Mechanism of chemical *O*-glycosylation: from early studies to recent discoveries

Laurel K. Mydock and Alexei V. Demchenko*

Received 10th August 2009
First published as an Advance Article on the web 12th November 2009
DOI: 10.1039/b916088d

The main focus of this perspective lies in the discussion of the recent mechanistic theories and supporting experimental evidences that have been put forth in an attempt to advance our understanding of the factors affecting chemical glycosylation.

1. Introduction and outline

The first glycosylation reactions were performed in the late 1800s. Since then, carbohydrate chemistry has evolved into a broad area of research that has persistently captured the interest of the scientific community. Existing as the most abundant class of organic compounds, carbohydrates are involved in a myriad of life-sustaining and life-threatening processes.[1] However, understanding the structure, reactivity and function of these bioorganic compounds has proven to be a remarkable challenge. Therefore, the unique molecular complexities of these molecules have attracted just as much attention as has their biological significance.

Department of Chemistry and Biochemistry, University of Missouri–St. Louis, One University Blvd, St. Louis, Missouri, 63121, USA. E-mail: demchenkoa@umsl.edu

The fundamental reaction performed between two monosaccharide units is the glycosylation reaction. Nature flawlessly and repeatedly executes this reaction to yield complex poly- and oligosaccharides.[2] Chemically, however, the installation of the glycosidic linkage remains cumbersome, even with the aid of modern technologies. However, owing to many recent breakthroughs in the field, the formation of most glycosidic bonds can be readily achieved.[3–16] Unfortunately, it is the inability to effectively predict and control the stereoselectivity of the reaction that has proven to be the synthetic hurdle. This is in part due to the lack of mechanistic understanding regarding a few key steps and intermediates within the glycosylation reaction. Optimization of this reaction has thereby remained an underlying theme throughout the history of carbohydrate chemistry. With recent advances in the rapidly expanding field of glycobiology,[17] the demand for reliable and stereocontrolled glycosylation methodologies has now increased, thus elevating the priority with which we improve our synthetic capabilities.

Laurel K. Mydock

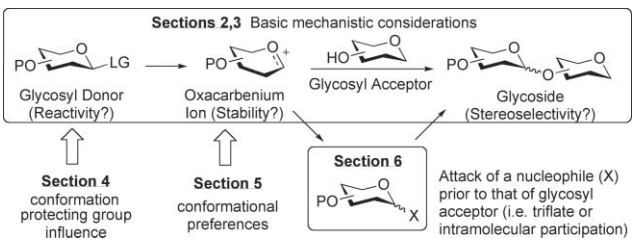
Laurel K. Mydock obtained her B.S. in Mathematics from Lindenwood University in 2002. In 2003, she began her journey into the world of chemistry and in 2005 she entered the PhD program at the University of Missouri–St. Louis, wherein her research is centered upon exploring the mechanism and reactivity of thioimidate and thioglycoside leaving groups in order to further expedite the synthesis of complex oligosaccharides. The main focus of her research is on the mechanistic aspects of the stereocontrolled synthesis of glycosides. She is currently working towards her doctoral degree under the supervision of Dr Alexei Demchenko.

Alexei Demchenko

Alexei Demchenko graduated from Mendeleev University of Chemical Technology of Russia with a Diploma in Chemical Engineering (1988) before joining the laboratory of Nikolay Kochetkov at Zelinski Institute of Organic Chemistry. In 1993 he was awarded a PhD and after two post-doctoral years under Kochetkov, he joined Geert-Jan Boons' group at the University of Birmingham, and then the Complex Carbohydrate Research Center. In 2001 he joined the faculty at the University of Missouri St. Louis. Professor Demchenko received a CAREER award from the National Science Foundation (2005) and a New Investigator Award from the Division of Carbohydrate Chemistry of the American Chemical Society (2007). He has co-authored over 85 articles and edited two books. Professor Demchenko's research program in the area of synthetic carbohydrate is currently funded by awards from National Science Foundation, National Institutes of Health, and American Heart Association.

In the past three decades, much scientific effort has been dedicated to refining the glycosylation reaction through the development of new leaving groups, new promoters (activators), and through the optimization of the general reaction conditions. However, as these enhancements were not able to adequately control the glycosylation outcome, studies have now begun to redirect the focus toward gaining a better understanding of the mechanisms and energies controlling the reaction. There are many factors that can have a profound effect on the reactivity of the glycosyl donor and acceptor and the selectivity of the glycosidic bond formation. It is not the primary intent of this account, however, to provide examples of specific glycosyl donors and acceptors that, when subjected to specific set of reaction conditions, will yield a particular product; but rather to draw a clearer picture of the structural and electronic effects governing the reaction en route to product formation. For a more extended coverage of different glycosylation techniques and practical applications the reader should be referred to the recent comprehensive handbook.[18] The focus herein will be to outline how the intrinsic properties of the glycosyl donor can affect the glycosylation reaction. In particular, current mechanistic studies related to the conformation, configuration, and stereoelectronics of glycosyl donors and their key reaction intermediates will be discussed. This includes glycosyl donor traits, such as: the conformation of the pyranose ring, the orientation of the attached substituents (axial *vs.* equatorial), and the type, number and location of the protecting groups. While modifying these attributes can lead to a wide variation in reaction outcomes, studies are often neglected due to the inherent difficulties in quantifying the resultant mechanistic, conformational and energetic consequences. Other factors, such as the influences of the leaving group,[18–22] temperature,[23–30] pressure,[31,32] promoter/additives[33–35] or reaction solvent[28,35–41] can also significantly affect glycosylation. For the discussion of these effects the reader should be referred to the original articles cited or recent reviews.[3,4,10,42–44]

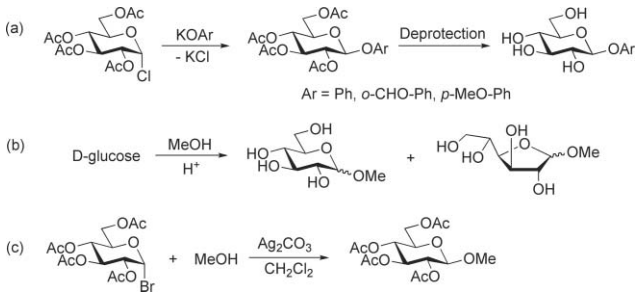
The outline of this perspective is depicted in Scheme 1, and will begin by acknowledging some relevant pioneering studies that helped shape our current mechanistic interpretation of the glycosylation reaction (Section 2). For a more detailed historical perspective, the reader is referred to the early reviews of the subject.[45,46] Upon establishment of the fundamental studies and basic mechanistic principles of the chemical glycosylation (Section 3), a more in depth discussion of modern theories and their supporting experimental evidence will follow. Section 4 will address some of the recent developments with regard to the factors that ultimately affect the reactivity of the glycosyl donor, including the electronic effects originating from the type, location and orientation of the ring substituents, as well as the torsional effects that can affect necessary conformational changes. Following this, Section 5 will embark upon discussions about the conformational preferences of the oxacarbenium ion intermediate, which differ greatly from that of the donor due to the change in the hybridization state of the anomeric carbon and the acquired positive charge. As such, this conformational knowledge can be instrumental in determining *from which* face the glycosyl acceptor will prefer to approach the oxacarbenium ion.[47] Finally, Section 6 will discuss other reaction intermediates that may form prior to the nucleophilic attack of the glycosyl acceptor. This discussion will focus on the electronic and conformational properties of the intermediate species en route to product formation, and the theoretical calculations backing these assertions.



Scheme 1 Outline of the review.

2. Historical perspective and important lessons from early work

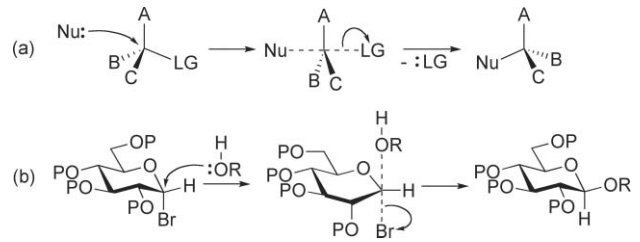
The first chemical glycosylation was reported by Arthur Michael some 130 years ago.[48] Just as in many modern methodologies, this reaction proceeded by the nucleophilic displacement of an anomeric leaving group (chlorine, Scheme 2a). Although there was still very little known about the structure and reactivity of carbohydrates, Michael's vision of how the anomeric substitution should proceed was fundamentally accurate. Inconveniently however, it was deemed necessary to first convert the glycosyl acceptor into its respective potassium salt. In 1893, Emil Fischer took a different approach to the glycosylation reaction.[49] In sharp contrast to the earlier protocol, Fischer perceived the unprotected monosaccharide unit as a hemiacetal. As such, the reaction was carried out under harsh acidic conditions in an excess of the desired glycosyl acceptor (most commonly low weight alcohols) (Scheme 2b). Being conceptually the simplest way to obtain glycosides, the Fischer method commonly leads to an equilibrium of inter-converting species, all of which are formed in addition to the product formation.



Scheme 2 (a) Michael, (b) Fischer and (c) Koenigs–Knorr glycosylation reactions.

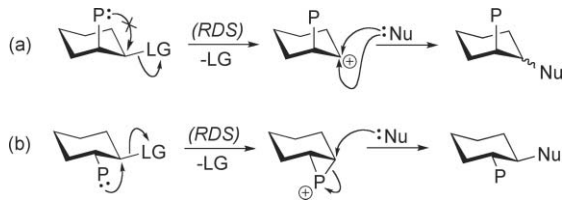
While these pioneering approaches were not broad in their applicability, some of the fundamentals necessary for carrying out a successful glycosylation reaction had already emerged. (1) In order to give the product a definite ring size, the use of temporary protecting groups appeared as a relatively simple and practical solution. (2) Michael's displacement of an anionic leaving group became prototypical in many modern glycosylation techniques. (3) It became clear that the glycosylation could not simply be regarded as a typical acetal formation. These elements created a solid base for developing a more practical and

versatile glycosylation approach. In 1901 Koenigs and Knorr[50] (and independently Fischer and Armstrong)[51] took the chemical glycosylation approach a step further by reacting glycosyl halides with conventional alcohol acceptors in the presence of Ag_2CO_3 or Ag_2O (Scheme 2c). While the latter were used as mild bases with the primary intention to scavenge the hydrogen halide byproduct, it was not until the early 1930s when it was realized that the silver salts play an active role by assisting in leaving group departure.[52] It was also noted that the Koenigs–Knorr glycosylation reaction was very selective, often providing complete inversion of the anomeric configuration. This phenomenon was rationalized by the occurrence of "Walden inversion",[53] otherwise known as concerted nucleophilic substitution.[54] Mechanistically, this requires an "opposite face" attack, meaning that the incoming nucleophile must approach from the reverse side of the departing leaving group (Scheme 3a). Thus, it was commonly assumed that the nucleophilic displacement at the anomeric center also proceeded *via* this mechanism (Scheme 3b).[55]



Scheme 3 (a) Walden inversion, (b) Inversion at the anomeric center.

