Vesparioside from the Marine Sponge *Spheciospongia vesparia*, the First Diglycosylceramide with a Pentose Sugar Residue[‡]

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The marine sponge *Spheciospongia vesparia* produces vesparioside (**1a**), a diglycosylated glycosphingolipid which is the first example of a natural diglycosylceramide with a pentose sugar residue. The structure of vesparioside was mostly determined by extensive spectroscopic analysis but determination of the nature of the alkyl chains and elucidation of the absolute stereochemistry of the sugars and of the ceramide required chemical degradation. In this respect, an improved and simplified procedure for the microscale chemical degradation of glycosphingolipids was employed here for the first time.

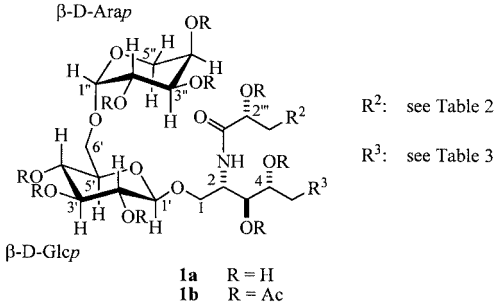
Introduction

Marine sponges are a rich source of novel glycosphingolipids which are often characterised by unprecedented structural features. Among them, and worthy of note, are plakosides from *Plakortis simplex*[1] which are immunosuppressive glycosphingolipids with a prenylated galactose as well as α-galactoglycosphingolipids[2−4] which are a new class of glycosphingolipids with an α-galactose as the first sugar of the carbohydrate chain. These have been found only in sponges of the genera *Agelas* and *Axinella* and are characterised by their immunostimulating[5] and antitumor[2,6] properties. Even though a relatively large number of papers on this topic has appeared in the literature in recent years, the study of glycolipids form sponges is far from being concluded and the structural variety of the isolated compounds is far from being fully explored.

In this paper, we report the isolation and structural elucidation of vesparioside (**1a**), a new diglycosylated glycosphingolipid from *Spheciospongia vesparia*, characterised by the presence of a β-arabinopyranoside linked at position 6 of the first sugar residue of the chain (a β-glucopyranoside). Vesparioside is the first example of a natural diglycosylceramide whose carbohydrate chain contains a pentose unit.

Results and Discussion

Spheciospongia vesparia was collected near Grand Bahamas Island (Bahamas) and kept frozen until extraction. The specimens were extracted with chloroform and methanol and the combined extracts were partitioned between water and BuOH. The organic phase was dried and, according to our standard procedure, a glycolipid fraction was obtained by subsequent reversed-phase and normal-phase column chromatography. The glycolipid fraction was acetylated and the peracetylated glycolipids were subjected to repeated HPLC on SiO_2 columns to give 4.5 mg of pure compound **1b**. Compound **1b** was deacetylated with MeOH/MeONa yielding the natural glycolipid **1a**.



Scheme 1

The ESI mass spectrum showed a series of sodiated pseudomolecular ion peaks at m/z = 986, 1000, 1014 and 1028, in accordance with the molecular formula $C_{52}H_{101}NO_{14} + nCH_2$ (n = 0−3). A high-resolution measurement performed on the most abundant ion at m/z = 1000.7265 confirmed the molecular formula $C_{53}H_{103}NO_{14}$ for the dominant homologue.

[‡] Glycolipids from Sponges, 14. Part 13: See ref.[4]

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A preliminary ^{1}H NMR spectroscopic analysis of the peracetyl derivative **1b** in $CDCl_3$ showed appropriate signals for a glycosphingolipid, i.e. the intense aliphatic chain signal at δ = 1.25 ppm, several signals of oxymethine and oxymethylene groups between δ = 5.4 ppm and 3.4 ppm and a characteristic amide NH doublet at δ = 6.76 ppm, suggesting the presence of a ceramide amide function. The ^{1}H NMR spectrum also showed, in the methyl region, a triplet at δ = 0.87 ppm (ethyl terminus) and a doublet at δ = 0.85 ppm (isopropyl terminus) the intensities of which were not in an integral ratio with respect to those of other signals in the spectrum. This showed that the alkyl chain mixture differs not only in the length but also in the branching of the alkyl chains.

