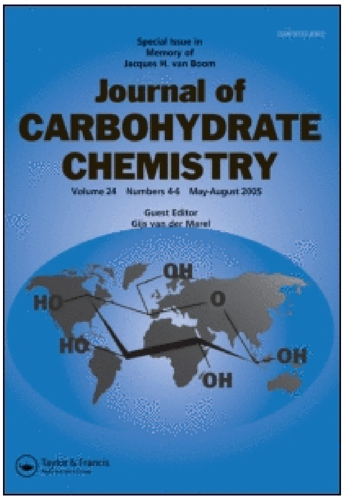


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Studies on the Thioglycosides of *N*-Acetylneuraminic Acid 10: Synthesis of *S*-(α-Sialosyl)-(2→6)-*O*-2-acetamido-2-deoxy-β-D-Hexopyranosyl Ceramide and Its Related Compounds

Akira Hasegawa[a]; Tomohiro Terada[a]; Hirotsugu Ogawa[a]; Makoto Kiso[a]
[a] Department of Applied Bioorganic Chemistry, Gifu University, Gifu, Japan

To cite this Article Hasegawa, Akira , Terada, Tomohiro , Ogawa, Hirotsugu and Kiso, Makoto(1992) 'Studies on the Thioglycosides of *N*-Acetylneuraminic Acid 10: Synthesis of *S*-(α-Sialosyl)-(2→6)-*O*-2-acetamido-2-deoxy-β-D-Hexopyranosyl Ceramide and Its Related Compounds', Journal of Carbohydrate Chemistry, 11: 3, 319 — 331
To link to this Article: DOI: 10.1080/07328309208017996
URL: http://dx.doi.org/10.1080/07328309208017996

J. CARBOHYDRATE CHEMISTRY, 11(3), 319-331 (1992)

STUDIES ON THE THIOGLYCOSIDES OF *N*-ACETYLNEURAMINIC ACID 10: SYNTHESIS OF *S*-(α-SIALOSYL)-(2→6)-*O*-2-ACETAMIDO-2-DEOXY-β-D-HEXOPYRANOSYL CERAMIDE AND ITS RELATED COMPOUNDS

Akira Hasegawa, Tomohiro Terada, Hirotsugu Ogawa, and Makoto Kiso

Department of Applied Bioorganic Chemistry, Gifu University,
Gifu 501-11, Japan

Received November 4, 1991 - Final form December 30, 1991

ABSTRACT

Sialosylglycolipids in which 2-thio-*N*-acetylneuraminic acid (2-thio-Neu5Ac) is linked as the α-glycoside at C-6 of *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) residue, and ceramide or 2-(2-tetradecylhexadecanamido)-ethanol is contained as the lipophilic part, have been synthesized. Coupling of the sodium salt (**11**) of α-2-thio-Neu5Ac with 2-azidoethyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-tosyl-β-D-glucoside (**6**) or -β-D-galactoside (**10**), which are prepared *via* condensation of the oxazoline derivative of GlcNAc or GalNAc with 2-azidoethanol, *O*-deacetylation, 6-*O*-tosylation and *O*-acetylation gave the corresponding β-thioglycosides (**12** and **16**), respectively. The β-thioglycosides obtained were converted *via* selective reduction of the azide group, condensation with 2-tetradecylhexadecanoic acid (**20**) and removal of the protecting groups, into the end products (**15** and **19**). On the other hand, glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**22**) with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide gave the β-glycoside, which was transformed *via O*-deacetylation, conversion of the phthalimide group to *N*-acetyl, selective 6-*O*-tosylation, coupling with **11**, reduction of the azide group, condensation with octadecanoic acid and removal of the protecting groups, into the title compound **29**.

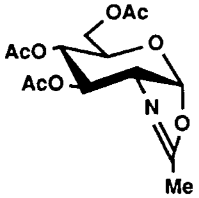
INTRODUCTION

As more and more biological functions[1-6] of sialoglycoconjugates have been reported, chemical synthesis of gangliosides and their various types of analogs is becoming stimulating and rewarding. Recently, we have synthesized[7-8] several ganglioside analogs containing α-thioglycosides of sialic acid and have observed that these analogs are potent inhibitors[9] of sialidase activities of different subtypes of influenza viruses. Further modification of the glycolipid molecule by replacement with different carbohydrate or lipophilic residues, should be carried out, not only to obtain sialidase inhibitors, but also for the purpose of elucidating the functions of sialosyl glycolipids at the molecular level. We describe here the synthesis of *S*-(α-sialosyl)-(2→6)-β-D-2-acetamido-2-deoxy-hexopyranosyl-(1→1)-ceramide or 2-(2-tetradecyl-hexadecanamido)-ethanol.

