(1′-^{13}C)-2′-Deoxyribonucleosides: Structural and Conformational Insights Derived from ^{13}C–^{1}H Spin Coupling Constants Involving C1′

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Received March 9, 1993 (Revised Manuscript Received May 17, 1993)

2′-Deoxyadenosine (1), 2′-deoxycytidine (2), and thymidine (3) have been prepared with ^{13}C-enrichment at C1′ (99 atom% ^{13}C) and studied by ^{1}H NMR spectroscopy at 500 MHz in $^{2}H_2O$. $^{1}J_{CH}$, $^{2}J_{CH}$ and $^{3}J_{CH}$ values were measured between C1′ and several protons in the furanose and base moieties of 1–3 and compared to related values observed in corresponding ribonucleosides and erythronucleosides. Results are consistent with the expected shift to south conformers on conversion of ribonucleosides to 2′-deoxyribonucleosides. The observation that C1′ of 1–3 couples more strongly to H2′*S* (~5.7 Hz) than to H2′*R* (≤0.4 Hz) has been explained using model compounds that mimic the C1′–C2′–H2′*R* and C1′–C2′–H2′*S* coupling pathways in pure north and south conformers of 1–3. Results suggest that the difference, $|^{2}J_{C1',H2'S}| - |^{2}J_{C1',H2'R}|$, may be a useful probe of N/S equilibria in 2′-deoxyribonucleosides in $^{2}H_2O$ solution. Model compounds have also been used to probe the effect of ring conformation on the chemical shifts of H2′*R* and H2′*S* in 1–3. For simple, unphosphorylated 2-deoxy-β-D-ribofuranosyl rings in aqueous solution, the difference, $\delta_{H2'R} - \delta_{H2'S}$, may be correlated with N/S distribution.

Introduction

In recent years stable isotopes have played a key role in the development of NMR spectroscopy as an integral tool to assess the conformations of macromolecules and their complexes in solution. For example, the combined use of ^{13}C- and/or ^{15}N-labeled proteins with multidimensional NMR methods has assisted in the collection and interpretation of otherwise complex spectra by providing enhanced sensitivity and/or spectral editing features.[1] The binding of small substrates to protein receptors has been studied by labeling either partner, usually uniformly with ^{13}C, to permit, via several approaches, the discrimination between the signals of the substrate and those of the receptor.[2a-f] While these applications are now commonly appreciated in the study of protein structure, related approaches to the study of nucleic acids have not been as rigorously developed, although a few studies of uniformly ^{13}C-labeled RNA and DNA have appeared recently.[2g-i] The paucity of applications in these latter systems likely results from the unavailability of ^{13}C- and ^{15}N-labeled nucleosides, and a limited knowledge of ^{13}C–^{1}H and ^{13}C–^{13}C spin coupling constants in these biomolecules. This latter information is critical to the proper implementation of many multipulse NMR methods used for sensitivity enhancement and/or spectral editing.

Nucleic acids are homophosphofuranose polysaccharides composed of conformationally flexible monomers (e.g., β-D-ribofuranose 5-phosphate, 2-deoxy-β-D-*erythro*-pentofuranose 5-phosphate) that are substituted at the anomeric carbon with nitrogen-containing heterocycles. Thus, studies of ^{13}C–^{1}H spin couplings within the furanosyl rings of these polymers are logical extensions of previous studies of carbohydrate systems in general,[3] and furanose rings in particular.[4] Using methods described previously,[5a,6a] three 2′-deoxyribonucleosides (2′-deoxyadenosine (1), 2′-deoxycytidine (2), thymidine (3) (Scheme I) have been prepared with ^{13}C-enrichment at C1′, and ^{13}C–^{1}H spin couplings involving C1′ have been examined within the furanosyl ring and across the *N*-glycoside linkage. These couplings have been compared to corresponding couplings in the ribonucleosides[5a] and erythronucleosides[5b] in order to validate their utility as conformational probes. The effect of furanose ring conformation on the chemical shifts of the C2 protons of 2-deoxyaldofuranosyl rings has also been examined with the use of model compounds.

Experimental Section

Materials. 2′-Deoxyadenosine, 2′-deoxycytidine, thymidine, 2-deoxy-D-glucose (2-deoxy-D-*arabino*-hexose) and 2-deoxy-D-allose (2-deoxy-D-*ribo*-hexose) were purchased from Sigma Chemical Co. [1′-^{13}C]-2′-Deoxyadenosine, [1′-^{13}C]-2′-deoxycytidine, [1′-^{13}C]thymidine, and [1′-^{13}C]ribothymidine (99 atom%)

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(3) (a) Bock, K.; Pedersen, C. *Acta Chem. Scand.* **1977**, *B31*, 354. (b) Schwarcz, J. A.; Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1975**, *53*, 1872. (c) Cyr, N.; Hamer, G. K.; Perlin, A. S. *Can. J. Chem.* **1978**, *56*, 297. (d) Schwarcz, J. A.; Perlin, A. S. *Can. J. Chem.* **1972**, *50*, 3667. (e) Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett.* **1973**, 1037. (f) Bock, K.; Pedersen, C. *J. Chem. Soc. Perkins Trans. 2* **1974**, 293. (g) Bock, K.; Pedersen, C. *Acta Chem. Scand.* **1975**, *B29*, 258.

