Supporting Information: Correlation of Thermal Stability and Structural Distortion of DNA Interstrand Cross-links Produced From Oxidized Abasic Sites With Their Selective Formation and Repair

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Contents:

General Methods. Syntheses of cross-linked oligonucleotides (**S1** and **S2**) 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).

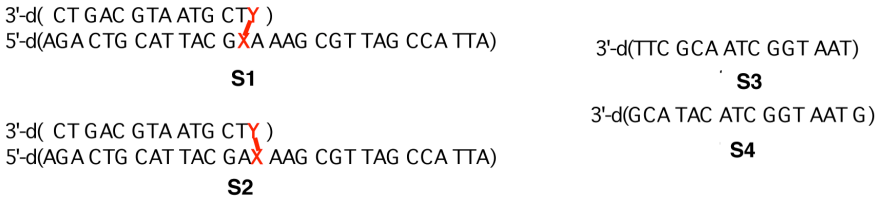
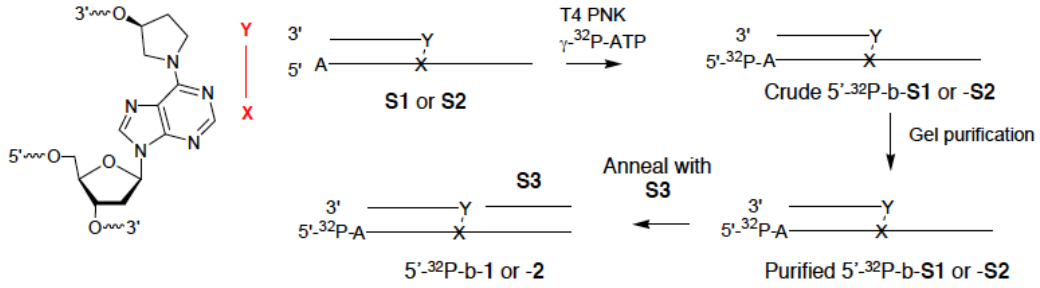


Figure S1. Oligonucleotides used to prepare **1** and **2**.

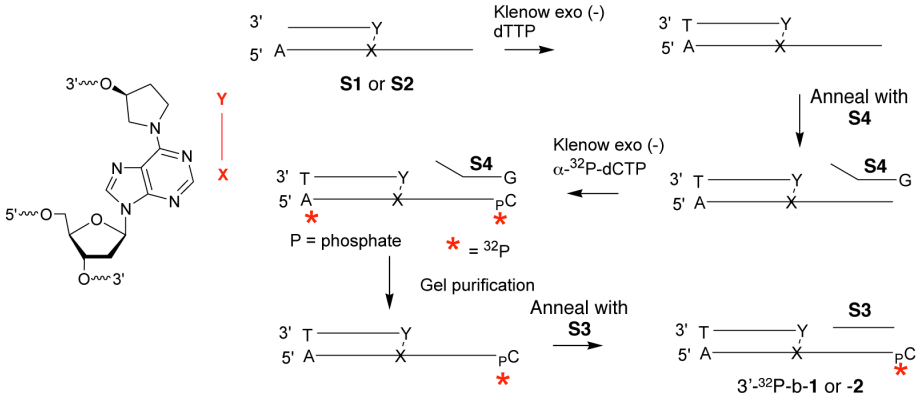
Scheme S1. Preparation of 5'-^{32}P-b-**1** and 5'-^{32}P-b-**2**.



*Preparation of 5'-^{32}P-b-**1** and 5'-^{32}P-b-**2** (Scheme S1).* Oligonucleotide **S1** or **S2** (50 pmol, Figure S1) 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 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 8 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 hybridized with **S3** (Figure S1) by slow cooling from 90 °C to 25 °C in 1 × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) to provide 5'-^{32}P-b-**1** and 5'-^{32}P-b-**2**. The yield (30-50%) was determined based on the specific activity of 5'-^{32}P-labeled **S1** and **S2**.

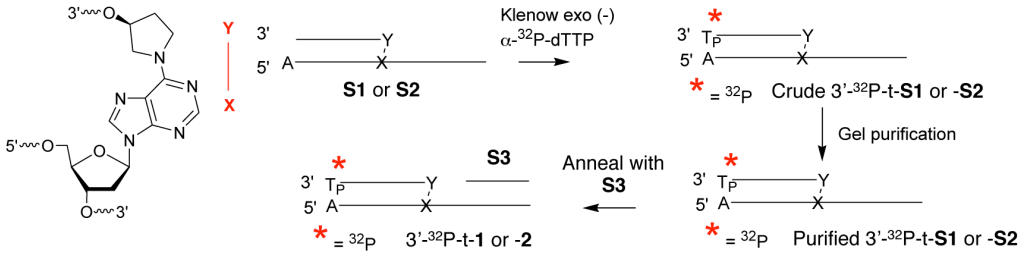
Scheme S2. Preparation of 3'-^{32}P-b-**1** and 3'-^{32}P-b-**2**.



*Preparation of 3'-^{32}P-b-**1** and 3'-^{32}P-b-**2** (Scheme S2).* Oligonucleotide **S1** or **S2** (50 pmol, Figure S1) was subjected to a "fill-in" reaction (30 μL) containing 1 × NEB 2.0 buffer (50 mM NaCl, 10 mM Tris•HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9), dTTP (1 mM), and 30 U of Klenow exo(-) at 37 °C for 3 h. The reaction was stopped by heating at 75 °C for 30 min. The reaction was then added and hybridized with **S4** (100 pmol, Figure S1) 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 annealed 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 $MgCl_2$, 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 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 8 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 hybridized with **S3** (Figure S1) by slow cooling from 90 °C to 25 °C in 1 × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) to provide 3'-^{32}P-b-**1** and 3'-^{32}P-b-**2**. The yields (30-50%) of 3'-^{32}P-b-**1** -**2** were determined based on the specific activity of 3'-^{32}P-labeled **S1** and **S2**.

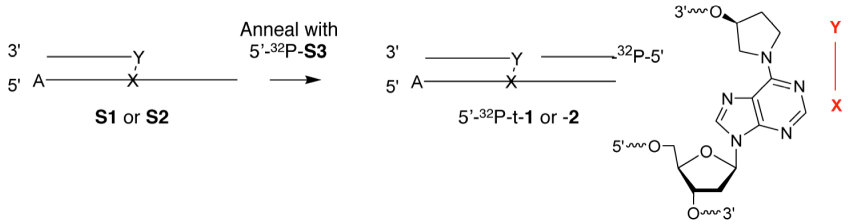
Scheme S3. Preparation of 3'-^{32}P-t-**1** and 3'-^{32}P-t-**2**.



*Preparation of 3'-^{32}P-t-**1** and 3'-^{32}P-t-**2** (Scheme S3).* Oligonucleotide **S1** or **S2** (50 pmol, Figure S1) was subjected to reaction (50 µL) containing 1 × NEB 2.0 buffer (50 mM NaCl, 10

mM Tris•HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9), 30 μCi α-^{32}P-dTTP, 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. 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 8 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 hybridized with **S3** (Figure 76) by slow cooling from 90 °C to 25 °C in 1 × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) to provide 3'-^{32}P-t-**1** and 3'-^{32}P-t-**2**. The yield (30-50%) of 3'-^{32}P-t-**1** and 3'-^{32}P-t-**2** were determined based on the specific activity of 3'-^{32}P-labeled **S1** and **S2**.

Scheme S4. Preparation of 5'-^{32}P-t-**1** and 5'-^{32}P-t-**2**.



*Preparation of 5'-^{32}P-t-**1** and 5'-^{32}P-t-**2** (Scheme S4).* Oligonucleotide **S3** (30 pmol, Figure S1) was 5'-^{32}P-labeled in a reaction (50 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 absorption (A_{260}) of 5'-^{32}P-**S3**. Oligonucleotide 5'-^{32}P-**S3** was hybridized with with **S1** or **S2** (60 pmol, Figure S1) by slow cooling from 90 °C to 25 °C in 1 × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) to provide 5'-^{32}P-t-**1** and 5'-^{32}P-t-**2**.

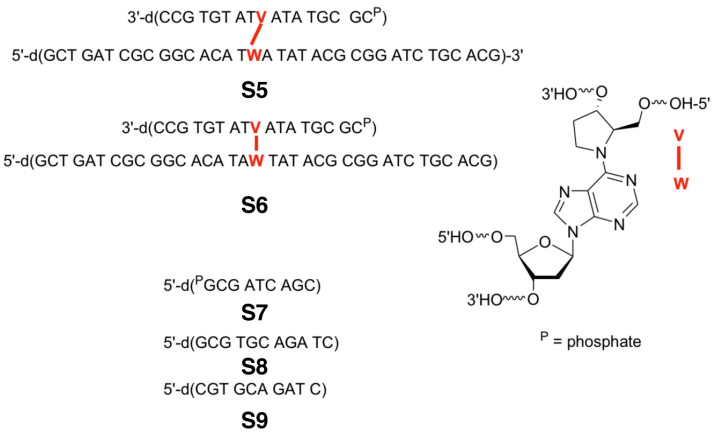
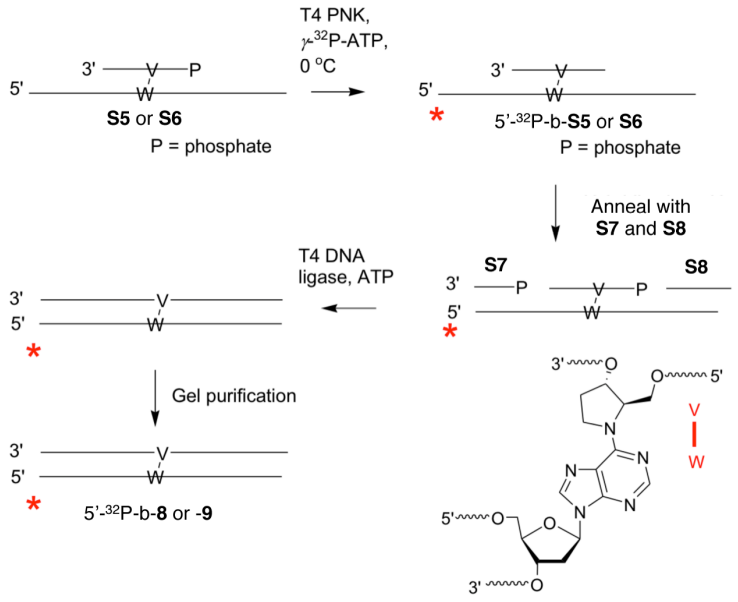


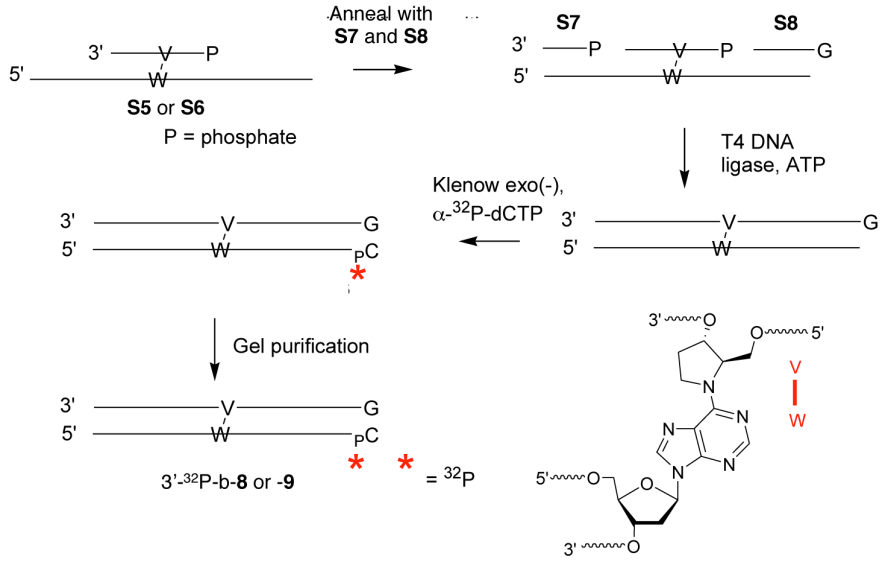
Figure S2. Oligonucleotides used to prepare **8** and **9**.

Scheme S5. Preparation of 5'-^{32}P-b-**8** and 5'-^{32}P-b-**9**.



