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Structural, Mechanistic, and Computational Analysis of the Effects of Anomeric Fluorines on Anomeric Fluoride Departure in 5-Fluoroxylosyl Fluorides

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Supporting Information

ABSTRACT: The effects of fluorine substitution at the C-5 center of pyranosyl fluorides on the reactivity at the C-1 anomeric center was probed by studying a series of 5-fluoroxylosyl fluoride derivatives. X-ray structures of their per-*O*-acetates detailed the effects on the ground-state structures. First-order rate constants for spontaneous hydrolysis, in conjunction with computational studies, revealed that changes in the stereochemistry of the 5-fluorine had minimal effects on the solvolysis rate constants and that the observed rate reductions were broadly similar to those caused by additional fluorine substitution at C-1 but significantly less than those due to substitution at C-2. Differences in the trapping behavior of 5- versus 2-fluoro-substituted glycosyl fluorides with α- and β-glycosidases arise more from differences in steric effects and hydrogen-bonding interactions than from intrinsic reactivity differences.

Understanding of the mechanisms of spontaneous and enzyme-catalyzed hydrolysis of glycosides and glycosyl phosphates has benefited considerably from insights obtained using deoxy and deoxyfluorinated substrates as probes.[1−3] The sterically conservative nature of the substitution of F or H for OH and the large differences in electronegativity have allowed changes in charge development between the ground and transition states to be probed.[4] Furthermore, covalent glycosyl−enzyme intermediates have been trapped on retaining glycosidases by judicious use of fluorine substituents at C-2 or C-5 along with appropriate leaving groups at the anomeric center.[5−7]

The effects of pyranoside fluorination at either the C-1 (anomeric)[3] or C-2[2,4] center on the rates of glycoside hydrolase trapping may be readily analyzed with reference to uncatalyzed rates of hydrolysis. The quantitation of effects due to C-5 fluorination in this manner is, however, more complex. The rate constants for hydrolysis presented in the first line of Table 1 confirm the ∼40-fold faster hydrolysis of β-D-glucosyl fluoride (β-GlcF) relative to α-GlcF reported previously[3] and attributed primarily to differences in the stabilities of the ground states that themselves arise from the anomeric effect. Since transition-state energies for dissociative (oxocarbenium ion-like) transition states are broadly similar for both anomers, it is the difference in ground-state energies that primarily dictates the relative rates. In this light, the observation of very similar rate constants (30 and 39 s^{-1}) for the α- and β-anomers of 5F-GlcF is consistent with initial departure of fluoride from C-5, since departure from C-1 would be expected to result in different rates.[8] Further support for the conclusion that departure of the C-5 fluorine precedes that at C-1 is drawn from the similar rate constants for the two *ido* anomers, 5F-α-IdoF and 5F-β-IdoF, for much the same reasons.

An understanding of the structural and kinetic consequences of C-5 fluorination could help explain puzzling enzymic reactivities, including the following: (1) both 5-fluoroglycosyl fluorides and 2-fluoroglycosyl fluorides may be used to trap the covalent intermediates of β-glycosidases, whereas α-glycosidases form stable intermediates only with 5-fluoroglycosyl fluorides;[9,10] and (2) the unnatural C-5 epimers (e.g., L-*ido*) of the 5-fluoroglycosyl fluorides better trap covalent intermediates than do those of the natural configuration (e.g., D-*gluco*).[11,12] However, since loss of fluoride in the uncatalyzed reaction most likely occurs first from the secondary center at C-5 rather than from C-1, a direct comparison of enzyme-catalyzed and uncatalyzed rates is misleading.

