Supporting Information for:

Synthesis and characterization of oligonucleotides containing a nitrogen mustard formamidopyrimidine mono-adduct of deoxyguanosine.

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Materials and Methods

Materials. dGuo was purchased from Chem Gene Corporation (Wilmington, MA). N,N-bis(2-chloroethyl)ethylamine hydrochloride was purchased from Aaron Chemistry GmbH (Munich, Germany). *E. coli* Endo IV and FPG were purchased from New England Biolabs.

NMR Spectra. $^1$H NMR spectra were recorded at 300 or 600 MHz in DMSO-$d_6$ and acetone-$d_6$.

Mass Spectrometry. FAB mass spectra (low and high resolution) were obtained at the Mass Spectrometry Facility at the University of Notre Dame, Notre Dame, IN.

Chromatography. HPLC analysis was carried out on a gradient instrument (Beckman Instruments: pump module 125, photodiode array detector module 168, and System Gold software). Thin-layer chromatography was performed on silica gel glass plates (Merck, Silica Gel 60 F254, layer thickness 250 µm). The chromatograms were visualized by staining with an anisaldehyde/sulfuric acid solution, followed by heating. Column chromatography was performed using silica gel (Merck, 70-230 mesh).

HPLC purification. A Phenomenex Gemini-C18 column (250 × 4.6 mm, flow rate 1.5 ml/min, 250 × 4.6 mm, flow rate 5 ml/min) was used to monitor reactions and for oligonucleotide purifications. Oligonucleotides were detected by their UV absorbance at 254 nm. The mobile phase consisted of acetonitrile and 100 mM aqueous ammonium formate.

Gradient 1: initial conditions were 1% acetonitrile; a linear gradient to 10% acetonitrile over 15 min; a linear gradient to 20% acetonitrile over 5 min; isocratic at 20% acetonitrile for 5 min; a linear gradient to 80% acetonitrile over 2.5 min; isocratic at 80% acetonitrile for 3 min; then a linear gradient to the initial conditions over 3 min.

Gradient 2: initial conditions were 1% acetonitrile; a linear gradient to 5% acetonitrile over 5 min; a linear gradient to 10% acetonitrile over 15 min; a linear gradient to 80% acetonitrile over 2 min; isocratic at 80% acetonitrile for 2 min; then a linear gradient back to the initial conditions over 3 min.

Gradient 3: initial conditions were 1% acetonitrile; a linear gradient to 5% acetonitrile over 5 min; a linear gradient to 11% acetonitrile over 15 min; a linear gradient to 80% acetonitrile over 2 min; isocratic at 80% acetonitrile for 2 min; then a linear gradient back to the initial conditions over 3 min.

Gradient 4: initial conditions were 1% acetonitrile; a linear gradient to 5% acetonitrile over 5 min; a linear gradient to 12% acetonitrile over 15 min; a linear gradient to 80% acetonitrile over 2 min; isocratic at 80% acetonitrile for 2 min; then a linear gradient back to the initial conditions over 3 min.

UPLC-MS/MS analysis of the Enzymatic Digestion of oligonucleotide (9). Enzymatic digestion of oligonucleotide 9 was performed as previously describe (Christov et al. *Chem. Res. Toxicol. 2008,* 21, 2324-2333). HPLC analysis (5 µL) was performed using solvent gradient 1.

MS analysis of the enzyme digest was performed in the Vanderbilt University facility on a Waters Acquity UPLC system (Waters, Milford, MA) connected to a Finnigan LTQ mass spectrometer (ThermoElectron) equipped with an Ion Max API source and a standard electrospray probe using an Acquity UPLC system BEH octadecysilane (C18) column (1.7 µm, 2.1 mm ×50 mm). LC conditions were as follows: buffer A contained 100 mM NH$_4$CO$_2$ and
buffer B contained acetonitrile The following gradient program was used with a flow rate of 80 µL/min: initially 0% B; 15 min linear gradient to 10% B; 5 min linear gradient to 20% B; 4 min linear gradient 100% B; isocratic at 100% B for 1 min; 2 min linear gradient to 0% B; isocratic at 0% B for 3 min. The temperature of the column was maintained at 50 °C and the samples (15 µL) were infused with an auto-sampler. The electrospray conditions were as follows: source voltage 5 kV, source current 100 µA, N₂ was used as the auxiliary gas and the flow-rate setting was 20, sweep gas flow-rate setting 5, sheath gas flow setting 34, capillary voltage -49 V, capillary temperature 350 °C, and tube lens voltage -30 V. No CID offset was employed. MS/MS conditions were as follows: normalized collision energy 35%, activation Q 0.250, and activation time 30 ms. The isolation width in MS/MS was 2. The automatic gain control (AGC) settings in full MS and MSⁿ were 10000. The maximum injection time in full MS and MSⁿ were 10 ms and 40 ms, respectively. The MS data were acquired in positive mode. Helium was used as the collision damping gas in the ion trap and was set at a pressure of 1 mTorr. The number of µscan used for data acquisition in full MS and MSⁿ modes was 2. A method consisting of six scan events was used: 1) full scan, 2 microscans, ion accumulation time 200 ms, m/z [150-500.00]; 2) selected reaction monitoring: 1 microscan, spectral width 2, ion accumulation time 50 ms, MS m/z 268 @ 25 [100-200]; 3) selected reaction monitoring: 1 microscan, spectral width 2, ion accumulation time 50 ms, MS m/z 251 @ 25 [120-220]; 4) selected reaction monitoring: 1 microscan, spectral width 2, ion accumulation time 50 ms, MS m/z 242 @ 25 [100-200]; 5) selected reaction monitoring: 1 microscan, spectral width 2, ion accumulation time 50 ms, MS m/z 228 @ 25 [100-200]; 5) selected reaction monitoring: 1 microscan, spectral width 2, ion accumulation time 50 ms, MS m/z 400 @ 25 [200-300].

