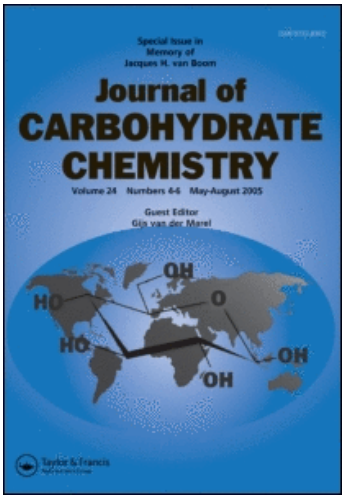

Journal of Carbohydrate Chemistry

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

Synthetic Studies on Sialoglycoconjugates 88: Synthesis of Ganglioside GM_3 and GM_4 Analogs Containing 2- OR 3-Branched Fatty-Alkyl Residues in Place of Ceramide

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To cite this Article Hasegawa, Akira , Suzuki, Naomi , Ishida, Hideharu and Kiso, Makoto(1996) 'Synthetic Studies on Sialoglycoconjugates 88: Synthesis of Ganglioside GM_3 and GM_4 Analogs Containing 2- OR 3-Branched Fatty-Alkyl Residues in Place of Ceramide', Journal of Carbohydrate Chemistry, 15: 5, 623 — 637
To link to this Article: DOI: 10.1080/07328309608005679
URL: http://dx.doi.org/10.1080/07328309608005679

J. CARBOHYDRATE CHEMISTRY, 15(5), 623-637 (1996)

SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 88: SYNTHESIS OF GANGLIOSIDE GM_3 AND GM_4 ANALOGS CONTAINING 2- OR 3-BRANCHED FATTY-ALKYL RESIDUES IN PLACE OF CERAMIDE

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Received January 27, 1996 - Final Form March 21, 1996

ABSTRACT

Each of four ganglioside GM_4 and GM_3 analogues containing 2- or 3-branched fatty alkyl residues in place of ceramide have been synthesized. Coupling of *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**13**) or *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-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-α-D-glucopyranosyl trichloroacetimidate (**14**) with 2- or 3-branched fatty-alkyl-1-ols (**9**-**12**), prepared from the corresponding branched fatty acids by methyl esterification and reduction, using BF_3•OEt_2 gave the corresponding ganglioside analogues (**15**, **17**, **19**, **21**, **23**, **25**, **27**, **29**) in good yields, which were coverted, via *O*-deacylation and de-esterification, into the title compounds.

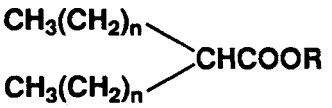
INTRODUCTION

Ganglioside GM3 was first isolated[1] from horse erythrocytes in 1952, and is the major ganglioside component in erythrocytes of many animal species.[2-5] Ganglioside GM3, as well as other gangliosides, is a polymorphous molecule at sialic acid and ceramide moieties, and it exhibits various important biological functions, serving as the influenza A virus receptor,[6] causing induction of monocytic differentiation of human myeloid cells,[7] enhancing or inhibiting protein kinase activity,[8] and exhibiting potent immunosuppressive activity[9] as well as GM4.[9a]

In view of these facts, it is an interesting substance for further investigation at the molecular level. In order to elucidate the role of the ceramide and sialic acid component in the functions of GM3 and GM4, we synthesized these gangliosides, and their analogues containing a variety of lipophilic moieties in place of the ceramide, and analogues having truncated (C7, C8) sialic acids, deoxy-*N*-acetylneuraminic acids and hydroxyl in place of the *N*-acetyl group at C-5 in *N*-acetylneuraminic acid.[10] We describe here the synthesis of ganglioside GM3 and GM4 analogues containing the 2- and 3-branched fatty alkyl residues in place of a ceramide, in order to clarify the structural features of the lipid moiety required for the immunosuppressive activity of GM3 and GM4, and to develop a new type of immunosuppresser.

RESULTS AND DISCUSSION

For the synthesis of the desired ganglioside GM4 and GM3 analogs, we employed *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate[10c] (**13**) and *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-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[10b] (**14**) as the glycosyl donors, and the 2- and 3-branched fatty-alkyl alcohols (**9-12**) prepared from the corresponding fatty acids as the glycosyl acceptors. The acceptors were coupled with the donors using boron



