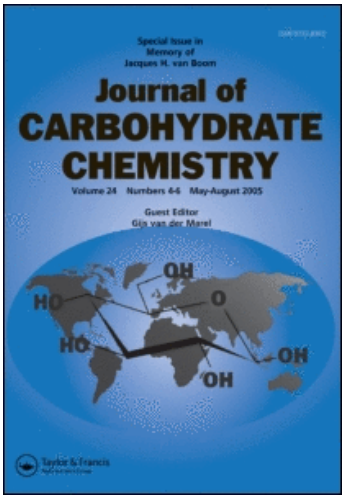

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STUDIES ON THE THIOGLYCOSIDES OF *N*-ACETYL-NEURAMINIC ACID 8: SYNTHESIS OF S-(α-SIALYL)-(2→6)-β-HEXOPYRANOSYL AND -(2→6')-β-LACTOSYL CERAMIDES CONTAINING β-THIOGLYCOSIDICALLY LINKED CERAMIDE

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ABSTRACT

Coupling of the sodium salt of *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-*galacto*-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-*O*-acetyl-1,6-dithio-β-D-glucopyranose (**5**), -β-D-galactopyranose (**8**), or *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-*O*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranose (**12**), which were prepared from the corresponding 1-hydroxy compounds, **1**, **2**, and **9**, *via* 1-chlorination, displacement with thioacetyl group, and *S*-deacetylation, with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-1-*O*-(p-toluenesulfonyl)-4-octadecene-1,3-diol (**13**), gave the corresponding β-thioglycosides **14**, **18** and **22**, respectively in good yields. The β-thioglycosides obtained were converted, *via* selective reduction of the azide group, condensation with octadecanoic acid, and removal of the protecting groups, into the title compounds.

INTRODUCTION

There has been a great deal of activity in recent years in the synthesis of glycolipids such as gangliosides and glycosphingolipids. These compounds are of interest, not only from the point of view of chemistry involved, but also for their various types of functions[1-6] (cell growth, differentiation, adhesion, oncogenesis, and receptor functions for viruses and bacterial toxins).

In view of these facts, we have synthesized a series of gangliosides[7-10] and their analogs[11,12] in order to elucidate the functions of the glycolipids at the molecular level, and have observed that the ganglioside analogs containing α–thioglycosides of sialic acid are potent inhibitors[13] of sialidase activities of different subtypes of influenza viruses. As part of a program on the synthesis of sialoglycoconjugates containing the thioglycoside linkages in the molecules, we describe here the synthesis of *S*-(α–*N*-acetylneuraminyl)-(2→6)-β-hexopyranosyl and *S*-(α–*N*-acetylneuraminyl)-(2→6')-β-lactosyl ceramides containing β-thioglycosidically linked ceramide.

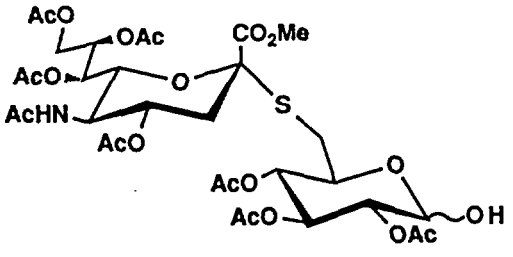
RESULTS AND DISCUSSION

For the synthesis of the target thioanalogs of sialyl glycosphingolipids, we set out to prepare the sodium salts of the per-*O*-acetylated-*S*–α–sialyl-(2–6)-1,6-dithio-β-hexopyranoses (**5** and **8**) and -(2-6')-1,6'-dithio-β-lactose (**12**) as the glycosyl donors, for coupling with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-1-*O*-(p-toluenesulfonyl)-4-octadecene-1,3-diol[12c] (**13**). The intermediates could then, by introduction of the ceramide moiety, be transformed to the end products.

Treatment of *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*–α–D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-*O*-acetyl-6-thio-D-glucopyranose[12b] (**1**) with methanesulfonyl chloride in dichloromethane in the presence of 2,4,6-collidine for 1 h at -15 °C, gave the 1-chloro derivative **3**, which was converted to the desired β-thioacetate **4** in 58% yield by the action of potassium thioacetate in acetone. Significant signals in the ^{1}H NMR spectrum of **4** were ten three-proton singlets at δ 1.87 (*N*-acetyl), 2.00, 2.01, 2.03, 2.05, 2.09, 2.13, 2.14 (7*O*-acetyl), 2.38 (*S*-acetyl), and 3.82 (methyl ester) and a one-proton doublet at δ 5.22 ($J_{1,2}$ = 10.3 Hz, H-1, Glc unit). Other ^{1}H NMR data are given in the Experimental consistent with the structure assigned.

By essentially the same way described for **4**, compounds **2**[12b] and **9**[12b] were transformed to the corresponding β-1-thioacetate derivatives **7** and **11** in 56 and 53% yields, *via* 1-chlorination and subsequent displacement with thioacetate.

