Absence of Reverse Anomeric Effect: Conformational Analysis of Glucosylimidazolium and Glucosylimidazole

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Tetrahydropyranyl derivatives with an electronegative group X at C1 show an increased preference for the axial conformer that opposes the steric preference for the equatorial conformer. This is known as the anomeric effect.[1] However, when X is positively charged, the conformational preference is claimed to be shifted toward the equatorial. This has been attributed to a so-called reverse anomeric effect.

The first examples were the conformational equilibria of *N*-(α-glycopyranosyl)pyridinium ions[2] and subsequently other such ions.[3] However, a pyridinium ring is quite bulky, and the results could be due simply to avoidance of steric repulsions associated with placing that group axial.

An imidazolyl group provides its own control for steric factors, since protonation at the distant nitrogen is considered not to change the size of the group. Yet on *N*-protonation or *N*-methylation of *N*-(tetra-*O*-acetyl-α-D-glucopyranosyl)imidazole or *N*-(tetra-*O*-acetyl-α-D-mannopyranosyl)imidazole, there is a shift toward the conformer with the imidazolyl group equatorial.[4] Similar behavior is shown by several *O*-acetylated glycosylimidazoles, although not by the unacetylated ones.[5] More quantitatively, in *N*-(tri-*O*-acetyl-α-D-xylopyranosyl)imidazole, there is 65% equatorial conformer, whereas in the presence of trifluoroacetic acid the proportion increases to >95%.[6] This is a substantial change, corresponding to a $\Delta\Delta G^\circ > 1.4$ kcal/mol. Such results have been accepted as the best evidence for the reverse anomeric effect.

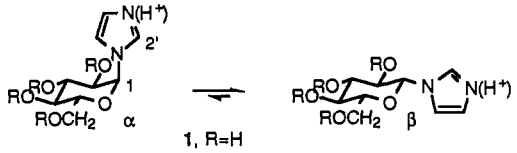
Nevertheless, this effect is not seen in a wide variety of glucopyranosylamines.[7] These have the advantage of NHR or NH_2R^+ groups of known steric preference. The data indicate that the shift of conformational equilibrium that occurs upon *N*-protonation is small and can be accounted for on the basis of steric effects and a small normal anomeric effect. Therefore, it was concluded that the reverse anomeric effect does not exist.

These results raised the question of whether the steric requirement of the imidazolyl group is really invariant to protonation. Even though the distant proton itself does not add much bulk, introduction of a positive charge changes the solvation shell and may increase the effective size. From an NMR titration of *cis*- and *trans*-*N*-(4-phenylcyclohexyl)imidazole in aqueous acetone, it was found[8] that the protonated imidazolyl group is indeed "larger" than the unprotonated group. When axial, it suffers as much as 0.089 kcal/mol of additional steric repulsions, depending on solvent. However, this difference is too small to account for the change from 65% to 95% on protonation of *N*-(tri-*O*-acetyl-α-D-xylopyranosyl)imidazole, so it would appear that this change is due to a reverse anomeric effect arising from the positive charge.[6]

Yet it must be noted that neither those populations nor the shifts of equilibrium were determined from direct observation of the separate conformers of xylosylimidazole at low temperature but rather from small changes in coupling constants. Since coupling constants are sensitive to substituents and to slight conformational deviations, small changes are difficult to interpret, and they may not afford reliable equilibrium constants.

It is important to understand the conformational behavior of sugar derivatives with cationic groups, especially since such derivatives often react via their protonated forms as intermediates. The preference for the β anomer of glycosyl onium ions does allow S_N2 synthesis of α glycosides.[9] This preference has also been invoked to account for relative reactivities or stereoselectivities[10] and to assign product as equatorial,[11] but this reasoning has been shown[12] to lead to error.

We therefore have undertaken to measure directly the effect of *N*-protonation on the anomeric equilibrium in *N*-(D-glucopyranosyl)imidazole (**1**). To permit study in a wider range of solvents, we have also studied its tetra-*O*-acetyl derivative **2**. In



1, R=H
2, R=Ac

principle, the magnitude of any reverse anomeric effect could be measured as the increase in the proportion of the β anomer on protonation of an equilibrating mixture of anomers. However, glycosylimidazoles are configurationally stable and do not equilibrate.[13] Nevertheless, the reverse anomeric effect can be measured instead from the difference in pK_a of the two anomers. To measure with high precision this difference, we have used an NMR titration method that is applicable to a mixture of α and β anomers. We now show that *N*-protonation does not shift the equilibrium strongly toward an equatorial imidazolyl but rather it shifts the equilibrium toward axial.

