The base-catalysed anomerization of dinitrophenyl glycosides: evidence for a novel reaction mechanism

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This paper is dedicated to Professor Ross Stewart on the occasion of his 65th birthday

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The mechanism of base-catalysed anomerization of per-*O*-acetylated 2,4-dinitrophenyl-β-D-glucopyranoside in dimethylsulfoxide has been investigated using a variety of techniques. A mechanism involving proton abstraction at C-1 was eliminated by the absence of proton exchange at that center and the measurement of a secondary deuterium kinetic isotope effect for the 1-deuterio substrate. A mechanism involving phenolate departure and recombination is rendered unlikely on the basis of remote substituent effects on the reaction rate and by the absence of any exchange of the phenyl moiety with added phenolate. A mechanism involving nucleophilic aromatic substitution initiated by an attack of the dimethylsulfinyl anion to generate a glucosyl oxyanion intermediate that anomerizes and recombines with the reactive aryl intermediate is consistent with the observations. This mechanism is further supported by the observation of a purple Meisenheimer complex intermediate and by the observed exchange between the substrate containing a labelled sugar moiety and added unlabelled 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose.

Key words: glycoside, anomerization, reaction mechanism.

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Faisant appel à diverses techniques, on a examiné le mécanisme de l'anomérisation catalysée par les bases du β-D-glucopyranoside de 2,4-dinitrophényle per-*O*-acétylé en solution dans le diméthylsulfoxyde. En se basant sur le fait qu'il n'y a pas d'échange de proton dans cette position et sur la mesure d'un effet isotopique cinétique secondaire du deutérium pour le substrat 1-deutéré on a éliminé un mécanisme impliquant l'enlèvement d'un proton de la position C-1. En se basant sur l'existence d'effets des substituants éloignés sur la vitesse de la réaction et sur l'absence de tout échange de la portion phénylée lorsqu'on ajoute des ions phénolates, on peut déduire qu'un mécanisme impliquant un départ et une recombinaison du phénolate est improbable. Un mécanisme impliquant une substitution aromatique nucléophile, initiée par une attaque de l'anion diméthylsulfinyle pour générer l'intermédiaire oxyanion glucosyle qui s'anomérise et se recombine avec l'intermédiaire aryle réactif, est en accord avec les données expérimentales. Ce mécanisme est aussi supporté par l'observation d'un complexe intermédiaire de Meisenheimer violet et par l'observation d'un échange entre le substrat contenant une portion sucrée marquée et du 2,3,4,6-tétra-*O*-acétyl-β-D-glucopyranose qui n'est pas marqué.

Mots clés : glycoside, anomérisation, mécanisme de réaction.

[Traduit par la revue]

Introduction

There are many examples of acid-catalysed glycoside anomerization and their mechanisms are reasonably well understood (1–7). In all cases studied to date, including simple alkyl glycosides (1–3), protected alkyl glycosides (4), or fully acetylated sugars (5–7), the mechanism has involved oxocarbonium ion intermediates, whether catalysed by a Lewis or a Brønsted acid. Usually, reaction proceeds via cleavage of the exocyclic carbon–oxygen bond, though in some cases it is suggested that the preferred mode involves endocyclic bond cleavage. Base-catalysed reactions involving the glycosidic linkage are less common and, consequently, little is known about the mechanisms of such reactions. The anomerization of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose and 2′,4′-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (β-2,4-DNPG)[2] has been reported using sodium hydroxide in anhydrous pyridine (8). Although yields were low, this reaction was one of the first methods used to transform a β- to the α-phenyl glycoside. Interestingly, anomerization of the β-glucosides of phenol, 2-nitrophenol, and 4-nitrophenol were unsuccessful. More recent studies (9) have shown that treatment of β-2,4-DNPG with potassium carbonate in dimethylsulfoxide (DMSO) afforded an equilibrium mixture of the α and β anomers (80:20, respectively) in good yield. Reaction of 2′,4′-dinitrophenyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside, however, was found to proceed approximately 70 times more slowly than the acetylated glycoside, leading to the suggestion that the acetyl group at C-2 may participate in the reaction.

Very little is known concerning the mechanism of this base-catalysed anomerization. The only published suggestion for a mechanism is that (10) involving initial abstraction of the proton from the anomeric centre, generating a glycoside anion that can then reprotonate from either side. However, there has been no published evidence in support of this, or any other mechanism. We have considered four possible mechanisms for the carbonate catalysed anomerization of 2′,4′-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (Scheme 1). The first (i) involves proton abstraction at C-1 as suggested by Ferrier (10), forming a carbanion intermediate that can reprotonate from either face. The second (ii) is initiated by phenolate departure, forming an oxocarbonium ion intermediate with possible neighboring group assistance of the C-2 acetoxy group. The phenolate and oxocarbonium ions then recombine to give α or β product. The third mechanism (iii) also involves phenolate departure, but is in this case an elimination reaction initiated by proton abstraction from C-2 with formation of an acetoxyglucal intermediate that subsequently recombines with phenolate. The

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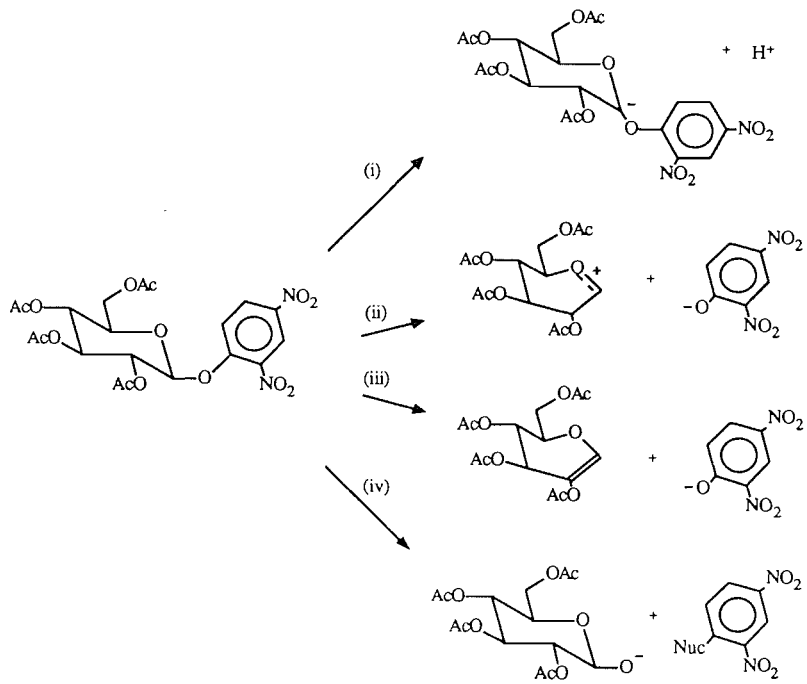
[2]Abbreviations: α-2,4-DNPG = 2′,4′-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside; β-2,4-DNPG = 2′,4′-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside; β-2,6-DNPG = 2′,6′-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside; DMSO = dimethylsulfoxide.