Treatment of the sodium salt **5**, freshly derived from **4** by selective *S*-deacetylation with sodium methoxide in methanol at -30 °C, with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-1-*O*-(p-toluenesulfonyl)-4-octadecene-1,3-diol (**13**) in *N*,*N*-dimethylformamide under a nitrogen atmosphere overnight at 45 °C, yielded *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*–α–D-*galacto*-2-nonulopyranosylonate)-(2→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-benzoyloxy-4-octadecene-1-thiol (**14**) in 39% yield, after column



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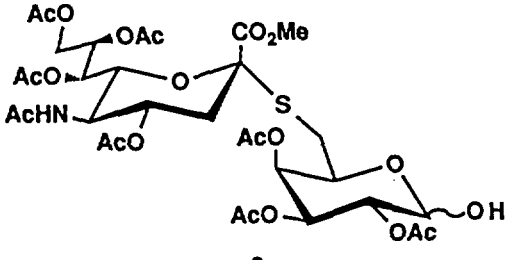

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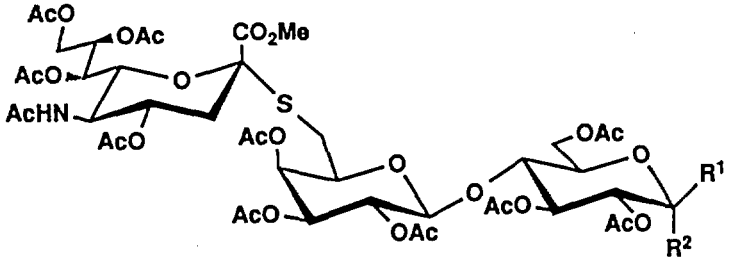
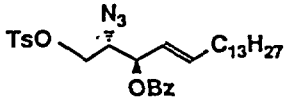
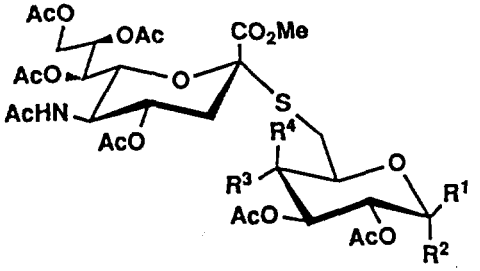




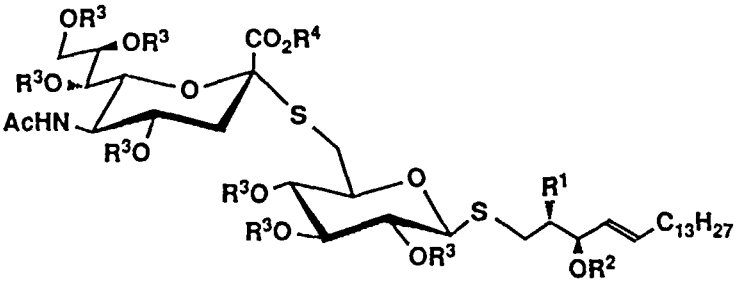
13

Ts = *p*-toluenesulfonyl
Bz = benzoyl

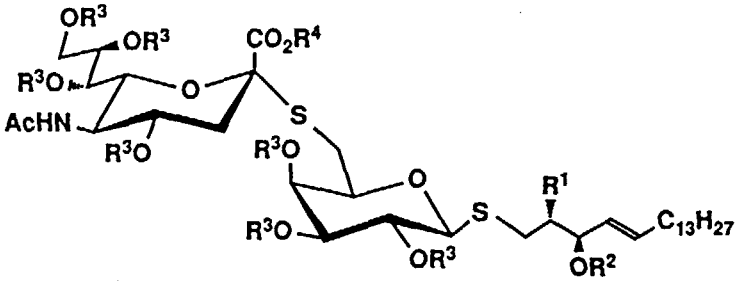


3 R^1=R^4=H, R^2=Cl, R^3=OAc
4 R^1=SAc, R^2=R^4=H, R^3=OAc
5 R^1=SNa, R^2=R^4=H, R^3=OAc
6 R^1=R^3=H, R^2=Cl, R^4=OAc
7 R^1=SAc, R^2=R^3=H, R^4=OAc
8 R^1=SNa, R^2=R^3=H, R^4=OAc

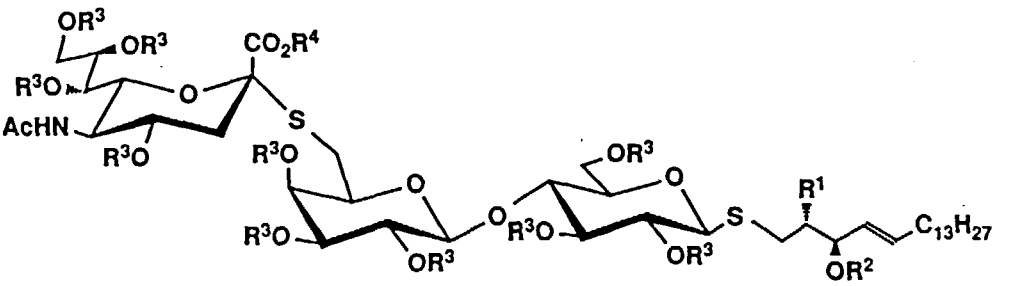
9 R^1,R^2=H, OH
10 R^1=H, R^2=Cl
11 R^1=SAc, R^2=H
12 R^1=SNa, R^2=H



14 R^1=N_3, R^2=Bz, R^3=Ac, R^4=Me
15 R^1=NH_2, R^2=Bz, R^3=Ac, R^4=Me
16 R^1=$NHCO(CH_2)_{16}Me$, R^2=Bz, R^3=Ac, R^4=Me
17 R^1=$NHCO(CH_2)_{16}Me$, R^2=R^3=R^4=H



18 R^1=N_3, R^2=Bz, R^3=Ac, R^4=Me
19 R^1=NH_2, R^2=Bz, R^3=Ac, R^4=Me
20 R^1=$NHCO(CH_2)_{16}Me$, R^2=Bz, R^3=Ac, R^4=Me
21 R^1=$NHCO(CH_2)_{16}Me$, R^2=R^3=R^4=H



