THE CARBONATE CATALYZED ANOMERIZATION OF PROTECTED 2,4-DINITROPHENYL GLUCOPYRANOSIDES: A MECHANISTIC STUDY

by

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ABSTRACT

The mechanism of the carbonate catalyzed conversion of 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside in DMSO to an equilibrated mixture of the α- and β-glycosides has been investigated using a variety of techniques. Pseudo-first-order rate constants (k) measured for the anomerization of the parent substrate and the 1-deuterio substrate indicated a secondary deuterium isotope effect of $^{kH}/_{kD}$ = 1.09 ± 0.06. Pseudo-first-order rate constants measured for several deoxy and deoxyfluoro derivatives of the parent sugar showed that the deoxyfluoro sugars react at least as fast as the parent sugar whereas the deoxy sugars reacted more slowly. In addition to the 2,4-dinitrophenyl glucoside, 2,6-dinitrophenyl glucoside also was found to anomerize, yet attempts to exchange the 2,4-dinitrophenolate groups of the glucoside with added 2,6-dinitrophenolate anion and vice versa were unsuccessful. Exchange of the proton at the anomeric carbon with a deuteron also does not occur when the anomerization is performed in the presence of a deuteron source (and vice versa). Exchange of the glucosyl residue was observed, however, when the 1-deuterio substrate was anomerized in the presence of non-deuterated 2,3,4,6-tetra-O-acetyl-D-glucopyranose. ^{1}H-n.m.r. of the 2,4-dinitrophenyl α-glucoside isolated from this reaction indicated that the α-glucoside possessed only 50% of the deuterium label at the anomeric center. These results along with the observation of a Meisenheimer intermediate indicate that

the anomerization proceeds via nucleophilic aromatic substitution and as such is novel mechanism for glycoside anomerization.

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ABBREVIATIONS

DMSO	=	dimethyl sulphoxide
Ac	=	acetyl
Me	=	methyl
DNP	=	dinitrophenyl
FDNB	=	1-fluoro-2,4-dinitrobenzene
DABCO	=	1,4-diazabicyclo[2.2.2]-octane
DMF	=	N,N-dimethylformamide
DNPG	=	dinitrophenyl glucopyranoside
Bn	=	benzyl
THF	=	tetrahydrofuran
t.l.c.	=	thin layer chromatography
n.m.r.	=	nuclear magnetic resonance
u.v./vis	=	ultraviolet/visible
t-BuOH	=	t-butanol or 2-methyl-2-propanol
KIE	=	kinetic isotope effect
MeCN	=	acetonitrile
dimsyl	=	dimethylsulfinyl
m.p.	=	melting point
b.p.	=	boiling point
ppm	=	parts per million
s	=	singlet
d	=	doublet
t	=	triplet
m	=	multiplet
Bz	=	benzoyl
c	=	concentration in mg/ml
eu	=	entropy units
M	=	molar
mmol	=	millimoles

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INTRODUCTION

Glycosides are cyclic acetals which are derived from the hemiacetal form of a sugar and an alcohol or phenol (Figure 1). Each glycoside has

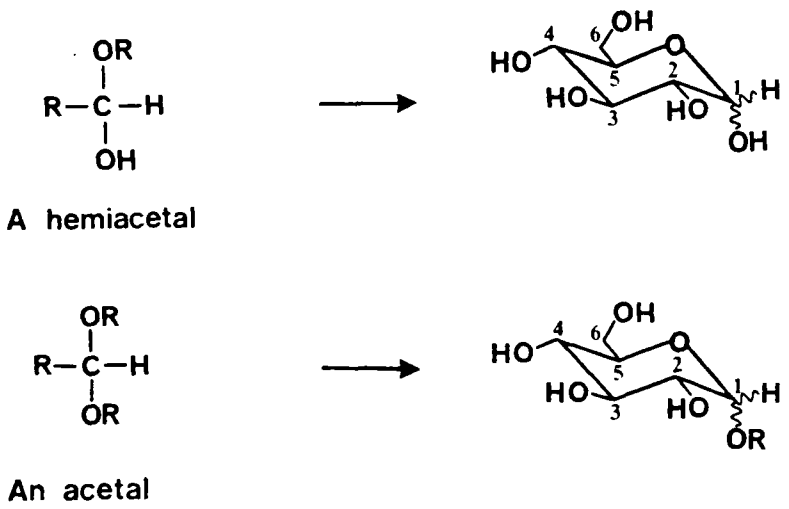
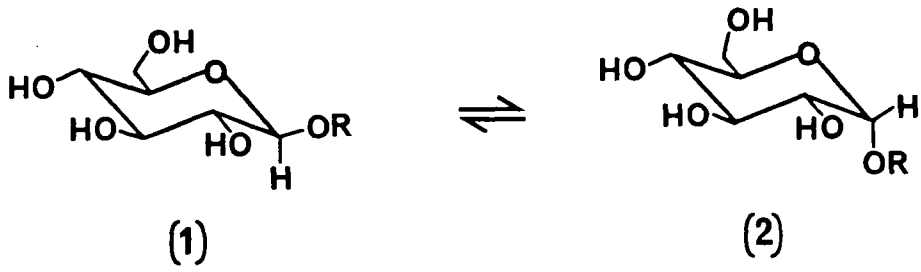


Figure 1: The structure of glycosides (R" = alkyl or aryl)

two possible isomers called anomers which differ only in the configuration at C(1) (the anomeric carbon). For D-glycopyranosides in the $^4C_1$ conformation, the α-anomer (2) has an axial substituent at C(1) whereas the β-anomer (1) has an equatorial substituent at C(1). Interconversion of the two isomers is termed anomerization (Figure 2). This introduc-

tion outlines the thermodynamic and kinetic aspects of anomerization for a variety of glycosides and simple sugars.



(1) (2)

Figure 2: The anomers of D-glucopyranoside (R = H, alkyl or aryl)

The elucidation of reaction mechanisms at glycosidic centers has attracted the interest of many investigators. One reason for this interest concerns the function of glycosides in plants and animals. Many carbohydrate-containing biopolymers and naturally occurring monosaccharides are glycosides and it is the glycosidic linkage which is preferentially cleaved in the breakdown of such compounds. One example is the enzymic breakdown of glycosides or oligosaccharides by glycosidases which hydrolyze the glycosidic linkage to produce a sugar residue and an alcohol (the aglycone) in the case of a simple glycoside, or two or more sugar residues in the case of an oligosaccharide. Since these types of biochemical transformations can often be related to the analogous chemical transformations, the mechanisms for reactions such as glycoside hydrolysis, anomerization, and glycosyl transfer are of

interest.

A second reason for studying anomerization is that the process often occurs during the preparations and reactions of simple sugars and glycosides, thus producing low yields and undesired by-products. Therefore, mechanistic studies can lead to the development of improved synthetic methods.

1. The Anomeric Effect

The anomeric effect is defined as the tendency of electronegative substituents at the anomeric center to adopt the axial position in pyranose rings. This preference for the axial position contrasts predictions based solely on steric interactions which favor the equatorial orientation.

Two theories have been proposed to explain the anomeric effect. One simple rationalization postulates electrostatic interactions existing between the anomeric substituent and the lone pair electrons on the acetal oxygen in the pyranose ring.[1,2] For the β-anomer in the $^4C_1$ conformation, the dipole associated with the C(1) substituent exactly eclipses the dipole from the lone pair electrons on the oxygen (Figure 3). Destabilization of the β-anomer is due to repulsion between the eclipsing dipoles. In the case of the α-anomer, this unfavorable dipole-dipole interaction does not occur as shown by the Newman projections in Figure 3.

A second explanation of the anomeric effect proposes the stabiliza-

tion of the α-anomer by electron delocalization through the molecular orbitals on the ring oxygen to the C(1) substituent.[3] In the case of

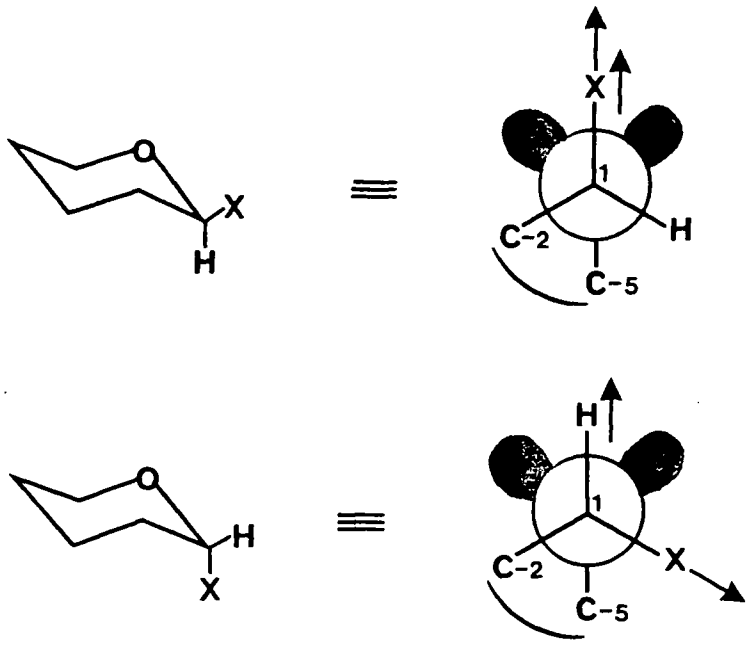


Figure 3: The anomeric effect: electrostatic interactions

the α-anomer, the electron pair on the ring oxygen is oriented antiperiplanar to the axial C-X bond. Stabilization results from partial electron transfer from this lone pair to the antibonding σ^* orbital of the bond to the electronegative substituent at the anomeric center (Figure 4). This explanation can also be illustrated by the idea of a "double bond - no bond resonance" structure in which the electronic delocalization is due to the overlap of an electron pair p-orbital on the oxygen with the antibonding σ^* orbital of the C-X bond (Figure 4).

Overlap of these orbitals is possible only in the axial orientation since in the equatorial orientation the orbitals are orthogonal.

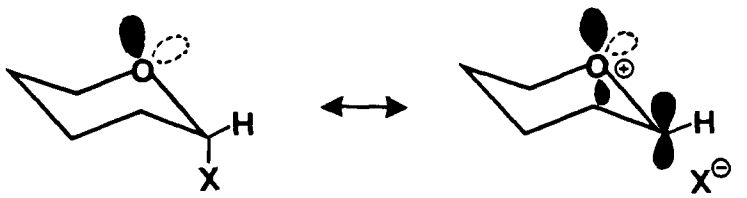


Figure 4: The anomeric effect: molecular orbital interactions. Figure at the right shows the "double bond - no bond resonance" structure

Evidence for the anomeric effect arising from these interactions is well established. For example, the extent of the preference for the axial position decreases in solvents of increasing polarity.[4] Solvents of relatively high polarity were found to decrease the anomeric effect since these solvents will stabilize the net dipole present in the β-anomer. Also, the anomeric effect is magnified with increasing electronegativity of the anomeric substituent X. This is particularly true for glycosyl halides: an equilibrium mixture of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl chloride in dry acetonitrile contains 93-95% of the α-anomer in the presence of added chloride ion.[5] Finally, groups which reverse the dipole of the bond attaching them to the anomeric

carbon show a "reverse anomeric effect". Lemieux studied the anomeric effect for N-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-4-methyl pyridinium bromide (3) and found that the positively charged pyridinium group prefers to adopt the equatorial orientation shown in Figure 5.[6]

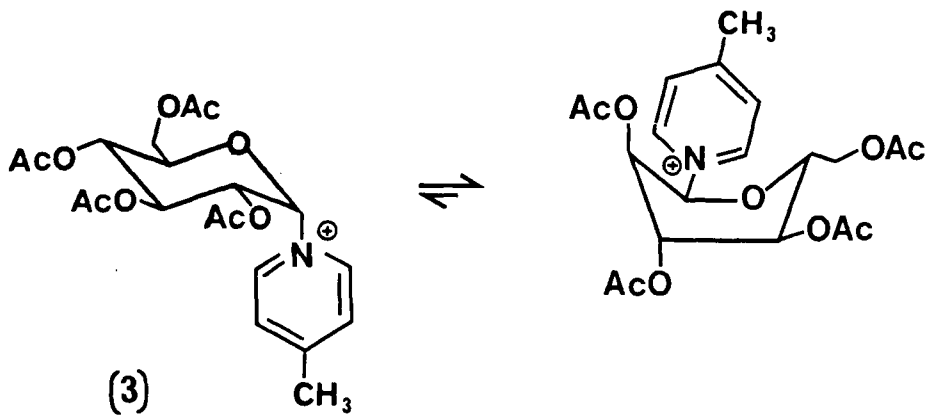


Figure 5: The reverse anomeric effect

It was also shown that this normally unfavored conformation was not simply a result of the steric bulk of the pyridinium group by comparison with the imidazole derivative (4) (Figure 6). Compound (4) prefers the $^4C_1$ chair conformation but upon protonation, flips to the boat conformation. Therefore, the preference for the equatorial orientation in (3) and (4) may be due to stabilization of the positive charge by an eclipsing interaction with the concentration of negative charge on the ring oxygen. This observation of a reverse anomeric effect is one major flaw in the "double bond - no bond resonance" argument for the anomeric effect since this theory would predict that the axial orientation in (3)

would still be preferred despite the positively charged pyridinium substituent.

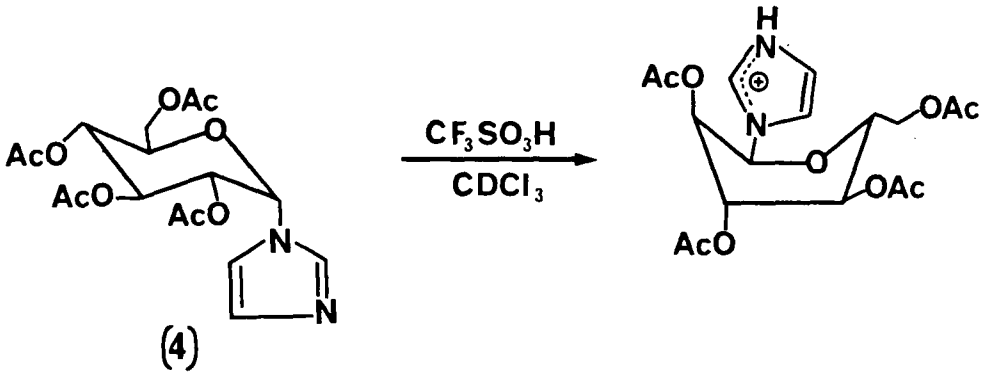


Figure 6: The protonation of 2,3,4,6-tetra-O-acetyl-α-glucosyl imidazole

2. Mutarotation of D-Glucose

When β-D-glucopyranose (1, R = H) is dissolved in water, a change in optical rotation is observed as it anomerizes to the α-form (2, R = H). This phenomenon is termed mutarotation. The reaction is well characterized for most reducing sugars such as D-glucose and the mechanism is considered to involve acid/base catalysis with the formation of an aldehydo-sugar intermediate as shown in Figure 7. The best evidence for the acyclic intermediate is the observation that [1-^{18}O]-D-glucose undergoes oxygen exchange with water 30 times more slowly than the mutarotation reaction itself.[7] This result excludes a possible alternative mechanism in which the hydroxyl group at C(1) undergoes

nucleophilic displacement by water and oxygen exchange would be as fast as mutarotation (Figure 8). The mechanism given in Figure 7 involves

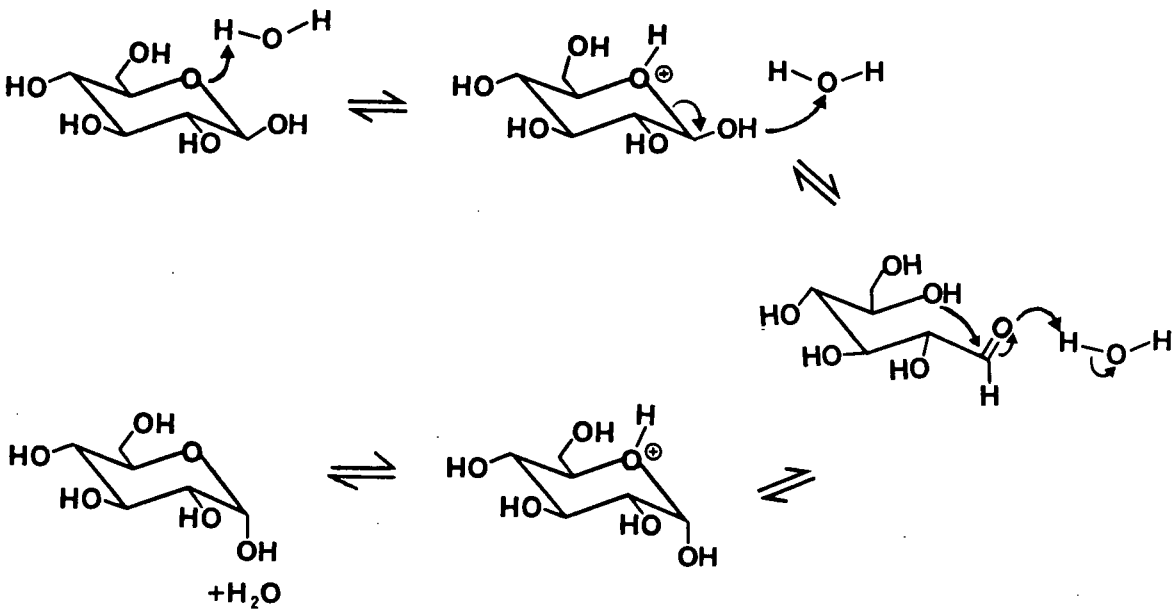


Figure 7: The mechanism for mutarotation of D-glucopyranose

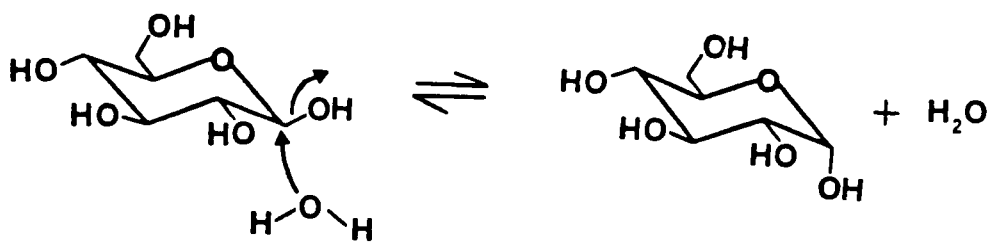


Figure 8: The mutarotation of D-glucopyranose via nucleophilic attack of water

both removal and addition of a proton. Indeed it has been determined that the reaction is catalyzed by the presence of both acid and base. Consequently, the mutarotation of D-glucose proceeds very slowly in aprotic solvents but is accelerated by the addition of water which then serves as a specific acid/base catalyst. In addition, it was found that 2,3,4,6-tetra-O-methyl-D-glucopyranose mutarotates in a mixture of pyridine and cresol where the pyridine is thought to act as a base and the cresol as an acid. However, virtually no mutarotation was observed in either pyridine or cresol alone since the proton donor and acceptor are not present simultaneously in the system.[8] Furthermore, 2-hydroxypyridine was found to be a very effective bifunctional catalyst. Although 2-hydroxypyridine is a weaker acid or base than either pyridine or cresol, it catalyzes the reaction even faster than a mixture of these two catalysts.[9]

3. Acid Catalyzed Mutarotation

Anomerization of glycosides and other simple carbohydrate derivatives is generally either acid or base catalyzed. Examples of acid catalyzed reactions are far more prevalent and therefore, more is known about the mechanisms of their reactions. Acid catalyzed reactions are therefore discussed in this introduction at greater length than base catalyzed reactions.

3.1 Unprotected Glucopyranosides

The final step in the Fischer synthesis of glycosides is the equilibration of α- and β- furanosides and pyranosides.[10] In systems where pyranoside formation is favored, an equilibrium mixture of methyl-D-glucopyranosides contains 73% of the α-anomer and 27% of the β-anomer.[11] The mechanism of this final equilibration step has been of much interest.

Capon has proposed three possible mechanisms for the equilibration of methyl α-and β-D-glucopyranosides (5).[11] The first suggests a cyclic oxocarbonium ion intermediate ((6), mechanism i), the second suggests an acyclic intermediate ((7), mechanism ii), and the third, an acetal intermediate ((8), mechanism iii) (Figure 9). By performing the acid catalyzed anomerization of (5) in CD_3OD, it was found that the methoxyl group completely exchanges with the solvent, thereby excluding mechanism (ii). Mechanism (iii) was excluded by synthesizing the dimethyl acetal (8) separately and subsequently subjecting it to the anomerization conditions. Here it was found that the methyl furanosides were formed, as opposed to pyranosides, and any ring expansion to form the pyranosides shown in Figure 9 went slowly compared to the rate of anomerization for (5-β) or (5-α). In addition, an earlier publication by Capon et al.[12] reported entropies of activation of +5.7 ± 1.0 eu for (5-β) and +7.7 ± 1.0 eu for (5-α) at 35°C, suggesting a unimolecular mechanism for the reaction. Recently, Jensen investigated the effects of methanol acting as a solvent as opposed to it acting as a reagent in this anomerization.[13] By measuring the effect of decreasing methanol concentration

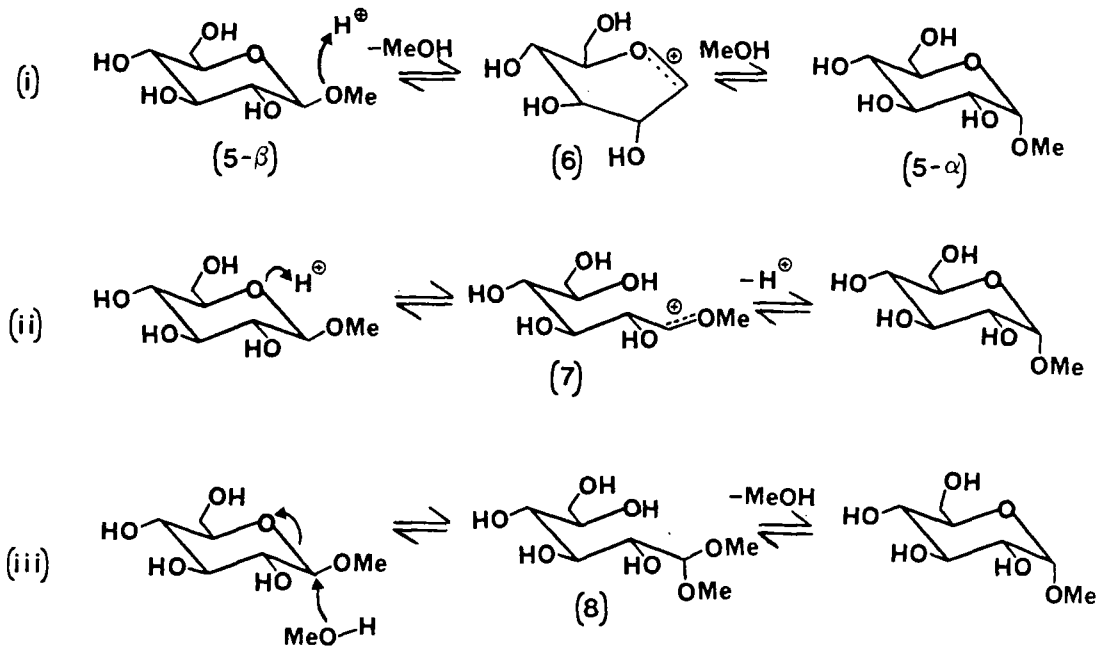


Figure 9: Mechanisms for acid catalyzed anomerization of methyl D-glucopyranoside

in CH_3OH/DMSO solutions on the rate of anomerization, it was determined that the reaction is zero order in methanol as is necessary for mechanism (i). However, it still requires methanol or a similar nucleophilic species for the transformation to occur. Jensen used this evidence to propose an "intermediate" of the type shown in Figure 10 where the oxocarbonium ion formed is not a free species but requires interaction with solvent molecules in the solvent cage for stabilization. It was

also suggested that the four hydroxyl groups on the pyranoside ring might interact with the solvent cage by delocalization of charge to provide further transition state stabilization.

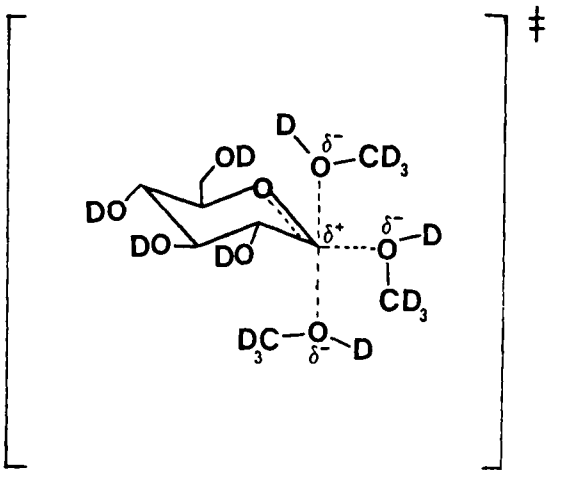


Figure 10: The stabilization of the oxocarbonium ion transition state (reaction performed in CD_3OD).

3.2. Acetylated Glycopyranosides

Anomerization of acetylated glycosides has been found to occur using strong Lewis acid catalysts such as stannic chloride,[14] titanium tetrachloride,[15] and boron trifluoride.[16] Examination of the reaction of various acetylated glucosides with boron trifluoride showed that the rate of anomerization depends on the nature of the aglycon: the reactivity of different glucosides decreases following the order isopropyl > ethyl > methyl ≃ allyl ≃ benzyl. Therefore, electron-donating substituents seem to accelerate the reaction. These results can be explained

in terms of a mechanism in which the acid catalyst coordinates with the ring oxygen atom, facilitating the formation of an open chain intermediate similar to (8).[17] Further evidence for the open chain intermediate was shown by the reaction of methyl tri-O-acetyl-β-D-arabinopyranoside (9) with either 8% zinc chloride or 0.16% sulfuric acid in a 7:3 acetic anhydride-acetic acid mixture.[18] The products isolated from this reaction were both anomeric forms of the O-acetylated methyl hemiacetal (10) in good and approximately equal yields (Figure 11).

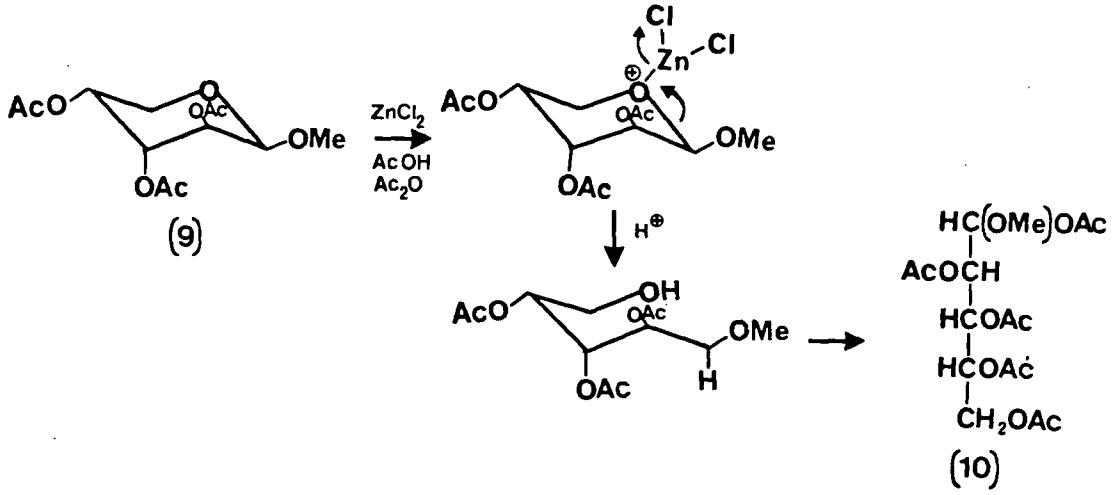


Figure 11: Lewis acid catalyzed reaction of glycosides

3.3 Penta-O-acetyl Glycopyranoses

Equilibration of 1,2,3,4,6-penta-O-acetyl-D-glucopyranose (11) in acetic anhydride with sulfuric acid produces a mixture containing 87% of the α-anomer and 13% of the β-anomer.[19] As with the anomerization of

methyl glucopyranosides, the mechanism is considered to involve unimolecular dissociation of the acetate at C(1) forming an oxocarbonium ion intermediate (12) (Figure 12). Investigations by Lemieux et al.,[20,21] and Bonner[22] support this mechanism by comparing the rates of anomerization with the rates of acetate exchange using compounds labelled with ^{14}C at the C(1) acetyl group. For the α-anomer (11-α) the rate of acetate exchange was almost the same as the rate of anomerization suggesting that these two processes occur via the same intermediate (1:1 acetic anhydride-acetic acid with 0.50 M sulfuric acid at 25°C, k_{exch} = 0.703 x 10^{-4}sec^{-1}, k_{anom} = 0.68 x 10^{-4} sec^{-1}). The β-peracetate (11-β), however, was found to exchange acetate approximately 15 times faster than it anomerized (same conditions, k_{exch} = 72.2 x 10^{-4} sec^{-1}, k_{anom} = 4.93 x 10^{-4} sec^{-1}). This difference in rates for (11-β) was explained

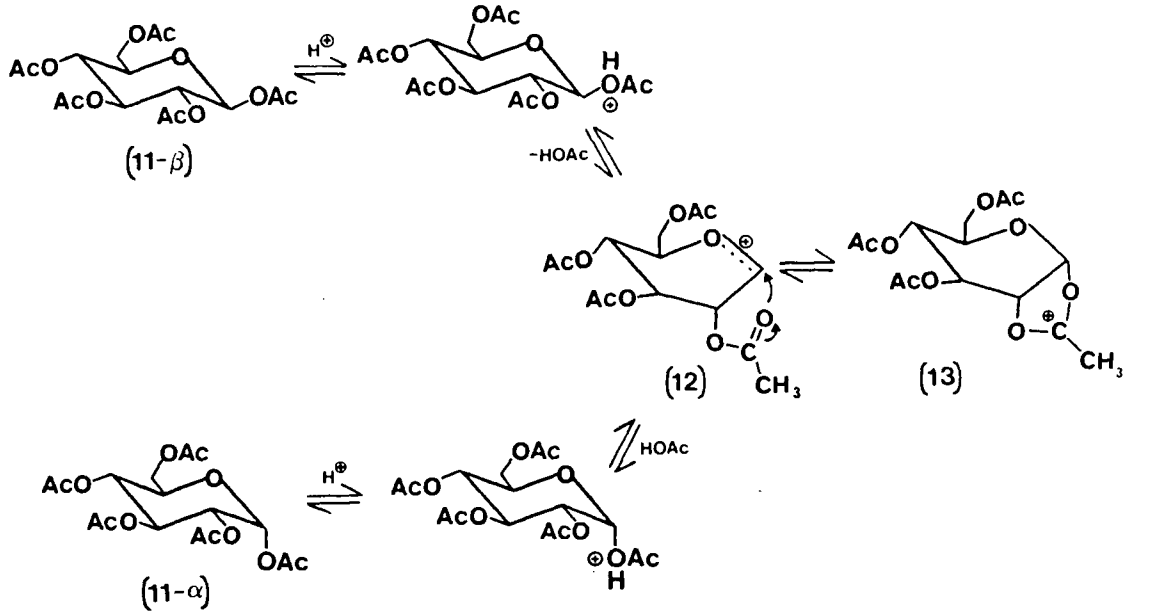


Figure 12: The mechanism for acid catalyzed anomerization of 1,2,3,4,6-penta-O-acetyl-D-glucopyranose

in terms of anchimeric assistance of the acetate group at C(2) to give the acetoxonium ion (13) shown in Figure 12. This intermediate can be attacked only on the β-face to reform (11-β). Therefore, many exchanges of the C(1) acetate group occur before the oxocarbonium ion is attacked by solvent on the α-face to form (11-α). Anchimeric assistance of this type requires that the groups at C(1) and C(2) have a <u>trans</u> relationship. Indeed, similar results were obtained with 1,2,3,4,6-penta-O-acetyl-D-mannopyranose (14). In this case, (14-α) exchanged faster than it anomerized since it now has a <u>trans</u> disposition with the axial acetyl group at C(2) and can form the acetoxonium ion readily (1:1 acetic acid-acetic anhydride, 0.50 M sulfuric acid at 25°C, k_{exch} (14-α) = 3.15 x 10^{-4} sec^{-1}, k_{anom} (14-α) = 0.33 x 10^{-4} sec^{-1}, k_{exch} (14-β) = 5.5 x 10^{-4} sec^{-1}, k_{anom} (14-β) = 5.23 x 10^{-4} sec^{-1}) (Figure 13).

