Electronic Supplementary Information

## **Templated Chemistry for Monitoring Damage and Repair Directly in Duplex DNA**

Seoung Ho Lee, Shenliang Wang, Eric T. Kool\*

Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

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**Scheme S1.** Synthesis of (A) imidazophenanthrene nucleobase analogue (4) and (B) imidazophenanthrene-appended nucleoside phosphoramidite derivative (8); (i) formaldehyde, NH<sub>4</sub>OAc, AcOH, 100 °C, 12 h. (85%); (ii) 4, NaH, CH<sub>3</sub>CN, r.t., 12 h (40%); (iii) NaOCH<sub>3</sub>, CH<sub>3</sub>OH, r.t., 4 h. (87%); (iv) DMTCl, DIPEA, pyridine, r.t., 4 h. (68%); (v) 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, DCM, r.t., 3 h. (98% crude yield).



Scheme S2. Synthesis of  $\beta$ -pyrene deoxyriboside phosphamidite derivative (13); (i) 1-Bromopyrene, *N*-methyldicyclohexylamine, (*t*-Bu<sub>3</sub>P)<sub>2</sub>Pd(0), dioxane, 90 °C, 16 h.; (ii) TBAF, THF, 0 °C, 2 h.; (iii) NaBH(OAc)<sub>3</sub>, THF/CH<sub>3</sub>CN (2/3, *v/v*), -10 °C (48% over 3 steps); (iv) DMTCl, DIPEA, pyridine, r.t., 4 h. (86%); (v) 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, DCM, r.t., 3 h.





**Figure S1.** The sequences of (A) TPP probe, (B) Q-STAR probes, and (C) DNA templates; (D) Scheme showing templated Staudinger reduction, resulting in release of quencher group.



Figure S2. ROESY spectrum of 6, confirming desired isomer of glycosidic attachment and beta anomeric geometry.







**Figure S4.** UV-vis absorption spectra of Q-STAR probes (I (blue trace) and Y (red)), showing presence of dabsyl and fluorescein chromophores as well as modified nucleobases.



**Figure S5.** HPLC traces of purified Q-STAR probes containing I and Y. Absorbance was monitored at 260 nm. Probes elute at 30-32 min.

Probes	Calcd.	Found	Target DNAs	Calcd.	Found
Q-STAR (I)	5505.06	5506.96	Undamaged	18467.86	18436.09
Q-STAR (Y)	5489.05	5489.18	THF	18334.75	18274.31
TPP	4608.07	4607.96	Abasic	18350.75	18321.74
			UG mismatched	18469.84	18419.53

 Table S1. MALDI-TOF MS data of Q-STAR probes and target DNAs.



**Fig. S6.** Thermal denaturation experiments showing evidence of selective recognition of abasic sites by triplex-forming QSTAR probes containing modified bases I and Y. Shown are thermal melting profiles (plotted as hyperchromicity) for probes containing I (A) and Y (B) with abasic duplexes and with non-damaged control duplex. Conditions: 2.0  $\mu$ M  $\psi$ C Q-STAR probe, 2.0  $\mu$ M DNA template, 70 mM trisacetate buffer (pH 7.0) containing 10 mM MgCl<sub>2</sub>, 25 °C. Absorbance changes were monitored at 260 nm; temperature was changed at 1.0 °C / min.



**Fig. S7.** Thermal denaturation control experiments. (A) Target duplexes alone; (B) TPP probe alone with target duplexes, showing added transitions at ~25-40 °C. Conditions: 2.0  $\mu$ M  $\psi$ C Q-STAR probe, 2.0  $\mu$ M DNA template, 70 mM tris-acetate buffer (pH 7.0) containing 10 mM MgCl<sub>2</sub>, 25 °C. Absorbance changes were monitored at 260 nm; temperature was changed at 1.0 °C / min.



**Fig. S8.** Extended timecourse (12 h reaction) showing detection of THF abasic site by fluorogenic chemistry. Data are for probe containing Y with THF target DNA. Conditions: 200 nM Q-STAR Y probe, 200 nM DNA template, 600 nM TPP probe, 70 mM tris-acetate buffer (pH 7.0) containing 10 mM MgCl<sub>2</sub>, 25 °C;  $\lambda_{ex}$ =494 nm,  $\lambda_{em}$ =521 nm.



**Fig. S9.** Turnover experiment, tested by detection of an abasic site by fluorogenic chemistry at two different template concentrations. Shown are timecourses (24 h) for reaction with Q-STAR probe Y with THF DNA (80 and 400 nM). 400 nM is one equivalent of probe per target; 80 nm is five equivalents probe per target. Conditions: 400 nM  $\psi$ C Q-STAR probe, 800 nM  $\psi$ C TPP probe, 70 mM tris-acetate buffer (pH 7.0) containing 10 mM MgCl<sub>2</sub>, 25 °C;  $\lambda_{ex}$ =494 nm,  $\lambda_{em}$ =521 nm.



