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2,3-DEHYDRO-4-EPI-*N*-ACETYLNEURAMINIC ACID; A NEURAMINIDASE INHIBITOR*

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ABSTRACT

Treatment of *N*-acetylneuraminic acid methyl ester with sulfuric acid and acetic anhydride at 50° followed by deacetylation gave 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid methyl ester and methyl 5-acetamido-2,6-anhydro-2,3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (2,3-dehydro-4-epi-NeuAc methyl ester) in equal yields (~40% each). The structure of the latter was ascertained primarily from analysis of its mass spectrum and ^{1}H- and ^{13}C-nuclear magnetic resonance spectra. The relative proportions of these two glycals in the foregoing reaction was dependent on temperature, as at 0°, the yield of 2,3-dehydro-4-epi-NeuAc was markedly diminished. A minor by-product of this acetylation reaction was 2-methyl-(methyl 7,8,9-tri-*O*-acetyl-2,6-anhydro-2,3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate)-[4,5-*d*]-2-oxazoline. Based upon this finding and additional interconversion experiments, a mechanism involving the intermediacy of the latter oxazoline to account for the epimerization is proposed. These glycals and their methyl esters are competitive inhibitors of *Arthrobacter sialophilus*, neuraminidase, suggesting that the 4-hydroxyl group must be equatorially oriented for maximal enzyme inhibition.

INTRODUCTION

We have recently suggested that 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (2,3-dehydro-NeuAc) and its methyl ester are transition-state analogs for *Arthrobacter sialophilus* neuraminidase, and that substrates are distorted upon binding to the enzyme[1,2]. The sterically appropriate inhibitor 2,3-dehydro-NeuAc and its derivatives were originally prepared by Meindl and Tuppy[3,4] by a synthetic route involving 4,7,8,9-tetra-*O*-acetyl-*N*-acetylneuraminic acid methyl ester chloride as a key intermediate. In this communication, we describe a more-direct synthesis of 2,3-dehydro-NeuAc utilizing acetic anhydride and sulfuric acid, which also leads to the biologically active diastereomer, 5-acetamido-2,6-anhydro-2,3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonic acid (2,3-dehydro-4-epi-NeuAc), as well as to 2-methyl-(methyl 7,8,9-tri-

*Part 1 of a series Synthesis of Glycal Antagonists.

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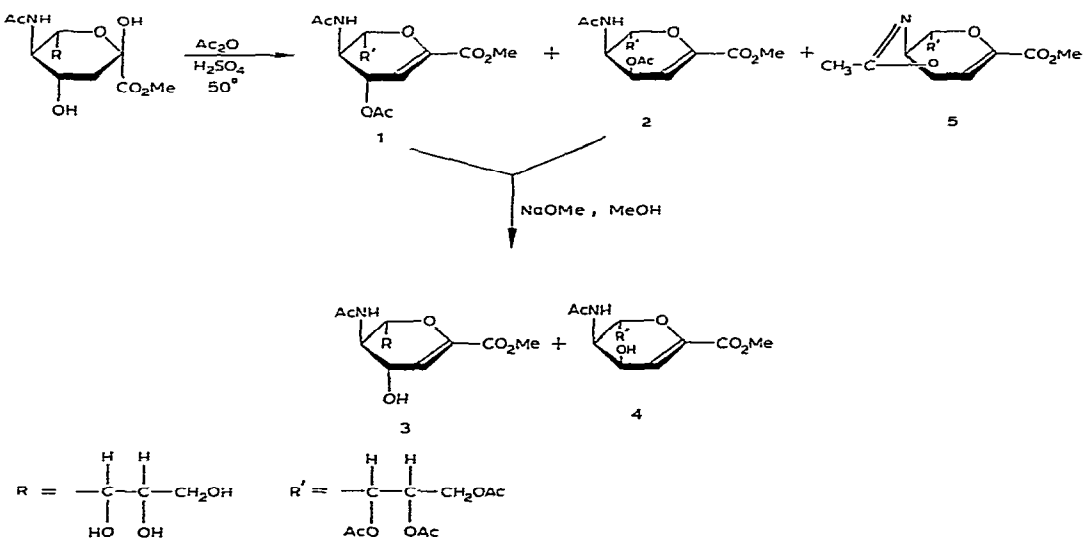
O-acetyl-2,6-anhydro-2,3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate)-[4,5-*d*]-2-oxazoline. We also report preliminary findings on the relative effectiveness of these glycals in inhibiting *A. sialophilus* neuraminidase.

