Supporting Information

2'-Fluorinated Hydantoins as Chemical Biology Tools for Base Excision Repair Glycosylases

Sheng Cao, John Patrick Rogers, Jongchan Yeo, Brittany Anderson-Steele, Jonathan Ashby, and Sheila S. David*

Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, California 95616

*To whom correspondence should be addressed:
Sheila S. David, Tel: (530) 754-4280; email, ssdavid@ucdavis.edu

Table of Contents:

(1) Table S1: Synthesized 2-F-OG and 2'-F-hydantoin oligonucleotides	**2**
(2) Biochemical Methods	**2-4**
(3) Compound Synthesis	**5-6**
(4) Compound NMR Spectra	**8-20**
(5) HPLC Purification of FGh and FSp Oligonucleotides	**1**
(6) References	**21**

(1) Supplementary Tables

Table 1. Synthesized modified 2'-F-OG, and 2'-F-hydantoin-containing oligonucleotides

Name	Sequence	MW (Da)	
		Calc.	*Observ.*
H30-2'-riboFOG (H30rFOG)	5' – TGT TCA TCA TGG GTC [**rFOG**]TC GGT ATA TCC CAT – 3'	9213.1	9213.6
H30-2'-arabinoFOG (H30aFOG)	5' – TGT TCA TCA TGG GTC [**aFOG**]TC GGT ATA TCC CAT – 3'	9213.1	9213.6
After Iridium Chloride oxidation			
H30-2'-riboFGh (H30rFGh)	5' – TGT TCA TCA TGG GTC [**rFGh**]TC GGT ATA TCC CAT – 3'	9203.1	9202.4
H30-2'-arabinoFGh (H30aFGh)	5' – TGT TCA TCA TGG GTC [**aFGh**]TC GGT ATA TCC CAT – 3'	9203.1	9202.4
H30-2'-riboFSp (H30rFSp)	5' – TGT TCA TCA TGG GTC [**rFSp**]TC GGT ATA TCC CAT – 3'	9229.1	9228.8
H30-2'-arabinoFSp (H30aFSp)	5' – TGT TCA TCA TGG GTC [**aFSp**]TC GGT ATA TCC CAT – 3'	9229.1	9228.8

(2) BIOCHEMICAL METHODS

General Materials and Instrumentation.

Fpg, Nei and NEIL1 (edited and unedited) were purified according to previously reported methods.[1-2] The activity assays and active fractions of Fpg, Nei and NEIL1 were also determined as previously described.[1-2] DNA oligonucleotides were synthesized at the core facility at the University of Utah Medical School. Mass analyses (ESI-MS) of DNA were performed at the University of Utah chemistry department or the University of California, Davis Mass Spectrometry core facility. Synthesized DNAs were purified via HPLC on a Beckman Gold Nouveau system with a Dionex DNAPac® PA-100 9 $\times$ 250 semipreparative column. Radiolabeling agent [γ-^{32}P] ATP $(3000\ \mathrm{ci/mmol})$ was purchased from GE Health Sciences and T4 polynucleotide kinase was obtained from New England BioLabs. Labeled oligonucleotides were purified using ProbeQuant G-50 spin columns from GE Health Sciences according to the manufacturer's protocol. A Milli-Q PF system was used to purify distilled deionized water that was used to make all of the buffers. All buffers were passed through a 0.45 μm filter before use. Storage phosphor autoradiography was performed

on a Typhoon 9400 phosphorimager system. Data analysis was performed using ImageQuant software (version 5.2a) and the equilibrium dissociation constants (K_d) were determined from fitting of the data with GraFit 5.0. All other chemicals used were purchased from Fisher Scientific, VWR, or Sigma and used directly without further treatment.

Synthesis of DNA Containing 2'-F-riboOG and 2'F-arabinoOG.

Oligonucleotides were synthesized from the synthetic phosphoramidites (compound synthesis detailed separately) and kept on the column with the 5'-dimethoxytrityl (DMT) group removed. The oligonucleotides were deprotected manually by incubation with concentrated ammonia containing 0.25 M 2-mercaptoethanol at 55 ^{o}C for over 17 h. The solution was evaporated to dryness by using a Savant Speed Vac®. Purification was accomplished via HPLC using a gradient (42 min) with the initial conditions of 30% buffer A (10% CH_3CN and 90% H_2O) and 70% buffer B (2.5 M NH_4OAc in CH_3CN: H_2O 9:1) and final conditions of 100% buffer B. Finally, the lyophilized oligonucleotides were desalted on a Waters Sep-Pak C18 Cartridge. The concentration of the single-stranded oligonucleotides was determined by an HP 8452A Diode Array Spectrophotometer at 260 nm, using the following molar extinction coefficients (Є) for each base: 15,000 (A), 11,700 (G), 8,800 (T), 7,800 (C), and 9,900 (OG) cm^{-1} M^{-1}. The extinction coefficients of the analogues (aFOG and rFOG) are not known and are approximated using the Є values for OG. All of the synthesized oligonucleotides are listed in Table S1.

Conversion of FOG to FGh and FSp with Na_2IrCl_6.

All FGh and FSp containing DNA strands (ribo or arabino configuration) were synthesized from the FOG containing 30 nt strands using procedures similar to those used with OG-oligonucleotides previously published.[3-4] Selective oxidation to prepare FGh or FSp was achieved at different reaction conditions. FGh was formed by treating 500 μl samples containing 12 μM of the FOG containing 30 nt sequence with 2 μl of 25 mM sodium hexachloroiridate (Na_2IrCl_6) at room temperature. Meanwhile, FSp was generated at 65 ^{o}C with the addition of 10 mM sodium phosphate buffer (pH 8) and 100 mM NaCl. Both reactions were quenched with 4 μl of 20 mM EDTA (pH 8.5) after 30 min. Oxidized products were dialyzed (3500 MWCO, Thermo Scientific) against MilliQ water for 24-48 h, followed by HPLC purification with the same column and buffer system as used previously for the purification of FOG containing oligonucleotides. Finally, the purified oligonucleotides were desalted on a Waters Sep-Pak C18 cartridge. Pure samples were stored in the freezer and were quantified by UV absorbance at 260 nm. The sequences of the synthesized oligonucleotides are listed in Table S1.

Substrate DNA Preparation.

For all experiments, 2.5 pmol of the X-containing strand was radiolabeled on the 5' end using [γ-^{32}P] ATP by T4 kinase at 37^{o}C. Excess [γ-^{32}P] ATP was removed using a Microspin G-50 spin column, according to the manufacturer's protocol. For the glycosylase/lyase activity assays, additional nonradioactive X-containing DNA was added to the labeled strand for a final concentration of 5% labeled DNA. This was then annealed to the

complementary strand (added at 20% excess) by heating at 90 °C for 5 min and cooling overnight in annealing buffer [20 mM Tris-HCl (pH 7.6), 10 mM EDTA, and 150 mM NaCl]. In the binding affinity assay and the borohydride trapping assay, only the labeled duplex was used with an estimated upper limit of duplex concentration based on 100% recovery from the labeling procedure.

Glycosylase/AP Lyase Assays.

Single-turnover experiments, where the enzyme concentration is greater than the DNA concentration ([Enz] > [DNA]), were performed using ^{32}P-labeled 30-bp duplexes containing fluorinated analogues opposite C to evaluate the glycosylase/AP lyase activity of the enzymes. The total reaction volume was 60 μL with a final duplex DNA concentration of 20 nM. The duplex was incubated with 100−300 nM active enzyme in an assay buffer [20 mM Tris-HCl (pH 7.6), 10 mM EDTA, and 0.1 mg/mL BSA] at 37 °C with 150 mM NaCl. Two sets of separate 5 μl aliquots were taken from the reaction mixture at different time points (5 and 60 min). One set of aliquots were immediately quenched by freezing with dry ice for the measurement of lyase activity. In order to measure the glycosylase activity of each enzyme, the second set of aliquots were quenched by the addition of 5 μL of 0.5 M NaOH, heated to 90 °C for 5 min, and then placed on dry ice. 5 μL of formamide denaturing dye (80% formamide, 0.025% xylene cyanol, and 0.025% bromophenol blue in TBE buffer) was added to all aliquots, followed by heating at 90 °C for 5 min again. The samples were run on a 15% denaturing polyacrylamide gel (19:1 acrylamide:bis-acrylamide ratio) in 1× TBE at 1600 V for 2 h. The separation of the 15-bp DNA fragment arising from the product and the 30-nt fragment originating from the substrate was visualized using autoradiography by exposure to an Amersham storage phosphor screen overnight. As a control, parallel reactions of enzyme with the substrates (OG:C containing duplex) were quenched with NaOH or with formamide dye alone. A correction was made for the amount of the strand scission mediated with or without the NaOH in the absence of enzyme. The extent of control cleavage (at 60 min without enzyme) was subtracted during quantitation of the storage phosphor autoradiograms. Quantitation was performed using ImageQuant software.

Equilibrium Dissociation Constant (K_d) Measurements.

