The results demonstrate the intermediacy of a completely free, symmetrically solvated monomeric metaphosphate intermediate in a phospho group transfer to a hindered nucleophile in a protic solvent and are consistent with the proposal that the formation of *tert*-butyl phosphate is, in such reactions, diagnostic of the metaphosphate intermediate.[11]

(11) **Note Added in Proof.** The results reported here are in gratifying agreement with those of Cullis and Nicholls (Cullis, P. M.; Nicholls, D. *J. Chem. Soc., Chem. Commun.* **1987**, in press) on the positional isotope exchange observed in adenosine 5′-[α,β-^{18}O]diphosphate trianion in neat *tert*-butyl alcohol.

Detailed Tautomeric Equilibrium of Aqueous D-Glucose. Observation of Six Tautomers by Ultrahigh Resolution Carbon-13 NMR

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The equilibrium tautomeric composition of aqueous reducing monosaccharides has been extensively studied for almost 150 years.[1] Each aldopentose or aldohexose exists as a mixture of at least six compounds: the aldehyde, the hydrated aldehyde (*gem*-diol), the two pyranoses, and the two furanoses.[2] However, the proportion of the *gem*-diol has not been reported for any unmodified aldopentose or aldohexose,[2] although the ^{13}C NMR signal of the *gem*-diol tautomer of D-[1-^{13}C]idose has been detected.[3]

The composition of aqueous D-glucose is the greatest challenge of the remaining gaps of knowledge about tautomeric equilibria. The reported proportions of β-D-glucofuranose and *aldehydo*-D-glucose are 0.14% (at 43 °C) and 0.002% (at 20 °C), respectively.[2] The *gem*-diol and α-furanose tautomers have not been detected. In this report we show that ultrahigh-resolution NMR methodology[4-9] yields resolved resonances for all six tautomers in ^{13}C NMR spectra of aqueous D-[1-^{13}C]glucose. We present equilibrium proportions as a function of temperature.

The sample was maintained at each temperature for at least 5 h prior to data acquisition, in order to establish tautomeric equilibrium.[1,10-14] Figure 1 shows the region of saturated C1 resonances in the ^{13}C NMR spectrum of 1.4 M D-[1-^{13}C]glucose[15] in H_2O (with 10% v/v dioxane-d_8 and 1% v/v dioxane), at 37 and 67 °C. The truncated peaks 3 and 4 are the resonances of β-D-glucopyranose and α-D-glucopyranose, respectively.[16] The peaks labeled with X are instrumental artifacts (see below). The peaks designated with C are the $^1J_{CC}$ satellites that arise from the pyranose tautomers of the 1.1% of molecules doubly ^{13}C labeled at C1 and C2. Spinning sidebands have been smeared out by spinner speed modulation.[17] We shall show that peaks 1, 2, and 5 arise from the β-D-glucofuranose, α-D-glucofuranose, and *gem*-diol tautomers, respectively. First, we rule out the possibility that these small peaks are artifacts or arise from impurities by noting that they exhibit large monotonic intensity increases as the temperature is raised. Peak 1 has already been assigned to the β-furanose tautomer.[18] Its chemical shift is consistent with those of anomeric carbons of furanoses with O1 trans to O2.[16] Peak 2 has a chemical shift consistent with a furanose ring which has O1 cis to O2,[16] and is therefore assigned to α-D-glucofuranose. We assign peak 5 to the *gem*-diol tautomer, because its chemical shift (90.66 ppm from Me_4Si at 37 °C) is almost identical with the values reported for the *gem*-diol tautomers of D-[1-^{13}C]-threose[13] and D-[1-^{13}C]idose.[3] Because peak 5 is extremely small relative to the resonances of the pyranose tautomers, it is particularly important to rule out the possibility that it is an artifact. Not only does the intensity of peak 5 increase with temperature but its line width also increases, a behavior consistent with an exchange contribution to the line width from *gem*-diol/aldehyde interconversion (see below).

