Supporting Information to

5-Fluoro Pyrimidines: Labels to Probe DNA and RNA Secondary Structures by 1D ^{19}F NMR Spectroscopy

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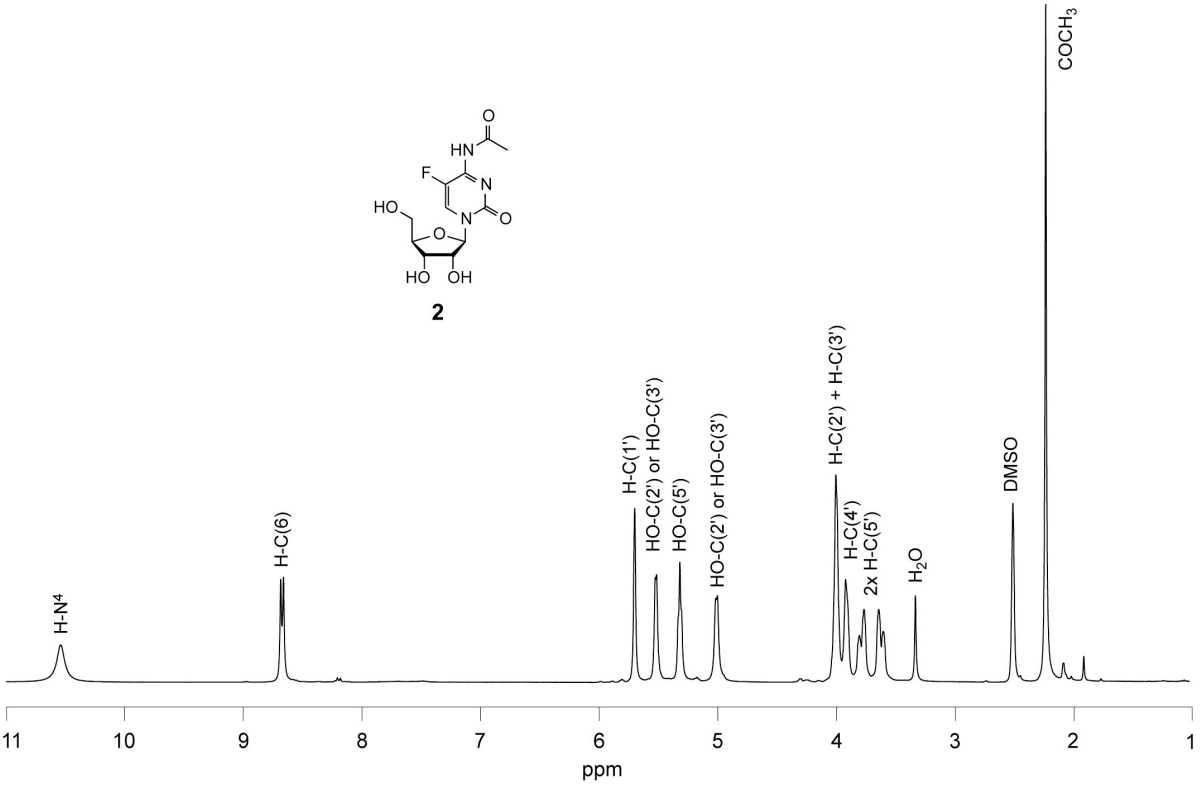
1. ^{1}H and ^{13}C NMR spectra of N^4-acyl-5-fluoro cytidine derivatives 2 - 9

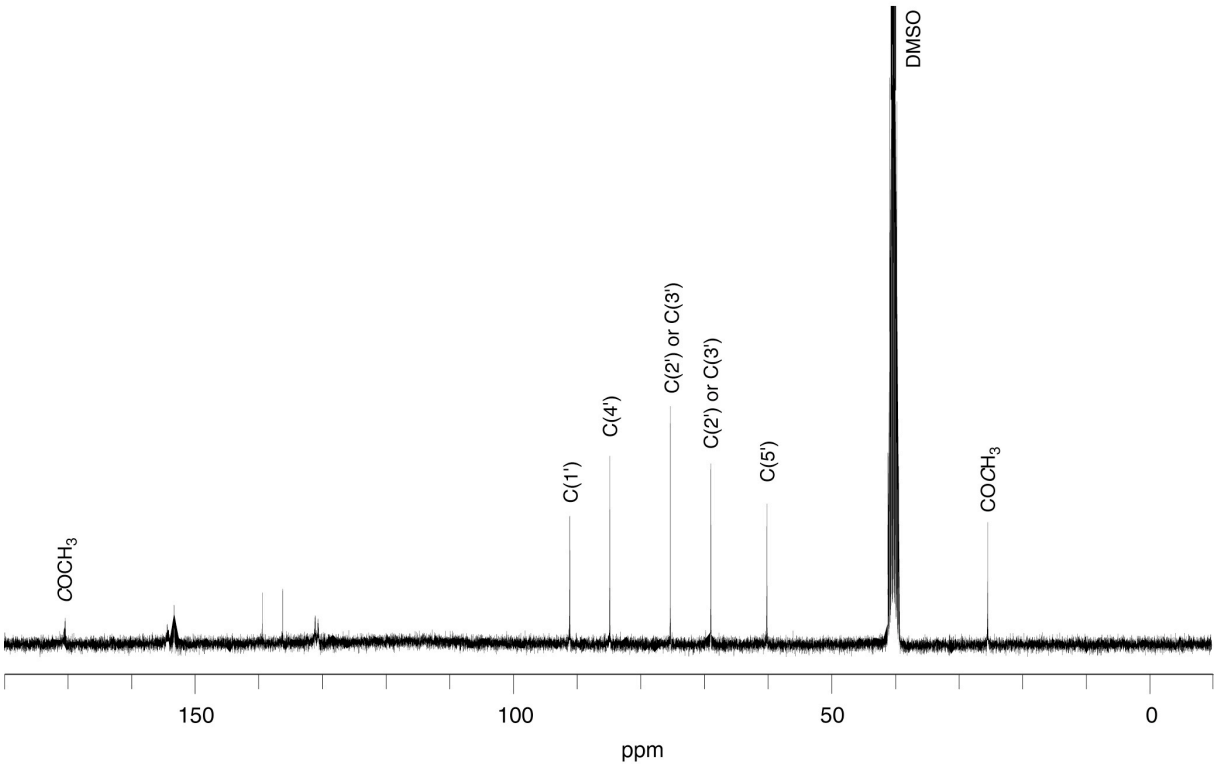
*N^4-Acetyl-5-fluoro cytidine **2***

^{1}H NMR (300 MHz, DMSO):



