Spirodi(iminohydantoin) products from oxidation of 2'-deoxyguanosine in the presence of NH_4Cl in nucleoside and oligodeoxynucleotide contexts

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Supporting Information

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Experimental Methods

HPLC Analysis. Analysis by HPLC was conducted by passing the sample down a C18 reversed-phase HPLC column (250 X 4.6 mm, 5 μm) running the following solvents: A = 10 mM NH_4OAc (pH 7.0) in ddH_2O, B = CH_3CN, running at 1 mL/min, while monitoring the absorbance at 240 nm. The run was initiated at 1% B then after 3 min B increased to 10% over 10 min following a linear gradient, after which 10% B was held isocratic for 4 min. Next, B was increased to 65% over 10 min along a linear gradient then held at 65% for 10 min followed by termination of the run. The void volume from this HPLC run was collected and lyophilized to dryness.

Analysis of the void volume from the previous run was achieved by passing the collected sample down a Hypercarb HPLC column (150 X 4.6 mm, 5 μm, Thermo Scientific) that was running the following solvent systems: A = 0.1% acetic acid in ddH_2O, and B = methanol, while running at a 1 mL/min flow rate, and monitoring absorbance at 240 nm. The run started at 0% B and after 10 min increased to 90% B following a linear gradient over 30 min.

Identification of the eluting peaks was initially determined by LC-ESI^+-MS for both HPLC runs (see data below). As a second method for product identification, the peaks were individually purified and submitted for ESI^+-MS/MS. For the MS/MS experiments the nucleoside samples were analyzed by positive ion electrospray ionization (ESI) on a Micromass Quattro II tandem mass spectrometer equipped with Zspray API source. Samples were dissolved in CH_3CN and 0.1% formic acid ddH_2O (1:1) and introduced via infusion at a flow rate of 7 μL/min. The source and desolvation temperatures were 80°C and 120°C, respectively. The capillary voltage was set to 3.25 KV, sampling cone voltage to 45 V, and the extractor cone to 3 V. The collision energy was set to 18 eV. Argon, used as a gas collision for CID experiments, was adjusted to a pressure of 1.7 X 10^{-4} mBar. The mass for the nucleoside base was set in the first scanning analyzer (MS-1) and the precursor ion was subjected to CID in the static quadrupole and the resulting spectrum of the products recorded by scanning the second scanning analyzer (MS-2) between 50 and 600 Da. The scan duration and interscan delay were 3.0 and 0.1 seconds, respectively. The instrument was operated and data accumulated with Micromass Masslynx software (version 4.0).

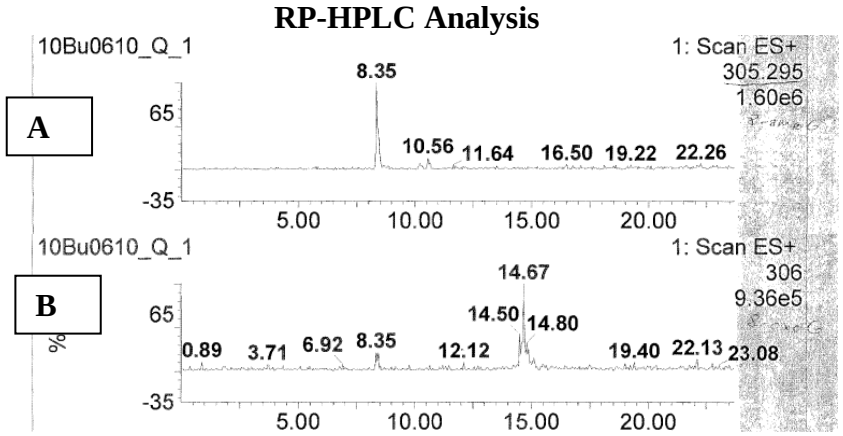
Quantification of the peaks was achieved by integrating the areas under the peaks. Next, the peaks were normalized so they could be directly compared via each compounds unique extinction coefficient at 240 nm (wavelength at which the HPLC runs were monitored). The ε_{240nm} ($L*mol^{-1}*cm^{-1}$) values used for each compound were as follows, (*R*)- and (*S*)-dSp (3275), dGh (2412), and dZ (1778). The extinction coefficients for the diastereomers of 5- and 8-dSi were determined after incubation of samples in formic acid to hydrolyze the imino groups leading to the known dSp nucleoside; the ε_{240nm} ($L*mol^{-1}*cm^{-1}$) values were found to be 3800 and 3500 for 5- and 8-dSi, respectively.

