Glycoconjugate Journal 18, 557–563, 2001

A one pot synthesis of mono- and di-lactosyl sphingosines

Yongmin Zhang*, Tatsushi Toyokuni†, Fuqiang Ruan** and Sen-itiroh Hakomori

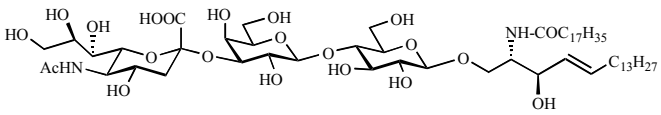
Division of Biomembrane Research, Pacific Northwest Research Institute, 720 Broadway, Seattle, Washington 98122-4327, Departments of Pathobiology and Microbiology, University of Washington, Seattle, Washington 98195, USA

The importance of analogues of lactosyl ceramides as basic structures of many natural glycosphingolipids provided a rationale for developing an efficient synthetic route to these compounds. We report herein a novel approach to synthesize several members of this family. Glycosylation of *N*-diphenylmethylene-spingosine, which exists in an imine–oxazolidine tautomeric mixture, with acetobromolactose under a modified Koenigs-Knorr condition yielded lactosyl β-(1 $\rightarrow$ 1) sphingosine, lactosyl β-(1 $\rightarrow$ 3) sphingosine and dilactosyl sphingosine in good yields. A similar glycosylation could be applicable to the synthesis of other glycosphingolipids.

Keywords: **glycosphingolipids, GM3, lactosyl ceramide, lactosyl sphingosine, glycosylation**

Introduction

Glycosphingolipids (GSLs) have been implicated as mediators of cell adhesion and as modulators of signal transduction [1]. Recent studies indicate that GSLs are clustered at the cell surface in close association with various signal transducer molecules, and are involved in initiation of signal transduction coupled with GSL-dependent cell adhesion [2,3]. A typical example is GM3 ganglioside clustering at the cell surface of mouse melanoma B16, forming a "glycosignaling domain (GSD)".



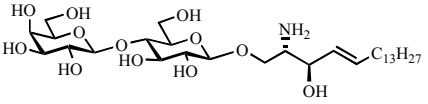
GM3

GM3 in B16 cells is also recognized as a melanoma-associated antigen [4], and may have a role in initiating adhesion of melanoma to endothelial cells, the first step in metastasis [5]. Antigenicity, mediation of adhesion, and initiation of signaling through GM3 ganglioside at the B16 cell surface are thought to be maintained by GM3 clustering in GSD. If GM3 clustering in GSD is inhibited, antigenicity, adhesion, and signaling through GM3 could be blocked. We therefore undertook a new approach to observe disruptive effects of chemically synthesized GSL analogues on GSD structure and function in B16 cells. Such compounds should have structural features analogous to those of GM3, destroy or reduce clustering of GM3 in GSD, and inhibit GM3-dependent adhesion and signaling [6]. On the other hand, lactosyl ceramide (LacCer) has been implicated as a stimulator of aortic smooth muscle cells [7], and claimed to induce Ras-GTP loading causing a variety of signal transductions [8]. Since smooth muscle cell proliferation in aorta is the initial event leading to atherosclerosis, the functional role of LacCer and its derivatives is of great pathobiological significance. Several analogues of GM3 have been synthesized and investigated for their biological activities, including sialyl α-(2 $\rightarrow$ 1) sphingosine [6], lyso-GM3 [9] and lactosyl β-(1 $\rightarrow$ 1) sphingosine (**1**), lactosyl β-(1 $\rightarrow$ 3) sphingosine (**2**) and dilactosyl sphingosine (**3**). Here we describe a one pot method to synthesize the derivatives **1**, **2** and **3**. These compounds have been involved in a variety of biological studies on the structure and function of GSD [6,9], and may have potential interest in relation to LacCer, which initiates signal transduction as a component of GSD, to activate neutrophils [10], including phagocytosis [11].

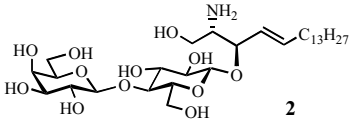
*To whom correspondence should be addressed: Ecole Normale Supérieure, Département de Chimie, UMR CNRS 8642, 24 rue Lhomond, 75231 Paris, Cedex 05, France. Tel.: 33-1-44323334; Fax: 33-1-44323397; E-mail: yongmin.zhang@ens.fr

†Present address: LA Tech Center, Department of Molecular and Medical Pharmacology, UCLA School of Medicine, 6140 Bristol Parkway, Culver City, CA 90320, USA.

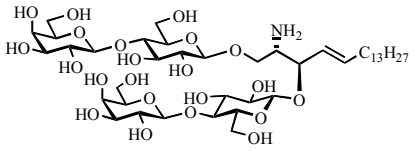
**Present address: ICOS Corporation, 22021 20th Avenue SE, Bothell, WA 98021, USA.



1



2



3

Results and discussion

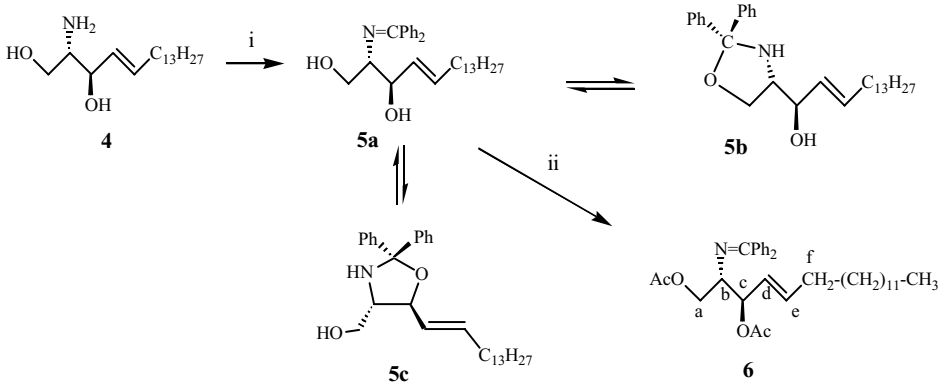
The importance of lactosyl ceramides as basic structures of many natural glycosphingolipids provided a rationale for developing an efficient synthetic route to these compounds. A common synthetic method to the glycosphingilipids consists in condensation of a glycosyl trichloroacetimidate with an appropriately protected ceramide or azidosphingosine [12,13]. For example, lactosyl $\beta 1 \rightarrow 1$ sphingosine **1** has been prepared by Zimmermann et al. [14] as an intermediate in the synthesis of lactosyl ceramide. The strategy used by these authors was the lactosylation of 3-*O*-benzoyl azidosphingosine with the *O*-acetyl protected lactosyl trichloroacetimidate, through *in situ* rearrangement of the intermediate ortho-ester, followed by *O*-deprotection and transformation of the azido group into an amino group. The *O*-glycosyltrichloroacetimidates as glycosyl donors have been largely employed in the synthesis of biologically important glycosphingolipids [15–18]. Another method for preparing the glycosphingolipids, using a glycal procedure, has been developed by Bilodeau et al. [19]. Glycosyl halides have not attracted much attention for glycosphingolipids preparation. However, recently the glycosyl chloride [20] and glycosyl fluoride [21] have been successfully applied to the glycosphingolipids synthesis.

