Absence of Reverse Anomeric Effect in Glycosylimidazoles

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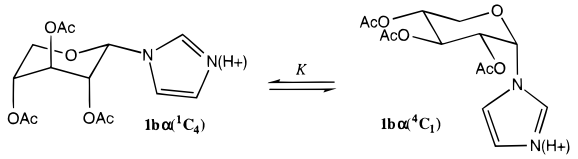
Abstract: The so-called reverse anomeric effect is the preference of cationic substituents for the equatorial position on a pyranose ring, but it is not consistent with theories of molecular structure. To reinvestigate this, we have measured the *N*-protonation-induced shifts of the anomeric equilibrium in *N*-(glycopyranosyl)imidazoles and their tetra-*O*-acetyl derivatives **1**−**3** with high precision through an NMR titration method that is applicable to a mixture of α and *β* anomers. We find a $\Delta\Delta G^\circ_{\beta\rightarrow\alpha}$ that is almost always negative, corresponding to a greater preference for the axial position of a protonated imidazolyl group than of an unprotonated group. This preference counters a small steric effect, arising from hindrance to ionic solvation, that has been measured independently in *N*-(4-*tert*-butylcyclohexyl)imidazoles **4**. These results are exactly opposite to what is expected from the reverse anomeric effect. We conclude that there is no firm evidence for this effect.

Introduction

The anomeric effect (AE) is the tendency for an electronegative X at C1 of a tetrahydropyran derivative to take the axial position.[1] However, when X is cationic, the equilibrium shifts toward equatorial, a phenomenon known as the reverse anomeric effect (RAE).[2] The first examples were with *N*-(α-glycosyl)-pyridinium ions.[2,3] However, a pyridinium ring is bulky, and these results could have been due simply to steric repulsions when that group is axial.

An imidazolyl group provides its own control for steric factors, since protonation at the distant nitrogen is not likely to increase the size. Nevertheless *N*-protonation or -methylation of *N*-(tetra-*O*-acetyl-α-gluco or -mannosyl)imidazole seems to shift the equilibrium toward the conformer with the imidazolyl group equatorial (often designated $^1C_4$),[4] although not in the unacetylated derivatives.[5] More quantitatively, in *N*-(tri-*O*-acetyl-α-xylopyranosyl)imidazole (**1bα**) there is 65% equatorial ($^1C_4$) conformer ($K = 0.5$), whereas on addition of trifluoroacetic acid the proportion reportedly increases to >95% ($K < 0.05$).[6] This is a substantial change, corresponding to a $\Delta\Delta G^\circ$ of >1.4 kcal/mol. This case has been widely accepted as the best evidence for the RAE. The data are reproducible and are not due to a change of solvent polarity arising from the addition of acid.[7]

Modern theories of molecular structure do not account for the RAE.[1a] Simply stated, if the AE is characteristic of an electronegative X, X^+ is even more electronegative and ought



to show an enhanced AE. Although the AE itself has been attributed to electrostatic interactions, which can account for the RAE,[2] a molecular orbital interpretation is currently favored.[8] Orbital overlap between an oxygen lone pair (*n*) and the C−X antibonding orbital (*σ**) stabilizes the axial conformer. Cationic X lowers the energy of the *σ** orbital so that it interacts more strongly. Alternatively, this interaction corresponds to a double-bond/no-bond resonance form, which contributes even more with cationic X since there is no penalty of charge separation. Either way the AE ought to increase, not reverse.

According to a review of the RAE,[9] the evidence is conflicting. The RAE is not seen in glucosylamines, with NHR or NH_2R^+ groups of known steric preference.[10] Instead there is only a small shift of the anomeric equilibrium upon *N*-protonation, consistent with an enhancement of the ordinary AE, countering a steric effect. Therefore it was concluded that the RAE does not exist. Additional experimental cases are sparse. No such effect is observed with 2-$PPh_n(CH_3)_{3-n}^+$ groups ($n = 0-3$) on a 1,3-dithiane,[11] nor is any seen in a 1,3-dioxane with a 2-trialkylammonio group.[12] The proportion of pseudoaxial conformation of *ψ*-isocytidine increases on *N*-protonation, counter to the RAE.[13]

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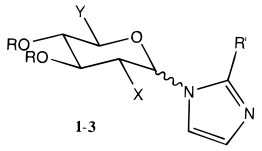
Even molecular orbital calculations are inconclusive. In general, most are consistent with the RAE, but it is difficult to distinguish this from hydrogen bonding between the ring oxygen and a protonated exocyclic group, which also favors the equatorial conformer.[9] According to MP2/6-31+G* calculations, protonated methoxymethanol shows no strong conformational preference.[14] Calculated bond length changes are uniformly consistent with an ordinary AE, not a reverse one.[9] Solvation effects on various $O-C-XH_n^+$ ions support an AE, and not a reverse one.[15] Other calculations on imidazole derivatives in a continuum dielectric suggest that the RAE diminishes with increasing solvent polarity.[16]

It is important to understand the conformational behavior of sugar derivatives with cationic groups. Many bioactive molecules have cationic or protonatable heterocyclic bases attached to a sugar, the most familiar examples being NAD^+ and the conjugate acids of nucleosides. Many other sugar derivatives and analogues react only when protonated, and it is desirable to know the conformation of such intermediates in order to address stereoelectronic effects.[17] The reactivity of glycosyl onium ions often allows the stereospecific S_N2 synthesis of glycosides,[18] regardless of the origin of the preference for the β anomer. This preference has also been invoked to account for relative reactivities or stereoselectivities[19] and to assign products,[20] but the reasoning has been shown[21] to lead to error.

We therefore have undertaken to measure the effect of *N*-protonation on the anomeric equilibrium in a series of *N*-(xylopyranosyl)imidazoles **1** and *N*-(glucopyranosyl)imidazoles **2**. This is a different approach from previous studies, which focused on the effect of *N*-protonation on the ring-inversion equilibrium of a single anomer. To intensify steric contributions, we have also studied the corresponding 2-methylimidazole derivatives. To permit access to a wide range of solvents and also to probe potential contributions from hydrogen bonding by the 2-OH, we have also studied their tri- or tetra-*O*-acetyl derivatives and the 2-deoxyglucosyl analogues **3**. The various glycosyl derivatives studied are distinguished in Table 1.



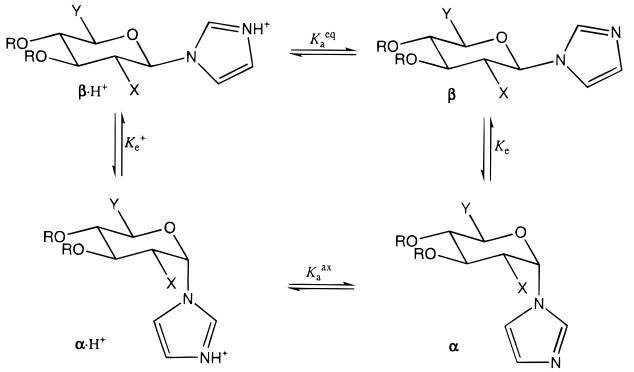
1-3

The RAE can be manifested as an increase in the proportion of the β anomer on protonation of an equilibrating mixture of glycosylimidazoles. This increase can be expressed as the ratio K_e^+/K_e in Scheme 1, where $K_e = [\beta]/[\alpha]$ and $K_e^+ = [\beta H^+]/[\alpha H^+]$. However, glycosylimidazoles are configurationally stable and do not equilibrate.[22] Nevertheless the increase can be evaluated indirectly from the difference in p*K* of the two anomers. Scheme 1 is a thermodynamic cycle, and it follows that K_e^+/K_e must equal the ratio of acidity constants, K_a^{ax}/K_a^{eq}. Then a further consequence of the RAE is that the β anomer must be more basic than the α anomer. To measure the difference in basicities, we have used an NMR titration method that is applicable without the necessity of separating the anomers.[23] The method is capable of high precision, and it succeeds across a wide range of solvents. We now show that *N*-protonation does not shift the equilibrium toward β but rather toward α.

