A Free-Energy Relationship between the Rate of Acidic Hydrolysis of Glycosides and the pK_a of Isofagomines**

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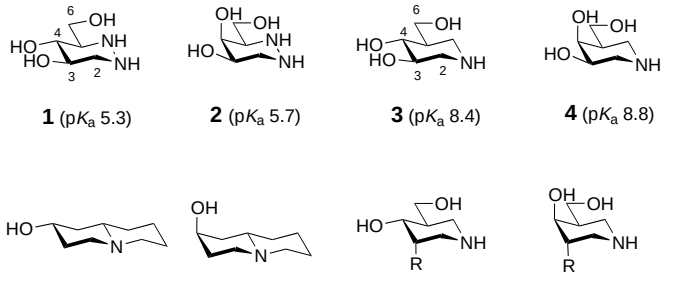
Carbohydrates, the most abundant biomolecules, consist mainly of monomers that are linked together by glycosidic bonds. Since each carbohydrate molecule owes many of its properties to the glycosidic linkages it contains, and because formation and cleavage of these bonds are involved in any carbohydrate synthesis or breakdown, the chemistry of the glycosidic bond has long been under intense scrutiny. As early as 1908 it was noted that the rates of acidic hydrolysis of glycosides depend on the configuration of the hydroxyl groups of the sugar, and that the more axial hydroxyl groups are present in the glycosyl unit, the more rapid is the acidic hydrolysis.[1] Edward proposed that the rate differences arise because the axial groups facilitate a conformational change necessary for the molecule to reach a half-chair transition-state structure.[1e] While recent work has suggested that differences in reaction rates may be caused by differences in substituent inductive effects in series with conserved stereochemistry,[2] Edward's proposal regarding the differences in reaction rates between glycosides with different stereochemistry has not been challenged.

Here we report on a surprising finding that has bearing on this issue. We recently observed[3] that the basicity of *galacto*-azafagomine (**2**) is slightly higher than that of the *gluco* isomer **1**[4] (Scheme 1). While this difference in basicity might appear insignificant, the only structural difference between the two hydrazines is whether the 4-hydroxyl group is equatorial or axial, and no difference in base strength was anticipated. We therefore also measured the pK_a of the protonated forms of isofagomine (**3**)[5] and its *galacto* analogue **4**.[6] Again the same difference in basicity was observed between the two stereoisomers (Scheme 1).



Scheme 1. Amines **1**–**10** and the pK_a values of their protonated forms at 25 °C.

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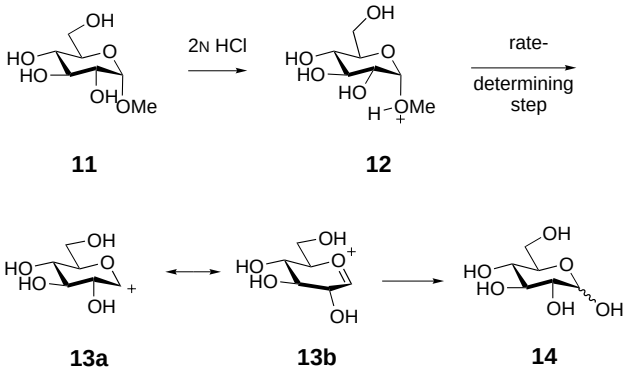
A literature search revealed that a similar effect was observed in less highly functionalized compounds.[7, 8] For example, in the conformationally restricted amines **5** and **6**, the isomer with an axial hydroxyl group was found to be the stronger base (Scheme 1).[7] We also measured the pK_a values of available[9, 10] isofagomine analogues **7**–**10** and found a considerable variation (Scheme 1). Furthermore, the pK_a of 1-deoxynojirimycin and its *manno* and *galacto* analogues were reported[11] to be 6.3, 7.2, and 7.1, respectively. These data show that changing a hydroxyl group from an equatorial to an axial position increases the pK_a of the amine by about 0.8 units when the OH group is β to the amino group, and by about 0.4 units when it is γ to the amino group.