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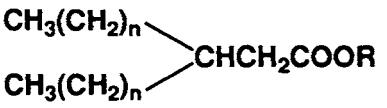
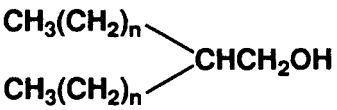
2 R = Me

n = 13, **3** R = H
4 R = Me

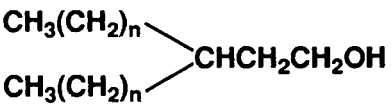


n = 8, **5** R = H
6 R = Me

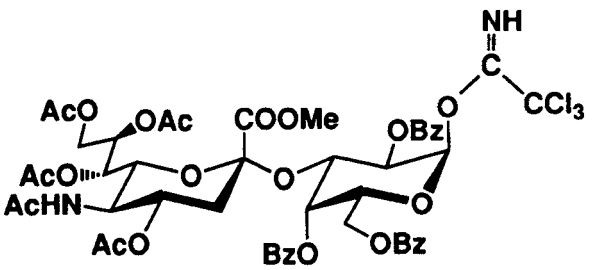
n = 12, **7** R = H
8 R = Me



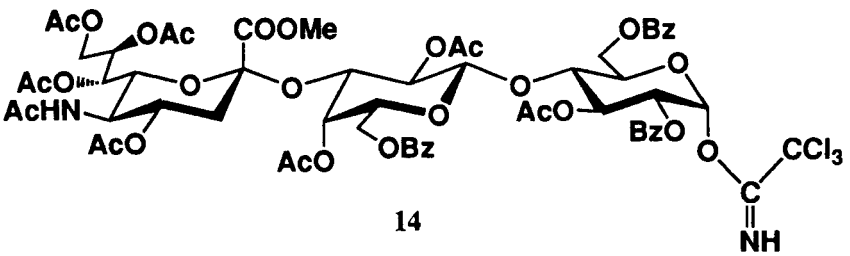
n = 9, **9**
n = 13, **10**



n = 8, **11**
n = 12, **12**



13



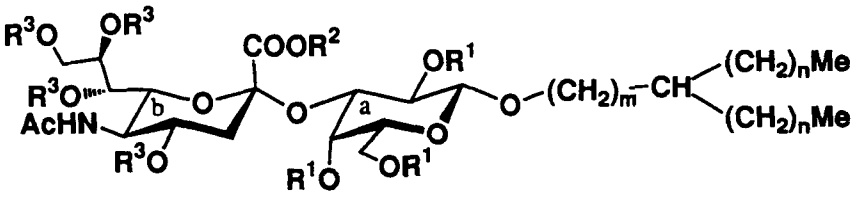
14

trifluoride etherate[11,12] as a promoter, and the removal of the protecting groups of the products could be converted into the title ganglioside GM4 and GM3 analogues containing the 2- and 3-branched fatty-alkyl residues in place of a ceramide.

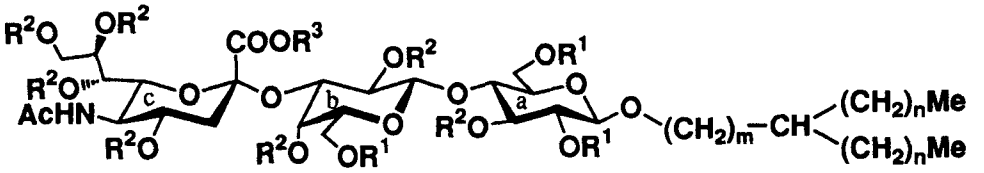
Previously,[9] we have observed that a ceramide structure for the immunosuppressive activity of gangliosides effectively can be replaced by other fatty-alkyl residue, and also the length and branched structure of the fatty-alkyl chain are critically important for the activity. Therefore, we have chosen here the β- and γ-branched alcohols consisting of 21, 22, 29, and 30 carbon atoms in order to know more detail about the structural features of the lipid parts for activity.

Treatment of 2-(decyl)dodecanoic acid (**1**) in methanol in the presence of concd H_2SO_4 overnight at 50 °C gave the methyl ester (**2**) in quantitative yield. In essentially the same way, methyl esterification of 2-(tetradecyl)hexadecanoic acid (**3**), 3-(nonyl)dodecanoic acid (**5**), and 3-(tridecyl)hexadecanoic acid (**7**) gave the corresponding methyl esters (**4**, **6**, and **8**) in almost quantitative yields. Reduction of the methyl esters (**2**, **4**, **6**, and **8**) with $LiAlH_4$ in dry ether gave the corresponding alcohols (**9**-**12**) in good yields, respectively.

The glycosylation[9,11] of **9** with **13** in dry dichloromethane overnight at 0 °C in the presence of BF_3•OEt_2 and powdered molecular sieves 4Å [MS-4Å (AW-300)] afforded exclusively the β-glycoside **15** in 71%. A significant signal in the ^1H NMR spectrum of **15** was a one-proton doublet at δ 4.88 ($J_{1,2}$ = 8.1 Hz, H-1a), showing the newly formed glycosidic linkage to be β. In essentially the same way, glycosylation of **9** with **14**, and **10**, **11**, or **12** with **13** and **14** gave the corresponding β-glycosides (**17**, **19**, **21**, **23**, **25**, **27**, and **29**) in good yields, respectively. The observed chemical shifts and coupling constants of compounds **17**, **19**, **21**, **23**, **25**, **27**, and **29**, (δ 4.59~4.88, $J_{1,2}$ = 7.7~8.3 Hz, for H-1a) are characteristic of the β-glycosidic linkage. *O*-Deacylation of **15**, **17**, **19**, **21**, **23**, **25**, **27**, and **29** with sodium methoxide in methanol, with subsequent saponification of the sialate methyl ester group, yielded the desired products **16**, **18**, **20**, **22**, **24**, **26**, **28**, and **30** in almost quantitative yields. The ^1H NMR data of the products thus obtained are consistent with the structures assigned.



