MAGNETIC RESONANCE IN CHEMISTRY, VOL. 35, 168–174 (1997)

Through-Space $^{7}J_{HF}$ and $^{6}J_{CF}$ Spin–Spin Couplings in 2′,3′-Dideoxy-4′-fluoroalkylnucleosides. The Role of Sugar Ring Conformation and Solvent Effect

Andrea Mele,[1]* Giuliana Salani,[1] Fiorenza Viani[2] and Pierfrancesco Bravo[2]
[1] Dipartimento di Chimica del Politecnico, Via Mancinelli 7, I-20131 Milan, Italy
[2] CNR—Centro Studio sulle Sostanze Organiche Naturali, Via Mancinelli 7, I-20131 Milan, Italy

^{1}H and ^{13}C NMR spectra of seven compounds of a new class of synthetic fluorinated nucleosides, 2′,3′-dideoxy-4′-fluoroalkylthymidine and 2′,3′-dideoxy-4′-fluoroalkylfluorouridine, showed long-range $^{7}J_{HF}$ and $^{6}J_{CF}$ couplings between the F of the fluoroalkyl moiety and H-6 (and C-6) of the nucleobase. All the couplings were selectively detected on α anomers only, and ^{1}H{^{19}F} NOE difference spectra indicated that the F nucleus and H-6 are also in spatial proximity, supporting the hypothesis of a through-space mechanism for the transmission of the nuclear spin information. Molecular mechanics calculations on α-2′,3′-dideoxy-4′-fluoroalkylthymidine as a model compound indicated that F···H-6 distance spans the range 2.58–2.73 Å irrespective of the sugar ring conformation and the F···H-6—C-6 angle is within the range 145–159°. Calculated data support the view of an attractive interaction between F and H-6 of the base, consistent with an intramolecular hydrogen bond. Experimental evidence to this hypothesis are provided by NMR measurement in different solvents: the observed values of $^{7}J_{HF}$ and $^{6}J_{CF}$ decrease with increasing dielectric constant of the solvent and with the increasing capability of the solvent to establish intermolecular hydrogen bonds in competition with the intramolecular ones, i.e. passing from $CDCl_3$ to acetone-d_6 and to DMSO-d_6.

Magn. Reson. Chem. **35**, 168–174 (1997) No. of Figures: 5 No. of Tables: 2 No. of References: 27

Keywords: NMR; ^{1}H NMR; ^{13}C NMR; ^{19}F NMR; through-space couplings; ^{1}H, ^{19}F coupling constants; ^{13}C, ^{19}F coupling constants; fluorinated nucleosides

Received 12 June 1996; accepted (revised) 2 October 1996

INTRODUCTION

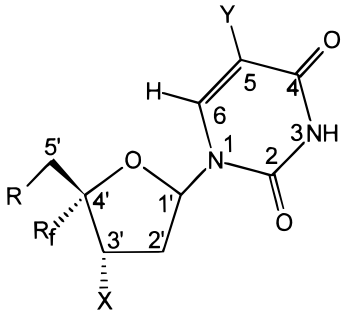
The growing interest in synthetic nucleosides incorporating fluorinated substituents is connected with the potential ability of those molecules to exhibit antiviral and antitumour activity. We undertook the synthesis of a novel class of fluorinated nucleosides, 2′,3′-dideoxy-4′-fluoroalkylthymidine and 2′,3′-dideoxy-4′-fluoroalkylfluorouridine, as part of a research programme aimed at the synthesis and testing of potentially active anti-HIV molecules.[1] The molecules **1**–**7** examined in this work are shown in Scheme 1, together with the atom numbering. Particular emphasis was paid to structural and conformational investigations of the newly synthesized molecules, as it is experimentally documented that the pharmacological activity of nucleoside-based drugs (AZT, ddI, ddC, etc.) is closely related to the preferred conformation of the molecule.[2] In the course of the structural characterization of **1**–**7** we observed the existence of long-range H,F and C,F coupling constants between F of the R_f residue and H-6 (and C-6) of the nucleobase. These long-range couplings are likely to be transmitted mainly via a through-space route. Through-space H,F coupling constants have been known since the pioneering work of Davis *et al.* in 1961[3] and are still the subject of theoretical and experimental investigations. The case of **1**–**7** was intriguing as we observed a large number of through-space couplings (see below) in molecular frames with a high degree of conformational flexibility, which are generally not compatible with the strict geometrical requirements for the long-range couplings to occur. We present here the results of some NMR measurements and molecular mechanics calculations on **1**–**7**, along with a discussion of the possible factors determining the couplings and a critical evaluation of the use of these NMR parameters for stereochemical and conformational investigations.

RESULTS

The seven pairs of α/β-anomers **1**–**7** differ in the fluorinated substituents at C-4′ ($R_f = CH_2F$, CHF_2 or CF_3), the R group attached to C-5′ [R = OH, $OCH_2C_6H_5$ or $PO(EtO)_2$] and heterocyclic base [thymine (Y = CH_3) or 5-fluorouracil (Y = F)]. Moreover, two of them, **7a**

* *Correspondence to*: A. Mele.

