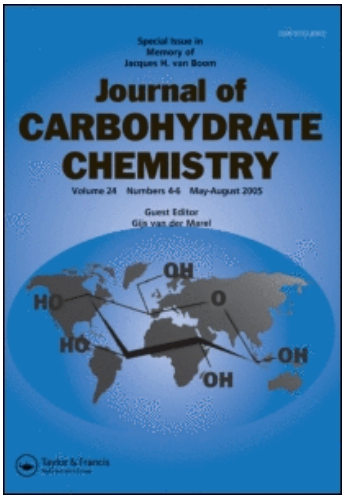


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis of α- and β-Methyl NEU5Ac α-(2⟶8) NEU5Ac Disaccharides

S. Z. Abbas; S. Sugiyama; J. Diakur; R. A. Pon; R. Roy

To cite this Article Abbas, S. Z. , Sugiyama, S. , Diakur, J. , Pon, R. A. and Roy, R.(1990) 'Synthesis of α- and β-Methyl NEU5Ac α-(2⟶8) NEU5Ac Disaccharides', Journal of Carbohydrate Chemistry, 9: 6, 891 — 901
To link to this Article: DOI: 10.1080/07328309008543882
URL: http://dx.doi.org/10.1080/07328309008543882

J. CARBOHYDRATE CHEMISTRY, 9(6), 891-901 (1990)

SYNTHESIS OF α- AND β-METHYL NEU5Ac α-(2→8) NEU5Ac DISACCHARIDES

S.Z. Abbas[a], S. Sugiyama[a], J. Diakur[a]*, R.A. Pon[b], and R. Roy[b]

[a]Biomira Inc., 9411 - 20 Avenue, Edmonton, Alberta, Canada, T6N 1E5.
[b] Department of Chemistry, University of Ottawa, Ottawa, Ontario, K1N 6N5.

Received January 4, 1990 - Final Form August 8, 1990

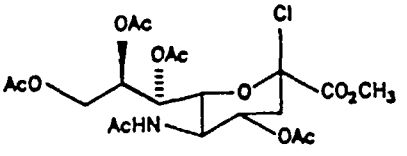
ABSTRACT

Acid hydrolysis of colominic acid, an α-(2→8)-linked oligomer of sialic acid, yielded Neu5Ac α-(2→8) Neu5Ac (di-Neu5Ac) **2** as one of the products. Starting from this disaccharide, it was possible to prepare two potential di-Neu5Ac donors, **5** and **8** , as their corresponding 2-chloro derivatives. Subsequent reaction of the donor **8** with methanol as a simple acceptor led to the α- and β-methyl Neu5Ac α-(2→8) Neu5Ac glycosides.

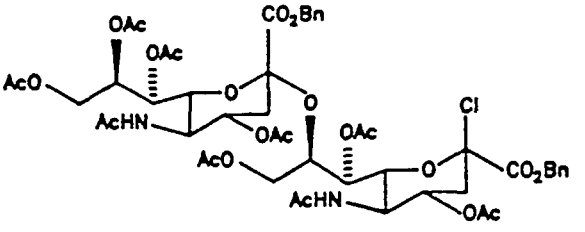
INTRODUCTION

Sialic acid (Neu5Ac) bearing glycolipids and glycoproteins are an important class of carbohydrate molecules which are involved in cell social events and biological recognition phenomena.[1] One important class of this diverse group of oligosaccharides possesses the Neu5Ac α-(2→8) Neu5Ac sequence found in group B meningococcal polysaccharides,[2] brain tissues,[3] and tumor-associated gangliosides.[4] Aside from the elegant synthesis of the α-2→8 linkage by Goto et al.,[5] synthetic endeavors in this area are still lacking.

Based on the fact that the most common Neu5Ac glycosyl donor is the 2β-chloro derivative **1**,[6] it was anticipated that Neu5Acα-(2→8) Neu5Ac donor **8** directly prepared from the dimer **2** would provide a convenient entry into this class of



