Supporting Information: NMR and Kinetic Analysis of DNA Interstrand Cross-link Formation.

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Contents:

General Methods. $^{13}CH_3I$ and $^{15}NH_4Cl$ were purchased from Cambridge Isotopes. Snake venom phosphodiesterase (100 Units, lyophilized powder) was purchased from USB. Calf intestinal alkaline phosphatase was purchased from New England BioLabs. Nuclease P1 was purchased from Sigma-Aldrich. Oligonucleotides were synthesized via standard automated DNA synthesis on an Applied Biosystems model 394 instrument. For synthesis of oligonucleotides containing 5-phenylselenylmethyl-2′-deoxyuridine (**2**)[1] and 5-(2,5-dimethoxy-phenylsulfanylmethyl-2′-deoxyuridine (**17**),[2] 1 M TBHP in toluene was used as the oxidation reagent. The coupling time for modified nucleotides was 5 min. Oligonucleotides were deprotected using 1:1 methylamine (40% in water) – concentrated NH_4OH at 25 °C for 75 min or concentrated NH_4OH at 25 °C for 8 h (for oligonucleotides containing PhSeT). Oligonucleotides were purified by 20 % denaturing polyacrylamide gel electrophoresis. HPLC purification was applied for oligonucleotides containing **2**, **17** (C18-Microsorb column (4.6 mm × 25 cm) or ^{13}C-**2** (Waters Delta Pak C18 column (300 × 7.8 mm). HPLC conditions: UV detection at 260 nm; solvent A: 0.5% acetonitrile, 0.05 M TEAA; solvent B: 50% acetonitrile,

0.05 M TEAA; linear gradient 0 to 25% B over 50 min at a flow rate of 1 mL/min for **2** and **17** or 0 to 10% B over 25 min at a flow rate of 4 mL/min for ^{13}C-**2**. Oligonucleotides were hybridized with 1.0 eq. of complementary oligonucleotides in 10 mM potassium phosphate (pH 7.2) and 100 mM NaCl at 65 °C for 1 min and cooled to room temperature. All anaerobic reactions were carried out in sealed Pyrex tubes, which were degassed and sealed using freeze-pump-thaw (three cycles, 3 min each) degassing cycles.

Large scale ICL formation.

The ^{13}C-**2**-containing oligonucleotide and its complementary ^{15}N-dA-containing strand (600 nmol of each) were hybridized in a mixture (3.0 mL) of 10 mM potassium phosphate (pH 7.2) and 100 mM NaCl. The sample was redistributed to 4 × 750 μL. To each sample, more 10 mM potassium phosphate (45 μL, pH 7.2) and 100 mM NaCl (45 μL) were added, followed by H_2O (60 μL) and 20 mM $NaIO_4$ (300 μL). The samples were incubated at 37 ^{o}C for 16 h, dried, resuspended in formamide loading buffer and subjected to 20% denaturing PAGE purification. The DNA ICL was collected, and its quantity was determined using UV (785 μM in 300 μL H_2O, 235.5 nmol; 39.3% yield).

Enzymatic digestion of oligonucleotides and isolation of ^{15}N-^{13}C-labeled dA-dT dimmer (14) by HPLC.

Nuclease P1 (235 μL, 1 U/μL) was reconstituted from lyophilized solid into H_2O. Snake venom phosphodiesterase (2.94 mL, 34 U/mL) was reconstituted from lyophilized solid into 110 mM Tris (pH 8.9), 110 mM NaCl, 15 mM $MgCl_2$ and 50 % glycerol. The digestion experiment was carried out in duplicate. A solution of the above 785 μM ICL (85 μL) was diluted with 1.0 M $MgCl_2$ (140 μL), 10 × NEB buffer 3 (140 μL; 1M NaCl, 500 mM Tris-HCl, 100 mM $MgCl_2$, and 10 mM dithiothreitol; pH 7.9) and H_2O (650 μL). Nuclease P1 (14 μL, 14 U) was added,