Table 1. Glycosylimidazoles **1**−**3**

cmpd	sugar	X	R	Y	R′
1a	Xylo	OH	H	H	H
1b	Ac_3Xylo	OAc	Ac	H	H
1c	Xylo	OH	H	H	CH_3
1d	Ac_3Xylo	OAc	Ac	H	CH_3
2a	Gluco	OH	H	CH_2OH	H
2b	Ac_4Gluco	OAc	Ac	CH_2OAc	H
2c	Gluco	OH	H	CH_2OH	CH_3
2d	Ac_4Gluco	OAc	Ac	CH_2OAc	CH_3
3a	DeoxyGlu	H	H	CH_2OH	H
3b	Ac_3DeoxyGlu	H	Ac	CH_2OAc	H
3c	DeoxyGlu	H	H	CH_2OH	CH_3
3d	Ac_3DeoxyGlu	H	Ac	CH_2OAc	CH_3

Scheme 1. Acid Dissociations of α- and *β*-*N*-(D-Glycopyranosyl)imidazolium Ions



A further question is whether the effective size of an imidazolyl group is truly invariant to protonation. The effective size of a substituent can be expressed by its *A* value,[24] the free-energy difference between axial and equatorial conformers (eq 1). Although protonation is on a distant nitrogen, the positive

$$A = G^\circ_{\text{axial}} - G^\circ_{\text{equatorial}} = RT \ln([\text{equatorial}]/[\text{axial}]) > 0 \quad (1)$$

charge requires additional solvation, which may be hindered when it is axial. Indeed, steric hindrance to ionic solvation is well established, as in the basicities of the methylated amines

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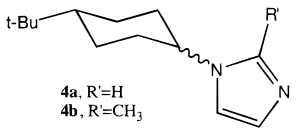
and the acidities of alcohols.[25] Therefore to compare the *A* values of protonated and unprotonated imidazolyls, we also subjected the two stereoisomers of *N*-(4-*tert*-butylcyclohexyl)imidazole (**4a**) and of 1-(4-*tert*-butylcyclohexyl)-2-methylimidazole (**4b**) to NMR titration.



4a, R'=H
4b, R'=CH_3

Experimental Section

Instrumentation. NMR spectra were recorded on a Varian Unity-500 spectrometer (499.8 MHz ^{1}H) using an indirect probe. Chemical shifts for ^{1}H spectra are referenced to TMS (δ 0.00), except for titrations in D_2O, which are referenced to *t*-BuOH (δ 1.17). Mass spectral analyses were conducted by The Scripps Research Institute Mass Spectrometry Facility (La Jolla, CA).

Materials. Deuterated solvents, 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide, and other reagents were obtained commercially and used as received. Dichloromethane was distilled from CaH_2 and stored under nitrogen. Acetylated glycopyranosylimidazoles were prepared as a mixture of α and β anomers by a standard method from the corresponding D-glycosyl bromide and either imidazole or 2-methylimidazole,[26] as described below. They were deacetylated according to a standard deprotection procedure,[27] also described below. Samples were purified by chromatography on silica gel with benzene−methanol (10:1) as eluent. No effort was made to separate the anomers. Anomers were assigned as α or β on the basis of a small (≤6 Hz) or large (>8 Hz) coupling constant J_{12}.[28]

2,3,4-Tri-*O*-acetyl-α-xylopyranosyl Bromide.[29] This was prepared from β-D-xylopyranose tetraacetate and PBr_3. ^{1}H NMR ($CDCl_3$) δ 6.57 (d, 1H, H1, J = 4 Hz), 5.56 (t, 1H, H3, J = 9.5 Hz), 5.06−5.00 (m, 1H, H4), 4.76 (dd, 1H, H2, J = 4, 10 Hz), 4.04 (dd, 1H, H5(5′), J = 6.5, 11.5 Hz), 3.87 (dd, 1H, H5(5′), J = 10.5, 11.5 Hz), 2.09 (s, 3H, Ac), 2.05 (s, 6H, Ac).

***N*-(2,3,4-Tri-*O*-acetylxylopyranosyl)imidazole (1b).** Imidazole (0.6 g, 8.8 mmol) was added to 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (1.0 g, 2.95 mmol) in freshly distilled dioxane (4 mL). The mixture was refluxed for 4 h and cooled to room temperature, diluted with dioxane (5 mL), filtered through Celite, concentrated by evaporation of solvent in a vacuum, and purified by chromatography, to produce the mixture **1b(α+*β*)** (418 mg, 43%) as an amorphous foam. ^{1}H NMR (α) (DMSO-d_6) δ 7.80 (s, 1H. H2′), 7.29 (s, 1H), 6.93 (s, 1H), 5.91 (d, 1H, H1, J = 2 Hz), 5.10−5.08 (m, 1H), 4.99−4.98 (m, 1H), 4.74−4.71 (m, 1H), 4.15 (dd, 1H, J = 2, 13.5 Hz), 4.04−4.00 (m, 1H), 2.16 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.06 (s, 3H, Ac). ^{1}H NMR (β) (DMSO-d_6) δ 7.82 (s, 1H, H2′), 7.36 (s, 1H), 6.92 (s, 1H), 5.70 (d, 1H, H1, J = 8.5 Hz), 5.47 (dd, 1H, J = 9, 9.5 Hz), 5.38 (dd, 1H, J = 9.9, 9.5 Hz), 5.16−5.10 (m, 1H), 4.07 (dd, 1H, J = 5, 11 Hz), 3.68 (dd, 1H, J = 11, 11.5 Hz), 2.02 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.81 (s, 3H, Ac). HRMS: calculated, MH^+ 327.1192; found, 327.1181.

1-(2,3,4-Tri-*O*-acetylxylopyranosyl)-2-methylimidazole (1d). This was prepared similarly from the same bromide (1.0 g, 2.95 mmol) and 2-methylimidazole (0.727 g, 8.85 mmol). Chromatography gave **1dα** (310 mg, 31%) as an amorphous solid, and **1d(α+*β*)** (116 mg, 12%) as an amorphous foam. ^{1}H NMR (α) ($CDCl_3$) δ 7.22 (d, 1H, J = 1.2 Hz), 6.96 (d, 1H, J = 1.2 Hz), 5.79 (d, 1H, H1, J = 3.5 Hz), 5.47 (dd, 1H, H3, J = 6, 6.5 Hz), 5.08 (dd, 1H, H2, J = 3.9, 6.5 Hz), 4.95−4.90 (m, 1H, H4), 3.99 (dd, 1H, H5, J = 3.5, 12.5 Hz), 3.80 (dd, 1H, H5, J = 5.5, 12.5 Hz), 2.45 (s, 3H, CH_3), 2.18 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.03 (s, 3H, Ac). ^{1}H NMR (β) ($CDCl_3$) δ 6.94 (s, 1H), 6.93 (s, 1H), 5.37 (t, 1H, J = 9.5 Hz), 5.30 (dd, 1H, J = 9, 9.5 Hz), 5.19 (d, 1H, J = 8.5 Hz), 5.18−5.13 (m, 1H), 4.28 (dd, 1H, J = 5.5, 11.5 Hz), 3.52 (t, 1H, J = 11 Hz), 2.46 (s, 3H, CH_3), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.89 (s, 3H, Ac). HRMS: calculated, MH^+ 341.1348; found, 341.1358.