1



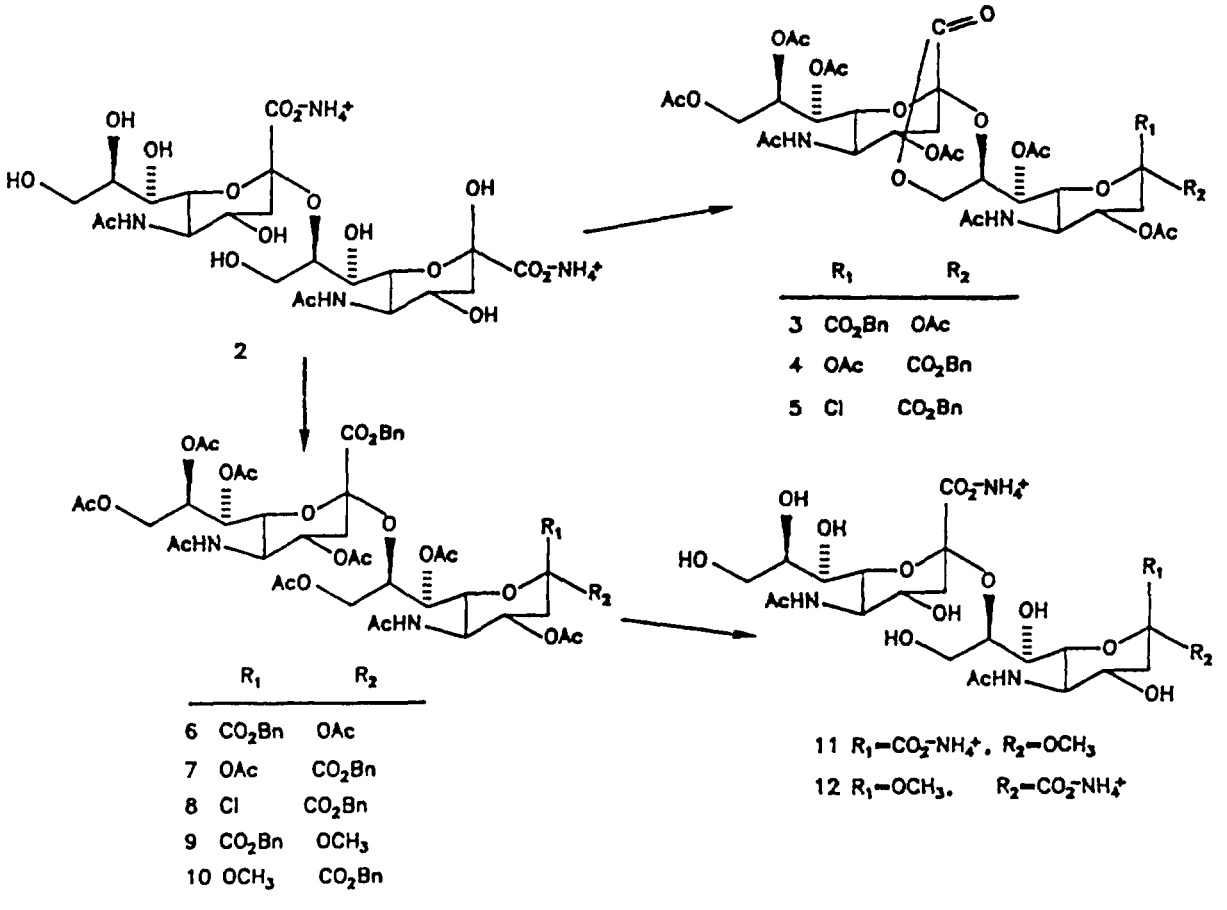
8

gangliosides.[5] Indeed, the starting α-(2→8) disialyl glycoside **2** can be prepared in large quantities from naturally occuring poly α-(2→8) sialic acid (colominic acid from E.coli) via graded acid hydrolysis.[7]

RESULTS AND DISCUSSION

Neu5Acα-(2→8) Neu5Ac dimer **2**,[8] obtained from polysaccharide degradation was first acetylated (Ac_2O-Py) then subsequently esterified (BnBr-KF in DMF) (Scheme 1). However, instead of the desired diester **7**, a mixture of lactones **3** and **4** (11% and 55% yield, respectively) resulted. The structural assignments of **3** and **4** were based on their ^1H NMR spectra which displayed only 5 protons in the aromatic region and 9 rather than 10 acetate signals. Furthermore, lactone formation has been previously observed in the group B meningococcal polysaccharide[9] and in the G_{D1b} ganglioside,[10] indicating a common feature of the α-(2→8) linkage. Conversion of the major β-acetate **4** into the chloride **5** was carried out with AcCl-CH_2Cl_2. The ^1H NMR spectrum of **5** showed a downfield shift for H-3a (δ≈2.3 ppm) which is consistent with that displayed in the corresponding 2β-chloro derivative **1**. The desired dibenzyl esters **6** and **7** along with some lactone **4** were obtained by p-toluenesulfonic acid catalyzed acetylation of **2** followed by esterification (BnBr-KF in DMF) in 3%, 41% and 12% yield, respectively. Treatment of the β-acetate **7** with AcCl-CH_2Cl_2 provided the crude chloride **8** (>85%, ^1H NMR) which again displayed a downfield shift in the ^1H NMR

SCHEME 1



spectrum for H-3a (δ=2.22 ppm). With the desired di-Neu5Ac donor now available, reaction of the chloride **8** with excess methanol using $AgClO_4$-TMU in THF[11] provided the α-methyl glycoside **9** as the major anomer together with some β-methyl glycoside **10** (α/β = 2.75:1) in 75% yield.

