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Cation Clock Permits Distinction Between the Mechanisms of α- and β-O- and β-C-Glycosylation in the Mannopyranose Series: Evidence for the Existence of a Mannopyranosyl Oxocarbenium Ion

Min Huang,[†] Pascal Retailleau,[†] Luis Bohé,[†] and David Crich*[,†,‡]

[†]Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, 1 Avenue de la Terrasse, 91190 Gif-sur-Yvette, France

[‡]Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202, United States

Supporting Information

ABSTRACT: The use of a cationic cyclization reaction as a probe of the glycosylation mechanism has been developed and applied to the 4,6-*O*-benzylidene-protected mannopyranoside system. Cyclization results in the formation of both cis- and trans-fused tricyclic systems, invoking an intermediate glycosyl oxocarbenium ion reacting through a boat conformation. Competition reactions with isopropanol and trimethyl(methallyl)silane are interpreted as indicating that β-O-mannosylation proceeds via an associative S_N2-like mechanism, whereas α-O-mannosylation and β-C-mannosylation are dissociative and S_N1-like. Relative rate constants for reactions going via a common intermediate can be estimated.

Glycosylation is the substitution of a leaving group in a glycosyl donor by an acceptor alcohol, often with the aid of a promoter, for which there are two extreme mechanisms, uni- and bimolecular nucleophilic substitution, that are bridged by a continuum of more or less tightly bound ion pairs.[1] Reaction mechanisms are typically based on combinations of stereochemical and kinetic evidence and are supported whenever possible by the characterization of any predicted intermediates and by computational work. In glycosylation, stereochemical evidence in terms of the anomeric selectivity of a coupling is readily available. On the other hand, kinetic evidence is rare,[1a,2] particularly when it is required for both anomers, and it is difficult to obtain because of the multicomponent nature of most glycosylation reactions. Furthermore, the most widely invoked mechanism for glycosylation involves the intermediacy of a glycosyl oxocarbenium ion, a species which, despite much effort,[3] has never been observed other than in silico[4] or in a mass spectrometer.[5] We seek to develop methods for the determination of reaction kinetics for individual glycosylations to facilitate their rational optimization and provide evidence for or against the involvement of glycosyl oxocarbenium ions.[2c] In view of the frequent difficulties faced in obtaining absolute kinetic data for glycosylations, we conceived that relative kinetics would be helpful and that such data might be obtained through the use of a competing cyclization reaction as a clock. We report on the implementation of such a competition kinetic scheme and through it on the distinction between the mechanisms of α- and β-O- and β-C-mannopyranosylation in the presence of a 4,6-*O*-benzylidene acetal group.

Mayr has developed a series of reference scales for the characterization of cationic electrophiles and neutral nucleophiles and has discussed their potential use to predict changes between S_N1 and S_N2 reactions.[6] These scales, however, make use of the intermolecular trapping of a series of chromophoric cations and consequently are not readily adaptable to our purposes. The diffusion-controlled azide clock reaction has been developed for the determination of the kinetics of acetal hydrolysis in aqueous solution and used to estimate the lifetimes of various glycosyl oxocarbenium ions under those conditions,[7] but this method has not been applied to actual glycosidic bond-forming reactions conducted at low temperature in organic solution. Cognizant of the impact of rearrangements as clocks for the determination of relative kinetics in the field of radical chemistry[8] and drawing on our experience with cyclizations of activated glycosyl donors onto protecting groups[9] and parallels with the intramolecular aglycone delivery method of glycosidic bond formation,[10] we considered that ring closure onto appropriately designed substituents would provide a suitable clock reaction for the determination of the relative kinetics in glycosylation reactions.

To avoid complications from the formation of stereogenic centers during the cyclization reaction, we designed a system based on an intramolecular Sakurai reaction[11] and thus on the use of 2-*O*-2-(trimethylsilylmethyl)allyl ethers. Accordingly, regioselective benzylation of diol **1** in the standard manner[12] gave 3-*O*-benzyl ether **2**,[13] which was alkylated with 2-chloromethyl-3-trimethylsilyl-1-propene and sodium hydride to give the desired trimethylsilylmethallyl ether **3** in moderate yield (Scheme 1). Since the use of glycosyl sulfoxides[14] over thioglycosides is preferred for mechanistic work because of the simpler activation protocol and cleaner reaction mixtures, **3** was then oxidized with *m*-chloroperoxybenzoic acid (*m*-CPBA) to give sulfoxide **4** as a 16:1 diastereomeric mixture in which the major isomer was assigned as the $(R)_S$ isomer consistent with precedent.[15] Activation of **4** at −72 °C in dichloromethane in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP)[16] gave two cyclization products after quenching at −72 °C (Scheme 1). The major product, **5**, was identified as the anticipated cis-

