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AGELASPHINS, NOVEL ANTITUMOR AND IMMUNOSTIMULATORY CEREBROSIDES FROM THE MARINE SPONGE *AGELAS MAURITIANUS*

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ABSTRACT: New glycosphingolipids, named agelasphins (**1-8**), have been isolated by antitumor and immunostimulatory bioassay-guided purification from an extract of a marine sponge, *Agelas mauritianus*. Strongly active agents in agelasphins had characteristic α-galactosylceramide structures, the isolation of which from natural products has not previously been reported. The absolute configurations of agelasphins were elucidated by the total synthesis.

INTRODUCTION

In the course of our screening for antitumor and immunostimulatory agents from marine organisms, it was found that a lipophilic extract from an Okinawan sponge, *Agelas mauritianus*, showed high *in vivo* antitumor activity against murine B16 melanoma and enhanced the mixed lymphocyte reaction (MLR)[1] *in vitro*. The bioactive compounds agelasines,[2] agelasidines,[3] and agelines[4] have been reported to be present in the genus *Agelas*. Agelasines were also obtained from the sample of *A. mauritianus* investigated here. However, these cytotoxic compounds showed no *in vivo* antitumor efficacy against B16 tumor. This finding prompted a search for the *in vivo* active substances in crude extract. This paper reports the isolation and the structures of these novel active substances, agelasphins (AGLs, **1-8**), and shows brief results of bioassays of these cerebrosides. Six of the AGLs have characteristic α-galactosylceramide structures which have no precedents. In a recent communication[5] the total synthesis of the cerebroside AGL-9b (**6**) was reported and the synthesis of AGL-9a (**5**) is also described here.

RESULTS AND DISCUSSION

A lipophilic extract of *A. mauritianus* was washed with 30% aqueous methanol to remove agelasines. The remaining extract was chromatographed to yield two bioactive fractions containing constituents which were

separated by conventional TLC. The less polar fraction showed stronger *in vivo* antitumor activity than the more polar fraction. Reversed-phase HPLC facilitated separation of the less polar fraction to give two major components, AGL-10 (**1**) and -12 (**2**). Additionally, HPLC separation of the other fraction gave six components AGL-7a (**3**), 7b (**4**), 9a (**5**), 9b (**6**), 11 (**7**) and 13 (**8**). The ^{13}C NMR data of all AGLs are summarized in Table 1.

Table 1 ^{13}C **NMR Spectral Data of Agelasphins**

carbon / compound	1	2	3	4	5	6	6*	7*	8
Long-chain base									
1 (t)	70.0	69.9	68.1	68.2	68.3	68.2	68.6	68.9	68.2
2 (d)	54.6	54.6	50.4	50.6	50.4	50.5	51.2	51.5	50.5
3 (d)	72.3	72.3	76.5	76.5	76.5	76.4	76.1	75.9	76.5
4 (d)	132.1	132.0	72.3	72.4	72.3	72.3	72.8	73.0	72.3
5 (t)(d)	131.9	131.9	---	---	---	---	---	---	---
6 (t)	32.7	32.6	---	---	---	---	---	---	---
7 (t)	28.3	28.3	---	---	---	---	---	---	---
8 (d)	129.9	129.9	---	---	---	---	---	---	---
9 (s)	134.2	134.2	---	---	---	---	---	---	---
10 (d)	135.3	135.5	---	---	---	---	---	---	---
11 (d)	127.8	127.8	---	---	---	---	---	---	---
19 (q)	12.7	12.6	---	---	---	---	---	---	---
normal (q)	14.5	14.2	14.2	---	14.2	---	---	---	---
iso (q)	---	---	---	22.8	---	22.7	23.0	---	---
(d)	---	---	---	28.2	---	28.2	28.5	---	---
(t)	---	---	---	39.2	---	39.2	39.6	---	---
anteiso (q)	---	---	---	11.6	---	---	---	12.0	11.5
(q)	---	---	---	19.3	---	---	---	19.9	19.3
(d)	---	---	---	34.6	---	---	---	35.1	34.5
(t)	---	---	---	36.8	---	---	---	37.4	36.8
Fatty acid									
1' (s)	175.5	175.5	175.0	175.0	175.0	175.0	175.9	176.4	175.0
2' (d)	72.5	72.5	72.4	72.4	72.4	72.4	72.8	73.0	72.4
3' (t)	35.6	35.6	35.5	35.5	35.5	35.5	35.7	35.8	35.5
4'(t)	25.8	25.8	25.8	25.8	25.8	25.8	26.1	26.1	25.8
-CH3	14.5	14.2	14.2	14.2	14.2	14.2	14.5	14.5	14.2
Sugar									
1" (d)	105.5	105.4	101.2	101.2	101.2	101.2	101.2	101.2	101.2
2" (d)	75.0	75.0	70.1	70.2	70.2	70.1	70.2	70.3	70.1
3" (d)	78.4	78.4	71.6	71.6	71.6	71.5	71.5	71.6	71.6
4" (d)	71.6	71.6	70.9	70.9	70.9	70.9	70.9	71.0	70.9
5" (d)	78.4	78.3	73.0	73.1	73.0	73.0	72.9	73.0	73.0
6" (t)	62.7	62.8	62.6	62.6	62.6	62.6	62.7	62.8	62.6
Aliphatic carbons									
$-CH_2-$ (t)	---	---	34.4	34.4	34.4	34.3	34.1	33.6	34.4
	33.1	33.1	---	---	---	---	---	---	---
	32.0	32.0	32.1	32.1	32.1	32.1	32.3	32.6	32.0
	---	---	30.3	30.3	30.3	30.3	30.6	30.8	30.3
	---	---	30.1	30.1	30.1	30.1	30.2	30.4	30.1
	---	---	30.0	30.0	30.0	30.0	30.2	30.4	30.0
	29.9	29.9	29.9	29.8	29.9	29.9	30.1	30.3	29.8
	29.8	29.8	---	29.7	---	---	---	30.2	29.7
	29.5	29.5	29.5	29.5	29.6	29.6	29.8	30.0	29.5
	29.4	29.4	---	---	---	---	---	---	---
	29.4	29.3	---	---	---	---	---	---	---
	---	---	---	27.8	---	27.7	28.0	---	---
	---	---	---	27.4	---	---	---	27.8	27.3
	---	---	26.4	26.4	26.4	26.4	26.8	26.9	26.4
	22.8	22.8	22.9	23.0	22.9	22.9	23.0	23.3	22.9

δ (ppm), in C_5D_5N, 125MHz, 27°C
* : 55°C

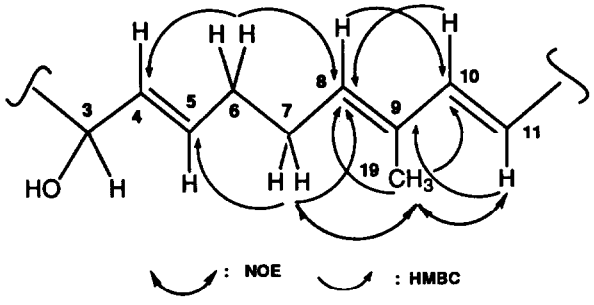
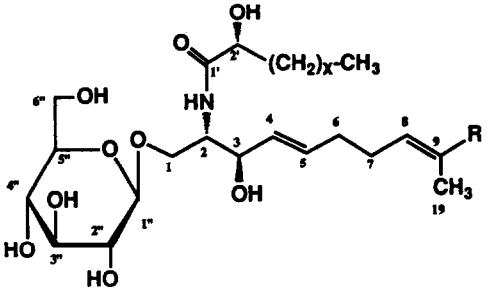


Fig. 1 NOE and HMBC experiments using AGL-10 (1)



	X	R
Agelasphin-10 (1)	21	$-CH=CH-(CH_2)_6-CH_3$
Agelasphin-12 (2)	22	$-CH=CH-(CH_2)_6-CH_3$
Sch-II	13	$-(CH_2)_8-CH_3$

