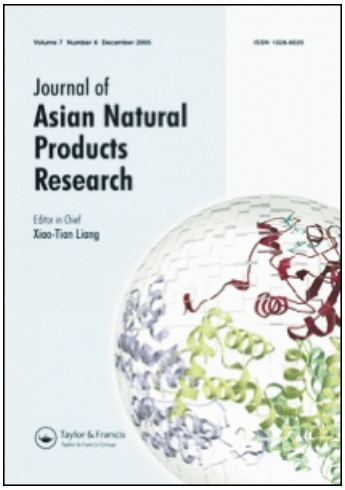

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Ceramides and cerebrosides from the marine bryozoan *Bugula neritina* inhabiting South China Sea

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From the marine bryozoan *Bugula neritina* inhabiting South China Sea, a new ceramide named (2*S*,3*R*,4*E*)-2-(14′-methyl-pentadecanoylamino)-4-octadecene-l,3-diol (**1**) and a new cerebroside named 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*)-2-(heptadecanoylamino)-4-octadecene-l,3-diol (**6**), together with one known ceramide (**2**) and three known cerebrosides (**3**, **4**, and **5**), were isolated. Their structures were deduced by extensive spectral analysis and chemical evidences. Compound **1** is branched with a methyl [—$CH(CH_3)_2$] in the fatty acid moiety, which is a rare structural feature among ceramides. Compound **6** is a new cerebroside with 17 carbons in the fatty acid moiety, while **5** is a new natural product which was isolated from a natural origin for the first time.

Keywords: *Bugula neritina*; cerebroside; ceramide; (2*S*,3*R*,4*E*)-2-(14′-methyl-pentadecanoylamino)-4-octadecene-l,3-diol; 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*)-2-(heptadecanoylamino)-4-octadecene-l,3-diol

1. Introduction

Bugula neritina Linnaeus is a common fouling organism in South China Sea. Previous chemical studies have shown that macrolides are the main antineoplastic components in this genus. In 1982, Pettit's group isolated the first milligram of bryostatin 1, which showed remarkable inhibiting activity against P388 cell [1]. To date, 20 active macrolides have been isolated and are named bryostatins 1–20 [2,3]. Ceramides and cerebrosides are important sphingosine derivatives isolated from some marine organisms such as starfish *Luidia maculata*, *Anthopleura pacifica*, *Dicliptera chinensis*, and so on [4–6]. Several ceramides and cerebrosides were also isolated from an unidentified *Bugula* sp. [7]. Most ceramides or cerebrosides were reported as mixtures because of separation difficulties [8]. In this paper, we report the isolation and structural elucidation of a new ceramide (**1**) and a new cerebroside (**6**), along with one known ceramide (**2**) and three known cerebrosides (**3**, **4**, and **5**) from *B. neritina* (Figure 1).

2. Results and discussion

Ceramide **1** was obtained as a white amorphous powder with mp 80–82°C (dec). The positive ion mode HR-ESI-MS showed a pseudomolecular ion peak at *m/z* 560.5018 $[M+Na]^+$, which, together with the pseudomolecular ion peak at *m/z* 536

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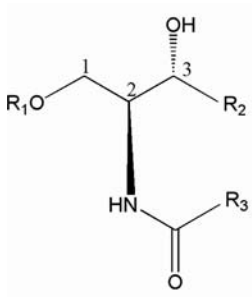

1 R_1=H, R_2=$CH_3(CH_2)_{12}CH^{E}{=}CH$-, R_3=$(CH_3)_2CH(CH_2)_{12}$-

2 R_1=H, R_2=$CH_3(CH_2)_8CH^{E}{=}CHCH_2CH_2CH^{E}{=}CH$-, R_3=$CH_3(CH_2)_{14}$-

3 R_1=glc, R_2=$CH_3(CH_2)_8CH^{E}{=}CHCH_2CH_2CH^{E}{=}CH$-, R_3=$CH_3(CH_2)_{14}$-

4 R_1=glc, R_2=$CH_3(CH_2)_{12}CH^{E}{=}CH$-, R_3=$CH_3(CH_2)_{14}$-

5 R_1=glc, R_2=$CH_3(CH_2)_{14}$-, R_3=$CH_3(CH_2)_{14}$-

6 R_1=glc, R_2=$CH_3(CH_2)_{12}CH^{E}{=}CH$-, R_3=$CH_3(CH_2)_{15}$-

Figure 1. Structures of compounds **1**–**6**.

