Supporting Information: Nucleotide Excision Repair of Chemically Stabilized Analogues of DNA Interstrand Cross-links Produced From Oxidized Abasic Sites

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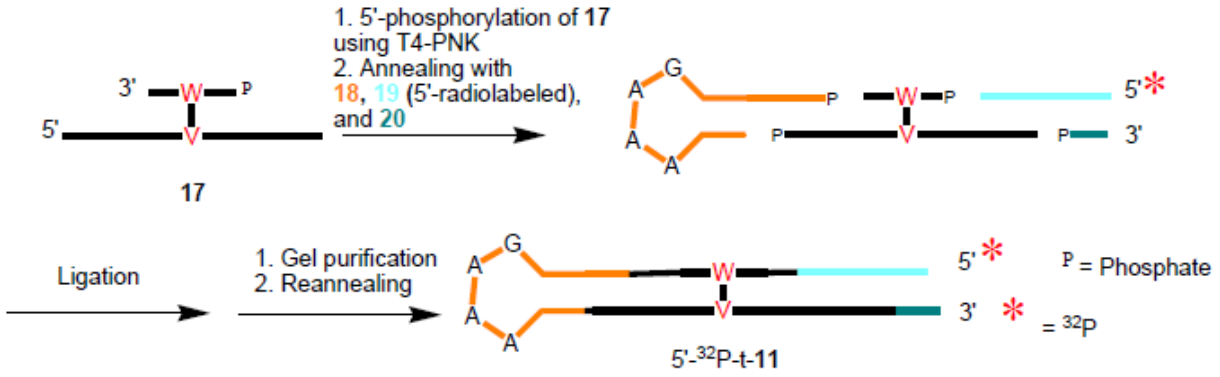
Contents:

General Methods. Syntheses of oligonucleotide precursors (**17** and **21**) to **8**, **9**, and **11** and general synthetic procedure for preparing 3'-^{32}P-t-**8**, 5'-^{32}P-t-**8**, 5'-^{32}P-b-**8**, and 3'-^{32}P-b-**8** was previously reported.[1] Oligonucleotides containing native nucleotides were deprotected using 1:1 mixture of aqueous methylamine (40%)-concentrated NH_4OH at 55 °C for 1 h. Oligonucleotides were purified by 20% denaturing polyacrylamide gel electrophoresis (PAGE), isolated by the crush and soak method, and desalted using C-18-Sep-Pak cartridges (Waters). Oligonucleotides were 5'-^{32}P-labeled by T4 polynucleotide kinase (New England Biolabs) and γ-^{32}P-ATP (Perkin-Elmer) using standard protocols. Radiolabeling of the 3'-termini was done using α-^{32}P-cordycepin triphosphate (Perkin-Elmer) and terminal deoxynucleotidyl transferase (NEB). Radioisotopic "fill in" experiments at 3'-termini were carried out with klenow (exo-) (NEB) and α-^{32}P-dCTP (Perkin-Elmer). CviQI, Fnu4HI, BsrBI, Taq$^{\alpha}$I, HpyCH4V, NEB 2.0, NEB 3.1, and CutSmart buffer were from NEB.

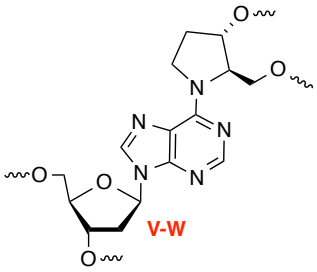
Preparation of 5'-^{32}P-t-11. The preparation of 5'-^{32}P-t-**11** is described below in **Scheme S1**. Oligonucleotide **19** (Figure S1) (80 pmol) was 5'-^{32}P-labeled in a reaction (80 μL) containing 1 × T4 PNK buffer (70 mM TRIS-HCl pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 50 μCi γ-^{32}P-ATP, and 30 U of T4 PNK at 37 °C for 2 h. Excess γ-^{32}P-ATP was removed by passing the reaction through a 1 mL Sephadex G25 column. The specific activity was determined by counting the radioactivity (using a liquid scintillation counter) and measuring the concentration of **19** (A_{260}). Separately, **17** (40 pmol) was 5'-phosphorylated on the template strand in a 25 μL reaction containing 1 × T4 PNK buffer, ATP (80 pmol), and 30 U of T4 PNK at 37 °C for 1 h. Both phosphorylation reactions were stopped by heating at 65 °C for 30 min. The phosphorylated products were hybridized with 160 pmol each of **18** and **20** in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution by slowly cooling from 90 °C to 16 °C. The reaction was

incubated overnight at 16 °C in presence of T4 DNA ligase (50 U) and 1 × ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP). Formamide loading buffer (50 µL, 90% formamide, 10 mM EDTA, pH 8.0) was added, followed by heating at 90 °C for 5 min and cooling on ice prior to purification by 20% denaturing PAGE (37 × 32 × 0.04 cm). The gel was run under limiting power (55 W) for 18 h. The product band was excised from the gel, crushed, and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The supernatant was desalted using a 100 mg C-18-Sep-Pak cartridge to provide 5'-^{32}P-t-**11**. The yield (10-20%) of 5'-^{32}P-t-**11** was determined based on the specific activity of 5'-^{32}P-**19**.

Scheme S1: Preparation of 5'-^{32}P-t-11

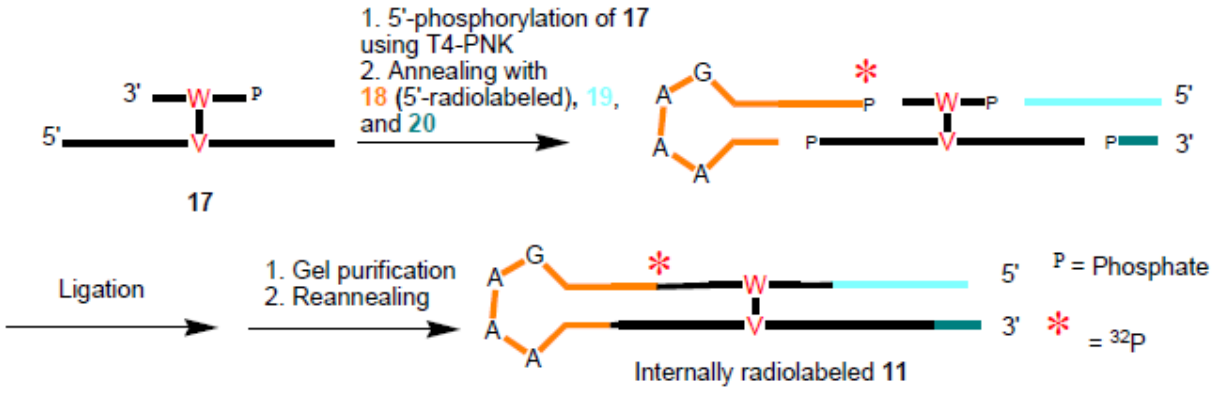


Preparation of 11 radiolabeled internally on 3'-side of abasic site analog (W). The preparation of internally radiolabeled **11** is described below in **Scheme S2**. Oligonucleotide **18** (80 pmol) was 5'-^{32}P-labeled in reaction (80 µL) containing 1 × T4 PNK exchange buffer buffer (50 mM imidazole-HCl pH 6.4, 18 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine, and 0.1 mM ADP), 6% PEG 8000 solution (w/v), 12 µM ATP, 50 µCi γ-^{32}P-ATP, and 30 U of T4 PNK at 37 °C for 2 h. Excess γ-^{32}P-ATP was removed by passing the reaction through a 1 mL Sephadex G25 column. The specific activity was determined by counting the radioactivity (using



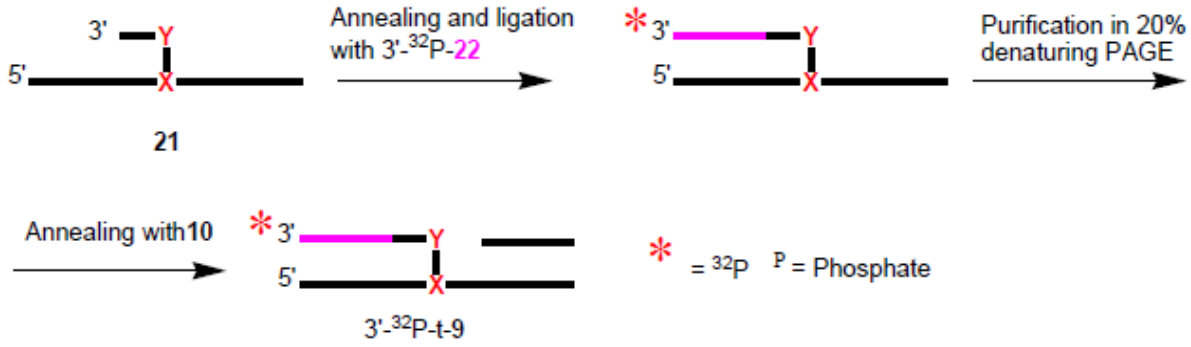
a liquid scintillation counter) and measuring the concentration of **18** (A_{260}). Separately, **17** (40 pmol) was 5'-phosphorylated on the template strand in a 25 µL reaction containing 1 × T4 PNK buffer, ATP (80 pmol), and 30 U of T4 PNK at 37 °C for 1 h. Both phosphorylation reactions were stopped by heating at 65 °C for 30 min. The phosphorylated products were hybridized with 160 pmol each of **19** and **20** in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution by slowly cooling from 90 °C to 16 °C. The reaction was incubated overnight at 16 °C in presence of T4 DNA ligase (50 U) and 1 × ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP). Formamide loading buffer (50 µL, 90% formamide, 10 mM EDTA, pH 8.0) was added, followed by heating at 90 °C for 5 min and cooling on ice prior to purification by 20% denaturing PAGE (37 × 32 × 0.04 cm). The gel was run under limiting power (55 W) for 18 h. The product band was excised from the gel, crushed, and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The supernatant was desalted using a 100 mg C-18-Sep-Pak cartridge to provide internally radiolabeled **11**. The yield (10-20%) of 5'-^{32}P-t-**11** was determined based on the specific activity of 5'-^{32}P-**18**.