N*-(2,3,4,6-Tetra-*O*-acetylglucopyranosyl)imidazole (2b).** This was prepared similarly from 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (1.65 g, 4 mmol) and imidazole (0.6 g, 8.8 mmol). Chromatography provided **2bα** (158 mg, 10%), mp 204−205 °C (lit[26] 213 °C), **2b*β (149 mg, 9%), mp 160−162 °C, and the mixture **2b(α+*β*)** (386 mg, 24%) as a white solid. The ^{1}H NMR chemical shifts agree with those previously reported.[22]

1-(2,3,4,6-Tetra-*O*-acetylglucopyranosyl)-2-methylimidazole (2d). This was prepared similarly from the same bromide (1.65 g, 4 mmol) and 2-methylimidazole (0.72 g, 8.8 mmol). Chromatography gave **2dα** (370 mg, 22%), **2d*β*** (230 mg, 14%), and the mixture **2d(α+*β*)** (321 mg, 19%), each as an amorphous solid. ^{1}H NMR (α) ($CDCl_3$) δ 7.33 (s, 1H), 6.97 (s, 1H), 6.11 (d, 1H, H1, J = 6 Hz), 5.78 (t, 1H, J = 9.6 Hz), 5.31 (dd, 1H, J = 6, 10.5 Hz), 5.15 (t, 1H, J = 9.6 Hz), 4.21 (dd, 1H, J = 4.8, 12.3 Hz), 3.94 (dd, 1H, J = 2, 13 Hz), 3.53 (m, 1H), 2.42 (s, 3H, CH_3), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.98 (s, 3H, Ac). ^{1}H NMR (β) ($CDCl_3$) δ 6.96 (s, 1H), 6.94 (s, 1H), 5.34 (m, 2H), 5.25 (m, 1H), 5.20 (m, 1H), 4.25 (dd, 1H, J = 5.5, 12 Hz), 4.14 (dd, 1H, J = 2, 12 Hz), 3.91 (m, 1H), 2.43 (s, 3H, CH_3), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.86 (s, 3H, Ac).

***N*-(3,4,6-Tri-*O*-acetyl-2-deoxyglucopyranosyl)imidazole (3b).** This was prepared similarly from 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucosyl bromide[30] (447 mg, 1.27 mmol) and imidazole (215 mg, 3.16 mmol). Chromatography gave the mixture **3b(α+*β*)** (245 mg, 57%) as an oil. ^{1}H NMR (mixture of α+β) (CD_3OD) δ 7.92 (s, 1H, H2′, α), 7.89 (s, 1H, H2′, β), 7.36 (s, 1H, Himid, α), 7.35 (s, 1H, Himid, β), 7.10 (s, 1H, Himid, α), 7.01 (s, 1H, Himid, β), 6.02−6.00 (m, 1H, H1, α), 5.72 (dd, 1H, H1, β, J = 1.5, 11 Hz), 5.29−5.23 (m, 1H), 5.20 (dd, 1H, J = 9.5, 10.5 Hz), 5.10 (dd, 1H, J = 9.5, 10 Hz), 4.42 (sept, 1H, J = 5.5, 11.5 Hz), 4.30 (dd, 1H, J = 5, 12 Hz), 4.26 (dd, 1H, J = 5, 12 Hz), 4.12 (dd, 1H, J = 2, 12 Hz), 4.00−3.96 (m, 1H), 3.93−3.89 (m, 1H), 3.67−3.62 (m, 1H), 2.94−2.88 (m, 1H), 2.79−2.75 (m, 1H), 2.55−2.50 (m, 1H), 2.32−2.24 (m, 1H), 2.12 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac). HRMS: calculated, MH^+ 341.1349; found, 341.1361.

1-(3,4,6-Tri-*O*-acetyl-2-deoxyglucopyranosyl)-2-methylimidazole (3d). This was prepared similarly from the same bromide (500 mg, 1.4 mmol) and 2-methylimidazole (296 mg, 3.6 mmol). Chromatography gave the mixture **3d(α+*β*)** (233 mg, 46%) as an oil. ^{1}H NMR (α) ($CDCl_3$) δ 7.27 (s, 1H), 6.99 (s, 1H), 5.85−5.82 (m, 1H, H1), 5.31−5.24 (m, 1H), 5.11 (t, 1H, J = 9 Hz), 4.31 (dd, 1H, J = 6, 12.5 Hz), 3.97 (dd, 1H, J = 2, 12.5 Hz), 3.58−3.52 (m, 1H), 2.81−2.75 (m, 1H), 2.49 (s, 3H, CH_3), 2.27−2.20 (m, 1H), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac). ^{1}H NMR (β) ($CDCl_3$) δ 6.95 (s, 1H), 6.90 (s, 1H), 5.31 (dd, 1H, H1, J = 2, 11 Hz), 5.18−5.12 (m, 1H), 5.07 (t, 1H, J = 10 Hz), 4.24 (dd, 1H, J = 5.5, 12.5 Hz), 4.10 (dd, 1H, J = 2.5, 12.9 Hz), 4.88−4.81 (m, 1H), 2.52−2.47 (m, 1H), 2.41 (s, 3H, CH_3), 2.17 (t, 1H, J = 11.5 Hz), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.03 (s, 3H, Ac). HRMS: calculated, MH^+ 355.1505; found, 355.1516.

***N*-(Xylopyranosyl)imidazole (1a).** Triacetate **1b** (100 mg, 0.306 mmol) and K_2CO_3 (4 mg, 0.03 mmol) were stirred in methanol (20 mL) for 4 h at room temperature, diluted with 80 mL of 1:1 ether/petroleum ether, filtered through Celite, and concentrated by evaporation in a vacuum. The oily residue was dried under vacuum to give **1a-(α+*β*)** as a colorless oil (56 mg, 91%). ^{1}H NMR (α) (D_2O) δ 7.84 (bs, 1H, H2′), 7.26 (bs, 1H), 6.92 (bs, 1H), 5.71 (d, 1H, H1, J = 3 Hz), 3.96−3.90 (m, 2H), 3.84−3.81 (m, 1H), 3.68−3.59 (m, 2H). ^{1}H NMR (β) (D_2O) δ 7.76 (bs, 1H, H2′), 7.21 (bs, 1H), 6.95 (bs, 1H), 5.14 (d, 1H, H1, J = 9.5 Hz), 3.92−3.90 (m, 1H), 3.78 (m, 1H), 3.67 (tt, 1H, J = 4.5, 11.5 Hz), 3.65−3.59 (m, 1H), 3.44 (tt, 1H, J = 4.5, 9 Hz). HRMS: calculated, MH^+ 201.0875; found, 201.0880.

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1-(Xylopyranosyl)-2-methylimidazole (1c). This was prepared similarly from triaacetate **1d** (100 mg, 0.24 mmol) and anhydrous K_2CO_3 (4 mg, 0.03 mmol) in methanol, to give **1c(α+*β*)** (57 mg, 90%) as a colorless oil. ^{1}H NMR (α) (D_2O) δ 7.33 (s, 1H), 6.80 (s, 1H), 5.63 (d, 1H, H1, $J = 2.5$ Hz), 4.10−3.91 (m, 2H), 3.84 (m, 1H), 3.68−3.61 (m, 2H), 2.35 (s, 3H, CH_3). ^{1}H NMR (*β*) (D_2O) δ 7.12 (s, 1H), 6.83 (s, 1H), 5.11 (d, 1H, H1, $J = 9$ Hz), 3.99 (t, 1H, $J = 5$ Hz), 3.68−3.61 (m, 2H), 3.48 (t, 1H, $J = 9$ Hz), 3.41 (t, 1H, $J = 11$ Hz), 2.31 (s, 3H, CH_3). HRMS: calculated, MH^+ 215.1032; found, 215.1040.