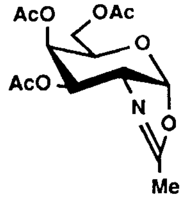
RESULTS AND DISCUSSION

For the synthesis of *S*-α-sialosyl-(2→6)-β-D-2-acetamido-2-deoxy-hexopyranosyl lipids, we set out to prepare the 2-azidoethyl 2-acetamido-2,3-di-*O*-acetyl-2-deoxy-6-*O*-tosyl-β-D-glucopyranoside (**6**), -β-D-galactopyranoside (**10**) and *O*-(2-acetamido-2-deoxy-6-*O*-tosyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-4-octadecene-1,3-diol (**25**) as the glycosyl acceptors for coupling with the sodium salt **11**[10] of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate. The intermediates could then, by introduction of the fatty acids after reduction of the azide group to the amine, be converted to the end products.

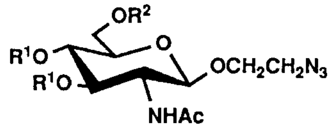
Treatment of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-d]-2-oxazoline (**1**) or 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-galactopyrano)-[2,1-d]-oxazoline (**2**) with 2-azidoethanol in dichloromethane in the presence of a small amount of sulfuric acid gave the corresponding β-glycosides **3** and **7** in good yields, respectively. When treated with *p*-toluenesulfonyl chloride in pyridine at 0 °C and acetylated with acetic anhydride, 2-azidoethyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**4**) or -β-D-galactopyranoside (**8**), which were derived by *O*-deacetylation of **3** and **7**, afforded the corresponding 6-*O*-tosyl derivatives **6** and **10** in high yields, respectively. The ^{1}H NMR spectra of **6** and **10** contained the H-3 and H-4 signals at δ 4.90 (t, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4 for **6**), 5.34 (dd, $J_{2,3}$ = 10.4 Hz, H-3 for **6**), and 5.28-5.38 (m, H-3,4 for **10**), and the H-1 at δ 4.80 (d, 8.3 Hz for **6** and 8.4 Hz for **10**), indicating the β-configuration of the glycosidic linkage and the position of *O*-tosyl group. Condensation of the sodium salt **11**,[10] freshly derived from methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate by selective *S*-deacetylation with sodium methoxide, with **6** or **10** in *N,N*-dimethylformamide (DMF) at 45 °C gave the desired α(2→6)-thioglycosides **12** and **16** in 76 and 55% yields. Selective reduction[12] of the azide group in **12** or **16** with H_2S in aqueous pyridine for 2



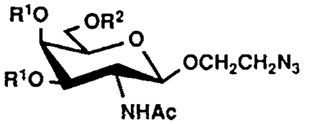
1



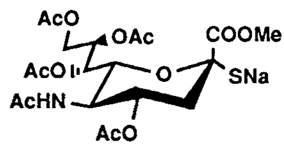
2



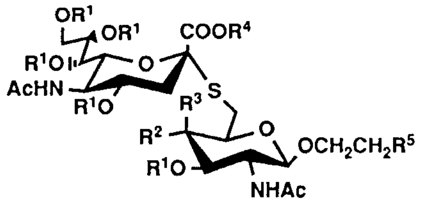
3 $R^1 = R^2 = Ac$
4 $R^1 = R^2 = H$
5 $R^1 = H, R^2 = Ts$
6 $R^1 = Ac, R^2 = Ts$



7 $R^1 = R^2 = Ac$
8 $R^1 = R^2 = H$
9 $R^1 = H, R^2 = Ts$
10 $R^1 = Ac, R^2 = Ts$



11

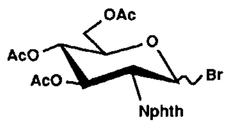


20

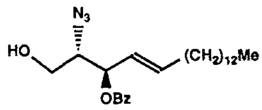
12 $R^1 = Ac$, $R^2 = OAc$, $R^3 = H$, $R^4 = Me$, $R^5 = N_3$
13 $R^1 = Ac$, $R^2 = OAc$, $R^3 = H$, $R^4 = Me$, $R^5 = NH_2$
14 $R^1 = Ac$, $R^2 = OAc$, $R^3 = H$, $R^4 = Me$, $R^5 = NHCOCH[(CH_2)_{13}Me]_2$
15 $R^1 = R^3 = R^4 = H$, $R^2 = OH$, $R^5 = NHCOCH[(CH_2)_{13}Me]_2$
16 $R^1 = Ac$, $R^2 = H$, $R^3 = OAc$, $R^4 = Me$, $R^5 = N_3$
17 $R^1 = Ac$, $R^2 = H$, $R^3 = OAc$, $R^4 = Me$, $R^5 = NH_2$
18 $R^1 = Ac$, $R^2 = H$, $R^3 = OAc$, $R^4 = Me$, $R^5 = NHCOCH[(CH_2)_{13}Me]_2$
19 $R^1 = R^2 = R^4 = H$, $R^3 = OH$, $R^5 = NHCOCH[(CH_2)_{13}Me]_2$

days at 0 °C gave the amines **13** and **17** which on condensation with 2-tetradecylhexadecanoic acid (**20**),[13] using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) in dichloromethane, gave the acylated products **14** and **18** in high yields, respectively. Finally, *O*-deacetylation of **14** and **18** with sodium methoxide in methanol and subsequent saponification of the methyl ester group yielded the desired products **15** and **19**, quantitatively.