Because the consequences of C-5 fluorination on the rate constants of anomeric (C-1) solvolyses cannot be determined by studying hexopyranosyl fluorides, an alternative means of quantitatively assessing the consequences of C-5 fluorination for the rates of glycoside hydrolysis was needed. One simple means would be to symmetrize the system to make both the C-1 and C-5 centers primary carbon centers. To this end, a series of 5-fluoroxylosyl fluoride derivatives were synthesized and structurally characterized by both X-ray crystallography and NMR spectroscopy; their rates of spontaneous hydrolysis were also determined, and ab initio quantum-chemical calculations on the ground- and transition-state structures were performed. Xylosyl fluorides **1** and **2** were synthesized as previously reported.[13] Key steps in the synthesis of difluorinated xylosides **4**−**6** and trifluorinated xyloside **3** were the photobromination of per-*O*-acetylated xylosyl fluorides (Scheme 1) and the subsequent substitution of bromine for fluorine using either silver tetrafluoroborate or silver fluoride.

X-ray crystal structures of the per-O-acetylated derivatives of compounds **3**−**6** revealed near $^4C_1$ (chair) conformations for **3**, **4**, and **5**, consistent with the conformations previously reported for per-O-acetylated **1**[14] and **2**.[15] The presence of a second fluorine atom in an equatorial position at C-5 of β-xylosyl fluoride causes the per-O-acetylated derivative of **6** to adopt a $^1S_3$ (skew-boat) conformation in the crystalline phase (Figure 1), a clear manifestation of the dipolar interactions underlying the anomeric effect. Presumably the destabilization afforded by three

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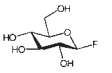
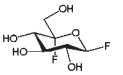
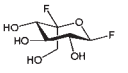
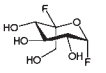
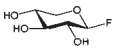
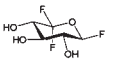
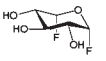
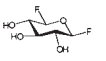
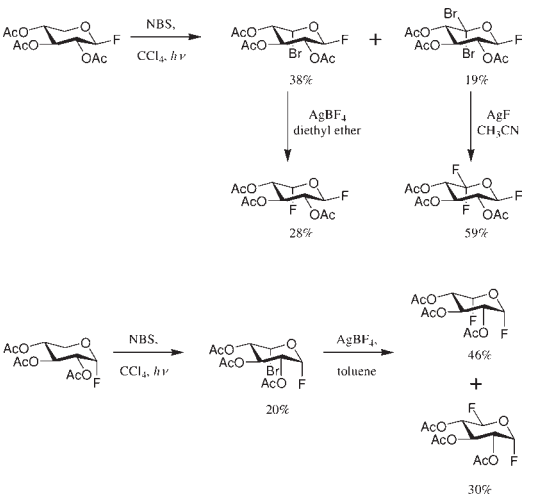
Table 1. Glucosyl and Xylosyl Fluorides Studied (Shown in $^4C_1$ Chair Conformations) and Their Solvolysis Rate Constants ($\times 10^{-8}$ s^{-1})

	β-GlcF	α-GlcF	5F-β-GlcF	5F-α-GlcF	5F-α-IdoF	5F-β-IdoF
Gluco-series						
	39000	1000	39	30	1750	1300
	β-XylF **1**	α-XylF **2**	DiF-β-XylF **3**	5aF-α-XylF **4**	5eF-α-XylF **5**	5eF-β-XylF **6**
Xylo-series						
	270000	11000	4.3	1.3	19	61

Scheme 1. Synthesis of Per-O-acetylated Fluorinated Xylosyl Fluorides



axial acetyl groups is sufficient to disfavor the $^1C_4$ conformation that the anomeric effects at C-1 and C-5 would otherwise dictate, as seen previously[16] for per-O-benzoylated **1** . Inspection of the bond lengths is enlightening, as the axial C−F bonds (∼1.39 Å) are always longer than the equatorial ones (∼1.36 Å), consistent with anomeric effects and the presence of a second fluorine (at C-1 or C-5) having little effect, as would again be anticipated since lone-pair donation from the endocyclic oxygen formally can occur with only one C−F σ^* orbital at any moment.