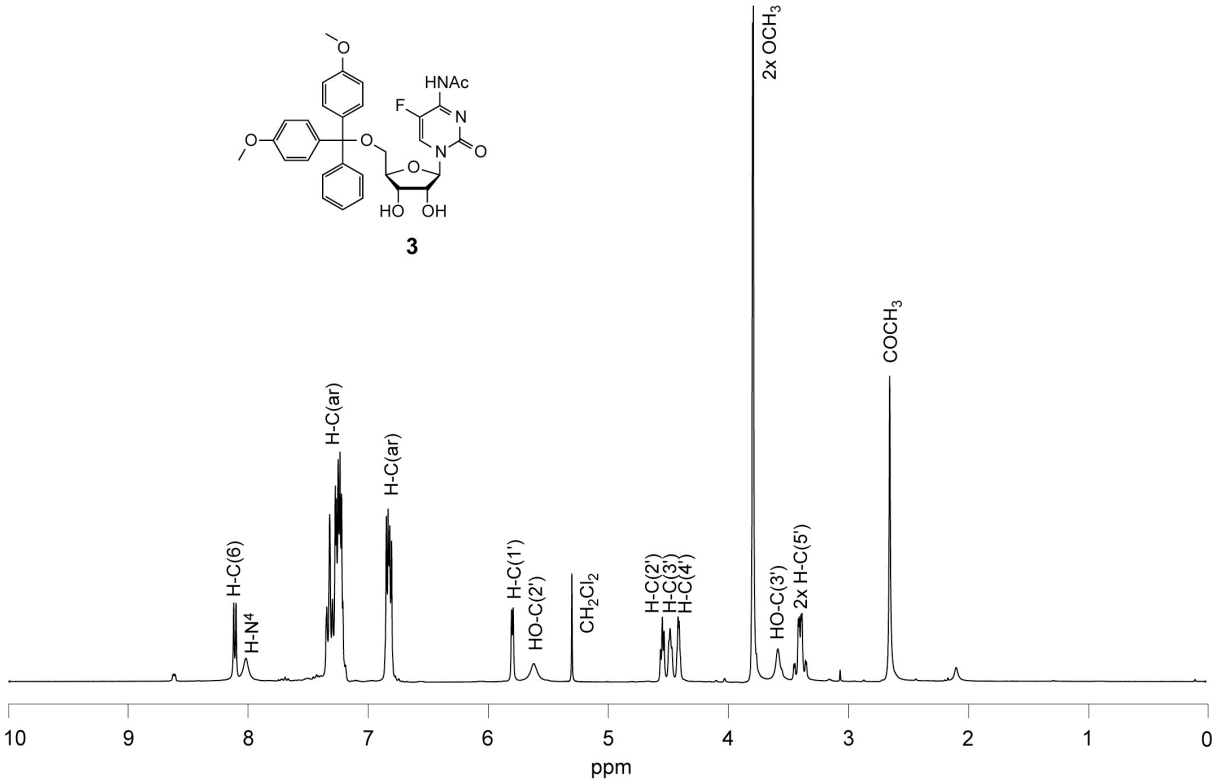
2

^{13}C NMR (75 MHz, DMSO):

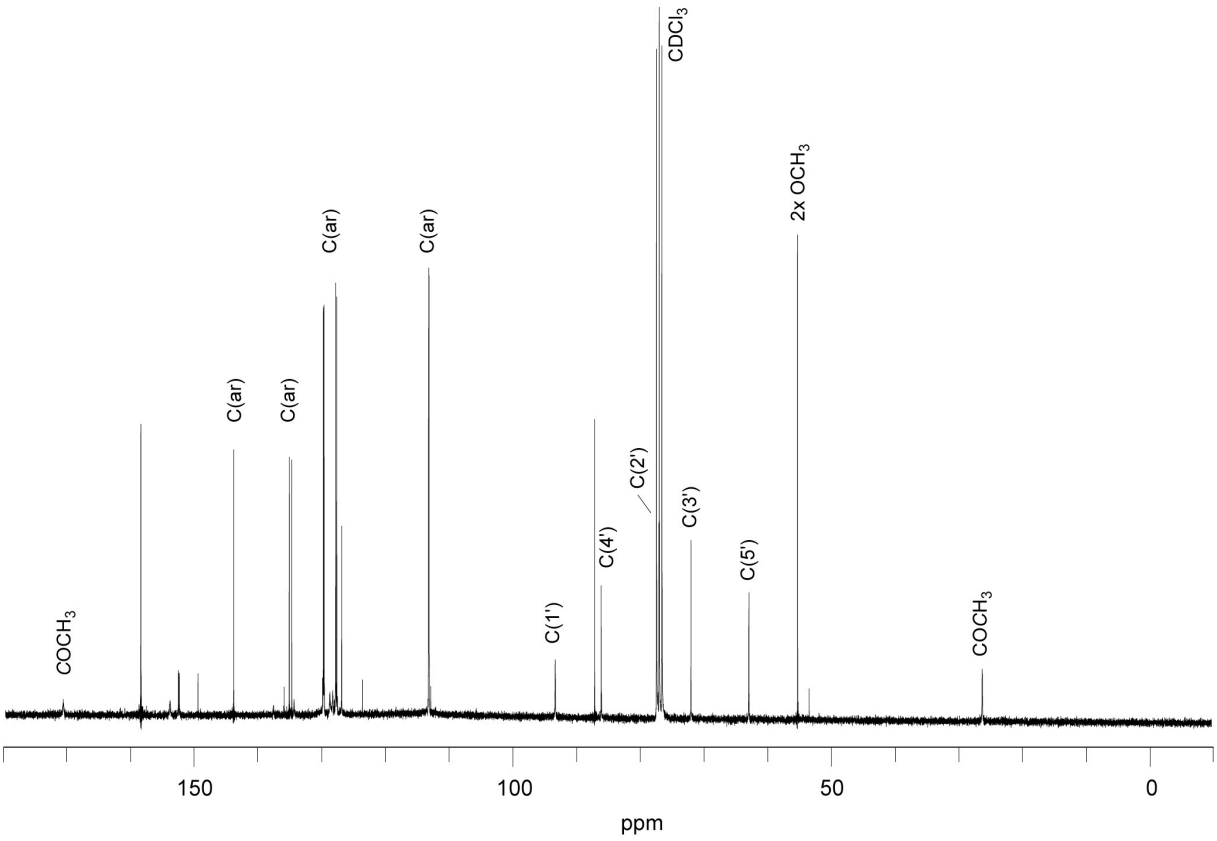


*N^4-Acetyl-5'-O-(4,4'-dimethoxytrityl)-5-fluoro cytidine **3***

^{1}H NMR (300 MHz, $CDCl_3$):

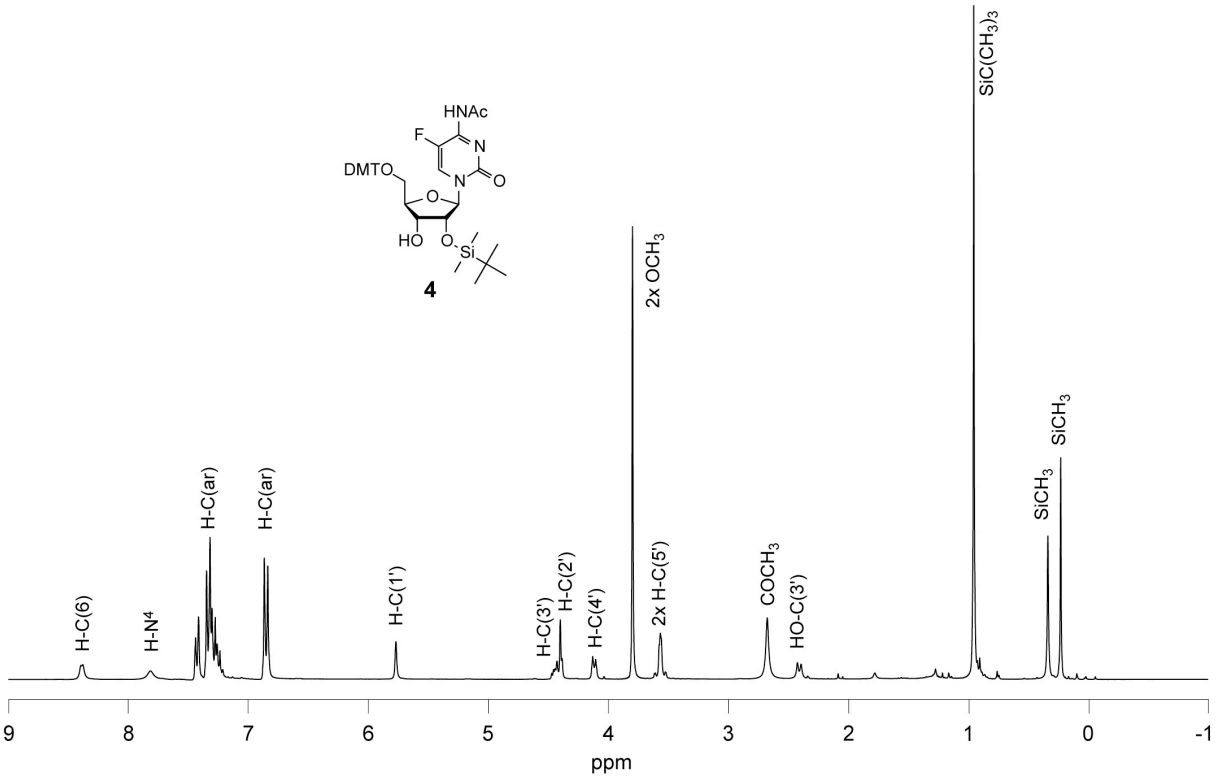


^{13}C NMR (75 MHz, $CDCl_3$):

