH	69.1, CH	72.0, CH	68.9, CH	68.9, CH	68.9, CH	69.6, CH	69.6, CH	72.2, CH
12	37.3, CH$_2$	37.3, CH$_2$	34.1, CH$_2$	37.2, CH$_2$	37.2, CH$_2$	37.2, CH$_2$	36.5, CH$_2$	36.5, CH$_2$	34.0, CH$_2$
13	121.0, CH	121.0, CH	119.5, CH	120.7, CH	120.7, CH	120.7, CH	120.0, CH	120.1, CH	119.5, CH
14	139.0, C	139.0, C	140.6, C	139.3, C	139.2, C	139.2, C	139.9, C	140.0, C	140.2, C
15	44.0, CH$_2$	44.0, CH$_2$	43.9, CH$_2$	44.0, CH$_2$	44.1, CH$_2$	44.1, CH$_2$	43.8, CH$_2$	44.0, CH$_2$	39.7, CH$_2$
16	26.4, CH$_2$	26.5, CH$_2$	26.6, CH$_2$	26.6, CH$_2$	26.4, CH$_2$	26.6, CH$_2$	26.3, CH$_2$	26.5, CH$_2$	24.8, CH$_2$
17	54.5, CH	54.3, CH	54.3, CH	54.4, CH	54.4, CH	54.4, CH	54.4, CH	54.3, CH	52.7, CH
18	42.1, C	41.7, C	41.8, C	41.8, C	42.1, C	41.8, C	42.0, C	41.8, C	42.0, C
19	89.0, CH	78.3, CH	78.3, CH	78.4, CH	88.9, CH	78.4, CH	88.9, CH	78.4, CH	88.4, CH
20	28.3, CH$_2$	28.7, CH$_2$	29.0, CH$_2$	29.0, CH$_2$	28.3, CH$_2$	29.0, CH$_2$	28.2, CH$_2$	29.0, CH$_2$	32.4, CH$_2$
21	36.3, CH$_2$	36.4, CH$_2$	36.4, CH$_2$	36.4, CH$_2$	36.4, CH$_2$	36.4, CH$_2$	36.2, CH$_2$	36.4, CH$_2$	34.0, CH$_2$
22	88.2, C	88.3, C	88.3, C	88.4, C	88.2, C	88.3, C	88.2, C	88.3, C	149.4, C
23	26.0, CH$_3$	25.9, CH$_3$	26.5, CH$_3$	26.4, CH$_3$	25.3, CH$_3$	25.2, CH$_3$	25.1, CH$_3$	25.1, CH$_3$	26.5, CH$_3$
24	21.6, CH$_3$	21.6, CH$_3$	21.7, CH$_3$	21.6, CH$_3$	22.1, CH$_3$	22.1, CH$_3$	21.8, CH$_3$	21.8, CH$_3$	21.7, CH$_3$
25	25.0, CH$_3$	24.9, CH$_3$	23.9, CH$_3$	24.1, CH$_3$	23.3, CH$_3$	23.3, CH$_3$	23.2, CH$_3$	23.1, CH$_3$	23.9, CH$_3$
26	13.0, CH$_3$	12.9, CH$_3$	13.0, CH$_3$	13.1, CH$_3$	13.1, CH$_3$	13.0, CH$_3$	12.8, CH$_3$	12.9, CH$_3$	13.0, CH$_3$
27	16.7, CH$_3$	16.6, CH$_3$	16.6, CH$_3$	16.7, CH$_3$	16.7, CH$_3$	16.7, CH$_3$	16.6, CH$_3$	16.7, CH$_3$	16.5, CH$_3$
28	28.6, CH$_3$	29.0, CH$_3$	28.8, CH$_3$	28.8, CH$_3$	28.6, CH$_3$	28.8, CH$_3$	28.5, CH$_3$	28.8, CH$_3$	26.7, CH$_3$
29	16.7, CH$_3$	16.0, CH$_3$	16.1, CH$_3$	16.1, CH$_3$	16.7, CH$_3$	16.1, CH$_3$	16.6, CH$_3$	16.0, CH$_3$	17.5, CH$_3$
30	20.5, CH$_3$	20.4, CH$_3$	20.4, CH$_3$	21.2, CH$_3$	20.4, CH$_3$	20.4, CH$_3$	20.5, CH$_3$	20.3, CH$_3$	108.6, CH$_2$
1′	107.3, CH				107.3, CH		107.2, CH		107.3, CH
2′	73.1, CH				73.1, CH		73.1, CH		73.2, CH
3′	75.2, CH				75.2, CH		75.2, CH		75.3, CH
4′	70.3, CH				70.3, CH		70.3, CH		70.3, CH
5′	76.5, CH				76.5, CH		76.4, CH		76.4, CH
6′	62.5, CH$_2$				62.5, CH$_2$		62.5, CH$_2$		62.4, CH$_2$
8-OAc			171.7, C	171.9, C	172.1, C	172.1, C			171.8, C
			21.1, CH$_3$	21.2, CH$_3$	21.3, CH$_3$	21.3, CH$_3$			21.1, CH$_3$
11-OAc			172.1, C						172.2, C
			21.2, CH$_3$						21.2, CH$_3$
22-OAc	172.2, C	172.1, C	172.1, C	172.1, C	172.1, C	172.1, C	172.1, C	172.1, C	
	23.0, CH$_3$	22.9, CH$_3$	23.0, CH$_3$	23.0, CH$_3$	23.0, CH$_3$	23.0, CH$_3$	22.9, CH$_3$	23.0, CH$_3$	