22 R^1=N_3, R^2=Bz, R^3=Ac, R^4=Me
23 R^1=NH_2, R^2=Bz, R^3=Ac, R^4=Me
24 R^1=$NHCO(CH_2)_{16}Me$, R^2=Bz, R^3=Ac, R^4=Me
25 R^1=$NHCO(CH_2)_{16}Me$, R^2=R^3=R^4=H

chromatography. The structure of **14** was unambiguously proved by 270 MHz ^1H NMR spectroscopy. The observed signals exhibited nine sharp singlets, each integrating for three protons, which demonstrated the presence of the following groups: one-*N*-acetyl (δ 1.87), seven *O*-acetyl (δ 2.00-2.12), and one methyl ester (δ 3.75). H-1 appeared at δ 4.59 ($J_{1,2}$ = 10.1 Hz), indicating the β-configuration of the newly formed glycosidic linkage.

According to the same way described for **14**, coupling of the sodium salts **8** and **12**, derived from the corresponding 1-*S*-acetyl derivatives **7** and **11** by selective *S*-deacetylation, with **13** afforded the expected β-glycosides **18** and **22** in 44 and 38% yields. Selective reduction[12a,14] of the azide group in **14** with hydrogen sulfide in aqueous 83% pyridine gave the amine **15**, which, on condensation with octadecanoic acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) in dichloromethane, gave the fully protected desired product **16** in 81% yield.

By essentially the same way just described above, selective reduction of the azide group in compounds **18** and **22**, and subsequent condensation with octadecanoic acid afforded the corresponding products **20** and **24** in high yields, respectively.

Finally, *O*-deacetylation of compounds **16**, **20**, and **24** with sodium methoxide in methanol, and subsequent saponification of the methyl ester group yielded almost quantitatively the end products **17**, **21**, and **25**, respectively.

EXPERIMENTAL

General Procedures. Specific rotations were determined with a Union PM-201 polarimeter at 25 °C, and IR spectra were recorded with a Jasco A-100 spectrophotometer. ^1H NMR spectra were recorded with a Jeol JNM-GX 270 spectrometer. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-β-D-glucopyranose (4). To a solution of *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-*O*-acetyl-6-thio-D-glucopyranose[12b] (**1**; 500 mg, 0.63 mmol) in dichloromethane (5 mL) was added 2,4,6-collidine (0.5 mL), and cooled to -15 °C. Methanesulfonyl chloride (0.25 mL) was added to the mixture, the mixture was stirred for 20 min at -15 °C, then for 1 h at room temperature, the progress of the reaction being monitored by TLC. Dichloromethane (50 mL) was added and the

solution was successively washed with 2M hydrochloric acid and water, dried (Na_2SO_4), and concentrated to the crude **3**, which was used for the next reaction without further purification. To a solution of **3** in acetone (10 mL) was added Drierite (2 g), and the mixture was stirred for 2 h at room temperature, then potassium thioacetate (430 mg) was added. The mixture was stirred overnight at 45 °C, filtered and the precipitate was washed with dichloromethane. The filtrate and washings were combined and concentrated to a syrup. The residue was chromatographed on a column of silica gel with 120:1 dichloromethane-methanol, to give **4** (310 mg, 58%) as an amorphous mass; $[\alpha]_D$ +38.0° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) Glc unit δ 2.38 (s, 3H, AcS), 2.88 (m, 2H, H-6,6'), 4.96 (t, 1H, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3), 5.07 (dd, 1H, H-2), and 5.22 (d, 1H, $J_{1,2} = 10.3$ Hz, H-1); Neu5Ac unit δ 1.87 (s, 3H, AcN), 2.71 (dd, 1H, $J_{3a,3e} = 12.5$ Hz, $J_{3e,4} = 4.2$ Hz, H-3e), 3.82 (s, 3H, MeO), 4.28 (dd, 1H, $J_{9,9'} = 12.8$ Hz, H-9), 4.87 (m, 1H, H-4), and 5.30 (m, 2H, H-7,8); *O*-acetyl groups δ 2.00, 2.01, 2.03, 2.05, 2.09, 2.13, and 2.14 (7s, 21H, 7AcO).

Anal. Calcd for $C_{34}H_{47}NO_{20}S_2$ (853.9): C, 47.82; H, 5.55; N, 1.64. Found: C, 47.69; H, 5.58: N, 1.53.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-β-D-galactopyranose (7). A solution of *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-*O*-acetyl-6-thio-galactopyranose[12b] (**2**; 1.44 g, 1.8 mmol) in dichloromethane (14 mL) and 2,4,6-collidine (1.4 mL) was cooled to -15 °C and methanesulfonyl chloride (0.7 mL) was dropwise added with stirring; the stirring was continued for 30 min at -15 °C, then for 2 h at room temperature. The mixture was extracted with dichloromethane, and the solution was successively washed with 2M hydrochloric acid and water, dried (Na_2SO_4), and concentrated.

