Carbohydrate Research, 224 (1992) 175–184
Elsevier Science Publishers B.V., Amsterdam

Synthesis of an *S*-(α-sialosyl)-(2→9)-*O*-(α-sialosyl)-(2→3′)-β-lactosylceramide*

Akira Hasegawa, Hirotsugu Ogawa, Hideharu Ishida, and Makoto Kiso
Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-11 (Japan)

(Received April 24th, 1991; accepted August 13th, 1991)

ABSTRACT

A ganglioside GD_3 analog has been synthesized having an *N*-acetylneuraminic acid (Neu5Ac) residue α-thioglycosidically linked to C-9 of the Neu5Ac residue in the ganglioside GM_3 structure. Glycosylation of 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside with methyl (methyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-bromo-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate, which was prepared from methyl (methyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate by 8,9-*O*-isopropylidenation, *O*-acetylation, hydrolysis of the isopropylidene group, selective bromination, and *O*-acetylation, using dimethyl(methylthio)sulfonium triflate (DMTST) as a promoter, gave the α-sialosyl-(2→3′)-lactoside **8**. Coupling of the *O*-acetyl derivative of **8** with the sodium salt of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate gave an α-thioglycosidically linked tetrasaccharide. This was converted, *via* selective removal of the 2-(trimethylsilyl)ethyl group, trichloroacetimidation, and glycosidation with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol into a ganglioside precursor. Finally the precursor on selective reduction of the azide group, coupling with octadecanoic acid, *O*-deacylation, and de-esterification gave the analog, *S*-(*N*-acetyl-α-neuraminosyl)-(2→9)-*O*-(*N*-acetyl-9-thio-α-neuraminosyl)-(2→3′)-β-lactosylceramide.

INTRODUCTION

Recently, we have synthesized several ganglioside analogs containing a sulfur atom in place of oxygen[1-4]. These analogs are not hydrolyzed by influenza-virus sialidases[5], and indeed constitute a new type of potent sialidase inhibitors. Thus, we believe further modifications of the ganglioside molecule should be carried out, not only to obtain enzyme inhibitors, but also to elucidate the functions of sialic acid-containing glycosphingolipids at the molecular level. We describe here the synthesis of a thioglycosidically linked, position-isomeric analog of ganglioside GD_3, *S*-(*N*-acetyl-α-neuraminosyl)-(2→9)-*O*-(*N*-acetyl-9-thio-α-neuraminosyl)-(2→3′)-β-lactosylceramide.

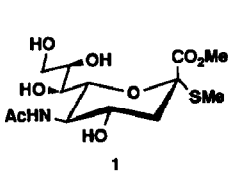
* Studies on the thioglycosides of *N*-acetylneuraminic acid, Part 9. For Part 8, see ref. 1.

0008-6215/92/$ 05.00

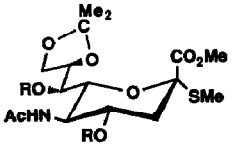
RESULTS AND DISCUSSION

For the synthesis of the target ganglioside analog we set out to synthesize the per-*O*-acylated 2-(trimethylsilyl)ethyl *S*-(methyl *N*-acetyl-α-neuraminosyl)-(2→9)-*O*-(methyl *N*-acetyl-9-thio-α-neuraminosyl)-(2→3′)-β-lactoside (**11**) as an intermediate. Compound **11** could then, by coupling to the ceramide moiety, be transformed into the end product.

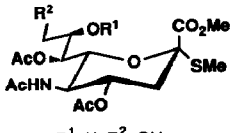
Treatment of methyl (methyl 5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**1**) with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid monohydrate gave the 8,9-*O*-isopropylidene derivative **2** in 70% yield, and this was acetylated with acetic anhydride in pyridine to give **3**. The ^{1}H-n.m.r. spectrum of **3** exhibited signals for one isopropylidene group at δ 1.34 and 1.36, one *N*-acetyl at δ 1.88, two *O*-acetyl and one *S*-methyl groups at 2.03, 2.15, and 2.17, a methyl ester group at 3.85, H-3*eq* at 2.78 (J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz), H-4 at 4.96 ($J_{3ax,4}$ 11.8, $J_{4,5}$ 10.4 Hz), and H-7 as a doublet of doublets at δ 5.39 ($J_{6,7}$ 2.0, $J_{7,8}$ 3.9 Hz), consistent with the assignment of the 8,9-*O*-isopropylidene structure. Removal of the isopropylidene group from **3** with aqueous 80% acetic acid gave compound **4** in 89% yield, without acetyl migration; in the n.m.r. spectrum, H-7 appeared as a doublet of doublets at δ 5.08 ($J_{6,7}$ 2.0, $J_{7,8}$ 9.3 Hz). Selective bromination[6] of **4** with carbon tetrabromide–triphenylphosphine in pyridine gave methyl (methyl 5-acetamido-4,7-di-*O*-acetyl-9-bromo-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**5**) in 72% yield, which was quantitatively converted into the 4,7,8-tri-*O*-acetyl derivative **6**. Characteristic signals in the n.m.r. spectrum were a one-proton multiplet at δ 4.92 ($J_{3eq,4}$ 4.8, $J_{3ax,4}$ 11.7, and $J_{4,5}$ 10.3 Hz, H-4), a two-proton multiplet at 5.35 (H-7,8), and six three-proton



