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Solution dynamics of the 1,2,3,4,6-penta-*O*-acetyl-*α*-D-idopyranose ring

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The anticoagulant properties of heparin are thought to derive from the inhibition of thrombin and other coagulation-related proteases by the binding of heparin to cofactors such as antithrombin III and heparin cofactor II. The apparent minimum native heparin sequence which can bind to antithrombin III is a highly sulfated pentasaccharide which contains a 2-*O*-sulfo-*α*-L-idopyranosyluronic acid residue. The idopyranosyl residue has the unusual property of existing in the solution state as a mixture of ring conformers. Whereas most hexopyranose sugars exist as a single chair conformer (*eg* D-glucose exists overwhelmingly as a $^4C_1$ chair), the idopyranosyl ring is known to rapidly exchange between at least two and often more distinct conformations, depending on type and number of substituents (hydroxyl, carboxyl, sulfate, *etc.*) and solvent conditions (solvent pH, salt concentration, temperature). It is believed that this flexibility of the idopyranosyl residue in heparin is related to its binding specificity.

In the past, coupling constants and molecular dynamics have been used to estimate the relative populations of conformers in iduronate and related compounds. Here we report extensive NMR measurements, including line shape analysis, $T_{1\rho}$ measurements, T_1 and NOE measurements and spectral density mapping, which have been used to study the dynamics of conformer interconversion in model compounds related to idose and glucose. The findings presented here indicate that 1,2,3,4,6-penta-*O*-acetyl-*α*-D-idopyranose can be reasonably well described as existing in a two-state equilibrium consisting of the $^4C_1$ and $^0S_2$ conformers. ^{13}C NMR line shape analysis yields a $\Delta H^{\dagger}$ of 40 kJ mol^{-1} and a $\Delta S^{\ddagger}$ of 31 J mol^{-1} K^{-1} for the $^4C_1 \rightarrow {}^0S_2$ interconversion and a $\Delta H^{\ddagger}$ of 31 kJ mol^{-1} and a $\Delta S^{\ddagger}$ of 13 J mol^{-1} K^{-1} for the $^0S_2 \rightarrow {}^4C_1$ interconversion. This corresponds to exchange rates of 22 and 128 MHz, respectively, at room temperature.

***Keywords*: heparin, 1,2,3,4,6-penta-*O*-acetyl-*α*-D-idopyranose, solution dynamics**

Introduction

The vast majority of hexopyranose sugars exist in the solution state in a single conformation, typically either a $^1C_4$ or $^4C_1$ chair. Idopyranose, however, is unusual in that it is known to exist in a rapid conformational equilibrium among several ring conformations. Evidence for this can be found from both energy calculations [1–3] and $^3J_{HH}$ coupling constant analyses [2, 4, 5].

Derivatives of idose, principally the non-sulfated and the 2-O-sulfated α-L-idopyranose uronic acid moieties, are found in heparin, heparan sulfate and dermatan sulfate, and it has been postulated that the flexible nature of the idopyranose ring may be related to the protein-binding specificity of these compounds [6]. The utility of heparin derivatives as antithrombotic agents, and the finding that the iduronate residue is a necessary component of an antithrombin III-specific pentasaccharide [7] has resulted in intense study of the conformational properties of the idopyranose ring.

Studies of the dynamics of the ring conformer interconversions are much less common. That only one resonance is observed for each nucleus (^{13}C or ^1H) under all solution conditions tested to date indicates that the conformation interconversions are occurring at a frequency sufficient to average chemical shifts. This places a lower limit on the interconversion rate of roughly 1 MHz at room temperature. An upper limit of approximately 10 gHz can be inferred from molecular dynamics studies [8] and order parameter analysis [9].

Here, we present the first experimental determination of a two-state interconversion rate for 1,2,3,4,6-penta-*O*-acetyl-α-D-idopyranose in acetone.

