Conformational Analysis of Glucopyranosylammonium Ions: Does the Reverse Anomeric Effect Exist?

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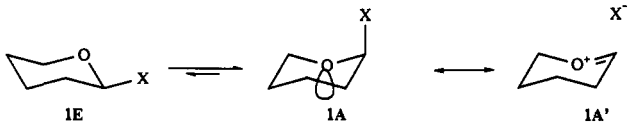
Abstract: Despite the generality of the anomeric effect (the axial tendency exhibited by many electronegative groups at C1 of a tetrahydropyran), some cationic substituents prefer the equatorial position. This preference is claimed to exceed that due to steric repulsion, and it has been called a "reverse anomeric effect". Such an effect is sometimes invoked to account for relative reactivities and stereoselectivities when there are cationic leaving groups. However, all such substituents have been bulky aromatic rings whose steric factors are difficult to judge, and it is advantageous to compare NHR and NH_2R^+ groups of known axial preference. Therefore the proportions of axial anomers of glucopyranosylamine and some of its *N*-alkyl, tetra-*O*-acetyl, and 4,6-benzylidene derivatives were determined by ^1H NMR in a variety of solvents, including acidic media. This proportion is small, and the assignments were confirmed by coupling constants, saturation transfer, reequilibration, and decoupling difference spectroscopy. The data indicate that the shift in the position of equilibrium that occurs upon N-protonation is small and can be accounted for on the basis of steric effects and a small normal anomeric effect. Therefore we conclude that the reverse anomeric effect does not exist.

Introduction

Reverse Anomeric Effect. The anomeric effect[1] is the tendency shown by tetrahydropyranyl derivatives **1** with an electronegative group X at C1 to take the axial conformer **1A**. It is the preference opposing that due to the steric repulsion, which creates a preference for the equatorial conformer **1E**. The quantitative relation is given in eq 1,

$$E_{An} = -RT \ln ([\mathbf{1E}]/[\mathbf{1A}])_{obs} + A_X \qquad (1)$$

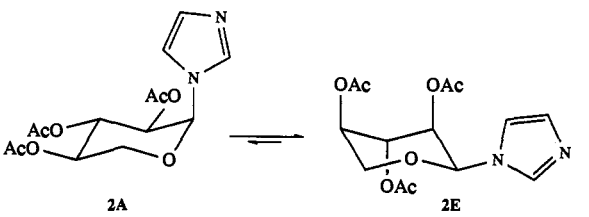
where E_{An} is the preference (energy > 0) for the axial position due to the anomeric effect and A_X is the steric preference for equatorial X as measured in a model compound such as a cyclohexane. However, when X is a positively charged nitrogen substitutent, the conformational preference is reversed so that the equilibrium lies toward **1E**. This has been attributed to a so-called reverse anomeric effect, contributing as much as 4–12 kJ mol^{-1} to the stabilization of the equatorial form.



1E 1A 1A'

The first examples of a reverse anomeric effect were the conformational equilibria of *N*-(α-glucopyranosyl)-4-methylpyridinium ion and *N*-(α-D-glucopyranosyl)- and *N*-(α-D-mannopyranosyl)imidazolium ions, in which the cationic heterocycle is equatorial despite the consequence that other substituent groups must be axial.[2] Similar behavior is shown by other such ions, although some may take a twist-boat conformation.[3] However, an aromatic heterocycle is quite bulky, especially with a positive charge that must be solvated, and all the results could be due simply to avoidance of prohibitive steric repulsions associated with placing that group in the axial position.

Yet it is claimed that the conformational preference in protonated *N*-(tri-*O*-acetyl-α-D-xylopyranosyl)imidazole **2** is greater than can be attributed to steric factors.[4] In $CDCl_3$ the steric bulk of the imidazolyl group favors conformer **2E** by 65:35 over **2A**. In the presence of trifluoroacetic acid the proportion of **2E** increases to >95%. Since protonation is considered not to change the size of the substituent, the shift of the equilibrium is attributed to the positive charge. However, protonation might change the bond length or the solvation, so that the effective size of the substituent may increase by more than just the size of a distant hydrogen.



2A 2E

The anomeric effect itself has been explained as the result of either dipole–dipole interactions or molecular-orbital interactions.[1] Orbital overlap between an oxygen lone pair (n) and the C–X antibonding (σ^*) orbital is a stabilizing interaction and is most effective in the axial conformer, where a lone pair is antiperiplanar to the C–X bond. This is the MO equivalent of including the resonance form **1A'**, which is not possible for **1E**, where the *p* orbital on oxygen is orthogonal to the C–X bond. Alternatively, according to the electrostatic interpretation, repulsion between the dipoles of the oxygen lone pairs and of the C–X bond is minimized in the axial conformer. Both of these kinds of interactions are probably operative, but the molecular-orbital interpretation is currently favored.[5]

Yet the reverse anomeric effect is better accounted for by electrostatics. Electrostatic forces do reverse upon introduction

(1) (a) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer: New York, 1983. (b) Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019.

(2) Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2205. Lemieux, R. U. *Pure Appl. Chem.* **1971**, *25*, 527.

(3) (a) Finch, P.; Nagpurkar, A. G. *Carbohydr. Res.* **1976**, *49*, 275. (b) Hosie, L.; Marshall, P. J.; Sinnott, M. L. *J. Chem. Soc., Perkin Trans. II* **1984**, 1121. (c) Dauben, W. G.; Köhler, P. *Carbohydr. Res.* **1990**, *203*, 47.

(4) Paulsen, H.; Györgydeák, Z.; Friedmann, M. *Chem. Ber.* **1974**, *107*, 1590.

(5) Praly, J.-P.; Lemieux, R. U. *Can. J. Chem.* **1987**, *65*, 213.

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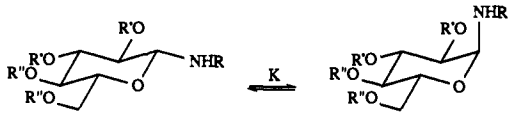
of a positive charge. This has been attributed to a reversal of the dipole moment of the C–N bond,[2] but it must be remembered that dipole moment is undefined when there is net charge, since it is not invariant to a change of origin.[6] Instead, it is proper to recognize that monopole–dipole attraction is maximal with the substituent in an equatorial position, where the positive charge is closer to the negative end of the dipole. Thus can the equatorial conformer be stabilized.

Question. The inability of the molecular–orbital or resonance interpretation to account for the reverse anomeric effect has been noted.[1a] The introduction of a positive charge makes a nitrogen substituent even more electronegative[3a] (not "electropositive"[7] or "less electronegative"[8] as has been stated in attempts to rationalize the reverse anomeric effect). Then resonance form **1A′** ought to contribute more and the anomeric effect ought to increase. Alternatively, the energy of the σ^* orbital is lowered so that it interacts more strongly with the n orbital. Since n–σ^* interaction is stabilizing, the proportion of axial conformer ought to increase, but that is not what is seen experimentally.

There is considerable evidence counter to the reverse anomeric effect. Ab initio calculations[9] on $HOCH_2OH_2^+$ and H_2NCH_2-NH_3^+ seem to support a reverse anomeric effect, but the stabilization of their synperiplanar conformers is probably due simply to hydrogen bonding. Indeed, calculations on $(HO)_2$-$CHNH_3^+$ show conformational stabilities that reflect hydrogen bonding but also show characteristic lengthening of the C–N bond when it is antiperiplanar to two oxygen lone pairs.[10] More recent calculations on various systems[11] show no evidence for a reverse anomeric effect. Also, reaction of 1-pent-4-enyl-D-glucopyranoside with *N*-bromosuccinimide in acetonitrile leads exclusively to *N*-(α-D-glucopyranosyl)acetamide,[12] consistent with a kinetic anomeric effect but not with a reverse anomeric effect. No reverse anomeric effect is seen in 2-(triphenylphosphonio)-1,3-dithiane[13] or in the crystal structure of a furanosylammonium ion.[14]

The previous interpretation of the conformational behavior of carbohydrates with cationic nitrogen substituents is therefore suspect. Since both steric and reverse-anomeric effects favor the equatorial conformer, it is essential to quantify steric effects. Pyridinium and imidazolium rings are too bulky to provide a reliable measure. We need a substituent whose steric sizes, both unprotonated and protonated, are known.

We have chosen to study the anomeric equilibrium in various glucopyranosylamines, with R′ = H = R″ (**3**) or R′ = Ac = R″ (**4**) or R′ = H and $(R'')_2$ = PhCH (**5**) and with R = H, Me, Et, and Bu, both unprotonated and protonated. Nuclear magnetic resonance (NMR) methods are quite powerful in such studies.[15] Glucose derivatives were chosen because these are most readily available, and the hydroxymethyl and three hydroxy substituents help maintain a chair conformation with all these groups equatorial. A further advantage is that the ^{1}H NMR chemical shifts of all the axial ring protons are well upfield of H1, which is nicely isolated in the spectrum. The tetraacetate was chosen to permit solubility in nonaqueous solvents, and the benzylidene acetal fixes the ring against ring inversion. In contrast to the previous studies of the reverse anomeric effect, which involved ring inversion, this equilibrium involves only the conversion of the amino substituent from equatorial to axial while all other substituents remain equatorial.



β R = H, Me, Et, Bu (±H⁺) α
3, R′ = H = R″
4, R′ = Ac = R″
5, R′ = H, $(R'')_2$ = PhCH

An estimate of the steric demands of NH_2 and NH_3^+ groups can be made from the conformational equilibria of cyclohexylamine and its conjugate acid. The *A* values, free-energy differences between equatorial and axial conformers, for both NH_2 and NH_3^+ have been determined.[16] These *A* values provide a measure of the steric effect on the anomeric equilibria in unprotonated and protonated glucopyranosylamines. Moreover, the slight but detectable increase in the *A* value of the amino group upon N–protonation is an estimate of the steric contribution to the shift in the anomeric equilibrium of glucopyranosylamine upon N–protonation. If a reverse anomeric effect exists, then N–protonation should increase the proportion of the equatorial anomers of **3–5** by more than the increase in *A* values would suggest.