RESULTS AND DISCUSSION

Treatment of *N*-acetylneuraminic acid methyl ester with acetic anhydride in the presence of a catalytic amount of concentrated sulfuric acid at 50° gave a mixture containing 4,7,8,9-tetra-*O*-acetyl-2,3-dehydro-2-deoxy-NeuAc methyl ester (**1**), and 4,7,8,9-tetra-*O*-acetyl-2,3-dehydro-2-deoxy-4-epi-NeuAc methyl ester (**2**), (Scheme 1). These peracetylated diastereomers were not resolved by t.l.c. or by l.c. (Whatman Partisil-10 silica gel column or Whatman Partisil-10-ODS-3 column) using a variety of solvent combinations, but were detected following analysis of the reaction products by 60- and 100-MHz n.m.r. spectroscopy. These determinations revealed two olefinic proton resonances at δ 6.10 and 6.28, which were taken to indicate an isomeric mixture. Deacetylation of this mixture with a catalytic amount of sodium methoxide in dry methanol, and resolution by l.c. (Whatman Partisil-10-ODS-3 column) with water as solvent, afforded 2,3-dehydro-NeuAc methyl ester (**3**, yield 40%), and 4-epi-2,3-dehydro-NeuAc methyl ester (**4**, yield 41%). Product **3** was identical in all physical and chemical characteristics to the substance prepared from 4,7,8,9-tetra-*O*-acetyl-NeuAc methyl ester chloride and triethylamine[3]. The mass spectra of **3** and **4** showed the identical parent peak (*m/z* 305), indicating that compound **4** is an isomer of **3**. We have also generated 2,3-dehydro-4-oxo-9-*O*-trityl-NeuAc by oxidation with manganese dioxide of either 2,3-dehydro-9-*O*-trityl-NeuAc or 2,3-dehydro-4-epi-9-*O*-



Scheme 1

TABLE I

^{1}H-N.M.R. DATA[a] FOR 2,3-DEHYDRO-NeuAc DERIVATIVES (3 AND 4)

Compound	*Chemical shifts (δ)*								
	H-3	*H-4*	*H-5*	*H-6*	*H-8*	*H-9a*	*H-7 H-9b*	*CH_3 (NAc)*	*CH_3*
3	6.265	4.732	4.308	4.503	4.143	4.098	3.872	2.82	4.037
	$J_{3,4}$ 2.44	$J_{3,4}$ 2.44	$J_{5,6}$ 9.15	$J_{5,6}$ 10.98					
		$J_{4,5}$ 9.15							
4	6.240	4.525	4.459	4.459	4.172	4.105	3.884	2.77	4.037
	$J_{3,4}$ 6.10								

[a]Measured in D_2O with sodium 4,4-dimethyl-4-silapentane-1-sulfonate as internal standard.

TABLE II

^{13}C-N.M.R. DATA[a] FOR 2,3-DEHYDRO-NeuAc DERIVATIVES (3 AND 4)

Compound	*Chemical shifts (p.p.m.)*											
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-8*	*C-9*	*C-10*	*C-11*	*C-12*
											(Me)	*(Ac)*
3	165.2	143.8	113.2	68.4	50.6	77.0	69.0	71.0	64.0	176.4	54.2	23.0
4	165.8	145.4	111.0	61.0	48.5	73.0	69.0	71.0	63.8	175.8	54.0	23.0

[a]Measured in D_2O with sodium 4,4-dimethyl-4-silapentane-1-sulfonate as internal standard.

