# Hydrolysis of (2-Deoxy-β-D-glucopyranosyl)pyridinium Salts

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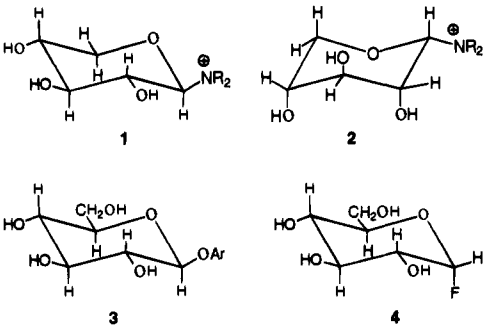
**Abstract:** The hydrolysis reactions of three (2-deoxy-β-D-glucopyranosyl)pyridinium salts exhibit first-order rate constants that are independent of pH in the range of 4.4−10.1 pH units. Derived second-order rate constants for the hydrolysis reactions of (2-deoxy-β-D-glucopyranosyl)-4′-bromoisoquinolinium bromide (**5b**) conducted in the presence of nucleophilic monoanions ($\mu$ = 2.0) including $AcO^-$, $Cl^-$, $Br^-$, and $N_3^-$ exhibit a Swain−Scott parameter ($s$) of 0.03 ± 0.05, indicating that these reactions show no sensitivity to the nature of the anion. However, a substantial quantity of the (2-deoxyglucopyranosyl)pyridinium salt hydrolysis product is formed as a result of a post-rate-limiting reaction involving a nucleophilic anion. Analysis of the product ratios indicates that the first-formed intermediate in the hydrolytic reaction is a solvent-separated ion pair:molecule encounter complex. The data allow a calculated estimate of greater than $2.5 \times 10^{-12}$ s for the lifetime of the glucopyranosyloxocarbenium ion in aqueous solution.

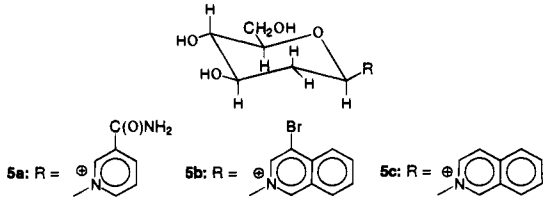
## Introduction

Specific acid-catalyzed hydrolysis of glucopyranosides is generally considered to occur with unassisted rate-limiting exocyclic C−O cleavage of the protonated glycoside to give a cyclic oxocarbenium ion intermediate and a neutral alcohol.[1] In principal, the rate-limiting C−O bond cleavage could occur with nucleophilic assistance from either the solvent or other solute molecules in an $S_N2$ $(A_ND_N)^2$ reaction.[3] Alternatively, C−O bond cleavage could be independent of such nucleophilic assistance, proceeding instead through an $S_N1$ $(D_N + A_N)^2$ reaction.[3] Several acyclic acetal derivatives have been shown to react in aqueous media via a mechanism involving a small nucleophilic interaction at the rate-limiting transition state for hydrolysis.[4] An extensive kinetic isotope effect study conducted on the acid-catalyzed hydrolysis of methyl α- and β-glucopyranosides supports the interpretation that glycoside hydrolysis occurs without nucleophilic involvement during the rate-limiting C−O bond cleavage step.[5] Furthermore, in this 1986 study, glycosides containing a neutral leaving group, 4-bromoisoquinoline (**1**, **2**), or the highly delocalized anionic leaving group 2,4-dinitrophenolate (**3**) displayed reaction rates that correlated better with the basicity than with the nucleophilicity of added salts.[5] Bennet and Sinnott suggested that these results were consistent with H-bonding of the added salts to the 2-OH group of the glycoside.[5] In this case, increasing the strength of the H-bonding interaction would be accompanied by a weakening of the hydroxyl group inductive effect, leading ultimately to less destabilization of the positively-charged transition state.[5] Commenting on the results obtained by Bennet and Sinnott, Amyes and Jencks[4c] suggested that the effect of added azide salt on the hydrolysis reaction of **2** was consistent with the known reluctance of equatorial cyclohexyl derivatives to undergo $S_N2$ reactions relative to a comparable acyclic derivative.[6] Consequently, Amyes and Jencks argued that glycoside **2** reacted with azide ion by an $A_ND_N$ mechanism.[4c] Recently, Banait and Jencks demonstrated that anionic nucleophiles react with α-glucopyranosyl fluoride (**4**) through a concerted $(A_ND_N)$




reaction.[7] The transition state for the reaction of **4** with anionic nucleophiles is characterized by a large degree of C−F bond cleavage, combined with a small sensitivity to the nucleophilicity of the incoming anion ($s$ = 0.18).[7]

In the present report, the effect of a C-2 hydroxyl group on the reactivity of glycosyl pyridinium salts was probed by studying the reactions of three (2-deoxyglucopyranosyl)pyridinium salts (**5a**−**c**) in the presence of anionic nucleophiles. In order to measure the Swain−Scott sensitivity parameter, $s$, the sensitivity of the rate of hydrolysis of **5b** to the nucleophilicity of various anions was studied.




## Materials and Methods

The buffers 2-(*N*-morpholino)ethanesulfonic acid (MES), 3-(*N*-morpholino)propanesulfonic acid (MOPS), 3-(*N*-tris(hydroxymethyl)-methyl)amino)propanesulfonic acid (TAPS), 2-(*N*-cyclohexylamino)-

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ethanesulfonic acid (CHES), and 3-(*N*-cyclohexylamino)propanesulfonic acid (CAPS) as well as 2-deoxyglucose were purchased from Sigma and used without further purification. Sodium azide was recrystallized from water and dried under vaccum (1 mmHg) for several hours.[8] All other salts used in the hydrolysis studies were of "Analar" grade and were used without further purification. Milli-Q water (18.2 MΩ $cm^{-1}$) was used for the kinetic experiments. The NMR spectra were acquired on a Bruker AMX-400 spectrometer. Melting points are reported as uncorrected values. Full experimental details for the synthesis of ([1-$^{13}$C]-2-deoxy-β-D-glucosyl)-4′-bromoisoquinolinium bromide (**12**) from D-arabinitol and the syntheses of α- and β-D-*arabino*-hexopyranosyl azides (**15** and **14**, respectively) from 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranosyl bromide are presented in the supporting information deposited with this manuscript.

**3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranosyl Bromide (7).** 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranose (**6**)[9] (1.00 g, 3 mmol) was added in one portion, with stirring, to a cooled solution (0 °C) of 30% w/v HBr in a mixture of dichloromethane (2 mL) and glacial acetic acid (4 mL) maintained under an inert atmosphere. After stirring for 90 min, the solution was diluted with dichloromethane (50 mL). The resulting solution was washed with ice-cold water (50 mL) and cold, saturated $NaHCO_3$ (30 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated at aspirator pressure to give a syrup (1.06 g, 100% crude yield). The $^1$H-NMR (400 MHz) spectrum obtained for this product was identical to that reported in the literature.[10] Due to the lability of this compound, no further purification was attempted.

**(3,4,6-Tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranosyl)-3′-carboxamidopyridinium Bromide (8a).** Hexopyranosyl bromide **7** (1.03 g) in dichloromethane (5 mL) was added to a solution of nicotinamide (2.0 g) in dry THF (100 mL) and was subsequently stirred at room temperature for 72 h. The resulting precipitate was allowed to settle, the solvent was removed, and after drying the product under vacuum, a white, hygroscopic solid (1.63 g) was obtained. Since recrystallization of the material proved to be unsuccessful, the dried product was immediately deprotected.

