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Supporting Information

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Supporting Information

Diazirine–Based DNA Photocross–Linking Probes for Studying Protein–DNA Interactions

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General: All reagents were obtained from commercial sources and used as received unless otherwise noted. Inert N_2 atmosphere was employed for all reactions and all solvents were distilled unless otherwise noted. Forced-flow chromatography on EM Science Geduran silica gel 60 (35-75 μm) was used to purify products. Thin layer chromatography was performed on EM Science silica gel 60 F254 plates (250 μm). Developed chromatogram was visualized by Ultra Violet lamp and/or by staining with aqueous potassium permanganate/K_2CO_3 solutions. Nuclear magnetic resonance (NMR) spectra for ^1H were acquired with $CDCl_3$ on Bruker DRX-500/400 operating at 500/400 MHz; TMS was used as internal standard for calibration purpose. Data for ^1H NMR are recorded as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet), integration, coupling constant (Hz). High-Resolution Mass was performed by Mass Spectrometry Facility at University of Norte Dame.

Table S1. Melting Temperatures (T_m) of Normal and Modified Oligonucleotides.

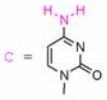
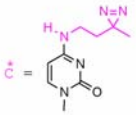
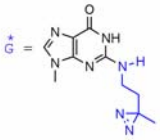
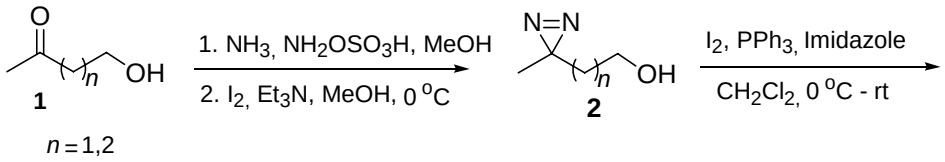
Oligonucleotide		Melting Temperature (^oC)
5'-ATCAAAGCGCAAGAC-3' 3'-TAGTTTCGCGTTCTG-5'	C = 	60.6
dsDNA-2 5'-ATCAAAG*CGCAAGAC-3' 3'-TAGTTTCGCGTTCTG-5'	*C = 	55.0
dsDNA-7 5'-ATCAAAGCGCAAGAC-3' 3'-TAGTTTCG*CGTTCTG-5'	*G = 	61.2

Table S2. Summary of the Probes that Most Efficiently Photocross–Linked with EcoDam.

Oligonucleotide	Modification	Better Carbon-length
dsDNA-2 5'-ATCAAAG*CGCAAGAC-3' 3'-TAGTTTCGCGTTCTG-5'	*C	2
dsDNA-3 5'-ATCAAAG*CGCAAGAC-3' 3'-TAGTTTCACGTTCTG-5'	*C	3
dsDNA-10 5'-ATCAAAG GCAAGAC-3' 3'-TAGTTTCG*CGTTCTG-5'	*G	3
dsDNA-11 5'-ATCAAA CGCAAGAC-3' 3'-TAGTTTCG*CGTTCTG-5'	*G	3

Experimental Procedure to Synthesize Diazirine Amines:



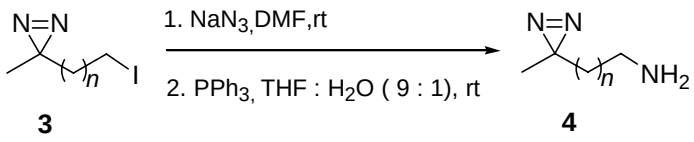


Figure S1. The synthetic route for making diazirine amines.

Two-carbon and three-carbon diazirine amine were made using similar procedure

2-(3-Methyl-3H-diazirin-3-yl)-ethanol (2, n=1)

4-hydroxy-2-butanone (45 g, 0.51 mol) was added to 200 mL of NH_3 and stirred for 3 h at -78 °C. Hydroxylamine O-Sulfonic acid (63.5 g, 0.56 mol) was dissolved in methanol and poured into the reaction mixture. After overnight stirring, the white precipitate was filtered and methanol was added to ice cold reaction mixture followed by triethylamine. Iodine was slowly added until the color of iodine persists. After 2 h methanol was evaporated and the reaction mixture was extracted with ether and dried over $MgSO_4$. Vacuum distillation was done to get the final pale yellow oil **2** (15 g, 30% overall yield).

3-(2-Iodo-ethyl)-3-methyl-3H-diazirine (3, n=1)

One equivalent of triphenylphosphine was dissolved in 50 mL of ice cold dichloromethane. After dissolving triphenylphosphine, iodine was added followed by imidazole. Once the color of iodine vanished compound **2** (6.06 g, 0.06 mol) was added and stirred overnight. The product was extracted with ether, washed with water and dried over $MgSO_4$. After solvent evaporation, the product was purified by column chromatography on silica gel (pure hexane) to give compound **3** (4.05 g, 33%).