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Methods

Samples were prepared as mixtures of 0.5 M each 1,2,3,4,6-penta-*O*-acetyl-α-D-idopyranose [α-D-Ido(OAc)$_5$] and 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose [α-D-Glc(OAc)$_5$] (Sigma, St Louis, MO) in 700 µl of (1) acetone-d$_6$, (2) a 65: 35 (by vol) mixture of acetone-d_6 and methanol-d_4 or (3) DMSO-d_6. All solvents were purchased from Cambridge Isotope Laboratories (Woburn, MA). Spectra were acquired over temperature ranges of 163 to 298 K (acetone), 133 to 183 K (acetone: methanol) or 288 to 363 K (DMSO) at 11.7 T on a Varian UNITY 500 NMR spectrometer. Data were processed off-line using standard Varian (VNMR 4.1 s) software and analysed using either the statistical functions of Axum 3.0 (Trimetrix, Seattle) or with software written in-house. MathCAD 3.1 (MathSoft, Cambridge, MA) was also used for modelling of the spectral density values.

^{13}C relaxation parameters were determined by direct observation of the ^{1}H-decoupled ^{13}C nuclei. Non-selective T_1 measurements were performed using the inversion-recovery experiment with a preacquisition delay of at least five times the longest T_1 of interest in the sample. Carbon-proton NOE's were measured by comparison of two ^{13}C spectra, one acquired with ^{1}H saturation during the preacquisition delay and one acquired with no ^{1}H saturation. T_2 measurements utilized the CPMG[10] sequence with additional ^{1}H π pulses inserted in the spin-lock on even ^{13}C echoes to minimize the effects of CSA-dipolar cross correlation [11]. Longitudinal and transverse two-spin order relaxation rates were determined by one-dimensional, direct-detected versions of the analogous sequences described by Peng and Wagner [12]. Proton decoupling was accomplished using the GARP1 [13] decoupling scheme with a field strength of 4000 Hz. A ^{1}H–^{1}H NOESY was acquired at 298 K using a mixing time of 300 ms and a recycle delay of 6 s. Measurement of $^3J_{HH}$ was aided by selective ^{1}H-{^{1}H} decoupling experiments as the majority of the three-bond coupling constants for α-D-Ido(OAc)$_5$were fairly small (2–4 Hz). ^{13}C-line widths for α-D-Glc(OAc)$_5$ and α-D-Ido(OAc)$_5$ were measured from one-dimensional, ^{1}H-decoupled, ^{13}C spectra. Exchange contributions to the α-D-Ido(OAc)$_5$ line width were obtained by subtracting the average α-D-Glc(OAc)$_5$ line width at the same temperature. The use of a mixture eliminates field inhomogeneity contributions to the determination of the exchange portion of the line width.

Monte Carlo simulations and molecular mechanics calculations were performed using the MM3*(90) force field with a GB/SA solvent approximation as implemented in MacroModel 3.5a [14] running on an IBM RS/6000-320 workstation as has been described previously [9]. $^3J_{HH}$ for the three lowest energy conformations of α-D-Ido(OAc)$_5$ (^{0}S$_2$ skew-boat and the ^{1}C$_4$ and ^{4}C$_1$ chairs) were calculated using the Karplus relationship developed for carbohydrates by Haasnoot *et al.* [2]. Our coupling constant results are comparable to those obtained by Bhacca *et al.* [15] for this same compound.

The relative populations at 298 K of the various conformational isomers of α-D-Ido(OAc)$_5$ were estimated by comparing experimental NOEs with those calculated for these conformers. Since the conformation exchange for idopyranose sugars is fast on the NMR relaxation time scale, one must use population-weighted relaxation matrices, rather than population-weighted NOE intensities, in such an analysis [8]. The H-1/H-2, H-2/H-3 and H-4/H-5 distances, which vary little among the three sugar conformers were used as distance standards. Because of the unknown rotameric distribution of the C-5/C-6 exocyclic bond, calculations of NOEs to the H-6 protons were made assuming a three-site jump among equally populated rotamers.

