Synthesis and Hydrolysis Kinetics of 1-O-[p-(N,N,N-Trimethylammonio)phenyl]-β-D-ribofuranoside

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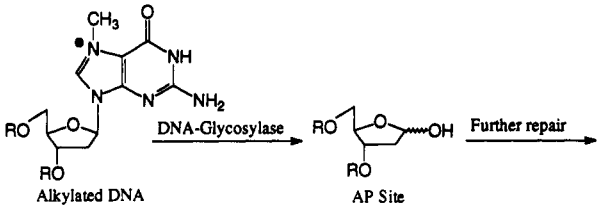
Glycosyl transferases, enzymes that catalyze the transfer of sugar moieties within the biosynthetic pathway, are key components in the anabolism and catabolism of carbohydrates such as cellulose, lactose, and starch. Glycosides containing *p*-nitrophenol groups have been utilized extensively in the assay and study of glycosyl transferase enzymes.[1] The ready availability, low cost, and ease of spectroscopic monitoring make *p*-nitrophenyl glycosides attractive and useful components in enzyme activity determinations. The mechanistic pathways involved in glycosidic hydrolysis have been the focus of considerable research.[2] *p*-Nitrophenol's leaving-group ability has been incorporated advantageously into these studies.

Mammalian DNA glycosylases are important in one of the repair systems for alkylated DNA bases.[3] Excision of methylated bases is accomplished by these enzymes via hydrolysis of the glycosidic bond (Scheme I). Complete reparation of the alkylated DNA can then be achieved through the action of an additional enzyme on the newly formed apurinic site. Because several glycosyl transferase enzymes act on substrates with cationic aglycons (e.g., 7-methylguanine-DNA-glycosylase and 3-methyladenine-DNA-glycosylase), we have sought a nonnucleosidic, nonpyridylic ribofuranoside possessing a reactive aglycon that is positively charged. Such a species would provide a new UV-active substrate for use in mechanistic studies. We now report the synthesis and hydrolysis kinetics of 1-O-[p-(N,N,N-trimethylammonio)phenyl]-β-D-ribofuranosyl iodide (3).

Results

As shown in Scheme II, the synthesis of 3 was achieved through a two-step procedure starting with 1-O-(*p*-nitrophenyl)-β-D-ribofuranoside (1).[4] Catalytic hydrogenation yielded 1-O-(*p*-aminophenyl)-β-D-ribofuranoside (2) in 87% yield after lyophilization. Exhaustive methylation of 2 was achieved by addition of methyl iodide

Scheme I



with gentle warming of the acetonitrile solution; the course of the reaction could be followed easily by TLC on silica gel (30% MeOH in $CHCl_3$). Purification by precipitation from isopropyl alcohol afforded a yellow film that upon lypohilization yielded a light brown solid. Both ^1H and ^{13}C NMR demonstrated the homogeneity of the product.

Hydrolysis kinetics were obtained by a variation of our previously reported method.[6] Riboside 3, dissolved in water, was injected into equilibrated refluxing buffered solutions. Aliquots (≈1 mL) was removed at timed intervals and cooled immediately. The pH was adjusted to ≥9 with strong base if necessary,[5] and the absorbance at 288 nm was measured. The data were then fit to a first-order equation and a rate constant calculated. The k_{obs}'s for pH's 2–11 were obtained and are shown in Table I and Figure 1. These data are compared to the hydrolysis kinetics data for the hydrolysis of 1.[2a] Reactions in the pH-independent region (4–8) were not monitored to completion, owing to the slow reaction rate; rather, all were followed to at least 25% completion and rate constants were based on calculated endpoints.

Discussion

Attempted crystallization of 3 proved to be unfruitful. The riboside's solubility in alcoholic or aqueous solution was very high. Attempts with a cosolvent system yielded a method of precipitation, but not crystallization. All impurities seen in the ^1H and ^{13}C NMR spectrums could be removed via selective precipitation.

The pH profile shown in Figure 1 clearly demonstrates a difference in reactivity between 3 and 1. The acidic regions show similar rates of hydrolysis, but the near-neutral and alkaline regions show that 1 hydrolyzes about 1 order of magnitude faster than 3. The pK_a's for *p*-nitrophenol and *p*-(hydroxyphenyl)trimethylammonium tosylate are 7.15[6] and 8.35,[7] respectively. This 1.2 unit difference in acidity led us to anticipate that 3 would hydrolyze more slowly in the pH-independent region. Riboside 3's pH-independent reactivity is slower than that of the *p*-nitroriboside, and the region has also been shifted nearly 1 pH unit more alkaline compared to 1.