Electrophoretic mobility shift assays (EMSA) were performed to determine the K_d values. Reaction volumes contained 10 pM duplex DNA, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM DTT, 10% glycerol, 0.1 mg/mL BSA, and enzyme concentrations ranging from 655 nM to 20 pM. The concentration of NaCl in reaction volumes was maintained at 150 mM. Enzyme solution was freshly prepared by diluting aliquots of enzyme at 4 °C with dilution buffer containing 20 mM Tris-HCl pH 7.5, 10 mM EDTA and 20% glycerol. Samples of the protein/DNA mixture were incubated at 25 °C for 30 min followed by the addition of 5 μL of nondenaturing loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol in 1× TBE). Bound versus unbound DNA was resolved using electrophoresis on a 6% nondenaturing polyacrylamide gel (29:1 acrylamide:bis-acrylamide ratio) at 4 °C in 0.5× TBE buffer at 120 V for 2 h. Gels were dried and exposed to a storage phosphor

screen overnight. K_d values were determined by fitting the data (percent bound substrate vs enzyme concentration) using a one-site binding isotherm.

Sodium borohydride trapping assay.
Reaction volumes contained 10 nM duplex DNA, 300 nM active enzyme, 25 mM sodium phosphate (pH 6.8), 1 mM EDTA, and 0.1 mg/mL BSA. The sample was supplemented with $NaBH_4$ at a final concentration of 90mM and NaCl to reach a final sodium concentration of 180mM. The final reaction volume was 10 uL. The reaction was allowed to proceed for 60 minutes at 37°C. After reaction with $NaBH_4$, the sample was mixed with an equal volume of SDS-page loading buffer (125 mM Tris-HCl [pH 8.0], 5% SDS, 25% glycerol, 0.025% bromophenol blue), and heated to 90°C for 10 minutes. After heating, the sample was loaded onto a 15% polyacrylamide SDS gel for resolution of free DNA from covalent DNA-enzyme complexes. The gels were exposed to a storage phosphor screen overnight for at least 12 hours before image scanning and quantification.

(3) COMPOUND SYNTHESIS

General Procedures: Glassware for all reactions was oven-dried at 120°C and cooled in a desiccator, or flame-dried and cooled under vacuum prior to use. Moisture sensitive solvents were transferred by syringe and all reactions were purged with nitrogen. Thin-layer chromatography was performed on Merck Kieselgel 60 F_{254} aluminum plates and visualized by a handheld lamp at 254 nm. Column chromatography was performed with Merck 60Å (230-400) silica gel. All ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were obtained with 300 MHz and 400 MHz spectrometer. The following abbreviations were used to designate the multiplicity of individual signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiple, dd = double of doublets, brs = broad singlet, dt = double of triplets; all coupling constant (J) are in hertz. High-resolution ESI-MS were recorded at University of California, Davis mass spectrometry facility on an Orbitrap mass spectrometer. Elemental analyses were performed at the Atlantic Microlab, Inc.

8-bromo-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (4). To a suspension of 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (**2**) (0.5 g, 1.75 mmol) in 10 ml water, saturated bromine-water was added in aliquots of *ca.* 0.5 ml at a rate that allowed for disappearance of the yellow color indicative of the reaction between each addition. The reaction was completed when the yellow color persisted. The reaction mixture was then filtered off, successively washed with 20 ml cold water and 20 ml cold acetone, and dried to provide **3** as white solid (0.6 g, 94% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 10.82 (brs, 1H), 6.56 (brs, 2H), 5.93 (dd, 1H, J = 2.9, 22), 5.78 (ddd, 1H, J = 5.2, 53.9), 5.54 (d, 1H, J = 6.7), 4.84 (t, 1H, J = 5.9), 4.49-4.61 (m, 1H), 3.84-3.88 (m, 1H), 3.47-3.71 (m, 2H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm): 156.1, 154.5, 152.6, 121.1, 117.9, 93.7 (d, J_{CF} =185), 88.5 (d, J_{CF} =36), 84.9, 69.5 (d, J_{CF} =16), 62.2. ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -200.7 (dt, J =19, 53). HRMS ($C_{10}H_{11}BrFN_5O_4$): calcd for $[M + H]^+$: 364.0056. obsd: 364.0036.

8-oxo-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (6). Compound **4** (0.6 g, 1.65 mmol) was dissolved in a mixture of Ac_2O-AcOH (each 10.5 ml) containing anhydrous NaOAc (2.48 g, 30.23 mmol). The mixture was heated under reflux for 5 h. The solvent was evaporated and the residue was evaporated with EtOH (15 ml × 3) to remove traces of Ac_2O. The residue was partitioned between $CHCl_3$ (85 ml) and water (85 ml). The organic layer was washed with sat. $NaHCO_3$ (30 ml × 2), water (30 ml × 2), dried over $MgSO_4$, and evaporated. The residue was dissolved in 7N NH_3/MeOH (20 ml), kept at room temperature for 20 h, and the solution was concentrated and purified by flash column chromatography using 15-100% MeOH in DCM. Evaporation of the fraction gave 0.31 g of **6** as a cream powder in 62% yield over two steps. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 10.76 (brs, 1H), 6.51 (brs, 2H), 5.78 (dd, 1H, *J* = 2.3, 23.4), 5.58 (ddd, 1H, *J* = 2.3, 4.6, 54.5), 5.45 (d, 1H, *J* = 6.7), 4.75 (t, 1H, *J* = 5.9), 4.38-4.48 (m, 1H), 3.77 (m, 1H), 3.40-3.66 (m, 2H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm): 154.2, 152.0, 151.9, 147.5, 99.4, 94.2 (d, J_{CF} =183), 84.3 (d, J_{CF} =35), 84.0, 69.8 (d, J_{CF} =16), 62.4. ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -200.5 (dt, *J* =19, 54). IR (neat, cm^{-1}): 1722, 1670, 1623, 1432, 1365, 1106, 1039. HRMS ($C_{10}H_{12}FN_5O_5$): calcd for $[M + H]^+$: 302.0900. obsd: 302.0889. Anal. Calcd. for $C_{10}H_{12}FN_5O \cdot 1.1H_2O$: C, 37.41; H, 4.46; N, 21.81. Found: C, 37.40; H, 4.38; N, 21.22.

N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-di-methoxytrityl)-β-D-ribofuranosyl]guanine (8). Compound **6** (0.25 g, 0.81 mmol) was suspended in DMF (10ml). To the stirred mixture, N, N-dimethylformamide dimethylacetal (0.4 ml, 3.7 mmol) was added and stirring was continued for 4 hours at room temperature. The solvent was evaporated to give a crude solid that was co-evaporated with pyridine and then dissolved in pyridine (10 ml) directly; 4, 4'-Dimethoxytrityl chloride (0.49 g, 1.4 mmol) was added and the reaction mixture was stirred at room temperature for 14 hours. After quenching with MeOH (1 ml), the mixture was partitioned between $CHCl_3$ and water. The organic layer was washed with water (2 × 30 ml), dried over Na_2SO_4, and concentrated to a small volume. The residue was purified by flash column chromatography with 0-6% MeOH in $CHCl_3$ containing 1% Et_3N. The fractions were combined and concentrated to afford 0.4 g of **8** as a white powder in 75% yield over two steps. R_f (SiO_2): 0.18 (19:1, $CHCl_3$/MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.63 (brs, 1H), 10.99 (brs, 1H), 8.44 (brs, 1H), 6.74-7.31(m, 13H), 5.90 (d, 1H, *J* = 25.2), 5.59 (dd, 1H, *J* = 4.2, 54.1), 5.53 (d, 1H, *J* = 6.6), 4.73 (m, 1H), 3.91 (m, 1H), 3.68 (s, 6H), 2.98-3.07 (m, 8H). ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -197.7 (dt, *J* = 24, 55). HRMS ($C_{34}H_{35}FN_6O_7$): calcd $[M + H]^+$: 659.2629, obsd: 659.2613.

[N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-di-methoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-ribo-furanosyl]guanine (10). Compound **8** (0.25 g, 0.38 mmol) was dissolved in anhydrous CH_2Cl_2 (5 ml), followed by the addition of diisopropylethylamine (145 μl, 0.83 mmol) and 2-cyanoethyl N, N-diisopropyl chlorophosphoramidite (103 μl, 0.45 mmol). After the reaction mixture was stirred at room temperature under argon for 3h, the mixture was

concentrated and the residue was purified by flash column chromatography with 0-2% MeOH in $CHCl_3$ containing 1% Et_3N. The fractions were collected and evaporated to give 0.20 g of **10** as white foam in 72% yield. R_f (SiO_2): 0.35 (19:1, $CHCl_3$/MeOH); ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -196.2 (m). ^{31}P NMR (300 MHz, DMSO-d_6) δ (ppm): 152.2 (d, J_{PF} = 5.9), 150.8 (d, J_{PF} = 10.4). HRMS ($C_{43}H_{52}FN_8O_8P$): calcd $[M + H]^+$: 859.3708, obsd: 859.3710.

