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# Reverse anomeric effect and steric hindrance to solvation of ionic groups

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Abstract: The proportions of axial anomers of various glucosylamines (**2**) and their conjugate acids were determined by $^1$H NMR. The change upon *N*-protonation is small and can be accounted for by steric effects, without any "reverse anomeric effect." To test whether *N*-protonation changes the steric bulk of an imidazolyl group, ring-inversion equilibria of *cis*-*N*-(4-alkylcyclohexyl)imidazoles (**3c**, **4c**) and of their conjugate acids were studied. The equatorial-to-axial free-energy change, $A_{Im}$ or $A_{ImH^+}$, is 2.2 ± 0.1 kcal/mol. To measure relative sizes more precisely, an NMR method was developed. The ratio of acidity constants of *cis* and *trans* **4** could be determined from chemical shifts as a 1:1 mixture was titrated. The *cis* isomer is 0.048 p*K* unit less basic, corresponding to a $\Delta A$ of 0.089 ± 0.004 kcal/mol, with protonated imidazolyl larger. To reinvestigate the effect of *N*-protonation on conformational equilibria in sugar derivatives, this NMR titration was applied to a mixture of α- and β-*N*-(glucosyl)imidazoles (**5**). The $\Delta A$ is -0.018 to -0.368 kcal/mol, exactly opposite to the reverse anomeric effect! This method has been applied to *t*-butylcyclohexanes **6** with acidic groups. Ions usually have a measurably lower preference for the axial position than do the corresponding neutrals.

**Introduction.** The favored chair conformer of a monosubstituted cyclohexane has its substituent equatorial. Were the substituent axial, it would suffer destabilizing steric repulsions with the axial hydrogens at C3 and C5. The *A* value, or free-energy difference between axial and equatorial conformers, as in eq 1, is

$$A = -RT\ln([\text{axial}]/[\text{equatorial}]) \quad (1)$$

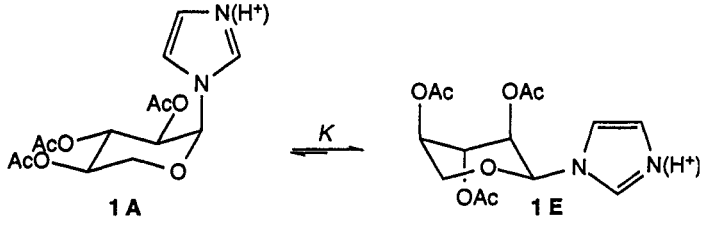
a quantitative measure of the effective size of a substituent (*1*). The topics addressed here involve how that conformational equilibrium may be modified by anomeric effects and by the need for solvation of ionic substituents.

Anomeric effects, especially with charged substituents, represent significant puzzles regarding molecular structure. They are important for understanding conformations of carbohydrates, simple organic heterocycles, and nucleosides and for understanding the reactivity of such molecules, which often react via their protonated forms as intermediates. More generally, the conformational behavior of charged substituents is a less well understood aspect of conformational analysis, and a new method for precise assessment of the effect of the charge can provide insight into the old problem of steric hindrance to solvation.

**Reverse Anomeric Effect.** Despite the generality of the anomeric effect (*2*) (the axial tendency exhibited by many electronegative groups at C1 of a tetrahydropyran or related heterocycle), some cationic groups, as in *N*-(α-glycosyl)pyridinium ions, prefer the equatorial position (*3*). This preference is called a "reverse anomeric effect", contributing as much as 1-3 kcal/mol to the stabilization of the equatorial form. However, all the substituents that produce this effect involve bulky aromatic rings, and the observations could be due simply to avoidance of prohibitive steric repulsions associated with placing that group axial.

A clever experiment was the study of conformational preferences of imidazolyl groups. Since protonation at the distant nitrogen is considered not to change the size of the group, an imidazolyl group provides its own control for steric factors. Indeed, on *N*-protonation or *N*-methylation of *N*-(tetra-*O*-acetyl-α-glucosyl) or -mannosyl)imidazole there is a shift toward the conformer with the imidazolyl group equatorial (*4*). More quantitatively, *N*-(tri-*O*-acetyl-α-xylopyranosyl)imidazole (**1**) exists as 65% equatorial conformer (**1E**) in $CDCl_3$, whereas in the presence of trifluoroacetic acid the proportion increases to >95% (*5*). This is a substantial change, corresponding to a free-energy change of >1.4 kcal/mol. If *N*-protonation does not change the size of the imidazolyl group, the shift of the equilibrium cannot be due to steric effects but must be presumed to be due to the positive charge. Such results have been accepted as the best evidence for the reverse anomeric effect. However, it must be recognized that the populations were not determined

from direct observation of the separate conformers but from small changes in coupling constants. Since these are sensitive to substituent and to slight conformational variations, they are difficult to interpret, and they may not provide reliable equilibrium constants.

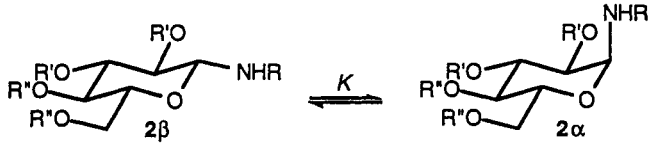



1A 1E

Various experimental and theoretical results have raised doubts about the reverse anomeric effect (*6*). The positive charge makes a nitrogen substituent even more electronegative, so that the anomeric effect ought to increase, according to the molecular-orbital/resonance interpretation. Only an electrostatic interpretation can account for a reverse anomeric effect (*1a*), although a recent suggestion (*7*) that electrostatics is more important for the ordinary anomeric effect is quite controversial.

