Mechanisms for nucleophilic aliphatic substitution at glycosides

NICOLE A. HORENSTEIN

Department of Chemistry, University of Florida, Gainesville, Florida, 32611 7200 USA

Abstract

Much of carbohydrate chemistry and biochemistry is centered on bond forming and bond breaking reactions at the anomeric carbon of glycosides. No single mechanism adequately covers the scope of these reactions, because differences in sugar substituents, stereochemistry, leaving groups, nucleophiles, and catalysts can influence the mechanistic pathway taken. The influence of solvent is only now beginning to become apparent in greater detail. Several methods exist to probe the mechanisms of these reactions; they include a variety of kinetic studies, including isotope effects, and computational methods. It has been found that typical reactions will uniquely utilize a mechanism somewhere within a continuum between A_ND_N and $D_N + A_N$ mechanisms. With knowledge of the factors that determine the mechanism, synthetic method development will be furthered and a deeper understanding of biological catalysis is likely to be gained.

ADVANCES IN PHYSICAL ORGANIC CHEMISTRY
VOLUME 41 ISSN 0065-3160 DOI: 10.1016/S0065-3160(06)41005-4

1 Scope and rationale for this review

This chapter is focused primarily on the mechanisms of non-enzymatic nucleophilic displacement reactions at the anomeric carbon of glycopyranosides. This includes solvolysis (primarily hydrolysis) reactions and reactions leading to creation of glycosides (glycosidation). Interestingly, a rich chemistry of the anomeric carbon functioning as a *nucleophile* is known, but will not be covered here.[1] Enzyme-catalyzed glycosyl transfer reactions are not the focus of this review, however excellent reviews are available that treat *N*- and *O*-glycohydrolase enzyme mechanisms.[2–4] This review is primarily focused on the literature from 2002. Interested readers are referred to the excellent reviews[5,6] by Bennet and Kitos, and Berti and Tanaka which cover work prior to 2002. Some of the key areas of coverage include the issue of the timing of steps in reactions at the anomeric carbon, the role of substituent identity and stereochemistry in reactivity and product stereochemistry at the anomeric center, lifetime of glycosyl oxocarbenium ions, computational analyses of reaction pathways and conformational analysis of oxocarbenium ions, and gas-phase studies of oxocarbenium ions. Despite some overlap with prior reviews, several highlights from the relevant literature are first presented to facilitate readers' perspectives.

There are several reasons for interest in the mechanistic chemistry of the glycosidic bond. First, carbohydrate chemistry is one of the oldest areas of inquiry in organic chemistry, yet still remains an extremely active field in synthetic, mechanistic, and biochemical studies. The driving force for this is a combination of different factors. In biology, complex carbohydrates play a critical role in cellular recognition events and disease states.[7,8] Mechanistic studies can impact many aspects of biological science by providing a solid basis for understanding factors important in the biosynthesis of glycans. The richness and diversity of carbohydrate structures begins at the level of the many variations in the monosaccharide building blocks. This is further expanded by the many regioisomers and stereoisomers that can be obtained when oligosaccharides and higher polymers are assembled by creation of the glycosidic linkage between monosaccharides. As will become apparent in later sections, the fundamental chemistry of the glycosidic carbon is exquisitely sensitive to the three-dimensional disposition, and identity of functional groups that surround it. Considerable work remains to characterize and understand these factors in detail. Further, synthetic chemists are pressed to assemble ever more complex saccharide structures. Mechanistic understanding of the basic process of glycoside hydrolysis and formation is likely to facilitate further synthetic method development[9] and continue to provide insights into enzyme catalytic mechanisms. Finally, as a purely

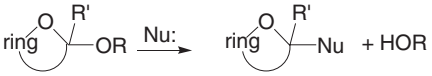
intellectual pursuit, the mechanisms of nucleophilic displacements at glycosidic carbon turn out to be rather interesting, because no single mechanism exists. Rather, a mechanistic continuum is operative, and the precise mechanism is a complex function of saccharide structure, leaving group properties, nucleophile properties, solvent, and the nature of catalysis. The tools of kinetic isotope effects (KIEs), computational methods, and even gas-phase chemistry are now being applied to more complex systems, often of direct synthetic relevance.

2 Introduction and general points

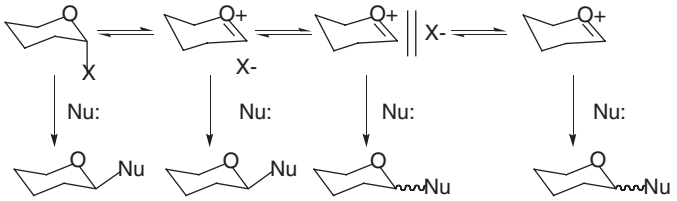
In the simplest sense, the reaction under discussion is the net exchange of a nucleophile for a leaving group, often an alkoxy substituent, at C-1 (the "anomeric" carbon) in a cyclic acetal or ketal as shown in Scheme 1.

An important structural distinction must be made between simple acetals and ketals versus their more complex carbohydrate homologs, the aldoses and ketoses. The rich hydroxylation patterns found in the naturally occurring saccharides can impact the mechanisms and outcome of nucleophilic displacement reactions. This review is primarily focused on nucleophilic displacements at the anomeric center of glycosides, though some reference to model acetal chemistries will be made. Aldofuranose and aldopyranose anomeric carbons have a secondary substitution pattern, one group being the ring alkoxy and the other the ring alkyl substituents. The cyclic forms of ketoses can be considered to have tertiary anomeric centers. The cleavage of a glycosidic bond only occurs significantly under acid catalysis; rates of hydrolysis are greatly diminished at neutral and alkaline pH values for alkyl aglycons,[10] though spontaneous or base-catalyzed loss of aglycon can be significant for leaving groups that are either highly stabilized or positively charged in the glycoside. Capon[11] has extensively reviewed the literature for acid-catalyzed hydrolysis and glycosylations prior to 1969, and Cordes and Bull[12] have reviewed the mechanisms for hydrolysis of acetals and related compounds.

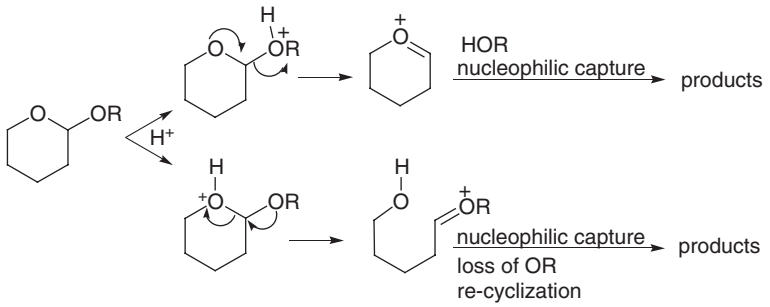
A closer look at glycoside chemistry, utilizing analyses of product stereochemistry, trapping experiments, and KIEs reveal that much like displacement reactions at secondary aliphatic carbon, a mechanistic continuum is possible ranging from dissociative loss of the aglycon, first producing a solvent-equilibrated glycosyl oxocarbenium ion, followed by nucleophilic capture, to an associative mechanism, featuring concomitant attack of a nucleophile and loss of the aglycon.[13] The IUPAC nomenclature will be favored here, thus the two limiting processes mentioned above are referred to as ($D_N + A_N$) and ($A_N D_N$), respectively.[14] These two extremes represent an oversimplification of the nature of possible intermediates; Scheme 2 illustrates an expanded view of the possible steps that may be relevant in a glycoside



Scheme 1.



Scheme 2.



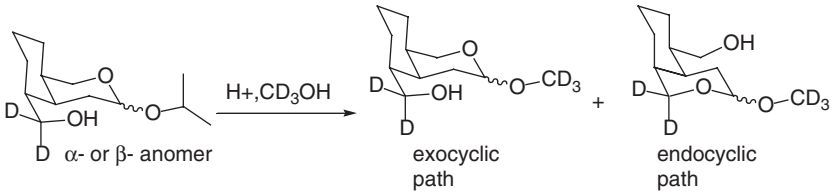
Scheme 3.

solvolysis or nucleophilic displacement reaction. Richard and coworkers have recently reviewed mechanistic considerations of ion pairing for solvolysis of non-glycoside compounds.[15]

Where the reacting sugar is finally captured in the mechanistic scheme, Scheme 2 will be determined by the nucleophilicities of the leaving group X and Nu, solvent effects, and the stability of the oxocarbenium ion. An early insight into the mechanism of substitution at the glycosidic carbon comes from the observation that few of these reactions are stereospecific; immediately arguing for mechanisms that will typically involve oxocarbenium ions. As will be discussed, oxocarbenium ion chemistry is dominant but not exclusively so, since there is good evidence to suggest that A_ND_N processes do occur.

The acid-catalyzed cleavage of a glycosidic bond can involve exocyclic cleavage of the protonated glycoside, or first involve endocyclic cleavage of the ring C–O bond, in a pathway that involves more steps to lead to the final substitution product (Scheme 3). Reactions of pyranosides have generally been considered to proceed with exocyclic C–O cleavage, however there are indications that the endocyclic pathway can be operative.[16–18]

In studies employing a pseudosymmetric deuterated acetal (Scheme 4), Anslyn and coworkers explored the preference for endocyclic versus exocyclic cleavage as a function of the starting acetal stereochemistry.[17] They found that the α-acetal with the leaving isopropoxyl group axial gave only products arising from the exocyclic pathway, while the β-configured anomer afforded products deriving from both pathways. An analysis of the effect of temperature on the product distribution for methanolysis of the β-acetal allowed calculation of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the endo- and



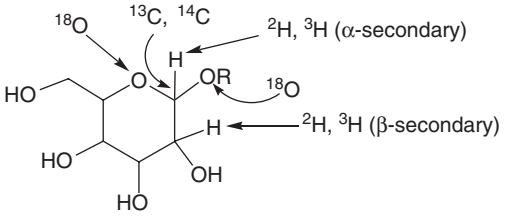
Scheme 4.

exocyclic paths. Increasing fractions of endocyclic cleavage were observed at decreasing temperatures. The enthalpy and entropy activation parameters for endocyclic cleavage were $19.2 \pm 1.4\,\text{kcal mol}^{-1}$ and -12.6 ± 6.1 e.u., while for exocyclic cleavage they were $22.8 \pm 1.1\,\text{kcal mol}^{-1}$ and 3.7 ± 3.8 e.u. While the respective enthalpic terms are quite similar there is a substantial difference in the entropic terms which accounts for the diminishing amount of exocyclic cleavage at lower temperatures. Further, the -12 e.u. observed for the endocyclic activation entropy was considered to be too negative to be solely accounted for by freezing of the exocyclic C–O bond in the transition state. Thus the large negative value was used to propose that nucleophilic participation (by solvent) is a larger component of the endocyclic pathway than found for the exocyclic pathway. These conclusions need to be considered cautiously since the differences in entropy of activation could reflect differences in solvation without requiring nucleophilic participation per se. For example, in the endocyclic model, liberation of the endocyclic oxygen could in principle involve a different change in solvent structure than for liberation of the exocyclic aglycon oxygen. The modest enthalpic advantage to the endocyclic route was rationalized on the basis of the endocyclic bond being weakened due to partial $n \rightarrow \sigma^*$ donation from the exocyclic O to the anomeric carbon. There are few confirmed reports of endocyclic cleavage of pyranosides, and it is generally assumed that the exocyclic pathway is operative. What is certain from these model studies is that an endocyclic pathway may be competitive with exocyclic pathways under the appropriate conditions, including a β-aglycon leaving group, and lower temperatures. This point is worth keeping in mind.

3 Key experimental methods

Two invaluable methodologies that can probe the structure of transition states and the lifetimes of short-lived glycosyl oxocarbenium ion intermediates are KIEs and competitive trapping methods. This section presents a brief overview and discussion of these methods; readers are referred to excellent reviews for more in depth commentary.[6,15] Sugars are extremely well suited to KIE studies because all of the atoms at and around the glycosidic bond can be isotopically labeled to report on changes in bonding that occur in proceeding from the ground to transition states. Scheme 5 illustrates the positions about a glycoside that can be labeled and are particularly useful.

The α-^{2}H (or ^{3}H) secondary KIE has seen extensive application in glycoside cleavage reactions. To a lesser degree, secondary β-^{2}H (or ^{3}H) and primary ^{13}C (or



Scheme 5.

^{14}C) isotope effects have also been employed. The α-effect arises when the force constants at the anomeric carbon differ in the rate-limiting transition state from the ground state. This KIE has been taken to provide a measure of the degree of rehybridization from sp_3 towards sp_2 at the transition state. Often, a sizeable isotope effect at H-1 has been used to suggest that the mechanism of the hydrolysis is a dissociative one, with the implication that an oxocarbenium ion intermediate is formed subsequent to the transition state reported on by the KIE. This may be in error however, because even in a concerted bimolecular process, the hybridization at the anomeric carbon is changing and therefore this alternate mechanism is not ruled out by the observation of an α-deuterium KIE. Probably for synthetic reasons, the β-deuterium and primary carbon effects have been employed less often. This is unfortunate, because they provide a clearer picture of the reaction mechanism. The origin of the β-KIE is primarily due to hyperconjugation between the β-hydron and the partially vacant orbital at C1. The isotope effect is sensitive to charge development at the anomeric carbon, and provides a more reliable estimate of charge than the α-effect can. The primary carbon isotope effect is complementary to the β-deuterium KIE. The origin of the KIE at carbon is related to the net loss of vibrational energy between ground state and transition state, along with a contribution to reaction coordinate motion. Concerted bimolecular displacements produce large KIEs ($^{13/12}k > 1.03$; $^{14/12}/k > 1.06$) while dissociative reactions that feature oxocarbenium ion-like transition states without nucleophilic participation have KIEs that are below 1.03 or 1.06, respectively, for ^{13}C or ^{14}C isotopes.