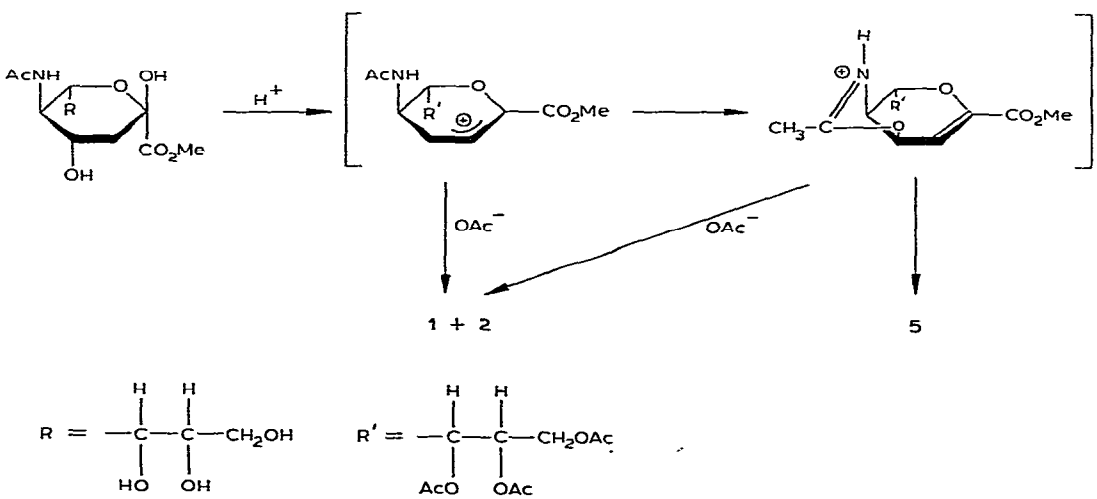
trityl-NeuAc. The ^{1}H-n.m.r. spectrum (100 MHz) of the enone showed a singlet at δ 6.20 instead of the doublet present in the starting material (see Table I), thereby confirming that **3** and **4** are epimers at C-4.

Compound **4** was identified by analysis of its 360-MHz ^{1}H-n.m.r. and ^{13}C-n.m.r. spectra (Tables I and II). Carbon chemical shifts of **4** were assigned relative to the literature values[5] of **3**. Proton assignments for **3** and **4** were made by single-frequency, decoupling experiments. The major differences between **3** and **4** were found to be the chemical shifts and coupling constants of H-3, -4, and -5. The $J_{3,4}$ value for **3** was 2.44 Hz, whereas $J_{3,4}$ for **4** was 6.10 Hz; this was interpreted as indicating epimerization at C-4. These coupling constants are similar to those reported by Sharma and Brown[6] for the $J_{2,3}$ allylic coupling in 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*arabino*-hex-1-enitol and its *ribo* epimer. In ^{1}H-n.m.r., the change in $J_{5,6}$ of **4**, the downfield shift of H-3, -4, -5, and -6, the upfield shift of H-2 as compared with **3** (see Table I and II), and the large difference in optical rotation of **3** ($[\alpha]_D^{25}$ +43.7°) and **4** ($[\alpha]_D^{27}$ −130.3°), are all consistent with a change in the conformation of the pyranose ring. Comparable structural analyses and assignment for 4-*O*-methyl-*N*-

acetylneuraminic and epi-4-*O*-methyl-*N*-acetylneuraminic acids were recently reported by Beau and Sinaÿ[7,8].

The relative proportions of **1** and **2** were dependent upon the temperature of the mixture. Treatment of NeuAc methyl ester with acetic anhydride and sulfuric acid at 0° also resulted in dehydration, as indicated by the presence of alkenic protons in the n.m.r. spectrum. However, this reaction condition resulted in only partial acetylation. Deacetylation of this mixture gave products **3** and **4** in the ratio of 4:1, suggesting that the extent of epimerization was under kinetic control. This conclusion was also supported by the observation that acetate **2** could be isomerized to the thermodynamically more-stable product **1** by refluxing in acetic acid for 3–4 days.

