DOI: 10.1002/ejoc.200801230

Amphiceramide A and B, Novel Glycosphingolipids from the Marine Sponge *Amphimedon compressa*[‡]

Valeria Costantino,[a] Ernesto Fattorusso,[a] Concetta Imperatore,[a] Alfonso Mangoni,*[a] and Roberta Teta[a]

Keywords: Amines / Carbohydrates / Glycolipids / Sponges / Structure elucidation

Glycolipid analysis of the Caribbean sponge *Amphimedon compressa* has shown it to contain two novel glycosphingolipids, amphiceramide A (**1a**) and B (**2a**), which possess an unusual Δ^6-phytosphingosine. The saccharide chain of amphiceramide A is composed of a β-glucose residue glycosylated at the 6-position by an *N*-acetyl-β-glucosamine and has never been found before in a natural product. The saccharide chain of amphiceramide B consists of an allolactose [Gal(1β→6)Glc] residue β-linked to the ceramide and is found here for the first time in a natural glycosphingolipid. In addition, the sponge contains a new molecular species, acetamidoglucosyl ceramide (**3a**), and the known glucosyl ceramide **4a** (halicerebroside A) and melibiosyl ceramide **5a** (amphimelibioside C).

Introduction

Glycosphingolipids (GSLs) from marine sponges represent an increasingly rich family of compounds that are significantly different from the GSLs found in mammals.[1–7] The most distinctive structural features of sponge GSLs, which make them unique among GSLs from animals, can be summarized as follows. (i) The ceramide is highly hydroxylated with an almost constant presence of trihydroxylated sphingosines (phytosphingosines) and α-hydroxy fatty acids. (ii) Sponge GSLs often occur as complex mixtures of homologues, sharing the same polar part and differing in the ceramide alkyl chains. These mixtures contain high numbers of methyl-branched (iso and anteiso) and odd-numbered carbon sphingosines, whereas fatty acids are usually unbranched. (iii) The oligosaccharide chains show a remarkable chemical diversity. Unusual structural features, like the α-anomeric configuration of the first sugar, the glycosylation at the 2-position of the first sugar, or the presence of hexofuranoses, make many of these saccharide chains unprecedented in natural glycoconjugates.

As a part of our continuing study of glycolipids from sponges, we have examined the glycolipid composition of the Caribbean sponge *Amphimedon compressa* and have shown it to contain two novel glycosphingolipids, which we have named amphiceramide A (**1a**) and B (**2a**) (Scheme 1). In addition, the sponge contains a new molecular species of acetamidoglucosyl ceramide (**3a**), and the known glucosyl ceramide (**4a**; halicerebroside A)[8] and melibiosyl ceramide (**5a**; amphimelibioside C).[3]

Amphiceramide A and B are diglycosyl ceramides with the same unusual Δ^6-phytosphingosine moiety that is present in amphimelibioside C. The saccharide chain of amphiceramide A (**1a**), composed of a β-glucose residue glycosylated at the 6-position by an *N*-acetyl-β-glucosamine, has never been found before in a natural product. The saccharide chain of amphiceramide B (**2a**) consists of an allolactose [Gal(1β→6)Glc] residue β-linked to the ceramide and is found here for the first time in a natural glycosphingolipid.

The elucidation of the stereostructures of the amphiceramides has been accomplished by a combination of extensive one- and two-dimensional NMR spectroscopy, MS and MS/MS analysis, and chemical degradation, as described below.

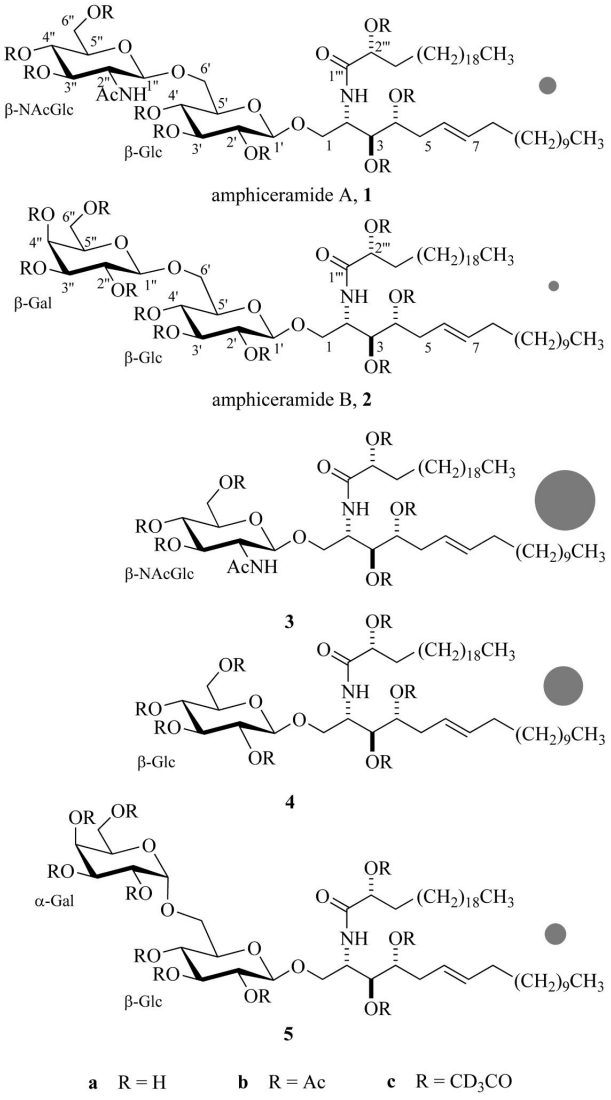
Results and Discussion

The BuOH-soluble fraction obtained from combined chloroform and methanol extracts of *A. compressa* was subjected first to reversed-phase and then to normal-phase silica gel column chromatography, which gave a fraction mainly composed of glycolipids. According to our usual procedure, the fraction was acetylated with Ac_2O in pyridine to give a peracetylated fraction, which is easier to purify by HPLC on an SiO_2 column. HPLC separation yielded six peracetylated glycolipids, each isolated as a mixture of homologues. Unlike our observations with other sponges,

[‡] Glycolipids from Sponges, 21. Part 20: Ref.[1]

[a] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II",
Via D. Montesano 49, 80131 Napoli, Italy
Fax: +39-081-678552
E-mail: alfonso.mangoni@unina.it

Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.



Scheme 1. Glycosphingolipids from *Amphimedon compressa*. The area of each circle is proportional to the amount of the relevant compound in the sponge.

these mixtures were relatively simple, with one single major homologue in each fraction, as judged by the MS and ^{1}H NMR data. Therefore, the six glycolipid mixtures were subjected to reversed-phase HPLC separation, which yielded the six pure peracetylated glycolipids **1b**–**5b** (Scheme 1).

