Supporting Information for

Unexpected Complexity in the Products Arising from NaOH-, Heat-, Amine-, and Glycosylase-Induced Strand Cleavage at an Abasic Site in DNA

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Figure S1

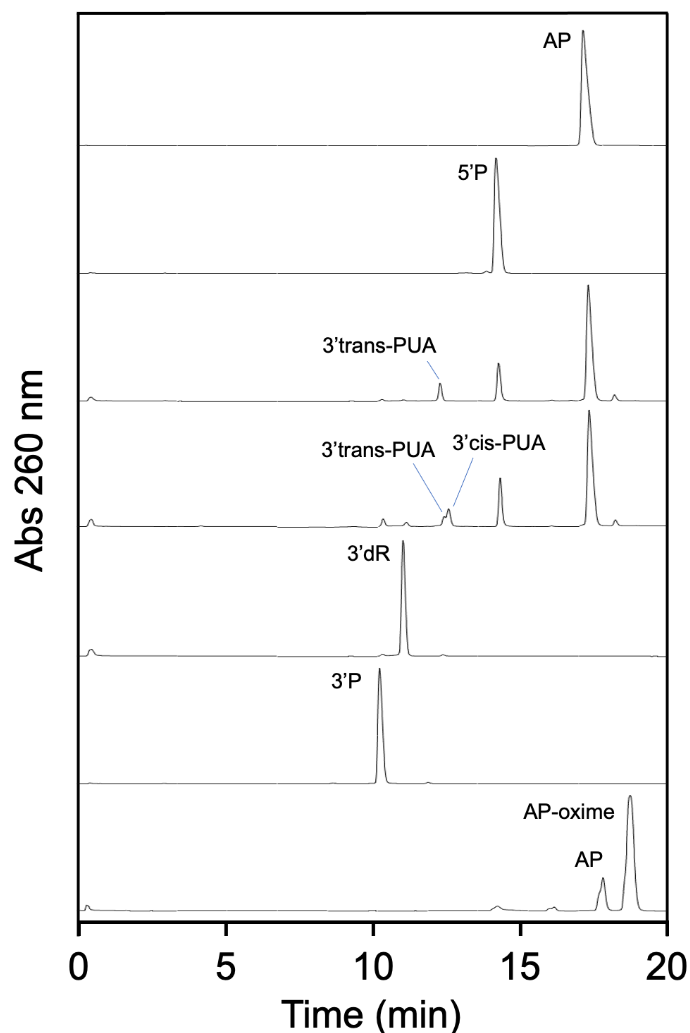

Figure S1. HPLC retention times of authentic standards for AP-oligonucleotide, 5'P, 3'*cis*- and *trans*-PUA, 3'dR, 3'P and AP-oxime. From top: the AP-containing oligonucleotide (5'-TTTTTXTTTTTTTTTT, where X=AP) was generated by treatment of the corresponding dU containing oligomer with UDG. The 5'-P product (5'-PTTTTTTTTTT, where P = a phosphoryl group) and 3'P (5'-TTTTTP-3') were purchased from IDT. The 3'*trans*-PUA product was generated by heating the AP-oligo at 85 ˚C in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) for 15 min (Sugiyama, *Chem. Res. Toxicol.* **1994**, *7*, 673-683). The mixture of 3'*cis*-PUA and 3'*trans*-PUA was generated by incubation of the AP-containing oligo in Tris-borate buffer according to the method of Kushida et al (*J. Am. Chem. Soc.* **1994**, *116*, 479-486). The 3'dR product (5'-TTTTT-dR-3') was generated by acid-catalyzed depurination of the adenine residue in the precursor 5'-TTTTTA-3' according to the conditions of Bailly and Verly (10 mM HCl, 65 ˚C, 1 h; *Nucleic Acids Res.* **1988**, *16*, 9489-9497). For comparison, the AP-oxime was generated by treatment of the AP-oligo with $CH_3ONH_2{\cdot}HCl$ (2 mM) at 37 °C in HEPES buffer (50 mM, pH 7) containing NaCl (100 mM) for 2 h.

Figure S2

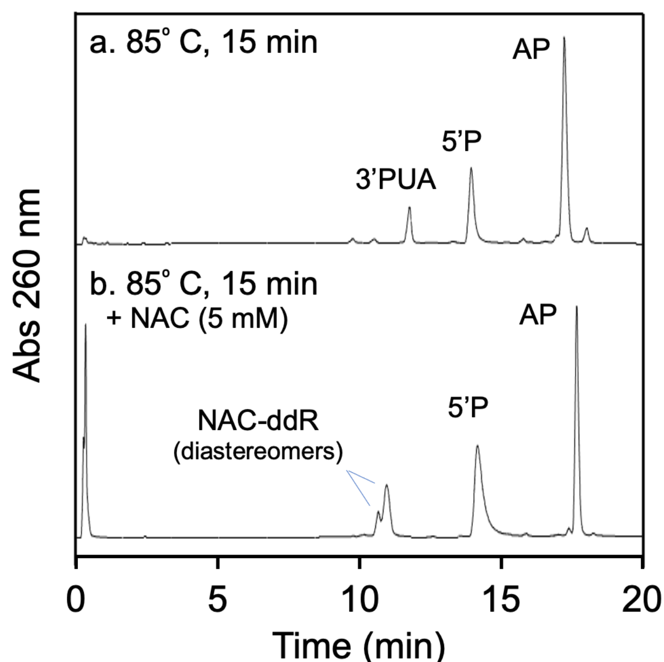

Figure S2. HPLC analysis of the reaction of *N*-acetylcysteine (NAC) with 3'*trans*-PUA generated by thermal treatment of the AP-containing oligodeoxynucleotide. Panel a. Heating the AP-containing oligonucleotide in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85 ˚C for 15 min generated two major cleavage products, 3'*trans*-PUA and 5'P. Panel b. Heating the AP-containing oligonucleotide in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85 ˚C for 15 min, followed by addition of NAC (5 mM) and incubation for 15 min at 37 ˚C, caused disappearance of the 3'*trans*-PUA peak and appearance of two new peaks, assigned as a diastereomeric mixture of 3-alkylthio-2,3-dideoxyribose (NAC-ddR) products on the 3'-terminus of the strand break. Note that the NAC-ddR products elutes at a different time (retention time near 11 min) than the diastereomeric mixture of 3-alkylthio-2,3-dideoxyribose products generated by 2-mercaptoethanol (with retention times near 13 min under these chromatographic conditions).