***N*-(Glucopyranosyl)imidazole (2a)**. This was prepared similarly from tetraacetate **2b** (100 mg, 0.25 mmol) and K_2CO_3 (4 mg, 0.03 mmol) in methanol, to give **2a(α+*β*)** (50 mg, 91%) as a colorless oil. The ^{1}H NMR chemical shifts agree with those previously reported.[22]

1-(Glucopyranosyl)-2-methylimidazole (2c). This was prepared similarly from tetraacetate **2d** (100 mg, 0.24 mmol) and K_2CO_3 (4 mg, 0.03 mmol) in methanol, to give **2c(α+*β*)** as a colorless oil (52 mg, 89%). ^{1}H NMR (α) (D_2O) δ 7.28 (s, 1H), 6.79 (s, 1H), 5.86 (d, 1H, H1, $J = 5.5$ Hz), 3.95 (m, 2H), 3.56 (m, 2H), 3.39 (t, 1H, $J = 8.5$ Hz), 3.1 (m, 1H), 2.29 (s, 3H, CH_3). ^{1}H NMR (*β*) (D_2O) δ 7.13 (s, 1H), 6.83 (s, 1H), 5.17 (d, 1H, H1, $J = 8.5$ Hz), 3.75 (m, 1H), 3.62 (m, 2H), 3.54 m, 2H), 3.43 (m, 1H), 2.27 (s, 3H, CH_3).

***N*-(2-Deoxyglucopyranosyl)imidazole (3a).** This was prepared similarly from triacetate **3b** (50 mg, 0.15 mmol) and K_2CO_3 (3 mg, 0.02 mmol) in methanol (10 mL) as a colorless oil **3a(α+*β*)** (34 mg, 88%). ^{1}H NMR (D_2O) δ 7.76 (s, 1H, H2′, α), 7.52 (s, 1H, H2′, *β*), 7.21 (s, 1H, Himid, α), 7.17 (s, 1H, Himid, *β*), 6.96 (s, 1H, Himid, α), 6.91 (s, 1H, Himid, *β*), 6.82 (bd, 1H, H1, α, $J = 5$ Hz,), 5.50 (dd, 1H, H1, *β*, $J = 2$, 11 Hz), 4.16−4.10 (m, 1H), 3.75−3.65 (m, 4H), 3.52−3.48 (m, 1H), 3.45−3.41 (m, 1H), 3.32 (q, 1H, $J = 9$, 14.5 Hz), 3.20−3.14 (m, 1H), 2.66−2.61 (m, 1H), 2.52 (m, 1H), 2.34−2.30 (m, 1H), 2.06−2.01 (m, 1H), 1.97 (dd, 1H, $J = 3.5$, 11.5 Hz). HRMS: calculated, MH^+ 215.1032; found, 215.1038.

1-(2-Deoxyglucopyranosyl)-2-methylimidazole (3c). This was prepared similarly from triacetate **3d** (54 mg, 0.15 mmol) and K_2CO_3 (3 mg, 0.02 mmol) in methanol as a colorless oil **3c(α+*β*)** (39 mg, 91%). ^{1}H NMR (mixture of α+*β*) (D_2O) δ 7.17 (s, 1H, Himid, α), 7.12 (d, 1H, Himid, *β*, $J = 1.5$ Hz), 6.82 (d, 1H, Himid, α, $J = 1.5$ Hz), 6.77 (s, 1H, Himid, *β*), 5.82 (bd, 1H, H1, α, $J = 4.5$ Hz,), 5.43 (dd, 1H, H1, *β*, $J = 2$, 11 Hz), 4.18−4.12 (m, 1H), 3.78−3.58 (m, 4H), 3.59−3.49 (m, 1H), 3.48−3.46 (m, 1H), 3.37−3.28 (m, 1H), 3.04−3.00 (m, 1H), 2.64−2.62 (m, 1H), 2.56−2.60 (m, 1H), 2.40−2.38 (m, 1H), 2.26 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.06−2.02 (m, 1H), 1.97 (m, 1H). HRMS: calculated, MH^+ 229.1188; found, 229.1195.

***N*-(4-*t*-Butylcyclohexenyl)imidazole.** Ogata's thionyldiimidazole procedure[31] was adapted to transfer a single imidazole to a cyclohexanone. Thionyl chloride (3.26 g, 27.4 mmol) was added dropwise to 5.99 g of imidazole (88 mmol) in 40 mL of dry CH_2Cl_2 at 0 °C. After 15 min the mixture was added dropwise to a solution of 4-*tert*-butylcyclohexanone (2.27 g, 14.7 mmol) in 30 mL of CH_2Cl_2. The mixture was stirred for 3 days, neutralized with aqueous $NaHCO_3$, extracted with CH_2Cl_2, and dried over Na_2SO_4. After evaporation of solvent, the residue was chromatographed and eluted with MeOH−CH_2Cl_2 (1:9). The product (0.73 g, 24.3%) was used in the following step without further purification: mp 61−65 °C. ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1H), 7.08 (s, 1H), 7.07 (s, 1H), 5.82 (t, $J = 2.8$ Hz, 1H), 2.47 (m, 2H), 2.21 (m, 1H), 2.0 (m, 2H), 1.35 (m, 2H), 0.90 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 23.6, 25.5, 27.1, 28.4, 32.1, 43.4, 116.6, 116.7, 129.1, 133.6, 134.5. From the less polar fraction 4-*tert*-butyl-1,1-di-(1-imidazolyl)cyclohexane (0.211 g, 16%) was also isolated. ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.34 (s, 1H), 7.10 (s, 1H), 7.04 (s, 1H), 6.93 (s, 1H), 6.71 (s, 1H), 2.85 (d, 2H, $J = 13$ Hz), 2.28−2.17 (m, 2H), 1.86 (d, 2H, $J = 12$ Hz), 1.25−1.10 (m, 3H), 0.74 (s, 9H, tBu).

***N*-(4-*t*-Butylcyclohexyl)imidazole (4a).** *N*-(4-*tert*-Butylcyclohexenyl)imidazole (0.3 g, 1.47 mmol), 0.6 mL of trifluoroacetic acid (7.78 mmol), 0.6 g of 10% Pd/C, and 20 mL of ethanol were shaken under 55 psi H_2. After 3 days the reaction had gone to completion, as judged by disappearance of the δ 5.8 ^{1}H NMR signal. Filtration, titration to alkaline pH with NaOH, extraction into CH_2Cl_2, drying over Na_2SO_4, and solvent evaporation produced a 1:4 mixture of *cis* and *trans*-*N*-(4-*tert*-butylcyclohexyl)imidazole (286 mg, 94.2%), according to integration of H1 signals; cis δ 4.24 (qn, $J = 2.8$ Hz), trans δ 3.85 (tt, $J = 12$, 4 Hz).

1-(4-*t*-Butylcyclohexenyl)-2-methylimidazole. A solution of 2-methylimidazole (8 g, 97.4 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C before thionyl chloride (2.2 mL, 30.2 mmol) was added dropwise. After 10 min of stirring, 4-*tert*-butylcyclohexanone (2.5 g, 16.2 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 2 days, then neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The extract was washed with water and dried over Na_2SO_4. The solvent was evaporated in a vacuum to a syrup, which was chromatographed on silica gel with CH_2Cl_2−methanol (50:1 to 20:1) to yield an oily yellow solid (1.1 g, 31%). ^{1}H NMR ($CDCl_3$) δ 6.92 (d, 1H, Himid, $J = 1.5$ Hz), 6.81 (d, 1H, Himid, $J = 1.5$ Hz), 5.71−5.75 (m, 1H, H2), 2.35 (s, 3H, CH_3), 2.38−2.28 (m, 1H), 2.28−2.20 (m, 2H), 2.01−1.93 (m, 2H), 1.40−1.34 (m, 2H), 0.91 (s, 9H, tBu). ^{13}C NMR ($CDCl_3$) δ; 126.97, 125.13, 119.00, 84.42, 43.34, 43.31, 32.03, 32.03, 30.40, 27.06 (tBu), 25.93, 13.35 (C_q, tBu).