Thus, a pattern of a large β-deuterium KIE and a small carbon KIE are indica tive of a dissociative transition state, while small β-deuterium isotope effects and a large carbon isotope effect provide the signature of an associative transition state involving both a leaving group and a nucleophile. Observation of the latter pattern then necessarily rules out an oxocarbenium ion intermediate, and strongly points towards an A_ND_N mechanism. On the other hand, KIEs that identify a dissociative transition state cannot rule out the case of a very weakly associated nucleophile, and cannot report on post-rate-determining events. Ring ^{18}O KIEs can report on bonding changes between the anomeric carbon and the ring oxygen (i.e. C1–O5 in a pyranose). In a mechanism involving oxocarbenium ion-like transition states, the participation of non-bonding electrons of the ring oxygen causes a net tightening of force constants around this atom, with an associated inverse KIE with values between 0.98 and 1.00. It is interesting to consider how

possible changes in solvation at O-5 between the ground and transition states might perturb the O-18 KIE.

For substitution mechanisms that involve formation of a discrete oxocarbenium ion intermediate, it is possible to trap the intermediate with nucleophiles that result in configurationally stable products. A stereochemical analysis leads to information about the concertedness of the reaction, and quantification of the ratio of products arising from the solvolytic capture to products arising from capture by added nucleophile can provide an estimate of the lifetime for the oxocarbenium ion. In the absence of other nucleophilic species, the solvolytic capture by water is not a useful stereochemical probe because the products often mutarotate at a rate that is far too great to allow identification of the initially formed product's stereochemistry. If stable product mixtures are obtained that reflect both retention and inversion with respect to the original glycosidic stereochemistry, this indicates that the reacting glycon has progressed to solvent-separated and/or -equilibrated oxocarbenium ions. If inversion is observed, this could indicate either an A_ND_N mechanism or reaction with nucleophile from an intimate ion pair. Depending on the relative values of the rate constants, multiple reaction modes could operate simultaneously. Trapping with powerful nucleophiles such as azide affords the possibility of "clocking" the lifetime of reactive oxocarbenium ion species. The rate diffusion controlled rate constant for capture of a carbenium ion by a strong negatively charged nucleophile is approximately $5 \times 10^9\,M^{-1}\,s^{-1}$.[15] In a competitive trapping experiment, in which the same oxocarbenium ion species can be captured by azide or solvent (typically water), product ratios for azide versus solvent trapped product yields the ratio of rate constants. The known rate constant for the azide reaction allows determination of the rate constant for the water reaction, and its reciprocal represents the lifetime of the cation. Note that highly stabilized carbenium ions may have an intrinsic barrier to capture by azide, and for such species the assumption of diffusion controlled capture will not be valid.[19] Sugar-derived oxocarbenium ions are on the order of stability of the *t*-butyl cation, and are sufficiently reactive to lend themselves to this methodology.

4 Studies of glucosides

SOLVOLYSIS AND TRAPPING EXPERIMENTS

A little over 30 years ago it was commonly thought that acid-catalyzed aldopyranoside substitutions at the anomeric carbon proceeded by dissociative mechanisms that involved oxocarbenium ion intermediates (formerly referred to as oxycarbonium ions).[11,12] However, evidence was available that suggested this generality might not be valid. Vernon and coworkers showed that methanolysis of phenyl glucopyranosides proceeds with considerable inversion of configuration, suggesting that the oxocarbenium ion species was not solvent equilibrated.[20] Interestingly, that same study presented an early use of ^{18}O leaving group KIEs, and used these data to argue in favor of a dissociative mechanism involving exocyclic glycosidic bond

cleavage. A quantitative sense of the degree of stability of oxocarbenium ion species was lacking however. In mixtures of ethanol/trifluoroethanol, solvolysis of glucopyranosides yielded product distributions that were sensitive to both leaving group and the starting anomeric configuration.[21] This result is inconsistent with a mechanism involving a solvent-equilibrated oxocarbenium ion. Interestingly, the proportion of ethyl glycoside to trifluoroethyl glycoside did not correlate with retention or inversion pathways – surprising, given the greater nucleophilicity of ethanol. This led to the conclusion that the transition state involved the solvent's facilitation of leaving group departure.[21]

In 1986, Bennet and Sinnott reported a comparison of salt effects for solvolysis of aldopyranosides versus methoxymethyl acetals in water, as part of a larger study that featured KIEs for glucoside hydrolysis.[22] It was found that dinitrophenyl (DNP) glucoside hydrolysis was nearly insensitive to addition of a variety of salts having a wide range of nucleophilicities and basicities, while the methoxymethyl acetals were clearly sensitive to the nucleophilicity of the added salt. The results led to the conclusion that the glucopyranosides did not involve a nucleophilic component at the transition state, possibly explained by the stricter steric requirements for attack at the more hindered secondary anomeric carbon of an aldopyranosyl ring as compared to the primary carbon of a methoxymethyl compound. Though not proof, the results suggested that an oxocarbenium ion intermediate with a very short lifetime was formed.

In 1989, Amyes and Jencks reported a study of azide common ion inhibition for solvolysis of a series of α-azido ethers (Fig. 1).[23] The rate constants k_{HOH} for addition of water to the oxocarbenium ions were determined with an assumed diffusion controlled second-order rate constant k_{az} of $5.0 \times 10^9\ M^{-1}\ s^{-1}$ for attack on the oxocarbenium ion by azide and the observed rate suppression by varying concentrations of added azide, as determined from Equation (1). With k_{HOH} in hand, the lifetime of the oxocarbenium ion was taken as k_{HOH}^{-1}

$$k_{obs} = k_{solv}k_{HOH}/k\left(k_{HOH} + k_{az}[N_3^-]\right) \tag{1}$$

From the estimated lifetimes for the series (Fig. 1), it was possible to propose a series of substituent effects on oxocarbenium ion lifetime, which were then used to

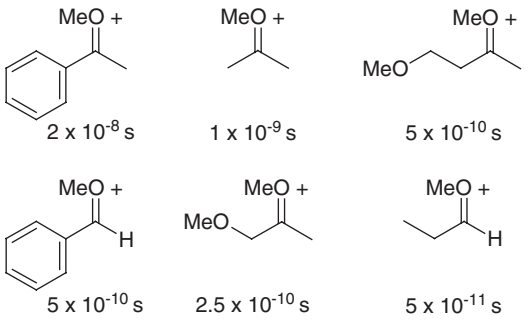


Fig. 1 Oxocarbenium ion lifetimes in aqueous solution. Adapted from Ref. [23].

estimate that the lifetime of a glycopyranosyl oxocarbenium was on the order of 10^{-12} s. Estimates of the equilibrium constants for formation of the oxocarbenium ion from the azido ether were also reported in this study. They were obtained by dividing the observed first-order rate constant for solvolysis by the apparent first-order rate constant for capture of the oxocarbenium ion by azide at a given concentration. The results are interesting and show that substituents have a greater effect on K_{eq} for oxocarbenium ion formation than for the rate at which water captures the oxocarbenium ion. A comparison pertinent to sugar chemistry is between the acetone *O*-methyl and the β-methoxy acetone *O*-methyl oxocarbenium ions which serve as models for the glucosyl and 2-deoxyglucosyl oxocarbenium ions, respectively. The observed rate constants for capture of these two species by water is differed by a factor of 4, but the ratio of the equilibrium constants for formation of these species is approximately 600. Thus, 2-deoxysugar oxocarbenium ions have a significantly greater intrinsic stability than their 2-hydroxy counterparts, but their reactivity is very much the same. This provides an indication that the transition states for their capture is early.

It has been shown that changes of leaving group and nucleophile can affect the nature of the substitution reaction in a profound way. The α-anomer of glucopyranosyl fluoride was examined in its reaction with a charged and uncharged nucleophiles in water.[24] Reactions were first order in concentration for anionic nucleophiles, but the rate was independent of concentration of uncharged (amine) nucleophiles. The Swain–Scott plot (Fig. 2) of the second-order rate constants for a given anionic nucleophile versus its *n* value had a modest slope of 0.18, indicating a weakly nucleophilic transition state. The stereochemical course of the reaction was complete inversion for azide as the nucleophile, consistent with a mechanism

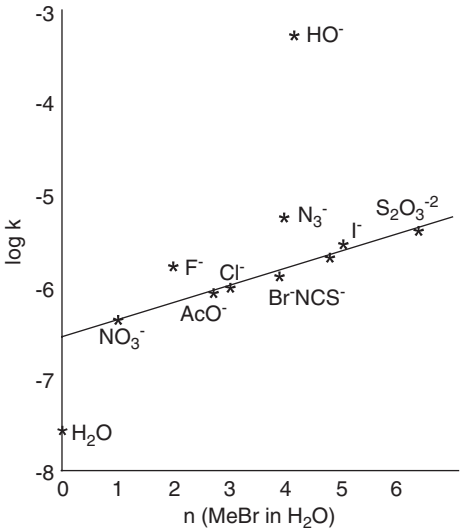


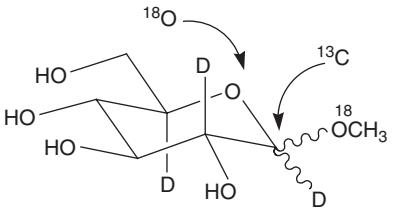
Fig. 2 Swain–Scott plot for reactions of nucleophiles with α-D-glucopyranosyl fluoride in water. Adapted from Ref. [24].

involving a concerted bimolecular substitution at the anomeric carbon. By product analysis, all of the β-azido glucoside observed could be accounted for by the observed second-order rate constant for the azide reaction. This excludes any significant contribution to the reaction from azide trapping of solvent-separated or -equilibrated oxocarbenium ion species. On the other hand, a modest rate dependence on [nuc^-] could be rationalized on the basis of a stepwise preassociation mechanism in which a tight ion pair reacts with the nucleophile. However, the Swain–Scott plot is linear over the region of nucleophiles stronger and weaker than fluoride. In the stepwise mechanism, there would be a break in the plot corresponding to a change in rate-determining step with nucleophiles better than the leaving group fluoride. This can be rationalized by the idea that loss of fluoride is slower than attack by a better nucleophile, but with weaker nucleophiles, fluoride loss is faster, leading to a non-linear free energy relationship. The results with the fluoro leaving group lead to another key aspect of nucleophilic substitution at the anomeric center, namely that the nature of the leaving group can determine whether or not an intermediate can exist. In this case, the fluoride leaving group is an effective nucleophile for the return reaction from the ion pair, so in the presence of fluoride, the ion does not exist in an experimentally detectable way. Leaving groups such as alkoxy are protonated at the transition state in acid-catalyzed hydrolysis and are much less effective nucleophiles from the ion pair, being uncharged. They are able to diffuse away more rapidly than recapture the oxocarbenium ion, allowing this species to exist as a discrete intermediate.

KIE STUDIES

The acid-catalyzed hydrolysis of α- and β-methyl glucopyranosides has been studied with multiple KIEs.[22,25] Scheme 6 presents a composite representation of the isotopically substituted positions used in the studies. Table 1 presents selected KIE results.

Several observations bear mention from this study. First, both anomers hydrolyze via transition states that have substantial oxocarbenium ion character (significant β-^{2}H effects) are lacking in nucleophilic participation (small ^{13}C KIEs), and are late with respect to loss of the methyl alcohol aglycon (significant leaving group, ^{18}O KIE). These results are consistent with a $D_N{}^*A_N$ or $D_N + A_N$ pathway. Closer inspection of the data reveals significant differences in the β-D KIEs and ring ^{18}O KIEs for each anomer. This led to the conclusion that there are differences in the



Scheme 6.

Table 1 Selected KIE results[22,25]

Isotope label	α-methyl glucoside KIE	β-methyl glucoside KIE
α-D	1.137	1.089
β-D	1.073	1.045
^{18}O-methoxyl	1.026	1.024
^{18}O-ring	0.996	0.991
^{13}C-anomeric	1.007	1.011

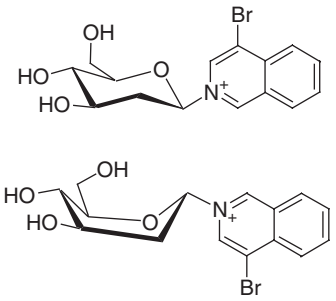
transition state conformation of each sugar. Namely, the α-anomer proceeds via a flattened $^{1}S_{3}$ skew boat transition state, while the β-anomer resembled a flattened $^{4}C_{1}$ chair at the transition state. The lack of any observed nucleophilic character (small primary ^{13}C KIEs) in these transition states supports the idea that subsequent oxocarbenium ion intermediates are formed as discussed above.

Following Banait and Jencks[24] work on azide trapping of fluorosugars, Sinnotts group measured the KIEs for this and related nucleophilic displacements of fluoroglucosides.[26] Perhaps the most important KIE was the primary ^{13}C KIE at the anomeric carbon. Hydrolysis of α-fluoroglucopyranoside resulted in large ^{13}C KIEs of 1.032, indicative of a transition state having bimolecular character and significant nucleophilic participation. The β-^{2}H KIE was still substantial at 1.067, indicating that the transition state had oxocarbenium ion character. In accord with the β-^{2}H KIE, the ^{18}O KIE for the ring oxygen atom was strongly inverse at 0.984 indicating that the ring oxygen was in a tighter environment at the transition state compared to the ground state. In terms of structure this corresponds to the endocyclic O-anomeric C bond as having partial double bond character, or being ''oxocarbenium ion like''. Taken together, the KIEs point to a transition state that is exploded with regard to bond order to the departing fluoride and incoming water, but still involves bonding between each of these groups and the anomeric carbon. The KIE data also point to another subtlety in the reactivity of glucose towards nucleophilic substitution. The β-D-fluoroglucopyranoside hydrolysis reactions show a smaller ^{13}C KIE (1.017) indicating less nucleophilic participation in this transition state. In the presence of azide, the ^{13}C KIE for substitution of α-D-glucopyranosyl fluoride rises dramatically to 1.085. This KIE is the signature for a concerted bimolecular displacement, and demonstrates that in the presence of a suitable nucleophile, and a nucleophilic leaving group, the oxocarbenium ion pathway is not followed.