SCHEME 1

fourth mechanism (iv) involves an initial nucleophilic substitution reaction at C-1′ of the aromatic ring by an added, or generated, nucleophile (most likely dimethylsulfinyl anion in the present work), displacing a glycosyl oxyanion and forming an activated aromatic intermediate. This glycosyl oxyanion then anomerizes by the usual mechanism (1, 2) under the basic conditions and subsequently recombines with the reactive aromatic species (see also Scheme 2 for mechanism (iv)). Each of these mechanisms has some attractive and unattractive features. Mechanism (i) is certainly consistent with the basic conditions of the reaction, but involves a most unusual deprotonation of the anomeric carbon. However, this would be consistent with the earlier observations (8) that only glycosides of extremely electron-deficient phenols undergo the anomerization. Mechanism (ii) is consistent with known carbohydrate chemistry and with the requirement for electron-deficient phenol leaving groups, and with the greater reaction rate for glycosides with potential participating groups at C-2. However, it is difficult to envisage a role for the base in such a reaction. Mechanism (iii) is also reasonably consistent with known carbohydrate chemistry and with the requirement for reactive leaving groups. In addition, it does suggest a clear role for the base. However, it seems doubtful that the glycal intermediate would be reactive enough to recombine with such a poor nucleophile as dinitrophenolate. Additional problems also arise concerning retention of stereochemistry at C-2 in the product. Mechanism (iv) is consistent with the requirement for an electron-deficient aromatic ring and suggests a role for the base either as the nucleophile or in generating the dimethylsulfinyl anion nucleophile. However, it suffers from a paucity of precedence since nucleophilic aromatic substitution reactions are rare in aryl glycoside chemistry. Nonetheless, an important precedent for this exists in the base-catalysed hydrolysis of nitrophenyl glycosides (11, 12). In this case reaction is initiated by base-catalysed intramolecular attack of the sugar 2-hydroxyl upon C-1′ of the aromatic moiety, resulting in migration of the nitrophenyl group to O-2. Subsequent further migration of the aromatic group to O-3 is followed by elimination, yielding nitrophenolate and saccharinic acids.

This paper describes our approaches to identifying the mechanism through a combination of kinetic and product identification studies. A preliminary account of this work has been published previously (13).

Results and discussion

Investigation of solvents, catalysts, and substrates

The first factors to be investigated were the solvents and the catalysts capable of effecting the transformation. This was of particular interest not only in terms of providing clues to the mechanism itself, but also in terms of finding a totally homogeneous system that would be suitable for kinetic studies. For these initial investigations β-2,4-DNPG was used as the substrate and potassium carbonate as the catalyst while thin-layer chromatography was used to determine whether or not reaction had occurred. The substrate, β-2,4-DNPG, was soluble in all the solvents tested, while the potassium carbonate was, at best, only sparingly soluble. Reaction failed to occur only in the least polar of the solvents tested, namely toluene, diethyl ether, and dichloromethane, while reaction was successful in a range of more polar solvents including dimethylsulfoxide, *N*,*N*-dimethylformamide, propylene carbonate, tetrahydrofuran, pyridine, chloroform, and acetonitrile. The reaction does not therefore appear to be especially solvent dependent. Indeed, the absence of reaction in the least polar solvents may simply reflect poor solubility of the catalyst. On the basis of these results dimethylsulfoxide (DMSO) was chosen as the solvent for subsequent studies due to the ease of reactions therein and due to its stability to bases.

A variety of other bases was tested for their ability to catalyse the anomerization of β-2,4-DNPG in DMSO. All the carbonate salts tested (potassium, barium, cesium, tetramethyl-, tetraethyl-, and tetrabutyl-ammonium) were effective, along with

other dianions or trianions such as trisodium phosphate and bis-tetramethylammonium hydrogen phosphate. The very weakly basic dianion potassium sulfate and the monoanion potassium perchlorate were ineffective. Neutral bases of comparable basicity[3] to carbonate (triethylamine and 1,8-bis(dimethylamino)naphthalene) were unable to support the reaction. However, relatively strong monoanionic bases such as potassium *tert*-butoxide, dimethylsulfinyl anion, and sodium hydride were quite effective at promoting anomerization. These observations were initially confusing since there appeared to be no clear correlation with base strength until it was realized that anionic bases, and particularly dianionic bases, are *much* stronger bases in DMSO than in water (14–16) while neutral bases are of comparable basicity in the two solvents. Indeed potassium *tert*-butoxide was shown previously (14) to be capable of generating a high concentration of dimethylsulfinyl anion when dissolved in DMSO, and it is probable that the carbonate dianion has a comparable basicity. It therefore appears that a strong base is required to bring about anomerization, possibly strong enough to generate significant quantities of dimethylsulfinyl anion. The fact that dimethylsulfinyl anion itself promotes anomerization supports this contention. The search for different catalysts also revealed several that were soluble in DMSO, these being the bis(tetra-alkylammonium) carbonates and dimethylsulfinyl anion. This finding is particularly relevant not only in terms of providing a completely soluble homogeneous system for kinetic studies, but also because it disproves an earlier hypothesis (8) that the reaction requires heterogeneous catalysis.