*Preparation of 5'-^{32}P-b-**8** and 5'-^{32}P-b-**9** (Scheme S5).* Oligonucleotide **S5** or **S6** (80 pmol, Figure S2) was 5'-^{32}P-labeled in a reaction (50 μL) containing 1 × T4 PNK buffer (70 mM Tris•HCl pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 6% PEG 8000 solution (w/v), 50 μCi γ-^{32}P-ATP, and 30 U of T4 PNK at 0 °C for 2 h. Excess γ-^{32}P-ATP was removed by passing the reaction through a 1 mL Sephadex G25 column. The phosphorylation reaction was stopped by heating at 65 °C for 30 min and the T4 PNK was removed by phenol-chloroform-isopropanol extraction. 5'-^{32}P-b-**S5** or 5'-^{32}P-b-**S6** was hybridized with **S7** and **S8** (160 pmol each) in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution (120 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 to the reaction, 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 11 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-b-**8** or 5'-^{32}P-b-**9**. The isolated final products were suspended in × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) and hybridized by slow cooling from 90 °C to 25 °C.

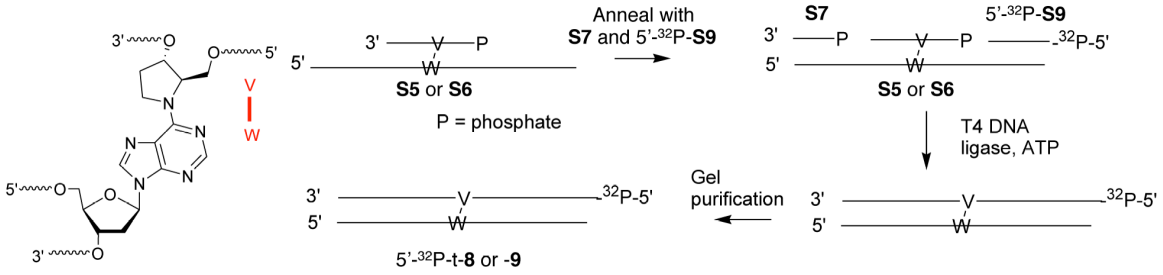
Scheme S6. Preparation of 3'-^{32}P-b-**8** and 3'-^{32}P-b-**9**.



*Preparation of 3'-^{32}P-b-**8** and 3'-^{32}P-b-**9** (Scheme S6).* Oligonucleotide **S5** or **S6** (50 pmol) was annealed with **S7** and **S8** (100 pmol) 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). The ligated substrates were subjected to a fill-in reaction (100 μL) containing 1 × NEB 2.0 buffer (50 mM NaCl, 10 mM Tris•HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9), dTTP (1 mM), 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 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 assuming hundred percent recovery from the Sephadex column. Formamide loading buffer (50 μL, 90% formamide, 10 mM EDTA, pH 8.0) was added the ^{32}P-labeled substrates, 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 11 h. The product band was excised from the gel, crushed, and the DNA was eluted overnight in 5 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 3'-^{32}P-b-**8** or 3'-^{32}P-b-**9**. The isolated final products were suspended in × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) and hybridized by slow cooling from 90 °C to 25 °C. The yields (4-10%) of purified 3'-^{32}P-b-**8** and 3'-^{32}P-b-**9** was determined based on the specific activity of crude substrates.

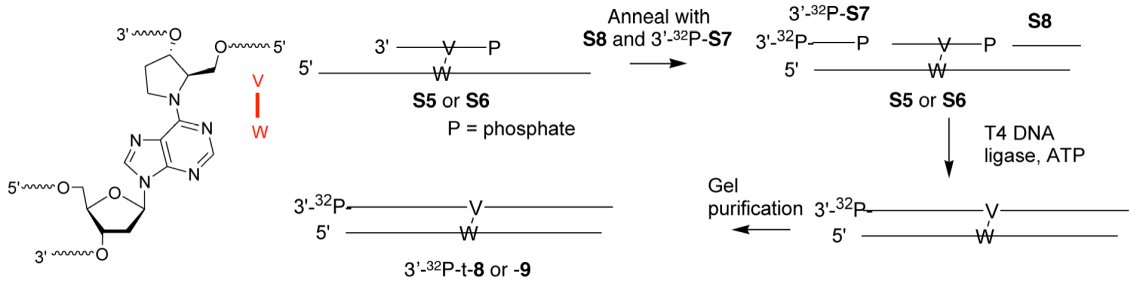
Scheme S7. Preparation of 5'-^{32}P-t-**8** and 5'-^{32}P-t-**9**.



*Preparation of 5'-^{32}P-t-**8** and 5'-^{32}P-t-**9** (Scheme S7).* **S9** (80 pmol) was 5'-^{32}P-labeled in a reaction (50 µL) containing 1 × T4 PNK buffer (70 mM Tris•HCl pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 6% PEG 8000 solution (w/v), 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 **S9** (A_{260}, Ɛ = 94900 L•mol^{-1}•cm^{-1}). 5'-^{32}P-**S9** was hybridized with 50 pmol of **S5** or **S6** and 160 pmol of **S7** in 10 mM sodium phosphate (pH 7.2) and 100 mM sodium chloride solution (120 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 to the reaction, 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 11 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-**8** or 5'-^{32}P-t-**9**. The isolated final products were suspended in × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) and hybridized by slow cooling from 90 °C to 25 °C. The yield (4–10%) of 5'-^{32}P-t-**8** and 5'-^{32}P-t-**9** was determined based on the specific activity of 5'-^{32}P-**S9**.

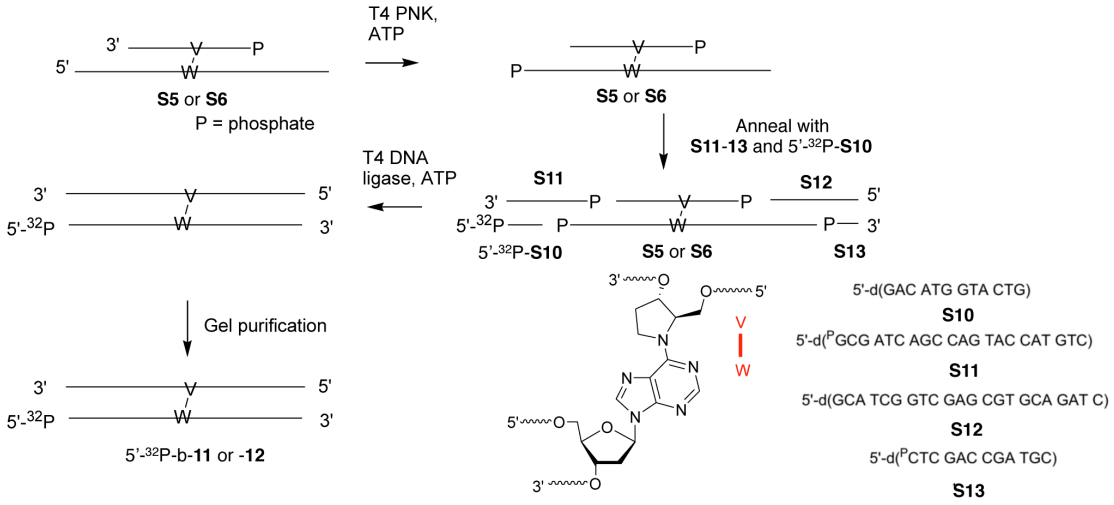
Scheme S8. Preparation of 3'-^{32}P-t-**8** and 3'-^{32}P-t-**9**.



*Preparation of 3'-^{32}P-t-**8** and 3'-^{32}P-t-**9** (Scheme S8).* **S7** (80 pmol) was 3'-^{32}P-labeled in a reaction (50 µL) containing 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. 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 **S7** (A_{260}, Ɛ = 86300 L•mol^{-1}•cm^{-1}). 3'-^{32}P-**S7** and **S8** were hybridized with **S5** or **S6** (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 to the reaction, 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 11 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 3'-^{32}P-t-**8** or 3'-^{32}P-t-**9**. The isolated final products were suspended in × PBS buffer (10 mM sodium phosphate, pH 7.2, 100 mM NaCl) and hybridized by slow cooling from 90 °C to 25 °C. The yield (4–10%) of 3'-^{32}P-t-**8** and 3'-^{32}P-t-**9** was determined based on the specific activity of 3'-^{32}P-**S7**.

Scheme S9. Preparation of 5'-^{32}P-b-**11** and 5'-^{32}P-b-**12**.



*Preparation of 5'-^{32}P-b-**11** and 5'-^{32}P-b-**12** (Scheme S9).* **S10** (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), 6% PEG 8000 solution (w/v), 50 μCi γ-^{32}P-ATP, and 30 U of T4 PNK at 37 °C for 1 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 5'-^{32}P-**S10** (A_{260}, Ɛ =119700 L•mol^{-1}•cm^{-1}). Separately, **S5** or **S6** (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 **S11**, **S12**, and **S13** 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 5 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-b-**11** or 5'-^{32}P-b-**12**. The yield (10-20%) of 5'-^{32}P-b-**11** or 5'-^{32}P-b-**12** was determined based on the specific activity of 5'-^{32}P-**S10**.

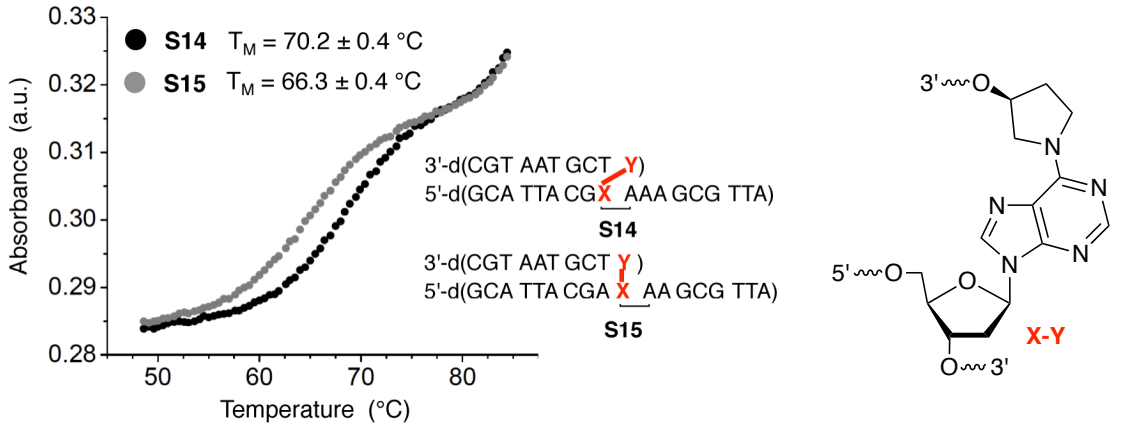


Figure S3. UV-melting of DOB ICL analogues **S14** and **S15**.