The reverse anomeric effect is therefore suspect. The preference for equatorial conformer could be due to steric effects of pyridinium and imidazolium rings, which are too bulky to be accounted for reliably. Instead we seek a substituent whose steric sizes, both unprotonated and protonated, are known. Such a substituent is $NH_2$. From the conformational equilibria of cyclohexylamine and of its conjugate acid the *A* value of an $NH_2$ group is 1.6 kcal/mol in $D_2O$ or 1.4 kcal/mol in aprotic solvents, and the corresponding values for $NH_3^+$ are slightly larger, 1.9 and 1.6 kcal/mol (*8*).

We have accordingly studied various glucosylamine derivatives (**2**, R = H, Me, Et, and Bu, R' = H = R" or R' = Ac = R" or R' = H and (R")$_2$ = PhCH), along with their conjugate acids (*9*). Glucose was chosen because the four hydroxylic substituents maintain a chair conformation with these groups equatorial, leaving all the axial ring protons well upfield of H1, which is nicely isolated in the $^1$H NMR spectrum. In contrast to previous studies of the reverse anomeric effect, which involved ring inversion, this equilibrium involves simply the anomerization of the amino group from equatorial to axial. The *A* values of $NH_2$ and $NH_3^+$ provide a measure of the pure steric effect to be expected in this equilibrium. What is more relevant is that the slight but detectable increase in the *A* value of the amino group upon *N*-protonation is a measure of the change in steric repulsions arising from *N*-protonation. If a reverse anomeric effect is operative, then *N*-protonation should increase the proportion of the equatorial anomers of **2** by more than the increase in *A* values would predict. It is surprising that such a study had never been done before, but the difficulty in verifying the small amount of α anomer makes the reason clear.




2β 2α

By $^1$H NMR integration of representative signals we have measured the equilibrium proportions of the α anomer of each of these glucosylamines (**2**) not only under basic conditions but also under conditions acidic enough to protonate the amino group. Signals were assigned by standard methods involving chemical-shift correlations and coupling constants, and by comparisons with spectra of α and β glucose. Most characteristic of the α anomer is its H1 doublet, on the average 0.65 ppm downfield of H1 of the β anomer, and with an average $J_{12}$ of 5.1 Hz, smaller than the 8.7 Hz for the β. The proportion of α anomer is small, so the assignments were carefully confirmed by coupling constants, $^{13}$C spectra, a two-dimensional CH correlation spectrum, saturation transfer, reequilibration, and decoupling difference spectra. Moreover, coupling constants verify that not only are the β anomers chair conformers but also none of the α distort significantly from the chair, even though the amino substituent must be axial.

For the amines the proportion of α anomer varies from 3 to 21%. The lower proportions are in aqueous media, where solvation makes the $NH_2$ group slightly larger. The higher proportions are for the *N*-alkyl derivatives, probably owing to a greater entropy from conformations about the exocyclic C-N bond. The proportions can be converted to the free-energy change for conversion of β isomer to α. On the average the β anomers of the primary glucosylamines (**2**, R = H) are more stable than the α by 1.6 kcal/mol across a wide range of solvents. If the *N*-alkylglucosylamines (**2**, R = alkyl) are corrected for the conformational entropy, the β anomers are again more stable than the α, but by an average of 1.5 kcal/mol. Both of these values are close to $A_{NH_2}$ in cyclohexanes. This means that the preference for equatorial $NH_2$ or NHR in

glucosylamines is largely due to steric bulk. Actually, $A_{NH_2}$ in cyclohexanes is an underestimate, since the C-O bond is shorter than a C-C bond. By comparison of 2-alkyltetrahydropyrans with the corresponding alkylcyclohexanes (*10*) $A_{NH_2}$ on a pyranose can be corrected to 2-2.5 kcal/mol, depending on solvent. The observed preference in glucosylamines is thus lower, corresponding to a small normal anomeric effect.

Our inquiry concerns the glucosylammonium ions. The key result (*9*) is that even for the protonated amines the proportion of α anomer is appreciable, varying from 3 to 12%. Again there is slightly less in aqueous media owing to the greater bulk of aquated substituents, but no appreciable solvent effect. There is no difference associated with the tetraacetates, which might have different hydrogen-bonding properties than the others. The data show clearly that there is only a small shift of equilibrium upon *N*-protonation. Were a reverse anomeric effect operative, there would have been a large shift and very little protonated α anomer.

In terms of free energies the β anomer of the *N*-protonated glucosylamines is more stable than the α by an average of 2.0 kcal/mol in water and 1.5 kcal/mol in other solvents. This preference is close to that in the neutral glucosylamines. It is also close to $A_{NH_3^+}$, which is 1.6-1.9 kcal/mol, depending on solvent. Therefore just as for the glucosylamines the anomeric equilibria of their conjugate acids can be accounted for almost entirely by steric effects. Actually, with the correction for the shorter C-O bond of a tetrahydropyran $A_{NH_3^+}$ becomes 2.4-2.9 kcal/mol. Therefore the preference of the $NH_3^+$ or $NH_2R^+$ group for the equatorial position is actually smaller than would be expected on the basis of steric bulk. The extra preference for the axial position is a small but significant 1 kcal/mol. This represents a weak anomeric effect, but not a reverse anomeric effect!

This reasoning may be clearer in terms of concentrations. If steric repulsions alone were operative, the estimated proportion of α anomer of an *N*-protonated glucosylamine would be 0.8% in water or 1.7% in nonaqueous solution. If there were any reverse anomeric effect favoring the β anomer, the proportion would be even lower. Yet the average proportions are found to be significantly greater, 3.5% and 7.3%, respectively. Therefore we conclude that the reverse anomeric effect does not exist.