The reverse anomeric effect represents a significant puzzle regarding molecular structure. It is important for our understanding of the conformations of carbohydrates, simple organic heterocycles, and nucleosides. It is also necessary for understanding the reactivity of such molecules, which often react via their protonated forms as intermediates. Certainly the preference for the β anomer of glycosyl onium ions allows the synthesis of α glycosides by S_N2 reaction.[7,17] A reverse anomeric effect is sometimes invoked to account for relative reactivities and stereoselectivities when there are cationic leaving groups[18] or to assign the product as equatorially substituted,[19] and this has been shown[12] to lead to error.

We have measured the equilibrium distribution of α and β anomers of glucopyranosylamine (**3a**), some of its *N*-alkyl (**3b–d**) derivatives, and its tetra-*O*-acetyl (**4**) and 4,6-*O*-benzylidene (**5a,b**) derivatives by ^{1}H NMR. The relative concentrations of the two anomers were measured through integration of representative signals not only under basic conditions but also under conditions acidic enough that the amino substituent was entirely protonated. It is surprising that such a study had never been done before. The closest are ones on 2-aminotetrahydropyrans (**1**, X = $N(CH_3)_2$, $NHCH_3$, or $NHCOCH_3$), which found 0–15% of **1A** at room temperature,[20] and another on glycosylaminoguanidinium ions, where only the equatorial conformer could be seen.[21] No quantitative studies on conjugate acids have been

(6) Daniels, F.; Williams, J. W.; Bender, P.; Alberty, R. A.; Cornwell, C. D. *Experimental Physical Chemistry*, 6th ed.; McGraw-Hill: New York, 1962; p 212.
(7) West, A. C.; Schuerch, C. *J. Am. Chem. Soc.* **1973**, *95*, 1333. Bailey, W. F.; Eliel, E. L. *J. Am. Chem. Soc.* **1974**, *96*, 1798.
(8) David, S.; Eisenstein, O.; Hehre, W. J.; Salem, L.; Hoffmann, R. *J. Am. Chem. Soc.* **1973**, *95*, 3806.
(9) Wipff, G. *Tetrahedron Lett.* **1978**, 3269. Kost, D.; Raban, M. *J. Am. Chem. Soc.* **1982**, *104*, 2960. Woods, R. J.; Szarek, W. A.; Smith, V. H., Jr. *J. Chem. Soc., Chem. Commun.* **1991**, 334.
(10) Lehn, J.-M.; Wipff, G. *J. Am. Chem. Soc.* **1980**, *102*, 1347.
(11) Anders, E.; Markus, F.; Meske, H.; Tropsch, J.; Maas, G. *Chem. Ber.* **1987**, *120*, 735. Wu, Y.-D.; Houk, K. N. *J. Am. Chem. Soc.* **1991**, *113*, 2353. Andrews, C. W.; Fraser-Reid, B.; Bowen, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 8293.
(12) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans. I* **1990**, 747.
(13) Mikolajczyk, M.; Graczyk, P.; Wieczorek, M. W.; Bujacz, G. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 578.
(14) Noe, C. R.; Knollmüller, M.; Steinbauer, G.; Jangg, E.; Völlenkle, H. *Chem. Ber.* **1988**, *121*, 1231.
(15) Inch, T. D. *Annu. Rev. NMR Spectrosc.* **1969**, *2*, 35.
(16) (a) Batchelor, J. G. *J. Chem. Soc., Perkin Trans. II* **1976**, 1585. (b) Booth, H.; Jozefowicz, M. L. *J. Chem. Soc., Perkin Trans. II* **1976**, 895.
(17) Kawakami, H. et al. *Chem. Lett.* **1989**, 1365. Mereyala, H. B.; Reddy, G. V. *Tetrahedron* **1991**, *47*, 6435.
(18) Sallam, M. A. E.; Whistler, R. L.; Markley, J. L. *Carbohydr. Res.* **1980**, *87*, 87. Taira, K.; Fanni, T.; Gorenstein, D. G. *J. Org. Chem.* **1984**, *49*, 4531. Bennet, A. J.; Sinnott, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 7287.
(19) Pougny, J.-R.; Sinaÿ, P. *Tetrahedron Lett.* **1976**, 4073. Schmidt, R. R.; Michel, J. *J. Carbohydr. Chem.* **1985**, *4*, 141.
(20) Tesse, J.; Glacet, C.; Couturier, D. *C. R. Acad. Sci., Paris* **1975**, *C280*, 1525. Zefirov, N. S.; Shekhtman, N. M. *J. Org. Chem. USSR (Engl. Transl.)* **1970**, *6*, 863. Booth, H.; Khedhair, K. A.; Readshaw, S. A. *Tetrahedron* **1987**, *43*, 4699.
(21) Szilágyi, L.; Györgydeák, Z.; Duddeck, H. *Carbohydr. Res.* **1986**, *158*, 67.

reported. The difficulties are the sensitivity of an aminal (**1**, X = NR_2) to hydrolysis in acid and the low concentration of its axial stereoisomer.

Experimental Section

Preparation of Glucopyranosylamines. All reagents and solvents were obtained commercially and used without further purification. Glucose was obtained from Merck, anhydrous ammonia from Linde (Union Carbide), methylamine (40% aqueous) from Fisher, ethylamine (70% aqueous), 4,6-*O*-benzylidene-α-D-glucose and tetra-*O*-acetyl-α-glucopyranosyl bromide from Sigma, and sodium azide, butylamine, and trifluoroacetic acid (TFA) from Aldrich. Dry methanolic methylamine was prepared by dropping the 40% solution onto NaOH pellets and directing the resulting gas through a CaO drying tube and into ice-cooled methanol. Deuterated solvents pyridine-d_5 and dimethyl sulfoxide-d_6 were purchased from Cambridge Isotope Labs, and DCl in D_2O (37%), ND_3 in D_2O (27%), chloroform-*d*, and D_2O were from Aldrich.

All of the glucopyranosylamine derivatives were made according to known procedures, from D-glucose or 4,6-*O*-benzylidene-D-glucose and the amine or by hydrogenating tetra-*O*-acetyl-β-glucopyranosyl azide:[22-27] D-glucopyranosylamine (**3a**), mp 125–127 °C (lit.[22] 125–127 °C); *N*-methyl-D-glucopyranosylamine (**3b**), mp 73–75 °C (lit.[23] 74–76 °C); *N*-ethyl-D-glucopyranosylamine (**3c**), mp 82–83 °C (lit.[24] 77–78 °C); *N*-butyl-D-glucopyranosylamine (**3d**), mp 85–87 °C (lit.[24] 87–89 °C), CMR δ 90.8 (major), δ 86.9 (minor) in DMSO-d_6; 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosylamine (**4**); mp 122–124 °C (lit.[25] 126 °C); 4,6-*O*-benzylidene-D-glucopyranosylamine (**5a**), mp 160–163 °C (lit.[26] 166–168 °C); 4,6-*O*-benzylidene-*N*-butyl-D-glucopyranosylamine (**5b**), mp 113–116 °C (lit.[27] 110 °C). D-Glucopyranosylamine-1-^{13}C was prepared from anhydrous ammonia and 12 mg of D-glucose-1-^{13}C (Isotech, 98.9 atom %) plus 50 mg of unlabeled D-glucose in methanol: mp 122–125 °C, CMR δ 85.7 (major), δ 81.9 (minor) in ND_3-D_2O, δ 82.0 (major), δ 81.1 (minor) in DCl-D_2O.

Sample Preparation. Aqueous samples (0.3 M) were prepared by the addition of 15–35 mg of the glucopyranosylamine to 0.7 mL of D_2O. Additional ammonia or *N*-alkylamine (20–50 μL) was added to inhibit hydrolysis and dimerization and to shift the HOD resonance away from any relevant ^{1}H NMR signals. In some cases minimization of the HOD peak required O,N-deuterated glucopyranosylamine, prepared by lyophilization from 10 volumes of D_2O. Aqueous pyridine was 1:1 (v/v) pyridine-d_5/D_2O. All aqueous samples included 10 μL of DMSO as internal standard (δ 2.49).

Acidic samples were ca. 0.7 M in DCl and ca. 0.3 M in the glucopyranosylamine. Catalysis of the anomerization of *N*-methylglucopyranosylamine was tested with a 2 M 1:1 $NaDCO_3/Na_2CO_3$ buffer 2 M in $MeND_2$.

Nonaqueous samples were 0.05–0.3 M in glucopyranosylamine. Most of the nonacidified samples contained ca. 20 μL of either ammonia or *N*-alkylamine, except for tetra-*O*-acetylglucopyranosylamine, which would be deacetylated. Acidic samples were 0.1–0.4 M, prepared by addition of the glucopyranosylamine to excess TFA or DCl/D_2O in 0.7 mL of solvent. A residual proton signal of the solvent was used as internal standard (DMSO-d_5, δ 2.49; $CHCl_3$, δ 7.26; C_6D_5H, δ 7.15; pyridine-d_4, δ 8.60).

General NMR Methods. Experiments were carried out on either a GE QE-300 NMR spectrometer (300 MHz ^{1}H, 75 MHz ^{13}C) or a Varian Unity-500 spectrometer (500 MHz ^{1}H, 125 MHz ^{13}C).