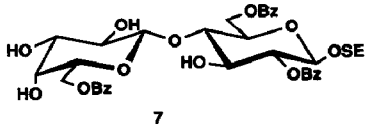
1



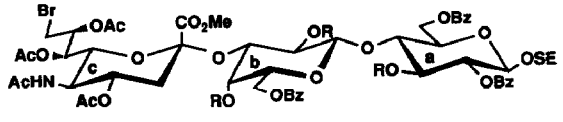
2 R=H
3 R=Ac



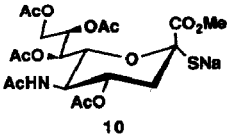
4 R^1=H, R^2=OH
5 R^1=H, R^2=Br
6 R^1=Ac, R^2=Br



7



8 R=H
9 R=Ac



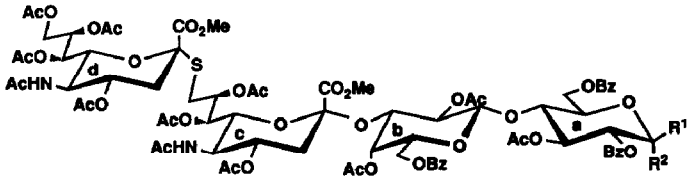
10

SE = $Me_3SiCH_2CH_2$-
Bz = benzoyl

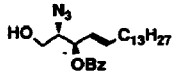
singlets at 1.85 (*N*-acetyl), 2.03, 2.15, 2.16, and 2.17 (three *O*-acetyl and one *S*-methyl), and 3.80 (methyl ester), substantiating the assigned structure.

The glycosylation[7,8] of 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside[7b] (**7**) with **6** in acetonitrile for 20 h at −15° in the presence of dimethyl(methylthio)sulfonium triflate (DMTST) as the glycosyl promoter gave a 37% yield of the expected α-glycoside **8**, in which the sialic acid is coupled to O-3′ of compound **7**. Acetylation of **8** with acetic anhydride in pyridine gave the acetate **9** in 93% yield. The structure of **9** was unambiguously proved by the ^{1}H-n.m.r. data. The observed chemical shifts and coupling constants of the sialic acid protons H-3*eq* (δ 2.59, J_{gem} 12.6, $J_{3eq,4}$ 5.0 Hz), H-4 (δ 5.07), and H-7 (δ 5.34, $J_{6,7}$ 2.8, $J_{7,8}$ 6.2 Hz) are characteristic[9–14] of α-linked sialic acids. Other ^{1}H-n.m.r. data, given in the Experimental section, are consistent with the assigned structure.

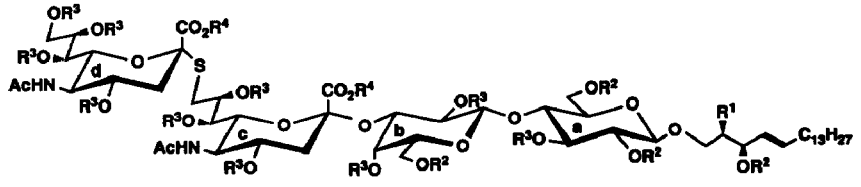
The sodium salt[12] **10**, freshly derived from methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate[12] by selective *S*-deacetylation with sodium methoxide, was condensed with compound **9** in *N*,*N*-dimethylformamide (DMF) under nitrogen to afford the desired tetrasaccharide **11** in 80% yield. Selective removal of the 2-(trimethylsilyl)ethyl group[15] in **11** was performed by treatment with boron trifluoride etherate in acetonitrile for 2.5 h at 0° to give **12** in 92% yield. When treated with trichloroacetonitrile[16] in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 2 h at 0°, **12** afforded the trichloroacetimidate **13** in quantitative yield. Signals in the ^{1}H-n.m.r. spectrum at δ 6.65 (d, $J_{1,2}$ 4.0 Hz)



11 R^1=OSE, R^2=H
12 R^1, R^2=H, OH
13 R^1=H, R^2=OC(=NH)CCl_3



14



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

and 8.56 (C=N*H*) showed the imidate to be α. The final glycosylation[2,16a] of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[2,17] (**14**) with **13** in CH_2Cl_2 in the presence of boron trifluoride etherate for 24 h at 0° afforded the corresponding β-glycoside **15** in 75% yield, after chromatography. Selective reduction[18] of the azide group in **15** with H_2S in aqueous 83% pyridine gave the amine **16** which, on condensation with octadecanoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in CH_2Cl_2, gave the acylated product **17** in 97% yield.