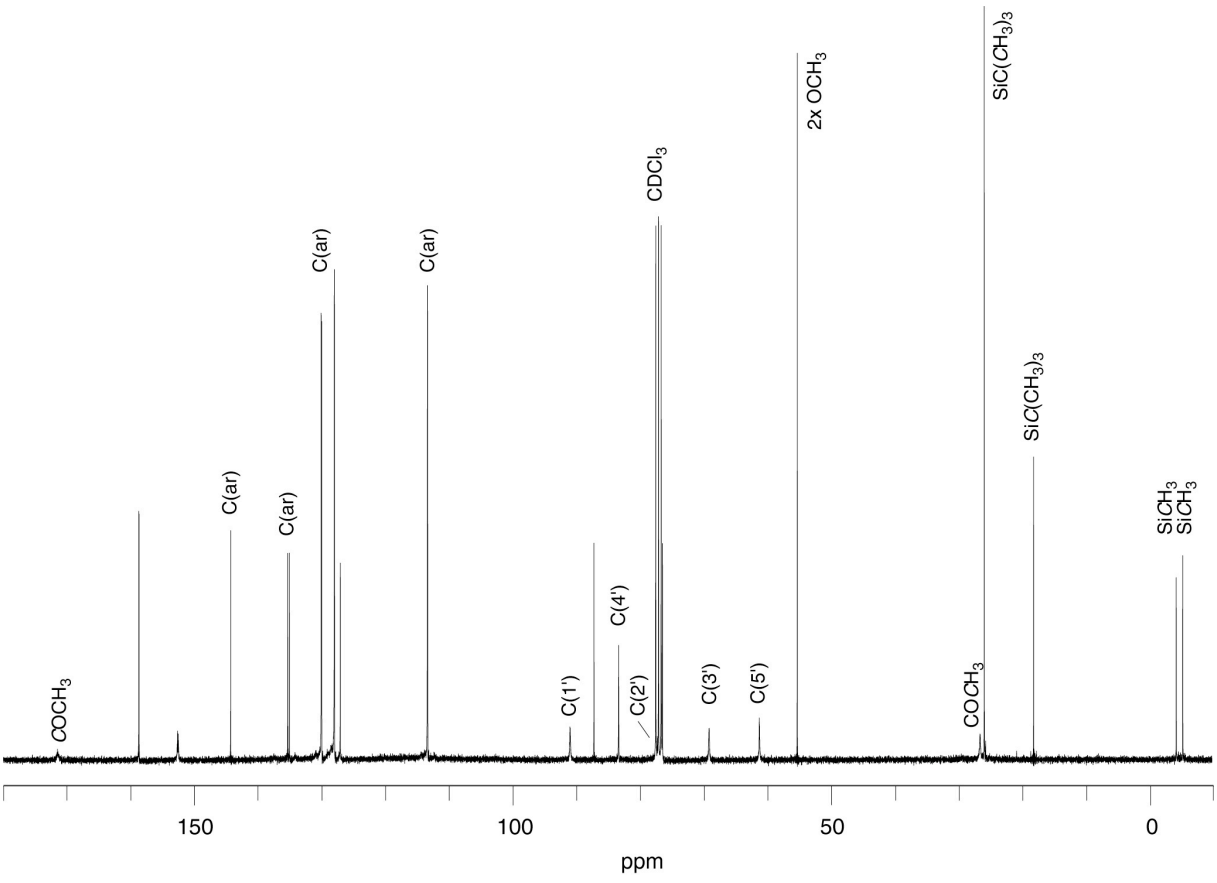


*N^4-Acetyl-2'-O-(tert.-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-5-fluoro cytidine **4***

^{1}H NMR (300 MHz, $CDCl_3$):

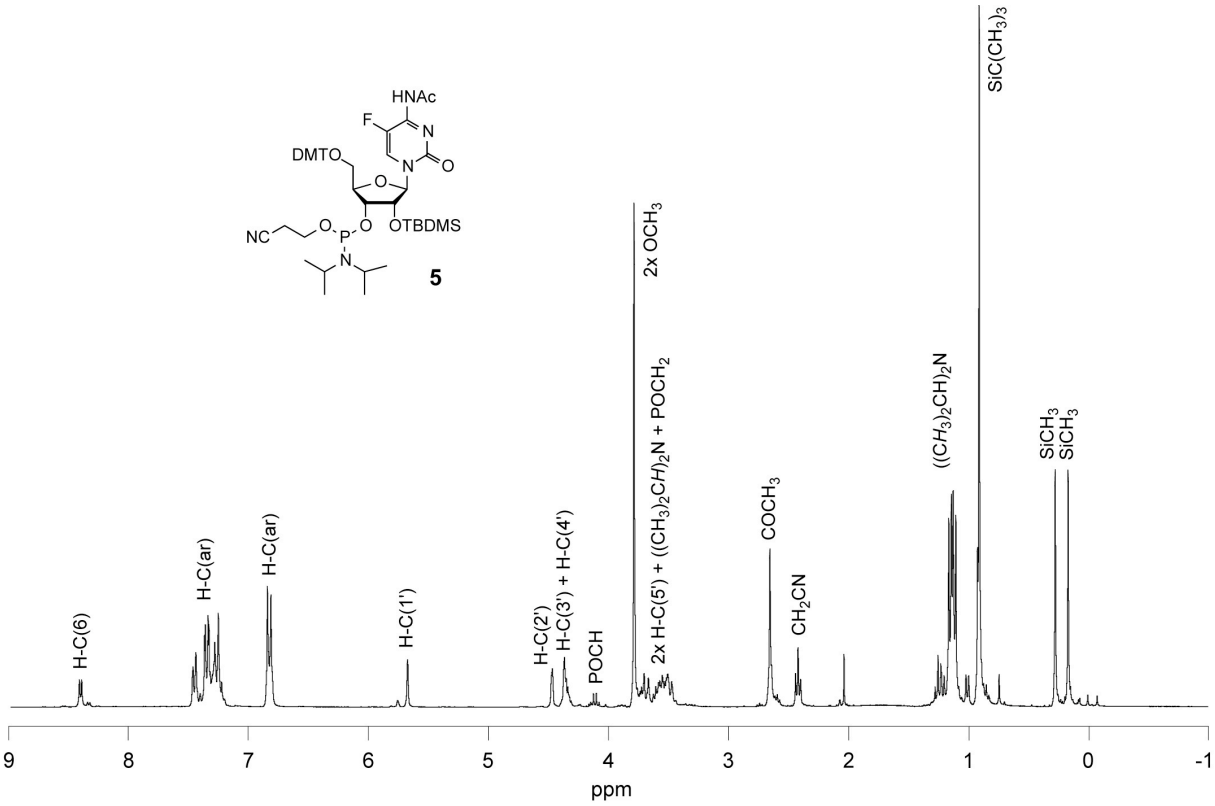


^{13}C NMR (75 MHz, $CDCl_3$):

