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FULL PAPER

Exploring atypical fluorine-hydrogen bonds and their effects on nucleoside conformations

Daniel O'Reilly, Robin S. Stein, Mihai Burai Patrascu, Sunit Kumar Jana, Jerry Kurian, Nicolas Moitessier, and Masad J. Damha*[a]

This paper is dedicated in memory of Anne Dyer, mother of Daniel O'Reilly.

Abstract: The ability of fluorine to serve as a hydrogen-bond acceptor has been debated for many years. Short fluorine-hydrogen contacts are thought to play a key role in stabilizing some complex supramolecular systems. To directly probe the existence of fluorine-hydrogen bonds, we have carried out NMR experiments and computational modeling studies in a series of C2′-fluorinated nucleosides. Specifically, quantum mechanics-molecular mechanics (QM/MM) analysis and [^{19}F-^{1}H]-HMBC NMR experiments provided direct evidence for a C-H···F hydrogen bond in a 2′-F,4′-C-α-alkyl-ribonucleoside analogue. This interaction was also supported by QTAIM and NBO analyses which confirmed a bond critical point for the C-H···F interaction (0.74 kcal/mol). In contrast, while conformational analysis and NMR experiments of 2′-deoxy-2′-fluoro-arabinonucleosides indicated a close proximity between the 2′-fluorine and the nucleobase's H6/8 protons, molecular simulations did not provide evidence for a C-H···F hydrogen bond.

Introduction

Scientists have debated the existence of organic fluorine hydrogen bonds (H-bonds) for decades,[1] mainly due to the fact that fluorine seldom accepts H-bonds.[1a, 2] Current views of what constitutes a H-bond range from purely electrostatic contact and polarization effects (or a combination of both), to considerable covalent bond character.[3] As fluorine has become widespread in medicinal applications, increasing evidence has been found for both the existence of C-F···H H-bonds and their energetic importance in small molecules and nucleic acid-based systems.[4] This has led IUPAC to define characteristics for the C-F···H H-bond, which include the presence of scalar J_{HF} coupling (detectable by NMR), C-F···H angles of 110°-180°, and H···F distances of 1.9 Å - 2.5 Å (Figure 1a),[5] although deviations from these values have been suggested.[5c, 6] In recent examples reported in the literature, NMR is utilized to give evidence for these interactions.[4b, 7]

NMR is a powerful tool for studying bonding networks and conformations of systems.[8] Particularly useful for solution studies of C-F···H H-bonds are chemical shifts, the nuclear Overhauser effect (nOe), and scalar couplings. ^{1}H chemical shifts, measured from simple 1D NMR experiments, are correlated with hydrogen bond strength.[9] Through-space interactions, such as cross-relaxation, enable heteronuclear Overhauser (HOESY) experiments;[4b, 6, 10] these allow the investigation of small molecules determination of inter- and intramolecular F-H distances.[10] However, proximity between atoms is not enough to establish the existence of a H-bond.[11] Here, scalar coupling is of great utility, as this type of coupling between ^{19}F and ^{1}H may arise from orbital overlap hence providing evidence of a fluorine H-bond interaction. Scalar coupling can be detected in 1D experiments as well as in 2D experiments such as HSQC and HMBC.[7b, 9, 12]

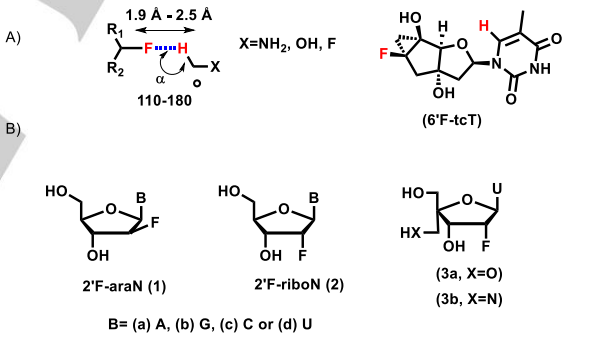

Figure 1. a) IUPAC characteristics of a C-F···H H-bond (left) and an example of a nucleoside that has an F···H interaction.[5a] b) Structures of nucleosides described in this study. Nucleosides **1** and **3b** are proposed to have C-F···H H-bonds.[7a, 13]

