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A SYNTHESIS OF *N*-ACETYLNEURAMINIC ACID AND [6-^{2}H]-*N*-ACETYLNEURAMINIC ACID FROM *N*-ACETYL-D-GLUCOSAMINE*

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ABSTRACT

N-Acetylneuraminic acid (Neu5Ac) and [6-^{2}H]-Neu5Ac were prepared from 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine). The Henry reaction of a 1-deoxy-1-nitro derivative of GlcNAc (protected 1-*C*-nitroanhydro-D-glucitol) with cyclohexylidene-D-glyceraldehyde, followed by successive acetylation and reductive denitration with Bu_3SnH, gave an anhydrononitol intermediate (**6**) diastereoselectively in high yields. Debenzylidenation of **6** freed its distal primary carbinol group, which was subjected to catalytic oxidation followed by hydrolysis, esterification (diazomethane), and acetylation to give a protected methyl nononate. This ester was transformed into the known methyl *N*-acetyl-4,7,8,9-tetra-*O*-acetyl-2,3-dehydroneuraminate (**15**), which was identical with a sample prepared from Neu5Ac. Neu5Ac was obtained from **15** by bromoetherification (NBS, methanol) followed by reductive debromination with Bu_3SnH and hydrolysis. Similarily, the [6-^{2}H]-derivative of **15** was transformed into [6-^{2}H]-Neu5Ac.

INTRODUCTION

Sialic acids, particularly *N*-acetylneuraminic acid (Neu5Ac, **1**), are constituents of many glycoconjugates, where they occupy the nonreducing ends of the oligosaccharide chains[1–3]. To study the relations existing on the one hand between the structure of sialic acid residues and the function of glycoconjugates containing them and, on the other hand, between the structure of these glycoconjugates and the mode of action of the enzymes involved in their biosynthesis and transformation, one needs a range of selectively modified analogs. Such compounds may be obtained by synthesis from sialic acid derivatives. Thus, several analogs have been prepared from Neu5Ac itself or by partial synthesis from other carbohydrates (for recent examples see refs. 4–16), and although a recently reported total synthesis[17] of racemic Neu5Ac may also prove useful in this respect, there is still a need for simple and general approaches to these compounds.

*Dedicated to the memory of Hermann O. L. Fischer on the centenary of his birth.
†To whom correspondence should be addressed.

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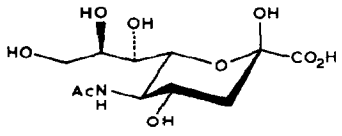

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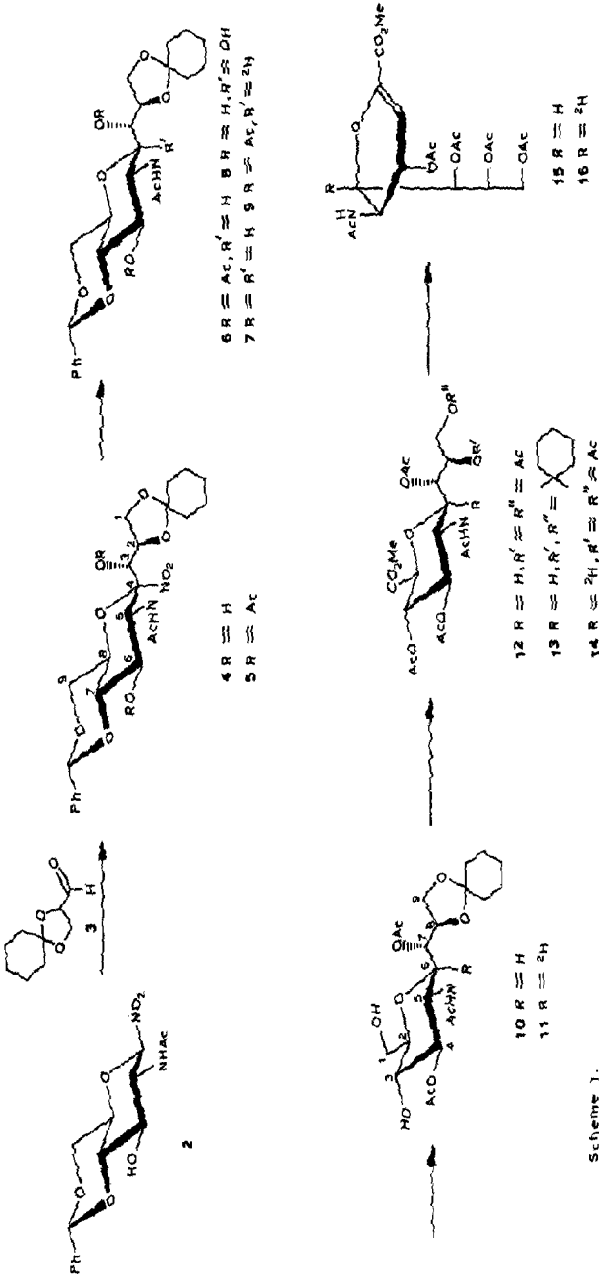
We have recently described a synthesis of Neu5Ac, 4-epi-Neu5Ac, and 4-deoxy-Neu5Ac based upon extension of the chain of a protected *N*-acetyl-1-deoxy-1-nitromannose by a C_3 unit corresponding to C-1–C-3 of Neu5Ac (refs. 18, 19). This synthesis should allow convenient modifications at C-1 to C-5 of Neu5Ac.

We now present a new synthesis of Neu5Ac designed to permit modifications particularly at C-6 to C-9. It is based upon an extension of the chain of the *N*-acetyl-1-deoxy-1-nitro-D-glucosamine (1-*C*-nitroanhydro-D-glucitol) **2** at C-1 by a C_3 unit corresponding to C-7–C-9 of Neu5Ac. Transformation of the hydroxymethyl group corresponding to C-6 of *N*-acetylglucosamine into an alkoxycarbonyl group, β-elimination, hydration of the ensuing enol ether corresponding to 2,3-dehydro-Neu5Ac (**15**), and deprotection should lead to Neu5Ac. The Henry reaction of the nitrosugar **2*** with 2,3-*O*-cyclohexylidene-D-glyceraldehyde[21] (**3**) could give rise to four diastereomeric 4-*C*-nitroanhydrononitols. Nucleophilic additions to 2,3-*O*-isopropylidene-D-glyceraldehyde in the absence of chelating agents, however, lead preferentially to *erythro* products[22–25]. An equatorial approach of **3** to the nitronate anion is expected from previous results[26,29], but is not relevant to the outcome of the synthesis, since the reductive denitration of the nitropyranoses of the *gluco* configuration may proceed diastereoselectively with substitution of the nitro group by an axial hydrogen, regardless of the anomeric configuration of the Henry product[26,27]. The above mentioned Henry reaction followed by reductive denitration should thus lead to an anhydrononitol having the desired configuration.

RESULTS AND DISCUSSION

The Henry reaction of **2** with **3**, catalysed by tetraethylammonium hydroxide, gave a single nitroalcohol **4** in yields of 85–90% (Scheme 1). The axial position of the nitro group was evidenced by an i.r. band at 1550 cm^{-1} (refs. 29, 30) and confirmed by the chemical shift differences ($\Delta\delta$ values) of the H_a and H_b signals of compounds **4–8** (Table I). Due to the coplanar arrangement of the nitro group and the adjacent C–O bond[30], H_a and H_b are exposed to the deshielding and shielding effect, respectively, of the magnetically anisotropic nitro group. The equatorial orientation of the newly introduced side chain may be the result of thermodynamic and/or kinetic control. The latter could be provided by a preferred pyramidalization of the nitronate anion upon equatorial approach of **3**, avoiding conjugative destabilization by interaction with the lone pair of electrons on the ring oxygen[34]

*Easily available in four steps from *N*-acetyl-D-glucosamine in 43% yield[20].

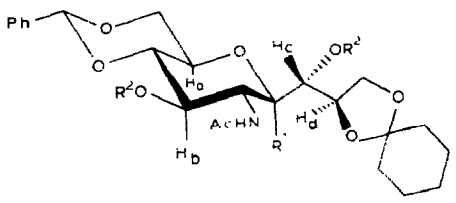


Scheme 1.

TABLE I

INFLUENCE OF R^1 ON THE ^{1}H-N.M.R. SHIFTS OF H_a AND H_b

Compound	R^1	R^2	δH_a	δH_b
4	NO_2	H	4.31	3.60[a]
7	H	H	3.25	3.65[a]
8	OH	H	3.80	3.80[a]
5	NO_2	Ac	4.03	5.09[b]
6	H	Ac	3.54	5.25[b]



[a]Spectra recorded in DMSO-d_6. [b]In $CDCl_3$.

