Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Excess Electron Transport through 5-Substituted Pyrimidines in DNA: Electron Affinities of Uracil and Cytosine Derivatives Differently Affect the Apparent Efficiencies

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METHODS

General. Reagents were purchased from Aldrich, Wako Pure Chemical Industries, Tokyo Chemical Industries, and Nacalai Tesque, and used as received. Phosphoramidites for DNA synthesis were purchased from Glen Research. High-performance liquid chromatography was performed with a Hitachi LaChrom Elite D-2000 system. Sample solutions were injected onto a reversed phase column (Inertsil ODS-3, GL Science Inc., ø 4.6 mm × 250 mm or ø 10 mm × 250 mm) at 40 °C. The column eluents were monitored through the UV absorbance at 260 nm at a flow rate of 0.6 (analytical) or 3.0 (preparative) mL min^{-1} with an appropriate gradient of acetonitrile/triethylamine acetate buffer (0.1 M, pH 7.0). ODNs containing PTZ were synthesized with an Applied Biosystems 3400 DNA Synthesizer following the same procedures previously reported.[8d] ODNs containing ^{x}C were purchased from Japan BioService and purified by 20% PAGE. UV-visible spectra were obtained using a JASCO V-630 UV/VIS spectrophotometer equipped with a temperature programmable cell block. ESI-mass spectrometry of ODNs was performed on a Bruker Daltonics microTOF mass spectrometer.

Melting Temperature (T_m) of ODNs. ODNs (2 μM) were dissolved in 10 mM sodium phosphate and 0.1 M sodium chloride buffer (pH 7.0). UV melting curves were recorded on a JASCO-V630 spectrophotometer equipped with a multi-cell block and a Peltier temperature controller. Melting curves were obtained by monitoring the UV absorbance at 260 nm as the temperature increased from 4 °C to 80 °C at a rate of 1 °C min^{-1}.

Table S1. Thermal melting properties of the synthesized DNA duplex (2.0 μM) in 10 mM phosphate buffer (pH 7.0) containing 0.1 M sodium chloride.

	Melting temperature T_m/°C		
X =	C	^{Me}C	^{F}C
DNA 1	55.5	55.0	54.0
DNA 2	54.0	53.7	54.2
DNA 3	-	55.5	54.0

Table S2. Thermal melting properties of DNA 4 (2.0 μM) in 10 mM phosphate buffer (pH 7.0) containing 0.1 M sodium chloride.

	Melting temperature T_m / °C				
XYZ =	^{Me}CC^{F}C	^{F}CC^{Me}C	CC^{F}C	C^{F}C^{F}C	CCC
DNA 4	57.0	57.4	56.6	57.0	55.0

Thermal Denaturation. 2-Aminopurine can substitute for A in an ODN. It is a naturally fluorescent base that is sensitive to the local environment making it a useful probe for monitoring the structure. To investigate the effect of ^{Me}C on the local structure of our sequences, ODN containing aminopurine (^{Am}P) near the ^{Me}C site was synthesized (AP/^{Me}C-ODN) and temperature-dependencies of the fluorescence and excitation spectra were compared to those for the C-containing DNA (AP/C-ODN). It has been reported that motions of ^{Am}P in duplex DNA are correlated to the spectral shift in the excitation spectra (O'Neill, M. A. and Barton, J, K. *J. Am. Chem. Soc.*, 2004, **126**, 11471.). However, as shown in Figures S1 and S2, significant difference was not observed between AP/C-ODN and AP/^{Me}C-ODN.

AP/C-ODN
5'- AG^{Am}P AGC ACT GGA -3'
3'- T**C** T TCG TGA CCT -5'

AP/^{Me}C-ODN
5'- AG ^{Am}P AGC ACT GGA -3'
3'- T^{Me}**C** T TCG TGA CCT -5'

^{Am}P: 2-aminopurine

^{Me}C: 5-methylcytosine

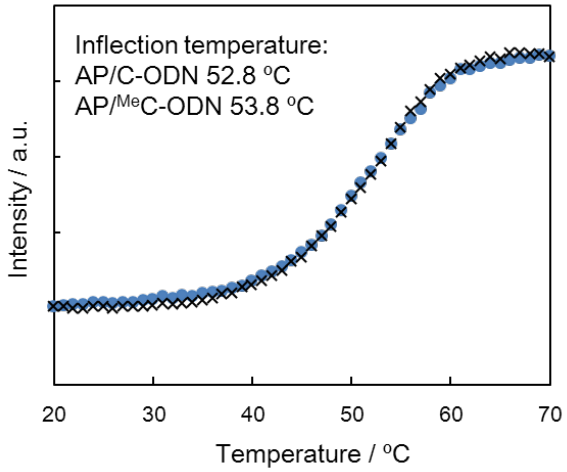


Figure S1. Temperature-dependent changes in emission from (●) AP/C-ODN and (x) AP/^{Me}C-ODN. Emission intensities at 380 nm were obtained upon photo-excitation (λ_{ex} = 325 nm) of DNA (5 μM) in phosphate buffer (10 mM, pH 7.0) containing NaCl (90 mM).