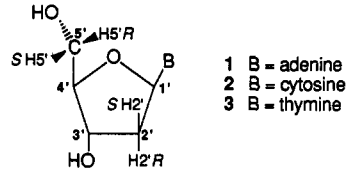
(4) (a) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504. (b) Serianni, A. S.; Barker, R. *J. Org. Chem.* **1984**, *49*, 3292. (c) Snyder, J. R.; Serianni, A. S. *J. Org. Chem.* **1986**, *51*, 2694. (d) Vuorinen, T.; Serianni, A. S. *Carbohydr. Res.* **1990**, *209*, 13. (e) Snyder, J. R.; Serianni, A. S. *Carbohydr. Res.* **1987**, *163*, 169.

(5) (a) Kline, P. C.; Serianni, A. S. *J. Am. Chem. Soc.* **1990**, *112*, 7373. (b) Kline, P. C.; Serianni, A. S. *J. Org. Chem.* **1992**, *57*, 1772. (c) Crystalline labeled nucleosides were analyzed by high-resolution mass spectrometry (Finnigan-MAT 8430), giving the following results: [1′-^{13}C]-2′-deoxyadenosine, $C_{10}H_{13}O_3N_5$, *m/e* 252.1052 calcd, 252.1070 found; [1′-^{13}C]-2′-deoxycytidine, $C_9H_{13}O_4N_3$, *m/e* 228.0940 calcd, 228.0935 found; [1′-^{13}C]thymidine, $C_9H_{14}N_2O_5$, *m/e* 243.0936 calcd, 243.0942 found; [1′-^{13}C]ribothymidine, $C_{10}H_{14}N_2O_6$, *m/e* 259.0885 calcd, 259.0876 found.

(6) (a) Kline, P. C.; Serianni, A. S. *Magn. Reson. Chem.* **1990**, *28*, 324. (b) Austin, P. W.; Hardy, F. E.; Buchanan, J. C.; Baddiley, J. *J. Chem. Soc.* **1963**, 5350. (c) Hodge, J. E.; Hofreiter, B. T. *Meth. Carbohydr. Chem.* **1962**, *1*, 380. (d) Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.

0022-3263/93/1958-5513$04.00/0

Scheme I



were prepared as described previously.[5a,c,6a] Deuterium oxide (^2H_2O, 99 atom% ^2H) was obtained from Cambridge Isotope Laboratories.

The methyl α- and β-pyranosides of 2-deoxy-D-glucose (2-deoxy-D-*arabino*-hexose) were prepared by Fischer glycosidation. 2-Deoxy-D-glucose (0.5 g) was dissolved in anhydrous methanol (30 mL), Dowex HCR-W2 (H^+) ion-exchange resin (1.0 g) was added, and the mixture was refluxed for 24 h. After cooling and removal of the resin by filtration, the glycoside mixture was chromatographed on a 70 × 3.5 cm column containing Dowex 1×2 (200–400 mesh) resin in the hydroxide form,[6b] using distilled water as the solvent. Fractions (20 mL) were collected and assayed with phenol–sulfuric acid.[6c] The α-pyranoside eluted before the β-pyranoside, at elution volumes of ~280 mL and ~400 mL, respectively. The pyranosides were identified by their characteristic ^{13}C chemical shifts.[6d]

Instrumentation. High-resolution ^1H NMR spectra were obtained at 500 MHz. Probe temperature was regulated at 30 °C, and sample solutions (0.6 mL, 10–20 mM in ^2H_2O) were analyzed in 5-mm NMR tubes. Spectra were obtained with sufficient digital resolution to permit the use of resolution-enhancement to improve the detection of small couplings.

Computer simulation of 500-MHz ^1H NMR spectra was performed using the LAOCN5 program as implemented in the FTNMR program (VAX version) available from Hare Research, Inc. of Woodinville, WA. The computations were conducted on a Digital VaxStation 3200 minicomputer equipped with a Tektronix CX4107 graphics terminal.

Results

A. Furanose ^1H Chemical Shift Assignments. Signal assignments for H1′, H3′, and H4′ of **1–3** were made straightforwardly, since these signals are well resolved at 500 MHz. A comparison between the ^1H NMR spectra of unlabeled and [1′-^{13}C]-labeled **1-3** permitted the direct determination of $^1J_{C1',H1'}$, $^3J_{C1',H3'}$, and $^3J_{C1',H4'}$ values (Table I). The stereochemical assignments of the diastereotopic protons at C5′ (H5′*R* and H5′*S*) of **1–3** (Scheme I) have been made previously[6a] by selective deuteration (H5′*R* is more shielded than H5′*S*), but these assignments are not critical to the present study since C1′ is not coupled to these protons. However, as shown in Table I, C1′ is selectively coupled to *one* of the C2′ protons in **1–3**, and thus their assignments are essential to a structural interpretation of this behavior.