Spectra were recorded on a Varian Unity-500 spectrometer. Chemical shifts at 24.5 °C were referenced to 0.5% tetramethylsilane or *tert*-butyl alcohol as internal standard. *N*-(2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl)imidazole (**2**) was prepared from tetra-*O*-acetyl-α-D-glucopyranosyl bromide and imidazole according to a standard procedure.[13] Chromatography provided a 1:2 mixture of **2**α and **2**β. Deacetylation was achieved with K_2CO_3 in methanol[14] to give a 1:2 mixture of **1**α and **1**β. Chemical shifts and coupling constants agree with published values.[13]

To the mixture of **1**α and **1**β (11.5 mg, 0.050 mmol) or **2**α and **2**β (15 mg, 0.038 mmol) dissolved in 1.0 mL of methanol-d_4 or DMSO-d_6 were added successive 5-μL portions of 0.25, 0.50, or 0.67 N trifluoroacetic acid (TFA) in the same solvent. The tetraacetate (**2**) was also titrated in CD_2Cl_2, and the parent (**1**)

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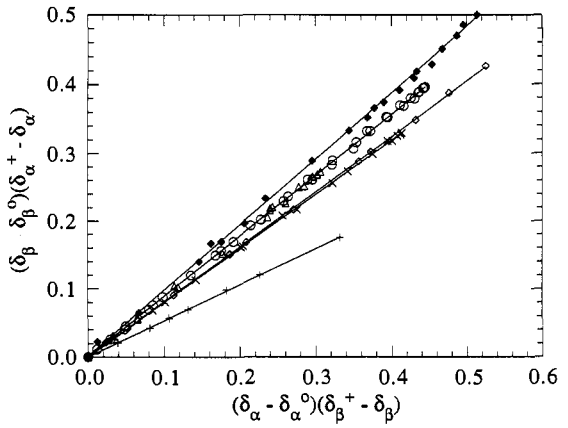

Figure 1. Linearized plot of H2′ chemical shift, $(\delta_\beta - \delta_\beta^\circ)(\delta_\alpha^+ - \delta_\alpha)$ vs $(\delta_\alpha - \delta_\alpha^\circ)(\delta_\beta^+ - \delta_\beta)$, during titration of *N*-D-glucopyranosylimidazole anomers **1** and *N*-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)imidazole anomers **2**: (◆) **1** in DMSO-d_6, (×) **1** in methanol-d_4, (+) **1** in D_2O, (◇) **2** in DMSO-d_6, (△) **2** in CD_2Cl_2, (○) **2** in methanol-d_4.

was also titrated in D_2O with DCl. The ^1H chemical shifts of H1 on the glucose ring and of H2′ on the imidazole ring were recorded after each addition.

These chemical shifts undergo sufficient change on *N*-protonation to permit NMR determination of the extent of protonation. Tables S1 and S2 (supplementary material) list the ^1H chemical shifts of **1** and **2** following each addition of acid. Addition of further acid did not change the chemical shifts further.

What is readily measurable is K_a^α/K_a^β, the ratio of acidity constants of α and β *N*-(glucopyranosyl)imidazolium ions. It can readily be shown[8] that the observed chemical shifts δ_α and δ_β are given by eq 1,where δ_α^+, δ_α°, δ_β^+, and δ_β° are limiting

$$(\delta_\beta - \delta_\beta^\circ)(\delta_\alpha^+ - \delta_\alpha) = (K_a^\alpha/K_a^\beta)(\delta_\alpha - \delta_\alpha^\circ)(\delta_\beta^+ - \delta_\beta) \quad (1)$$

chemical shifts of protonated and unprotonated α and β forms, respectively. Thus a plot of $(\delta_\beta - \delta_\beta^\circ)(\delta_\alpha^+ - \delta_\alpha)$ vs $(\delta_\alpha - \delta_\alpha^\circ)(\delta_\beta^+ - \delta_\beta)$ ought to be linear, with slope K_a^α/K_a^β and zero intercept.

Figures 1 and S2 show such plots, with chemical shifts from Tables S1 and S2. The excellent linearity is confirmed by correlation coefficients always >0.999. The intercepts, which are all properly zero, are given in Table S3, as well as the slopes, which are K_a^α/K_a^β.

