ELSEVIER

Carbohydrate Research 284 (1996) 51–60

CARBOHYDRATE RESEARCH

Assignment of the ^{1}H, ^{19}F, and ^{13}C NMR spectra of 2-deoxy-2-fluoro-D-ribose and characterisation of the isomeric equilibrium

Paul N. Sanderson, Brian C. Sweatman, R. Duncan Farrant, John C. Lindon *

Department of Physical Sciences, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK

Received 3 November 1995; accepted 12 January 1996

Abstract

The assignment of the ^{1}H, ^{19}F, and ^{13}C NMR chemical shifts and coupling constants of 2-deoxy-2-fluoro-D-ribose, an important intermediate in the synthesis of antiviral nucleoside drugs, is reported and the NMR spectra are used to determine the proportions of the pyranose and furanose forms together with the anomeric ratios in acetone-d_6 solution. The β-pyranose isomer is shown to exist at equilibrium with both $^{4}C_{1}$ and $^{1}C_{4}$ conformations in approximately equal proportions in fast exchange. The α-pyranose isomer at equilibrium is predominantly in the $^{4}C_{1}$ form but the $^{1}C_{4}$ conformer is also present in solution, the two forms being in intermediate exchange on the ^{19}F NMR timescale but in fast exchange on the ^{1}H and ^{13}C NMR timescales. For both the pyranose and furanose forms, the β-anomer predominates. The results are similar to those for D-ribose.

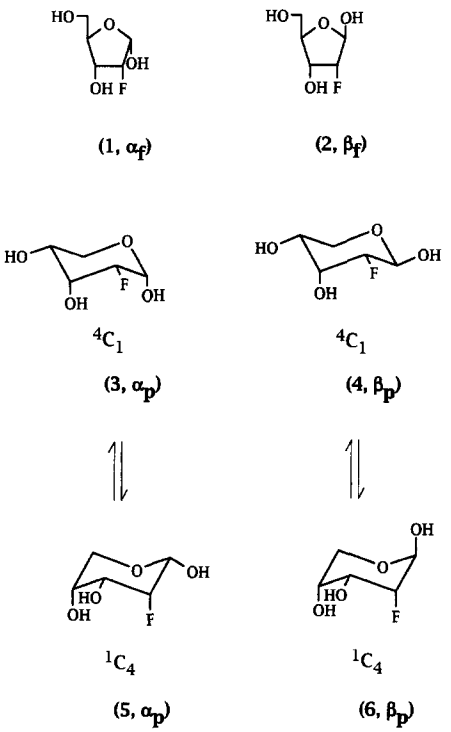
Keywords: NMR; Fluorosugars; Ribose

1. Introduction

Modified sugars have been used frequently as mimics of natural carbohydrates in many drug design studies and for probing biochemical processes [1]. In the area of chemotherapy, for drugs which inhibit nucleic acid synthesis, analogues of the natural

* Corresponding author. Present address: Department of Chemistry, Birkbeck College, University of London, Gordon House, 29 Gordon Square, London WC1H 0PP, UK.

SSDI S0008-6215(96)00020-1



Scheme 1.

aldopentoses, and in particular derivatives of ribose, have been used extensively [2]. One type of sugar modification has been to replace a hydroxyl group by a fluorine as in the use of 2-deoxy-2-fluoro-D-ribose.

Although the NMR spectra of 2-deoxy-2-fluoroarabinose and 2-deoxy-2-fluoroxylose have been reported [3], there appear to be no published studies of the NMR spectra of 2-deoxy-2-fluoro-D-ribose nor of its anomeric or ring isomer proportions. The ^1H spectra of the arabinose and xylose derivatives were reported without assignments and the ^{13}C NMR spectrum of the former compound was assigned but only α- and β-pyranose forms were considered. The synthesis of 2-deoxy-2-fluororibose has been reported [4,5] as has the synthesis of the arabinose [5] and lyxose [6] analogues and the ^{19}F NMR chemical shifts in a series of fluoromonosaccharides have been reported and analysed in terms of structural dependency [7].

The ^1H, ^{19}F, and ^{13}C NMR spectra of 2-deoxy-2-fluoro-D-ribose have therefore now been measured and assigned using a combination of one-dimensional and two-dimensional NMR spectroscopy. Furthermore, the isomeric equilibrium has been determined and compared with that of ribose.

