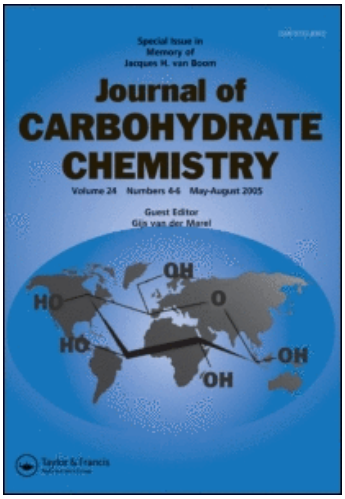

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SYNTHESIS OF NEU5Ac α-(2→8)NEU5Ac α-(2→3)GALβ1→$OCH_2CH_2CH_3$, A DETERMINANT EPITOPE OF GD_3 GANGLIOSIDE

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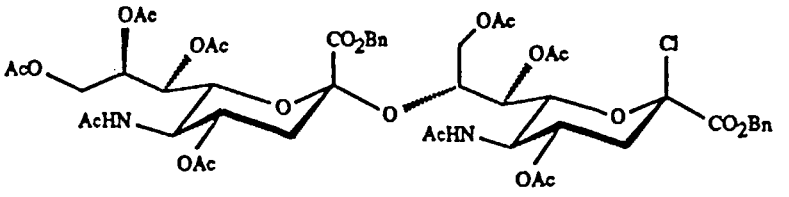
ABSTRACT

Synthesis of the terminal trisaccharide sequence of the ganglioside GD_3, α-D-Neu*p*5Ac-(2→8)-α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer (**2**) was achieved by employing an α-(2→8) disialyl glycosyl donor (**1**). Condensation of **1** with the glycosyl acceptor **6**, propyl 4,6-*O*-benzylidene-β-D-galactopyranoside, gave the desired protected trisaccharide **10** (14%) as well as the elimination and hydrolysis products of **6**, compounds **8** and **9** respectively. *O*-Deacetylation and debenzylation of **10** gave the final trisaccharide **11**, as its propyl glycoside.

INTRODUCTION

Sialic acid-containing glycolipids, or gangliosides, are a group of biologically active cell-surface carbohydrate molecules.[1] One branch of this diverse group of oligosaccharides includes those possessing the Neu5Ac α-(2→8)Neu5Ac (di-Neu5Ac), unit such as the disialogangliosides GD_2 and GD_3, which are found in fetal brain[2] and in tumors of neuroectodermal origin.[3] Although these gangliosides are not tumor-specific antigens, it appears that they display a relatively restricted distribution in



1

α-D-Neu*p*5Ac-(2→8)-α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer

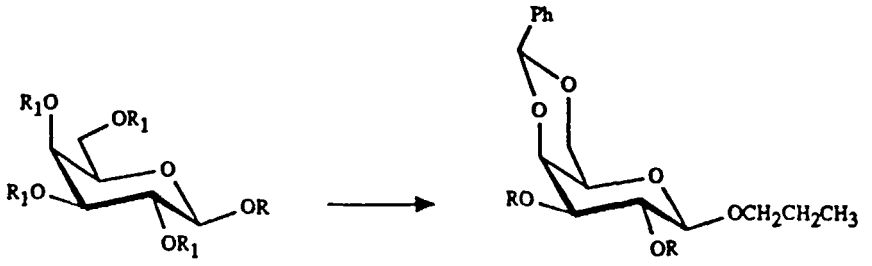
2

normal tissues and it has been suggested that these carbohydrate antigens may serve as potential targets for active specific immunotherapy.[4]

A key step in our approach to the synthesis of disialylgangliosides involves the introduction of the di-Neu5Ac unit with α-stereoselectivity onto the glycosyl acceptor. We have recently demonstrated the utility of di-Neu5Ac synthon **1** with methanol[5] and now wish to report the reaction of this donor with a galactose derivative, thereby providing the terminal trisaccharide portion of the GD_3 ganglioside **2**.