Another measure of any reverse anomeric effect is the difference in free energies between protonated and unprotonated glucosylamines. This is the extent to which *N*-protonation increases the preference of the amino substituent for the equatorial position. All the values are quite small, averaging 0.3 kcal/mol. This is quite close to $\Delta A$, the difference in *A* values of $NH_3^+$ and $NH_2$, which is 0.3 kcal/mol, or 0.4 kcal/mol for a tetrahydropyran. Therefore the shift in the anomeric equilibrium upon *N*-protonation can be accounted for simply by the increased steric demands of a protonated amino group. Alternatively, with correction for the conformational entropy of the *N*-alkylglucosylamines, the average difference in free energies is only 0.1 ± 0.1 kcal/mol, which is not significantly different from zero. This is even less than that expected from the increase in steric bulk. Even though $NH_3^+$ is certainly bulkier than $NH_2$ the proportion of axial isomer does not decrease on *N*-protonation. In opposition to the increased bulk there appears to be a slight extra tendency for cationic nitrogen to be axial, not equatorial. This corresponds to a small normal anomeric effect, and there is certainly no need to invoke a reverse anomeric effect.

How general is our conclusion that the reverse anomeric effect does not exist? *N*-Protonated glucosylamines are not the same as the original examples with quaternary nitrogens. However, the major difference between the two kinds of nitrogens is the possibility of hydrogen bonding with the *N*-protonated ones, and this does not seem to influence the anomeric equilibrium. Therefore we conclude that there is probably no reverse anomeric effect with any cationic nitrogen substituent.

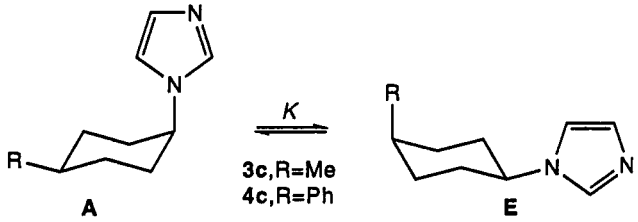
**Comparison of Effective Steric Sizes of Imidazolium and Imidazole.** This conclusion raises a question about the original evidence for the reverse anomeric effect (*5*). It was assumed that the steric requirements of the imidazolyl substituent do not change on protonation. Yet even though the distant proton itself does not add much bulk, introduction of a positive charge is likely to change the solvation shell about the substituent, and the associated counterion may also influence the equilibrium. Besides, the C1-N bond does shorten. All these effects ought to increase the effective size of the imidazolyl substituent. The assumption that there is no change is therefore worth testing.

What is known about $\Delta A$, the difference in *A* values of corresponding protonated and unprotonated substituents? To what extent does steric hindrance to solvation of an axial ion increase its *A* value? There are two conflicting comparisons. The first is that of $NH_2$ above, where *N*-protonation increases *A* by 0.2-0.3 kcal/mol, depending on solvent (*8*). However, part of this increase is due simply to the extra proton of $NH_3^+$, which makes it isosteric to $CH_3$, whereas $NH_2$ may direct its lone pair toward the axial hydrogens and avoid the 1,3-diaxial repulsions. Therefore in this case the energy cost of solvating the hindered cation must be less than 0.3 kcal/mol. The second comparison is of COOH and $CO_2^-$. According to p$K_a$s of stereoisomeric 4-*t*-butylcyclohexanecarboxylic acids (*11*), $A_{CO_2^-} - A_{COOH}$ is large, 0.7 kcal/mol, even though the lack of a proton might have reduced the steric repulsions of the anion.

An *N*-imidazolyl substituent is simpler than these two. It undergoes protonation at a distant atom and its charge is delocalized. This allows us to focus on the charge effect alone without introducing a steric effect due to the proton itself. Therefore we have chosen to evaluate the *A* values of imidazolyl and of *N*-protonated imidazolyl. To what extent does the necessity for solvation of the charge increase the size of this latter substituent? To what extent does such an increase depend on solvent?

Since the relatively large imidazolyl substituent strongly favors the equatorial position, the proportion of axial conformer in the parent cyclohexylimidazole may not be easily or reliably measured. A remedy is to use a *cis* 1,4-disubstituted cyclohexane, since the additional substituent makes the conformational equilibrium more balanced.

Accordingly we have measured the conformational equilibria for both *cis*-*N*-(4-methylcyclohexyl)-imidazole (**3c**) and *cis*-*N*-(4-phenylcyclohexyl)imidazole (**4c**), as well as for their *N*-protonated derivatives (*12*). These could be prepared from 4-methyl- or 4-phenyl-cyclohexanone plus thionyldiimidazole, to produce the *N*-(cyclohexenyl)imidazole, followed by catalytic hydrogenation and separation of the *cis* isomer if necessary. *N*-Protonation was achieved by addition of trifluoroacetic or *p*-toluenesulfonic acid.




As the temperature is lowered, the NMR signals broaden and below -80 °C each decoalesces into two signals with different intensities. For *cis*-*N*-(4-phenylcyclohexyl)imidazole (**4c**) the major isomer has its H1 signal near δ 4 and downfield of that of the minor isomer, and its H4 signal upfield of that of the minor isomer. Since axial protons are generally upfield of their equatorial counterparts, the major isomer can be identified as **4cA**, with imidazolyl axial and phenyl equatorial. In contrast, for *cis*-*N*-(4-methylcyclohexyl)-imidazole (**3c**) H1 of the major isomer is upfield of H1 of the minor isomer, and its H4 is downfield. Therefore for **3c** conformer **E**, with imidazolyl equatorial and methyl axial, is favored. Representative $^1H$ or $^{13}C$ NMR signals may then be integrated to provide the proportions of the two conformers, the equilibrium constant *K*, and $\Delta G^\circ$, the free-energy difference between conformers **A** and **E**. For **3c** $\Delta G^\circ$ is 0.45 kcal/mol; for **4c** it is -0.68 kcal/mol. Within experimental error these values are the same in either dichloromethane or methanol.

Since **3c** exists predominantly as conformer **E** whereas **4c** exists primarily as conformer **A**, the imidazolyl substituent must be larger than a methyl but smaller than a phenyl. That imidazolyl is larger than methyl is certainly not surprising. However, the observation that it is smaller than phenyl may be surprising inasmuch as the shorter C-N bonds might bring its ortho hydrogens closer to the hydrogens on the cyclohexane. On the other hand, the CNC angle in the five-membered ring is smaller than the ipso CCC angle of the phenyl. Indeed, molecular-mechanics (MMX) calculations (*12*) verify that the ortho hydrogens of an axial imidazolyl have longer distances to H2,6 on the cyclohexane and thus a lower steric destabilization.

