Supporting Information

Enthalpy-entropy Contribution to Carcinogen-induced DNA Conformational Heterogeneity

Fengting Liang and Bongsup P. Cho

Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI 02881

Supporting Information

Enthalpy-entropy Contribution to Carcinogen-induced DNA Conformational Heterogeneity

Fengting Liang and Bongsup P. Cho

Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI 02881

Adduct Synthesis. Crude oligodeoxynucleotides in 15 mmol desalted form were obtained from Sigma-Genosys (The Woodlands, TX). All high performance liquid chromatography (HPLC) solvents were purchased from Fisher, Inc. (Pittsburgh, PA). Following the published procedures (10, 23), we prepared FABP- and FAF-modified 11-mer oligonucleotides d(5′-CCATCG*CAACC-3, in which G* is either an FABP- or FAF-modified dG adduct (Figure 1a). The HPLC system used for purification of the oligos consisted of a Hitachi EZChrom Elite unit (Ibaraki-ken, Japan) with a L2450 diode array as a detector and employed a Phenomenex Luna C18 column (10 x 150 mm, 2.5 mm) (Torrance, CA) with a 40-min gradient system involving 3 to 15% acetonitrile in pH 7.0 ammonium acetate buffer (0.10 M) with a flow rate of 2.0 mL/min. The modified oligos were characterized by ESI-TOF-MS analysis as reported previously and annealed individually with appropriate complementary strand to produce the desired duplexes (Figure 1). An identical set of unmodified duplexes was also prepared as controls.

UV-Melting. UV-melting data were obtained using a Beckman DU 800 UV/VIS spectrophotometer equipped with a 6-chamber, 1-cm path-length Tm cell. Sample cell temperatures were controlled by a Peltier mechanism. Duplexes with a total concentration in the range of 0.2 - 14 μM were prepared in solutions containing 0.2 M NaCl, 10 mM Na_3PO_4, and 0.2 mM EDTA at pH 7.0. Thermomelting curves were constructed by varying the temperature of the sample cell (1°C/min) and monitoring the absorbance of the sample at 260 nm. A typical melting experiment consisted of forward/reverse scans and was repeated three times. Thermodynamic parameters were calculated using the program MELTWIN® version 3.5, as described previously (10, 31).

Circular Dichroism (CD). CD measurements were conducted on a Jasco J-810 spectropolarimeter equipped with a Peltier mechanism. Duplex samples (2 ODS single strand concentration) in 400 μL of the UV buffer (described above) were placed in a 1-mm path-length cell and heated at 85°C for 5 min and then cooled to 15°C over a 10 min period to ensure complete duplex formation. Spectra were then acquired every 0.2 nm with a 2-s response time from 200 to 400 nm at a rate of 50 nm/min and were averaged from 10 accumulations and smoothed using the 17 point adoptive algorithms provided by Jasco.

Dynamic NMR. Duplex samples (20 ~ 40 ODS) were pelleted in an ultracentrifuge using a Pall Microsep MF centrifugal device (Yellow, MW cutoff = 1,000). The centrifuged samples were dissolved in 300 µL of a pH 7.0 buffer (100 mM NaCl, 10 mM Na3PO4, and 100 µM EDTA in 10% D_2O/90% H_2O) and filtered into through a Shigemi tube using a 0.2 µm membrane filter.