Fig. 2 Structures of AGL-10, -12 and the cerebroside from *S. commune*

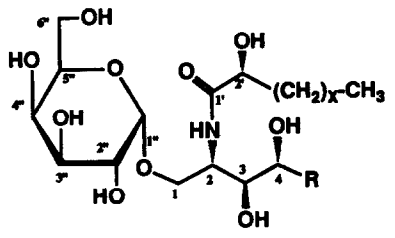
Compounds **1** and **2** showed almost the same IR absorption (KBr 3350, 2920, 2850, 1640, 1530, 1470, and 1080 cm^{-1}) and ^1H and ^{13}C NMR data except for some minor differences. The structural elucidation of compound **1** is described as follows. The molecular formula of **1**, $C_{49}H_{90}NO_9$ [(M-H)$^-$, *m/z* 836.6589, Δ3.2 mmu], was determined by negative HR FABMS. The IR spectrum exhibited strong absorption bands for hydroxyl and amide groups and the ^{13}C NMR spectrum in C_5D_5N showed the presence of an anomeric carbon (δ 105.5) and a number of carbinol and aliphatic carbons in addition to an amide functionality (δ 175.5 and 54.6). These data indicated that **1** was a cerebroside. The ^1H NMR spectrum in C_5D_5N exhibited seven exchangeable proton signals due to one NH [δ 8.33 (d, J = 9.2 Hz)] and six OH (broad singlets or doublets in the range of δ 6-8). The assignment of ^1H and ^{13}C NMR signals for the core portion of the molecule was carried out by 2D NMR and decoupling experiments. In methanolysis using aq. HCl-MeOH,[6] **1** gave methyl 2-(*R*)-hydroxytetracosanoate (**9**), a long-chain base and a methyl glucoside. The structure of the ester **9** which could be isolated from the reaction mixture by extraction with n-hexane was identified from spectroscopic data and by comparison with a synthetic sample.[7] The remaining portion of the reaction mixture was separated using an Amberlite CG-400 column eluted with MeOH and the base was acetylated by the conventional method. Spectroscopic data suggested this to be 2-acetoamino-1,3-diacetoxy-9-methyl-4,8,10-octadecanetriene (**10**). In the ^1H NMR of **1**, the coupling constants ($J_{4\text{-}5}$ = 15.9 Hz, $J_{10\text{-}11}$ = 15.3 Hz) between C4 and C5 and between

C10 and C11 protons indicated *E* geometry of the doublebonds. The chemical shift (δ 12.7) of a methyl carbon suggested that the double bond at position 8 was also *E* as demonstrated by a comparison with the chemical shifts of *E* and *Z* isomers of 3-methyl-3-hexene.[8] Furthermore, NOEs between the C19 methyl proton and C7 methylene protons were observed but NOE between C19 methyl proton and the C8 olefinic proton was not observed (Fig. 1). HMBC experiments (Fig. 1) showed the connectivity of the C4-C11 segment which contains the three double bonds. It is clear that **1** possesses a sphingosine moiety with (4*E*, 8*E*, 10*E*) geometry. Analysis of the sugar component[9] showed it to be glucose in the D-form according to optical rotation measurement ($[\alpha]_D^{23}$ +55.4°, H_2O) of the free sugar. The ^1H NMR spectrum of **1** in C_5D_5N showed the anomeric proton as a doublet (J = 7.9 Hz) at δ 4.89 indicating the α-orientation of the proton in the glucose moiety. The overall structure of **1** was established as shown in Fig. 2. The structure of AGL-12 (**2**) was shown to contain 2-(*R*)-hydroxypentacosanoyl instead of 2-(*R*)-hydroxytetracosanoyl in the fatty acid moiety of compound **1** as determined using the same analytical method.

A tri-unsaturated long-chain base moiety of these cerebrosides was already detected by Irie *et al.* from a starfish, *Asterias amurensis*.[10] The dihydro derivative of **10** (**11**) was observed from a basidiomycete, *Schizophyllum commune*.[11]

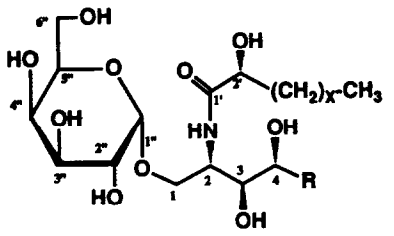
All other AGLs (**3-8**) gave essentially the same IR absorption (KBr 3400, 2950, 2870, 1645, 1535, 1475, and 1080 cm^{-1}) and ^1H and ^{13}C NMR data except for some minor differences. The structural elucidation of compound **6** is described as an example. The molecular formula $C_{48}H_{94}NO_{10}$ of **6** was deduced from negative HR FABMS[$(M-H)^-$, *m/z* 844.6948, Δ6.5 mmu]. The IR spectrum exhibited strong absorption bands for hydroxyl and amide groups and the ^{13}C NMR spectrum in C_5D_5N showed the presence of an anomeric carbon (δ 101.2) and a number of carbinol and aliphatic carbons in addition to an amide functionality (δ 175.0 and 50.6), suggesting **6** to also be a cerebroside. The ^1H NMR spectrum indicated the absence of an olefinic moiety in compound **6**. Methanolysis of each of **3-8**, as above, yielded three components. Analysis of the sugar components[9] of **6** showed it to be a galactose and optical rotation ($[\alpha]_D^{23}$ +78.2°, H_2O) of the free sugar revealed it to be in the D-form. The ^1H NMR signal at δ 5.59 (d, J = 3.7 Hz) clearly pointed that the galactose of **6** had an α-linkage. The fatty acid and base moieties were similarly identified as in **1**. Thus the structure of **6** was established. The structures of **3**, **5**, **7**, and **8** were similarly identified as illustrated in Fig. 3.

Negative HR FABMS of AGL-7b (**4**) gave a single molecular ion peak at *m/z* 830.6730 [$(M-H)^-$ calcd. for $C_{47}H_{92}NO_{10}$ 830.6726 (Δ 0.4 mmu)] which implied a molecular formula of $C_{47}H_{93}NO_{10}$, and suggested a total 41 carbon atoms for the fatty acid and the phytosphingosine moieties. The ^{13}C NMR spectrum showed characteristic signals for both the iso-terminal (δ 22.8, 28.2 and 39.2 ppm) and the anteiso-terminal (δ 11.6, 19.3, 34.6 and 36.8 ppm). The former three signals were about five times more intensive than the latter ones, indicating **4** to be a mixture of two or more compounds having the same molecular formula. In fact, methanolysis of **4** gave fatty acid components whose molecular ion peaks in FDMS were observed at *m/z* 384 and 398 corresponding to the formulae $C_{24}H_{48}O_3$ and $C_{25}H_{50}O_3$ of methyl 2-hydroxytricosanoate and methyl 2-hydroxytetracosanoate, respectively. Further separation of each component of **4** could not be achieved. Thus, AGL-7b (**4**) may be depicted by the structures shown in Fig. 4.



	X	R
Agelasphin-7a (3)	21	$-(CH_2)_{11}-CH_3$
Agelasphin-9a (5)	21	$-(CH_2)_{12}-CH_3$
Agelasphin-9b (6)	21	$-(CH_2)_{11}-CH(CH_3)_2$
Agelasphin-11 (7)	21	$-(CH_2)_{11}-CH(CH_3)-C_2H_5$
Agelasphin-13 (8)	22	$-(CH_2)_{11}-CH(CH_3)-C_2H_5$

Fig. 3 Structures of AGL-7a, -9a, -9b, -11 and -13



R: $-(CH_2)_Y-CH(CH_3)-C_2H_5$
or
$-(CH_2)_Z-CH(CH_3)_2$

4 (
Agelasphin 7b-1 (X=20 Y=10 and / or X=20 Z=11)
Agelasphin 7b-2 (X=21 Y=9 and / or X=21 Z=10)
)

Fig. 4 Structures of the components of AGL-7b (4)

The absolute stereochemical configuration of AGL-9a (**5**) and -9b (**6**) were established by total synthesis. AGL-9a (**5**) was synthesized using 3,4,6-tri-*O*-benzyl-D-galactopyranose as a starting material as shown in Fig. 5. This route mainly follows that described by Ogawa *et al.*[12] The (2*R*,3*S*,4*R*)-2,3,5-tribenzyloxy-4-hydroxypentanal (**17**)[13] was treated with dodecylidene triphenylphosphorane in tetrahydrofuran, yielding a mixture of *E* and *Z* olefin **18** at 52% yield. The double bond of the mixture **18** was selectively reduced over 10% palladium-carbon in tetrahydrofuran to yield **19** in a quantitative yield and was converted to mesylate **20** at 92% yield. S_N2 displacement of the mesylate group in **20** using sodium azide yielded the desired **21** at 75% yield. Selective reduction of the azide group in **21** to amine **22** was achieved at 79.4% yield over 10% palladium-carbon in tetrahydrofuran. Amine **22** was acylated to **24** at 85% yield with (*R*)-2-acetoxytetracosanoic acid (**23**) and EEDQ in tetrahydrofuran. Hydrogenolysis of the benzyl groups of **24** over 10% palladium-carbon in tetrahydrofuran-n-propanol gave crystalline (2*S*,3*S*,4*R*)-*N*-[(*R*)-2-acetoxytetracosanoyl]-2-amino-1,3,4-heptadecanetriol **25** at 83% yield. Ceramide **25** thus obtained was converted to glycosyl acceptor **28** using the conventional method[13] at 59% overall yield. The α-galactosidation process of compound **28** to **29** was performed under the conditions of Mukaiyama's glycosidation.[14] Furthermore, deprotection of benzyl and acetyl groups in **29** in 2 steps in the conventional way yielded **5** at 79% overall yield. All spectral data including ^1H NMR[15] and optical rotations of synthetic product were identical with those of the natural product. The result indicated the absolute configuration of **5**

to be 2*S*, 2'*R*, 3*S*, and 4*R*. We have recently reported the total synthesis of AGL-9b (6)[5] and have shown that **6** also had the same stereochemistry.