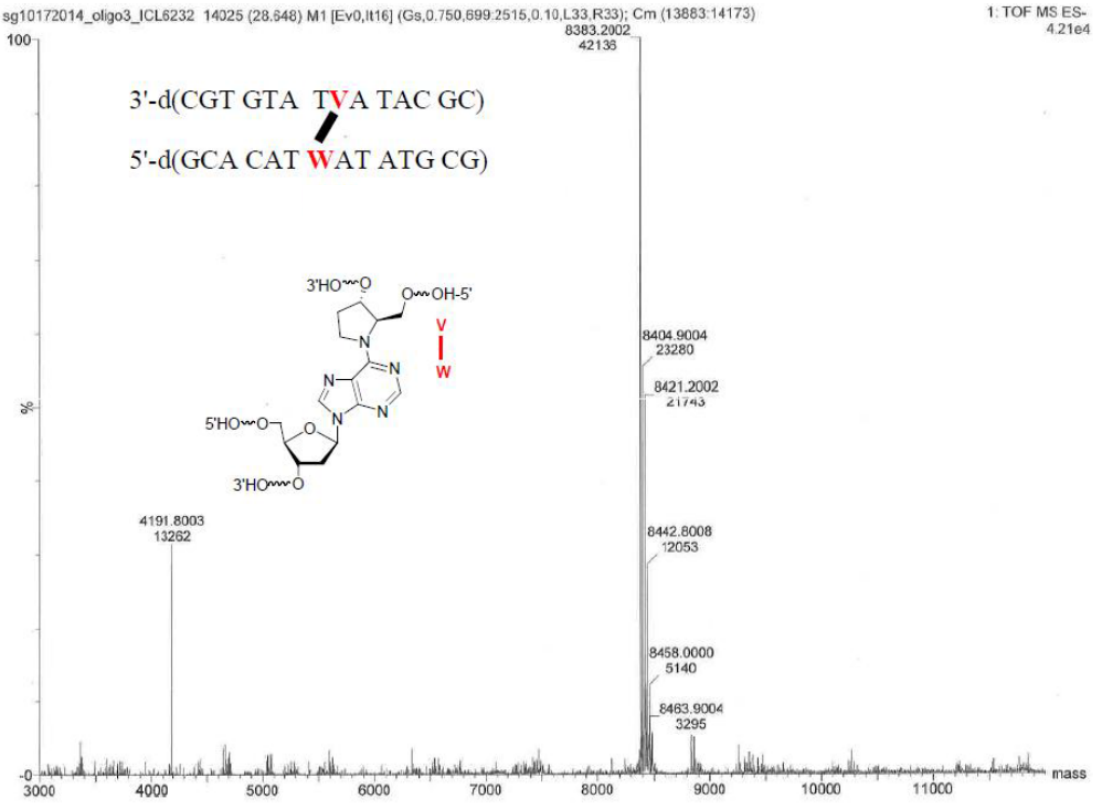


Figure S4. ESI-MS of **13**. Calc'd mass 8383.464, observed mass 4191.800 (z = -2), 8383.200, 8404.900 $[M + Na]^+$, 8421.200 $[M + K]^+$, 8442.800 $[M + Na + K]^+$, 8458.000 8421.200 $[M + 2K]^+$, 8463.900 $[M + 2Na + K]^+$.

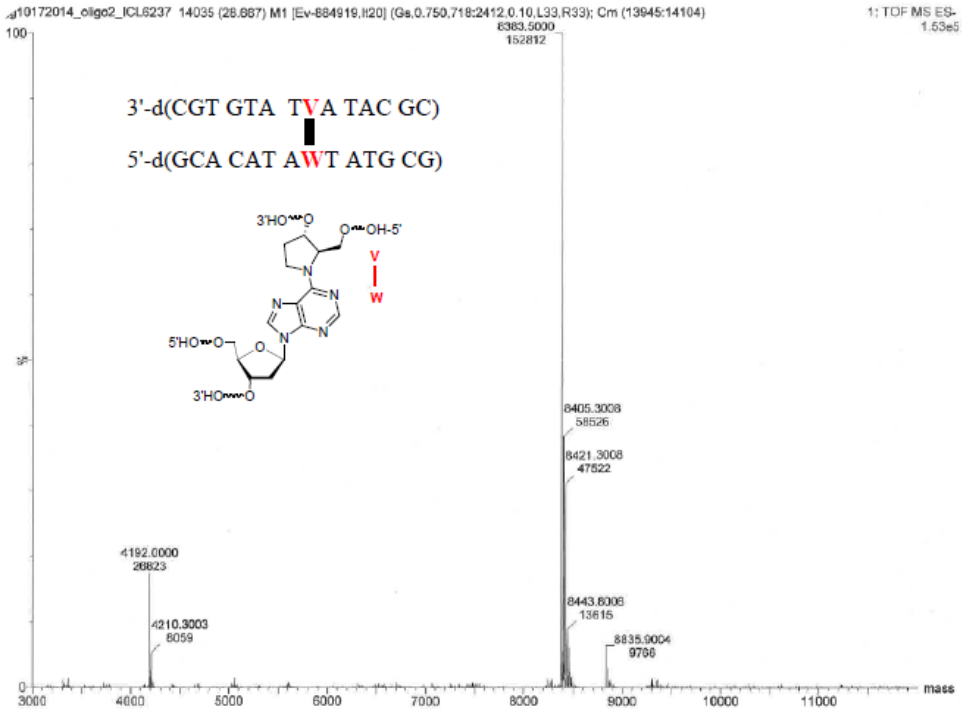


Figure S5. ESI-MS of **14**. Calc'd mass 8383.464, observed mass 4192.000 (z = -2), 8383.500, 8405.300 $[M + Na]^+$, 8421.300 $[M + K]^+$, 8443.800 $[M + Na + K]^+$.

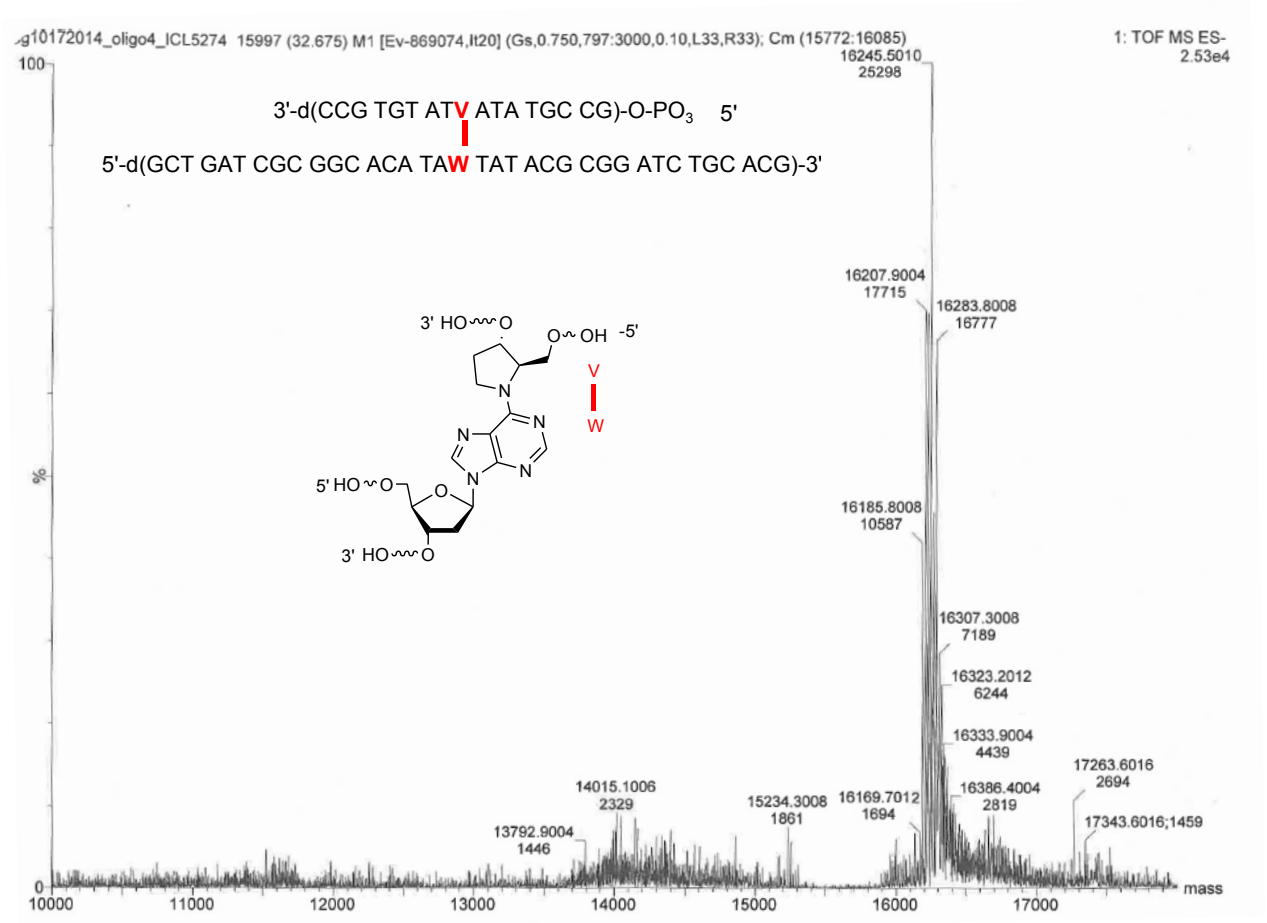


Figure S6. ESI-MS of **S6**. Calc'd mass 16182.485, observed mass 16185.800, 16207.900 $[M + Na]^+$, 16245.501 $[M + Na + K]^+$, 16283.801 $[M + Na + 2K]^+$, 16307.300 $[M + 2Na + 2K]^+$, 16323.201 $[M + Na + 3K]^+$, 16333.900 $[M + 3Na + 2K]^+$, 16386.400 $[M + 2Na + 4K]^+$.

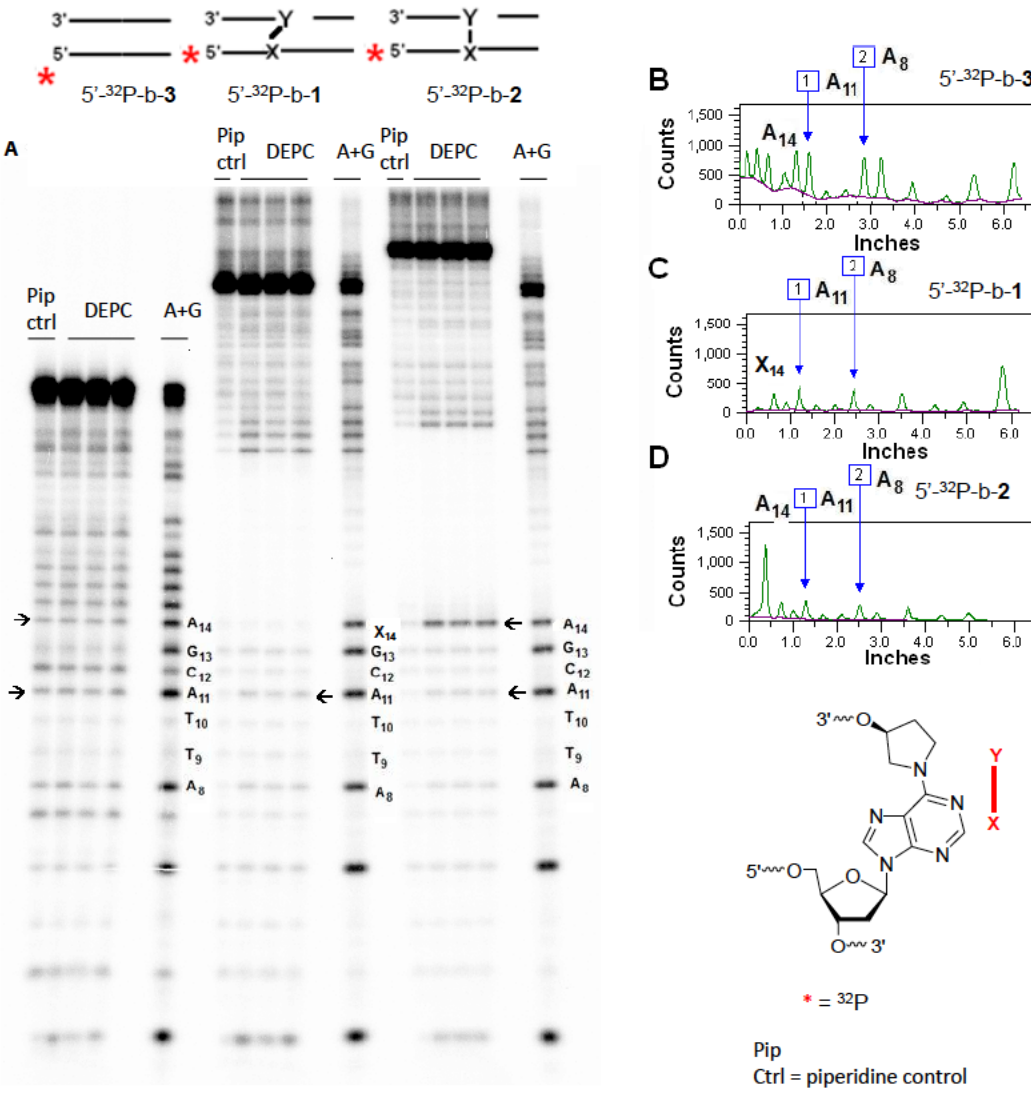


Figure S7. Representative autoradiogram of DEPC reaction with 5'-^{32}P-b-**1**-**3**.