All ^{1}H and ^{19}F NMR results were recorded using a dedicated 5-mm $^{19}F/^{1}H$ dual probe on a Bruker DPX400 Avance spectrometer operating at 400.0 and 376.5 MHz, respectively. Imino proton spectra were obtained using phase sensitive jump-return sequences at 5°C and referenced relative to DSS (2,2-dimethyl-2-silapentane-5-sulfonate). ^{19}F NMR spectra were acquired in the ^{1}H-decoupled mode and referenced to $CFCl_3$ by assigning external hexafluorobenzene in C_6D_6 at -164.90 ppm. One-dimensional ^{19}F NMR spectra were measured between 0°C and 60°C using increments of 5~10°C. Additional temperatures were used as needed to clarify the signal exchange process. Temperatures were maintained by a Bruker VT unit by adding liquid N_2 to the probe. Spectra were obtained by collecting 65,536 points using a 37,664-Hz sweep width and a recycle delay of 1.0 s. A total of 1200 scans were acquired for each dynamic NMR spectrum. All free induction decays were processed by zero-filling, exponential multiplication using a 20 Hz line broadening factor, and Fourier transformation. NOESY/exchange ^{19}F NMR spectra were obtained in the phase-sensitive mode using the following parameters: sweep width 4529 Hz, number of complex data points in t_2 1024; number of complex FIDs in t_1 256; number of scans 96; dummy scans 16; recycle delays 1.0 s; and, mixing time 400 ms. The data were apodized with sine function using 2 Hz line broadening in both dimensions and Fourier transformed with the 1024 × 256 data matrix.

