Supplementary Material (ESI) for Chemical Communications

Supporting Information

Effect of 2' Fluorine Substitutions on DNA i-motif Structure and Stability

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Experimental Details.

Oligonucleotide Synthesis and Purification.

Oligonucleotide strands were synthesised on a solid phase support using phosphoramidite chemistry with an Applied Biosystems ABI 391 PCR-MateTM EP oligonucleotide synthesiser. Phosphoramidites purchased from Glen Research (UK). Standard procedures were adopted for cleavage from CPG and deprotection[1] except for the modified sequences for which the reaction time was reduced to 5 hours to prevent further reaction at the fluorine modifications.

Samples were purified by reverse phase and ion-exchange High Performance Liquid Chromatography (HPLC) as required [1]. Samples were then desalted using a pre-packed sephadex column.

In preparation for UV and NMR experiments samples were heated to 95 ^{0}C for 5 minutes and allowed to cool slowly to room temperature. They were then stored for 1 week at 4 ^{o}C prior to data collection.

UV Absorbance Melting Experiments.

Ultraviolet absorbance melting experiments were performed on a Hewlett Packard 8452A UV diode array spectrometer with a computer controlled Peltier device..Samples of 5 µM concentration were heated from 19 °C to 90 °C at intervals of 0.5 °C, with each temperature interval maintained for 5 minutes. T_m values were taken from the first derivative of the signmoidal absorbance versus temperature plots.

NMR spectroscopy.

^{1}H NMR spectra were recorded for samples in 90% H_2O/ 10% D_2O solutions (of 50 mM citrate buffer) at 10 ^{0}C using a Bruker DRX 500. 2D NOESY spectra were collected with a mixing time of 200 ms and in 2048 data points. Water suppression was achived using the WATERGATE sequence. Prior to Fourier transformation a window function with gb= 0.02 and lb= -10 was applied in both dimensions.

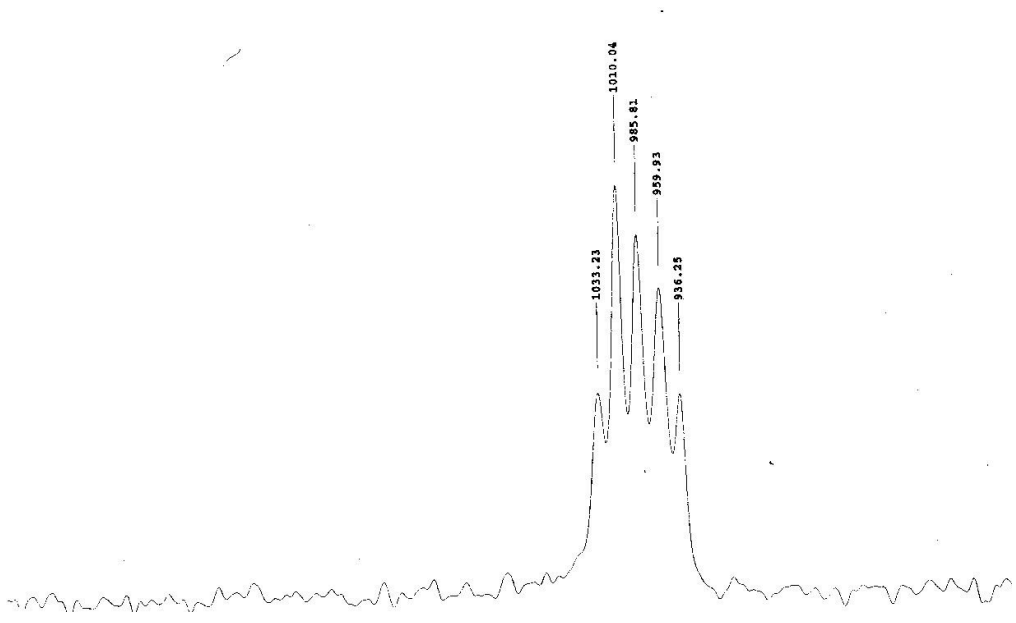
^{19}F NMR experiments were run on a Varian Unity 500 Inova spectrometer using an H/F/C probe. The ^{19}F operating frequency was 470 MHz. A spectral width of 2000 Hz collected in 8192 pairs of complex points was used, giving an acquisition time of 4.1 s. Prior to Fourier transformation a line broadening of -

1 Hz and a Gaussian parameter of 0.06 was applied. The solvent induced isotope shift experiments were performed on samples in 5 mm diameter NMR tubes with a co-axial insert containing approximately 60 ul of D_2O external lock. The field position was not changed between samples/experiments.