Analysis of $^3J_{HH}$ and the NOESY indicated that only the ^{4}C$_1$ and ^{0}S$_2$ conformers were present to any significant degree. Consequently, conformer populations over the temperature range of 263 to 353 K were estimated by minimizing the differences between the observed $^3J_{HH}$ for H-2/H-3 and H-3/H-4 and those calculated using these two conformers only. These calculated populations yield an equilibrium constant (K_{eq}) for each of the sampled temperatures. Equilibrium thermodynamic parameters (ΔH° and ΔS°) were obtained from a least squares fit of $\ln(K_{eq})$ vs. 1/T. These values for ΔH° and ΔS° were then used to estimate populations at lower temperatures where line widths became too broad to allow measurement of $^3J_{HH}$. Because we were unable to observe, at any temperature, separate resonances for the various isomers, we were unable to measure the chemical shift differences (δ) for each nucleus between conformers. We have assigned a value of 1 kHz for the chemical shift difference between isomers for C-4 and scaled δ for the other carbon atoms based on their line broadening relative to C-4 (a $\Delta\delta$ of 1 kHz is typical of the difference in ^{13}C chemical shifts between axially and equatorially substituted cyclohexyl compounds). The activation enthalpies ($\Delta H^\ddagger$) reported here are independent of the magnitude of this chemical shift difference; only the activation entropies ($\Delta S^\ddagger$) will be affected. Exchange rate constants were extracted from the exchange contribution to the α-D-Ido(OAc)$_5$ ^{13}C line widths using the analytical solutions to the McConnell-modified Bloch equations [16] as presented by Sandstrom [17]. $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were then determined *via* the Eyring equation.

Error estimates were determined for the spectral density values as described by Peng and Wagner [12] using as errors in the relaxation rates those reported by the VNMR data analysis software. Errors in the thermodynamic parameters were determined by the Monte Carlo approach [18]. Five hundred sets of $^3J_{HH}$ coupling constants and exchange-broadened line widths were constructed from Gaussian distributions centered on the observed values with standard deviations of 0.2 Hz and the standard deviation in

the average α-D-Glc(OAc)$_5$ line width ($\sim$5%), respectively. For each set, ΔH° and ΔS° were calculated as described above and populations at lower temperatures were estimated. Forward and reverse rate constants were then calculated and $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were obtained. The reported errors in the parameters are one standard deviation from the average value of each parameter.

Results

The NOESY spectrum is indicative of conformer populations for α-D-Ido(OAc)$_5$ of 82% $^4C_1$, 14% $^0S_2$ and 4% $^1C_4$ at 298 K (Table 1). $^3J_{HH}$ data at 298 K are consistent with these conformer populations (data not shown). Given this, the kinetic analysis of the conformer interconversion was carried out using a two-state model. K_{eq} for the $^4C_1 \rightleftharpoons {}^0S_2$ equilibrium was estimated from the $^3J_{HH}$ at nine temperatures between 263 and 353 K. From a least squares fit of $\ln K_{eq}$ *vs.* 1/T we have obtained $\Delta H^\circ = 10\ \text{kJ mol}^{-1}$ and $\Delta S^\circ = 19\ \text{J mol}^{-1}\ \text{K}^{-1}$ (Figure 1).

We have observed no differences between α-D-Ido(OAc)$_5$ and α-D-Glc(OAc)$_5$ T_1s and NOEs over temperatures ranging from 163 to 313 K (Figure 2). This indicates that conformational reorientation is not occurring significantly faster than molecular tumbling. This is in accord with previous studies of L-idopyranose [9] which have indicated that the ring conformation interconversion is too slow to affect order parameter values. Likewise, no significant differences were observed between the spectral density maps for the two sugars. Figure 3 shows the spectral densities determined for α-D-Ido(OAc)$_5$ and α-D-Glc(OAc)$_5$. They are both consistent with isotropic motion and a correlation time of approximately 100 ps, and are indistinguishable from each other within experimental error. This again indicates that, at frequencies faster than molecular reorientation, the two sugars exhibit similar dynamic behavior and places a lower limit, on the order of 100 ps, on any conformational state life time.