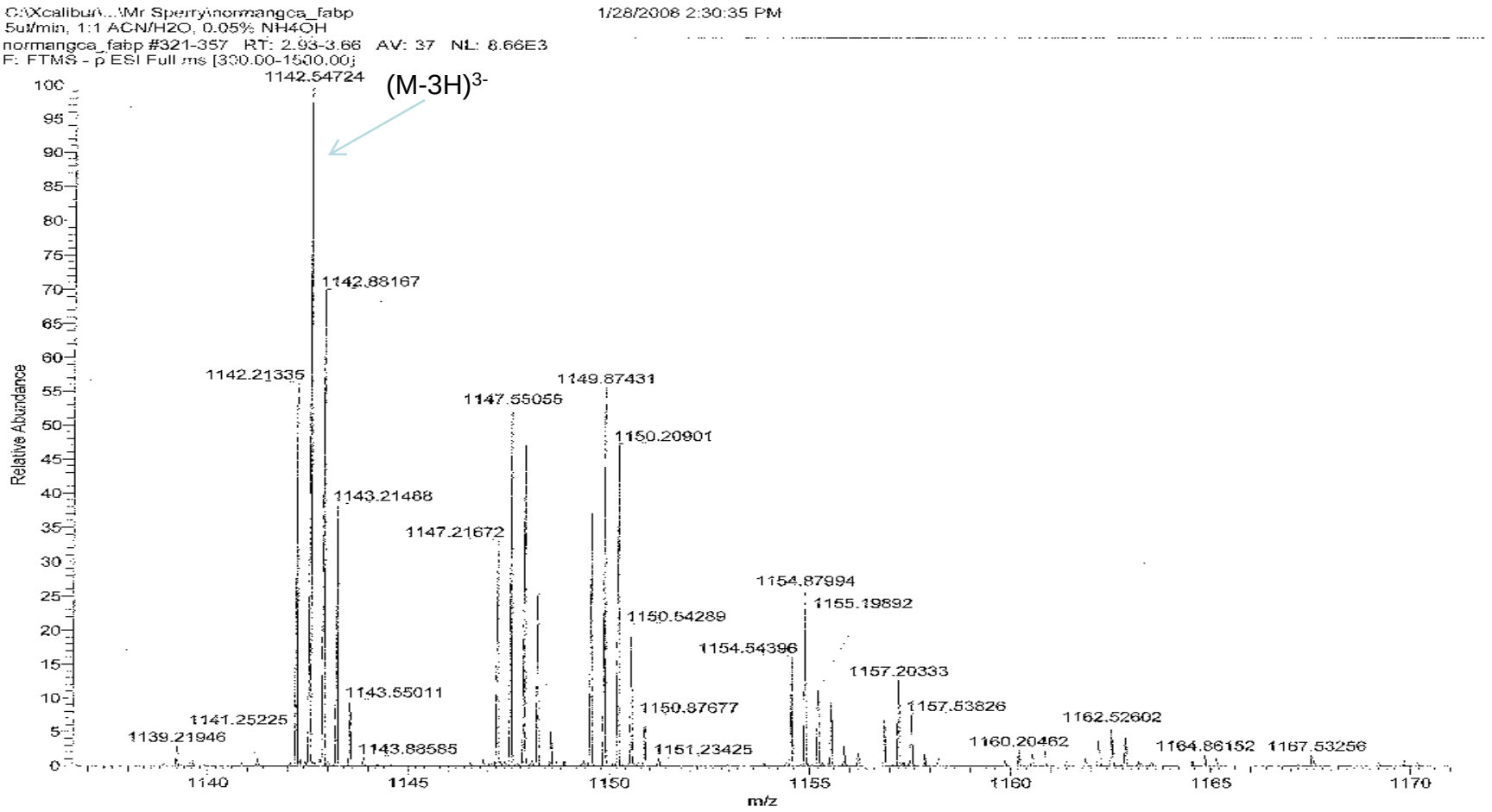

Figure S1. The results for the ESI-linear ion trap/FTMS spectrum of FABP-modified **CCATCG*CAACC.** For the $(M-3H)^{3-}$ ion the measured mass is m/z 1142.547. The theoretical mass is m/z 1142.546. This is a difference of 0.9 ppm. This corresponds to theoretical and experimental molecular masses of 3430.662 and 3430.665 daltons, respectively.