 CCC 0749–1581/97/030168–07 $17.50



Compound	R	Rf	X	Y	anomer
1a	OH	CH_2F	H	CH_3	α
1b	OH	CH_2F	H	CH_3	β
2a	OH	CHF_2	H	CH_3	α
2b	OH	CHF_2	H	CH_3	β
3a	OH	CF_3	H	CH_3	α
3b	OH	CF_3	H	CH_3	β
4a	OBzl	CH_2F	H	CH_3	α
4b	OBzl	CH_2F	H	CH_3	β
5a	OBzl	CH_2F	H	F	α
5b	OBzl	CH_2F	H	F	β
6a	OBzl	CHF_2	H	F	α
6b	OBzl	CHF_2	H	F	β
7a	PO(EtO)2	CH_2F	SO2pTol	CH_3	α
7b	PO(EtO)2	CH_2F	SO2pTol	CH_3	β

Scheme 1

and **7b**, also carry a substituent attached to C-3′ of the sugar ring. The relative configuration of the anomeric centre was assessed by using $^1H\{^1H\}$, $^1H\{^{19}F\}$ steady-state NOE and 2D-NOESY techniques, as reported previously for some compounds whose synthesis and routine spectral data were already published.[1] Long-range scalar couplings between the fluorine nucleus of the R_f residue and H-6 ($^7J_{HF}$) or C-6 ($^6J_{CF}$) of the base appeared in the simple ^1H and ^{13}C (with proton broad-band decoupling) spectra as additional and unexpected splittings of the signals of H-6 and C-6. The main results of the NMR experiments in $CDCl_3$ solution and the observed values of $^7J_{HF}$ and $^6J_{CF}$ are collected in Table 1, together with a selection of other NMR parameters for **1**–**7**. Most of the measurements were repeated in different solvents for reasons discussed below.

In the ^1H NMR spectra, the H-6 multiplet of the β-anomers of thymine derivatives displayed, when resolved, a quartet multiplicity, due to the allylic coupling with CH_3 attached to C-5 (e.g. $^4J_{HH} = 1.5$ Hz in **1b**), whilst the same signals of the corresponding α-anomers are quintets, as a result of the further splitting of each line of the original quartet with a second J of the same value (e.g. $^4J_{HH} = {}^7J_{HF} = 1.5$ Hz in **1a**). For the 5-fluorouracil derivatives **5** and **6** the observed multiplicities of H-6 are a doublet (due to ^3J between F-5 and H-6) and doublet of doublets (due to ^3J between F-5 and H-6 and ^7J between F of the R_f residue and H-6) for the β- and α-anomers, respectively. For all of the compounds, the existence of ^7J with fluorine was checked by recording ^1H spectra with selective ^{19}F decoupling. Under these experimental conditions, the H-6 quintet of the α-anomers of 4′-fluoroalkylthymidines turned into a quartet, and the doublet of doublets of the α-anomers of 4′-fluoroalkylfluorouridines collapsed into a doublet. Figure 1 shows, as an example, the results of selective heteronuclear double resonance experiments on a mixture of **5a** and **5b**. Trace (a) shows the expansion of the reference ^1H spectrum including the doublet assigned to H-6 of the β-anomer (δ 8.05 ppm, $J = 6.4$ Hz) and the corresponding doublet of doublet of the α-anomer (δ 7.68, $J = 6.4$ and 2.8 Hz). Trace (b) shows that saturation of the ^{19}F signal assigned to CH_2F of the β-anomer (δ −228.5 ppm) does not affect the proton spectrum. By contrast, saturation of the ^{19}F resonance assigned to CH_2F of the α-anomer (δ −233.4 ppm) caused the proton multiplet at 7.68 ppm to collapse into a doublet by removing the scalar coupling between the remote fluorine nucleus of the fluoroalkyl moiety and H-6 [trace (c)]. Eventually, saturation of F-5 of both the α- and β-anomers turns the H-6 signals into a singlet (β-anomer) and a doublet (α-anomer), respectively, the latter with a small splitting due to coupling with the

