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TOTAL SYNTHESIS OF SIALOSYLCEREBROSIDE, GM_4*

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ABSTRACT

Described are total syntheses of *O*-[sodium (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-β-D-galactopyranosyl-(1→1)-(2*R*,3*S*,4*E*)-2-*N*-tetracosanoylsphingenine, *O*-[sodium (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-α-D-galactopyranosyl-(1→1)-(2*R*,3*S*,4*E*)-2-*N*-tetracosanoylsphingenine, *O*-[sodium (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-β-D-galactopyranosyl-(1→1)-(2*R*,3*S*,4*E*)-2-*N*-tetracosanoylsphingenine, and *O*-[sodium (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-α-D-galactopyranosyl-(1→1)-(2*R*,3*S*,4*E*)-2-*N*-tetracosanoylsphingenine by using *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,3,4,6-tetra-*O*-acetyl-D-galactopyrano-syl trichloroacetimidate and *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-D-galactopyranosyl trichloroacetimidate as key glycosyl donors, and (2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-*N*-tetracosanoylsphingenine as a key glycosyl acceptor.

INTRODUCTION

In view of the biological functions[2] attributable to gangliosides as components of cell membranes, development of an efficient synthetic route to them, especially in the case of minor gangliosides present in only minute amounts in Nature, has been desired. Synthetic gangliosides and their analogs having precisely defined structures may be regarded as most useful in attemps to uncover their biological functions on the molecular level.

As part of a project on the synthesis of glycosphingolipids, we now describe the first total synthesis of sialosylcerebroside or GM_4 (**1**), the simplest ganglioside. GM_4 (**1**) has been isolated as a minor component of gangliosides from brain, rat kidney, mouse erythrocytes, and hen-egg yolk[3], and its structure determined by ozonolysis and enzymic studies[4].

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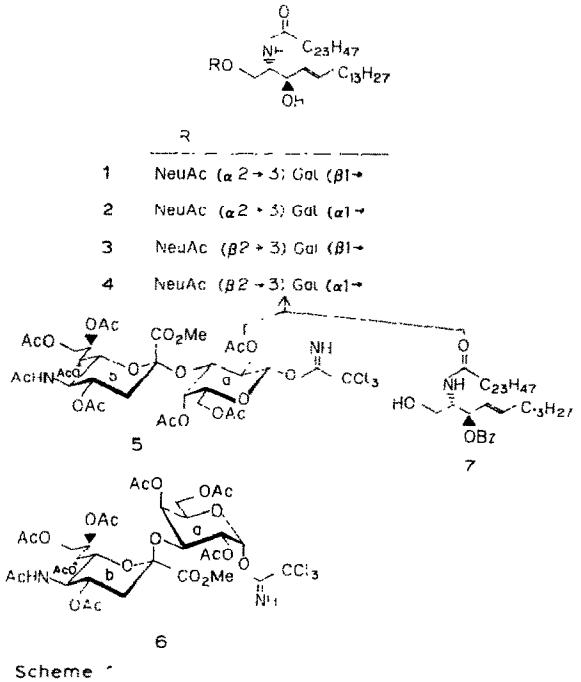
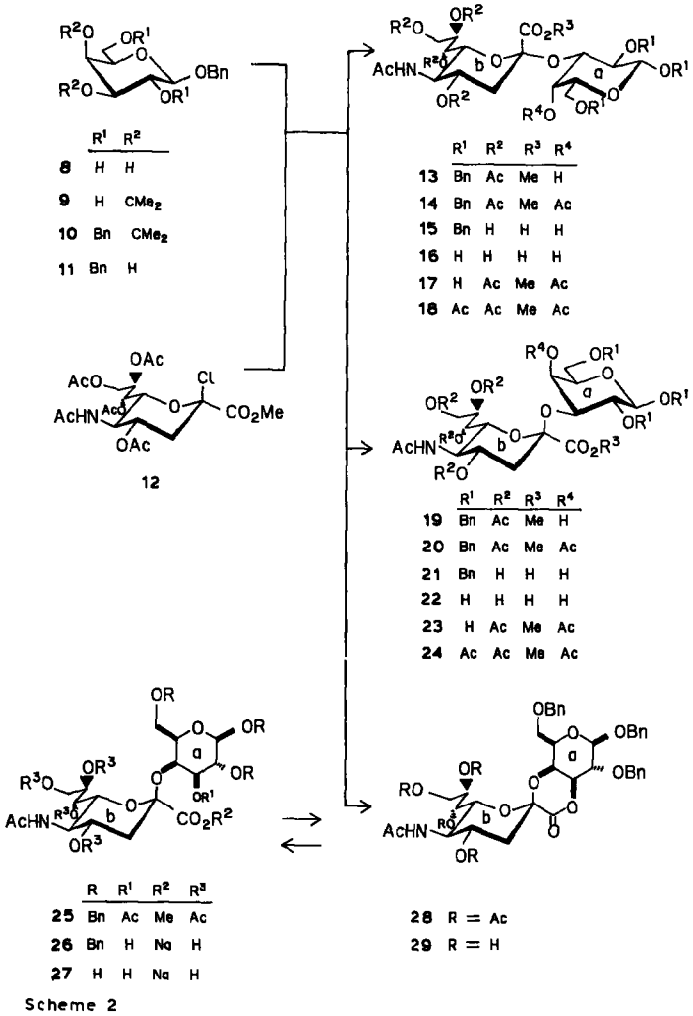


Scheme

RESULTS AND DISCUSSION

In planning a synthetic route, target structures GM_4 (**1**) and its stereoisomers **2–4** were "disconnected" into glycosyl donors **5** and **6** and benzoylceramide (**7**), which had been prepared from D-glucose in an efficient way[5]. Recently, several groups have described glycosylation with sialosyl halides at primary hydroxyl groups[6], but only a few reports[7] have appeared on the synthesis of glycosides between a sialosyl group and secondary hydroxyl groups. We now describe the first synthesis of glycosyl donors **5** and **6**, to be used for the crucial glycosylations.

Benzyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (**11**; ref. 8), readily obtainable from benzyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**9**; ref. 8) in two steps in 60% overall yield, was treated with sialosyl donor **12** (ref. 9) in the presence of mercuric bromide, mercuric cyanide, and molecular sieves 4A in dichloroethane. The reaction products were first submitted to flash chromatography, to remove the excess of glycosyl acceptor **11** and the 2,3-dehydro by-product formed by elimination of hydrogen chloride from the glycosyl donor **12**. The crude mixture of glycosylated products thus obtained was then chromatographed in a Lobar column (LiChroprep Si-60) to give, first, a 2:1 mixture of (2→4)-linked product **25** and lactone **28** in 10% yield. On standing at room temperature, this mixture was quantitatively transformed into crystalline lactone **28**. Owing to the closeness of the R_F values of **25** and **28**, and also to facile lactonization of **25** into **28**, isolation of pure compound **25** was found to be difficult.



Scheme 2

Further elution afforded the (2→3)-linked products **19** and then **13**, in 20 and 12% yield, respectively. The regiochemistry of the (2→3)-glycosidic linkage in both compounds **13** and **19** was determined from the ^{1}H-n.m.r. data for their acetates, **14** and **20**, which respectively showed deshielded signals for H-4a at δ 5.058 and 5.385 as characteristic doublets. The configuration at C-2b of glycosylation products **13** and **19** was assigned as α and β, respectively, by observing a characteristic signal for H-4b at δ 4.855 and 5.106, respectively, in their ^{1}H-n.m.r. spectra[6e,6g]. The structures of **13** and **19** were confirmed by their conversion into free disaccharides **16** and **22**. The ^{1}H-n.m.r. spectrum of **16** in D_2O contained two signals for equatorial H-3b, in the ratio of 2:1, at δ 2.763 and 2.735 as double doublets, as well as two signals for axial H-3b, in the ratio of 2:1, at δ 1.804 and 1.788 as triplets,

corresponding to the β- and α-D configuration at C-1a, respectively. The ^{1}H-n.m.r. spectrum of **22** in D_2O showed two signals for equatorial H-3b, in the ratio of 2:1, at δ 2.469 and 2.424 as double doublets corresponding to the β- and α-D configuration at C-1a, respectively, as well as a signal for axial H-3b for both anomers at δ 1.691 as a triplet. These observed chemical shifts for H-3b are in agreement with the assigned stereochemistry[10] at C-2b.

