

Supporting Information: DNA Double Strand Cleavage via Interstrand Hydrogen Atom Abstraction.

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General Methods. Phosphoramidite **2** and oligonucleotides containing it were synthesized as previously described.^{1,2} Oligonucleotides were synthesized on an Applied Biosystems Incorporated 394 oligonucleotide synthesizer. Oligonucleotide synthesis reagents were purchased from Glen Research (Sterling, VA.). C-18-Sep-Pak cartridges were obtained from Waters. Expression and purification of all core histone proteins, as well as refolding and purification of the histone octamer, were carried out as previously described². T4 polynucleotide kinase and T4 DNA ligase were obtained from New England Biolabs (NEB). γ -³²P-ATP was from Perkin Elmer. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager 840 equipped with ImageQuant TL software. Photolyses of oligonucleotides were carried out in a Rayonet photoreactor (RPR-100) fitted with 16 lamps having an output maximum at 350 nm. ESI-MS was carried out on a Thermoquest LCQDeca. LC/MS were carried out with a Waters Xevo G2 Q-ToF mass spectrometer.

General procedure for preparation of 145 mer single stranded DNA. The oligonucleotides used for preparing each 145 mer ssDNA are shown in Figure S1. Chemically synthesized oligonucleotides were enzymatically phosphorylated at their 5'-termini, each in a separate 100 μ L reaction containing 1.5 nmol DNA, 1 \times T4 DNA ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 10 mM DTT, 1 mM ATP), and 50 U of T4 polynucleotide kinase (T4 PNK) at 37 °C for 4 h. T4 PNK was deactivated by heating at 95°C for 5 min. The phosphorylated oligonucleotides were combined with the unphosphorylated 5'-terminal oligonucleotides (1.5 nmol) together with two or three scaffold strands (2 nmol) and hybridized by heating at 95 °C for 2 min, followed by slow cooling to room temperature. T4 DNA ligase (1200 U) was added and the mixture was incubated at 16 °C overnight. The reaction was ethanol precipitated prior

to purification by 8 % denaturing PAGE (20 × 16 × 0.1 cm). The gel was run under limiting power (15 W) until the bromophenol blue migrated to the bottom of the gel. The product band was excised from the gel and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The slurry was ethanol precipitated to give 145 mer ssDNA.

General procedure for preparing ³²P labeled 145 bp double stranded 9. One of the 145 nucleotide long strands of DNA synthesized above (either the strand containing **2** or its complementary strand) were 5'-³²P-labeled in a 50 μL reaction containing 20 pmol DNA, 1 × T4 PNK buffer (70 mM TRIS-HCl pH 7.6, 10 mM MgCl₂, 5 mM DTT), 50 μCi γ-³²P-ATP, and 50 U of T4 PNK at 37 °C for 3 h. The reaction was stopped by heating at 65 °C for 20 min and then 30 pmol of complementary DNA was added. The mixture was heated at 95 °C for 2 min, followed by slow cooling to room temperature. The reaction was ethanol precipitated prior to purification by 8 % nondenaturing PAGE (20 × 16 × 0.1 cm). The gel was run at constant power (7 W) until the xylene cyanol band migrated to the bottom of the gel. The product band was excised from the gel and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The gel was removed by filtration. The eluted DNA was then evaporated up to 200 μL and precipitated by addition of 600 μL of EtOH. Finally it was washed twice with 70 % EtOH to give the 145 bp dsDNA **9**.

Reconstitution of nucleosome core particles (NCPs) containing 145 mer duplex 9.³ Salmon sperm DNA (10 μg, ~ 85 pmol) and 5'-³²P-**9** (~ 1 pmol) were combined in a small siliconized tube to a final volume of 10 μL containing 2 M NaCl. The appropriate amount of histone octamer (~ 85 pmol) (also in 2 M NaCl) was added and the sample (total volume around 11-12 μL) was incubated at 30 °C in the heat block of a thermal cycler for 30 min before beginning a series of dilutions using nucleosome buffer (10 mM HEPES, 1 mM EDTA, and 0.1 mM PMSF, pH 7.5). Dilution # (volume of buffer added in μL, incubation time in minutes): 1: 12, 60; 2: 6, 60; 3: 6, 60; 4: 10, 30; 5: 10, 30; 6: 20, 30; 7: 50, 30; 8: 100,

30. After the final dilution (total volume ~225 μ L), the sample was cooled to room temperature and any precipitate was pelleted via a brief (5 min) spin at 15,000 g. The solution was then transferred to a fresh siliconized tube, a small aliquot was removed and analyzed by nucleoprotein gel electrophoresis to determine the extent of reconstitution (10 \times 8 \times 0.15 cm, 5 %, acrylamide/bisacrylamide, 59:1, 0.6 \times TBE buffer, run at 4 $^{\circ}$ C using 0.2 \times TBE buffer. The gel was run under limiting voltage (150 V) until the bromophenol blue band migrated to the bottom of the gel. All reconstituted nucleosome core particles were stored at 4 $^{\circ}$ C and under this condition no dissociation was detected for up to two weeks.

Photolysis of NCP containing 9. NCPs prepared above were used directly in these experiments without adjustments in their concentrations following reconstitution (10 mM HEPES, 100 mM NaCl, 1 mM EDTA, and 0.1 mM PMSF, pH 7.5). NCPs containing the radical precursor were photolyzed in the Rayonet photoreactor equipped with 16 lamps (λ_{max} = 350 nm, 4 h) at room temperature. The samples were treated with proteinase K (1 μ L (20 μ g, 10 min, 25 $^{\circ}$ C) and analyzed by 12 % non denaturing PAGE (20 \times 16 \times 0.1 cm). The gel was run at 250 V, 30 mA, and 7 W until the bromophenol blue band migrated to the bottom of the gel. Additional analysis of photolysed NCP was performed by 10 % denaturing PAGE after being subjected to the chemical treatments described below..

General procedure for photolysis of free 9, 35 bp duplexes, and ternary complex 17. The strand of interest was 5'-labeled with γ - 32 P-ATP using T4 PNK in T4 PNK buffer (70 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 5 mM DTT, 45 min, 37 $^{\circ}$ C). Radiolabeled oligonucleotides were purified by gel filtration using Sephadex G-25. Prior to photolysis, labeled strands were hybridized to the complementary strand in PBS (0.1 M NaCl, 10 mM sodium phosphate pH 7.2) by heating at 90 $^{\circ}$ C for 2 min and slow cooling to room temperature. DNA was photolyzed (350 nm, Rayonet fitted with 16 lamps) under aerobic or anaerobic conditions. Samples were degassed by freeze-pump-thaw degassing (3 X) and sealed under

vacuum using a high vacuum line. Chemically treated samples (as described below) were analyzed by 10% denaturing PAGE (145 bp duplexes) or 20% denaturing page PAGE (35 bp duplexes).

Chemical and enzymatic treatments. Aliquots were treated with NaOH (0.1 M, 30 min, 37°C; neutralized with 0.1 M HCl after base treatment) or piperidine (1 M, 30 min, 90°C). Alternatively, Ir⁴⁺ (0.1 mM of Na₂IrCl₆•6H₂O, 1 h, 25 °C) treated samples were quenched (2 mM Hepes, 10 mM EDTA, pH 7) and treated with piperidine (1 M, 30 min, 90 °C).⁴ Additional treatments involved PNK treatment using T4 PNK (3 U) in T4 PNK buffer (30 min, 37°C) or hydrazine treatment using freshly prepared 100 mM hydrazine (1 h, 25 °C).

LC-MS characterization of photolyzed oligonucleotides. Duplexes (20 μM) were hybridized and photolyzed as describe above. An orthogonal ESI-Q-TOF mass spectrometer (Xevo-G2, Waters) was connected in-line with an ACQUITY H-class UPLC. Mass spectra were acquired in negative ion mode using an ESI-MS capillary voltage of 2.5 kV, a sample cone voltage of 35 V and an extraction cone voltage of 4 V. The cone gas flow was set up to 0 L/h and desolvation gas flow was 400 L/h. Desolvation temperature and source temperature were set to 350 and 120 °C, respectively. The acquisition range was m/z 500–3000. The LC/MS system was operated by the Mass Lynx software. Raw summed spectra were deconvoluted using the MaxEnt1 algorithm.

LC was performed using a linear gradient containing mobile phase A (15 mM TEA, 200 mM HFIP) to mobile phase B (50 % MeOH, 50 % mobile phase A) on a ACQUITY UPLC®BEH C18 Column (17 μm, 21 x 50 mm) using the following linear gradient (Table 1). The built in column heater of the UPLC system was set to 60 °C and mobile phase flow rate was 0.2 ml/min.

