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Chiral Hydroxymethyl Groups: ^{1}H NMR Assignments of the Prochiral C-5′ Protons of 2′-Deoxyribonucleosides

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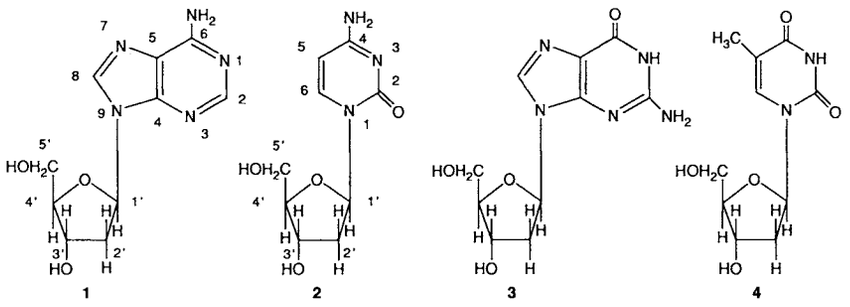
2′-Deoxyadenosine, 2′-deoxycytidine, 2′-deoxyguanosine and 2′-deoxyuridine were prepared with stereoselective deuteriation at C-5′ and used to assign the prochiral C-5′ protons in 300 MHz ^{1}H NMR spectra obtained in ^2H_2O. In all cases, the more shielded C-5′ proton was found to be the pro-*R* proton. From these assignments, C-4′—C-5′ rotamer populations were determined using three previously published methods based on the spin couplings, ^3J(H-4′,H-5′*R*) and ^3J(H-4′,H-5′*S*), and the errors associated with these methods were assessed. The effects of base structure, furanose and *N*-glycoside bond conformation on the relative populations of hydroxymethyl rotamers in nucleosides are discussed.

KEY WORDS ^{1}H NMR 2′-Deoxyribonucleosides Prochiral C-5′ proton assignment Rotamer populations Hydroxymethyl rotamers

INTRODUCTION

The conformational properties of the biologically important 2′-deoxyribonucleosides [2′-deoxyadenosine (**1**), 2′-deoxycytidine (**2**), 2′-deoxyguanosine (**3**) and thymidine (**4**)] (Scheme 1) have been examined extensively by NMR,[1] x-ray crystallography[2] and calculational methods.[3] The data suggest that furanose, hydroxymethyl and *N*-glycoside conformations in these biomolecules are correlated.[4,5] Although a complete analysis of hydroxymethyl conformation in **1–4** by ^{1}H NMR spectroscopy depends on the unambiguous signal assignments of the prochiral C-5′ methylene protons, only indirect techniques have been used previously to make these assignments; the most common technique is based on the differential deshielding effects of 2′- and 3′-phosphorylation on the chemical shifts of the C-5′ protons.[6,7] These assignments, however, are made most reliably in 2′-deoxyribonucleosides that have been stereoselectively deuteriated at either H-5′*R* or H-5′*S*. To date, hydroxymethyl assignments in **1–4** based on selective C-5′ deuteriation have not been reported.

We recently prepared four ribonucleosides [adenosine (**5**), cytidine (**6**), guanosine (**7**) and uridine (**8**)] (Scheme 2) in which the H-5′*S* proton was partially replaced with deuterium.[8] ^{1}H NMR spectra (300 MHz) of deuteriated **5–8** showed that H-5′*S* resonated downfield of H-5′*R*. In this study, **5–8** were stereoselectively deuteriated at H-5′*S* and chemically converted to the corresponding deuteriated 2′-deoxyribonucleosides by the method of Robins *et al.*[9] The hydroxyl groups at C-3′ and C-5′ were protected with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TPDS-Cl_2), and the free 2′-OH group was converted to the phenyl thionocarbonate ester with phenyl chlorothionoformate. After deoxygenation with tributyltin hydride and removal of the TPDS group, the crude ^{2}H-enriched 2′-deoxyribonucleosides were purified by chromatography,[10] crystallized, and analyzed by ^{1}H NMR spectroscopy. A comparison of the ^{1}H NMR spectra of natural and deuteriated 2′-deoxyribonucleosides has provided the unambiguous resonance assignments of H-5′*R* and H-5′*S* required to evaluate hydroxymethyl conformation in these biologically important compounds.