The basicities of amines are known to be affected by a) substituent effects, b) resonance, c) steric hindrance to protonation, d) solvation, and e) internal hydrogen bonding.[12] In this case, b) and c) can immediately be ruled out of playing any role in explaining the differences in basicity between **1** and **2**. Solvation is mainly significant when the space around the hydrogen atoms attached to the basic center varies, but this is not the case between compounds **3** and **4** and **7**–**10**.[13] Internal hydrogen bonding cannot explain the difference in basicity between **3** and **4**, but could possibly affect the basicity of compounds with an axial 3-hydroxyl group, though this is unlikely in water. So the differences in basicity can be attributed mainly to substituent effects. This suggests a difference in electron-withdrawing power between the axial and equatorial 4-hydroxyl groups of **4** and **3**, respectively, whereby the former is apparently de facto less electron withdrawing. This can be explained by differences in charge–dipole interactions in the two molecules. An equatorial hydroxyl group in the β- or γ-position (**A**) will pull electrons away from the charged nitrogen atom to a greater extent than a corresponding axial substituent (**B**), since its vector component in the plane of the charged nitrogen atom is larger (see Scheme 3).[14] Note that NMR spectroscopic data of **1** and **2** at neutral and acidic pH clearly revealed that the $^4C_1$ conformation was predominant, regardless of pH.

This difference in substituent effects of an axial and equatorial hydroxyl group must be expected to play a role in other compounds of similar structure, particularly in the carbocation **13** (Scheme 2) or in a transition state resembling **13**. The kinetics of acid-catalysed hydrolysis of simple glycosides were studied in detail by Overend et al.[1f] They found



Scheme 2. The mechanism of acid-catalyzed hydrolysis of glycosides in 2 N hydrochloric acid (taken from ref. [1f]).

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that the reaction follows an A-1 mechanism with a rapid preequilibrium protonation step (**11** → **12**) followed by rate-determining fission of the glycosyl–oxygen bond. Since the transition state was expected to be late in this reaction, it resembles the cation **13a** quite closely, or possibly its oxocarbenium ion resonance form **13b**.

We therefore compared the free-energy differences $\Delta\Delta G$[15], calculated from the differences in pK_a value between the amines **3**, **4**, and **7**–**10**, with the differences in free energy of activation $\Delta\Delta G^{\neq}$[16] for the hydrolysis of the corresponding glycosides (Table 1).[17] The values show the effect on acidity and rate of hydrolysis of changes in the 3- and 4-hydroxyl groups and are plotted in Figure 1. There is a remarkable

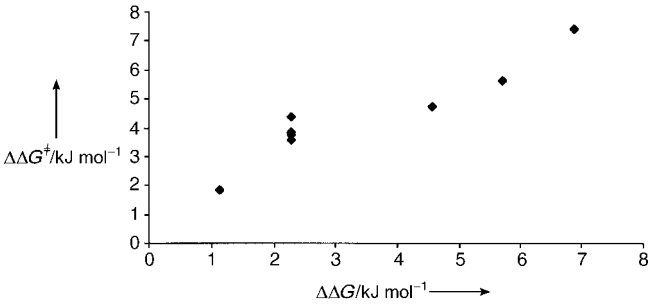
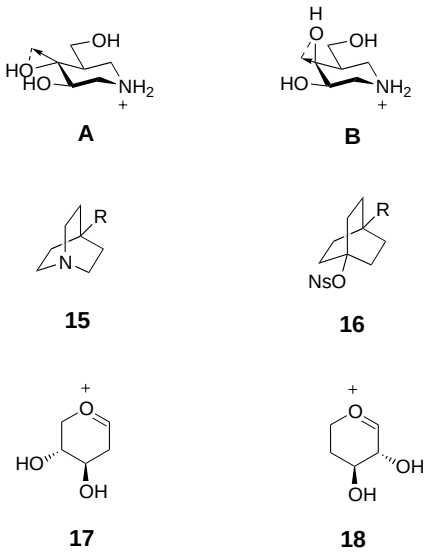


Figure 1. A plot of the differences in activation energy for acidic hydrolysis of glycosides $\Delta\Delta G^{\neq}$ versus the differences in free energy of protonation between isofagomines $\Delta\Delta G$.

correlation between $\Delta\Delta G$ and $\Delta\Delta G^{\neq}$ as the result of inverting or removing a hydroxyl group, and the best fit was obtained for the equation $\Delta\Delta G^{\neq} = \rho\Delta\Delta G$ with $\rho = 0.86$ ($r^2 = 0.94$).[18] This shows that substituent effects can explain the difference in rate of hydrolysis of stereoisomeric glycosides. It also suggests that positive charge development on the anomeric center is more important in the hydrolysis reaction than previously conceived.