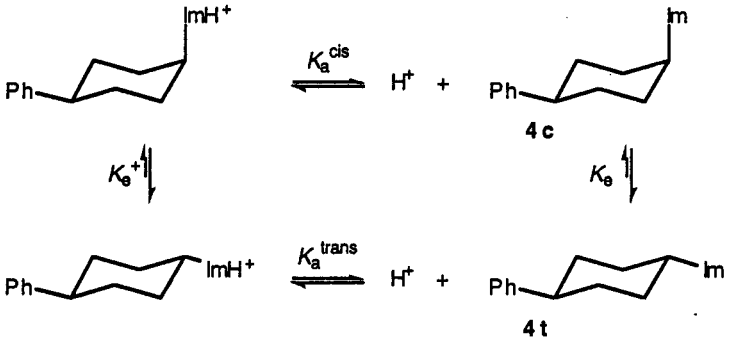
The observed free-energy difference between **A** and **E** conformers of **3c** or **4c** is the difference between the *A* values of imidazolyl and methyl or phenyl. Therefore from the known (*13*) $A_{Me}$ of 1.74 ± 0.07 kcal/mol and $A_{Ph}$ of 2.9 ± 0.1 kcal/mol it is possible to calculate the *A* value of the imidazolyl group or of its *N*-protonated form. The average *A* value for an imidazolyl group is 2.2 kcal/mol, from either **3c** or **4c** and independent of whether the solvent is dichloromethane or methanol. It should be noted that MMX parametrization would suggest that $A_{N\text{-Imidazolyl}}$ = 2.9 kcal/mol, which is an overestimate.

The real question is what happens in acid. The answer is that the conformational equilibria for **3c** and **4c** hardly change on going to acidic media. The *A* value of *N*-protonated imidazolyl is also 2.2 kcal/mol, regardless of solvent or counterion. Within experimental error the cationic substituent is the same size as the neutral. However, it must be admitted that the experimental error is a substantial 0.1 kcal/mol. We would like to reduce this error and obtain a better estimate of the difference in size between cation and neutral.

**Titration Method for Determining Steric Difference between Imidazolyl and Protonated Imidazolyl.** To measure more precisely the change in $A_{N\text{-Imidazolyl}}$ upon *N*-protonation, an NMR titration method was developed (*12*). A convenient feature of this method is that it is applicable to a mixture of isomers, without any necessity for separation. What makes it feasible is that the $^1H$ chemical shifts of H1 undergo sufficient change on *N*-protonation to permit NMR determination of the extent of

protonation, especially with a 500-MHz instrument. If one isomer is more basic than the other, then during a titration its spectral characteristics will change earlier than those of the other isomer.

To a 1.0-mL sample containing 51.4 mg of the 1:1 mixture of *cis* and *trans* *N*-(4-phenylcyclohexyl)-imidazoles and *t*-butyl alcohol or tetramethylsilane as internal standard, successive microliter portions of 3 M DCl in the same solvent were added. Solvents were 1:1 acetone-$d_6$/$D_2O$, $CD_2Cl_2$, and DMSO-$d_6$. The 500-MHz $^1H$ NMR chemical shifts of H1 in both isomers at 24.5 °C were recorded after each addition. The merit of this method is that the ratio of acidity constants of *cis* and *trans* *N*-(4-phenylcyclohexyl)imidazoles could then be determined from the variation of H1 chemical shifts as a 1:1 mixture was titrated with DCl. Those acidity constants are defined in Scheme 1.




Scheme 1. Acid Dissociations of *cis*- and *trans*- (4-phenylcyclohexyl)imidazolium ions.

What is readily measurable is $K$, the ratio of those acidity constants, related to concentrations of protonated and unprotonated forms as in eq 2. The observed chemical shift of the *cis* isomer **4c** is given by

$$K = \frac{K_a^{cis}}{K_a^{trans}} = \frac{[cis][trans{\cdot}H^+]}{[cis{\cdot}H^+][trans]} \tag{2}$$

eq 3, where $\delta_{CH^+}$ and $\delta_C$ are limiting chemical shifts of protonated and unprotonated forms, respectively,

$$\delta_{cis} = \frac{\delta_C[cis] + \delta_{CH^+}[cis{\cdot}H^+]}{[cis] + [cis{\cdot}H^+]} \tag{3}$$

which can be measured at the ends of the titration. A similar equation holds for $\delta_{trans}$, the chemical shift of the *trans* isomer, **4t**. Algebraic manipulations of eq 2, eq 3, and the equivalent equation for $\delta_{trans}$ lead to eq 4, which is nonlinear whenever $K \neq 1$. Such a plot is shown in Fig. 1a. There is a slight but systematic

$$\delta_{cis} = \delta_C + \frac{(\delta_{CH^+}-\delta_C)(\delta_{trans}-\delta_T)}{(1-K)(\delta_{trans}-\delta_T) + K(\delta_{TH^+}-\delta_T)} \tag{4}$$

upward curvature, indicating that the *trans* isomer is more readily protonated than the *cis*. However, this nonlinear equation is difficult to fit, and the fitting parameters can depend on the initial guesses. Alternative manipulations lead to the linearized form in eq 5, relating observed chemical shifts to the desired $K$. The

$$(\delta_{trans} - \delta_T)(\delta_{CH^+} - \delta_{cis}) = K(\delta_{cis} - \delta_C)(\delta_{TH^+} - \delta_{trans}) \tag{5}$$

simplicity of this expression means that a plot of $(\delta_{trans} - \delta_T)(\delta_{CH^+} - \delta_{cis})$ vs. $(\delta_{cis} - \delta_C)(\delta_{TH^+} - \delta_{trans})$ ought to be a straight line, with slope $K$ and zero intercept. (This may be a well-known equation for evaluating a ratio of acidity constants, but it was easier to derive it than to find it in the journals. The closest method that I know of is the NMR determination of isotope effects on acidity constants (*14*), but this requires accurate aliquots or pH measurement and a nonlinear fit.)