6.35 showed cross-peaks at 8-OH/H-6, H-7a, H-8, H-10, H-24, H-25, and H-26. Among these, the cross-peak at 8-OH/H-6 was crucial for ruling out the alternative 6S* and 8S* configurations (Figure 1). Similarly the NOESY data of the cyclohexane moiety showed conspicuous cross-peaks at H-17/H-19, H-17/H-21β (δ_H 1.77), H-17/H-28, H-19/H-21β, H-19/H-28, H-20α (δ_H 1.57)/H-21α (δ_H 2.48), H-20α/H-29, H-21α/H-30, and H-29/H-30. These 1,3-diaxial-type cross-peaks readily established the 17S*, 19S*, 22S* configurations (Figure 1). The absolute configurations at these centers and the remaining C-11 were determined by a series of Mosher esterification reactions and are discussed below along with those of the other compounds.

The molecular formula of pouogenin A (5) was C$_{32}$H$_{52}$O$_6$ on the basis of HRFABMS data. The ^{1}H and ^{13}C NMR spectra of this compound were similar to those of 4 except, most noticeably, for the absence of signals corresponding to the galactose unit. This interpretation was confirmed by a combination of 2-D NMR experiments and further supported by the shift of the H-19 oxymethine proton (4: δ_C 89.0, δ_H 3.30; 5: δ_C 78.3, δ_H 3.27). Thus, 5 was structurally defined as the aglycone of 4.

A structurally related compound, pouogenin B (6), was isolated as a colorless oil with the molecular formula C$_{36}$H$_{56}$O$_8$ as determined by HRFABMS. As for 5, the absence of a sugar unit was apparent from the NMR data, implying that 6 is a triterpene triacetate. A combination of 2-D NMR data showed that this compound was the aglycone of 1. This conclusion is consistent with noticeable shifts of the H-19 oxymethine proton (1: δ_C 89.0, δ_H 3.30; 6: δ_C 78.3, δ_H 3.27) as well as downfield shifts of the H-8 and H-11 oxymethine protons (5: δ 3.95 and 4.32; 6: δ 4.97 and 5.41 for H-8 and H-11, respectively) from that of 5.

Table 2. ^{1}H MMR (δ, mult (J in Hz)) Assignments for Compounds 4–7 in CD$_3$OD

position	4	5	6	7
3	2.89, ddq (22.5, 4.5, 1.0)	2.89, ddq (22.5, 4.5, 1.0)	2.93, ddq (22.5, 4.5, 1.0)	2.93, ddq (22.5, 4.5, 1.0)
	2.71, ddq (22.5, 3.0, 1.0)	2.72, ddq (22.5, 3.0, 1.0)	2.72, ddq (22.5, 3.0, 1.0)	2.72, ddq (22.5, 3.0, 1.0)
4	5.50, m	5.49, m	5.47, m	5.48, m
6	2.27, dd (9.5, 4.5)	2.27, dd (9.5, 4.5)	2.12, dd (6.5, 5.0)	2.16, dd (8.0, 4.5)
7	1.68, ddd (13.5, 10.0, 4.5)	1.68, ddd (13.5, 10.0, 4.5)	1.88, ddd (14.5, 9.0, 5.0)	1.92, ddd (14.5, 10.0, 4.5)
	1.14, ddd (13.5, 9.5, 3.0)	1.15, ddd (13.5, 9.5, 3.0)	1.42, ddd (14.5, 6.5, 4.5)	1.42, ddd (14.5, 8.0, 3.0)
8	3.95, dd (10.0, 3.0)	3.95, dd (10.0, 3.0)	4.97, dd (9.0, 4.5)	4.96, dd (10.0, 3.0)
10	5.37, br d (9.0)	5.37, br d (9.0)	5.32, br d (9.0)	5.33, br d (9.0)
11	4.32, ddd (9.0, 7.0, 6.5)	4.32, ddd (9.0, 7.0, 6.5)	5.41, ddd (9.0, 7.0, 6.5)	4.30, ddd (9.0, 7.0, 6.5)
12	2.29, ddd (14.0, 7.0, 6.5)	2.29, ddd (14.0, 7.0, 6.5)	2.39, ddd (14.0, 7.0, 6.5)	2.29, ddd (14.0, 7.0, 6.5)
	2.14, ddd (14.0, 7.0, 7.0)	2.15, ddd (14.0, 7.0, 7.0)	2.24, ddd (14.0, 7.0, 7.0)	2.13, ddd (14.0, 7.0, 7.0)
13	5.17, br t (7.0)	5.18, br t (7.0)	5.08, br t (7.0)	5.12, br t (7.0)
15	2.14, m	2.15, m	2.14, ddd (12.5, 12.5, 5.0)	2.15, m
	2.01, ddd (12.5, 12.5, 5.0)	2.01, ddd (12.5, 12.5, 5.0)	2.00, m	1.98, ddd (12.5, 12.5, 5.0)
16	1.55, m	1.55, m	1.51, m	1.56, m
	1.46, m	1.46, m	1.44, m	1.46, m
17	1.60, m	1.57, m	1.56, m	1.57, m
19	3.30, dd (11.5, 4.0)	3.27, dd (11.5, 4.0)	3.27, dd (11.5, 4.0)	3.27, dd (11.5, 4.0)
20	2.06, dddd (13.5, 4.0, 4.0, 4.0)	1.68, dddd (13.5, 4.0, 4.0, 4.0)	1.69, dddd (13.5, 4.0, 4.0, 4.0)	1.69, dddd (13.5, 4.0, 4.0, 4.0)
	1.57, dddd (13.5, 11.5, 11.5, 4.0)	1.53, dddd (13.5, 11.5, 11.5, 4.0)	1.54, dddd (13.5, 11.5, 11.5, 4.0)	1.53, dddd (13.5, 11.5, 11.5, 4.0)
21	2.48, ddd (13.5, 4.0, 4.0)	2.51, ddd (13.5, 4.0, 4.0)	2.52, ddd (13.5, 4.0, 4.0)	2.52, ddd (13.5, 4.0, 4.0)
	1.77, ddd (13.5, 11.5, 4.0)	1.77, ddd (13.5, 11.5, 4.0)	1.76, ddd (13.5, 11.5, 4.0)	1.77, ddd (13.5, 11.5, 4.0)
23	1.19, s	1.19, s	1.21, s	1.21, s
24	1.06, s	1.06, s	1.07, s	1.09, s
25	1.91, d (1.0)	1.91, d (1.0)	1.79, d (1.0)	1.81, d (1.0)
26	1.59, d (1.0)	1.60, d (1.0)	1.66, d (1.0)	1.61, d (1.0)
27	1.64, br s	1.64, br s	1.65, br s	1.63, br s
28	1.10, s	1.02, s	1.02, s	1.02, s
29	0.88, s	0.80, s	0.81, s	0.81, s
30	1.47, s	1.47, s	1.47, s	1.47, s
1′	4.28, d (7.5)			
2′	3.50, dd (7.5, 9.5)			
3′	3.43, dd (9.5, 3.5)			
4′	3.81, dd (3.5, 0.5)			
5′	3.48, dt (0.5, 6.0)			
6′	3.71, d (6.0)			
8-OAc			2.01, s	1.99, s
11-OAc			1.98, s	
22-OAc	1.93, s	1.93, s	1.93, s	1.93, s

