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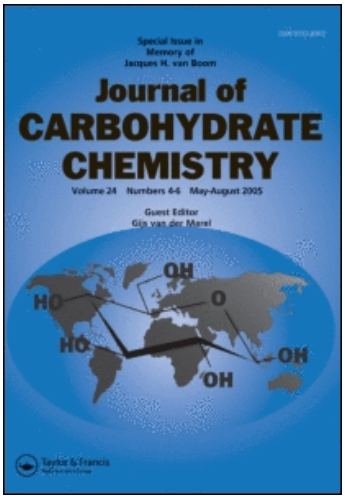

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
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A Novel Approach to N-Acetyl-neuraminic Acid-Containing Oligosaccharides. Synthesis of a Glycosyl Donor Derivative of α-N-Acetyl-D-neuraminyl- (2-6) -D-galactose

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To cite this Article Pozsgay, Vince , Jennings, Harold J. and Kasper, Dennis L.(1987) 'A Novel Approach to N-Acetyl-neuraminic Acid-Containing Oligosaccharides. Synthesis of a Glycosyl Donor Derivative of α-N-Acetyl-D-neuraminyl-(2-6) -D-galactose', Journal of Carbohydrate Chemistry, 6: 1, 41 — 55
To link to this Article: DOI: 10.1080/07328308708058859
URL: http://dx.doi.org/10.1080/07328308708058859

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J. CARBOHYDRATE CHEMISTRY, 6(1), 41-55 (1987)

A NOVEL APPROACH TO N-ACETYL-NEURAMINIC ACID-CONTAINING OLIGOSACCHARIDES. SYNTHESIS OF A GLYCOSYL DONOR DERIVATIVE OF α-N-ACETYL-D-NEURAMINYL-(2→6)-D-GALACTOSE*

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Received August 25, 1986 - Final Form December 1 1986

ABSTRACT

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-(2→6)-(3,4-di-O-benzyl-2-O-(4-nitrobenzoyl)-α-D-galactopyranosyl bromide was synthesized and used as a disaccharide glycosyl donor in the synthesis of sialylactose α-D-Neup5Ac-(2→6)-β-D-Galp-(1→4)-D-Glc.

INTRODUCTION

N-Acetyl-α-D-neuraminic acid (Neu5Ac) is a frequent, terminal glycose unit in the carbohydrate portion of a variety of mammalian glycoconjugates, such as glycoproteins and glycolipids[1] and in several bacterial, capsular polysaccharides[2], and is involved in a number of biological phenomena.

In spite of their enormous biological significance, there are few publications available on the chemical synthesis of α-D-Neup-5Ac-containing oligosaccharides[3-7]. Recently, enzymatic

*Presented at the 13th International Carbohydrate Symposium, Ithaca, New York, USA, August 10-15, 1986.

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synthesis has been suggested[8], as an alternative approach to the chemical one. To date, the chemical synthesis of only three trisaccharides[4,5,7] and one tetrasaccharide[4] have been reported which incorporate terminal, α-linked Neup5Ac. The syntheses of these trisaccharides were achieved by coupling the acetochloro methyl ester derivative (1)[5] of Neup5Ac under controlled conditions to a suitably protected disaccharide aglycon. In this way both α-(1→6)-[4,5] and α-(2→3)-[7] linkages could be reconstructed. However, the product mixture contained an equimolar amount of the unnatural, β-linked isomer, making the isolation of the required isomer tedious, with yields ranging from 25% for the α-(2→6)-[5] to 6% for the α-(2→3)-[7] isomer. This approach is obviously less practical in case of larger oligosaccharides.

To overcome the difficulties associated with the stereoselective introduction of a Neup5Ac-derived glycosyl donor into an oligosaccharide "aglycon", the availability of a disaccharide glycosyl donor, incorporating Neup5Ac in the natural, α glycosidic linkage is clearly desirable. In the Neup5Ac-containing polysaccharides the penultimate, non-sialic acid carbohydrate moiety is frequently D-galactose, to which Neup5Ac is α-(2→6)-linked. In the present paper we describe the synthesis of an α-D-Neup5Ac-(2→6)-D-Galp glycosyl donor and demonstrate its utilization in the synthesis of α-D-Neup5Ac-(2→6)-β-D-Galp-(1→4)-D-Glc, which has been isolated from human, bovine and ovine milk and colostrum[1b].