Scheme S2: Preparation of 11 labeled internally at top strand



Preparation of 3'-^{32}P-t-9. The preparation of 3'-^{32}P-t-**9** is described below in **Scheme S3**. Oligonucleotide **22** (80 pmol) was 3'-^{32}P-labeled in reaction (80 μL) containing 1 × TdT buffer buffer (50 mM KOAc, 20 mM Tris-OAc, 10 mM $MgCl_2$, pH 7.9), 40 μCi α-^{32}P-cordycepin triphosphate, and 30 U of terminal deoxynucleotidyl transferase at 37 °C for 2 h. The reaction was stopped by heating at 75 °C for 30 min. Excess α-^{32}P-cordycepin triphosphate was removed by passing the reaction through a 1 mL Sephadex G25 column. The specific activity was determined by counting the radioactivity (using a liquid scintillation counter) and measuring the concentration of **22** (A_{260}). The radiolabeled **22** was hybridized with **21** (50 pmole) in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution (100 μL) by slowly cooling from 90 °C to 16 °C. The reaction was incubated overnight at 16 °C in presence of T4 DNA ligase (50 U) and 1 × ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP). Formamide loading buffer (50 μL, 90% formamide, 10 mM EDTA, pH 8.0) was added, followed by heating at 90 °C for 5 min and cooling on ice prior to purification by 20% denaturing PAGE (37 × 32 × 0.04 cm). The gel was run under limiting power (55 W) for 18 h. The product band was excised from the gel, crushed, and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The supernatant was desalted using a 100 mg C-18-Sep-Pak cartridge, concentrated and then thermally annealed with **10** to provide 3'-^{32}P-t-**9**. The yield (10-20%) of 3'-^{32}P-t-**9** was determined based on the specific activity of 3'-^{32}P-**22**.

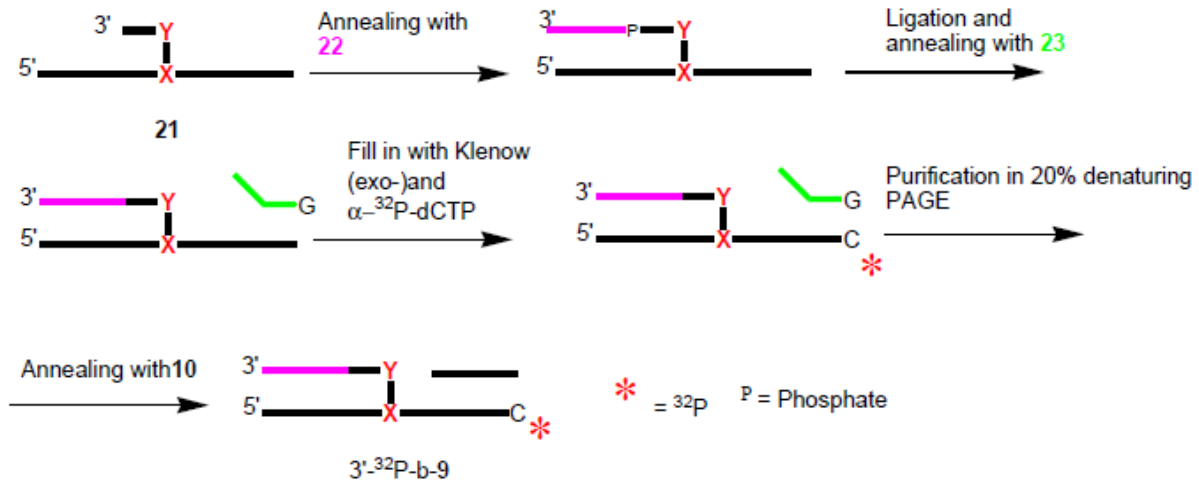
Scheme S3: Preparation of 3'-^{32}P-t-9



Preparation of 3'-^{32}P-b-9. The preparation of 3'-^{32}P-b-**9** is described below in **Scheme S4**. Oligonucleotide **22** (160 pmol) was hybridized with **21** (50 pmol) in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution (20 μL) by slowly cooling from 90 °C to 16 °C. The reaction was incubated overnight at 16 °C in presence of T4 DNA ligase (50 U) and 1 × ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP). The ligation was stopped by heating at 65 °C for 30 min. After addition of **23** (160 pmol) to the solution, it was hybridized with the cross-link by slowly cooling down from 90 °C to 16 °C. The cross-linked substrate was then 3'-^{32}P-labeled in its bottom strand using a "fill in" reaction (150 μL) containing 1 × NEB 2.0 buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl2, 1 mM dTT, pH 7.9), 30 μCi α-^{32}P-dCTP, and 30 U of klenow exo(-) at 37 °C for 3 h. The "fill in" reaction was stopped by heating at 75 °C for 30 min. Excess α-^{32}P-dCTP was removed by passing the reaction through a 1 mL Sephadex G25 column. The specific activity was determined by counting the radioactivity (using a liquid scintillation counter) and assuming hundred percent recovery of the radiolabeled cross-linked substrate. Formamide loading buffer (50 μL, 90% formamide, 10 mM EDTA, pH 8.0) was added to the recovered substrate, followed by heating at 90 °C for 5 min and cooling on ice prior to purification by 20% denaturing PAGE (37 × 32 × 0.04 cm). The gel was

run under limiting power (55 W) for 18 h. The product band was excised from the gel, crushed, and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The supernatant was desalted using a 100 mg C-18-Sep-Pak cartridge, concentrated and then thermally annealed with **10** to provide 3′-^{32}P-b-**9**. The yield (10-20%) of 3′-^{32}P-b-**9** was determined based on the specific activity of 3′-^{32}P-radiolabeled cross-link.

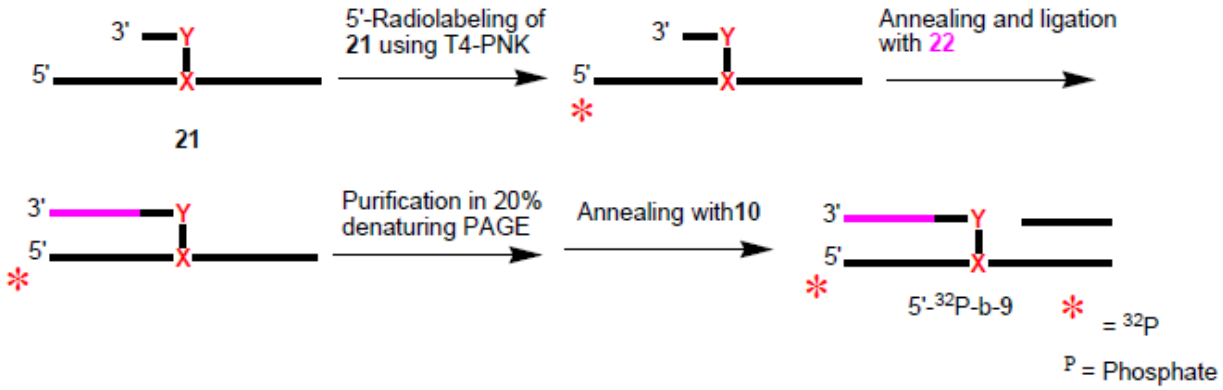
Scheme 4: Preparation of 3'-^{32}P-b-9



Preparation of 5′-^{32}P-b-9. The preparation of 5′-^{32}P-b-**9** is described in **Scheme S5**. Oligonucleotide **21** (50 pmol) was 5′-^{32}P-labeled in reaction (80 µL) containing 1 × T4 PNK buffer (70 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 50 µCi γ-^{32}P-ATP, and 30 U of T4 PNK at 37 °C for 2 h. The phosphorylation reaction was stopped by heating at 65 °C for 30 min. Excess γ-^{32}P-ATP was removed by passing the reaction through a 1 mL Sephadex G25 column. The specific activity was determined by counting the radioactivity (using a liquid scintillation counter) and measuring the concentration of **21** (A_{260}). The ^{32}P-radiolabeled **21** were hybridized with **22** (160 pmol) in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution by slowly cooling from 90 °C to 16 °C. The reaction was incubated overnight at 16 °C in

presence of T4 DNA ligase (50 U) and 1 × ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP). Formamide loading buffer (50 μL, 90% formamide, 10 mM EDTA, pH 8.0) was added, followed by heating at 90 °C for 5 min and cooling on ice prior to purification by 20% denaturing PAGE (37 × 32 × 0.04 cm). The gel was run under limiting power (55 W) for 18 h. The product band was excised from the gel, crushed, and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The supernatant was desalted using a 100 mg C-18-Sep-Pak cartridge, concentrated and then thermally annealed with **10** to provide 5′-^{32}P-b-**9**. The yield (10-20%) of 5′-^{32}P-b-**9** was determined based on the specific activity of 5′-^{32}P-**21**.