Quantum-mechanical optimizations of solvated ground states for xylopyranosyl fluorides **1**−**6** were conducted in which various xylopyranoside ring conformations and hydroxyl group rotamers were considered [see the Supporting Information (SI)].[17] The lowest-energy conformations determined agreed well with those found in the solid and aqueous phases: compounds **1**− **5** adopt $^4C_1$ conformations, while low-energy $^4C_1$ and $^1S_3$ conformations (depending on the solvent model) were determined for compound **6**. The computed bond lengths agreed well with those determined crystallographically for all cases except difluoroxylosyl fluoride **3**, in which both equatorial C−F bonds are much shorter (1.33 and 1.35 Å) yet the axial C−F bond is normal at 1.39 Å. Proton NMR spectra were recorded for compounds **1**−**6** in water, and the xylopyranose ring conformations were estimated from ^3J couplings using Haasnoot's empirical generalization of the Karplus equation.[18] The ring-proton dihedral

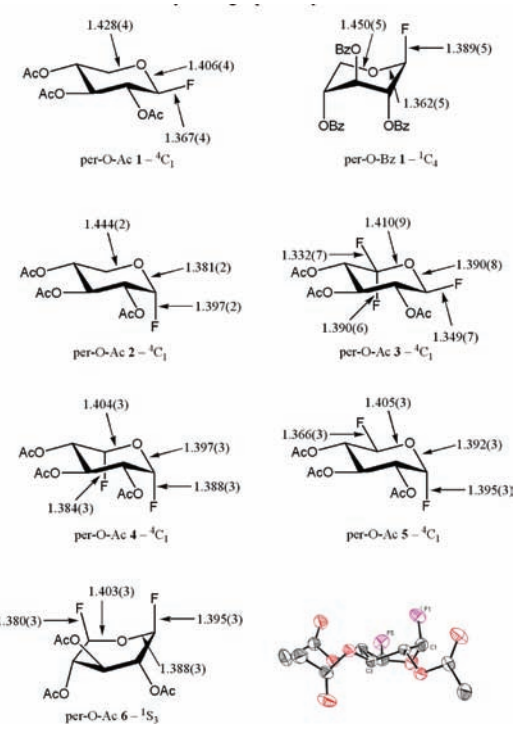


Figure 1. Crystallographically determined conformations and key bond lengths for O-protected xylosyl fluorides **1**−**6**.

angles for compounds **1**−**5** were all consistent with dominant $^4C_1$ conformations (with no predicted dihedral angle deviating more than 15° from its canonical value for a chair conformation), as seen in the solid state. The ring-proton dihedral angles estimated for difluoride **6** in solution are consistent with a $B_{O,3}$ (boat) conformation, which is close to the $^1S_3$ conformation determined crystallographically.

pH-independent rates of hydrolysis of fluoroxylosyl fluorides were determined at 50 °C (Table 1).[4] β-Xylosyl fluoride **1** is hydrolyzed 24-fold faster than the α-anomer **2**, consistent with the 40-fold difference noted earlier for the glucosyl fluorides.[3] The addition of a second fluorine atom to α-xylosyl fluoride and β-xylosyl fluoride, producing symmetric difluorides **4** and **6**, respectively, decreases the rate constants 8500- and 4500-fold, corresponding to very similar increases in the associated activation free energies (25.2 kJ mol^{-1} comparing **2** and **4**; 24.6 kJ mol^{-1} comparing **1** and **6**). If it is assumed that the equatorial fluoride is always the more labile, comparison of the rate constants for

1 and **6** indicates that an equatorial fluorine slows the loss of an equatorial fluoride by 4500-fold, while comparison of the values for **1** and **5** indicates that an axial fluorine slows that reaction somewhat more, by 14 000-fold. Furthermore, as noted above, an axial fluorine at C-5 slows the departure of an axial fluorine at C-1 8500-fold (**2** vs **4**). The general similarity of these effects on the departure of axial and equatorial leaving groups from C-1 strongly suggests that differences in inductive effects are probably not the explanation for the differences in trapping behavior of 5-fluorosugar inactivators with α- and β-glycosidases.[19]

