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C2-Oxyanion Neighboring Group Participation: Transition State Structure for the Hydroxide-Promoted Hydrolysis of 4-Nitrophenyl α-D-Mannopyranoside

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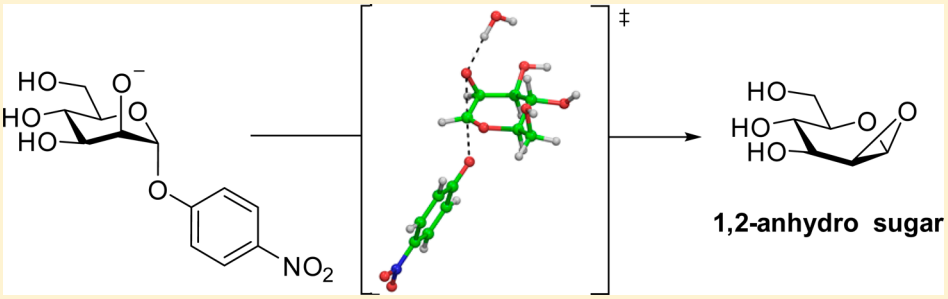
Supporting Information



ABSTRACT: The hydroxide-catalyzed hydrolysis of aryl 1,2-*trans*-glycosides proceeds through a mechanism involving neighboring group participation by a C2-oxyanion and rate-limiting formation of a 1,2-anhydro sugar (oxirane) intermediate. The transition state for the hydroxide-catalyzed hydrolysis of 4-nitrophenyl α-D-mannopyranoside in aqueous media has been studied by the use of multiple kinetic isotope effect (KIE) measurements in conjunction with *ab initio* theoretical methods. The experimental KIEs are C1-^{2}H (1.112 ± 0.004), C2-^{2}H (1.045 ± 0.005), anomeric 1-^{13}C (1.026 ± 0.006), C2-^{13}C (0.999 ± 0.005), leaving group oxygen 2-^{18}O (1.040 ± 0.012), and C2-^{18}O (1.044 ± 0.006). The transition state for the hydrolysis reaction was modeled computationally using the experimental KIE values as constraints. Taken together, the reported kinetic isotope effects and computational modeling are consistent with the reaction mechanism involving rate-limiting formation of a transient oxirane intermediate that opens in water to give α-D-mannopyranose. The transition state has significant nucleophilic participation by the C2-alkoxide, an essentially cleaved glycosidic bond, and a slight shortening of the endocyclic C1−O5 bond. The TS is late, consistent with the large, normal C2-^{18}O isotope effect.

INTRODUCTION

The natural world contains an abundance of glycoconjugates, which are characterized by the presence of a glycosidic bond connecting monosaccharides to one another, or to non-carbohydrate species. A wide range of processes capable of cleaving glycosidic bonds has been identified, with acid-catalyzed hydrolysis reactions the most commonly encountered and studied. However, for over 100 years it has been recognized that certain naturally occurring glycosides, such as picein, salicin, and coniferin, undergo facile alkaline solvolyses.[1] Under these conditions, the anomeric group undergoes nucleophilic substitution by a C2-oxyanion forming a 1,2-anhydro sugar intermediate, which then undergoes ring opening through nucleophilic attack.[2,3] In the case of β-glucosides and β-galactosides the major product is the 1,6-anhydro sugar.[4] McCloskey and Coleman[5] were the first to recognize that the 1,6-anhydro-β-glucopyranose formed in these alkaline hydrolyses likely results from reaction of 1,2-anhydro-α-glucose intermediates.[6,7] Such observations extend to 1,2-*trans*-related glycosyl fluorides.[8,9] In the case of β-xylosides,[6] which lack the 6-OH group, and α-mannosides,[10] which proceed through a β-configured 1,2-anhydro sugar, the major products are xylose and mannose, respectively. Reaction rates are dramatically reduced for substrates bearing a 2-*O*-methyl group, which prevents formation of the pivotal C2-oxyanion.[10]

The aforementioned base-promoted processes constitute glycosyl transfer through neighboring group participation, wherein a group with an unshared pair of electrons is located adjacent to the leaving group and participates to form a discrete reaction intermediate. The effect of neighboring group participation by 2-acetamido groups on *N*-acetylhexosaminides has been studied extensively, with strong evidence for carbonyl participation in chemical solvolytic processes, through oxazoline (or oxazolinium ion) intermediates,[11,12] and there is compelling evidence that neighboring group participation

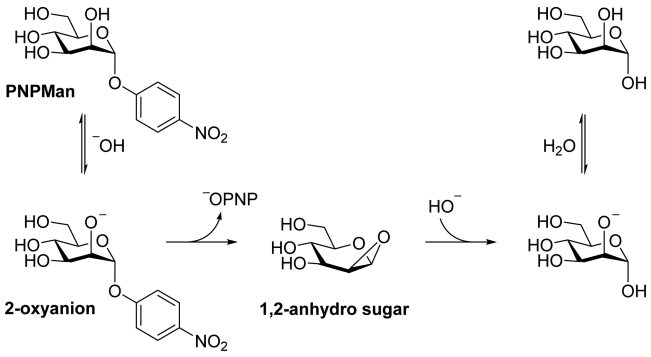
Received: July 31, 2016
Published: October 10, 2016