Finally, *O*-deacylation of **17** with sodium methoxide in methanol and subsequent saponification of the methyl ester groups yielded the desired *S*-(α-*N*-acetylneuraminosyl)-(2→9)-*O*-(α-*N*-acetyl-9-thioneuraminosyl)-(2→3′)-*O*-β-lactosyl-(1→1)-ceramide (**18**) quantitatively. The ^{1}H-n.m.r. spectrum of **18** in 49:1 $(CD_3)_2SO$–D_2O contained two doublets due to anomeric protons at δ 4.17 ($J_{1,2}$ 7.2 Hz, H-1, Glc unit) and 4.43 ($J_{1,2}$ 6.2 Hz, H-1, Gal unit). The further ^{1}H-n.m.r. data are consistent with structure **18**.

The work here described shows that the use of thioglycosides of sialic acids as glycosyl donors is effective for the synthesis of ganglioside analogs having various types of sialic acid units or sialic acid in thioglycosidic linkage. The 2-(trimethylsilyl)ethyl group is useful for protecting the anomeric hydroxyl group of the glycosyl acceptor because of its easy and selective removal and its stability toward many reagents used in carbohydrate synthesis.

EXPERIMENTAL

General methods. — Optical rotations were determined with a Union PM-201 polarimeter at 25° and i.r. spectra were recorded with a Jasco IRA-100 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a JEOL JNM-GX 270 spectrometer. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Evaporative concentrations were conducted *in vacuo*.

*Methyl (methyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio*-D-glycero-α-D-galacto-*2-nonulopyranosid)onate* (**2**). — To a solution of methyl (methyl 5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**1**, 500 mg, 1.4 mmol) in dry DMF (5 mL) were added 2,2-dimethoxypropane (0.87 mL), *p*-toluenesulfonic acid monohydrate (15 mg), and Drierite (1.0 g), and the mixture was stirred for 1 h at 0°, and then neutralized with $NaHCO_3$. The precipitate was filtered off and washed with MeOH. The filtrate and washings were combined and concentrated. The residue was chromatographed on a column of silica gel (50 g) with 40:1 CH_2Cl_2–MeOH to give compound **2** (390 mg, 70%) as an amorphous mass, $[\alpha]_D$ + 12.6° (*c* 0.58, $CHCl_3$); ν 3400 (OH, NH), 1750 and 1220 (ester), 1660 and 1550 (amide), and 850 cm^{-1} (Me_2C); ^{1}H-n.m.r. ($CDCl_3$): δ 1.34, 1.36 (2 s, 6 H, Me_2C), 1.72 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.6 Hz, H-3*ax*), 1.98 (s, 3 H, AcN), 2.16 (s, 3 H, MeS), 2.72 (dd, 1 H, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 3.38 (dd, 1 H, $J_{5,6}$ 10.4, $J_{6,7}$ 1.3 Hz, H-6), 3.56 (dd, 1 H, $J_{7,8}$ 6.4 Hz, H-7), 3.64 (ddd, 1 H, $J_{4,5}$ 10.1 Hz, H-4), 3.79 (s, 3 H, MeO), 3.97 (dd, 1 H, $J_{8,9}$ 6.4, $J_{9,9'}$ 8.2 Hz, H-9), 4.07 (dd, 1 H, $J_{8,9'}$ 6.4 Hz, H-9′), and 4.25 (m, 1 H, H-8).

Anal. Calc. for $C_{16}H_{27}NO_8S$ (393.5): C, 48.83; H, 6.92; N, 3.56. Found: C, 48.74; H, 6.95; N, 3.40.

*Methyl (methyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-*D*-glycero-α-*D*-galacto-2-nonulopyranosid)onate* (**3**). — Compound **2** (390 mg, 0.99 mmol) was acetylated with Ac_2O (2 mL) in pyridine (4 mL) overnight at room temperature. The product was purified by chromatography on silica gel (60 g) with 60:1 CH_2Cl_2–MeOH to give **3** (470 mg, quantitative) as an amorphous mass, $[\alpha]_D$ + 14.6° (*c* 0.8, $CHCl_3$); *v* 3400 (NH), 1750 and 1230 (ester), 1670 and 1550 (amide), and 860 cm^{-1} (Me_2C); ^{1}H-n.m.r. ($CDCl_3$): δ 1.34, 1.36 (2 s, 6 H, Me_2C), 1.88 (s, 3 H, AcN), 2.03, 2.15, 2.17 (3 s, 9 H, 2 AcO, MeS), 2.78 (dd, 1 H, $J_{3ax,3eq}$ 12.8, $J_{3eq,4}$ 4.8 Hz, H-3*eq*), 3.73 (dd, 1 H, $J_{5,6}$ 10.6, $J_{6,7}$ 2.0 Hz, H-6), 3.85 (s, 3 H, MeO), 4.62 (ddd, 1 H, $J_{7,8}$ 3.9, $J_{8,9}$ 7.0, $J_{8,9'}$ 6.8 Hz, H-8), 4.96 (ddd, $J_{3ax,4}$ 11.8, $J_{4,5}$ 10.4 Hz, H-4), 5.39 (dd, 1 H, H-7), and 5.45 (d, 1 H, $J_{NH,5}$ 9.9 Hz, N*H*).

