Acyclic Forms of [1-^{13}C]Aldohexoses in Aqueous Solution: Quantitation by ^{13}C NMR and Deuterium Isotope Effects on Tautomeric Equilibria

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High-resolution ^{13}C NMR spectra (150 MHz) have been obtained on the complete series of D-aldohexoses (D-allose **1**, D-altrose **2**, D-galactose **3**, D-glucose **4**, D-gulose **5**, D-idose **6**, D-mannose **7**, D-talose **8**) selectively labeled with ^{13}C at C1 in order to detect and quantify the percentages of acyclic forms, and to measure and/or confirm percentages of furanoses and pyranoses, in aqueous solution. Aldehyde and hydrate signals were detected for all aldohexoses, and percentages of these forms at 30 °C ranged from 0.006 to 0.7% (hydrate) and 0.0032 to 0.09% (aldehyde). Aldehyde percentages are largest for the *altro*, *ido*, and *talo* configurations, ranging from 0.01 to 0.09%; the *ido* configuration yielded the most hydrate (0.74%). Hydrate/aldehyde ratios vary with aldohexose configuration, ranging from 1.5 to 13, with *gluco* exhibiting the smallest ratio and *gulo* the largest. ^{2}H Equilibrium isotope effects (EIEs) on aldohexose anomerization were measured in D-galactose **3** and D-talose **8** selectively ^{13}C- and ^{2}H-labeled at C1 and H1. The ^{2}H isotope effect on ^{13}C chemical shift, and broadband ^{1}H- and ^{2}H-decoupling, were exploited to permit simultaneous observation and quantitation of the protonated and deuterated molecules in NMR samples containing equimolar mixtures of D-[1-^{13}C]aldose and D-[1-^{13}C; 1-^{2}H]aldose. Small ^{2}H EIEs were observed for **8**, but were undetectable for **3**. These results suggest that configuration at C2 influences the magnitude of the ^{2}H isotope effect at H1 and/or that the observed effect cannot be reliably interpreted due to complications arising from the involvement of acyclic aldehyde forms as intermediates in the interconversion of cyclic forms. The observed ^{2}H isotope effects on aldohexose tautomeric equilibria provide new insights into the important question of whether ^{2}H substitutions can alter aldofuranose ring conformation, and lead to the identification of an optimal ^{2}H- and ^{13}C-substituted 2-deoxyribofuranose isotopomer on which to investigate this potential effect.

Introduction

It is well-known that aldohexoses **1**−**8** (Scheme 1) generate complex tautomeric equilibria in aqueous solution involving cyclic pyranoses and furanoses and acyclic hydrates and aldehydes (Scheme 2).[1−3] Septanose forms are also possible in substituted aldohexoses,[4] but have not been identified unequivocally in solutions of **1**−**8**. NMR studies using saturation-transfer and line-broadening methods[5−13] have demonstrated that aldose anomerization proceeds via acyclic or pseudoacyclic forms and have yielded unidirectional rate constants (k_{open}, k_{close}) indicating that furanose−furanose exchange is kinetically favored over furanose−pyranose and pyranose−pyranose exchange. While rates of aldofuranose ring-opening appear related to ring configuration (e.g., 1,2-cis effect), structural factors influencing aldopyranose ring-opening rates remain incompletely understood. Tautomeric equilibria of aldoses and k_{close} are influenced by Thorpe−Ingold effects;[12] thus, aqueous solutions of **1**−**8** contain substantially less acyclic forms than those of parent structures such as 5-hydroxypentanal **9**.[14]

While quantitation of the more abundant cyclic forms of **1**−**8** has received much attention,[1−3] percentages of the acyclic forms, especially of the hydrates, have not been fully investigated. This void in the literature is especially noteworthy in light of the fact that the chemical and biological reactivities of aldoses are governed, in part, by tautomeric equilibria in solution, and frequently by the propensity of the aldose to assume an acyclic form. For example, enzymes utilizing aldohexose substrates or inhibitors often discriminate between tautomers,[15] and thus the determination of kinetic constants such as K_m

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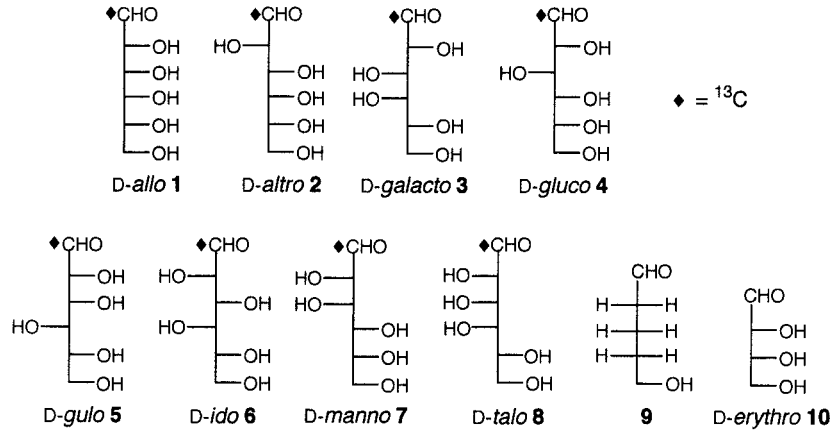
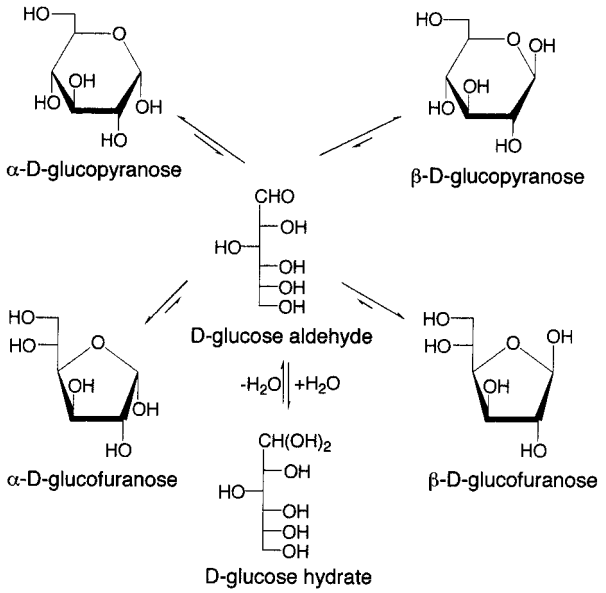
Scheme 1



Scheme 2



and K_i depend on accurate quantitation of forms in solution. Likewise, information on the percentages of acyclic hydrates and aldehydes of **1**−**8** under identical solution conditions, which is presently unavailable, may lead to a better understanding of the factors controlling ring-opening, ring-closing, and hydration/dehydration in saccharides, and provide data essential to the modeling of solution equilibria by computational methods.