RESULTS AND DISCUSSION

Our synthetic strategy was to couple 1 to HO-6 of a D-galactopyranose derivative, having a substituent at C-1 which is either a good leaving group in the subsequent glycosylation or can be converted to such a group. Also, HO-2 of this galactose moiety had to be substituted with either a "participating" group or one which can be converted to it with minimal chemical manipulation, whereas HO-3 and -4 had to be protected by "persistent" protecting groups which would also impart increased reactivity to HO-6. A

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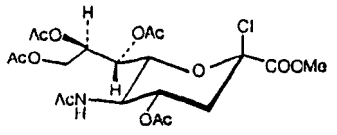
further requirement was high solubility in the solvent of glycosylation. Based on the highly successful use[3] of benzyl 2,3,4-tri-O-benzyl-β-D-galactopyranoside in glycosylation reaction with 1, 4-methoxybenzyl 3,4-di-O-benzyl-2-O-(4-methoxybenzyl)-β-D-galactopyranoside (11) has been selected. Compound 2 was prepared from acetobromo-galactose and 4-methoxybenzyl alcohol, under Helferich conditions[9], followed by Zemplén-deacetylation. The structure of 2 was assigned by 500 MHz 1-H NMR and 50 MHz 13-C NMR spectroscopy. 2 was converted to the mixed acetal 3 with an excess of 2,2-dimethoxypropane, in the presence of a catalytic amount of p-toluenesulfonic acid, in 63% yield after column chromatography. In this reaction also the 3,4-O (5, 19.0%) and 4,6-O-isopropylidene derivative (8, 5.0%) was formed. A similar mixed acetalization reaction has been described first by Lipták *et al.*[10], the difference in the product ratios reported[10] and obtained by us is easily explained by the higher molar ratio of 2,2-dimethoxypropane used by us. 3 was converted to 4 with 4-methoxybenzyl chloride/NaH in N,N-dimethylformamide. 4 was selectively deblocked to 6 by brief treatment with trifluoroacetic acid. Allylation of 6 gave 7. Treatment of 7 with aqueous HBF_4 in methanol selectively removed the isopropylidine group to give 9. Other methods of de-isopropylidination (e.g. hydrolysis with aqueous solutions of acetic acid or trifluoroacetic acid) were accompanied by extensive loss of the methoxybenzyl functions. Conventional benzylation of 9 followed by de-allylation using tris(triphenylphosphine)rhodium chloride-catalyzed isomerisation[11] followed by hydrolysis with aqueous $HgCl_2$ gave crystalline 11.

Reaction of 1 and 11 in toluene, under catalysis by silver salicylate[3], gave disaccharide 12 in 45% yield. The α-configuration of the interglycosidic linkage is evidenced by the appearance of a one-proton doublet of doublets at 2.611 ppm in the 200 MHz 1-H NMR spectrum, which is characteristic for $H\text{-}3_{eq}$ of α-(2→6)-linked Neup5Ac. Also, the 50 MHz 13-C NMR spectrum of 12 was in agreement with the published[3], 20 MHz 13-C NMR spectrum of



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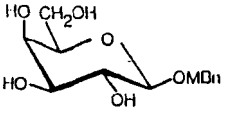

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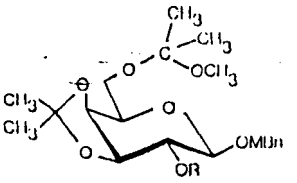
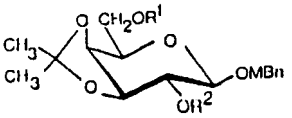
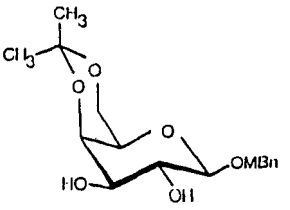


3 R=H
4 R=MBn



5 $R^1=R^2=H$
6 $R^1=H$ $R^2=MBn$
7 $R^1=All$ $R^2=MBn$



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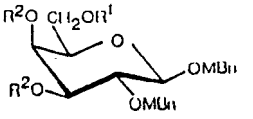
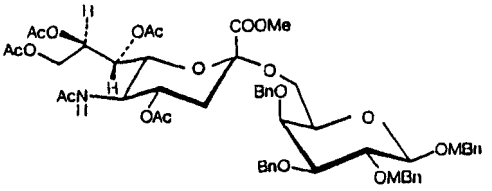


9 $R^1=All$ $R^2=H$
10 $R^1=All$ $R^2=Bn$
11 $R^1=H$ $R^2=Bn$



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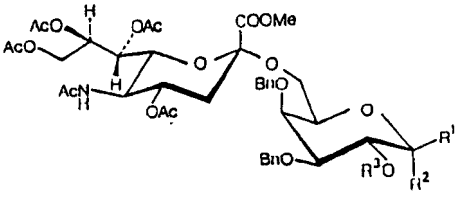
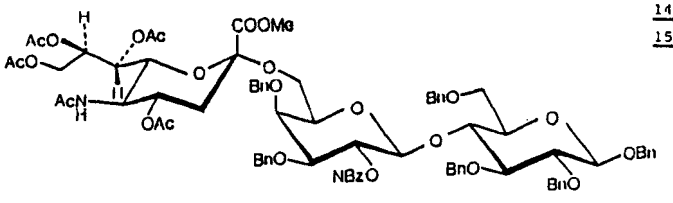


13α $R^1=H$ $R^2=OH$ $R^3=H$
13β $R^1=OH$ $R^2=H$ $R^3=H$
14α $R^1=H$ $R^2=ONBz$ $R^3=NBz$
14β $R^1=ONBz$ $R^2=H$ $R^3=NBz$
15 $R^1=H$ $R^2=Br$ $R^3=NBz$