Having D-*erythro*-sphingosine readily available [22], we envisaged that the lactosylation of the amino-protected sphingosine could provide the desired three compounds in one pot. Thus the D-*erythro*-sphingosine (**4**) was reacted with diphenylketimine using the published procedure of O'Donnell and Polt [23] to give the Schiff base **5a**, with 10% of sphingosine being recovered (Scheme 1). The isolated product **5a** was proven by ^{1}H NMR to exist in a tautomeric equilibrium with the oxazolidine forms **5b** and **5c** in the ratio of 21:23:56, respectively. This phenomenon has been observed previously by other authors [24] for the β-hydroxy Schiff base. The structure was characterized by acetylation to compound **6**, which exists as a single compound according to the ^{1}H and ^{13}C NMR data.

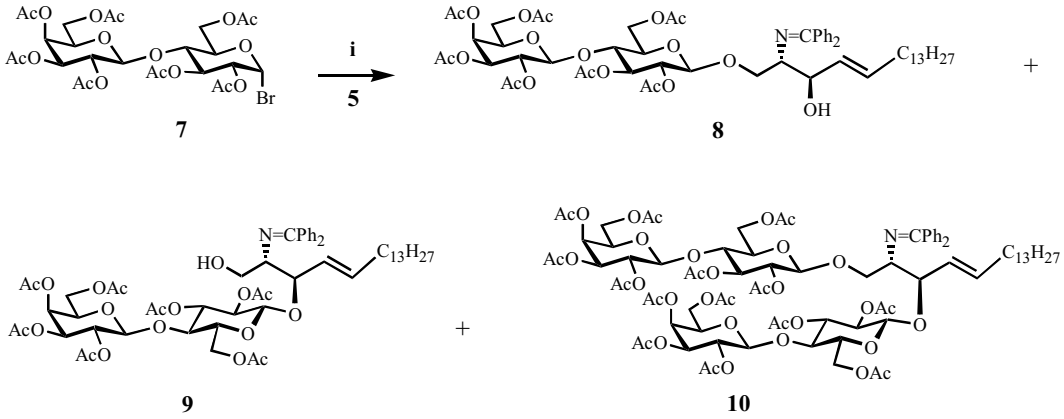
The glycosylation of **5** with known per-*O*-acetyl lactosyl bromide (**7**) [25], promoted by AgOTf as described by Polt [26], gave mono-lactosyl sphingosine derivatives **8** and **9** as an inseparable mixture in 65% yield, together with di-lactosyl sphingosine derivative **10** in 12% yield (Scheme 2). Separation of **8** and **9** by silica-gel column chromatography was not feasible due to their similar chromatographic behavior as well as their tautomerization to the corresponding oxazolidine forms.

Separation of **8** and **9** was therefore carried out as the amino-deprotected derivatives **11** and **12**, respectively (Scheme 3). After acid hydrolysis, flash column chromatography allowed isolation of **11** and **12** in 56% and 39% yield, respectively. Their ^{1}H NMR spectra ($J_{1,2} = 8.2$ Hz for **11** and **12**) indicated that the newly formed glycosidic linkage was β. Their glycosylation site was assigned based on examination of their acetates, **13** and **14**.

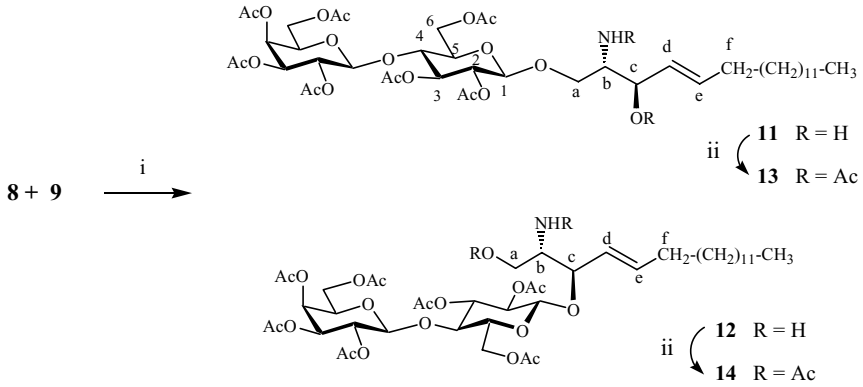
The ^{1}H NMR spectra of **13** and **14** revealed the deshielded signal of H-c (for **13**, δ 5.25, dd, $J_{b,c} = J_{c,d} = 7.2$ Hz) and H-a, H-a′ (for **14**, δ 4.29, m), respectively, supporting the structure shown in Scheme 3.



Scheme 1. Reagents and conditions: i. $Ph_2C{=}NH$, CH_2Cl_2, rt, 15 h, 78%; ii. Ac_2O, Py, rt, 20 h, 99%.



Scheme 2. Reagents and conditions: i. 4Å molecular sieves, dry CH_2Cl_2, AgOTf, rt, 15 h.



Scheme 3. Reagents and conditions: i. 5% trifluoroacetic acid solution (prepared from 49.5:49.5:1 THF-CH_2Cl_2-H_2O), rt, 10 min; ii. Ac_2O, Py, rt, 13 h, quantitative.

Deprotection of the Schiff base **10** was effected by acid hydrolysis as described above for preparation of **11** and **12**, yielding the free amino compound **15** in 93% yield (Scheme 4). De-*O*-acetylation of **11**, **12** and **15** under Zemplèn condition gave **1**, **2** and **3**, respectively, in quantitative yields.