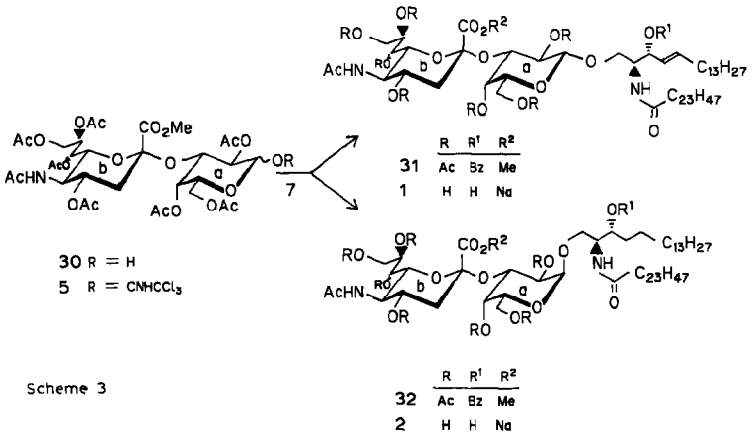
The β configuration at C-2b of the minor product **25**, formed by the glycosylation at OH-4 of the glycosyl acceptor **11**, was assigned from the ^{1}H-n.m.r. spectrum of deacetylation product **26**, which contained the signal for equatorial and axial H-3b at δ 2.614 and 1.656, respectively, in agreement with the data for the β compound **21**, but not with the data for the α compound **15**. The corresponding acid of sodium salt **26** could not be obtained, as treatment of **26** with Amberlyst 15 afforded only lactone **29**.

Compound **26** was hydrogenolyzed to give free disaccharide **27**. The ^{1}H-n.m.r. spectrum of **27** contained three signals for equatorial H-3b, at δ 2.605, 2.550, and 2.497, in the ratios of 4:3:3, indicating that **27** was accompanied by an unidentified product which was formed in the course of the Pd–C-catalyzed hydrogenolysis of **26**. Attempted purification of **27** was not successful.

Stereoselective formation of the thermodynamically more stable β anomer in glycosylation at OH-4 of the glycosyl acceptor **11** is in contrast to the glycosylation at OH-3 which gave α (**13**) and β anomer (**19**) in the ratio of 3:5. These results may be attributable to the lower reactivity of OH-4 which led to the selective formation[11] of the β anomer **25**. Because, in this Koenigs–Knorr glycosylation, the unnatural β anomer **19** was a major product, reaction conditions were examined in an attempt to improve the ratio of α to β product in favor of the former.

When glycosylation was performed in the presence of silver triflate and powdered molecular sieves 4A in tetrahydrofuran (THF), the ratio of α (**13**) to β anomer (**19**) was changed to 2:1, but the yield was decreased to 15%. However, addition of stannous chloride to the silver triflate–molecular sieves 4A system in THF increased the yield to 28% and the α to β ratio was also improved to 4:1. Under these conditions, formation of the (2→4) regioisomer **25** could not be detected by t.l.c. examination of the crude reaction-mixture.

Conversion of the glycosylation product **13** into glycosyl donor **5** was achieved in 5 steps in 48% overall yield, as follows. Acetylation of **13** afforded compound **14** in 73% yield. Hydrogenolysis of compound **14** to give compound **17** and then acetylation of compound **17** afforded an 85% yield of peracetate **18** as a 2:1 mixture of β and α anomers. Selective deacetylation at the anomeric position of compound **18**, according to Excoffier *et al.*[12], with hydrazine and acetic acid afforded a 97% yield of compound **30**. Treatment of compound **30** with trichloroacetonitrile in the presence of DBU gave a 79% yield of trichloroacetimidate **5** as a 1:8 mixture of α and β anomers. It is to be noted that in our hands use of DBU as a base afforded a higher yield of trichloroacetimidate than the use of sodium hydride or potassium carbonate as originally proposed by Schmidt and Michel[13]. By using a similar



Scheme 3

reaction sequence, compound **19** was also converted into the glycosyl donor **6** as a 5:1 mixture of α and β anomers in 5 steps in 35% overall yield.

The crucial glycosylation of benzoylceramide **7** with a 1:8 mixture of α and β trichloroacetimidate **5** in the presence of boron trifluoride etherate[14] and molecular sieves AW-300 in chloroform afforded a 3:1 mixture of β- and α-glycosylated products **31** and **32** in 40% yield. The newly formed glycosidic linkage of compound **31** was assigned as β-D by ^{1}H-n.m.r. data, which contained a signal for H-1a at δ 4.577 as a doublet with a coupling constant of 8.3 Hz. Compound **31** was deacylated, and the product saponified, to give GM_4 (**1**) in 60% yield. Similarly, compound **32** was transformed into the GM_4 isomer **2**. The ^{1}H-n.m.r. data of **2** in 49:1 Me_2SO-d_6–D_2O at 30° showed a signal for H-1a at δ 4.661 as a singlet which became a doublet with a coupling constant of 1.5 Hz when measured at 65°, thus demonstrating the α-D configuration at C-1a in **2**. It is to be noted that a signal for equatorial H-3b of GM_4 (**1**) was observed at δ 2.760, whereas that of the isomer **2** was observed at δ 2.684, as shown in Fig. 1.

Similar glycosylation of benzoylceramide **7** with a 5:1 mixture of α and β trichloroacetimidate **6** afforded a 13% yield of a mixture of products **34** and **35** which, in our hands, could not be separated into pure products. After deprotection, however, the stereoisomers were separated by preparative t.l.c., to give pure GM_4 isomers **3** and **4** in the ratio of 3:1. The ^{1}H-n.m.r. data of both **3** and **4** were in agreement with the assigned structure (see Fig. 1). Because both β-rich imidate **5** and α-rich imidate **6** afforded a 3:1 mixture of glycosylated products with β and α configuration at C-1a, the stereochemistry of the trichloroacetimidates in this case was not found to be crucial for control of the stereochemical outcome of protected GM_4 and isomers.

In conclusion, GM_4 (**1**) and three possible stereoisomers (**2, 3,** and **4**) were synhesized for the first time by using peracetylated trichloroacetimidates **5** and **6** as the key glycosyl donors and benzoylceramide **7** as the key glycosyl acceptor.