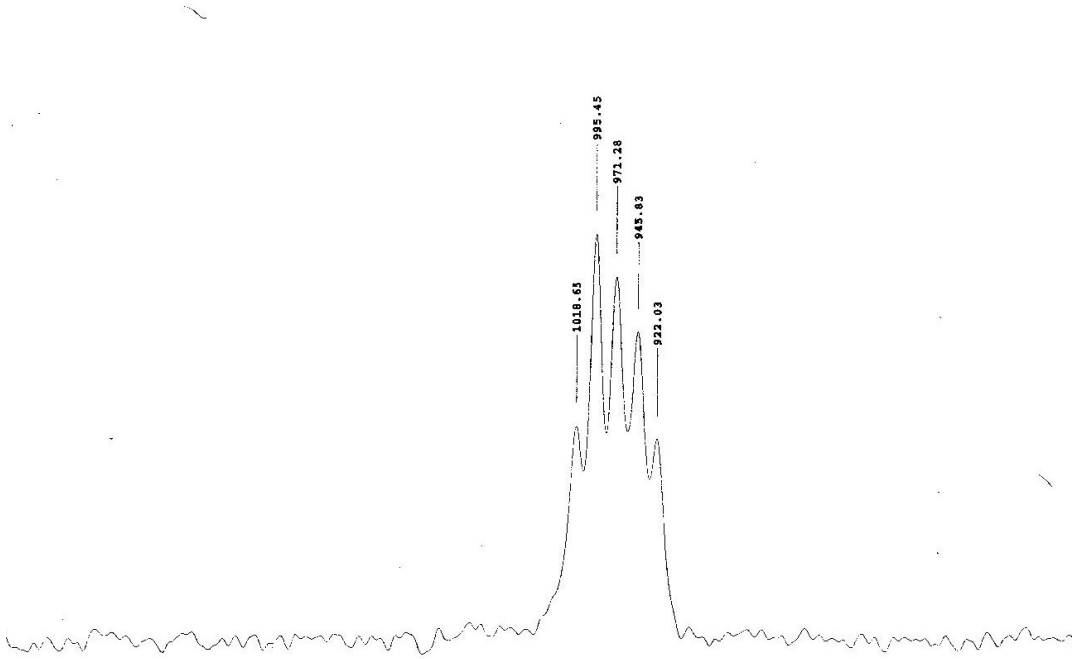
Table S1. Imino proton shifts for d(TCCCCC) and d(TCC**Cf**CC)

Imino	TCCCCC δ/ppm	TCC**Cf**CC δ/ppm
T1	11.431	11.431
C2	15.880	15.831
C3	15.646	15.548
C4	15.438	15.258
C5	15.646	15.625

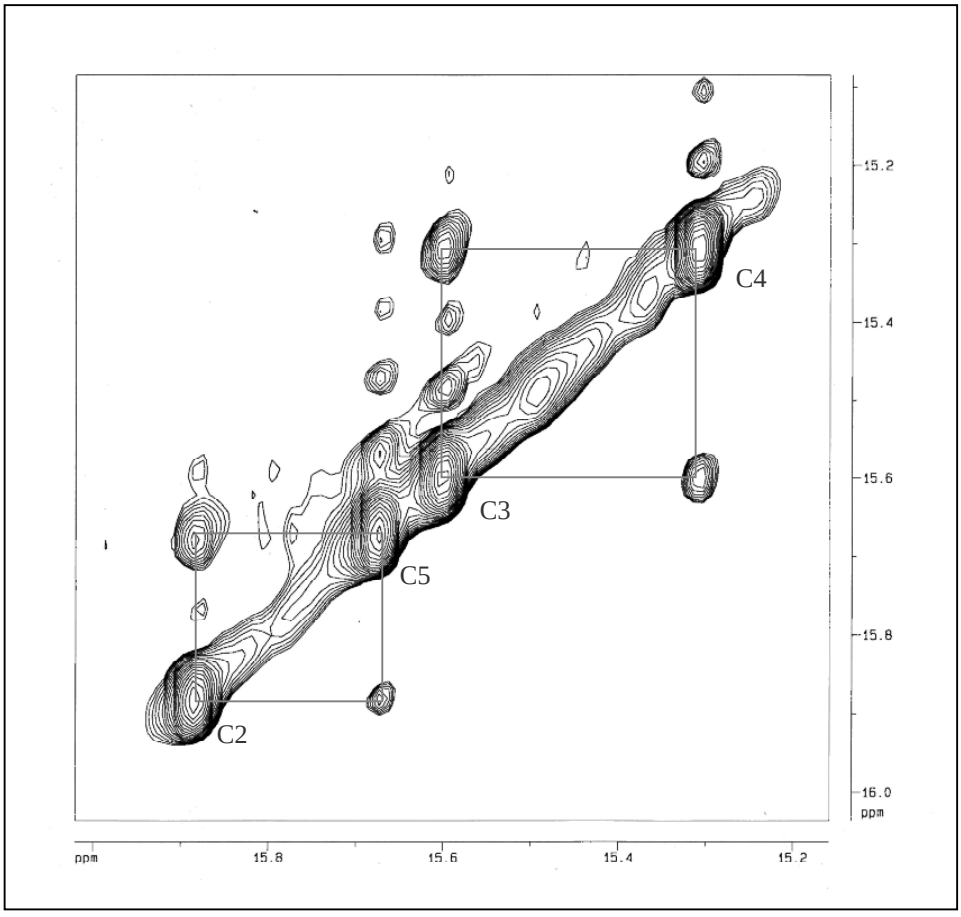
Spectrum 1: ^{19}F NMR spectrum of d(TCC**Cf**CC) in H_2O at 20 °C.