The previous assertion (8) that the only aryl glycosides to undergo this transformation are those with aglycone moieties of low pK_a was investigated further by synthesis and testing of a series of protected aryl glycosides to see which underwent anomerization. Thus the per-*O*-acetylated β-D-glucopyranosides of the following phenols were synthesized: phenol, 4-nitrophenol, pentafluorophenol, 3,4-dinitrophenol, 2,3-dinitrophenol, 2,5-dinitrophenol, 2,6-dinitrophenol, and 2,6-dichloro-4-nitrophenol. These glycosides were dissolved in DMSO containing potassium carbonate and stirred for several days. Analysis of reaction products by tlc and nmr revealed that only the 2,6-dinitrophenyl and 2,6-dichloro-4-nitrophenyl glucosides anomerized at a comparable rate to β-2,4-DNPG. These are the only three phenols that have pK_a's less than 4.0 (17, 18), confirming that the reaction depends on the electron-withdrawing effects of substituents on the aryl ring.

The search for intermediates. Exchange reactions

All four possible mechanisms proposed for the anomerization reaction occur via bond cleavage at either C-1—H-1 (proton abstraction), C-1—O-1 (phenolate departure), or O-1—C-1′ (nucleophilic aromatic substitution). In all cases, the intermediates generated by bond cleavage should be exchangeable with appropriate equivalent substances added to the reaction mixture. Therefore a series of exchange reactions was performed to determine which bond is cleaved during anomerization.

Proton abstraction (mechanisms (i) or (iii)) was investigated by anomerizing [1-^{2}H]-β-2,4-DNPG (19) in DMSO-d_6 containing tetramethylammonium carbonate in the presence of *tert*-butanol as a proton source (100-fold mole excess). The ^{1}H nmr analysis of the isolated product mixture indicated that although complete anomerization had occurred, there had been no exchange of the deuteron at C-1 since no resonance due to an anomeric proton was observed. Similarly, anomerization of non-labelled β-2,4-DNPG in the presence of a deuteron source (^{2}H-*tert*-butanol) showed no exchange at either C-1 or C-2. These observations are strong evidence against mechanisms (i) or (iii).

Demonstration of a dinitrophenolate intermediate was attempted by looking for exchange of added dinitrophenolate anion with that which might be generated during the reaction. Ideally this could be achieved using appropriately labelled (e.g., deuterated), 2,4-dinitrophenolate in the presence of β-2,6-DNPG. However, since such material was not readily available, and since β-2,6-DNPG had also been shown to undergo anomerization, experiments were performed in which β-2,4-DNPG was anomerized in the presence of added potassium 2,6-dinitrophenolate and β-2,6-DNPG in the presence of potassium 2,4-dinitrophenolate. In both cases, ^{1}H nmr analysis of the product mixture indicated that no exchange had occurred. The lack of phenolate exchange is significant evidence against mechanisms (ii) and (iii). However, it is just possible, though unlikely, that exchange might not occur if the phenolate was not released from the immediate solvent shell before recombining with the sugar moiety.

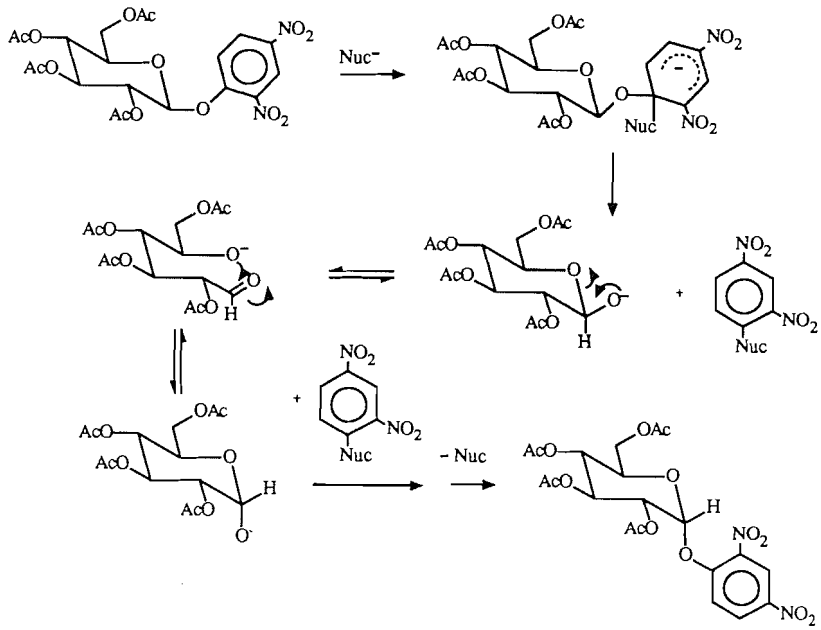
To test mechanism (iv), a third exchange reaction was performed in which [1-^{2}H]-β-2,4-DNPG was anomerized in the presence of one equivalent of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose. In the presence of base, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose is deprotonated to produce the glycosyl oxyanion intermediate proposed in the nucleophilic aromatic substitution mechanism (Scheme 2). If this is indeed an intermediate in the reaction, then the added, unlabelled, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose should be capable of exchanging with the deuterated species generated from the substrate during reaction. The ^{1}H nmr spectrum of the isolated α-2,4-DNPG revealed that exchange had indeed occurred since an anomeric proton resonance integrating to approximately half a proton was evident in the spectrum (Fig. 1). Had there been no exchange, no anomeric proton resonance whatsoever would have been seen. The glycosyl oxyanion must therefore be an intermediate in the reaction mechanism, and this strongly supports mechanism (iv).

A final experiment performed to probe mechanism (iii) involved the independent synthesis of 2,3,4,6-tetra-*O*-acetyl-D-glucal, the postulated intermediate, and attempted reaction of this with potassium 2,4-dinitrophenolate in DMSO in the presence of potassium carbonate. No dinitrophenyl glycoside product was observed, providing additional evidence against mechanism (iii).

Kinetic studies

Two kinds of kinetic studies were performed to attempt to distinguish between mechanisms. One of these involved measurement of the kinetic isotope effect associated with the anomerization of 1-[^{2}H]-β-2,4-DNPG in an attempt to distinguish between the anomeric proton abstraction mechanism (i), which should show a primary kinetic isotope effect, and the other mechanisms, which would likely be subject to a secondary deuterium kinetic isotope effect. The second study involved comparison of the rates of anomerization of a series of remotely substituted 2′,4′-dinitrophenyl glycosides in an attempt to distinguish between cationic and anionic mechanisms. At-

[3]Basicity in H_2O.



