New Acylated Sulfoglycolipids and Digalactolipids and Related Known Glycolipids from Cyanobacteria with a Potential To Inhibit the Reverse Transcriptase of HIV-1

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Five novel diacylated sulfoglycolipids (**1**−**5**) were isolated from the cyanobacterium *Scytonema* sp. (TAU strain SL-30-1-4) and four novel acylated diglycolipids (**6**−**9**) were isolated from the cyanobacterium *Oscillatoria raoi* (TAU strain IL-76-1-2). These two groups of glycolipids and related known glycolipids isolated from these two and three other strains of cyanobacteria, *Phormidium tenue* (TAU strain IL-144-1), *O. trichoides* (TAU strain IL-104-3-2), and *O. limnetica* (TAU strain NG-4-1-2), were found to inhibit HIV-1 RT enzymatic activity to different extents. The structure elucidation of the various compounds is based on the selective hydrolysis of the glycerol ester moieties, GCMS analysis of the methyl ester derivatives of the liberated fatty acids, homo- and heteronuclear-2D-NMR techniques, and MS. The use of negative-ion FABMS for analyzing the combination and distribution of the fatty acids in glycolipids is demonstrated.

Glycolipids are widespread in photosynthetic eucaryotic[1] and procaryotic[2] organisms, where they are associated with the thylakoid membranes. In cyanobacteria, glycolipids are also associated with the heterocystous cell walls.[3,4] Several studies on glycolipids reported them as having specific biological activities, e.g., antitumor-promoting,[5] antiinflammatory,[6] antialgal,[7] hemolytic,[8] and antiviral properties.[9,10]

As part of our program to discover new biologically active agents from cultured cyanobacteria, we initiated a survey for inhibitors of the human immunodeficiency virus type 1 (HIV-1), the major causative agent of AIDS, focusing on the inhibition of the viral reverse transcriptase (RT).[11,12] RT is known to be a key enzyme in the life cycle of the HIV and thus is one of the main targets for AIDS therapy. In fact, most of the currently approved anti-AIDS agents are potent inhibitors of HIV-1 RT.[13] Our initial screening process yielded several extracts of cyanobacteria with the capacity to inhibit the polymerase activity of HIV-1 RT. Here, we report the isolation of three related groups of HIV-1 RT inhibitors derived from lipophilic extracts of five cyanobacteria strains. A group of novel diacylated sulfoglycolipids (**1**−**5**) and a group of novel acylated diglycolipids (**6**−**9**) were isolated from the lipophilic extracts of *Scytonema* sp. (Nostocales, Scytonemaceae) (TAU strain SL-30-1-4) and *Oscillatoria raoi* De Toni (Nostocales, Oscillatoriaceae) (TAU strain IL-76-1-2), respectively. The known glycolipids **11**−**18** were obtained from *Scytonema* sp. (TAU strain SL-30-1-4); **10**−**13** and **15**−**21** from *Oscillatoria raoi* (TAU strain IL-76-1-2); **11**−**13**, **15**, **16**, **20**, and **22** from *O. trichoides* Sazafer (Nostocales, Oscillatoriaceae) (TAU strain IL-104-3-2); **19**, **20**, **22**, and **23** from *O. limnetica* Lemmermann (Nostocales, Oscillatoriaceae) (TAU strain NG-4-1-2) and **20** and **22** from *Phormidium tenue* Gomont (Nostocales, Oscillatoriaceae) (TAU strain IL-144-1).

Results and Discussion

Both groups of sulfoglycolipids and glycolipids were purified following their capacity to inhibit HIV-1 RT-associated DNA polymerase activity at the different purification steps. Isolation commenced with normal-phase (silica gel) chromatography of the crude lipophilic extracts, followed by gel filtration on Sephadex LH-20. The mixtures of the active glyco- and sulfoglycolipids obtained from the gel filtration were further fractionated by normal-phase (silica gel) chromatography. Finally, the major components of the sulfoglycolipid or glycolipid mixtures were purified by reversed-phase HPLC. The structure elucidation of the various compounds was based on selective hydrolysis of the ester moieties attached to the glycerol at positions 1 and 2. Free acids were converted to the methyl ester derivatives and analyzed by GCMS to afford the acid acylating each glycerol position. The glycoglycerol structure, e.g., sulfoquinovopyranosyl glycerol (SQG), monogalactopyranosyl glycerol (MGG), or digalactopyranosyl glycerol (DGG), was determined by spectroscopic analysis (including; MS, 1D- and 2D-NMR techniques) of the mixtures, the pure compounds, and the hydrolysis products.

The lipophilic extract of *Scytonema* sp. (TAU strain SL-30-1-4) contains a mixture of five sulfoglycolipids: **1**−**5**. The NMR data of the mixture was characteristic of a sulfoglycolipid, namely, a 6‴-methylene carbon signal at δ_C 52.4 ppm and corresponding proton signals at δ_H 3.39 and 3.06 ppm.[10] The chromatographic behavior, ^{1}H and ^{13}C NMR data, and negative-ion FABMS data of this mixture strongly suggest that these sulfoglycolipids are substituted by two additional fatty acid units. They were somewhat less polar than the sulfoglycolipids mentioned bellow (**19**−**23**). In the ^{1}H NMR spectrum, the sugar H-2‴ and H-3‴ are shifted downfield (4.78 and 5.35 ppm, respectively) relative to

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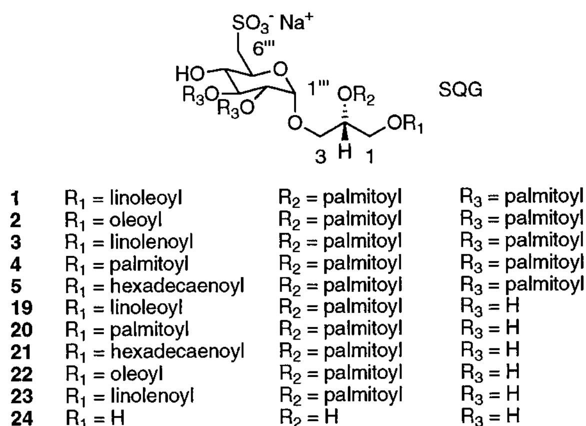
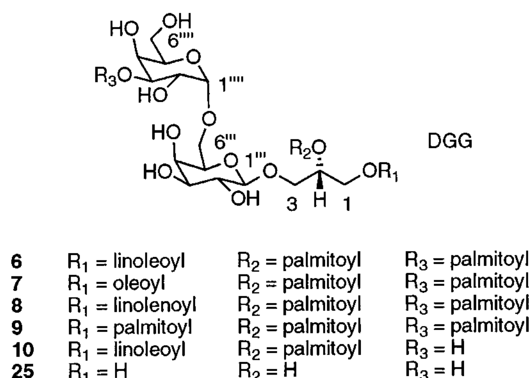
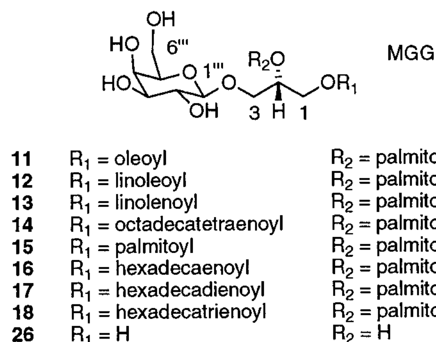






the same protons in **19** (3.53 and 3.70 ppm, respectively). In the ^{13}C NMR spectrum, four instead of two ester carbon signals are evident (see Table 1). In the negative-ion FABMS (APD as the matrix) five different quasimolecular ion peaks were observed at *m/z* 1295.9, 1293.9, 1291.9, 1269.9, and 1267.9. The crude sulfoglycolipid mixture was subjected to a regioselective enzymatic deacylation using Lipase type XI from *Rhizopus arrhizus*,[14] which liberated the fatty acid residue from position 1 of the glycerol and afforded triacyl-3-*O*-α-(6‴-sulfoquinovopyranosyl)glycerol, which remained soluble in the aqueous solution. The fatty acid mixture was treated with diazomethane and the methyl ester mixture was subject to GCMS analysis. The fatty acid mixture was determined as a mixture (ratio) of: hexadecaenoic (1), palmitic (1), oleic (2), linoleic (3), and linolenic (1) acids. Subsequent alkaline treatment (KOH−MeOH) of the triacyl-3-*O*-α-(6‴-sulfoquinovopyranosyl)glycerol gave the sulfoquinovopyranosylglycerol, **24**, and a single fatty acid, which was identified by the GCMS analysis of its methyl ester derivative as palmitic acid. These findings were supported by the fragment

Table 1. NMR Data of Compound **1** in $CDCl_3$ + CD_3OD[a]

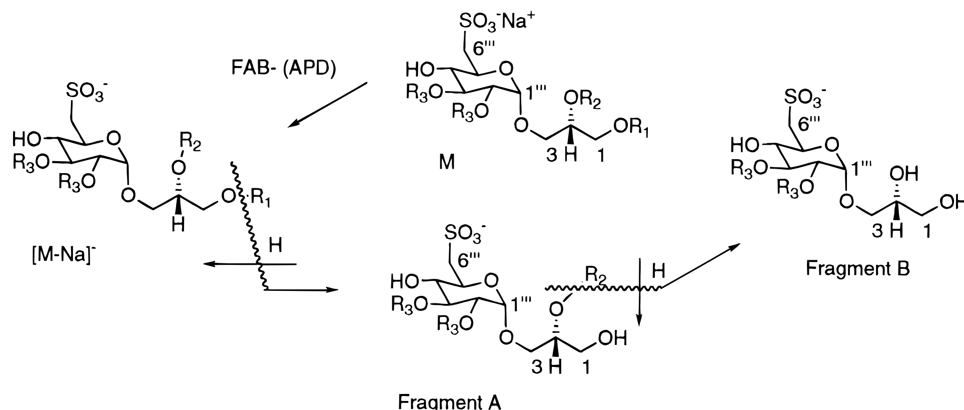
position	δ_C, mult[b]	δ_H (mult, *J* in Hz)	H−C correlations[c]
1	62.5 t	4.16 (dd, 12.0, 6.2)	3
		4.37 (dd, 12.0, 5.8)	
2	69.9 d	5.34 (m)	1,3
3	65.6 t	3.60 (dd, 10.8, 6.8)	
		4.04 (dd, 10.8, 4.5)	
1′ [d]	173.6 s		1
1″ [e]	173.4 s		2
1‴	95.5 d	5.01 (d, 3.5)	3, 3‴, 5‴
2‴	70.6 d	4.78 (dd, 3.5, 10.1)	3‴
3‴	71.6 d	5.35 (dd, 10.1, 9.8)	1‴, 2‴, 4‴
4‴	71.5 d	3.39 (dd, 9.8, 9.6)	2‴, 3‴, 6‴
5‴	67.9 d	4.16 (m)	1‴, 6‴
6‴	52.4 t	3.06 (dd, 14.6, 8.0)	4‴
		3.39 (dd, 14.6, 4.5)	
1⁗ [f]	173.0 s		2‴
1′′′′′ [g]	173.3 s		3‴
2′, 2″	33.9 t	2.32 (t, 7.3)	2′, 3′, 2″, 3″
2⁗, 2′′′′′	33.7 t	2.34 (t, 7.3)	2⁗, 3⁗, 2′′′′′, 3′′′′′
3′, 3″, 3⁗, 3′′′′′, 3′′′′′	24.5 t	1.62 (m)	(2)′, ″, ⁗, ′′′′′
(4−13)′, ″, ⁗, ′′′′′	29.3 t, 29.1 t, 28.9 t, 28.8 t	1.31−1.26 (brs)	
(14)′, ″, ⁗, ′′′′′	31.5 t	1.26 (brs)	(16)′, ″, ⁗, ′′′′′
(15) ′, ″, ⁗, ′′′′′	22.2 t	1.26 (brs)	(16)′, ″, ⁗, ′′′′′
(16) ′, ″, ⁗, ′′′′′	13.5 q	0.89 (t, 6.3)	