The α-methyl glycoside **9** was deblocked in two steps (1: H_2, 5% Pd-C, 2: NaOMe in MeOH) to give **11** as the di-ammonium salt after chromatography over silica gel using 2-propanol-ammonia-water 8:1:1 as eluant. Likewise, treatment of the β-anomer **10** to the same reaction sequence provided **12**. The structural assignments of **11** and **12** are based on the chemical shift of H-3e and are in good agreement with those predicted from empirical rules.[5,12] Our reasoning was as follows: (1) in aqueous solutions, sialic acid exists almost exclusively (92-95%) in the β-configuration and the shift of H-3e(α) at 2.75 ppm is downfield from that of H-3e(β) at 2.2 ppm;[13] (2) likewise, Neu5Ac α-(2→8) Neu5Ac **2** displayed signals at 2.75 ppm, for H-3'e and 2.2 ppm for H-3e and; (3) the shift of H-3e for the corresponding α- and β-methyl glycosides of Neu5Ac were shown to resonate at 2.69 and 2.36 ppm respectively.[14] As can be seen in FIG. 1, compound **11** showed downfield shifts for both H-3e and H-3'e at 2.78 and 2.61 ppm corresponding to the α-methyl glycoside, while compound **12**, which showed shifts at 2.76 ppm, for H-3'e and 2.30 ppm for H-3e, was assigned the β-methyl glycoside. These assignments are in agreement with recent high field ^{1}H NMR studies on α-(2→8)-linked homosialooligosaccharides.[15]

In conclusion, preparation of di-Neu5Ac donor **8** and subsequent transformations to methyl glycosides has been demonstrated. This donor should prove useful in the preparation of gangliosides such as GD_2 and GD_3. Furthermore, the lactone chloride **6** could be useful in a block synthesis approach to gangliosides. We are currently investigating the reaction of donor **8** with secondary alcohols along with the utility of chloride **6**.

EXPERIMENTAL

General Procedures. ^{1}H 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. Analytical TLC was performed on Silica Gel 60 F_{254} (Merck, Darmstadt). Column chromatography was

