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SYNTHESIS OF 6-*O*-(5-ACETAMIDO-3,5-DIDEOXY-α-D-*glycero*-D-*galacto*-2-NONULOPYRANOSYLONIC ACID)-D-GALACTOSE [6-*O*-(*N*-ACETYL-α-D-NEURAMINYL)-D-GALACTOSE]

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ABSTRACT

Condensation of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate with benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside, using silver salicylate as promoter, gave 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 (**11**) as the main product in 65% yield. Furthermore, the following by-products were formed: methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-salicyloyl-D-*glycero*-D-*galacto*-2-nonulopyranosonate, methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate, and an impure compound that gave, after *O*-deacetylation and catalytic hydrogenolysis, 6-*O*-(methyl 5-acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-D-galactose. *O*-Deacetylation of **11** gave benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-β-D-galactopyranoside, which was converted into 6-*O*-(methyl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-D-galactose (**13**) by catalytic hydrogenolysis. Saponification of **13** gave the title compound as its potassium salt.

INTRODUCTION

N-Acetyl-D-neuraminic acid (Neu5Ac), which is the most common of the sialic acids, plays a role in several biological processes, in particular when located at the non-reducing ends of the carbohydrate chains of gangliosides and glycoproteins[1–4]. It has been found that Neu5Ac can be linked to various positions of D-galactose[2–5], 2-acetamido-2-deoxy-D-galactose[3,6,7], 2-acetamido-2-deoxy-D-glucose[3,6,8], and sialic acid[3,4]. In all cases investigated so far, only α-glycosidic linkages are involved, which are equatorial in the observed $^2C_5$(D) conformation of the Neu5Ac ring[9,10].

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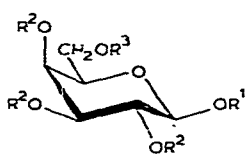
In view of the biological significance of carbohydrate chains containing sialic acids, the chemical synthesis of such structures is important, because it provides pure compounds for structural and biochemical investigations. Our initial concern has been to develop convenient methods for the synthesis of sialodisaccharides with Neu5Ac in non-reducing positions. The synthesis of sialodisaccharides is complicated by the steric effects associated with the ketose character of Neu5Ac and the extreme lability of activated Neu5Ac derivatives towards elimination under current glycosylation conditions.

Some reports on the synthesis of this type of saccharide have appeared. Starting from methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**5**) and partially protected derivatives of D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose, a number of (2→6)-α- and (2→3)-α-linked sialodisaccharides have been synthesised[11] under silver carbonate-promoted, Koenigs–Knorr conditions in moderate yields (8–18%). Similarly, condensation of methyl 4,5,7,8-tetra-*O*-acetyl-2-chloro-2,3-dideoxy-α-D-*manno*-2-octulopyranosonate, for which the structural situation around the anomeric centre is comparable to that of **5**, with methyl 2,3-di-*O*-acetyl-β-D-ribofuranoside gave a low yield of methyl 2,3-di-*O*-acetyl-5-*O*-(methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-D-*manno*-2-octulopyranosylonate)-β-D-ribofuranoside[12]. Condensation of **5** or its free acid **4** with partially protected derivatives of Neu5Ac, D-galactose, and D-glucose, in the presence of a polymer-bound silver salt instead of silver carbonate, afforded[13] sialodisaccharides in yields of 10–40%.

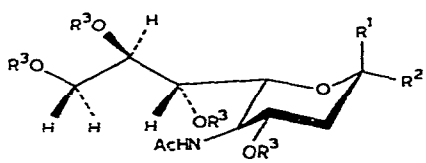
Recently, we have shown that, by use of silver salicylate, **5** can be effectively converted into alkyl α-glycosides of Neu5Ac methyl ester[14]. We now report on the applicability of this approach to the synthesis of 6-*O*-(*N*-acetyl-α-D-neuraminyl)-D-galactose (**14**).

RESULTS AND DISCUSSION

For the synthesis of disaccharide **14**, benzyl 2,3,4-tri-*O*-benzyl-β-D-galacto-



1 R^1 = Bzl, R^2 = R^3 = H
2 R^1 = R^2 = Bzl, R^3 = H



3 R^1, R^2 = OAc, CO_2Me; R^3 = Ac
4 R^1 = Cl, R^2 = CO_2H, R^3 = Ac
5 R^1 = Cl, R^2 = CO_2Me, R^3 = Ac
6 R^1 = CO_2Me, R^2 = $OCHMe_2$, R^3 = Ac
7 R^1, R^2 = CO_2Me, Sal; R^3 = Ac
8 R^1 = CO_2Me, R^2 = OMe, R^3 = H
9 R^1 = OMe, R^2 = CO_2Me, R^3 = H