Scheme 1

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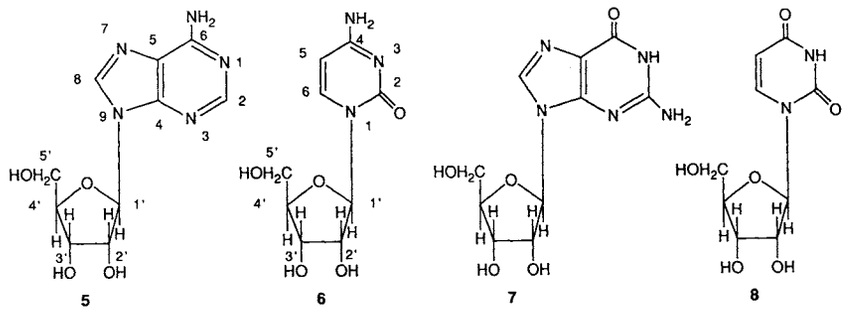


Scheme 2

EXPERIMENTAL

2′-Deoxyadenosine, 2′-deoxycytidine, 2′-deoxyguanosine, 2′-deoxyuridine and thymidine were purchased from Sigma. Deuterium oxide (^2H_2O, 99 at.-% ^2H) was purchased from Cambridge Isotope Laboratories. Isopropyl bromide, trichlorosilane, 4-*N*,*N*-dimethylaminopyridine (DMAP), phenyl chlorothionoformate (PTC-Cl) and tributyltin hydride were obtained from Aldrich. 2,2′-Azobis(2-methylpropionitrile) (AIBN) was purchased from Chemical Dynamics. Chlorine gas was purchased from Linde Specialty Gases. All chemicals were of analytical-reagent grade and were used without further purification.

1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (TPDS-Cl_2) was synthesized according to the procedure of Van Boom and Wreesmann.[11] A solution of isopropyl bromide (270 ml, 2.9 mol) in anhydrous diethyl ether (400 ml) was added dropwise to a stirred suspension of magnesium curls (64 g, 2.6 mol) in anhydrous diethyl ether (200 ml). The mixture was refluxed for 3.5 h, and a solution of trichlorosilane (100 ml, 1.0 mol) in 400 ml of anhydrous diethyl ether was added dropwise. The mixture was refluxed for 12 h and the reaction was quenched by dropwise addition of 800 ml of 0.1 M HCl. The mixture was refluxed for an additional 3.5 h, the organic layer was removed and the aqueous layer extracted three times with 300 ml of diethyl ether. The combined organic fractions were dried over anhydrous magnesium sulfate overnight. The magnesium sulfate was removed by vacuum filtration and the solution was concentrated *in vacuo* at 35 °C to a colorless oil, which was further distilled under reduced pressure (b.p. 83–89 °C, 10 mm Hg) to give 1,1,3,3-tetraisopropyldisiloxane (92 g, 373 mmol) as a colorless oil.

The distilled product was dissolved in 500 ml of anhydrous methylene chloride and H_2SO_4-dried chlorine gas was bubbled through the solution (7 ml min^{-1}). When the temperature rose to 27–30 °C, the reaction mixture was cooled to 18 °C in an ice-bath. An aliquot was withdrawn every hour and analyzed by IR spectrophotometry. The reaction was complete when the absorption band at 2100 cm^{-1} disappeared. The volatile compounds were removed *in vacuo* at 30 °C and the residue was distilled under reduced pressure (b.p. 87–90 °C, 2 mm Hg). The pure 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (61 g, 193 mmol) was stored under nitrogen at 0 °C.

Methyl β-D-erythrofuranoside[12] was synthesized from D-erythrose.[13] The glycoside was refluxed in deuterium oxide with deuteriated Raney nickel[12] to yield methyl β-D-[2,3,4*S*-$^2H_3$]erythrofuranoside (**9**), which was determined by ^{1}H NMR to be completely deuteriated at H-3 and partially deuteriated (*ca.* 40%) at H-2 and H-4*S*. Compound **9** was converted to 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-[3,4,5*S*-$^2H_3$]ribofuranoside (**10**) as described previously.[8] [3′,4′,5′*S*-$^2H_3$]Ribonucleosides were synthesized from **10** using a modification of the trimethylsilyl method of Vorbrüggen *et al.*[14,15]

[3′,4′,5′*S*-$^2H_3$]Cytidine (**11**) was converted to N^4-acetyl[3′,4′,5′*S*-$^2H_3$]cytidine (**12**) using the method of Otter and Fox.[16] Compound **11** (500 mg, 2.1 mmol) was refluxed in methanol (50 ml) and acetic anhydride (500 μl, 5.3 mmol) was added in six hourly additions. After refluxing for an additional 1 h, the mixture was concentrated at 30 °C *in vacuo* and the residue was dried by evaporation twice from toluene (30 ml). Methanol (50 ml) was added and the solution refluxed a second time. Two further hourly additions of acetic anhydride were made, after which the solution was concentrated at 30 °C *in vacuo* and dried by evaporation from toluene. The product **12** was dried *in vacuo* at 50 °C overnight.