The structures of the four ring forms of 2-deoxy-2-fluoro-D-ribose are shown in Scheme 1, as the α-furanose (α_f) (**1**), β-furanose (β_f) (**2**), α-pyranose (α_p) (**3**, **5**), and β-pyranose (β_p) (**4**, **6**) forms, respectively. In addition, the possibility of the acyclic aldehyde form of the compound needs to be considered. The pyranose form could exist

in either or both chair conformations, i.e. $^4C_1$ (**3**, **4**) or $^1C_4$ (**5**, **6**) as shown in Scheme 1, or in various boat and twist-boat forms.

2. Experimental

NMR spectroscopy was performed on a solution of 2-fluoro-2-deoxy-D-ribose in acetone-d_6 on a Bruker AMX600 spectrometer equipped with an inverse detection 5 mm broadband probe with z-field gradient coils operating at 600.13 MHz for ^{1}H and 150.90 MHz for ^{13}C. ^{19}F detection was at 564.69 MHz on a dual ^{19}F/^{1}H probe. Chemical shift referencing was to the solvent acetone-d_5 resonance at δ 2.05 for ^{1}H, to the solvent acetone-d_6 resonance at 29.5 ppm for ^{13}C and to external $CFCl_3$ at 0.0 ppm for ^{19}F. Unless otherwise mentioned, all experiments were performed at 303 K. Resolution enhancement of the one-dimensional NMR spectra was by the Lorentzian–Gaussian transformation method.

Homonuclear correlation experiments, COSY-45 [8] and TOCSY [9], were typically acquired with a spectral width of 2100 Hz. For COSY-45 (magnitude mode) spectra, 32 transients of 2048 complex data points were acquired for each of 512 FIDs. Data were processed with a sine-bell function prior to Fourier transformation in both dimensions. The TOCSY data were acquired in phase sensitive mode with States-TPPI phase cycling with a spin lock period of 75 ms and 80 transients of 4096 complex data points were acquired for each of 400 FIDs. Data were processed with a sine-bell squared function prior to Fourier transformation in both dimensions.

Two-dimensional ^{1}H–^{13}C HMQC spectra (magnitude mode) [10] were obtained with the use of field gradients for coherence selection and GARP ^{13}C decoupling [11]. A ^{1}H spectral width of 2512 Hz was used with data acquired into 2048 complex data points in F2. A delay of 3.85 ms (corresponding to $1/2J_{CH}$ for J_{CH} 130 Hz) was used and 64 transients were collected for each of 256 increments in F1. Data were processed with a sine-bell squared function prior to Fourier transformation in both dimensions. A ^{19}F-detected ^{19}F–^{1}H correlation spectrum was obtained with a spectrum width of 16,667 Hz and 64 transients of 2048 complex data points were acquired for each of 512 FIDs. Data were processed with a sine-bell squared function prior to Fourier transformation in both dimensions.

2-fluoro-2-deoxy-D-ribose was synthesised in-house [12] as a white powder; mp 123–125 °C. Anal. Calcd for $C_5H_9F_1O_4$: C, 39.48; H, 5.96; F, 12.49. Found: C, 39.38; H, 6.00; F, 12.18.

3. Results and discussion

The 600 MHz ^{1}H NMR spectrum of 2-deoxy-2-fluoro-D-ribose in acetone-d_6 at 303 K with resolution enhancement is shown in Fig. 1. Assignment of resonances to the various isomers, as indicated in Fig. 1, was based on the following premises: (*i*) the chemical shifts of the anomeric protons of furanose isomers are deshielded by around 0.3–0.5 ppm relative to pyranose isomers for ribose [13]; (*ii*) the β to α anomeric ratio