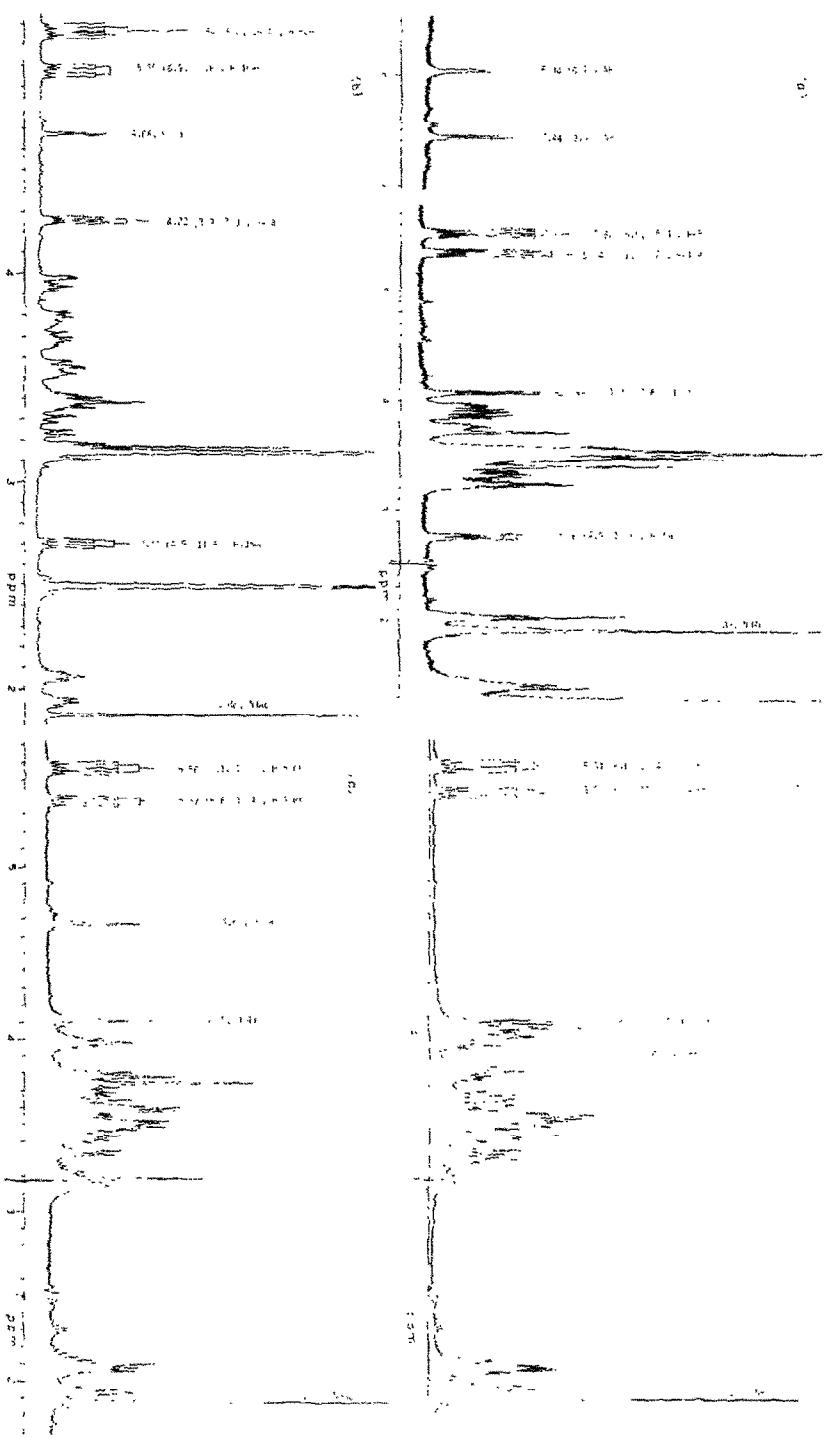
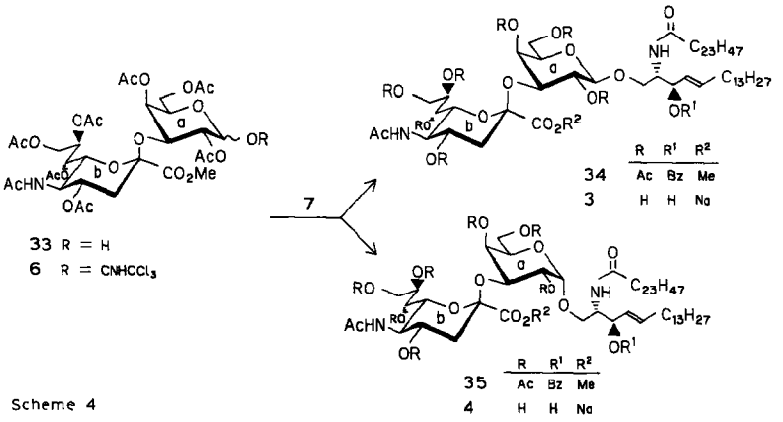


Fig. 1. 400-MHz, ^{1}H-n.m.r. spectra of synthetic glycosphingolipids in 49:1 Me_2SO-d_6–D_2O: (a) GM_4 (**1**) at 30°, (b) compound **2** at 30°, (c) compound **3** at 65°, and (d) compound **4** at 65°. The values in parentheses are *J* values in Hz.



Scheme 4

EXPERIMENTAL

General. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter, for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on columns of Silica Gel (Merck, 70–230 mesh). Flash chromatography was performed on columns of Wako gel C-300 (200–300 mesh). T.l.c. and high-performance t.l.c. were performed on Silica Gel 60 F_{254} (Merck, Darmstadt). Molecular sieves were purchased from Nakarai Chemicals, Ltd. I.r. spectra were recorded with an EPI-G2 Hitachi spectrophotometer, using KBr pellets for the crystalline samples, and films for the liquid samples. ^{1}H-N.m.r. spectra were recorded with either a JNM-GX400 or a JNM-FX90Q n.m.r. spectrometer. ^{13}C-N.m.r. spectra were recorded with a JNM-FX 100FT n.m.r. spectrometer operated at 25.05 MHz. The values of δ_C and δ_H are expressed in p.p.m. downward from the signal for internal Me_4Si, for solutions in $CDCl_3$, unless noted otherwise. Values of δ_H (D_2O) and δ_C (D_2O) are expressed in p.p.m. downward from Me_4Si, by reference to internal standards of Me_2CO (2.225) or Me_3COH (1.230), and 1,4-dioxane (67.4) or MeOH (49.8), respectively.

*Benzyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranoside* (**10**). — To a suspension of NaH (50%, 46 mg, washed with hexane) in DMF (1 mL) was added dropwise a solution of compound **9** (100 mg, 320 μmol)[8b] in DMF (2 mL), and the mixture was stirred for 30 min at 20°, and then cooled in an ice bath. To this mixture was added, dropwise, benzyl bromide (165 mg) at −5 to 0° and the mixture was stirred for 1 h at 20°. Processing, and chromatography of the residue on SiO_2 in 10:1 toluene–EtOAc afforded **10** (111 mg, 71%); $[\alpha]_D$ +7.3° (*c* 1.0); lit.[8a] $[\alpha]_D$ +8.0°; R_F 0.55 in 1:1 toluene–EtOAc; n.m.r. data: δ_H 4.392 (d, 1 H, *J* 7.9 Hz, H-1), 1.360 (s, 3 H, CH_3), and 1.309 (s, 3 H, CCH_3); δ_C 109.8 (CMe_2), 101.7 ($^1J_{CH}$ 155.0 Hz, C-1), 79.8 (C-2), 79.1 (C-3), 27.7 (CCH_3), and 26.3 (CCH_3).

Anal. Calc. for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99. Found: C, 73.38; H, 6.98.

Benzyl 2,6-di-O-benzyl-β-D-galactopyranoside (**11**). — A solution of compound **10** (6.88 g, 14 mmol) in 80% aq. AcOH (50 mL) was stirred for 3 h at 60°, and evaporated *in vacuo*. Crystallization of the residue from Et_2O afforded **11** (5.2 g, 82%); m.p. 107–108°, $[\alpha]_D$ −15.1° (*c* 1.0); lit.[8a] m.p. 107–108°, $[\alpha]_D$ −17.1°; R_F 0.17 in 10:1 toluene–EtOAc; n.m.r. data: δ_C 102.5 ($^1J_{CH}$ 156.3 Hz, C-1), 79.2 (C-2), 69.4 (C-6), and 69.0 (C-4).

Anal. Calc. for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 72.41; H, 6.75.

Benzyl O-[*methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-*α*-D-galacto-2-nonulopyranosyl)onate*]-*(2→3)-2,6-di-*O*-benzyl-β-D-galactopyranoside* (**13**) *and benzyl* O-[*methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-*β*-D-galacto-2-nonulopyranosyl)onate*]-*(2→3)-2,6-di-*O*-benzyl-β-D-galactopyranoside* (**19**). — (*A*) To a mixture of compound **11** (5.40 g, 12 mmol), powdered molecular sieves 4A (15 g), $Hg(CN)_2$ (3.03 g), and $HgBr_2$ (1.44 g) in $Cl(CH_2)_2Cl$ (6 mL) was added dropwise, one-third of a solution of **12** (2.13 g, 4.2 mmol)[9] in $Cl(CH_2)_2Cl$ (6 mL) three times with 1-h intervals, while stirring was continued under Ar. The mxiture was stirred for 2 days at 20°, diluted with EtOAc, and filtered. The filtrate was successively washed with water and aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 1:2 toluene–EtOAc afforded a mixture of glycosylated products (R_F 0.68–0.62 in EtOAc), as well as recovered **11** (R_F 0.77) and the elimination product, *methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3-didehydro-3,5-dideoxy-*D-glycero-D-galacto-*2-nonulopyranosonate* (929 mg, 49%, R_F 0.53). The fraction containing glycosylated products was chromatographed on Lichroprep (size C) in 1:9 MeOH–toluene, to afford **25** (313 mg, 8.4%), **28** (60 mg, 1.6%), **19** (715 mg, 19.4%), and **13** (427 mg, 11.6%). Compound **25** was isolated as a syrup, which still contained ~25% of **28** as judged by ^{1}H-n.m.r. spectroscopy. Compound **25** was unstable, and upon standing at 20° it gradually changed quantitatively into crystalline **28**.