DOI: 10.1021/jacs.6b07935
J. Am. Chem. Soc. 2016, 138, 14012−14019

mechanisms are used by certain β-hexosaminidases.[13−15] In contrast to the existence of enzyme-catalyzed processes that operate through neighboring group participation mechanisms involving a 2-acetamido group, there are no clear-cut examples of enzymatic processes that utilize neighboring group participation mechanisms involving a 2-oxyanion or 2-hydroxyl group. Early proposals for 2-oxygen neighboring group participation in the stereochemically retaining LacZ β-galactosidase-catalyzed hydrolysis of β-galactosides[16,17] failed to withstand detailed scrutiny. Evidence now supports a classical Koshland retaining mechanism involving nucleophilic attack by an enzymic residue and formation of a glycosyl enzyme intermediate that subsequently undergoes hydrolysis in two inverting steps, and which explains the net retention of reaction stereochemistry.[18,19] Likewise, early proposals for the formation of a 1,2-anhydro sugar in the mechanisms of base-catalyzed solvolysis and enzyme-catalyzed hydrolysis of NAD^+ have failed to withstand detailed mechanistic scrutiny.[20−22]

Recently, on the basis of structural studies that failed to identify a candidate enzymatic nucleophile for a stereochemically retaining glycoside hydrolase (GH) family 99 (for family classification see: www.cazy.org; www.cazypedia.org)[23] *endo*-1,2-α-mannosidase/*endo*-1,2-α-mannanase, a neighboring group participation mechanism proceeding through a 1,2-anhydro sugar was proposed.[24] In order to understand the nature of the transition state for C2-oxyanion participation, and to assist in identifying the kinetic signatures of this reaction, we chose to study the well characterized specific base-catalyzed hydrolysis of *p*-nitrophenyl α-D-mannopyranoside (PNPMan; Scheme 1).[10,25] This reaction was chosen owing to its close relationship

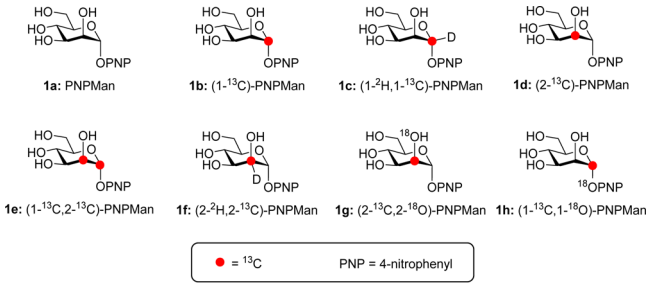
Scheme 1. Neighboring Group Participation by a 2-Oxyanion in the Alkaline Hydrolysis of 4-Nitrophenyl α-D-Mannopyranoside



to the reaction proposed for *endo*-1,2-α-mannosidase/*endo*-1,2-α-mannanase, as well as the tremendous rate acceleration observed under basic conditions relative to the β-anomer, a hallmark of neighboring group participation. Accordingly, we measured a complete set of secondary deuterium and heavy-atom kinetic isotope effects (KIEs) on the base-promoted hydrolysis of PNPMan, and these values were used as constraints for an *ab initio* modeling study to identify possible transition states.

RESULTS AND DISCUSSION

Advances in NMR spectroscopy have enabled the development of a highly sensitive competitive ^{13}C NMR method that allows the high precision measurement of KIEs.[26] The method requires milligram quantities of light and heavy isotopologues, with each possessing an NMR-active nucleus at a probe site (in this case ^{13}C), and an adjacent site labeled with light and heavy isotopes. Application of this method for the measurement of KIEs at positions C1, H1, O1, C2, H2, O2 of PNPMan required the synthesis of just seven isotopologues owing to redundancy in the use of probe positions (experimental details and Table S1, Supporting Information). PNPMan isotopologues were synthesized from isotopically labeled D-mannose. In a typical procedure, acetylation of D-mannose with Ac_2O and catalytic H_2SO_4 afforded mannose pentaacetate, which after workup was used to glycosylate 4-nitrophenol (or 4-$NO_2C_6H_4$-^{18}OH) in the presence of $BF_3{\cdot}Et_2O$. Deprotection with NaOMe/MeOH and recrystallization afforded the PNPMan isotopologues.