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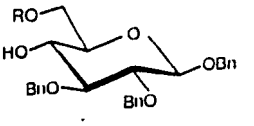
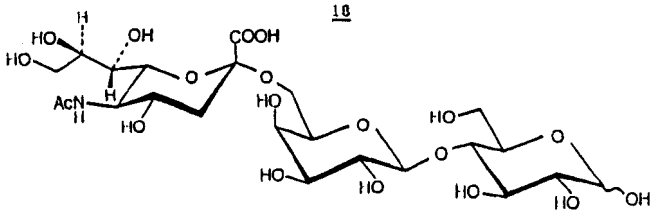


16 R=H
17 R=Bn



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the closely related benzyl 2,3,4-tri-O-benzyl-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranoside, with additional signals for the 4-methoxybenzyl groups. Our assignments agree with those proposed by Vleugel et al.[3], except for the relative assignments of the resonances between 73.44-70.51 p.p.m. Based on DEPT experiments, which unequivocally establish the identities of CH_2 carbons, the resonances at 73.0 and 70.5 p.p.m. are assigned to CH_2Ph carbons. Assignments of the remaining resonances are to be found in the Experimental. Removal of the 4-methoxybenzyl groups by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone[12] gave diol 13 (71%), as a ca. 10:1 mixture of the α and β anomers. A noteworthy feature of the 13-C NMR spectrum of 13 is the dramatic shift of C-3 of the galactose unit from 82.2 p.p.m. in 12 to 79.1 p.p.m. and that of C-5 from 73.4 p.p.m. to 69.0 p.p.m., reflecting 1,3-cis diaxial interactions between HO-1α-H-3 and HO-1α-H-5. Subsequent reaction of 13 with 4-nitrobenzoyl chloride in pyridine gave an anomeric mixture of 4-nitrobenzoates (14), which upon a short treatment with anhydrous hydrogen bromide in dichloromethane gave bromide 15, without any apparent cleavage of the interglycosidic linkage. 15 was reacted without purification immediately after isolation with the D-glucose derivative 17 under silver carbonate catalysis, to give the fully protected trisaccharide 18 in 63.9% yield, based on 14. In the glycosidation reaction, as expected, only the formation of the new, β-interglycosidic linkage was observed. 17 was obtained in 82% yield without chromatography from the easily available 16 by dibutyltin oxide-assisted regioselective benzylation.[13] Treatment of 18 with sodium hydroxide solution removed the methyl ester, O-(4-nitrobenzoyl) and O-acetyl functions. Finally, catalytic hydrogenolysis removed the benzyl protecting groups to give the free, α-(2→6)-linked sialyllactose 19 as a ca. 2:1 mixture of the β and α anomers. The 13-C NMR spectrum of 19 agrees with that published earlier[14].

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EXPERIMENTAL

General procedures. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 243 automatic polarimeter. Thin layer chromatography was made on pre-coated, 0.25 mm silica gel plates (Kieselgel 60 F_{254}, E. Merck), detection was made by spraying with ammonium molybdate-ceric sulfate-sulfuric acid reagent[15], followed by heating at 120°. Preparative medium pressure column chromatography[16] was made on Kieselgel 60 (230-400 mesh, E. Merck) columns. 1-H NMR spectra were recorded with Bruker AM-500 (500 MHz) and AM-200 (200 MHz) spectrometers, at 300°K using internal standard Me_4Si (δ = 0 p.p.m.) or acetone (δ = 2.225 p.p.m.). The assignments for compounds 3, 5 and 8 were corroborated by H,H COSY-90 experiments. 13-C NMR spectra were recorded at 50 MHz with a Bruker AM-200 spectrometer (300° K) (in $CDCl_3$ solutions δ = 77.0 p.p.m. for the central line of $CDCl_3$, in D_2O solutions δ = 31.07 p.p.m. for internal acetone). Assignments were aided by gated-decoupling and DEPT experiments.

4-Methoxybenzyl β-D-galactopyranoside (2). A mixture of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (59 g, 14.4 mM), 4-methoxybenzyl alcohol (20.3 g, 18.4 mL, 16.6 mM), 4 Å molecular sieves (19 g), and dichloromethane (300 mL) was stirred at room temperature for 2 h. Then yellow mercuric oxide (31 g) was added and the mixture was further stirred under ice-water cooling for 1 h. Mercuric bromide (4.5 g) was added, followed by further stirring under ice-water cooling for 1 h. The mixture was then allowed to warm up to 20° and was further stirred for 4 h. The mixture was filtered, the filter cake was washed with dichloromethane (3 x 50 mL). The combined dichloromethane solution was washed with 20% aqueous potassium iodide solution (3 x 100 mL) then with water (100 mL), was then dried (Na_2SO_4) and concentrated. The residual syrup was dissolved in methanol (300 mL) followed by the addition of triethylamine (100 mL) and water (50 mL). After 16 h, the solution was concentrated to give a semi-solid which was recrystallized from ethyl acetate.