RESULTS AND DISCUSSION

"Disconnection" of the terminal trisaccharide sequence revealed the possibility of a synthetic route employing glycosyl donor **1** and glycosyl acceptor **6**. Preparation of the galactose acceptor was carried out in a straightforward manner in four steps as shown in Scheme 1. Reaction of allyl alcohol and β-D-galactose pentaacetate in presence of tin (IV) chloride, according to the procedure of Banoub and Bundle[6] provided the allyl glycoside **3** which upon subsequent Pd-C-catalyzed hydrogenation yielded the propyl derivative **4**. De-O-acetylation of **4** (sodium methoxide in dry methanol) led to tetraol **5** which was then selectively blocked at the 4,6-O-positions by treatment with α,α-dimethoxytoluene and catalytic p-toluenesulfonic acid in 2:1 acetonitrile - *N,N*-dimethylformamide to give the acceptor **6**. For future comparison



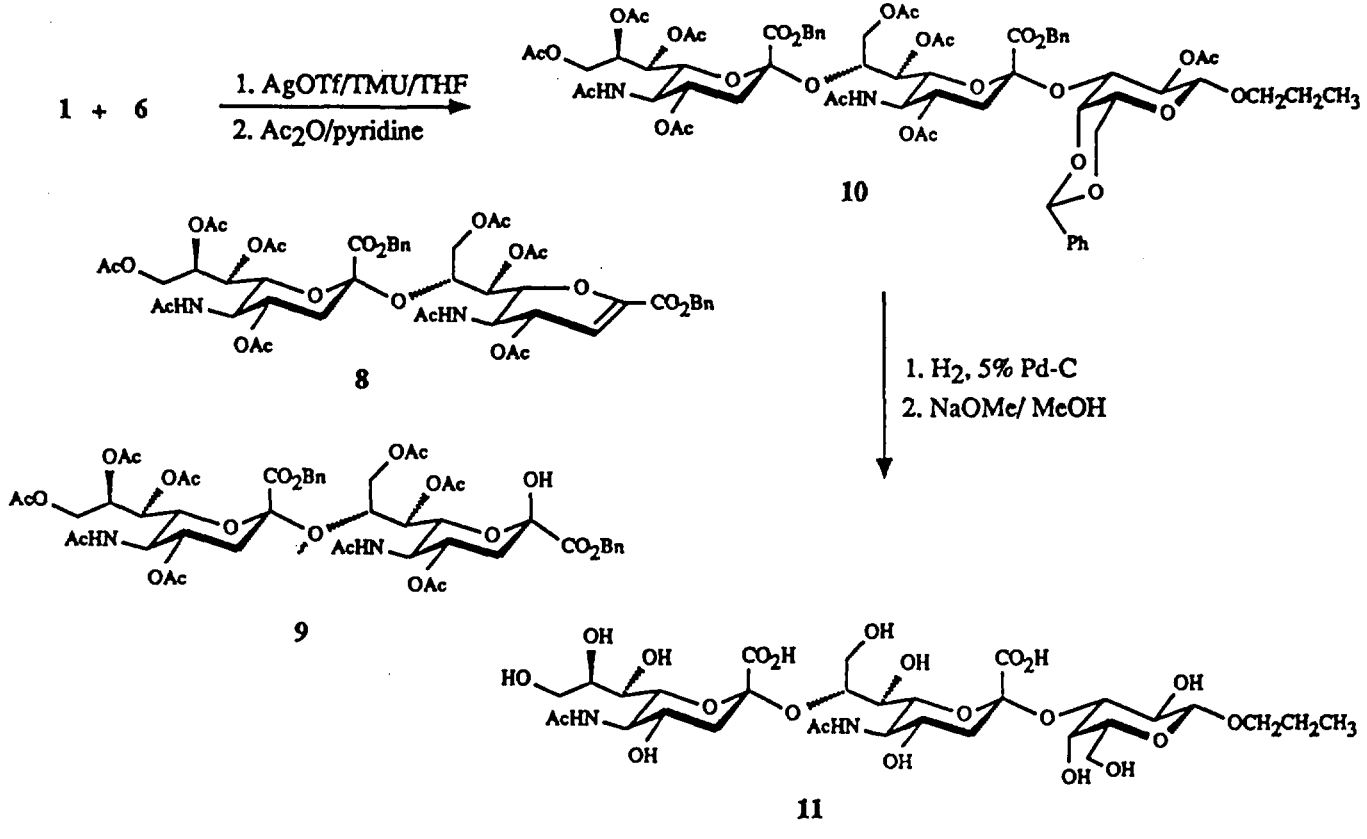
3 R= -$CH_2CH{=}CH_2$, R_1= Ac
4 R= -$CH_2CH_2CH_3$, R_1= Ac
5 R= -$CH_2CH_2CH_3$, R_1= H

6 R= H
7 R= Ac

Scheme 1

purposes, a small amount of **6** was acetylated (2:1 pyridine-acetic anhydride) to give **7**, which upon ^{1}H NMR analysis, displayed characteristic deshielded doublet of doublets at δ 5.39 ($J_{2,3}$ = 10.5 Hz, $J_{2,1}$ = 8.0 Hz) and 4.96 ($J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz) for H-2 and H-3 respectively.