Structure of the Ceramide

The ceramide portion of molecule is that commonly found in glycolipids from marine sponges, i.e. it is composed of a trihydroxylated, saturated sphinganine and an α-hydroxy fatty acid residue. The amide NH doublet at δ = 6.76 ppm (2-NH) allowed us to assign all the protons of the polar part of the sphinganine unit up to 6-H_2 through the COSY spectrum. The α-hydroxy substitution of the fatty acid residue was revealed by the absence, from the ^{1}H NMR spectrum of **1b**, of the characteristic triplet at δ = 2.3 ppm for the fatty acid α-protons, whereas a signal at δ = 5.12 (2′′′-H) was present in the spectrum and showed an intense correlation peak with the amide NH doublet in the ROESY spectrum. In addition, both 2′′′-H and 2-NH were shown, by the HMBC spectrum, to be coupled with the CO carbon atom at δ = 170.0 (C-1′′′) ppm. The length of the alkyl chains and the configurations of the stereogenic centres of the ceramide were established by chemical degradation (see below).

Structure of the Sugar Chain

The nature of the sugar unit was unambiguously determined by NMR spectroscopic analysis. The presence of two sugar units was revealed by two doublets at δ = 4.46 ppm (J = 8.1 Hz, 1′-H) and 5.03 ppm (J = 3.6 Hz, 1′′-H) which were assigned to the anomeric protons on account of the chemical shift of the relevant carbon atoms (δ = 100.5 ppm and 97.1 ppm, respectively) identified through the HMQC spectrum. These protons were used in the analysis of the COSY spectrum as starting points for the sequential assignment of all the protons in the disaccharide unit. Four more oxymethine protons and a couple of oxymethylene protons were assigned to the first sugar (the one with the anomeric proton at δ = 4.46 ppm), suggesting a hexose. The other sugar, in addition to the anomeric proton at δ = 5.03 ppm, only contained three oxymethine protons and a couple of oxymethylene protons and is therefore a pentose unit.

The hexose sugar residue could be readily recognised as a β-glucopyranoside because of the large coupling constants between all the ring protons (see Table 1) indicating their axial orientations. In addition, the deshielded chemical shifts of the oxymethine protons at positions 2′, 3′ and 4′ indicated that the relevant hydroxyl groups were acetylated and therefore not glycosylated. In contrast, the chemical shifts below δ = 4.0 ppm for the protons at C-6′ suggested glycosylation at this position and this was confirmed by three intense correlation peaks in the ROESY spectrum of the pentose anomeric proton, namely 1′′-H with 5′-H, 6′-Ha and 6′-Hb. Evidence of the linkage of this sugar residue to the ceramide primary hydroxyl group was provided by the ROESY spectrum which displayed correlation peaks of 1′-H with 1-Ha and 1-Hb, and by the HMBC spectrum which indicated a three-bond coupling between 1′-H and C-1.

The nature of the pentose residue was determined on the basis of the coupling constants between the ring protons. The 3′′-H signal displayed one large (10.8 Hz) and one small (3.5 Hz) coupling constant, showing 3′′-H to be an axial proton flanked by one axial and one equatorial proton. The signal of 4′′-H was a broad singlet indicating that this proton is clearly equatorial and that 2′′-H is therefore axial (the multiplicity of the 2′′-H signal could not be

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of vesparioside peracetate **1b** ($CDCl_3$)

Position		δ_H [mult., J (Hz)][a]	δ_C (mult.)[b]
1	a	3.84 (dd, 10.9, 2.9)	66.6 (CH_2)
	b	3.67[c]	
2		4.26 (m)	48.3 (CH)
2-N*H*		6.76 (d, 8.7)	–
3		5.11[c]	72.0 (CH)
4		4.85 (m)	73.2 (CH)
5		1.59 (m)	28.4 (CH_2)
6	a	1.32 (m)	25.6 (CH_2)
	b	1.19 (m)	
1′		4.46 (d, 8.1)	100.5 (CH)
2′		4.87 (dd, 9.6, 8.1)	71.3 (CH)
3′		5.18 (t, 9.6)	73.0 (CH)
4′		5.07 (t, 9.6)	68.8 (CH)
5′		3.63 (ddd, 9.6, 4.5, 2.5)	73.0 (CH)
6′	a	3.80 (dd, 11.8, 2.5)	66.3 (CH_2)
	b	3.48 (dd, 11.9, 4.5)	
1′′		5.03 (d, 3.6)	97.1 (CH)
2′′		5.14[c]	68.2 (CH)
3′′		5.29 (dd, 10.8, 3.5)	67.1 (CH)
4′′		5.36 (br. s)	69.0 (CH)
5′′	a	3.94 (br. d, 13.4)	60.5 (CH_2)
	b	3.67[c]	
1′′′		–	170.0 (C)
2′′′		5.12[c]	74.0 (CH)
3′′′		1.83 (m)	31.8 (CH_2)
4′′′		1.33 (m)	24.9 (CH_2)
Ac's	CH_3	2.24, 2.14, 2.12, 2.10, 2.04, 2.04, 2.01, 2.00, 1.98	21.0–20.5 (CH_3)
	CO	–	171.0–169.2

