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SYNTHESIS OF A FULLY PROTECTED DERIVATIVE OF *O*-(*N*-ACETYL-α-D-NEURAMINYL)-(2→3)-*O*-β-D-GALACTOPYRANOSYL-(1→3)-*O*-[(*N*-ACETYL-α-D-NEURAMINYL)-(2→6)]-*O*-(2-ACETAMIDO-2-DEOXY-α-D-GALACTOPYRANOSYL)-(1→3)-L-SERINE*

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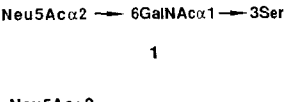
ABSTRACT

N-(Benzyloxycarbonyl)-*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→3)-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate-(2→6)]-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine benzyl ester was synthesized 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)-*O*-(2,4,6-tri-*O*-acetyl-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-nonulopyranosyl)onate-(2→6)]-4-*O*-acetyl-2-azido-2-deoxy-α- and -β-D-galactopyranosyl trichloroacetimidate as a key glycotetraosyl donor which, upon reaction with *N*-(benzyloxycarbonyl)-L-serine benzyl ester, afforded a 44% yield of a mixture of the α- and β-glycosides in the ratio of 2:5.

INTRODUCTION

Such sialic acid-containing, mucin-type glycopeptides as **1, 2,** and **3** appear in the biosynthetic pathway at the branching points for the extension of oligosaccharide chains glycosidically linked either to L-serine or L-threonine[2]. In 1984, it was proposed that glycotetraosyl-L-serine **3** is one of the sialyl glycopeptides[3] isolated



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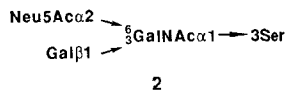

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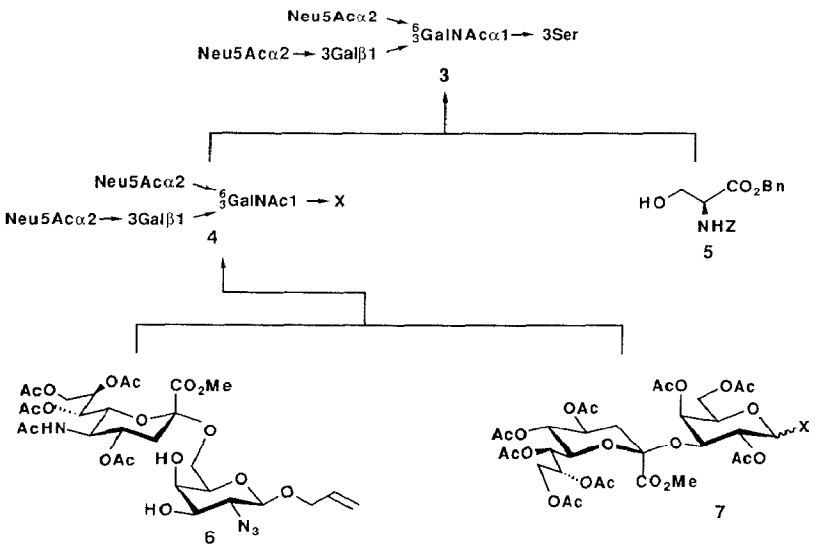
from the urine of a patient suffering from mucolipidosis I. The corresponding tetrasaccharide-alditol α-NeuAc-(2→3)-β-Gal-(1→3)-[α-NeuAc-(2→6)]-GalNAc-ol was obtained from various sources[4], and the structure was determined through methylation analysis and ^{1}H-n.m.r.-spectral study. Synthetic approaches to the glycosylserines **1** (ref. 5) and **2** (ref. 6) have already been reported. We now describe a synthesis of glycotetraosyl-L-serine **3** in the completely blocked structure **19**.

RESULTS AND DISCUSSION

In planning a synthetic route to the glycotetraosyl-L-serine **3**, a direct glycosylation of the L-serine derivative **5** with glycotetraosyl donor **4** was chosen, in order to examine the efficiency of this type of coupling (see Scheme 1). The key intermediate **4**, in turn, may be obtainable from the condensation of the known glycobiosyl acceptor **6** (ref. 5) and the glycobiosyl donor **7**. As the synthetic equivalent of the donor **7**, both the known trichloroacetimidate **8** (ref. 7) and the thioglycoside **13** were employed.

First, boron trifluoride etherate-promoted reaction of **8** with **6** afforded the desired tetrasaccharide **9** and the hexasaccharide **11** in 48 and 8% yield, respectively. The structure of compound **9** was determined by conversion into completely acetylated derivative **10**, and inspection of the ^{1}H-n.m.r. data of **10** revealed a signal for H-1c at δ 4.918 as a doublet with a $J_{1,2}$ value of 7.8 Hz, as well as a deshielded signal for H-4a at δ 5.359 as a doublet with a $J_{3,4}$ value of 3.0 Hz. The anomeric configuration at C-1c of **9** was further confirmed by the two-step transformation of **10** into deblocked propyl glycoside **15** [by (*i*) palladium-on-carbon and hydrogen in



Scheme 1

methanol, (*ii*) sodium hydroxide in methanol], and by observation of a signal for both H-1a and H-1c at δ 4.480 as a doublet with a $J_{1,2}$ value of 8.1 Hz. On the other hand, the structure of diglycosylated compound **11** was readily assigned by conversion into deblocked compound **12**, which showed, in its ^{1}H-n.m.r. spectrum, three signals, for H-1a, H-1c, and H-1d, as three pairs of doublets at δ 4.497, 4.824, and 4.616 with $J_{1,2}$ values of 7.8, 7.6, and 7.6 Hz, respectively.