Anal. Calc. for $C_{20}H_{31}NO_{10}S$ (477.5): C, 50.30; H, 6.54; N, 2.93. Found: C, 50.21; H, 6.48; N, 2.88.

*Methyl (methyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-*D*-glycero-α-*D*-galacto-2-nonulopyranosid)onate* (**4**). — A solution of **3** (2.12 g, 4.44 mmol) in aqueous 80% acetic acid (20 mL) was heated for 3.5 h at 45°, and evaporated. The residue was chromatographed on a column of silica gel (100 g) with 80:1 CH_2Cl_2–MeOH to give **4** (1.73 g, 89%) as an amorphous mass, $[\alpha]_D$ + 33.8° (*c* 0.7, $CHCl_3$); *v* 3700–3200 (OH, NH), 1740 and 1230 (ester), and 1670 and 1560 cm^{-1} (amide); ^{1}H-n.m.r. ($CDCl_3$): δ 1.89 (s, 3 H, AcN), 2.02, 2.15, 2.16 (3 s, 9 H, 2 AcO, MeS), 2.81 (dd, 1 H, $J_{3ax,3eq}$ 12.8, $J_{3eq,4}$ 4.8 Hz, H-3*eq*), 3.49 (dd, 1 H, $J_{8,9}$ 3.7, $J_{9,9'}$ 12.6 Hz, H-9), 3.70 (s, 3 H, MeO), 3.77 (dd, 1 H, $J_{5,6}$ 10.3, $J_{6,7}$ 2.2 Hz, H-6), 4.24 (q, $J_{4,5}$ 10.4, $J_{5,NH}$ 8.4 Hz, H-5), 4.87 (ddd, 1 H, $J_{3ax,4}$ 11.4 Hz, H-4), 5.08 (dd, 1 H, $J_{7,8}$ 9.3 Hz, H-7), and 6.06 (d, 1 H, N*H*).

Anal. Calc. for $C_{17}H_{27}NO_{10}S$ (437.5): C, 46.67; H, 6.22; N, 3.20. Found: C, 46.51; H, 6.36; N, 3.15.

*Methyl (methyl 5-acetamido-4,7-di-*O*-acetyl-9-bromo-3,5,9-trideoxy-2-thio-*D*-glycero-α-*D*-galacto-2-nonulopyranosid)onate* (**5**). — A solution of **4** (2.0 g, 4.57 mmol) in pyridine (40 mL) was treated with carbon tetrabromide (3.03 g, 9.14 mmol), and cooled to 0°. Triphenylphosphine (2.4 g, 9.15 mmol) was added, and the mixture was stirred for 5 h at room temperature. Methanol (1 mL) was added to the mixture, and it was concentrated to a syrup, which was chromatographed on a column of silica gel (100 g) with 2:1 EtOAc–hexane to give **5** (1.65 g, 72%) as an amorphous mass, $[\alpha]_D$ + 38.5° (*c* 0.94, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.89 (s, 3 H, AcN), 2.05, 2.15, 2.16 (3 s, 9 H, 2 AcO, MeS), 2.81 (dd, 1 H, $J_{3ax,4eq}$ 12.9, $J_{3eq,4}$ 4.9 Hz, H-3*eq*), 3.33 (dd, 1 H, $J_{8,9}$ 7.0, $J_{9,9'}$ 11.0 Hz, H-9), 3.51 (dd, 1 H, $J_{8,9'}$ 2.8 Hz, H-9'), 3.79 (dd, 1 H, $J_{5,6}$ 10.6, $J_{6,7}$ 2.4 Hz, H-6), 3.89 (s, 3 H, MeO), 4.14 (m, 1 H, H-8), 4.86 (ddd, 1 H, $J_{3ax,4}$ 11.5, $J_{4,5}$ 10.3 Hz, H-4), 5.09 (dd, 1 H, $J_{7,8}$ 8.6 Hz, H-7), and 5.59 (d, 1 H, $J_{NH,5}$ 10.3 Hz, N*H*).

Anal. Calc. for $C_{17}H_{26}BrNO_9S$ (500.4): C, 40.80; H, 5.24; N, 2.80. Found: C, 40.63; H, 5.30; N, 2.76.

*Methyl (methyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-bromo-3,5,9-trideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosid)onate* (**6**). — Compound **5** (1.35 g, 2.7 mmol)

was acetylated with Ac_2O (5 mL) in pyridine (10 mL) overnight at room temperature. The product was purified by chromatography on silica gel (60 g) with 2:1 EtOAc–hexane to give **6** (1.45 g, quantitative) as an amorphous mass, $[\alpha]_D$ +28.0° (*c* 0.88, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.85 (s, 3 H, AcN), 2.03, 2.15, 2.16, 2.17 (4 s, 12 H, 3 AcO, MeS), 2.76 (dd, 1 H, $J_{3ax,3eq}$ 12.6, $J_{3eq,4}$ 4.8 Hz, H-3*eq*), 3.38 (dd, 1 H, $J_{8,9}$ 6.1, $J_{9,9'}$ 11.4 Hz, H-9), 3.80 (s, 3 H, MeO), 3.83 (dd, 1 H, $J_{8,9'}$ 2.8 Hz, H-9'), 3.90 (dd, 1 H, $J_{5,6}$ 10.6, $J_{6,7}$ 1.8 Hz, H-6), 4.92 (ddd, 1 H, $J_{3ax,4}$ 11.7, $J_{4,5}$ 10.3 Hz, H-4), 5.35 (m, 2 H, H-7,8), and 5.84 (d, 1 H, $J_{NH,5}$ 9.7 Hz, N*H*).