Summary

The synthesis of 1-O-[p-(N,N,N-trimethylammonio)-phenyl]-β-D-ribofuranosyl iodide has been achieved through the reduction of 1-O-(*p*-nitrophenyl)-β-D-ribofuranoside and exhaustive methylation of the resulting amine. The

(1) Recent examples: (a) Boretto, L. J.; Villette, J. R.; Fontaine, I. F.; Sicard, P. J.; Bouquelet, S. J.-L. *Biotechn. Appl. Biochem.* **1992**, *15*, 59–68. (b) Gopalan, V.; Van der Jagt, D. J.; Libell, D. P.; Glew, R. H. *J. Biol. Chem.* **1992**, *267*, 9629–9638. (c) Chauvaux, S.; Béguin, P.; Aubert, J.-P.; *J. Biol. Chem.* **1992**, *267*, 4472–4478. (d) Alonso, J. M.; Santa-Cecilia, A.; Calvo, P. *Biochem. J.* **1991**, *278*, 721–727. (e) Ware, C. E.; Lachke, A. H.; Gregg, K. *Biochem. Biophys. Res. Commun.* **1990**, *171*(2), 777–786. (f) Pulvin, S.; Friboulet, A.; Thomas, D. *Biochim. Biophys. Acta* **1990**, *1041*(2), 97–100.

(2) For an overview, see: (a) Cherian, X. M.; Van Arman, S. A.; Czarnick, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 4490–4498. (b) Sinnott, M. I. In *The Chemistry of Enzyme Action*; Page, M. I., Ed.; Elsevier: Amsterdam, 1984; pp 389–432.

(3) (a) Gallagher, P. E.; Brent, T. P. *Biochim. Biophys. Acta* **1984**, *782*, 394–401. (b) Pegg, A. E.; Bennett, R. A. In *Enzymes of Nucleic Acid Synthesis and Modification*; Jacob, S. T., Ed.; CRC Press: Boca Raton, 1983; Vol. I, pp 180–200.

(4) Previously synthesized in our group using the known method: Honma, K.; Nakazima, K.; Uematsu, T.; Heimada, A. *Chem. Pharm. Bull.* **1976**, *24*, 394.

(5) The quench to pH 9 is done at room temperature, and the UV is taken within 30 s after the quench. At 100 °C, the hydrolysis reaction at pH 9 would have proceeded about 0.15% after 30 s. At room temperature, there is no observable reaction at pH 9 after even several hours.

(6) *Lange's Handbook of Chemistry*, 13th ed.; Dean, J. A., Ed.; McGraw-Hill: New York, 1985; pp 5–51.

(7) Bordwell, F. G.; Boutan, P. J. *J. Am. Chem. Soc.* **1956**, *78*, 87–91.

0022-3263/93/1958-2343$04.00/0

Scheme II

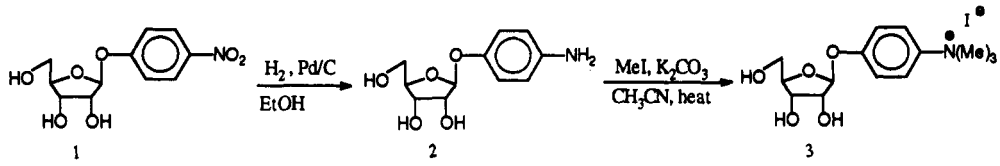


Table I. Observed Rate Constants for the Hydrolysis of 3 at 100 °C and Various pH's

pH	k_{obs} (min^{-1})	log k	pH	k_{obs} (min^{-1})	log k
1	7.0×10^{-1}	−0.15	7	1.7×10^{-4}	−3.8
2	6.8×10^{-2}	−1.2	8	1.7×10^{-4}	−3.8
3	4.0×10^{-3}	−2.4	9	2.9×10^{-3}	−2.5
4	2.7×10^{-4}	−3.6	10	3.8×10^{-3}	−2.4
5	1.3×10^{-4}	−3.9	11	1.2×10^{-2}	−1.9
6	1.2×10^{-4}	−3.9			

pH–rate profile demonstrates rapid acidic and alkaline hydrolysis as well as a slower pH-independent region from 4.5 to 8.0.

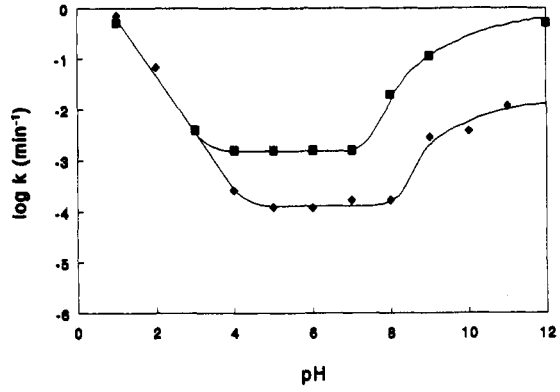


Figure 1. pH–rate profiles for the hydrolysis of 1 (■) and 3 (♦) at 100 °C.