15	m = 1, n = 9	R^1 = Bz,	R^2 = Me,	R^3 = Ac
16	m = 1, n = 9	$R^1 = R^2 = R^3$ = H		
17	m = 1, n = 13	R^1 = Bz,	R^2 = Me,	R^3 = Ac
18	m = 1, n = 13	$R^1 = R^2 = R^3$ = H		
19	m = 2, n = 8	R^1 = Bz,	R^2 = Me,	R^3 = Ac
20	m = 2, n = 8	$R^1 = R^2 = R^3$ = H		
21	m = 2, n = 12	R^1 = Bz,	R^2 = Me,	R^3 = Ac
22	m = 2, n = 12	$R^1 = R^2 = R^3$ = H		



23	m = 1, n = 9	R^1 = Bz,	R^2 = Ac,	R^3 = Me
24	m = 1, n = 9	$R^1 = R^2 = R^3$ = H		
25	m = 1, n = 13	R^1 = Bz,	R^2 = Ac,	R^3 = Me
26	m = 1, n = 13	$R^1 = R^2 = R^3$ = H		
27	m = 2, n = 8	R^1 = Bz,	R^2 = Ac,	R^3 = Me
28	m = 2, n = 8	$R^1 = R^2 = R^3$ = H		
29	m = 2, n = 12	R^1 = Bz,	R^2 = Ac,	R^3 = Me
30	m = 2, n = 12	$R^1 = R^2 = R^3$ = H		

EXPERIMENTAL

General Procedures. Specific rotations were determined with a Union PM-201 polarimeter at 25 °C. ^{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*.

Methyl 2-(Decyl)dodecanoate (2). To a solution of 2-(decyl)dodecanoic acid (**1**, 300 mg, 0.88 mmol) in MeOH (25 mL) was added concd H_2SO_4 (0.05 mL) and the mixture was heated overnight at 50 °C, neutralized with aq 10% NaOH, concentrated, then extracted with CH_2Cl_2. The extract was washed with H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (1:50 EtOAc-hexane) of the residue on silica gel gave compound **2** (308 mg, 98%) as a syrup: IR (film) 2930 and 2860 (Me, methylene), and 1740 and 1230 cm^{-1} (ester); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 6H, 2*Me*CH_2), 1.31 (s, 36H, 18CH_2), 2.36 (m, 1H, CH), and 3.70 (s, 3H, MeO).

Anal. Calcd for $C_{23}H_{46}O_2$ (354.6): C, 77.90; H, 13.08. Found: C, 77.79; H, 13.14.

Methyl 2-(Tetradecyl)hexadecanoate (4). Methyl esterification of 2-(tetradecyl)hexadecanoic acid (**3**, 300 mg, 0.67 mmol) as described for **2** gave **4** (300 mg, 96%) as an amorphous mass: IR (KBr) 2930 and 2860 (Me, methylene), and 1750 and 1230 cm^{-1} (ester); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 6H, 2*Me*CH_2), 1.30 (s, 52H, 26CH_2), 2.36 (m, 1H, CH), and 3.70 (s, 3H, MeO).

Anal. Calcd for $C_{31}H_{62}O_2$ (466.8): C, 79.76; H, 13.39. Found: C, 79.70; H, 13.33.

Methyl 3-(Nonyl)dodecanoate (6). Esterification of 3-(nonyl)dodecanoic acid (**5**, 322 mg, 0.98 mmol) as described for **2** gave **6** (319 mg, 95%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 32H, 16CH_2), 1.84 (m, 1H, CH), 2.22 (d, 2H, CH_2CO), and 3.64 (s, 3H, MeO).

Anal. Calcd for $C_{22}H_{44}O_2$ (340.6): C, 77.58; H, 13.02. Found: C, 77.41; H, 13.23.

Methyl 3-(Tridecyl)hexadecanoate (8). Esterification of 3-(tridecyl)hexadecanoic acid (**7**, 310 mg, 0.7 mmol) as described for **2** gave **8** (310 mg,

quantitative) as an amorphous mass: ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 6H, 2*Me*CH_2), 1.33 (s, 48H, 24CH_2), 1.91 (m, 1H, CH), 2.30 (d, 2H, CH_2CO), and 3.71 (s, 3H, MeO).