Compound **25**: R_F 0.29 in 1:10 MeOH–toluene; n.m.r. data: δ_H 7.45–7.10 (m, 15 H, aromatic), 4.502 (d, 1 H, *J* 7.6 Hz, H-1a), 3.663 (s, 3 H, OMe), 2.549 (dd, 1 H, *J* 4.6 and 12.9 Hz, H-3beq), 1.129 (s, 3 H, Ac), 2.087 (s, 3 H, Ac), 2.025 (s, 3 H, Ac), 1.959 (s, 3 H, Ac), 1.788 (t, 1 H, *J* 12.7 Hz, H-3bax), and 1.456 (s, 3 H, Ac).

Compound **28**: m.p. 147–152°, $[\alpha]_D$ +11.3° (*c* 1.8); R_F 0.29 in 1:10 MeOH–toluene; n.m.r. data: δ_H 7.50–7.25 (m, 15 H, aromatic), 4.483 (d, 1 H, *J* 7.8 Hz, H-1a), 2.476 (t, 1 H, *J* 12.0 Hz, H-3beq), 2.134 (s, 3 H, Ac), 2.040 (s, 3 H, Ac), 2.014 (s, 3 H, Ac), 1.992 (s, 3 H, Ac), and 1.839 (s, 3 H, Ac).

Anal. Calc. for $C_{46}H_{53}NO_{17}\cdot 0.5\ H_2O$: C, 61.32; H, 6.04; N, 1.55. Found: C, 61.30; H, 5.99; N, 1.53.

Compound **13**: $[\alpha]_D$ −21.7° (*c* 1.2); R_F 0.24 in 1:10 MeOH–toluene; n.m.r. data: δ_H 7.40–7.20 (m, 15 H, aromatic), 5.383 (dt, 1 H, *J* 2.7 and 7.6 Hz, H-8b), 5.375 (d, 1 H, *J* 8.8 Hz, N*H*), 5.305 (dd, 1 H, *J* 2.2 and 7.8 Hz, H-7b), 4.960 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.855 (m, 1 H, H-4b, overlapped with a signal for CH_2Ph), 4.844 (d, 1 H, *J* 1.7 Hz, CH_2Ph), 4.722 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.603 (s, 2 H,

CH_2Ph), 4.553 (d, 1 H, *J* 7.8 Hz, H-1a), 3.773 (s, 3 H, OMe), 2.530 (dd, 1 H, *J* 4.6 and 13.2 Hz, H-3beq), 2.094 (s, 3 H, Ac), 1.996 (s, 3 H, Ac), 1.984 (s, 3 H, Ac), 1.953 (s, 3 H, Ac), and 1.860 (s, 3 H, Ac); δ_C 102.8 (C-1a), 98.4 (C-2b), 77.7 (C-3a), 75.9 (C-2a), 62.5 (C-9b), 52.9 (O*C*H$_3$), 49.2 (C-5b), 36.8 (C-3b), 23.0 (NCO*C*H$_3$), 21.1 (OCO*C*H$_3$), and 20.6 (3 OCO*C*H$_3$).

Anal. Calc. for $C_{47}H_{57}NO_{18}$: C, 61.09; H, 6.21; N, 1.52. Found: C, 60.92; H, 6.25; N, 1.54.

Compound **19**: $[\alpha]_D$ −25.4° (*c* 1.4); R_F 0.28 in 1:10 MeOH–toluene; n.m.r. data: δ_H 7.4–7.2 (m, 15 H, aromatic), 5.281 (dd, 1 H, *J* 2.7 and 4.9 Hz, H-7b), 5.210 (ddd, 1 H, *J* 2.7, 4.9 and 7.5 Hz, H-8b), 5.106 (dt, 1 H, *J* 3.7 and 7.8 Hz, H-4b), 5.016 (d, 1 H, *J* 11.0, C*H*$_2$Ph), 4.977 (d, 1 H, *J* 12.0 Hz, C*H*$_2$Ph), 4.744 (dd, 1 H, *J* 2.7 and 12.5 Hz, C*H*$_2$Ph), 4.667 (d, 1 H, *J* 9.3 Hz, N*H*), 4.663 (d, 1 H, *J* 12.0 Hz, C*H*$_2$Ph), 4.608 (s, 2 H, C*H*$_2$Ph), 4.603 (d, 1 H, *J* 11.0 Hz, C*H*$_2$Ph), 4.576 (d, 1 H, *J* 7.6 Hz, H-1a), 3.593 (s, 3 H, OMe), 2.547 (dd, 1 H, *J* 4.6 and 13.9 Hz, H-3beq), 2.125 (s, 3 H, Ac), 2.089 (s, 3 H, Ac), 2.037 (s, 3 H, Ac), 1.990 (s, 3 H, Ac), and 1.707 (s, 3 H, Ac); δ_C 102.8 (C-1a), 99.5 (C-2b), 77.7 (C-3a), 77.0 (C-2a), 62.6 (C-9b), 52.8 (O*C*H$_3$), 48.7 (C-5b), 36.1 (C-3b), 23.0 (NHCO*C*H$_3$), and 20.7 (4 OCO*C*H$_3$).

Anal. Calc. for $C_{47}H_{57}NO_{18}$: C, 61.09; H, 6.21; N, 1.52. Found: C, 60.90; H, 6.27; N, 1.48.

(*B*) To a stirred mixture of compound **11** (25.9 g, 57 mmol), compound **12** (5 g, 9.8 mmol), and powdered molecular sieves 4A (15 g) in tetrahydrofuran (15 mL) was added, dropwise, a solution of $AgOSO_2CF_3$ (2.5 g, 29 mmol) in THF (5 mL) at −15 to −10°. The mixture was stirred for 2 h at −10°; then a solution of **12** (5 g, 9.8 mmol) in THF (5 mL) was added dropwise at −10°. After being stirred for 16 h at 20°, the mixture was diluted with EtOAc, filtered through Celite, and the filtrate successively washed with aq. $NaHCO_3$ and aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2, first in 2:1 EtOAc–toluene and then in 1:10 MeOH–*i*Pr$_2$O, afforded **13** (1.920 g, 10.6%) and **19** (0.835 g, 4.6%). Neither compound **25** nor **28** could be isolated in this case.

(*C*) To a mixture of compound **11** (1.4 g, 3.1 mmol), compound **12** (250 mg, 460 μmol), and powdered molecular sieves 4A (1.5 g) in THF (3 mL) were successively added, dropwise, a solution of $AgOSO_2CF_3$ (1.0 g, 6 mmol) in THF (1 mL) and a solution of $SnCl_2$ (378 mg, 2 mmol) in THF (1 mL) at −15 to −10°. The mixture was stirred for 1 h at −10°, a further solution of compound **12** (250 mg, 460 μmol) in THF (1 mL) was added dropwise, and, after being stirred for 16 h at 20°, the mixture was diluted with EtOAc, filtered through Celite, and the filtrate successively washed with aq. $NaHCO_3$ and aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 as described in (*B*) afforded **13** (187 mg, 22.1%) and **19** (47.5 mg, 5.6%). T.l.c. examination of the reaction mixture could detect only a trace of the regioisomer **25** or **28**.

Benzyl O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)-4*-O-*acetyl-2,6-di*-O-*benzyl*-β-D-

galactopyranoside (**14**). — A solution of compound **13** (195 mg, 211 μmol) in Ac_2O (10 mL)–pyridine (10 mL) was stirred for 24 h at 20°, and evaporated *in vacuo*. Chromatography of the residue on Lobar LiChroprep Si-60 (size B) in 1:2 toluene–EtOAc afforded **14** (148 mg, 73%), $[\alpha]_D$ −30.7° (*c* 1.3): R_F 0.62 in EtOAc; n.m.r. data: δ_H 5.521 (ddd, 1 H, *J* 3.0, 5.5, and 8.2 Hz, H-8b), 5.331 (dd, 1 H, *J* 2.4 and 7.8 Hz, H-7b), 5.195 (d, 1 H, *J* 10.3 Hz, N*H*), 5.058 (d, 1 H, *J* 2.9 Hz, H-4a), 4.956 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 4.907 (ddd, 1 H, *J* 4.9, 10.6, and 11.5 Hz, H-4b), 4.869 (d, 1 H, *J* 12.2 Hz, C*H*$_2$Ph), 4.816 (d, 1 H, *J* 12.4 Hz, C*H*$_2$Ph), 4.684 (d, 1 H, *J* 12.0 Hz, C*H*$_2$Ph), 4.679 (d, 1 H, *J* 7.6 Hz, H-1a), 4.557 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 4.526 (dd, 1 H, *J* 3.4 and 9.8 Hz, H-3a), 4.482 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 4.384 (dd, 1 H, *J* 2.7 and 12.7 Hz, H-9b), 4.108 (q, 1 H, *J* 10.5 Hz, H-5b), 3.999 (dd, 1 H, *J* 5.4 and 12.7 Hz, H-9b), 3.841 (s, 3 H, OMe), 3.725 (dd, 1 H, *J* 2.7 and 10.7 Hz, H-6b), 3.559 (dd, 1 H, *J* 6.1 and 10.0 Hz, H-6a), 3.547 (dd, 1 H, *J* 7.6 and 9.8 Hz, H-2a), 3.491 (dd, 1 H, *J* 6.1 and 10.0 Hz, H-6a), 2.582 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3beq), 2.120 (s, 3 H, Ac), 2.038 (s, 3 H, Ac), 2.009 (s, 3 H, Ac), 1.992 (s, 3 H, Ac), 1.855 (s, 3 H, Ac), and 1.830 (s, 3 H, Ac).