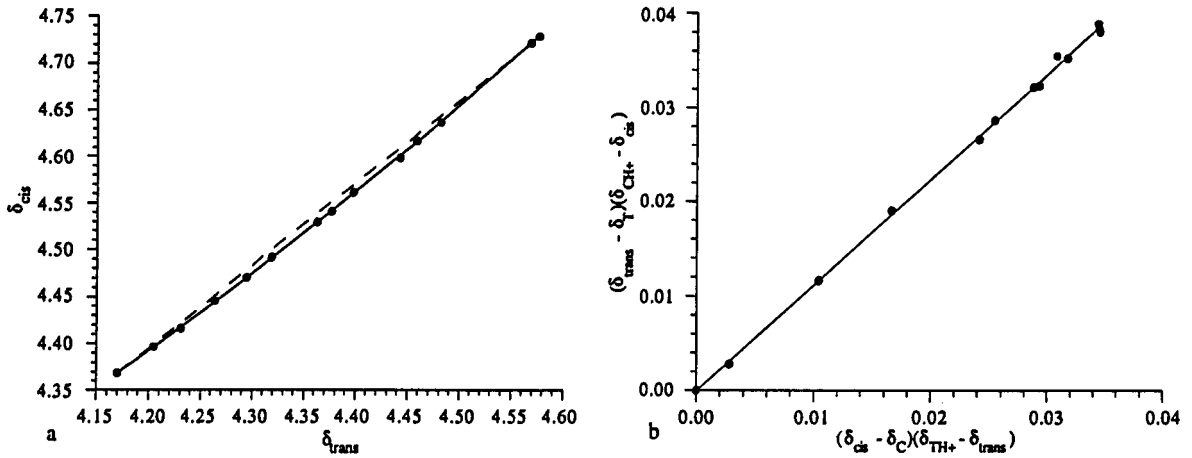



Fig. 1 Chemical shifts relative to TMS during titration of 1:1 mixture of *cis* and *trans* *N*-(4-phenylcyclohexyl)imidazoles (**4**) with DCl in 1:1 acetone-$d_6$/$D_2O$. (a) Nonlinear plot of $\delta_{cis}$ vs. $\delta_{trans}$ (linear - - - for comparison). (b) Linearized plot of $(\delta_{trans} - \delta_T)(\delta_{CH+} - \delta_{cis})$ vs. $(\delta_{cis} - \delta_C)(\delta_{TH+} - \delta_{trans})$.

Figure 1b shows such a plot. The excellent linearity is confirmed by a correlation coefficient of 0.9996. The slope is 1.122 ± 0.009, and the intercept is -0.0001 ± 0.0002, which is properly zero. A repeat titration using *t*-butyl alcohol as internal standard gave the same slope, 1.114 ± 0.006. The average is 1.118 ± 0.005. This slope is the ratio of acidity constants of *cis* and *trans* **4** and corresponds to a $\Delta pK_a$ = $-\log(K_a^{cis}/K_a^{trans})$ of -0.048 ± 0.002. In $CD_2Cl_2$ and DMSO-$d_6$ these slopes are 1.03 ± 0.02 and 0.89 ± 0.01, respectively. It is remarkable that this ratio of acidity constants can be measured with such precision, higher than the acidity constants themselves.

Scheme 1 is a thermodynamic cycle that relates the acid-dissociation equilibria of *cis*- and *trans*- *N*-(4-phenylcyclohexyl)imidazolium ions to the epimerization equilibrium between *cis*- and *trans*- *N*-(4-phenylcyclohexyl)imidazole **4c** and **4t** and to the corresponding equilibrium between their conjugate acids. These latter two equilibria have equilibrium constants, [axial imidazole]/[equatorial imidazole], designated as $K_e$ and $K_e^+$, respectively, in Scheme 1. These equilibrium constants are governed by the energy cost required to place an imidazolyl group in the axial position. Therefore they can be defined in terms of the free-energy differences between the *cis* and *trans* stereoisomers even though there is no catalyst that can equilibrate them. What we want is $\Delta A$, the difference in $A$ values between protonated and unprotonated imidazolyl groups. This is given in eq 6.

$$\Delta A = A_{N\text{-ImidazolylH}^+} - A_{N\text{-Imidazolyl}} = RT\ln(K_e/K_e^+) \quad (6)$$

It follows from the thermodynamic cycle of Scheme 1 that the desired ratio $K_e/K_e^+$ must equal $K_a^{cis}/K_a^{trans}$. But this is the $K$ that was obtained from the plots in Fig. 1. Actually, there is a small correction that must be made to account for the ring-inverted conformers of **4c** and of its conjugate acid, which contribute to $K_a^{cis}$. Then by correcting the above $K$, $K_e^+/K_e$ becomes 1.161 ± 0.008, corresponding to a $\Delta A$ of 0.089 ± 0.004 kcal/mol.

This result means that the repulsion energy of a protonated imidazolyl substituent in the axial position of a cyclohexane is detectably greater than that of the unprotonated. This is consistent with the previous conclusion that the repulsion energies are the same, within the experimental error of ±0.1 kcal/mol. This additional repulsion energy is an effect solely due to the positive charge, since the site of protonation of an *N*-cyclohexylimidazole is remote from the hydrogens on the cyclohexane. The size of the imidazolyl substituent does not change, but its effective size does.