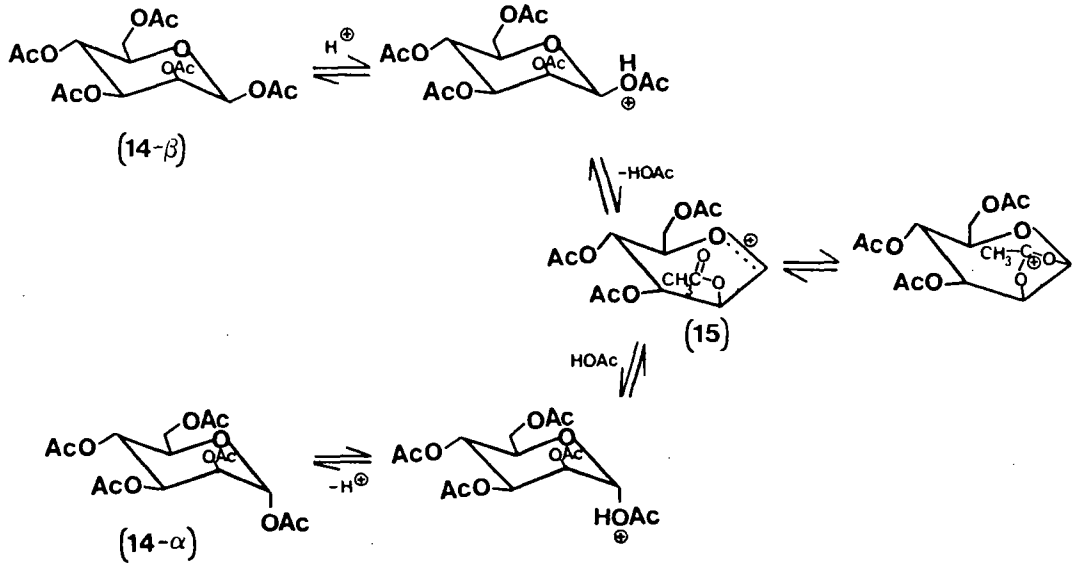


Figure 13: The mechanism for acid catalyzed anomerization of 1,2,3,4,6-penta-O-acetyl-D-mannopyranose

Further evidence for the above mechanism is given by successive replacement of the hydrogen atoms on the C(2) acetoxy group by chlorine atoms which causes a decrease in the rates of both anomerization and exchange.[20] This constitutes evidence for a carbonium ion intermediate or transition state since the greater electron-withdrawing power of chlorine relative to hydrogen will destabilize the positive charge at the anomeric carbon and thereby slow the rate of the anomerization. Furthermore, the rate of the exchange reaction is retarded since the electron-withdrawing chlorine atoms decrease the nucleophilicity of the carbonyl oxygen on the C(2) acetate group.

4. Halide Ion Catalyzed Anomerization

Koenigs-Knorr glycosidation is a method of preparing 1,2-trans-aryl glycopyranosides from glycosyl halides.[23] For example, treatment of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (16) with 2-nitrophenol in the presence of an electrophilic catalyst (Ag_2CO_3) yields 2-nitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (17). The reaction occurs via unimolecular dissociation of the bromide ion forming an oxocarbonium ion which is then subject to nucleophilic attack. The stereospecificity for the formation of the β-anomer is thought to be due to participation by the acetyl group at C(2) forming an acetoxonium ion intermediate (18) which can only be substituted on the β-face (Figure 14). Because of this stereoselectivity and the relative stability of α-glycosyl halides, this procedure provides a good method for preparing aryl β-glucopyranosides.

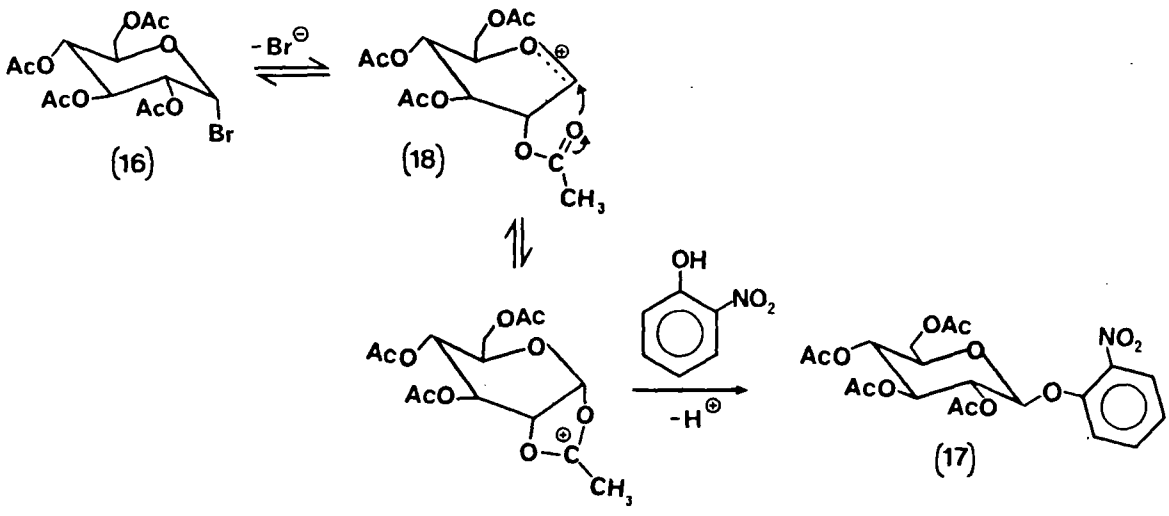


Figure 14: The Koenigs-Knorr glycosidation

Studies by Lemieux et al.[24] showed that the above reaction can be modified with the addition of halide ion to produce the α-glycoside in excess. This was termed "halide ion catalyzed glycosidation". In the initial studies of the reaction between (16-α) and pyridine,[25] both anomers of the pyridinium bromide (19) were obtained in nearly equal amounts. Under the same reaction conditions but with addition of tetra-n-butylammonium perchlorate, a 10% excess of the α-anomer (19-α) was obtained. With addition of tetra-n-butylammonium bromide, the amount of the α-anomer (19-α) was increased to >90%. These results were used to propose the mechanism shown in Figure 15. The glycosyl halide (16-α) initially undergoes nucleophilic attack by pyridine to produce the β-pyridinium bromide (19-β). The bromide ion liberated catalyzes the anomerization of the α-glycosyl bromide (16-α). The β-glycosyl

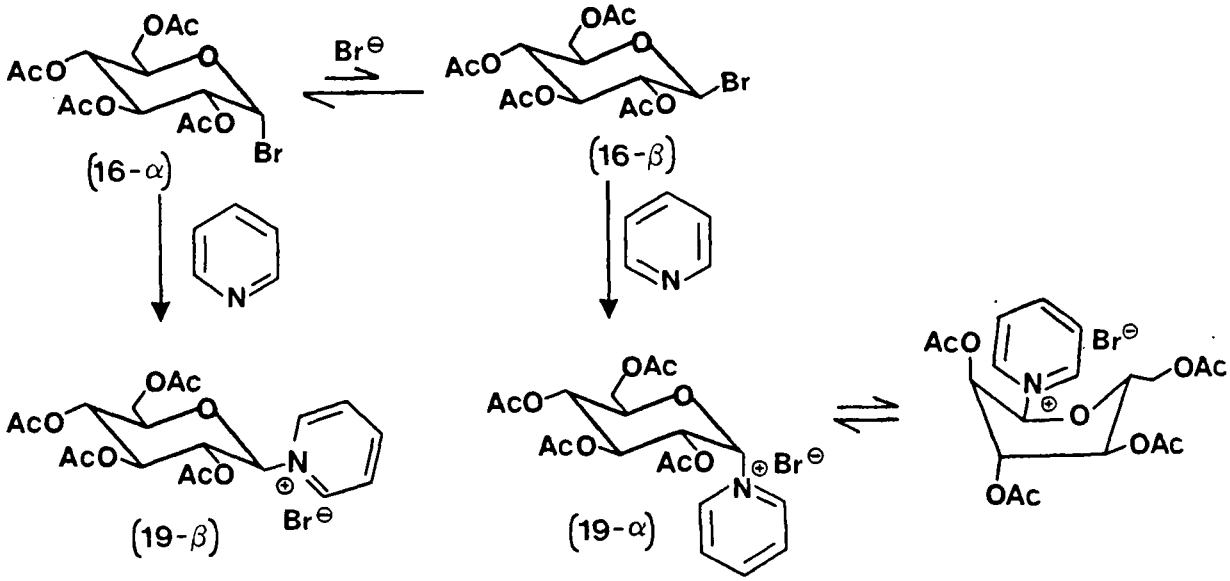


Figure 15: The halide ion catalyzed glycosidation.
The α-pyridinium bromide has been found to prefer the boat conformation shown due to the reverse anomeric effect and the steric bulk of the pyridinium group[5]

bromide (16-β) formed undergoes nucleophilic attack faster than the more stable α-anomer and hence the α-anomer (19-α) is obtained. Furthermore, the effect of added halide ion is so strong that the participation of the acetate at C(2), which facilitates β-glycoside formation (Figure 14), becomes insignificant. In fact, α-glycosyl halides with non-participating groups at C(2) are commonly used in halide ion catalyzed glycosidations to form the α-glycoside.[26] Thus, reaction of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl chloride with methanol in the presence of tetraethylammonium chloride yields 83% of the methyl α-D-glycoside.

Measurement of first order rate constants for 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl chloride (20-β) showed that the rate is proportional to the added chloride concentration.[5] Also, the rate of exchange of the α-anomer (20-α) with radioactive chloride was nearly equal to the rate of anomerization (k_{exch} = 2.0 x 10^{-4} min^{-1}, k_{anom} = 2.3 ± 0.4 x 10^{-4} min^{-1}, [20] = 0.2 M, [Me_4NCl] = 0.2 M, 30°C). These results suggest an S_N2-type process. As was seen with the acid catalyzed anomerization of penta-O-acetyl-D-glucopyranose (11), the rate of exchange of the β-glucosyl chloride (20-β) was about four times greater than its rate of anomerization. This observation suggests anchimeric assistance by the C(2) acetoxy group. The acetoxonium ion thus produced reacts with the chloride ion to reform the β-anomer (20-β) (Figure 16).

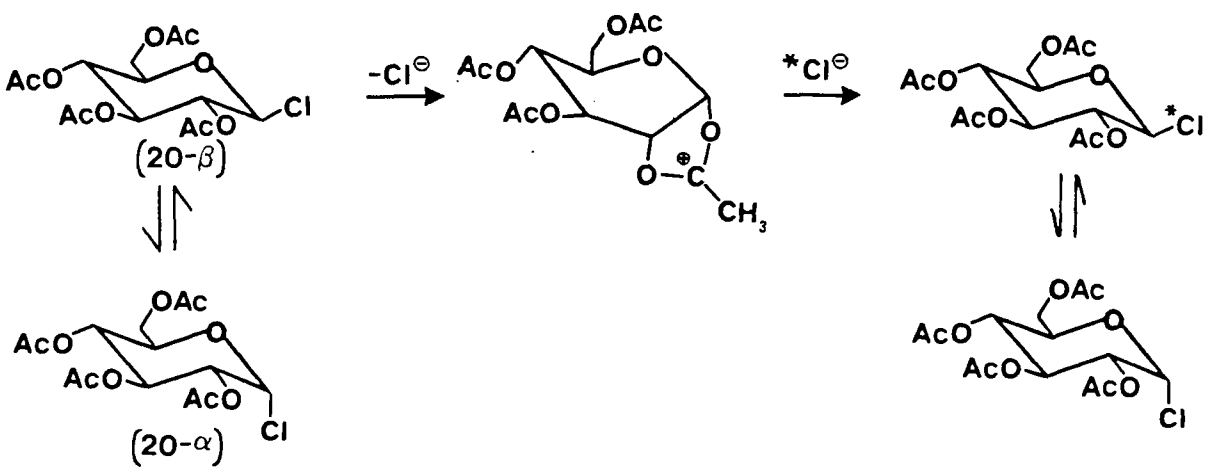


Figure 16: The mechanism of halide exchange

5. Base Catalyzed Reactions of Glycopyranosides

5.1 Hydrolysis

Base catalyzed reactions involving the glycosidic linkage are much less common than acid catalyzed reactions. Consequently, very little is known about the mechanism and kinetics of base catalyzed transformations. Alkaline hydrolysis and degradation of glycosides perhaps have been the most extensively studied in this area, yet the mechanisms are still very unclear. Generally, the glycosidic linkage is more sensitive to basic conditions when the aglycon is electron-withdrawing. For example, the alkaline hydrolysis of 4-nitrophenyl α-D-glucopyranoside is approximately 600,000 times faster than phenyl α-D-glucopyranoside ([glycoside] = 0.002 M, [NaOH] = 3.9 M, 70°C).[27] It has also been suggested that in some cases aryl-D-glucopyranosides are cleaved by alkali with aryl-oxygen fission. An unusual case of this reaction is the alkaline hydrolysis of 4-nitrophenyl α-D-glucopyranoside (21).[28] The mechanism is considered to involve O(1) to O(2) migration of the 4-nitrophenyl group followed by O(2) to O(3) migration of the aryl group to give 3-O-(4-nitrophenyl)-D-glucopyranose. Subsequent hydrolysis liberates the 4-nitrophenoxide anion via a β-elimination pathway and produces the sugar residue as a mixture of saccharinic acids (23) (Figure 17). The mechanism is supported by the isolation and characterization of the 2-O-(4-nitrophenyl)-D-glucopyranose (22). This migration pattern is unusual since it may involve an intramolecular, nucleophilic, aromatic substitution reaction and cleavage of the aryl-oxygen bond.

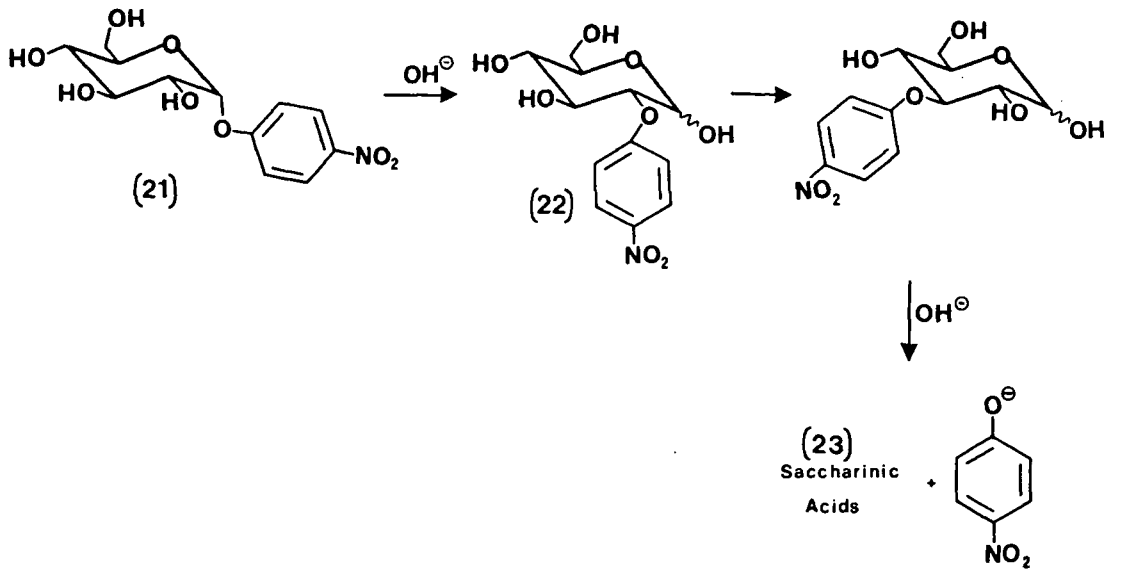


Figure 17: The base catalyzed hydrolysis of 4-nitrophenyl α-D-glucopyranoside

Other mechanisms for alkali fission of glycosides are varied and often depend on the orientation of hydroxyl groups around the pyranose ring.[29] β-Glycosides with the C(2) hydroxyl trans to the aglycon frequently yield the 1,6 anhydro compound via initial formation of the 1,2-epoxide. Thus, 4-nitrophenyl-β-D-galactopyranoside (24) reacts with sodium methoxide in methanol to yield 76% of 1,6-anhydrogalactose (25) and 8% methyl β-D-galactopyranoside (26) (Figure 18). Evidence for participation of the C(2) hydroxyl and the initial formation of the 1,2-epoxide was obtained by performing the same reaction with 4-nitrophenyl-α-D-mannopyranoside (27).[30] Here, only methyl α-D-mannopyranoside (28) was produced since the 1,2-epoxide initially formed has the wrong configuration for reaction with the C(6) hydroxyl group (Figure 19).

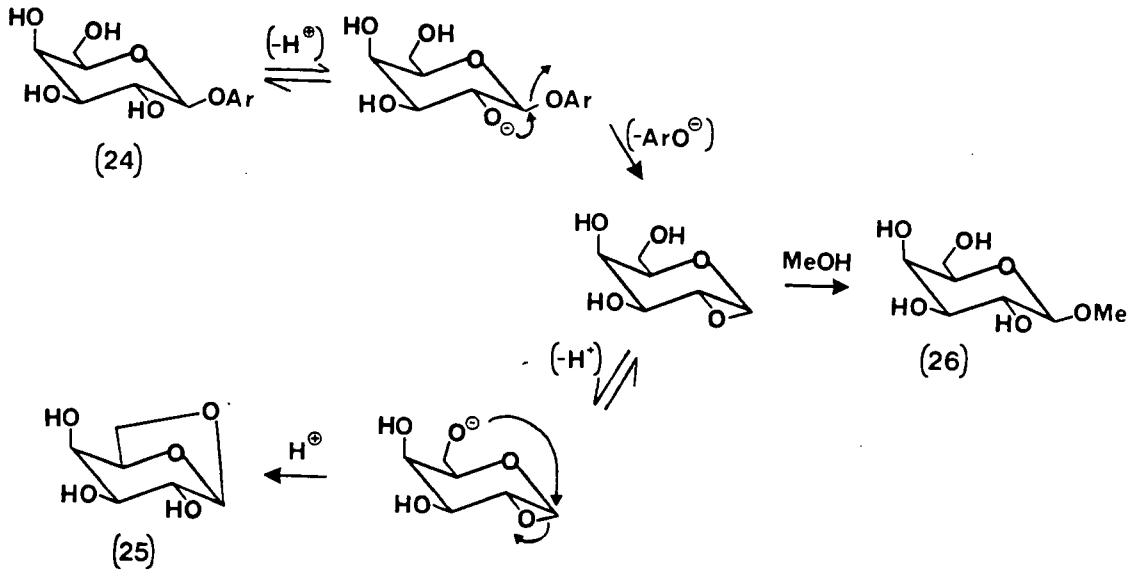


Figure 18: The hydrolysis of 4-nitrophenyl β-D-galactopyranoside, Ar = 4-nitrophenyl

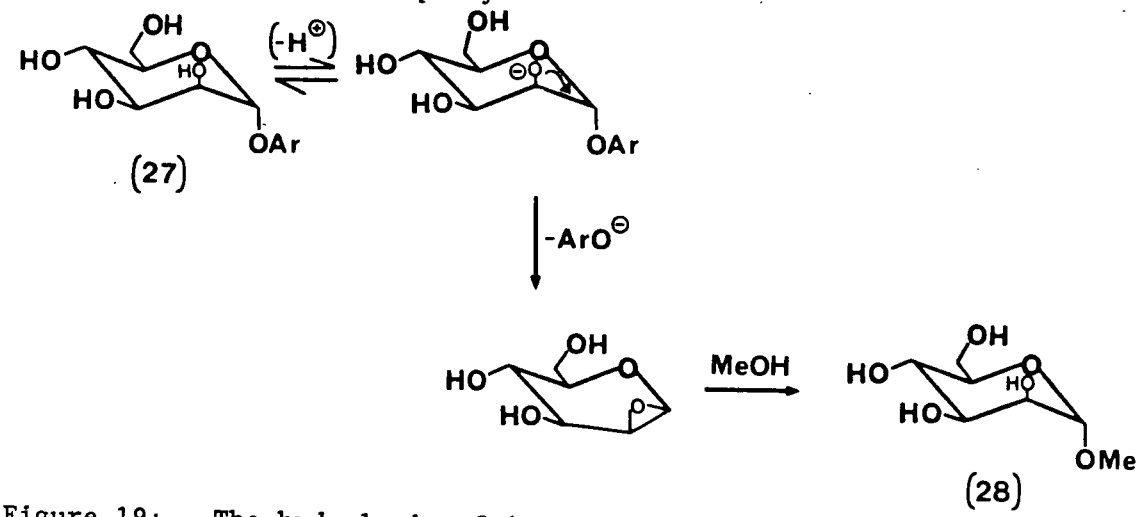


Figure 19: The hydrolysis of 4-nitrophenyl α-D-mannopyranoside, Ar = 4-nitrophenyl

5.2 Anomerization

The precedence for base catalyzed anomerization reactions is very limited. Up to 1937, the only example of base catalyzed anomerization

was the mutarotation of non-substituted reducing sugars such as glucose. As explained earlier, this transformation is effected by both acid and base catalysis.

Wolfrom and Husted were the first to report the anomerization of a fully protected sugar using alkali instead of acid.[31] They converted 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (11-β) to the α-form using a suspension of the sugar and solid sodium hydroxide in anhydrous ether. The method was not an improvement over acid catalyzed preparations since it was accompanied by at least a small amount of deacetylation. Still, it provided an unusual example of the base catalyzed interconversion of anomeric sugars.

Lindberg suggested that the above reaction involves heterogeneous catalysis.[32] He reported that when a mixture of solid sodium hydroxide and Drierite was shaken in ether, filtered, and the filtrate used as the reagent for the anomerization of (11-β), there was no trace of base in the solution and the transformation from β to α did not occur. Lindberg also showed that the anomerization of 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (29-β) could be effected using Ascarite in anhydrous pyridine. Although the yield of the α-anomer (29-α) was only 30%, this reaction was one of the first methods found to transform a phenyl β-glycoside into the α-glycoside. Interestingly, anomerization of the β-glucosides of phenol, 2-nitrophenol and 4-nitrophenol by the same method were unsuccessful.

In 1980, van Boom et al. reported further studies on the anomerization of (29-β).[33] They prepared the 2,4-dinitrophenyl (DNP) β-D-glucoside (29-β) from 2,3,4,6-tetra-O-acetyl-D-glucopyranose (30)

and fluorodinitrobenzene (FDNB) in DMF using 1,4-diazabicyclo-[2.2.2]-octane (DABCO) as a base catalyst (Figure 20). Exclusive production of the β-glucoside is due to kinetic control of the glycosidation reaction. Because of the steric bulk of the dinitrophenyl group, the reaction of FDNB with the β-anomer, which has a less hindered equatorial hydroxyl at C(1), proceeds faster than the reaction with the α-anomer. Subsequent re-equilibration of (30-α) and (30-β) drives the reaction towards exclusive production of the less thermodynamically stable β-anomer (29-β). Furthermore, no anomerization or rearrangement of the DNP ether group was observed, and a high yield (82%) of (29-β) was obtained. In comparison, previous routes to the DNP-glycoside involved treatment of tetra-O-acetyl glucosyl halides with 2,4-dinitrophenol in the presence of potassium carbonate to afford the protected 1,2-trans glycosides in relatively low yields (8-50%).[34,35]

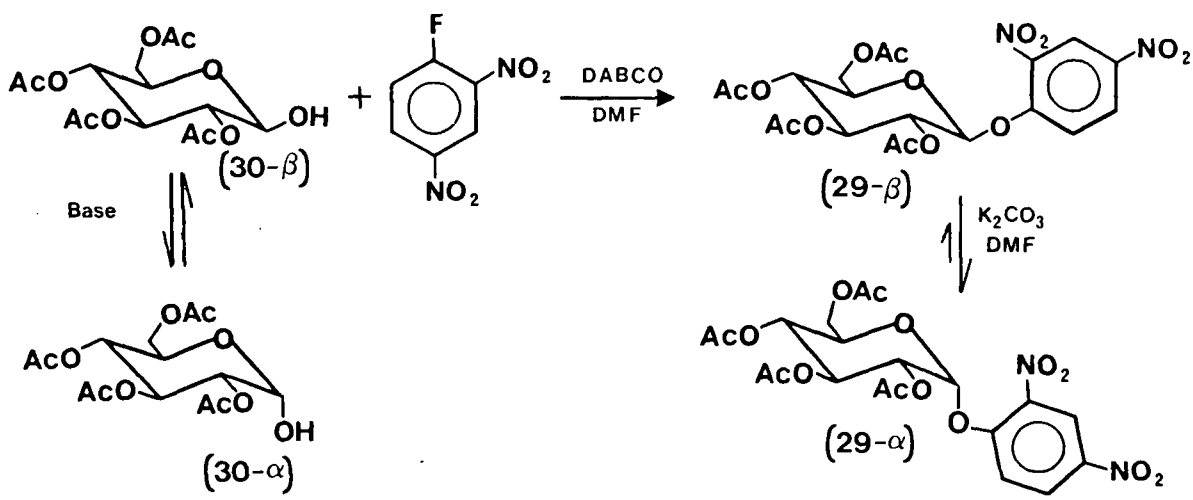


Figure 20: The preparation and anomerization of 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

Treatment of (29-β) with solid potassium carbonate in DMF afforded an equilibrium mixture of the α- and β- anomers (80:20 respectively) in good yield (Figure 20). A clue to the mechanism of the anomerization was obtained when they reacted 2,4-dinitrophenyl 2,3,4,6-tetra-O-benzyl-β-D-glucopyranoside (31) under the same reaction conditions and found the reaction to proceed approximately 70 times more slowly than the acetylated DNP sugar (29). Hence it was suggested that the acetyl group at C(2) may provide some neighboring group assistance to the reaction. Further studies towards the mechanism of this anomerization and investigation into the role of the potassium carbonate are the subject of this thesis.

RESULTS

1. Synthesis

1.1 2,4-Dinitrophenyl β-D-glucopyranosides

Per-O-acetylated 2,4-dinitrophenyl β-D-glucopyranosides were routinely prepared using the method of van Boom et. al.[33] in which the appropriate sugar was reacted with FDNB in DMF using DABCO as a base catalyst. All of the β-2,4-DNPG derivatives were highly crystalline and were recrystallized from warm ethanol. Yields for the derivatization reaction were greater than 80%.

1.2 Deuterium Labelled 2,4-Dinitrophenyl β-D-glucopyranosides

The route for the preparation of 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-[1-^{2}H]-glucopyranoside (32) is given in Figure 21.[36] Lewis acid catalyzed acetylation D-glucono-1,5-lactone was performed essentially according to Nelson[37] using acetic anhydride and zinc chloride. Removal of traces of acetic anhydride was achieved by stirring the reaction mixture in saturated sodium bicarbonate. Simple work-up and evaporation of the solvent produced the tetra-O-acetyl lactone (33) as a colorless gum in 92% yield. The lactone was reduced with sodium borodeuteride, producing a mixture of the α- and β-[1-^{2}H]-tetraacetates (34)

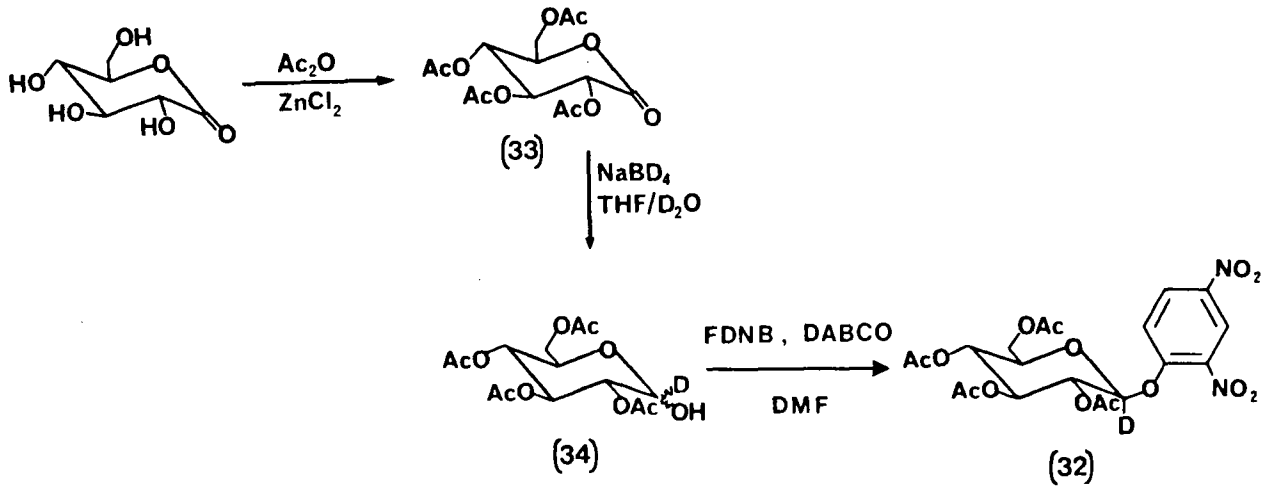


Figure 21: Synthesis of 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-[1-^{2}H]-glucopyranoside

which were derivatized to the β-2,4-DNPG compound (32). The crystalline β-2,4-DNPG derivative was obtained in 66% overall yield from the lactone. Alternative methods of deuterium substitution reported in the literature[38,39,40] which were considered here include direct reduction of D-glucono-1,5-lactone in D_2O solution with sodium amalgam in the presence of phosphoric acid-d_3, followed by acetylation, or reduction of the tetrahydropyranyl derivative of D-glucono-1,5-lactone with sodium borodeuteride in THF, acid-catalyzed hydrolysis in D_2O of the protecting groups, and acetylation with acetic anhydride and sodium acetate. Yields for both of these approaches, however, are reported to be only about 30%.

The deuterated tetra-O-benzyl derivative (35) was prepared according to the scheme in Figure 22. The oxidation of 2,3,4,6-tetra-O-

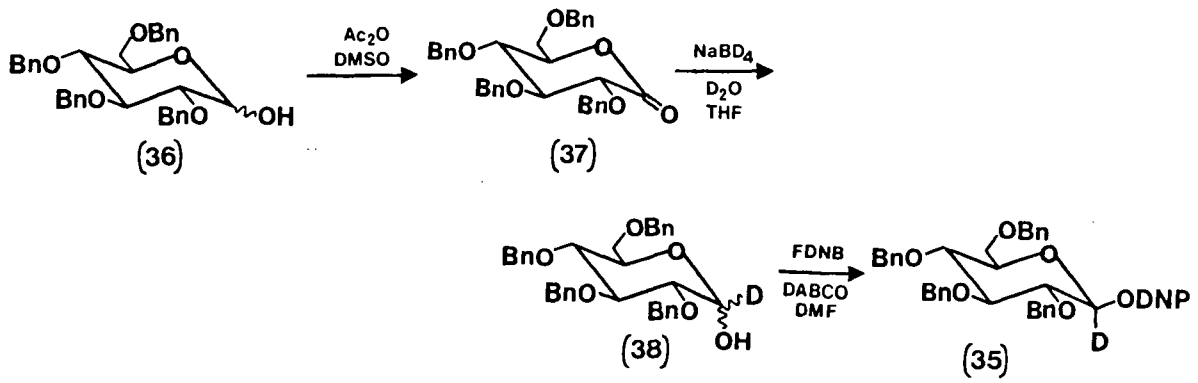


Figure 22: Synthesis of β-2,4-dinitrophenyl 2,3,4,6-tetra-O-benzyl-β-D-[1-^{2}H]-glucopyranose

benzyl-D-glucopyranose (36) was performed by the method of Kuzuhara and Fletcher[41] and the lactone (37) was isolated as a colorless syrup in high yield. Subsequent reduction to (38) and derivatization afforded the deuterated DNP derivative (35) in 62% overall yield.