5 Studies of deoxyglucosides

MECHANISMS

The solvolysis of 2-deoxyglucopyranosides has been studied extensively by the Bennet laboratory with emphasis on the progression through the dissociative mechanistic continuum and characterization of the lifetime of the 2-deoxyglucosyl oxocarbenium ion.



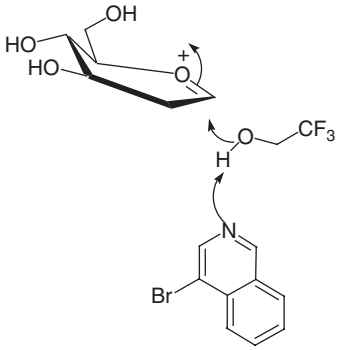
Scheme 7.

Solvolysis of both α- and β-2-deoxyglucopyranosyl-isoquinolinium salts allowed generation of oxocarbenium ions with a poorly nucleophilic leaving group (Scheme 7). It was observed that solvolysis of the β-anomer in the presence of the series of nucleophilic monoanions AcO^-, Cl^-, Br^-, and N_3^- gave a Swain–Scott parameter of 0.03 ± 0.05. Similarly, solvolysis of the α-compound in the presence of the same series of salts afforded a Swain–Scott parameter of 0.03 ± 0.10. The results indicate little or no sensitivity to anion nucleophilicity and therefore point to a mechanism that does not involve an A_ND_N transition state.[27,28]

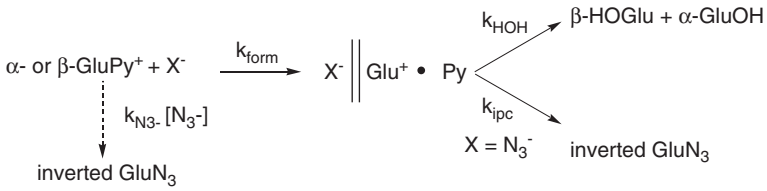
Later work concluded that while the 2-deoxyglucosyl isoquinolinium compounds do not react by an A_ND_N mechanism, neither do they solvolyze via a free oxocarbenium ion.[29] The key result was the observation that in aqueous/alcohol mixtures, solvolysis of 2-deoxyglucopyranosyl-isoquinolinium anomers affords diastereomeric product mixtures whose composition depended on the starting anomeric configuration of the glycoside. This ruled out a solvent-equilibrated oxocarbenium ion intermediate for the solvolysis of the diastereomeric glycosides. This narrows the focus of the mechanistic framework for the formation of substitution products at the stage of an ion–molecule pair and a solvent-separated ion–molecule pair. In methanol/water and ethanol/water systems the α-anomer showed a modest preference for formation of inverted products, while the β-anomer showed a strong preference for inversion. The observation of some retained product in all cases demonstrates that the reaction manifold must include the solvent-separated ion–molecule complexes. Interestingly, in trifluoroethanol/water mixtures, the α-anomer afforded the retention products as the major component, and following this same trend, the β-anomer showed reduced preference for inversion. The model invoked to explain this pattern (Scheme 8) involves general base catalysis from isoquinoline in the solvent-separated ion–molecule complex, providing a bias towards retention.

LIFETIMES OF OXOCARBENIUM IONS

Solvolysis of 2-deoxyisoquinolinium salts in the presence of azide does not show A_ND_N kinetic behavior, and the 2-deoxyglucosyl oxocarbenium ion is not solvent



Scheme 8.



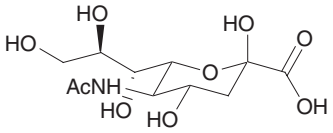
Scheme 9.

equilibrated. Its reactions will occur from solvent-separated ion or ion–molecule pairs in the $D_N{}^*A_N$ mechanism.[29] In the presence of the good nucleophile azide, competitive trapping of the oxocarbenium ion yields a mixture of 2-deoxyglucose and inverted 1-azido 2-deoxyglucoside. That the inverted azido product predominated indicates that the collapse of the solvent-separated ion pair of azide and oxocarbenium ion to intimate ion pair is faster than reorganization of the solvent-separated ion pair to place azide on the other face of the oxocarbenium ion (Scheme 9). The net rate constant of diffusion of a leaving group from the oxocarbenium ion and solvent reorganization, k_{form}, can be estimated to be on the order of 1×10^{-11} s, as described.[15] Thus, the 2-deoxyglucosyl oxocarbenium ion is very much on the border of having a real lifetime, but can be detected.

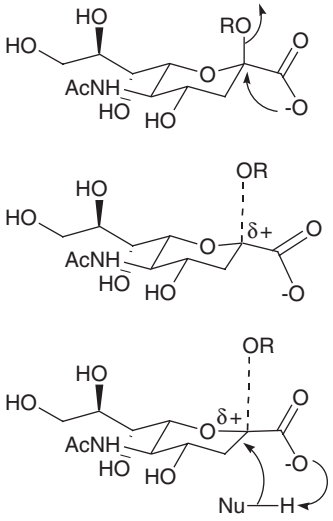
As a result of these studies, and the approximately 4-fold greater stability attributed to 2-deoxyglycosyl oxocarbenium ions over their 2-hydroxy counterparts, Zhu and Bennet estimated that the lifetime for the 2-deoxyglucosyl oxocarbenium ion is on the order of 2×10^{-11} s, and $\sim 10^{-12}$ s for the glucosyl oxocarbenium ion in water, consistent with prior results.[29]

6 Studies of *N*-acetyl neuraminic acids

N-acetyl neuraminic acid (NeuAc) is a saccharide of unusual structure compared to glucose, fructose, and other common monosaccharides (Scheme 10). The sugar is



Scheme 10.

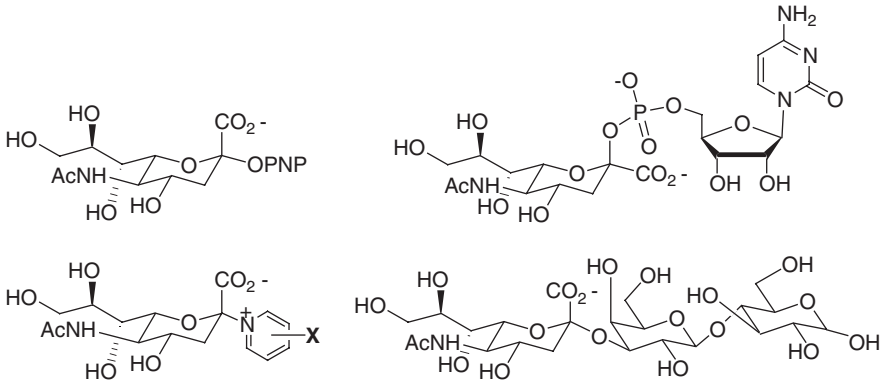


Scheme 11.

formally a 2-deoxyketose, and additionally has a carboxylic acid residue attached to the anomeric center. The carboxyl group is highly acidic with a pK_a of approximately 2.7 for *O*-glycosides and 0.36–0.74 for cationic *N*-glycosides.[30–32] Within the context of nucleophilic substitution at the anomeric carbon, the chemistry is potentially richer than for glucose and other aldoses. As discussed earlier for 2-deoxyglucose, the absence of a hydroxyl substituent adjacent to the anomeric carbon is stabilizing for oxocarbenium ion-like transition states and for the intermediates which could be subsequently formed. NeuAc is not a typical ketose; the anomeric center is substituted with an electron-rich and potentially nucleophilic carboxylate group. One could consider the potential of this group to participate in displacement reactions at the anomeric center, or functioning to stabilize an oxocarbenium ion intermediate, and/or acting as an intramolecular general base to facilitate capture of the oxocarbenium ions by nucleophiles as indicated in Scheme 11.

KIE STUDIES

β-dideuterium, primary ^{14}C, and primary ^{13}C KIEs have been measured for NeuAc glycoside hydrolysis containing α-*p*-nitrophenyl, β-cytidine monophosphate, and



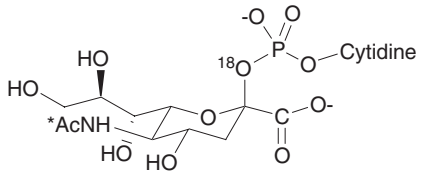
Scheme 12.

α-lactosyl glycosides (Scheme 12). Note that the structure of NeuAc precludes measurement of α-secondary hydrogen isotope effects. A primary ^{14}C KIE of 1.030 was observed for solvolysis of CMP–NeuAc at pH 5.0.[31] As discussed earlier, a carbon KIE of this low is indicative of a transition state lacking significant nucleophilic assistance. Acid-catalyzed solvolysis of NeuAc α (2→3) lactose afforded a ^{13}C KIE of 1.016, which compares well to the ^{14}C for solvolysis of CMP–NeuAc.[33] Again, the results indicated that the transition state involved insignificant nucleophilic participation. From the limited results available, the degree of nucleophilic participation is the same for solvolysis of NeuAc glycosides whether the starting configuration is α or β, and with leaving groups of low and high reactivity. Though the C2 anomeric carbon of NeuAc has three non-hydrogen substituents and is somewhat hindered, it is interesting that "A_ND_N-like" chemistry has been identified for NeuAc in the case of the *trans*-sialidase enzyme. *Trans*-sialidase-catalyzed substitution of NeuAc α (2→3) lactose affords large (1.03) primary ^{13}C KIEs, which led to the conclusion that a covalent intermediate was formed on the enzyme.[33] This was confirmed by isolation of radioactive enzyme substrate adducts in quench experiments[34] and subsequent kinetic and structural studies with fluoro NeuAc substrate analogs.[35,36] One interesting lesson in all of this is that while solution studies of NeuAc solvolysis mechanisms have thus far shown no indication of a propensity towards A_ND_N chemistry, an enzyme can utilize this pathway, despite the bulky environment at the anomeric center. Finally, with regard to carbon isotope effects, KIEs have not been measured for α-aryl or pyridinium glycosides of Neuac. This is of some importance, because as will be discussed below, there are conflicting opinions regarding the likelihood of nucleophilic participation of the carboxylate group.

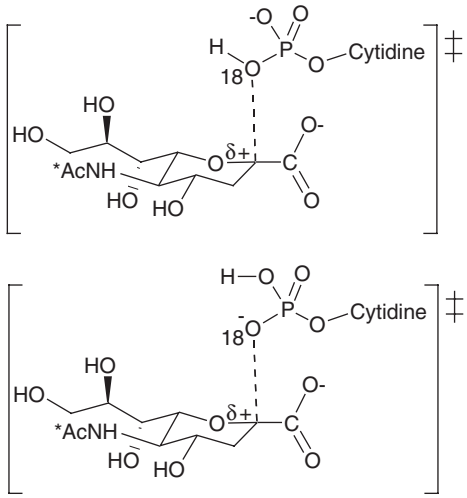
β-deuterium isotope effects point to varying degrees of oxocarbenium ion development in the transition state, depending on the leaving group. Solvolysis of CMP–NeuAc[31] at pHs 4.0, 5.0, and 6.0 proceeded with observed β-dideuterium KIEs of 1.25 ± 0.02, 1.276 ± 0.008, and 1.354 ± 0.008. Some elimination to 2,3-dehydro NeuAc[37] accompanied solvolysis at pH 5.0 and 6.0 (but not at pH 4.0) so it was necessary to account for the contribution that β-elimination makes to the

observed isotope effect.[31] Studies of the effect of buffer concentration on elimination argued against buffer functioning as a general base in the elimination reaction. Further, production of 2,3-dehydro NeuAc from CMP–NeuAc is nearly pH independent between pH 8.0 and 10.0.[37] Solution of simultaneous equations for the KIE data at pH 5.0 and 6.0 with 0.02 and 0.095 mol fractions for elimination, respectively, yielded values of 5.1 and 1.26 for the primary and secondary KIEs. Solvolysis of CMP–NeuAc at pH 4.0 proceeds without detectable β-elimination, and affords a β-^{2}H KIE of 1.25 ± 0.02, in excellent agreement with the estimated value above. The large value observed for the dideuterium KIE is significant, because it indicates that the transition state is late with respect to loss of the leaving group and the anomeric carbon bears considerable positive charge. The β-deuterium isotope effects are lower for alkyl or aryl NeuAc glycosides; note however that these substrates (Scheme 12) have the α-configuration, whereas CMP–NeuAc is a β-glycoside. Sinnott and coworkers[30] reported a series of β-^{2}H isotope effects for solvolysis of α-*p*-nitrophenyl NeuAc. At pH 1.00, the carboxyl group of α-*p*-nitrophenyl NeuAc (pK_a 2.69) is largely protonated, and the observed β-dideuterium KIE was 1.10. Interestingly, the isotope effect drops as the carboxyl group ionizes. At pH 2.69 the observed KIE was 1.08, and at pH 6.67 it was 1.07. The interpretation of these data involved a late transition state with respect to departure of *p*-nitrophenolate, but involved nucleophilic participation once the carboxyl group was ionized. Solvolysis of NeuAc-α(2→3) lactose at pH 1.0, 37 °C, afforded a β-deuterium KIE of 1.11,[33] virtually identical to the value measured for the *p*-nitrophenyl NeuAc glycoside.[30] At this pH the leaving groups could be considered to be of approximately equal reactivity (as protonated oxonium ions) and so the similarity of the two isotope effects argues for similar transition states. Taken with the primary ^{13}C data, the results indicate that the transition state at low pH does not involve nucleophilic participation. Once again, primary carbon isotope effects for *p*-nitrophenyl substituted NeuAc were not measured at any pH, but would be of considerable interest to further probe for nucleophilic character in the transition state. The axial β-CMP is approximately 10 times more reactive as a leaving group than an equatorial α-*p*-nitrophenyl, and it is conceivable that even poorer leaving groups could require nucleophilic assistance.