Anal. Calc. for $C_{19}H_{28}BrNO_{11}S$ (542.4): C, 42.07; H, 5.20; N, 2.58. Found: C, 42.02; H, 5.33; N, 2.49.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-9-bromo-3,5,9-trideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-2,6-di*-O-*benzoyl-β*-D-*glucopyranoside* (**8**). — To a solution of 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside[7b] (**7**, 800 mg, 1.06 mmol) and **6** (1.15 g, 2.12 mmol) in dry MeCN (10 mL) was added molecular sieves 3A (MS-3A, 2.5 g). The mixture was stirred for 10 h at room temperature and then cooled to −25°. To the cooled mixture was added, with stirring, a mixture (2.94 g, 75% DMTST by weight) of dimethyl(methylthio)sulfonium triflate[7,19] (DMTST) and MS-3A, and the stirring was continued for 20 h at −15°. The solids were filtered off and washed thoroughly with CH_2Cl_2. The filtrate and washings were combined and the solution was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and evaporated to a syrup that was chromatographed on a column of silica gel (80 g) with 3:1 EtOAc–hexane to give **8** (490 mg, 37%) as an amorphous mass, $[\alpha]_D$ +9.1° (*c* 0.78, $CHCl_3$); ^{1}H-n.m.r. (1:1 CD_3OD–$CDCl_3$): δ 0.88 (m, 2 H, Me_3SiCH_2), 1.88 (s, 3 H, AcN), 2.03, 2.11, 2.16 (3 s, 9 H, 3 AcO), 2.69 (dd, 1 H, $J_{3ax,3eq}$ 13.0, $J_{3eq,4}$ 4.8 Hz, H-3c*eq*), 3.59, 4.60 (2 ddd, 2 H, $Me_3SiCH_2CH_2O$), 3.82 (s, 3 H, MeO), 4.60 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 4.66 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 4.91 (ddd, 1 H, $J_{3ax,4}$ 12.1, $J_{4,5}$ 10.1 Hz, H-4c), 5.23 (dd, 1 H, $J_{6,7}$ 2.4, $J_{7,8}$ 8.1 Hz, H-7c), 5.27 (dd, 1 H, H-2a), 5.40 (m, 1 H, H-8c), and 7.30–8.08 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{56}H_{70}BrNO_{24}Si$ (1249.2): C, 53.84; H, 5.65; N, 1.12. Found: C, 53.82; H, 5.63; N, 1.14.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-9-bromo-3,5,9-trideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-3*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*glucopyranoside* (**9**). — Compound **8** (540 mg, 0.43 mmol) was acetylated with Ac_2O (3 mL) in pyridine (6 mL) overnight at room temperature. The product was purified by chromatography on silica gel (50 g) with 2:1 EtOAc–hexane to give **9** (550 mg, 93%) as an amorphous mass, $[\alpha]_D$ +5.0° (*c* 0.6, $CHCl_3$); ^{1}H-n.m.r. (1:1 CD_3OD–$CDCl_3$): δ 0.87 (m, 2 H, Me_3SiCH_2), 1.65 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.6 Hz, H-3c*ax*), 1.83 (s, 3 H, AcN), 1.98, 2.00, 2.03, 2.09, 2.12, and 2.22 (6 s, 18 H, 6 AcO), 2.59 (dd, 1 H, $J_{3eq,4}$ 5.0 Hz, H-3c*eq*), 3.42 (dd, 1 H, $J_{8,9}$ 6.2, $J_{9,9'}$ 11.7 Hz, H-9c), 3.63 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 2.8 Hz, H-6c), 3.77 (s, 3 H, MeO), 4.59 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.3 Hz, H-3b), 4.69 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1a), 4.87 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1b), 5.06 (d, 1 H, H-4b), 5.07 (m, 1 H, H-4c), 5.22 (dd, 1 H, $J_{2,3}$

9.5 Hz, H-2a), 5.34 (dd, 1 H, $J_{7,8}$ 6.2 Hz, H-7c), 5.42 (m, 1 H, H-8c), 5.45 (t, 1 H, H-3a), and 7.40–8.07 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{62}H_{76}BrNO_{27}Si$ (1375.3): C, 54.14; H, 5.57; N, 1.02. Found: C, 54.03; H, 5.69; N, 1.05.