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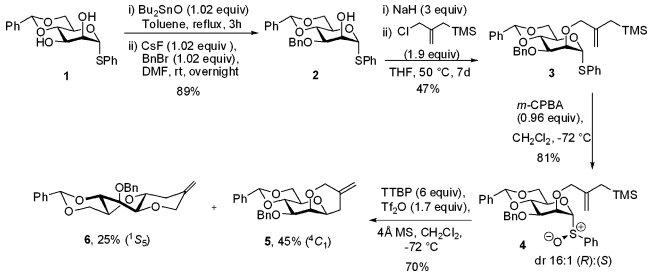
Scheme 1. Preparation and Activation of Sulfoxide 4 and Formation of Tricyclic Compounds 5 and 6



fused system, whereas the minor isomer was the unexpected trans-fused product **6**. Both cyclization products were confirmed by X-ray crystallography (Figure 1), which revealed that the pyranose ring of **5** adopts the $^4C_1$ chair conformation while that of the minor trans-fused isomer **6** takes up the $^1S_5$ twist boat conformation.

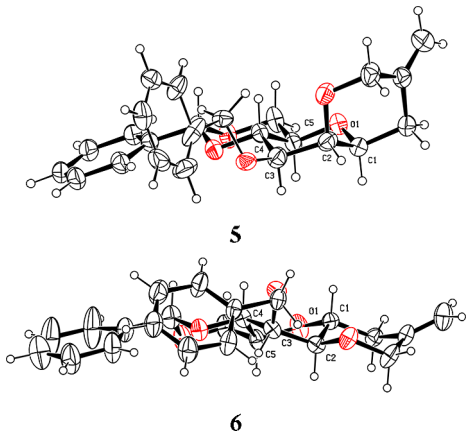


Figure 1. X-ray crystallographic structures of tricycles **5** and **6**.

We rationalize the formation of the trans-fused product **6** by invoking a mannosyl oxocarbenium ion, **8**, that exists in equilibrium with α-glycosyl triflate **7**[17] and accesses the $B_{2,5}$ conformation previously computed.[2c,18] In this conformation, the 2-*O*-silylmethylallyl ether can access both the β-face of the cation, leading to the cis-fused product **5**,[19] and the α-face, resulting in the formation of the trans isomer **6** initially as the $^OS_2$ twist boat, which then relaxes to the observed $^1S_5$ conformer (Scheme 2). The observation of product **6** provides very strong evidence in support of the existence of a mannosyl oxocarbenium ion in equilibrium with the covalent glycosyl triflate.

With the concept established, we turned to deploying the cyclization of sulfoxide **4** as a clock for a glycosylation reaction. Activation of **4** at −72 °C in the presence of 1-octene as a scavenger of the various electrophilic byproducts was followed by the rapid addition of isopropanol, which led to the formation of the β- and α-*O*-mannosides **9** and **10**, respectively, along with the two cyclization products **5** and **6** (Table 1). In a series of experiments, the ratio of the individual amount of glycoside **9** or **10** formed to the combined amount of the cyclization products **5** and **6** was determined as a function of the amount of isopropanol added, resulting in the data presented in Table 1 and plotted in Figure 2.

Scheme 2. Mechanism of Formation of Tricycles 5 and 6

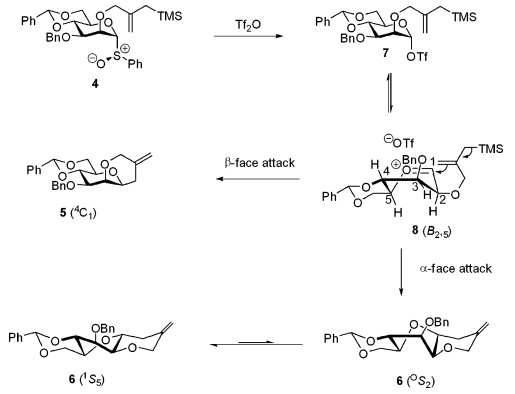
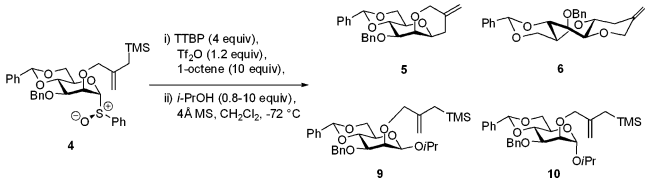