$[M-H]^-$ in the negative ion mode ESI-MS, enabled the determination of the molecular formula as $C_{34}H_{67}NO_3$, with the help of NMR spectral data. The ^1H and ^{13}C NMR spectral data of **1** indicated the presence of an amide linkage and two long-chain aliphatic moieties, suggesting the sphingosine nature of the molecule (Table 1).

A carbonyl carbon signal at δ 174.1 and a tertiary carbon at δ 54.6 in the ^{13}C NMR spectrum, along with a downfield nitrogen proton signal at δ 6.22 (1H, d, $J = 7.1$ Hz) in the ^1H NMR spectrum, suggested a sphingosine skeleton. The conclusion was confirmed by the observations of a broad singlet at δ 2.64 (two hydroxyl proton signals) in the ^1H NMR spectrum and two oxocarbon signals at δ 62.7 (CH_2) and 74.9 (CH) in the ^{13}C NMR spectrum. In the ^1H NMR spectrum of compound **1**, a doublet at δ 0.86 (6H, d,

Table 1. NMR spectral data of **1** in $CDCl_3$ (500/125 MHz, 25°C)[a].

No.	δ_H, m (*J* in Hz)	δ_C, m	^1H–^1H COSY	HMBC
NH	6.22 d (7.1)		H-2	C-1′
1-OH, 3-OH	2.64 brs			
1	3.71 dd (11.0, 3.4), 3.95 dd (11.0, 3.4)	62.7 t	H-2	
2	3.91 dt (7.1, 3.5)	54.6 d	NH, H-1, 3	
3	4.32 brt (5.0)	74.9 d	H-2, 4	
4	5.53 dd (15.4, 6.4)	129.0 d	H-3, 5	C-3, 6
5	5.79 dt (15.4, 6.6)	134.5 d	H-4, 6	C-3, 6, 7–15
6	2.06 q-like (6.6)	32.4 t	H-5	C-4, 5, 7–15
7–15	1.26 brs	29.3–30.1 t		
16	1.26 brs	32.1 t	H-17	
17	0.86 m	22.9 t	H-16, 18	
18	0.89 t (7.0)	14.3 q	H-17	C-16, 17
1′		174.1 s		
2′	2.23 t (7.5)	37.0 t	H-3′	C-1′, 3′, 4′–10′
3′	1.63 m	25.9 t	H-2′	C-2′, 1′, 4′–10′
4′–10′	1.26 brs	29.3–30.1 t		
11′	1.26 brs	30.1 t		
12′	1.26 brs	27.6 t	H-13′	
13′	1.14 m	39.2 t	H-12′, 14′	C-11′, 12′, 15′
14′	1.51 m	28.1 d	H-13′, 15′	14′-CH_3, C-15′
15′	0.86 d (7.0)	22.8 q	H-14′	C-13′, 14′,14′-CH_3
14′-CH_3	0.86 d (7.0)	22.8 q	H-15′	C-13′, 14′, 15′

Note: [a]Assignments aided by the ^1H–^1H COSY, TOCSY, HSQC, HMBC, and NOESY experiments.

