The Agminosides: Naturally Acetylated Glycolipids from the New Zealand Marine Sponge *Raspailia agminata*

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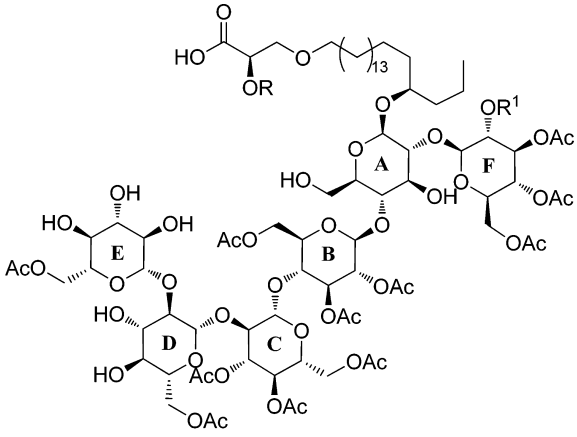
Examination of the New Zealand sponge *Raspailia agminata* resulted in the isolation of five members of a novel family of glycolipids, agminosides A−E (**1**−**5**). These large and complex molecules contain up to six partially acetylated glucose residues. The chromatographic separation of these compounds was a challenge due to the similarity of the congeners and their lack of a chromophore. MS-guided isolation followed by extensive NMR analysis and chemical derivatization eventually led to the purification and identification of **1**−**5**.

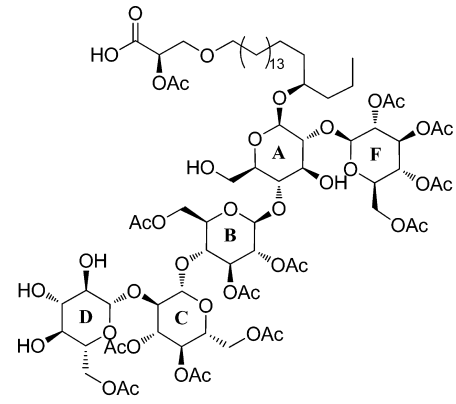
Among sponge metabolites, glycolipids often display a fairly conserved pattern of sphingoid or ceramide aglycon and one or two sugars. In recent times, several sponge glycolipids have been isolated that show unusual features. These include such complex molecules as axinelloside A, a highly sulfated lipopolysaccharide with multiple lipids attached to 12 sugars.[1] Although acetylation is a common technique used to aid isolation and structure elucidation of glycolipids,[2] natural acylation of sugars remains a rarity and to our knowledge is restricted to caminosides A−D,[3,4] pachymoside,[5] and erylusamine.[6,7] In this paper we report the isolation and structure elucidation of a new group of complex, partially acetylated glycolipids from the New Zealand sponge *Raspailia agminata*.



1 R = Ac R^1 = Ac
2 R = Ac R^1 = H
3 R = H R^1 = Ac
4 R = H R^1 = H

Results and Discussion

A sample of *R. agminata*, collected off the coast of Northland, New Zealand, was extracted with MeOH, and the extracts were partitioned on reversed-phase polystyrene divinyl benzene resin with mixtures of acetone and water. The 60% acetone fraction was further purified by size exclusion chromatography to yield a mixture of related glycolipids. Due to their similarity, the congeners were difficult to distinguish by TLC, and the absence of a chromophore precluded the use of a UV detector for HPLC. Therefore, fractions from flash chromatography or HPLC were subjected to MS analysis



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and were pooled on the basis of molecular ions observed. Final separation was achieved by repetitive chromatography on silica gel (guided by MS) using CH_2Cl_2/MeOH/H_2O mixtures to give the agminosides A−E (**1**−**5**) as colorless glasses.

The positive-ion HRMALDI-TOF spectrum of **1** showed a $[M + Na]^+$ pseudomolecular ion peak at 1943.7707, consistent with a molecular formula of $C_{85}H_{132}O_{48}$ requiring 20 degrees of unsaturation. Analysis of the ^1H, ^{13}C, and HSQC spectra revealed the presence of six hemiacetals, as well as 26 oxymethines and eight oxymethylenes, sufficient for six hexopyranose sugar moieties. The presence of a series of aliphatic methylene resonances between δ_H 1.3 and 1.6 (including a large unresolved CH_2 envelope at δ_H 1.29 typical of a long saturated alkyl chain) was suggestive of a linear hydrocarbon chain, indicating agminoside A (**1**) was a glycolipid. Additionally, 13 acetate methyls and carbonyl carbons were observed, indicating the presence of natural acetylation.