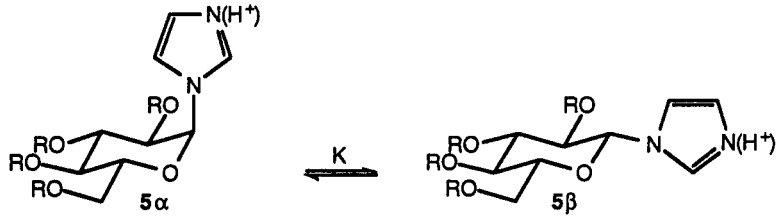
This change in effective size is genuinely a solvation phenomenon, since in nonaqueous solvents the change is different. In $CD_2Cl_2$ and DMSO-$d_6$ the above values for $K$ lead to $\Delta A$ of 0.024 ± 0.013 and -0.089 ± 0.009 kcal/mol, respectively. In $CD_2Cl_2$ the effect diminishes, and in DMSO-$d_6$ it reverses, with the protonated imidazolyl more stable in the axial position than is the unprotonated.

The changes in effective size on *N*-protonation of the imidazolyl substituent are not large. This result is reasonable since even $A_{NH_2}$ increases by only 0.3 kcal/mol upon *N*-protonation (*8*). The site of protonation of an *N*-cyclohexylimidazole is more remote than in cyclohexylamine, so the change of $A_{N\text{-Imidazolyl}}$ upon *N*-protonation must be smaller. Yet even this small change can be measured with remarkably high precision. Moreover, it can be measured without using the monosubstituted *N*-cyclohexylimidazole, without equilibrating the *cis* and *trans* isomers of *N*-(4-phenylcyclohexyl)imidazole, and without requiring ring inversion, which would place the phenyl axial.

**Reinvestigation of Glycosylimidazolium Ions and the Reverse Anomeric Effect.** Now that the change of steric bulk on *N*-protonation can be assessed, we can return to the evidence for the reverse anomeric effect, namely, the increase from 65% to 95% in the proportion of equatorial conformer upon *N*-protonation of *N*-(xylosyl)imidazole (*5*). We now admit that this cannot be attributed simply to an increased steric bulk of the substituent, since even the maximum 0.089 kcal/mol of additional steric repulsions is too small to account for this increase. There are no large solvation effects on the conformational equilibrium of imidazole derivatives, just as suggested by Paulsen and Lemieux, so that it would appear that the change is due to a reverse anomeric effect.

Nevertheless there is a contradiction, since no reverse anomeric effect was seen with glucosylamines. Yet it must be remembered that the populations of xylosylimidazole and the change of populations on *N*-protonation were determined from NMR coupling constants rather than from direct observation of the separate conformers at low temperature.

To reinvestigate the conformational equililbria in glycosylimidazoles we have undertaken to determine the effect of *N*-protonation on the anomeric equilibrium in *N*-(D-glucosyl)imidazole (**5**, R = H) and its tetra-*O*-acetyl derivative (**5**, R = Ac). In principle this could be evaluated from the change in the proportion of the β anomer on protonating an equilibrating mixture of anomers. However, glycosylimidazoles happen to be configurationally stable, so that they do not equilibrate (*15*). Fortunately the reverse anomeric effect can be evaluated by an alternative method, which measures the difference in p$K_a$ of the two anomers.




To measure with high precision this difference, we have used (*16*) the above NMR titration method, which is applicable to an anomeric mixture of sugar derivatives. *N*-(D-Glucosyl)imidazole (**5**, R = H) or its 2,3,4,6-tetraacetate (**5**, R = Ac) was readily prepared as a 1:2 mixture of α and β anomers. To the mixture, dissolved in an appropriate solvent, successive microliter portions of trifluoroacetic acid (TFA) in the same solvent were added. After each addition the $^1$H chemical shifts of H1 on the glucose ring and of H2' on the imidazole ring were recorded.

Again what is readily measurable is $K_a^{\alpha}/K_a^{\beta}$, the ratio of the acidity constants of α and β *N*-(glucosyl)-imidazolium ions. Scheme 1 and equations 1-6 are readily adapted to the observed chemical shifts $\delta_{\alpha}$ and $\delta_{\beta}$ of H1 or H2' and limiting chemical shifts $\delta_{\alpha}^{+}$, $\delta_{\alpha}^{o}$, $\delta_{\beta}^{+}$, and $\delta_{\beta}^{o}$ of protonated and unprotonated α and β forms. Thus a plot of $(\delta_{\beta} - \delta_{\beta}^{o})(\delta_{\alpha}^{+} - \delta_{\alpha})$ vs. $(\delta_{\alpha} - \delta_{\alpha}^{o})(\delta_{\beta}^{+} - \delta_{\beta})$ ought to be linear with zero intercept and with slope equal to $K_a^{\alpha}/K_a^{\beta}$. The experimental results from such plots are given in Table 1. Intercepts are all properly zero. The slopes $K_a^{\alpha}/K_a^{\beta}$ have been converted to $\Delta A$ according to eq 7. The values are the

$$\Delta A = A_{N\text{-ImidazolylH}^+} - A_{N\text{-Imidazolyl}} = RT\ln(K_e/K_e^{+}) = RT\ln(K_a^{\alpha}/K_a^{\beta}) \quad (7)$$

same from both H1 and H2', so we are indeed measuring the differential extent of protonation of α and β anomers, rather than some artifact of chemical shifts. It should be noted that these values are obtained without equilibrating the anomers and also without interchanging equatorial and axial imidazolyls via ring inversion. It is remarkable that this $\Delta A$ can be measured with such precision, higher than the *A* values themselves.