2-(3-Methyl-3H-diazirin-3-yl)-ethylamine (4, n=1)

One equivalent of NaN_3 was added to **3** (4.05 g, 0.02 mol) and the reaction mixture was stirred at room temperature overnight. The product mixture was extracted with ether and after ether evaporation tetrahydrofuran:water (9:1) was added followed by triphenylphosphine. After 2 h 1N HCl was added to the product mixture and extracted with ether to remove the byproducts. Subsequently 1N NaOH was added to the mixture and after ether extraction yielded the final product **4.** (1.6 g, 80% crude). ^{1}H-NMR ($CDCl_3$) ∗ 2.57 (t, 2H, *J* = 7.0 Hz), 1.54 (t, 2H, *J* = 7.0 Hz), 1.04 (s, 3H).

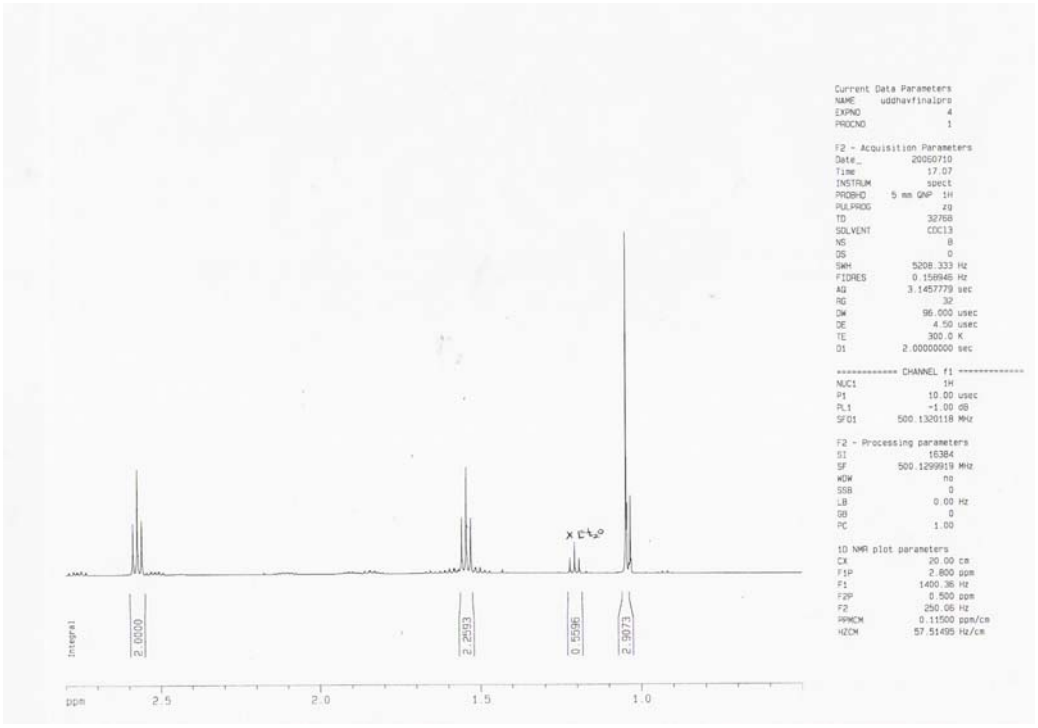


Figure S2. ^{1}H-NMR of 2-(3-Methyl-3H-diazirin-3-yl)-ethylamine (**4, n=1**).

3-(3-Methyl-3H-diazirin-3-yl)-propylamine (4, n=2) was made with 3-acetyl-1-propanol as a starting material following the same procedure as making **(4, n=1)**.

^{1}H-NMR ($CDCl_3$) ∗ 2.66 (t, 2H, *J* = 6.9 Hz), 1.38 (m, 2H), 1.32 (m, 2H), 1.01 (s, 3H); FABMS *m/z* $[MH]^+$; calculated for $C_5H_{11}N_3$ 114.1031 found 114.1021.