Another glycobiosyl donor, compound **13**, was readily prepared in 89% yield by treatment of the imidate **8** with tributyltin methyl sulfide[8] in the presence of boron trifluoride etherate. The β-D-configuration at C-1a of compound **13** was assigned by the presence, in the ^{1}H-n.m.r. spectrum, of a signal for H-1a at δ 4.557 with a $J_{1,2}$ value of 10.0 Hz. However, compared to the imidate procedure, cupric bromide–tetrabutylammonium bromide–silver triflate-promoted[9] glycosylation of **6** by use of the donor **13** gave an inferior result, affording, after acetylation, compound **10** in ~17% yield.

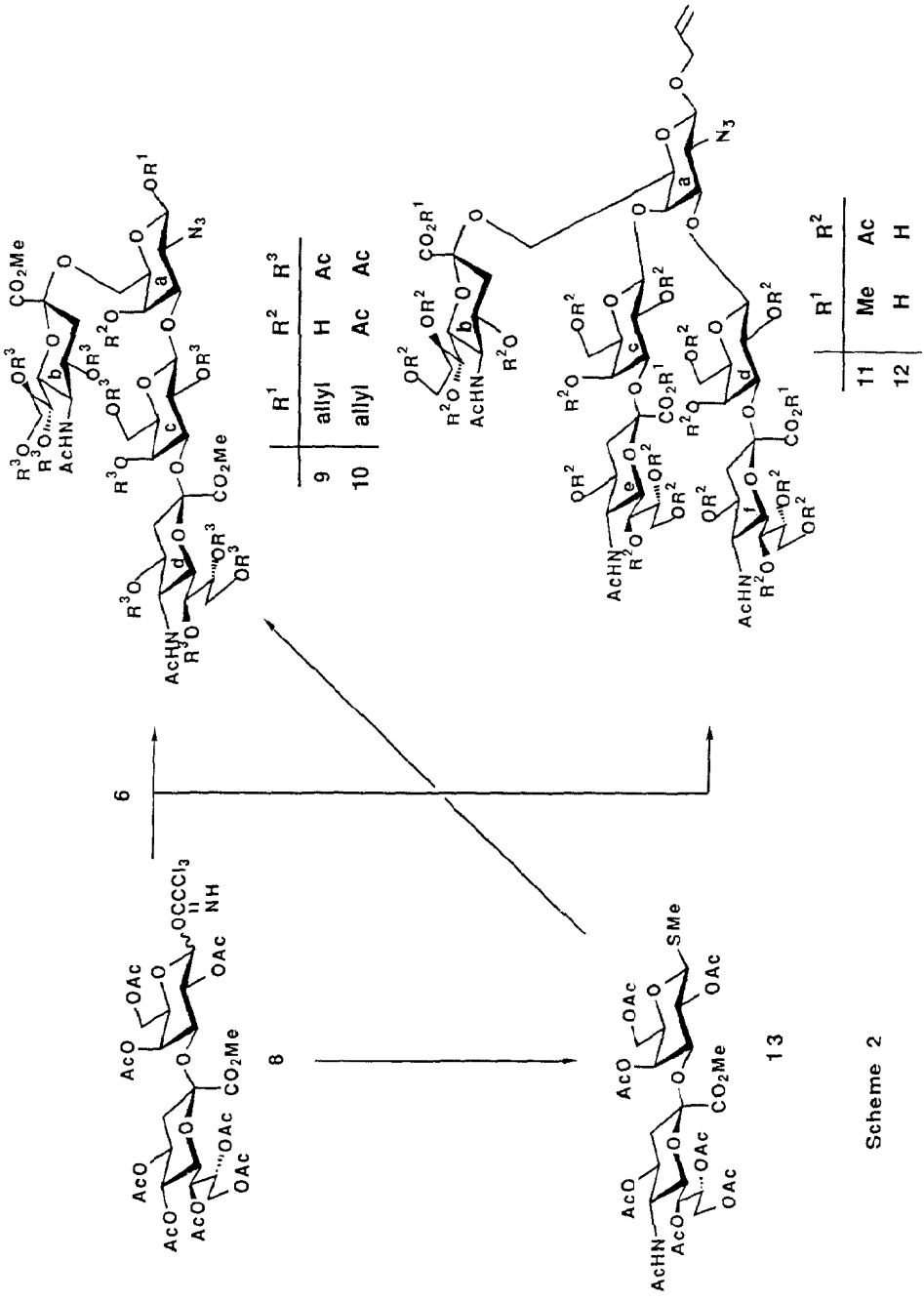
Having the tetrasaccharide intermediate **10** at our disposal, the glycotetraosyl imidate **17** was designed as a synthetic equivalent of the glycosyl donor **4** in Scheme 1. Deallylation[10] of compound **10** with palladium(II) chloride–sodium acetate in aq. acetic acid gave compound **16**, which was treated with trichloroacetonitrile and DBU to afford crude trichloroacetimidate **17** in 49% overall yield.