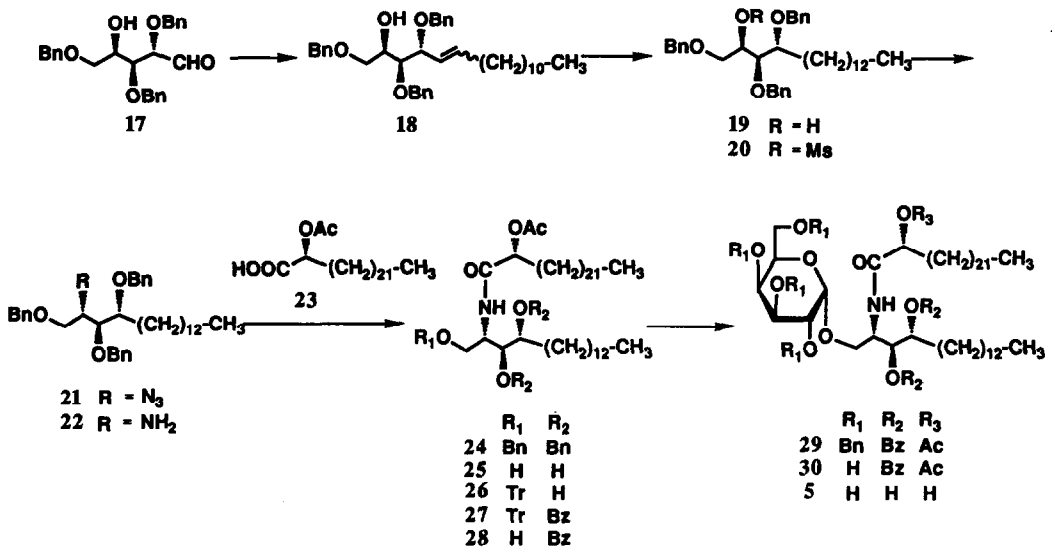


Fig. 5 Scheme for total synthesis of 5

The *in vivo* antitumor activity of AGLs was examined using intraperitoneally implanted B16 murine melanoma. All AGLs, especially those with the α-galactosyl linkage (**3**-**8**) showed high activity with T/C ranging from 160 to 190%. Furthermore, in an *in vitro* MLR assay these compounds showed 200-400% relative ^{3}H-TdR incorporation at concentrations <1 μg/ml. In contrast, the *in vitro* cytotoxicity of AGLs was weak and no activity was observed against B16 melanoma cells at 20 μg/ml. The acute toxicity of AGLs in rats (intravenous injection) was also weak (LD50 > 10 mg/kg).

Their low toxicity and easy synthesis make AGLs promising antitumor agents.

EXPERIMENTAL

Instrumentation Optical rotations were determined using a JASCO DIP-140 Digital Polarimeter. Melting points were measured with Yanagimoto micro-melting point apparatus. IR spectra were measured on a JASCO A-3 IR Spectrometer. Mass spectral measurements were obtained with a JEOL JMS SX/SX-102A and a Hitachi M-80 Mass Spectrometer. UV spectra were recorded using a Hitachi U-3200 UV Spectrophotometer. ^{1}H NMR, ^{1}H-^{1}H COSY, ^{13}C NMR, ^{1}H-^{13}C COSY and HMBC spectra were obtained using a JEOL JNM-GX500 FT NMR Spectrometer.

Preparation of AGLs The sponge *Agelas mauritianus* was collected at the depth of 15-25 m in Kume Shima, Okinawa, Japan. A sample was lyophilized to give a dry powder (346.5 g) which was extracted with chloroform-methanol (1:1). The extract was then concentrated *in vacuo* to give 43.67 g of residue. The

residue was distributed between ethyl acetate and water. The upper layer was dried over anhydrous sodium sulfate and the lower aqueous layer was extracted with 1-butanol. The ethyl acetate and 1-butanol layers were combined and concentrated *in vacuo* to give 36.77 g of residue which was washed with 30% aqueous methanol to remove agelasines. The remaining material was extracted with methanol to give 4.66 g of a brown solid. The solid was applied to a silica gel column (Wako Gel C-200, 400 g) and eluted with a step gradient of chloroform-methanol. Elution with chloroform containing 5-8% methanol yielded an active fraction I (224.3 mg), and 7-10% of fraction II. Fraction I was extracted with a small amount of methanol to give a crude solid. This crude solid was chromatographed on a Toyopearl HW-40 column (chloroform-methanol 1:1) to give an active fraction (161.8 mg). This fraction was further purified by reversed-phase HPLC using a D-ODS-5 column (YMC Co., 20ϕ×250 mm) eluting with methanol at the flow rate of 11 ml/min to afford **1** (31.7 mg, retention time 54 min) and **2** (9.9 mg, 65 min).

The same procedure using 1,077.6 g lyophilized sponge gave 371.5 mg fraction II which, on separation by HPLC, yielded **3** (24.0 mg, retention time 39 min), **4** (29.5 mg, 41 min), **5** (20.9 mg, 46 min), **6** (86.6 mg, 50 min), **7** (103.8 mg, 59 min) and **8** (9.8 mg, 74 min).

Agelasphin-10 (1): $[\alpha]_D^{24}$ +3.0° (*c* 1.0, Pyr); $[\alpha]_D^{28}$ -1.6° (*c* 1.0, 1-propanol); m.p. 141.0-142.0 °C; negative HR FABMS *m/z* 836.6589 [(M-H)$^-$, calcd. for $C_{49}H_{90}NO_9$ 836.6621]; UV : λ_{max} (methanol) 236 nm (ε 5800); ^{1}H NMR (500 MHz, C_5D_5N) δ 8.33 (1H, d, *J* = 9.2 Hz, NH), 7.61 (1H, d, *J* = 5.5 Hz, OH), 7.22 (1H, bs, OH), 7.19 (1H, bs, OH) 7.12 (1H, bs, OH), 6.86 (1H, d, *J* = 4.9 Hz, OH), 6.35 (1H, bs, OH), 6.19 (1H, d, *J* = 15.3 Hz, H10), 5.99 (1H, dd, *J* = 6.7, 15.9 Hz, H4), 5.90 (1H, dt, *J* = 6.1, 15.9 Hz, H5), 5.64 (1H, dt, *J* = 7.3, 15.3 Hz, H11), 5.49 (1H, bt, *J* = 6.7 Hz, H8), 4.89 (1H, d, *J* = 7.9 Hz, H1"), 4.79 (1H, m, H2), 4.74 (1H, m, H3), 4.69 (1H, dd, *J* = 6.1, 10.4 Hz, H1a), 4.56 (1H, m, H2'), 4.49 (1H, bd, *J* = 11.0 Hz, H6"a), 4.34 (1H, m, H6"b), 4.21 (1H, m, H1b), 4.19 (2H, m, H4", H3"), 4.01 (1H, m, H2"), 3.88 (1H, m, H5"), 2.21 (2H, m, H7), 2.18 (1H, m, H3'a), 2.15 (2H, m, H6), 2.11 (2H, m, H12), 2.00 (1H, m, H3'b), 1.79 (1H, m), 1.75 (3H, s, H19), 1.70 (1H, m), 1.20-1.40 (48H, m), 0.84 (3H, t, *J* = 6.7 Hz, terminal methyl) and 0.84 (3H, d, *J* = 6.7 Hz, terminal methyl).