To distinguish any acetyl group possibly present in the natural glycolipid from those introduced during the acetylation reaction, the isolation procedure was repeated by using deuteriated acetic anhydride in the acetylation step, which gave the pertrideuterioacetylated derivatives **1c**–**5c**. The ^{1}H NMR spectra of compounds **2c**, **4c**, and **5c** were identical to those of the corresponding peracetylated derivatives, except that no acetyl methyl singlets were present in the spectrum. This showed that all the acetyl groups came from the acetylation reaction. The ^{1}H NMR spectrum of compound **1c** showed one acetyl methyl singlet, indicating that one acetyl group is present in the natural compound **1a**. The subsequent structure elucidation showed that this acetyl group is part of an amide function. Likewise, the ^{1}H NMR spectrum of compound **3d** also contained one acetyl signal, which showed that the natural compound **3a** contains an acetamido group. Finally, the peracetylated glycolipids were deacetylated by using MeONa/MeOH (the acetamido groups present in compounds **1a** and **3a** survive these reaction conditions) to give the corresponding natural glycolipids **1a**–**5a**.

Amphiceramide A (1a)

The positive-ion ESI mass spectrum of amphiceramide A (**1a**) showed a sodiated pseudomolecular ion peak at *m*/*z* = 1041, in accord with the molecular formula $C_{72}H_{120}N_2NaO_{24}$. This was confirmed by a high-resolution measurement at *m*/*z* = 1041.7183. The ESI mass spectrum of the peracetyl derivative **1b** showed an $[M + Na]^+$ pseudomolecular ion peak at *m*/*z* = 1419, which indicates the presence of nine additional acetyl groups. Compound **1b** was used for the subsequent NMR analysis.

The ^{1}H NMR spectral features of compound **1b** showed it to be a glycosphingolipid, that is, the NH doublet at δ = 6.94 ppm of the ceramide amide function, a series of overlapped signals in the region between δ = 5.4 and 3.4 ppm, nine signals at $\delta \approx 2.0$ ppm from the acetyl groups, an intense signal at δ = 1.24 ppm indicative of a long aliphatic chain, and a methyl (6 H) triplet at δ = 0.88 ppm as the sole signal in the high-field region of the spectrum, which indicates that the alkyl chains of the ceramide are both unbranched. A second amide NH doublet at δ = 6.48 ppm suggested the presence of an amino sugar in the molecule.

The absence of the characteristic triplet of the fatty acid α protons at δ = 2.3 ppm suggested the presence in the ceramide moiety of an α-hydroxy acid, as usually found in GSLs from marine sponges. In addition, the presence of two methine groups resonating at δ = 134.9 and 123.7 ppm evinced from the ^{13}C NMR and HSQC spectra indicated a disubstituted C=C double bond. Starting from the NH doublet at δ = 6.94 ppm (2-H), the COSY spectrum allowed sequential assignment of all the protons of the sphingosine (Table 1). In particular, the scalar coupling of the two diastereotopic methylene protons with signals at δ = 2.37 and 2.24 ppm (5-H_2) with the oxymethine proton with signal at δ = 4.94 ppm (4-H) and the olefinic proton with signal at δ = 5.24 ppm (6-H) located the double bond on the sphingosine at the 6-position. In addition, the coupling constant between 6-H and 7-H (15.2 Hz) indicated the (*E*) stereochemistry of the double bond. The HMBC correlation peaks for the coupling of 2-H with C-1′′′ (δ = 170.2 ppm) and C-2 (δ = 48.9 ppm) confirmed the amide linkage between the sphingosine and the fatty acid.

The key step in the structure elucidation of a GSL is the identification of the nature of the sugars composing the saccharide chain. This can be achieved by collecting data from COSY, TOCSY, and HSQC experiments to identify the spin system of each sugar unit and to assign the protons within each spin system. If the sugars are in the pyranose form, the nature of each sugar can then be easily identified

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for **1b** and **2b** ($CDCl_3$).

Position	**1b**		**2b**	
	δ_H [ppm] (mult., J [Hz])	δ_C [ppm] (mult.)	δ_H [ppm] (mult., J [Hz])	δ_C [ppm] (mult.)
1a	3.80 (dd, 10.9, 4.0)	65.8 (CH_2)	3.90[a]	66.9 (CH_2)
1b	3.69 (dd, 10.9, 4.2)		3.61[a]	
2	4.28[a]	48.9 (CH)	4.32 (m)	48.4 (CH)
2-NH	6.94 (d, 8.4)	–	6.88 (d, 8.9)	–
3	5.04 (dd, 6.2, 4.9)	72.5 (CH)	5.09 (dd, 7.3, 4.1)	72.1 (CH)
4	4.94 (ddd, 8.9, 4.9, 3.5)	72.4 (CH)	4.91 (ddd, 9.3, 4.1, 3.6)	72.6 (CH)
5a	2.37 (m)	32.7 (CH_2)	2.36 (m)	32.3 (CH_2)
5b	2.24[a]		2.24[a]	
6	5.24 (ddd, 15.2, 6.9, 6.9)	123.7 (CH)	5.26 (ddd, 15.2, 6.7, 6.7)	124.1 (CH)
7	5.48 (ddd, 15.2, 6.8, 6.8)	134.9 (CH)	5.47 (ddd, 15.2, 6.8, 6.8)	134.8 (CH)
8	1.96[a]	32.6 (CH_2)	1.95 (m)	32.7 (CH_2)
9–15, 4′′′–19′′′	1.24[a]	29.7 (CH_2)	1.24[a]	29.8 (CH_2)
16, 20′′′	1.25[a]	31.9 (CH_2)	1.24[a]	32.0 (CH_2)
17, 21′′′	1.28[a]	22.7 (CH_2)	1.28[a]	22.8 (CH_2)
18, 22′′′	0.88 (t, 6.8)	14.1 (CH_3)	0.88 (t, 6.8)	14.2 (CH_2)
1′	4.46 (d, 8.0)	100.1 (CH)	4.44 (d, 8.0)	100.6 (CH)
2′	4.85 (dd, 9.6, 8.0)	71.2 (CH)	4.84 (dd, 9.7, 8.0)	71.5 (CH)
3′	5.18 (t, 9.5)	72.8 (CH)	5.15 (t, 9.6)	72.9 (CH)
4′	4.98 (dd, 10.0, 9.5)	68.5 (CH)	4.93 (t, 9.9, 9.5)	69.0 (CH)
5′	3.65 (ddd, 10.0, 5.5, 1.7)	73.6 (CH)	3.64 (m)	73.4 (CH)
6′a	3.95[a]	67.6 (CH_2)	3.88 (dd, 11.2, 1.7)	68.0 (CH_2)
6′b	3.52 (dd, 11.9, 5.5)		3.60[a]	
1′′	4.61 (d, 8.4)	101.4 (CH)	4.52 (d, 8.0)	101.3 (CH)
2′′	3.95[a]	54.2 (CH)	5.17 (t, 10.5, 8.0)	68.8 (CH)
2′′-NH	6.48 (d, 8.9)	–	–	–
2′′-NAc	1.95	23.1 (CH_3)	–	–
	–	170.7 (C)	–	–
3′′	5.24 (dd, 10.5, 9.4)	72.7 (CH)	5.04 (dd, 10.5, 3.4)	71.0 (CH)
4′′	5.06 (dd, 10.0, 9.4)	68.7 (CH)	5.38 (dd, 3.4, 1.2)	67.2 (CH)
5′′	3.70[a]	71.8 (CH)	3.92 (ddd, 6.7, 6.7, 1.2)	70.8 (CH)
6′′a	4.28 (dd, 12.3, 4.7)	62.1 (CH_2)	4.20 (dd, 11.3, 6.5)	61.3 (CH_2)
6′′b	4.10 (dd, 12.3, 2.3)		4.10 (dd, 11.3, 6.9)	
1′′′	–	170.2 (C)	–	169.8 (C)
2′′′	5.11 (dd, 7.5, 4.6)	74.1 (CH)	5.13 (dd, 7.3, 4.8)	74.1 (CH)
3′′′	1.84 (m)	31.7 (CH_2)	1.84[a]	31.9 (CH_2)
OAc groups	2.22, 2.10, 2.09, 2.06, 2.04, 2.03, 2.02, 2.01, 1.98	21.1–20.6 (CH_3)	2.22, 2.15, 2.09, 2.07, 2.06, 2.04, 2.04, 2.02, 1.98, 1.98	21.1–20–7 (CH_3)
	–	171.1–169.3 (C)	–	170.7–169.3