Protection of the 3′- and 5′-OH groups

[3′,4′,5′*S*-$^2H_3$]Adenosine (**13**), [3′,4′,5′*S*-$^2H_3$]uridine (**14**) or N^4-acetyl-[3′,4′,5′*S*-$^2H_3$]cytidine (**12**) (2 mmol) was suspended in 20 ml of dry pyridine, TPDS-Cl_2 (640 μl, 2 mmol) was added and the mixture was stirred for 3 h at room temperature. The pyridine was removed at 30 °C *in vacuo* and the residue was dissolved in 50 ml of ethyl acetate and extracted with 50 ml of distilled water. The organic phase was washed twice and consecutively with 20 ml of cold 1 M HCl, distilled water, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the solvent removed at 30 °C *in vacuo*. The residue was dried at 30 °C *in vacuo* overnight and used in the next step without further purification.

[3′,4′,5′*S*-$^2H_3$]Guanosine (**15**) (2 mmol) was suspended in 30 ml of *N*,*N*-dimethylformamide and 2 ml of anhydrous pyridine. TPDS-Cl_2 (650 μl, 2 mmol) was added and the mixture stirred for 5 h. The reaction mixture was poured into 500 ml of stirred ice–water and the precipitate was collected by filtration and dried *in vacuo* at 30 °C overnight.

Activation of the 2′-OH group

The crude TPDS-blocked deuteriated adenosine, guanosine or uridine was added to 30 ml of dry acetonitrile and 500 mg (4.1 mmol) of DMAP and 400 μl (2.2 mmol) of PTC-Cl were added. The solution was stirred for 16 h at room temperature and the solvent removed at 30 °C *in vacuo*. The residue was dissolved in 50 ml of ethyl acetate and extracted with 50 ml of distilled water. The organic phase was washed twice and consecutively with 40 ml of cold 1 M HCl, distilled water, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. After drying the organic phase over anhydrous sodium sulfate, the solvent was removed at 30 °C *in vacuo* and the residue dried overnight at 37 °C *in vacuo*.

Deuteriated N^4-acetyl-3′,5′-*O*-TPDS-cytidine was dissolved in 20 ml of dry acetonitrile, DMAP (2.0 g, 16.4 mmol) was added and the mixture was cooled in an ice-bath. PTC-Cl (400 μl, 2.2 mmol) was added, and the solution was stirred for 5 min in the ice-bath and for an additional 45 min at room temperature. The solvent was evaporated *in vacuo* at 30 °C and the residue dissolved in 50 ml of ethyl acetate. The organic phase was extracted with water and washed twice and consecutively with 40 ml of cold 1 M HCl, distilled water, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. The organic phase was evaporated *in vacuo* at 30 °C and the residue dried by evaporation three times from 10 ml of toluene, 20 ml of chloroform and 15 ml of diethyl ether. The residue was dried *in vacuo* at room temperature.

Deoxygenation of the 2′-OH group

The vacuum-dried residue was dissolved in 40 ml of anhydrous toluene, and oxygen-free nitrogen was bubbled through the solution for 20 min. AIBN (100 mg, 0.4 mmol) and tributyltin hydride (1.2 ml, 3.0 mmol) were added and the mixture was heated at 75 °C for 3 h (14 h for cytidine).

Removal of protecting groups

A 2-ml volume of 1 M TBAF/THF was added directly to the above mixture and the solution was heated at 75 °C for 1 h. The solvent was removed at 30 °C *in vacuo*, the residue was dissolved in 100 ml of distilled water and the solution was washed with 100 ml of diethyl ether and concentrated *in vacuo* at 30 °C.

Removal of the *N*-acetyl group from N^4-acetyl-2′-deoxycytidine was achieved by treatment with saturated methanolic ammonia (25 ml) at room temperature overnight. The solvent was evaporated *in vacuo* at 30 °C and the residue dissolved in distilled water. The aqueous phase was extracted once with 50 ml of diethyl ether and evaporated *in vacuo* at 30 °C to a syrup.

The crude 2′-deoxyribonucleosides were purified by column chromatography (21 × 2.5 cm i.d. column) on Dowex 1-X2 resin (200–400 mesh) in the hydroxide form.[10] Compound **16** was eluted with 3:7 methanol–CO_2-free distilled water and **17** with CO_2-free distilled water. The columns for **18** and **19** were washed with 400 ml of CO_2-free distilled water and eluted with 250 mM triethylammonium hydrogencarbonate (TEAB) buffer at pH 9.0. The column effluent was monitored by measuring the UV absorbance at 254 nm with an ISCO UA-5 absorbance/fluorescence detector with a Type 6 optical unit. The main peak was pooled and concentrated at 30 °C *in vacuo*. The residues containing **18** or **19** were repeatedly evaporated at 30 °C from distilled water to remove the residual TEAB. Compounds **16** and **19** were recrystallized from diethyl ether–ethanol, **17** from methanol and **18** from distilled water. The crystals were harvested and dried *in vacuo* at 45 °C.