(compare also the results of Beau and Sinaÿ[31-33]). The value of $J_{c,d}$ (7.8 Hz) in the ^{1}H-n.m.r. spectrum of **4** is very similar to the coupling constants found by Brandstetter and Zbiral[12] for sialic acid derivatives possessing a similarily protected side chain. An *erythro* configuration has also been reported for the product of the addition to 2,3-*O*-isopropylidene-D-glyceraldehyde of the anion derived from a glycopyranosyl phenyl sulfone[32].

The acetylation of **4** with acetic anhydride and pyridine gave the diacetate **5** in high yields. Treatment of **5** with tri-*n*-butyltin hydride and azoisobutyronitrile (AIBN) in refluxing benzene gave the desired anhydrononitol **6** in yields of 97%. The signal of the newly introduced H-6* appears at 3.68 p.p.m. as a doublet of doublets with $J_{5,6}$ 10.5 Hz, while H-7 showed $J_{7,8}$ 5.3 Hz and $J_{6,7}$ 3.2 Hz, values which again agree well with those of similar Neu5Ac derivatives[12]. Although encouraging at this stage of the synthesis, these comparisons are at best weak evidence of the *erythro* configuration, since the values of $J_{7,8}$ depend strongly upon structural parameters (compare $J_{c,d}$ for **4**, **5**, and **6**, see also Table III). Attempts to reductively denitrate the nitrodiol **4** in an analogous way (in toluene at 90°), gave the reduction product **7** in only 30% yield, together with 50% of the ulose **8**, even under anhydrous conditions. The mechanism of formation of **8** has not been studied, but in the absence of tributyltin hydride and AIBN, **4** appears to be considerably more stable to heat.

Hydrogenolytic cleavage of the benzylidene group of **6** proceeded smoothly in the presence of palladium hydroxide on carbon, at a slightly elevated pressure, to give the diol **10** in almost quantitative yield. According to a procedure of Paulsen[37], **10** was oxidized (O_2, Pt) at pH 8 and elevated temperature. This led to concomitant hydrolysis of the acetate groups. Liberation of the intermediate acid and cleavage of the acetal were combined†. Finally, esterification with

*Numbers refer to systematic nomenclature (see Experimental part).

†Treatment of the oxidation product with acid under milder conditions, followed by esterification and acetylation, led to a mixture of **12** and **13**.

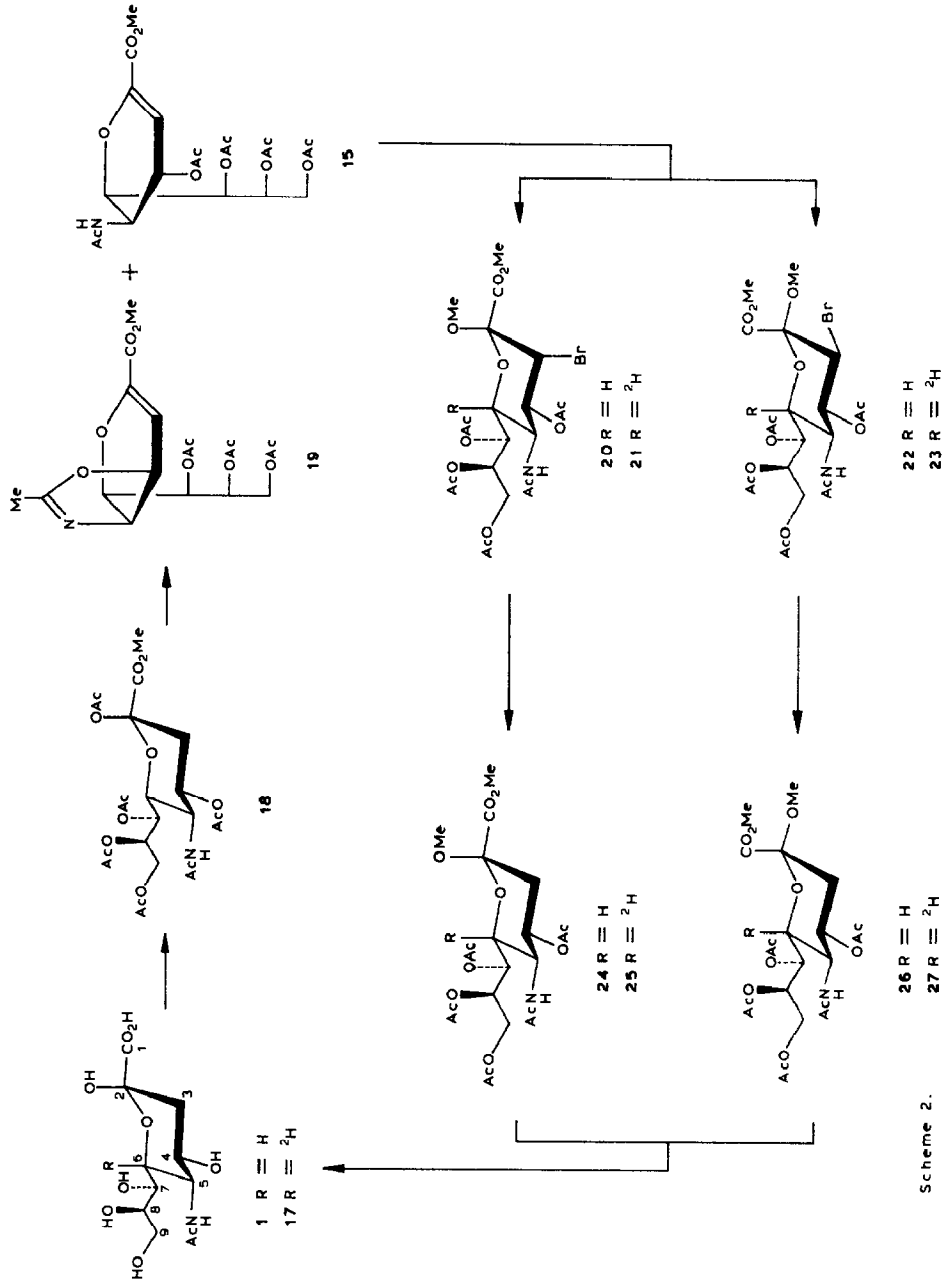
diazomethane and acetylation gave the crystalline ester **12** in 60% yield. Prolonged treatment of the β-acetoxyester **10** with diazabicycloundecene[38] (DBU) gave the enol ether **15** in (95%) yield. This enol ether was also prepared from Neu5Ac *via* the acetylated methyl ester[35] **18** (Scheme 2). Treatment of **18** with 2.0 eq. of trimethylsilyl triflate in nitromethane gave **15** in a yield of 69%, together with 15% of the oxazoline **19**‡. The ^{1}H-n.m.r., ^{13}C-n.m.r., i.r., and u.v. spectra, and the specific rotations of the two samples of **15** prepared from **2** and from **1**, respectively, could not be distinguished from each other.

To transform **15** into Neu5Ac, it was first treated with *N*-bromosuccinimide (NBS) and methanol to give a 97% yield of the two diastereomers **22** and **20** in a 1:1 ratio. In the less polar product **22** the newly introduced bromo substituent at C-3 is equatorial, as evidenced by the ^{1}H-n.m.r. spectrum in which H-3 appears as a doublet at 3.97 p.p.m. with $J_{3,4}$ 10.5 Hz. In **20** the axial position of the bromo substituent at C-3 was also evident from the ^{1}H-n.m.r. signal for H-3, which is seen as a doublet at 4.61 p.p.m., $J_{3,4}$ 3.6 Hz. The chemical shifts of C-3 also confirm this assignment. In the ^{13}C-n.m.r. of **22**, C-3 appears as a doublet at 49.55 p.p.m., and in the spectrum of **20** at 45.46 p.p.m. Higher shifts are usually observed for carbons carrying equatorial substituents. The configuration at C-2 could be assigned after reductive debromination of **22** and **20** with tributyltin hydride. The known glycosides **26** (refs. 40–42), and **24** (refs. 13, 40) were obtained in almost quantitative yields from **22** and **20** respectively. Complete saponification, followed by acid hydrolysis of the methyl glycosides, gave **1** in yields of 77% from **26** and 70% from **24**.

The ^{1}H-n.m.r. data for the acetylated and esterified neuraminic acid derivatives studied in this work are collected in Tables II and III. The values of the coupling constant $J_{7,8}$ (in $CDCl_3$) depend strongly upon the anomeric configuration (Table III). For α anomers, $J_{7,8}$ is ~8–9 Hz, and for β anomers, ~4–5 Hz, showing differences in side-chain conformation for the two groups. This dependency on anomeric configuration might prove useful for the characterization of additional derivatives (see also ref. 40).