The C2′ proton in **1–3** that is *cis* to H3′ is defined as H2′*S*, whereas that which is *cis* to O3′ is H2′*R* (Scheme I). In **1**, C1′ is coupled strongly to the *less shielded* C2′ proton (defined arbitrarily as H2′), whereas in **2**, C1′ is coupled to the *more shielded* C2′ proton (defined arbitrarily as H2″) (Table I). The remaining C2′ proton in **1** and **2** exhibits little or no coupling to C1′ (Table I). Fraser-Reid and Radatus[7] have previously prepared **2** with stereospecific deuteration at H2′*R* and H2′*S* and found that the H2′*S* signal is more shielded than that of H2′*R*. Thus, in **2**, $^2J_{C1',H2'S}$ = 5.7 Hz, whereas $^2J_{C1',H2'R}$ = 0 Hz.

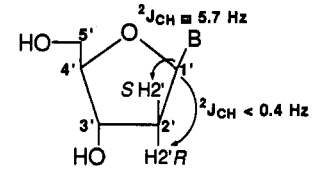
(7) Fraser-Reid, B.; Radatus, B. *J. Am. Chem. Soc.* **1971**, *93*, 6342.

Table I. ^1H–^1H and ^{13}C–^1H Spin Coupling Constants[a] in 2′-Deoxyribonucleosides 1–3

coupled nuclei[b]	compound 1	2	3[c]
H1′, H2′	7.7	~6.5	6.8
H1′, H2″	6.3	~6.7	6.7
H2′, H2″	−14.1	−14.2	−14.2
H2′, H3′	6.1	4.1	4.1
H2″, H3′	3.3	~6.7	6.7
H3′, H4′	~3.1	~4.0	3.9
H4′, H5′	3.3	3.6	3.9
H4′, H5″	4.3	5.3	5.2
H5′, H5″	−12.7	−12.5	−12.5
H5, H6		7.6	
H1′, H3′		0.6	~0.5
H1′, H5		0.4	
H6, C*H*$_3$[d]			1.3
C1′, H1′	167.4 (165.6)[d]	170.8 (170.3)	170.1 (169.0)
C1′, H2′	~5.7[e]	0	~0
C1′, H2″	~0.4	5.7[e]	~5.7[e]
C1′, H3′	5.3 (~5.1)	4.5 (3.0)	4.6 (3.7)
C1′, H4′	2.9 (1.3)	2.4 (0.6)	2.2 (<1)
C1′, H6		2.9 (2.8)	2.8

[a] In Hz, ±0.1 Hz; coupling signs were not determined. [b] For the prochiral C2′ and C5′ sites, the more shielded proton is denoted by the double prime (″) symbol. [c] Data obtained by computer simulation. [d] Values in parentheses pertain to the corresponding ribonucleoside;[5a] data for ribothymidine have not been reported previously. [e] The sign of this coupling is probably negative. [f] Nuclei in italics coupled to H6.

Scheme II



Furthermore, Wood et al.[8a] concluded from the inverse complementarity of $^3J_{H2',H3'}$ and $^3J_{H2'',H3'}$ in **1** and **2** (Table I) that the H2′*S* signal in **1** is *less* shielded than that of H2′*R*. This conclusion is confirmed by the magnitudes of $^2J_{C1',H2'}$ and $^2J_{C1',H2''}$ in **1**; a larger coupling is observed to the less-shielded proton, which like **2**, is *cis* to H3′. In addition, recent studies of site-specifically deuterated **1** have indicated that H2′*R* is more shielded than H2′*S*.[8b] Thus, in **1** and **2**, C1′ is strongly coupled to the C2′ proton *cis* to H3′ (~5.7 Hz) and weakly coupled (<0.4 Hz) to the C2′ proton *cis* to O3′ (Scheme II). The signs of these couplings are discussed below.

$^2J_{C1',H2'R}$ and $^2J_{C1',H2'S}$ in **3** were more difficult to obtain since the 500-MHz ^1H NMR spectrum of **3** is not first-order. The chemical shifts of H2′*R* and H2′*S* are very similar, giving rise to a deceptively simple quartet (Figure 1A), which in [1′-^{13}C]-**3** is converted to a nine-line multiplet (Figure 1B). Using J_{HH} and J_{CH} values observed in **2** as initial parameters, the ^1H NMR spectrum of [1′-^{13}C]-**3** was simulated (Figure 1, parts C and D), and the calculated couplings are given in Table I. Like **2**, the H2′*S* proton of **3** is more shielded than H2′*R*, but the chemical shift difference is small (1.5 Hz at 500 MHz); again only H2′*S* is coupled to C1′ (Table I, Scheme II).

