Carbohydrate Research, 172 (1988) 183–193
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

TOTAL SYNTHESIS OF 3-*O*-[2-ACETAMIDO-6-*O*-(*N*-ACETYL-α-D-NEURAMINYL)-2-DEOXY-α-D-GALACTOSYL]-L-SERINE AND A STEREOISOMER*

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(Received January 2nd, 1987; accepted for publication, May 14th, 1987)

ABSTRACT

O-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine, a structural unit occurring in various submaxillary mucins, was synthesized for the first time 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→6)-3,4-di-*O*-acetyl-2-azido-2-deoxy-D-galactopyranosyl trichloroacetimidate (**13**) and *N*-(benzyloxycarbonyl)-L-serine benzyl ester as the key intermediates. The trichloroacetimidate **13** was prepared by starting from two monosaccharide synthons, namely, allyl 2-azido-2-deoxy-β-D-galactopyranoside and methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate, which were coupled in the presence of silver triflate in tetrahydrofuran to give the desired α-(2→6)-linked disaccharide in moderate selectivity.

INTRODUCTION

Various submaxillary mucins contain[2] the structural unit **1**, where the disaccharide α-NeuAc-(2→6)-GalNAc in the α-D-configuration is linked either to L-serine or to L-threonine. Because, in the course of structure determination of these mucin-type glycopeptides, base-catalyzed β-elimination of saccharide portions from a peptide backbone has been required, a disaccharide-serine or -threonine structure such as **1** has not been isolated from natural sources.

For the purpose of supplying structurally well defined compounds as models for natural mucin glycopeptides, several approaches toward mucin-type glycopeptides carrying neutral mono- or di-saccharides have been reported[3]. We now describe a synthetic approach toward target structure **1**.

*Part 52 in the series "Synthetic Studies on Cell-Surface Glycans". For Part 51, see ref. 1.
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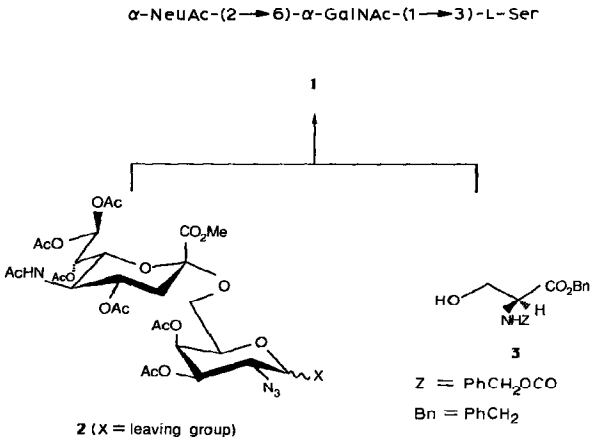

Scheme 1

RESULTS AND DISCUSSION

The target structure **1** was retrosynthesized to give the glycosyl donor **2** and the known[4] glycosyl acceptor **3** as shown in Scheme 1. As a synthetic equivalent of the glycosyl donor **2**, we designed the trichloroacetimidate **13**. A synthetic route to compound **13** by use of an open strategy, starting from the known[5] triol glycosyl acceptor **4**, was straightforward.

Glycosylation of compound **4** with the glycosyl donor[6] **6** in the presence of silver triflate in THF afforded a 5:1 mixture of the α-(2→6) (**7**) and the β-(2→6) glycoside (**11**) in 68% yield based on the acceptor **4** consumed. Use of a 2:1 mixture of mercuric cyanide and mercuric bromide instead of silver triflate in the same solvent gave a slightly higher yield (78%), but the ratio of **7** to **11** became 3:2. In close connection with this glycosylation reaction, Paulsen and his coworkers[7] recently reported that the glycosylation of compound **5** with the glycosyl donor **6** in the presence of a 5:2 mixture of mercuric cyanide and mercuric bromide in dichloromethane afforded a 7:6 mixture of α-(2→6) and β-(2→6) glycosylated products in 78% yield, the result is in good agreement with our observation just described. The use of silver triflate instead of mercuric salts was found to increase the ratio of α anomer **7** *versus* β anomer **11** in the products.