Each compound was characterized by ^{1}H NMR. Integrations were done at 25 °C, except when coincidence with a broadened signal from an exchangeable proton in the molecule required 40 °C, which sharpened the interfering signal and shifted it away from the peak being measured. The relative proportions of the two anomers were usually measured by integration of the characteristic ^{1}H NMR signals from H1 of each anomer. In some cases, where an H1 signal overlaps other signals in the spectrum, the resolved, isolated signal of a different ring proton was integrated instead. A linear baseline correction was utilized. The free-energy changes for the anomeric equilibria were calculated from the observed ratio according to $\Delta G^\circ_{\beta\to\alpha} = -RT \ln ([\alpha]/[\beta])$.

Decoupling. Decoupler power was kept at a minimum and was on during both the acquisition time and the preacquisition delay. To establish connectivity, incomplete decoupling was utilized, but when the coupling constant was under investigation, decoupler power was higher, to ensure complete decoupling. To reduce artifacts, every experiment was repeated with the decoupler at a nearby frequency where there was no signal. Decoupling difference spectra[28a] were obtained by subtraction of the decoupled spectrum from the off-resonance spectrum. Such spectra were obtained primarily to locate H2 of α anomers and to measure J_{23}. Since only H2 is affected by decoupling of H1, the difference spectrum contains only these two signals, with all the intense signals removed. The coupling constant J_{23} was measured directly from this spectrum, assuming first-order behavior. Since each peak of a decoupled doublet is centered between two undecoupled peaks, there are no inaccuracies due to the peak shifts that can arise from overlap of positive and negative signals.[29]

Decoupling difference spectra were also obtained to confirm the assignment of any signals other than H1 that were used to measure the anomeric ratio. The signal in question was irradiated, and once a difference spectrum was generated, the newly isolated signal, inverted in the difference spectrum, was chosen as the new irradiation site. A second difference spectrum was generated, and the process was repeated until the difference spectrum revealed H1. This then confirmed the connectivity of the molecule and associated the integrated signal with the ring carrying the established H1 signal.

Saturation Transfer. All saturation-transfer studies were done with decoupler power as low as possible and on only during the relaxation delay between pulses. The on-resonance and off-resonance irradiation sites were equidistant to the observed signal. The saturated site was generally the H1 signal of the major β anomer (or H3 in the case of tetra-*O*-acetylglucopyranosylamine), and the observed site was the corresponding site of the α anomer. Saturation transfer was seen as an inverted α H1 (or H3) signal in the difference spectrum.

Quantitative estimates of the saturation were made according to the equation $t_i(j) = (I_i^0 - I_i\{j\})/I_i^0$, where $I_i\{j\}$ is the intensity of site i when the decoupler is directed at site j, and I_i^0 is its intensity when the decoupler is off resonance. These values were corrected for incomplete saturation of site j by dividing $t_i(j)$ by $(M_j^0 - M_j)/M_j^0$, where M_j is the residual magnetization of site j, and M_j^0 is its equilibrium magnetization.

Two-Dimensional Heteronuclear Correlation. ^{13}C–^{1}H heteronuclear correlation spectra[28b] were obtained using standard pulse sequence CSCMB4 of the QE300 spectrometer, with labeling times incremented by 10 ms before and after the 36-μs ^{13}C 180° pulse, an 18-μs ^{13}C 90° pulse, 3.3- and 2-ms delays preceeding and following the 42.5-ms ^{1}H 90° pulse, a 1.8-s interpulse delay, and a 1.36-s post-aquisition delay. All pulse widths were calibrated, and a spin–lattice relaxation time was measured to select the delay between pulses.

Results

Signal Assignments. Assignment began by comparison with the ^{1}H NMR spectrum of glucose,[30] which shows H1 doublets at δ 5.23, with a 3.6-Hz gauche coupling constant $^3J_{12}$, due to the α anomer, and at δ 4.65, with a 7.8-Hz anti $^3J_{12}$, due to the β anomer. Extrapolation next provided estimates of all the coupling constants and chemical shifts expected for a glucopyranosylamine. Since its equilibrium lies far toward the β anomer (**3–5β**), these signals were easy to identify. In all cases β H1 appears as the only prominent doublet. Representative spectra are shown in Figure 1. In the free amine there is a distinctive 8.7-Hz doublet at δ 3.92, assignable to H1, and there are other characteristic peaks assignable to other hydrogens around the ring, with chemical shifts quite similar to those of β-D-xylopyranosylamine.[31]

The signals of the α anomer are weaker and less apparent, even its H1. Except for tetra-*O*-acetylglucopyranosylamine, α H1 appears as a characteristic doublet well downfield of all other peaks. Figure 1a shows a weak doublet 0.5 ppm downfield of the

(22) Isbell, H. S.; Frush, H. L. *J. Org. Chem.* **1958**, *23*, 1309.
(23) Masciorini, E.; Thiel, I. M. E.; DeFarrari, J. O. *An. Quim.* **1976**, *72*, 946.
(24) Stepanenko, B. N.; Grehnykh, R. D. *Dokl. Akad. Nauk (Engl. Transl.)* **1966**, *170*, 860.
(25) Bertho, A.; Maier, J. *Justus Liebigs Ann. Chem.* **1932**, *498*, 50.
(26) Pigman, W.; Cleveland, E. A.; Couch, D. H.; Cleveland, J. H. *J. Am. Chem. Soc.* **1951**, *73*, 1976.
(27) Micheel, F.; Frowein, A. *Chem. Ber.* **1957**, *90*, 1599.
(28) Sanders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy*; Oxford University Press: New York, 1987; (a) p 54 and (b) p 100.
(29) Oschkinat, H.; Freeman, R. *J. Magn. Reson.* **1984**, *60*, 164.
(30) DeBruyn, A.; Anteunis, M.; Kováč, P. *Coll. Czech. Chem. Comm.* **1977**, *42*, 3057.
(31) De Bruyn, A.; Anteunis, M.; Claeyssens, M.; Saman, E. *Bull. Soc. Chim. Belg.* **1976**, *85*, 605.

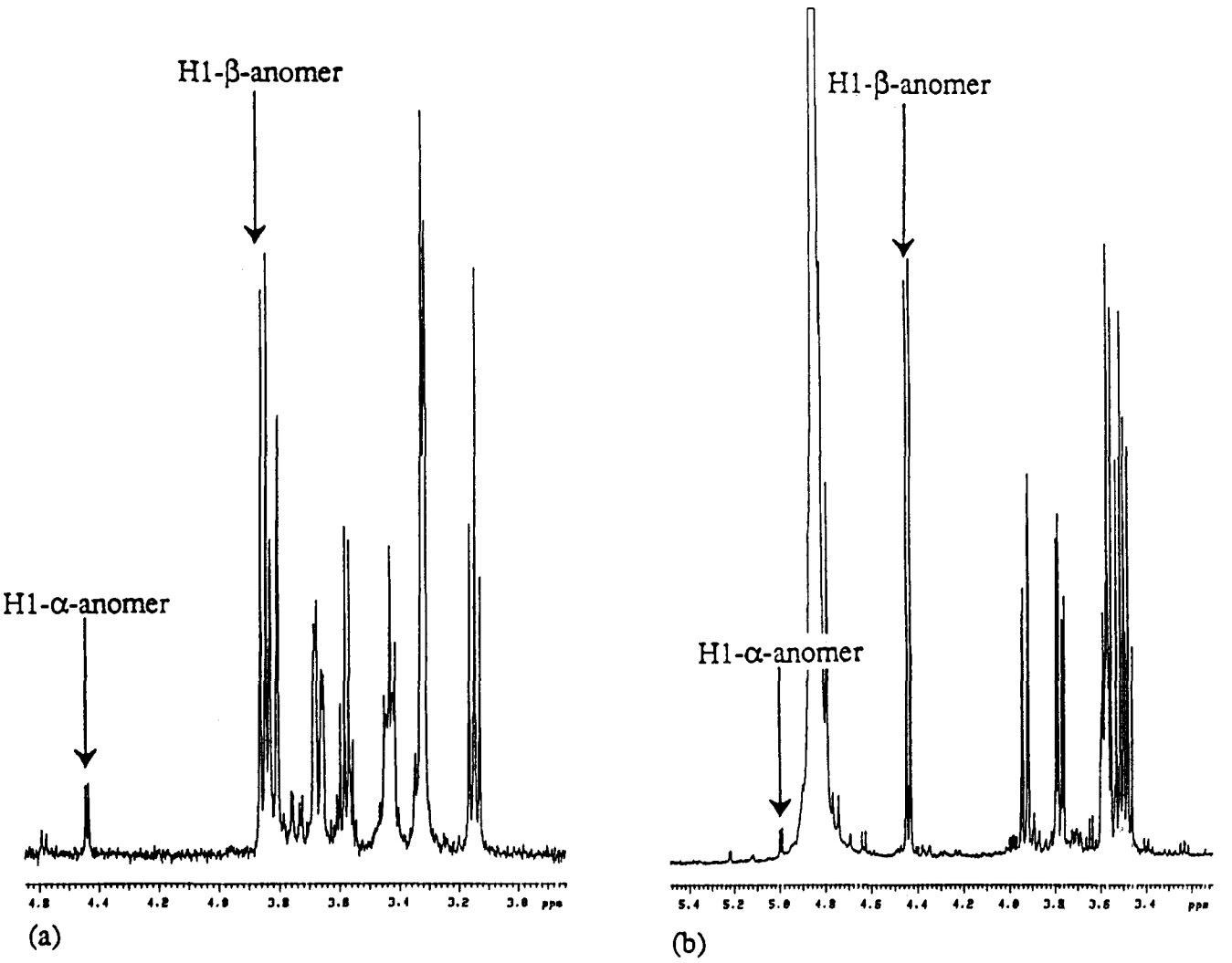


Figure 1. ^1H NMR spectrum of *N*-methyl-D-glucopyranosylamine (a) in D_2O and (b) in D_2O/DCl.