TABLE 1. Slope, intercept, and correlation coefficient ρ of linearized plots of chemical shifts (eq 5) and derived $\Delta A$ (eq 7), from titrations of mixture of α and β anomers of *N*-(glucosyl)imidazole (**5**, R = H) or its tetraacetate (**5**, R = Ac).

| R | Signal | Solvent | Slope | Intercept | ρ | $\Delta A$, kcal/mol |
|---|---|---|---|---|---|---|
| H | H1 | $D_2O$ | 0.520±0.006 | 0.00007±0.00004 | 0.99906 | -0.386±0.007 |
| H | H2' | $D_2O$ | 0.530±0.002 | 0.0001±0.0004 | 0.99993 | -0.375±0.003 |
| H | H1 | $CD_3OD$ | 0.798±0.002 | -0.0003±0.0007 | 0.99993 | -0.134±0.002 |
| H | H2' | $CD_3OD$ | 0.798±0.004 | -0.00007±0.00005 | 0.99979 | -0.133±0.003 |
| H | H1 | DMSO-$d_6$ | 0.970±0.012 | 0.0002±0.0002 | 0.99845 | -0.018±0.007 |
| H | H2' | DMSO-$d_6$ | 0.965±0.007 | 0.0015±0.0024 | 0.99937 | -0.021±0.004 |
| Ac | H1 | $CD_3OD$ | 0.882±0.004 | 0.00000±0.00003 | 0.99971 | -0.074±0.002 |
| Ac | H2' | $CD_3OD$ | 0.892±0.002 | -0.0003±0.0007 | 0.99989 | -0.068±0.001 |
| Ac | H1 | DMSO-$d_6$ | 0.803±0.006 | -0.00005±0.00004 | 0.99970 | -0.130±0.004 |
| Ac | H2' | DMSO-$d_6$ | 0.801±0.008 | 0.001±0.003 | 0.99942 | -0.131±0.006 |
| Ac | H2' | $CD_2Cl_2$ | 0.889±0.008 | -0.0000±0.0016 | 0.99939 | -0.069±0.005 |

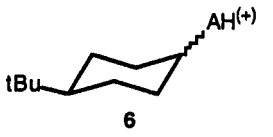
This $\Delta A$ is the difference in *A* values between protonated and unprotonated imidazolyl in these glycosylimidazoles, including both steric and anomeric effects. It represents the extra energy cost to place a protonated imidazolyl in the axial position of the α anomer, relative to the energy cost of an unprotonated imidazolyl. All the values are small but significantly less than zero. This means that there is a greater preference of the protonated imidazolyl group for the axial position than of the unprotonated. In other words, *N*-protonation does not shift the equilibrium toward an equatorial imidazolyl but instead it shifts the equilibrium toward axial. This is exactly opposite to what is expected from the reverse anomeric effect!

These results cannot be due merely to steric effects. The results above show that in aqueous acetone or dichloromethane *N*-protonation of an imidazolyl group on a cyclohexane increases its effective steric bulk. This increase ought to reduce the proportion of α anomer, contrary to what is seen. Therefore the protonated imidazolyl shows an enhanced anomeric effect, rather than a reverse anomeric effect. There are variations with solvent and with acetylation, but such small variations are more readily measured than interpreted.

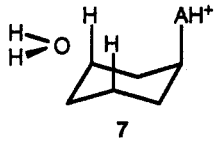
**Steric Hindrance to Solvation of Ionic Groups.** The NMR titration method is quite general. It permits the determination of the relative acidities of closely related species, including stereoisomeric ones. For axial and equatorial epimers the relative acidities can be converted to the extra energy cost required to place the charged substituent in the axial position of a cyclohexane. It is "extra" in that it is compared to the corresponding neutral, which has the same overall size, except for exchange of a proton for a lone pair. All that is needed to apply the method is a mixture, not necessarily 1:1, of the two stereoisomers, and a reporter nucleus whose chemical shift changes appreciably with the state of protonation.

It is thus possible to determine how the solvation of a wide range of charged substituents, both cationic and anionic, affects their size, as compared to that of the corresponding uncharged substituent. This is also equivalent to the extent to which the axial environment exerts a steric hindrance to solvation. Alternatively, a charged substituent might even favor the axial position, if the nearby groups provide an internal solvation. The roles of internal solvation and steric hindrance to solvation have been the key to understanding the relative basicities of the various methylamines (*17*).

We have applied this method to a series of 4-*t*-butylcyclohexane derivatives **6**. These are readily available as a mixture of *cis* and *trans* stereoisomers. The *t*-butyl group has the advantage over the phenyl that there is no need to apply a correction for the ring-inverted conformer of the *cis* form. Our preliminary data (*18*) are in Table 2, where $\Delta A$ is the *A* value of the ionic group minus that of the neutral.




**6**




**7**

These results are quite intriguing. The value for COOH agrees well with those obtained by direct titration of the individual 4-*t*-butylcyclohexanecarboxylic acids (*11*). The values for $NH_2$ and $N(CH_3)_2$ are somewhat smaller than the previous estimates (*8*), including the 0.3 kcal/mol that was repeatedly used above. The value for $N(CH_3)_2$ in protic medium is surprisingly small for a substituent that directs its lone pair toward the axial hydrogens but cannot avoid 1,3-diaxial repulsions on protonation. Values in DMSO are very different from those in protic solvents and sometimes even negative, so this is genuinely a solvation

TABLE 2. Extra energy, $\Delta A$, required to place ionic substituent $AH^+$ or $A^-$ axial