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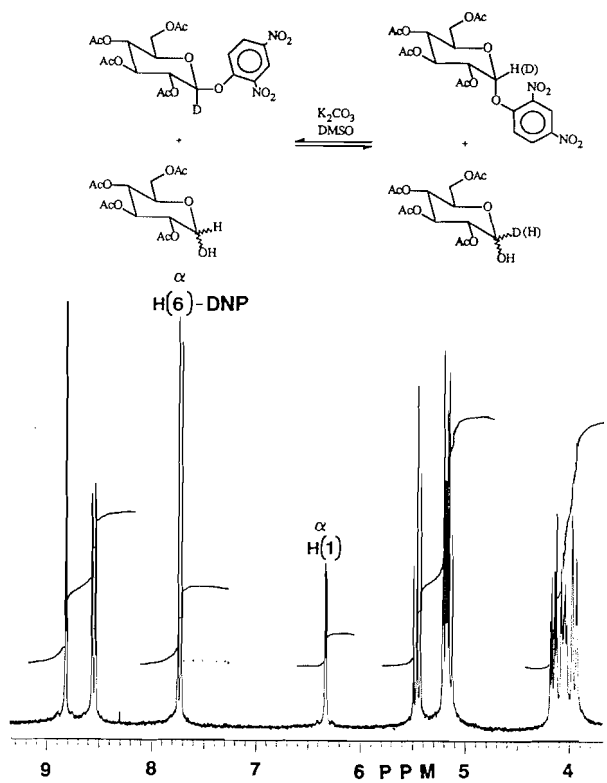


FIG. 1. The ^{1}H nmr spectrum of α-2,4-DNPG isolated from the reaction mixture shown. Integration of the anomeric proton resonance reveals that exchange has occurred.

tempts were made initially to monitor the reaction by use of a polarimeter since there is a significant difference in optical rotation between β-2,4-DNPG and α-2,4-DNPG. Unfortunately all such attempts were unsuccessful because of the relatively rapid appearance of small amounts of a highly chromophoric by-product, identified as 2,4-dinitrophenolate, even under anhydrous conditions. This rendered the use of polarimetry unreliable and forced the use of ^{1}H nmr to follow the reaction. This was more suitable since the reaction could be followed directly using sealed tubes under scrupulously anhydrous conditions. The quantity of 2,4-dinitrophenolate formed was too small to be observable by ^{1}H nmr, and hence it did not interfere with this technique. Direct monitoring by ^{1}H nmr allowed determination of the amounts of product and starting materials at any time by careful integration of the appropriate resonances. It should also allow the observation of any intermediate species generated if they are formed at significant concentrations.

Deuterium kinetic isotope effects

The deuterium kinetic isotope effect measured for the anomerization of 1-substituted β-2,4-DNPG was $k_H/k_D = 1.09 \pm 0.06$. This is a small, but real, isotope effect, but is clearly not a primary kinetic isotope effect since much larger values (up to 7) might have been expected. This finding argues strongly against the proton abstraction mechanism (i) since it is reasonable to assume that the initial proton abstraction step would be rate determining for this mechanism, and thus a primary kinetic isotope effect would be expected. The value measured is, however, in the range expected for a secondary (α or β) deuterium kinetic isotope effect. Either of the other three mechanisms (ii, iii, or iv) could be expected to show such an effect.

Remote substituent effects

It has long been known that substitution of sugar ring hydroxyl groups by hydrogen or other substituents can have a pronounced effect upon the rate of hydrolysis of such substituted glycosides when reaction proceeds via an oxocarbonium ion mechanism (2, 20–22). These changes in rate have generally been considered to be the result of some combination of steric and electronic effects that affects the stability of the transition state. More recent studies on the hydrolysis rates of a complete series of deoxy- and deoxyfluoro-α-D-glucopyranosyl phosphates (23, 24) have suggested that, in these cases at least, the effect is entirely electronic in origin, with the deoxysugars hydrolysing faster than the parent compound and the deoxyfluorosugars hydrolys-

TABLE 1. Substituent effects on anomerization rate

β-2,4-DNPG Substrate	[DNPG] (M)	$[(Me_4N)_2CO_3]$ (M)	Rel. Rate (disappearance of β-anomer)
β-2,4-DNPG	0.0138	0.0167	1.00*
2,3,6-Tri-*O*-acetyl-4-deoxy	0.0138	0.0167	0.13
2,3,4-Tri-*O*-acetyl-6-deoxy	0.0153	0.0167	0.40
2,3,6-Tri-*O*-acetyl-4-deoxy-4-fluoro	0.0136	0.0167	1.05
β-2,4-DNPG	0.0121	0.0182	1.00
3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro	0.0134	0.0182	0.90

*Apparent first-order rate constant at 25°C = 3.63×10^{-3} min^{-1}.

ing more slowly. It should therefore be possible to measure anomerization rates for several deoxy- and deoxyfluoro-β-2,4-DNPG substrates and determine whether the reaction proceeds via an anionic mechanism or a cationic mechanism. Such data are presented in Table 1. As can be seen, the two deoxy-β-2,4-DNPG substrates anomerize considerably more slowly than the parent substrate while the two fluorinated substrates anomerize at comparable rates to β-2,4-DNPG. This supports an anionic rather than a cationic mechanism and is therefore inconsistent with mechanism (ii) involving phenolate departure from an oxocarbonium ion.

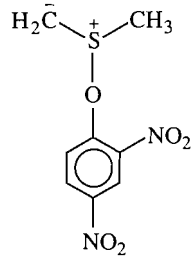
Conclusion

The only mechanism to emerge unscathed from this investigation is the nucleophilic aromatic substitution mechanism (iv), Scheme 2. The kinetic studies are consistent with a mechanism such as this, which involves anionic intermediates and exhibits a secondary deuterium kinetic isotope effect. In this case the isotope effect would be a β-kinetic isotope effect if the nucleophilic attack is rate limiting (most likely) and an α-kinetic isotope effect if the anomerization of the hemiacetal anion is rate limiting. Either could be consistent with the observed effect of $k_H/k_D = 1.09$. The best evidence, however, is the observation of exchange between [1-^{2}H]-β-2,4-DNPG and added unlabelled 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose. This provides unequivocal evidence for the intermediacy of the protected sugar oxyanion. Additional support for this mechanism comes from the observation that when the anomerization is performed under scrupulously anhydrous conditions a deep purple coloration ($\lambda_{max} = 574$ nm) is observed initially in the reaction mixture. Similar observations have been described previously in nucleophilic aromatic substitution reactions and attributed to the formation of Meisenheimer complexes (25); thus it seems likely that the colored species generated in this reaction is the initial adduct formed upon attack o sthe nucleophile onto β-2,4-DNPG.