The ^1H NMR spectrum of an equimolar mixture of **1** and **2** shows that H2′*R* and H2′*S* in **1** are deshielded relative

(8) (a) Wood, D. J.; Hruska, F. E.; Ogilvie, K. K. *Can. J. Chem.* **1974**, *52*, 3353. (b) Huang, W.-C.; Orban, J.; Kintanar, A.; Reid, B. R.; Drobny, G. P. *J. Am. Chem. Soc.* **1990**, *112*, 9059. (c) The ^1H NMR spectrum of 2′-deoxyguanosine contains well-resolved multiplets for H2′*R* and H2′*S* similar to those observed for **1** in terms of chemical shifts and ^1H–^1H spin couplings. By analogy to **1**, therefore, it is reasonable to assume that H2′*R* is the more shielded C2′ proton in this nucleoside.

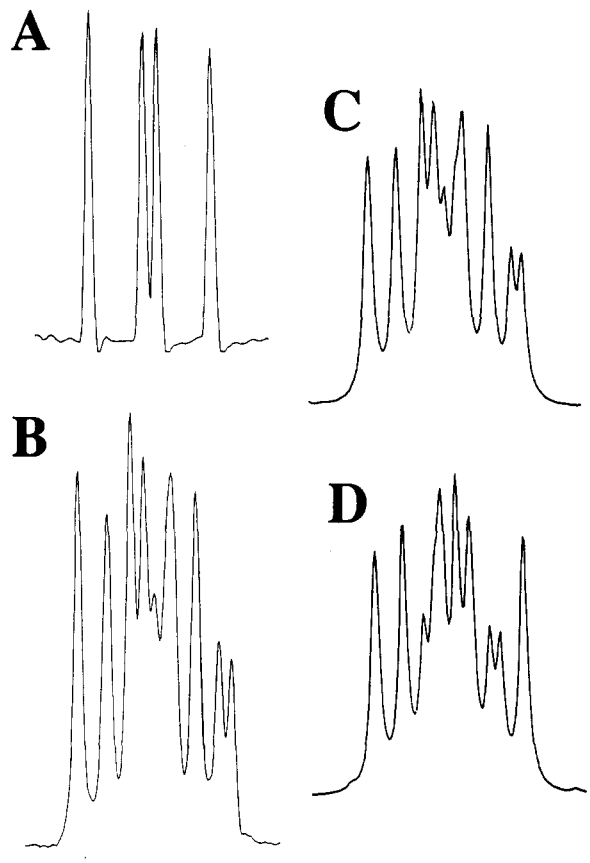


Figure 1. (A) The partial 500-MHz ^{1}H NMR spectrum (resolution-enhanced) of thymidine (**3**) showing a deceptively simple quartet due to H2′*R* and H2′*S*. (B) The same signals as in A observed in the ^{1}H NMR spectrum of [1′-^{13}C]-**3**. (C and D) The same signals as in B obtained by spectral simulation with the assumption that H2′*S* is coupled to C1′ by about −5.7 Hz and is more shielded (C) and less shielded (D) than H2′*R*, with the latter not coupled to C1′; data in C is more consistent with the experimental data in B.