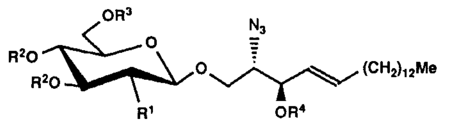
On the other hand, glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**22**)[12b,14] with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl



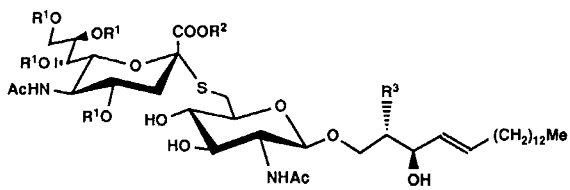
21



22



23 R^1 = Nphth, $R^2 = R^3$ = Ac, R^4=Bz
24 R^1 = NHAc, $R^2 = R^3 = R^4$ = H
25 R^1 = NHAc, $R^2 = R^4$ = H, R^3 = Ts



26 R^1 = Ac, R^2 = Me, $R^3 = N_3$
27 R^1 = Ac, R^2 = Me, $R^3 = NH_2$
28 R^1 = Ac, R^2 = Me, $R^3 = NHCO(CH_2)_{16}Me$
29 $R^1 = R^2$ = H, $R^3 = NHCO(CH_2)_{16}Me$

bromide (**21**)[15] in dichloromethane in the presence of silver carbonate and silver perchlorate gave the desired β-glycoside **23** in 59% yield. Significant signals of the GlcN unit in the ^{1}H NMR spectrum were at δ 5.44 (d, $J_{1,2}$ = 8.2 Hz, H-1), 5.19 (t, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), and 5.78 (dd, $J_{4,5}$ = 11.0 Hz, H-4), indicating the newly formed glycosidic linkage to be β. *O*-Deacylation of **23** with sodium methoxide, followed by heating with hydrazine hydrate in aqueous 95% ethanol, and subsequent *N*-acetylation afforded *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-4-octadecene-1,3-diol (**24**) in 94% yield. Treatment of **24** with *p*-toluenesulfonyl chloride in pyridine at 0 °C gave the 6-*O*-tosyl derivative **25** which, on coupling with **11** in DMF as described in the synthesis of **12**, afforded **27** in 76% yield.

Selective reduction of the azide group in **27**, subsequent condensation with octadecanoic acid, and removal of the protecting groups, according to the method described above, yielded *S*-(α-sialosyl)-(2→6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→1)-ceramide (**29**), quantitatively.

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. ^{1}H 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*.

2-Azidoethyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (3). To a stirred solution of **1** (3.3 g, 1 mmol) and 2-azidoethanol (1.3 g, 1.5 mmol), was added molecular sieves 4Å (3 g) and the mixture was stirred for 1.5 h at room temperature. A catalytic amount of sulfuric acid was added to the mixture and it was stirred for 8 h at room temperature, then extracted with dichloromethane. The extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:1 ethyl acetate-hexane) of the residue on silica gel (200 g) gave **3** (3.87 g, 93%) as an amorphous mass: $[\alpha]_D$ -43.5° (*c* 0.8, $CHCl_3$); IR (KBr) 2100 (N_3), 1750 and 1230 (ester), and 1650 and 1550 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 1.92 (s, 3H, AcN), 2.03, 2.04, 2.09 (3s, 9H, 3 AcO), 3.25, 3.28 (2m, 2H, $CH_2CH_2N_3$), 4.05 (m, 1H, H-5), 4.16 (dd, 1H, $J_{5,6'}$ = 2.3 Hz, $J_{6,6'}$ = 12.3 Hz, H-6'), 4.26 (dd, 1H, $J_{5,6}$ = 4.8 Hz, H-6), 4.85 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 5.07 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 5.37 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-3), and 5.89 (d, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_9$ (416.4): C, 46.15; H, 5.81; N, 13.46. Found: C, 46.30; H, 5.83; N, 13.49.

2-Azidoethyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (4). To a solution of **3** (2.5 g, 6 mmol) in methanol (25 mL) was added sodium methoxide (20 mg), and the mixture was stirred for 10 min at room temperature, neutralized with Amberlite IR-120 (H^+) resin and filtered. The resin was washed with methanol, and the combined filtrate and washings were concentrated. The residue was crystallized from ether to give **4** (1.60 g, quantitative) as needles: mp 153-155 °C, $[\alpha]_D$ -51.2° (*c* 1.1, methanol); IR (KBr) 3600-3300 (OH, NH), 2100 (N_3), and 1650 and 1550 cm^{-1} (amide); ^1H NMR (CD_3OD) δ 1.97 (s, 3H, AcN) and 4.49 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1).

Anal. Calcd for $C_{10}H_{18}N_4O_6$ (290.3): C, 41.37; H, 6.25; N, 19.30. Found: C, 41.33; H, 6.34; N, 19.15.