2-(Trimethylsilyl)ethyl S-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-3*-O-*acetyl-2,4-di*-O-*benzoyl-β*-D-*glucopyranoside* (**11**). — A solution of **9** (400 mg, 0.29 mmol) and the sodium salt[12] **10** (308 mg, 0.58 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 room temperature under N_2, and concentrated. The residue was chromatographed on a column of silica gel (60 g) with EtOAc to give **11** (421 mg, 80%) as an amorphous mass, $[\alpha]_D$ +9.0° (*c* 0.58, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 0.90 (m, 2 H, Me_3SiCH_2), 1.63 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.5 Hz, H-3c*ax*), 1.85 (2 s, 6 H, 2 AcN), 2.00 (2), 2.02 (2), 2.06, 2.09, 2.10, 2.12, 2.21 (2), (7 s, 30 H, 10 AcO), 2.57 (dd, 1 H, $J_{3eq,4}$ 12.3 Hz, H-3c*eq*), 2.62 (dd, 1 H, $J_{3ax,3eq}$ 12.5, $J_{3eq,4}$ 4.2 Hz, H-3d*eq*), 3.71, 3.77 (2 s, 6 H, 2 MeO), 4.66 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1a), 4.79 (m, 2 H, H-4c,4d), 4.86 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1b), 5.04 (d, 1 H, H-4b), 5.20 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2a), 5.34 (m, 4 H, H-7c,7d, 8c,8d), 5.46 (t, 1 H, H-3a), and 7.39–8.09 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{82}H_{104}N_2O_{39}SSi$ (1801.9): C, 54.66; H, 5.82; N, 1.55. Found: C, 54.49; H, 6.03; N, 1.49.

S-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-3*-O-*acetyl-2,6-di*-O-*benzoyl*-D-*glucopyranose* (**12**). — To a solution of **11** (300 mg, 0.17 mmol) in CH_3CN (2 mL), cooled to 0°, $BF_3{\cdot}OEt_2$ (0.2 mL) was added dropwise. The mixture was stirred for 2.5 h at 0°. Dichloromethane (50 mL) was added, and the solution was successively washed with M $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (30 g) with 40:1 CH_2Cl_2–MeOH to give **12** (260 mg, 92%) as an amorphous mass, $[\alpha]_D$ +28.8° (*c* 0.66, $CHCl_3$); *v* 3600–3300 (OH, NH), 1740 and 1230 (ester), 1660 and 1540 (amide), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 1.84, 1.85 (2 s, 6 H, 2 AcN), 1.99 (2), 2.01, 2.03 (2), 2.04, 2.06, 2.14, 2.19, 2.20 (8 s, 30 H, 10 AcO), 2.57 (dd, 1 H, $J_{3ax,3eq}$ 12.6, $J_{3eq,4}$ 4.4 Hz, H-3c*eq*), 2.62 (m, 1 H, H-3d*eq*), 3.74, 3.76 (2 s, 6 H, 2 MeO), 4.66 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.3 Hz, H-3b), 4.80 (m, 2 H, H-4c,d), 4.96 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 5.04 (br. d, 1 H, H-4b), 5.30–5.35 (m, 4 H, H-7c,d and 8c,d), 5.79 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3a), and 7.39–8.08 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{77}H_{92}N_2O_{39}S$ (1701.6): C, 54.35; H, 5.45; N, 1.65. Found: C, 54.20; H, 5.58; N, 1.56.

S-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5,-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-

acetyl-6-O-benzoyl-β-D-galactopyranosyl)-(1→4)-3-O-acetyl-2,6-di-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**13**). — To a stirred solution of **12** (200 mg, 0.12 mmol) in dry CH_2Cl_2 (2 mL), cooled to 0°, were added Cl_3CCN (0.12 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.018 mL). The mixture was stirred for 2 h at 0°, the progress of the reaction being monitored by t.l.c., then directly chromatographed on a column of silica gel (50 g) with 30:1 CH_2Cl_2–MeOH to give **13** (216 mg, quantitative) as an amorphous mass, $[\alpha]_D$ + 29.5° (*c* 0.8, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.65 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.5 Hz, H-3c*ax*), 1.87 (2 s, 6 H, 2 AcN), 2.00 (2), 2.03, 2.04, 2.05, 2.08 (2), 2.14, 2.18, and 2.22 (8 s, 30 H, 10 AcO), 2.58 (dd, 1 H, $J_{3eq,4}$ 4.4 Hz, H-3c*eq*), 2.63 (dd, 1 H, $J_{3ax,3eq}$ 12.5, $J_{3eq,4}$ 4.6 Hz, H-3d*eq*), 2.83 (m, 2 H, H-9c,9'c), 3.75, 3.78 (2 s, 6 H, 2 MeO), 4.66 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.3 Hz, H-3b), 4.84 (m, 2 H, H-4c,d), 4.93 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1b), 5.06 (br. d, 1 H, H-4b), 5.36 (m, 4 H, H-7c,d and 8c,d), 5.63 (d, 1 H, $J_{NH,5}$ 9.9 Hz, N*H*), 5.83 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3a), 6.65 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1a), 7.38–8.09 (m, 15 H, 3 Ph), and 8.56 (s, 1 H, C = N*H*).

Anal. Calc. for $C_{79}H_{92}Cl_3N_3O_{39}S$ (1846.0): C, 51.40; H, 5.02; N, 2.28. Found: C, 51.55; H, 5.13; N, 2.09.