Crucial glycosylation of the L-serine derivative **5** with the glycosyl donor **17** was performed in the presence of boron trifluoride etherate, to give the desired product **18** and the undesired stereoisomer **21** in 12 and 32% yield, respectively. The newly introduced configuration at C-1a of compound **18** was assigned from ^{1}H-n.m.r.-spectral data, which contained a signal for H-1a as a doublet at δ 4.851 with a $J_{1,2}$ value of 3.4 Hz, along with a signal for H-2a as a double doublet at δ 3.671 with $J_{1,2}$ and $J_{2,3}$ values of 3.4 and 11.2 Hz, respectively. On the other hand, for compound **21**, signals for H-1a and H-2a were observed as a doublet and a double doublet at δ 4.237 with $J_{1,2}$ value of 7.8 Hz, and at δ 3.487 with $J_{1,2}$ and $J_{2,3}$ values of 8.1 and 10.3 Hz, respectively. Azido functions of both compounds **18** and **21** were now transformed into acetamido groups in two steps [(*i*) sodium borohydride–nickel chloride[11] and (*ii*) acetic anhydride–pyridine], to give compounds **19** and **22**. The attempted deblocking of compound **19** into the glycotetraosyl-L-serine **3** afforded insufficient of the free compound **3** for it to be well characterized by ^{1}H-n.m.r. spectroscopy.

In conclusion, the completely protected glycotetraosyl-L-serine derivative **19** has been synthesized by employing glycotetraosyl trichloroacetimidate **17** as a key intermediate. The low stereoselectivity observed for the coupling between compounds **17** and **5** gravely detracts from the utility of this synthetic approach.

EXPERIMENTAL

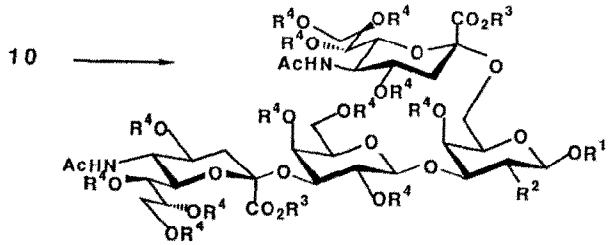
General. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a



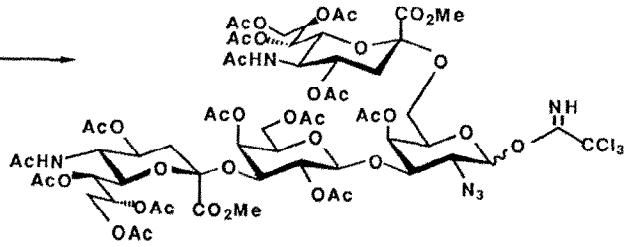
	R^1	R^2	R^3
9	allyl	H	Ac
10	allyl	Ac	Ac

	R^1	R^2
11	Me	Ac
12	H	H

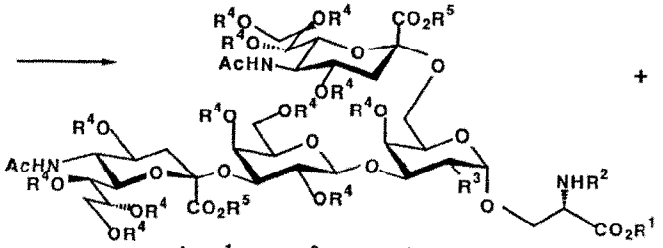
Scheme 2



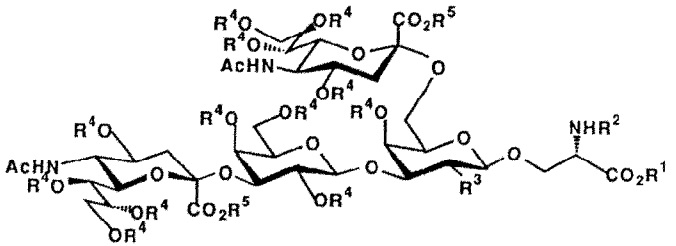
	R^1	R^2	R^3	R^4
14	Propyl	NHAc	Me	Ac
15	Propyl	NHAc	Na	H
16	H	N_3	Me	Ac



17



	R^1	R^2	R^3	R^4	R^5
18	Bn	CO_2Bn	N_3	Ac	Me
19	Bn	CO_2Bn	NHAc	Ac	Me
20	H	H	NHAc	Ac	Me
3	H	H	NHAc	H	H



	R^1	R^2	R^3	R^4	R^5
21	Bn	CO_2Bn	N_3	Ac	Me
22	Bn	CO_2Bn	NHAc	Ac	Me

Scheme 3

Perkin–Elmer Model 241 MC polarimeter, for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on 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 (h.p.) t.l.c. were performed on Silica Gel 60 F_{254} (Merck). Molecular sieves were purchased from Nakarai Chemicals. N.m.r. spectra were recorded with either a JEOL GX400 [^1H (400 MHz)] or an FX90Q [^{13}C (22.50 MHz)] spectrometer. The values of δ_H and δ_C 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 the signal for Me_4Si, by reference to internal Me_2CO (2.225) or Me_3COH (1.230), and 1,4-dioxane (67.4) or MeOH (49.8), respectively.