[a] Additional ^{1}H signals: δ = 1.25 (broad band, alkyl chain protons), 0.87 (t, J = 7.0, *n*-chain Me groups), 0.85 (d, J = 6.5, *iso*-chain Me groups) ppm. [b] Additional ^{13}C signals: δ = 31.9 (CH_2, ω-2), 22.7 (CH_2, ω-1), 22.7 (CH_3, *iso*-chain Me groups), 14.1 (CH_3, ω) ppm. [c] Submerged by other signals.

analysed due to the overlapping of signals). Finally, the anomeric proton 1′′-H is equatorial based on its small (3.6 Hz) coupling constants with 2′′-H. On the basis of the above data, the pentose residue is clearly a β-arabinopyranoside. The pyranose ring closure was confirmed by the coupling between 5′′-Hb and C-1′′ indicated by the HMBC spectrum, as well as by the low-field chemical shift of 4′′-H indicating acetylation of the relevant OH group.

Once the structure of the peracetyl derivative **1b** was assessed, the 1- and 2-D NMR spectra of the natural GSL **1a** were also recorded and analysed. The information provided by the COSY and HMQC NMR spectra of **1a** confirmed all the structural features determined so far and allowed the assignments of all the resonances in its ^{1}H and ^{13}C NMR spectra (see Experimental Section).

Chemical Degradation

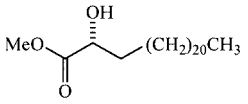
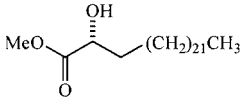
The remaining structural features of compound **1a** were determined by microscale chemical degradation. In spite of the huge progress of NMR spectroscopy and mass spectrometry, chemical degradation is still an essential step in the study of glycosphingolipids. This is particularly true for marine glycosphingolipids which are often present in the organisms as inseparable mixtures of homologues and are characterised by branched and odd-carbon alkyl chains. Many marine glycosphingolipids can only be isolated in an amount of 1 mg or less so that even a microscale degradation procedure can destroy a significant portion of the sample. Therefore, it is very important to try to increase the sensitivity of this analytical procedure.

The degradation procedure we used for vesparioside, which is quite complex and somewhat different from that used in our previous papers, is summarised in Scheme 2. Compound **1a** (100 μg) was subjected to acidic methanolysis with HCl in MeOH and the reaction products were partitioned between $CHCl_3$ and H_2O/MeOH (8:2) giving an aqueous layer consisting of methyl glycosides (fraction A) and an organic layer composed of sphinganines and fatty acid methyl esters (fraction B).

The methyl glycoside fraction was used to determine the absolute configuration of the two sugars.[4] Fraction A was perbenzoylated with BzCl in pyridine and the reaction mixture was separated by HPLC. The chromatogram contained two peaks which were identified as methyl tetra-*O*-benzoyl-β-D-glucopyranoside (**2**) and methyl tri-*O*-benzoyl-β-D-arabinopyranoside (**3**) since they each showed the same retention times, ^{1}H NMR spectra and CD spectra as those obtained from authentic samples prepared with the same procedure from, respectively, D-glucose and D-arabinose.