Nuclease Digestion of ODN Samples. The ODN samples were digested with a suite of nucleases to liberate the oxidized nucleotides as follows: (1) The antioxidant butylated hydroxytoluene (2.0 mM) along with the deaminase inhibitors pentostatin (100 μM) and tetrahydrouridine (100 μM) were added before commencement of the digestion. Note: Ammonium salts were used throughout the digestion process to minimize deamination of the

ammonia adducts. The ODN samples were lyophilized to dryness and then resuspended in DNase I reaction buffer (20.0 mM Tris (pH 8.4), 2.0 mM $MgCl_2$, 50.0 mM NH_4Cl) followed by addition of DNase I (2.0 U), and incubated at 37 ^oC for 3 h. (2) 10-μL of a NH_4OAc buffer solution (100.0 mM, pH 5.3) containing zinc acetate (10.0 mM) was added to the digestion solution, followed by nuclease P1 (2.0 U). The reaction was incubated at 45 ^oC for 9 h, followed by addition of more nuclease P1 (2.0 U) and incubation for another 9 h. (3) 11-μL of Tris buffer (100.0 mM, pH 7.8) with $MgCl_2$ (10.0 mM) and snake venom phosphodiesterase (2.0 U) was added to the digestion mixture. The reaction was incubated at 45 ^oC for 9 h after which snake venom phosphodiesterase (2.0 U) and calf intestinal phosphatase (16.0 U) were added and allowed to react for 9 h to liberate the damaged and undamaged nucleosides from the reacted ODNs. The digestion proteins were removed before HPLC analysis by passing the sample through a 10,000 molecular weight cutoff filter (Millipore), and then analyzed by HPLC as follows below.

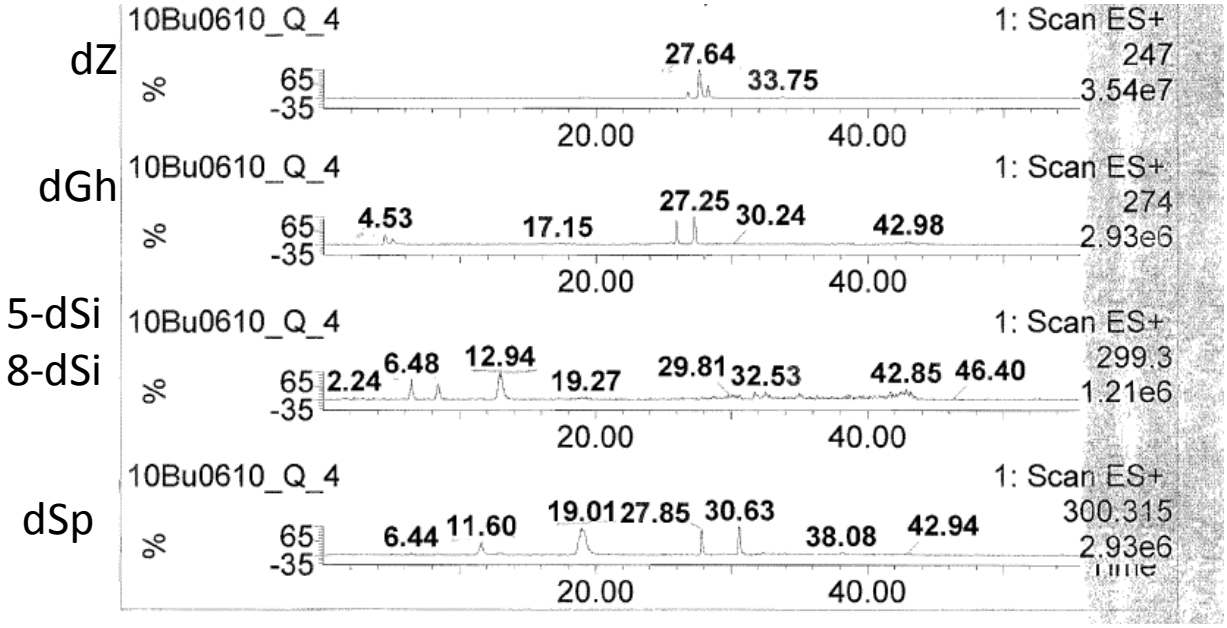
Purification of ODNs for Polymerase Studies. The individual diastereomers were purified using an ion-exchange HPLC column running 1.5 mM NaOAc as the resolving salt. The method was initiated at 15% B followed by a linear increase to 100% B over 30 min where A = 9:1 ddH_2O:CH_3CN, and B = 1.5 M NaOAc pH 7 with 10% CH_3CN. The run was conducted with a flow rate = 1 mL/min while monitoring the abs at 260 nm on a Dionex DNApac PA100 column.

Figure S1. LC-ESI$^+$-MS analysis of a reaction mixture



Panel A provides a mass for 8-amino-dG [$(M+Na)^+$ = 305.3] and panel B provides a mass for dOG [$(M+Na)^+$ = 306.3]

Hypercarb HPLC Analysis



Calculated masses for these compounds are $(M+H)^+$, dZ = 247.3, dGh = 274.3, dSp = 300.3, 5-dSi and 8-dSi = 299.3. The LCMS run was conducted on a (100 X 2.1 mm) column; therefore, the retention times do not lineup with those obtained from our analytical column. However, the elution order remained the same.