Experimental Section

General. Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Microanalyses were carried out at Canadian Microanalytical Services, New Westminster, BC, or Atlantic Microlab, Inc., Norcross, GA. FT-NMR were obtained at 7.0 T (300 MHz). UV spectra were obtained on a Hewlett-Packard Model 8451A diode-array spectrophotometer; all wavelength data reported are ±1 nm. Most of the chemicals used in this study were obtained from Aldrich Chemical Co., Milwaukee, WI. Biological buffers CAPS and HEPES were obtained from Sigma Chemical Co., St. Louis, MO.

1-*O*-(*p*-Aminophenyl)-β-D-ribofuranoside (2). 1-*O*-(*p*-Nitrophenyl)-β-D-ribofuranoside (1.5 g, 5.6 mmol) was dissolved in ethanol (100 mL), and then 10% Pd/C was added and the system evacuated and pressurized with hydrogen (3 × 50 psi). The system was shaken for 24 h, filtered over Celite and concentrated in vacuo. Lyophilization afforded a white solid (1.2 g, 87%): mp 63–75 °C; ^{1}H NMR (D_2O) δ 3.52 (dd, 1, 5-H), 3.68 (dd, 1, 5-H), 3.93–3.99 (m, 1, 4-H), 4.14 (d, 1, 2-H), 4.20 (t, 1, 3-H), 5.42 (s, 1, 1-H), 6.69 (d, 2, Ar-H), 6.81 (d, 2, Ar-H); ^{13}C NMR (D_2O) 62.7 (t, 5-C), 70.7 (d, 4-C), 74.6 (d, 2-C), 83.5 (d, 3-C), 106.1 (d, 1-C), 117.7 (d, Ar-C), 118.5 (d, Ar-C), 141.6 (s, Ar-C), 149.0 (s, Ar-C); FAB mass spectrum *m/e* 242 (M^+).

Anal. Calcd for $C_{11}H_{15}N_1O_5$: C, 54.77; H, 6.27; N, 5.81. Found: C, 54.36; H, 6.11; N, 5.71.

1-*O*-[*p*-(Trimethylammonio)phenyl]-β-D-ribofuranosyl Iodide (3). Into a 100-mL round-bottom flask was placed 1-*O*-(*p*-aminophenyl)-β-D-ribofuranoside (0.098 g, 0.41 mmol), iodomethane (0.4 mL, 4 mmol), 2,4,6-lutidine (94 mg, 0.89 mmol), and anhydrous acetonitrile (50 mL). The system was warmed to 45 °C and the reaction monitored by TLC (30% MeOH/$CHCl_3$). Upon completion, the solution was filtered and concentrated in vacuo. The resulting yellow solid was taken up in isopropyl alcohol (60 mL) and the volume reduced (40 mL). Upon refrigeration, a solid appeared on the surface of the flask. The solution was decanted, and the solid was again precipitated from isopropyl alcohol (35 mL). Lyophilization of the precipitate afforded a light brown solid (30 mg, 18%): ^{1}H NMR (D_2O) δ 3.46–3.58 (m, 10, Me and 5-H), 3.70 (dd, 1, 5-H), 4.00–4.07 (m, 1, 4-H), 4.24–4.31 (m, 2, 3-H and 2-H), 5.67 (s, 1, 1-H), 7.13 (d, 2, Ar-H), 7.69 (d, 2, Ar-H); ^{13}C NMR (D_2O) δ 60.0 (q, Me), 65.2 (t, C-5), 73.4 (d, C-4), 77.4 (d, C-3), 86.7 (d, C-2), 107.5 (d, C-1), 120.5 (d, ArC), 124.2 (d, ArC), 143.7 (s, ArCO), 158.4 (s, ArCN); FAB mass spectrum *m/e* 284 (M^+), 152 (phenol).

Anal. Calcd for $C_{14}H_{22}INO_5H_2O$: C, 39.17; H, 5.64; N, 3.26. Found: C, 39.19; H, 5.50; N, 3.36.

Kinetic Measurements. The following buffers (50 or 100 mM) were used for all kinetic studies: pH 1–2, HCl/KCl; pH 4–5, CH_3CO_2H; pH 6, sodium phosphate; pH 7, potassium phosphate; pH 8, HEPES; pH 9–10, sodium borate; pH 11, CAPS. The hydrolysis of 3 was accomplished as follows. A solution of the appropriate buffer (60 mL) was equilibrated at 100 ± 0.5 °C for a minimum of 1 h, and then a solution of 3 in water (0.30 M) was injected (83 μL, 0.41 mM overall). Aliquots (≈1 mL) were withdrawn at timed intervals and placed into iced water until cooled to room temperature (1 min). A 2 N solution of NaOH (40 μL) was added to a constant volume of those solutions with pH's <9 to effect complete deprotonation of the phenoxide. The increase in absorbance at 288 nm was used to monitor the formation of the phenoxide product. Because of the very slow reactions for pH's 4–8, these reactions were not followed to completion; A_∞ values were calculated on the basis of the known concentration of the starting material. The determination of first-order rate constants (min^{-1}) was accomplished by using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, UK.

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