Table 1. Selection of NMR data in $CDCl_3$ for compounds 1–7

Compound	H-1′[a]	H-6[a]	C-6[a]	$^7J_{F,H-6}$[b]	$^6J_{F,C-6}$[b]	NOE (%)[c]
1a	6.24 dd, 6.7; 6.4	7.37 qn, 1.5; 1.5	135.11 d, 6.7	1.5	6.5	1.5
1b	6.07 dd, 6.8; 5.9	7.35 q, 1.5	136.60 s	—	—	—
2a	6.35 dd, 6.4; 7.1	7.72 br, s	134.76 d, 4.9	<0.5	4.9	5.9
2b	6.05 dd, 5.5; 7.0	7.28 q, 1.2	137.18 s	—	—	—
3a	6.45 dd, 5.5; 9.2	7.17 q, 1.2	134.20 s	<0.5	—	6.4
3b	6.03 dd, 6.9; 6.9	7.36 q, 1.2	137.34 s	—	—	—
4a[e]	6.23 dd, 6.3; 6.3	—[d]	135.34 d, 6.4	—[d]	6.4	6.8
4b[e]	6.21 dd, 6.5; 6.5	7.57 q, 1.3	135.88 s	—	—	—
5a[e]	6.23–6.16 m	7.69 dd, 2.8; 6.4	123.79 dd, 34.1; 7.9	2.8	7.9	6.0
5b[e]	6.23–6.16 m	8.05 d, 6.4	124.48 d, 34.6	—	—	—
6a	6.31–6.23 m	7.53 dd, 1.1; 5.9	123.44 ddd, 33.6; 2.2; 5.0	1.1	2.2; 5.0	5.4
6b	6.25 ddd 6.6; 6.6; 1.8	7.92 d, 6.3	124.31 d, 34.1	—	—	—
7a	6.29 dd, 5.3; 9.2	7.51 qn, 1.4; 1.4	135.36 d, 7.2	1.4	7.2	6.7
7b	6.51 dd, 3.6; 8.5	7.82 q, 1.2	136.25 s	—	—	—

[a] δ (ppm), multiplicity and value(s) in Hz of detected J couplings(s).
[b] In Hz.
[c] Steady-state NOE observed on H-6 after saturation of fluorine(s) of R_f.
[d] Not detectable.
[e] In a mixture with the other anomer.

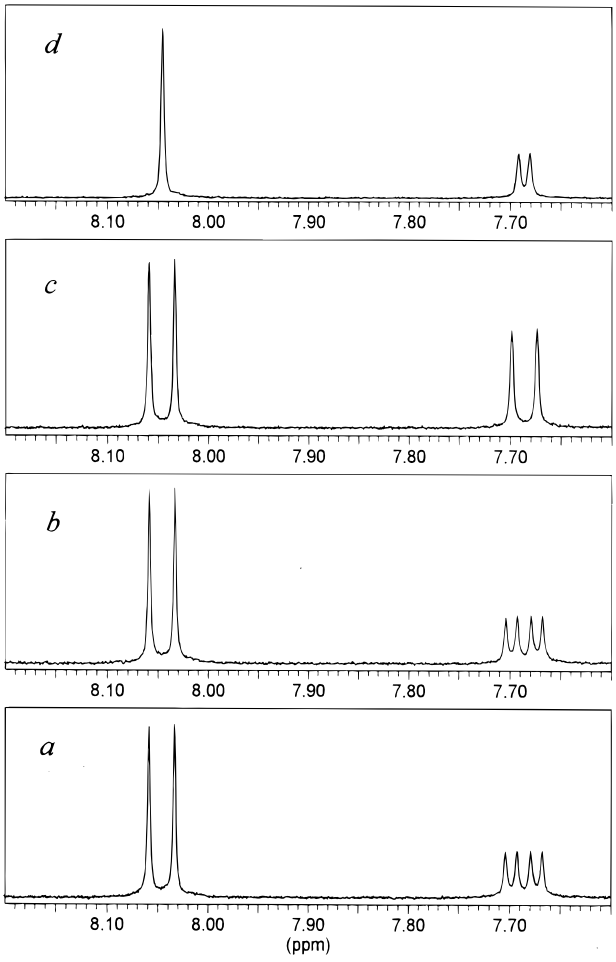


Figure 1. (a) Expansion of ^1H reference spectrum of **5** (mixture of α- and β-anomers) showing the resonances of H-6 (8.05 ppm for β-anomer; 7.68 ppm for α-anomer); (b) spectrum obtained after selective saturation of ^{19}F resonance of CH_2F of the β-anomer; (c) spectrum obtained after selective saturation of ^{19}F resonance of CH_2F of the α-anomer; (d) spectrum obtained after saturation of ^{19}F resonances of F-5 of the β- and α-anomers (see Scheme 1 for numbering).