$J = 7.0$ Hz), a triplet at δ 0.89 (3H, t, $J = 7.0$ Hz), and a broad singlet at δ 1.26, together with a number of overlapped methylene signals at δ 29.3–30.1 in the ^{13}C NMR spectrum, indicated the presence of a long aliphatic chain and a branched aliphatic chain. Furthermore, compound **1** was proposed to possess both *normal* and $-CH(CH_3)_2$-type side chains because the carbon signals for the terminal methyl groups were observed at δ 14.3 (*normal*) and δ 22.8 [$-CH(\mathbf{C}H_3)_2$] in the ^{13}C NMR spectra (Table 1), respectively [9]. The signal of δ_C 22.8 [$-CH(\mathbf{C}H_3)_2$] may be assigned to the terminus of either a sphingoid base or a fatty acid moiety. Methanolysis of **1** by hydrochloric acid/methanol gave fatty acid methyl ester (FAM) as the major product, and the ^{1}H NMR spectrum of FAM displayed two methyl signals at δ 0.89 (3H, d, $J = 7.0$ Hz) and 0.86 (3H, d, $J = 6.0$ Hz) in the upfield, suggesting that the carbon at δ_C 14.3 (*normal*) is in the sphingoid base and the carbon at δ_C 22.8 [$-CH(\mathbf{C}H_3)_2$] is in the fatty acid moiety.

The protons of *trans*-olefinic bonds appeared at δ 5.53 (1H, dd, $J = 15.4$, 6.4 Hz) and 5.79 (1H, dt, $J = 15.4$, 6.6 Hz). The positions of the double bonds and the key functionalities and their connectivities of ceramide **1** were determined by the HSQC, ^{1}H–^{1}H COSY, and HMBC experiments. The HSQC spectral data indicated that the proton H-3 (δ 4.32) was connected to the carbon at δ 74.9 (C-3) and H-2 (δ 3.91) was connected to the carbon at δ 54.6 (C-2), while the protons H_2-1 (δ 3.95, 3.71) were connected to the carbon at δ 62.7 (C-1). The observation of a nitrogen proton signal at δ 6.22 (1H, d, $J = 7.1$ Hz) was coupled to H-2, which was in turn coupled to H-3 (δ 4.32) and H-1 (δ 3.95, 3.71). The methine proton H-3 was coupled to the olefinic proton H-4 (δ 5.53), which, together with the coupling relationship of H-5 (δ 5.79) with H-6 (δ 2.06) in the ^{1}H–^{1}H COSY spectrum, indicated that the double bonds were in C-4 (δ 129.0) and C-5 (δ 134.5). This was also supported by the key cross-peaks H-4 with C-3 (δ 74.9) and H-5 with C-3 and C-6 (δ 32.4) in the HMBC experiment. Similarly, H-2′ (δ 2.23) exhibited HMBC correlations with C-1′ (δ 174.1) and C-3′ (δ 25.9), nitrogen proton signal at δ 6.22 with C-1′ (δ 174.1), suggesting the key connectivities of ceramide **1** (Table 1).

The geometry of the C-4/C-5 alkenyl bond was found to be *trans* based on the vicinal coupling constant ($J_{4,5} = 15.4$ Hz). The *trans* configuration was also deduced from the chemical shift of C-6 at δ 32.4. The literature reported that the carbons next to a *trans* double bond appeared between δ 32 and 33, while those next to a *cis* double bond appeared upfield between δ 27 and 28 [10].

The absolute stereochemistry of **1** was determined to be a D-(−)-*erythro*-2*S*,3*R* configuration on the basis of ^{13}C NMR spectral data. The chemical shifts of C-2 (δ 54.6) and C-3 (δ 74.9) of **1** were in good agreement with those of the reported natural product *N*-palmitoyl-D-*erythro*-(2*S*,3*R*)-octadecasphinga-4(*E*)-ene (δ 54.5 and 74.7). The result was also supported by the comparison of the optical rotation of **1** ($[\alpha]_D^{22}$ −5.9) with that of the synthetic ceramide possessing the same configuration ($[\alpha]_D^{22}$ −4.6) [11], whereas the optical rotation of ceramide with other configurations such as *N*-lauroyl-L-*erythro*-(2*R*,3*S*)-docosasphinga-4,8-dienine was $[\alpha]_D^{22}$ +2.9 [12,13].

After mapping all of the signals for each moiety, the lengths of the fatty acid moiety and the sphingoid base were determined by negative ESI-MS, which showed important fragment peaks at *m*/*z* 237, 255, 263, 280, and 296 (Figure 2). From these analyses, the structure of **1** was deduced as (2*S*,3*R*,4*E*)-2-(14′-methylpentadecanoylamino)-4-octadecene-l,3-diol.