Figure S3

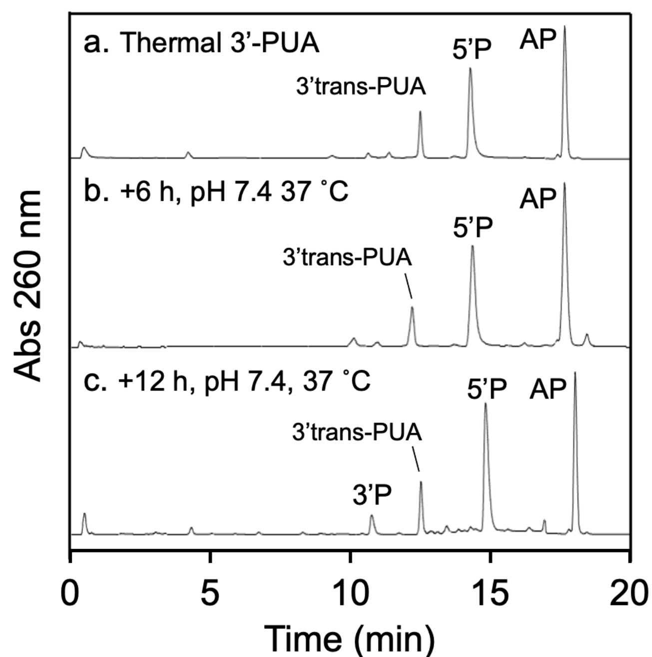

Figure S3. 3'*trans*-PUA is stable in pH 7.4 buffer (in the absence of amines and thiol). Panel a. 3'trans-PUA was generated by incubation of the AP-containing oligonucleotide at 85 ˚C in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) for 15 min. Panel b. The product was then incubated at 37 ˚C for 6 h prior to HPLC analysis. Panel c. Incubation of 3'*trans*-PUA for 12 h. The 3'trans-PUA end group is relatively stable, with only small amounts of the 3'P product produced by γ-elimination over the course of 12 h.

Figure S4

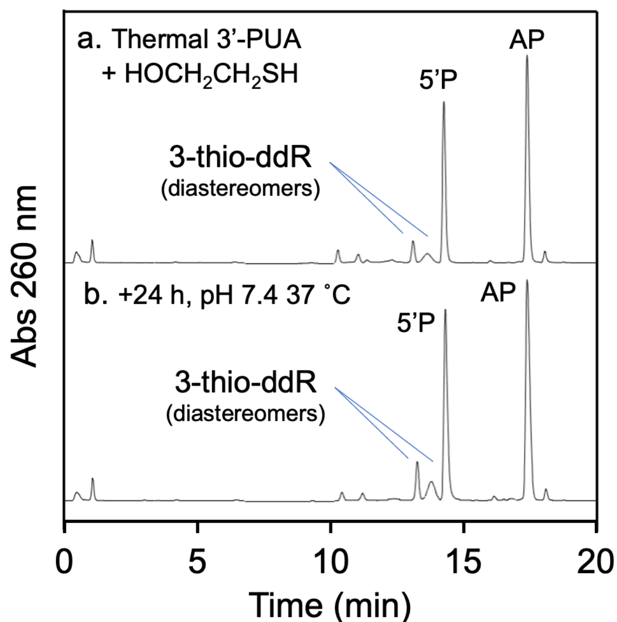

Figure S4. The 3-thio-2,3-dideoxyribose thiol adducts on the 3'-terminus in pH 7.4 buffer are stable (in the absence of amines and thiol). Panel a. Thermal generation of the 3'*trans*-PUA cleavage product, followed by addition of 2-mercaptoethanol (5 mM) and incubation at 37 ˚C for 15 min generated a diastereomeric mixture of 3-alkylthio-2,3-dideoxyribose (3-thio-ddR) products on the 3'-terminus of the strand break. Panel b. Incubation at 37 ˚C for 24 h did not cause significant degradation of the of the 3-alkylthio-2,3-dideoxyribose (3-thio-ddR) products.

Figure S5

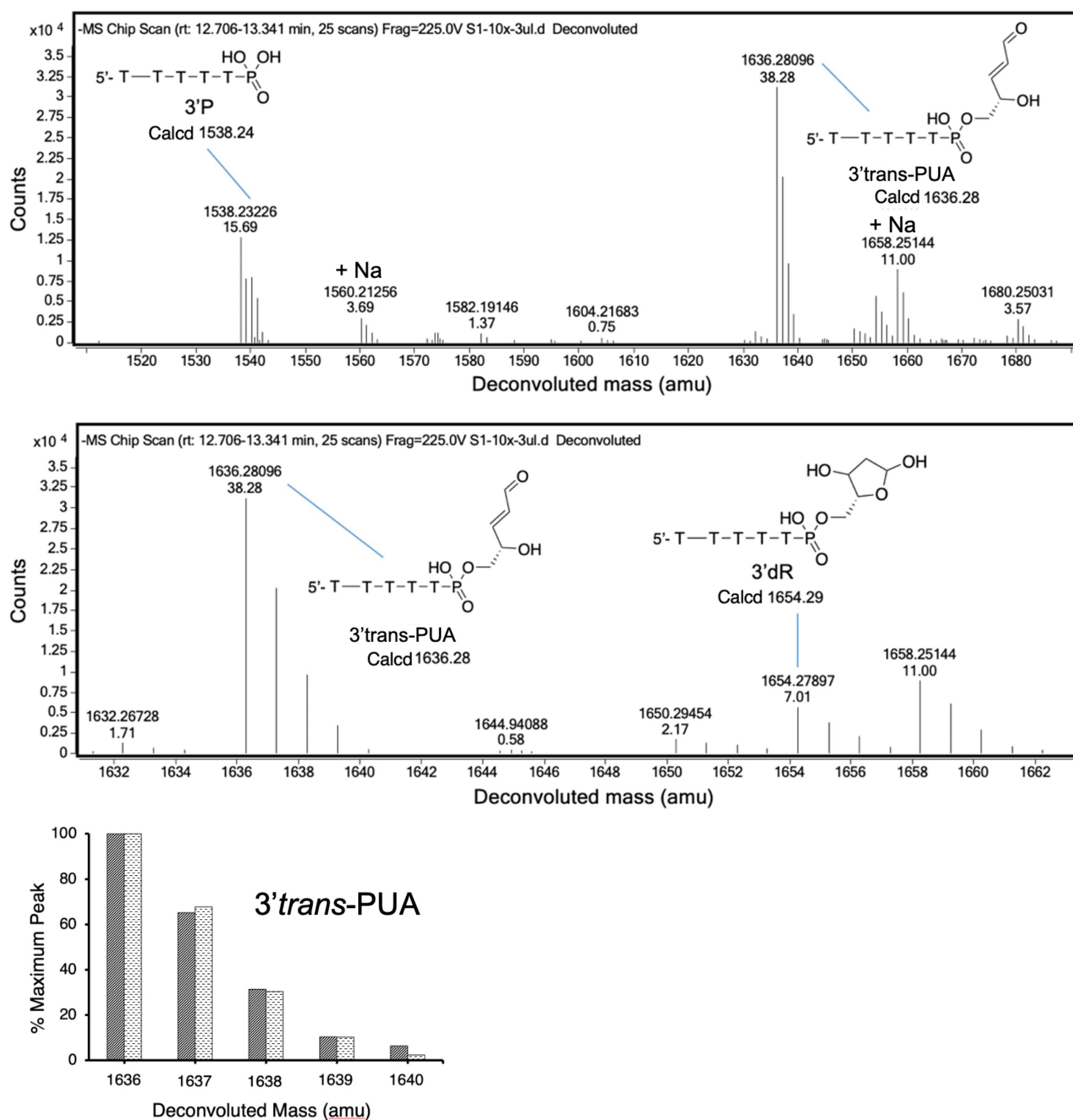

Figure S5. ESI(-)-QTOF-LC-MS analysis of the products generated by thermolysis of the AP-containing oligonucleotide after 15 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'*trans*-PUA, 3'dR (minor) and 3'P generated by thermal cleavage of the AP-containing oligo in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85 ˚C for 15 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'*trans*-PUA cleavage product.