[a] Field strength was 500 MHz for protons and 125 MHz for carbons. [b] Multiplicity was assigned by DEPT experiment. [c] Based on HMBC experiment. [d] First carbon of acyl substituent at position 1. [e] First carbon of acyl substituent at position 2. [f] First carbon of acyl substituent at position 2‴. [g] First carbon of acyl substituent at position 3‴.

ion at *m/z* 1031.7 in the negative-ion FABMS of the crude mixture. This fragment was derived from the loss of the fatty acid attached to C-1 of the glycerol, as a ketene (see fragment A in Scheme 1). On the basis of the specific rotation ([α]$_D$ +74.0°), compound **24** was identified as (2′*R*)-1-glyceryl-*O*-α-D-6-sulfoquinovopyranose.[6] Compound **1** was obtained in a pure state by reversed-phase HPLC; the other components of the mixture were not resolved. Its negative-ion FABMS (APD as the matrix) gave a quasimolecular ion peak at *m/z* 1293.9 (M − Na)$^-$ corresponding to a molecular formula of $C_{75}H_{137}NaO_{14}S$. Extensive NMR studies (^{1}H and ^{13}C NMR and 2D COSY, HMQC and HMBC experiments, see Table 1) allowed us to suggest a 1,2,2‴,3‴-tetraacyl-6‴-sulfo-α-quinovopyranosylglycerol structure for compound **1**. Enzymatic hydrolysis, using Lipase type XI from *Rhizopus arrhizus*,[14] regioselectively liberated the acyl moiety at the 1 position. This fatty acid was esterified and identified, by GCMS, as linoleic acid methyl ester. Alkaline hydrolysis of the 1-O-deacylated-**1** yielded compound **24** and a single fatty acid, which after methylation was determined by GCMS to be palmitic acid methyl ester. A (2*S*)-1-linoleoyl-2,2‴,3‴-tripalmitoyl-3-*O*-α-D-6‴-sulfoquinovopyranosylglycerol structure is thus proposed for **1**. The data described above and the NMR data of the mixture (which is essentially identical with that of **1**) allowed us to assign structures **1**−**5** to the sulfoglycolipid mixture of *Scytonema* sp. (TAU strain SL-30-1-4).

The lipid fraction of the lipophilic extract of *O. raoi* (TAU strain IL-76-1-2)[15] comprises of four novel monoacylated digalactosyl diacylglycerols, **6**−**9**, a known digalactosyl diacylglycerol, **10**, seven monogalactosyl diacylglycerols, **11**−**13**, **15**−**18**, and three sulfoglycolipids **19**−**21**. The fraction that contained the less polar digalactosyl glycerides exhibited negative-ion FABMS quasimolecular ions at *m/z* 1127.8, 1149.8, 1151.8, and

Scheme 1. Negative FABMS Fragmentation Pattern of Sulfoquinovopyranosyl Diglycerides



1153.8, a downfield shift of H-3′′′′ (4.83 ppm, relative to 3.59 ppm in compound **10**), and three carboxyl signals (173.4 s, 173.4 s, and 173.6 s) in the ^{13}C NMR spectrum, suggesting that these digalactoacylglycerides are substituted at positions 1, 2, and 3′′′′ by three fatty acid residues. The crude diglycolipid mixture was subject to a regioselective enzymatic deacylation as described above, and the acid mixture liberated from position 1 of the glycerol was determined as a mixture of palmitic (1), linolenic (3), linoleic (8), and oleic (1) acids. Palmitic acid was the sole acid attached to the other two acylated positions. Compound **6** was purified by reversed-phase HPLC from the mixture of the monoacylated digalactosyl diacylglycerol. The quasimolecular ion of the FABMS of **6** at *m*/*z* 1175.8 (M + Na)$^+$ corresponds to a molecular formula of $C_{65}H_{116}O_{16}$. Extensive NMR studies (^{1}H and ^{13}C NMR and 2D COSY, HMQC and HMBC experiments, see Table 2) of **6** and its hexaacetate, **6a**, established the 1,2,3′′′′-triacyl-3-*O*-[α-galactopyranosyl-(1′′′′-6′′′)-*O*-β-galactopyranosyl]glycerol structure of compound **6**. Enzymatic deacylation of **6** as described above, liberated linoleic acid from position 1 of the glycerol while subsequent alkaline hydrolysis of delinoleoyl-**6** afforded palmitic acid and the digalactosylglycerol **25**.[16] The chirality of the digalactosylglycerol was tentatively established by measuring the optical rotation of **25** and comparing it to literature data.[16] Accordingly, the structure of **6** was determined as (2*S*)-1-linoleoyl-2-palmitoyl-3-*O*-[α-D-galactopyranosyl-(1′′′′−6′′′)-3′′′′-palmitoyl-*O*-β-D-galactopyranosyl]glycerol. On the basis of the data given above, the other three constituents of this mixture were assigned as **7**−**9**. Compound **10** was subjected to the selective hydrolysis described above, which afforded linoleic and palmitic acids as the respective substituents of the glycerol positions 1 and 2 and compound **25**. Compound **10** was acetylated to give the heptaacetate, **10a**, which allowed a complete proton-coupling constant analysis and the assignment of the α-galactopyranosyl-(1′−6)-*O*-β-galactopyranosyl structure to the glycosidic portion of **10**. Comparison of the NMR data and the specific rotation of compound **25** with those from the literature identified compound **10** as (2*S*)-1-linoleoyl-2-palmitoyl-3-*O*-[α-D-galactopyranosyl-(1′′′′−6′′′)-*O*-β-D-galactopyranosyl]glycerol, previously isolated from the cyanobacterium *P. tenue*.[16]

The negative-ion FABMS (APD as matrix) of the monoglycolipids mixture of *O. raoi* (TAU strain IL-76-1-2) exhibited seven weak quasimolecular ion peaks ([M − H]$^-$) at *m*/*z* 755.6, 753.6, 751.6, 729.6, 727.6, 725.6, and 723.6 and five strong fragment ions at *m*/*z*

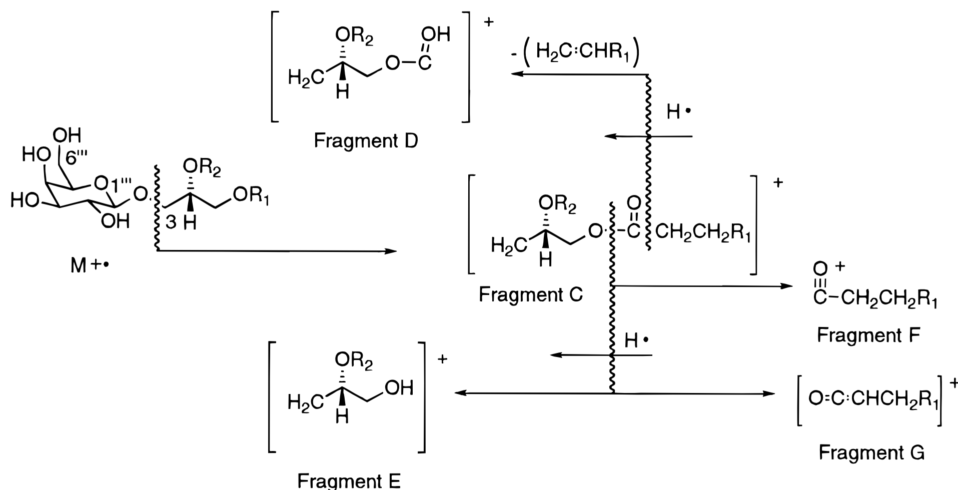
Table 2. NMR Data of Compound **6** in $CDCl_3$ + CD_3OD[a]

position	δ_C, mult[b]	δ_H (mult, *J* in Hz)	H−C correlations[c]
1	62.4 t	4.20 (dd, 12.0, 9.1) 4.32 (dd, 12.0, 2.6)	2, 3
2	70.0 d	5.24 (m)	1, 3
3	67.5 t	3.66 (m), 3.92 (m)	1, 2
1′ [d]	173.4 s		1, 2′
1′′ [e]	173.3 s		2, 2′′
1′′′	103.6 d	4.19 (d, 7.1)	3, 2′′′, 3′′′
2′′′	70.9 d	3.49 (m)	3′′′, 4′′′
3′′′	72.9 d	3.47 (m)	1′′′, 2′′′, 4′′′, 5′′′
4′′′	69.9 d	3.93 (m)	5′′′, 6′′′
5′′′	72.8 d	3.66 (m)	3′′′, 4′′′, 6′′′
6′′′	66.2 t	3.68 (m), 3.88 (m)	5′′′
1′′′′	99.0 d	4.92 (d, 3.8)	5′′′′
2′′′′	66.2 d	4.02 (dd, 3.8,10.5)	1′′′′, 3′′′′, 4′′′′
3′′′′	72.7 d	4.95 (dd, 10.5,3.0)	1′′′′, 4′′′′
4′′′′	68.0 d	4.08 (br d, 3.0)	5′′′′, 6′′′′
5′′′′	70.0 d	3.84 (t, 5.7)	3′′′′, 6′′′′
6′′′′	61.5 t	3.72 (m), 3.76 (m)	5′′′′
1′′′′′ [f]	173.6 s		3′′′′, 2′′′′′
2′	33.9 t	2.31 (t, 7.3)	
3′	24.4 t	1.59 (m)	
4′−6′	29.3−28.7 t × 3	1.25−1.30 (br s)	
7′	28.4 t	1.35 (m)	
8′	26.8 t	2.04 (q, 7.3)	
9′	129.6 d	5.36 (m)	
10′	127.8 d	5.31 (m)	
11′	25.2 t	2.76 (t, 6.6)	
12′	127.6 d	5.31 (m)	
13′	129.9 d	5.36 (m)	
14′	26.8 t	2.04 (q, 7.3)	
15′	28.4 t	1.35 (m)	
16′	31.2 t	1.25 (m)	
17′	22.2 t	1.25 (m)	
18′	13.6 q	0.88 (t, 6.8)	
2′, ′′′′′	33.8 t	2.32, 2.34 (t, 6.8)	
3′, ′′′′′	24.5 t	1.59 (m)	
(4−13)′, ′′′′′	29.3, 29.1, 29.0, 28.9, 28.8, 28.7, t × 22	1.25 (m)−1.30 (m)	
14′, ′′′′′	31.6 t × 2	1.25 (m)	
15′, ′′′′′	22.3 t	1.25 (m)	
16′, ′′′′′	13.6 q	0.87 (t, 6.6)	