Agelasphin-12 (2): $[\alpha]_D^{24}$ +2.2° (*c* 1.0, Pyr); $[\alpha]_D^{28}$ -2.0° (*c* 1.0, 1-propanol); m.p. 158.5-159.5 °C; negative HR FABMS *m/z* 850.6742 [(M-H)$^-$, calcd. for $C_{50}H_{92}NO_9$ 850.6777]; UV : λ_{max} (methanol) 236 nm (ε 5500); ^{1}H NMR (500 MHz, C_5D_5N) δ 8.34 (1H, d, *J* = 9.2 Hz, NH), 7.62 (1H, d, *J* = 5.5 Hz, OH), 7.22 (1H, d, *J* = 3.7 Hz, OH), 7.19 (1H, bs, OH) 7.13 (1H, bs, OH), 6.87 (1H, d, *J* = 4.9 Hz, OH), 6.36 (1H, bt, *J* = 6.1 Hz, OH), 6.19 (1H, d, *J* = 15.3 Hz, H10), 5.99 (1H, dd, *J* = 6.7, 15.9 Hz, H4), 5.89 (1H, dt, *J* = 6.1, 15.9 Hz, H5), 5.63 (1H, dt, *J* = 7.3, 15.3 Hz, H11), 5.49 (1H, bt, *J* = 6.7 Hz, H8), 4.89 (1H, d, *J* = 7.9 Hz, H1"), 4.78 (1H, m, H2), 4.74 (1H, m, H3), 4.69 (1H, dd, *J* = 6.1, 10.4 Hz, H1a), 4.56 (1H, m, H2'), 4.49 (1H, m, H6"a), 4.34 (1H, m, H6"b), 4.21 (1H, m, H1b), 4.19 (2H, m, H4", H3"), 4.01 (1H, m, H2"), 3.88 (1H, m, H5"), 2.21 (3H, m, H7, H3'a), 2.15 (2H, m, H6), 2.11 (2H, m, H12), 2.00 (1H, m, H3'b), 1.79 (1H, m), 1.75 (3H, s, H19), 1.70 (1H, m), 1.20-1.40 (57H, m), 0.84 (3H, t, *J* = 6.7 Hz, terminal methyl), 0.84 (3H, d, *J* = 6.7 Hz, terminal methyl).

Agelasphin-7a (3): $[\alpha]_D^{24}$ +52.3° (*c* 1.0, Pyr); m.p. 193.5-195.0 °C; negative HR FABMS *m/z* 816.6619 [(M-H)$^-$, calcd. for $C_{46}H_{90}NO_{10}$ 816.6557]; ^{1}H NMR (500 MHz, C_5D_5N), δ 8.49 (1H, d, *J* = 9.2 Hz, NH), 7.53 (1H, bs, OH), 7.04 (1H, bs, OH), 6.71 (1H, d, *J* = 6.7 Hz, OH), 6.68 (1H, bs, OH), 6.52 (1H, bs, OH), 6.32 (1H, bs, OH), 6.09 (1H, d, *J* = 6.1 Hz, OH), 5.58 (1H, d, *J* = 3.7 Hz, H1"), 5.26 (1H, m, H2), 4.62 (2H, m, H2', H2"), 4.57 (1H, m, H1a), 4.52 (1H, bs, H4"), 4.48 (2H, m, H5", H3"), 4.37 (1H, m, H3), 4.34 (2H, m, H1b, H6"a), 4.32 (1H, m, H6"b), 4.26 (1H, m, H4), 2.28 (1H, m), 2.18 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.73 (1H, m), 1.66 (2H, m), 1.10-1.40 (56H, m) and 0.85 (6H, t, *J* = 7.3 Hz, terminal methyl).

Agelasphin-7b (4): $[\alpha]_D^{24}$ +51.9° (*c* 0.5, Pyr); m.p. 203.0-205.0 °C; negative HR FABMS *m/z* 830.6730 [(M-H)$^-$, calcd. for $C_{47}H_{92}NO_{10}$ 830.6726]; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.49 (1H, d, *J* = 9.2 Hz, NH), 7.53 (1H, bs, OH), 7.04 (1H, bs, OH), 6.71 (1H, d, *J* = 6.7 Hz, OH), 6.68 (1H, bs, OH), 6.52 (1H, bs,

OH), 6.32 (1H, bs, OH), 6.09 (1H, d, J = 6.1 Hz, OH), 5.58 (1H, d, J = 3.7 Hz, H1"), 5.26 (1H, m, H2), 4.62 (2H, m, H2', H2"), 4.57 (1H, m, H1a), 4.51 (1H, bs, H4"), 4.48 (2H, m, H5", H3"), 4.36 (1H, m, H3), 4.33 (3H, m, H1b, H6"a, b), 4.25 (1H, m, H4), 2.29 (1H, m), 2.18 (1H, m), 1.99 (1H, m), 1.88 (2H, m), 1.73 (1H, m), 1.66 (2H, m), 1.46 (2H, m), 1.10-1.42 (53H, m), 0.84 (9H, m, terminal methyl).

Agelasphin-9a (5): $[\alpha]_D^{24}$ +49.9° (*c* 1.0, Pyr); m.p. 201.0-203.5 °C; negative HR FABMS *m/z* 830.6747 [(M-H)$^-$, calcd. for $C_{47}H_{92}NO_{10}$ 830.6726]; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.48 (1H, d, J = 9.2 Hz, NH), 7.53 (1H, bs, OH), 7.03 (1H, bs, OH), 6.71 (1H, d, J = 6.7 Hz, OH), 6.67 (1H, bs, OH), 6.53 (1H, bs, OH), 6.32 (1H, bs, OH), 6.09 (1H, bs, OH), 5.59 (1H, d, J = 3.7 Hz, H1"), 5.27 (1H, m, H2), 4.63 (2H, m, H2', H2"), 4.58 (1H, m, H1a), 4.52 (1H, bs, H4"), 4.47 (2H, m, H5", H3"), 4.38 (1H, m, H3), 4.32 (3H, m, H1b, H6"a, b), 4.26 (1H, m, H4), 2.27 (1H, m), 2.18 (1H, m), 1.98 (1H, m), 1.88 (2H, m), 1.73 (1H, m), 1.65 (2H, m), 1.10-1.46 (58H, m), 0.85 (6H, t, J = 7.3 Hz, terminal methyl).

Agelasphin-9b (6): $[\alpha]_D^{24}$ +55.0° (*c* 1.0, Pyr); m.p. 211.0-212.0 °C; negative HR FABMS *m/z* 844.6948 [(M-H)$^-$, calcd. for $C_{48}H_{94}NO_{10}$ 844.6883]; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.50 (1H, d, J = 9.2 Hz, NH), 7.55 (1H, bs, OH), 7.01 (1H, bs, OH), 6.69 (1H, d, J = 6.7 Hz, OH), 6.65 (1H, bs, OH), 6.52 (1H, bs, OH), 6.30 (1H, bs, OH), 6.08 (1H, d, J = 6.1 Hz, OH), 5.59 (1H, d, J = 3.7 Hz, H1"), 5.28 (1H, m, H2), 4.64 (2H, m, H2', H2"), 4.59 (1H, m, H1a), 4.53 (1H, m, H4"), 4.48 (2H, m, H5", H3"), 4.39 (1H, m, H3), 4.34 (2H, m, H1b, H6"a), 4.32 (1H, m, H6"b), 4.27 (1H, m, H4), 2.29 (1H, m), 2.19 (1H, m), 1.99 (1H, m), 1.88 (2H, m), 1.73 (1H, m), 1.66 (2H, m), 1.46 (2H, m), 1.20-1.40 (55H, m), 0.84 (3H, t, J = 6.1 Hz, terminal methyl), 0.84 (6H, d, J = 6.7 Hz, terminal methyl); ^{1}H NMR (500 MHz, C_5D_5N+D_2O 95:5, 55°C) δ 5.47(1H, d, J = 3.7 Hz, H1"), 5.14 (1H, m, H2), 4.62 (1H, m, H2'), 4.59 (1H, m, H2"), 4.58 (1H, m, H1a), 4.46 (2H, m, H4", H5"), 4.43 (1H, dd, J = 3.7, 10.4 Hz, H3"), 4.32(2H, m, H3, H1b), 4.30 (2H, m, H6"a, b), 4.26 (1H, m, H4), 2.21 (2H, m, H5a, H3'a), 2.01 (1H, m, H3'b), 1.90 (2H, m, H5b), 1.75 (1H, m), 1.70 (2H, m), 1.55 (2H, m), 1.20-1.40 (55H, m), 0.91 (3H, t, J = 7.3 Hz, terminal methyl), 0.91 (6H, d, J = 6.7 Hz, terminal methyl).