1.3 Deoxy and Deoxyfluoro 2,4-Dinitrophenyl β-D-glucopyranosides

The synthetic route for the preparation of the 4-deoxy-4-fluoro derivative (40) is given in Figure 23. Treatment of 1,2,3,6-tetra-O-acetyl-4-deoxy-4-fluoro-β-D-glucopyranose (41) with 45% hydrobromic acid in acetic acid and acetic anhydride[42] afforded the bromide (42) as a colorless syrup in 85% yield. Hydrolysis of the bromide (42) with silver carbonate and water in acetone[43] yielded a brown syrup which consisted primarily of the hydrolysis product (43) (~70%) and was not

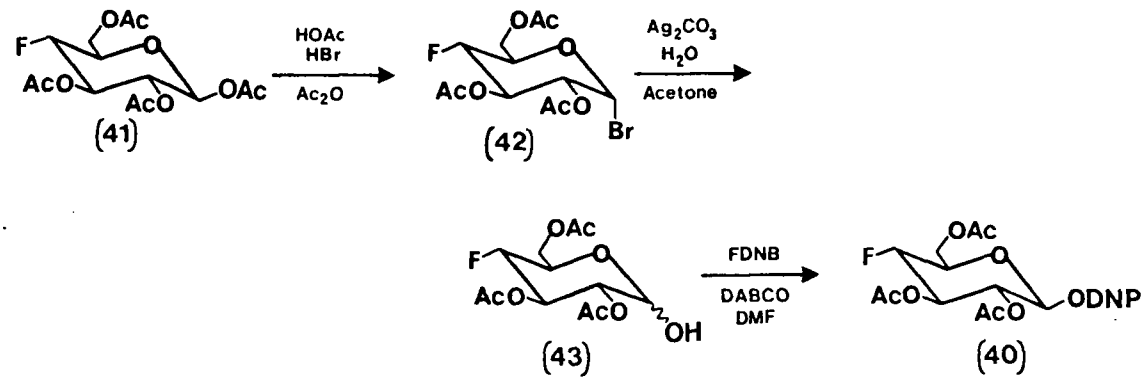


Figure 23: Synthesis of 2,4-dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy 4-fluoro-β-D-glucopyranoside

purified. Derivatization by the standard method gave the 4-deoxy-4-fluoro DNPG (40) as yellow crystals in 34% overall yield.

Synthesis of the 4-deoxy DNPG derivative (44) was achieved from methyl 2,3,6-tri-O-benzoyl-4-deoxy-α-D-glucopyranoside (45) as shown in Figure 24. Deprotection of (45) with sodium methoxide in methanol[44] followed by acetic anhydride/pyridine acetylation afforded (46) as a white solid. Formation of the glycosyl chloride (47) was achieved by the method of Kovac et.al.[45] using zinc chloride and dichloromethyl methyl ether to obtain a brown syrup which consisted of only one component by t.l.c. (presumably the chloro sugar). Subsequent hydrolysis[43] to form (48) and derivatization afforded the 4-deoxy DNPG (44) as yellow crystals in 51% overall yield.

Synthesis of the 6-deoxy DNPG (49) was achieved by the same route used for the preparation of the 4-deoxy-4-fluoro derivative (40) (Figure

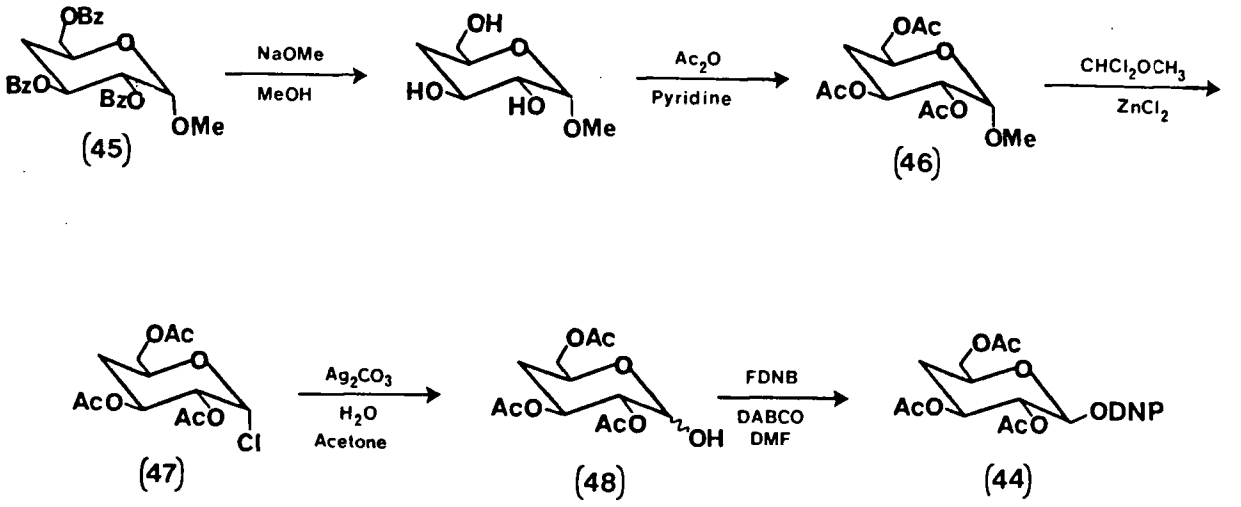


Figure 24: Synthesis of 2,4-dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy-β-D-glucopyranose

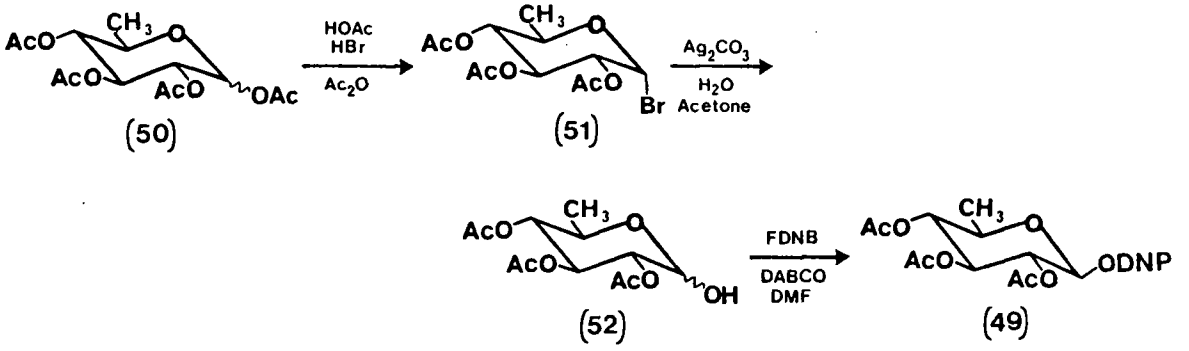


Figure 25: Synthesis of 2,4-dinitrophenyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-glucopyranoside

25). The 6-deoxy DNPG (49) was obtained as yellow crystals in 83% overall yield.

1.4 Peracetylated Substituted Phenyl Glucopyranosides

The substituted phenyl β-D-glucopyranosides listed in Table 1 were prepared by condensation of the sodium salt of the phenol with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (16) in aqueous acetone.[46] The β-D-glucopyranoside was formed exclusively in each case. However, yields were generally low due to the formation of 2,3,4,6-tetra-O-acetyl-D-glucopyranose by hydrolysis of the bromide (Figure 26).

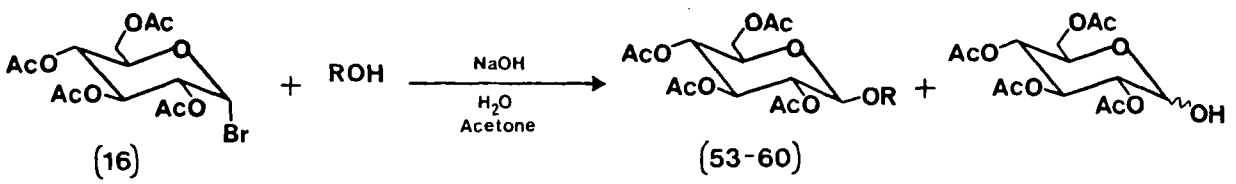


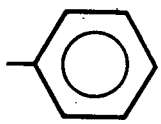
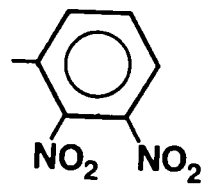
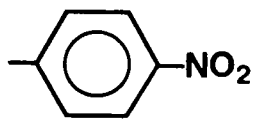
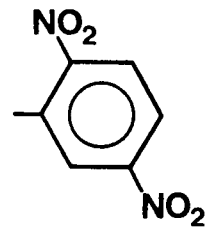
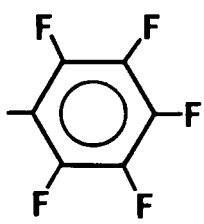
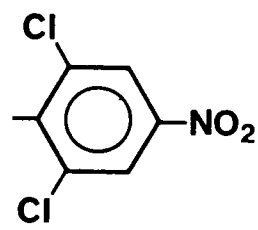
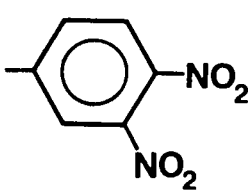
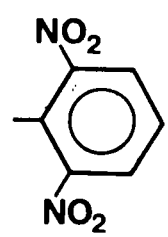
Figure 26: Synthesis of substituted phenyl β-D-glucopyranosides

2. Mechanistic Investigations

2.1 General Anomerization Procedure

The β-2,4-DNPG derivatives (29,31,32,35,39,40,44,49) as well as the substituted phenyl glucopyranosides (53-60) were anomerized by the method of van Boom et al.[33] using potassium carbonate as a catalyst. Due to the possible decomposition of DMF under basic conditions,[47] however, DMSO was substituted as the solvent in most experiments. In general, the reaction mixture was stirred overnight or longer to obtain

Table 1: Substituted phenyl glucopyranosides and obtained yields

	R	% yield		R	% yield
(53)		46	(57)		51
(54)		35	(58)		33
(55)		51	(59)		58
(56)		57	(60)		55

an equilibrium mixture of the anomers. Separation of the mixture of α- and β-anomers was achieved by fractional crystallization in warm ethanol.

The anomerization reactions were routinely monitored by t.l.c. in which the α- and β-glucopyranosides were detected by u.v. light or charring and the α- and β-anomers typically had R_f values which differed by about 0.16 (standard t.l.c. conditions, $R_f(\beta) = 0.36$, $R_f(\alpha) = 0.52$). When accurate determination of the amounts of each anomer was necessary, the reaction mixture was worked-up without separation of the products. The amount of each anomer formed was determined by integration of the ^{1}H-n.m.r. spectrum of the product mixture. When dissolved in $CDCl_3$, the ratio of α- and β-anomers generally was determined by integration of the resonance for H(6) of the phenyl ring (Figure 27) in which the resonance for the β-anomer is shifted about 0.10 ppm upfield from that of the α-anomer. In DMSO-d_6, resonances for the sugar ring protons were more distinct and the α/β ratio could be determined by integration of the anomeric resonance for each compound (Figure 28).

Initially minimal care was taken to remove water from the reaction mixture. In general, solvents were distilled and reactants were dried overnight in vacuo, but the reaction itself was not performed in an anhydrous environment. Later it was determined that the presence of even a small amount of water has a pronounced effect on the anomerization, particularly on rate measurements. This effect was originally believed to be due to a hydrolysis side reaction since t.l.c., and n.m.r. and u.v./vis spectroscopy showed the presence of the 2,4-dinitrophenolate anion after anomerization of (29). In order to

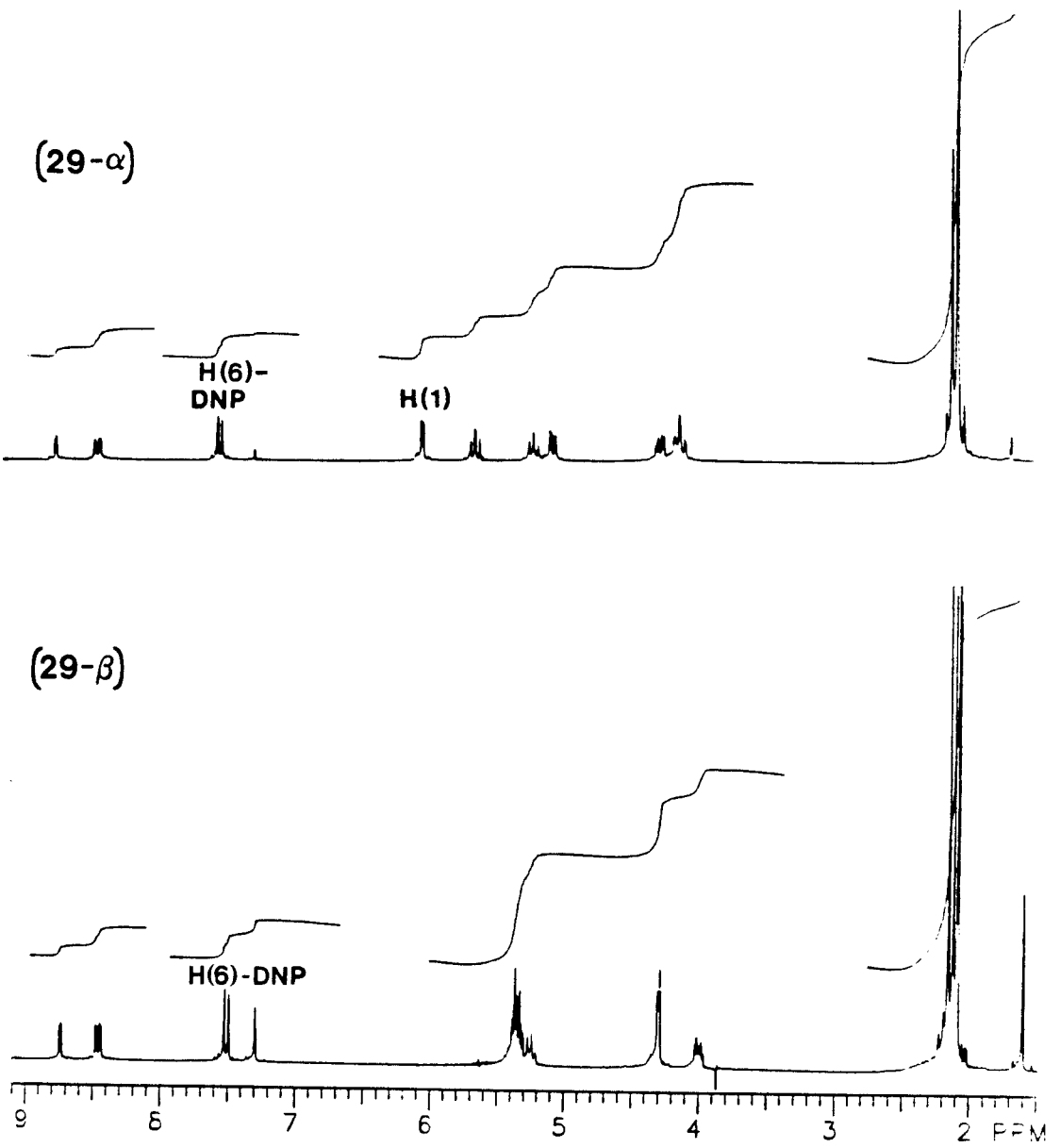


Figure 27: ^{1}H-N.m.r. spectrum of β-2,4-DNPG (29-β) and α-2,4-DNPG (29-α) in $CDCl_3$

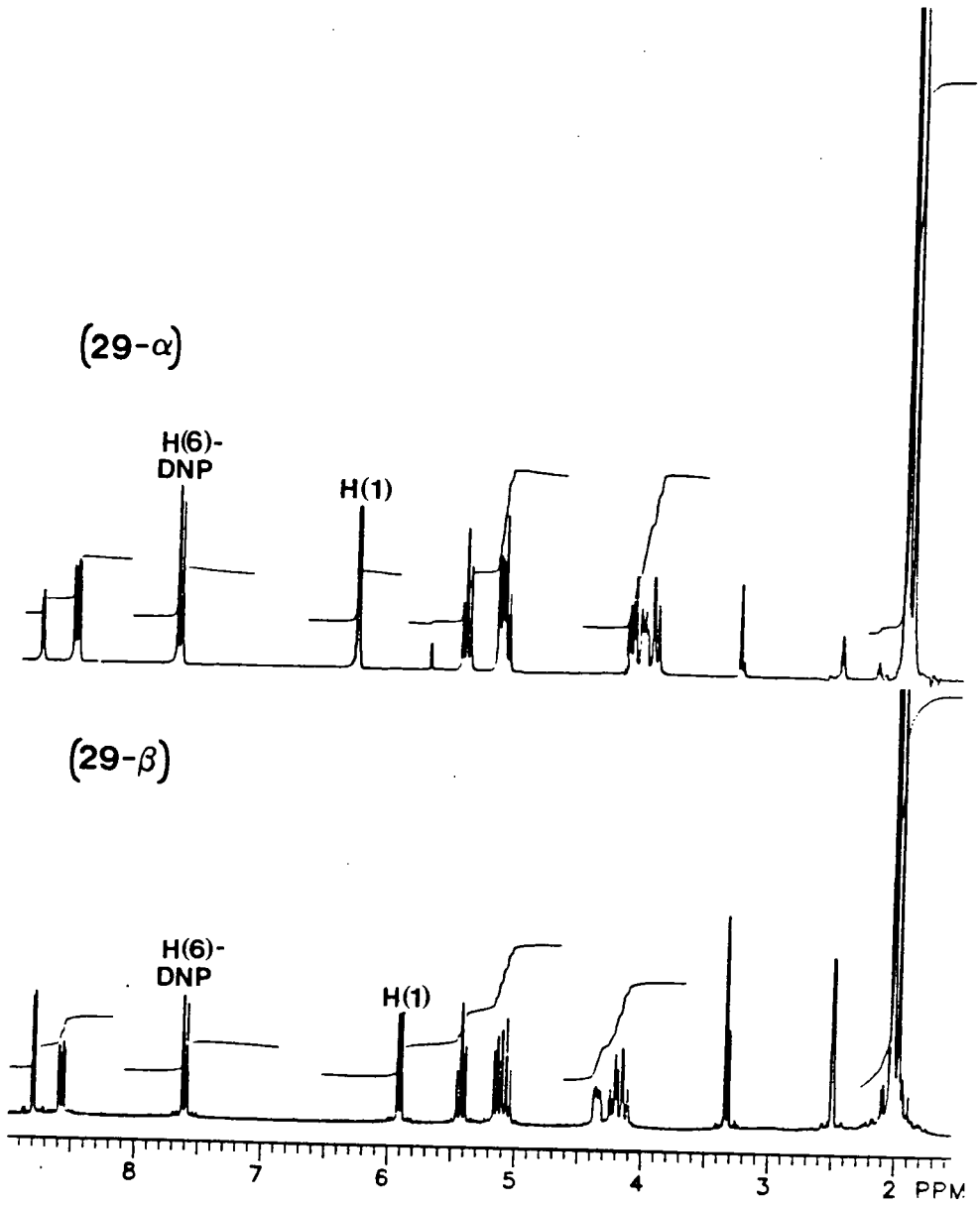


Figure 28: ^{1}H-N.m.r. spectrum of β-2,4-DNPG (29-β) and α-2,4-DNPG (29-α) in DMSO-D_6

determine the effect of water on the anomerization and on the generation of the 2,4-dinitrophenolate anion, the reaction was monitored by means of u.v./vis spectrophotometry at the wavelength of the 2,4-dinitrophenolate absorbance (λ_{max} = 429 nm). These results are shown in Figure 29. As can be seen, the addition of water resulted in a significant decrease in the rate of generation of dinitrophenolate, whereas scrupulous removal of water resulted in a dramatic increase in the rate. Simultaneous t.l.c. of these reaction mixtures showed that although addition of water apparently slows the reaction, it does not prevent anomerization entirely. These results suggest that formation of the dinitrophenolate anion is not a consequence of hydrolysis, but perhaps is formed by some other side reaction. In any case, water dramatically affects the rate of the reaction and the reason for this observation is not entirely clear. As a result, greater care was necessary to eliminate water from the system, particularly with kinetic measurements.

2.2 Anomerization of Substituted Phenyl Glucopyranosides

The per-O-acetylated aryl glucopyranosides in Table 2 were submitted to the anomerization conditions (potassium carbonate, DMSO) to determine the effect of substitution of the phenyl group on the reaction. Only the 2,6-dinitrophenyl glucopyranoside (60) and the 2,6-dichloro-4-nitrophenyl glucopyranoside (59) anomerized and the rates of anomerization were considerably slower than that for the 2,4-dinitrophenyl glucopyranoside (29) as determined by t.l.c. of the reaction

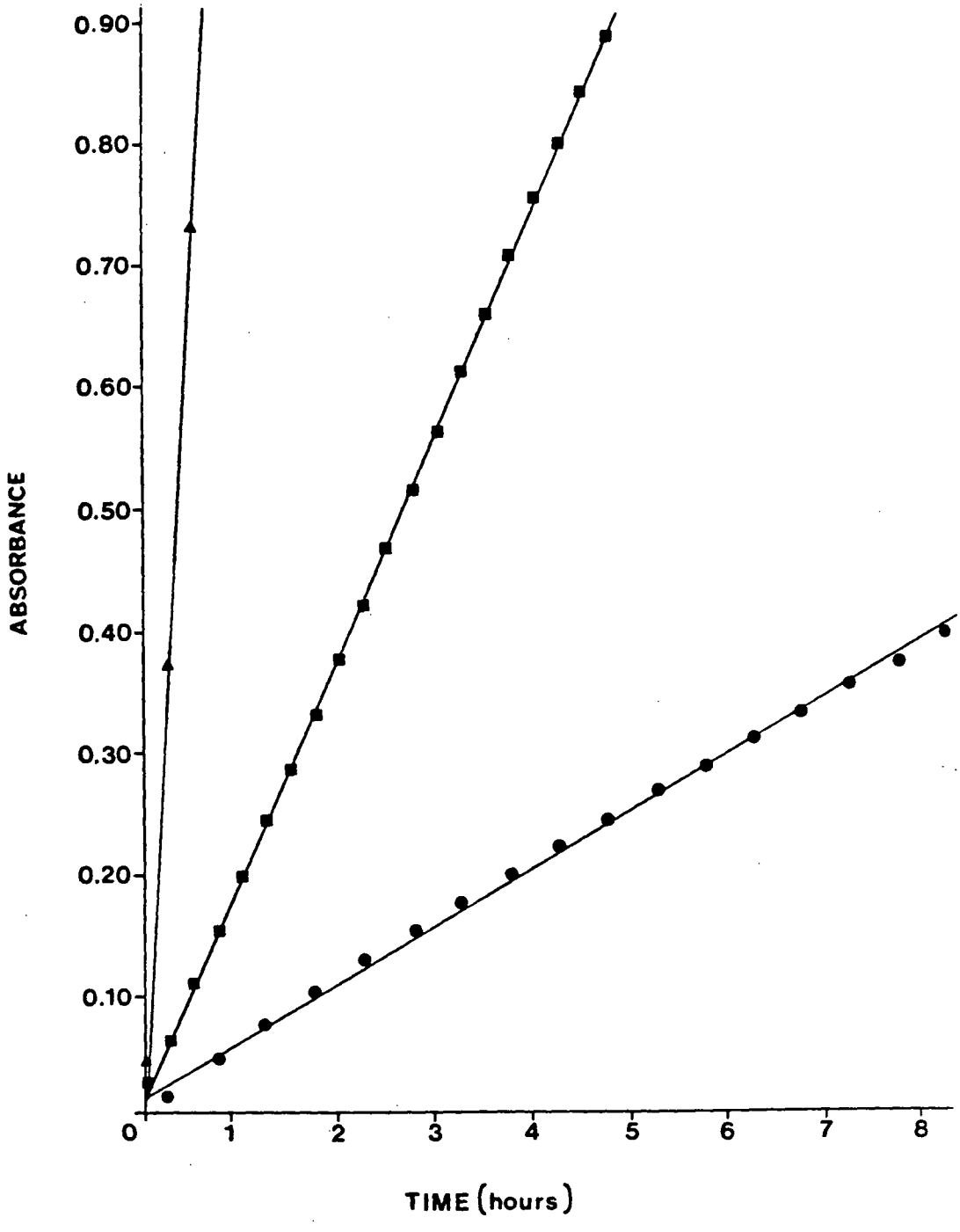
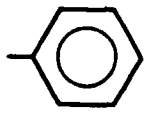
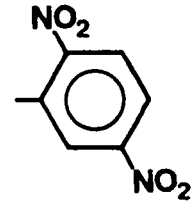
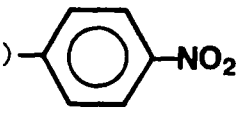
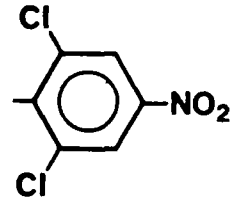
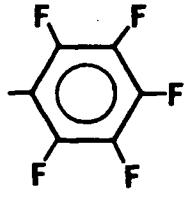
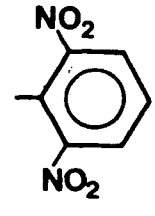
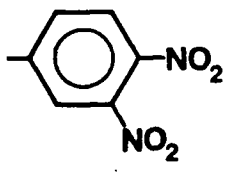
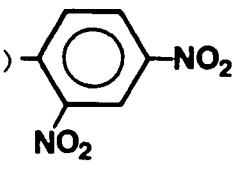
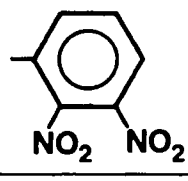
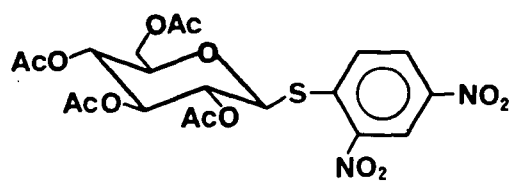


Figure 29: Absorbance vs time for generation of 2,4-dinitrophenolate (λ_{max} = 429 nm) during the anomerization of (29); ● water added, ■ no water added, no special drying precautions, ▲ all reagents dried <u>in vacuo</u>

Table 2: Anomerization of substituted phenyl glucopyranosides. pK_a values are those for the corresponding phenol and were determined in water.[48,49]

Glucoside	R	pK_a	$\beta \rightarrow \alpha$	Glucoside	R	pK_a	$\beta \rightarrow \alpha$
(53)		9.98	no	(58)		5.15	no
(54)		7.15	no	(59)		3.15	yes
(55)		5.32	no	(60)		3.77	yes
(56)		5.36	no	(29)		4.00	yes
(57)		4.89	no				

mixture and n.m.r. analysis of the product mixture. Since 2,6-dichloro-4-nitrophenol and 2,6-dinitrophenol are the only two aglycones which have pKa's lower than that of 2,4-dinitrophenol, then the reaction appears to depend on the relative electron-withdrawing power of the substituted phenyl group. This effect may be combined with inhibitory steric factors associated with the extra ortho substitution in the phenyl group which are not present with 2,4-DNPG (29) and thus may explain the relatively slow rates of anomerization for (59) and (60). In addition, the sulfur analogue (61) was submitted to the anomerization conditions and was found to be unreactive.



(61)

2.3 Exchange Reactions

The anomerization of β-2,4-DNPG (29) may proceed via three possible mechanisms involving either (i) C(1)-H(1) bond cleavage (proton abstraction), (ii) C(1)-O(1) bond cleavage (phenolate departure), or (iii) O(1)-aryl bond cleavage (nucleophilic aromatic substitution) (Figure 30). (Details of these mechanisms are considered in the Discussion

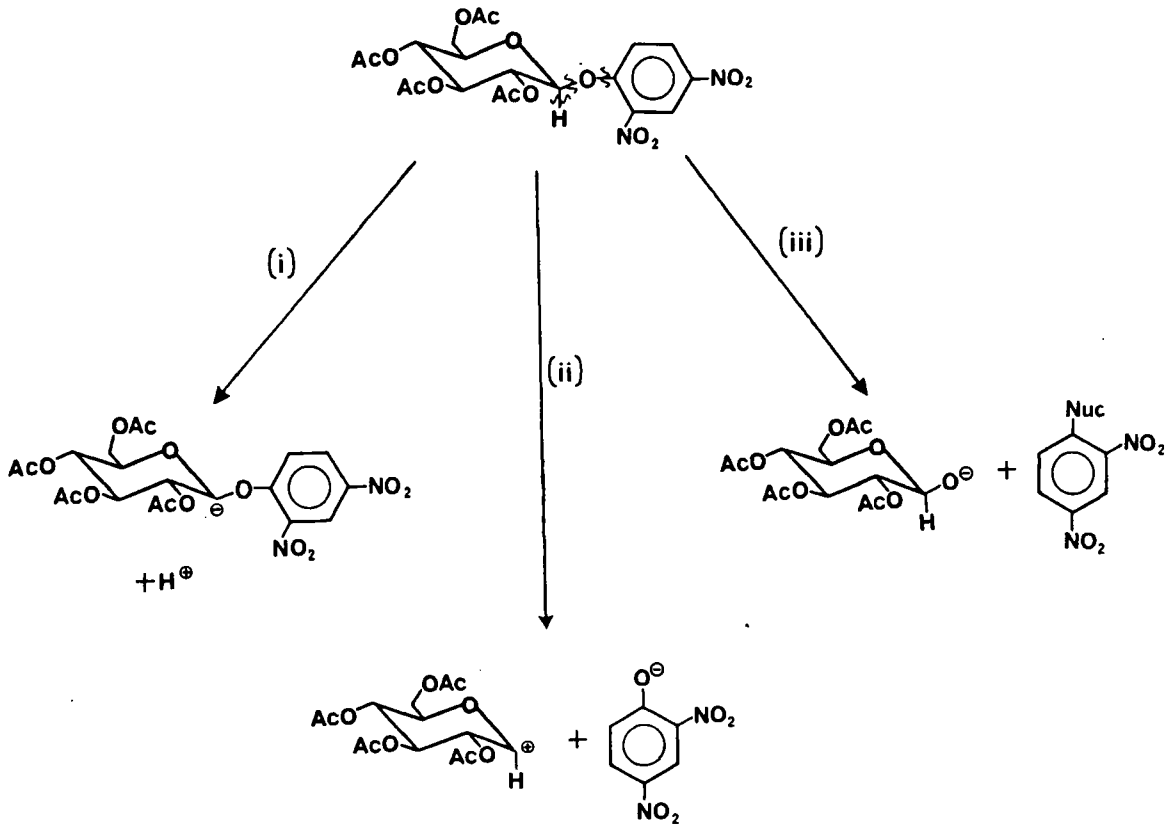


Figure 30: Possible sites of bond cleavage

section). In all three 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 in an attempt to determine which bond is cleaved during anomerization.

Proton abstraction, case (i), was tested in two experiments. The first involved reacting β-2,4-DNPG (29) under normal anomerization conditions, but with the addition of t-BuOD (prepared from t-BuOH and D_2O). Exchange between the deuteron and H(1) on β-2,4-DNPG (29) should occur if the reaction proceeds via anomeric proton abstraction. Deuterated t-butanol was chosen as a deuteron source since t-butoxide is a relatively poor nucleophile and therefore, would be unlikely to cause side reactions. The reaction was carried out in the presence of a 100 fold mole excess of t-BuOD and the mixture was stirred for 2 days, after which time approximately 50% conversion to the α-anomer had occurred. ^{1}H-N.m.r. analysis involving a comparison of the integration values for the α-, β- aromatic resonances with those of the α-,β-anomeric resonances showed that no exchange had occurred (Figure 31).