[a] Field strength was 500 MHz for protons and 125 MHz for carbons. [b] Multiplicity was assigned by DEPT experiment. [c] Based on HMBC experiment. [d] First carbon of acyl substituent at position 1. [e] First carbon of acyl substituent at position 2. [f] First carbon of acyl substituent at position 3′′′′.

281.2, 279.2, 277.2, 255.2, and 253.2 derived from dissociation of the ester bonds. The EIMS of the mixture reveiled seven type C (see Scheme 2) fragment ions at *m*/*z* 577.5 (5), 575.5 (17), 573.5 (7), 551.5 (5), 549.5 (5), 547.5 (2), and 545.5 (1), and strong type D and E fragment ions at *m*/*z* 341.2 and 313.2, respec-

Scheme 2. EIMS Fragmentation Pattern of Galagctopyranosyl Diglycerides



tively. The crude monoglycolipid mixture was subject to a regioselective enzymatic deacylation as described above, and the acid mixture liberated from position 1 of the glycerol was determined to be a mixture of hexadecatrienoic (1), hexadecadienoic (1), hexadecaenoic (2), palmitic (4), linolenic (6), linoleic (26), and oleic (8) acids. On the basis of the structure of the products of the alkaline hydrolysis and the fragment ions of type D and E in the EIMS of the mixture, palmitic acid was determined as the substituent at position 2 of the glycerol. Compound **12** was purified by reversed-phase HPLC from the monoglycolipid mixture of *O. raoi* (TAU strain IL-76-1-2). The quasimolecular ion of the negative-ion FABMS of **12** at *m/z* 753.5 $(M - H)^-$ and the type-C fragment ion in its HREIMS *m/z* 575.5025 $(M^{\bullet+} - C_6H_{11}O_6)$ calcd for $C_{37}H_{67}O_4$ (+1.4 mDa) correspond to a molecular formula of $C_{43}H_{78}O_{10}$. The ^1H and ^{13}C NMR data for the sugar moiety suggested a 1-substituted *O*-β-D-galactopyranose structure.[17] Enzymatic hydrolysis of **12**, followed by alkaline treatment, afforded compound **26**. Linoleic acid was assigned as the substituent at position 1 of the glycerol and palmitic acid as the substituent at position 2 by GCMS analysis of the methyl esters obtained from selective hydrolysis. Compound **26** was determined to be (2′*R*)-1-glyceryl-*O*-β-D-galactopyranose by comparing its specific rotation and NMR data with reported values.[16,18] The molecular formula, ^1H and ^{13}C NMR data, and the results from the selective hydrolysis of **12**, suggested that it is identical with (2*S*)-1-linoleoyl-2-palmitoyl-3-*O*-β-D-galactopyranosyl glycerol previously isolated from the cyanobacterium *P. tenue*.[16] On the basis of the structure of **12**, the molecular weights obtained from the MS and the fatty acid composition, structures **11**−**13** and **15**−**18** were assigned to the monoglycolipid mixture of *O. raoi* (TAU strain IL-76-1-2).

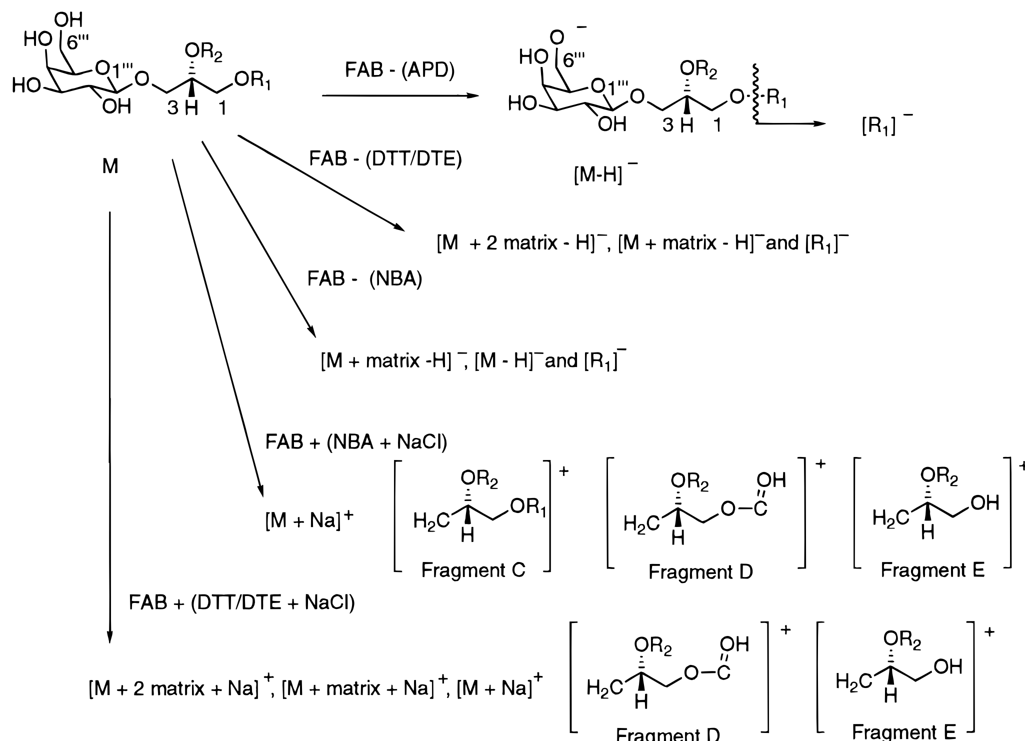
In a similar fashion, the composition of the monogalactolipid mixture was determined for: *O. trichoides* (TAU strain IL-104-3-2) as a 6:3:3:2:1 mixture of compounds **11**−**13** and **15**, and **16** and that of *Scytonema* sp. (TAU strain SL-30-1-4) as a 3:7:6:1:10:3:4:6 mixture of compounds **11**, **12**, **13**, **14**, **15**, **16**, **17**, and **18**. Compounds **11** and **15** were purified from these mixtures. Comparison of their physiochemical data with data from the literature revealed that compound **11** is identical with (2*S*)-1-oleoyl-2-palmitoyl-3-*O*-β-D-galactopyranosylglycerol previously isolated from the cyanobacterium *Fischerella ambigua*,[19] and compound **15** is identical with (2*S*)-1,2-dipalmitoyl-3-*O*-β-D-galactopyranosylglycerol previously isolated from the Okinawan sponge *Phyllospongia foliascens*.[6]

In a similar way, as described above for compounds **1**−**5**, the composition of the sulfoglycolipid mixture was determined for *Phormidium tenue* (TAU strain IL-144-1) as a 1:2 mixture of compounds **20** and **22**; *O. limnetica* (TAU strain NG-4-1-2) as a 4:1:3:2 mixture of compounds **19**, **20**, **22**, and **23**; *O. raoi* (TAU strain IL-76-1-2) as a 1:4:2 mixture of compounds **19**, **20**, and **21**; and *O. trichoides* (TAU strain IL-104-3-2) as a 1:2 mixture of compounds **20** and **22**. Compounds **19**, **20**, and **22** were isolated from these mixtures by HPLC and their spectroscopic data were found to be identical with those found in the literature.[10]

EIMS of acetylated glycodiglycerides has been used for the analysis of the combination and distribution of the fatty acids in plant[20] and cyanobacteria[3] glycolipids. FABMS, on the other hand, has only been used for determination of the molecular formula.[6,7,10] Comparison with the results from selective hydrolysis revealed that the negative-ion FABMS of glycolipids provides full evidence for the combination and distribution of the fatty acid substituents of the glycerol moiety. The combination of the fatty acids substituting the glycolipids (i.e., $C_{16:0} + C_{16:0}$, $C_{18:1} + C_{16:0}$, ...) was deduced from the mass weight of the quasimolecular ions, while the distribution of the different fatty acids in the acylation position is deduced from the fragment ions (see Schemes 1 and 3).

Negative-ion FABMS of sulfoglycolipids, using aminopropanediol (APD) as the matrix, gives a spectrum that includes all of the information needed for analyzing the combination and distribution of the fatty acids in both positions 1 and 2 of the glycerol; strong $[M - Na]^-$ and ions derived first from the fragmentation of the C-1 substituent and then from both C-1 and C-2 substituents (fragments A and B, respectively, in Scheme 1). When dithiothritol/dithioerythritol (DTT/DTE) is used as the matrix, additional quasimolecular ions that contain the matrix are evident in the spectrum. A good quality positive FABMS of the sulfoglycolipids is obtained only upon adding alkaline salt to the matrix. *m*-Nitrobenzyl alcohol (NBA) as the matrix gives better results than DTT/DTE, which usually produces few adduct ions.

Scheme 3. FABMS Fragmentation Pattern of Galactopyranosyl Diglycerides



The EIMS of glycolipids usually lacks the molecular ion but displays important fragment ions, derived from the loss of the sugar unit and the subsequent loss of the C-1 residues (fragments C−F in Scheme 2). Negative-ion FABMS of glycolipids gives a simple spectrum that is easily interpreted, especially using APD as the matrix. Other matrix substances usually give additional adduct ions (see Scheme 3). These spectra contain a quasimolecular $[M - H]^-$ ion and strong (base peaks) fragment ions derived from the fatty acid substituent at C-1. Positive-ion FABMS of glycolipids is obtained only when the sample is contaminated with, or upon the addition of alkali salt (NaCl) to the matrix. In addition to the quasimolecular ion $[M + Na]^+$, and adduct ions, fragment ions similar to the EIMS fragment ions, C, D, and E, are observed. With DTT/DTE as matrix, adduct ion with the matrix appears only for those compounds that contain unsaturated fatty acid residues.