Wiesner and co-workers[16] first reported the percentage of D-glucose aldehyde in aqueous solution (0.0026 ± 0.0002% at 25 °C) using polarography. More recently, quantitation of the cyclic *and* acyclic forms of D-glucose **4**,[17,18] D-idose **6**,[7] and D-talose **8**[10] has been reported using ^{13}C NMR spectroscopy and [1-^{13}C]-labeled aldoses. The use of selectively ^{13}C-labeled aldoses facilitated the latter measurements by enhancing the detection of the C1 signal, which is the most convenient carbon for aldose tautomer identification, thereby permitting the detection of forms present in low abundance. The availability of high-field NMR spectrometers with enhanced sensitivity and dynamic range further enables the detection of very minor forms. Aqueous solutions of short-chain aldoses such as D-erythrose **10** contain acyclic forms in relatively high abundance (1−10%),[5] but chain extension to aldopentoses and aldohexoses substantially reduces these forms to well below 0.1%. For example, recent NMR solution studies of D-[1-^{13}C]aldopentoses yielded hydrate and aldehyde percentages of 0.06−0.1% and 0.01−0.04%, respectively.[19] Having demonstrated the ability to detect and quantify these small solution constituents in aldopentoses, our attention turned to the D-[1-^{13}C]aldohexoses **1**−**8**, with the expectation that even lower abundances of acyclic forms were likely within this series. The results of these studies are reported herein. In addition, the effects of ^{2}H-substitution at H1 on aldohexose tautomeric equilibria for D-galactose **3** and D-talose **8** were investigated with the use of selective ^{13}C- and ^{2}H-labeling at C1 and H1. The relatively high sensitivity conferred by selective ^{13}C-labeling enabled the detection of small changes in cyclic tautomer percentages, the magnitudes of which appear to depend on both C-^{2}H bond orientation (axial vs equatorial) and on ring configuration. The *configurational* dependence of the ^{2}H equilibrium isotope effects observed in these conformationally stable aldopyranosyl rings yielded new insights into the optimal design of deuterated aldofuranoses with which to investigate potential ^{2}H isotope effects on furanose *conformational* equilibria. The existence of the latter effects could be of substantial importance to NMR structural studies of oligonucleotides.

Experimental Section

Synthesis of ^{13}C- and ^{2}H-Labeled Compounds. [1-^{13}C]-Aldohexoses **1**−**8** were prepared by cyanohydrin reduction using D-arabinose, D-lyxose, D-ribose, and D-xylose as the starting aldoses and K^{13}CN (99 atom-% ^{13}C).[20,21] The resulting four mixtures of C2-epimeric [1-^{13}C]aldose pairs were separated by chromatography on Dowex 50 × 8 (200−400 mesh) in the Ca^{2+} form;[22] the *gluco*, *galacto*, *altro*, and *gulo* configurations within each pair eluted first. Crystalline samples of the [1-^{13}C]aldohexoses were used for NMR sample preparation, except for D-idose, which was available as an aqueous solution of known concentration.

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D-[1-^{13}C;1-^{2}H]Galactose **11** and D-[1-^{13}C;1-^{2}H]talose **12** were prepared from D-lyxose and K^{13}CN by cyanohydrin reduction in $^{2}H_2O$ solvent; reduction of the intermediate C2-epimeric aldononitriles was accomplished with $^{2}H_2$ gas in the presence of a Pd/$BaSO_4$ catalyst.[23,24] Separation of **11** and **12** was achieved by chromatography on Dowex 50 × 8 (200−400 mesh) in the Ca^{2+} form[22] as described above.

NMR Measurements. Solutions of [1-^{13}C]aldoses (2 M in $^{2}H_2O$) were transferred to 3 mm NMR tubes, and quantitative ^{13}C NMR spectra, obtained on a Varian Unity*Plus* NMR spectrometer (599.887 MHz for ^{1}H; 150.860 MHz for ^{13}C) equipped with a Nalorac ^{1}H/^{13}C dual microprobe, were collected, processed, and integrated following an experimental protocol developed in prior work.[19] Three spectra were recorded on each sample at 30 °C, and each spectrum was obtained from the averaging of 10,000−22,000 transients using a recycle time of 5 x T_1. Signal integration was performed using spectrometer software; each signal was fit to a Lorentzian curve after treatment with a 5 Hz line-broadening function, and the resulting curve was integrated electronically.[19] Percentages obtained from the three data sets were averaged and are reported ± 1 STD (standard deviation).

Deuterium equilibrium isotope effects (^{2}H EIEs) were measured at 150.86 MHz (^{13}C) in samples containing D-[1-^{13}C]-aldose and D-[1-^{13}C;1-^{2}H]aldose in $^{2}H_2O$, each at ∼1 M concentration at 30 °C. Spin−lattice relaxation times (T_1) were measured by inversion−recovery,[25] and the raw data treated using a three-parameter fitting program supplied by Varian to extract the relaxation times for both the protonated and deuterated C1 carbons. Based on these measured ^{13}C relaxation times, quantitative ^{13}C NMR spectra were acquired with a 51 s recycle time, and both ^{1}H and ^{2}H broadband decoupling, in the presence of a ^{2}H lock, were applied during data acquisition. To suppress heteronuclear ^{13}C−^{1}H NOE, ^{1}H-decoupling was applied only during data acquisition (gated-decoupling). Three data sets were acquired for each aldose (D-galactose **3** and D-talose **8**), each containing ∼2000 transients, and spectra were processed and signals integrated as described above.

Results and Discussion

1. Quantitation of Forms in Aqueous Solution. The assignments of the ^{13}C-labeled C1 signals of cyclic pyranoses of **1**−**8** were made based on C1 chemical shifts assigned previously.[26] Furanose signal assignments were made based either on previous reports and/or by applying empirical ^{13}C chemical shift rules.[26] Identification of the acyclic aldehyde C1 signals was straightforward; although their intensities were weak, these signals exhibit characteristic chemical shifts (206.2 ± 1.2 ppm for aldopentoses)[19] in a region devoid of other carbohydrate signals. In contrast, the assignments of the hydrate C1 signals were, in some cases, problematic (notably for *allo* **1**, *galacto* **3**, *gluco* **4**, and *manno* **7**) since their chemical shifts (∼91 ppm) lie in a region where signal artifacts arising from the intense cyclic C1 signals and/or signals arising from very minor sample impurities occur. To assist in their assignment, proton-coupled ^{13}C NMR spectra were obtained in order to observe $^{1}J_{C1,H1}$ values. Recent studies of aldopentoses yielded average δ_{C1} and $^{1}J_{C1,H1}$ values in hydrates of 91.3 ± 0.3 ppm and 164.3 ± 0.4 Hz, respectively;[19] in the present cases where multiple weak signals were observed in the 91 ppm region, this *J*-coupling was helpful in identifying the true hydrate signal. The average $^{1}J_{C1,H1}$ for **1**, **3**, **4**, and **7** was 164.6 ± 1.2 Hz, in good agreement with the prior aldopentose data.

