Structure–Activity Relationship of α-Galactosylceramides against B16-Bearing Mice

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Agelasphin-9b, (2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-16-methyl-2-[*N*-((*R*)-2-hydroxytetracosanoyl)-amino]-1,3,4-heptadecanetriol, is a potent antitumor agent isolated from the marine sponge *Agelas mauritianus*. Various analogues of agelasphin-9b (a lead compound) were synthesized, and the relationship between their structures and biological activities was examined using several assay systems. From the results, KRN7000, (2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-2-(*N*-hexacosanoylamino)-1,3,4-octadecanetriol, was selected as a candidate for clinical application.

Introduction

Monoglycosylated ceramides such as galactosylceramide (GalCer) are important surface molecules found in virtually all cells, and glycosphingolipids related to GalCer and their metabolites play important roles in promoting the regulation of nerve cells,[1] regulating protein kinase C activities,[2] and modulating the function of the hormone receptor.[3] Recently, GalCer was identified as essential components of the neural receptor for type 1 human immunodeficiency virus (HIV) surface glycoprotein gp120.[4,5] Thus, GalCer is considered to be an important component for organisms and an interesting compound for researchers. Furthermore, several types of β-GalCers have been isolated from marine organisms[6,7] and organ tissues,[8,9] although any marked biological activities have not been reported yet. In addition, it was reported that the injection of GalCer shows no antitumor activity against tumor-bearing mice.[10]

In contrast to the findings, six kinds of agelasphins (AGLs) having α-galactosylceramide (α-GalCer) structures were isolated from the marine sponge *Agelas mauritianus* as active substances prolonging the life span of mice intraperitoneally inoculated with B16 mouse melanoma cells.[11,12] Since agelasphin-9b (AGL-9b which is AGL-502 in Table 1), a main compound of these AGLs, showed potent antitumor activities against *in vivo* models of several murine tumor cells, we considered that this compound may be a useful agent in cancer treatment. Since the sponge contains little AGL-9b, it is quite difficult to obtain a large amount of this compound from the sponge. We succeeded in the total synthesis of AGL-9b,[13] although the scale-up synthesis of this compound would be difficult owing to its troublesome procedures caused by structural complexities such as an isotype terminal chain and chiral constituents.

Various analogues of AGL-9b were synthesized to search for another candidate which possesses antitumor activity similar to that of AGL-9b and can be synthesized more easily than AGL-9b on a large scale. In this report, we describe the relationship between their structures and antitumor activities against mice subcutaneously inoculated with B16 cells and the selection of the most desirable candidate for clinical application using several assay systems.

Chemistry

1. Syntheses of AGL Analogues Containing a Phytosphingosine Moiety. AGLs were composed of three parts, sugar, fatty acid (FA), and a phytotype long chain base (LCB). In the synthetic approach to AGLs, we considered that how to construct stereoselectively a phytosphingosine moiety which has three successive chiral centers in 2*S*,3*S*,4*R* configurations was a key point. With regard to the synthesis of phytosphingosine, the asymmetric synthesis of phytosphingosine by Komori *et al.*[14] using Katsuki–Sharpless epoxidation and the stereoselective syntheses of phytoshingosines starting from several chiral precursors[15–23] were known. We chose the latter strategy using sugars as the starting materials because there was no necessity to consider diastereomeric yield.

According to the method by Ogawa *et al.*[19] using a D-galactose derivative, **A1** (Scheme 1), as a starting material, AGL-9b and AGL-9a (AGL-519 in Table 1) were synthesized to determine their absolute configurations previously[11,13] and were designated AGL-502 and AGL-519 in this report, respectively. As shown in Scheme 1, in the same manner, other phytotype analogues having different components, AGL-509 (LCB, C-11, FA, C′-24), AGL-510 (C-15, C′-24), AGL-512 (C-18, C′-24), AGL-548 (C-18, C′-26), AGL-549 (C-19, C′-26), and AGL-550 (C-20, C′-26), were synthesized using ylids and fatty acids having appropriate alkyl chain lengths, respectively. Also, as 2′-deoxy analogues, AGL-525 (2′-deoxy, C-18, C′-24) and AGL-582 (2′-deoxy, C-18, C′-26) were synthesized using tetracosanoic acid and hexacosanoic acid in each amidation step.

2. Syntheses of AGLs Containing a Sphinganine Moiety. Various synthetic approaches to optically active sphingosines and the related compounds have been reported.[24–30] Here, we synthesized several AGL analogues lacking the hydroxyl groups at C′2 and/or C4 by two methods as shown below.

(1) Syntheses from Sphingosine (Scheme 2). Since sphingosine is convertible to sphinganine by the hydrogenation of the olefinic portion, we had planned to synthesize several AGL analogues containing the

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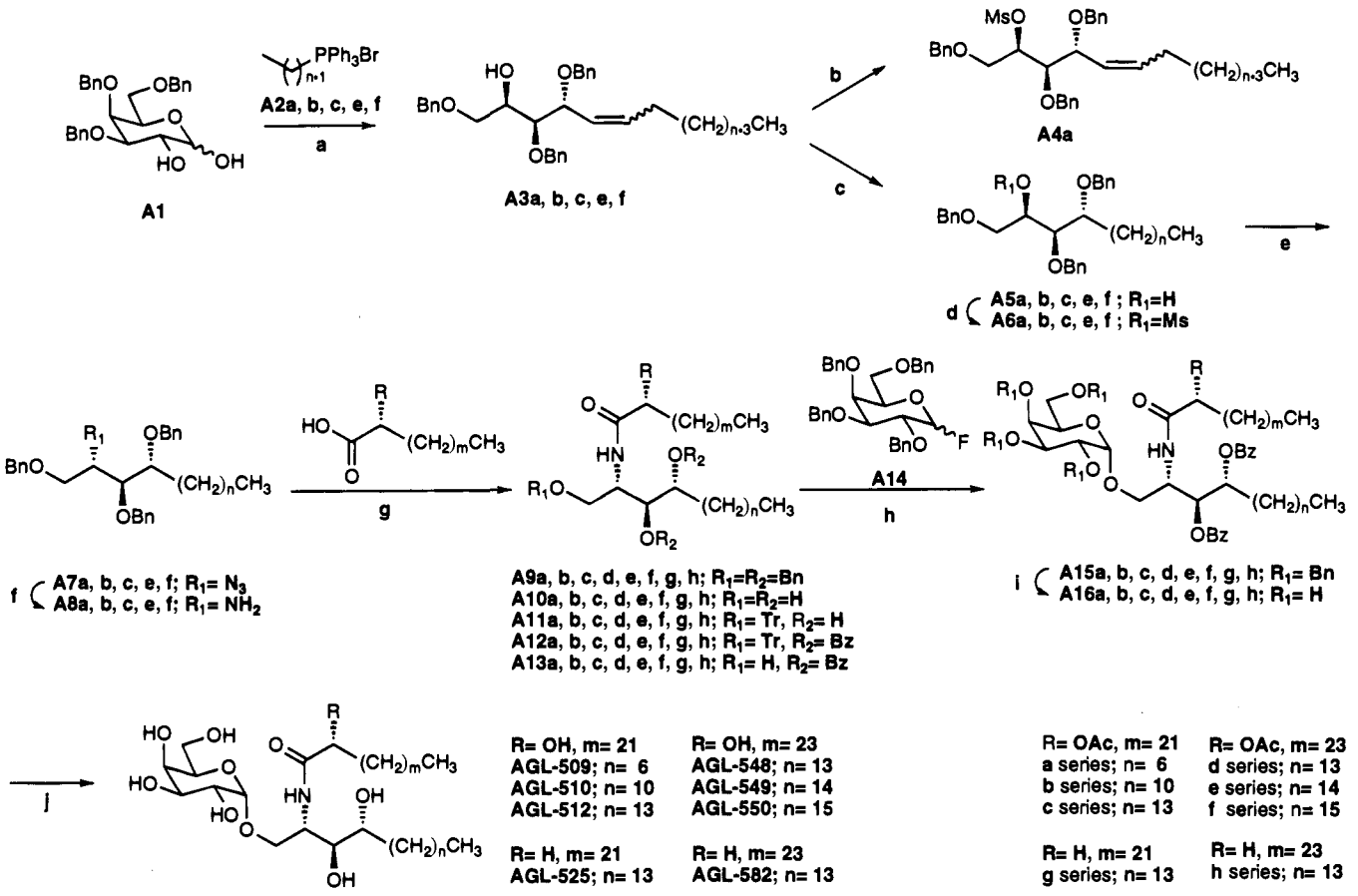
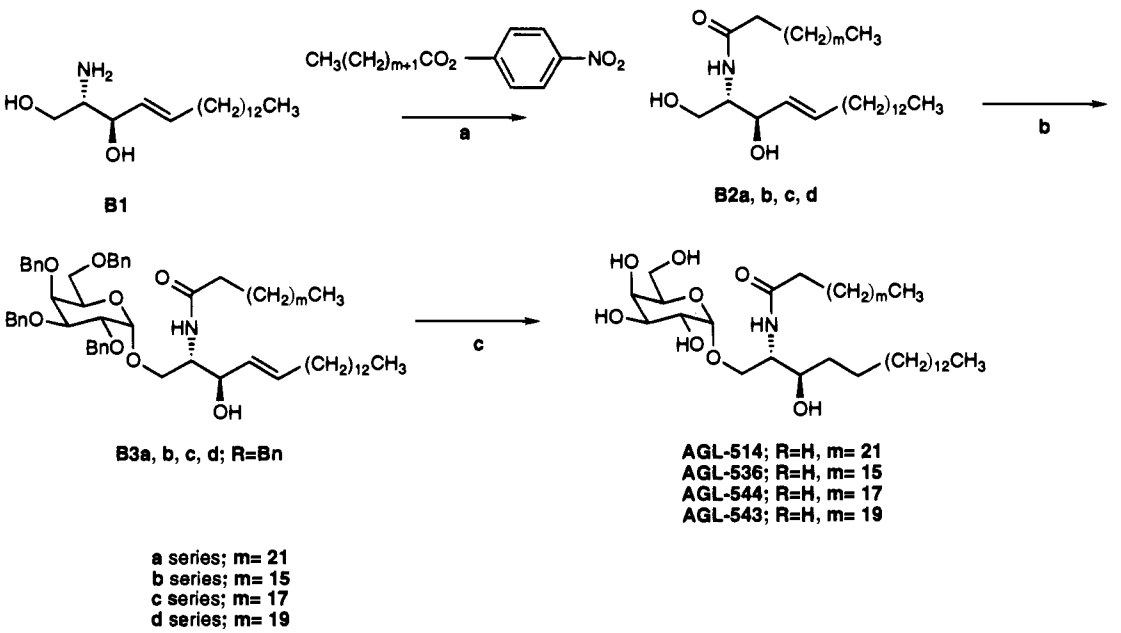
Scheme 1[a]



[a] (a) (1) $NaIO_4$/aq EtOH, −5 °C to rt 10 h, (2) *n*-BuLi/THF, −10 °C to rt, 16 h; (b) MsCl/pyridine, 0 °C to rt, 1.5 h; (c) H_2, Pd−C/THF, 16 h; (d) MsCl/pyridine, 0 °C to rt, 1.5 h; (e) NaN_3/DMF, 100 °C, 16 h; (f) H_2, Pd−C/THF, 16 h; (g) (1) 2-chloro-1-methylpyridinium iodide, *n*-Bu_3N/CH_2Cl_2, reflux, 2 h, (2) H_2, Pd/THF, 1-PrOH, 40 °C, 16 h, (3) TrCl, DMAP/pyridine, 40 °C, 3 h, (4) BzCl, DMAP/pyridine, 12 h, (5) TsOH−H_2O/MeOH, CH_2Cl_2, 2 h; (h) $SnCl_2$, $AgClO_4$, MS4A/THF, −10 °C to rt, 2 h; (i) H_2, Pd/EtOAc, 6 h; (j) NaOMe/MeOH, THF.