The configuration at C-2b of compounds **7** and **11** was assigned from their ^{1}H-n.m.r. data[8]. In the case of compound **7**, a signal for H-4b was observed at δ 4.88; for compound **11**, it was observed at δ 5.40, The high site-selectivity of glycosylation for OH-6 of glycosyl acceptor **4**, expected from the steric requirement around the electrophilic C-2 of the reactive intermediate derived from the glycosyl donor **6**, was confirmed by ^{1}H-n.m.r. data of the acetate **8** (derived from compound **7**), which contained two deshielded signals, for H-3a and H-4a, at δ 4.83 and 5.38.

All = H2C=CHCH2
Scheme 2

Compound **7** was further transformed into the deblocked product **10** in three steps, (*i*) 10% Pd–C and H_2, (*ii*) Ac_2O and pyridine, and (*iii*) NaOH in MeOH. ^{1}H-N.m.r. data of compound **10** were in good agreement with the related data[9] of the compounds isolated from natural sources.

Deallylation[10] of compound **8** with $PdCl_2$–AcONa gave a 79% yield of hemiacetal **12** as a 1:1 mixture of α and β anomers. Treatment of compound **12** with trichloroacetonitrile and potassium carbonate under the conditions of Grundler and Schmidt[11] was expected to give the β-trichloroacetimidate as the major product. However, a 1:1 mixture of the α- and β-trichloroacetimidates was obtained in 70% yield. The loss of stereoselectivity observed for the transformation of disaccharide **12** into the imidate **13** was in contrast with the case[11] of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-galactopyranose, which gave a 1:4 mixture of the α and β anomers of the trichloroacetimidate under the same condition.

Crucial glycosylation of the L-serine derivative **3** was examined by using the glycosyl donor **13** as a 1:1 mixture of the α and β anomers in the presence of trimethylsilyl triflate, to give a 3:2 mixture of compounds **14** and **18** which could not be separated by column chromatography. Reduction of the azido group of a mixture of **14** and **18** gave a mixture of the amino compounds **15** and **19**, and acetylation thereof, followed by chromatography on silica gel, gave a 42% yield of the desired product **16**, as well as a 25% yield of the β anomer **20**. The configuration at C-1a of compounds **16** and **20** was assigned as α-D and β-D from ^{1}H-n.m.r. data which showed the signals for H-1a of compounds **16** and **20** at δ 4.75 as a doublet with J 3.7 Hz, and at δ 4.56 as a doublet with J 8.3 Hz, respectively. Deprotection of compounds **16** and **20** in two steps, namely, (*i*) 10% palladium-on-carbon–H_2

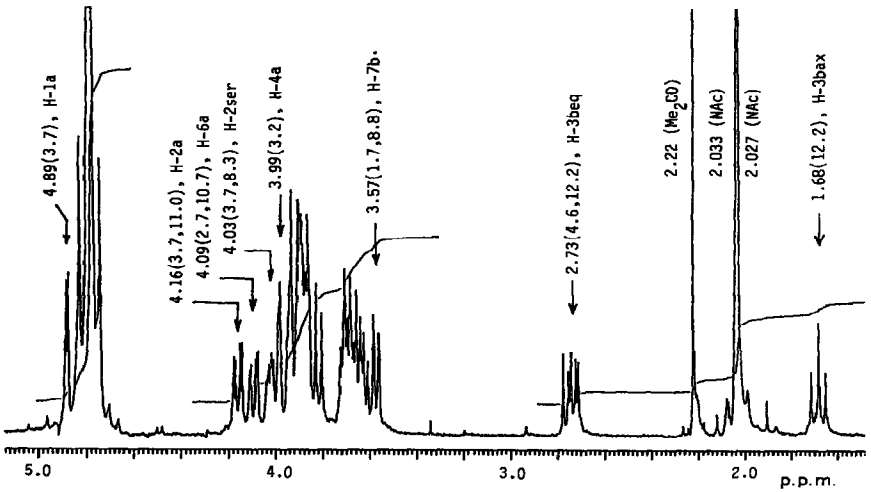

Fig. 1. 400-MHz ^{1}H-n.m.r. spectrum of synthetic compound **1**, recorded in D_2O at 20°. The values of δ_H are expressed in p.p.m. downward from Me_4Si by reference to an internal standard of Me_2CO (2.225). The values in parentheses are $^3J_{HH}$ in Hz.

and (*ii*) NaOH, afforded the target compound **1** and the stereoisomer **22**, respectively. No racemization of the serine residue was observed during sodium hydroxide saponification of C-1b methyl ester of both compounds **17** and **21**, because of the prior hydrogenolysis of the benzyl ester function of the serine residue[12].