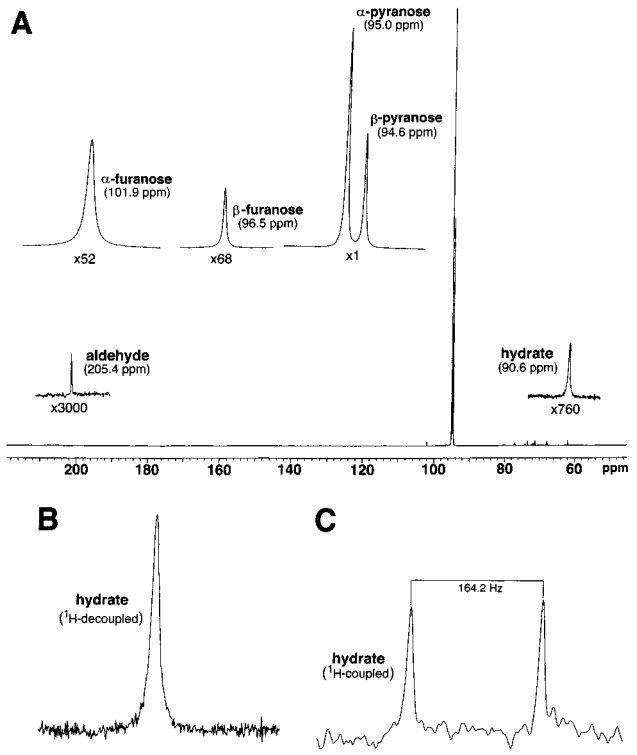

Figure 1. (A) The 150.86 MHz ^{13}C{^{1}H} NMR spectrum (18,216 transients, 7.8 s recycle time) of D-[1-^{13}C]mannose **7**, showing signals arising from the labeled C1 carbons and vertical scale expansions of these signals (inset). Four cyclic (α-pyranose, *β*-pyranose, α-furanose, *β*-furanose) and two acyclic (hydrate, aldehyde) forms are detectable at equilibrium in $^{2}H_2O$ at 30 °C. Signals arising from the natural abundance C2−C6 carbons are observed between 60 and 80 ppm. Signal line widths after application of an 5 Hz exponential processing filter: aldehyde, 8.8 Hz; hydrate, 7.1 Hz; α*f*, 6.9 Hz; *βf*, 6.5 Hz; α*p*, 7.7 Hz; *βp*, 7.5 Hz. (B) An expansion of the signal in (A) arising from C1 of D-[1-^{13}C]mannose hydrate (^{1}H-decoupled). (C) The [1-^{13}C]mannose hydrate C1 signal observed under conditions of ^{1}H-coupling. The observed $^{1}J_{C1,H1}$ (164.2 Hz) is consistent with couplings reported previously for aldopentoses.[19]

The ^{13}C NMR spectrum of D-[1-^{13}C]mannose **7** contains six enriched C1 signals arising from both cyclic and acyclic forms (Figure 1). Despite the low intensities of the latter, the combination of isotope labeling, signal averaging, and high magnetic field produced signal-to-noise ratios sufficient for accurate and reproducible signal integration. C1 Chemical shifts of the cyclic and acyclic forms of **1**−**8** are given in Table 1. Within **1**−**8**, δ_{C1} for the hydrate and aldehyde forms were 90.6 ± 0.3 ppm and 205.9 ± 0.9 ppm, respectively, which are similar to corresponding chemical shifts observed in aldotetroses[5] and aldopentoses.[19] Solution compositions for **1**−**8** are found in Table 2, along with percentages reported previously. Total percentages of pyranose, furanose and acyclic forms, and hydrate/aldehyde ratios, are given in Table 3.

Total pyranose percentages of **1**−**8** varied from 68 to 99%, with *gluco* yielding the most pyranose and *altro* yielding the least. Total furanose percentages varied from 0.4 to 32%, with *altro* yielding the most and *gluco* yielding

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Table 1. ^{13}C Chemical Shifts[a] for C1 of the Cyclic and Acyclic Forms of D-[1-^{13}C]Aldohexoses in Aqueous Solution[b]

	C1 chemical shift[c]						
aldohexose	αp	βp	αf	βf	hydrate	aldehyde	ref
D-allose **1**	93.7	94.3	96.8	101.6	90.4	207.1	
	(93.7)	(94.3)	(96.8)	(101.6)			26
D-altrose **2**	94.7	92.6	102.2	96.2	90.8	206.2	
	(94.7)	(92.6)	(102.2)	(96.2)			26
D-galactose **3**	93.2	97.3	95.8	101.8	91.1	206.4	
	(93.2)	(97.3)	(95.8)	(101.8)			26
D-glucose **4**	92.9	96.7	97.6	103.2	90.3	205.9	
					(90.66)		18
	(92.9)	(96.7)		(103.8)			26
D-gulose **5**	93.7	94.7	96.0	101.4	90.3	206.3	
	(93.6)	(94.6)	(97.3)	(101.4)			26
D-idose **6**	94.0	93.2	102.5	96.4	90.6	205.3	
	(94.4)	(93.7)	(103.1)	(96.8)	(91.2)	(205.7)	7
	(93.2)	(93.9)	(102.5)	(96.3)			26
D-mannose **7**	95.0	94.6	101.9	96.5	90.6	205.4	
	(95.0)	(94.6)					26
D-talose **8**	95.5	95.0	101.8	97.3	91.0	204.3	
	(96.2)	(95.7)	(102.5)	(98.0)	(91.2)	(204.2)	10
	(95.5)	(95.0)	(101.8)	(97.3)			26

[a] In ppm, referenced (external) to the C1 chemical shift of α-D-[1-^{13}C]mannopyranose (95.0 ppm). [b] 30 °C in ^2H_2O solvent. [c] αp = α-pyranose; βp = β-pyranose; αf = α-furanose; βf = β-furanose. Values reported previously are given in parentheses.

the least. Good agreement is observed between these new data and previous data for pyranose and furanose forms. Significant differences, however, are observed for the aldehyde percentages (Table 2); for example, solutions of **1**−**3** contain considerably less aldehyde than reported previously.[2] Aldehyde percentages are greatest for the *altro*, *ido*, and *talo* configurations, varying from 0.01 to 0.09%, and are comparable to those determined recently for aldopentoses under similar solution conditions.[19] Considerably less aldehyde is observed for the remaining configurations, ranging from 0.003 to 0.006%.