The residue **6** was dissolved in acetone (30 mL), Drierite (3 g) was added, and the mixture was stirred for 1 h at room temperature and overnight at 45 °C. Processing described in the preparation of **4** gave compound **7** (870 mg, 56%) as an amorphous mass; $[\alpha]_D$ +17.8° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) Gal unit δ 2.38 (s, 3H, AcS), 2.64 (dd, 1H, $J_{5,6} = 7.5$ Hz, $J_{5',6} = 6.6$ Hz, $J_{6,6'} = 14.3$ Hz H-6), 2.87 (dd, 1H, H-6'), 5.21(dd, 1H, $J_{2,3} = 9.7$ Hz, $J_{3,4} = 3.1$ Hz, H-3); 5.29 (near t, $J_{2,3} = 9.7$ Hz, H-2), 5.42 (d, 1H, $J_{1,2} = 9.9$ Hz, H-1), and 5.59 (d, 1H, H-4); Neu5Ac unit δ 1.89 (s, 3H, AcN), 2.72 (dd, 1H, $J_{3a,3e} = 12.6$ Hz, $J_{3e,4} = 4.6$ Hz, H-3e), 4.15 (dd, 1H, $J_{8,9} = 3.3$ Hz, $J_{9,9'} = 11.9$ Hz, H-9), 4.46 (dd, 1H, H-9'), 4.91 (ddd, 1H, $J_{3a,4} = 11.5$ Hz, $J_{4,5} = 10.3$ Hz, H-4), and 5.31 (m, 2H, H-7,8); *O*-acetyl groups δ 1.97, 2.03(2), 2.04, 2.15, 2.17, and 2.19 (7s, 21H, 7AcO).

Anal. Calcd for $C_{34}H_{47}NO_{20}S_2$ (853.9): C, 47.82; H, 5.55; N, 1.64. Found: C, 47.69; H, 5.63; N, 1.63.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-(2,3,4-tri-O-acetyl-6-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-1-S-acetyl-1-thio-β-D-glucopyranose (11). Treatment of **9**[12b] (500 mg, 0.46 mmol) with methanesulfonyl chloride (0.3 mL, 3.88 mmol) in dichloromethane (6 mL) in the presence of 2,4,6-collidine (0.6 mL), and subsequent replacement of the 1-chloro derivative **10** with potassium thioacetate (315 mg, 2.76 mmol) in acetone (10 mL), as described for **4**, gave compound **11** (279 mg, 53%) as an amorphous mass; $[\alpha]_D$ +0.26° (*c* 0.75, $CHCl_3$); ^1H NMR ($CDCl_3$) lactose unit δ 2.37 (s, 3H, AcS), 2.55 (dd, 1H, $J_{5',6'a}$ = 7.2 Hz, $J_{6'a,6'b}$ = 14.5 Hz, H-6'a), 2.81 (dd, 1H, $J_{5',6'b}$ = 7.2 Hz, H-6'b), 4.70 (d, 1H, $J_{1',2'}$ = 7.3 Hz, H-1'), 5.00 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 2.9 Hz, H-3'), 5.06 (dd, 1H, $J_{1,2}$ = 10.4 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 5.07 (t, 1H, H-3), 5.23 (d, 1H, H-1), and 5.52 (broad d, 1H, H-4'); Neu5Ac unit δ 1.90 (s, 3H, AcN), 2.73 (dd, 1H, $J_{3e,4}$ = 4.6 Hz, $J_{3a,3e}$ = 12.8 Hz, H-3e), 3.86 (s, 3H, MeO), 4.26 (dd, 1H, $J_{8,9}$ = 2.9 Hz, $J_{9,9'}$ = 12.4 Hz, H-9), 4.47 (dd, 1H, H-9'), 4.93 (m, 1H, H-4), 5.23 (m, 1H, H-8), and 5.39 (d, 1H, $J_{NH,5}$ = 8.1 Hz, NH); *O*-acetyl groups δ 1.94, 2.03(2), 2.04, 2.05(2), 2.08, 2.14, 2.18, and 2.19 (10 s, 30H, 10AcO).

Anal. Calcd for $C_{46}H_{63}NO_{28}S_2$ (1142.1): C, 48.37; H, 5.56; N; 1.23. Found: C, 48.20; H, 5.69; N, 1.25.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→1)-(2R,3R,4E)-2-azido-3-benzoyloxy-4-octadecene-1-thiol (14). To a stirred solution of **4** (300 mg, 0.35 mmol) in dry methanol (2 mL), cooled to -30 °C, was added a solution of sodium metal (8 mg) in dry methanol (0.3 mL). Stirring was continued for 5 min at -30 °C, and the mixture was concentrated to give **5** as an amorphous mass, which was used for the next reaction without purification. A solution of **5** and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-1-(*p*-toluenesulfonyl)-4-octadecene-1,3-diol[12c] (**13**; 410 mg, 0.7 mmol) in dry *N,N*-dimethylformamide (DMF; 4.5 mL) was stirred overnight at 45 °C under nitrogen. Acetic anhydride (2 mL) and pyridine (4 mL) were added to the mixture, which was stirred overnight at room temperature, and concentrated. The residue was chromatographed on a column of silica gel (50 g) with 100:1 dichloromethane-methanol, to give **14** (165 mg, 38.5%) as an amorphous mass; $[\alpha]_D$ -6.4° (*c* 0.95, $CHCl_3$); IR (KBr) 3500-3400 (NH), 2950 and 2850 (Me, methylene), 2100 (N_3), 1750 and 1230 (ester), 1670 and 1550 (amide), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) Glc unit δ 2.85 - 2.92 (m, 2H, H-

6,6'), 4.59 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 4.98 (near t, 1H, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3) 5.01 (dd, 1H, H-2), and 5.24 (dd, 1H, H-4); Neu5Ac unit δ 1.87 (s, 3H, AcN), 2.60 (dd, 1H, $J_{3a,3e}$ = 13.0 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.75 (s, 3H, MeO), 4.10, 4.27 (dd, 2H, H-9,9'), 4.83 (ddd, 1H, $J_{3a,4}$ = 11.2 Hz, $J_{4,5}$ = 10.6 Hz, H-4), and 5.29 (m, 2H, H-7,8); sphingosine unit δ 0.88 (t, 3H, MeCH$_2$), 1.24 (s, 22H, 11 CH$_2$), 2.63 (dd, 1H, $J_{1,1'}$ = 14.5 Hz, $J_{1,2}$ = 8.8 Hz, H-1), 2.85-2.92 (m, 1H, H-1'), 5.55 (dd, 1H, $J_{3,4}$ = 8.1 Hz, $J_{4,5}$ = 15.4 Hz, H-4), 5.68 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-3), 5.97 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.7 Hz, H-5), and 7.44 - 8.10 (m, 5H, Ph); *O*-acetyl groups δ 2.00, 2.03, 2.04, 2.05, 2.10, and 2.12 (2) (7s, 21H, 7AcO).