Table 1. O-Glycosylation in Competition with Cyclization[a]



entry	*i*-PrOH[b]	**9**/(**5** + **6**)[c]	**10**/(**5** + **6**)[c]
1	0.8 (0.014)	2.17	0.15
2	1.2 (0.020)	3.66	0.28
3	1.5 (0.026)	5.36	0.44
4	2.5 (0.043)	10.99	0.99
5	3 (0.051)	13.09	1.28
6	4 (0.068)	15.75	1.14
7	5 (0.085)	19.38	1.53
8	8 (0.136)	24.34	1.60

[a]Experimental conditions: TTBP (4 equiv), 1-octene (10 equiv), 4 Å molecular sieves, and Tf_2O (1.2 equiv) at −72 °C. [b]Number of equivalents (concentration in mol/L). [c]Molar ratios were determined by UHPLC/UV/MS.

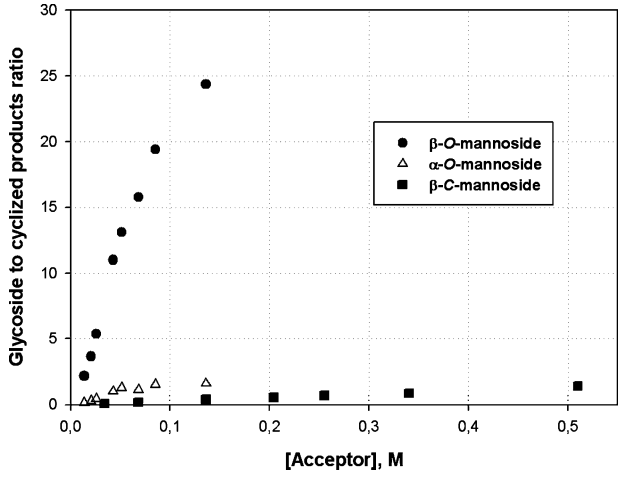


Figure 2. Ratios of *O*- and *C*-glycosides to cyclized products as functions of nucleophile concentration.

Comparable experiments were also conducted using trimethyl(methallyl)silane as an external nucleophile, resulting in a competition between cyclization and *C*-glycoside formation (Table 2 and Figure 2). Consistent with earlier results from our laboratory on the reaction of strong C-nucleophiles with 4,6-*O*-

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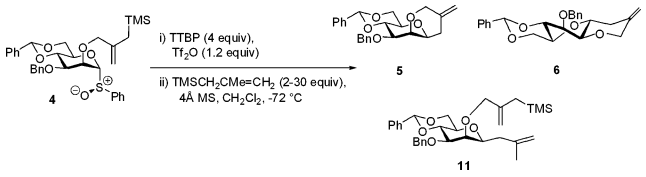
Table 2. C-Glycosylation in Competition with Cyclization[a]



entry	$TMSCH_2C(Me)=CH_2$[b]	11/(5 + 6)[c]
1	2 (0.034)	0.06
2	4 (0.068)	0.18
3	8 (0.136)	0.40
4	12 (0.204)	0.55
5	15 (0.255)	0.69
6	20 (0.34)	0.87
7	30 (0.51)	1.40

[a]Experimental conditions: TTBP (4 equiv), 4 Å molecular sieves, and Tf_2O (1.2 equiv) at −72 °C. [b]Number of equivalents (concentration in mol/L). [c]Molar ratios were determined by UHPLC/UV/MS.

benzylidene-protected mannopyranosyl donors,[20] only a single β-anomer of *C*-glycoside **11** was formed in the course of these experiments.

From the graphical representation of the competition experiments presented in Figure 2, it is clear that the rate of formation of β-*O*-mannoside **9** shows a strong and more or less linear dependence on the concentration of the nucleophile, isopropanol, at least over the initial range of concentrations. The rates of formation of α-*O*-mannoside **10** and β-*C*-mannoside **11**, on the other hand, both exhibit a much weaker dependence on the nucleophile concentration. These results are consistent with the formation of **9** being first order in nucleophile, while those of **10** and **11** are zeroth order overall in nucleophile. Accordingly, a highly associative mechanism for the formation of β-*O*-mannoside **9** that approximates an S_N2-like displacement of the triflate anion from the covalent intermediate **7** by isopropanol or the functionally indistinguishable β-face attack by isopropanol on a contact ion pair (CIP) derived by ionization of **7** is indicated, in agreement with the conclusion derived recently from ^{13}C primary kinetic isotope effect (KIE) studies.[2c] The formation of α-*O*-mannoside **10** or β-*C*-mannoside **11**, on the other hand, is clearly the result of a dissociative S_N1-like mechanism involving the formation and subsequent trapping of mannopyranosyl oxocarbenium ion **8**, either in a solvent-separated ion pair (SSIP) or as the free ion, which is also consistent with primary KIE measurements for the formation of α-*O*-mannosides. The nonzero concentration dependence of the rates of formation of α-*O*-mannoside **10** and β-*C*-mannoside **11** arises from the product-forming step, in which intermolecular nucleophilic attack competes with cyclization for capture of the transient oxocarbenium ion **8**.