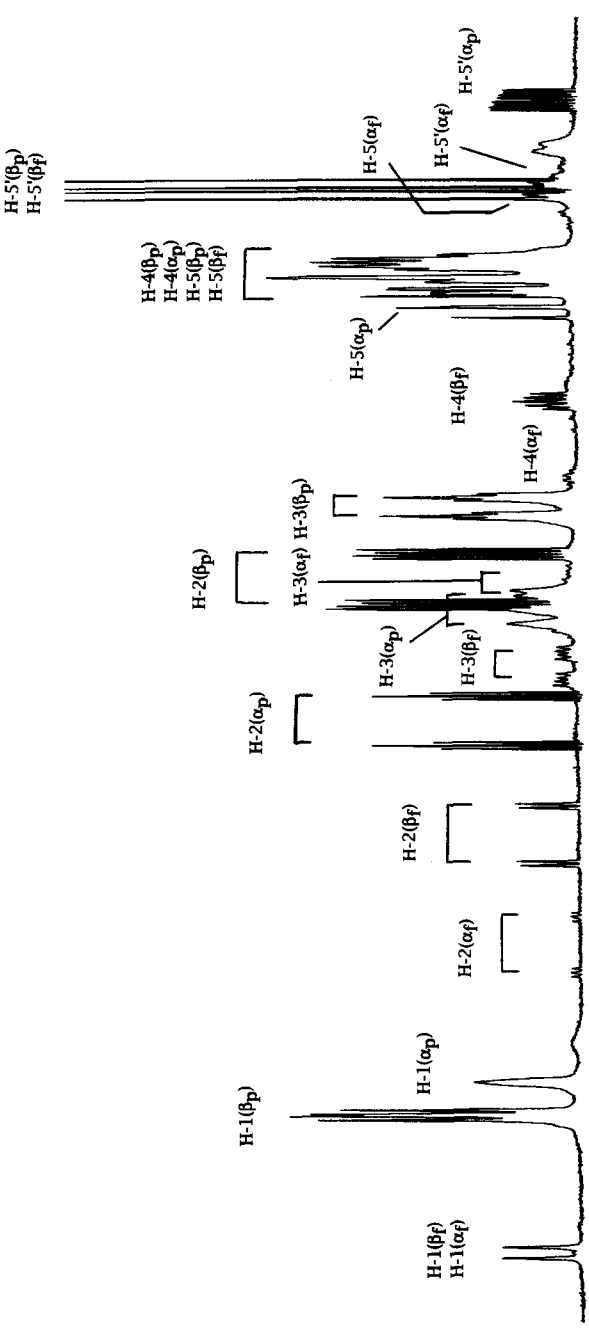


Fig. 1. Resolution enhanced 600 MHz ^{1}H NMR spectrum of 2-deoxy-2-fluoro-D-ribose in acetone-d_6 showing all assignments. Key: α_f, β_f, α_p, and β_p denote the α-furanose, β-furanose, α-pyranose, and β-pyranose forms, respectively.

is always greater than one for aldopentoses [14]; (*iii*) the ^{13}C chemical shift of C-4 in pentofuranoses is around 85 ppm whereas for pentopyranose forms it is near 70 ppm [15]. Full assignments then followed from coupling connectivities determined from correlation experiments. ^{1}H chemical shifts were obtained from COSY-45 and TOCSY NMR spectra. The ^{19}F chemical shifts were obtained from a one-dimensional spectrum (Fig. 2) and were assigned using a ^{19}F-detected heteronuclear correlation experiment. ^{13}C NMR chemical shifts were measured from ^{1}H-detected HMQC experiments, based on one-bond $^{13}C-^{1}H$ coupling constants. The low level of the α-furanose form precluded the detection of $^{1}H-^{13}C$ correlations and thus the assignment of the ^{13}C resonances for this isomer was not possible. The chemical shift data are shown in Table 1 together with all of the measured coupling constants.

The relative proportions of the four main isomers of 2-deoxy-2-fluoro-D-ribose in acetone-d_6 at 303 K were measured from the ^{19}F NMR spectrum and were 66 : 21 : 11 : 2. The two most abundant species are the pyranose forms which have more shielded anomeric proton ^{1}H chemical shifts and, perhaps most diagnostically, more shielded ^{13}C chemical shifts for C-4. The β-form is known to be the major pyranose form in aldopentoses [14]. Similarly, the two furanose forms are assigned based on ribose, assuming that the β-isomer is the more abundant [13]. A further distinguishing feature between the pyranose and furanose forms is the $^{2}J_{HF}$ coupling constant known to be around 50 Hz [16]. For the furanoses, this is observed here to be around 54 Hz whereas it is 6–8 Hz smaller for the pyranose forms. In each case, the $^{2}J_{HF}$ value for the β-form is slightly higher than that for the α-form.

