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Aqueous methanolysis of an α-D-*N*-acetylneuraminyl pyridinium zwitterion: solvolysis occurs with no intramolecular participation of the anomeric carboxylate group†

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ABSTRACT: The synthesis of 3,4-dihydro-*2H*-pyrano[3,2-*c*]pyridinium α-D-*N*-acetylneuraminoate (**4**), the subsequent rate constants for aqueous methanolysis and the associated reaction products are reported. When compared with the analogous 2-deoxyglucopyranosyl 4′-bromoisoquinolinium salt (**11**), the rate of solvolysis of **4** displays a reduced sensitivity towards the ionizing power of the solvent. Solvolysis product analysis showed that substitution occurs predominantly with inversion of configuration. Hence it can be concluded that the methanolysis reactions of **4** proceed via dissociative transition states with no intramolecular nucleophilic participation by the anomeric carboxylate group.

KEYWORDS: nucleophilic substitution; hydrolysis; methanolysis; carbohydrates; oxacarbenium ion; sialoside; zwitterion

INTRODUCTION

Over the years, innumerable researchers have probed nucleophilic substitution mechanisms for phosphoryl, acyl and acetal transfer reactions.[1] One of the most prominent research groups in this area of biological physical organic chemistry is that of Jencks. Indeed, several reaction mechanism probes in current use originated from this group. For example, diffusion-limited trapping, usually by azide ion, of a carbenium ion intermediate is used to provide a 'clock' from which the intermediate's lifetime can be estimated.[2–4] For the specific case of acetal hydrolysis, Young and Jencks used this methodology to predict that the archetypal methoxymethyl oxacarbenium ion ($CH_3OCH_2^+$) has no existence in water and, as a consequence, the reactions of methoxymethyl acetals occur via concerted A_ND_N mechanisms.[2] This prediction has subsequently been shown to be correct.[5,6]

In 1980, Sinnott and Jencks' extensive study on the solvolyses of several glucopyranosyl derivatives led to the conclusion that these reactions were dissociative in nature but that the cationic intermediate was not solvent equilibrated.[7] In accord with these results, the lifetime in aqueous solution of the glucopyranosylium ion (**1**) has been estimated to be around $(1.0–2.5) \times 10^{-12}$ s,[8,9] which is too short to allow solvent equilibration.[10] Based on this estimated lifetime for the glucopyranosylium ion, one would expect that many reactions of glucopyranosides should occur via concerted mechanisms. However, only reactions in which the aglycon departs as an anion have been shown to occur via A_ND_N mechanisms.[11–13] In comparison with glucopyranosides, sialosides (*N*-acetylneuraminides) are a distinct subfamily of carbohydrates because of the carboxylate group that is attached to the anomeric centre. It has been proposed that this pendant carboxylate group stabilizes the oxacarbenium ion (**2**) via an electrostatic interaction.[14] As a result, it is expected that reactions at the tertiary anomeric centre in *N*-acetylneuraminides, as reported by Chou *et al.* for the spontaneous hydrolysis of pyridinium *N*-acetylneuraminyl zwitterions (**3a**),[15] should occur via $D_N + A_N$ mechanisms. However, it is also possible that intramolecular nucleophilic participation could become a viable pathway during the reactions of *N*-acetylneuraminides when departure of an anionic leaving group is not catalyzed. This alternative pathway has been proposed to occur during the spontaneous hydrolysis of **3b**.[16] It should be noted that because of the rapid mutarotation of *N*-acetylneuraminic acid[17] it is impossible to probe the stereochemical outcome for hydrolysis reactions of these carbohydrates. Hence in the present study, the synthesis and aqueous methanolysis of 3,4-dihydro-*2H*-pyrano[3,2-*c*]pyridinium α-D-*N*-acetylneuraminoate (**4**)

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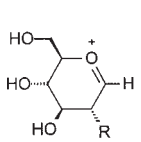
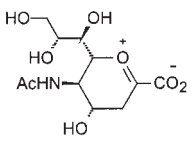
was undertaken in order to investigate the stereochemical outcome for the reactions of pyridinium *N*-acetylneuraminyl zwitterions.