Allyl 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→3)*-O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate-(2→6)*]-*2-azido-2-deoxy*-β-D-*galactopyranoside* **9** *and allyl* 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→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)*-O-*(2,4,6-tri*-O-*acetyl*-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-nonulopyranosyl)onate-(2→6)*]-*2-azido-2-deoxy*-β-D-*galactopyranoside* (**11**). — To a stirred mixture of compound **6** (368 mg, 511 μmol) and powdered molecular sieves AW-300 (1.2 g) in $Cl(CH_2)_2Cl$ (2.5 mL) was added a solution of compound **8** (α:β = 1:6; 394 mg, 426 μmol) in $Cl(CH_2)_2Cl$ (2.5 mL), and then $BF_3 \cdot Et_2O$ (64 μL, 511 μmol) at −15° under Ar. After stirring for 30 min at −15°, the mixture was diluted with EtOAc and filtered through Celite. The filtrate was successively washed with aq. $NaHCO_3$ and aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed over SiO_2 in 15:5:2 EtOAc–toluene–MeOH, to give **9** (300 mg, 48%) and **11** (67 mg, 8%).

Compound **9** had $[\alpha]_D$ −5.0° (*c* 0.3); R_F 0.29 in 8:8:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 5.932 (m, 1 H, $CH{=}CH_2$), 4.609 (dd, 1 H, *J* 3.4 and 10.0 Hz, H-3c), 3.862 and 3.806 (2 s, 6 H, 2 OCH_3), 2.590 (m, 2 H, H-3b*eq* and H-3d*eq*), 2.245, 2.195, 2.136, 2.129, 2.115, 2.079, 2.074, 2.049, 2.037, 2.028, 2.017, 1.881, and 1.864 (13 s, 39 H, 11 $OCOCH_3$ and 2 $NCOCH_3$).

Anal. Calc. for $C_{61}H_{89}N_5O_{39}$: C, 48.32; H, 5.91; N, 4.62. Found: C, 48.33; H, 5.53; N, 4.49.

Compound **11** had $[\alpha]_D$ −9.0° (*c* 1.4); R_F 0.07 in 8:8:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 5.930 (m, 1 H, $CH{=}CH_2$), 4.684 (dd, 1 H, *J* 3.7 and 9.8 Hz, H-3c*), 4.617 (dd, 1 H, *J* 3.7 and 9.8 Hz, H-3d*), 3.847 (s, 6 H, 2 OCH_3), 3.824 (s, 3 H, OCH_3), 2.61–2.51 (m, 3 H, H-3b*eq*, 3e*eq*, and 3f*eq*), 2.194, 2.186, 2.166, 2.151, 2.129, 2.123, 2.117, (6 H), 2.074, 2.070, 2.062, 2.059, 2.055, 2.027, 2.019, 2.014, (9 H), 1.884, 1.866, and 1.857 (18 s, total 63 H, 21 $COCH_3$), and 1.769 and 1.693 (2 t, 2 H, *J* 12.7 Hz, two of H-3b*ax*, 3e*ax*, and 3f*ax*).

*Assignments marked with an asterisk may have to be interchanged.

Anal. Calc. for $C_{77}H_{104}N_6O_{49}$: C, 48.74; H, 5.52; N, 4.43. Found: C, 48.93; H, 5.46; N, 3.57.

Allyl 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→3)*-O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate-(2→6)*]-*4*-O-*acetyl-2-azido-2-deoxy*-β-D-*galactopyranoside* (**10**). — [*A*] A solution of compound **9** (160 mg, 108 μmol) in Ac_2O (2 mL) and pyridine (3 mL) was stirred for 24 h at 20°, and evaporated *in vacuo*. The residue was chromatographed over SiO_2 in 30:1 $CHCl_3$–MeOH, to give **10** (163 mg, 98%); $[\alpha]_D$ −4.1° (*c* 0.3); R_F 0.36 in 20:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 5.960 (m, 1 H, C*H*=CH_2), 5.569 (ddd, m, 1 H, *J* 3.0, 5.5, and 9.5 Hz, H-8d), 5.359 (d, 1 H, *J* 3.0 Hz, H-4a), 5.097 (d, 1 H, *J* 10.3 Hz, N*H*), 4.986 (dd, 1 H, *J* 7.8 and 10.0 Hz, H-2c), 4.913 (d, 1 H, *J* 7.8 Hz, H-1c), 4.891 (d, 1 H, *J* 2.0 Hz, H-4c), 4.91–4.81 (m, 2 H, H-4b,4d), 4.546 (dd, 1 H, *J* 3.4 and 10.0 Hz, H-3c), 4.321 (d, 1 H, *J* 7.8 Hz, H-1a), 3.853 (s, 3 H, OCH_3), 3.789 (s, 3 H, OCH_3), 3.582 (dd, 1 H, *J* 7.8 and 10.3 Hz, H-2a), 3.324 (dd, 1 H, *J* 5.6 and 10.0 Hz, H-6a), 2.588 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3d*eq*), 2.568 (dd, 1 H, *J* 4.4 and 12.9 Hz, H-3b*eq*), 2.233, 2.186, 2.134, 2.112, 2.100, 2.096, 2.076, 2.068, 2.048, 2.035, 2.020, 2.012, 1.875, and 1.857 (14 s, 42 H, 12 OCOCH_3 and 2 NCOCH_3), 1.921 (t, 1 H, *J* 12.5 Hz, H-3b*ax*), and 1.716 (t, 1 H, *J* 12.5 Hz, H-3d*ax*); δ_C 100.5 (C-1a,1c), 98.6 (C-2b), and 96.8 (C-2d).