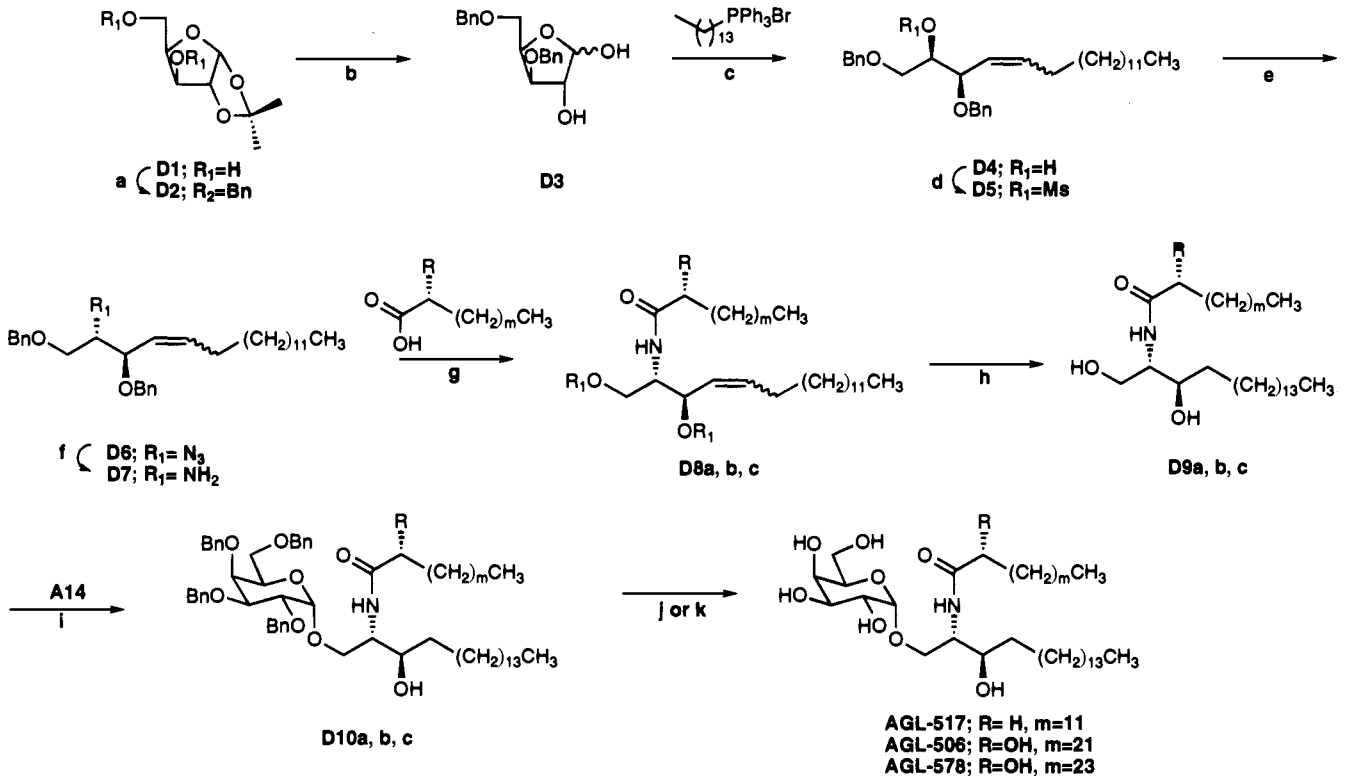
Scheme 2[a]



[a] (a) DMAP/THF, 40 °C, 12 h; (b) A14, $SnCl_2$, $AgClO_4$, MS4A/THF, −10 °C to rt, 2 h; (c) H_2, Pd−$BaSO_4$/THF, 16 h.

sphinganine moiety starting from a commercially available sphingosine (Scheme 2). The sphingosine **B1** was treated with the *p*-nitrophenyl ester of tetracosanoic acid to give **B2a**. The ceramide **B2a** was glycosylated[31] in the same manner as described in Scheme 1 except for the protection of a hydroxyl group of the LCB part to afford an α-galactosylceramide, **B3a**. Hydrogenolysis of the benzyl groups of **B3a** over palladium−$BaSO_4$ in THF, accompanied with hydrogenation of the olefinic portion of the LCB part, gave AGL-514 (2′,4-dideoxy,

Scheme 3[a]



[a] (a) BnCl, NaH/DMF, 16 h; (b) 5% aq H_2SO_4, *t*-BuOH, 45 °C, 15 h; (c) (1) $NaIO_4$/aq EtOH, 10 h, (2) *n*-BuLi/THF, −10 °C to rt, 15 h; (d) MsCl/pyridine, 0 °C to rt, 1.5 h; (e) NaN_3/DMF, 100 °C, 12 h; (f) H_2, Pd−C/THF, *n*-PrOH, 16 h; (g) 2-chloro-1-methylpyridinium iodide, *n*-Bu_3N/CH_2Cl_2, reflux, 2 h; (h) HCO_2H, Pd−C/THF, *n*-PrOH, 45 °C, 16 h; (i) $SnCl_2$, $AgClO_4$, MS4A/THF, −10 °C to rt, 2 h; (j) H_2, Pd−$BaSO_4$/THF, 16 h; (k) (1) H_2, Pd/EtOAc, 6 h, (2) NaOMe/MeOH, THF.

C-18, C′-24). In the same manner, the AGL analogues containing the sphinganine with various FA lengths, AGL-536 (2′,4-dideoxy, C-18, C′-18), AGL-544 (2′,4-dideoxy, C-18, C′-20), and AGL-543 (2′,4-dideoxy, C-18, C′-22), were synthesized using an appropriate fatty acid in each amidation step.

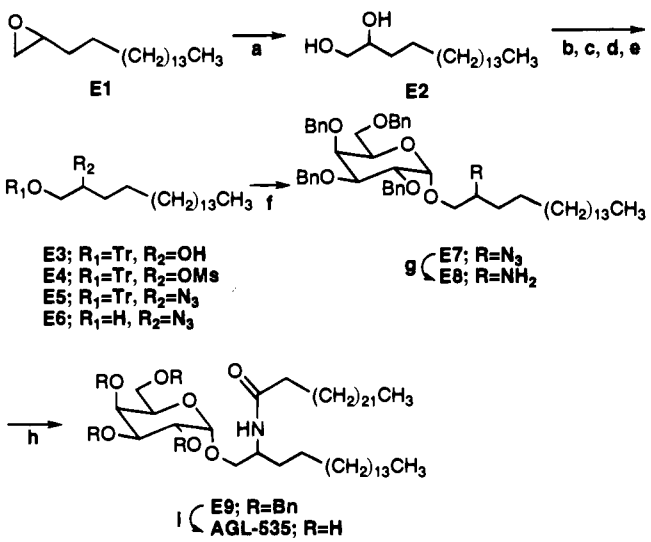
(2) Syntheses from D-Xylose (Scheme 3). Although the syntheses of 4-deoxy AGL analogues starting from sphingosine are very simple as mentioned above, sphingosine is too expensive as a starting material for the syntheses of AGL analogues on a gram scale. Therefore, we attempted to develop another approach to 4-deoxy analogues. As a chiral precursor for sphinganines, D-xylose was employed,[32] the C3 and C4 of which have the same configurations as the C4 and C5 of D-galactose. 1,2-Diol **D3**, which corresponded to compound **A1** in Scheme 1, was easily accessible from commercially available 1,2-*O*-isopropylidene-D-xylofuranose **D1** via **D2**[33] as shown in Scheme 3. The compound **D3** was convertible to ceramide **D9a** in a manner similar to that described for **A10a**. In this reaction sequence, the azidation of an unsaturated mesylate, **D5**, progressed smoothly to give the desired azide **D6** in good yield, while that of **A4a** was unsuccessful under the same condition. Since the neighboring groups of the mesyloxy group are benzyloxy groups in both **D5** and **A4a**, the difference in the reactivity is due to the distance between the olefinic portion and the mesyloxy group. The ceramide **D9a** was directly glycosylated to **D10a** under the same condition as described for **A15a**, and then benzyl groups of **D10a** were hydrogenolized to give the 4-deoxy AGL analogue AGL-517 (2′,4-dideoxy, C-18, C′-14). In the same way, AGL-506 (4-deoxy, C-18, C′-24) and AGL-578 (4-deoxy, C-18, C′-26) were synthesized using an appropriate fatty acid in each amidation step.

3. Synthesis of the 2′,3,4-Trideoxy Analogue. AGL-535, an AGL analogue containing a 3-deoxysphinganine moiety, was synthesized as follows (Scheme 4). Oxirane **E1** was hydrolyzed in 0.5 M H_2SO_4 to give racemic diol **E2**. The tritylation of **E2** followed by mesylation gave mesylate **E4**. The mesyloxy group of **E4** was substituted by an azido group to afford **E5** in high yield. The azide **E5** was deprotected to an alcohol **E6** and then glycosylated to give an α-galactoside, **E7**. Compound **E7** was converted to amine **E8** which was subjected to amidation and deprotection to give the desired compound AGL-535 (2′,3,4-trideoxy, C-18, C′-24) as a C2 epimeric mixture. All of these AGL analogues exhibited the expected physical and spectroscopic properties (see Table 1 and the Experimental Section).

Results and Discussion

AGL-9b (100 μg/kg) prolonged the life span of mice intraperitoneally inoculated with B16 (T/C = 149%) and stimulated the lymphocyte proliferation (LP) on allogeneic mixed lymphocyte reaction (MLR).[11,12] We undertook the total synthesis of AGL-9b to confirm the absolute configuration of AGL-9b and designated the synthesized compound as AGL-502. Since the possibility that the antitumor activity of AGL-9b was caused

Scheme 4[a]



[a] (a) 0.5 M H_2SO_4/*t*-BuOH, reflex, 12 h; (b) TrCl/pyridine, CH_2Cl_2, 16 h; (c) MsCl/pyridine, 0 °C to rt, 1.5 h; (d) NaN_3/DMF, 100 °C, 16 h; (e) 10% HCl–meOH/CH_2Cl_2, rt 3 h; (f) A14, $SnCl_2$, $AgClO_4$, MS4A/ THF, −10 °C to rt, 2 h; (g) H_2, Pd–C/THF, 16 h; (h) tetracosanoic acid, WSC/CH_2Cl_2, reflux, 1 h; (i) H_2, Pd–$BaSO_4$/ THF, 16 h.

by some contamination remaining in it, we compared the biological activities between AGL-9b and AGL-502 using several assay systems.

We compared the antitumor activities of two compounds. As shown in Figure 1, when AGL-9b or AGL-502 was intravenously administered to mice inoculated with B16 subcutaneously, both compounds showed a similar degree of tumor growth inhibitory effect, and additive tumor growth inhibitions were observed in the combination with these compounds and adriamycin (a chemotherapeutic agent). Furthermore, similar LP stimulatory activities of spleen cells or allogeneic MLR were observed by treatment with AGL-9b or AGL-502 (Table 2). These findings demonstrated that the antitumor activities of AGL-9b were caused not by some impure components contained in it but by AGL-9b. However, the total synthesis of AGL-9b is quite difficult; we, therefore, synthesized various analogues of AGL-9b to search for another candidate for clinical application.

To evaluate the role of the side chain in LCB of AGL-502, the antitumor activities of AGL-502 and AGL-519 which has no side chain were examined. As shown in Table 3, these compounds showed a similar tumor growth inhibition ratio (TGIR) against B16-bearing mice, and the side chain in LCB was demonstrated to play little or no important role in the antitumor activities of AGLs.

To evaluate the relationship between the length of LCB of AGLs and their biological activities, comparisons were undertaken among AGL-509 (C-11), AGL-510 (C-15), and AGL-512 (C-18) that possess the same FA (C′-24) using two assay systems. Among these three analogues, AGL-512 showed the most potent LP stimulatory activities and the highest TGIR in combination with a chemotherapeutic agent, mitomycin C (MMC) (Tables 3, 4). These findings suggested that the longer the LCB the more potent the biological activity tended to be. Furthermore, we examined the biological activities of other analogues, AGL-548 (C-18), AGL-549 (C-19), and AGL-550 (C-20), which have the same FA (C′-26) but longer LCBs than AGL-512 and found that these compounds showed nearly equal TGIRs and LP stimulatory activities (Table 3, 4). These findings demonstrated that the C-18 is the optimal length of LCB.

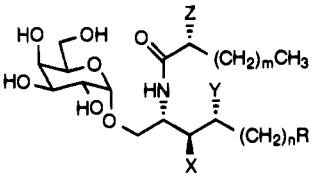
Since AGL-512 has four chiral centers in its ceramide portion, we examined the role of the three hydroxyl groups in biological activities. We synthesized four kinds of analogues of AGL-512, which are AGL-525 (2′-deoxy), AGL-506 (4-deoxy), AGL-514 (2′,4-dideoxy), and AGL-535 (2′,3,4-trideoxy), and their biological activities were compared with that of AGL-512. As shown in Tables 3 and 4, AGL-535 showed little or no LP stimulatory activities, and its antitumor activity was weaker than that of AGL-512, although the three other analogues showed similar biological activities to that of AGL-512. These findings demonstrated that only the 3-hydroxyl group plays an important role in the biological activities of AGLs.

On the basis of these findings, we synthesized the analogues (C-18) of AGL-514 having different FA lengths, AGL-517 (C′-14), AGL-536 (C′-18), AGL-544 (C′-20), and AGL-543 (C′-22), and compared their biological activities with that of AGL-514 (C′-24). As shown in Table 3, these analogues showed potent antitumor activities, and among them, a longer FA showing a more potent activity was observed. Therefore, we concluded that the optimal lengths in LCB and FA are C-18 and C′-24 or longer, respectively, that the 3-hydroxyl group is essential to show potent antitumor activities of AGLs, and that AGL-514 is the most desirable candidate for clinical application among the analogues mentioned above.

Since to confirm whether this conclusion applies to other species such as a human being was important, we compared the biological activities of AGL-514 and AGL-525 using human umbilical cord blood (hUCB) and found that AGL-525 stimulated the LP of hUCB more potently than AGL-514. This indicated that reevaluation of the role of the three hydroxyl groups in biological activities using human cells is necessary. Considering the tendency that a longer FA showed more potent antitumor activity in the case of 2′,4-dideoxy analogues, and that AGL-548 (C′-26) showed more potent antitumor activity than AGL-512 (C′-24) (Table 3), AGL-578 (4-deoxy, C-18, C′-26) and AGL-582 (2′-deoxy, C-18, C′-26) were synthesized, and the effects of the two compounds on hUCB were compared with that of AGL-548. As shown in Table 5, AGL-548 and AGL-582 stimulated the LP of hUCB similarly and more markedly than AGL-578. Furthermore, AGL-548 and AGL-582 showed similar LP-stimulating effects on human peripheral blood (hPB) (Table 5) and allogeneic MLR (Table 6) and similar antitumor activities against mice subcutaneously inoculated with B16 cells (Table 3) or other murine tumor cells (data not shown). Taking these findings and the total scale-up syntheses of these two compounds into consideration, AGL-582 was selected as the most desirable candidate for clinical application and was designated KRN7000.

