Glycosylation Mechanism

Mechanism of 4,6-*O*-Benzylidene-Directed β-Mannosylation as Determined by α-Deuterium Kinetic Isotope Effects**

David Crich and N. Susantha Chandrasekera*

Glycosylation is one of the most fundamental reactions in organic chemistry and one that is absolutely critical to the science of glycobiology.[1] Many diverse types of glycosidic bonds are found in nature,[2] and an absolutely bewildering array of methods exist to access them.[3,4] In spite of this, studies on the mechanism of chemical glycosylation, important prerequisites for any rational development of new and improved methods, are extremely sparse. Most mechanistic thinking in the area is shaped by the seminal paper of Lemieux and co-workers advocating an array of contact and solvent-separated ion pairs as reactive intermediates,[5] but even that paper, prescient as it may be, contains no quantitative data. The kinetics of displacement of anomeric halides by simple amines, alcohols, and thiolates were measured in the 1950s and 60s,[6] but very few studies have been conducted with the inclusion of an actual promoter.[7,8] Thus, "much of the evidence used to substantiate proposed inter-glycosidic coupling mechanisms is anecdotal or circumstantial".[8d] To some extent this is understandable as, until recent years, many glycosylation reactions were heterogeneous, in other words, used an insoluble promoter. Additionally there is the possibility that the transition state for the actual glycosylation step is termolecular bringing together the acceptor alcohol and an activated complex of the donor and promoter. The enzymic formation and/or cleavage of glycosidic bonds, however, has been very thoroughly studied for a number of different enzymes, both by kinetic methods and through the determination of kinetic isotope effects.[9]

Recently, we discovered a very rapid, direct preparation of β-mannosides,[10] in which a 4,6-*O*-benzylidene-protected mannosyl sulfoxide is first activated with triflic anhydride to give a covalent α-mannosyl triflate.[11] This is then displaced by the acceptor to give the β-mannoside with excellent yield and selectivity. In a more recent version, the α-mannosyl triflate is preformed from a mannosyl thioglycoside and the combination of triflic anhydride and 1-benzenesulfinyl piperidine before addition of the acceptor.[12] This clean, homogeneous coupling reaction, which proceeds without promoter, provides the opportunity to study an actual glycosidic bond-

[*] Prof. Dr. D. Crich, N. S. Chandrasekera
Department of Chemistry
University of Illinois at Chicago
845 West Taylor Street, Chicago, IL 60607-7061 (USA)
Fax: (+1) 312-996-4439
E-mail: dcrich@uic.edu

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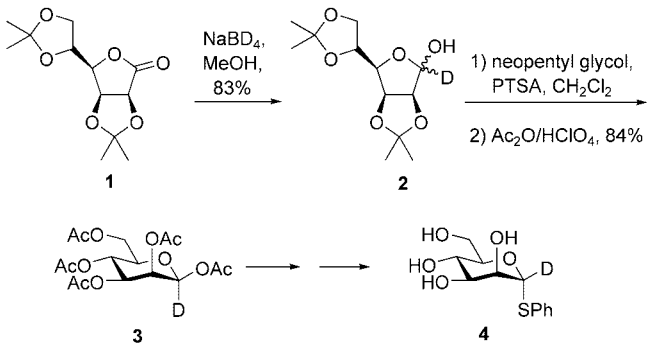
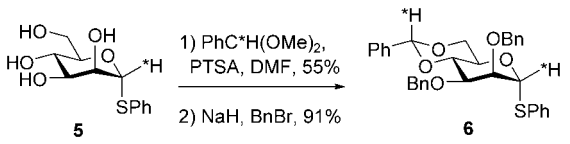
forming reaction with the possibility of differentiating between direct S_N2 displacement and mechanisms involving transient contact ion pairs. We report here on the execution of such a study.

We elected to address the problem by the determination of kinetic isotope effects (KIEs),[13–15] and, so, synthesized a thiomannoside **4** >95% enriched in ^2H (D) at C1 by the method outlined in Scheme 1.[16] Mannoside **4** was then



Scheme 1. Preparation of labeled thioglycoside **4**. PTSA = *p*-toluenesulfonic acid.