**(2-Deoxy-β-D-*arabino*-hexopyranosyl)-3′-carboxamidopyridinium Bromide (5a).** The acetylated salt **8a** (1.63 g) was dissolved in methanol (8 mL), and the solution was cooled to −10 °C and, following dropwise addition of acetyl bromide (0.9 mL), was kept in a freezer (−10 °C) for 3 days. Precipitation of the crude product was accomplished by the addition of DME (250 mL). The compound was filtered, washed with DME (50 mL), and dried under vacuum to give a light yellow solid (1.14 g). An appreciable fraction of this material was identified as nicotinamide hydrobromide, a byproduct of the deprotection step. For use in the kinetic studies, the product (377 mg) was first dissolved in a minimum volume of ice-cold 10% aqueous ammonia. The solution was then immediately shell frozen and lyophilized. The resulting solid was dissolved in a minimum quantity of anhydrous ethanol, and DME (100 mL) was added to precipitate **5a** (45 mg). The resulting solid contained less than 5% nicotinamide hydrobromide ($^1$H-NMR). However, numerous attempts to recrystallize the material failed due to the sensitivity of **5a** to solvolysis in alcoholic medium. Therefore, a satisfactory elemental analysis was not obtained. The $^1$H-NMR spectrum showed no peaks other than those expected for compound **5a**. $^1$H-NMR (400 MHz, $D_2O$): δ 1.98 (q, 1 H, $J_{1,2a} \approx J_{2a,2e} \approx J_{2a,3} \approx 12$ Hz, H-2a), 2.68 (ddd, 1 H, $J_{2e,3} = 5$ Hz, $J_{1,2e} = 2$ Hz, H-2e), 3.53 (t, 1 H, $J_{3,4} + J_{4,5} = 20$ Hz, H-4), 3.67−3.75 (m, 3 H, H-5, H-6a, H-6b), 3.9−4.0 (m, 1 H, H-3), 6.15 (dd, 1 H, H-1), 8.23 (dd, 1 H, H-5′), 8.95 (m, 1 H, H-4′), 9.20 (m, 1 H, H-6′), 9.48 (bs, 1 H, H-2′).

**(3,4,6-Tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranosyl)-4′-bromoisoquinolinium Bromide (8b).** Hexopyranosyl bromide **7** (1.06 g, 3 mmol) was stirred for 10 min with 4-bromoisoquinoline (1.0 g) and *m*-cresol (0.5 mL) in dichloromethane (10 mL). After evaporation of the solvent, the addition of ether (50 mL) to the reaction mixture yielded a solid residue. The solid was purified by flash chromatography using silica gel and an initial mobile phase of 2% methanol in dichloromethane, followed by 5% methanol in dichloromethane, to give a light yellow hygroscopic solid (1.4 g, 82%).

**(2-Deoxy-β-D-*arabino*-hexopyranosyl)-4′-bromoisoquinolinium Bromide (5b).** The acetylated salt **8b** (1.2 g) was dissolved in methanol (10 mL), the solution was cooled to 0 °C, acetyl bromide (0.5 mL) was added in a dropwise manner, and the resulting solution was stored in a refrigerator (5 °C). After 4 days, the resulting precipitate was collected (0.65 g, 69%) and recrystallized from water/acetone to give white crystals. Mp: 120 °C (dec). $^1$H-NMR (400 MHz, $D_2O$): δ 2.08 (q, 1 H, $J_{1,2a} \approx J_{2a,2e} \approx J_{2a,3} \approx 12$ Hz, H-2a), 2.76 (ddd, 1 H, $J_{2a,2e} = 12$ Hz, $J_{2e,3} = 5$ Hz, $J_{1,2e} = 2$ Hz, H-2e), 3.62 (t, 1 H, $J_{3,4} + J_{4,5} = 19$ Hz, H-4), 3.81 (ddd, 1 H, $J_{4,5} = 10$ Hz, $J_{5,6a} = 5$ Hz, $J_{5,6b} = 2$ Hz, H-5), 3.95 (dd, 1 H, $J_{6a,6b} = 12$ Hz, H-6a), 4.00−4.08 (m, 2 H, H-3, H-6b), 6.21 (dd, 1 H, H-1), 8.16−8.48 (m, 4 H, ArH), 9.08 (bs, 1 H, ArH-3), 9.98 (bs, 1 H, ArH-1). Anal. Calcd for $C_{15}H_{17}Br_2NO_4$: C, 41.41; H, 3.94; N, 3.22. Found: C, 41.40; H, 3.96; N, 3.11.

**(3,4,6-Tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranosyl)isoquinolinium Bromide (8c).** Hexopyranosyl bromide **7** (1.06 g, 3 mmol) was stirred for 10 min with isoquinoline (0.60 g) and *m*-cresol (0.5 mL) in dichloromethane (10 mL). A light yellow hygroscopic solid (1.30 g, 87%) was obtained, following the work-up procedure outlined above for **8b**.

**(2-Deoxy-β-D-*arabino*-hexopyranosyl)isoquinolinium Bromide (5c).** The acetylated salt **8c** (1.3 g) was dissolved in methanol (10 mL), cooled to 0 °C, and following the dropwise addition of acetyl bromide (1.0 mL) to the solution, was stored in a refrigerator (5 °C). After 2 days, the resulting precipitate was collected (0.5 g, 60%) and recrystallized from methanol to give white crystals. Mp: 124 °C (dec). $^1$H-NMR (400 MHz, $D_2O$): δ 2.08 (q, 1 H, $J_{1,2a} \approx J_{2a,2e} \approx J_{2a,3} \approx 12$ Hz, H-2a), 2.75 (ddd, 1 H, $J_{1,2e} = 2$ Hz, $J_{2e,3} = 5$ Hz, H-2e), 3.62 (t, 1 H, $J_{3,4} + J_{4,5} = 19$ Hz, H-4), 3.80 (ddd, 1 H, $J_{4,5} = 10$ Hz, $J_{5,6b} = 5$ Hz, $J_{5,6a} = 2$ Hz, H-5), 3.94 (dd, 1 H, $J_{6a,6b} = 12$ Hz, H-6b), 4.00−4.08 (m, 2H, H-3, H-6a), 6.20 (dd, 1 H, H-1), 7.98−8.65 (m, 6 H, ArH), 9.92 (bs, 1 H, ArH-1). Anal. Calcd for $C_{15}H_{18}BrNO_4$: C, 50.58; H, 5.09; N, 3.93. Found: C, 50.74; H, 5.14; N, 4.07.

**Kinetics.** Hydrolysis of **5b** (80−150 μM) at 65 °C was monitored by observing the rate of decrease in absorbance at 345 nm using a Cary-3E UV−vis spectrophotometer equipped with the Cary six-cell Peltier constant-temperature accessory. The reaction was initiated by injection of an aqueous stock solution of the glucoside (30 μL, 8−15 mM) into a 1-cm quartz cuvette containing 3.0 mL of the required buffer that had been equilibrated for 30 min at 65 °C. Clean isosbestic points were observed at 261, 294, 299, and 326 nm, and the rate constant for hydrolysis was calculated by performing a standard nonlinear least-squares fit of the absorbance (345 nm) versus time data. Kinetic data for the hydrolysis of **5a** was obtained in an analogous fashion, except that 20 μL of a 34 mM stock solution was used, and the reaction was monitored at 273 nm. A high-performance liquid chromatography (HPLC) methodology was utilized to monitor the hydrolysis of **5c**. In a typical experiment, 100 μL of a stock solution (56 mM) of **5c** was diluted into 4.0 mL of the required buffer solution that had been pre-equilibrated for 30 min at 65 °C. At appropriate time intervals, samples (0.4 mL) were removed and cooled in an ice/water bath. A portion of each cooled sample (8−25 μL) was then analyzed by HPLC, using a Water's "Nova-Pak" 6-μm particle size, 8 × 100 mm C18 column, a solvent system of 20% MeOH in $H_2O$ that contained 0.5% $CF_3CO_2H$, and a flow rate of 3 mL/min. Absorbance of the column effluent was monitored at 235 nm. The rate constant for **5c** hydrolysis was obtained by a standard nonlinear least-squares fit of the starting material concentration versus sampling time data. It was not feasible to calculate hydrolytic rate constants for **5c** by using the amount of isoquinoline product formed because the extended time period (up to 14 days) required for the hydrolysis reaction of **5c** at 65 °C led to a substantial loss of this product.

**Solvent Kinetic Isotope Effect.** The solvent kinetic isotope effect ($k_{H_2O}/k_{D_2O}$) for the hydrolysis of **5b** was measured (see the previous section for full details) using 0.01 M phosphate buffer (1:1 $NaH_2PO_4$:$Na_2HPO_4$) in $H_2O$ or $D_2O$ (final deuterium content >98%) that contained either no added salt ($\mu$ = 0.02 M) or 1.98 M NaOAc.