Yields based on the starting deuteriated ribonucleosides were as follows: 2′-[3′,4′,5′*S*-$^2H_3$]deoxyadenosine (**16**), 0.35 g, 1.4 mmol, 72%; 2′-[3′,4′,5′*S*-$^2H_3$]deoxycytidine (**17**), 0.25 g, 1.1 mmol, 55%; 2′-[3′,4′,5′*S*-$^2H_3$]deoxyguanosine (**18**), 0.33 g, 1.2 mmol, 62%; 2′-[3′,4′,5′*S*-$^2H_3$]deoxyuridine (**19**), 0.3 g, 1.3 mmol, 65%.

Instrumentation

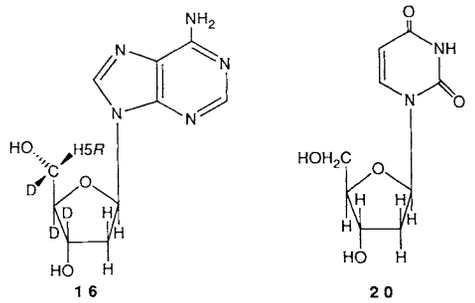
High-resolution ^1H NMR spectra were obtained at *ca.* 25 °C on a General Electric GN-300 300 MHz superconducting FT-NMR spectrometer equipped with quadrature phase detection and a 293B pulse programmer. Partially relaxed ^1H NMR spectra were obtained by the inversion–recovery method.[17] Sample concentrations were approximately 50 mM in ^2H_2O with the exception of 2′-deoxyguanosine, which was approximately 5 mM in ^2H_2O.

DISCUSSION

We have shown recently that ^2H-enriched[8] and ^{13}C-enriched[15] ribonucleosides can be prepared in good yield by the trimethylsilyl method of Vorbrüggen *et al.*[14,15] This study shows that ^2H-labeled ribonucleosides can be converted in good yield to corresponding ^2H-labeled 2′-deoxyribonucleosides using the method of Robins *et al.*[9] Thus, chemical methods provide good access to stable isotopically labeled ribo- and 2′-deoxyribo-nucleosides that may be useful for *in vivo* NMR studies of biological metabolism and NMR studies of mono- and oligo-nucleotide structure and reactivity in solution.

Partial 300 MHz ^1H NMR spectra of 2′-deoxyadenosine (**1**) and 2′-[3′,4′,5′*S*-$^2H_3$]deoxyadenosine (**16**) are shown in Fig. 1. Integration of the hydroxymethyl proton signals of the starting ribonucleoside **13** showed the H-5′*S* proton to be *ca.* 40% exchanged with deuterium. Integration of the hydroxymethyl proton signals in **16** showed the downfield C-5′ proton to be *ca.* 40% exchanged. The same result was obtained for 2′-[3′,4′,5′*S*-$^2H_3$]deoxycytidine (**17**), 2′-[3′,4′,5′*S*-$^2H_3$]deoxyguanosine (**18**) and 2′-[3′,4′,5′*S*-$^2H_3$]deoxyuridine (**19**). Therefore, in the 2′-deoxyribonucleosides **1**, **2**, **3** (Scheme 1) and 2′-deoxyuridine (**20**) and the ribonucleosides **5–8** (Scheme 2) studied previously,[8] the downfield C-5′ proton signal can be assigned unequivocally to the H-5′*S* proton. Previous deuteriation studies[12] of the eight methyl D-pentofuranosides (*arabino*, *lyxo*, *ribo*, *xylo*) also showed

that H-5′*S* is more deshielded than H-5′*R*; apparently, *N*-glycosidation does not affect the relative chemical shifts of hydroxymethyl protons in pentofuranosyl rings. In the following discussion, we assume that thymidine (**4**) (Scheme 1), which was not deuteriated in this study, behaves like 2′-deoxyuridine (**20**) with respect to H-5′*R* and H-5′*S* signal assignments.



The partial deuterium exchange at H-5′*S* in **16–19** complicates their ^{1}H NMR spectra owing to the presence of overlapping subspectra. Partially relaxed spectra of **16–19** resolve these subspectra and confirm the site of deuteriation at C-5′ (Fig. 2). The H-5′*R* proton in molecules containing ^{2}H at H-5′*S* will have a longer spin–lattice relaxation time (T_1) than the H-5′*R* proton in molecules lacking ^{2}H at H-5′*S*. In addition, the H-5′*R* resonance will be shifted upfield in molecules containing ^{2}H at the geminal H-5′*S* site.[12] Clearly, the upfield singlet relaxes more slowly than the other hydroxymethyl proton signals and is assigned to H-5′*R* in molecules deuteriated at H-5′*S*. Hence the difference in T_1 values between H-5′*R* in H-5′*S* non-deuteriated and deuteriated molecules can be exploited to resolve overlapping subspectra and assign the site of deuteriation (Fig. 2).