**Fig. S10.** Detection of base excision repair by fluorogenic templated chemistry. Target DNA sequence containing U-G mismatch is shown at top. The U-G target was incubated with uracil DNA glycosylase (UDG) (5 units) at 37 °C for 30 min. Then Q-STAR and TPP probes were added and fluorescence changes monitored over 3.5 h. Conditions: 200 nM DNA template, 200 nM Q-STAR probe, 600 nM  $\psi$ C TPP probe, 70 mM tris-acetate buffer (pH 7.0) containing 10 mM MgCl<sub>2</sub>, 25 °C;  $\lambda_{ex}$ =494 nm,  $\lambda_{em}$ =521 nm. (A) Results with I-containing probe at left; (B) Y-containing probe at right. Controls show results with no UDG enzyme (black traces) and with undamaged DNA containing an intact A-T pair (blue traces).

## Experimental

Compounds **3**, **5**, and **9** were prepared as described in the literature, respectively.<sup>1-3</sup>

1*H*-phenanthro[1,2-*d*]imidazole (4): A mixture of phenanthren-1,2-dione (3) (0.3 g, 1.4 mmol), formaldehyde (0.23 mL, 37 wt%), glacial acetic acid (5.6 mL), and ammonium acetate (2.3 g, 30.0 mmol) was stirred at 100 °C for 12 h. The reaction mixture was cooled to room temperature. It was then diluted with water (600 mL) and neutralized with concentrated aqueous ammonia (20 ~ 30% wt) to pH 7.0. A light cream precipitate was filtered and washed with water, acetone, dichloromethane, and ethyl ether. Yield: 85%; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>,  $\delta_{ppm}$ ): 8.84 (d, 1H), 8.65 (d, 1H), 8.48 (br, 1H), 8.40 (s, 1H), 8.00 (m, 2H), 7.90 (d, 1H), 7.70 (t, 1H), 7.60 (t, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>,  $\delta_{ppm}$ ): 141.0, 130.8, 130.5, 128.8, 127.0, 125.5, 123.0, 120.8; HR-MS (*m/z*): M+H<sup>+</sup> calcd for C<sub>15</sub>H<sub>11</sub>N<sub>2</sub>, 219.0917; found, 219.0914.

**Compound 6**: To a solution of **4** (0.20 g, 0.92 mmol) and **5** (0.32 g, 0.82 mmol) in CH<sub>3</sub>CN (5.0 mL), NaH (0.066 g, 2.76 mmol) was added at 0 °C. The reaction mixture was then stirred at r.t. for 12 h. The reaction was quenched with a few drops of MeOH, and then the solvent was removed. The mixture was extracted with DCM (20 mL), washed with water (20 mL × 3), and the organic layer was separated. It was dried over anhydrous magnesium sulfate, and then the solvent was removed in *vacuo*. The residue was purified by column chromatography on a silica gel. Yield: 40%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>,  $\delta_{ppm}$ ): 8.62 (d, 2H), 8.41(d, 1H), 8.22 (s, 1H), 8.01 (d, 2H), 7.95 (m, 2H), 7.91 (d, 2H), 7.81 (d, 1H), 7.66 (t, 1H), 7.59 (t, 1H), 7.32 (d, 2H), 7.22 (d, 2H), 6.52 (m, 1H), 5.80 (m, 1H), 4.80-4.66 (m, 3H), 3.05 (m, 1H), 2.81 (m, 1H), 2.42 (s, 3H), 2.38 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>,  $\delta_{ppm}$ ): 166.3, 166.2, 145.0, 144.6, 142.0, 139.0, 134.0, 132.0, 130.2, 130.1, 130.0, 129.8, 129.2, 128.0, 127.0, 126.8, 126.4, 126.0, 125.5, 123.0, 122.2,

119.0, 110.6, 86.0, 83.0, 75.0, 64.2, 38.3, 30.0, 22.0; HR-MS (*m/z*): M+H<sup>+</sup> calcd for C<sub>36</sub>H<sub>31</sub>O<sub>5</sub>N<sub>2</sub>, 571.2227; found, 571.2220.