In summary, negative-ion FABMS was found to be superior to positive-ion FABMS and EIMS for determination of the molecular formula and for the analysis of the combination and distribution of the fatty acids in glycolipids. The negative-ion FABMS presents a simple spectrum from which the required information is easily obtained. In negative-ion FABMS the fragment ions that appear in the spectrum contain the sugar residue(s) and are derived from the loss of acyl moieties (from the glycerol residue). In EIMS and positive-ion FABMS the fragment ions, which are important for analysis of the combination and distribution of the fatty acids in the examined compound, are all derived from loss of the sugar and acyl moieties (Schemes 2 and 3). Thus, in the case of acylation on the sugar unit (i.e., in the case of compounds **1**−**9**), only negative-ion FABMS will enable a correct analysis of the combination and distribution of the fatty acids in the examined compoumd. Four matrix substances (glycerol, NBA, DTT/DTE, and APD) were examined for determination of the negative-ion FABMS of the glycolipids in this study. The best results were obtained with DTT/DTE and APD but the mass spectra obtained with APD are easier to interpret because they do not present any adduct ions with the matrix.

Initial screening for the inhibitory activity of the DNA polymerase function of HIV-1 RT[21,22] revealed that compounds **1**, **19**, **20**, and **22**, all of which are sulfoglycolipids, are potent inhibitors. At a final concentration of 10 μM, these compounds totally abolished the initial enzymatic activity (by almost 100%). On the other hand, under the same assay conditions, the glycolipids, **12**, **6**, **11**, and **15** exhibited only a moderate to weak inhibitory capacity. The RT-associated DNA polymerase activity was inhibited by these compounds, by 65%, 42%, 33%, and 8%, respectively. These results are in line with a previous report on the anti-HIV-1 activity of similar sulfoglycolipids in a cell-based assay system.[10] Indeed, it could well be that the inhibitory activity by these compounds in tissue culture results from their potential to abolish the HIV-1 RT-associated DNA polymerase activity. It was, also, shown by others that crude extracts of cyanobacteria are capable of inhibiting avian myeloblastosis virus (AMV) and HIV RTs.[23] However, the nature of these inhibitors is unknown, and it seems they are different from the glycolipids in the present study, since they have been isolated from polar extracts. The mode of inhibition of these compounds is under investigation.

Experimental Section

Instrumentation. FTIR spectra were measured on a Nicolet FTIR in $CHCl_3$ or neat. High-resolution MS and GCMS were obtained on a Pisons VG AutoSpec Q instrument equipped with a Hewlett-Packard 5890 Series II gas chromatograph. UV spectra were measured on a Kontron 931 plus spectrophotometer. NMR

spectra were measured on a Bruker ARX-500 spectrometer at 500.1 MHz for ^{1}H and 125.8 MHz for ^{13}C. ^{1}H, ^{13}C, DEPT, COSY-45, HMQC, and HMBC spectra were obtained using standard Bruker pulse sequences. HPLC separations were performed with an Applied Biosystem Inc. instrument equipped with two Model 150 pumps and a 893 programmable UV detector.

Culture Conditions. An edaphic form of *Scytonema* sp. designated TAU strain number SL-30-1-4, was isolated from a soil sample collected on Praslin Island, Seychelles. An edaphic form of *O. raoi* De Toni (Nostocales, Oscillatoriaceae),[24] designated Tel Aviv University (TAU) strain no. IL-76-1-2, was isolated from a soil sample collected at a greenhouse in Kfar Azar, Israel. An edaphic form of *O. trichoides*, designated TAU strain no. IL-104-3-2, was isolated from a soil sample collected at a greenhouse in Kfar Azar, Israel. An edaphic form of *O. limnetica* designated TAU strain no. NG-4-1-2, was isolated from a soil sample collected in Lagos, Nigeria. An edaphic form of *Phormidium tenue*, designated TAU strain no. IL-144-1, was isolated from a soil sample collected at a greenhouse in Rishon Le Zion, Israel. All five strains are deposited at TAU cyanobacteria culture collection. Clonal strains of these isolates were purified on a BG-11 agar medium.[25] The cyanobacteria were cultured in 20 L glass bottles containing a BG-11 medium.[25] Cultures were illuminated continuously at an intensity of 100 mein/M^{2}/s from fluorescent tubes and aerated with 0.5% CO_2 in air (1 L/min) at an incubation temperature of 25 °C for 30−35 days. Yields of lyophilized cells typically ranged from 0.4 to 0.6 g/L of culture.

Enzymatic Assay. All purified dried compounds were dissolved in 100% dimethyl sulfoxide to a final concentration of 10 μM. The final dimethyl sulfoxide concentration in the enzymatic assay was adjusted to 1%, a concentration that did not affect the RT-associated activity. HIV-1 RT is a recombinant enzyme expressed in *E. coli* and purified to homogeneity as described previously.[26] The HIV-1 RT expression plasmid was derived from BH-10 proviral isolate.[27] The purified enzyme is a heterodimer composed of p66 and p51 subunits. DNA polymerase activity of the HIV-1 RT was assayed by following the poly(rA)$_n$·oligo(dT)$_{12-18}$-directed incorporation of [^{3}H]dTTP into DNA, as described in detail previously.[21] In all inhibition experiments, the enzyme was preincubated for 5 min at 30 °C in the absence or in the presence of inhibitor at various concentrations.[22] The enzymatic reactions were initiated by adding the substrate ([^{3}H]dTTP) followed by an incubation for 30 min at 37 °C. Inhibition of enzyme activity was calculated relative to the initial linear reaction rates observed under identical conditions when no inhibitor was added.

General Isolation Procedure. Freeze-dried bacterial cells were first extracted with 7:3 MeOH/H_2O (3 × 0.5 L) and then with 1:1 $CHCl_3$/MeOH (3 × 0.5 L). The filtered extracts were combined and concentrated under reduced pressure. The crude $CHCl_3$/MeOH extract was flash-chromatographed over a normal-phase (silica gel 60H, Merck) column (150 mm × 100 mm, i.d. × h). The material was eluted from the column with solvents of increasing polarity (200 mL each): petroleum ether (PE), 9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7, 2:8, 1:9 PE/EtOAc, EtOAc, and MeOH. Guided by the inhibition of HIV-1 RT, the EtOAc and MeOH fractions were combined and loaded on a Sephadex LH-20 column (25 × 300 mm, i.d. × h) and eluted with 1:1 $CHCl_3$/MeOH. Fractions of 20 mL were collected. The active fractions (usually 1−4) were combined and flash-chromatographed again on a normal-phase (silica gel 60H, Merck) column (20 × 100 mm, i.d. × h). A 5% step gradient from $CHCl_3$ to MeOH (20 mL each) was used to elute the different crude mixtures (SGG, MGG, or DGG) from the column. A few of the compounds (**1**, **6**, **10**, **11**, **12**, **15**, **19**, **20**, and **22**) were purified from the latter fractions by RP HPLC (Phenomenex, Ultracarb, 5 μm, ODS, 10 × 300 mm) using 1:9 or 1:19 H_2O/CH_3CN mixtures.

Strain SL-30-1-4 was grown in two 20 L carboys for 30 days in a BG-11 medium affording 22.9 g of dry cells. The lipophilic extract (1:1 $CHCl_3$/MeOH) afforded 3.9 g of a brownish gum that inhibited 98% of the HIV-1 RT activity at 10 μg/mL. From this crude extract, 16.9 mg of the sulfoglycolipid mixture (**1**−**5**) was obtained by the procedure given above. Negative-ion FABMS (APD) of the mixture *m*/*z* (relative intensity) 1295.9 (15), 1293.9 (30), 1291.9 (20), 1269.9 (5), 1267.9 (15) $[M - Na]^-$, 1031.7 (13) [fragment A]$^-$, 793.5 (10) [fragment B]$^-$, 225.0 (100) $[C_6H_{11}O_8S]^-$. The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **1** except for some signals that derived from the unsaturation presented in the fatty acids of the other compounds. Compound **1** (1.9 mg) was separated from this mixture by HPLC (1:9 H_2O/CH_3CN). From the latter crude extract, 98.1 mg of the monoglycolipid mixture (**11**−**18**) was obtained by the procedure given above. Positive-ion FABMS (DTT/DTE) of the mixture *m*/*z* (relative intensity) 1085.7 (5), 1083.7 (13), 1081.7 (10), 1057.4 (7), 1055.4 (14) $[M + 2\ matrix + Na]^+$, 933.7 (20), 931.7 (32), 929.7 (49), 927.7 (38), 905.4 (30), 903.4 (45), 901.4 (40) $[M + matrix + Na]^+$, 779.6 (25), 777.6 (35), 775.6 (60), 773.6 (50), 753.6 (100), 751.6 (45), 749.6 (55), 747.6 (75) $[M + Na]^+$, 515.2 (73), 313.3 (52), 198.9 (58),179.0 (56); positive-ion EIMS *m*/*z* (relative intensity) 577.5 (1), 575.5 (3), 573.5 (5), 571.5 (4), 551.5 (12), 549.5 (4), 547.5 (6), 545.5 (7) $[M - C_6H_{11}O_6]^+$, 341.3 (65), 313.3 (85), 239.2 (100). The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **15**. Compound **15** (2.4 mg) was separated from this mixture by HPLC (1:19 H_2O/CH_3CN).