[a] Overlapped signal.

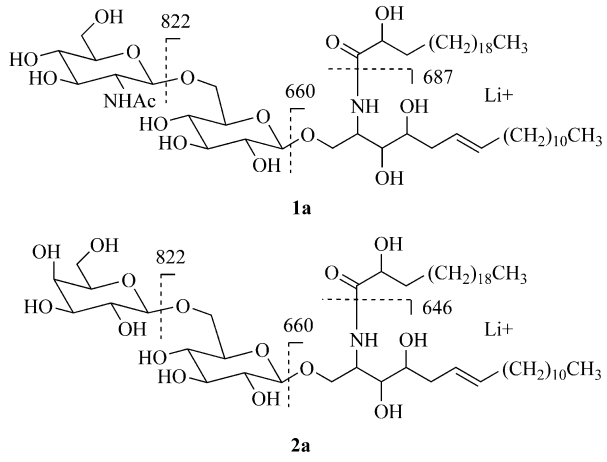
by coupling-constant analysis. Finally, the positions of the sugar–ceramide and sugar–sugar linkages are identified on the basis of HMBC and/or ROESY data.

According to this protocol, the two anomeric protons (signals at δ = 4.61 and 4.46 ppm) were identified from their HMQC correlation peaks with the corresponding anomeric carbon signals (resonances at δ = 101.4 and 100.1 ppm) in the ^{13}C NMR spectrum. Then, starting from each anomeric proton resonance, each sugar spin system was assigned on the basis of the TOCSY and COSY spectra (Table 1). Both sugar residues contain, in addition to the anomeric CH group, four methine groups and one methylene group, and were therefore designated hexoses. The upfield chemical shifts of the 5′-H and 5′′-H signals showed that the corresponding oxymethine groups are not involved in an ester function, which indicates that the two sugar residues are both in the pyranose form. Coupling-constant analysis allowed us to establish that all the ring protons of the sugar, whose anomeric proton resonates at δ = 4.46 ppm, are axial and therefore that this sugar residue is a β-glucopyranoside. As for the second unit, the chemical shift values of C-2′′ (δ = 54.2 ppm) and 2′′-H (δ = 3.95 ppm) are indicative of an *N*-linked methine carbon atom, and this was confirmed by the coupling of 2′′-H with the NH doublet at δ = 6.48 ppm. Coupling-constant analysis also showed for this sugar that the ring protons are all axial, and therefore the sugar residue was identified as a 2-amino-2-deoxyglucopyranoside. Finally, the HMBC correlation peak of 1′-H with C-1 showed that the glucose is directly linked to ceramide, whereas the correlation peak of 1′′-H with C-6′ showed that the glucosamine is glycosylating the glucose at the 6′-position. Once the gross structure of the peracetyl derivative **1b** had been elucidated, NMR spectra of **1a** were collected and fully assigned, providing further support for the proposed structure (see Exp. Sect.).

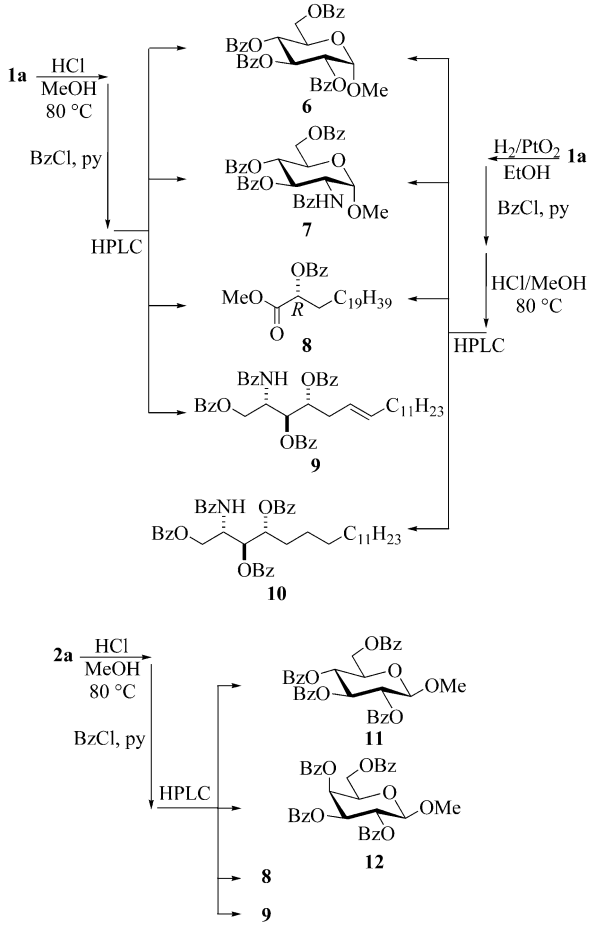
The absolute configuration of the two sugars, the configuration at C-2′′′ of the α-hydroxy fatty acid, and the configurations at C-2, C-3, and C-4 of the sphingosine were determined by chemical degradation. We used a simplified version of the microscale procedure that we developed for the analysis of glycolipids isolated as mixtures of homologues (Scheme 2).[9] Compound **1a** was subjected to acidic meth-