2-Azidoethyl 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(*p*-toluenesulfonyl)-β-D-glucopyranoside (6). To an ice-cooled solution of **4** (500 mg, 1.7 mmol) in pyridine (8 mL) was added *p*-toluenesulfonyl chloride (430 mg, 2.26 mmol), and the mixture was stirred for 4 h at 0 °C. Methanol (1 mL) was added and concentrated to a syrup which was acetylated with acetic anhydride (3 mL) in pyridine (6 mL) overnight at room temperature. The reaction mixture was concentrated and extracted with dichloromethane. The extract was successively washed with 2 M HCl, M Na_2CO_3, and water, dried (Na_2SO_4) and concentrated. Column chromatography (2:1 ethyl acetate-hexane) of the residue on silica gel (50 g) gave **6** (620 mg, 70%) as an amorphous mass: $[\alpha]_D$ -7.2° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.93 (s, 3H, AcN), 1.99, 2.02 (2s, 6H, 2AcO), 2.46 (s, 3H, Me), 3.24, 3.26, 3.69, 4.00 (4m, 4H, $CH_2CH_2N_3$), 4.04-4.13 (m, 2H, H-6,6'), 4.80 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.90 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4), 5.34 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-3), 5.86 (d, 1H, NH), and 7.27-7.80 (m, 4H, Ph).

Anal. Calcd for $C_{21}H_{28}N_4O_{11}S$ (528.5): C, 47.72; H, 5.34; N, 10.60. Found: C, 47.60; H, 5.59; N, 10.34.

2-Azidoethyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (7). To a stirred solution of **2** (3.3 g, 1 mmol) and 2-azidoethanol (1.3 g, 1.5 mmol) was added molecular sieves 4Å (2 g), and the mixture was stirred for 1.5 h at room temperature. A catalytic amount of sulfuric acid was added and the mixture was stirred for 8 h at room temperature; it was then processed as already described for **3** to give **7** (3.96 g, 95%) as an amorphous mass: $[\alpha]_D$ -29.6° (*c* 0.9, $CHCl_3$); IR (KBr) 2100 (N_3), 1750 and 1230 (ester), and 1650 and 1550 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 1.97 (s, 3H, AcN), 2.01, 2.06, 2.15 (3s, 9H, 3AcO), 3.28, 3.53, 3.73 (m, 4H, $CH_2CH_2N_3$), 4.15-4.22 (m, 2H, H-6,6'), 4.87 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 5.35-5.40 (m, 2H, H-3,4), and 5.79 (d, 1H, NH).

Anal. Calcd for $C_{16}H_{24}N_4O_9$ (416.4): C, 46.15; H, 5.81; N, 13.46. Found: C, 46.08; H, 5.93; N, 13.32.

2-Azidoethyl 2-Acetamido-2-deoxy-β-D-galactopyranoside (8). *O*-Deacetylation of **7** (1.6 g, 3.5 mmol) in methanol (5 mL) with sodium methoxide (20 mg) as described for **4**, gave **8** (1.1 g, quantitative) as needles: mp 154-157 °C; $[\alpha]_D$ -32.1° (*c* 0.7, methanol); ^1H NMR (CD_3OD) δ 1.98 (s, 3H, AcN) and 4.47 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1).

Anal. Calcd for $C_{10}H_{18}N_4O_6$ (290.3): C, 41.37; H, 6.25; N, 19.30. Found: C, 41.36; H, 6.31; N, 19.18.

2-Azidoethyl 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(*p*-toluenesulfonyl)-β-D-galactopyranosides (10). To a solution of **8** (1.0 g, 3.44 mmol) in pyridine (5 mL) cooled to 0 °C was added *p*-toluenesulfonyl chloride (1.3 g, 6.8 mmol), and the mixture was stirred for 4.5 h at 0 °C. Methanol (1 mL) was added to the mixture and the mixture was concentrated. The residue was acetylated with acetic anhydride (5 mL) in pyridine (10 mL) overnight at room temperature. It was then processed as described for **6** to give **10** (853 mg, 68%) as an amorphous mass: $[\alpha]_D$ -26.0° (*c* 0.3, $CHCl_3$); IR (KBr) 3340 (NH), 2100 (N_3), 1750 and 1230 (ester), and 1650 and 1550 (amide), and 750 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 1.96 (s, 3H, AcN), 1.99, 2.05 (2s, 6H, 2AcO), 2.46 (s, 3H, Me), 3.26, 3.50, 3.68, 3.92 (4m, 4H, $CH_2CH_2N_3$), 4.80 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 5.28-5.38 (m, 2H, H-3,4), and 7.28-7.81 (m, 4H, Ph).

Anal. Calcd for $C_{21}H_{28}N_4O_{10}S$ (528.5): C, 47.72; H, 5.34; N, 10.60. Found: C, 47.66; H, 5.49; N, 10.58.