Representative HPLC chromatogram obtained from the Hypercarb column.

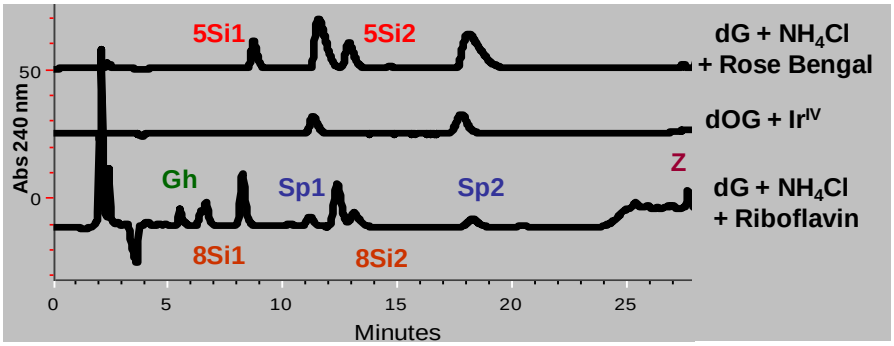
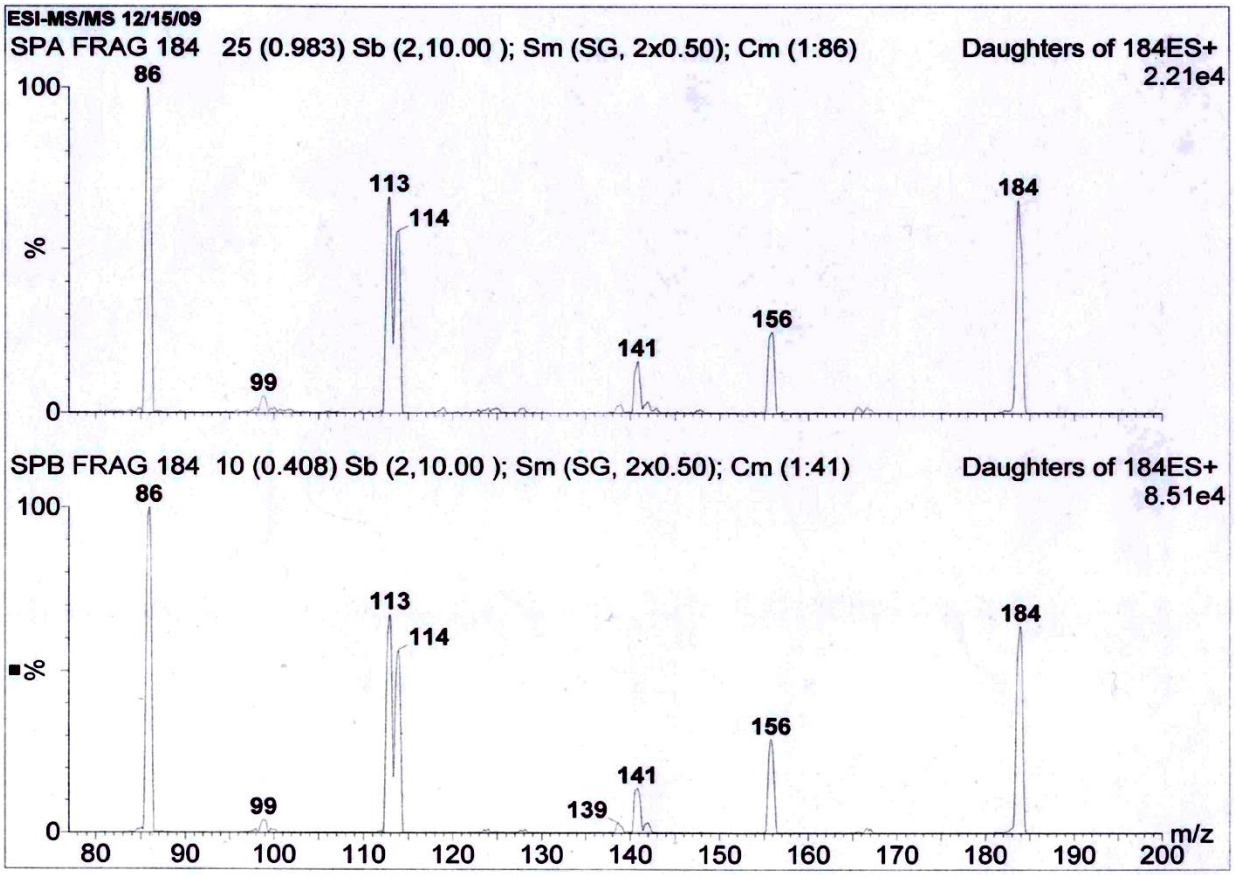
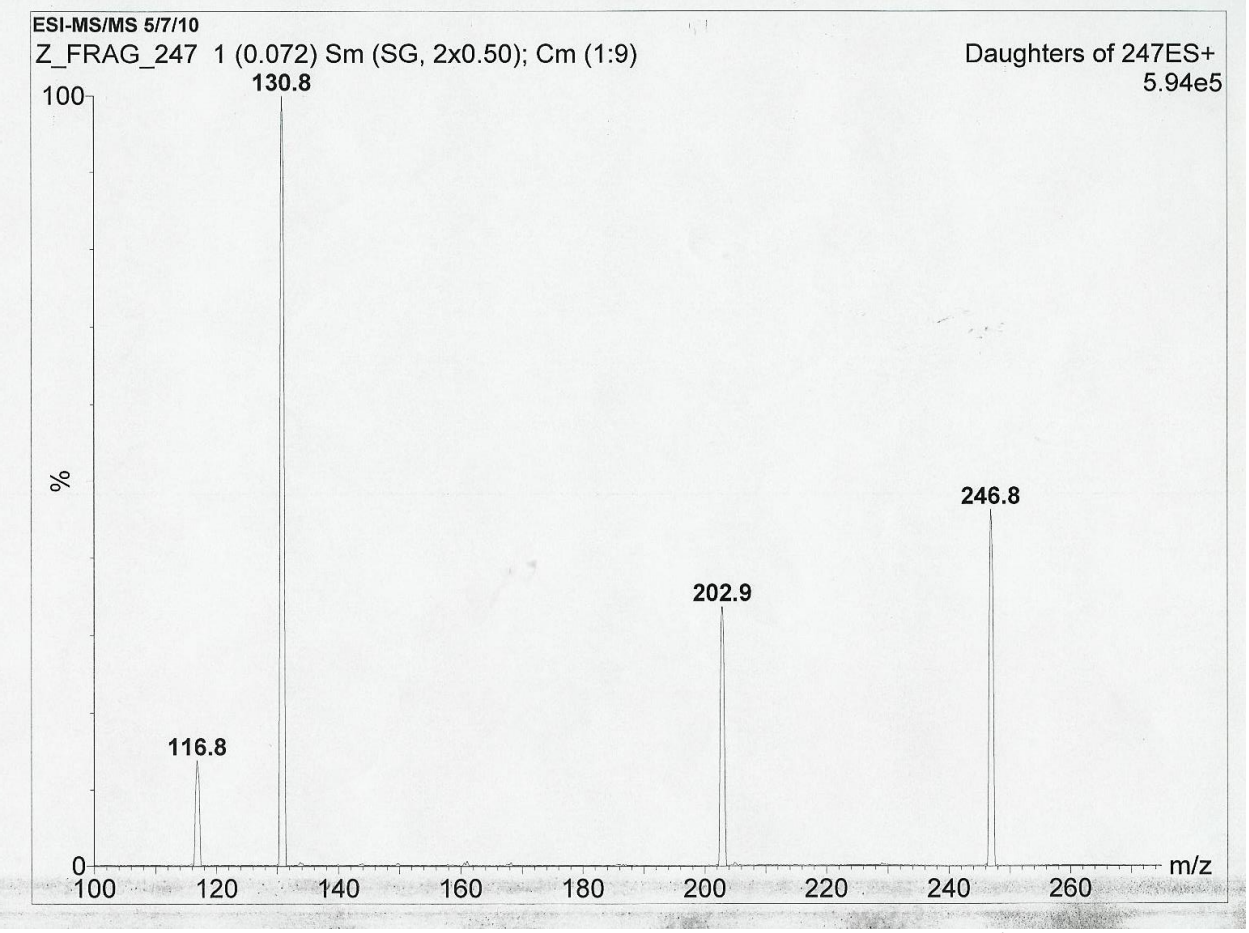