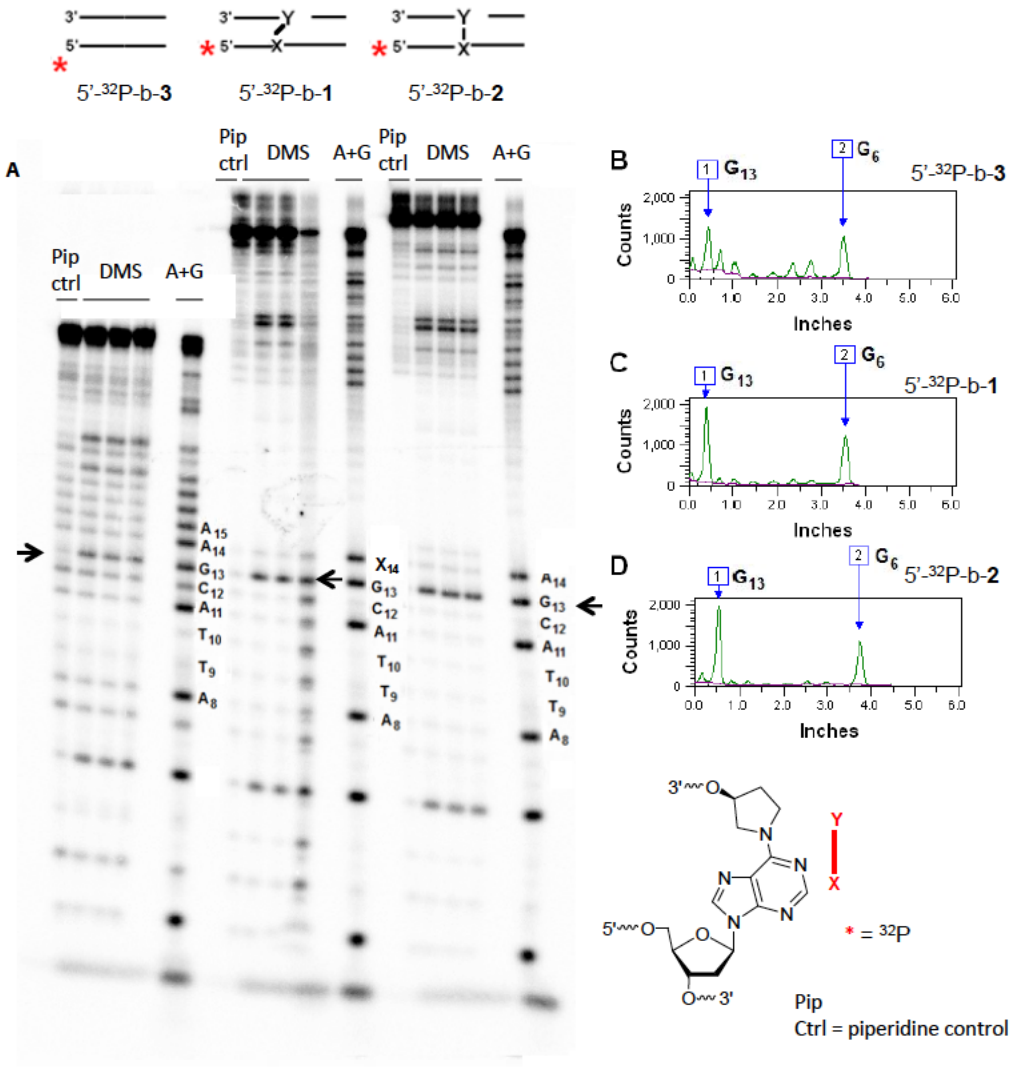


Figure S8. Representative autoradiogram of DMS reaction with 5'-^{32}P-b-**1**-**3**.

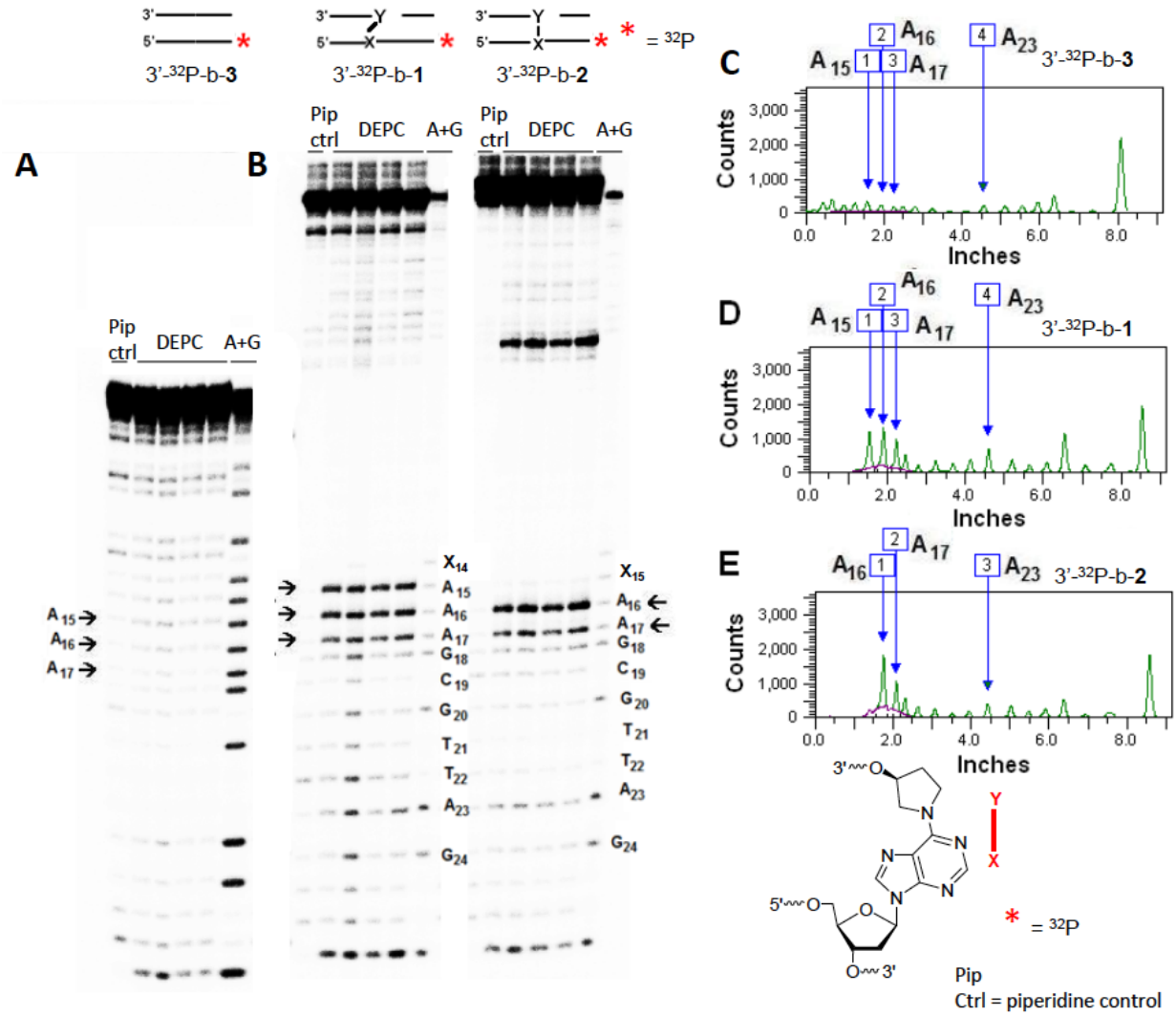


Figure S9. Representative autoradiogram of DEPC reaction with 3'-^{32}P-b-**1**-**3**.

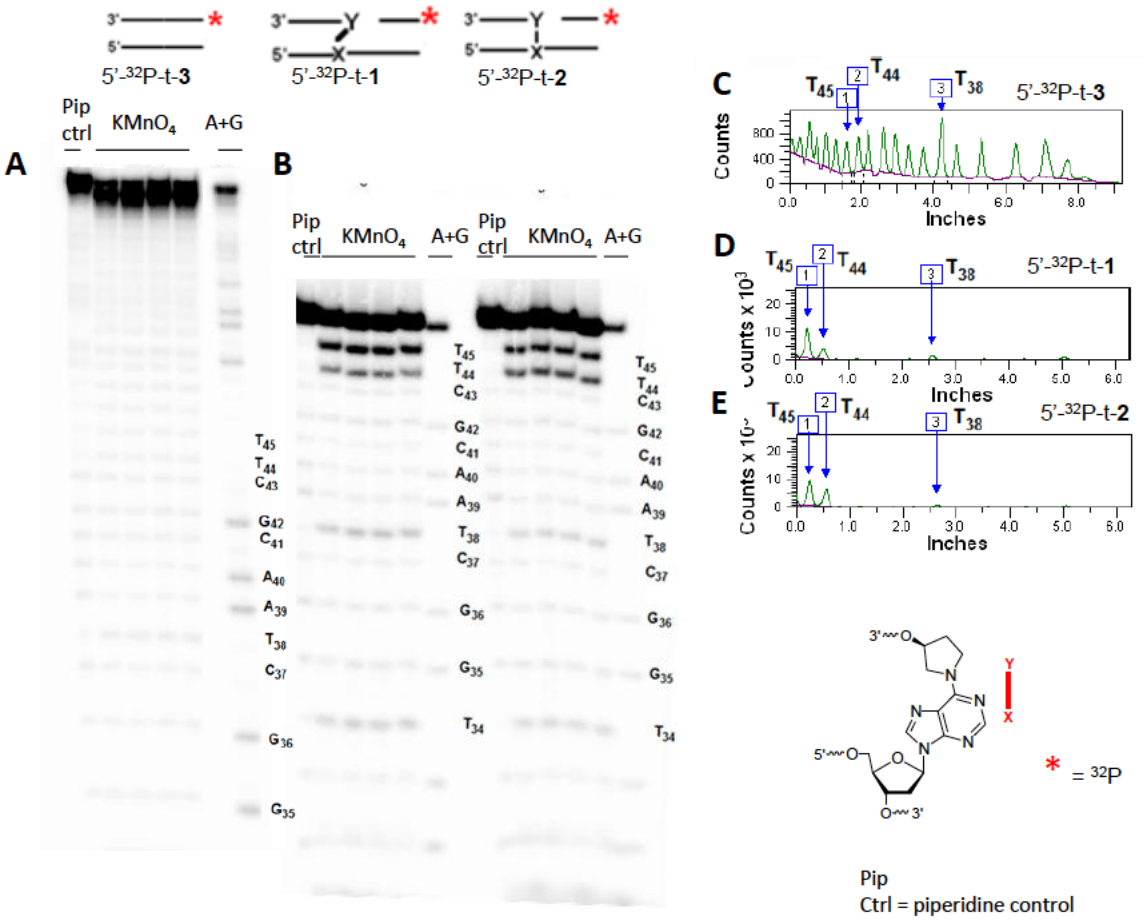


Figure S10. Representative autoradiogram of $KMnO_4$ reaction with 5'-^{32}P-t-**1**-**3**.