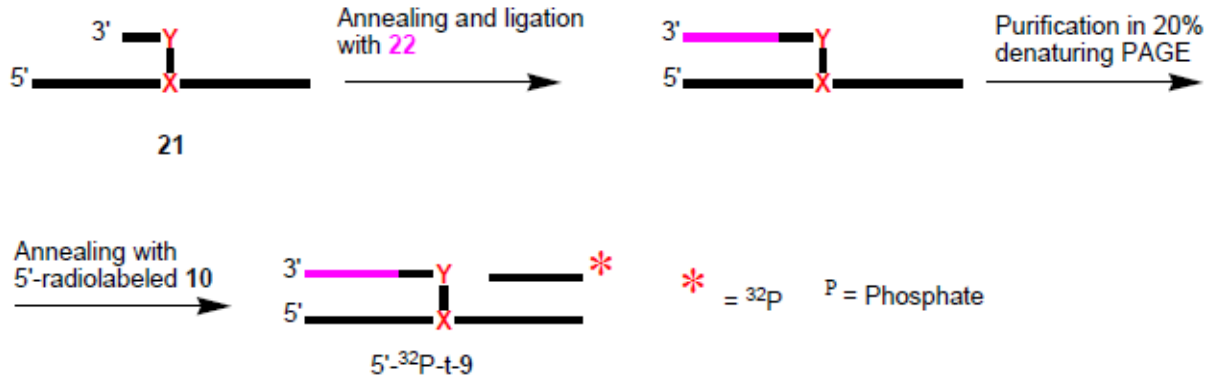
Scheme S5: Preparation of 5'-^{32}P-b-9



Preparation of 5′-^{32}P-t-9. The preparation of 5′-^{32}P-t-**9** is described below in **Scheme S6**. Oligonucleotide **22** (1.5 nmole) was hybridized with **21** (3 nmole) in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution (50 μL) by slowly cooling from 90 °C to 16 °C. The reaction was incubated overnight at 16 °C in presence of T4 DNA ligase (50 U) and 1 × ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP). Formamide loading buffer (50 μL, 90% formamide, 10 mM EDTA, pH 8.0) was added, followed by heating

at 90 °C for 5 min and cooling on ice prior to purification by 20% denaturing PAGE (37 × 32 × 0.1 cm). The gel was run under limiting power (45 W) for 14 h. The product band was detected using UV, excised from the gel, crushed, and the DNA was eluted overnight in 1 mL of elution buffer (0.2 M NaCl and 1 mM EDTA). The supernatant was concentrated by repeatedly mixing with 1-butane and then desalted by precipitation. The yield of ligation (22%) was determined from the concentration of isolated product (A_{260}). In a separate reaction **10** (20 pmol) was 5′-^{32}P-labeled in reaction (80 µL) containing 1 × T4 PNK buffer (70 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 50 µCi γ-^{32}P-ATP, and 30 U of T4 PNK at 37 °C for 2 h. The 5′-^{32}P-**10** was then hybridized with the ligated cross-link to obtain 5′-^{32}P-t-**9.**

Scheme S6: Preparation of 5'-^{32}P-t-9



Restriction Enzyme Treatment for Characterization of 5′-^{32}P-t-11. TaqαI (10 U) and 5′-^{32}P-t-**11** were incubated in an 8 µL reaction containing of 1 × CutSmart buffer (50 mM potassium acetate, 20 mM Tris•acetate, 10 mM magnesium acetate, 100 µg/mL BSA, pH 7.9) at 65°C (TaqαI) for 1 h. In two independent experiments, CviQI was incubated with 5′-^{32}P-t-**8** or 5′-^{32}P-t-**11** in 8 µL reactions containing of 1 × NEB 3.1 buffer (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 µg/mL BSA, pH 7.9) at room temperature for 1 h. After 1 h, formamide loading

buffer (2 μL, 90% formamide, 10 mM EDTA, pH 8.0) was added to these reactions, followed by heating at 90°C (2 min), and cooling on ice. The reactions were analyzed by 15% denaturing PAGE.

Restriction Enzyme Treatment for Characterization of Internally Radiolabeled 11. The appropriate restriction enzyme (Fnu4HI and HpyCH4V) (10 U) and internally radiolabeled **11** were incubated in an 8 μL reaction containing of 1 × CutSmart buffer (50 mM potassium acetate, 20 mM Tris•acetate, 10 mM magnesium acetate, 100 μg/mL BSA, pH 7.9) at 37°C for 1 h, at which time formamide loading buffer (2 μL, 90% formamide, 10 mM EDTA, pH 8.0) was added, followed by heating at 90°C (2 min), and cooling on ice. The reactions were analyzed by 15% denaturing PAGE.

Restriction Enzyme Treatment of 3'-^{32}P-b-9, 3'-^{32}P-t-9, 5'-^{32}P-b-9, and 5'-^{32}P-t-9. The restriction enzyme BsrBI (10 U) and **9** were incubated in a 8 μL reaction containing of 1 × CutSmart buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg/mL BSA, pH 7.9) at 37°C for 1 h. In a separate reaction, CviQI and **9** were incubated in a 8 μL reaction containing of 1 × NEB 3.1 buffer (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/mL BSA, pH 7.9) at room temperature for 1 h. The reactions were added with formamide loading buffer (2 μL, 90% formamide, 10 mM EDTA, pH 8.0) followed by heating at 90°C (2 min) and cooling on ice. The reactions were analyzed by 12% denaturing PAGE.

Determination of thermal stability of 5'-^{32}P-t-9 under nucleotide excision repair conditions. Oligonucleotide 5'-^{32}P-t-**9** (2 nM) was incubated with **10** (10 nM) for 1 h in 60 μL reaction containing 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM dTT, and ATP 1 mM) at 55 °C for 3 h **.** Aliquots (9 μL) were taken at 0, 5, 10, 15, 30 and 60 min and

mixed with glycerol loading buffer (2 μL, 80% glycerol in water, v/v). The exchange was analyzed by 20% non-denaturing PAGE.

Determination of thermal stability of 13 under nucleotide excision repair conditions. Oligonucleotide 5'-^{32}P-**13** (0.26 nM) was incubated with **15** (0.26 nM or 2.6 nM) in a 50 μL reaction containing 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM dTT, and ATP 1 mM) at 55 °C for 3 h. The experiment was cooled to 4 °C and mixed with glycerol loading buffer (5.5 μL, 80% glycerol in water, v/v). The exchange was analyzed by 15% non-denaturing PAGE.

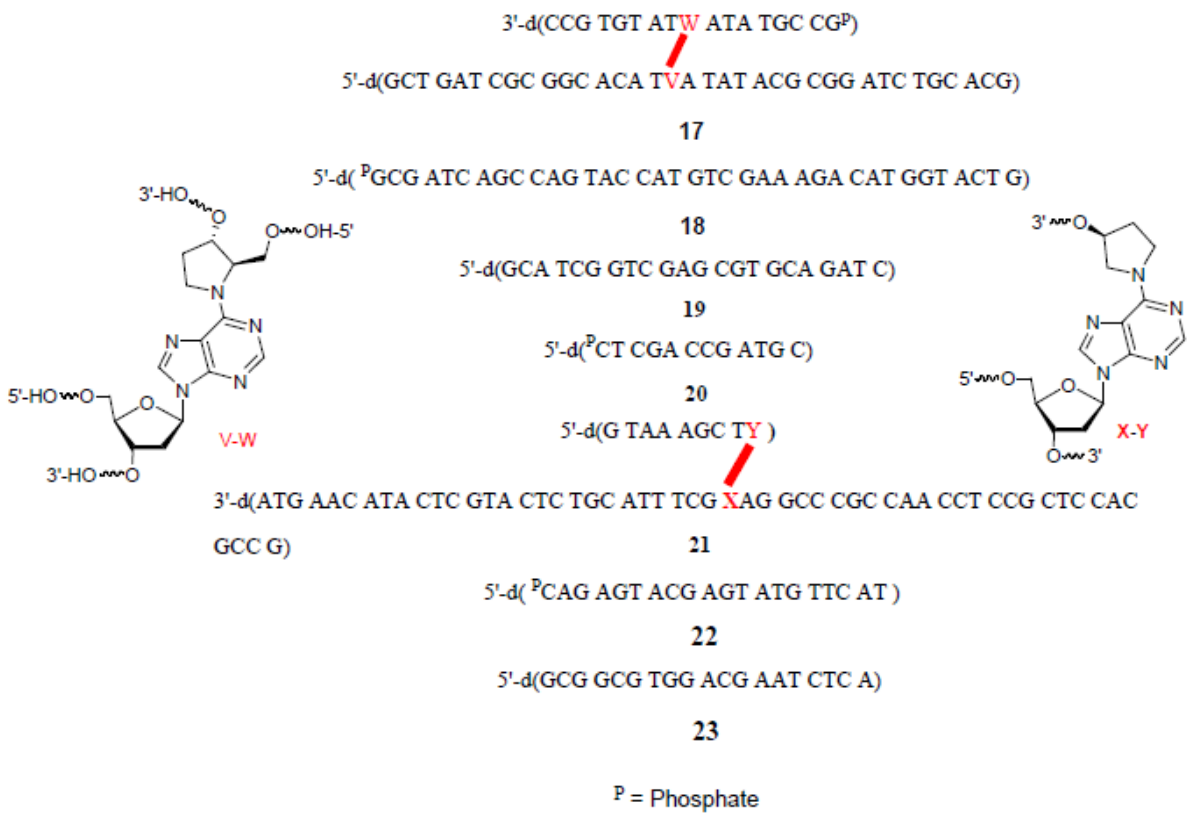


Figure S1. Cross-link precursors and oligonucleotides used to make **8, 9,** and **11**.