Figure S6

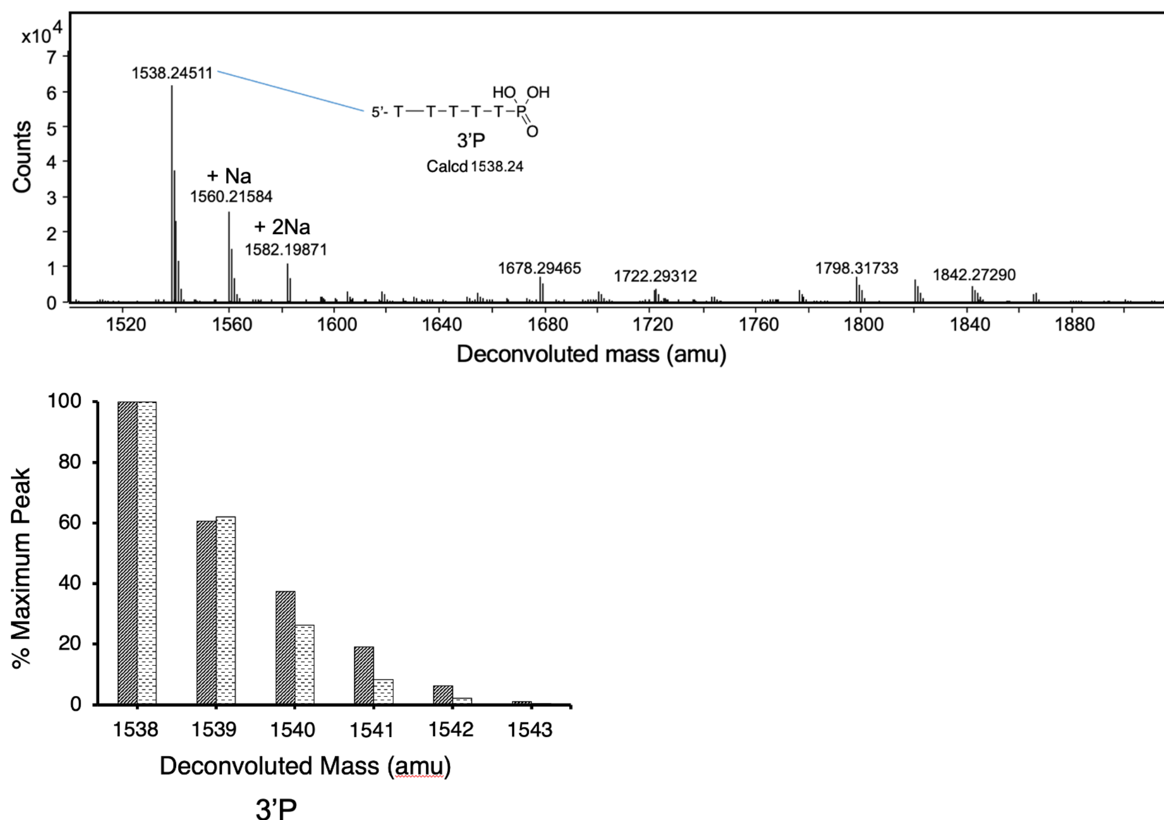

Figure S6. ESI(-)-QTOF-LC-MS analysis of the products generated by thermolysis of the AP-containing oligonucleotide after 45 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage product 3'P generated by thermal cleavage of the AP-containing oligo in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85 ˚C for 45 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'P cleavage product.

Figure S7

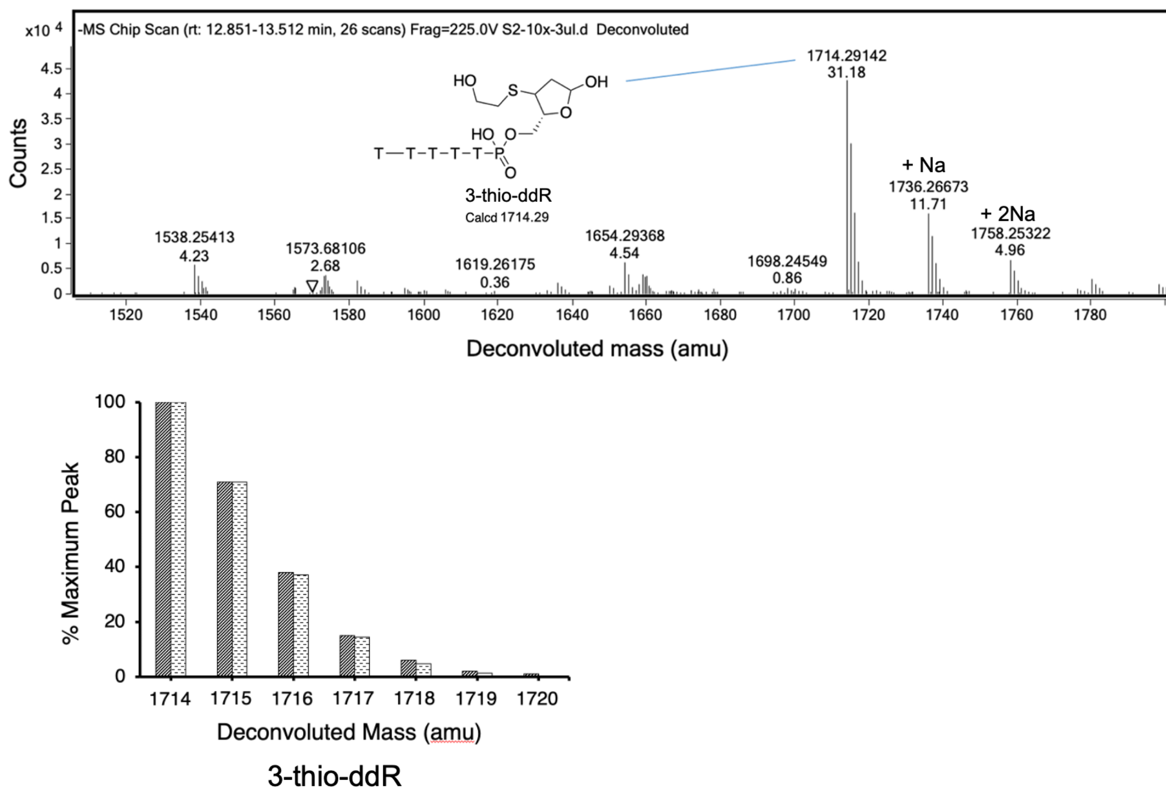

Figure S7. ESI(-)-QTOF-LC-MS analysis of products generated by thermolysis of the AP-containing oligonucleotide in the presence of 2-mercaptoethanol. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3-alkylthio-2,3-dideoxyribose (3-thio-ddR) products on the 3'-terminus of the strand break generated by thermal cleavage of the AP-containing oligo in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85 ˚C for 15 min, followed by incubation with 2-mercaptoethanol (5 mM) for 15 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the cleavage product bearing the 3-thio-ddR end group on the 3'-terminus of the strand break.