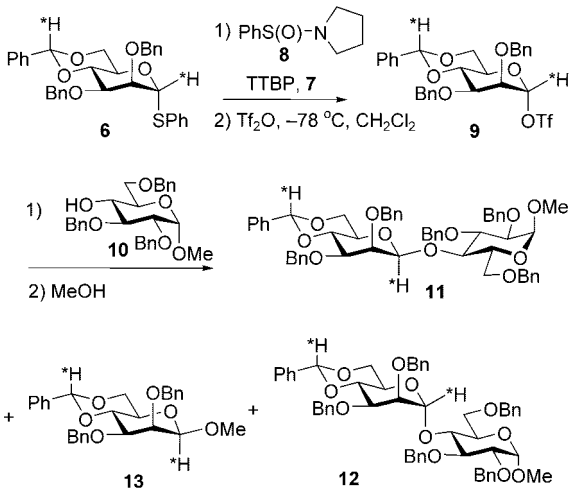
admixed with an equal quantity of the nondeuteriated material to give **5**, enriched to approximately 50%. This substance was converted to the benzylidene acetal by transacetalization with benzaldehyde dimethyl acetal, 50% enriched with deuterium at the acetal position. Standard benzylation then gave donor **6** incorporating approximately 50% deuterium at the anomeric and, as an internal standard, the benzylidene acetal position (Scheme 2).



Scheme 2. Preparation of doubly labeled donor **6**. Bn = benzyl, *H = proton with approximate enrichment of 50% in ^2H.

Donor **6** and approximately 50 mol% of 4,4,5,5-tetramethyl-2-(1-naphthyl)-1,3-dioxolane (**7**), a convenient internal standard with which to determine conversion, were dissolved in $CDCl_3$, the ^1H NMR spectrum was recorded, and the singlets corresponding to the benzylidene acetal and anomeric hydrogens were integrated against the signals of **7**.[17] After removal of the $CDCl_3$, the mixture was taken up in CH_2Cl_2 and admixed with tri-*tert*-butylpyrimidine (TTBP),[18] and 140 mol% of **8**.[19,20] The solution was then cooled to −78 °C and treated with 150 mol% of Tf_2O to give the α-mannosyl triflate **9**. Acceptor **10** (50 mol%) was added[21] and then 1.5 h later MeOH was added before the reaction mixture was quenched (Scheme 3).

The ^1H NMR spectrum of the reaction mixture revealed the formation of the β-mannoside **11**, whose yield was determined by integration of the corresponding benzylidene



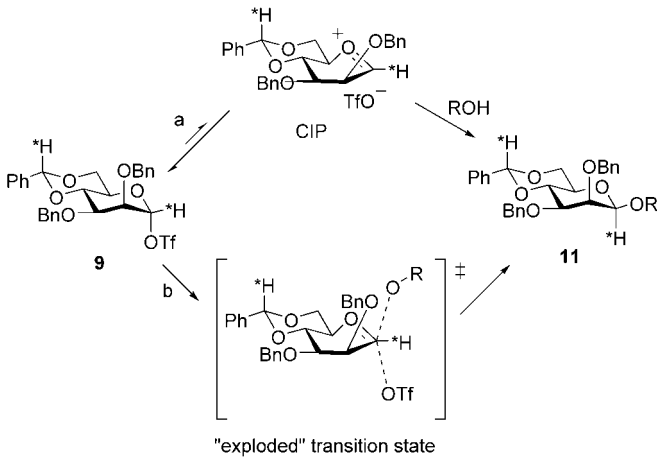
Scheme 3. The kinetic isotope effect experiment. TTBP = 2,4,6-tri-*tert*-butylpyrimidine, Tf_2O = trifluoromethanesulfonic anhydride).

acetal resonance against the internal standard **7**, a trace of the α-mannoside **12**,[22,23] and the methyl β-mannoside **13**. After separation by preparative HPLC, the ^1H NMR spectrum of **11** was again recorded and the ratio of the benzylidene acetal to anomeric protons determined by careful integration. The complete sequence was repeated three times, giving three independent sets of data.

The data were processed according to Equation (1), for the determination of KIEs from reaction products,[13,15] wherein F is the fractional conversion of the triflate **9** (yield of **11**) and R_{11} and R_6 the ratios of the benzylidene and anomeric resonances in the product **11** and the thioglycoside **6**, corresponding to the D/H ratios in **11** and **6**. In this manner the α-deuterium KIE at −78 °C for each of three independent runs was found to be 1.20, 1.21, and 1.16 (or 1.13, 1.13, and 1.10 after conversion to 25 °C)[24] the assumption being that the conversion of **6** to **9** is quantitative.