Agelasphin-11 (7): $[\alpha]_D^{24}$ +51.9° (*c* 1.0, Pyr); m.p. 189.5-190.5 °C; negative HR FABMS *m/z* 858.7032 [(M-H)$^-$, calcd. for $C_{49}H_{96}NO_{10}$ 858.7040]; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.47 (1H, d, J = 9.2 Hz, NH), 7.51 (1H, d, J = 4.9 Hz, OH), 6.96 (1H, d, J = 4.9 Hz, OH), 6.64 (1H, d, J = 6.7 Hz, OH), 6.60 (1H, d, J = 5.0 Hz, OH), 6.48 (1H, bt, J = 5.1 Hz, OH), 6.24 (1H, d, J = 3.7 Hz, OH), 6.04 (1H, d, J = 6.1 Hz, OH), 5.56 (1H, d, J = 3.7 Hz, H1"), 5.24 (1H, m, H2), 4.60 (2H, m, H2', H2"), 4.56 (1H, m, H1a), 4.50 (1H, bs, H4"), 4.45 (2H, m, H5", H3"), 4.35 (1H, m, H3), 4.31 (3H, m, H1b, H6"a, b), 4.24 (1H, m, H4), 2.26 (1H, m), 2.18 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.73 (1H, m), 1.66 (2H, m), 1.20-1.40 (59H, m), 0.88 (9H, m, terminal methyl). ^{1}H(500 MHz, C_5D_5N+D_2O 95:5, 55°C) δ 5.43 (1H, d, J = 3.7 Hz, H1"), 5.04 (1H, m, H2), 4.67 (1H, dd, J = 4.0, 7.4 Hz, H2'), 4.56 (1H, m, H2"), 4.54 (1H, m, H1a), 4.46 (1H, m, H4"), 4.45 (1H, m, H5"), 4.44 (1H, dd, J = 3.7, 10.4 Hz, H3"), 4.38 (1H, dd, J = 4.9, 5.0 Hz, H3), 4.33 (1H, m, H1b), 4.31 (1H, m, H6"a), 4.28 (1H, m, H6"b), 4.25 (1H, m, H4), 2.19 (2H, m, H5a, H3'a), 2.07 (1H, m, H3'b), 1.95 (1H, m, H5b), 1.75 (2H, m), 1.20-1.50 (61H, m), 1.00 (9H, m, terminal methyl).

Agelasphin-13 (8): $[\alpha]_D^{24}$ +48.8° (*c* 0.5, Pyr); m.p. 215.5-218.0 °C; negative HR FABMS *m/z* 872.7202 [(M-H)$^-$, calcd. for $C_{50}H_{98}NO_{10}$ 872.7196]; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.50 (1H, d, J = 9.2 Hz, NH), 7.53 (1H, bs, OH), 7.02 (1H, bs, OH), 6.71 (1H, d, J = 6.7 Hz, OH), 6.66 (1H, bs, OH), 6.52 (1H, bs, OH), 6.31 (1H, bs, OH), 6.09 (1H, d, J = 3.9 Hz, OH), 5.59 (1H, d, J = 3.7 Hz, H1"), 5.27 (1H, m, H2), 4.62 (2H, m, H2', H2"), 4.58 (1H, m, H1a), 4.52 (1H, bs, H4"), 4.47 (2H, m, H5", H3"), 4.38 (1H, m, H3), 4.33 (3H, m, H1b, H6"a, b), 4.26 (1H, m, H4), 2.28 (1H, m), 2.18 (1H, m), 1.99 (1H, m), 1.87 (2H, m), 1.73 (1H, m), 1.66 (2H, m), 1.10-1.42 (61H, m), 0.85 (9H, m, terminal methyl).

Methanolysis of cerebrosides (1-8) and acetylation of the long-chain base Each compound was treated with 0.9 N HCl in 82% aqueous methanol (1-3 ml) for 18 hr at 80 °C. The reaction mixture was extracted with n-hexane and the hexane layer was concentrated and chromatographed using silica gel (n-

hexane-ethyl acetate 7:3) to give a methyl ester of fatty acid. The aqueous methanol layer was neutralized and chromatographed using Amberlite CG-400 eluted with methanol to give a long-chain base and a 1-*O*-methylated sugar. The long-chain base fraction was dried and heated with acetic anhydride / pyridine (1:1) for 1.5 hr at 70 °C. The reaction mixture was diluted with H_2O and extracted with ethyl acetate. The residue of the ethyl acetate layer was chromatographed using silica gel (n-hexane-ethyl acetate 3:2) to give an acetate of the long-chain base. The yields of each compound are shown as follows.

compound	ester	long-chain base acetate	methyl glycoside
AGL-10 (1) (90.2 mg)	**9** (37.4 mg)	**10** (11.6 mg)	glucoside (16.4 mg)
AGL-12 (2) (17.4 mg)	**12** (6.4 mg)	**10** (0.6 mg)	glucoside (3.5 mg)
AGL-7a (3) (10.7 mg)	**9** (4.2 mg)	**13** (1.0 mg)	galactoside (2.4 mg)
AGL-7b (4) (11.4 mg)	(4.4 mg)	(1.2 mg)	galactoside (2.6 mg)
AGL-9a (5) (10.7 mg)	**9** (3.2 mg)	**14** (1.0 mg)	galactoside (2.8 mg)
AGL-9b (6) (21.3 mg)	**9** (9.0 mg)	**15** (3.4 mg)	galactoside (4.2 mg)
AGL-11 (7) (19.5 mg)	**9** (7.4 mg)	**16** (0.6 mg)	galactoside (3.9 mg)
AGL-13 (8) (7.2 mg)	**12** (3.1 mg)	**17** (0.6 mg)	galactoside (1.1 mg)

AGL-7b (4) (11.4 mg) gave a mixture (4.4 mg) of methyl 2-hydroxytricosanoate and **9**, a mixture (1.2 mg) of 2-acetoamino-1,3,4-triacetoxy-15-methylheptadecane and / or 2-acetoamino-1,3,4-triacetoxy-16-methylheptadecane and 2-acetoamino-1,3,4-triacetoxy-14-methylhexadecane and / or 2-acetoamino-1,3,4-triacetoxy-15-methylhexadecane and methyl galactoside (2.6 mg). The mixture of esters exhibited M^+ in FDMS at *m/z* 384 and 398. ^1H NMR spectrum mixture of esters was almost the same as that of **9**. The mixture of long-chain base acetate showed $(M+H)^+$ in FDMS at *m/z* 472 and 486, and almost the same ^1H NMR spectrum as that of **13**.

Methyl 2-(*R*)-hydroxytetracosanoate (9): $[\alpha]_D^{23}$ -2.4° (*c* 3.0, $CHCl_3$); FDMS *m/z* 398 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 4.19 (1H, dd, *J* = 4.3, 7.3 Hz, H2), 3.79 (3H, s, OCH_3), 2.74 (1H, bs, OH), 1.77 (1H, m), 1.63 (1H, m), 1.18-1.49 (40H, m) and 0.88 (3H, t, *J* = 7.3 Hz, CH_3). The ester **9** was identical with an authentic sample according to TLC, optical rotation, FDMS, and ^1H NMR spectrum.

(4*E*,8*E*,10*E*,2*S*,3*R*)-2-Acetoamino-1,3-diacetoxy-9-methyl-4,8,10-octadecanetriene (10): $[\alpha]_D^{23}$ -11.6° (*c* 0.39, $CHCl_3$); FDMS *m/z* 435 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 6.21 (1H, dd, *J* = 10.9, 14.6 Hz, H10), 5.79 (2H, m, H5, H11), 5.65 (1H, d, *J* = 9.1 Hz, NH), 5.58 (1H, m, H8), 5.41 (1H, m, H4), 5.28 (1H, m, H3), 4.43 (1H, m, H2), 4.30 (1H, dd, *J* = 6.1, 11.6 Hz, H1a), 4.04 (1H, dd, *J* = 3.4, 11.6 Hz, H1b), 1.95-2.15 (15H, m, Ac×3, H6, H7, H12), 1.71 (3H, s, H19), 1.22-1.62 (10H, m) and 0.88 (3H, t, *J* = 6.1 Hz, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ 170.9 (s), 169.9 (s), 169.6(s), 137.0 (d), 135.6 (s), 132.8 (d), 126.5 (d), 125.2 (d), 124.6 (d), 73.9 (d), 62.6 (t), 50.8 (d), 39.2 (t), 32.9 (t), 31.8 (t), 31.8 (t), 29.6 (t), 28.9 (t), 27.1 (t), 23.3 (q), 22.6 (t), 21.1 (q), 20.8 (q), 16.4 (q) and 14.1 (q).

Methyl 2-(*R*)-hydroxypentacosanoate (12): $[\alpha]_D^{23}$ -2.2° (*c* 0.5, $CHCl_3$); FDMS *m/z* 413 $[(M+H)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ 4.19 (1H, dd, *J* = 4.3, 7.3 Hz, H2), 3.79 (3H, s, OCH_3), 2.74 (1H, bs, OH), 1.77 (1H, m), 1.63 (1H, m), 1.18-1.49 (40H, m) and 0.88 (3H, t, *J* = 7.3 Hz, CH_3).

2-Acetoamino-1,3,4-triacetoxyhexadecane (13): FDMS *m/z* 458 $[(M+H)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, d, *J* = 9.2 Hz, NH), 5.10 (1H, dd, *J* = 8.5, 3.1 Hz, H3), 4.93 (1H, dt, *J* = 9.8, 3.1 Hz, H4), 4.47 (1H, m, H2), 4.29 (1H, dd, *J* = 11.6, 4.3 Hz, H1a), 4.00 (1H, dd, *J* = 11.6, 3.1 Hz, H1b), 2.08 (3H, s, 3-OAc), 2.05 (6H, s, 1-OAc, 4-OAc), 2.03 (3H, s, NHAc), 1.12-1.70 (22H, m) and 0.88 (3H, t, *J* = 6.1 Hz, CH_3).