Sal = salicyloyl

TABLE I

CARBON-13 CHEMICAL SHIFTS (δ)[a] OF **2**, **6**, AND **11**

Carbon	**2**	**11**	**6**[c]
Neu5Ac residue			
C=O (C-1, NAc, OAc)		170.7, 170.4, 170.1, 169.9, 169.7, 167.8	170.7, 170.4, 170.0, 169.9, 169.9, 168.7
C-2		98.6	98.5
C-3		37.7	38.2
C-4		67.3[b]	67.2[b]
C-5		49.2	49.0
C-6		72.6[b]	72.3[b]
C-7		68.9[b]	68.8[b]
C-8		68.9[b]	68.3[b]
C-9		62.2	62.2
CH_3 (CO_2Me)		52.6	52.2
CH_3 (NAc)		23.0	22.8
CH_3 (OAc)		20.9, 20.6 (3×)	20.8, 20.5 (3×)
Galactopyranoside residue			
C-1	102.7	102.5	
C-2	79.3	79.3	
C-3	82.0	82.0	
C-4	70.8	70.6	
C-5	72.9	72.8	
C-6	61.6	62.6	
Ph-CH_2	74.9, 74.4, 74.0, 72.9	75.0, 74.2, 73.3, 72.6	

[a]Assignments were made with the aid of refs. 20, 21, 34–38, and additive increment rules (refs. 39–41). [b]Assignments may be interchanged. [c]Taken from ref. 14.

pyranoside (**2**) and the glycosyl chloride[15] **5** were chosen as synthons. The "aglycon" **2** was selected, because benzyl groups are stable in the glycosylation step and can easily be removed by catalytic hydrogenolysis under mild conditions without rupture of the glycosidic linkage. Compound **2** was prepared from benzyl β-D-galactopyranoside[16] (**1**) in 66% yield as described earlier[17], using a modified detritylation procedure[18].

Condensation of **5** and **2** in benzene in the presence of silver salicylate gave a mixture of five components (t.l.c.). The formation of the side-products methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-salicyloyl-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**7**) and methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (**10**) could be largely suppressed by the presence of high concentrations of **2**, and therefore benzene solutions saturated with **2** were used. After work-up, the reaction mixture was fractionated by column chromatography on silica gel 60 H. The main product was the fully blocked disaccharide **11**, which was obtained in 65% yield. ^{13}C-N.m.r. and 360-MHz, ^{1}H-

TABLE II

^{1}H-N.M.R. DATA FOR **2**, **6**, AND **11** AT 360 MHz IN CHLOROFORM-*d*

Reducing unit

Compound	Chemical shifts[a], δ (and multiplicities)							First-order coupling constants, Hz[a]						
	H-1 (d)	H-2 (dd)	H-3 (dd)	H-4 (dd)	H-5 (m)	H-6 (dd)	H-6′ (dd)	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
2	4.480	3.912	3.525	—[d]	3.357	—[d]	3.48[e]	7.8	9.7	2.7	~1	6.1	6.1	—[d,e]
11	4.498	3.878	3.535	—[d]	—[d]	—[d]	—[d]	7.6	9.7	2.9	—[d]	—[d]	—[d]	—[d]
12[b]	4.468	3.82	3.551	3.899	3.57	3.952	3.480	7.9	9.5	2.4	~1	5.2	7.7	−8.8

Non-reducing unit

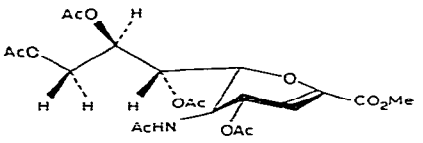
Compound	Chemical shifts[a], δ (and multiplicities)									First-order coupling constants, Hz[a]									
	II-3eq (dd)	H-3ax (dd)	H-4 (m)	II-5 (m)	II-6 (dd)	II-7 (dd)	II-8 (m)	H-9 (dd)	II-9′ (dd)	$J_{3eq,3ax}$	$J_{3eq,4}$	$J_{3ax,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,NH}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$
11	2.614	1.969	4.864	—[d]	4.093	5.317	5.356	4.332	4.083	−12.4	4.7	12.4	9.8	10.0	9.7	2.0	8.4	2.5	6.0	−12.4
12[b]	2.745	1.826	3.64	3.75	3.418	—[d]	—[d]	3.876	—[d]	−12.5	4.7	12.5	10.1	10.1	—	~1.0	—[d]	~2.5	—[d]	−13.1
6[c]	2.554	1.903	4.802	—[d]	4.051	5.290	5.352	4.297	4.091	−12.5	4.6	12.5	10.2	9.8	—[d,f]	2.0	8.2	2.8	5.9	−12.5

Other groups

Compound	Chemical shifts, δ (and multiplicities)				
	Me-ester (s)	NH (d)	OAc, NAc (s)	PhCh$_2$ (m)	Ph (m)
2	—	—	—	4.63–5.02	7.22–7.44
11	3.634	5.484	2.148, 2.113, 2.029, 1.991, 1.877	4.64–4.99	7.21–7.43
12[b]	3.615	—	2.021	4.54–5.03	7.12–7.46
6[c]	3.761	~5.35[f]	2.130, 2.114, 2.018, 2.000, 1.853	—	—