The ratio of isomers determined for 2-deoxy-2-fluoro-D-ribose is in general agreement with data previously reported for equilibria of D-ribose itself in D_2O solutions. In the following, the numbers refer to the ratio β-pyranose : α-pyranose : β-furanose : α-furanose, respectively. The first reported data, based upon anomeric proton resonances were 54 : 18 : 16 : 12 at 70 °C [13] and values, derived by the same method, of 56 : 20 : 18 : 6 were reported for the equilibrium at 35 °C [17]. Breitmaier and Hollstein [14], using ^{13}C NMR at 30 °C, found a ratio of 62.0 : 20.3 : 11.6 : 6.1 and Horton and Walaszek [18], also using ^{13}C NMR at 30 °C, produced values of 64 : 19 : 11 : 6. Later Angyal [19], using ^{1}H NMR at 31 °C, reported proportions of 48.5 : 21.5 : 13.5 : 6.5 and, most recently, Franks et al. [20] published values of 55 : 23 : 14 : 8 obtained by ^{1}H NMR at 30 °C. For 2-deoxy-2-fluoro-D-ribose solutions at equilibrium therefore, it can be seen that there is a higher ratio of pyranose : furanose isomers than is found for D-ribose itself; also the ratio of β- to α-pyranose appears to be slightly higher for the fluoro derivative. No evidence was found in this study for the presence of the acyclic form.

^{19}F NMR spectra at 564 MHz were recorded at 5° intervals between 298 and 318 K. The spectra recorded at 298, 308, and 318 K are shown in Fig. 2. The relative proportions of each species at each temperature were determined by comparison of the areas of the fluorine resonances. Over this temperature range there is a small decrease in the level of the β-pyranose form (from 69 to 61% of the total) and slight increase in each of the other three species. Such a relative change in proportions was also observed on heating ribose in D_2O [17]. Each of the fluorine resonances also undergoes a change in chemical shift with temperature in that they all move to low frequency by varying amounts as the temperature is increased. This is most likely due to the inherent