NMR experiments can also be used in conjunction with computational modeling to elucidate the energetics of systems. [7b, 9] Computational modeling has become a widely used tool for chemists, providing insights into protein-ligand binding, nucleic acid structures and sugar puckering, as well as C-F···H interactions.[14] Therefore with the objective of describing sugar puckering in nucleosides, we recently developed a protocol based on molecular dynamics (MD) simulations,[15] which was successfully applied to several molecules, including fluorine-containing nucleosides.[15-16] Subsequently, our group became interested in investigating whether fluorine hydrogen bonds exist in nucleosides and whether this protocol was robust enough to shed light upon the energetics and geometry of F···H bonding

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(Figure 1b). Generally, computational modeling utilizes crystal structures and NMR measurements to model a system.[4b] In the past, short contacts crystal structures have been the main source of evidence for fluorine H-bonds,[1b, 17] but this does not always relate to a direct bonding interaction being present in solution.[18]

It is of critical importance to gain an understanding of factors influencing the stability of fluorinated nucleosides as many are potent anti-cancer (2′,2′-diF-N) and anti-viral agents, particularly against: Hepatitis B (L-2′-F-ara-N), Hepatitis C (2'-C-Me-2′-F-ribo-N) and HIV (2′F-d4N).[19] They are also key building blocks in the production of oligonucleotides, and are ubiquitous in the development of therapeutic antisense, aptamers, siRNA,[20] and gene editing (CRISPR/Cas9) agents.[21] It is currently understood that C-F···H H-bonds are present in oligonucleotides and can offer additional stability to the overall structure.[19a] For example, the hybridization of 2′-F-ANA oligonucleotides with complementary RNA strands results in the formation of close C-F···H contacts.[13a, 13c, 19a, 22] Evidence for these bonds arises from C-F···H contacts (nOe), conformational analysis, and theoretical calculations of binding free energies.[13a, 23] On the other hand, 2′-F-ara-A nucleoside (**1a**) is also hypothesized to have a C2′-F···H8-C, interaction based on ^{1}H-NMR and deuterium exchange studies.[13a, 13c] Several nucleosides,[13c, 24] and more recently, 6′-F-tricyclo-thymidine (**6′F-tcT**),[7a] have been proposed to engage in a similar interaction (C6′-F···H-C6; Figure 1a). Methods for detecting these interactions in non-nucleic acid based systems are well developed.[4b, 6] However, it is less well understood why these weak intramolecular interactions form and how they influence nucleoside and nucleic acid structure and stability.

Herein, we examine three fluorinated nucleoside systems, namely the C2′-epimeric nucleosides 2′-F-ara-N (**1**) and 2′-F-ribo-N (**2**) and the known 2′,4′-modified nucleoside **3a**, *via* NMR, crystallography, and computational studies (Figure 1b). Understanding whether C-F···H H-bonds play a role in the conformation and stability adopted by these systems is important and could lead to the design of new modified nucleosides that incorporate these interactions.

Results and Discussion

Conformations of 2′-F-arabino versus 2′-F-ribo nucleosides

The conformation of nucleoside sugar rings is readily assessed by measuring the torsion angles of the furanose ring. In turn, these torsion angles can be used to calculate a pseudorotational phase angle value which can be represented on a so-called “pseudorotational wheel” (Figure 2) that provides an intuitive means for describing nucleotide sugar “puckering” or conformation.[25] Generally, nucleosides exist in a rapid equilibrium between two puckered forms, the *North* (C3′-*endo*, C2′-*exo*, *P* = ~0-18º), and the *South* (C2′-*endo*, C3′-*exo*, *P* = ~144-180º), and pass through the *East* (O4′-*endo*) form when moving between these conformational minima (Figure 2).[26] 2′-Deoxyribonucleosides have a mild preference for the *South* conformation (~65%), while ribonucleosides are almost evenly distributed between the *North* (~51%) and *South* puckers (~49%). 2′F-ribonucleosides (including 2′,4′-diF) have a strong preference for the *North* pucker (~80-100%).[27] The observed $J_{H1'-H2'}$ and $J_{H3'-H4'}$ coupling constant values obtained from basic ^{1}H NMR experiments can be used to calculate an approximation of the *North/South* conformational distribution in solution.[25a, 28] This utilizes the Karplus equation, which relates the dihedral angle between CH bonds and ^{3}J coupling constant. [29] For nucleosides that exist predominately in the *North* conformation, the angle between H1′-H2′ is close to 90º, and therefore $^{3}J_{H1'-H2'}$ is close to 0 Hz; meanwhile, for the *South* conformation, the angle is >90º (generally 150º -180º) and therefore the $^{3}J_{H1'-H2'}$ is closer to 10 Hz. The *East* conformation is most clearly established through H1′-H4′ nOe contacts and can cause reduced $^{3}J_{H1'-H2'}$.[30] The compounds utilized in this study are representative of both the *North (N)* and *South (S)* conformations, with corresponding N/S ratios shown in Table 1.