Table S1. Linear gradient used in LC/MS analysis.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	90	10
2.0	90	10
30.0	55	45
31.0	10	90
33.0	10	90
33.1	90	10

References:

- (1) Marx, A., Erdmann, P., Senn, M., Korner, S., Jungo, T., Petretta, M., Imwinkelreid, P., Dussy, A., Kulicke, K. J., Macko, L., Zenhder, M., Giese, B., *Helvetica Chimica Acta*, **1996**, 79, 1980-1994.
- (2) a) Meggers, E., Kusch, D., Spichy, M., Wille, U., Giese, B., *Angew. Chem. Int. Ed.*, **1998**, 37, 459-461. b) Meggers, E., Dussy, A., Schäfer, T., Giese, B. *Chem. Eur. J.* **2000**, 6, 485-492.
- (3) Dyer, P.D., Edayathumangalam, R., White, K., Bao, Y., Chakravarthy, S., Muthurajan, U., Luger, K., *Methods Enzymol.* **2004**, 375, 23-44.
- (4) Muller, J., Duarte, V., Hickerson, R., Burrows, C, *Nucleic Acid Res.*, **1998**, 26, 2247-2249.

(S1) ATCGATGTATATATCTGACACCTGCCTGGA
 (S2) GACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCG
 (S3) GGTGACAGCTATTGATCTTATT²TTGTGATGCTAG
 (S4) AGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGGATTCTGAT
 (S5) ATCAGAATCCCGGTGCCGAGGCCGCTCAATTGGTC
 (S6) GTAGACAGCTCTAGCATCACAAAATAAGATCAATAGCTGTCACCCGCGTTTTAA
 (S7) CCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGT GTCAGATATATACATCGAT
 (S8) CTCCCTAGTCTCCAGGCACG
 (S9) AGCTGTCACCCGCGTTTTAA
 (S10) GTAGACAGCTCTAGCATCAC
 (S11) GCG ATG CTA G GAC CAA TTG A
 (S12) CCC TTG GCG G GGT GAC AGC T

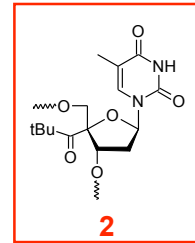


Figure S1. Oligonucleotides synthesized for preparing 145mer single stranded oligonucleotides (601).

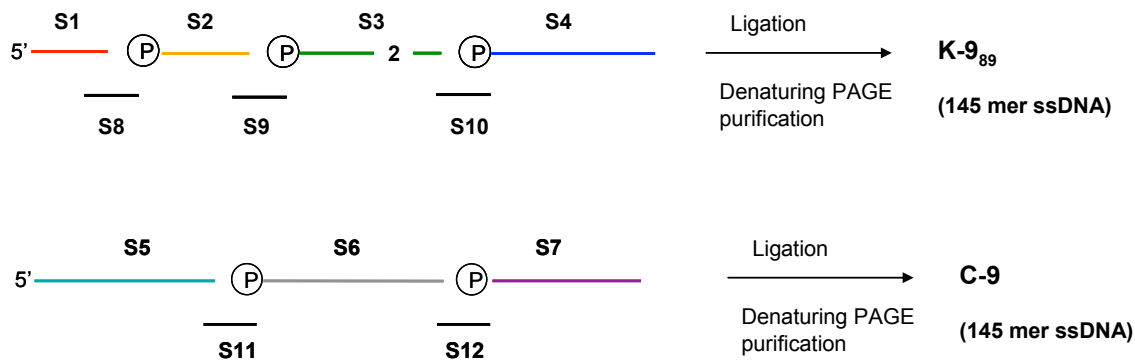


Figure S2. Preparation of 145mer ssDNA by ligation.

5'-d(ATCGATGTATATATCTGACACCTGCCTGGAGACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCGGGTGACAGCT-
 3'-d(TAGCTACATATATAGACTGTGGACGGACCTCTGATCCCTCATTAGGGGAACCGCCAATTTTGCGCCCACTGTCGA-
 -ATTGATCTT ATT²₈₉TT GTGATGCTAGAGCTGTCTACGACCAATTGAGCGG CCTCGGCACCGGGATTCTGAT)3'
 -TAACTAGAATAAA AACACTACGATCTCGACAGATGCTGGTAACTCGCC GGAGCCGTGGCCCTAAGACTA)5'

Figure S3. Sequence of 145 bp DNA containing **2** (9).

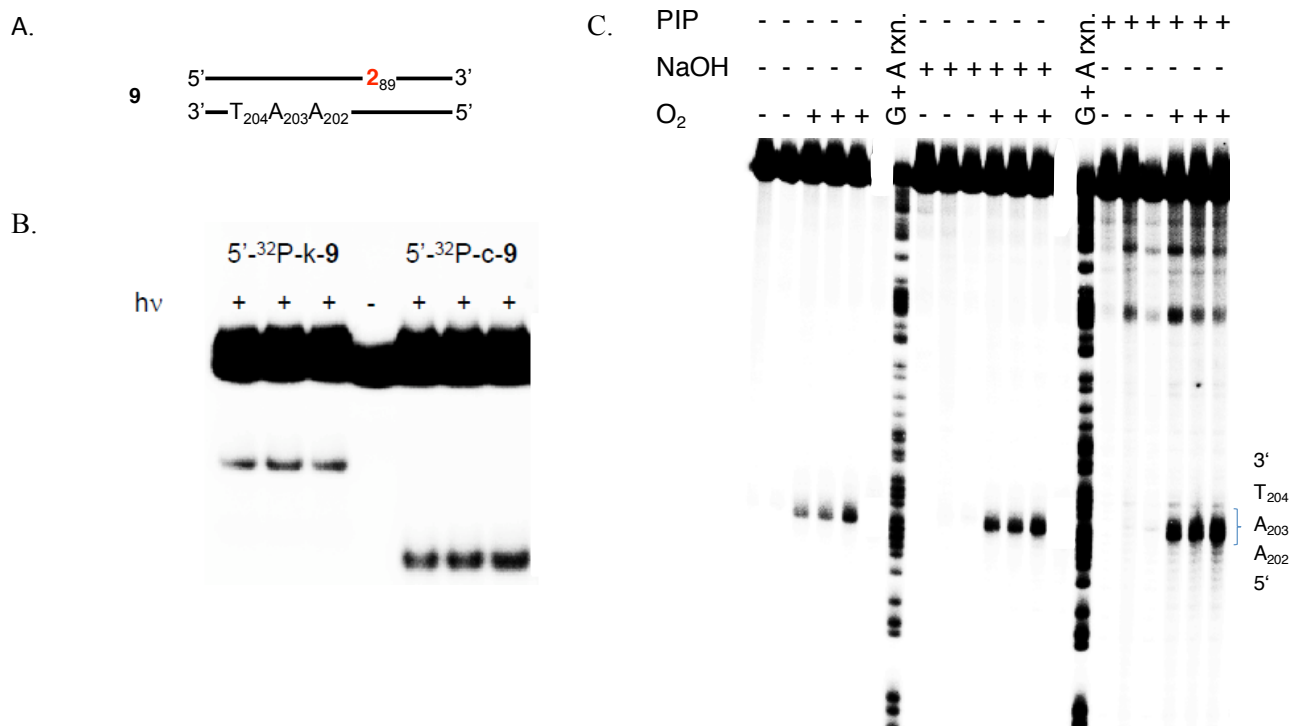


Figure S4. DSB formation upon photolysis of free **9**. A. Portion of **9** containing **2** at position 89. B. 12 % native PAGE showing DSB formation. C. 10 % denaturing PAGE autoradiogram showing direct strand scission, NaOH, and piperidine-induced strand cleavage of aerobic and anaerobic photolysis of 5'-³²P-c-**9**.

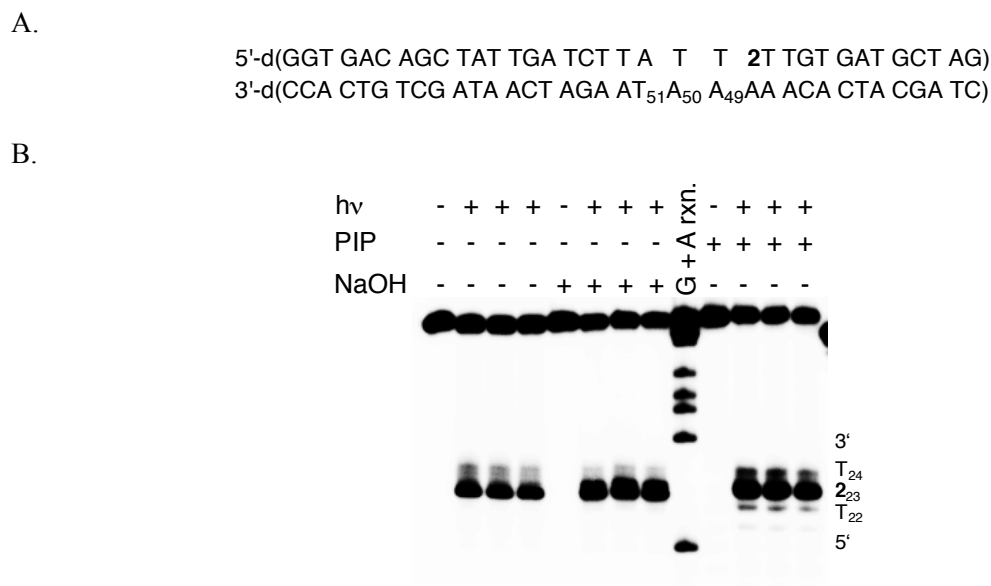


Figure S5. 20 % denaturing PAGE analysis of photolysis of 5'-³²P-k-**10**. A. Sequence of **10** containing **2**. B. 20% denaturing PAGE autoradiogram showing direct strand scission, NaOH, and piperidine-induced strand cleavage of aerobic photolysis of 5'-³²P-k-**10**.