Spectrum 2: ^{19}F NMR spectrum of d(TCC**Cf**CC) in D_2O at 20 °C

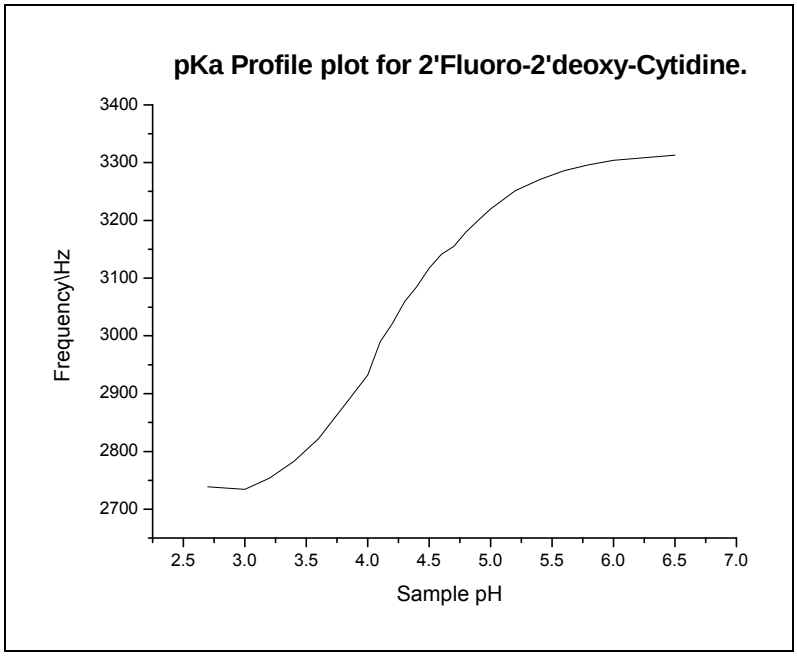


Spectrum 3: The imino-imino region of the 2D NOESY spectrum recorded for d(TCC**Cf**CC) in 90% H_2O/ 10% D_2O, (50 mM citrate buffer) , at 10 ^{0}C.



pKa data for 2' Fluorocytidine

pH	Frequency\Hz
2.7	2738.85
3.0	2734.62
3.2	2753.97
3.4	2782.68
3.6	2821.9
3.8	2877.15
4.0	2932.52
4.1	2989.43
4.2	3021.3
4.3	3059.16
4.4	3084.92
4.5	3117.22
4.6	3140.8
4.7	3154.78
4.8	3179.9
4.9	3200.41
5.0	3219.5
5.2	3251.29
5.4	3270.77
5.6	3286.41
5.8	3296.02
6.0	3304.23
6.5	3313



pH was adjusted using NaOD (40 wt. %, 100 µl) in RNase free water (900 µl) and DCl (35 wt. %, 100 µl) in RNase free water (900 µl), even though the sample was in 90% H_2O /10 % D_2O.

Spectra were recorded using an HFC triple resonance probe with a ^{19}F operating frequency of 470 MHz. A Boltzman fit of this data calculates the pKa of the N3 proton to be 4.18±0.02. The pKa in the

oligonucleotide sequence is likely to be a little higher, in line with observations made for each of the natural nucleosides/nucleotides [2].

[1] Murray, J.B.; Collier, A.K; Arnold, J.R.P. *Analytical Biochemistry* **1994**, *218*, 177.
[2] Blackburn, G.M.; Gait, M.J.; Loakes, D.; Williams, D.M. Ed. , *Nucleic Acids in Chemistry and Biology*. 3rd Ed. RSCPublishing, Cambridge, 2006. pg 16.

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