Anal. Calcd for $C_{57}H_{82}N_4O_{11}S_2$ (1223.4): C, 55.96; H, 6.76; N, 4.58. Found: C, 55.90; H, 6.73; N, 4.60.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→1)-(2R,3R,4E)-3-benzoyloxy-2-octadecanamido-4-octadecene-1-thiol (16). Hydrogen sulfide was bubbled through a solution of **14** (165 mg, 0.135 mmol) in pyridine (10 mL) and water (2 mL) for 3 days while the solution was stirred at 0-10 °C. The mixture was concentrated to give the amine **15**, which was used for the next reaction without purification. To a solution of **15** in dichloromethane (10 mL) were added octadecanoic acid (115 mg, 0.4 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 78 mg, 0.41 mmol), and the mixture was stirred for 3 h at room temperature; the progress of the reaction was monitored by T L C. After completion of the reaction, dichloromethane (50 mL) was added to the mixture , and the solution was washed with water, dried (Na_2SO_4), and concentrated to a syrup that was chromatographed on a column of silica gel (20 g) with 110:1 dichloromethane-methanol, to afford compound **16** (160 mg, 81%) as an amorphous mass; $[\alpha]_D$ + 14.7° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) Glc unit δ 2.84-2.92 (m, 2H, H-6,6'), 4.51 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 4.93 (near t, 1H, $J_{2,3}$ = 9.7, $J_{3,4}$ = 9.4 Hz, H-3), 4.98 (dd, 1H, H-2); Neu5Ac unit δ 1.87 (s, 3H, AcN), 2.64 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.2 Hz, H-3e), 3.79 (s, 3H, MeO), 4.09 (dd, 1H, $J_{8,9}$ = 4.6 Hz, $J_{9,9'}$ = 13.6 Hz, H-9), 4.28 (dd, 1H, H-9'), 4.86 (ddd, 1H, $J_{3a,4}$ = 11.5 Hz, $J_{4,5}$ = 11.0 Hz, H-4), and 5.29 (m, 2H, H-7,8); Cer unit δ 0.88 (t, 6H, 2MeCH$_2$), 1.24 (s, 50H, 25CH$_2$), 2.83 (dd, 1H, $J_{1,1'}$ = 10.3 Hz, $J_{1,2}$ = 9.2 Hz, H-1), 3.08 (dd, 1H, $J_{1',2}$= 3.7 Hz, H-1'), 4.46 (m, 1H, H-2), 5.49 (dd, 1H, $J_{3,4}$= 6.8 Hz, $J_{4,5}$ = 15.4 Hz, H-4), 5.57 (t, 1H, $J_{2,3}$ = $J_{3,4}$= 6.8 Hz, H-3), 5.85 (d, 1H, $J_{2,NH}$ = 8.8 Hz, NH), 5.87 (m, 1H, H-5), and 7.43-8.07 (m, 5H, Ph); *O*-acetyl groups δ 1.99, 2.00, 2.03, 2.04, 2.09, 2.12, and 2.13 (7s, 21H, 7AcO).

Anal. Calcd for $C_{75}H_{118}N_2O_{22}S_2$ (1463.9): C, 61.53; H, 8.13; N, 1.91. Found: C, 61.42; H, 8.33; N, 1.95.

S-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-S-(6-thio-β-D-glucopyranosyl)-(1→1)-(2R,3R,4E)-3-hydroxy-2-octadecanamido-4-octadecene-1-thiol (17). To a solution of **16** (100 mg, 63.8 μmol) in dry methanol (3 mL) was added sodium methoxide (10 mg). The mixture was stirred for 7 h at room temperature. After completion of the reaction, 0.2M potassium hydroxide (2 mL) was added to the mixture, and the solution was stirred overnight at room temperature, and then treated with Amberlite IR-120 (H^+) resin to remove the base. The resin was filtered off, and washed with 10:10:1 chloroform-methanol-water. The filtrate and washings were combined and concentrated to a syrup. The residue was chromatographed on a column of Sephadex LH-20 (50 g) with 10:10:1 chloroform-methanol-water, to give **17** (68 mg, 95%) as an amorphous mass; $[\alpha]_D$ +15.9° (*c* 0.44, $CHCl_3$); ^1H NMR [49:1 $(CD_3)_2SO$-D_2O] Glc unit δ 2.91 (dd, 1H, $J_{5,6}$ = 2.9 Hz, $J_{6,6'}$ = 13.2 Hz, H-6), and 4.20 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1); Neu5Ac unit δ 1.88 (s, 3H, AcN) and 2.75 (dd, 1H, H-3e); Cer unit δ 0.85 (t, 6H, 2MeCH$_2$), 1.24 (s, 50H, 25CH$_2$), 2.04 (m, 2H, COCH$_2$CH$_2$), 5.35 (dd, 1H, $J_{3,4}$ = 5.7 Hz, $J_{4,5}$ = 14.8 Hz, H-4), and 5.55 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5).