Figure S2. ESI$^+$-MS/MS spectrum for the Sp free base



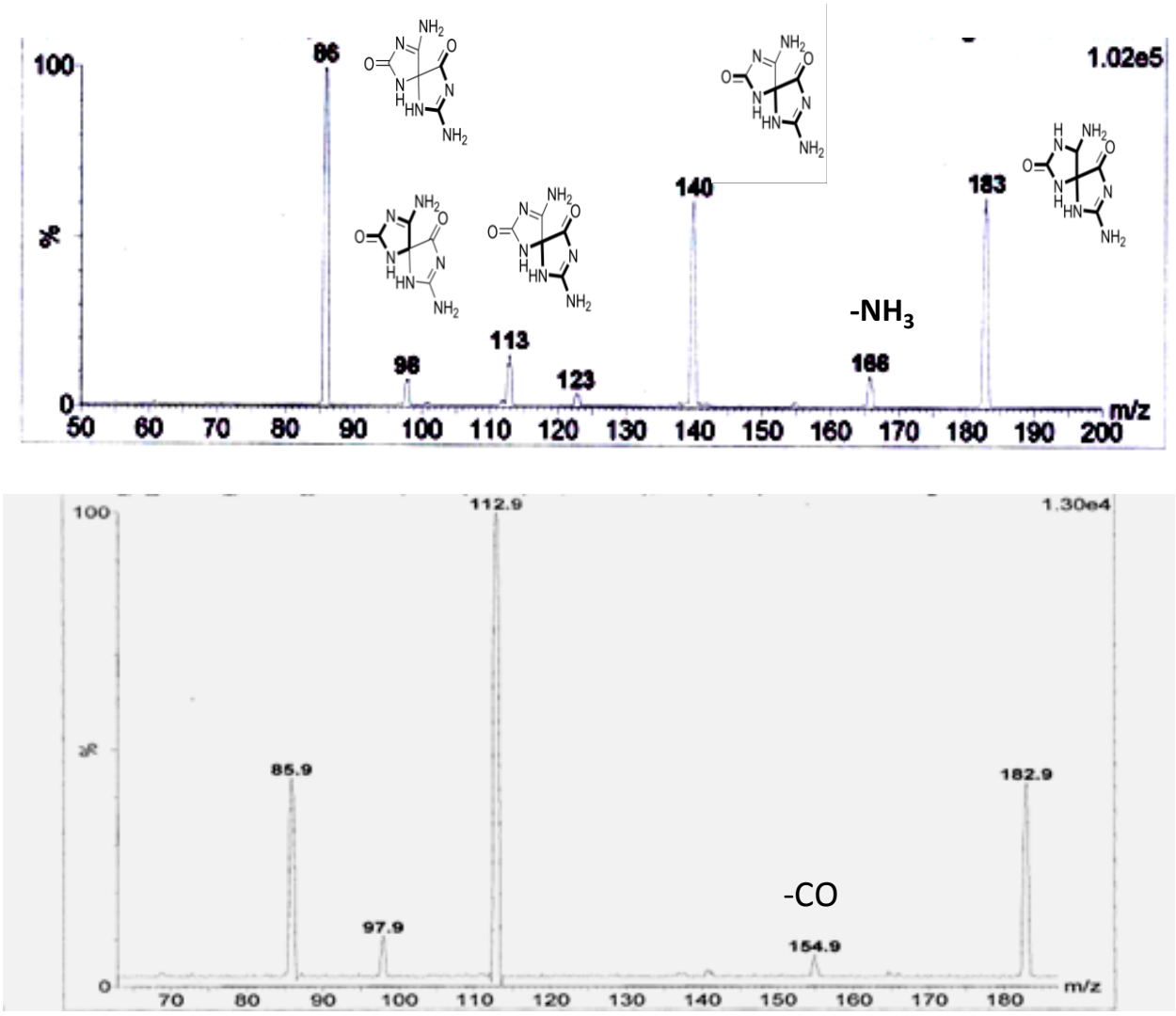
The established MS/MS fragmentation pattern for the Sp free base [$(M+H)^+$ = 186] gave daughter fragments with masses $(M+H)^+$ = 184, 156, 141, 113, 114, 99, and 86 (Burrows Laboratory-Org. Lett. 2000, 2, 613). All fragments were observed in the current data.

Figure S3. ESI$^+$-MS/MS spectrum for the free base of Z.