From the proton of each of the hemiacetals, HSQC-TOCSY correlations clearly identified four oxymethines and an oxymethylene belonging to each of the six sugar spin systems. The assignment of their positions was established through COSY and HMBC correlations. On the basis of large (7−9 Hz) axial−axial coupling constants between neighboring protons and several 1−3 diaxial NOE correlations, all sugars were established as β-glucose.

Seven of the oxymethines (δ_H 5.27, 5.18, 5.08, 5.00, 4.88, 4.86, 4.81) and five of the oxymethylenes (δ_H 4.31/4.81, 4.07/4.33, 4.24/4.51, 4.14/4.78, 4.11/4.30) had proton chemical shifts 1−2 ppm downfield from normal. These data, together with the HMBC correlations from the oxymethylene and oxymethine protons to acetate carbonyls, indicated acetylation at these positions and accounted for 12 of the 13 acetates present in the molecule. The six saccharide units are 6-*O*-acetyl-glucose (rings A, D, and E), 2,3,6-tri-*O*-acetyl-glucose (ring B), 3,4,6-tri-*O*-acetyl-glucose (ring C), and 2,3,4,6-tetra-*O*-acetyl-glucose (ring F).

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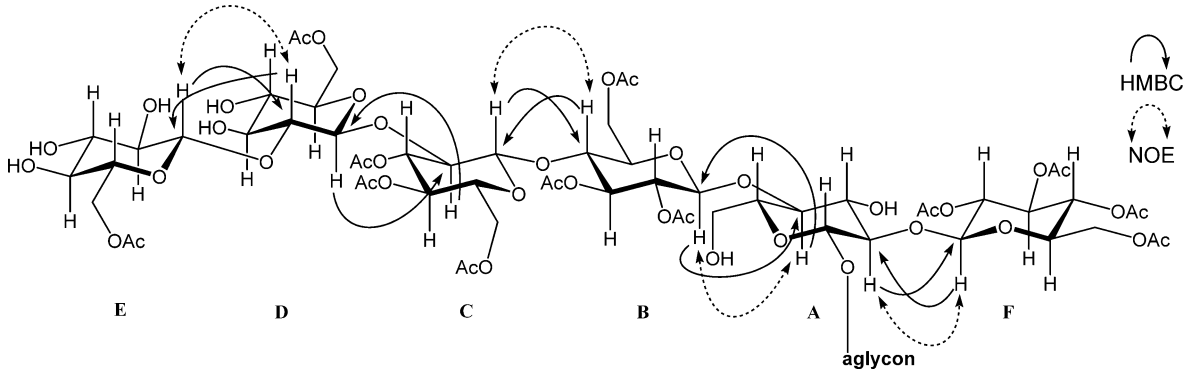

Figure 1. Key HMBC and NOE correlations establishing the linkages between the glucose sugars of agminoside A (**1**).

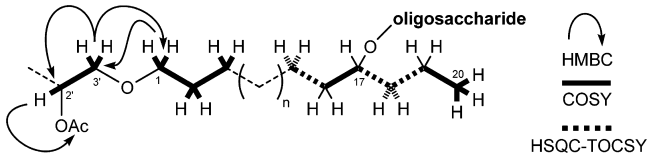

Figure 2. Key correlations establishing the aglycon substructure.