$$\mathrm{KIE} = \ln(1-F)/\ln[1-(FR_{11}/R_6)] \quad (1)$$

α-Deuterium KIEs of this magnitude correspond well to those observed in acid-catalyzed hydrolysis of simple methyl glycosides,[25] and in the hydrolysis of glycosyl fluorides,[26] leading to the conclusion that the displacement of the triflate from **9** by the typical carbohydrate acceptor **10** to give **11** proceeds with the development of substantial oxacarbenium ion character. This may be interpreted either by a fully dissociative mechanism involving the intermediacy of a transient contact ion pair (CIP) (Scheme 4, path a), or by a functionally equivalent mechanism involving an "exploded" transition state (Scheme 4, path b).[27] In the CIP mechanism the triflate anion is necessarily closely associated with face of the oxacarbenium ion from which it has just departed and shields that face against attack by the incoming alcohol. In the alternative mechanism there is a loose association of the nucleophile with the anomeric center as the leaving group departs. The minor amount of α-anomer **12** formed in these reactions most likely arises through the intermediacy of a looser, perhaps solvent-separated, ion pair (SSIP), which is in



Scheme 4. The proposed glycosylation mechanism.

equilibrium with an initial CIP. The function of the torsionally disarming[28] benzylidene group is oppose rehybridization at the anomeric carbon and, so, to shift the complete set of equilibria toward the covalent triflate and away from the SSIP, thereby minimizing α-glycoside formation.[29] The expected chemical shift of an oxacarbenium ion carbon is $\delta_{C1} \sim 250$ ppm,[30] whereas that measured[11] for the covalent triflate, the only observable species by NMR spectroscopy, is $\delta_{C1} = 104.5$ ppm. It is apparent, therefore, that the complete set of equilibria between the covalent triflate **9**, the CIP, and SSIP lie very heavily toward **9** in complete agreement with known lifetimes of oxacarbenium ions.[31,32]

The development of significant oxacarbenium ion character even in the highly stereoselective 4,6-*O*-benzylidene-directed β-mannosylation strongly suggests that other, less selective glycosylation reactions will be similarly dissociative.[33] The application of the current technique to other glycosylation methods and stereochemical series is currently underway.

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[14] Displacement of triflate from preformed glycosyl triflates is complete in minutes −78 °C,[11] ruling out the use of kinetic measurements, at least by the NMR methods used to characterize these intermediates.
[15] On grounds of the amount of substrate needed, the substrate solubility, and the instrument time needed, direct determination of kinetic isotope effects by NMR spectroscopy at natural abundance (as in a) D. A. Singleton, A. A. Thomas, J . *Am. Chem. Soc.* **1995**, *117*, 9357; b) D. A. Singleton, M. J. Szymanski, *J. Am. Chem. Soc.* **1999**, *121*, 9455; c) J. K. Lee, A. D. Bain, P. J. Berti, *J. Am. Chem. Soc.* **2004**, *126*, 3769) was deemed impractical.
[16] The critical feature is the liberation of the deuteriated mannose from **2** with neopentyl glycol and PTSA which minimizes isomerization to glucose.
[17] ^{2}H NMR spectroscopy was investigated but the linewidths and chemical shifts were such that the integration was compromised.
[18] D. Crich, M. Smith, Q. Yao, J. Picione, *Synthesis* **2001**, 323.
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[20] An excess of **8** and Tf_2O was employed to ensure complete conversion of **6** to **9**, as verified on the NMR spectrum of the crude reaction mixture following glycosylation, such that any isotope effects observed do not result from the initial activation step.
[21] P. J. Garegg, H. Hultberg, S. Wallin, *Carbohydr. Res.* **1982**, *108*, 97.
[22] The yield of **12** was <5%, ruling out determination of the KIE for its formation, but allowing it to be neglected in the calculation of the KIE of **11**.
[23] Ideally, comparison of the KIEs on the α- and β-products in an unselective coupling would be informative. However, this is very difficult to achieve as the method employed requires baseline resolution of the various anomeric and benzylidene acetal signals. In practice, this has proven to be a limitation with numerous substrates assayed, particularly for unselective reactions when the number of similar signals is multiplied. Second, while the reaction conditions can be manipulated[10a,b] to give

predominantly the α-product it is doubtful whether measurements made under such circumstances are relevant as they presumably do not proceed via the intermediate glycosyl triflate.
[24] Assuming $T(\ln k_H/k_D)$ is constant and no tunnelling occurs.
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[32] The fact that the covalent α-triflate **9** is so heavily favored argues against the possibility that the effects measured here result from an equilibrium isotope effect. Likewise, the highly stereoselective nature of the coupling argues against the effects measured arising from any significant shift in the equilibrium position as this would necessarily be associated with reduced selectivity.
[33] On the grounds that the reaction studied is highly stereoselective, and alcohol **10** is a typical carbohydrate the results obtained here may reasonably be considered as representative of this class of reactions. Of course exceptions may exist, particularly with extremely selective cases using highly reactive alcohols, but they are not likely to be representative of the formation of true interglycosidic bonds.