8-bromo-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (3). The same methodology for the synthesis of **4** was utilized. Starting with compound **1** (0.73 g, 1.75 mmol) provided 0.87 g of **3** as white solid in 94% yield. ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 10.82 (brs, 1H), 6.53 (brs, 2H), 6.23 (dd, 1H, *J* = 6.4, 10.0), 5.13 (dt, 1H, *J* = 54.5), 4.63 (d, 1H, *J* = 22.5), 3.68-3.78 (m, 3H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm): 156.1, 154.2, 153.0, 120.3, 117.6, 95.8 (d, J_{CF} = 195), 82.7 (d, J_{CF} = 9), 82.5, 74.0 (d, J_{CF} = 21), 62.2. ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -197.5 (ddd, *J* = 8, 20, 53). HRMS ($C_{10}H_{11}BrFN_5O_4$): calcd for $[M + H]^+$: 364.0056. obsd: 364.0061.

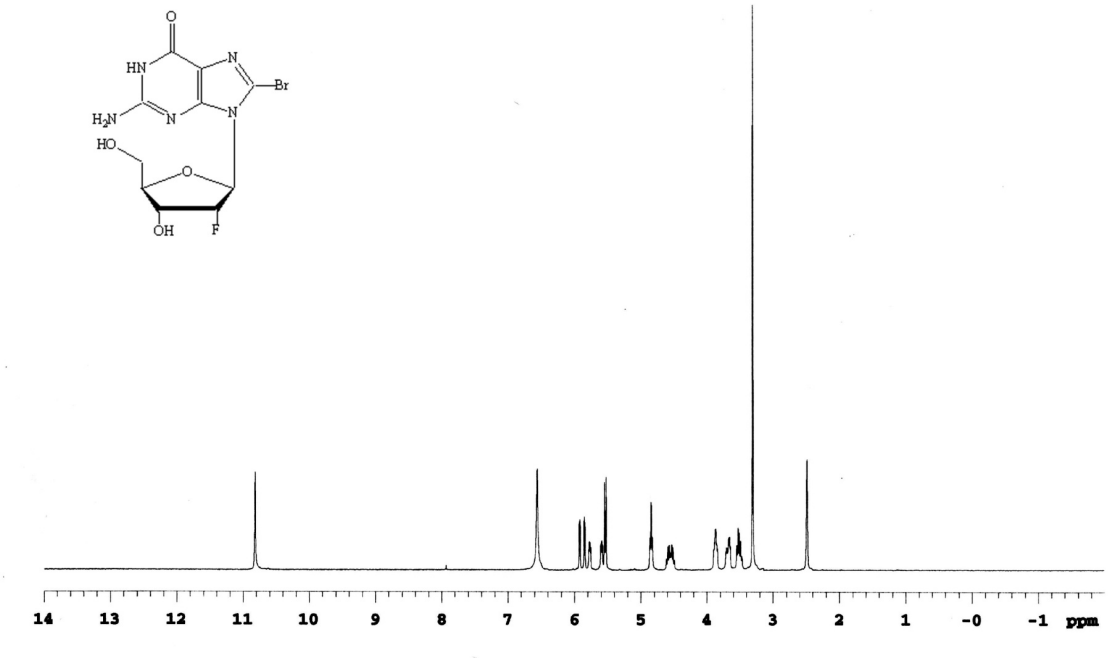
8-oxo-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (5). The same methodology for the synthesis of **6** was utilized. Thus, compound **3** (0.6 g, 1.65 mmol) was reacted to provide 0.36 g of **5** as cream powder in 72% yield over two steps. ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 10.73 (brs, 2H), 6.41 (brs, 2H), 6.01 (dd, 1H, *J* = 4.1, 7.0), 5.65 (d, 1H, *J* = 5.3), 5.14 (dt, 1H, *J* = 54.8), 4.75 (m, 2H), 3.65 (m, 3H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm): 153.8, 152.2, 151.9, 147.8, 99.3, 95.5 (d, J_{CF} = 195), 81.9 (d, J_{CF} = 11), 78.0 7 (d, J_{CF} = 18), 73.3 (d, J_{CF} = 20), 62.5. ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -200.1 (dd, *J* = 22, 55). HRMS ($C_{10}H_{12}FN_5O_5$): calcd for $[M + H]^+$: 302.0900. obsd: 302.0885.

N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-di-methoxytrityl)-β-D-arabinofuranosyl]guanine (7). The same methodology for the synthesis of **8** was utilized. Starting with compound **5** (0.25 g, 0.81 mmol) provided 0.4 g of **7** as white powder in 75% yield over two steps. R_f (SiO_2): 0.18 (19:1, $CHCl_3$/MeOH); ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.63 (brs, 1H), 10.99 (brs, 1H), 8.44 (brs, 1H), 7.14-7.31(m, 13H), 5.87 (d, 1H, *J* = 25.2), 5.53 (dd, 1H, *J* = 4.2, 54.1), 5.51 (d, 1H, *J* = 6.6), 4.73 (m, 1H), 3.91 (m, 1H), 3.68 (s, 6H), 4.49-4.61 (m, 1H), 3.87 (m, 1H), 3.47-3.71 (m, 2H). ^{13}C NMR (101 MHz, Methylene Chloride-*d*2) δ 158.02, 157.98, 157.74, 157.67, 156.97, 154.69, 151.63, 151.03, 146.65, 144.53, 144.28, 135.28, 135.19, 134.81, 134.66, 129.70, 129.64, 129.30, 129.12, 112.66 – 111.97 (m), 102.23, 86.50, 85.10, 63.54, 39.72, 33.87, 28.93. ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -202.6 (dd, *J* = 16, 53). HRMS ($C_{34}H_{35}FN_6O_7$): calcd for $[M + H]^+$: 659.2629. obsd: 659.2636.

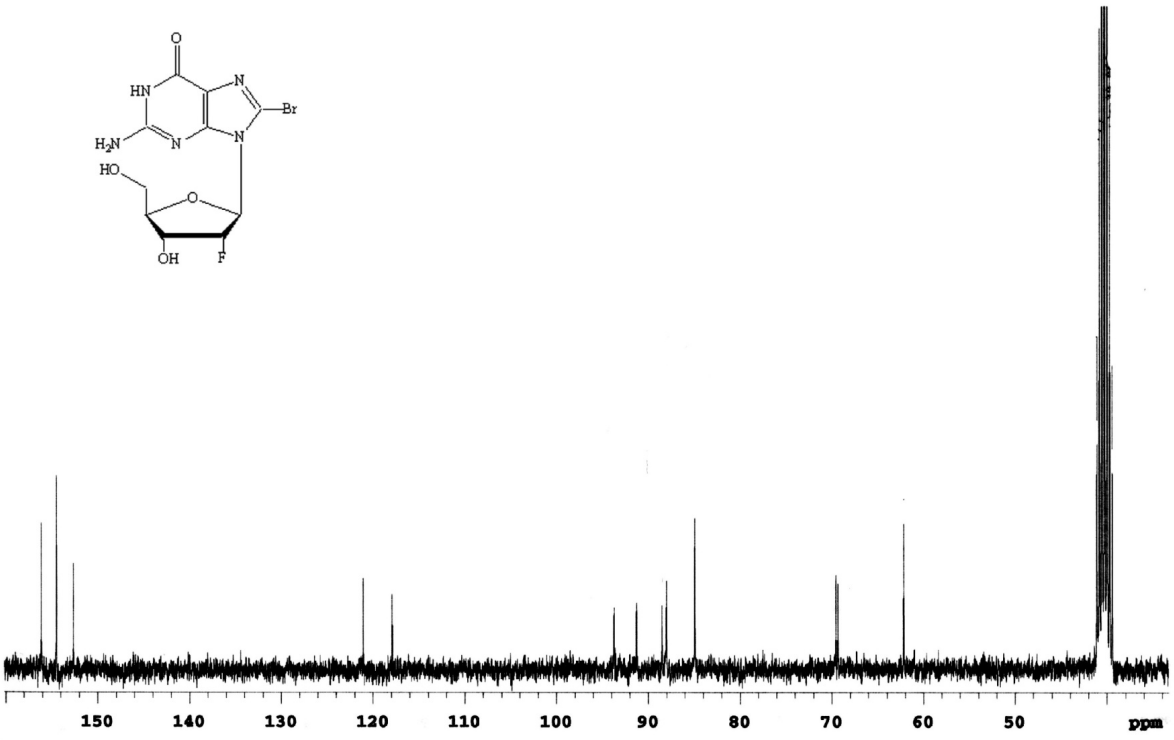
N^2-(N,N-dimethylaminomethylene)-8-oxo-9-{2-deoxy-2-fluoro-5-O-(4,4'-di-methoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-arabino-furanosyl}guanine (9). The same methodology as for the synthesis of **10** was utilized. Starting with compound **7** (0.25 g, 0.38 mmol) provided 0.26 g of **9** as white powder in 80% yield. R_f (SiO_2): 0.27 (19:1, $CHCl_3$/MeOH); ^{19}F NMR (300 MHz, DMSO-d_6) δ (ppm): -201.3 (dd, J_{PF} = 16, 53).

^{31}P NMR (300 MHz, DMSO-d_6) δ (ppm): 151.3 (d, J_{PF} = 30). HRMS ($C_{43}H_{52}FN_8O_8P$): calcd for $[M + H]^+$: 859.3708. obsd: 859.3707.