It was observed during the preparation of **1** and **2** that a small proportion (2–4%) of an additional product, identified as 2-methyl-(methyl 7,8,9-tri-*O*-acetyl-2,6-anhydro-2,3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate)-[4,5-*d*]-2-oxazoline (**5**) accumulated. This compound was assigned structure **5** primarily on the basis of its ^{1}H-n.m.r. (100 MHz), i.r., and mass spectra. The coupling constants ($J_{4,5}$ 7.0; $J_{5,6}$ 6.0 Hz) are consistent with the *talo* configuration for the oxazoline. Treatment of **5** with sulfuric acid and acidic anhydride, followed by deacetylation, gave **3** and **4** in equal quantities (total yield, 70%) analogous to the observations reported by Salo and Fletcher[9]. Conversely, treatment of acetates **1** and **2** with acetic anhydride and sulfuric acid, as already described, gave a small amount of **5** (yield 9%).



Scheme 2

The formation of the oxazoline during the synthesis of **1** and **2** suggests a possible mechanism for epimerization of NeuAc methyl ester (Scheme 2). The initial step in this mechanism is similar to the one postulated by Priebe and Zamojski[10] for the acid-catalyzed reaction of thiols with glycals, and involves the formation of

TABLE III

INHIBITION CONSTANTS (K_I) OF 2,3-DEHYDRO-NEUAC AND 2,3-DEHYDRO-4-EPI-NEUAC AND THEIR METHYL ESTERS FOR *Arthrobacter sialophilus* NEURAMINIDASE

Inhibitor	*Inhibition constant* (K_I)[a] (M)
2,3-Dehydro-NeuAc	1.6×10^{-6}
2,3-Dehydro-NeuAc methyl ester	2.1×10^{-4}
2,3-Dehydro-4-epi-NeuAc	4.8×10^{-5}
2,3-Dehydro-4-epi-NeuAc methyl ester	7.1×10^{-4}

[a]Calculated from secondary plots of $K_{m\ app}$ *vs.* inhibitor concentration obtained by determining enzyme-reaction velocities at 37° for six substrate concentrations ranging from 0.3 K_m to 2 K_m, and four inhibitor concentrations ranging from 0 to 2 K_I. Substrate: sialyllactose (K_m, 0.90mM).

an allyl cation from **1**. This cation is postulated to undergo intramolecular attack by the *N*-acetyl group, generating the oxazolinium ion. The allyl cation or the oxazolinium ion can each then be attacked by acetate anion to generate **1** and **2**. The loss of a proton from the oxazolinium ion would lead to **5**. Furthermore, Priebe and Zamojski[10] have reported formation of epimers from an allyl cation of similar stereochemistry.

The K_I values of 2,3-dehydro-NeuAc and 2,3-dehydro-4-epi-NeuAc and their methyl esters, as determined from their respective double-reciprocal plots as recommended by Segel[11], are given in Table III. Each of these compounds was a competitive inhibitor of the *A. sialophilus* neuraminidase, consistent with our previous finding that the methyl ester of 2,3-dehydro-NeuAc binds to the enzyme[1]. The relative K_I values of these epimers and their methyl esters as inhibitors for the *A. sialophilus* neuraminidase underscores the necessity for the quasi-equatorial configuration of the hydroxyl group at C-4 in maintaining maximal inhibitory activity.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. Proton n.m.r. spectra were recorded with Varian A-60 and XL-100 spectrometers. ^{13}C-N.m.r. spectra were measured at a frequency of 25.2 MHz with a Varian XL-100 spectrometer. The 360-MHz spectra were obtained through the courtesy of Dr. G. McDonald, Dept. of Biochemistry and Biophysics, the university of Pennsylvania. For ^{1}H-n.m.r. spectra, the chemical shifts are expressed in p.p.m. relative either to internal tetramethylsilane or to sodium 4,4-dimethyl-4-silapentane-1-sulfonate. For ^{13}C-n.m.r. spectra, the chemical shifts are expressed in p.p.m. relative to internal sodium 4,4-dimethyl-4-silapentane-sulfonate. Mass spectra were obtained with a Hitachi Model RMU-GE spectrometer using electron impact. Compounds were routinely purified by l.c. using a Perkin–Elmer 2/2 chromatograph with Whatman Partisil-10 silica gel or Whatman

Partisil-10-ODS-3 semi-preparative columns. For the separation of acetylated compounds on columns of silica gel, the solvent was ethyl acetate; for separation of deacetylated methyl esters on ODS-reverse phase column, the eluant was water. Precoated plates of silica gel P.F. 254 (E. Merck) were used for t.l.c. Compounds were detected either by their u.v. absorption (254 nm) or by charring with 10% concentrated sulfuric acid in ethanol at 110°. Optical rotations were recorded with a Perkin–Elmer Model 141 automatic polarimeter. Microanalyses were performed by Micro-Analysis Inc., P.O. Box 5088, Wilmington, DE 19808.