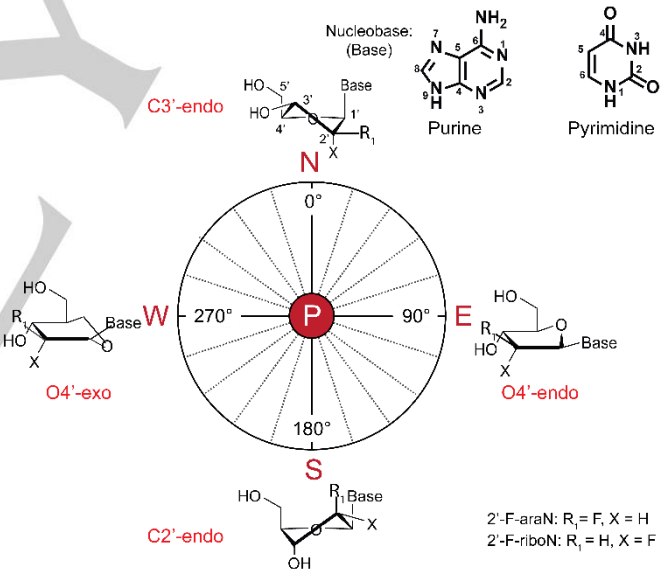

Figure 2. Nucleoside sugar conformations, with positions numbered, arranged on a pseudorotational wheel.

In D_2O, 2′-F-arabinonucleosides (**1a-d**) exhibit similar sugar conformations, with a *North:South* pucker ratio of approximately 2:3 in all cases (Table 1). Evidence for the existence of the *East* conformation was obtained from nOe experiments; for example, 2′-F-ara-C (**1c**) exhibits stronger H1′-H4′ nOe contacts than 2′-F-ara-U (**1d**), and thus exhibits a stronger preference for the *East* conformation.[13c, 30] These calculations, together with ^{1}H-^{19}F HOESY NMR experiments, confirmed that for the 2′-F-

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arabinonucleosides, the H6/8 protons are in close proximity to 2′-fluorine, with distances within 2.4 and 2.7 Å respectively; these values are in close agreement with those calculated in solution (Table 1; Figure 3). The calculation (Equation S1) utilizes the F2′-H2′ distance, from the crystal structure as a reference.[10] While our simulations predicted that minimum energy structures for the 2′-F-arabinonucleosides would be found in the *East-South* sugar pucker conformation,[15] the *North* conformation was found for all the 2′-F-ribonucleosides (**2a-d**). This was expected given the increased strength of the gauche effect from the 2′-fluorine.

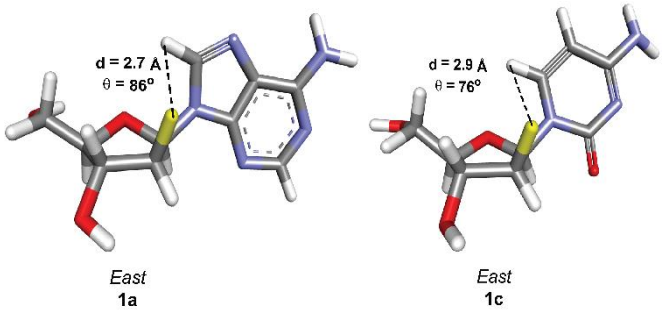

Figure 3. Predicted low-energy conformations of 2′-F-ara-A (left) and 2′-F-ara-C (right).