1-(4-*t*-Butylcyclohexyl)-2-methylimidazole (4b). 1-(4-*tert*-Butylcyclohexenyl)-2-methylimidazole (0.5 g, 2.29 mmol) and 10% Pt/C (0.5 g) in acetic acid (20 mL) were shaken for 3 days at 60 psi H_2 at room temperature and then filtered through Celite. Solvent was removed in a vacuum. The residue was dissolved in a small amount of water, mixed with saturated $NaHCO_3$, and extracted repeatedly with CH_2Cl_2. The combined organic extract was dried over Na_2SO_4. The solvent was evaporated in a vacuum to a syrup, which was chromatographed on silica gel with CH_2Cl_2−methanol (50:1 to 20:1) to yield a yellow oil (313 mg, 62%) that was characterized as a 1:1 mixture of cis and trans stereoisomers. ^{1}H NMR ($CDCl_3$) δ 7.13 (bs, 1H, Himid), 6.90 (bs, 1H, Himid), 6.89 (bs, 1H, Himid), 6.85 (bs, 1H, Himid), 4.23 (h, 1H, $H1_{eq}$, $J = 2.5$ Hz), 4.23 (tt, 1H, $H1_{ax}$, $J = 11.5$ Hz), 3.37 (s, 6H, CH_3), 2.10−1.98 (m, 3H), 1.95−1.88 (m, 2H), 1.87−1.77 (m, 2H), 1.70−1.64 (m, 2H), 1.64−1.52 (m, 2H), 1.39−1.29 (m, 2H) 1.27−1.05 (m, 5H), 0.87 (s, 18H, tBu). HRMS: calculated, MH^+ 221.2018; found, 221.2024.

NMR Titrations. Samples were prepared with 1.00 mL of solvent, 5 μL of tetramethylsilane (TMS), and 0.03−0.1 mmol of the stereoisomeric mixture of glycosyl- or cyclohexyl-imidazoles. Samples of cyclohexylimidazoles in DMSO-d_6 and CD_3OD/D_2O were prepared with trace alkali to suppress hydrolysis to imidazolium ions. An initial ^{1}H NMR spectrum of the sample was taken. Stock acid solutions were prepared as 10% (v/v) trifluoroacetic acid-*d* (TFA-*d*) in CD_3OD, DMSO-d_6, or CD_2Cl_2, or by diluting TFA-*d* or 35% DCl with D_2O. Aliquots of 3−10 μL of the stock acid were continually added, and ^{1}H NMR chemical shifts were recorded until they no longer changed. At least 10 points were obtained for each titration. The H1 and H2′ signals (on sugar or cyclohexane and imidazole, respectively) undergo large shifts upon protonation, ∼0.3 and ∼1.0 ppm, respectively. These are large enough to accurately monitor the extent of protonation of each stereoisomer during the course of a titration. In some cases imidazole H4′, H5′, or 2′-CH_3 was also monitored.

Data Analysis. The ratio of acidity constants K_a^{ax}/K_a^{eq} is obtained as the slope of a linearized plot created from the chemical shifts of the neutral (δ_{α°,δ_{β°) and ionic forms (δ_{α^+},δ_{β^+}) and the observed chemical shifts (δ_α,δ_β), according to eq 2. A similar equation holds for the ratio of acidity constants of the cis and trans stereoisomers of 4-*tert*-butylcyclohexylimidazole. It should be noted that this method differs from the more direct NMR method of following the pH dependence of chemical shifts, which has been applied to measuring the basicities of the two epimers of 2-glycosamines.[32] In our method no pH measurement is necessary.

$$(\delta_\beta - \delta_{\beta^\circ})(\delta_{\alpha^+} - \delta_\alpha) = (K_a^{ax}/K_a^{eq})(\delta_\alpha - \delta_{\alpha^\circ})(\delta_{\beta^+} - \delta_\beta) \quad (2)$$

The ratio was converted to $\Delta\Delta G^\circ_{\beta\rightarrow\alpha}$, the change upon *N*-protonation of the free-energy difference between α and *β* anomers at 298 K (eq 3). A similar equation holds for 4-*tert*-butylcyclohexylimidazoles, except that α and *β* become axial and equatorial, and $\Delta\Delta G^\circ$ becomes ΔA (eq 4), the difference between the *A* values (eq 1) of protonated and

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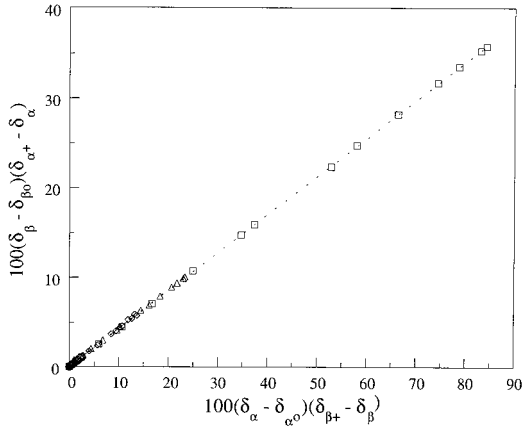

Figure 1. Linearized plots (eq 2) of chemical shifts of (triacetoxyxylosylimidazole (**1b**) during titration in DMSO-d_6: (○) H1, (□) H2′, (◇) H4′, (△) H5′.

unprotonated *N*-imidazolyl groups.

$$\Delta\Delta G^\circ_{\beta\rightarrow\alpha} = \Delta G^\circ_{\text{ImidazolylH}^+} - \Delta G^\circ_{\text{Imidazolyl}} = RT\ln(K_a^{ax}/K_a^{eq}) \quad (3)$$

$$\Delta\Delta G^\circ_{\text{eq}\rightarrow\text{ax}} = \Delta A_{\text{Im}} = A_{\text{ImidazolylH}^+} - A_{\text{Imidazolyl}} = RT\ln(K_a^{ax}/K_a^{eq}) \quad (4)$$

Force-Field Calculations. Dihedral angles in the energy-minimized structures of axial and equatorial conformers of neutral and protonated glycosylimidazoles and their tri- or tetraacetates were calculated with a Macintosh version of MMX (PCMODEL).[33]

Results

The α and *β* anomers of *N*-(D-glycopyranosyl)imidazoles and their tri- or tetra-*O*-acetyl derivatives (**1**-**3**) were submitted to NMR titrations and analyzed according to eq 2. For all the plots the average correlation coefficient was >0.999, indicative of excellent linearity. The average of all the intercepts was <0.0003, or properly zero within a very small experimental error. Figure 1 shows a typical set of plots. The slopes K_a^{ax}/K_a^{eq} from all the titrations are presented in Tables 2−4, along with the $\Delta\Delta G^\circ_{\beta\rightarrow\alpha}$ values (eq 3). For the xyloses the slopes are strictly K_a^{α}/K_a^{β}, owing to the mixture of two α conformations, as explained below. A few of these results were published in a brief communication.[34] Table 5 lists the ratio of acidity constants for *N*-(4-*tert*-butylcyclohexyl)imidazoles (**4**) and the resulting Δ*A* values (eq 4).

The values in Tables 2−5 are remarkably accurate, as can also be judged from the excellent linearity of Figure 1. The same values are obtained regardless of which reporter nucleus (H1, H2′, H4′, H5′, or 2′-CH_3) is used, although it can be seen from Figure 1 that the H1 chemical shift is the least sensitive of these to imidazole protonation. The errors in $\Delta\Delta G^\circ_{\beta\rightarrow\alpha}$ or Δ*A* are usually only a few calories per mole, not kilocalories per mole. This high accuracy is a consequence of the precision in measuring ^{1}H chemical shifts with a 500 MHz spectrometer, without any need to integrate NMR signals or even to measure pK_a or pH with a pH meter.

Key HCCH dihedral angles in selected xylosylimidazoles, glucosylimidazoles, their tri- and tetraacetates, and their protonated forms, as calculated by MMX, are listed in Table 6. An axial imidazole is more stable when the C2′NCH1 dihedral angle is near ±90° rather than near 0° or 180°.

