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Total synthesis of a cholinergic neuron-specific ganglioside GT1aα: A high affinity ligand for myelin-associated glycoprotein (MAG)†

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An efficient total synthesis of a cholinergic neuron-specific ganglioside GT1aα (IV^{3}NeuAcIII^{6}NeuAcII^{3}NeuAc-GgOse4Cer) is described. The suitably protected sialyl-α(2→6)-gangliotriose (III^{6}NeuAc-GgOse3) derivative was glycosylated with the phenyl 2-thioglycoside of sialic acid in the presence of N-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) in acetonitrile medium, giving the disialogangliotriose (III^{6}NeuAcII^{3}NeuAc-GgOse3) derivative which contains both sialyl-α(2→6)-GalNAc and sialyl-α(2→3)-Gal structures (Route I). This pentasaccharide was efficiently synthesized also by the coupling of (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-D-galactopyranosyl trichloroacetimidate with 2-(trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside, followed by conversion of the phthalimido group to the acetamido group (Route II). *O*-Deisopropylidenation and further glycosylation with methyl (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-1-thio-β-D-galactopyranoside, promoted by dimethyl(methylthio)sulfonium triflate (DMTST), gave the desired trisialogangliotetraose (IV^{3}NeuAcIII^{6}NeuAcII^{3}NeuAc-GgOse4) derivative, which was converted stepwise into the title ganglioside GT1aα by the introduction of the ceramide part and then complete deprotection. The ganglioside obtained was shown to be identical with the native GT1aα on TLC-immunostaining.

Keywords: **ganglioside GT1aα, sialic acid, cholinergic neuron-specific antigen, myelin-associated glycoprotein**

Introduction

Gangliosides, sialic acid-containing glycosphingolipids, are a class of structurally diverse molecules commonly present in cell-surface membranes and are particularly rich in tissues of the central nervous system. It has been widely recognized that gangliosides are involved in many biological processes such as cell growth, cell differentiation, cell adhesion, immune responses, oncogenesis, and many other receptor-mediated reactions [2–5]. The sialo-oligosaccharide parts of gangliosides are considered to be exposed as ligands to the external environment, capable of expressing biological functions which are congruent with the chemical structure of the ganglioside.

A new class (α-series) of gangliosides, GT1aα and GQ1bα, have been identified [6–11] as cholinergic neuron-specific antigens in brains. These gangliosides have in common one sialic acid residue α(2→6)-linked to the GalNAc moiety in the gangliotetraose core structure (Fig. 1). We have reported the first total syntheses of α-series gangliosides, GM1α [12], GD1α [13], and GQ1bα [14] to elucidate their biological functions. Recently, it has been found [15–17] that the synthetic GQ1bα has the extremely high potency as the ligand of myelin-associated glycoprotein (MAG), a member of sialic acid-dependent immunoglobulin lectin (siglec) family, previously termed sialoadhesins or I-type lectins [18–20]. The binding activity of GQ1bα to MAG was much higher than that of GT1b, suggesting a special importance of the sialic acid residue α(2→6)-linked to the GalNAc moiety for the MAG binding. In view of these facts, we describe herein an efficient total synthesis of ganglioside GT1aα, a cholinergic neuron-specific antigen.

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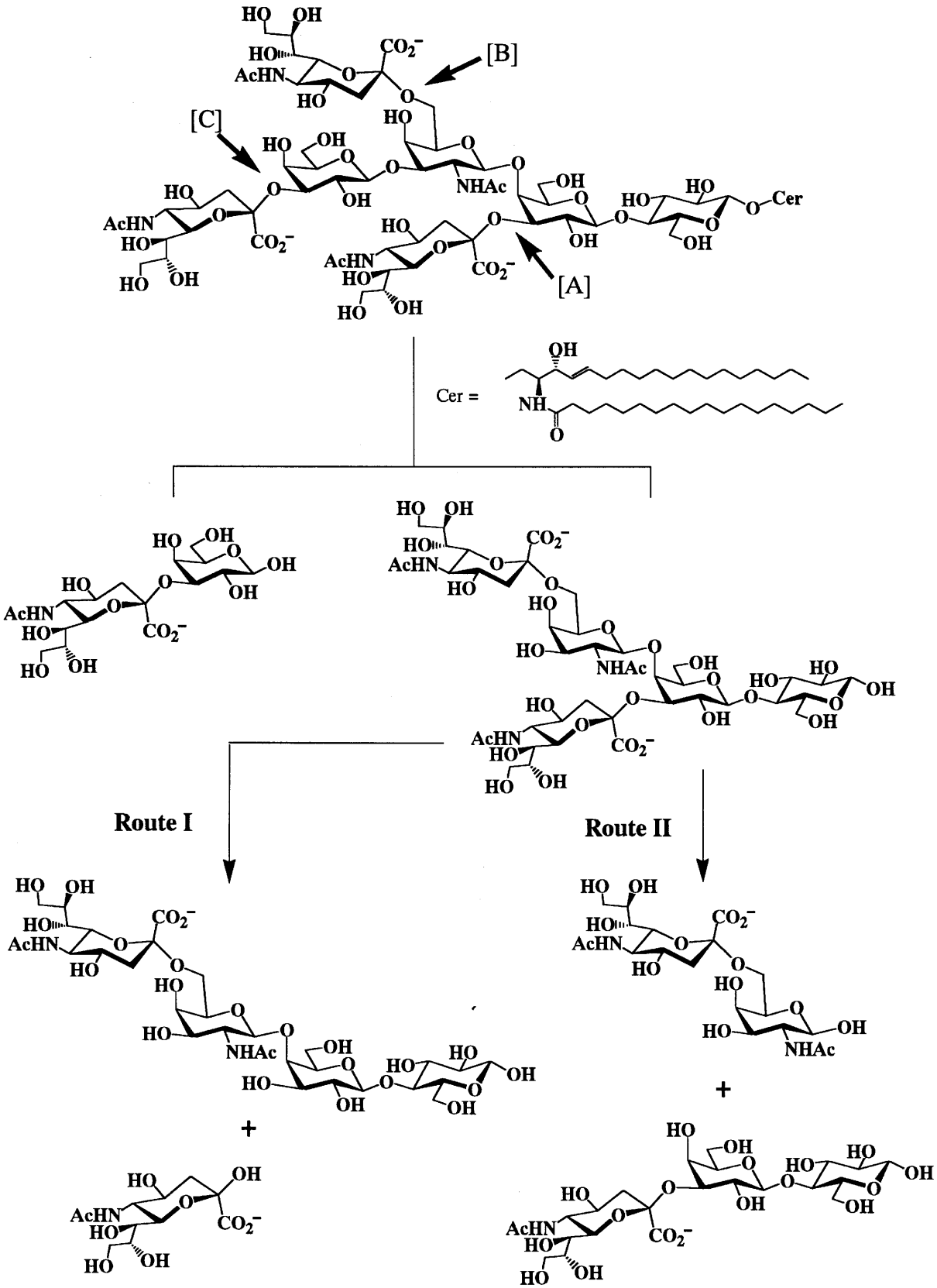

Figure 1. Retrosynthetic analysis of anglioside GT1aα

Results and discussion

The most important problems in the total synthesis of the title trisialoganglioside GT1aα are a) regio- and α-stereoselective sialylations at *O*-3 of both internal and external Gal residues (arrows [A] and [C] in Fig. 1), and at *O*-6 of the GalNAc residue (arrow [B] in Fig. 1), b) efficient constructions of the gangliotriose and gangliotetraose core structures, and c) convenient transformation of the protected heptasaccharide chain (IV^{3}NeuAcIII^{6}NcuAcII^{3}NeuAc-GgOse4) into the target ganglioside. We employed two synthetic routes (I and II) to construct the key intermediate, III^{6}NeuAcII^{3}NeuAcGgOSe3. In the route I [21], we selected the suitably protected sialyl-α(2→6)-gangliotriose derivative **1** as a key glycosyl acceptor, which had served as the synthetic intermediate for GQ1bα [14] and the phenyl 2-thioglycoside of sialic acid (**2**) as the sialyl donor [22] (Fig. 2). The glycosylations of **1** with **2** was performed in the presence of N-iodosuccinimide (NIS)-trimethylsilyl trifluoromethanesulfonate(TMSOTf) [22,25] in acetonitrile medium [22–29], to give **3** in 40% yield. Due to the low yield of the above mentioned reaction, an alternative synthetic route, route II, was developed for the synthesis of GT1aα as shown in Figure 3.

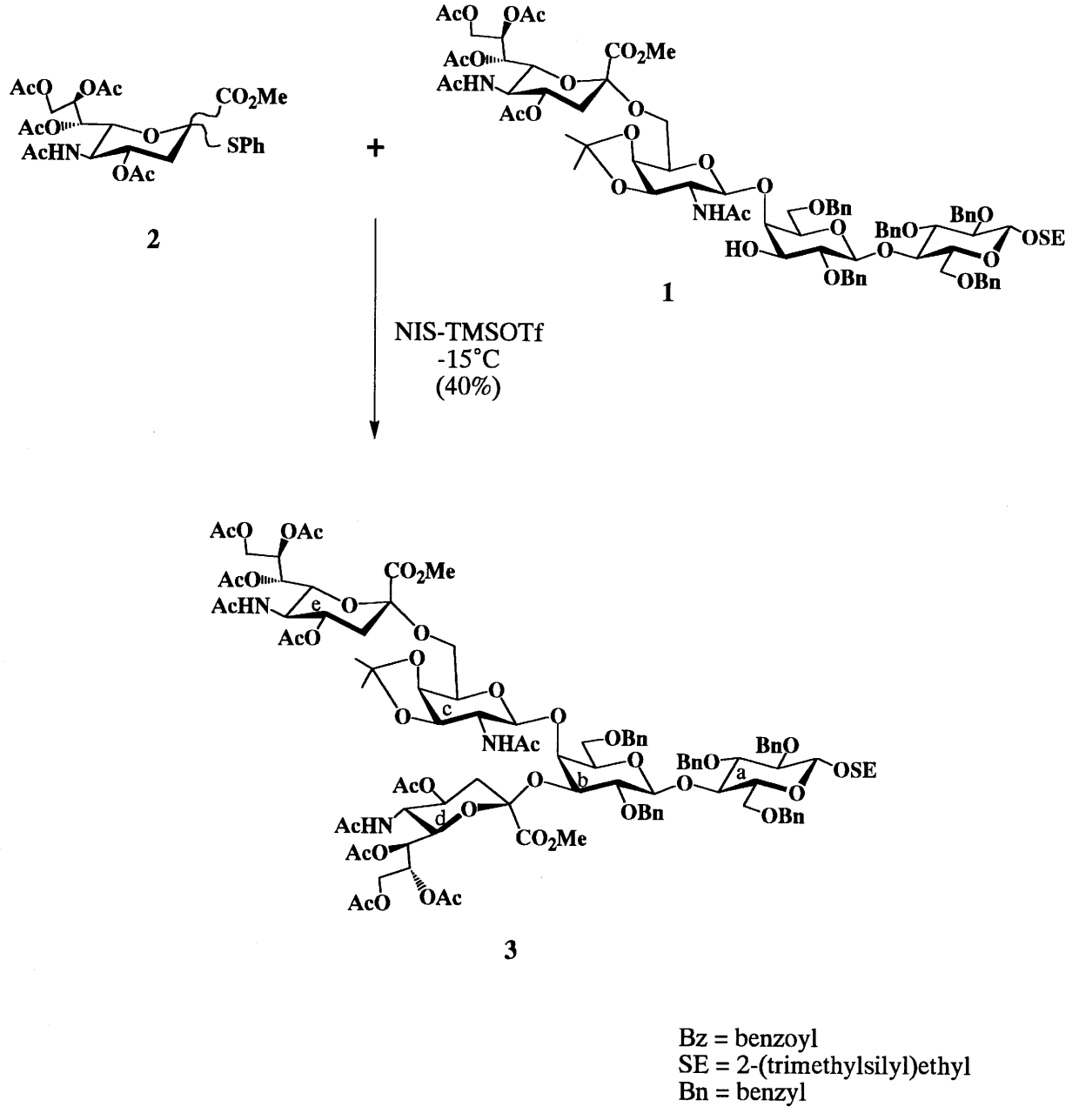

Figure 2. Synthesis of disialogangliotriose derivative by Route I

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-D-galactopyranose, which was readily prepared by selective 1-*O*-deacetylation of 1,3,4,6-tera-*O*-acetyl-2-deoxy-2-phthalimido-D-galactopyranose [28,32], was treated with *t*-butyldimethylsilyl (TBDMS) chloride and imidazole in *N*,*N*-dimethylformamide (DMF) to give **4** in 92% yield. The characteristic ^{1}H-NMR signal at δ 5.48 (d, $J_{1,2} = 8.1$ Hz, H-1) showed the anomeric configuration of **4** to be β. *O*-Deacetylation of **4** and the following 3,4-*O*-isopropylidenation gave **6**, which was glycosylated with NeuAc donor **2** in the presence of NIS-TfOH in acetonitrile at -30°C to afford the desired sialyl-$\alpha(2\rightarrow6)$ glycoside **7** in 81% yield; no β-glycoside was isolated. In the ^{1}H-NMR spectrum of **7**, the characteristic H-3*ax* (δ 1.98, t, $J_{gem} = J_{3ax,4} = 12.8$ Hz), H-3*eq* (δ 2.63, dd, $J_{gem} - 12.8$, $J_{3eq,4} = 4.6$ Hz) and a three-proton singlet of *Me*O (δ 3.84) of the NeuAc residue were clearly observed indicating the structure assigned. Removal of the TBDMS group in **7** (83%) and trichloroacetimidate formation (99%) yielded the disaccharide donor **9** (the major compound was β-imidate, $J_{1,2} = 9.2$ Hz), which was coupled with the sialyl-$\alpha(2\rightarrow3)$ lactose derivative **10** [28] to give the desired pentasaccharide (**11**, 76%). The characteristic one-proton doublet of H-1c (δ 5.22, $J_{1,2} = 8.7$ Hz) in the ^{1}H-NMR spectrum of **11** indicated the newly formed glycosidic linkage to be β. The phthalimido group in **11** was then converted to the acetamido group. The physicochemical properties and spectral data of **3** thus obtained were identical with those of **3** separately synthesized by the coupling of **1** and **2** as described above. In the ^{1}H-NMR spectrum of **3**, H-3*eq* of the NeuAc $\alpha(2\rightarrow6)$-linked to the GalNAc residue was observed at δ 2.61 (dd, $J_{gem} = 12.6$, $J_{3eq,4} = 4.6$ Hz), while H-3*eq* of the NeuAc $\alpha(2\rightarrow3)$-linked to the Gal residue at δ 2.23 showing significant up-field shift from δ 2.47 observed for **10**. The two, three-proton singlets of *Me*O appeared at δ 3.83 and δ 3.85, respectively.

The isopropylidene group in 3 was cleaved by treatment with 80% acetic acid for 3 h at 40°C to give **13** in 85% yield. Regioselective glycosylation of **13** with methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-$(2\rightarrow3)$-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**14**) [33] in dichloromethane for 24 h at 0°C in the presence of DMTST and molecular sieves 4 Å (MS-4 Å), gave the protected GT1aα heptasaccharide **15** in 95% yield (Fig. 4). Hydrogenolytic removal of the benzyl groups in **15** over $Pd(OH)_2$ in 9:1 ethanol-acetic acid, followed by complete acetylation of the resulting free hydroxyl groups with Ac_2O-pyridine, afforded the fully acylated oligosaccharide **16** in 81% yield. Selective removal of the 2-(trimethylsilyl)ethyl (SE) group was achieved [34,35] by treatment of **16** with trifluoroacetic acid in dichloromethane to give the 1-hydroxy compound **17** (92%), which upon further treatment [35] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane, gave the trichloroacetimidate **18** in high yield.

Coupling of **18** with (2*S*,3*R*,4*E*)-2-azido-3-*O*-(*tert*-butyldiphenylsilyl)-4-octadecanamido-1,3-diol (**19**) [36,37] was carried out in the presence of TMSOTf and MS-4 Å (AW300) in dichloromethane to give **20** in 62% yield. Selective reduction [38] of the azido group in **20** with triphenylphosphine in 5:1 benzene–water gave the amine, which on condensation with stearic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane, afforded the fully protected ganglioside GT1aα **21** in 76% yield. Finally, removal [36] of the *tert*-butyldiphenylsilyl group in **21** with 1.0 M tetrabutylammonium fluoride in acetonitrile, *O*-deacetylation with sodium methoxide in methanol, and subsequent saponification of the methyl ester group gave the title ganglioside GT1aα as amorphous mass in quantitative yield, after chromatography on a column of Sephadex LH-20 with 5:5:1 chloroform–methanol–water.

The structure of ganglioside GT1aα thus obtained was characterized by FAB MS (negative ion mode) and ^{1}H-NMR [6] spectrometry. The molecular ion species of the synthetic GT1aα were clearly detected at *m/z* 2172.04 $[M–Na]^-$ ($C_{95}H_{162}N_5Na_2O_{47}$ MW, Exact 2171.0236, Ave. 2172.3161), 2150.06, 2149.07 $[M–2Na]^-$, and 2127.06 $[M–3Na]^-$, accompanied by the significant fragment ions at *m/z* 1858.0, 1857.0 $[M–2Na–NeuAc]^-$, 1696.0 $[M–2Na–NeuAc–Gal]^-$, and 1545.0 $[M–3Na–2NeuAc]^-$; providing unambiguous evidences for the structure assigned. The characteristic fragment ions at *m/z* 888.7 $[lactosylceramide]^-$, 726.6 $[glucosylceramide]^-$, and 564.6 $[ceramide]^-$, provides further evidences for the assigned structure including the ceramide moiety. In the ^{1}H-NMR spectrum (500 MHz) of the synthetic GT1aα in DMSO-d_6-D_2O, the characteristic three H-3*eq* of NeuAc (δ 2.73–2.89), four anomeric protons due to the β glycosidic linkages in the GgOse4 core structure (δ 4.15, 4.30, 4.43 and 4.84), and two olefinic protons of ceramide (δ 5.32 and 5.75) were clearly observed. In addition, TLC-immunostaining of the synthetic GT1aα was performed in comparison with the native GT1aα. As shown in Figure 6, the two compounds were found to be identical.

MAG is a quantitatively minor protein constituent of central and peripheral nervous system myelin, and has been considered to be implicated in myelin–neuron interactions [39]. Therefore, detailed structure–function relationship study for carbohydrate ligands that support MAG binding may provide opportunities for intervention in the control of neurite outgrowth and myelination. In binding experiments with the MAG-transfected COS cell for the immobilized gangliosides including synthetic GT1aα, it was demonstrated that GT1aα can efficiently support the adhesion as strong as GD1α [1]. The hierarchy of binding strengths of a series of gangliosides with MAG were GQ1bα > GT1aα, GD1α > GT1b, GD1a > GM3, GM4 (GQ1b, GD1b, GM1 and GD3 failed to support binding) [39,40]. This result indicates that not only the terminal sialic acid, which is essential for MAG binding [15–17], but also the internal sialic acids play important roles for expressing higher binding activity for MAG.

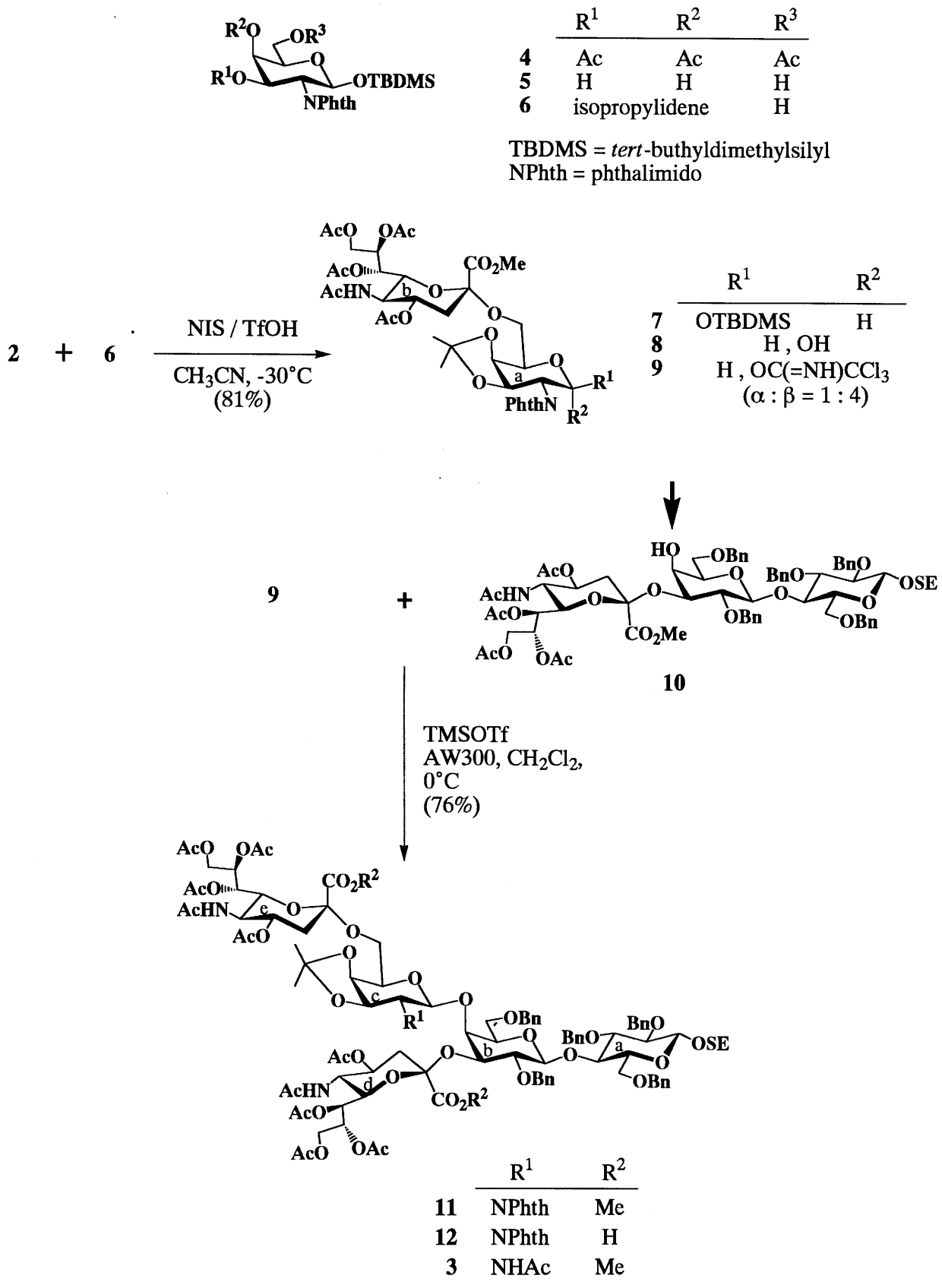

Figure 3. Synthesis of disialogangliotriose derivative by Route II

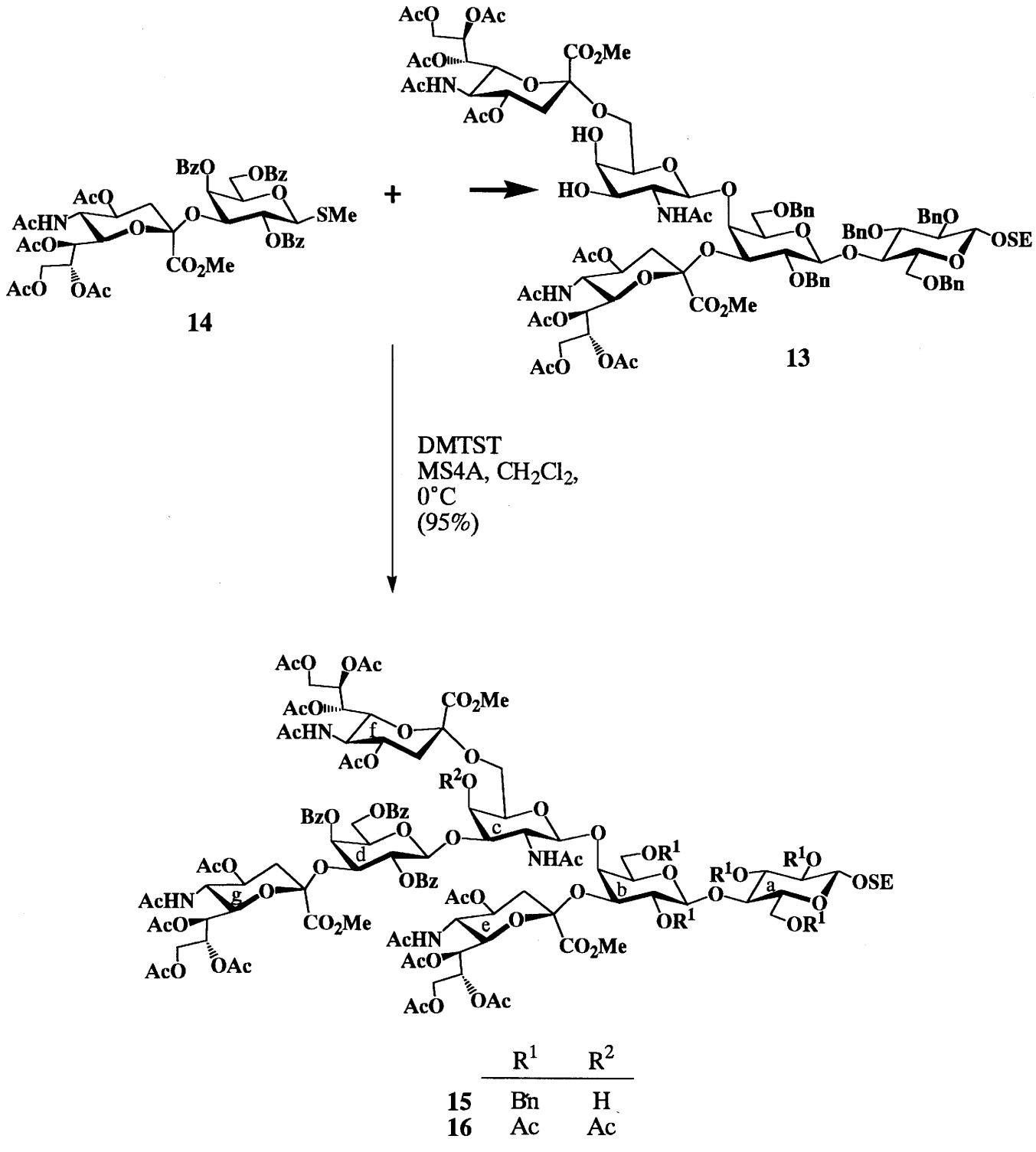

Figure 4. Synthesis of trisialogangliotetraose derivative

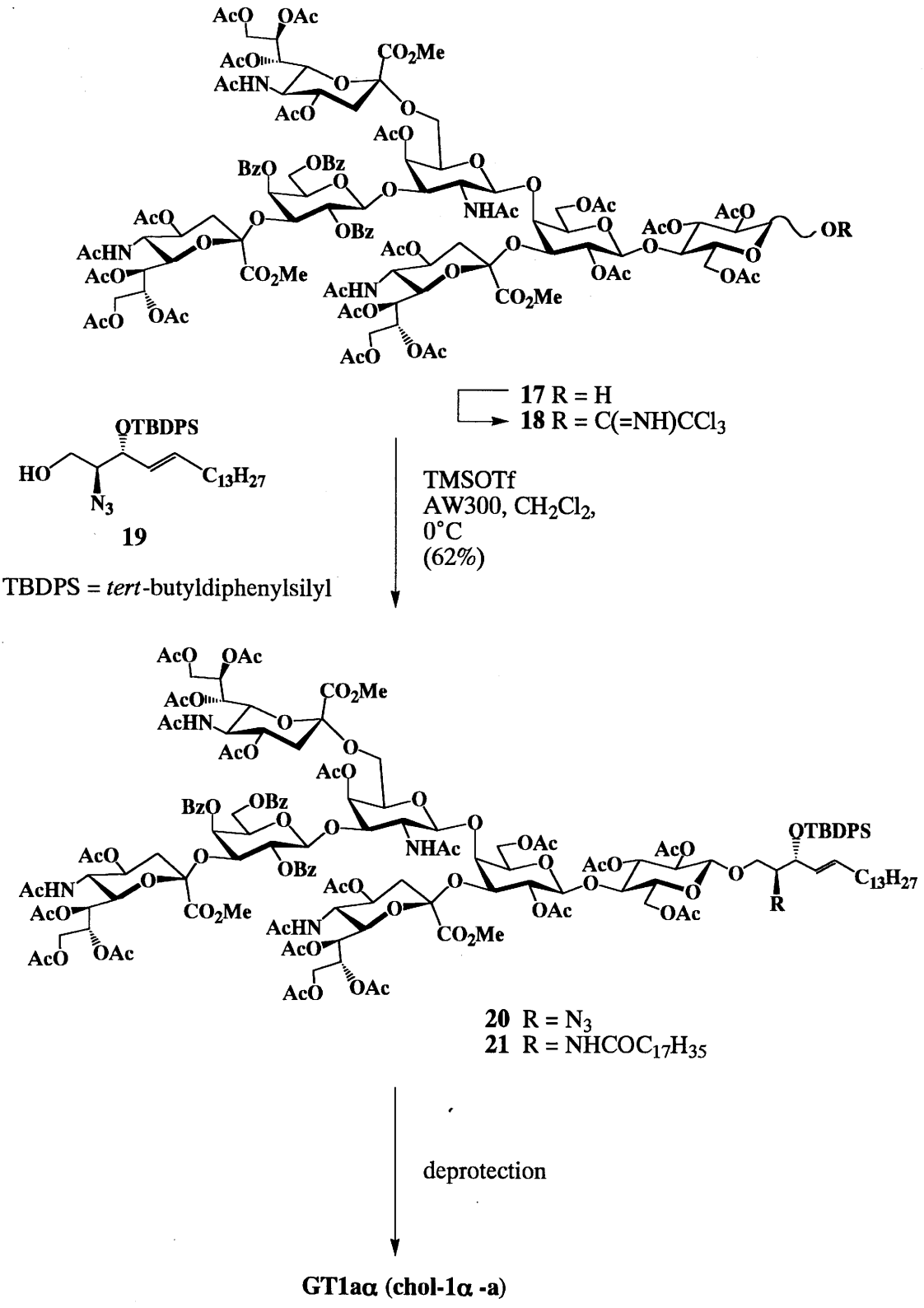

Figure 5. Total synthesis of ganglioside GT1aα

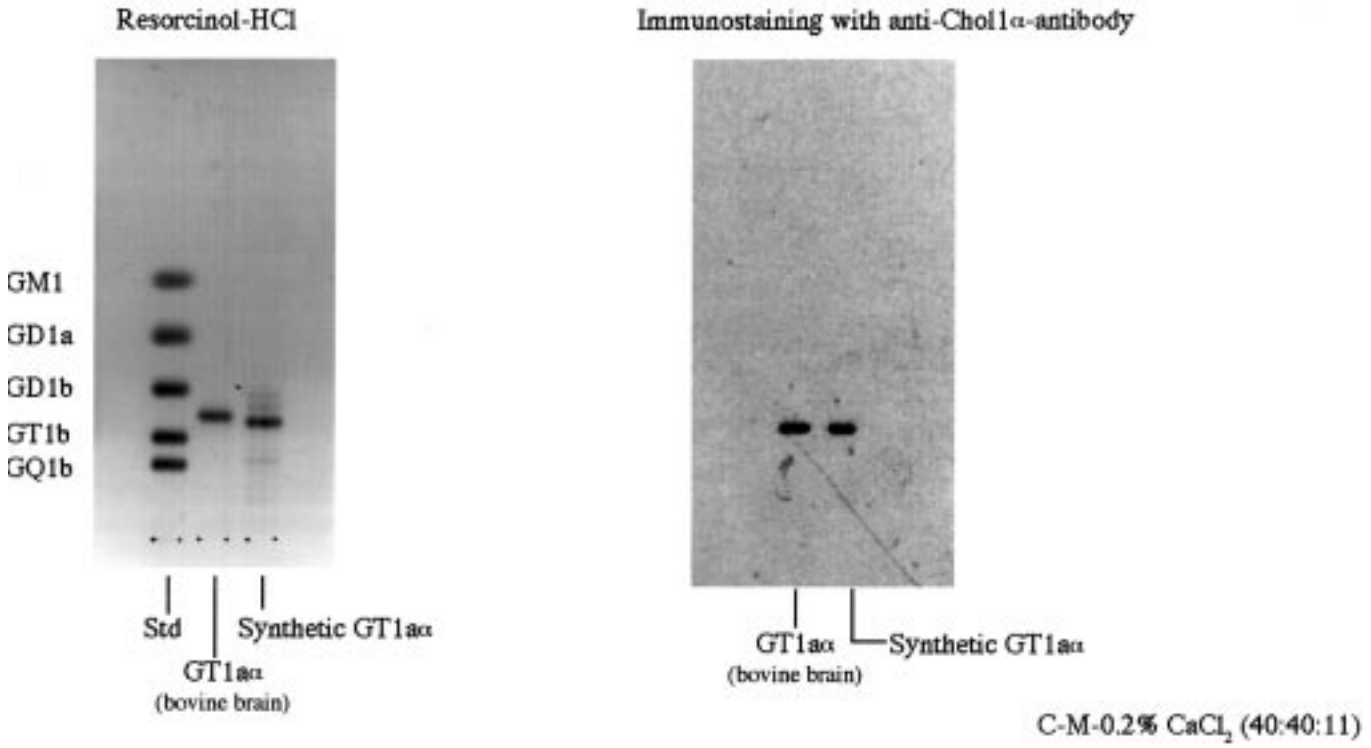

Figure 6. TLC-immunostaining of synthetic and native GT1aα

Materials and methods

Chemical synthesis

General methods

Optical rotations were determined with a Union PM-201 polarimeter at 25°C and IR spectra were recorded with a Jasco IRA-100 spectrophotometer. ^{1}H-NMR spectra were recorded at 400 or 500 MHz with a Varian UNITY Inova-400 and a Varian UNITY Inova-500 spectrometers, respectively. FAB-MS were recorded on a Jeol JMS-SX 120 A mass spectrometer/JMA-DA 7000 data system. Preparative chromatography was performed on silica gel (Fuji Silysia Co., 300 mesh) with the solvent systems specified. Concentrations were conducted in vacuo.

2-(Trimethylsilyl)ethyl O-*(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-3,4*-O-*isopropylidene-β-*D*-galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β-*D*-glucopyranoside* (**3**)

Route (I):
To a solution of 2-(trimethylsilyl)ethyl *O*-(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-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1→4)-*O*-(2,6-di-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-*O*-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside [14] (**1**; 800 mg, 0.50 mmol) and methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate [30] (**2**; 580 mg, 1.0 mmol) in acetonitrile (6 mL) were added molecular sieves 3 Å (1 g) and the mixture was stirred for 7 h at room temperature, then cooled to −20°C. To the solution were added *N*-iodosuccinimide (NIS; 675 mg, 3.0 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf; 58 µL, 0.3 mmol), and the stirring was continued for 72 h at −15°C. After reaction completed, the precipitates were filtered off and washed with chloroform. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3 and M $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 toluene–methanol) of the residue on silica gel gave **3** (450 mg, 40%) as an amorphous mass.

Route (II):
To a solution of **12** (500 mg, 0.23 mmol) in aq. 95% ethanol (15 mL) was added hydrazine monohydrate (130 µL, 2.3 mmol), and the solution was stirred for 15 h at 80°C. The solids were filtered and washed with ethanol, and the combined filtrate and washings was concentrated to a syrup. To a solution of the residue in methanol (10 mL) was added acetic anhydride (0.3 mL), and the solution was stirred for 6 h

at room temperature. Pyridine (0.6 mL) was added to the solution, and which was concentrated. To a solution of the residue in methanol (10 mL) was added large excess of diazomethane in diethyl ether (3 mL) at room temperature. After completion of the reaction, acetic acid (1 mL) was added to the solution, and which was concentrated and acetylated with pyridine (6 mL) and acetic anhydride (3 mL) for 24 h at 40°C. The product was purified by column chromatography (30:1 chloroform-methanol) of the syrup on silica gel to give **3** (344 mg, 71%) as an amorphous mass: $[\alpha]_D$ + 2.77° (*c* 0.7, $CHCl_3$); IR (KBr) 3300 (NH), 3100–2900 (CH), 1750 and 1220 (ester), 1660 and 1530 (amide), 860 and 840 (Me_3Si), and 700 cm^{-1} (phenyl); ^{1}H-NMR ($CDCl_3$) δ 1.03 (m, 2H, $Me_3SiCH_2CH_2$), 1.34 and 1.50 (2s, 6H, Me_2C), 1.66–2.12 (11s, 33H, AcN and 10AcO), 2.23 (m, 1H, H-3d*eq*), 2.61 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3e*eq*), 3.83 and 3.85 (2s, 6H, 2*Me*O), 4.86 (m, 1H, H-4e), 5.10 (m, 1H, H-4d), and 7.16–7.57 (m, 25H, 5Ph).

Anal. Calcd for $C_{103}H_{135}N_3O_{40}Si$ (2083.28): C, 59.38; H, 6.53; N, 2.02. Found: C, 59.37; H, 6.27; N, 2.01.

t-Butyldimethylsilyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside* (**4**)

To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-galactopyranose (3.30 g, 7.6 mmol) in *N*,*N*-dimethylformamide (DMF; 10 mL) were added *t*-butyldimethylsilyl chloride (1.37 g, 9.1 mmol) and imidazole (1.24 g, 18.2 mmol) at 0°C. The mixture was stirred for 4 h at room temperature, and methanol (5 mL) was then added. The solution was concentrated to a syrup, and which was extracted with ethyl acetate. The organic layer was successively washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 ethyl acetate–hexane) of the residue on silica gel gave **4** (3.82 g, 92%) as an amorphous mass: $[\alpha]_D$ −12.66° (*c* 0.6, $CHCl_3$); IR (KBr) 3000–2870 (CH), 1730 and 1240 (ester), 1700 (imide), and 720 cm^{-1} (Ph); ^{1}H-NMR ($CDCl_3$) δ −0.05 and 0.05 (2s, 6H, Si*Me*$_2$), 0.70 (s, 9H, SiC*Me*$_3$), 1.86, 2.06 and 2.21 (3s, 9H, 3AcO), 4.09 (m, 1H, H-5), 4.15 (dd, 1 H, J_{gem} = 11.0 Hz, H-6), 4.23 (dd, 1 H, J_{gem} = 11.0 Hz, H-6′), 4.50 (dd, 1 H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 11.7 Hz, H-2), 5.48 (d, 1 H, $J_{1,2}$ = 8.1 Hz, H-1), 5.49 (d, 1H, H-4), 5.85 (dd, 1H, $J_{2,3}$ = 11.7 Hz, $J_{3,4}$ = 3.7 Hz, H-3), and 7.74–7.88 (m, 4H, Ph).

Anal. Calcd for $C_{26}H_{35}NO_{10}Si$ (549.65): C, 56.82; H, 6.42; N, 2.55. Found: C, 56.81; H, 6.33; N, 2.44.

t-Butyldimethylsilyl 2-deoxy-2-phthalimido-β-D-galactopyranoside (**5**)

To a solution of **4** (3.80 g, 6.9 mmol) in 3:2 methanol–tetrahydrofuran (25 mL) was added sodium methoxide (20 mg), and the mixture was stirred for 2 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered and washed with methanol, and the combined filtrate and washings was concentrated. Column chromatography (10:1 chloroform–methanol) of the residue on silica gel gave **5** (2.75 g, 94%) as an amorphous mass: $[\alpha]_D$ −9.35° (*c* 0.6, $CHCl_3$); IR (KBr) 3500–2850 (OH, CH), 1700 (imide), and 710 cm^{-1} (Ph); ^{1}H-NMR ($CDCl_3$) δ −0.09 and 0.04 (2s, 6H, Si*Me*$_2$), 0.68 (s, 9H, SiC*Me*$_3$), 3.69 (m, 1H, H-5), 3.89–3.93 (m, 2H, H-6,6′), 4.16 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 4.30 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 11.0 Hz, H-2), 4.45 (dd, 1H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 2.9 Hz, H-3), 5.36 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), and 7.70–7.82 (m, 4H, Ph).

Anal. Calcd for $C_{20}H_{29}NO_7Si$ (423.54): C, 56.72; H, 6.90; N, 3.31. Found: C, 56.43; H, 6.89; N, 3.20.

t-Butyldimethylsilyl 2-deoxy-3,4-O-*isopropylidene-2-phthalimido-β-D-galactopyranoside* (**6**)

To a solution of **5** (3.70 g, 8.7 mmol) in DMF (20 mL) was added 2,2-dimethoxypropane (2.6 mL, 21.7 mmol) and drierite (4.0 g), and the mixture was stirred for 1 h at room temperature. After addition of (±)-10-Camphorsulfonic acid (100 mg), the mixture was stirred for 4 h at 80°C, then neutralized with triethylamine and concentrated. Column chromatography (1:2 ethyl acetate–hexane) of the residue on silica gel gave **6** (3.03 g, 75%) as an amorphous mass: $[\alpha]_D$ + 14.58° (*c* 0.5, $CHCl_3$); IR (KBr) 3600–3400 (OH), 3050–2850 (CH), 1700 (imide), and 720 cm^{-1} (Ph); ^{1}H-NMR ($CDCl_3$) δ −0.10 and 0.03 (2s, 6H, Si*Me*$_2$), 0.64 (s, 9H, SiC*Me*$_3$), 1.29 and 1.61 (2s, 6H, C*Me*$_2$), 3.84 (m, 1H, H-5), 3.98–4.03 (m, 2H, H-6, 6′), 4.19 (d, 1H, $J_{3,4}$ = 5.0 Hz, H-4), 4.21 (dd, 1H, H-2), 4.83 (dd, 1H, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 5.0 Hz, H-3), 5.28 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), and 7.68–7.81 (m, 4H, Ph).

Anal. Calcd for $C_{23}H_{33}NO_7Si$ (463.60): C, 59.59; H, 7.18; N, 3.02. Found: C, 59.43; H, 6.98; N, 2.94.

t-Butyldimethylsilyl O-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2-deoxy-3,4*-O-*isopropylidene-2-phthalimido-β-D-galactopyranoside* (**7**)

To a solution of **6** (1.20 g, 2.6 mmol) and **2** (3.02 g, 5.2 mmol) in acetonitrile (15 mL) were added molecular sieves 3 Å (4.0 g) and the mixture was stirred for 5 h at room temperature, then cooled to −30°C. To the mixture were added, with stirring, NIS (2.33 g, 10.4 mmol) and TfOH (92 μL, 1.0 mmol), and the stirring was continued for 24 h at −30°C. The solids were removed by filtration and washed with chloroform. The combined filtrate and washings was successively washed with

M Na_2CO_3 and M $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated. Column chromatography (30:1 toluene–methanol) of the residue on silica gel gave **7** (1.95 g, 81%) as an amorphous mass: $[\alpha]_D$ +4.73° (*c* 0.8, $CHCl_3$); IR (KBr) 3500–2900 (NH, CH), 1740 and 1240 (ester), 1700 (imide), 1650 and 1550 (amide), and 740 cm^{-1} (Ph); ^{1}H-NMR ($CDCl_3$) δ − 0.04 and 0.08 (2s, 6H, Si*Me*$_2$), 0.66 (s, 9H, SiC*Me*$_3$), 1.33 and 1.64 (2s, 6H, C*Me*$_2$), 1.89, 2.04, 2.05, 2.15 and 2.16 (5s, 15H, AcN and 4AcO), 1.98 (t, 1H, $J_{gem} = J_{3ax,4} = 12.8$ Hz, H-3b*ax*), 2.63 (dd, 1H, $J_{gem} = 12.8$ Hz, $J_{3eq,4} = 4.6$ Hz, H-3b*eq*), 3.84 (s, 3H, *Me*O), 4.08 (m, 1H, H-5b), 4.19 (dd, 1H, $J_{gem} = 12.4$ Hz, H-9b), 4.22 (dd, 1H, $J_{1,2} = 8.5$ Hz, $J_{2,3} = 9.4$ Hz, H-2a), 4.37 (dd, 1H, $J_{gem} = 12.4$ Hz, H-9′b), 4.81 (dd, 1H, $J_{2,3} = 9.4$ Hz, $J_{3,4} = 5.0$ Hz, H-3a), 4.91 (m, 1H, H-4b), 5.27 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1a), 5.34 (m, 1H, H-7b), 5.40 (m, 1H, H-8b), and 7.69–7.82 (m, 4H, Ph).

Anal. Calcd for $C_{43}H_{60}N_2O_{19}Si$ (937.04): C, 55.12; H, 6.45; N, 2.99. Found: C, 54.85; H, 6.36; N, 2.97.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→6)-2-deoxy-3,4*-O-*isopropylidene-2-phthalimido-*β-D-*galactopyranosyl trichloroacetimidate* (**9**)

To a solution of **7** (1.90 g, 2.0 mmol) in tetrahydrofuran (15 mL) were added acetic acid (150 μL) and M tetrabutylammonium fluoride in tetrahydrofuran (3 mL) at −15°C, and the mixture was stirred for 17 h at −15°C. The mixture was diluted with chloroform and the solution was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 chloroform–methanol) of the residue on silica gel gave (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-*β*-D-galactopyranose (**8**; 1.38 g, 83%) as an amorphous mass: IR (KBr) 3600–3100 (OH, NH), 1730 and 1240 (ester), 1700 (imide), 1660 and 1550 (amide), and 720 cm^{-1} (Ph). The ^{1}H-NMR date showed the complete loss of the TBDMS group at *O*-1.

To a solution of **8** (560 mg, 0.068 mmol) in dichloromethane (1.5 mL) and trichloroacetonitrile (3 mL, 2.0 mmol) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 120 μL, 0.082 mmol) at 0°C, and the mixture was stirred for 30 min at 0°C, then concentrated. Column chromatography (35:1 chloroform–methanol) of the residue on silica gel gave **9** (656 mg, 99%, $\alpha:\beta = 1:4$) as an amorphous mass: $[\alpha]_D$ +25.77° (*c* 0.5, $CHCl_3$); IR (KBr) 3400 (NH), 1720 and 1220 (ester), 1700 (imide), 1680 and 1550 (amide), and 720 cm^{-1} (Ph); ^{1}H-NMR for *β* isomer ($CDCl_3$) δ 1.36 and 1.67 (2s, 6H, C*Me*$_2$), 1.90, 2.03, 2.04, 2.14 and 2.17 (5s, 15H, AcN and 4AcO), 2.00 (t, 1H, $J_{gem} = J_{3ax,4} = 12.8$ Hz, H-3b*ax*), 2.64 (dd, 1H, $J_{gem} = 12.8$ Hz, $J_{3eq,4} = 4.6$ Hz, H-3b*eq*), 3.83 (s, 3H, *Me*O), 4.90 (m, 1H, H-4b), 5.33 (m, 1H, H-7b), 5.49 (m, 1H, H-8b), 6.36 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1a), 7.69–7.82 (m, 4H, Ph), and 8.60 (s, 1H, C = N*H*).

Anal. Calcd for $C_{39}H_{46}Cl_3N_3O_{19}Si$ (967.16): C, 48.43; H, 4.79; N, 4.34. Found: C, 48.27; H, 4.55; N, 4.14.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→6)*-O-*(2-deoxy-3,4*-O-*isopropylidene-2-phthalimido-*β-D-*galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*benzyl-*β-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-*β-D-*glucopyranoside* (**11**)

To a solution of 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,6-di-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (**10**; 612 mg, 0.45 mmol) and **9** (656 mg, 0.68 mmol) in dichloromethane (5 mL) was added molecular sieves 4 Å (AW-300; 1.0 g), and the mixture was stirred for 15 h at room temperature, then cooled to 0°C. TMSOTf (13 μL, 0.068 mmol) was added, and the mixture was stirred for 24 h at 0°C, then filtered. The insoluble materials were washed with M $NaHCO_3$ and water, and the conbined filtrate and washings was dried (Na_2SO_4) and concentrated. Column chromatography (25:1 chloroform–methanol) of the residue on silica gel gave **11** (734 mg, 76%) as an amorphous mass: $[\alpha]_D$ +16.92° (*c* 0.5, $CHCl_3$); IR (KBr) 3500–3200 (NH), 1750 and 1230 (ester), 1700 (imide), 1670 and 1550 (amide), 860 and 840 (Me_3Si), and 700 cm^{-1} (Ph); ^{1}H-NMR ($CDCl_3$) δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.34 and 1.48 (2s, 6H, C*Me*$_2$), 1.68, 1.85, 1.86, 1.92, 2.01, 2.02, 2.03, 2.08, 2.09 and 2.11 (10s, 30H, 2AcN and 8AcO), 1.78 (t, 1H, $J_{gem} = J_{3ax,4} = 12.8$ Hz, H-3d*ax*), 1.88 (t, 1H, $J_{gem} = J_{3ax,4} = 12.8$ Hz, H-3e*ax*), 2.55 (dd, 1H, $J_{gem} = 12.8$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3e*eq*), 2.84 (dd, 1H, $J_{1,2} = 7.3$ Hz, $J_{2,3} = 9.6$ Hz, H-2b), 2.88 (dd, 1H, $J_{gem} = 12.8$ Hz, $J_{3eq,4} = 4.3$ Hz, H-3d*eq*), 3.31 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.2$ Hz, H-2a), 3.53 (m, 1H, H-3a), 3.62 (d, 1H, $J_{3,4} = 2.3$ Hz, H-4b), 3.76 (t, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4a), 3.82 and 3.87 (2s, 6H, 2*Me*O), 4.03 (m, 2H, H-5d and H-5e), 4.10 (m, 1H, H-3b), 4.28 (dd, 1H, $J_{3,4} = 5.3$ Hz, $J_{4,5} = 11.9$ Hz, H-4c), 4.30 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1a), 4.33 (m, 1H, H-2c), 4.47 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1b), 4.80 (m, 1H, H-4d), 4.95 (m, 1H, H-4e), 5.06 (dd, 1H, $J_{2,3} = 8.9$ Hz, $J_{3,4} = 5.3$ Hz, H-3c), 5.22 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1c), 5.27 (dd, 1H, $J_{6,7} = 2.5$ Hz, $J_{7,8} = 8.2$ Hz, H-7e), 5.3 (m, 2H, H-7d and H-8d), 5.44 (m, 1H, H-8e), and 6.99–7.84 (m, 29H, 6Ph).

Anal. Calcd for $C_{109}H_{135}N_{41}O_{19}Si$ (2171.35): C, 60.29; H, 6.27; N, 1.94. Found: C, 60.01; H, 6.06; N, 1.85.

2-(Trimethylsilyl)ethyl O-*(5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonic acid)-(2→6)*-O-*(2-deoxy-3,4*-O-*isopropylidene-2-phthalimido-β-*D*-*galactopyranosyl)-(1→4)*-O-[(5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonic acid)-(2→3)]*-O-*(2,6-di*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β-*D*-glucopyranoside* (**12**)

To a solution of **11** (600 mg, 0.28 mmol) in pyridine (15 mL) was added lithium iodide (384 mg, 2.96 mmol), and the mixture was stirred for 24 h under reflux and nitrogen atmosphere in the dark. The mixture was concentrated, and a solution of the residue in chloroform was successively washed with 2M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (10:1 chloroform-methanol) of the residue on silica gel gave **12** (500 mg, 85%) as an amorphous mass: $[\alpha]_D$ −3.70° (*c* 0.6, $CHCl_3$); the complete cleavage of the methyl ester was confirmed by ^{1}H-NMR.

Anal. Calcd for $C_{107}H_{131}N_3O_{41}Si$ (2143.29): C, 59.96; H, 6.16; N, 1.96. Found: C, 59.85; H, 5.92; N, 1.87.

2-(Trimethylsilyl)ethyl O-*(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-β-*D*-galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β-*D*-glucopyranoside* (**13**)

A solution of **3** (450 mg, 0.22 mmol) in aqueous 80% acetic acid (10 mL) was heated, with stirring, for 3 h at 40°C, then concentrated. Column chromatography (20:1 toluene-methanol) of the residue on silica gel gave **13** (375 mg, 85%) as an amorphous mass: $[\alpha]_D$ −7.18° (*c* 0.6, $CHCl_3$); IR (KBr) 3600–3100 (OH and NH), 1760 and 1220 (ester), 1680 and 1560 (amide), 860 and 840 (Me_3Si), and 710 cm^{-1} (phenyl); ^{1}H-NMR ($CDCl_3$) δ 1.03 (m, 2H, $Me_3SiCH_2CH_2$), 1.70–2.12 (11s, 33H, AcN and 10AcO), 1.84 (t, 1H, $J_{gem} = J_{3ax,4} =$ 12.6 Hz, H-3e*ax*), 2.11 (m, 1H, H-3d*ax*), 2.21 (m, 1H, H-3d*eq*), 2.60 (dd, 1H, $J_{gem} = 12.6$ Hz, $J_{3eq,4} = 4.6$ Hz, H-3e*eq*), 3.80 and 3.84 (2s, 6H, 2*Me*O), 4.83 (m, 1H, H-4e), 5.11 (m, 1H, H-4d), and 7.20–7.46 (m, 25H, 5Ph).

Anal. Calcd for $C_{100}H_{131}N_3O_{40}Si$ (2043.22): C, 58.78; H, 6.46; N, 2.06. Found: C, 58.72; H, 6.19; N, 1.98.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)*-O-*(2,4,6-tri*-O-*benzoyl-β-*D*-galactopyranosyl)-(1→3)*-O-*[(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-β-*D*-galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β-*D*-glucopyranoside* (**15**)

To a solution of **13** (500 mg, 0.24 mmol) and methyl *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-1-thio-*β*-D-galactopyranoside [33] (**14**; 488 mg, 0.48 mmol) in dichloromethane (6 mL) was added molecular sieves 4 Å (660 mg), and the mixture was stirred for 6 h at room temperature then cooled to 0°C. DMTST [22,25] (504 mg, 1.2 mmol), was added to the mixture, and the stirring was continued for 24 h at 0°C. The precipitates were removed by filtration, and washed thoroughly with chloroform. The combined filtrate and washings was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (15:1 toluene–methanol) of the residue on silica gel gave **15** (696 mg, 95%) as an amorphous mass: $[\alpha]_D$ + 0.43° (*c* 0.6, $CHCl_3$); IR (KBr) 3600–3100 (OH and NH), 1740 and 1220 (ester), 1660 and 1530 (amide), 860 and 840 (Me_3Si), 750 and 700 cm^{-1} (phenyl); ^{1}H-NMR ($CDCl_3$) δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.55–2.13 (16s, 48H, 4AcN and 12AcO), 1.59 (t, 1H, $J_{gem} = J_{3ax,4} = 12.6$ Hz, H-3g*eq*), 1.84 (m, 1H, H-3e*ax*), 2.05 (m, 1H, H-3f*ax*), 2.42 (dd, 1H, $J_{gem} = 12.6$ Hz, $J_{3eq,4} = 4.6$ Hz, H-3g*eq*), 2.54 (m, 2H, H-3e*eq* and H-3f*eq*), 3.71, 3.77, and 3.81 (3s, 9H, 3*Me*O), 4.80 (m, 1H, H-4g), 4.83 (m, 1H, H-4c), 4.97 (m, 1H, H-4f), 5.04 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1d), 5.43 (d, 1H, $J_{3,4} = 3.2$ Hz, H-4d), 5.51 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.3$ Hz, H-2d), and 7.17–8.16 (m, 40H, 8 Ph).

Anal. Calcd for $C_{147}H_{180}N_4O_{60}Si$ (2991.12): C, 59.03; H, 6.07; N, 1.87. Found: C, 58.77; H, 5.78; N, 1.84.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)*-O-*(2,4,6-tri*-O-*benzoyl-β-*D*-galactopyranosyl)-(1→3)*-O-*[(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-4*-O-*acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*acetyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl-β-*D*-glucopyranoside* (**16**)

A solution of **15** (600 mg, 0.20 mmol) in ethanol (15 mL) was hydrogenated in the presence of 20% $Pd(OH)_2$ (600 mg) for 72 h at room temperature, the catalyst removed by filtration and the solution concentrated. The residue was treated with acetic anhydride (3 mL) and pyridine (6 mL) for 24 h at 35°C.

The mixture was concentrated, and a solution of the residue in chloroform was successively washed with 2M HCl and M Na_2CO_3, dried (Na_2SO_4) and concentrated. Column chromatography (15:1 chloroform–methanol) of the residue on silica gel gave **16** (563 mg, quantitative) as an amorphous mass: $[\alpha]_D$ $-12.50°$ (*c* 0.6, $CHCl_3$); IR (KBr) 3300 (NH), 1740 and 1220 (ester), 1670 and 1550 (amide), 860 and 840 (Me_3Si), and 710 cm^{-1} (phenyl); ^{1}H-NMR ($CDCl_3$) δ 0.92 (m, 2H, $Me_3SiCH_2CH_2$), 1.53–2.20 (22s, 66H, 4AcN and 18AcO), 1.59 (t, 1H, $J_{gem} = J_{3ax,4} = 12.2$ Hz, H-3g*ax*), 1.71 (t, 1H, $J_{gem} = J_{3ax,4} = 12.4$ Hz, H-3e*ax*), 1.90 (m, 1H, H-3fax), 2.45 (m, 2H, H-3feq, and H-3g*eq*), 2.85 (dd, 1H, $J_{gem} = 12.4$ Hz, $J_{3eq,4} = 4.3$ Hz, H-3e*eq*), 3.76, 3.78, and 3.81 (3s, 9H, 3Me*O*), 4.98 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1d), 5.40 (d, 1H, $J_{3,4} = 3.7$ Hz, H-4d), and 7.33–8.18 (m, 15H, 3Ph).

Anal. Calcd for $C_{124}H_{162}N_4O_{66}Si$ (2792.72): C, 53.33; H, 5.85; N, 2.01. Found: C, 54.96; H, 5.87; N, 1.88.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→3)*-O-*(2,4,6-tri*-O-*benzoyl-*β-D-*galactopyranosyl)-(1→3)*-O-*[(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-4*-O-*acetyl-2-deoxy-*β-D-*galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra-O*-acetyl-3,5-dideoxy-D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*acetyl-*β-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl-D-glucopyranose* (**17**)

To a solution of **16** (170 mg, 0.060 mmol) in dichloromethane (1 mL) was added trifluoroacetic acid (2 mL) at 0°C, and the mixture was stirred for 3 h at room temperature and concentrated. Column chromatography (15:1 chloroform–methanol) of the residue on silica gel gave **17** (150 mg, 92%) as an amorphous mass: IR (KBr) 3600–3100 (OH, NH), 1740 and 1220 (ester), 1670 and 1550 (amide), and 710 cm^{-1} (Ph).

Anal. Calcd for $C_{119}H_{150}N_4O_{66}$ (2692.48): C, 55.09; H, 5.62; N, 2.08. Found: C, 52.89; H, 5.46; N, 1.85.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→3)*-O-*(2,4,6-tri*-O-*benzoyl-*β-D-*galactopyranosyl)-(1→3)*-O-*[(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-4*-O-*acetyl-2-deoxy-*β-D-*galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*acetyl-*β-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl-D-glucopyranosyl trichloroacetimidate* (**18**)

Treatment of **17** (480 mg, 0.18 mmol) with trichloroacetonitrile (780 μL, 5.4 mmol) and DBU (32 μL, 0.22 mmol) in dichloromethane (2 mL), then work up as described for **9** followed by column chromatography (20:1 chloroform–methanol) of the residue on silica gel gave **18** (505 mg, quantitative) as an amorphous mass: $[\alpha]_D$ $-1.4°$ (*c* 0.6, $CHCl_3$); IR (KBr) 3200 (NH), 1740 and 1220 (ester), 1650 and 1540 (amide), and 710 cm^{-1} (phenyl); ^{1}H-NMR for α isomer ($CDCl_3$) δ 1.53–2.19 (22s, 66H, 4AcN and 18AcO), 2.45 (m, 2H, H-3f*eq*, and H-3g*eq*), 2.87 (dd, 1H, $J_{gem} = 13.0$ Hz, $J_{3eq,4} = 4.3$ Hz, H-3e*eq*), 3.77, 3.79, and 3.81 (3s, 9H, 3*Me*O), 3.87 (t, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4a), 5.00 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1d), 5.05 (dd, 1H, $J_{1,2} = 3.9$ Hz, $J_{2,3} = 10.3$ Hz, H-2a), 5.47 (t, 1H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3a), 6.48 (d, 1H, $J_{1,2} = 3.9$ Hz, H-1a), 7.39–8.18 (m, 15H, 3Ph), and 8.65 (s, 1H, C=NH).

Anal. Calcd for $C_{121}H_{150}Cl_3N_4O_{66}$ (2836.87): C, 51.23; H, 5.33; N, 2.47. Found: C, 51.03; H, 5.14; N, 2.25.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→3)*-O-*(2,4,6-tri*-O-*benzoyl-*β-D-*galactopyranosyl)-(1→3)*-O-*[(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-4*-O-*acetyl-2-deoxy-*β-D-*galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-*D-*glycero-*α-D-*galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*acetyl-*β-D-*galactopyranosyl)-(1→4)-(2,3,6-tri*-O-acetyl-β-D-*glucopyranosyl)-(1→1)-(2S,3R,4E)-2-azido-3*-O-*(butyldiphenylsilyl)-4-octadecene-1,3-diol* (**20**)

To a solution of **18** (100 mg, 0.035 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-(butyldiphenylsilyl)-4-octadecene-1,3-diol [36,37] (**19**; 40 mg, 0.070 mmol) in dichloromethane (1.5 mL) were added molecular sieves 4 Å (AW-300; 1.2 g), and the mixture was stirred for 12 h at room temperature, then cooled to 0°C. TMSOTf (1.4 μL, 7.0 μmol) was added, and the mixture was stirred for 45 h at 0°C, then filtered. The insoluble materials were washed with chloroform, and the combined filtrate and washings was washed with M $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 chloroform-methanol) of the residue on silica gel gave **20** (71 mg, 62%) as an amorphous mass: $[\alpha]_D$ $-14.25°$ (*c* 0.8, $CHCl_3$); IR (KBr) 3300 (NH), 3100–2900 (CH), 2100 (azide), 1740 and 1220 (ester), 1670 and 1540 (amide), 710 and 700 cm^{-1} (phenyl); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, $J_{Me,CH2} = 6.6$ Hz, *Me*CH_2), 1.04 (s, 9H, *Me*$_3$C), 1.27 (s, 22H, 11CH_2), 1.73–2.22 (22s, 66H, 4AcN and 18AcO), 2.45 (m, 2H, H-3f*eq*, and H-3g*eq*), 2.85 (m, 1H, H-3e*eq*), 3.73, 3.77 and 3.80 (3s, 9H, 3*Me*O), 5.85 (m, 1H, H-5 of sphingosinc), and 7.30–8.21 (m, 25H, 5Ph).

Anal. Calcd for $C_{153}H_{201}N_7O_{66}Si$ (3238.36): C, 56.75; H, 6.26; N, 3.03. Found: C, 56.54; H, 6.11; N, 2.87.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)*-O-*(2,4,6-tri*-O-*benzoyl-β-D-galactopyranosyl)-(1→3)*-O-*[(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-4*-O-*acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)*-O-*[(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)]*-O-*(2,6-di*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri*-O-*acetyl-βD-glucopyranosyl)-(1→1)-(2S,3R,4E)-3*-O-*(butyldiphenylsilyl)-2-octadecanamido-4-octadecene-1,3-diol* (**21**)

To a solution of **20** (83 mg, 0.025 mmol) in benzene (1 mL) and water (0.04 mL) was added triphenylphosphine (14 mg, 0.053 mmol), and the mixture was stirred for 24 h at 30°C and concentrated. To a solution of the residue in dichloromethane (1 mL) were added octadecanoic acid (22 mg, 0.077 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (16 mg, 0.083 mmol), and the mixture was stirred for 24 h at 30°C. The mixture was diluted with chloroform and the solution was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 chloroform–methanol) of the residue on silica gel gave **21** (68 mg, 76%) as an amorphous mass: $[\alpha]_D$ −2.20° (*c* 1.2, $CHCl_3$); IR (KBr) 3200 (NH), 3100–2950 (CH), 1740 and 1240 (ester), 1650 and 1540 (amide), 710 and 700 cm^{-1} (phenyl); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2} = 6.9$ Hz, 2*Me*CH_2), 1.03 (s, 9H, *Me*$_3$C), 1.26 (s, 52H, 26CH_2), 1.77–2.18 (22s, 66H, 4AcN and 18AcO), 2.37–2.75 (m, 3H, H-3e*eq*, H-3f*eq*, and H-3g*eq*), 3.71, 3.77 and 3.81 (3s, 9H, 3*Me*O), 5.90 (m, 1H, H-5 of ceramide), and 7.36–8.25 (m, 25H, 5Ph).

Anal. Calcd for $C_{171}H_{237}N_5O_{68}Si$ (3478.83): C, 59.04; H, 6.87; N, 2.01. Found: C, 58.91; H, 6.64; N, 1.75.

Ganglioside GT1aα

To a solution of **21** (18 mg, 0.019 mmol) in acetonitrile (1.5 mL) was added M tetrabutylammonium fluoride in tetrahydrofuran (0.3 mL), and the mixture was stirred for 48 h at room temperature, then concentrated. To a solution of the residue in methanol (1 mL) was added a catalytic amount of sodium methoxide, and the mixture was stirred for 24 h at room temperature. Water (0.5 mL) was added and the solution was stirred for 24 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1:1 chloroform–methanol, and the combined filtrate and washings was concentrated. Column chromatography (5:5:1 chloroform–methanol–water) of the residue on Sephadex LH-20 gave the ganglioside GT1aα (8.8 mg, 84%) as an amorphous mass: $[\alpha]_D$ +2.0° (*c* 0.6, 5:5:1 $CHCl_3$–MeOH–H_2O); ^{1}H-NMR ($(CD_3)_2SO$-D_2O) δ 0.88 (t, 6H, $J_{Me,CH2} = 6.9$ Hz, 2*Me*CH_2), 1.24 (s, 52H, 26CH_2), 2.00–2.02 (4s, 12H, 4AcN), 2.73–2.89 (m, 3H, H-3e*eq*, H-3f*eq*, and H-3g*eq*), 4.15 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1a), 4.30 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1b), 4.43 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1d), 4.84 (m, 1H, H-1c), 5.32 (m, 1H, H-4 of ceramide), and 5.75 (m, 1H, H-5 of ceramide); FAB MS (negative ion mode, triethanolamine matrix): 2172.04 $[M\text{-}Na]^-$ ($C_{95}H_{162}N_5Na_2O_{47}$ MW, Exact 2171.0236, Ave. 2172.3161), 2150.06, 2149.07 $[M\text{-}2Na]^-$ ($C_{95}H_{163}N_5NaO_{47}$ MW, Exact 2149.0416, Ave. 2150.3343), 2127.06 $[M\text{-}3Na]^-$ ($C_{95}H_{164}N_5O_{47}$ MW, Exact 2127.0597, Ave. 2128.3525), 1858.0, 1857.0 [M-2Na-NeuAc]$^-$; 1696.0 [M-2Na-NeuAc-Gal]$^-$, 1545.0 [M-3Na-2NeuAc]$^-$, 888.7 [lactosylceramide]$^-$, 726.6 [glucosylceramide]$^-$, and 564.6 [Ceramide]$^-$.

TLC-immunostaining of gangliosides

TLC of the gangliosides was carried out with a solvent system of chloroform/methanol/0.2% $CaCl_2$ (40:40:11) using an HPTLC plate (E. Merk, Darmstadt, Germany). GT1aα isolated from bovine brain was developed in parallel with ganglioside. Immunostaining of samples were performed by the methods previously described [41]. The developed plate was dried in vacuo for 20 min, and dipped in 0.4% polyisobutylmethacrylate in a solution of chloroform/*n*-hexane (16:84) for 1 min. Organic solvents were removed from the plate. The plate was covered with anti-Chol-1α antibody (GGR41, ascites form) [42], and was incubated at room temperature overnight. The antibody was diluted with Tris-buffered saline (pH 7.4) (TBS) containing 0.3% gelatin. The plate was washed with TBS, and then covered with horseradish peroxidase-conjugated anti-mouse IgG. An enzyme-linked second antibody was diluted with TBS containing 0.3% gelatin and 5% skim milk. The plate was incubated at room temperature for 90 min, and then was washed with TBS. For visualization, the plate was incubated with ECL Western blotting detection reagents (Amersham, Buckinghamshire, England). Chemiluminescence was recorded on an X-ray film.

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