Figure S8

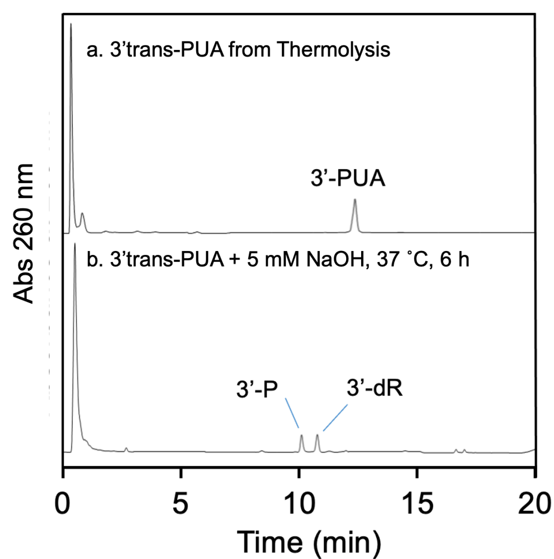

Figure S8. 3'*trans*-PUA is converted to 3'dR by treatment with 5 mM NaOH. The 3'*trans*-PUA product isolated from the thermolysis of the AP-containing oligonucleotide (upper panel) was converted to a mixture of 3'P and 3'dR by treatment with 5 mM NaOH, 37 ˚C for 6 h (lower panel).

Figure S9

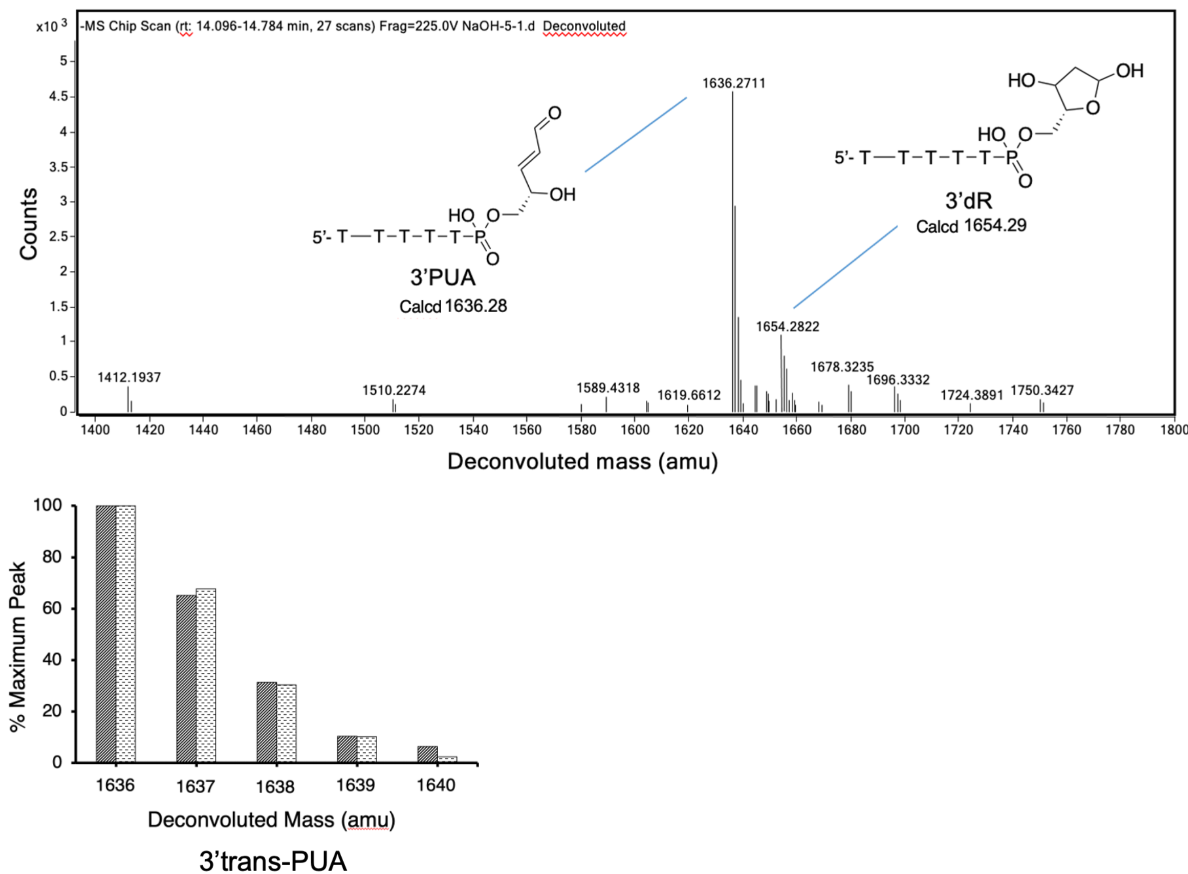

Figure S9. ESI(-)-QTOF-LC-MS analysis of products generated treatment of the AP-containing oligonucleotide with 5 mM NaOH. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'*trans*-PUA (major) and 3'dR (minor) generated by treatment of the AP-containing oligonucleotide with mild NaOH (5 mM, 1 h, 37 ˚C). Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'*trans*-PUA cleavage product.

Figure S10

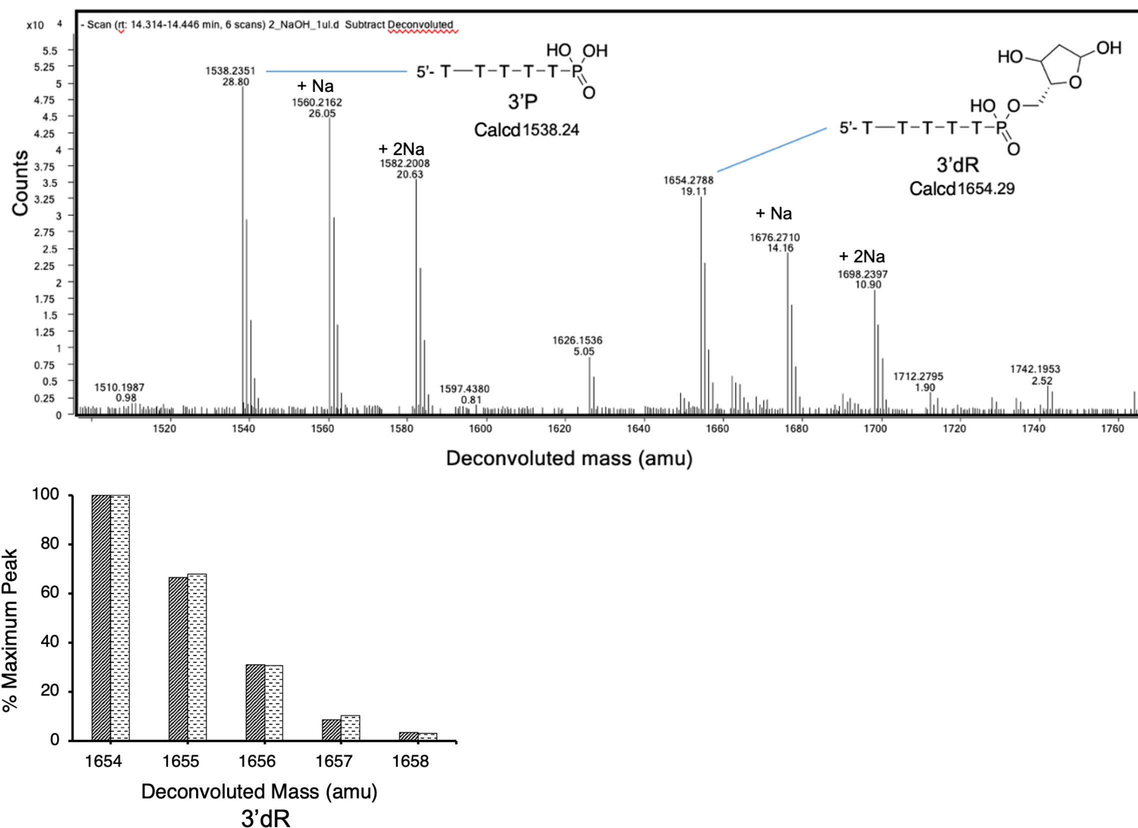

Figure S10. ESI(-)-QTOF-LC-MS analysis of products generated treatment of the AP-containing oligonucleotide with NaOH (200 mM). Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'dR and 3'P generated by treatment of the AP-containing oligonucleotide with NaOH (200 mM, 20 min, 37 ˚C). Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'dR cleavage product.