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Yield: 24 g. Concentration of the mother liquor gave an additional 6 g. Total yield: 30.0 g (69.6%). Mp 104-106°C, $[\alpha]_D$ - 24.2 (c 7.5, MeOH), 1-H NMR (500 MHz, D_2O): 7.34, 6.88 (2d, 2 x 2H, H-2, H-6 and H-3, H-5 of aromatic ring,), 4.847 (m, 2H $C\underline{H}_2Ph$), 4.284 (d, 1H $J_{1,2}$ = 7.74 Hz, H-1), 3.829 (dd, 1H, $J_{3,4}$ = 3.42 Hz, $J_{4,5}$ = 1.06 Hz, H-4), 3.792 (dd, 1H, $J_{5,6}$ = 7.00 Hz, $J_{6,6}$ = 11.45 Hz, H-6), 3.776 (s 3H, $C\underline{H}_3$) 3.740 (dd, 1H $J_{5,6}$ = 5.30 Hz, H-6'), 3.556 (dd, 1H, $J_{2,3}$ = 9.71 Hz, H-2), 3.492 (ddd, 1H, H-5), 3.444 (dd, 1H, H-3). 13-C NMR (D_2O-acetone, 5:1): 159.6, 131.1, 114.6, (aromatic carbons), 102.5 (C-1), 75.8 (C-5), 73.7 (C-3), 71.6 (C-2), 69.4 (C-4), 61.6 (C-6), 55.9 ($O\underline{C}H_3$).

Anal. calcd. for $C_{14}H_{20}O_7$: C, 55.99; H, 6.71. Found: C, 56.12; H, 6.74.

4-Methoxybenzyl 3,4-O-isopropylidene-6-O-(methoxydimethyl)-methyl-β-D-galactopyranoside (3). A mixture of 2 (10.0 g), 2,2-dimethoxypropane (250 mL) and p-toluenesulfonic acid monohydrate (100 mg) was stirred at room temperature for 3 h. Triethylamine (2 mL) was added and the solution was concentrated. The residual syrup was chromatographed on a silica gel 60 column (70-230 mesh) with 1:1 ethyl acetate-hexane. First was eluted syrupy 3 (8.7 g, 63.4%), $[\alpha]_D$ - 16.0 (c 0.5, $CHCl_3$). 1-H NMR (500 MHz, $CDCl_3$): 7.29, 6.86 (2d, 2 x 2H, H-2, H-6 and H-3, H-5 of aromatic ring,), 4.850, 4.562 (2d, 2 x 1H $C\underline{H}_2Ph$), 4.207 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.152 (dd, 1H, $J_{3,4}$ = 5.43 Hz, $J_{4,5}$ = 2.07 Hz, H-4), 4.029 (dd, 1H, $J_{2,3}$ = 7.39 Hz, H-3), 3.835 (m, 1H, $J_{5,6}$ = $J_{5,6}$ = 6.07 Hz, H-5), 3.805 (s, 3H, $O\underline{C}H_3$), 3.736 (m, 2H, H-6,6'), 3.259 (s, 3H, $O\underline{C}H_3$), 1.517, 1.396, 1.384, 1.332 (4s, 4 x 3H, $2C(C\underline{H}_3)_2$). 13-C NMR ($CDCl_3$): 159.3, 130.0, 113.7 (aromatic carbons), 109.9 [$\underline{C}(CH_3)_2$], 100.4 (C-1), 100.0 ($\underline{C}(CH_3)_2OCH_3$), 78.7 (C-3), 73.7, 73.5 (C-2,5), 72.4 (C-4), 70.1 ($\underline{C}H_2Ph$), 60.3 (C-6), 55.1 ($\underline{C}H_3OPh$), 48.4 ($O\underline{C}H_3$), 28.0, 26.1, 24.3(2 x C) ($2(\underline{C}H_3)_2C$). Eluted was next 5 (2.15 g, 19.0%), mp 139-140° $[\alpha]_D$ - 6.3 (c 0.6 $CHCl_3$). 1-H NMR (500 MHz, $CDCl_3$): 7.28, 6.88 (2d, 2 x 2H, H-2, H-6 and H-3, H-5 of aromatic ring), 4.85, 4.58 (2d, 2 x 1H, $C\underline{H}_2Ph$), 4.259 (d, 1H, $J_{1,2}$ = 8.32 Hz, H-1), 4.145 (dd, 1H, $J_{3,4}$ = 5.53 Hz, $J_{4,5}$ = 2.09 Hz, H-4),

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4.075 (dd, 1H, $J_{2,3}$ = 7.30 Hz, H-3), 4.00 (m, 1H, H-6), 3.84 (m, 1H, H-6'), 3.83 (m, 1H, H-5), 3.808 (s, 3H, OCH_3), 3.598 (m, 1H, H-2), 1.517, 1.304 (2s, 2 x 3H, $C(CH_3)_2$). 13-C NMR ($CDCl_3$): 159.0, 129.9, 113.9 (aromatic carbons), 110.4 ($C(CH_3)_2$), 100.9 (C-1), 78.8 (C-3), 73.8, 73.5 (C-4,5), 73.5 (C-2), 70.8 (CH_2Ph), 62.3 (C-6), 55.2 (OCH_3), 28.0, 26.2 ($C(CH_3)_2$).