anolysis, and the reaction products were perbenzoylated and subjected to HPLC to give compounds **6**–**9**. The methyl glycoside **6** was identified as methyl tetra-*O*-benzoyl-α-D-glucopyranoside by comparison of its ^{1}H NMR and CD spectra with those reported in the literature.[10] The methyl glycoside **7** was identified as methyl tri-*O*-benzoyl-2-benzamido-2-deoxy-α-glucopyranoside by comparison of its ^{1}H NMR and CD spectra with those of an authentic sample prepared from *N*-acetyl-D-glucosamine according to the same procedure used to prepare compound **1a**. The fatty ester **8** was identified as methyl (*R*)-2-benzoyloxydocosanoate from the EI mass spectrum ([M$^+$] peak at *m*/*z* = 474) and from the ^{1}H NMR and CD spectra, which matched those reported previously.[9] The stereochemistry of the perbenzoylated Δ^6-phytosphingosine **9** could not be determined directly from its spectra because reference data were not available in the literature. In the ^{1}H NMR spectrum of **9**, the chemical shifts and coupling constants of the protons at C-1 to C-4 were close to those of D-*ribo*-phytosphingosine perbenzoate (**10**), but the presence of the additional double bond significantly affected the CD spectrum of **9**, which was remarkably different from that of the reference compound[9] (see Supporting Information) and did not allow a reliable assignment of its configuration. Therefore, compound **1a** was subjected to catalytic reduction with H_2/PtO_2, and the reaction product was subjected to the degradation procedure described above. HPLC separation gave a compound whose ^{1}H NMR and CD spectra were identical to those of *ribo*-phytosphingosine perbenzoate (**10**), thus also defining the configuration of the perbenzoylated Δ^6-phytosphingosine **9**.

The structure of **1a** was further supported by MS/MS data recorded by dissolving the sample in MeOH with 1 mM LiCl, as previously reported,[11] and by using the [M + Li]$^+$ pseudomolecular ion (*m*/*z* = 1025) as the precursor ion. The spectrum contained three peaks at *m*/*z* = 822, 660, and 687 arising from the loss of the dehydrated *N*-acetylglucosamine, the loss of the dehydrated saccharide chain, and the loss of a C_{22} 2-hydoxyacyl group, respectively (Scheme 3).



Scheme 3. MS/MS fragmentation pathways of the lithiated adduct of amphiceramides A (**1a**) and B (**2a**).



Scheme 2. Degradation scheme of amphiceramides A (**1a**) and B (**2a**).

Amphiceramide B (2a)

Compound **2a** showed an [M + Na]$^+$ pseudomolecular ion peak in the high-resolution ESI mass spectrum at *m*/*z* = 1000.6931, in accord with the molecular formula $C_{53}H_{101}NO_{14}$. The ^{1}H NMR spectrum of the peracetate **2b** was similar to that of **1b**, but only one NH doublet was present. This data suggested a diglycosyl ceramide with no amino sugar present in the structure. The ceramide part of the molecule appeared to be the same as that of **1b**, each proton signal showing a chemical shift close to those observed in **1b** and nearly identical coupling constants (Table 1).

The structure of the saccharide chain of **2b** was studied by the same methods used for **1b**. The sugar linked to the ceramide is, again, a β-glucopyranoside, glycosylated at the 6′-position by the second sugar, which was shown to be a β-galactopyranoside on the basis of the following evidence. The 2′′-H proton showed a large coupling constant with both 1′′-H and 3′′-H, and these three protons are therefore axial. In contrast, the coupling constant between 3′′-H and 4′′-H (3.4 Hz) showed the latter to be equatorial. As for 5′′-H, its axial orientation was demonstrated by its intense

correlation peak with the axial 3′′-H in the ROESY spectrum.

The absolute configuration of the sugar was determined by the same degradation scheme described for **1a** (Scheme 2). In this case, however, the benzoylated α-glycosides of glucose and galactose (which are the major products of the reaction sequence) co-eluted from the HPLC column and could not be obtained in pure form. The corresponding β-glycosides **11** and **12**, although less abundant, gave rise to distinct peaks in the chromatogram. The structures and absolute configurations of compounds **11** and **12** were identified by comparison of their ^{1}H NMR and CD spectra with those reported in the literature.[1]

Finally, the structure of the ceramide of **2a**, and in particular the lengths of the two alkyl chains, was confirmed by MS/MS data. The $[M + Li]^+$ pseudomolecular ion of **2a** (m/z = 984) showed the same fragmentation pattern as **1a** (Scheme 3) with the loss of a C_{22} α-hydroxyacyl group leading to a fragment ion at m/z = 646.

Compounds 3a, 4a, and 5a

The ^{1}H and ^{13}C NMR spectroscopic data of the peracetate **3b** are very similar to those reported for amphicerebroside B heptaacetate,[8] a monoglycosyl ceramide composed of a Δ^6-unsaturated phytosphingosine and an *N*-acetyl-β-glucosamine. The only difference between the two compounds, that is, the unbranched C_{18} sphingosine of **3b** instead of the iso C_{19} sphingosine of amphicerebroside B, is reflected in the methyl triplet (6 H) at δ = 0.88 ppm in the ^{1}H NMR spectrum of **3b**. This was confirmed by the mass spectrum of the natural compound **3a**, which showed a lithiated $[M + Li]^+$ pseudomolecular ion peak at m/z = 863 indicative of a C_{40} ceramide. The fragment ion peak at m/z = 525 in the MS/MS data ($[M + Li - C_{22}H_{42}O_2]^+$) indicated a C_{22} α-hydroxy fatty acid and, consequently, a C_{18} sphingosine.

The ESI mass spectrum of compound **4a** ($[M + Na]^+$ at m/z = 838) was consistent with the molecular formula $C_{46}H_{89}NO_{10}$ and indicative of a monohexosyl ceramide with the same ceramide as compounds **1a** and **2a**. Examination of the ^{1}H NMR spectrum of the peracetate **4b** evidenced the presence of the signals of a β-glucopyranoside (chemical shifts and coupling constants matched well those reported for a β-glucosyl ceramide peracetate),[12] whereas the signals of the ceramide proton were very similar to those of **1b** and **2b**. Degradation analysis as described above led to the formation of the methyl glycoside **6**, the fatty ester **8**, and the sphingosine **9**, thus defining completely the structure of **4a**. Compound **4a** had previously been reported as halicerebroside A from the Red Sea sponge *Halilona* sp,[8] but due to the limited spectroscopic data present in the original paper identification by spectral comparison was not possible.

Finally, compound **5a** was identified by comparison of its MS and ^{1}H and ^{13}C NMR data with those reported for amphimelibioside C from the Japanese sponge *Amphimedon* sp.[3]

Conclusions

The structures of the glycosphingolipids produced by the marine sponge *Amphimedon compressa*, all characterized by an unusual Δ^6-phytosphingosine, have been elucidated. The NAcGlc(1β→6)Glc disaccharide of amphiceramide A (**1a**) has never been found before as a part of a natural compound, whereas the saccharide chain of amphiceramide B (**2a**) is found for the first time in a glycosphingolipid.