One question for both solution and enzymatic reactions of the natural NeuAc donor, CMP–NeuAc, is if acid catalysis is operative, whether it is general or specific, and the site at which the proton is delivered. Studies of pH versus rate for solvolysis of CMP–NeuAc were consistent with a mechanism for solvolysis at pH 5.0 that featured specific acid catalysis of the dianion. At the time of the original studies,[31] it was suggested that the departing CMP could be either at the former glycosidic oxygen, or one of the more basic non-bridging phosphoryl oxygens. Subsequently it was suggested[38] that the bridging glycosidic oxygen was the likely protonation site on the basis of inverse solvent deuterium isotope effects. As pointed out by Bennet, microscopic reversibility would argue in favor of protonation occurring at the non-bridging position, which would allow a negatively charged phosphate oxygen to attack the oxocarbenium ion, rather than a much less nucleophilic protonated oxygen.[5] A productive approach to this question involved ^{18}O leaving group isotope effects[39] (Scheme 13).



Scheme 13.



Scheme 14.

Solvolysis of [2-^{18}O] CMP NeuAc at pH 5.0 in acetate buffer afforded a KIE of 1.003 ± 0.005. The ab initio modeling (6-31G* basis set) of the KIE predicted an isotope effect of 1.023 for protonation at the bridging position, while protonation at the non-bridge position gave a predicted KIE of 1.017. Thus, the data is best fit by the non-bridge protonation model (Scheme 14).

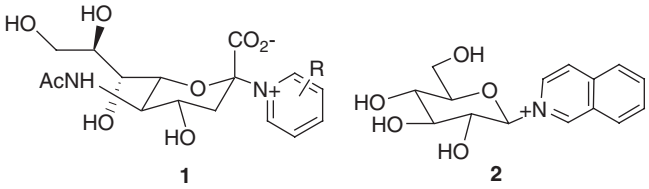
TRAPPING STUDIES AND OXOCARBENIUM ION LIFETIMES

If solvolysis of CMP–NeuAc required nucleophilic participation of the carboxylate, this would proceed via a transient α-lactone, and in a second step give net retention to afford the β-anomer. A direct displacement of CMP by solvent without carboxylate participation would yield the α-anomer, while a transition state that resulted in formation of a sialyl oxocarbenium ion would proceed with results ranging from inversion to racemization depending on whether the oxocarbenium ion lived long enough to equilibrate with solvent. Solvolysis of CMP–NeuAc in methanol/water mixtures at pH 5.0, resulted in ~1:1 ratios of α- and β-methyl glycosides of NeuAc as determined by ^{1}H-NMR.[31] The α/β-methyl glycoside ratio was invariant when the mol fraction of methanol was increased by over 50%, which shows

that there is no methanol concentration dependence over this range. The results are consistent with near or complete solvent equilibration of the sialyl cation; preassociation pathways would be expected to be sensitive to the methanol mol fraction. The selectivity for water versus methanol attack was approximately 1.3. The selectivity for methanol attack in water/methanol is rather low at 1.3:1; high ratios favoring methanol trapping are associated with stable cations, low ratios are typically associated with highly reactive, short-lived carbenium ion pairs. For a series of cumyl cations, the corresponding methanol/trifluoroethanol trapping ratios revealed a limiting minimum ratio of 2 for cations with a lifetime shorter than 10^{-10} s.[40] Such cations were described as ion pairs which reacted with solvent before diffusion of the leaving group could occur. Alternative factors which could favor a low methanol/water trapping ratio might include steric effects disfavoring methanol attack at a bulky tertiary center, or a localized polarity effect which could favor water for solvation of a charge-separated transition state or tight ion pair.

Solvolysis of CMP–NeuAc in 1.8 M acetate buffer at pH 5.0 containing 0.9 M azide results in the formation of both anomers of 2-deoxy-2-azido NeuAc in addition to NeuAc as determined by ^{1}H-NMR product analysis.[38] The relative percentages of α- and β-NeuAc and α- and β-N_3–NeuAc were 5, 68, 25, and 2, respectively. A modest rate dependence on [azide] was observed with an apparent bimolecular rate constant of $(2.1 \pm 0.3) \times 10^{-3}\,M^{-1}\,min^{-1}$ which could only account for half of the α-azido-NeuAc formed. Comparison of rate, product ratio, and stereochemical data indicate that concurrent pathways for formation of N_3–NeuAc are operative, with 17% of product forming from reaction of azide and the tight ion pair, 12% via the solvent-separated ion pair, and 6% from the free NeuAc oxocarbenium ion. From the corrected product ratio data, the lifetime of the oxocarbenium ion was estimated to be $\geq 3 \times 10^{-11}$ s. Taken in conjunction with the data for trapping in methanol, the neuraminyl oxocarbenium ion is probably able to equilibrate with solvent in the absence of a strong nucleophile-like azide, but in its presence only a fraction is able to proceed past the ion pair stage.

Bennet and coworkers studied the spontaneous hydrolysis of a series of pyridinium glycosides of NeuAc[32] (Scheme 15, **1**). These compounds simplify the acid–base considerations for the hydrolytic mechanism by fixing a positive charge on the reactant-state leaving group, leaving only the anomeric carboxyl group as a consideration. Study of the observed rate versus pH led to a rate law for solvolysis that had two rate constants corresponding to spontaneous hydrolysis of the zwitterions (carboxylate) and spontaneous hydrolysis of the cation (carboxyl protonated).



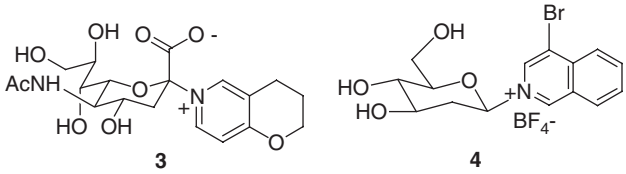
1 **2**

Scheme 15.

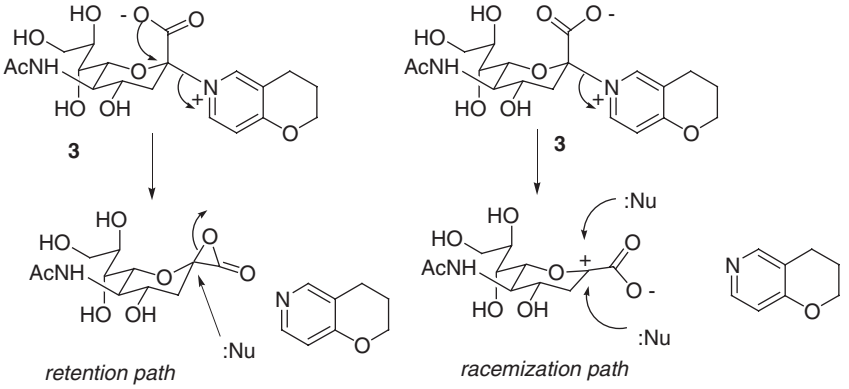
For the series of pyridinium substrates, the rate constant for hydrolysis of the zwitterions was only about 3 times greater than for the protonated form. The relative nucleophilicity of the acetic acid/acetate pair spans a considerably greater range, so this was taken as an indication that the ionized carboxyl would show a greater rate difference from the protonated form if it was functioning as a nucleophile. The comparison with acetate may have its shortcomings – acetate does not have the ring strain penalty associates with α-lactone formation, so in the case of a hypothetical α-lactone-like transition state, the enhanced nucleophilicity of a carboxylate group might be tempered by the ring strain. The β_{lg} values for the two processes were identical at −1.22, indicating a similar and late transition state structure with respect to the leaving group, whether or not the carboxylic group was protonated or not. The reported activation parameters $\Delta H^{\ddagger} = 112\,\mathrm{kJ\,mol^{-1}}$ and $\Delta S^{\ddagger} = 28\,\mathrm{J\,mol^{-1}\,K^{-1}}$ for the unsubstituted pyridinium glycoside was considered most consistent with a transition state not involving nucleophilic participation of the carboxyl group. However, the $\Delta S^{\ddagger} = +48\,\mathrm{J\,mol^{-1}\,K^{-1}}$ for pH independent solvolysis of α-PNP NeuAc was considered[30] to be circumstantially supportive of a nucleophilic role for the carboxylate. The large positive $\Delta S^{\ddagger}$ term was rationalized on the basis of liberation of water solvating the carboxylate at the nucleophilic transition state.[30] These two reports are difficult to reconcile on their own, but as discussed earlier, the issue of carboxylate nucleophilicity might be further addressed by application of carbon KIEs at the anomeric center. Also, data obtained for product analyses of solvolyses of pyridinium *N*-glycosides of NeuAc (discussed below) argue against nucleophilic carboxylate participation as the *sole* reaction manifold.

Armed with earlier correlations of reactivity and oxocarbenium ion lifetimes, Bennet and coworkers[32] used the 2-deoxyisoquinolinium salt **2** (Scheme 15) as a model to make a correlation between its reactivity and known cation lifetime and the neuraminyl pyridinium ion's reactivity to estimate the lifetime of the neuraminyl oxocarbenium ion to be 3×10^{-11} s, in agreement with studies utilizing the azide trapping method.[38]

In 2004, Knoll and Bennet[41] reported a study of the aqueous methanolysis of the α-3,4-dihydro-2H-pyrano[3,2-c]pyridinium *N*-glycoside of NeuAc **3** (Scheme 16). This study was aimed at addressing the possibility that the NeuAc carboxylate group plays a nucleophilic role in the overall displacement process at the anomeric center. One would predict that overall retention stereochemistry would be observed in a two-step displacement process involving initial intramolecular attack by the carboxylate group, followed by backside attack from solvent (Scheme 17). Solvolysis of



Scheme 16.

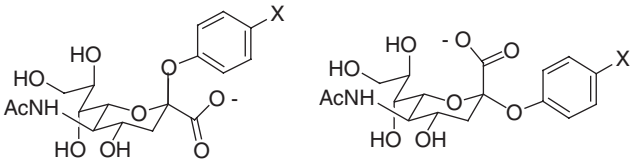


Scheme 17.

3 in either 100% or 50% aqueous MeOH afforded β-methyl glycoside (in 50% MeOH over 50% of the product was the hemiketal product). This result is consistent with a mechanistic model not involving the carboxylate as a nucleophile, at least when presented with the presence of a methanol nucleophile. In that same work, a study of the effect of solvent polarity on k_{obs} was reported. Variation of the %MeOH content between 0% and 100% resulted in only a 10-fold increase in k_{obs} for the 100% MeOH runs. This relative insensitivity to solvent polarity change was considered a good indication that an intramolecular carboxylate pathway was not operative, since such a pathway would have a much lesser charge distribution at the transition state and should have been more sensitive to the decreasing polarity of the reaction mixture than was observed. In comparison, the authors compared the data for **3** to that previously reported for **4**, a 2′-deoxy-3-bromiisoquinolinium glucosyl salt. This compound, which cannot experience nucleophilic participation by a carboxylate would have been expected to be *less sensitive to solvent polarity than the hypothetical nucleophilic carboxylate reactions of* **3**, however it was found to be slightly more sensitive to solvent polarity.

As a final point in these experiments, it was noted that the selectivity ratio for formation of methyl glycoside via MeOH attack versus hemiketal via water attack (k_{MeOH}/k_{H_2O}) was about 1.6 whereas the ratio for solvolysis of CMP–NeuAc was about 1.3.[31] Higher ratios reflect methanol's greater selectivity for stabilized carbenium ion species, and the relatively low values seen for either **3** or CMP–NeuAc provide an indication that the oxocarbenium ion species derived from either substrate are of limited lifetime (i.e. stability). One must be careful when comparing data from differing leaving groups of different glycosidic bond stereochemistry, as discussed below.

In 2005, Dookhun and Bennet reported a study of the stability of β-aryl *N*-acetylneuraminides (**5**, Scheme 18).[42] Note that the natural glycosyltransfer substrate, CMP–NeuAc and the compounds in this study were of the β-configuration, whereas prior studies of solvolysis of NeuAc aryl glycosides had been conducted on the α-glycosides **6**. Bennet concluded that the β-aryl glycosides are



Scheme 18.

approximately 10-fold more stable than expected, and attributed their increased stability relative to the α-glycosides as deriving from ground-state steric strain present in the α-compounds, and absent in the β-compounds. The α-compounds place the carboxylate group in the axial position, a position considered to be less favored thermodynamically. The spontaneous hydrolysis of the α-compound is 100 times faster than that of the β-compound. As had been proposed for the solvolysis of the natural CMP–NeuAc, it was also suggested that the β-aryl glycosides showed no evidence for intramolecular participation of the carboxylate group. The evidence constituting this conclusion included the observation that during aqueous alcoholysis, products of both inversion and retention were observed. Another piece of supporting evidence was presented in that the β_{lg} values for either anomer of PNP–NeuAc were quite similar, yet their reactivities differ 100-fold. Given this, it would seem unlikely that carboxylate assistance was operative. Another piece of supporting information was found in the experimentally estimated activation entropies, which were approximately $+60$ and $+30$ e.u. for the β- and α-anomers of PNP–NeuAc, respectively. Such strongly positive values are consistent with transition states that are dissociative, without an intramolecular nucleophilic component, with any effect of transition state desolvation not withstanding. The smaller activation entropy for the α-anomer was attributed to a ground-state effect, whereby in the ground state, the less well-solvated α-anomer and the better solvated β-anomer, reach similarly dissociative and similarly solvated transition states.