Anal. Calc. for $C_{40}H_{50}NO_{19}$: C, 60.92; H, 6.16; N, 1.45. Found: C, 60.65; H, 6.28; N, 1.33.

Deprotection of **13**. — A solution of compound **13** (260 mg, 280 μmol) in 0.2M NaOMe–MeOH (6 mL) was stirred for 24 h at 20°, the base neutralized with Amberlyst 15, and the suspension filtered. The filtrate was evaporated *in vacuo*, and chromatography of the residue on Lobar LiChroprep RP-18 (size B) in 3:1 MeOH–H_2O afforded *benzyl O-(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)-2,6-di-O-benzyl-β*-D-*galactopyranoside* (**15**; 120 mg, 58%); $[\alpha]_D$ −25.9° (*c* 1.6, MeOH); R_F 0.64 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (CD_3OD) 4.486 (d, 1 H, *J* 7.8 Hz, H-1a), 4.169 (dd, 1 H, *J* 3.2 and 9.8 Hz, H-3a), 4.031 (d, 1 H, *J* 2.9 Hz, H-4a), 3.892 (ddd, 1 H, *J* 2.4, 5.1, and 11.8 Hz, H-4b), 2.827 (dd, 1 H, *J* 4.6 and 11.0 Hz, H-3beq), 2.006 (s, 3 H, NAc), and 1.807 (t, 1 H, *J* 11.0 Hz, H-3bax); δ_C (CD_3OD), 104.2 (C-1a), 79.5 (C-3a), 76.9 (C-2a), 64.6 (C-9b), and 23.0 (NCO*C*H$_3$).

A mixture of compound **15** (108 mg, 146 μmol) and 10% Pd–C (200 mg) in MeOH (5 mL) was stirred for 24 h at 20° under H_2. The usual processing afforded a quantitative yield of *O-(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)-*D-*galactopyranose* (**16**); $[\alpha]_D$ +23.1° (*c* 1.2, H_2O); R_F 0.37 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O) 5.291 (d, 0.3 H, *J* 3.9 Hz, H-1aα), 4.631 (d, 0.7 H, *J* 8.1 Hz, H-1aβ), 4.327 (dd, 0.3 H, *J* 2.9 and 10.3 Hz, H-3aα), 4.077 (dd, 0.7 H, *J* 3.2 and 10.0 Hz, H-3aβ), 4.015 (d, 0.3 H, *J* 2.9 Hz, H-4aα), 3.945 (d, 0.7 H, *J* 3.2 Hz, H-4aβ), 2.763 (dd, 0.7 H, *J* 4.9 and 12.5 Hz, H-3bβeq), 2.735 (dd, 0.3 H, *J* 5.0 and 12.0 Hz, H-3bαeq), 2.033 (d, 3 H, Ac), 1.804 (t, 0.7 H, *J* 12.0 Hz, H-3bβax), and 1.788 (t, 0.3 H, *J* 12.0 Hz, H-3bαax); δ_C (D_2O) 100.8 (C-2bα), 100.7 (C-2bβ), 97.1 ($^1J_{CH}$ 161 Hz, C-1aβ), 93.1 ($^1J_{CH}$ 170 Hz, C-1aα), 76.7 (C-3aβ), 75.7 (C-5aβ), 63.4 (C-9b), 62.0 (C-6aα), 61.8 (C-6aβ), 52.6 (C-5b), 40.5 (C-3bβ), 40.3 (C-3bα), and 22.9 (NCO*C*H$_3$).

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)-2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl acetate* (**18**) *and its α anomer.* — A mixture of compound **14** (658 mg, 680 μmol) and 10% Pd–C (320 mg) in MeOH (25 mL) was stirred for 24 h at 20° under H_2, and then filtered through Celite. Evaporation of the filtrate *in vacuo* afforded crude **17** (463 mg, 98%), R_F 0.72 in 2:1:1 BuOH–EtOH–H_2O. A solution of crude **17** (463 mg) in Ac_2O (2 mL) and pyridine (2 mL) was stirred for 24 h at 20°, and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 1:4 toluene–EtOAc afforded a 2:1 mixture of **18** and the α anomer (545 mg, 85%); $[\alpha]_D$ +7.6° (*c* 1.0); R_F 0.38 in EtOAc; n.m.r. data: δ_H 6.293 (d, 0.33 H, *J* 3.8 Hz, H-1aα) and 5.826 (d, 0.67 H, *J* 8.3 Hz, H-1aβ).

Anal. Calc. for $C_{34}H_{47}NO_{22}$: C, 49.69; H, 5.77; N, 1.70. Found: C, 49.73; H, 5.78; N, 1.57.

Conversion of **18** *into trichloroacetimidate* **5**. — To a stirred solution of a 2:1 mixture (452 mg, 550 μmol) of **18** and the α anomer in DMF (1.0 mL) was added $H_2NNH_2 \cdot AcOH$ (56 mg) at 50°. The mixture was stirred for 5 min at 50°, cooled to 20°, and diluted with EtOAc (20 mL). The organic layer was washed with water, dried ($MgSO_4$), and evaporated *in vacuo*, to give crude **30** (414 mg, 97%); R_F 0.32 in EtOAc, which was used for the next step without purification.

To a solution of compound **30** (133 mg, 170 μmol) in CH_2Cl_2 (1.0 mL) was added CCl_3CN (358 μL, 3.57 mmol), and DBU (12 μL, 85 μmol) at −10 to −5°. The mixture was stirred for 2 h at −5 to 0°, and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 1:2 Me_2CO–CCl_4 gave O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)-onate*]-*(2→3)-2,3,4,6-tetra*-O-*acetyl*-D-*galactopyranosyl trichloroacetimidate* (**5**) as a 1:8 mixture of the α and β anomers (125 mg, 79%); R_F 0.31 in 1:2 Me_2CO–CCl_4; n.m.r. data: δ_H 8.685 (s, 1 H, C=N*H*), 6.528 (d, 0.11 H, *J* 3.4 Hz, H-1aα), 5.944 (d, 0.89 H, *J* 8.3 Hz, H-1aβ), 5.004 (d, 1 H, *J* 3.2 Hz, H-4a), 3.874 (s, 3 H, OMe), 2.614 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3beq), 2.197 (s, 3 H, Ac), 2.183 (s, 3 H, Ac), 2.132 (s, 3 H, Ac), 2.075 (s, 3 H, Ac), 2.054 (s, 3 H, Ac), 2.048 (s, 3 H, Ac), 2.019 (s, 3 H, Ac), 1.859 (s, 3 H, Ac), and 1.739 (t, 1 H, *J* 12.5 Hz, H-3bax).