Six of the oxymethines showed lower field carbon chemical shifts (δ_C 83.8, 82.4, 81.7, 81.1, 79.8, 79.6), characteristic of carbons in a glycosidic bond. The sugar linkages were established through key HMBC and NOE correlations (depicted in Figure 1). Reciprocal HMBC correlations were observed between positions A2 and F1 (H-A2 to C-F1 and H-F1 to C-A2), B1 and A4, C1 and B4, D1 and C2, and E1 and D2, implying the linkages between the carbohydrate monomers. The interglycosidic linkages were further confirmed by NOE cross-peaks between H-B1 and H-A4, H-C1 and H-B4, H-E1 and H-D2, and H-F1 and H-A2. The anomeric proton doublets all had large coupling constants (7.8−8.2 Hz), consistent with β-glycosidic linkages. $^1J_{CH}$ coupling constants of about 160 Hz further supported the axial positioning of the anomeric protons.[8]

The remaining two oxymethylenes, an acetylated oxymethine, another oxymethine, an acetate, a methyl triplet, and a series of aliphatic methylenes were attributed to the aglycon. Starting from a terminal methyl, COSY and HSQC-TOCSY correlations allowed the construction of a linear six carbon chain, CH_3-20 to CH_2-15, including an oxymethine at CH-17. CH_2-15 showed correlations to the unresolved CH_2 envelope. Oxymethylene CH_2-1 showed COSY correlations to CH_2-2, which in turn showed both COSY and TOCSY correlations to methylene CH_2-3. CH_2-3 also correlated to the CH_2 envelope, establishing a continuous linear chain. The integration of the CH_2 envelope suggested a further 11 methylenes.

Oxymethine CH-2′ (δ_C 74.3, δ_H 5.14) showed a correlation to the last remaining oxymethylene CH_2-3′ as an isolated spin system in the COSY spectrum. The ^{1}H chemical shift of H-2′ was once again indicative of acetylation, and indeed, a correlation from H-2′ to the acetate carbonyl δ_C 172.4 was observed in the HMBC spectrum and from the acetate methyl to the same carbonyl. This final fragment was connected to the rest of the aglycon through observations of reciprocal HMBC correlations between centers C-1 and C-3′, establishing an ether linkage between these two parts (Figure 2).

This substructure accounted for all the remaining visible signals in the NMR spectra. One further carbon, two oxygens, a hydrogen, and a double-bond equivalent from the molecular formula remained unaccounted for and were not readily apparent in the NMR spectra, and so the molecule was subjected to chemical derivatization reactions. Methanolysis of **1** with HCl and MeOH yielded the aglycon methyl ester (**6**) and the methyl glycoside of glucose. A similar reaction on a mixture of the glycolipids also yielded compound **6**, indicating the glycolipids possessed only one type of aglycon. Compound **6** showed a $[M + Na]^+$ pseudomolecular ion at 439.3381 in positive-ion mode HRESIMS, indicating a molecular formula of $C_{24}H_{48}O_5$.

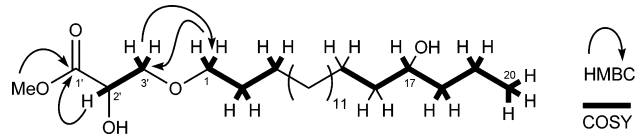

Figure 3. Key COSY and HMBC correlations establishing the structure of the aglycon methyl ester (**6**).

Analysis of the ^{1}H and ^{13}C NMR spectra revealed the presence of a carbonyl carbon (which accounted for the one double-bond equivalent indicated by the molecular formula), two oxymethines, two oxymethylenes, a series of methylenes, a methyl triplet, and a new *O*-methyl resonance. Three spin systems were constructed through COSY correlations as for the aglycon above and were linked together through HMBC correlations (see Figure 3). The salient feature was the presence of a new OMe moiety (δ_C 52.7, δ_H 3.79), which displayed an HMBC correlation to an ester carbonyl at δ_C 173.3. The oxymethine CH-2′, now resonating slightly upfield at δ_H 4.30 as it had lost the acetate during the methanolysis, also correlated to the carbonyl. This established a methyl ester as the other terminus of compound **6**. As the OMe resonance was not observed in the original NMR spectra of the natural product and is a product of the methanolysis, the original functional group present in the aglycon was established as a carboxylic acid. The carboxylic acid carbon resonance may not have been apparent in the NMR spectra of **1** due to overlap with one of the 13 acetate carbonyl resonances.