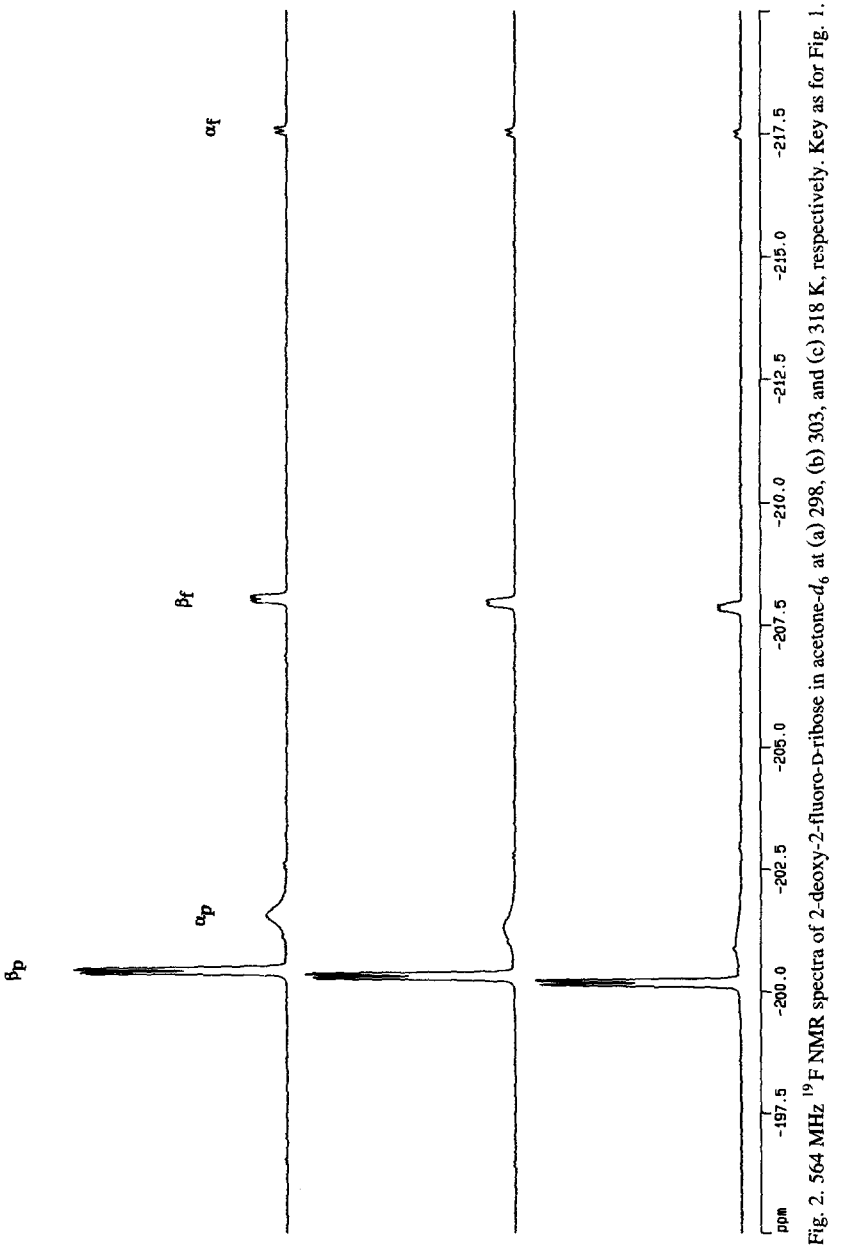


Fig. 2. 564 MHz ^{19}F NMR spectra of 2-deoxy-2-fluoro-D-ribose in acetone-d_6 at (a) 298, (b) 303, and (c) 318 K, respectively. Key as for Fig. 1.

Table 1
NMR parameters for 2-deoxy-2-fluoro-D-ribose[a]

	α-Furanose (1)	β-Furanose (2)	α-Pyranose (3)+(5)	β-Pyranose (4)+(6)
Proportions	2	11	21	66
^{19}F	−217.5	−207.9	−201.1	−200.2
δH-1	5.28	5.29	5.01	5.07
δH-2	4.79	4.62	4.44	4.22
δH-3	4.20	4.36	4.26	4.10
δH-4	4.06	3.94	3.70	3.71
δH-5	3.65	3.72	3.79	3.73
δH-5′	3.56	3.59	3.46	3.60
C-1	nd[b]	99.49	92.14	92.64
C-2	nd	96.01	87.18	91.42
C-3	nd	70.45	71.62	68.62
C-4	nd	83.40	67.08	68.26
C-5	nd	≈ 62.5	58.99	64.08
J_{12}(HF)	12.5	10.8	3.1	4.0
J_{32}(HF)	20.6	25.2	3.1	17.8
$J_{22'}$(HF)	53.3	54.1	46.6	48.4
J_{12}(HH)	3.4	0.6	3.1	5.7
J_{23}(HH)	4.6	4.1	3.1	2.9
J_{34}(HH)	~ 8.5	7.3	nd	3.0
J_{45}(HH)	~ 4	3.5	9.8	nd
$J_{45'}$(HH)	~ 3	3.5	4.8	7.2
$J_{55'}$(HH)	11.4	nd	11.1	11.0
J_{22}(CF)	nd	174	197	182
J_{12}(CF)	nd	28	nd	nd
J_{32}(CF)	nd	~ 18	~ 10	~ 20

[a] In acetone-d_6 at 303 K.
[b] nd = not detected.

temperature dependence of ^{19}F NMR chemical shifts [21] rather than due to small changes in the relative amounts of multiple conformations.

Evidence for the nature of the pyranose ring conformations can be obtained by analysis of the vicinal $^3J_{HH}$ coupling constants. These were obtained by first-order analysis by direct measurement of the splittings. This represents an approximation in that there is a degree of second-order nature to the spectrum as can be seen from the ^1H chemical shifts given in Table 1. However, this is only significant for the H-4 and H-5 resonances of the β-pyranose form and it is accepted that the value for J_{45} for this form might be in error and thus its value must be treated with circumspection for the determination of the conformation. For the α-pyranose form, the large value of 9.8 Hz for J_{45} demonstrates that H-4 must be axial and therefore is indicative of the $^4C_1$ conformer **(3)**. Although this indicates that this is the predominant conformer, there is also evidence that other conformations may be populated to some degree as the ^{19}F and

H-1 ^{1}H resonances in particular are broadened and do not show resolved couplings. The ^{19}F resonance for this form becomes appreciably sharper as the temperature is raised from 298 to 318 K (see Fig. 2) providing further evidence that this resonance is reflecting conformational exchange at an ''intermediate'' rate.