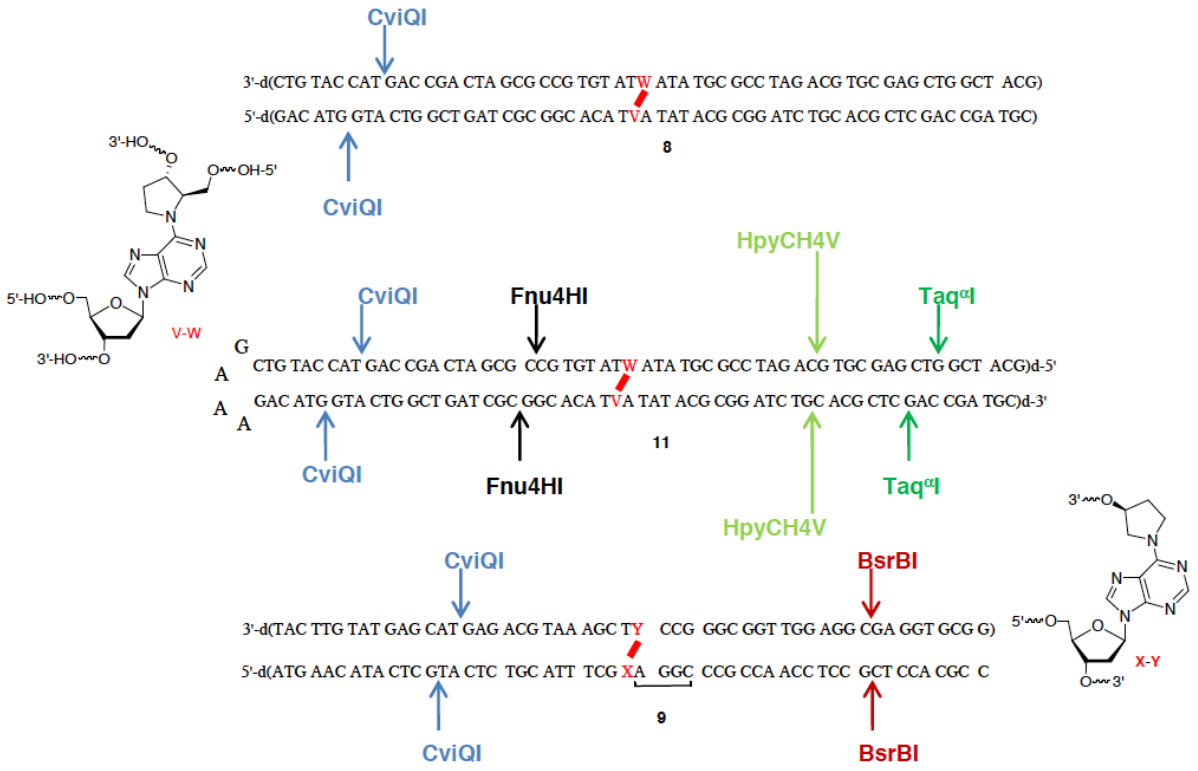


Figure S2. Restriction enzyme sites present on **8, 9** and **11**.

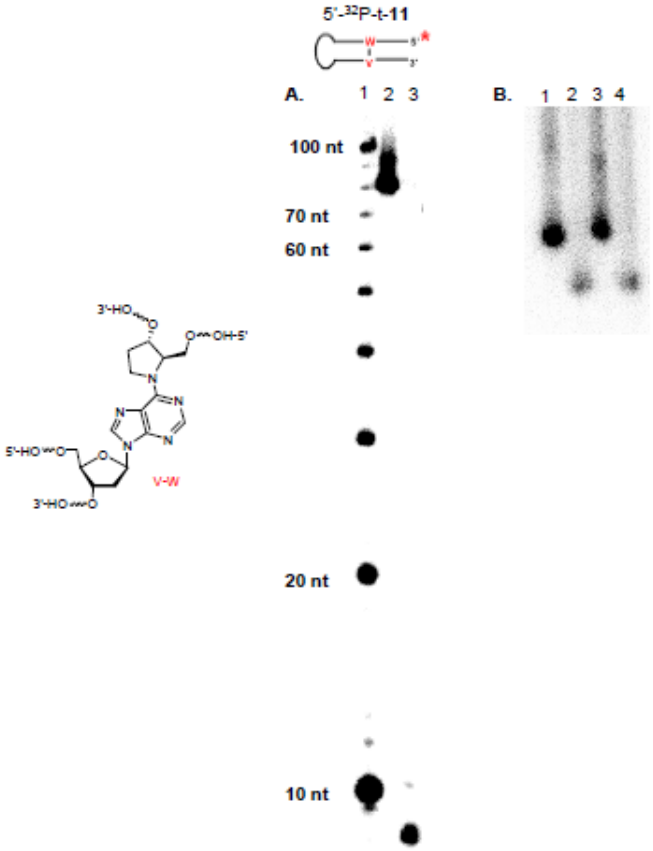


Figure S3. Restriction enzyme treatment on 5'-^{32}P-t-**11** and 5'-^{32}P-t-**8**. (A.) Denaturing PAGE gel (15%) analysis of restriction enzyme treatment of 5'-^{32}P-t-**11**. Lane 1, 10 base pair DNA ladder. Lane 2, 5'-^{32}P-t-**11** without any treatment. Lane 3, 5'-^{32}P-t-**11** treated with with Taq$^{\alpha}$I. (B.) Denaturing PAGE gel (15%) analysis of restriction enzyme treatment of 5'-^{32}P-t-**11** and 5'-^{32}P-t-**8**. Lane 1, 5'-^{32}P-t-**8** without any treatment. Lane 2, 5'-^{32}P-t-**8** treated with CviQI. Lane 3, 5'-^{32}P-t-**11** without any treatment. Lane 2, 5'-^{32}P-t-**11** treated with CviQI.

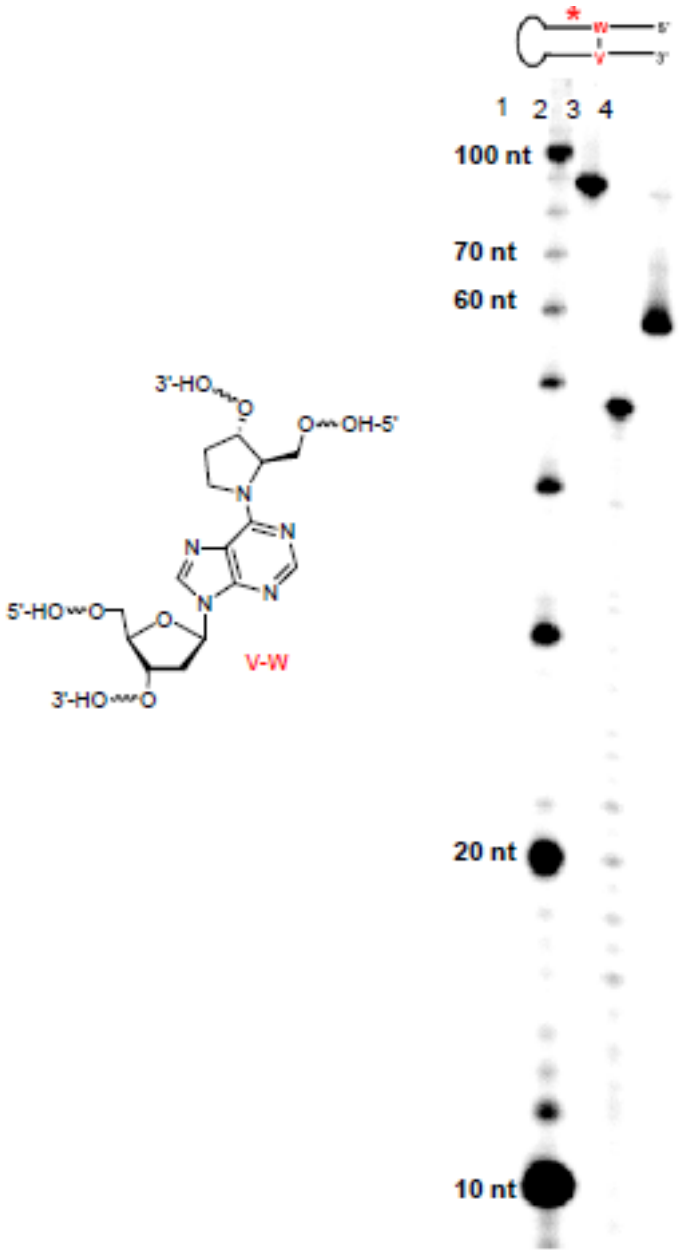


Figure S4. Representative 12% denaturing PAGE gel analysis of restriction enzyme treatment on **11** internally labeled at 3'-side of the abasic site analog (W). Lane 1, 10 base pair DNA ladder. Lane 2, internally labeled **11** without any treatment. Lane 3, internally labeled **11** treated with Fnu4HI. Lane 3, internally labeled **11** treated with HpyCH4V.