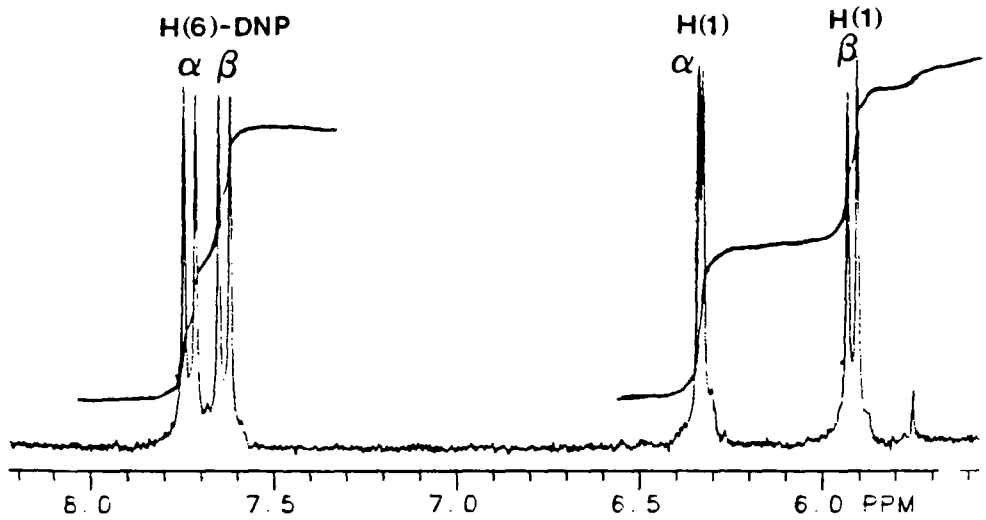


Figure 31: ^{1}H-N.m.r. of H/D exchange experiment (DMSO-d_6)

The converse H/D exchange experiment also was performed. In this experiment, deuterium labelled β-2,4-DNPG (32) was reacted under the same anomerization conditions in the presence of normal t-BuOH (100 fold mole excess). ^{1}H-N.m.r. analysis in this case would show H/D exchange very clearly since the anomeric resonance absent in the ^{1}H-n.m.r. spectrum of [1-^{2}H]-2,4-DNPG would be readily apparent if the deuterium atoms are exchanged for hydrogen. Again, no exchange was observed (Figure 32).

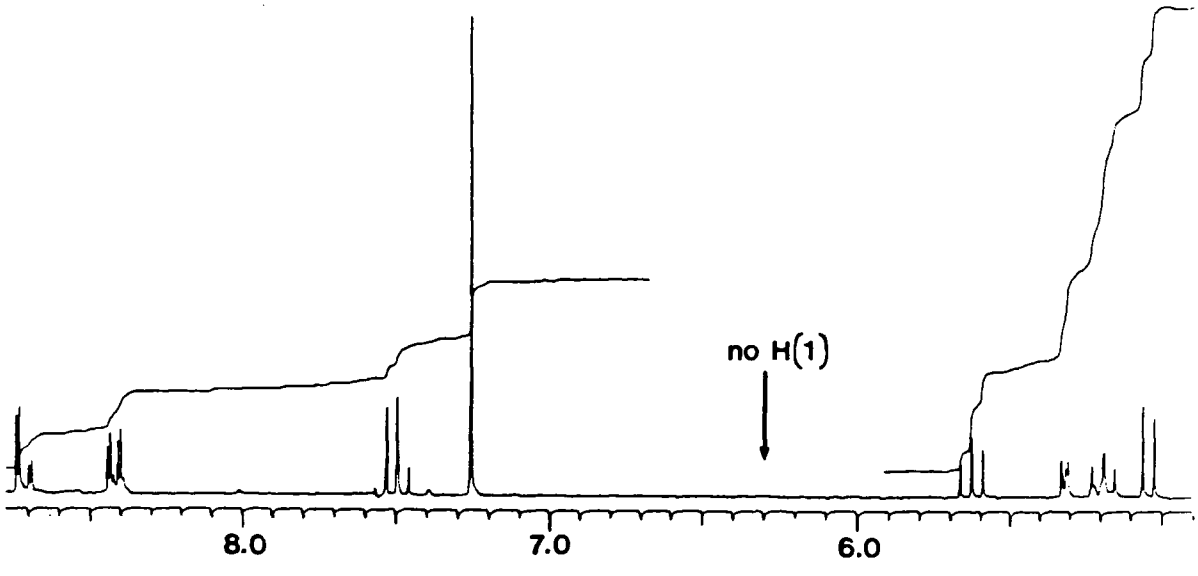


Figure 32: ^{1}H-N.m.r. of D/H exchange experiment ($CDCl_3$)

The possibility of phenolate departure and recombination, case (ii), was investigated by attempting to exchange the 2,4-DNP group for

the 2,6-DNP group. (Recall that of all the glycosides studied in Table 2, β-2,6-DNPG (60) was one of only three glycosides found to anomerize). If the reaction occurs via cleavage of the C(1)-O(1) bond, then 2,4-dinitrophenolate is generated, and if another phenolate of similar pKa such as 2,6-dinitrophenolate is present, then a mixture of α-,β-2,4-DNPG (29) and α-,β-2,6-DNPG (60) should result. ^{1}H-N.m.r. analysis of the product mixture would indicate if exchange had occurred. Again, two experiments were performed. The first involved reacting β-2,4-DNPG (29) under the normal anomerization conditions in the presence of added potassium 2,6-dinitrophenolate (one equivalent). The second involved reacting β-2,6-DNPG (60) under the same conditions in the presence of added potassium 2,4-dinitrophenolate (one equivalent). The reactions were stirred for three days after which time t.l.c. indicated that 80% conversion to the α-anomer (either 2,6-DNPG or 2,4-DNPG) had occurred in the first reaction, and 50% conversion in the second reaction. In both cases, ^{1}H-n.m.r. analysis of the product mixture indicated that no exchange had occurred (Figure 33). The reaction of the β-2,6-DNPG (60) was repeated with 10 fold mole excess of the 2,4-dinitrophenolate. Again ^{1}H-n.m.r. indicated that no exchange had occurred.

An alternative mechanism leading to the cleavage of the C(1)-O(1) bond would involve proton abstraction at C(2) and result in generation of a protected glucal intermediate via elimination of 2,4-dinitrophenolate (Figure 34). However, this route should also lead to exchange between 2,6-DNP and 2,4-DNP. Furthermore, proton abstraction at C(2) should lead to H/D exchange at C(2) and perhaps some epimerization to

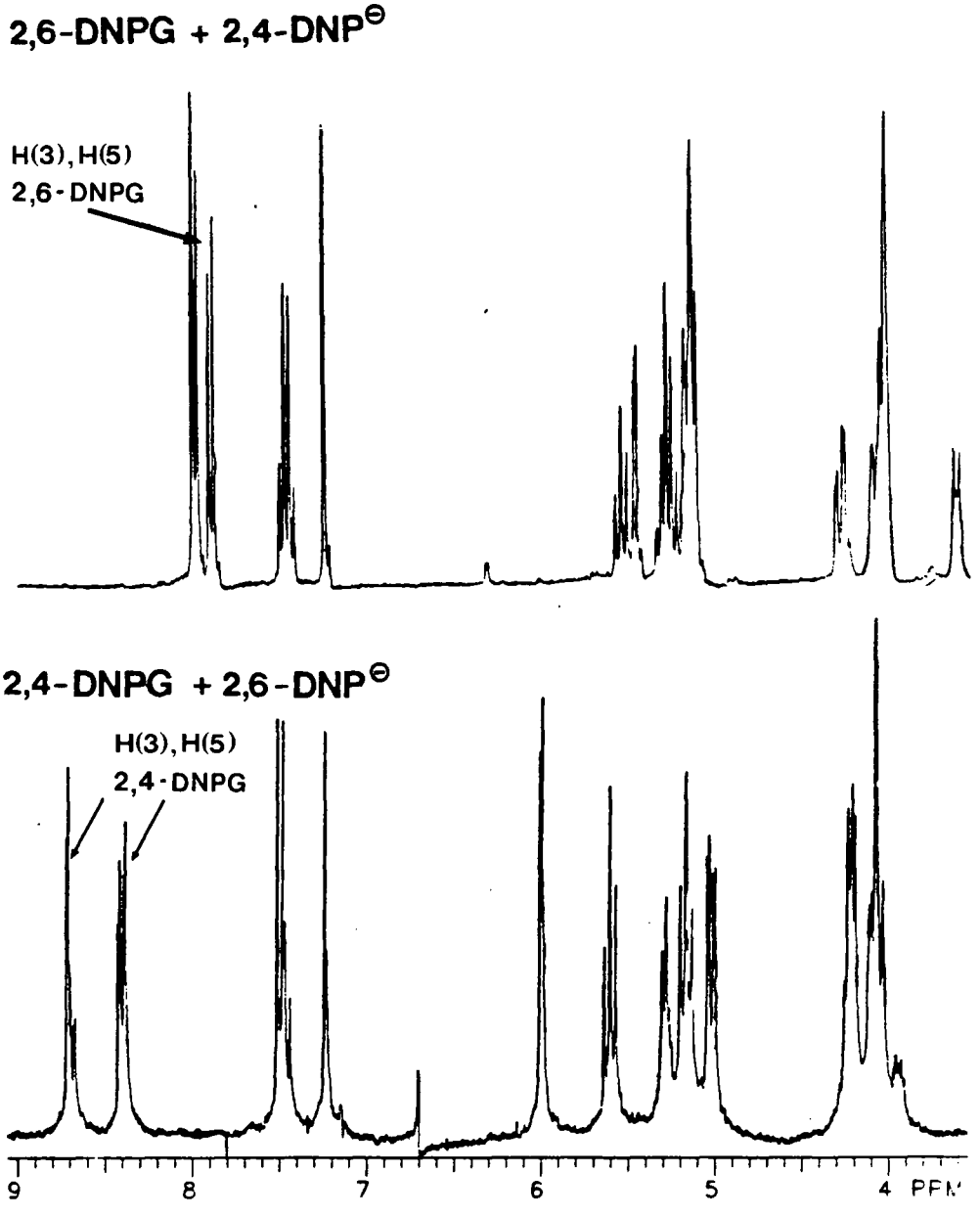


Figure 33: ^{1}H-N.m.r. spectra of attempted phenolate exchange experiments ($CDCl_3$)

the mannoside derivative. Again, ^{1}H-n.m.r. spectra of the product mixture from the anomerization of β-2,4-DNPG (29) in the presence of a deuteron source showed no H/D exchange or epimerization at C(2). Furthermore, when 2,3,4,6-tetra-O-acetyl-D-glucal (62) was reacted with 2,4-dinitrophenol in potassium carbonate/DMSO, no DNPG was formed, also providing evidence against the glucal (62) as an intermediate.

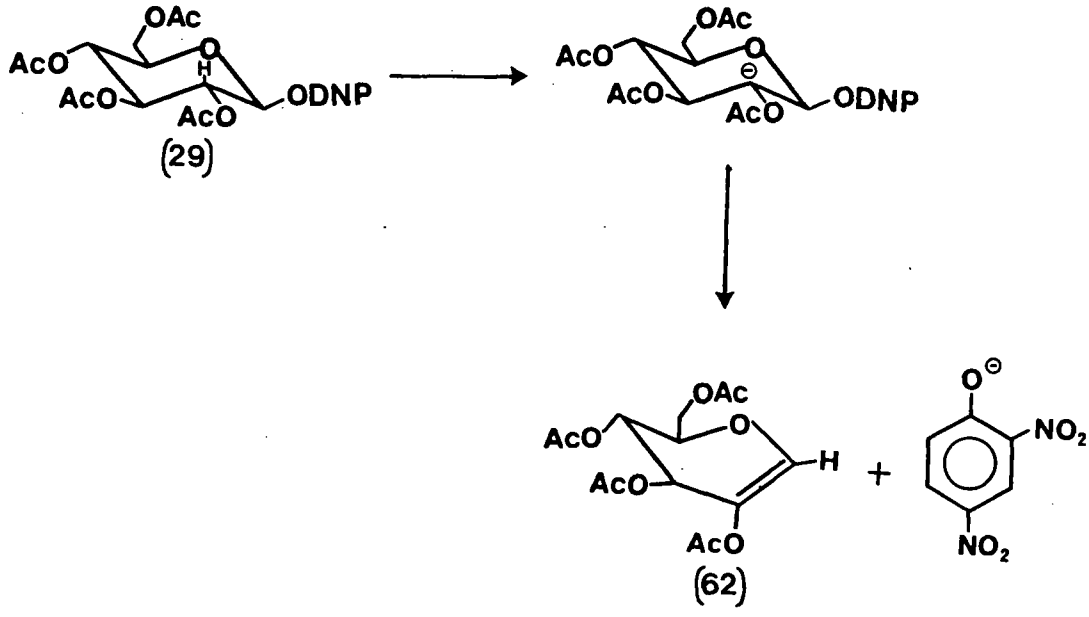


Figure 34: Alternative route to C(1)-O(1) bond cleavage

Cleavage of the O(1)-aryl bond or case (iii) is possible if the reaction proceeds via nucleophilic substitution on the aromatic ring, perhaps with the carbonate anion acting as a nucleophile. The β-glycosyl anion which is generated could anomerize to the α-glycosyl anion and then re-attack the aromatic intermediate via nucleophilic substitution.

To test case (iii), β-2,6-DNPG (60) was anomerized in the presence of 1-fluoro-2,4-dinitrobenzene (FDNB) under standard conditions. If the reaction involves O(1)-aryl bond cleavage, exchange of the aryl substituents should occur via attack of the intermediate glycosyl anion on the FDNB. Indeed, exchange was observed as shown by the ^{1}H-n.m.r. spectrum of the product mixture (Figure 35). Although only about 30% of the β-2,6-DNPG (60) had reacted as judged by both t.l.c. and ^{1}H-n.m.r. after stirring for 8 days, the ^{1}H-n.m.r. spectrum ($CDCl_3$) showed at least a small amount of the α-2,4-DNPG (29-α) and possibly some of the β-2,4-DNPG (29-β) had been formed. Furthermore, no α-2,6-DNPG (60-α) was formed as judged by the absence of the H(6)-DNP resonance of the α-2,6-DNPG at 7.87 ppm. Although the aglycon exchange observed here had occurred only to a small extent, it supports a mechanism involving O(1)-aryl bond cleavage.

A second exchange experiment was performed to provide further evidence for the nucleophilic substitution mechanism. In this case, β-2,4-DNPG which was deuterated at C(1) (32) was anomerized as usual but in the presence of one equivalent of 2,3,4,6-tetra-O-acetyl-D-glucopyranose (30). Under the basic conditions, the anomeric hydroxyl of (30) will be deprotonated to produce the same glucosyl anion as that formed by nucleophilic aromatic substitution on (32). Therefore, the glucosyl anion intermediate will consist of both deuterium labelled and unlabelled species. Subsequent anomerization of the anion and recombination with the aromatic intermediate should produce α-2,4-DNPG which has lost at least some of its deuterium label (Figure 36). Indeed, the ^{1}H-n.m.r. spectrum of isolated α-2,4-DNPG showed a resonance for the

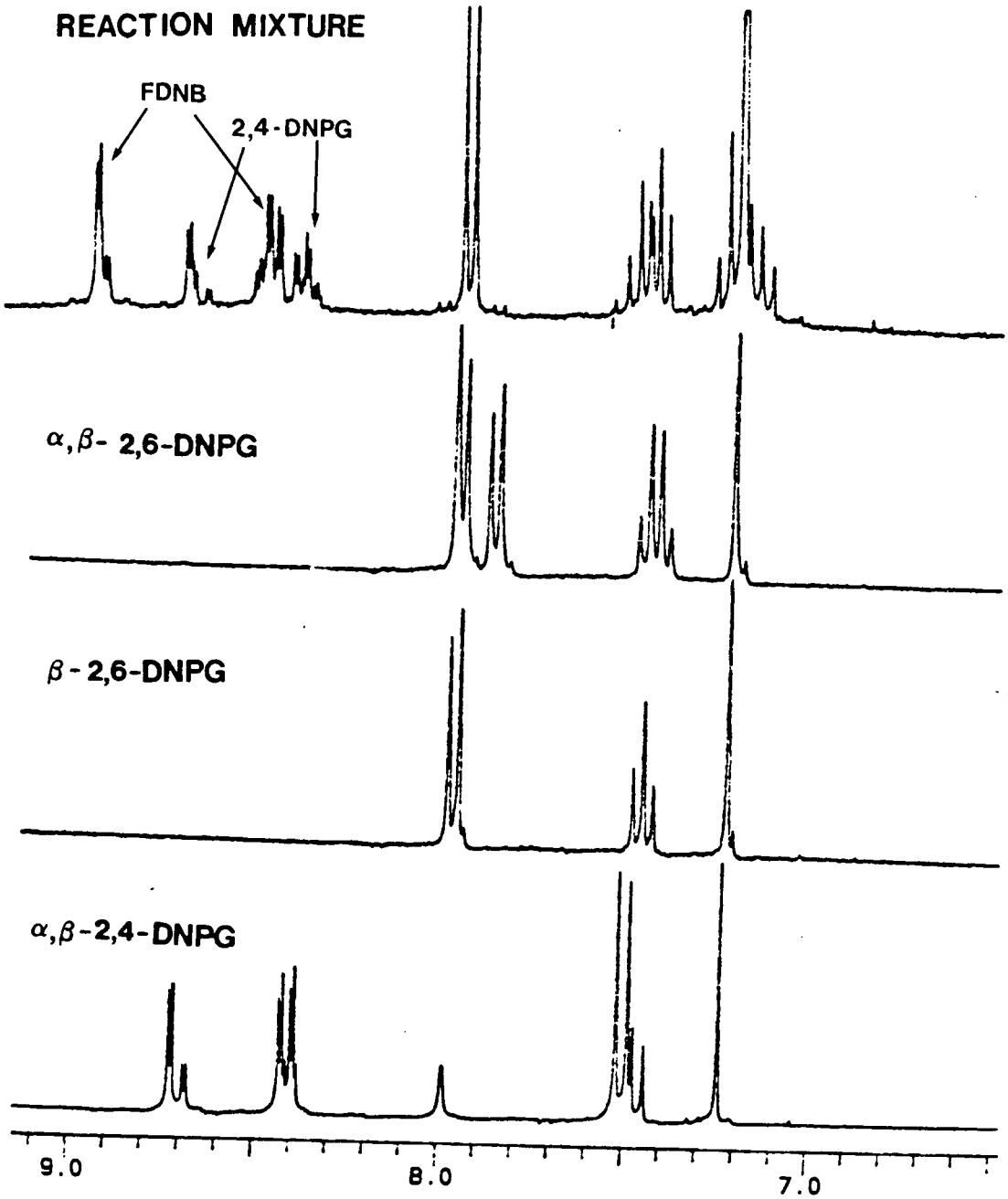


Figure 35: ^{1}H-N.m.r. for exchange via O(1)-aryl bond cleavage (experiment 1) ($CDCl_3$)

anomeric hydrogen and comparison between the integrated area of this peak and that for H(6) of the aromatic group indicated that the α-2,4-DNPG was only 50% deuterated (Figure 37).

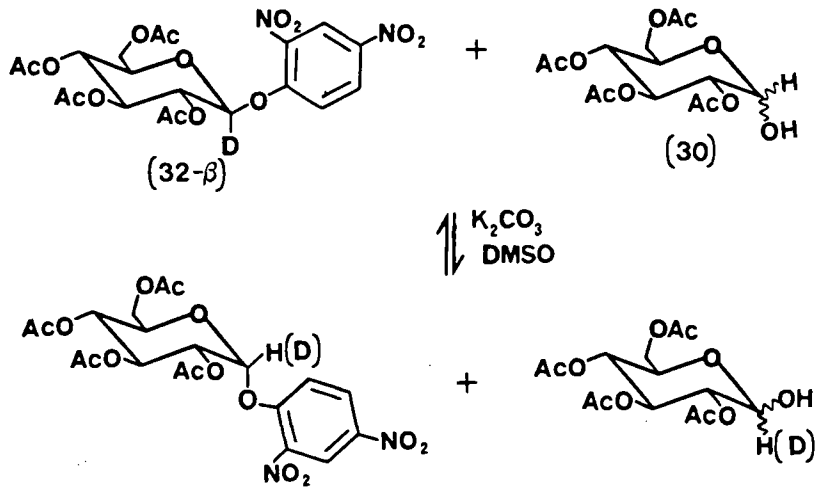


Figure 36: Exchange experiment for O(1)-aryl bond cleavage (experiment 2)

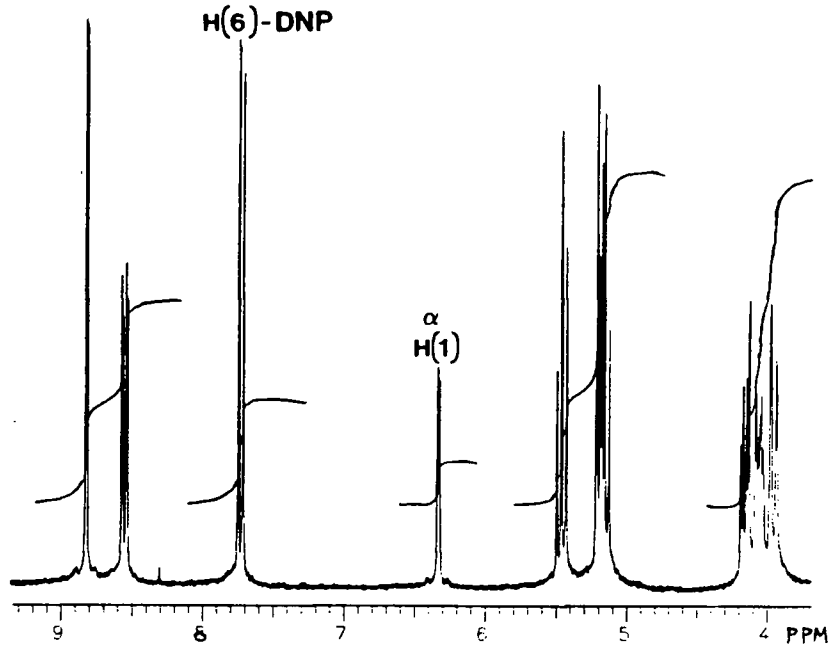


Figure 37: ^{1}H-N.m.r. for exchange via O(1)-aryl bond cleavage (experiment 2) (DMSO-d_6)

2.4 Kinetic Isotope Effects

Kinetic isotope effects (KIEs) were measured as another method to probe the site of bond cleavage for the anomerization reaction. (See appendix 1 for an outline of the theory behind KIEs.) Deuterium was substituted at the anomeric center for the per-O-acetylated DNPG (32) and the per-O-benzylated DNPG (35). For both of these substrates, the magnitude of the isotope effect should indicate which bond is cleaved at the anomeric center. If the reaction proceeds via C(1)-H(1) bond cleavage, then a primary KIE would be expected in which $^{kH}/_{kD}$ = 2-7. On the other hand, if the reaction goes via C(1)-O(1) bond cleavage, then a secondary KIE would be expected in which $^{kH}/_{kD}$ = 1.1-1.2. The absence of an isotope effect or an isotope effect of less than 1.1 would suggest an alternative mechanism, in which the bond being cleaved does not involve the anomeric center, or that some step in the mechanism other than bond cleavage is rate-limiting.

Anomerization by the method of van Boom[33] results in a heterogenous mixture since potassium carbonate is virtually insoluble in DMF. Reaction rates for this system may be measured by removing aliquots from the reaction mixture at specified times, performing a mini-work-up, and finally analyzing for products by ^{1}H-n.m.r. spectroscopy. This method does not ensure, however, that products are not lost during the work-up consequently affecting the measured rates. In order to monitor the reaction directly and therefore obtain more accurate kinetic data, it was necessary to find a homogeneous system. This investigation entailed a thorough survey of catalysts which effect the anomerization and

solvents in which both the sugar and the catalyst are soluble.

2.4.1 Survey of Solvents

The anomerization of the acetylated β-2,4-DNPG (29) was performed in each of the solvents listed in Table 3. The sugar was soluble in most relatively polar solvents but potassium carbonate was virtually insoluble in all of the solvents. However, in cases where anomerization did occur, it was presumed that the potassium carbonate must be at least sparingly soluble in order to catalyze the reaction.

2.4.2 Survey of Catalysts

In order to increase the solubility of the carbonate catalyst, the potassium cation was replaced by the tetraalkylammonium cation. Tetramethyl, tetraethyl and tetrabutylammonium carbonate were synthesized by bubbling carbon dioxide through a solution of the tetraalkylammonium hydroxide in water. The solution was saturated with carbon dioxide and the tetraalkylammonium carbonate salt was precipitated. Although the tetramethylammonium carbonate was the most hygroscopic, it was the easiest to purify by recrystallization and so was used most extensively for the anomerization. All three carbonates, however, were found to be at least somewhat soluble in DMSO, DMF, THF, and MeCN. Anomerization with the tetraalkylammonium salts in these solvents proceeded to the usual product mixture.

Other catalysts employed for the anomerization as part of the

Table 3: Investigation of solvents for anomerization of (29) with K_2CO_3. "Dec" indicates decomposition of (29)

Solvent	Dielectric constant $(\epsilon)^{50}$	K_2CO_3	β-2,4-DNPG	$\beta \rightarrow \alpha$
DMSO	49	sl. sol.	soluble	yes
DMF	36.7	sl. sol.	soluble	yes
Propylene carbonate	65.1		soluble	yes
Tetrahydrofuran	7.32		soluble	yes
Ethyl acetate	6.02		soluble	yes (dec)
Pyridine	12.3		soluble	yes
N-methyl-2-pyrrolidinone	32.0		soluble	yes
Chloroform	4.7		soluble	yes
t-Butanol	10.9		soluble	no
Toluene	2.38		soluble	no
Diethyl ether	4.34		soluble	no
Methanol	32.6		soluble	dec
1,4-Dioxane	2.21		soluble	yes (dec)
Dichloromethane	8.9		soluble	no
Acetone	20.7		soluble	yes (dec)
Acetonitrile	36.2		soluble	yes

search for a soluble system are listed in Table 4. The pK_a values listed are determined in water[51] and these values are known to be highly dependent on the solvent system in which they are determined.[52] Therefore, the values listed can only be used to compare reactivities of DNPG substrates and basicity of the catalyst as a rough approximation. Dissociation constants have been investigated in DMSO solutions and for charged anions, such as alcohols and carboxylic acids, the pK_a has been predicted to increase by as much as 7 pK_a units relative to values determined in water.[53,54] Therefore, it is presumed that the basicity of catalysts such as carbonate and phosphate, which are both highly charged delocalized anions, is considerably higher in DMSO than in water.

As can be seen in Table 4, the reaction does appear to depend on the basicity of the catalyst used since weakly acidic compounds such as $SO_4^=$ and ClO_4^- and neutral bases such as 1,8-bis(dimethylamino)-naphthalene do not catalyze the anomerization. This suggests a base catalyzed type mechanism involving proton abstraction at the anomeric carbon (case (i), in Figure 30). The anions which catalyze the reaction might be considered to be relatively nucleophilic, particularly in aprotic solvents such as DMSO. Alternatively, these anions may be basic enough to deprotonate DMSO and form the dimethylsulfinyl anion which also catalyzes the reaction. Therefore, the results found in Table 4 may also suggest a mechanism via nucleophilic aromatic substitution (case (iii) in Figure 30).

Table 4: Survey of catalysts for anomerization of β-2,4-DNPG (29). All were reactions performed in DMSO except where otherwise noted.

Catalysts	pK_a^{51} (H_2O)	β → α
Carbonates	10.33	Yes
K_2CO_3, Na_2CO_3, $BaCO_3$, $CsCO_3$, $(Me_4N)_2CO_3$, $(Et_4N)_2CO_3$, $(Bu_4N)_2CO_3$		
$KHCO_3$	6.35	Yes
Phosphates (tribasic)	12.67	
$Na_3PO_4 \cdot H_2O$		Yes
$(Me_4N)_3PO_4$		No
Phosphates (dibasic)	7.21	
$(Me_4N)_2HPO_4$		Yes
$KClO_4$	~-8	No
K_2SO_4	1.92	No
Amines		
tri-n-butylamine	~10	No
1,8-bis(dimethylamino)naphthalene	12.37	No
Et_4NBr		No
Dimethylsulfinyl anion	31.3	Yes
Sodium hydride	35	Yes
Potassium t-butoxide	19	Yes
n-Butyllithium in hexanes	>40	No

2.4.3 Measurement of Rates

Polarimetry is the most common method used to measure rates of anomerization reactions. Since anomerization results in an inversion of stereochemistry at C(1), the optical rotation of the reaction mixture changes dramatically as the concentrations of the α-anomer increase and the β-anomer decrease. A disadvantage of this method is that side reactions cannot be readily detected so the measured change in optical rotation may be due in part to the formation of side products. This was found to be the problem in the anomerization of β-2,4-DNPG (29).

DMSO was used as a solvent for this study since it is relatively inert under basic conditions and since bis(tetramethylammonium) carbonate is relatively soluble in DMSO. The reaction was monitored simultaneously by polarimetry and t.l.c. After several hours, the anomerization had proceeded to about 50% as judged by t.l.c. of the product mixture but the optical rotation of the solution indicated that the reaction had proceeded to only approximately 10% of the α-anomer. At this point the solution was dark brown, presumably due to the formation of 2,4-dinitrophenolate as shown by comparison of the u.v./visible spectrum of the reaction mixture and that of a standard solution of potassium 2,4-dinitrophenolate. Therefore, it was concluded that a side reaction involving the formation of the dinitrophenolate ion, perhaps due to the presence of water in the mixture, was affecting the optical rotation measurement. Since complete elimination of water from the polarimetry cell is difficult, polarimetry was found to be an unsuitable method to measure kinetic data.

Because of the potential problems with side reactions, ^{1}H-n.m.r. spectroscopy was found to be the most suited for kinetic measurements for several reasons. Firstly, the relative amounts of α- and β-anomers formed are easily determined by integration of either anomeric resonances or the resonances of H(6) of the DNP ring (Figures 27 and 28). Secondly, the reaction can be performed in DMSO-d_6, in which both the sugar and the catalyst are soluble, so that the reaction can be monitored directly in the n.m.r. tube. Also, anhydrous reaction mixtures can be maintained in sealed n.m.r. tubes. Finally, n.m.r. spectroscopy also allows easy detection of any side reactions as well as identification and quantification of products from these reactions.

Sample ^{1}H-n.m.r. spectra taken over the course of the reaction for anomerization of β-2,4-DNPG (29) and [1-^{2}H]-2,4-DNPG (32) are shown in Figures 38 and 39. Integration values in all spectra were standardized to the sum of the -OAc peak at δ 2.0-2.4 (not shown) for which the integral did not change during the reaction. In the spectra for [1-^{2}H]-DNPG, the signal at δ 6.3 was not due to the anomeric resonance of α-2,4-DNPG but was the signal for H(6) of the 2,4-dinitro-phenolate anion which was formed to a small extent.