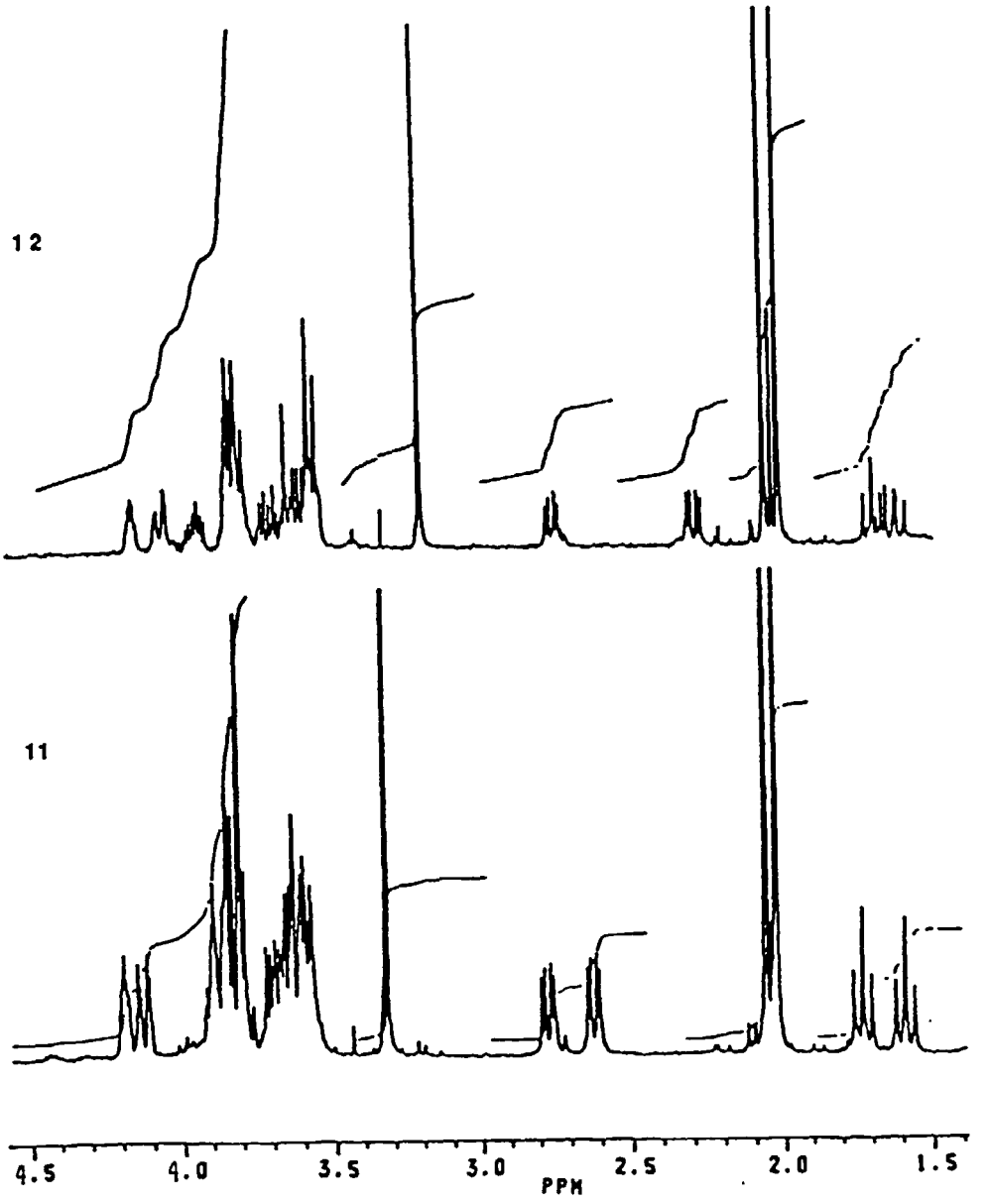


FIG. 1 400 MHz ^{1}H NMR spectrum of **11** and **12** in D_2O (293 °K, HOD δ= 4.8 ppm).

performed using Kieselgel 60H (Merck, Darmstadt). Toluene and THF were distilled from sodium benzophenone ketyl. Evaporations were conducted *in vacuo* .

Lactones (3) and (4). A solution of **2** (29 mg, 0.046 mmol) in 2:1 pyridine-acetic anhydride (3 mL) was stirred at room temperature for 18 h. The solvents were removed and the residue was coevaporated with toluene, taken up in ethyl acetate, deionized with Amberlite™ IR 120(H^+) resin (180 mg), filtered and the solvents were removed to give 29 mg of an amorphous powder. A solution of this material (26 mg) in dry *N,N*-dimethylformamide (1 mL) containing anhydrous potassium fluoride (10 mg, 0.16 mmol) and benzyl bromide (24 mg, 0.14 mmol) was stirred at room temperature for 24 h. The solvents were then removed under high vacuum and the residue taken up in dichloromethane, washed with water, saturated sodium bicarbonate, saturated sodium chloride, dried (sodium sulfate), filtered and the solvents were removed. Column chromatography over silica gel (2.5 g) using 10:10:1 hexane-ethyl acetate-ethanol and then changing to 6:6:1 gave first 3 mg (11%) of material (possibly **3**) followed by 15 mg (55%) of **4**. Lactone **4** was an amorphous powder: ^{1}H NMR δ 7.4 (m, 5 H, aromatic), 5.84 (m, 1 H, NH), 5.42 (d, 1 H, J =10.5 Hz, NH), 5.32 (m, 3 H), 5.22 (s, 2 H, $CH_2C_6H_5$), 5.21 (m, 1 H), 5.17 (dd, 1 H, J =8.0 and 1.5 Hz), 4.45 (dd, 1 H, J =12.5 and 9.5 Hz), 4.35 (m, 2 H), 4.29 (dd, 1 H J = 12.5 and 2.5 Hz), 4.17 (m, 3 H), 4.07 (dd, 1 H, J =10.5 and 2.5 Hz), 4.01 (dd, 1 H, J =12.5 and 5.5 Hz), 2.51 (dd, 1 H, J =13.0 and 4.5 Hz), 2.42 (dd, 1 H, J =13.0 and 5.0 Hz), 1.98 (dd, 1 H, J =13.0 and 11.5 Hz), 1.8 (dd,1 H,J =13.0 and 115 Hz), 2.18 (s,3 H, Ac), 2.16 (s, 3 H, Ac), 2.13 (s, 3 H, Ac), 2.1 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.04 (s, 3 H, Ac), 2.0 (s, 3 H, Ac), 1.92 (s, 3 H, Ac), 1.89 (s, 3 H, Ac).

Anal. Calcd for $C_{43}H_{54}O_{23}N_2$: C, 53.42; H, 5.63; N, 2.9. Found; C, 53.52; H, 5.81; N, 3.01.

Lactone Chloride (5). A solution of **4** (9 mg, 9.3 μmol) in 1:1 dichloromethane-acetyl chloride (1 mL) was cooled to 5 °C and water (25 μL) was added. The mixture was stirred at room temperature for 18 h, then diluted with dichloromethane, treated with ice-water and the layers separated. The organic layer was washed with water, saturated aqueous sodium bicarbonate, dried (sodium sulfate), filtered and the solvents were removed to give 8 mg of **5** as a white solid after lyophilization from benzene: ^{1}H NMR δ7.4 (m, 5 H, aromatic), 5.6 (d, 1 H, NH), 5.22-5.48 (m, 7 H), 5.15 (ddd, 1 H, J = 8.0, 5.0 and 3.0 Hz), 4.55 (dd, 1 H, J = 12.0 and 10.5 Hz), 4.48 (dd, 1 H, J = 10.5 and 2.0 Hz), 4.36 (dd, 1 H, J =12.0 and 3.0 Hz), 4.3 (dd, 1 H, J =12.5 and 2.75 Hz), 4.15-4.28 (m, 3 H), 4.02 (dd, 1 H, J =12.5 and 5.0 Hz), 3.79 (dd, 1 H, J =10.5 and 2.0 Hz), 2.82 (dd, 1 H, J =13.5 and