The enzymatic digestion reaction mixture was also treated with hydrochloric acid (6N, 3 µL) at 70° C for 3h. After cooling down, the mixture was neutralized with sodium hydroxide (3N). UPLC-MS analysis of the reaction mixture was performed as described for the enzyme digestion of 9 with the following modifications. A method consisting of two scan events was used: 1) full scan, 2 microscans, ion accumulation time 200 ms, m/z [200-1000]; 2) selected reaction monitoring: 1 microscan, spectral width 2, ion accumulation time 50 ms, MS m/z 285 @25 [174-274]. The proposed fragmentation of the parent ion is shown below.

![Proposed fragmentation of the parent ion](image-url)
Figure S1. $^1$H NMR spectra of $N^2$-[(Dimethylamino)methylene]-$O^6$-[2-(trimethylsilyl)-ethyl]-2'-deoxyguanosine (2).
Figure S2. $^1$H NMR spectra of $N^2$-[(Dimethylamino)methylene]-[5'-O-(bis(4-methoxy-phenyl)phenyl-methyl)-O$^6$-(trimethylsilylethy)]-2'-deoxy-guanosine (3).
Figure S3. $^1$H NMR and MS spectra (inset) of $N^6$-[4-[5-O-[bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-D-erythro-pentofuranosyl]amino]-2-[[[(dimethyl-amino)methylene]amino]-$O^4$-[2-(trimethylsilyl)ethyl]-5-$N$-[2-(2-chloroethyl)ethylamino]ethylformamidopyrimidine (5). The isotopic distribution matched that calculated for $C_{45}H_{63}ClN_7O_7Si$ at FWHM of 0.05 (htt://www.chemcalc.org)
Figure S4. $^1$H NMR spectra of $N^6$-[4-[5-$O$-[bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-D-erythro-pentofuranosyl]amino]-2-[[dimethyl-amino)methylene]amino]-$O^A$-[2-(trimethylsilyl)ethyl]-5-$N$-[2-(2-acetoxyethyl)ethylamino]ethylformamidopyrimidine (6).
**Figure S5.** $^1$H NMR spectra of $N^6$-[4-[5-$O$-[bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-D-erythro-pentofuranosyl]amino]-2-[[[dimethyl-amino)methylene]amino]-1,4-dihydro-4-oxo-5-$N$-[2-(2-hydroxyethyl)ethylamino]ethylformamidopyrimidine (7).
Figure S6. $^1$H NMR spectra of $N^6$-[4-[5-O-[bis(4-methoxyphenyl)phenylmethyl]-3-O-[(N,N-diisopropylamino)(2-cyanoethoxy)phosphino]-2-deoxy-D-erythro-pentofuranosyl]amino]-2-[[dimethyl-amino)methylene]amino]-1,4-dihydro-4-oxo-5-$N$-[2-(2-hydroxyethyl)ethylamino]ethylformamidopyrimidine (8).
Figure S7. MALDI-TOF (HPA) mass spectrum of the NM-Fapy-dGuo containing 12mer (9).
Figure S8. MALDI-TOF (HPA) mass spectrum of the NM-Fapy-dGuo containing 24mer (10).
Figure S9. MALDI-TOF (HPA) mass spectrum of the 8-oxo-dGuo containing 24mer.
5’-GCTAGC-(NM-Fapy-dGuo)-AGTCC-3’ (9)

“short” deprotection

“long” deprotection

Furanose form of (9) (purified)

Pyranose form of (9) (purified)

Figure S10. HPLC analysis of the oligonucleotide (9).
Figure S11. Steady-state kinetics of the FPG excision of the NM-FapyG, MeFapyG, and 8-oxo-G containing 24mer (10) and representative gel analyses of the excision reaction.
5′-ACC ACG CTA GCX AGT CCT AAC AAC-3′
3′-TGG TGC GAT CGC TCA GGA TTG TTG-5′

Incision at 15 nM of NM-Fapy by Endo IV

Figure S12. Steady-state kinetics of the Endo IV incision of the NM-FapyG containing 24mer (10) and representative gel analysis of the incision reaction.
Figure S13. Anomerization of the NM-Fapy-dGuo adduct at pH 7.0, 7.5, and 8.0. Aliquots were taken at ~1, 3, and 5 days and subjected to Endo IV incision.