[a]The proton designated H-x′ resonates at higher field than that designated H-x. [b]A few drops of methanol-d_4 were added to the solution. [c]Taken from ref. 14. [d]Not assigned owing to the complexity of the spectrum. [e]H-6′ appears as a broad multiplet without fine structure. [f]Overlapped by H-8 resonances.

n.m.r. spectral data for **11** are presented in Tables I and II, respectively. For comparison, the data for **2** and methyl (isopropyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[14] (**6**) are included. The anomeric purity of **11** was demonstrated by the presence of only one double-doublet (δ 2.614) characteristic for H-3*eq* of the sialic acid moiety[14,19] in the region between δ 2 and 3 of the ^{1}H-n.m.r. spectrum. This was further substantiated by the appearance of only two resonances (δ 102.5, galactose C-1; δ 98.2, Neu5Ac C-2) in the anomeric region of the ^{13}C-n.m.r. spectrum. Comparison of the resonance positions of the galactose C-6 in the ^{13}C-n.m.r. spectra of **2** and **11** showed that sialylation of position 6 of **2** is attended by a 1.0 p.p.m. downfield shift for C-6. This value is smaller than the usually observed sialylation shifts (2–3 p.p.m. downfield) for unblocked sialo-oligosaccharides[20–22]. Owing to the lack of ^{1}H-n.m.r. reference data for solutions in chloroform-*d*, the anomeric configuration of the interglycosidic linkage of **11** cannot easily be derived at this stage.



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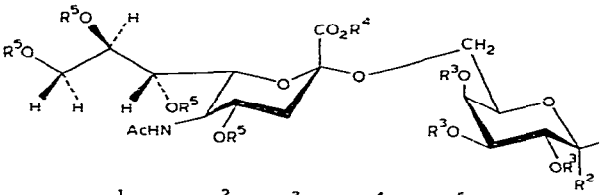


11 R^1 = OBzl, R^2 = H, R^3 = Bzl, R^4 = Me, R^5 = Ac
12 R^1 = OBzl, R^2 = R^5 = H, R^3 = Bzl, R^4 = Me
13 R^1, R^2 = H, OH; R^3 = R^5 = H, R^4 = Me
13a R^1, R^2 = H, $OSiMe_3$; R^3 = R^5 = $SiMe_3$, R^4 = Me
14 R^1, R^2 = H, OH; R^3 = R^5 = H; R^4 = K

O-Deacetylation of **11** afforded crystalline **12** in 85% yield. The ^{13}C-n.m.r. and 360-MHz, ^{1}H-n.m.r. data are summarised in the Experimental and Table II, respectively. The benzyl groups were removed from **12** by palladium-catalysed hydrogenolysis in methanol, to give amorphous **13** in almost quantitative yield. The presence of an α-glycosidic linkage in **13** was indicated by the 360-MHz, ^{1}H-n.m.r. spectrum (D_2O) which showed signals for H-3*eq* and H-4 of Neu5Ac at δ 2.708 and 3.77, respectively (ranges for α-linked Neu5Ac derivatives[23]: H-3*eq*, δ 2.6–2.8; H-4, δ 3.6–3.8). The galactose moiety of **13** occurs almost exclusively in the pyranoid form (H-1α: $J_{1,2}$ 3.6 Hz; H-1β: $J_{1,2}$ 7.9 Hz; αβ-ratio, 3:7). The effect of anomerisation of the galactose residue is also expressed in the doubling of the resonance signal of the Neu5Ac H-3*ax* (~0.007 p.p.m.) without influencing the signal of H-3*eq* (see also ref. 24). Further structural evidence for **13** was obtained by mass spectrometry of its per-*O*-trimethylsilyl (Me_3Si) derivative **13a**. Some significant fragment ions are presented in Table III. The intense peak at *m/z* 726, which is the analogue of the peak at *m/z* 583 in Me_3Si-aldohexosyl-(1→6)-aldohexoses[25], demonstrates the presence of the (2→6)-linkage in **13**.