2-Azidoethyl *S*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-thio-β-D-glucopyranoside (12). A solution of **6** (300 mg, 0.57 mmol) and the sodium salt **11** (450 mg, 0.85 mmol) of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate in DMF (5 mL) was stirred overnight at 45 °C under N_2. Acetic anhydride (2.5 mL) and pyridine (5 mL) were added to the mixture and the mixture was stirred for 6 h at room temperature and concentrated. The residue was chromatographed on a column of silica gel (50 g) with 3:1 ethyl acetate-hexane to give **12** (370 mg, 76%) as an amorphous mass: $[\alpha]_D$ +19.5° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) Neu5Ac unit δ 2.71 (dd, 1H, $J_{3a,3e}$ = 13.0 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e), 3.81 (s, 3H, MeO), 4.83 (m, 1H, H-4), and 5.23-5.32 (m, 2H, H-7,8); GlcNAc unit δ 2.77-2.98 (m, 2H, H-6,6'), 4.72 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), and 4.92 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.3 Hz, H-4); other groups δ 1.88, 1.94 (2s, 6H, 2AcN), 2.02, 2.04, 2.08, 2.09, 2.15, 2.18 (6s, 18H, 6AcO).

Anal. Calcd for $C_{34}H_{49}N_5O_{19}S$ (863.9): C, 47.27; H, 5.72; N, 8.11. Found: C, 47.09; H, 5.83; N, 8.05.

***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-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-thio-β-D-glucopyranosyl)-(1→1)-2-(2-tetradecyl-hexa-decanamido)ethanol (14).** Hydrogen sulfide was bubbled through a stirred solution of **12** (200 mg, 0.23 mmol) in aqueous 83% pyridine (12 mL) for 48 h at 0 ~10 °C with the reaction being monitored by TLC. The mixture was concentrated to give the syrupy amine **13** which was stirred with 2-tetradecyl-hexadecanoic acid (**20**, 130 mg, 0.42 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (WSC, 50 mg) in dry dichloromethane (5 mL) for 3 h at room temperature. Dichloromethane (50 mL) was added, and the mixture was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (4:1 ethyl acetate-hexane) of the residue on silica gel (60 g) gave **14** (235 mg, 80%) as an amorphous mass: $[\alpha]_D$ +17.8° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) Neu5Ac unit δ 2.71 (dd, 1H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.2 Hz, H-3e), 3.98 (s, 3H, MeO), 4.84 (m, 1H, H-4), 5.25 (m, 1H, H-7) and 5.37 (ddd, $J_{7,8}$ = 9.5 Hz, H-8); GlcNAc unit δ 2.89, 2.93 (2m, 2H, H-6,6'), 4.59 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.93 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), and 5.20 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3); lipophilic unit δ 0.88 (t, 6H, 2*Me*CH_2), 1.25 (s, 52H, 26 CH_2), and 3.40-3.65 (m, 4H, OCH_2CH_2NHCO); other groups δ 1.88, 1.99 (2s, 6H, 2AcN), 2.03, 2.05, 2.06, 2.08, 2.15, 2.17 (6s, 18H, 6AcO).

Anal. Calcd for $C_{64}H_{109}N_3O_{20}S$ (1272.6): C, 60.40; H, 8.63; N, 3.30. Found: C, 60.22; H, 8.69; N, 3.20.

S-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-(2-acetamido-2-deoxy-6-thio-β-D-glucopyranosyl)-(1→1)-2-(tetradecylhexadecanamido)ethanol (15). To a solution of **14** (100 mg, 0.078 mmol) in methanol (5 mL) was added sodium methoxide (30 mg), and the mixture was stirred for 3 h at room temperature, and water (0.5 mL) was added. The solution was stirred for 3 h at room temperature, neutralized with Amberlite IR-120 (H^+) resin and filtered. The resin was washed with methanol, and the combined filtrate and washings were concentrated. Column chromatography (1:1 chloroform-methanol) of the residue on Sephadex LH-20 (40 g) gave **15** (84 mg, quantitative): $[\alpha]_D$ +6.8° (*c* 1.6, $CHCl_3$); ^1H NMR (CD_3OD) δ 0.98 (t, 6H, 2$MeCH_2$), 1.28 (s, 52H, 26CH_2), 1.98, 2.00 (2s, 6H, 2AcN), 2.78-2.95 (m, 3H, H-3e for Neu5Ac and H-6,6' for GlcNAc), and 4.36 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1 for GlcNAc).

Anal. Calcd for $C_{51}H_{95}N_3O_{14}S$ (1006.4): C, 60.86; H, 9.52; N, 4.18. Found: C, 60.59; H, 9.64; N, 3.93.

2-Azidoethyl 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-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-thio-β-D-galactopyranoside (16). A solution of **10** (300 mg, 0.57 mmol) and **11** (450 mg, 0.84 mmol) in DMF (3 mL) was heated overnight at 45 °C. Acetic anhydride (2 mL) and pyridine (5 mL) were added to the mixture and, after 8 h at room temperature, the mixture was concentrated to a syrup that was chromatographed on a column of silica gel (60 g) with 40:1 dichloromethane-methanol to give **16** (268 mg, 55%) as an amorphous mass: $[\alpha]_D$ -9.7° (*c* 0.7, $CHCl_3$); IR (KBr) 3400 (NH), 2100 (N_3), 1750 and 1220 (ester), and 1650 and 1550 cm^{-1} (amide); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 2.56 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.2 Hz, H-3e), 3.80 (s, 3H, MeO), 4.59 (dd, 1H, $J_{8,9'}$ = 11.9 Hz, $J_{9,9'}$ = 13.0 Hz, H-9'), 4.90 (m, 1H, H-4), 5.14 (broad d, 1H, $J_{7,8}$ = 9.9 Hz, H-7), and 5.31 (m, 1H, H-8); GalNAc unit δ 2.56 (dd, 1H, $J_{5,6'}$ = 8.8 Hz, $J_{6,6'}$ = 14.6 Hz, H-6'), 2.79 (dd, 1H, $J_{5,6}$ = 5.9 Hz, H-6), 4.74 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 5.24 (dd, 1H, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.1 Hz, H-3), and 5.64 (broad d, 1H, H-4); other groups δ 1.90, 1.96 (2s, 6H, 2AcN), and 2.03, 2.06, 2.08, 2.10, 2.14, and 2.18 (6s, 18H, 6AcO).