The glycosidation step (Scheme 2) was carried out with chloride donor **1**[5] (1 equiv.) and diol acceptor **6** (2 equiv) in the presence of silver trifluoromethane-sulfonate and 1,1,3,3-tetramethylurea in tetrahydrofuran at -30 °C under inert atmosphere.[7] As expected, the major product of the reaction mixture was the 2,3-dehydro compound **8**, formed by elimination of HCl from **1**, along with some hydrolysis product **9**. However, upon separation of the complex mixture by column chromatography over silica gel, followed by acetylation (2:1 pyridine-acetic anhydride) and rechromatography, the desired trisaccharide **10** was obtained in 14% yield (based on starting chloride). The ^{1}H NMR spectrum of **10** displayed doublet of doublet signals at δ 2.76 and 2.81 for the H_{3e}'s of the sialic acid moieties as well as ten acetate singlets. Although the downfield signal for the galactose H-2 proton (δ 5.22) was partially obscured by the benzyl proton resonances, the regiochemistry of the (2→3) glycosidic linkage was confirmed by decoupling the clearly visible galactose H-3 and H-1 signals at δ 4.55 and 4.41 ppm respectively.



Scheme 2

Routine deblocking of **10** (1: H_2, 5%Pd-C, MeOH, trace HOAc, 2: NaOMe in MeOH) proceeded in 68% overall yield to give 13 mg of **11** after chromatography over silica gel using isopropanol:ammonia:water 8:1:1 as eluant. The ^1H NMR spectrum of **11** showed two doublet of doublets at δ 2.81 and 2.65 for the H_{3e}'s of the sialic acid residues. In the spectra of the methyl glycosides of di-Neu5Ac in D_2O,[5a] the corresponding H_{3e} resonances for the α-anomer were at δ 2.78 and 2.61, and those for the β-anomer were at δ 2.76 and 2.30. Thus, it appears that the trisaccharide **11** possesses the correct α-stereoselectivity. The same pattern is observed in the spectra of other sialic acid derivatives in D_2O; the chemical shift of H_{3e} is greater than 2.5 ppm in α-monosialosides[8] but less than 2.5 ppm in the β-analogues.[9] Similar observations have also been reported for α-(2→8)-linked sialic acid oligosaccharides.[10]

Previously, we had shown the utility of donor **1** with a simple primary alcohol (methanol). We have now expanded our approach to include a secondary alcohol (the galactose 3 position) yielding the terminal trisaccharide portion of GD_3 **2**. Recently, it has been suggested that this epitope represents an important binding region for certain anti-GD_3 monoclonal antibodies.[11] Recent results[12] have also indicated that the free oligosaccharides of GD_3 and GM_3 could be directly transformed into polymer and protein conjugates for antibody production and screening.

Further, this approach may be applicable to lactose which will give rise to the complete GD_3 carbohydrate sequence. Since virtually no β-(2→3) glycoside was detected, it may be postulated that participation of the solvent (THF) may in part be responsible for the α-stereoselectivity observed. Moreover, due to the low glycosylation yield obtained using the silver triflate - tetramethylurea procedure,[7] it may be anticipated that further glycosidation using either the thioglycoside[13] or the *S*-xanthate[14] approaches would allow for better glycosidation yields. The 2,3-dehydro derivative **8** may be a useful starting material for strategies employing C-3 stereocontrolling functionalities such as phenylseleno and phenylthio auxiliaries.[15]

EXPERIMENTAL

General Procedures. Melting points were determined with a Gallenkamp MFB-585 melting point apparatus and are uncorrected. Specific rotations were

determined with an Optical Activity Ltd. AA-100 polarimeter. Proton NMR spectra were recorded with a Bruker AM 400 spectrometer, in solutions of $CDCl_3$, unless noted otherwise. The values of δ are expressed in ppm downfield from the signal for internal Me_4Si. Fast atom bombardment (FAB) mass spectra (positive ion mode) were recorded with a Kratos Concept II H spectrometer. Analytical TLC was performed on Silica Gel 60 F_{254} (Merck, Darmstadt). Column chromatography was performed using Kieselgel 60H (Merck, Darmstadt). Toluene and tetrahydrofuran were distilled from sodium benzophenone ketyl. Evaporations were conducted *in vacuo*.

Allyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (3).[16] To a solution of β-D-galactose pentaacetate (3.9 g, 10 mmol) in dry dichloromethane (80 mL) cooled to -10 °C, was added a solution of tin (IV) chloride (1.3 mL, 11 mmol) in dichloromethane (10 mL) and the mixture was stirred at this temperature for 10 min. To this mixture was added a solution of allyl alcohol (890 μL, 13 mmol) in dichloromethane (10 mL) and the mixture was stirred at -10 °C for 3 h, then at room temperature overnight. The reaction mixture was then diluted with dichloromethane, washed with water, saturated sodium bicarbonate, dried (sodium sulfate), filtered and the solvents were removed. Column chromatography over silica gel, using 4:1 hexane-ethyl acetate as eluant, gave 1.74 g (45%) of **3** as an oil: $[\alpha]_D$ -17.7° (*c* 1.0, chloroform); ^{1}H NMR δ 5.90 (m, 1H, allyl CH), 5.41 (dd, 1H, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 5.30 (dq, 1H, J_d = 17.5 Hz, J_q = 1.5 Hz, allyl CH), 5.24 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 5.22 (dq, 1H, J_d = 10.5 Hz, J_q = 1.5 Hz, allyl CH), 5.03 (dd, 1H, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.53 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.37 (m, 1H, allyl CH), 4.16 (dd, 1H, $J_{6,6}$ = 12.0 Hz, $J_{6,5}$ = 7.0 Hz, H-6), 4.13 (dd, 1H, $J_{6,6}$ = 12.0 Hz, $J_{6,5}$ = 7.0 Hz, H-6), 4.10 (m, 1H, allyl CH), 3.92 (dt, 1H, $J_{5,6}$ = 7.0 Hz, $J_{5,4}$ = 1.5 Hz, H-5), 2.16 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.99 (s, 3H, Ac).

Anal. Calcd for $C_{17}H_{24}O_{10}$: C, 52.57; H, 6.23. Found: C, 52.58; H, 6.08.

Propyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (4).[17] A mixture of allyl glycoside **3** (1.14 g, 2.94 mmol) and 5% palladium-on-carbon (2 g, 55% moisture content) in methanol (30 mL) was hydrogenated at 1 atm, room temperature for 2 h. The mixture was filtered through Celite® and the solvents were removed to give 1.03 g (90%) of **4** as an oil: $[\alpha]_D$ -14.6° (*c* 1.0, chloroform, Lit.[17] -13.5°).

Anal. Calcd for $C_{17}H_{26}O_{10}$: C, 52.30; H, 6.71. Found: C, 52.09; H, 6.61.

Propyl β-D-galactopyranoside (5).[18] Crude **4** (1.03 g, 2.6 mmol) was dissolved in methanol (20 mL) containing sodium (90 mg, 3.9 mmol) and the mixture was stirred at room temperature overnight. The mixture was then deionized with Amberlite® IR 120 (H^+) resin (2 g), filtered, and the solvents were removed to give 586 mg (quantitative) of **5**: mp 113 - 114 °C; $[\alpha]_D$ -21° (*c* 1.0, methanol); Lit.[18]: mp 110 - 112 °C; $[\alpha]_D$ -20.3° (*c* 1.0, methanol).

Anal. Calcd for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 47.83; H, 8.27.