Herein, we mentioned the relationship between the structures of the analogues of AGL-9b and their biological activities using several assay systems. This is the

Table 1. Physical Properties of AGL Analogues[a]



compd	X	Y	Z	R	*m*	*n*	mp (°C)	$[\alpha]_D$[b]	formula[c]
AGL-502	OH	OH	OH	$CH(CH_3)_2$	21	11	221–222.5	+56.3	$C_{48}H_{95}NO_{10}$
AGL-519	OH	OH	OH	CH_3	21	12	201–203.5	+49.9	$C_{47}H_{93}NO_{10}$
AGL-509	OH	OH	OH	CH_3	21	6	145–147	+67.1	$C_{41}H_{81}NO_{10}$
AGL-510	OH	OH	OH	CH_3	21	10	193–194	+59.2	$C_{45}H_{89}NO_{10}$
AGL-512	OH	OH	OH	CH_3	21	13	202–204	+48.0	$C_{48}H_{95}NO_{10}$
AGL-548	OH	OH	OH	CH_3	23	13	198–199	+45.2	$C_{50}H_{99}NO_{10}$
AGL-549	OH	OH	OH	CH_3	23	14	205–206	+46.5	$C_{51}H_{101}NO_{10}$
AGL-550	OH	OH	OH	CH_3	23	15	200–201	+46.0	$C_{52}H_{103}NO_{10}$
AGL-525	OH	OH	H	CH_3	21	13	146–147	+28.2	$C_{48}H_{95}NO_9$
AGL-506	OH	H	OH	CH_3	21	13	92–95	+50.2	$C_{48}H_{95}NO_9$
AGL-514	OH	H	H	CH_3	21	13	184.5–186.5	+50.0	$C_{48}H_{95}NO_8$
AGL-535[d]	H	H	H	CH_3	21	13	102–104	+41.6	$C_{48}H_{95}NO_7$
AGL-517	OH	H	H	CH_3	11	13	159–161	+57.8	$C_{38}H_{75}NO_8$
AGL-536	OH	H	H	CH_3	15	13	157.5–159.5	+55.5	$C_{42}H_{83}NO_8$
AGL-544	OH	H	H	CH_3	17	13	151.5–153	+47.3	$C_{44}H_{87}NO_8$
AGL-543	OH	H	H	CH_3	19	13	147–149.5	+50.7	$C_{46}H_{91}NO_8$
AGL-578	OH	H	OH	CH_3	23	13	105.5–108	+54.8	$C_{50}H_{99}NO_9$
AGL-582	OH	OH	H	CH_3	23	13	189.5–190.5	+43.6	$C_{50}H_{99}NO_9$

[a] ^1H NMR, ^{13}C NMR, and infrared spectra were obtained for all compounds, and the data are in the Experimental Section for all compounds. [b] $[\alpha]_D$ were measured at 23 or 24 °C at $c = 0.3–1.0$ in pyridine solution. [c] Anal. for C, H, N. [d] C2 epimeric mixture.

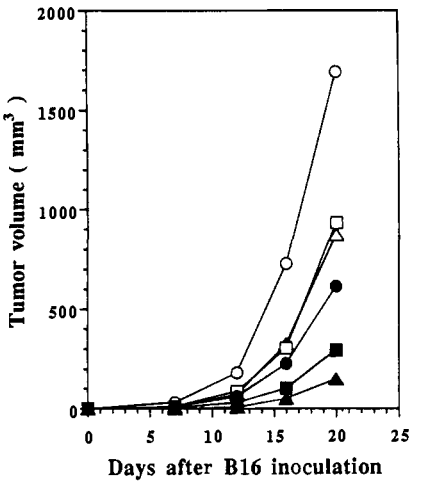
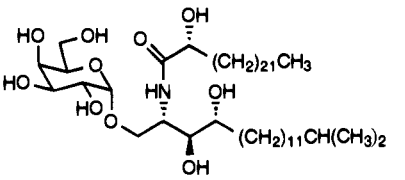


Figure 1. Tumor growth inhibitory effects of AGL-9b and AGL-502 in combination with or without adriamycin on mice subcutaneously inoculated with B16 cells. B16 cells (1×10^6) were subcutaneously inculated into female BDF_1 mice on day 0. AGL-9b and AGL-502 were intravenously administered on days 1, 5, and 9, and ADR was intraperitoneally administered on day 1. The mean of six mice is shown here: ○, control; △, AGL-9b (100 μg/kg); □, AGL-502 (100 μg/kg); ●, ADR (10 mg/kg); ▲, ADR + AGL-9b; ■, ADR + AGL-502.

first finding that the biological activities of α-GalCers differ depending on the structures of the ceramide portion.

Since KRN7000 showed a potent antitumor activity and stimulated the LP on allogeneic MLR in the mouse, KRN7000 is considered to be a nonspecific immunostimulating agent[34] which is a biological response modifier.[35] In addition, KRN7000 also stimulated the LP of human blood. These findings demonstrated that KRN-7000 may be a clinically useful agent for the treatment of cancer.

Table 2. Lymphocyte Proliferation Stimulatory Effect of AGL-9b and AGL-502 on Mouse Spleen Cell (A) or Allogeneic MLR (B)



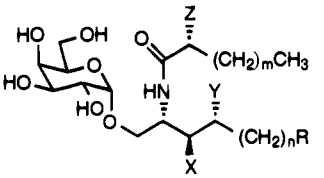
	[^{3}H]TdR uptake (cpm)		
compd	1 ng/mL	10 ng/mL	100 ng/mL
(A) vehicle	1507 ± 227	1187 ± 397	897 ± 327
AGL-9b	8230 ± 3842	19 985 ± 503	24 264 ± 2133
AGL-502	7935 ± 1583	20 746 ± 4179	23 968 ± 2086
(B) vehicle	9368 ± 1583	8723 ± 3209	8407 ± 2472
AGL-9b	36 022 ± 10 658	36 713 ± 4055	32 240 ± 4412
AGL-502	29 143 ± 2815	38 668 ± 3488	31 219 ± 5562

Experimental Section

Column chromatography was performed on silica gel (WAKO-gel C200, particle size 0.075–0.150 mm). TLC analyses were done on silica gel plates (Merck, Art.5554). All melting points were measured on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded by a JASCO A-3 IR or JEOL JIR-DS 20 FT-IR spectrometer on KBr disks or thin films. Mass spectra were measured on a JEOL JMS SX/SX-102 or Hitachi M-80 mass spectrometer. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. Elemental analyses were recorded with a Perkin-Elmer 240C elemental analyzer, and the results indicated by element symbols and within ±0.4% of the theoretical values. ^1H and ^{13}C NMR spectra were obtained using a JEOL JNM-GX-500 FT NMR spectrometer, chemical shifts are expressed in δ units from tetramethylsilane (TMS) as an internal standard, and coupling constants (*J*) are reported in hertz (Hz).

(2*R*,3*S*,4*R*)-1,3,4-Tri-*O*-benzyl-5-undecene-1,2,3,4-tetrol (A3a). To a stirred solution of 3,4,6-tri-*O*-benzyl-D-galactose (**A1**)[19] (4.95 g, 11.0 mmol) in ethanol and water (4:

Table 3. Tumor Growth Inhibitory Effects of AGL Analogues on Mice Subcutaneously Inoculated with B16 Cells



compd	X	Y	Z	R	m	n	maximum TGIR (%) single	MMC	AGLs + MMC
AGL-502	OH	OH	OH	$CH(CH_3)_2$	21	11	86.8	61.5	94.6
AGL-519	OH	OH	OH	CH_3	21	12	82.4	61.5	90.6
AGL-509	OH	OH	OH	CH_3	21	6	46.2	46.5	85.1
AGL-510	OH	OH	OH	CH_3	21	10	94.1	46.5	77.4
AGL-512	OH	OH	OH	CH_3	21	13	57.9	64.0	91.9
AGL-548	OH	OH	OH	CH_3	23	13	92.8	87.3	97.7
AGL-549	OH	OH	OH	CH_3	23	14	72.3	87.3	97.1
AGL-550	OH	OH	OH	CH_3	23	15	92.8	87.3	94.3
AGL-512	OH	OH	OH	CH_3	21	13	57.9	64.0	91.9
AGL-525	OH	OH	H	CH_3	21	13	65.0	64.8	90.8
AGL-506	OH	H	OH	CH_3	21	13	81.3	70.7	98.5
AGL-514	OH	H	H	CH_3	21	13	68.9	64.0	99.1
AGL-535[a]	H	H	H	CH_3	21	13	40.7	80.0	91.4
AGL-517	OH	H	H	CH_3	11	13	54.2	64.1	84.4
AGL-536	OH	H	H	CH_3	15	13	78.1	64.1	80.3
AGL-544	OH	H	H	CH_3	17	13	53.1	39.4	90.4
AGL-543	OH	H	H	CH_3	19	13	56.9	39.4	85.0
AGL-514	OH	H	H	CH_3	21	13	68.9	64.0	99.1
AGL-548	OH	OH	OH	CH_3	23	13	69.8	67.6	85.0[b]
AGL-582	OH	OH	H	CH_3	23	13	53.8	67.6	86.7[b]

[a] C2 epimeric mixture. [b] AGL-548 and AGL582 were administered at days 6, 10, and 14. Tumor volume of each mouse was measured on days 8, 12, 16, and 20, and maximum TGIRs are shown in this table.

Table 4. Lymphocyte Proliferation Stimulatory Effects of AGL Analogues on Mouse Spleen Cells

compd	[^{3}H]TdR uptake (% of control) 1 ng/mL	10 ng/mL	100 ng/mL
AGL-502	282	524	879
AGL-519	520	1491	1202
AGL-509	196	541	936
AGL-510	330	676	838
AGL-512	1060	1405	1552
AGL-548	203	437	620
AGL-549	221	488	673
AGL-550	153	482	481
AGL-512	1060	1405	1552
AGL-525	198	642	790
AGL-506	397	898	872
AGL-514	1115	1432	1494
AGL-535	90	100	147
AGL-517	249	1010	1664
AGL-536	155	383	645
AGL-544	177	791	1009
AGL-543	378	992	875
AGL-514	1115	1432	1494

Table 5. Lymphocyte Proliferation Stimulatory Effects of AGL-548, AGL-578, and AGL582 on Human Umbilical Cord Blood (A) or Human Peripheral Blood (B)

compd	[^{3}H]TdR uptake (% of control) 1 ng/mL	10 ng/mL	100 ng/mL
(A) AGL-578	210	238	362
AGL-548	382	582	586
AGL-582	398	663	581
(B) AGL-548	332	352	470
AGL-582	356	389	590

1) was added portionwise sodium metaperiodate (3.79 g, 17.7 mmol) at −5 °C, and the resulting mixing was stirred at −5 °C to room temperature for 16 h. The mixture was then filtered, and the filtrate was diluted with water and extracted with CH_2Cl_2 (three times). The extract was successively washed with water and brine, dried over $MgSO_4$, and then concentrated. The resulting crude aldehyde was used for the next step without further purification. To a suspension of hexyltriphenylphosphonium bromide (**A2a**; 10.68 g, 25.0 mmol, prepared by heating 1-bromohexane and triphenylphosphine for 12 h at 150 °C) in THF (40 mL) was added dropwise a hexane solution of *n*-butyllithium (8.9 mL of 2.8 M solution, 25.0 mmol) at −10 °C under an argon atmosphere. After the addition was complete, stirring was continued for an additional 30 min. To this mixture was added dropwise a solution of the above aldehyde in THF (30 mL). After the addition, the resulting mixture was allowed to warm to room temperature and stirred for 16 h. After the reaction was quenched with methanol (10 mL), an 80% aqueous methanol solution was added. The mixture was extracted with hexane (three times), washed with brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (300 g) using hexane−EtOAc (9:1) as the eluent to give compound **A3a** as colorless oil: 3.36 g (63%); $[\alpha]^{23}_D$ −37.2° (*c* 0.8, $CHCl_3$); FDMS *m/z* 489 $(M + 1)^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22−7.36 (15H, m), 5.72 (1H, dt, *J* = 7.3, 11.0 Hz), 5.46 (1H, dd, *J* = 9.8, 11.0 Hz), 4.32−4.69 (7H, m), 4.07 (1H, m), 3.51−3.57 (3H, m), 2.99 (1H, m), 1.88−1.99 (2H, m), 1.23−1.32 (6H, m), 0.87 (3H, t, *J* = 7.3 Hz). Anal. ($C_{32}H_{40}O_4$) C, H.

Table 6. Lymphocyte Proliferation Stimulatory Effects of AGL-548 and AGL582 on Allogeneic MLR

compd	[^{3}H]TdR uptake (% of control) 0.1 ng/kg	1 ng/mL	10 ng/mL	100 ng/mL
AGL-548	118	180	250	280
AGL-582	125	200	251	283

Similar, compounds **A3b,c,e,f** were prepared from **A1** using the appropriate 1-bromoalkanes: 1-bromodecane, 1-bromotridecane, 1-bromotetradecane, and 1-bromopentadecane, respectively.