The reductive denitration of **5** with tri-*n*-butyltin [^{2}H]-hydride offered a convenient way to introduce a deuterium atom at C-6. The monodeuterated reduction product **9** showed almost the same specific rotation as **6**. The ^{1}H-n.m.r. spectrum of **9** (as compared to that of **6**) is characterised by the disappearence of the H-6 signal at 3.68 p.p.m. and the simplification of the signals of H-5 at 4.25 p.p.m. (q→t, *J* 10.2 Hz) and of H-7 at 5.25 p.p.m. (dd→d, *J* 5.9 Hz). Deuteration also facilitates the assignment to C-6 of the signal at 78.12 p.p.m. in the ^{13}C-n.m.r. spectrum of **6**. Following the procedure described above, **17** was easily obtained from **9** *via* the intermediates **11, 14, 16, 21, 23, 25,** and **27**. All the deuterated intermediates possess almost the same melting points and specific rotations as their nondeuterated analogs. The simplifications of the signals of H-5 and H-7 in the

‡In our hands, **18** did not react under the conditions described by Claesson and Luthman[35].



Scheme 2.

TABLE II

^{1}H-N.M.R. SHIFTS MEASURED IN $CDCl_3$[a]

Compound	*H-2*	*H-3a*	*H-3e*	*H-4*	*H-5*	*H-6*	*H-7*	*H-8*	*H-9*	*H-9'*	*NH*	*COOCH$_3$*	*OCH$_3$*	*CH$_3$CO*	*CH$_3$CON*
12	3.90	5.20	—	5.20	4.10	3.82	5.31	5.23	4.50	4.10	5.39	3.75	—	2.15, 2.08, 2.04, 2.03, 2.02	1.89
13[b]	3.99	5.18	—	5.29	4.05	3.89	5.23	4.28	3.98	3.89	5.48	3.75	—	2.14, 2.03, 2.02	1.90
14	3.93	5.20	—	5.20	4.10	—	5.30	5.22	4.50	4.10	5.40	3.75	—	2.14, 2.07, 2.03, 2.02, 2.01	1.89
16	—	6.01		5.48	4.38	—	5.50	5.36	4.58	4.19	5.54	3.80	—	2.12, 2.08, 2.06, 2.05	1.93
17[c]	—	1.86	2.28	4.05	3.91	—	3.53	3.74	3.83	3.60	—	—	—	—	2.04
20	—	—	4.61	5.46	4.36	4.08	5.36	5.30	4.86	4.19	5.33	3.84	3.30	2.18, 2.08, 2.08, 2.04	1.91
21	—	—	4.61	5.46	4.34	—	5.35	5.30	4.85	4.18	5.32	3.84	3.29	2.18, 2.08, 2.08, 2.03	1.91
22	—	3.97	—	5.48	4.26	4.65	5.27	5.34	4.23	4.04	5.50	3.84	3.53	2.13, 2.08, 2.06, 2.03	1.90
23	—	3.97	—	5.48	4.25	—	5.26	5.34	4.23	4.04	5.42	3.84	3.53	2.14, 2.08, 2.07, 2.03	1.90
25	—	1.88	2.43	5.25	4.12	—	5.40	5.25	4.79	4.12	5.27	3.81	3.26	2.15, 2.07, 2.03, 2.01	1.88
27	—	1.94	2.56	4.85	4.06	—	5.33	5.43	4.33	4.10	5.13	3.81	3.32	2.15, 2.14, 2.04, 2.03	1.88

[a] δ Values (p.p.m.). [b] Cyclohexylidene 1.3–1.7 (5 CH_2). [c] In D_2O.

TABLE III

^{1}H-N.M.R. COUPLING CONSTANTS[a]

Compound	$J_{2,3a}$	$J_{3a,3e}$	$J_{3a,4}$	$J_{3e,4}$	$J_{4,5}$	$J_{5,NH}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$
12	ABX	—	ABX	—	ABX	9.8	10.4	2.2	5.7	2.5	6.8	12.5
13	9.8	—	9.6	—	9.8	10.0	10.4	1.9	6.0	6.3	6.2	8.9
14	ABX	—	ABX	—	ABX	9.7	—	—	5.7	2.5	6.7	12.5
16	—	—	3,4		7.2	9.3	—	—	4.8	3.4	6.9	12.3
17	—	13.0	11.5	4.9	10.1	—	—	—	9.2	2.7	6.4	11.8
20	—	—	—	3.7	10.4	10.0	10.4	1.9	4.6	2.5	7.3	12.5
21	—	—	—	3.7	10.4	10.1	—	—	4.5	2.4	7.5	12.5
22	—	—	10.5	—	10.3	10.3	10.5	2.1	8.8	2.6	6.1	12.4
23	—	—	10.6	—	10.4	10.3	—	—	8.8	2.7	6.1	12.5
25	—	12.6	12.2	4.9	10.0	10.3	—	—	4.1	2.5	7.7	12.4
27	—	12.7	12.5	4.6	10.5	10.3	—	—	8.5	2.8	5.6	12.5

[a]Values given in Hz.

^{1}H-N.m.r. spectra confirmed the assignment of these signals in the intermediates of the protio series, and a comparison of the ^{13}C-n.m.r. spectra of the two series allowed the unambigous assignment of the C-6 signals.

EXPERIMENTAL

General methods. — Solvents were distilled before use. All reagents were obtained from Fluka. Solutions were evaporated at or below 50° on a Büchi rotary evaporator. Qualitative t.l.c. was performed with Merck precoated silica gel 60 F-254 plates, and compounds were detected by spraying the plates either with a solution of 0.02M I_2 and 0.30M KI in 10% aqueous H_2SO_4 or with phosphomolybdic acid (10% in EtOH), followed in both cases by heating at about 200°. Flash chromatography was carried out on silica gel Merck 60 (40–63 μm). Melting points (uncorrected) were determined with a Büchi 510 apparatus. Optical rotations were measured on a Perkin–Elmer 241 spectrometer at 25°, in a 1 dm cell, at 365, 436, 546, 578, and 589 nm. The specific rotations at 589 nm were determined with the help of a regression curve. ^{1}H-N.m.r. spectra were recorded at 400 MHz on a Brucker AM-400 spectrometer and ^{13}C-n.m.r. spectra at 50 MHz on a Varian XL-200 spectrometer. The chemical shifts are given in p.p.m. relative to tetramethylsilane as internal standard, and the coupling constants are given in Hz. I.r. spectra were recorded in KBr on a Perkin–Elmer 298 spectrometer. U.v. spectra were measured on a Perkin–Elmer 555 spectrometer using a 1 cm cell. Mass spectra were determined using a Varian 112S (e.i., 70 eV; c.i., isobutane) spectrometer.

Henry reaction of **2** *with* **3**. — To a solution of the nitrosugar **2** (4.0 g, 11.8 mmol) in *N*,*N*-dimethylformamide (DMF) (30 mL) were added tetraethylammonium hydroxide (0.3 mL; 1.5M in methanol), and 2,3-*O*-cyclohexylidene-D-glyceraldehyde (**3**) (1.5 g, 8.8 mmol) (ref. 21.). These additions were repeated three more times at intervals of 45 min. The reaction mixture was stirred for a further 1 h, the solvent was removed *in vacuo*, and the residue was purified by chromatography on SiO_2 (500 g; 1:1 AcOEt–hexane) to yield (4*R*)-5-acetamido-4,8-anhydro-7,9-*O*-benzylidene-1,2-*O*-cyclohexylidene-5-deoxy-4-*C*-nitro-D-*gluco*-L-*erythro*-nonitol (**4**) (5.1 g, 85%) as a foam; R_F 0.51 (AcOEt), $[\alpha]_D^{20}$ +17.4° (*c* 1.1, chloroform); ν_{max} 3400 sb, 2940 m, 2860 w, 1660 m, 1550 s, 1450 m, 1375 m, 1285 w, 1210 w, 1160 m, 1090 s, 1045 m, 925 m, 850 w, 760 w, and 700 m cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 7.3–7.5 (m, 5 H, Ph-H), 6.26 (d, 1 H, $J_{5,NH}$ 9.6 Hz, N*H*), 5.55 (s, 1 H, PhC*H*), 5.52 (d, 1 H, $J_{3,OH}$ 5.4 Hz, O*H*-3), 4.66 (t, 1 H, $J_{5,6} = J_{5,NH}$ 10.0 Hz, H-5), 4.50 (dd, 1 H, $J_{9a,9e}$ 10.1, $J_{9e,8}$ 4.9 Hz, H-9e), 4.41 (dt, 1 H, $J_{8,9a}$ 9.5, $J_{8,9e}$ 4.9 Hz, H-8), 4.22 (dt, 1 H, $J_{1,2} = J_{1',2}$ 5.6, $J_{2,3}$ 7.8 Hz, H-2), 4.10 (m, 2 H, 2 H-1), 4.01 (dd, 1 H, $J_{3,4}$ 7.8 Hz, $J_{3,OH}$ 5.4 Hz, H-3), 3.85 (t, 1 H, $J_{6,7} = J_{7,8}$ 9.9 Hz, H-7), 3.71 (t, 1 H, $J_{5,6} = J_{6,7}$ 10.0 Hz, H-6), 3.67 (t, 1 H, $J_{9a,9e} = J_{8,9a}$ 9.3 Hz, H-9a), 2.9 (m, 1 H, O*H*-7), 2.18 (s, 3 H, CH_3), and 1.3–1.7 (m, 10 H, 5 CH_2); ^{13}C-n.m.r. ($CDCl_3$): δ 173.15 (s), 136.43 (s), 129.59 (d), 128.41 (d, 2 C), 126.33 (d, 2 C), 112.90 (s), 110.59 (s), 102.25 (d), 80.78 (d), 72.79 (d), 68.25 (d), 68.25 (t), 67.81 (d), 66.45 (t),