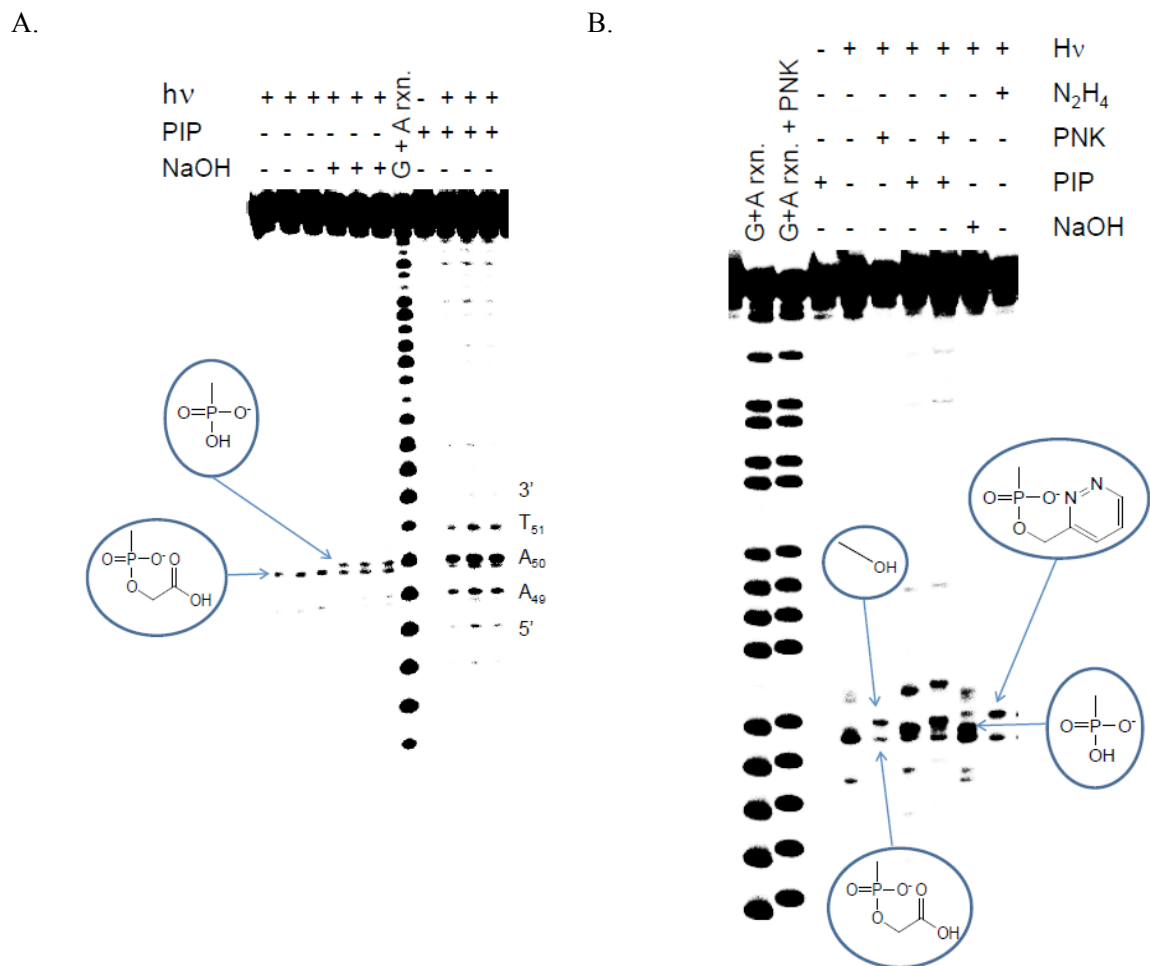


Figure S6. Denaturing PAGE (20%) analysis of aerobic photolysis of duplex 5'-³²P-c-10. A. 20% PAGE autoradiogram showing direct strand scission, NaOH, and piperidine-induced strand cleavage of aerobic photolysis of 5'-³²P-c-10. B. Representative 20% PAGE denaturing showing the effect of various post-photolysis treatments on strand cleavage of 5'-³²P-c-10. The direct strand scission band moves slightly faster than the Maxam-Gilbert “G+A” sequencing marker (3'-phosphate) and corresponds to a DNA fragment bearing a 3'-phosphoglycolate terminus. NaOH and piperidine treatments revealed the formation of an additional cleavage band, which co-migrate with the “G+A” sequencing marker (3'-phosphate). PNK treatment does not affect the migration of the direct strand scission product (3'-phosphoglycolate). The formation of 3'-pyridazine terminus is evidenced by formation of a slower migrating product upon treatment with hydrazine, indicating the presence of C4-AP.

5'-d(GGT GAC AGC TAT TGA TCT T A T T 2T TGT GAT GCT AG)
 3'-d(CCA CTG TCG ATA ACT AGA AT₅₁A₅₀ A₄₉AA ACA CTA CGA TC)
10

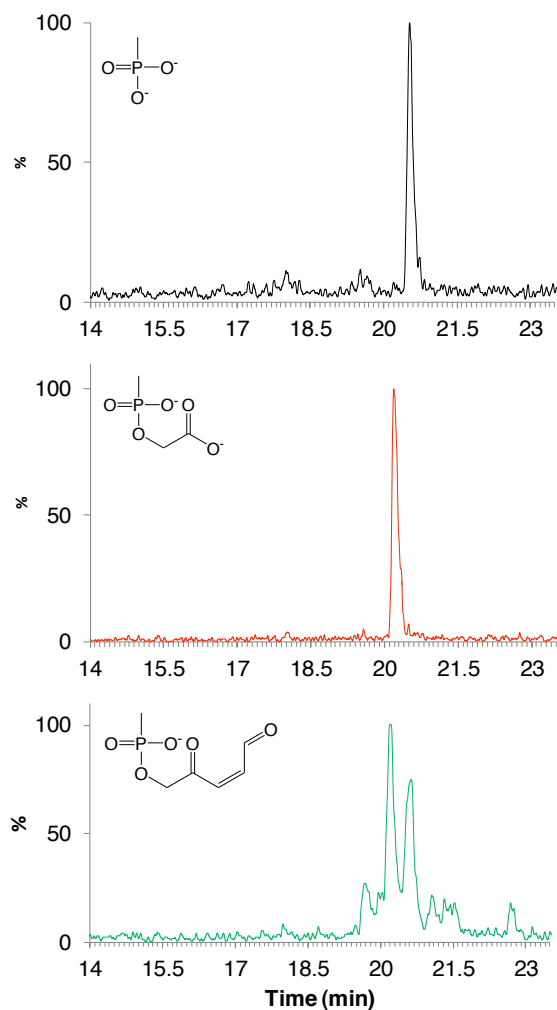
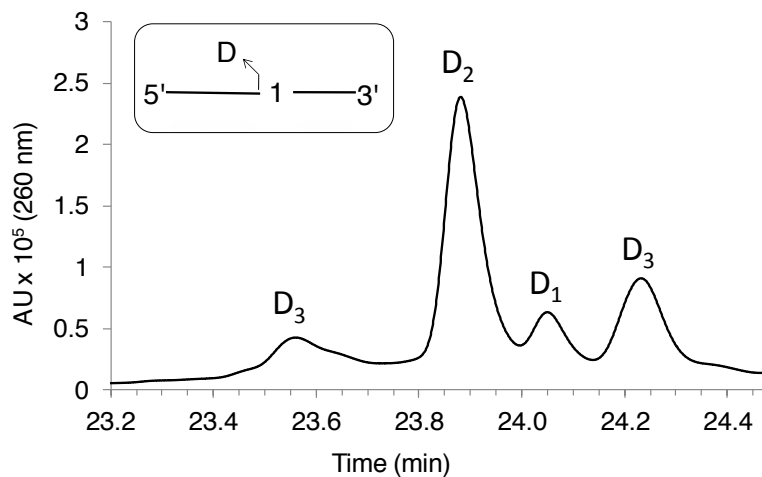


Figure S7. LC/MS analysis of the complementary strand of photolyzed **10**. Extracted ion chromatograms (XIC) of the masses 4304.5 (Top), 4363.3 (Middle), 4399.5 (Bottom) are shown. These masses correspond to DNA fragments bearing the different terminus generated from cleavage at position A50 (structures of the corresponding 3'-terminal products are shown in the top left of each chromatogram).

5'-d(GGT GAC AGC TAT TGA TCT T A T T 2T TGT GAT GCT AG)
 3'-d(CCA CTG TCG ATA ACT AGA AT₅₁A₅₀ A₄₉AA ACA CTA CGA TC)
10



Peak	Terminus	Experimental mass (Da)	Calculated mass (Da)
D ₁	3'-OPO ₃ ⁻²	6836.2	3991.6
D ₂	3'-OPO ₃ CH ₂ COO ⁻	6894.4	4362.8
D ₃	3'- OPO ₃ -X	6931.2	4304.8

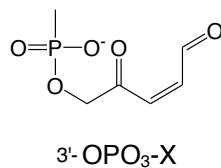


Figure S8. LC/MS analysis of the ketone containing strand of photolyzed **10**.