4.5 Hz), 2.36 (dd, 1H,J =13.5 and 5.5 Hz), 2.3 (dd,1H, $J_{3a,3e}$ =13.5 Hz, $J_{3a,4}$ =11.0 Hz, H-3a), 2.18 (s, 3 H, Ac), 2.15 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 1.96 (s, 3 H, Ac), 1.90 (s, 3 H, Ac).

Benzyl 5-Acetamido-2,4,7,9-tetra-O-acetyl-8-0-(benzyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate (7). A suspension of **2** (63 mg, 0.1 mmol) and acetic anhydride (2 mL) was cooled to -5 °C and a solution of p-toluenesulfonic acid monohydrate (47 mg, 0.247 mmol) in acetic anhydride (1 mL) was added. The reaction was stirred at this temperature for 48 h after which time pyridine (7 mL) was added and stirring was continued at -5 °C for an additional 18 h. The solvents were removed and the residue was coevaporated with toluene. Flash chromatography over silica gel (10 g) using 95:15:5 ethyl acetate-methanol-water gave 104 mg of crude material. This material was dissolved in *N,N*-dimethylformamide (2 mL) containing anhydrous potassium flouride (35 mg, 0.6 mmol) and benzyl bromide (137 mg, 0.8 mmol) and the mixture was stirred at room temperature for 48 h. The solvents were removed and the residue was then taken up in chloroform, washed with water, dried (sodium sulfate), filtered and the solvents were removed. Column chromatography over silica gel (6.5 g) using chloroform then 75:1 chloroform-methanol gave; first 3 mg of material (possibly **6**), followed by 46 mg of β-acetate **7** and finally 13 mg of lactone **5** (59% total recovery). The β-acetate **7** was obtained as a white solid after lyophilization from benzene: ^{1}H NMR δ 7.38 (m, 10 H, aromatic), 6.08 (d, 1 H, J =9.5 Hz, NH), 5.42 (ddd, 1 H, $J_{8',7'}$ =9.5 Hz, $J_{8',9'}$ =5.5 Hz, J =2.5 Hz, H-8'), 5.35 (m, 1 H, H-4), 5.33 (d, 1 H, J =12.0 Hz, $CH_2C_6H_5$), 5.28 (t, 1 H, $J_{7,6}$ =$J_{7,8}$ =2.5 Hz, H-7), 5.27 (dd,1H, $J_{7',8'}$ =?, $J_{7',6'}$ =2.0 Hz, H-7'), 5.22 (d, 1 H, J =12.0 Hz, $CH_2C_6H_5$), 5.19 (d, 1 H, J =12.0 Hz, $CH_2C_6H_5$), 5.11 (d, 1 H, J =12.0 Hz, $CH_2C_6H_5$), 5.01 (d, 1 H, $J_{NH',H5}$ =10.0 Hz, NH'), 4.85 (ddd, 1 H, $J_{4,3a}$ =13.0 Hz, $J_{4,5}$ =10.5 Hz and $J_{4,3e}$ =4.5 Hz,H-4'), 4.69 (dt, 1 H, $J_{8,9}$ = 9.5 Hz, $J_{8,9}$ = $J_{8,7}$ =2.5 Hz, H-8), 4.58 (dd, 1 H, $J_{9,9}$ = 12.5 Hz, $J_{9,8}$ =2.5 Hz, H-9), 4.23 (dd, 1 H, $J_{9',9'}$ =12.5 Hz, $J_{9',8'}$ =2.5 Hz, H-9'), 4.18 (dd, 1 H, $J_{6,5}$ =10.5 Hz, $J_{6,7}$ =2.0 Hz, H-6), 4.12 (dd, 1 H, $J_{9,9}$ =12.5 Hz, $J_{9,8}$ =9.0 Hz, H-9), 4.08 (dd, 1 H, $J_{9',9'}$ =12.5 Hz, $J_{9',8'}$ =5.5 Hz, H-9'), 4.02 (q, 1 H, J =10.5 Hz, H-5') 3.95 (q, 1 H, J =10.5 Hz, H-5), 3.66 (dd, 1 H, $J_{6',5'}$ =10.5 Hz, $J_{6',7'}$ =2.5 Hz, H-6'), 2.67 (d, 1 H, $J_{3'e,3'a}$ =13.0 Hz, $J_{3'e,4'}$ =4.5 Hz, H-3'e), 2.53 (dd, 1 H, $J_{3e,3a}$ =13.0 Hz, $J_{3e,4}$ =4.5 Hz, H-3e), 1.98 (t, 1 H, J =13.0 Hz, H-3a), 1.79 (t, 1 H, J =13.0 Hz, H-3'a), 2.19 (s, 3 H, Ac), 2.18 (s, 3 H, Ac), 2.13, (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.06 (s, 3

H, Ac), 2.05 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 2.0 (s, 3 H, Ac), 1.97 (s, 3 H, Ac), 1.87 (s, 3 H, Ac).