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Table 2. Slopes of Linearized Plots of Chemical Shifts and $\Delta\Delta G^\circ_{\beta\rightarrow\alpha}$ for *N*-(Glucosyl)imidazoles

cmpd	solvent	signal	K_a^{ax}/K_a^{eq}	$-\Delta\Delta G^\circ$, cal/mol
2a	D_2O	H1	0.520 ± 0.006	386 ± 7
2a	D_2O	H2′	0.530 ± 0.002	375 ± 3
2a	CD_3OD	H1	0.798 ± 0.004	134 ± 2
2a	CD_3OD	H2′	0.798 ± 0.002	133 ± 3
2a	DMSO-d_6	H1	0.970 ± 0.011	18 ± 7
2a	DMSO-d_6	H2′	0.965 ± 0.007	21 ± 4
2b	CD_3OD	H1	0.882 ± 0.004	74 ± 2
2b	CD_3OD	H2′	0.892 ± 0.002	68 ± 1
2b	DMSO-d_6	H1	0.803 ± 0.006	130 ± 4
2b	DMSO-d_6	H2′	0.801 ± 0.008	131 ± 6
2b	CD_2Cl_2	H2′	0.880 ± 0.004	69 ± 5
2c	D_2O	H1	0.798 ± 0.006	133 ± 4
		H4′	0.819 ± 0.007	118 ± 5
		H5′	0.812 ± 0.004	123 ± 3
		CH_3	0.801 ± 0.006	132 ± 5
2d	CD_3OD	H1	1.090 ± 0.005	−51 ± 2
		H4′	1.088 ± 0.005	−50 ± 3
		H5′	1.089 ± 0.002	−50 ± 1
		CH_3	1.091 ± 0.008	−51 ± 4
2d	DMSO-d_6	H1	1.097 ± 0.006	−55 ± 3
		H4′	1.087 ± 0.006	−49 ± 3
		H5′	1.097 ± 0.003	−55 ± 2
		CH_3	1.121 ± 0.014	−68 ± 7
2d	CD_2Cl_2	H5′	0.9979	1 ± 5
		CH_3	1.004 ± 0.005	−2 ± 3

Discussion

Δ*A* of Imidazolyl. The data in Table 5 show that Δ*A* (eq 4) of imidazolyl is uniformly positive. This result means that the repulsion energy of a protonated imidazolyl substituent in the axial position of a cyclohexane is greater than that of the unprotonated substituent. This is due solely to the positive charge, since the site of imidazole protonation is remote from the hydrogens on the cyclohexane. The size of the imidazolyl substituent does not change, but its effective size does, through the change of the solvation shell and perhaps through the decrease of the C1−N bond length.

This change in effective size is genuinely an effect of solvation, since it depends on solvent, being smallest in CD_2Cl_2. The positive Δ*A* in DMSO-d_6 refutes the apparent negative value derived from 4-phenylcyclohexylimidazole, where the need for correction for the proportion of ring-inverted conformer rendered Δ*A* less certain.[35]

All of the values in Table 5 are small. Earlier investigators were quite correct in their supposition that protonation at the distant nitrogen of an imidazole is not likely to increase its size. An increase is certainly detectable, but it is never greater than 0.1 kcal/mol for imidazole itself and reaches only 0.25 kcal/mol for 2-methylimidazole. This is much less than the 1.4 kcal/mol that corresponds to the change from 65% equatorial conformer to >95% on protonation of **1b**.[6] Thus the increased steric bulk of the protonated imidazole cannot account for this change.

In all solvents Δ*A* of 2-methylimidazolyl is greater than that of imidazolyl. This is not simply because of the steric bulk of the additional methyl, which presumably increases *A* itself. Instead it is because steric effects are intrinsically nonlinear. The positive charge creates additional demand for solvation of a substituent that is already bulkier because of the methyl.

Δ*G*° in Glycosylimidazoles. The observed $\Delta\Delta G^\circ_{\beta\rightarrow\alpha}$ in glycosylimidazoles is a composite of three possible contributions. There is a positive one from the increased steric bulk of

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Table 3. Slopes of Linearized Plots of Chemical Shifts and $\Delta\Delta G°_{\beta\rightarrow\alpha}$ for *N*-(Xylosyl)-imidazoles

cmpd	solvent	signal	K_a^{α}/K_a^{β}	$-\Delta\Delta G°$, cal/mol
1a	D_2O	H1	0.323 ± 0.010	668 ± 18
		H2′	0.3045 ± 0.006	703 ± 12
		H4′	0.309 ± 0.001	695 ± 1
		H5′	0.314 ± 0.004	685 ± 8
1a	CD_3OD	H1	0.410 ± 0.003	528 ± 4
		H2′	0.400 ± 0.001	542 ± 1
		H4′	0.401 ± 0.003	541 ± 4
		H5′	0.393 ± 0.003	553 ± 4
1b	CD_3OD	H2′	0.577 ± 0.012	326 ± 12
		H4′	0.585 ± 0.005	317 ± 5
		H5′	0.575 ± 0.015	328 ± 15
1b	DMSO-d_6	H1	0.440 ± 0.004	487 ± 5
		H2′	0.428 ± 0.001	502 ± 1
		H4′	0.438 ± 0.005	488 ± 7
		H5′	0.432 ± 0.003	497 ± 4
1b	acetone-d_6	H1	0.379 ± 0.003	575 ± 4
		H2′	0.396 ± 0.007	549 ± 11
		H4′	0.390 ± 0.006	557 ± 9
		H5′	0.414 ± 0.011	522 ± 15
1b	CD_2Cl_2	H2′	0.404 ± 0.007	536 ± 10
		H4′	0.409 ± 0.004	529 ± 5
		H5′	0.454 ± 0.010	467 ± 13
1c	D_2O	H1	0.484 ± 0.007	430 ± 8
		H4′	0.440 ± 0.005	486 ± 7
		H5′	0.427 ± 0.003	504 ± 5
1c	acetone-d_6	H1	0.687 ± 0.008	222 ± 7
		H4′	0.675 ± 0.012	232 ± 10
		H5′	0.648 ± 0.011	257 ± 10
1d	CD_3OD	H1	0.370 ± 0.002	589 ± 4
		H4′	0.361 ± 0.002	602 ± 3
		H5′	0.364 ± 0.002	598 ± 3
		CH_3	0.367 ± 0.002	594 ± 3
1d	DMSO-d_6	H1	0.484 ± 0.005	429 ± 7
		H4′	0.477 ± 0.004	438 ± 5
		H5′	0.480 ± 0.003	434 ± 4
		CH_3	0.487 ± 0.003	426 ± 3
1d	acetone-d_6	H1	0.449 ± 0.010	474 ± 13
		H4′	0.445 ± 0.009	479 ± 12
		H5′	0.454 ± 0.010	468 ± 13
1d	CD_2Cl_2	H4′	0.479 ± 0.002	436 ± 3
		H5′	0.435 ± 0.021	493 ± 29
		CH_3	0.410 ± 0.007	528 ± 9

Table 4. Slopes of Linearized Plots of H1 Chemical Shifts and $\Delta\Delta G°_{\beta\rightarrow\alpha}$ for *N*-(2-Deoxyglucosyl)imidazoles

cmpd	solvent	K_a^{ax}/K_a^{eq}	$-\Delta\Delta G°$, cal/mol
3a	D_2O	0.691 ± 0.010	219 ± 9
3b	CD_3OD	0.929 ± 0.013	44 ± 8
3b	DMSO-d_6	0.691 ± 0.010	219 ± 9
3b	acetone-d_6	0.843 ± 0.005	101 ± 4
3b	CD_2Cl_2	0.777 ± 0.007	150 ± 5
3c	D_2O	0.679 ± 0.013	229 ± 11
3d	CD_3OD	1.008 ± 0.021	−5 ± 12
3d	DMSO-d_6	1.002 ± 0.027	−1 ± 16
3d	acetone-d_6	0.878 ± 0.003	77 ± 2
3d	CD_2Cl_2	0.653 ± 0.006	252 ± 5

a protonated imidazolyl substituent, relative to unprotonated, owing to the need for solvation of the positive charge, as in cyclohexylimidazoles **4**. According to the values in Table 5, this contribution is expected to be small, especially for imidazole itself. There may be a further positive contribution from the RAE, which shifts the anomeric equilbrium toward β upon *N*-protonation. There may also be a negative contribution from enhancement of the ordinary AE, which shifts the equilibrium toward α. The net $\Delta\Delta G°$ is the sum of all of these contributions. The sign of $\Delta\Delta G°$ is then diagnostic of the relative importance of the steric and RAEs, as compared to the enhancement of the ordinary AE.