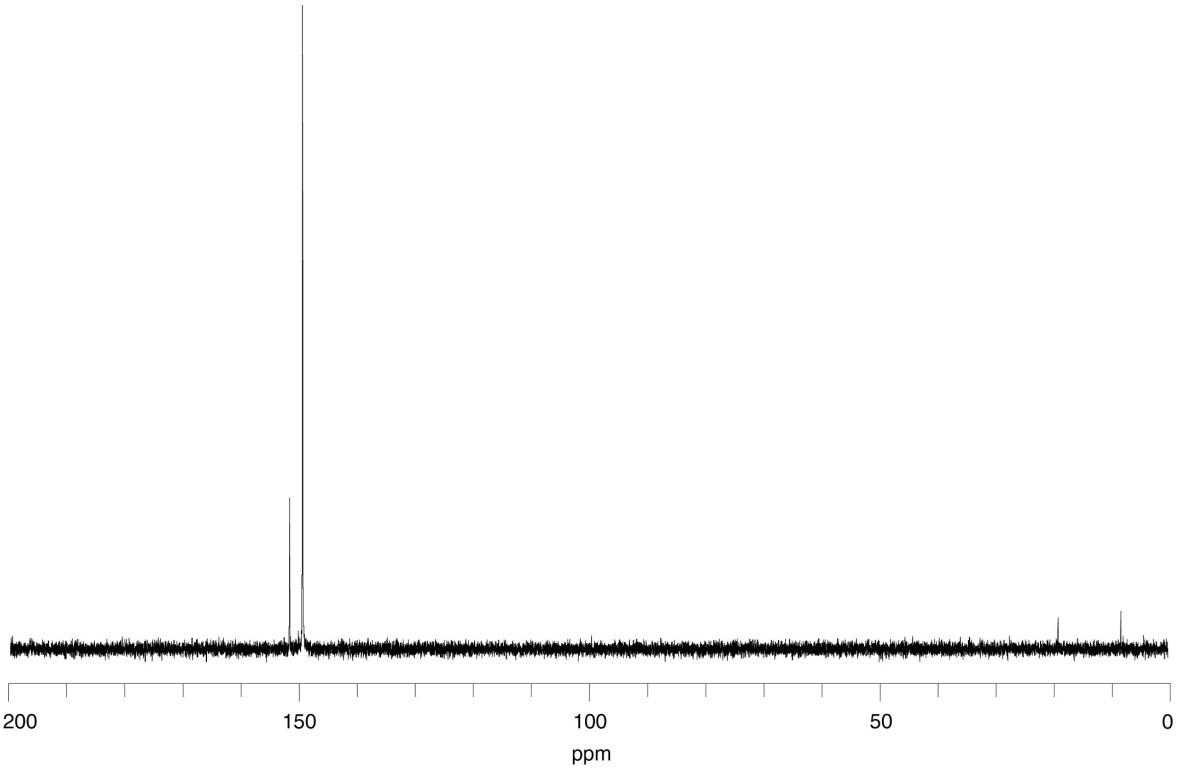


*N^4-Acetyl-2'-O-(tert.-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-5-fluoro cytidine 3'-(2-cyanoethyl)-N,N-diisopropylphosphoramidite **5***

^{1}H NMR (600 MHz, $CDCl_3$):

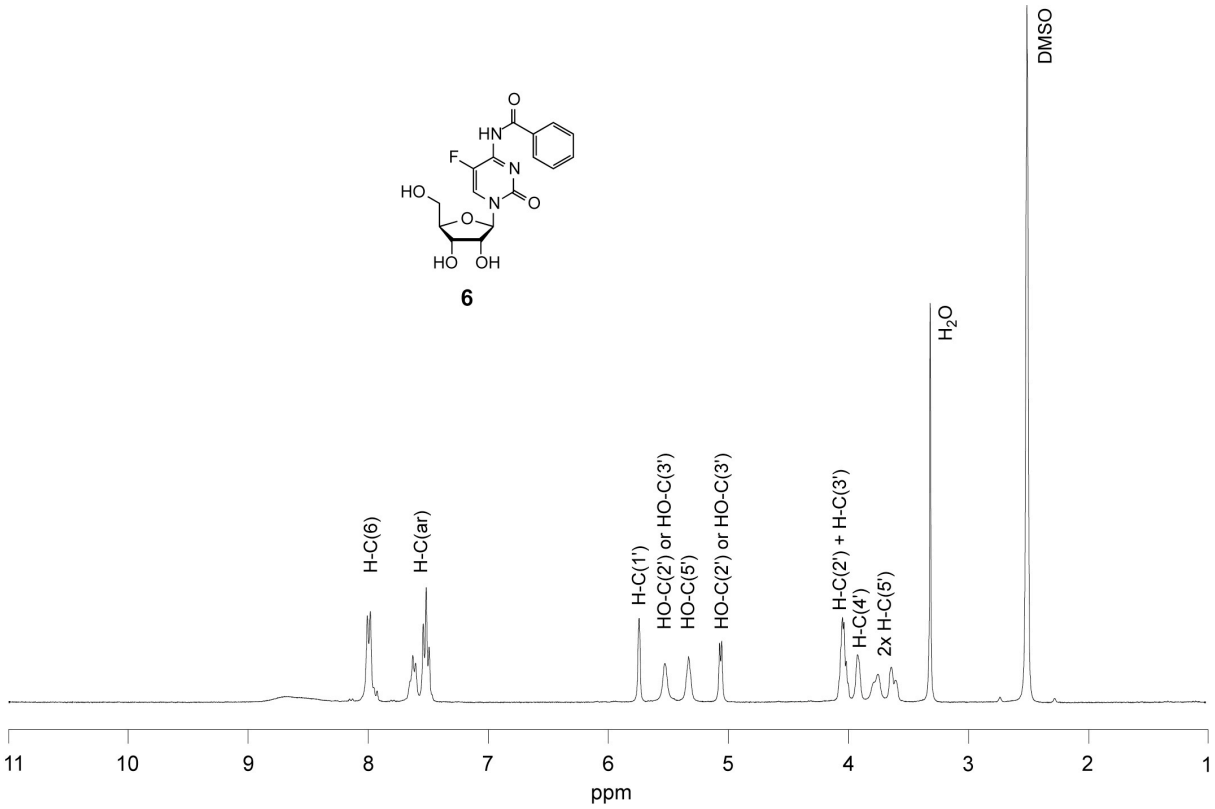


^{31}P NMR (121 MHz, $CDCl_3$):

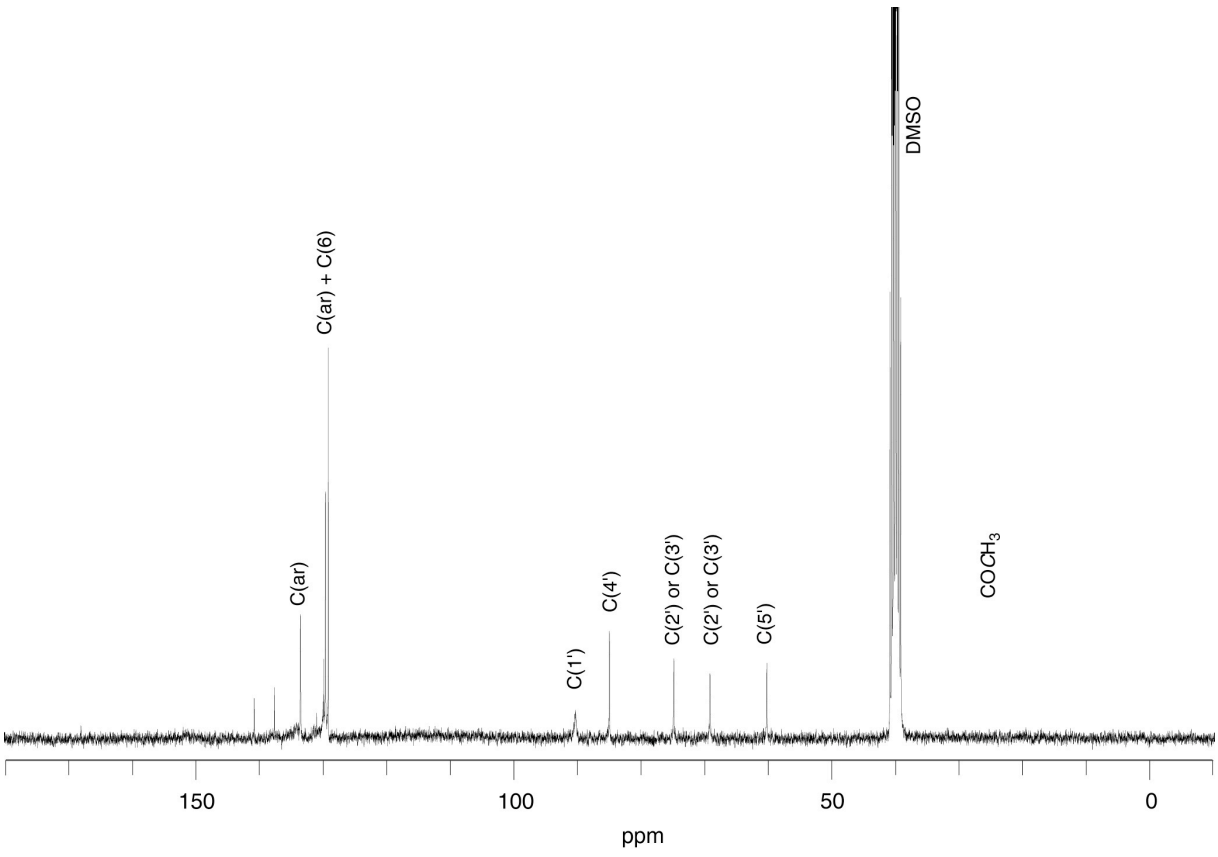


*N^4-Benzoyl-5-fluoro cytidine **6***

^{1}H NMR (300 MHz, DMSO):