**Compound 1**: To a solution of **6** (0.10 g, 0.18 mmol) in MeOH (5.0 mL), NaOCH<sub>3</sub> (1 mL, 0.5 M) was added. The reaction mixture was stirred at r.t. for 4 h, and then the solvent was removed. The mixutre was extracted with DCM (20 mL), washed with water (20 mL × 3), and the organic layer was separated. It was dried over anhydrous magnesium sulfate, and then the solvent was removed in *vacuo*. The residue was purified by column chromatography on a silica gel. Yield: 87%; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD,  $\delta_{ppm}$ ): 8.76 (d, 1H), 8.70 (d, 1H), 8.63 (s, 1H), 8.51 (d, 1H), 7.96 (m, 3H), 8.63 (s, 1H), 7.68 (t, 1H), 7.58 (t, 1H), 6.55 (t, 1H), 4.60 (m, 1H), 4.08 (m, 1H), 3.80 (m, 2H), 2.75 (m, 1H), 2.55 (m, 1H); <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>,  $\delta_{ppm}$ ): 141.8, 141.5, 131.8, 131.7, 131.0, 129.0, 128.0, 127.8, 127.6, 126.6, 126.5, 126.0, 125.9, 125.0, 123.6, 121.3, 119.0, 112.4, 92.0, 88.5, 85.5, 71.2, 68.0, 66.0, 64.0, 62.0, 46.0, 37.2, 34.0, 29.5, 23.2, 15.5, 9.0; HR-MS (*m/z*): M+H<sup>+</sup> calcd for C<sub>20</sub>H<sub>19</sub>O<sub>3</sub>N<sub>2</sub>, 335.1390; found, 335.1387.

**Compound 7**: To a solution of **1** (0.03 g, 0.09 mmol) in pyridine (5.0 mL), DMTCl (0.27 mmol) and DIPEA (0.27 mmol) were added. The reaction mixture was stirred at r.t. for 4 h, and then the solvent was removed. The residue was purified by column chromatography on a silica gel. Yield: 68%; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD,  $\delta_{ppm}$ ): 9.12 (d, 1H), 8.94 (d, 1H), 8.36 (d, 1H), 8.22 (m, 2H), 8.12 (d, 1H), 7.84 (t, 1H), 7.78 (t, 1H), 7.76 (m, 1H), 7.64 (m, 1H), 7.42 (m, 3H), 7.30 (m, 5H), 7.21 (m, 1H), 6.86 (m, 4H), 6.79 (t, 1H), 4.68 (m, 1H), 4.25 (m, 1H), 3.90 (m, 3H), 3.80 (s, 6H), 3.20 (m, 1H), 2.85 (m, 1H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD,  $\delta_{ppm}$ ): 158.0, 145.5, 136.0, 132.0, 130.0, 129.0, 127.2, 126.0, 123.4, 122.2, 118.5, 112.8, 111.9, 89.0, 88.0, 70.0, 68.0, 61.0, 54.8,

46.2, 41.0, 39.0, 34.3, 30.5, 29.5, 24.0, 23.5, 14.0, 10.0, 9.0; HR-MS (m/z): M+H<sup>+</sup> calcd for C<sub>41</sub>H<sub>37</sub>O<sub>5</sub>N<sub>2</sub>, 637.2702; found, 637.2694.

**Compound 8**: To a solution of **1** (0.03 g, 0.05 mmol) in DCM (5.0 mL), 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.27 mmol) and DIPEA (0.27 mmol) were added. The reaction mixture was stirred at 0 °C for 3 h, and then the solvent was removed. The product was used in the DNA synthesis without further purification. Crude yield: 98%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>,  $\delta_{ppm}$ ): 8.60 (m, 2H), 8.35 (m, 1H), 8.15 (s, 1H), 7.90 (m, 3H), 7.62 (m, 1H), 7.55, (m, 3H), 7.40 (m, 2H), 7.25 (m, 8H), 6.72 (m, 4H), 6.40 (m, 1H), 4.30, (m, 2H), 4.15 (m, 2H), 4.00 (m, 2H), 3.88 (m, 2H), 3.68 (s, 6H), 3.48 (m, 2H), 2.64 (m, 2H), 1.20 (m, 18H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>,  $\delta_{ppm}$ ): 159.5, 144.2, 141.0, 140.0, 135.5, 132.0, 131.0, 130.5, 129.0, 128.0, 127.0, 126.2, 125.5, 118.0, 117.5, 117.2, 113.5, 113.2, 112.0, 86.0, 64.0, 58.0, 55.5, 46.5, 46.0, 44.0, 30.0, 24.0, 23.0, 22.5, 20.0; <sup>31</sup>P NMR (160 MHz, CDCl<sub>3</sub>,  $\delta_{ppm}$ ): 150.0, 16.0; HR-MS (*m*/*z*): M+H<sup>+</sup> calcd for C<sub>50</sub>H<sub>54</sub>O<sub>7</sub>N<sub>4</sub>P, 853.3725; found, 853.3717.