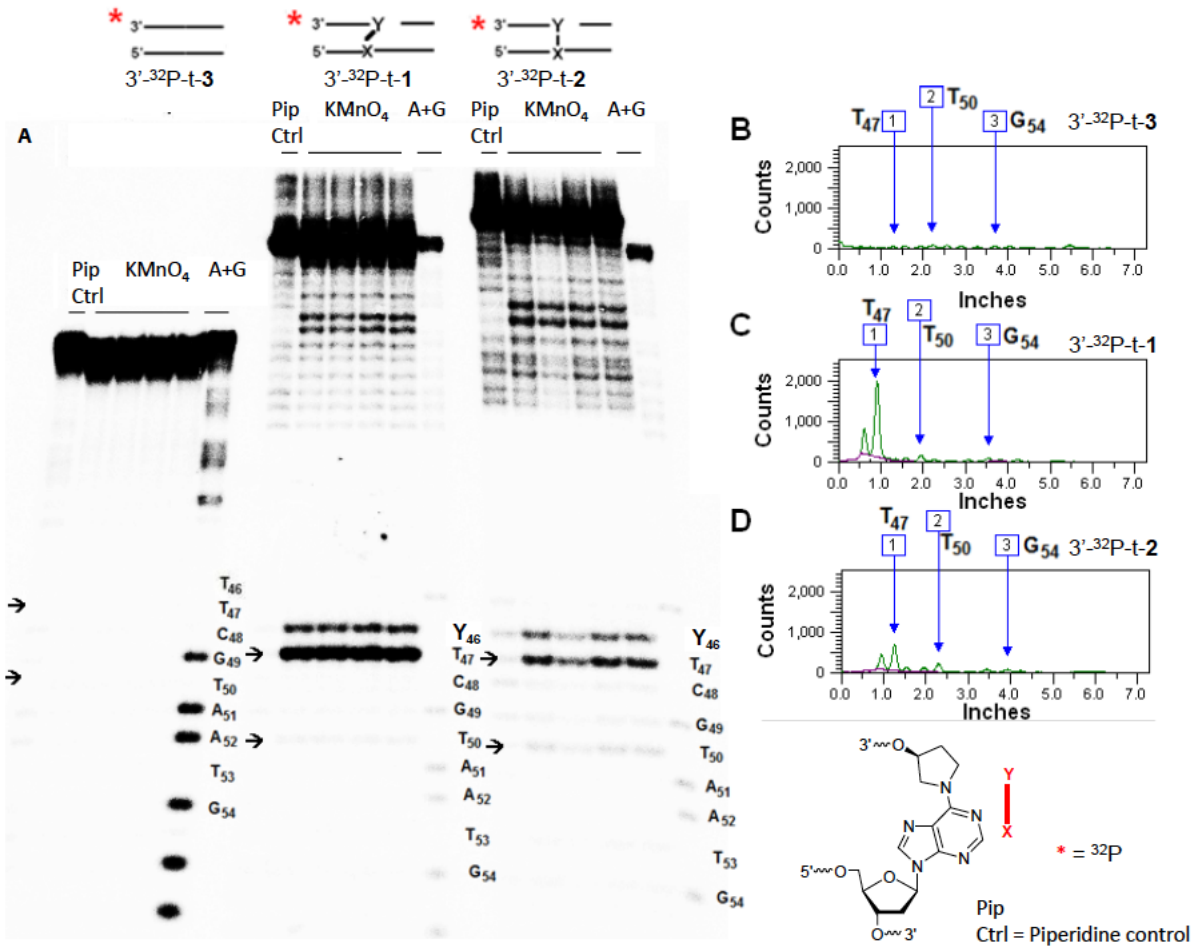


Figure S11. Representative autoradiogram of $KMnO_4$ reaction with 3'-^{32}P-t-**1**-**3**.

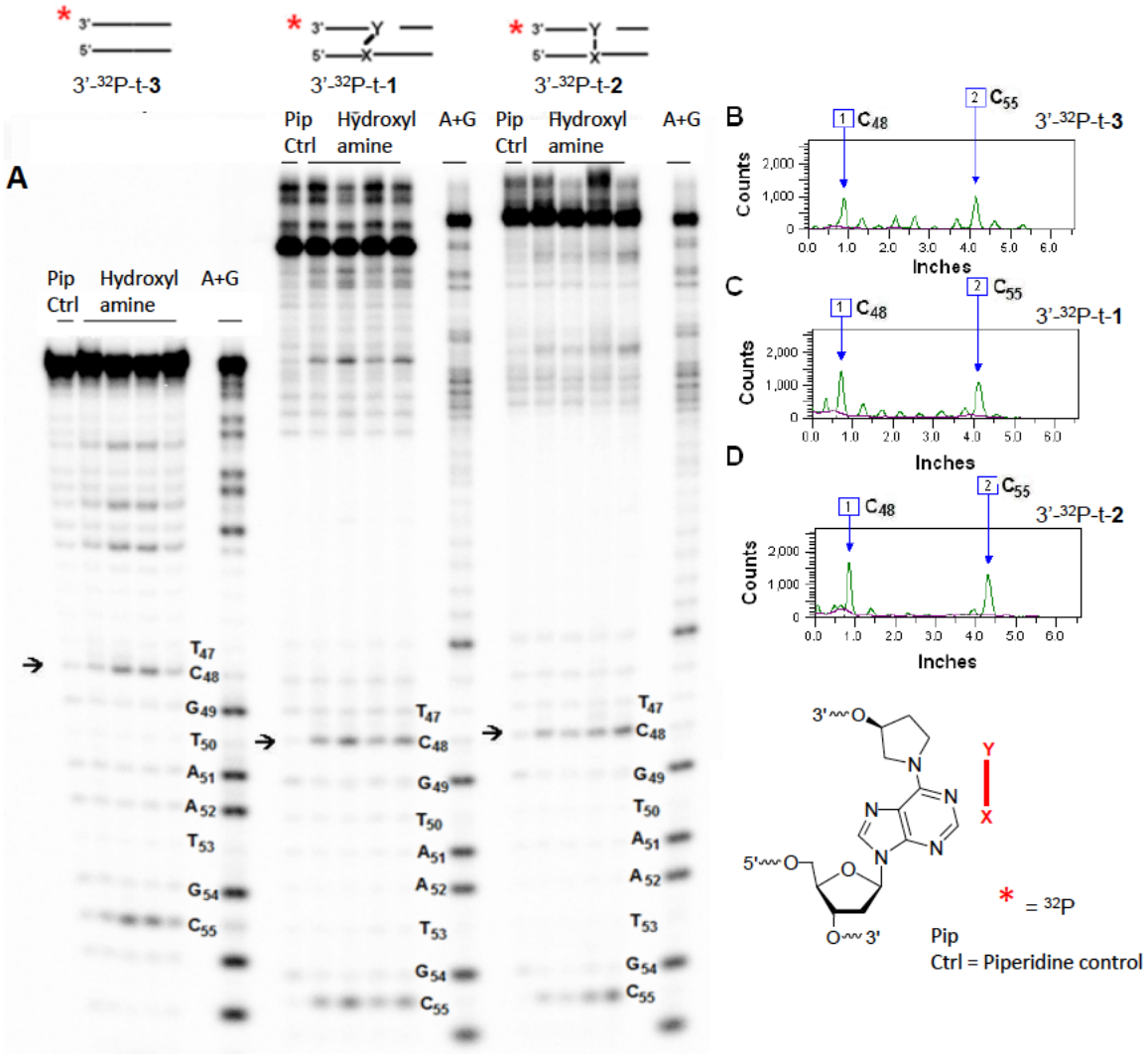


Figure S12. Representative autoradiogram of hydroxylamine reaction with 3'-^{32}P-t-**1**-**3**.

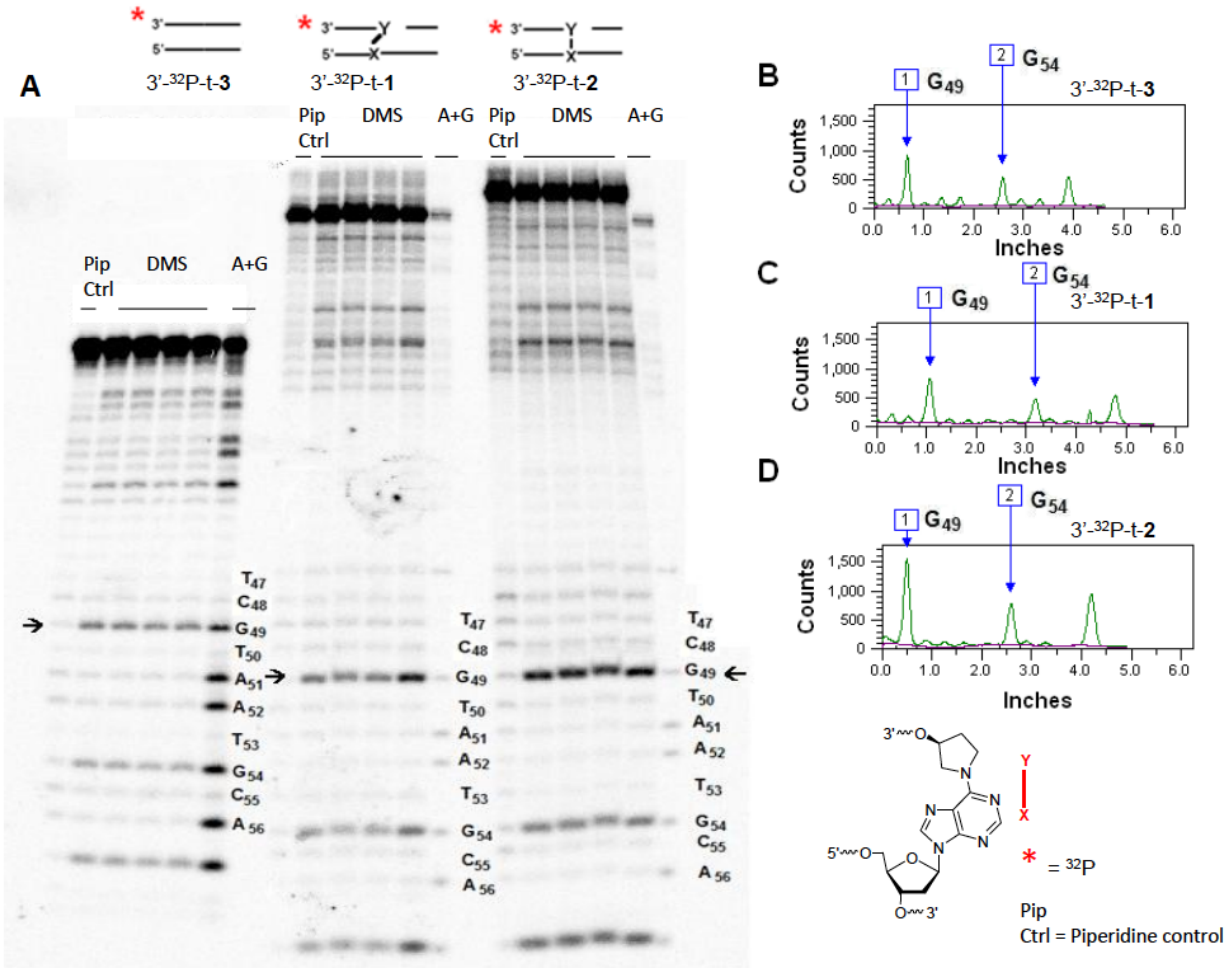


Figure S13. Representative autoradiogram of DMS reaction with 3'-^{32}P-t-**1**-**3**.

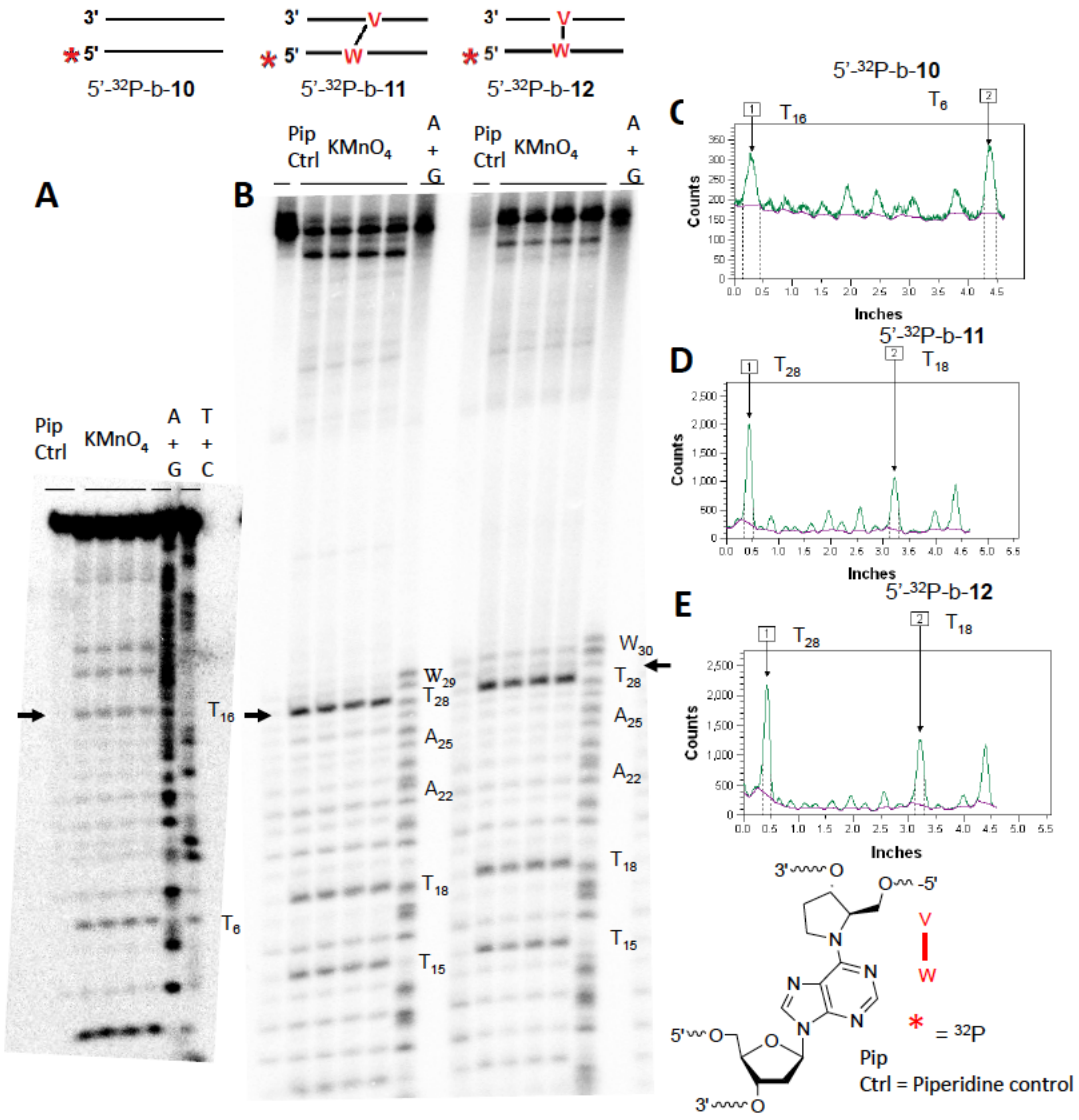


Figure S14. Representative autoradiogram of $KMnO_4$ reaction with 5'-^{32}P-b-**10-12**.