^{1}H-N.m.r. data (20°) of compound **1** (see Fig. 1) showed characteristic signals for H-1a, H-2a, H-6a, H-4a, H-7b, H-3b*e*, two NAc, and H-3b*a* protons at δ 4.89, 4.16, 4.09, 3.99, 3.57, 2.73, 2.03, 2.03, and 1.68, respectively. These data for synthetic compound **2** are in reasonable agreement with the data[13] for natural, ovine submaxillary mucin, which showed the corresponding signals at δ 4.90, 4.13, 4.12, 3.98, 3.58, 2.72, 2.03, 2.03, and 1.63. ^{13}C-N.m.r. data of synthetic compound **1** (see Fig. 2) were also in good agreement with the data reported[14] for natural, ovine submaxillary mucin.

In conclusion, the typical disaccharide–serine structure **1**, which constitutes various submaxillary mucins, was synthesized by use of the key trichloroacetimidate **13** as a glycosyl donor and the protected serine **3** as a glycosyl acceptor. The n.m.r. data of synthetic compound **1** provided supporting evidence for the proposed structure of ovine submaxillary mucin.

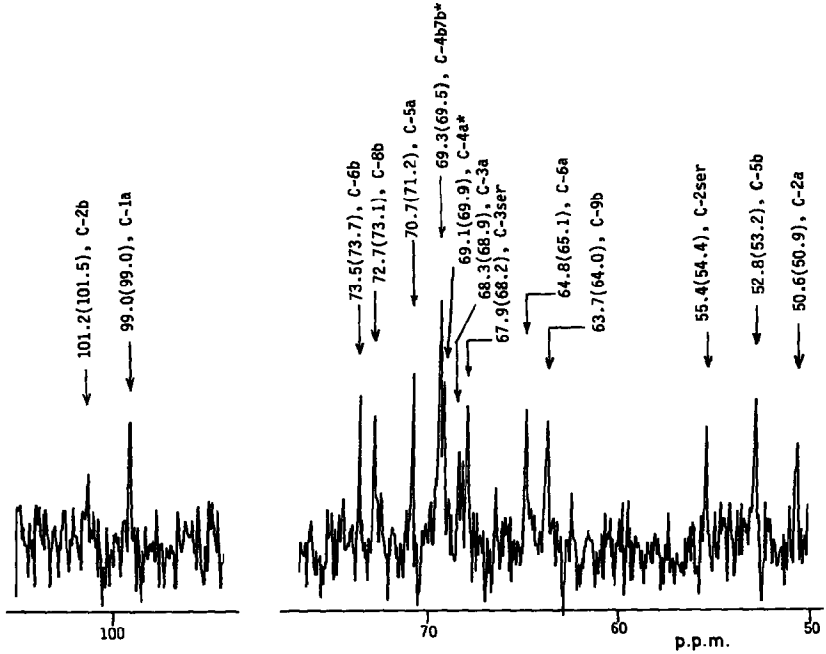

Fig. 2. 22.5-MHz ^{13}C-n.m.r. spectrum of synthetic compound **1**, recorded in D_2O at 20°. The values of δ_C are expressed in p.p.m. downward from Me_4Si by reference to an internal standard of MeOH (49.8). The values in parentheses are δ_C values reported for natural ovine submaxillary mucin[13].

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. was performed on Silica Gel 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 JNM-GX400 or JNM-FX90Q n.m.r. spectrometers. ^{13}C-N.m.r. spectra were recorded with a JNM-FX90Q n.m.r. spectrometer operated at 22.50 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 $\delta_H(D_2O)$ and $\delta_C(D_2O)$ 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.