The carboxylic acid moiety was unequivocally proven through methylation of the mixture of the glycolipids with TMS-diazomethane. NMR analysis of the resulting product mixture revealed the presence of a new signal at δ_C 51.6 and δ_H 3.74. A correlation from δ_H 3.74 to C-1′ was observed in the HMBC spectrum, indicating the successful creation of a methyl ester. The connectivity between the aglycon and the oligosaccharide was established through observation of an HMBC correlation from the anomeric proton of glucose A (H-A1) to C-17 of the aglycon and was confirmed by an NOE enhancement between H-17 and H-A1.

As the aglycon possessed two secondary alcohols, Mosher's method was used to assign their absolute configuration. Two portions of **6** were esterified with (*R*)- and (*S*)-MTPA chloride, respectively. The reactions did not go to completion (resulting in a mixture of the two monoesters as well as the diester and starting material), but as the two stereogenic centers are quite remote and therefore have little effect on each other, they were assigned fully by NMR without further purification. The difference between the chemical shifts of the (*S*)- and the (*R*)-MTPA esters **6b** and **6a** was calculated (see Figure 4), yielding the absolute configuration of the aglycon as 17*R*, 2′*R*. The aglycon is therefore assigned as (*R*)-2-hydroxy-3-(((*R*)-17-hydroxyicosyl)oxy)propanoic acid.

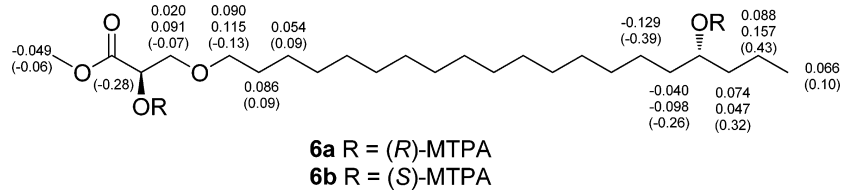

Figure 4. $\Delta\delta_H$ ($\delta_S - \delta_R$) values (in ppm) for (*S*)- and (*R*)-MTPA esters **6b** and **6a**. $\Delta\delta_C$ values are given in parentheses.

A mixture of the glycolipids was subjected to acid hydrolysis, and the hydrolyzed sugars were subjected to reductive amination with (*S*)-1-amino-2-propanol.[9] GC analysis of the resulting alditol product and comparison with a D-glucose standard derivatized in a similar fashion established that the sugars were indeed D-glucose. Therefore the structure (and absolute configuration) of agminoside A (**1**) is as drawn.

During the MS-guided purification of agminoside A (**1**), multiple molecular ions varying by combinations of 42 (an acetate) and 162 (a sugar) mass units were observed, indicative of a series of related congeners. As degradation experiments on the glycolipid mixture resulted in the generation of only one type of aglycon and only glucose sugars, the congeners could only differ in the number and position of acetates, number of sugars, and potentially the sugar linkages. Further MS-guided chromatographic separation on silica gel resulted in the isolation of agminosides B−E (**2**−**5**). Their structures were elucidated in a similar fashion to **1** (see Supporting Information for full NMR data).

Negative-ion mode HRESIMS analysis of agminoside B (**2**) (1877.7734 [M − H]$^-$) indicated a molecular formula of $C_{83}H_{130}O_{47}$. The loss of 42 Da implied a loss of one acetate with respect to agminoside A. Indeed, only 12 acetate signals were observed in the ^{1}H and ^{13}C NMR spectra. The chemical shifts of the aglycon and most of the sugar positions were within 0.02 ppm of those for agminoside A. Significant differences were observed for glucose F, the largest being at H-F2 (δ_H 3.43 vs δ_H 4.86 in **1**). This indicated the loss of the acetate at that position and explained the changes in chemical shift for that sugar. Indeed, the HMBC correlation from H-F2 to an acetate carbonyl was absent, while all other correlations remained essentially the same as in **1**.

Agminoside C (**3**) also had a molecular formula of $C_{83}H_{130}O_{47}$ and only 12 acetate signals in the NMR spectra. However, it had a different retention time on the HPLC, implying it was an isomer of agminoside B (**2**). Close examination of the NMR data revealed that the sugar portion of **3** was identical (within 0.02 ppm) to that of agminoside A (**1**). The major difference was the absence of the signals characteristic of an acetate at oxymethine CH-2′ of the aglycon. A resonance at δ_H 4.34 was present instead, indicating the acetate was replaced with a hydroxy group.