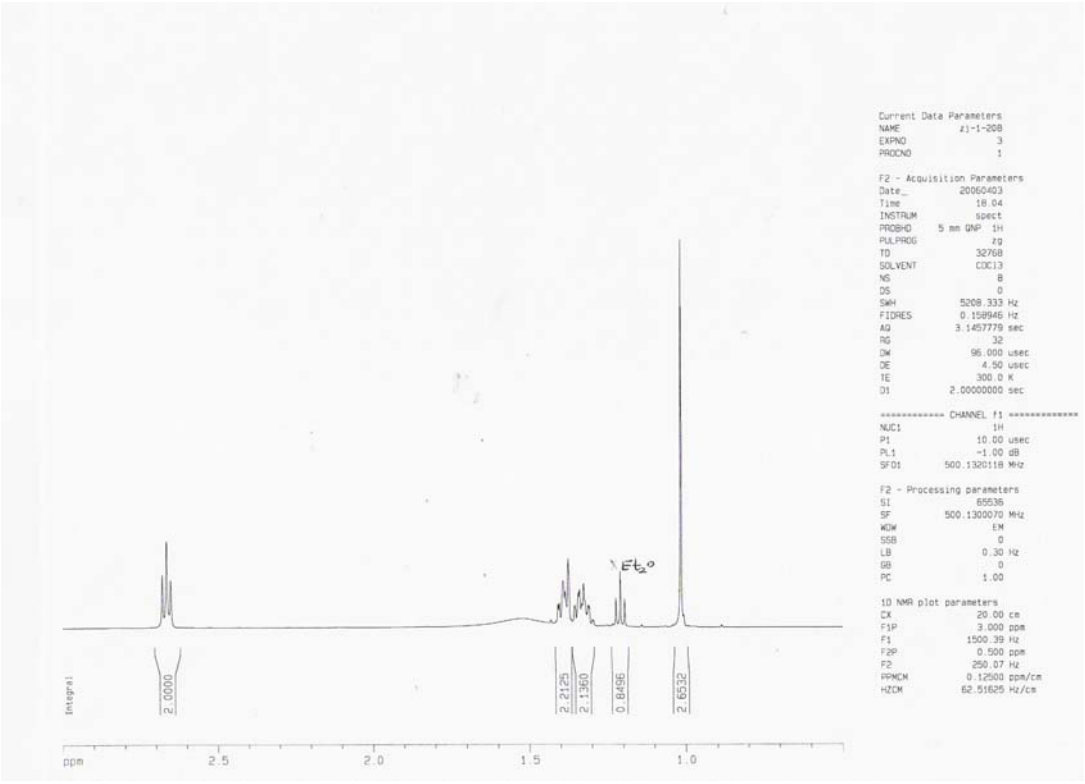


Figure S3. ^{1}H-NMR of 3-(3-Methyl-3H-diazirin-3-yl)-propylamine (**4, n=2**).

Expression and Purification of Human AGT. Human AGT was purified as described.[2d]

Expression and Purification of DNA Adenine Methyltransferase. *E. coli* cells containing a construct of DNA adenine methyltransferase was grown aerobically with chloramphenicol (30 μg/mL) at 37 °C until the OD_{600} reached 0.7. IPTG (1 mM) was added and the cells were grown at 30 °C for 4 h. The cells were harvested by centrifugation and stored at -80 °C. All subsequent steps were performed at 4 °C. The cell pellet was resuspended in 20 mL of lysis buffer (10 mM Tris-HCl [pH 7.4], 300 mM NaCl, 5% glycerol, 2 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol), disintegrated by sonication, and centrifuged at 12,000 rpm for 20 min. The supernatant was loaded onto HisTrap FF (GE Healthcare) that had been equilibrated with buffer A (10 mM Tris-HCl [pH 7.34], 150 mM NaCl, 10 mM 2-mercaptoethanol, 5% glycerol), and was washed with 20% buffer B (10 mM Tris-HCl [pH 7.34], 150 mM NaCl, 400 mM imidazole, 10 mM 2-mercaptoethanol, 5% glycerol) and finally eluted with a linear

gradient of imidazole (0-400 mM). Fractions containing the protein were concentrated by ultrafiltration (Centricon YM10 membrane; Amicon, Millipore Corporation, Bedford, MA) and purified further with a Mono-S cation exchange column (Amersham Biosciences) using a linear gradient of NaCl (0-1.0 M).

Oligonucleotide Synthesis. Oligodeoxynucleotides were synthesized on an Expedite Nucleic Acid Synthesizer from PE biosystems. Major groove modifications were made by incorporating O^4-triazolyl-dU-CE phosphoramidite (Glen Research) at the modified positions during solid-phase synthesis. For minor groove modification 2F-dI-CE phosphoramidite (Glen Research) was used. The diazirine amine tether was added via a postsynthetic modification/deprotection method as described.[6] For oligos containing 2-F-dI modification, after deprotection with diazirine amine, 50 μL of DBU (1,8-diazabicyclo [5.4.0] undec-7-ene) and 300 μL formamide (heated to 55 °C) was used to remove the O6-protecting group. All oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis. Concentrations of oligonucleotides were estimated by UV absorption at 260 nm.

Cross–linking Reactions and Analysis. For all cross–linking reactions, 10 mM Tris-HCl [pH 7.4] and 100 mM NaCl was used as the buffer. Typically, the protein (0.3 nmol) and modified DNA oligos (0.9 nmol) were incubated at 4 °C for 16 h. After incubation the samples were irradiated with mercury vapor lamp (Ace Glass Incorporated, Vineland, New Jersey, 450 W power supply; lights below 300 nm were cut off with a filter) for 10 min. Coomassie Blue stained SDS-PAGE was used to analyze the cross–linked product.