Anal. Calcd for $C_{52}H_{64}O_{25}N_2$: C, 55.91; H, 5.77; N, 2.51. Found: C, 56.15; H, 5.95; N, 2.51.

Benzyl 5-Acetamido-4,7,9-tri-O-acetyl-8-O-(benzyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-2-chloro-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosonate (8). Compound 7 (10 mg, 8.95 mmol) was dissolved in 1:1 dichloromethane-acetyl chloride (1 mL). The mixture was cooled to 5 °C, water (25 μL) added and the mixture was then stirred at room temperature for 18 h. After this time, the reaction mixture was diluted with chloroform (5 mL), cooled to 5 °C and ice added portionwise until the bubbling subsided. The layers were separated and the organic layer was washed with water, saturated aqueous sodium bicarbonate, water, dried (sodium sulfate), filtered and the solvents were removed to give 10 mg of 8 after freeze-drying from benzene: ^{1}H NMR δ 7.4 (m, 10 H, aromatic), 6.6 (d, 1 H, $J_{NH,H5}$ =10.5 Hz, NH), 5.41 (ddd, 1 H, $J_{4,3a}$ =11.0 Hz, $J_{4,5}$ =10.5 Hz, $J_{4,3e}$ =4.5 Hz, H-4), 5.38 (t, 1 H, J =2.0 Hz, H-7), 5.33 (m, 1 H, H-8'), 5.33 (d, 1 H, J =12.0 Hz, $CH_2C_6H_5$), 5.3 (d,1H, J =12.0 Hz, $CH_2C_6H_5$), 5.28 (dd,1H, $J_{7',6'}$ =?, $J_{7',8'}$ =2.0 Hz, H-7'), 5.27 (d, 1 H, J = 12.0 Hz, $CH_2C_6H_5$) 5.18 (d, 1 H, J =12.0 Hz, $CH_2C_6H_5$), 5.02 (d, 1 H, $J_{NH',5'}$ =10.0 Hz, NH'), 4.87 (ddd, 1 H, $J_{4',3a}$ =12.5 Hz, $J_{4',5'}$ =10.5 Hz, $J_{4',3e'}$ =4.5 Hz, H-4'), 4.65 (dt, 1 H, $J_{8,9}$ =8.0 Hz, $J_{8,9}$ =2.0 Hz, H-8), 4.5 (dd, 1 H, $J_{6,5}$ =10.5 Hz, $J_{6,7}$ =2.0 Hz, H-6), 4.45 (dd, 1 H, J =12.5 and 2.5 Hz, H-9 or H-9'), 4.36 (dd, 1 H, J =12.5 and 2.5 Hz, H-9 or H-9'), 4.13 (q, 1 H, J =10.5 Hz, H-5 or H-5'), 4.1 (m, 2 H, H-9 and H-9'), 4.05 (q, 1 H, J =10.5 Hz, H-5 or H-5'), 3.8 (dd, 1 H, $J_{6',5'}$ =10.5 Hz, $J_{6',7'}$ =2.0 Hz, H-6'), 2.85 (dd, 1 H, $J_{3e,3a}$ =13.5 Hz, $J_{3e,4}$ =4.5 Hz, H-3e), 2.68 (dd, 1 H, $J_{3'e,3'a}$ =12.5 Hz, $J_{3'e,4'}$ =4.5 Hz, H-3'e), 2.22 (dd, 1 H, $J_{3a,3e}$ =13.5 Hz, $J_{3a,4}$ =11.0 Hz, H-3a), 2.15 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 1.97 (s, 6 H, Ac), 1.95 (s, 3 H, Ac), 1.87 (s, 3 H, Ac). Position of H-3'a was obscured by acetate signals.