Allyl 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* (**7**) *and allyl* O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-*β*-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)-2-azido-2-deoxy-β*-D-*galactopyranoside* (**11**). — To a stirred mixture of compound **4** (727 mg, 1.96 mmol), powdered molecular sieves 4A (6.0 g), and $AgOSO_2CF_3$ (715 mg, 2.94 mmol) in THF (5 mL) was added dropwise a solution of compound **6** (999 mg, 1.96 mmol) in THF (2 mL) during 1 h at −10°. The mixture was stirred for 20 h at 20°, 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*. Chromatography of the residue on SiO_2 in 10:10:1 EtOAc–toluene–MeOH gave **7** (852 mg, 41%), **11** (170 mg, 8%), and unreacted **5** (220 mg).

Compound **7** had $[\alpha]_D$ −6.5° (*c* 1.6): R_F 0.32 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 5.94 (m, 1 H, C*H*=CH_2), 4.88 (ddd, 1 H, *J* 5.0, 9.8, and 12.4 Hz, H-4b), 4.32 (d, 1 H, *J* 7.8 Hz, H-1a), 3.97 (d, 1 H, *J* 3.4 Hz, H-4a), 3.82 (s, 3 H, CO_2Me), 2.57 (dd, 1 H, *J* 4.6 and 12.9 Hz, H-3b*e*), 2.15, 2.14, 2.04, 2.03, and 1.89 (5 s, 15 H, 5 Ac).

Anal. Calc. for $C_{29}H_{42}N_4O_{17}$: C, 48.47; H, 5.89; N, 7.80. Found: C, 48.66; H, 5.88; N, 7.50.

Compound **11** had $[\alpha]_D$ −6.9° (*c* 0.5); R_F 0.36 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 6,10 (d, 1 H, *J* 9.1 Hz, N*H*), 5.92 (m, 1 H, C*H*=CH_2), 5.40 (m, 1 H, H-4b), 4.33 (d, 1 H, *J* 7.8 Hz, H-1a), 3.82 (s, 3 H, CO_2Me), 2.49 (dd, 1 H, *J* 4.9 and 12.9 Hz, H-3b*e*), 2.15, 2.11, 2.05, 2.02, and 1.93 (5 s, 15 H, 5 Ac), and 1.81 (t, 1 H, *J* 12.2 Hz, H-3b*a*).

Anal. Calc. for $C_{29}H_{42}N_4O_{17}$: C, 48.47; H, 5.89; N, 7.80. Found: C, 48.70; H, 5.84; N, 7.47.

Allyl O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)-3,4-di*-O-*acetyl-2-azido-2-deoxy*-β-D-*galactopyranoside* (**8**). — A solution of compound **7** (403 mg, 560 μmol) in 1:1 Ac_2O–pyridine (8 mL) was stirred for 12 h at 20° and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 50:1 $CHCl_3$–MeOH afforded **8** (441 mg, 98%); $[\alpha]_D$ −33.0° (*c* 0.1); R_F 0.29 in 50:1 $CHCl_3$–MeOH; n.m.r. data: δ_H 5.97 (m, 1 H, C*H*=CH_2), 5.38 (dd, 1 H, *J* 1.2 and 3.4 Hz, H-4a), 5.09 (d, 1 H, *J* 9.5 Hz, N*H*), 4.85 (m, 1 H, H-4b), 4.83 (dd, 1 H, *J* 3.4 and 10.9 Hz, H-3a), 4.50 (d, 1 H, *J* 8.1 Hz, H-1a), 3.88 (dt, 1 H, *J* 1.2 and 7.6 Hz, H-5a), 3.79 (dd, 1 H, *J* 5.6 and 11.0 Hz, H-6a), 3.78 (s, 3 H, CO_2Me), 3.69 (dd, 1 H, *J* 8.1 and 10.9 Hz, H-2a), 3.38 (dd, 1 H, *J* 7.6 and 10.3 Hz, H-6a'), 2.52 (dd, 1 H, *J* 4.6 and 12.9 Hz, H-3b*e*), 2.18, 2.14, 2.12, 2.04, 2.03, 2.02, and 1.88 (7 s, 21 H, 7 Ac).

Anal. Calc. for $C_{33}H_{46}N_4O_{19}$: C, 49.38; H, 5.78; N, 6.98. Found: C, 49.14; H, 5.72; N, 6.61.