(2*R*,3*R*,4*R*)-1,3,4-Tri-*O*-benzyl-2-*O*-(methylsulfonyl)-4-undecene-1,2,3,4-tetrol (A4a). To a stirred solution of **A3a** (650 mg, 1.33 mmol) in pyridine (5mL) was added dropwise methanesulfonyl chloride (0.2 mL, 2.7 mmol) at 0 °C. After

the addition, the mixture was allowed to warm to room temperature and stirred for 1.5 h. The resulting mixture was concentrated, and the residue was dissolved in Et_2O. The solution was successively washed with water and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel colunm (30 g) using hexane–EtOAc (5:1) as the eluent to give **A4a**: 730 mg (97%); $[\alpha]^{23}_D$ +5.0° (*c* 0.8, $CHCl_3$); FDMS *m/z* 567 $(M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25–7.36 (15H, m), 5.81 (1H, dt, J = 7.3, 11.0 Hz), 5.49 (1H, dd, J = 9.8, 11.0 Hz), 5.05–5.08 (1H, m), 4.77 (1H, d, J = 11.6 Hz), 4.53 (1H, d, J = 11.6 Hz), 4.52 (1H, d, J = 11.6 Hz), 4.67 (1H, d, J = 11.6 Hz), 4.35–4.48 (3H, m), 3.76 (1H, m), 3.67 (1H, dd, J = 6.8, 11.0 Hz), 3.50 (1H, dd, J = 3.6, 11.0 Hz), 2.95 (3H, s), 1.96–2.10 (2H, m), 1.25–1.44 (6H, m), 0.88 (3H, t, J = 6.7 Hz). Anal. ($C_{33}H_{42}O_6S$) C, H.

(2*R*,3*S*,4*R*)-1,3,4-Tri-*O*-benzyl-1,2,3,4-undecanetetrol (A5a). To a solution of **A3a** (1.60 g, 3.28 mmol) in THF (15 mL) was added 10% palladium on charcoal (0.15 g). After the reaction vessel was purged with hydrogen, the mixture was stirred at room temperature for 16 h and filtered through Celite. Then the filtrate was concentrated. The residue was purified by chromatography on a silica gel column (60 g) using hexane–EtOAc (9:1) as the eluent to give **A5a**: 1.41 g (88%); $[\alpha]^{23}_D$ −34.4° (*c* 0.9, $CHCl_3$); FDMS *m/z* 491 $(M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25–7.46 (15H, m), 4.69 (1H, d, J = 11.6 Hz), 4.65 (1H, d, J = 11.6 Hz), 4.56 (1H, d, J = 11.0 Hz), 4.53 (1H, d, J = 11.6 Hz), 4.50 (1H, d, J = 11.0 Hz), 4.48 (1H, d, J = 12.2 Hz), 4.03 (1H, m), 3.68 (1H, dt, J = 4.3, 7.9 Hz), 3.61 (1H, dd, J = 3.1, 4.3 Hz), 3.52–3.57 (2H, m), 3.17 (1H, bd, J = 4.9 Hz), 1.24–1.69 (12H, m), 0.88 (3H, t, J = 6.7 Hz). Anal. ($C_{32}H_{42}O_4$) C, H.

Similarly, compounds **A5b,c,e,f** were prepared from **A3b,c,e,f**, respectively.

(2*R*,3*R*,4*R*)-1,3,4-Tri-*O*-(methylsulfonyl)-1,2,3,4-undecanetetrol (A6a). The title compound was prepared from saturated alcohol **A5a** (1.30 g, 2.65 mmol) in a manner similar to that described for **A4a**: 1.49 g (92%); $[\alpha]^{23}_D$ +5.8° (*c* 0.8, $CHCl_3$); FDMS *m/z* 569 $(M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25–7.32 (15H, m), 4.92 (1H, m), 4.77 (1H, d, J = 11.6 Hz), 4.62 (1H, d, J = 11.6 Hz), 4.58 (1H, d, J = 11.6 Hz), 4.55 (1 H, d, J = 11.6 Hz), 4.49 (1H, d, J = 11.6 Hz), 4.48 (1H, d, J = 11.6 Hz), 3.88 (1H, dd, J = 4.4, 5.1 Hz), 3.71 (2H, m), 3.60–3.62 (1H, m), 2.91 (3H, s), 1.23–1.72 (12H, m), 0.88 (3H, t, J = 6.7 Hz). Anal. ($C_{33}H_{44}O_6S$) C, H.

Using the same method, compounds **A6b,c,e,f** were prepared from **A5b,c,e,f**, respectively.

(2*S*,3*S*,4*R*)-2-Azido-1,3,4-tri-*O*-benzyl-1,3,4-undecanetriol (A7a). To a solution of **A6a** (2.96 g, 5.21 mmol) in DMF (30 mL) was added sodium azide (2.70 g, 41.6 mmol). After stirring at 100 °C for 16 h, the mixture was diluted with EtOAc, successively washed with water and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (100 g) using hexane–EtOAc (98:2) as the eluent to give **A7a**: 2.12 g (79%); $[\alpha]^{23}_D$ +7.4° (*c* 0.8, $CHCl_3$); FDMS *m/z* 516 $(M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.27–7.36 (15H, m), 4.69 (1H, d, J = 11.0 Hz), 4.60 (1H, d, J = 11.6 Hz), 4.56 (1H, d, J = 11.6 Hz), 4.48–4.52 (3H, m), 3.75–3.80 (2H, m), 3.66–3.70 (2H, m), 3.60 (1H, dt, J = 3.7, 7.9 Hz), 1.25–1.70 (12H, m), 0.88 (3H, t, J = 6.7 Hz). Anal. ($C_{32}H_{41}N_3O_3$) C, H, N.

Using the same method, compounds **A7b,c,e,f** were prepared from **A6b,c,e,f**, respectively.

(*R*)-2-Acetoxytetracosanoic Acid. This compound was kindly provided by Prof. Ohta of Keio University: $[\alpha]^{20}_D$ +8.5° (*c* 1.0, $CHCl_3$).

Preparation of (*R*)-2-Acetoxyhexacosanoic Acid. The title compound was prepared from cerebrine-E [a yeast extract which contains a mixture of ceramides (LCB, C-18, C-19, C-20), with (*R*)-2-hydroxyhexacosanoic acid as the sole fatty acid moiety; purchased from The Sigma-Aldrich Library of Rare Chemicals] as follows: To a suspension of cerebrine-E (2.0 g, *ca.* 6.6 mmol as ceramide) in MeOH and water (8:1, 45 mL) was added hydrochloric acid (4 mL), and the mixture was stirred at reflux for 10 h. After cooling to room temperature, it was extracted with hexane (three times), washed with saturated sodium hydrogen carbonate and brine, dried over $MgSO_4$, and then concentrated. The residue was purified through a short column of silica gel (40 g) using hexane–EtOAc (9:1) as the eluent and concentrated. The residue was dissolved in 95% aqueous ethanol (50 mL), and then potassium hydroxide (400 mg) was added. The resulting mixture was stirred at reflux for 1.5 h and cooled to room temperature. Then, 0.1 M hydrogen chloride aqueous solution (20 mL) was added, and the mixture was extracted with Et_2O. The extract was washed with saturated sodium hydrogen carbonate and brine and then concentrated. The residue was subjected to acetylation by a standard method (Ac_2O/pyridine) and crystallized from hexane to give the title compound: 420 mg (33%); $[\alpha]^{23}_D$ +8.2° (*c* 0.4, $CHCl_3$); mp 65–66 °C; FDMS *m/z* 455 $(M + 1)^+$; IR (cm^{-1}, KBr) 3400, 2940, 2860, 1740, 1470, 1380, 1240; ^1H NMR (500 MHz, $CDCl_3$) δ 5.02 (1H, t, J = 6.1 Hz), 2.15 (3H, s), 1.84–1.89 (2H, m), 1.19–1.44 (44H, m), 0.88 (3H, t, J = 6.7 Hz). Anal. ($C_{28}H_{54}O_4$) C, H.

(2*S*,3*S*,4*R*)-2-[*N*-((*R*)-2-Acetoxytetracosanoyl)amino]-1,3,4-tri-*O*-benzyl-1,3,4-undecanetriol (A9a). To a solution of **A7a** (140 mg, 0.27 mmol) in THF (5 mL) was added 10% palladium on charcoal (15 mg). After the reaction vessel was purged with hydrogen, the mixture was stirred at room temperature for 16 h and filtered through Celite. The filtrate was concentrated to give a crude syrup of **A8a** [FDMS *m/z* 490 $(M + 1)^+$]. The resulting crude amine was used for the next step without further purification. To a solution of the crude syrup of **A8a** in CH_2Cl_2 (5 mL) was added (*R*)-2-acetoxytetracosanoic acid (120 mg, 0.31 mmol), 2-chloro-1-methylpyridinium iodide (92 mg, 0.36 mmol), and *n*-tributylamine (0.84 mL, 0.36 mmol), and the mixture was stirred at reflux for 2 h. After being cooled to room temperature, the mixture was successively washed with 5% aqueous sodium thiosulfate solution, 5% aqueous sodium hydrogen carbonate solution, and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (10 g) using hexane–acetone (10:1) as the eluent to give **A9a**: 208 mg (86% from **A7a**); $[\alpha]^{24}_D$ +1.8° (*c* 0.8, $CHCl_3$); FDMS *m/z* 899 $(M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24–7.35 (15H, m), 6.49 (1H, d, J = 9.9 Hz), 5.05 (1H, dd, J = 4.9, 7.3 Hz), 4.82 (1H, d, J = 11.6 Hz), 4.62 (1H, d, J = 11.6 Hz), 4.55 (1H, d, J = 11.6 Hz), 4.51 (1H, d, J = 11.6 Hz), 4.42 (2H, s), 4.21–4.23 (1H, m), 3.81–3.85 (2H, m), 3.47–3.51 (2H, m), 1.99 (3H, s), 1.23–1.79 (54H, m), 0.88 and 0.87 (each 3H, t, J = 6.7 Hz). Anal. ($C_{58}H_{91}NO_6$) C, H, N.

Similarly, compounds **A9b,c** were prepared from **A7b,c** in the same manner as described for **A9a**, compounds **A9d–f** were prepared from **A7c,e,f** using (*R*)-2-acetoyhexacosanoic acid, and compounds **A9g** and **A9h** were prepared from **A7c** using tetracosanoic acid and hexacosanoic acid, respectively.

(2*S*,3*S*,4*R*)-2-[*N*-((*R*)-2-Acetoxytetracosanoyl)amino]-1,3,4-undecanetriol (A10a). To a solution of **A9a** (560 mg, 0.62 mmol) in THF and 1-propanol (1:1, 8 mL) was added palladium black (55 mg), and the reaction vessel was purged with hydrogen. After being stirred at 40 °C for 16 h, the mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by chromatography on a silica gel column (20 g) using chloroform–methanol (20:1) as the eluent to give **A10a**: 343 mg (88%); $[\alpha]^{23}_D$ +5.6° (*c* 0.1, $CHCl_3$); FDMS *m/z* 629 $(M + 1)^+$; ^1H NMR (500 MHz, C_5D_5N) δ 8.68 (1H, d, J = 8.5 Hz), 5.54 (1H, dd, J = 5.5, 7.3 Hz), 5.07 (1H, m), 4.46 (1H, m), 4.41 (1H, m), 4.38 (1H, m), 4.28 (1H, m), 2.20 (1H, m), 2.07 (2H, m), 2.04 (3H, s), 1.90 (2H, m), 1.68 (1H, m), 1.15–1.60 (48H, m), 0.88 (6H, t, J = 6.7 Hz). Anal. ($C_{37}H_{73}NO_6$) C, H, N.

Similarly, compounds **A10b–h** were prepared from **A9b–h**, respectively.

(2*S*,3*S*,4*R*)-2-[*N*-((*R*)-2-Acetoxytetracosanoyl)amino]-1-*O*-(triphenylmethyl)-1,3,4-undecanetriol (A11a). To a solution of **A10a** (390 mg, 0.62 mmol) in pyridine (5 mL) were added triphenylchloromethane (1.76 g, 6.3 mmol) and 4-(dimethylamino)pyridine (5 mg, 0.04 mmol), and the mixture was stirred at 40 °C for 3 h. After being diluted with chloroform, the mixture was washed with brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (20 g) using chloroform–acetone (50:1) as the eluent to give **A11a**: 460 mg (85%); $[\alpha]^{24}_D$ −11.9°

(*c* 0.1, $CHCl_3$); FDMS *m/z* 870 M^+; ^1H NMR (500 MHz, $CDCl_3$) δ 7.23–7.38 (15H, m), 6.86 (1H, d, J = 8.6 Hz), 5.22 (1H, dd, J = 4.9, 7.9 Hz), 4.26–4.28 (1H, m), 3.58–3.61 (1H, m), 3.43 (1H, dd, J = 3.1, 9.8 Hz), 3.34–3.39 (2H, m), 2.06 (3H, s), 1.87 (1H, m), 1.75 (1H, m), 1.25–1.62 (52H, m), 0.88 (6H, t, J = 7.3 Hz). Anal. ($C_{56}H_{87}NO_6$) C, H, N.