A pseudomolecular ion peak observed in negative-ion mode HRESIMS for agminoside D (**4**) indicated a molecular formula of $C_{81}H_{128}O_{46}$ (1835.7599 [M − H]$^-$), suggesting a loss of two acetates compared to agminoside A. Only 11 acetate signals were observed in the NMR spectra, supporting this hypothesis. Changes in chemical shift similar to those of **2** and **3** indicated a loss of the F2 and the 2′-acetates.

The final glycolipid, agminoside E (**5**), displayed a pseudomolecular ion peak of 1715.7176 in negative-ion mode HRESIMS. This indicated a formula of $C_{77}H_{120}O_{42}$, consistent with a loss of one sugar and one acetate. Observation of only five anomeric signals and 12 acetates in the NMR spectra supported this assumption. Again, the majority of signals remained similar to that of agminoside A, although signals characteristic for glucose E were totally absent and some differences were apparent for glucose D. The most distinct change was in the chemical shift of the carbon at position D2. In agminoside A (**1**) C-D2 is at δ_C 83.8, being a glycosidic-linked carbon, while in **5** it is at δ_C 74.7, consistent with the loss of glucose E. The absence of glucose E also accounts for the loss of one acetate (E6-Ac); the remaining acetate pattern is identical to agminoside A.

The agminosides are unusual compared to most other glycolipids isolated from sponges in that they possess only one type of aglycon, whereas sponge glycolipid aglycons are often complex mixtures of homologues differing only in the length and branching of the alkyl chain (e.g., ref 10). In addition, few examples of natural acetylation have been reported. Acetylation is in fact often used to facilitate isolation of glycolipids, as this reduces their amphiphilic nature, resulting in better chromatographic separation. In the case of the agminosides, peracetylation would have resulted in the loss of vital information about acetylation position necessary to distinguish the congeners present in the mixture. The agminoside congeners reported here differ only in the level of acetylation and sugar number. Being so similar, their separation was achieved only after repetitive normal-phase chromatograpy, which was made more difficult by the lack of a chromophore for HPLC monitoring; thus mass spectrometry of resulting fractions was crucial to the final purification of the agminosides.

Experimental Section

General Experimental Procedures. Optical rotations were performed on a Perkin-Elmer 241 polarimeter. NMR spectra were obtained at 600 MHz for ^{1}H and 150 MHz for ^{13}C; ^{1}H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak.[11] Normal-phase HPLC was performed using Rainin Instrument Microsorb-MV analytical (4.6 × 250 mm, 5 μm) or Dynamax semipreparative (10 × 250 mm, 8 μm) silica columns. GC analysis was performed with an HP5 column (30 m × 0.32 nm, 0.25 μm film thickness) with N_2 as the carrier gas (1 mL/min) and a head pressure of 12 psi. The oven temperature program was as follows: start at 180 °C (1 min hold), then increase 2 °C/min to 220 °C (2 min hold), then 4 °C/min to 250 °C (5 min hold). Normal-phase flash chromatography was carried out using Kieselgel 60 (230−400 mesh ASTM) silica gel. Size exclusion chromatography was performed using Sephadex LH-20. Reversed-phase chromatography was carried out using Supelco Diaion HP20 and HP20SS PSDVB chromatographic resin.

Animal Material. The sponge *Raspailia agminata* was collected by hand using scuba from Taheke Reef, Cavalli Islands, New Zealand (34°57.8′ S, 173°59.0′ E at a depth of 10−15 m) in December 2003. The sample was frozen immediately and kept at −18 °C until extraction. A voucher specimen (PTN2_79D) has been deposited at the School of Chemical and Physical Sciences, Victoria University of Wellington, New Zealand.