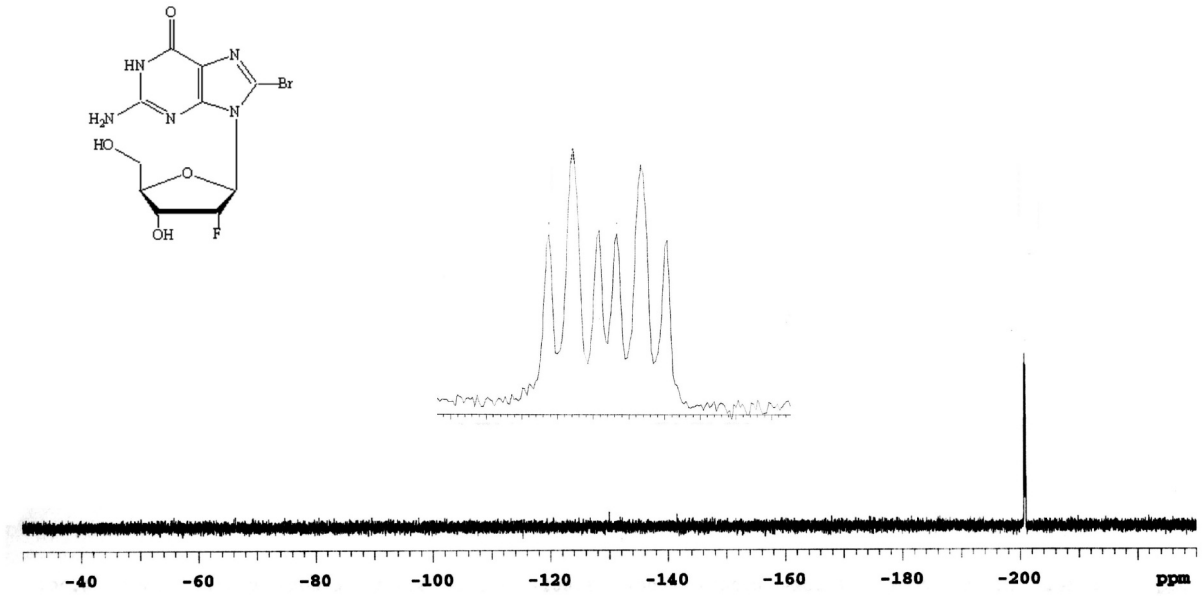
(4) Compound NMR Spectra



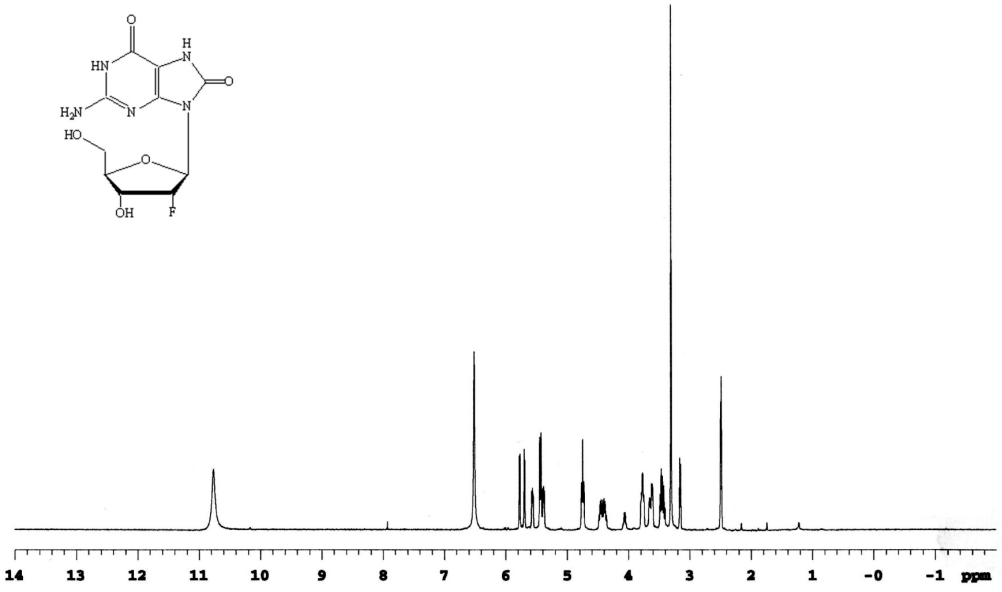
S1. ^{1}H NMR of 8-bromo-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (4)



S2. ^{13}C NMR of 8-bromo-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (**4**)



S3. ^{19}F NMR of 8-bromo-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (**4**)



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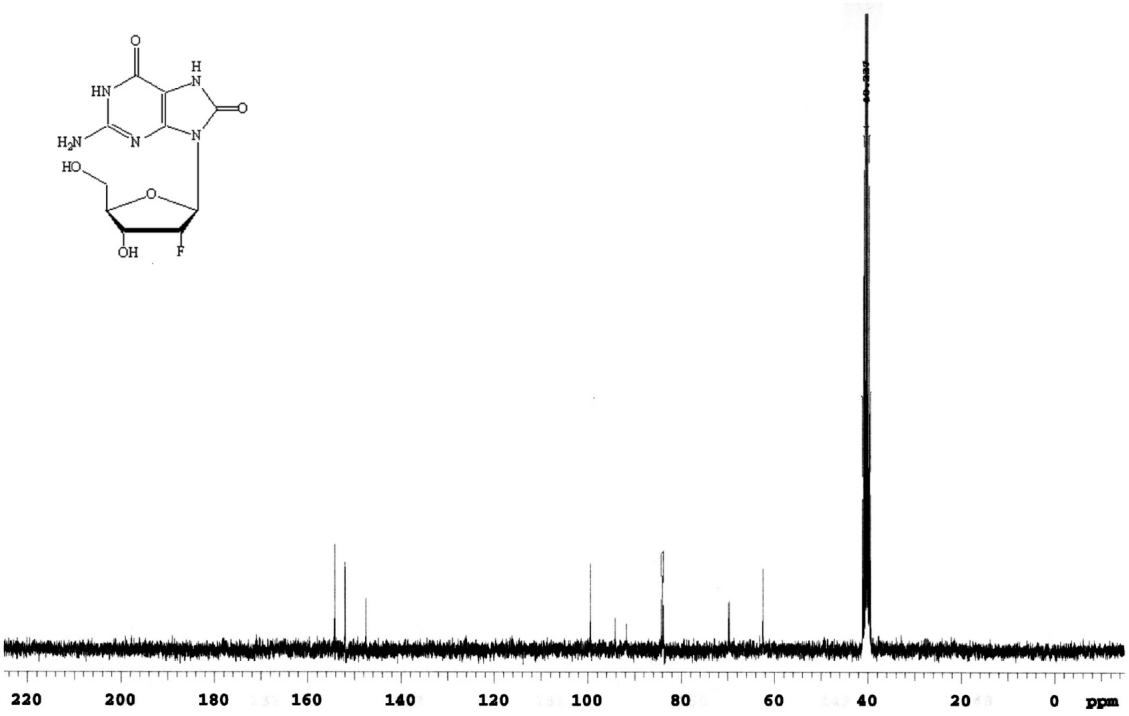
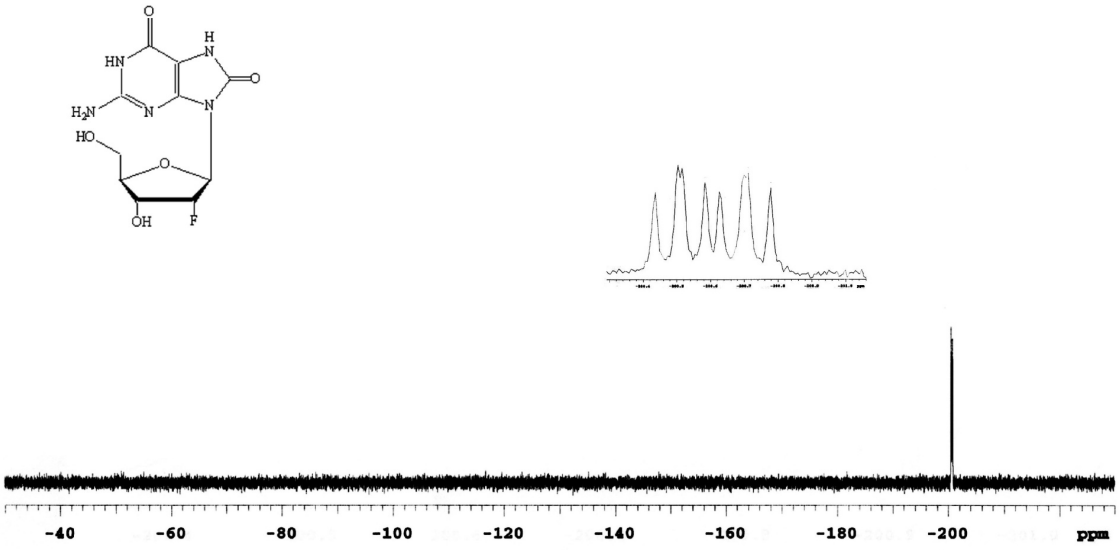
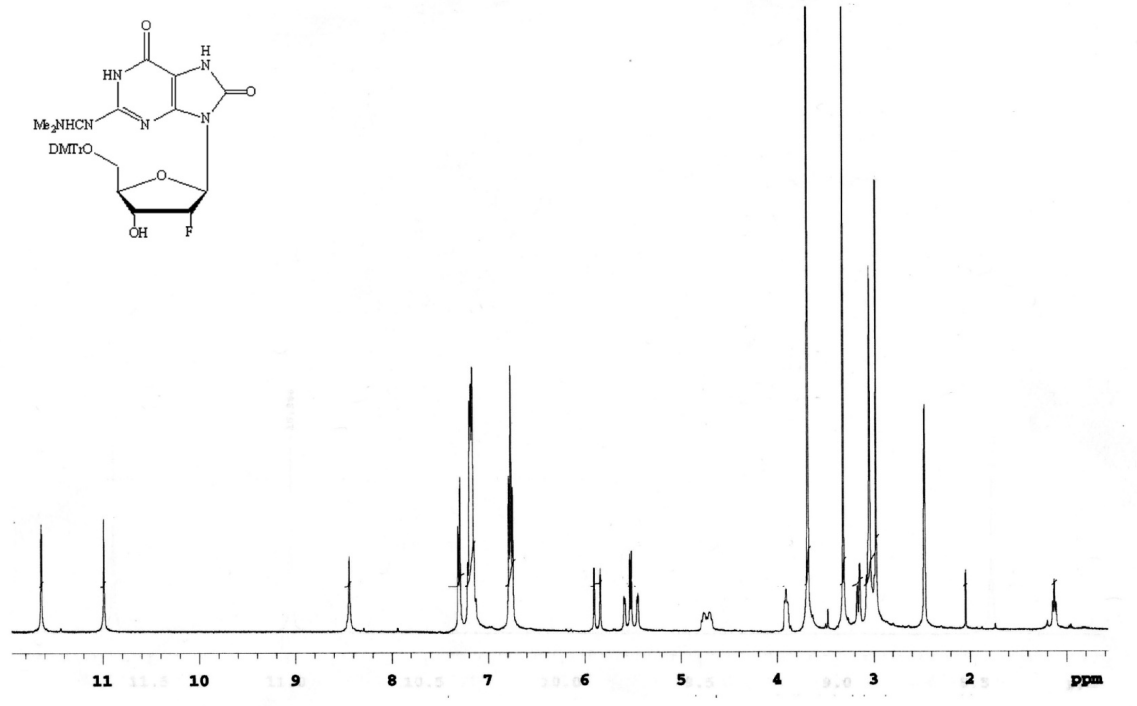