The NMR spectroscopic features of another aglycone, pouogenin C (**7**) (C$_{34}$H$_{54}$O$_7$), were similar to those of the other aglycones (Tables 1 and 2). 2-D NMR analyses revealed that this compound possessed the same carbon framework as **5** and **6** with structural differences occurring only in the oxygenated functionalities at C-8, C-11, or C-22. Proton chemical shifts and HMBC correlation analyses placed a hydroxy group at C-11 (δ_H 4.30) and acetoxy groups at C-8 and C-22, respectively. Thus, **7** was structurally defined as the aglycone of pouoside B (**3**), which is consistent with the chemical shifts of the oxymethine group at C-19 (**3**: δ_C 89.0, δ_H 3.30; **7**: δ_C 78.4, δ_H 3.27).

The molecular formula of pouoside G (**8**) was deduced as C$_{40}$H$_{64}$O$_{13}$ by HRFABMS. A detailed examination of its ^{13}C and ^{1}H NMR spectra revealed that this compound is another triterpene galactoside. However, significant differences were found in the carbon and proton signals of the cyclohexenone moiety (C-1−C-6). The carbon signals corresponding to the C-4 trisubstituted double bond were replaced with those of a disubstituted double bond [δ_C 158.0 (CH) and 125.3 (CH)]. A

corresponding difference was also found in the ^{1}H NMR data, in which new signals appeared at δ_H 6.73 (1H, d, J = 10.0 Hz) and 5.80 (1H, d, J = 10.0 Hz). In addition, the C-3 methylene carbon of **1**−**7** disappeared and there was a new C-5 quaternary carbon atom (δ_C 72.0). These changes were accommodated by a migration of the C-4 double bond to C-3 and concomitant attachment of a hydroxy group at C-5. These changes were confirmed by 2-D NMR data including key long-range correlations at H-3/C-1, C-5, H-4/C-2, C-6, and H-25/C-4, C-5, C-6 in the gHMBC data. The relative configurations at C-5 and C-6 were established as 5S* and 6S* on the basis of cross-peaks at H-6/H-8, H-6/H-23 (δ_H 1.21), H-7a (δ_H 1.95)/ H-24, H-7a/H-25, H-7b (δ_H 1.78)/H-23, H-7b/H-24 (δ_H 1.02), H-8/H-25, H-8/H-26, H-23/8-OAc, H-24/H-25, and H-25/H-26 in the NOESY data (Figure 1). Thus the structure of pouoside G (**8**) was that of a 3-ene-5-hydroxy derivative of pouoside B (**2**).

The aglycone of compound **8**, pouogenin D (**9**), was also isolated as a colorless oil with a molecular formula of C$_{34}$H$_{54}$O$_8$. With the exception of signals derived from the galactose
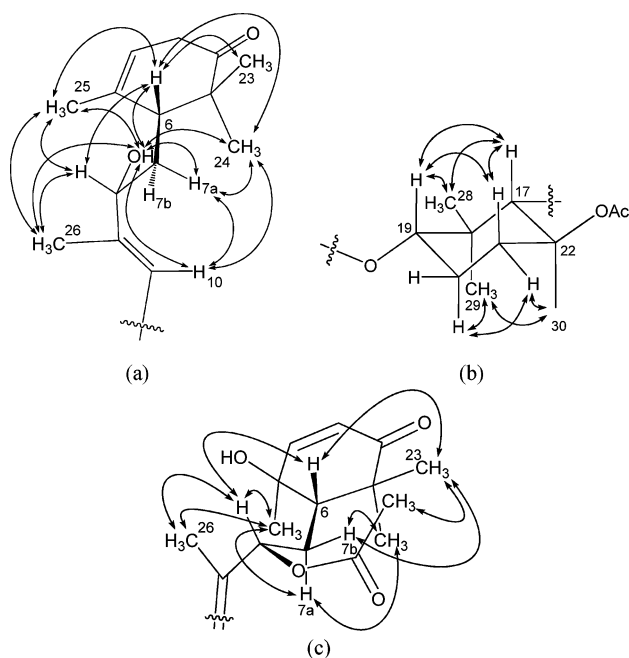
Figure 1. Selected NOESY correlations for compounds **4** (a, b) and **8** (c).