After saponification of **13** with aqueous potassium hydroxide, the title product

TABLE III

INTERPRETATION OF SOME IMPORTANT FRAGMENT IONS PRESENT IN THE MASS SPECTRA OF THE Me_3Si DERIVATIVES OF THE METHYL ESTERS OF 6-*O*-(*N*-ACETYL-α-D-NEURAMINYL)-D-GALACTOSE (**13a**) AND ITS β ANOMER (**15a**)

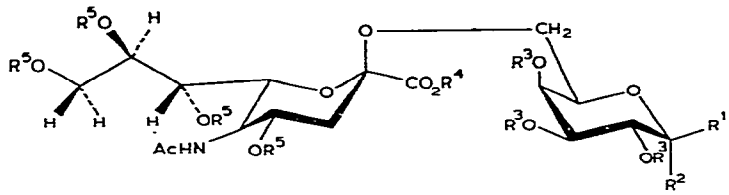
m/z	*Fragment*[a]	*Intensities*[b]	
		13a	**15a**
1061	M	0.8	0.7
1046	M − CH_3	2.9	7.8
1002	M − $COOCH_3$	4.1	2.7
856	M − $CH(OSiMe_3)CH_2OSiMe_3$	6.5	28.3
726	Neu5Ac-$OCH_2CH{=}OSiMe_3$	15.2	12.8
624	Neu5Ac-$OSiMe_3$ − $COOCH_3$	7.6	16.0
594	[Neu5Ac]	4.2	8.3
504	594 − Me_3SiOH	9.0	29.8
451	[Gal]	3.8	10.0
361	451 − Me_3SiOH	3.5	5.5
300	$AcNH{=}CHC(OSiMe_3){=}CHCH{=}CHOSiMe_3$ $AcNH{=}CHCH{=}C(OSiMe_3)CH{=}CHOSiMe_3$	14.9	46.0
298	M − HO-Gal − $CH(OSiMe_3)CH_2OSiMe_3$ − Me_3SiOH	16.7	31.0
217	$Me_3SiOCH{=}CHCH{=}OSiMe_3$	75.3	38.1
204	$Me_3SiO{=}CHCHOSiMe_3$	100.0	100.0
186	$AcNHCH{=}CHCHOSiMe_3$ $AcNH{=}CHC(OSiMe_3){=}CH_2$ $NH_2{=}CHCOCH_2CH{=}CHOSiMe_3$	23.1	20.0
173	$AcNHCHCH{=}OSiMe_3$	4.6	5.3

[a]For a proper description of the fragment ions, the disaccharides **13a** and **15a** are considered to be Neu5Ac-O-Gal. [b]The intensities of the ions are given relative to that of *m/z* 204.

was obtained in 87% yield in the form of its stable potassium salt **14**. As described for **13**, the galactopyranose form of **14** is indicated by its 360-MHz, ^{1}H-n.m.r. spectrum (H-1α: $J_{1,2}$ 3.7 Hz; H-1β: $J_{1,2}$ 7.9 Hz; αβ-ratio, 3:7). Again, a doubling of the resonance signal of the Neu5Ac H-3*ax* (~0.008 p.p.m.) was observed and no effect on the signal of H-3*eq* was detectable. The resonance position of H-3*eq* at δ 2.721 and the fact that **14** was cleaved by *Clostridium perfringens* neuraminidase proved the α configuration of the glycosidic linkage in **14**. Finally, the presence of the anticipated (2→6)-linkage in **14** was confirmed *via* methylation analysis of borodeuteride-reduced **14**.

As mentioned above, the condensation reaction gives rise to few side-products. Upon chromatography of the crude reaction mixture, two compounds were eluted from the column prior to **11**. The fast-moving was excess of **2**, which could be easily recovered. An impure compound was eluted second, which, after *O*-deacetylation, purification by column chromatography, and catalytic hydrogenolysis, gave disaccharide **15** in 3% yield based on the precursor of **5**, methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**3**). The pres-

ence of a β-glycosidic linkage in **15** was indicated by the 360-MHz, ^{1}H-n.m.r. spectrum (D_2O) which showed signals for H-3*eq* and H-4 of Neu5Ac at δ 2.453 and 4.098, respectively (ranges for β-linked Neu5Ac derivatives[23]: H-3*eq*, δ 2.1–2.5; H-4, δ 3.9–4.2). As shown in Table IV, the difference in anomeric configuration of the glycosidic linkage in **13** and **15** is also reflected by the resonance positions of the Neu5Ac H-7 and the protons of the *N*-acetyl group. For comparison, the corresponding data for methyl (methyl 5-acetamido-3,5-dideoxy-α-[14] and -β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[23] (**8** and **9**) are included. Although the absolute differences in the resonance positions are subtle, the data demonstrate the structural relationship of **15** and **9** on the one hand, and of **13** and **8** on the other. The statements regarding the pyranosid form of the galactose moiety of **13** also hold for **15** (H-1α: $J_{1,2}$ 3.4 Hz; H-1β: $J_{1,2}$ 7.6 Hz; αβ-ratio, 3:7). The doubling of the resonance signal of H-3*eq* was small (~0.001 p.p.m.) but significant. In contrast to **13**, no doubling of



15 $R^1, R^2 = H,OH; R^3 = R^5 = H; R^4 = Me$

15a $R^1, R^2 = H,OSiMe_3; R^3 = R^5 = SiMe_3; R^4 = Me$

the resonance signal of H-3*ax* was observed. Mass-spectral data of per-*O*-trimethylsilylated **15** (**15a**), showing, *inter alia*, the presence of a (2→6)-linkage in **15**, are summarised in Table III.