Propyl 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-3,4-di*-O-*acetyl-2-deoxy*-β-D-*galactopyranoside* (**9**), *and conversion of* **9** *into* **10**. — A mixture of compound **7** (32 mg, 39 μmol) and 10% Pd–C (15 mg) in MeOH (3 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 1:1 Ac_2O–pyridine (2 mL) was stirred for 3 h at 20°, and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 5:5:1 EtOAc–toluene–MeOH gave **9** (23 mg, 64%); $[\alpha]_D$ −36.3° (*c* 0.8); R_F 0.16 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 6.25 (d, 1 H, *J* 9.5 Hz, N*H*–C-2a), 5.49 (d, 1 H, *J* 3.4 Hz, H-4a), 5.28 (dd, 1 H, *J* 3.7 and 11.0 Hz, H-3a), 5.18 (d, 1 H, *J* 9.5 Hz, N*H*–C-5b), 4.81 (ddd, 1 H, *J* 4.4, 10.0, and 12.5 Hz, H-4b), 4.56 (d, 1 H, *J* 8.5 Hz, H-1a), 3.81 (s, 3 H, CO_2Me), 2.53 (dd, 1 H, *J* 4.5 and 12.8 Hz, H-3b*e*), 2.23, 2.17, 2.14, 2.02, 1.99 1.95, 1.89 (7 s, in the ratios of 1:1:1:2:1:1:1, 24 H, 8 Ac), 1.60 (m, 2 H, $CH_2CH_2CH_3$), and 0.90 (t, 3 H, $CH_2CH_2CH_3$).

Anal. Calc. for $C_{35}H_{52}N_2O_{20}$: C, 51.22; H, 6.39; N, 3.41. Found: C, 50.88; H, 6.51; N, 3.16.

A solution of compound **9** (14 mg) in MeOH (2 mL) and M aq. NaOH (0.24 mL) was stirred for 3 h at 20°, made neutral with Amberlyst-15, and the suspension filtered through Celite. The filtrate was evaporated *in vacuo*. Purification of the residue by Sephadex G-10 in H_2O gave *propyl* O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy*-β-D-*galactopyranoside* (**10**; 10 mg, quantitative yield); m.p. 203–204°, $[\alpha]_D$ −6.0° (*c* 0.32, H_2O); R_F 0.46 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data (D_2O): δ_H 4.43 (d, 1 H, *J* 8.3 Hz, H-1a), 2.72 (dd, 1 H, *J* 4.4 and 12.2 Hz, H-3b*e*), 2.03 and 2.02 (2 s, 6 H, 2 NAc), 1.68 (t, 1 H, *J* 12.2 Hz, H-3b*a*), 1.54 (sex, 2 H, *J* 7.3 Hz, $CH_2CH_2CH_3$), and 0.86 (t, 3 H, *J* 7.3 Hz, $CH_2CH_2CH_3$.

O-[*Methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)-3,4-di*-O-*acetyl-2-azido-2-deoxy*-D-*galactopyranose* (**12**) *and the trichloroacetimidate* (**13**). — A mixture of compound **8** (378

mg, 470 μmol), $PdCl_2$, (121 mg, 660 μmol), and AcONa (164 mg, 2.0 mmol) in 20:1 AcOH–H_2O (15 mL) was stirred for 12 h at 20°, and diluted with EtOAc (80 mL). The organic layer was successively washed with aq. $NaHCO_3$ and aq. NaCl, dried ($MgSO_4$), and filtered through Celite. The filtrate was evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 40:40:3 EtOAc–toluene–MeOH gave **12** (285 mg, 79%); $[\alpha]_D$ −14.1° (*c* 0.85); R_F 0.37 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 3.79 and 3.78 (2 s, in the ratio of 1:1, 3 H, CO_2Me); δ_C 98.7 (C-2b), 96.3 (C-1aβ), and 92.4 (C-1aα).

Anal. Calc. for $C_{30}H_{42}N_4O_{19}$: C, 47.25; H, 5.55; N, 7.35. Found: C, 47.25; H, 5.50; N, 6.79.