The molecular formula of compound **6** was assigned as $C_{41}H_{79}NO_8$ on the basis of the pseudomolecular ion peak at *m*/*z* 736.5709 $[M+Na]^+$ in the positive ion

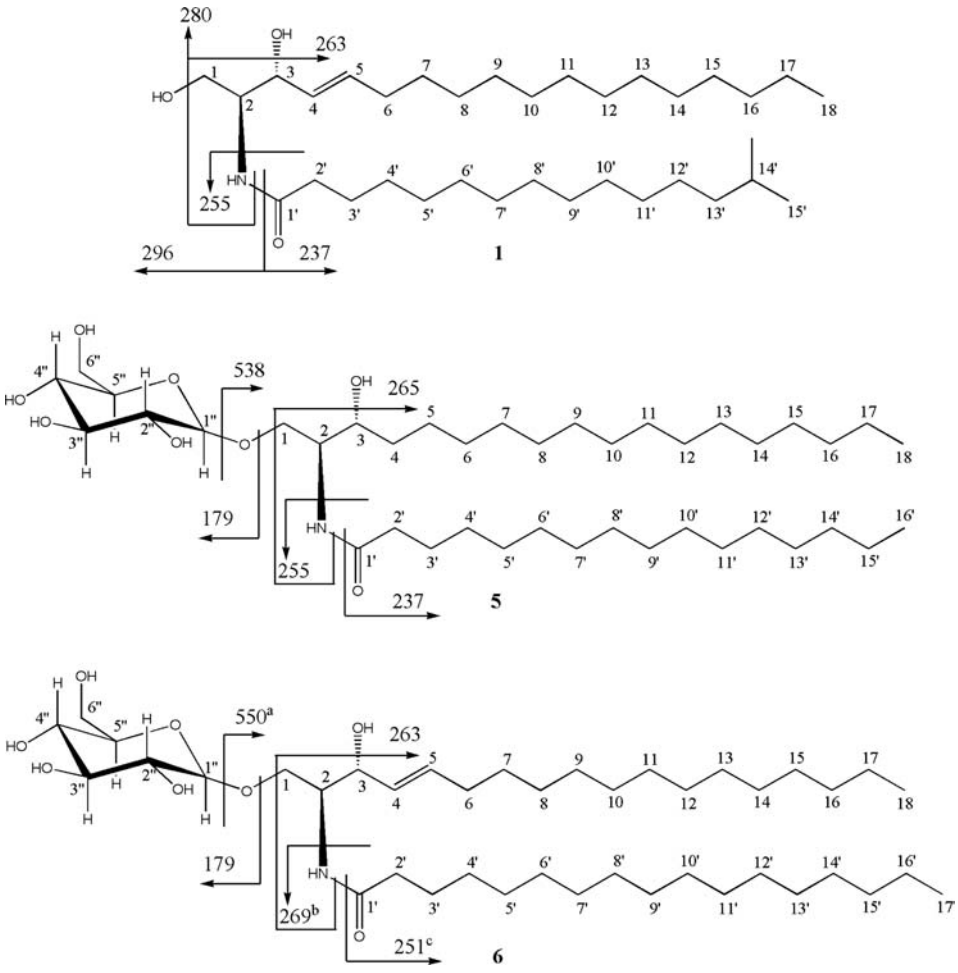

Figure 2. Key negative ESI-MS fragment ions of ceramide **1** and cerebrosides **4**–**6**.
Note: a, b, c were replaced, respectively, by *m/z* 536, 255, and 237 in the negative ESI-MS fragment ions of cerebroside **4**.

mode HR-ESI-MS and the pseudomolecular ion peak at *m/z* 712 $[M-H]^-$ in the negative ion mode ESI-MS, with the help of NMR spectral data. The NMR spectra of **6** showed a close resemblance to those of ceramide **1**, except for the additional glycoside signals and the type of the fatty acid moiety, which confirmed that **6** was a cerebroside.