Anal. calcd. for $C_{17}H_{24}O_7$: C, 59.98; H, 7.10. Found: C, 59.86; H, 7.26.

Subsequently 8 was eluted (0.57 g, 5.0%), mp 120-121°, $[\alpha]_D$ - 53.0 (c 1, $CHCl_3$). 1-H NMR (500 MHz, $CDCl_3$): 7.30, 6.88 (2d, 2 x 2H, H-2, H-6 and H-3, H-5 of aromatic ring), 4.90, 4.52 (2d, 2 x 1H, CH_2Ph), 4.262 (d, 1H, $J_{1,2}$ = 7.63 Hz, H-1), 4.140 (m, 1H, $J_{4,5}$ ~ 3.2 Hz, H-4), 4.090 (dd, 1H, $J_{5,6}$ = 5.6 Hz, $J_{6,6}$ = 12.8 Hz, H-6), 3.988 (dd, 1H, $J_{5,6}$ = 1.3 Hz, H-6'), 3.799 (s, 3H, OCH_3) 3.729 (d, 1H, $J_{2,3}$ ~ 8 Hz, H-2), 3.56 (m, 1H, H-3), 3.31 (b, 1H, H-5), 1.466 (s, 6H, $C(CH_3)_2$). 13-C NMR ($CDCl_3$): 159.0, 129.6, 113.5 (aromatic carbons), 101.1 (C-1), 98.7, ($C(CH_3)_2$), 72.1 (C-3), 70.9, 67.9, 66.2, (C-2,4,5), 70.2 (CH_2Ph), 62.3 (C-6), 54.9 (OCH_3), 28.9, 18.4 $C(CH_3)_2$).

Anal. calcd. for $C_{17}H_{24}O_7$: C, 59.98; H, 7.10. Found: C, 59.55; H, 7.20.

4-Methoxybenzyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)--β-D-galactopyranoside (6). To a stirred solution of 3 (8.2 g) in anhydrous N,N-dimethylformamide (50 mL) NaH (4 g) was added in small portions. The mixture was stirred for 1 h at room temperature, was then cooled to ~4°C and 4-methoxybenzyl chloride (3.0 mL) was added dropwise. The mixture was stirred overnight at room temperature. Anhydrous methanol (20 mL) was added dropwise, under stirring, followed by water (100 mL). The solution was extracted with chloroform (150 mL), the chloroform layer was washed with water (2 x 50 mL), was dried (Na_2SO_4) and concentrated under reduced pressure. A small portion of the syrupy residue (4) was purified by column chromatography with 1:2 ethyl acetate-hexane. $[\alpha]_D$ + 6.6 (c 1, $CHCl_3$). 13-C NMR ($CDCl_3$): 159.2, 159.0, 129.7, 113.7, 113.5 (aromatic carbons), 109.7 ($C(CH_3)_2$), 101.2 (C-1) 100.0

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($\underline{C}(CH_3)_2OCH_3$), 79.4, 79.1 (C-2,3), 73.8, (C-5), 72.1 (C-4), 73.2, 70.2 (2 $\underline{C}H_2Ph$), 60.3 (C-6), 55.2 ($\underline{C}H_3OPh$), 48.4 ($O\underline{C}H_3$), 27.8, 26.2, 24.3(2 x C) ($2C(\underline{C}H_3)_2$). The syrup was dissolved in CH_2Cl_2 (150 mL), the solution was cooled to ~10°C, was then shaken with ice-cold 5% CF_3COOH (20 mL) until TLC (3:1 ethyl acetate-hexane) showed complete conversion. The dichloromethane layer was washed with water (3 x 50 mL), was dried (Na_2SO_4) and concentrated. The residue was chromatographed (1:1 ethyl acetate-hexane) to give 6 as a yellow syrup. Yield: 8.5 g (92.9% from 3). $[\alpha]_D$ + 35.5 (c 0.8, $CHCl_3$). 13-C NMR ($CDCl_3$): 159.3, 159.1, 130.3, 129.7, 129.5, 113.7, 113.6 (aromatic carbons), 110.0 ($\underline{C}(CH_3)_2$), 101.7 (C-1), 79.2 (C-2,3), 73.9 (C-5), 73.0 (C-4), 73.2, 70.7 (2 $\underline{C}H_2Ph$), 62.4 (C-6), 55.2 ($O\underline{C}H_3$), 27.7, 26.3 ($C(\underline{C}H_3)_2$).