moiety, the carbon and proton NMR data were very similar to those of **8**. All of the proton–proton and proton–carbon correlations observed in the 2-D NMR experiments were also virtually identical to those of **8**, and the removal of the galactose unit from C-19 was further confirmed by characteristic shifts of the methine (**8**: δ_C 88.9, δ_H 3.29, **9**: δ_C 78.4, δ_H 3.27).

The molecular formula of pouoside H (**10**) was established as $C_{38}H_{60}O_{12}$ by HRFABMS. The NMR spectra of this compound were reminiscent of those of other pouosides, and particularly with those of **8**, with a galactose unit and two six-membered rings as the most apparent structural features. However, the ^{13}C NMR data indicated oxidation of a hydroxymethine to a ketone (δ 203.8) that was consistent with the shift [**8**: δ_H 5.45 (1H, dd, J = 9.0, 5.0 Hz); **10**: δ_H 6.57 (1H, br d, J = 8.0 Hz)] of a proton signal in the ^{1}H NMR data. The ketone was placed at C-8 on the basis of long-range correlations at H-6/C-8, H-7/C-8, H-10/C-8, C-26, and H-26/ C-8, C-9, C-10 in the gHMBC data. Thus, the structure of pouoside H (**10**) was determined to be that of an 8-oxo analogue of pouoside G (**8**).

The aglycone of pouoside H (**10**), pouogenin E (**11**), was isolated as a colorless oil with a molecular formula of $C_{32}H_{50}O_7$ based on HR-negative-FABMS analysis. As found for the other triterpene galactosides and corresponding aglycones, the NMR data of this compound were highly reminiscent of those obtained from pouoside H (**10**). On the basis of the results of combined NMR analyses, all of the carbons and protons are in the same locations as in **10** (Tables 2 and 3).

The molecular formula of another triterpene galactoside, pouoside I (**12**), was determined as $C_{40}H_{62}O_{11}$ by mass analysis. The spectroscopic data of this compound were very similar to those of the other triterpene galactosides. However, the ^{13}C NMR spectrum revealed an exo-double bond [δ 149.4 (C), 108.6 (CH_2)] that coincided with the exo-methylene protons [δ 4.84 (1H, br s), 4.55 (1H, br s)] in the ^{1}H NMR data. This group was securely established at C-22 (30) based on long-range correlations H-16/C-22, H-17/C-22, C-30, H-20/

C-22, H-21/C-22, C-30, and H-30/ C-21, C-22 in the gHMBC data. The remainder of the structure, including the galactose moiety, was identical to that of **1** according to combined 2-D NMR analyses. Thus, the structure of pouoside I (**12**) was that of a 22-deacetoxy derivative of pouoside A (**1**).

A comparison of ^{13}C and ^{1}H NMR data revealed nearly identical chemical shifts for both carbons and protons among the core triterpenes of all the compounds **1**−**12** (Tables 1−3). Thus, the results of NOESY experiments indicated the same 5S*, 6R* (6S* for **8**−**11** but the same orientation with others), 8S*, 17S*, 19S*, and 22S* relative configurations at the same locations for these compounds. On the basis of a comparison of chemical shifts, the relative configuration at C-11, which was not determinable by NMR experiments, is also thought to be the same among these compounds.

Absolute configurations were assigned using Mosher's method on selected compounds. First, MTPA esterification of **3** with both (−)- and (+)-MTPA chloride unambiguously established the 11R configuration (Figure 2a). The same reactions with **4** resulted in the formation of bis-MTPA esters at the aglycone. Despite the proximity of the C-8 and C-11 asymmetric centers, the results clearly established 8S and 11R configurations based on differences in proton chemical shifts at H-10 and H-26. These two protons are located between two ester centers, and their chemical shifts were significantly different from those of the **3**-MTPA esters. The chemical shifts of protons at the remaining locations were not significantly shifted (Figure 2b). Finally, a 19S configuration was established at the galactoside position by MTPA esterification of pouogenin C (**7**), and an 11R configuration was again confirmed by this reaction (Figure 2c). Thus, the absolute configurations of the asymmetric centers were unambiguously determined as 6R, 8S, 11R, 17S, 19S, and 22S (except **12**) for **1**−**7** and **12** and 5S, 6S, 8S (except **10** and **11**), 11R, 17S, 19S, and 22S for **8**−**11**.