53.19 (d), 35.78 (t), 34.55 (t), 25.07 (t), 23.88 (t), 23.73 (t), and 23.16 (q); c.i.-m.s.: *m/z* 461 (65, $M^+ + 1 - 48$), 443 (82).

(4R)-5-Acetamido-3,6-di-O-acetyl-4,8-anhydro-7,9-O-benzylidene-1,2-O-cyclohexylidene-5-deoxy-4-C-nitro-D-gluco-L-erythro-*nonitol* (**5**). — A solution of **4** (5.15 g, 10.1 mmol) was acetylated overnight in 1:2 acetic anhydride–pyridine. The solvent was removed *in vacuo* and the residue was purified by chromatography on SiO_2 (300 g, 1:1 AcOEt–hexane) to yield **5** (5.50 g, 92%) as a white powder. The compound was crystallized from ether–hexane, m.p. 148–149°. R_F 0.62 (AcOEt), $[\alpha]_D^{20}$ −3.8° (*c* 3.1, chloroform), ν_{max} 3420 b, 2940 m, 2860 w, 1760 s, 1695 m, 1565 m, 1500 m, 1450 w, 1370 s, 1220 s, 1160 s, 1095 s, 1060 w, 1040 s, 1000 w, 930 w, 845 w, 760 w, and 700 w cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 7.3–7.5 (m, 5 H, Ph-H), 6.46 (d, 1 H, $J_{5,NH}$ 10.6 Hz, N*H*), 5.73 (d, 1 H, $J_{3,4}$ 4.5 Hz, H-3), 5.53 (s, 1 H, PhC*H*), 5.09 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 10.4 Hz, H-6), 4.60 (t, 1 H, $J_{5,6} = J_{5,NH}$ 10.7 Hz, H-5), 4.45 (dd, 1 H, $J_{9a,9e}$ 10.1, $J_{8,9e}$ 4.6 Hz, H-9e), 4.24 (dt, 1 H, $J_{1,2} = J_{1',2}$ 6.5, $J_{2,3}$ 4.6 Hz, H-2), 4.03 (dt, 1 H, $J_{7,8} = J_{8,9a}$ 9.8, $J_{8,9e}$ 4.7 Hz, H-8), 4.00 (dd, 1 H, $J_{1,1'}$ 8.6, $J_{1,2}$ 6.5 Hz, H-1), 3.92 (t, 1 H, $J_{6,7} = J_{7,8}$ 9.7 Hz, H-7), 3.88 (t, 1 H, $J_{9a,9e} = J_{8,9a}$ 10.0 Hz, H-9), 3.85 (dd, 1 H, $J_{1,1'}$ 8.5, $J_{1',2}$ 6.9 Hz, H-1'), 2.15 (s, 3 H, CH_3CO), 2.03 (s, 3 H, CH_3CO), 1.97 (s, 3 H, CH_3CON), and 1.3–1.6 (m, 10 H, 5 CH_2); ^{13}C-n.m.r. ($CDCl_3$): δ 170.54 (s), 170.36 (s), 168.95 (s), 136.15 (s), 129.39 (d), 128.27 (d, 2 C), 126.16 (d, 2 C), 113.93 (s), 109.88 (s), 101.95 (d), 77.71 (d), 72.60 (d), 69.80 (d), 69.23 (d, 2 C), 67.84 (t), 64.73 (t), 50.79 (d), 35.64 (t), 34.63 (t), 24.95 (t), 23.72 (t), 23.66 (t), 23.03 (q), 20.85 (q), and 20.47 (q); c.i.-m.s.: *m/z* (23, $M^+ + 1$), 564 (100), 547 (20), 546 (70), 504 (13), 431 (11), 241 (23), 203 (12), 171 (11), and 149 (35).

Anal. Calc. for $C_{28}H_{36}N_2O_{12}$ (592.73): C, 56.8; H, 6.1; N, 4.7. Found: C, 56.7; H, 6.3; N, 4.6.

5-Acetamido-4,7-di-O-acetyl-2,6-anhydro-1,3-O-benzylidene-8,9-O-cyclohexylidene-5-deoxy-D-arabino-L-gulo-*nonitol* (**6**). — To acetylated compound **5** (7.56 g, 12.8 mmol) in dry benzene (140 mL), under N_2, were added AIBN (500 mg) and Bu_3SnH (6.5 mL), and the solution was heated to reflux. After 2 h, AIBN (340 mg; total 5.1 mmol) and Bu_3SnH (2 mL; total 32 mmol) were added, and the solution was again heated to reflux for 3 h. The solvent was removed *in vacuo* and the residue was purified by chromatography on SiO_2 (400 g, 3:2 AcOEt–hexane) to yield 6.72 g (96%) of **6** as a white powder; R_F 0.36 (AcOEt), $[\alpha]_D^{25}$ −43.7° (*c* 1.1, chloroform), ν_{max} 3380 b, 2940 s, 2860 m, 1750 s, 1690 m, 1670 m, 1540 m, 1450 w, 1430 w, 1370 s, 1290 m, 1225 s, 1165 w, 1100 s, 1040 s, 930 m, 910 w, 850 w, 750 w, and 700 m cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 7.3–7.5 (m, 5 H, Ph-H), 5.80 (d, 1 H, $J_{5,NH}$ 10.2 Hz, N*H*), 5.51 (s, 1 H, PhC*H*), 5.25 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.25 (dd, 1 H, $J_{7,8}$ 5.0 Hz, $J_{6,7}$ 2.5 Hz, H-7), 4.25 (dd, 1 H, $J_{1a,1e}$ 10.3, $J_{1e,2}$ 4.4 Hz, H-1e), 4.25 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH}$ 10.3 Hz, H-5), 4.05 (dt, 1 H, $J_{7,8}$ 5.6, $J_{8,9} = J_{8,9'}$ 6.0 Hz, H-8), 3.81 (m, 2 H, 2 H-9), 3.75 (t, 1 H, $J_{1a,1e} = J_{1a,2}$ 10.2 Hz, H-1a), 3.70 (t, 1 H, $J_{2,3} = J_{3,4}$ 10.0 Hz, H-3), 3.68 (dd, $J_{5,6}$ 10.5, $J_{6,7}$ 3.2 Hz, H-6), 3.54 (dt, $J_{1a,2} = J_{2,3}$ 9.7, $J_{1e,2}$ 4.8 Hz, H-2), 2.13, 2.08 (2 s, ca. 3 H, CH_3CO), 1.89 (s, 3 H, CH_3CON).

and 1.3–1.6 (m, 10 H, 5 C*H*$_2$); ^{13}C-n.m.r. ($CDCl_3$): δ 171.72 (s), 170.21 (s), 170.14 (s), 136.98 (s), 129.07 (d), 128.20 (d, 2 C), 125.91 (d, 2 C), 109.12 (s), 101.18 (d), 78.79 (d), 78.12 (d), 74.64 (d), 73.74 (d), 71.03 (d), 68.91 (d), 68.38 (t), 65.34 (t), 49.35 (d), 36.12 (t), 35.08 (t), 25.05 (t), 23.87 (t), 23.78 (t), 23.05 (q), and 20.95 (q, 2 C); c.i.-m.s.: *m/z* 548 (100, $M^+ + 1$), 504 (17), 488 (12), 450 (33), 442 (12), and 284 (8).

Anal. Calc. for $C_{28}H_{37}NO_{10}$ (547.61): C, 61.4; H, 6.8; N, 2.6. Found: C, 61.1; H, 7.1; N, 2.5.