2-Acetoamino-1,3,4-triacetoxyheptadecane (14): FDMS *m/z* 472 $[(M+H)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, d, *J* = 9.2 Hz, NH), 5.10 (1H, dd, *J* = 8.5, 3.1 Hz, H3), 4.93 (1H, dt, *J* = 9.8, 3.1 Hz, H4),

4.47 (1H, m, H2), 4.29 (1H, dd, J = 11.6, 4.3 Hz, H1a), 4.00 (1H, dd, J = 11.6, 3.1 Hz, H1b), 2.08 (3H, s, 3-OAc), 2.05 (6H, s, 1-OAc, 4-OAc), 2.03 (3H, s, NHAc), 1.12-1.70 (22H, m) and 0.88 (3H, t, J = 6.1 Hz, CH_3).

2-Acetoamino-16-methyl-1,3,4-triacetoxyheptadecane (15): $[\alpha]_D^{23}$ +23.4° (c 0.3, $CHCl_3$); FDMS m/z 486 $[(M+H)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, d, J = 9.2 Hz, NH), 5.10 (1H, dd, J = 8.5, 3.1 Hz, H3), 4.93 (1H, dt, J = 9.8, 3.1 Hz, H4), 4.47 (1H, m, H2), 4.29 (1H, dd, J = 11.6, 4.3 Hz, H1a), 4.00 (1H, dd, J = 11.6, 3.1 Hz, H1b), 2.08 (3H, s, 3-OAc), 2.05 (6H, s, 1-OAc, 4-OAc), 2.03 (3H, s, NHAc), 1.12-1.70 (22H, m) and 0.88 (3H, t, J = 6.1 Hz, CH_3).

2-Acetoamino-16-methyl-1,3,4-triacetoxyoctadecane (16): FDMS m/z 500 $[(M+H)^+]$; ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, d, J = 9.2 Hz, NH), 5.10 (1H, dd, J = 8.5, 3.1 Hz, H3), 4.93 (1H, dt, J = 9.8, 3.1 Hz, H4), 4.47 (1H, m, H2), 4.29 (1H, dd, J = 11.6, 4.3 Hz, H1a), 4.00 (1H, dd, J = 11.6, 3.1 Hz, H1b), 2.08 (3H, s, 3-OAc), 2.05 (6H, s, 1-OAc, 4-OAc), 2.03 (3H, s, NHAc), 1.12-1.70 (22H, m) and 0.88 (3H, t, J = 6.1 Hz, CH_3).

(2*R*,3*S*,4*R*)-1,3,4-Tri-*O*-benzyl-5-heptadecene-1,2,3,4-tetraol (18): To a solution of dodecyl triphenylphosphonium bromide (32.1 g) in tetrahydrofuran (40 ml) under an argon atmosphere a 2 N solution of n-butyllithium in n-hexane (30 ml) was added dropwise at -10 °C and the mixture was stirred at -10 °C for 15 min. To this mixture a solution of (2*R*,3*S*,4*R*)-2,3,5-tribenzyloxy-4-hydroxypentanal (**17**)[12] (13.18 g) in tetrahydrofuran (20 ml) was added dropwise at -10 °C and allowed to warm to room temperature and stirred for 16 hr. After the mixture had been quenched with methanol it was dissolved in 80% aqueous methanol, extracted with n-hexane, washed with brine and then concentrated. Purification of the residue on a silica gel column (Wako Gel C-200, 200 g), eluted with hexane-ethyl acetate (9:1), yielded 9.31 g (51.9%) of **18**. $[\alpha]_D^{23}$ -38.5° (c 1.0, $CHCl_3$); FDMS m/z 573 $[(M+H)^+]$, 301; positive HR FABMS m/z 595.3731 $[(M+Na)^+$, calcd. for $C_{38}H_{52}O_4Na$ 595.3766]; ^1H NMR (500 MHz, $CDCl_3$) δ 7.22-7.35 (15H, m, aromatic-H), 5.72 (1H, dt, J = 7.9, 11.0 Hz, H6), 5.46 (1H, dd, J = 9.8, 11.0 Hz, H5), 4.68 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.60 (1H, d, J = 12.2 Hz, -OC$\underline{H}_2$Ph), 4.47-4.52 (3H, m, -OC$\underline{H}_2$Ph), 4.43 (1H, dd, J = 5.5, 9.8 Hz, H4), 4.33 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.08 (1H, m, H2), 3.57 (1H, dd, J = 2.5, 5.5 Hz, H3), 3.51 (2H, d, J = 6.1 Hz, H1a, b), 3.01 (1H, d, J = 5.5 Hz, OH), 1.87-2.01 (2H, m, H7a, b), 1.18-1.38 (18H, m) and 0.88 (3H, t, J = 6.7 Hz, terminal methyl).

(2*R*,3*S*,4*R*)-1,3,4-Tri-*O*-benzyl-1,2,3,4-heptadecanetetraol (19): To a solution of **18** (9.31 g) in tetrahydrofuran (30 ml), 10% Pd-C (0.53 g) was added. After the reaction vessel was purged with hydrogen, the mixture was stirred at room temperature for 16 hr, filtered through celite, and the filtrate was concentrated to give 9.34 g (100%) of **19**. $[\alpha]_D^{24}$ -35.1° (c 0.5, $CHCl_3$); FDMS m/z 575 $[(M+H)^+]$; positive HR FABMS m/z 575.4138 $[(M+H)^+$, calcd. for $C_{38}H_{55}O_4$ 575.4103]; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24-7.34 (15H, m, aromatic-H), 4.69 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.65 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.56 (1H, d, J = 11.0 Hz, -OC$\underline{H}_2$Ph), 4.53 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.50 (1H, d, J = 11.0 Hz, -OC$\underline{H}_2$Ph), 4.48 (1H, d, J = 12.2 Hz, -OC$\underline{H}_2$Ph), 4.04 (1H, m, H2), 3.69 (1H, m, H4), 3.60 (1H, dd, J = 3.1, 4.3 Hz, H3), 3.54 (2H, d, J = 6.1 Hz, H1a, b), 3.17 (1H, d, J = 4.9 Hz, OH), 1.85 (3H, m), 1.65 (2H, m), 1.56 (1H, m), 1.20-1.47 (18H, m) and 0.88 (3H, t, J = 6.7 Hz, terminal methyl).

(2*R*,3*S*,4*R*)-1,3,4-Tri-*O*-benzyl-2-*O*-methanesulfonyl-1,2,3,4-heptadecanetetraol (20): To a solution of **19** (9.34 g) in pyridine (70 ml), methanesulfonyl chloride (2.52 ml) was added dropwise at 0 °C and allowed to warm to room temperature and stirred for 2 hr. The mixture was concentrated and the residue was dissolved in diethyl ether and washed with water and brine then concentrated. Purification of the residue on a silica gel column (Wako Gel C-200, 200 g) eluted with hexane-ethyl acetate (9:1) yielded 9.74 g (91.8 %) of **20**. $[\alpha]_D^{24}$ +6.5° (c 1.0, $CHCl_3$); FDMS m/z 653 $[(M+H)^+]$; positive HR FABMS m/z 653.3868 $[(M+H)^+$, calcd. for $C_{39}H_{57}O_6S$ 653.3879]; ^1H NMR (500 MHz, $CDCl_3$) δ 7.28-7.34 (15H, m, aromatic-H), 4.91 (1H, m, H2), 4.77 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.62 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.58 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.55 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.49 (1H, d, J = 11.6 Hz, -OC$\underline{H}_2$Ph), 4.48 (1H, d, J = 11.6 Hz,

-OC$\underline{H_2}$Ph), 3.88 (1H, t, J = 3.9 Hz, H3), 3.72 (2H, d, m, H1a, b), 3.61 (1H, m, H4), 2.90 (1H, s, -OSO$_2$CH$_3$), 1.72 (1H, m, H5a), 1.54 (1H, m, H5b), 1.18-1.47 (22H, m) and 0.89 (3H, t, J = 7.3 Hz, terminal methyl).