Compounds 1a−1h. Pseudo-first-order rate constants for the base-promoted hydrolysis of PNPMan in aqueous solution at 25 °C were estimated from the absorbance (400 nm) versus time data at hydroxide ion concentrations of 0.1, 0.5, and 1.0 M. The half-life for hydrolysis of PNPMan in 0.1 M NaOH at 25 °C was 13 h, a value similar to that of 16 h extrapolated from the reported second-order rate constants at higher temperatures.[10] To assess the contribution of an S_NAr mechanism to the hydrolytic reaction, PNPMan was treated with $Na^{18}OH$ in $H_2{}^{18}O$, and with unlabeled NaOH in H_2O, and the released 4-nitrophenol was analyzed by mass spectrometry. In both cases, the mass spectrum of the isolated 4-nitrophenol revealed isotope ratios identical to those obtained from natural abundance material, indicating that the S_NAr pathway was insignificant.

KIE measurements were performed in 0.1 M aqueous sodium hydroxide (I = 0.4, NaCl) in NMR tubes containing a Teflon insert, which is inert to base. The ^{13}C atom probe nucleus was used to report on changes in the isotopologue ratios for the residual starting materials. Individual ^{13}C probe nucleus signals were integrated, and the relative ratio (R) for each isotopologue was calculated. The fraction of reaction (F_1) was determined by comparing the integration for the light isotopologue with an inert internal standard [methyl α-D-(1-^{13}C)glucopyranoside]. A representative overlay of NMR spectra at commencement ($F_1 = 0$) and at $F_1 = 0.79$ is shown in Figure 1, and typical examples of the nonlinear least-squares fit for the experimental KIE data are illustrated in Figure 2. Table 1 lists the mean and standard deviation values for each KIE derived from three replicate experiments, and the individual KIE values that were derived from the nonlinear fits to eq 1 along with the associated standard error are provided in the Supporting Information section (Table S2).

Carbon-13 KIEs. Anomeric carbon KIEs (k_{12}/k_{13}) for the reactions of glycopyranosides in solution are typically in the range of 1.005 to 1.030. Reactions occurring via dissociative transition states exhibit anomeric ^{13}C-KIE values that are closer to unity;[27−30] for instance, the reported ^{13}C-KIEs for

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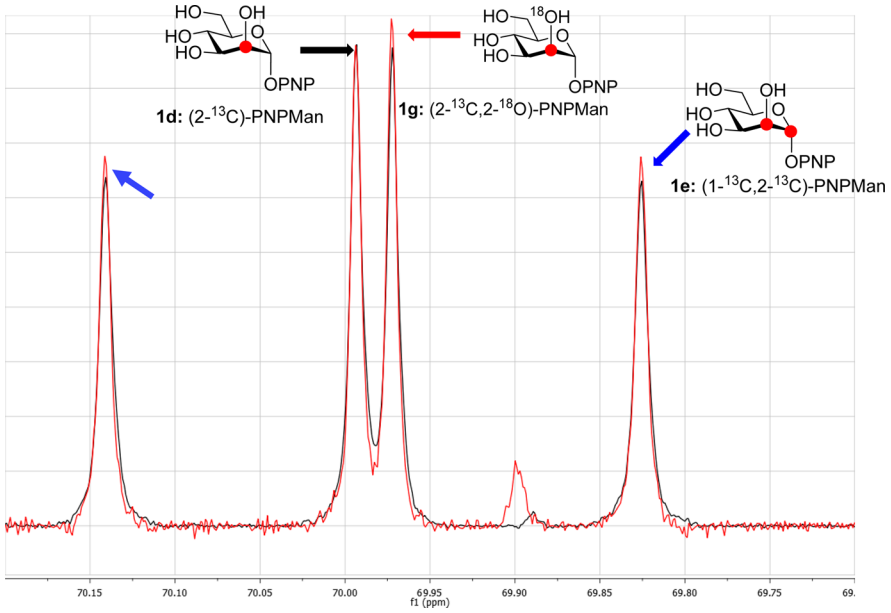

Figure 1. Overlaid proton decoupled ^{13}C NMR spectra containing a mixture of **1d**, **1e**, and **1g** in NaOH (0.1 M; I = 0.4 at fraction of reactions (F_1) = 0.00 (black) and 0.79 (red)) that have normalized peak heights for the probe isotopologue (**1d**; black arrow). Note the relative increase in peak heights for the 1-^{13}C-PNPMan (**1e**; blue arrows) and 2-^{18}O-PNPMan (**1g**; red arrow) isotopologues, which have increased in relative intensity during reaction.