Table I. Relevant ^1H NMR Parameters[a] of *N*-Alkylglucopyranosylamines (3)

R	solvent	δ_1^β	J_{12}^β	δ_2^β	δ_1^α	J_{12}^α	δ_2^α	J_{23}^α	$n(\beta)$[b]
H	D_2O	3.85	8.7	2.91	4.59	*c*	3.39	*c*	1
H	Py-d_5/D_2O	4.2	8.7	3.40	5.02	5.1	*d*	*d*	1
H	Py-d_5	4.49	8.6	3.75	5.41	5.3	4.42	9.4	2
H	DMSO-d_6	3.73	8.5	2.77	4.63	4.8	3.20	9.5	2
$H_2^+Cl^-$	D_2O	4.37	8.7	3.27	5.05	5.4	3.89	9.8	2
$H_2^+Cl^-$	DMSO-d_6	4.24	8.6	3.14	4.88	5.0	3.49	9.6	2
Me	D_2O	3.92	8.7	3.21	4.43	4.8	3.60	9.8	1 + 6(3.89)
Me	DMSO-d_6	3.57	8.8	2.86	4.17	4.8	3.27	9.5	1
Me	Py-d_5	4.26	8.5	3.80	4.85	5.0	4.24	9.2	1
MeH^+Cl^-	D_2O	4.30	8.7	3.34	4.88	5.4	3.93	9.3	1
MeH^+Cl^-	DMSO-d_6	4.18	*c*	2.65	4.71	4.8	3.56	9.3	1
Et	D_2O	3.81	8.8	3.00	4.44	4.6	3.46	9.0	1
Et	DMSO-d_6	3.64	8.8	2.84	4.30	4.8	3.25	9.3	1 + 6(3.62)
EtH^+Cl^-	D_2O	4.32	8.9	3.35	4.90	5.6	3.83	10.0	1
EtH^+Cl^-	DMSO-d_6	4.19	8.6	2.92	4.75	5.4	3.56	9.7	1
Bu	D_2O	3.84	8.7	3.04	4.48	*c*	3.52	*c*	1
Bu	DMSO-d_6	3.63	8.5	2.65	4.28	4.9	3.25	9.5	1
Bu	Py-d_5	4.35	8.8	3.80	4.99	4.8	4.22	9.3	2
Bu	Py-d_5/D_2O	4.05	8.7	3.42	4.72	5.0	*d*	*d*	1 + 6(4.02)
BuH^+Cl^-	D_2O	4.32	8.7	3.05	4.92	5.5	4.30	9.7	1
BuH^+Cl^-	DMSO-d_6	4.19	8.8	3.06	4.75	5.3	3.58	9.5	1

[a] Chemical shifts δ ±0.05 ppm, coupling constants J ±0.3 Hz. [b] β-Anomer signal integrated, with chemical shift if not H1 or H2. [c] Indeterminate owing to virtual coupling. [d] Too weak.

prominent β H1 doublet and with a 4.8-Hz coupling constant. Such an α H1 doublet could be seen, either directly or in a decoupling difference spectrum, in every sample.

Tables I–III contain the relevant spectral parameters of all the glucopyranosylamines. Chemical shifts are uncertain to ±0.05 ppm and coupling constants are ±0.3 Hz. As with glucose itself, H1 of the α anomer is found an average of 0.65 ± 0.2 ppm downfield of H1 of the β anomer. Also H1 of the α anomer exhibits an average J_{12} of 5.1 ± 0.4 Hz, smaller than the 8.7 ± 0.2 Hz for the β anomer. These coupling constants are within

Table II. Relevant ^{1}H NMR Parameters[a] of Tetra-*O*-acetylglucopyranosylamine (**4**)

R	solvent	δ_1^β	J_{12}^β	δ_2^β	δ_1^α	J_{12}^α	δ_2^α	J_{23}^α	$n(\beta)^a$	$n(\alpha)^b$
H	Py-d_5	4.54	8.6	3.80	5.25	5.3	4.24	9.4	3(5.71)	3(6.06)
H	DMSO-d_6	4.28	8.9	4.62	4.85	5.0	4.90	10.1	3(5.22)	3(5.42)
H	$CDCl_3$	4.19	9.2	4.82	5.13	5.1	4.99	10.0	3(5.24)	3(5.43)
H	C_6D_6	3.65		4.90	4.85	4.9	5.08	10.1	3(5.38)	3(5.63)
$H_2^+Cl^-$	DMSO-d_6	4.98	8.8	4.92	5.18	5.4	5.07	9.9	3(5.38)	3(5.66)
$H_2^+CF_3CO_2^-$	DMSO-d_6	5.03	9.0		5.34	5.5	5.15	9.8	3(5.37)	3(5.66)
$H_2^+CF_3CO_2^-$	$CDCl_3$	4.93	9.0	5.15	5.52	4.6	5.33	9.6	3(5.40)	2(5.33)
$H_2^+CF_3CO_2^-$	C_6D_6	4.18		4.80	4.96	4.5	5.04		3(5.13)	3(5.42)

[a] Chemical shifts δ ±0.05 ppm, coupling constants J ±0.3 Hz. [b] β- or α-anomer signal integrated, with chemical shift if not H1 or H2.

Table III. Relevant ^{1}H NMR Parameters[a] of *N*-Alkyl-4,6-*O*-benzylideneglucosylamines (**5**)

R	solvent	δ_1^β	J_{12}^β	δ_2^β	δ_1^α	J_{12}^α	δ_2^α	J_{23}^α	$n(\beta)^b$
H	Py-d_5/D_2O	4.38	8.6	3.68	5.17	5.2	4.07	9.4	1
H	DMSO-d_6	3.95	8.5	2.94	4.70	5.2	3.37	9.2	1
H	Py-d_5	4.52	8.7	4.15	5.40	5.1	4.70	9.5	1
$H_2^+CF_3CO_2^-$	DMSO-d_6	4.46	8.6	3.33	4.99	5.4	3.75	9.2	1
Bu	Py-d_5/D_2O	4.27	8.7	3.72	4.83	5.5	4.02	9.5	CH_2N(2.90)
Bu	DMSO-d_6	3.88	8.7	3.02	4.40	5.0	4.70	9.9	1
Bu	$CDCl_3$	3.97	8.8	3.21	4.60	5.3	3.76	10.0	2
$BuH^+CF_3CO_2^-$	DMSO-d_6	4.48	8.7	3.43	4.94	5.4	3.75	9.2	1

[a] Chemical shifts δ ±0.05 ppm, coupling constants J ±0.3 Hz. [b] β-Anomer signal integrated, with chemical shift if not H1 or H2.

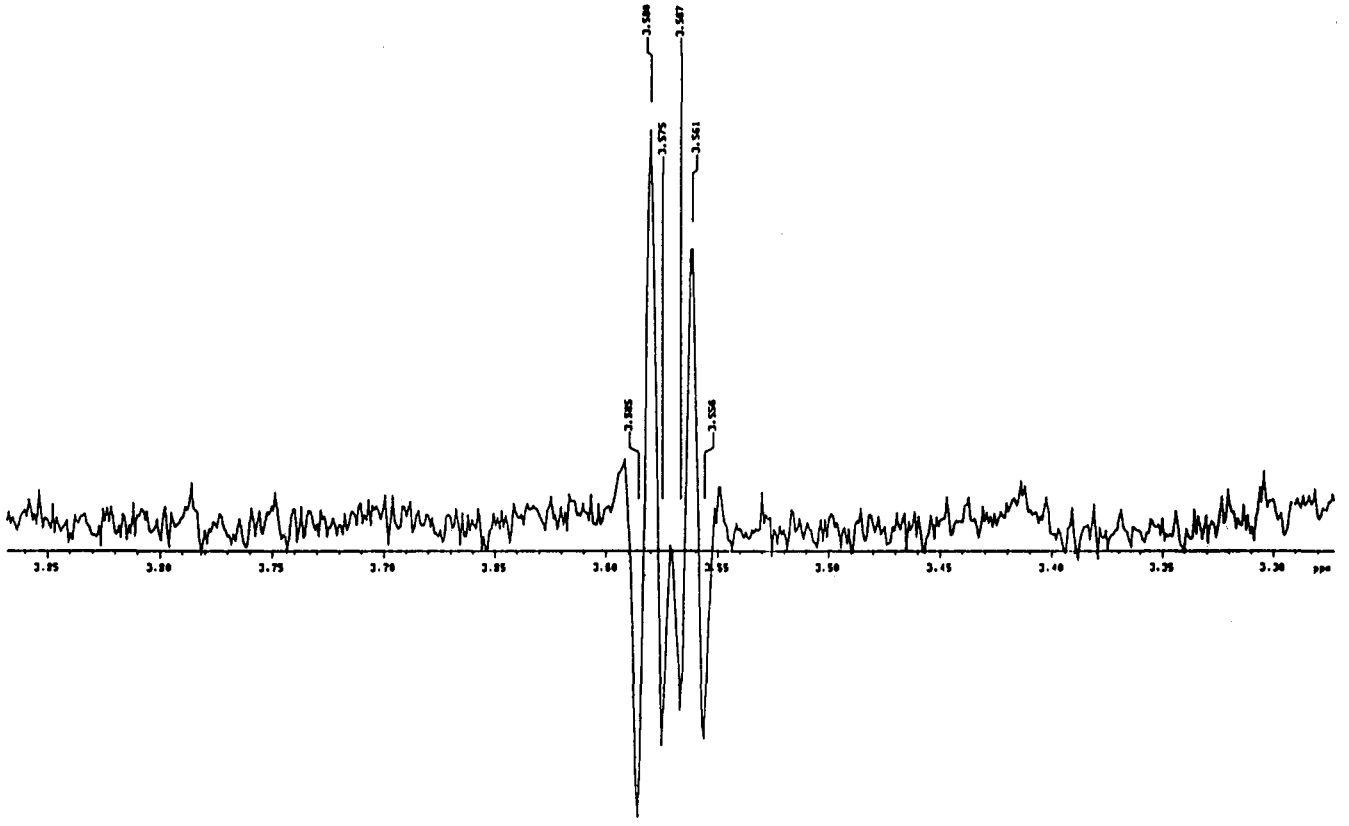


Figure 2. Decoupling difference spectrum of α H2 region of glucopyranosylamine in pyridine-d_5.