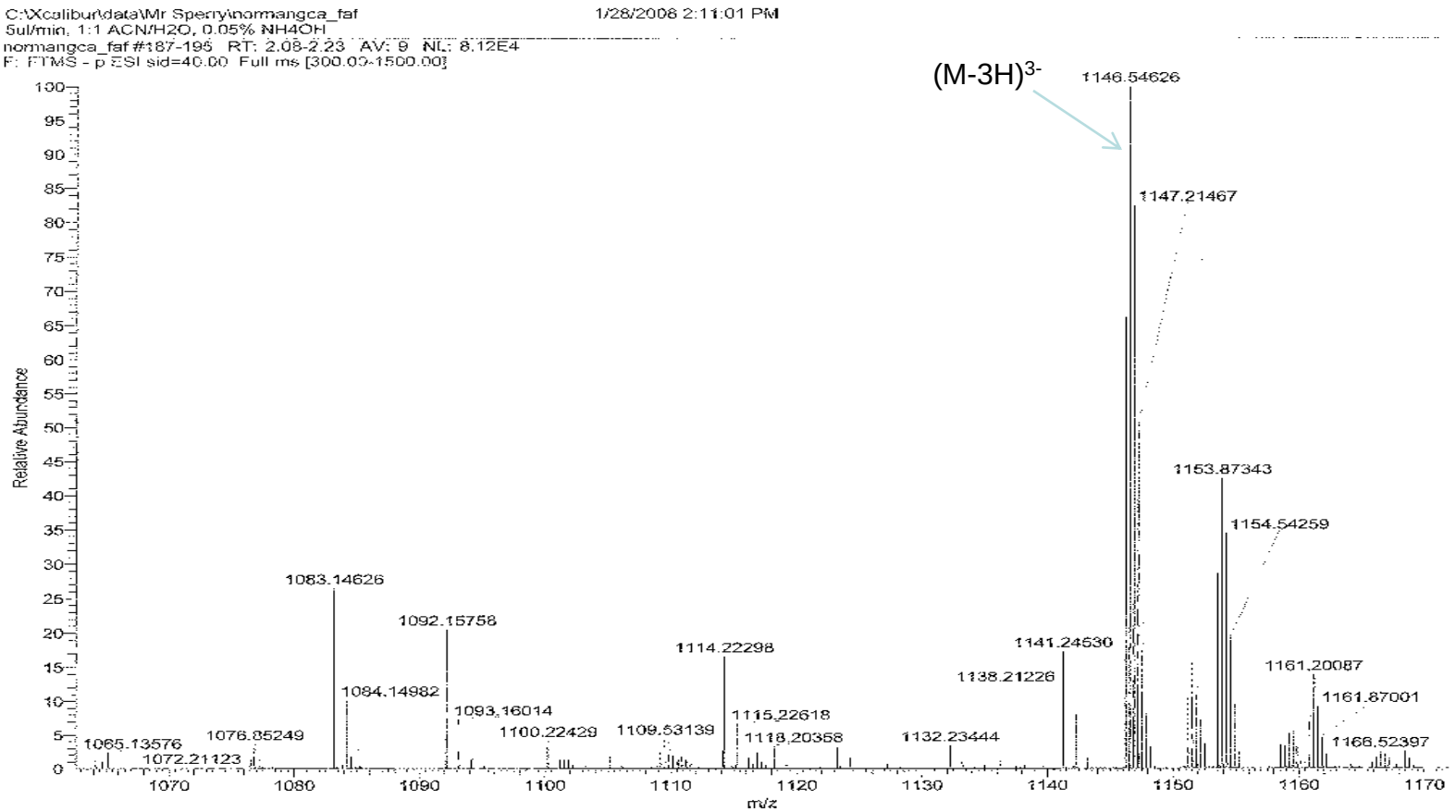

Figure S2. The results for the ESI-linear ion trap/FTMS spectrum of FAF-modified **CCATCG*CAACC.** For the $(M-3H)^{3-}$ ion the measured mass is m/z 1146.546. The theoretical mass is m/z 1146.545. This is a difference of 0.9 ppm. This corresponds to theoretical and experimental molecular masses of 3442.659 and 3442.662 daltons, respectively.

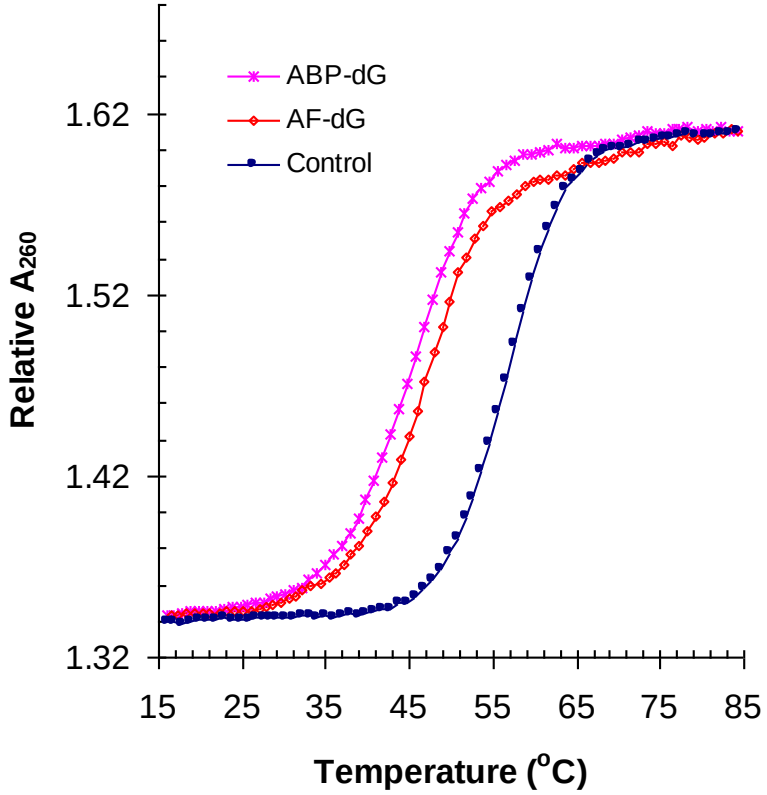

Figure S3. UV melting profiles at 260 nm for the FABP- (pink), AF-adduct (red) and the control 11-mer duplexes at 7.6 μM duplex concentration. The melting curves were normalized for clarity.