Anal. Calcd for $C_{30}H_{60}O_2$ (452.8): C, 79.57; H, 13.36. Found: C, 79.51; H, 13.34.

2-(Decyl)dodecan-1-ol (9). To a solution of **2** (290 mg, 0.82 mmol) in dry ether (25 mL), cooled to 0 °C, was added $LiAlH_4$ (50 mg, 1.3 mmol) and the mixture was stirred overnight at room temperature. After completion of the reaction, the mixture was cooled to 0 °C and water (0.3 mL) was added. The mixture was filtered through celite-545 and washed with CH_2Cl_2. The combined filtrate and washings were concentrated. Column chromatography (1:20 acetone-hexane) of the residue on silica gel (60 g) gave **9** (205 mg, 77%) as an amorphous mass: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 36H, 18CH_2), 1.96 (br s, 1H, OH), and 3.51 (d, 2H, C*H*$_2$OH).

Anal. Calcd for $C_{22}H_{46}O$ (326.6): C, 80.90; H, 14.20. Found: C, 80.78; H, 14.31.

2-(Tetradecyl)hexadecan-1-ol (10). Reduction of **4** (467 mg, 1.0 mmol) with $LiAlH_4$ (61 mg, 1.6 mmol) in ether (30 mL) as described for **9** gave **10** (405 mg, 92%) as an amorphous mass: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 52H, 26CH_2), 2.01 (br s, 1H, OH), and 3.50 (d, 2H, C*H*$_2$OH).

Anal. Calcd for $C_{30}H_{62}O$ (438.8): C, 82.11; H, 14.28. Found: C, 82.15; H, 14.32.

3-(Nonyl)dodecan-1-ol (11). Reduction of **6** (294 mg, 0.86 mmol) with $LiAlH_4$ (52 mg, 1.4 mmol) in ether (25 mL) as described for **9** gave **11** (212 mg, 79%) as an amorphous mass: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 32H, 16CH_2), 1.51 (dd , 2H, CH_2), 2.22 (br s, 1H, OH), and 3.62 (t, 2H, C*H*$_2$OH).

Anal. Calcd for $C_{21}H_{44}O$ (312.6): C, 80.69; H, 14.19. Found: C, 80.71; H, 14.35.

3-(Tetradecyl)hexadecan-1-ol (12). Reduction of **8** (290 mg, 0.64 mmol) with $LiAlH_4$ (39 mg, 1.0 mmol) in ether (25 mL) as described for **9** gave **12** (240 mg, 88%) as an amorphous mass: ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 6H, 2*Me*CH_2), 1.19 (s, 48H, 24CH_2), 1.42 (dd , 2H, CH_2), 1.86 (br s, 1H, OH), and 3.56 (t, 2H, C*H*$_2$OH).

Anal. Calcd for $C_{29}H_{60}O$ (424.8): C, 81.99; H, 14.24. Found: C, 81.78 H, 14.29.

2-(Decyl)dodecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (15). To a solution of the trichloroacetimidate[9c] (**13**, 160 mg, 0.14 mmol) and **9** (70.5 mg, 0.21 mmol) in dry CH_2Cl_2 (2 mL) were added molecular sieves 4Å [MS-4Å (AW-300), 2 g] and the mixture was stirred for 3 h at room temperature, then cooled to 0 °C. Boron trifluoride etherate (0.09 mL) was added and the mixture was stirred overnight at 0 °C then filtered. Dichloromethane (30 mL) was added, and the solution was washed with M Na_2CO_3 and H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 CH_2Cl_2 -MeOH) of the residue on silica gel (40 g) gave **15** (131 mg, 71%) as an amorphous mass: $[\alpha]_D$ +27.6° (*c* 2.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.24 (s, 36H, 18CH_2), 1.63~2.16 (5s, 15H, 4AcO, AcN), 1.68 (t, 1H, J_{gem} = $J_{3ax,4}$ = 12.6 Hz, H-3b*ax*), 2.46 (dd, 1H, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.35 (dd, 2H, CH_2O), 3.60 (dd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 2.6 Hz, H-6b), 3.83 (s, 3H, MeO), 3.99 (dd, 1H, $J_{5,6}$ = 5.7 Hz, $J_{6,6'}$ = 12.6 Hz, H-6a), 4.50 (dd, 1H, $J_{5,6'}$ = 6.4 Hz, H-6'a), 4.80 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1a), 4.86 (dd, 1H, H-4b), 5.22 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 2.5 Hz, H-3a), 5.38 (dd, 1H, $J_{7,8}$ = 8.6 Hz, H-7b), 5.43 (dd, 1H, H-2a), 5.61 (m, 1H, H-8b), and 7.39-8.18 (m, 15H, 3Ph).

Anal. Calcd for $C_{69}H_{95}NO_{21}$ (1274.5): C, 65.02; H, 7.51; N, 1.10. Found: C, 65.15; H, 7.60; N, 1.09.