S-*(Methyl* to-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3*-O-*benzoyl-4-octadecene-1,3-diol* (**15**). — To a solution of **13** (247 mg, 0.134 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecane-1,3-diol[2,17] (**14**, 172 mg, 0.40 mmol) in dry CH_2Cl_2 (6 mL) was added MS-4A (AW-300, 2 g), and the mixture was stirred for 1.5 h at room temperature then cooled to − 15°. Boron trifluoride etherate (0.02 mL) was added to the mixture, and this was stirred for 4 h at − 15°, the progress of the reaction being monitored by t.l.c. The solids were filtered off and washed with CH_2Cl_2. The solution was washed successively with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated to a syrup that was chromatographed on a column of silica gel (20 g) with 40:1 CH_2Cl_2–MeOH to give **15** (215 mg, 75%) as an amorphous mass, $[\alpha]_D$ + 1.65° (*c* 0.61, $CHCl_3$); *v* 3500 (NH), 2100 (azide), 1740 and 1230 (ester), 1640 and 1540 (amide), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 3 H, C*H*$_3$CH$_2$), 1.25 (s, 22 H, 11 C*H*$_2$), 1.65 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.6 Hz, H-3c*ax*), 1.87 (s, 6 H, 2 AcN), 1.99, 2.00, 2.01, 2.02, 2.05, 2.07, 2.10, 2.12, 2.17, 2.23 (10 s, 30 H, 10 AcO), 2.58 (dd, 1 H, $J_{3eq,4}$ 4.4 Hz, H-3c*eq*), 2.64 (dd, 1 H, $J_{3ax,3eq}$ 12.8, $J_{3eq,4}$ 4.4 Hz, H-3d*eq*), 2.82 (m, 2 H, H-9c,9'c), 3.74, 3.78 (2 s, 6 H, MeO), 4.64 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 2.9 Hz, H-3b), 4.67 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1b), 4.82 (m, 2 H, H-4c,d), 4.85 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1a), 5.00 (dd, 1 H, H-2b), 5.05 (br. d, 1 H, H-4b), 5.17, 5.62 (2 d, 2 H, $J_{NH,5}$ 10.3 Hz, 2 N*H*), 5.23 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2a), 5.36 (m, 4 H, H-7c,d and 8c,d), 5.40 (dd, 1 H, $J_{3,4}$ 8.1, $J_{4,5}$ 15.4 Hz, H-4 of sphingosine), 5.44 (t, 1 H, H-3a), 5.51 (dd, 1 H, $J_{2,3}$ 3.7 Hz, H-3 of sphingosine), 5.65 (dt, 1 H, $J_{5,6} = J_{5,6'}$ = 6.8 Hz, H-5 of sphingosine) and 7.32–8.09 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{102}H_{129}N_5O_{41}S$ (2113.2): C, 57.97; H, 6.15; N, 3.31. Found: C, 59.77; H, 6.30; N, 3.15.

S-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→1)-(2*S,*3*R,*4*E*)-3*-O-*benzoyl-2-octadecanamido-4-octadecene-1,3-diol* (**17**). — Hydrogen sulfide was bubbled through a solution of **15** (180 mg, 0.085mmol) in pyridine (10 mL) and water (2 mL) for 3 days while the solution was stirred at 0°, the course of the reaction being monitored by t.l.c. The mixture was concentrated to give the syrupy amine **16**, which was used without further purification in the next reaction. For this, a solution of **16** in dry CH_2Cl_2 (8 mL) was treated with octadecanoic acid (73 mg, 0.256 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 49 mg, 0.256 mmol), and the mixture was stirred overnight at room temperature. After completion of the reaction CH_2Cl_2 (30 mL) was added to the mixture, and the solution was washed with water, dried (Na_2SO_4), and evaporated to a syrup that was chromatographed on a column of silica gel (30 g) with 50:1 CH_2Cl_2–MeOH to give compound **17** (193 mg, 97%) as an amorphous mass, $[\alpha]_D$ + 12.8° (*c* 0.6, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 6 H, 2 C*H*$_3$CH$_2$), 1.26 (s, 50 H, 25 CH$_2$), 1.64 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.8 Hz, H-3c*ax*), 1.75, 1.87 (2 s, 6 H, 2 AcN), 1.88, 2.00 (2), 2.01, 2.06, 2.08, 2.11, 2.12, 2.14, 2.23 (9 s, 30 H, 10 AcO), 2.57 (dd, 1 H, $J_{3eq,4}$ 4.4 Hz, H-3c*eq*), 2.65 (dd, 1 H, $J_{3ax,3eq}$ 12.6, $J_{3eq,4}$ 4.6 Hz, H-3d*eq*), 2.80 (dd, 1 H, $J_{8,9}$ 9.3, $J_{9,9'}$ 13.6 Hz, H-9d), 2.90 (dd, 1 H, H-9'd), 3.75, 3.77 (2 s, 6 H, 2 MeO), 4.60 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1a), 4.65 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 2.7 Hz, H-3b), 4.83 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1b), 4.87 (m, 2 H, H-4c,d), 5.02 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2a), 5.19 (d, 1 H, N*H*), 5.37 (m, 4 H, H-7c,d and 8c,d), 5.76 (dt, 1 H, $J_{4,5}$ 14.7, $J_{5,6} = J_{5,6'}$ = 7.0 Hz, H-5 of ceramide), and 7.24–8.09 (m, 20 H, 5 Ph).