A mixture of compound **12** (104 mg, 136 μmol), Cl_3CCN (100 mg, 692 μmol), and K_2CO_3 (20 mg, 144 μmol) in CH_2Cl_2 (0.8 mL) was stirred for 3 h at 20°. The reaction mixture was directly subjected to chromatography on SiO_2 in 10:15:1 EtOAc–toluene–MeOH to give O-[*methyl (5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)-3,4-di*-O-*acetyl-2-azido-2-deoxy-α- and β*-D-*galactopyranosyl trichloroacetimidate* (**13**) as a 1:1 mixture (87 mg, 70%), as well as unreacted **12** (15 mg).

Compound **13** had R_F 0.63 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 8.80 (s, 1 H, C=N*H*), 6.52 (d, 0.5 H, *J* 3.4 Hz, H-1aα), 5.81 (d, 0.5 H, *J* 8.6 Hz, H-1aβ), 5.51 (d, 0.5 H, *J* 2.9 Hz, H-4a), 5.46 (d, 0.5 H, *J* 2.7 Hz, H-4a), 4.86 (m, 1 H, H-4b), 3.77 (s, 3 H, CO_2Me), 2.52 (dd, 0.5 H, *J* 4.6 and 12.9 Hz, H-3b*e*), and 2.50 (dd, 0.5 H, *J* 4.4 and 12.7 Hz, H-3b*e*).

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-*(3,4-di*-O-*acetyl-2-azido-2-deoxy-α- and -β*-D-*galactopyranosyl)-(1→3)*-N-*(benzyloxycarbonyl)*-L-*serine benzyl ester* (**14** *and* **18**). — To a stirred mixture of powdered molecular sieves AW 300 (320 mg), compound **3** (95 mg, 288 μmol), and compound **13** (87 mg, 96 μmol) in $Cl(CH_2)_2Cl$ (2 mL) was added dropwise $Me_3SiOSO_2CF_3$ (22.0 μL, 115 μmol) at −15° under Ar. The mixture was stirred for 40 min at −15°, 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*. Chromatography of the residue on SiO_2 afforded a 3:2 mixture of **14** and **18** (84 mg, 82%); R_F 0.35 in 25:25:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 5.87 (d, 1 H, *J* 8.5 Hz, N*H*Ser), 4.87 (d, 0.6 H, *J* 3.7 Hz, H-1aα), 4.43 (d, 0.4 H, *J* 7.8 Hz, H-1aβ), 3.77 (s, 1.2 H, OMe), 3.74 (s, 1.8 H, OMe), 2.54 (dd, 0.6 H, *J* 4.6 and 12.9 Hz, H-3b*e*), and 2.51 (dd, 0.4 H, *J* 4.6 and 11.5 Hz, H-3b*e*).

Anal. Calc. for $C_{46}H_{55}N_5O_{23}$: C, 52.82; H, 5.30; N, 6.70. Found: C, 52.53; H, 5.11; N, 6.41.

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-3,4-di*-O-*acetyl-2-deoxy-α- and -β*-D-*galactopyranosyl)-(1→3)*-N-*(benzyloxycarbonyl)*-L-*serine benzyl ester* (**16** *and* **20**). — A solution of compounds **14** and **18** (ratio 3:2; 78 mg) in MeOH (2.5 mL) was stirred in the presence of added Lindlar catalyst (100 mg) for 6.5 h at 40°

under H_2. T.l.c. examination in 15:5:1 EtOAc–toluene–MeOH showed the disappearance of compounds **14** and **18** (R_F 0.76) and the formation of **15** and **19** (R_F 0.57 and 0.61). The mixture was diluted with MeOH, filtered through Celite, and the filtrate evaporated *in vacuo*. A solution of the residue in 2:1 pyridine–Ac_2O (1.5 mL) was stirred for 12 h at 20° and evaporated *in vacuo*. Chromatography of the residue on SiO_2 in 40:40:3 EtOAc–toluene–MeOH afforded **16** (33 mg, 42%) and **20** (20 mg, 25%).