1a, R = OH
1b, R = H



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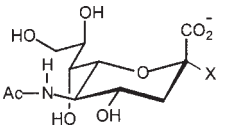
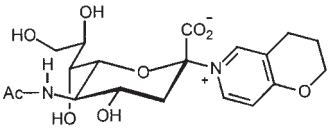


3a, X = pyridine
3b, X = OAr



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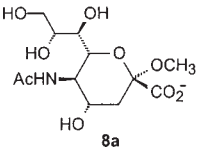
EXPERIMENTAL

Methanol was dried by distillation from its magnesium alkoxide salt. Deionized water was further purified by use of a Milli-Q ultra-pure water system. NMR spectra were acquired at operating frequencies of 400 and 100 MHz for ^{1}H and ^{13}C NMR, respectively, using either $CDCl_3$ or D_2O as the solvent and internal reference. Coupling constants (J) are reported in hertz. The two anomeric methyl *N*-acetylneuraminides (**8a** and **8b**) were purchased from Toronto Research Chemicals (TRC), and *N*-acetylneuraminic acid (**9**) from Rose Chemicals.

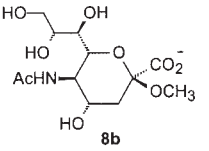
Syntheses

N-[Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-3′,4′-dihydro-2′H-pyrano[3′,2′-c]pyridinium tetrafluoroborate (7). The fully protected *N*-acetylneuraminyl chloride **6** (1.00 g, 1.96 mmol)[18] was added to a suspension of dried 4 Å molecular sieves (3.0 g) in anhydrous THF (40 ml). This mixture was stirred under a nitrogen atmosphere for 10 min, following which 3,4-dihydro-2*H*-pyrano[3,2-*c*]pyridine (**5**)[19] (6.63 g, 49.0 mmol) was added and the reaction mixture was cooled using an ice–water bath in the dark. Subsequently, silver tetrafluoroborate (0.05 g, 2.56 mmol) was added to the stirred solution, which was then allowed to warm to room temperature, and the reaction mixture was kept in the dark at this temperature for 60 h. The crude product, which was obtained after a standard work-up, was purified by flash chromatography [silica gel, MeOH–CH_2Cl_2 (1:10)] to give a white solid (0.56 g, 44%): R_F 0.24 [MeOH–CH_2Cl_2 (1:7)]. ^{1}H NMR (400 MHz, $CDCl_3$), δ 1.89 (m, 4 H, CH_3, H-3a), 2.01 (s, 3 H, CH_3), 2.05 (s, 3 H, CH_3), 2.12–2.17 (m, 5 H, CH_3, H-3′), 2.27 (s, 3 H, CH_3), 3.00 (m, 2 H, H-4′), 3.48 (dd, 1 H, $J_{3a,3e} = 14.0$ Hz, $J_{3e,4} = 7.0$ Hz, H-3e), 4.04 (dd, 1 H, $J_{9a,9b} = 12.5$ Hz, $J_{9a,8} = 5.8$ Hz, H-9a), 4.21–4.29 (m, 2 H, H-5, H-9b), 4.49 (t, 2 H, $J_{2',3'} = 5.2$ Hz, H-2′), 5.00 (dd, 1 H, $J_{6,5} = 11.4$ Hz, $J_{6,7} = 3.1$ Hz, H-6), 5.42 (dd, 1 H, $J_{4,3e} = 7.0$ Hz, $J_{4,3a} = 2.6$ Hz, H-4), 5.49–5.55 (m, 2 H, H-8, H-7), 7.11 (d, 1 H, $J_{7',8'} = 7.4$ Hz, H-8′), 8.62 (d, 1 H, H-7′), 8.83 (s, 1 H, H-5′). ^{13}C NMR (400 MHz, D_2O) δ 20.1, 20.8, 20.9, 21.5, 22.0, 23.1, 29.7, 37.9, 48.7, 63.1, 67.0, 67.1, 69.4, 70.2, 73.5, 94.1, 114.0, 121.8, 139.2, 142.0, 165.7, 167.4, 169.9, 170.0, 170.8, 171.1, 171.6.