Strain IL-76-1-2 was grown in six 20 L carboys for 33 days in a BG-11 medium affording 77 g of dry cells. The lipophilic extract (1:1 $CHCl_3$/MeOH) afforded 4.9 g of a brownish gum that inhibited 92% of the HIV-1 RT activity at 10 μg/mL. From this crude extract, 38.6 mg of the acylated diglycolipid mixture (**6**−**9**) was obtained by the procedure given above. Positive-ion EIMS *m*/*z* (relative intensity) 577.5 (1), 575.5 (3), 573.5 (1), 551.5 (<1); negative-ion FABMS (APD) of the mixture *m*/*z* (relative intensity) 1153.8 (1), 1151.8 (2), 1149.8 (1), 1127.8 (<1) $[M - H]^-$, 279.2 (25), 255.2 (100), 253.2 (55). The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **6**. Compound **6** (3.4 mg) was separated from this mixture by HPLC (1:19 H_2O/CH_3CN). From this crude extract, 19.4 mg of the sulfoglycolipid mixture (**19**−**21**) was obtained by the procedure given above. Negative-ion FABMS of the mixture *m*/*z* (relative intensity) 817.5 (10), 793.5 (45), 791.5 (15) $[M - Na]^-$, 555.3 (100) [fragment A]$^-$, 299.0 (40) [fragment B]$^-$, 283.2 (40), 255.3 (60), 225.0 (40)

$[C_6H_{11}O_8S]^-$. The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **20**. Compound **20** (2.1 mg) was separated from this mixture by HPLC (1:9 H_2O/CH_3CN). From this crude extract, 144.7 mg of the monoglycolipid mixture (**11**−**13** and **15**−**18**) was obtained by the procedure given above. Negative-ion FABMS (APD) of the mixture *m*/*z* (relative intensity) 755.6 (1), 753.6 (4), 751.6 (<1), 729.6 (<1), 727.6 (<1), 725.6 (<1), 723.6 (<1) $[M - H]^-$, 281.2 (25), 279.2 (85), 277.2 (30), 255.2 (100), 253.2 (30); positive-ion EIMS *m*/*z* (relative intensity) 577.4 (5), 575.4 (17), 573.4 (7), 551.4 (5), 549.4 (5), 547.4 (2), 545.4 (1), 341.2 (40), 313.2 (90), 179 (100). The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **12**. Compound **12** (7.5 mg) was separated from this mixture by HPLC (1:19 H_2O/CH_3CN). This crude extract yielded 6.7 mg of the diglycolipid, **10**, by the procedure given above.

Strain IL-104-3-2 was grown in six 20 L carboys for 30 days in a BG-11 medium affording 74.5 g of dry cells. The lipophilic extract (1:1 $CHCl_3$/MeOH) afforded 9.5 g of a brownish gum that inhibited 70% of the HIV-1 RT activity at 10 μg/mL. From this crude extract, 5.4 mg of the sulfoglycolipid mixture (**20** and **22**) was obtained by the procedure given above. Negative-ion FABMS (DTT/DTE) of the mixture *m*/*z* (relative intensity) 819.5 (100), 793.5 (47) $[M - Na]^-$, 555.3 (75) [fragment A]$^-$, 299.0 (57) [fragment B]$^-$, 225.0 (35) $[C_6H_{11}O_8S]^-$. Positive-ion FABMS (DTT/DTE) of the mixture *m*/*z* (relative intensity) 865.5 (50), 839.5 (10) $[M + Na]^+$, 897.3 (100), 873.3 (20) $[M + K]^+$. From this crude extract, 84.7 mg of the monoglycolipid mixture (**11**−**13**, **15**, and **16**) was obtained by the procedure given above. Negative-ion FABMS (NBA) of the mixture *m*/*z* (relative intensity) 909.7 (35), 907.7 (50), 905.7 (30), 883.7 (5), 881.7 (3) $[M + matrix - H]^-$, 755.6 (15), 753.6 (20), 751.6 (18), 729.6 (5), 727.6 (4) $[M - H]^-$, 281.2 (90), 279.2 (90), 277.2 (15), 255.2 (92), 253.2 (100); positive-ion EIMS *m*/*z* (relative intensity) 577.5 (10), 575.5 (14), 573.5 (8), 551.5 (10), 549.5 (5), 341.2 (20), 313.2 (25), 264.2 (20), 262.2 (25), 238.2 (20), 236.2 (20), 179 (100). The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **11**. Compound **11** (5.4 mg) was separated from this mixture by HPLC (1:19 H_2O/CH_3CN).

Strain NG-4-1-2 was grown in two 13 L carboys for 30 days in a BG-11 medium affording 4.3 g of dry cells. The lipophilic extract (1:1 $CHCl_3$/MeOH) afforded 1.57 g of a brownish gum that inhibited 92% of the HIV-1 RT activity at 10 μg/mL. From this crude extract, we obtained 33 mg of the sulfoglycolipid mixture (**19**, **20**, **22**, and **23**) by the procedure given above. Negative-ion FABMS of the mixture *m*/*z* (relative intensity) 819.5 (65), 817.5 (85), 815.5 (40), 793.5 (20) $[M - Na]^-$, 555.3 (25) [fragment A]$^-$, 299.0 (90) [fragment B]$^-$, 225.0 (100) $[C_6H_{11}O_8S]^-$. The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **19**. Compound **19** (3.5 mg) was separated from this mixture by HPLC (1:9 H_2O/CH_3CN).

Strain IL-144-1 was grown in a 13 L carboy for 32 days in a BG-11 medium affording 7.35 g of dry cells. The lipophilic extract (1:1 $CHCl_3$/MeOH) afforded 467 mg of a greenish gum that inhibited 95% of the HIV-1 RT activity at 10 μg/mL. From this crude extract, 15.8 mg of the sulfoglycolipid mixture (**20** and **22**) was obtained by the procedure given above. Negative-ion FABMS of the mixture *m*/*z* (relative intensity) 819.5 (40), 793.5 (15) $[M - Na]^-$, 555.3 (50) [fragment A]$^-$, 299.0 (40) [fragment B]$^-$, 79.9 (100) $[SO_3]^-$. The ^{1}H and ^{13}C NMR data of this mixture were virtually identical with that of compound **22**. Compound **22** (4.2 mg) was separated from this mixture by HPLC (1:9 H_2O/CH_3CN).

Compound 1: colorless oil; $[\alpha]_D$ +13.0° (c = 0.75, MeOH); negative-ion FABMS (APD) *m*/*z* (relative intensity) 1293.9 (28) $[M - Na]^-$, 1031.7 (10) [fragment A]$^-$, 793.7 (9) [fragment B]$^-$, 299.0 (70), 255.0 (100) $[C_{16}H_{31}O_2]^-$; IR (neat) ν_{max} 3520 (br), 2955, 1736, 1450, 1166, 1073 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1.

Compound 6: colorless oil; $[\alpha]_D$ +37.2° (c = 0.49, MeOH); negative-ion FABMS (APD) *m*/*z* (relative intensity) 1151.8 (5) $[M - H]^-$, 279.2 (80) $[C_{18}H_{31}O_2]^-$, 255.2 (100) $[C_{16}H_{31}O_2]^-$; positive-ion FABMS (DTT/DTE) *m*/*z* (relative intensity) 1329.8 (<1) $[M + matrix + Na]^+$, 1175.8 (<1) $[M + Na]^+$, 575.5 (10) $[M - C_{12}H_{21}O_{11}]^+$, 341.2 (35) [D]$^+$, 313.2 (100) [E]$^+$, 239.2 (40); IR (neat) ν_{max} 3450 (br), 2956, 1736, 1450, 1150, 1061 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2.

Compound **6** was acetylated, in Ac_2O/pyridine at rt, to give the hexaacetate **6a**: colorless oil; ^{1}H NMR (500 MHz) δ ($CDCl_3$) 4.31 (1H, dd, J = 3.1, 12.0 Hz, H-1a), 4.14 (1H, dd, J = 7.3, 12.0 Hz, H-1b), 5.12 (1H, dddd, J = 3.1, 4.8, 5.6, 7.3 Hz, H-2), 3.98 (1H, dd, J = 4.8, 10.8 Hz, H-3a), 3.56 (1H, dd, J = 5.6, 10.8 Hz, H-3b), 4.48 (1H, d, J = 7.9 Hz, H-1‴), 5.19 (1H, dd, J = 7.9, 10.5 Hz, H-2‴), 5.00 (1H, dd, J = 10.5, 3.3 Hz, H-3‴), 5.42 (1H, br d, J = 3.3 Hz, H-4‴), 3.85 (1H, dd J = 7.0, 5.9 Hz, H-5‴), 3.44 (1H, dd, J = 10.3, 7.0 Hz, H-6a‴), 3.78 (1H, dd, J = 10.3, 5.9 Hz, H-6b‴), 4.94 (1H, d, J = 3.7 Hz, H-1⁗), 5.12 (1H, dd, J = 3.7, 10.9 Hz, H-2⁗), 5.30 (1H, dd, J = 10.9, 3.4 Hz, H-3⁗), 5.46 (1H, br d, J = 3.4 Hz, H-4⁗), 4.21 (1H, dd, J = 7.1, 6.3 Hz, H-5⁗), 4.09 (1H, dd, J = 11.2, 7.0 Hz, H-6a⁗), 4.12 (1H, dd, J = 11.2, 6.3 Hz, H-6b⁗), 2.77 (2H, t, J = 6.6 Hz, H-11′), 2.32 (2H, t, J = 7.6 Hz, H-2′), 2.30 (2H, t, J = 7.7 Hz, H-2″), 2.29 (2H, t, J = 7.3 Hz, H-2″‴), 2.14 (3H, s, *Me*-CO), 2.13 (3H, s, *Me*-CO), 2.09 (3H, s, *Me*-CO), 2.08 (3H, s, *Me*-CO), 2.07 (3H, s, *Me*-CO), 2.06 (4H, q, J = 7.4 Hz, H-8′ and H-14′), 1.99 (3H, s, *Me*-CO), 1.61 (6H, m, H-3′, H-3″ and H-3″‴), 1.30−1.25 (62H, m, H-4′-H-7′, H-15′-H-17′, H-4″-H-15″, H-4″‴-H-15″‴), 5.37 (2H, m, H-9′ and H-13′), 5.34 (2H, m, H-10′ and H-12′), 0.88 and 0.89 (9H, t, J = 7.1 Hz, H-18′, H-16″ and H-16″‴).