$T_{1\rho}$ experiments detected no field-strength dependence of the ^{13}C rotating frame relaxation rates. This arises because the conformational exchange rate regime which yields r.f. field strength sensitive relaxation rates falls within a temperature range where exchange-induced line broadening renders accurate peak integration difficult.

Substantial differences in ^{13}C line widths between α-D-Ido(OAc)$_5$ and α-D-Glc(OAc)$_5$ were observed over temperatures ranging from 273 K to 168 K. The exchange contributions to the line width for each ring carbon are shown in

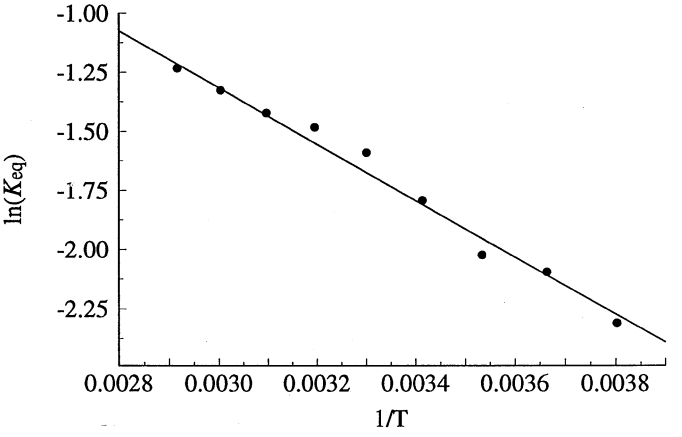

Figure 1. ln K_{eq} vs. 1/T for the two-state $^4C_1 \rightleftharpoons {}^0S_2$ equilibrium. The reciprocal temperature abscissa corresponds to 353–263 K. The best-fit line has a slope of $-\Delta H^0/R$ and an intercept of $-\Delta S^0/R$.

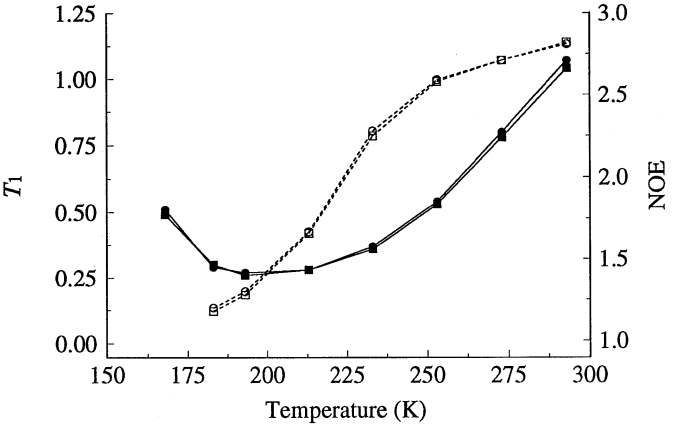

Figure 2. Comparison of ^{13}C T_1 (solid symbols) and NOE (empty symbols) between α-D-Glc(OAc)$_5$ (circles) and α-D-Ido(OAc)$_5$ (squares) over the temperature range of 168 to 293 K. Values are averaged over all ring carbons (standard deviations ranged from 0.01 to 0.07).

Table 1. Calculated distances for three conformers of α-D-Ido(OAc)$_5$ † best-fit population-weighted final distance ($\langle 1/r \rangle^6 = (p_1/r_1^6 + p_2/r_2^6 + p^3/r_3^6)$ for ring populations $p_1 : p_2 : p_3$ of 0.82 : 0.14 : 0.04 ($^4C_1 : {}^0S_2 : {}^1C_4$); and observed and calculated negative percent NOE for these populations.