Having assigned the C-5′ protons unambiguously, the conformational properties of the C-4′—C-5′ bond, γ, in **1**, **2**, **3** and **20** may be evaluated. Hydroxymethyl group conformation may be described in terms of three staggered rotamers about γ (*gg*, *gt* and *tg*) (Scheme 3). The observed ^3J(H-4′,H-5′*R*) and ^3J(H-4′,H-5′*S*) values (Table 1) represent a weighted average of these three rotamers, and the populations (*P*) of each rotamer may be calculated from the equations

$$0.9P_{gg} + 10.7P_{gt} + 5.0P_{tg} = {}^3J(\text{H-4}',\text{H-5}'R) \quad (1)$$

$$0.9P_{gg} + 2.8P_{gt} + 10.7P_{tg} = {}^3J(\text{H-4}',\text{H-5}'S). \quad (2)$$

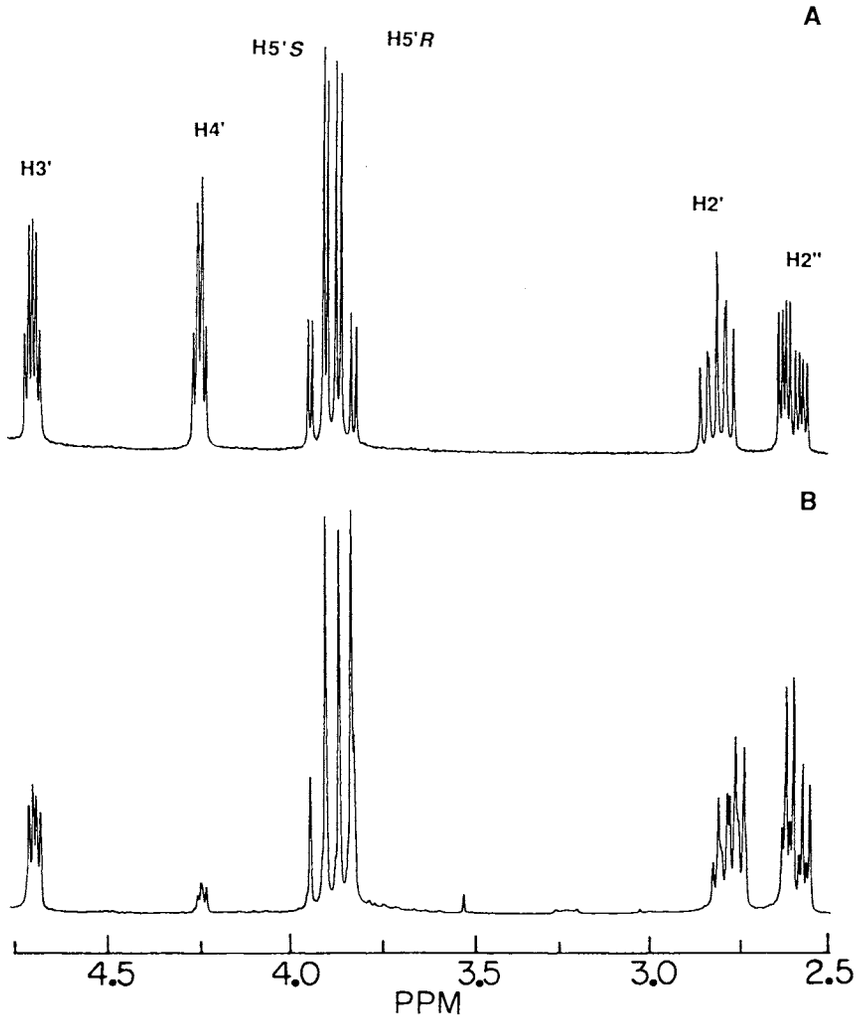


Figure 1. (A) Partial 300 MHz ^{1}H NMR spectrum of 2′-deoxyadenosine (**1**) in ^2H_2O showing the 2′-deoxyribose signal assignments. (B) Partial 300 MHz ^{1}H NMR spectrum of 2′-[3′,4′,5′*S*-$^2H_3$]deoxyadenosine (**16**) in ^2H_2O. Deuterium exchange is not complete at H-3′, H-4′ and H-5′*S* (see text). The hydroxymethyl proton signals appear as a superimposed pair of doublets (due to molecules deuteriated at H-4, and protonated at H-5′*S* and H-5′*R*) and a singlet (due to molecules deuteriated at H-4 and H-5′*S*, and protonated at H-5′*R*).