It is noteworthy that, in spite of the very large number of natural glycoconjugates that have been isolated so far, marine sponges continue to provide new examples of oligosaccharide chains. The biological role of this wide array of glycoconjugates in sponges is still unknown, although the clear species and taxon specificities of most of them suggest that they may be involved in cell-recognition processes.

Experimental Section

General Methods: High-resolution ESI mass spectra were recorded with a Bruker APEX II FT-ICR mass spectrometer. ESI mass spectra were recorded with an Applied Biosystem API 2000 triple-quadrupole mass spectrometer. All the mass 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. ^{1}H and ^{13}C NMR spectra were recorded with Varian UnityInova 700 MHz and 500 MHz NMR spectrometers; chemical shifts are referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm; [D_5]pyridine: δ_H = 8.71, 7.56, and 7.19 ppm; δ_C = 149.9, 135.6, and 123.6 ppm). For an accurate measurement of the coupling constants, the one-dimensional ^{1}H NMR spectra were transformed at 64K points (digital resolution: 0.09 Hz). Homonuclear ^{1}H connectivities were determined by COSY and TOCSY (mixing time 100 ms) experiments. Through-space ^{1}H connectivities were evidenced by using a ROESY experiment with a mixing time of 450 ms. The reverse single-quantum heteronuclear correlation (HSQC) spectra were optimized for an average $^1J_{CH}$ of 145 Hz. The multiple-bond heteronuclear correlation (HMBC) experiments were optimized for a $^3J_{CH}$ of 8 Hz. GC/MS was performed with an Agilent 6850 gas chromatograph with an MSD HP 5975B mass-selective detector, a split/splitless injector, and a fused-silica column (25 m × 0.20 mm HP-5, cross-linked 25% PhMe silicone, 0.33 mm film thickness). After a delay of 3 min from the injection, the temperature of the column was varied from 150 to 280 °C with a gradient of 10 °C min^{-1}. Quantitative determination was based on the area 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 Procedures: Specimens of *Amphimedon compressa* were collected in the summer of 2005 along the coast of Key Largo (Florida). They were frozen immediately after collection and kept frozen until extraction. The sponge (290 g dry weight after extraction) was homogenized and extracted with methanol (3 × 5 L) and then with chloroform (3 × 5 L). The combined extracts were partitioned between H_2O and *n*BuOH. The organic layer was concentrated in vacuo and afforded 53.3 g of a dark oil, which was purified by chromatography on a column packed with RP-18 silica gel. A fraction eluted with $CHCl_3$ (4.7 g) was further purified by chromatography on an SiO_2 column, giving a fraction [1.01 g, eluent: EtOAc/MeOH (7:3)] mainly composed of

glycolipids. This fraction was peracetylated with Ac_2O in pyridine for 12 h. The acetylated glycolipids were subjected to HPLC separation on an SiO_2 column [eluent: *n*-hexane/EtOAc (6:4)]. Five major fractions were obtained, each mainly composed of one of the compounds **1b**–**5b**. Axiceramide A (**1b**, 5.6 mg) and B (**2b**, 1.6 mg) were obtained in pure form from the corresponding fractions after a further normal-phase HPLC purification [eluent: *n*-hexane/*i*PrOH (85:15)] followed by reversed-phase HPLC (eluent: MeOH). Fractions containing compounds **3b**–**5b** were homogeneous as far as the polar parts of the molecules are concerned, and therefore reversed-phase HPLC (eluent: MeOH) was sufficient to separate them from their minor homologues, the new molecular species **3b** (81 mg) and known compounds **4b** (33 mg) and **5b** (8.8 mg).

Amphiceramide A Peracetate (1b): Amorphous solid. $[\alpha]_D^{25}$ = +2 (*c* = 1.5, $CHCl_3$). MS (ESI, positive ion mode, MeOH): *m*/*z* = 1419 $[M + Na]^+$. For ^1H and ^{13}C NMR data, see Table 1.

Amphiceramide B Peracetate (2b): Amorphous solid. $[\alpha]_D^{25}$ = +4 (*c* = 0.5, $CHCl_3$). MS (ESI, positive ion mode, MeOH): *m*/*z* = 1420 $[M + Na]^+$. For ^1H and ^{13}C NMR data, see Table 1.

Compound 3b: Amorphous solid. MS (ESI, positive ion mode, MeOH): *m*/*z* = 1131 $[M + Na]^+$. ^1H NMR ($CDCl_3$): δ = 0.88 (t, *J* = 6.7 Hz, 6 H, 18-H_3 and 22′′′-H_3), 1.28 (alkyl chain methylene protons), 1.92 (s, 3 H, *N*-acetyl Me group), 1.93 (overlapped, 8-H_2), 1.99–2.19 (6 s, 18 H, *O*-acetyl Me groups), 2.22 (overlapped, 1 H, 5-Hb), 2.34 (m, 1 H, 5-Ha), 3.46 (m, 1 H, 2′-H), 3.72 (overlapped, 1-H_2), 3.72 (overlapped, 5′-H), 4.10 (br. d, *J* = 12.4 Hz, 1 H, 6′-Hb), 4.24 (dd, *J* = 12.4, 4.1 Hz, 1 H, 6′-Ha), 4.30 (m, 1 H, 2-H), 4.85 (d, *J* = 8.2 Hz, 1 H, 1′-H), 4.95 (overlapped, 2 H, 3-H, 4-H), 5.00 (t, *J* = 9.6 Hz, 1 H, 4′-H), 5.07 (dd, *J* = 4.5, 7.3 Hz, 1 H, 2′′-H), 5.23 (ddd, *J* = 15.2, 6.9, 6.9 Hz, 1 H, 6-H), 5.37 (t, *J* = 9.6 Hz, 1 H, 3′-H), 5.44 (ddd, *J* = 15.2, 6.9, 6.9 Hz, 1 H, 7-H), 6.07 (d, *J* = 8.6 Hz, 1 H, 2′-NH), 7.10 (d, *J* = 8.6 Hz, 1 H, 2-NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.2 (CH_3, C-18, C-22′′), 20.5–23.2 (several CH_3, acetyl Me groups), 22.7–31.8 (several CH_2, alkyl chain methylene groups), 32.7 (CH_2, C-5), 32.7 (CH_2, C-8), 47.8 (CH, C-2), 55.5 (CH, C-2′), 61.9 (CH_2, C-6′), 66.2 (CH_2, C-1), 68.8 (CH, C-4′), 71.9 (CH, C-5′), 72.2 (CH, C-3′), 72.6 (CH, C-4), 72.6 (CH, C-3), 98.9 (CH, C-1′), 123.7 (CH, C-6), 134.7 (CH, C-7), 170.1 (C, C-1′′), 171.2 (C, *N*-acetyl CO group), 169.6–177.1 (several C, *O*-acetyl CO groups) ppm.