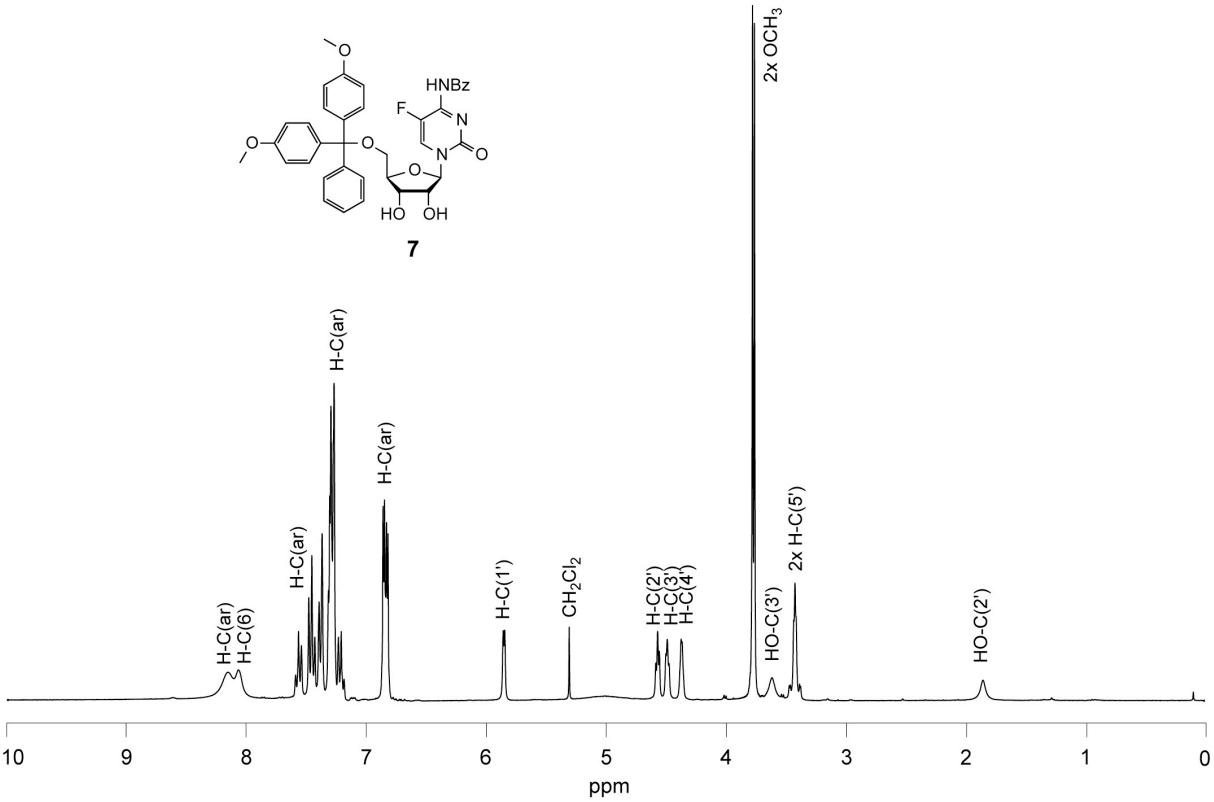


^{13}C NMR (75 MHz, $CDCl_3$):

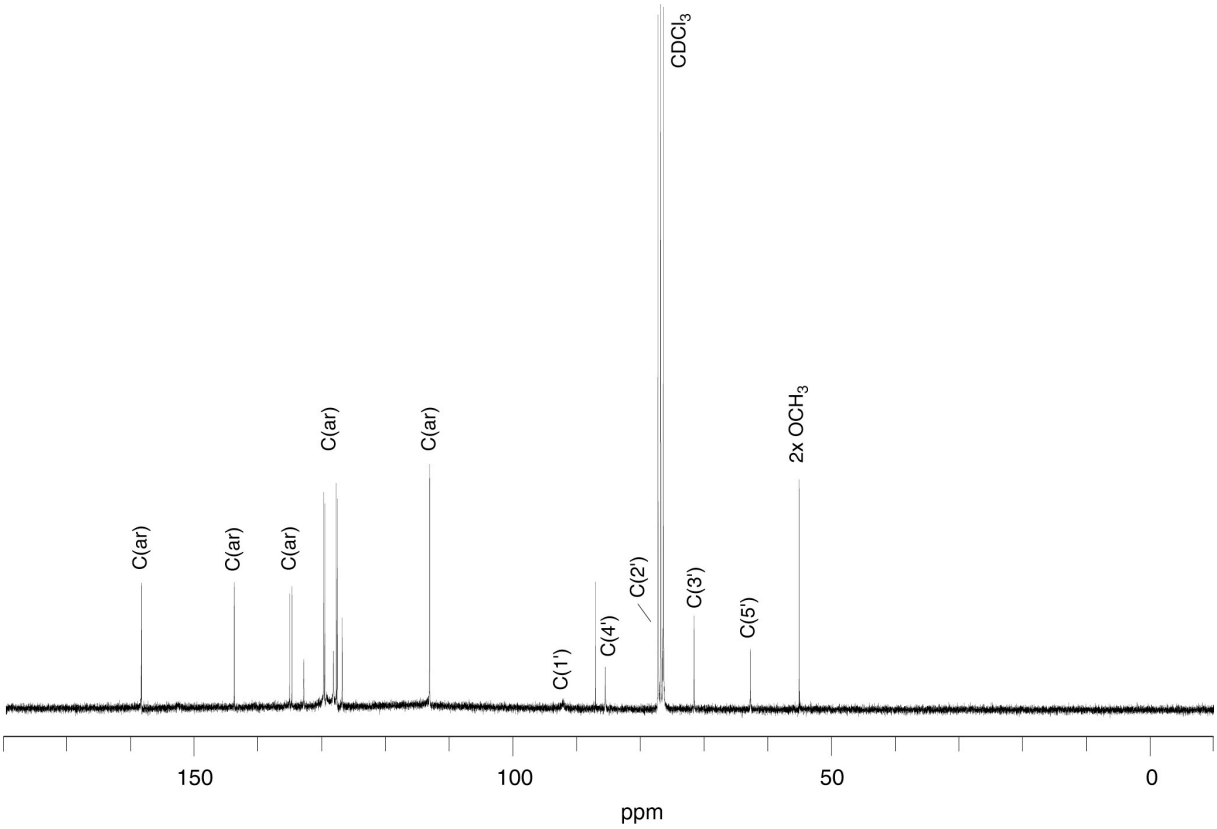


*N^4-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-5-fluoro cytidine **7***

^{1}H NMR (300 MHz, $CDCl_3$):