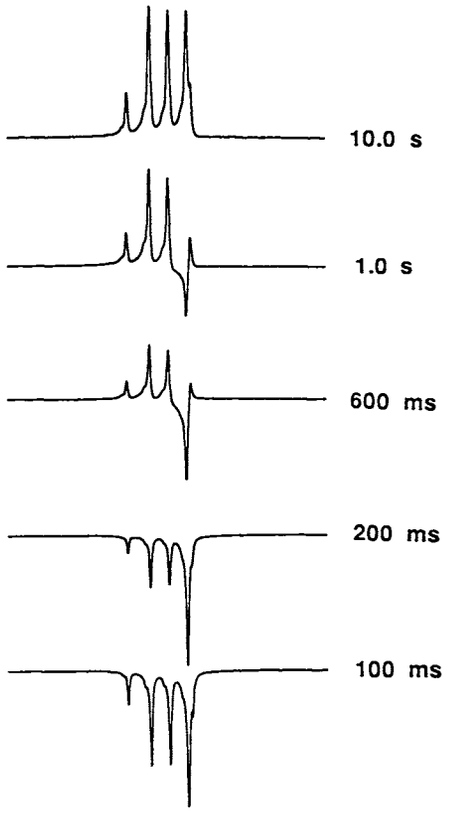


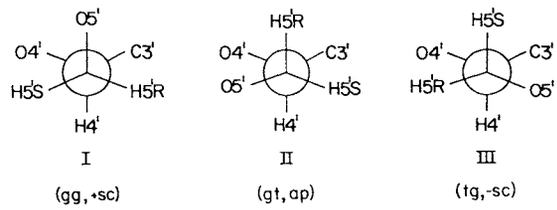
Figure 2. Partially relaxed 300 MHz ^{1}H NMR spectra of **16** resolve the superimposed hydroxymethyl subspectra shown in Fig. 1. The more rapidly relaxing doublets can be distinguished from the more slowly relaxing singlet, confirming the assignment of the more shielded hydroxymethyl proton to H-5′*R*. These data also show the large upfield isotope shift of the H-5′*R* signal in molecules containing deuterium at H-5′*S*. Indicated times are τ values in the 180°–τ–90° inversion–recovery pulse sequence.[17]

Table 1. ^{1}H–^{1}H spin coupling constants[a] between H-4′ and the C-5′ protons in ^2H_2O at *ca.* 25 °C

Compound	^3J(H-4′,H-5′*S*)	^3J(H-4′,H-5′*R*)	$\Delta\delta^b$ (ppm)
1	3.3	4.3	0.078
2	3.6	5.2	0.091
3	3.7	4.7	0.067
20	3.6	5.1	0.089
4	3.6	5.0	0.088
21	4.3[c] (4.6)[d]	7.0[c] (7.0)[d]	
22[e]	4.1	7.2	
23[f]	3.1	6.6	

[a] Coupling constants are expressed in Hz and are accurate to ±0.1 Hz.
[b] $\Delta\delta = \delta_{H\text{-}5'S} - \delta_{H\text{-}5'R}$.
[c] From Ref. 24.
[d] From Ref. 22.
[e] From Ref. 23.
[f] From Ref. 8.

This treatment (method 1) assumes that the three rotamers are perfectly staggered and requires standard ^3J(HH) couplings for the 60° and 180° torsion angles. These standard couplings were calculated using a modified Karplus equation developed by Haasnoot *et al.*[18]



Scheme 3

Crystallographic studies of nucleosides[19] have suggested, however, that rotamers I–III (Scheme 3) may not be perfectly staggered in solution, and alternate treatments [Eqns (3) and (4) (method 2[19]) and Eqns (5) and (6) (method 3[20])] of coupling data have been proposed to accommodate this situation.

$$1.3P_{gg} + 11.5P_{gt} + 5.8P_{tg} = {}^3J(\text{H-4}',\text{H-5}'R) \quad (3)$$

$$1.3P_{gg} + 2.7P_{gt} + 11.7P_{tg} = {}^3J(\text{H-4}',\text{H-5}'S) \quad (4)$$

$$1.4P_{gg} + 10.5P_{gt} + 3.8P_{tg} = {}^3J(\text{H-4}',\text{H-5}'R) \quad (5)$$

$$2.3P_{gg} + 2.6P_{gt} + 10.6P_{tg} = {}^3J(\text{H-4}',\text{H-5}'S) \quad (6)$$

Equations (1)–(6) and $P_{gg} + P_{gt} + P_{tg} = 1$ were used to compare calculated hydroxymethyl rotamer populations in 2′-deoxyribonucleosides. Similar calculations were also performed for the corresponding ribonucleosides using ^3J(H-4′,H-5′*R*) and ^3J(H-4′,H-5′*S*) values reported previously.[8] These results are given in Table 2. Although the three methods predict different absolute rotamer populations, the general trends discussed below are consistent with data obtained by each method. Consequently, the data from the three methods were averaged to obtain an estimate of the extent to which the methods differ (Table 3). The average values have standard deviations of *ca.* 4%, a reasonable value considering the fundamental assumptions underlying all three methods (see below). The following discussion is based on the average populations given in Table 3.