Figure S11

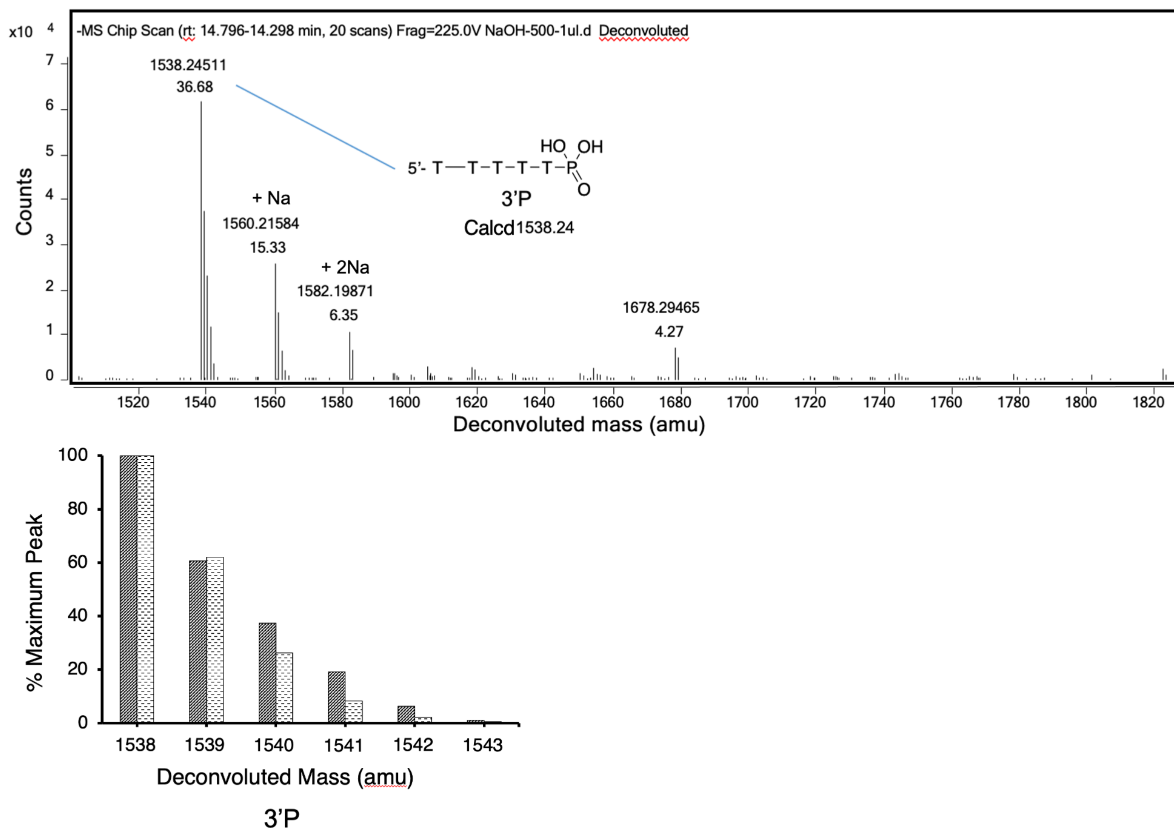

Figure S11. ESI(-)-QTOF-LC-MS analysis of products generated by addition of an equal volume of 500 mM NaOH to the AP-oligonucleotide in 20 mM HEPES pH 7.4 containing 100 mM NaCl, followed by heating at 95 ˚C for 2 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage product 3'P generated by treatment of the AP-containing oligonucleotide with NaOH (500 mM, 2 min, 95 ˚C). Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'P cleavage product.

Figure S12

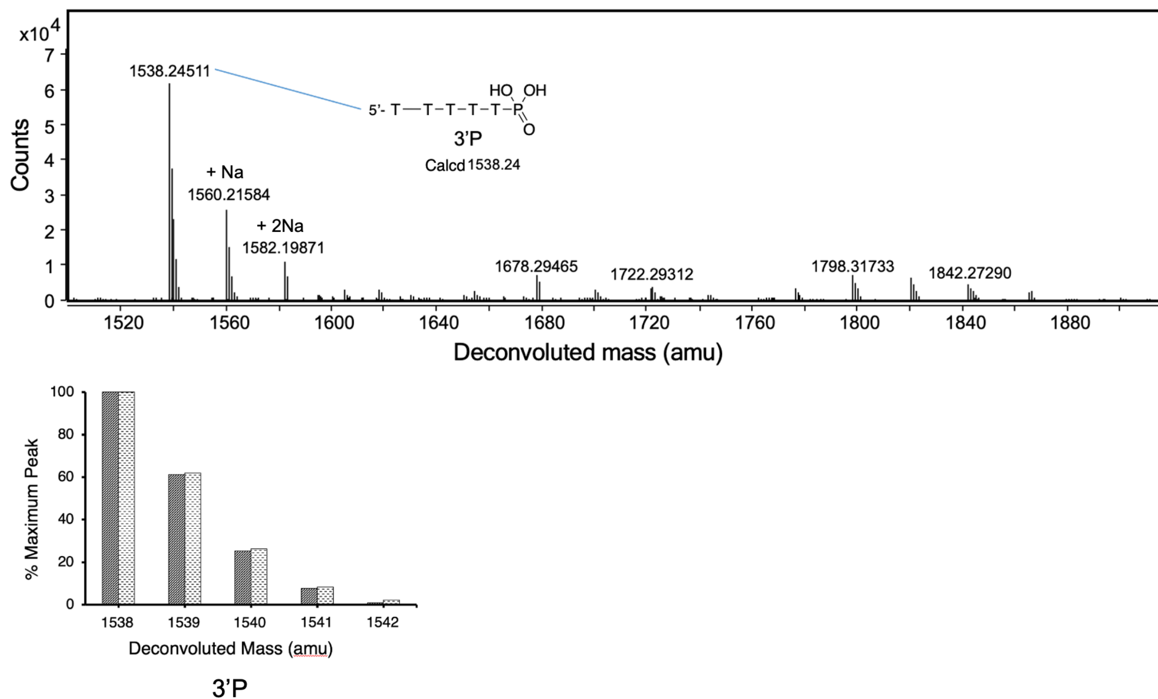

Figure S12. ESI(-)-QTOF-LC-MS analysis of products generated by treatment of the AP-oligonucleotide with piperidine (1 M) at 95 ˚C for 30 min (Maxam-Gilbert workup). Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'P generated by treatment of the AP-containing oligonucleotide with piperidine (1 M) at 95 ˚C for 30 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'P cleavage product.