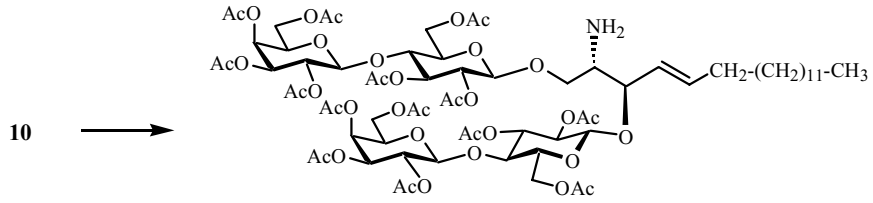
In summary, we have presented a one pot method for preparation of the lactosyl sphingosine (**1**), and its analogues **2** and **3**. A similar glycosylation could be applicable to the synthesis of other glycosphingolipids.

Materials and methods

General methods

Optical rotations were measured at 589 nm (Na line) at 20 ± 2°C with a Perkin Elmer Model 241MC digital polarimeter. Mass spectra were obtained on a JEOL JMS-HX 110 mass spectrometer in a fast atom bombardment (FAB) mode using *m*-nitrobenzyl alcohol (NBA) as matrix. High resolution mass spectra (HRMS) were recorded with a JMS-700 spectrometer in FAB mode using NBA as matrix. ^{1}H NMR spectra were recorded with a Bruker DRX 400 spectrometer or a Bruker WM-500 spectrometer. ^{13}C NMR spectra were measured on a Bruker AM-300 (75.5 MHz) spectrometer or a Bruker DRX 400 (100.6 MHz) spectrometer. Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated plate of silica gel 60 F_{254} (Merck, 0.2 mm). Flash column chromatography was performed on silica gel 60 (230–400 mesh).

(2*S*,3*R*,4*E*)-1,3-Dihydroxy-2-[*N*-(Diphenylmethylene)-amino]-4-octadecene (5a), (4*S*)-2,2-Diphenyl-4-[(1*R*,2*E*)-1-hydroxy-hexadec-2-en-1-yl)]oxazolidine (5b) and **(4*S*,5*S*)-2,2-Diphenyl-4-hydroxymethyl-5-[(*E*)-pentadec-1-en-1-yl]-oxazolidine (5c).** Diphenylketimine (181 mg, 1 mmol) was added at room temperature to a stirred suspension of D-*erythro*-sphingosine (248 mg, 0.83 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred overnight under argon. The mixture was concentrated, and the resulting syrup was purified by flash chromatography (4:1 hexane-EtOAc) to give **5** (362 mg, 78%) as a syrup: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.16 (m, 10 H, 2 Ph), 5.88 (dt, 0.21 H, $J_{e,f} = 6.8$, $J_{d,e} = 15.4$ Hz, H-e), 5.74 (2 dt, 0.79 H, $J_{e,f} = 6.8$, $J_{d,e} = 15.4$ Hz, H-e), 5.56 (ddt, 0.21 H, $J_{d,f} < 1$, $J_{c,d} = 7.5$, $J_{d,e} = 15.4$ Hz, H-d), 5.47 (ddt, 0.56 H, $J_{d,f} < 1$, $J_{c,d} = 6.5$, $J_{d,e} = 15.4$ Hz, H-d), 5.39 (ddt, 0.23 H, $J_{d,f} < 1$, $J_{c,d} = 7.0$, $J_{d,e} = 15.4$ Hz, H-d), 4.50



Scheme 4. Reagents and conditions: 5% trifluoacetic acid solution (prepared from 49.5:49.5:1 THF-CH_2Cl_2-H_2O), rt, 10 min, 93%.

(dd, 0.21 H, $J_{b,c} = 7.2$ Hz, H-c), 4.39 (dd, 0.23 H, $J_{b,c} = 6.3$ Hz, H-c), 4.29 (dd, 0.56 H, $J_{b,c} = 4.4$ Hz, H-c), 3.93–3.88 (m, H-a, H-a′), 3.68 (dd, $J_{a,b} = 4.4$, $J_{a,a'} = 11.2$ Hz, H-a), 3.62–3.39 (m, H-a′, H-b), 2.9–2.6 (br, NH), 2.15–2.01 (m, 2 H, H-f), 1.38–1.26 (m, 22 H, 11 CH_2), 0.94 (t, 3 H, $J = 6.8$ Hz, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.26 (C=N), 144.62, 144.39, 144.11, 143.94, 139.63, 136.67 (6 quaternary C), 135.11, 133.69, 133.43 (C-e), 129.63, 129.53, 126.17 (C-d), 130.27, 128.60, 128.47, 128.44, 128.22, 128.14, 128.12, 128.02, 127.95, 127.52, 127.49, 127.32, 126.31, 125.99, 125.81, 125.51 (aromatic CH), 78.98, 74.93, 71.83 (C-c), 67.43, 62.61, 62.43 (C-b), 65.57, 64.36, 60.90 (C-a), 32.33, 32.31, 32.18 (C-f), 31.89, 29.64, 29.63, 29.57, 29.50, 29.48, 29.44, 29.33, 29.19, 29.10, 29.07, 29.01, 22.66 (CH_2), 14.10 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{31}H_{46}NO_2$ $(M+H)^+$ m/z 464.3529, found 464.3543.

(2*S*,3*R*,4*E*)-1,3-Di-acetoxy-2-[*N*-(diphenylmethylene)-amino]-4-octadecene (6). Compound **5** (6 mg, 0.013 mmol) was acetylated with Ac_2O (0.2 mL) in pyridine (0.3 mL) for 20 h at room temperature. After concentration, the residue was co-evaporated with toluene, and the resulting syrup was purified by flash chromatography to afford **6** (7 mg, 99%) as an amorphous solid. TLC (5:1 hexane-EtOAc): Rf = 0.3; $[\alpha]_D = -7.6$ (c = 0.4, chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.62–7.13 (m, 10 H, 2 Ph), 5.77 (dt, 1 H, $J_{e,f} = 6.7$, $J_{d,e} = 15.4$ Hz, H-e), 5.56 (dd, 1 H, $J_{c,d} = 8.0$ Hz, H-d), 5.39 (dd, 1 H, $J_{b,c} = 5.2$ Hz, H-c), 4.27 (dd, 1 H, $J_{a,b} = 4.5$, $J_{a,a'} = 11.0$ Hz, H-a), 4.18 (dd, 1 H, $J_{a',b} = 7.9$ Hz, H-a′), 3.86 (ddd, 1 H, H-b), 2.01–2.00 (m, 8 H, 2 H-f, 2 OAc), 1.35–1.25 (m, 22 H, 11 CH_2), 0.89 (t, 3 H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.65, 170.33, 169.73 (2 C=O, C=N), 136.98 (C-e), 124.73 (C-d), 75.59 (C-c), 65.06 (C-a), 63.14 (C-b), 32.36, 31.93, 29.68, 29.56, 29.47, 29.37, 29.17, 28.92, 22.69 (12 CH_2), 21.31 (OAc), 20.95 (OAc), 14.12 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{35}H_{50}NO_4$ $(M+H)^+$ m/z 548.3740, found 548.3749.