Table 1. Properties of nucleosides under study. N/S ratios are calculated from $J_{H\text{-}F}$ scalar couplings.[32] HOESY distances were calculated according to ref.[10] (supplemental information). Predicted distances are from molecular modelling

Nucleoside	N/S ratio	Atom pairs	$J_{H\text{-}F}$ Coupling (Hz)[a]	HOESY distance (Å)	Predicted distance H8/6-F (Å)
araF-A (1a)	41:59	H8-2′F	2.5	2.5	2.7
araF-G (1b)	41:59	H8-2′F	2.6	2.4	2.7
araF-C (1c)	38:62	H6-2′F	1.8	2.7	2.9
araF-U (1d)	40:60	H6-2′F	1.5	2.7	2.7
rF-A (2a)	80:20	H8-2′F	0	-	-
rF-G (2b)	80:20	H8-2′F	0	-	-
rFC (2c)	80:20	H6-2′F	0	-	-
rFU (2d)	80:20	H6-2′F	0	-	-
3a	70:30	H6′-2′F	1.0[b]	2.3[c]	2.3
4	87:13	H8-2′F	0.5[d]	2.6[d]	-

[a] Calculated using the method described in ref. [27a]. [b] In D_2O using a 800 MHz spectrometer. [c] Performed in MeOD-d_4 at -80 ºC to favour the formation of the *North* conformation, providing a more accurate distance measurement for the F2′···H8 distance. [d] In DMSO-d_6 due to poor solubility in D_2O.

Evidence for and against C-H···F H-bonding in 1a-d

The H8 and H6 ^{1}H-NMR signals of compounds **1a-d**, showed a small but distinct splitting of approximately 2.5 and 1.5 Hz respectively, which may suggest H-bond-mediated coupling with fluorine as previously proposed for nucleosides, duplexes and quadruplexes.[7d, 13a, 31] Markiewicz 5′,3′-silylation of 2′-F-ara-A (**1a**) to obtain **4** causes a ring conformational change from *South* to *North*, an increase of the H8···F2′ distance, and concomitant reduction in $J_{H8,2'F}$ from 2.5 Hz to ~0.5 Hz , consistent with the disruption of a C-H···F interaction in the *North* form (Figure 4).[13a] Conversely, the 2′-F-ribonucleosides (**2a-d**) investigated herein exhibit a *North:South* ratio of 80:20 and show no coupling between the 2′-F and H6 or H8 of the nucleobase.

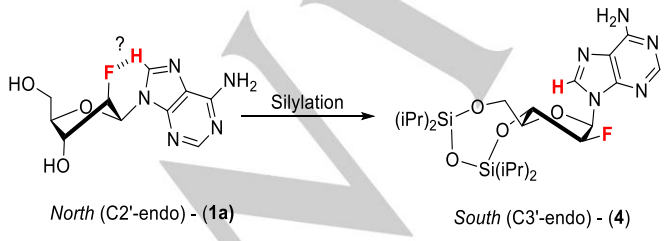

Figure 4. Silylation of 2′-F-ara-A (**1a**) yields **4**, switches the furanose sugar pucker from *South* to *North*, and and increases the distance between H8 and F.[13a]

Next, we examined the splitting of ^{13}C signals of the purine/pyrimidine bases by fluorine (**1a-d**). The purine C8/C4 and the pyrimidine C6/C2 carbons are four bonds away from the 2′-fluorine. If the interactions were purely long-range in nature, similar $^4J_{2'F\text{-}C}$ coupling values would be expected. However, in 2′-F-ara-A (**1a**), only the C8 shows significant splitting (4 Hz) from ^{19}F (Figure 5). All other ^{13}C signals showed a splitting of 0.5 Hz or less, (Figure S10). The same was true for 2′-F-ara-G (**1b**, Figure S17). For 2′-F-ara-C (**1c**, S23) and 2′-F-ara-U (**1d**, S28), we compared the C-2 and C-6 signals; splitting of only the latter was observed. The epimeric nucleosides (**2a-d,** Figures S32, S36, S42 and S47) exhibited no such couplings.

C-H-F bond angles for 2′-F-ara-A, G, C, and T[15] were calculated to be 86º, 54º, 76º and 75º (Tables S2, S5, S8 and S11), respectively, which fall outside the typical range of 110º-180º. In the crystal structure of 2′-F-ara-T, the 2′-F-H6 distance and C-F-H angles were 2.90 Å and 95.7º, respectively. 2′-F-ara-A (**1a**) crystallized in the '*North*' conformation, therefore no C-H-F bond distance or angle was calculated as the atoms are far apart from each other.