Anal. Calcd for $C_{34}H_{49}N_5O_{19}S$ (863.9): C, 47.27; H, 5.72; N, 8.11. Found: C, 47.15; H, 5.77; N, 8.01.

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-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-thio-β-D-galactopyranosyl)-(1→1)-2-(2-

tetradecylhexa-decanamido)ethanol (18). Selective reduction of the azide group in **16** (115 mg, 0.13 mmol) and subsequent coupling with **20** (130 mg, 0.47 mmol), as described for **14**, afforded **18** (128 mg, 76%) as an amorphous mass: $[\alpha]_D$ -10.1° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 2.72 (dd, 1H, $J_{3a,3e}$ = 13.2 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e), 3.81 (s, 3H, MeO), 4.61 (dd, 1H, $J_{8,9}$ = 3.3 Hz, $J_{9,9'}$ = 13.7 Hz, H-9), 4.89 (ddd, 1H, $J_{3a,4}$ = 13.5 Hz, $J_{4,5}$ = 10.3 Hz, H-4), and 5.10 (dd, 1H, $J_{6,7}$ = 1.6 Hz, $J_{7,8}$ = 10.2 Hz, H-7); GalNAc unit δ 2.52 (dd, 1H, $J_{5,6'}$ = 9.2 Hz, $J_{6,6'}$ = 14.8 Hz, H-6'), 2.74 (dd, 1H, $J_{5,6}$ = 7.7 Hz, H-6), 4.64 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 5.22 (dd, 1H, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.5 Hz, H-3), and 5.67 (broad d, 1H, $J_{3,4}$ = 3.5 Hz, H-4); lipophilic unit δ 0.85 (t, 6H, 2*Me*CH_2), 1.24 (s, 52H, 26CH_2); other groups δ 1.90, 1.97 (2s, 6H, 2AcN), and 1.99, 2.00, 2.04, 2.10, 2.13, and 2.14 (6s, 18H, 6AcO).

Anal. Calcd for $C_{64}H_{109}N_3O_{20}S$ (1272.6): C, 60.40; H, 8.63; N, 3.30. Found: C, 60.33; H, 8.90; N, 3.25.

***S*-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-(2-acetamido-2-deoxy-6-thio-β-D-galactopyranosyl)-(1→1)-2-(2-tetradecylhexadecanamido)ethanol (19).** Deacetylation and saponification of **18** (39 mg, 0.03 mmol), as described for **15**, gave **19** (31 mg, quantitative) as an amorphous mass; $[\alpha]_D$ -1.4° (*c* 0.4, $CHCl_3$); ^1H NMR (CD_3OD) δ 0.94 (t, 6H, 2*Me*CH_2), 1.23 (s, 52H, 26CH_2), 1.83, 1.91 (2s, 6H, 2AcN), 2.80 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e for Neu5Ac), 2.85-3.10 (2m, 2H, H-6,6' for GalNAc), and 4.27 (d, 1H, $J_{1,2}$ = 9.0 Hz, H-1).

Anal. Calcd for $C_{51}H_{95}N_3O_{14}S$ (1006.4): C, 60.86; H, 9.52; N, 4.18. Found: C, 60.69; H, 9.79; N, 4.05.

***O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (23).** To a solution of **22** (200 mg, 0.47 mmol) in dichloromethane (2 mL) were added silver carbonate (260 mg), silver perchlorate (200 mg), and powdered molecular sieves 4Å (100 mg), and the mixture was stirred for 20 h at room temperature in the dark (mixture A). A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide[15] (**21**; 470 mg, 0.94 mmol) in dichloromethane (1 mL) was treated with powdered molecular sieves 4Å (100 mg) as above and then added to mixture A at room temperature. After vigorous stirring overnight, the precipitate was collected and washed with dichloromethane, and the combined filtrate and washings were concentrated. Column chromatography (1:2 ethyl acetate-hexane) of the residue on silica gel (40 g) afforded **23** (230 mg, 58.5%) as an amorphous mass: $[\alpha]_D$ +0.3° (*c* 0.7, $CHCl_3$); IR (KBr) 2100 (N_3), 1750 and 1230 (ester), and 720 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) sugar unit δ 1.87, 2.03, 2.07 (3s, 9H, 3AcO), 4.15 (dd, 1H, $J_{5,6'}$ = 2.4 Hz, $J_{6,6'}$ = 12.5 Hz, H-6'), 4.28 (dd, 1H, $J_{5,6}$ = 4.6 Hz, H-6), 4.37 (dd, 1H, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 5.19 (t , $J_{3,4}$ = 9.5 Hz, H-3), 5.44 (d, 1H, H-1), and 5.78 (dd, 1H, $J_{4,5}$ = 11.0 Hz); sphingosine unit δ 0.88 (t, 3H, *Me*CH_2), 1.22-1.26 (m, 22H, 11CH_2), 5.42 (m, 1H, H-4), and 5.70 (m, 1H, H-5); other groups δ 7.40-8.04 (m, 9H, aromatic protons).