Compound 10: colorless oil; $[\alpha]_D$ +56.0° (c = 1.0, MeOH); negative-ion FABMS (APD) *m*/*z* (relative intensity) 915.6 (5) $[M - H]^-$, 279.2 (100) $[C_{18}H_{31}O_2]^-$, 255.2 (100) $[C_{16}H_{31}O_2]^-$; positive-ion FABMS (NBA) *m*/*z* (relative intensity) 955.5 (30) $[M + K]^+$, 939.1 (45) $[M + Na]^+$, 917.1 (5) $[M + H]^+$, 575.3 (30) $[M - C_{12}H_{21}O_{11}]^+$, 341.2 (60) [D]$^+$, 313.2 (100) [E]$^+$; IR (neat) ν_{max} 3352 (br), 2924, 1730, 1470, 1150, 1068 cm^{-1}; ^{1}H NMR (500 MHz) δ ($CDCl_3$ + CD_3OD) 4.23 (1H, dd, J = 3.0, 12.0 Hz, H-1a), 4.10 (1H, dd, J = 6.8, 12.0 Hz, H-1b), 5.12 (1H, dddd, J = 3.0, 5.5, 6.2, 6.8 Hz, H-2), 3.81 (1H, dd, J = 5.5, 11.0 Hz, H-3a), 3.56 (1H, m, H-3b), 4.09 (1H, d, J = 7.1 Hz, H-1‴), 3.40 (1H, dd, J = 7.1, 9.9 Hz, H-2‴), 3.39 (1H, dd, J = 9.9, 3.1 Hz, H-3‴), 3.79 (1H, br d, J = 3.1 Hz, H-4‴), 3.52 (1H, dd, J = 6.6, 6.0 Hz, H-5‴), 3.57 (1H, m, H-6a‴), 3.77 (1H, dd, J = 9.6, 5.5 Hz, H-6b‴), 4.79 (1H, d, J = 3.8 Hz, H-1⁗), 3.69 (1H, m, H-2⁗), 3.59 (1H, m, H-3⁗), 3.81 (1H, br, J = 3.0 Hz, H-4⁗), 3.70 (1H, m, H-5⁗), 3.68 (2H, m, H-6a⁗, H-6b⁗), 2.19

(2H, t, $J = 7.3$ Hz, H-2′), 2.18 (2H, t, $J = 7.7$ Hz, H-2″), 1.93 (4H, q, $J = 6.9$ Hz, H-8′ and H-14′), 1.48 (4H, m, H-3′ and H-3″), 2.64 (2H, t, $J = 6.6$ Hz, H-11′), 1.24–1.17 (38H, m, H-4′-H-7′, H-15′-H-17′, H-4″-H-15″), 5.24 (2H, m, H-9′ and H-13′), 5.20 (2H, m, H-10′ and H-12′), 0.75 (3H, t, $J = 7.0$ Hz, H-18′), 0.76 (3H, t, $J = 6.6$ Hz, H-16″); ^{13}C NMR (125 MHz) δ ($CDCl_3$ + CD_3OD) 62.4 (t, C-1), 69.9 (d, C-2), 67.5 (t, C-3), 103.6 (d, C-1‴), 70.9 (d, C-2‴), 72.9 (d, C-3‴), 67.9 (d, C-4‴), 72.8 (d, C-5‴), 66.0 (t, C-6‴), 99.0 (d, C-1⁗), 68.7 (d, C-2⁗), 69.9 (d, C-3⁗), 69.5 (d, C-4⁗d, C-5⁗), 61.6 (t, C-6⁗), 173.7 (s, C-1′), 173.4 (s, C-1″), 33.9 (t, C-2′), 33.7 (t, C-2″), 24.5 (t × 2, C-3′ and C-3″), 28.7, 28.9, 28.9, 29.1 and 29.3 (t × 15, C-4′-C-7′, C15′, C-4″-C-13″), 26.8 (t × 2, C-8′ and C-14′), 129.8 and 129.6 (d × 2, C-9′ and C-13′), 127.7 and 127.6 (d × 2, C-10′ and C-12′), 25.3 (t, C-11′), 31.5 (t × 2, C-16′ and C-14″), 22.3 (t × 2, C-17′ and C-15″), 13.5 (q × 2, C-18′ and C-16″).

Compound **10** was acetylated, in Ac_2O/pyridine at rt, to give the heptaacetate **10a**: colorless oil; ^{1}H NMR (500 MHz) δ ($CDCl_3$) 4.31 (1H, dd, $J = 3.1$, 12.0 Hz, H-1a), 4.14 (1H, dd, $J = 7.3$, 12.0 Hz, H-1b), 5.12 (1H, dddd, $J = 3.1$, 4.8, 5.5, 7.3 Hz, H-2), 3.98 (1H, dd, $J = 4.8$, 10.7 Hz, H-3a), 3.56 (1H, dd, $J = 5.5$, 10.7 Hz, H-3b), 4.48 (1H, d, $J = 7.9$ Hz, H-1‴), 5.19 (1H, dd, $J = 7.9$, 10.5 Hz, H-2‴), 5.00 (1H, dd, $J = 10.5$, 3.1 Hz, H-3‴), 5.42 (1H, br d, $J = 3.1$ Hz, H-4‴), 3.84 (1H, dd, $J = 7.4$, 5.3 Hz, H-5‴), 3.44 (1H, dd, $J = 10.0$, 7.4 Hz, H-6a‴), 3.78 (1H, dd, $J = 10.0$, 5.3 Hz, H-6b‴), 4.95 (1H, d, $J = 3.6$ Hz, H-1⁗), 5.11 (1H, dd, $J = 3.6$, 10.9 Hz, H-2⁗), 5.29 (1H, dd, $J = 10.9$, 3.3 Hz, H-3⁗), 5.45 (1H, br d, $J = 3.3$ Hz, H-4⁗), 4.20 (1H, br dd, $J = 7.1$, 6.3 Hz, H-5⁗), 4.09 (1H, dd, $J = 11.2$, 7.0 Hz, H-6a⁗), 4.12 (1H, dd, $J = 11.2$, 6.3 Hz, H-6b⁗), 2.77 (2H, t, $J = 6.6$ Hz, H-11′), 2.30 (2H, t, $J = 7.6$ Hz, H-2′), 2.29 (2H, t, $J = 7.7$ Hz, H-2″), 2.14 (3H, s, *Me*-CO), 2.13 (3H, s, *Me*-CO), 2.09 (3H, s, *Me*-CO), 2.07 (3H, s, *Me*-CO), 2.06 (3H, s, *Me*-CO), 2.06 (4H, q, $J = 7.4$ Hz, H-8′ and H-14′), 1.98 (3H, s, *Me*-CO), 1.97 (3H, s, *Me*-CO), 1.61 (4H, m, H-3′ and H-3″), 1.30–1.25 (38H, m, H-4′-H-7′, H-15′-H-17′, H-4″-H-15″), 5.37 (2H, m, H-9′ and H-13′), 5.34 (2H, m, H-10′ and H-12′), 0.87 (6H, t, $J = 7.1$ Hz, H-18′ and H-16″).

Compound 11: colorless oil; $[\alpha]_D$ +13.8° (c = 0.19, MeOH); negative-ion FABMS (NBA) m/z (relative intensity) 908.7 (50) [M + matrix − H]$^-$, 755.5 (35) [M − H]$^-$, 281.0 (90) $[C_{18}H_{33}O_2]^-$, 255.0 (100) $[C_{16}H_{31}O_2]^-$, (DTT/DTE) m/z (relative intensity) 1063.6 (25) [M + 2 matrix − H]$^-$, 909.7 (100) [M + matrix − H]$^-$, 341.2 (20), 313.2 (25), 281.0 (50) $[C_{18}H_{33}O_2]^-$, 255.0 (65) $[C_{16}H_{31}O_2]^-$; IR (neat) ν_{max} 3380 (br), 2924, 1735, 1220, 1167 cm^{-1}; ^{1}H NMR (500 MHz) δ ($CDCl_3$) 4.39 (1H, dd, $J = 3.2$, 12.0 Hz, H-1a), 4.21 (1H, dd, $J = 6.4$, 12.0 Hz, H-1b), 5.31 (1H, m, H-2), 3.91 (1H, dd, $J = 5.4$, 11.2 Hz, H-3a), 3.73 (1H, dd, $J = 6.4$, 11.2 Hz, H-3b), 4.27 (1H, d, $J = 7.5$ Hz, H-1‴), 3.65 (1H, dd, $J = 7.5$, 9.2 Hz, H-2‴), 3.58 (1H, dd, $J = 9.2$, 3.27 Hz, H-3‴), 4.00 (1H, br s, H-4‴), 3.53 (1H, br s, H-5‴), 3.85 (1H, dd, $J = 2.3$, 10.5 Hz, H-6a‴), 3.94 (1H, br d, $J = 10.5$ Hz, H-6b‴), 2.33 (2H, t, $J = 6.8$ Hz, H-2′), 2.31 (2H, t, $J = 8.3$ Hz, H-2″), 1.60 (4H, m, H-3′ and H-3″), 1.31–1.27 (44H, m, H-4′-H-7′, H-10′-H-17′, H-4″-H-15″), 0.88 (6H, t, $J = 7.0$ Hz, H-18′ and H-16″); ^{13}C NMR (125 MHz) δ ($CDCl_3$) 62.6 (t, C-1), 70.2 (d, C-2), 68.3 (t, C-3), 104.0 (d, C-1‴), 71.6 (d, C-2‴), 73.4 (d, C-3‴), 69.4 (d, C-4‴), 74.5 (d, C-5‴), 62.7 (t, C-6‴), 173.8 (s, C-1′), 173.4 (s, C-1″), 34.2 (t, C-2′), 34.1 (t, C-2″), 24.8 (t × 2, C-3′ and C-3″), 29.1, 29.1, 29.2, 29.3, 29.5, 29.6 and 29.7 (t × 18, C-4′-C-7′, C-10′-C15′, C-4″-C-13″), 27.2 (t × 2, C-8′ and C-11′), 130.3 and 130.0 (d × 2, C-9′ and C-10′), 31.9 (t × 2, C-16′ and C-14″), 22.7 (t × 2, C-17′ and C-15″), 14.1 (q × 2, C-18′ and C-16″); positive-ion HREIMS m/z (relative intensity) 577.5198 (21) $[M - C_6H_{11}O_6]^+$ (calcd for $C_{37}H_{69}O_4$, 577.5195), 341.2 (20) [D]$^+$, 313.2777 (25) [E]$^+$ (calcd for $C_{19}H_{37}O_3$, 313.2742), 264.2404 (20) [G]$^+$ (calcd for $C_{18}H_{32}O$, 264.2453).