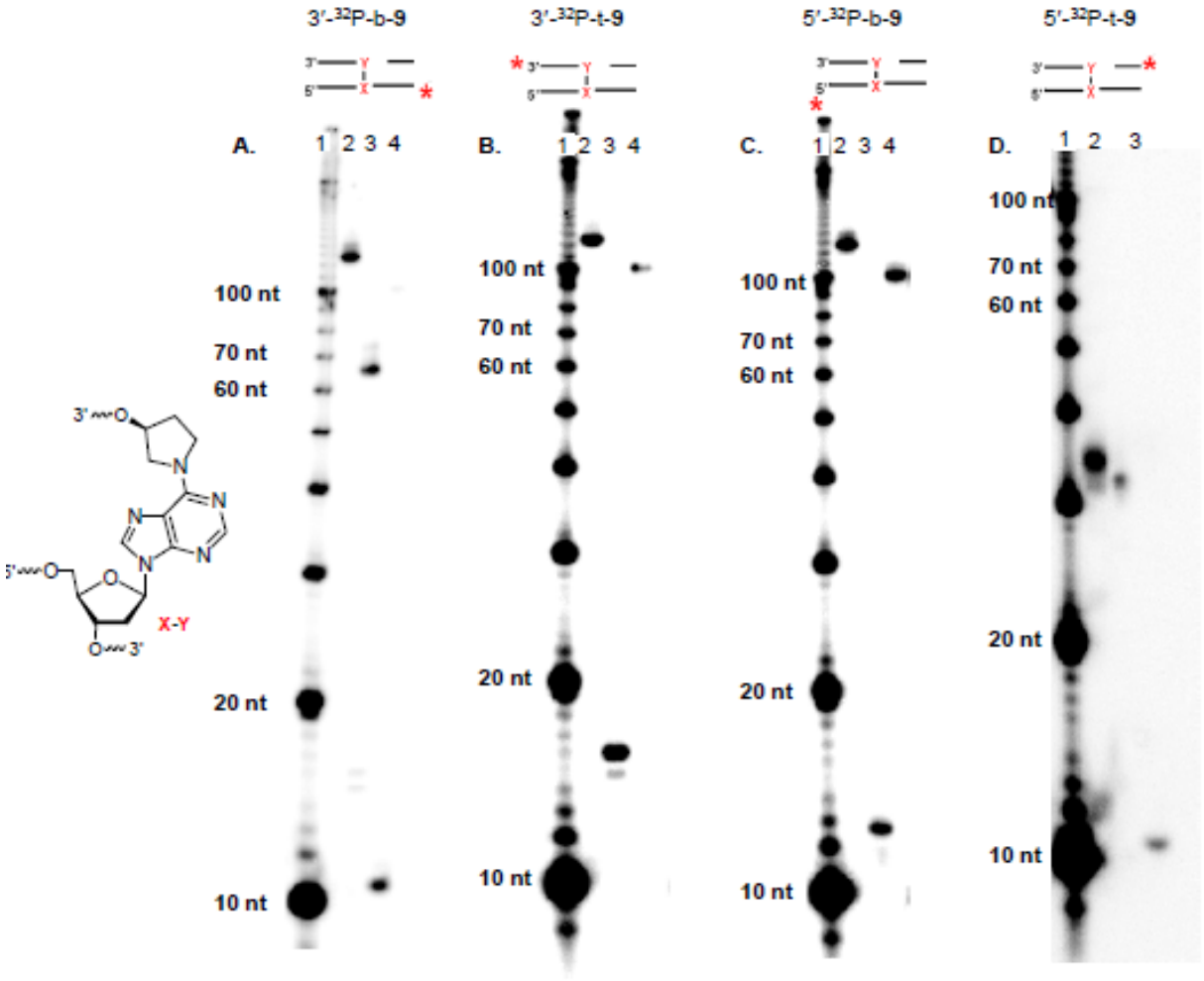


Figure S5. Restriction enzyme treatment on 3'-^{32}P-b-**9**, 3'-^{32}P-t-**9**, 5'-^{32}P-b-**9**, and 5'-^{32}P-t-**9**. (A.) Representative 15% denaturing PAGE gel analysis of restriction enzyme treatment of 3'-^{32}P-b-**9**. Lane 1, 10 base pair DNA ladder. Lane 2, 3'-^{32}P-b-**9** without any treatment. Lane 3, 3'-^{32}P-b-**9** treated with CviQI. Lane 4, 3'-^{32}P-b-**9** treated with BsrBI. (B.) Representative 15% denaturing PAGE gel analysis of restriction enzyme treatment of 3'-^{32}P-t-**9**. Lane 1, 10 base pair DNA ladder. Lane 2, 3'-^{32}P-t-**9** without any treatment. Lane 3, 3'-^{32}P-t-**9** treated with CviQI. Lane 4, 3'-^{32}P-t-**9** treated with BsrBI. (C.) Representative 15% denaturing PAGE gel analysis of restriction enzyme treatment of 5'-^{32}P-b-**9**. Lane 1, 10 base pair DNA ladder. Lane 2, 5'-^{32}P-b-**9** without any treatment. Lane 3, 5'-^{32}P-b-**9** treated with CviQI. Lane 4, 5'-^{32}P-b-**9** treated with BsrBI. (D.) Representative 15% denaturing PAGE gel analysis of restriction enzyme treatment of 5'-^{32}P-t-**9**. Lane 1, 10 base pair DNA ladder. Lane 2, 5'-^{32}P-t-**9** without any treatment. Lane 3, 5'-^{32}P-t-**9** treated with BsrBI.

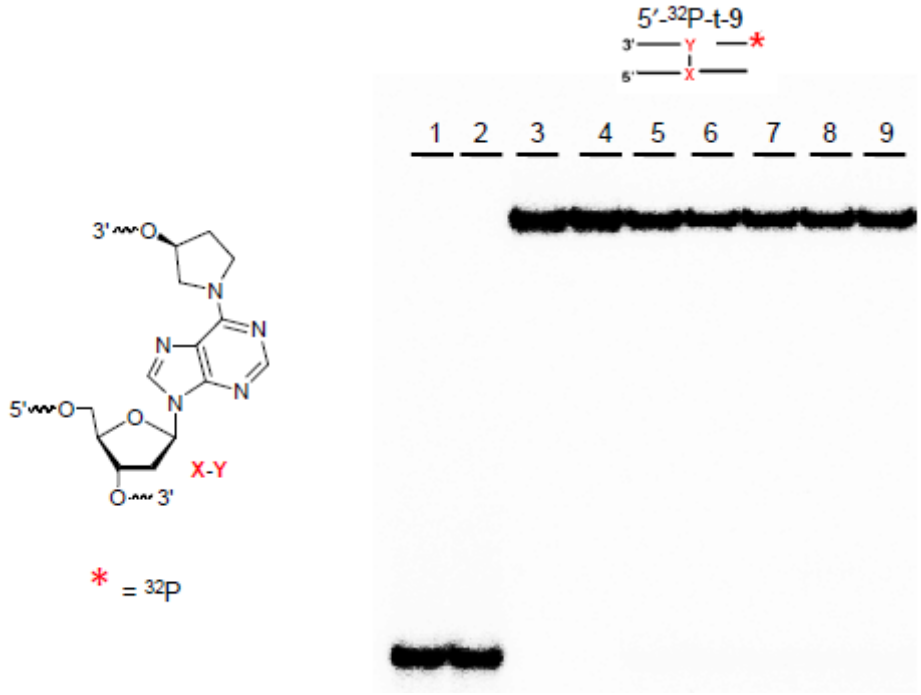


Figure S6. Thermal stability of 5'-^{32}P-t-**9** analyzed using non-denaturing (20%) PAGE. Lane 1 and 2, aliquots at 0 and 60 min from single stranded 5'-^{32}P-**10**, respectively. Lane 3, hybridized 5'-^{32}P-t-**9** without any treatment. Lanes 4 to 9, aliquots taken at 0, 5, 10, 15, 30 and 60 min from 5'-^{32}P-t-**9** (2 nM) incubated with non-radiolabeled **10** (10 nM) in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at 55 °C.

