Note

The synthesis of *N*-acetylneuraminyl-(2→3)- and -(2→6)-hexoses

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The development of syntheses of oligosaccharides containing terminal sialic acid moieties is of importance in connection with structural investigations of sialobiopolymers and with mechanistic studies of neuraminidases (E.C. 3.2.1.18). Recently[1], we described a synthesis of "*O*-, *S*, and *N*-glycosides" of *N*-acetylneuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid, NANA), which were used as substrates and inhibitors of bacterial and viral neuraminidases[2]. We now report on the synthesis of the dissaccharides α-*N*-acetylneuraminyl-(2→6)-D-glucose (**1**), α-*N*-acetylneuraminyl-(2→6)-D-galactose (**2**), α-*N*-acetylneuraminyl-(2→6)-2-acetamido-2-deoxy-D-glucose (**3**), α-*N*-acetylneuraminyl-(2→3)-D-glucose (**4**), and α-*N*-acetylneuraminyl-(2→3)-2-acetamido-2-deoxy-D-glucose (**5**), and their behaviour as substrates for the neuraminidase of *Vibrio cholerae* and influenza virus.

Disaccharides **1–5** were prepared by condensation of the acetylated glycosyl chloride (**6**) of methyl *N*-acetylneuraminate with the corresponding, partially protected monosaccharides (see Experimental), followed by conventional removal of the protecting groups. Final purification was achieved by preparative paper chromatography to give disaccharides **1–5** in 8–18% yield.

In addition to the use of elemental analysis and periodate-oxidation data, the structures of **1–5** were confirmed as follows. Each disaccharide gave a colour reaction typical of sialic acids with the Svennerholm and Ehrlich reagents, and for reducing sugars with aniline hydrogen phthalate. Acid hydrolysis of **1–5** gave rise to NANA and the corresponding hexose. Disaccharides **1–5** were completely hydrolysed with bacterial or viral neuraminidases, thereby establishing the α-D configuration of the ketoside linkage. Hydrolyses with neuraminidase from *V. cholerae* were performed by incubation in 0.1M acetate buffer (pH 5.9) containing 0.9% of sodium chloride and 0.1% of calcium chloride at 37°. At a mM substrate concentration, the rate of enzymic hydrolysis (measured by NANA release[10]) was a linear function of enzyme concentration in the range 0.3–1.0 mg/ml (300–1000 units of neuraminidase activity[3]). The dependence of the initial rate of neuraminidase-catalysed hydrolysis, calculated by extrapolation to zero-time for an enzyme concentration of 0.5 mg/ml, was in accordance with Michaelis–Menten kinetics[4]. A Lineweaver–Burk plot gave values for K_m and V_{max} (see Table I). By analogy with natural *N*-acetylneuraminyl-lactoses,

the hydrolysis of the (2→3)-linked-isomers **4** and **5** proceeded at a greater rate than for the (2→6)-linked isomers **1–3** (*cf.* Refs. 11 and 12). The stereochemistry of the reduced monosaccharides in the disaccharides **1–5** and the nature of their substituents at C-2 do not effect the values of K_m and V_{max}.

TABLE I

MICHAELIS CONSTANT (K_m) AND MAXIMAL VELOCITY (V_{max}) VALUES FOR HYDROLYSIS OF THE SYNTHETIC DISACCHARIDES **1–5** WITH *V. cholerae* NEURAMINIDASE

Disaccharide	K_m (M × 10^3)	V_{max} (M × 10^5.min^{-1})
NANA-(2→6)-D-Glc (**1**)	6.7	3.3
NANA-(2→6)-D-Gal (**2**)	5.9	5.0
NANA-(2→6)-D-GlcNAc (**3**)	5.2	2.0
NANA-(2→3)-D-Glc (**4**)	3.3	4.0
NANA-(2→3)-D-GlcNAc (**5**)	2.9	3.5

Viral neuraminidase also exhibited a greater specificity for the (2→3)-neuraminoside bonds in disaccharides **4** and **5**. This was shown by the hydrolysis of compounds **1–5** with a purified preparation from the A2 (U.S.S.R. 0395/69) strain of influenza virus (Table II).

TABLE II

RELEASE[a] OF *N*-ACETYLNEURAMINIC ACID FROM DISACCHARIDES **1–5** BY INFLUENZA VIRAL NEURAMINIDASE

Disaccharide (2mM)	*Quantity of released NANA* (*moles* × 10^4)
NANA-(2→6)-D-Glc (**1**)	4.45
NANA-(2→6)-D-Gal (**2**)	2.66
NANA-(2→6)-D-GlcNAc (**3**)	5.30
NANA-(2→3)-D-Glc (**4**)	8.00
NANA-(2→3)-D-GlcNAc (**5**)	11.00

[a]After incubation for 30 min in 0.3M phosphate buffer (pH 5.8) at 37°.