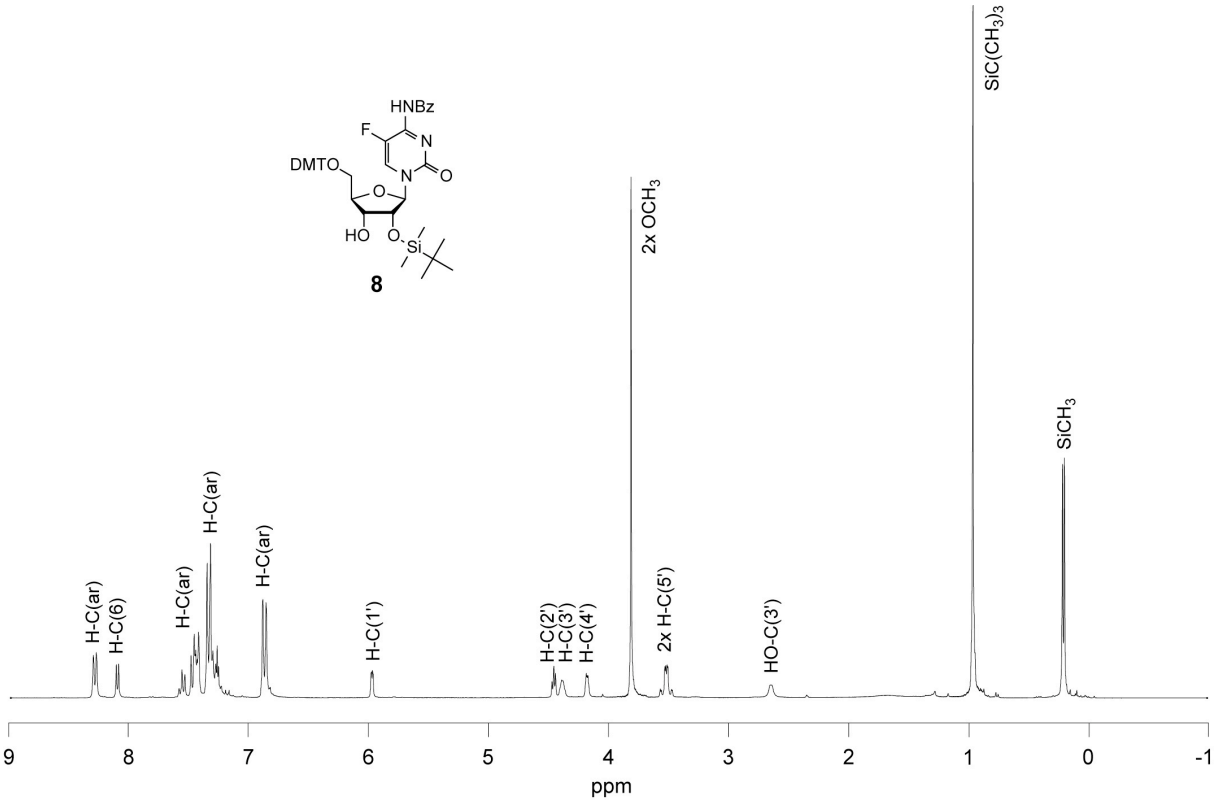


^{13}C NMR (75 MHz, $CDCl_3$):

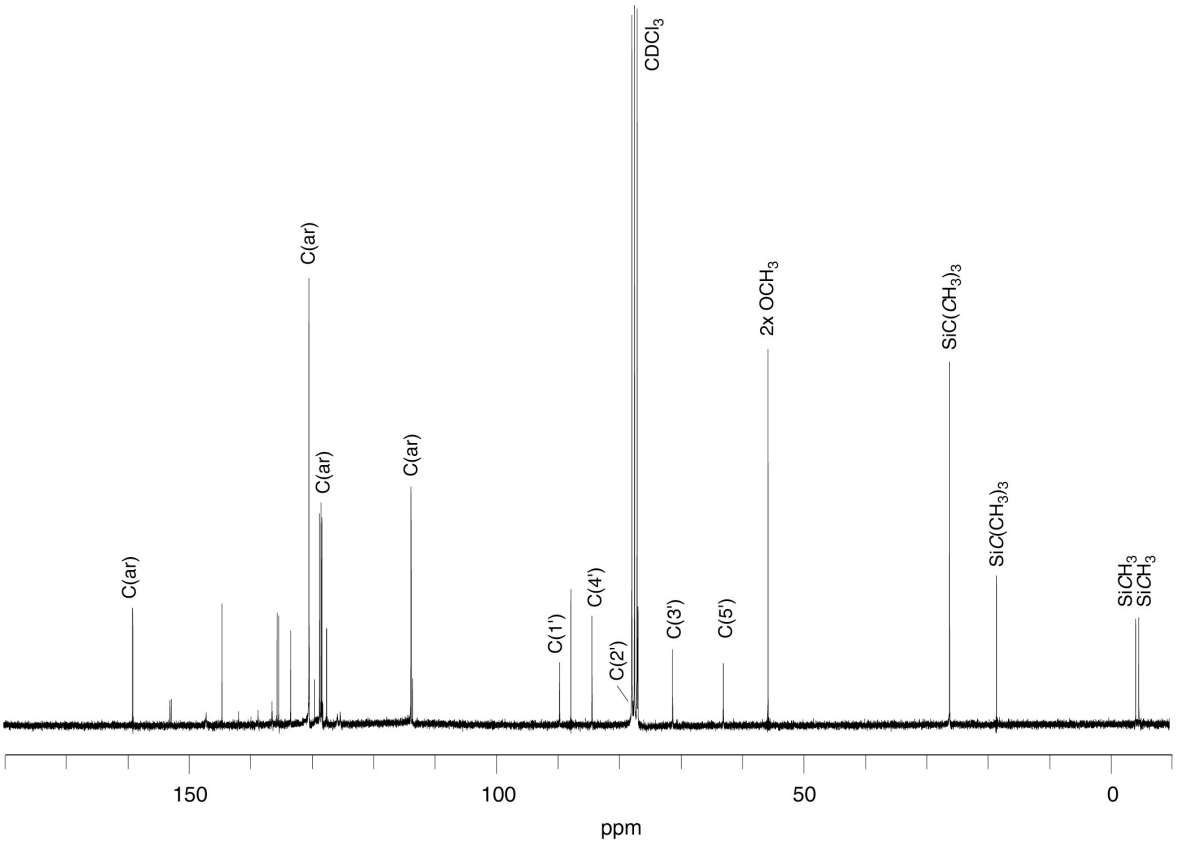


*N^4-Benzoyl-2'-O-(tert.-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-5-fluoro cytidine **8***

^{1}H NMR (300 MHz, $CDCl_3$):

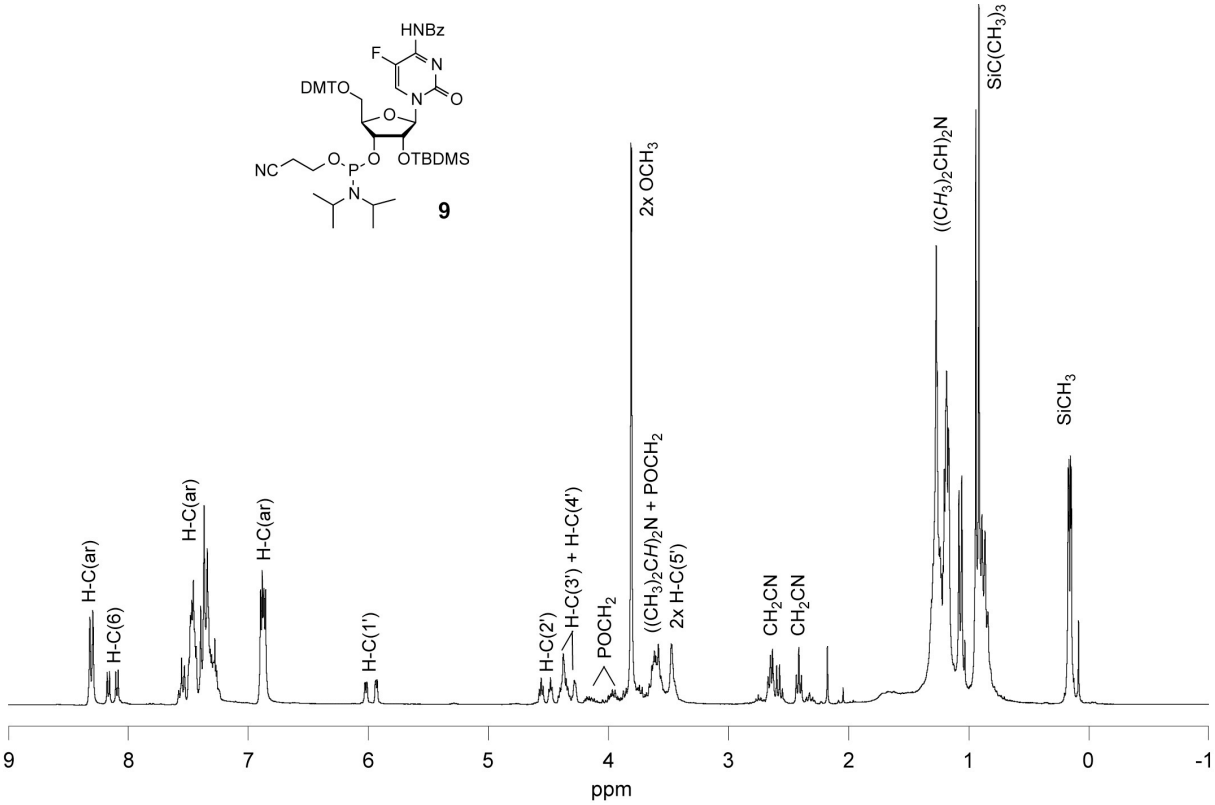


^{13}C NMR (75 MHz, $CDCl_3$):