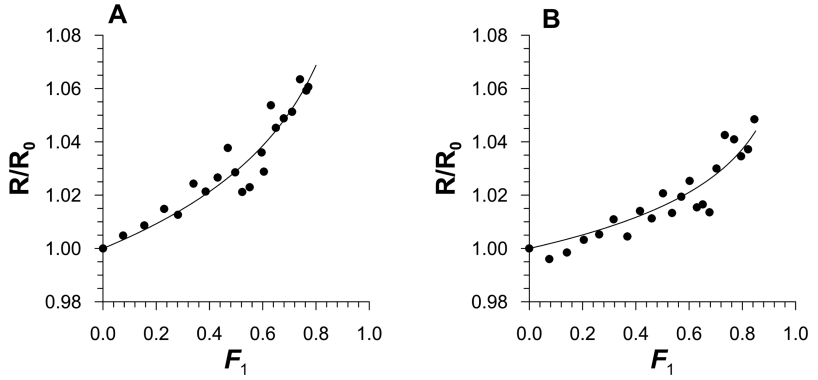

Figure 2. Plots of the change in integrated peak intensity ratios (R/R_0) versus fraction of reaction for the light isotopologue (F_1) for the measurement of competitive KIE values: (A) data from an experimental measurement of $k_{(2\text{-}^{16}O)}/k_{(2\text{-}^{18}O)}$ using **1d** and **1g**; (B) data from an experimental measurement of $k_{(1\text{-}^{12}C)}/k_{(1\text{-}^{13}C)}$ using **1d** and **1e**.

Table 1. Kinetic Isotope Effects on the Base-Promoted Hydrolysis of 4-Nitrophenyl α-D-Mannopyranoside in 100 mM NaOH (I = 0.4, NaCl) at T = 25 °C, and the Calculated KIEs for the *gauche-gauche* C6-CH$_2$OH conformer at the B3LYP/6-31G* Level of Theory

Position of KIE	Experimental KIEs	Calculated KIEs		
	Mean ± SD	Initial TS	Unconstrained TS	Constrained TS
1-^{2}H	1.112 ± 0.004	1.1648	1.1583	1.1128
2-^{2}H	1.045 ± 0.005	1.0926	1.0727	1.0700
1-^{13}C	1.026 ± 0.006	1.0417	1.0360	1.0442
2-^{13}C	0.999 ± 0.005	1.0068	1.0061	1.0074
1-^{18}O	1.040 ± 0.012	1.0364	1.0452	1.0331
2-^{18}O	1.044 ± 0.006	1.0354	1.0338	1.0478

spontaneous hydrolysis of α-D-glucopyranosyl 4-bromo-isoquinolinium bromide and the acid-catalyzed reaction of the anomeric methyl D-xylopyranosides are 1.005 ± 0.002[31] and 1.006 ± 0.001,[30] respectively. In contrast, the anomeric ^{13}C-KIE for the base-catalyzed hydrolysis of PNPMan is 1.026 ± 0.006 (Table 1), which is in the range typically associated with S_N2 reactions on glycosides that proceed via 'exploded' transition states.[32] The secondary ^{13}C-KIE that we measured for C-2 (**1b** versus **1e**) is within experimental error equal to unity (k_{12}/k_{13} = 0.999 ± 0.005, Table 1), a result consistent with essentially no change between the ground and transition states in vibrational frequencies associated with isotopic substitution at C2.

Oxygen-18 KIEs. We measured primary oxygen-18 KIEs for the nucleophilic oxyanion at C2 and the nitrophenolate aglycone. Effects on k_{16}/k_{18} for both the leaving group oxygen (1.040 ± 0.012) and the C2-oxygen (1.044 ± 0.006) are substantially greater than unity. The magnitude of 1-^{18}O-KIE values for the hydrolysis of 4-nitrophenyl glycosides is known to be strongly dependent on the degree of proton catalysis during aglycone departure. That is, as the degree of proton transfer at the transition state decreases there is a concomitant

DOI: 10.1021/jacs.6b07935
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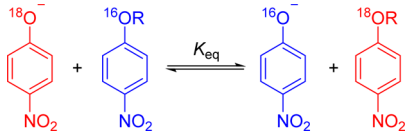
increase in the measured KIE (k_{16}/k_{18}) value. The smallest oxygen-18 KIE reported (k_{16}/k_{18} = 1.023) is for the specific-acid catalyzed hydrolysis of 4-nitrophenyl β-D-(1-^{18}O)-glucopyranoside, a mechanism that involves complete protonation of the substrate prior to rate-limiting aglycone departure.[33] In contrast, for the general-acid catalyzed reaction of 4-nitrophenyl β-D-(1-^{18}O)glucopyranoside, which involves simultaneous aglycone departure and oxygen protonation, a larger KIE value was reported (k_{16}/k_{18} = 1.0355 ± 0.0015).[34] Lastly, for reactions in which the leaving group departs as a 4-nitrophenoxide ion, the base-promoted reaction of 4-nitrophenyl β-D-(1-^{18}O)glucopyranoside[34] and the spontaneous hydrolysis of the 4-nitrophenyl *N*-acetyl-α-(2-^{18}O)neuraminide anion,[35] the corresponding ^{18}O-KIEs are 1.0386 ± 0.0032 and 1.053 ± 0.002, respectively. We therefore conclude that the measured value (1.040 ± 0.012, for **1b** versus **1h**) is in line with expectations for a base-catalyzed intramolecular nucleophilic substitution reaction that gives 4-nitrophenoxide and a sugar oxirane intermediate following rate-limiting glycoside bond cleavage.