Acetolysis at 40-50°: *methyl 5-acetamido-2,6-anhydro-2,3,5-trideoxy*-D-glycero-D-galacto-*non-2-enonate* (**3**) *and methyl 5-acetamido-2,6-anhydro-2,3,5-trideoxy*-D-glycero-D-talo-*non-2-enonate* (**4**). — A slurry of NeuAc methyl ester (0.323 g, 1 mmol), prepared from "edible bird's nest" as previously described[12], was stirred and heated to 40° in 10 mL of acetic anhydride. Acetic anhydride (1 mL) containing concentrated sulfuric acid (2 drops) was slowly added. After 2 h, an additional drop of concentrated sulfuric acid was added and the temperature was raised to 50°. After an additional 5 h, starting material was undetectable by t.l.c. in 7:3 1-propanol–water, whereas a new, u.v.-absorbing compound having R_F 0.75 was observed. The mixture was poured into ice–water, stirred, saturated with solid ammonium chloride, and then extracted with chloroform (3 × 100 mL). The combined extracts were washed with cold, saturated sodium hydrogencarbonate, dried, and evaporated to a syrup containing **1** and **2** (0.432 g, 91%), together with a small amount of **5** (18 mg, 4%). Compound **5** was separated from **1** and **2** by l.c. with a Whatman Partisil column and ethyl acetate as solvent. Compound **5** was identified from its i.r. and ^{1}H-n.m.r. spectra (see later). To a mixture of **1** and **2** (0.59 g, 1.37 mmol) in freshly distilled, dry methanol (25 mL) was added 2mM sodium methoxide in methanol (5 mL). The mixture was stirred for 1 h at 25°, and then for 18 h at 0°. The yellow solution was made neutral with dry Dowex 50W-8 (H^+) resin. The resin was filtered off, washed thoroughly with methanol, and the combined filtrate evaporated under diminished pressure to yield 0.323 g of a semi-solid mixture of **3** and **4** (85% yield). T.l.c. in 7:3 chloroform–methanol revealed two products. This mixture was resolved by l.c. with a Whatman Partisil-10-ODS-3 reverse-phase column and water as eluant. The first fraction (**3**) was isolated after lyophilization and was crystallized from 1:1 methanol–ether to yield 128 mg (40%); m.p. 230–231°, $[\alpha]_D^{25}$ +43.7° (*c* 5.5, water) [lit.[3] m.p. 225–227°, $[\alpha]_D^{25}$ +42.3° ±0.5° (*c* 5.5, water)]. The second fraction (**4**) was isolated as a white powder after lyophilization and crystallization from 1:1 methanol–ether to yield 132 mg (41%); m.p. 190–193°, $[\alpha]_D^{27}$ −130.3° (*c* 1.0, water); details of 360-MHz ^{1}H-n.m.r. and ^{13}C-n.m.r. spectra are presented in Results and Discussion.

Anal. Calc. for $C_{12}H_{19}NO_8 \cdot 0.5\ H_2O$ (**4**): C, 45.86; H, 6.42; N, 4.45. Found: C, 45.67; H, 6.57; N, 4.32.

Glycals **3** and **4** were hydrolysed to their corresponding oxides by exposure to 1 M NaOH at 0°.

Acetolysis at 0°. — NeuAc methyl ester (0.358 g, 1.08 mmol) was acetylated

as already described, except that the temperature was maintained for 24 h at 0°. Analysis by t.l.c. (7:3 1-propanol–water) indicated that starting material was no longer present. Dry pyridine (20 mL) was then added, and the mixture was stirred for an additional 24 h in the cold. After processing and deacetylation, the syrupy product (0.235 g, 81%) was resolved by l.c. on a ODS-3 reverse-phase column to give **3** and **4** in 4:1 ratio.