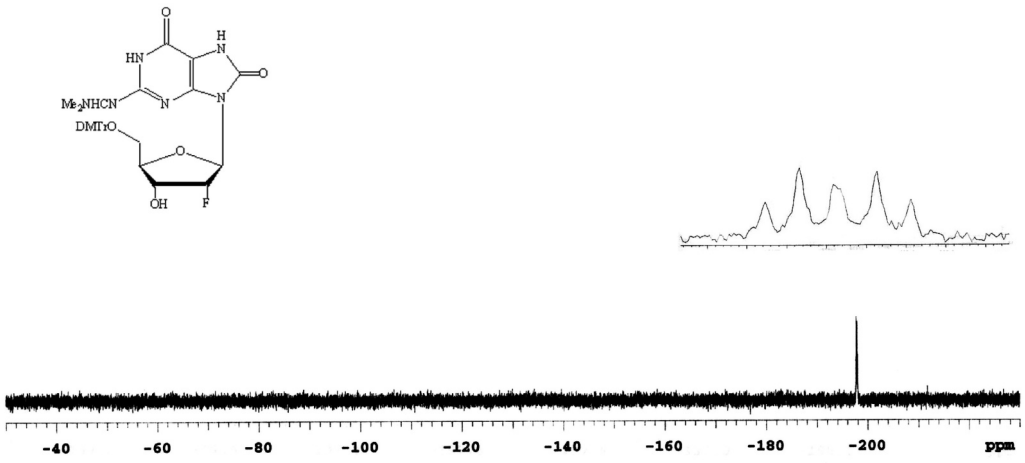
S5. ^{13}C NMR of 8-oxo-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (**6**)



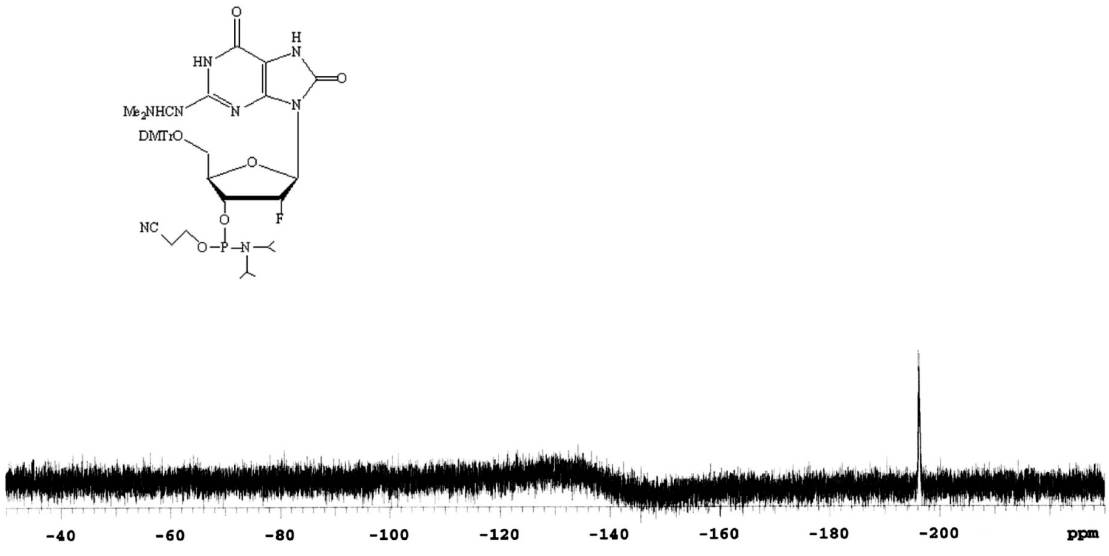
S6. ^{19}F NMR of 8-oxo-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)guanine (**6**)



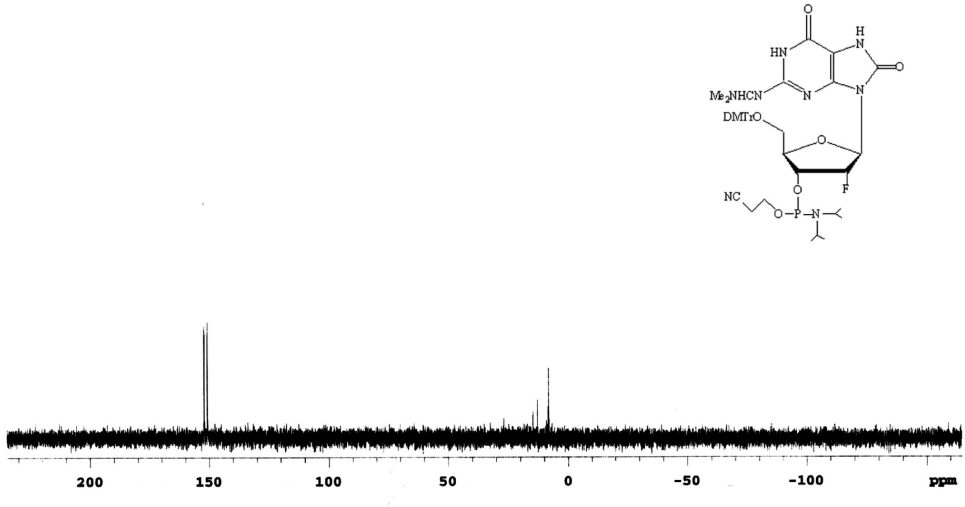
S7. ^{1}H NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]guanine (**8**)



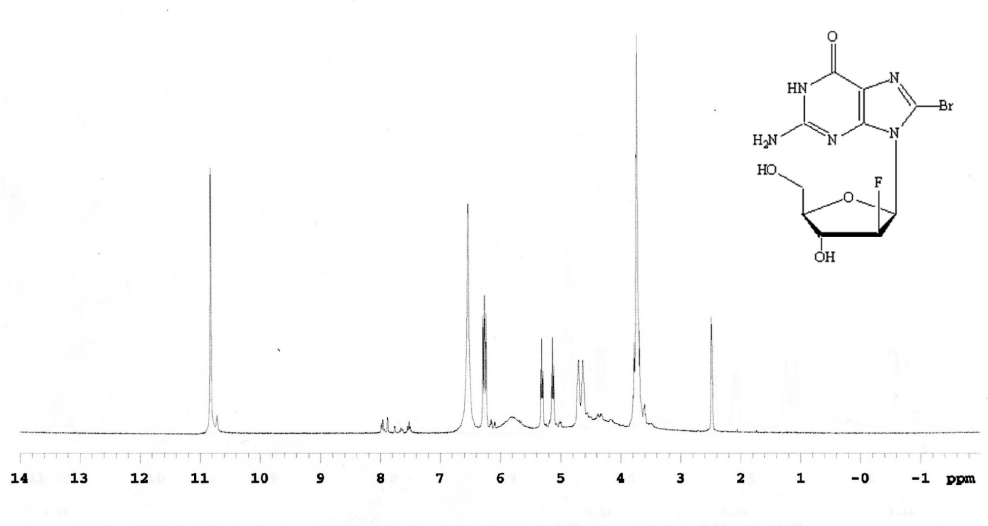
S8. ^{19}F NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]guanine (**8**)