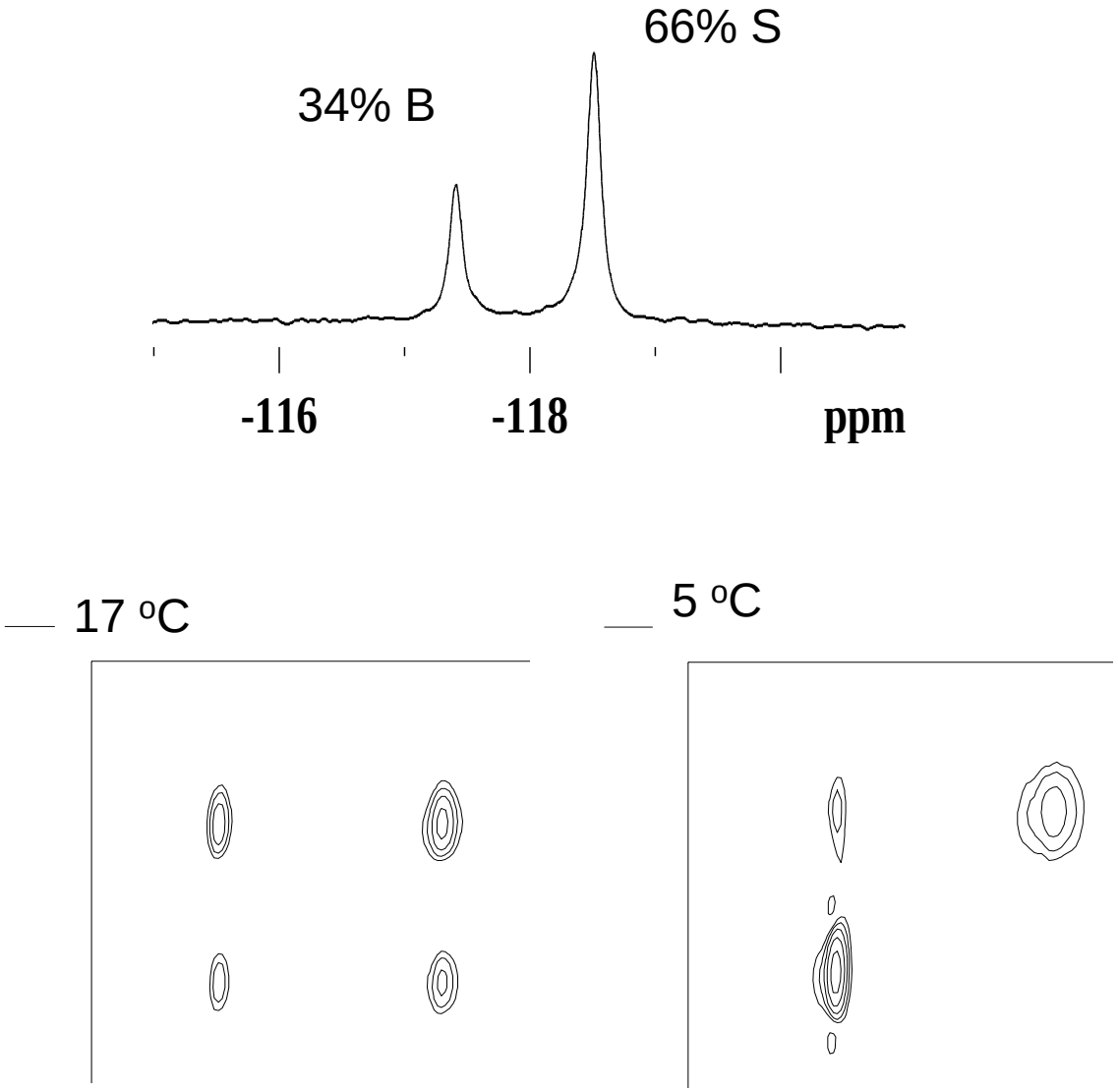

Figure S4. Temperature dependence of NOESY/EXSY ^{19}F NMR of FAF-modified DNA.