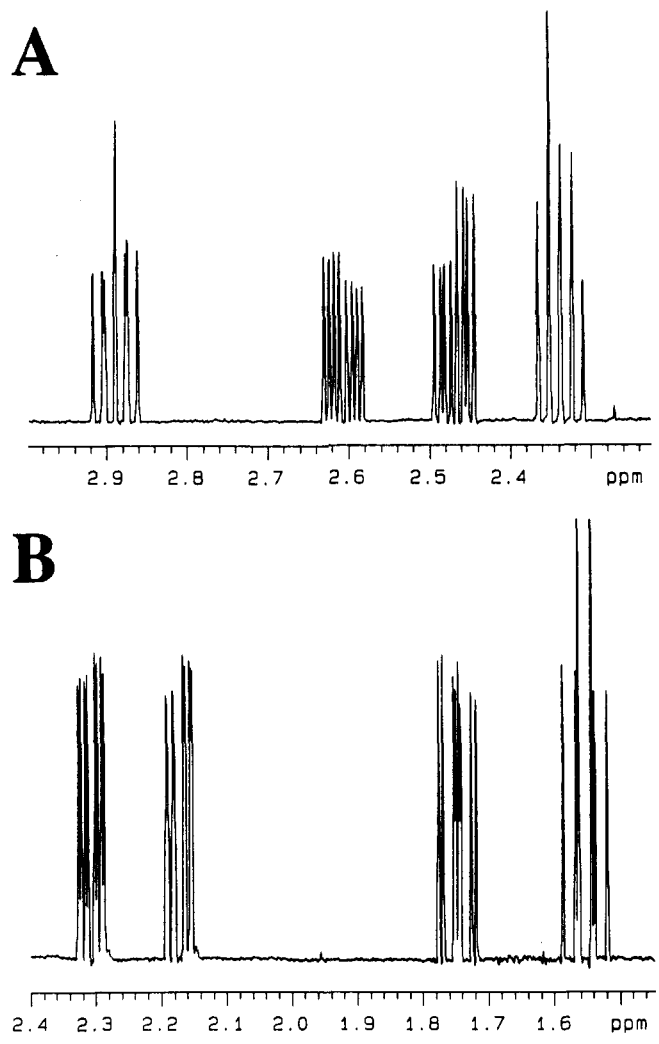


Figure 2. (A) The partial 500-MHz ^{1}H NMR spectrum of a 1:1 mixture of 2′-deoxyadenosine (**1**) and 2′-deoxycytidine (**2**) in ^2H_2O. Signal assignments are as follows: 2.34 ppm, H2′*S*, **2**; 2.47 ppm, H2′*R*, **2**; 2.60 ppm, H2′*R*, **1**; 2.89 ppm, H2′*S*, **1**. (B) The partial 500-MHz ^{1}H NMR spectrum of **7** and **8** in ^2H_2O. Signal assignments are as follows: 1.55 ppm, H2*S*, **8**; 1.75 ppm, H2*S*, **7**; 2.17 ppm, H2*R*, **7**; 2.30 ppm, H2*R*, **8**.

to the corresponding protons in **2**, but the deshielding effect on H2′*S* is significantly greater (+0.55 ppm) than that of H2′*R* (+0.13 ppm) (Figure 2A). Thus the larger downfield shift in the C2′ proton *cis* to the base substitution (H2′*S*) is responsible for the inverted assignments of the C2′ protons in **1** and **2**. Ring current effects from the purine base are mainly responsible for the deshielding of H2′*S* in **1**, as discussed previously by Wood et al.,[8a] but other factors may play a role in determining $\delta_{H2'R}$ and $\delta_{H2'S}$ in 2′-deoxyribonucleosides, as discussed below.[8c]

B. ^{13}C–^{1}H Spin Coupling Constants in 1–3. As observed in ribonucleosides[5a] and erythronucleosides,[5b] $^1J_{C1',H1'}$ is larger in pyrimidine 2′-deoxyribonucleosides than in purine 2′-deoxyribonucleosides, although the difference is small (Table I). Comparisons between 2′-deoxyribonucleosides and corresponding ribonucleosides (e.g., **1** and adenosine (**5**), **2** and cytidine (**6**)) show $^1J_{C1',H1'}$ to be slightly larger in the former (Table I). This difference may be attributed to changes in *N*-glycoside torsions, different (averaged) orientations of the C1′–H1′ bond (quasiaxial, quasiequatorial), substituent effects (2′-oxy, 2′-deoxy), and/or other factors.[5a]

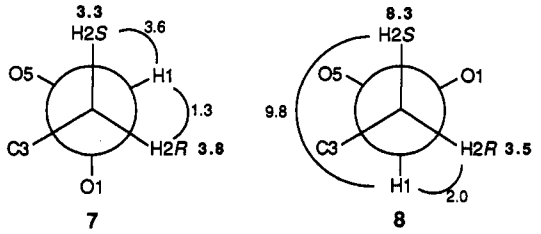
Previous analyses of ^{1}H–^{1}H couplings in **1** and **2** have indicated that these compounds have a greater preference in aqueous solution for south (S) conformers with **5** and **6**.[6,9] This conclusion is confirmed by the behavior of $^3J_{C1',H4'}$, which should increase in magnitude as S conformers become more favored.[10] In **1–3**, $^3J_{C1',H4'}$ ranges from 2.2–2.9 Hz, whereas in the corresponding ribonucleosides, couplings of 0.6–1.3 Hz are observed. Likewise, $^3J_{C1',H3'}$ should increase as S conformers become more preferred, but a direct comparison of these couplings is not strictly valid since the coupling pathways are dissimilar. Studies of $^3J_{CH}$ in aliphatic compounds have shown that an internal electronegative substituent appended to the central carbon in a H–C–C–C coupling pathway truncates the magnitude of $^3J_{CH}$.[11a] Nevertheless, $^3J_{C1',H3'}$ is larger (4.8±0.4 Hz) in 2′-deoxyribonucleosides than in ribonucleosides (3.9±1.1 Hz) (Table I).[11b]

$^2J_{C1',H2'R}$ and $^2J_{C1',H2'S}$ are significantly different in **1–3** as might be expected, since the disposition of electrone-

(9) (a) Davies, D. B. *Prog. Nucl. Magn. Reson. Spectrosc.* **1978**, *12*, 135. (b) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York 1984.

(10) An inspection of Figure 6B in ref 5a suggests that the extent to which $^3J_{C1',H4'}$ increases as the N/S equilibrium shifts towards S forms will depend on the specific S forms favored. Thus, a greater increase is expected when the favored forms lie near $^2E/E_3$ (pure south) than when southeast (e.g., E_1) forms are preferred.