Melting Temperature (T_m) Measurement.[10] Melting temperatures of normal and modified oligonucleotides were measured by using MicroCal VP Differential Scanning Calorimeter (DSC) (Northampton, MA) with 5 μM concentration of DNA in 10 mM Tris-HCl [pH 7.4] and 100 mM NaCl buffer. Samples were scanned over the temperature range of 20-75 °C at a rate of 90 K/h. The DSC curves were adjusted from the reference buffer and T_m values were determined directly from the curves.

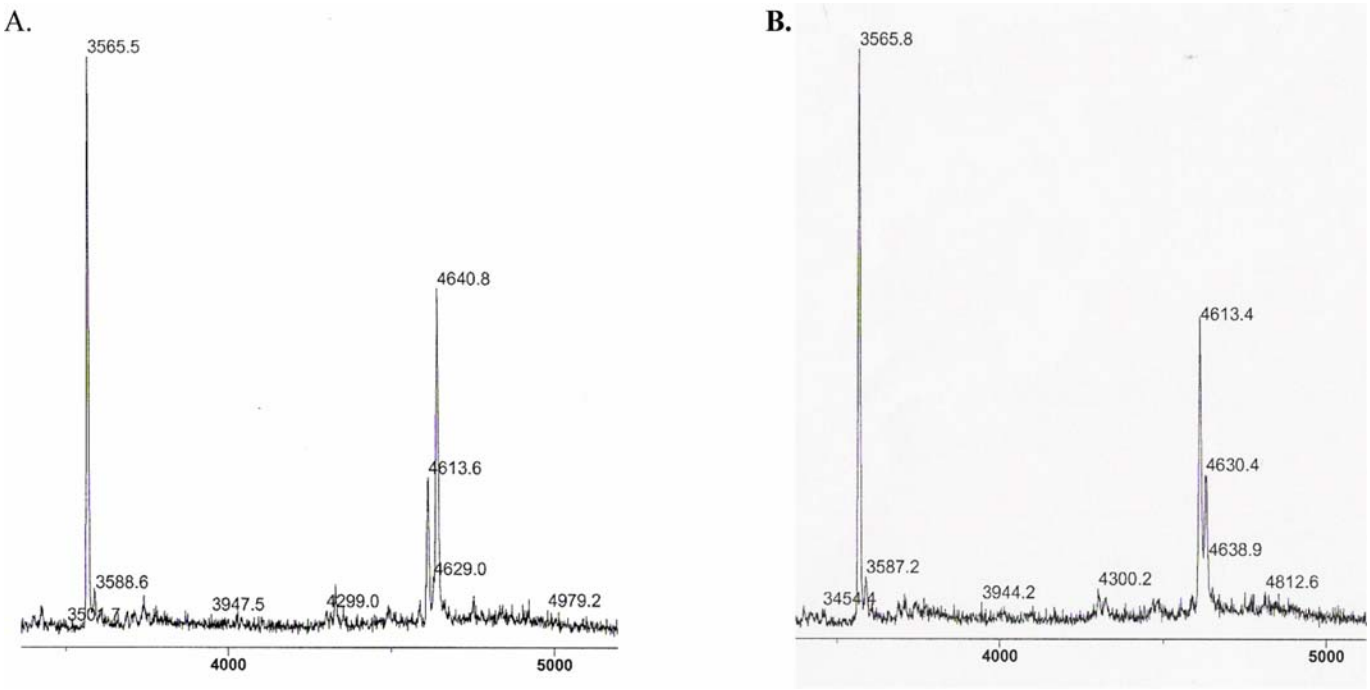


Figure S4. MALDI-TOF MS spectra of a diazirine–containing oligonucleotide before (Figure S4A) and after UV irradiation (Figure S4B). The ssDNA-1 probe containing a diazirine was UV irradiated and the mass difference (*m/z*) (4640.8 - 4613.4) of the oligonucleotide before and after irradiation is ~28, which is consistent with the loss of a dinitrogen and insertion of carbene into ∃ or (C–H bond to yield an olefin or cyclopropane as described previously for aliphatic diazirines.[I] The mass difference (*m/z*) (4640.8 - 4630.4) of ~10 is the insertion of water molecule into the carbene. The peak at mass (*m/z*) 4613.6 in Figure S4A is the release of dinitrogen when MALDI is taken. The peak at mass (*m/z*) 3565.5 is the mass of an oligonucleotide used as an internal standard.

[I]. Kirmse, W. Carbene Chemistry, 2nd ed.; Academic Press: New York, 1971.