**Compound 12**: 1-Bromopyrene (1.59 g, 5.6 mmol), 3'-O-(tert-butyldiphenylsilyl)-1,2-dehydro-2-deoxy-*d*-ribofuranose (**9**) (2 g, 5.6 mmol), *N*-methyldicyclohexylamine (1.8 mL, 8.5 mmol) and *bis(tri-tert*-butylphosphine)palladium(0) (0.29 g, 0.56 mmol) were charged into a flame dried round-bottom flask equipped with a septum. Anhydrous dioxane (30 mL), previously bubbled with argon for 1 h, were added via syringe. The mixture was bubbled with argon for 1 h and heated at 90 °C for 16 h. After cooling, the mixture was concentrated to dryness and then partitioned between water and ethyl acetate. The organic layer was evaporated to dryness and then dissolved in THF (30 mL). After cooling to 0 °C, acetic acid (0.5 mL) and tetrabutylammonium fluoride (1M, 7.5 mL) were sequentially added. The reaction mixture was stirred at 0 °C for 2 h. After concentration under vacuum, the residue was partitioned between water and ethyl acetate. The organic layer was evaporated to dryness and then dissolved in THF (20 mL) and acetonitrile (30 mL). After cooling to -10 °C, acetic acid (1 mL) and sodium triacetoxyborohydride (1.60 g, 7.5 mmol) were added. The reaction mixture was stirred at -10°C for 4 h. Ammonium hydroxide (10 mL) was then added dropwise to quench the reaction. The mixture was concentrated and then partitioned between water and ethyl acetate. After drying with sodium sulfate, the organic layer was evaporated under vacuum and the residue was purified by column chromatography (MeOH/CH<sub>2</sub>Cl<sub>2</sub> 1:20) to yield the product as a white foam (0.87 g, 48%). The pyrene diol nucleoside (0.5 g, 1.57 mmol) was coevaporated with pyridine (25 mL) 3 times and then dried on vacuum pump for 4 h. 4,4'-Dimethoxytrityl chloride (0.64 g, 1.89 mmol), DIEA (0.30 g, 2.35 mmol) was added and anhydrous pyridine (50 mL) was added via syringe. The reaction was stirred under argon in the dark and was monitored by TLC. Upon completion of reaction (approximately 1.5 h), methanol (5 mL) was added to quench the leftover 4,4'-dimethoxytrityl chloride. After evaporation of the solvents, the product was purified by column chromatography (ethyl acetate/hexanes 1:2) to yield the product as a white foam (0.84 g, 86%). 1H-NMR(500 MHz, CDCl3): δ=8.337-8.289(dd, J= 16Hz, J'= 9Hz, 2H), 8.186-8.165(m, 2H), 8.160-8.144(d, J = 8 Hz, 1H), 8.084-8.066(d, J = 9Hz, 1H), 8.046(s, 2H), 7.998(t, 1H), 7.536-7.521(d, J= 7.5 Hz, 2H), 7.421-7.401(q, 4H), 7.302 (t, 2H), 7.227 (t, 1H), 6.841-6.813(q, 4H), 6.227-6.195(q, 1H), 4.568-4.562(m, 1H), 4.257-4.232(m, 1H), 3.775(s, 3H), 3.770(s, 3H), 3.538-3.450(m, 2H), 2.628-2.585(m, 1H), 2.286-2.228(m, 1H). 13C-NMR(125 MHz, CDCl3):  $\delta = 158.749, 145.152, 136.295, 135.686, 131.619, 130.937, 130.869, 130.400, 128.501, 128.152, 136.295, 135.686, 131.619, 130.937, 130.869, 130.400, 128.501, 128.152, 130.869, 130.400, 128.501, 128.152, 130.869, 130.86$ 127.812, 127.771, 127.363, 127.102, 126.148, 125.447, 125.279, 125.106, 124.994, 123.147,

122.999, 113.417, 86.602, 86.378, 74.969, 64.605, 55.476, 44.063. HR-MS (*m/z*): calculated for  $C_{42}H_{36}O_5$ : 620.2564, found 620.2565. The product was further characterized by 2D ROESY experiment as the β-anomer.



Figure S11. <sup>1</sup>H NMR of 4.



Figure S12. <sup>13</sup>C NMR of 4.



Figure S13. <sup>1</sup>H NMR of 6.



Figure S14. <sup>13</sup>C NMR of 6.



**Figure S15.** <sup>1</sup>H NMR of 1 (I).



**Figure S16.** <sup>13</sup>C NMR of **1** (I).



Figure S17. <sup>1</sup>H NMR of 7.



**Figure S18.** <sup>13</sup>C NMR of 7.





Figure S19. <sup>1</sup>H NMR of 8.



Figure S20. <sup>13</sup>C NMR of 8.



**Figure S21.** <sup>31</sup>P NMR of **8**.

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