followed by snake venom phosphodiesterase (14 μL, 0.48 U) and calf intestinal alkaline phosphatase (14 μL, 140 U). The samples were incubated at 37 ^{o}C for 20 h. The reaction mixture was diluted to 2 mL and passed through a Microcon filter (YM 3) by centrifugation at 14,000 × g. The filter was washed with water (2 × 100 μL). The combined filtrate was purified by HPLC with UV detection at 260 nm. HPLC conditions: Waters Delta Pak C18 column (300 × 7.8 mm); solution A: water; solution B: acetonitrile; increase of B from 2% to 20% in 25 min at a flow rate of 4 mL/min. The retention time of the product is 19.8 min. The peaks of dA-dT dimmer (**14**) were collected and lyophilized to give 27 nmol of the ^{15}N-^{13}C-**14**.

Photoreaction for NMR study.

Photoreaction of the duplex (**8**) was carried out in a Shigemi NMR tube in a Rayonet photoreactor fitted with 16 lamps having a maximum output at 350 nm. The duplex (1 mM) was in a mixture (400 μL) of 10 mM potassium phosphate (pH 7.2), 100 mM NaCl and 10% D_2O. The irradiation time was 90 min. The sample was analyzed directly by NMR.

NMR Study-Interstrand Cross-Link formation by $NaIO_4$ treatment.

The reaction was carried out in a Shigemi NMR tube. Duplex **8** (1 mM) was in a mixture (400 μL) of 10 mM potassium phosphate (pH 7.2), 100 mM NaCl and 10% D_2O. $NaIO_4$ (2 mg, 0.009 mmol, 22.5 mM) was added to the solution. After 15 min, the sample was analyzed directly by NMR.

All heteronuclear, two-dimensional NMR experiments were carried out on a Bruker, Avance II spectrometer operating at a ^{1}H frequency of 600 MHz (14.1 T) and equipped with a TCI, triple resonance (^{1}H, ^{13}C, ^{15}N), z-gradient cryoprobe. Flip-back watergated HSQC pulse sequences were used for the ^{1}H-^{15}N spectra shown in Figures 1 - 3 and Supporting Information Figure 1. INEPT delays corresponding to $1/(4\times J_{NH})$ periods/^{15}N carrier positions/solvent/pH

were 12 ms/220 ppm/D_2O/7.0 for the spectra in Figures 1, 2, and Supporting Information Figure 1, and 2.5 ms/80 ppm/H_2O/5.0 for the spectrum in Figure 3. All data were processed with nmrPipe/nmrDraw software.

Determination of k_{ISC}.

Samples of the duplexes containing **2** or **17** (5 nM) were prepared in 10 mM potassium phosphate (pH 7.2) and 100 mM NaCl in the presence of varying concentrations of BME or GSH (0 to 5 mM). Samples were degassed and sealed. Photoreactions were carried out for 90 min, at which time they were precipitated from 0.3 M NaOAc (pH 5.3) and calf thymus DNA (0.1 mM bp). Pellets were dried, resuspended in formamide loading buffer, and subjected to 20% denaturing PAGE analysis. The ratio of single stranded product (ssDNA) to ICL was calculated and plotted versus BME or GSH concentration.

NaN_3 trapping kinetics.

Kinetic studies were carried out in 1 cm pathlength quartz cells. A cocktail of **11** was prepared in a cuvette by mixing 1.9 mM **11** (5.8 μL), 100 mM KH_2PO_4 (50 μL, pH 7.2), 1 M NaCl (25 μL) and water (162.5 μL). A solution of 20 mM $NaIO_4$ (6.2 μL) was added to the cocktail. After 15 min, proper concentration of NaN_3 stock (0.5 μL) was added to adjust the final concentration of NaN_3 to 1, 2, 3, and 4 mM, respectively. UV absorbance of the sample was recorded at 260 nm every 8 min over a period of 720 min. Rate constants were calculated by using the computer program Origin to fit the absorbance with respect to time, using first-order exponential growth fitting. Experiments were carried out in triplicate.