Compound 12: olorless oil; $[\alpha]_D$ +4.8° (c = 0.62, MeOH); negative-ion FABMS (APD) m/z (relative intensity) 753.5 (5) [M − H]$^-$, 515.4 (4) $[M - C_{16}H_{30}O]^-$, 491.4 (3) $[M - C_{18}H_{30}O]^-$, 279.0 (85) $[C_{18}H_{31}O_2]^-$, 255.0 (100) $[C_{16}H_{31}O_2]^-$; IR (neat) ν_{max} 3350 (br), 2955, 1740, 1465, 1166, 1117 cm^{-1}; ^{1}H NMR (500 MHz) δ ($CDCl_3$ + CD_3OD) 4.39 (1H, dd, $J = 3.0$, 12.0 Hz, H-1a), 4.24 (1H, dd, $J = 6.7$, 12.0 Hz, H-1b), 5.31 (1H, dddd, $J = 3.0$, 5.3, 6.2, 6.7 Hz, H-2), 3.96 (1H, dd, $J = 5.3$, 10.9 Hz, H-3a), 3.73 (1H, dd, $J = 6.2$, 10.9 Hz, H-3b), 4.23 (1H, d, $J = 7.4$ Hz, H-1‴), 3.56 (1H, dd, $J = 7.4$, 9.6 Hz, H-2‴), 3.51 (1H, dd, $J = 9.6$, 3.0 Hz, H-3‴), 3.90 (1H, d, $J = 3.0$ Hz, H-4‴), 3.51 (1H, dd, $J = 6.5$, 5.7 Hz, H-5‴), 3.76 (1H, br d, $J = 11.6$, 5.7 Hz, H-6a‴), 3.83 (1H, dd, $J = 11.6$, 6.5 Hz, H-6b‴), 2.33 (2H, t, $J = 7.4$ Hz, H-2′), 2.32 (2H, t, $J = 7.7$ Hz, H-2″), 2.06 (4H, q, $J = 6.9$ Hz, H-8′ and H-14′), 1.61 (4H, m, H-3′ and H-3″), 2.78 (2H, t, $J = 6.6$ Hz, H-11′), 1.31–1.26 (38H, m, H-4′-H-7′, H-15′-H-17′, H-4″-H-15″), 5.38 (2H, m, H-9′ and H-13′), 5.35 (2H, m, H-10′ and H-12′), 0.89 (3H, t, $J = 6.7$ Hz, H-18′), 0.88 (3H, t, $J = 7.0$ Hz, H-16″); ^{13}C NMR (125 MHz) δ ($CDCl_3$ + CD_3OD) 62.5 (t, C-1), 70.1 (d, C-2), 67.5 (t, C-3), 103.7 (d, C-1‴), 70.9 (d, C-2‴), 73.1 (d, C-3‴), 68.5 (d, C-4‴), 74.7 (d, C-5‴), 61.1 (t, C-6‴), 173.6 (s, C-1′), 173.4 (s, C-1″), 33.9 (t, C-2′), 33.8 (t, C-2″), 24.5 (t × 2, C-3′ and C-3″), 28.8, 28.9, 29.0, 29.1, 29.2 and 29.3 (t × 15, C-4′-C-7′, C15′, C-4″-C-13″), 26.8 (t × 2, C-8′ and C-14′), 129.8 and 129.6 (d × 2, C-9′ and C-13′), 127.7 and 127.6 (d × 2, C-10′ and C-12′), 25.2 (t, C-11′), 31.6 (t × 2, C-16′ and C-14″), 22.3 (t × 2, C-17′ and C-15″), 13.6 (q × 2, C-18′ and C-16″); positive-ion HREIMS m/z (relative intensity) 575.5025 (15) $[M - C_6H_{11}O_6]^+$ (calcd for $C_{37}H_{67}O_4$, 575.5039), 341.2681 (35) [D]$^+$ (calcd for $C_{20}H_{37}O_4$, 341.2691), 313.2749 (85) [E]$^+$ (calcd for $C_{19}H_{37}O_3$, 313.2742), 262.2318 (50) [G]$^+$ (calcd for $C_{18}H_{30}O$, 262.2296).

Compound 15: colorless oil; $[\alpha]_D$ +26.4° (c = 1.09, MeOH); negative-ion FABMS (APD) m/z (relative intensity) 729.5 (2.5) [M − H]$^-$, 491.3 (8) $[M - C_{16}H_{30}O]^-$, 255.2 (100) $[C_{16}H_{31}O_2]^-$, 179.0 (15) $[C_6H_{11}O_6]^-$; IR (neat) ν_{max} 3352 (br), 2955, 2855 1736, 1470, 1166, 1073 cm^{-1}; ^{1}H NMR (500 MHz) δ ($CDCl_3$ + CD_3OD) 4.39 (1H, dd, $J = 3.0$, 12.1 Hz, H-1a), 4.24 (1H, dd, $J = 6.6$, 12.1 Hz, H-1b), 5.31 (1H, dddd, $J = 3.0$, 5.3, 6.1, 6.6 Hz, H-2), 3.95 (1H, dd, $J = 5.3$, 10.9 Hz, H-3a), 3.72 (1H, dd, $J = 6.1$, 10.9 Hz, H-3b), 4.23 (1H, d, $J = 7.3$ Hz, H-1‴), 3.57 (1H, dd, $J = 7.3$, 9.6 Hz, H-2‴), 3.52 (1H, dd, $J = 9.6$, 2.4 Hz, H-3‴), 3.90 (1H, br d, $J = 2.4$ Hz, H-4‴), 3.51 (1H, br dd, $J = 6.4$, 5.5 Hz, H-5‴), 3.76 (1H, br d, $J = 11.6$, 5.5 Hz, H-6a‴), 3.83 (1H, dd, $J = 11.6$, 6.4 Hz, H-6b‴), 2.33 (2H, t, $J = 7.2$ Hz, H-2′), 2.32 (2H, t, $J = 7.5$ Hz, H-2″), 1.61 (4H, m, H-3′ and H-3″), 1.31–1.26 (48H, m H-4′-H-15′, H-4″-H-15″), 0.88 (6H, t, $J = 6.9$ Hz, H-16′ and H-16″); ^{13}C NMR (125 MHz) δ ($CDCl_3$ + CD_3OD) 62.6 (t, C-1), 70.1 (d, C-2), 67.6 (t, C-3), 103.7

(d, C-1‴), 71.0 (d, C-2‴), 73.2 (d, C-3‴), 68.6 (d, C-4‴), 74.8 (d, C-5‴), 61.2 (t, C-6‴), 173.6 (s, C-1′), 173.4 (s, C-1″), 34.0 (t, C-2′), 33.8 (t, C-2″), 24.6 (t × 2, C-3′ and C-3″), 28.8, 28.9, 29.1, 29.2 and 29.3 (t × 20, C-4′-C-13′, C-4″-C- 13″), 31.6 (t × 2, C-14′ and C-14″), 22.3 (t × 2, C-15′ and C-15″), 13.6 (q × 2, C-16′ and C-16″); positive-ion HREIMS *m/z* (relative intensity) 551.5052 (5) $[M - C_6H_{11}O_6]^+$ (calcd for $C_{37}H_{67}O_4$, 551.5039), 341.2647 (35) $[D]^+$ (calcd for $C_{20}H_{37}O_4$, 341.2691), 313.2700 (85) $[E]^+$ (calcd for $C_{19}H_{37}O_3$, 313.2742), 239.2394 (50) $[F]^+$ (calcd for $C_{16}H_{32}O$, 239.2374).

Compound 19: colorless oil; $[\alpha]_D$ +49.3° (c = 0.22, MeOH); negative-ion FABMS *m/z* (relative intensity) 817.5 (85) $[M - Na]^-$, 555.3 (25) [fragment A]$^-$, 299.0 (90) [fragment B]$^-$, 225.0 (95), 79.9 (100) $[SO_3]^-$; IR (neat) ν_{max} 3355 (br), 2955, 1734, 1220, 1165, 1090, 1035 cm^{-1}; ^{1}H NMR (500 MHz) δ ($CDCl_3$ + CD_3OD) 4.43 (1H, dd, J = 2.5, 12.1 Hz, H-1a), 4.16 (1H, dd, J = 6.9, 12.1 Hz, H-1b), 5.32 (1H, m, H-2), 3.95 (1H, dd, J = 5.2, 10.7 Hz, H-3a), 3.62 (1H, dd, J = 6.4, 10.7 Hz, H-3b), 4.84 (1H, d, J = 3.5 Hz, H-1‴), 3.53 (1H, dd, J = 3.5, 9.7 Hz, H-2‴), 3.70 (1H, dd, J = 9.4, 9.7 Hz, H-3‴), 3.45 (1H, t, J = 9.4 Hz, H-4‴), 4.01 (1H, br dd, J = 9.4, 5.2 Hz, H-5‴), 3.37 (1H, br d, J = 14.6 Hz, H-6a‴), 3.19 (1H, dd, J = 5.2, 14.6 Hz, H-6b‴), 2.34 (2H, t, J = 6.8 Hz, H-2′), 2.32 (2H, t, J = 8.3 Hz, H-2″), 2.06 (4H, q, J = 7.0 Hz, H-8′ and H-14′), 1.61 (4H, m, H-3′ and H-3″), 2.78 (2H, t, J = 6.7 Hz, H-11′), 1.31−1.27 (38H, m, H-4′-H-7′, H-15′-H-17′, H-4″-H-15″), 5.36 (2H, m, H-9′ and H-13′), 5.33 (2H, m, H-10′ and H-12′), 0.89 (6H, t, J = 7.0 Hz, H-18′ and 16″); ^{13}C NMR (125 MHz) δ ($CDCl_3$ + CD_3OD) 62.9 (t, C-1), 70.2 (d, C-2), 66.2 (t, C-3), 98.8 (d, C-1‴), 71.5 (d, C-2‴), 73.3 (d, C-3‴), 72.8 (d, C-4‴), 68.0 (d, C-5‴), 52.9 (t, C-6‴), 173.9 (s, C-1′), 173.8 (s, C-1″), 34.1 (t, C-2′), 33.9 (t, C-2″), 24.7 and 24.7 (t × 2, C-3′ and C-3″), 29.0, 29.1, 29.2, 29.4, and 29.5 (t × 15, C-4′-C -7′, C15′, C-4″-C-13″), 27.0 (t × 2, C-8′ and C-14′), 130.0 and 129.8 (d × 2, C-9′ and C-13′), 128.1 and 127.9 (d × 2, C-10′ and C-12′), 31.7 (t × 2, C-16′ and C-14″), 22.5 (t × 2, C-17′ and C-15″), 13.8 (q × 2, C-18′ and C-16″).