Table 5. Slopes of Linearized Plots of Chemical Shifts and ΔA_{Im} for *N*-(4-*tert*-Butylcyclohexyl)imidazoles

cmpd	solvent	signal	K_a^{ax}/K_a^{eq}	ΔA, cal/mol
4a	CD_3OD/D_2O	H1	1.181 ± 0.004	98 ± 2
4a	DMSO	H1	1.051 ± 0.003	30 ± 2
4a	CD_2Cl_2	H1	1.05 ± 0.03	29 ± 17
		H2′	1.028 ± 0.009	16 ± 5
4b	CD_3OD	H1	1.355 ± 0.010	180 ± 4
		H4′	1.334 ± 0.005	171 ± 2
		H5′	1.315 ± 0.010	162 ± 4
		CH_3	1.324 ± 0.006	166 ± 3
4b	DMSO-d_6	H1	1.539 ± 0.011	255 ± 4
		H4′	1.357 ± 0.048	181 ± 21
4b	CD_2Cl_2	H1	1.094 ± 0.007	53 ± 4
		H4′	1.091 ± 0.020	52 ± 11
		H5′	1.074 ± 0.016	42 ± 9

Table 6. Calculated Dihedral Angles in Glycosylimidazoles

cmpd	sugar	H1−C−C−H2	H4−C−C−$H5_{eq}$	H4−C−C−$H5_{ax}$
***β*-1a**	β-Xylo	176°	55°	175°
***β*-1a**·H^+	β-Xylo·H^+	178°	53°	173°
***β*-2a**	β-Gluco	178°	—	173°
***β*-2a**·H^+	β-Gluco·H^+	180°	—	172°
α-1a	α-Xylo	52°	54°	174°
α-1a·H^+	α-Xylo·H^+	49°	55°	174°
α-1b	α-Ac_3Xylo	53°	54°	177°
α-1b·H^+	α-Ac_3Xylo·H^+	54°	49°	168°
α-2a	α-Gluco	54°	—	172°
α-2a·H^+	α-Gluco·H^+	49°	—	177°
α-2b	α-Ac_4Gluco	52°	—	170°
α-2b·H^+	α-Ac_4Gluco·H^+	55°	—	166°

Xylosylimidazoles have the further complication of ring inversion, which was the basis for the original study.[6] The β anomer has its imidazole equatorial, but that of the α anomer is not necessarily axial, as implied in Scheme 1. Indeed, crystalline *N*-(tri-*O*-acetyl-α-xylopyranosyl)imidazole (**1bα**) takes the $^1C_4$ conformation, with imidazole equatorial and three acetoxy groups axial.[36] The conformational heterogeneity of the α anomer means that its observed K_a is not simply K_a^{ax}, as in Scheme 1, but $(K_a^{ax} + K_e^+K_a^{eq})/(1 + K_e^+)$,[35] where K_a^{eq} of the ring-inverted α anomer is assumed to be identical to that of the β. This represents a reduction of the magnitude of $\Delta\Delta G°$ relative to that for a conformationally fixed pair, so that the observed value is a lower limit to the extent to which the equilibrium shifts to axial on protonation. Fortunately this complication does not affect glucosylimidazole, since it can be estimated from the known *A* values of the substituents[24] that less than 6% of its α anomer is ring-inverted.

With very few exceptions the $\Delta\Delta G°$ values (eq 3) for all the glycosylimidazoles in Tables 2−4 are negative. Even though all of the values are small, they are highly accurate. Therefore we can reliably conclude that the α anomer is more basic than the β or, equivalently, that the anomeric equilibrium shifts toward the α anomer on protonation of the imidazole.

Reverse Anomeric Effect vs Enhancement of Normal Anomeric Effect. The sign of $\Delta\Delta G°$ permits us to distinguish whether the RAE or the enhancement of the ordinary AE is dominant. The negative values in Tables 2−4 represent a protonation-induced shift of the anomeric equilibrium toward the α anomer. Such results are not consistent with the RAE, according to which the equilibrium should have shifted toward β anomer. The results are consistent only with an enhancement of the normal AE. Regardless of whether the AE is interpreted in terms of n−σ* overlap or in terms of resonance,[1a] it ought

(36) Luger, P.; Kothe, G.; Paulsen, H. *Chem. Ber.* **1974**, *107*, 2626.

to be enhanced by protonation of the imidazole, which increases its electronegativity. This is exactly what we observe.

Steric effects do not account for these results. *N*-Protonation of an imidazolyl group increases its effective steric bulk because of the need for solvation of the ion, but the extent is small, according to Table 5. Besides, any such increase would represent a positive contribution to $\Delta\Delta G°$. It would reduce the proportion of α anomer, contrary to the increase that is seen. Therefore the protonated imidazolyl is subject to an enhanced AE, not the RAE.

The only exceptions are **2d** in CD_3OD and DMSO-d_6, where $\Delta\Delta G°_{\beta\rightarrow\alpha}$ is < 0, and **2d** in CD_2Cl_2 and **3d** in CD_3OD and DMSO-d_6, where $\Delta\Delta G°_{\beta\rightarrow\alpha}$ is very close to 0. These are all 2-methylimidazole derivatives, where ΔA is larger, according to the data for **4b** in Table 5. It is likely that this steric contribution is sufficient to offset the enhancement of the AE. Indeed, $\Delta\Delta G°$ is generally, but not always, larger for the 2-methylimidazole derivatives than for the parent. This is not consistent with molecular-orbital calculations that predicted a greater shift to axial on protonation of the 2-methylimidazole.[16]

These results do not agree with any of the claims of an increased proportion of the $^1C_4$ conformation on protonating pyranosylimidazoles. In methanol or DMSO there is no significant trend associated with acetylation, as had been claimed for some hexoses.[5] Both glucosylimidazoles and xylosylimidazoles show negative $\Delta\Delta G°$s, even though the latter represented the primary evidence for the RAE.[6] The similarity renders unnecessary the suggestion that the existence of the RAE in the latter is compatible with its absence in the former, owing to differing contributions to conformational energy.[7]

Solvent Effects. The data in Tables 2−4 show small variations with solvent. The only substantial divergence is that $\Delta\Delta G°$ for **2a** and perhaps **1a**, **1c**, and **2c** is more negative in water than in other solvents. Although an increase in solvent polarity usually leads to a decrease of the AE,[37] this effect of *N*-protonation corresponds to an enhancement of the AE, or a reduction of the RAE, in water. This is consistent with one calculation,[16] but not with another,[15] where $-NH_3^+$ solvation introduces an additional factor.

It was suggested that the unusually large negative value of $\Delta\Delta G°$ in water is due to an enhanced AE,[34] combined with a reduction of the monopole−dipole attraction that stabilizes the equatorial form and leads to a RAE.[9] In most solvents the net effect of the AE and the RAE, along with the slight increase in steric bulk due to *N*-protonation (Table 5), leads to the very small $\Delta\Delta G°_{\beta\rightarrow\alpha}$ values observed. In water the electrostatic interaction and the RAE are reduced, producing a more negative $\Delta\Delta G°_{\beta\rightarrow\alpha}$. This result is in agreement with that calculated[16] and is consistent with the conclusion that the RAE is of electrostatic origin, as originally proposed.[2] Indeed, this small solvent dependence may be the lone evidence in support of the RAE.