Anal. Calc. for $C_{63}H_{91}N_5O_{40}$: C, 48.55; H, 5.88; N, 4.49. Found: C, 48.62; H, 5.60; N, 4.76.

[*B*] To a stirred mixture of $CuBr_2$ (30 mg, 135 μmol), powdered molecular sieves 4A (230 mg), Bu_4NBr (9 mg, 27 μmol), $AgOSO_2CF_3$ (35 mg, 135 μmol), and compound **6** (65 mg, 90 μmol) in $Cl(CH_2)_2Cl$ (1.0 mL) was added dropwise a solution of compound **13** (73 mg, 90 μmol) in $Cl(CH_2)_2Cl$ (1.0 mL) at 20° under Ar. After stirring for 2 h at 20°, the mixture was diluted with EtOAc and filtered through Celite. The filtrate was successively washed with aq. $NaHCO_3$ and aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 in 8:8:1 EtOAc–toluene–MeOH, to give an inseparable mixture (50 mg) of compound **9** and an unknown by-product; R_F 0.17 in 10:10:1 EtOAc–toluene–MeOH. This mixture (50 mg) was dissolved in 1:1 pyridine–Ac_2O (1.0 mL). The solution was stirred for 12 h at 20° and evaporated *in vacuo*. The residue was chromatographed over SiO_2 in 15:5:1 CCl_4–acetone–MeOH to give a 1:1 mixture (32 mg) of compound **10** (~12%) and the unknown product (R_F 0.39 in 15:5:1 CCl_4–acetone–MeOH), and pure compound **10** (6 mg, 5%); R_F 0.38 in 15:5:1 CCl_4–acetone–MeOH; the ^{1}H-n.m.r. data were identical with those for **10** obtained by method [*A*].

Deprotection of compound **11**. — A solution of compound **11** (6.5 mg) in 0.05M NaOMe–MeOH (0.6 mL) was stirred for 2 d at 20°. To this solution was added M aq. NaOH (100 μL). The mixture was stirred for 4 h at 20°, made neutral with Amberlyst-15 (H^+) resin, and filtered through Celite. The filtrate was evapo-

rated *in vacuo*, and the residue was purified by gel chromatography over Sephadex G-25 in H_2O, to give, quantitatively, allyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→3)-*O*-[(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)]-*O*-[(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)]-2-azido-2-deoxy-β-D-galactopyranoside (**12**): m.p. 186–187° (dec.), $[\alpha]_D$ −4.8° (*c* 0.25, H_2O); R_F 0.36 in 2:1:1 BuOH–EtOH–H_2O; δ_H (D_2O, *t*-BuOH, 30°) 4.824 (d, 1 H, *J* 7.6 Hz, H-1c), 4.616 (d, 1 H, *J* 7.6 Hz, H-1d), and 4.497 (d, 1 H, *J* 7.8 Hz, H-1a).

Methyl O-{*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*}-*(2→3)-2,4,6-tri*-O-*acetyl-1-thio*-β-D-*galactopyranoside* (**13**). — To a stirred mixture of compound **8** (α:β = 1:6; 96 mg, 103 μmol) and Bu_3SnSMe (42 mg, 124 μmol) in $Cl(CH_2)_2Cl$ (1 mL) was added $BF_3 \cdot Et_2O$ (17 mg, 124 μmol) at 0°. The mixture was stirred for 3 h at 0°, diluted with EtOAc, washed with aq. KF, and filtered through Celite. The organic layer was washed with aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed over SiO_2 in 10:10:1 EtOAc–toluene–MeOH, to give **13** (75 mg, 89%); $[\alpha]_D$ −6.3° (*c* 0.57); R_F 0.37 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 5.548 (ddd, 1 H, *J* 2.7, 5.4, and 9.0 Hz, H-8b), 5.397 (dd, 1 H, *J* 2.7 and 9.0 Hz, H-7b), 5.108 (t, 1 H, *J* 9.8 Hz, H-2a), 5.086 (d, 1 H, *J* 10.3 Hz, N*H*), 4.972 (d, 1 H, *J* 2.4 Hz, H-4a), 4.896 (ddd, 1 H, *J* 4.6, 10.3, and 12.0 Hz, H-4b), 4.630 (dd, 1 H, *J* 9.8 and 3.4 Hz, H-3a), 4.557 (d, 1 H, *J* 10.0 Hz, H-1a), 4.364 (dd, 1 H, *J* 2.7 and 12.5 Hz, H-9b), 3.922 (t, 1 H, *J* 6.8 Hz, H-5a), 3.862 (s, 3 H, OCH_3), 3.655 (dd, 1 H, *J* 2.7 and 10.7 Hz, H-6a), 2.600 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3b*eq*), 2.241, 2.199, 2.191, 2.101, 2.084, 2.065, 2.052, 2.018, and 1.863 (9 s, 27 H, 7 $OCOCH_3$, $NCOCH_3$, and SCH_3), and 1.721 (t, 1 H, *J* 12.5 Hz, H-3b*ax*).

Anal. Calc. for $C_{33}H_{49}NO_{21}S$: C, 47.88; H, 5.97; N, 1.69. Found: C, 47.89; H, 5.80; N, 1.74.