limits predicted using a modified Karplus equation.[32] They are close to the J_{anti} of 8–13 Hz and J_{gauche} of 2–6 Hz seen in similar compounds,[3a,31] among which the most unambiguously assigned are some *N*-aryl-D-glucopyranosylamines, where $^3J_{12}$'s are near 4 Hz for the α anomers and near 8 Hz for the β.[33]

Although α H2 was obscured by the intense signals of the predominant β anomer, decoupling difference spectra[28a] provided J_{23} in most cases. Figure 2 shows an example. For aqueous glucopyranosylamine and *N*-butylglucopyranosylamine the signals isolated in this manner were distorted. This is probably due to virtual coupling,[34] and simulated difference spectra could reproduce the distortions. Addition of pyridine is sufficient to change the chemical shifts and eliminate the complication. Thus the H1 signal of α-*N*-butylglucopyranosylamine changes from a distorted 3.9-Hz doublet to a clean 4.8-Hz doublet.

Other signals provide further verification of the assignments for α-glucopyranosylamines. Although the H1 signal is usually the only one resolved, decoupling H1 may locate H2. This was done in nearly every sample. In some cases H2 can then be

(32) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon Press: Oxford, 1969; p 280ff. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.

(33) Capon, B.; Connett, B. E. *J. Chem. Soc.* **1965**, 4492. Ellis, G. P.; Williams, J. M. *Carbohydr. Res.* **1981**, *95*, 304.

(34) Becker, E. D. *High Resolution NMR*, 2nd ed.; Academic Press: New York, 1980; p 163.

decoupled to locate H3, and so on. The chemical shift and coupling pattern of each new signal are consistent with those expected for the α anomer.

A ^{13}C NMR spectrum of glucopyranosylamine-1-^{13}C further substantiates the ^1H NMR assignments. Again comparison with glucose, whose α C1 is 4.0 ppm upfield of β C1,[35] is predictive. The ^{13}C spectrum of glucopyranosylamine-1-^{13}C is dominated by the β C1 signal at δ 85.7, which agrees with a reported[36] δ 85.6. Moreover, there is a small additional signal at δ 81.9, which is assigned to C1 of the α anomer. Our values differ from the δ 80.4 and δ 77.3 previously assigned[37] to a poorly characterized material. *N*-Butylglucopyranosylamine likewise shows C1 signals separated by 3.9 ppm, with sufficient α anomer so that ^{13}C enrichment was not necessary. The chemical shift of the β anomer agrees with one previously reported.[38]

A two-dimensional ^{13}C–^1H correlation spectrum[28b] of glucopyranosylamine-1-^{13}C, which maps out C–H one-bond connections, contains a strong crosspeak between the ^{13}C signal at δ 85.7 and the ^1H signal at δ 3.85 as well as a weak crosspeak between the δ 81.9 and δ 4.59 signals. The latter is the signal assigned above to α H1. This spectrum thus correlates the α H1 signal with a ^{13}C peak of appropriate chemical shift. Such a correlation between the ^{13}C and ^1H signals was also confirmed for glucopyranosylamine in aqueous acid.

The chemical shifts in Tables I–III verify that these amines have been converted to their conjugate acids under the acidic conditions used. In acid all show a downfield shift of 0.1–0.7 ppm for H1 of both the α and β forms. Complete protonation is consistent with a reported pK_a of 5.57[39] and with an estimated pK_a of 7.5, based on substituent effects.[40]

The chemical shifts and coupling constants in Tables I–III are consistent with the assignments. For several β anomers the resonances of every proton and every carbon could be resolved. This information, along with published parameters on β-glucopyranosylamine itself and various similar molecules, was sufficient to establish conclusively the spectral assignments made for the β anomer. Yet the only resolved resonances of the α anomer are those listed in Tables I–III. Since these signals are weak and therefore conceivably due to impurities, further evidence was sought.

Interconvertibility of Anomers. The best evidence for the assignment of the weak signals to an α-glucopyranosylamine is the observation that it is in rapid exchange with the dominant species, which is known to be the β anomer. Under conditions of acid catalysis, transfer of magnetization could be detected from the β H1 signal to the α H1 for all of the protonated glucopyranosylamines (except **3b** in DMSO-d_6/DCl). An example of a saturation-transfer difference spectrum is shown in Figure 3. More quantitatively, the extents of saturation transfer $t_{\alpha 1}(\beta 1)$ for glucopyranosylamine and *N*-methylglucopyranosylamine in D_2O/DCl at 40 °C are ca. 0.5 and 0.35, respectively, corresponding to lifetimes for chemical interconversion on the order of 1 s. The fact that no saturation transfer was observed for either molecule at room temperature shows that the observed saturation transfer is not an artifact.

General acid catalysis was observed qualitatively for the anomerization of *N*-methylglucopyranosylamine in bicarbonate buffer. The ^1H NMR spectrum was observed immediately following dissolution of crystalline β-glucopyranosylamine and every 30–40 s thereafter. As anomerization proceeded, the α H1 signal appeared and grew, and equilibrium was established within

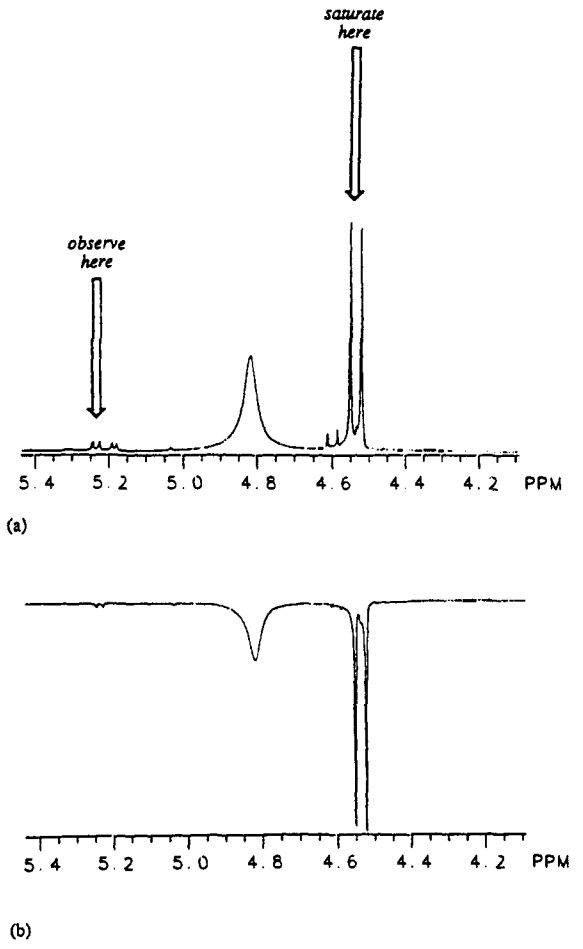


Figure 3. Saturation-transfer difference spectrum of H1 region of 4,6-*O*-benzylideneglucopyranosylamine in DMSO-d_6/DCl.

5 min. In an unbuffered sample of the same concentration equilibration required more than 30 min.

Anomerization in basic media is too slow to lead to detectable magnetization transfer, even at 40 °C. An indirect method was successful in establishing a connection between the β H1 and α H1 signals in aqueous base. *N*-Methylglucopyranosylammonium ion, whose α H1 signal at δ 4.88 was positively identified through saturation transfer from β H1, shows 4.8% of the α anomer in D_2O/DCl. A ^1H spectrum acquired immediately after addition of enough methylamine to deprotonate the ion shows that same α H1 signal, shifted upfield but still present at the 4.8% characteristic of the anomeric equilibrium in acid. Over the course of about an hour the amine equilibrium, with 8.5% α anomer, was then established. This observation supports the assignment of the signal at δ 4.43 in basic D_2O to α H1, since it exchanges with H1 of β-*N*-methylglucopyranosylamine, whose assignment is certain.

Trace Impurities. Once enough time had elapsed for the anomeric equilibrium to be established (up to several days), additional small signals sometimes appeared in the α H1 region of the spectrum. Likely impurities include glucose from hydrolysis and *N*,*N*-di(glucopyranosyl)amines from dimerization.[31,41] Addition of excess ammonia or *N*-alkylamine was sufficient to reduce signals due to glucose or dimer below those assigned as α H1. Glucose was easily identified from its distinctive downfield α H1 signal and its J_{12} and by addition of authentic glucose to the sample. Signals attributable to dimers were less easily identified. As with di-(β-D-xylopyranosyl)amine, where H1 is 0.15 ppm downfield of monomer H1,[31] a signal attributable to H1 of di-(β-D-glucopyranosyl)amine sometimes appeared ca. 0.1–0.2 ppm

(35) Horton, D.; Walaszek, A. *Carbohydr. Res.* **1982**, *105*, 145.
(36) DeGroot, A. W.; Carroll, F. I.; Cuculo, J. A. *J. Polymer Sci. A., Polymer Chem.*, **1986**, *24*, 673.
(37) Sawaki, M.; Takeda, T.; Ogihara, Y.; Shibata, S. *Chem. Pharm. Bull.* **1984**, *32*, 3698.
(38) Funcke, W.; Klemer, A. *Justus Liebigs Ann. Chem.* **1975**, 1232.
(39) Inouye, S. *Chem. Pharm. Bull.* **1968**, *16*, 1134.
(40) Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436.
(41) Hodge, J. E.; Moy, B. F. *J. Org. Chem.* **1963**, *28*, 2784.

downfield of the β monomer H1 signal and with the same J_{12}. Similarly, glucopyranosylamine and tetra-*O*-acetylglucopyranosylamine in pyridine-d_5 show an H1 doublet or H3 triplet, respectively, 0.2 ppm downfield of the corresponding signal of the α monomer and attributable to the α,β dimer.