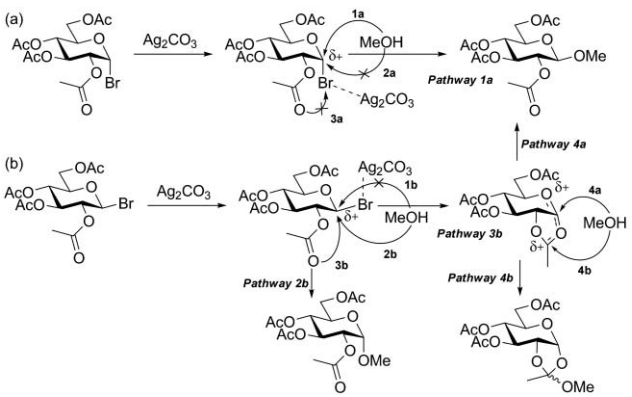
Later on, however, several research groups began to notice that the ester protecting group at C-2 seemed to effect both the stereochemical outcome and the byproduct formation of the glycosylation reaction.[56] For instance, Pigman and Isbell observed that the 1,2-*trans* configuration was a prerequisite to both 1,2-anhydro and 1,2-orthoester formation,[57] and insightfully drew upon this information to re-evaluate the mechanistic pathway of the Koenigs–Knorr reaction.[58] At the time, the mechanistic details of how and why orthoesters formed were still sketchy;[59] however, their existence helped to substantiate the intramolecular reaction pathways within the sugar ring. This in turn, provided a solid mechanistic scaffold for which the fundamental theories of C-2 participation could be built upon, ultimately providing further insight into understanding and rationalizing the end products of the glycosylation reaction. Isbell's findings were further substantiated through Winstein's kinetic studies on neighboring group participation. This approach involved calculating the energy required for a nucleophilic substitution to occur in the absence or presence of participation in various 1,2-disubstituted cyclohexanes. Ultimately, this led to the conclusion that the unassisted departure of a leaving group to yield a free ion species (S_N1 mechanism, Scheme 4a) would require much more energy than a concerted nucleophilic displacement that occurs *via* intramolecular participation (S_N2 mechanism, Scheme 4b).[60,61] As a consequence, 1,2-*trans* species were found to react efficiently through concerted S_N2 mechanisms, while their analogous 1,2-*cis* counterparts were forced to proceed *via* the higher energy S_N1 pathway, making them sluggish in comparison. Although these model studies were not conducted at the anomeric center, the knowledge acquired proved invaluable in application



Scheme 4 Rate-determining ionization pathways for (a) S_N1 and (b) S_N2 mechanisms.

to carbohydrates, ultimately giving rise to the current standard protocol for introducing the 1,2-*trans* linkage through utilization of neighboring group participation.

With this knowledge of neighboring group participation, Isbell also proposed two distinct pathways of glycosylation based upon the configuration of the C-1 substituent relative to C-2, being either 1,2-*cis* or 1,2-*trans* (Scheme 5).[58] Initially, the activation pathway is the same for both glycosyl donor configurations; the anomeric bromide complexes with the silver salt, which decreases the electron density at the anomeric center, making it more susceptible to nucleophilic attack. Subsequent to this point, however, the pathways diverge. In the case of the 1,2-*cis* glycosyl donor, wherein both the anomeric bromide and the 2-*O*-acetyl substituent are on the same side of the ring, only the expected inversion product was obtained (pathway 1a). The lack of the 1,2-orthoester formation (pathway 3a), was rationalized by the fact that the approach of the 2-*O*-acetyl group is blocked, making participation impossible. It then follows, that the 1,2-*cis* glycoside is not observed because there is no plausible mechanism that would lead to this product (pathway 2a). *The high stereoselectivity and lack of an observed 1,2-orthoester byproduct from 1,2-cis bromides, serves as evidence that the Koenigs–Knorr reaction is one of the rare examples wherein a concerted bimolecular displacement (S_N2 mechanism) occurs*. Conversely, the 1,2-*trans* bromide yielded three distinct products: two diastereomeric glycosides and an orthoester. Following activation, the expected 1,2-*cis* product was obtained *via* direct nucleophilic displacement from the bottom (opposite) face of the ring (pathway 2b). Additionally, the intramolecular attack from the adjacent carbonyl oxygen leads to the formation of a reactive acyloxonium (*i.e.* dioxalenium) intermediate (pathway 3b). Then, depending on the site of nucleophilic attack on the acyloxonium intermediate, two products are possible; a 1,2-*trans* glycoside (pathway 4a) and a



Scheme 5 Bimolecular mechanism of the Koenigs–Knorr reaction (a) 1,2-*cis* glycoside, (b) 1,2-*trans* glycoside.

1,2-orthoester (pathway 4b). It should be noted that the 1,2-*trans* glycoside cannot be obtained directly (pathway 1b).

As studies on the unique reactivity of the anomeric center became more prevalent, it was further revealed that there existed an unconventional inclination for anomeric substituents to reside in an axial configuration. This phenomenon was first observed by Edward[62] and later defined as the *"anomeric effect"* by Lemieux.[63] Although the anomeric effect is well recognized in the field, its rationalization is often the subject of much deliberation. Typically, in cyclic six-membered hydrocarbons, *equatorial* substituents are energetically preferred over *axial* substituents, due to the unfavorable 1,3-diaxial interactions that arise (Fig. 1a). With sugar structures, however, the six-membered ring differs in that it contains an endocyclic oxygen atom adjacent to C-1. As the attached leaving group is also a heteroatom, the combined inductive effects produce a considerable electron deficiency at C-1, leading to some unique electronic characteristics. The rationale for the observed phenomenon, is often a unification of both electrostatic and hyperconjugation effects. Electrostatically, the anomeric effect is explained in terms of dipole–dipole interactions (Fig. 1b). Thus, when the leaving group X resides equatorially, the lone pair electrons on its heteroatom exhibit strong repulsive electrostatic interactions with electrons on the ring oxygen (O-5). These destabilizing electrostatic interactions do not exist when X is in the axial orientation. Additionally, electron-withdrawing axial substituents are further stabilized through hyperconjugation (Fig. 1c), as the lone-pair electrons at O-5 and the antibonding orbital of C-1 are in an *anti*-periplanar alignment. This stabilization cannot be achieved when X is equatorial, as the respective orbitals of O-5 and C-1 are in different planes. It then follows, that as the electronegativity of X increased, so does its axial proclivity.[64] This rationalization is supported by the observed shortening of the C-1–O-5 bond and a concomitant lengthening of the C-1–X bond.

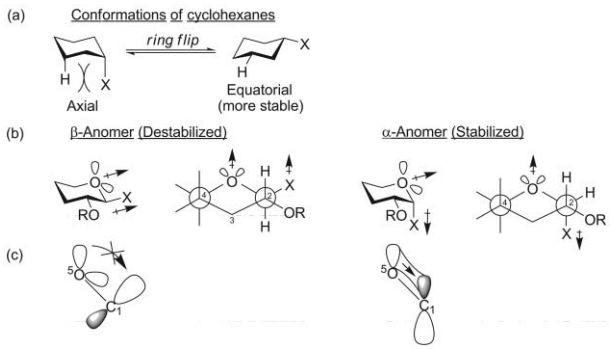


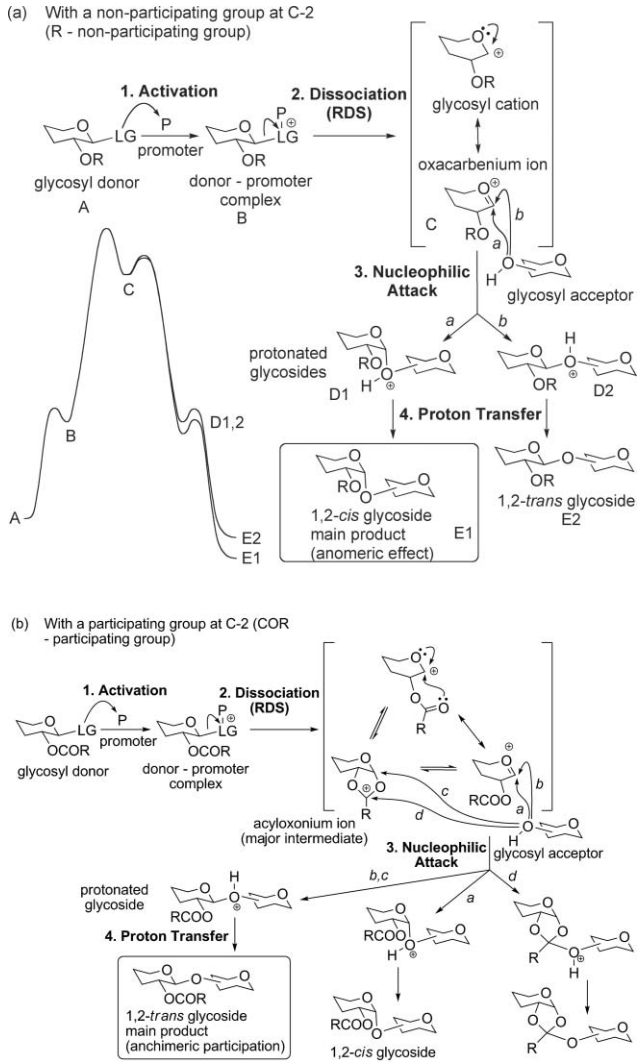
Fig. 1 Anomeric effect.

In terms of the reactivity of the anomeric center, it has often been observed that one anomer is often more reactive that the other. While several theories have emerged to justify this, the *anti*-periplanar lone pair hypothesis, also known as the kinetic anomeric effect, is the most well known.[65,66] This theory expounds upon the hyperconjugation model, owing a greater lability of axial glycosides to a lengthening, and therefore weakening, of the axial C-1–X bond. However, often the opposite reactivity is also encountered, and so alternative theories, namely the *syn*-periplanar lone pair hypothesis[67] and the principle of least nuclear motion,[68] have been developed to explain this contradictory observation.

3. General considerations and basic mechanisms of glycosylation

There are many complexities to consider when depicting the mechanism of the glycosylation reaction, and often a clear delineation between S_N1 and S_N2 nucleophilic substitution reactions is obscured.[69] Nevertheless, nowadays it is generally presumed that the reaction conditions favor that of a unimolecular S_N1 mechanism. However, one can always find counterarguments; Paulsen's glycosyl donor–acceptor match–mismatch concept[70] that was recently explored by Fraser-Reid and Lopez *et al.*,[71–75] and the double stereodifferentiation phenomenon.[76] In theory, the S_N1 mechanism implies that the rate determining step (RDS) is unimolecular, and is independent of the glycosyl acceptor. As such, this also implies that there is at least one intermediate step prior to product formation. Consequently, the reaction is thought to proceed through a total of four distinct steps (Scheme 6):[69] (1) formation of the donor–promoter complex, which can be reversible or irreversible depending on the system involved; (2) ionization of the glycosyl donor, a typically irreversible act, and the slowest step (RDS) of the reaction; (3) nucleophilic attack by the glycosyl acceptor; and (4) proton transfer to give a neutral glycoside.