^1H pair	Distances (Å)				NOE	
	$^4C_1$	$^0S_2$	$^1C_4$	$\langle r \rangle^\dagger$	Obs.	Pred.
H-1/H-2	2.55	3.04	3.02	2.60	1.32	1.07
H-1/H-3	4.27	2.75	2.70	3.48	0.15	0.19
H-1/H-4	4.91	4.50	3.99	4.77	0.04	0.03
H-1/H-5	3.67	3.66	3.72	3.67	0.11	0.14
H-1/H-6	4.67	4.62	2.69	4.14	0.13	0.13
H-2/H-3	2.54	3.02	3.03	2.59	1.10	1.10
H-2/H-5	4.08	2.51	3.97	3.32	0.40	0.25
H-2/H-6	5.78	4.84	4.82	5.50	0.00	0.02
H-3/H-5	3.85	3.77	3.91	3.84	0.15	0.10
H-3/H-6	5.12	4.57	2.93	4.48	0.08	0.08
H-4/H-5	2.42	2.23	2.39	2.39	1.37	1.79
H-5/H-6	3.06	3.23	3.81	3.10	0.59	0.76

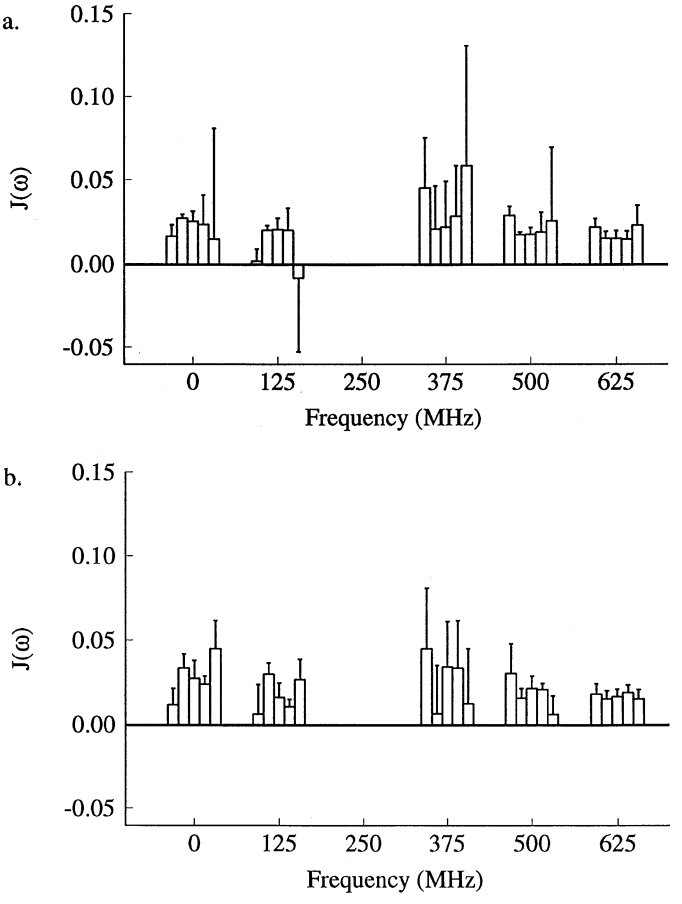

Figure 3. Spectral density maps. a. *α*-D-Glc(OAc)$_5$, b. *α*-D-Ido(OAc)$_5$. Ring carbons 1–5 are represented left to right within each cluster. Error was calculated as described in text.