Benzyl O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)-4*-O-*acetyl-2,6-di*-O-*benzyl*-β-D-*galactopyranoside* (**20**). — Compound **19** (577 mg, 625 μmol) was treated, as described for the preparation of compound **14**, to give **20** (477 mg, 79%); $[\alpha]_D$ −23.9° (*c* 1.0); R_F 0.56 in 17:3 iPr_2O–MeOH; n.m.r. data: δ_H 7.4–7.2 (m, 15 H, aromatics), 5.653 (d, 1 H, *J* 10.2 Hz, N*H*), 5.385 (d, 1 H, *J* 2.4 Hz, H-4a), 5.382 (t, 1 H, *J* 2.4 Hz, H-7b), 5.289 (dt, 1 H, *J* 9.3 and 2.4 Hz, H-8b), 5.054 (dd, 1 H, *J* 2.4 and 12.2 Hz, H-9b), 5.052 (m, 1 H, H-4b), 4.980 (d, 1 H, *J* 11.5 Hz, C*H*$_2$Ph), 4.782 (d, 1 H, *J* 11.0 Hz, C*H*$_2$Ph), 4.075 (t, 1 H, *J* 10.5 Hz, H-5b), 4.044 (t, 1 H, *J* 6.6 Hz, H-5a), 3.907 (dd, 1 H, *J* 9.3 and 12.2 Hz, H-9b), 3.250 (s, 3 H, OMe), 2.582 (dd, 1 H, *J* 4.6 and 13.2 Hz, H-3beq), 2.175 (s, 3 H, Ac), 2.133 (s, 3 H, Ac), 2.028 (s, 3 H, Ac), 2.018 (s, 3 H, Ac), 2.006 (s, 3 H, Ac), 1.922 (s, 3 H, Ac), and 1.801

(dd, 1 H, *J* 12.2 and 13.2 Hz, H-3bax); δ_C 102.8 ($^1J_{CH}$ 160 Hz, C-1a), 99.2 (C-2b), 77.8 (C-3a), 62.9 (C-9b), 52.4 (C-5b), 48.2 (OCH_3), 37.3 (C-3b), 23.3 ($NCOCH_3$), 21.0 ($OCOCH_3$), and 20.7 ($OCOCH_3$).

Anal. Calc. for $C_{49}H_{59}NO_{19}$: C, 60.92; H, 6.16; N, 1.45. Found: C, 60.69; H, 6.18; N, 1.41.

Deprotection of **19**. — Compound **19** (106 mg, 110 μmol) was treated as described for the preparation of compound **15**, to give *benzyl* O-*(5-acetamido-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosylonic acid)-(2→3)-2,6-di*-O-*benzyl*-β-D-*galactopyranoside* (**21**; 60 mg, 71%); R_F 0.63 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (CD_3OD) 4.502 (d, 1 H, *J* 7.6 Hz, H-1a), 4.108 (dt, 1 H, *J* 4.4 and 11.4 Hz, H-4b), 2.710 (dd, 1 H, *J* 4.6 and 13.2 Hz, H-3beq), 1.972 (s, 3 H, $NHCOCH_3$), and 1.683 (t, 1 H, *J* 12.0 Hz, H-3bax); δ_C (CD_3OD) 103.7 (C-1a), 80.1 (C-3a), 78.8 (C-2a), 64.5 (C-9b), and 23.0 ($NCOCH_3$).

Compound **21** (63 mg) was treated as described for the preparation of compound **17**, to give O-*(5-acetamido-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosylonic acid)-(2→3)*-D-*galactopyranose* (**22**); $[\alpha]_D$ +11.0° (*c* 0.3, H_2O); R_F 0.26 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O) 5.260 (d, 0.33 H, *J* 3.2 Hz, H-1aα), 4.593 (d, 0.67 H, *J* 7.6 Hz, H-1aβ), 4.315 (d, 0.33 H, *J* 3.0 Hz, H-4aα), 4.246 (d, 0.67 H, *J* 3.2 Hz, H-4aβ), 4.182 (dt, 1 H, *J* 4.4 and 12.0 Hz, H-4b), 2.469 (dd, 0.67 H, *J* 4.6 and 12.9 Hz, H-3bβeq), 2.424 (dd, 0.33 H, *J* 5.1 and 13.4 Hz, H-3bαeq), 2.051 (s, 3 H, $NHCOCH_3$), and 1.691 (t, 1 H, *J* 12.7 Hz, H-3bax): δ_C (D_2O) 103.7 (C-2bβ), 103.6 (C-2bα), 97.5 ($^1J_{CH}$ 161.6 Hz, C-1aβ), 93.2 ($^1J_{CH}$ 169.7 Hz, C-1aα), 78.2 (C-3aβ), 75.7 (C-5aβ), 74.8 (C-3aα), 64.2 (C-9b), 61.8 (C-6aα), 61.6 (C-6aβ), 52.9 (C-5b), 41.5 (C-3b), and 23.0 ($NCOCH_3$).

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)-2,4,6-tri*-O-*acetyl*-β-D-*galactopyranosyl acetate* (**24**) *and the α anomer.* — Compound **20** (1.145 g, 1.65 mmol) was treated as described for the preparation of compound **18**, to give a 2:1 mixture of **24** plus the α anomer in 73% yield *via* compound **23** (R_F 0.64 in 4:2:1 BuOH–EtOH–H_2O).

Compound **24** plus the α anomer (2:1) had $[\alpha]_D$ +26.6° (*c* 1.0); R_F 0.26 in 10:1 toluene–MeOH; n.m.r. data: δ_H 6.217 (d, 0.33 H, *J* 3.6 Hz, H-1aα), 5.631 (d, 0.67 H, *J* 8.3 Hz, H-1aβ), 3.761 (s, 1 H, OMe), 3.748 (s, 2 H, OMe), 2.585 (dd, 0.67 H, *J* 4.6 and 13.2 Hz, H-3bβeq), 2.568 (dd, 0.33 H, *J* 4.6 and 13.4 Hz, H-3bαeq), 1.829 (t, 0.33 H, *J* 13.4 Hz, H-3bαax), and 1.799 (t, 0.67 H, *J* 13.4 Hz, H-3bβax).

Anal. Calc. for $C_{34}H_{47}NO_{22} \cdot 0.5\ C_6H_5CH_3$: C, 51.90; H, 5.92; N, 1.61. Found: C, 51.71; H, 5.91; N, 1.69.

Conversion of **24** *into trichloroacetimidate* **6**. — To a stirred solution of a 2:1 mixture (96 mg, 120 μmol) of **24** plus the α anomer in DMF (1 mL) was added $H_2NNH_2 \cdot AcOH$ (12 mg) at 50°. The mixture was stirred for 5 min at 50°, cooled to 20°, and diluted with EtOAc (20 mL). The organic layer was washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2

in 1:1 Me_2CO–CCl_4 afforded compound **33** (70 mg, 77%); $[\alpha]_D$ +36° (*c* 1.1); R_F 0.51 in 1:1 Me_2CO–CCl_4.

To a stirred solution of compound **33** (133 mg, 170 μmol) in CH_2Cl_2 (1 mL) were added Cl_3CCN (358 μL, 3.6 mmol) and DBU (12 μL, 85 μmol) at −10 to −5°. The mixture was stirred for 3 h at −5 to 0°, and then directly chromatographed on SiO_2 in 1:2 Me_2CO–CCl_4, to give O-[*methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)-2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl trichloroacetimidate* (**6**) as a 5:1 mixture (122 mg, 78%) of the α and β anomer; $[\alpha]_D$ +35.2° (*c* 1.0); R_F 0.39 in 1:2 Me_2CO–CCl_4; n.m.r. data: δ_H 8.683 (s, 0.84 H, C=N*H*α), 8.672 (s, 0.16 H, C=N*H*β), 6.463 (d, 0.84 H, *J* 3.4 Hz, H-1aα), 5.800 (d, 0.16 H, *J* 8.5 Hz, H-1aβ), 5.631 (d, 1 H, *J* 10.3 Hz, N*H*CO), 5.568 (d, 0.84 H, *J* 2.4 Hz, H-4aα), 5.373 (t, 1 H, *J* 2.0 Hz, H-7b), 3.853 (s, 0.48 H, OCH_3β), 3.822 (s, 2.52 H, OCH_3α), 2.515 (dd, 0.84 H, *J* 4.6 and 13.4 Hz, H-3bαeq), 2.503 (dd, 0.16 H, *J* 4.6 and 13.4 Hz, H-3bβeq), 2.321 (s, 2.5 H, Ac), 2.155 (s, 2.5 H, Ac), 2.065 (s, 2.5 H, Ac), 2.060 (s, 2.5 H, Ac), 2.055 (s, 2.5 H, Ac), 2.052 (s, 2.5 H, Ac), 2.014 (s, 2.5 H, Ac), 1.927 (s, 2.5 H, Ac), and 1.822 (t, 0.84 H, *J* 13.4 Hz, H-3bαax).