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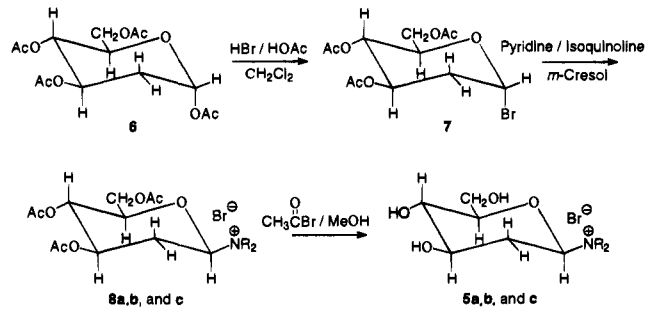
**Scheme 1**




**Product Studies.** The UV−vis spectrum (240−380 nm) of a completely hydrolyzed (>10 half-lives) sample of **5b** ($2.02 \times 10^{-4}$ M) in 0.01 M phosphate buffer (1:1 $NaH_2PO_4$:$Na_2HPO_4$, $\mu = 0.02$) at 65 °C was identical within 0.016 au (maximum absorbance = 1.03 au) of a spectrum of 4-bromoisoquinoline ($2.00 \times 10^{-4}$ M) and 2-deoxyglucose ($2.04 \times 10^{-4}$ M) acquired under identical conditions. Identification of the hydrolysis products from the carbohydrate portion of **5b** was accomplished by $^{13}$C-NMR analysis. For this procedure, the glucoside **5b** (4−8 mg) was dissolved in 0.01 M phosphate buffer (0.75 mL, 1:1 $NaH_2PO_4$:$Na_2HPO_4$, $\mu = 2.0$ M) containing $NaClO_4$, NaOAc, or $NaN_3$. Each sample was heated at 65 °C for a period of time that corresponded to five half-lives for hydrolysis. The solutions were then extracted with $CH_2Cl_2$ (1 × 2 mL), $D_2O$ (0.5 mL) was added to the aqueous layer, the samples were transferred to NMR tubes, and the $^{13}$C spectra were acquired using a standard pulse sequence. Similar product studies were performed with **12** (**5b** that was enriched (99%) with $^{13}$C at C-1). In these experiments, 0.01 M phosphate buffer solutions (1:1 $NaH_2PO_4$:$Na_2HPO_4$) containing 0.99 M $NaN_3$ were made up to an ionic strength of 2.0 M with $NaN_3$, NaOAc, NaCl, NaBr, KF, or KCl. Compound **12** (≈3 mg) was dissolved in the buffer (250 μL) and heated to 65 °C for 2 h. After cooling to room temperature, $D_2O$ (250 μL) and $CH_2Cl_2$ (≈1 mL) were added, the mixture was shaken, and following separation of the aqueous and organic phases, the aqueous layer was filtered through a glass wool plug into an NMR tube. The $^{13}$C-NMR spectra were acquired using an inverse-gated pulse sequence with a 50° pulse and a 2-s delay time between pulses.

Reactivity of the 2-deoxyglucosyl azides toward hydrolysis and substitution was measured using $^{13}$C-NMR spectroscopy. The 2-deoxyglucosyl azide **14** or **15** (≈20 mg) was dissolved in 0.01 M phosphate buffer (0.40 mL, 1:1 $NaH_2PO_4$:$Na_2HPO_4$, $\mu = 2.0$ M; $NaN_3$) and heated for 2 h at 65 °C. After the sample was cooled to room temperature, $D_2O$ (0.40 mL) was added. The resulting solution was transferred to an NMR tube, and the $^{13}$C spectrum was acquired using the same inverse-gated pulse sequence mentioned above.

## Results

Preparation of the desired (2-deoxyglucopyranosyl)pyridinium salts from readily available tetra-*O*-acetyl-2-deoxy-α-D-glucose (**6**) followed the synthetic route outlined in Scheme 1.[9] *In situ* conversion of **7** to its β-anomer (which can lead to either partial or exclusive formation of the α-pyridinium salt) was prevented by the addition to the reaction mixture of *m*-cresol as a free bromide ion complexing agent.[11] Deprotection of the acetylated salts was accomplished in a methanolic solution of hydrogen bromide that was generated *in situ* by the reaction of acetyl bromide and methanol.

Table 1 presents the observed rate constants ($k_{obsd}$) obtained as a function of pH for the hydrolysis of three (2-deoxyglucopyranosyl)isoquinolinium and -pyridinium salts (**5a−c**).[12]

Experimental conditions similar to those reported by Bennet and Sinnott[5] were employed in the present study in order to compare the effect of added salts on the reaction rate of **5b** and the previously reported rate[5] of β-xylopyranosyl-4′-bromoisoquinolinium bromide (**1**). Table 2 lists the observed rate constants ($k_{obsd}$) obtained for hydrolysis of **5b** under the experimental conditions of 0.98 M salt, 0.01 M phosphate buffer ($\mu = 1.0$), and 65 °C. The Swain−Scott nucleophilic parameter *n* is included for each anion studied.

Solvolysis of **5b** in water exhibited a negative salt effect: the observed rate constant for hydrolysis decreased by about 70% when the ionic strength of the medium was increased from 0.02 to 2.0 M ($NaClO_4$). A comparable increase in ionic strength using sodium acetate yielded a 25% increase in the observed rate constant for the hydrolysis of **5b**. The experimentally determined rate constants for the hydrolysis of **5b** with respect to ionic strength changes are given in Table 3.

In order to compare the effect of added salts on the reaction rates of **5b** to the published data for α-D-glucopyranosyl fluoride hydrolysis,[7] measurements for **5b** were made at an ionic strength of 2.0 M maintained with $NaClO_4$. Presented in Figure 1 are plots of the experimentally determined relationship between $k_{obsd}$ and the salt concentration for four of the seven anionic nucleophiles studied. A complete record of the experimental data obtained for the hydrolysis of **5b** ($\mu = 2.0$, $NaClO_4$) is given in Table S1 of the supporting information.

When the effect of fluoride ion on the solvolysis of **5b** was examined, $KNO_3$ was used to maintain the ionic strength at 2.0 M. A complete listing of the experimental data for the

**Table 1.** Observed Rate Constants ($10^6 \times k_{obsd}$ ($s^{-1}$)) for the Hydrolysis of (2-Deoxy-β-D-*arabino*-hexopyranosyl)pyridinium and -isoquinolinium Bromides as a Function of pH at 65.0 °C and an Ionic Strength of 2 M ($NaClO_4$)

| buffer[a] | pH | **5a**[b] | **5b**[b] | **5c**[c] |
|---|---|---|---|---|
| acetate | 4.41 | 340 ± 2 | 151 ± 2 | 2.3 ± 1 |
| MES | 6.46 | 338 ± 2 | 150 ± 1 | 1.6 ± 1 |
| MOPS | 7.44 | 333 ± 3 | 151 ± 2 | 1.6 ± 1 |
| TAPS | 8.33 | 329 ± 3 | 148 ± 1 | 1.6 ± 1 |
| CHES | 9.44 | 338 ± 3 | 153 ± 1 | 1.8 ± 1 |
| CAPS | 10.11 | 341 ± 6 | 149 ± 1 | 1.8 ± 1 |

[a] $[buffer]_{total} = 10.0$ mM. [b] Mean value of three kinetic runs; quoted error = $\sigma_{n-1}$. [c] Calculated nonlinear least squares fit from a single kinetic run.

**Table 2.** Observed Rate Constants for the Hydrolysis of (2-Deoxy-β-D-*arabino*-hexopyranosyl)-4′-bromoisoquinolinium Bromide with Added Salts (0.98 M) at 65.0 °C in 0.01 M Phosphate Buffer ($\mu = 1.0$)

| added salt | $10^4 \times k_{obsd}$ ($s^{-1}$)[a] | *n* (MeBr in $H_2O$)[b] |
|---|---|---|
| $NaClO_4$ | 2.46 × 0.07 | 0[c,d] |
| $NaNO_3$ | 3.73 ± 0.10 | 1.0 |
| NaF | 5.33 ± 0.15 | 2.0 |
| NaOAc | 5.40 ± 0.20 | 2.7 |
| NaCl | 4.41 ± 0.13 | 3.0 |
| NaBr | 4.03 ± 0.10 | 3.9 |
| $NaN_3$ | 4.92 ± 0.18 | 4.0 |
| NaSCN | 3.54 ± 0.08 | 4.77 |

[a] Mean value of three kinetic runs; quoted error = $\sigma_{n-1}$. [b] Swain−Scott parameter for reactions of methyl bromide in water.[24b] [c] Swain−Scott constant for $H_2O$. [d] Rate constant in the absence of added salts = $(4.84 \pm 0.20) \times 10^{-4}$ $s^{-1}$ ($\mu = 0.02$ M).