Deacetylation of Compounds 1b–4b: Compounds **1b**–**4b** were each dissolved in MeOH (1 mL), and a solution of MeONa in MeOH (0.4 M, 50 μL) was added. The reactions were carried out at 25 °C for 18 h, and then the reaction mixtures were dried under nitrogen and the residues partitioned between water and chloroform. After removal of the solvent, the organic layers gave the corresponding native glycosphingolipids **1a** (1.5 mg), **2a** (0.9 mg), **3a** (11.3 mg), **4a** (2.8 mg), and **5a** (5.7 mg).

Amphiceramide A (1a): Colorless solid. $[\alpha]_D^{25}$ = –4 (*c* = 0.9, $CHCl_3$). HRMS (ESI, positive ion mode, MeOH): *m*/*z* = 1041.7183 $[M + Na]^+$ (calcd. for $C_{54}H_{102}N_2NaO_{15}^+$ 1041.7172). ^1H NMR (C_5D_5N): δ = 0.86 (2 overlapping t, *J* = 6.7 Hz, 6 H, 18-H_3, 22′′′-H_3), 1.27 (alkyl chain methylene protons), 1.75 (m, 1 H, 4′′′-Hb), 1.67 (m, 1 H, 4′′′-Ha), 1.94 (m, 1 H, 3′′′-Hb), 2.03 (m, 2 H, 8-H_2), 2.17 (s, 3 H, Ac), 2.18 (overlapped, 3′′′-Ha), 2.68 (m, 1 H, 5-Hb), 3.02 (m, 1 H, 5-Ha), 3.91 (overlapped, 5′′-H), 3.93 (overlapped, 4′-H), 3.94 (overlapped, 2′-H), 3.95 (overlapped, 5′-H), 4.11 (m, 1 H, 3′-H), 4.14 (dd, *J* = 11.2, 6.3 Hz, 1 H, 6′-Hb), 4.21 (m, 1 H, 4′′-H), 4.28 (m, 1 H, 4-H), 4.32 (overlapped, 6′′-Hb), 4.33 (overlapped, 3-H), 4.40 (m, 1 H, 3′′-H), 4.47 (m, 1 H, 6′′-Ha), 4.50 (dd, *J* = 10.3, 3.5 Hz, 1 H, 1-Hb), 4.53 (q, *J* = 8.7 Hz, 1 H, 2′′-H), 4.57 (m, 1 H, 2′′′-H), 4.75 (overlapped, 1-Ha), 4.75 (overlapped, 6′-Ha), 4.85 (d, *J* = 7.7 Hz, 1 H, 1′-H), 5.25 (d, *J* = 8.3 Hz, 1 H, 1′′-H), 5.27 (overlapped, 1 H, 2-H), 5.70 (ddd, *J* = 15.2, 6.9, 6.9 Hz, 1 H, 7-H), 5.97 (ddd, *J* = 15.2, 6.9, 6.9 Hz, 1 H, 6-H), 8.55 (d, *J* = 8.9 Hz, 1 H, 2-NH), 8.92 (d, *J* = 7.5 Hz, 1 H, 2′′-NH) ppm. ^{13}C NMR (C_5D_5N): δ = 14.3 (CH_3, C-18, C-22′′′), 23.0 (CH_2, C-17, C-21′′′), 23.4 (CH_3, acetyl Me), 25.9 (CH_2, C-4′′′), 29.7–30.3 (several CH_2, alkyl chain methylene groups), 32.1 (CH_2, C-16, C-20′′′), 33.2 (CH_2, C-8), 35.5 (CH_2, C-3′′′), 37.7 (CH_2, C-5), 51.6 (CH, C-2), 58.1 (CH, C-2′′), 62.7 (CH_2, C-6′′), 70.1 (CH_2, C-6′), 70.3 (CH_2, C-1), 71.8 (CH, C-4′), 72.5 (CH, C-4′′), 72.5 (CH, C-2′′′), 72.9 (CH, C-4), 75.2 (CH, C-2′), 75.6 (CH, C-3), 76.7 (CH, C-3′′), 77.3 (CH, C-5′), 78.4 (CH, C-3′), 78.6 (CH, C-5′′), 103.2 (CH, C-1′′), 105.3 (CH, C-1′), 128.4 (CH, C-6), 133.0 (CH, C-7), 171.2 (C, acetyl CO), 175.7 (C, C-1′′′) ppm.

Amphiceramide B (2a): Colorless solid. $[\alpha]_D^{25}$ = –7 (*c* = 0.5, $CHCl_3$). HRMS (ESI, positive ion mode, MeOH): *m*/*z* = 1000.6931 $[M + Na]^+$ (calcd. for $C_{52}H_{99}NNaO_{15}^+$ 1000.6907). ^1H NMR (C_5D_5N): δ = 0.85 (2 overlapping t, *J* = 6.7 Hz, 6 H, 18-H_3 and 22′′′-H_3), 1.27 (alkyl chain methylene protons), 1.75 (m, 1 H, 4′′′-Hb), 1.67 (m, 1 H, 4′′′-Ha), 1.97 (m, 1 H, 3′′′-Hb), 2.03 (m, 2-H, 8-H_2), 2.18 (m, 1 H, 3′′′-Ha), 2.69 (m, 1 H, 5-Hb), 3.02 (m, 1 H, 5-Ha), 3.91 (m, 1 H, 2′-H), 3.95 (m, 1 H, 5′-H), 4.06 (overlapped, 5′′-H), 4.08 (overlapped, 4′-H), 4.10 (overlapped, 3′-H), 4.17 (m, 1 H, 3′′-H), 4.25 (dd, *J* = 11.3, 6.1 Hz, 1 H, 6′-Hb), 4.28 (overlapped, 4-H), 4.35 (m, 1 H, 3-H), 4.41 (overlapped, 6′′-H_2), 4.43 (overlapped, 1-Hb), 4.49 (t, *J* = 8.3 Hz, 1 H, 2′′-H), 4.53 (br. s, 1 H, 4′′-H), 4.58 (m, 1 H, 2′′′-H), 4.75 (dd, *J* = 10.7, 5.9 Hz, 1 H, 1-Ha), 4.81 (br. d, *J* = 11.3 Hz, 1 H, 6′-Ha), 4.85 (d, *J* = 7.9 Hz, 1 H, 1′-H), 4.99 (d, *J* = 7.8 Hz, 1 H, 1′′-H), 5.26 (overlapped, 2-H), 5.71 (ddd, *J* = 15.1, 6.7, 6.7 Hz, 1 H, 7-H), 5.98 (ddd, *J* = 15.1, 6.9, 6.9 Hz, 1 H, 6-H), 8.57 (d, *J* = 9.3 Hz, 1 H, 2-NH) ppm. ^{13}C NMR (C_5D_5N): δ = 14.3 (CH_3, C-18, C-22′′′), 22.9 (CH_2, C-17, C-21′′′), 29.7–30.3 (several CH_2, alkyl chain methylene groups), 32.2 (CH_2, C-16, C-20′′′), 33.2 (CH_2, C-8), 35.5 (CH_2, C-3′′′), 37.3 (CH_2, C-5), 51.3 (CH, C-2), 62.4 (CH_2, C-6′′), 69.8 (CH_2, C-6′), 70.2 (CH, C-4′′), 70.5 (CH_2, C-1), 71.4 (CH, C-4′), 72.3 (CH, C-2′′′), 72.6 (CH, C-2′′), 72.6 (CH, C-4), 74.9 (CH, C-2′), 75.1 (CH, C-3′′), 75.3 (CH, C-3), 76.9 (CH, C-5′′), 77.1 (CH, C-5′), 78.2 (CH, C-3′), 105.3 (CH, C-1′), 105.8 (CH, C-1′′), 128.2 (CH, C-6), 132.7 (CH, C-7), 175.7 (C, C-1′′′) ppm.