Scheme III



gative substituents along C–C–H coupling pathways has been shown to be an important determinant of $^2J_{CCH}$.[3a–d] However, in **1–3**, these dispositions can vary due to the inherent conformational flexibility of the furanose ring. To assist in the structural interpretation of these couplings, $^2J_{C1,H2R}$ and $^2J_{C1,H2S}$ were examined in the conformationally rigid 2-deoxy-α- and β-D-[1-^{13}C]glucopyranoses **7** and **8**. At 500 MHz, the H2*R* and H2*S* signals of **7** and **8** are essentially first-order (Figure 2B), thereby permitting measurements of $^2J_{C1,H2R}$ and $^2J_{C1,H2S}$ directly from the spectrum. Since **7** and **8** highly prefer the $^4C_1$ conformation, assignment of the H2*R* and H2*S* signals can be made as follows. The characteristic H1 signals of **7** and **8** were well resolved (5.0–5.5 ppm), with the upfield signal containing $^3J_{HH}$ values of 2.0 and 9.8 Hz and the downfield signal containing values of 1.3 and 3.6 Hz. In **7**, H1 is gauche to both C2 protons, while H1 is gauche and anti to the C2 protons in **8** (Scheme III). Thus, the upfield H1 signal can be assigned to H1 of **8**. Correlation of these couplings to those found in the four H2 multiplets (Figure 2B), and the fact that H2*S* in **7** and **8** should show a large *trans* coupling to H3 (12.0 Hz), yielded H2*R* and H2*S* signal assignments for **7** and **8** (**7**, δ_{H2R} 2.17 ppm, δ_{H2S} 1.75 ppm; **8**, δ_{H2R} 2.30 ppm, δ_{H2S} 1.55 ppm). Thus in **7** and **8**, H2*S* is axial and more shielded than H2*R*. $^2J_{C1,H2R}$ and $^2J_{C1,H2S}$ values determined from the ^{1}H NMR spectrum of [1-^{13}C]-**7** and **8** were as follows: $^2J_{C1,H2R}$ = 3.8 Hz and $^2J_{C1,H2S}$ = 3.3 Hz for **7**; $^2J_{C1,H2R}$ = 3.5 Hz and $^2J_{C1,H2S}$ = 8.3 Hz for **8**. These couplings were analyzed by the projection rule[3a] and the vector resultant method,[3b–d] both of which predict a large *negative* value for $^2J_{C1,H2S}$ in **8** (since the resultant vector produced by the O5 and O1 bonds eclipses the C2–H2*S* bond; Scheme III) and similar but less-negative values for the remaining three couplings.[11c]

The above data may now be applied to $^2J_{C1',H2'R}$ and $^2J_{C1',H2'S}$ in **1–3** by noting that the C1–C2 bond projections for **7** and **8** correlate *approximately* with the C1′–C2′ bond projections for north (N) (E_2) and south (^2E) furanose conformations of **1–3**, respectively (Scheme IV). An analysis of the C1′–C2′–H2′*R*(*S*) coupling pathways in **1–3** (Table II) predicts $^2J_{C1',H2'R}$ and $^2J_{C1',H2'S}$ to be similar in magnitude in N conformers and substantially different in S conformers, in agreement with experiment. Thus, the difference, $|^2J_{C1',H2'S}| - |^2J_{C1',H2'R}|$, should be a useful probe of N/S equilibria in **1–3** and should increase in magnitude as S forms become more preferred.[12a] Recent NMR studies[12b] of the DNA octamer, d($A_1GCCA_5A_6TA_8$), selectively enriched with ^{13}C at C1′ of the A_1 residue have shown that C1′ is coupled to H2′*S* (6.5 Hz) but not to H2′*R*, suggesting that the furanose ring of A_1 highly prefers a south conformation. Indeed, an analysis of $^3J_{HH}$ values in this residue indicated a preference for the C2′-*endo* conformation (~80%).

Scheme IV

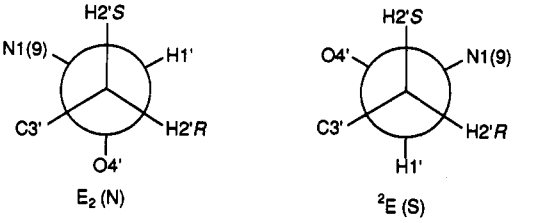


Table II. Analysis of $^2J_{C1',H2'R}$ and $^2J_{C1',H2'S}$ in 1–3 via Projection Sum[a] and Vector[b] Methods

C2′ proton (conformer)	projections (sums),[c] deg	vector resultant angle,[c] deg
H2′*S* (E_2, north)	0, 120 (0.5)	120
H2′*S* (^2E, south)	120, 120 (−1.0)	0
H2′*R* (E_2, north)	0, 120 (0.5)	120
H2′*R* (^2E, south)	0, 120 (0.5)	120

[a] Described in ref 3a. [b] Described in ref 3b–d. [c] Values based on furanose rings with τ_m = 60° (Scheme IV). Analysis with τ_m = 35° (expected in solution) led to similar conclusions about the conformational dependence of $^2J_{C1',H2'R(S)}$ in **1–3** (see text).

C. Chemical Shifts of H2′*R* and H2′*S* and Furanose Ring Conformation. Data collected on **7** and **8** provide some insight into the chemical shift behavior of H2′*R* and H2′*S* in **1–3**. In **7** and **8**, the *more* shielded C2 proton (H2*S*) lies in an *axial* orientation. By analogy, H2′*S* is expected to be more shielded than H2′*R* in **1–3**, if south (^2E) conformers are preferred. In contrast, H2′*R* is expected to be more shielded than H2′*S* if north (E_2) conformers are preferred (in E_2, H2′*R* is axial). Since $^3J_{HH}$ data indicate that **1–3** prefer S conformations, **1** deviates significantly from the predicted pattern, and **3** less so, and these deviations are probably caused mainly by deshielding effects by the base on H2′*S* as discussed above.