7 Substituent effects on glycoside reactivity

POSITION ON THE RING

The hydroxylation pattern of a saccharide affects its chemical reactivity and behavior. Deoxy aldopyranosides lacking the hydroxyl group at the 2′-position are more reactive towards hydrolysis by approximately three orders of magnitude[11] and form oxocarbenium ion intermediates that are estimated to be 4-fold more stable than their 2-hydroxy counterparts. Withers and coworkers reported on the kinetics for the pH independent hydrolysis of a series of substituted β-2,4-DNP glycopyranosides.[43] The glycon series consisted of the ''native'' saccharides glucose, galactose, allose, and mannose and their deoxy- and fluoro-substitutions at the 2,3,4, and 6 positions. Based on measured solvolytic rate constants, Hammett plots were

constructed that revealed the rate constant correlated well with σ_I. Further, the sensitivity to the substituent was greatest for the 2-position and least for the 6-position, in general agreement with a model that involves the substituent interacting with the reaction center via field effects. When the observed rate constants were compared with the relative rates calculated by a Kirkwood–Westheimer analysis, excellent correlations were obtained. This indicates that the oxocarbenium ion-like transition state stability is under the strong influence of field effects from the substituents. Another aspect of reactivity is the impact of glycosyl substituents on conformation and the ensuing stereocontrol for product formation (Fig. 3).

SUBSTITUENT STEREOCHEMISTRY

While it has been known[44] that equatorial substituents on the glycon afford slower hydrolysis rates than axial ones, the source of this phenomenon was originally attributed by Edward to steric interactions.[45] Recent studies suggest that substituent effects can often be largely attributed to electronic factors. Experimental and computational studies[46] of the acetolysis of gluco- and galacto-2,3,6 tri-*O*-methyl pyranosides supported this idea (Scheme 19). The 4-substituent was either methoxy, acetoxy, or acetamido, and showed that whereas the glucoside was only modestly sensitive to variation of the 4-substituent (ca. 3-fold rate variation) the galactosides having the 4-substituent axial showed ca. 50-fold variation in k_{obs}. Methoxy provided the fastest rates and the acetamido substituted compound was the slowest. Interestingly, ab initio analysis of a model (*trans*-4-acetamido-1-methoxy pyran) for the 4-axial acetamido oxocarbenium ion showed that the nitrogen was pyrimidalized, with its lone pair directed towards the positive charge. The ground-state methyl pyranoside did not show this effect.

In 2002, Kirby and coworkers[47] compared hydrolytic rate constants for methyl homologs of tetrahydropyrans and found a range of rate constants differing by a factor of approximately 4; whereas acyclic analogs hydrolyzed faster, as indicated in Scheme 20 which presents structures and values for k_{obs}. The data were compared to the observation that the *hydroxylated* THP ring (i.e. glycopyranosides) can lead to rate reductions of a million-fold relative to the parent THP acetal. After the counterbalancing electron-releasing effects of the methyl substituted THP acetals were taken into consideration, it was estimated that the steric or torsional effects of equatorial substituents in glycopyranosides is only worth two orders of magnitude for the rate reduction, the balance primarily deriving from electronic factors of the hydroxyl substituents.

Bols and coworkers reported linear free energy relationships for the hydrolysis of glycopyranosides.[48] Axial and equatorial substituent effects, determined from amine basicity of similarly configured compounds, were employed.[49] The two reactions studied were the spontaneous hydrolysis of 2,4-DNP glycosides, and the specific acid-catalyzed hydrolysis of methyl glycosides (Scheme 21).

Axial groups are less retarding than equatorial ones at the same position. Outstanding correlations of $-\log k_{obs}$ and substituent effect were observed, with near

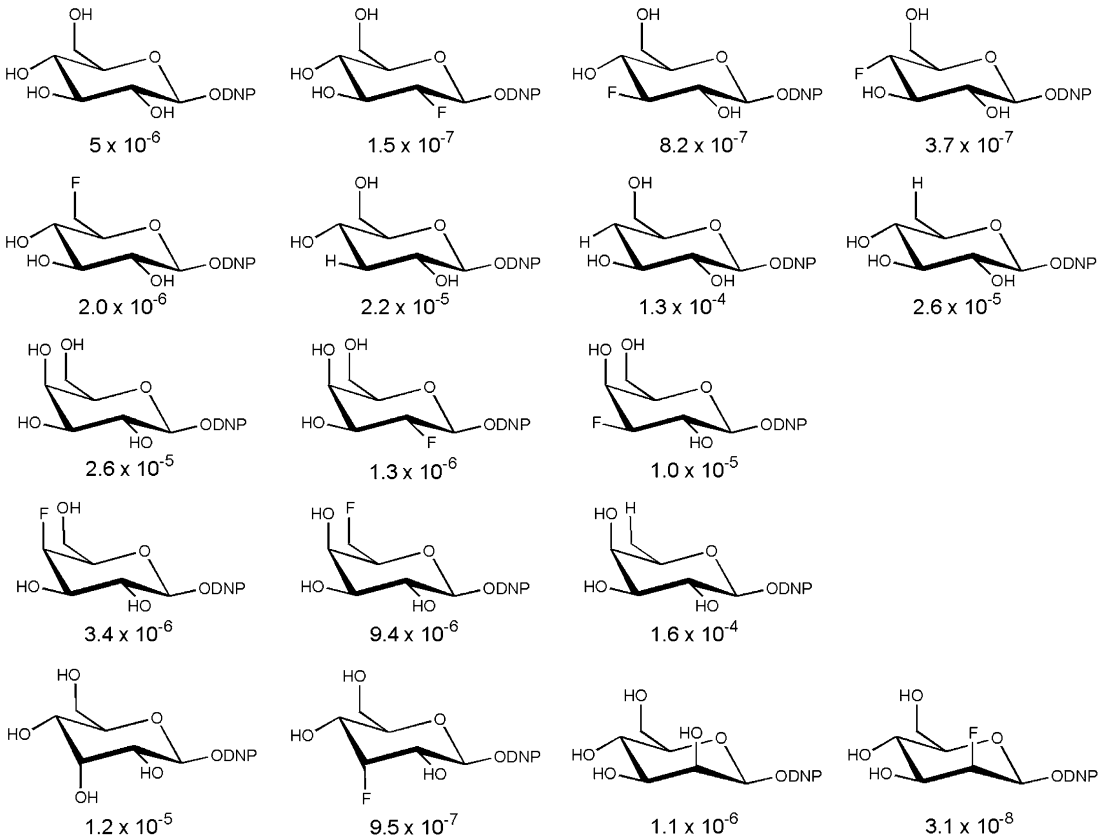
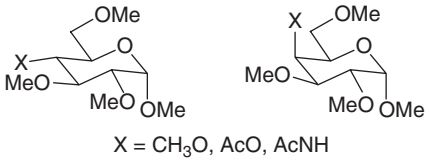
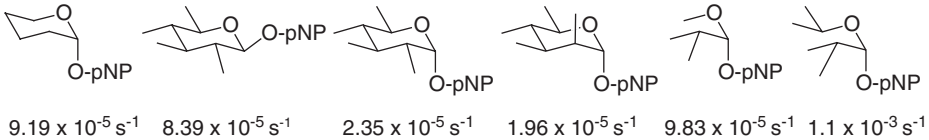


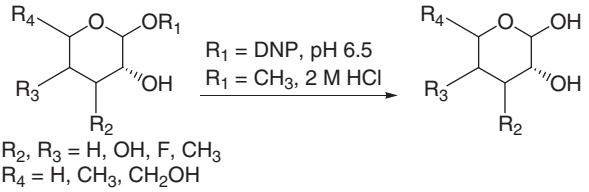
Fig. 3 Series of DNP glycosides used to determine substituent effects. Numbers under each structure represent k_{obs} for hydrolysis; units are s^{-1}. Adapted from Ref. [43].



Scheme 19.



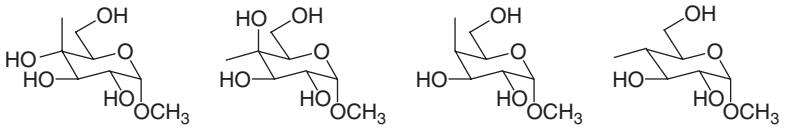
Scheme 20.



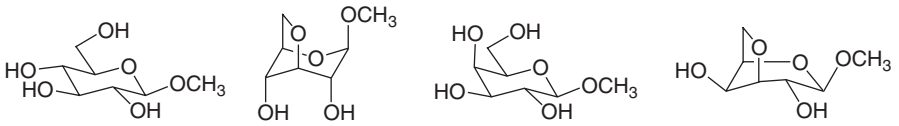
Scheme 21.

unity slopes. The analysis led to the conclusion that electronic effects of substituents are a primary factor in influencing hydrolytic rate. In a subsequent study[50] it was shown that in the series presented in Scheme 22, bulkier groups in the axial position are not predictive for increased rates over compounds placing similar substituents in the equatorial one. Of course, some care must be placed in the above analyses, since the assumption that the chemistry is occurring from $^4C_1$ conformations is subject to interpretation given that the transition states must feature flattened pyranoside rings.

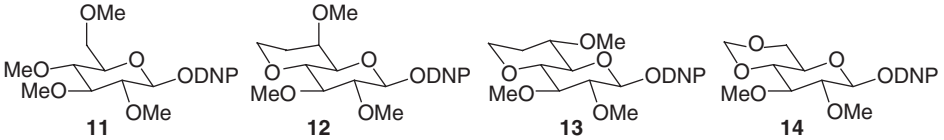
That axially placed, electron-withdrawing groups have a higher reactivity led to the proposal that in general, glycosides able to place a hydroxyl substituent axially would hydrolyze faster than those that could not. As a test, conformationally locked anhydrosugar glycosides in the $^1C_4$ conformation were synthesized and the kinetics for hydrolysis determined and compared to data for the $^4C_1$ methyl pyranosides[44,51] (Scheme 23). For example, a 248-fold acceleration for hydrolysis of anhydro **8** versus monocyclic **7** was observed. The mechanistic interpretation was that axially disposed hydroxyl groups in **8** were not destabilizing compared to the all-equatorial hydroxyl groups in methyl glucoside **7**. An alternate explanation was that relief of ring strain contributes to the reactivity of the anhydro sugar. It is noteworthy that the methyl galactoside **9** and its anhydro derivative **10** (which exists in the boat conformation) have similar rate constants for hydrolysis, suggesting that simple relief of strain does not account for the dramatic rate difference between **7** and **8**. An added complexity



Scheme 22.



Scheme 23.



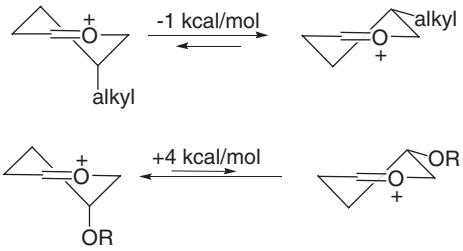
Scheme 24.

arises when one considers that hydrolysis of **8** does not lead to the anhydroglucose, but instead affords the bicyclic furanose which indicates endocyclic cleavage. As a result, it is difficult to directly apply the data to address the role that axial hydroxyls may play in the exocyclic cleavage of pyranosides. Perhaps computational analyses can be used to shed light on the basis for reactivity in the anhydro series with axially disposed hydroxyl groups.

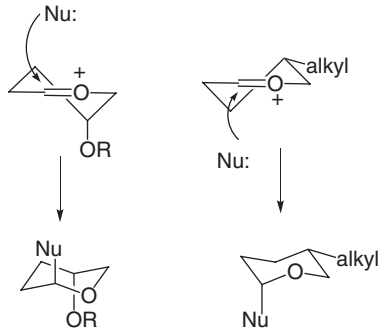
In addition to considering the impact of the stereochemistry of ring substituents on glycoside reactivity, one may consider the conformational itinerary available to the hydroxymethyl group. In a recent investigation, Bols and coworkers reported a study of the spontaneous rate of hydrolysis of DNP glycosides of glucose and analogs as presented in Scheme 24.[52] Clearly, the data support the idea that the tg conformation is the least reactive, with compound **14** being some 14 times less reactive than monocyclic **11** with the hydroxymethyl group free. One can rationalize the data based on the dipole for the C6–O6 bond either stabilizing or destabilizing an oxocarbenium ion-like transition state depending upon its conformation. As a practical matter, "disarmed" glycosyl donors[53,54] bearing a 4,6-acetal-protecting group can be considered to be both electronically, and torsionally disarmed, since neither **12** or **13** (Scheme 24) recover full reactivity relative to the unrestrained **11**.

8 Pyranosyl oxocarbenium ion conformations and reactivity

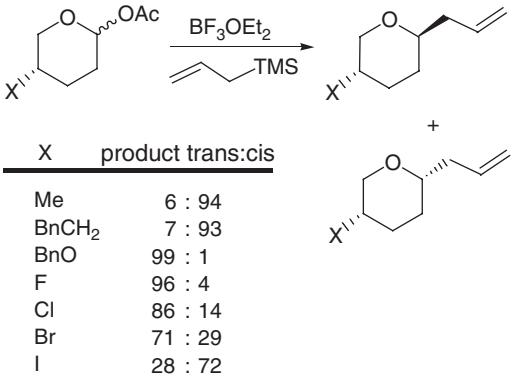
Oxocarbenium ions are typically key intermediates of nucleophilic substitution at glycosidic carbon. A better understanding of the subtle interplay of steric and electronic factors that dictate final product diastereoselectivity requires an in-depth understanding of how substituents on a pyranosyl ring influence conformation and reactivity. The design of synthetic methods that are highly diastereoselective for glycoside conformation is one area in which new developments may be furthered through understanding of oxocarbenium ion conformations. A pyranosyl oxocarbenium ion ring is flattened, and commonly resides in a half-chair conformation. Molecular mechanics[55] and ab initio calculations[46] revealed that whereas alkyl substituents at the 3 or 4 position are modestly favored in the pseudoequatorial position, the hydroxyl group is strongly favored when in the pseudoaxial position (Scheme 25). The rationale for this stems from the idea that electrostatic interaction between non-bonding electron on the hydroxyl and the positively charged atoms of the oxocarbenium ion is facilitated when axial, simply because the charged sites are closer in this conformation. The second point concerns the trajectory for nucleophilic attack. The two faces of the oxocarbenium ion are diastereomeric, and axial attack on one face leads to a twist boat-like transition state, whereas attack on the other face gives a sterically favored chair-like transition state. As shown in Scheme 26, the attack on a half-chair with its 4-substituent in the pseudoequatorial



Scheme 25.