(2*S*,3*R*,4*E*)-1-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-[*N*-(diphenylmethylene)amino]-octadec-4-en-3-ol (8), (2*S*,3*R*,4*E*)-3-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-[*N*-(diphenylmethylene)amino]-octadec-4-en-1-ol (9) and **(2*S*,3*R*,4*E*)-1,3-Bis-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-[*N*-(diphenylmethylene)amino]-octadec-4-ene (10).** A mixture of hepta-*O*-acetyl-α-D-lactopyranosyl bromide **7** (133 mg, 0.19 mmol), acceptor **5** (79 mg, 0.17 mmol), and 4-Å powdered molecular sieves (200 mg) in dry CH_2Cl_2 (4 mL) was stirred at room temperature for 30 min. To this mixture was added AgOTf (49 mg, 0.19 mmol) portionwise over 15 min at room temperature, and the mixture was stirred overnight under argon. After the addition of Et_3N (0.1 mL), the mixture was filtered through celite and the filtrate was washed with aqueous $Na_2S_2O_3$, water and brine. After drying over $MgSO_4$ the mixture was concentrated, and the residue was flash chromatographed (1:1 hexane-EtOAc) to afford first recovered **5** (12 mg, 15%), then a mixture of **8** and **9** (120 mg, 65%). FABMS ($C_{57}H_{79}NO_{19}$): 1082.6 $(M+1)^+$. The NMR spectra were very complicated, and the confirmation of the structures was made as the corresponding acetates **13** and **14** (see below). The third fraction yielded **10** (34 mg, 12%). Rf = 0.46 (2:1 CH_2Cl_2-EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57–7.54 (m, 2 H, Ar H), 7.42–7.27 (m, 6 H, Ar H), 7.17–7.13 (m, 2 H, Ar H), 5.65 (dt, 1 H, $J_{e,f} = 7.5$, $J_{d,e} = 15.5$ Hz, H-e), 5.39 (dd, $J_{c,d} = 8.8$ Hz, H-d), 5.34 (dd, 1 H, $J_{3'A,4'A} = 2.6$, $J_{4'A,5'A} < 1$ Hz, H-4′A), 5.31 (dd, 1 H, $J_{3'B,4'B} = 2.7$, $J_{4'B,5'B} < 1$ Hz, H-4′B), 4.51–3.45 (m, 30 H), 2.18–1.97 (m, 44 H, 2 H-f, 14 OAc), 1.39–1.25 (m, 22 H, 11 CH_2), 0.89 (t, $J = 6.5$ Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.39, 170.32, 170.27, 170.14, 170.06, 169.79, 169.74, 169.64, 169.46, 169.09 (14 C=O, C=N), 140.14, 138.10, 136.68, 129.96, 128.61, 128.56, 128.15, 127.93, 125.67 (C-d, C-e, Ar C), 101.19, 101.15, 100.14, 96.33 (C-1A, C-1A′, C-1B, C-1B′), 79.56, 76.46, 76.24, 73.28, 73.12, 72.48, 72.38, 71.96, 71.49, 70.98, 70.66, 70.58, 69.13, 69.05, 66.60 (ring C, C-a, C-c), 64.17 (C-b), 62.24, 62.06, 60.77, 60.76 (C-6A, C-6A′, C-6B, C-6B′), 32.49, 31.93, 29.72, 29.67, 29.62, 29.47, 29.37, 22.69 (12 CH_2), 20.88, 20.77, 20.63, 20.50, 20.22 (14 OAc), 14.12 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{83}H_{113}NO_{36}$ $(M+H)^+$ m/z 1700.7121, found 1700.7115.

(2*S*,3*R*,4*E*)-1-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-amino-octadec-4-en-3-ol (11) and **(2*S*,3*R*,4*E*)-3-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-amino-octadec-4-en-1-ol**

(12). A mixture of **8** and **9** (32 mg, 0.0296 mmol) in 5% TFA solution (0.8 mL, prepared from 49.5:49.5:1 THF-CH_2Cl_2-H_2O) was stirred at room temperature for 10 min. The reaction mixture was diluted with CH_2Cl_2 (4 mL) and poured into saturated aqueous $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2×15 mL) and the combined organic layers were dried over $MgSO_4$. After filtration, the filtrate was concentrated to dryness. The residue was flash chromatographed (13:1 CH_2Cl_2-MeOH) to afford first **11** (15.3 mg, 56%) as a white amorphous solid. Rf = 0.30 (11:1 CH_2Cl_2-MeOH); $[\alpha]_D = -5.8$ (c = 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.73 (dt, 1 H, $J_{e,f} = 6.8$, $J_{d,e} = 15.4$ Hz, H-e), 5.42 (dd, 1 H, $J_{c,d} = 6.9$ Hz, H-d), 5.34 (dd, 1 H, $J_{3',4'} = 3.5$, $J_{4',5'} < 1$ Hz, H-4′), 5.19 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 5.11 (dd, 1 H, $J_{1',2'} = 7.9$, $J_{2',3'} = 10.3$ Hz, H-2′), 4.97 (dd, 1 H, H-3′), 4.89 (dd, 1 H, $J_{1,2} = 8.2$ Hz, H-2), 4.49 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1), 4.48 (d, 1 H, $J_{1',2'} = 7.9$ Hz, H-1′), 2.16 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.07–2.03 (m, 11 H, 2 H-f, 3 OAc), 1.97 (s, 3 H, OAc), 1.37–1.25 (m, 22 H, 11 CH_2), 0.88 (t, 3 H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.57, 170.41, 170.20, 169.99, 169.87, 169.73, 169.22 (7 C=O), 134.37 (C-e), 128.17 (C-d), 100.42, 100.20 (C-1, C-1′), 75.68, 72.51, 72.28, 71.39, 70.69, 70.13, 68.86, 66.50 (C-2,3,4,5, C-2′,3′,4′,5′, C-a, C-c), 61.60, 60.51 (C-6, C-6′), 54.40 (C-b), 31.90, 31.42, 29.17, 29.14, 29.00, 28.85, 28.80, 28.74, 22.16 (12 CH_2), 20.13, 20.01, 19.93, 19.89, 19.84, 19.74 (7 OAc), 13.38 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{44}H_{71}NO_{19}$ $(M+H)^+$ m/z 918.4699, found 918.4684.