Similarly, compounds **A11b–h** were prepared from **A10b–h**, respectively.

(2*S*,3*S*,4*R*)-2-[*N*-((*R*)-2-Acetoxytetracosanoyl)amino]-3,4-di-*O*-benzoyl-1-*O*-(triphenylmethyl)-1,3,4-undecanetriol (A12a). To a solution of **A11a** (440 mg, 0.51 mmol) in pyridine (5 mL) were added benzoyl chloride (0.35 mL, 3.0 mmol) and 4-(dimethylamino)pyridine (5 mg, 0.04 mmol). After the mixture was stirred at room temperature for 12 h, the reaction was quenched with cooled water and the mixture was extracted with chloroform. The extract was dried over $MgSO_4$ and then concentrated to a residue which was purified on a silica gel column (10 g) eluting with hexane–EtOAc (10:1) to give **A12a**: 491 mg (90%); $[\alpha]^{23}_D$ +6.8° (*c* 0.5, $CHCl_3$); negative FABMS *m/z* 1076 ($M - H)^-$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.04–8.16 (25H, m), 5.80 (1H, dd, J = 3.1, 8.5 Hz), 5.36 (1H, dt, J = 3.1, 9.8 Hz), 5.29 (1H, t, J = 6.1 Hz), 4.55–4.60 (1H, m), 3.28 (1H, dd, J = 3.7, 9.8 Hz), 3.22 (1H, dd, J = 3.7, 9.8 Hz), 1.98 (3H, s), 1.20–1.90 (54H, m), 0.88 (6H, t, J = 7.3 Hz). Anal. ($C_{70}H_{95}NO_8$) C, H, N.

Using the same method, compounds **A12b–h** were prepared from **A11b–h**, respectively.

(2*S*,3*S*,4*R*)-2-[*N*-((*R*)-2-Acetoxytetracosanoyl)amino]-3,4-di-*O*-benzoyl-1,3,4-undecanetriol (A13a). To a solution of **A12a** (230 mg, 0.21 mmol) in CH_2Cl_2 and methanol (2:1, 5 mL) was added *p*-toluenesulfonic acid monohydrate (20 mg, 0.1 mmol), and the mixture was stirred at room temperature for 2 h. After being diluted with EtOAc, the mixture was successively washed with 5% aqueous sodium hydrogen carbonate and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (10 g) using hexane–EtOAc (3:1) as the eluent to give **A13a**: 156 mg (88%); $[\alpha]^{24}_D$ +25.0° (*c* 0.5, $CHCl_3$); FDMS *m/z* 838 ($M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37–8.14 (10H, m), 6.99 (1H, d, J = 9.2 Hz), 5.44 (1H, dd, J = 2.4, 9.2 Hz), 5.37 (1H, dt, J = 3.1, 9.8 Hz), 5.19 (1H, t, J = 6.1 Hz), 4.32–4.36 (1H, m), 3.56–3.68 (2H, m), 2.21 (3H, s), 1.23–2.09 (54H, m), 0.83–0.89 [6H (0.88, t, J = 6.7 Hz) and (0.85, t, J = 6.7 Hz)]. Anal. ($C_{51}H_{81}NO_8$) C, H, N.

Similarly, compounds **A13b–h** were prepared from **A12b–h**, respectively.

(2*S*,3*S*,4*R*)-2-[*N*-((*R*)-2-Acetoxytetracosanoyl)amino]-3,4-di-*O*-benzoyl-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-1,3,4-undecanetriol (A15a). To a stirred solution of **A13a** (155 mg, 0.19 mmol) in THF (5 mL) were added stannous chloride (83 mg, 0.45 mmol), silver perchlorate (91 mg, 0.45 mmol), and molecular sieves (4 Å, power, 580 mg), and the mixture was stirred at room temperature for 30 min. The mixture was cooled to −10 °C, and solution of benzylgalactosyl fluoride[36] (**A14**; 152 mg, 0.29 mmol) in THF (2 mL) was added dropwise. Then the resulting mixture was allowed to warm to room temperature and stirred for an additional 2 h. The mixture was diluted with acetone and filtered through Celite. Then the filtrate was concentrated. The residue was dissolved in EtOAc, washed with brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (10 g) using hexane–EtOAc (6:1) as the eluent to give α-galactoside **A15a**: 168 mg (67%); $[\alpha]^{23}_D$ +18.4° (*c* 0.5, $CHCl_3$); FDMS *m/z* 1358 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 8.02 (2H, d, J = 7.3 Hz), 7.89 (2H, d, J = 7.3 Hz), 7.82 (1H, d, J = 9.8 Hz), 7.14–7.61 (26H, m), 5.79 (1H, dd, J = 2.4, 9.8 Hz), 5.37–5.40 (1H, m), 5.08 (1H, dd, J = 4.9, 7.9 Hz), 4.87 (1H, d, J = 11.6 Hz), 4.50–4.76 (7H, m), 4.48 (1H, d, J = 11.6 Hz), 4.40 (1H, d, J = 11.6 Hz), 4.09 (1H, t, J = 7.2 Hz), 3.99 (1H, dd, J = 3.3, 10.4 Hz), 3.93 (1H, bs), 3.82 (2H, dd, J = 2.4, 9.8 Hz), 3.59 (1H, dd, J = 2.3, 10.4 Hz), 3.53 (1H, dd, J = 7.1, 9.2 Hz), 3.45 (1H, dd, J = 6.7, 9.2 Hz), 2.01 (3H, s), 1.82–1.93 (4H, m), 1.10–1.40 (50H, m), 0.83–0.89 [6H (0.88, t, J = 6.7 Hz) and (0.85, t, J = 6.7 Hz)]. Anal. ($C_{85}H_{115}NO_{13}$) C, H, N.

Using the same method, compounds **A15b–h** were prepared from **A13b–h**, respectively.

(2*S*,3*S*,4*R*)-2-[*N*-((*R*)-2-Acetoxytetracosanoyl)amino]-3,4-di-*O*-benzoyl-1-*O*-(α-D-galactopyranosyl)-1,3,4-undecanetriol (A16a). To a solution of **A15a** (165 mg, 0.12 mmol) in EtOAc (3 mL) was added palladium black (17 mg), and the reaction vessel was purged with hydrogen. After being stirred at room temperature for 6 h, the mixture was filtered through Celite, and the filtrate was then concentrated. The residue was purified by chromatography on a silica gel column using hexane–acetone (3:2) as the eluent to give **A16a**: 110 mg (91%); $[\alpha]^{24}_D$ +22.2° (*c* 0.3, $CHCl_3$); FDMS *m/z* 999 ($M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.99 (2H, d, J = 7.3 Hz), 7.90 (2H, d, J = 6.7 Hz), 7.75 (1H, d, J = 9.8 Hz), 7.61 (1H, t, J = 7.3 Hz), 7.53 (1H, t, J = 7.3 Hz), 7.47 (2H, t, J = 7.3 Hz), 7.37 (2H, t, J = 7.3 Hz), 5.78 (1H, dd, J = 2.4, 9.8 Hz), 5.24–5.27 (1H, m), 5.07 (1H, t, J = 6.7 Hz), 4.71 (1H, d, J = 3.7 Hz), 4.58 (1H, m), 4.00 (1H, bs), 3.65–3.95 (6H, m), 3.46 (1H, t, J = 6.7 Hz), 2.18 (3H, s), 1.84–1.93 (4H, m), 1.17–1.45 (54H, m), 0.82–0.89 [6H (0.88, t, J = 6.7 Hz) and (0.84, t, J = 7.3 Hz)]. Anal. ($C_{57}H_{91}NO_{13}$) C, H, N.

Using the same method, compounds **A16–h** were prepared from **A15b–h**, respectively.

(2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-[*N*-((*R*)-2-hydroxytetracosanoyl)amino]-1,3,4-undecanetriol (AGL-509). To a solution of **A16a** (88 mg, 88 μmol) in methanol and THF (1:1, 6 mL) was added dropwise a methanol solution of sodium methoxide (0.5 mL of 1 M solution, 0.5 mmol). After the mixture was stirred at room temperature for 2 h, a cation exchange resin (Dowex 50W X8; Dow Chemical Co.) was added to neutralize it. The resulting mixture was filtered and then concentrated. The residue was purified by chromatography on a silica gel column (5 g) using chloroform–methanol (9:1) as the eluent to give AGL-509: 53 mg (82%); $[\alpha]^{23}_D$ +67.1° (*c* 1.0, pyridine); FDMS *m/z* 748 (M^+); IR (cm^-, KBr) 3300, 2870, 2800, 1630, 1605, 1515, 1455, 1060; ^1H NMR (500 MHz, C_5D_5N) δ 8.50 (1H, d, J = 9.2 Hz), 5.61 (1H, d, J = 3.7 Hz), 5.29 (1H, m), 4.64–4.67 (2H, m), 4.59 (1H, m), 4.54 (1H, bs), 4.47–4.51 (2H, m), 4.32–4.43 (4H, m), 4.26 (1H, m), 2.29 (1H, m), 2.18 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.74 (1H, m), 1.66 (2H, m), 1.15–1.46 (54H, m), 0.87 and 0.82 (each 3H, t, J = 6.8 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 175.0 (s), 101.2 (d), 76.5 (d), 73.0 (d), 72.4 (d), 72.3 (d), 71.6 (d), 70.9 (d), 70.1 (d), 68.1 (t), 62.6 (t), 50.4 (d), 35.5 (t), 34.4 (t), 32.0 (t), 30.2 (t), 29.9 (t), 29.8 (t), 29.7 (t), 29.5 (t), 26.3 (t), 25.8 (t), 22.9 (t), 22.8 (t), 14.21 (q), 14.18 (q).

Similarly, the following AGL analogues were obtained.

(2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-[*N*-((*R*)-2-hydroxytetracosanoyl)amino]-1,3,4-pentadecanetriol (AGL-510): from A16b (87%); $[\alpha]^{24}_D$ +59.2° (*c*, 0.3, pyridine); FDMS *m/z* 805 ($M + 1)^+$; IR (cm^{-1}, KBr) 3400, 2950, 2870, 1645, 1535, 1475, 1080; ^1H NMR (500 MHz, C_5D_5N) δ 8.50 (1H, d, J = 9.2 Hz), 5.59 (1H, d, J = 3.7 Hz), 5.28 (1H, m), 4.64 (2H, m), 4.58 (1H, m), 4.53 (1H, m), 4.48 (2H, m), 4.30–4.42 (4H, m), 4.27 (1H, m), 2.29 (1H, m), 2.18 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.74 (1H, m), 1.66 (2H, m), 1.15–1.46 (54H, m), 0.84 (6H, t, J = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 174.9 (s), 101.2 (d), 76.5 (d), 73.0 (d), 72.4 (d), 72.3 (d), 71.6 (d), 70.9 (d), 70.1 (d), 68.1 (t), 62.6 (t), 50.4 (d), 35.5 (t), 34.4 (t), 32.1 (t), 30.3 (t), 30.0 (t), 29.9 (t), 29.5 (t), 26.4 (t), 25.8 (t), 22.9 (t), 14.2 (q).

(2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-[*N*-((*R*)-2-hydroxytetracosanoyl)amino]-1,3,4-octadecanetriol (AGL-512): from A16c (82%); $[\alpha]^{23}_D$ +48.0° (*c* 0.3, pyridine); FDMS *m/z* 847 ($M + 1)^+$; IR (cm^{-1}, KBr) 3400, 2950, 2870, 1645, 1535, 1475, 1080; ^1H NMR (500 MHz, C_5D_5N) δ 8.50 (1H, d, J = 9.2 Hz), 5.59 (1H, d, J = 3.7 Hz), 5.27 (1H, m), 4.64 (2H, m), 4.58 (1H, m), 4.53 (1H, m), 4.48 (2H, m), 4.30–4.42 (4H, m), 4.27 (1H, m), 2.29 (1H, m), 2.18 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.74 (1H, m), 1.67 (2H, m), 1.15–1.46 (60H, m), 0.84 (6H, t, J = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 174.9 (s), 101.2 (d), 76.5 (d), 73.0 (d), 72.4 (d), 72.3 (d), 71.6 (d), 70.9 (d), 70.1 (d), 68.1 (t), 62.6 (t), 50.4 (d), 35.5 (t), 34.4 (t), 32.1 (t), 30.3 (t), 30.1 (t), 30.0 (t), 29.9 (t), 29.5 (t), 26.4 (t), 25.8 (t), 22.9 (t), 14.2 (q).

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(N-hexacosanoylamino)-1,3,4-octadecanetriol (AGL-548): from A16d (83%); $[\alpha]^{23}_D$ +45.2° (*c* 1.0, pyridine); FDMS *m/z* 875 (M + 1)$^+$; IR (cm^{-1}, KBr) 3400, 2950, 2870, 1645, 1535, 1475, 1080; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.49 (1H, d, *J* = 9.2 Hz), 5.57 (1H, d, *J* = 3.7 Hz), 5.26 (1H, m), 4.62 (2H, dd, *J* = 4.9, 10.4 Hz), 4.58 (1H, m), 4.51 (1H, bs), 4.46 (2H, m), 4.28–4.41 (4H, m), 4.26 (1H, m), 2.27 (1H, m), 2.17 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.74 (1H, m), 1.66 (2H, m), 1.16–1.46 (64H, m), 0.85 (6H, t, *J* = 6.1 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 175.0 (s), 101.2 (d), 76.5 (d), 73.0 (d), 72.4 (d), 72.3 (d), 71.6 (d), 70.9 (d), 70.1 (d), 68.2 (t), 62.6 (t), 50.5 (d), 35.5 (t), 34.4 (t), 32.1 (t), 30.3 (t), 30.1 (t), 29.9 (t), 29.9 (t), 29.6 (t), 26.4 (t), 25.8 (t), 22.9 (t), 14.2 (q).