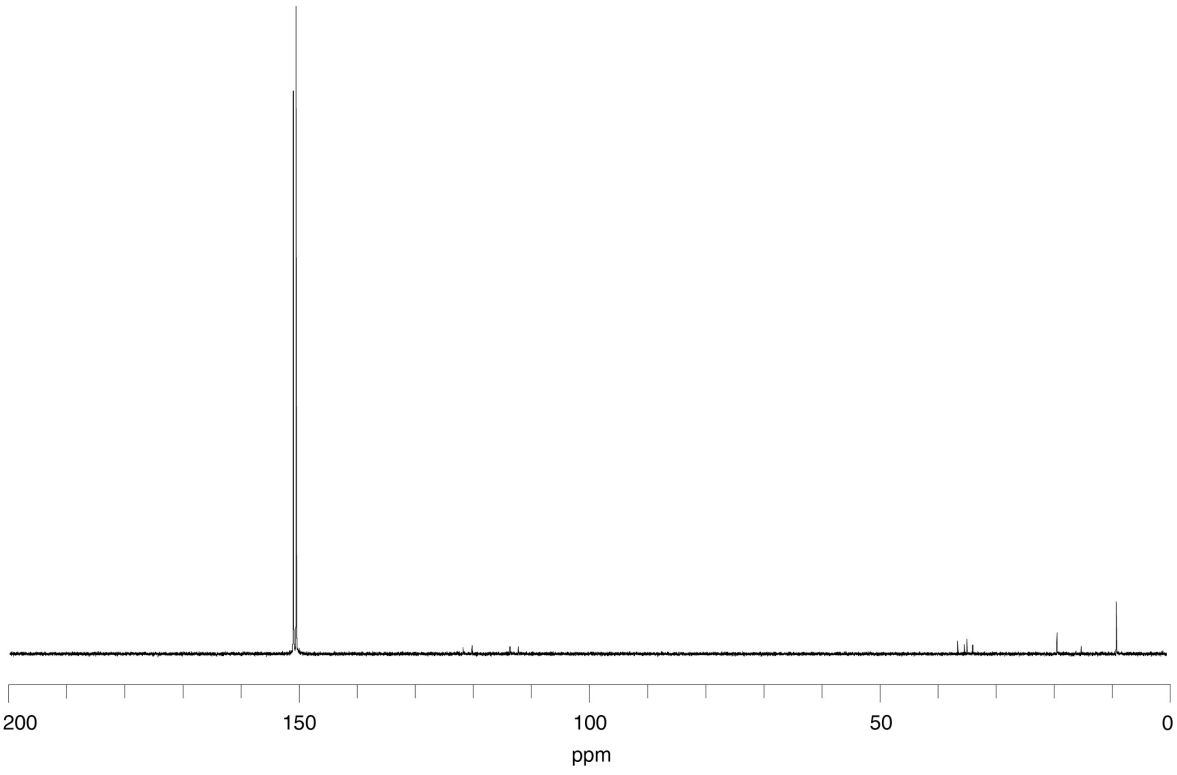


*N^4-Benzoyl-2'-O-(tert.-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-5-fluoro cytidine 3'-(2-cyanoethyl)-N,N-diisopropylphosphoramidite **9***

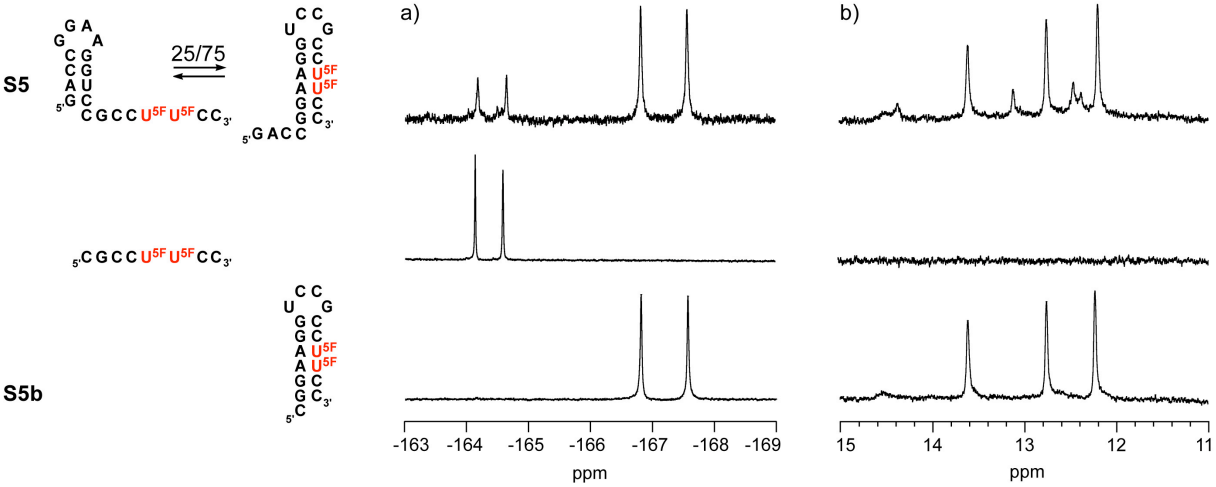
^{1}H NMR (600 MHz, $CDCl_3$):



^{31}P NMR (121 MHz, $CDCl_3$):

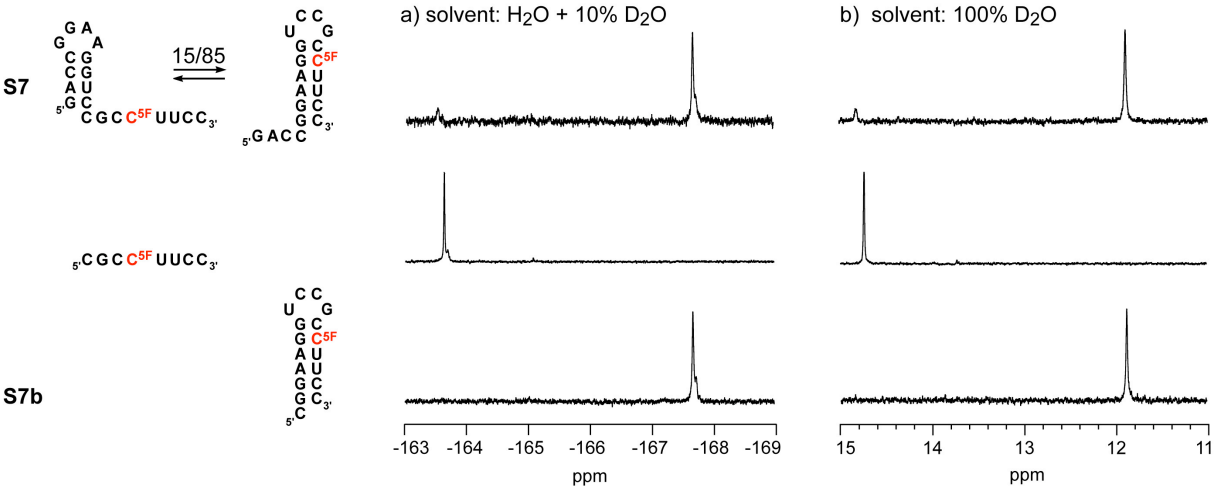


2. ^{1}H and ^{19}F NMR spectra of RNA S5 ($U^{5F}U^{5F}$) and reference sequences



Double U^{F5}-labeled RNA **S5** and reference sequences, 5'-CGCCU^{5F}U^{5F}CC-3' and **S5b**: a) ^{1}H decoupled ^{19}F NMR spectra; b) ^{1}H NMR spectra, imino proton region. Conditions: 0.3 mM RNA, 25 mM sodium arsenate buffer, H_2O/D_2O 9/1, pH 7.0, 300 K.