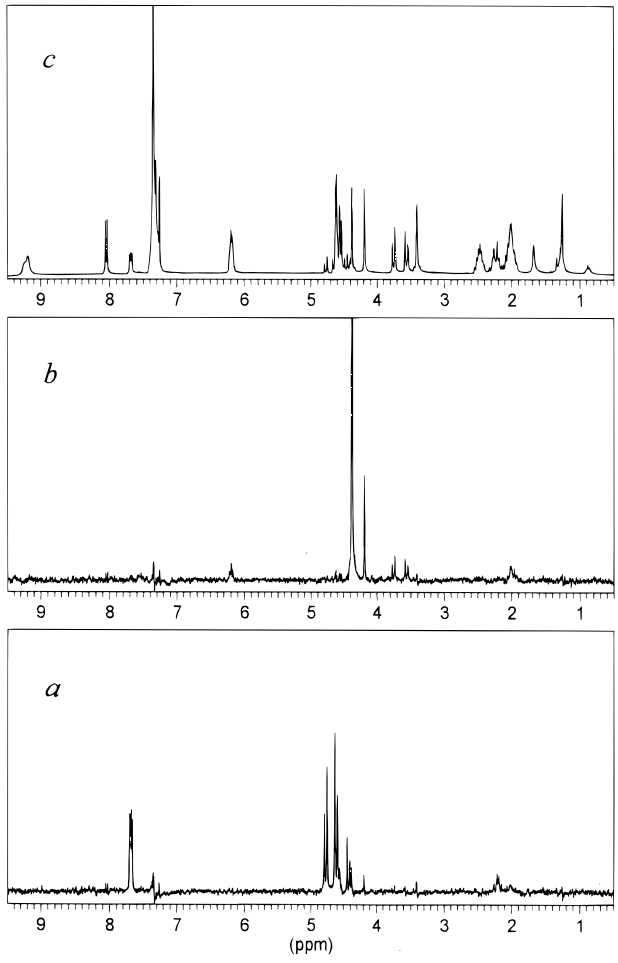


Figure 2. $^1H\{^{19}F\}$NOE difference experiments on a $CDCl_3$ solution of a mixture of α- and β-anomers of **5**. (a) NOE difference spectrum obtained after selective saturation of ^{19}F of CH_2F of α-anomer. The vertical scale is enhanced 64-fold with respect to the off-resonance. The multiplet at 7.68 ppm (H-6 of the α-anomer) undergoes 6% enhancement. (b) NOE difference spectra after selective saturation of ^{19}F of CH_2F of the β-anomer. (c) Off-resonance spectrum.

remote F [trace (d)]. A set of $^1H\{^{19}F\}$ steady-state NOE difference experiments were also carried out on all the nucleosides examined in this work, with the purposes of working out the relative configurations of the anomeric centres and of double-checking them in the case of those compounds already published.[1] The observed enhancements on H-6 of the base upon selective saturation of F of the R_f residue are summarized in Table 1. An example of a heteronuclear $^1H\{^{19}F\}$ steady-state NOE difference experiment is illustrated in Fig. 2 for the mixture of **5a** and **5b**.

The heteronuclear ^{19}F–^{13}C long-range couplings $^6J_{CF}$ were detected by simply examining the proton

Table 2. Through-space *J* couplings (Hz) of compounds 1–7 in different solvents

Compound	$^7J_{F,H\text{-}6}(CDCl_3)$	$^7J_{F,H\text{-}6}$(acetone-d_6)	$^7J_{F,H\text{-}6}$(DMSO-d_6)	$^6J_{F,C\text{-}6}(CDCl_3)$	$^6J_{F,C\text{-}6}$(acetone-d_6)	$^6J_{F,C\text{-}6}$(DMSO-d_6)
1a	1.5	—[a]	—[a]	6.5	—[a]	—[a]
2a	<0.5	0.0	0.0	4.9; 0.0	2.2; 2.2	1.9; 1.9
3a	0.0	—[a]	—[a]	0.0; 0.0	—[a]	—[a]
4a[b]	—[c]	1.5	0.0	6.4	4.1	3.0
5a[b]	2.8	1.8	<0.5	7.9	5.5	0.0
6a	1.1	<0.5	0.0	2.2; 5.0	2.7; 2.7	1.0 < *J* < 1.5[d]
7a	1.4	—[c]	—[c]	7.2	6.3	3.9

[a] Data not available in this solvent.
[b] In a mixture with the other anomer.
[c] Not detectable owing to spectral overlap.
[d] For both *J*.

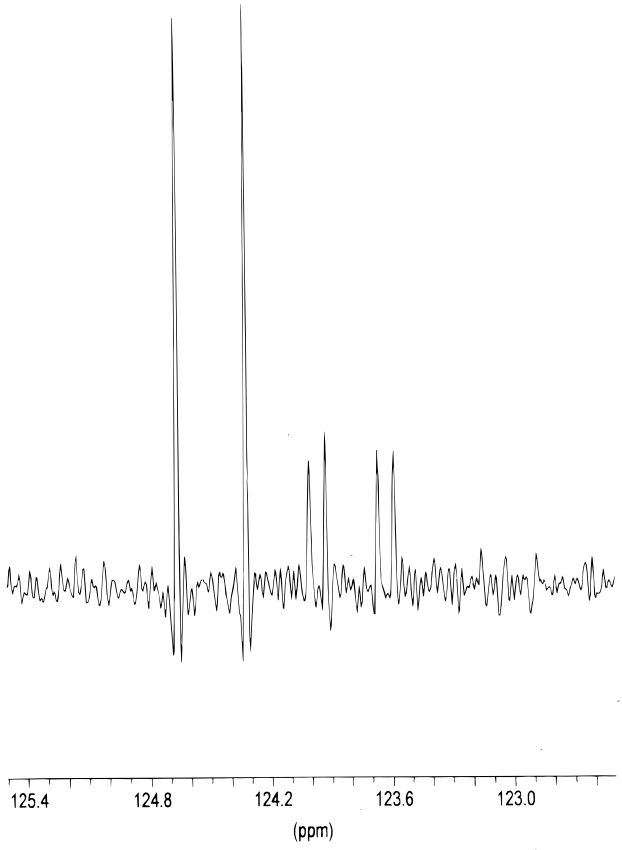


Figure 3. Resolution-enhanced expansion of the ^{13}C proton broadband decoupled spectrum ($CDCl_3$) of a mixture of α- and β-anomers of **5**. The signals of C-6 of the β-anomer (d, 124.48 ppm) and the α-anomer (dd, 123.79 ppm) are shown.