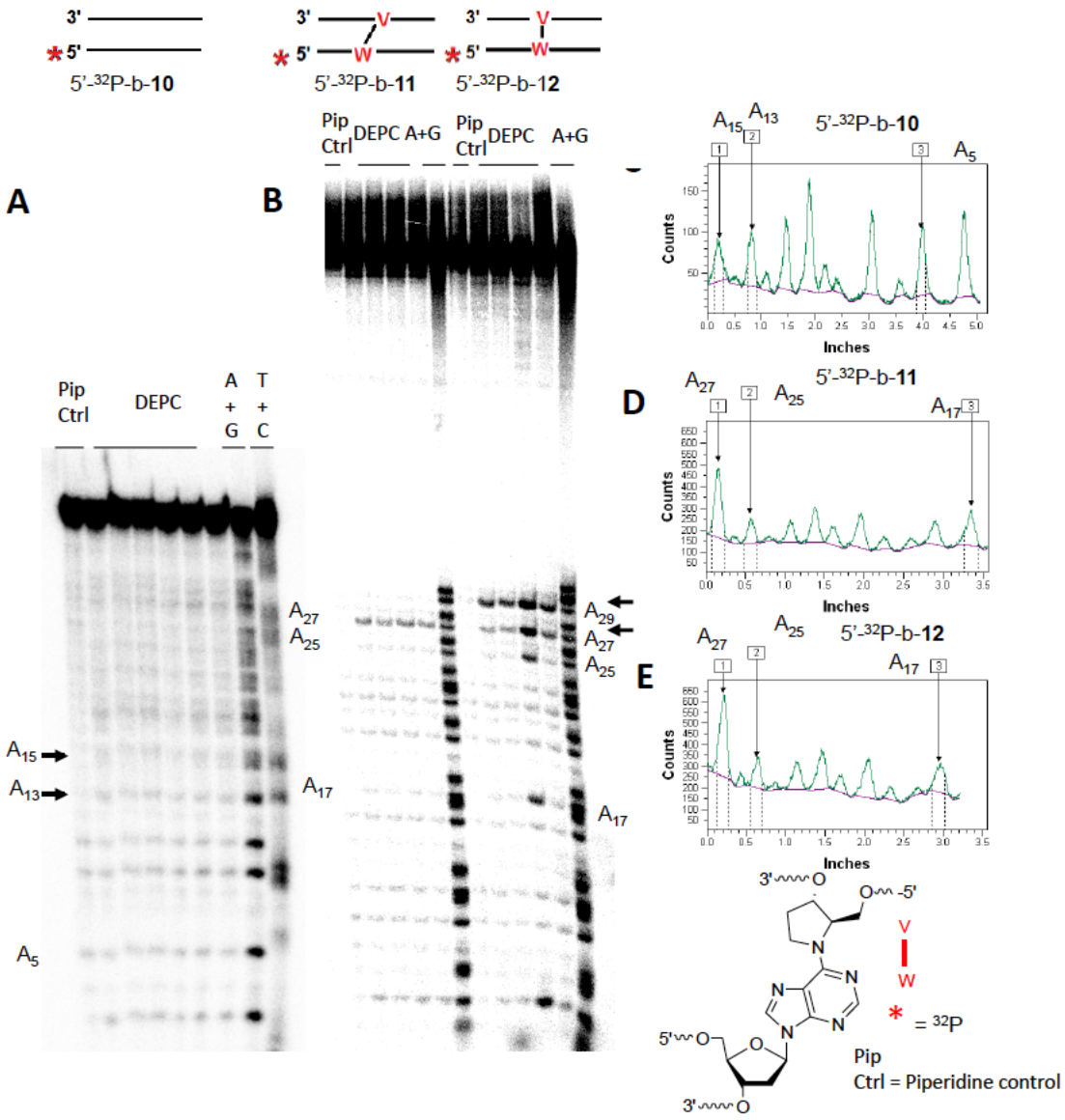


Figure S15. Representative autoradiogram of DEPC reaction with 5'-^{32}P-b-**10-12**.

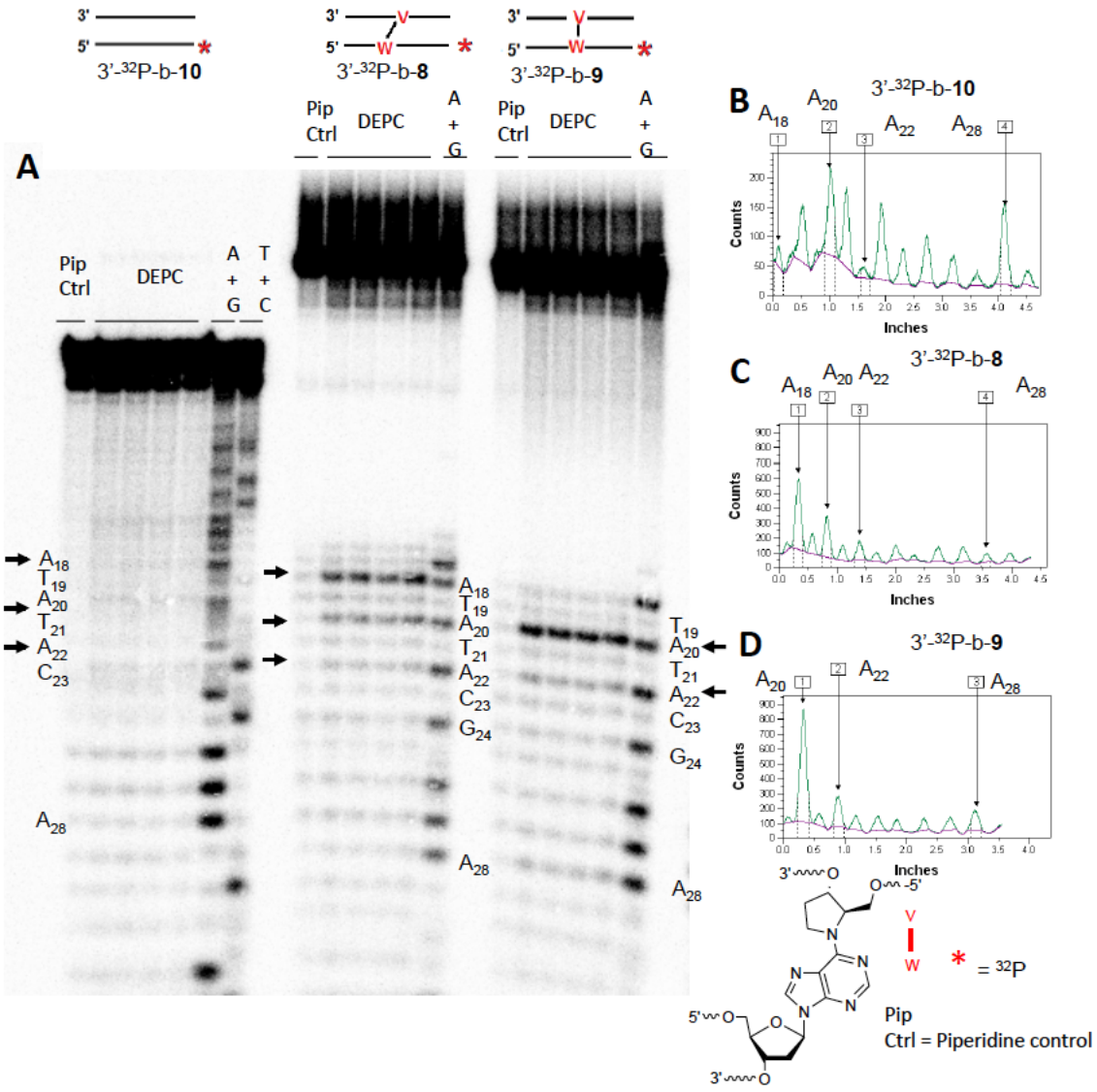


Figure S16. Representative autoradiogram of DEPC reaction with 3'-^{32}P-b-**10**-**12**.

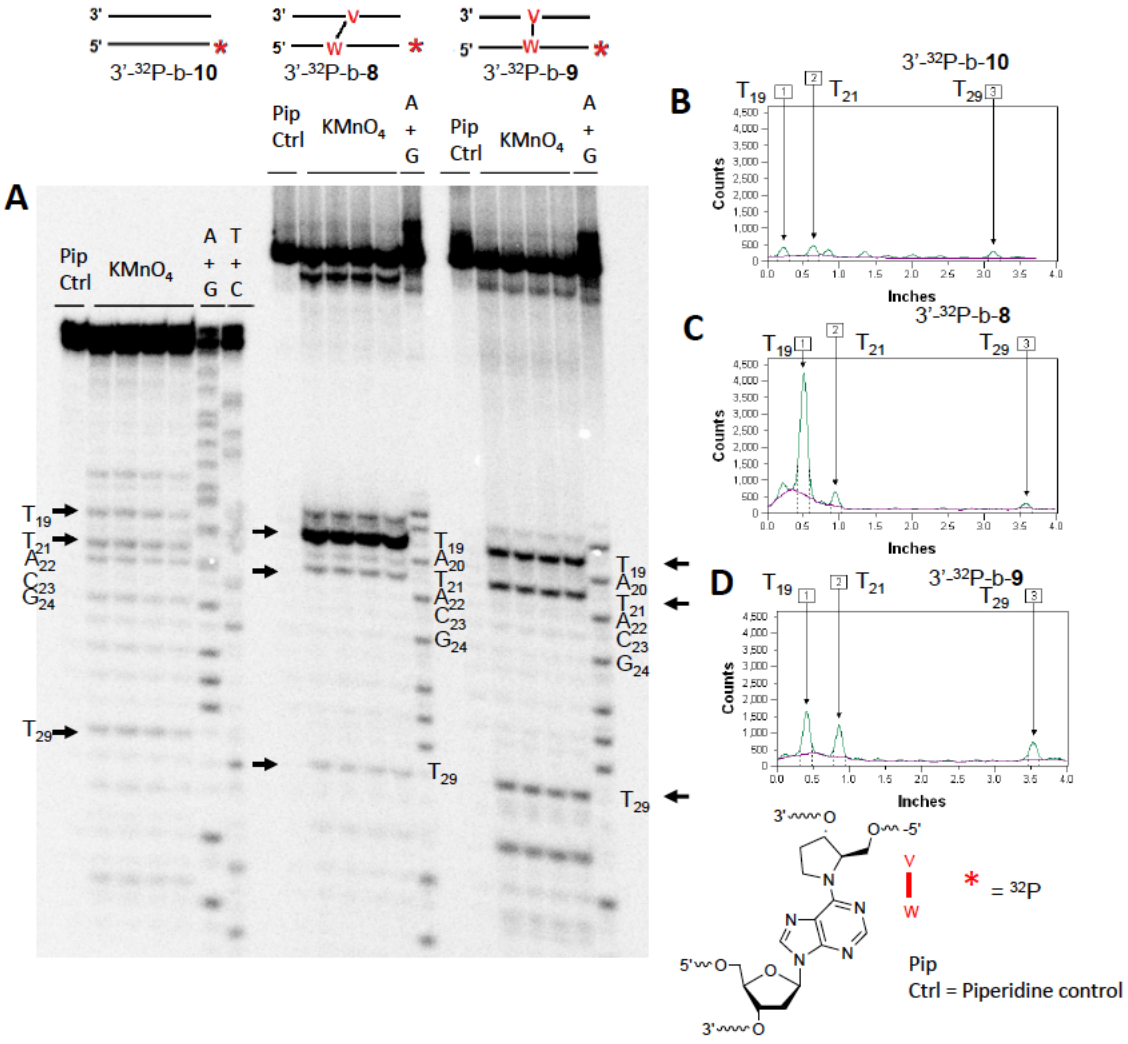


Figure S17. Representative autoradiogram of $KMnO_4$ reaction with 3'-^{32}P-b-**10**-**12**.