The identity of the nucleophile and therefore of the activated aryl intermediate is, as yet, unproven. Likely candidates are the added nucleophile itself (carbonate or phosphate) or dimethylsulfinyl anion generated in the solution by addition of the anionic base. Clearly, in the reaction where dimethylsulfinyl anion is used as the base, there is no other candidate. In all the other cases studied, as stated previously, it is either known, or at least probable, that the bases employed are strong enough to generate significant quantities of dimethylsulfinyl anion *in situ*.

The possibility that phosphate is the nucleophile was eliminated by independent synthesis (26) of 2,4-dinitrophenyl phosphate, the putative intermediate in that case, and reacting it with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in DMSO in the presence of trisodium phosphate. No dinitrophenyl glucoside product was observed. Attempts to synthesize 2,4-dinitrophenyl carbonate were, not surprisingly, unsuccessful. Such an intermediate would be extremely unstable and subject to rapid spontaneous decarboxylation, liberating 2,4-dinitrophenolate. This could explain formation of dinitrophenolate, even under strictly anhydrous conditions. Such an intermediate therefore remains a possibility.

There are two likely candidates for the intermediate if dimethylsulfinyl anion is the nucleophile, either **1** or **2**.



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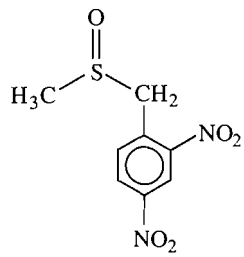

2

Compound **2** was synthesized by an independent route involving oxidation of the methyl dinitrobenzyl thioether formed by reaction of methane thiol with 2,4-dinitrobenzyl chloride (27, 28). Reaction of this with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in DMSO in the presence of bis(tetramethylammonium) carbonate yielded no dinitrophenyl glucoside product, thus eliminating compound **2** as a possible candidate.

All of our attempts to synthesize compound **1** have been unsuccessful. The most promising approach, involving direct substitution of fluoro-2,4-dinitrobenzene with dimethylsulfinyl anion in DMSO failed as the reaction proved difficult to control and products formed decomposed upon work-up and purification. Interestingly, the same compound was suggested to be an intermediate in the reaction of chloro-2,4-dinitrobenzene with DMSO at high temperature (29), as well as in the reaction of 2,4-dinitrophenol with dicyclohexylcarbodiimide and DMSO in the presence of acid/base catalysts (30, 31). Considerable effort was expended by these authors in attempts to isolate and characterize this intermediate, but without success. Suggestive evidence for the identity of the intermediate as compound **1** is provided by the observation that, even under anhydrous conditions, some 2,4-dinitrophenolate by-product is formed in these reactions. This could be explained readily by the collapse of **1**, yielding 2,4-dinitrophenolate and the sulfonium derivative. It was of concern to us initially that the generation of this type of intermediate had previously been shown to be accompanied by *ortho*-alkylation of the aromatic ring, yet no such reaction has been observed in our hands. However, it has

been shown (31) that no such reaction occurs with the 2,4-dinitrophenyl derivative, presumably because of the electron-deficient nature of the system.

In summary, the anomerization of protected 2,4-dinitrophenyl-β-D-glucopyranosides has been shown to proceed via a novel mechanism involving an initial nucleophilic aromatic substitution reaction. The identity of the aromatic intermediate involved has not been proven, but is in all likelihood the aryldimethylsulfinyl species generated by attack of dimethylsulfinyl anion on the substrate. It also suggests that a likely mechanism for the pyridine-catalysed anomerization of per-*O*-acylated sugars (8) involves initial attack of the pyridine on the acyl carbon of the anomeric substituent, resulting in formation of the acyl pyridinium species and the protected sugar oxyanion. This could then mutarotate and react with the acyl pyridinum salt by an entirely analogous process to that described in this work.

Experimental

Thin-layer chromatography (tlc) was performed on Merck Silica Gel 60 F_{254} aluminum backed plates using a solvent system of 1:1 (v/v) ethyl acetate/petroleum ether unless otherwise stated. Chromatograms were visualized by uv irradiation or by charring with 10% sulfuric acid in methanol. Flash chromatography was carried out on Merck Silica Gel (180–230 mesh). The ^{1}H nmr spectra were recorded on a 300 MHz Varian XL-300 instrument using $CDCl_3$ solutions and tetramethylsilane as an internal standard. Chemical shifts are expressed in ppm and coupling constants (J) are given in hertz (s = singlet, d = doublet, t = triplet, m = multiplet). Kinetic measurements were carried out in DMSO-d_6 solutions using residual proton resonances from DMSO (δ = 2.49) as a reference.

Syntheses

With the exception of the 2,4-dinitrophenyl glycosides, commercially unavailable acetylated phenyl glycosides were prepared by condensation of the sodium salt of the phenol with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (32). Protected 2,4-dinitrophenyl glucosides were prepared from the corresponding protected glycoside hemiacetals using fluoro 2,4-dinitrobenzene according to the method of van Boom *et al.* (9). Physical properties were in agreement with those previously reported (32, 33).

The following new compounds were prepared.

*Pentafluorophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside:* mp 144–145°C; ^{1}H nmr data: δ ($CDCl_3$) 5.34–5.12 (m, 3H, H-2, H-3, H-4), 4.97 (d, 1H, $J_{1,2}$ 8 Hz, H-1), 4.25–4.10 (2 dd, $J_{6,5}$ 4, $J_{6',5}$ 2, $J_{6,6'}$ 12 Hz, H-6, H-6'), 3.70 (m, 1H, H-5), 2.10–2.02 (4 s, 12H, 4 × CH_3CO). Anal. calcd. for $C_{20}H_{17}F_5O_{10}$: C 46.71, H 3.72; found: C 45.90, H 3.56.