Propyl 4,6-O-benzylidene-β-D-galactopyranoside (6). The crude tetrol **5** (311 mg, 1.4 mmol) was benzylidenated in 2:1 acetonitrile - *N,N* -dimethylformamide (12 mL) containing α,α'-dimethoxytoluene (462 mg, 2.8 mmol) and p-toluenesulfonic acid monohydrate (18 mg, 0.09 mmol). After stirring at room temperature for 16 h, the reaction mixture was neutralized with excess triethylamine and the solvents were removed. The residue was dissolved in chloroform, washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), filtered and the solvents were removed. Column chromatography over silica gel, using chloroform then 100:1 chloroform-methanol as eluant, gave 263 mg (61%) of **6** as an amorphous solid: mp 162 - 164 °C; $[\alpha]_D$ -41.1° (*c* 1.0, chloroform); ^1H NMR δ 7.55-7.30 (m, 5H, aromatic), 5.55 (s, 1H, benzylidene), 4.34 (dd, 1H, $J_{6,6}$ = 12.5 Hz, $J_{6,5}$ = 1.5 Hz, H-6), 4.21 (dd, 1H, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.0 Hz, H-4), 4.08 (dd, 1H, $J_{6,6}$ = 12.5 Hz; $J_{6,5}$ = 1.75 Hz, H-6), 3.94 (dt, 1H, J_d = 9.5 Hz, J_t = 6.5 Hz, OCH_2), 3.80-3.70 (m, 2H), 3.48 (m, 1H), 3.47 (dt, 1H, J_d = 9.5 Hz, J_t = 6.5 Hz, OCH_2), 2.60 (bm, OH), 1.67 (m, 2H, CH_2), 0.95 (t, 3H, J_t = 7.5 Hz, CH_3). *m/z*: 310.9 [CI, calc. for $C_{16}H_{22}O_6$: 310.34].

Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.15. Found: C, 61.40; H, 7.10.

Propyl 2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranoside (7). A small portion of **6** (28 mg) was acetylated in 2:1 pyridine-acetic anhydride and purified by column chromatography over silica gel using 3:1 hexane - ethyl acetate as eluant to provide **7** as an amorphous solid: mp 92 - 93 °C; $[\alpha]_D$ +61.1° (*c* 1.0, chloroform); ^1H NMR δ 7.55-7.35 (m, 5H, aromatic), 5.50 (s, 1H, benzylidene), 5.39 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 4.96 (dd, 1H, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.49 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.38 (dd, 1H, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 0.5 Hz, H-4), 4.33 (dd, 1H, $J_{6,6}$ = 12.5 Hz, $J_{6,5}$ = 1.5 Hz, H-6), 4.06 (dd, 1H, $J_{6,6}$ = 12.5 Hz, $J_{6,5}$ =

1.75 Hz, H-6), 3.90 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, OCH_2), 3.51 (q, 1H, J_q = 1.5 Hz, H-5), 3.44 (dt, 1H, J_d = 9.5 Hz, J_t = 6.5 Hz, OCH_2), 2.08 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.60 (m, 2H, CH_2), 0.91 (t, 3H, J_t = 7.5 Hz, CH_3).

Anal. Calcd for $C_{20}H_{26}O_8$: C, 60.90; H, 6.65. Found: C, 60.62; H, 6.65.

Propyl O-[benzyl (5-acetamido-4,7,9-tri-O-acetyl-3,5-dideoxy-8-O-(benzyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-(2→3)-2-O-acetyl-4,6-O-benzylidene-β-D-galactopyranoside 10. To a mixture of diol **6** (50 mg, 0.16 mmol), silver trifluoromethanesulfonate (50 mg, 0.19 mmol), 1,1,3,3-tetramethylurea (24 mg, 0.2 mmol), Drierite® (250 mg) and dry tetrahydrofuran (1.5 mL), at -30 °C, was added a solution of chloride **1**[5] (85 mg, 0.079 mmol) in toluene (200 μL). The solution was stirred at this temperature for 30 minutes, then at -5 °C overnight. The reaction mixture was then diluted with chloroform, filtered through Celite® and the combined organic filtrate washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), filtered and the solvents were removed. Column chromatography over silica gel, using 15:10:1 hexane-ethyl acetate-ethanol followed by rechromatography with 115:1 chloroform:methanol, gave 2,3-dehydro compound **8**, then hydrolysis product **9**, and finally a crude mixture which was acetylated (2:1 pyridine-acetic anhydride, 3 mL). The product was chromatographed over silica gel using 115:1 chloroform:methanol to give **10** (14 mg, 14% based on chloride >85% as estimated by ^{1}H NMR) as a powder after lyophilization from benzene: ^{1}H NMR δ 7.45-7.25 (m, 15H, aromatic), 6.25 (d, 1H, J_d = 10.0 Hz, NH"), 5.47 (ddd, 1H, $J_{8',7'}$ = 10.0 Hz, $J_{8',9'}$ = 5.0 Hz, $J_{8',9'}$ = 3.0 Hz, H-8), 5.37 (bs, 1H, H-7'), 5.33 (d, 1H, J_d = 12.0 Hz, benzyl), 5.31 (dd, 1H, $J_{7',8'}$ = 10.0 Hz, $J_{7',6'} \leq$ 1.5 Hz, H-7"), 5.24 (s, 2H, benzyl), 5.23 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 5.19 (d, 1H, J_d = 12.0 Hz, benzyl), 5.1 (d, 1H, $J_{NH',5'}$ = 10.5 Hz, NH'), 5.099 (dd, 1H, $J_{9',9'}$ = 12.0 Hz, $J_{9',9'}$ = 2.0 Hz, H-9'), 4.99 (bm, 1H, H-4'), 4.95 (dd, 1H, J_d = 9.5 Hz, J_d = 2.0 Hz, H-8'), 4.93 (s, 1H, benzylidene), 4.87 (ddd, 1H, $J_{4',3a'}$ = 13.5 Hz, $J_{4',5'}$ = 10.5 Hz, $J_{4',3e'}$ = 4.5 Hz, H-4"), 4.55 (dd, 1H, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.41 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.23 (dd, 1H,$J_{9',9'}$ = 12.5 Hz, $J_{9',8'}$ = 5.0 Hz, H-9"), 4.19 (dd, 1H, $J_{9',9'}$ = 12.5 Hz, $J_{9',8'}$ = 3.0 Hz, H-9"), 4.18 (dd, 1H, $J_{6,6}$ = 12.5, $J_{6,5}$ < 1.0 Hz, H-6), 4.09 (q, 1H, J_q = 10.5