Extraction and Isolation. The sponge (251 g wet weight) was macerated and extracted twice overnight with MeOH (900 mL). The second, then the first extract were passed through a column of HP20 (200 mL, 5.5 × 8.5 cm). The eluent was diluted with H_2O (2 L) and passed through the column. The resulting eluent was further diluted with H_2O (4 L) and passed through the column again. The column was then washed with H_2O (600 mL) and eluted with 600 mL fractions of (1) 20% Me_2CO/H_2O, (2) 40% Me_2CO/H_2O, (3) 60% Me_2CO/H_2O, (4) 80% Me_2CO/H_2O, and (5) Me_2CO. Fraction 3 yielded 450 mg of material, which was chromatographed in three portions on LH20 (2 × 67 cm) in MeOH. Early eluting fractions were combined to afford a mixture of glycolipids (344 mg). Portions of this were repeatedly chromatographed on benchtop and HPLC silica columns using CH_2Cl_2/MeOH/H_2O mixtures (such as 100:10:1) to yield agminoside A (**1**) (11.0 mg), agminoside B (**2**) (2.5 mg), agminoside C (**3**) (1.3 mg), agminoside D (**4**) (3.1 mg), and agminoside E (**5**) (0.5 mg).

Table 1. NMR Data (600 MHz for ^{1}H, 150 MHz for ^{13}C, CD_3OD) for Agminoside A (**1**)

	δ_C		δ_H		δ_C		δ_H
pos.	(ppm)	mult	(ppm) mult, *J* (Hz)	pos.	(ppm)	mult	(ppm) mult, *J* (Hz)
			Aglycon				Glucose C
1a	72.8	CH_2	3.54, m	C1	103.6	CH	4.53, d (7.8)
1b			3.47, m	C2	79.8	CH	3.50, t (8.4)
2	30.7	CH_2	1.56, m	C3	76.5	CH	5.08, t (9.5)
3	27.2	CH_2	1.34, m	C4	69.9	CH	4.81, t (10.0)
4−14	30.7	CH_2	1.29, m	C5	72.5	CH	3.90, ddd (10.0, 3.6, 2.3)
15	26.2	CH_2	1.37, m	C6a	63.1	CH_2	4.33, dd (12.6, 4.2)
16a	35.9	CH_2	1.57, m	C6b			4.07, d (12.0)
16b			1.49, m	C3-Ac	171.2[c]	C	
17	81.7	CH	3.63, m		21.3	CH_3	1.99, s
18	37.2	CH_2	1.48, m	C4-Ac	171.5	C	
19	18.9	CH_2	1.48, m		20.8[b]	CH_3	1.93, s
20	14.8	CH_3	0.92, t (6.9)	C6-Ac	172.2[e]	C	
3′a	70.9	CH_2	3.83, dd (11.2, 5.6)		20.6	CH_3	2.04, s
3′b			3.78, m				Glucose D
2′	74.3	CH	5.14, dd (5.9, 2.6)	D1	103.1	CH	4.46, d (7.8)
1′	[a]	C		D2	83.8	CH	3.22, m
2′-Ac	172.4	C		D3	77.9	CH	3.49, t (7.9)
	20.8[b]	CH_3	2.13, s	D4	71.5	CH	3.23, m
			Glucose A	D5	75.3	CH	3.44, ddd (9.4, 7.0, 1.5)
A1	101.9	CH	4.39, d (7.8)	D6a	64.4	CH_2	4.51, dd (11.0, 7.5)
A2	81.1	CH	3.46, t (8.3)	D6b			4.24, dd (10.3, 1.6)
A3	77.3	CH	3.58, m	D6-Ac	173.0	C	
A4	82.4	CH	3.53, m		21.4	CH_3	2.19,[f] s
A5	75.7	CH	3.32, m				Glucose E
A6a	61.6	CH_2	3.76, d (11.9)	E1	106.1	CH	4.40, d (7.9)
A6b			3.62, dd (11.9, 3.8)	E2	75.2	CH	3.19, t (8.1)
			Glucose B	E3	78.2	CH	3.34, t (8.6)
B1	102.0	CH	4.81, d (8.2)	E4	70.7	CH	3.46, t (9.7)
B2	73.0	CH	4.88, t (8.2)	E5	75.6	CH	3.36, m
B3	74.5	CH	5.27, t (9.4)	E6a	63.5	CH_2	4.78, m
B4	79.6	CH	3.78, t (9.4)	E6b			4.14, m
B5	74.2	CH	3.94, ddd (9.6, 7.2, 1.8)	E6-Ac	173.1	C	
B6a	64.6	CH_2	4.80, m		21.6	CH_3	2.19,[f] s
B6b			4.31, m				Glucose F
B2-Ac	171.2[c]	C		F1	102.1	CH	5.07, d (7.9)
	20.7	CH_3	2.06, s	F2	73.3	CH	4.86, dd (9.8, 7.9)
B3-Ac	171.7	C		F3	74.2	CH	5.18, t (9.4)
	20.8[b]	CH_3	2.01,[d] s	F4	69.8	CH	5.00, t (9.7)
B6-Ac	172.5	C		F5	72.7	CH	3.78, ddd (9.6, 4.1, 2.7)
	21.2	CH_3	2.21, s	F6a	63.2	CH_2	4.30, m
				F6b			4.11, m
				F2-Ac	171.3	C	
					20.6	CH_3	2.00, s
				F3-Ac	171.6	C	
					20.5	CH_3	1.98, s
				F4-Ac	171.2[c]	C	
					20.9	CH_3	2.01,[d] s
				F6-Ac	172.2[e]	C	
					20.8[b]	CH_3	2.05, s