Methyl Benzyl 5-acetamido-4,7,9-tri-O-acetyl-8-O-(benzyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (9) and β-OCH_3 isomer (10). A solution of methanol(13mg,0.41mmol),Drierite® (100 mg), 1,1,3,3-tetramethylurea (19 mg, 0.16 mmol) and dry tetrahydrofuran (400 μL) was stirred at room temperature for 2

h. To the mixture was added chloride **8** (40 mg, 0.037 mmol) and the mixture was cooled to -30 °C. A solution of silver perchlorate (15 mg, 0.073 mmol) in 2:1 tetrahydrofuran-toluene (300 μL) was added dropwise and the reaction was stirred at this temperature for 15 min then slowly warmed to 0 °C and stirred overnight. The reaction mixture was diluted with chloroform, filtered through Celite®, then washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), filtered and the solvents were removed. Column chromatography over silica gel (10 g) using first chloroform, then 100:1 chloroform-methanol gave 22 mg (55%) of **9** followed by 8 mg (20%) of **10**. Compound **9** was obtained as a white solid after lyophilization from benzene: ^{1}H NMR δ 7.4 (m, 10 H, aromatic), 6.42 (d, 1 H, $J_{NH,5}$ =9.5 Hz, NH), 5.48 (dd, 1 H, J = 9.5, 5.0 and 3.0 Hz), 5.33 (d, 1 H, J =12.5 Hz, $CH_2C_6H_5$), 5.3 (m, 2 H), 5.28 (s, 2 H, $CH_2C_6H_5$), 5.18 (d, 1 H, J =12.5 Hz, $CH_2C_6H_5$), 5.12 (d, 1 H, $J_{NH',5'}$ =10.0 Hz, NH'), 4.97 (ddd, 1 H, J =13.0, 10.0 and 4.5 Hz), 4.93 (m, 1 H), 4.84 (ddd, 1 H, J =13.0, 10.5 and 4.5 Hz), 4.61 (dd, 1 H, J =12.0 and 2.5 Hz), 4.24 (m, 3 H), 4.0-4.15 (m, 3 H), 3.84 (dd, 1 H, J =10.5 and 2.0 Hz), 3.4 (s, 3 H, OCH_3) , 2.71 (dd, 1 H, J =13.0 and 4.5 Hz), 2.70 (dd, 1 H, J =13.0 and 4.5 Hz), 2.18 (s, 3 H, Ac), 2.15 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 2.025 (s, 3 H, Ac), 2.015 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 1.9 (s, 3 H, Ac), 1.92 (t, 1 H, J =13.0 Hz), 1.89 (t, 1 H, J =13.0 Hz). Similarily, lyophilization of **10** from benzene gave a white solid: ^{1}H NMR δ 7.4 (m, 10 H, aromatic), 6.18 (bd, 1 H, J =9.5 Hz), 5.37 (ddd, 1 H, J =9.5, 6.0 and 2.5 Hz), 5.35-5.25 (m, 5 H), 5.22 (d, 1 H, J =12.5 Hz, $CH_2C_6H_5$), 5.15 (d, 1 H, J =12.5 Hz, $CH_2C_6H_5$), 5.1 (d, 1 H, J =10.0 Hz), 4.9 (dd, 1 H, J =12.0 and 2.5 Hz), 4.82 (m, 2 H), 4.26 (dd, 1 H, J =12.5 and 2.5 Hz), 4.05 (m, 4 H), 3.72 (dd, 1 H, J =10.5 and 1.5 Hz), 3.48 (s, 3 H, OCH_3), 2.7 (dd, 1 H, J =13.0 and 4.5 Hz), 2.47 (dd, 1 H, J =13.0 and 5.0 Hz), 2.19 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.01 (s, 6 H, Ac), 1.9 (s, 3 H, Ac), 1.87 (s, 3 H, Ac), 1.84 (t, 1 H, J =13.0 Hz), 1.79 (t, 1 H, J =13.0 Hz).

Methyl 5-Acetamido-8-O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-3,5-dideoxy-D-glycero-α- D - galacto-2-nonulpyranosylonic acid (11). A mixture of **9** (18 mg) in 3:1 toluene-methanol (4 mL) and 5% palladium-on-carbon (50 mg, 55% moisture content) was hydrogenated at room temperature, 1 atm for 2 h. The mixture was filtered through Celite® and the solvents were removed. The resulting material was dissolved in methanol (3 mL) containing sodium metal (2.5 mg) and the mixture was

stirred at room temperature overnight. The reaction was neutralized with acetic acid and the solvents were removed. Column chromatography over silica gel (5 g) using 8:1:1 2-propanol-ammonia-water gave 11 mg of **11**, presumably as the di-ammonium salt: ^{1}H NMR (D_2O, HOD) δ 4.2 (m, 1 H), 4.18 (dd, 1 H, J =12.0 and 3.5 Hz), 3.88-3.98 (m, 6 H), 3.5-3.75 (m, 6 H), 3.25 (s, 3 H, OCH_3), 2.78 (dd, 1 H, J =12.0 and 4.5 Hz), 2.61 (dd, 1 H, J =12.5 and 4.5 Hz), 2.17 (s, 3 H, Ac), 2.12 (s, 3 H, Ac), 1.73 (t, 1 H, J =12.0 Hz), 1.58 (t, 1 H, J =12.5 Hz).