Hz, H-5"), 4.04 (dd, 1H, $J_{9',9'}$ = 12.0 Hz, $J_{9',8'}$ = 9.5 Hz, H-9'), 4.01 (m, 2H, H-5' and H-6'), 3.83 (dd, 1H, $J_{6",5"}$ = 10.5 Hz, $J_{6",7"}$ = 1.5 Hz, H-6"), 3.81 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, propyl CH), 3.78 (dd, 1H, $J_{6,6}$ = 12.5 Hz, $J_{6,5}$ < 1.0 Hz, H-6), 3.60 (d, 1H, $J_{4,3}$ = 3.5 Hz, H-4), 3.41 (dt, 1H, J_d = 9.5 Hz, J_t = 6.5 Hz, propyl CH), 3.31 (bs, 1H, H-5), 2.81 (dd, 1H, $J_{3e',3a'}$ = 13.0 Hz, $J_{3e',4'}$ = 5.0 Hz, H-3e'), 2.76 (dd, 1H, $J_{3e",3a"}$ = 13.0 Hz, $J_{3e",4"}$ = 4.5 Hz, H-3e"), 2.18 (s, 3H, Ac), 2.17 (s, 3H, Ac), 2.16 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.049 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.88 (t, 1H, J_t = 13.0 Hz, H-3a"), 1.86 (t, 1H, J_t = 13.0 Hz, H-3a'), 1.55 (m, 2H, propyl CH_2), 0.88 (t, 3H, J_t = 7.5 Hz, CH_3). The assignments for some signals are tentative. Data for 2,3-dehydro di-Neu5Ac **8**: ^{1}H NMR δ 7.40-7.30 (m, 10H, aromatic), 6.61 (d, 1H, $J_{NH,5}$ = 10.0 Hz, NH or NH'), 5.97 (d, 1H, J_d = 2.5 Hz), 5.61 (dd, 1H, J_d = 9.5 J_d = 2.5 Hz), 5.30 (s, 2H, benzyl), 5.25-5.28 (m, 4H), 5.18 (d, 1H, J_d = 12.5 Hz, benzyl), 5.11 (d, 1H, $J_{NH,5}$ = 10.5 Hz, NH or NH'), 4.85 (ddd, 1H, $J_{4',3a'}$ = 13.0 Hz, $J_{4',5'}$ = 9.5 Hz, $J_{4',3a'}$ = 4.5 Hz, H-4'), 4.79 (ddd, 1H, J_d = 8.0 Hz, J_d = 5.5 Hz, J_d = 3.0 Hz), 4.54 (dd, 1H, J_d = 12.0 Hz, J_d = 2.5 Hz), 4.36-4.44 (m, 3H), 4.18 (dd, 1H, J_d = 12.5 Hz, J_d = 8.5 Hz), 4.00 (q, 1H, J_q = 10.5 Hz), 3.95 (m, 1H), 3.81 (dd, 1H, J_d = 10.5, J_d = 2.5 Hz), 2.68 (dd, 1H, $J_{3e',3a'}$ = 13.0 Hz, $J_{3e',4'}$ = 4.5 Hz, H-3e'), 2.15 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.85 (s, 3H, Ac). Data for 2-OH di-Neu5Ac **9**: ^{1}H NMR δ 7.50-7.30 (m, 10H, aromatic), 5.71 (d, 1H, J_d = 2.5 Hz), 5.54 (d, 1H, $J_{NH,5}$ = 10.0 Hz, NH or NH'), 5.45 (ddd, 1H, $J_{8',7'}$ = 9.5 Hz, $J_{8',9'}$ = 5.5 Hz, $J_{8',9'}$ = 2.5 Hz, H-8'), 5.34 (d, 1H, J_d = 12.5 Hz, benzyl), 5.31 (dd, 1H, J_d = 9.5 Hz, J_d = 2.5 Hz), 5.30 (m, 1H), 5.26 (s, 2H), 5.13 (m, 2H), 5.05 (d, 1H, $J_{NH,5}$ = 10..5 Hz, NH or NH'), 4.86 (ddd, 1H, J_d = 13.0 Hz, J_d = 10.5 Hz, J_d = 4.5 Hz), 4.55 (dt, 1H, J_d = 8.0 Hz, J_t = 2.5 Hz, H-8), 4.38-4.32 (m, 2H), 4.14-4.08 (m, 2H), 4.04 (q, 1H, J_q = 10.5 Hz), 3.98 (dd, 1H, J_d = 12.5 Hz, J_d = 5.5 Hz), 3.65 (dd, 1H, J_d = 10.5 Hz, J_d = 2.5 Hz), 2.71 (dd, 1H, J_d = 13.5 Hz, J_d = 5.0 Hz), 2.28 (dd, 1H, J_d = 13.5 Hz, J_d = 5.0 Hz), 2.21 (s, 3H, Ac), 2.105 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.85 (s, 3H, Ac), 1.77 (s, 3H, Ac).