Propyl 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→3)*-O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate-(2→6)*]-*2-acetamido-4*-O-*acetyl-2-deoxy*-β-D-*galactopyranoside* (**14**). — A mixture of compound **10** (20 mg, 13 μmol) and 10% Pd–C (7 mg) in MeOH (1 mL) was stirred for 10 h at 20° under H_2, and then filtered through Celite. The filtrate was evaporated *in vacuo*. A solution of the residue in pyridine (1 mL) and Ac_2O (0.5 mL) was stirred for 3 h at 20°, and then evaporated *in vacuo*, and chromatography of the residue on SiO_2 in 20:1 $CHCl_3$–MeOH gave **14** (14 mg, 69%); $[\alpha]_D$ +1.8° (*c* 0.34); R_F 0.20 in 5:5:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 5.786 (d, 1 H, *J* 7.3 Hz, N*H*-2a), 5.540 (ddd, 1 H, *J* 3.1, 6.4, 8.7 Hz, H-8d), 5.466 (d, 1 H, *J* 3.2 Hz, H-4a), 5.042 (d, 1 H, *J* 8.3 Hz, H-1c), 5.002 (dd, 1 H, *J* 8.1 and 10.3 Hz, H-2c), 4.908 (d, 1 H, *J* 3.2 Hz, H-4c), 4.733 (d, 1 H, *J* 7.8 Hz, H-1a), 4.508 (dd, 1 H, *J* 3.4 and 10.0 Hz, H-3c), 3.848 (s, 3 H, OCH_3), 3.792 (s, 3 H, OCH_3), 3.634 (dd, 1 H, *J* 2.7 and 10.7 Hz, H-6d), 2.583

(dd, 1 H, *J* 4.6 and 12.5 Hz, H-3b*eq**), 2.567 (dd, 1 H, *J* 4.9 and 13.2 Hz, H-3d*eq**), 2.251, 2.173, 2.144, 2.107, 2.093, 2.089, 2.082, 2.074, 2.048, 2.035, 2.022, 2.007, 1.971, 1.876, 1.856 (15 s, 45 H, 12 OCOCH_3 and 3 NCOCH_3), and 0.91 (t, 3 H, *J* 7.5 Hz, CH_2CH_3).

Anal. Calc. for $C_{60}H_{85}N_5O_{39}$: C, 48.03; H, 5.71; N, 4.67. Found: C, 47.65; H, 5.37; N, 4.47.

Propyl O-{*sodium (5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*}-*(2→3)*-O-α-D-*galactopyranosyl-(1→3)*-O-[*sodium (5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate-(2→6)*]-*2-acetamido-2-deoxy*-β-D-*galactopyranoside* (**15**). — A solution of compound **14** (10 mg, 6 μmol) in MeOH (2 mL) and M aq. NaOH (240 μL) was stirred for 5 h at 20°, made neutral with Amberlyst-15 (H^+) resin, diluted with H_2O, and the suspension filtered through Celite. The filtrate was evaporated *in vacuo*, and the residue was purified by gel chromatography over Sephadex G-25 in H_2O, to give **15** (6.5 mg, quantitative); m.p. 227–229° (dec.), $[\alpha]_D$ −2.1° (*c* 0.14, H_2O); R_F 0.36 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, *t*-BuOH, 20°) 4.480 (d, 2 H, *J* 8.1 Hz, H-1a,1c), 4.176 (d, 1 H, *J* 3.4 Hz, H-4a), 4.056 (dd, 1 H, *J* 2.9 and 10.0 Hz, H-3c), 3.981 (dd, 1 H, *J* 8.1 and 11.0 Hz, H-2a), 2.734 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3d*eq**), 2.702 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3b*eq**), 2.017, 2.014, 1.999 (3 s, 9 H, 3 NCOCH_3), 1.781 (t, 1 H, *J* 12.5 Hz, H-3d*ax*), 1.673 (t, 1 H, *J* 12.1 Hz, H-3b*ax*), 1.539 (sex, 2 H, *J* 7.20, $CH_2CH_2CH_3$), and 0.856 (t, 3 H, *J* 7.3 Hz, $CH_2CH_2CH_3$).

Anal. Calc. for $C_{39}H_{63}N_3Na_2O_{27}\cdot 2\,H_2O$: C, 43.06; H, 6.20; N, 3.86. Found: C, 42.92; H, 5.72; N, 3.71.

Deallylation of compound **10**. — A mixture of compound **10** (107 mg, 70 μmol), $PdCl_2$ (18 mg, 98 μmol), and NaOAc (23 mg, 287 μmol) in 20:1 AcOH–H_2O (1 mL) was stirred for 12 h at 20°, and then diluted with EtOAc, and filtered through Celite. The filtrate was successively washed with aq. $NaHCO_3$ and aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed over SiO_2 in 5:5:1 EtOAc–toluene–MeOH, to give **16** (66 mg, 64%); $[\alpha]_D$ +0.8° (*c* 0.51); R_F 0.26 in 5:5:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 3.850 (s, 3 H, OCH_3), 3.780 (s, 1.5 H, OCH_3), and 3.770 (s, 1.5 H, OCH_3).

Transformation of compound **16** *into trichloroacetimidate* **17**. — To a stirred solution of compound **16** (96 mg, 65 μmol) in CH_2Cl_2 (1 mL) were added Cl_3CCN (45 mg, 325 μmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (4.8 μL, 33 μmol) at 0°. After stirring for 1 h at 0°, the mixture was directly chromatographed over SiO_2 in 5:5:1 EtOAc–toluene–MeOH, to give crude trichloroacetimidate **17** (80 mg, 76%); $[\alpha]_D$ +11.7° (*c* 0.5); R_F 0.39 in 5:5:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 6.490 (d, 1 H, *J* 3.7 Hz, H-1a), and 3.860 and 3.790 (2 s, 6 H, 2 CH_3O).