2-(Decyl)dodecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (16). To a solution of **15** (106.5 mg, 0.08 mmol) in MeOH (10 mL) was added NaOMe (10 mg) and the mixture was stirred overnight at 40 °C. Water (0.5 mL) was added and the mixture was stirred for an additional 6 h at 40 °C, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1:1 $CHCl_3$-MeOH. The filtrate and washings were combined and concentrated. Column chromatography (1:1 $CHCl_3$-MeOH) of the residue on Sephadex LH-20 (30 g) gave **16** (64.5 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -3.6° (*c* 1.3, 1:1 $CHCl_3$-MeOH); ^1H NMR

(2:1 $CDCl_3$-CD_3OD) δ 0.88 (t, 6H, 2*Me*CH_2), 1.25 (s, 36H, 18CH_2), 1.59 (br t, 1H, H-3b*ax*), 2.02 (s, 3H, AcN), 2.65 (br dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.4~4.6 Hz, H-3b*eq*), and 4.28 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a).

Anal. Calcd for $C_{39}H_{73}NO_{14}$ (780.0): C, 60.05; H, 9.43; N, 1.80. Found: C, 59.88; H, 9.65; N, 1.59.

2-(Tetradecyl)hexadecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (17). Coupling of **13** (102 mg, 0.09 mmol) with **10** (60.2 mg, 0.14 mmol), as described for **15**, gave **17** (107 mg, 84%) as an amorphous mass: $[\alpha]_D$ +23.0° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.96 (t, 6H, 2*Me*CH_2), 1.34 (s, 52H, 26CH_2), 1.55~2.25 (5s, 15H, 4AcO, AcN), 1.76 (t, 1H, J_{gem} = $J_{3ax,4}$ = 12.3 Hz, H-3b*ax*), 2.52 (dd, 1H, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.43 (dd , 2H, CH_2O), 3.72 (dd, 1H, $J_{5,6}$ = 10.6 Hz, $J_{6,7}$ = 2.6 Hz, H-6b), 3.91 (s, 3H, MeO), 4.88 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1a), 4.91 (m, 1H, H-4b), 5.28 (dd, 1H, $J_{7,8}$ = 9.5 Hz, H-7b), 5.46 (dd, 1H, H-3a), 5.53 (dd, 1H, H-2a), 5.70 (m, 1H, H-8b), and 7.35-8.25 (m, 15H, 3Ph).

Anal. Calcd for $C_{77}H_{111}NO_{21}$ (1386.7): C, 66.69; H, 8.07; N, 1.01. Found: C, 66.80; H, 8.05; N, 1.06.

2-(Tetradecyl)hexadecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (18). Deacylation and saponification of **17** (107 mg, 0.08 mmol), as described for **16**, yielded **18** (67 mg, 98%) as an amorphous mass: $[\alpha]_D$ -3.0° (*c* 1.3, 1:1 $CHCl_3$-MeOH); ^1H NMR (2:1 $CDCl_3$-CD_3OD) δ 0.89 (t, 6H, 2*Me*CH_2), 1.28 (s, 52H, 26CH_2), 1.40 (br t, 1H, H-3b*ax*), 2.03 (s, 3H, AcN), 2.87 (br dd, 1H, H-3b*eq*), and 4.24 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a).

Anal. Calcd for $C_{47}H_{89}NO_{14}$ (892.2): C, 63.27; H, 10.05; N, 1.57. Found: C, 63.05; H, 10.19; N, 1.51.

3-(Nonyl)dodecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (19). Coupling of **13** (170 mg, 0.15 mmol) with **8** (72 mg, 0.23 mmol), as described for **15**, gave **19** (136 mg, 70%)

as an amorphous mass: $[\alpha]_D$ +27.0° (*c* 2.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.24 (s, 32H, 16CH_2), 1.46-2.16 (5s, 15H, 4AcO, AcN), 1.72 (t, 1H, J_{gem} = $J_{3ax,4}$ = 12.5 Hz, H-3b*ax*), 2.46 (dd, 1H, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.60 (dd , 2H, CH_2O), 3.64 (dd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 2.4 Hz, H-6b), 3.83 (s, 3H, MeO), 4.84 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.86 (m, 1H, H-4b), 5.20 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 2.6 Hz, H-3a), 5.38 (dd, 1H, H-7b), 5.43 (dd, 1H, H-2a), 5.63 (m, 1H, H-8b), and 7.39-8.18 (m, 15H, 3Ph).

Anal. Calcd for $C_{68}H_{93}NO_{21}$ (1260.6): C, 64.79; H, 7.44; N, 1.11. Found: C, 64.88; H, 7.34; N, 1.01.