A previous paper on pouosides A−E reported moderate cytotoxicity of pouoside A toward a P388 cell-line (ED_{50} 1.5 μg/mL).[2] The compounds reported herein exhibited weak to no activity against a K562 human erythroleukemia cell line (IC_{50} 17.0, 12.5, >100, >100, 14.8, 38.9, 14.6, >100, 27.5, >100, 18.9, and >100 μM for **1**−**12**, respectively, n = 3; IC_{50} 13.6 μM for doxorubicin). With the exception of **1** and **2**, the aglycones were far more cytotoxic than the triterpene galactosides. Compounds **5**, **7**, and **11**, for example, showed inhibition properties comparable to those of doxorubicin against this cell line. None of these compounds showed significant antimicrobial activity against selected Gram-positive and Gram-negative bacteria and pathogenic fungi.[10]

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. UV spectra were recorded on a Hitachi U-3010 spectrophotometer, and IR spectra were recorded on a JASCO 300E FT-IR spectrometer. NMR spectra were recorded in MeOH-d_4 containing Me_4Si as an internal standard on Bruker Avance 500 and 600 spectrometers. Proton and carbon NMR spectra were measured at 600 and 150 MHz (**4**, **6**, **8**, **9**, **11**, and **12**) and 500 and 125 MHz (**5**, **7**, and **10**), respectively. 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. Gas chromatography was performed on a Younglin YL6100 Plus

Table 3. ^{1}H MMR (δ, mult (J in Hz)) Assignments for Compounds 8−12 in CD$_3$OD

position	8	9	10	11	12
3	5.80, d (10.0)	5.80, d (10.0)	5.81, d (10.0)	5.82, d (10.0)	2.89, ddq (22.5, 4.5, 1.0)
					2.68, ddq (22.5, 3.0, 1.0)
4	6.73, d (10.0)	6.73, d (10.0)	6.79, d (10.0)	6.77, d (10.0)	5.46, m
6	2.06, dd (7.0, 4.0)	2.06, dd (7.0, 4.0)	2.86, dd (8.0, 3.5)	2.86, dd (8.0, 3.5)	2.11, dd (6.5, 5.0)
7	1.95, ddd (15.0, 7.0, 5.0)	1.94, ddd (15.0, 7.0, 5.0)	3.00, dd (17.5, 8.0)	3.02, dd (17.5, 8.0)	1.87, ddd (14.5, 9.0, 5.0)
	1.78, ddd (15.0, 9.0, 4.0)	1.78, ddd (15.0, 9.0, 4.0)	2.85, dd (17.5, 3.5)	2.84, dd (17.5, 3.5)	1.42, ddd (14.5, 6.5, 4.5)
8	5.45, dd (9.0, 5.0)	5.45, dd (9.0, 5.0)			4.97, dd (9.0, 4.5)
10	5.39, br d (9.0)	5.39, br d (9.0)	6.57, br d (8.0)	6.57, br d (8.0)	5.31, br d (9.0)
11	4.34, ddd (9.0, 7.0, 6.0)	4.34, ddd (9.0, 7.0, 6.0)	4.54, ddd (8.0, 7.5, 6.5)	4.54, ddd (8.0, 7.5, 6.5)	5.39, ddd (9.0, 7.0, 6.5)
12	2.31, ddd (14.0, 7.0, 6.0)	2.30, ddd (14.0, 7.0, 6.0)	2.41, ddd (14.0, 7.5, 6.5)	2.41, ddd (14.0, 7.5, 6.5)	2.38, ddd (14.0, 7.0, 6.5)
	2.16, ddd (14.0, 7.0, 7.0)	2.15, ddd (14.0, 7.0, 7.0)	2.29, ddd (14.0, 7.5, 7.5)	2.28, ddd (14.0, 7.5, 7.5)	2.22, ddd (14.0, 7.0, 7.0)
13	5.15, br t (7.0)	5.16, br t (7.0)	5.19, br t (7.5)	5.19, br t (7.5)	5.03, br t (7.0)
15	2.14, m	2.14, m	2.14, ddd (12.5, 12.5, 5.0)	2.15, ddd (12.5, 12.5, 5.0)	2.04, m
	2.00, m	1.99, m	2.04, ddd (12.5, 12.5, 4.5)	2.02, ddd (12.5, 12.5, 4.5)	1.77, m
16	1.54, m	1.56, m	1.56, dddd (12.5, 12.5, 12.5, 5.0)	1.53, m	1.66, m
	1.47, m	1.46, m	1.44, m	1.44, dddd (12.5, 12.5, 5.0, 3.0)	1.55, m
17	1.61, m	1.57, m	1.59, m	1.55, m	1.67, m
19	3.29, dd (11.5, 4.0)	3.27, dd (11.5, 4.0)	3.28, dd (11.5, 4.0)	3.26, dd (11.5, 4.0)	3.35, dd (9.5, 4.5)
20	2.04, dddd (13.5, 4.0, 4.0, 4.0)	1.68, dddd (13.5, 4.0, 4.0, 4.0)	2.05, dddd (13.5, 4.0, 4.0, 4.0)	1.69, dddd (13.5, 4.0, 4.0, 4.0)	2.02, dddd (12.5, 4.5, 4.5, 4.5)
	1.59, dddd (13.5, 11.5, 11.5, 4.0)	1.53, dddd (13.5, 11.5, 11.5, 4.0)	1.58, dddd (13.5, 11.5, 11.5, 4.0)	1.52, dddd (13.5, 11.5, 11.5, 4.0)	1.64, m
21	2.47, ddd (13.0, 4.0, 4.0)	2.51, ddd (13.0, 4.0, 4.0)	2.48, ddd (13.5, 4.0, 4.0)	2.52, ddd (13.5, 4.0, 4.0)	2.31, ddd (12.5, 4.5, 4.5)
	1.78, ddd (13.0, 11.5, 4.0)	1.76, ddd (13.0, 11.5, 4.0)	1.76, ddd (13.5, 11.5, 4.0)	1.76, ddd (13.5, 11.5, 4.0)	1.95, ddd (12.5, 11.5, 4.5)
23	1.21, s	1.21, s	1.01, s	1.01, s	1.20, s
24	1.02, s	1.02, s	1.06, s	1.06, s	1.07, s
25	1.35, s	1.35, s	1.34, s	1.34, s	1.79, d (1.0)
26	1.71, d (1.0)	1.71, d (1.0)	1.80, d (1.0)	1.79, d (1.0)	1.66, d (1.0)
27	1.65, br s	1.65, br s	1.66, br s	1.66, br s	1.61, br s
28	1.10, s	1.02, s	1.09, s	1.01, s	1.07, s
29	0.89, s	0.81, s	0.88, s	0.80, s	0.79, s
30	1.47, s	1.47, s	1.47, s	1.46, s	4.84, br s
					4.55, br s
1′	4.27, d (7.5)		4.28, d (7.5)		4.27, d (7.5)
2′	3.49, dd (7.5, 9.5)		3.50, dd (7.5, 9.5)		3.51, dd (7.5, 9.5)
3′	3.42, dd (9.5, 3.5)		3.43, dd (9.5, 3.5)		3.43, dd (9.5, 3.5)
4′	3.81, dd (3.5, 0.5)		3.82, dd (3.5, 0.5)		3.82, d (3.5, 0.5)
5′	3.48, dt (0.5, 6.0)		3.48, dt (0.5, 6.0)		3.47, dt (0.5, 6.0)
6′	3.70, d (6.0)		3.71, d (6.0)		3.72, dd (6.0, 11.0)
					3.69, dd (6.0, 11.0)
8-OAc	2.02, s	2.02, s			2.01, s
11-OAc					1.99, s
22-OAc	1.93, s	1.93, s	1.92, s	1.91, s	