Scheme 26.



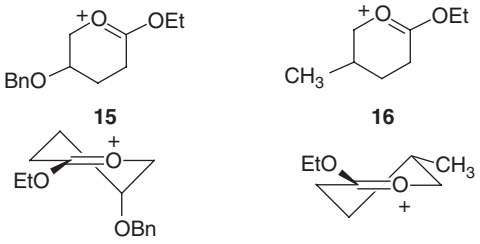
X	product trans:cis
Me	6 : 94
$BnCH_2$	7 : 93
BnO	99 : 1
F	96 : 4
Cl	86 : 14
Br	71 : 29
I	28 : 72

Scheme 27.

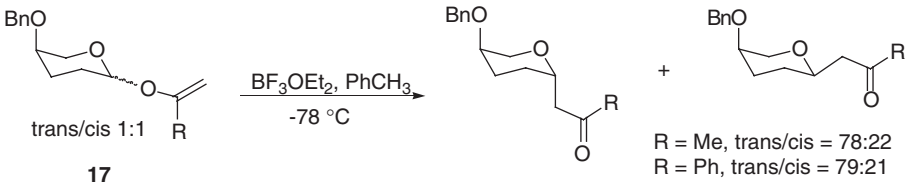
position provides products with 1,4-*cis* stereochemistry, whereas attack on a half-chair with its substituent in the pseudoequatorial position gives products with 1,4-*trans*-stereochemistry.

With these considerations in mind, Woerpel and coworkers[56] reported a study of the substituent effects for nucleophilic addition to tetrahydropyranyl oxocarbenium ions, substituted at the 2,3, or 4 positions with alkyl, hydroxyl, alkoxy, and halogen substituents. The starting THP acetates were used as an anomeric mixture in these experiments. Scheme 27 presents selected data for the 4-substituted THP acetate reactions. A dramatic reversal of selectivity from *cis* to *trans* is realized in changing X from alkyl to heteroatom. The halogen series is interesting because it presents good experimental evidence against the idea that polar 4-substituents can promote *trans*-selectivity via anchimeric assistance. If this was the dominant mode, one would have anticipated that the larger halogens would have been more effective at promoting *trans*-selectivity. The results are consistent with an axially disposed substituent providing through space electronic stabilization of an oxocarbenium ion intermediate. One assumption implicit in these experiments is that the anomeric mixture of acetates proceeds to the same solvent-equilibrated oxocarbenium ion. While not demonstrated here, work on similar systems (mentioned below) argues that this is indeed the case. One nice feature of the studies with X at the 4-position is that it is too remote from the oxocarbenium ion carbon to influence the transition state trajectory of the nucleophile. In the case of the 2- and 3-substituted compounds studied, this is not the case. Superposition of the aforementioned electronic effects and transition state steric interactions seems to be operative.

An interesting study that provided compelling evidence for axial electronegative groups being stabilizing to oxocarbenium ions utilized NMR, crystallographic, and computational studies of dioxocarbenium ions that derived from 4-substituted 1-ethoxy THP.[57] Unlike oxocarbenium ions derived from sugars or tetrahydropyrans, these compounds are sufficiently stable to isolate and characterize spectroscopically. The dioxocarbenium ion $SbCl_6$ salts were crystallized and unambiguously shown to place the benzyloxygroup of **15** and the methyl group of **16** in the pseudoaxial and



Scheme 28.



Scheme 29.

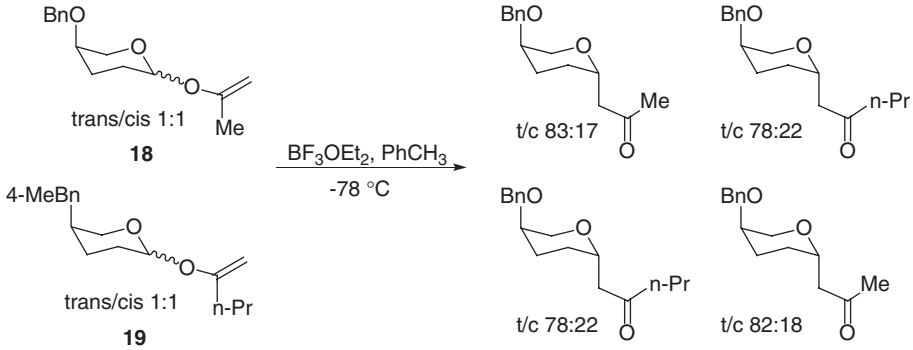
pseudoequatorial positions, respectively (Scheme 28). In the solution phase, the same conformation for **15** was determined from ^{1}H-NMR on the basis of coupling constants to the methine hydrogen at C-4. Similarly, **16** displayed a spectrum with two large diaxial coupling constants (11.2, 14.1 Hz) as part of the splitting of H-4, consistent with it being pseudoaxial, and the methyl group pseudoequatorial.

Calculations (MP2/6-31G*) allowed an estimate of the relative preference for **15** and **16** to place the alkoxy and methyl groups pseudoaxially and pseudoequatorially, respectively. Compound **15** placed the benzyloxy group pseudoaxial, with a 5.3 kcal mol^{-1} preference, whereas **16** resided in a conformation placing the methyl pseudoequatorial with a modest 1.0 kcal mol^{-1} preference.

In a study aimed at probing the reactivity of pyranosyl oxocarbenium ions, Shenoy and Woerpel reported on the diastereoselectivity associated with the intramolecular O to C rearrangement of 4-alkoxy 1-vinyl ethers **17** (Scheme 29).[58]

The authors were interested in using this system to establish the importance of tight ion pair intermediates in dictating product stereochemistry. The observation that either configuration of starting enol ether acetal afforded essentially the same "anomeric" ratio of C-alkylated product led to the conclusion that tight ion pairing was not operative in this system. As a further check, a crossover experiment (Scheme 30) was performed with molecules **18** and **19**.

The results provide excellent evidence that an oxocarbenium ion is generated, and that this and the generated Lewis acid–enolate complexes are able to equilibrate. Hence, in this system, ion pairing is not a factor in determining facial selectivity for capture of the oxocarbenium ion. One important factor is the nature of the leaving group in this system. As the BF_3 complex, its nucleophilicity is likely tempered and this would promote progress to the equilibrated oxocarbenium ion. Most



Scheme 30.

importantly, the roughly 4:1 diastereoselectvity observed in this system may be attributed to the propensity of the oxocarbenium ions to react from the half-chair conformation that places the 4-alkoxy group axially, and that it serves as a determinant of facial selectivity for capture of the oxocarbenium ion.

It is clear from the substituent effect studies conducted over the last few years that the identity and stereochemistry of pyran substituents can dramatically influence both the rate for creation of the oxocarbenium ion, the subsequent conformation of the oxocarbenium ion, and the stereoselectivity for product formation. As the system becomes more substituted (e.g. compare galactose to 4-hydroxy THP) the interplay of the different substituents becomes exceptionally complex. Further, in cases where specific solvation/desolvation events and hydrogen bonding may occur, a higher level of complexity arises. Sorting through these effects is incredibly difficult, and two areas which hold considerable promise in helping to do this are computational methods and gas-phase chemistry. Computational methods are becoming sufficiently inexpensive such that synthetically realistic molecules may be employed, rather than simple models. Methodologies including ab initio techniques, hybrid QM/MM models, and dynamics are all finding application in the area of mechanistic carbohydrate chemistry. Gas-phase chemistry affords the opportunity to do two things that can not be done in solution: (1) remove solvation effects and (2) allow for detection and kinetic studies on unstable intermediates (i.e. oxocarbenium ions). The following sections present some of the most recent studies that have helped shed light on aspects of glycosyl bond chemistry that would be difficult to do otherwise.

9 Computational analyses of oxocarbenium ion reactions

FISCHER GLYCOSYLATION

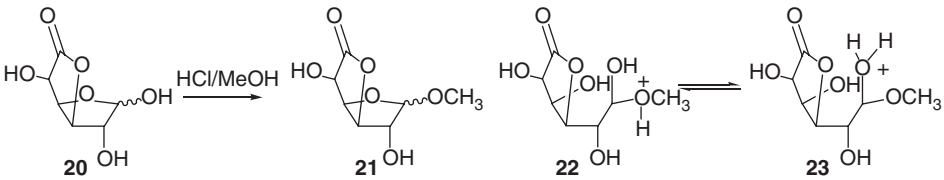
An interesting semi-empirical study[59] (PM3/COSMO) explored the mechanistic manifolds open to the Fisher glycosylation of **20** in HCl/methanol to provide **21**

(Scheme 31). It was concluded that the reaction proceeded by exocyclic loss of the anomeric hydroxyl, followed by oxocarbenium ion capture by methanol, as opposed to a mechanism involving endocyclic ring opening. This was in agreement with experimental work in which only furanosides were reported as products.[60] Surprisingly the key energetic barrier that excluded the ring-opening pathway did not involve C–O bond formation or breakdown, but instead involved a proton transfer step from the acyclic intermediate exchanging the position of the proton in the hemiacetal from the methoxyl group to the hydroxyl group, as in 22 → 23. It seems possible that other factors such as strain and conformational biasing against pyranoside formation could also have been influential in determining the experimentally observed lack of pyranosides.

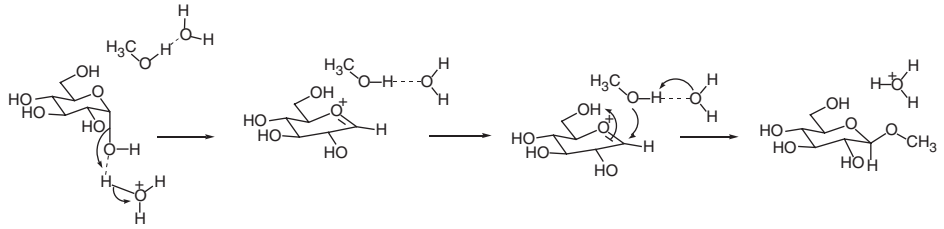
MOLECULAR DYNAMICS

Stubbs and Marx reported Car–Parrinello ab initio molecular dynamics studies for the specific-acid-catalyzed glycosidic bond formation between α-D-glucopyranose and methanol (which corresponds to the cleavage reaction by microscopic reversibility)[61,62] (Scheme 32). In this work, particular attention was paid to the role of the aqueous solvent, and as a computational study is particularly noteworthy for explicit consideration of solvent molecules. The dynamics simulations required constraints on the reaction coordinate (the methanol oxygen/oxocarbenium ion carbon distance) to ensure driving it along a productive pathway over the short time-course of the dynamics simulation, over a time period of approximately 15 ps.

The reaction between α-D-glucopyranose and methanol was shown to proceed by a $D_N{}^*A_N$ mechanism as outlined in Scheme 32. The initial dissociation step included



Scheme 31.

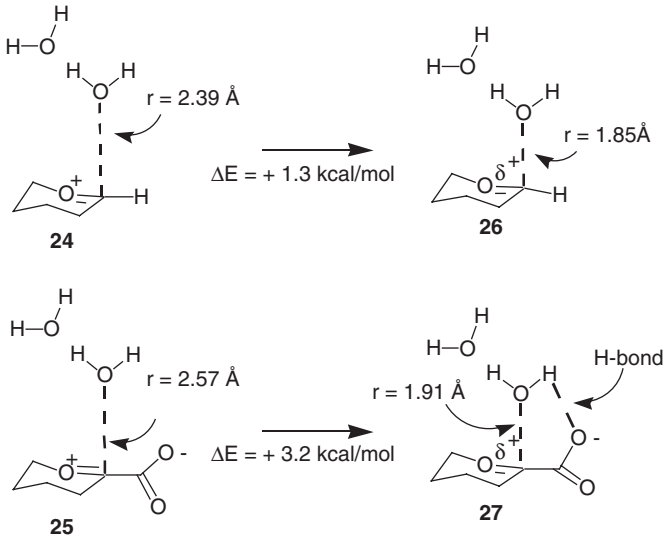


Scheme 32.

the concerted protonation of the O_1 hydroxyl leaving group, and formation of double bond character between the ring oxygen and former anomeric carbon. This step resulted in formation of a very short-lived oxocarbenium ion. In the second step, the glycosidic bond is formed between methanol and C_1 of the oxocarbenium ion ring, with associated loss of double bond character between C_1 and O_5, and proton transfer from the methanol-derived glycosidic oxygen. The authors concluded that the oxocarbenium ion was not solvent equilibrated, in agreement with experimental estimates[23,24,27,28] that the lifetime of the glucosyl oxocarbenium ion is also on the ps time scale. When the latent nucleophile methanol was *not* constrained to be proximate to the nascent oxocarbenium ion, protonations of the leaving group hydroxyl were found to be unproductive. In other words, only runs featuring intimate association of methanol led to productive dissociation of the protonated hydroxyl that then resulted in oxocarbenium ion formation. This is consistent with the idea that the oxocarbenium ion is too unstable and short lived to exist without participation of solvent. Another intriguing result of the dynamics study involved the observation of the transient desolvation of the pyranose ring oxygen over the existence of the oxocarbenium ion intermediate. This makes some sense, given that hydrogen bonding to the oxygen would render it a poorer electron donor to the electron deficient oxocarbenium ion carbon. It was also pointed out that one strength of the calculations was that only with solvation included would this feature be observed, raising a possible caveat for calculations on glycosyltransfer systems that fail to include solvation effects in an explicit manner. The predicted desolvation of the ring oxygen also raises the question as to what impact this could have on ^{18}O KIEs; it would be interesting to model these isotope effects in the presence and absence of solvation to see if they might be of some value in detecting solvation changes at the ring oxygen.