N-*(Benzyloxycarbonyl)*-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→3)*-O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5- dideoxy* - D - glycero - α - D - galacto - *2 - nonulopyranosyl)onate*

-(2→6)]-O-(4-O-acetyl-2-azido-2-deoxy-α-and-β-D-galactopyranosyl)-(1→3)-L-serine benzyl ester (**18** *and* **21**). — To a stirred mixture of compound **5** (56 mg, 171 μmol), compound **17** (92 mg, 57 μmol), and powdered molecular sieves AW-300 (200 mg) in $Cl(CH_2)_2Cl$ (0.8 mL) was added dropwise $BF_3 \cdot Et_2O$ (8.3 μL, 68 μmol) at −15° under Ar. After stirring for 30 min at −15°, the mixture was diluted with EtOAc, and filtered through Celite. The filtrate was washed successively with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed over SiO_2 in 2:1 CH_2Cl_2–acetone, to give **18** (12 mg, 12%) and **21** (33 mg, 32%).

Compound **18** had $[\alpha]_D$ +43.8° (*c* 0.08); R_F 0.34 in 2:1 $CHCl_3$–acetone; n.m.r. data: δ_H 7.39–7.33 (m, 10 H, aromatic), 6.103 (d, 1 H, *J* 9.0 Hz, N*H*-2Ser), 5.674 (dt, 1 H, *J* 3.2 and 8.5 Hz, H-8d), 5.361 (d, 1 H, *J* 2.9 Hz, H-4a), 5.233 (d, 1 H, *J* 12.5 Hz, C*H*$_2$Ph), 5.158 (d, 1 H, *J* 12.5 Hz, C*H*$_2$Ph), 5.148 (d, 1 H, *J* 12.5 Hz, C*H*$_2$Ph), 5.054 (d, 1 H, *J* 12.5 Hz, C*H*$_2$Ph), 4.882 (d, 1 H, *J* 3.4 Hz, H-4c), 4.851 (d, 1 H, *J* 3.4 Hz, H-1a), 4.89–4.80 (m, 2 H, H-4b,4d), 4.673 (d, 1 H, *J* 7.8 Hz, H-1c), 4.627 (dt, 1 H, *J* 3.2 and 9.0 Hz, H-2Ser), 4.535 (dd, 1 H, *J* 3.4 and 10.3 Hz, H-3c), 4.480 (dd, 1 H, *J* 3.2 and 12.1 Hz, H-9b or 9d), 4.267 (dd, 1 H, *J* 3.2 and 12.1 Hz, H-9b or d), 4.221 (dd, 1 H, *J* 3.4 and 11.1 Hz, H-9b or 9d), 3.884 (t, 1 H, *J* 6.1 Hz, H-5c), 3.845 (s, 3 H, OC*H*$_3$), 3.735 (s, 3 H, OC*H*$_3$), 3.671 (dd, *J* 3.4 and 11.2 Hz, H-2a), 3.643 (dd, 1 H, *J* 2.9 and 11.2 Hz, H-6d), 3.251 (dd, 1 H, *J* 4.2 and 10.3 Hz, H-6a), 2.586 and 2.537 (2 dd, 2 H, *J* 4.6 and 12.5 Hz, and *J* 4.4 and 12.7 Hz, H-3b*eq* and 3d*eq*), 2.247, 2.172, 2.144, 2.112, 2.096, 2.092, 2.076, 2.038, 2.020 (6 H), 2.016, 1.906, 1.872, 1.864 (13 s, total 42 H, 12 OCOC*H*$_3$ and 2 NCOC*H*$_3$), and 1.890 and 1.720 (2 t, 2 H, *J* 12.5 Hz, H-3b*ax* and 3d*ax*).

Compound **21** had $[\alpha]_D$ −15.3° (*c* 0.15); R_F 0.31 in 2:1 CH_2Cl_2–acetone; n.m.r. data: δ_H 7.36–7.31 (m, 10 H, aromatic), 5.883 (d, 1 H, *J* 8.1 Hz, N*H*-2Ser), 5.570 (ddd, 1 H, *J* 2.9, 5.9, and 9.0 Hz, H-8d), 5.387 (dd, 1 H, *J* 2.9 and 8.8 Hz, H-7d), 5.342 (d, 1 H, *J* 2.9 Hz, H-4a), 4.855 (d, 1 H, *J* 7.8 Hz, H-1c), 4.237 (d, 1 H, *J* 7.8 Hz, H-1a), 3.853 and 3.769 (2 s, 6 H, 2 OC*H*$_3$), 3.487 (dd, 1 H, *J* 8.1 and 10.3 Hz, H-2a), 3.319 (dd, 1 H, *J* 6.0 and 10.1 Hz, H-6a), 2.590 and 2.556 (2 dd, 2 H, *J* 4.6 and 12.7 Hz, and *J* 4.6 and 12.7 Hz, H-3b*eq* and 3d*eq*), 2.274, 2.221, 2.182, 2.107, 2.102, 2.096, 2.068, 2.048, 2.038, 2.015, 2.010, 1.998, 1.872, 1.860 (14 s, 42 H, 12 OCOC*H*$_3$ and 2 NCOC*H*$_3$), 1.943 and 1.715 (2 t, 2 H, *J* 12.9 Hz, H-3b*ax* and 3d*ax*).