[a] Not observed. [b−f] Entries with the same letter can be interchanged.

Agminoside A (1): clear glass; $[\alpha]^{20}_D$ −3.0 (*c* 1.0, MeOH); NMR data see Table 1 and Table S2, Supporting Information; HRMALDI-TOF-MS *m/z* 1943.7707 $[M + Na]^+$ (calcd for $C_{85}H_{132}O_{48}Na$, 1943.7780; Δ 3.8 ppm).

Agminoside B (2): clear glass; NMR data see Table S3, Supporting Information; HRESIMS *m/z* 1877.7734 $[M - H]^-$ (calcd for $C_{83}H_{129}O_{47}$, 1877.7704; Δ 1.6 ppm).

Agminoside C (3): clear glass; NMR data see Table S4, Supporting Information; HRESIMS *m/z* 1877.7721 $[M - H]^-$ (calcd for $C_{83}H_{129}O_{47}$, 1877.7704; Δ 0.9 ppm).

Agminoside D (4): clear glass; NMR data see Table S5, Supporting Information; HRESIMS *m/z* 1835.7599 $[M - H]^-$ (calcd for $C_{81}H_{127}O_{46}$, 1835.7599; Δ 0.4 ppm).

Agminoside E (5): clear glass; NMR data see Table S6, Supporting Information; HRESIMS *m/z* 1715.7176 $[M - H]^-$ (calcd for $C_{77}H_{119}O_{42}$, 1715.7176; Δ 1.2 ppm).

Methanolysis of a Crude Mixture of Glycolipids. A portion of the crude mixture of glycolipids (60 mg) was heated in MeOH (1 mL) with 3 drops of concentrated HCl in a screw-cap vial at 80−90 °C for 48 h. The sample was cooled and then diluted with H_2O (1 mL) and loaded onto a column of HP20SS (1 × 2 cm). The eluent was diluted with H_2O and passed through the column again. The column was then eluted with 6 mL of H_2O and then MeOH. The MeOH fraction was evaporated to dryness to yield 12.0 mg of aglycon methyl ester (**6**). The H_2O fraction was concentrated under reduced pressure to yield 50.8 mg of 1-*O*-methyl-α-D-glucose and 1-*O*-methyl-β-D-glucose (49:1).