chromatograph equipped with an FID. 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 by hand using scuba equipment off the shore of Weno Island, Chuuk state, Federated States of Micronesia, at a depth of 15 m on February 18, 2010. The sponge was globular in shape and beige in alcohol, measured 130 × 170 mm, and was 45 mm thick. This structure was attached to the sea bottom by a few to several stalks up to 5 cm long and 1 cm in diameter. The surface was warty but smooth, and the texture was elastic in alcohol. The consistency was cartilaginous. The skeleton was a network of oxea and strongyle (400−1000 × 3−20 μm). The specimens were similar to those of *Lipastrotethya rhaphoxea* de Laubenfels, 1954, in general morphology except that *L. rhaphoxea* had larger spicules, reaching 5000 × 15 μm, with smaller spicules in the dermis measuring only 50 × 1 μm. A voucher specimen (registry No. Spo. 57) was deposited at

the Natural History Museum, Hannam University, 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 (2 L × 3) and CH$_2$Cl$_2$ (2 L × 3). The combined extracts (112.7 g) were successively partitioned between H$_2$O (99.3 g) and n-BuOH (24.8 g); the latter fraction was repartitioned between H$_2$O−MeOH (15:85) (13.72 g) and n-hexane (7.72 g). An aliquot of the former layer (5.70 g) was separated by C$_{18}$ reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H$_2$O as eluents (six fractions in gradient, H$_2$O−MeOH, from 50:50 to 0:100), acetone, and finally EtOAc.

On the basis of the results of ^{1}H NMR and cytotoxicity analyses, the fractions eluted with H$_2$O−MeOH (30:70) (0.33 g), H$_2$O−MeOH (20:80) (0.74 g), and H$_2$O−MeOH (10:90) (1.10 g) were chosen for separation. The fraction eluted with H$_2$O−MeOH (30:70) was
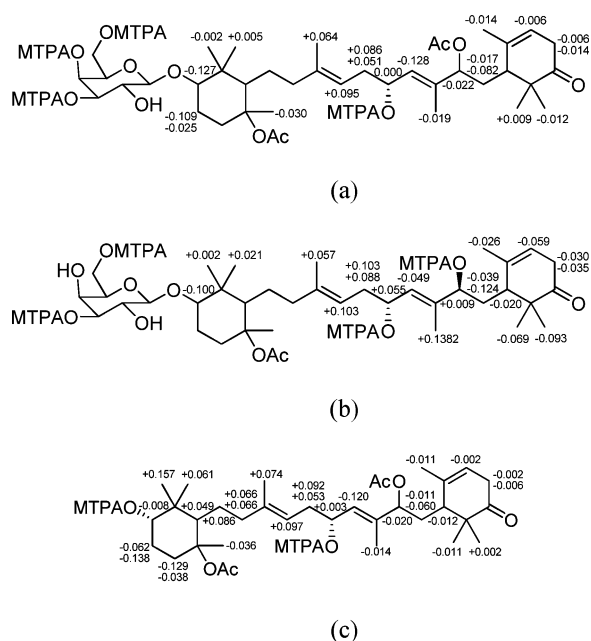
Figure 2. Results of MTPA esterification for compound **3**, **4**, and **7** (a−c).

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) provided compound **10** as a colorless oil, while purification of the second peak by reversed-phase HPLC (H_2O−MeOH, 60:40) yielded compound **11**.