N-[(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-3′,4′-dihydro-2′H-pyrano[3′,2′-c]-pyridinium (4). Deprotection of **7** by use of the method described by Chou *et al.*,[15] followed by reversed-phase HPLC purification [mobile phase: 5% aqueous MeOH containing 1% (v/v) HOAc on a C-18 column) gave **4** as a white solid in 25% yield. ^{1}H NMR (400 MHz, D_2O), δ 1.89 (t, 1 H, $J_{3a,3e} + J_{3a,4} = 22.9$ Hz, H-3a), 2.07 (m, 2 H, H-3′), 2.90 (t, 1 H, $J_{3',4'} = 6.4$ Hz, H-4′), 3.23 (dd, 1 H, $J_{3e,3a} = 12.2$ Hz, $J_{3e,4} = 3.9$ Hz, H-3e) 3.62–3.67 (m, 2 H, H-7, H-9a), 3.84–4.04 (m, 5 H, H-4, H-5, H-6, H-8, H-9b), 4.51 (t, 2 H, $J_{2',3'} = 5.1$ Hz, H-2′), 7.24 (d, 1 H, $J_{7',8'} = 7.4$ Hz, H-8′), 8.57 (dd, 1 H, $J_{5',7'} = 2.0$ Hz, H-7′), 8.66 (d, 1 H, H-5′). ^{13}C NMR (400 MHz, D_2O), δ 22.2, 24.2, 24.7, 43.1, 53.8, 65.4, 70.6, 70.7, 72.6, 73.7, 77.2, 96.5, 117.5, 126.1, 140.7, 142.4, 171.4, 172.3, 177.7. HRMS (ESI): calcd for $C_{19}H_{27}N_2O_9$ [$M + H^+$], 427.1717; found, 427.1717.



8a



8b

Kinetics

The solvolysis reactions of **4** were conducted at 65 °C and were monitored by following the decrease in absorbance at 266 nm using a Cary 3E UV–visible spectrophotometer equipped with the Cary six-cell Peltier constant-temperature accessory. Reactions were initiated by the injection of a stock solution of **4** (10 µl, 3 mM) into a methanol–water mixture (1.00 ml) containing *N*-methylmorpholine (3 equiv.). Rate constants were calculated by non-linear least-squares regression of the absorbance versus time data to a standard first-order rate equation.

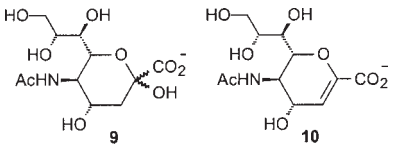
Determination of pK_a

The pK_a of the conjugate acid of 3,4-dihydro-2*H*-pyrano[3,2-*c*]pyridine was calculated from absorbance versus pH data measured in buffered solutions (10 mM) of

the pyridine (1×10^{-4} M) between pH 5.5 and 9.0 (no ionic strength adjustment).

Product studies

The solvolytic product studies were performed by heating for $7 \times t_{½hyd}$ at 65 °C in a sealed vial that contained a solution of **4** (1.05–1.12 mg) and *N*-methylmorpholine (3 equiv.) in a binary solvent mixture (10 ml). After cooling, the reaction vials were opened and the solvent was removed under reduced pressure. After drying under vacuum (~0.01 mm Hg) for 48 h, the resulting solid residues were dissolved in D_2O (0.5 ml) and their ^{1}H NMR spectra were acquired. The reaction products formed were identified by comparing the newly acquired NMR spectra with those of two methyl sialosides (**8a** and **8b**), sialic acid (**9**) and the glycal **10**.



RESULTS

After synthesis of 3,4-dihydro-*2H*-pyrano[3,2-*c*]pyridine (**5**),[19] the pK_a of its conjugate acid was calculated as 7.18 ± 0.02 by fitting the measured absorbance (245 nm) versus pH data to a standard titration equation. The synthetic route devised by Chou *et al.*[15] was used to couple the basic pyridine (**5**) to a fully protected *N*-acetylneuraminyl chloride (**6**), resulting in **7**, which, after deprotection, gave **4** (Scheme 1; see Experimental section for full details).

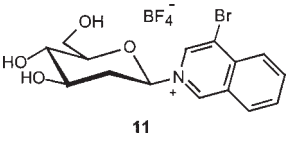
Listed in Table 1 are the observed first-order rate constants for the aqueous methanolysis of **4** at 65 °C.

In order to facilitate a direct comparison with the published data for the solvolytic reaction of **11**, the methanolysis reactions of **4** were performed without the addition of ionic strength adjusting salts.[20] Figure 1 shows a plot of $\log(k_{obs})_{\mathbf{4}}$ versus $\log(k_{obs})_{\mathbf{11}}$. Given that the slope of this plot is 0.71, it is clear that a reduction in the solvent's polarity produces a smaller rate-accelerating effect for the solvolysis of **4** relative to the reported effect for **11**.[20]

Table 1. Observed rate constants for the aqueous methanolysis of **4** at 65.0 °C[a]

[MeOH] (%, v/v)	$10^5 \times k_{obs}$ (s^{-1})
100	10.8 ± 0.3
80	4.58 ± 0.29
60	2.85 ± 0.02
50	2.18 ± 0.01
40	1.64 ± 0.01
20	1.10 ± 0.04
0	0.868 ± 0.028

[a] Mean value of three runs; quoted error = σ_{n-1}.