Compound 3a: White solid. MS (ESI, positive ion mode, 1 mM LiCl in MeOH): *m*/*z* = 863 $[M + Li]^+$. ^1H NMR (C_5D_5N): δ = 0.86 (2 overlapping t, *J* = 6.7 Hz, 6 H, 18-H_3, 22′′′-H_3), 1.27 (alkyl chain methylene protons), 1.75 (m, 1 H, 4′′′-Hb), 1.67 (m, 1 H, 4′′′-Ha), 1.97 (m, 1 H, 3′′-Ha), 2.02 (m, 2 H, 8-H_2), 2.17 (s, 3 H, Ac), 2.18 (overlapped, 3′′-Hb), 2.67 (m, 1 H, 5-Hb), 3.00 (m, 1 H, 5-Ha), 3.82 (m, 1 H, 5′-H), 4.18 (t, *J* = 9.2 Hz, 1 H, 4′-H), 4.23 (overlapped, 4-H), 4.24 (overlapped, 3′-H), 4.29 (overlapped, 6′-Hb), 4.29 (overlapped, 3-H), 4.44 (m, 1 H, 6′-Ha), 4.48 (m, 1 H, 2′-H), 4.54 (dd, *J* = 10.9, 3.9 Hz, 1 H, 1-Hb), 4.59 (overlapped, 1-Ha), 4.59 (overlapped, 2′′-H), 5.12 (d, *J* = 8.3 Hz, 1 H, 1′-H), 5.24 (m, 1 H, 2-H), 5.70 (ddd, *J* = 15.2, 6.6, 6.6 Hz, 1 H, 7-H), 5.94 (ddd, *J* = 15.2, 6.8, 6.8 Hz, 1 H, 6-H), 8.5 (d, *J* = 9.3 Hz, 1 H, 2′-NH), 8.85 (d, *J* = 7.7 Hz, 1 H, 2-NH) ppm. ^{13}C NMR (C_5D_5N): δ = 14.3 (CH_3, C-18, C-22′′′), 23.0 (CH_2, C-17, C-21′′′), 23.4 (CH_3, acetyl Me), 25.9 (CH_2, C-4′′′), 29.7–30.3 (several CH_2, alkyl chain methylene groups), 32.1 (CH_2, C-16, C-20′′′), 33.2 (CH_2, C-8), 35.5 (CH_2, C-3′′′), 37.7 (CH_2, C-5), 50.6 (CH, C-2), 57.8 (CH, C-2′), 62.4 (CH_2, C-6′), 69.0 (CH_2, C-1), 72.3 (CH, C-4′), 72.6 (CH, C-2′′), 72.8 (CH, C-3′), 75.4 (CH, C-3), 77.1 (CH, C-4), 78.5 (CH,

C-5′), 102.0 (CH, C-1′), 128.1 (CH, C-6), 132.9 (CH, C-7), 172.2 (C, acetyl CO), 175.7 (C, C-1′′) ppm.

Methyl Tri-*O*-benzoyl-2-benzamido-2-deoxy-α-glucopyranoside (7): *N*-Acetyl-D-glucosamine (11.2 mg) was subjected to acidic methanolysis as described below. The resulting methyl glycosides were benzoylated with benzoyl chloride (200 μL) in pyridine (2 mL) at 25 °C for 16 h. The reaction was quenched with MeOH, and, after 30 min, the mixture was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum with an oil pump for 24 h. The residue was purified by HPLC [column: Luna SiO_2, 5 μm; eluent: *n*-hexane/*i*PrOH (97:3); flow: 1 mL min^{-1}; UV detector: 280 nm] and afforded the methyl glycoside **7** (t_R = 15 min). ^{1}H NMR ($CDCl_3$): δ = 3.50 (s, 3 H, OMe), 4.37 (ddd, J = 9.9, 4.9, 2.8 Hz, 1 H, 5-H), 4.48 (dd, J = 12.1, 4.9 Hz, 1 H, 6-Hb), 4.62 (dd, J = 12.1, 2.8 Hz, 1 H, 6-Ha), 4.76 (ddd, J = 10.6, 9.1, 3.5 Hz, 1 H, 2-H), 5.02 (d, J = 3.5 Hz, 1 H, 1-H), 5.76 (t, J = 9.7 Hz, 1 H, 4-H), 5.83 (dd, J = 10.6, 9.5 Hz, 1 H, 3-H), 6.62 (d, J = 9.1 Hz, 1 H, 2-NH), 7.28–7.59 (overlapping signals, 12 H, benzoyl protons), 7.68 (d, J = 7.8 Hz, 2 H, benzoyl *ortho* protons), 7.90 (d, J = 7.9 Hz, 2 H, benzoyl *ortho* protons), 7.93 (d, J = 7.9 Hz, 2 H, benzoyl *ortho* protons), 8.06 (d, J = 8.0 Hz, 2 H, benzoyl *ortho* protons) ppm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 231 (+4) nm.

Methanolysis of Amphiceramide A (1a): Compound **1a** (1.2 mg) was dissolved in 1 N HCl in 91% MeOH (500 μL), and the obtained solution was kept at 80 °C for about 12 h. The reaction mixture was dried under nitrogen and benzoylated with benzoyl chloride (50 μL) in pyridine (500 μL) at 25 °C for 16 h. The reaction was then quenched with MeOH. After 30 min, the reaction mixture was dried under nitrogen. Most of the methyl benzoate was removed by keeping the residue under vacuum with an oil pump for 24 h. The residue was then purified by HPLC [column: Luna SiO_2, 5 μm; eluent: *n*-hexane/*i*PrOH (99:1); flow: 1 mL min^{-1}]. The chromatogram showed four peaks: methyl glycoside **6**, identified as methyl tetra-*O*-benzoyl-α-D-glucopyranoside (t_R = 10.8 min) by comparison of its ^{1}H NMR and CD spectra with those reported in the literature,[9] methyl tri-*O*-benzoyl-2-benzamido-2-deoxy-α-glucopyranoside (**7**; t_R = 43.3 min), identified by comparison of its ^{1}H NMR and CD spectra with those of an authentic sample of **7**, the benzoylated fatty acid methyl ester **8** (t_R = 4.0 min), recognized as methyl (*R*)-2-benzoyloxydocosanoate because of its EI mass, ^{1}H NMR, and CD spectra, which matched those reported previously,[9] and the perbenzoylated Δ^6-phytosphingosine **9** (t_R = 10.5 min).