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 **4**, **8**, **3**, **5**, and **9**. These metabolites were then purified by reversed-phase HPLC (YMC-Pack CN column; H_2O−MeOH, 65:35, for **4** and **8**, and H_2O−MeOH, 60:40, for **3**, **5**, and **9**, respectively).

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 **7**, **1**, **2**, **6**, and **12**. These metabolites were then purified by HPLC (YMC-Pack CN column; H_2O−MeOH, 50:50, for **1** and **7**, H_2O−MeCN, 62:38, for **2**, and H_2O−MeOH, 65:35, for **6** and **12**, respectively). The purified metabolites were isolated in the following amounts: 48.0, 3.7, 72.4, 24.6, 17.0, 3.8, 20.2, 16.2, 17.8, 3.8, 4.6, and 3.5 mg of **1**−**12**, respectively.

Pouoside A (1): colorless oil; $[\alpha]_D^{25}$ −26.2 (c 0.50, MeOH).

Pouoside D (2): colorless oil; $[\alpha]_D^{25}$ −25.8 (c 0.48, MeOH).

Pouoside B (3): colorless oil; $[\alpha]_D^{25}$ −40.8 (c 0.65, MeOH).

Pouoside F (4): colorless oil; $[\alpha]_D^{25}$ −29.0 (c 0.35, MeOH); IR (ZnSe) ν_{max} 3400 (br), 1730, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 717.4194 [M + Na]$^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190).

Pouogenin A (5): colorless oil; $[\alpha]_D^{25}$ −49.5 (c 0.40, MeOH); IR (ZnSe) ν_{max} 3420 (br), 1730, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 555.3663 [M + Na]$^+$ (calcd for $C_{32}H_{52}O_6Na$, 555.3662).

Pouogenin B (6): colorless oil; $[\alpha]_D^{25}$ −24.0 (c 0.40, MeOH); IR (ZnSe) ν_{max} 3400 (br), 1733, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 639.3876 [M + Na]$^+$ (calcd for $C_{36}H_{56}O_8Na$, 639.3873).

Pouogenin C (7): colorless oil; $[\alpha]_D^{25}$ −26.3 (c 0.60, MeOH); IR (ZnSe) ν_{max} 3450 (br), 1730, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 597.3771 [M + Na]$^+$ (calcd for $C_{34}H_{54}O_7Na$, 597.3767).

Pouoside G (8): colorless oil; $[\alpha]_D^{25}$ −13.4 (c 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.42), 273 (1.45) nm; IR (ZnSe) ν_{max} 3400 (br), 1729, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 775.4247 [M + Na]$^+$ (calcd for $C_{40}H_{64}O_{13}Na$, 775.4245).

Pouogenin D (9): colorless oil; $[\alpha]_D^{25}$ −25.9 (c 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.41), 275 (1.47) nm; IR (ZnSe) ν_{max} 3430, 1730, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 613.3719 [M + Na]$^+$ (calcd for $C_{34}H_{54}O_8Na$, 613.3716).

Pouoside H (10): colorless oil; $[\alpha]_D^{25}$ −11.5 (c 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.43), 275 (1.47) nm; IR (ZnSe) ν_{max} 3400 (br), 1728, 1711 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 731.3987 [M + Na]$^+$ (calcd for $C_{38}H_{60}O_{12}Na$, 731.3982).

Pouogenin E (11): colorless oil; $[\alpha]_D^{25}$ −5.0 (c 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.43), 2.73 (1.46) nm; IR (ZnSe) ν_{max} 3420 (br), 1729, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 545.3484 [M − H]$^-$ (calcd for $C_{32}H_{49}O_7$, 545.3478).

Pouoside I (12): colorless oil; $[\alpha]_D^{25}$ −11.5 (c 0.35, MeOH); IR (ZnSe) ν_{max} 3430 (br), 1732, 1710 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS m/z 741.4195 [M + Na]$^+$ (calcd for $C_{40}H_{62}O_{11}Na$, 741.4190).