broadband decoupled ^{13}C spectra of **1**–**7** (Table 1). Figure 3 displays the spectral region of the ^{13}C spectrum of the mixture of **5a** and **5b** where C-6 resonances fall. The two signals were assigned to C-6 of the β-anomer (δ 124.48 ppm) and C-6 of the α-anomer (δ 123.79 ppm). They are both characterized by $^2J_{CF}$ (34.1 and 34.4 Hz for the α- and β-anomers, respectively), but the multiplet at 123.79 ppm shows a further splitting of 7.9 Hz due to $^6J_{CF}$ with fluorine of the fluoromethyl group. The signals of C-6 of the α-anomers of those derivatives carrying $R_f = CHF_2$, namely **2a** and **6a**, display a multiplicity (doublet of doublets) consistent with a scalar coupling of the C nucleus with two diastereotopic fluorines. No $^7J_{HF}$ or $^6J_{CF}$ could be detected in the only CF_3 derivative, **3**, examined. The NMR measurements were repeated in $CDCl_3$, acetone-d_6 and DMSO-d_6 and the values of the long-range H,F and C,F coupling constants under these experimental conditions are summarized in Table 2. In all cases the observed values of both $^7J_{HF}$ and $^6J_{CF}$ decreased with increasing polarity of the solvent (see Discussion).

DISCUSSION

From the experimental data collected in Table 1 we can infer the following: (i) the long-range couplings are selectively detected on H-6 and C-6 and not on any intermediate nucleus of a possible through-bond pathway, e.g. C-1′; this finding is consistent with a through-space route for the transmission of the nuclear spin information from remote fluorine to H-6 and an indirect through-space coupling mechanism to C-6; (ii) the coupling constants $^7J_{HF}$ and $^6J_{CF}$ exist in those nucleosides with an α-configuration of the anomeric centre only, thus showing a relationship between the detectability of these NMR parameters and the relative configuration of C-1′ and C-4′ in this class of compounds; and (iii) there is a neat parallelism between the existence of long-range F–H-6 couplings (and consequently F–C-6) and the observed $^1H\{^{19}F\}$ steady-state NOEs for all of the anomeric pairs **1**–**7**: detectable through-space J_{HF} and J_{CF} always occur where NOE is observed on H-6 after saturation of F of the R_f residue (see Table 1 for details). This latter point is consistent with a remote F scalar coupled to H-6 (and C-6) and also in spatial proximity to H-6.

The above data indicate that there are stereochemical and geometrical requirements for the long-range couplings to occur in this class of compounds and therefore they could be conveniently exploited as novel tools for stereochemical and conformational studies on synthetic fluorinated nucleosides, along with the more traditional vicinal *J* couplings and homo- and heteronuclear NOE data. However, the general applicability of this type of long-range couplings is questionable since, in general, long-range couplings, transmitted either via a through-bond or a through-space mechanism, are stereospecific and highly sensitive to the steric environment of the coupled nuclei.[4] This hampers their general use and very often limits the information to conformationally stiff systems only. The major applications of long-range couplings so far reported are stereochemical and structural assessments on rigid and cyclic molecular frames.[5–7] In the specific field of C–F and H–F long-range spin–spin couplings, mainly transmitted through space, most of the examples reported in the literature refer to conformationally rigid systems such as aromatic rings,[8] aromatic polycyclic fused rings[9–11] and fluorinated corticosteroids.[12] The exploitation of through-space H–F or C–F couplings in structural investigations of conformationally free molecules has been reported in only a very limited number of cases.[13–15]

Nucleosides **1**–**7** exhibit considerable flexibility of the five-membered ring, as shown in Table 1 by the values of the vicinal coupling constants of H-1′ with the two diastereotopic protons attached to C-2′, with the remarkable exceptions of **3a**, **7a** and **7b**. Both anomers of **7** are pushed into a single conformation by the strong tendency of the *p*-toluensulphonate group attached to C-3′ to assume a pseudo-axial position, in such a way that the exocyclic C—S bond assumes a *gauche* relationship with respect to the endocyclic C4′—O bond (*gauche* effect[16]). A detailed conformational analysis of the 2′,3′-dideoxyribose ring in terms of puckering amplitude (Ψ_m) and pseudo-rotation phase[17] (*P*) would require the quantitative evaluation of all the vicinal homonuclear coupling constants within the ring (in the case of **1**–**7** this implies the analysis of a strongly coupled ABCDE spin system). However, the splitting pattern of H-1′ can be analysed by first-order rules and affords two values of *J* couplings that can be conveniently used as descriptors of the ring flexibility. Indeed,

similar values of $J_{1',2'}$ and $J_{1',2''}$ (see, for instance, **1a**) and centred on 6.5 Hz are diagnostic of two extreme conformations nearly equally populated and in fast exchange on the NMR time-scale.[18] These limiting conformations generally belong to the north (N) and south (S) segment of the pseudo-rotation circle. In contrast, $J_{1',2'}$ and $J_{1',2''}$ of 5.5 and 9.2 Hz, as in **3a**, are typical of a single pseudo-rotamer prevalently populated. We undertook molecular mechanics calculations on the reference compound **1a** in order to model the north and south conformations with the purpose of finding a rationale for the presence of a set of through-space couplings in such a highly flexible molecular frame. Details on the calculations and the conformational search protocol are included in the Experimental section.