The carbon signals for the terminal methyl groups of **6** were observed at δ 14.3, which, together with a triplet at δ 0.85 (6H, t, $J = 8.0$ Hz) in the ^{1}H NMR spectrum, suggested that **6** possesses *normal* side chains [8]. Methanolysis of **6** revealed the presence of glucose by TLC comparison with an authentic sample. The signals at δ 105.9 (C-1″), 75.3 (C-2″), 78.6 (C-3″), 71.6 (C-4″), 78.6 (C-5″), and 62.7 (C-6″) in the ^{13}C NMR spectrum and the coupling constant of H-1″ ($J = 7.5$ Hz) revealed the presence of a β-D-glucopyranoside in **6** [14]. The absolute configuration of glucose residue was chosen in keeping with the fact that this was most commonly encountered among natural cerebrosides. In the HMBC spectrum, a cross-peak between C-1 of the sphingoid base (δ 70.6) and H-1″ of the

glucose moiety (δ 4.93) indicated that glucose was connected to C-1 of **6**. The stereochemistry at C-2 and C-3 was also proposed as 2*S* and 3*R* on the basis of the chemical shifts of C-2 (δ 55.1) and C-3 (δ 72.7), which was close to those of synthetic glucosyl–*erythro*–ceramide (δ 53.8, 73.6) [15]. This was supported by the optical rotation of **6** ($[\alpha]_D^{22}$ +10.7) in comparison with that of 1-*O*-(β-D-glucopyranosyl)-D-(+)-(2*S*,3*R*)-2-(docosanoylamide)-1,3-eicosanediol ($[\alpha]_D^{22}$ +8.6) [16]. In fact, the absolute configurations of natural cerebrosides at C-2 and C-3 were all reported as 2*S* and 3*R* to date [17]. Fragment peaks at *m*/*z* 550 $[M-H\text{-}162]^-$, 269, 263, and 251 in the negative ESI-MS (Figure 2) indicated that the number of carbons in the sphingoid base was 18 and in the fatty acid moiety was 17. On the basis of the above evidences, **6** was finally assigned as 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*)-2-(heptadecanoylamino)-4-octadecene-l,3-diol. The aglycone of **6** was an intermediate to synthesize [3,6-di-*O*-(sodiumsulfonato)-β-D-galactopyranosyl]-(1 → 1)-(2*S*,3*R*,4*E*)-2-heptadecanoylamino-4-octadecene-1,3-diol, a synthetic sulfogalactosylceramide used for the mass spectrometric quantitative urinary determination in metachromatic leukodystrophies [18]. Cerebroside **6**, not reported in the literature, is a new cerebroside with 17 carbons in the fatty acid moiety.

By comparing the NMR and MS spectral data with those previously reported in the literature, ceramide **2** was identified as (2*S*,3*R*,4*E*,8*E*)-2-(hexadecanoylamino)-4,8-octadecadiene-l,3-diol, and cerebroside **3** was assigned as 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*)-2-(hexadecanoylamino)-4,8-octadecadiene-l,3-diol [19,20]. In fact, ceramide **2** was the aglycone of cerebroside **3** (Figure 1). Cerebroside **4** was identified as 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*)-2-(hexadecanoylamino)-4-octadecene-l,3-diol on the basis of its MS and NMR spectral data compared with the literature data and by methanolysis followed by TLC analysis of the corresponding sugar derivative. Cerebroside **4** exhibited significant cancer cell growth inhibition activity against the murine P388 lymphocytic leukemia and a panel of human cancer cell lines (GI_{50} 0.15–2.6 μg/ml) [21]. Cerebroside **5** was assigned as 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*)-2-(hexadecanoylamino)-octadecane-l,3-diol, which has been analyzed as a semisynthetic cerebroside by HPLC-MS [22], but reported as a natural product for the first time.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT5-XMT apparatus and are uncalibrated. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. The 1D and 2D NMR spectra were measured in pyridine-d_5 or $CDCl_3$ on a Bruker AVANCE-500 spectrometer, with TMS as the internal standard. ESI-MS and HR-ESI-MS were taken on a Micromass Quattro mass spectrometer. Semi-preparative HPLC was carried out on a Dionex P680 liquid chromatograph equipped with a UV170 UV/vis detector using a YMC-Pack R&D ODS-A column (250 × 20 mm i.d.) and monitored at 206 nm. Chromatographic materials were silica gel (10–40 μm; Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Pharmacia, Inc., Piscataway, NJ, USA). TLC detection was achieved by spraying the silica gel plates with 20% H_2SO_4 followed by heating.

