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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**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\(_4\)OH 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).

![Diagram](image)

**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 \(^{32}\)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 \(^{32}\)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'-32P-b-1 and 5'-32P-b-2. The yield (30-50%) was determined based on the specific activity of 5'-32P-labeled S1 and S2.

Scheme S2. Preparation of 3'-32P-b-1 and 3'-32P-b-2.

Preparation of 3'-32P-b-1 and 3'-32P-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 MgCl2, 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'-32P-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-32P-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-32P-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'-32P-b-1 and 3'-32P-b-2. The yields (30-50%) of 3'-32P-b-1 -2 were determined based on the specific activity of 3'-32P-labeled S1 and S2.

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

**Preparation of 3'-32P-t-1 and 3'-32P-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 MgCl₂, 1 mM DTT, pH 7.9), 30 μCi α-³²P-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'-³²P-t-1 and 3'-³²P-t-2. The yield (30-50%) of 3'-³²P-t-1 and 3'-³²P-t-2 were determined based on the specific activity of 3'-³²P-labeled S1 and S2.

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

![Scheme S4](image)

*Preparation of 5'-³²P-t-1 and 5'-³²P-t-2 (Scheme S4).* Oligonucleotide S3 (30 pmol, Figure S1) was 5'-³²P-labeled in a reaction (50 μL) containing 1 × T4 PNK buffer (70 mM Tris•HCl pH 7.6, 10 mM MgCl₂, 5 mM DTT), 50 μCi γ-³²P-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-³²P-
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'-32P-S3. Oligonucleotide 5'-32P-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'-32P-t-1 and 5'-32P-t-2.

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

**Scheme S5.** Preparation of 5'-32P-b-8 and 5'-32P-b-9.
Preparation of 5'-32P-b-8 and 5'-32P-b-9 (Scheme S5). Oligonucleotide S5 or S6 (80 pmol, Figure S2) was 5'-32P-labeled in a reaction (50 µL) containing 1 × T4 PNK buffer (70 mM Tris•HCl pH 7.6, 10 mM MgCl₂, 5 mM DTT), 6% PEG 8000 solution (w/v), 50 µCi γ-32P-ATP, and 30 U of T4 PNK at 0 °C for 2 h. Excess γ-32P-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'-32P-b-S5 or 5'-32P-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 MgCl₂, 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'-32P-b-8 or 5'-32P-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'-32P-b-8 and 3'-32P-b-9.

Preparation of 3'-32P-b-8 and 3'-32P-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 x ligase buffer (50 mM Tris•HCl pH 7.5, 10 mM MgCl₂, 10 mM DTT, 1 mM ATP). The ligated substrates were subjected to a fill-in reaction (100 µL) containing 1 x NEB 2.0 buffer (50 mM NaCl, 10 mM Tris•HCl, 10 mM MgCl₂, 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-32P-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 32P-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'-32P-b-8 or 3'-32P-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'-32P-b-8 and 3'-32P-b-9 was determined based on the specific activity of crude substrates.

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

*Preparation of 5'-32P-t-8 and 5'-32P-t-9 (Scheme S7).* S9 (80 pmol) was 5'-32P-labeled in a reaction (50 µL) containing 1 × T4 PNK buffer (70 mM Tris•HCl pH 7.6, 10 mM MgCl2, 5 mM DTT), 6% PEG 8000 solution (w/v), 50 µCi γ-32P-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-32P-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 (A260, ε = 94900 L•mol⁻¹•cm⁻¹). 5'-32P-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 x ligase buffer (50 mM Tris•HCl pH 7.5, 10 mM MgCl₂, 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 x 32 x 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'-32P-t-8 or 5'-32P-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'-32P-t-8 and 5'-32P-t-9 was determined based on the specific activity of 5'-32P-S9.

Scheme S8. Preparation of 3'-32P-t-8 and 3'-32P-t-9.

Preparation of 3'-32P-t-8 and 3'-32P-t-9 (Scheme S8). S7 (80 pmol) was 3'-32P-labeled in a reaction (50 µL) containing containing 1 x TdT buffer buffer (50 mM KOAc, 20 mM Tris•OAc, 10 mM MgCl₂, pH 7.9), 40 µCi α-32P-cordycepin triphosphate, and 30 U of terminal deoxynucleotidyl transferase at 37 °C for 2 h. Excess α-32P-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}, \varepsilon = 86300 \text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}). 3'\text{-}^{32}\text{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 \mu L) 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 x ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM MgCl_2, 10 mM DTT, 1 mM ATP). Formamide loading buffer (50 \mu L, 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 x 32 x 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'\text{-}^{32}\text{P-t-8} or 3'\text{-}^{32}\text{P-t-9}. The isolated final products were suspended in x 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'\text{-}^{32}\text{P-t-8} and 3'\text{-}^{32}\text{P-t-9} was determined based on the specific activity of 3'\text{-}^{32}\text{P-S7}.

**Scheme S9.** Preparation of 5'\text{-}^{32}\text{P-b-11} and 5'\text{-}^{32}\text{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 MgCl₂, 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 (A₂₆₀, ε =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 MgCl₂, 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 S5. ESI-MS of 14. Calc’d mass 8383.464, observed mass 4192.000 ($\varepsilon = -2$), 8383.500, 8405.300 [M + Na]$^+$, 8421.300 [M + K]$^+$, 8443.800 [M + Na + K]$^+$. 
Figure S7. Representative autoradiogram of DEPC reaction with 5'-32P-b-1-3.
Figure S8. Representative autoradiogram of DMS reaction with 5'-32P-b-1-3.
Figure S9. Representative autoradiogram of DEPC reaction with 3'-32P-b-1-3.
Figure S10. Representative autoradiogram of KMnO₄ reaction with 5'-³²P-t-1-3.
Figure S11. Representative autoradiogram of KMnO₄ reaction with 3′-³²P-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'-32P-t-1-3.
Figure S14. Representative autoradiogram of KMnO₄ reaction with 5'-³²P-b-10-12.
Figure S15. Representative autoradiogram of DEPC reaction with 5'-32P-b-10-12.
Figure S16. Representative autoradiogram of DEPC reaction with $3'$$32P$-b-10-12.
Figure S17. Representative autoradiogram of KMnO$_4$ reaction with 3'-$^32$P-b-10-12.
Figure S18. Representative autoradiogram of DEPC reaction with 5'-'32P-t-8-10.
Figure S19. Representative autoradiogram of KMnO₄ reaction with 5′-³²P-t-8-10.
Figure S20. Representative autoradiogram of KMnO₄ reaction with 3'-³²P-t-8-10.
Figure S21. Representative autoradiogram of DEPC reaction with 3'-32P-t-8-10.
Figure S22. Representative autoradiogram of OH• reaction with 3'-32P-t-8-10.

Ctrl = No hydroxyl radical treatment
Figure S23. Representative autoradiogram of OH\textsuperscript{•} reaction with 3\textsuperscript{'}-\textsuperscript{32}P-t-8-10.