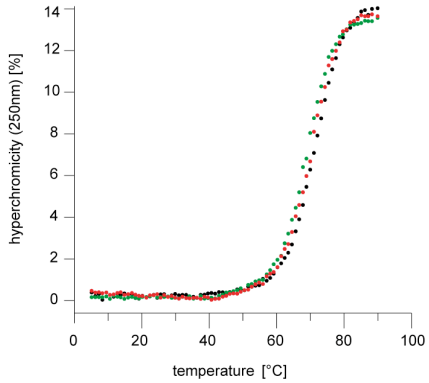
3. ^{19}F NMR spectra of RNA S7 (C^{5F})



5-Fluoro cytosine modified RNA; a) ^{1}H decoupled ^{19}F spectra of **S7** and reference sequences, 5'-CGCC^{5F}UUCC-3' and **S7b** in H_2O/D_2O 9/1; b) same as a) but in 100% D_2O. Conditions: 0.3 mM RNA, 25 mM sodium arsenate buffer, pH 7.0, 300 K.

4. UV melting profiles of DNA S1b, S2b, S6b and RNA S3b, S4b, S5b, S7b

a)



S1b

T_m = 73.6°C

ΔG^0 = -6.6 kcal mol^{-1}

S2b

T_m = 71.1°C

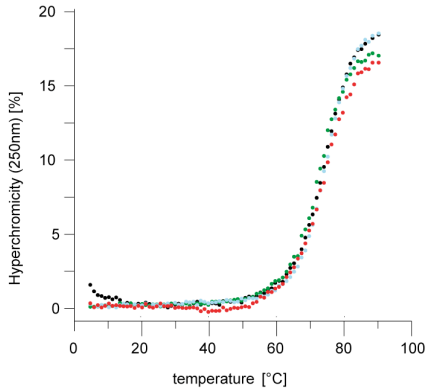
ΔG^0 = -7.6 kcal mol^{-1}

S6b

T_m = 72.5°C

ΔG^0 = -6.6 kcal mol^{-1}

b)



S3b

T_m = 76.7°C

ΔG^0 = -7.1 kcal mol^{-1}

S4b

T_m = 74.4°C

ΔG^0 = -7.4 kcal mol^{-1}

S5b

T_m = 75.6°C

ΔG^0 = -6.9 kcal mol^{-1}

S7b

T_m = 75.6°C

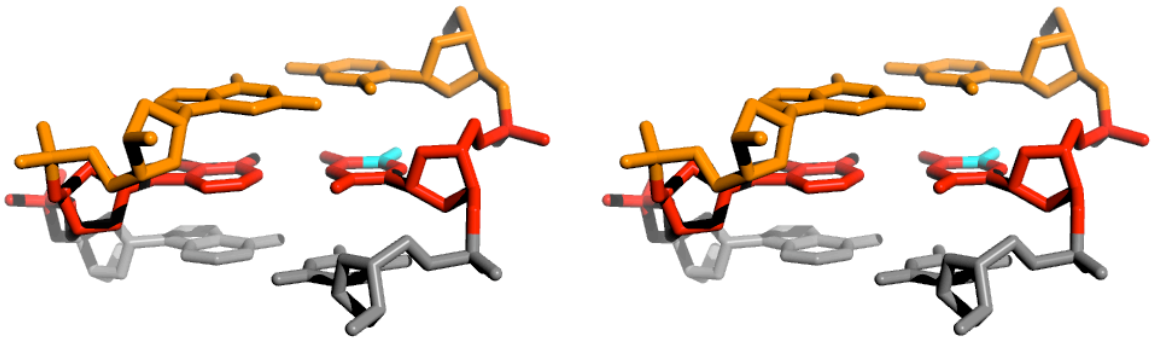
ΔG^0 = -6.7 kcal mol^{-1}

UV melting profiles comparing 5-fluoro uridine-, 5-fluoro cytidine- and unmodified DNA and RNA oligonucleotides; a) hyperchromicity versus temperature overlay plot of **S1b** (unmod), **S2b** (dU^{5F}) and **S6b** (dC^{5F}); b) hyperchromicity versus temperature overlay plot of **S3b** (unmod), **S4b** (U^{5F}), **S5b** ($U^{5F}U^{5F}$) and **S7b** (C^{5F}); conditions: 2 µM RNA, 150 mM sodium chloride, 10 mM sodium phosphate buffer, pH 7.0.

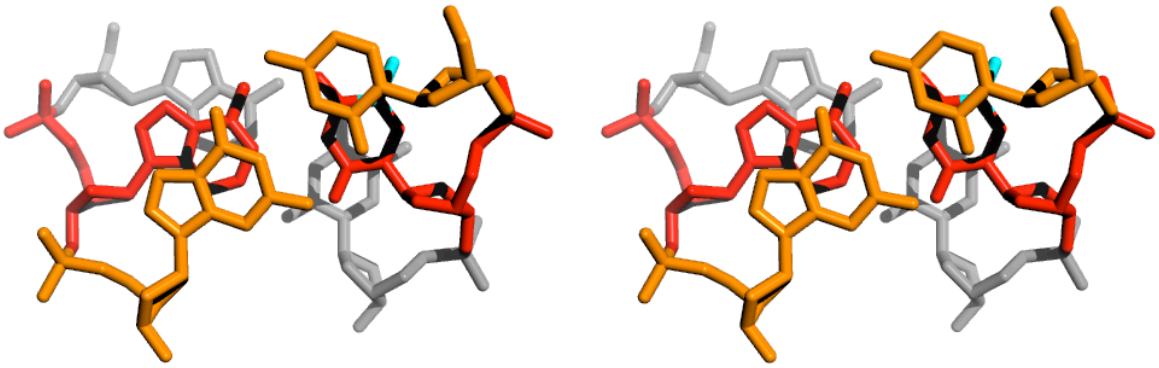
5. Stereo views of B-form DNA and A-form RNA according to Figure 4 in the main text

Pyrimidine C5 positions are indicated in cyan color.

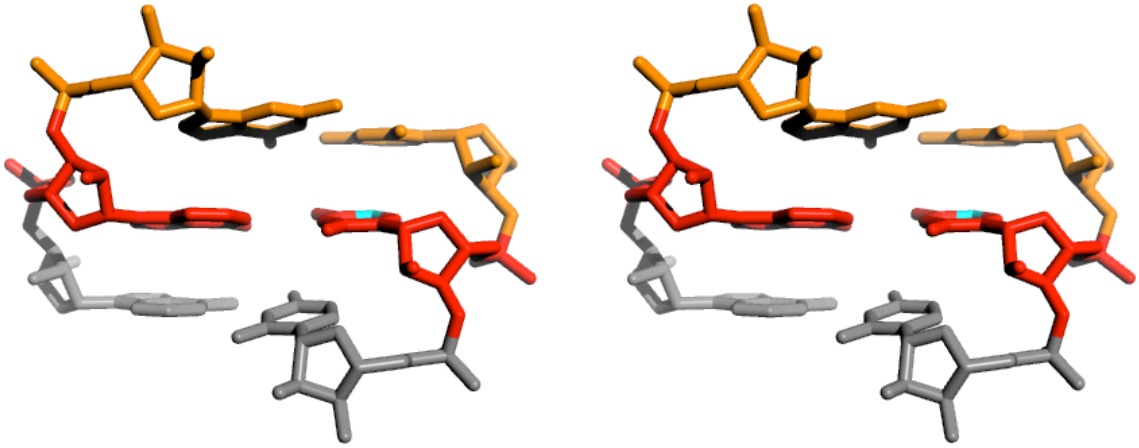
Stereo view of Figure 4A:



Stereo view of Figure 4A:



Stereo view of Figure 4C:



Stereo view of Figure 4D: