iron-sulfur center present in these enzymes[27] and that electron transfer occurs between the two iron sites.[25] It has been recently suggested[12b] that the iron-sulfur center may act as an electron reservoir which can deliver electrons to the iron chlorin active site to facilitate the multiple-electon reduction of bound nitrite. The $H_2OFe^{II}XW_{11}O_{39}^{(n+1)-}$ complexes behave in a similar fashion, with the tungsten-oxo framework acting like the iron-sulfur electron reservoir and the coordinated iron center acting like the active iron chlorin site in the enzyme. Thus, in a number of respects, the iron-substituted heteropolytungstates act as totally inorganic enzyme mimics.

(27) Aparicio, P. J.; Knaff, D. B.; Malkin, R. *Arch. Biochem. Biophys.* **1975**, *169*, 102.

Concluding Remarks

The electrocatalytic chemistry we have described for the iron-substituted heteropolytungstates in solutions of nitrite and nitric oxide represents the first example of a durable, entirely inorganic electrocatalyst that exhibits both binding of a substrate and subsequent multiple-electron reduction. The unique pattern of catalytic activity displayed by these transition-metal-substituted heteropolyanions, especially their ability to store and deliver multiple electrons to a bound substrate, seems likely to be useful in additional contexts that are the subjects of continuing investigations.

Acknowledgment. This work was supported by the National Science Foundation.

Effects of Solvent and Anomeric Configuration upon the Circular Dichroism, Temperature Dependence of Amide Proton Chemical Shifts, Amide Proton Exchange Rates, and Infrared Absorption of Methyl 2-Acetamido-2-deoxy-D-glucopyranosides

L. A. Buffington,* D. W. Blackburn, C. L. Hamilton, T. C. Jarvis, J. J. Knowles, P. A. Lodwick, L. M. McAllister, D. J. Neidhart, and J. L. Serumgard

Contribution from the Department of Chemistry, Carleton College, Northfield, Minnesota 55057.
Received August 17, 1987

Abstract: Explanations were sought for the dependence upon anomeric configuration of the temperature dependence of the NMR chemical shifts of amide protons, $\Delta\delta/\Delta T$, in 2-acetamido-2-deoxy-D-glucopyranosides. Measurements in several solvents were made of $\Delta\delta/\Delta T$, of amide circular dichroism, of amide infrared absorption frequencies, and of rates of exchange of amide protons with solvent protons. The effect of solvent upon the relative $\Delta\delta/\Delta T$ values of the two anomers varies and correlates with the effect of solvent upon the relative molar ellipticities of the two anomers. The α amide I frequencies and the α rates of exchange with solvent, however, are less than those of the β anomer in all solvents studied. The "normal" (for peptide conformational analyses) correlation between high $|\Delta\delta/\Delta T|$ values and high solvent exchange rates is not observed. It is suggested that the "conventional" interpretation of high $|\Delta\delta/\Delta T|$ values in terms of solvent exposure does not apply and, more tentatively, that the two anomers differ in intramolecular hydrogen bonding.

The temperature dependence, $\Delta\delta/\Delta T$, of the NMR[1] chemical shifts of the exchangeable protons of biomolecules is often used in conformational studies as an indicator of exposure of the proton to the solvent. The most widely used interpretation of $\Delta\delta/\Delta T$ values is that a relatively large dependence upon temperature means that the proton is relatively exposed to the solvent while a lesser dependence upon temperature suggests that the proton is involved in a hydrogen bond or is otherwise sequestered from the solvent. This interpretation was established through the measurement of $\Delta\delta/\Delta T$ values in peptides of known conformation and has been applied to amide protons in peptides[2] and carbohydrates[3] and to hydroxyl protons in carbohydrates.[3,4] The association of high $|\Delta\delta/\Delta T|$ values with solvent exposure can be rationalized as follows: A solvent-exposed proton is likely to hydrogen bond with the solvent and as the temperature is increased this hydrogen bonding is disrupted, decreasing the chemical shift. It has been recognized, however, that exchangeable protons that are not solvent exposed can exhibit high $|\Delta\delta/\Delta T|$ values if they are involved in other temperature-dependent interactions, especially in nonpolar solvents.[3,5]

We previously reported that, for some sugars in DMSO and water, the $|\Delta\delta/\Delta T|$ values of exchangeable protons of carbon 2 (Figure 1) substituents were greater for α anomers than for β anomers.[6] Here we focus on a pair of anomeric glucopyranosides and report that the dependence upon anomeric configuration is solvent dependent. We also use CD, hydrogen to deuterium exchange rates, and IR absorption to seek an understanding of the factors affecting the $\Delta\delta/\Delta T$ values. The importance of such an understanding is manyfold. It should help to (1) determine how $\Delta\delta/\Delta T$ values should be used in conformational studies of carbohydrates, (2) learn more about the conformational properties

(1) Abbreviations used: NMR, nuclear magnetic resonance; DMSO, dimethyl sulfoxide; CD, circular dichroism; IR, infrared; UV, ultraviolet; α, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside; β, methyl 2-acetamido-2-deoxy-β-D-glucopyranoside; HFIP, hexafluoroisopropyl alcohol; HFB, heptafluorobutanol; TFE, trifluoroethanol; TMS, trimethylsilane; DMF, dimethylformamide.

(2) (a) Llinas, M.; Klein, M. P. *J. Am. Chem. Soc.* **1975**, *97*, 4731-4737, and references therein. More recent examples are: (b) Kessler, H.; Bernd, M.; Kogler, H.; Zarbock, J.; Sorensen, O. W.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1983**, *105*, 6944-6958. (c) Pope, M.; Mascagni, P.; Gibbons, W. A.; Ciufetti, L. M.; Knoche, L. M. *J. Am. Chem. Soc.* **1984**, *106*, 3863-3864. (d) Kartha, G.; Bhandary, K. K.; Kopple, K.; Go, A.; Zhu, P. *J. Am. Chem. Soc.* **1984**, *106*, 3844-3850.

(3) Heatley, F.; Scott, J. E.; Jeanloz, R. W.; Walker-Nasir, E. *Carbohydr. Res.* **1982**, *99*, 1-11, and references therein.

(4) St.-Jacques, M.; Sundararajan, P. R.; Taylor, K. J.; Marchessault, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 4386-4391.

(5) (a) Stevens, E. S.; Sugarawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048-7050. (b) Pease, L. G.; Watson, C. *J. Am. Chem. Soc.* **1978**, *100*, 1279-1286.

(6) Buffington, L.; Crusius, J.; Nachbor, M.; Reven, L. *J. Am. Chem. Soc.* **1983**, *105*, 6745-6747.

0002-7863/89/1511-2451$01.50/0

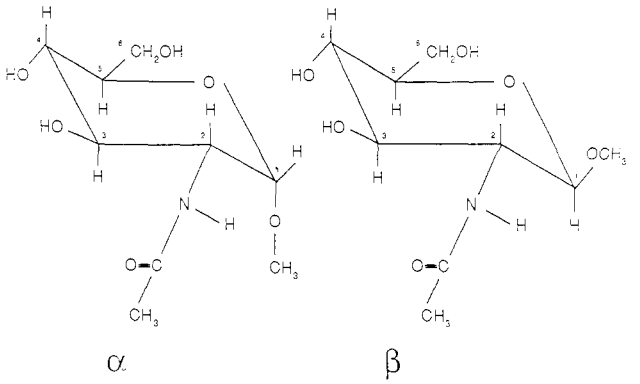


Figure 1. α (methyl 2-acetamido-2-deoxy-α-D-glucopyranoside) and β (methyl 2-acetamido-2-deoxy-β-D-glucopyranoside).

Table I. Temperature Dependencies of Amide Proton Chemical Shifts[a]

solvent	α anomer	β anomer	Δ[b]
n-butyl alcohol	−12.1[c] (0.01 M)	−9.3[c] (0.02)	−2.8
isopropyl alcohol	−11.5[c] (0.02 M)	−8.7[c] (0.02 M)	−2.8
ethanol	−10.2[c] (0.04 M)	−7.5[c] (0.03 M)	−2.7
methanol	−8.9[c] (0.07 M)	−6.6[c] (0.08 M)	−2.3
H_2O[d]	−9.6[e] (0.1 M)	−7.7[e] (0.1 M)	−1.9
HFIP	−3.8[c,f] (0.2 M)	−7.1[c,f] (0.2 M)	3.3
	−4.1[f] (0.009 M)	−7.2[c,f] (0.009 M)	3.1
HFB	−9.1[c] (0.09 M)	−11.1[c] (0.05 M)	2.0
TFE	−6.7[f] (0.2 M)	−9.6[f] (0.2 M)	2.9
	−6.3[c] (0.04 M)	−9.4[c] (0.03 M)	3.1
DMSO-d_6[h]	−6.6[g] (0.2 M)	−3.9[g] (0.2 M)	−2.7
	−6.7[g] (0.008 M)	−4.0[g] (0.001 M)	−2.7
DMF	−8.4[c] (0.06 M)	−5.6[c] (0.03 M)	−2.8
dioxane-d_8	−5.7[c] ( )[i]	−4.7[c] ( )[i]	−1.0
acetonitrile-d_3	−3.7[g] (0.004 M)	−3.3[g] (0.004 M)	−0.4

[a] In ppb/deg. Values in parentheses are concentrations in molarity. In most cases the amide peak was a doublet and the reported values are the average of the two peaks. In five cases the measurements were repeated two or three times; reproducibility was ±0.2 ppb/deg. [b] Value for α minus value for β. [c] Chemical shifts referenced against internal TMS. [d] D_2O/H_2O (50/50) adjusted with DCl to an apparent pH of 3.7. [e] Chemical shifts referenced against TSP, sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4. [f] Chemical shifts were referenced by setting the chemical shift of a nonexchangeable solvent or solute proton to the value found against TMS in a solution of the same sugar in the same solvent at the same temperature. [g] Chemical shifts referenced against literature value for residual protons of deuterated solvent. [h] Previously[6] we reported slightly higher values for α in DMSO: −7.1 (0.01 M). We attribute the discrepancy to the fact that in the earlier work we erroneously used the temperature correction determined at 298 K for all five temperatures. [i] Concentrations were less than 0.02 M and close to saturating.

of amide-substituted sugars, sugars that are components of a variety of important biomolecules,[7] and (3) improve our knowledge of the effects of solvent upon conformation. In addition to elucidating our $\Delta\delta/\Delta T$ values, knowledge of any dependence of exchange rates upon anomeric configuration in monosaccharides is also of prime importance to the interpretation of exchange rates in conformational studies of more complex carbohydrates.[8] The possibility of such a dependence has been noted in one such study.[8a]

Experimental Procedures and Results

Materials. All compounds were from commercial sources. Non-deuterated solvents were of spectrophotometric grade.

NMR $\Delta\delta/\Delta T$ Values. All spectra were taken on an IBM Instruments, Inc. 200-MHz AF spectrometer with an Aspect 3000 computer and a variable-temperature unit. Measurements were made at five temperatures from 298 to 318 K. Actual temperatures were determined with a sealed ethylene glycol standard sample.[9] The actual temperatures differed from the nominal temperatures, but repeated calibrations were unnecessary because the deviations were reproducible. Calibration was essential as use of the nominal temperatures gave errors in $\Delta\delta/\Delta T$ values of as much as 2 ppb/deg. Measurements with non-deuterated solvents were made unlocked; good reproducibility of $\Delta\delta/\Delta T$ values showed that field drift was not a problem. In some non-deuterated solvents, signal to noise was improved by presaturating the solvent peak or peaks. Digital

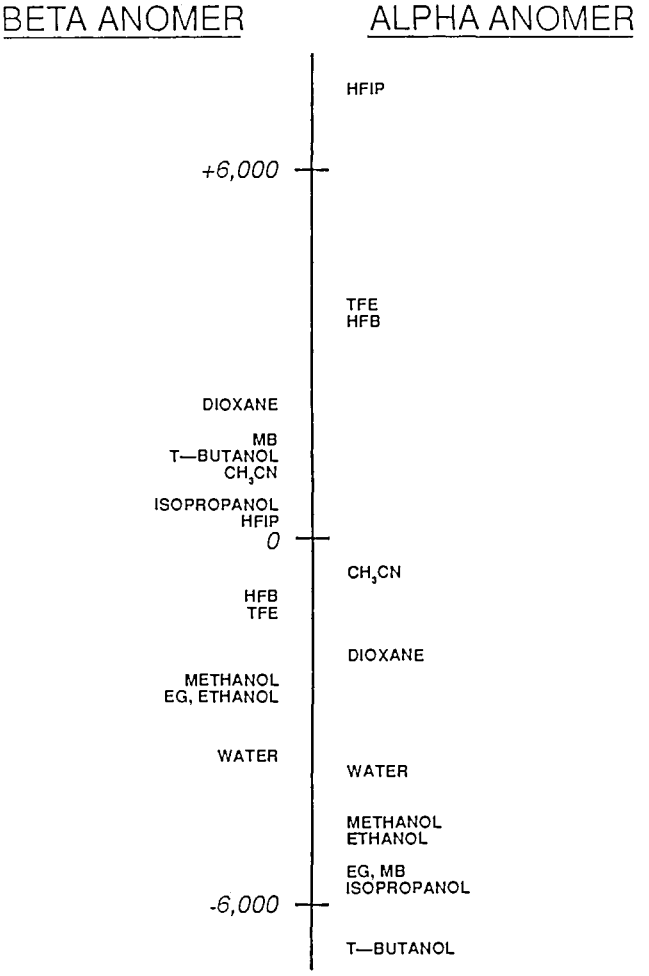


Figure 2. Molar ellipticities (vertical axis) of α (right) and β (left) in various solvents. EG, ethylene glycol; MB, 3-methyl-2-butanol.

Table II. Molar Ellipticities × 10^{-3} (deg cm^2 dmol^{-1})[a]

solvent	α anomer	β anomer	Δ[b]
tert-butyl alcohol	−6.8 (2, 216 nm)	+1.3 (2, 213 nm)	−8.1
isopropyl alcohol	−5.7 (3, 214 nm)	+0.8 (2, 214 nm)	−6.5
3-methyl-2-butanol	−5.5 (2, 214 nm)	+1.6 (2, 214 nm)	−7.1
ethylene glycol	−5.5 (3, 214 nm)	−2.4 (3, 208 nm)	−3.1
ethanol	−4.9 (2, 212 nm)	−2.4 (2, 209 nm)	−2.5
methanol	−4.7 (3, 214 nm)	−2.1 (2, 211 nm)	−2.6
water	−3.7 (3, 211 nm)	−3.4 (3, 209 nm)	−0.3
HFIP	+7.5 (2, 209 nm)	+0.4 (5, 209 nm)[c]	+7.1
HFB	+3.2 (2, 209 nm)	−0.6 (2, 213 nm)	+3.8
TFE	+3.6 (2, 211 nm)	−0.8 (2, 211 nm)	+4.4
dioxane	−1.8 (5, 219 nm)	+2.5 (2, 215 nm)	−4.3
acetonitrile	−0.5 (3, 230 nm)	+1.1 (4, 215 nm)	−1.6

[a] Values are the average of measurements on two or more solutions; the number of solutions is in parentheses and is followed by the wavelength of the extremum; solution concentrations ranged from 0.001 to 0.011 M. Reproducibility was ±0.5 for the first seven solvents and ±0.2 for the remaining solvents. [b] Molar ellipticity of α anomer minus molar ellipticity of β anomer. [c] At high concentrations (0.1 M and greater) there was an extremum at 225 nm with a molar ellipticity of about −0.01. No significant changes with concentration were observed in $\Delta\delta/\Delta T$ or in the coupling constant.

(7) Ginsburg, V.; Robbins, P. W. *Biology of Carbohydrates*; Wiley: New York, 1984; Vol. 2.

(8) (a) Oberholtzer, J. C.; Englander, S. W.; Horwitz, A. F. *Biochemistry* **1981**, *20*, 4785–4792. (b) Huang, D. H.; Pritchard, D. G.; Sakai, T. T.; Krishna, N. R. *Biochem. Biophys. Res. Commun.* **1987**, *143*, 685–690.

(9) Raiford, D. S.; Fisk, C. L.; Becker, E. D. *Anal. Chem.* **1979**, *51*, 2050–2051.

Table III. Hydrogen to Deuterium Exchange Rates

solvent	α anomer k_{ps}[a]	α anomer k[b]	β anomer k_{ps}[a]	β anomer k[b]	ratio[c]
ethanol, 5 °C[d]	0.48	2.8×10^{-2}	0.94	5.5×10^{-2}	2.0
H_2O, pD 2.5, 25 °C	0.71	2.2×10^{2}	1.1	3.4×10^{2}	1.5
H_2O, pD 5.0, 25 °C	1.4	1.0×10^{10}	3.3	2.4×10^{10}	2.4
TFE, 0 °C	0.0047	3.3×10^{-4}	0.023	1.6×10^{-3}	4.9

[a] In min^{-1}; k_{ps} is the pseudo-first-order rate constant. Values were reproducible to within 8% for α and β at pD 2.5 and for α at pD 5.0 but were to within 40% for β at pD 5.0, for α and β in ethanol, and for α in TFE, and to within 55% for β in TFE. Values in ethanol are the average of seven to nine trials, those in water of two to three trials, and those in TFE of three trials. [b] In M^{-1} min^{-1}. k the is second-order rate constant: for ethanol and TFE $k = k_{ps}$/(solvent molarity), for D_2O of pD 2.5 $k = k_{ps}/10^{-pd}$, for D_2O of pD 5.0 $k = k_{ps}/10^{(-14.87+pD)}$.[10,21] Plots of log k_{ps} versus pD had slopes of about −1 at pD 2.5 and +1 at pD 5.0 establishing[8a] that catalysis at pD 2.5 is predominantly acid catalysis while that at pD 5.0 is predominantly base catalysis (assuming $k_{ps} = k_{Acid}[D^+] + k_{Base}[OD^-] + k_{Water}$). [c] (k of β)/(k of α). [d] The isotope effect associated with the fact that exchange in ethanol was D → H while that in water and trifluoroethanol was H → D is of little concern because we are interested in comparisons between α and β under a given set of conditions.

resolution was usually 0.12 Hz/point but was sometimes 0.3 Hz/point.

$\Delta\delta/\Delta T$ values were determined by a least-squares fit of data to $\delta = (\Delta\delta/\Delta T)T + b$. Differences between the δ calculated and δ observed were usually less than twice the digital resolution. Results are summarized in Table I.

Circular Dichroism. Measurements (Table II, Figure 2) were at room temperature on a Jasco J-41C spectrometer using the digital controller. Cell path lengths were either 1 or 0.1 mm. The number of scans ranged from 4 to 32. Base lines were recorded for each sensitivity by using a cell with solvent or with no cell in the sample chamber.

Hydrogen to Deuterium Exchange Rates. UV Method. In water and in ethanol the UV method of Englander[8a,10] was used. The spectrophotometer was a Perkin-Elmer Lambda 5 with a Model 3000 Data Station. Buffers were 0.01 M glycine (pH 2.0–3.5), 0.01 M acetate (pH 4.0–6.0), and 0.1 M NaCl. pD was adjusted with DCl. About 3 mL of ethanol or deuterated buffer was brought to thermal equilibrium in the spectrophotometer's thermostated cell holder. The reaction was initiated with the addition from a Finnpipette of 0.10–0.15 mL of a 0.04–0.15 M solution of α or β in H_2O or ethanol-d_1. About 15 s elapsed from initiation to first reading. A BASIC data acquisition program was written to read and store 50–100 absorbance values at 220 nm at intervals of 1–3 s. Total changes in absorbance were from 0.03 to 0.06. A nonlinear regression program, FLEXFIT, was used to fit the data to the equation $A = A_0 + (A_0 - A_\infty)e^{-k_{ps}t}$, where A is the absorbance at time t, A_0 and A_∞ are the absorbances at zero and infinite time and k_{ps} is the pseudo-first-order rate constant. In the fit A_0, A_∞, and k_{ps} were all adjustable parameters. The results are in Table III.

Hydrogen to Deuterium Exchange Rates. NMR Method. One gram of the solvent, TFE-d_3, and 1–2 mg of sugar (dry or dissolved in 0.1 mL of TFE) were separately brought to thermal equilibrium in a −20 °C NaCl/ice bath. After being mixed in an NMR tube, the sample was placed in the probe, which was at 0 °C. The time elapsed from the beginning of mixing to the acquisition of the first spectrum was ~1 min. About 20 spectra of eight scans each were taken over a 60–90-min time period. The first spectrum was not used in the calculation of rate constants because the chemical shift of the solvent hydroxyl showed that the temperature was not yet 0 °C. A linear least-squares fit of the data to $\ln I/I_0 = -k_{ps}t$ (t, time; I, height of amide peak; I_0, height of amide methyl peak; k_{ps}, first-order rate constant) yielded the rate constants given in Table III.

IR Measurements. All spectra were taken on a Nicolet 5MX FT-IR spectrometer. Concentrations were generally from 0.01 to 0.6 M. Cells were either sealed CaF_2 (paths of 0.015 and 0.05 mm) or IRTRAN-2 (ZnS) demountable (0.015-mm spacers). When necessary, obscuring solvent peaks were removed by subtracting a spectrum of the solvent with the subtraction mode of the spectrometer. Table IV lists the absorption frequencies.

Table IV. Amide I and II Vibrational Frequencies for α and β[a]

solvent	amide I α	amide I β	amide II α	amide II β
H_2O	1624–1637	1630–1637	br, 1564	br, 1570
D_2O	1626–1628	1630–1632	1478	1478–1480
HFIP	1628–1630	1630–1636	1535	1543–1545
DMSO-d_6	1667	1672	1551	1555
dioxane-d_8	1676[b]	1680[b]	1520–1541	br, 1545
acetonitrile-d_3	1676[b]	1682[b]	1525	1549

[a] Values are in cm^{-1}. Data in ethylene glycol consisted of broad amide I peaks centered at 1655 cm^{-1} in both α and β. The amide II positions in ethylene glycol were not determined. [b] Additional absorptions at slightly lower frequencies were also observed and may be due to water and/or multiple carbonyl environments.

Discussion

The $\Delta\delta/\Delta T$ measurements (Table I) suggest two categories of solvents: (1) most solvents examined, in which $|\Delta\delta/\Delta T|_\alpha > |\Delta\delta/\Delta T|_\beta$, and (2) the fluorinated alcohols in which $|\Delta\delta/\Delta T|_\alpha < |\Delta\delta/\Delta T|_\beta$.

The variation of $\Delta\delta/\Delta T$ values with solvent could result from solvent-induced changes in conformation, or from differences in solvent properties that do not affect conformation of the sugar, or from some combination of these. CD can help to distinguish between the possibilities because a solvent-induced change in the molar ellipticity signifies a solvent-induced conformational change. The CD data (Table II, Figure 2) for the amide group n → π* transition clearly show that conformation is affected by solvent. The dramatic effect of HFIP upon the CD of amide-substituted sugars has previously been reported[11] and analyzed theoretically.[12] Our data show that in the CD, just as in the $\Delta\delta/\Delta T$ values, the fluorinated alcohols differ from the other solvents; $[\theta]_\alpha > [\theta]_\beta$ in the fluorinated alcohols but $[\theta]_\alpha < [\theta]_\beta$ in the other solvents. This correlation of the effect of solvent upon $\Delta\delta/\Delta T$ values with that upon the CD shows that the $\Delta\delta/\Delta T$ values reflect, at least in part, changes in conformation. In addition, the range of CD values (Figure 2) shows that the two solvent groups suggested by the $\Delta\delta/\Delta T$ values do not correspond to two narrowly defined conformations but rather to a continuum of conformations. Indeed, the conformational flexibility evident in the continuum of CD values suggests that changes in CD with solvent could reflect changes in relative populations of conformers rather than a change from one conformer to another.

With it established by CD that the variation of $\Delta\delta/\Delta T$ with solvent is a function of conformation, the question arises "does the conventional interpretation that greater $|\Delta\delta/\Delta T|$ values signify greater solvent exposure apply here?". Hydrogen to deuterium exchange rates address this question because greater exchange rates are interpreted as meaning greater solvent exposure in carbohydrates[8] and, in a much greater number of studies, in peptides and proteins.[2c,13] The results in Table III show, however, that in all four solvents examined the amide proton of β has a greater exchange rate than does the amide proton of α even though in three of the four solvents β exhibits smaller $|\Delta\delta/\Delta T|$ values than does α.

The lack of a correspondence between greater $|\Delta\delta/\Delta T|$ values and greater exchange rates implies that the difference in exchange rates between α and β and/or the difference in $\Delta\delta/\Delta T$ values between α and β is dominated by some factor other than solvent exposure. We will first consider the relative exchange rates, these being somewhat more directly interpretable than the $\Delta\delta/\Delta T$ values. As described below, this will lead us to suggest that the relative exchange rates but not the relative $\Delta\delta/\Delta T$ values are substantially affected by solvent exposure.

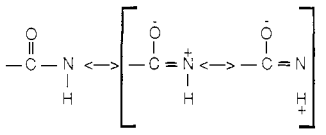
What is the explanation for the faster rate of exchange in the β anomer? The effects upon exchange rates of the inductive and electrostatic properties of amino acid side chains have been long recognized, and these substituent effects are used in the interpretation of exchange rates in peptides and proteins.[13,14] One

(10) Englander, J. J.; Calhoun, D. B.; Englander, S. W. *Anal. Biochem.* **1979**, *92*, 517–524.

(11) Dickinson, H. R.; Coduti, P. L.; Bush, C. A. *Carbohydr. Res.* **1977**, *56*, 249–257.

(12) Yeh, C. Y.; Bush, C. A. *J. Phys. Chem.* **1974**, *78*, 1829–1833.

(13) Barksdale, A. D.; Rosenberg, A. *Methods Biochem. Anal.* **1982**, *28*, 1–113, and references therein.



A B

Figure 3. Amide group resonance structures.[2a]

might consider the entire glucopyranoside ring as a substituent of the amide group and propose that the electron-withdrawing characteristics of the ring differ between α and β. If so, the anomer with the greater acid-catalyzed exchange rate should have the lesser base-catalyzed exchange rate,[13,15] but this is not observed. Another source of explanation for the consistently greater exchange rate of β could be differences between α and β in the electronic structure of the amide group itself due to interactions in α between the amide group and the carbon 1 methoxy group. A greater contribution of the resonance structures B (Figure 3) to β would be consistent with its greater rates. Such an explanation, however, is not consistent with the infrared data.

We suggest instead that in α a hydrogen bond exists between the amide proton and the oxygen of the axial methoxy group at carbon 1. The amide proton is approximately trans to the carbon 2 proton,[16] an orientation consistent with such a hydrogen bond. A similar hydrogen bond is not possible in β, with its equatorial carbon 1 methoxy group, and thus the amide proton of β would be more exposed to solvent and exchange more quickly than that of α.

This hypothesis is consistent with the IR data (Table IV). The IR results resemble those of the exchange studies (and differ from those of the $\Delta\delta/\Delta T$ and CD studies) in that the qualitative behavior of α relative to that of β is the same for all the solvents examined. The α amide proton exchanges more slowly than that of the β anomer and the α anomer amide I and II vibrations are at a lower frequency than those of the β anomer. If the proposed hydrogen bond occurs in α but not in β, then resonance forms B (Figure 3) should contribute more to α than to β. The decreased carbonyl bond order in α would explain the lower frequency of the amide I (carbonyl stretch)[17] in α. The changes in the amide II bond (carbon–nitrogen stretch and nitrogen–hydrogen bend[17]) are more difficult to interpret.[18]

The proposed hydrogen bond could exist within a range of specific geometries and thus be consistent with the variation in conformation with solvent manifest in the CD. The fact that the $|\Delta\delta/\Delta T|$ values are higher than those generally found in hydrogen bonds means that the proposed hydrogen bond must be one that is disrupted or modified by temperature and/or one in which the amide group simultaneously participates in a temperature-dependent interaction with the solvent or carbon 3 hydroxyl. One such temperature-dependent interaction would be hydrogen bonding of the solvent with the amide carbonyl. The lower frequencies of amide I in the hydrogen-bonding solvents water and HFIP compared to those in DMSO, dioxane, and acetonitrile are consistent with such an interaction.