Scheme 6a profiles a typical glycosylation reaction. Generally, the leaving group (LG) employed at the anomeric carbon of a glycosyl donor (A, *herein and below is pertained to the* D-*glucopyranose series*) is nucleophilic in nature (halogen, SR, OR, *etc.*). Therefore, upon adding an electrophilic promoter (activator, *P*), it will activate the leaving group to form donor–promoter complex B (step 1). The next step is considered to be the unimolecular RDS, wherein the transformation of complex B into the glycosyl carbocation occurs (step 2). This intermediate exists in its stabilized resonance form, oxacarbenium ion (C). As a consequence, the anomeric carbon is sp^2-hybridized, which results in a flattened half chair conformation. Thus, the subsequent nucleophilic attack (step 3) of the glycosyl acceptor is possible from both the bottom (pathway a) and the top (pathway b) face of the sugar ring, leading to the formation of α-(1,2-*cis*) or β-(1,2-*trans*) linkages, respectively. Finally, the loss of the proton results in the formation of the neutral 1,2-*cis* and 1,2-*trans* glycosides E1 and E2 (step 4). Once proton transfer occurs, the formation of the glycosidic bond is irreversible, and as such can be thought of as the termination step in the glycosylation reaction. It should be noted that step 4 is often neglected in mechanistic discussions with the belief that it has no effect on the outcome of glycosylation. However, there has been accumulating evidence that this simple assumption is inaccurate,[77] and that the effects of hydrogen bonding and proton transfer may have great influence. For example, H-bonding has been found to occur at or near the transition state associated with the approach of the nucleophile, and as such, can affect the transition state energy corresponding to a specific facial approach. Furthermore, it has been proposed that intramolecular proton transfer may also be involved in the mechanism by which neighboring group participation proceeds.

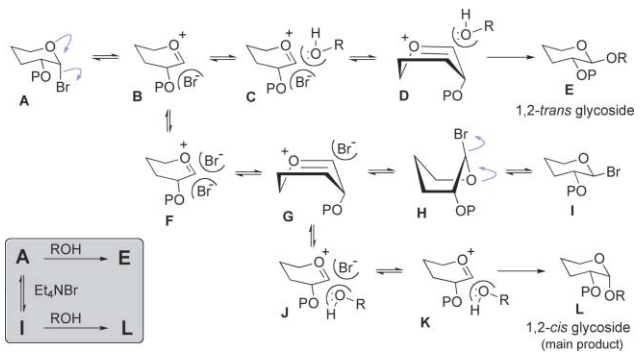


Scheme 6 General mechanisms of glycosylation.

As depicted in Scheme 6b, the glycosylation mechanism becomes slightly more complicated when a glycosyl donor bearing a participating group at C-2 is utilized. While the underlying philosophy dictating product formation remains the same, the number of potential intermediate species and plausible mechanistic pathways increases (discussed more thoroughly in Section 6). Again, a promoter is employed to assist in leaving group departure. Upon dissociation of the leaving group, a short lived positively charged species is formed and it is generally assumed that an intramolecular attack immediately occurs to form the more stable, lower-energy acyloxonium ion. From this point, it is unclear whether the incoming nucleophile directly attacks this species (in an S_N2 fashion), or if a more complex pathway involving additional intermediates is followed. However, it is generally presumed that the direct nucleophilic attack on C-1 is the route to the 1,2-*trans* glycoside product (pathway c), and that direct attack at the carbonyl carbon is responsible for the formation of the orthoester product (pathway d).

At this point, it seems appropriate to draw attention to the more critical and controversial points of the reaction mechanism related to our topic of discussion. Thus, Section 4 will cover *Steps 1 and 2 (Activation and Dissociation)*. Although the initial promoter complexation seems to serve as a reflection of the glycosyl donor's reactivity, it is actually the dissociation of the leaving group that is the RDS. Consequently, the observed reaction rate is largely dependent upon the stability of the resulting oxacarbenium ion. As such, many of the mechanistic discussions pertaining to the reactivity of the glycosyl donor will be conceptually approached through assessing the stability of the oxacarbenium ion intermediate. Section 5 will expand upon *Step 3 (Nucleophilic Attack)*. Given that the nucleophilic attack of the glycosyl acceptor occurs after the RDS, it is not the rate with which this step proceeds, but rather the selectivity of this step that is of significance. In other words, it is the facial preference of the approaching nucleophile that is largely responsible for the observed stereoselectivity, as reflected in intermediate D, and is then presumed to be carried through to the glycosidic product E, forming the kinetic product. This preferential attack is thought to arise from the stability of the transition state associated with each approach (α or β), and is in part, due to the conformation of the oxacarbenium ion intermediate, as the various electronic and steric factors can give each approach a different energy. Additional product selectivities can arise from the stabilization provided by the anomeric effect, which is thought to be responsible for the thermodynamic product of the reaction. We are aware of the existence of the non-kinetically controlled glycosylations, in which the initially formed β-glycoside is then anomerized into its thermodynamically more stable α-counterpart. Without diminishing the importance and versatility of this approach, we choose to direct the reader to the recent authentic publications.[78,79]

The first application of this accrued mechanistic and kinetic knowledge was the halide ion-catalyzed glycosylation developed by Lemieux *et al*.[80] Through careful consideration of the reaction intermediates and conformations thereof, and through extensive theoretical studies, it was found that a rapid equilibrium could be established between a relatively stable α-halide **A** and its far more reactive β-counterpart **I**, by adding tetraalkylammonium bromide (Et_4NBr, Scheme 7). Initially, the expulsion of the α-halide **A** results in the formation of ion-pair **B**. Since no inverted product (**E**) is formed herein, it can be concluded that the ion-pair **F** leading to the anomerized β-linked bromide **I** is a more energetically favorable pathway. Note the existence of alternative conformations for intermediates **G** and **H**. These are presumed to be necessary in order to form/activate the equatorial bond, and are in accordance with the *syn*-periplanar lone pair hypothesis,[67] wherein an axial-like stabilization is achieved when the sugar ring adopts a

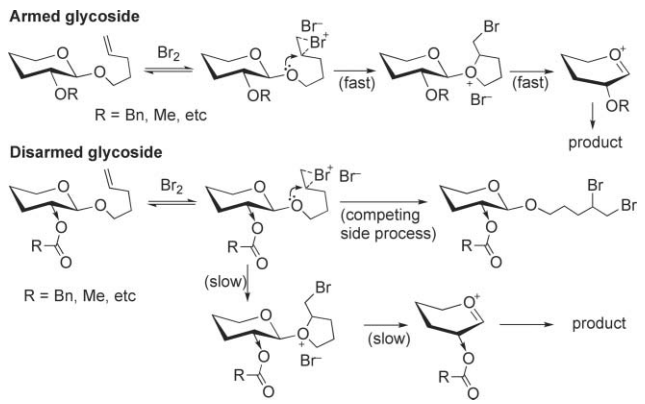


Scheme 7 Mechanism of Lemieux's *in situ* anomerization procedure.

conformation where the equatorial anomeric substituent becomes axial (or pseudo-axial). At this point, the highly unstable β-halide dissociates back into its ion pair (**I**→**G**), whereupon it quickly undergoes nucleophilic attack (**G**→**K**) to form the 1,2-*cis* product **L**. As an end result, nucleophilic substitution of the β-bromide **I** occurs favorably, whereas the α-bromide **A** quickly anomerizes before glycosylation can occur. The observed stereoselectivity is additionally reinforced by the Curtin–Hammett principle[47] in that when two compounds are in rapid equilibrium, the ratio of product formation is often controlled by the standard Gibbs energies of the respective transition states, and is not a reflection of their respective equilibrium populations, as equilibrium favors the α-bromide and would therefore yield the 1,2-*trans* glycoside.

4. Reactivity of glycosyl donor and formation of the oxacarbenium ion intermediate

Protecting groups were initially applied to reduce unwanted side reactions, by masking additional sites of reactivity. However, it soon became evident that the inherent properties of the protecting groups themselves could significantly affect the outcome of the glycosylation. One of the more salient effects observed and capitalized upon in carbohydrate synthesis, was neighboring group participation. Furthermore, it was noticed that the steric bulk accompanying a variety of the groups could have a profound impact on the stereochemical outcome of the reaction.[10] Keeping with this trend, in 1988 Fraser-Reid *et al.* described a new manner by which to exploit the properties of protecting groups. Known as the "armed–disarmed strategy,"[81] this approach took advantage of the different electronic effects among the various functional groups (Scheme 8). It was noticed that ester-type protecting groups (OAc, OBz, *etc.*) strongly reduced "disarmed" the reactivity of the *n*-pentenyl glycosyl donor, in comparison to the effects of ether-type protecting groups (OBn, OMe, *etc.*). One justification for such an observation, is that the increased electron-withdrawing ability of ester protecting groups decreases the electron density and, hence, the nucleophilicity of the leaving group. In the case of *n*-pentenyl glycosides, which are activated at the remote double bond, the arming/disarming effect is noticed in the intramolecular cyclization step. Thus, the less reactive disarmed glycosyl donor yields a vicinal dihalide byproduct that is not observed with the ether-protected armed analog. Another consequence of the decreased electron density at the anomeric center, which is highly



Scheme 8 Arming and disarming effects by protecting groups.

relevant to the topic of this account, is that upon departure of the leaving group, the resulting oxacarbenium ion is destabilized by the electron withdrawal.

Although this discovery was made using *n*-pentenyl glycosides, this electronic effect ultimately proved to be of a general nature, and can be applied to nearly any class of glycosyl donor. Furthermore, the usefulness of this approach was found in application towards expeditious oligosaccharide synthesis, as it circumvents the need for protecting group manipulations at the anomeric center.[9] In an attempt to facilitate the armed–disarmed strategy in oligosaccharide synthesis, Ley *et al.* developed a new approach wherein the reactivity of glycosyl donors and acceptors could be "tuned".[82] Wong *et al.* further devised a mathematical approach, assigning relative reactivity values (RRVs) to a wide library of over fifty *S*-tolyl donors and acceptors, each containing a different set of protecting groups.[83] In a further expansion of the basic armed–disarmed theory, Schmidt and Madsen were able to achieve a disarming effect through the strategic placement of a single powerful electron-withdrawing ester group (pentafluorobenzoyl) on the C-6 position of an ether-protected phenyl thioglycoside.[84] Related studies also revealed that the arming/disarming ability of the protecting groups was highly dependent upon both their location and their core donor structure.[82,83] Crich and Vinogradova have also investigated the influence of the electron withdrawal at the C-6 position on the stereoselectivity of the glycosylation. In exploring a series of 6-deoxy mono-, di-, and trifluoro *S*-phenyl rhamnosyl donors,[85] they found a clear correlation between the electron withdrawing ability at C-6 and the stability of the glycosyl triflate reaction intermediate. While common glycosyl triflates undergo rapid decomposition at temperatures above –60 °C, it was shown that their trifluorinated counterparts were stable up to +10 °C.