The second fraction yielded **12** (10.7 mg, 39%) as a white amorphous solid. Rf = 0.23 (11:1 CH_2Cl_2-MeOH); $[\alpha]_D = -11.7$ (c = 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.72 (dt, 1 H, $J_{e,f} = 6.7$, $J_{d,e} = 15.4$ Hz, H-e), 5.35 (dd, 1 H, $J_{3',4'} = 3.5$, $J_{4',5'} < 1$ Hz, H-4′), 5.26 (dd, 1 H, $J_{c,d} = 7.7$ Hz, H-d), 5.18 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 5.11 (dd, 1 H, $J_{1',2'} = 8.0$, $J_{2',3'} = 10.3$ Hz, H-2′), 4.97 (dd, 1 H, H-3′), 4.88 (dd, 1 H, $J_{1,2} = 8.2$ Hz, H-2), 4.50 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1), 4.49 (d, 1 H, $J_{1',2'} = 8.0$ Hz, H-1′), 2.14 (s, 3 H, OAc), 2.13 (s, 3 H, Ac), 2.10–2.00 (m, 14 H, 2 H-f, 4 OAc), 1.96 (s, 3 H, OAc), 1.33–1.22 (m, 22 H, 11 CH_2), 0.88 (t, 3 H, $J = 7.0$ Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.48, 170.32, 170.09, 170.02, 169.69, 169.58, 169.03 (7 C=O), 137.82 (C-e), 125.57 (C-d), 101.05, 97.63 (C-1, C-1′), 76.21, 72.60, 72.56, 71.39, 70.87, 70.61, 69.00, 66.51 (C-2,3,4,5, C-2′,3′,4′,5′, C-a, C-c), 61.56, 60.73 (C-6, C-6′), 54.47 (C-b), 32.36, 31.87, 29.66, 29.63, 29.57, 29.41, 29.32, 29.24, 29.09, 22.65 (12 CH_2), 20.83, 20.77, 20.65, 20.62, 20.49 (7 OAc), 14.10 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{44}H_{71}NO_{19}$ $(M+H)^+$ m/z 918.4699, found 918.4690.

(2*S*,3*R*,4*E*)-1-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1$\rightarrow$4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-acetamido-3-acetoxy-octadec-4-ene (13). Compound **11** (2.1 mg, 0.0023 mmol) was acetylated with Ac_2O (0.3 mL) in pyridine (0.2 mL) for 13 h at room temperature. After concentration, the residue was co-evaporated with toluene and flash chromatographed (15:1 CH_2Cl_2-MeOH) to afford **13** (2.3 mg, 100%) as an amorphous solid: TLC : Rf = 0.3 (15:1 CH_2Cl_2-MeOH), or Rf = 0.35 (1:2 CH_2Cl_2-EtOAc); $[\alpha]_D = -16.9$ (c = 0.23, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.77 (dt, 1 H, $J_{e,f} = 6.7$, $J_{d,e} = 15.4$ Hz, H-e), 5.71 (d, 1 H, $J_{b,NH} = 9.1$ Hz, NH), 5.35 (dd, 1 H, $J_{c,d} = 7.7$ Hz, H-d), 5.35 (dd, 1 H, $J_{3',4'} = 3.7$, $J_{4',5'} < 1$ Hz, H-4′), 5.25 (dd, 1 H, $J_{b,c} = 6.6$ Hz, H-c), 5.19 (dd, 1 H, $J_{2,3} = 9.5$, $J_{3,4} = 9.1$ Hz, H-3), 5.11 (dd, 1 H, $J_{1',2'} = 7.9$, $J_{2',3'} = 10.4$ Hz, H-2′), 4.96 (dd, 1 H, H-3′), 4.87 (dd, 1 H, $J_{1,2} = 7.8$ Hz, H-2), 4.53 (dd, 1 H, $J_{5,6a} = 2.0$, $J_{6a,6b} = 12.0$ Hz, H-6a), 4.49 (d, 1 H, H-1′), 4.43 (d, 1 H, H-1), 4.28 (m, 1 H, H-b), 4.15–4.09 (m, 2 H, H-6′a, H-6′b), 4.06 (dd, 1 H, $J_{5,6b} = 4.7$ Hz, H-6b), 3.90–3.86 (m, 2 H, H-5′, H-a), 3.80 (dd, 1 H, $J_{4,5} = 9.9$ Hz, H-4), 3.59 (ddd, 1 H, H-5), 3.55 (dd, 1 H, $J_{a,a'} = 10.1$, $J_{a',b} = 4.3$ Hz, H-a′), 2.15 (s, 3 H, Ac), 2.13 (s, 3 H, Ac), 2.09–2.00 (m, 17 H, 2 H-f, 5 Ac), 1.96 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 1.36–1.25 (m, 22 H, 11 CH_2), 0.88 (t, 3 H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.37, 170.36, 170.14, 170.08, 169.93, 169.80, 169.70, 169.58, 169.05 (9 C=O), 137.34 (C-e), 124.54 (C-d), 101.05, 100.32 (C-1, C-1′), 75.95, 73.46, 72.66, 72.45, 71.70, 70.93, 70.68, 69.03, 66.56 (C-2,3,4,5, C-2′,3′,4′,5′, C-c), 67.11 (C-a), 61.67, 60.76 (C-6, C-6′), 50.72 (C-b), 32.29 (C-f), 31.90, 29.68, 29.67, 29.66, 29.64, 29.59, 29.45, 29.34, 29.18, 28.97, 22.67 (11 CH_2), 21.07, 20.83, 20.78, 20.76, 20.65–20.63, 20.51 (9 Ac), 14.11 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{48}H_{76}NO_{21}$ $(M+H)^+$ m/z 1002.4910, found 1002.4944.