4-Methoxybenzyl 6-O-allyl-2-O-(4-methoxybenzyl)-β-D-galactopyranoside (9). To a solution of 6 (7.2 g) in anhydrous N,N-dimethylformamide (50 mL) NaH (4g) was added in small portions, under stirring. The mixture was further stirred for one hour at room temperature, was then cooled with ice-water followed by dropwise addition of allyl bromide (5 mL). The mixture was allowed to reach room temperature, was then further stirred for 4 h. Work-up was similar to that of 4. The syrupy residue was purified by chromatography (with 2:1 ether-hexane, then with ether) to give syrupy 7 (7.3 g, 93.4%). $[\alpha]_D$ + 15.1 (c 1.5, $CHCl_3$). 13-C NMR ($CDCl_3$): 159.2, 129.7, 113.7, 113.5 (aromatic carbons), 134.4 ($\underline{C}H$= allyl), 116.6 ($\underline{C}H_2$= allyl), 109.8 ($\underline{C}(CH_3)_2$), 101.2 (C-1), 79.2, 79.1 (C-2,3) 73.6 (C-4), 73.0, 72.1 [$\underline{C}H_2Ph$, $\underline{C}H_2$ (allyl)], 72.0 (C-5), 70.2 ($\underline{C}H_2Ph$), 69.2 (C-6), 55.0 ($\underline{C}H_3O$), 27.5, 26.1 ($C(\underline{C}H_3)_2$). 7 (7.1 g) was dissolved in ethanol (100 mL). 50% aqueous HBF_4 (2 mL) was added and the solution was stirred. Disappearance of 7 (monitored by TLC with 1:1 ether-hexane), was immediately followed by addition of solid $NaHCO_3$ (~4 g) to neutralize the mixture. The mixture was concentrated under reduced pressure, the residue was partitioned between chloroform (100 mL) and water (50 mL), the organic layer was dried (Na_2SO_4), and concentrated to give 9 as a chromatographically (ether, or 2:1 ether-hexane) homogeneous syrup

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which crystallized on standing. Yield: 6.1 g (93.4%). Mp 70-71°C, $[\alpha]_D$ - 12.7 (c 1.2, $CHCl_3$). 13-C NMR ($CDCl_3$): 159.2, 129.6, 113.8 (aromatic carbons), 134.4 (CH= allyl), 117.2 (CH_2= allyl), 102.2 (C-1), 78.6 (C-2), 74.1, 72.5, 70.6, [2 CH_2Ph, CH_2(allyl)], 73.3 (C-5), 73.1 (C-3), 69.3 (C-6), 68.9 (C-4).

4-Methoxybenzyl 6-O-allyl-3,4-di-O-benzyl-2-O-(4-methoxybenzyl)-β-D-galactopyranoside (10). 9 (4 g) in anhydrous N,N-dimethylformamide (20 mL) was treated with NaH (3 g), followed by addition of benzyl bromide (5 mL). Conditions of reaction and work-up were similar to those for 4. The syrupy crude product was stirred with 1:1 ether-hexane, to give crystalline 10 (3.45 g). Chromatography of the mother liquor with 1:1 ether-hexane gave an additional 1.15 g of 10, total yield 4.6 g (82.6%). Mp 88-89°C (ethanol), $[\alpha]_D$ - 27.5 (c 0.5, $CHCl_3$). 13-C NMR ($CDCl_3$): 159.1, 138.6, 130.9, 129.9, 129.7, 128.3, 128.1, 127.5, 113.7, 113.6 (aromatic carbons), 134.4 (CH= allyl), 117.2 (CH_2= allyl), 102.6 (C-1), 82.3 (C-3), 79.2 (C-2), 73.4 (C-4,5), 74.8, 74.4, 73.0, 72.3, 70.6 [4 CH_2Ph, CH_2 (allyl], 68.8 (C-6), 55.2 (2 CH_3O).

4-Methoxybenzyl 3,4-di-O-benzyl-2-O-(4-methoxybenzyl)-β-D-galactopyranoside (11). 10 (3 g) $(PPh_3)_3RhCl$[11] (200 mg), and diazabicyclo[2.2.2]octane (200 mg) was stirred in 90% aqueous ethanol (100 mL) under reflux. The solution was then cooled to 20°, filtered, concentrated, and the residual syrup was dissolved in acetone (20 mL). Yellow HgO (2 g) and a solution of $HgCl_2$ (1 g) in water (5 mL) was added and the mixture was stirred for 30 min. The mixture was filtered, the filtrate evaporated. A solution of the residue in dichloromethane (50 mL) was extracted with aqueous, 20% KI solution (3 x 10 mL) then water (3 x 10 mL), was dried (Na_2SO_4) and concentrated. The residue crystallized spontaneously. Recrystallization from cyclohexane gave 11 as fine needles. Yield: 2.4 g (86.4%). Mp 95-96°C, $[\alpha]_D$ - 42 (c 0.2, $CHCl_3$). 13-C NMR ($CDCl_3$): 159.2, 138.5, 138.2, 130.8, 129.8-127.6, 113.7, 113.6 (aromatic carbons), 102.8, (C-1), 82.3 (C-3), 79.4 (C-2), 74.9, 74.1, 73.4, 70.9 (4 CH_2Ph), 74.5 (C-5), 72.9 (C-4), 62.0 (C-6), 55.2 (2 OCH_3).

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Anal. calcd. for $C_{36}H_{40}O_8$: C, 71.97; H, 6.71. Found: C, 71.70; H, 6.59.