Aglycon Methyl Ester (6): white solid; $[\alpha]^{20}_D$ −19.5 (*c* 0.99, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.30 (1H, t, *J* = 3.5 Hz, H-2′), 3.79 (3H, s, 1′-OMe), 3.70 (2H, d, *J* = 3.5 Hz), 3.59 (1H, m, H-17), 3.49 (1H, dt, *J* = 9.4, 6.6 Hz, H-1a), 3.42 (1H, dt, *J* = 9.4, 6.6 Hz, H-1b), 1.54 (2H, quin, *J* = 6.7 Hz, H-2), 1.44 (2H, m, H-19), 1.41 (4H, m, H-16 and H-18), 1.40 (2H, m, H-15), 1.26 (2H, m, H-3), 1.24 (20H, m, H4−H14), 0.92 (3H, t, *J* = 6.9 Hz, H-20); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 173.3 (C-1′), 72.1 (CH_2-3′), 72.0 (CH_2-1), 71.9 (CH-17), 71.0 (CH-1′), 52.7 (1′-OMe), 39.8 (CH_2-18), 37.6 (CH_2-16), 29.7 (CH_2-4−14), 29.5 (CH_2-2), 26.1 (CH_2-3), 25.8 (CH_2-15), 19.0 (CH_2-19), 14.3 (CH_3-20); HRESIMS *m/z* 439.3381 $[M + Na]^+$ (calcd for $C_{24}H_{48}O_5Na$, 439.3394; Δ 3.0 ppm).

1-*O*-Methyl-D-Glucose: white solid; $[\alpha]^{20}_D$ +209 (*c* 0.65, H_2O).

Methylation of a Crude Mixture of Glycolipids. $TMSCHN_2$ (50 μL, 2 M in hexanes) was added to 8.8 mg of a crude mixture of

glycolipids dissolved in dry MeOH (1 mL) under argon at 0 °C. After 1 h, the reaction was quenched with 2% (v/v) acetic acid in H_2O (1 mL) and was allowed to warm to room temperature. After the disappearance of all yellow color from the solution (30 min), the sample was loaded onto a column of HP20SS (1 × 2 cm). The column was then eluted with 6 mL fractions of (1) H_2O, (2) 50% Me_2CO/H_2O, (3) 80% Me_2CO/H_2O, and (4) Me_2CO. Fraction 3 was evaporated to dryness to yield 7.4 mg of methylated glycolipids.

Preparation of (*R*)-MTPA Ester (6a) and (*S*)-MTPA Ester (6b). To a solution of **6** (2 mg, 4.8 μmol) in CH_2Cl_2 (2 mL) was added DMAP (4.5 mg, 36.8 μmol) and either (*R*)- or (*S*)-MTPACl (3 μL, 16.1 μmol) under argon, and the mixture was stirred overnight. The reaction was quenched with H_2O (1 mL), and the solution was washed with 4 mL portions of 1 M HCl, 1 M NaOH, and finally brine. The organic layer was concentrated to yield the *R*- (**6a**) (5.0 mg) and *S*-Mosher's ester (**6b**) (4.7 mg) derivatives.

(*R*)-MTPA Ester (6a): white solid; NMR data see Table S1, Supporting Information.

(*S*)-MTPA Ester (6b): white solid; NMR data see Table S1, Supporting Information.

Determination of the Absolute Configuration of the Sugars. A portion of the crude mixture of glycolipids (7.3 mg) was heated in 2 M TFA (2 mL) in a screw-cap vial at 100 °C overnight. The aglycon was extracted with CH_2Cl_2, and the aqueous residue was concentrated under reduced pressure. A 1.7 mg portion of the hydrolyzed sugars was subjected to reductive amination with (*S*)-1-amino-2-propanol, as previously described.[9] The following reagents were added: 40 μL of 1:8 (*S*)-1-amino-2-propanol in MeOH, 34 μL of 1:4 glacial acetic acid in MeOH, and 26 μL of 3% $NaBH_3CN$ in MeOH. The capped vial was allowed to react for 1 h at 65 °C. TFA (2 M) was added dropwise until pH 1−2. The mixture was dried and co-evaporated with H_2O (3 × 0.5 mL) and MeOH (5 × 0.5 mL). The sample was dried under high vacuum, then treated with 2 mL of 1:1 pyridine/Ac_2O (2 h, 90 °C). The reaction was quenched with H_2O (2 mL) and loaded onto a column of HP20SS (1 × 2 cm). The column was then eluted with 6 mL of H_2O and MeOH. The MeOH fraction was evaporated to dryness to yield the 1-deoxy-1-(2-hydroxypropylamino)alditol acetate derivative, which was subjected to GC analysis with a D-glucose standard prepared under the same derivatization conditions (retention time 16.5 min).

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Supporting Information Available: Additional NMR data tabulation and spectra for agminosides A-E are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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