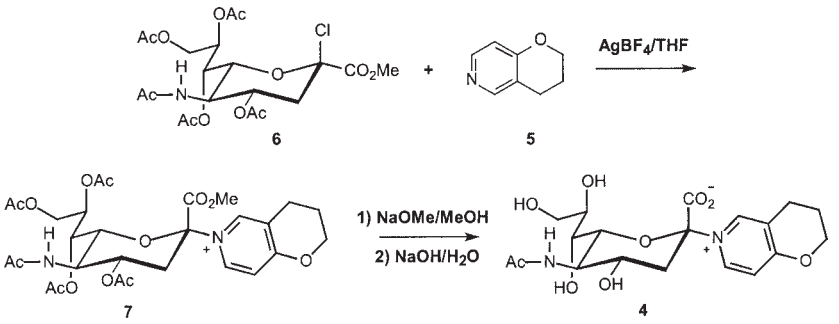


Product studies on the solvolysis reactions of **4** were performed in the presence of the sterically hindered base *N*-methylmorpholine to ensure that the acid-labile products (**8a** and **8b**) were stable to the solvolytic conditions. Listed in Table 2 are the observed products for the reactions of **4** in both methanol and 50% (v/v) aqueous methanol.

DISCUSSION

The central question addressed in this work is whether zwitterions such as **4** react with (pathway a; Scheme 2) or without (pathway b; Scheme 2) anchimeric assistance by the anomeric carboxylate group.

It is known that positively charged glycosyl pyridinium salts hydrolyse via transition states that have significant C—N bond cleavage.[21–23] As a result, the expected effects of solvent polarity changes on the two possible pathways shown in Scheme 2 are different. Pathway a is analogous to a 'type III' S_N2 reaction where charge at the transition state is greatly reduced in comparison with



Scheme 1

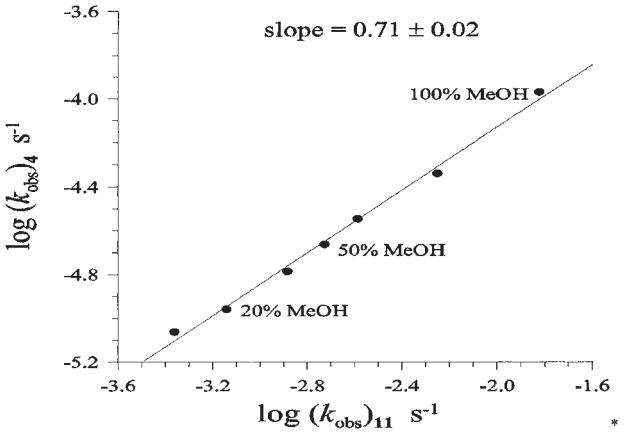


Figure 1. Plot of $\log(k_{obs})_{\mathbf{4}}$ versus $\log(k_{obs})_{\mathbf{11}}$ for the aqueous methanolyses of **4** and **11** at 65 °C. The kinetic data for **11** were taken from Ref. 20. The line shown is the best linear least-squares fit through the data points

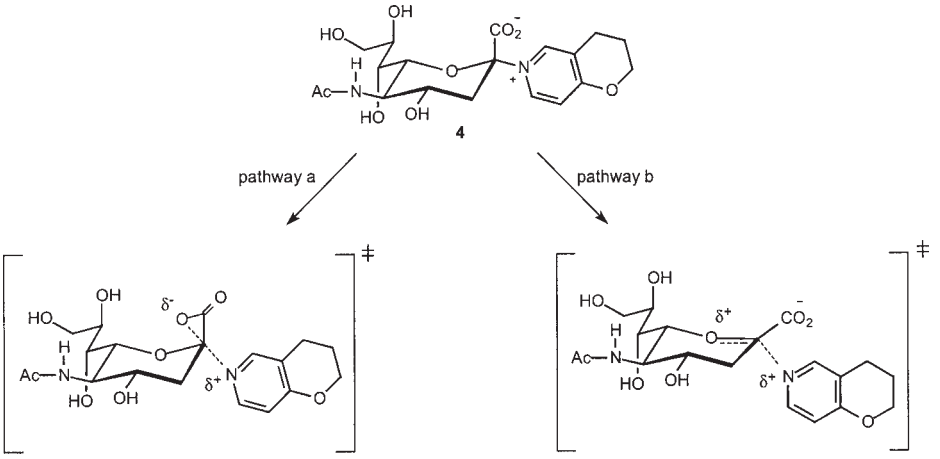
Table 2. Observed products formed during the reactions of **4** in aqueous methanol at 65 °C[a,b]