Monomeric dA-dT coupling product (13) formation by oxidation of ^{13}C-2 (11).

A solution of **11** (5 mM) in MeOH (2 mL) and **12** (10 mM) in MeOH (1 mL) was concentrated and redissolved in a mixture (500 μL) of 10 mM potassium phosphate (pH 7.2),

100 mM NaCl and 10% D_2O. The sample was treated with $NaIO_4$ (10.7 mg, 0.05 mmol). After standing at 25 °C for 5 h, it was analyzed directly by NMR.

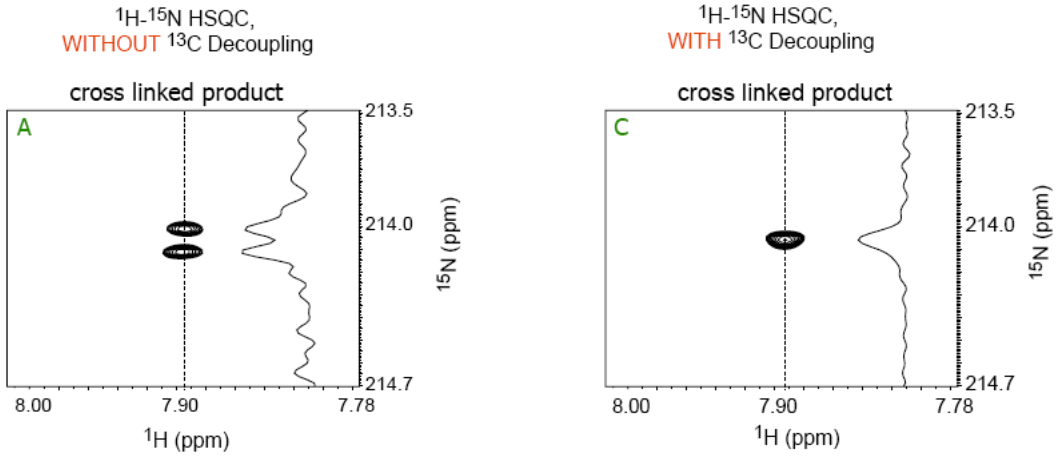
Rearrangement of the dA-dT coupling product (13) to 14 by piperidine treatment.

The above sample was evaporated to dryness and incubated with 1 M piperidine (1 mL) at 90 °C for 20 min, dried under vacuum, resuspended in H_2O, and dried again. It was dissolved in H_2O and purified by HPLC as described above to give **14** (1.1 μmol).