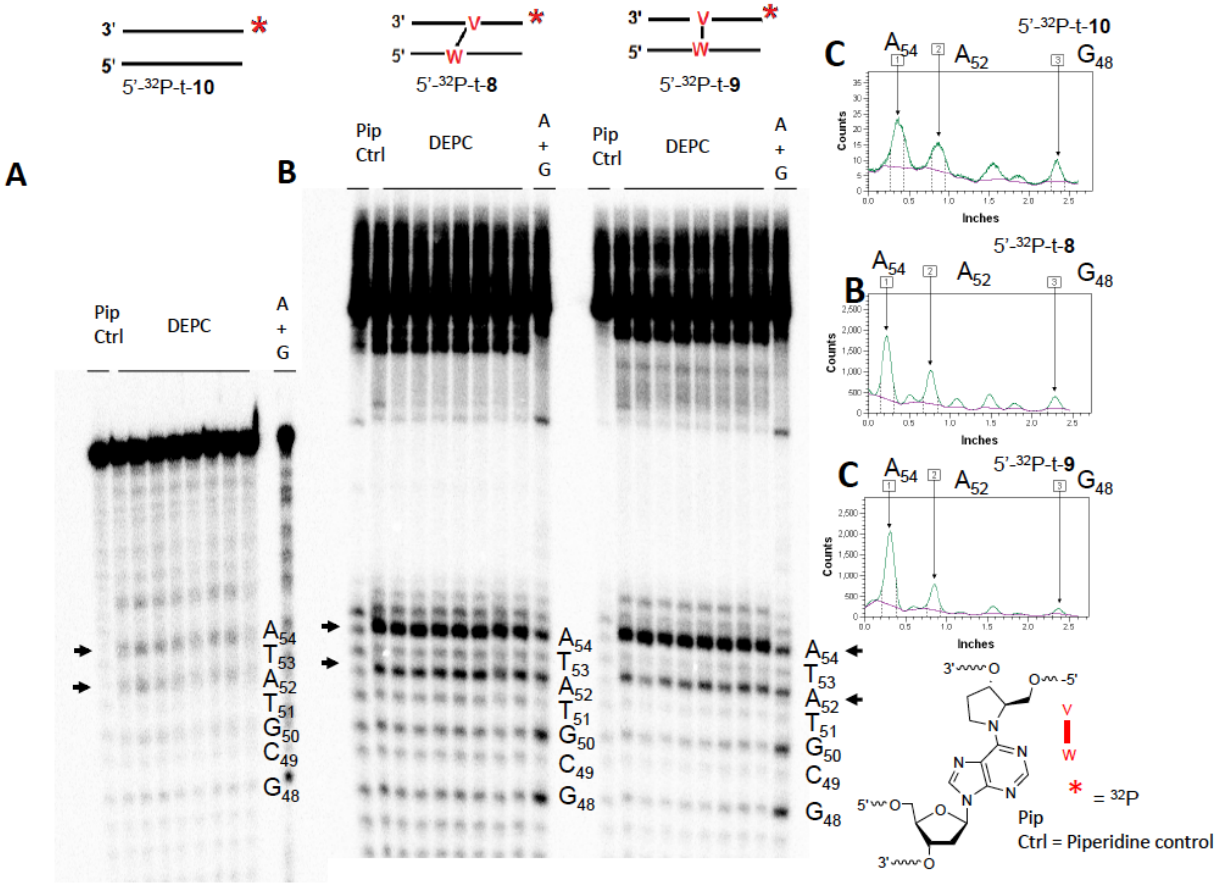


Figure S18. Representative autoradiogram of DEPC reaction with 5'-^{32}P-t-**8**-**10**.

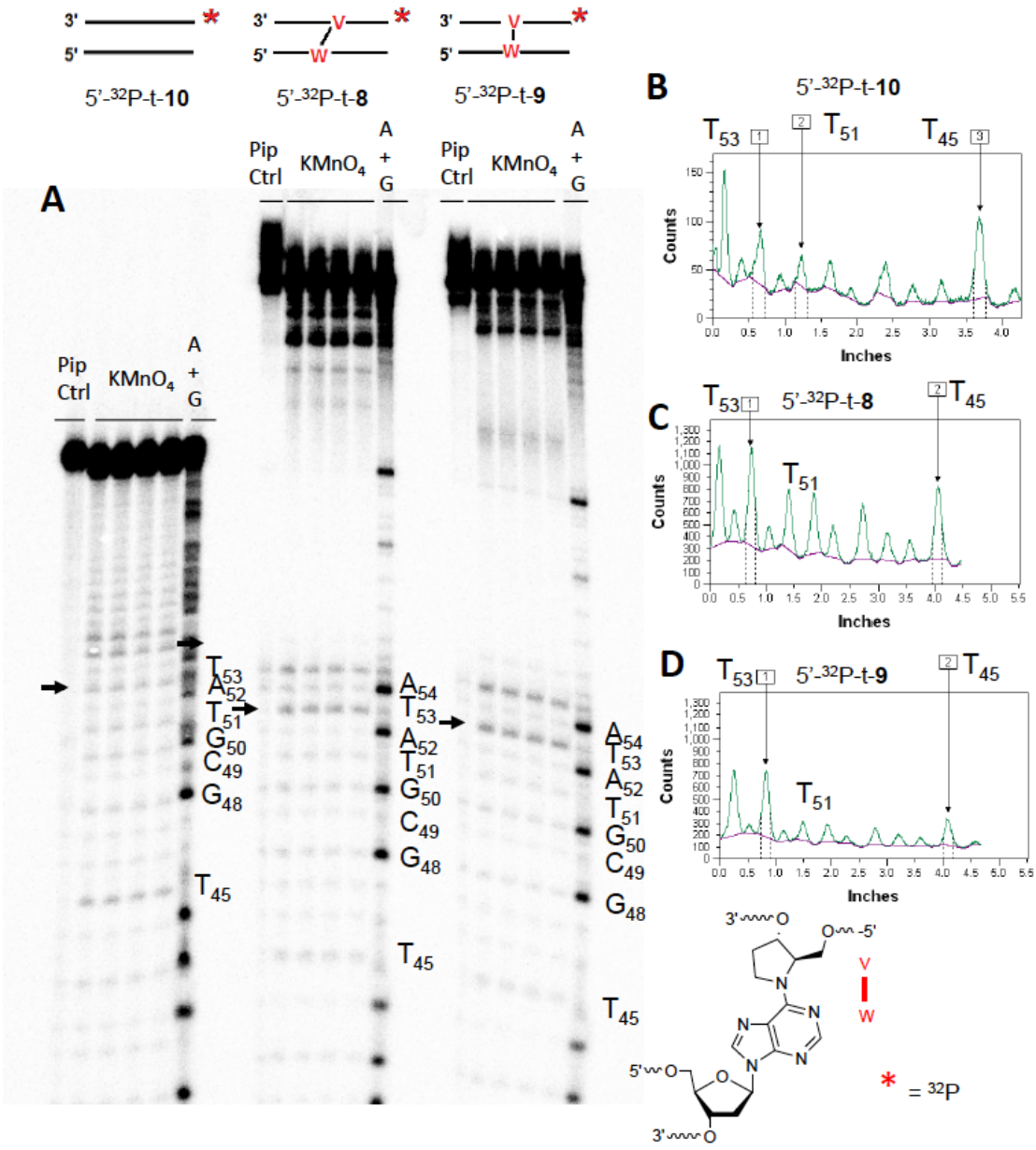


Figure S19. Representative autoradiogram of $KMnO_4$ reaction with 5'-^{32}P-t-**8**-**10**.

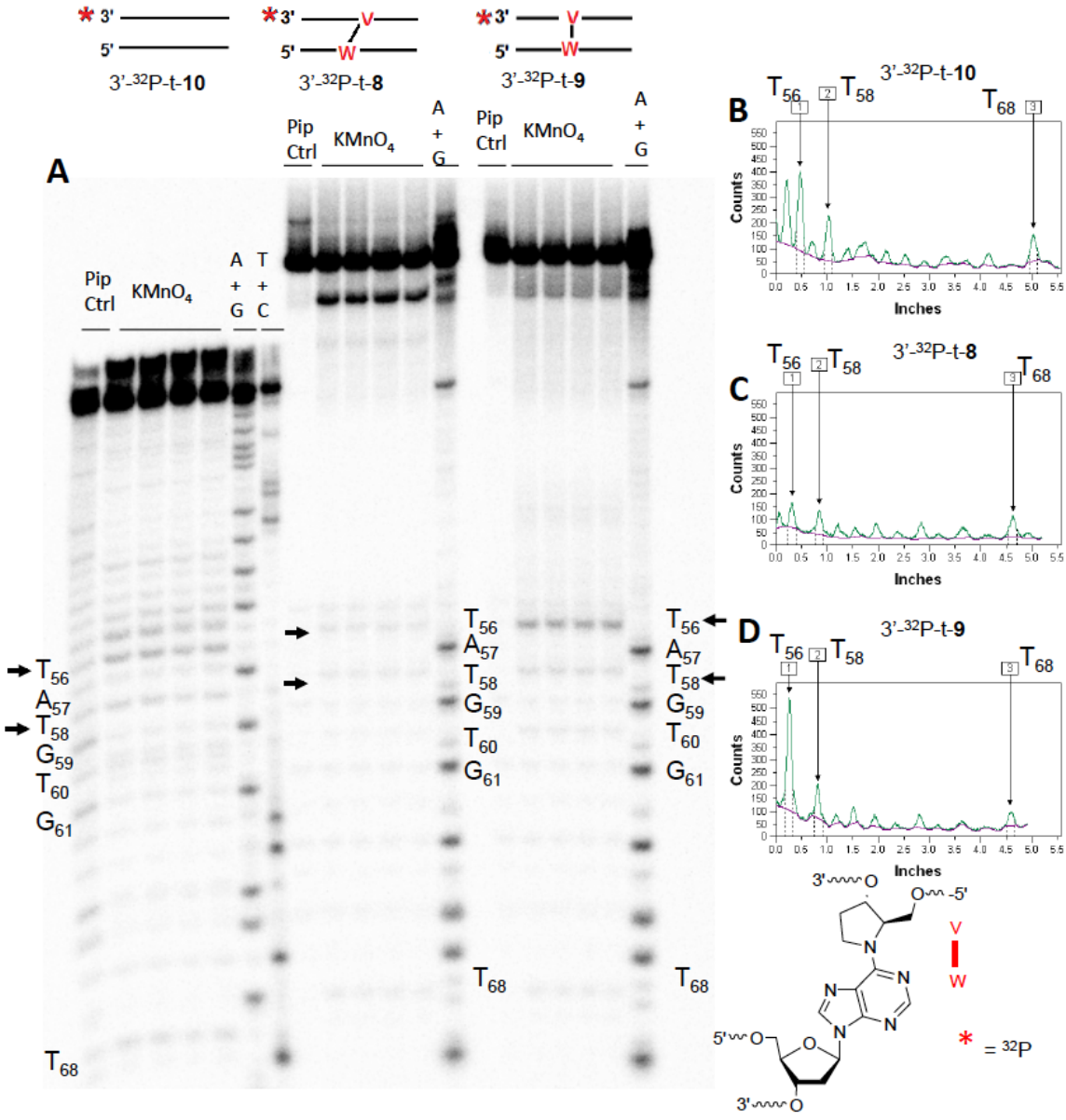


Figure S20. Representative autoradiogram of $KMnO_4$ reaction with 3'-^{32}P-t-**8**-**10**.