Anal. Calcd for $C_{45}H_{58}N_4O_{14}$ (847.0): C, 63.81; H, 6.90; N, 6.62. Found: C, 63.66; H, 7.10; N, 6.58.

***O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-4-octadecene-1,3-diol (24).** A solution of **23** (250 mg, 0.3 mmol) in methanol (5 mL) was stirred with sodium methoxide (20 mg) for 1 h at 45 °C. The mixture was treated with Amberlite IR-120 (H^+) resin and concentrated, and a solution of the residue in aqueous 95% ethanol (3 mL) was treated with hydrazine hydrate (0.057 mL, 1.12 mmol) for 5 h under reflux. The precipitate was collected and washed with ethanol, and the combined filtrate and washings were concentrated. The residue was treated with acetic anhydride (1 mL) in methanol (5 mL) overnight at room temperature, and concentrated. Column chromatography (10:1 dichloromethane-methanol) of the residue on silica gel (40 g) gave **24** (147 mg, 94%) as an amorphous mass: $[\alpha]_D$ +14.0° (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD) GlcNAc unit δ 1.98 (s, 3H, AcN), 3.32 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.0 Hz, H-3), 3.65 (m, 1H, H-5), 3.88 (dd, 1H, $J_{5,6'}$ = 1.8 Hz, $J_{6,6'}$ = 10.6 Hz, H-6'), 3.93 (dd, 1H, $J_{5,6}$ = 6.4 Hz, H-6), 4.13 (t, 1H, $J_{4,5}$ = 7.0 Hz, H-4), and 4.44 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1); sphingosine unit δ 0.90 (t, 3H, *Me*CH_2), 1.28 (s, 22H, 11CH_2), 5.49 (dd, 1H, $J_{3,4}$ = 7.3 Hz, $J_{4,5}$ = 15.2 Hz, H-4), and 5.74 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5).

Anal. Calcd for $C_{26}H_{48}N_4O_7$ (528.7): C, 59.06; H, 9.15; N, 10.60. Found: C, 59.11; H, 9.23; N, 10.48.

***O*-2-Acetamido-2-deoxy-6-*O*-(*p*-toluenesulfonyl)-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-4-octadecene-1,3-diol (25).** To a solution of **24** (100 mg, 0.19 mmol) in pyridine (3 mL) cooled to 0 °C, was added *p*-toluenesulfonyl chloride (80 mg, 0.42 mmol), and the mixture was stirred for 4 h at 0 °C. MeOH (1 mL) was added to the mixture and the mixture was concentrated to a syrup which was chromatographed on a column of silica gel (30 g) with 15:1 dichloromethane-methanol to give **25** (83 mg, 64%) as an amorphous mass: $[\alpha]_D$ -21.5° (*c* 0.53, $CHCl_3$); ^{1}H NMR (CD_3OD) GlcNAc unit δ 1.98 (s, 3H, AcN), 2.47 (s, 3H, Me), 4.42 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), and 7.44-7.83 (m, 4H, Ph); sphingosine unit δ 0.91 (t, 3H, *Me*CH_2), 1.29 (s, 22H, 11CH_2), 5.51 (dd, 1H, $J_{3,4}$ = 7.4 Hz, $J_{4,5}$ = 15.4 Hz, H-4), and 5.75 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5).

Anal. Calcd for $C_{33}H_{54}N_4O_9S$ (682.9): C, 58.04; H, 7.97; N, 8.21. Found: C, 58.11; H, 8.09; N, 8.21.

***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-acetamido-2-deoxy-6-thio-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-4-octadecene-1,3-diol (26).** A solution of **25** (83 mg, 0.12 mmol) and **11** (130 mg, 0.25 mmol) in DMF (3 mL) was heated overnight at 45 °C under N_2, and concentrated. Column chromato-graphy (20:1 dichloromethane-methanol) of the residue on silica gel (40 g) gave **26** (94 mg, 76%) as an amorphous mass: $[\alpha]_D$ +13.5° (*c* 0.8, $CHCl_3$); IR (KBr) 3600-3200 (OH, NH), 2100 (N_3), 1730 and 1230 (ester), and 1650 and 1550 cm^{-1} (amide); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 2.03, 2.05, 2.16, 2.19 (4s, 12H, 4AcO), 2.71 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e), 3.81 (s, 3H, MeO), 4.85 (ddd, 1H, $J_{3a,4}$ = 11.4 Hz, $J_{4,5}$ = 10.3 Hz, H-4), 5.30 (broad d, 1H, $J_{7,8}$ = 8.8 Hz, H-7), and 5.42 (m, 1H, H-8); GlcNAc unit δ 2.87 (dd, 1H, $J_{5,6}$ = 9.0 Hz, $J_{6,6'}$ = 13.9 Hz, H-6), and 4.56 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1); sphingosine unit δ 0.88 (t, 3H, *Me*CH_2), 1.26 (s, 22H, 11CH_2), 5.51 (dd, 1H, $J_{3,4}$ = 7.3 Hz, $J_{4,5}$ = 15.4 Hz, H-4), and 5.79 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5); other group δ 1.87, 2.02 (2s, 6H, 2AcN).