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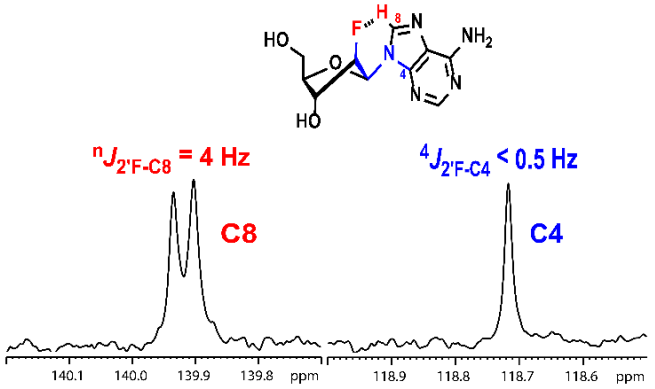

Figure 5. Portion of the $^{13}C\{^{1}H\}$ spectrum of 2-F-ara-A (**1a**) in DMSO-d_6 showing the region where the C8 and C4 signals appear.

There was no indication in the potential of mean force (PMF) energies obtained by MD simulations of an attractive interaction between H8 and 2F'. This was also supported by natural bond orbital (NBO) and quantum theory of atoms in molecules (QTAIM) analysis, which showed no interaction between H8 and F2′. While these data argue against the presence of C-F··H H-bonding interactions, angles of 90º have been suggested in other systems which exhibit C-F··H H-bonding interactions, such as intermolecular interactions of *tert*-butyl alcohol with difluoromethane and in highly ordered structures such as protein-substrate complexes.[7c, 12b, 33]

Branched nucleoside (3a)

Next, we turned our attention to nucleoside **3a.** This nucleoside is analogous to the previously reported branched nucleoside **3b**,[13b] and was predicted by DFT calculations and molecular dynamics simulations to engage in a weak intramolecular C(sp^3)-H···F bond between the hydrogen atom of the 4′-C-CH_2 group and F2′.[13b, 34] Given their similarity and the greater electronegativity of O vs N, **3a** was expected to exhibit the same interaction.

Nucleoside **3a** was synthesised using a modification of a previously reported procedure.[34] This compound crystallized in its most stable *North* conformation (*P*=49.5º), as predicted by our modeling (Figure S65, *P*=51º). Overlay of the two structures revealed a heavy-atom RMSD of 0.78 Å (Figure S68). NMR analysis indicated a predominant *North* conformation (70%) in aqueous solution (Table 1). Interestingly, the *North* and *South* puckers become equally populated in organic solvents (DMSO-d_6 and MeOD-d_3). Cooling the solution to -80 ºC (MeOD-d_3) enriched the *North* conformer over the *South* conformer (70:30). Intrigued by these findings, we computed the *N/S* ratio as described in our protocol.[15] Our simulations predicted a *North-East* pucker as the lowest energy conformation, with 40% of the population also residing in the *South* pucker (Figure 6).

To probe potential H-bonding interactions in the sugar moiety, the distance between the hydrogen atom of the α-CH_2 linked to 4′-C and F2′ was measured from the conformations generated during the MD simulations. For the lowest energy (*North-East)* conformer, the distance between hydrogen and fluorine was found to be ~2.3 Å, with a C−H−F angle of ~125°, similar to those reported for 3b.[13b] This led us to posit that a weak C−H···F H-bonding interaction was present (Figure 6a).

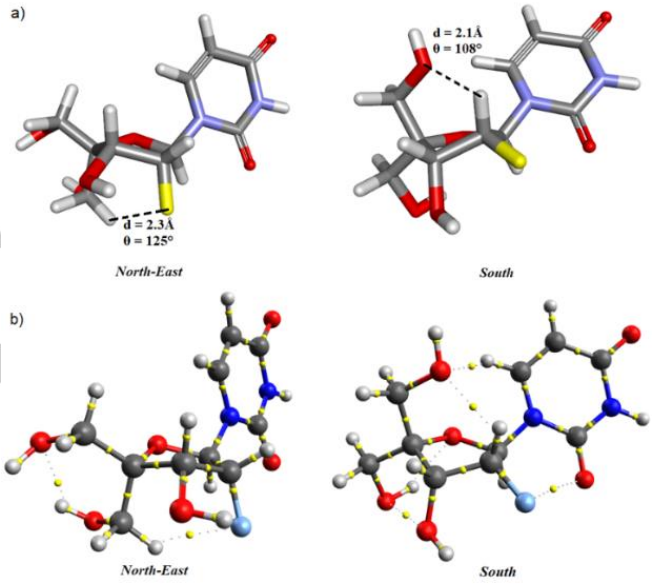

Figure 6. a) The two different conformations found in solution and predicted by molecular modelling. *North-East* predicted to have an F--H interaction (left) and *South* predicted to have a O--H bond (right). b) QTAIM bond critical points (yellow) showing the CF···H (left) and CO···H interactions (right).