Compound 20: colorless oil; $[\alpha]_D$ +35.5° (c = 0.4, MeOH); negative-ion FABMS (APD) *m/z* (relative intensity) 793.5 (30) $[M - Na]^-$, 555.3 (100) [fragment A]$^-$, 299.0 (20) [fragment B]$^-$; IR (neat) ν_{max} 3350 (br), 2955, 1735, 1220, 1167, 1096 1035 cm^{-1}; ^{1}H NMR (500 MHz) δ ($CDCl_3$ + CD_3OD) 4.44 (1H, dd, J = 2.8, 12.0 Hz, H-1a), 4.16 (1H, dd, J = 7.0, 12.0 Hz, H-1b), 5.34 (1H, m, H-2), 3.99 (1H, dd, J = 5.2, 10.7 Hz, H-3a), 3.60 (1H, dd, J = 6.5, 10.7 Hz, H-3b), 4.81 (1H, d, J = 3.6 Hz, H-1‴), 3.47 (1H, dd, J = 3.6, 9.7 Hz, H-2‴), 3.65 (1H, dd, J = 9.6, 9.7 Hz, H-3‴), 3.30 (1H, t, J = 9.6 Hz, H-4‴), 4.03 (1H, ddd, J = 9.6, 7.2, 5.9 Hz,, H-5‴), 3.34 (1H, dd, J = 2.9, 14.5 Hz, H-6a‴), 3.09 (1H, dd, J = 7.2, 14.5 Hz, H-6b‴), 2.33 (2H, t, J = 6.8 Hz, H-2′), 2.32 (2H, t, J = 8.3 Hz, H-2″), 1.60 (4H, m, H-3′ and H-3″), 1.31−1.27 (48H, m, H-4′-H-15′, H-4″-H-15″), 0.87 (6H, t, J = 6.7 Hz, H-18′ and H-16″); ^{13}C NMR (125 MHz) δ ($CDCl_3$ + CD_3OD) 62.8 (t, C-1), 70.1 (d, C-2), 65.9 (t, C-3), 98.6 (d, C-1‴), 71.6 (d, C-2‴), 73.8 (d, C-3‴), 73.1 (d, C-4‴), 67.9 (d, C-5‴), 52.9 (t, C-6‴), 174.0 (s, C-1′), 173.7 (s, C-1″), 34.0 (t, C-2′), 33.8 (t, C-2″), 24.6 (t × 2, C-3′ and C-3″), 28.8, 29.0, 29.2 and 29.3 (t × 20, C-4′ − C-13′, C-4″-C-13″), 31.6 (t × 2, C-14′ and C-14″), 22.3 (t × 2, C-15′ and C-15″), 13.6 (q × 2, C-16′ and C-16″).

Compound 22: olorless oil; $[\alpha]_D$ +36.0° (c = 0.76, MeOH); negative-ion FABMS *m/z* (relative intensity) 819.5 (50) [M-Na]$^-$, 555.3 (75) [fragment A]$^-$, 299.0 (50) [fragment B]$^-$, 79.9 (100) $[SO_3]^-$; IR (neat) ν_{max} 3360 (br), 2970, 1730, 1224, 1162, 1096 1030 cm^{-1}; ^{1}H NMR (500 MHz) δ ($CDCl_3$ + CD_3OD) 4.44 (1H, dd, J = 2.6, 12.1 Hz, H-1a), 4.17 (1H, dd, J = 6.9, 12.1 Hz, H-1b), 5.32 (1H, m, H-2), 3.98 (1H, dd, J = 5.0, 10.7 Hz, H-3a), 3.61 (1H, dd, J = 6.4, 10.7 Hz, H-3b), 4.82 (1H, d, J = 3.5 Hz, H-1‴), 3.50 (1H, dd, J = 3.5, 9.6 Hz, H-2‴), 3.66 (1H, dd, J = 9.6, 9.0 Hz, H-3‴), 3.34 (1H, dd, J = 9.0, 9.6 Hz, H-4‴), 3.99 (1H, m, H-5‴), 3.34 (1H, br d, J = 14.5 Hz, H-6a‴), 3.12 (1H, dd, J = 6.6, 14.5 Hz, H-6b‴), 2.34 (2H, t, J = 7.7 Hz, H-2′), 2.32 (2H, t, J = 8.2 Hz, H-2″), 2.02 (4H, m, H-8′ and H-11′), 1.61 (4H, m, H-3′ and H-3″), 1.31−1.27 (44H, m, H-4′-H-7′, H-10′-H-17′, H-4″-H-15″), 5.35 (2H, m, H-9′ and H-10′), 0.88 (6H, t, J = 6.8 Hz, H-18′ and H-16″); ^{13}C NMR (125 MHz) δ ($CDCl_3$ + CD_3OD) 63.4 (t, C-1), 70.6 (d, C-2), 66.4 (t, C-3), 99.1 (d, C-1‴), 72.0 (d, C-2‴), 73.8 (d, C-3‴), 73.7 (d, C-4‴), 68.5 (d, C-5‴), 53.4 (t, C-6‴), 174.5 (s, C-1′), 174.3 (s, C-1″), 34.5 (t, C-2′), 34.3 (t, C-2″), 25.1 and 25.0 (t × 2, C-3′ and C-3″), 28.4, 29.5, 29.5, 29.7, 29.8, and 29.9 (t × 18, C-4′-C-7′, C-10′-C15′, C-4″-C-13″), 27.4 (t × 2, C-8′ and C-11′), 130.2 and 129.9 (d × 2, C-9′ and C-10′), 32.1 (t × 2, C-16′ and C-14″), 22.9 (t × 2, C-17′ and C-15″), 14.1 (q × 2, C-18′ and C-16″).

Enzymatic Hydrolysis.[14] A solution of sulfoglycolipid (5 mg) and Triton X-100 (2.5 mg) in boric acid buffer (1.65 mL, pH 7.7) was stirred for 1 h at 38 °C. Lipase type XI (700 units) was then added to the stirred solution and allowed to incubate for 1 h at 38 °C. The reaction was quenched with acetic acid (0.1 mL) and MeOH (1 mL) added. The resultant solution was extracted with 3 × 5 mL of hexane. Evaporation of the hexane, under reduced pressure, afforded the fatty acid mixture which was analyzed by GCMS. The aqueous solution contained a mixture of 1-deacylglycolipid and Triton X-100. This mixture was used for the alkaline hydrolysis. Monoglycolipids and diglycolipids were treated in the same maner.

Alkaline Hydrolysis. The aqueous solution from the enzymatic hydrolysis of the sulfoglycolipids was evaporated under reduced pressure. The residue was dissolved in 5 mL of 5% aqueous KOH/MeOH (1:1) and stirred at room temperature for 2 h. The reaction mixture was acidified with 1% HCl to pH 5 and extracted with 3 × 5 mL of hexane. The aqueous methanol solution was evaporated under reduced pressure, redissolved in water and passed over an ion-exchange cartridge (Baker SPE-Quaternary Amine 500 mg, 100% H_2O then 10% $NaHSO_4$ pH 3) and than over a reversed-phase (C_{18}) cartridge (Baker SPE-Octadecyl 200 mg, H_2O) to yield compound **24** as an amorpous solid. In case of the mono- and diglycolipids a similar scheme was used excluding the ion-exchange step. Compounds **25** and **26** were isolated from the aqueous phase of the hydrolysis reactions of the mono- and diglycolipids, respectively.

Compound 24: amorphous solid; $[\alpha]_D$ +74.1° (c = 1.0, MeOH); FABMS (NBA) *m/z* (relative intensity) 363 $(M + Na)^+$; ^{1}H NMR δ (D_2O) 4.93 (1H, d, J = 3.2 Hz, H-1′), 4.05 (1H, dd, J = 9.8, 9.4 Hz, H-5′), 3.99 (1H, m, H-2), 3.93 (1H, dd, J = 3.2, 9.4 Hz, H-2′), 3.74 (1H, dd, J = 9.4, 9.6 Hz, H-3′), 3.70 (1H, dd, J = 10.4, 4.0 Hz,

H-3), 3.63 (2H, dd, $J = 6.2$, 9.7 Hz, H-1, H-1), 3.45 (1H, dd, $J = 10.4$, 7.2 Hz, H-3), 3.38 (1H, dd, $J = 14.8$, 1.2 Hz, H-6′), 3.26 (1H, dd, $J = 9.4$, 9.6 Hz, H-4′), 3.07 (1H, dd, $J = 14.8$, 9.8 Hz, H-6′).

Compound 25: colorless oil; $[\alpha]_D$ +75.2° ($c = 0.12$, H_2O); FABMS (NBA) m/z (relative intensity) 433 (7) $[M + H]^+$; ^1H NMR (500 MHz) δ (C_5D_5N) 5.46 (1H, d, $J = 3.7$ Hz, H-1″), 4.80 (1H, d, $J = 7.8$ Hz, H-1′), 4.67 (2H, m, H-2″, H-4″), 4.58 (2H, m, H-1a, H-3″), 4.48 (1H, m, H-2), 4.46 (1H, d, $J = 3.3$ Hz, H-4′), 4.40 (3H, m, H-2′, H-3a, H-3b), 4.32 (1H, m, H-1b), 4.22 (2H, m, H-5′, H-5″), 4.10 (5H, m, H-3′, H-6a′, H-6b′, H-6a″, H-6b″).

Compound 26: colorless oil; $[\alpha]_D$ −7.0° ($c = 0.3$, H_2O); FABMS (glycerol) m/z (relative intensity) 277 (20) $[M + Na]^+$, 255 (12) $[M + H]^+$; ^1H NMR (500 MHz) δ (C_5D_5N) 4.91 (1H, d, $J = 7.6$ Hz, H-1′), 4.56 (1H, d, $J = 3.5$ Hz, H-4′), 4.52 (1H, dd, $J = 7.6$, 9.3 Hz, H-2′), 4.47 (1H, m, H-2), 4.42 (3H, m, H-6a′, H-6b′, H-3a), 4.25 (1H, dd, $J = 3.9$, 9.6 Hz, H-3b), 4.17 (1H, dd, $J = 3.0$, 9.3 Hz, H-3′), 4.13 (2H, d, $J = 5.4$ Hz, H-1a, H-1b), 4.08 (1H, dd, $J = 5.3$, 6.5 Hz, H-5′).

Fatty Acid Analysis. Fatty acid methyl esters were prepared by adding an ether solution of CH_2N_2 to a methanol solution of the acids. A hexane solution of the methyl esters was analyzed by GCMS using a capillary column (DB-5, J&W 0.25 mm × 30 m, 0.25 mm film thickness). Injector temperature: 250 °C; injector mode: splitless; carrier gas: He. The oven temperature program was 3 min at 100 °C, 5 °C min^{-1} to 270 °C, and 30 °C min^{-1} to 300 °C. The fatty acid methyl esters t_R's were compared to those of the standard mixture obtained from Sigma. The retention times of the acid methyl esters were: methyl hexadecadienoate, 24:21 min; methyl hexadecatrienoate, 24:29 min; methyl hexadecaenoate, 24:38 min; methyl palmitoate, 25:05 min; methyl octadecatetraenoate, 28:16 min; methyl linleoate, 28:27 min; methyl oleate, 28:30 min; and methyl linolenoate, 28:34 min.

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