Two other by-products of the condensation reaction were eluted after **11**. The fast-moving component was identified as **7** by ^{1}H-n.m.r. spectroscopy. Apart from resonances of the Neu5Ac moiety, the spectrum showed the characteristic features of a salicyloyl group. The formation of **7** proceeds from the direct reaction between **5** and silver salicylate[26]. The slow-moving compound was identified as **10**, which is

TABLE IV

CHEMICAL SHIFTS (δ)[a] OF H-7 AND THE *N*-ACETYL PROTONS IN THE ^{1}H-N.M.R. SPECTRA OF **8**, **9**, **13**, AND **15** AT 360 MHz

	9[b]	15	8[c]	13
NAc	2.050	2.050	2.034	2.034
H-7	3.581	3.586	3.559	3.547

[a]Measured in deuterium oxide. [b]Values obtained from ref. 23. [c]Values obtained from ref. 14.

formed from **5** by elimination of HCl. The 360-MHz, ^{1}H-n.m.r. spectrum of **10** showed the absence of the resonance signals of H-3*eq* and H-3*ax*, and the appearance of a one-proton doublet at δ 5.987 originating from the olefinic H-3.

EXPERIMENTAL

Materials. — *N*-Acetyl-D-neuraminic acid (Neu5Ac) was isolated from the urine of a patient with sialuria[27]. Methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**3**) was prepared from Neu5Ac according to Kuhn *et al.*[15]. The product was crystallised from chloroform–ether; m.p. 154–155°, $[\alpha]_D^{20}$ −4.6° (*c* 1.2, chloroform); lit.[15] m.p. 156–157°, $[\alpha]_D^{20}$ −3.3° (*c* 1.0, chloroform).

General methods. — Melting points were determined with a Meopta melting-point microscope and are uncorrected. Evaporations were conducted *in vacuo* at <40° (bath). Elemental analyses were carried out at the Institute for Organic Chemistry TNO, Utrecht, The Netherlands. Specific rotations were measured with a Perkin–Elmer 241 polarimeter, using a 10-cm micro-cell. I.r. spectra (KBr discs) were recorded with a Perkin–Elmer Model 457 spectrophotometer.

^{1}H-N.m.r. spectra were recorded with a Varian EM-390 (90 MHz) spectrometer and a Bruker HX-360 (360 MHz) spectrometer, operating in the Fourier-transform mode, at probe temperatures of 25°. Chemical shifts (δ) for solutions in chloroform-*d* are given relative to tetramethylsilane as internal standard. For solutions in deuterium oxide, sodium 4,4-dimethyl-4-silapentane-1-sulphonate was used (indirectly, acetone in deuterium oxide: δ 2.225). Prior to spectral analysis, solutions in deuterium oxide were exchanged three times with intermediate lyophilisation. ^{13}C-N.m.r. spectra were recorded at ~30° with a Varian CFT-20 spectrometer operating at 20 MHz in the Fourier-transform mode with complete proton-decoupling. Chemical shifts (δ) are given relative to tetramethylsilane as internal standard for solutions in chloroform-*d*.

Trimethylsilylation of 1-mg samples of sugars was performed with hexamethyldisilazane and chlorotrimethylsilane in pyridine[28]. Sugar analysis by methanolysis, followed by g.l.c. of the trimethylsilylated methyl glycosides, was performed as indicated previously[29,30].

G.l.c. was carried out on a Varian Aerograph 2740–30-01, equipped with a flame-ionisation detector. The injection-port and detector temperatures were 210° and 230°, respectively. The nitrogen flow-rate was 35 mL/min. For partially methylated alditol acetates, a glass column (1.60 m × 4.0 mm i.d.) packed with 3% of OV-225 on Chromosorb W HP (100–120 mesh) and an oven temperature of 180° were used. A glass column (2.00 m × 4.0 mm i.d.) packed with 3.8% of SE-30 on Chromosorb W HP (80–100 mesh) was used for the analysis of per-*O*-trimethylsilyl derivatives of sugars; the oven temperature was 290° for disaccharide samples and was programmed from 135→220° at 1°/min for monosaccharide methyl glycosides.

G.l.c.–m.s. was performed with a combined Hewlett–Packard 5710A gas

chromatograph/Jeol JMS-D300 mass spectrometer/Jeol JMA-2000 mass data analysis system. 70-eV Mass spectra were recorded for an ion-source temperature of 225°, an accelerating voltage of 3 kV, and an ionising current of 300 μA. The same stationary phases were used as described above. 70-eV Mass spectra of disaccharide samples were recorded on a ZAB-2F VG Micromass mass spectrometer (ion-source temperature, 180°; probe temperature, 130–150°; accelerating voltage, 8 kV; ionising current, 100 μA).

