Communication

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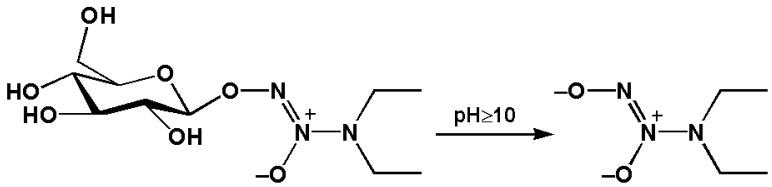

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Diazeniumdiolate Ions as Leaving Groups in Anomeric Displacement Reactions: A Protection−Deprotection Strategy for Ionic Diazeniumdiolates

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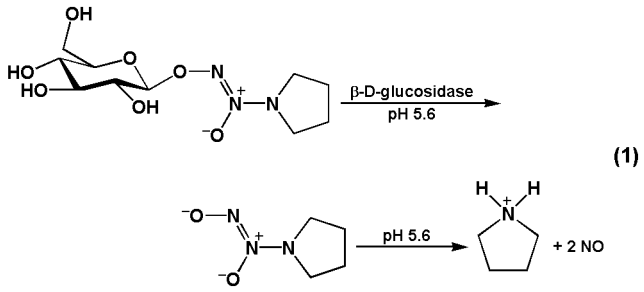
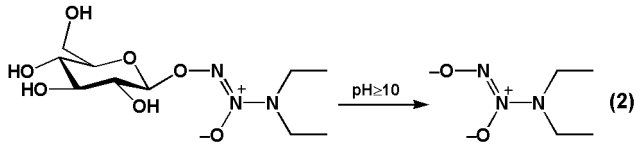
Carbohydrates substituted at the anomeric position with nitric oxide-releasing diazeniumdiolate ions are a promising new class of prodrug, having been reported to be stable at physiological pH but readily hydrolyzed by glycosidases, as in eq 1.[1] Thus, Wu et al. have introduced them as a means of selectively generating bioactive nitric oxide in cell types that possess the appropriate enzymatic activity.[1]



(1)

We have discovered a second, synthetically and mechanistically significant feature of their chemistry: an unexpected sensitivity to base- (but not acid-) induced cleavage of the anomeric substituent. Hydrolysis of glucoside **1** was found to proceed as outlined in eq 2. The observed reactivity is described by a rate expression of the form $k_{obs} = k_o + k_{OH^-}[OH^-]$. The hydrolysis rate displayed a linear dependence on $[OH^-]$ between pH 12.9 and 14.0. The mean value of k_{OH^-} was 5.3×10^{-3} M^{-1} s^{-1}. Diazeniumdiolate ion **2** was confirmed as a primary product in 0.1 M sodium hydroxide, with clean isosbestic behavior being observed as the starting material's peak at 228 nm gave way to that of **2** at 250 nm.[2]



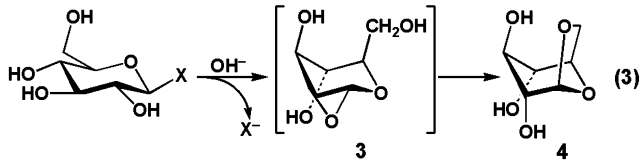
(2)

1 **2**

Hydrolysis of **1** in acid was very slow. The mean value of k_o, $(7.8 \pm 2.4) \times 10^{-7}$ s^{-1}, obtained from NO generation rates followed over 8 days by chemiluminescence[2] at 37 °C and pH values of 7.4, 5.6, and 2.6, was almost 800-fold slower than that in 0.1 M NaOH. Rate constants estimated from NO release profiles at pH 5.6 (citrate) and 7.4 (phosphate) showed the hydrolysis rate to be only marginally (ca. 50%) slower than that at 2.6 (citrate).

Inverse pH rate behavior was also observed for the hydrolysis of the β-fluoro[3,4] and β-*p*-nitrophenoxy[5] glucosides. The half-life of **1** was >25-fold shorter than that for the *p*-nitrophenoxide, as measured by following the decrease in the respective anomeric proton NMR signals. That of the fluoro analogue proved to be too fast to follow under these conditions, bracketing the nucleofugality of the diazeniumdiolate as being between those of fluoride and *p*-nitrophenoxide. Rate constants (k_{obs}) for dissociation of the diazeniumdiolate, the fluoride, and the *p*-nitrophenoxide were 8.8 $\times 10^{-5}$, $>1.2 \times 10^{-3}$, and 3.3×10^{-6} s^{-1}, respectively, at 37 °C and pH 12.4.

Base-induced hydrolysis of β-D-glucosyl fluoride and related saccharides that have a potentially nucleophilic C-2 substituent trans to a good leaving group at the anomeric position has been rationalized[4−7] as proceeding via anchimeric assistance by a C-2 heteroatom as shown in eq 3. According to this mechanism, the substrates are postulated to convert initially to glycal oxide **3**, an intermediate whose oxirane ring is rapidly opened by attack of the C-6 hydroxyl group at C-1 to form 1,6-anhydroglucose (**4**), as outlined in eq 3.



3 **4** (3)

Consistent with this prediction, **4** was the major product of all three pH 12.4 reactions, with glucose being produced in low yield in each case. There was no evidence for conversion of glucose to **4** under these conditions, indicating that **4** was indeed a primary product of these reactions.