A similar comparison between amine basicity and transition state energy was made by Grob et al., who compared a series of substituted amines **15** with the rate of S_N1 solvolysis of a series of nosylates **16** (Scheme 3).[19] They found a similar relationship to the above with a ρ value close to unity (ρ = 1.02).



Scheme 3. Structures of **A** and **B**, which show the difference in charge–dipole interaction for an axial and an equatorial 4-hydroxyl group, the amines **15** and nosylates **16** (R represents a range of different substituents), and the oxocarbenium ions **17** and **18**, obtained by hydrolysis of methyl 2-deoxy- and 4-deoxyxylopyranoside, respectively.

As mentioned above, the differences in rate between glycosides of different sugars have been explained as being due to the substrate's changing conformation to a half-chair transition state, and the conformational change supposedly becomes easier with an increasing number of axial substituents.[1e,f] However, it is clear from the present results that the differing effects of substituents on charge development can adequately explain a large fraction of the differences in reactivity observed. This is in agreement with a recent study, which found that field effects largely dictate the rate of hydrolysis of dinitrophenyl glycosides with conservation of stereochemistry.[2b] It does not directly agree with the postulate that the faster hydrolysis of galactosides is due to a through-space electrostatic interaction between the axial 4-OH group and an oxocarbenium ion,[2a] but it is possible that both this effect and those reported here could contribute to the difference in rate of hydrolysis between glucosides and galactosides.

The present work also suggests that the transition state has considerable positive charge on the anomeric carbon atom,

Table 1. A comparison of the differences in free energy of protonation between isofagomines ($\Delta\Delta G$) with differences in activation energy for acidic hydrolysis of glycosides ($\Delta\Delta G^{\neq}$), both at 25 °C.

Compared amines	$\Delta\Delta G$ [kJ mol^{-1}]	Compared glycosides	Relative hydrolysis rates[a]	$\Delta\Delta G^{\neq}$ [kJ mol^{-1}]
3 and **4**	2.28	methyl α-pyranoside, *galacto-*/*gluco-*	4.7	3.84
3 and **4**	2.28	phenyl α-pyranoside, *galacto-*/*gluco-*	4.6	3.76
3 and **4**	2.28	methyl β-pyranoside, *galacto-*/*gluco-*	5.8	4.37
3 and **4**	2.28	phenyl β-pyranoside, *galacto-*/*gluco-*	4.2	3.57
3 and **7**	4.56	methyl α-pyranoside, *altro-*/*manno-*	6.8	4.74
3 and **8**	6.84	methyl α-pyranoside, 3-*deoxygluco-*/*gluco-*	19.8[b]	7.41
3 and **9**	5.70	methyl α-pyranoside, *ido-*/*manno-*	9.7[b]	5.62
7 and **9**	1.14	methyl α-pyranoside, *ido-*/*altro-*	2.09[b]	1.83

[a] The relative hydrolysis rates are $k_1(1)/k_1(2)$ at 25 °C. The rate constants for acidic glycoside hydrolysis at 60 °C with 2 N HCl were obtained from ref. [1f] and recalculated at 25 °C by using the Arrhenius equation and the activation energies and preexponential factors given in that paper. [b] The rate constant for 3-deoxyglucoside was only available at 58.4 °C, and that for the idoside only at 58.1 °C. The ratios were therefore calculated for these temperatures as it was assumed that the activation energy was identical for the glycosides compared. This is a reasonable assumption.[14]