**Table 3.** Observed Rate Constants ($10^6 \times k_{obsd}$ ($s^{-1}$)) for the Hydrolysis of **5b** as a Function of Added Salts at 65 °C, in 0.01 M Phosphate Buffer ($\mu = 0.02$ + [salt])

| [salt] (M) | NaOAc | $NaClO_4$ |
|---|---|---|
| 0.00 | 460 ± 1 | 460 ± 1 |
| 0.33 | 479 ± 1 | 337 ± 1 |
| 0.66 | 496 ± 2 | 275 ± 1 |
| 0.99 | 512 ± 2 | 231 ± 1 |
| 1.50 | 538 ± 3 | 186 ± 1 |
| 1.98 | 568 ± 4 | 152 ± 1 |

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(12) Reaction of **5b** with hydroxide ion (pH > 12) displayed complex non-first-order behavior.

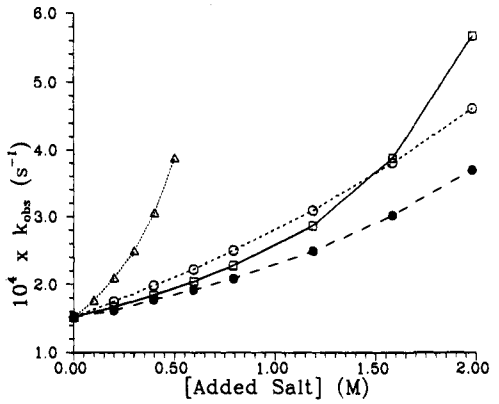



**Figure 1.** Plots of $k_{obsd}$ versus added salt concentration for **5b**, $T$ = 65 °C, 0.01 M phosphate buffer (1:1 $NaH_2PO_4$:$Na_2HPO_4$), $\mu$ = 2.0 ($NaClO_4$): ●, NaCl; □, NaOAc; ○, $NaN_3$; △, $Na_2S_2O_3$. Error limits are encompassed within the symbol diameter. Lines drawn through the points are for visual aid only.

**Table 4.** Calculated Second-Order Rate Constants ($10^6 \times k_2^{calcd}$ ($M^{-1}$ $s^{-1}$)) for the Reaction of **5b** with Various Anions at 65 °C, in 0.01 M Phosphate Buffer, $\mu$ = 2.0 M

| anion | $Na^+$ [a] | $K^+$ [b] |
|---|---|---|
| $NO_3^-$ | 53 ± 2 | |
| $F^-$ | | 72 ± 4 |
| $Cl^-$ | 69 ± 6 | 34 ± 2 |
| $Br^-$ | 66 ± 4 | 21 ± 4 |
| $AcO^-$ | 88 ± 5 | |
| $N_3^-$ | 118 ± 2 | |
| $SCN^-$ | 59 ± 2 | |
| $S_2O_3^{2-}$ | 290 ± 29[c] | |

[a] Linear fit of the data from Table S1, with [NaX] ≤ 0.594 M; ionic strength maintained with $NaClO_4$. [b] Linear fit of the data from Table S2, with [salt] ≤ 0.33 M; ionic strength maintained with $KNO_3$. [c] Linear fit of the data from Table S1, with $[Na_2S_2O_3]$ ≤ 0.2 M.

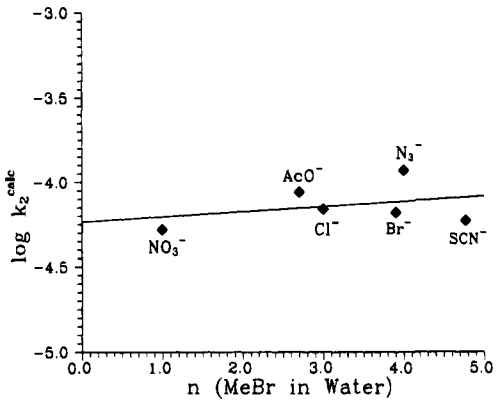


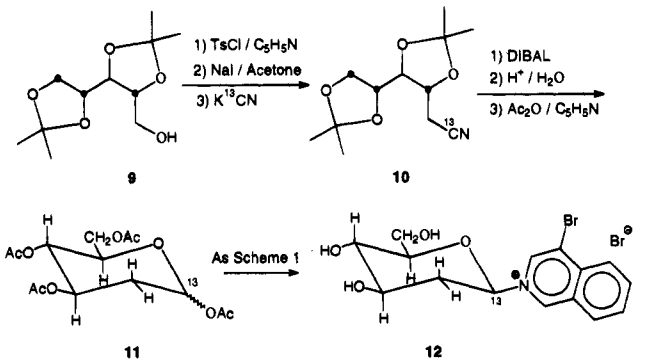

**Figure 2.** Plot of the Swain–Scott correlation of the calculated second-order rate constants log $k_2^{calcd}$ versus $n$ ($CH_3Br$ in $H_2O$)[24b] for **5b**, $T$ = 65 °C, 0.01 M phosphate buffer (1:1 $NaH_2PO_4$:$Na_2HPO_4$), $\mu$ = 2.0 ($NaClO_4$). Error limits are encompassed within the symbol diameter. The line shown is the least-squares fit through the data points (see Discussion). The calculated slope of the line is 0.03 ± 0.05.

hydrolysis of **5b** ($\mu$ = 2.0, $KNO_3$) is given in Table S2 of the supporting information.

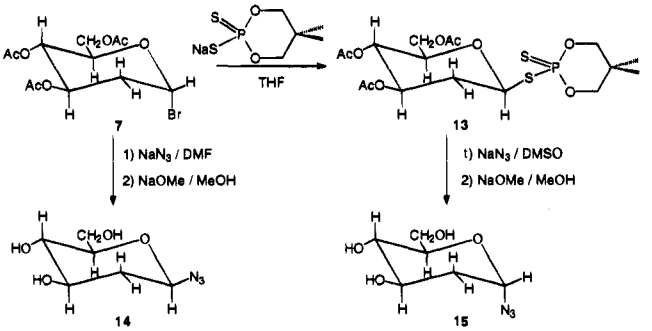
The second-order rate constants obtained for the hydrolysis of **5b** in the presence of various anions are compiled in Table 4. These values were calculated using a standard linear regression. The data from Table 4 were utilized to generate the Swain–Scott plot presented in Figure 2. In addition, Figure 2 illustrates the calculated best-line fit to the data obtained for the monoanions (slope = 0.030 ± 0.046).

The solvent deuterium kinetic isotope effect ($k_{H_2O}/k_{D_2O}$) for the hydrolysis reactions of **5b** at 65 °C was measured to be 1.18 ± 0.01 in the presence of 1.98 M NaOAc ($\mu$ = 2.0) and 1.19 ± 0.01 when no salt was added to the buffer solution (0.01 M phosphate buffer; 1:1 $NaH_2PO_4$:$Na_2HPO_4$, $\mu$ = 0.02).

**Scheme 2**




**Scheme 3**




$^{13}$C-Labeled 4-bromoisoquinolinium salt **12** was synthesized from the starting material 2,3:4,5-di-*O*-isopropylidene-D-arabinitol[13] using the multistep synthetic pathway outlined in Scheme 2. In this synthetic route, conversion of **11** to **12** utilized the same reaction pathway presented in Scheme 1.

Scheme 3 illustrates the two reaction sequences employed in the synthesis of both anomeric 2-deoxyglucopyranosyl azides (**14** and **15**) from hexopyranosyl bromide **7**.

Product analysis by UV–vis spectroscopy showed that the aromatic product from the hydrolysis of **5b** was 4-bromoisoquinoline, while $^{13}$C-NMR spectral resonances for the carbohydrate reaction products of **5b** in perchlorate- and acetate-containing buffers coincided with those of an authentic sample of 2-deoxyglucose. Two additional products that formed in the presence of azide ion were identified as 2-deoxy-α- and 2-deoxy-β-D-glucopyranosyl azide (**15** and **14**, respectively; spectra not shown).