A.

5'-d(GGT GAC AGC TAT TGA TCT T T T T 2T TGT GAT GCT AG)
 3'-d(CCA CTG TCG ATA ACT AGA AA₅₁A₅₀ A₄₉AA ACA CTA CGA TC)
11

5'-d(GGT GAC AGC TAT TGA TCT T T A A 2T TGT GAT GCT AG)
 3'-d(CCA CTG TCG ATA ACT AGA AA₅₁T₅₀ T₄₉ AA ACA CTA CGA TC)
12

5'-d(GGT GAC AGC TAT TGA TCT A A T T 2A AGT GAT GCT AG)
 3'-d(CCA CTG TCG ATA ACT AGA TT₅₁A₅₀ A₄₉ AT TCA CTA CGA TC)
13

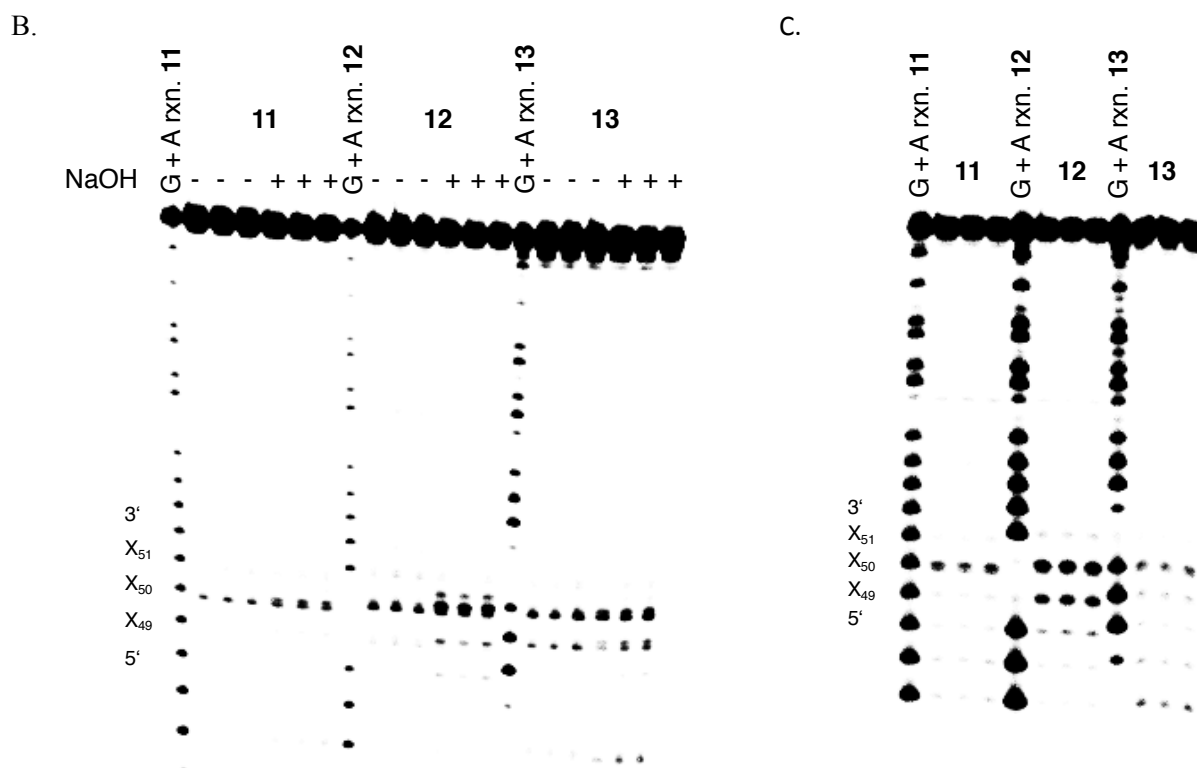
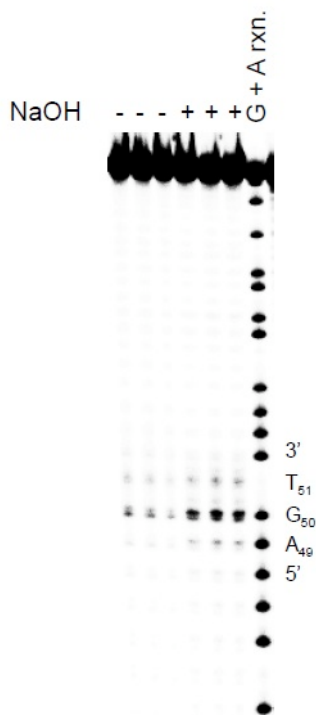


Figure S9. 20 % denaturing PAGE analysis of aerobic photolysis of 5'-³²P-c-**11**, 5'-³²P-c-**12**, and 5'-³²P-c-**13**. A. Sequence of duplexes **11**, **12** and **13** containing **2**. B. Autoradiogram showing direct strand scission and NaOH-induced strand cleavage. C. Autoradiogram showing piperidine-induced strand cleavage.

A.

5'-d(GGT GAC AGC TAT TGA TCT T A C T 2T TGT GAT GCT AG)
3'-d(CCA CTG TCG ATA ACT AGA AT₅₁G₅₀A₄₉AA ACA CTA CGA TC)
15

B.



C.

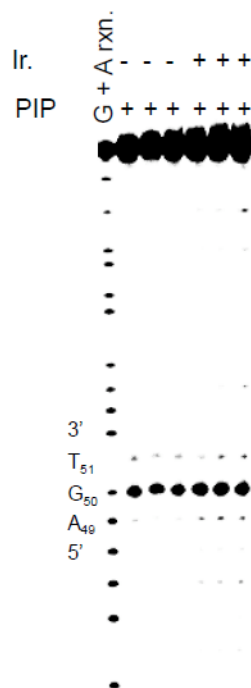
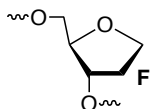


Figure S10. Denaturing PAGE (20%) of aerobic photolysis of 5'-³²P-c-15. A. Sequence of duplex **15** containing **2**. B. Autoradiogram showing direct strand scission and NaOH-induced strand cleavage. C. Autoradiogram showing piperidine and iridium-induced strand cleavage. Iridium treatment was used to check for the formation of 8-oxo-2'-deoxyguanosine, which is not piperidine labile (See: Muller, J. G.; Duarte, V.; Hickerson, R. P.; Burrows, C. J. *Nucleic Acids Res.* **1998**, *26*, 2247-49.).

A.

5'-d(GGT GAC AGC TAT TGA TCT T A T T 2T TGT GAT GCT AG)
3'-d(CCA CTG TCG ATA ACT AGA AT₅₁A₅₀ A₄₉AA ACA CTA CGA TC)
10

5'-d(GGT GAC AGC TAT TGA TCT T A T F 2T TGT GAT GCT AG)
3'-d(CCA CTG TCG ATA ACT AGA AT₅₁A₅₀ A₄₉AA ACA CTA CGA TC)
16



B.

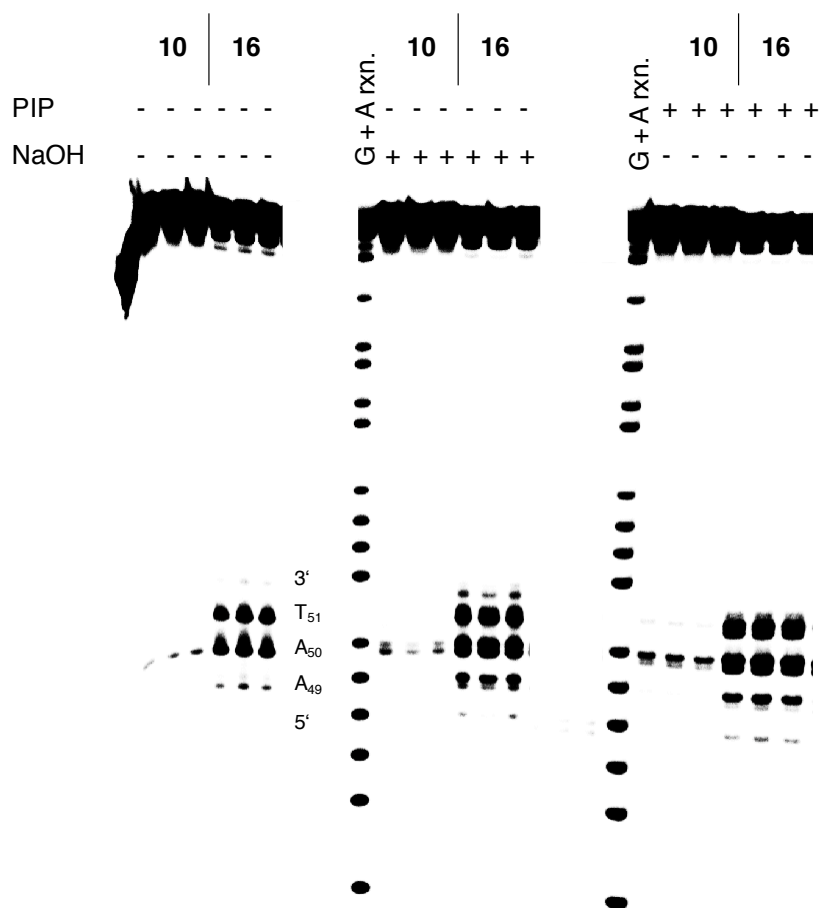


Figure S11. Denaturing PAGE (20%) of aerobic photolysis of 5'-³²P-c-**10** and 5'-³²P-c-**16**. A. Sequence of duplexes **10** and **16** containing **2**. B Autoradiogram showing direct strand scission, NaOH, and piperidine-induced strand cleavage.