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(N-hexacosanoylamino)-1,3,4-nonadecanetriol (AGL-549): from A16e (86%); $[\alpha]^{23}_D$ +46.5° (*c* 0.7, pyridine); FDMS *m/z* 889 (M + 1)$^+$; IR (cm^{-1}, KBr) 3400, 2950, 2870, 1645, 1535, 1475, 1080; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.50 (1H, d, *J* = 9.2 Hz), 5.58 (1H, d, *J* = 3.7 Hz), 5.27 (1H, m), 4.63 (2H, m), 4.58 (1H, m), 4.52 (1H, bs), 4.47 (2 H, m), 4.28–4.41 (4H, m), 4.27 (1H, m), 2.27 (1H, m), 2.18 (1H, m), 1.99 (1H, m), 1.88 (2H, m), 1.74 (1H, m), 1.66 (2H, m), 1.16–1.46 (66H, m), 0.85 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 175.0 (s), 101.2 (d), 76.5 (d), 73.0 (d), 72.4 (d), 72.3 (d), 71.6 (d), 70.9 (d), 70.1 (d), 68.1 (t), 62.6 (t), 50.4 (d), 35.5 (t), 34.4 (t), 32.1 (t), 30.3 (t), 30.1 (t), 29.9 (t), 29.9 (t), 29.5 (t), 26.4 (t), 25.8 (t), 22.9 (t), 14.2 (q).

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(N-hexacosanoylamino)-1,3,4-icosanetriol (AGL-550): from A16f (84%); $[\alpha]^{23}_D$ +46.0° (*c* 0.8, pyridine); FDMS *m/z* 903 (M + 1)$^+$; IR (c^{-1}, KBr) 3400, 2950, 2870, 1645, 1535, 1475, 1080; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.49 (1H, d, *J* = 9.2 Hz), 5.57 (1H, d, *J* = 3.7 Hz), 5.25 (1H, m), 4.62 (2H, dd, *J* = 4.9, 10.4 Hz), 4.57 (1H, m), 4.51 (1H, bs), 4.46 (2H, m), 4.28–4.40 (4H, m), 4.26 (1H, m), 2.26 (1H, m), 2.17 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.73 (1H, m), 1.65 (2H, m), 1.16–1.46 (68H, m), 0.86 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 175.0 (s), 101.2 (d), 76.4 (d), 73.0 (d), 72.4 (d), 72.3 (d), 71.5 (d), 70.9 (d), 70.1 (d), 68.1 (t), 62.6 (t), 50.5 (d), 35.5 (t), 34.3 (t), 32.1 (t), 30.3 (t), 30.1 (t), 29.9 (t), 29.6 (t), 26.4 (t), 25.8 (t), 22.9 (t), 14.2 (q).

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(N-tetracosanoylamino)-1,3,4-octadecanetriol (AGL-525): from A16g (90%); $[\alpha]^{24}_D$ +28.2° (*c* 0.3, pyridine); FDMS *m/z* 831 (M + 1)$^+$; IR (cm^{-1}, KBr) 3400, 2950, 2870, 1645, 1535, 1475, 1080; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.45 (1H, d, *J* = 8.5 Hz), 5.55 (1H, d, *J* = 3.7 Hz), 5.24 (1H, m), 4.64–4.67 (2H, m), 4.52 (1H, m), 4.48 (1H, m), 4.38 (4H, m), 4.28 (2H, bs), 2.41 (2H, t, *J* = 6.3 Hz), 2.24 (1H, m), 1.88 (1H, m), 1.78 (2H, m), 1.64 (1H, m), 1.10–1.45 (62H, m), 0.85 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 173.2 (s), 101.5 (d), 76.7 (d), 73.0 (d), 72.5 (d), 71.6 (d), 71.0 (d), 70.3 (d), 68.7 (t), 62.7 (t), 51.5 (d), 36.8 (t), 34.3 (t), 32.1 (t), 30.4 (t), 30.1 (t), 30.0 (t), 29.9 (t), 29.8 (t), 29.7 (t), 29.6 (t), 26.5 (t), 26.4 (t), 22.9 (t), 14.3 (q).

(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-2-(N-hexacosanoylamino)-1,3,4-octadecanetriol (AGL-582): from A16h (88%); $[\alpha]^{23}_D$ +43.6° (*c* 1.0, pyridine); negative FABMS *m/z* 857 (M − H)$^-$; IR (cm^{-1}, KBr) 3300, 2930, 2850, 1640, 1540, 1470, 1070; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.47 (1H, d, *J* = 8.5 Hz), 5.58 (1H, d, *J* = 3.7 Hz), 5.27 (1H, m), 4.63–4.70 (2H, m), 4.56 (1H, m), 4.52 (1H, t, *J* = 6.1 Hz), 4.37–4.47 (4H, m), 4.33 (2H, m), 2.45 (2H, t, *J* = 7.3 Hz), 2.25–2.34 (1H, m), 1.87–1.97 (2H, m), 1.78–1.85 (2H, m), 1.62–1.72 (1H, m), 1.26–1.45 (66H, m), 0.88 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (125 MHz, MHz, C_5D_5N) δ 173.2 (s), 101.5 (d), 76.7 (d), 73.0 (d), 72.5 (d), 71.6 (d), 71.0 (d), 70.3 (d), 68.7 (t), 62.7 (t), 51.4 (d), 36.8 (t), 34.4 (t), 32.1 (t), 30.4 (t), 30.2 (t), 30.03 (t), 30.00 (t), 29.93 (t), 29.87 (t), 29.81 (t), 29.76 (t), 29.6 (t), 26.5 (t), 26.4 (t), 22.9 (t), 14.3 (q).

(2S,3R,4E)-2-(N-Tetracosanoylamino)-4-octadecene-1,3-diol (B2a). To a solution of sphingosine **B1** (100 mg, 0.33 mmol) in THF (10 mL) were added *p*-nitrophenyl tetracosanoate (242 mg, 0.50 mmol), prepared from tetracosanoic acid and *p*-nitrophenol in the presence of dicyclohexylcarbodiimide by a standard method, and 4-(dimethylamino)pyridine (2.5 mg, 0.02 mmol). After the mixture was stirred at 40 °C for 12 h, it was evaporated under reduced pressure. The residue was purified by chromatography on a silica gel column (20 g) using chloroform–methanol (4:1) as the eluent to give ceramide **B2a**, 103 mg (45%). All of the spectral data of the title compound were identical with those obtained by Hasegawa *et al.*[32]

Using the same method, compounds **B2b–d** were prepared from **B1** with *p*-nitrophenyl octadecanoate, *p*-nitrophenyl icosanoate, and *p*-nitrophenyl docosanoate, respectively.

(2S,3R,4E)-1-O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-2-(N-tetracosanoylamino)-4-octadecene-1,3-diol (B3a). The title compound was prepared from **B2a** (40.0 mg, 0.06 mmol) in a manner similar to that described for **A15a**: 37.5 mg (52%); $[\alpha]^{23}_D$ +25.1° (*c* 0.5, $CHCl_3$); mp 63.0–64.5 °C; FDMS *m/z* 1173 (M + 1)$^+$; IR (cm^{-1}; KBr) 3210, 2920, 2850, 1640, 1590, 1545, 1495, 1465, 1450, 1335, 1290, 1110; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23–7.37 (20H, m), 6.40 (1H, d, *J* = 7.9 Hz), 5.62–5.69 (1H, m), 5.42 (1H, dd, *J* = 6.1, 15.3 Hz), 4.91 (1H, d, *J* = 11.6 Hz), 4.86 (1H, d, *J* = 11.6 Hz), 4.74–4.75 (3H, m), 4.71 (1H, d, *J* = 11.6 Hz), 4.55 (1H, d, *J* = 11.6 Hz), 4.47 (1H, d, *J* = 11.6 Hz), 4.38 (1H, d, *J* = 11.6 Hz), 4.09–4.16 (1H, m), 3.94–4.06 (3H, m), 3.79–3.92 (3H, m), 3.68 (1H, dd, *J* = 3.7, 10.4 Hz), 3.40–3.57 (2H, m), 2.12 (2H, dt, *J* = 3.4, 7.6 Hz), 1.90–2.01 (3H, m), 1.10–1.60 (63H, m), 0.88 (6H, t, *J* = 6.7 Hz). Anal. ($C_{76}H_{117}NO_8$) C, H, N.

Similarly, compounds **B3b-d** were prepared from **B2b-d**, respectively.

(2S,3R)-1-O-(α-D-Galactopyranosyl)-2-(N-tetracosanoylamino)-1,3-octadecanediol (AGL-514). To a solution of **B3a** (30.0 mg, 25.6 μmol) in THF (5 mL) was added 5% palladium on barium sulfate (5 mg). After the reaction vessel was purged with hydrogen, the mixture was stirred at room temperature for 16 h. The catalyst was removed by filtration with Celite, and then the filtrate was concentrated. The residue was purified by chromatography on a silica gel column (10 g) using chloroform–methanol (10:1) as the eluent to give AGL-514: 9.4 mg (90%); $[\alpha]^{23}_D$ +50.0° (*c* 0.3, pyridine); FDMS *m/z* 814 (M$^+$); IR (cm^{-1}, KBr) 3260, 2910, 2850, 1645, 1545, 1470, 1350, 1125, 1065; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.52 (1H, d, *J* = 8.6 Hz), 5.46 (1H, d, *J* = 3.7 Hz), 4.74 (1H, m), 4.66 (1H, dd, *J* = 3.6, 9.8 Hz), 4.54–4.60 (2H, m), 4.40–4.52 (4H, m), 4.37 (1H, dd, *J* = 5.5, 10.4 Hz), 4.29 (1H, m), 2.48 (2H, t, *J* = 7.3 Hz), 1.80–2.00 (4H, m), 1.58 (1H, m), 1.20–1.45 (65H, m), 0.881 and 0.887 (each 3H, t, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 173.4 (s), 102.2 (d), 73.1 (d), 71.9 (d), 71.7 (d), 71.0 (d), 70.5 (d), 69.7 (t), 62.7 (t), 54.9 (d), 36.8 (t), 35.1 (t), 32.1 (t), 30.2 (t), 30.1 (t), 30.0 (t), 29.9 (t), 29.83 (t), 29.76 (t), 29.6 (t), 26.6 (t), 26.4 (t), 22.9 (t), 14.3 (q).

Using the same method, the following AGL analogues were obtained.

(2S,3R)-1-O-(α-D-Galactopyranosyl)-2-(N-octadecanoylamino)-1,3-octadecanediol (AGL-536): from **B3b** (88%); $[\alpha]^{24}_D$ +55.5° (*c* 0.8, pyridine); FDMS *m/z* 731 (M + 1)$^+$; IR (cm^{-1}, KBr) 3230, 2940, 2830, 1640, 1540, 1465, 1345, 1120, 1090, 1060; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.52 (1H, d, *J* = 8.6 Hz), 5.46 (1H, d, *J* = 3.7 Hz), 4.73 (1H, m), 4.66 (1H, dd, *J* = 4.5, 10.1 Hz), 4.57 (1H, d, *J* = 2.5 Hz), 4.55 (1H, t, *J* = 6.1 Hz), 4.40–4.51 (4H, m), 4.37 (1H, dd, *J* = 5.8, 10.7 Hz), 4.29 (1H, m), 2.48 (2H, t, *J* = 7.3 Hz), 1.80–1.96 (4H, m), 1.59 (1H, m), 1.20–1.44 (53H, m), 0.88 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 173.4 (s), 102.1 (d), 73.1 (d), 71.9 (d), 71.7 (d), 71.0 (d), 70.5 (d), 69.7 (t), 62.7 (t), 54.9 (d), 36.8 (t), 35.1 (t), 32.1 (t), 30.2 (t), 30.1 (t), 30.0 (t), 29.9 (t), 29.8 (t), 29.7 (t), 29.6 (t), 26.6 (t), 26.6 (t), 26.4 (t), 22.9 (t), 22.8 (t), 14.3 (q).

(2S,3R)-1-O-(α-D-Galactopyranosyl)-2-(N-icosanoylamino)-1,3-octadecanediol (AGL-544): from **B3c** (87%); $[\alpha]^{24}_D$ +47.3° (*c* 1.0, pyridine); FDMS *m/z* 759 (M + 1)$^+$; IR (cm^{-1}, KBr) 3390, 3220, 2880, 2810, 1635, 1535, 1455, 1080, 1055; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.52 (1H, d, *J* = 8.6 Hz), 5.46 (1H, d, *J* = 4.3 Hz), 4.73 (1H, m), 4.66 (1H, dd, *J* = 4.5, 10.1 Hz), 4.40–4.60 (6H, m), 4.37 (1H, dd, *J* = 5.5, 10.4 Hz), 4.29 (1H, m), 2.48 (2H, t, *J* = 7.3 Hz), 1.80–1.97 (4H, m), 1.58 (1H, m), 1.20–1.42 (57H, m), 0.879 and 0.876 (each 3H, t, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 173.4 (s), 102.1 (d), 73.1 (d), 71.9 (d), 71.6 (d), 71.0 (d), 70.5 (d), 69.7 (t), 62.7 (t), 54.9

(d), 36.8 (t), 35.1 (t), 32.1 (t), 30.2 (t), 30.1 (t), 30.0 (t), 29.9 (t), 29.8 (t), 29.7 (t), 29.6 (t), 26.6 (t), 26.4 (t), 22.9 (t), 14.3 (q).