Perhaps the biggest surprise was found for trifluoride **3**, which reacts only 5−15-fold more slowly than difluorides **5** and **6** and 3-fold more rapidly than difluoride **4**. It is tempting to ascribe this to displacement occurring at C-5 instead, assisted by mesomeric effects of fluorine. However, the observed 1000-fold lower solvolysis rate of 1,1-difluoroglucose relative to α-glucosyl fluoride renders this unlikely.[3,4] Instead, this observation must be a reflection of the much greater reactivity of the β-fluoride leaving group. Indeed, it is noteworthy that within the difluoroxylose derivatives, the more equatorial fluorines are present, the faster is the solvolysis. This is again consistent with the ground-state destabilization that results from destabilizing anomeric effects playing a significant role in determining the rates of hydrolysis, and the absolute potential energies computed for the ground-state structures of fluoroxylosyl fluorides **4**−**6** support this proposal: difluorides **4**−**6** possess computed lowest-energy conformers with relative energies of 0, 3.9, and 11.7 kJ mol^{-1} [computed at the SMD/M06-2X/6-31+G(d,p)//PCM/M06-2X/6-31+G(d,p) level of theory including correction for zero-point energies; for further details, see the SI].

These observed reactivity patterns and the conclusions drawn above were further investigated through construction of ab initio quantum-mechanical models combined with continuum descriptions of bulk solvent effects. The minimal transition-state model (Figure 2A,B) for the hydrolysis of xylosyl fluorides **1**−**6** included the xylosyl fluoride, a nucleophilic water molecule, and one additional water molecule (mediating a proton transfer between the incoming water nucleophile and the outgoing fluoride ion). In these models, the 2-hydroxyl group (for the displacement of fluorine atoms at C-1) or the 4-hydroxyl group (for the displacement of fluorine atoms at C-5) also mediates the proton shuttle between the water nucleophile and the leaving group, consistent with the "internal solvation" proposal of Sinnott and Jencks.[20]

The reactivity trends determined computationally broadly reproduced those observed experimentally (Figure 2C), with the only deviation being the misordering of the reactivities of trifluoro- and difluoroxylose derivatives **3** and **4**. The computed activation energies are lower than those determined experimentally because of the omission of solute entropic contributions in these calculations. Such contributions are overestimated using the standard rigid-rotor/harmonic-oscillator approach and may ultimately best be incorporated using a restraint release method.[21] In any event, while these contributions are significant in determining the magnitude of the computed reaction rates, they do not seem to be important in determining the trend among homologous compounds. The determined transition-state structures directly linked the xylosyl fluoride reactants and hemiacetal products. These concerted (S_N2) reaction pathways modeled are consistent with those inferred from anomeric ^{13}C kinetic isotope effect studies of glucosyl fluoride hydrolyses.[22]

The computational modeling of these transition states considered which (nonequivalent) fluorine atom is likely first displaced for difluoride **5** and trifluoride **3**. The calculations show that