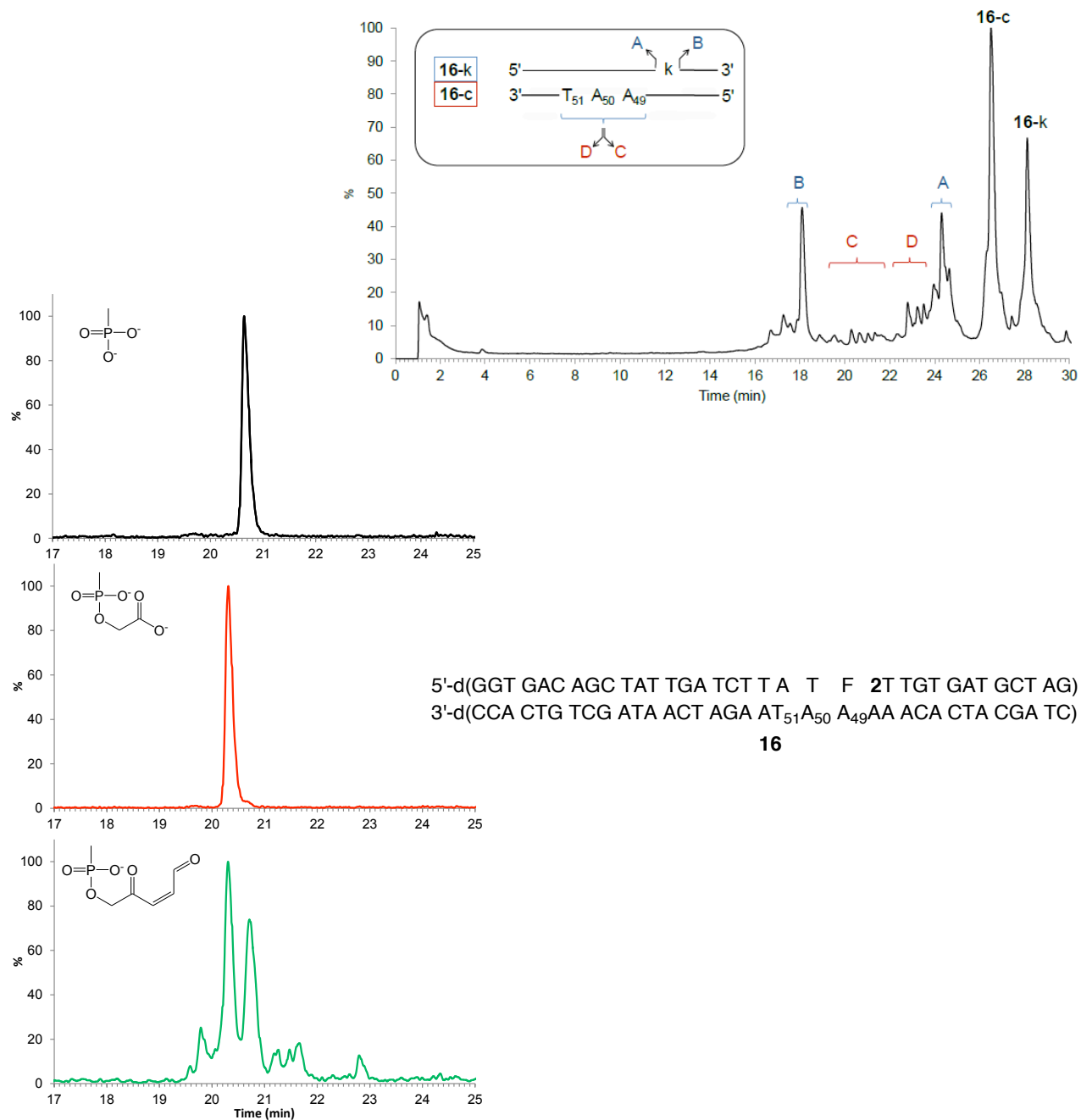
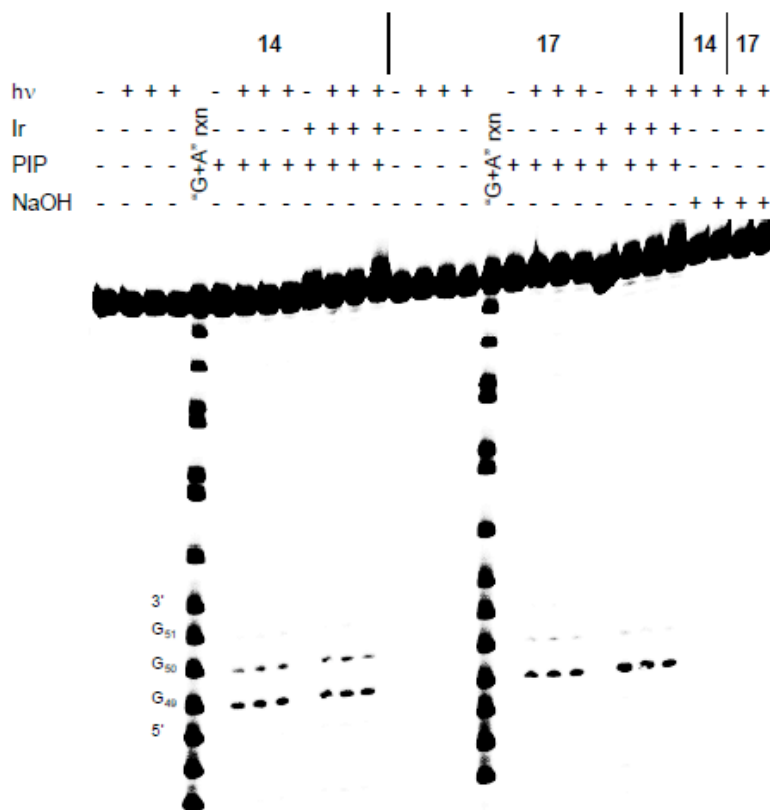


Figure S12. LC/MS analysis of photolyzed **16**. Top: Total Ion Chromatogram scaled relative to the MS scan with the greatest ion count. Groups of peaks are named as follows. A: 3'-cleavage fragments of the ketone containing strand B: 5'-cleavage fragments of the ketone containing strand, C: 3'-cleavage fragments of the complementary strand, D: 5'-cleavage fragments of the complementary strand. Bottom: Complementary strand product analysis. Extracted ion chromatograms of the masses 4305.2 (Black), 4363.2 (Red). 4400.1 (Green). These masses correspond to DNA fragments bearing the respective termini generated from cleavage at A₅₀.

A. 5'-d(GGT GAC AGC TAA TTG ATA T C C C T2 TGC GAT GCT AG)
 3'-d(CCA CTG TCG ATT AAC TAT AG₅₁G₅₀ G₄₉ AA ACG CTA CGA TC)
 14

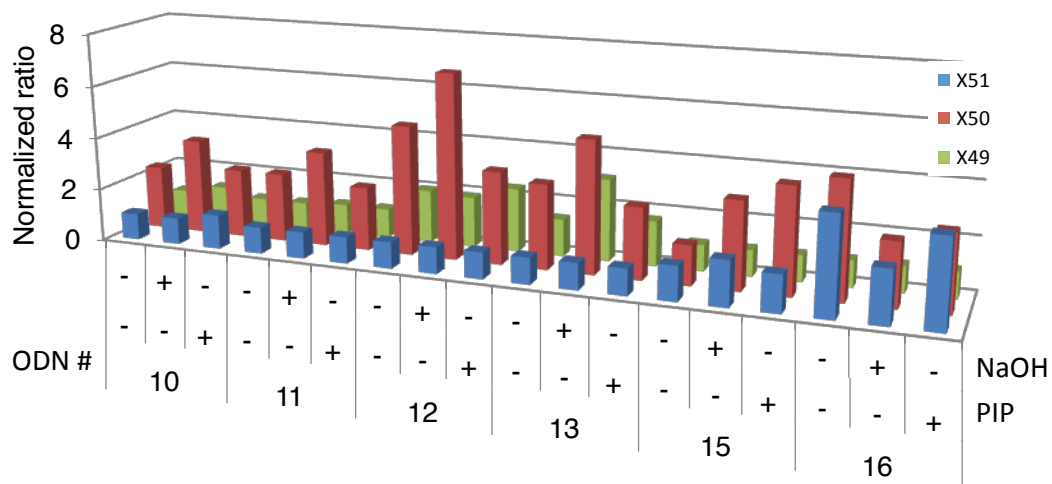
5'-d(GGT GAC AGC TAA TTG ATA T C C C T2-OH TGC GAT GCT AG)
 3'-d(CCA CTG TCG ATT AAC TAT AG₅₁G₅₀ G₄₉ AA _____ ACG CTA CGA TC)
 17

B.