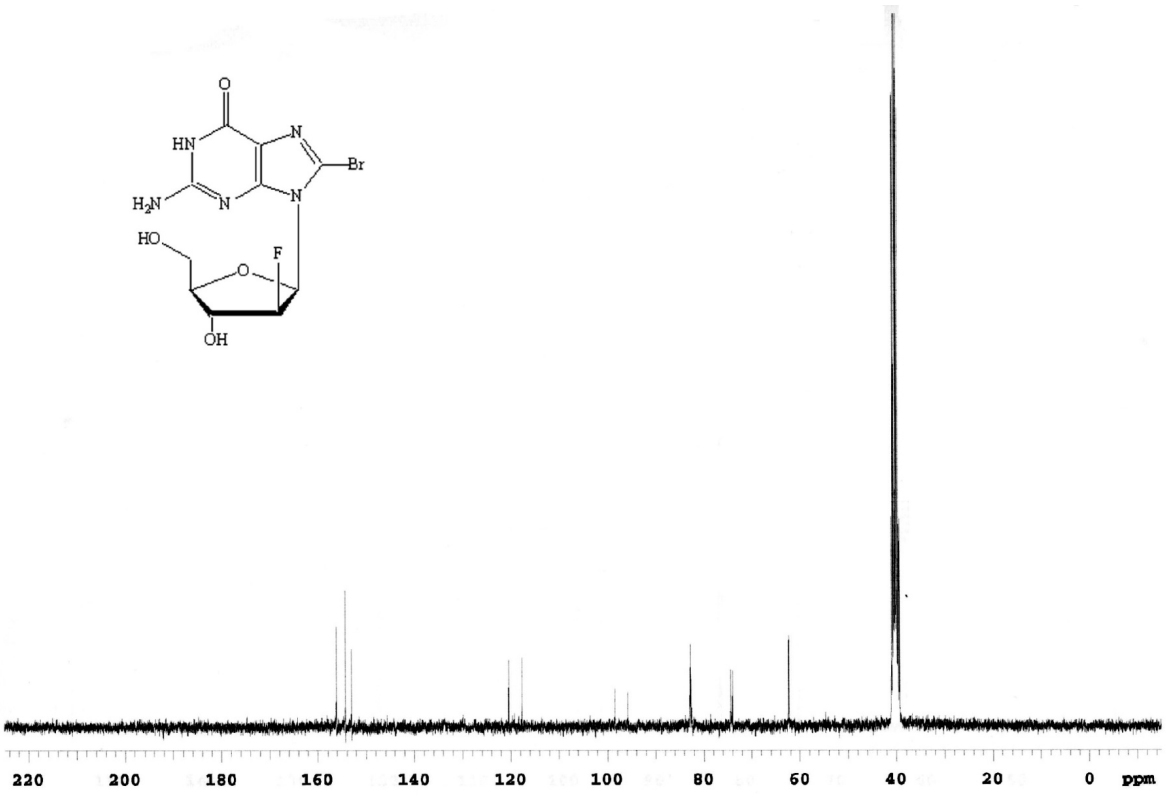
S9. ^{19}F NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-ribo-furanosyl]guanine (**10)**



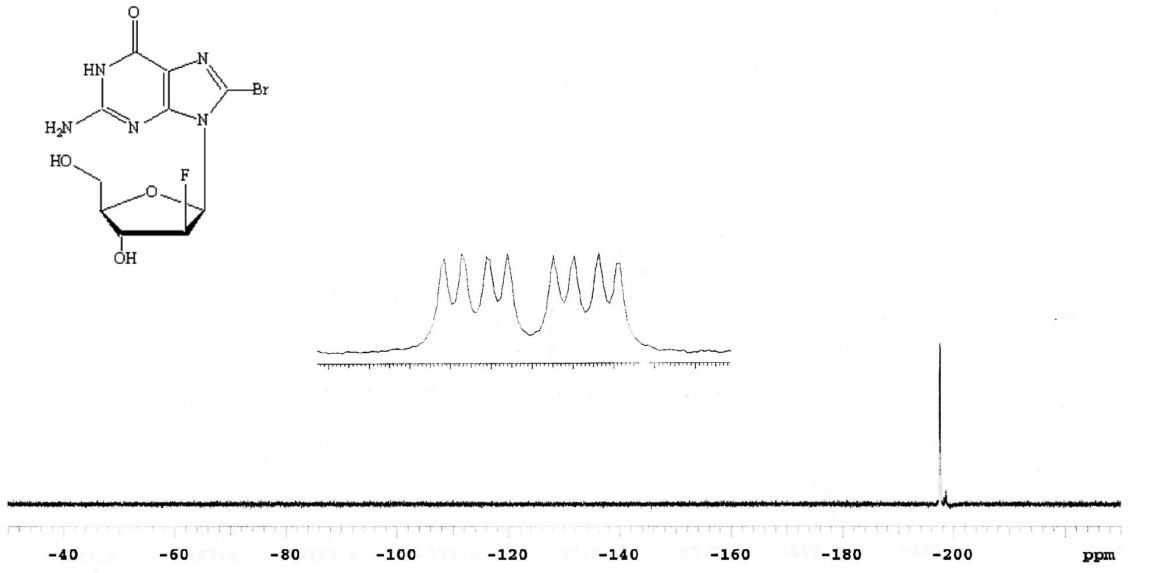
S10. ^{31}P NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-ribofuranosyl]guanine (**10**)



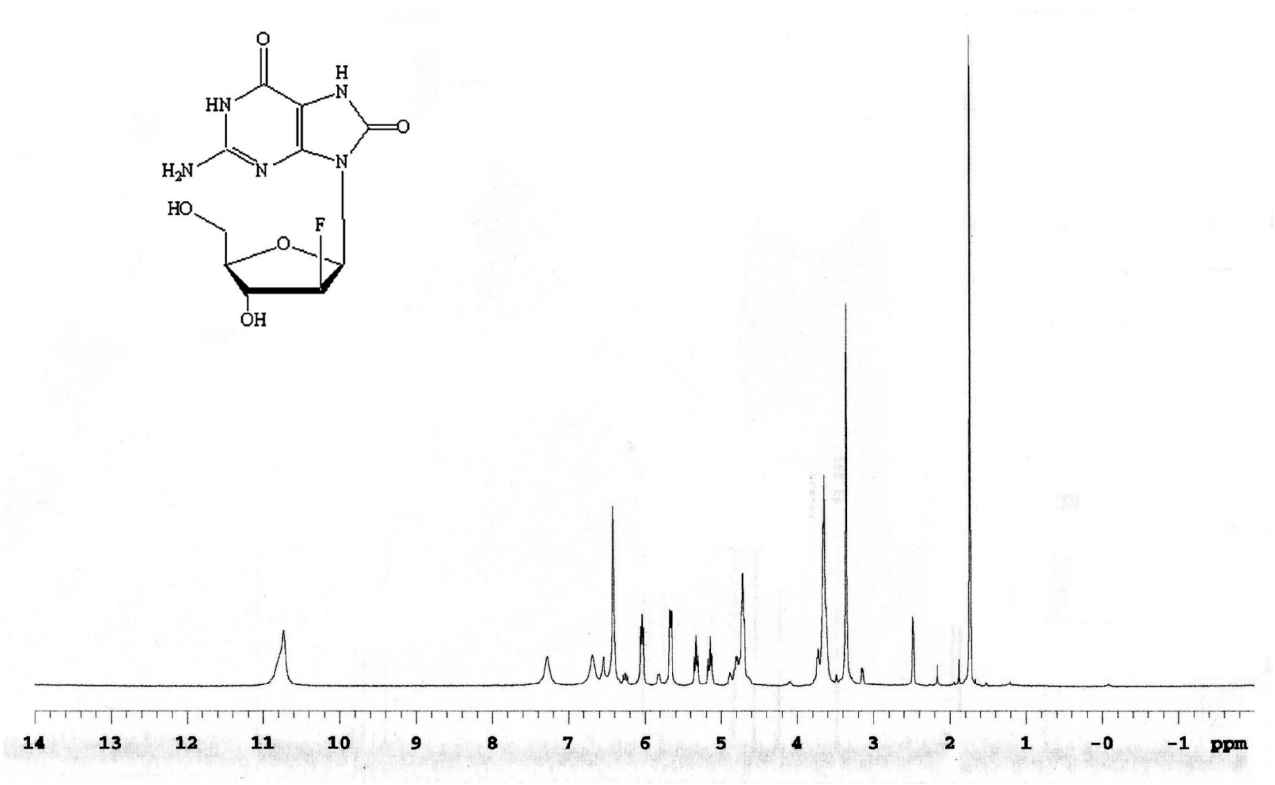
S11. ^{1}H NMR of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-8-bromo-guanine (**3**)