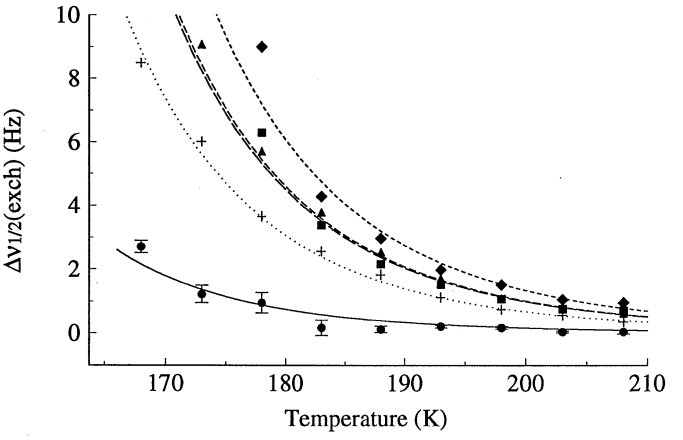

Figure 4. Observed (symbols) and calculated (lines) exchange component of the *α*-D-Ido(OAc)$_5$ line width as a function of temperature. C-1, ● and solid line; C-2, ■ and long dashed line; C-3, ▲ and medium dashed line; C-4, ◆ and short dashed line; C-5, + and dotted line. Error bars plotted with C-1 represent the standard deviation of the *α*-D-Glc(OAc)$_5$ ring carbons at the corresponding temperature.

Table 2. Thermodynamic and kinetic parameters determined from $^3J_{HH}$ and line shape analysis of *α*-D-Ido(OAc)$_5$ over the temperature range of 353 to 173 K.

Parameter	$^0S_2 \rightarrow {}^4C_1$	$^4C_1 \rightarrow {}^0S_2$
$\Delta H^{\ddagger}$ (kJ mol^{-1})	31 (2)	40 (5)
$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	13 (8)	31 (16)
k (273 K)	128×10^6	22×10^6
ΔH° (kJ mol^{-1})	− 10 (2)	10 (2)
ΔS° (J mol^{-1} K^{-1})	− 19 (7)	19 (7)

Figure 4. Each value was determined by subtracting the average line width of the α-D-Glc(OAc)$_5$ ring carbons, which are not affected by exchange processes. As these measurements represent the difference in line width between sugars in a single, mixed sample, they are independent of contributions from B_0 inhomogeneity or sample differences and are a good estimate of the exchange contribution to the line widths. Energetic parameters and errors, based on an estimated error of 0.2 Hz for the $^3J_{HH}$, are presented in Table 2.

Discussion

This work represents the first experimental determination of the rate of conformational interconversion in idopyranose derivatives. Previous investigations have suggested conformational equilibria between multiple ring conformations [3, 5] and have placed limits on the allowed time scales for interconversion [9], but no kinetic parameters have been reported. Dynamics calculations have suggested that the time scale for interconversion may be in the order of nanoseconds [8]. The results presented here indicate a major conformer life time in the order of 50 ns at ambient temperatures.

The determination of both type and population of idopyranose ring conformations has been the subject of some controversy [19]. This situation exists because of the possibility of interconversion among the two chair forms and numerous skew-boat conformers [8, 20, 21] and the dearth of useful NMR parameters: a maximum of four vicinal ^{1}H coupling constants and NOE data which are compromized by motional averaging [20]. Further, the conformer equilibrium is very sensitive to ring substituent type and solvent conditions, and both the Karplus relationship between coupling constants and dihedral angles and the available molecular mechanics force fields are relatively ill-parameterized for charged carbohydrates. Published estimates for the $^3J_{HH}$ for the three ring conformations considered here can vary by as much as 0.5 Hz.

We have attempted to integrate multiple methodologies to deal with some of these difficulties. Only the three

low-energy conformations from the molecular mechanics calculations were considered in the population analysis. These three conformers encompassed all conformers found within 25 kJ mol^{-1} of the overall energy minimum. Coupling constant data were combined with NOE data to ascertain the relative populations of the conformers. Both of these data sets are consistent with a $^4C_1 \rightleftharpoons {}^0S_2$ equilibrium. It is worth noting that these experiments were carried out on D-sugars. This will have no effect on the thermodynamic parameters determined, but will change the labelling of the conformations involved in the exchange. The L-sugar equivalent to the $^4C_1 \rightleftharpoons {}^0S_2$ equilibrium is $^1C_4 \rightleftharpoons {}^2S_0$.