(2*S*,3*R*,4*E*)-3-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1$\rightarrow$4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-acetamido-1-acetoxy-octadec-4-ene (14). A mixture of compound **11** and **12** (14 mg) was treated with Ac_2O (0.3 mL) in pyridine (0.6 mL) for 15 h at room temperature. TLC indicated disappearance of the starting material, and a new spot with Rf = 0.3 in 15:1 CH_2Cl_2-MeOH, but this spot could be separated into two spots using 1:2 CH_2Cl_2-EtOAc; the first one had Rf = 0.35 which corresponds the compound **13** and the second one had Rf = 0.31. After concentration, the residue was co-evaporated with toluene and purified by flash chromatography (2:3 CH_2Cl_2-EtOAc) to afford **13** (1.3 mg) and **14** (4.8 mg), together with a mixture of **13** and **14** (7.5 mg). **14**: $[\alpha]_D = -11.7$ (c = 0.48, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.02 (d, 1 H, $J_{b,NH} = 9.0$ Hz, NH), 5.74 (dt, 1 H, $J_{e,f} = 6.8$, $J_{d,e} = 15.5$ Hz, H-e), 5.38 (dd, 1 H, $J_{3',4'} = 3.4$, $J_{4',5'} = 0.8$ Hz, H-4′), 5.33 (dd, 1 H, $J_{c,d} = 7.3$ Hz, H-d), 5.21 (dd, 1 H, $J_{2,3} = 9.6$, $J_{3,4} = 9.1$ Hz, H-3), 5.14 (dd, 1 H, $J_{1',2'} = 7.9$, $J_{2',3'} = 10.4$ Hz, H-2′), 4.99 (dd, 1 H, H-3′), 4.94 (dd, 1 H, $J_{1,2} = 8.0$ Hz, H-2), 4.54 (dd, 1 H, $J_{5,6a} = 2.1$, $J_{6a,6b} = 11.8$ Hz, H-6a), 4.52 (d, 1 H, H-1′), 4.46 (d, 1 H, H-1), 4.28 (m, 1 H, H-b), 4.23–4.09 (m, 6 H, H-a, H-a′, H-6b, H-c, H-6′a, H-6′b), 3.91 (dt, $J_{5',6'a} = J_{5',6'b} = 7.0$ Hz, H-5′), 3.83 (dd, $J_{4,5} = 9.8$ Hz, H-4), 3.58 (ddd, 1 H, $J_{5,6b} = 5.0$ Hz, H-5),

2.19 (s, 3 H, Ac), 2.17 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 2.09–2.06 (m + 4 s, 14 H, H-f, H-f′, 4 Ac), 2.01 (s, 3 H, Ac), 2.00 (s, 3 H, Ac), 1.31–1.28 (m, 22 H, 11 CH_2), 0.92 (t, 3 H, $J = 7.0$ Hz, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.97, 170.37, 170.33, 170.14, 170.09, 169.75, 169.74, 169.52, 169.03 (9 C, 9 C=O), 136.93 (C-e), 124.84 (C-d), 101.04 (C-1′), 97.97 (C-1), 80.23 (C-c), 72.67 (2 C, C-3, C-5), 71.34 (C-2), 70.91 (C-3′), 70.67 (C-5′), 69.06 (C-2′), 66.54 (C-4′), 62.08, 61.63, 60.76 (C-a, C-6, C-6′), 51.32 (C-b), 32.31 (C-f), 31.91, 29.69, 29.68, 29.66, 29.64, 29.59, 29.45, 29.35, 29.24, 29.07, 22.68 (11 CH_2), 23.22, 20.83, 20.82, 20.80 (4 Ac), 20.65 (4 C, 4 Ac), 20.52 (Ac), 14.12 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{48}H_{76}NO_{21}$ $(M+H)^+$ m/z 1002.4910, found 1002.4892.

(2*S*,3*R*,4*E*)-1,3-Bis-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-2-amino-octadec-4-ene (15). A mixture of **10** (25 mg, 0.0147 mmol) in 5% TFA solution (0.6 mL, prepared from 49.5:49.5:1 THF-CH_2Cl_2-H_2O) was stirred at room temperature for 30 min. The reaction mixture was diluted with CH_2Cl_2 (2 mL) and poured into saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were dried over $MgSO_4$. After filtration, the filtrate was evaporated to dryness. The residue was flash chromatographed (12:1 CH_2Cl_2-MeOH) to afford first **15** (21 mg, 93%) as a white amorphous solid. Rf = 0.45 (11:1 CH_2Cl_2-H_2O); $[\alpha]_D = -14.6$ (c = 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.68 (dt, 1 H, $J_{e,f} = 6.7$, $J_{d,e} = 15.4$ Hz, H-e), 5.35 (2 dd, 2 H, $J_{3',4'} = 3.5$, $J_{4',5'} < 1$ Hz, H-4A′, H-4B′), 5.24 (dd, 1 H, $J_{c,d} = 8.2$ Hz, H-d), 5.18, 5.16 (2 dd, 2 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3A, H-3B), 5.11, 5.10 (2 dd, 2 H, $J_{1',2'} = 8.0$, $J_{2',3'} = 10.5$ Hz, $J_{1',2'} = 7.8$, $J_{2',3'} = 10.5$ Hz, H-2A′, H-2B′), 4.96 (2 dd, 2 H, H-3A′, H-3B′), 4.88 (dd, 1 H, $J_{1,2} = 8.0$ Hz) and 4.87 (dd, 1 H, $J_{1,2} = 8.1$ Hz) (H-2A and H-2B), 4.49, 4.48, 4.47, 4.44 (4 d, 4 H, H-1A, H-1A′, H-1B, H-1B′), 2.14 (s, 6 H, 2 OAc), 2.13 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.09–2.01 (m, 26 H, 2 H-f, 8 OAc), 1.96 (s, 6 H, OAc), 1.39–1.25 (m, 22 H, 11 CH_2), 0.88 (t, 3 H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.35, 170.29, 170.09, 170.02, 169.71, 169.51, 169.01 (14 C=O), 138.18 (C-e), 124.80 (C-d), 101.07, 101.06, 100.63, 97.14 (C-1A, C-1A′, C-1B, C-1B′), 80.27, 76.34, 76.21, 72.83, 72.78, 72.57, 72.06, 71.74, 71.58, 70.95, 70.64, 69.08, 66.59 (C-2A,3A,4A,5A, C-2A′,3A′,4A′,5A′, C-2B,3B,4B,5B, C-2B′,3B′,4B′,5B′, C-a, C-c), 61.96, 60.75 (C-6A, C-6A′, C-6B, C-6B′), 53.77 (C-b), 32.41, 31.87, 29.65, 29.61, 29.41, 29.31, 29.20, 22.64 (12 CH_2), 20.84, 20.77, 20.70, 20.67, 20.59, 20.46 (14 OAc), 14.08 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{70}H_{106}NO_{36}$ $(M+H)^+$ m/z 1536.6495, found 1536.6504.