Demchenko's group reported that a mixed protecting group pattern can also unexpectedly and profoundly affect the glycosyl donor reactivity.[86] Upon investigating *S*-benzoxazolyl (SBox) glycosides that contained an "arming" benzyl group at C-2 and "disarming" acyl groups on the remaining positions, it was expected that reactivity should fall somewhere between that of the armed (per-benzylated) and the disarmed (per-benzoylated) glycosyl donors. However, results revealed that these "mixed-pattern" donors were the least reactive amongst the building blocks investigated.[86] Additionally, a glycosyl donor containing a participating benzoyl group at C-2 and electron donating groups at the remaining positions, was also investigated. Resultantly, these glycosyl donors proved to be even more reactive (superarmed) than their armed (per-benzylated) counterparts.[87,88] Together, these findings implicate that a complex system of electronic effects may exist beyond the recognized inductive effects of the C-2 protecting group. The observed reactivity dichotomy was rationalized by the occurrence of the "O-2/O-5 cooperative effect." This states that, in addition to the "arming" and "disarming" nature of the protecting group at C-2, the stabilization of the related oxacarbenium ion intermediate must also be taken into consideration when justifying glycosyl donor reactivity. As such, it was proposed that stabilization for the glycosylation intermediate could be achieved through two possible sources of lone electron pair donation. The first, comes from a lone electron pair on the neighboring endocyclic ring oxygen, O-5. However, if electron withdrawing protecting groups are placed near the O-5 ring oxygen, they will decrease

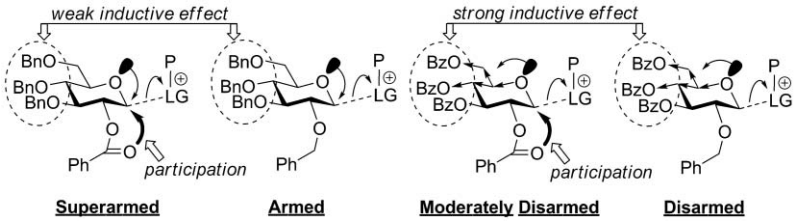


Fig. 2 Mixed protecting groups and O-2/O-5 cooperative effect.

the electron density at this oxygen, effectively suppressing the oxacarbenium ion formation (Fig. 2).

Additionally it was thought that the second source of reactivity arose from the availability of a lone electron pair existing on an acyl type protecting group at C-2, providing stabilization by way of an acyloxonium ion intermediate. However, upon revisiting this theory, Crich and Li suggested that anchimeric assistance provided the additional reactivity, thus revealing this as a highly underestimated source of reactivity.[89] With this established, it then follows that glycosyl donors possessing participating groups at C-2 will display a higher reactivity than analogous donors possessing non-participating groups at C-2.

In 2001, Bols *et al.* began investigating the influence that substituent orientation can have on the reactivity of a molecule.[90] While these studies were performed using substituted heterocyclic amines, the resultant findings proved to be extremely useful with respect to the reactivity of carbohydrates. Thus, it was found that the pK_a of protonated amines (conjugate acids) could be used to directly measure the electronic effects of various ring substituents. Ultimately, a correlation emerged between the acidity of the molecule and the configuration of the substituent, finding equatorial substituents to be significantly more electron withdrawing (destabilizing) than their axial counterparts (Fig. 3). The numerical values (substituent constants) shown are in pH units, and reflect the amount by which the pH decreases with respect to its unsubstituted parent amine (piperidine). Alternative explanations, such as steric hindrance, resonance, induction, solvation and internal hydrogen bonding were all ruled out, leaving a strong case in favor of stereoelectronic substituent effects.[91]

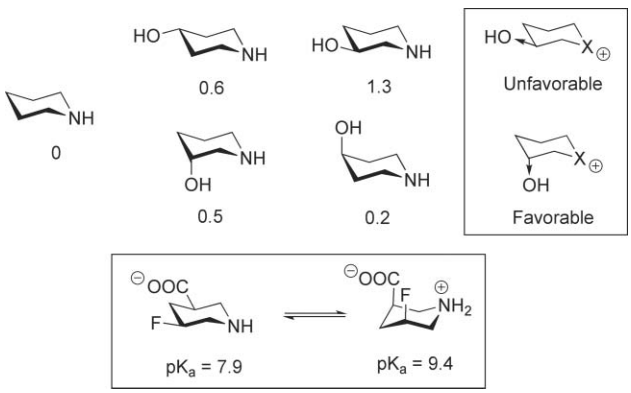
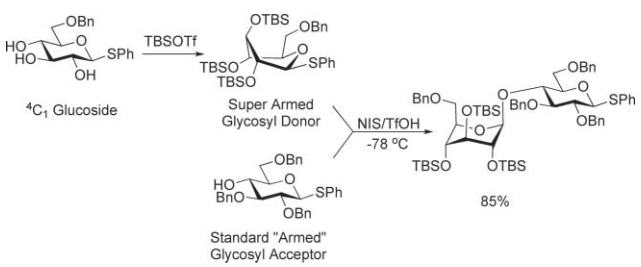


Fig. 3 Substituent effect and conformational preferences of substituted piperidines.

Further revealed by these findings was that a perturbation of the equilibrium conformations also occurred upon protonation of the heterocyclic amine.[92] This was found to result from the desire for substituents to reside axially, as they have a greater ability to provide charge stabilization through charge–dipole interactions. For example, after protonation of the fluoropiperidine derivative in Fig. 3, it was found to exist solely in the conformation where the electron-withdrawing substituents were axial. Furthermore, in viewing these compounds as analogs for similar cationic structures, they were easily likened to oxacarbenium ion intermediates. This could suggest that positively charged glycosylation intermediates will spontaneously undergo conformational changes in an attempt to maximize the number of axial substituents, which could impact the reactivity and stereoselectivity of the reaction.

In further application toward carbohydrates, it was subsequently established that a glycosyl donor possessing axial substituents at the C-3 and C-4 position had a more stabilized oxacarbenium ion intermediate, relative to an analogous glycosyl donor with all equatorial substituents. Accordingly, this configurational modification proved to increase the reactivity of the glycosyl donor, and also provided further insight into the reactivity difference between the various sugar derivatives (gluco-, manno-, galacto-, *etc.*), thus bringing to light the profound impact that subtle electronic changes can have on the reactivity of the glycosyl donor. In turn, this led to the concept of conformationally superarming the glycosyl donors.[93] It was previously found that introducing steric congestion at the equatorial C-3 and C-4 positions would cause conformational changes wherein the typical $^4C_1$ conformation would flip to the less common $^1C_4$ conformer.[94] This concept was utilized by Matsuda and Shuto *et al.*,[95] in which bulky and robust *tert*-butyldimethylsilyl protecting groups were installed on xylopyranose derivatives.[96] However, when applied to glucose analogs, they were found to exist in more of a skew-boat conformation (as shown for the superarmed glycosyl donor in Scheme 9),[97] perhaps due to the added bulk of the substituent at C-5. Nevertheless, this general approach sufficiently induced the conformational change necessary to reconfigure the substituents perpendicularly to the sugar ring. As a result, these conformationally armed (ring flipped) glucosyl donors have shown a dramatic increase in reactivity relative to the traditional



Scheme 9 Conformationally superarmed glycosyl donors.

armed, benzylated derivatives (Scheme 9).[98] This increase in reactivity was further verified through kinetic studies, wherein the conformationally armed donor was found to react 20-fold faster than its armed counterpart, and could be successfully coupled with armed acceptors.[99] Similar observations have been made with glycosyl donors of the manno, rhamno, and galacto series.[100]

In contrast to conformational arming, Fraser-Reid and co-workers discovered that locking the pyranose ring in the $^4C_1$ chair conformation disarms the glycosyl donor.[101] This deactivation is attributed to the increased rigidity of the fused ring system, calculating that the oxacarbenium ion intermediate is not able to achieve the requisite planar geometry (about the C-2–C-1–O-5–C-5 atoms) in the half-chair transition state. This concept was expanded upon by Ley and co-workers in their exploration of 1,2-diacetal systems.[102] In further mechanistic probing, Bols and co-workers proposed that the source of the disarming effect may not be solely conformational, but may also be partially due to the orientation of the C-6 substituent.[103] Ingeniously, a series of torsionally restricted substrates were designed wherein each one was varied with respect to the orientation of its C-6 substituent (Fig. 4, rotamers **b–d**). The reactivities of these analogs were then compared to that of the unrestricted compound **a**. It was found that a basic torsional disarming effect *does* exist, as all of the conformationally restricted analogs exhibited a much lower reactivity towards acidic hydrolysis. However, the data suggests that the stereoelectronic effect[104] of the substituent configuration *also* plays a significant role in the overall level of disarming (electronic effect). As seen in Fig. 4, the torsionally disarmed rotamer **b**, wherein the methoxy substituent is perpendicular to the ring, is 1.5 times more reactive than rotamer **c**, and 3.5 times more reactive than rotamer **d**, which is the conformation adopted in 4,6-acetal-protected glucosyl donors. Thus, it was concluded that both conformational restriction and stereoelectronics (charge–dipole interactions) were equally responsible for the observed disarming effect.

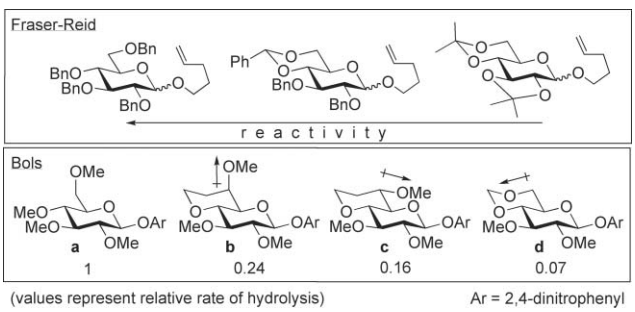
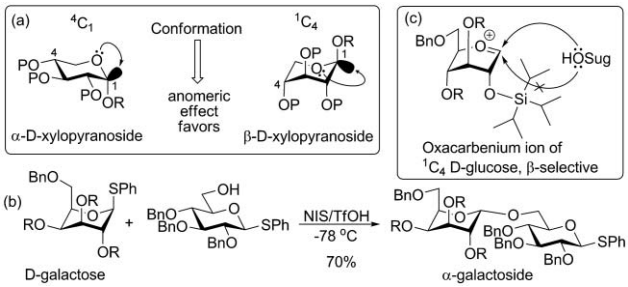


Fig. 4 Torsional or electronic disarming.