Figure 2 shows the effect of temperature on the C1 resonance of the aldehyde tautomer. Because the resonance is very broad, each spectrum was processed with 10-Hz digital Lorentzian

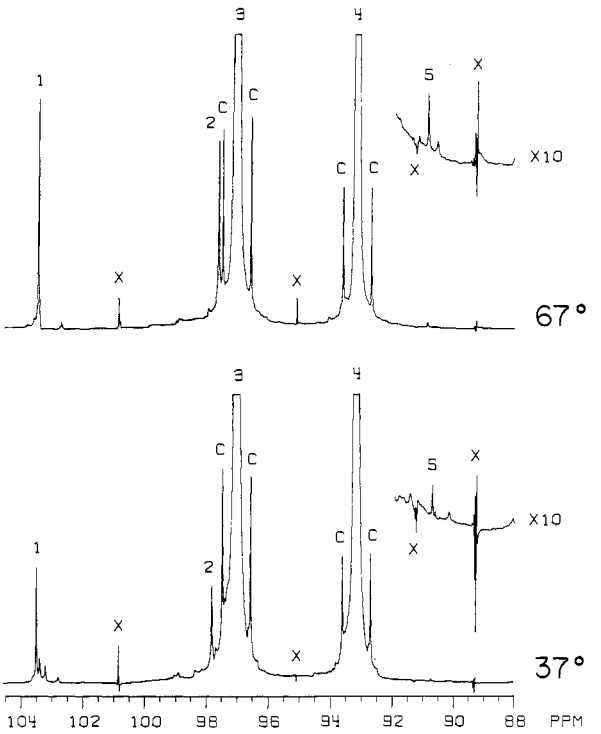

Figure 1. Region of the saturated anomeric carbons in the proton-decoupled ^{13}C NMR spectrum of 1.4 M D-[1-^{13}C]glucose in H_2O, with 10% v/v dioxane-d_8 and 1% v/v dioxane, recorded at 50.3 MHz and temperatures of 37 °C (pH 6.0) and 67 °C (pH 4.8), with an acquisition time of 3.28 s, a spectral width of ±10 000 Hz (quadrature detection), and 128K time-domain points. Homogeneity (Z_1 gradient only) was automatically adjusted prior to each batch of 200 scans. Each batch was automatically added to a double-precision (40-bit) data file which contained the sum of all prior batches. 140 and 376 batches were recorded at 37 and 67 °C, respectively. Each final time-domain spectrum was processed in the floating-point mode with 0.5-Hz digital broadening and Fourier transformation. Chemical shifts are expressed in parts per million from Me_4Si, but they were measured relative to internal dioxane, taken to have a chemical shift of 67.47 ppm at 37 °C. The β-pyranose resonance of the spectrum at 67 °C was aligned with the corresponding one in the spectrum at 37 °C. In each spectrum, the β-pyranose resonance is truncated at 0.8% of its full peak height.

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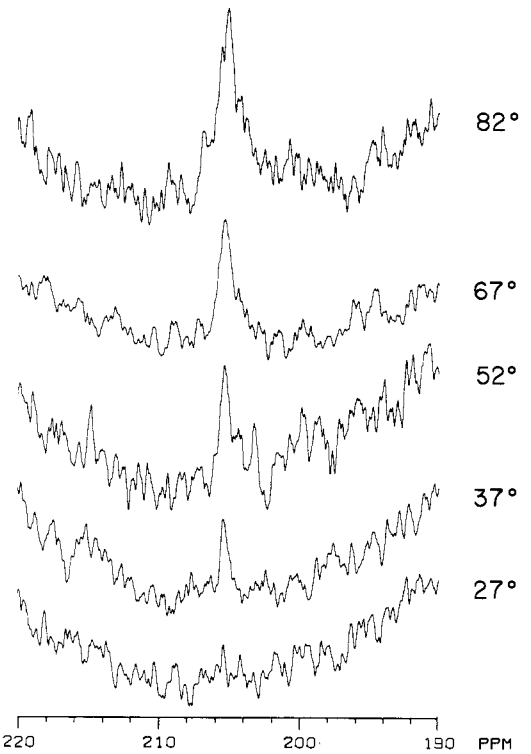

Figure 2. Temperature dependence of the aldehyde region in the proton-decoupled ^{13}C NMR spectrum of D-[1-^{13}C]glucose. Sample and spectral conditions are those of Figure 1, except that the pH was 4.8 for all spectra, the digital Lorentzian broadening was 10 Hz, and the number of 200-scan batches was 101 (27 °C), 177 (37 °C), 113 (52 °C), 376 (67 °C), and 161 (82 °C). Vertical amplitudes have been normalized so that integrated intensities are proportional to aldehyde content.

broadening instead of the 0.5 Hz used for Figure 1. After subtraction of the 10-Hz digital broadening, the line width goes from about 20 Hz at 37 °C to about 70 Hz at 82 °C. It is dominated by the exchange contribution (W_{ex}) from interconversion of aldehyde into all the other tautomers. The other tautomers exhibit relatively small W_{ex} values, but those of the α-furanose and *gem*-diol are measurable. We plan to report on kinetic data extracted from W_{ex} measurements in a future publication.