The above-described similarities between anomerically substituted **2**, fluoride, and *p*-nitrophenoxide suggest that diazeniumdiolate ions can be considered halide-like, but there are important differences that may make them uniquely useful. For example, ion **2**

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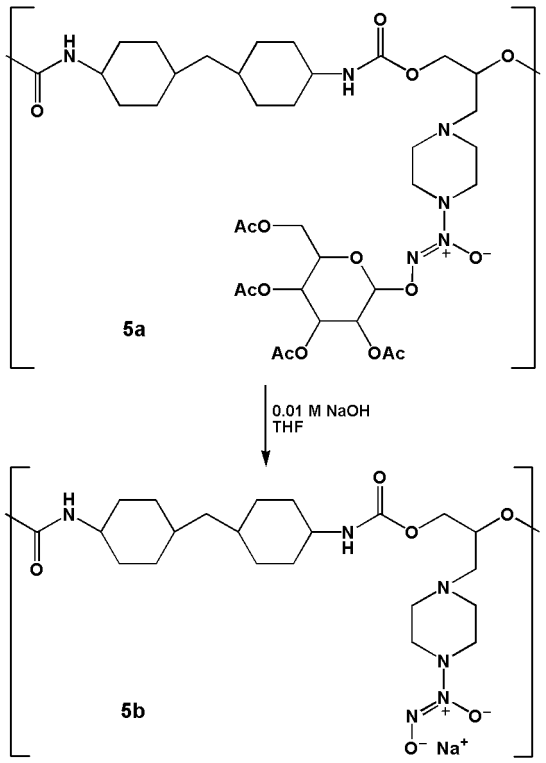
Scheme 1. Preparation of Diazeniumdiolated Poly(urethane) **5b** from **5a** (Partial Structural Unit)



lies between fluoride and chloride as an S_NAr nucleofuge,[8] but it is much less reactive than either halide in anomeric displacement reactions. Indeed, the fluoride is seen above to hydrolyze much faster than **1** in base and in acid (see Supporting Information), and the chloride is so unstable that it has yet to be characterized.

The stability of **1** at neutral pH, combined with the relative ease of preparing such glycosides, suggests a significant applicability of this chemistry in the synthetic as well as biomedical realm. As one example, we are exploiting this pH-dependent susceptibility to hydrolysis as a protection−deprotection strategy for preparing NO-releasing substances that are otherwise difficult or impossible to access synthetically. A case in point is poly(urethane) **5** (Scheme 1), which contains diazeniumdiolated piperazine moieties attached to its diol component. Poly(urethane) **5** was prepared by reacting tetraacetyl glucose-protected *N*′-(2,3-dihydroxy)piperazine diazeniumdiolate with 4,4′-methylene-bis-cyclohexyldiisocyanate, as described in the Supporting Information. No NO release was observed when a film of tetra-O-acetylglucosylated derivative **5a** was soaked for several days in pH 7.4 phosphate. When **5a** was exposed to base to convert it to its ionic form **5b**, however, smooth release of NO (42% of theoretical; 5 wt % of protected monomer) at surface fluxes of ≥ 4 pmol min^{-1} mm^{-2} was observed for more than 10 h on exposing the recast film (10 × 10 mm and approximately 25−75 μm thick) to physiological buffer (Figure 1). This rate of NO generation matches or exceeds that produced by normal vascular endothelium to prevent adhesion of blood cells, particularly platelets, to the vessel wall.[9]

Preliminary studies have indicated that **5a** is stable at temperatures up to 210 °C, making it a promising candidate for fabricating biomedical devices requiring processing under conditions that would destroy ionic diazeniumdiolate groups. As one example, tubing might be manufactured by extruding **5a**, then treating it with a suitable base to remove the saccharide residues. By thus converting the composition to NO-releasing and hence thromboresistant form

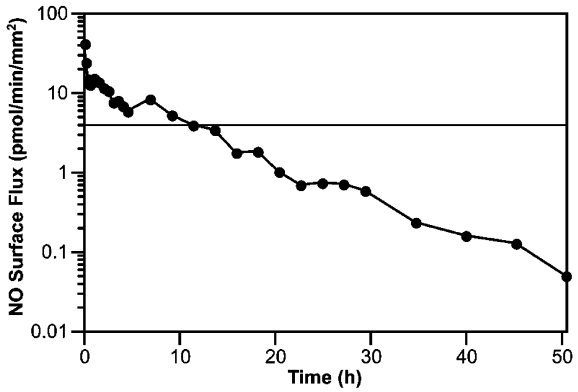


Figure 1. Flux of NO generated from the surface of a film of polymer **5b** in 0.1 M phosphate buffer (pH 7.4) at 37 °C. NO release was measured by chemiluminescence. The horizontal line at 4 pmol min^{-1} mm^{-2} represents the rate of NO generation by the normal vascular endothelium.[9]

5b, loss of platelets from blood that contacts the tubing (for example, that of an extracorporeal membrane oxygenation circuit[10]) should be minimized, allowing the clinical procedure to be conducted without the need for potentially risky systemic anticoagulation. Such a product would have the advantage of covalently binding all components of the NO-releasing moiety to the polymer, such that only NO can be released at the surface.

Further work is underway to explore the technical issues (maximizing loading of the NO donor, optimizing the deprotection of the sugar, and tuning the physical properties of the polymer) relevant to the possible clinical utility of such materials, as well as to establish the full chemical profile of the O^2-glycosylated diazeniumdiolates.

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Supporting Information Available: Preparations of **1**, its tetra-*O*-acetate, **5a**, and **5b**; kinetic plots and the ultraviolet and proton spectra from which they were derived; details of the chemiluminescence and thermogravimetric analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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