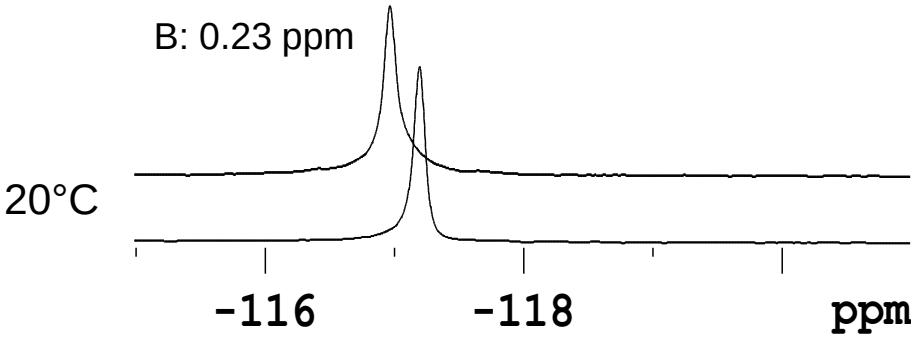

Figure S5. The ^{19}F NMR measurement of D_2O effect of FABP-modified [5'-CCATCG*CAACC-3']:[3'-GGTAGCGTTGG] at 20°C.

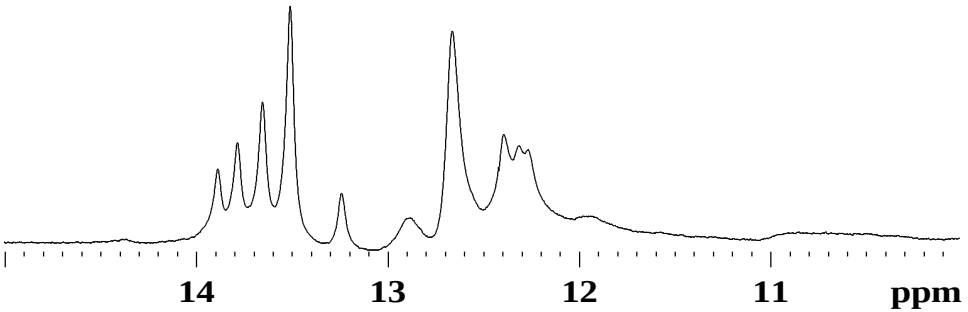

Figure S6. The imino ^{1}H NMR spectrum of FABP-modified [5'-CCATCG*CAACC-3']:[3'-GGTAGCGTTGG] at 20°C.

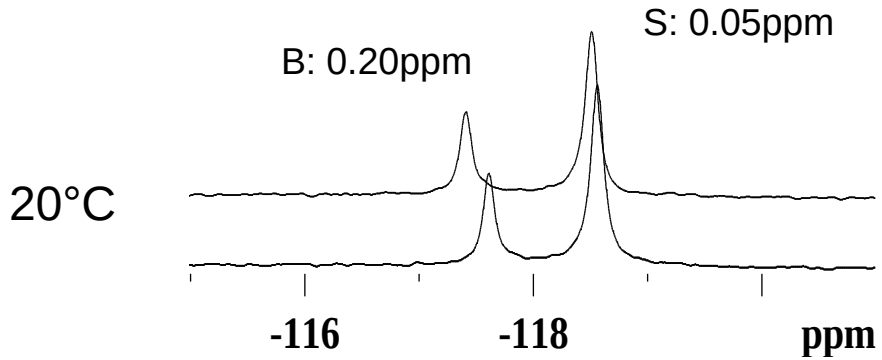

Figure S7. The ^{19}F NMR measurement of D_2O effect of FAF-modified [5'-CCATCG*CAACC-3']:[3'-GGTAGCGTTGG] duplex at 20ºC.