(2*S*,3*R*,4*E*)-1-[β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]-2-amino-octadec-4-en-3-ol (1). NaOMe (2 mg) was added to a solution of **11** (16 mg, 0.0174 mmol) in MeOH-CH_2Cl_2 (3:1, 1 mL) at room temperature under argon. The mixture was stirred at room temperature for 15 h. The reaction mixture was neutralized with Amberlite IR-120 resin (H^+), filtered and evaporated under reduced pressure. The residue was purified by passing through a Sephadex LH-20 column (1:1 CH_2Cl_2-MeOH) to afford **1** (10.5 mg, 96%) as a white amorphous solid. Rf = 0.53 (5:4:1 CH_2Cl_2-MeOH-H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ 5.83 (dt, 1 H, $J_{e,f} = 6.7$, $J_{d,e} = 15.3$ Hz, H-e), 5.45 (dd, 1 H, $J_{c,d} = 6.6$ Hz, H-d), 4.32, 4.31 (2 d, 2 H, $J = 7.9$ Hz, $J = 7.8$ Hz, H-1, H-1′), 4.27 (m, 1 H, H-c), 3.96–3.26 (m, 15 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6′a, H-6′b, H-a, H-a′, H-b), 2.07 (m, 2 H, 2 H-f), 1.41–1.22 (m, 22 H, 11 CH_2), 0.87 (t, 3 H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ 136.64 (C-e), 128.52 (C-d), 105.11, 103.82 (C-1, C-1′), 80.42, 77.14, 76.61, 76.29, 74.83, 74.54, 72.52, 71.19, 70.28 (C-2,3,4,5, C-2′,3′,4′,5′, C-c), 67.50 (C-a), 62.52, 61.66 (C-6, C-6′), 56.63 (C-b), 33.37, 33.06, 30.78, 30.75, 30.63, 30.46, 30.39, 30.19, 23.73 (12 CH_2), 14.43 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{30}H_{58}NO_{12}$ $(M+H)^+$ m/z 624.3959, found 624.3937.

(2*S*,3*R*,4*E*)-3-[β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]-2-amino-octadec-4-en-1-ol (2). Reaction was carried out in a similar manner as that described for **1**, using **12** (15 mg) to provide **2** (10 mg, 98%); TLC: Rf = 0.37 (5:4:1 CH_2Cl_2-MeOH-H_2O); $[\alpha]_D = -24.6$ (c = 1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.97 (dt, 1 H, $J_{e,f} = 6.8$, $J_{d,e} = 15.4$ Hz, H-e), 5.39 (dd, 1 H, $J_{c,d} = 7.7$ Hz, H-d), 4.51 (dd, 1 H, $J_{b,c} = 3.7$ Hz, H-c), 4.33 (2 d, 2 H, $J = 7.7$ Hz, H-1, H-1′), 3.96–3.29 (m, 15 H, H-2, H-3, H-4, H-5, 2 H-6, H-2′, H-3′, H-4′, H-5′, 2 H-6′, 2 H-a, H-b), 2.13 (m, 2 H, 2 H-f), 1.46–1.22 (m, 22 H, 11 CH_2), 0.90 (t, 3 H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ 140.38 (C-e), 124.28 (C-d), 105.11, 100.42 (C-1, C-1′), 80.54, 77.14, 76.88, 76.56, 76.35, 74.83, 74.42, 72.50, 70.27 (C-2,3,4,5, C-2′,3′,4′,5′, C-c), 62.52, 61.76 (C-6, C-6′), 59.64 (C-a), 56.63 (C-b), 33.40, 33.06, 30.79, 30.75, 30.59, 30.45, 30.36, 30.04, 23.72 (12 CH_2), 14.43 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{30}H_{58}NO_{12}$ $(M+H)^+$ m/z 624.3959, found 624.3954.

(2*S*,3*R*,4*E*)-1,3-Bis-[β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]-2-amino-octadec-4-ene (3). Reaction was carried out similarly to that described for **1**, except that MeOH-CH_2Cl_2 (7:3) was used as solvent. Thus 15 mg of compound **15** gave 9.2 mg of **3** (98%); TLC: Rf = 0.2 (3:3:2 EtOAc-iPrOH-H_2O); $[\alpha]_D = -23.3$ (c = 1 , MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 6.00 (dt, 1 H, $J_{e,f} = 6.8$, $J_{d,e} = 15.4$ Hz, H-e), 5.41 (dd, 1 H, $J_{c,d} = 7.7$ Hz, H-d), 4.53 (m, 1 H, H-c), 4.37–4.32 (4 d, 4 H, $J = 7.7$ Hz, $J = 8.0$ Hz, H-1A, H-1A′, H-1B, H-1B′), 2.13 (m, 2 H, 2 H-f), 1.46–1.22 (m, 22 H, 11 CH_2), 0.90 (t, 3 H, $J = 6.8$ Hz, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ 140.17 (C-e), 123.82 (C-d), 104.85, 104.85, 103.56, 100.31 (C-1A, C-1A′, C-1B, C-1B′), 80.29, 80.18, 76.88, 76.67, 76.38, 76.08, 74.56, 74.23, 74.14, 72.23, 70.00 (C-2A,3A,4A,5A, C-2A′,3A′,4A′,5A′, C-2B,3B,4B,5B,

C-2B′,3B′,4B′,5B′, C-c), 67.19 (C-a), 62.26, 60.75 (C-6A, C-6A′, C-6B, C-6B′), 55.52 (C-b), 33.16, 32.17, 30.54, 30.49, 30.32, 30.19, 30.14, 29.77, 23.45 (12 CH_2), 14.15 (CH_3).

HRMS (FAB$^+$): Calcd for $C_{42}H_{78}NO_{22}$ $(M+H)^+$ m/z 948.5015, found 948.5002.

Acknowledgment

This work was supported in part by a NIH grant (CA80054-02).