Figure 4 shows ball-and-stick models of the energy minimized north and south conformations of **1a**. The most striking feature visualized by the graphical output of the calculations is that the non-bonded distance between F and H-6 is in good approximation independent of the tetrahydrofuran ring puckering (2.73 and 2.58 Å for the north and south conformations, respectively). More interestingly, the angle defined by F, H-6 and C-6 is 145° and 159° in the two conformers, respectively. The calculated F···H distances and

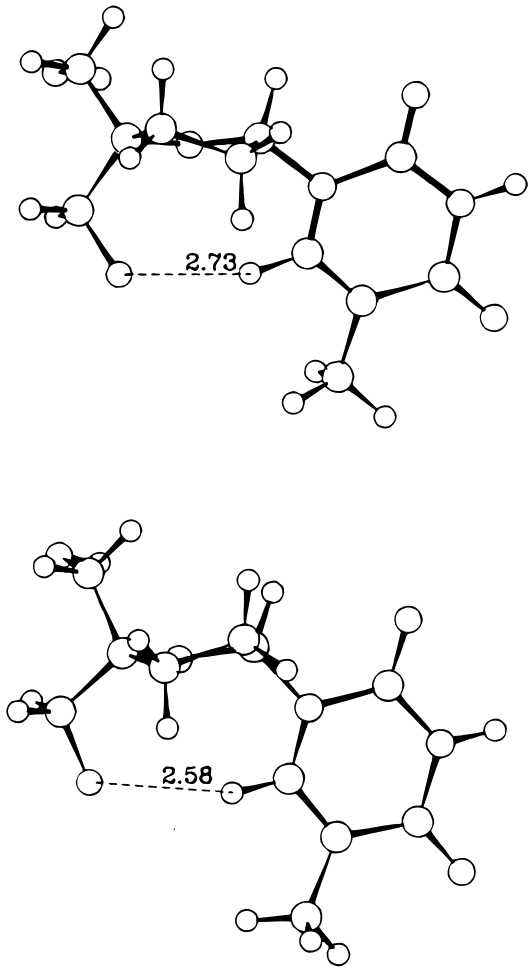


Figure 4. Ball-and-stick representation of the energy minima found for **1a** (molecular mechanics calculations, CVFF force field). Top, north conformer; bottom south conformer.

F···H—C(sp^2) angles in both conformations fulfil the geometric requirements for the existence of an intramolecular hydrogen bond.[19] Energy calculations on **1a** as a function of the torsion angle χ, defined as the dihedral angle O—C-1′—N—C-6, indicated that C-6 can be in either a *syn* or an *anti* position with respect to R_f [$\chi = -36.6°$ (*syn*) and 138.9° (*anti*) for N; $\chi = -41.5°$ (*syn*) and 125.5° (*anti*) for S). The energy differences $\Delta E(syn - anti)$ are -1.14 and -1.33 kcal mol^{-1} for N and S in that order, indicating that C-6 is prevalently facing the R_f residue. The same calculations carried out on the derivative of **1a** obtained by replacing CH_2F with CH_3 gave similar values of χ angles, but in this case $\Delta E(syn\text{-}anti)$ were -0.202 and -0.436 kcal mol^{-1} for N and S, respectively. Substitution of CH_2F with CH_3 decreases the $syn - anti$ energy gaps, consistent with a possible attractive interaction of F and H-6.

The C—F bond is commonly believed to be able to interact significantly with proton donors, as indicated by single-crystal x-ray diffraction data, although the interaction is regarded as weak and the typical proton donors belong to the H—X type (X = O, N).[20] In the specific field of fluoronucleosides, an attractive interaction of type F···H—C(sp^2) has been postulated on the basis of the following data: (i) MINDO/3 calculations on 3′,3′-difluoro-3′-deoxythymidine[21] suggested an 'attractive interaction' between the F atom *syn* to the base and H-6 of the base itself; and (ii) 2′,3′-difluorodideoxycytosine carrying the fluorine atoms in a *xylo* configuration showed a fixed north conformation, stabilized by the attractive interaction between F and H-6 of the base. When cytosine was replaced with 6-azacytosine, the F···H-6 attraction was replaced by F···N-6 repulsion, resulting in a complete loss of conformational rigidity.[22]