5. Conformation of the oxacarbenium ion and stereoselectivity of glycosylation

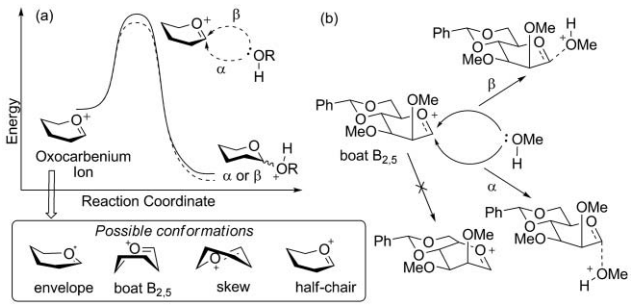
In 2000, Matsuda and Shuto began investigating various silylated xylopyranosyl donors that existed in the ring-flipped $^1C_4$ conformation.[95] They found that through this conformational modification, excellent β-stereoselectivity could be achieved, even in the absence of neighboring group participation. This was proposed to be a consequence of the anomeric effect, wherein formation of the axial anomer is favored (Scheme 10a). On this premise, experiments were designed wherein various xylose derivatives were inverted to their $^1C_4$ conformations, thus altering the anomeric effect, and thereby reversing their stereoselectivities.[96] However, a further study by Bols and co-workers revealed that the ring-flipped glycosyl donors of the D-manno-, D-galacto-, and L-rhamno series lead to nearly complete α-stereoselectivity (Scheme 10b), a stark contradiction to the anticipated influence of the anomeric effect. Interestingly, only the D-gluco analog provided excellent β-stereoselectivity (see Scheme 9).[100] Thus, it was proposed that steric factors are the underlying basis for the selectivity of these reactions. Yamada *et al.*, further reinforced this observation, attributing the β-selectivity in glucose derivatives to the steric environment created by the near $^1C_4$ (skew-boat) conformation (Scheme 10c).[97]



Scheme 10 Attempts to reverse the anomeric effect with conformationally inverted glycosyl donors. (a) Influence of conformation on the anomeric effect, (b) Glycosylation using conformationally inverted D-galactosyl donor, (c) Steric factors affecting transition state of a ring inverted D-glucosyl donor.

Whitfield *et al.* also investigated the stereoselectivity with which glycosylation reactions proceed; however, they attributed the glycosylation outcome to the conformational preference of the oxacarbenium ion intermediate.[69] This rationale was based upon the energy differences of the transition states associated with the transformation of the oxacarbenium ion intermediate to the glycoside product. Accordingly, each face of attack (α or β) will possess a different transition state energy and therefore, the major glycosylation product will be associated with the lower energy transition state (Scheme 11a). As various factors can contribute to the energy inequalities in this transition state, theoretical calculations had to consider several effects, including: solvation, hydrogen bonding, bonding interactions between the incoming nucleophile and the oxacarbenium ion, ring strain induced by the incoming nucleophile or by hydrogen bonding, and differential ion pairing.

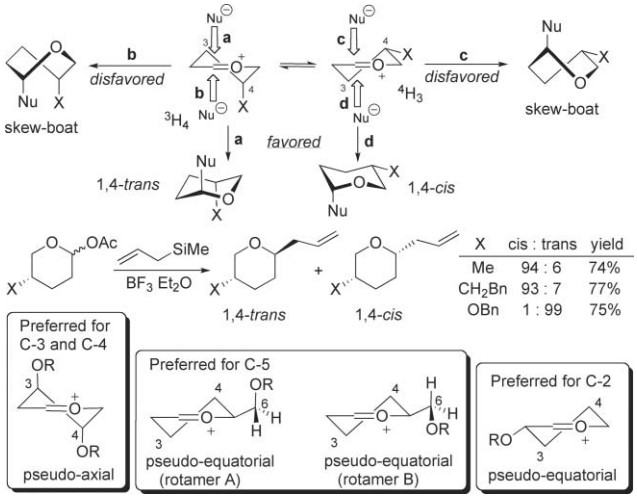
Before the relative energies of the transition states could be calculated, it followed that the conformation of the oxacarbenium ion intermediate needed to be established. Previously, it had been proposed that low-energy conformations, such as half-chairs, were the most likely, as they mimic the flattened sp^2 geometry of the electron deficient anomeric center (C-5–O-5–C-1–C-2).[105] However, the ensuing calculations revealed that the flexibility of the pyranose ring actually allowed for a wider variety of intermediates. As such, the boat, skew, and envelope conformations were added to the pool of low-energy intermediate conformations (Scheme 11a). This required that the likely oxacarbenium ion conformations, corresponding to each and every glycosyl donor,



Scheme 11 (a) Reaction profile of oxacarbenium ion transition-state, (b) Plausible reaction pathways of 4,6-*O*-benzylidene-2,3-di-*O*-methyl-mannopyranosyl cation.

be individually calculated.[106] It was thus found that each glycosyl donor gives rise to two possible series of low-energy oxacarbenium ion conformations,[69,107] one series being the ring-flipped version of the other. To simplify the study, one series of conformers was prevented from forming by introducing a rigid 4,6-acetal protecting group to the glycosyl donor. For example, the 4,6-*O*-benzylidene-2,3-di-*O*-methyl-mannopyranosyl cation can only exist in the series corresponding to the $B_{2,5}$ conformation, but not in the family of conformers represented by ring inversion (Scheme 11).[69] With this simplification, it was calculated that the transition state formed from the β-attack of the glycosyl acceptor (MeOH) was 38 kJ mol^{-1} lower in energy than its α-approach, and thus the β-glycoside was predicted to be the major product. While the theoretical calculations of these simplified donor–acceptor systems were in good correlation with the experimental results, it is not to be expected that this method can be used to generally predict the diastereomeric product ratio of any glycosylation. However, it does reinforce the proposed theory that the stereoselectivity arises from the conformational preferences of the oxacarbenium ion intermediate. Furthermore, it implies that the relative energies of the transition states corresponding to α- and β-attack play an important role in defining the final product selectivity. It is thus anticipated that this knowledge will be instrumental in designing future glycosyl donors, wherein conformational restrictions may be implemented to generate a high degree of facial selectivity.

Possessing a similar viewpoint, Woerpel and co-workers also reported on the adopted conformations of oxacarbenium ions, and their effect on the facial preferences of incoming nucleophiles. Their approach utilized substituted tetrahydropyrans as model substrates, wherein the steric and electronic effects of the attached substituents could be methodically studied.[108] An anomeric acetate was used as the leaving group, and to ensure irreversibility of the glycoside formation, allyltrimethylsilane was employed as the nucleophile. Subsequently, systematic changes were made to the substituted tetrahydropyran glycosyl donor and the resulting *cis*/*trans* ratios of the *C*-glycoside products were recorded. These ratios were then used to determine how the various protecting group modifications affected the conformation of the ensuing oxacarbenium ion intermediate. As depicted in Scheme 12, Woerpel initially presumed that oxacarbenium ions exist in rapid equilibrium between two diastereomeric half-chair conformations, either $^4H_3$ or $^3H_4$. As dictated by the location and type of substituent(s) attached to the ring, one of these conformers should be generally more preferred. Furthermore, because orbital



Scheme 12 Investigation with C-4 substituted tetrahydropyrans.

interactions favor a pseudo-axial attack on the sp^2 carbon, there are only two possible trajectories of attack on each half-chair conformer, each leading to a different product stereoselectivity (α or β).[108] However, one of these facial approaches can always be excluded, due to the high energy skew-boat transition state that is encountered en route to product formation (disfavored pathways **b** or **c**, Scheme 12). Thus, the alternative facial approach, wherein the more stable chair-like transition state occurs (favored pathways **a** or **d**), always predominates.[109]

As the $^4H_3$ or $^3H_4$ half-chairs are diastereomers, the allowed facial attack on one diastereomer will result in an α-glycoside, while the same allowed attack of the other will lead to a β-glycoside. Thus, the major glycoside product will also reveal which oxacarbenium ion conformer predominates. For example, the experimental results shown in the table in Scheme 12, revealed opposite stereochemical outcomes for an alkyl *vs.* alkoxy substituent. The product route associated with the 1,4-*cis* formation was traced back to the $^4H_3$ conformation of the oxacarbenium ion, whereas the 1,4-*trans* product resulted from the $^3H_4$ conformation.[110] Using this method, they found that alkoxy substituents at the C-3 and C-4 positions preferred to adopt the half-chair conformation wherein they could exist pseudo-axially, ultimately giving rise to 1,4-*trans* products. Conversely, alkyl substituents preferred conformations wherein they could reside pseudo-equatorially, and thus gave rise to 1,4-*cis* products. These opposing preferences are thought to be a product of electrostatic interactions[110] similar to those of the charge–dipole effect proposed by Bols (Section 4 Fig. 3).[100] Therefore, in alkyl substituents, wherein there can be no electrostatic stabilization, sterics predominate and so the pseudo-equatorial configuration is preferred. Further revealed, was the preference of the flexible C-5 alkoxymethyl group to reside in a pseudo-equatorial position, and that the orientation (rotamer) of the attached C-6 alkoxy group always pointed back over the ring (Scheme 12, rotamer A).[110] Lastly, the C-2 alkoxy substituent was found to prefer the pseudo-equatorial orientation, as it is thought to be involved in a stabilizing electronic interaction with the anomeric center (Scheme 12).[110]

Additionally, van der Marel and co-workers have begun studying the influence of the C-5 position on glycosylation

stereoselectively.[111–113] It was shown that a carboxylic acid functionality at C-5 (uronic acids) displays an extremely strong axial preference in its oxacarbenium ion transition state, much higher than that of an ether or alkyl protecting group at C-5. Again, the primary motivation for this preference is electrostatic charge stabilization of the oxacarbenium ion. Thus, in the case of mannuronate esters, wherein all substituents occupy their preferred transition state configurations, a completely β-selective glycosylation was achieved.

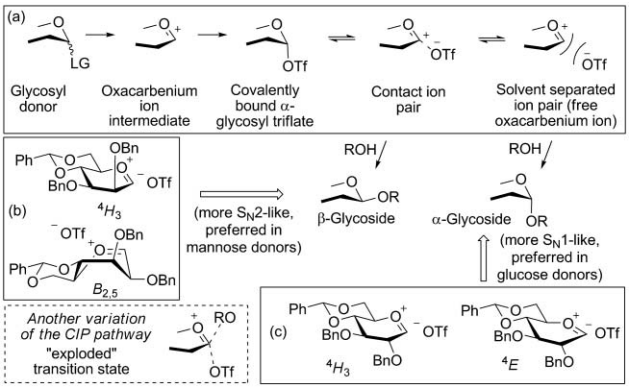
Armed with this comprehensive knowledge, the preferred half-chair conformation for the model substrates was accurately predicted. The established preferences of these simplified systems, however, does not take into account the additional steric (and possibly electronic) factors that are present in actual sugars. Thus, in more complicated systems, the stereoelectronic and steric complexities can compound rather quickly and may alter the established trends.[114] Ultimately, however, both Whitfield and Woerpel reached the same conclusion, finding the configuration of the oxacarbenium ion intermediate to be highly influential in determining the diastereoselectivity of the glycosylation reaction. As such, the observed product stereoselectivities can ultimately be attributed to a delicate balance between steric and stereoelectronic effects influencing the transition state.