To verify this, we plotted the electron density and molecular orbitals (Figures S66 and S69b) of the *North-East* pucker and quantified the C-H···F interaction by NBO analysis (0.74 kcal/mol). This interaction is also supported by QTAIM analysis:[35] we identified a bond path (BP), bond critical point (BCP) and an interatomic surface (IAS) between C-H···F, indicating an attractive interaction that is electrostatic in nature (Figure 6b left).[35c, 36] NBO and QTAIM analysis[37]of the *South* pucker (Fig. 6b right) also revealed an intramolecular FC-H···O5' interaction (0.92 kcal/mol) (Figures 6b, S67 and S69a, Table S16), with a C-O-H angle of ~108º and CO···H distance of ~2.14 Å, providing a possible explanation for the significant *South* conformer population observed for this nucleoside.

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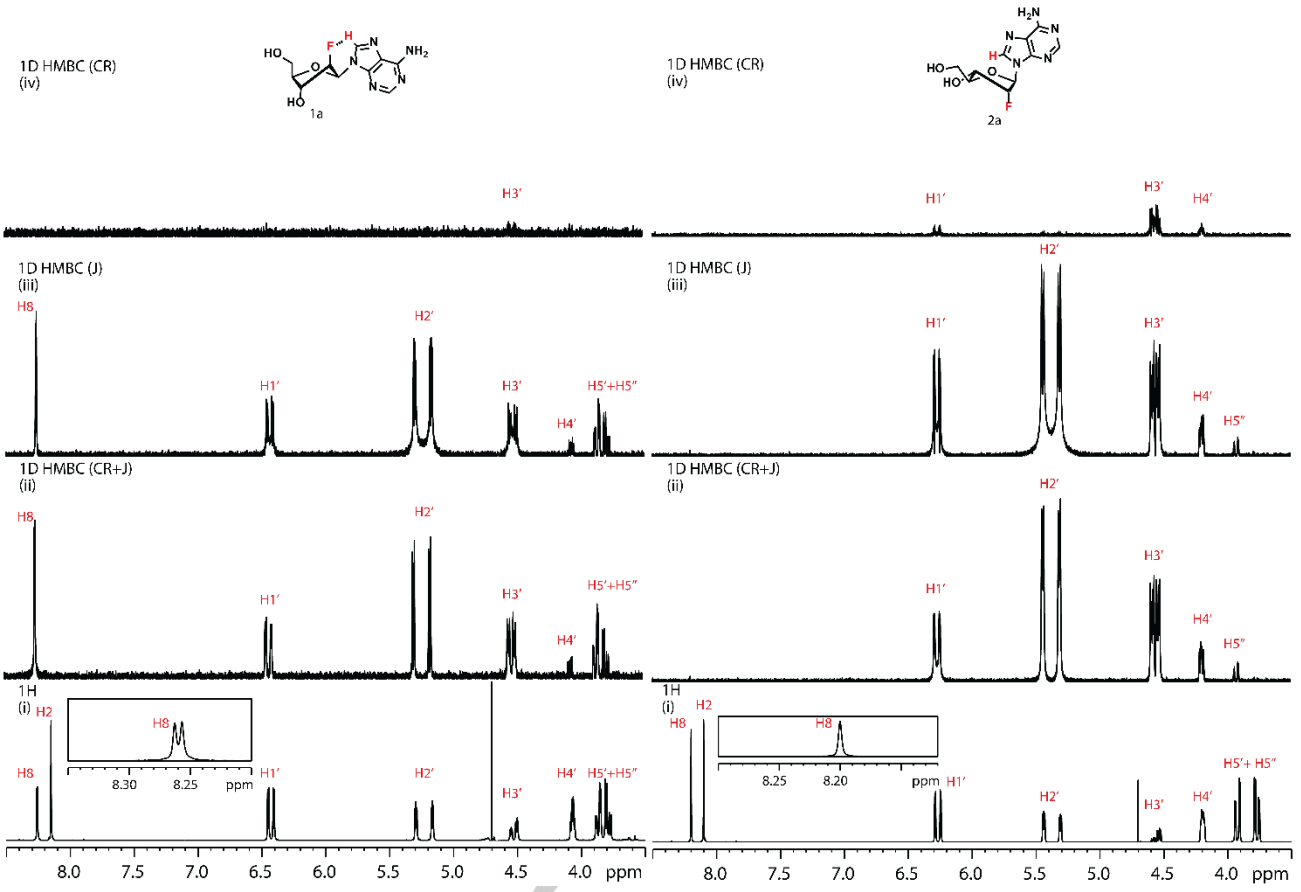