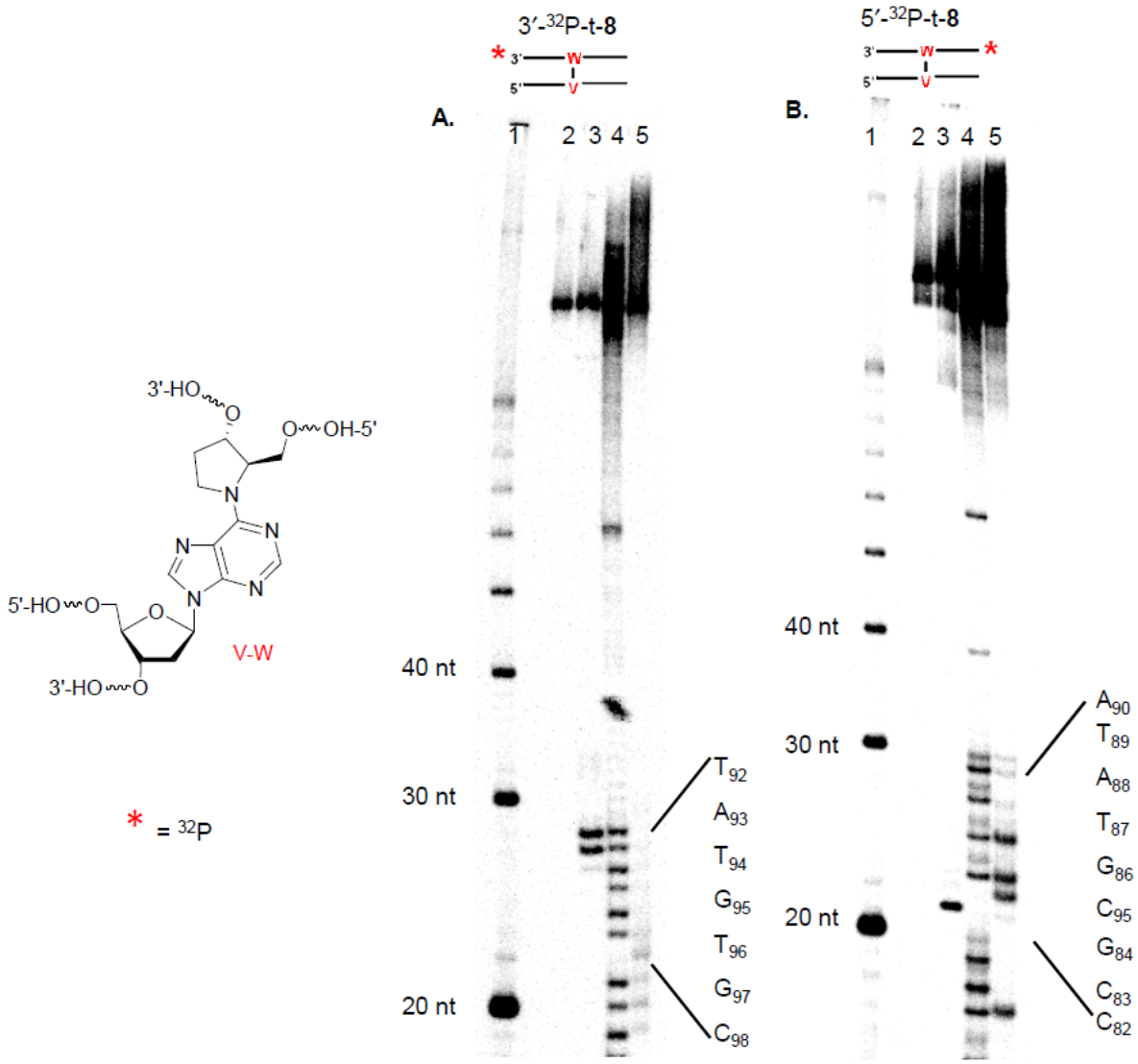


Figure S7. Denaturing (20%) PAGE analysis of UvrABC incision on the top strand (t) of **8**. (A.) Strand containing abasic site analog (W) labeled at its 3'-terminus. (B.) Strand containing abasic site analog (W) labeled at its 5'-terminus. Lane 1, 10 base pair DNA ladder. Lane 2, 3'-^{32}P-t-**8** (A.) or 5′-^{32}P-t-**8** (B.). Lane 3, 3'-^{32}P-t-**8** (Panel A) or 5'-^{32}P-t-**8** (B.) treated with UvrABC for 8 h. Lane 4, A+G sequencing of 3'-^{32}P-t-**8** (A.) or 5'-^{32}P-t-**8** (B.). Lane 5, T+C sequencing of 3'-^{32}P-t-**8** (A.) or 5'-^{32}P-t-**8** (B.).

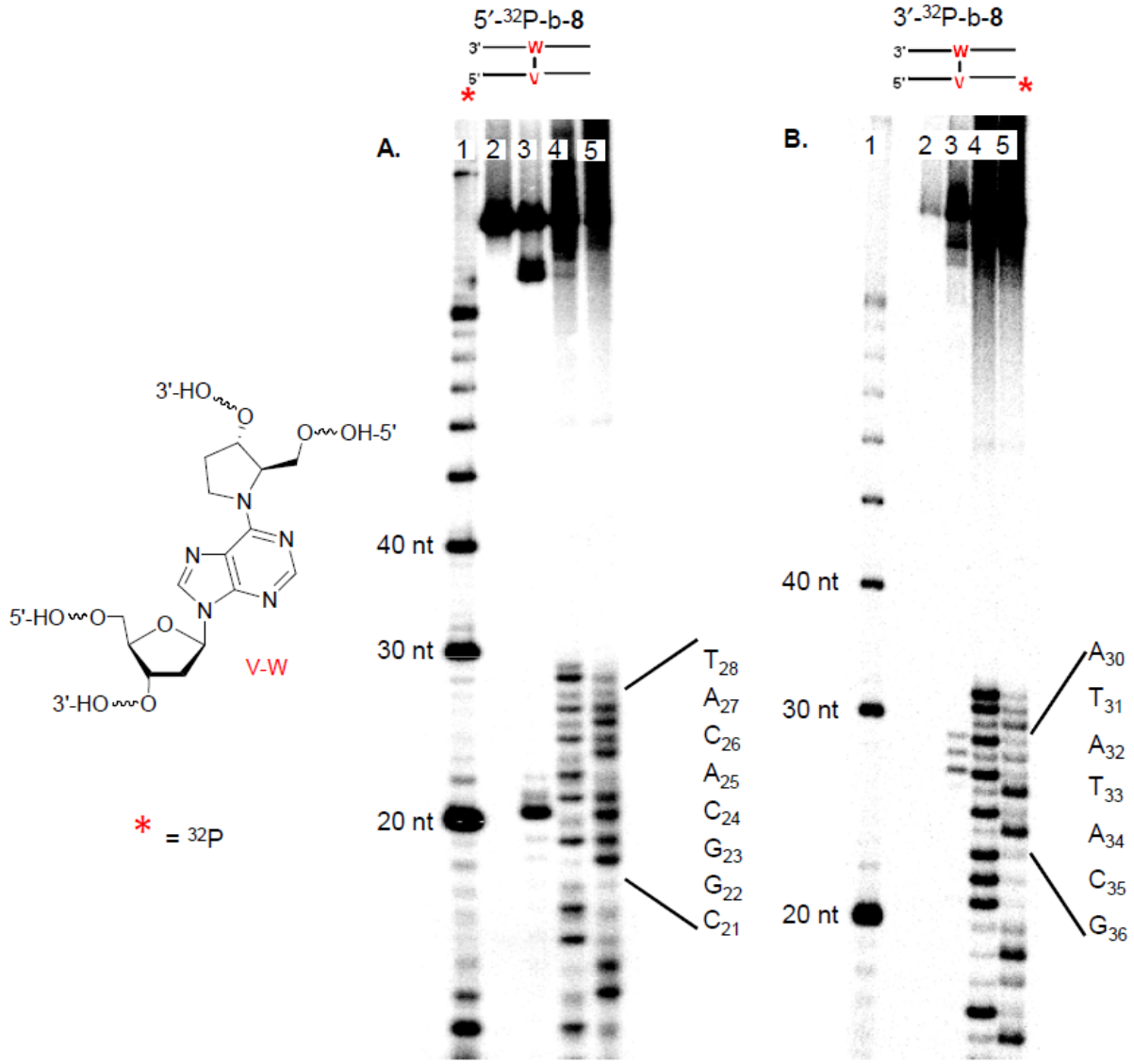


Figure S8. Denaturing (20%) PAGE showing UvrABC incision on bottom strand (b) of **8**. (A.) Strand containing deoxyadenosine analog (V) labeled at its 5'-terminus. (B.) Strand containing abasic site analog (W) labeled at its 3'-terminus. Lane 1, 10 base pair DNA ladder. Lane 2, 5'-^{32}P-b-**8** (A.) or 3'-^{32}P-b-**8** (B.). Lane 3, 5'-^{32}P-b-**8** (A.) or 3'-^{32}P-b-**8** (B.) treated with UvrABC for 8 h. Lane 4, A+G sequencing of 5'-^{32}P-b-**8** (A.) or 3'-^{32}P-b-**8** (B.). Lane 5, T+C sequencing of 5'-^{32}P-b-**8** (A.) or 3'-^{32}P-b-**8** (B.).

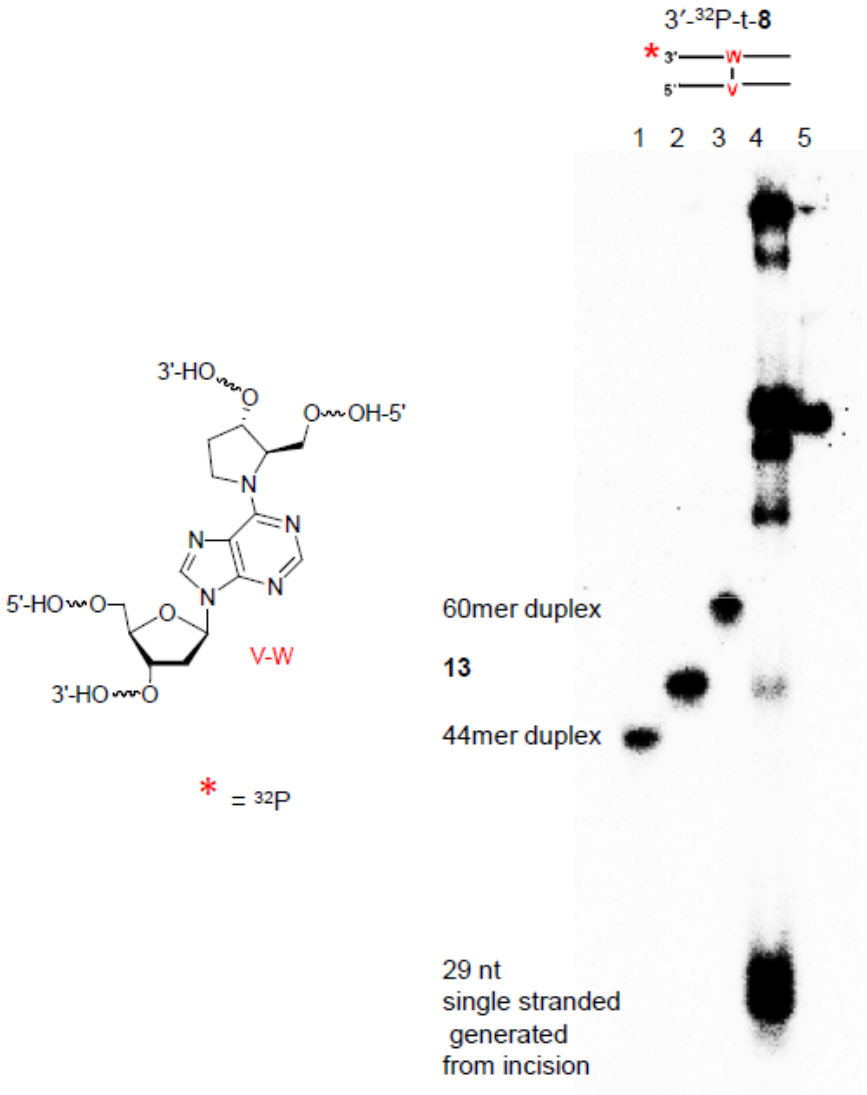


Figure S9. Migration of products from UvrABC incision on 3'-^{32}P-t-**8** in a 20% non-detaturing PAGE. Lane 1, independently synthesized 44mer duplex. Lane 2, oligonucleotide **13** labeled at 5'-side of shorter strand. Lane 3, independently synthesized 60mer duplex. Lane 4, aliquot from UvrABC proteins incubated with 3'-^{32}P-t-**8** in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at 55 °C for 8 h. Lane 5, 3'-^{32}P-t-**8** without any treatment.