Table 5 reports some typical results for the anomerization of (29) and (32). The data provide an example of the raw integral values obtained and the calculated amounts of β-anomer remaining at the noted intervals of the reaction. The rates were determined by plotting the integration value for the β-anomer versus time (i.e., the raw data) and by plotting the calculated $\log(\alpha_t-\alpha_e)$ versus time[13] to obtain a pseudo-first-order rate constant k (α_e = amount of β-DNPG present at

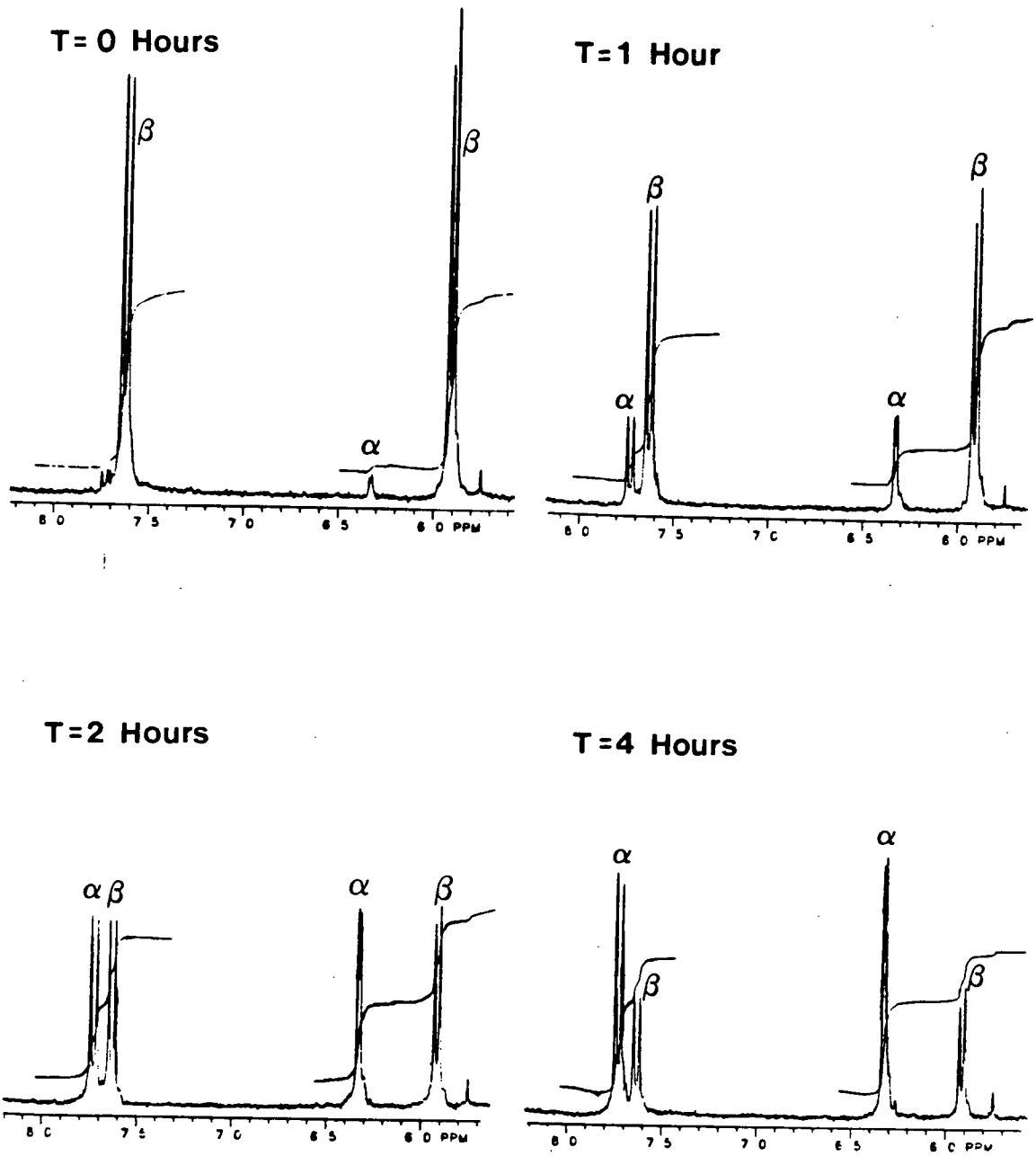


Figure 38: ^{1}H-N.m.r. for KIE measurements: non-deuterated substrate (29) (DMSO-d_6)

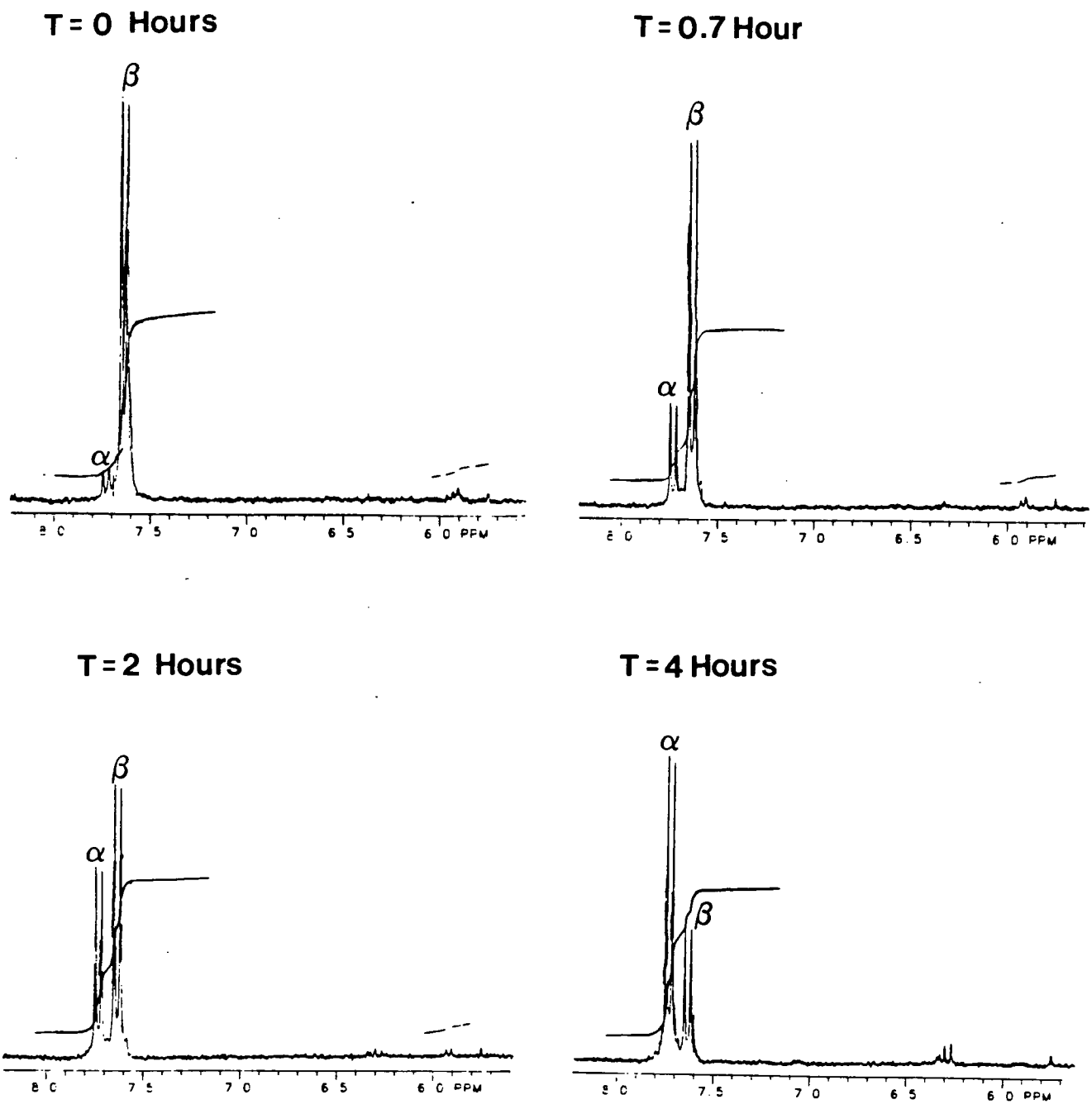


Figure 39: ^{1}H-N.m.r. for KIE measurements: deuterated substrate (32) (DMSO-d_6)

Table 5: Kinetic data for KIE measurement. Data for one trial only are given. $[(Me_4N)_2CO_3]$ = 0.0182 M (DMSO-d_6). Temperature = 25°.

1-H-β-D-DNPG (29), [DNPG] = 0.0121 M (DMSO-d_6)

Integral Area for H(6)-DNP for β-anomer	β-DNPG (mmol x 10^3)	$\log(\alpha_t - \alpha_e)$	time (min)
5.94	6.78	-2.27	6
4.93	6.08	-2.33	20
4.55	5.57	-2.38	40
3.82	4.78	-2.48	60
3.32	4.13	-2.57	80
2.72	3.43	-2.70	100
2.58	3.21	-2.76	120
2.29	2.89	-2.84	140
2.03	2.53	-2.97	160
1.86	2.35	-3.05	180
1.63	2.04	-3.28	240
1.40	1.76	-3.63	284

[1-^{2}H]-β-D-DNPG (32), [DNPG] = 0.0127 M (DMSO-d_6)

Integral Area for H(6)-DNP for β-anomer	β-DNPG (mmol x 10^3)	$\log(\alpha_t - \alpha_e)$	time (min)
6.20	7.65	-2.21	5
5.17	6.93	-2.27	20
4.60	6.18	-2.33	40
3.95	5.38	-2.41	60
3.55	4.77	-2.49	75
3.03	4.08	-2.59	100
2.60	3.58	-2.69	120
2.65	3.59	-2.69	140
2.36	3.17	-2.78	160
1.80	2.50	-3.01	200
1.68	2.26	-3.13	220
1.45	1.92	-3.40	260
1.40	1.93	-3.42	280

equilibrium, α_t = amount of β-2,4-DNPG present at time t, Figure 40). The KIE for anomerization of β-2,4-DNPG from both methods was calculated to be 1.09 ± 0.06. The results obtained from four repeated determinations are summarized in Table 6. The relatively small isotope effect might be representative of a secondary KIE but more importantly, it is too small to be a primary KIE. This suggests that the reaction does not proceed via proton abstraction at C(1).

The per-O-benzylated DNPG (31) anomerized much more slowly than (29) and the isotope effect could not be determined due to a large amount of decompositon to the 2,4-dinitrophenolate anion which complicated the ^{1}H-n.m.r. spectrum. The isotope effect for the production of the dinitrophenolate anion, however, was measured to be 1.09. This is in accordance with the KIE for the anomerization of (29) and therefore, the anion may be formed by a similar mechanism.

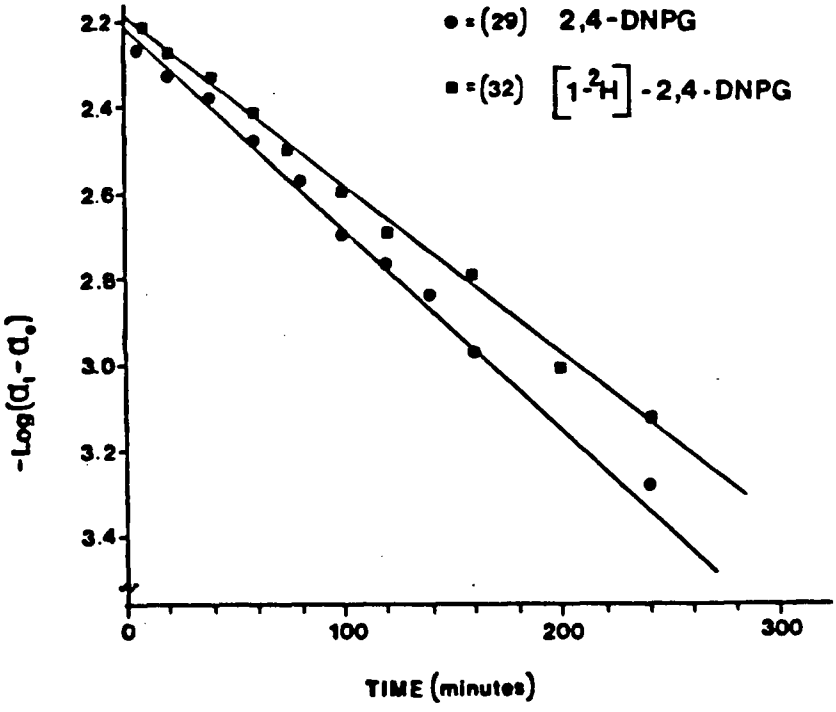


Figure 40: KIE for anomerization of β-2,4-DNPG

Table 6: Summary of results: Pseudo-first-order rate constants calculated for the disappearance of β-2,4-DNPG (29) and (32)

kH 1-H-β-2,4-DNPG (min^{-1})	Correlation Coefficient	kD [1-^{2}H]-β-2,4-DNPG (min^{-1})	Correlation Coefficient
-4.81×10^{-3}	-.9945	-4.47×10^{-3}	-.9917
-4.96×10^{-3}	-.9980	-4.47×10^{-3}	-.9934
-4.87×10^{-3}	-.9895	-4.65×10^{-3}	-.9982
-4.72×10^{-3}	-.9982	-4.15×10^{-3}	-.9975

Average kH = $-4.84 \times 10^{-3} \pm 1.27 \times 10^{-4}$

Average kD = $-4.44 \times 10^{-3} \pm 1.16 \times 10^{-4}$

KIE = kH/kD = 1.09 ± 0.06

Interestingly, under these anhydrous conditions, the reaction mixture takes on a deep violet color which eventually becomes red-orange (1 hour) and then very dark brown (8-10 hours). The dark brown color was attributed to the 2,4-dinitrophenolate anion formed by a side reaction. The presence of the anion was apparent in the ^{1}H-n.m.r. spectrum (H(6) δ 6.3). Attempts to identify the species causing the deep purple color were unsuccessful by ^{1}H-n.m.r. spectroscopy since the spectrum showed no distinct anomalous peaks. The u.v./visible spectrum, however, showed an unusual charge transfer absorption at 570 nm. (DNPG λ_{max} = 275 nm, potassium 2,4-dinitrophenolate λ_{max} = 429 nm in DMSO). Furthermore, a mixture of β-2,4-DNPG in DMSO and a small amount of dimethylsulfinyl (dimsyl) anion, which may be present in the anomerization mixture due to the high basicity of carbonate in DMSO, showed the same purple color and an absorption at 574 nm. The reaction of dimsyl anion with FDNB in DMSO also produced the purple color and an absorption at 548 nm (along with absorptions at 642 nm, 436 nm, and 370 nm) (Figure 41). The purple color of this solution disappeared within 2-3 minutes producing a brown solution whereas the purple color of the DNPG solution persisted for several hours before decomposing to the brown color. The probable origin of this unusual coloration is proposed in the Discussion section.

2.5 Remote Substituent Effects

Substitution of electronegative substituents around the sugar ring is known to cause pronounced effects on the rates of glycoside hydroly-

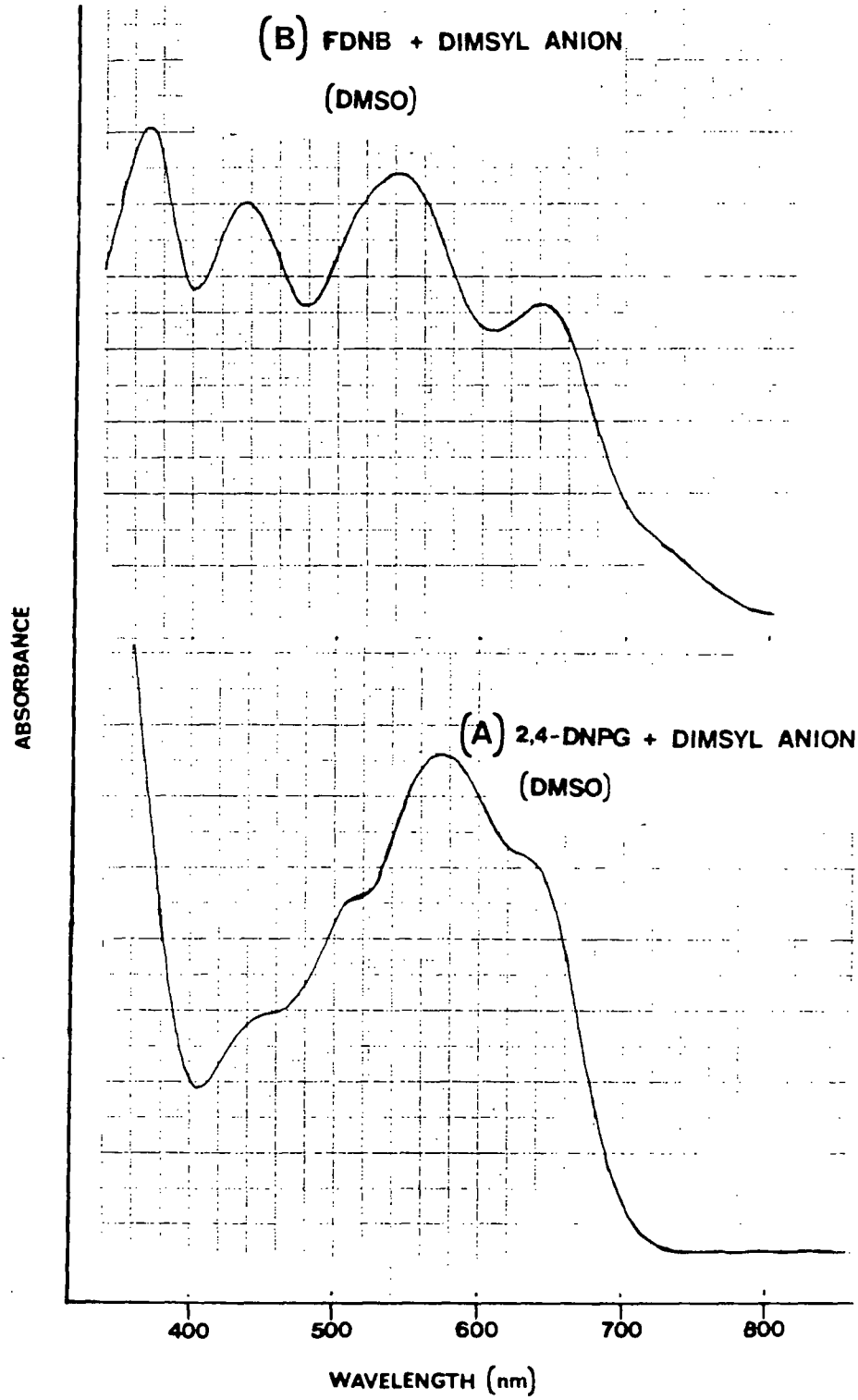


Figure 41: U.v./visible absorbance spectra for anomerization reaction (A) 2,4-DNPG with dimsyl anion and (B) reaction of FDNB with dimsyl anion

sis.[55,56] For example, it was recently shown that substitution of fluorine for hydroxyls at the 2,3,4, or 6-position of α-D-glucopyranosyl phosphate lowered the rates of acid catalyzed hydrolysis. The electronegative fluorine destabililzes the electron deficient intermediate and slows the reaction. In this series of deoxyfluoro sugars, the 2-deoxy-2-fluoro and the 4-deoxy-4-fluoro derivatives showed the most dramatic effect. Therefore, it was believed that similar effects might be observed with the anomerization of dinitrophenyl glucopyranosides if the reaction proceeds via an oxocarbonium ion formed by cleavage of the C(1)-O(1) bond. Alternatively, the opposite effect might be observed if the reaction involves a carbanion intermediate formed by a mechanism involving proton abstraction at the reaction center. In this case, an electronegative substituent such as fluorine will stabilize the anionic intermediate and thereby increase the rate of anomerization.

To probe the effect of substituents around the sugar ring, pseudo-first-order rate constants (k) for the 4-deoxy-4-fluoro, 4-deoxy, and 6-deoxy DNPG (40, 44, and 49, respectively) along with the parent sugar (29), were measured as described previously. The results are summarized in Table 7 (experiment 1; plot shown in Figure 42). As can be seen, the 4-deoxy-4-fluoro DNPG (40) showed a slight rate enhancement over the rate of anomerization of (29), whereas the 4-deoxy DNPG (44) anomerized an order of magnitude slower than (29). The rate measured for the 6-deoxy DNPG (49) is similar to the rate measured for (44) since this also showed a slight decrease in rate compared to (29).

In addition, a separate experiment was performed in which the rate of anomerization of 2-deoxy-2-fluoro DNPG (39) was compared to that of

Table 7: Remote substituent effects of anomerization: Calculated pseudo-first-order rate constants for the disappearance of β-2,4-DNPG derivatives. Temperature = 25°

Experiment 1: $[(Me_4N)_2CO_3]$ = 0.0167 M

Substrate	Concentration (M)	$k \times 10^3$ (min^{-1})	Correlation Coefficient
4-Deoxy-4-fluoro (40)	0.0138	-3.82	-.9923
4-Deoxy (44)	0.0150	-0.456	-.9952
6-Deoxy (49)	0.0153	-1.45	-.9897
DNPG (29)	0.0136	-3.63	-.9901

Experiment 2: $[(Me_4N)_2CO_3]$ = 0.0182 M

Substrate	Concentration (M)	$k \times 10^3$ (min^{-1})	Correlation Coefficient
2-Deoxy-2-fluoro (39)	0.0121	-3.56	-.9881
DNPG (29)	0.0134	-3.97	-.9969

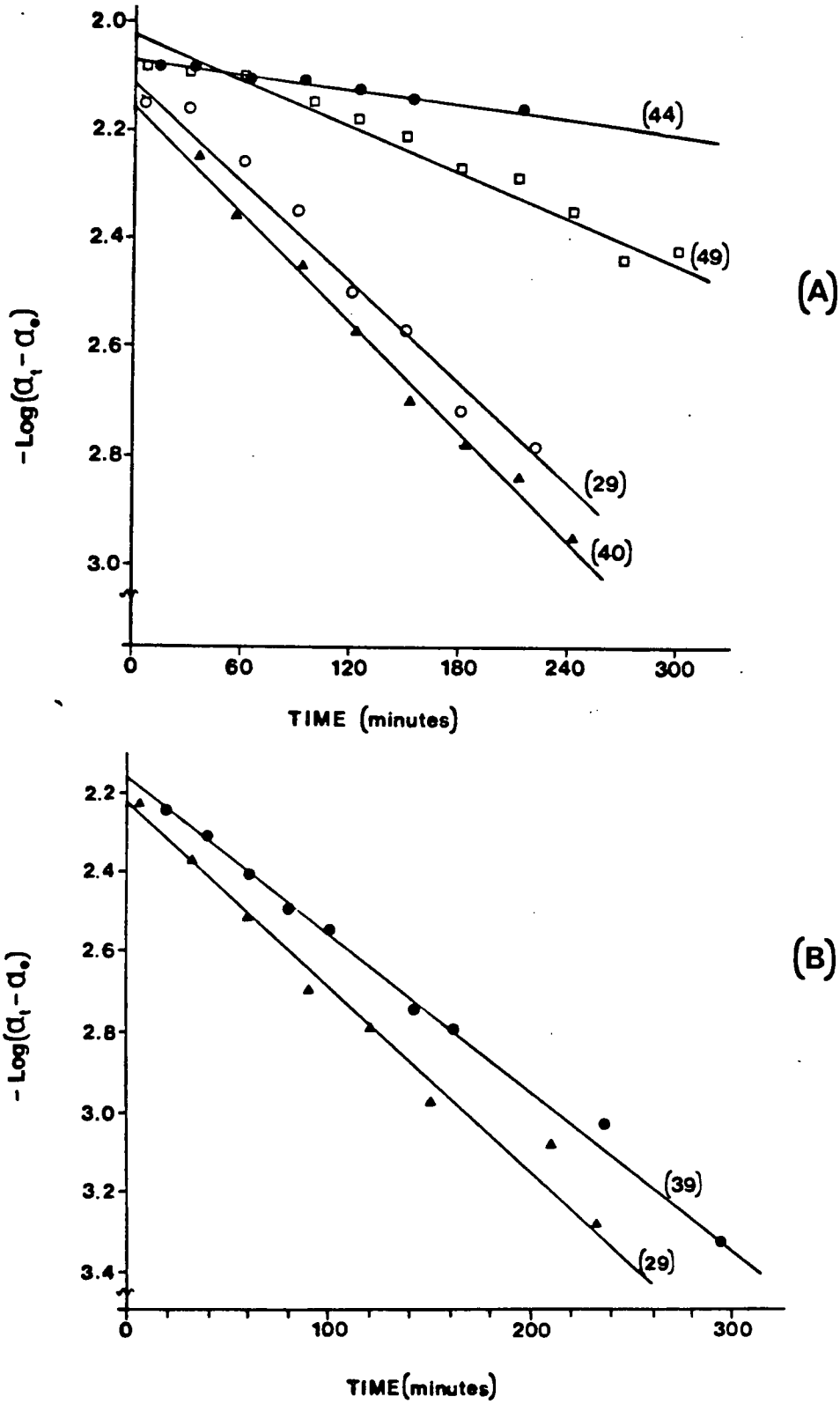


Figure 42: Reaction rates measured for remote substituent effects (A) experiment 1; (B) experiment 2.

(29) (experiment 2). Similar to (40), the 2-deoxy-2-fluoro DNPG reacted at nearly the same rate as the parent sugar (29) (Figure 42). Mechanistic implications of these results will be considered in the Discussion section.

DISCUSSION

Four mechanisms can be proposed for the carbonate catalyzed anomerization of protected 2,4-dinitrophenyl β-D-glucopyranosides. Briefly, these are (i) proton abstraction at C(1); (ii) phenolate abstraction; (iii) proton abstraction at C(2); and (iv) nucleophilic attack on the aromatic ring.

Mechanism (i) (Figure 43) was suggested by Ferrier[57] as an unusual yet viable pathway to the α-anomer. The mechanism involves base catalyzed removal of the anomeric proton and formation of a carbanion intermediate (63). Although the pK_a of the anomeric proton would be expected to be high (>20), the acidity of H(1) may be increased by the presence of the strongly electron-withdrawing dinitrophenyl group which

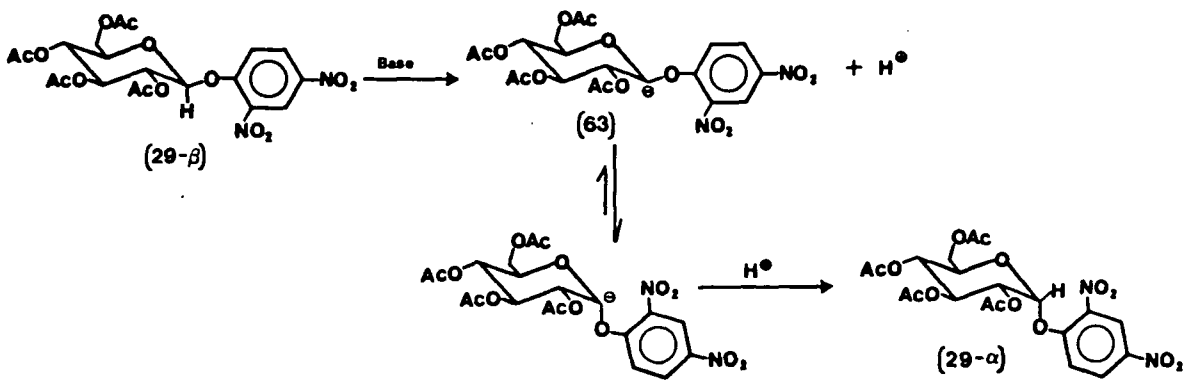


Figure 43: Mechanism (i). Base catalyzed proton abstraction

would facilitate this mechanism. Furthermore, the carbonate anion is probably a much stronger base in DMSO[53] than in water (pK_a = 10.33) and may be strong enough to remove the relatively non-acidic H(1).

The total lack of H/D exchange at C(1) when the anomerization of β-2,4-DNPG (29) is performed in the presence of a deuteron source, or when the anomerization of [1-^{2}H]-β-2,4-DNPG (32) is performed in the presence of a proton source, is strong evidence against mechanism (i). This absence of H/D exchange might be rationalized through the formation of an intimate ion pair intermediate in which the proton is removed then re-attached without ever leaving the solvent cage surrounding the reaction center. This explanation is unlikely, however, since an ion pair intermediate would be expected to exchange to at least a small extent.

The measurement of a small isotope effect ($^{kH}/_{kD}$ = 1.09) is also evidence against mechanism (i). Proton abstraction at C(1) would give a primary isotope effect in the order of $^{kH}/_{kD}$ = 2-7 if bond cleavage is rate-determining, as would be expected. Although primary isotope effects can be suppressed by factors such as proton tunneling or non-linearity in the transition state,[58] these factors can be precluded in light of the absence of H/D exchange.

Mechanism (ii) (Figure 44) involves the removal of the phenolate anion and formation of an oxocarbonium ion intermediate (64) which may be further stabilized by the participation of the C(2) acetoxy group and the formation of an acetoxonium ion (65). This mechanism initially was considered to be the most likely route for several reasons. Early experiments in this study showed the release of the dinitrophenolate anion by the orange color, t.l.c., and the u.v./visible spectrum of the

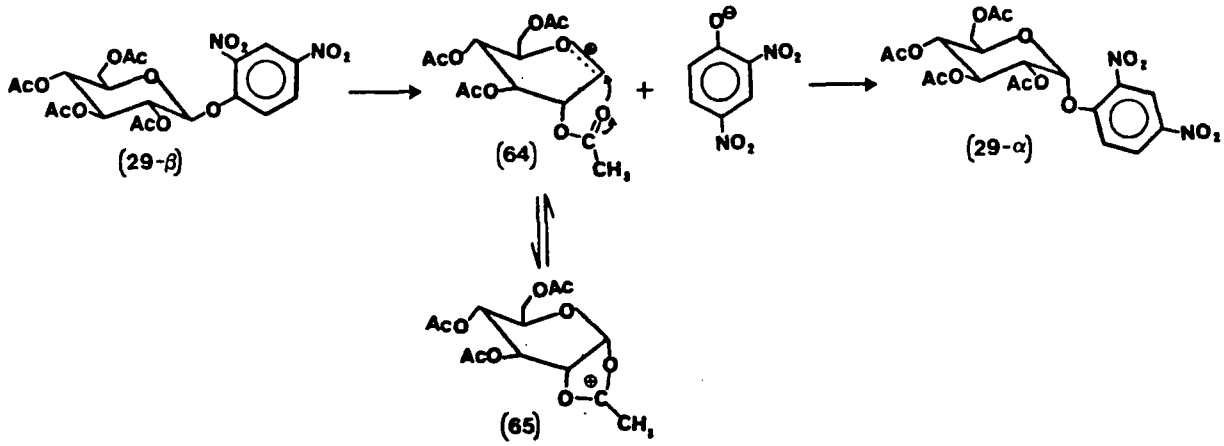


Figure 44: Mechanism (ii). Phenolate abstraction and formation of an oxocarbonium ion intermediate

reaction mixture. The measurement of a small isotope effect and lack of H/D exchange are in accord with mechanism (ii). Finally, van Boom et. al.[33] reported a 70 fold decrease in the rate of anomerization for the non-participating tetra-O-benzyl DNPG (31), suggesting formation of the acetoxonium ion which assists phenolate departure. Our study confirmed this result. There is strong evidence, however, which may be used to discount mechanism (ii). Firstly, only substrates with aglycons having pK_a values lower than 4.00 (2,4-DNPG (29), 2,6-DNPG (60), and 2,6-dichloro-4-nitrophenyl glucopyranoside (59)) were found to anomerize, although to a small extent, under these conditions. This dramatic influence of electron-withdrawing substituents on the phenyl ring is surprising since studies on the acid catalyzed hydrolysis of glycopyra-

nosides (also considered to occur via an oxocarbonium ion mechanism) show only a small dependence of rate on the electronegativity of the aglycon.[59] Secondly, phenolate exchange experiments in which the 2,6-DNPG (60) was anomerized in the presence of added 2,4-dinitrophenolate indicated that no exchange of the phenolate substituents occurred. Similarly, no exchange was observed when 2,4-DNPG (29) was anomerized in the presence of added 2,6-dinitrophenolate. Thirdly, the rate of anomerization measured for the 2-deoxy-2-fluoro DNPG (39) is in the same order as the rate of normal DNPG (29). It is unlikely, therefore, that the C(2)-acetoxy group participates in this mechanism as suggested earlier, assuming there is no change in mechanism for the two substrates. Furthermore, substituent effects at C(2), C(4) and C(6) provide evidence against the formation of an oxocarbonium ion since the relative rates of the 4-deoxy and the 6-deoxy derivatives (44 and 49, respectively) are lower than that for the parent sugar (29), but the 4-deoxy-4-fluoro derivative (40), like the 2-deoxy-2-fluoro derivative (39), anomerized as fast as (29). If an oxocarbonium ion is involved in the mechanism, the electronegative fluorine substituent should destabilize the transition state leading to the intermediate, and slow the reaction. Finally, it is difficult to find a role for the carbonate catalyst in such a mechanism.