Propyl O-[5-acetamido-3,5-dideoxy-8-O-(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)-α-D-glycero-D-galacto-2-nonulopyranosylonic acid]-(2→3)-β-D-galactopyranoside (11). A solution of **10** (30 mg) and 5% palladium-on-carbon (60 mg, 55% moisture content) in methanol containing a few drops of acetic acid was hydrogenated at 1 atm, room temperature for 18 h. The reaction yielded one major product by TLC (Rf=0.085, 80:15:5 ethyl acetate-methanol-water) along with a minor product (Rf=0.15). The mixture was filtered through Celite® and the solvents were removed. Column chromatography over silica gel (4.5 g), using 100:15:5 ethyl acetate-methanol-water as eluant, gave 18 mg of material which was de-O-acetylated under Zemplen conditions, neutralized and finally purified by column chromatography over silica gel (1.5 g) using 8:1:1 isopropanol-ammonia-water as eluant to give 13 mg of **11** (68% overall yield from **10**, presumably as the di-ammonium salt) as a white solid after lyophilization from water: ^{1}H NMR (D_2O, HOD=4.80, 297°K) δ 4.47 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.11 (dd, 1H obscured by a second proton, J_d = 10.0 Hz, J_d = 3.0 Hz, possibly H-3), 4.01 (d, 1H, J_d = 3.0 Hz, possibly H-4), 3.95-3.5 (m), 2.81 (dd, 1H, $J_{3e,3a}$ = 13.0 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e' or H-3e"), 2.65 (dd, 1H, $J_{3e,3a}$ = 12.5 Hz, $J_{3e,4}$ = 4.0 Hz, H-3e' or H-3e"), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.85 (t, 1H, J_t = 12.5 Hz, H-3a' or H-3a"), 1.75 (t, 1H, J_t = 13.0 Hz, H-3a' or H-3a"), 1.65 (m, 2H,CH_2CH_3), 0.91 (t, 3H, J_t = 7.5 Hz, CH_3). Molecular weight for $C_{31}H_{52}O_{22}N_2$: calcd 804.6, found (FAB, M^++1): 805.2.

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