| $AH^{(+)}$ | T, °C | Solvent | $\Delta A$, kcal/mol |
|---|---|---|---|
| $NH_3^+$ | 15.0 | 3:1 $CD_3OD/D_2O$ | 0.165±0.004 |
| $NH_3^+$ | 22.3 | 3:1 $CD_3OD/D_2O$ | 0.170±0.009 |
| $NH_3^+$ | 24.5 | 3:1 $CD_3OD/D_2O$ | 0.174±0.004 |
| $NH_3^+$ | 32.5 | 3:1 $CD_3OD/D_2O$ | 0.184±0.008 |
| $NH_3^+$ | 40.2 | 3:1 $CD_3OD/D_2O$ | 0.191±0.005 |
| $NH_3^+$ | 56.7 | 3:1 $CD_3OD/D_2O$ | 0.214±0.006 |
| $NH_3^+$ | 30.0 | DMSO-$d_6$ | 0.326±0.012 |
| $NH(CH_3)_2^+$ | 24.5 | 3:1 $CD_3OD/D_2O$ | 0.083±0.007 |
| $NH(CH_3)_2^+$ | 24.5 | DMSO-$d_6$ | 1.111±0.008 |
| $NH_2Ph^+$ | 24.5 | 3:1 $CD_3OD/D_2O$ | 0.82±0.02 |
| COOH | 24.5 | 2:1 $CD_3OD/D_2O$ | 0.619±0.007 |
| COOH | 15.0 | 3:1 $CD_3OD/D_2O$ | 0.588±0.005 |
| COOH | 22.3 | 3:1 $CD_3OD/D_2O$ | 0.587±0.014 |
| COOH | 24.5 | 3:1 $CD_3OD/D_2O$[a] | 0.595±0.007 |
| COOH | 32.5 | 3:1 $CD_3OD/D_2O$ | 0.583±0.011 |
| COOH | 40.2 | 3:1 $CD_3OD/D_2O$ | 0.591±0.013 |
| COOH | 56.7 | 3:1 $CD_3OD/D_2O$ | 0.599±0.011 |
| COOH | 24.5 | $CD_3OD$ | 0.544±0.007 |
| COOH | 24.5 | DMSO-$d_6$ | -0.072±0.015 |
| SH | 24.5 | 3:1 $CD_3OD/D_2O$ | 0.908±0.013 |
| $NHSO_2Ph$ | 24.5 | 1:1 $(CD_3)_2CO/D_2O$ | 0.800±0.009 |
| $NHSO_2Ph$ | 24.5 | DMSO-$d_6$ | 0.02±0.01 |
| $NHSO_2p$Tol | 24.5 | 1:1 $(CD_3)_2CO/D_2O$ | 0.688±0.006 |

[a]Sodium counterion (others potassium).

phenomenon. However, all these data are so precise that they provide such small changes as are probably beyond our ability to interpret.

It is protic solvents that are most interesting, and the data suggest that there is a remarkable dependence on charge type, namely that except for $NH_2Ph^+$ $\Delta A$ seems to be larger for anions than for cations. Certainly the large $\Delta A$ for SH or $NHSO_2Ar$ is quite striking. The large $\Delta A$ for NHPh is probably a special case, since the phenyl is aligned so as to overlap with the lone pair on the nitrogen, and on protonation it rotates and changes its interactions with the cyclohexane hydrogens.

The temperature dependences correspond to $\Delta\Delta H^\circ$ = 0.51 kcal/mol and $\Delta\Delta S^\circ$ = -0.06 cal/mol-deg for COOH and -0.18 kcal/mol and -1.2 cal/mol-deg, respectively, for $NH_3^+$. These values mean that the difference between these two lies more in the enthalpy than in the entropy. This is unusual for a solvation phenomenon. The negative $\Delta\Delta H^\circ$ is also unusual, although in DMSO it is positive for the ammonium ions.

The possible difference between cations and anions in protic solvents deserves further study. According to simple electrostatics, positive and negative charges are equivalent, so a continuum dielectric cannot account for the difference. The general direction is opposite to what might have been expected, since cations are ordinarily smaller than anions and demand greater solvation. Our current hypothesis is that this is a consequence of the "hydrophobic interaction", which orients protons away from a hydrocarbon surface (7). If so, a solvent molecule can still stabilize a positive charge even though these are separated by a region of low dielectric constant, but it cannot solvate the negative charge as well. To test this hypothesis it is still necessary to investigate additional groups. Molecular-mechanics simulations may also be able to reproduce these solvent effects and thereby provide some insight into their origin.

**Summary & Conclusions.** The proportions of axial ($\alpha$) anomers of several *N*-alkylglucosylamine derivatives were determined by $^1H$ NMR in a variety of solvents, including acidic media. Assignments were confirmed by coupling constants, saturation transfer, reequilibration, and decoupling difference spectra. The proportions of axial anomers of the neutral amines can be accounted for simply on the basis of the steric bulk of $NH_2$ and NHR substituents. The reduction in the proportion of axial anomers that occurs upon *N*-protonation is also small. It can be accounted for largely, but not entirely, on the basis of the slightly greater steric bulk of solvated $NH_3^+$ and $NH_2R^+$ substituents. However, there is also a small enhancement of the normal anomeric effect. There is no increased tendency, beyond that due to a greater steric bulk, for a positively charged substituents to prefer the equatorial position. Therefore we conclude that the so-called reverse anomeric effect does not exist.

The *A* value of an *N*-imidazolyl group is 2.2 ± 0.1 kcal/mol, intermediate between that of methyl and phenyl. The *A* value is solvent independent. The *A* value of a protonated imidazolyl is 2.2 ± 0.1 kcal/mol. Thus within experimental error, there is no change in the *A* value of an imidazolyl group upon *N*-protonation. However, by a more accurate NMR titration method the protonated imidazolyl substituent is detectably "larger" than the unprotonated. In aqueous acetone the additional energy required to place the protonated substituent axial is 0.089 ± 0.004 kcal/mol. In $CD_2Cl_2$ this energy is lower, and in DMSO-$d_6$ it reverses.

This NMR titration method has been used to measure with high precision the shift of anomeric equilibrium on protonation of *N*-(glucosyl)imidazole and its tetraacetate. We find a $\Delta A$ of -0.018 to -0.368 kcal/mol. This means that the protonated imidazolyl group has a small but significantly greater preference for the axial position than does the unprotonated, which is exactly opposite to what is claimed for the reverse anomeric effect.

The NMR titration method is a powerful one for precise determination of small differences in acid-dissociation constants or of small differences in conformational equilibria between protonated and corresponding unprotonated substituents. In a series of *t*-butylcyclohexane derivatives it provides a quantitative measure of the steric hindrance to solvation of charged axial substituents, but further research is necessary.

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