If it is assumed that the cyclization products **5** and **6** and the α-*O*-mannoside and β-*C*-mannosides **10** and **11**, respectively, are formed via the intermediacy of a common transient cation (i.e., **8**), then, with the use of the usual steady-state approximation, the cyclization may be employed as a unimolecular clock for the determination of relative rate constants of bimolecular additions. Thus, division of the slopes for the relative rates of formation of **10** and **11** (see the Supporting Information) leads to the conclusion that the pseudo-first-order unimolecular rate constant for trapping of transient oxocarbenium ion **8** by isopropanol (k_{10}) is ∼6 times greater than that for trapping of the same intermediate by trimethyl(methallyl)silane (k_{11}).

Transient oxocarbenium ion **8** shows different face selectivities toward isopropanol and trimethyl(methallyl)silane, being apparently α-selective or at worst unselective[21] toward the former and β-selective toward the latter. This may reflect the fact that the transition states for attack by π-type carbon nucleophiles and σ-type alcohol nucleophiles have different steric requirements and thus necessarily result in different selectivities. Indeed, the transition states for O and C attack on oxocarbenium ion **8** do not necessarily even involve the same conformation of the electrophile.[22] Alternatively, it may be considered that this difference in selectivity arises from differing degrees of association with the counterion in the transition states for the two processes.[23]

In conclusion, the concept of using cationic cyclization reactions as probes of the mechanism of glycosylation has been developed and illustrated by application to a 4,6-*O*-benzylidene-protected mannopyranosyl donor. Cyclization takes place via an intramolecular Sakurai reaction and results in the formation of both cis- and trans-fused tricyclic products, a fact that is best interpreted by invocation of a glycosyl oxocarbenium intermediate reacting through a $B_{2,5}$ conformer. Competition experiments with external nucleophiles indicated that the β-*O*-mannopyranoside is formed by an associative S_N2-like mechanism whereas the α-mannoside is the result of a dissociative S_N1-like process. This conclusion, which is a departure from the common rationalization according to which diastereomeric ratios are analyzed in terms of two competing diastereomeric transition states, provides an obvious means of optimization for the β-isomer; it also explains why β-mannosylation of polymer-supported acceptors is relatively unselective[24] while the reaction of polymer-supported β-mannosylation donors with an excess of acceptor retains good selectivity.[25] This approach for the determination of relative kinetics of glycosylation reactions, which gives results that agree with recent results based on KIE measurements, is straightforward and potentially applicable to a broad range of glycosyl donors.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental procedures, spectral data for all unknown compounds, and crystallographic data (CIF) for **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

dcrich@chem.wayne.edu

Notes

The authors declare no competing financial interest.

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(19) The cis-fused compound **5** is not necessarily formed exclusively from the $B_{2,5}$ boat conformer of oxocarbenium ion **8**. Other conformers unable to achieve trans cyclization leading to **6** (e.g., the $^4H_3$ half-chair) are well-suited to undergo concomitant cis cyclization to give **5**.

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(21) The possibility that a minor portion of *β*-*O*-mannoside **9** is formed by the dissociative mechanism cannot be excluded.

(22) This difference in transition states and selectivity does not preclude the use of a clock reaction to determine the relative rates, as demonstrated by the classical use of the 5-hexenyl radical cyclization reaction to estimate the rate of H atom abstraction from tributylstannane.[8]

(23) It could also be considered that *β*-*O*-mannoside **9** arises from reaction with oxocarbenium ion **8**, in which case the latter would exhibit a strong inherent *β*-selectivity toward both C and O nucleophiles. While we recognize this possibility, we consider it unlikely because the KIE studies[2c] indicated that the *β*-*O*-mannosides are formed by a highly associative pathway that is different from the one for the formation of the *α*-*O*-mannosides, at least in the 4,6-*O*-benzylidene series.

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