Anal. Calcd for $C_{46}H_{75}N_5O_{18}S$ (1018.2): C, 54.26; H, 7.42; N, 6.88. Found: C, 54.26; H, 7.49; N, 6.63.

***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-acetamido-2-deoxy-6-thio-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol (28).** Selective reduction of the azide group in **26** (94 mg, 0.09 mmol) and subsequent coupling with octadecanoic acid (61.8 mg, 0.21 mmol) as described for **14**, afforded **28** (71 mg, 61%) as an amorphous mass: $[\alpha]_D$ -4.0° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 2.69 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e), 3.82 (s, 3H, MeO), 4.87 (ddd, 1H, $J_{3a,4}$ = 12.2 Hz, $J_{4,5}$ = 11.2 Hz, H-4), 5.31 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 11.0 Hz, H-7) and 5.45 (m, 1H, H-8); GlcNAc unit δ 2.85 (2m, 2H, H-6,6') and 4.46 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1); Cer unit δ 0.87 (t, 6H, 2*Me*CH_2), 1.25 (s, 52H, 26CH_2), 5.45 and 5.75 (2m, 2H, H-4,5); other groups δ 1.88, 2.03 (2s, 6H, 2AcN), 2.04, 2.06, 2.16, and 2.19 (4s, 12H, 4AcO), and 6.35, 6.54, and 6.92 (3d, 3H, NH).

Anal. Calcd for $C_{64}H_{111}N_3O_{19}S$ (1258.7): C, 61.07; H, 8.90; N, 3.34. Found: C, 61.00; H, 9.14; N, 3.39.

***S*-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-(2-acetamido-2-deoxy-6-thio-β-D-gluco-**

pyranosyl)-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol (29). Deacetylation and saponification of **28** (31 mg, 0.02 mmol), as described for **15**, yielded **29** (26 mg, quantitative) as an amorphous mass; $[\alpha]_D$ -0.6° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, 6H, 2*Me*CH_2), 1.26 (s, 52H, 26CH_2), 1.82, 1.84 (2s, 6H, 2AcN), 2.75 (m, 1H, H-3e for Neu5Ac), 2.85 (2m, 2H, H-6,6' for GlcNAc), 4.29 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1 for GlcNAc), and 5.35, 5.72 (2m, 2H, H-4,5 for Cer).

Anal. Calcd for $C_{55}H_{101}N_3O_{15}S$ (1076.5): C, 61.36; H, 9.46; N, 3.90. Found: C, 61.10; H, 9.65; N, 3.86.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 03255207) for the Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

REFERENCES AND FOOTNOTES

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

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

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

4. M. J. Polley, M. L. Phillips, E. Wayner, E. Nudelman, A. K. Singhal, S. Hakomori, and J. C. Paulson, *Proc. Natl. Acad. Sci. USA*, **88**, 6224 (1991).

5. H. Nojiri, M. Stroud, and S. Hakomori, *J. Biol. Chem.*, **266**, 4531 (1991).

6. I. Eggens, B. Fenderson, T. Toyokuni, B. Dean, M. Stroud, and S. Hakomori, *J. Biol. Chem.*, **264**, 9476, 20159 (1989).

7. A. Hasegawa, M. Morita, Y. Kojima, H. Ishida, and M. Kiso, *Carbohydr. Res.*, **214**, 43 (1991).

8. A. Hasegawa, H. Ogawa, H. Ishida, and M. Kiso, *Carbohydr. Res.*, in press, and the references cited therein.

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

10. A. Hasegawa, J. Nakamura, and M. Kiso, *J. Carbohydr. Chem.*, **5**, 21 (1986).

11. A. Hasegawa, J. Nakamura, and M. Kiso, *J. Carbohydr. Chem.*, **5**, 11 (1986).

12. a) T. Adachi, Y. Yamada, I. Inoue, and M. Saneyoshi, *Synthesis*, 45 (1977); b) Y. Ito, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 285 (1989).

13. This acid was purchased from Wako Chemical Co., (Osaka, Japan).

14. R. R. Schmidt and P. Zimmermann, *Angew. Chem. Int. Ed. Engl.*, **29**, 725 (1986).

15. R. U. Lemieux, T. Takeda, and B. Y. Chung, *ACS Symp Ser.*, **39**, 90 (1976).