Compound **16** had $[\alpha]_D$ +22.1° (*c* 0.9); R_F 0.41 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 7.36 (bs, 10 H, aromatic), 5.93 (d, 1 H, *J* 8.3 Hz, N*H*Ser), 5.70 (d, 1 H, *J* 9.8 Hz, N*H*-C2a), 5.03 (dd, 1 H, *J* 11.5 and 3.2 Hz, H-3a), 4.84 (m, 1 H, H-4b), 4.75 (d, 1 H, *J* 3.7 Hz, H-1a), 3.76 (s, 3 H, OMe), 3.29 (dd, 1 H, *J* 6.4 and 10.3 Hz, H-6a), 2.53 (dd, 1 H, *J* 4.9 and 12.9 Hz, H-3b*e*), 2.15, 2.12, 2.09, 2.02, 2.01, 1.98, 1.91, and 1.87 (8 s, 24 H, 8 Ac).

Anal. Calc. for $C_{50}H_{63}N_3O_{24}\cdot 0.5\ H_2O$: C, 54.64; H, 5.87; N, 3.82. Found: C, 54.68; H, 5.74; N, 3.88.

Compound **20** had $[\alpha]_D$ −28.6° (*c* 0.2); R_F 0.36 in 10:10:1 EtOAc–toluene–MeOH; n.m.r. data: δ_H 7.4–7.2 (m, 10 H, aromatic), 6.35 (d, 1 H, *J* 9.3 Hz, N*H*), 5.85 (d, 1 H, *J* 8.3 Hz, N*H*), 5.48 (d, 1 H, *J* 3.4 Hz, H-4a), 4.80 (m, 1 H, H-4b), 4.56 (d, 1 H, *J* 8.3 Hz, H-1a), 3.79 (s, 3 H, OMe), 2.51 (dd, 1 H, *J* 4.4 and 12.9 Hz, H-3b*e*), 2.22, 2.14, 2.13, 2.02, 1.98, 1.93, 1.88, and 1.87 (8 s, 24 H, 8 Ac).

Anal. Calc. for $C_{50}H_{63}N_3O_{24}\cdot H_2O$: C, 54.19; H, 5.91; N, 3.79. Found: C, 54.37; H, 5.76; N, 3.74.

O-*(5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→6)*-O-*(2-acetamido-2-deoxy-α*-D-*galactopyranosyl)-(1→3)*-L-*serine* (**1**) *and the isomer* (**22**). — A mixture of compound **16** (19 mg) and 10% Pd–C (8 mg) in MeOH (2 mL) was stirred for 3.5 h at 20° under H_2. The mixture was diluted with MeOH, filtered through Celite, and the filtrate evaporated *in vacuo*, to give **17** (15 mg); $[\alpha]_D$ +28.7° (*c* 0.80, MeOH); R_F 0.60 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (CD_3OD): 5.42 (d, 1 H, *J* 2.9 Hz, H-4a), 5.37 (m, 1 H, H-8b), 5.32 (dd, 1 H, *J* 2.2 and 9.3 Hz, H-7b), 5.17 (dd, 1 H, *J* 2.9 and 11.5 Hz, H-3a), 4.94 (d, 1 H, *J* 3.4 Hz, H-1a), 4.80 (m, 1 H, H-4b), 3.81 (s, 3 H, OMe), 2.60 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3b*e*), 2.16, 2.13, 2.10, 2.01, 1.97, 1.97, 1.94, 1.83 (8 s, 14 H, 8 Ac), and 1.79 (t, 1 H, *J* 12.7 Hz, H-3b*a*). A solution of compound **17** (15 mg) in MeOH (2 mL) and M aq. NaOH (0.24 mL) was stirred for 2 h at 20°, diluted with H_2O (18 mL), made neutral with Amberlyst-15, and the suspension filtered through Celite. The filtrate was evaporated *in vacuo*, and the residue was purified by passage through a column of Sephadex G-10 in H_2O, to give **1** (10 mg, quantitative yield); $[\alpha]_D$ +65.0° (*c* 0.1, H_2O); R_F 0.37 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O, 60°) 4.89 (d, 1 H, *J* 3.9 Hz, H-1a), 4.18 (dd, 1 H, *J* 3.7 and 11.0 Hz, H-2a), 4.09 (dd, 1 H, *J* 2.4 and 10.5 Hz, H-6a), 4.02 (dd, 1 H, *J* 4.1 and 7.7 Hz, H-2Ser), 4.00 (d, 1 H, *J* 3.4 Hz, H-4a), 3.57 (dd, 1 H, *J* 1.7 and 8.6 Hz, H-7b), 2.74 (dd, 1 H, *J* 4.6 and 12.2 Hz, H-3b*e*), 2.04 (s, 3 H, NHCOC*H*$_3$), 2.03 (s, 3 H, NHCOC*H*$_3$), and 1.67 (t, 1 H, *J* 12.2 Hz, H-3b*a*); δ_C (D_2O–CH_3OH) 100.7 (C-2b), 98.5 (C-1a),