Figure 7. (i) 1D ^{1}H and (ii, iii, iv) 1D [^{19}F,^{1}H]-HMBC spectra of nucleosides **1a** (left) and **2a** (right) (400 MHz, D_2O). (i) 1D ^{1}H spectrum with solvent suppression. Highlighted is the H8 signal to show coupling (**1a**) or no coupling (**2a**) to the 2′-fluorine. (ii) Signals arising from both scalar and cross correlations (*J*+CR). (iii) Signals arising from scalar coupling (*J*) only. (iv) Signals arising from cross relaxation (CR) only. Nucleosides **1a** and **2a** (as are **1b** and **2b**) are poorly soluble in D_2O and yield poor signal to noise in the CR spectrum. The same experiments in DMSO-d_6 (Figures S12 (**1a**) and S34 (**1b**)) also display minimal CR character.

In the crystal structure, the α-CH_2OH arm linked to 4′-C was rotated away to engage in an intermolecular H···O interaction. From these results it is apparent that crystal packing and intermolecular H-bonding interactions between molecules outcompete the putative intramolecular C-F···H-C interactions observed in solution. Interestingly, the QTAIM analysis for the *South* conformer also shows several more interactions (Table S16), the most intriguing being the ones between F···O=C and 3′O···4′O. In both cases we were able to identify a BP, BCP and IAS between the atoms.[38] These weak attractive interactions[35c] are likely afforded by the differences in the computed atomic charges (F = -0.646 a.u., O = -1.142 a.u.; 3'O = -1.018 a.u., 4'O = -0.984 a.u).

[^{19}F,^{1}H]-HMBC NMR experiments

To further investigate ^{19}F to ^{1}H/^{13}C couplings observed in the NMR spectra, we used 1D [^{19}F-^{1}H]-HMBC experiments.[11] The 1D HMBC pulse sequence can be varied to obtain NMR signals that arise exclusively from cross relaxation (CR, i.e., "through space" coupling arising from the combination of dipolar interactions and chemical shift anisotropy),[11] exclusively from scalar (*J*) couplings (through bond), and from both scalar and cross correlation interactions (*J*+CR), respectively (Figure 7).[11b] These variations have been used to study proteins and organometallic complexes.[11, 39] Determining the CR contribution allowed Dingley *et al.* to establish that hydrogen bonds in Watson-Crick base pairs exist in solution and have significant *J* (through bond character).[39] CR contribution to the splitting is small but detectable, generally falling in the 0.5-1 Hz range, only slightly smaller than the *J* coupling values detected in **1a-d** (Table 1). To validate these experiments, we used a 2-fluoro-N-(2-fluorophenyl)benzamide standard, which participates in the formation of a C-F···H-N intramolecular interaction (Figure S6).[7b, 40]

The [^{19}F-^{1}H]-HMBC spectra of 2′-F-ara-A (**1a**) and 2′-F-ribo-A (**2a**) are shown in Figure 7; those of 2′-F-ara-G, C, and U can be found in the supporting information (Figures S18, S24, S29).

In the case of (**1a-d**), the ^{1}H(6/8) and most sugar signals exhibit significant *J* (through-bond) character and little CR (dipolar) character. As expected, for the epimeric 2′-F-ribonucleosides (**2a-d,** Figures 7, S38, S44 and S49 respectively), our negative controls, no *J* or CR were observed for the H8/6 signals.

As observed for the 2′-F-arabinonucleosides, the [^{19}F-^{1}H]-HMBC spectra of **3a** suggest the J_{HF} couplings ($J_{H6\text{-}F}$ and $J_{H6'\text{-}F}$) arose primarily through-bond (*J*) rather than through dipole-dipole (CR) interactions (Figure S56). Furthermore, we detected coupling between F2′ and one of the geminal H6′ protons (J_{HF} = 1 Hz, Figure S56). No coupling with the geminal protons at C5′ was observed. This supports the modeling predictions of a F2′—H6′ interaction.