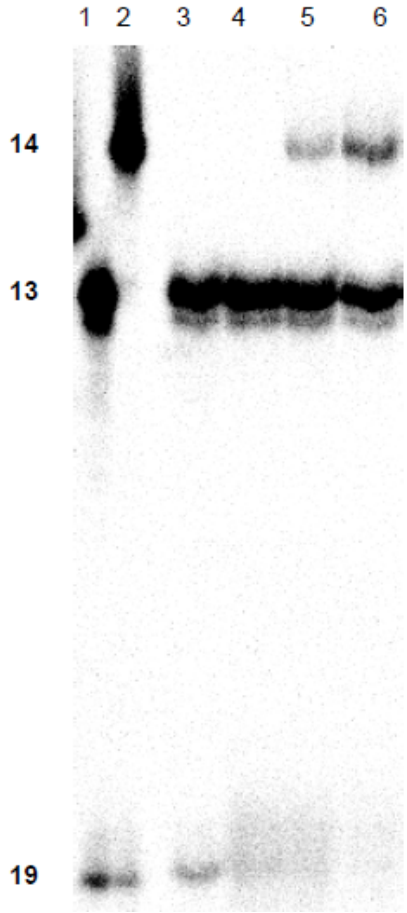


Figure S10. Non-denaturing (16%) PAGE analysis of thermal stability of putative UvrABC double strand cleavage product of **8**. Lane 1, oligonucleotide **13** radiolabeled at 5'-termini of shorter strand. Lane 2, oligonucleotide **14** radiolabeled at 5'-termini of shorter strand. Lane 3, oligonucleotide **13** (0.26 nM) incubated in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at room temperature for 3 h. Lane 4, oligonucleotide **13** (0.26 nM) incubated in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at 55 °C for 3 h. Lane 5, oligonucleotide **13** (0.26 nM) incubated with **15** (0.26 nM) in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at 55 °C for 3 h. Lane 6, oligonucleotide **13** (0.26 nM) incubated with **15** (1.3 nM) in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at 55 °C for 3 h.

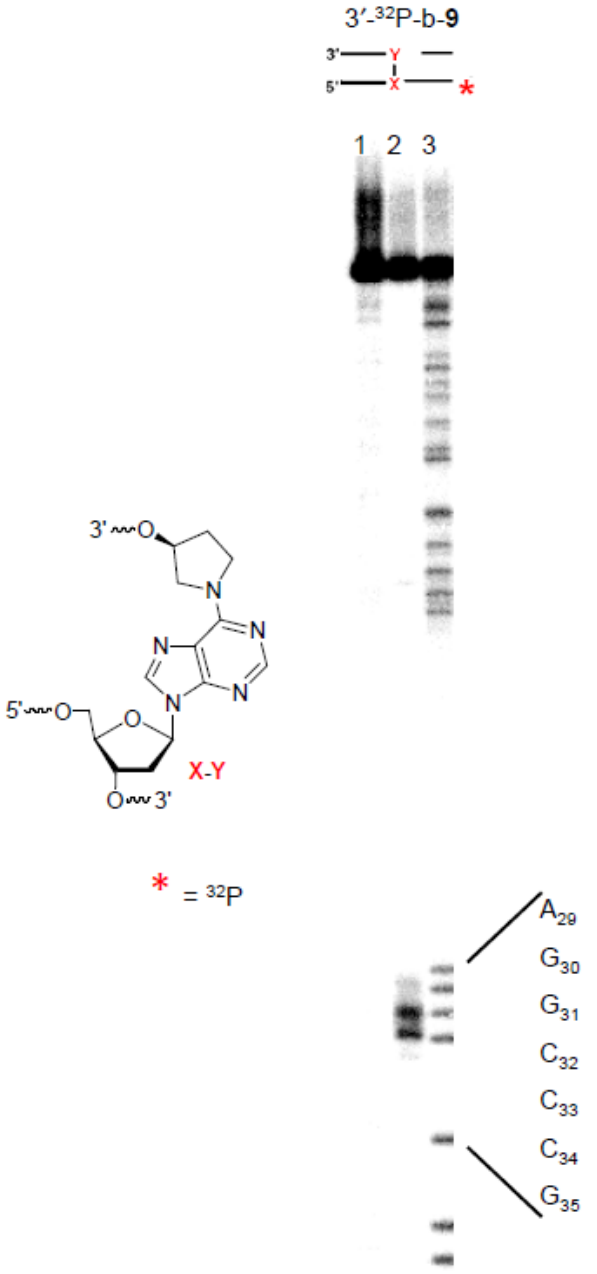


Figure S11. Denaturing PAGE gel (20%) analysis of UvrABC incision on 3'-^{32}P-b-**9**. Lane 1, 3'-^{32}P-b-**9** without any treatment. Lane 2, 3'-^{32}P-b-**9** incubated with UvrABC proteins in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at 55 °C for 1 h. Lane 3, A+G sequencing experiment on 3'-^{32}P-b-**9**.

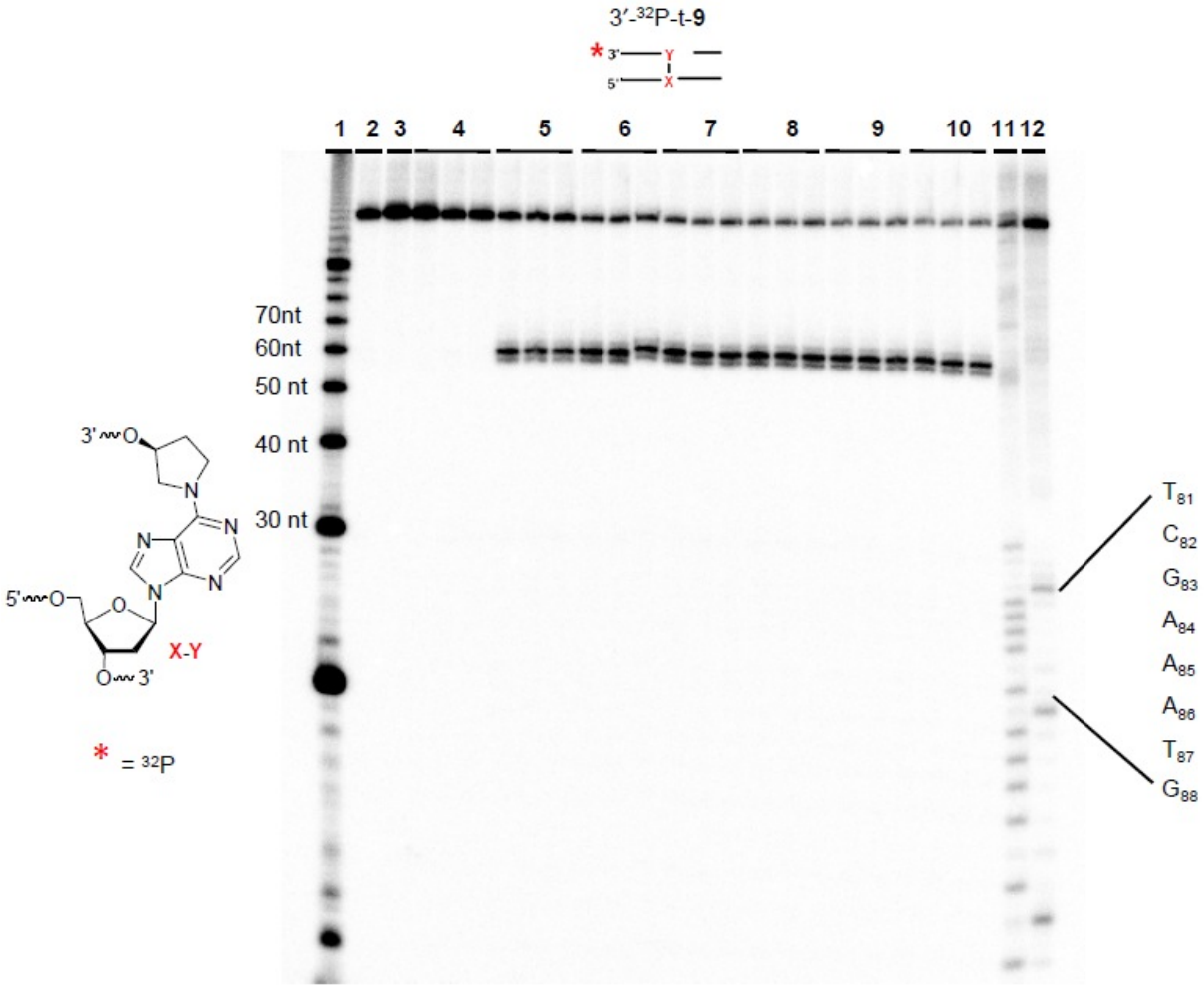


Figure S12. Denaturing PAGE (20%) analysis of 8 h time course study of UvrABC incision on 3'-^{32}P-t-**9**. Lane 1, 10 base pair DNA ladder. Lane 2, 3'-^{32}P-t-**9** incubated in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) without any treatment at 55 °C for 0 h. Lane 3, 3'-^{32}P-t-**9** incubated in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) without any treatment at 55 °C for 8 h. Lane 4 to 10, aliquots (in triplicate) taken at 0, 1, 2, 3, 4, 6, and 8 h from incubation of 3'-^{32}P-t-**9** incubated with UvrABC in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, and ATP 1 mM) at 55 °C. Lane 11, A+G sequencing reaction on 3'-^{32}P-t-**9.** Lane 12, T+C sequencing reaction on 3'-^{32}P-t-**9.**