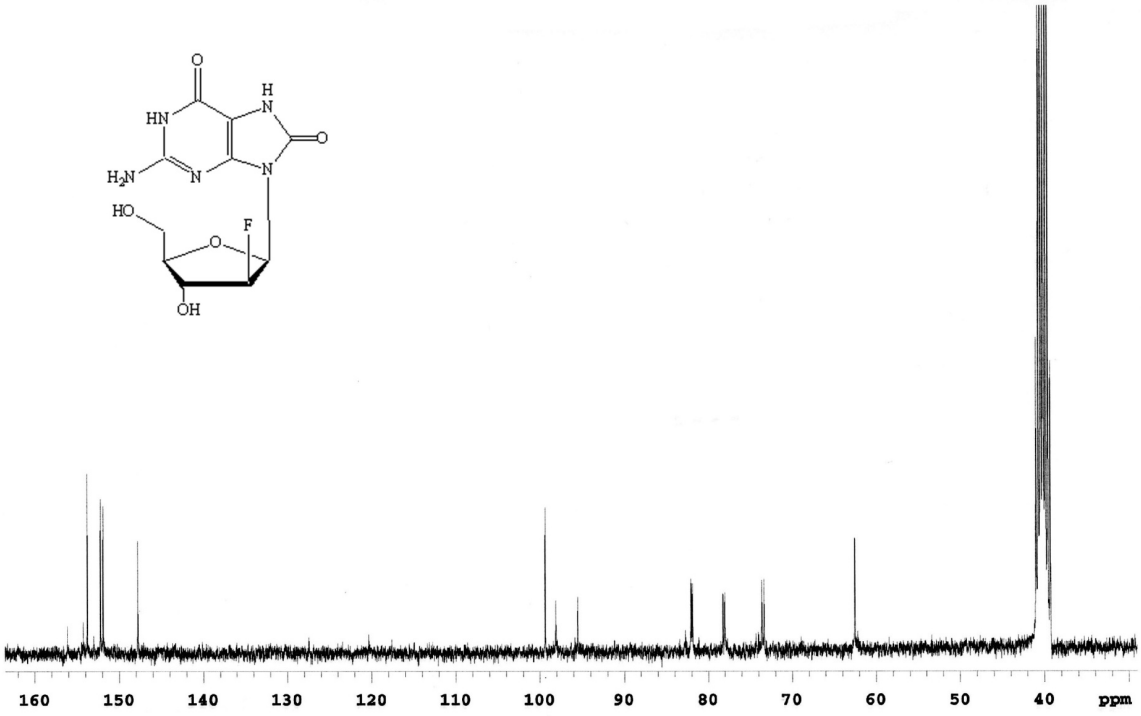
S12. ^{13}C NMR of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-8-bromo-guanine (**3**)



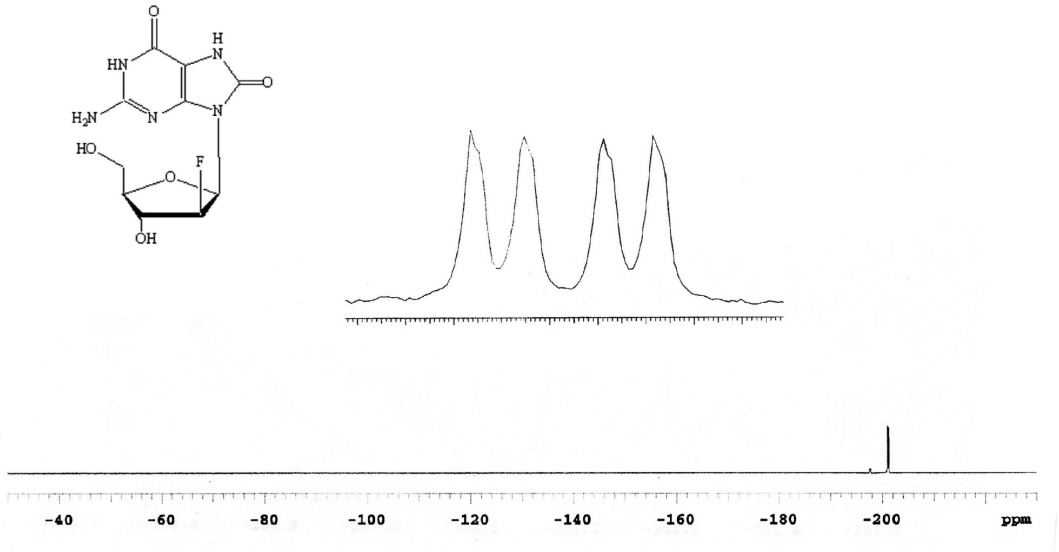
S13. ^{19}F NMR of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-8-bromo-guanine (**3**)



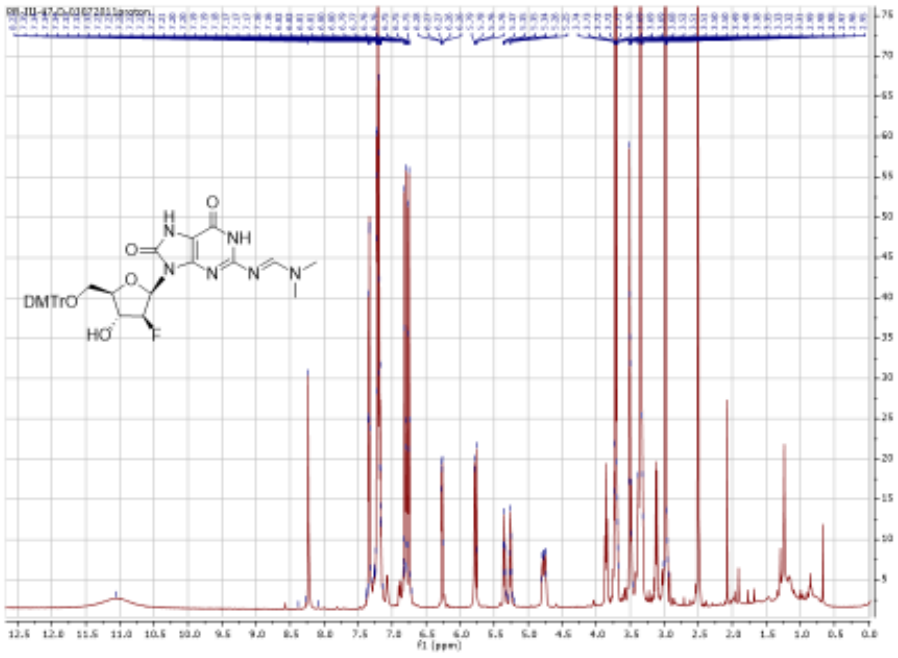
S14. ^{1}H NMR of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-8-oxo-guanine (**5**)



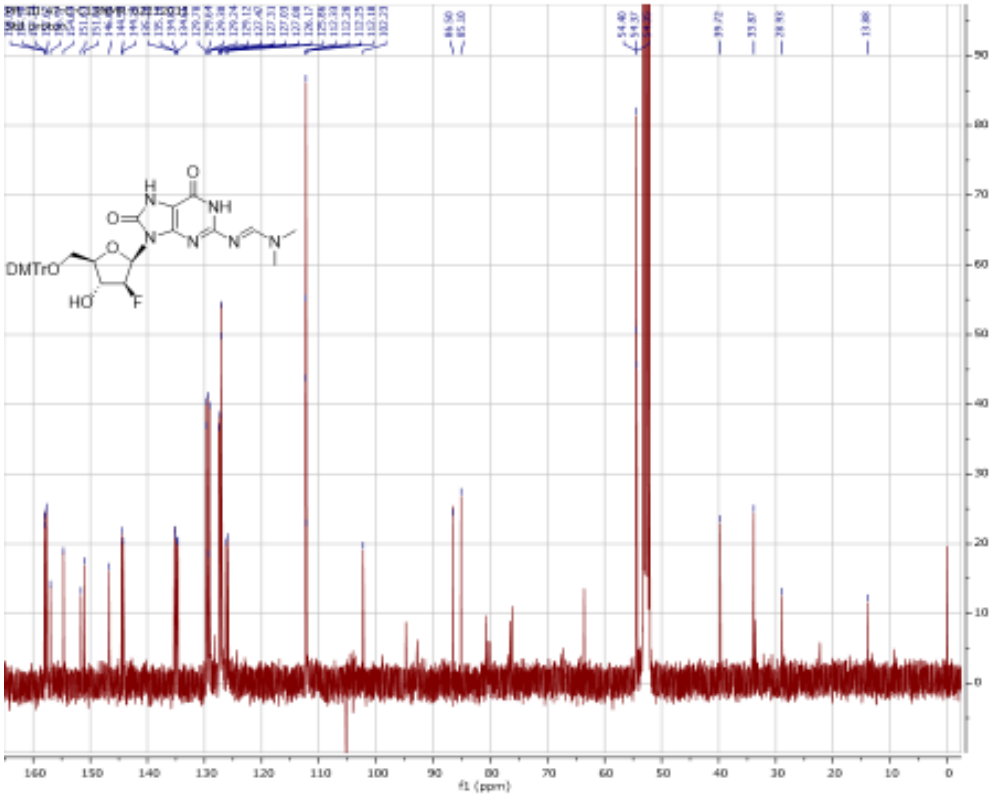
S15. ^{13}C NMR of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-8-oxo-guanine (**5**)