EXPERIMENTAL

Optical rotations were measured with a Hilger M-142 polarimeter. Paper chromatography was carried out on "Goznak" paper with butyl alcohol saturated with water–pyridine–water (6:4:6, v/v). Detection was made by aniline hydrogen phthalate and Ehrlich's reagent.

α-N-Acetylneuraminyl(2→6)-D-glucopyranose (**1**). — 1,2,3,4-Tetra-*O*-acetyl-*β*-D-glucopyranose[6] (2 mmoles) was dissolved in chloroform (5 ml), silver carbonate (0.7 g) and Drierite (2 g) were added, and the mixture was stirred for 12 h in the

dark. To the stirred mixture was added, dropwise during 1 h, a solution of the acetylated glycosyl chloride **6** (2 mmoles) in chloroform (3 ml), and stirring was continued overnight. The precipitate was filtered off and washed with chloroform, and the combined filtrates and washings were evaporated. The residue was dissolved in methanol (5 ml), and M methanolic sodium methoxide (0.5 ml) was added. After 3 h at room temperature, 2M sodium hydroxide (0.5 ml) was added, and the mixture was left overnight. After deionisation with Amberlite IR-120 (H^+) resin at −30°, the solution was evaporated to dryness. A solution of the residue in water (2 ml) was freeze-dried to yield a crude product which was purified by preparative paper chromatography, to give **1** as an amorphous powder (17%), R_{NANA} 0.25, $[\alpha]_D^{20}$ +21° (*c* 0.9, water); it consumed 5.2 moles of periodate per mole.

Anal. Calc. for $C_{17}H_{29}NO_{14}$: C, 43.31; H, 6.20; N, 2.98. Found: C, 43.62; H, 6.24; N, 3.20.

α-N-*Acetylneuraminyl-(2→6)*-D-*galactopyranose* (**2**). — This was prepared, as described above, from 1,2,3,4-tetra-*O*-acetyl-β-D-galactopyranose[7] and chloride **6**. Yield, 18.3%; R_{NANA} 0.23, $[\alpha]_D^{20}$ +23° (*c* 0.2, water). It consumed 5.1 moles of periodate per mole.

Anal. Calc. for $C_{17}H_{29}NO_{14}$: C, 43.31; H, 6.20; N, 2.98. Found: C, 43.74; H, 6.35; N, 2.70.

α-N-*Acetylneuraminyl-(2→6)-2-acetamido-2-deoxy*-D-*glucose* (**3**). — This was prepared, as described above, from 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-D-glucose[8] and chloride **6**. Yield, 16%; R_{NANA} 0.6, $[\alpha]_D^{20}$ +20.5° (*c* 0.2, water). It consumed 3.2 moles of periodate per mole.

Anal. Calc. for $C_{19}H_{32}N_2O_{14}$: C, 44.53; H, 6.29; N, 5.47. Found: C, 44.85; H, 6.42; N, 5.30.

α-N-*Acetylneuraminyl-(2→3)*-D-*glucopyranose* (**4**). — 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose[6] (0.7 g) was condensed with chloride **6** (1.4 g), and the product was deacetylated and saponified as described previously. The mixture was treated with Amberlite IR-120 (H^+) resin to pH 3.5, the resin was filtered off and washed with aqueous methanol, and the combined filtrates and washings were evaporated. The residue was purified by paper chromatography to give the title compound (11%), R_{NANA} 0.65, $[\alpha]_D^{20}$ +19.5° (*c* 0.2, water); it consumed 3.5 moles of periodate per mole.

Anal. Calc. for $C_{17}H_{29}NO_{14}$: C, 43.31; H, 6.20; N, 2.98. Found: C, 43.68; H, 5.78; N, 2.85.

α-N-*Acetylneuraminyl-(2→3)-2-acetamido-2-deoxy*-D-*glucose* (**5**). — 2-Acetamido-1-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-glucose[9] (0.8 g) was condensed with chloride **6** (1 g), and the product was deacetylated and saponified as described above. The residue was treated with hydrogen over 5% palladium-on-carbon in methanol (10 ml) for 2 days at room temperature. The catalyst was filtered off and washed with methanol (4 × 30 ml) and the combined filtrates and washings were evaporated. The residue was purified by paper chromatography to give **5** (7.9%), R_{NANA} 0.4, $[\alpha]_D^{20}$ +18° (*c* 0.2, water); it consumed 2.3 moles of periodate per mole.

Anal. Calc. for $C_{19}H_{32}N_2O_{14}$: C, 44.53; H, 6.29; N, 5.47. Found: C, 44.21; H, 6.37; N, 5.25.

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