What we want is $\Delta\Delta G^\circ_{\beta\to\alpha}$, the difference in $\Delta G^\circ_{\beta\to\alpha}$ between protonated and unprotonated imidazolyl groups. It follows[8] from a thermodynamic cycle that this is given by eq 2. The $\Delta\Delta G^\circ_{\beta\to\alpha}$

$$\Delta\Delta G^\circ_{\beta\to\alpha} = \Delta G^\circ_{N\text{-ImidazolylH}^+} - \Delta G^\circ_{N\text{-Imidazolyl}} = RT \ln (K_a^\alpha/K_a^\beta) \quad (2)$$

values are listed in Table 1. The values are the same from either H1 or H2′. Notice that these values are obtained without equilibrating the anomers and also without interchanging axial and equatorial imidazolyl through ring inversion. It is remarkable that this $\Delta\Delta G^\circ$ can be measured with such precision, higher than the ΔG° values themselves.

All the $\Delta\Delta G^\circ$ values are small but significantly less than zero. This means that a protonated imidazolyl group has a greater preference for the axial position than does the unprotonated. This is exactly opposite to what is claimed for the reverse anomeric effect!

Table 1. $\Delta\Delta G^\circ_{\beta\to\alpha} = \Delta G^\circ_{N\text{-ImidazolylH}^+} - \Delta G^\circ_{N\text{-Imidazolyl}}$, from Linearized Plots of Chemical Shifts in Titrations of *N*-(D-Glucopyranosyl)imidazole Anomers **1**α and **1**β and *N*-(2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl)imidazole Anomers **2**α and **2**β

compd	signal	solvent	acid	$\Delta\Delta G^\circ$, kcal/mol
1	H1	D_2O	DCl	−0.387 ± 0.007
1	H2′	D_2O	DCl	−0.375 ± 0.003
1	H1	CD_3OD	TFA	−0.134 ± 0.002
1	H2′	CD_3OD	TFA	−0.133 ± 0.003
1	H1	DMSO-d_6	TFA	−0.018 ± 0.007
1	H2′	DMSO-d_6	TFA	−0.021 ± 0.004
2	H1	CD_3OD	TFA	−0.074 ± 0.002
2	H2′	CD_3OD	TFA	−0.068 ± 0.001
2	H1	DMSO-d_6	TFA	−0.130 ± 0.004
2	H2′	DMSO-d_6	TFA	−0.131 ± 0.006
2	H2′	CD_2Cl_2	TFA	−0.069 ± 0.005

Steric effects cannot account for these results. *N*-Protonation of the imidazolyl group increases its effective steric bulk[8] because of the need for solvation of the ion and perhaps because of pairing with the counterion. This would reduce the proportion of α anomer, contrary to what is seen. Therefore, the protonated imidazolyl is subject to an enhanced anomeric effect, not a reverse anomeric effect. There are small variations with solvent and with acetylation. The only larger value is in water, perhaps due to an enhanced anomeric effect.

To avoid complications due to ring inversion, we chose glucosylimidazoles, with bulky OH and CH_2OH groups that remain equatorial. A previous estimate,[5] assuming that imidazolyl is as large as phenyl, suggested that the ring inversion equilibrium of the α anomer is more balanced. However, $\Delta G_{N\text{-Imidazolyl}}$, the energy cost to place an imidazolyl group axial, is now known to be only 2.2 kcal/mol,[8] so that with ΔG_{OAc}, ΔG_{OH}, and ΔG_{CH_3} of 0.71, 0.97,[15] and 1.74[16] kcal/mol, respectively, we can estimate that <6% of the ring-inverted α anomer is present. Besides, even if there is more α anomer, its presence could not make it more basic than the β.

In summary, we have used an NMR titration method to measure with high precision the shift of anomeric equilibrium on protonation of *N*-(D-glucopyranosyl)imidazole **1** and its tetra-*O*-acetyl derivative **2**. We find a $\Delta\Delta G^\circ_{\beta\to\alpha} = \Delta G_{N\text{-ImidazolylH}^+} - \Delta G_{N\text{-Imidazolyl}}$ of −0.018 to −0.368 kcal/mol. This result means that the protonated imidazolyl group has a small but significantly greater preference for the axial position than does the unprotonated group. This is exactly opposite to what is claimed for the reverse anomeric effect!

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Supplementary Material Available: Figure S2, a plot of H1 chemical shifts; tables of chemical shifts of **1** and **2** during titrations; complete table of slopes, intercepts, and correlation coefficients from plots (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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