Anal. Calcd for $C_{53}H_{98}N_2O_{14}S_2$ (1051.5): C, 60.54; H, 9.39; N, 2.66. Found: C, 60.31; H, 9.63; N, 2.51.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-galactopyranosyl)-(1→1)-(2R,3R,4E)-2-azido-3-benzoyloxy-4-octadecene-1-thiol (18). Condensation of **8**, derived from **7** (300 mg, 0.35 mmol) by treatment with sodium methoxide (15 mg) in dry methanol (2 mL), with **13** (410 mg, 0.7 mmol) in DMF (4.5 mL) as described for **14**, afforded compound **18** (189 mg, 44%) as an amorphous mass; $[\alpha]_D$ -26.2° (*c* 0.83, $CHCl_3$); IR (KBr) 3500-3400 (NH), 2950 and 2850 (Me, methylene), 2100 (N_3), 1750 and 1230 (ester), 1660 and 1540 (amide), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) Gal unit δ 2.60 (dd, 1H, $J_{5,6}$ = 7.7 Hz, $J_{6,6'}$ = 14.7 Hz, H-6), 2.78 (dd, 1H, $J_{5,6'}$ = 6.6 Hz, H-6'); 4.92 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 5.15 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 5.22 (dd, 1H, H-2), and 5.66 (broad d, 1H, H-4); Neu5Ac unit δ 1.89 (s, 3H, AcN), 2.66 (dd, 1H, $J_{3a,3e}$ = 13.0 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e), 3.71 (s, 3H, MeO), 4.27 (dd, 1H, H-9), 4.88 (m, 1H, H-4), 5.27 (m, 2H, H-7,8), and 5.38 (d, 1H, $J_{5,NH}$ = 8.9 Hz, NH); sphingosine unit δ 0.88 (t, 3H, MeCH$_2$), 1.24 (s, 22H, 11CH$_2$), 2.71 (m, 1H, H-1), 2.93 (dd, 1H, $J_{1,1'}$ = 14.0 Hz, $J_{1',2}$ = 5.8 Hz, H-1'), 5.56 (dd, 1H, $J_{3,4}$ = 8.2 Hz, $J_{4,5}$ = 15.4 Hz, H-4), 5.76 (dd, 1H, $J_{2,3}$ = 3.5 Hz, H-3), 5.96 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.8 Hz, H-5), and 7.42-8.09 (m, 5H, Ph); *O*-acetyl groups δ 1.96, 2.02, 2.03, 2.07, 2.13, 2.15, and 2.19 (7s, 21H, 7AcO) .

Anal. Calcd for $C_{57}H_{82}N_4O_{11}S_2$ (1223.4): C, 55.96; H, 6.76; N, 4.58. Found: C, 55.91; H, 6.70; N, 4.53.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-galactopyranosyl)-(1→1)-(2R,3R,4E)-3-benzoyloxy-2-octadecanamido-4-octadecene-1-thiol (20). Selective reduction of the azide group in **18** (134 mg, 0.11 mmol) with hydrogen sulfide, and subsequent condensation of the amine **19** with octadecanoic acid (94 mg, 0.33 mmol) using WSC (63 mg, 0.33 mmol), as described for **16**, gave compound **20** (135 mg, 84%) as an amorphous mass; $[\alpha]_D$ -7.9° (*c* 0.56, $CHCl_3$); ^1H NMR ($CDCl_3$) Gal unit δ 2.84 (dd, 1H, $J_{5,6}$ = 6.8Hz, $J_{6,6'}$ = 12.5 Hz, H-6), 2.88 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, H-6'), 4.70 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 5.12 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 5.18 (dd, 1H, H-2), and 5.61 (broad d, 1H, H-4); Neu5Ac unit δ 1.89 (s, 3H, AcN) and 2.67 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.2 Hz, H-3e), 3.79 (s, 3H, MeO), 4.13 (dd, 1H, H-9), 4.25 (dd, 1H, H-9'), 4.90 (m, 1H, H-4), and 5.27 (m, 2H, H-7,8); Cer unit δ 0.88 (t, 6H, 2 $\underline{Me}CH_2$), 1.24 (s, 50H, $25CH_2$), 2.59, (dd, 1H, $J_{1,1'}$= 14.3 Hz, $J_{1,2}$ = 7.7 Hz, H-1), 3.15 (dd, 1H, $J_{1',2}$ = 3.8 Hz, H-1'), 4.46 (m, 1H, H-2), 5.49 (dd, 1H, $J_{3,4}$ = 7.3 Hz, $J_{4,5}$ = 15.2 Hz, H-4), 5.85 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.8 Hz, H-5), 6.06 (d, 1H, $J_{5,NH}$ = 8.8 Hz, NH), and 7.41-8.06 (m, 5H, Ph); *O*-acetyl groups δ 1.96, 2.03 (2), 2.04, 2.11, 2.12, and 2.18 (7s, 21H, 7AcO) .

Anal. Calcd for $C_{75}H_{118}N_2O_{22}S_2$ (1463.9): C, 61.53; H, 8.13 N, 1.91. Found: C, 61.33; H, 8.25; N, 1.89.