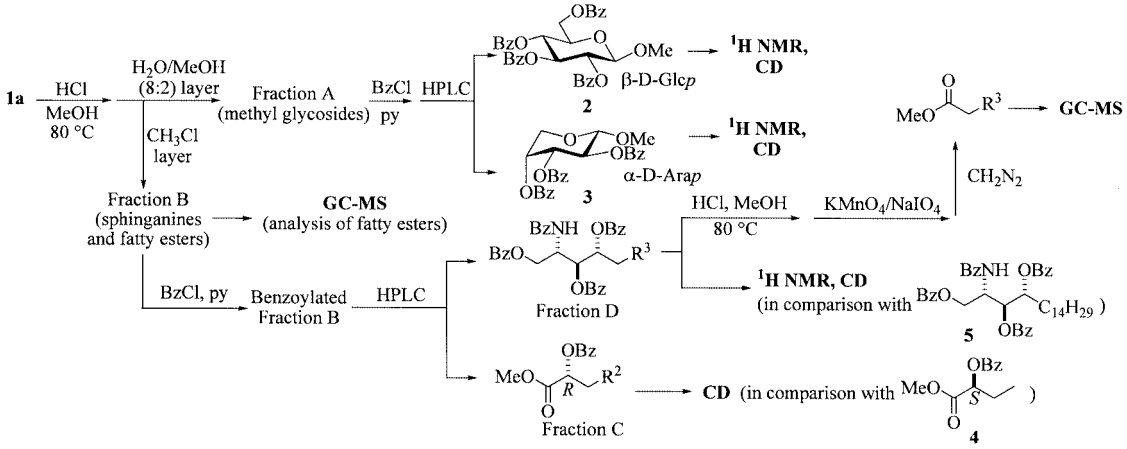
Fraction B, containing the sphinganines and the fatty acid methyl esters from the methanolysis, was analysed by GC-MS and was shown to contain two different unbranched 2-hydroxy fatty acids, identified by comparison of their retention times and mass spectra with those of authentic samples (Table 2). Following this, fraction B was perbenzoylated as described above and the reaction mixture was separated by normal-phase HPLC. This led to a fraction composed of 2-benzoyloxy fatty acid methyl esters (fraction C) and a fraction composed of perbenzoylated 4-hydroxysphinganines (fraction D).

Table 2. Fatty acyl composition of vesparioside **1a**

Fatty acid methyl ester	
	83.2 %
	16.8 %

The CD spectrum of fraction C showed a negative Cotton effect at λ_{max} = 232 nm ($\Delta\varepsilon$ = −4.4). Since the CD spectrum of methyl (*S*)-2-benzoyloxybutyrate (**4**), prepared from commercial (*S*)-2-hydroxybutyric acid, showed a positive Cotton effect at $\lambda_{max.}$ = 231 nm ($\Delta\varepsilon$ = +4.7) the (*R*) configuration of the 2-hydroxy fatty acids from compound **1a** could be deduced.

The *ribo* relative configurations of the benzoylated sphinganines were determined by recording the ^{1}H NMR spec-

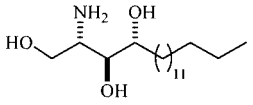
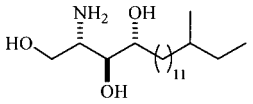
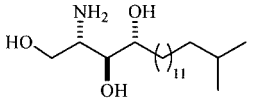
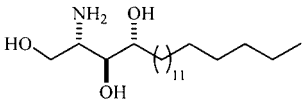


Scheme 2. The new microscale degradation procedure for glycolipids

trum of fraction D which was identical (apart from the methyl region) to that of an authentic sample of D-*ribo*-phytosphingosine perbenzoate (**5**).[4] Their absolute configurations were deduced from the CD spectrum in MeCN solution which matched that of compound **5** recorded in the same solvent.[4]

After this, fraction D was subjected to acidic methanolysis to remove the benzoyl groups and then to Lemieux oxidation with $KMnO_4/NaIO_4$ to convert the 4-hydroxysphinganines to carboxylic acids with three less carbon atoms. The obtained fatty acids were methylated with CH_2N_2 and analysed by GC-MS. The results are reported in Table 3, in the form of the structures of the corresponding sphinganines.

Table 3. Sphinganine composition of vesparioside **1a**

Sphinganine	
	60.4 %
	9.4 %
	25.5 %
	4.7 %

Conclusion

The novel GSL vesparioside **1a** is the first example of a natural diglycosylceramide containing a pentose unit in the sugar head. The structure of vesparioside was determined by the combined use of spectroscopic analysis and a new and improved procedure for microscale chemical degradation.

The new degradation procedure described here allowed us: (a) to confirm the nature of the sugars in the carbohydrate chain and determine their absolute configurations, (b) to determine the relative and absolute configuration of the sphinganines as well as the nature of their alkyl chains and (c) to determine the configuration at C-2 of the 2-hydroxy fatty acids and the nature of their alkyl chains. The modifications we made to the method, in comparison with those previously reported, were aimed at improving sensitivity and reducing manipulation of the sample and thus the consequent risk of contamination.