*2'3'-Dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside:* mp 191–192°C; ^{1}H nmr data: δ ($CDCl_3$) 7.96 (dd, 1H, $J_{4',5'}$ 8, $J_{4'6'}$ 2 Hz, H-4), 7.68 (dd, 1H, $J_{6',5'}$ 8, $J_{6',4'}$ 2 Hz, H-6), 7.62 (dd, 1H, $J_{5',4'}$ 8, $J_{5',6'}$ 8 Hz, H-5), 5.32–5.05 (m, 4H, H-1, H-2, H-3, H-4), 4.24 (d, 2H, $J_{6,5'} = J_{6',5}$ 4 Hz, H-6, H-6'), 3.86 (dt, 1H, $J_{5,4}$ 10, $J_{5,6'}$ 4 Hz, H-5), 2.14–2.03 (4 s, 12H, 4 CH_3CO). Anal. calcd. for $C_{20}H_{22}N_2O_{14}$: C 46.69, H 4.31, N 5.47; found: C 46.32, H 4.51, N 5.42.

*2'5'-Dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside:* mp 145–147°C; ^{1}H nmr data: δ ($CDCl_3$) 8.23 (d, 1H, $J_{6',4'}$ 2 Hz, H-6), 8.06 (dd, 1H, $J_{4',3'}$ 9, $J_{4',6'}$ 2 Hz, H-4'), 7.89 (d, 1H, $J_{3',4'}$ 9 Hz, H-3'), 5.37–5.06 (m, 4H, H-1, H-2, H-3, H-4), 4.30 (dd, 1H, $J_{6',6}$ 12, $J_{6',5}$ 3 Hz, H-6'), 4.19 (dd, 1H, $J_{6,6'}$ 12, $J_{6,5}$ 6 Hz, H-6), 4.02 (m, 1H, H-5), 2.13–2.02 (4 s, 12H, 4 CH_3CO).

*2'6'-Dichloro-4'-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside:* mp 181–182°C; ^{1}H nmr data: δ $CDCl_3$) 8.20 (s, 2H, H-3', H-5'), 5.46–5.13 (m, 4H, H-1, H-2, H-3, H-4), 4.18 (dd, 1H, $J_{6,6'}$ 11 Hz, H-6), 4.07 (dd, 1H, $J_{6',6}$ 11, $J_{6',5'}$ 2 Hz, H-6'), 3.65 (m, 1H, H-5), 2.09–2.02 (3 s, 12H, 4 CH_3CO). Anal. calcd. for $C_{20}H_{21}Cl_2NO_8$: C 44.62, H 3.93, N 2.61; found: C 44.39, H 4.06, N 2.79.

*2'6'-Dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside:* mp 190–191°C; ^{1}H nmr data: δ ($CDCl_3$) 7.98 (d, 2H, $J_{3',5'}$ 8 Hz, H-3', H-5'), 7.47 (t, 1H, $J_{4',5'} = J_{4',3'}$ 8 Hz, H-4'), 5.36–5.08 (m, 4H, H-1, H-2, H-3, H-4), 4.03 (d, 2H, $J_{6,5} = J_{6',5}$ 4 Hz, H-6'), 3.62 (dt, 1H, $J_{5,4}$ 10, $J_{5,6} = J_{5,6'}$ 4 Hz, H-5), 2.13–2.00 (4 s, 12H, CH_3CO). Anal. calcd. for $C_{20}H_{22}N_2O_{14}$: C 46.69, H 4.31, N 5.47, found: C 46.45, H 4.23; N 5.29.

*2',4'-Dinitrophenyl 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-β-D-glucopyranoside*

A solution of 1,2,3,6-tetra-*O*-acetyl-4-deoxy-4-fluoro-β-D-glucopyranose (23) (0.100 g, 0.29 mmol) in 45% HBr/HOAc (3.0 mL) and acetic anhydride (0.30 mL) was stirred for 2 h at room temperature, then cooled to 0°C, diluted with dichloromethane (30 mL), washed five times with ice-cold water, and the organic phase dried with sodium sulfate. Removal of the solvent produced 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-α-D-glucopyranosyl bromide as a colourless syrup, which was crystallized from diethyl ether/hexanes; 90 mg, 0.24 mmol, 85%.

A solution of 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-α-D-glucopyranosyl bromide (90 mg, 0.24 mmol) was dissolved in acetone (0.20 mL) and cooled to 0°C. Water (10 μL) was added, followed by addition of silver carbonate (0.10 g, 0.36 mmol) in small portions. The solution was stirred for 20 h at room temperature in the dark, warmed to 50°C, filtered, and washed with two portions of warm acetone. The solvent was removed from the combined filtrates to produce a brown syrup of 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-D-glucopyranose (64 mg, 0.21 mmol).

Derivatization of 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-D-glucopyranose was performed according to van Boom *et al.* (9) giving the title compound as yellow needles (45 mg, 0.091 mmol); mp 171–173°C; ^{1}H nmr data: δ ($CDCl_3$) 8.72 (d, 1H, $J_{3',5'}$ 3 Hz, H-3'), 8.44 (dd, 1H, $J_{5',6'}$ 10, $J_{5',3'}$ 3 Hz, H-5'), 7.47 (d, 1H, $J_{6',5'}$ 10 Hz, H-6'), 5.50–5.23 (m, 3H, H-1, H-2, H-4), 4.68 (dt, 1H, $J_{F,4}$ 50, $J_{3,4} = J_{4,5}$ 9 Hz, H-4), 4.55 (dd, 1H, $J_{6,6'}$ 12 Hz, H-6), 4.28 (dd, 1H, $J_{6',6}$ 12 Hz, H-6'), 4.20 (m, 1H, H-5), 2.14 (s, 6H, 2CH_3CO), 2.09 (s, 3H, CH_3CO). Anal. calcd. for $C_{18}H_{19}FN_2O_{12}$: C 45.57, H 4.04, N 5.93; found: C 45.50, H 3.82, N 6.01

*2',4'-Dinitrophenyl 2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucopyranoside*

To a suspension of methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-glucopyranoside (24) (0.20 g, 0.41 mmol) in methanol (1.0 mL) was added 1 M sodium methoxide in methanol (0.010 mL). The mixture was stirred for 2 days at room temperature, neutralized with Dowex 50W-X8 (H^+) ion exchange resin, and the suspension filtered. The filtrate was concentrated *in vacuo* and the concentrate was purified by column chromatography (9:1 v/v ethyl acetate/methanol). The resulting syrup (72 mg, 88%) was immediately acetylated by addition of a cold (0°C) solution of acetic anhydride (0.37 mL, 3.9 mmol) and pyridine (0.47 mL), warmed to room temperature, and stirred overnight. This was cooled to 0°C, then quenched by addition of methanol and stirred at 0°C for 1 h. The solution was diluted with dichloromethane, washed three times with ice-cold water, and the organic phase was dried with sodium sulfate. Removal of the solvent *in vacuo* afforded methyl 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-glucopyranoside as a colourless syrup, which was crystallized from diethyl ether (87 mg, 0.29 mmol, 71%).