Methyl 5-Acetamido-8-O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-3,5-dideoxy-D-glycero-β- D - galacto-2-nonulopyranosylonic acid (12). A mixture of **10** (6 mg) in 1:1 ethyl acetate-methanol (1mL), and 5% palladium-on-carbon (30 mg, 55% moisture content), was hydrogenated at 1 atm, and room temperature for 1 h. The reaction mixture was then filtered through Celite® and the solvents were removed. The residue was dissolved in methanol (1mL) containing sodium metal (1 mg) and the reaction was stirred at room temperature for 2 h. The reaction mixture was then neutralized with acetic acid and the solvents were removed. Column chromatography over silica gel (1.5 g) using 8:1:1 2-propanol-ammonia-water as eluant gave 2 mg of **12**, presumably as the di-ammonium salt: ^{1}H NMR (D_2O, HOD) δ 4.17 (m, 1 H), 4.07 (dd, 1 H, J =12.0 and 2.5 Hz), 3.95 (ddd, 1 H, J =13.0, 10.5 and 5.0 Hz), 3.8-3.86 (m, 5 H), 3.55-3.75 (m, 6 H), 3.2 (s, 3 H, OCH_3), 2.76 (dd, 1 H, J =12.5 and 4.5 Hz), 2.30 (dd, 1 H, J =13.0 and 4.75 Hz), 2.05 (s, 3 H, Ac), 2.0 (s, 3 H, Ac).

Acknowledgements:

This work was funded in part by the National Research Council of Canada under the IRAP program. We thank G. Bigam, T. Brisbane, L. Kong and Dr. T. T. Nakashima, Department of Chemistry, Unviersity of Alberta, for their valuable NMR spectral services, and Shauna Attewell for preparation of the manuscript.

References and Footnotes

1. R. Schauer, Sialic Acids, *Chemistry, Metabolism and Function, Cell Biology Monographs Vol.* **10**, Springer, Wien, 1982.

2. A. K. Bhattachargee, H. J. Jennings, C. P. Kenny, A. Martin, and I. C. P. Smith, *J. Biol. Chem.*, **250**, 1926 (1975); H. J. Jennings, E. Katzenellenbogen, C. Lugowski, F. Michon, R. Roy, and D. L. Casper, *Pure and Appl. Chem.*, **56**, 893 (1984).

3. J. Finne, *Trends Biochem. Sci.*, **10**, 129 (1985).

4. L. D. Cahn, R. F. Irie, R. Singh, A. Cassidenti, and J. C. Paulson, *Proc. Natl. Acad. Sci. USA*, **79**, 7629 (1982).

5. K. Okamoto, , T. Kondo, and T. Goto, *Tetrahedron. Lett.*, **27**, 5229 (1986); K. Okamoto, T. Kondo, and T. Goto, *Tetrahedron*, **44**, 1291 (1988).

6. R. Kuhn, P. Lutz, and D. L. MacDonald, *Chem. Ber.*, **99**, 611 (1966).

7. H. J. Jennings, R. Roy, and F. Michon, *J. Immunol.*, **134**, 2561 (1985); H. Nomoto, M. Iwasaki, T. Endo, S. Inoue, Y. Inoue, and G. Matsumura, *Arch. Biochem. Biophys.*, **218**, 335 (1982).

8. R. Roy, and R. A. Pon, *Glycocojugate J.*, **7**, 3 (1990).

9. M. R. Lifely, A. S. Gilbert and C. Moreno, *Carbohydr. Res.*, **134**, 229 (1984).

10. D. Acquotti, G. Fronza, L. Riboni, S. Sonnino, and G. Tettamanti, *Glycoconjugate J.*, **4**, 119 (1987).

11. S. Z.Abbas, J. Diakur, S. Sugiyama, and D. Fleming in *Sialic Acids, 1988, Proceedings Japanese-German Symposium on Sialic Acids*, R. Schauer and T. Yamakawa, Eds.; Kieler Verlag Wissenschaft and Bildung, Kiel, (1988), p 20; S. Z. Abbas, S. Sugiyama, J. Diakur, and P. Rudnew, *ibid.*, p 22.

12. H. Paulsen and U. von Deessen, *Carbohydr. Res.*, **146**, 147 (1986); Y. Itoh and T. Ogawa, *Tetrahedron Lett.*, **28**, 6221 (1987); T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, **188**, 71 (1989) and ref. cited therein.

13. H. Friebolin, M. Supp, R. Brossmer, G. Keilich, and D. Ziegler, *Angew. Chem. Int. Ed. Engl.*, **19**, 208 (1980).

14. U. Dabrowski, H. Friebolin, R. Brossmer, and M. Supp, *Tetrahedron Lett.*, **48**, 4637 (1979).

15. F. Michon, J. R. Brisson, and H. J. Jennings, *Biochemistry.*, **26**, 8399 (1987).