In previous methods,[4,7] the sphinganine fraction was separated from the fatty acid fraction using SiO_2 column chromatography. This is no longer necessary because sphinganines and fatty acids can be now benzoylated together and, after the introduction of the benzoate chromophore, they can be separated using an HPLC instrument equipped with a sensitive UV detector. This can dramatically improve the purity of the samples. In addition, the configurations of 2-hydroxy fatty acids can now be determined using the CD spectra of their benzoylated methyl esters instead of those of the underivatised methyl esters. As a consequence, the measured Cotton effect is more than three times stronger ($\Delta\varepsilon = -4.4$ vs. -1.3) and at a longer wavelength (232 vs. 212 nm), making the measurement easier.

The only step that still requires extensive manipulation of the sample is the Lemieux oxidation of the sphinganines. We could not avoid this because of the concurrent presence of *iso* and *anteiso* alkyl chains in the sphinganines. In such a case, conversion of the sphinganines to fatty acid methyl esters and subsequent GC-MS analysis is mandatory for a successful separation and quantitative analysis. In fact, fatty acid methyl esters can be separated very effectively by GC and their mass spectra have been very well studied allowing location of a branching methyl at any position.

In simpler cases, Lemieux oxidation could, in future work, be replaced by reversed-phase LC-MS analysis of the benzoylated sphinganines. This would enable a further scaling-down of this analytical method making it suitable for a detailed structural analysis of glycosphingolipids other than those of marine origin, those from mammalian tissues for example.

Experimental Section

General Remarks: High-Resolution ESI-MS spectra were performed with a Micromass QTOF Micro mass spectrometer by dissolving the sample in MeCN/H_2O (1:1) with 0.1% TFA. ESI MS experiments were performed on a Applied Biosystem API 2000 triple-quadrupole mass spectrometer. The spectra were recorded by infusion into the ESI source using MeOH as the solvent. Optical rotations were measured at 589 nm with a Perkin−Elmer 192 polarimeter using a 10-cm microcell. ^1H and ^{13}C NMR spectra were determined with a Varian UnityInova spectrometer at 500.13 and 125.77 MHz, respectively. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: $\delta_H = 7.26$ ppm, $\delta_C = 77.0$ ppm; [D_5]pyridine: $\delta_H = 8.73$ ppm, 7.56 ppm and 7.21 ppm; $\delta_C = 149.9$ ppm, 135.6 ppm and 123.6 ppm). For an accurate measurement of the coupling constants, the 1-D ^1H NMR spectra were transformed with 64 K points (digital resolution: 0.09 Hz). Homonuclear ^1H connectivities were determined by COSY experiments. Through-space ^1H connectivities were highlighted using a ROESY experiment with a mixing time of 500 ms. The reverse multiple-quantum heteronuclear correlation (HMQC) spectra were recorded using a pulse sequence with a BIRD pulse of 0.5 s before each scan to suppress the signal originating from protons not directly bound to ^{13}C nuclei. The interpulse delays were adjusted for an average $^1J_{C,H}$ of 142 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiment was optimised for a $^3J_{C,H}$ of 8.3 Hz. GC-MS spectra were performed with a Hewlett−Packard 5890 gas chromatograph with a mass selective detector MSD HP 5970 MS, a split/splitless injector and an HP-5 fused-silica column of dimensions 25 m × 0.20 mm (cross-linked 25% Ph Me silicone,

0.33-mm film thickness). The temperature of the column was varied, after a delay of 3 min from the injection, from 150 °C to 280 °C with a rate of 10 °C·min^{-1}. Quantitative determination was based on the areas of the GLC peaks. High performance liquid chromatography (HPLC) was performed with a Varian Prostar 210 apparatus equipped with a Varian 350 refractive index detector or a Varian 325 UV detector.

Collection, Extraction and Isolation: Specimens of *Spheciospongia vesparia* were collected in the summer of 2000 along the coast of Grand Bahamas Island (Bahamas) and identified by Prof. M. Pansini (University of Genoa). They were frozen immediately after collection and kept frozen until extraction. The sponge (100 g dry weight after extraction) was homogenised and extracted with methanol (3 × 1 L) and then chloroform (3 × 1 L). The combined extracts were partitioned between H_2O and *n*BuOH. The organic layer was concentrated in vacuo to afford 12.1 g of a dark green oil which was chromatographed on a column packed with RP-18 silica gel. A fraction eluted with $CHCl_3$ (3.6 g) was further chromatographed on an SiO_2 column giving a fraction [285 mg, eluent EtOAc/MeOH (7:3)] mainly composed of glycolipids. This fraction was peracetylated with Ac_2O in pyridine at 25 °C for 12 h. The acetylated glycolipids were subjected to HPLC separation on an SiO_2 column [eluent: *n*-hexane/EtOAc (6:4)], thus affording a mixture (11.7 mg) containing **1b** and other glycolipids. Further normal-phase HPLC purification [eluent: *n*-hexane/*i*PrOH (95: 5)] gave 4.5 mg of vesparioside peracetate (**1b**).