In contrast to the many reported ^{18}O-KIEs for 4-nitrophenol leaving groups, few nucleophile ^{18}O-KIEs have been reported, especially for cases in which the oxygen nucleophile is anionic. Several isotope effects have been reported in substitution reactions when ^{18}O-water is the nucleophile, and these KIEs fall in the range of 1.023−1.030.[36,37] For the base-catalyzed hydrolysis of PNPMan the nucleophile is an oxyanion rather than a neutral water molecule, so the equilibrium isotope effect (EIE) for ionization and KIE for ring closure contribute to the observed ^{18}O-KIE. Although we are not aware of any example of a primary oxygen-18 EIE on the ionization of an alkanol (as they are generally unable to be titrated in water because of their low acidity), an ^{18}O-EIE ($^{16}K_a/^{18}K_a$ = 1.0153 ± 0.002) has been reported for the deprotonation of 4-nitrophenol (Scheme 2; R = H, K_{eq} = $^{16}K_a/^{18}K_a$).[38]

Scheme 2. Isotopologue Equilibria for the Ionization of 4-Nitrophenol (R = H) and Transfer of an Acetyl Group [R = C(O)CH$_3$]



A much larger ^{18}O-EIE was measured for the transfer of an acetyl group between 4-nitrophenolate isotopologues (Scheme 2; R = CH_3CO, K_{eq} = 1.0277 ± 0.0007), an effect that allows calculation of the ^{18}O-EIE between 4-nitrophenol and 4-nitrophenyl acetate as $^{16}K_a/^{18}K_a$ = 1.0277/1.0153 = 1.0122.[38] Although we conclude that a greater fraction of 2-^{13}C-PNPMan (**1d**) will be ionized in the basic media relative to 2-^{13}C,2-^{18}O-PNPMan (**1g**), it is implausible that the magnitude of the EIE on either deprotonation or oxygen transfer (between a hydrogen and a carbon atom) is the primary contributor to the magnitude of the measured KIE. Nevertheless, it is clear that the strikingly large magnitude of the KIE value for the C-2 oxygen of 1.044 ± 0.006 requires significant changes in vibrational frequencies between the ground and transition states during oxirane formation. That is, the crucial difference between these model reactions and equilibria is that the reaction of PNPMan in base involves the formation of a strained three-membered ring. Oxirane rings have strain energies of ∼27 kcal/mol,[39,40] and the transition state for ring closure must incorporate a significant degree of strain, which predominantly resides in the ring σ-bonds. Based on the principle of microscopic reversibility (that the transition state for ring closure is the same as that for ring opening) and the report by Hoz and co-workers who proposed, based on mechanical principles, that strain energy is lost earlier during ring opening of oxiranes than oxetanes (four-membered ring ethers),[41,42] we conclude that the ring closing transition state must be late and results in a significant weakening of the C2−oxygen bond, which leads to the large normal ^{18}O-KIE for this reaction.

β-Secondary Deuterium KIEs (β-SDKIEs). β-SDKIEs for substitution reactions on glycosides usually originate from a hyperconjugative weakening of the C−H/(D) bond through interaction with the developing empty *p*-orbital at the anomeric center.[27,43] In this case, the observed rate of hydrolysis will be complex to interpret because the hydrolysis of PNPMan is specific-base catalyzed, and thus the measured β-SDKIE should be interpreted in terms of contributions from negative hyperconjugation associated with the EIE for ionization of the C2-hydroxyl group (to give the anion) and the KIE for ring closing of the C2-oxyanion intermediate to form an oxirane (i.e., the rate at which the anion reacts). There are only a few secondary deuterium EIEs reported for the ionization of an alcohol, primarily because they are not sufficiently acidic to be titratable in water;[44] thus, we used the EIE ($^{H}K_a/^{D}K_a$ = 1.067 per deuterium) measured for the ionization of 2,2,2-trifluoroethanol and 2,2,2-trifluoro(1,1-$^2H_2$)ethanol in aqueous solution.[45] Under the conditions of the NMR reaction, for which the pH is lower than the pK_a value of a C2-sugar hydroxyl group,[46] a greater fraction of the more acidic protiated substrate (**1d**) is ionized; however, the anion of the deuterated substrate (**1f**) must be more nucleophilic; i.e., the anion of **1f** should react faster than the corresponding anion from **1d**. Thus, we conclude that the measured β-SDKIE (k_H/k_D = 1.045 ± 0.005, Table 1) for the hydroxide-promoted hydrolysis of PNPMan is consistent with a normal equilibrium isotope effect (K_H/K_D > 1) for ionization of the C-2 hydroxyl group that is attenuated by an inverse KIE (k_H/k_D < 1) on oxirane ring formation.