Listed in Table 5 are the relative peak heights observed in the $^{13}$C-NMR spectra (between 85 and 105 ppm) of the water soluble products obtained from the hydrolysis of labeled **5b** (**12**) in the presence of added salts. The $^{13}$C-NMR resonance data obtained for the hydrolysis of **12** indicated that the reaction products were 2-deoxy-β-D-glucose ($\delta$ = 96.2 ppm), 2-deoxy-α-D-glucose ($\delta$ = 94.1 ppm), 2-deoxy-α-D-glucopyranosyl azide (**15**) ($\delta$ = 89.8 ppm), and 2-deoxy-β-D-glucopyranosyl azide (**14**) ($\delta$ = 89.4 ppm). The corresponding $^{13}$C-NMR spectra (100 MHz, $H_2O$:$D_2O$(2:1)) are shown in Figure S1 of the supporting information.

Shown in Figure S2a,b of the supporting information are the $^{13}$C-NMR spectra of the pure 2-deoxy-α- and 2-deoxy-β-D-glucopyranosyl azides (**15** and **14**). Both Table 6 and Figures S2c,d (supporting information) present the relative peak heights observed in the anomeric region of the $^{13}$C-NMR spectra for a sample of glucosyl azide that was exposed to the same reaction

(13) Nakagawa, T.; Tokuoka, H.; Shinoto, K.; Yoshimura, J.; Sato, T. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2150–2154.

**Table 5.** Relative Peak Heights from the $^{13}C$-NMR (100 MHz, $H_2O:D_2O$ (2:1)) of the Carbohydrate-Based Products Obtained from the Hydrolysis of $^{13}C$-Labeled **5b** (**12**) in 0.01 M Phosphate Buffer, $[NaN_3] = 0.99$ M, and [salt] = 0.99 M at 65 °C[a]

| entry | salt | 2-deoxy-β-D-glucose | 2-deoxy-α-D-glucose | 2-deoxy-α-D-glucopyranosyl azide (**15**) | 2-deoxy-β-D-glucopyranosyl azide (**14**) | $n^b$ |
|---|---|---|---|---|---|---|
| 1 | $NaN_3$ | 0.46 | 0.41 | 1.00 | 0.09 | 4.0 |
| 2 | KF | 0.99 | 0.89 | 1.00 | 0.10 | 2.0 |
| 3 | KCl | 1.03 | 0.91 | 1.00 | 0.19 | 3.0 |
| 4 | NaCl | 0.96 | 0.87 | 1.00 | 0.18 | 3.0 |
| 5 | NaBr | 1.01 | 0.95 | 1.00 | 0.27 | 3.9 |
| 6 | NaOAc | 0.84 | 0.80 | 1.00 | 0.10 | 2.7 |

[a] Peak heights are relative to that of 2-deoxy-α-D-glucopyranosyl azide (**15**). [b] Swain–Scott parameter for the reaction of $CH_3Br$ in $H_2O$ taken from ref 24b.

**Table 6.** Relative Peak Heights Measured from the $^{13}C$-NMR Spectra (100 MHz, $H_2O:D_2O$ (2:1)) of the Carbohydrates Present after Reacting Either **14** or **15** for 2 h in 0.01 M Phosphate Buffer Containing $[NaN_3] = 1.98$ M at 65 °C[a]

| entry | compound | 2-deoxy-β-D-glucose | 2-deoxy-α-D-glucose | 2-deoxy-α-D-glucopyranosyl azide (**15**) | 2-deoxy-β-D-glucopyranosyl azide (**14**) |
|---|---|---|---|---|---|
| 1 | **14** | 0.05 | 0.06 | 0.15 | 1.00 |
| 2 | **15** | 0.03 | 0.02 | 1.00 | 0.09 |

[a] Actual spectra are given in the supporting information as Figure S2c,d.

conditions utilized in the **5b** hydrolysis product studies (0.01 M phosphate buffer, $[NaN_3] = 1.98$ M, $t = 65$ °C, 2h).

## Discussion

The derived rate constants for the hydrolysis reactions of **5a–c** (Table 1) were all independent of pH between 4.4 and 10.1 pH units. This indicates that these reactions are neither acid nor base catalyzed. Due to the slow reaction rates exhibited for the hydrolysis of **5c** at 65 °C, each rate constant was measured only once at each pH condition tested. Therefore, the deviation observed in the pH versus rate profile (acetate buffer, Table 1) is probably the result of error associated with using singly determined data points.[14] Data from Table 1 and the literature values for the leaving group $pK_a$'s[15] can be used to calculate a $\beta_{lg}$ of $1.0 \pm 0.1_6$ for the hydrolysis of (2-deoxy-β-glucopyranosyl)pyridinium salts. Although this value is derived from only three data points, it is similar to the calculated values reported for the hydrolysis of both (β-galactopyranosyl)-pyridinium ions (five data points, $1.26 \pm 0.12$, 25 °C)[16] and (β-xylopyranosyl)pyridinium ions (three data points, $1.2 \pm 0.2$, 25 °C).[11] All three $\beta_{lg}$ values are consistent with the hydrolysis reactions having "late" transition states that involve substantial C–N bond cleavage.

**Effect of the C-2 Hydroxyl Group and Added Salts on Reactivity.** Using the data of Hosie *et al.*,[11] an estimated rate constant of $3.6 \times 10^{-7}$ $s^{-1}$ can be calculated for hydrolysis of β-D-glucopyranosyl-4′-bromoisoquinolinium bromide at 65 °C. When both this value and the corresponding rate constant for hydrolysis of **5b** (Table 2) are considered, a rate-retarding effect of approximately 680-fold at 65 °C can be correlated to the presence of the equatorial 2-OH group. However, when the observed rate constants for hydrolysis of **5b** ($I = 1.0$, Table 2) and the 2-hydroxyl-containing compound β-D-xylopyranosyl-4′-bromoisoquinolium bromide (**1**)[5] are compared, the data reveal that, as the identity of the added salt is varied, the relative changes in the rate constant are greater for **5b** than for β-D-xylopyranosyl-4′-bromoisoquinolium bromide (**1**).[17] Therefore, hydrogen-bonding to the 2-OH group cannot be the major cause of the reactivity pattern observed for glycopyranosyl pyridinium ions in the presence of added salts. In addition, the solvent kinetic isotope effect ($k_{H_2O}/k_{D_2O}$) of 1.18 for the hydrolysis of **5b** in 1.98 M sodium acetate is inconsistent with a proton being transferred at the transition state.[18] Therefore, the observed reactivity pattern for hydrolysis of **5b** cannot be attributed to a general-base-catalyzed reaction induced by the higher basicity of small "hard" anions.

Other possible explanations for the particular reactivity pattern observed for **5b** hydrolysis include the occurrence of a nonspecific salt effect and/or the existence of a small nucleophilic component to the reaction. In order to discern whether a measurable nucleophilic factor contributes to the reaction, it is essential that the ionizing power of the medium be kept relatively constant. To determine a suitable hydrolysis medium in which to probe for a nucleophilic component to the reactions of **5b**, the effect of ionic strength on the hydrolysis rate of **5b** was studied using $NaClO_4$. Addition of $NaClO_4$ to the reaction media caused a pronounced negative salt effect on the rate of hydrolysis of **5b**. Specifically, the rate constant decreased nonlinearly to about 33% of its initial value in response to an increase in the ionic strength of the medium from 0.02 to 2.0 M (Table 3). However, as the higher ionic strength limit was approached, there were correspondingly smaller changes in the observed rate constants (Table 3). Therefore, the nucleophilic effects of various anions were measured at high ionic strength ($I = 2.0$ M; $NaClO_4$ or $KNO_3$) and the second-order rate constants ($k_2^{calcd}$) were calculated using data obtained from samples that contained a $NaClO_4$ or a $KNO_3$ concentration of greater than 1.40 or 1.65 M, respectively.