Vesparioside Peracetate (1b): Colourless oil, $[\alpha]_D^{25} = -18$ ($CHCl_3$, $c = 0.3$). ^{1}H and ^{13}C NMR: Table 1. Composition in fatty acids: Table 2. Composition in sphinganines: Table 3.

Deacetylation of 1b: Compound **1b** (3.0 mg) was dissolved in MeOH (950 μL) and a 0.4 M solution of MeONa in MeOH was added (50 μL). The reaction was allowed to proceed for 18 h at 25 °C and the mixture was then dried under nitrogen and the residue partitioned between water and chloroform. After removal of the solvent, the organic layer gave 2.1 mg of the native glycosphingolipid **1a**.

Vesparioside (1a): Colourless amorphous solid, $[\alpha]_D^{25} = -12$ (c = 0.1 in MeOH). ESI MS (positive ion mode, MeOH): m/z = 986, 1000, 1014, and 1028 ([M + Na]$^+$ series). HRESI MS (positive ion mode, MeOH): m/z = 1000.7265 ([M + Na]$^+$, $C_{53}H_{103}NNaO_{14}^+$ gives 1000.7276). ^{1}H NMR ([D$_5$]pyridine): δ = 0.86 (*n*- and *iso*-chain Me groups), 1.25 (large band, alkyl chains), 1.67 (m, 2 H, 4′′′-H$_2$), 1.71 (m, 2 H, 6-H$_2$), 1.90 (m, 1 H, 5-Hb), 1.99 (m, 1 H, 3′′′-Hb), 2.21 (m, 1 H, 3′′′-Ha), 2.23 (m, 1 H, 5-Ha), 4.00 (ddd, J = 7.9, 7.9, and 3.2 Hz, 1 H, 2′-H), 4.04 (overlapped, 5′-H), 4.07 (overlapped, 5′′-Hb), 4.11 (overlapped, 4′-H), 4.18 (overlapped, 3′-H), 4.18 (overlapped, 6′-Hb), 4.25 (m, 1 H, 4-H), 4.30 (overlapped, 5′′-Ha), 4.32 (overlapped, 3-H), 4.38 (br. s, 1 H, 4′′-H), 4.55 (overlapped, 3′′-H), 4.56 (overlapped, 1-Hb), 4.58 (overlapped, 2′′′-H), 4.63 (overlapped, 2′′-H), 4.64 (br. d, J = 10.5 Hz, 1 H, 6′-Ha), 4.73 (dd, J = 10.7, 6.0 Hz, 1 H, 1-Ha), 4.97 (d, J = 7.9 Hz, 1 H, 1′-H), 5.28 (m, 1 H, 2-H), 5.55 (d, J = 3.1 Hz, 1 H, 1′′-H), 6.01 (d, J = 6.5 Hz, 1 H, 4-OH), 6.30 (d, J = 2.9 Hz, 1 H, 4′′-OH), 6.50 (d, J = 7.3 Hz, 1 H, 2′′-OH), 6.61 (d, J = 5.4 Hz, 1 H, 3′′-OH), 6.79 (d, J = 6.3 Hz, 1 H, 3-OH), 7.31 (2 H, overlapped, 2′-OH and 4′-OH), 7.38 (d, J = 4.0 Hz, 1 H, 3′-OH), 7.66 (d, J = 5.1 Hz, 1 H, 2′′′-OH), 8.58 (d, J = 9.2 Hz, 1 H, 2-NH). ^{13}C NMR ([D$_5$]pyridine, ppm): δ = 14.2 (CH$_3$, *n*-chain Me groups), 22.8 (CH$_3$, *iso*-chain Me groups), 23.0 (CH$_2$, *n*-chain ω-1 CH$_2$ groups), 25.9 (CH$_2$, C-6), 26.5 (CH$_2$, C-4′′′), 30.5−29.5 (several CH$_2$, alkyl chains), 32.1 (CH$_2$, *n*-chain ω-2 CH$_2$ groups), 33.9 (CH$_2$, C-5), 35.6 (CH$_2$, C-3′′′), 51.2 (CH, C-2), 64.3 (CH$_2$, C- 5′′), 69.0 (CH$_2$, C-6′), 70.0 (CH, C-4′′), 70.6 (CH$_2$, C-1), 70.7 (CH, C-2′′), 70.7 (CH, C-3′′), 71.7 (CH, C-4′), 72.2 (CH, C-4), 72.3 (CH, C-2′′′), 75.0 (CH, C-2′), 75.7 (CH, C-3), 76.8 (CH, C-5′), 78.3 (CH, C-3′), 101.7 (CH, C-1′′), 105.3 (CH, C-1′), 175.3 (C, C-1′′′) ppm; Composition in fatty acids: Table 2. Composition in sphinganines: Table 3.