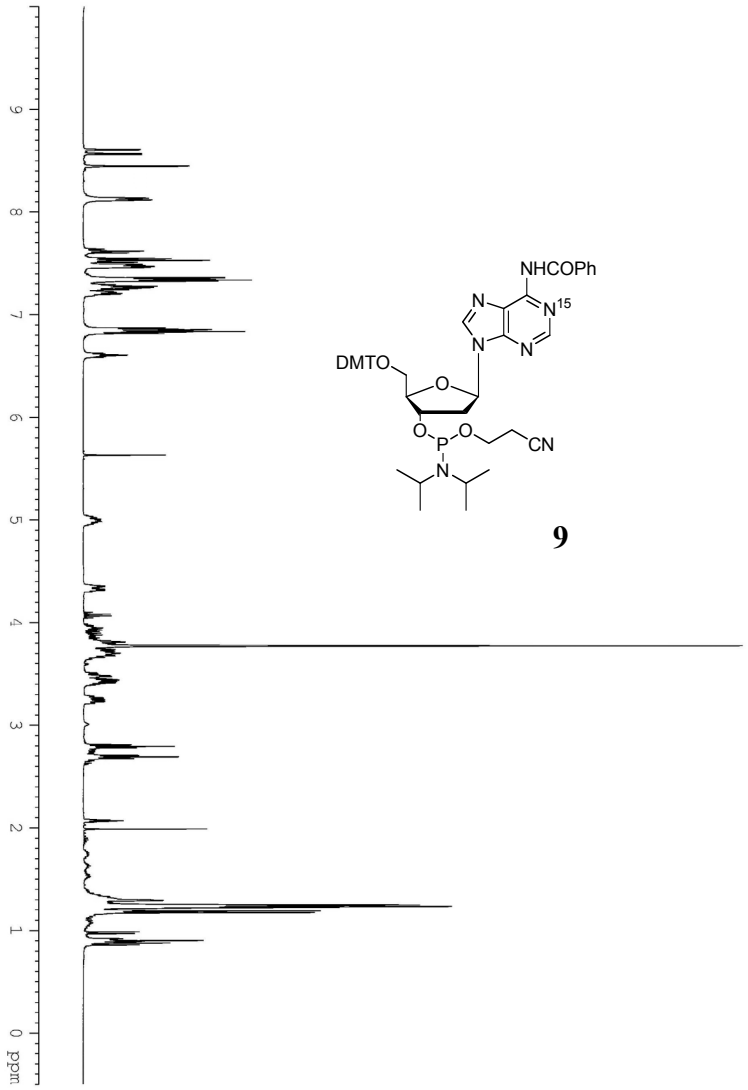
References:

1. Hong, I.; Greenberg, M. M. *Org. Lett.* **2004**, *6*, 5011-5013.

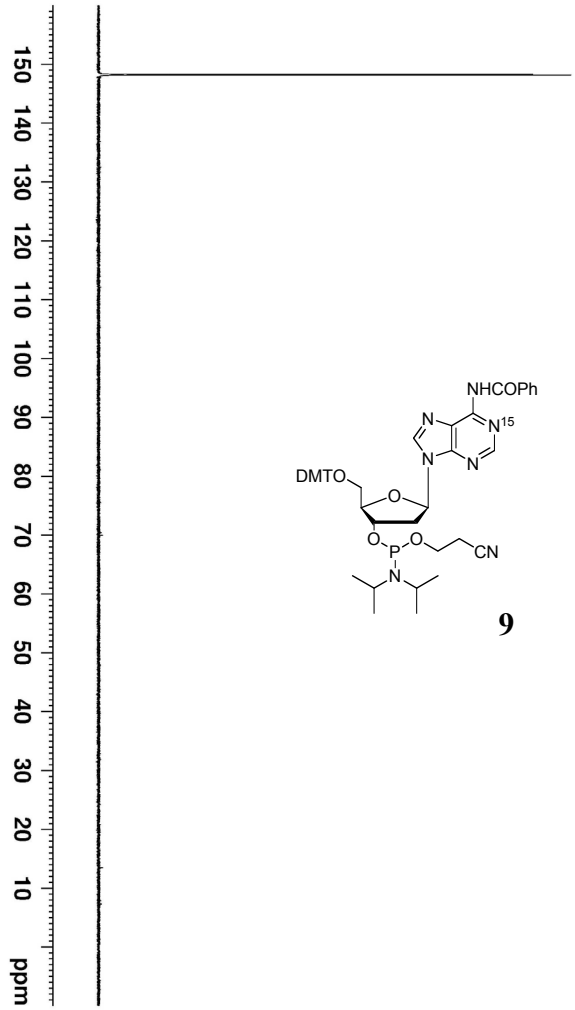
2. Hong, I. Ding, H. Greenberg, M. M. *J. Am. Chem. Soc.* **2006**, *128*, 485-491.