Isomerization of **2** *in acetic acid.* — A solution of **2** (0.3 g, 0.63 mmol, prepared from **4**) in acetic acid (10 mL) was heated for 4 days at 110–115°. The dark-brown mixture was then evaporated under diminished pressure with additions of toluene. The residue was extracted into chloroform, and the extract was washed with cold, saturated sodium hydrogencarbonate, saturated salt solution, and dried over anhydrous sodium sulfate. Removal of solvent gave a dark-brown gum. After dissolution in ethyl acetate, treatment with activated charcoal, and filtration, the resultant light-yellow extract was evaporated to a syrup, (0.260 g, 86%). T.l.c. in 9:1 chloroform–methanol indicated primarily one spot, with several minor compounds of higher R_F value. Deacetylation with sodium methoxide in methanol gave a syrup (0.130 g, 78%). T.l.c. in 7:3 chloroform–methanol showed the presence of a mixture of **3** and **4**. The compounds were separated by l.c. with the reverse-phase column with water as eluant; their retention times were identical to those of **3** and **4**.

Treatment of **1** *and* **2** *with acetic anhydride and sulfuric acid.* — A mixture of **1** and **2** (0.250 g, 0.53 mmol) in acetic anhydride (7 mL) was heated to 50°. Acetic anhydride (1 mL) containing concentrated sulfuric acid (1 drop) was slowly added. The mixture was stirred for 8 h. Analysis by t.l.c. (ethyl acetate) revealed the formation of **5** in admixture with unreacted starting material. Additional stirring for 12 h did not improve the yield of **5**. The mixture was processed as already described to yield a syrup (0.235 g). These products were resolved by l.c. on a Whatman Partisil column (ethyl acetate) to yield a mixture of **1** and **2** (191 mg, 75%) and **5** (21 mg, 9%); *m/z* 414 $(M + 1)^+$, 354 $(M - CO_2CH_3)^+$; ν_{max}^{neat} 1740 (OAc) and 1690 cm^{-1} (C=N); ^{1}H-n.m.r. (100 MHz): δ 6.4 (d, $J_{3,4}$ 6 Hz, H-3), 5.65 (dd, $J_{5,6}$ 6, $J_{6,7}$ 2 Hz, H-6), 5.46 (m, $J_{4,5}$ 7.0, $J_{5,6}$ 5.8 Hz, H-5), 4.82 (q, $J_{3,4}$ 5, $J_{4,5}$ 8 Hz, H-4), 4.6 (m, $J_{7,8}$ 10, $J_{8,9b}$ 4, $J_{8,9a}$ 4 Hz, H-8), 4.20 (q, $J_{8,9}$ 6, $J_{9a,9b}$ 12 Hz, H-9a), 3.95 (d, $J_{7,8}$ 10 Hz, H-7), 3.84 (s, OCH_3), 3.45 (dd, $J_{8,9a}$ 2, $J_{9a,9b}$ 12 Hz) and 2.10 (ms, 3 × OAc and N-CH_3 protons). The product has an identical R_F value in t.l.c., and a retention time in l.c., to **5**.

Anal. Calc. for $C_{18}H_{22}NO_{11}$ (**5**): C, 52.30; H, 5.61; N, 3.39. Found: C, 52.56; H, 5.85; N, 3.08.

Treatment of oxazoline **5** *with acetic anhydride and sulfuric acid.* — To a solution of **5** (75 mg, 181 mmol) in acetic anhydride (5 mL) at 50° was added a drop of concentrated sulfuric acid. The mixture was stirred for 8 h at this temperature. T.l.c. revealed only a small amount of unreacted **5**. The mixture was processed as already described, and the crude products were separated by l.c. on a column of Whatman Partisil with ethyl acetate as solvent to yield a mixture of **1** and **2** (60 mg, 70%). Deacylation resulted in products **3** and **4** in almost identical yields.

Enzyme studies. — Inhibition studies on a homogeneous preparation of *A. sialophilus* neuraminidase (specific activity, 110 units/mg of protein)[13] were performed in 10mM citrate–phosphate buffer, pH 6.0, using sialyllactose as substrate. These assay conditions have been described earlier[1].

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