As far as **1**–**7** are concerned, intramolecular hydrogen bonds provide a rationale for NOE data and through-space couplings, as the latter are known to occur only if the distance of the coupled nuclei is less than the sum of their van der Waals radii, 2.5–2.6 Å, for HF couplings[9] and to decay exponentially with increasing distance.[23] Moreover, Rae *et al.*,[24] assuming the existence of a C—F···H—N intramolecular hydrogen bond in 2-fluoro-*N*-methylbenzamide, showed via NMR and MO calculations that this intramolecular hydrogen bond may transmit the spin information. In the present work, our experimental results support the view of an intramolecular hydrogen bond as a possible factor for the through-space transmission of spin couplings in **1**–**7**, by variable solvent NMR measurements. Table 2 shows that changing solvent causes large variations in the values of both $^7J_{HF}$ and $^6J_{CF}$, and constantly in the direction of decreasing value of the couplings with increasing polarity and ability of the solvent to establish intermolecular hydrogen bonds in competition with intramolecular hydrogen bonds: $J(CDCl_3) > J(\text{acetone-}d_6) > J(\text{DMSO-}d_6)$. Noticeably, the corresponding values $J_{H1',H2'}$ and $J_{H1',H2''}$ do not change significantly (data not reported), indicating that the conformational equilibrium of the compounds is not strongly influenced by the polarity of the solvent.

As a final point, it must be noted that the absolute values of both $^7J_{HF}$ and $^6J_{CF}$ are sensitive to the degree of fluorination of the residue R_f: the higher the number

of fluorine atoms, the lower is the corresponding value of the through-space coupling constants. The number of compounds taken into account in this work is not large enough to infer a general trend and, at present, no literature data on the comparison of the different hydrogen bonding donor properties of a C—F bond belonging to CH_2F, CHF_2 and CF_3 are available. Further investigations on model compounds are in progress in order to find a rationale for the different behaviour R_f group as a function of the fluorine content.

CONCLUSION

The 2′,3′-dideoxy-4′-fluoroalkylnucleosides examined exhibit long-range $^7J_{HF}$ and $^6J_{CF}$ mainly transmitted via a through-space mechanism. The couplings are observed in the α-anomers of all the compounds except those carrying a CF_3 substituent, despite the conformational flexibility exhibited by nearly all the terms of the series. A possible rationale for this behaviour is in the attractive interaction of F belonging to the R_f residue with H-6 of the nucleobase. The geometric feature deduced from molecular mechanics calculations show that this interaction is consistent with an intramolecular hydrogen bond. This interaction forces the fluorine nucleus and H-6 into spatial proximity independently of the sugar ring conformation. The strong solvent dependence of the couplings provides experimental evidence for such an interpretation. The independence of the through-space couplings reported here of the sugar ring conformation allows a convenient exploitation of these NMR parameters as stereochemical tools in the structural and conformational analysis of this class of compounds.

EXPERIMENTAL

Proton and carbon NMR measurements were carried out on a Bruker ARX 400 spectrometer. All the experiments involving ^{19}F were carried out on an AC250L spectrometer equipped with a supplementary BM1 broadband modulator operating at 235 MHz. Normally, 10–25 mg of **1**–**7** were dissolved in 700 μl of $CDCl_3$ (or another deuterated solvent) containing 0.05% of $(CH_3)_4Si$ as internal standard. Typical acquisition parameters were as follows: for ^1H spectra (400.13 MHz), 7.0 μs 90° pulse (2 dB attenuation on full transmitter power), spectral window 4700 Hz, 16K data points and zero filling to 32K prior to Fourier transformation; for ^{13}C spectra (100.13 MHz), 11.6 μs 90° pulse (2 dB attenuation on full transmitter power), spectral window 17 850 Hz, 32K data points, zero filling to 64K and Gaussian multiplication (LB = −2.5 Hz; GB = 0.28) prior to Fourier transformation. Proton broadband decoupling was achieved by a WALTZ16 pulse train (^1H 90° pulse: 110 μs). ^{19}F spectra were acquired at 235.35 MHz, using the following parameters: 3.0 μs 90° pulse (full transmitter power), spectral width 14 000 Hz and 32K data points zero filled to 64K prior to Fourier transformation. For $^1H\{^{19}F\}$NOE difference spectra and $^1H\{^{19}F\}$ decoupled spectra, the fluorine frequency was provided by a supplementary BM1 broadband modulator.

The synthesis and spectral data of **1**–**6** were reported previously[1] and those of **7a** and **7b** will be reported elsewhere.[25]

All the calculations were carried out on a Silicon Graphics Personal Iris 4D-35 using Insight and Discover software (Biosym Technologies, San Diego, CA, USA). The input geometries were generated from scratch using the fragment library of the Builder module of Insight. A preliminary conformational search was performed by a short molecular dynamics simulation at 300 K *in vacuo* (20 ps equilibration, 100 ps dynamics, trajectory sampling every 1 ps). The north and south conformers obtained in this way were minimized according to the following protocol: (i) determination of partial atomic charges by the semiempirical quantum mechanical AM1 method via a single-point calculation, using the MOPAC module of Insight (the full optimization of nucleosides at the AM1 level yields unrealistically flat furanose rings,[26] and therefore was not performed); (ii) full optimization of the molecular geometry using molecular mechanics (CVFF force field, part of the Discover package); and (iii) reiteration of the steps (i) and (ii) until a constant energy value in the molecular mechanics minimization. The conformational space was explored by independently varying the dihedral angles α (torsion around the C—F bond), β (torsion around the C5′—C4′ bond) and χ (torsion of the nucleobase) in 30° steps and minimizing the structure until a maximum energy gradient $\leqslant 10^{-3}$ kcal $Å^{-1}$. As commercially available force fields do not include a correct parametrization of the *gauche* effect,[27] we arbitrarily considered only those conformers showing the C—F bond of the R_f moiety *gauche* with respect to the endocyclic C(4′)—O bond.