Anal. Calc. for $C_{120}H_{165}N_3O_{42}S$ (2353.7): C, 61.23; H, 7.07; N, 1.79. Found: C, 61.11; H, 7.20; N, 1.74.

S-*(5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→9)*-O-*(5-acetamido-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)*-O-*β*-D-*galactopyranosyl-(1→4)*-O-*β*-D-*glucopyranosyl-(1→1)-(2*S,*3*R,*4*E*)-2-octadecanamido-4-octadecene-1,3-diol* (**18**). — To a solution of **17** (130 mg, 0.055 mmol) in MeOH (4 mL) was added NaOMe (25 mg), and the mixture was stirred overnight at room temperature. Potassium hydroxide (0.2M, 2 mL) was added and the mixture was again stirred overnight at room temperature, then neutralized with Amberlite IR-120 (H^+) resin, and filtered. The resin was thoroughly washed with 10:10:1 $CHCl_3$–MeOH–H_2O, and the combined filtrate and washings were concentrated. Column chromatography (10:10:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 (40 g) gave **18** (81 mg, quantitative) as an amorphous mass, $[\alpha]_D$ +24.5° (*c* 1:1 $CHCl_3$–MeOH); ^{1}H-n.m.r. [49:1 $(CD_3)_2SO$–D_2O]: δ 0.85 (t, 6 H, 2 C*H*$_3$CH$_2$), 1.24 (s, 50 H, 25 CH$_2$), 1.66, 1.89 (2 s, 6 H, 2 AcN), 2.04 (t, 2 H, *J* 6.6 Hz, COC*H*$_2$CH$_2$), 2.68, 2.75 (2 m, 2 H, H-3c*eq*,3d*eq*), 4.17 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1a), 4.43 (d, 1 H, $J_{1,2}$ 6.2 Hz, H-1b), 5.34 (dd, 1 H, $J_{3,4}$ 5.9, $J_{4,5}$ 15.4 Hz, H-4 of ceramide), and 5.54 (dt, 1 H, H-5 of ceramide).

Anal. Calc. for $C_{70}H_{125}N_3O_{28}S$ (1488.8): C, 56.47; H, 8.46; N, 2.82. Found: C, 56.31; H, 8.68; N, 2.75.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 02259206) for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1 A. Hasegawa, H. Ogawa, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, (1991) in press.
2 Y. Ito, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 285–294.
3 A. Hasegawa, M. Morita, Y. Ito, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, 9 (1990) 369–392.
4 A. Hasegawa, M. Morita, Y. Kojima, H. Ishida, and M. Kiso, *Carbohydr. Res.*, 214 (1991) 43–53.
5 Y. Suzuki, K. Sato, M. Kiso, and A. Hasegawa, *Glycoconjugate J.*, 7 (1990) 349–356.
6 A. K. M. Anisuzzaman and R. L. Whistler, *Carbohydr. Res.*, 61 (1978) 511–518.
7 (a) T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 184 (1988) c1–c4; (b) *ibid.*, 188 (1989) 71–80; (c) T. Murase, A. Kamayama, K. P. R. Kartha, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 265–283.
8 A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, and M. Kiso, *Carbohydr. Res.*, 212 (1991) 277–281.
9 O. Kanie, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 7 (1988) 501–506.
10 K. Okamoto, T. Kondo, and T. Goto, *Tetrahedron Lett.*, 27 (1986) 5229–5232, 5233–5236.
11 Y. Ito and T. Ogawa, *Tetrahedron Lett.*, 28 (1987) 6221–6224.
12 A. Hasegawa, J. Nakamura, and M. Kiso, *J. Carbohydr. Chem.*, 5 (1986) 11–19.
13 K. Okamoto and T. Goto, *Tetrahedron*, 46 (1990) 5835–5857.
14 A. Hasegawa, T. Murase, K. Adachi, M. Morita, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, 9 (1990) 181–199.
15 K. Jansson, T. Frejd, J. Kihlberg, and G. Magnusson, *Tetrahedron Lett.*, 27 (1986) 753–756.
16 (a) R. R. Schmidt and G. Grundler, *Synthesis*, (1981) 885–887; (b) M. Numata, M. Sugimoto, K. Koike, and T. Ogawa, *Carbohydr. Res.*, 163 (1987) 209–222.
17 R. R. Schmidt and P. Zimmermann, *Angew. Chem. Int. Ed. Engl.*, 29 (1986) 725–726.
18 T. Adachi, Y. Yamada, I. Inoue, and M. Saneyoshi, *Synthesis*, (1977) 45–46.
19 P. Fügedi and P. J. Garegg, *Carbohydr. Res.*, 149 (1986) c9–c12.