Interconversion between **7** and **8** changes the relative orientations of the substituents on C1 and C2, but not between C2 and C3. In both anomers, O3 is gauche with respect to H2*R* and H2*S* and thus its effect on the chemical shift of these protons should be roughly similar. The latter, however, is not true for N/S interconversion in **1–3** (i.e., the gauche orientation of H2′*S* and O3′ in N conformers changes to anti in S conformers). Thus, *in the absence of base effects*, the chemical shift *difference*, $\delta_{H2R} - \delta_{H2S}$, observed in **7** ($\Delta$ = 0.42 ppm) should be a reasonable approximation of the difference found in N conformers of **1–3**. The related difference observed in **8** (Δ = 0.75 ppm) is probably larger than that expected in S conformers of

(11) (a) Marshall, J. L. *Carbon-Carbon and Carbon-Proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis*, Verlag Chemie International: Deerfield Beach, FL, 1983, pp 20–21. (b) It is unclear whether the predicted increase in $^3J_{C1',H3'}$ for the deoxy coupling pathway, which is based on observations made on aliphatic compounds,[11a] is valid in carbohydrate systems. $^3J_{C1,H3}$ = 1.3 Hz in methyl β-D-glucopyranoside and $^3J_{C1,H3}$ < 0.8 Hz in methyl 2-deoxy-β-D-glucopyranoside, whereas $^3J_{C1,H5}$ is essentially the same (2.5 Hz), as expected, in both compounds. $^3J_{C1,H3}$ is essentially zero in both methyl α-D-glucopyranoside and methyl 2-deoxy-α-D-glucopyranoside. These limited data suggest that $^3J_{C1',H3'}$ in 2-deoxyribonucleosides may be the same or *smaller* than $^3J_{C1',H3'}$ in ribonucleosides, at least for dihedral angles of ~60°. (c) $^2J_{C1,H2R}$ and $^2J_{C1,H2S}$ in **7** and $^2J_{C1,H2R}$ in **8** may be positive in sign.

(12) (a) The value of $|^3J_{C1',H2'S}| - |^3J_{C1',H2'R}|$ may also be a useful probe of N/S equilibrium in simple 2-deoxyaldofuranosides. For β-D-anomers, this value should increase in magnitude (become more positive) as S forms become more preferred, as predicted for **1–3**. In α-D-anomers, this value should also increase in magnitude (become less negative) as S conformers become more preferred (in N forms of this anomer, H2*R* should couple more strongly to C1 than H2*S*). (b) Wu, J.; Serianni, A. S. 203rd American Chemical Society National Meeting, San Francisco, CA, April, 1992, Division of Biological Chemistry, Abstr. 116.

1-3 (a five-membered ring proton is more shielded when syn to an OH group than when anti to it; the *syn*-upfield rule).[4b,13] However, the value of $\delta_{H2'R} - \delta_{H2'S}$ for S conformers can be estimated experimentally using 2-deoxy-β-D-allopyranose (2-deoxy-β-D-*ribo*-hexopyranose) **9** as a model compound. In **9**, the relative orientations of the substituents on C1 and C2, and C2 and C3, are similar to those found in S conformers. Analysis of the 500-MHz ^{1}H NMR spectrum of **9** shows H2*S* (axial) is more shielded by 0.36 ppm than H2*R* (equatorial).[14]

Results obtained on **7** and **9** suggest that the difference, $|\delta_{H2'R} - \delta_{H2'S}|$, may be similar in *pure* N and S conformers of unphosphorylated 2-deoxy-β-D-aldofuranosyl rings lacking aromatic substituents at C1, and that $\delta_{H2'R}$ and $\delta_{H2'S}$ exchange positions during N/S interconversion due to complementary changes in the quasiaxial/quasiequatorial orientation of the C-H bonds. As a consequence, N/S conformational averaging will significantly affect the value of $\delta_{H2'R} - \delta_{H2'S}$, with large positive values observed when S conformers are preferred, large negative values when N conformers are preferred, and zero or small values when N and S forms exist in comparable proportions. Thus, for simple 2-deoxy-β-D-aldofuranosyl rings *in which base effects are absent* (e.g., in reducing sugars and simple alkyl glycosides), the value of $\delta_{H2'R} - \delta_{H2'S}$ may be used to assess N/S distributions.[15] However, in purine 2'-deoxyribonucleosides (e.g., **1**) where base effects are significant, an interpretation of $\delta_{H2'R}$ and $\delta_{H2'S}$ in terms of N/S equilibrium is not straightforward.[17a] In pyrimidine 2'-deoxyribonucleosides **2** and **3**, similar $^3J_{HH}$ values for the furanose ring protons suggest similar N/S equilibria (Table I), yet the magnitudes of $\delta_{H2'R} - \delta_{H2'S}$ for these compounds differ significantly,[17b] implying that pyrimidine base effects are not neglible.