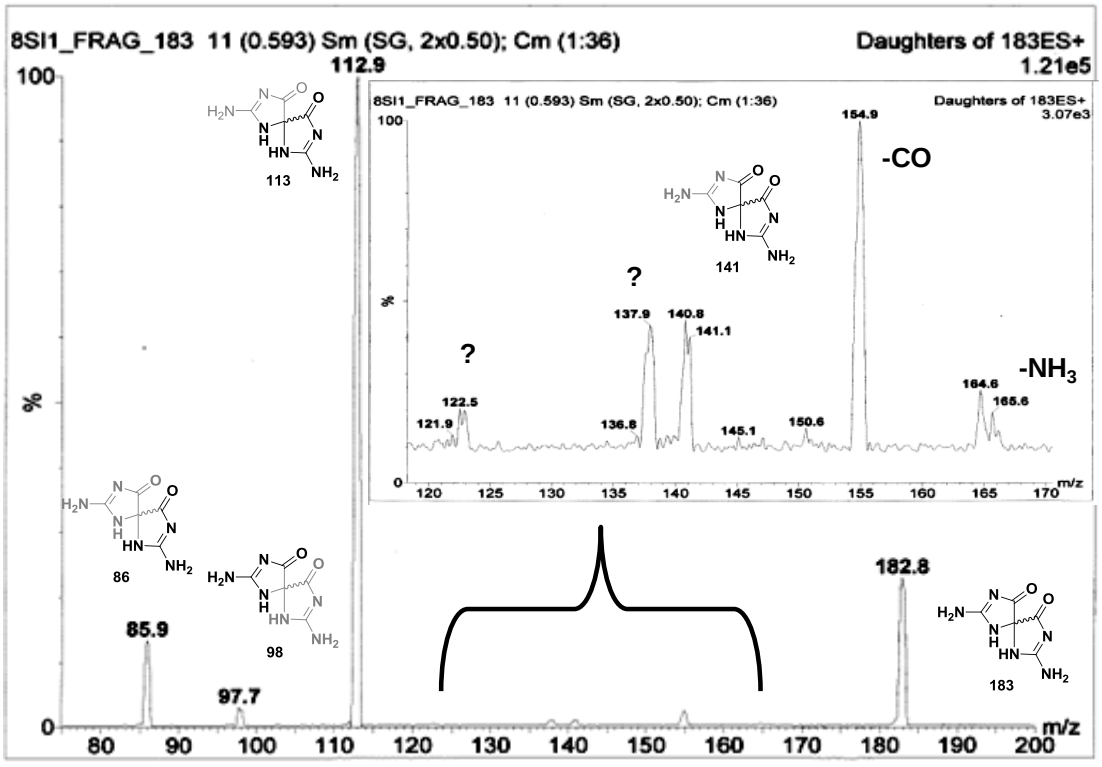
The data was compared to the values found in the following reference. $(M+H)^+$ = 247, 203, and 131. Matter, B.; Malejka-Giganti, D.; Csallany, A.S.; Tretyakova, N. *Nucleic Acids Res.* **2006**, *34*, 5449-5460.

Figures S4. ESI$^+$-MS/MS spectra for the free bases of 5-dSi.



The top spectrum was obtained from the first eluting 5-dSi isomer from a Hypercarb HPLC column, and the bottom spectrum was from the second eluting 5-dSi isomer from a Hypercarb HPLC column.

Figures S5. ESI$^+$-MS/MS spectra for the free bases of 8-dSi.