*5-Acetamido-2,6-anhydro-1,3-*O*-benzylidene-8,9-*O*-cyclohexylidene-5-deoxy-*D-arabino-L-gulo-*nonitol* (**7**) *and 5-acetamido-7,9-*O*-benzylidene-1,2-*O*-cyclohexylidene-5-deoxy-α-*D-gluco-L-erythro-*4-nonulo-4,8-pyranose* (**8**). — To a solution of **5** (370 mg, 0.73 mmol) in dry toluene (10 mL) at 100° were added AIBN (15 mg) and Bu_3 SnH (0.1 mL, 0.38 mmol). These additions were repeated 4 times at intervals of 30 min. The solution was heated for a further 3 h, then the solvent was distilled off, and the residue was purified by chromatography on SiO_2 (150 g, 9:1 AcOEt–hexane) to yield 104 mg (31%) of **7** and 217 mg (62%) of **8**. Compound **7** had m.p. 248°, R_F 0.31 (9:1 AcOEt–hexane), $[\alpha]_D^{22}$ −33.0° (*c* 0.75, DMF); ν_{max} 3400 sb, 3280 s, 3100 w, 2940 s, 2890 s, 1625 s, 1570 s, 1450 s, 1390 m, 1320 m, 1300 w, 1240 w, 1170 w, 1110 s, 1070 s, 1030 s, 1010 s, 970 m, 920 m, 860 w, 830 w, 800 w, 770 s, and 710 s cm^{-1}; ^{1}H-n.m.r. (DMSO-d_6): δ 8.07 (d, 1 H, $J_{5,NH}$ 8.3 Hz, N*H*), 7.3–7.5 (m, 5 H, Ph-H), 5.59 (s, 1 H, PhC*H*), 5.25 (d, 1 H, $J_{4,OH}$ 5.5 Hz, O*H*-4), 4.77 (d, 1 H, $J_{7,OH}$ 5.7 Hz, OH-7), 4.16 (dd, 1 H, $J_{1e,1a}$ 10.2, $J_{1e,2}$ 4.9 Hz, H-1e), 4.03 (dt, 1 H, $J_{7',8}$ 7.3, $J_{8,9} = J_{8,9'}$ 5.7 Hz, H-8), 3.89 (dd, 1 H, $J_{9,9'}$ 8.4, $J_{8,9}$ 6.1 Hz, H-9), 3.83 (dd, 1 H, $J_{9,9'}$ 8.3, $J_{8,9'}$ 5.5 Hz, H-9'), 3.74 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH}$ 9.5 Hz, H-5), 3.68 (t, 1 H, $J_{1a,1e} = J_{1a,2}$ 10.1 Hz, H-1a), 3.65 (m, 1 H, H-4), 3.39 (br d, 1 H, $J_{5,6}$ 10.3 Hz, H-6), 3.39 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 3.28 (br t, 1 H, $J_{7,8} = J_{7,OH}$ 6.6 Hz, H-7), 3.25 (dt, 1 H, $J_{1a,2} = J_{2,3}$ 9.6, $J_{1e,2}$ 5.0 Hz, H-2), 1.86 (s, 3 H, C*H*$_3$CON), and 1.3–1.7 (m, 10 H, 5 C*H*$_2$); ^{13}C-n.m.r. (DMSO-d_6): δ 171.08 (s), 137.73 (s), 128.64 (d), 127.84 (d, 2 C), 126.23 (d, 2 C), 108.29 (s), 100.55 (d), 81.51 (d), 78.16 (d), 74.27 (d), 70.89 (d), 70.21 (d), 69.10 (d), 67.84 (t), 65.77 (t), 52.22 (d), 36.14 (t), 34.52 (t), 24.58 (t), 23.53 (t), 23.34 (t), and 22.57 (q).

Anal. Calc. for $C_{28}H_{33}NO_8$ (463.53): C, 62.1; H, 7.2; N, 3.0. Found: C, 62.1; H, 7.0; N, 2.9.

The data for compound **8** were: R_F 0.19 (9:1 AcOEt–hexane), $[\alpha]_D^{22}$ +4.0° (*c* 0.90, DMF); ν_{max} 3920 sb, 3280 s, 3080 w, 2930 s, 2860 m, 1630 s, 1620 s, 1450 m, 1420 m, 1380 s, 1335 w, 1300 w, 1235 w, 1210 w, 1170 m, 1145 m, 1090 s, 1040 s, 1000 s, 925 m, 850 m, 760 m, 700 m, and 615 m cm^{-1}; ^{1}H-n.m.r. (DMSO-d_6): δ 8.22 (d, 1 H, $J_{5,NH}$ 8.7 Hz, N*H*), 7.3–7.5 (m, 5 H, Ph-H), 5.77 (s, 1 H, OH-4), 5.60 (s, 1 H, PhC*H*), 5.39 (d, 1 H, $J_{3,OH}$ 4.8 Hz, OH-3), 5.13 (d, 1 H, $J_{6,OH}$ 5.9 Hz, OH-3), 4.32 (dt, 1 H, $J_{1,2} = J_{1',2}$ 6.5, $J_{2,3}$ 4.1 Hz, H-2), 4.08 (dd, 1 H, $J_{9a,9e}$ 9.6, $J_{8,9e}$ 4.4 Hz, H-9e), 3.89 (t, 1 H, $J_{5,6} = J_{5,NH}$ 9.7 Hz, H-5), 3.8 (m, 4 H, H-8,6, 2 H-1), 3.69 (t, 1 H, $J_{9a,9e} = J_{8,9a}$ 9.8 Hz, H-9a), 3.42 (t, 1 H, $J_{2,3}$ 4.6 Hz, H-3), 3.40 (t, 1 H, $J_{6,7} = J_{7,8}$ 9.3 Hz, H-7), 1.94 (s, 3 H, C*H*$_3$CON), and 1.3–1.7 (m, 10 H, 5 C*H*$_2$); ^{13}C-n.m.r.

(DMSO-d_6): δ 172.14 (s), 137.28 (s), 128.77 (d), 127.95 (d, 2 C), 126.35 (d, 2 C), 107.93 (s), 100.77 (d), 99.52 (s), 81.86 (d), 73.78 (d), 71.06 (d), 68.12 (t), 67.83 (t), 64.57 (d), 63.14 (d), 54.56 (d), 34.56 (t), 34.54 (t), 23.60 (t), 23.46 (t), 22.41 (t), 22.29 (t).

Anal. Calc. for $C_{24}H_{33}NO_9$ (479.53): C, 60.1; H, 6.7; N, 2.9. Found: C, 60.0; H, 6.8; N, 2.7.

*5-Acetamido-4,7-di-*O*-acetyl-2,6-anhydro-1,3-*O*-benzylidene-8,9-*O*-cyclohexylidene-5-deoxy-[6-^{2}H]*-D-arabino-L-gulo-*nonitol* (**9**). — Compound **9** was obtained as described for **6**, using Bu_3Sn^2H (obtained from Bu_3SnCl and $LiAl^2H_4$ according to ref. 39) instead of Bu_3SnH for the reductive denitration. The product had R_F 0.36 (AcOEt), $[\alpha]_D^{25}$ −42.5° (*c* 1.0, chloroform), ν_{max} 3380 b, 2940 s, 2860 m, 1750 s, 1670 m, 1540 m, 1450 w, 1430 w, 1370 s, 1230 s, 1160 w, 1095 s, 1040 s, 1005 m, 930 m, 910 w, 850 w, 750 w, and 700 m cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 7.3–7.5 (m, 5 H, Ph-H), 5.62 (d, 1 H, $J_{5,NH}$ 10.0 Hz, N*H*), 5.51 (s, 1 H, PhC*H*), 5.23 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.22 (d, 1 H, $J_{7,8}$ 5.9 Hz, H-7), 4.28 (dd, 1 H, $J_{1a,1e}$ 10.4, $J_{1e,2}$ 4.9 Hz, H-1e), 4.22 (t, 1 H, $J_{4,5} = J_{5,NH}$ 10.2 Hz, H-5), 4.13 (q, 1 H, $J_{7,8} = J_{8,9} = J_{8,9'}$ 6.2 Hz, H-8), 3.85 (m, 2 H, 2 H-9), 3.77 (t, 1 H, $J_{1a,1e} = J_{1a,2}$ 10.2 Hz, H-1a), 3.71 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3), 3.53 (dt, $J_{1a,2} = J_{2,3}$ 9.7, $J_{1e,2}$ 4.8 Hz, H-2), 2.13, 2.08 (2 s, ca. 3 H, C*H*$_3$CO), 1.90 (s, 3 H, C*H*$_3$CON), and 1.3–1.6 (m, 10 H, 5 C*H*$_2$); ^{13}C-n.m.r. ($CDCl_3$): δ 171.76 (s), 170.19 (s), 170.13 (s), 136.95 (s), 129.04 (d), 128.17 (d, 2 C), 125.84 (d, 2 C), 109.02 (s), 101.08 (d), 78.77 (d), 74.72 (d), 73.74 (d), 70.88 (d), 68.72 (d), 68.33 (t) 65.21 (t), 49.14 (d), 46.05 (t), 35.07 (t), 25.01 (t), 23.83 (t), 23.75 (t), 23.02 (q), and 20.94 (q, 2 C); c.i.-m.s.: *m/z* 549 (100, $M^+ + 1$).