6. Exploration of anomeric inversion and participation-assisted mechanistic pathways

In the previous section, discussions involved oxacarbenium ion intermediates that were transformed directly into their respective glycoside products upon nucleophilic attack by the glycosyl acceptor. However, there are often many other reactive species present in the reaction mixture, such as the counter anion of the electrophilic promoter, the leaving group, additives (such as bases), the solvent, or even the intramolecular participation of protecting groups.[77] This creates an opportunity for other reactions to occur at the anomeric center prior to the attack of the glycosyl acceptor. As such, the resulting intermediate species can also affect the product stereoselectivity. Therefore, investigating such species can provide further insight into the general mechanistic pathways and preferences of the glycosylation reaction. Herein, we will predominantly discuss a few chosen intermediates, and the pathways and conformational changes that they incur en route to product formation. Reaction intermediates of both intermolecular (glycosyl triflate) and intramolecular (neighboring group participation) character will be considered. Often, these intermediate species exert a profound influence upon the stereoselectivity of the glycosylation reaction. Therefore, it is conjectured that these reactions may proceed *via* a concerted nucleophilic displacement.[5] However, the probability of an actual S_N2 mechanism occurring at the anomeric center is proposed to be highly unlikely, even in completely stereoselective reactions.[115] Such claims have been attributed to the electron–electron repulsions that are encountered upon nucleophile approach,[116] as well as the weakness of typical nucleophiles used in glycosylation. Based upon this assumption, an intermediate glycosylation species that is formed must first transform back into a cationic species before glycosyl acceptor attack occurs. As such, comparisons can be made between the factors that affect the transition of a glycosyl donor directly into a glycoside product and those which affect the transformation of a secondary intermediate into the observed glycoside product.

First, we will start by addressing the glycosyl triflate. This species was brought to light when Crich *et al.* found that the stereoselectivity of a glycosidation reaction utilizing glycosyl sulfoxides, triflic anhydride and a pyridine-derived base was completely dependent upon the order of reagent addition.[117] Through spectroscopic studies, it was determined that when the reagents were added prior to the glycosyl acceptor ("pre-activation" conditions), a covalently bound triflate species would form *in situ*.[118] Furthermore, the characteristics of the glycosidic bond formation reflected that of the intermediate triflate, and were independent of the original leaving group employed.[119] Probing this mechanism revealed that the stereoselectivity with which the reaction proceeded was strongly dependent upon the core monosaccharide structure and selected protecting groups.[120,121] Thus, the pre-activation of a mannosyl donor, possessing the conformationally restrictive 4,6-benzylidene acetal, with Tf_2O and DTBMP (di-*tert*-butyl-4-methylpyridine), yielded a very stable α-triflate. Thereafter, the addition of a nucleophile often resulted in complete β-selectivity. In contrast, mannosyl donors lacking the rigid benzylidene protecting group were much less selective. One could presume that torsional disarming enhances the stability of the α-triflate, which then allows for the inversion product to form *via* concerted bimolecular displacement. Against expectations, however, the use of torsionally disarmed glucosyl donors preferentially led to the formation of α-glucosides.[120] Thus, the probability of the reaction proceeding *via* a true S_N2 mechanism is highly questionable. Additionally perplexing was that the NMR spectra of the 4,6-benzylidene manno- and glucosyl donors revealed that only the α-triflate was present, diminishing the likelihood of an isomerization pathway (akin to Lemieux's halide ion promoted *in situ* anomerization protocol).[80]

In order to discriminate between the possible S_N1 and S_N2 pathways, a kinetic isotope effect study was carried out using the benzylidene-protected α-mannosyl triflate.[122] By matching the experimentally determined results with already known kinetic isotope effects of simple glycoside hydrolysis, it was ascertained that the results were consistent with that of an S_N1 mechanism. This study led to a mechanistic interpretation wherein the covalently bound triflate first dissociates into a continuum of ionic species prior to nucleophilic attack (Scheme 13a). Consequently, the stereoselectivity of these reactions arises from the dominant ionic species through which the product formation occurs. Accordingly, it was concluded that the α-selectivity seen with the 4,6-benzylidene glucosyl donors must have occurred *via* a solvent separated ion pair (*i.e.* free oxocarbenium ion), whereas the β-selectivity seen in 4,6-benzylidene mannosyl donors occurred through a contact ion pair. The rationalization is that the solvent separated ion pair can allow for attack to occur from either face, whereas the contact ion pair will inhibit the bottom face attack. This can either be due to a shielding effect or a remaining loose attachment (*i.e.* "exploded transition state") as the triflate anion departs from the donor (Scheme 13). In order to bolster this mechanistic interpretation, a study of the various conformations of the corresponding oxacarbenium intermediate species was embarked upon. Therein, it was assumed that the more stable the oxacarbenium ion intermediate was, the more likely its existence. As a consequence, the equilibrium will shift from the



Scheme 13 Proposed participation–dissociation pathway in a glycosylation reaction: glycosyl triflates. (a) Continuum ion ionic species, (b) Preferred oxacarbenium ion species for 4,6-*O*-benylidene protected D-mannosyl donor, (c) Preferred oxacarbenium ion species for 4,6-*O*-benylidene protected D-glucosyl donor.

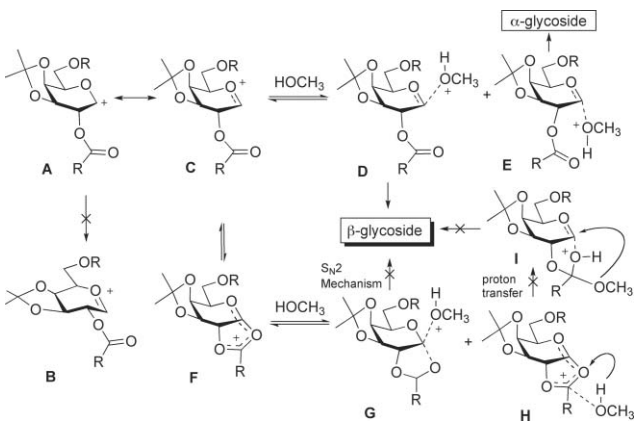
covalently bonded α-triflate toward the solvent separated ion pair, thus decreasing the β-selectivity. Therefore, it was surmised that the energy required for the mannosyl donor to proceed to its cationic intermediate was higher than that of its glucosyl counterpart.

Seeing as the only structural difference between the two glycosyl donors is the configuration about the C-2 position, the torsional angle about this bond was examined. To begin these studies, a conformational model of the oxacarbenium ion was needed. Taking into consideration the theoretical calculations of prior studies,[69,107,114] plausible conformations were considered to be the $^4H_3$ half-chair, the $B_{2,5}$ boat, and the ^4E envelope (Scheme 13b,c). As shown in Table 1, there is a greater compression of the O-2–C-2–C-3–O-3 torsional angle upon going from the mannosyl triflate to its proposed oxacarbenium intermediates, as compared to the relaxation of this torsional angle upon transition of the glucosyl species. It was thereby postulated that the rehybridization of the anomeric carbon causes unfavorable changes in the case of the mannosyl donor, whereas this transformation is much more favored in the case of the glucosyl donor.[123] Therefore, the instability of the mannosyl oxacarbenium ion intermediate, causes the equilibrium to shift toward the covalently bound glycosyl triflate, leading to a more S_N2-like displacement, and thus higher β-selectivity. The opposite is true for the glucosyl donor, wherein equilibrium will shift toward the free ion pair, resulting in a more S_N1-like mechanism. In related study by Huang and Whitfield *et al.*,[124] anomeric triflates equipped with a C-2 participating group were investigated. Therein, it was found that the more electron-deficient the sugar ring was, the more apt the species was to form the covalently bound anomeric triflate. Conversely, the more electron-rich the ring was, the more likely it was to form the positively charged acyloxonium ion, again, reinforcing the notion that the reactivity and selectivity of the reaction was found to be strongly dependent upon the stability of their respective glycosylation intermediates.

Table 1 Torsional angle values (and change) for glycosyl triflates and the oxacarbenium conformers

Man	O2–C2–C3–O3	Glc	O2–C2–C3–O3
α-OTf	60°	α-OTf	60°
$^4H_3$	45° (−15°)	$^4H_3$	75° (+15°)
$B_{2,5}$	60° (0°)	^4E	90° (+30°)

Whitfield *et al.* further probed the role that auxiliary species may play in the glycosylation reaction. They studied the mechanism by which intramolecular neighboring group participation occurs. These studies uncovered an array of challenges similar to those of the intermolecular glycosyl triflate participation. As aforementioned, the probability of an actual S_N2 mechanism occurring at the anomeric center is highly unlikely, even in highly stereoselective reactions, such as those with the neighboring group participation.[115] If true, then the acyloxonium intermediate must first dissociate prior to nucleophilic attack. Consequently, a resulting contact ion pair must be responsible for the observed stereoselectivity. While it is commonly assumed that the bicyclic acyloxonium ion intermediate is solely responsible for the high (and often complete) stereoselectivity achieved with 2-acyl derivatives, Whitfield *et al.* have provided a viable alternative.[115] First, they were able to limit the number of possible intermediate conformations to two (oxacarbenium ion **C**, and acyloxonium ion **F**, Scheme 14), through the use of conformationally restricted glycosyl donors. Subsequently, low-energy pathways connecting these key intermediates to the other plausible species (*i.e.* **D**, **E**, **G**, **H** and **I**) en route to the anticipated 1,2-*trans* and 1,2-*cis* product, were calculated. It was assumed that acyloxonium ion **F** can form only after the formation of oxacarbenium ion **C**. Although **F** was calculated to be a lower energy intermediate, the C-2 substituent must adopt a pseudo-axial orientation in order to bond with the anomeric center. Therefore, these conformational changes create a small energy barrier that must first be overcome.[106] Further still, was the problem that once **F** did form, calculations could not find a reasonable low-energy pathway linking its subsequent intermediates (**G** or **H**) to the observed β-glycoside product.[115] While it seems counterintuitive, protonated orthoester **H** was actually calculated to be the preferred intermediate. Hence, if the reaction mechanism does proceed by this route, it would likely have to involve a proton transfer to form a higher energy intermediate **I**, before formation of the β-linked product could occur. Because this seemed improbable, they presented the possibility that the stereoselectivity may instead emanate from a face-discriminated attack upon the monocyclic oxacarbenium ion **C**.[106] To test this hypothesis, the relative energies of adducts **D** and **E** were calculated, wherein the β-methanol adduct **D** was found to be



Scheme 14 Plausible mechanism of neighboring group-assisted formation of 1,2-*trans* glycosides.

of lower energy.[69] The energy disparity in these calculations was shown to be highly influenced by both anomeric and hydrogen bonding preferences. Resultantly, it was reasoned that the pathway involving intermediate **D** could, in fact, be responsible for the observed β-stereoselectivity; however, the mechanistic possibility of attack occurring *via* the bicyclic species **G** or **H** could not be completely ruled out.