Methanolysis of 1a: Compound **1a** (100 μg) was dissolved in 1 N HCl in 91% MeOH (500 μL) and the resultant solution was kept for about 12 h at 80 °C in a sealed tube. The reaction mixture was dried under nitrogen and partitioned between $CHCl_3$ and H_2O/MeOH (8:2). The aqueous layer was concentrated to give a mixture of methyl glycosides (fraction A), whereas the organic layer contained a mixture of α-hydroxy acid methyl esters and sphinganines (fraction B).

Methyl Tetra-*O*-benzoyl-β-D-glucopyranoside (2): D-Glucose (2.0 mg) was subjected to acidic methanolysis as described above. The resultant methyl glycosides were benzoylated with benzoyl chloride (50 μL) in pyridine (500 μL) at 25 °C for 16 h. The reaction was then quenched with MeOH and after 30 min was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum for 24 h with an oil pump. The residue was purified by HPLC (column: Luna SiO_2, 5 μ; eluent: *n*-hexane/*i*PrOH, 99:1, flow 1 mL/min, UV dedector) affording the glycoside **2** (t_R = 10.2 min). ^{1}H NMR ($CDCl_3$, ppm): δ = 3.53 (s, 3 H, OMe), 4.14 (m, 1 H, 5-H), 4.49 (dd, J = 12.0, 5.4 Hz, 1 H, 6-Hb), 4.63 (dd, J = 12.0, 3.1 Hz, 1 H, 6-Ha), 4.75 (d, J = 7.8, 1 H, 1-H), 5.50 (dd, J = 9.8, 7.8 Hz, 1 H, 2-H), 5.66 (t, J = 9.8 Hz, 1 H, 4-H), 5.89 (t, J = 9.8, 1 H, 3-H), 7.55−7.24 (12 H, overlapping signals, benzoyl protons), 7.80 (t, J = 8.1 Hz, 2 H, benzoyl *ortho* protons), 7.94 (d, J = 8.1 Hz, 2 H, benzoyl *ortho* protons), 7.88 (d, J = 8.1 Hz, 2 H, benzoyl *ortho* protons), 8.00 (d, J = 8.1 Hz, 2 H, benzoyl *ortho* protons). CD (MeCN): $\lambda_{max.}$ ($\Delta\varepsilon$) = 235 nm (+15), 220 nm (−2).

Methyl Tri-*O*-benzoyl-α-D-arabinopyranoside (3): D-Arabinose (2.0 mg) was subjected to acidic methanolysis followed by benzoylation as described above. HPLC purification under the same conditions as above afforded the glycoside **3** (t_R = 12.5 min). ^{1}H NMR ($CDCl_3$, ppm): δ = 3.54 (s, 3 H, OMe), 3.89 (br. d, J = 12.9 Hz, 1 H, 5-Hb), 4.31 (dd, J = 12.9 and J = 3.7 Hz, 1 H, 5-Ha), 4.64 (d, J = 6.3 Hz, 1 H, 1-H), 5.58 (dd, J = 9.0, 3.5 Hz, 1 H, 3-H), 5.73−5.65 (overlapping signals, 2 H, 2-H and 4-H), 7.58−7.28 (overlapping signals, 9 H, benzoyl protons), 7.89 (d, J = 7.9 Hz, 2 H, benzoyl *ortho* protons), 8.00 (d, J = 7.9 Hz, 2 H, benzoyl *ortho* protons), 8.03 (d, J = 7.9 Hz, 2 H, benzoyl *ortho* protons). CD (MeCN): $\lambda_{max.}$ ($\Delta\varepsilon$) = 237 nm (−52), 221 nm (+10).