Anal. Calc. for $C_{28}{}^1H_{36}{}^2HNO_{10}$ (548.61): C, 61.3; ^{1}H + ^{2}H, 7.0; N, 2.6. Found: C, 61.3; ^{1}H + ^{2}H, 7.2; N, 2.5.

Debenzylidenation of **6**. — A mixture of **6** (6.5 g, 11.9 mmol), methanol (150 mL), and $Pd(OH)_2$/C (1.5 g) was hydrogenated under pressure (5 atm) overnight. Filtration through Celite and evaporation of the solvent afforded 5-acetamido-4,7-di-*O*-acetyl-2,6-anhydro-8,9-*O*-cyclohexylidene-5-deoxy-D-*arabino*-L-*gulo*-nonitol (**10**) (5.40 g, 99%) as a foam. The compound was crystallized from CH_2Cl_2–ether; m.p. 196–198°, R_F 0.51 (7:2:1 AcOEt–MeOH–H_2O), $[\alpha]_D^{20}$ −14.8° (*c* 0.99, chloroform), ν_{max} 3430 sb, 2940 m, 2860 w, 1700 s, 1665 s, 1550 m, 1450 w, 1370 m, 1285 w, 1230 s, 1165 w, 1095 m, 1035 m, 980 w, 930 m, 910 w, 850 w, and 755 w cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 5.97 (d, 1 H, $J_{5,NH}$ 9.8 Hz, N*H*), 5.22 (dd, $J_{6,7}$ 5.9, $J_{7,8}$ 1.9 Hz, H-7), 5.08 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 4.26 (q, 1 H, $J_{8,9} = J_{8,9'} = J_{7,8}$ 6.2 Hz, H-8), 3.99 (dd, 1 H, $J_{1,1}$ 8.5, $J_{1,2}$ 5.9 Hz, H-1), 3.98 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH}$ 10.2 Hz, H-5), 3.80 (m, 3 H, 2 H-9, H-1'), 3.79 (dd, 1 H, $J_{5,6}$ 10.4, $J_{6,7}$ 2.0 Hz, H-6), 3.68 (dt, 1 H, $J_{3,4} = J_{2,3}$ 9.3 Hz, $J_{3,OH}$ 5.3 Hz, H-3), 3.43 (dt, 1 H, $J_{2,3} = J_{1,2}$ 9.4, $J_{1,2}$ 4.1 Hz, H-2), 3.0 (m, 1 H, O*H*), 2.3 (m, 1 H, O*H*), 2.10, 2.09 (2 s, ca. 3 H, C*H*$_3$CO), 1.88 (s, 3 H, C*H*$_3$CON), and 1.3–1.6 (m, 10 H, 5 C*H*$_2$); ^{13}C-n.m.r. ($CDCl_3$): δ 172.15 (s), 170.61 (s), 170.50 (s), 109.50 (s), 77.90 (d), 77.45 (d), 76.81 (d), 74.37 (d), 69.77 (d), 69.60 (d), 65.78 (t), 62.71 (t), 49.69 (d), 36.26 (t), 35.62

(t), 25.08 (t), 23.93 (t), 23.79 (t), 23.10 (q), and 20.90 (q, 2 C); c.i.-m.s.: *m/z* 460 (100, $M^+ + 1$), 363 (10), and 352 (64).

*5-Acetamido-4,7-di-*O*-acetyl-2,6-anhydro-8,9-*O*-cyclohexylidene-5-deoxy-[6-^2H]-*D*-arabino-*L*-gulo-nonitol* (**11**). — As described for **10**, **11** was obtained from **9**; m.p. 196–198°, R_F 0.51 (7:2:1 AcOEt–MeOH–H_2O), $[\alpha]_D^{20}$ −14.0° (*c* 1.02, chloroform), ν_{max} 3400 sb, 2940 s, 2860 m, 1700 s, 1660 s, 1550 s, 1450 m, 1430 w, 1370 s, 1235 s, 1165 m, 1090 s, 1040 s, 940 m, 930 m, 910 w, 850 w, 830 w, and 700 w, cm^{-1}; ^1H-n.m.r. ($CDCl_3$): δ 5.53 (d, 1 H, $J_{5,NH}$ 9.8 Hz, N*H*), 5.17 (dd, $J_{7,8}$ 6.0 Hz, H-7), 5.06 (dd, 1 H, $J_{3,4}$ 10.2, $J_{4,5}$ 9.3 Hz, H-4), 4.26 (q, 1 H, $J_{8,9} = J_{8,9'} = J_{7,8}$ 6.3 Hz, H-8), 4.01 (t, 1 H, $J_{4,5} = J_{5,NH}$ 10.2 Hz, H-5), 3.98 (dd, 1 H, $J_{1,1'}$ 8.7, $J_{1,2}$ 6.1 Hz, H-1), 3.93 (dd, 1 H, $J_{9,9'}$ 11.9, $J_{8,9}$ 6.3 Hz, H-9), 3.86 (dd, 1 H, $J_{1,1'}$ 8.5, $J_{1',2}$ 6.1 Hz, H-1′), 3.84 (dd, 1 H, $J_{9,9'}$ 11.6, $J_{8,9'}$ 5.3 Hz, H-9′), 3.74 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 3.44 (dt, 1 H, $J_{1,2} = J_{2,3}$ 9.7, $J_{1,2}$ 4.1 Hz, H-2), 2.8–3.0 (m, 2 H, 2 O*H*), 2.11, 2.10 (2 s, ca. 3 H, CH_3CO), 1.90 (s, 3 H, CH_3CON), and 1.3–1.6 (m, 10 H, 5 CH_2); ^{13}C-n.m.r. ($CDCl_3$): δ 172.17 (s), 170.46 (s), 170.42 (s), 109.54 (s), 79.82 (d), 77.41 (d), 74.22 (d), 69.78 (d), 69.56 (d), 65.85 (t), 62.72 (t), 49.60 (d), 36.29 (t), 35.00 (t), 25.08 (t), 23.95 (t), 23.79 (t), 2315 (q), and 20.91 (q, 2 C); c.i.-m.s.: *m/z* 461 (100, $M^+ + 1$), and 363 (34).

Anal. Calc. for $C_{21}{}^1H_{32}{}^2HNO_{10}$ (460.50): C, 54.8; $^1H + {}^2H$, 7.4; N, 3.0. Found: C, 54.9; $^1H + {}^2H$, 7.7; N, 3.1.

*Methyl 5-acetamido-3,4,7,8,9-penta-*O*-acetyl-2,6-anhydro-5-deoxy-*D*-arabino-*L*-gulo-nononate* (**12**). — To a solution of **10** (1.0 g, 2.2 mmol) and $NaHCO_3$ (0.7 g, 8.1 mmol) in water (70 mL) was added Pt (from 690 mg of PtO_2). Oxygen (50 L/h) was bubbled through the vigorously agitated (Vibromixer) solution at 90–100°. After 15 h the catalyst was filtered off and the solution was passed through a column of Dowex HCR-W, 16–40 mesh, H^+ form. The eluate was mixed with 0.5M HCl (50 mL) and evaporated to dryness. A solution of diazomethane in ether was added to a solution of the residue in MeOH (60 mL) until gas evolution stopped. The solvent was evaporated and the residue was acetylated overnight in 1:2 acetic anhydride–pyridine. Evaporation of the solvent and purification of the residue by chromatography on SiO_2 (150 g, AcOEt) gave **12** (700 mg; 60%) as a white powder, which was crystallized from CH_2Cl_2–ether. The compound melted at 195–196°, R_F 0.30 (AcOEt), $[\alpha]_D^{20}$ +23.3° (*c* 1.1, chloroform), ν_{max} 3380 b, 2960 w, 1755 s, 1665 m, 1540 w, 1440 w, 1370 m, 1295 m, 1230 s, 1095 m, 1045 m, and 975 w cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.99 (s), 170.49 (s), 170.28 (s), 170.20 (s), 170.17 (s), 169.28 (s), 167.06 (s), 77.00 (d), 76.44 (d), 73.44 (d), 71.09 (d), 69.21 (d), 67.65 (d), 62.28 (t), 52.78 (q), 49.30 (d) 23.04 (q), 20.85 (q), 20.74 (q, 2 C), 20.60 (q), and 20.49 (q); c.i.-m.s.: *m/z* 534 (100, $M^+ + 1$), and 474 (10).