Mechanism (iii) (Figure 45) also involves the removal of the phenolate anion via proton abstraction at C(2) forming a glucal intermediate (62) or an open chain intermediate (66). This mechanism is supported by the small isotope effect which, in this case, would be a β-secondary isotope effect. Also, the relative rates measured for the

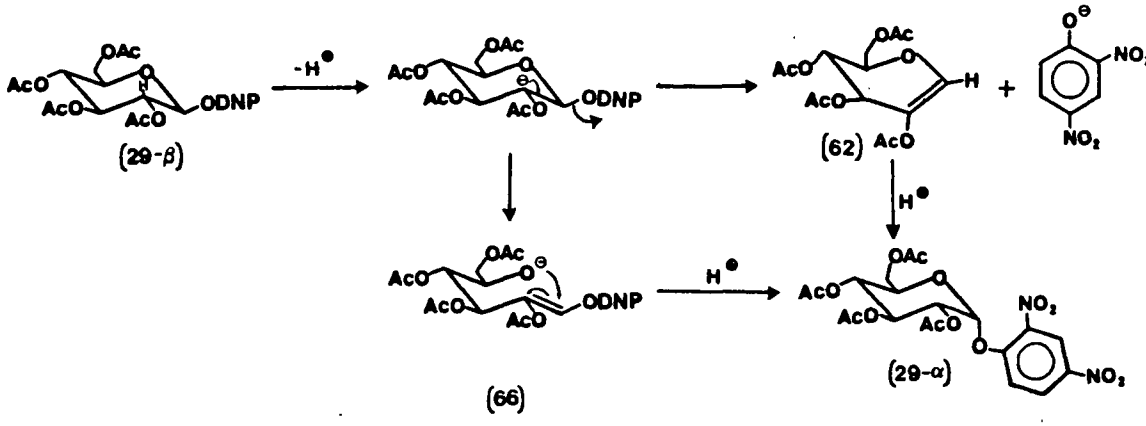


Figure 45: Mechanism (iii). Proton abstraction at C(2) and formation of a glucal intermediate

2-deoxy-2-fluoro (39) and the 4-deoxy-4-fluoro (40) derivatives favor the mechanism since the electronegative fluorine substituents would stabilize the carbanion intermediate.

Against this mechanism, however, is the complete lack of exchange between phenolate substituents as explained for mechanism (ii) and which would also be expected in this case. In addition, there was no H/D exchange at C(2) when the anomerization of (29) was performed in the presence of a deuteron source, nor was there epimerization to the D-mannoside derivative. Finally, 2,3,4,6-tetra-O-acetyl-glucal (62), a possible intermediate in this mechanism, did not react with potassium 2,4-dinitrophenolate to form the α- or β-glucopyranoside. Therefore,

mechanism (iii) can also be discounted as a reasonable pathway for anomerization.

Mechanism (iv) (Figure 46) is perhaps the most unusual pathway and initially was not considered in this study. The mechanism proceeds via nucleophilic aromatic substitution on the phenyl ring and formation of the glycosyl oxyanion (68) which can anomerize via the open chain

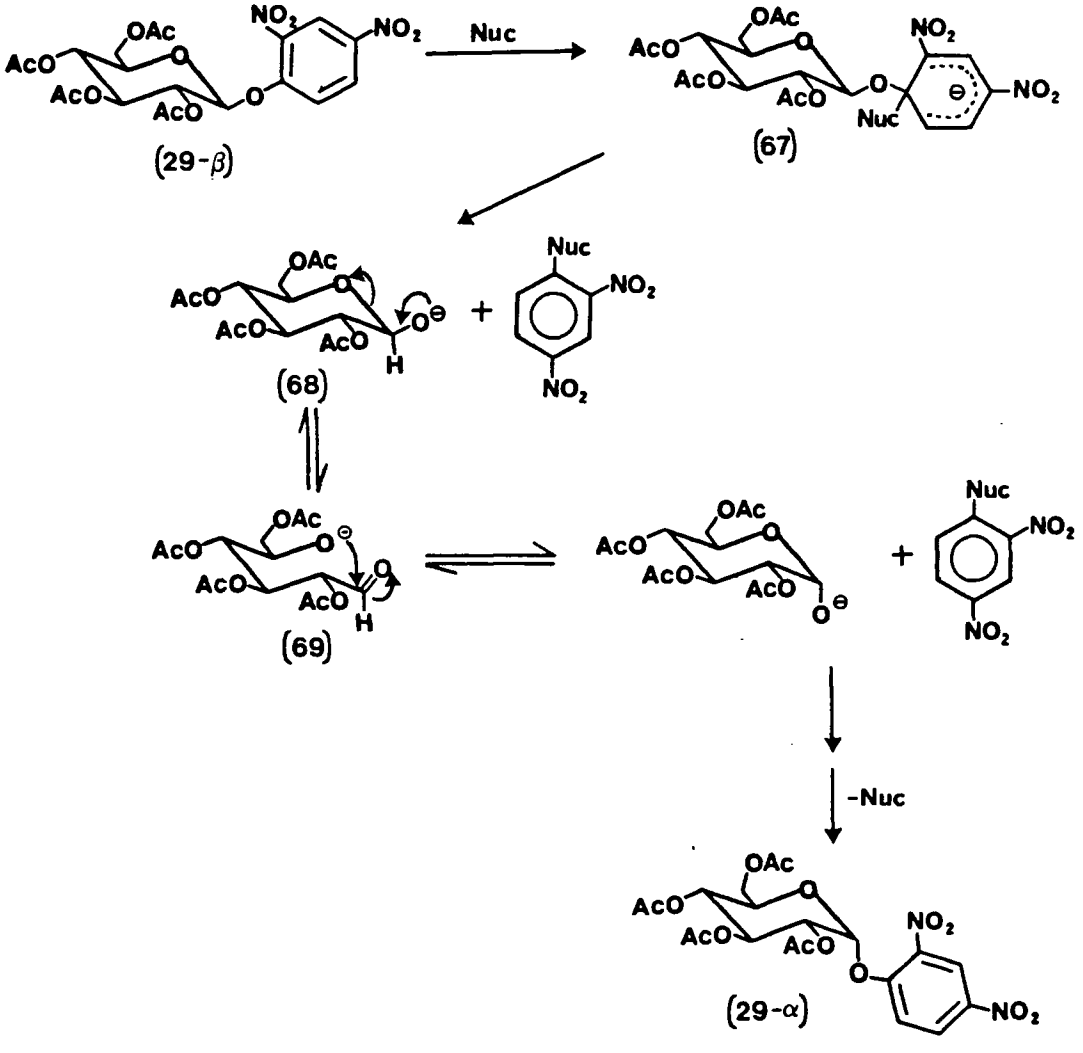


Figure 46: Mechanism (iv). Aromatic nucleophilic substitution. Nuc = nucleophile ($CO_3^=$ or $CH_3SOCH_2^-$)

aldehyde form (69) of the sugar. The anomerization process itself is mechanistically similar, therefore, to the acid/base catalyzed mutarotation of D-glucose. In addition, this mechanism involves the formation of a highly delocalized charged intermediate (67), which is known as a Meisenheimer intermediate. The occurrence of Meisenheimer intermediates is common for nucleophilic reactions involving highly activated aromatic compounds.[60] For example, nucleophilic aromatic substitution reactions of 1-substituted 2,4-dinitrobenzenes in DMSO are well characterized.[61] In a study of the reaction of the 1-halo-substituted compounds with methoxide in methanolic DMSO (Figure 47), the reaction rates followed the expected order: fluoro >> chloro > bromo > iodo. In each case, the product was 2,4-dinitroanisole which was isolated quantitatively and characterized. A particularly interesting observation in this investigation was the color change which occurred during the course of the

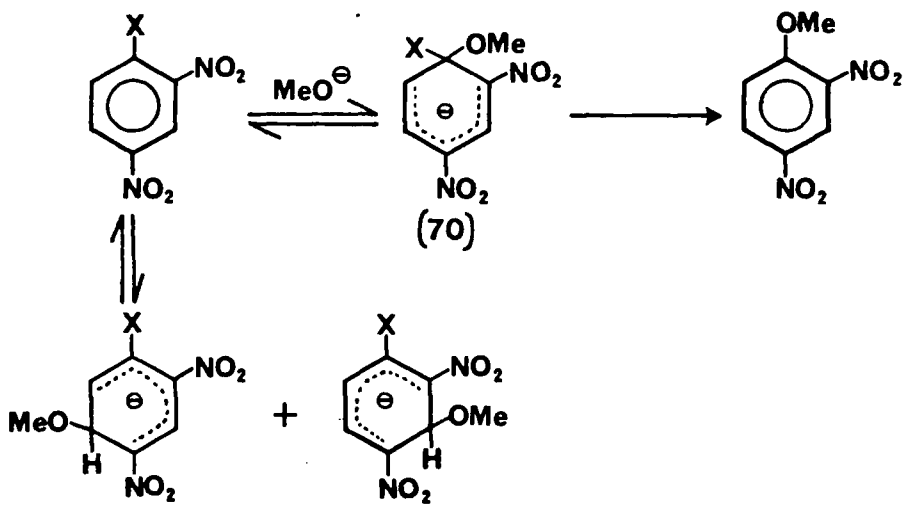


Figure 47: Meisenheimer intermediate (70) formed by the reaction of 1-substituted 2,4-dinitrobenzenes and methoxide in DMSO (X = F, Cl, Br, I)

reaction. In a solvent mixture containing >55 mol% DMSO, the mixture instantly became purple upon addition of the substrate and then gradually became more red to brown. The u.v./visible spectrum of this colored solution showed an absorption at 295 nm due to the product, 2,4-dinitroanisole, but also a large absorbance at 500 nm. This new absorbance was attributed to the formation of the Meisenheimer complex (70) shown in Figure 47. Nucleophilic attack at the 3- and the 5-positions of these highly activated benzene rings also has been reported.[62] The reaction of 1-chloro-2,4,6-trinitrobenzene with sodium hydroxide in DMSO-d_6/D_2O solutions showed rapid hydrogen exchange of the aromatic protons by ^{1}H-n.m.r. spectroscopy and isolated starting material after 30 seconds reaction time showed full exchange of these protons by mass spectrometry.

Nucleophilic aromatic substitution reactions are rare in aryl glucopyranoside chemistry. In fact, only one example was found in the literature which reports a mechanism similar to the mechanism proposed here. This is the base catalyzed hydrolysis of 4-nitrophenyl α-D-glucopyranoside (21) which was described previously in the Introduction.[28] In short, the hydrolysis involved base catalyzed O(1) to O(2) migration of the 4-nitrophenyl group via an intramolecular, nucleophilic, aromatic substitution mechanism (Figure 17). The evidence presented here suggests a similar mechanism (but with no intramolecular component) for the anomerization of protected 2,4-dinitrophenyl glucopyranosides.

All of the evidence used in supporting or opposing the previous three mechanisms supports or at least does not discount mechanism (iv). The absence of H/D exchange at C(1), the absence of phenolate exchange,

and the measurement of a small isotope effect are consistent with this mechanism, but by no means prove it. The marked influence of electron-withdrawing groups on the ortho- and para- positions of the phenyl ring supports this mechanism since substitution at these positions has the largest effect on the reactivity of the aromatic group. The relatively slow reaction rate for the 2,6-dinitrophenyl and the 2,6-dichloro-4-nitrophenyl glucopyranosides (60 and 59, respectively) may be explained by the increase in steric bulk at the reaction center when the aryl group is substituted at both the 2- and the 6-positions. The unreactivity of the 2,4-dinitrophenyl 1-thio-glucopyranoside (61) is consistent with the observed general trend that 1-O-substituted compounds are more reactive to nucleophilic aromatic substitution than are 1-S-substituted compounds.[63] The reaction rates measured for the 4-deoxy-4-fluoro (40) and the 2-deoxy-2-fluoro (39) derivatives are consistent with this mechanism since the electronegative fluorine substituents will stabilize the glycosyl oxyanion intermediate. Furthermore, the 4-deoxy (44) and the 6-deoxy (49) compounds showed the expected decrease in rate resulting from the absence of electron-withdrawing groups at C(4) or C(6). The large decrease in rate observed for 2,3,4,6-tetra-O-benzyl DNPG (31) may be due to the greater bulk of the benzyl groups or possibly to slower anomerization of the tetra-O-benzyl glycosyl oxyanion. This is consistent with other findings from this laboratory involving the Wittig reaction of 2,3,4,6-tetra-O-benzyl-D-glucopyranose (36) with various ylides in which (36) was found to react very slowly and only in the presence of very strong base. The mechanism of the Wittig reaction is similar to mechanism (iv) since it also proceeds via the open chain form

of the sugar. It is possible, therefore, that the slowness of both reactions may be due to the relative inability of the benzyl group to stabilize the oxyanion compared to the acetate group which is considerably more electron-withdrawing.

The most conclusive evidence for mechanism (iv) comes from the exchange reactions where β-2,6-DNPG (60) was anomerized in the presence of FDNB, and where [1-^{2}H]-β-2,4-DNPG (32) was anomerized in the presence of 2,3,4,6-tetra-O-acetyl-D-glucopyranose (30). In the first experiment, the 2,6-DNPG (60) reacted only to a small extent but the ^{1}H-n.m.r. spectrum (Figure 35) of the product mixture showed that α- and β-2,4-DNPG (29) were formed, yet no α-2,6-DNPG was formed. In the second experiment, [1-^{2}H]-2,4-DNPG (32) lost 50% of its deuterium label upon anomerization. This second experiment conclusively demonstrates that the oxyanion of 2,3,4,6-tetra-O-acetyl-D-glucopyranose must be an intermediate in the reaction mechanism.

Additional evidence for mechanism (iv) are the color changes observed during the anomerization which are characteristic of those found in the formation of the previously described Meisenheimer complexes. U.v./visible absorption spectra of the purple reaction mixture resulting from mixing β-2,4-DNPG (29) with dimsyl anion or with bis(tetramethyl-ammonium) carbonate in DMSO showed an absorbance at about 574 nm. As mentioned earlier, nucleophilic aromatic substitution reactions of 1-substituted 2,4-dinitrobenzenes also exhibit this purple color and the u.v./visible spectrum showed an absorbance at 500 nm.[61] Although it is unclear whether the carbonate or the dimsyl anion acts as a nucleophile in the anomerization reaction, it seems more likely that

it is the dimsyl anion since this has been shown previously to be nucleophilic in aromatic substitution reactions.[64] In reactions where the anomerization was performed with the carbonate catalyst in DMSO, the carbonate must be basic enough to deprotonate DMSO and form the dimsyl anion <u>in situ</u>. Predictions of the basicity of highly charged anions in polar aprotic solvents suggest that carbonate may be much more basic in DMSO than in water,[53] and therefore <u>in situ</u> formation of the dimsyl anion is not unreasonable. The alternative, involving the carbonate anion acting as the nucleophile, is less likely but possible in an aprotic solvent such as DMSO. The formation of the dinitrophenolate anion which is apparent from the ^{1}H-n.m.r. spectral data of the anomerization reaction mixture (Figure 39), can be rationalized by the action of carbonate as a nucleophile (Figure 48). The intermediate (71) presumably would eliminate carbon dioxide quite readily forming the

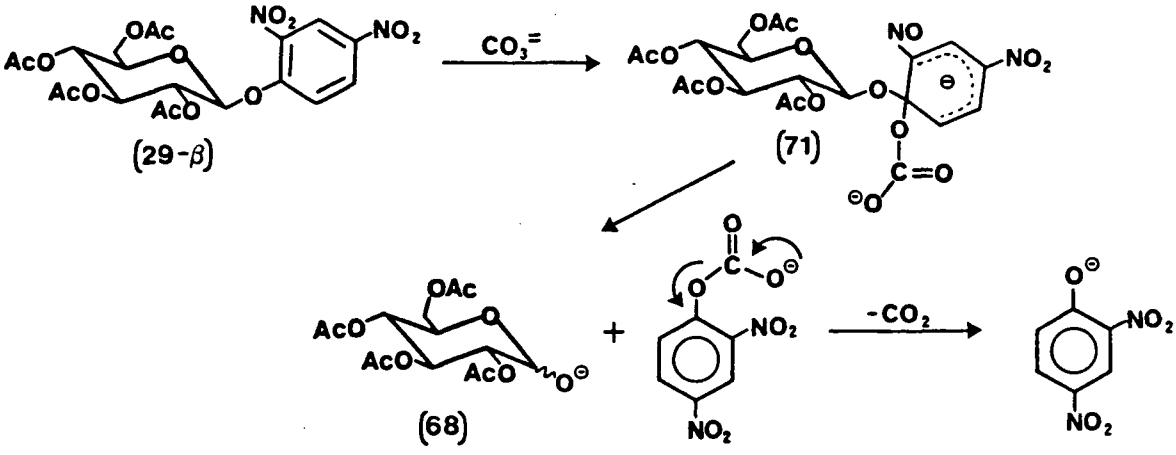


Figure 48: Formation of the 2,4-dinitrophenolate anion during anomerization of (29)

2,4-dinitrophenolate anion. Although the resultant protected glucose residue was not apparent by t.l.c. or ^{1}H-n.m.r. spectra of the reaction mixture, it is believed that the acetate groups on the glucosyl oxyanion (68) could easily rearrange and cause decomposition of the glucose residue to other sugars. Indeed, this type of decomposition was observed with the previously described base catalyzed hydrolysis of 4-nitrophenyl α-D-glucopyranoside in which saccharinic acids were the final carbohydrate products of the reaction.

Finally, the mechanism proposed here for the anomerization of β-2,4-DNPG (29) is consistent with the early reports by Lindberg on the anomerization of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (11) using alkali in pyridine.[32] In this case and in accordance with mechanism (iv), pyridine acts as a nucleophile and attacks the carbonyl group of the anomeric acetate, forming the highly reactive acylating reagent (72) shown in Figure 49. Anomerization of the resulting glucosyl anion (68) and reacylation affords the thermodynamically favored α-pentaacetate. Lindberg reported that the anomerization of the β-pentaacetate (11) is 6-7 times slower when the reaction is performed using alkali in ether-dioxane in place of pyridine. This is not surprising since these conditions are more likely to cause deacetylation at the anomeric center via nucleophilic attack of hydroxide forming the glucosyl residue (68) and the acetate anion. Deacetylation and relatively low yields were found in Wolfrom and Hudsted's early study on anomerization of fully acetylated sugars under basic conditions.[31]

Further work towards proving mechanism (iv) should include synthesizing the aromatic intermediates thought to be formed in the reaction

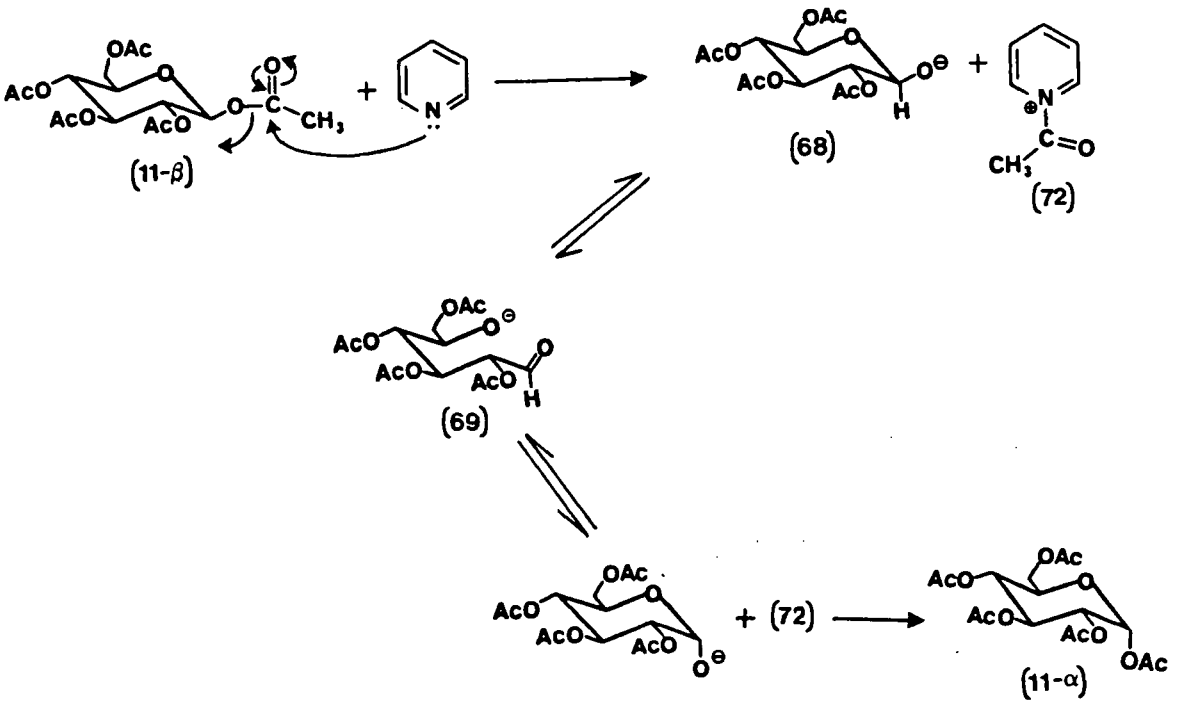


Figure 49: Pyridine catalyzed anomerization of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose

by a separate route, then characterization of these intermediates (by u.v./visible and ^{1}H-n.m.r. spectroscopy) and finally, determining that they are present in the anomerization mixture. It should also be determined if these intermediates react with 2,3,4,6-tetra-O-acetyl-glucopyranose (30) to form the expected equilibrium mixture of the α- and β- 2,4-DNPG. Measurements of rates of this glycosidation reaction and comparison with measured rates of anomerization would be required to prove the kinetic competence of these species as possible intermediates in the anomerization.

MATERIALS AND METHODS

1. Synthesis

1.1 General Procedures and Materials

Melting points (m.p.) were determined with a Bristoline melting-point apparatus and are uncorrected.

^{1}H-nuclear magnetic resonance (n.m.r.) spectra were recorded with a 300 MHz Varian XL-300 instrument. Chemical shifts are given in the delta (δ) scale. Samples which were dissolved in $CDCl_3$ are referenced to internal tetramethylsilane (δ = 0.00). Samples which were dissolved in DMSO-d_6 are referenced to the residual proton resonances from DMSO (δ = 2.49). Signal multiplicity, integrated area, coupling constants (where necessary), and resonance assignments are indicated in parentheses.

^{13}C-n.m.r. spectra were recorded on a Varian XL-300 MHz instrument. Samples dissolved in DMSO-d_6 were referenced using the DMSO resonances as the internal reference (39.5 ppm relative to TMS). Chemical shifts are given in ppm.

^{19}F-n.m.r. spectra were recorded at 254 MHz on a Bruker model HX7-270 spectrometer equipped with a Nicolet 1180 computer, a Diablo disk drive and a 5 mm ^{19}F probe. ^{19}F-Chemical shifts were measured against external trifluoroacetic acid and are given in ppm upfield from $CFCl_3$. Trifluoroacetic acid resonates 76.53 ppm upfield from $CFCl_3$.

Infrared spectra were recorded on a Nicolet 5DX fourier transform spectrophotometer using NaCl plates.

Chemical-ionization mass spectra were recorded with a KRATOS MS50 spectrometer.

U.v./visible absorbance spectra were recorded on a Pye Unicam PU 8800 u.v./visible spectrophotometer using quartz cells.

Micro-analyses were performed by Mr. P. Borda, Micro-analytical laboratory, University of British Columbia, Vancouver.

Solvents and reagents used were either reagent grade, certified or spectral grade. Dry solvents were prepared as follows. THF was refluxed over calcium hydride, distilled, then refluxed with sodium benzophenone and distilled. DMF was stirred overnight with potassium hydroxide then distilled at reduced pressure from calcium oxide. DMSO was predried overnight with sodium hydroxide pellets then distilled at reduced pressure from sodium hydroxide. Distilled DMF and DMSO were stored over 3A molecular sieves. Pyridine was predried overnight with potassium hydroxide pellets followed by distillation from barium oxide. Methanol was distilled from magnesium methoxide prepared in situ by the reaction of methanol with magnesium turnings in the presence of iodine. Acetone was dried over potassium carbonate. Anhydrous diethyl ether was used as supplied.

DABCO, FDNB, D-glucono-1,5-lactone and 2,4-dinitrophenol were obtained from Sigma Chemical Co. Sodium borodeuteride was obtained from Aldrich Chemicals and was stored under nitrogen. The following substituted phenols were obtained from Fluka Chemical Co.: pentafluorophenol, 3,4-dinitrophenol, 2,3-dinitrophenol, 2,5-dinitrophenol, 2,6-dichloro-4-

nitrophenol. 2,6-Dinitrophenol was obtained from J.T. Baker Chemical Co. All of the above chemicals were used as received without further purification.

The following compounds and precursors were synthesized by other workers in this laboratory: 2,3,4,6-tetra-O-benzyl-D-glucopyranose (36); 1,2,3,6-tetra-O-acetyl-4-deoxy-4-fluoro-β-D-glucopyranose (41); methyl 2,3,6-tri-O-benzoyl-4-deoxy-α-D-glucopyranoside (45); 1,2,3,4-tetra-O-acetyl-6-deoxy-D-glucopyranose (50); 2,3,4,6-tetra-O-acetyl-D-glucal (62); 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (61); 2,4-dinitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranoside (39). Dimethylsulfinyl anion was prepared from sodium hydride and DMSO and was kindly provided by the laboratory of Dr. G.G.S. Dutton.

Thin layer chromatography (t.l.c.) separations were performed using Kieselgel 60 F-254 (Merck) analytical plates. The spots were detected with u.v. light when possible, or by charring with 10% sulfuric acid in methanol. The solvent system was 1:1 (V/V) ethyl acetate/petroleum ether unless otherwise stated and the R_f of the product in this solvent system is noted. Column chromatography was performed on Merck silica gel 60 (180-230 mesh). Solvents were evaporated <u>in vacuo</u> at <50°. Products were dried <u>in vacuo</u> and were generally stored in a vacuum dessicator over phosphorous pentoxide.

<u>2-methyl-2-propan(ol-d) (t-butanol)</u> —— <u>t</u>-Butanol was deuterated by dissolving <u>t</u>-butanol (3.9 g, 53 mmol) in D_2O (5.0 ml, 250 mmol), mixing well, and extracting out the deuterated <u>t</u>-butanol with pentane. The

organic extracts were dried over calcium oxide for 20 hours and a small amount of deuterated t-butanol was isolated by distillation; b.p. 81-82° (lit. b.p. 82°); ^{1}H-n.m.r.: δ ($CDCl_3$) 1.21 (s, 3 x CH_3), no signal δ 2.40 due to -OH; IR: (neat) no -OH absorbance at 3600 cm^{-1}.

Bis(tetraalkylammonium)carbonates —— Tetramethyl-, tetraethyl-, and tetrabutylammonium carbonate salts were prepared from the tetraalkylammonium hydroxide and carbon dioxide as described here for bis(tetramethylammonium) carbonate. Carbon dioxide was bubbled into 25% tetramethylammonium hydroxide in water (25 ml) over a period of several hours during which time a white precipitate formed. The water was removed in vacuo and a white solid was obtained which was crystallized from warm acetonitrile; m.p. >300°, ^{1}H-n.m.r. δ (DMSO-d_6) 3.10 (s, CH_3). ^{13}C-n.m.r.: δ (DMSO-d_6) 158.6 (s, $CO_3^=$), 56.0 (q, CH_3). Anal. Calcd. for $C_9H_{24}N_2O_3$: C, 51.87; H, 11.61; N, 13.50. Found: C, 51.47; H, 11.29; N, 13.44. The other carbonate salts were prepared in a similar manner but were not characterized.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (16) —— α-D-glucose (10 g, 56 mmol) was acetylated using pyridine and acetic anhydride by the method of Wolfrom and Thompson[65] to afford the α-pentaacetate (11) as a crystalline solid which was recrystallized from 95% ethanol; 16.5 g, 42.3 mmol, 76%; m.p. 110-111° (lit.[65] m.p. 112-113°). The per-acetate (11) (10.0 g, 25.6 mmol) was brominated by the method of Haynes and Newth[42] using 45% HBr in acetic acid. 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide was obtained as a colorless syrup and

was crystallized from diethyl ether/petroleum ether. The pure (16) was stored at 0-5° in the presence of potassium hydroxide pellets (8.47 g, 20.6 mmol, 79%) m.p. 89-90° (lit.[66] m.p. 88-89°).

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranose (30) — Compound (30) was prepared by hydrolysis of the bromide (16) (5.5 g, 3.0 mmol) using silver carbonate in aqueous acetone[43] and was isolated as a crystalline solid (3.07 g, 8.8 mmol, 68%); m.p. 131-134° (lit.[43] m.p. 132-134°).

1.2 General Preparation of 2,4-Dinitrophenyl β-D-Glucopyranosides

The method of van Boom[33] was employed for the syntheses of 2,4-dinitrophenyl glucopyranosides and is described here for the preparation of 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (29). 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (30) (1.25 g, 3.6 mmol) and DABCO (1.37 g, 12.2 mmol) were dissolved in DMF and the solution stirred over 3A molecular sieves to remove traces of water. FDNB (0.78 g, 4.2 mmol) was added and the solution stirred for 2 hours at which time t.l.c. showed one u.v. active, charring component (R_f = 0.36). The solution was concentrated in vacuo to a syrup, then dissolved in chloroform, washed with 10% aqueous sodium bicarbonate and water, respectively, and finally the organic phase was dried with magnesium sulphate. Removal of the solvent produced the product (29) as a yellow syrup which crystallized immediately upon addition of 95% ethanol. Repeated recrystallization afforded the pure (29) as white needles (1.50 g, 2.9 mmol,

81%); m.p. 177-178° (lit.[33] m.p. 174-175°); ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.68 (d, 1H, H(3) of DNP), 8.40 (dd, 1H, H(5) of DNP), 7.49 (d, 1H, H(6) of DNP), 5.4-5.1 (m, 3H, H(2), H(3), H(4)), 4.25 (m, 2H, H(6), H(6')), 4.04 (m, 1H, H(5)) and 2.2-2.0 (4S, 12H, $4CH_3CO$); δ (DMSO-d_6) 8.81 (d, 1H, H(3) of DNP), 8.58 (dd, 1H, $J_{5,6}$ 10 Hz, $J_{5,3}$ 3 Hz, H(5) of DNP), 7.66 (d, 1H, $J_{6,5}$ 10 Hz, H(6) of DNP), 5.93 (d, 1H, $J_{1,2}$ 9 Hz, H(1)), 5.43 (dd, 1H, $J_{3,4}$ 9 Hz, $J_{3,2}$ 9 Hz, H(3)), 5.20-5.00 (m, 2H, H(2) and H(4)), 4.38 (m, 1H, H(5)), 4.23 (dd, 1H, $J_{6,6}$ - 12 Hz, $J_{6,5}$ - 6 Hz, H(6)), 4.13 (dd, 1H, $J_{6,6}$ 12 Hz, $J_{6,5}$ 3 Hz, H(6')), 2.05 (s, 3H CH_3CO), 2.00 (s, 6H, 2 x CH_3CO), 1.97 (s, 3H, CH_3CO).