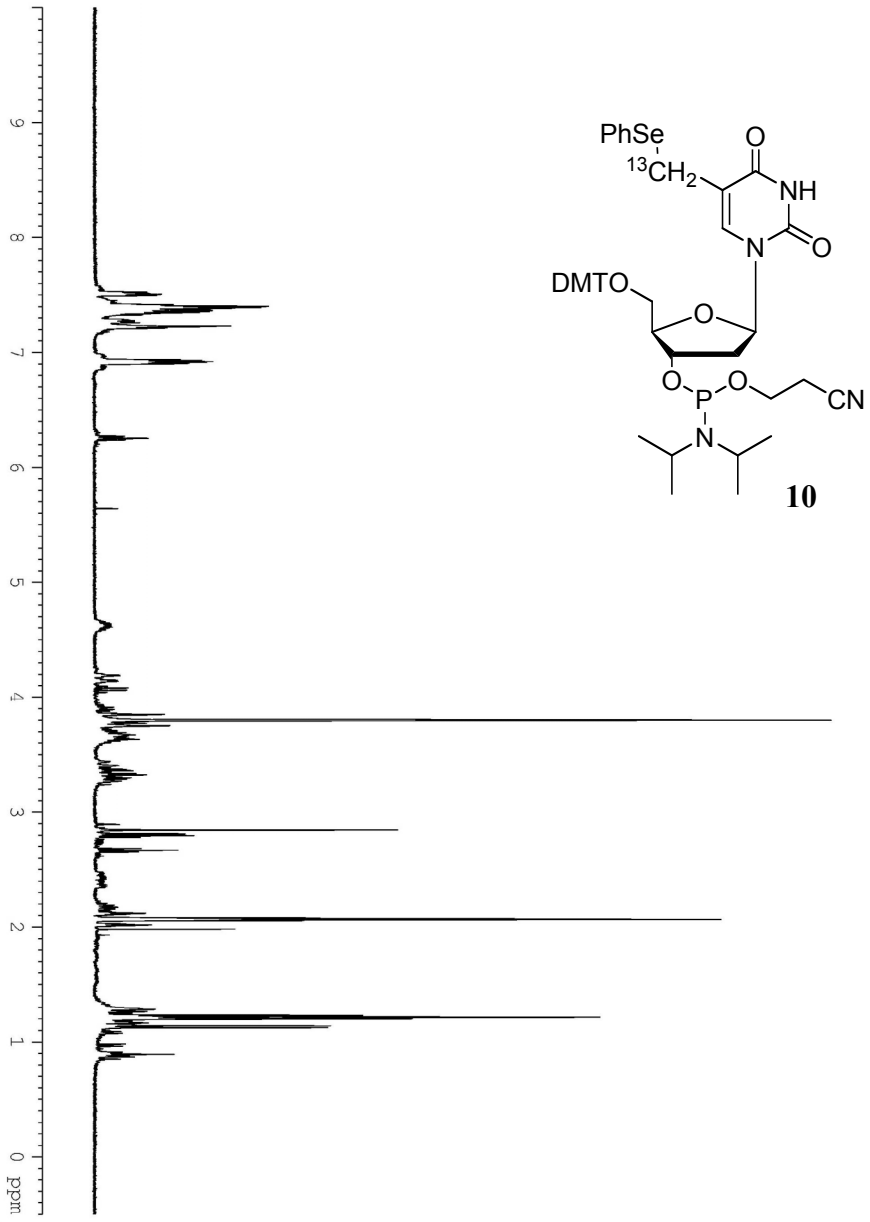
Supporting Information Figure 1. NMR analysis following photolysis (350 nm) of **8**. (A, C): 2D H2, N1 HSQC of the crude reaction mixture acquired in high resolution (0.47 Hz/pt) in the ^{15}N dimension, in the presence (left) and absence (right) of ^{13}C-decoupling in the ^{15}N dimension. Peaks belonging to unreacted starting material are not shown. The contour thresholds are not identical in the two figures.