α-Secondary Deuterium KIEs (α-SDKIEs). α-SDKIEs for substitution reactions resulting in glycosidic bond cleavage originate predominantly from changes in bending vibrations as the sp^3 anomeric carbon undergoes rehybridization to an sp^2-like center as the reaction coordinate passes over either a dissociative S_N1 or an 'exploded' S_N2 transition state. α-SDKIEs for reactions involving α-glycosides include the spontaneous hydrolyses of 4-nitrophenyl tetrahydropyran (k_H/k_D = 1.17)[47,48] and α-D-glucopyranosyl 4-bromoisoquinolinium bromide (k_H/k_D = 1.19),[31] the acid-catalyzed hydrolysis of methyl α-D-glucopyranoside (k_H/k_D = 1.14),[27] and the S_N2 reaction of α-D-glucopyranosyl fluoride with an azide ion (k_H/k_D = 1.19).[32] In the current study, the anomeric carbon atom undergoes conversion into an oxirane carbon and the bending C−H vibration changes are expected to govern the magnitude of the α-SDKIE. Indeed, based on the reported α-SDKIE (k_H/k_D = 0.95 per deuterium) for the epoxidation of ethylene[49] where the sp^2 carbons are converted into an oxirane ring we expected the α-SDKIE for the base-catalyzed hydrolysis of PNPMan (**1b** versus **1c**) to be smaller in magnitude than those for the formation of glycopyranosylium ion intermediates listed above,

DOI: 10.1021/jacs.6b07935
J. Am. Chem. Soc. 2016, 138, 14012−14019

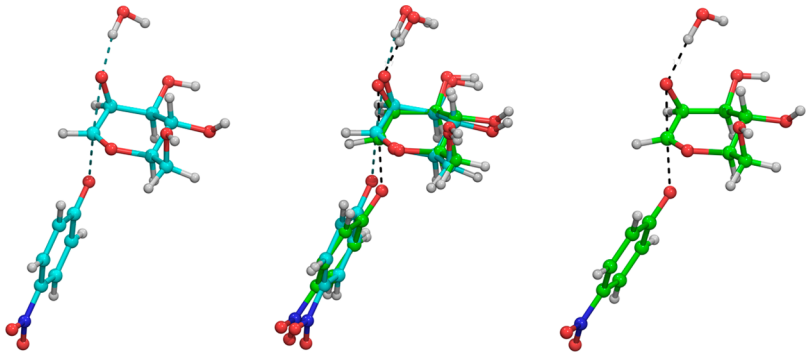


Figure 3. Calculated transition state structures for the formation of the 1,2-anhydro sugar intermediate during the base-promoted hydrolysis of 4-nitrophenyl α-D-mannopyranoside, from the *gauche*−*gauche* C6-CH_2OH conformer. The unconstrained TS is shown in teal (left-hand structure), the constrained TS, which was obtained by systematic variation of the two reaction coordinate C−O bonds, is shown in green (right-hand structure) and an overlay of the two calculated transition states (center). The unconstrained TS structure calculated at the B3LYP6311++G (d, p) level of theory with the SCRF method and IEF-PCM water dielectric environment and the constrained TS structure was calculated at the B3LYP631+G (d, p) level of theory with the SCRF method and IEF-PCM water dielectric environment and Bondi Atomic Radii.

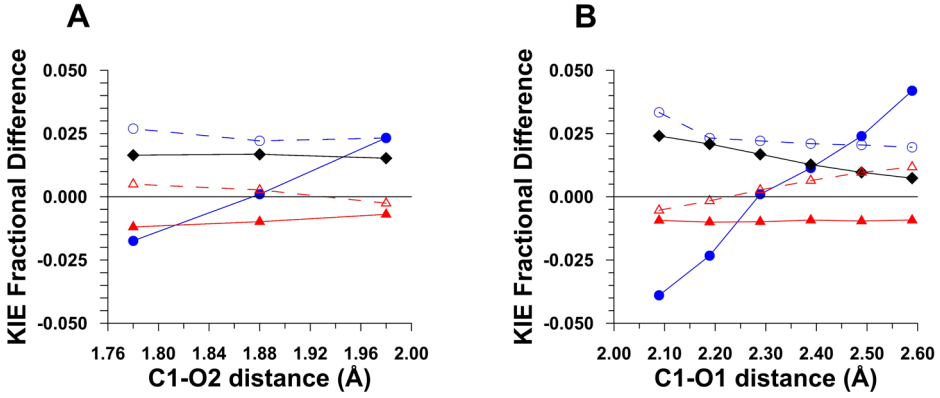


Figure 4. Calculated fractional errors between the measured kinetic isotope effects on the base-promoted hydrolysis of 4-nitrophenyl α-D-mannopyranoside (T = 25 °C) and those calculated for the *gauche*−*gauche* conformer at the B3LYP/6-31G* level of theory; a negative value indicates that the calculated KIE is smaller than the experimental value. (A) The interatomic distance between the anomeric carbon and the leaving group oxygen (C1−O1) was fixed at 2.2893 Å. (B) The interatomic distance between the anomeric carbon and the nucleophilic oxygen (C1−O2) was fixed at 1.8824 Å. Secondary deuterium KIEs are in blue α-SDKIE (filled circle, solid line); β-SDKIE (hollow circle, dashed line), ^{18}O-KIE are in red; leaving group-^{18}O (filled triangle, solid line), nucleophilic-^{18}O (hollow triangle, dashed line), and anomeric-^{13}C KIEs are in black (diamond).