Significant differences in hydrate percentages (Table 2) are observed in **1**−**8**, with values ranging from 0.006 to 0.7%. In contrast to aldehyde percentages which span an ∼30-fold range, a considerably broader range of percentages exists for the hydrates (∼125-fold). The maximal hydrate percentage is observed for *ido* (0.74%), followed by *altro* (0.079%) and *gulo* (0.077%); the least hydrate is found for *allo* and *gluco* (∼0.006%). Thus, the enhanced range of hydrate percentages is caused by the *ido* configuration, whose percentage is uniquely high within the series.

Hydrate/aldehyde ratios for **1**−**8** ranged from 1.5 to 13, with *gluco* exhibiting the smallest ratio and *gulo* exhibiting the largest (Table 3). Hayward and Anygal[27] proposed previously that aldoses might exhibit hydrate/aldehyde ratios of ∼10 regardless of their structures. Although relatively large errors in the aldehyde percentages introduce significant errors in the reported ratios in some cases, the present data suggest that aldohexose configuration affects this ratio. This result is not surprising, given that different acyclic hydrate configurations will lead to different conformations containing unequal numbers and strengths of 1,3 and other types of destabilizing interactions involving the hydroxyl groups at C1, thus leading to different relative stabilities of the acyclic hydrates.

In the three reported cases where percentages of all six aldohexose forms have been determined (i.e., *gluco*, *ido*, *talo*), the present data are in good agreement with these prior results.

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2. ^{2}H Equilibrium Isotope Effects. The solution compositions of D-galactose **3** and D-talose **8** selectively ^{13}C- and ^{2}H-labeled at C1 and H1 were examined in order to evaluate ^{2}H equilibrium isotope effects (EIEs) on tautomeric equilibria. ^{2}H EIEs were measured by preparing solutions containing ∼1 M D-[1-^{13}C]aldose and ∼1 M D-[1-^{13}C;1-^{2}H]aldose and exploiting the ^{2}H isotope effect on ^{13}C chemical shift to resolve the signals arising from the protonated and deuterated species (Figure 2). Thus, potential experimental sources of error arising from the use of two different samples were eliminated. At 150 MHz (^{13}C), ^{2}H isotope shifts ranged from 49 to 58 Hz (0.33−0.39 ppm) and their magnitudes depend on tautomeric form, with larger shifts found for pyranoses than for furanose and acyclic forms. Signal multiplicity caused by ^{13}C−^{2}H spin-coupling, and the resultant compounding errors caused by the need for multiple signal integration, were eliminated with the use of a broadband ^{2}H-decoupling accessory, which allowed simultaneous ^{1}H and ^{2}H decoupling during data acquisition. Complications arising from differential ^{13}C−^{1}H NOE at C1 in the protonated and deuterated molecules were eliminated by collecting ^{13}C spectra in the presence of gated ^{1}H-decoupling (^{1}H decoupling applied only during data acquisition).

The optimal recycle time was determined by measuring ^{13}C T_1 values in the sample by inversion recovery (Table 4). Assuming C1 of the [1-^{13}C]aldose and the [1-^{13}C;1-^{2}H]-aldose relaxes solely via a dipole−dipole mechanism and aldohexoses tumble isotropically in the extreme narrowing limit as rigid rotors, then a theoretical ratio, $T_1(^2H)/T_1(^1H)$, for C1 may be calculated from the following equations[28a] (expressed in SI units):

$$1/T_1\,(^1\mathrm{H}) = (\mu_o/4\pi)^2\,\gamma_H{}^2\gamma_C{}^2\hbar^2\tau_c\,(\Sigma\, r_{CH}{}^{-6}) \qquad (1)$$

$$1/T_1\,(^2\mathrm{H}) = (8/3)\,(\mu_o/4\pi)^2\,\gamma_D{}^2\gamma_C{}^2\hbar^2\tau_c\,(r_{C1-D1}{}^{-6}) + (\mu_o/4\pi)^2\,\gamma_H{}^2\gamma_C{}^2\hbar^2\tau_c\,(\Sigma\, r_{CH}{}^{-6}) \qquad (2)$$

where $\mu_o/4\pi$ is the magnetic permeability of vacuum, γ_C,

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Table 2. Percentages of Cyclic and Acyclic Forms of D-[1-^{13}C]Aldohexoses in Aqueous Solution[a]