(2*S*,3*S*,4*R*)-1,3,4-*O*-Benzoyl-2-benzoylaminooctadec-6-ene-1,3,4-triol (9): ^{1}H NMR ($CDCl_3$): δ = 0.87 (d, J = 6.9 Hz, 3 H, 18-H_3), 1.34–1.16 (br., alkyl chain), 1.88 (m, 2 H, 8-H_2), 2.62 (m, 1 H, 5-Hb), 2.74 (m, 1 H, 5-Ha), 4.59 (dd, J = 12.0, 6.1 Hz, 1 H, 1-Hb), 4.67 (dd, J = 12.0, 6.0 Hz, 1 H, 1-Ha), 5.15 (m, 1 H, 2-H), 5.41 (ddd, J = 15.2, 7.2, 7.2 Hz, 1 H, 6-H or 7-H), 5.52 (ddd, J = 15.2, 7.0, 7.0 Hz, 1 H, 7-H or 6-H), 5.56 (m, 1 H, 4-H), 5.72 (t, J = 5.2 Hz, 1 H, 3-H), 7.14 (d, J = 9.1 Hz, 1 H, 2-NH), 7.33–7.63 (overlapping signals, 12 H, benzoyl protons), 7.83 (d, J = 7.7 Hz, 2 H, benzoyl *ortho* protons), 7.93 (d, J = 7.7 Hz, 2 H, benzoyl *ortho* protons), 8.01 (d, J = 7.7 Hz, 2 H, benzoyl *ortho* protons), 8.04 (d, J = 7.7 Hz, 2 H, benzoyl *ortho* protons) ppm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 248 (–1), 222 (+8) nm.

Catalytic Reduction and Methanolysis of Compound 1a: Compound **1a** (0.5 mg) was dissolved in EtOH (1 mL), and a small amount of PtO_2 was added. The suspension was kept under H_2 at 1 atm for 12 h, then filtered through a Titan PTFE membrane disc filter (0.2 μm), and dried. The residue was dissolved in 1 N HCl in 92% MeOH (1 mL), and the solution obtained was kept at 80 °C for 12 h. The resulting mixture was dried under nitrogen, benzoylated with benzoyl chloride (100 μL) and pyridine (1 mL), and purified by HPLC [column: Luna SiO_2, 5 μm; eluent: *n*-hexane/*i*PrOH (99:1); flow: 1 mL min^{-1}].

(2*S*,3*S*,4*R*)-1,3,4-*O*-Benzoyl-2-benzoylaminooctadecane-1,3,4-triol (10): CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 233 (–8), 221 (+2) nm. The ^{1}H NMR spectrum was identical to that of an authentic sample of D-*ribo*-phytosphingosine perbenzoate.[13]

Methanolysis of Amphiceramide B and Analysis of Methyl Glycosides from 2a: Amphiceramide B (**2a**; 0.7 mg) was subjected to acidic methanolysis as described above for compound **1a**. The reaction mixture was dried under nitrogen and benzoylated with benzoyl chloride (100 μL) and pyridine (1 mL) at 25 °C for 16 h. The reaction was quenched with MeOH and dried under nitrogen. The residue was then purified by HPLC [column: SiO_2, 4.6 × 250 mm, 5 μm; eluent: *n*-hexane/*i*PrOH (99.5:0.5); flow: 1 mL min^{-1}; UV detector: 260 nm], and the two peaks observed were identified as methyl tetra-*O*-benzoyl-β-D-glucopyranoside (**11**; t_R = 28.8 min) and methyl tetra-*O*-benzoyl-β-D-galactopyranoside (**12**; t_R = 30.7 min) by a comparison of their retention times and ^{1}H NMR and CD spectra with those reported previously.[1]

Supporting Information (see footnote on the first page of this article): ESI mass spectra and ^{1}H NMR spectra of compounds **1a** and **2a**; ^{1}H, ^{13}C, COSY, ROESY, HSQC, and HMBC NMR spectra of compound **1b**; ^{1}H and HSQC NMR spectra of compound **2b**; ^{1}H NMR and CD spectra of compounds **7** and **9**.

Acknowledgments

This work was supported by the Italian MIUR PRIN 2007 (grant no. 2007KZ9W5J). We thank Prof. J. R. Pawlik (University of North Carolina) for giving us the opportunity to join the expedition to the Caribbean Sea during which the sponge *A. compressa* was collected and Professor S. Zea (Departamento de Biología y Centro de Estudios en Ciencias del Mar – CECIMAR, Universidad National de Colombia) for identifying the sponge. NMR and mass spectra were recorded at the Centro Interdipartimentale di Analisi Strumentale, Università di Napoli "Federico II"; the assistance of the staff is gratefully acknowledged.

[1] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *J. Org. Chem.* **2008**, *73*, 6158–6165.
[2] E. Fattorusso, A. Mangoni in *Progress in the Chemistry of Organic Natural Products* (Eds.: W. Hertz, G. W. Kirby, R. E. Moore, W. Steiglich, Ch. Tamm), Springer, Vienna, **1997**, pp. 215–301.
[3] C. Emura, R. Higuchi, T. Miyamoto, R. W. M. VanSoest, *J. Org. Chem.* **2005**, *70*, 3031–3038.
[4] N. Borbone, S. De Marino, M. Iorizzi, F. Zollo, C. Debitus, A. Ianaro, B. Pisano, *Eur. J. Org. Chem.* **2001**, 4651–4656.
[5] G. R. Pettit, J. P. Xu, D. E. Gingrich, M. D. Williams, D. L. Doubek, J. C. Chapuis, J. M. Schmidt, *Chem. Commun.* **1999**, 915–916.
[6] H. Li, S. Matsunaga, N. Fusetani, *Tetrahedron* **1995**, *51*, 2273–2280.
[7] T. Natori, Y. Koezuka, T. Higa, *Tetrahedron Lett.* **1993**, *34*, 5591–5592.
[8] S. Hirsch, Y. Kashman, *Tetrahedron* **1989**, *45*, 3897–3906.
[9] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *Eur. J. Org. Chem.* **2005**, 368–373.

[10] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, S. Freigang, L. Teyton, *Bioorg. Med. Chem.* **2008**, *16*, 2077–2085.
[11] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *Eur. J. Org. Chem.* **2003**, 1433–1437.
[12] S. S. Kang, J. S. Kim, Y. N. Xu, Y. H. Kim, *J. Nat. Prod.* **1999**, *62*, 1059–1060.
[13] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *J. Org. Chem.* **2004**, *69*, 1174–1179.

Received: December 11, 2008
Published Online: March 18, 2009