Recently, a variety of alternative neighboring participating groups have also been investigated. For instance, Boons and co-workers have demonstrated that an (*S*)-1-phenyl-2-thiophenylethyl group at the C-2 position of a glycosyl donor is capable of an efficient neighboring group participation *via* a quasi-stable anomeric *trans*-decalin sulfonium ion (Fig. 5a).[125,126] Displacement of the sulfonium ion by a hydroxyl group leads to the stereoselective formation of 1,2-*cis* glycosides. This study was recently reinforced by showing that thioether additives can increase the α-stereoselectivity of the glycosylation reaction by forming an anomeric β-sulfonium ion.[127] The preference for the formation of the β-species was attributed to a minimization of steric interactions, as opposed to the typical stereoelectronic justification of the reverse anomeric effect. Additionally, Demchenko and co-workers studied 2-picolinyl derivatives which provided a stable 1,2-*cis* participation intermediate, leading to a completely stereoselective 1,2-*trans* glycosylation (Fig. 5b).[128,129] NMR experiments were employed to show the presence of the proposed reaction intermediates shown in Fig. 5a and 5b. Very recently, Fairbanks showed the versatility of 2-(thiophen-2-yl)methyl derivatives capable of stereoselective 1,2-*cis* glycosylation *via* the proposed intermediate shown in Fig. 5c.[130]

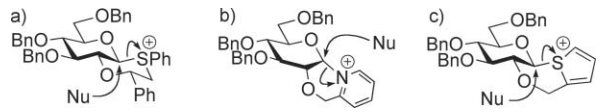
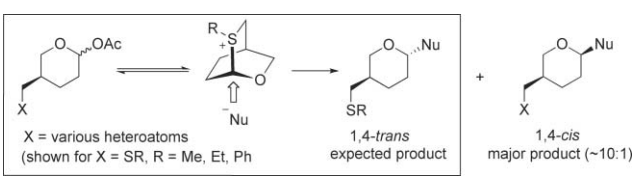


Fig. 5 Alternative participating groups.

Both α- and β-sulfonium species were recently studied by Yoshida and co-workers, wherein the authors suggest that glycosidation of the sulfonium intermediates may proceed *via* glycosyl cation (S_N1).[131] Woerpel *et al.*[132,133] also proposed that the mechanisms for neighboring group participation may actually proceed through the open cation. Investigations were initially carried out on C-4-sulfur-substituted tetrahydropyrans, wherein it was revealed that the resultant 1,4-*cis* product did not correspond to a pathway involving participation from a sulfonium ion species as expected (Scheme 15). Mathematical calculations verified the ring-closed sulfonium ion to be the lowest energy intermediate, and the existence of the sulfonium-ion species resulting from C-4 participation was confirmed by NMR. This phenomenon was further probed by investigating additional C-4-substituted tetrahydropyrans, containing a variety of heteroatoms (selenium, sulfur, oxygen and halogens), yet all analogous species revealed a selectivity preference in favor of the 1,4-*cis* product. External factors such as solvent, promoter and nucleophile were additionally investigated, and unexpectedly, the stereoselectivity got worse as the nucleophilicity was increased. These surprising findings strongly suggest that prudence should be administered when justifying the product formation. Although it is common practice to base reaction outcomes on calculated low-energy intermediates, it does not necessarily mean that these species are involved in the pathway of product formation, an idea reinforced by the Curtin–Hammett kinetic scenario,[47] which states that product formation does not necessarily have to occur *via* the lowest energy intermediates.



Scheme 15 Model study of the neighboring group participation.

7. Conclusions and outlook

It seems that with the inherent complexities of the glycosylation reaction, scientists have not yet been able to fully predict reaction outcomes, rather their investigations have only made it easier to rationalize them. Therefore, while the studies and examples surveyed herein cannot definitively answer many of the mechanistic questions remaining about the glycosylation reaction, they have offered a unique perspective with which the problem can be approached. As such, it is likely that only after: having a complete understanding of the relative populations and energy minimization of the ground state, and possessing a plausible model for the various transition state(s) that also includes an energy minimum; can the true source of product formation be identified.

Thus, as more is learned about the underlying and unwavering fundamental principles governing the glycosylation reaction mechanism, the better able we will be to understand and justify the decisions we make regarding how to control the outcome of the reaction; helping to eliminate unnecessary trial and error methods. When coupled with our existing knowledge about the glycosylation reaction, the studies herein, and the future studies inspired by these works, can only serve to enhance our synthetic capabilities in the challenging field of carbohydrate chemistry.

Acknowledgements

This work was supported by NSF-CAREER award (CHE-0547566)

References

1 A. Varki, *Glycobiology*, 1993, **3**, 97–130.
2 E. J. Hehre, *Carbohydr. Res.*, 2001, **331**, 347–368.
3 K. C. Nicolaou and H. J. Mitchell, *Angew. Chem., Int. Ed.*, 2001, **40**, 1576–1624.
4 B. G. Davis, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2137–2160.
5 D. M. Whitfield and S. P. Douglas, *Glycoconjugate J.*, 1996, **13**, 5–17.
6 K. Toshima and K. Tatsuta, *Chem. Rev.*, 1993, **93**, 1503–1531.
7 P. H. Seeberger and D. B. Werz, *Nature*, 2007, **446**, 1046–1051.
8 D. P. Galonic and D. Y. Gin, *Nature*, 2007, **446**, 1000–1007.
9 J. T. Smoot and A. V. Demchenko, *Adv. Carbohydr. Chem. Biochem.*, 2009, **62**, 161–250.
10 A. V. Demchenko, *Curr. Org. Chem.*, 2003, **7**, 35–79.
11 P. H. Seeberger and W. C. Haase, *Chem. Rev.*, 2000, **100**, 4349–4393.
12 O. J. Plante, E. R. Palmacci and P. H. Seeberger, *Science*, 2001, **291**, 1523–1527.
13 O. J. Plante, E. R. Palmacci and P. H. Seeberger, *Adv. Carbohydr. Chem. Biochem.*, 2003, **58**, 35–54.