Derivatization of 2,3,4,6-tetra-O-benzyl-D-glucopyranose (0.81 g, 1.5 mmol) by the foregoing procedure produced 2,4-dinitrophenyl 2,3,4,6-tetra-O-benzyl-β-D-glucopyranoside (31) (1.70 g, 1.2 mmol, 81%); m.p. 99-100° (lit.[33] m.p. 99-100°); ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.64 (d, 1H, $J_{3,5}$ 3 Hz, H(3) of DNP), 8.11 (dd, 1H, $J_{5,6}$ 10 Hz, $J_{5,3}$ 3 Hz, H(5) of DNP), 7.27-7.20 (m, 21H, H(6) of DNP and aromatic H of benzyl groups), 5.15 (d, 1H, $J_{1,2}$ 7 Hz, H(1)), 5.04-4.46 (m,, 10H, methylene H), 3.87-3.40 (m, 6H, H(2), H(3), H(4), H(5), H(6) and H(6'); δ (DMSO-d_6) 8.80 (d, 1H, $J_{3,5}$ 3 Hz, H(3) of DNP), 8.42 (dd, 1H, $J_{5,6}$ 10 Hz, $J_{5,3}$ 3 Hz, H(5) of DNP), 7.67 (d, 1H, $H_{6,5}$ 10 Hz, H(6) of DNP), 7.60-7.02 (m, 20H, aromatic H of benzyl groups), 5.71 (d, 1H, $J_{1,2}$ 6 Hz, H(1)), 4.95-4.33 (m, 10H, methylene), 3.96 (m, 1H, H(5)), 3.87-3.47 (m, 6H, H(2), H(3), H(4), H(5), H(6) and H(6').

1.3 Deuterium Labelled Glucopyranosides

1.3.1 2,4-Dinitrophenyl 2,3,4,6-tetra-O-acetyl-[1-^{2}H]-β-D-gluco pyranoside (32)

2,3,4,6-Tetra-O-acetyl-β-D-[1-^{2}H]-glucopyranose (34) — The procedure of Nelson[37] was used for the acetylation of D-glucono-1,5-lactone. A mixture of D-glucono-1,5-lactone (5.0 g, 28 mmol), and zinc chloride (2.5 g, 1.8 mmol) in acetic anhydride (25 ml) was stirred at room temperature for 1 h, poured into a cold, saturated sodium bicarbonate solution (250 mL), stirred for 1 h, extracted three times with chloroform (100 mL) and the extracts combined, washed twice with cold water (100 mL), dried with magnesium sulphate, and evaporated to a colorless syrup which consisted of one component by t.l.c. (R_f = 0.52, H_2SO_4 spray). Reduction of the lactone (33) was performed by the method of Hosie and Sinnott.[38] The syrup was dissolved in tetrahydrofuran (60 mL) and cooled to 0°. A cold solution of sodium borodeuteride (0.44 g, 11 mmol) in D_2O (2.1 mL) was added dropwise with stirring, and the mixture stirred for 2 h, the base was neutralized with Dowex 50-W (H^+) ion-exchange resin, the suspension filtered, and the filtrate evaporated *in vacuo*. The resulting syrup was washed with methanol to remove boric acid. The product (34) was obtained as a colorless syrup which was a mixture of the anomers (8.02 g, 23 mmol, 82%), R_f = 0.42 (H_2SO_4 spray).

2,4-Dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-[1-^{2}H]-glucopyranoside (32) — The tetra acetate (34) was derivatized by the method described for (29). The deuterated β-DNPG (32) was isolated as white needles (9.62 g, 19 mmol, 66% from the lactone); m.p. 176-177° (lit.[33] m.p. 174-175°); ^{1}H-n.m.r. showed an identical spectrum to (29) except peaks at δ 5.1-5.4 integrated for 3 (not 4) protons in $CDCl_3$ and there was no resonance at δ 5.93 (H(1)) in DMSO-d_6 indicating loss of H(1). Isotopic purity at the anomeric center was confirmed by mass spectrometry of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose which was prepared using a modification of the foregoing procedure;[36] m/z (rel. intensity) 332 [5.51, [^{2}H] (M^+-OAc) = $C_{14}H_{18}DO_9$] and 331 [0.15, (M^+-OAc) = $C_{14}H_{19}O_9$].

1.3.2 2,4-Dinitrophenyl 2,3,4,6-tetra-O-benzyl-β-D-[1-^{2}H]-glucopyranoside (35)

2,3,4,6-Tetra-O-benzyl-D-[1-^{2}H]-glucopyranose (38) — Oxidation of 2,3,4,6-tetra-O-benzyl-D-glucopyranose (36) (1.23 g, 2.3 mmol) with a solution of acetic anhydride (4.7 mL) and DMSO (7.0 mL)[41] afforded the benzylated lactone (37) as a colorless syrup; R_f = 0.74 (7:1 V/V benzene/ether, H_2SO_4 spray and u.v.); IR (neat) 1757 cm^{-1} (C=O, 1,5-lactone), no -OH absorption. Reduction of the lactone by the method employed for reduction of (33) produced the product (38) as a white crystalline solid which was recrystallized from warm ethanol; (1.06 g, 2.0 mmol, 87% from (36)); m.p. 150-151° (lit.[67] m.p. 151-152° for nondeuterated (38)).

2,4-Dinitrophenyl 2,3,4,6-tetra-O-benzyl-β-D-[1-^{2}H]-glucopyranoside (35) — Compound (38) was derivatized according to the general method to afford the benzylated β-DNPG (35) as a yellow syrup which was crystallized from 95% ethanol; (1.30 g, 1.8 mmol, 79% from (36)); R_f = 0.71 (98:2 V/V chloroform/methanol); m.p. 99-100° (lit.[33] m.p. 99-100°); ^{1}H-n.m.r. data: identical spectrum to (31) except there was no resonance for H(1) at δ 5.15 in $CDCl_3$ or at δ 5.71 in DMSO-d_6.

1.4 Deoxy and Deoxyfluoro Glucopyranosides

1.4.1 2,4-Dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy-4-fluoro-β-D-glucopyranoside (40)

2,3,6-Tri-O-acetyl-4-deoxy-4-fluoro-α-D-glucopyranosyl bromide (42) — The bromide (42) was prepared essentially by the method of Haynes and Newth[42] from 1,2,3,6-tetra-O-acetyl-4-deoxy-4-fluoro-β-D-glucopyranose (41). A solution of (41) (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°, diluted with dichloromethane (30 mL), washed five times with ice cold water, and the organic phase dried with sodium sulphate. Removal of the solvent produced the product as a colorless syrup which was crystallized from diethyl ether/hexanes; R_f = 0.61 (H_2SO_4 spray); 90 mg, 0.24 mmol, 85%.

2,3,6-Tri-O-acetyl-4-deoxy-4-fluoro-D-glucopyranose (43) — Hydrolysis of the bromide (42) was performed according to the method of McCloskey and Coleman.[43] A solution of (42) (90 mg, 0.24 mmol) was dissolved in acetone (0.20 mL) and cooled to 0°. Water (10 μL) was added followed by addition of silver carbonate (0.10 g, 0.36 mmol) (freshly prepared from sodium carbonate and silver nitrate in small portions. The solution was stirred for 20 h at room temperature in the dark, warmed to 50°, and the silver salts were filtered and washed with two portions of warm acetone. The solvent was removed from the combined filtrates to produce a brown syrup which was not purified; 64 mg, 0.21 mmol.

2,4-Dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy-4-fluoro-β-D-gluco-pyranoside (40) —— Derivatization of the unpurified tri-acetate (43) was performed by the general method to afford (40) as yellow needles (45 mg, 0.091 mmol, 34% from tetra-acetate (41)); R_f = 0.74 (H_2SO_4 spray, u.v.); m.p. 171-173°; ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.72 (d, 1H, $J_{3,5}$ 3 Hz, H(3) of DNP), 8.44 (dd, 1H, $J_{5,6}$ 10 Hz, $J_{5,3}$ 3 Hz, H(5) of DNP), 7.47 (d, 1H, $J_{6,5}$ 10 Hz, H(6) of DNP), 5.50-5.23 (m, 3H, H(1), H(2), H(4)), 4.68 (dt, 1H, $J_{F,4}$ 50, J 9 Hz, H(4)), 4.55 (dd, 1H, $J_{6,6}$ 12 Hz, H(6) or H(6')), 4.28 (dd, 1H, $J_{6,6'}$ 12 Hz, H(6) or H(6')), 4.20 (m, 1H, H(5)), 2.14 (s, 6H, 2 x CH_3CO), 2.09 (s, 3H, CH_3CO); δ (DMSO-d_6) 7.62 (d, 1H, $J_{6,5}$ 10 Hz, H(6) of DNP), 5.93 (d, 1H, $J_{1,2}$ 8 Hz, H(1); ^{19}F-n.m.r. data: δ ($CDCl_3$) 199.8 (dd, $J_{F,4}$ 50.2 Hz, $J_{F,3}$ 15.3 Hz). 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.

1.4.2 2,4-Dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy-β-D-glucopyranoside (44)

Methyl 2,3,6-tri-O-acetyl-4-deoxy-α-D-glucopyranoside (46) — To a suspension of methyl 2,3,6-tri-O-benzoyl-4-deoxy-α-D-glucopyranoside (45) (0.20 g, 0.41 mmol) in methanol (1.0 mL) was added 1 M sodium methoxide in methanol (0.010 mL).[44] 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 (90:10 V/V ethyl acetate/methanol). The resulting syrup (72 mg, 88%) was immediately acetylated essentially by the method of Wolfrom and Thompson.[65] A solution of acetic anhydride (0.37 mL, 3.9 mmol) and pyridine (0.47 mL) was cooled to 0° and added to the syrup. The mixture was stirred for 1 h at 0° then warmed to room temperature and stirred overnight. The reaction was quenched by addition of methanol to the cooled mixture and stirred at 0° for 1 h. The solution was diluted with dichloromethane, washed three times with ice cold water and the organic phase was dried with sodium sulphate. Removal of the solvent in vacuo afforded the product (46) as a colorless syrup which was crystallized from diethyl ether (87 mg, 0.29 mmol, 71%); R_f = 0.50 (H_2SO_4 spray).

2,3,6-tri-O-acetyl-4-deoxy-α-D-glucopyranosyl chloride (47) — Chlorination of (46) was performed by the method of Kovac et.al.[45] The methyl glucopyranoside (46) 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° for 1 hr at which time t.l.c. showed only one component, R_f = 0.59 (H_2SO_4 spray). The solution was concentrated in vacuo, diluted with chloroform, washed twice with 10 mL of cold saturated sodium bicarbonate solution. The organic phase was dried with sodium sulphate and removal of the solvent produced a brown syrup (65 mg, 74% from (45)).

2,3,6-Tri-O-acetyl-4-deoxy-D-glucopyranose (48) —— The chloro sugar (47) was hydrolyzed by the method employed for (42) to produce (48) as a yellow syrup (82 mg).

2,4-Dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy-β-D-glucopyranoside (44) – Compound (48) was reacted with FDNB by the usual method to produce (44) as a yellow crystalline solid (96 mg, 0.21 mmol, 51% from the tri-O-benzoate (45)); R_f = 0.45 (H_2SO_4 spray, u.v.); m.p. 180-181°; ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.71 (d, 1H, $J_{3,5}$ 3 Hz, H(3) of DNP), 8.42 (dd, 1H, $J_{5,6}$ 10 Hz, $J_{5,3}$ 3 Hz, H(5) of DNP), 7.48 (d, 1H, $J_{6,5}$ 10 Hz, H(6) of DNP), 5.32-5.05 (m, 3H, H(1), H(2), H(3)), 4.22 (d, 2H, $J_{6,6}$ 7 Hz, H(6) and H(6'), 4.40 (m, 1H, H(5)); δ (DMSO-d_6) 7.60 (d, 1H, $J_{6,5}$ 10 Hz, H(6) of DNP), 5.72 (d, 1H, $J_{1,2}$ 8 Hz, H(1)). 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.

1.4.3 2,4-Dinitrophenyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-glucopyranoside (49)

2,3,4-Tri-O-acetyl-6-deoxy-α-D-glucopyranosyl bromide (51) — 1,2,3,4-tetra-O-acetyl-6-deoxy-D-glucopyranose (50) (0.40 g, 1.2 mmol) was brominated by the method employed for the preparation of (42). After stirring for 2 h at room temperature, t.l.c. showed one component, R_f = 0.69 (H_2SO_4 spray). The product was isolated as a white solid (420 mg, 1.06 mmol, 99%).

2,3,4-Tri-O-acetyl-6-deoxy-α-D-glucopyranose (52) — Hydrolysis of the bromide (51) was performed exactly as for (43), yielding (52) as a colorless gum (306 mg, 1.05 mmol, 99% from bromide); R_f = 0.42 (H_2SO_4 spray).

2,4-Dinitrophenyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-glucopyranoside (49) — Compound (52) was derivatized by the general method to afford (49) as white needles (424 mg, 0.93 mmol, 77% from the tetra-acetate (50)); R_f = 0.58 (H_2SO_4 spray, u.v.); m.p. 198-199°; (lit.[35] m.p. 152-156°); ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.70 (d, 1H, $J_{3,5}$ 3 Hz, H(3) of DNP), 8.43 (dd, 1H, $J_{5,6}$ 10 Hz, $J_{5,3}$ 3 Hz, H(5) of DNP), 7.45 (d, 1H, $J_{6,5}$ 10 Hz, H(6) of DNP), 5.46-5.20 (m, 3H, H(1), H(2), H(3)), 4.95 (dd, 1H, $J_{4,5}$ $J_{4,3}$ 10 Hz, H(4)), 3.83 (m, 1H, H(5)), 2.12 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.35 (d, 3H, $J_{6,5}$ 4 Hz, 3 x H(6)); δ (DMSO-d_6) 7.66 (d, 1H, $J_{6,5}$ 10 Hz, J(6) of DNP), 5.86 (d, 1H, $J_{1,2}$ 7 Hz, H(1)). 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.

1.5 Peracetylated Substituted Phenyl Glucopyranosides

Preparations of acetylated glucopyranosides (53-60) were carried out by the method of Sinnott and Viratelle[46] as follows. 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (16) (1.0 g, 2.4 mmol) was dissolved in acetone (6.0 mL) and the solution mixed with the appropriate phenol (4.0 mmol) dissolved in 1.0 M sodium hydroxide (4.0 mL). The mixture was stirred at room temperature for 20 hours at which time t.l.c. showed one u.v. active, charring component and some proportion of a component corresponding to 2,3,4,6-tetra-O-acetyl-D-glucopyranose (R_f = 0.42, H_2SO_4 spray). The acetone was removed in vacuo and the mixture diluted with water (20 mL). The solution was extracted 3 times with dichloromethane (25 mL) and the organic phase was then washed twice with 50 mL of 2 M sodium hydroxide, washed once with 50 mL of water and dried with magnesium sulphate. Evaporation of the solvent afforded the glucopyranoside as a yellow syrup which crystallized immediately upon addition of 95% ethanol. The products were recrystallized from warm ethanol. Compounds and characterization data are listed below.

Phenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (53) — (0.25 g, 0.60 mmol, 46%), R_f = 0.50 (H_2SO_4 spray, u.v.), m.p. 124-126° (lit.[68] m.p. 125-126°) ^{1}H-n.m.r. data: δ ($CDCl_3$) 7.38-6.80 (m, 5H, aromatic), 5.35-4.93 (m, 4H, H(1), H(2), H(3), H(4)), 4.25 (dd, 1H, $H_{6,6'}$, 12 Hz,

$J_{6,5}$ 4 hz, H(6)), 4.10 (dd, 1H, $J_{6,6}$ 12 Hz, $J_{6',5}$ 2 Hz, H(6')), 2.08 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO).

4-Nitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (54) — (0.39 g, 0.83 mmol, 35%); R_f 0.40 (H_2SO_4 spray, UV); m.p. 178-180° (lit.[68] m.p. 174-175°); ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.15 (dd, 2H, $J_{3,2}$ and $J_{5,6}$ 11 Hz, $J_{3,5}$ 4 Hz, H(3) and H(5), aromatic), 5.35-5.15 (m, 4H, H(1), H(2), H(3), H(4)), 4.30-4.15 (m, 2H, H(6) and H(6')) 3.88 (m, 1H, H(5)), 2.08-1.95 (4S, 12H, 4 x $OCOCH_3$).

Pentafluorophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (55) — (0.64 g, 1.24 mmol, 51%); R_f = 0.51 (H_2SO_4 spray, u.v.); m.p. 144-145°; ^{1}H-n.m.r. 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 (2dd, $J_{6,5}$ 4 Hz, $J_{6',5}$ 2 Hz, $J_{6,6'}$ 12 Hz, H(6) and H(6')), 3.70 (m, 1H, H(5)), 2.10 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.02 9s, 6H, 2 x CH_3CO); ^{19}F-n.m.r. data: δ ($CDCl_3$) 166.73 (t, 1F, $J_{F4,F3}$ and J_{F4-F5} 21 Hz, F(4)), 163.30 (m, 2F, F(3) and F(5)). 147.32 (dd, 2F, J_{F2-F3} and J_{F6-F5} 19 Hz, F(2) and F(6)), Anal. Calcd. for $C_{20}H_{19}F_5O_{10}$: C, 46.71; H, 3.72. Found: C, 45.90; H, 3.56.

3,4-Dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (56) — (0.71 g, 1.38 mmol, 57%); R_f = 0.63 (H_2SO_4 spray, u.v.) m.p. 171-172°; ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.03 (d, 1H, $J_{5,6}$ 8 Hz, H(5) of DNP) 7.42 (d, 1H, $J_{2,6}$ 2 Hz, H(2) of DNP), δ 7.25 (1H, H(6) of DNP, hidden by $CHCl_3$ isotopic impurity peak) 5.42-5.22 (m, 4H, H(1), H(2), H(3), H(4)), 4.22

(d, 2H, $J_{6,5}$ and $H_{6',5}$ 4 Hz, H(6) and H(6')), 3.97 (dt, 1H, $J_{,4}$ 11 Hz, $J_{5,6}$ and $H_{5,6'}$ 5 Hz H(5)), 2.08-1.98 (4S, 12H, 4 x CH_3CO). Anal. Calcd. for $C_{20}H_{22}N_2O_{14}$: C, 46.69; H, 4.31; N, 5.47. Found: C, 46.49; H, 4.22; N, 5.52.

2,3 Dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (57) — (0.33 g, 0.64 mmol, 51%); R_f = 0.35 (H_2SO_4 spray, u.v.); m.p. 191-192°; ^{1}H-n.m.r. data: δ ($CDCl_3$) 7.96 (dd, 1H, $J_{4,5}$ 8 Hz, $J_{4,6}$ 2 Hz, H(4) of DNP), 7.68 (dd, 1H, $J_{6,5}$ 8 Hz, $J_{6,4}$ 2 Hz, H(6) of DNP), 7.62 (dd, 1H, $J_{5,4}$ 8 Hz, $J_{5,6}$ 8 Hz, H(5) of DNP), 5.32-5.05 (m, 4H, H(1), H(2), H(3), H(4)), 4.24 (d, 2H, $J_{6,5}$ and $J_{6',5}$ 4 Hz, H(6) and H(6')), 3.86 (dt, 1H, $J_{5,4}$ 10 Hz, $J_{5,6}$ and $J_{5,6'}$ 4 Hz, H(5)), 2.14 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.03 (s, 3H, 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 (58) — (0.41 g, 0.80 mmol, 33%); R_f = 0.60 (H_2SO_4 spray, u.v.); m.p. 145-147°; ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.23 (d, 1H, $J_{6,4}$ 2 Hz, H(6) of DNP), 8.06 (dd, 1H, $J_{4,3}$ 9 Hz, $J_{4,6}$ 2 Hz, H(4) of DNP), 7.89 (d, 1H, $J_{3,4}$ 9 Hz, H(3) of DNP), 5.37-5.06 (m, 4H, H(1), H(2), H(3), H(4)), 4.30 (dd, 1H, $J_{6',6}$ 12 Hz, $J_{6',5}$ 3 Hz, H(6')), 4.19 (dd, 1H, $J_{6,6'}$ 12 Hz, $J_{6,5}$ 6 Hz, H(6)), 4.02 (m, 1H, H(5)), 2.13 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO).

2,6-Dichloro-4-nitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (59) —— (0.75 g, 1.4 mmol, 58%); R_f = 0.60 (H_2SO_4 spray, u.v.); m.p. 181-182°; ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.20 (s, 2H, H(3) and H(4) of aromatic group), 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 Hz, $J_{6',5}$, 2 Hz, H(6')), 3.65 (m, 1H, H(5), 2.09 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.02 (6H, 2 x 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 (60) —— (0.34 g, 0.66 mmol, 55%); R_f = 0.40 (H_2SO_4 spray, u.v.); m.p. 190-191°; ^{1}H-n.m.r. data: δ ($CDCl_3$) 7.98 (d, 2H, $J_{3,5}$ 8 Hz, H(3) and H(5) of DNP), 7.47 (t, 1H, $J_{4,5}$ and $J_{4,3}$ 8 Hz, H(4) of DNP), 5.36-5.08 (m, 4H, H(1), H(2), H(3), H(4)), 4.03 (d, 2H, $J_{6,5}$ and $J_{6',5}$ 4 Hz, H(6) and H(6'), 3.62 (dt, 1H, $H_{5,4}$ 10 Hz, $J_{5,6}$ and $J_{5,6'}$ 4 Hz, H(5)), 2.13 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.00 (s, 3H, 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. Anomerization Reactions

2.1 Preparative Scale Anomerization

2.1.1 2,4-Dinitrophenyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (29-α)

The α-anomer (29-α) was prepared essentially by the method of van Boom[33] as follows. 2,4-Dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (29-β) (0.453 g, 0.88 mmol) was dissolved in DMF (1.7 mL) containing 3A molecular sieves to remove traces of water. Anhydrous potassium carbonate (0.320 g, 2.3 mmol) was added and the mixture was stirred for 20 h at room temperature after which time t.l.c. (H_2SO_4 spray, u.v.) indicated that the mixture contained the α- and β- anomers (~80:20) (R_f (α) = 0.52, R_f (β) = 0.36, H_2SO_4 spray and u.v.). The mixture was diluted with chloroform, filtered and concentrated in vacuo to a red syrup. The syrup was redissolved in chloroform (50 mL), washed twice with 10% sodium bicarbonate solution (50 mL), then twice with water (50 mL). The organic phase was dried with magnesium sulphate and evaporated in vacuo to a yellow syrup. The syrup was dissolved in warm 95% ethanol and the α-anomer fractionally crystallized out from the solution (211 mg, 0.41 mmol, 47%). The crystals were filtered and the filtrate concentrated and cooled to produce crystals which were a mixture of α- and β- anomers (200 mg, 0.39 mmol, 44%). This mixture was not purified further, but separation of the anomers could be achieved readily by column chromatography as described by van Boom. α-2,4-DNPG

(29-α): m.p. 184-185° (lit.[33] m.p. 181-183°); ^{1}H-n.m.r. data: δ ($CDCl_3$) 8.76 (d, 1H, $J_{3,5}$ 4 Hz, H(3) of DNP), 8.45 (dd, 1H, $J_{5,6}$ 9 Hz, $J_{5,3}$ 4 Hz, H(5) of DNP), 7.54 (d, 1H, $J_{6,5}$ 9 Hz, H(6) of DNP), 6.02 (d, 1H, $J_{1,2}$ 3 Hz, H(1)), 5.67 (dd, 1H, J 8 Hz, H(3), assigned by decoupled ^{1}H at δ 5.09, H(2)), 5.23 (dd, 1H, J 8 Hz, H(4)), 5.09 (dd, $J_{2,3}$ 8 Hz, $J_{1,2}$ 4 Hz, H(2)), 4.24-4.10 (m, 3H, H(6), H(6'), H(5)), 2.03 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.98 (s, 6H, 2 x CH_3CO); δ (DMSO-d_6) 8.82 (d, 1H, $J_{3,5}$ 4 Hz, H(3) of DNP), 8.56 (dd, 1H, $J_{5,6}$ 10 Hz, $J_{5,3}$ 3 Hz, H(5) of DNP), 7.74 (d, 1H, $J_{6,5}$ 10 Hz, H(6) of DNP), 6.32 (d, 1H, $J_{1,2}$ 4 Hz, H(1), 5.48 (dd, 1H, J Hz, H(3)), 5.25-5.12 (m, 2H, H(2), H(4)), 4.19 (dd, $J_{6,6'}$ 12 Hz, $J_{6',5}$ 4 Hz, H(6)), 4.09 (m, 1H, H(5)), 3.97 (dd, $J_{6,6'}$ 12 Hz, $J_{6',5}$ 2 Hz, H(6')), 2.02 (s, 6H, 2 x CH_3CO), 2.00 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO).

[1-^{2}H]-β-2,4-DNPG (32) (447 mg, 0.87 mmmol) was anomerized by the foregoing procedure to produce the pure α-anomer (180 mg, 0.35 mmol, 40%) and a mixture of anomers (225 mg, 0.44 mmol, 50%); R_f (α) = 0.56 (H_2SO_4 spray, u.v.); m.p. 183-185°; ^{1}H-n.m.r. data: spectrum identical to non-deuterated α-2,4-DNPG (29-α) except no signal for H(1) at δ 6.02 in $CDCl_3$ and δ 6.33 in DMSO-d_6.

2.1.2 Peracetylated Substituted Phenyl Glucopyranosides

The preceding anomerization method was modified slightly to determine the α/β product composition for the aryl glucopyranosides (53-60). Thus, the β-glucopyranoside (0.036 mmol) was stirred with anhydrous

potassium carbonate (0.10 mmol) in DMSO (0.20 mL) containing 3A molecular sieves. The mixture was stirred for 3-5 days at room temperature at which time the reaction was worked-up as previously described except no attempt was made to separate the anomers. T.l.c. and n.m.r. analyses of the product mixture indicated when anomerization had occurred since the α-anomers have very characteristic t.l.c. and n.m.r. properties. Compounds 53-58 showed no change by t.l.c. or n.m.r. analyses after being submitted to the above conditions and therefore, anomerization had not occurred.

2,6-Dichloro-4-nitrophenyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (59-α) — R_f = 0.70 (H_2SO_4 spray, u.v.); ^{1}H-n.m.r. data: δ ($CDCl_3$) 7.88 (d, $J_{3,4}$ $J_{5,4}$ 9 Hz, H(3) and H(5) of DNP), 6.32 (d, $J_{1,2}$ 3 Hz, H(1)).

2,6-Dinitrophenyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (60-α) — R_f = 0.52 (H_2SO_4 spray, u.v.); ^{1}H-n.m.r. data: δ ($CDCl_3$) 7.87 (d, H(3) and H(5) aromatic).

2.1.3 Small Scale Reactions: Variation of Solvents and Catalysts

Attempted anomerization of 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (29) using various catalysts and solvent were performed using 10 mg (0.018 mmol) of (29), 0.05 mmol of catalyst and 0.10 mL of solvent. Mixtures were stirred for 1-3 days and the extent of anomerization (if any) was determined by t.l.c. only ($R_f(\beta)$ = 0.36,

$R_f(\alpha)$ = 0.52, H_2SO_4 spray and u.v.).

2.2 Exchange Reactions

2.2.1 Exchange of H or D at Anomeric Carbon

2,4-Dinitrophenyl glucopyranoside (29) with t-butan(ol-d) — A 0.12 mM solution of (29) in DMSO-d_6 (0.200 mL) was mixed with a 0.18 mM solution of $(Me_4N)_2CO_3$ in DMSO-d_6 (0.400 mL) and t-butan(ol-d) (0.100 mL, 0.84 mmol) in a n.m.r. tube and the tube was sealed off using an oxygen torch (see also preparation of samples for kinetic studies). After 2 days at room temperature the ^{1}H-n.m.r. spectrum showed that anomerization had occurred to 50% and the amount of α-anomer present determined by integration of H(1) (δ 6.32, DMSO-d_6) was equal to the amount determined by integration of H(6)-DNP (δ 7.74, DMSO-d_6) (53% α-anomer by H(1), 52% α-anomer by H(6) of DNP). Therefore, no H/D exchange had occurred.

2,4-Dinitrophenyl [1-^{2}H]-glucopyranoside (32) with t-butanol — 2,4-Dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-[1-^{2}H]-glucopyranoside (32) (45 mg, 0.087 mmol) and $(Me_4N)_2CO_3$ (31 mg, 0.23 mmol) was stirred in DMSO (0.20 mL) containing t-butanol (0.70 g, 9.4 mmol) for 7 days. The mixture was worked-up according to the procedure given for anomerization of substituted phenyl glucopyranosides. ^{1}H-n.m.r. of the product mixture indicated that anomerization had occurred to 70% by the integration of the resonance of H(6)-DNP at δ 7.54 of the α-anomer ($CDCl_3$) but there

was clearly no resonance at δ 6.02 p.p.m. ($CDCl_3$) corresponding to a proton at the aromatic center.

The preceding H/D exchange experiments were repeated using dimsyl anion as a catalyst where DMSO is the only proton or deuteron source. [1-^{2}H]-β-D-2,4-DNPG (32) (20 mg, 0.039 mmol) was anomerized with 0.064 M dimsyl anion in DMSO (1.0 mL), and non-deuterated β-2,4-DNPG (29) (20 mg, 0.039 mmol) was anomerized with 0.064 M dimsyl anion in DMSO-d_6 (1.0 mL). ^{1}H-n.m.r. spectra for both experiments showed that anomerization had occurred with no H/D exchange at the anomeric center or with any other sugar ring protons.

2.2.2 Exchange of Phenolate

2,4-Dinitrophenyl glucopyranoside (29) with 2,6-dinitrophenolate — A mixture containing β-2,4-DNPG (29) (45 mg, 0.087 mmol), anhydrous potassium carbonate (32 mg, 0.23 mmol), and potassium 2,6-dinitrophenolate (19 mg, 0.087 mmol) in DMF (0.20 mL) was stirred at room temperature for 3 days. T.l.c. of the mixture after this time showed the presence of approximately 80% of a component with an R_f of 0.56 (H_2SO_4 spray, u.v.) corresponding either to the α-2,4-DNPG or the α-2,6-DNPG. the mixture was worked-up according to the procedure for anomerization of aryl glucopyranosides. ^{1}H-n.m.r. ($CDCl_3$) of the product mixture showed only the presence of the 2,4-dinitrophenyl anomers. There was no resonance δ 7.87 corresponding to H(3) and H(5) of the aromatic group for the α-2,6-DNPG (60-α).

2,6-Dinitrophenyl glucopyranoside (60) with 2,4-dinitrophenolate — A mixture containing β-2,6-DNPG (60) (20 mg, 0.039 mmol), anhydrous potassium carbonate (15 mg, 0.11 mmol) and potassium 2,4-dinitrophenolate (9 mg, 0.041 mmol) in DMF (0.20 mL) was stirred at room temperature for 3 days. T.l.c. of this product mixture indicated that approximately 50% of the β-2,6-DNPG (60-β) had reacted to form either the α-2,6-DNPG (60-α) or the α-2,4-DNPG (29-α). ^{1}H-n.m.r. of the worked-up reaction mixture showed the presence of only α- and β-2,6-DNPG and no signals at positions corresponding to the 2,4-DNPG anomers.

2,3,4,6-Tetra-O-acetyl glucal (62) and 2,4-dinitrophenolate — The glucal (10 mg, 0.030 mmol) was mixed with 0.60 mL of potassium 2,4-dinitrophenolate (8 mg, 0.036 mmol) in DMSO-d_6. The ^{1}H-n.m.r. spectrum of the sample after several days indicated the presence of only starting materials.