Anal. Calc. for $C_{78}H_{100}N_6O_{42} \cdot H_2O$: C, 51.71; H, 5.67; N, 4.64. Found (for a 1:1 mixture of compound **18** and **21**): C, 51.53; H, 5.39; N, 4.58.

N-(Benzyloxycarbonyl)-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→3)-O-[methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate-(2→6)]-O-(2-acetamido-4-O-acetyl-2-deoxy-α-and-β-D-galactopyranosyl)-(1→3)-L-serine benzyl ester (**19** *and* **22**). — [*A*] Compound **18** (4.0 mg, 2.2 μmol) was dissolved in a solution of $NiCl_2 \cdot 6\ H_2O$ (8 mg, 34 μmol) and H_3BO_3 (4 mg, 65

μmol) in EtOH (0.2 mL). To this solution was added $NaBH_4$ (2 mg, 53 μmol) at 20°. After being stirred for 15 min at 20°, AcOH (0.2 mL) and EtOH (0.4 mL) were added, and the mixture was evaporated *in vacuo*. The residue was extracted with $CHCl_3$, and the extract successively washed with H_2O and aq. NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. A solution of the residue in Ac_2O (0.15 mL) and pyridine (0.3 mL) was stirred for 4 h, and then evaporated *in vacuo*. The residue was chromatographed over SiO_2 in 2:1 THF–toluene, to give **19** (3.7 mg, 92%); $[\alpha]_D$ +31.4° (*c* 0.11); R_F 0.33 in 2:1 THF–toluene; n.m.r. data: δ_H 7.42–7.28 (m, 10 H, aromatic), 6.044 (d, 1 H, *J* 8.1 H, N*H*), 6.017 (d, 1 H, *J* 9.3 Hz, N*H*), 5.683 (dt, 1 H, *J* 1.7 and 9.3 Hz, H-8d), 4.870 (d, 1 H, *J* 3.7 Hz, H-1a), 4.605 (d, 1 H, *J* 8.1 Hz, H-1c), 4.385 (dt, 1 H, *J* 3.7 and 10.0 Hz, H-2a), 3.852 (s, 3 H, OCH_3), 3.748 (s, 3 H, OCH_3), 3.270 (dd, 1 H, *J* 4.2 and 10.5 Hz, H-6a), 2.589 and 2.540 (2 dd, 2 H, *J* 4.9 and 13.2 Hz, *J* 4.6 and 12.7 Hz, H-3b*eq* and 3d*eq*), 2.313, 2.274, (9 H), 2.188, 2.117, 2.098, 2.067, 2.027, 2.022, 2.018, 1.969, 1.959, 1.871, and 1.864 (13 s, total 45 H, 12 $OCOCH_3$ and 3 $NCOCH_3$).

[*B*] Compound **21** (9.7 mg, 5.4 μmol) was treated as described in [*A*], to give **22** (6.3 mg, 64%); $[\alpha]_D$ −2.5° (*c* 0.16); R_F 0.39 in 2:1 THF–toluene; n.m.r. data: δ_H 7.39–7.28 (m, 10 H, aromatic), 6.041 (d, 1 H, *J* 8.3 Hz, N*H*-2Ser), 5.758 (d, 1 H, *J* 6.8 Hz, N*H*-2a), 5.540 (ddd, 1 H, *J* 2.9, 6.4, and 9.0 Hz, H-8d), 5.434 (d, 1 H, *J* 3.2 Hz, H-4a), 5.362 (dd, 1 H, *J* 2.4 and 8.8 Hz, H-7d), 5.209 (bs, 2 H, CH_2Ph), 5.156 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 5.107 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 5.052 (d, 1 H, *J* 10.3 Hz, N*H*), 4.968 (d, 1 H, *J* 7.8 Hz, H-1c), 4.900 (d, 1 H, *J* 2.9 Hz, H-4c), 4.701 (d, 1 H, *J* 7.6 Hz, H-1a), 4.540 (m, 1 H, H-2Ser), 4.500 (dd, 1 H, *J* 3.4 and 10.3 Hz, H-3c), 3.848 (s, 3 H, OCH_3), 3.773 (s, 3 H, OCH_3), 3.333 (dd, 1 H, *J* 4.2 and 9.8 Hz, H-6a), 2.583 and 2.553 (2 dd, 2 H, *J* 4.6 and 12.5, *J* 4.4 and 11.7 Hz, H-3b*eq*, and 3d*eq*), 2.230, 2.176, 2.172, 2.125, 2.099, 2.096, 2.078, 2.071, 2.064, 2.043, 2.012, (6 H), 2.007, 1.875, and 1.859 (14 s, total 45 H, 12 $OCOCH_3$ and 3 $NCOCH_3$), 1.961 and 1.679 (2 t, 2 H, *J* 12.9 and 12.7 Hz, H-3b*ax* and 3d*ax*).

Anal. Calc. for $C_{80}H_{104}N_4O_{43}$: C, 53.10; H, 5.79; N, 3.10. Found (for a 1:1 mixture of **19** and **22**): C, 53.39; H, 5.69; N, 3.13.

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