Anal. Calc. for $C_{22}H_{31}NO_{14}$ (533.49): C, 49.5; H, 5.9; N, 2.6. Found: C, 49.3; H, 6.1; N, 2.8.

Minor product of the oxidation reaction. — A solution of **10** (1.0 g) was treated as just described but HCl was omitted after the ion-exchange chromatography. This gave **12** (505 mg) together with methyl 5-acetamido-3,4,7-tri-*O*-acetyl-

2,6-anhydro-8,9-*O*-cyclohexylidene-5-deoxy-D-*arabino*-L-*gulo*-nononate (**13**) (85 mg), R_F 0.31 (AcOEt), $[\alpha]_D^{20}$ +12.5° (*c* 0.99, chloroform); ν_{max} 3380 b, 2940 m, 2860 w, 1755 w, 1665 m, 1540 m, 1440 m, 1370 m, 1290 w, 1225 s, 1165 w, 1100 m, 1035 m, 980 w, and 930 w cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.84 (s), 170.37 (s), 170.31 (s), 169.18 (s), 167.26 (s), 109.02 (s), 76.63 (d), 75.99 (d), 74.41 (d), 73.36 (d), 69.38 (d), 68.66 (d), 65.09 (t), 52.20 (d), 48.97 (d), 35.92 (t), 34.77 (t), 24.81 (t), 23.66 (t), 23.55 (t), 22.80 (q), 20.78 (q), 20.49 (q), and 20.27 (q).

*Methyl 5-acetamido-3,4,7,8,9-penta-*O*-acetyl-2,6-anhydro-5-deoxy-[6-^2H]-*D*-arabino-L-gulo-nononate* (**14**). — As described for **12**, **14** was obtained from **11**; m.p. 197–198°, R_F 0.30 (AcOEt); $[\alpha]_D^{25}$ +23.8° (*c* 1.03, chloroform); ν_{max} 3400 b, 2960 s, 1755 s, 1660 m, 1540 w, 1444 w, 1370 m, 1295 m, 1225 s, 1095 m, 1040 w, and 980 w cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.72 (s), 170.32 (s), 170.25 (s), 170.16 (s), 170.10 (s), 169.19 (s), 167.09 (s), 76.06 (d), 73.33 (d), 71.28 (d), 69.31 (d), 67.56 (d), 62.22 (t), 52.55 (q), 48.99 (d), 22.78 (q), 20.69 (q), 20.54 (q), 20.44 (q, 2 C), 20.32 (q); c.i.-m.s.: *m/z* 535 (100, $M^+ + 1$), and 475 (15, $M^+ + 1 - 60$).

Anal. Calc. for $C_{22}{}^1H_{30}{}^2HNO_{14}$ (534.48): C, 49.4; $^1H + {}^2H$, 6.0; N, 2.6. Found: C, 49.2; $^1H + {}^2H$, 6.0; N, 2.6.

*Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-D-galacto-non-2-enonate* (**15**). — A solution of crystalline **12** (600 mg, 1.12 mmol), dry $CHCl_3$ (15 mL), and DBU (0.9 mL, 4.5 mmol) was heated to reflux. After 24 h another portion of DBU (0.9 mL) was added. After refluxing again for 24 h, the solution was poured on a column of SiO_2 (100 g). Elution with AcOEt afforded **15** (506 mg; 95%) as a white powder. All spectral data were identical with those of a sample prepared from Neu5Ac (**1**) (ref. 47).

*Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-[6-^2H]-*D*-glycero-D-galacto-non-2-enonate* **16**). — As described for **15**, **16** was obtained from **14**; R_F 0.31 (AcOEt), $[\alpha]_D^{25}$ +68.7° (*c* 1.09, chloroform); u.v. (EtOH): λ_{max} 238 (ε 6000) and 203 (ε 4700) nm; ν_{max} 3400 b, 1750 s, 1660 m, 1540 m, 1440 m, 1370 s, 1225 s, 1150 w, 1110 w, 1050 m, 960 w, and 770 w cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.65 (s), 170.46 (s), 170.12 (s), 170.05 (s), 169.98 (s), 161.49 (s), 144.89 (s), 107.86 (d), 70.70 (d), 67.97 (d), 67.45 (d), 61.85 (t), 52.39 (q), 46.13 (d), 22.89 (q), 20.67 (q, 2 C), and 20.55 (q, 2 C); c.i.-m.s.: *m/z* 416 (24, $M^+ + 1 - 59$) and 415 (100, $M^+ + 1 - 60$).

Anal. Calc. for $C_{20}{}^1H_{26}{}^2HNO_{12}$ (474.43): C, 50.6; $^1H + {}^2H$, 6.0; N, 3.0. Found: C, 50.7; $^1H + {}^2H$, 6.1; N, 2.9.

Enol ether **15** *and oxazoline* **19** *from* N-*acetylneuraminic acid*. — To a solution of **18*** (7.15 g, 13.4 mmol) in dry CH_3NO_2 under N_2 at 4°, trimethylsilyl trifluoromethanesulfonate (TMSOTf) (4.86 mL, 26.8 mmol) was added within 2–3 min. After the mixture had been stirred for 15 h at 4°, 50 g of K_2CO_3 was added, and the salts were removed by filtration through Celite. Concentration of the filtrate left a residue which was dissolved in CH_2Cl_2 (100 mL), and the solution was washed with

*Obtained from **1** according to a described procedure[36].

water (3 × 50 mL). After drying over $MgSO_4$ and evaporation of the solvent, purification of the residue by chromatography on SiO_2 (1:1→1:4 gradient, toluene–AcOEt) afforded **15** (4.37 g; 69%) (ref. 46) and **19** (0.83 g; 15%) (ref. 11).

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-bromo-3,5-dideoxy-*D*-erythro-β-*L*-gluco-nonulopyranosid)onate* (**22**), *and methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-bromo-3,5-dideoxy-*D*-erythro-α-*L*-manno-nonulopyranosid)onate* (**20**). — A solution of **15** (300 mg, 0.63 mmol), NBS (170 mg, 0.96 mmol), and methanol (10 mL) was stirred for 2 h at room temperature. The solvent was evaporated and the two isomers were separated by chromatography on SiO_2 (150 g, 1:1, 2:1, 3:1, and finally 4:1 AcOEt–hexane as eluants) to give **22** (180 mg; 49%) and **20** (178 mg; 48%). Compound **22** had m.p. 156–158°, R_F 0.33 (AcOEt), $[\alpha]_D^{25}$ −71.3° (*c* 0.97, chloroform); ν_{max} 3400 b, 3250 m, 3060 w, 2980 w, 2930 w, 1750 s, 1690 w, 1660 m, 1550 m, 1430 w, 1370 m, 1310 m, 1220 s, 1175 w, 1100 w, 1050 m, 1030 m, 950 w, and 800 w cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.48 (s), 170.38 (s), 170.26 (s), 169.80 (s), 169.35 (s), 167.92 (s), 99.19 (s), 72.79 (d), 72.41 (d, C-6), 68.37 (d), 66.81 (d), 62.21 (t), 52.42 (q), 52.26 (q), 50.19 (d), 49.55 (d), 22.75 (q), 20.67 (q), 20.63 (q), 20.52 (q), and 20.43 (q); c.i.-m.s.: *m/z* 586 (100, M^+ + 3) and 584 (90, M^+ + 1).

Anal. Calc. for $C_{21}H_{30}BrNO_{13}$ (584.38): C, 43.2; H, 5.2; Br, 13.7; N, 2.4. Found: C, 43.3; H, 5.2; Br, 13.5; N, 2.6.

Compound **20** melted at 177–179°, R_F 0.27 (AcOEt), $[\alpha]_D^{25}$ +26.5° (*c* 1.03, chloroform); ν_{max} 3400 b, 2960 w, 1750 s, 1690 w, 1665 m, 1540 m, 1440 m, 1370 m, 1300 m, 1225 s, 1160 m, 1130 w, 1100 w, 1050 s, 800 w, and 710 w cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.69 (s), 170.59 (s), 170.46 (s), 170.34 (s), 169.19 (s), 165.34 (s), 100.37 (s), 71.78 (d), 71.44 (d, C-6), 68.40 (d), 68.21 (d), 62.36 (t), 52.85 (q), 52.37 (q), 51.16 (d), 45.62 (d), 23.03 (q), 20.91 (q), 20.67 (q, 2 C), and 20.51 (q); c.i.-m.s.: *m/z* 586 (100, M^+ + 3) and 584 (90, M^+ + 1).