Does hydrogen bonding contribute to these results? For example, hydrogen bonding involving the 1-OH has been proposed to affect the relative basicities of the two anomers of 2-glucosylamine.[38] Here the 2-OH may donate a (weak) hydrogen bond to the imidazole, and it may preferentially favor one of the anomers. We sought to probe this by comparing the acetates, which have no hydrogen to donate and whose oxygens are geometrically incapable of accepting a hydrogen bond from the imidazolium group. Yet there is no distinct pattern discernible from the comparison of acetates with parent, $\Delta\Delta G°$ being slightly more negative for **2b** in CD_3OD or DMSO and substantially so for **1b** in CD_3OD, but not for **1d** in acetone.

For the deoxyglucosylimidazoles **3a**−**d**, $\Delta\Delta G°$ is slightly more negative (Table 4) than for the corresponding glucosylimidazole in nearly all solvents, with the exception of water. This may be due to an even greater enhancement of the normal AE, owing to the absence of the electron-withdrawing 2-OH.

Reliability of Conformational Shifts Deduced from Coupling Constants. Our findings are inconsistent with the RAE, in sharp contrast to earlier results.[5−7] We find that *N*-protonation does not shift the anomeric equilibrium toward β (equatorial imidazole), but rather toward α. The previous results led to the conclusion that *N*-protonation shifts the conformational equilibrium of the α anomer from 65% equatorial imidazole to >95%.[6]

However, it must be noted that neither those populations nor the shift of conformational equilibrium was determined from direct observation of the separate conformers at low temperature. Instead they were inferred by comparing coupling constants with those in model compounds. Even a re-examinaton that confirmed the previous results confirmed only the coupling constants.[7] It is true that coupling constants will change with the position of the conformational equilibrium, but this is not the only determinant. Coupling constants are also sensitive to substituent electronegativity,[39] which is different for imidazolyl and protonated imidazolyl from that for the acetamido and pyridinium substituents that were used as models. However, it must be acknowledged that we observe no significant changes in J_{12} on protonation of xylosylimidazoles, except for a slight diminution (or loss of resolution) in some α anomers.

Moreover, coupling constants can change if there are distortions from the ideal chair conformation, as might be expected with such bulky groups. The calculated geometries in Table 6 suggest that such changes can occur upon protonation of the imidazole. There is no significant change in the dihedral angles of the β anomers, which are rigid. In contrast, there is a small decrease in the H_1CCH_2 dihedral angle of α-xylosylimidazole and a larger decrease in the H_4CCH_{5ax} dihedral angle of its triacetate. With α-glucosylimidazole the former angle decreases and the latter increases, but these changes are reversed in the tetraacetate. All of these correspond to a flattening or twisting of the ring. The calculated changes are too small to account fully for the observed changes in coupling constants. This may be a defect of the parametrization, which includes CN torsions and the AE of an OCOC fragment but does not include any OCN.[40] The variations are an indication that the dihedral angles do change with protonation, as well as with substituent electronegativity. Therefore we conclude that conformational proportions derived from coupling constants in α-glycopyranosylimidazoles are not reliable.

It must be recognized that two different measures of the RAE are being invoked. Both are shifts of equilibrium in response to *N*-protonation. The earlier one was of the conformational equilibrium, which was claimed to shift toward equatorial. Ours is of the anomeric equilibrium, which shifts toward axial. Scheme 2 demonstrates the contradiction involved. The ring inversion at the top is described by $K_{inv} = [\alpha(^1\mathbf{C_4})]/[\alpha(^4\mathbf{C_1})]$. The anomeric equilibrium at the left is described by $K_{anom} = [\alpha(^4\mathbf{C_1})]/[\boldsymbol{\beta}]$. (Strictly $K_{anom}{}^{obs} = [\alpha]/[\boldsymbol{\beta}] = ([\alpha(^4\mathbf{C_1})]+[\alpha(^1\mathbf{C_4})])/[\boldsymbol{\beta}] = K_{anom}(1 + K_{inv})$, but this does not affect the argument.) The isomerization at the bottom, which exchanges all substituents except imidazole from equatorial to axial, is described by K_{isom}. The equilibrium at the right is an enantiomerization,

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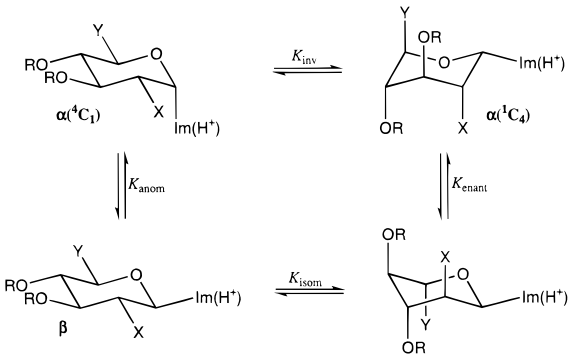
Scheme 2. Anomerization, Ring Inversion, and Isomerization Reactions of *N*-(Glycosyl)imidazolium Ions



for which $K_{enant} = 1$, unquestionably. Since this scheme too is a thermodynamic cycle, $K_{anom}K_{inv} = K_{isom}K_{enant} = K_{isom}$.

How do these equilibrium constants change upon *N*-protonation? According to the earlier evidence, $K_{inv}(H^+) > K_{inv}^0$, by >1.4 kcal/mol of free energy.[6] According to the data in Tables 2−4, $K_{anom}(H^+) > K_{anom}^0$, by up to 0.7 kcal/mol. From these two inequalities $K_{inv}(H^+)K_{anom}(H^+) > K_{inv}^0K_{anom}^0$, by ∼2 kcal/mol. But then the equality above requires $K_{isom}(H^+) > K_{isom}^0$, again by ∼2 kcal/mol. However, there is no AE on isomerization, so $K_{isom}(H^+)$ should equal K_{isom}^0. This contradicts the difference of 2 kcal/mol.

In view of this contradiction, we must judge the reliability of each of its parts. Our evidence on K_{anom} is indirect, from NMR titration, but secured by a thermodynamic cycle. The invariance of K_{isom} to protonation may not be exact, since there are steric interactions between Im and gauche X. Nevertheless these should be the same in both isomers and independent of protonation. They are certainly not responsible for a difference of 2 kcal/mol. Therefore we conclude that the earlier evidence on K_{inv} is not reliable. Above we have expressed doubts about this evidence, since it was based on coupling constants.

Of the two different measures of the RAE, we conclude that the change of K_{anom} is more reliable. Moreover, this represents a "simpler" equilibrium since only one group changes between axial and equatorial, whereas the previous study of K_{inv} requires all to change.

Conclusions

NMR titrations of a mixture of α and *β* anomers of *N*-(α-glycosyl)imidazoles **1**−**3** and their tri- or tetra-*O*-acetates, to measure the change of the anomeric equilibrium on protonation, give $\Delta\Delta G°_{\beta\rightarrow\alpha}$ values of −0.70 to +0.05 kcal/mol, corresponding, with rare exceptions, to a greater preference of the protonated substituent for the axial position than the neutral position. This counters the increased steric bulk of a protonated imidazole, as expressed by its Δ*A*. These Δ*A* values have been measured by a corresponding NMR titration of **4**, but they are quite small. The shift of anomeric equilibrium to axial on protonation is consistent with an enhancement of the normal AE, as expected from the increased electronegativity of the protonated imidazole. It is exactly opposite to what is claimed for the RAE. We conclude that previous evidence for this effect is not reliable. These results demonstrate the applicability and versatility of this methodology to examine chemical phenomena.

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