	percent form in solution[b]						
aldohexose	α*p*	β*p*	α*f*	β*f*	hydrate	aldehyde	reference[d]
D-allose **1**	14.754	77.028	2.949	5.258	0.0064	0.0042	
	14.597	77.097	2.983	5.313	0.0064	0.0029	
	14.557	77.071	3.033	5.330	0.0062	0.0026	
	14.6_4	**77.06_5**	**2.98_8**	**5.30_0**	**0.0063**	**0.0032**	
	$\pm 0.1_0$	**$\pm 0.03_5$**	**$\pm 0.04_2$**	**$\pm 0.03_8$**	**±0.0001**	**±0.0009**	
	(14)	(77.5)	(3.5)	(5)		(0.01)	2 (31 °C)
D-altrose **2**	26.943	41.012	18.578	13.376	0.077	0.014	
	27.007	40.968	18.585	13.343	0.080	0.014	
	26.672	41.182	18.650	13.400	0.080	0.015	
	26.8_7	**41.0_5**	**18.60_4**	**13.37_3**	**0.079**	**0.014**	
	$\pm 0.1_8$	**$\pm 0.1_1$**	**$\pm 0.04_0$**	**$\pm 0.02_9$**	**±0.002**	**±0.001**	
	(27)	(43)	(17)	(13)		(0.04)	2 (22 °C)
D-galactose **3**	31.467	62.515	2.325	3.636	0.052	0.0060	
	30.982	62.929	2.333	3.709	0.043	0.0037	
	31.160	62.826	2.247	3.717	0.042	0.0086	
	31.2_0	**62.7_6**	**2.30_2**	**3.68_7**	**0.046**	**0.006_1**	
	$\pm 0.2_5$	**$\pm 0.2_2$**	**$\pm 0.04_8$**	**$\pm 0.04_5$**	**±0.006**	**$\pm 0.002_5$**	
	(30)	(64)	(2.5)	(3.5)		(0.02)	2 (31 °C)
D-glucose **4**	37.672	61.956	0.100	0.263	0.0059	0.0033	
	37.666	61.944	0.118	0.261	0.0063	0.0041	
	37.577	61.992	0.107	0.316	0.0055	0.0045	
	37.63_8	**61.96_4**	**0.108**	**0.28_0**	**0.0059**	**0.0040**	
	$\pm 0.05_3$	**$\pm 0.02_5$**	**±0.009**	**$\pm 0.03_1$**	**±0.0004**	**±0.0006**	
	(38.8)	(60.9)	(0.14)	(0.15)	(0.0045)	(0.0024)	18 (27 °C)
	(38)	(62)		(0.14)[e]		(0.002)	2 (31 °C)
D-gulose **5**	12.160	83.740	0.957	3.067	0.072	0.0047	
	12.117	83.902	0.889	3.007	0.077	0.0082	
	12.334	83.548	0.974	3.057	0.081	0.0048	
	12.2_0	**83.7_3**	**0.94_0**	**3.04_4**	**0.077**	**0.005_9**	
	$\pm 0.1_2$	**$\pm 0.1_8$**	**$\pm 0.04_5$**	**$\pm 0.03_2$**	**±0.005**	**$\pm 0.002_0$**	
	(16)	(81)		(3)			2 (22 °C)
D-idose **6**	33.443	37.206	12.188	16.096	0.977	0.089	
	33.940	37.602	12.140	16.164	0.604	0.096	
	33.745	37.319	12.105	16.100	0.634	0.096	
	33.7_1	**37.3_8**	**12.14_4**	**16.12_0**	**0.7_4**	**0.094**	
	$\pm 0.2_5$	**$\pm 0.2_0$**	**$\pm 0.04_2$**	**$\pm 0.03_8$**	**$\pm 0.2_1$**	**± 0.004**	
	[33.817][c]	[37.270]	[12.118]	[16.211]	[0.583]	[0.087]	
	(35.9)	(33.4)	(13.5)	(16.5)	(0.5)	(0.1)	7 (30 °C)
	(38.5)	(36)	(11.5)	(14)		(0.2)	2 (37 °C)
D-mannose **7**	66.237	32.915	0.617	0.205	0.022	0.0044	
	66.283	32.831	0.618	0.243	0.022	0.0040	
	66.191	32.803	0.690	0.288	0.023	0.0047	
	66.23_7	**32.85_0**	**0.64_2**	**0.24_5**	**0.022**	**0.0044**	
	$\pm 0.04_6$	**$\pm 0.05_8$**	**$\pm 0.04_2$**	**$\pm 0.04_2$**	**±0.001**	**±0.0004**	
	(65.5)	(34.5)	(0.6)	(0.3)		(0.005)	2 (44 °C)
D-talose **8**	41.739	28.516	18.279	11.383	0.050	0.032	
	42.377	28.864	17.731	10.947	0.053	0.027	
	42.399	28.755	17.747	11.017	0.054	0.028	
	42.1_7	**28.7_1**	**17.9_2**	**11.1_2**	**0.052**	**0.029**	
	$\pm 0.3_8$	**$\pm 0.1_8$**	**$\pm 0.3_1$**	**$\pm 0.2_3$**	**± 0.002**	**±0.003**	
	(41.0	(29.0	(18.5	(11.6	(0.03	(0.03	
	±1.1)	±0.8)	±0.5	±0.3)	±0.01)	±0.01)	10 (28 °C)
	(42)	(29)	(16)	(13)		(0.03)	2 (22 °C)

[a] 30 °C in ^2H_2O. [b] α*p* = α-pyranose; β*p* = β-pyranose; α*f* = α-furanose; β*f* = β-furanose. Each entry contains data from three separate determinations on the same sample followed, in bold type, by the average ± 1 STD. Literature values appear in parentheses. [c] Data collected with a recycle time = $10T_1$. [d] Temperature of measurement in parentheses. [e] At 43 °C.

γ_H, and γ_D are the magnetogyric ratios for ^{13}C, ^{1}H, and ^{2}H, respectively, $\hbar$ is Planck's constant divided by 2π, r_{C1-D1} is the C1−D1 bond length, $\Sigma\, r_{CH}^{-6}$ is the sum of all of the inverse sixth distances between ^{13}C1 and ^{1}H nuclei in the molecule, and τ_c is the correlation time characterizing overall molecular reorientation in solution. Theoretical ratios were calculated using neutron diffraction crystallographic data reported for methyl α- and β-D-galactopyranosides;[28b] contributions from the methyl and hydroxyl protons to C1 relaxation were ignored, in the latter case because measurements were made in ^2H_2O (thus truncating potential contributions from these protons) and because contributions caused by C−O bond rotational averaging would be difficult to treat quantitatively. The calculated ratios, $T_1(^2H)/T_1(^1H)$, were found to be ∼11 for the α- and β-galactopyranosyl rings, respectively, which is larger than the observed experimental ratios (8.1−8.9) for galacto- and talopyranoses (Table 4). The difference may be due to the fact that calculations were carried out assuming the same correlation times and bond lengths in the protonated and deuterated forms. Also, some of the above assumptions may not be completely fulfilled, especially the rigid body approximation and isotropic tumbling. Nonetheless, $T_1(^2H)/T_1(^1H)$ ratios of ∼10 can be expected for C1 of aldohexoses upon deuteration at H1; thus, in ^{2}H EIE NMR experiments, relaxation delays of 50 s were applied (recycle time of 51 s). Given this long recycle time,

Table 3. Total Percentages of Cyclic and Acyclic Forms of D-[1-^{13}C]Aldohexoses in ^2H_2O

	percent (%) in solution[a]			
aldohexose	pyranoses	furanoses	acyclics[b]	hydrate/ aldehyde
D-allose **1**	91.7 ± 0.1	8.3 ± 0.1	0.010 ± 0.001	2.0 ± 0.6
D-altrose **2**	67.9 ± 0.2	32.0 ± 0.1	0.093 ± 0.002	5.6 ± 0.4
D-galactose **3**	94.0 ± 0.3	6.0 ± 0.1	0.052 ± 0.007	7.5 ± 3.2
D-glucose **4**	99.6 ± 0.1	0.39 ± 0.03	0.010 ± 0.001	1.5 ± 0.2
D-gulose **5**	95.9 ± 0.2	4.0 ± 0.1	0.083 ± 0.005	13 ± 4.5
D-idose **6**	71.1 ± 0.3	28.3 ± 0.1	0.8 ± 0.2	7.9 ± 2.3
D-mannose **7**	99.1 ± 0.1	0.9 ± 0.1	0.026 ± 0.001	5.0 ± 0.5
D-talose **8**	70.9 ± 0.4	29.0 ± 0.4	0.081 ± 0.004	1.8 ± 0.2

[a] 30 °C. [b] Hydrate + aldehyde. Errors were computed from the standard treatments of error propagation using the standard deviations in the percentages reported in Table 2.