Conclusions

^{1}H NMR properties of several [1'-^{13}C]-2'-deoxyribonucleosides (**1-3**) have been reported in this study. The stereochemical assignments of the prochiral C2' protons have been confirmed through an analysis of $^2J_{C1',H2'R}$ and $^2J_{C1',H2'S}$. Previous rules devised to interpret $^2J_{CH}$ in carbohydrates[3a-d] have been used to show that C1' is more highly coupled to H2'*S* than to H2'*R*, behavior which is consistent with $^3J_{HH}$ and $^3J_{CH}$ data suggesting that the furanose rings of **1-3** prefer south conformations. More importantly, the magnitude of $|^2J_{C1',H2'S}| - |^2J_{C1',H2'R}|$ appears to be a useful parameter to assess N/S distribution in 2'-deoxyribonucleosides as free entities or as residues in DNA oligomers and may complement $^1J_{CH}$[18] in some instances.

The chemical shift behavior of H2'*R* and H2'*S* in **1-3** was also studied in an attempt to correlate this behavior with ring conformation. Results suggest that, in simple unphosphorylated 2-deoxyaldofuranosyl rings (e.g., reducing sugars, alkyl glycosides), the value of $\delta_{H2'R} - \delta_{H2'S}$ may reflect N/S distribution. However, this correlation cannot be readily extended to 2'-deoxyribonucleosides in which base effects on ^{1}H chemical shifts (especially H2'*S*) are significant. Similar complications are likely to arise in oligodeoxyribonucleotides in which both intra- and interresidue base effects and phosphate effects will determine $\delta_{H2'R} - \delta_{H2'S}$ for a specific residue. It is interesting to note, however, that H2'*R* is commonly found downfield of H2'*S* in oligodeoxyribonucleotides,[19] as observed in **2** and **3** but not in **1**.

The assessment of furanose ring behavior in DNA and RNA oligomers in solution by NMR is complicated by increased resonance line widths which may prevent the measurements of some NMR parameters diagnostic of ring conformation (e.g., the relatively small two- and three-bond ^{1}H-^{1}H and ^{13}C-^{1}H couplings[5a,b]). As oligomers increase in size, structural information derived from the larger $^1J_{CH}$[20] and $^1J_{CC}$[21] (and ^{13}C chemical shifts[22]) increases in importance, since these parameters are easier to measure in more complex molecules. Further study of the effect of molecular structure on these couplings will be needed in order to assess their full potential as conformational probes.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM 33791) and Omicron Biochemicals, Inc. of South Bend, IN. The authors thank Carol Podlasek for obtaining the ^{1}H NMR spectra of **7-9**, Cambridge Isotope Laboratories for providing a sample of 2-deoxy-D-[1-^{13}C]glucose (99 atom% ^{13}C), and Dr. Bruce Plashko for obtaining high-resolution mass spectra on the labeled nucleosides.

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(14) Data for **9** are as follows: δ_{H2R}, 2.13 ppm; δ_{H2S}, 1.77 ppm; $^3J_{H1,H2R}$, 2.2 Hz; $^3J_{H1,H2S}$, 9.9 Hz; $^3J_{H2R,H3}$, 3.8 Hz; $^3J_{H2S,H3}$, 2.8 Hz; $^2J_{H2R,H2S}$, -14.1 Hz.

(15) Recent results[16] support this prediction. The ^{1}H NMR spectrum of 2-deoxy-5-*O*-methyl-β-D-ribofuranose in ^2H_2O at 25 °C shows that $\delta_{H2'R} - \delta_{H2'S}$ is slightly negative, indicating a small excess of N conformers in solution. An analysis of $^3J_{HH}$ data for this compound indicates a slight preference for *N* forms (58%).

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(17) (a) Giessner-Prettre, C.; Pullman, B. *Quart. Rev. Biophys.* **1987**, *20*, 113-172. (b) The ^{1}H NMR spectrum of an equimolar mixture of **2** and **3** in ^2H_2O shows that the H2'*R* and H2'*S* signals of **3** fall between those of **2**. The effect of pyrimidine base structure on the C2' protons appears greater for that proton *cis* to the base (H2'*S*), which experiences a greater (downfield) shift in converting **2** to **3** than H2'*R* (upfield).

(18) The magnitude of $^1J_{CH}$ in pyranosyl rings has been shown to depend on the orientation of the C-H bond (axial vs equatorial),[3a-c] with axial orientations giving couplings about 10 Hz smaller than equatorial orientations. Similar obeservations have been made in furanosyl rings.[4d,20]

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(21) Preliminary experimental and calculational results suggest a dependence of $^1J_{CC}$ on the C-C torsion angle in O-C-C-O molecular fragments that may be valuable as a conformational probe in carbohydrate systems: Carmichael, I.; Chipman, D. M.; Podlasek, C.; Serianni, A. S. *J. Am. Chem. Soc.* **1993**, in press.

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