2.2.3 Exchange via a Nucleophilic Attack Mechanism

2,6-Dinitrophenyl glucopyranoside (60) with FDNB — β-2,6-DNPG (60) (50 mg, 0.097 mmol) was mixed with FDNB (165 mg, 0.99 mmol) and potassium carbonate (70 mg, 0.51 mmol) in DMSO (0.50 mL) containing 3A molecular sieves. The mixture was stirred for 8 days at which time the reaction was worked-up according to the procedure for anomerization of aryl glucopyranosides. ^{1}H-n.m.r. analysis in $CDCl_3$ of the product mixture showed the presence of predominantly β-2,6-DNPG (60-β) (~60% by

H(6)-aromatic at δ 7.98) and a small amount of α-2,4-DNPG (29) (~10% by H(1) at δ 6.02), but no α-2,6-DNPG (60-α) no signals for (H(3) and H(5) of DNP at δ 7.87). All other aromatic peaks could be assigned to FDNB, β-2,6-DNPG (60) or α-,β-2,4-DNPG (29).

2,4-Dinitrophenyl [1-^{2}H]-glucopyranoside (32) with 2,3,4,6-tetra-O-acetyl-D-glucopyranose (30) — [1-^{2}H]-β-D-2,4-DNPG (32) (40 mg, 0.078 mmol) and the tetra-acetate (30) (27 mg, 0.078 mmol) were stirred with potassium carbonate (57 mg, 0.41 mmol) in DMSO (0.80 mL) containing 3A molecular sieves for 2 days at which time t.l.c. indicated that the β-2,4-DNPG had anomerized. The mixture was worked-up as usual. ^{1}H-n.m.r. analysis of the crude product mixture in DMSO-d_6 showed the α-and β-DNPG anomers in approximately equilibrium proportions (80:20 respectively). The area of the peak at δ 6.33 (H(1) of α-2,4-DNPG) integrated to only about one half the area of the peak at δ 7.74 (H(6)-aromatic of α-2,4-DNPG). The α-2,4-DNPG was fractionally crystallized from warm 95% ethanol and ^{1}H-n.m.r. of the isolated pure α-anomer in DMSO-d_6 clearly confirms this result (26 mg, 0.051 mmol, 64%); m.p. 183-184° (lit. m.p. 181-183°); ^{1}H-n.m.r. data: δ (DMSO-d_6) 8.82 (d, 1H, H(3) of DNP), 8.56 (dd, 1H, H(5) of DNP), 7.74 (d, 1H, H(6) of DNP), 6.32 (d, 0.5H, H(1)), 5.48 (dd, 1H, H(3)), 5.25-4.12 (m, 2H, H(2) and H(4)), 4.19 (dd, 1H, H(6)), 4.09 (m, 1H, H(5)), 3.97 (dd, 1H, H(6')), 2.02 (s, 6H, 2 x CH_3CO), 2.00 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO).

There was no observed loss of the deuterium label when [1-^{2}H]-β-D-

2,4-DNPG (32) was anomerized with potassium carbonate and DMSO in a separate, control experiment.

3. KINETICS

3.1 Polarimetric Studies

Optical rotations were measured with a Perkin Elmer 141 polarimeter using a sodium lamp. A 1-dm, 1.0 ml, water-jacketed cell was used and temperature of the solution was maintained at 25°. The optical rotations, $[\alpha]_D^{25}$, of β- and α-2,4-DNPG (29) measured in DMSO were +12.3° (c 5.7) and +187.7° (c 9.3), respectively.

After several modifications, the following procedure was used in an attempt to measure rates of anomerization. Bis(tetramethylammonium) carbonate (0.156 g, 0.702 mmol) was dissolved in DMSO (10 mL) by stirring overnight at room temperature. This solution (2.0 mL) was added to β-2,4-DNPG (29) (0.335 g, 0.651 mmol) in a 2.0 mL volumetric test tube. The solution was mixed and transferred to the polarimeter cell as quickly as possible to prevent the solution from coming into contact with the atmosphere and to measure the optical rotation as soon as possible after mixing the reagents. Optical rotation readings were taken at 10, 20 or 30 minute intervals. After 90 minutes, optical rotation indicated that anomerization had occurred to only 5 to 10% whereas t.l.c. of the same reaction mixture showed that 30 to 40% of the α-anomer had formed. After 5 hours, there was no longer any change

in optical rotation but t.l.c. of the mixture indicated that anomerization was nearly complete. The reaction mixture at this time was red to brown in color possibly due to the formation of 2,4-dinitrophenolate. These erroneous optical rotation measurements do not appear to be an artifact of the highly absorbing background since a solution of β-2,4-DNPG (29) with added potassium 2,4-dinitrophenolate in DMSO showed an essentially identical optical rotation to that of a standard b-2,4-DNPG solution ($[\alpha]_D^{25}$ = +14.89°, c 14.2, DMSO).

3.2 ^{1}H-N.m.r. Studies

The kinetic solutions were prepared in sealed n.m.r. tubes and the following precautions were taken to avoid contamination by water. The β-2,4-DNPG (29) and bis(tetramethylammonium) carbonate were weighed out in approximate amounts into separate pre-weighed vials then dried <u>in vacuo</u>. The vials were stoppered with rubber septa in a glove box then reweighed to obtain the accurate amounts of dried substrate and catalyst. The vials were transferred again to the glove box and the appropriate amount of DMSO-d_6 (sealed under nitrogen and used as received) was added. The bis(tetramethylammonium) carbonate was dissolved after stirring overnight at room temperature. N.m.r. tubes (5 mm) with a 14/20 ground glass joint at the top and were oven-dried (>100°) before use, then cooled while flowing anhydrous nitrogen through the tube. The appropriate amount of the catalyst solution was added via micro-syringe and the solution was frozen with liquid nitrogen, followed by addition

of the appropriate amount of substrate solution via micro-syringe and again freezing with liquid nitrogen. In this way the reagents are frozen in layers to prevent mixing until the mixture is warmed to room temperature. While frozen, the n.m.r. tubes were evacuated and sealed using an oxygen torch. Reactions were initiated by warming the tube to room temperature as quickly as possible (over a period of about 3 minutes by rubbing the outside of the tube) and mixing thoroughly. The ^{1}H-n.m.r. spectra were immediately recorded using the Varian XL-300 spectrometer at a probe temperature of 25°. Progress of the reaction was followed by recording spectra at appropriate intervals.

Rates of anomerization were obtained by following the disappearance of the β-anomer or by following the appearance of the α-anomer, the former method giving more accurate results. The H(6) resonances of the DNP group and the anomeric resonances were used to determine the amounts of each anomer and the chemical shifts of these resonances for each substrate are summarized in Table 8. The H(1) signal and the H(6) signal of the DNP group were integrated using acetate resonances at δ 2.2-2.0 as the internal reference. For the deuterium labelled substrate, the reaction could be followed only by integration of the H(6) signal of the DNP group. The relative integrations were measured at various times, t, and the amount of remaining β-anomer was denoted α_t (mmol). The equilibrium amount of the β-anomer, α_e (mmol), was determined after a reaction time of 8-12 hours.

Reaction rate plots of log $(\alpha_t - \alpha_e)$ against time[13] were linear for 2 half lives (correlation coefficient, $r \geq 0.98$). Reaction rates

Table 8: Chemical shifts (δ) for H(6)-DNP group and H(1) of the 2,4-DNPG derivatives in DMSO-d_6. Peaks are referenced internally to DMSO-d_6 at δ 2.49 relative to TMS

Compound	β-anomer		α-anomer	
	H(6) of DNP ($d, J_{6,5}$ 10Hz)	H(1) ($d, J_{1,2}$ 8Hz)	H(6) of DNP ($d, J_{6,5}$ 10Hz)	H(1) ($d, J_{1,2}$ 3Hz)
(29)	7.64	5.93	7.74	6.33
(32)	7.67	5.71	7.72	6.33
(39)	7.73	6.57	7.65	6.09
(40)	7.62	5.93	7.74	6.32
(44)	7.60	5.72	7.70	6.26
(49)	7.66	5.86	7.72	6.27

reported are the rates of disappearance of the β-anomer and were calculated using linear regression analysis. Error in the measured integration values is ± 5%. Kinetic isotope effects were calculated from the ratio of reaction rates for the isotopically substituted and unsubstituted substrates, or Rate (H)/Rate (D).

APPENDIX 1

KINETIC ISOTOPE EFFECTS[58]

1. Definition of Deuterium Isotope Effects

An isotope effect is the observed difference in rate of a reaction when a reactant is isotopically substituted. Therefore, the kinetic isotope effect (KIE) is given by the ratio of rates for the isotopically unsubstituted and substituted substrates or $^{kH}/_{kD}$ for deuterium effects. Since a change in the rate of a reaction reflects a change in the energy of the transition state for that reaction, isotope effects provide a method for probing the structure of the transition state and the formation of intermediates during the reaction. Use of an isotope effect in this way is based upon the fact that substitution of an isotope in the substrate molecule does not appreciably perturb the potential energy surface of the reaction. That is, the electronic structure of the molecule is essentially insensitive to differences in nuclear masses (the Born-Oppenheimer principle). Therefore, the isotope effect may be treated only in terms of vibrational energies, making interpretation simpler. The following sections will review types of KIEs and give a basic introduction to the theory behind these effects. The scope of this discussion will be limited to deuterium isotope effects. It should be noted, however, that heavier atom KIEs, such as $^{13}C/^{14}C$, $^{14}N/^{15}N$, and $^{16}O/^{18}O$ which have been difficult to measure accurately are being used more commonly as methods to measure them become more precise.

2. Theory

2.1 Primary Isotope Effects

Primary isotope effects can be measured when there is isotopic substitution in a bond which is being cleaved in a reaction. The difference in observed rates relates to changes in the vibrational frequency of a bond upon isotopic substitution. For a deuterium KIE, this is most easily explained by comparing the ground state energies of C-H and C-D bonds. If the bonds are assumed to act as simple harmonic oscillators, then the vibrational frequency ν of the bond is given by $\nu = \frac{1}{2\pi}\sqrt{k/\mu}$ where μ is the reduced mass and k is the force constant. If k is constant for isotopically substituted and unsubstituted molecules and frequency and therefore the energy (given by $E = \frac{1}{2} h\nu$ where h = Plancks constant) depend only on the reduced mass. The C-D bond, having a higher reduced mass, will have a lower ground state energy. As a result, the C-D bond will require a higher activation energy for bond cleavage than the C-H bond. This is shown schematically in Figure 50. For the primary KIE it is considered that the isotopically substituted and unsubstituted molecules go through the same transition state or intermediate so that the energy difference between the C-H and C-D bonds disappears as the reaction approaches the transition state. The magnitude of the isotope effect can be used to estimate the relative position of the transition state. If there is complete bond cleavage at the transition state, a larger KIE would be expected than if the transition state more nearly resembled the reactants where very little bond

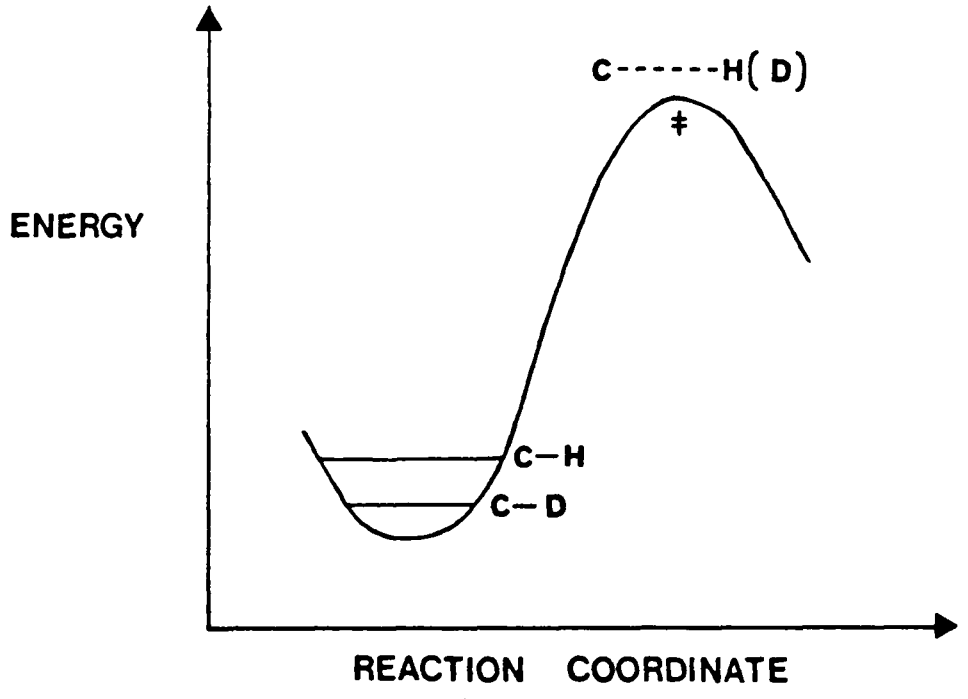


Figure 50: **The reaction coordinate diagram for a primary isotope effect**

cleavage has occurred. The maximum KIE possible for deuterium is 7.0 which corresponds to the total disappearance of the C-H or C-D stretching vibration in the transition state. In general, a deuterium KIE ranging from 2 to 7 is a primary isotope effect.

2.2 Secondary Isotope Effects

Secondary isotope effects arise from changes in the bonding of the isotopically labelled carbon, but the isotope is substituted in a bond which is not being cleaved in the reaction. Two types of secondary KIEs

exist: α-secondary KIEs where the isotope is substituted directly at the reaction center; or β-secondary KIEs where the isotope is substituted on an adjacent atom to the reaction center.

a. α-Secondary KIEs

α-Secondary KIEs may be observed when the reactive carbon undergoes a change in hybridization in the rate controlling step of the reaction. A change from sp^3 to sp^2 hybridization at the transition state produces a change in the zero-point energies of the C-H and C-D bonds as a result of changes in the bonding vibrations in reaching the transition state (Figure 51). Again, because of the lower reduced mass of the C-H bond

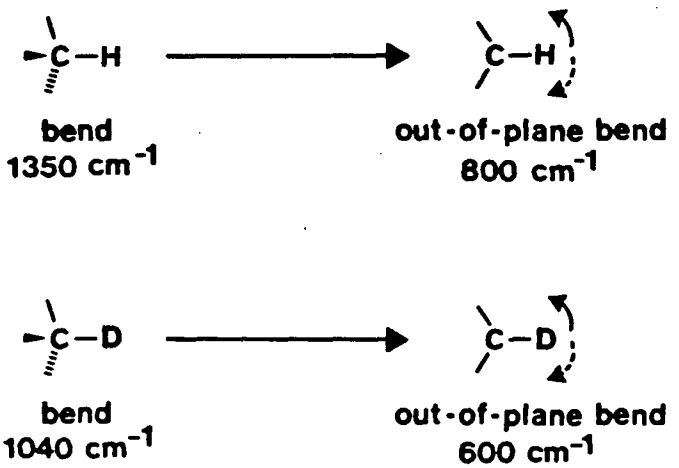


Figure 51: The bending vibrational frequencies of sp^3 and sp^2 hybridized carbon

the vibrational frequency of the C-H bond is generally higher. In going from sp^3 to sp^2 hybridization, there is an overall lowering of the vibrational frequencies and the lowering is greatest for the C-H bond (550 cm^{-1} for C-H versus 440 cm^{-1} for C-D). The rate of the reaction of the isotopically unsubstituted molecule will be greater since the activation energy will be lower. Therefore, the isotope effect of $^{kH}/_{kD}$ will be greater than unity. The energy diagram for an α-secondary KIE is shown in Figure 52.

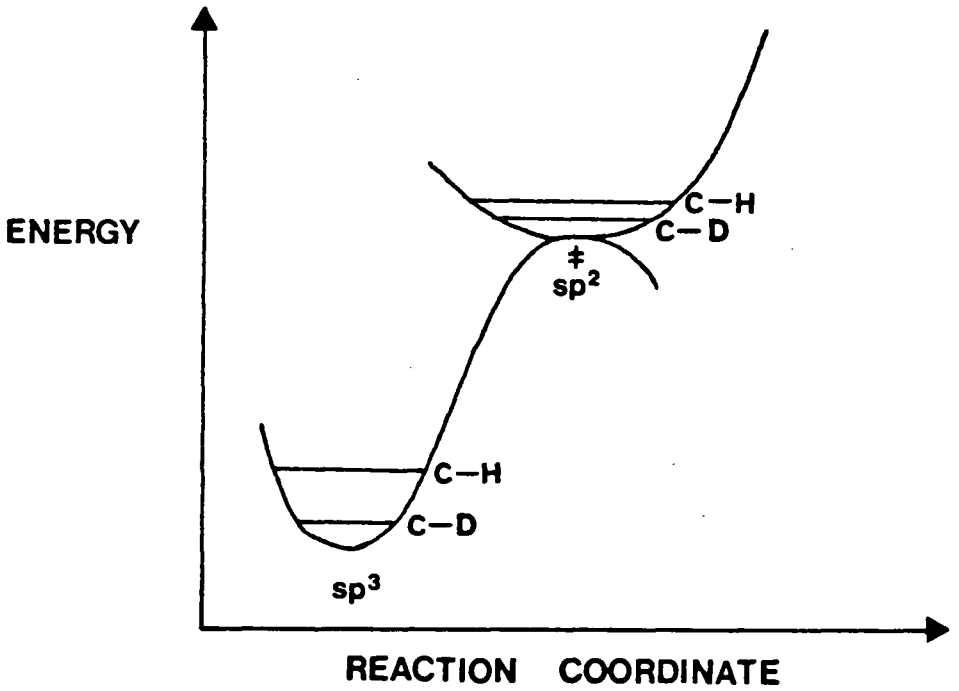


Figure 52: The reaction coordinate diagram for an α-secondary isotope effect

The converse is true for a reaction going from sp^2 to sp^3 hybridization in the rate-controlling step. In this case there is an overall increase in the vibrational frequencies of the C-H and C-D

bonds, and the increase is greater for the C-H bond. Thus, the reaction involving the C-H bond will have a lower rate since it will require a higher activation energy. The isotope effect or $^{kH}/_{kD}$ will be less than unity and is therefore termed an inverse isotope effect.

In both cases, the difference in rates or the magnitude of the isotope effect will reflect the nature of the transition state. Rate differences for α-secondary KIEs are generally in the order of 10-20% but may reach a maximum of 40%. S_N1-type reactions which involve the formation of a carbonium ion intermediate and therefore involve a transition state with carbonium ion character typically give an α-secondary KIE about $^{kH}/_{kD} = 1.2$. On the other hand, an S_N2 type mechanism, where there is no net change in the hybridization of the reaction center during the reaction, would give an isotope effect of nearly unity.

b. <u>β-Secondary KIEs</u>

β-Secondary deuterium isotope effects also arise from changes in hybridization and are generally observed when the reaction involves the formation of a carbonium ion. When the adjacent carbon is deuterium substituted, the difference in rate between the protio- and deutero-substrates is due to the relative ability of the C-H or C-D bond to stabilize the electron deficient reaction center through hyperconjugation. That is, the difference in rate depends on the ability of an adjacent C-H or C-D σ bond to donate electron density to an electron deficient p-orbital on the reactive carbon. For example, acid catalyzed

glycoside hydrolysis is considered to occur via an oxocarbonium ion intermediate. Hyperconjugative stabilization of this intermediate or the transition state leading to it is represented by the structures shown in Figure 53. The structure at the right shows the possible influence of resonance interactions on hyperconjugation. Since the deuterium atom is slightly electron donating relative to hydrogen, a C-D bond is stronger and the electrons on the deuterium atom are less available for hyperconjugation. Therefore, deuterium substitution at C(2) of the structure in Figure 53 causes a decrease in reaction rate because its transition state will be less stable and higher in energy. The hyperconjugative component of a secondary isotope effect is, however, geometry dependent and will have no influence if the C-H or C-D bonds are orthogonal to the electron deficient p-orbital on the reactive carbon. In this case, it is possible that the C-D bond may show an inductive effect. Here the C-D bond, being slightly more electron

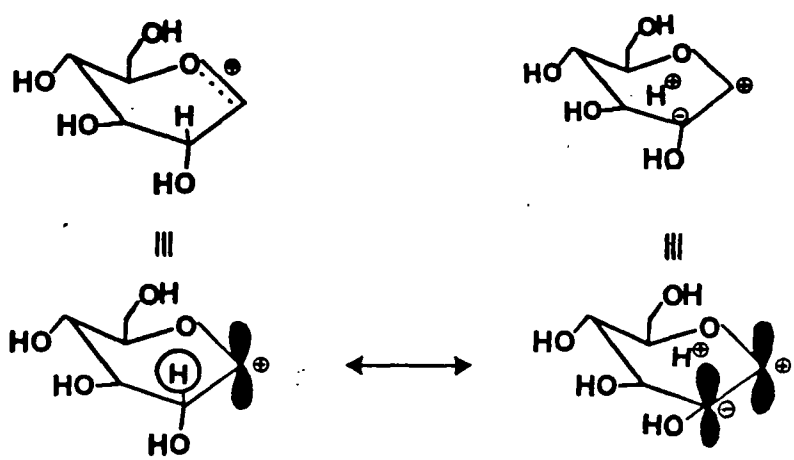


Figure 53: Hyperconjugation in an oxocarbonium ion

donating, will stabilize the charge at the reaction center relative to the C-H bond. The deuterated species then reacts faster and an inverse isotope effect will result. β-Secondary KIEs are generally no greater than about 10% and until recently, with the development of more accurate methods of measurement, have been used very little.

3. Use in Studying Reaction Mechanisms

A recent publication by Sinnott and Bennet[69] exemplifies the power of isotope effects as a method of studying reaction mechanisms. Here a number of isotope effects on the acid catalyzed hydrolysis of methyl α-and β-glucopyranosides (5) were measured. The positions of substitution for each isotope effect are shown in Figure 54. Some of the results of this study in consideration of the prevailing mechanism on the acid catalyzed hydrolysis (Figure 55) are summarized as follows. (All KIEs measured in 2.0 M $HClO_4$ at 80°C).

(i) Leaving group ^{18}O effects (primary KIE) were determined to be $k_{16_O}/k_{18_O} = 1.026$ for (5-α) and $k_{16_O}/k_{18_O} = 1.024$ for (5-β). The magnitude of the KIEs were found to be very close to isotope effects calculated for the dissociation of 4-nitrophenyl β-glucopyranoside where 4-nitrophenol and a glucopyranosyl cation are generated. Thus, it was concluded that there is a high degree of glycosyl-oxygen bond cleavage in the transition state leading to the oxocarbonium ion.

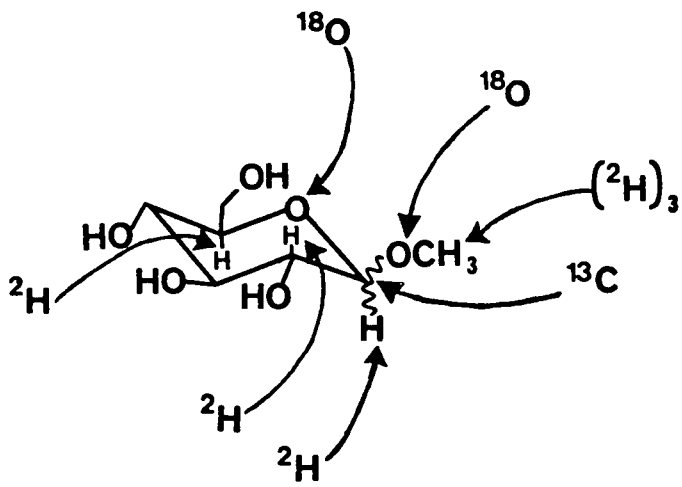


Figure 54: The isotope effects measured for the acid catalyzed hydrolysis of methyl α- and β-D-glucopyranoside

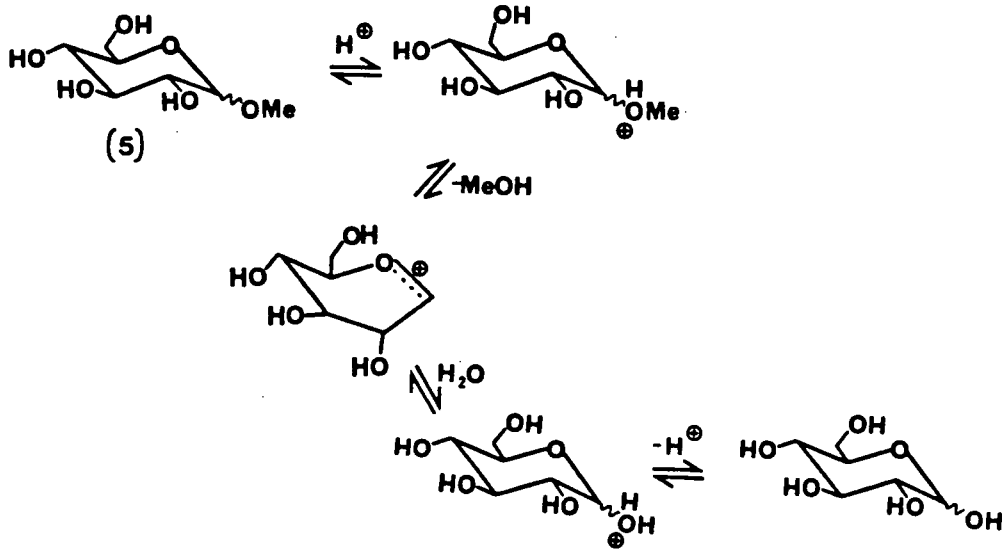


Figure 55: The acid catalyzed hydrolysis of methyl α- and β-D-glucopyranosides

(ii) α-Secondary deuterium effects were determined to be $^{kH}/_{kD}$ = 1.137 for (5-α) and $^{kH}/_{kD}$ = 1.089 for (5-β). Allowing for the inductive effect of deuterium which decreases the KIE, the magnitude of these isotope effects suggests that the transition state is nearly sp^2 hybridized and C(1)-O(1) bond cleavage is essentially complete.

(iii) Ring ^{18}O effects (secondary KIE) were found to be inverse for both anomers ($^{k16_O}/^{k18_O}$ (5-α) = 0.996, $^{k16_O}/^{k18_O}$ (5-β) = 0.991). The inverse KIE reflects the double bond character of the C(1)-O(5) bond in the transition state. Since ^{18}O is slightly more electron-donating than ^{16}O, delocalization by the oxygen lone pair is greater for the isotopically substituted glycoside, thus stabilizing the transition state for this substrate. The magnitude of these effects was correlated with the amount of orbital overlap between C(1) and the ring oxygen in the transition state in order to derive the dihedral angle about the C(1)-O(5) bond.

(iv) β-Secondary deuterium effects were measured to be $^{kH}/_{kD}$ = 1.073 for the α-anomer and $^{kH}/_{kD}$ = 1.045 for the β-anomer. As with the ring ^{18}O effects, the secondary β KIE was correlated with orbital overlap of the C(2)-H(2) or C(2)-D(2) bond with the electron deficient p-orbital at C(1) and the dihedral angle between the p-orbital and the C(2)-H(2) or C(2)-D(2) bond was calculated. These results in conjunction with results from the ring ^{18}O effects were used to propose probable conformations of the transition state in the reaction.

As seen with the above example, measurement of isotope effects is a powerful tool in the mechanistic investigations of chemical reactions. It should be noted that isotope effects alone cannot give a complete picture of a reaction mechanism. Therefore, other aspects such as products, stereochemistry, kinetics, and salt or solvent effects should be considered.

APPENDIX 2

KINETIC DATA FOR ANOMERIZATION REACTIONS

Reaction rate data obtained for the anomerization reactions are given in the following tables. All measurements were performed by the method described in Materials and Methods. Concentrations of substrate and catalyst are indicated.

Table 9: Remote substituent effects: 2,4-dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy-4-fluoro-β-D-glucopyranoside (40). [DNPG] = 0.0138 M. $[(Me_4N)_2CO_3]$ = 0.0167 M. $\alpha_e = 1.42 \times 10^{-3}$ mmol

Integral Area for H(1) of β-anomer	β-DNPG (mmol x 10^{-3})	log ($\alpha_t - \alpha_e$)	time (min)
7.80	7.08	-2.25	37
7.75	5.77	-2.36	56
6.73	4.96	-2.45	93
5.50	4.09	-2.57	123
3.58	3.33	-2.72	153
4.08	3.06	-2.78	183
3.72	2.89	-2.84	213

Table 10: Remote substituent effects: 2,4-dinitrophenyl 2,3,6-tri-O-acetyl-4-deoxy-β-D-glucopyranoside (44). [DNPG] = 0.0150 M. $[(Me_4N)_2CO_3]$ = 0.0167 M. $\alpha_e = 2.08 \times 10^{-3}$ mmol

Integral Area for H(1) of β-anomer	β-DNPG (mmol x 10^{-3})	log ($\alpha_t - \alpha_e$)	time (min)
12.90	10.4	-2.08	14
12.85	10.4	-2.08	34
12.50	10.2	-2.09	64
12.05	9.89	-2.11	94
11.65	9.59	-2.12	124
11.35	9.30	-2.14	154
10.32	8.52	-2.16	214
10.10	8.44	-2.20	319
9.83	7.80	-2.24	369

Table 11: Remote substituent effects: 2,4-dinitrophenyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-glucopyranoside (49). [DNPG] = 0.0153 M. $[(Me_4N)_2CO_3]$ = 0.0167 M. $\alpha_e = 2.06 \times 10^{-3}$ mmol

Integral Area for H(1) of β-anomer	β-DNPG (mmol x 10^{-3})	log ($\alpha_t - \alpha_e$)	time (min)
13.72	10.3	-2.08	6
13.60	10.1	-2.09	30
13.42	10.0	-2.10	62
12.53	9.10	-2.15	97
11.68	8.72	-2.18	123
10.82	8.21	-2.21	150
10.05	7.37	-2.29	180
9.60	7.17	-2.35	212
8.70	6.51	-2.44	242
7.55	5.65	-2.52	270

Table 12: Remote substituent effects: 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (29). [DNPG] = 0.0136 M. $[(Me_4N)_2CO_3]$ = 0.0167 M. $\alpha_e = 1.76 \times 10^{-3}$ mmol

Integral Area for H(1) of β-anomer	β-DNPG (mmol x 10^{-3})	log ($\alpha_t - \alpha_e$)	time (min)
12.30	8.78	-2.15	5
12.15	8.42	-2.18	30
10.40	7.28	-2.26	60
8.90	6.22	-2.35	90
7.00	4.95	-2.50	120
6.10	4.32	-2.59	150
5.03	3.59	-2.74	180
4.15	2.95	-2.92	210
3.62	2.57	-3.09	240
3.40	2.42	-3.18	270
3.30	2.38	-3.21	355

Table 13: Remote substituent effects: 2,4-dinitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranoside (39). [DNPG] = 0.0134 M. $[(Me_4N)_2CO_3]$ = 0.0182 M. $\alpha_e = 9.85 \times 10^{-3}$ mmol

Integral Area for H(1) of β-anomer	β-DNPG (mmol x 10^{-3})	log ($\alpha_t - \alpha_e$)	time (min)
5.10	6.61	-2.24	6
4.00	5.89	-2.30	32
3.20	4.72	-2.42	60
2.50	3.69	-2.55	90
1.90	3.42	-2.60	120
1.45	2.48	-2.80	150
1.20	2.07	-2.93	190
1.12	1.76	-3.06	212
0.80	1.56	-3.17	291

Table 14: Remote substituent effects: 2,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside. [DNPG] = 0.0121 M. $[(Me_4N)_2CO_3]$ = 0.01821 M. $\alpha_e = 1.45 \times 10^{-3}$ mmol

Integral Area for H(1) of β-anomer	β-DNPG (mmol x 10^{-3}	log $(\alpha_t - \alpha_e)$	time (min)
6.55	7.91	-2.19	4
5.62	7.06	-2.25	20
4.95	6.31	-2.31	40
4.04	5.24	-2.41	60
3.62	4.68	-2.49	80
3.40	4.35	-2.54	100
3.20	4.13	-2.57	120
2.61	3.24	-2.75	142
2.40	3.12	-2.79	160
2.30	2.98	-2.82	180
1.53	1.99	-3.03	237
1.32	1.73	-3.33	293
1.25	1.62	-3.68	375

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