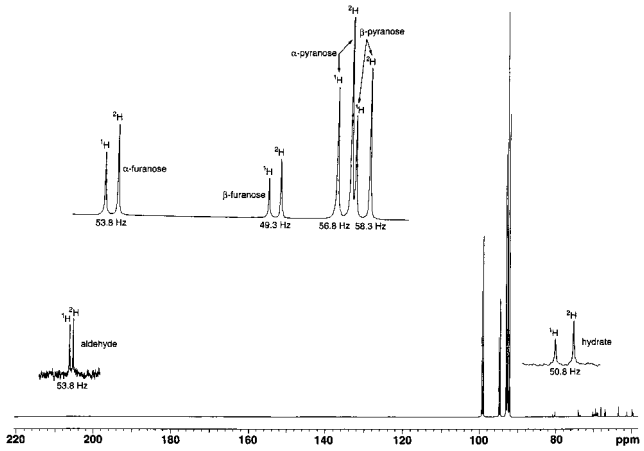

Figure 2. The 150.86 MHz $^{13}C\{^1H,^2H\}$ NMR spectrum (2,300 transients, 51 s recycle time) of a sample of 1 M D-[1-^{13}C]talose and 1 M D-[1-^{13}C,^{2}H]talose in ^2H_2O at 30 °C, showing the enriched C1 signals. Pairs of signals for each of the six monomeric forms are observed, with the downfield and upfield signals within each pair assigned to C1 of the protonated (^{1}H) and deuterated (^{2}H) molecule, respectively. The ^{2}H isotope effect on the C1 chemical shift ranges from ~49−58 Hz (0.33−0.39 ppm), which is consistent with the ~5−6 Hz shifts observed at 15 MHz.[23] Signals arising from the natural abundance C2−C6 carbons are observed between 60 and 80 ppm.

Table 4. ^{13}C Spin−Lattice Relaxation Times[a] for the Cyclic Forms of D-[1-^{13}C]Galactose 3, D-[1-^{13}C;1-^{2}H]Galactose 11, D-[1-^{13}C]Talose 8, and D-[1-^{13}C; 1-^{2}H]Talose 12

	solution form[b]			
cpd	α*p*	β*p*	α*f*	β*f*
3	0.838 (0.005)	0.853 (0.006)	0.984 (0.009)	1.06 (0.01)
11	6.86 (0.05)	6.92 (0.04)	8.00 (0.08)	8.73 (0.08)
8	1.004 (0.008)	1.007 (0.005)	1.017 (0.002)	1.08 (0.02)
	1.00 (0.01)	1.049 (0.002)	1.010 (0.004)	1.07 (0.02)
	[1.002 ± 0.006]	[1.028 ± 0.003]	[1.014 ± 0.002]	[1.075 ± 0.014]
12	8.57 (0.02)	8.78 (0.03)	9.04 (0.05)	9.47 (0.07)
	8.54 (0.02)	8.73 (0.01)	9.01 (0.03)	9.45 (0.04)
	[8.56 ± 0.01]	[8.76 ± 0.02]	[9.03 ± 0.03]	[9.46 ± 0.04]

[a] In seconds. Tau (τ) values used in these determinations were 0.003, 0.03, 0.3, 3, 9, 40, and 90 s; the overall recycle time was 31 s. Errors are given in parentheses, and average values with their associated errors (talose only) are given in brackets. Only one set of determinations was made for **3** and **11**. [b] α*p* = α-pyranose, β*p* = β-pyranose, α*f* = α-furanose, β*f* = β-furanose.

substantial signal averaging became impractical, thus limiting the S/N for signals arising from acyclic forms achievable within a practical data collection time. In these experiments, ~2000 transients were averaged per spectrum, and three spectra were collected on each sample.

Table 5. Tautomeric Composition[a] of Equimolar Solutions of D-[1-^{13}C]Talose 8 and D-[1-^{13}C;1-^{2}H]Talose 12, and of D-[1-^{13}C]Galactose 3 and D-[1-^{13}C;1-^{2}H]Galactose 11

	solution form[b]					
cpd	α*p*	β*p*	α*f*	β*f*	hydrate	aldehyde
8	41.550	28.954	17.993	11.417	0.0527	0.0298
	41.239	29.391	17.946	11.340	0.0405	0.0134
	41.204	29.484	17.928	11.326	0.0395	0.0192
	41.3$_3$	**29.2$_8$**	**17.95$_6$**	**11.36$_1$**	**0.044$_2$**	**0.020$_8$**
	±0.1$_9$	**±0.2$_8$**	**±0.03$_4$**	**±0.04$_9$**	**±0.007$_3$**	**±0.008$_3$**
12	42.519	27.653	18.399	11.350	0.0501	0.0291
	42.413	27.712	18.442	11.356	0.0442	0.0322
	42.393	27.709	18.449	11.360	0.0492	0.0393
	42.44$_2$	**27.69$_1$**	**18.43$_0$**	**11.355**	**0.047$_8$**	**0.033$_5$**
	±0.06$_8$	**±0.03$_3$**	**±0.02$_7$**	**±0.005**	**±0.003$_2$**	**±0.005$_2$**
3	32.413	61.690	2.299	3.556	0.0415	
	32.571	61.539	2.273	3.560	0.0579	
	32.209	61.679	2.453	3.595	0.0654	
	32.4$_0$	**61.63$_6$**	**2.34$_2$**	**3.57$_0$**	**0.05$_5$**	
	±0.1$_8$	**±0.08$_4$**	**±0.09$_7$**	**±0.02$_1$**	**±0.01$_2$**	
11	32.599	61.409	2.364	3.602	0.0271	
	33.706	60.372	2.313	3.580	0.0285	
	32.522	61.445	2.433	3.566	0.0336	
	32.9$_4$	**61.0$_8$**	**2.37$_0$**	**3.58$_3$**	**0.029$_7$**	
	±0.6$_6$	**±0.6$_1$**	**±0.06$_0$**	**±0.01$_8$**	**±0.003$_4$**	

[a] In percent at 30 °C. Recycle time for these determinations was 51 s. Data were collected with broadband ^{1}H decoupling (gated/no NOE) and ^{2}H-decoupling. Each entry contains data from three separate determinations on the same sample followed, in bold type, by the average ± 1 STD. [b] α*p* = α-pyranose, β*p* = β-pyranose, α*f* = α-furanose, β*f* = β-furanose.