The effect of bisulfite dianion on the reactivity of **5b** to hydrolysis was larger than that of the other anions studied. However, a concentration increase of bisulfite from 0.0 to 0.2 M required a concomitant decrease in the perchlorate concentration from 1.98 to 1.38 M in order to keep the ionic strength of the medium constant. From the data in Table S1 it is clear that, under conditions where $[X^-] = 0.594$ M ($[ClO_4^-] = 1.406$ M), the observed rate constants are very similar to the rate constant measured when $[S_2O_3^{2-}] = 0.2$ M ($[ClO_4^-] = 1.38$ M). Consequently, alterations in the rate-retarding perchlorate ion concentration have the potential to cause changes of a similar magnitude to the observed rate constant. Thus, the enhanced hydrolytic reactivity of **5b** in the presence of bisulfite dianion

(14) At 75 °C the observed rate constants (UV–vis) obtained in the pH range 4.4–8.5 were identical within experimental error ($(1.3 \pm 0.1) \times 10^{-5}$ $s^{-1}$).

(15) Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965.

(16) Jones, C. C.; Sinnott, M. L.; Souchard, I. J. L. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1191–1198.

(17) The rate ratios $k_X/k_{ClO_4}$ for the reactions of **5b** (65 °C) are 2.20, 2.17, 2.00, 1.79, and 1.64 for X = OAc, F, $N_3$, Cl, and Br respectively, whereas the corresponding ratios for the β-D-xylopyranosyl salt **1** are 1.47, 2.08, 1.33, 1.21, and 1.07 (80 °C).

(18) Alverez, F. J.; Schowen, R. L. In *Isotopes in Organic Chemistry*; Elsevier: Amsterdam, 1987; Vol. 7, pp 1–60.

cannot be analyzed solely in terms of a nucleophilic component to the reaction. Accordingly, for the purpose of comparing reactivity with nucleophilicity, only the calculated second-order rate constants for monoanions were used in the estimation of the Swain−Scott sensitivity parameter $s$. The calculated value for the nucleophilic sensitivity parameter $s$ of 0.03 ± 0.05 ($\mu$ = 2.0, $NaClO_4$) shows that, in a medium of approximately constant ionizing strength, the reaction rate of **5b** is insensitive to the nature of the anion. Although it was not possible to evaluate the effect of fluoride ion on the reactivity of **5b** in perchlorate solutions, it can be seen from the data in Table 4 that $k_{obsd}(F^-) > k_{obsd}(Cl^-) > k_{obsd}(Br^-)$. Consequently, the calculated value of $s$ would be reduced even further if a point for fluoride ion was included on the graph shown in Figure 2.

The absence of a detectable nucleophilic component to the reactions of the (2-deoxy-$\beta$-D-glucopyranosyl)-4′-bromoisoquinolinium salt (**5b**) indicates that a stepwise $D_N + A_N$ ($S_N1$) mechanism is followed. The observed similarity in behavior of the $\beta$-D-xylopyranosyl salt (**1**)[5] to that of **5b** implies that carbohydrates that contain a 2-hydroxyl group in addition to a pyridinium leaving group also react via a stepwise mechanism.

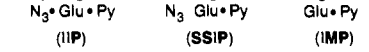
**Reaction Products Formed in the Presence of Azide Ion.** The carbohydrate products formed during the hydrolysis reactions of **5b** in aqueous media provide additional evidence that these reactions occur through a stepwise process. Hydrolysis of $^{13}C$-labeled **5b** (**12**) in the presence of 1.98 M $ClO_4^-$ or $AcO^-$ gave 2-deoxy-α- and $\beta$-glucopyranose as the sole carbohydrate products of the reaction (spectra not shown). However, in the presence of 1.98 M $N_3^-$, an appreciable quantity of both 2-deoxy-α- and $\beta$-glucopyranosyl azides were formed (Table 5, Figure S1a, supporting information). To quantify the relative proportions of the two anomeric azide products, $^{13}C$-NMR spectral acquisition parameters were selected so that NOE enhancements of $^{13}C$-signal intensity did not occur. Under these conditions, all of the signals detected for the methine carbons in the $^{13}C$-NMR spectra of both pure anomeric azides displayed equal peak intensities (Figure S2a,b, supporting information).

The inverted substitution product (2-deoxy-α-glucopyranosyl azide (**15**)) comprised about one-half of the total products formed from the reaction of **12** ($^{13}C$-labeled **5b**) in a solution containing azide ion (1.98 M; $I$ = 2.0; Table 5). In comparison, when the reaction of **12** was run in the presence of both azide (0.99 M) and another anion ($F^-$, $Cl^-$, $Br^-$, or $AcO^-$ at 0.99 M), the quantity of **15** produced was reduced to approximately one-half the amount generated in the sample that contained azide ion alone (Table 5). More importantly, however, is the trend in the quantity of retained, relative to inverted, azide product (**14** and **15**, respectively) formed in the presence of 0.99 M salt and 0.99 M azide ion. The relative amount of retained product (**14**) formed in the presence of azide plus a panel of different monoanions can be ordered as follows: $AcO^- \approx F^- < Cl^- < Br^-$ (Table 5). In contrast, the order of observed rate constants for the reactions of **5b** in the presence of 0.98 M salt (Table 2) is $k_{obsd}(AcO^-) \approx k_{obsd}(F^-) > k_{obsd}(Cl^-) > k_{obsd}(Br^-)$. The trend in the amount of retained azide product formed with respect to the anion present correlates with the nucleophilicity of the added ion ($Br^- > Cl^- > F^-$), rather than with the experimentally observed effect of these anions on the reaction rate. Therefore, in the substitution reactions of **5b**, a step subsequent to the rate-limiting step of the reaction must be determining the relative anomeric azide product ratios.

**Reactivity of 2-Deoxy-α- and β-glucopyranosyl Azides with Azide Ion.** To elucidate whether the 2-deoxyglucosyl azide products react appreciably during the hydrolysis of **5b**, pure samples of **14** and **15** were exposed to conditions identical to those used for the reaction of azide ion with $^{13}C$-labeled **5b** (0.01 M phosphate buffer; $[NaN_3]$ = 1.98 M; 65 °C for 2 h). The $^{13}C$-NMR spectral intensities (Table 6) indicate that both anomers react with azide to give measurable quantities (≈10%) of the diastereomeric azide, as well as smaller quantities of two other products. These two other products, which constituted almost 50% of the carbohydrate-based material when the reaction of 2-deoxy-$\beta$-glucopyranosyl azide was allowed to continue for 20 h, were identified as 2-deoxy-α- and $\beta$-glucose (Figure S2e, supporting information).

**Mechanism for the Reactions of 2-Deoxy-α- and β-glucosyl Azides with Azide Ion.** If either anomer of 2-deoxyglucopyranosyl azide reacts with azide ion in a concerted, nucleophilic displacement reaction $A_ND_N$ ($S_N2$), then according to the principle of microscopic reversibility, both anomers must react with azide via an $A_ND_N$ mechanism. The procedure of Banait and Jencks affords an estimated rate of $10^{16}-10^{18}$ $s^{-1}$ for the collapse of an intimate ion pair containing (2-deoxyglucopyranosyl)oxocarbenium and azide ions to give 2-deoxyglucopyranosyl azide.[7] Since the value of this estimated rate constant is much greater than the frequency of the C−N bond vibration (≈$10^{13}$ $s^{-1}$), collapse of the intimate ion pair will occur without an activation barrier to produce 2-deoxyglucopyranosyl azide. Hence, the intimate ion pair cannot be an intermediate, and the reactions of both anomers of 2-deoxyglucopyranosyl azide with azide ion will proceed through a concerted mechanism $A_ND_N$ ($S_N2$).

**Mechanism for the Hydrolysis of (2-Deoxyglucosyl)pyridinium Salts. (a) Reactions in the Presence of Azide Ion.** Since the hydrolysis reactions of **5b** follow a stepwise $D_N + A_N$ ($S_N1$) mechanism (*vide supra*), there are three possible structures for the intermediate that forms directly following the rate-limiting C−N cleavage step of the reaction. As shown below, the three structures are (1) an intimate ion pair:molecule complex (IIP); (2) a solvent-separated ion pair:molecule complex (SSIP); and (3) an ion−molecule pair (IMP).