The β-pyranose form of 2-deoxy-2-fluoro-D-ribose in acetone-d_6 solution at 303 K exhibits small couplings of approximately 3 Hz for both J_{23} and J_{34}; these values are small for both chair conformers of this isomer and are therefore not diagnostic for identifying the pyranose conformation. The measured values for J_{12} and J_{45} are 5.7 Hz and 7.2 Hz, respectively, and are intermediate between those expected for a *trans*-diaxial coupling and an axial–equatorial or di-equatorial coupling. The observed values are therefore consistent with an equilibrium mixture containing significant levels of the two chair conformers. The relative amounts of each conformer can be estimated with knowledge of the coupling constants for each of the individual chair conformers. These have been determined using an extended Karplus equation taking into account the electronegativity and orientation of neighbouring substituents [22]. The group electronegativities used were taken from the literature [23] with the exception of a fluorine substituent which was assumed to have a value of 1.70 relative to hydrogen [22]. The ring oxygen was approximated by an OCH_2 group and ring carbons were considered as CH_2OH groups because electronegativities for the specific groups required in these molecules have not been reported. These calculations give values for J_{12} of 8.9 and 3.2 Hz for the $^{4}C_1$ and $^{1}C_4$ conformers, respectively, and for J_{45} they give values of 10.8 and 2.7 Hz, respectively, for the same conformers. On the basis of these calculations, the measured values of J_{12} and J_{45} indicate, respectively, that the $^{4}C_1$ conformer comprises 56 or 45% of the equilibrium mixture. The closeness of these two values suggests that the value of J_{45} derived by first-order analysis is a good approximation. Thus, it would appear that the β-pyranose form of 2-deoxy-2-fluoro-D-ribose exists as an approximately 50 : 50 mixture of the $^{4}C_1$ and $^{1}C_4$ conformers at equilibrium.

From the earliest studies of Rudrum and Shaw [17] it has been recognised that the pyranose form of β-D-ribose, in D_2O solution, exists as an equilibrium mixture of both chair forms. α-D-Ribopyranose, on the other hand, in aqueous solution existed predominantly in a single chair conformation which was shown [13] to be $^{4}C_1$. More recently, Franks et al. [20] have also shown that β-D-ribopyranose exists as a mixture of chair conformers whilst the α-anomer is present in solution predominantly as the $^{4}C_1$ conformer. In the present study, the coupling constant data indicate that, at equilibrium, 2-deoxy-2-fluoro-D-ribose exhibits similar conformational behaviour to D-ribose. Furthermore, as both the ^{19}F and ^{1}H resonances for the β-pyranose form of 2-deoxy-2-fluoro-D-ribose are sharper than those for the α-pyranose, it would appear that interconversion between the two chair conformers is faster on the NMR timescale for the β-pyranose. The comparison of the conformational results for 2-deoxy-2-fluororibose and ribose given here rely on the assumption that the NMR parameters of carbohydrates are closely similar in D_2O and acetone-d_6 solutions. To confirm this point, there is recent evidence given in an NMR spectroscopic study on 4-deoxy-4-fluoroglucose which has been examined in both solvents. In that case the vicinal coupling constants which were used to determine the ring conformations were very similar in the two solvents, with values differing by only 0.1–0.3 Hz [24].

Conformational equilibria have also been demonstrated for various ribose derivatives. In a study of aldopentopyranose tetraacetates in chloroform solution [25], it was concluded that these exist almost entirely in the $^4C_1$ (D) conformation, with the exception of the β-D-ribose derivative which appeared to contain substantial proportions of each chair form. Similarly, in chloroform solutions α-D-ribopyranose tetrabenzoate was found to exist as the $^4C_1$ conformer, but β-D-ribopyranose tetrabenzoate existed as a 2 : 1 ratio of conformers with the $^1C_4$ form predominating [26]. The relative proportions of the conformers was determined in these studies by analysis of coupling constants as weighted means of those from the individual forms present. In subsequent studies of β-D-ribopyranose tetraacetate, the two conformers have been "frozen out" at low temperatures in acetone [27] and the rates of conformational inversion and coalescence temperatures have also been reported [28].