Methyl 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-glucopyranoside was dissolved in dichloromethyl methyl ether (0.40 mL) and a catalytic amount of freshly fused zinc chloride was added. The solution was stirred at 65–70°C for 1 h, then concentrated *in vacuo*, diluted with chloroform, and washed twice with 10 mL of cold saturated aqueous sodium bicarbonate. The organic phase was dried with sodium sulfate and removal of the solvent produced a brown syrup of 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-glucopyranosyl chloride (65 mg).

2,3,6-Tri-*O*-acetyl-4-deoxy-α-D-glucopyranosyl chloride was hydrolysed exactly as described previously for the 4-fluoro derivative to yield a yellow syrup, which was reacted directly with fluoro-2,4-dinitrobenzene according to van Boom *et al.* (9) to yield the title compound as a yellow crystalline solid; mp 180–181°C; ^{1}H nmr data:

δ ($CDCl_3$) 8.71 (d, 1H, $J_{3',5'}$ 3 Hz, H-3'), 8.42 (dd, 1H, $J_{5',6'}$ 10, $J_{5',3'}$ 3 Hz, H-5'), 7.48 (d, 1H, $J_{6',5'}$ 10 Hz, H-6'), 5.32–5.05 (m, 3H, H-1, H-2, H-3), 4.22 (d, 2H, $J_{6,6'}$ 7 Hz, H-6, H-6'), 4.40 (m, 1H, H-5). Anal. calcd. for $C_{18}H_{20}N_2O_{12}$: C 47.36, H 4.42, N 6.16; found: C 47.27, H 4.40, N 6.18.

*2',4'-Dinitrophenyl 2,3,4-tri-*O*-acetyl-6-deoxy-β-*D*-glucopyranoside*

1,2,3,4-Tetra-*O*-acetyl-6-deoxy-D-glucopyranose (24) (0.40 g, 1.2 mmol) was brominated at the anomeric centre as described previously for the 4-deoxy sugar. The product was isolated as a white solid (420 mg, 1.06 mmol, 99%), and directly hydrolysed as described previously, using silver carbonate, yielding 2,3,4-tri-*O*-acetyl-6-deoxy-D-glucopyranose as a colourless gum (306 mg, 1.05 mmol). This was derivatized using fluoro-2,4-dinitrobenzene by the general method to afford 2'4'-dinitrophenyl 2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranoside as white needles (424 mg, 0.93 mmol); mp 198–199°C (lit. (34) mp 152–156°C); ^{1}H nmr data: δ ($CDCl_3$) 8.70 (d, 1H, $J_{3',5'}$ 3 Hz, H-3'), 8.43 (dd, 1H, $J_{5',6'}$ 10, $J_{5',3'}$ 3 Hz, H-5'), 7.45 (d, 1H, $J_{6',5'}$ 10 Hz, H-6'), 5.46–5.20 (m, 3H, H-1, H-2, H-3), 4.95 (m, 1H, H-5), 2.12, 2.08, 2.05 (3 s, 9H, CH_3CO), 1.35 (d, 3H, $J_{6,5}$ 4 Hz, 3 × H-6). Anal. calcd. for $C_{18}H_{20}N_2O_{12}$: C 47.36, H 4.42, N 6.16; found: C 47.34, H 4.35, N 6.10.

*2',4'-Dinitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-*D*-glucopyranoside*

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (35) (1.4 g) was hydrolysed using silver carbonate, as described previously, yielding 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranose as a colourless gum. This was dissolved in DMF (10 mL) containing DABCO (1.5 g), stirred over 4A molecular sieves for 15 min, then a solution of fluoro-2,4-dinitrobenzene (0.9 g) in DMF (5 mL) added. After 1.5 h the solution was filtered, and the solvent removed by evaporation under vacuum. The residual oil was redissolved in chloroform, washed twice with saturated aqueous sodium bicarbonate, then twice with water, and dried over sodium sulfate. Evaporation of the solvent yielded an oil, which crystallized spontaneously upon addition of ethanol giving a mixture of the α(20%)- and β(80%)-glycosides (0.99 g). This solid was dissolved in a minimal volume of hot ethyl acetate and, upon cooling to room temperature, the pure α-glucoside crystallized out. After filtration, petroleum ether was added to induce crystallization of the β-glycoside. This process was repeated twice, giving a mixture of the α(5%)- and β(95%)-glycosides as a crystalline solid. Pure β-glycoside was obtained by preparative thin-layer chromatography (silica; 1.2:1 ethyl acetate/petroleum ether) and recrystallized from ethyl acetate/petroleum ether; mp 145–147°C; ^{1}H nmr data: δ ($CDCl_3$) 8.77 (d, 1H, $J_{3',5'}$ 2.6 Hz, H-3'), 8.50 (dd, 1H, $J_{5',6'}$ 9.3, $J_{5',3'}$ 2.5 Hz, H-5'), 7.41 (d, 1H, $J_{6',5'}$ 9.3 Hz, H-6'), 5.52–5.39 (m, 2H, H-3, H-1), 5.15 (t, 1H, $J_{4,3} = J_{4,5}$ 9.7 Hz, H-4), 4.73 (ddd, 1H, $J_{2,F}$ 49.5, $J_{2,3}$ 8.9, $J_{2,1}$ 7.4 Hz, H-2), 4.28 (dd, 1H, $J_{6,6'}$ 14.6, $J_{6,5}$ 5.4 Hz, H-6), 4.20 (dd, 1H, $J_{6',6}$ 14.6, $J_{6',5}$ 2.9 Hz, H-6'), 3.95 (ddd, 1H, $J_{5,4}$ 9.7, $J_{5,6}$ 5.4, $J_{5,6'}$ 2.9 Hz, H-5), 2.14 (s, 3H, CH_3CO), 2.08 (s, 6H, CH_3CO). Anal. calcd. for $C_{18}H_{19}N_2O_{12}F$: C 45.48, H 4.04, N 5.91; found: C 45.10, H 4.18, N 5.69.