(2S,3R)-2-(N-Docosanoylamino)-1-O-(α-D-galactopyranosyl)-1,3-octadecanediol (AGL-543): from **B3d** (83%); $[\alpha]^{24}_D$ +50.7° (*c* 0.8, pyridine); FDMS *m/z* 786 (M^+); IR (cm^{-1}, KBr) 3390, 3220, 2870, 2810, 1635, 1535, 1455, 1080, 1055; ^1H NMR (500 MHz, C_5D_5N) δ 8.53 (1H, d, *J* = 8.6 Hz), 5.46 (1H, d, *J* = 3.1 Hz), 4.74 (1H, m), 4.66 (1H, dd, *J* = 3.6, 9.8 Hz), 4.40–4.60 (6H, m), 4.37 (1H, dd, *J* = 5.8, 10.1 Hz), 4.29 (1H, m), 2.48 (2H, t, *J* = 7.3 Hz), 1.80–1.97 (4H, m), 1.58 (1H, m), 1.20–1.45 (61H, m), 0.880 and 0.876 (each 3H, t, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 173.4 (s), 102.2 (d), 73.1 (d), 72.0 (d), 71.7 (d), 71.0 (d), 70.6 (d), 69.7 (t), 62.7 (t), 54.9 (d), 36.8 (t), 35.1 (t), 32.1 (t), 30.2 (t), 30.1 (t), 30.0 (t), 29.95 (t) 29.92 (t), 29.83 (t), 29.76 (t), 29.62 (t), 29.61 (t), 26.6 (t), 26.4 (t), 22.9 (t), 14.3 (q).

3,5-Di-O-benzyl-D-xylofuranose (D3). To a solution of **D2**[33] (2.89 g, 7.8 mmol) in 2-methyl-2-propanol (25 mL) was added 5% aqueous sulfuric acid solution (25 mL), and the mixture was stirred at 45 °C for 15 h. After being cooled, the reaction mixture was neutralized with powdery sodium hydrogen carbonate, and 2-methyl-2-propanol was evaporated under reduced pressure. To the residual solution was added additional water (30 mL). It was then extracted with EtOAc (three times), dried over $MgSO_4$, and concentrated. The residue was purified by chromatography on a silica gel column (100 g) using hexane–acetone (2:1) as the eluent to give diol **D3** as an anomeric mixture: 2.28 g (89%); FDMS *m/z* 330 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.27–7.46 (10H, m), 5.49 (0.6H, bs), 5.09 (0.4H, d, *J* = 11.6 Hz), 4.47–4.69 (4.6H, m), 4.42 (0.4H, q, *J* = 4.9 Hz), 4.25 (0.4H, m), 4.20 (0.6H, m), 3.98–4.02 [1H (3.99, dd, *J* = 3.1, 5.5 Hz) and (4.01, dd, *J* = 3.1, 5.5 Hz)], 3.85–3.90 (1H, m), 3.77 (0.4H, dd, *J* = 4.9, 9.8 Hz), 3.73 (0.4H, dd, *J* = 6.7, 9.8 Hz), 3.65–3.70 (1.2H, m), 2.81 (0.6H, bs), 2.17 (0.4H, bs). Anal. ($C_{19}H_{22}O_5$) C, H.

(2R,3R)-1,3-Di-O-benzyl-4-octadecene-1,2,3-triol (D4). The title compound was prepared from **D3** (2.48 g, 7.5 mmol) with **A2e** in a manner similar to that described for **A3a** to give alcohol **D4**: 2.28 g (70%); FDMS *m/z* 481 ($M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.26–7.46 (10H, m), 5.69–5.78 (1H, m), 5.31–5.38 (1H, m), 4.34–4.63 (4H, m), 4.28 (0.7H, dd, *J* = 6.7, 9.2 Hz), 3.85 (0.3H, t, *J* = 7.3 Hz), 3.73–3.78 (1H, m), 3.56–3.60 (1H, m), 3.47 (1H, dd, *J* = 5.5, 10.4 Hz), 1.98–2.13 (2H, m), 1.26–1.34 (22H, m), 0.88 (3H, t, *J* = 6.7 Hz). Anal. ($C_{32}H_{48}O_3$) C, H.

(2R,3R)-1,3-Di-O-benzyl-2-O-(methylsulfonyl)-4-octadecene-1,2,3-triol (D5). The title compound was prepared from **D4** (5.03 g, 10.5 mmol) in a manner similar to that described for **A4a**: 5.20 g (89%); FDMS *m/z* 558 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.23–7.35 (10H, m), 5.77–5.83 (1H, m), 5.26–5.35 (1H, m), 4.71–4.77 (1H, m), 4.33–4.62 (5H, m), 4.06 (0.3H, t, *J* = 8.1 Hz), 3.74 (0.7H, dd, *J* = 3.1, 11.0 Hz), 3.65–3.70 (1H, m), 2.96 [3H (2.964, s) and (2.956, s)], 1.99–2.17 (2H, m), 1.26–1.37 (22H, m), 0.88 (3H, t, *J* = 6.8 Hz). Anal. ($C_{33}H_{50}O_5S$) C, H.

(2S,3R)-2-Azido-1,3-di-O-benzyl-4-octadecene-1,3-diol (D6). The title compound was prepared from **D5** (1.52 g, 2.72 mmol) in a manner similar to that described for **A7a**: 1.07 g (78%); FDMS *m/z* 505 (M^+); IR (cm^{-1}) 2870, 2810, 2050, 1490, 1440; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25–7.35 (10H, m), 5.69–5.82 (1H, m), 5.35–5.43 (1H, m), 4.30–4.61 (4H, m), 3.89 (0.3H, dd, *J* = 5.5, 8.5 Hz), 3.55–3.70 (3.7H, m), 1.97–2.10 (2H, m), 1.25–1.36 (22H, m), 0.88 (3H, t, *J* = 6.8 Hz). Anal. ($C_{32}H_{47}N_3O_2$) C, H, N.

(2S,3R)-1,3-Di-O-benzyl-2-(N-tetradecanoylamino)-4-octadecene-1,3-diol (D8a). The title compound was prepared from **D6** (3.90 g, 7.72 mmol) via an amine [**D7**: FDMS *m/z* 480 ($M + 1)^+$] using tetradecanoic acid in a manner similar to that described for **A9a**: 3.22 g (66% from **D6**); FDMS *m/z* 691 ($M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.26–7.32 (10H, m), 5.64–5.73 (2H, m), 5.33–5.41 (1H, m), 4.19–4.59 (6H, m), 3.79–3.89 (1H, m), 3.51–3.58 (1H, m), 1.98–2.13 (3H, m), 1.26–1.58 (45H, m), 0.88 (6H, t, *J* = 6.7 Hz). Anal. ($C_{46}H_{75}NO_3$) C, H, N.

Similarly, **D8b,c** were prepared from **D6** using (*R*)-2-acetoxytetracosanoic acid and (*R*)-2-acetoxyhexacosanoic acid, respectively.

(2S,3R)-2-(N-Tetradecanoylamino)-1,3-octadecanediol (D9a). To a solution of **D8a** (3.50 g, 5.1 mmol) in THF and 1-propanol (1:1, 30 mL) were added 10% palladium on charcoal (1.20 g) and formic acid (3.0 mL, 0.8 mol). The mixture was stirred at 45 °C for 16 h under a nitrogen atmosphere. Then the catalyst was removed by filtration with Celite, and the filtrate was concentrated. Crystallization of the residue from chloroform–acetone afforded ceramide **D9a**: 2.08 g (80%); $[\alpha]^{24}_D$ +3.5° (*c* 1.9, pyridine); mp 104–105 °C; FDMS *m/z* 513 ($M + 1)^+$; ^1H NMR (500 MHz, C_5D_5N) δ 8.35 (1H, d, *J* = 9.2 Hz), 6.36 (1H, t, *J* = 4.9 Hz), 6.24 (1H, d, *J* = 6.1 Hz), 4.62–4.67 (1H, m), 4.46 (1H, dt, *J* = 4.9, 11.0 Hz), 4.25–4.33 (2H, m), 2.47 (2H, dt, *J* = 1.8, 7.3 Hz), 1.80–2.00 (4H, m), 1.25–1.63 (46H, m), 0.88 (6H, t, *J* = 6.7 Hz). Anal. ($C_{32}H_{65}NO_3$) C, H, N.

Using the same method, **D9b,c** were prepared from **D8b,c**, respectively.

(2S,3R)-2-(N-Tetradecanoylamino)-1-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyanosyl)-1,3-octadecanediol (D10a). The title compound was prepared from **D9a** (1.0 g, 1.9 mmol) in a manner similar to that described for **A15a**: 646 mg (33%); FDMS *m/z* 1035 ($M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.23–7.37 (20H, m), 6.49 (1H, d, *J* = 7.9 Hz), 4.92 (1H, d, *J* = 11.3 Hz), 4.84 (1H, d, *J* = 12.2 Hz), 4.73–4.78 (3H, m), 4.67 (1H, d, *J* = 11.6 Hz), 4.56 (1H, d, *J* = 11.6 Hz), 4.46 (1H, d, *J* = 11.6 Hz), 4.37 (1H, d, *J* = 11.6 Hz), 4.03 (1H, dd, *J* = 3.7, 9.8 Hz), 3.96 (1H, bs), 3.83–3.92 (4H, m), 3.70 (1H, dd, *J* = 3.1, 10.4 Hz), 3.47–3.58 (3H, m), 2.12 (2H, dt, *J* = 1.8, 7.9 Hz), 1.25–1.61 (50H, m), 0.88 (6H, t, *J* = 6.7 Hz). Anal. ($C_{66}H_{99}NO_8$) C, H, N.

Similarly, **D10b,c** were prepared from **D9b,c**, respectively.

(2S,3R)-2-(N-Tetradecanoylamino)-1-O-(α-D-galactopyranosyl)-1,3-octadecanediol (AGL-517). The title compound was prepared from **D10a** (1.59 g, 1.54 mmol) in a manner similar to that described for AGL-514: 984 mg (95%); $[\alpha]^{24}_D$ +57.8° (*c* 1.0, pyridine); FDMS *m/z* 674 (M^+); IR (cm^{-1}, KBr) 3400, 3270, 2920, 2850, 1640, 1550, 1465, 1135, 1075, 1045; ^1H NMR (500 MHz, C_5D_5N) δ 8.52 (1H, d, *J* = 8.6 Hz), 5.45 (1H, d, *J* = 3.7 Hz), 4.73 (1H, m), 4.65 (1H, m), 4.40–4.58 (6H, m), 4.36 (1H, dd, *J* = 5.5, 10.0 Hz), 4.28 (1H, m), 2.48 (2H, t, *J* = 7.0 Hz), 1.80–1.95 (4H, m), 1.57 (1H, m), 1.18–1.43 (45H, m), 0.88 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 173.4 (s), 102.2 (d), 73.1 (d), 71.9 (d), 71.7 (d), 71.0 (d), 70.5 (d), 69.7 (t), 62.7 (t), 54.9 (d), 36.8 (t), 35.1 (t), 32.1 (t), 30.2 (t), 30.1 (t), 30.02 (t), 29.97 (t), 29.91 (t), 29.87 (t), 29.80 (t), 29.7 (t), 29.6 (t), 26.6 (t), 26.4 (t), 22.9 (t), 14.3 (q).

Similarly, the following AGL analogues were obtained.

(2S,3R)-1-O-(α-D-Galactopyranosyl)-2-[N-((R)-2-hydroxytetracosanoyl)amino]-1,3-octadecanediol (AGL-506): from **D10b** (83%); $[\alpha]^{23}_D$ +50.2° (*c* 1.0, pyridine); FDMS *m/z* 831 ($M + 1)^+$; IR (cm^{-1}, KBr) 3230, 2940, 2830, 1640, 1540, 1465, 1345, 1120, 1090, 1060; ^1H NMR (500 MHz, C_5D_5N) δ 8.45 (1H, d, *J* = 9.2 Hz), 5.44 (1H, d, *J* = 3.7 Hz), 4.71 (1H, m), 4.64 (2H, m), 4.53 (3H, m), 4.40 (3H, m), 4.25 (1H, m), 2.22 (1H, m), 2.09 (1H, m), 1.70–1.95 (4H, m), 1.54 (1H, m), 1.20–1.45 (63H, m), 0.884 and 0.876 (6H, each t, *J* = 6.7 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 175.1 (s), 101.9 (d), 73.2 (d), 72.4 (d), 71.7 (d), 71.0 (d), 70.5 (d), 69.4 (t), 62.7 (t), 54.1 (d), 35.6 (t), 35.2 (t), 32.1 (t), 30.3 (t), 30.04 (t), 29.97 (t), 29.9 (t), 29.64 (t), 29.61 (t), 26.5 (t), 25.8 (t), 22.9 (t), 14.3 (q).