An indication that the position of the equilibrium between the chair forms of 2-deoxy-2-fluoro-D-ribose in acetone-d_6 solution changes with temperature can be obtained from the observation that the chemical shifts of the ^{1}H resonances of this species change with temperature. The most readily observed signals are from H-1, H-2, and H-3. Of these, the resonances from H-1 and H-3 become less shielded at higher temperatures, whereas that from H-2 moves in the opposite direction. As the two chair conformers are in fast exchange, the observed chemical shift represents a weighted average of the chemical shifts of equivalent protons in the two conformers. Thus, using one of the earliest recorded rules for assignment of ^{1}H NMR spectra of carbohydrates [29], which states that "axial protons. usually appear at higher field than equatorial protons in chemically similar environments", the observed changes in chemical shift are consistent with there being a greater proportion of the $^1C_4$ conformer of 2-deoxy-2-fluoro-D-ribose at higher temperatures.

References

[1] N.F. Taylor (Ed.), *Fluorinated Carbohydrates, Chemical and Biochemical Aspects*, ACS Syposium Series, No. 374, ACS, Washington, DC, USA, 1988.
[2] P. Oksman, H. Hakala, S. Zavgorodny, M. Polianski, A. Azhayev, A. van Aerschot, P. Herdewijn, and H. Lönnberg, *J. Phys. Org. Chem.*, 5 (1992) 741–747.
[3] M. Bols and I. Lundt, *Acta Chem. Scand.*, 44 (1990) 252–256.
[4] J.T. Welch and S. Eswarakrishnan, *J. Chem. Soc., Chem. Commun.*, (1985) 186–188.
[5] E.L. Albano, R.L. Tolman, and R.K. Robins, *Carbohydr. Res.*, 19 (1971) 63–70.
[6] C.G. Butchard and P.W. Kent, *Tetrahedron*, 27 (1971) 3457–3463.
[7] P.W. Kent, R.A. Dwek, and N.F. Taylor, *Tetrahedron*, 27 (1971) 3887–3891.
[8] W.P. Aue, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229–2246.
[9] A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
[10] A. Bax, R.H. Griffey, and B.L. Hawkins, *J. Magn. Reson.*, 55 (1983) 301–315.
[11] A.J. Shaka, P.B. Barker, and R. Freeman, *J. Magn. Reson.*, 64 (1985) 547–552.
[12] J.V. Tuttle and T.A. Krenitsky, private communication.
[13] R.U. Lemieux and J.D. Stevens, *Can. J. Chem.*, 44 (1966) 249–262.
[14] E. Breitmaier and U. Hollstein, *Org. Magn. Reson.*, 8 (1976) 573–575 (1976).
[15] K. Bock and H. Thøgersen, *Annu. Rep. NMR Spectroscopy*, 13 (1982) 1–57.
[16] E. Pretsch, T. Clerc, J. Seibl, and W. Simon, *Tables of Spectral Data for Structure Determination of Organic Compounds*, 2nd ed., Springer, Berlin, Germany, 1989.

[17] M. Rudrum and D.F. Shaw, *J. Chem. Soc.*, (1965) 52–57.
[18] D. Horton and Z. Walaszek, *Carbohydr. Res.*, 105 (1977) 145–153.
[19] S.J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 15–68.
[20] F. Franks, P.J. Lillford, and G. Robinson, *J. Chem. Soc., Faraday Trans. 1*, 85 (1989) 2417–2426.
[21] J.W. Emsley and L. Phillips, *Prog. NMR Spectrosc.*, 7 (1971) 1–520.
[22] L.A. Donders, F.A.A.M. de Leeuw, and C. Altona, *Magn. Reson. Chem.*, 27 (1989) 556–563.
[23] N. Inamoto and S. Masuda, *Chem. Lett.*, (1982) 1003–1006.
[24] R.J. Abraham, E.J. Chambers, and W.A. Thomas, *Magn. Reson. Chem.*, 32 (1994) 248–254.
[25] R.U. Lemieux and J.D. Stevens, *Can. J. Chem.*, 43 (1965) 2059–2070.
[26] B. Coxon, *Tetrahedron*, 22 (1966) 2281–2302.
[27] P.L. Durette, D. Horton, and N.S. Bhacca, *Carbohydr. Res.*, 10 (1969) 565–577.
[28] P.L. Durette and D. Horton, *J. Org. Chem.*, 36 (1971) 2658–2669.
[29] R.U. Lemieux, R.K. Kullnig, H.J. Bernstein, and W.G. Schneider, *J. Am. Chem. Soc.*, 80 (1958) 6098–6105.