2,4-Dinitrobenzylchloride

A mixture of nitric acid (7 mL) and sulfuric acid (12 mL) was added with stirring to a suspension of 4-nitrobenzylchloride (11.4 g, 55 mmol) in sulfuric acid (52 mL) at 0°C, stirred at 0°C for 30 min, then at room temperature for 1 h. The mixture was poured onto ice and extracted with chloroform; the combined chloroform extracts were washed with cold water until the aqueous layer was neutral to pH paper, then dried with sodium sulfate and the chloroform removed *in vacuo*, producing a red oil. The oil was purified by flash chromatography (silica; 3:1 petroleum ether/ethyl acetate) to afford the product as a yellow oil, which solidified upon cooling (11.6 g, 43 mmol, 81%); ^{1}H nmr data: δ ($CDCl_3$) 8.91 (d, 1H, H-3), 8.53 (dd, 1H, H-5), 8.05 (d, 1H, H-6), 5.10 (s, 2H, $-CH_2-$).

2,4-Dinitrobenzylmethyl sulfide

The sulfide was prepared by the method of Russell and Pecoraro (27) as follows. 2,4-Dinitrobenzylchloride (8.02 g, 37 mmol) in methanol (100 mL) was added slowly, under nitrogen, to a 2.5 M solution of sodium (70 mmol) and methyl mercaptan in methanol (28 mL) at 0°C (28). After stirring for 15 min, the mixture was acidified with 1 M hydrochloric acid, diluted with water, and extracted with ether. The combined ether extracts were washed with water until the aqueous layer was neutral, dried with magnesium sulfate, and concentrated *in vacuo* to produce a red solid. Purification by flash chromatography (silica; 5:1 petroleum ether/ethyl acetate) afforded 2,4-dinitrobenzylmethyl sulfide as yellow crystals (5.78 g, 25 mmol, 68%); ^{1}H nmr data: δ ($CDCl_3$) 8.83 (d, 1H, H-3), 8.41 (dd, 1H, H-5), 7.72 (d, 1H, H-6), 4.11 (s, 1H, $-CH_2-$), 2.05 (s, 3H, $-CH_3$).

2,4-Dinitrobenzylmethyl sulfoxide

2,4-Dinitrobenzylmethyl sulfide (0.830 g, 3.64 mmol) in methylene chloride (1.0 mL) was cooled to −20°C and a solution of *m*-chloroperbenzoic acid (0.628 g, 3.64 mmol) in methylene chloride (6.0 mL) was added under nitrogen. The mixture was stirred for 20 h, at which time tlc (silica; ethyl acetate) showed one major product (R_f = 0.14) plus a very small amount of a component later identified as 2,4-dinitrobenzylmethyl sulfone (R_f = 0.54). The sulfoxide product was separated by flash chromatography, eluting first with ethyl acetate to remove the sulfone by-product and any residual starting material, then with methanol. The sulfoxide was isolated as a yellow solid, which was crystallized from warm ethanol (0.650 g, 2.66 mmol, 73%); mp 125–127°C; ir data: (KBr pellet) 1049 cm^{-1} (S=O stretch); ^{1}H nmr data: δ ($CDCl_3$) 9.02 (d, 1H, H-3), 8.54 (dd, 1H, H-5), 7.80 (d, 1H, H-6), 4.48 (m, 2H, $-CH_2-$), 2.73 (s, 3H, $-CH_3$); ms data: *m/z* 244, M^+ for $C_8H_8N_2O_5S$. Anal. calcd. for $C_8H_8N_2O_5S$: C 39.32, H 3.31, N 11.52; found: C 39.85, H 3.28, N 11.40.

Anomerization reactions

Tests for anomerization of substrates were performed by dissolving the aryl β-glycoside (0.036 mmol) over 3A molecular sieves in anhydrous DMSO (0.2 mL) containing anhydrous potassium carbonate (0.10 mmol). Reaction was monitored by tlc (1:1 ethyl acetate/petroleum ether) and product composition was confirmed by ^{1}H nmr of the isolated product mixture. Typically, anomerization was complete in approximately 20 h. Exchange reactions were also carried out by this method, but with the addition of appropriate potentially exchangeable compounds.

Isolation of the product α-2,4-DNPG, when necessary, was achieved by the following method. The reaction mixture was diluted with chloroform, filtered, and concentrated *in vacuo* to a red syrup, which was redissolved in chloroform, washed twice with 10% aqueous sodium bicarbonate, and twice with water. The organic phase was dried over magnesium sulfate, concentrated to a yellow syrup, dissolved in warm 95% ethanol, and allowed to cool to room temperature when the α-2,4-DNPG crystallized out. Further cooling yielded a mixture of α and β anomers. If necessary, separation of the anomers could be achieved readily by flash chromatography (silica; 97:3 chloroform/acetone).

Kinetic studies

All kinetic studies were performed by using ^{1}H nmr to follow the reaction. Reactions were performed in DMSO-d_6 solutions containing bis(tetramethylammonium) carbonate as catalyst. Solutions of both the aryl glycoside and the carbonate catalyst were prepared separately under anhydrous conditions. The solution of carbonate was introduced into the 5-mm nmr tube and frozen at −70°C, then the solution of glycoside was layered on top and frozen. Samples were stored in sealed tubes at −70°C until needed, then reaction initiated by warming to 25°C and mixing. The ^{1}H nmr spectra were recorded at 25°C at appropriate time intervals. Rates of disappearance of the β-glycoside and appearance of the α-glycoside were estimated by integration of both the anomeric proton resonances and the aromatic proton H-6' resonance. The total integral measured for the acetate resonances (δ 2.2–2.0) was used for standardization. Rate constants were calculated using a plot of log ($B_t - B_e$) against time, where B_t is the con-

centration of β anomer remaining at time t and B_e is the concentration of β anomer at equilibrium. Such plots were linear ($p > 0.98$) for at least two half-lives. A linear regression analysis was used to extract rate constants. Errors in reported values are ± 5% on single runs. The kinetic isotope effect reported was measured in the same manner, using four pairs of substrates (protiated and deuterated). The value reported is calculated from the average of the four rate constants determined for protiated material and the four rate constants for the deuterated material.

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