(2*S*,3*S*,4*R*)-1,3,4-Tri-*O*-benzyl-2-azide-1,3,4-heptadecanetriol (21): To a solution of **20** (9.74 g) in *N*,*N*-dimethylformamide (100 ml), sodium azide (9.70 g) was added. After stirring at 120 °C for 16 hr, the mixture was diluted with ethyl acetate and successively washed with water and brine and then concentrated. Purification of the residue on a silica gel column (Wako Gel C-200, 200 g) eluted with hexane-ethyl acetate (98:2) yielded 6.75 g (75.4%) of **21**. $[\alpha]_D^{24}$ +8.2° (*c* 1.0, $CHCl_3$); FDMS *m/z* 600 [(M+H)$^+$], 573, 450; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.35 (15H, m, aromatic-H), 4.69 (1H, d, J = 11.0 Hz, -OC$\underline{H_2}$Ph), 4.60 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.56 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.48-4.52 (3H, m, -OC$\underline{H_2}$Ph), 3.75-3.81 (2H, m, H1a, H2), 3.65-3.71 (2H, d, m, H1b, H3), 3.60 (1H, dt, J = 3.7, 7.3 Hz, H4), 1.65 (1H, m, H5a), 1.56 (1H, m, H5b), 1.20-1.45 (22H, m) and 0.88 (3H, t, J = 6.7 Hz, terminal methyl).

(2*S*,3*S*,4*R*)-2-Amino-1,3,4-tri-*O*-benzyl-1,3,4-heptadecanetriol (22): To a solution of **21** (605.5 mg) in tetrahydrofuran (6 ml), 10% Pd-C (60 mg) was added. After the reaction vessel was purged with hydrogen, the mixture was stirred at room temperature for 16 hr, filtered through celite, and the filtrate was concentrated. Purification of the residue on a silica gel column (Wako Gel C-200, 20 g) eluted with hexane-ethyl acetate (6:4) yielded 459.9 mg (79.4%) of **22**. $[\alpha]_D^{24}$ -7.0° (*c* 0.5, $CHCl_3$); FDMS *m/z* 574 [(M+H)$^+$]; positive HR FABMS *m/z* 574.4226 [(M+H)$^+$, calcd. for $C_{38}H_{56}NO_3$ 574.4263]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.35 (15H, m, aromatic-H), 4.74 (1H, d, J = 11.0 Hz, -OC$\underline{H_2}$Ph), 4.62 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.53 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.52 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.48 (2H, d, J = 1.8 Hz, -OC$\underline{H_2}$Ph), 3.69-3.72 (2H, m, H4, H3), 3.58 (1H,dd, J = 3.7, 6.7 Hz, H1a), 3.49 (1H,dd, J = 7.3, 9.2 Hz, H1b), 3.16 (1H,dt, J = 3.1, 7.3 Hz, H2), 1.82 (1H, m, H5a), 1.69 (1H, m, H5b), 1.58 (1H, m), 1.49 (1H, m), 1.20-1.35 (20H, m) and 0.88 (3H, t, J = 7.3 Hz, terminal methyl).

(*R*)-2-Acetoxytetracosanoic acid (23): A sample of **23** synthesized by the method described in reference 8 was kindly provided by Prof. Ohta of Keio University. $[\alpha]_D^{20}$ +8.5° (*c* 1.0, $CHCl_3$).

(2*S*,3*S*,4*R*)-2-Amino-1,3,4-tri-*O*-benzyl-*N*-[(*R*)-2-hydroxytetracosanoyl]-1,3,4-heptadecanetriol (24): Compound **22** (153.3 mg) and **23** (113.8 mg) were dissolved in tetrahydrofuran (4 ml), and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, 99.0 mg) was added to the solution. The mixture was stirred at room temperature for 60 hr and then concentrated and purified on a silica gel column (Wako Gel C-200, 10 g, hexane-ethyl acetate 9:1) to give compound **24** at a yield of 205.6 mg (78.3%). $[\alpha]_D^{23}$ +2.1° (*c* 0.6, $CHCl_3$); FDMS *m/z* 983 [(M+H)$^+$]; negative HR FABMS *m/z* 980.7702 [(M-H)$^-$, calcd. for $C_{64}H_{102}NO_6$ 980.7713]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22-7.36 (15H, m, aromatic-H), 6.50 (1H, d, J = 9.2 Hz, NH), 5.05 (1H, dd, J = 4.9, 7.3 Hz, H2'), 4.82 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.62 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.55 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.52 (1H, d, J = 11.6 Hz, -OC$\underline{H_2}$Ph), 4.42 (2H, s, -OC$\underline{H_2}$Ph), 4.23 (1H, m, H2), 3.84 (2H, m, H3, H1a), 3.51 (1H, m, H4), 3.48 (1H, dd, J = 3.7, 9.8 Hz, H1b), 1.98 (3H, s, Ac), 1.60-1.82 (2H, m), 1.50 (1H, m), 1.20-1.35 (63H, m) and 0.88 (6H, t, J = 7.3 Hz, terminal methyl).

(2*S*,3*S*,4*R*)-*N*-[(*R*)-2-Acetoxytetracosanoyl]-2-amino-1,3,4-heptadecanetriol (25): To a solution of **24** (317.7 mg) in tetrahydrofuran-n-propanol (1:1, 6 ml), 10% palladium on charcoal (167.4 mg) and formic acid (0.6 ml) were added. After the reaction vessel was purged with hydrogen, the mixture was stirred at 40 °C for 5 hr. The reaction mixture was then diluted with chloroform (10 ml), filtered through celite, and the filtrate was concentrated. Purification on a silica gel column (Wako Gel C-200, 15 g) eluted with chloroform-methanol (98:2) yielded 191.6 mg (83.2%) of **25**. $[\alpha]_D^{23}$ +6.0° (*c* 0.1, $CHCl_3$); FDMS *m/z* 712 [(M+H)$^+$]; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.63 (1H, d, J = 8.5 Hz, NH), 6.56 (2H, m, OH), 6.13 (1H, bd, J = 5.7 Hz, OH), 5.54 (1H, dd, J = 5.5, 7.3 Hz, H2'), 5.07 (1H, m, H2), 4.47 (1H, m, H1a), 4.43 (1H, m, H1b), 4.38 (1H, m, H3), 4.28 (1H, m, H4), 2.20 (1H, m), 2.07 (2H, m), 2.04 (3H, s, Ac), 1.90 (2H, m), 1.68 (1H, m), 1.15-1.60 (60H, m), and 0.85 (6H, t, J = 6.7 Hz, terminal methyl).

(2*S*,3*S*,4*R*)-*N*-[(*R*)-2-Acetoxytetracosanoyl]-2-amino-1-*O*-triphenylmethyl-1,3,4-heptadecanetriol (26): To a solution of **25** (99.7 mg) in pyridine (3 ml), triphenylmethyl chloride (390.3 mg) and 4-dimethylaminopyridine (5.0 mg) were added and the mixture was stirred at 60 °C for 3 hr. After dilution with chloroform (30 ml), the mixture was washed with brine then concentrated. Purification on a silica gel column (Wako Gel C-200, 5 g) eluted with chloroform yielded 111.7 mg (83.6%) of **26**. $[\alpha]_D^{23}$ -13.3° (*c* 0.1, $CHCl_3$); negative HR FABMS *m/z* 952.7328 [(M-H)$^-$, calcd. for $C_{62}H_{98}NO_6$ 952.7399]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21-7.40 (15H, m, aromatic-H), 6.89 (1H, d, *J* = 8.6 Hz, OH), 5.21 (1H, dd, *J* = 5.1, 6.6 Hz, H2'), 4.27 (1H, m, H2), 3.60 (1H, m, H3), 3.43 (1H, dd, *J* = 3.2, 7.1 Hz, H1a), 3.36 (1H, dd, *J* = 4.2, 7.1 Hz, H1b), 3.34 (1H, m, H4), 2.08 (1H, m), 2.05 (3H, s, Ac), 1.85 (1H, m), 1.75 (1H, m), 1.68 (1H, m), 1.10-1.50 (62H, m), and 0.88 (6H, t, *J* = 7.3 Hz, terminal methyl).

(2*S*,3*S*,4*R*)-*N*-[(*R*)-2-Acetoxytetracosanoyl]-2-amino-3,4-di-*O*-benzoyl-1-*O*-triphenylmethyl-1,3,4-heptadecanetriol (27): To a solution of **26** (166.5 mg) in pyridine (3 ml), benzoyl chloride (0.18 ml) and 4-dimethylaminopyridine (5.0 mg) were added. After stirring at room temperature for 36 hr, the mixture was diluted with brine, extracted with chloroform then the extract was concentrated. Purification of the residue on a silica gel column (Wako Gel C-200, 15 g) eluted with hexane-ethyl acetate (95:5) yielded 193.9 mg (95.6%) of **27**. $[\alpha]_D^{23}$ +7.3°(*c* 0.5, $CHCl_3$); FDMS *m/z* 1162 (M^+), 920; negative HR FABMS *m/z* 1160.7910 [(M-H)$^-$, calcd. for $C_{76}H_{106}NO_8$ 1160.7924]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04-8.16 (25H, m, aromatic-H), 5.91(1H, dd, *J* = 2.4, 9.0 Hz, H3), 5.45 (1H, dt, *J* = 2.9, 9.8 Hz, H4), 5.37 (1H, t, *J* = 7.3 Hz, H2') 4.68 (1H, m, H2), 3.34 (1H, dd, *J* = 3.7, 9.8 Hz, H1a), 3.26 (1H, dd, *J* = 2.9, 9.8 Hz, H1b), 2.02 (3H, s, Ac), 1.12-2.02 (66H, m) and 0.87 (6H, m, terminal methyl).