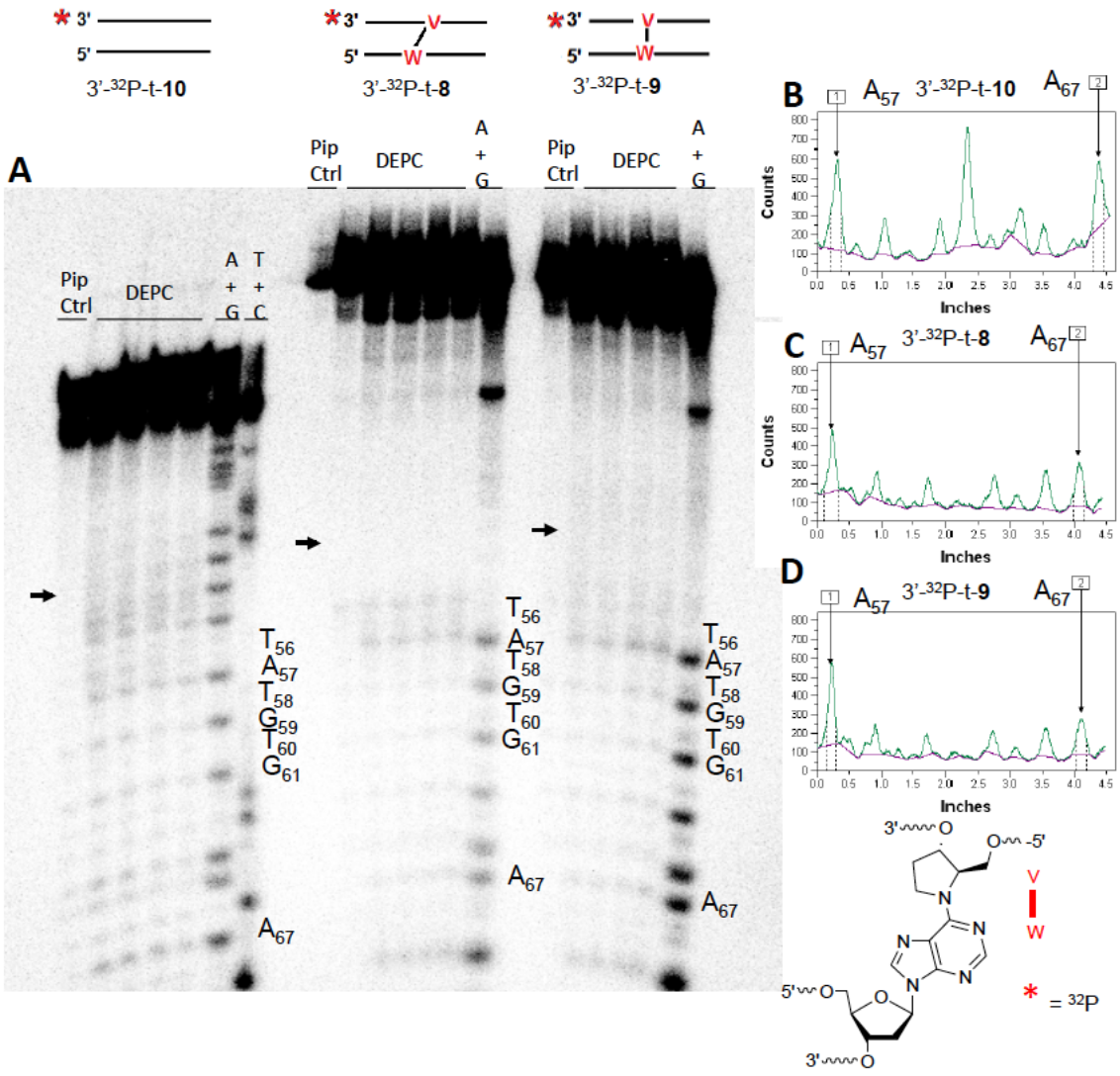


Figure S21. Representative autoradiogram of DEPC reaction with 3'-^{32}P-t-**8**-**10**.

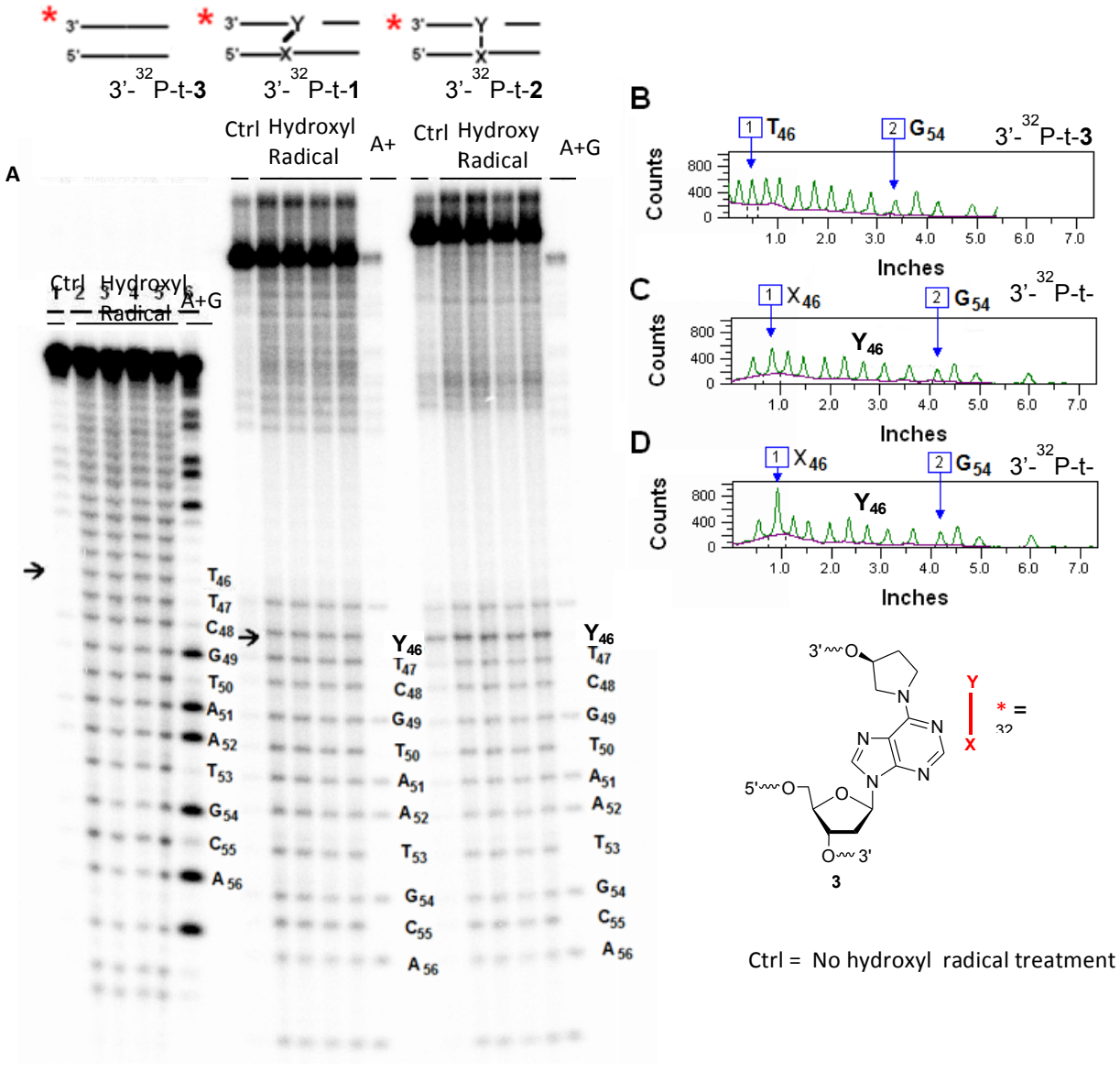


Figure S22. Representative autoradiogram of OH• reaction with 3'-^{32}P-t-**8**-**10**.

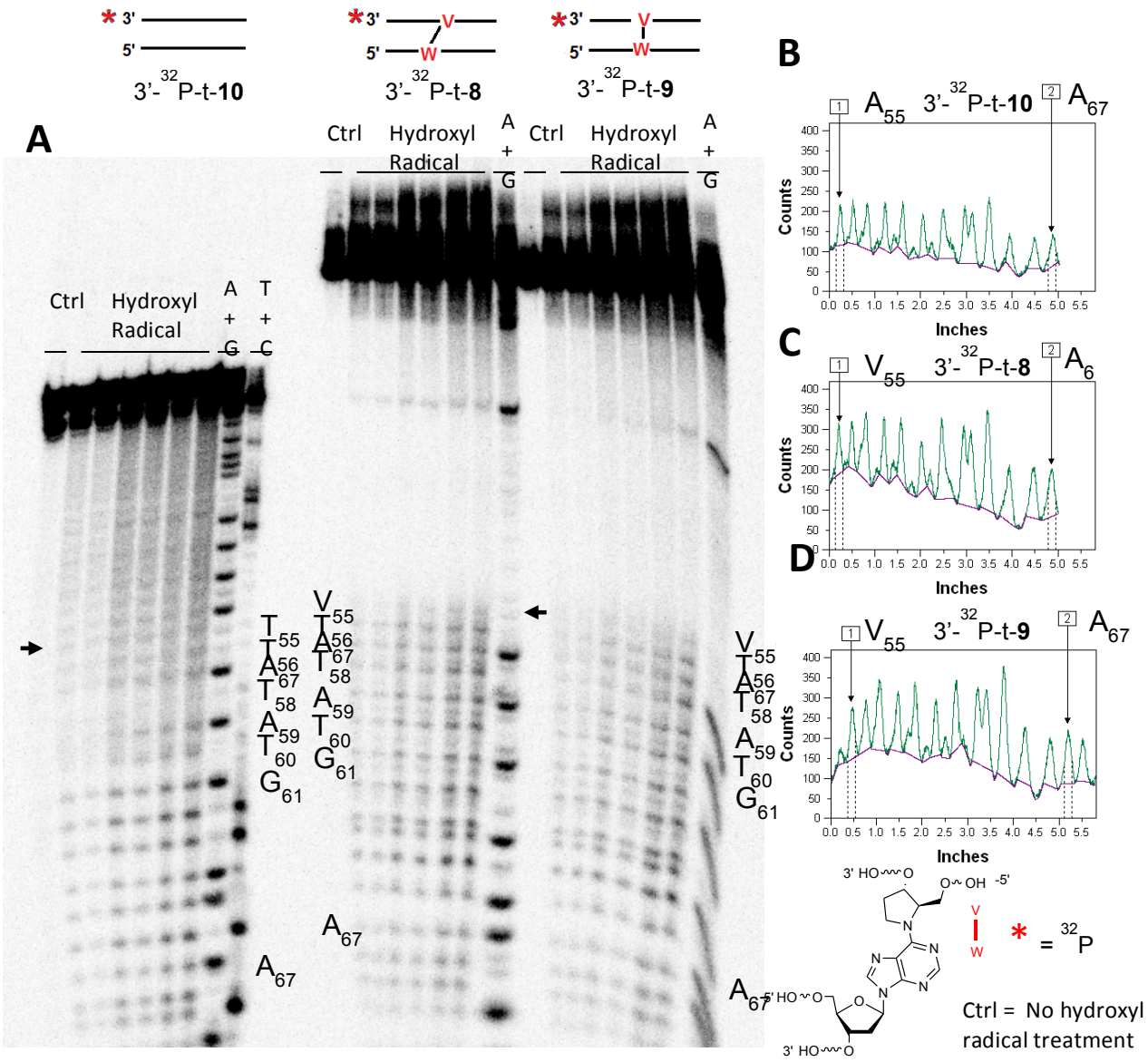


Figure S23. Representative autoradiogram of OH• reaction with 3'-^{32}P-t-**8**-**10**.