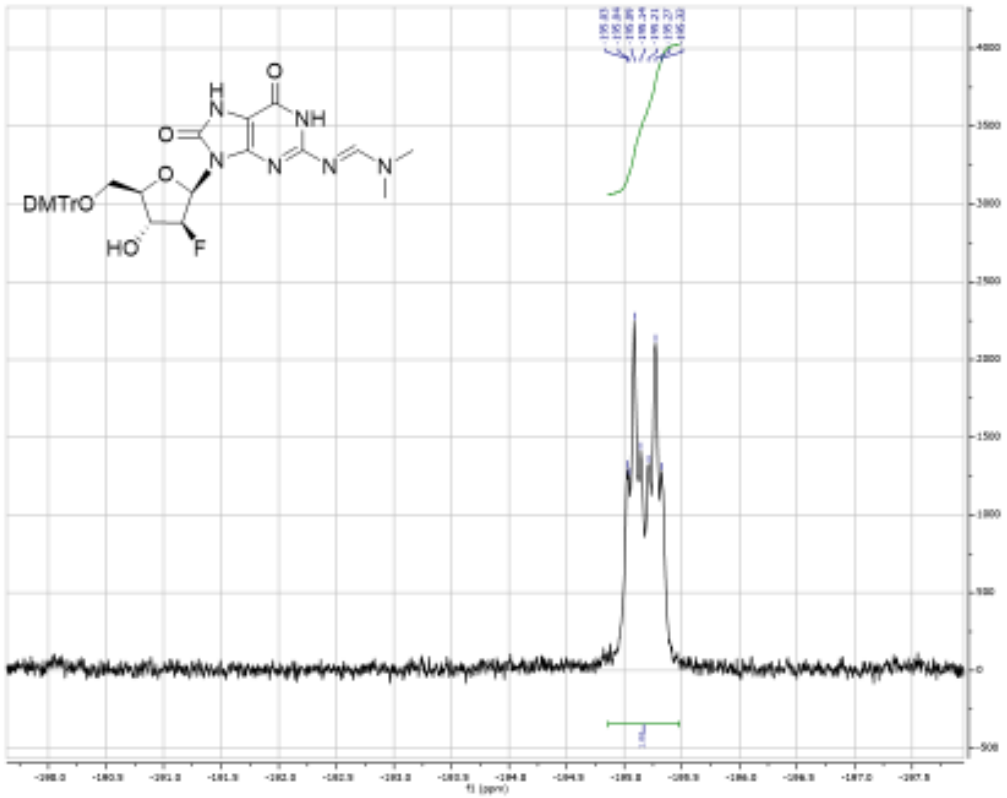
S16. ^{19}F NMR of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-8-oxo-guanine (**5**)



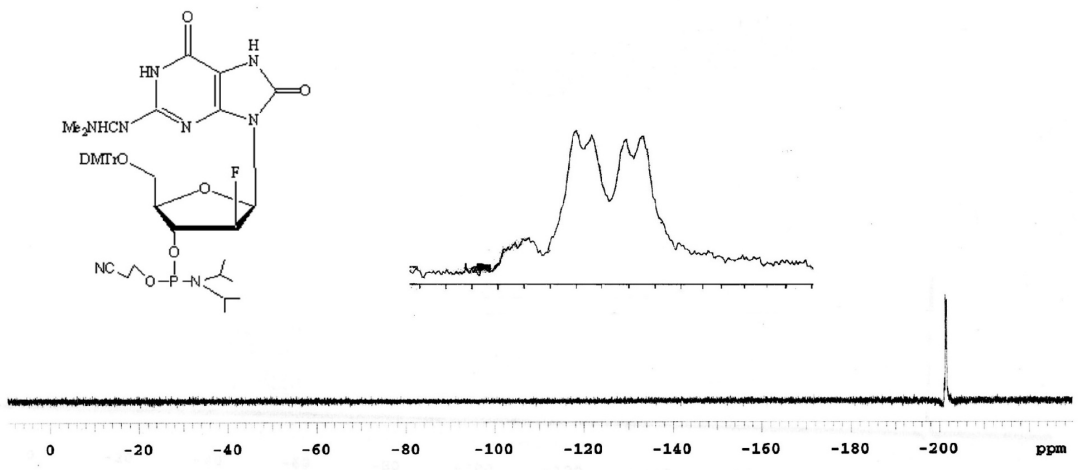
S17. ^{1}H NMR of N^{2}-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-arabino-furanosyl]guanine (**7**)



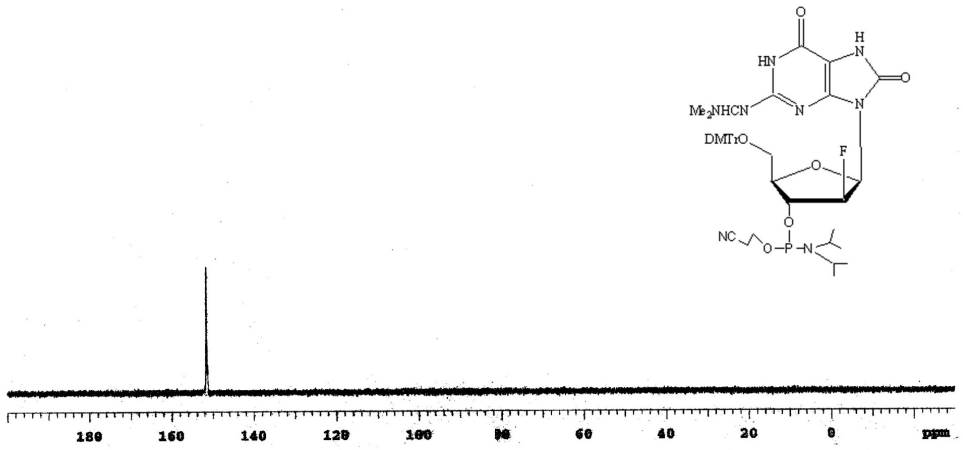
S18. ^{13}C NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-arabinofuranosyl]guanine (**7**)



S19. ^{13}C NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-arabinofuranosyl]guanine (**9)**

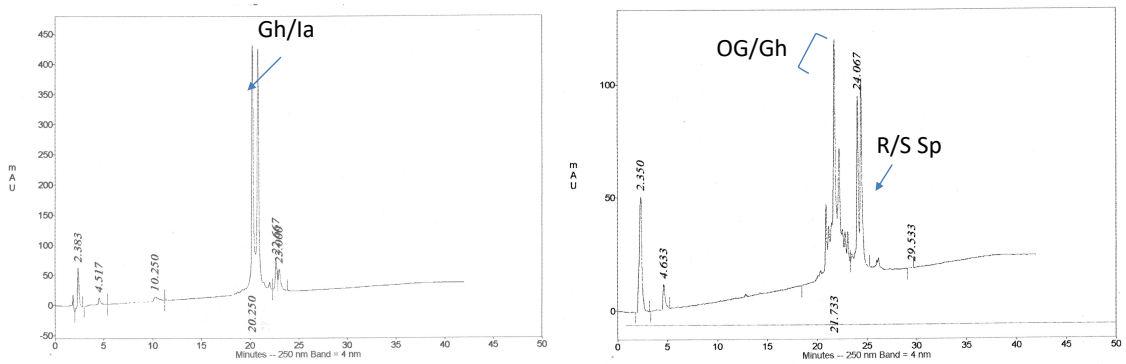


S20. ^{19}F NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-arabino-furanosyl]guanine (**9**)



S21. ^{31}P NMR of N^2-(N,N-dimethylaminomethylene)-8-oxo-9-[2-deoxy-2-fluoro-5-O-(4,4'-dimethoxytrityl)-3-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]-β-D-arabi-nofuranosyl}guanine (**9**)

(5) **HPLC PURIFICATION OF FGH AND FSP OLIGONUCLEOTIDES**



S22: HPLC Profiles of FGh and FSp oligonucleotides (H30)
*Using Dionex DNApac PA-100 9 × 250 mm column (as described by Zhao, X[4]).

(6) REFERENCES

1. Krishnamurthy, N.; Zhao, X.; Burrows, C. J.; David, S. S., Superior removal of hydantoin lesions relative to other oxidized bases by the human DNA glycosylase hNEIL1. *Biochemistry* **2008,** *47* (27), 7137-46.
2. Leipold, M. D.; Muller, J. G.; Burrows, C. J.; David, S. S., Removal of hydantoin products of 8-oxoguanine oxidation by the Escherichia coli DNA repair enzyme, FPG. *Biochemistry* **2000,** *39* (48), 14984-92.
3. Luo, W.; Muller, J. G.; Rachlin, E. M.; Burrows, C. J., Characterization of hydantoin products from one-electron oxidation of 8-oxo-7,8-dihydroguanosine in a nucleoside model. *Chemical research in toxicology* **2001,** *14* (7), 927-38.
4. Zhao, X.; Muller, J. G.; Halasyam, M.; David, S. S.; Burrows, C. J., In vitro ligation of oligodeoxynucleotides containing C8-oxidized purine lesions using bacteriophage T4 DNA ligase. *Biochemistry* **2007,** *46* (12), 3734-44.