Anal. Found: C, 43.4; H, 5.4; Br, 13.4; N, 2.2.

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-bromo-3,5-dideoxy-[6-^2H]-*D*-erythro-β-*L*-gluco-nonulopyranosid)onate* (**23**) *and methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-bromo-3,5-dideoxy-[6-^2H]-*D*-erythro-α-*L*-manno-nonulopyranosid)onate* (**21**). — As described for **22** and **20**, **23** and **21** were obtained from **16**. For **23** we found m.p. 150–152°, R_F 0.36 (AcOEt), $[\alpha]_D^{25}$ −70.0° (*c* 0.99, chloroform); ν_{max} 3400 b, 3250 m, 3060 w, 2980 w, 2850 w, 1750 s, 1690 w, 1660 m, 1550 m, 1435 m, 1370 s, 1310 m, 1225 s, 1170 m, 1050 s, and 790 cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.58 (s), 170.44 (s), 170.33 (s), 169.87 (s), 169.41 (s), 167.97 (s), 99.22 (s), 72.86 (d), 68.44 (d), 66.81 (d), 62.24 (t), 52.45 (q), 52.28 (q), 50.14 (d), 49.52 (d), 22.78 (q), 20.69 (q), 20.64 (q), 20.55 (q), and 20.46 (q); c.i.-m.s.: *m/z* 587 (100, M^+ + 3) and 585 (80, M^+ + 1).

Anal. Calc. for $C_{21}{}^1H_{29}{}^2HBrNO_{13}$ (585.37): C, 43.1; ^1H + ^2H, 5.3; Br, 13.7; N, 2.4. Found: C, 43.1; ^1H + ^2H, 5.0; Br, 13.5; N, 2.5.

Compound **21** had m.p. 177–179°, R_F 0.27 (AcOEt), $[\alpha]_D^{25}$ +24.1° (*c* 1.1, $CHCl_3$); ν_{max} 3400 b, 1750 s, 1690 w, 1665 m, 1550 w, 1440 w, 1370 m, 1305 w, 1225

s, 1160 w, 1100 w, and 1050 m cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.64 (s), 170.54 (s), 170.39 (s), 170.25 (s), 170.22 (s), 165.31 (s), 100.32 (s), 71.74 (d), 68.40 (d), 68.09 (d), 62.32 (t), 52.80 (q), 52.30 (q), 51.14 (d), 45.36 (d), 22.92 (q), 20.84 (q), 20.76 (q), 20.59 (q), and 20.43 (q); c.i.-m.s. *m/z* 587 (100, $M^+ + 3$) and 585 (90, $M^+ + 1$).

Anal. Found: C, 43.1; $^1H + ^2H$, 5.4; Br, 13.5; N, 2.4.

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-β-*D*-galacto-nonulopyranosid)onate* (**24**). — Compound **24** was obtained from **20** in a yield of 95%, as described below for the conversion of **22** into **26**. The spectral data for **24** agreed with those recorded in the literature (^1H-n.m.r. see refs. 13, 40). The compound had R_F 0.25 (AcOEt), $[\alpha]_D^{25}$ −12.0° (*c* 1.05, chloroform).

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-[6-^2H]-*D*-glycero-β-*D*-galacto-nonulopyranosid)onate* (**25**). — Obtained from **21**, the deutero analog **25** had R_F 0.25 (AcOEt), $[\alpha]_D^{25}$ −12.9° (*c* 1.06, chloroform); ν_{max} 3380 b, 2960 w, 1750 s, 1690 m, 1660 m, 1545 m, 1440 m, 1370 s, 1320 m, 1230 s, 1165 w, 1115 m, 1075 w, 1045 s, 955 m, and 920 w cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.93 (s), 170.78 (s), 170.53 (s), 170.33 (s), 170.10 (s), 167.25 (s), 98.75 (s), 72.06 (d), 68.85 (d), 68.23 (d), 62.29 (t), 52.63 (q), 51.16 (q) 48.85 (d), 37.14 (t), 22.93 (q), 20.90 (q), 20.76 (q), and 20.67 (q, 2 C); c.i.-m.s.: *m/z* 507 (16, $M^+ + 1$), 474 (21, $M^+ + 1 - 32$), 447 (7, $M^+ + 1 - 60$), and 415 (100, $M^+ + 1 - 92$).

Anal. Calc. for $C_{21}{}^1H_{30}{}^2HNO_{13}$ (506.47): C, 49.8; $^1H + ^2H$, 6.4; N, 2.8. Found: C, 49.6; $^1H + ^2H$, 6.1; N, 2.7.

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-nonulopyranosid)onate* (**26**). — A solution of 83 mg **22** (0.14 mmol), Bu_3SnH (200 μL, 0.76 mmol), AIBN (5 mg), and dry toluene (2 mL) was heated for 1 h at 90°. After cooling, the solvent was removed *in vacuo*, and the residue was purified by chromatography on SiO_2 (40 g, 1:1 AcOEt–hexane, then AcOEt) to yield **26** (69 mg; 97%) as a white powder. The spectral data for **26** agreed with the data in the literature (^1H-n.m.r. and ^{13}C-n.m.r. see refs. 40, 41). The compound had R_F 0.27 (AcOEt), $[\alpha]_D^{25}$ −19° (*c* 1.04, methanol), −24.9° (*c* 1.05, chloroform); lit.[42] −19.0° (MeOH).

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-[6-^2H]-*D*-glycero-α-*D*-galacto-nonulopyranosid)onate* (**27**). — As described for **26**, **27** was obtained from **23**; R_F 0.27 (AcOEt), $[\alpha]_D^{25}$ −23.5° (*c* 1.04, chloroform); ν_{max} 3400 b, 1750 s, 1660 m, 1550 w, 1450 w, 1370 m, 1220 s, 1140 m, 1115 w, and 1050 m cm^{-1}; ^{13}C-n.m.r. ($CDCl_3$): δ 170.91 (s), 170.61 (s), 170.23 (s), 170.08 (s, 2 C), 168.12 (s), 98.88 (s), 69.01 (d), 68.56 (d), 67.23 (d), 62.32 (t), 52.67 (q), 52.35 (q), 49.20 (d), 37.78 (t), 23.07 (q), 21.03 (q), 20.76 (q, 2 C), and 20.69 (q); c.i.-m.s.: *m/z* 507 (61, $M^+ + 1$), 475 (23, $M^+ + 1 - 32$), 447 (72, $M^+ + 1 - 60$), and 415 (100, $M^+ + 1 - 92$).

Anal. Calc. for $C_{21}{}^1H_{30}{}^2HNO_{13}$ (506.47): C, 49.8; $^1H + ^2H$, 6.4; N, 2.8; Found: C, 49.5; $^1H + ^2H$, 6.4; N, 2.7.

N-*Acetylneuraminic acid* (**1**). — According to the procedure of Zbiral[13], **26** and **24** were converted into **1**. Starting from **26** (180 mg), freeze-dried **1** (85 mg;

77%) was obtained. Crystallization[43–45] from H_2O (0.3 mL) and AcOH (4.5 mL) gave pure **1** (39 mg). Similarly, **24** gave freeze-dried **1** in 70% yield, m.p. 178–180°, $[\alpha]_D^{25}$ −31.5° (*c* 1.05, water); lit.[45] m.p. 181–183°, $[\alpha]_D^{20}$ −32.1° (*c* 1.3, water).

*5-Acetamido-3,5-dideoxy-[6-^2H]-*D*-galacto-nonulopyranosonic acid* (**17**). — As described for **1**, **17** was obtained from **27** or **25**; m.p. 175–177°, R_F 0.39 (7:3 (*n*-PrOH–H_2O), $[\alpha]_D^{25}$ −31.6° (*c* 1.02, water); ν_{max} 3400–2500 b, 2940 w, 1730 m, 1630 s, 1550 m, 1380 w, 1320 w, 1230 w, 1140 m, 1070 m, 1030 m, 970 w, 940 w, and 850 cm^{-1}; ^{13}C-n.m.r. (D_2O): δ 175.26 (s), 173.51 (s), 95.64 (s), 70.56 (d), 68.59 (d), 67.08 (d), 63.60 (t), 52.43 (d), 39.24 (t), and 22.51 (q).

Anal. Calc. for $C_{11}{}^1H_{18}{}^2HNO_9$ (310.27): C, 42.6; ^1H + ^2H, 6.5; N, 4.5. Found: C, 42.2; ^1H + ^2H, 6.3; N, 4.4.

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