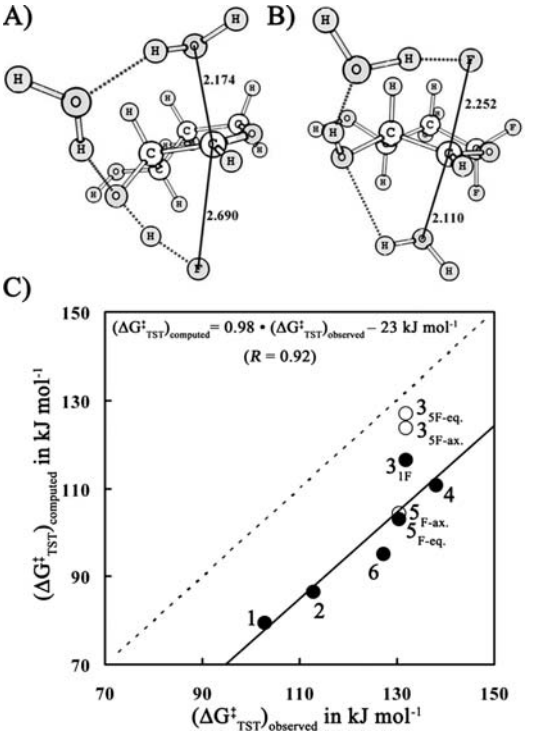


Figure 2. (A, B) Computed transition-state structures for (A) the hydrolysis of α-xylosyl fluoride **2** and (B) the displacement of equatorial C1 fluoride from trifluoride **3**. (C) Correlation between computed and observed activation energies.

displacement of either axial or equatorial fluorine from difluoride **5** yields very similar activation energies: precisely which transition state is lowest in energy depends on the solvent model used. This is consistent with the dominance of ground-state effects in determining trends in reactivity and our hypothesis above that inductive destabilization of the transition state for hydrolysis at C-1 is relatively insensitive to the orientation of the spectator fluoride at C-5. The displacement of the lone C-1 equatorial fluoride from trifluoride **3** was found to be favored by some 11 kJ mol^{-1} over displacement of the equatorial C-5 fluoride and by 7 kJ mol^{-1} over the displacement of the axial C-5 fluoride, strongly suggesting that the initial displacement occurs from the monofluorinated C-1 center.

Taken together, these kinetic and computational data highlight the range of reactivities that may be achieved through fluorination of the C-1 and C-5 centers of pyranosides. The effect of substitution by a single fluorine atom at C-5 is slightly smaller than that found upon introduction of additional fluorine substituents at C-1 (1,1-difluorides, 40 000-fold)[3] or substitution for hydrogen at C-2, where a 70 000-fold rate reduction is observed.[4] Furthermore, the relative configurations of fluorine substituents at C-1 and C-5 do not seem to be dominant inherent factors in determining the reaction rates. The previously reported differences in trapping behavior of 2- and 5-fluoroglycosyl fluorides with α- and β-glycosidases are therefore more likely due to the differential removal of key hydrogen-bonding interactions than to inherent differences in reactivity.[23,24]

The lower intrinsic reactivity of all-axial difluoroxylose derivative **4** relative to the axial−equatorial difluoroxylose derivative **5** provides further insight into the origins of the better trapping of glycosidases by 5-epimeric reagents (e.g., 5F-β-IdoF) than by

reagents possessing the natural C-5 configuration (e.g., 5F-α-GlcF). It is unlikely that this trapping is due to differences in the intrinsic reactivities of 5F-α-GlcF and 5F-β-IdoF. Indeed, the higher reactivity of difluoroxylose derivative **4** relative to **5** would suggest that the covalent glycosyl−enzyme intermediate derived from 5F-α-GlcF should be hydrolyzed less rapidly than that derived from 5F-β-IdoF. Furthermore, X-ray crystal structures have revealed that for both an α-mannosidase[12] and an α-amylase,[25] the hydroxymethyl group of the covalent glycosyl−enzyme intermediate derived from the 5-fluoro-*ido* (or *gulo*) substrate is well accommodated in the enzyme active site: the C-6 hydroxyl groups of both 5-fluoro-*gluco*- and 5-fluoro-*ido* (or *gulo*)-derived intermediates interact with the same enzymic residues. The effective trapping of these α-glycosidases by 5-epimeric reagents is therefore most likely due to the selective destabilization of the transition states for glycosylation and deglycosylation. In these cases, steric and noncovalent interactions between the enzyme and inhibitor are dominant in fine-tuning the relative rates of glycosylation and deglycosylation.

■ ASSOCIATED CONTENT

Supporting Information. Complete synthetic details, compound characterization, crystallographic details, description of the computational models constructed, and CIF files. This material is available free of charge via the Internet at http://pubs.acs.org.

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