Methyl (*S*)-2-Benzolyoxybutanoate (4): (*S*)-2-Hydroxybutanoic acid (Fluka, 10 mg) was treated with an excess of an ethereal solution of CH_2N_2 until the yellow colour persisted and then benzoylated as described above. The crude reaction mixture was purified under the same conditions as above to give 7.5 mg of the ester **4** (t_R = 5.5 min). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.09 (t, J = 7.4, 3 H, 4-H$_3$). 2.03 (m, 2 H, 3-H$_2$), 3.77 (s, 3 H, OMe), 5.21 (t, J = 6.1 Hz, 1 H, 2-H), 7.46 (t, J = 7.6 Hz, 2 H, benzoyl *meta* protons), 7.59 (t, J = 7.5 Hz, 1 H, benzoyl *para* proton), 8.09 (d, J = 8.0 Hz, 2 H, benzoyl *ortho* protons). CD (MeCN): $\lambda_{max.}$ ($\Delta\varepsilon$) = 231 nm (+4.7).

Absolute Stereochemistry of Methyl Glycosides from Compound 1a: Fraction A from methanolysis of compound **1a** was benzoylated with benzoyl chloride (20 μL) in pyridine (200 μL) at 25 °C for 16 h. The reaction was then quenched with MeOH and after 30 min was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum for 24 h with an oil pump. The residue was purified by HPLC (column: Luna SiO_2, 5 μ; eluent: *n*-hexane/

*i*PrOH, 99:1, flow 1 mL/min). The chromatogram contained two peaks which were identified as compounds **2** and **3** by a comparison of their retention times, ^{1}H NMR spectra and CD spectra with those of the authentic samples prepared from D-glucose and D-arabinose.

Analysis of Fatty Acid Methyl Esters: Fraction B from methanolysis of compound **1a** was analysed by GLC-MS and its components identified by a comparison of their retention times and mass spectra with those of authentic samples. The results are compiled in Table 2.

Analysis of Fraction B: Fraction B from methanolysis of compounds **1a** was benzoylated as described above and the crude reaction mixture was purified by HPLC (column: Luna SiO_2, 5 μ; eluent: *n*-hexane/*i*PrOH, 99:1, flow 1 mL/min). The chromatogram contained two peaks which were identified as being due to a mixture of homologous benzoylated fatty acid methyl esters (fraction C, t_R = 4.0 min) and a mixture of perbenzoylated sphinganines (fraction D, t_R = 7.2 min) on the basis of their respective ^{1}H NMR spectra.

Methyl (*R*)-2-Benzoyloxyalkanoate: (Fraction C, mixture of homologues) ^{1}H NMR ($CDCl_3$, ppm): δ = 0.87 (ω Me group), 1.25 (large band, alkyl chain), 1.99 (m, 2 H, 3-H_2), 3.78 (s, 3 H, OMe), 5.23 (t, J = 6.0 Hz, 1 H, 2-H), 7.44 (t, J = 7.6 Hz, 2 H, benzoyl *meta* protons), 7.58 (t, J = 7.5 Hz, 1 H, benzoyl *para* proton), 8.10 (d, J = 8.0 Hz, 2 H, benzoyl *ortho* protons). CD (MeCN): $\lambda_{max.}$ ($\Delta\varepsilon$) = 231 nm (−4.4).

(2*S*,3*S*,4*R*)-1,3,4-*O*-Benzoyl-2-benzamidoamino-1,3,4-alkanetriol: (Fraction D, mixture of homologues): CD (MeCN): $\lambda_{max.}$ ($\Delta\varepsilon$) = 233 nm (−7), 220 nm (+1); the ^{1}H NMR spectrum was identical (apart from the methyl region) to that of an authentic sample of D-*ribo*-phytosphingosine perbenzoate (**5**).[4]

Oxidative Cleavage and GC-MS Analysis of Sphinganines: Fraction D was debenzoylated by acidic methanolysis as described above, subjected to oxidative cleavage with $KMnO_4$/$NaIO_4$ as described[8] and the resultant carboxylic acids were methylated with CH_2N_2. The esters obtained were analysed by GC-MS and the results are compiled in Table 3, expressed in terms of the original sphinganines.

Supporting Information Available: (See also footnote on the first page of this article). ^{1}H NMR and ESI MS spectra of compound **1a**. 1-D and 2-D NMR spectra of compound **1b**. ^{1}H NMR and CD spectra of the degradation products **2**−**5**.

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