3-(Nonyl)dodecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (20). Deacylation and saponification of **19** (108 mg, 0.09 mmol), as described for **16**, gave **20** (63 mg, 96%) as an amorphous mass: $[\alpha]_D$ -2.7° (*c* 1.3, 1:1 $CHCl_3$-MeOH); ^1H NMR (2:1 $CDCl_3$-CD_3OD) δ 0.88 (t, 6H, 2*Me*CH_2), 1.25 (s, 32H, 16CH_2), 1.59 (br t, 1H, H-3b*ax*), 2.04 (s, 3H, AcN), 2.97 (br dd, 1H, H-3b*eq*), and 4.30 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a).

Anal. Calcd for $C_{38}H_{71}NO_{14}$ (765.5): C, 59.62; H, 9.40; N, 1.83. Found: C, 59.40; H, 9.63; N, 1.83.

3-(Tridecyl)hexadecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (21). Coupling of **13** (161 mg, 0.15 mmol) with **12** (92 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) in the presence of BF_3•OEt_2 (0.09 mL), as described for **15**, gave **21** (144 mg, 72%) as an amorphous mass: $[\alpha]_D$ +25.8° (*c* 2.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 48H, 24CH_2), 1.67 (t, 1H, J_{gem} = $J_{3ax,4}$ = 12.5 Hz, H-3b*ax*), 2.44 (dd, 1H, $J_{3eq,4}$ = 4.4 Hz, H-3b*eq*), 3.60 (m, 2H, CH_2O), 3.64 (dd, 1H, $J_{5,6}$ = 11.0 Hz, $J_{6,7}$ = 2.4 Hz, H-6b), 3.83 (s, 3H, MeO), 4.84 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.87 (m, 1H, H-4b), 5.22 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 2.6 Hz, H-3a), 5.39 (dd, 1H, H-7b), 5.43 (dd, 1H, H-2a), 5.56 (m, 1H, H-8b), and 7.39-8.18 (m, 15H, 3Ph).

Anal. Calcd for $C_{76}H_{109}NO_{21}$ (1372.7): C, 66.50; H, 8.00; N, 1.02. Found: C, 66.41; H, 8.09; N, 1.18.

3-(Tridecyl)hexadecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (22). Deacylation and saponification of **21** (144 mg, 0.1 mmol), as described for **16**, gave **22** (87 mg, 94%) as an amorphous mass: $[\alpha]_D$ -0.7° (*c* 1.7, 1:1 $CHCl_3$-MeOH); ^1H NMR (2:1 $CDCl_3$-CD_3OD) δ 0.88 (t, 6H, 2*Me*CH_2), 1.25 (s, 48H, 24CH_2), 2.04 (s, 3H, AcN), 2.88 (br dd, 1H, H-3b*eq*), and 4.26 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1a).

Anal. Calcd for $C_{46}H_{87}NO_{14}$ (878.2): C, 62.91; H, 9.99; N, 1.60. Found: C, 62.76; H, 10.20; N, 1.58.

2-(Decyl)dodecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-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 (23). Coupling of **14**[9b] (155 mg, 0.11 mmol) with **9** (53 mg, 0.17 mmol) in CH_2Cl_2 (2 mL) in the presence of BF_3•OEt_2 (0.07 mL) and MS-4Å (AW-300, 2.0 g), as described for **15**, yielded **23** (82 mg, 47%) as an amorphous mass: $[\alpha]_D$ +5.3° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.23 (s, 36H, 18CH_2), 1.67 (t, 1H, J_{gem} = $J_{3ax,4}$ = 11.4 Hz, H-3c*ax*), 1.82-2.22 (8s, 24H, 7AcO, AcN), 2.56 (dd, 1H, $J_{3eq,4}$ = 4.5 Hz, H-3c*eq*), 3.26 (t, 2H, CH_2O), 3.70 (s, 3H, MeO), 4.37 (dd, 1H, $J_{2,3}$ = 8.8 Hz, $J_{3,4}$ = 3.0 Hz, H-3b), 4.60 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1a), 4.88 (d, 1H, $J_{1,2}$ = 9.3 Hz, H-1b), 4.99 (m, 1H, H-4c), 5.04 (br t, 1H, $J_{2,3}$ = 8.8 Hz, H-2b), 5.21 (br t, 1H, $J_{2,3}$ = 10.1 Hz, H-2a), 5.35 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 8.1 Hz, H-7c), 5.48 (t, 1H, $J_{3,4}$ = 9.4 Hz, H-3a), 5.50 (m, 1H, H-8c), and 7.39-8.17 (m, 15H, 3Ph).

Anal. Calcd for $C_{81}H_{111}NO_{29}$ (1562.8): C, 62.25; H, 7.16; N, 0.90. Found: C, 62.31; H, 7.33; N, 0.96.