Finally, we note that the trends in $\Delta\delta/\Delta T$ values and in CD correlate with some properties to the solvents. Surely the variation between the CD in HFIP on the one hand and in TFE and HFB on the other is related to the relative hydrogen bond donating abilities of these solvents; the pK_a's are 9.3 for HFIP, 11.4 for HFB, and 12.4 for TFE.[19] The fact that HFIP is a secondary alcohol while the other two fluorinated alcohols are primary may also be important according to the extent that steric factors play a role in the solute–solvent interactions. The trends in $\Delta\delta/\Delta T$ and in $[\theta]$ as solvent changes through the regular alcohols from water to the larger alcohols may be related to dielectric constant or to steric factors. The role of dielectric constant has been suggested by Cohen and Stevens in a recent theoretical calculation of the CD of the 2-acetamido-2-deoxy-D-glucopyranoses and application of the results to our data.[20] We defer detailed discussion of conformational features responsible for these trends until completion of further investigations.

In conclusion, perhaps our most significant result is that the conventional interpretation of high $\Delta\delta/\Delta T$ values (as indicators of exposure to solvent) does not apply to these simple amide-substituted sugars in most solvents, including water, and, therefore, probably does not apply to the many amide-substituted sugars present as components of much larger biomolecules.

Acknowledgment. We acknowledge the support of the Research Corporation and the donors of the Petroleum Research Fund, administered by the American Chemical Society. The purchase of the NMR spectrometer was supported by the National Science Foundation under Grant No. CHE-8409822 and by the 3M Chemistry Research Fund Grant to Carleton College. We also thank Dr. Richard Ramette of Carleton College, the author of FLEXFIT, a nonlinear regression and plotting program, Dr. Steve Philson of the University of Minnesota Department of Chemistry for preliminary NMR measurements, and the University of Minnesota Department of Biochemistry for the use of their CD spectrometer. Preliminary presentation of portions of this work was through a poster at the XIII International Carbohydrate Symposium, August 1986.

Registry No. H_2, 1333-74-0; methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, 6082-04-8; methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, 3946-01-8.

(14) (a) Roder, H.; Wagner, G.; Wuthrich, K. *Biochemistry* **1985**, *24*, 7407–7411. (b) Krishna, N. R.; Heavner, G. A.; Vaughn, J. B., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 6930–6934.

(15) Perrin, C. L.; Arrhenius, G. M. L. *J. Am. Chem. Soc.* **1982**, *104*, 6693–6696.

(16) (a) Bush, C. A.; Duben, A.; Ralapati, S. *Biochemistry* **1980**, *19*, 504–512, and references therein. (b) Cowman, M. K.; Cozart, D.; Nakanishi, K.; Balazs, E. A. *Arch. Biochem. Biophys.* **1984**, *230*, 203–212.

(17) (a) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; W. H. Freeman: San Francisco, CA, 1980; Part II, p 468. (b) Joesten, M. D.; Schaad, L. J. *Hydrogen Bonding*; Marcel Dekker: New York, 1974; pp 3, 269–270. (b) Pimentel, G. C.; McClellan, A. L. *The Hydrogen Bond*; W. H. Freeman: San Francisco, CA, 1960; pp 118, 124–125.

(18) The carbon–nitrogen stretch contribution would cause resonance forms b, and therefore the α anomer, to have a higher frequency amide II vibration, contrary to our results. Another apparent contradiction between the hypothesized hydrogen bond in α and the amide II IR data is that a hydrogen bond is thought to increase amide II frequency through increased difficulty of N–H bending due to the linearity of the hydrogen bond.[17] In a bifurcated hydrogen bond (amide proton as donor to methoxy oxygen and to solvent oxygen), perhaps the amide II shifts to lower frequency due to easier N–H bending.

(19) Fasman, G. D., Ed. *CRC Handbook of Biochemistry and Molecular Biology*; CRC Press: Cleveland, OH, 1976; Vol. 1, p 315.

(20) Cohen, A. H.; Stevens, E. S. *J. Phys. Chem.* **1987**, *91*, 4466–4470.

(21) Weast, R. C., Ed. *CRC Handbook of Chemistry and Physics*, CRC Press: Boca Raton, FL, 1981; p D-145.