[MeOH] (%, v/v)	Glycal (**10**) (%)	GlyOH (**9**) (%)	α-GlyOMe (**8a**) (%)	β-GlyOMe (**8b**) (%)
100	10	—	ND[c]	90
50[d]	14	50[e]	ND[c]	35

[a] All solvents contained 3 mol equiv. of *N*-methylmorpholine.
[b] Percentages do not necessarily add up to 100 because of rounding.
[c] Not detected; it was estimated that <5% methyl α-D-*N*-acetylneuraminide (**8a**) was formed.
[d] $[H_2O]/[MeOH] = 2.25$ in this solvent mixture.
[e] Integral for β-D-sialic acid corrected assuming that 7% of the total sialic acid present was in the α-form.

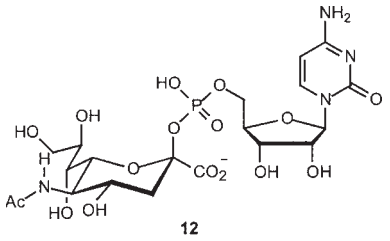
the ground state, and thus a reduction in solvent polarity should lead to a very large increase in reaction rate.[1] The Taft α value of 1.17 for water[24] suggests that water is a better H-bond donor than methanol ($\alpha = 0.93$). Therefore, a reaction proceeding via pathway a should increase in rate in a lower polarity solvent since H-bonding to the carboxylate group should be weaker at the transition state relative to that at the ground state. In contrast, a reaction proceeding via pathway b will have the positive charge that originates on the pyridinium ring being delocalized at the transition state and, as a result, only a modest increase in reaction rate is expected. For the pathway b scenerio, H-bonding to the carboxylate group should be similar at the transition and ground states. Therefore, based on the observation that the solvolytic rate constants for **4** display a smaller sensitivity to changes in solvent polarity than do the corresponding reactions of **11**, a compound that lacks the appended carboxylate group, it can be concluded that the carboxylate group in **4** is not directly involved as a nucleophile during these solvolysis reactions.

The observed solvolysis products for the reactions of **4** are also consistent with reactions proceeding via pathway b (Scheme 2). Specifically, in 100% methanol the only substitution product observed is the inverted methyl β-D-*N*-acetylneuraminide (**8b**), not **8a**, the product expected for reaction via pathway a. In contrast, it has been reported that the aqueous methanolysis of **12** in 20% (v/v) MeOH–H_2O, at pH 5.0 and 6.0, yields an approximately 1:1 mixture of methyl α- and β-D-*N*-acetylneuraminides (**8a** and **8b**).[14,25] Clearly, the reactions of these two different *N*-acetylneuraminides do not proceed via a common, solvent-equilibrated, intermediate. It is likely that at least some of the reaction products formed from **12** are made at the solvent-separated ion pair stage: reactions of glycosides in which the leaving group is anionic, such as α-glucopyranosyl fluoride,[11,12] are more prone to react via an A_ND_N (S_N1) mechanism than are the corresponding reactions of analogous neutral leaving group, yielding substrates such as α-glucopyranosyl pyridinium salts.[21,22] The nucleophilic selectivity for the reaction of **4** in 50% (v/v) MeOH–H_2O ($k_{MeOH}/k_{HOH} = 1.58$), calculated according to Eqn (1),[26] is larger than the



Scheme 2

corresponding value reported for the reactions of **11** in the same solvent ($k_{MeOH}/k_{HOH} = 1.07$).[20]



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$$\frac{k_{MeOH}}{k_{HOH}} = \frac{[Gly - OMe][HOH]}{[Gly - OH][MeOH]} \quad (1)$$

This greater selectivity of the *N*-acetylneuranimyl carbenium ion (**2**) relative to that of the 2-deoxyglucopyranosylium ion (**1b**) for the more nucleophilic methanol is consistent with cation **2** having a longer lifetime than cation **1b** in solution. The reported lifetimes for the cations **1b**[9] and **2**[14] in water are 1.0×10^{-11} and $\geq 3 \times 10^{-11}$ s, respectively.

As a final point, glycal **10** formation probably occurs with proton-abstraction assistance provided by the leaving group pyridine at the point of the ion–molecule complex. As such, this proposed a mechanism is similar to that put forward by Thibblin and Saeki for the solvolysis reactions of 1-(1-methyl-1-phenylethyl)pyridinium cations.[27]

Acknowledgements

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