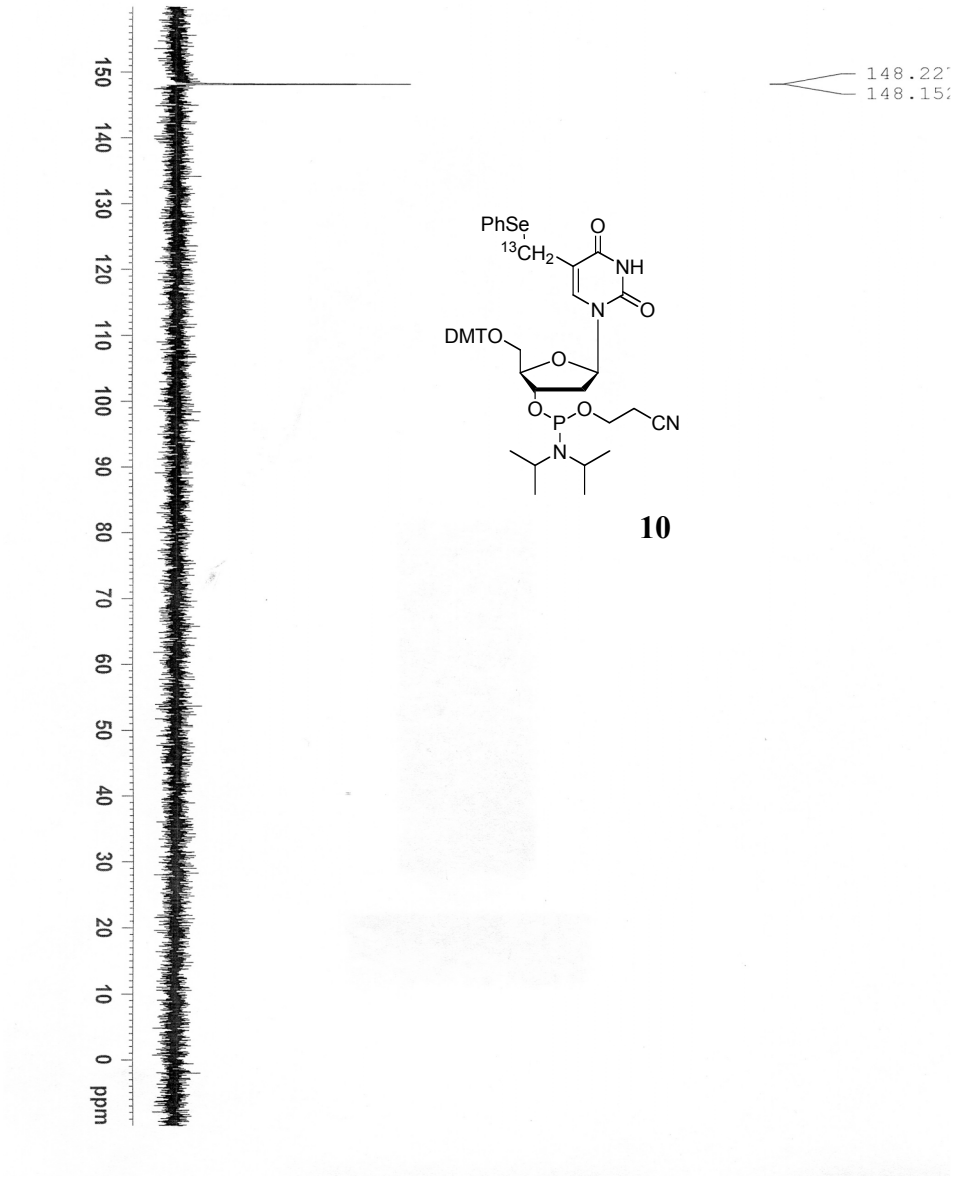
Supporting Information Figure 2. ^{1}H NMR spectrum of **9**.



Supporting Information Figure 3. ^{31}P NMR spectrum of **9**.



Supporting Information Figure 4. ^{1}H NMR spectrum of **10**.



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Supporting Information Figure 5. ^{31}P NMR spectrum of **10**.

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