The changes in the percentages of D-talose **8** tautomers in aqueous solution upon substitution with ^{2}H at H1 are small (Table 5). Given the associated errors, the changes observed in the percentages of β-furanose and both acyclic forms are not statistically significant. However, the percentages of α-talopyranose and α-talofuranose increase upon C1 deuteration, whereas that of β-talopyranose decreases. The ratios, β*p*/α*p*, are 0.708 ± 0.008 and 0.652 ± 0.001 for **8** and **12**, respectively, yielding a ^{2}H EIE (defined as K_H/K_D) of 1.086 ± 0.012. The corresponding ratios, β*f*/α*f*, are 0.633 ± 0.003 and 0.616 ± 0.001, yielding an EIE of 1.028 ± 0.005. The ^{2}H EIE thus appears to be >1.0 for the reactions, α-talopyranose → β-talopyranose and α-talofuranose → β-talofuranose, with a smaller effect observed for the latter reaction. These values bracket recently reported data for D-glucose and D-[1-^{2}H]glucose, where a ^{2}H equilibrium isotope effect of 1.043 was measured.[29] An EIE of 1.086 for the talopyranoses suggests that the C1−H1/D1 bond has a slightly "looser" binding potential in the β-pyranose than in the α-pyranose. The latter state may be caused by the axial orientation of the C−H(D) bond in β-talopyranose; an antiperiplanar O5 lone-pair not only induces bond-lengthening (anomeric effect) but also causes reductions in the vibrational and bending frequencies of the C−H(D) bond. The latter behavior might be tested experimentally from an analysis of C−H or C−D infrared absorptions[30] or possibly from studies of ^{1}H chemical shift.

The observed ^{2}H EIE on talopyranose tautomeric equilibrium is secondary in nature; the interconversion

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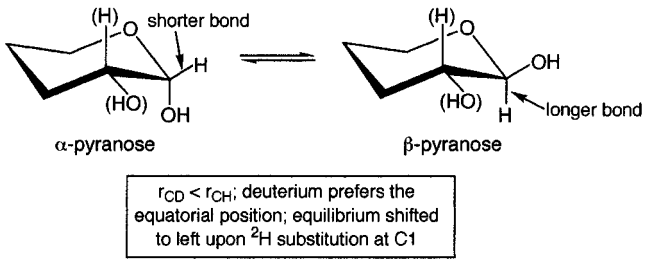
Scheme 3



of α- and *β*-talopyranoses occurs via the acyclic aldehyde and involves the breaking and forming of the nearby endocyclic C1−O5 bond. For the reaction, α*p*→*βp*, hybridization change occurs at C1, from $sp^3 \rightarrow sp^2$ for the initial ring-opening reaction to the acyclic aldehyde and $sp^2 \rightarrow sp^3$ for the subsequent ring-closing reaction to the *β*-pyranose. Prior work has shown that, for $sp^3 \rightarrow sp^2$ conversions, the principal difference in vibrational energy between the reactant and product is a reduction in frequency of one bending mode. For these reactions, a secondary *kinetic* isotope effect (SKIE), k_H/k_D, is calculated to be ∼1.4. The reciprocal of this value, ∼0.71, is expected for the ring-closing process. However, since an EIE is observable for α-talopyranose → *β*-talopyranose, *symmetric* SKIEs must not exist for the complex interconversion, α-pyranose ↔ aldehyde ↔ *β*-pyranose, otherwise an overall EIE of unity would be observed. The forward and reverse processes must involve different constituent SKIEs, and presumably a different reaction coordinate.

The effect of ^2H substitution at C1 on the talopyranose anomeric equilibrium appears consistent with expected correlations between C−^{1}H and C−^{2}H bond lengths and preferred bond orientation in chair conformations. Anet and Kopelevich[31] showed previously that ^2H substitution in cyclohexane-d_1 and cyclohexane-d_{11} shifts conformational equilibria toward chair structures containing equatorial C−^{2}H bonds. The effect was more pronounced in 5,5-dimethyl-1,3-dioxane-2-d_1, where both ring oxygens produce an enhanced anomeric effect and thus a greater difference between the axial and equatorial C−H bond lengths at C2.[32] These effects are apparently mediated by the preference of the shorter C−^{2}H bond (relative to the corresponding C−^{1}H bond) to assume an equatorial orientation in chair structures where equatorial C−H bonds are normally shorter than axial C−H bonds. By analogy to these non-carbohydrate examples, the C1−^{2}H1 bond in **8** is expected to prefer an equatorial (α-pyranose) rather than an axial orientation (*β*-pyranose), resulting in an increased α*p*/*βp* ratio (Scheme 3).

The above rationale, however, does not appear applicable to D-galactopyranoses, where substitution of ^2H at H1 does not effect tautomeric equilibria (Table 5). This result may be related to the fact that an EIE determined from considerations of cyclic forms only is a complex parameter, as discussed above, being composed of individual isotope effects for the constituent unidirectional ring-opening and -closing reactions in which the acylic aldehyde participates as a central intermediate (Scheme 2). The isotope effects for the individual ring-opening and -closing reactions for the conversion of α-galactopyranose to *β*-galactopyranose could cancel, giving rise to the observed result. Our original aim was to determine ^2H EIEs for these unidirectional reactions in order to address this problem, but errors in measuring the percentages of aldehyde forms in the presence and absence of ^2H substitution proved too large (Table 5), at least in aldohexoses where these percentages are very small (note that aldehyde percentages for **3** and **11** are absent in Table 5 because they could not be measured reliably under the present experimental conditions). However, it is clear from the present data that ^2H equilibrium isotope effects at C1 in aldopyranoses are not identical in the D-aldohexose series, and that configuration at C2 may play a role in determining their magnitudes.