3.2 Animal material

The samples of the marine bryozoan *B. neritina* were collected in March 2008 in Daya Bay, Shenzhen, Guangdong Province, China, and identified by Prof. H.W. Lin. A voucher specimen (No. 20080312) has been deposited in the Herbarium of the Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai, China.

3.3 Extraction and isolation

Fresh animals (about 100 kg, dry weight after extraction) were extracted with 95% EtOH (4 × 500 liters, 1 week/time) at ambient temperature. The EtOH extract was concentrated and the residue (2.0 kg) was suspended in water, and then partitioned successively with EtOAc (5 × 10 liters). The EtOAc phase was evaporated under reduced pressure to give a dark gummy residue (600 g). The EtOAc extract (200 g) was subjected to column chromatography over silica gel (1200 g, 10–40 μm) eluting with the $CHCl_3$–MeOH (100:1 to 1:1) gradient to give nine major fractions (A–I) based on TLC analysis. Fractions D (18.6 g) and G (21.4 g) mainly contained ceramides and cerebrosides, respectively. Fraction D was subjected to column chromatography over silica gel (300 g, 10–40 μm) eluting with the petroleum ether–EtOAc (20:1 to 1:1) gradient to give four major fractions (D1–D4). Fraction D4 (432.4 mg) was eluted with $CHCl_3$–MeOH (1:1) on Sephadex LH-20 and then further purified by semi-preparative HPLC to afford pure ceramides **1** (5.4 mg, $t_R = 43.6$ min) and **2** (19.8 mg, $t_R = 39.2$ min), using MeOH as the mobile phase at a flow rate of 8 ml/min. Fraction G (10.0 g) was subjected to column chromatography over silica gel (300 g, 10–40 μm) eluting with the petroleum ether–EtOAc (20:1, 18:1, 15:1) gradient to give four major fractions (G1–G4). Fraction G2 (3.0 g) was eluted with $CHCl_3$–MeOH (1:1) on Sephadex LH-20 repeatedly to give the major fraction CA (364.6 mg). CA was further purified by semi-preparative HPLC to afford pure cerebrosides **3** (37.6 mg, $t_R = 33.8$ min), **4** (85.9 mg, $t_R = 45.8$ min), **5** (11.7 mg, $t_R = 47.1$ min), and **6** (11.0 mg, $t_R = 55.9$ min), using MeOH as the mobile phase at a flow rate of 8 ml/min.

3.3.1 *Ceramide* **1**

A white amorphous powder, mp 80–82°C (dec), $[\alpha]_D^{22}$ −5.9 ($c = 0.11$, $CHCl_3$); ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS (positive ion mode) *m/z*: 560 $[M+Na]^+$, 1098 $[2M+Na]^+$; ESI-MS (negative ion mode) *m/z*: 536 $[M-H]^-$, 506 $[M-2CH_3—H]^-$, 572 $[M+Cl]^-$, 1073 $[2M-H]^-$, other important fragments, see Figure 2; HR-ESI-MS (positive ion mode) *m/z*: 560.5018 $[M+Na]^+$ (calcd for $C_{34}H_{67}NO_3Na$, 560.5021).

3.3.2 *Ceramide* **2**

A white amorphous powder, mp 85–87°C (dec); ESI-MS, ^1H and ^{13}C NMR spectral data were consistent with the literature [19].