Other extraneous signals were more mysterious. In pyridine-d_5, both 4,6-*O*-benzylideneglucopyranosylamine and *N*-butylglucopyranosylamine displayed a 4–5-Hz doublet, 0.2 ppm downfield or 0.14 ppm upfield, respectively, of α H1. Acidic samples of glucopyranosylamine and each of its *N*-alkyl derivatives in DMSO-d_6 showed an extra 3.8-Hz doublet 0.1 ppm downfield of α H1. Various methods were utilized to rule out these extraneous signals as due to α H1 and to substantiate the assignment of the characteristic α H1 signals. For example, the decoupling difference spectrum of the extra doublet in DMSO-d_6 solutions of the *N*-alkylglucopyranosylammonium chlorides is coupled only to a proton not coupled to any other protons. Therefore it cannot be coupled to α H2 and it cannot be α H1, although it may be from the α,β-dimer if second-order effects are operative. As there was sufficient independent evidence to identify the α H1 signal of every glucopyranosylamine in every sample, any extraneous peaks could be ignored.

Ring Conformation. The coupling constants J_{12} and J_{23} are diagnostic for the HCCH dihedral angles. If both anomers are chair conformers, J_{12} should be 8–9 Hz for the β and 4–5 Hz for the α, and J_{23} should be >9 Hz for both. If the α anomer were to exist as its inverted conformer, with the amino substituent equatorial and all other substituents axial, J_{23} would be small. If it were to adopt a twist-boat conformation as in *N*-(α-D-mannopyranosyl)pyridinium chloride, both J_{12} and J_{23} would be smaller.[3b]

According to the values in Tables I–III, the coupling constant J_{12} is 5.1 ± 0.3 Hz for all the α anomers and 8.7 ± 0.2 Hz for all the β anomers. There seems to be a slight increase of J_{12} from 5.0 in the α amines to 5.2 Hz in their protonated derivatives, perhaps due to the change in the substituent electronegativity or to small changes in conformation, but the difference is smaller than our ability to measure (±0.3 Hz). The coupling constant J_{23} is not only 9–10 Hz for those β anomers for which it was measured (values not tabulated) but also an average of 9.6 ± 0.3 Hz for the α. These coupling constants then confirm that the α and β anomers are the chair conformers depicted as **3–5**. This result may be attributed to the steric bulk of the hydroxymethyl and three hydroxy substituents, since it contrasts to the behavior of ribopyranosylamine,[42] where the α anomer undergoes ring inversion so that the bulky amino group can be equatorial.

It is entirely expected that a β-glucopyranosylamine would be a chair conformer, since this permits every substituent to take the equatorial position. To verify that the α-glucopyranosylamines are also chair conformers, 4,6-*O*-benzylideneglucopyranosylamine (**5a**) and its *N*-butyl derivative (**5b**) were investigated. Because of the bicyclic system these cannot undergo ring inversion. The data in Table III show that within experimental error not only the β anomers but also the α display the same coupling constants as **3–4**. Again there is an average increase of 0.2 Hz in $J_{12}{}^{\alpha}$ on protonation, but this is less than the experimental error, and it cannot be due to gross changes in conformation. Therefore we may be confident that none of the α-glucopyranosylamines distorts significantly from the chair conformation, even when the amino substituent must be axial.

Exocyclic C–N Conformation. Figure 4 depicts the conformers about the C1–N bond that might be populated. For the β anomer we neglect all conformers but **6β**, since this is the only one that avoids destabilizing steric interactions of the *N*-alkyl and also

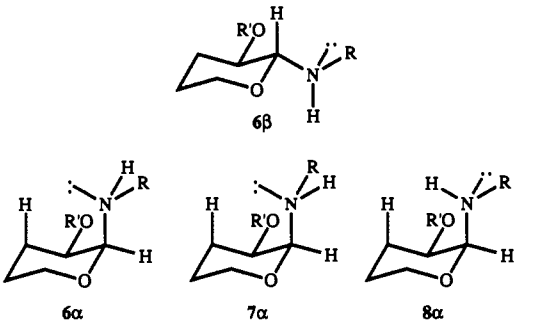


Figure 4. C–N rotational conformers of glucopyranosylamines.

gains the stabilization provided by the exo anomeric effect.[43] For the α anomer we neglect the two conformers with the alkyl group directed inward, since steric repulsions with axial H3 and H5 are prohibitive. The two conformers in which hydrogen is directed inward are also subject to steric repulsions, but the question is whether the exo anomeric stabilization in **8α** might overcome them. Thus there remain three conformers to consider, **6α**, **7α**, and **8α**. The conformers may be distinguished by their coupling constants, since J_{gauche} and J_{anti} in an HCNH system are 3 and 12 Hz, respectively.[44] In derivatives of the epimeric 2-(methylamino)tetrahydropyrans (**1E**, **1A**, X = $NHCH_3$) coupling constants of 12 and 9–12 Hz indicate that the dominant conformers are **6β** and **8α**.[45]

We have measured $^3J_{\text{NH-H1}}$ for both α- and β- tetra-*O*-acetylglucopyranosylamine (**4**) in toluene-d_8. Rapid C–N rotation and nitrogen inversion interchanges the NH protons, so that the measured coupling is an average over the two. To retard intermolecular NH exchange, it was necessary to lower the temperature to −35 °C. Both stereoisomers show H1 as an apparent quartet, indicating that the average $^3J_{\text{NH-H1}}$ for each is nearly the same as its $^3J_{12}$. Analysis of the patterns gives $^3J_{\text{NH-H1}}$'s of 8.5 Hz in the β isomer and 4.8 Hz in the α. The 8.5-Hz coupling is close to the average of J_{gauche} and J_{anti}, verifying that the β anomer is predominantly **6β** (R = H), consistent with the behavior of **1E** (X = $NHCH_3$).

The 4.8-Hz averaged $^3J_{\text{NH-H1}}$ of the α anomer is significantly lower than that of the β. If the α anomer were predominantly conformer **8α** (R = H), it would display the same 8.5-Hz average of J_{gauche} and J_{anti}. Instead the observed 4.8 Hz is closer to J_{gauche}. Although there must be some **8α** (R = H) present, for simplicity we neglect it and conclude that the α isomer is predominantly conformer **6α** or **7α**, which are identical when R = H. Moreover, this implies that **6α** and **7α** are the preferred conformers when R = alkyl. The predominance of these conformers indicates that the exo anomeric effect is insufficient to overcome the steric repulsions in **8α**. The contrast between the aminotetrahydropyran and the glucopyranosylamine is puzzling but may be due to changes in electronic and steric interactions due to the hydroxylic substituents in the latter.

Anomeric Ratios. Results of the integrations are listed in Table IV, along with the calculated standard deviations for those samples subjected to at least four separate measurements. When an H1 signal overlaps other signals in the spectrum, another signal or pair of signals was integrated instead, and that signal, with its chemical shift, is listed in Tables I–III. In many cases measurements were made on more than one sample. This provides a general estimate of reproducibility, so it was not necessary to do so in every case. The precision with which each signal was

(42) Chavis, C.; de Gourey, C.; Dumont, F.; Imbach, J.-L. *Carbohydr. Res.* **1983**, *113*, 1.

(43) Lemieux, R. U.; Pavia, A. A.; Martin, J. C.; Watanabe, K. A. *Can. J. Chem.* **1969**, *47*, 4427. Lemieux, R. U.; Koto, S. *Tetrahedron* **1979**, *30*, 1933. Lemieux, R. U.; Koto, S.; Voisin, D. In *The Anomeric Effect: Origin and Consequences*; Szarek, W. A., Horton, D., Eds.; ACS Symposium Series No. 87; Washington, DC 1979.

(44) Booth, H.; Lemieux, R. U. *Can. J. Chem.* **1971**, *49*, 777.

(45) Booth, H.; Dixon, J. M.; Khedhair, K. A.; Readshaw, S. A. *Tetrahedron* **1990**, *46*, 1625.

Table IV. Equilibrium Percentage of α-Glucopyranosylamines (25 °C)[a]

solvent	3a, R = H	3b, R = Me	3c, R = Et	3d, R = Bu	4, R = H	5a, R = H	5b, R = Bu
D_2O	2.7(1)	8.5(13)	7.2	6.1(4)			
DMSO-d_6	7.0(5)	14.4(10)	21	21.4(15)	10.9(4)	11.5(10)	18.4(9)
Py-d_5	8.4	18(1)	19(1)	21(1)	10.7	10.5	23
Py/D_2O	2.9(5)			8.7		3.0	7.1
$CDCl_3$					7.3(5)		11.5(10)
C_6D_6					7.4(10)		
D_2O/DCl[c]	3.2(4)	4.8(5)	3.0	3.2			
DMSO/DCl	6.5	12.1(7)	7.1	6.3(4)	7.1(5)	8.1(8)	9.5(10)
DMSO/TFA					7.4(5)	6.9	7.2
$CDCl_3$/TFA					6.5		
C_6D_6/TFA					5.9(7)		

[a] Standard deviation of last digit in parentheses, from at least four measurements. [b] At 40 °C.