Figure S13

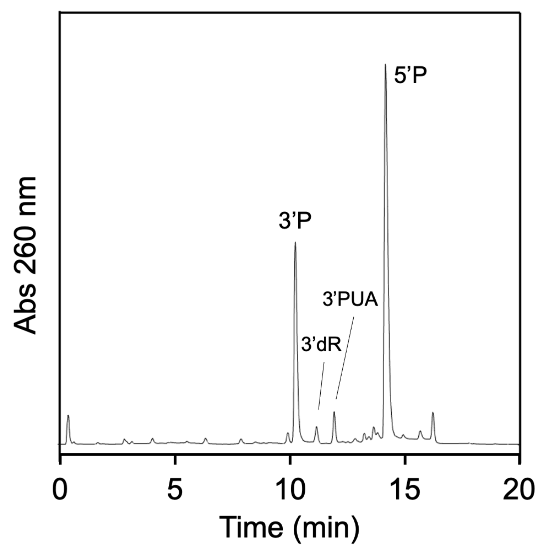

Figure S13. HPLC analysis of a control reaction involving heating the AP oligonucleotide at 95 ˚C for 30 min (no piperidine). Heating the AP-containing DNA oligomer at 95 ℃ for 30 min in water without piperidine generated a mixture of 3'PUA, 3'dR, and 3'P.

Figure S14

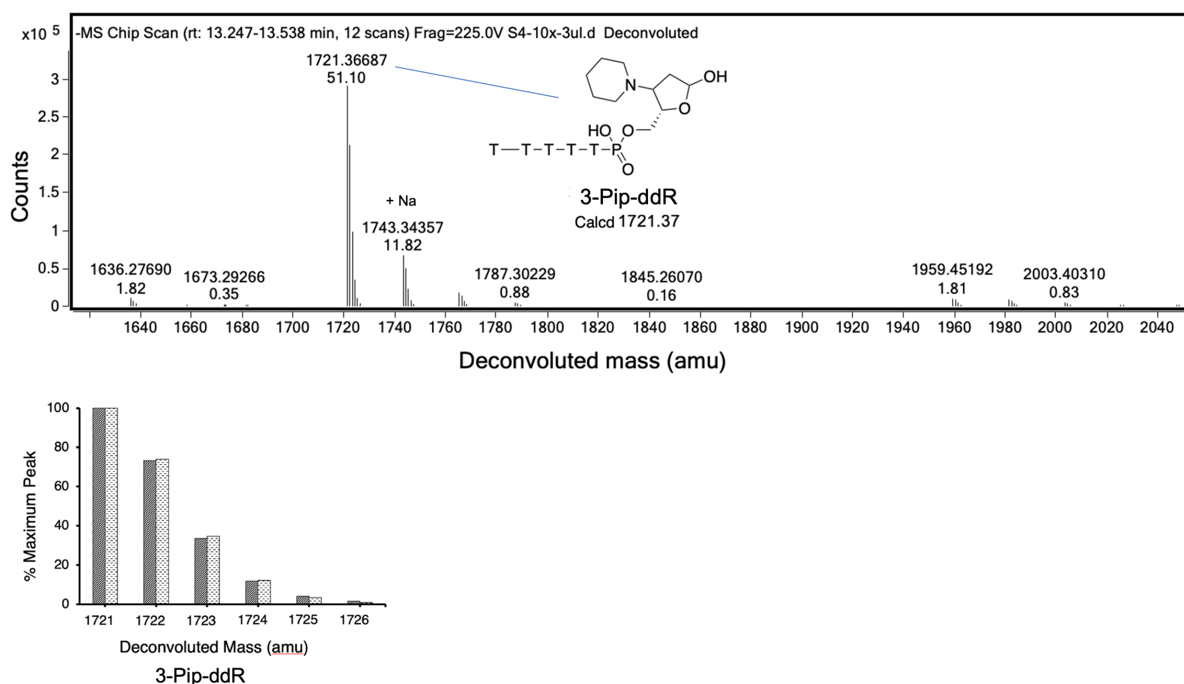

Figure S14. ESI(-)-QTOF-LC-MS analysis products generated by treatment of the AP oligonucleotide with piperidine (1 M) at 50 ˚C for 20 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage product bearing a 3-piperidinyl-2,3-dideoxyribose adduct (3-pip-ddR) on the 3'-terminus of the strand break generated by treatment of the AP-containing oligonucleotide with piperidine (1 M) at 50 °C for 30 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3-pip-ddR cleavage product.

Figure S15

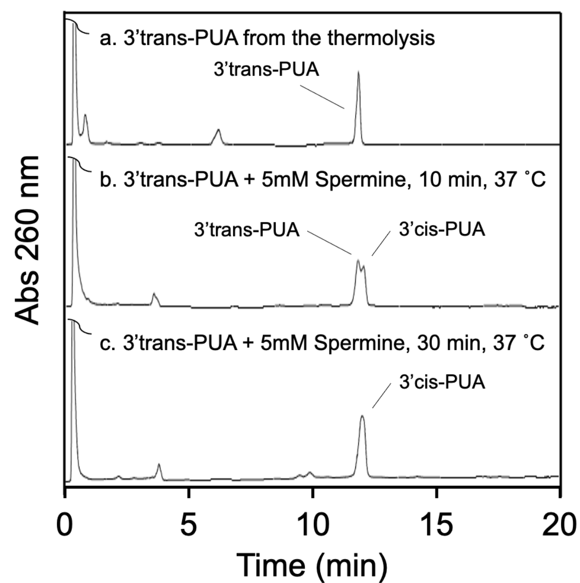

Figure S15. 3'*trans*-PUA is converted to a mixture of 3'*trans*-PUA and 3'*cis*-PUA by treatment with spermine (5 mM). The 3'trans-PUA product isolated from the thermolysis of the AP-containing DNA oligomer (panel a) was converted to a mixture of 3'*trans*-PUA and 3'*cis*-PUA by treatment with 5 mM Spermine, 37 ˚C for 10 min (panel b) and to a single peak ascribed to the 3'*cis*-PUA cleavage product after 30 min at 37 ˚C (panel c).