3.3.3 *Cerebroside* **3**

A white amorphous powder, mp 174–176°C (dec); ESI-MS, ^1H and ^{13}C NMR spectral data were consistent with the literature [20].

3.3.4 *Cerebroside* **4**

A white amorphous powder, mp 172–174°C (dec); ^1H and ^{13}C NMR spectral data, see Table 2; ESI-MS (positive ion mode) *m/z*: 722 $[M+Na]^+$, 1421 $[2M+Na]^+$; ESI-MS (negative ion mode) *m/z*: 698 $[M-H]^-$, 734 $[M+Cl]^-$, 1397 $[2M-H]^-$, other important fragments, see Figure 2.

3.3.5 *Cerebroside* **5**

A white amorphous powder, mp 160–162°C (dec), $[\alpha]_D^{22}$ +11.4 ($c = 0.20$, pyridine); ^1H NMR spectral data of aglycone (500 MHz, C_5D_5N): δ 8.39 (1H, d, $J = 7.5$ Hz, NH), 4.94 (1H, m, H-2), 4.76 (2H, m, H-1b, H-3), 4.20 (1H, m, H-1a), 2.45 (4H, m, H-4, H-2′), 1.83 (4H, m, H-5, H-3′), 1.25 (46H, m, H-7–H-17, H-4′–H-15′), 0.85 (6H, t, $J = 8.0$ Hz, H-18, H-16′). ^{13}C NMR spectral data of

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of cerebrosides **4** and **6**.[a]

	4		**6**	
No.	δ_H, m (*J* in Hz)	δ_C, m	δ_H, m (*J* in Hz)	δ_C, m
Ceramide				
NH	6.37 d (7.0)		8.33 d (7.5)	
1	3.71 dd (10.0, 3.0), 4.03 dd (10.0, 3.1)	69.7 t	4.21 dd (11.0, 3.5), 4.76 brs	70.6 t
2	4.10 m	54.0 d	4.81 m	55.1 d
3	4.20 brs	72.8 d	4.76 brs	72.7 d
4	5.46 m	128.9 d	5.99 m	132.2 d
5	5.75 m	134.9 d	5.91 m	132.6 d
6	2.03 m	32.6 t	2.04 m	32.7 t
7	1.27 brs	32.1 t	2.12 m	32.9 t
8–15	1.27 brs	29.5–30.0 t	1.25 brs	29.6–30.0 t
16	1.27 brs	32.1 t	1.25 brs	32.1 t
17	1.27 brs	22.8 t	1.25 brs	22.9 t
18	0.88 t (7.0)	14.2 q	0.85 t (8.0)	14.3 q
1′		173.5 s		173.4 s
2′	2.17 m	37.0 t	2.42 m	36.9 t
3′	1.57 m	26.0 t	1.81 m	26.4 t
4′–13′	1.27 brs	29.5–30.0 t	1.25 brs	29.6–30.0 t
14′	1.27 brs	32.1 t	1.25 brs	29.6–30.0 t
15′	1.27 brs	22.8 t	1.25 brs	32.1 t
16′	0.88 t (7.0)	14.2 q	1.25 brs	22.9 t
17′			0.85 t (8.0)	14.3 q
Glucosyl				
1″	4.33 d (7.5)	103.8 d	4.93 d (7.5)	105.9 d
2″	3.39 d (7.6)	73.7 d	4.05 m	75.3 d
3″	3.54 m	76.8 d	4.21 m	78.6 d
4″	3.56 m	70.8 d	4.21 m	71.6 d
5″	3.35 m	76.3 d	3.93 m	78.6 d
6″	3.84 dd (11.0, 2.5), 3.86 dd (11.0, 6.0)	62.0 t	4.35 dd (11.5, 2.5), 4.51 dd (11.5, 6.0)	62.7 t

Note: [a] Cerebroside **4** was measured in $CDCl_3$ at 45°C and **6** in pyridine-d_5 at 25°C. Assignments aided by the ^{1}H–^{1}H COSY, TOCSY, HSQC, HMBC, and NOESY experiments.