2-(Decyl)dodecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (24). Deacylation and saponification of **23** (82 mg, 0.05 mmol), as described for **16**, gave **24** (42 mg, 84%) as an amorphous mass: $[\alpha]_D$ +0.3° (*c* 0.8, 1:1 $CHCl_3$-MeOH); ^1H NMR (2:1 $CDCl_3$-CD_3OD) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 36H, 18CH_2), 2.04 (s, 3H, AcN), 2.92 (br dd, 1H, H-3c*eq*), 4.17 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1a), and 4.31 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1b).

Anal. Calcd for $C_{45}H_{83}NO_{19}$ (942.2): C, 57.37; H, 8.88; N, 1.49. Found: C, 57.20; H, 8.96; N, 1.46.

2-(Tetradecyl)hexadecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-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 (25). Coupling of **14** (150 mg, 0.11 mmol) with **10** (120 mg, 0.27 mmol), as described for **15**, gave **25** (160 mg, 89%) as an amorphous mass: $[\alpha]_D$ +7.3° (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, 6H, 2*Me*CH_2), 1.25 (s, 52H, 26CH_2), 1.84-2.22 (8s, 24H, 7AcO, AcN), 2.57 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3c*eq*), 3.24 (m, 1H, CH), 3.59 (dd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 2.8 Hz, H-6c), 3.71 (s, 3H, MeO), 4.59 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1a), 4.88 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.98 (m, 1H, H-4c), 5.03 (dd, 1H, $J_{2,3}$ = 10.1 Hz, H-2b), 5.22 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-3a), 5.35 (dd, 1H, $J_{7,8}$ = 9.0 Hz, H-7c), 5.48 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3a), 5.54 (m, 1H, H-8c), and 7.38-8.06 (m, 15H, 3Ph).

Anal. Calcd for $C_{89}H_{127}NO_{29}$ (1675.0): C, 63.81; H, 7.63; N, 0.84. Found: C, 63.69; H, 7.73; N, 0.91.

2-(Tetradecyl)hexadecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (26). Deacylation and saponification of **25** (75 mg, 0.045 mmol), as described for **16**, gave **26** (47 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -0.4° (*c* 1.5, 1:1 $CHCl_3$-MeOH); ^1H NMR (1:1 $CDCl_3$-CD_3OD) δ 0.88 (t, 6H, 2*Me*CH_2), 1.25 (s, 52H, 26CH_2), 1.93 (s, 3H, AcN), 2.80 (br dd, 1H, H-3c*eq*), 3.10 (m, 1H, CH), 4.15 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), and 4.27 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1b).

Anal. Calcd for $C_{53}H_{99}NO_{19}$ (1054.4): C, 60.37; H, 9.46; N, 1.33. Found: C, 60.29; H, 9.51; N, 1.35.

3-(Nonyl)dodecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-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 (27). Coupling of **14** (160

mg, 0.11 mmol) with **11** (52.4 mg, 0.17 mmol), as described for **15**, gave **27** (95 mg, 54%) as an amorphous mass: $[\alpha]_D$ +4.7° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.24 (s, 32H, 16CH_2), 1.72 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12.8 Hz, H-3c*ax*), 2.56 (dd, 1H, $J_{3eq,4}$ = 4.4 Hz, H-3c*eq*), 3.47 (m, 2H, CH_2O), 3.60 (dd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 2.7 Hz, H-6c), 3.70 (s, 3H, MeO), 4.61 (dd, 1H, $J_{2,3}$ = 8.6 Hz, $J_{3,4}$ = 2.9 Hz, H-3b), 4.64 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1a), 4.88 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.99 (m, 1H, H-4c), 5.07 (dd, 1H, H-2b), 5.21 (dd, 1H, $J_{2,3}$ = 7.7 Hz, H-2a), 5.35 (dd, 1H, $J_{7,8}$ = 9.6 Hz, H-7c), 5.49 (br t, 1H, H-3a), 5.54 (m, 1H, H-8c), and 7.39-8.17 (m, 15H, 3Ph).

Anal. Calcd for $C_{80}H_{109}NO_{29}$ (1548.8): C, 62.04; H, 7.09; N, 0.90. Found: C, 62.11; H, 7.06; N, 1.10.

3-(Nonyl)dodecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (28). Deacylation and saponification of **27** (92 mg, 0.06 mmol), as described for **16**, gave **28** (53 mg, 97%) as an amorphous mass: $[\alpha]_D$ -4.8° (*c* 1.1, 1:1 $CHCl_3$-MeOH); ^1H NMR (2:1 $CDCl_3$-CD_3OD) δ 0.89 (t, 6H, 2*Me*CH_2), 1.25 (s, 32H, 16CH_2), 2.04 (s, 3H, AcN), 2.84 (br dd, 1H, H-3c*eq*), 4.20 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1a), and 4.32 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1b).

Anal. Calcd for $C_{44}H_{81}NO_{19}$ (928.1): C, 56.94; H, 8.80; N, 1.51. Found: C, 56.71; H, 8.99; N, 1.48.