References

1 Hakomori S, Bifunctional role of glycosphingolipids: Modulators for transmembrane signaling and mediators for cellular interactions, *J Biol Chem* **265**, 18713–16 (1990).
2 Iwabuchi K, Yamamura S, Prinetti A, Handa K, Hakomori S, GM3-enriched microdomain involved in cell adhesion and signal transduction through carbohydrate-carbohydrate interaction in mouse melanoma B16 cells, *J Biol Chem* **273**, 9130–38 (1998).
3 Iwabuchi K, Handa K, Hakomori S, Separation of "glycosphingolipid signaling domain" from caveolin-containing membrane fraction in mouse melanoma B16 cells and its role in cell adhesion coupled with signaling, *J Biol Chem* **273**, 33766–73 (1998).
4 Nores GA, Dohi T, Taniguchi M, Hakomori S, Density-dependent recognition of cell surface GM3 by a certain anti-melanoma antibody, and GM3 lactone as a possible immunogen: Requirements for tumor-associated antigen and immunogen, *J Immunol* **139**, 3171–76 (1987).
5 Kojima N, Shiota M, Sadahira Y, Handa K, Hakomori S, Cell adhesion in a dynamic flow system as compared to static system: Glycosphingolipid-glycosphingolipid interaction in the dynamic system predominates over lectin- or integrin-based mechanisms in adhesion of B16 melanoma cells to non-activated endothelial cells, *J Biol Chem* **267**, 17264–70 (1992).
6 Zhang Y, Iwabuchi K, Nunomura S, Hakomori S, Effect of synthetic sialyl $2 \rightarrow 1$ sphingosine and other glycosylsphingosines on the structure and function of the "glycosphingolipid signaling domain (GSD)" in mouse melanoma B16 cells, *Biochemistry* **39**, 2459–68 (2000).
7 Chatterjee S, Lactosylceramide stimulates aortic smooth muscle cell proliferation, *Biochem Biophys Res Commun* **181**, 554–61 (1991).
8 Bhunia AK, Han H, Snowden A, Chatterjee S, Lactosylceramide stimulates Ras-GTP loading, kinases (MEK, Raf), p44 mitogen-activated protein kinase, and c-fos expression in human aortic smooth muscle cells, *J Biol Chem* **271**, 10660–66 (1996).
9 Iwabuchi K, Zhang Y, Handa K, Withers DA, Sinaÿ P, Hakomori S, Reconstitution of membranes simulating "glycosignaling domain" and their susceptibility to lyso-GM3, *J Biol Chem* **275**, 15174–81 (2000).
10 Symington FW, CDW17—A neutrophil glycolipid antigen regulated by activation, *J Immunol* **142**, 2784–90 (1989).
11 Lund-Johansen F, Olweus J, Horejsi V, Skubitz KM, Thompson JS, Vilella R, Symington FW, Activation of human phagocytes through carbohydrate antigens (CD15, Sialyl-CD15, CDW17, and CDW65), *J Immunol* **148**, 3221–29 (1992).
12 Schmidt RR, Zimmermann P, Synthesis of glycosphingolipids and psychosines, *Angew Chem Int Ed Engl* **25**, 725–26 (1986).
13 Schmidt RR, Bär T, Apell HJ, Lactosylceramides with unsaturated fatty acids—synthesis and use in the generation of bilayer membranes, *Angew Chem Int Ed Engl* **26**, 793–94 (1987).
14 Zimmermann P, Bommer R, Bär T, Schmidt RR, Azidosphingosine glycosylation in glycosphingolipid synthesis, *J Carbohydr Chem* **7**, 435–52 (1988).
15 Numata M, Sugimoto M, Shibayama S, Ogawa T, A total synthesis of hematoside, α-NeuGc-$(2 \rightarrow 3)$-β-Gal-$(1 \rightarrow 4)$-β-Glc-$(1 \rightarrow 1)$-Cer, *Carbohydr Res* **174**, 73–85 (1988).
16 Terada T, Kiso M, Hasegawa K, Synthesis of KDN-lactotetraosylceramide, KDN-neolactotetraosylceramide, and KDN-Lewis X ganglioside, *Carbohydr Res* **259**, 201–18 (1994).
17 Ehara T, Kameyama A, Yamada Y, Ishida H, Kiso M, Hasegawa A, Total synthesis of VIM-2 ganglioside isolated from human chronic myelogenous leukemia cells, *Carbohydr Res* **281**, 237–52 (1996).
18 Gege C, Oscarson S, Schmidt RR, Synthesis of fluorescence labeled sialyl Lewis^x glycosphingolipids, *Tetrahedron Lett* **42**, 377–80 (2001).
19 Bilodeau MT, Park TA, Hu S, Randolph JT, Danishefsky SJ, Livingston PO, Zhang S, Total synthesis of a human breast tumor associated antigen, *J Am Chem Soc* **117**, 7840–41 (1995).
20 Murakami T, Taguchi K, Stereocontrolled synthesis of novel phytosphingosine-type glucosaminocerabrosides, *Tetrahedron* **55**, 989–1104 (1999).
21 Nicolaou KC, Li J, Zenke G, Total synthesis and biological evaluation of glycolipids plakosides A, B and their analogs, *Helv Chim Acta* **83**, 1977–2006 (2000).
22 Ruan F, Yamamura S, Hakomori S, Igarashi Y, Synthesis of sphingosine conjugate with controlled pore glass beads, *Tetrahedron Lett* **36**, 6615–18 (1995).
23 O'Donnell MJ, Polt RL, A mild and efficient route to schiff base derivatives of amino acids, *J Org Chem* **47**, 2663–66 (1982).
24 Garner P, Park JM, Malecki E, A stereodivergent synthesis of *D*-erythro-sphingosine and *D*-threo-sphingosine from *L*-serine, *J Org Chem* **53**, 4395–98 (1988); Polt R, Szabo L, Treiberg J, Li Y, Hruby VJ, General methods for α- or β-*O*-Ser/Thr glycosides and glycopeptides. Solid-phase synthesis of *O*-glycosyl cyclic enkephalin analogues, *J Am Chem Soc* **114**, 10249–58 (1992).
25 Hudson CS, Kunz A, Relations between rotatory power and structure in the sugar group. X. The chloro-, bromo- and iodo-acetyl derivatives of lactose, *J Am Chem Soc* **47**, 2052–55 (1925).
26 Peterson MA, Polt R, *N*-Diphenylmethylene-protected glycosyl acceptors. Selective β-*O*-glycosylation to form lactosyl-threo-ceramides, *J Org Chem* **58**, 4309–14 (1993).

Received 5 March 2002; revised 20 May 2002;
accepted 22 May 2002