Acknowledgements

We thank Dr Giovanni Fronza and Alberto Arnone for helpful discussion.

REFERENCES

1. (a) P. Bravo, A. Mele, G. Salani, F. Viani and P. La Colla, *Gazz. Chim. It.* **125**, 295 (1995); (b) P. Bravo, A. Mele, G. Salani and F. Viani, *Bioorg. Med. Chem. Lett.* **4**, 713 (1994).
2. C. K. Chu and D. C. Baker (Eds), *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*. Plenum Press, New York (1993).
3. D. R. Davies, R. P. Lutz and J. D. Roberts, *J. Am. Chem. Soc.* **83**, 246 (1961).
4. R. H. Contreras and J. C. Facelli, *Annu. Rep. NMR Spectrosc.* **27**, 256 (1993).
5. N. Platzer, N. Goasdoue and D. Davoust, *Magn. Reson. Chem.* **25**, 311 (1987).

6. Z. Paryzek, J. Martynow and T. Shimo, *Magn. Reson. Chem.* **30**, 579 (1992).
7. H. Schröder and E. Haslinger, *Magn. Reson. Chem.* **32**, 12 (1994), and references cited therein.
8. J. Hilton and L. H. Sutcliffe, *Prog. Nucl. Magn. Reson. Spectrosc.* **10**, 27 (1975), and references cited therein.
9. D. J. Sardella and E. Boger, *Magn. Reson. Chem.* **27**, 13 (1989).
10. Li C. Hsee and D. J. Sardella, *Magn. Reson. Chem.* **28**, 688 (1990).
11. I. D. Rae, J. A. Weingold, R. H. Contreras and G. Yamamoto, *Magn. Reson. Chem.* **30**, 1047 (1992).
12. D. W. Hughes, A. D. Bain and V. J. Robinson, *Magn. Reson. Chem.* **29**, 187 (1991).
13. T. Miyake and Y. Koyama, *Carbohydr. Res.* **258**, 11 (1994).
14. Y. Takagi, H. Sohtome, T. Tsuchiya and S. Umazawa, *J. Antibiot.* **45**, 355 (1992).
15. J. W. Lyga, R. N. Hernie, II, G. A. Meier, R. W. Creekmore and R. M. Patera, *Magn. Reson. Chem.* **31**, 323 (1993).
16. (a) W. Olson, *J. Am. Chem. Soc.* **104**, 278 (1982); (b) T. Iimori, Y. Murai, Y. Wakizaka, Y. Ohtsuka, S. Ohuchi, Y. Kodama and T. Oichi, *Chem. Pharm. Bull.* **41**, 775 (1993).
17. C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.* **95**, 2333 (1973).
18. C. Thibaudeau, J. Plavec, N. Garg, A. Papchikhin and J. Chattopadhyaya, *J. Am. Chem. Soc.* **116**, 4038 (1994).
19. Z. Berkowitch-Yellin and L. Leiserowitz, *Acta Crystallogr., Sect. B* **40**, 159 (1984).
20. (a) P. Murray-Rust, W. C. Stallings, C. T. Monti, R. K. Preston and J. P. Glusker, *J. Am. Chem. Soc.* **105**, 3206 (1983); (b) L. H. Takahashi, R. Radhakrishnan, R. E. Rosenfield Jr, E. F. Meyer, Jr and D. A. Trainor, *J. Am. Chem. Soc.* **111**, 3268 (1989).
21. D. E. Bergstrom, D. J. Swartling, A. Wisor and M. R. Hoffmann, *Nucleosides Nucleotides* **10**, 693 (1991).
22. V. E. Marquez, B. B. Lim, J. J. Barchi, Jr and M. C. Nicklaus, in *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*, edited by C. K. Chu and D. C. Baker, p. 265. Plenum Press, New York (1993).
23. (a) M. A. Natiello and R. H. Contreras, *Chem. Phys. Lett.* **104**, 568 (1984); (b) R. H. Contreras, C. G. Giribet, M. A. Natiello, J. Perez, I. D. Rae and J. A. Weingold, *Aust. J. Chem.* **38**, 1779 (1985).
24. I. D. Rae, J. A. Weingold, R. H. Contreras and R. R. Biekofsky, *Magn. Reson. Chem.* **31**, 836 (1993).
25. A. Arnone, M. Frigerio, G. Salani, F. Viani and P. Bravo, submitted for publication.
26. P. Van Roey, E. Will Taylor, C. K. Chu and R. F. Schinazi, *J. Am. Chem. Soc.* **115**, 5365 (1993).
27. R. J. Abraham, E. J. Chambers and W. A. Thomas, *J. Chem. Soc., Perkin Trans. 2* 949 (1994).