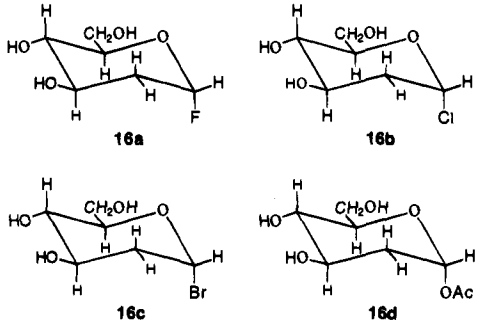



As discussed earlier, the collapse of an azide and (deoxyglucosyl)carbenium ion pair to form product occurs without an activation barrier (*vide supra*) and the reaction proceeds in a concerted fashion. Therefore, the intimate ion pair:molecule complex (IIP) can be ruled out as a potential intermediate that forms during the reactions of glycosylpyridinium salts with azide ion. In the case of the ion−molecule pair intermediate (IMP), diffusional separation to give a free oxocarbenium ion and pyridine would occur faster ($10^{11}$ $s^{-1}$)[19] than would the diffusional reaction of azide ion with the complex ($10^{10}$ $s^{-1}$).[20] Therefore, the predominant reaction pathway for (IMP) complexes would involve dissociation of the ion−molecule pair to give free solvent-equilibrated oxocarbenium ions. However, in the reactions of (2-deoxyglucopyranosyl)pyridinium salts with azide ion, the initial azide product is predominantly the α-anomer. This indicates that the first-formed intermediate reacts with $N_3^-$ and that most of these reactions must occur before solvent-equilibrated oxocarbenium ions are formed. Therefore, the reactions of **5b** with azide ion cannot involve the (IMP) intermediate. Accordingly, the first-formed intermediate that yields the glycosyl azide product must be a solvent-separated ion pair that is in contact with the pyridine leaving group (SSIP).

**(b) Reactions in the Presence of Azide Ion and Other Anions.** Formation of a solvent-separated ion pair:molecule complex as an intermediate in the **5b** azide substitution reactions

(19) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1−19.
(20) Calculated at 2.0 M azide ion with a diffusional rate constant of 5 × $10^9$ $M^{-1}$ $s^{-1}$.

is in accordance with the observed trend in the quantity of retained azide product that is formed with respect to the presence of other anions. A solvent-separated ion pair:molecule complex that contains an anion other than azide should also collapse to yield both substitution and hydrolysis products. By analogy to the azide ion reaction, the substitution reactions of **5b** with $F^-$, $Cl^-$, $Br^-$, or $AcO^-$ will predominantly occur with inversion of configuration to give compounds **16a–d**.




On the basis of the known reactivity of peracetylated glycosyl halides to nucleophilic attack,[21] susceptibility of the above halogen derivatives to hydrolysis should be **16a** < **16b** < **16c**. It is estimated that the least reactive halide compound in this group (2-deoxy-α-D-glucopyranosyl fluoride (**16a**)) has a 10-s half-life for hydrolysis at 65 °C.[22] Therefore, analysis of the relative quantities of 2-deoxyglucosyl halide products formed *in situ* by the reactions of **5b** in the presence of $N_3^-$ and the corresponding halide ion is based on the amount of retained azide product generated during the reaction.

It has been concluded that an intimate ion pair between a (2-deoxyglucopyranosyl)oxocarbenium ion and an azide ion cannot exist and as such has been ruled out as a viable intermediate (*vide supra*) in these reactions. In this case the nucleophilic reactions of 2-deoxy-β-glucopyranosyl azide are required to be concerted. Therefore, due to microscopic reversibility, **16a–c** would react with azide ion to produce 2-deoxy-β-glucopyranosyl azide through a concerted displacement ($A_ND_N$) mechanism. For 2-deoxyglucosyl halides, the ratio of substitution to hydrolysis products formed ($k_{N_3^-}/k_{H_2O}$) is expected to decrease as the nucleofugality of the leaving group increases.[4c] However, with respect to the leaving group nucleofugality, the predicted changes in $k_{N_3^-}/k_{H_2O}$ are expected to be fairly minor. For example, in the bimolecular reactions involving 1-methoxy-3-(4-methoxyphenyl)propane derivatives in which the nucleofuge has been altered from *p*-nitrophenolate ($I$ = 2.0 M; $t$ = 41 °C) to the better leaving group acetate ($I$ = 1.0 M; $t$ = 25 °C), the selectivity ratio ($k_{N_3^-}/k_{H_2O}$) changes from 1.2 to 0.8 $M^{-1}$.[4c] Therefore, if equal amounts of the respective 2-deoxyglucopyranosyl halide products (**16a–c**) were formed during the reaction of **5b** in the presence of the corresponding halide ion, the resulting quantity of 2-deoxy-β-glucopyranosyl azide product formed should be greatest for the reaction of **5b** with fluoride ion and least for the reaction with bromide ion. In reality, the 2-deoxyglucopyranosyl halides appear to be produced in quantities that parallel the order of nucleophilicity in water of the halide ions.[24] That is, **16c** ($Br^-$) > **16b** ($Cl^-$) > **16a** ($F^-$). In an hydroxylic medium, solvation of solute anions occurs by way of hydrogen-bonding of the solute to the hydroxylic protons of the solvent. This type of interaction in hydroxylic solvents is the primary factor contributing to the reduced nucleophilicity of the small fluoride ion as compared to the larger bromide ion. Whereas, in dipolar, aprotic solvents, fluoride is the stronger nucleophile.[25] Therefore, the *in situ* formation of more **16c** than **16a** is consistent with the reaction involving an intermediate such as the solvent-separated ion pair: molecule complex (SSIP), in which the anion is fully solvated. In the reactions containing fluoride ion, formation of a smaller amount of 2-deoxy-β-glucopyranosyl azide (**14**) product cannot be attributed to the particular cation used in the reaction. When tested in the presence of chloride ion, the cation ($K^+$ or $Na^+$) had no significant effect on the quantity of **14** produced (Table 5; Figure S1c,d, supporting information).

**Scheme 4**

$$Glu^{\oplus} \cdot N_3^{\ominus} \xrightarrow{k_{nuc}} GluN_3$$

$$Glu^{\oplus} \cdot Py \xrightarrow{k_{nuc}} {}^{\oplus}GluPy$$

The order of the rate constants for hydrolysis of **5b** in the presence of halide ions (Table 2; $k_{obsd}(F^-) > k_{obsd}(Cl^-) > k_{obsd}(Br^-)$) can be understood when examined in terms of the relative stabilities of the various SSIP's involved. The smaller, more basic anions form stronger hydrogen-bonds to the aqueous solvent. As a result, increased polarization of the water lowers the energy of the SSIP intermediate. Therefore, the rate constants associated with the smaller anions are greater than the larger, more nucleophilic anions.

**Effect of the Leaving Group Charge on the Mechanism of Reaction.** The above discussion indicates that, despite having leaving groups with similar $pK_a$'s,[26] 2-deoxy-β-glucopyranosyl azide and (2-deoxy-β-glucopyranosyl)-4′-bromoisoquinolinium salts undergo nucleophilic substitution reactions that occur by different mechanisms ($A_ND_N$ and $D_N + A_N$, respectively). The concept that these substitution reactions proceed by different mechanistic routes is consistent with the conclusion that, although α-glucopyranosyl fluoride reacts via a concerted mechanism with azide ion, the reaction gives no β-glucopyranosylpyridinium salt when it is conducted in the presence of pyridine.[7] Consequently, the reverse of these reactions (that is, substitution of β-glucopyranosyl azide and β-glucopyranosylpyridinium salt with fluoride ion) must also proceed through contrasting mechanistic pathways. Scheme 4 shows a possible explanation for the different behavior exhibited by glycopyranosyl azide and glycopyranosylpyridinium salts in nucleophilic substitution reactions.

The rate of collapse ($k_{nuc}$) of the ion pair $Glu^+ \cdot N_3^-$, which is estimated to be approximately $10^{16}$–$10^{18}$ $s^{-1}$ (*vide supra*), dictates that the reactions of glycosyl azides proceed by a concerted mechanism.[7] Since the aqueous reactions of glycosylpyridinium salts occur in a stepwise manner, the collapse ($k_{nuc}$) of the ion–molecule complex ($Glu^+ \cdot Py$) cannot be significantly faster than diffusional separation, a process that occurs with a rate constant of approximately $10^{11}$ $s^{-1}$.[19] The difference in the rates of collapse for the two reactions shown in Scheme 4 can be attributed to the strong, attractive, electrostatic force of the ion pair which, in the case of the ion–molecule pair ($Glu^+ \cdot Py$), is replaced by a weaker ion-dipole force.