(2S,3R)-1-O-(α-D-Galactopyranosyl)-2-[N-((R)-2-hydroxyhexacosanoyl)amino]-1,3-octadecanediol (AGL-578): from **D10c** (85%); $[\alpha]^{23}_D$ +54.8° (*c* 1.0, pyridine); FDMS *m/z* 859 ($M + 1)^+$; IR (cm^{-1}, KBr) 3400, 2920, 2850, 1640, 1550, 1465, 1075, 1045; ^1H NMR (500 MHz, C_5D_5N) δ 8.46 (1H, d, *J* = 9.2 Hz), 5.45 (1H, d, *J* = 3.7 Hz), 4.71 (1H, m), 4.64 (2H, m), 4.35–4.60 (6H, m), 4.26 (1H, m), 4.12 (1H, t, *J* = 6.7 Hz), 2.15–2.28 (2H, m), 1.70–2.00 (4H, m), 1.57 (1H, m), 1.18–1.43 (67H, m), 0.86–0.90 [6H (0.885, t, *J* = 6.7 Hz) and (0.887, t, *J* = 7.3 Hz)]; ^{13}C NMR (125 MHz, C_5D_5N) δ 175.2 (s), 101.9 (d), 73.2 (d), 72.4 (d), 71.7 (d), 71.0 (d), 70.5 (d), 69.4 (t), 62.7 (t), 54.1 (d), 35.7 (t), 35.2 (t), 32.1 (t), 30.1 (t), 30.0 (t),

29.9 (t), 29.83 (t), 29.78 (t), 29.64 (t), 29.61 (t), 26.5 (1), 25.8 (t), 22.9 (t), 14.3 (q).

1,2-Octadecanediol (E2). To a suspension of **E1** (10.0 g, 37.2 mmol) in *t*-BuOH (100 mL) was added 0.5 M aqueous H_2SO_4 solution (200 mL), and the mixture was stirred at reflux for 16 h. After being cooled to room temperature, the mixture was diluted with water (500 mL), and the precipitation formed was filtered. The precipitation was dissolved with EtOAc, washed with water, dried over $MgSO_4$, and then concentrated. The residue was crystallized in EtOAc and hexane to give **E2**: 6.44 g (60%); mp 70–71 °C; FDMS *m/z* 287 $(M + 1)^+$; ^1H NMR (500 MHz, CDCl3) δ 3.69–3.75 (1H, m), 3.67 (1H, dd, J = 3.1, 11.0 Hz), 3.44 (1H, dd, J = 7.9, 11.0 Hz), 1.15–1.50 (30H, m), 0.89, (3H, t, J = 6.7 Hz). Anal. ($C_{18}H_{38}O_2$) C, H.

1-*O*-(Triphenylmethyl)-1,2-octadecanediol (E3). To a solution of **E2** (6.0 g, 21.0 mmol) in pyridine and CH_2Cl_2 (4:1, 75 mL) was added triphenylchloromethane (7.02 g, 25.2 mmol), and the mixture was stirred at 30 °C for 16 h. The usual workup procedure and purification by chromatography on a silica gel column (300 g) using hexane–EtOAc (10:1) as the eluent gave **E3**: 10.04 g (91%); mp 62–63 °C; FDMS *m/z* 528 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.23–7.44 (15H, m), 3.52–3.58 (1H, m), 3.17–3.92 (1H, dd, J = 3.1, 9.2 Hz), 3.02 (1H, dd, J = 7.3, 9.2 Hz), 1.23–1.36 (30H, m), 0.88 (3H, t, J = 7.3 Hz). Anal. ($C_{37}H_{52}O_2$) C, H.

2-*O*-(Methylsulfonyl)-1-*O*-(triphenylmethyl)-1,2-octadecanediol (E4). The title compound was prepared from **E3** (7.63 g, 15.8 mmol) in a manner similar to that described for **A4a** and purified by chromatography on a silica gel column (200 g) using hexane–EtOAc (15:1) as the eluent: 8.68 g (91%); FDMS *m/z* 607 $(M + 1)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24–7.44 (15H, m), 4.73–4.78 (1H, m), 3.33 (1H, dd, J = 3.1, 10.9 Hz), 3.26 (1H, dd, J = 6.4, 11.0 Hz), 3.01 (3H, s), 1.20–1.72 (30H, m), 0.88, (3H, t, J = 7.3 Hz). Anal. ($C_{38}H_{54}O_4S$) C, H.

2-Azido-1-*O*-(triphenylmethyl)octadecanol (E5). The title compound was prepared from **E4** (8.40 g, 13.9 mmol) in a manner similar to that described for **A5a** and purified by chromatography on a silica gel column (200 g) using hexane–EtOAc (50:1) as the eluent: 7.55 g (98%); FDMS *m/z* 553 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.23–7.47 (15H, m), 3.35–3.42 (1H, m), 3.19 (1H, dd, J = 3.7, 9.8 Hz), 3.15 (1H, dd, J = 3.7, 9.8 Hz), 1.16–1.44 (30H, m), 0.88 (3H, t, J = 6.7 Hz). Anal. ($C_{37}H_{51}N_3O$) C, H, N.

2-Azidooctadecanol (E6). To a solution of **E5** (7.45 g, 13.5 mmol) in CH_2Cl_2 and methanol (4:1, 90 mL) was added 10% HCl–methanol solution (1.5 mL), and the mixture was stirred at room temperature for 3 h. After being diluted with CH_2Cl_2, the mixture was washed in water, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (200 g) using hexane–EtOAc (9:1) as the eluent to give **E6**: 3.90 g (93%); FDMS *m/z* 311 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 3.68–3.72 (1H, m), 3.53–3.57 (1H, m), 3.44–3.49 (1H, m), 1.22–1.55 (30H, m), 0.88 (3H, t, J = 7.3 Hz). Anal. ($C_{18}H_{37}N_3O$) C, H, N.

2-Azido-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)octadecanol (E7). The title compound was prepared from **E6** (430 mg, 1.38 mmol) in a manner similar to that described for **A15a** and purified by chromatography on a silica gel column (20 g) using hexane–EtOAc (9:1) as the eluent: 714 mg (62%); $[\alpha]^{24}_D$ +16.1° (*c* 0.5, $CHCl_3$); FDMS *m/z* 834 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.26–7.38 (20H, m), 4.94 (1H, dd, J = 4.9, 11.6 Hz), 4.55–4.85 (4H, m), 4.48 (1H, dd, J = 7.3, 12.2 Hz), 4.40 (1H, dd, J = 7.9, 12.2 Hz), 3.95–4.06 (4H, m), 3.40–3.72 (5H, m), 1.20–1.53 (30H, m), 0.88 (6H, t, J = 6.7 Hz). Anal. ($C_{52}H_{71}N_3O_6$) C, H, N.

1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(*N*-tetracosanoylamino)octadecanol (E9). To a solution of **E7** (420 mg, 0.50 mmol) in THF (10 mL) was added 10% palladium on charcoal (40 mg). After the reaction vessel was purged with hydrogen, the mixture was stirred at room temperature for 16 h and filtered through Celite, and the filtrate was concentrated to give a crude syrup of **E8** [FDMS *m/z* 808 (M^+)]. The resulting crude amine was used for the next step without further purification. To a solution of the crude syrup of **E8** in CH_2Cl_2 (15 mL) was added tetracosanoic acid (212 mg, 0.58 mmol) and WSC-HCl (114 mg, 0.58 mmol), and the mixture was stirred at reflux for 1 h. After being cooled to room temperature, the mixture was successively washed with 0.1 M aqueous hydrogen chloride solution and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by chromatography on a silica gel column (40 g) using hexane–acetone (9:1) as the eluent to give **E9**: 420 mg (73% from **E7**); $[\alpha]^{24}_D$ +19.9° (*c* 0.34, $CHCl_3$); FDMS *m/z* 1158 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.26–7.39 (20H, m), 5.98–6.17 [1H (6.13, d, J = 9.2 Hz) and (5.98, d, J = 9.2 Hz)], 4.36–4.95 (5H, m), 3.88–4.10 (3H, m), 3.74–3.79 (1H, m), 3.52–3.62 (2H, m), 3.34–3.38 (1H, m), 1.85–2.07 (4H, m), 1.15–1.58 (70H, m), 0.88 (6H, t, J = 6.7 Hz). Anal. ($C_{76}H_{119}NO_7$) C, H, N.

1-*O*-(α-D-Galactopyranosyl)-2-(*N*-tetracosanoylamino)-octadecanol (AGL-535). The title compound was prepared from **E9** (180 mg, 0.16 mmol) as a C-2 epimeric mixture in a manner similar to that described for AGL-514: 112 mg (90%); $[\alpha]^{23}_D$ +41.6° (*c* 1.0, pyridine); FDMS 798 (M^+); IR (cm^{-1}, KBr) 3415, 3300, 2915, 2850, 1645, 1540, 1470, 1360, 1100, 1070, 1040; ^1H NMR (500 MHz, C_5D_5N) δ 8.28–8.30 [1H (8.29, d, J = 8.6 Hz), and (8.27, d, 8.6 Hz)], 5.37–5.39 [1H (5.39, d, J = 3.7 Hz) and (5.37 d, J = 3.7 Hz)], 4.36–4.68 (7H, m), 4.05–4.15 [1H (4.13, dd, J = 4.9, 9.8 Hz) and (4.07, dd, J = 4.9, 9.8 Hz)], 3.74–3.89 [1H (3.87, dd, J = 5.5, 9.8 Hz) and (3.74, dd, J = 5.5, 9.8 Hz)], 2.37–2.44 [1H (2.43, t, J = 7.3 Hz) and (2.39, t, J = 7.3 Hz)], 1.14–1.88 (72H, m), 0.80–0.85 [6H (0.820, t, J = 7.3 Hz) and (0.815, t, J = 6.7 Hz)]; ^{13}C NMR (125 MHz, C_5D_5N) δ 173.1 (s), 101.67 (d), 101.56 (d), 73.1 (d), 71.8 (d), 71.7 (d), 71.1 (d), 71.0 (t), 70.69 (t), 70.60 (t), 62.8 (t), 62.7 (t), 49.7 (d), 49.5 (d), 36.8 (t), 32.3 (t), 32.1 (t), 30.0 (t), 29.91 (t), 29.88 (t), 29.8 (t), 29.7 (t), 29.6 (t), 26.7 (t), 26.6 (t), 26.5 (t), 22.9 (t), 14.3 (q).

Biological Methods. Materials and Animals. Mitomycin C (MMC) and adriamycin (ADR) were purchased from Kyowa Hakko Kogyo Co., Ltd. (Japan). Female BDF_1, BALB/c, and C57BL/6 mice were purchased from Nippon SLC Co. Ltd. at 5 weeks old and used at 6–10 weeks old. All other materials were reagent grade.

Tumor Growth Inhibition Assay. According to Watanabe *et al.*,[37] a tumor growth inhibition assay was done. In brief, six female BDF_1 mice were used as a group, and murine melanoma B16 (1×10^6 cells/mouse) was inoculated subcutaneously on day 0. AGL analogues (100 μg/kg) were administered intravenously on days 1, 5, and 9, and MMC (5 mg/kg) or ADR (10 mg/kg) was given intraperitoneally on day 1. The control group received no treatment. The tumor volume (length × width × height/2) of each mouse was measured on days 8, 12, 16, and 20 using callipers, and the tumor growth inhibition ratio (TGIR) was calculated by the following formula: TGIR (%) = [1 − (mean tumor volume of test group/mean tumor volume of control group)] × 100.

Allogeneic MLR. According to Hart and Mckenzie,[38] an allogeneic MLR assay was done. In brief, spleen cells obtained from BALB/c mice were used as responder cells, and MMC (50 μg/mL, 30 min)-treated spleen cells from C57BL/6 mice were used as stimulator cells in an allogeneic MLR assay. The same volumes (1×10^5 cells/50 μL/mL) of responder cells and stimulator cells suspended in 10% FCS RPMI 1640 medium were plated in a 96-well plate. At the same time, AGLs at various concentrations (10 μL/well) were added into each well, and these cell suspensions were cultured at 37 °C, in the presence of 5% CO_2. Two or three days after, a 6 h [^{3}H]TdR (tritiumthymidine) pulse was done, and [^{3}H]TdR uptaken into cells was counted using a liquid scintillation counter (β-plate, Pharmacia). Experiments were undertaken in triplicate.

Lymphocyte Proliferation (LP) of Mouse Spleen Cell Assay. This assay was done similarly to the allogeneic MLR assay except that spleen cells (2×10^5 cells/100 μL/well) from BALB/c mice and no stimulator cells were used.

LP of Human Peripheral Blood (hPB) or Umbilical Cord Blood (hUCB) Assay. According to Leary and Ogawa,[39] mononuclear cells were collected from hUCB or hPB. Mononuclear cells (1×10^5 cells/100 μL/well) suspended in 10% self-plasma RPMI 1640 medium were plated and incubated for 4 days. Other processes were done according to LP on the mouse spleen cell assay.

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Supplementary Material Available: Addtitional experimental descriptions of intermediates (**Ab-h** series in Scheme 1, **Bb-d** series in Scheme 2, and **Db,c** series in Scheme 3) and their spectral data (20 pages). Ordering information is given on any current masthead page.

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