	14			17		
PIP	-	-	+	-	-	+
NaOH	-	+	-	-	+	-
% cleavage GGG	0.4 ± 0.1	0.5 ± 0.1	4.0 ± 0.5	0.3 ± 0.1	0.4 ± 0.1	3.2 ± 0.4

Figure S13. Denaturing PAGE (20%) of aerobic photolysis of 5'-³²P-c-14 and 5'-³²P-c-17. A. Sequence of duplex **14** and ternary complex **17** containing **2**. B. Autoradiogram showing direct strand scission, NaOH, piperidine and iridium-induced strand cleavage. Iridium treatment was used to check for the formation of 8-oxo-2'-deoxyguanosine, which is not piperidine labile (See: Muller, J. G.; Duarte, V.; Hickerson, R. P.; Burrows, C. J. *Nucleic Acids Res.* **1998**, *26*, 2247-49.).

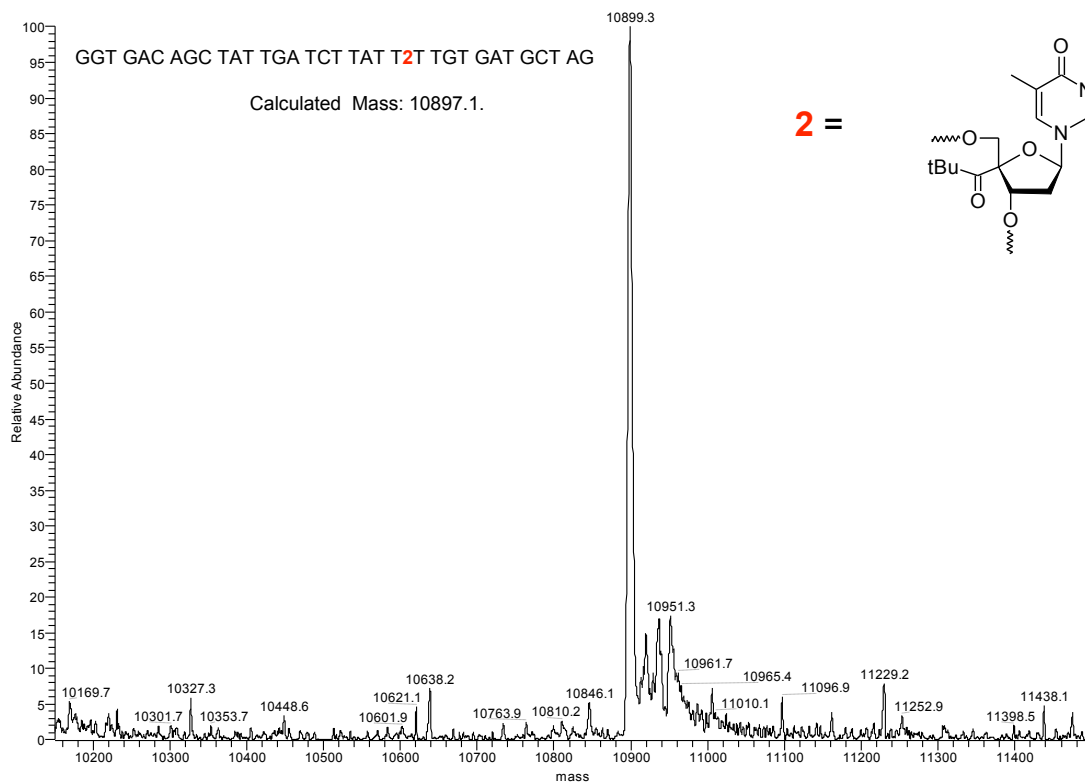


	10			11			12		
PIP	-	-	+	-	-	+	-	-	+
NaOH	-	+	-	-	+	-	-	+	-
X ₅₁	1.0	1.0	1.3	1.0	1.0	1.0	1.0	1.0	1.0
X ₅₀	2.4	3.6	2.6	2.6	3.6	2.4	4.9	7.0	3.5
X ₄₉	1.0	1.3	1.0	1.0	1.1	1.1	2.0	1.9	2.4

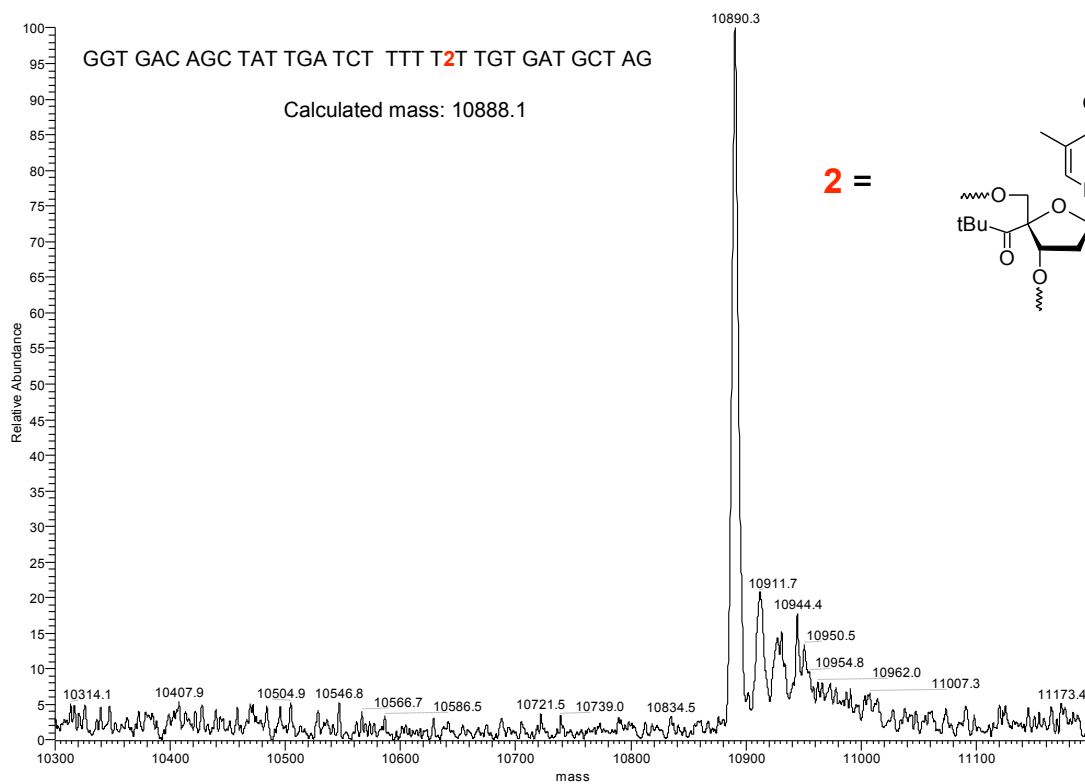
	13			15			16		
PIP	-	-	+	-	-	+	-	-	+
NaOH	-	+	-	-	+	-	-	+	-
X ₅₁	1.0	1.0	1.0	1.3	1.7	1.4	3.7	2.0	3.3
X ₅₀	3.2	5.0	2.7	1.5	3.3	4.0	4.4	2.4	2.9
X ₄₉	1.4	3.1	1.7	1.0	1.0	1.0	1.0	1.0	1.0

Figure S14. Ratios of cleavage at positions X₅₁, X₅₀ and X₄₉ in the complementary strand for oligonucleotides **10-13, 15** and **16** by direct strand scission, NaOH, and piperidine-induced strand scission. The values are normalized to the site where strand scission is weakest.