14 F. R. Carrel, K. Geyer, J. D. C. Codée and P. H. Seeberger, *Org. Lett.*, 2007, **9**, 2285–2288.
15 P. H. Seeberger, *Chem. Soc. Rev.*, 2008, **37**, 19–28.
16 M. A. Oberli, P. Bindschadler, D. B. Werz and P. H. Seeberger, *Org. Lett.*, 2008, **10**, 905–908.
17 *Essentials of Glycobiology*, ed. A. Varki, R. Cummings, J. Esko, H. Freeze, G. Hart and J. Marth, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y., 1999.
18 *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*, ed. A. V. Demchenko, Wiley-VCH, Weinheim, 2008.
19 B. A. Garcia, J. L. Poole and D. Y. Gin, *J. Am. Chem. Soc.*, 1997, **119**, 7597–7598.
20 B. A. Garcia and D. Y. Gin, *J. Am. Chem. Soc.*, 2000, **122**, 4269–4279.
21 H. M. Nguyen, Y. N. Chen, S. G. Duron and D. Y. Gin, *J. Am. Chem. Soc.*, 2001, **123**, 8766–8772.
22 T. A. Boebel and D. Y. Gin, *J. Org. Chem.*, 2005, **70**, 5818–5826.
23 F. Andersson, P. Fugedi, P. J. Garegg and M. Nashed, *Tetrahedron Lett.*, 1986, **27**, 3919–3922.
24 B. Wegmann and R. R. Schmidt, *J. Carbohydr. Chem.*, 1987, **6**, 357–375.
25 M. Nishizawa, W. Shimomoto, F. Momii and H. Yamada, *Tetrahedron Lett.*, 1992, **33**, 1907–1908.
26 H. Shimizu, Y. Ito and T. Ogawa, *Synlett*, 1994, 535–536.
27 H. K. Chenault, A. Castro, L. F. Chafin and J. Yang, *J. Org. Chem.*, 1996, **61**, 5024–5031.
28 S. Manabe, Y. Ito and T. Ogawa, *Synlett*, 1998, 628–630.
29 R. R. Schmidt and E. Rucker, *Tetrahedron Lett.*, 1980, **21**, 1421–1424.
30 H. Dohi, Y. Nishida, H. Tanaka and K. Kobayashi, *Synlett*, 2001, 1446–1448.
31 E. M. Klimov, N. N. Malysheva, A. V. Demchenko, Z. G. Makarova, V. M. Zhulin and N. K. Kochetkov, *Dokl. Akad. Nauk*, 1989, **309**, 110–114 and references therein.
32 M. Sasaki, Y. Gama, M. Yasumoto and Y. Ishigami, *Tetrahedron Lett.*, 1990, **31**, 6549–6552.
33 R. R. Schmidt, and K. H. Jung, in *Preparative Carbohydrate Chemistry*, ed. S. Hanessian, Marcel Dekker, Inc., New York, 1997, pp. 283–312.
34 T. Mukaiyama and K. Matsubara, *Chem. Lett.*, 1992, 1041–1044.
35 K. Fukase, A. Hasuoka, I. Kinoshita, Y. Aoki and S. Kusumoto, *Tetrahedron*, 1995, **51**, 4923–4932.
36 R. Eby and C. Schuerch, *Carbohydr. Res.*, 1974, **34**, 79–90and references therein.
37 G. Wulff and G. Rohle, *Angew. Chem., Int. Ed. Engl.*, 1974, **13**, 157–170.
38 A. Demchenko, T. Stauch and G. J. Boons, *Synlett*, 1997, 818–820.
39 S. Hashimoto, M. Hayashi and R. Noyori, *Tetrahedron Lett.*, 1984, **25**, 1379–1382.
40 A. Ishiwata, Y. Munemura and Y. Ito, *Tetrahedron*, 2008, **64**, 92–102.
41 M. Adinolfi, A. Iadonisi and A. Ravida, *Synlett*, 2006, 583–586.
42 A. V. Demchenko, *Handbook of Chemical Glycosylation*, 2008, pp. 1–27.
43 X. Zhu and R. R. Schmidt, *Angew. Chem., Int. Ed.*, 2009, **48**, 1900–1934.
44 S. Manabe and Y. Ito, *Curr. Bioact. Compd.*, 2008, **4**, 258–281.
45 R. U. Lemieux, *Adv. Carbohydr. Chem.*, 1954, **9**, 1–57 and references therein.
46 B. Capon, *Chem. Rev.*, 1969, **69**, 407–496.
47 J. I. Seeman, *Chem. Rev.*, 1983, **83**, 83–134.
48 A. Michael, *Am. Chem. J.*, 1879, **1**, 305–312.
49 E. Fischer, *Ber. Dtsch. Chem. Ges.*, 1893, **26**, 2400–2412.
50 W. Koenigs and E. Knorr, *Ber. Dtsch. Chem. Ges.*, 1901, **34**, 957–981.
51 E. Fischer and E. F. Armstrong, *Ber. Dtsch. Chem. Ges.*, 1901, **34**, 2885.
52 K. Igarashi, *Adv. Carbohydr. Chem. Biochem.*, 1977, **34**, 243–283.
53 P. Walden, *Ber. Dtsch. Chem. Ges.*, 1896, **29**, 133.
54 A. Streitweiser Jr, *Chem. Rev.*, 1956, **56**, 571–752.
55 A. F. Bochkov, and G. E. Zaikov, *Chemistry of the O-glycosidic bond: formation and cleavage*, Pergamon Press, 1979.
56 L. Goodman, *Adv. Carbohydr. Chem. Biochem.*, 1967, **22**, 109–175.
57 W. Pigman and H. Isbell, *J. Res. Nat. Bur. Stand.*, 1937, **19**, 189–213 and references therein.
58 H. S. Isbell, *Annu. Rev. Biochem.*, 1940, **9**, 65–92.
59 H. S. Isbell and H. L. Frush, *J. Res. Nat. Bur. Stand.*, 1949, **43**, 161–171.
60 S. Winstein, E. Grunwald, R. E. Buckles and C. Hanson, *J. Am. Chem. Soc.*, 1948, **70**, 816–821.
61 S. Winstein and E. Grunwald, *J. Am. Chem. Soc.*, 1948, **70**, 828–837.
62 J. T. Edward, *Chem. Ind.*, 1955, 1102–1104.
63 R. U. Lemieux, *Pure Appl. Chem.*, 1971, **25**, 527–548 and references therein.
64 I. Tvaroska and T. Bleha, *Adv. Carbohydr. Chem. Biochem.*, 1989, **47**, 45–123.
65 P. Deslongchamps, *Tetrahedron*, 1975, **31**, 2463–2490.
66 P. Deslongchamps, *Stereolectronic Effects in Organic Chemistry*, Pergamon Press, Oxford, 1983.
67 C. W. Andrews, B. Fraser-Reid and J. P. Bowen, *J. Am. Chem. Soc.*, 1991, **113**, 8293–8298.
68 M. L. Sinnott, *Adv. Phys. Org. Chem.*, 1988, **24**, 113–204.
69 T. Nukada, A. Berces and D. M. Whitfield, *Carbohydr. Res.*, 2002, **337**, 765–774.
70 H. Paulsen, in *Selectivity - a Goal for Synthetic Efficiency*, ed. W. Bartmann and B. M. Trost, Verlag Chemie, Weinheim, 1984, pp. 169–190.
71 C. Uriel, A. M. Gomez, J. C. Lopez and B. Fraser-Reid, *Eur. J. Org. Chem.*, 2009, 403–411.
72 K. N. Jayaprakash, J. Lu and B. Fraser-Reid, *Angew. Chem., Int. Ed.*, 2005, **44**, 5894–5898.
73 C. Uriel, A. M. Gomez, J. C. Lopez and B. Fraser-Reid, *Synlett*, 2003, 2203–2207.
74 M. Mach, U. Schlueter, F. Mathew, B. Fraser-Reid and K. C. Hazen, *Tetrahedron*, 2002, **58**, 7345–7354.
75 B. Fraser-Reid, J. C. Lopez, K. V. Radhakrishnan, M. V. Nandakumar, A. M. Gomez and C. Uriel, *Chem. Commun.*, 2002, 2104–2105.
76 N. M. Spijker and C. A. A. van Boeckel, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 180–183.
77 D. M. Whitfield, *Adv. Carbohydr. Chem. Biochem.*, 2009, **62**, 83–159.
78 W. Pilgrim and P. Murphy, *Org. Lett.*, 2009, **11**, 939–942.
79 J. D. M. Olsson, L. Eriksson, M. Lahmann and S. Oscarson, *J. Org. Chem.*, 2008, **73**, 7181–7188.
80 R. U. Lemieux, K. B. Hendriks, R. V. Stick and K. James, *J. Am. Chem. Soc.*, 1975, **97**, 4056–4062and references therein.
81 D. R. Mootoo, P. Konradsson, U. Udodong and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1988, **110**, 5583–5584.
82 N. L. Douglas, S. V. Ley, U. Lucking and S. L. Warriner, *J. Chem. Soc., Perkin Trans. 1*, 1998, 51–65.
83 Z. Zhang, I. R. Ollmann, X. S. Ye, R. Wischnat, T. Baasov and C. H. Wong, *J. Am. Chem. Soc.*, 1999, **121**, 734–753.
84 T. Schmidt and R. Madsen, *Eur. J. Org. Chem.*, 2007, 3935–3941.
85 D. Crich and O. Vinogradova, *J. Am. Chem. Soc.*, 2007, **129**, 11756–11765.
86 M. N. Kamat and A. V. Demchenko, *Org. Lett.*, 2005, **7**, 3215–3218.
87 L. K. Mydock and A. V. Demchenko, *Org. Lett.*, 2008, **10**, 2107–2110.
88 L. K. Mydock and A. V. Demchenko, *Org. Lett.*, 2008, **10**, 2103–2106.
89 D. Crich and M. Li, *Org. Lett.*, 2007, **9**, 4115–4118.
90 H. H. Jensen, L. Lyngbye and M. Bols, *Angew. Chem.*, 2001, **113**, 3555–3557.
91 H. H. Jensen, L. Lyngbye, A. Jensen and M. Bols, *Chem.–Eur. J.*, 2002, **8**, 1218–1226.
92 H. H. Jensen and M. Bols, *Acc. Chem. Res.*, 2006, **39**, 259–265.
93 C. McDonnell, O. Lopez, P. Murphy, J. G. F. Bolanos, R. Hazell and M. Bols, *J. Am. Chem. Soc.*, 2004, **126**, 12374–12385.
94 T. Hosoya, Y. Ohashi, T. Matsumoto and K. Suzuki, *Tetrahedron Lett.*, 1996, **37**, 663–666.
95 S. Shuto, Y. Yahiro, S. Ichikawa and A. Matsuda, *J. Org. Chem.*, 2000, **65**, 5547–5557.
96 H. Abe, S. Shuto and A. Matsuda, *J. Am. Chem. Soc.*, 2001, **123**, 11870–11882.
97 Y. Okada, O. Nagata, M. Taira and H. Yamada, *Org. Lett.*, 2007, **9**, 2755–2758.
98 C. M. Pedersen, L. U. Nordstrom and M. Bols, *J. Am. Chem. Soc.*, 2007, **129**, 9222–9235.
99 C. M. Pedersen, L. G. Marinescu and M. Bols, *Chem. Commun.*, 2008, 2465–2467.
100 H. H. Jensen, C. M. Pedersen and M. Bols, *Chem.–Eur. J.*, 2007, **13**, 7576–7582.
101 B. Fraser-Reid, Z. Wu, C. W. Andrews and E. Skowronski, *J. Am. Chem. Soc.*, 1991, **113**, 1434–1435.
102 S. V. Ley, D. K. Baeschlin, D. J. Dixon, A. C. Foster, S. J. Ince, H. W. M. Priepke and D. J. Reynolds, *Chem. Rev.*, 2001, **101**, 53–80.

103 H. H. Jensen, L. U. Nordstrom and M. Bols, *J. Am. Chem. Soc.*, 2004, **126**, 9205–9213.
104 A. J. Kirby, *Stereoelectronic effects*, Oxford University Press, 1996.
105 R. U. Lemieux, *Chem. Can.*, 1964, **16**, 14–22.
106 A. Bérces, G. Enright, T. Nukada and D. M. Whitfield, *J. Am. Chem. Soc.*, 2001, **123**, 5460–5464.
107 T. Nukada, A. Berces, L. Wang, M. Z. Zgierski and D. M. Whitfield, *Carbohydr. Res.*, 2005, **340**, 841–852.
108 J. A. C. Romero, S. A. Tabacco and K. A. Woerpel, *J. Am. Chem. Soc.*, 2000, **122**, 168–169.
109 L. Ayala, C. G. Lucero, J. A. C. Romero, S. A. Tabacco and K. A. Woerpel, *J. Am. Chem. Soc.*, 2003, **125**, 15521–15528.
110 M. T. Yang and K. A. Woerpel, *J. Org. Chem.*, 2009, **74**, 545–553.
111 J. D. C. Codee, L. J. van den Bos, A.-R. de Jong, J. Dinkelaar, G. Lodder, H. S. Overkleeft and G. A. van der Marel, *J. Org. Chem.*, 2009, **74**, 38–47.
112 J. Dinkelaar, A. R. de Jong, R. van Meer, M. Somers, G. Lodder, H. S. Overkleeft, J. D. C. Codee and G. A. van der Marel, *J. Org. Chem.*, 2009, **74**, 4982–4991.
113 M. T. C. Walvoort, G. Lodder, J. Mazurek, H. S. Overkleeft, J. D. C. Codee and G. A. van der Marel, *J. Am. Chem. Soc.*, 2009, **131**, 12080–12081.
114 C. G. Lucero and K. A. Woerpel, *J. Org. Chem.*, 2006, **71**, 2641–2647.
115 T. Nukada, A. Berces, M. Z. Zgierski and D. M. Whitfield, *J. Am. Chem. Soc.*, 1998, **120**, 13291–13295.
116 *The PMO Theory of Organic Chemistry*, ed. M. J. S. Dewar and R. C. Dougherty, Plenum, New York, 1975.
117 D. Crich and S. Sun, *J. Org. Chem.*, 1996, **61**, 4506–4507.
118 D. Crich and S. Sun, *J. Am. Chem. Soc.*, 1997, **119**, 11217–11223.
119 D. Crich and S. Sun, *J. Am. Chem. Soc.*, 1998, **120**, 435–436.
120 D. Crich and W. Cai, *J. Org. Chem.*, 1999, **64**, 4926–4930.
121 D. Crich, *J. Carbohydr. Chem.*, 2002, **21**, 663–686.
122 D. Crich and N. S. Chandrasekera, *Angew. Chem., Int. Ed.*, 2004, **43**, 5386–5389 and references therein.
123 D. Crich and O. Vinogradova, *J. Org. Chem.*, 2006, **71**, 8473–8480.
124 Y. Zeng, Z. Wang, D. Whitfield and X. Huang, *J. Org. Chem.*, 2008, **73**, 7952–7962.
125 J. H. Kim, H. Yang, J. Park and G. J. Boons, *J. Am. Chem. Soc.*, 2005, **127**, 12090–12097.
126 J. H. Kim, H. Yang and G. J. Boons, *Angew. Chem., Int. Ed.*, 2005, **44**, 947–949.
127 J. Park, S. Kawatkar, J. H. Kim and G. J. Boons, *Org. Lett.*, 2007, **9**, 1959–1962.
128 J. T. Smoot, P. Pornsuriyasak and A. V. Demchenko, *Angew. Chem., Int. Ed.*, 2005, **44**, 7123–7126.
129 J. T. Smoot and A. V. Demchenko, *J. Org. Chem.*, 2008, **73**, 8838–8850.
130 D. J. Cox and A. J. Fairbanks, *Tetrahedron: Asymmetry*, 2009, **20**, 773–780.
131 T. Nokami, A. Shibuya, S. Manabe, Y. Ito and J. Yoshida, *Chem.–Eur. J.*, 2009, **15**, 2252–2255.
132 S. B. Billings and K. A. Woerpel, *J. Org. Chem.*, 2006, **71**, 5171–5178.
133 M. G. Beaver, S. B. Billings and K. A. Woerpel, *J. Am. Chem. Soc.*, 2008, **130**, 2082–2086.