T.l.c. was performed on silica gel (Schleicher and Schüll TLC Ready Plastic Foil FR-1500) and detection was effected with u.v. light or by spraying with 20% conc. sulphuric acid in methanol followed by charring at 130° for 5–10 min. The following solvents were used: *A*, chloroform–methanol (25:1); *B*, chloroform–methanol (10:1); *C*, ethyl acetate–2-propanol–water (2:2:1); *D*, 1-propanol–water (7:3).

Incubations with *Clostridium perfringens* neuraminidase (EC 3.2.1.18) were performed at 37° and pH 5.4 (0.1M Na/K phosphate buffer) in a total volume of 0.1 mL, containing 1.7 mU of enzyme and 0.2 μmol of substrate. Free sialic acid was determined by Warren's method[31].

*Benzyl 2,3,4-tri-*O*-benzyl-β-*D*-galactopyranoside* (**2**). — Compound **2** was obtained from benzyl β-D-galactopyranoside[16] (**1**; 15.8 g, 58.5 mmol) by tritylation and benzylation[17], followed by detritylation[18]. The resulting syrup was chromatographed on a column of silica gel (Merck Kieselgel 60, 70–230 mesh) with chloroform and crystallised from chloroform–hexane, to give **2** (21.0 g, 66%), m.p. 94.5–95°, $[\alpha]_D^{20}$ −46° (*c* 1, chloroform); lit.[17] m.p. 96–96.5°, $[\alpha]_D^{20}$ −46.1° (*c* 3.0, chloroform); lit.[32] m.p. 96°, $[\alpha]_D$ −49° (*c* 1.3, chloroform); ν_{max}^{KBr} 3300 (broad, OH), 732, and 695 cm^{-1} (Ph). For ^{13}C- and ^1H-n.m.r. data, see Tables I and II, respectively.

Condensation of **2** *and* **5**. — Compound **5**, freshly prepared[15] from **3** (1.22 g, 2.29 mmol), was dissolved in dry benzene (14 mL). After the addition of **2** (9.20 g, 17.06 mmol) and silver salicylate[14] (1.10 g, 4.56 mmol), the mixture was stirred for 17 h at room temperature in the dark with exclusion of moisture. T.l.c. (solvent *A*) revealed five spots having R_F values of 0.60, 0.55, 0.40, 0.32, and 0.27. The mixture was diluted with chloroform and filtered through a bed of diatomaceous earth. The inorganic solids were washed extensively with chloroform. The combined filtrates and washings were evaporated *in vacuo* and the resulting syrupy residue was fractionated on a column (37 × 4 cm) of silica gel (Merck Kieselgel 60 H) equipped with an air pump for flow-rate regulation (40 mL/h). The excess of **2** (8.02 g; R_F 0.60, solvent *A*) was eluted with chloroform, and the other carbohydrate fractions were eluted with chloroform–methanol (100:1).

The first fraction (161 mg) contained one main component (R_F 0.55, solvent *A*). After evaporation, the residue was dissolved in dry methanol (20 mL) containing a catalytic amount of potassium *tert*-butoxide. The solution was stirred at room temperature until t.l.c. (solvent *B*) revealed that the reaction was complete (2–3 h), deionised with Dowex 50W-X8 (H^+) resin at 0°, and evaporated. The resulting syrup was chromatographed on a column (16 × 1.2 cm) of silica gel (Merck Kieselgel 60,

70–230 mesh) with chloroform (20 mL) and then chloroform–methanol (25:1). The main product was obtained as a syrup (61 mg) that was homogeneous in t.l.c. (solvents *B* and *C*). It was dissolved in dry methanol (10 mL) and hydrogenolysed over Pd/C (10%, 25 mg) until t.l.c. (solvent *C*) showed that the reaction was complete (1.5 h). The mixture was filtered and concentrated, and an aqueous solution of the residue was lyophilised, to give the amorphous, β-linked sialodisaccharide methyl ester **15** (35 mg, 3% based on **3**). The product was homogeneous in t.l.c. (solvents *C* and *D*), and had $[\alpha]_D^{20}$ −2.6° (*c* 0.33, methanol) and a Neu5Ac/galactose ratio of 1.00:1.02. The m.s. data of per-*O*-trimethylsilylated **15** (**15a**) are given in Table III. ^{1}H-N.m.r. data (360 MHz, D_2O): Neu5Ac unit: δ 1.788 (dd, 1 H, $J_{3ax,3eq}$ −12.5, $J_{3ax,4}$ 12.5 Hz, H-3*ax*), 2.050 (s, 3 H, NAc), 2.452, 2.453 [2dd, 1 H, $J_{3ax,3eq}$ −12.5, $J_{3eq,4}$ ~4.7 Hz, H-3*eq* (α and β anomer of galactopyranose)], 3.586 (dd, 1 H, $J_{6,7}$ ~1, $J_{7,8}$ 9.7 Hz, H-7), 3.664 (dd, 1 H, $J_{8,9}$ 6.1, $J_{9,9'}$ −13.1 Hz, H-9'), 3.868 (s, 3 H, CO_2Me), 3.895 (dd, 1 H, $J_{4,5}$ ~10, $J_{5,6}$ ~10 Hz, H-5), and 4.098 (m, 1 H, $J_{3ax,4}$ 12.5, $J_{3eq,4}$ ~4.7, $J_{4,5}$ ~10 Hz, H-4); galactopyranose unit: δ 3.474 [dd, 0.7 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.4 Hz, H-2 (β anomer)], 4.182 [broad m, 0.3 H, H-5 (α anomer)], 4.570 (d, 0.7 H, $J_{1,2}$ 7.6 Hz, H-1β), and 5.253 (d, 0.3 H, $J_{1,2}$ 3.4 Hz, H-1α).