**Lifetime of the Glucosyloxocarbenium Ion.** Illustrated in Scheme 5 are the possible reactions of the solvent-separated

(21) Stanek, J.; Cerný, M.; Kocourek, J.; Pacák, J. *The Monosaccharides*; Academic Press: New York, 1963; pp 198–199.

(22) Assuming an increase of 500-fold in the rate of hydrolysis compared to α-D-glucopyranosyl fluoride, which has a calculated hydrolytic rate constant of $1.29 \times 10^{-4}$ $s^{-1}$ ($\mu$ = 1.0) at 65 °C.[23]

(23) Zhang, Y.; Bommuswamy, J.; Sinnott, M. L. *J. Am. Chem. Soc.* **1994**, *116*, 7557–7563.

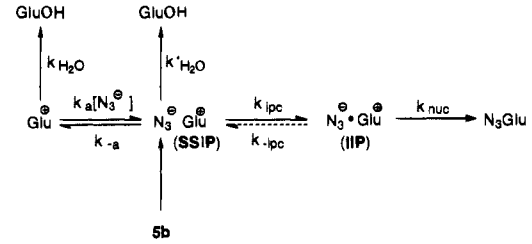
(24) (a) Swain, C. G.; Scott, C. B. *J. Am. Chem. Soc.* **1953**, *75*, 141–147. (b) Ibne-Rase, K. M. *J. Chem. Educ.* **1967**, *44*, 89–94. (c) Koivurinta, J.; Kyllönen, A.; Leinonen, L.; Valaste, K.; Koskikallio, J. *Finn. Chem. Lett.* **1974**, 239–243.

(25) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1–32.

(26) $pK_a$(4-bromoisoquinoline–$H^+$) = 3.31,[15] $pK_a$(pyridine–$H^+$) = 5.25,[15] $pK_a(HN_3)$ = 4.70.[27]

(27) Sillén, L. G.; Martell, A. E. *Stability Constants of Metal-Ion Complexes*, Supplement No. 1; The Chemical Society: London, 1971.

**Scheme 5**




ion pair (SSIP) formed during the reactions of glycosylpyridinium salts such as **5b**. The pyridine leaving group has been omitted from the scheme, since there is no stereochemical information available to indicate the initial configuration of the 2-deoxyglucose hydrolysis product.[28]

The data from Tables 5 and 6 allow a quantitative estimate for the amount of β-azide product (**14**) formed directly from the reaction of $^{13}$C-labeled **5b** via the free oxocarbenium ion ($Glu^+$) and indirectly by way of 2-deoxy-α-glucopyranosyl azide (**15**) (0.01 M phosphate buffer; 1:1 $NaH_2PO_4$:$Na_2HPO_4$, [$NaN_3$] = 1.98 M at 65 °C for 2 h). A correction to the experimentally measured product distribution is required because the average exposure time of 2-deoxy-α-glucopyranosyl azide (**15**) to the reaction conditions varies depending on the particular starting material involved. When **15** is the starting material (Table 6), the length of time each molecule of **15** resides in the reaction medium is about 120 min (<10% of **15** has reacted during the 2-h time period). Whereas, when **5b** is subjected to the reaction conditions (Table 5), every product molecule of **15** resides in the aqueous medium an average of about 85 min.[29] Therefore, when both the relative quantity of β-azide product (**14**) formed and the shorter reaction time are taken into account, an estimated 65−70% of the azide product **14** that is generated during the reaction of **5b** originates indirectly from an $A_ND_N$ reaction involving the α-azide product (**15**), while the remaining 30−35% (≈3% of the total azide product) arises directly from the reaction of **5b** via the free $Glu^+$ cation. Since a small fraction of the azide product from the reactions of **5b** has been generated via the free $Glu^+$ cation, it is expected that some of the hydrolysis product (2-deoxyglucose) will also have been formed from the free $Glu^+$ cation. However, experimental results have shown that, in the reactions of **5b**, most of the azide product generated has an inverted configuration. Therefore, this product must be the result of the direct, irreversible conversion of the solvent-separated ion pair (SSIP) to the intimate ion pair (IIP, $k_{nuc} \gg k_{-ipc}$).

Solvent reorganization is required for the conversion of SSIP to IIP, and this process ($k_{ipc}$) is anticipated to occur at a rate of about $10^{11}$ $s^{-1}$.[19] Under conditions where only azide ion is present, approximately one-half of the reaction product results from the collapse of the SSIP via $k_{ipc}$ to give azide product. Consequently, the rate constant $k'_{H_2O}$ must be $\leq k_{ipc}$. Therefore, the lifetime of the (2-deoxyglucosyl)oxocarbenium ion ($1/k'_{H_2O}$) at the stage of the SSIP is greater than $1/k_{ipc} = 1 \times 10^{11}$ s. Thus, using an estimated factor of four for destabilization of an oxocarbenium ion caused by a β-substituted hydroxyl group,[31] a lifetime of greater than $1/(1 \times 10^{11} \times 4) = 2.5 \times 10^{-12}$ s can be predicted for the glucosyloxocarbenium ion at the stage of the SSIP. Although an estimate for the reaction rate of the free (2-deoxyglucosyl)carbenium ion with water ($k_{H_2O}$) cannot be made from the present data, it appears reasonable that the two rate constants $k_{H_2O}$ and $k'_{H_2O}$ will be similar in magnitude. In that case, the free glucosylcarbenium ion would also have a lifetime of greater than $2.5 \times 10^{-12}$ s. This value is slightly larger than the estimated lifetime of $1 \times 10^{-12}$ $s^{-1}$ for the glucosyloxocarbenium ion in water reported by Amyes and Jencks.[31] In their study, Amyes and Jencks derived an estimated lifetime for the glucopyranosyloxocarbenium ion by extrapolating from the experimentally-measured lifetimes of acyclic oxocarbenium ions.

The transition states elucidated for the specific acid-catalyzed hydrolyses of methyl α- and β-D-glucopyranoside indicate that ring C−O bond strengthening lags behind leaving group departure.[5] Therefore, when methanol (and by analogy, water) reacts with the glucosyloxocarbenium ion, a distortion of the pyranosyl ring is required before addition of methanol can occur. In all probability, the discrepancy between the estimated value for the glucopyranosyloxocarbenium ion lifetime in water reported in this paper and the corresponding value obtained by Amyes and Jencks can be attributed to a difference in the reactivity of the cyclic versus acyclic compounds. Specifically, it is likely that the cyclic glucopyranosyloxocarbenium ion has a longer lifetime in $H_2O$ than previously estimated based on acyclic ions because of the requisite distortion of the pyranosyl ring that must precede the attack by water.

## Conclusions

The work presented in this paper shows that of the three small "hard" anions used as nucleophiles in the hydrolysis of the (2-deoxy-β-glucopyranosyl)-4′-bromoisoquinolinium salt (**5b**), only azide produces an appreciable quantity of inverted 2-deoxyglucopyranosyl product. The mechanism of glycoside hydrolysis depends not only on the $pK_a$ of the conjugate acid of the leaving group but on the leaving group charge. The glucosyloxocarbenium ion has an estimated lifetime of greater than $2.5 \times 10^{-12}$ s, indicating that it is a viable intermediate in the solvolysis of glucosyl derivatives that contain an uncharged leaving group.

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**Supporting Information Available:** Text giving experimental details, tables listing observed rate constants for the hydrolysis of **5b**, and $^{13}$C-NMR spectra (19 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(28) Preliminary results in a methanol:water (20:80 v/v) mixture show that methanolysis occurs with attack at both faces of the oxocarbenium ion. Therefore, the leaving group has at least partially freed the β-face for reaction with the solvent.

(29) This is calculated by subtracting the average time before a molecule of **5b** reacts (i.e., $1/k$)[30] from the total time of the reaction.

(30) Wilkinson, L. *Chemical Kinetics and Reaction Mechanisms*; Van Nostrand Reinhold: New York, 1980; p 17.

(31) Amyes, T. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7888−7900.