NEURAMINIC ACID TRANSITION STATES

Another area where computational studies have provided interesting insights is with regard to the oxocarbenium ion derived from NeuAc. As mentioned earlier, this sugar possess a carboxylate group that has been the subject of scrutiny with regards to its impact on glycoside breaking or formation. Intuitively, an oxocarbenium ion with an α-carboxylate might be predicted to be an especially stable one, due to the very proximate electrostatic interaction. Models for the 2-deoxyglucosyl oxocarbenium ion and the neuraminyl oxocarbenium ion were compared at the RHF/6-31G** level with regards to stable complex formation with water and the subsequent transition state barrier for capture of the oxocarbenium ion by the proximate water molecules.[63] The microsolvated structures are shown in Scheme 33. The two ion–molecule complexes **24** and **25** have distances of 2.39 and 2.57 Å between the nucleophilic water oxygen and the oxocarbenium ion carbon. The closer distance for **24** may be a reflection of the lack of carboxylate stabilization, i.e. greater ion–dipole interaction is required to stabilize complex **24**.



Scheme 33.

The barrier for capture of the 2-deoxyglucosyl oxocarbenium ion (**24**→**26**) is 1.3 kcal mol^{-1} while for the neuraminyl ion (**25**→**27**) the barrier is 3.2 kcal mol^{-1} both of these values were corrected for zero-point energy. These results agree with experiments that indicated the NeuAc oxocarbenium ion is marginally more stable than the 2-deoxyglucosyl oxocarbenium ion.

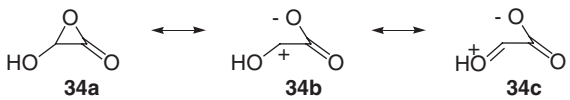
The ab initio calculated transition structure **27** (Scheme 33) features a strong hydrogen bond between the incoming nucleophile and the α-carboxylate group. Given that positive charge is passed into the attacking nucleophile as it bonds with the oxocarbenium ion, an estimate was made of the amount of stabilization a hydrogen bond might provide.[63] If the carboxylate stabilizes the transition state for nucleophilic attack on the oxocarbenium ion, this would shorten the "intrinsic" lifetime of the oxocarbenium ion. In single point calculations that either rotated the water cluster away from the carboxylate, or when the carboxylate was rotated to remove the hydrogen bond, an estimate for the hydrogen bond strength of ~8 kcal mol^{-1} was obtained. The lack of a fully solvated model argues however that this estimate is likely to be too high. Even with lower values attributed to the energetics of this hydrogen bond, the calculations supported the idea that the barrier for capture of the NeuAc oxocarbenium ion is lowered via the hydrogen bond. In any case, it is hard to rationalize why an α-carboxylate would not *intrinsically* stabilize the oxocarbenium ion – the only question, and it remains open, is to what extent? This provides a hypothesis to explain why the oxocarbenium ion derived from NeuAc is not significantly more long lived than the glucosyl oxocarbenium ion: the ability of the carboxylate to interact with attacking water in the transition state provides a considerable amount of catalysis via the hydrogen bond, which may proceed to bona fide general base catalysis as the glycosidic bond is formed. The

issues of base catalysis for nucleophilic capture of oxocarbenium ions of varying stability has been discussed by Richard and Jencks.[64]

α-LACTONES

The neuraminic acids have a carboxylate group immediately adjacent to the anomeric center begs the question as to if and how it might participate in displacement reactions. As presented in earlier sections, there is no overwhelming experimental evidence to indicate α-lactone intermediates are the *major* pathway for NeuAc glycoside hydrolysis, yet neither has the data ruled out if it might be a component of an overall reaction's manifolds. The α-lactone compounds are indeed known[65] but are too unstable to exist in nucleophilic media at room temperature. Hence, computational techniques are well suited to examine carboxylate function in neuraminic acid glycosyltransfer reactions. Williams has addressed the energetic issues surrounding intramolecular participation of the carboxyl group by computational methods, utilizing AIM analysis, with and without inclusion of solvent effects (ICPM models).[66] High-level computations on hydroxyoxiranone **28a** (Scheme 34) indicated that it is better represented electronically by **28b** and **28c**, whereas its geometry is α-lactone like in terms of it still maintaining an acute angle with respect to the lactone oxygen–lactone carbonyl carbon and α-carbon.

The nature of bonding in this system shows sensitivity to solvation. With the ICPM model and a dielectric of 78, the zwitterionic character of **34** increased over the gas phase. While one may criticize the non-explicit nature of the solvation model used in these calculations, subsequent work[67] on related oxiranone supported this conclusion with calculations employing QM/MM techniques that explicitly account for water (DFT/TIP3). In a recent study[68] that utilized time resolved IR spectroscopy and DFT calculations, it was reported that α-lactones are sensitive to medium polarity, with more polar media favoring zwitterionic forms. Further, electron-withdrawing substituents on the α-carbon favor the cyclic structure, but electron release favors the zwitterionic form. Hence, experimental and computational studies of model α-lactones provide the strong sense that an α-lactone intermediate during NeuAc glycoside bond changes would be disfavored by polar aqueous environments and the electron-releasing NeuAc ring oxygen. Just as a number of experimental studies of NeuAc glycosyl transfer have suggested the possibility of α-lactone intermediacy, but never found strong experimental support, the model work in this field also is not strongly supportive of the α-lactone hypothesis for the solvolysis of NeuAc glycosides.



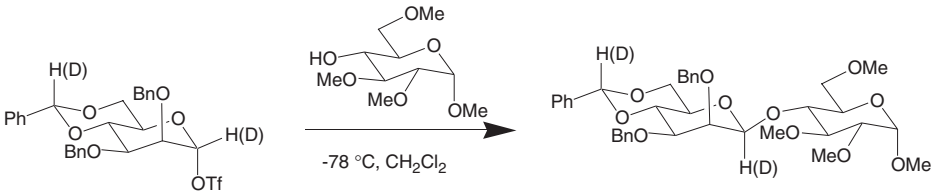
Scheme 34.

10 Mechanistic studies of ''synthetic'' reactions and glycon donors

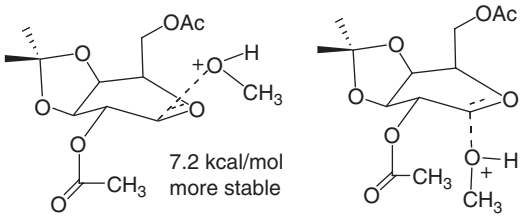
Mechanistic study of the hydrolysis reactions of unsubstituted sugars reveals considerable complexity in the mechanisms of these reactions. Studies aimed at the very practical and important problem of understanding glycosylation mechanisms with synthetic glycon donors bearing their requisite protecting groups face a considerably more difficult challenge due to the additional perturbations that the protecting groups can cause via steric, conformational, and electronic factors. Experimentally, the nature of glycon-protecting group can exert a profound outcome on the glycosylation reaction via conformational and electronic factors.[53,54] Another point is that modern synthetic methods[9] often utilize ''latent'' leaving groups that typically require activation; the mechanisms of the reactions need not resemble those of acid-catalyzed glycoside hydrolysis (or Fischer glycosylation). Relatively inexpensive and accurate computational methods are now available to address these issues, and these approaches may ultimately provide information of predictive utility to optimize stereo- and regio-selective syntheses.

Crich and Chandrasekera recently reported a KIE study of 4,6-*O*-benzylidine, 2,3-benzyl mannosyl triflate in its reaction with a saccharide donor as shown in Scheme 35.[69] The competitive method was used, and relied on ^{1}H-NMR integrals of the ratios for the remote *O*-benzylidine acetal hydrogen versus the anomeric hydrogen. After correcting the KIE to 25 °C, the average for three independent runs was 1.12. This value was taken as evidence for a transition state that was highly dissociative, however the discrimination between a mechanism involving nucleophilic association and one involving a tight ion pair was not possible. This system would be well suited for application of primary ^{13}C KIEs, which would help discriminate between these two mechanistic possibilities.

Whitfield and coworkers,[70,71] discussed interesting computational studies of the anomeric selectivity for glycosylation pathways of galactosyl, glucosyl, and mannosyl compounds, differing in the flexibility of the pyran ring as controlled by the presence or absence of cyclic acetal-protecting groups (Scheme 36). Of particular note is that the work was centered on saccharide compounds that could be employed in synthetic work. The methodology employed was DFT with a continuum dielectric model. The key outcome of this work was the suggestion that differing product anomeric selectivity as a function of the nature of the sugar and protecting groups is largely derived from conformational effects on the oxocarbenium ion intermediates and hydrogen bonding interactions between the incipient nucleophile and substituents



Scheme 35.



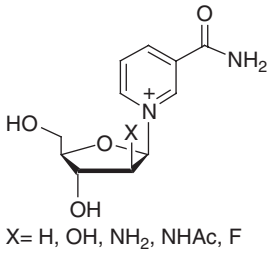
Scheme 36.

on the oxocarbenium ion. It was suggested that a rigid ring will limit the accessible conformations for the oxocarbenium ion, and in conjunction with the presence of a hydrogen bonding interaction with nucleophile could render one face of the oxocarbenium ion more accessible than the other.

For example, in the case of the 2,3-isopropylidene galactose addition reaction, attack on the β-face is favored by 7.2 kcal mol^{-1} largely driven by hydrogen bonding between the acetate at O-6 and the incoming methanol. As pointed out, it is likely that the importance of hydrogen bonding is overestimated, since the α-attack mode would still involve hydrogen bonding to solvent, despite the lack of the intramolecular hydrogen bond found for β-attack. In 2005 this line of inquiry was extended and developed into the "two-conformer hypothesis".[72] This essentially states that glycosyl oxocarbenium ions exist in two (or more) related energy conformations. Analysis of the conformational itinerary available to tetramethyl glucosyl and mannosyl oxocarbenium ions, and their ion–dipole complexes with methanol were carried out with DFT methods. In short, ion–dipole complexes of the oxocarbenium ion and nucleophile that will capture it can exist in a total of at least four species (when both α- and β-complexes are considered). The energetics of which face is most favorably added to are dominated by hydrogen bonding interactions between the nucleophile and sugar substituents, and whether attack from the α- or β-face forces the transition state to go through chair-like or boat-like structures. $^{4}H_{3}$ conformations for both gluco- and manno-oxocarbenium ions were identified as the low-energy minimum. However, close in energy were the ^{3}E for the mannosyl ion ($+1.9$ kJ mol^{-1}) and $^{5}S_{1}$ for the glucosyl ion ($+4.7$ kJ mol^{-1}). While these studies do not explicitly account for solvation, they illustrate the importance of conformational analysis in coming to understand oxocarbenium ion capture.

11 Experimental gas-phase studies

The study of glycoside formation and breaking in the gas phase is not as esoteric as it might appear. The very short lifetimes of glycosyl oxocarbenium ions in solution require that their study be via indirect competitive kinetic experiments. Knowledge of the barrier for capture of these species, whether as free oxocarbenium ions or as ion–molecule pairs, is difficult to obtain by direct methods. On the other

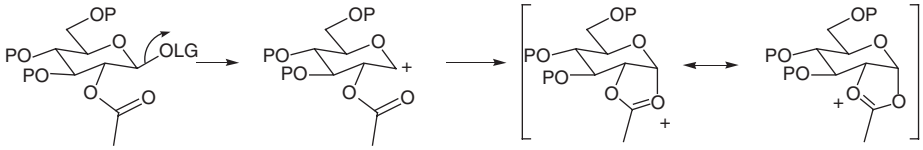


Scheme 37.

hand, gas-phase studies, such as those conducted in a mass spectrometer would in principle allow direct observation and experimentation with these species.

In 1994, Oppenheimer and coworkers[73] reported a study of the gas-phase generation of arabinosyl oxocarbenium ions via use of tandem positive-ion liquid secondary ion mass spectrometry (LSIMS) (Scheme 37). Plots of log ([oxocarbenium ion]/([M^+] + [oxocarbenium ion])) versus σ_F values for the 2′-substituents X showed excellent correlation with $\rho_F = -0.75$. In solution, the rate constants for solvolysis of the same series afford a ρ_I of –6.7. While the basis for this difference in ρ is not clear, it is reasonable to propose that the reaction in the gas phase is similar in nature to the solution reaction. With ion trap or FTICR instrumentation, studies such as these might be extended to following the fate of the oxocarbenium ion as it reacted with nucleophiles introduced into the mass spectrometer.

Denekamp and Sandlers reported electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) studies of protected gluco- and galacto-pyranosides to determine the factors that influence oxocarbenium ion generation and stability.[74,75] The protecting groups employed were acetyl, benzoyl, methyl, benzyl, and trimethylsilyl groups. Benzoyl or arylthio leaving groups at the anomeric center were used, and the ammoniated parent ion was the initial species isolated in the mass spectrometer, prior to CID. Oxocarbenium ions were observed after CID of ammoniated parent ions when the protecting groups were Bz or Ac, but not for Bn or Me groups. It was concluded that while participating and electron-withdrawing protecting groups, i.e. Bz, Ac facilitate oxocarbenium ion formation, electron-releasing alkyl groups disallow oxocarbenium ion formation. On this basis it was suggested that saccharides with alkyl protection may not proceed by the "S_N1" ($D_N + A_N$) mechanism, and possibly proceed by an "S_N2" mechanism. This conclusion could only apply to the gas phase, since alkyl-protected glycosyl donors do not show rigid adherence to inversion of configuration, as would be anticipated for an A_ND_N reaction. Further, in the gas phase the oxocarbenium ion species may indeed be generated, but more rapidly fragment rendering its detection difficult. It may well be that the neighboring group participation of the esters with the ability to extensively delocalize charge (e.g. see Scheme 38) yields a much more stabilized, and hence detectable ion. It is quite possible that the actual structure observed in the MS was the intramolecular adducts of the ester-protecting group at C2, bonding to C1. Secondly, it is clear that at least



Scheme 38.

in solution, KIE studies have revealed that with good leaving groups, hydrolysis of *unsubstituted* (and hence, electron-releasing and non-participatory) glucosides typically proceeds by dissociative $D_N{*}A_N$ mechanisms.[5,25] Of course in the gas phase the lack of solvation could well change the landscape for oxocarbenium ion chemistry, lending itself towards pathways involving elimination and fragmentation. It will be interesting to see if with more labile leaving groups, and gentler fragmentation methods, it might be possible to generate and isolate glycosyl oxocarbenium ions in the FTICR when the saccharide is substituted with alkyl-protecting groups, or even without protection. Another approach which might prove informative, if necessary, is to utilize simpler analogs of glycosyl oxocarbenium ions, which may be less prone to decomposition, should this prove to be a problem with the glycosyl compounds.