Figure S16

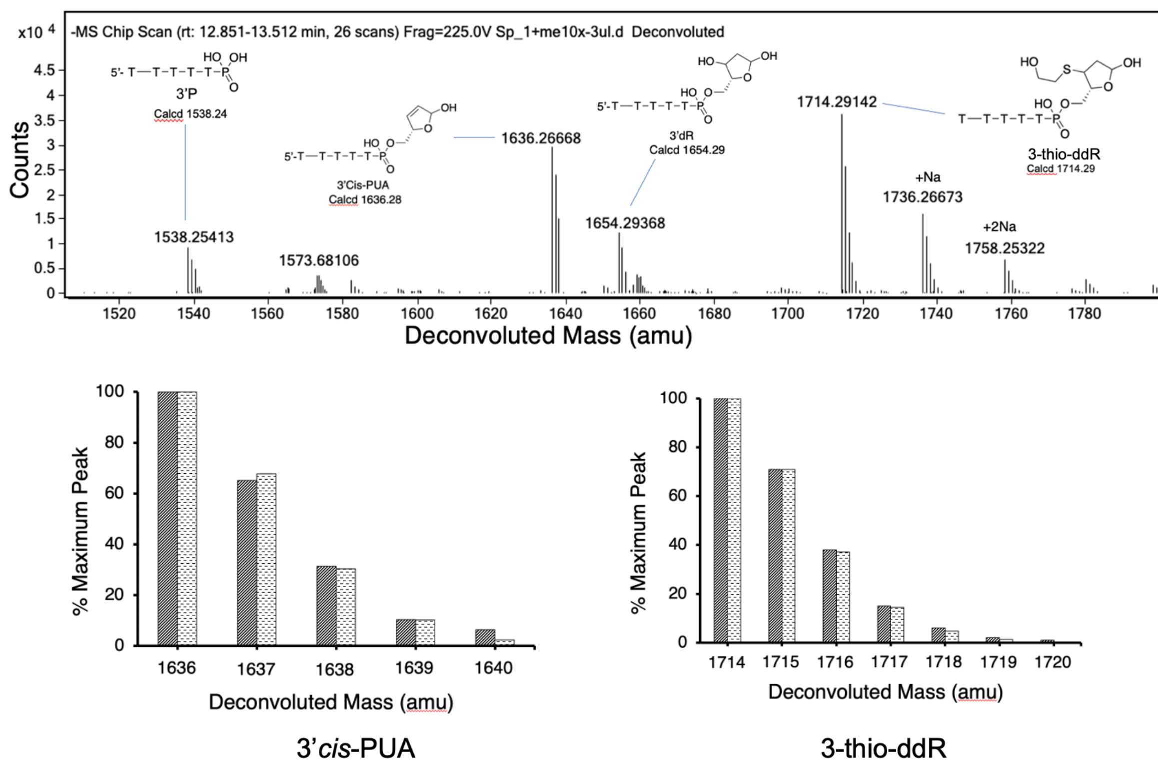

Figure S16. ESI(-)-QTOF-LC-MS analysis of the products generated by spermine-mediated cleavage of the AP oligonucleotide in the presence of 2-mercaptoethanol. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'P, 3'*cis*-PUA, 3'dR, and 3-thio-ddR generated by treatment of the AP-containing DNA oligomer with spermine (5 mM) in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 37 ˚C for 30 min, followed by addition of 2-mercaptoethanol (5 mM) and incubation for 15 min. Lower panel left side. Comparison of experimentally measured peak intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'*cis*-PUA cleavage product. Lower panel right side. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3-thio-ddR adduct.

Figure S17

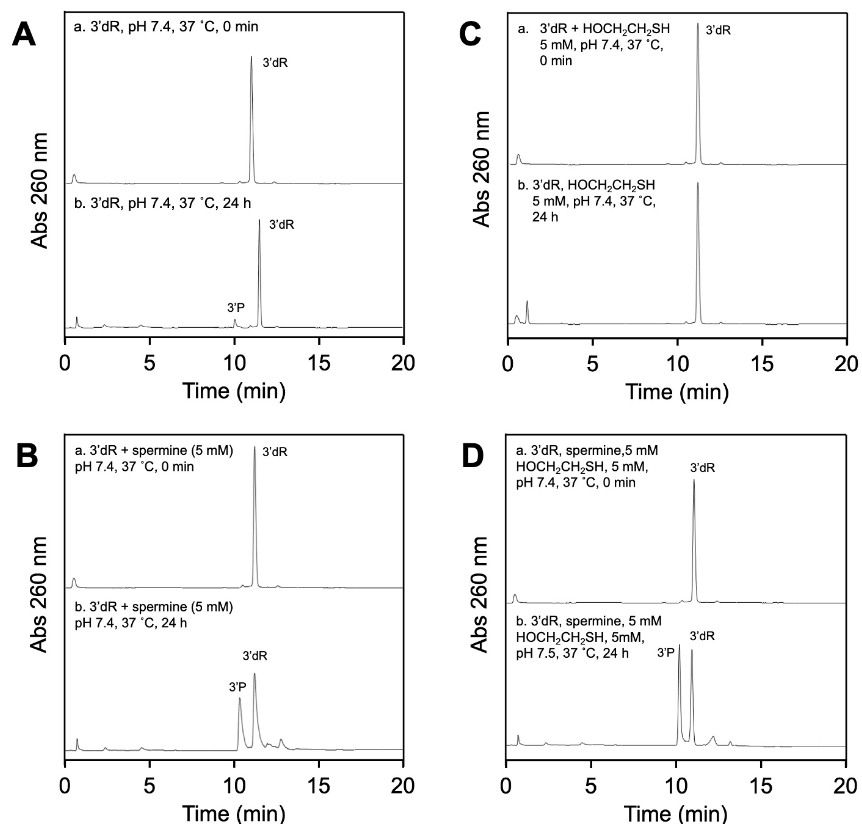

Figure S17. Reactivity of 3'dR under various conditions. Panel A, upper: Stability of authentic 3'dR in pH 7.4 buffer. The authentic 3'dR material was generated from by acid-catalyzed depurination (10 mM HCl, 65 ˚C, 1 h) of the adenine residue in the precursor DNA oligomer 5'-TTTTTA. Panel A, lower: Incubation of 3'dR at 37 ˚C in pH 7.4 HEPES buffer (50 mM, containing 100 mM NaCl) for 24 h showed generation of only a small amount of the 3'P product. Panel B: Incubation of 3'dR at 37 ˚C in pH 7.4 HEPES buffer (50 mM, with 100 mM NaCl) containing spermine (5 mM) for 24 h showed significant conversion of 3'dR to the 3'P product. Panel C: Incubation of 3'dR at 37 ˚C in pH 7.4 HEPES buffer (50 mM, with 100 mM NaCl) containing 2-mercaptoethanol (5 mM) for 24 h, showed no significant conversion of 3'dR to new products. Panel D: Incubation of 3'dR at 37 ˚C in pH 7.4 HEPES buffer (50 mM, with 100 mM NaCl) containing 2-mercaptoethanol (5 mM) and spermine (5 mM) for 24 h, showed significant conversion of 3'dR to the 3'P product.