In the 2′-deoxyribonucleosides the *gg* conformer is dominant, followed by *gt*; presumably these rotamers are stabilized relative to the *tg* rotamer by the '*gauche* effect.'[21] The rotamer distribution may also depend on base structure; the *gg* conformer appears to be more favored in purine nucleosides, with the effect being more pronounced in 2′-deoxyadenosine (**1**).

2′-Deoxygenation also appears to alter rotamer distribution. The *gg* population decreases by *ca.* 10% and the *gt* and *tg* populations each increase by *ca.* 5% when ribonucleosides **5**–**8** are converted to the corresponding 2′-deoxyribonucleosides **1**, **2**, **3** and **20**. Although the *gg* rotamer remains dominant in all 2′-deoxyribonucleosides, its proportion is reduced in both purine and pyrimidine nucleosides.

As observed previously in ribonucleosides,[8] rotamer populations are significantly different in β-nucleosides and structurally related furanosides. In 2′-deoxyribonucleosides the *gg* conformer is dominant, whereas in methyl 2-deoxy-β-D-ribofuranoside[22,24] (**21**) the *gt* rotamer dominates and the *gg* and *gt* conformers are populated almost equally (Table 3). Interestingly, the rotamer distribution in **21** is similar to that found for 5-*O*-methyl-2-deoxy-β-D-ribofuranose (**22**), despite the presence of the methyl ether substituent at O-5.[23] In methyl β-D-ribofuranoside (**23**) the *gt* conformer also

Table 2. C-4′–C-5′ rotamer populations in nucleosides determined by methods 1–3[a]

Compound	P_{gg} M1	P_{gg} M2	P_{gg} M3	P_{gt} M1	P_{gt} M2	P_{gt} M3	P_{tg} M1	P_{tg} M2	P_{tg} M3
				2′-Deoxyribonucleosides					
1	0.54	0.62	0.60	0.26	0.22	0.29	0.19	0.16	0.11
2	0.44	0.52	0.47	0.35	0.30	0.39	0.21	0.18	0.14
3	0.48	0.56	0.53	0.29	0.24	0.32	0.23	0.20	0.15
20	0.46	0.53	0.49	0.34	0.29	0.37	0.20	0.18	0.14
4	0.46	0.53	0.51	0.33	0.28	0.35	0.21	0.18	0.14
21	0.21	0.23	0.20	0.50	0.52	0.55	0.27	0.25	0.25
				Ribonucleosides					
5	0.66	0.71	0.74	0.20	0.17	0.22	0.14	0.11	0.04
6	0.56	0.63	0.63	0.30	0.26	0.32	0.14	0.11	0.05
7	0.60	0.67	0.68	0.25	0.21	0.27	0.14	0.11	0.05
8	0.56	0.62	0.62	0.30	0.25	0.31	0.14	0.12	0.06
23	0.35	0.42	0.37	0.53	0.47	0.55	0.12	0.11	0.08

[a] M1, M2 and M3 refer to the three methods described in the text for calculating hydroxymethyl rotamer populations.

Table 3. C-4′–C-5′ rotamer populations in nucleosides determined by averaging values calculated by methods 1–3 (Table 2)[a]

Compound	P_{gg}	P_{gt}	P_{tg}
	2′-Deoxyribonucleosides		
1	0.59 (0.04)	0.26 (0.04)	0.15 (0.04)
2	0.48 (0.04)	0.35 (0.05)	0.18 (0.04)
3	0.52 (0.04)	0.28 (0.04)	0.19 (0.04)
20	0.49 (0.04)	0.33 (0.04)	0.17 (0.03)
4	0.50 (0.04)	0.32 (0.04)	0.18 (0.04)
21	0.21 (0.02)	0.52 (0.03)	0.26 (0.01)
	Ribonucleosides		
5	0.70 (0.04)	0.20 (0.03)	0.10 (0.05)
6	0.61 (0.04)	0.29 (0.03)	0.10 (0.05)
7	0.65 (0.04)	0.24 (0.03)	0.10 (0.05)
8	0.60 (0.03)	0.29 (0.03)	0.11 (0.04)
23	0.38 (0.04)	0.52 (0.04)	0.10 (0.02)

[a] Values in parentheses are standard deviations obtained from the averaging of data in Table 2.

predominates, followed by *gg*; the *tg* conformer is least stable. Thus, conversion of **23** to **21** does not significantly affect the *gt* population, but causes a decrease in the *gg* and an increase in the *tg* populations.