S-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-S-(6-thio-β-D-galactopyranosyl)-(1→1)-(2R,3R,4E)-3-hydroxy-2-octadecanamido-4-octadecene-1-thiol (21). *O*-Deacylation of **20** (100 mg, 68.3 μmol) and saponification of the methyl ester group, as described for **17**, gave compound **21** (71 mg, quantitative) as an amorphous mass; $[\alpha]_D$ +4.3° (*c* 0.47, $CHCl_3$); ^1H NMR [49:1 $(CD_3)_2SO$-D_2O] Gal unit δ 2.76 (dd, 1H, $J_{5,6}$ = 6.1Hz, $J_{6,6'}$ = 12.1 Hz, H-6), 2.89 (dd, 1H, $J_{5,6'}$ = 5.0 Hz, H-6'), and 4.20 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1); Neu5Ac unit δ 1.89 (s, 3H, AcN) and 2.74 (dd, 1H, $J_{3a,3e}$ = 11.7 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e); Cer unit δ 0.85 (t, 6H, 2 $\underline{Me}CH_2$), 1.24 (s, 50H, $25CH_2$), 2.04 (t, 2H, $J_{CH2,CH2}$ = 7.2 Hz, $COC\underline{H_2}CH_2$), 2.70 (dd, 1H, $J_{1,1'}$= 13.2 Hz, $J_{1,2}$ = 7.0 Hz, H-1), 2.97 (dd, 1H, H-1'), 5.37 (dd, 1H, $J_{3,4}$ = 6.2 Hz, $J_{4,5}$ = 15.2 Hz, H-4), and 5.55 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5) .

Anal. Calcd for $C_{53}H_{98}N_2O_{14}S_2$ (1051.5): C, 60.54; H, 9.39; N, 2.66. Found: C, 60.39; H, 9.51; N, 2.65.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-(2,3,4-tri-O-acetyl-6-thio-β-D-galactopyranosyl)-(1→4)-S-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2R,3R,4E)-2-azido-3-benzoyloxy-4-octadecene-1-thiol (22). Condensation of **12**, derived from **11** (250 mg, 0.22 mmol) by treatment with sodium methoxide (15 mg) in methanol (0.2 mL), with **13** (255 mg, 0.44 mmol), as described for **14**, afforded compound **22** (124 mg, 38%) as an amorphous mass; $[\alpha]_D$ -35.0° (*c* 0.62, $CHCl_3$); IR (KBr) 3500-3400 (NH), 2950 and 2850 (Me, methylene), 2100 (N_3), 1760 and 1230 (ester), 1670 and 1540 (amide), and 720 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) lactose unit δ 2.62 (dd, 1H, $J_{5',6'}$ = 8.4 Hz, $J_{6'a,6'b}$ = 14.3 Hz, H-6'a), 2.76 (m, 1H, H-6'b), 4.54 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 4.68 (d, 1H, $J_{1',2'}$ = 6.8 Hz, H-1'), 4.97 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 5.21 (t, 1H, $J_{3,4}$ = 9.3 Hz, H-3), and 5.51 (broad d, 1H, H-4'); Neu5Ac unit δ 1.87 (s, 3H, AcN), 2.71 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.83 (s, 3H, MeO), 4.15 (dd, 1H, $J_{8,9}$ = 4.2 Hz, $J_{9,9'}$ = 12.6 Hz, H-9), 4.26 (dd, 1H, $J_{8,9'}$ = 2.9 Hz, H-9'), and 4.93 (m, 1H, H-4); sphingosine unit δ 0.85 (t, 3H, $\underline{Me}CH_2$), 1.21 (s, 22H, $11CH_2$), 2.52 (dd, 1H, $J_{1,1'}$ = 14.5 Hz, $J_{1,2}$ = 7.7 Hz, H-1), 2.79 (dd, 1H, $J_{1',2}$ = 6.4 Hz, H-1'), 5.52 (dd, 1H, $J_{3,4}$ = 8.3 Hz, $J_{4,5}$ = 15.4 Hz, H-4), 5.67 (dd, 1H, $J_{2,3}$ = 3.7 Hz, H-3), 5.93 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5), and 7.40-8.05 (m, 5H, Ph); *O*-acetyl groups δ 1.90, 1.91, 2.01 (3), 2.03 (2), 2.11, 2.15, and 2.16 (10s, 30H, 10AcO).

Anal. Calcd for $C_{69}H_{98}N_4O_{29}S_2$ (1511.6): C, 54.82; H, 6.54; N, 3.71. Found: C, 54.66; H, 6.51; N, 3.63.

S-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-(2,3,4-tri-O-acetyl-6-thio-β-D-galactopyranosyl)-(1→4)-S-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2R,3R,4E)-3-benzoyloxy-2-octadecanamido-4-octadecene-1-thiol (24). Selective reduction of the azide group in **22** (108 mg, 0.07 mmol) with hydrogen sulfide, and subsequent coupling of the amine **23** with octadecanoic acid (61 mg, 0.21 mmol) in the presence of WSC (41 mg, 0.21 mmol), as described for **16**, gave compound **24** (96 mg, 77%) as an amorphous mass; $[\alpha]_D$ -11.7° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) lactose unit δ 2.54 (dd, 1H, $J_{5',6a}$ = 7.3 Hz, $J_{6'a,6'b}$ = 14.5 Hz, H-6'a), 2.81 (dd, 1H, $J_{5',6b}$ = 6.8 Hz, H-6'b), 4.49 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.68 (d, 1H, $J_{1',2'}$ = 7.1 Hz, H-1'), 4.95 (t, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 5.03 (m, 2H, H-2',3'), 5.21 (t, 1H, H-3), and 5.52 (broad d, 1H, H-4'); Neu5Ac unit δ 1.90 (s, 3H, AcN), 2.73 (dd, 1H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.3 Hz, H-3e), 3.85 (s, 3H, MeO), 4.16 (dd, 1H, $J_{8,9}$ = 4.3 Hz, $J_{9,9'}$ = 12.6 Hz, H-9), 4.28 (dd, 1H, H-9'), 4.95 (m, 1H, H-4), and 5.27 (m, 2H, H-7,8); Cer unit δ 0.88 (t, 6H, $2\underline{Me}CH_2$),