3-(Tridecyl)hexadecyl *O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-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 (29). Coupling of **14** (168.5 mg, 0.12 mmol) with **12** (75 mg, 0.18 mmol), as described for **15**, gave **29** (108 mg, 54%) as an amorphous mass: $[\alpha]_D$ +4.5° (*c* 2.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, 6H, 2*Me*CH_2), 1.25 (s, 48H, 24CH_2), 1.67 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12.3 Hz, H-3c*ax*), 1.87-2.22 (8s, 24H, 7AcO, AcN), 2.56 (dd, 1H, $J_{3eq,4}$ = 4.1 Hz, H-3c*eq*), 3.47 (m, 2H, CH_2O), 3.60 (dd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 2.8 Hz, H-6c), 3.70 (s, 3H, MeO), 4.59 (dd, 1H, $J_{2,3}$ = 7.7 Hz, $J_{3,4}$ = 3.3 Hz, H-3b), 4.62 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1a), 4.88 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1b), 4.99 (m, 1H, H-4c), 5.07

(dd, 1H, H-2b), 5.21 (dd, 1H, $J_{2,3}$ = 8.3 Hz, H-2a), 5.35 (dd, 1H, $J_{7,8}$ = 8.6 Hz, H-7c), 5.48 (br t, 1H, $J_{3,4}$ = 9.5 Hz, H-3a), 5.56 (m, 1H, H-8c), and 7.28-8.06 (m, 15H, 3Ph).

Anal. Calcd for $C_{88}H_{125}NO_{29}$ (1661.0): C, 63.63; H, 7.59; N, 0.83. Found: C, 63.70; H, 7.48; N, 0.88.

3-(Tridecyl)hexadecyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (30). Deacylation and saponification of **29** (100 mg, 0.06 mmol), as described for **16**, gave **30** (60 mg, 96%) as an amorphous mass: $[\alpha]_D$ -4.3° (*c* 0.8, 1:1 $CHCl_3$-MeOH); ^1H NMR (2:1 $CDCl_3$-CD_3OD) δ 0.88 (t, 6H, 2*Me*CH_2), 1.25 (s, 48H, 24CH_2), 1.58 (br t, 1H, H-3c*ax*), 2.03 (s, 3H, AcN), 2.80 (br dd, 1H, H-3c*eq*), 4.18 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1a), and 4.28 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1b).

Anal. Calcd for $C_{52}H_{97}NO_{19}$ (1040.4): C, 60.03; H, 9.40; N, 1.35. Found: C, 59.75; H, 9.67; N, 1.34.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 05274102 and No. 07273226) for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. T. Yamakawa and S. Suzuki, *J. Biochem.* (Tokyo), **39**, 383 (1952).
2. G. J. M. Hooghwinkel, P. F. Barri and G. W. Bruyn, *Neurology*, **16**, 934 (1966).
3. N. Handa and S. Handa, *J. Exp. Med. (Jpn)*, **35**, 331 (1965).
4. K. Uemura, M. Yuzawa and T. Takemori, *J. Biochem.* (Tokyo), **83**, 463 (1978).
5. A. Gorio, G. Carmingnoto, F. Facci and M. Finnesso, *Brain Res.*, **197**, 236 (1980).
6. Y. Suzuki, M. Matsunaga and M. Matsumoto, *J. Biol. Chem.*, **260**, 1362 (1985).

7. H. Nojiri, F. Takaku, Y. Terui, Y. Miura and Y. Saito, *Proc. Natl. Acad. Sci. USA*, **83**, 782 (1986).
8. E. Bremer, J. Schlessinger and S. Hakomori, *J. Biol. Chem.*, **261**, 2434 (1986).
9. a) S. Ladisch, A. Hasegawa, R. Li and M. Kiso, *Biochemistry*, **34**, 1197 (1995); b) S. Ladisch, A. Hasegawa, R. Li and M. Kiso, *Biochem. Biophys. Res. Commun.*, **203**, 1102 (1994).
10. a) A. Hasegawa, T. Murase, K. Adachi, M. Morita, H. Ishida and M. Kiso, *J. Carbohydr. Chem.*, **9**, 181 (1990); b) T. Murase, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **188**, 71 (1989); c) T. Murase, A. Kameyama, K. P. R. Kartha, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 265 (1989); d) A. Hasegawa, T. Murase, M. Morita, H. Ishida and M. Kiso, *J. Carbohydr. Chem.*, **9**, 201 (1990); e) A. Hasegawa, K. Adachi, M. Yoshida and M. Kiso, *Carbohydr. Res.*, **230**, 273 (1992); f) A. Hasegawa, K. Adachi, M. Yoshida and M. Kiso, *Biosci. Biotechnol., Biochem.*, **56**, 445 (1992); g) T. Terada, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **12**, 425 (1993).
11. R. R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, **19**, 731 (1980).
12. Y. Ito, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 285 (1989).