The observed effect of ^2H substitution at H1 of aldohexoses on *tautomeric* equilibria leads to new insights into the design of experimental systems to investigate potential ^2H isotope effects on aldofuranose *conformational* equilibria. While the present data show that *talofuranose* tautomeric equilibrium is affected by ^2H substitution at H1, an interpretation of this effect is complicated by ring conformational flexibility. It is likely that a shift in *tautomeric* equilibrium would not be generally observed in aldofuranoses because C1−O1 bond orientation (and thus C1−H1 bond orientation) is conserved between anomers (i.e., the C1−O1 bond assumes a quasi-axial orientation regardless of anomeric configuration due to the anomeric effect). In cases where this orientation is not conserved between anomers, however, an isotope effect might be observed. Importantly, quantum chemical calculations on aldofuranosyl rings show that ring C−H bonds vary systematically with bond orientation, and that a given C−H bond will be minimal and maximal in length when quasi-equatorial and quasi-axial, respectively.[33−35] Taken collectively, these facts bear on the question of whether aldofuranose *conformational* shifts can be induced by one or more ^2H substitutions, a matter of significant importance for NMR structural studies of DNA and RNA where ^2H substitution can be useful in simplifying spectral analysis.[36] An inspection of the *β*-D-*ribo* and 2-deoxy-*β*-D-*ribo* rings suggests that the conformation of the latter may be more susceptible to isotope effects, because ^2H substitutions at H2*R*, H3, and H4 are *reinforcing* (i.e., the C2−H2*R*, C3−H3, and C4−H4 bonds are all either quasi-axial or quasi-equatorial in N and S forms, respectively). The conformational equilibrium for this trideuterated molecule might, therefore, shift slightly toward S forms relative to that of the fully protonated form. While small shifts in N/S equilibria may not be biologically significant for small molecules

(30) (a) In cyclohexane rings, it is generally held[30b] that an equatorial group X will show a C−X stretching vibration at a higher frequency than will the corresponding axial group (exceptions to this correlation have been observed in some substituted rings). From Badger's rule,[30c,d] the equatorial C−X bond length is expected to be shorter than the corrresponding axial C−X bond length. (b) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. *Conformational Analysis*; Interscience Publishers: New York, 1965; pp. 143−144 (c) Badger, R. M. *J. Chem. Phys.* **1934**, *2*, 128−131. (d) Badger, R. M. *J. Chem. Phys.* **1935**, *3*, 710−714.

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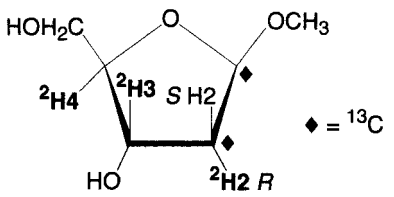
Scheme 4



methyl 2-deoxy-β-D-[1,2-$^{13}C_2$;H2*R*,H3,H4-$^{2}H_3$]-*erythro*-pentofuranoside (**13**)

such as nucleotides, the additive effects in larger oligomers could potentially change solution conformation and dynamics sufficiently to affect biological function. In contrast, only dual ^{2}H substitutions are reinforcing in β-D-*ribo* rings, and thus a smaller effect is expected. These considerations lead to the suggestion that methyl 2-deoxy-β-D-[1,2-$^{13}C_2$;H2*R*,H3,H4-$^{2}H_3$]*erythro*-pentofuranoside **13** (Scheme 4) would be an optimal structure on which to test for the existence of a ^{2}H EIE on ring conformation by NMR. In addition to the $^3J_{H1,H2S}$ which remains available, ^{13}C labeling will allow $^3J_{C1,C5}$ and $^3J_{C2,C5}$ values to be determined and used as additional conformational probes.[37] Efforts are underway in this laboratory to investigate this problem.

Conclusions

The results of this investigation demonstrate that acyclic forms of aldohexoses can be detected by ^{13}C NMR using compounds selectively ^{13}C-labeled at C1, thereby allowing assessments of the percentages of cyclic *and* acyclic tautomers under identical solution conditions for the first time. Tautomer percentages as low as 0.004% were detected and quantified using commercial high-field NMR instrumentation and [1-^{13}C]aldoses (99 atom-% ^{13}C-labeled) without the need for hardware modification. These new data advance NMR investigations of aldohexose tautomeric equilibria initiated over three decades ago, when cyclic forms were the focus of attention, by resolving the important, fundamental question of the abundance of the more elusive acyclic forms in aqueous solution. Since direct observation of the acyclic aldehyde forms of aldohexoses facilitates NMR studies of anomerization kinetics by saturation-transfer methods,[5−13] the present results imply that the latter studies are technically feasible.

^{2}H Equilibrium isotope effects on aldose tautomerization can be measured using [1-^{13}C]/[1-^{13}C;1-^{2}H] aldose mixtures and exploiting the ^{2}H isotope effect on ^{13}C chemical shifts to observe separate signals arising from both the protonated and deuterated molecules in the same NMR sample. Spectral data are simplified with the use of simultaneous ^{1}H and ^{2}H broadband decoupling, in the presence of a ^{2}H lock, which eliminates signal multiplicity and the compounding errors associated with multiple signal integration. However, the accuracy of these measurements is significantly affected by the achievable S/N, and the latter is difficult to maximize given the slow relaxation rates experienced by the deuterated carbon (T_1 increased by ~10-fold upon deuteration). Thus, such measurements are best conducted on high-field spectrometers and/or at high saccharide concentrations in order to reduce the need for extended signal averaging.[38] While ^{2}H isotope effects at C1 were discernible for D-talose **8**, no effects were observed for D-galactose **3**. An analysis of this difference was hampered by the inability to determine ^{2}H isotope effects for the unidirectional reactions, due to the large errors associated with quantification of aldehyde forms. The latter is a key limitation of the NMR approach used here; future studies at higher aldose concentrations and/or higher magnetic fields may yield S/N ratios for the acyclic signals sufficient to assess these unidirectional ^{2}H isotope effects. These measurements may reveal the origin of the different EIEs observed in **3** and **8**.

The observed shift in the $\alpha p \rightleftharpoons \beta p$ equilibrium for **8** appears consistent with previous observations made on the effect of ^{2}H-substitution on cyclohexane ring conformational equilibria ($^4C_1/^1C_4$). Unlike the latter system, however, hydroxyl group substitution may modulate this effect, especially configuration at C2. Observations on **8** combined with effects of ring conformation on C−H bond lengths in furanoses predicted recently from quantum mechanical calculations lead to coherent and testable predictions of the effect of specific multiple ^{2}H-substitutions on β-D-*ribo* and 2-deoxy-β-D-*ribo* ring conformation which may impact NMR structural studies of oligonucleotides.

Acknowledgment. This work was supported by a grant from Omicron Biochemicals, Inc. of South Bend, IN.

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(38) It is possible that relaxation (paramagnetic) reagents may be employed to enhance T_1 and thereby facilitate measurements of ^{2}H EIEs. However, use of these reagents cannot be made without first determining whether their presence in solution perturbs tautomeric equilibria.