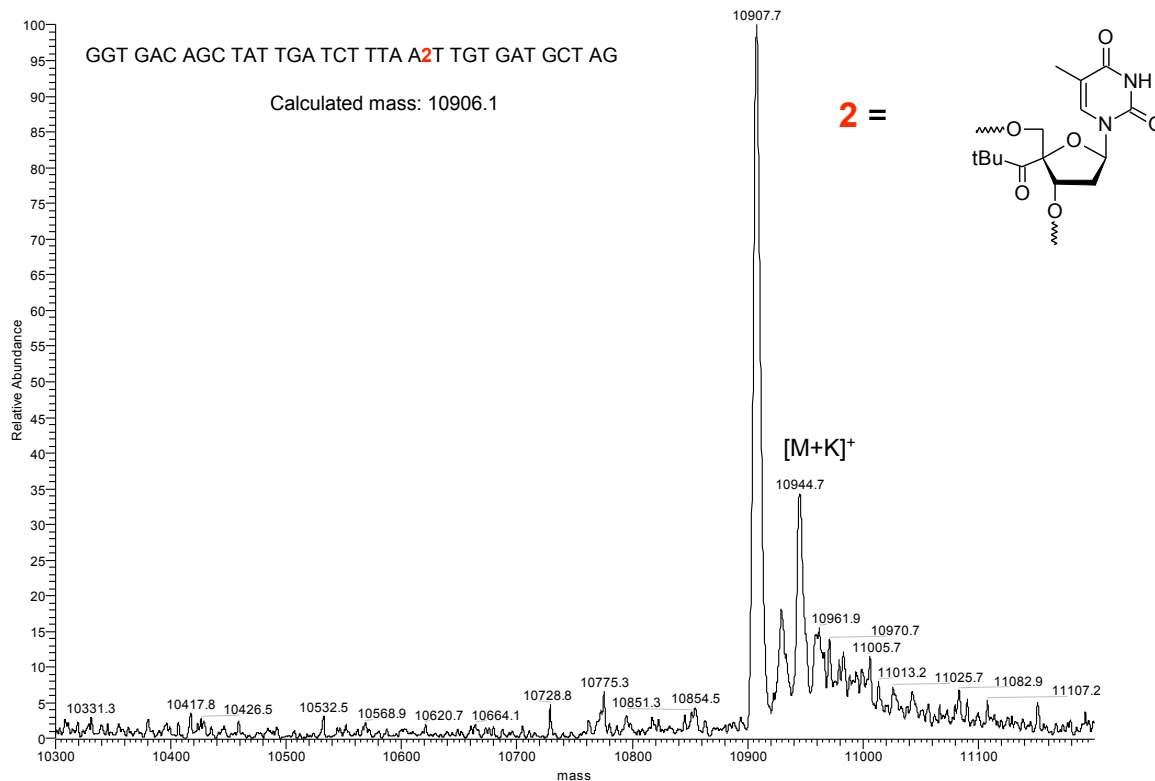
ODN5 #1 RT: 0.01 NL: 2.46E7
F: -p ESI Full ms [150.00-2000.00]



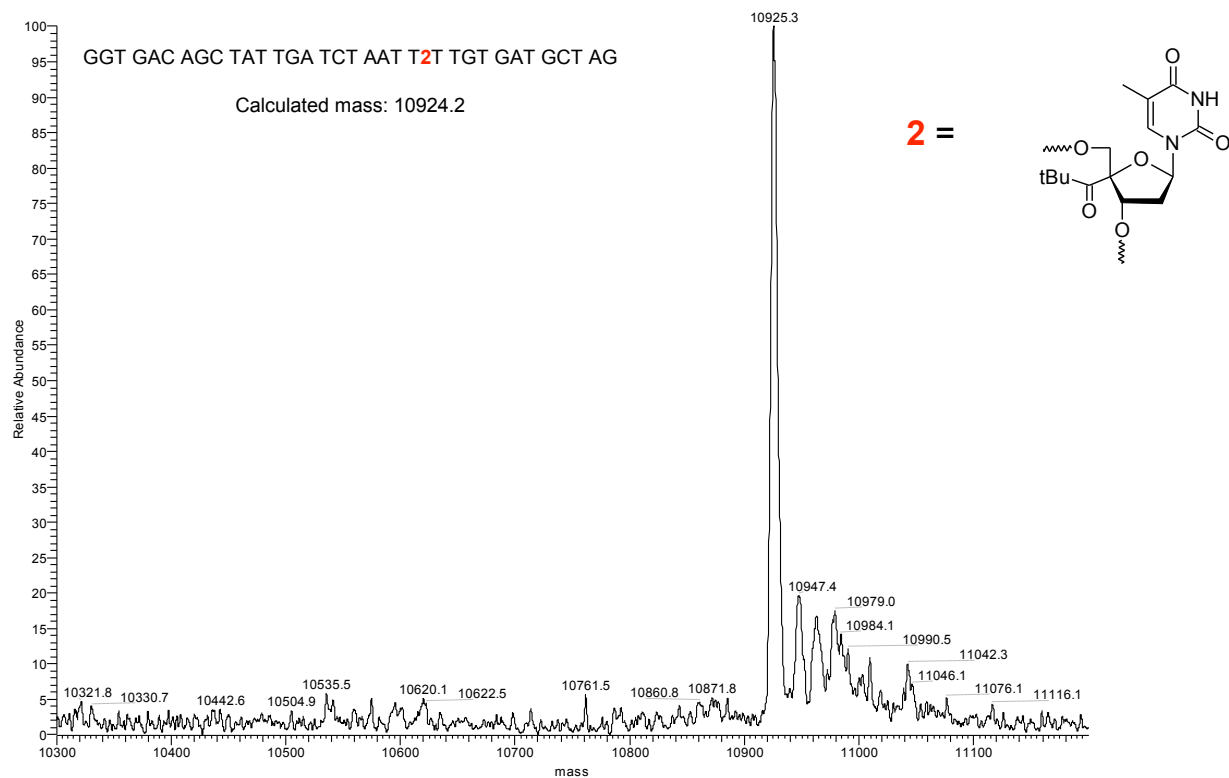
MTP-3-25 #1 RT: 0.03 NL: 6.62E7
F: -p ESI Full ms [50.00-2000.00]



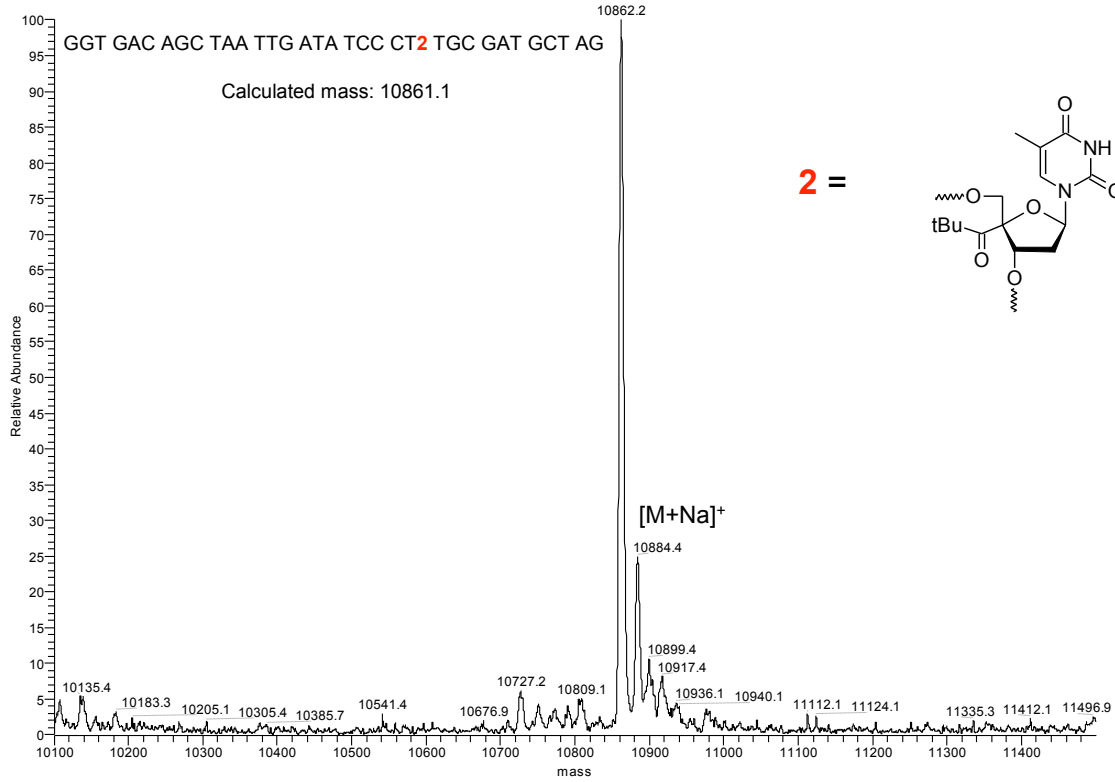
Mp-3-26A_130527192237 #1 RT: 0.02 NL: 5.51E5
F: -p ESI Full ms [100.00-2000.00]