We have been unable to observe separate resonances for any conformer at any temperature in the 200 K range studied (133–363 K). This is problematic, as the relationship of line width and exchange rate is dependent on the chemical shift difference between exchanging conformers. The use of 1 kHz as the maximum $\Delta\delta$ is a reasonable estimate, given prior studies on carbohydrates and other hexocyclic compounds. As it is assumed constant over temperature, the precise value chosen for $\Delta\delta$ will affect the calculated value for $\Delta S^{\ddagger}$ but not $\Delta H^{\ddagger}$. Use of a $\Delta\delta$ for C-4 of 500 Hz, for instance, results in a $\Delta S^{\ddagger}$ of 19 J mol^{-1} K^{-1} for the $^4C_1 \rightarrow {}^0S_2$ interconversion. It will, as such, affect the calculated value of $\Delta G^{\ddagger}$ to an extent dependent on the magnitude of $T\Delta S^{\ddagger}$.

Of greater concern is the effect of the application of the two-state equilibrium model when it is possible that the system exists as a combination of three conformers. Although this introduces an uncertainty into the reported results, two factors serve to possible minimize the impact of the third conformer. First, the population of this conformer is small compared to the populations of the two other conformers. This will decrease the effect of exchange on line width, as the exchange contribution to the line width is dependent on the square of the populations. Second, exchange between the $^0S_2$ and $^1C_4$ conformers will only affect the observed line width if it is occurring at the same frequency as or faster than the exchange between the $^4C_1$ and $^0S_2$ conformers. Calculations (not shown) have indicated that such exchange may decrease the observed $\Delta H^{\ddagger}$ by 2–4 kJ mol^{-1}, implying that the values listed in Table 4 may be at most several kJ mol^{-1} too low.

The parameters determined here can be compared with those determined for similar compounds. The activation enthalpy is smaller than those determined for cyclohexane ($\Delta H^{\ddagger} = 45$ kJ mol^{-1} [22]), oxane ($\Delta H^{\ddagger} = 43$ kJ mol^{-1} [23]) and dioxane ($\Delta H^{\ddagger} = 43.1$ kJ mol^{-1} [24]) but similar to that observed for 4-hydroxytetrahydropyran ($\Delta H^{\ddagger} = 37$ kJ mol^{-1}; Hajduk and Horita, unpublished results), 2,2-dimethoxyoxane ($\Delta G^{\ddagger} = 36.4$ kJ mol^{-1} [25]) and other 2-alkoxy- and 2-aryloxytetrahydropyrans ($\Delta G^{\ddagger}$ between 34.3 and 37.2 kJ mol^{-1}, depending on substituent [26]). The 2-alkoxy- and 2-aryloxytetrahydropyrans are the only compounds of those listed which have an anomeric effect. It has been suggested that the anomeric effect can decrease the barrier to interconversion in oxane systems via stabilization of the transition state [25, 26]. These data are consistent with this suggestion. However, the number and orientation of the substituents in ringed systems can significantly affect (by more than 10 kJ mol^{-1}) the free energies of activation [27]. A more detailed investigation of other carbohydrate systems would be required to investigate the dynamical effects of substituents on the idopyranose ring.

Conclusions

This work represents the first experimental determination of the rate of conformational interconversion in idopyranose derivatives. Previous investigations have placed limits on the allowed time scales for interconversion, but no kinetic parameters have been reported. Dynamics calculations have suggested that the time scale for interconversion may be in the order of nanoseconds [8]. The results presented here indicate a major conformational life time in the order of 50 ns at ambient temperatures.

Molecular mechanics calculations indicate that three low energy ring conformations exist for α-D-Ido(OAc)$_5$: the $^4C_1$ and $^1C_4$ chair forms and the $^0S_2$ skew-boat form. ^{1}H-NOE's and $^3J_{HH}$ suggest an equilibrium consisting mainly of the $^4C_1$ and $^0S_2$ forms, and only these two conformers were considered in the analysis of $^3J_{HH}$ and line broadening data.

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