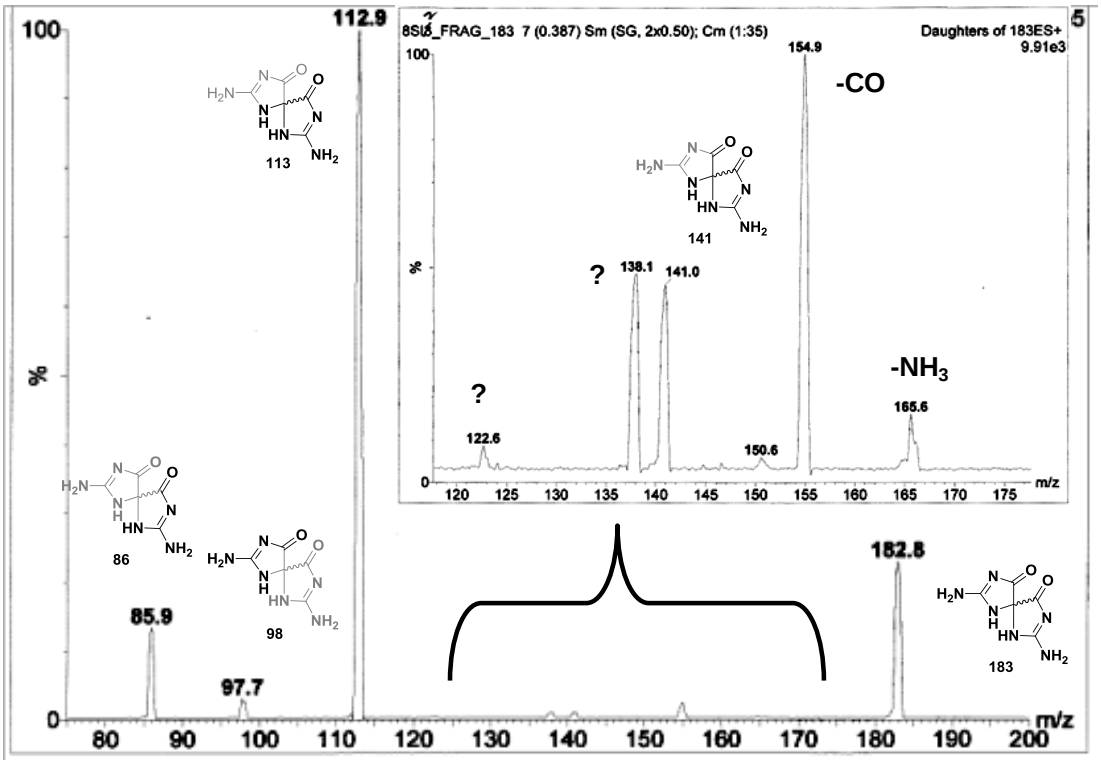


Figure S6. Hypercarb HPLC chromatogram for deamination of 5-dSi

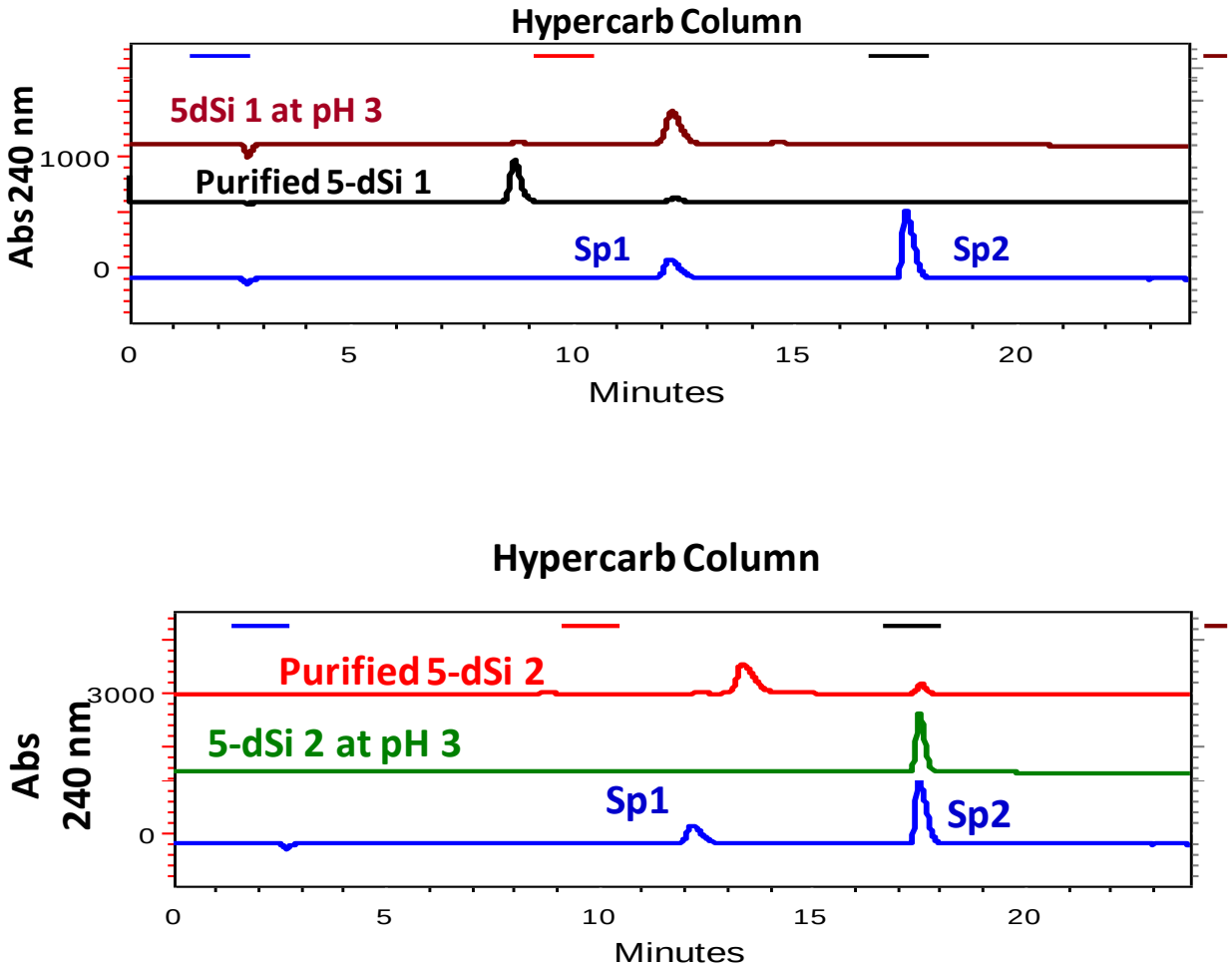
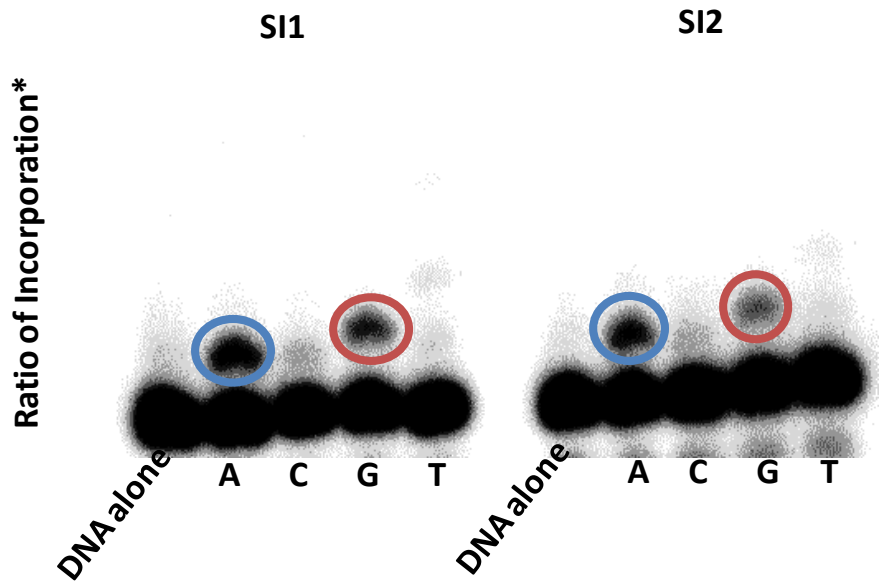
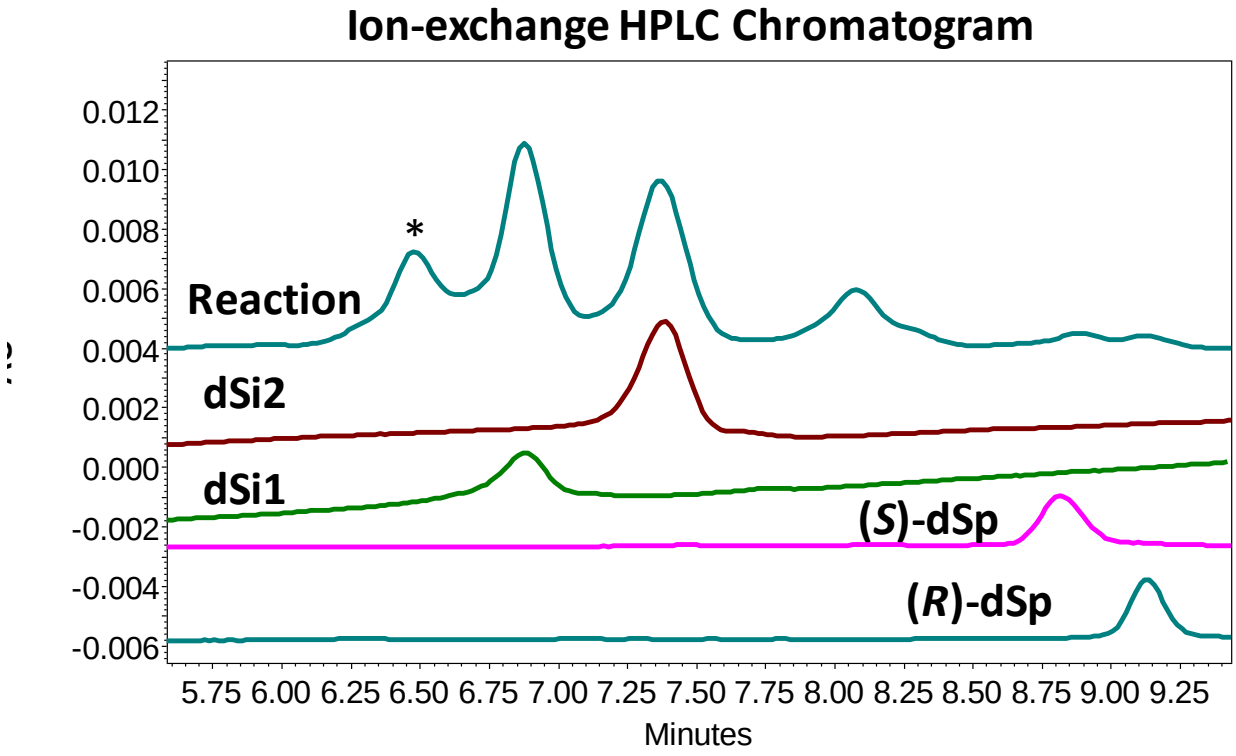


Figure S7. Polymerase insertion studies opposite 5-dSi



Oligo: 5’-CGT TAX GGC GCA ACT GGA AA-3’
Primer: 3’-CCG CGT TGA CCT TT-5’

Figure S8. Synthesis of dSi and dSp in an oligodeoxynucleotide.



Hypercarb HPLC Traces Obtained After Digestion of 5-dSi-containing ODNs