Conclusions

In conclusion, our work highlights the application of computational, crystallographic and solution-phase NMR experiments in the investigation of C−H···F H-bond formation as they provide complementary, useful insight. In the case of 2′-F-arabinonucleosides, these H-bonds were not supported by computer modelling techniques, although conformational analysis and NMR experiments clearly established a close proximity between F2′ and the nucleobase H6/8 protons and a *J* coupling. In the second system (**3a**), the bond angles and distances fall within the IUPAC guidelines which our computational techniques can predict. This type of hydrogen bond can be regarded as 'cooperative' [41] in the sense that its formation can be expected to be favored in a *North* conformation stabilized by a combination of steric and stereoelectronic (gauche) effects.[15] By determining that these bonding interactions are present and play a role in stabilising nucleic acid structure, our studies open the possibility of designing new nucleosides analogues that utilize these subtle but important interactions to favour particular conformations and tune their biological activity.

Experimental Section

2′-F-arabinonucleosides and 2′-F-ribonucleosides were purchased from Metkinen and ChemGenes Inc., respectively. Nucleoside **3a** was prepared following slight modification of a previously reported published procedure (Scheme S1). [34]

Nuclear magnetic resonance: All NMR experiments were performed at 298 K on a Bruker 400 MHz (BBFO+ SmartProbe), 500 MHz (BBFO+ SmartProbe) or 800 MHz (TCI cryoprobe). For each sample, 10 mg of sample was dissolved in 0.75 mL of the stated solvent. Peak assignments were based on a combination of NOESY, COSY and ^{1}H experiments.

2D ^{19}F-^{1}H HOESY experiments[42] were performed with a mixing time of 0.5 ms. Distance calculations are described in the SI (Equation S1).[10] 1D HMBC experiments used pulse sequences depicted in Figure S1.[11] All experiments were ^{1}H-detected and were performed with an evolution time Δ=62.5 ms (corresponding to J_{HF}=8 Hz).

Computer modelling: MD simulations were performed according to our previously established protocol.[15] Relevant angle values and bond distances were calculated in Avogadro[43] as well as the plotting of molecular orbitals. Visual QTAIM analysis [35a] was performed in Avogadro using wavefunction files obtained with Gaussian16[35b] at the M06L/def2-TZVP level of theory. Quantitative QTAIM analysis was performed using the AIMALL[44] program on the same wavefunction files used for visual QTAIM analysis. NBO analysis was performed using the NBO6[45] program. Electron densities were plotted using Molekel,[46] while the lowest energy conformation figures were made with Discovery Studio 4.5. [47]

Crystallography: Single crystals of each nucleoside were slowly crystallized in solutions of methanol and dichloromethane. A suitable crystal was selected and measured on a Bruker Venture Metaljet diffractometer. The crystal was kept at 150 K during data collection. Using Olex2,[48] the structure was solved with the ShelXT[49] structure solution program using Intrinsic Phasing and refined with the XL[50] refinement package using Least Squares minimisation. Crystal structures for nucleosides 2′-F-ara-A **1a** (CCDC = 1576520), 2′-F-ara-T **1d** (CCDC = 1576722) and **3a** (CCDC = 1576721) were deposited with the Cambridge Crystallographic database.

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[37] The Laplacian of the electronic(∇2BCP) and energy density(HBCP) at this point are > 0, while the |VC|/GC ratio is < 1, suggesting an interaction that is electrostatic in nature. QTAIM analysis also revealed an intramolecular H-bond between O5′ · · · HO-CH_2 that has the same description as the C-H· · · F interaction (Table S17).

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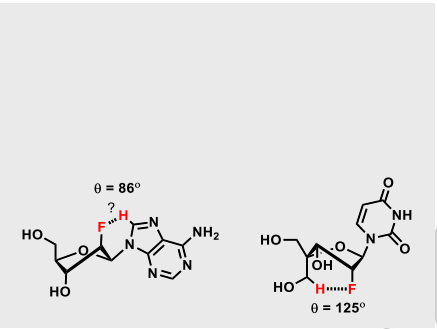
Detecting the Atypical: direct evidence for $^{\delta+}$C-H---F$^{\delta-}$ bonds that play a subtle but important role in conformational stability.



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Exploring atypical fluorine-hydrogen bonds and their effects on nucleoside conformations