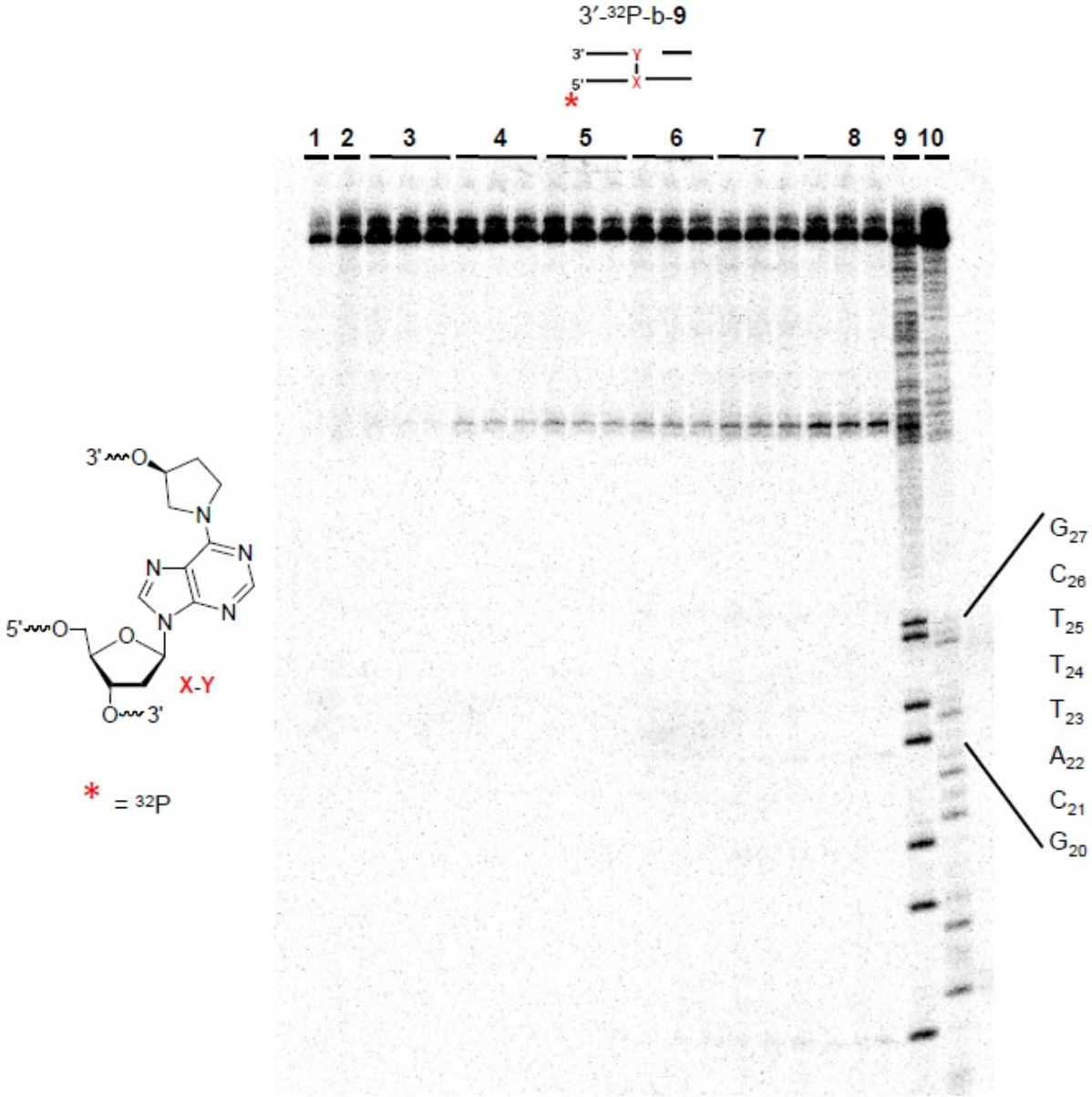


Figure S13. Denaturing (20%) PAGE analysis of 1 h time course study of UvrABC incision on 5'-^{32}P-b-**9**. Lane 1, 5'-^{32}P-b-**9** incubated in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM dTT, and ATP 1 mM) without any treatment at 55 °C for 0 min. Lane 2, 5'-^{32}P-b-**9** incubated in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM dTT, and ATP 1 mM) without any treatment at 55 °C for 60 min. Lane 3 to 9, aliquots (in triplicate) taken at 0, 5, 10, 15, 30, and 60 min from incubation of 5'-^{32}P-b-**9** incubated with UvrABC in 1 × NER buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM dTT, and ATP 1 mM) at 55 °C. Lane 11, A+G sequencing reaction on 5'-^{32}P-b-**9.** Lane 12, T+C sequencing reaction on 5'-^{32}P-b-**9.**

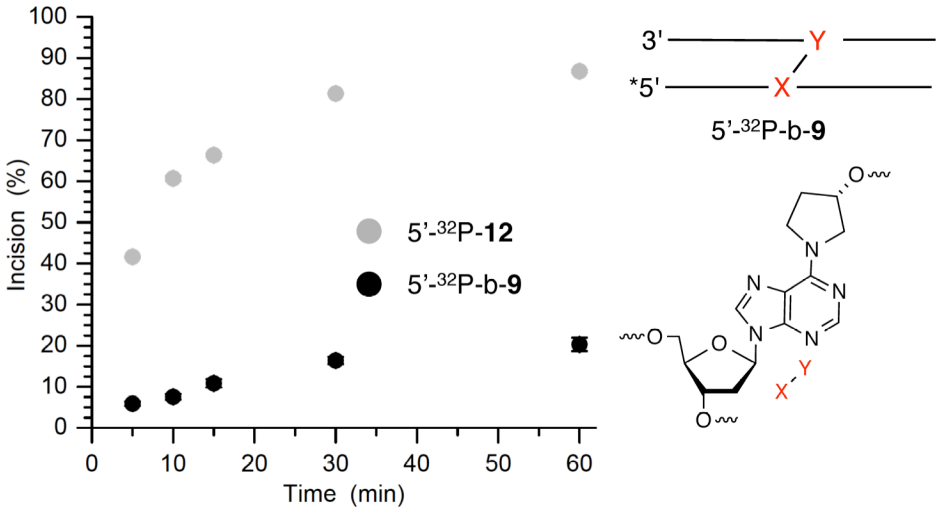


Figure S14. Time dependence of UvrABC incision of DOB ICL analogue **7** in 5'-^{32}P-b-**9** compared to that in 5'-^{32}P-**12**. (Total amount of incision at all nucleotides in region between ^{32}P-label and cross-link is plotted.)

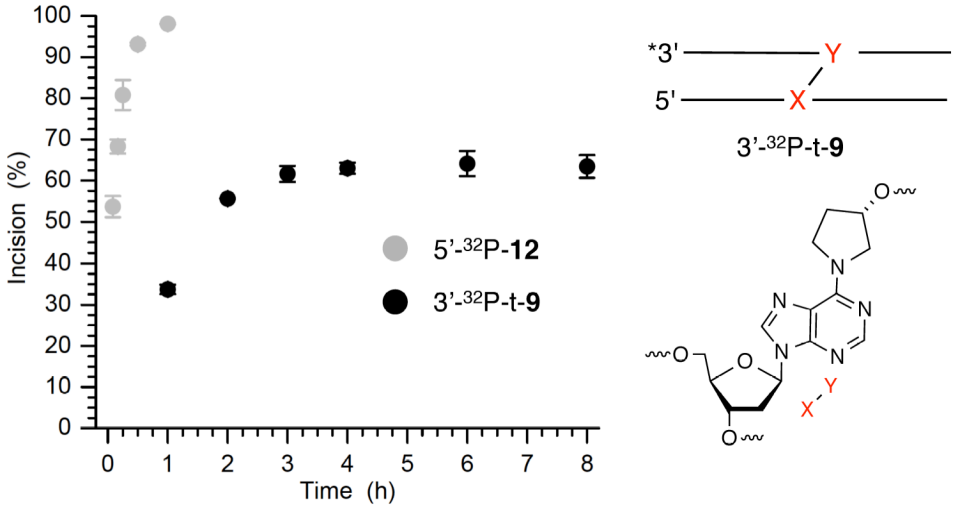


Figure S15. Time dependence of UvrABC incision of DOB ICL analogue **7** in 3'-^{32}P-t-**9** compared to that in 5'-^{32}P-**12**. (Total amount of incision at all nucleotides in region between ^{32}P-label and cross-link is plotted.)

1. Ghosh, S., and Greenberg, M. M. (2014) Synthesis of cross-linked DNA containing oxidized abasic site analogues. *J. Org. Chem. 79*, 5948-57.