73.0 (C-6b), 72.2 (C-8b), 70.2 (C-5a), 68.8 (C-4b, C-7b), 68.6 (C-4a), 67.8 (C-3a), 67.4 (C-3Ser), 64.3 (C-6a), 63.2 (C-9b), 54.9 (C-2Ser), 52.3, (C-5b), 50.1 (C-2a), 40.6 (C-3b), and 22.4 (2 $COCH_3$).

Anal. Calc. for $C_{22}H_{37}N_3O_{16}\cdot 3.5\ H_2O$: C, 39.82; H, 6.68; N, 6.33. Found: C, 39.90; H, 6.28; N, 5.95.

A mixture of compound **20** (3.0 mg) and 10% Pd–C (6 mg) in MeOH (0.3 mL) was stirred for 2.5 h at 20° under H_2, diluted with MeOH, and filtered through Celite. The filtrate was evaporated *in vacuo*, to give **21** (2.6 mg); $[\alpha]_D$ −30.8° (*c* 0.1, MeOH); R_F 0.51 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (CD_3OD) 5.42 (d, 1 H, *J* 2.9 Hz, H-4a), 5.38 (m, 1 H, H-8b), 5.27 (dd, 1 H, *J* 2.2 and 9.3 Hz, H-7b), 5.05 (dd, 1 H, *J* 11.5 and 2.9 Hz, H-3a), 4.81 (m, 1 H, H-4b), 4.60 (d, 1 H, *J* 8.3 Hz, H-1a), 4.35 (dd, 1 H, *J* 2.2 and 12.4 Hz, H-9b), 3.80 (s, 3 H, OMe), 2.57 (dd, 1 H, *J* 4.6 and 12.7 Hz, H-3b*e*), 2.16, 2.14, 2.13, 2.01, 1.97, 1.97, 1.96, and 1.84 (8 s, 24 H, 8 Ac).

A solution of compound **21** (2.6 mg) in MeOH (0.3 mL) and 0.1M aq. NaOH (30 μL) was stirred for 6 h at 20°. The mixture was diluted with H_2O (10 mL), made neutral with Amberlyst 15, and the suspension filtered through Celite. The filtrate was evaporated *in vacuo*, and the residue was purified by passage through a column of Sephadex G-10 in H_2O, to give *O-(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→6)*-O-*(2-acetamido-2-deoxy-β-*D-*galacto-pyranosyl)-(1→3)*-L-*serine* (**22**; 1.7 mg); $[\alpha]_D$ +31.7° (*c* 0.1, H_2O); R_F 0.21 in 2:1:1 BuOH–EtOH–H_2O; n.m.r. data: δ_H (D_2O at 20°) 4.49 (d, 1 H, *J* 8.3 Hz, H-1a), 2.73 (dd, 1 H, *J* 4.5 and 12.6 Hz, H-3b*e*), 2.05 (s, 3 H, NAc), 2.03 (s, 3 H, NAc), and 1.70 (t, 1 H, *J* 12.1 Hz, H-3b*a*).

ACKNOWLEDGMENTS

We are indebted to Mr. Y. Shitori of MECT Co. for a generous supply of *N*-acetylneuraminic acid. This work was partly supported by a Grant-in-aid for Scientific Research of the Ministry of Education, Science, and Culture and also by the SANKYO Foundation of Life Science. We thank Dr. J. Uzawa, Mrs. T. Chijimatsu, and Dr. T. Nukada for recording and measuring the n.m.r. spectra, and Dr. H. Yamazaki and his staff for the elemental analyses. We also thank A. Takahashi and K. Moriwaki for their technical assistance.

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