Finally, considerable progress has been made with regard to gas-phase conformational analysis of monosaccharides and their hydrated complexes by comparison of their IR spectra with that predicted by ab initio calculations.[76–78] Many of the tools are therefore available to place a sugar in the gas phase, cleave the glycosidic bond, isolate the oxocarbenium ion, and then have the opportunity to study the kinetics for nucleophilic capture by *direct* kinetic techniques. Experiments such as competitive KIEs for capture of the oxocarbenium ion also seem possible. Such work may allow direct experimental determination of oxocarbenium ion lifetimes, barriers for capture, and transition state structures. Differences observed between gas-phase results and those in solution may reveal the role that solvent plays in the reaction.

12 Conclusions

A fairly detailed mechanistic understanding is available for acid-catalyzed hydrolytic reactions of glucosides, deoxyglucosides, and NeuAc. The nucleophilic displacement reaction mechanisms can range from A_ND_N to $D_N + A_N$, heavily influenced by the leaving group and nucleophile combination employed. The lifetimes of any glycosyl oxocarbenium ion characterized thus far are all sub-nanosecond. Direct experimental measurements of the barrier height for capture of the oxocarbenium species are not yet reported. Further, the role of substituents on oxocarbenium ion lifetimes are limited to either H, OH, or F at what would be C2 of an aldopyranose. Many opportunities exist to further the knowledge based on monosaccharides other than those derived from glucose or NeuAc. Reactions using modern glycosidation methods are comparatively uncharacterized; work here is likely to facilitate new synthetic

method development. Finally, the prospects for mechanistic study of the glycosidic bond in the gas phase, in conjunction with computational methods, are excellent and relatively untapped, with the offer of insights into this chemistry that would be available in no other way.

Acknowledgments

This work was supported by grants MCB-9501866, MCB0091881 from the National Science Foundation, and GM 059322 from the National Institutes of Health.

References

1. Somsak, L. (2001). *Chem. Rev.* **101**, 81–135
2. Berti, P.J. and McCann, J.A.B. (2006). *Chem. Rev.* **106**, 506–555
3. Vasella, A., Davies, G.J. and Bohm, M. (2002). *Curr. Opin. Chem. Biol.* **6**, 619–629
4. Zechel, D.L. and Withers, S.G. (2000). *Accounts Chem. Res.* **33**, 11–18
5. Bennet, A.J. and Kitos, T.E. (2002). *J. Chem. Soc. Perk. Trans.* **2**, 1207–1222
6. Berti, P.J. and Tanaka, K.S.E. (2002). *Adv. Phys. Org. Chem.* **37**, 239–314
7. Bucior, I. and Burger, M.M. (2004). *Curr. Opin. Struc. Biol.* **14**, 631–637
8. Dube, D.H. and Bertozzi, C.R. (2005). *Nat. Rev. Drug Discov.* **4**, 477–488
9. Garegg, P.J. (2004). *Adv. Carbohyd. Chem. Biochem.* **59**, 69–134
10. Wolfenden, R., Lu, X.D. and Young, G. (1998). *J. Am. Chem. Soc.* **120**, 6814–6815
11. Capon, B. (1969). *Chem. Rev.* **69**, 407–498
12. Cordes, E.H. and Bull, H.G. (1974). *Chem Rev.* **74**, 581–603
13. Winstein, S., Klinedinst, P.E. and Robinson, G.C. (1961). *J. Am. Chem. Soc.* **83**, 885–895
14. Guthrie, R.D. and Jencks, W.P. (1989). *Accounts Chem. Res.* **22** (10), 343–349
15. Richard, J.P., Amyes, T.L., Toteva, M.M. and Tsuji, Y. (2004). *Adv. Phys. Org. Chem.* **39**, 1–26
16. McPhail, D.R., Lee, J.R. and Fraser-Reid, B. (1992). *J. Am. Chem. Soc.* **114**, 1905–1906
17. Liras, J.L. and Anslyn, E.V. (1994). *J. Am. Chem. Soc.* **116**, 2645–2646;Liras, J.L., Lynch, V.M. and Anslyn, E.V. (1997). *J. Am. Chem. Soc.* **119**, 8191–8200
18. Deslongchamps, P., Li, S.G. and Dory, Y.L. (2004). *Org. Lett.* **6**, 505–508
19. Richard, J.P. (1995). *Tetrahedron* **51**, 1535–1573
20. Banks, B.E.C., Meinwald, Y., Rhind-Tutt, A.J., Sheft, I., Vernon, C.A., *J. Chem. Soc.* (1961) 3240–3246
21. Sinnott, M.L. and Jencks, W.P. (1980). *J. Am. Chem. Soc.* **102**, 2026–2032
22. Bennet, A.J. and Sinnott, M.L. (1986). *J. Am. Chem. Soc.* **108**, 7287–7294
23. Amyes, T.L. and Jencks, W.P. (1989). *J. Am. Chem. Soc.* **111**, 7888–7900
24. Banait, N.S. and Jencks, W.P. (1991). *J. Am. Chem. Soc.* **113**, 7951–7958
25. Lee, J.K., Bain, A.D. and Berti, P.J. (2004). *J. Am. Chem. Soc.* **126**, 3769–3776
26. Zhang, Y., Bommuswamy, J. and Sinnott, M.L. (1994). *J. Am. Chem. Soc.* **116**, 7557–7563
27. Huang, X., Surry, C., Hiebert, T. and Bennet, A.J. (1995). *J. Am. Chem. Soc.* **117**, 10614–10621
28. Zhu, J. and Bennet, A.J. (1998). *J. Am. Chem. Soc.* **120**, 3887–3893
29. Zhu, J. and Bennet, A.J. (2000). *J. Org. Chem.* **65**, 4423–4430
30. Ashwell, M., Guo, X. and Sinnott, M.L. (1992). *J. Am. Chem. Soc.* **114**, 10158–10166
31. Horenstein, B.A. and Bruner, M. (1996). *J. Am. Chem. Soc.* **118**, 10371–10379

32. Chou, D.T.H., Watson, J.N., Scholte, A.A., Borgford, T.J. and Bennet, A.J. (2000). *J. Am. Chem. Soc.* **122**, 8357–8364
33. Yang, J.S., Schenkman, S. and Horenstein, B.A. (2000). *Biochemistry* **39**, 5902–5910
34. Yang, J. (2001). *Doctoral Dissertation*. University of Florida, Gainesville, FL
35. Watts, A.G., Damager, I., Amaya, M.L., Buschiazzo, A., Alzari, P., Frasch, A.C. and Withers, S.G. (2003). *J. Am. Chem. Soc.* **125**, 7532–7533
36. Amaya, M.F., Watts, A.G., Damager, T., Wehenkel, A., Nguyen, T., Buschiazzo, A., Paris, G., Frasch, A.C., Withers, S.G. and Alzari, P.M. (2004). *Structure* **12**, 775–784
37. Beau, J., Schauer, R., Haverkamp, J., Kamerling, J.P., Dorland, L. and Vliegenthart, J.F.G. (1984). *Eur. J. Biochem.* **140**, 203–208
38. Horenstein, B.A. and Bruner, M. (1998). *J. Am. Chem. Soc.* **120**, 1357–1362
39. Burke, E., Bruner, M., and Horenstein, N.A. Unpublished results
40. Richard, J.P., Amyes, T.L. and Vontor, T. (1991). *J. Am. Chem. Soc.* **113**, 5871–5873
41. Knoll, T.L. and Bennet, A.J. (2004). *J. Phys. Org. Chem.* **17**, 478–482
42. Dookhun, V. and Bennet, A.J. (2005). *J. Am. Chem. Soc.* **127**, 7458–7465
43. Namchuk, M.N., McCarter, J.D., Becalski, A., Andrews, T. and Withers, S.G. (2000). *J. Am. Chem. Soc.* **122**, 1270–1277
44. Overend, W.G., Rees, C.W., Sequeira, J.S., (1962). *J. Chem. Soc*. 3429–3440
45. Edward, J.T., (1955). *Chem. Ind*. 1102–1104
46. Miljkovic, M., Yeagley, D., Deslongchamps, P. and Dory, Y.L. (1997). *J. Org. Chem.* **62**, 7597–7604
47. Dean, K.E.S., Kirby, A.J. and Komarov, I.V., (2002). *J. Chem. Soc. Perk. Trans. 2* 337–341
48. Bols, M., Liang, X. and Jensen, H.H. (2002). *J. Org. Chem.* **67**, 8970–8974
49. Jensen, H.H., Lyngbye, L., Jensen, A. and Bols, M. (2002). *Chem. Eur. J.* **8**, 1218–1226
50. Jensen, H.H. and Bols, M. (2003). *Org. Lett.* **5**, 3419–3421
51. McDonnell, C., Lopez, O., Murphy, P., Fernandez Bolanos, J.G., Hazell, R. and Bols, M. (2004). *J. Am. Chem. Soc.* **126**, 12374–12385
52. Jensen, H.H., Nordstrom, L.U. and Bols, M. (2004). *J. Am. Chem. Soc.* **126**, 9205–9213
53. Fraser-Reid, B., Wu, Z., Udodong, U.E. and Ottosson, H. (1990). *J. Org. Chem.* **55**, 6068–6070
54. Fraser-Reid, B., Wu, Z., Andrews, C.W. and Skowronski, E. (1991). *J. Am. Chem. Soc.* **113**, 1434–1435
55. Woods, R.J., Andrews, C.W. and Bowen, J.P. (1992). *J. Am. Chem. Soc.* **114**, 859–864
56. Ayala, L., Lucero, C.G., Romero, J.A.C., Tabacco, S.A. and Woerpel, K.A. (2003). *J. Am. Chem. Soc.* **125**, 15521–15528
57. Chamberland, S., Ziller, J.W. and Woerpel, K.A. (2005). *J. Am. Chem. Soc.* **127**, 5322–5323
58. Shenoy, S.R. and Woerpel, K.A. (2005). *Org. Lett.* **7**, 1157–1160
59. Nowacki, A., Blazejowski, J. and Wisniewski, A. (2003). *J. Mol. Struc.* (*Theochem*) **664–665**, 217–228
60. Phillips, D.D. (1954). *J. Am. Chem. Soc.* **76**, 3598–3599
61. Stubbs, J.M. and Marx, D. (2003). *J. Am. Chem. Soc.* **125**, 10960–10962
62. Stubbs, J.M. and Marx, D. (2005). *Chem. Eur. J.* **11**, 2651–2659
63. Horenstein, B.A., (1999). *ACS Sym. Ser.* 721, Truhlar, D. and Morokuma, K. (eds), 411–423
64. Richard, J.P. and Jencks, W.P. (1984). *J. Am. Chem. Soc.* **106**, 1396–1401
65. Adam, W., Liu, J.-C. and Rodriguez, O. (1973). *J. Org. Chem.* **38**, 2269–2270
66. Firth-Clark, S., Rodriquez, C.F. and Williams, I.H., (1997). *J. Chem. Soc. Perk. Trans. 2* 1943–1948
67. Buchanan, J.G., Charlton, M.H., Mahon, M.F., Robinson, J.J., Ruggiero, G.D. and Williams, I.H. (2002). *J. Phys. Org. Chem.* **15**, 642–646
68. Showalter, B.M. and Tosacano, J.P. (2004). *J. Phys. Org. Chem.* **17**, 743–748

69. Crich, D. and Chandrasekera, N.S. (2004). *Angew. Chem. Int. Edit.* **43**, 5386–5389
70. Nukada, T., Berces, A., Zgierski, M.Z. and Whitfield, D.M. (1998). *J. Am. Chem. Soc.* **120**, 13291–13295
71. Nukada, T., Berces, A. and Whitfield, D.M. (2002). *Carbohyd. Res.* **337**, 765–774
72. Nukada, T., Berces, A., Wang, L.J., Zgierski, M.Z. and Whitfield, D.M. (2005). *Carbohyd. Res.* **340**, 841–852
73. Buckley, N., Handlon, A.L., Maltby, D., Burlingame, A.L. and Oppenheimer, N.J. (1994). *J. Org. Chem.* **59**, 3609–3615
74. Denekamp, C. and Sandlers, Y. (2005). *J. Mass Spectrom.* **40**, 765–771
75. Denekamp, C. and Sandlers, Y. (2005). *J. Mass Spectrom.* **40**, 1055–1063
76. Talbot, F.O. and Simons, J.P. (2002). *Phys. Chem. Chem. Phys.* **4**, 3562–3565
77. Jockusch, R.A., Talbot, F.O. and Simons, J.P. (2003). *Phys. Chem. Chem. Phys.* **5**, 1502–1507
78. Simons, J.P., Jockusch, R.A., Carcabal, P., Hunig, I., Kroemer, R.T., Macleod, N.A. and Snoek, L.C. (2005). *Int. Rev. Phys. Chem.* **24** (3–4), 489–531