Esterification with (−)-(R)-α-Methyoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) Chloride. Esterifications of compounds **3**, **4**, and **7** were carried out as follows. A total of 20 μL of (−)-(R)-MTPA chloride was added to a solution containing 1.1−2.0 mg of the alcoholic compound in 100 μL of dry pyridine. The mixture was allowed to stand under N_2 at room temperature for 2 h. After the consumption of starting material was confirmed by thin-layer chromatography, 0.5 mL of H_2O−MeOH (10:90) was added. The solvents were removed under vacuum, and the residue was separated by reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O−MeOH, 10:90) to give 1.0−2.1 mg of (S)-MTPA esters (**3a**, **4a**, and **7a**). The corresponding (R)-MTPA esters (**3b**, **4b**, and **7b**) were also obtained from the same esterification reactions with (+)-(S)-MTPA chloride. Selected ^{1}H NMR (CD$_3$OD, 500 MHz) of **3a**: δ_H 5.675 (1 H, ddd, J = 9.0, 7.0, and 7.0 Hz, H-11), 5.279 (1 H, d, J = 9.0 Hz, H-10), 5.150 (1 H, dd, J = 7.0 and 7.0 Hz, H-13), 4.935 (1 H, dd, J = 9.5 and 3.5 Hz, H-8), 2.488 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.337 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.112 (1 H, m, H-6), 1.881 (1 H, m, H-7), 1.728 (3 H, s, H-26), 1.635 (3 H, s, H-27), and 1.366 (1 H, m, H-7); LR-ESIMS m/z 1624.7 [M + Na]$^+$ (calcd for $C_{80}H_{92}F_{12}O_{20}Na$, 1624.6). Selected ^{1}H NMR (CD$_3$OD, 500 MHz) of **3b**: δ_H 5.675 (1 H, ddd, J = 9.0, 6.5, and 6.5 Hz, H-11), 5.407 (1 H, d, J = 9.0 Hz, H-10), 5.0554 (1 H, dd, J = 7.0 and 7.0 Hz, H-13), 4.958 (1 H, dd, J = 9.5 and 3.4 Hz, H-8), 2.403 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.285 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.140 (1 H, dd, J = 6.5 and 5.0 Hz, H-6), 1.899 (1 H, m, H-7), 1.747 (3 H, s, H-26), 1.571 (3 H, s, H-27), and 1.447 (1 H, m, H-7); LR-ESIMS m/z 1624.7 [M + Na]$^+$ (calcd for $C_{80}H_{92}F_{12}O_{20}Na$, 1624.6). Selected ^{1}H NMR (CD$_3$OD, 500 MHz) of **4a**: δ_H 5.719 (1 H, ddd, J = 9.0, 6.0, and 6.0 Hz, H-11), 5.432 (1 H, d, J = 9.0 Hz, H-10), 5.422 (1 H, br s, H-4), 5.270 (1 H, dd, J = 9.0 and 4.0 Hz, H-8), 5.176 (1 H, dd, J = 7.6 and 7.6 Hz, H-13), 2.906 (1 H, m, H-3), 2.681 (1 H, m, H-3), 2.494 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.371 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.278 (1 H, m, H-6), 1.871 (1 H, m, H-7), 1.748 (3 H, s, H-25), 1.748 (3 H, s, H-26), 1.630 (3 H, s, H-27), 1.338 (1 H, m, H-7), 1.067 (3 H, s, H-23), and 0.925 (3 H, s, H-24); LR-ESIMS m/z 1582.4 [M + Na]$^+$ (calcd for $C_{78}H_{90}F_{12}O_{19}Na$, 1582.6). Selected ^{1}H NMR (CD$_3$OD) of **4b**: δ_H 5.664 (1 H, ddd, J = 9.0, 6.0, and 6.0), 5.480 (1 H, br s, H-4), 5.480 (1 H, d, J = 9.0 Hz, H-10), 5.261 (1 H, dd, J = 8.5 and 4.5 Hz, H-8), 5.073 (1 H, dd, J = 7.0 and 7.0 Hz, H-13), 2.936 (1 H, m, H-3), 2.716 (1 H, m, H-3), 2.391 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.298 (1 H, m, H-6), 2.282 (1 H, m, H-12), 1.910 (1 H, m, H-7), 1.774 (3 H, s, H-25), 1.610 (3 H, s, H-26), 1.573 (3 H, s, H-27), 1.462 (1 H, m, H-7), 1.136 (3 H, s, H-23), and 1.018 (3 H, s, H-24); LR-ESIMS m/z 1582.4 [M + Na]$^+$ (calcd for $C_{78}H_{90}F_{12}O_{19}Na$, 1582.6). Selected ^{1}H NMR (CD$_3$OD, 500 MHz) of

7a: δ_H 4.775 (1 H, m, H-19), 2.442 (1 H, m, H-21), 1.895 (1 H, m, H-20), 1.843 (1 H, m, H-21), 1.743 (1 H, m, H-17), 1.582 (1 H, m, H-20), 1.472 (2 H, m, H-16), 1.458 (3 H, s, H-30), 0.983 (3 H, s, H-28), and 0.860 (3 H, s, H-29); LR-ESIMS m/z 1030.4 $[M + Na]^+$ (calcd for $C_{54}H_{68}F_6O_{11}Na$, 1030.5). Selected ^1H NMR (CD$_3$OD, 500 MHz) of **7b**: δ_H 4.784 (1 H, m, H-19), 2.571 (1 H, br s, H-21), 1.957 (1 H, m, H-20), 1.881 (1 H, m, H-21), 1.720 (1 H, m, H-20), 1.694 (1 H, m, H-17), 1.493 (3 H, s, H-30), 1.387 (2 H, m, H-16), 0.826 (3 H, s, H-28), and 0.799 (3 H, s, H-29); LR-ESIMS m/z 1030.4 $[M + Na]^+$ (calcd for $C_{54}H_{68}F_6O_{11}Na$, 1030.5).

Acid Hydrolysis and GC Analysis of Compound 4. A solution of **4** (4.0 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 redissolved in a solution of L-cysteine methyl ester hydrochloride (2.3 mg) in pyridine (0.5 mL), and the solution was stirred at 60 °C for 1.5 h. After being cooled and concentrated, the mixture was treated with hexamethyldi-silazane (HMDS) in 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$_2$O and CH$_2$Cl$_2$ (1 mL, v/v = 1:1). The CH$_2$Cl$_2$ 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. A peak corresponding to a hydrolysate sugar derivative was detected at 31.89 min. Retention times for authentic samples after being treated simultaneously with HMDS/TMSCl were 31.87 (D-galactose) and 30.58 min (L-galactose), respectively. Co-injection of the hydrolysate with the authentic silylated D-galactose gave single peaks at 31.90 min.

Biological Assays. Cytotoxicity assays were performed in accordance with the protocols in refs 11 and 12.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra of compounds **4−12** are available free of charge via the Internet at http://pubs.acs.org.

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