Anal. Calc. for $C_{18}H_{31}NO_{14} \cdot H_2O$: C, 42.94; H, 6.61; N, 2.78. Found: C, 42.82; H, 6.40; N, 2.85.

The second fraction gave the protected, α-linked sialodisaccharide **11** (1.50 g, 65% based on **3**), which was homogeneous in t.l.c. (R_F 0.40, solvent *A*). A sample was crystallised from carbon tetrachloride; m.p. 79–80°, $[\alpha]_D^{20}$ −5.0° (*c* 1.7, methanol); ν_{max}^{KBr} 3380 (NH), 1770–1730 (OAc, CO_2Me), 1665 (Amide I), 1545 (Amide II), 735, and 698 cm^{-1} (Ph). For ^{13}C- and ^{1}H-n.m.r. data, see Tables I and II, respectively.

Anal. Calc. for $C_{54}H_{63}NO_{18}$: C, 63.96; H, 6.26; N, 1.38. Found: C, 63.69; H, 6.31; N, 1.36.

For deblocking of **11**, see below.

The third fraction (44 mg) consisted mainly of the 2-*O*-salicyloyl derivative **7** (R_F 0.32, solvent *A*); ^{1}H-n.m.r. data of **7** (90 MHz, chloroform-*d*): Neu5Ac unit: δ 2.73 (dd, 1 H, $J_{3ax,3eq}$ −13.5, $J_{3eq,4}$ 4.8 Hz, H-3*eq*), 3.82 (s, 3 H, CO_2Me), and 4.48 (dd, 1 H, $J_{8,9}$ ~2.9, $J_{9,9'}$ −12.9 Hz, H-9); salicyloyl unit: δ 6.80–7.10 (m, 2 H, H-3,5), 7.51 (m, 1 H, J_{vic} 9.0 and 7.5, $J_{4,6}$ ~2 Hz, H-4), 7.86 (dd, 1 H, $J_{5,6}$ 7.5, $J_{4,6}$ ~2 Hz, H-6), and 10.17 (s, 1 H, OH; intramolecularly bridged proton).

The fourth fraction was a mixture of mainly **7** and the unsaturated compound **10** (R_F 0.32 and 0.27, respectively; solvent *A*) as shown by t.l.c., and was not collected.

The fifth fraction (45 mg) contained mainly **10** as judged by t.l.c. (R_F 0.27, solvent *A*) and ^{1}H-n.m.r. spectroscopy; ^{1}H-n.m.r. data of **10** (360 MHz, chloroform-*d*): δ 1.921, 2.051, 2.062, 2.075, 2.124 (5 s, 15 H, NAc and 4 OAc), 3.799 (s, 3 H, CO_2Me), 4.197 (dd, 1 H, $J_{8,9'}$ 6.7, $J_{9,9'}$ −12.5 Hz, H-9'), 4.654 (dd, 1 H, $J_{8,9}$ ~3.3, $J_{9,9'}$ −12.5 Hz, H-9), 5.987 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-3), and 6.086 (d, 1 H, $J_{5,NH}$ 9.7 Hz, NH).

*Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 5-acetamido-3,5-dideoxy-*α*-*D*-glycero-*D*-galacto-2-nonulopyranosylonate)-*β*-*D*-galactopyranoside* (**12**). — A solution of **11**

(1.38 g, 1.36 mmol) in dry methanol (40 mL) containing a catalytic amount of potassium *tert*-butoxide was stirred at room temperature until t.l.c. (solvent *B*) showed that *O*-deacetylation was complete (5 h). After treatment with Dowex 50W-X8 (H^+) resin at 0°, and evaporation, the slowly crystallising residue was recrystallised from carbon tetrachloride, to give **12** (981 mg, 85%), m.p. 183–184°, $[\alpha]_D^{20}$ −1.3° (*c* 0.95, methanol); ν_{max}^{KBr} 3570, 3510, 3350 (OH, NH), 1750 (CO_2Me), 1635 (Amide I), 1590 (Amide II), 750, 736, and 700 cm^{-1} (Ph); ^{13}C-n.m.r. data (20 MHz, chloroform-*d*): Neu5Ac unit: δ 173.9 (carbonyl, NAc), 169.3 (C-1), 98.5 (C-2), 53.2 (C-5), 52.9 (CH_3, ester), 40.2 (C-3), and 22.8 (CH_3, NAc); galactopyranoside unit: δ 102.8 (C-1), 81.9 (C-3), and 79.3 (C-2); ^{1}H-n.m.r. data: Table II.