Integrated intensities yielded the tautomeric composition at temperatures in the range 27-82 °C (Table I). The proportions of the pyranose tautomers are almost independent of temperature; those of the two furanoses and the *gem*-diol increase by a factor of about 5; the proportion of aldehyde increases by an order of magnitude. Our results for the aldehyde are in reasonable agreement with values obtained from circular dichroism spectra by Hayward and Angyal.[19] It is of interest that, within experimental error, we observe equal amounts of α-furanose and β-furanose at all temperatures. About equal proportions of the two furanoses have been observed for 5-*O*-methyl-D-glucose[20] and D-glucose 5,6-carbonate.[21] D-Idose, the other unmodified hexose with a *xylo* configuration, exhibits a β-furanose/α-furanose ratio (structurally analogous to the α-furanose/β-furanose ratio of D-glucose) of 1.2 at 30 °C.[3]

Finally, we wish to comment on the question of artifacts (labeled with X in Figure 1). It is well-known that very large signal-to-noise ratios (for the large resonances) reveal spurious peaks caused by imperfect accumulation and processing.[22-26] We believe that a detailed understanding of the origin of the artifacts will yield methods for reducing their size to levels even lower than in Figure 1 and also systematic procedures for distinguishing residual artifacts from real peaks. One specific procedure applicable to studies of tautomeric composition of monosaccarides is the use of $^1J_{CH}$ values.[3]

Table I. Tautomeric Composition (Percent) of Aqueous D-Glucose[a]

temp, °C	αP	βP	αF	βF	$CH(OH)_2$	CHO
27[b]	38.8	60.9	0.14	0.15	0.0045	c
37[b]	39.4	60.2	0.20	0.21	0.0077	0.0024
37[d]	39.1	60.5	0.19	0.22	0.0062	c
52[b]	39.7	59.7	0.32	0.31	0.0090	0.0051
67[b]	39.9	59.2	0.40	0.46	0.016	0.0096
82[b]	40.1	58.5	0.60	0.69	0.022	0.019

[a] αP, βP, αF, βF, $CH(OH)_2$, and CHO designate α-D-glucopyranose, β-D-glucopyranose, α-D-glucofuranose, β-D-glucofuranose, the *gem*-diol tautomer, and the aldehyde tautomer, respectively. Sample and typical spectral conditions are given in the captions of Figures 1 and 2. For all tautomers except the aldehyde, each integrated intensity was taken as the product of the peak height and the line width of the best-fit Lorentzian curve obtained from a computer fit of the spectra processed with 0.5-Hz digital broadening (see Figure 1). For the aldehyde, the time-domain spectra were reprocessed with 10-Hz digital broadening (see Figure 2); the integrated intensities of the aldehyde, β-furanose, and β-pyranose resonances, together with the proportions of the latter two obtained from the spectra with 0.5-Hz digital broadening, yielded the proportion of aldehyde. Estimated precision, as a *percentage of the given value*, is ±2 for αP and βP, ±10 for αF and βF, and ±25 for CHO and $CH(OH)_2$. [b] pH 4.8. [c] Not detected. [d] pH 6.0.

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Nitrogen-15-Labeled Oligodeoxynucleotides. Characterization by ^{15}N NMR of d[CGTACG] Containing $^{15}N^6$- or $^{15}N^1$-Labeled Deoxyadenosine†

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The use of nitrogen NMR to probe DNA structure has been limited by the quadrupolar nature of the most abundant isotope of nitrogen, ^{14}N, and by the insensitivity and low natural abundance (0.37%) of the spin-$^1/_2$ isotope, ^{15}N.[1] We recently developed a synthetic route for the transformation of deoxyadenosine into both the $^{15}N^6$ and $^{15}N^1$ derivatives.[2] We now report the first syntheses of ^{15}N-labeled oligonucleotides and the first examples of the use of ^{15}N NMR to monitor and to characterize an oligonucleotide helix-to-coil transition.

The syntheses of d[CGT($^{15}N^1$)ACG] and d[CGT($^{15}N^6$)ACG] were carried out by a large-scale phosphoramidite prodecure similar to that reported previously.[3] In the present work, however, the 2-cyanoethyl group was used as the phosphate triester protecting group,[4,5] and deoxyadenosine (^{15}N labeled) was N protected

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