1.25 (s, 50H, 25CH_2), 2.87 (dd, 1H, $J_{1,1'}$ = 13.7 Hz, $J_{1,2}$ = 7.5 Hz, H-1), 3.00 (dd, 1H, $J_{1',2}$ = 4.9 Hz, H-1'), 5.48 (dd, 1H, $J_{3,4}$ = 7.1 Hz, $J_{4,5}$ = 15.2 Hz, H-4), 5.61 (t, 1H, $J_{2,3}$ = 7.0 Hz, H-3), 5.86 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5), 5.88 (d, 1H, $J_{NH,2}$ = 8.9 Hz, NH), and 7.29-8.05 (m, 5H, Ph); *O*-acetyl groups δ 1.94, 1.99, 2.02, 2.04 (3), 2.05, 2.13, and 2.18 (2) (10s, 30H, 10AcO) .

Anal. Calcd for $C_{87}H_{134}N_2O_{30}S_2$ (1752.1): C, 59.64; H, 7.71; N, 1.60. Found: C, 59.73; H, 7.90; N, 1.62.

S-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-O-(6-thio-β-D-galactopyranosyl)-(1→4)-S-β-D-glucopyranosyl-(1→1)-(2R,3R,4E)-3-hydroxy-2-octadecanamido-4-octadecene-1-thiol (25). *O*-Deacylation of **24** (70 mg, 40 μmol) and subsequent saponification of the methyl ester group, as described for **17**, gave **25** (48 mg, quantitative) as an amorphous mass; $[\alpha]_D$ -12.5° (*c* 0.4, 1:1 $CHCl_3$-MeOH); ^1H NMR [49:1 $(CD_3)SO$-D_2O] δ 0.85 (t, 6H, 2MeCH_2, Cer), 1.24 (s, 50H, 25CH_2), 1.89 (s, 3H, AcN, Neu5Ac), 2.05 (t, 2H, $J_{CH2,CH2}$ = 7.1 Hz, COCH_2CH_2, Cer), 2.64 - 2.79 (m, 3H, H-3e of Neu5Ac, H-1 of Cer, and H-6 of Gal), 2.91 - 3.00 (m, 2H, H-1' of Cer, H-6' of Gal), 4.25 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1, lactose), 4.30 (d, 1H, $J_{1',2'}$ = 9.9 Hz, H-1', lactose), 5.36 (dd, 1H, $J_{3,4}$ = 5.9 Hz, $J_{4,5}$ = 15.6 Hz, H-4, Cer), and 5.54 (dt, 1H, H-5, Cer).

Anal. Calcd for $C_{59}H_{108}N_2O_{19}S_2$ (1213.6): C, 58.39; H, 8.97; N, 2.31. Found: C, 58.21; H, 9.19; N, 2.27.

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REFERENCES

1. *Gangliosides*; New Comprehensive Biochemistry, Vol. **10**; H. Wiegandt Ed., Elsevier, Amsterdam, 1985.

2. *Gangliosides and Modulation* of *Neuronal Functions*, NATO ASI Series H; Cell Biology Vol. **7**; H. Rahman Ed.; Springer-Verlag, Berlin-Heiderberg, 1987.

3. *Sialic Acids* 1988, Proceeding of the Japanese-German Symposium on Sialic Acids; R. Schauer, T. Yamakawa Eds.; Barbel Mende, Kiel, 1988.

4. G. Walz, A. Aruffo, W. Kolanus, M. Bevilacqua, and S. Seed, *Science*, **250**, 1132 (1990).

5. M. L. Phillips, E. Nudelman, F. C. A. Graeta, M. Perez, A. K. Singhal, S. Hakomori, and J. C. Paulson, *Science*, **250**, 1130 (1990).

6. J. B. Lowe, L. M. Stoolman, R. P. Nair, R. D. Larsen, T. L. Berhend, and R. M. Marks, *Cell*, **63**, 475 (1990).

7. a) A. Kameyama, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, in press (1991); b) *ibid.*, **8**, 799 (1989).

8. a) A. Kameyama, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, **193**, c1 (1989); b) *ibid.*, **200**, 269 (1990); c) *ibid.*, **209**, c1 (1991).

9. a) T. Murase, A. Kameyama, K. P. R. Kartha, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 265 (1989); b) T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, **188**, 71 (1989).

10. H. Prabhanjan, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, **211**, c1 (1991).

11. a) A. Hasegawa, T. Murase, K. Adachi, M. Morita, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, **9**, 181 (1990); b) A. Hasegawa, T. Murase, M. Morita, H. Ishida, and M. Kiso, *J. Carbohydr Chem.*, **9**, 201 (1990).

12. a) Y. Ito, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 285 (1989); b) A. Hasegawa, M. Morita, Y. Ito, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, **9**, 369 (1990); c) A. Hasegawa, M. Morita, Y. Kojima, H. Ishida, and M. Kiso, *Carbohydr. Res.*, in press (1991).

13. Y. Suzuki, K. Sato, M. Kiso, and A. Hasegawa, *Glycoconjugate J.*, **7**, 349 (1990).

14. T. Adachi, Y. Yamada, I. Inoue, and M. Saneyoshi, *Synthesis*, 45 (1977).