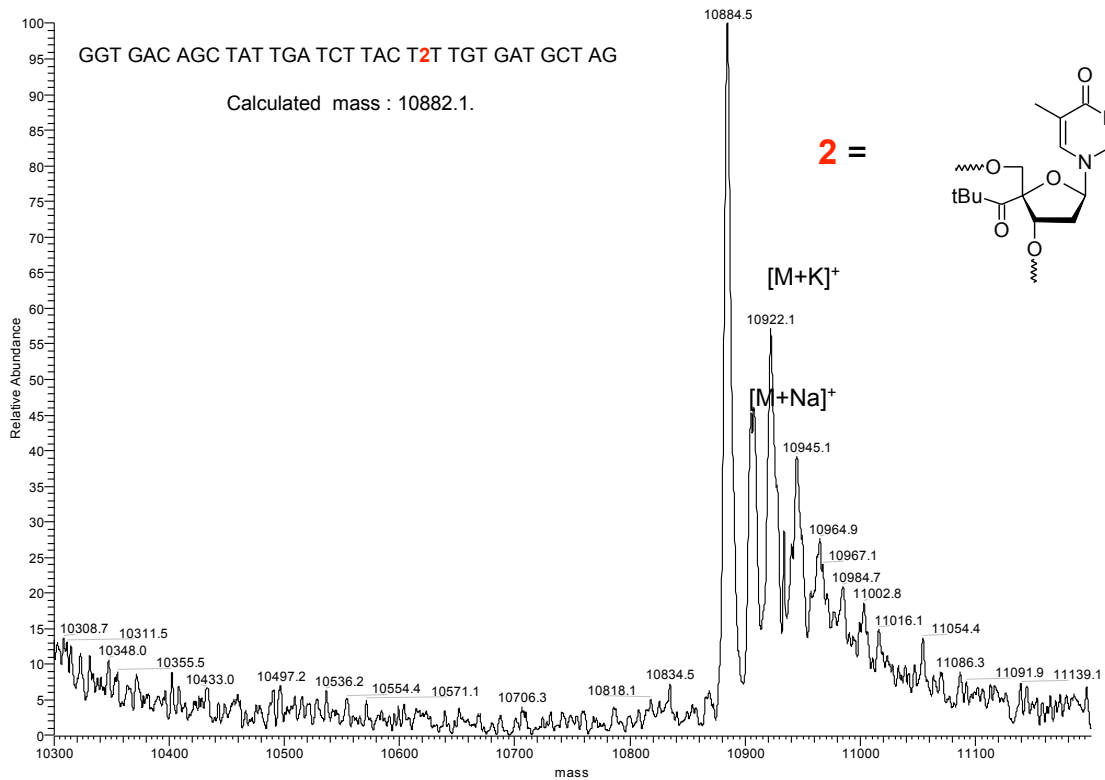
MTP-3-27 #1 RT: 0.01 NL: 2.73E7
F: -p ESI Full ms [50.00-2000.00]



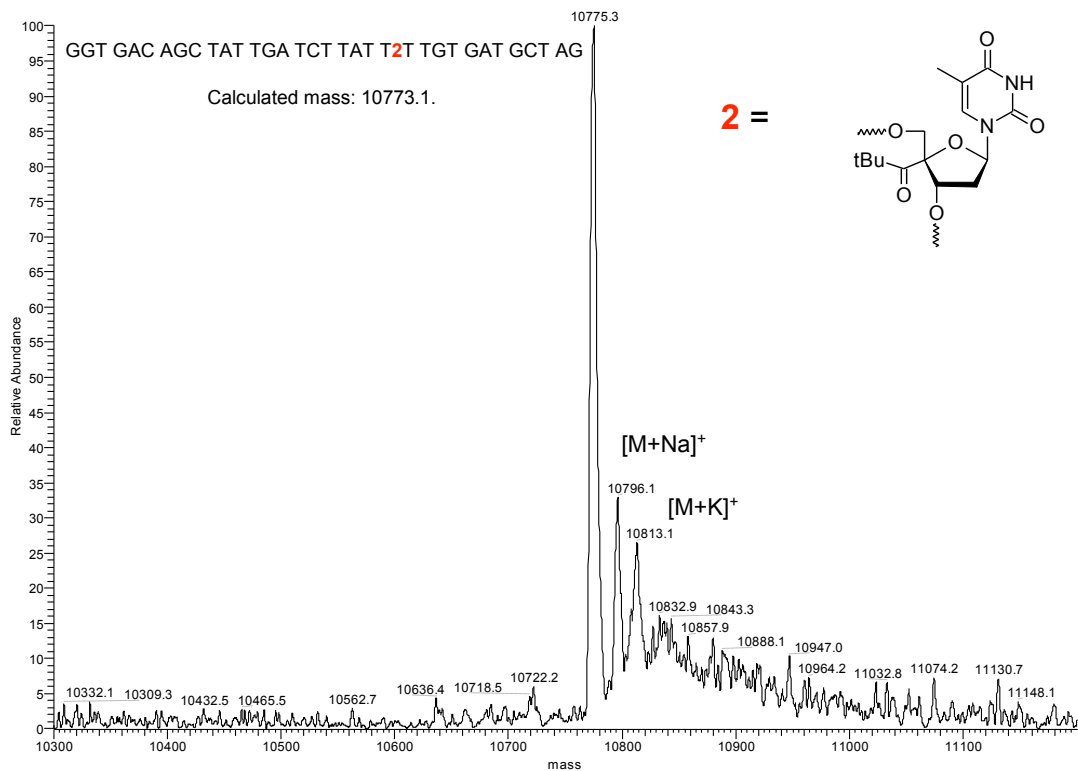
MTP95-ON-2 #30 RT: 0.01 NL: 1.18E6
F: -p ESI Full ms [150.00-2000.00]



86 #1 RT: 0.01 NL: 6.01E7
F: -p ESI Full ms [150.00-2000.00]



3-93 #1 RT: 0.01 NL: 1.35E7
F: -p ESI Full ms [150.00-2000.00]



mtp-3-58 #1 RT: 0.02 NL: 1.92E7
F: -p ESI Full ms [200.00-2000.00]

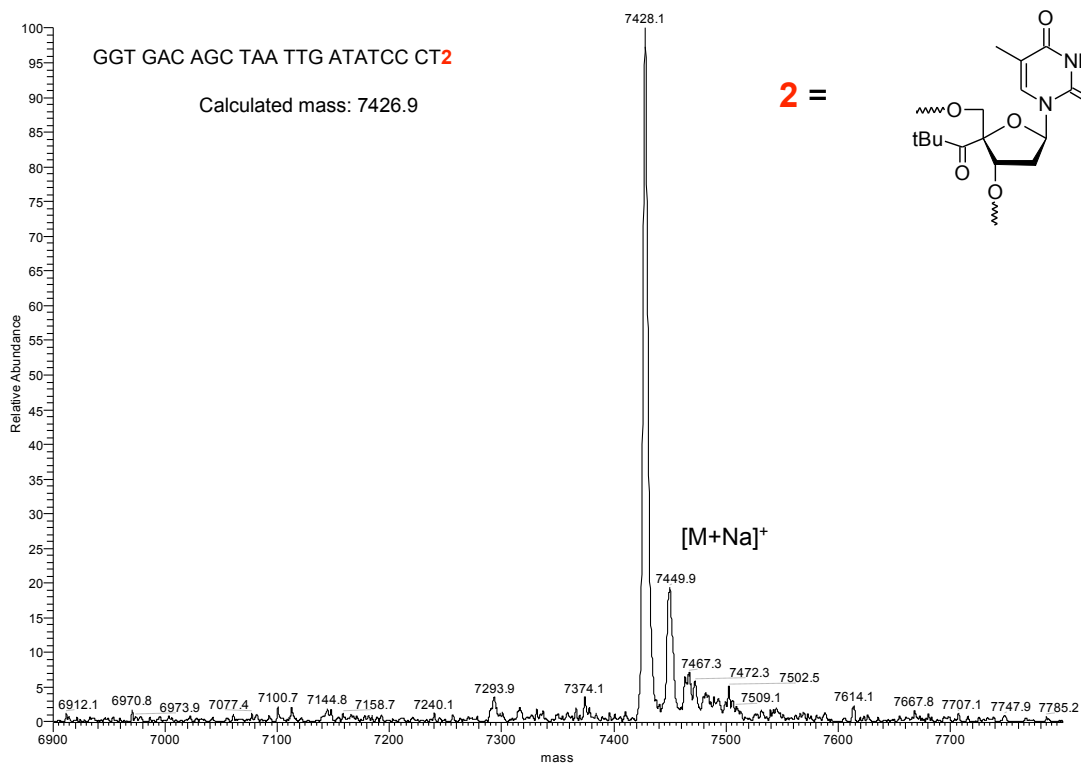


Figure S15. ESI-MS spectra of modified oligonucleotides.

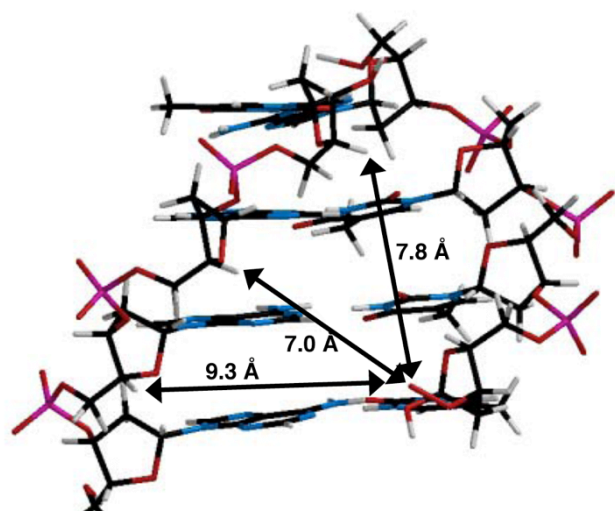


Figure S16. Molecular model of the distance between the peroxy radical oxygen of **8** and the C4'-hydrogen atoms of nucleotides on the complementary strand in 5'-d(AAAT)/3'-d(**8**TTA).