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although it is reasonable to assume that charge is also present on the ring oxygen atom. The fact that the greatest deviation in the free-energy relationship was seen for the *galacto*/*gluco* hydrolysis rate seems to suggest this. The finding that a significant amount of the positive charge is located on the anomeric carbon directly contradicts the recent postulate that 97% of the positive charge in the transition state lies on the ring oxygen atom.[2b] However, if through-space field effects control the rate of hydrolysis, it is not possible that so much charge could be located on the ring oxygen atom, because then 2- and 4-substituents should affect the rate of hydrolysis to a similar extent. Thus the transition states for hydrolysis of methyl 2- and 4-deoxyxylopyranoside **17** and **18** (Scheme 3), respectively, which have a high degree of symmetry, would be affected by substituent field effects of similar size. However since the rate of hydrolysis of 2-deoxyglycosides is about 50 times greater than that of 4-deoxyglycosides, this cannot be the case.[20] Therefore, if through-space field effects control the rate of hydrolysis, a substantial amount of positive charge must also be present on the anomeric carbon atom.

The following conclusions can be made: The greater rate of hydrolysis of galactosides can be explained on the basis of a smaller electron-withdrawing effect of an axial 4-OH group. Similarly, the inductive effects can explain the differences in rate of hydrolysis of other glycosides. Furthermore, it must be expected that other reactions at the anomeric center involving unimolecular mechanisms and charge development should be affected similarly. Secondly, the study shows that isofagomines are quite good transition-state analogues of nonenzymatic glycoside hydrolysis.

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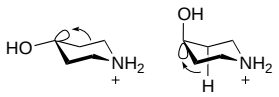
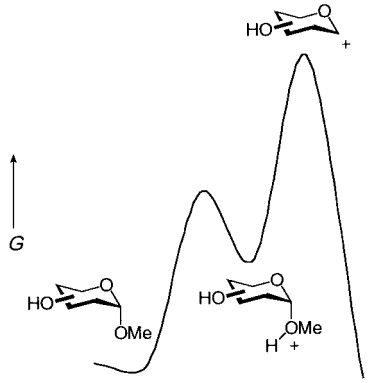
[12] J. Clark, D. D. Perrin, *Q. Rev.* **1964**, *17*, 295–320.
[13] It is known that the solvation energies of monosaccharides can differ, and particularly that the solvation energy of glucose and galactose differs (S. A. Galema, M. J. Blandamer, J. B. F. N. Engberts, *J. Am. Chem. Soc.* **1990**, *112*, 9665–9666; S. A. Galema, M. J. Blandamer, J. B. F. N. Engberts, *J. Org. Chem.* **1992**, *57*, 1995–2001). If such a difference exists between **1** and **2** or **3** and **4**, it would be expected to also exist in the protonated amines and hence not affect pK_a. The difference in solvolysis rates of glycosides does not appear to be an effect of solvation, because galactosides react faster than glucosides in solvents other than water as well.[2a]
[14] A referee suggested the following interesting explanation for the difference in electron-withdrawing power of equatorial and axial OH groups: With an equatorial OH group the antibonding orbital of the C–O bond can overlap with the adjacent C–C bond and thus remove electronic charge from the amine (see below left). With an axial OH group the overlap will occur with the adjacent C–H bond, and will not remove charge from the amine (below right).



[15] Calculated from $\Delta\Delta G = RT\ln[K_a(1)/K_a(2)]$ at 25 °C.
[16] Calculated from $\Delta\Delta G^{\neq} = RT\ln[k_1(1)/k_1(2)]$ at 25 °C. Since no rate constants were available at this temperature the Arrhenius equation $k_1 = A\exp(-E_a/RT)$ and data from ref. [1e] were used to calculate the rate constants at 25 °C. However, the ratios $k_1(1)/k_1(2)$ did not change much with temperature, as the activation energy E_a only varies by 10% between the different glycosides. Therefore, employing the ratio of rate constants at 60 °C gave a very similar result.
[17] The observed rate constant k_{obs} of Overend et al. is equal to an equilibrium constant K for exocyclic protonation multiplied by a rate constant k for conversion of the conjugate acid to the transition state (i.e., $k_{obs} = K \times k$). Our activation energies were derived from k_{obs}, which is the rate constant for going from the unprotonated methyl glycoside to the transition state, and we thereby obtain the activation energy for the entire process, as can be seen from the following energy diagram.



[18] Only the data for the five methyl α-glycosides were used in this regression.
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