Figure S18

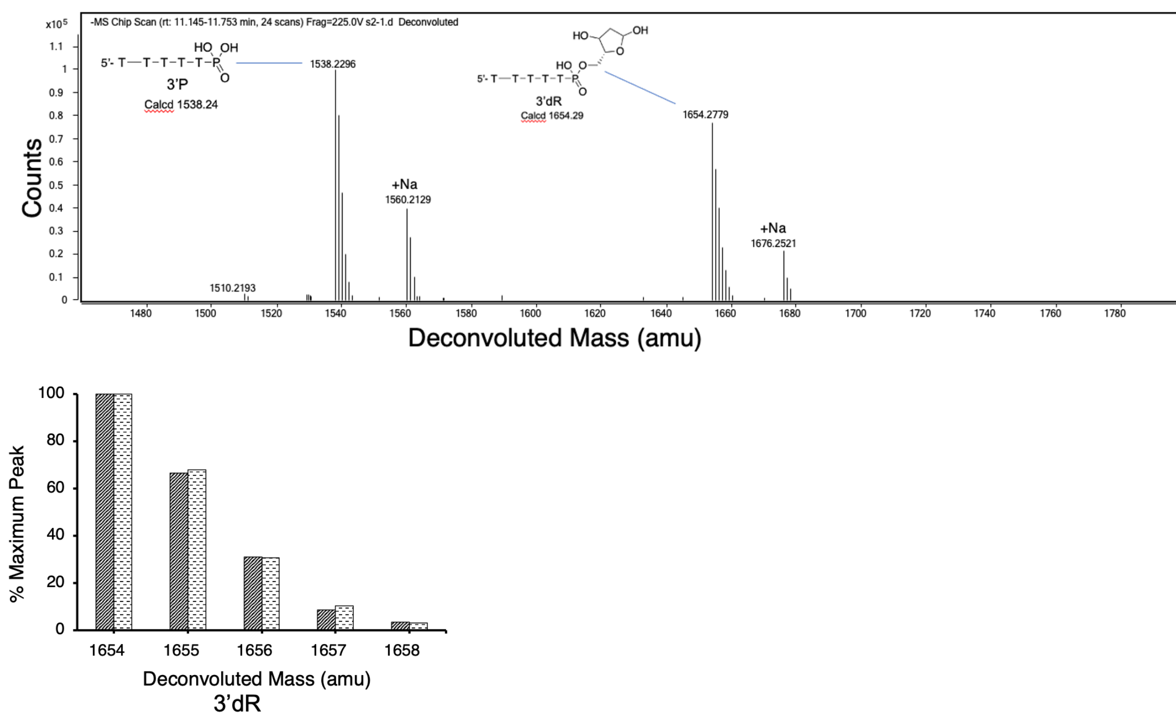

Figure S18. ESI(-)-QTOF-LC-MS analysis products generated by spermine-mediated cleavage of the AP-containing oligonucleotide at 24 h. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'dR and 3'P generated by treatment of the AP-containing DNA oligomer in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) with spermine (5 mM) at 37 ˚C for 24 h. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'dR cleavage product.

Figure S19

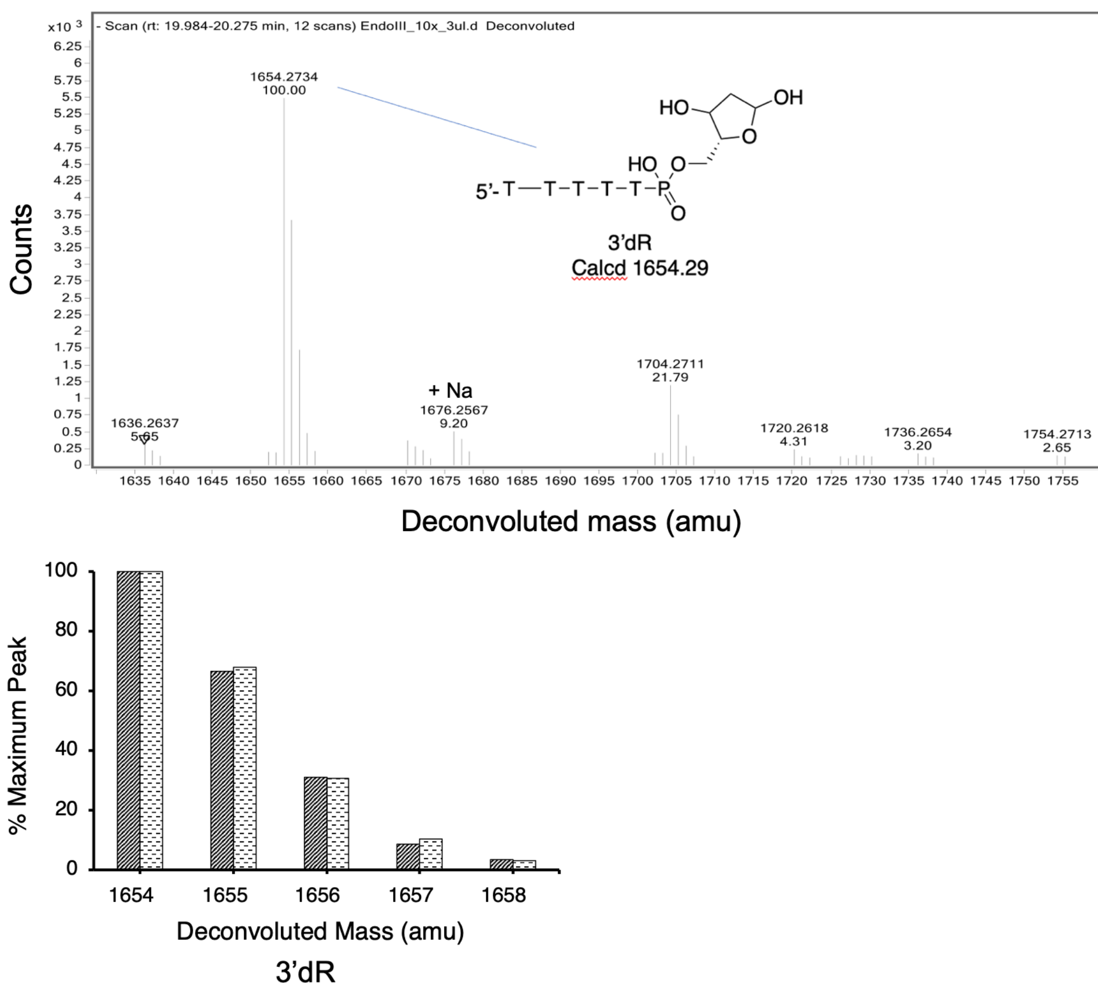

Figure S19. ESI(-)-QTOF-LC-MS analysis of the products generated by cleavage of the AP-containing oligonucleotide duplex by Endo III. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'dR and 3'P generated by treatment of the AP-containing DNA duplex in Endo III (20 units) in 20 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, at 37 ˚C for 2 h. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'dR cleavage product. The duplex was composed of an AP-containing strand 5'TTTTTXTTTTTTTTTT3', where X=AP and the complementary strand, 5'TTA$_{16}$TT.

Figure S20

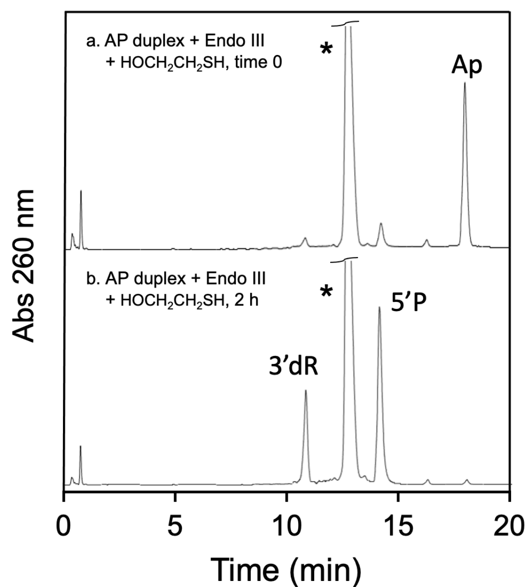

Figure S20. HPLC analysis of products generated by cleavage of the AP-containing oligonucleotide by Endo III in presence of 2-mercaptoethanol. When the AP-containing DNA duplex was incubated with Endo III in the presence of 2-mercaptoethanol for 2 h, 3'dR was observed as the major product. No significant amounts of 3'PUA or 3-thio-ddR products were seen. The AP-containing strand is 5'TTTTTXTTTTTTTTTT3', where X=AP and the large peak labeled with (*) corresponds to the complementary strand, 5'TTA$_{16}$TT.