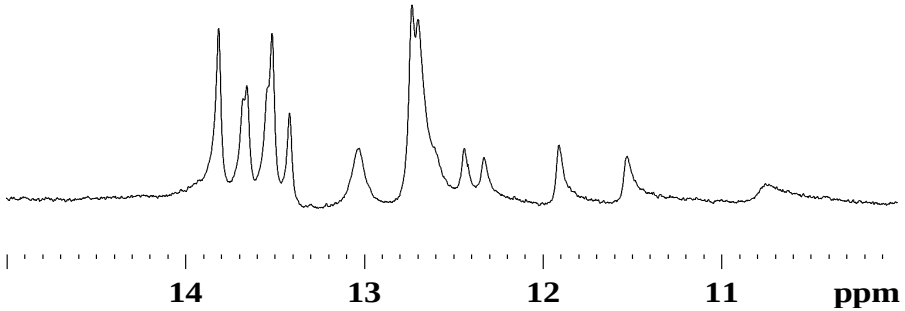

Figure S8. The imino ^1H NMR spectrum of FAF-modified [5'-CCATCG*CAACC-3']:[3'-GGTAGCGTTGG] duplex at 5ºC.

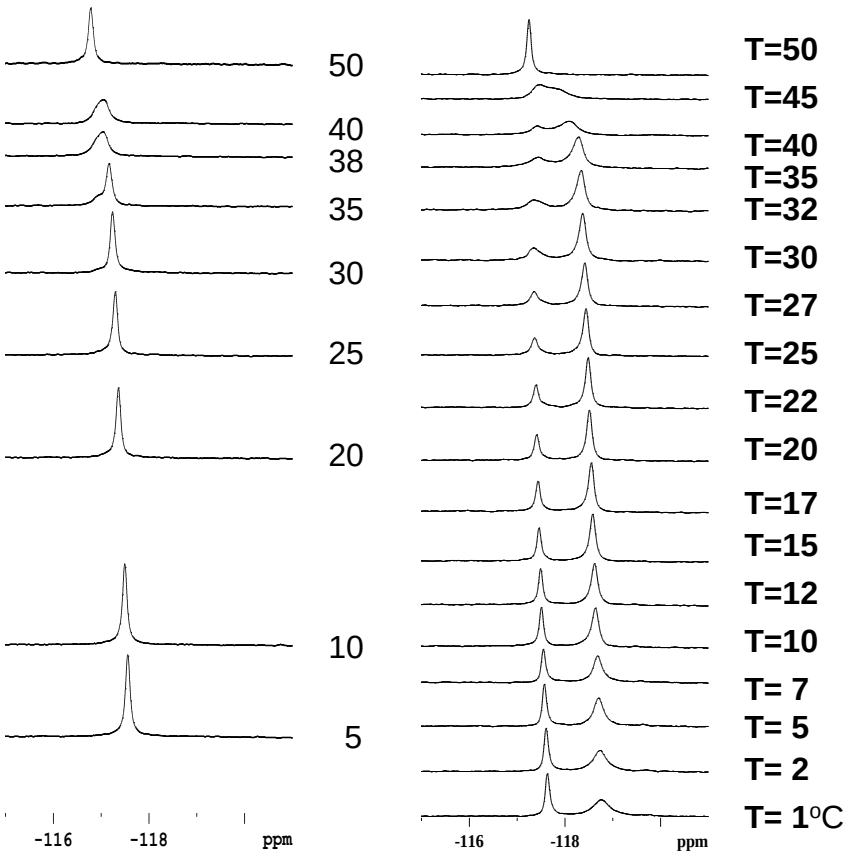

Figure S9. Dynamic ^{19}F NMR of the FABP- and FAF-modified DNA duplexes as a function of temperatures.

Supporting Table S1: UV Melting-derived thermodynamics

CCATC**G**CAACC
GGTAGCGTTGG

	Fits of individual melting curves		1/Tm vs ln Ct/4	
	ΔG^o (37C) (kcal/mol)	T_m (^{o}C)	ΔG^o (37C) (kcal/mol)	T_m (^{o}C)
Control	-12.7±0.2	60.6	-12.7±0.2	60.5
FABP	-9.4±0.1	49.7	-9.4±0.1	49.8
FAF	-9.7±0.2	51.0	-9.5±0.1	52.0