Deprotection of **28**. — A solution of compound **28** (200 mg) in 0.1M NaOMe–MeOH (13 mL) was stirred for 24 h at 20°, the base neutralized with Amberlite CG-50, and the suspension filtered through Celite. The filtrate was evaporated *in vacuo,* and the residue was chromatographed on silanized Kieselgel 60 (70–230 mesh) in 1:2 MeOH–H_2O, to give *benzyl* O-[*sodium (5-acetamido-3,5-dideoxy-*D-glycero-β-D-galacto-*2-nonulopyranosyl)onate*]-*(2→4)-2,6-di-*O*-benzyl-β-D-galacto-pyranoside* (**26**; 109 mg, 64%); R_F 0.69 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O) 7.4–6.9 (m, 15 H, aromatic), 4.618 (s, 2 H, CH_2Ph), 4.579 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.515 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.472 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.268 (d, 1 H, *J* 12.2 Hz, CH_2Ph), 2.614 (dd, 1 H, *J* 4.0 and 12.0 Hz, H-3beq), 2.019 (s, 3 H, NHCOCH_3), and 1.656 (t, 1 H, *J* 11.9 Hz, H-3bax); δ_C (CD_3OD) 103.4 (C-1a), 81.7 (C-2a), 65.3 (C-9b), and 23.4 (NHCOCH_3).

A mixture of compound **26** (108 mg) and 10% Pd–C (150 mg) in 9:1 MeOH–H_2O (5 mL) was stirred under H_2 for 24 h at 20° and then for 5 h at 60°. Filtration of the mixture through Celite, evaporation of the filtrate *in vacuo,* and chromatography of the residue on Lobar LiChroprep RP-8 (size A) in 80:1 MeOH–H_2O afforded O-[*sodium (5-acetamido-3,5-dideoxy-*D-glycero-β-D-galacto-*2-nonulo-pyranosyl)onate*]-*(2→4)-*D-*galactopyranose* (**27**; 23 mg, 33%); R_F 0.34 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 60°) 5.265 (d, 0.3 H, *J* 2.7 Hz, H-1aα), 4.620 (d, 0.4 H, *J* 6.9 Hz, H-1aβ), 2.605 (dd, 0.4 H, *J* 4.3 and 12.9 Hz, H-3beq), 2.550 (dd, 0.3 H, *J* 4.6 and 13.2 Hz, H-3beq), 2.497 (dd, 0.3 H, *J* 4.6 and 13.2 Hz, H-3beq), 2.042 (s, 3 H, NHCOCH_3), 1.666 (t, 0.4 H, *J* 12.3 Hz, H-3bax), 1.645 (t, 0.3 H, *J* 12.2 Hz, H-3bax), and 1.633 (t, 0.3 H, *J* 12.3 Hz, H-3bax).

Benzyl O-*(5-acetamido-3,5-dideoxy-*D-glycero-β-D-galacto-*2-nonulopyrano sylonic acid)-(2→4)-2,6-di-*O*-benzyl-β-D-galactopyranoside-(1b→3a)-lactone* (**29**). — A solution of compound **28** (60 mg) in 0.1M NaOMe–MeOH (3 mL) was stirred

for 24 h at 20°, the base neutralized with Amberlyst 15, and the suspension filtered. Evaporation of the filtrate *in vacuo*, and chromatography of the residue on Lobar LiChroprep Si-60 (size A) in 1:20 MeOH–EtOAc gave **29** (33 mg, 68%); $[\alpha]_D$ +54.5° (*c* 0.6, MeOH); R_F 0.88 in 2:1:1 BuOH–EtOH–H_2O; crystals from MeOH, m.p. 207–209°; n.m.r. data: δ_H (CD_3OD) 7.4–7.2 (m, 15 H, aromatic), 4.933 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.850 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.761 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 4.688 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.619 (s, 2 H, CH_2Ph), 4.566 (d, 1 H, *J* 4.4 Hz, H-4a), 4.551 (d, 1 H, *J* 7.8 Hz, H-1a), 4.489 (dd, 1 H, *J* 3.4 and 9.3 Hz, H-3a), 2.143 (dd, 1 H, *J* 11.3 and 13.2 Hz, H-3bax), and 2.023 (s, 3 H, NHCOCH_3).

Anal. Calc. for $C_{38}H_{45}NO_{13}\cdot H_2O$: C, 61.53; H, 6.39; N, 1.89. Found: C, 61.52; H, 6.29; N, 1.82.

O-[*Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-(2→*3*)-*O*-*(2,4,6-tri-*O*-acetyl-β-*D-*galactopyranosyl)-(1→*1*)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-*N*-tetracosanoylsphingenine* (**31**) *and α anomer* (**32**). — To a stirred mixture of compound **7** (51 mg, 67 μmol), compound **5** (62 mg, 67 μmol), and powdered molecular sieves AW 300 (1 g) in $CHCl_3$ (2 mL) was added dropwise $BF_3\cdot Et_2O$ (10 μL, 80 μmol) at −5 to 0°. The mixture was stirred for 1 h at −5° and then for 16 h at 20°, and filtered. The filtrate was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 1:2 toluene–EtOAc afforded **31** (28 mg, 28%) and **32** (11 mg, 11%).

Compound **31**: $[\alpha]_D$ −0.4° (*c* 1.4); R_F 0.25 in 1:2 toluene–EtOAc; n.m.r. data: δ_H 8.030 (d, 2 H, *J* 7.1 Hz, Bz), 7.550 (t, 1 H, *J* 7.6 Hz, Bz), 7.436 (t, 2 H, *J* 7.6 Hz, Bz), 5.875 (dt, 1 H, *J* 15.4 and 7.8 Hz, H-5), 5.843 (d, 1 H, *J* 10.0 Hz, N*H*CO-2), 5.559 (t, 1 H, *J* 7.3 Hz, H-3), 5.528 (td, 1 H, *J* 2.7 and 9.3 Hz, H-8b), 5.487 (dd, 1 H, *J* 7.3 and 15.4 Hz, H-4), 5.380 (dd, 1 H, *J* 2.7 and 9.0 Hz, H-7b), 5.075 (d, 1 H, *J* 10.3 Hz, C5b-N*H*CO), 4.997 (dd, 1 H, *J* 8.0 and 10.2 Hz, H-2a), 4.885 (dt, 1 H, *J* 3.4 and 10.3 Hz, H-4b), 4.885 (d, 1 H, *J* 3.4 Hz, H-4a), 4.577 (d, 1 H, *J* 8.3 Hz, H-1a), 3.845 (s, 3 H, OCH_3), 2.586 (dd, 1 H, *J* 4.4 and 12.5 Hz, H-3beq), 2.183 (s, 3 H, Ac), 2.163 (s, 3 H, Ac), 2.102 (s, 3 H, Ac), 2.074 (s, 3 H, Ac), 2.039 (s, 3 H, Ac), 2.012 (s, 3 H, Ac), 1.920 (s, 3 H, Ac), 1.858 (s, 3 H, Ac), 1.708 (t, 1 H, *J* 12.5 Hz, H-3bax), and 0.878 (t, 6 H, *J* 6.6 Hz, 2 CH_2CH_3).

Anal. Calc. for $C_{81}H_{130}N_2O_{24}\cdot 0.5\ C_6H_5CH_3$: C, 64.98; H, 8.65; N, 1.79. Found: C, 65.07; H, 8.77; N, 2.05.

Compound **32**: $[\alpha]_D$ −17.2° (*c* 0.9); R_F 0.41 in 1:2 toluene–EtOAc; n.m.r. data: δ_H 8.030 (d, 2 H, *J* 7.2 Hz, Bz), 7.566 (t, 1 H, *J* 7.0 Hz, Bz), 7.456 (t, 2 H, *J* 7.5 Hz, Bz), 5.954 (d, 1 H, *J* 10.0 Hz, N*H*CO-2), 5.906 (td, 1 H, *J* 7.3 and 14.6 Hz, H-5), 3.829 (s, 3 H, OCH_3), 2.670 (dd, 1 H, *J* 4.9 and 13.4 Hz, H-3beq), 2.165 (s, 3 H, Ac), 2.155 (s, 3 H, Ac), 2.104 (s, 3 H, Ac), 2.075 (s, 3 H, Ac), 2.046 (s, 3 H, Ac), 2.037 (s, 3 H, Ac), 1.947 (s, 3 H, Ac), 1.915 (s, 3 H, Ac), and 0.876 (t, 6 H, *J* 6.6 Hz, 2 CH_2CH_3).

Anal. Calc. for $C_{81}H_{130}N_2O_{24}$: C, 64.18; H, 8.64; N, 1.85. Found: C, 64.14; H, 8.71; N, 1.87.

O-[*Methyl (5-acetamido-4,6,7,8-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→1)-(2*S,*3*R,*4*E)-3*-O-*benzoyl-2*-N-*tetracosanoylsphingenine* (**34**) *and the α anomer* (**35**). — A mixture of compound **7** (83 mg, 109 μmol), compound **6** (100 mg, 108 μmol), and powdered molecular sieves AW-300 (1 g) in $CHCl_3$ (3 mL) was treated with $BF_3 \cdot Et_2O$ (15 μL, 124 μmol) as described for the preparation of compounds **31** and **32**, to give a mixture (21 mg, 13%) of **34** and **35**; R_F 0.25 in 1:2 toluene–EtOAc; n.m.r. data: δ_H 8.033 (d, 2 H, *J* 7.3 Hz, Bz), 7.558 (t, 1 H, *J* 7.6 Hz, Bz), 7.443 (t, 2 H, *J* 7.6 Hz, Bz), 3.834 (s, 3 H, OCH_3), 2.474 (dd, 1 H, *J* 4.6 and 13.4 Hz, H-3beq), 2.300 (s, 3 H, Ac), 2.145 (s, 3 H, Ac), 2.050 (s, 3 H, Ac), 2.034 (s, 3 H, Ac), 2.032 (s, 3 H, Ac), 2.000 (s, 3 H, Ac), 1.966 (s, 3 H, Ac), 1.910 (s, 3 H, Ac), 1.789 (t, 1 H, *J* 13.4 Hz, H-3bax), and 0.878 (t, 6 H, *J* 6.6 Hz, 2 CH_2CH_3).

Anal. Calc. for $C_{81}H_{130}N_2O_{24}$: C, 64.18; H, 8.64; N, 1.85. Found: C, 64.03; H, 8.50; N, 1.80.

O-[*Sodium (5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)*-O-*β*-D-*galactopyranosyl-(1→1)-(2*R,*3*S,*4*E)-2*-N-*tetracosanoylsphingenine* (**1**) *and the isomer* **2**. — A solution of compound **31** (10 mg, 66 μmol) in 1:1 THF–MeOH (2 mL) containing M NaOMe (25 μL) was stirred for 16 h at 20°, and evaporated *in vacuo*. The residue was dissolved in 2:2:1 THF–MeOH–H_2O (2.5 mL), and the solution was stirred for 16 h and evaporated *in vacuo*. Purification of the residue by chromatography on Sephadex LH-20 in 60:30:4.6 $CHCl_3$–MeOH–H_2O afforded **1** (5 mg, 69%); $[\alpha]_D$ −2.5° (*c* 0.17, 2:1 $CHCl_3$–MeOH); R_F 0.39 in 60:30:4.6 $CHCl_3$–MeOH–H_2O; n.m.r. data: δ_H (49:1 Me_2SO-d_6-D_2O, 30°) 8.042 (d, 1 H, *J* 6.1 Hz, *N*H), 7.438 (d, 1 H, *J* 9.0 Hz, *N*H), 5.524 (dt, 1 H, *J* 15.4 and 5.9 Hz, H-5), 5.343 (dd, 1 H, *J* 7.1 and 15.2 Hz, H-4), 4.072 (d, 1 H, *J* 7.6 Hz, H-1a), 3.959 (dd, 1 H, *J* 4.6 and 10.0 Hz, H-1), 3.862 (t, 1 H, *J* 8.3 Hz, H-3), 3.765 (m, 1 H, H-4b), 3.711 (d, 1 H, *J* 2.7 Hz, H-4a), 2.760 (dd, 1 H, *J* 4.2 and 12.0 Hz, H-3beq), 2.027 (t, 2 H, *J* 7.6 Hz, $COCH_2CH_2$), 1.893 (s, 3 H, $NHCOCH_3$), and 0.851 (t, 6 H, *J* 6.6 Hz, CH_2CH_3); lit.[14] δ_H (49:1 Me_2SO-d_6-D_2O, 30°) 5.546 (H-5), 5.346 (H-4), 4.062 (H-1a), 3.962 (H-1), 3.926 (H-3), 2.658 (H-3beq), 1.888 (NAc), and 1.536 (H-3bax).

Compound **32** (25 mg, 165 μmol) was treated as described for the preparation of compound **1**, to give O-[*sodium (5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→3)*-O-*α*-D-*galactopyranosyl-(1→1)-(2*R,*3*S,*4*E)-2*-N-*tetracosanoylsphingenine* (**2**; 12 mg, 66%); $[\alpha]_D$ −22.0° (*c* 0.38, 2:1 $CHCl_3$–MeOH); R_F 0.34 in 60:30:4.6 $CHCl_3$–MeOH–H_2O; n.m.r. data: δ_H (49:1 Me_2SO-d_6-D_2O, 30°) 5.544 (dt, 1 H, *J* 6.0 and 15.4 Hz, H-5), 5.349 (dd, 1 H, *J* 7.0 and 15.4 Hz, H-4), 4.661 (s, 1 H, H-1a), 4.221 (dd, 1 H, *J* 3.4 and 7.1 Hz, H-3a), 3.981 (d, 1 H, *J* 2.5 Hz, H-4a), 3.897 (t, 1 H, *J* 7.0 Hz, H-3), 3.786 (dd, 1 H, *J* 3.0 and 7.3 Hz, H-2a), 2.684 (dd, 1 H, *J* 4.3 and 11.0 Hz, H-3beq), 2.045 (q, 2 H, *J* 7.0 Hz, $COCH_2CH_2$), 1.877 (s, 3 H, $NHCOCH_3$), and 0.852 (t, 6 H, *J* 6.4 Hz, CH_2CH_3); δ_H (49:1 Me_2SO-d_6-D_2O, 65°) 4.676 (d, 1 H, *J* 1.5 Hz, H-1a), 2.705 (dd, 1 H, *J* 4.6 and 11.6 Hz, H-3beq), and 1.876 (s, 3 H, $NHCOCH_3$).

Deprotection of **34** *and* **35**. — A mixture of compounds **34** and **35** (15 mg, 99 μmol) was treated as described for the preparation of compound **1**, to give a mixture of **3** and **4** which was separated by preparative t.l.c. on Silica gel 60 F_{254} in 60:30:4.6 $CHCl_3$–MeOH–H_2O. O-[*Sodium (5-acetamido-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosyl)onate*]-(2→*3)*-O-β-D-*galactopyranosyl-(1→1)-(2S, 3R,4E)-2*-N-*tetracosanoylsphingenine* (**3**) *and the α anomer* (**4**) were separately extracted from the silica gel by 5:5:1 $CHCl_3$–MeOH–H_2O and chromatographed on Sephadex LH-20 in 60:40:4.6 $CHCl_3$–MeOH–H_2O, to give **3** (7.3 mg, 67%) and **4** (2.5 mg, 23%).

Compound **3**: $[\alpha]_D$ −13.3° (*c* 0.37, 2:1 $CHCl_3$–MeOH); R_F 0.33 in 60:30:4.6 $CHCl_3$–MeOH–H_2O; n.m.r. data: δ_H (49:1 Me_2SO-d_6–D_2O, 65°) 5.541 (dt, 1 H, *J* 15.4 and 6.1 Hz, H-5), 5.386 (dd, 1 H, *J* 6.8 and 15.6 Hz, H-4), 4.064 (d, 1 H, *J* 7.6 Hz, H-1a), 4.032 (bs, 1 H, H-4a), 1.869 (s, 3 H, $NHCOCH_3$), and 0.855 (t, 6 H, *J* 7.1 Hz, CH_2CH_3).

Compound **4**: $[\alpha]_D$ +15.6° (*c* 0.13, 2:1 $CHCl_3$–MeOH); R_F 0.41 in 60:30:4.6 $CHCl_3$–MeOH–H_2O; n.m.r. data: δ_H (49:1 Me_2SO-d_6–D_2O, 65°) 5.564 (td, 1 H, *J* 7.1 and 15.1 Hz, H-5), 5.373 (dd, 1 H, *J* 6.6 and 15.4 Hz, H-4), 4.667 (s, 1 H, H-1a), 4.104 (s, 1 H, H-4a), 1.875 (s, 3 H, $NHCOCH_3$), and 0.854 (t, 6 H, *J* 6.8 Hz, 2 CH_2CH_3).

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