4- Methoxybenzyl 3,4-di-O-benzyl-2-O-(4-methoxybenzyl)-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranoside (12). 1 (100 mg), 11 (1000 mg), powdered 4 Å molecular sieves (1 g) was stirred in anhydrous toluene (2 mL) under nitrogen for 1 h. Silver salicylate[17] (95 mg) was added and the mixture was stirred in the dark overnight. The mixture was filtered, the filter cake was washed with toluene (5 x 2 mL), the combined solution was concentrated and the residue chromatographed with 100:1 dichloromethane-methanol. 11 (900 mg) was recovered, followed by a mixture containing 12, which upon rechromatography with the above solvent gave 12 (95.4 mg, 45.2%). $[\alpha]_D$ - 15.7 (c 0.5, $CHCl_3$). 1-H NMR (500 MHz, $CDCl_3$): 4.457 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 3.794, 3.782 (2 x 3H, 2 $PhOCH_3$), 3.634 (3H, $COOCH_3$), 2.611 (dd, $J_{3'eq,3'ax}$ = 12.8 Hz, $J_{3'eq,4'}$ = 4.7 Hz, H-3'$_{eq}$), 2.145, 2.115, 2.030, 1.997, 1.877 (5 x 3H, 5 CH_3CO). 13-C NMR ($CDCl_3$): 102.4 (C-1), 98.8 (C-2'), 82.2 (C-3), 79.2 (C-2), 74.7, 74.3, 73.0, 70.5 (4 CH_2Ph), 73.4 (C-5), 72.6 (C-4, C-6'), 69.0, 68.7, 67.4, (C-4', 7', 8'), 62.7 (C-6), 62.3 (C-9'), 55.2(2 x C) (2 $PhOCH_3$), 52.8 ($COOCH_3$), 49.5 (C-5'), 37.8 (C-3'), 23.2 ($NCOCH_3$), 21.0, 20.8 (3 x C) (4 $OCOCH_3$).

Anal. calcd. for $C_{56}H_{67}NO_{20}$: C, 62.62; H, 6.28. Found: C, 62.30; H, 6.32.

3,4-Di-O-benzyl-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl-onate)-α,β-D-galactopyranose (13). A mixture of 12 (370 mg), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (320 mg), dichloromethane (50 mL), and water (5 mL) was stirred for 3 h at room temperature. The mixture was extracted with 5% $NaHCO_3$ (3 x 5 mL), then with water (3 x 5 mL), was concentrated and the residue chromatographed with ethyl acetate. 13 (204 mg, 71%) was obtained as an amorphous solid. 13-C NMR ($CDCl_3$)*: 98.7 (C-2'), 97.3 (C-1β, $J_{C-1,H-1}$ = 159

*α and β indicate the configuration of C-1 of the galactose moiety.

Hz), 92.8 (C-1α, $J_{C-1,H-1}$ = 171 Hz), 82.0 (C-3β), 79.1 (C-3α), 74.5, 72.3 (2 $\underline{C}H_2Ph$), 73.3(2 x C) (C-4,6'), 69.2, 69.0, 68.0 (C-4',7',8'), 69.0 (C-5), 63.4 (C-6α), 62.9 (C-6β), 62.5 (C-9'), 52.8 ($COO\underline{C}H_3$), 49.2 (C-5'), 38.0 (C-3'), 23.1 ($NCO\underline{C}H_3$), 21.0, 20.8(3 x C) (4 $OCO\underline{C}H_3$).

3,4-Di-O-benzyl-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-1,2-di-O-(4-nitrobenzoyl)-α,β-D-galactopyranose (14). 13 (180 mg) in anhydrous pyridine (2 mL) was treated at 0°C with 4-nitrobenzoyl chloride (400 mg) for 16 h. The mixture was poured into ice-water (30 mL) and was extracted with dichloromethane (10 x 5 mL), the extracts were combined, dried (Na_2SO_4), and concentrated. Chromatography of the residue with 5:1 ethyl acetate-hexane gave amorphous 14. Yield: 192 mg (88.4%). 13-C NMR ($CDCl_3$)*: 98.8 (C-2'β), 98.5 (C-2'α), 93.3 (C-1β, $J_{C-1,H-1}$ = 169 Hz), 91.7 (C-1α, $J_{C-1,H-1}$ = 179 Hz), 79.3 (C-3β), 75.1 (C-3α), 73.9 (C-5β), 74.5, 71.6 (2 $\underline{C}H_2Ph$), 67.3 (C-5α), 62.5 (C-6α) 62.1 (C-9'), 60.2 (C-6β), 52.6 ($COO\underline{C}H_3$), 48.8 (C-5'), 37.7 (C-3'β), 37.6 (C-3'α), 27.8 ($NCO\underline{C}H_3$), 20.8, 20.5, 20.3(2 x C), (4 $CO\underline{C}H_3$).