The hydroxymethyl conformation in nucleosides will be modulated by the conformation of the furanose ring. Previous NMR studies indicated that **21** and **23** prefer north conformations (^3E), compared with the furanose rings of the corresponding β-nucleosides which assume a higher percentage of south (^2E) conformations; 'north' and 'south' refer to the ring forms located in the northern and southern hemispheres of the pseudorotational itinerary.[25] The reduced *gg* population in **21** and **23** relative to the β-nucleosides suggests that the *gg* rotamer is more favored in south conformers. This conclusion is consistent with predictions by Raap *et al.*[22] that the *gg* population will be very small or zero in furanose rings existing exclusively in north conformations.

It is generally held that the north–south furanose equilibrium is slightly shifted towards south conformers in 2′-deoxyribonucleosides and towards north conformers in ribonucleosides. Indeed, this fact may explain why *tg* conformers appear to be less favored in ribonucleosides than in 2′-deoxyribonucleosides (Table 3). In north conformers (e.g., ^3E), a destabilizing 1,3-interaction exists between O-3 and O-5 in the *tg* conformer, whereas this interaction is absent in south conformers. Remin[4] has suggested that the *tg* population will be negligible or zero in north conformers. However, a disparity is observed with respect to the relative populations of *gg* rotamer in ribo- and 2′-deoxyribonucleosides. If south conformers are more favored in the latter, then more *gg* rotamer would be predicted in the latter (see above discussion). The opposite is observed (Table 3). Hence, it appears that factors in addition to furanose conformation affect the hydroxymethyl conformation, the most obvious being the *syn–anti* dynamics of the *N*-glycoside bond. The *gg* conformer may be particularly affected by *N*-glycoside conformation, as in this conformation O-5 orients 'over' the furanose ring and may participate in potential interactions with the base.

The treatment of ^3J(HH) values to define the hydroxymethyl conformation in 2′-deoxyribonucleosides is subject to the same errors as in ribonucleosides.[8] These errors fall into three categories. The first is the error associated with the conformational model. It is assumed that the three staggered (or near-staggered) rotamers define the most stable conformations, and that only these rotamers contribute to the observed ^3J(HH) values. Errors also accrue from the choice of standard ^3J(HH) values used in Eqns (1)–(6) to estimate rotamer populations. The Karplus equation of Haasnoot *et al.*,[18] which takes into account the relative orientations and electronegativities of α- and β-substituents along the H–C–C–H coupling pathway, is the most comprehensive and reliable equation at present to calculate standard ^{1}H–^{1}H couplings.

Errors will also arise in the measurement of ^3J(HH) due to potential non-first-order behavior of the ^{1}H NMR spectra. Because the difference in the chemical shifts of the H-5′*S* and H-5′*R* protons ($\Delta\delta$) in **1**, **2**, **3**, **4**

and **20** (Table 1) is smaller than that found in **5–8**,[8] we evaluated this source of error by partially simulating the 300 MHz ^{1}H NMR spectrum of 2′-deoxyadenosine (**1**). The chemical shift difference between the H-4′ and H-5′*S* signals was held constant while that between the H-5′*S* and H-5′*R* signals was altered. The measured and true coupling constants began to differ significantly (>0.1 Hz) when $\Delta\delta$ between H-5′*R* and H-5′*S* was $\leqslant$0.05 ppm. The H-5′*R* and H-5′*S* signals are closest in 2′-deoxyguanosine (**3**) ($\Delta\delta$ = 0.067 ppm) (Table 1), yet are still far enough apart to prevent second-order effects on measured couplings. Therefore, the measured ^3J(H-4′,H-5′*R*) and ^3J(H-4′,H-5′*S*) values obtained directly from the ^{1}H NMR spectra of **1**, **2**, **3**, **4** and **20** at 300 MHz (Table 1) should be within 0.1 Hz of the true couplings.

In summary, this study has provided unambiguous stereochemical assignments of the hydroxymethyl protons in several important 2′-deoxyribonucleosides, thereby placing previous and future structural correlations that depend on these assignments on a firm experimental foundation. It remains to be established, however, whether the H-5′*S* protons are the more deshielded C-5′ protons in ribonucleotide and 2′-deoxyribonucleotide residues of RNA and DNA oligomers.

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