aglycone (125 MHz, C_5D_5N): δ 173.3 (C-1′), 70.9 (C-1), 71.7 (C-3), 55.1 (C-2), 36.9 (C-2′), 35.0 (C-4), 32.1 (C-16, C-14′), 29.6–30.2 (C-7–17, C-4′–13′), 26.5 (C-5), 26.4 (C-3′), 22.9 (C-17, C-15′), 14.3 (C-18, C-16′); ^{1}H and ^{13}C NMR spectral data of glucosyl (500/125 MHz, C_5D_5N): δ 106.1 (C-1″)/4.94 (1H, d, $J = 7.5$ Hz, H-1″), 75.3 (C-2″)/4.05 (1H, m, H-2″), 78.6 (C-3″)/4.20 (1H, m, H-3″), 71.3 (C-4″)/4.20 (1H, m, H-4″), 78.6 (C-5″)/3.94 (1H, m, H-5″), 62.8 (C-6″)/4.35 (1H, m, H-6″a), and 4.52 (1H, m, H-6″b); ESI-MS (positive ion mode) *m/z*: 724 $[M+Na]^+$, 1425 $[2M+Na]^+$; ESI-MS (negative ion mode) *m/z*: 700 $[M-H]^-$, 736 $[M+Cl]^-$, other important fragments, see Figure 2.

3.3.6 *Cerebroside* **6**

A white amorphous powder, mp 174–176°C (dec), $[\alpha]_D^{22}$ +10.7 ($c = 0.20$, pyridine); ^{1}H and ^{13}C NMR spectral data, see Table 2; ESI-MS (positive ion mode) *m/z*: 736 $[M+Na]^+$, 1449 $[2M+Na]^+$; ESI-MS (negative ion mode) *m/z*: 712 $[M-H]^-$, 748 $[M+Cl]^-$, 1425 $[2M-H]^-$, other important fragments, see Figure 2; HR-ESI-MS (positive ion mode) *m/z*: 736.5709 $[M+Na]^+$ (calcd for $C_{41}H_{79}NO_8Na$, 736.5706).

3.4 Methanolysis of 1, 4, and 6

Ceramide **1** (3 mg) was dissolved in 5% HCl–MeOH (2 ml) and refluxed for 10 h (80°C). The reaction mixture was extracted with *n*-hexane thrice. The *n*-hexane layer was washed with H_2O and concentrated *in vacuo* to yield the major methyl 14-methylpentadecanoate. EI-MS *m/z* (rel. int.): 270 $[M]^+$ (15), 213 $[M-COOCH_3+2H]^+$ (34), 242 $[M-2CH_3+2H]^+$ (70), 239 $[M-OCH_3]^+$ (63). ^1H NMR (500 MHz, $CDCl_3$): δ 0.86 (3H, d, $J = 6.0$ Hz, CH_3), 0.89 (3H, d, $J = 7.0$ Hz, CH_3), 1.27 (20H, brs, long chain $-CH_2-$), 1.51 (1H, m, H-14), 1.62 (2H, m, H-3), 2.30 (2H, t, $J = 7.5$ Hz, H-2), 3.67 (3H, s, OCH_3).

Each cerebroside (**4**: 30 mg, **6**: 5 mg) was dissolved in 5% HCl–MeOH (20 and 5 ml, respectively) and refluxed for 12 h (80°C). The reaction mixture was extracted with *n*-hexane thrice. The *n*-hexane layer was concentrated *in vacuo* to yield methyl palmitate or methyl heptadecanoate. The aqueous MeOH layer of the hydrolysate was evaporated under reduced pressure to remove residual HCl. The resulting residue was partitioned between H_2O and EtOAc. The H_2O layer was concentrated to afford the major D-methyl glucopyranoside, which was subjected to co-TLC analysis with authentic sugars and developed with *n*-BuOH–pyridine–H_2O (6:4:3). Detection was carried out with aniline phthalate spray. Methyl-β-D-glucopyranoside was identified by TLC comparison ($R_f = 0.63$) for both **4** and **6**, while the R_f value of methyl-β-D-galactosylpyranoside was 0.54.

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