3,4-Di-O-benzyl-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-2-O-(4-nitrobenzoyl)-α-D-galactopyranosyl bromide (15). A solution of 14 (160 mg) in anhydrous dichloromethane (5 mL) was cooled to 0°. Anhydrous hydrogen bromide was passed through the solution at 0° for 3 min. 4-Nitrobenzoic acid was removed by filtration, the solvent was removed by a stream of nitrogen. The residue was dissolved in anhydrous toluene, the solution was filtered, the filtrate was evaporated to give syrupy 15 which was used immediately for the synthesis of 18. 1-H NMR (200 MHz, $CDCl_3$): 6.80 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 5.49 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2), 5.34 (dd, 1H, H-3), 3.75 (s, 3H $COO\underline{C}H_3$), 2.76 (dd, 1H, $J_{3'eq,3'ax}$ = 12.3 Hz, $J_{3'eq,4'}$ = 3.8 Hz, H-3'$_{eq}$), 2.43 (s, 3H,

*α and β indicate the configuration of C-1 of the galactose moiety.

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$NCOCH_3$), 2.17, 2.15, 2.02(2x) (4 x 3H, 4 $OCOCH_3$), 1.87 (t, 1H, $J_{3'ax,4}$ - 12 Hz, H-3ax).

Benzyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (17). A mixture of 16[18] (3.15 g), dibutyltin oxide (1.75 g) and toluene (200 mL) was refluxed for 4 h under stirring, under a Dean-Stark trap. Toluene (~100 mL) was distilled from the mixture, followed by cooling to ~50°C. Tetrabutylammonium bromide (2.35 g) and benzyl bromide (1.4 mL) was added and the mixture was stirred overnight at 50-55°. The cooled solution was washed with water (5 x 30 mL), dried (Na_2SO_4) and concentrated. The residue was triturated with hexane (3 x 20 mL). Addition of seed crystals followed by trituration with hexane (20 mL) gave 17 (2.35 g, 62%). The hexane solutions were combined, concentrated, the residue was kept at -20°C overnight. The semisolid obtained was washed with cold hexane on a filter to give additional 17 (0.75 g, 19.8%). Total yield: 3.1 g (81.8%). Mp 63-65°C, $[\alpha]_D$ - 43.5 (c 0.5, $CHCl_3$). Lit.[19] mp 64-65°, $[\alpha]_D$- 44.2 ($CDCl_3$). 13-C NMR ($CDCl_3$): 102.4 (C-1), 83.9 (C-3), 81.6 (C-2), 75.1, 74.6, 73.5, 71.0 (4 CH_2Ph), 74.1 (C-5), 71.3 (C-4), 70.0 (C-6).

Benzyl 2,3,6-tri-O-benzyl-4-O-[3,4-di-O-benzyl-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonate)-2-O-(4-nitrobenzyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (18). A solution of 15 as obtained above, in dichloromethane (3 mL) was added to a stirred mixture of 17 (385 mg), silver carbonate (250 mg), 4 A molecular sieves (1 g) and dichloromethane (5 mL). After stirring for 4 h, the mixture was filtered, the filtrate concentrated and the residue chromatographed with 2:1 ethyl acetate-hexane. 18 was obtained as a glassy solid. Yield: 136 mg (63.9% from 14). $[\alpha]_D$ + 8.2 (c 0.2, $CHCl_3$). 13-C NMR ($CDCl_3$): 102.3 (C-1), 100.0 (C-1'), 98.9 (C-2"), 82.7, 81.8 (C-3, C-3'), 79.8 (C-2), 77.7 (C-4), 74.9, 74.6, 74.3, 73.3, 71.3, 70.9 (6 CH_2Ph), 73.5 (C-5), 72.7 (C-5'), 68.4 (C-6), 69.0, 68.6, 67.3 (C-4",7",8"), 62.2 (C-6',9"), 52.8 ($COOCH_3$), 49.2 (C-5"), 37.5 (C-3"), 23.1 ($NCOCH_3$), 20.9, 20.7 (3 x C), (4 $OCOCH_3$).

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Anal. calcd. for $C_{80}H_{88}N_2O_{26}$: C, 64.50; H, 5.95. Found: C, 64.24; H, 5.97.

O-(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)-(2→6)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (19). A solution of 18 (63 mg) in methanol (6 mL) was treated with 1 N sodium hydroxide (4 mL) at 40° for 30 min, was then cooled to ~5° and was acidified with 10% acetic acid. Methanol was removed in vacuo, the solution was extracted with chloroform (10 x 5 mL). The extracts were combined, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on Kieselgel 60 (60-230 mesh) with 3:1 ethyl acetate-methanol to give a glassy solid which was hydrogenated in 90% ethanol (5 mL) in the presence of 10% Pd-C (100 mg) at atmospheric pressure and room temperature, for 24 h. Removal of catalyst and solvents followed by Sephadex G-15 chromatography with water gave 19[21] as an amorphous solid after freeze-drying. Yield: 19 mg (71%). $[\alpha]_D$ + 25 (c 0.4, H_2O). Lit.[20] $[\alpha]_D^{25}$ + 27.9 (c 1, H_2O).

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ACKNOWLEDGEMENT

This work was supported in part by a National Institutes of Health grant #AI20626-01.

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21. After completion of this work Professor R. Brossmer informed us about his synthesis of 19 in a different way (XIth International Carbohydrate Symposium, Vancouver, Canada (August, 1982), Abstracts I-68).

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