a prediction in accord with the measured KIE (k_H/k_D) of 1.112 ± 0.004 (Table 1).

The α-SDKIE measured for base-promoted hydrolysis of PNPMan differs markedly from the reported value for the base-promoted methanolysis of phenyl β-D-glucopyranoside, which also proceeds via an oxirane intermediate. However, Dahlquist et al. measured their α-SDKIE (k_H/k_D = 1.03 ± 0.01) using a radiolabeling methodology with [^{3}H]phenyl β-D-glucopyranoside as a surrogate for the anomeric C−H isotopologue.[50,51] At that time, the effect of tritium substitution on the pK_a value of phenol and thus its leaving group ability was not considered during analysis of the KIE. It is now appreciated that tritiated phenols are less acidic than the parent phenol, a conclusion that is based on the reported pK_a perturbation for deuterium substituted phenols,[52] and therefore are poorer leaving groups, which should result in a lower KIE value due to greater nucleophilic participation at the transition state. As a result, we conclude that the α-SDKIE value of Dahlquist et al. must be considered to be a lower limit for formation of the diastereomeric oxirane intermediate (1,2-anhydro-α-D-glucopyranose) compared to the one formed in the current study.

Computational Modeling of Transition State Structures. Modeling of the transition state structure for the base-catalyzed hydrolysis of PNPMan was performed at the B3LYP/6-31G+G(d,p) level with the ground state and transition states using a self-consistent reaction field (SCRF) with an integral equation formalism polarizable continuum model (IEF-PCM), with the dielectric constant set to 78.3553, and with a Bondi atomic radii for the molecular solvation cavity.[53] The ground state geometry of PNPMan was optimized without constraints in a $^4C_1$ chair conformation with two distinct conformers for the C6-CH_2OH group (Figures S1 and S2, Supporting Information). Although the theoretical modeling of TS geometry was performed using a polarizable continuum model, we incorporated an explicit water molecule that was used to 'solvate' the oxyanion at carbon-2, so as to prevent the *cis*-hydroxyl group at C-3 from engaging in a strong intramolecular H-bond to O2 and as a result unduly hinder neighboring group participation at the anomeric center. Following location of transition state structures, where the *gauche*−*gauche* TS (Figure 3) is slightly lower in energy than the *trans*−*gauche* TS (Figure S3), the associated KIE values were calculated using the computer program ISOEFF98 (the 'Initial' TS; Tables 1 and S3).[54] Of note, our initially located TSs only provided a good match to the experimental value for the leaving group ^{18}O-KIE. We therefore included Bondi

DOI: 10.1021/jacs.6b07935
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atomic radii, which improved the resulting associated KIEs (the 'Unconstrained' TS; Tables 1 and S3). Additional calculations were executed to improve the TS model by fixing the critical C−O reaction coordinate bond distances (C1−O1 and C1−O2), energy minimizing the restricted structure, and then by calculating the KIE values. This process was performed repeatedly with systematic variation of the two C−O bonds in 0.1 Å increments. The KIE values associated with all of these TSs are listed in Tables S4−S7 (Supporting Information), while the best TS match to the experimental KIE data is included in Tables 1 and S3 (the 'Constrained' TS). Figure 3 also presents the refined TS model that best fits the experimental KIE values (C1−O1 = 2.2893 Å and C1−O2 = 1.8824 Å). Listed in Tables S8−11 (Supporting Information) are bond lengths, bond, and torsional angles calculated for the ground state, unconstrained and constrained transition states, and the oxirane intermediates.

Our analysis identifies several interesting trends when certain distances are held constant while others are allowed to vary systematically. We note that when the C−O bond between the anomeric carbon and the 4-nitrophenoxide leaving group was fixed at 2.2893 Å and the distance between the nucleophilic oxygen and anomeric center varied during computational modeling, the calculated KIEs values for the anomeric-^{13}C and β-secondary deuterium are almost invariant (Figures 4 and S4, panel A). In addition, the two critical ^{18}O-KIEs show opposite trends as the nucleophile−electrophile distance is increased at the oxirane forming TS (Figures 4 and S4, panel A). Shortening the nucleophile-anomeric carbon distance (C1−O2) at the transition state without changing the glycosidic bond distance results in the following trends: (i) the leaving group ^{18}O KIE decreases; (ii) the nucleophile ^{18}O KIE increases; and (iii) the anomeric ^{13}C-KIE displays a slight increase.