Anal. Calc. for $C_{46}H_{55}NO_{14}$: C, 65.31; H, 6.55; N, 1.66. Found: C, 64.99; H, 6.45; N, 1.66.

6-O-(*Methyl 5-acetamido-3,5-dideoxy*-α-D-glycero-D-galacto-*2-nonulopyranosylonate*)-D-*galactose* (**13**). — A solution of **12** (550 mg, 0.65 mmol) in dry methanol (80 mL) was hydrogenolysed over Pd/C (10%, 230 mg) until t.l.c. (solvent *C*) showed that the reaction was complete (1 h). The mixture was filtered, concentrated, and finally lyophilised as an aqueous solution, to give amorphous **13** (326 mg, 99%). The product was homogeneous in t.l.c. (solvent *E*), and had $[\alpha]_D^{20}$ +5.4° (*c* 0.8, methanol); ^{1}H-n.m.r. data (360 MHz, D_2O): Neu5Ac unit: δ 1.833, 1.840 [2 dd, 1 H, $J_{3ax,3eq}$ −12.4, $J_{3ax,4}$ 12.4 Hz, H-3*ax* (α and β anomer of galactopyranose)], 2.034 (s, 3 H, NAc), 2.708 (dd, 1 H, $J_{3ax,3eq}$ −12.4, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 3.547 (dd, 1 H, $J_{6,7}$ ~1, $J_{7,8}$ 8.7 Hz, H-7), 3.77 (m, 1 H, H-4), and 3.879 (s, 3 H, CO_2Me); galactopyranose unit: δ 3.474 [dd, 0.7 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.6 Hz, H-2 (β anomer)], 4.555 (d, 0.7 Hz, $J_{1,2}$ 7.9 Hz, H-1β), and 5.231 (d, 0.3 H, $J_{1,2}$ 3.6 Hz, H-1α). The m.s. data of per-*O*-trimethylsilylated **13** (**13a**) are given in Table III.

Anal. Calc. for $C_{18}H_{31}NO_{14} \cdot H_2O$: C, 42.94; H, 6.61; N, 2.78; O, 47.67. Found: C, 42.77; H, 6.32; N, 2.82; O, 47.56.

6-O-(*Potassium 5-acetamido-3,5-dideoxy*-α-D-glycero-D-galacto-*2-nonulopyranosylonate*)-D-*galactose* (**14**). — A solution of **13** (57 mg, 0.11 mmol) in 0.02M potassium hydroxide (6.3 mL) was kept at 5° until t.l.c. (solvents *C* and *D*) showed that the reaction was complete (17 h). The mixture was concentrated by lyophilisation and freed from alkali by elution from a column (35 × 2 cm) of Bio-Gel P-2 (200–400 mesh) with water. The product was further purified on a column (66 × 2.5 cm) of Dowex 50W-X8 (200–400 mesh, K^+ form) resin by elution with water, and lyophilised, to give amorphous **14** (52 mg, 87%). The product was homogeneous in t.l.c. (solvents *C* and *D*), and had $[\alpha]_D^{25}$ +16.7° (*c* 0.35, water); Neu5Ac/galactose ratio of 1.00:0.99. Methylation analysis[33] of borodeuteride-reduced **14** afforded 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methyl-hexitol-1-*d* as the neutral, partially methylated alditol acetate. ^{1}H-N.m.r. data of **14** (360 MHz, D_2O): Neu5Ac unit: δ 1.685, 1.693 [2dd, 1 H, $J_{3ax,3eq}$ −12.2, $J_{3ax,4}$ 12.2 Hz, H-3*ax* (α and β anomer of galactopyranose)], 2.033 (s, 3 H, NAc), 2.721 (dd, 1 H, $J_{3ax,3eq}$ −12.2, $J_{3eq,4}$ 4.5 Hz, H-3*eq*), and 3.575 (dd, 1 H, $J_{6,7}$ 1.5, $J_{7,8}$ 8.6 Hz, H-7); galactopyranose unit: δ 3.469 [dd, 0.7 H, $J_{1,2}$ 7.9,

$J_{2,3}$ 9.9 Hz, H-2 (β anomer)], 4.566 (d, 0.7 H, $J_{1,2}$ 7.9 Hz, H-1β), and 5.237 (d, 0.3 H, $J_{1,2}$ 3.7 Hz, H-1α).

Anal. Calc. for $C_{17}H_{28}NO_{14}K \cdot H_2O$: C, 38.71; H, 5.73; N, 2.66. Found: C, 38.53; H, 5.74; N, 2.66.

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