Table V. Free Energy of Anomerization of Glucopyranosylamines, $\Delta G°_{\beta\to\alpha}$, kJ mol^{-1} (25 °C)[a]

solvent	3a, R = H	3b, R = Me	3c, R = Et	3d, R = Bu	4, R = H	5a, R = H	5b, R = Bu
D_2O	8.9(1)	5.9(4)	6.3	6.8(2)			
DMSO-d_6	6.4(2)	4.4(2)	3.3	3.2(2)	5.2(1)	5.1(2)	3.7(1.5)
Py-d_5	5.9	3.8(2)	3.6(2)	3.3(1.5)	5.3	5.3	2.95
Py/D_2O	9.1(5)[b]			5.8		8.6	6.4
$CDCl_3$					6.3(2)		5.1(2)
C_6D_6					6.3(4)		
D_2O/DCl[c]	8.5(3)	7.4(3)	8.6	8.5			
DMSO/DCl	6.9[b]	5.2(2)[b]	6.4	6.7(2)	6.4(2)	6.0(3)	5.6(3)
DMSO/TFA					6.3(2)	6.5	6.3
$CDCl_3$/TFA					6.6		
C_6D_6/TFA					6.9(3)		

[a] Standard deviation of last digit in parentheses. [b] At 40 °C.

integrated depended strongly upon the degree of crowding in its region of the spectrum, which varied from sample to sample. In cases where the signal being integrated lies on the shoulder of a solvent peak or immediately adjacent to another signal, larger errors resulted. Despite the variability of these and other effects on the quality of the spectra, the percentages reported in Table IV are conservatively judged as reliable to within ±20% of the value listed.

The observed values of $\Delta G°_{\beta\to\alpha}$, the free-energy change for conversion of β isomer to α, for each entry in Table IV, are listed in Table V. Error margins were calculated from propagation of the errors in Table IV.

Discussion

Reliability of Assignments. All the spectral characteristics are fully consistent with assignment of the minor peaks to α-glucopyranosylamine. Chemical shifts and coupling constants match those expected from model compounds, especially glucose itself. In every sample a prominent 9-Hz doublet was clearly assignable as H1 of the β anomer, and a weaker 5-Hz doublet 0.5–0.8 ppm downfield was then assignable as H1 of the α anomer. The downfield shifts of α H1 and α C1 are clearly inconsistent with a 2-keto derivative, the product of Amadori rearrangement. The large J_{23} is inconsistent with an inverted chair or with a furanose derivative. Moreover, the uniformity of spectral characteristics across the entire series of glucopyranosylamines not only facilitated identification but also corroborates the assignments. Once any of these signals can be assigned as α, consistency supports this same assignment in all other samples.

The strongest evidence for assignment as α-glucopyranosylamines is the observation of rapid exchange with the β anomer. Anomerization is known to be slow in alkaline solution, fast in strongly acidic solution, and fastest in neutral and weakly acidic solutions. The reaction proceeds by reversible protonation of the ring oxygen, ring opening to an iminium ion, rotation about the C1–C2 bond, and reclosure.[22] Consistent with this mechanism, rapid exchange is seen in acidic samples, and general acid catalysis is seen in basic samples. The only species that would rapidly interconvert with the β anomer are the α anomer, a furanose, and perhaps a 2-keto derivative, but only the first is consistent with the spectral characteristics.

Anomeric Equilibrium in Base. According to the $\Delta G°_{\beta\to\alpha}$ values in Table V, the β anomer of the primary glucopyranosylamines (3–5, R = H) is favored by an average of 6.6 ± 1.5 kJ mol^{-1} across a wide range of solvents. To gauge the steric effect, the simplest model is cyclohexylamine, for which A_{NH_2} is 6.7 ± 0.3 kJ mol^{-1} in D_2O and 5.0 ± 0.3 or 6.15 ± 0.1 kJ mol^{-1} in nonpolar solvents (average 5.6 ± 0.6 kJ mol^{-1}).[16] These are underestimates, since the C–O bond is shorter than a C–C bond, leading to more severe steric repulsions in a tetrahydropyran. They can be corrected through the linear relation, $A^{THP} = 1.53A + 0.08$, that holds between A values of alkylcyclohexanes and A values of the corresponding 2-alkyltetrahydropyrans.[46] Thus A_{NH_2} on a pyranose ring can be estimated as 8.6–10.3 kJ mol^{-1}, depending on solvent. The observed preference for the equatorial position is close enough to this estimate that we may conclude that the preference is predominantly due to the steric bulk of the NH_2. Moreover, the variation with solvent parallels that for cyclohexylamine, in that hydrogen bonding can increase an A value.[47]

According to the data in Table IV, the *N*-alkylglucopyranosylamines (3, 5, R = Me, Et, Bu) exhibit an average proportion of α anomer 2.4-fold that of the primary glucopyranosylamines (3, 5, R = H). This is surprising, since an NHR group is slightly bulkier than an NH_2 and ought to be subject to a greater steric repulsion in the axial position. Actually, A_{NHCH_3} on a cyclohexane is slightly less than A_{NH_2}, for obscure reasons.[16b] However, in the case of the glucopyranosylamines the factor near 2 may be accounted for through consideration of the rotational conformers about the C–N bond, shown in Figure 4. According to the observed $^3J_{NH-H1}$'s, there are two α conformers, **6α** and **7α**, available to the molecule when R = alkyl, but only one, **6α** = **7α**, when R = H and only one, **6β**, for the β conformers. If those two α conformers are equally populated, the resulting entropy of mixing for the *N*-alkyl-α-glucopyranosylamines is then R ln 2. Unfortunately it is not possible to verify this ΔS from the

(46) Franck, R. W. *Tetrahedron* **1983**, *39*, 3251.
(47) Eliel, E. L.; Della, E. W.; Williams, T. H. *Tetrahedron Lett.* **1963**, 831. Ford, R. A.; Allinger, N. L. *J. Org. Chem.* **1970**, *35*, 3178.

temperature dependence of the anomeric ratio, since experimental errors are too large.

If the free-energy changes for the *N*-alkylglucopyranosylamines are corrected by this entropy term, the β anomer is favored by an average of 6.3 ± 1.3 kJ mol^{-1}. This is again close to A_{NH_2} in cyclohexanes. Therefore we conclude that the preference for equatorial NH_2 or NHR in glucopyranosylamines is largely due to steric bulk. Actually, with the correction for the larger steric effects in a tetrahydropyran, there is a small additional preference for axial NH_2 or NHR that has been noted before and attributed to a normal (endo) anomeric effect.[46] It is perhaps surprising that the exo anomeric effect[43] does not lead to a greater preference for equatorial NH_2 or NHR, but anomeric effects can be smaller for nitrogen than for oxygen.[48] The weakness of the exo-anomeric effect is consistent with the low $^3J_{NH-H1}$ in **4**α, which indicates that the dominant conformer is not **8**α.

Anomeric Equilibrium in Acid. The real question is the anomeric equilibrium of the glucopyranosylammonium ions. The data in Table IV show that N-protonation does not greatly reduce the proportion of α anomer of a glucopyranosylamine. Even in acid there is a substantial proportion, up to 10–12%. Were a reverse anomeric effect operative, there would have been very little.

According to the $\Delta G°_{\beta\to\alpha}$ values in Table V, the β anomer of the N-protonated glucopyranosylamines is preferred by an average of 8.2 ± 0.5 kJ mol^{-1} in D_2O and 6.3 ± 0.5 kJ mol^{-1} in other solvents. This preference is not much different from that of the neutral glucopyranosylamines. It is also close to $A_{NH_3^+}$, which is 7.9 ± 0.3 kJ mol^{-1} in aqueous solution,[16a] or 6.7 ± 0.7 kJ mol^{-1} in nonaqueous solvents if it simply decreases by the same factor as A_{NH_2}. As for the glucopyranosylamines the anomeric equilibrium of their conjugate acids can be accounted for almost entirely by steric effects. However, with the correction[46] for the shorter C–O bond of a tetrahydropyran $A_{NH_3^+}$ becomes 12 kJ mol^{-1} in aqueous solution and ca. 10 kJ mol^{-1} in nonaqueous solution. Therefore the preference of the NH_3^+ or NH_2R^+ group for the equatorial position is *less* than can be expected on the basis of steric bulk. According to eq 1, E_{An}, representing the extra preference for the axial position, is a small but significant 4 kJ mol^{-1}. This represents an anomeric effect, albeit weak, but not a reverse anomeric effect!

This reasoning may be clearer if rephrased in terms of concentrations. On the basis of steric repulsions ($A_{NH_3^+}$ on a tetrahydropyran), the proportion of the α anomer of an N-protonated glucopyranosylamine ought to be 0.8% in water or 1.7% in nonaqueous solution. With any reverse anomeric effect favoring the β anomer, the proportion would be even lower. Yet experimentally the average proportions are significantly greater, 3.5% and 7.3%, respectively. The increased proportion of α anomer is evidence against the reverse anomeric effect and consistent with a small normal anomeric effect. However, this conclusion depends crucially on the ability to detect those weak signals and to assign them as α anomer.

Entropy Effects in Acid. According to the data in Table IV, the proportion of α anomer of N-protonated glucosylamines is the same for *N*-alkyl as for N-unsubstituted derivatives. In contrast to the *N*-alkylamines there is no entropy effect apparent. Figure 5 shows the rotational conformers about the C–N bond, including **7**′α, **7**′β, and **8**′β. Although these are subject to repulsions involving the *N*-alkyl group, the repulsions may be alleviated through partial rotation about the C–N bond. (The analogous conformers of the unprotonated derivatives were excluded, since the exo anomeric effect restricts rotation.) With the simplification that all the axial conformers in Figure 5 are equally populated, the resulting entropy of mixing is then R ln 2 when R = alkyl but zero when R = H. Likewise the entropy of mixing for the equatorial isomer when R = alkyl is R ln 3. As a result, there ought to be 1.5 times as much equatorial isomer when R = alkyl than when R = H, but less if conformers **6**′α and **6**′β predominate. This is too small to detect with confidence, and we neglect it.

(48) Perrin, C. L.; Armstrong, K. B.; Fabian, M. A., to be published.

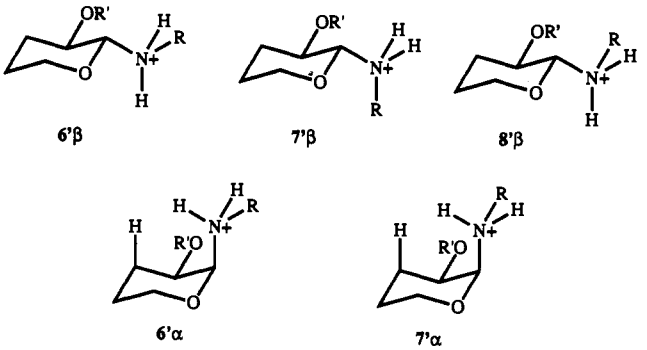


Figure 5. C–N rotational conformers of glucopyranosylammonium ions.

Table VI. Effect of N-Protonation on Anomeric Ratio, $\Delta\Delta G°_{N\to N^+}$, kJ mol^{-1}

amine	solvent	$\Delta\Delta G°_{N\to N^+}$	$\Delta\Delta G°_{N\to N^+}$ [a]
3a, R = H	D_2O	−0.4 ± 0.3	−0.4 ± 0.3
3a, R = H	DMSO-d_6	0.5	0.5
3b, R = Me	D_2O	1.5 ± 0.5	−0.2 ± 0.5
3b, R = Me	DMSO-d_6	0.8 ± 0.3	−0.9 ± 0.3
3c, R = Et	D_2O	2.3	0.6
3c, R = Et	DMSO-d_6	3.1	1.4
3d, R = Bu	D_2O	1.7	0.0
3d, R = Bu	DMSO-d_6	3.5 ± 0.3	1.8 ± 0.3
4, R = H	DMSO-d_6	1.2 ± 0.2	1.2 ± 0.2
4, R = H	$CDCl_3$	0.3	0.3
4, R = H	C_6D_6	0.6 ± 0.5	0.6 ± 0.5
5a, R = H	DMSO-d_6	1.1 ± 0.4	1.1 ± 0.4
5b, R = Bu	DMSO-d_6	1.9 ± 0.3	0.2 ± 0.3

[a] Corrected by RT ln 2 for entropy contribution to *N*-alkylglucopyranosylamines.

Protonation Effect on Equilibrium. Table VI lists $\Delta\Delta G°_{N\to N^+} = \Delta G°_{\beta\to\alpha}(NH^+) - \Delta G°_{\beta\to\alpha}(N)$, evaluated from the data in Tables IV and V. Also included are values of $\Delta\Delta G°_{N\to N^+}$ corrected at 25 °C by RT ln 2 for the entropy of axial *N*-alkylglucopyranosylamines. These are measures of the extent to which N-protonation increases the preference of an amino substituent for the equatorial position. The values are quite small, averaging 1.4 ± 1.1 kJ mol^{-1}. For comparison, the A values for aqueous NH_2 and NH_3^+ [16,46] predict a $\Delta\Delta G°_{N\to N^+}$ of 1.7 ± 0.5 kJ mol^{-1} for a tetrahydropyran. This means that the shift in the position of the anomeric equilibrium upon N-protonation can be accounted for simply by the increased steric demands of a protonated amino group. *There is no need to invoke a reverse anomeric effect.*

Alternatively, if the entropy correction is included, the average $\Delta\Delta G°_{N\to N^+}$ is only 0.5 ± 0.7 kJ mol^{-1}, which is not significantly different from zero. N-Protonation leads to *no* decrease in the average proportion of axial isomer, even though the increased steric repulsions ($\Delta A_{N\to N^+}$ = 1.7 kJ mol^{-1}) would have predicted a 50% reduction and the reverse anomeric effect would have produced an additional reduction. Therefore we conclude that the reverse anomeric effect is not operative. The observed value of $\Delta\Delta G°_{N\to N^+}$ is so small that this conclusion holds independently of any uncertainty about A values.

The crucial result is that the average $\Delta\Delta G°_{N\to N^+}$, corrected for entropy, is less than that expected from the increase in steric bulk. Although this increase is uncertain because A values are uncertain, NH_3^+ is certainly bulkier than NH_2. Yet the proportion of axial isomer does not decrease on N-protonation. This represents a slight tendency for cationic nitrogen to be axial, not equatorial. Rather than a reverse anomeric effect there appears to be a small normal anomeric effect!

Solvent Effects and Hydrogen Bonding. According to the data in Tables IV and V aqueous solvents show a slightly lower proportion of α anomer, both for the amines and for their conjugate

acids. The reduction corresponds to an average $\Delta\Delta G^{\circ}_{\beta\rightarrow\alpha}$ of 2.3 kJ mol^{-1}. This is close to the difference between 10.3 and 8.6 kJ mol^{-1}, the A_{NH_2} on a pyranose ring in aqueous and nonaqueous solvents, respectively. That A_{NH_2} and A_{OH} are larger in polar solvents is due to the stronger solvation or hydrogen bonding, which increases the effective size of the substituent.[49] Therefore the lower proportion of α anomer in aqueous solution is due predominantly to the fact that an NH_2 or NH_3^+ substituent is larger in aqueous solution, where it is more strongly solvated.

Other than this small effect due to the greater bulk of these substituents in aqueous solution, there is no appreciable solvent effect on the anomeric equilibrium. Nor is there any difference associated with the tetra-*O*-acetyl derivatives (**4**), which would have different hydrogen-bonding properties than the others. Therefore the anomeric equilibrium is not being affected by intramolecular hydrogen bonding between the NH_2 or NH_3^+ substituent and the OH or OAc substituent on C2. Of course such hydrogen bonding would not be expected to exert much of an effect, since the relationship between the substituents is gauche for both anomers.

How general is the conclusion that there is no reverse anomeric effect? Our results for N-protonated glucopyranosylamines cannot necessarily be extrapolated to the glycosides with quaternary ammonium substituents, which are the original examples of the effect. However, the major difference between the two kinds of substituents is the possibility of hydrogen bonding with the N-protonated ones, and this does not seem to influence the anomeric equilibrium. The other difference is that the original substituents were aromatic, and it has been suggested[3a] that the reverse anomeric effect arises from stabilization of the equatorial conformer by a homoallylic overlap between the oxygen lone pair and the π^* orbital of the aromatic. However, this is unprecedented and there is no evidence in UV spectra[50] for such an interaction. Therefore we conclude that there is probably no reverse anomeric effect with any cationic nitrogen substituent.

Anomeric and Reverse Anomeric Effects. These results have implications for the relative importance of the electrostatic and molecular–orbital interactions. Had a reverse anomeric effect been observed here, it would have been evidence of an electrostatic origin for the anomeric effect. The small anomeric effect that we observe is a reasonable consequence of n–σ^* overlap, which stabilizes the axial conformer. However, it does not follow that electrostatics is never responsible for anomeric effects. This system is particularly favorable to the orbital interactions. The positive charge makes the nitrogen more electronegative, lowers the energy of the σ^* orbital, and strengthens the interaction.

Reappraisal of Previous Investigations. Since these findings repudiate the reverse anomeric effect, a closer look at the previous evidence is warranted. In many cases the preference for the equatorial conformer could be due simply to the steric bulk of a heterocyclic substituent. In the study of xylopyranosylimidazole[4] the populations were not determined from direct observation of the separate conformers but from small changes in coupling constants, determined from NMR spectra where near coincidences of chemical shifts led to second-order behavior. In contrast, coupling constants confirmed by simulation[3a] show no large decrease in the proportion of axial conformer on N-protonation of *N*-(tetra-*O*-acetyl-α-D-mannopyranosyl)imidazole or *N*-(α-D-glucopyranosyl)imidazole.

Coupling constants are sensitive to substituent and to slight conformational deviations, and small changes are difficult to interpret. For example, the data in Tables I–III show small changes in J_{12} and J_{23} upon N-protonation or with solvent, even though no conformational change can occur with the 4,6-*O*-benzylidene derivatives (**5**). Even larger variations of the coupling constants of neutral xylopyranosylimidazole (**2**) were seen,[4] but these may be due to small distortions from the pure chair conformer rather than to changes of the anomeric ratio with solvent. We therefore conclude that reliable equilibrium constants cannot be obtained from such coupling constants.

Summary and Conclusions

The proportions of axial anomers of several *N*-alkylglucopyranosylamines and some of their tetra-*O*-acetyl and 4,6-benzylidene derivatives were determined by ^{1}H NMR in a variety of solvents, including acidic media. These proportions are all quite small, so the assignments were confirmed by coupling constants, saturation transfer, reequilibration, and decoupling difference spectroscopy. The values for the neutral amines can be accounted for simply on the basis of the steric bulk of NH_2 and NHR substituents, measured from model compounds and corrected for a small conformational entropy effect.

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. This arises from the greater electronegativity of these cationic substituents, which lowers the energy of the C–N σ^* orbital. There is definitely no increased tendency, beyond that due to a greater steric bulk, for a positively charged substituent to prefer the equatorial position. Therefore we conclude that the so-called reverse anomeric effect does not exist.

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(49) Jensen, F. R.; Bushweller, C. H. *Adv. Alicyclic Chem.* **1971**, *3*, 139. Franklin, N. C.; Feltkamp, H. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 774.
(50) Srivastava, S. L.; Prasad, M.; Singh, R. *Ind. J. Phys.* **1987**, *61B*, 361. Handa, T.; Utena, Y.; Yajima, H.; Ishii, T.; Morita, H. *J. Phys. Chem.* **1986**, *90*, 2589.