(2*S*,3*S*,4*R*)-*N*-[(*R*)-2-Acetoxytetracosanoyl]-2-amino-3,4-di-*O*-benzoyl-1,3,4-heptadecanetriol (28): To a solution of **27** (193.9 mg) in methylene chloride-methanol (2:1, 3 ml), p-toluenesulfonic acid monohydrate (63.4 mg) was added. After stirring at room temperature for 1.5 hr, the mixture was concentrated. The residue was dissolved in ethyl acetate and washed with aqueous sodium hydrogen carbonate and brine, and then concentrated. Purification of the residue on a silica gel column (Wako Gel C-200, 15 g) eluted with hexane-ethyl acetate (8:2) yielded 113.1 mg (73.7%) of **28**. $[\alpha]_D^{23}$ + 27.3°(*c* 0.1, $CHCl_3$); FDMS *m/z* 921 [(M+H)$^+$]; negative HR FABMS *m/z* 918.6777 [(M-H)$^-$, calcd. for $C_{57}H_{92}NO_8$ 918.6828]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (2H, d, *J* = 7.3 Hz, aromatic-H), 7.96 (2H, d, *J* = 7.3 Hz, aromatic-H), 7.64 (1H, t, *J* = 7.3 Hz, aromatic-H), 7.54 (1H, t, *J* = 7.6 Hz, aromatic-H), 7.50 (2H, t, *J* = 7.9 Hz, aromatic-H), 7.39 (2H, t, *J* = 7.9 Hz, aromatic-H), 7.06 (1H, d, *J* = 9.2 Hz, NH), 5.48 (1H, dd, *J* = 2.4, 9.1 Hz, H3), 5.38 (1H, dt, *J* = 3.1, 9.8 Hz, H4), 5.19 (1H, t, *J* = 6.1 Hz, H2'), 4.37 (1H, m, H-2), 3.57-3.68 (2H, m, H1), 2.20 (3H, s, Ac), 2.02 (2H, m), 1.92 (2H, m), 1.16-1.50 (62H, m) and 0.88 (6H, m, terminal methyl).

(2*S*,3*S*,4*R*)-*N*-[(*R*)-2-Acetoxytetracosanoyl]-2-amino-3,4-di-*O*-benzoyl-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-1,3,4-heptadecanetriol (29): To a solution of **28** (113.1 mg) in tetrahydrofuran (2 ml), stannous chloride (54.8 mg), silver perchlorate (59.9 mg) and Molecular Sieve 4A powder (500 mg) were added, and the resulting mixture was stirred at room temperature for 30 min. After cooling to -10 °C, a solution of benzylgalactosyl fluoride (313.4 mg) in tetrahydrofuran (2 ml) was added. The resulting mixture was allowed to warm to room temperature, stirred for 2 hr, diluted with acetone, and then filtered through celite. The filtrate was concentrated and the residue was dissolved in ethyl acetate, washed with brine and concentrated. Purification of the residue on a silica gel column (Wako Gel C-200, 10 g) eluted with hexane-ethyl acetate (19:1) yielded 148.0 mg (83.5%) of **29**. $[\alpha]_D^{23}$ +21.0° (*c* 0.1, $CHCl_3$); FDMS *m/z* 1443 (M^+); negative HR FABMS *m/z* 1440.9229 [(M-H)$^-$, calcd. for $C_{91}H_{126}NO_{13}$ 1440.9236]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (2H, d, *J* = 7.9 Hz, aromatic-H), 7.90 (2H, d, *J* = 7.9 Hz, aromatic-H), 7.73 (1H, d, *J* = 8.3 Hz, NH), 7.59 (1H, t, *J* = 6.4 Hz, aromatic-H), 7.50 (1H, t, *J* = 6.4 Hz, aromatic-H), 7.45 (2H, t, *J* = 7.6 Hz, aromatic-H), 7.15-7.40 (22H, m, aromatic-H), 5.78 (1H, dd, *J* = 2.6, 9.8 Hz, H3), 5.40 (1H, m, H4), 5.10 (1H, dd, *J* = 5.2, 7.6 Hz, H2'), 4.88 (1H, d, *J* = 11.3 Hz, -OCH_2Ph), 4.53-4.76 (7H, m, -OCH_2Ph×5, H1", H2),

4.48 (1H, d, J = 11.8 Hz, -OC$\underline{H_2}$Ph), 4.40 (1H, d, J = 11.8 Hz, -OC$\underline{H_2}$Ph), 4.09 (1H, bt, J = 7.2 Hz, H2"), 3.99 (1H, dd, J = 3.3, 10.4 Hz, H1a), 3.93 (1H, bs, H4"), 3.90 (1H, m, H5"), 3.82 (1H, dd, J = 2.4, 9.8 Hz, H3"), 3.59 (1H, dd, J = 2.3, 10.4 Hz, H1b), 3.53 (1H, dd, J = 7.1, 9.2Hz, H6"a), 3.45 (1H, dd, J = 6.7, 9.2 Hz, H6"b), 2.02 (3H, s, Ac), 1.89 (3H, m), 1.40 (2H, m), 1.10-1.35 (61H, m) and 0.88 (6H, m, terminal methyl).

(2*S*,3*S*,4*R*)-*N*-[(*R*)-2-Acetoxytetracosanoyl]-2-amino-3,4-di-*O*-benzoyl-1-*O*-(α-D-galacto-pyranosyl)-1,3,4-heptadecanetriol (30): To a solution of the **29** (147.1 mg) in ethyl acetate (3 ml), palladium black (15 mg) was added. After the reaction vessel was purged with hydrogen, the mixture was stirred at room temperature for 4 hr, filtered through celite, and the filtrate was then concentrated to give 106.6 mg (96.6%) of **30**. $[\alpha]_D^{23}$ +26.0° (*c* 0.1, $CHCl_3$); FDMS *m/z* 1083 (M^+) and 921; negative HR FABMS *m/z* 1080.7289 [$(M-H)^-$, calcd. for $C_{63}H_{102}NO_{13}$ 1080.7357]; ^1H NMR (500 MHz, $CDCl_3$), δ 7.99 (2H, d, J = 7.9 Hz, aromatic-H), 7.90 (2H, d, J = 7.9 Hz, aromatic-H), 7.75 (1H, d, J = 8.3 Hz, NH), 7.60 (1H, t, J = 6.4 Hz, aromatic-H), 7.53 (1H, t, J = 6.4 Hz, aromatic-H), 7.48 (2H, t, J = 7.6, aromatic-H), 7.38 (2H, t, J = 7.6 Hz, aromatic-H), 5.78 (1H, dd, J = 2.4, 9.8 Hz, H3), 5.26 (1H, m, H4), 5.07 (1H, t, J = 6.7 Hz, H2'), 4.70 (1H, d, J = 3.7 Hz, H1"), 4.57 (1H, m, H2), 3.40-3.98 (8H, m, H2", 3", 4", 5", 6"a, b, H1a, b), 2.18 (3H, s, Ac), 1.81-1.95 (4H, m), 1.41 (2H, m), 1.16-1.35 (60H, m) and 0.88 (6H, m, terminal methyl).

(2*S*,3*S*,4*R*)-2-Amino-1-*O*-(α-D-galactopyranosyl)-*N*-[(*R*)-2-hydroxytetracosanoyl]-1,3,4-hepta-decanetriol (5): To a solution of **30** (105.5 mg) in methanol (5 ml) 1 N methanolic sodium methoxide solution (1 ml) was slowly added, and the mixture was stirred at room temperature for 30 min. A cation exchange resin (Dowex 50W X8) was added to neutralize the mixture and the resulting mixture was filtered. The filtrate was concentrated and purified on a silica gel column (Wako Gel C-200, 5 g) eluted with chloroform-methanol-water (90:10:1) to yield 66.7 mg (82.2%) of **5**. This product was identical to AGL-9a extracted from the sponge in all respects including TLC, optical rotation, melting point, IR, MS, and NMR (^1H and ^{13}C) spectroscopy.

Bioassays The extracts, fractions and isolated compounds were routinely evaluated by an *in vivo* antitumor assay using mice intraperitoneally implanted with B16 cells and by MLR assay.[1]

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