On the other hand, lengthening the anomeric carbon-leaving group oxygen distance (C1−O1) at the transition state without changing the nucleophile−anomeric carbon distance results in the following trends: (i) the leaving group ^{18}O-KIE is almost unaffected; (ii) the nucleophilic ^{18}O-KIE increases substantially; and (iii) the anomeric ^{13}C-KIE decreases substantially (Figures 4 and S4, panel B). Notably, tight transition states (shorter C−O bonds) are associated with larger ^{13}C-KIEs and small ^{18}O-KIEs for both the nucleophile and leaving group. Interestingly, the C2-^{18}O-KIE, which is characteristic for oxirane formation, can be computationally modeled by a wide range of transition states.

EIEs were also calculated for the reaction using the local ground state and oxirane structures optimized with B3LYP 6-311++G(d,p). The resulting isotope effects for all isotopologues were found to be close to unity except for the C1-D and C2-D isotopologues that had calculated EIEs of ∼1.10 and 1.08, respectively (Tables S12 and S13, Supporting Information).

Finally, we note that Kamerlin and co-workers have shown that energetically similar but mechanistically distinct processes may occur for phosphate monoester hydrolysis and have highlighted the difficulty of calculating the preferred mechanism for *p*-nitrophenyl phosphate owing to the limitations in compressing multiple bonding changes onto the two-dimensional reaction energy surface of a More O'Ferrall Jencks plot.[55] In their case systematic variation of critical bond distances allowed identification of dissociative and associative transition states of similar energies, for which only a solvent-assisted dissociative pathway provided good agreement with experimentally determined KIE values.

CONCLUSIONS

Substitution reactions at the anomeric center of glycosides and other glycosyl derivatives occur through a spectrum of mechanisms.[3,56] Owing to the ability of the lone pair on oxygen to stabilize developing charge, reactions that tread the borderline of S_N1 (A_N*D_N)[29,30,57−59] and S_N2 (A_ND_N)[32,43,60,61] processes are most common, with the existence of a discrete pyranosylium ion intermediate contingent upon the degree of nucleophile and solvent participation. An S_Ni ('internal return') mechanism (D_N*D_h*A_N) has been reported for cases involving an intimate complex of the substrate's leaving group and a preassociated nucleophile.[28,62] Reactions proceeding through such processes have been studied extensively in solution, and closely related counterparts have been identified in various enzymic processes, catalyzed by glycosidases, glycosyltransferases, and carbohydrate phosphorylases (e.g., S_N1,[63,64] S_N2,[63] and S_Ni[65]).

The hydrolysis reaction of 4-nitrophenyl α-D-mannopyranoside in basic aqueous media occurs via a transient oxirane intermediate with rate limiting C−O bond cleavage that is coupled to neighboring group participation from a preionized oxyanion on carbon 2. The present work reveals that this reaction is associated with a large nucleophilic KIE (k_{16}/k_{18}), the magnitude of which appears to be related to the timing of build-up in ring strain upon formation of oxirane of the 1,2-anhydro sugar, which leads to a late transition state. Initial theoretical modeling of the reaction transition state structure resulted in a computational model that gave poor agreement with the experimental KIE values. However, upon systematic variation of critical transition state bond distances, better agreement between theory and experiment was obtained, an approach that has been successfully applied by Kamerlin and co-workers.[55] While the alkaline solvolysis of PNPMan is a specific base-catalyzed process, it is to be expected that a slightly attenuated nucleophilic KIE should also manifest in a general base-catalyzed process (where the negative charge on the nucleophile at the TS is reduced), such as that proposed for the mechanism of hydrolysis of GH99 *endo*-1,2-α-mannosidase/*endo*-1,2-α-mannanase.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b07935.

Full author list for ref 53, experimental procedures, spectroscopic data, ball and stick diagram for the local ground state, Cartesian coordinates and the sum of electronic and zero-point energies for all TSs, ground states and oxirane intermediates (PDF)

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Notes

The authors declare no competing financial interest.

DOI: 10.1021/jacs.6b07935
J. Am. Chem. Soc. 2016, 138, 14012−14019

■ ACKNOWLEDGMENTS

A.J.B. thanks the Natural Sciences and Engineering Council of Canada (NSERC) for financial support (Discovery Grant 121348-2012). S.J.W. thanks the Australian Research Council for financial support (FT130100103; DP120101396). M.F.-D. thanks NSERC for a graduate scholarship (PGS-M). The authors thank Prof. Vern Schramm and Dr. Zhen Wang (Albert Einstein College of Medicine) for providing computational training to M.F.-D.

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J. Am. Chem. Soc. 2016, 138, 14012−14019