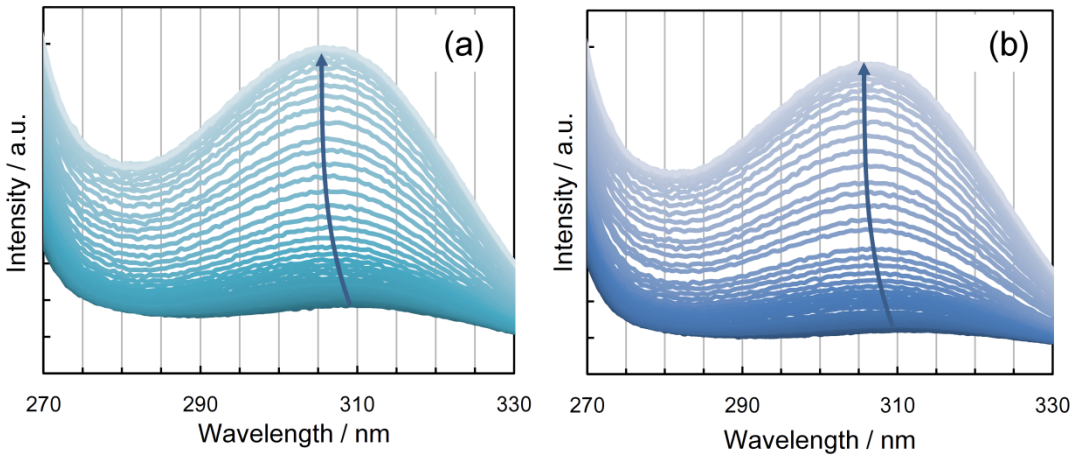


Figure S2. Temperature-dependent changes in excitation spectra of (a) AP/C-ODN and (b) AP/MeC-ODN (5 μM DNA, respectively, in 10 mM phosphate buffer containing 90 mM NaCl). Excitation spectra were obtained by monitoring emission at 380 nm as the temperature increased from 15 °C to 70 °C. Arrows indicate the shift of peak wavelength of the spectra.

pKa Measurement. The pKa values of nucleosides were determined by measuring the changes in their UV absorption spectra in aqueous solution as a function of the pH. Aqueous solutions of nucleosides (0.1 mM) were prepared and aliquots of a dilute, standard HCl solution were added. After each addition, the pH of the solution was measured by using a HORIBA pH-meter (Model F-52) and the absorption spectra were recorded.

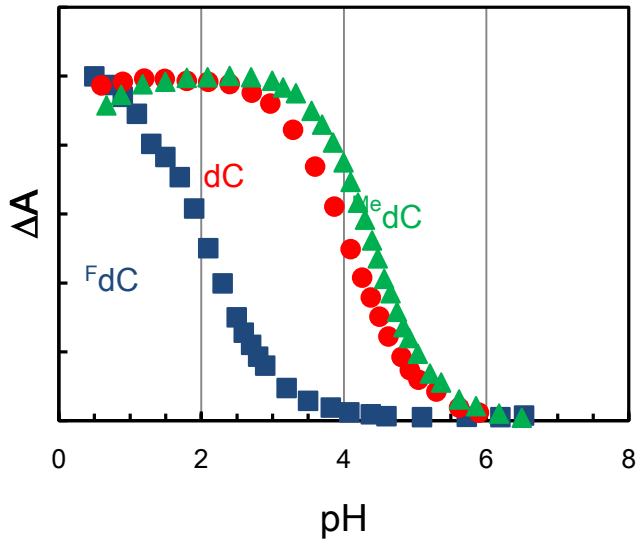


Figure S3. pH-Responses of UV-absorption at 280 nm by 2'-deoxycytidine derivatives.

X-Radiolysis. Dilute aqueous solutions of C, ^{F}C, and ^{Me}C (0.2 mM) containing 20 mM isopropyl alcohol in 10 mM phosphate buffer (pH 7.0) were bubbled with Ar gas for 15 min and exposed to X-ray with a Rigaku RADIOFLEX-350 at a dose rate of 5 Gy min^{-1} under Ar-atmosphere. Aliquots of the solutions were subjected to high-performance liquid chromatography analysis.

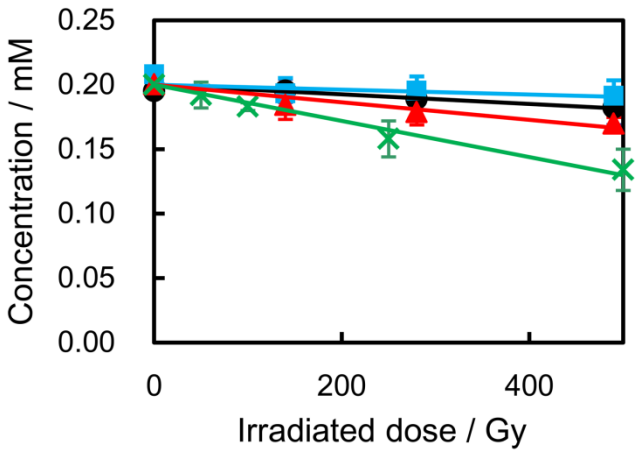


Figure S4. Reductive decomposition of 5-substituted cytosine derivatives (^{x}C) by hydrated electrons. Aqueous buffered solutions of ^{x}C (0.2 mM, pH 7.0) containing isopropyl alcohol (20 mM) were exposed to X-ray (5 Gy min^{-1}) under an Ar-atmosphere; (■) ^{Me}C, (●) C, (▲) ^{F}C, and (×) ^{Br}U. *G*-values (nmolJ^{-1}) for the decomposition of ^{x}C and ^{Br}U were calculated to be 26.6 (C), 34.2 (^{Me}C), 62.6 (^{F}C), and 135 (^{Br}U), respectively.

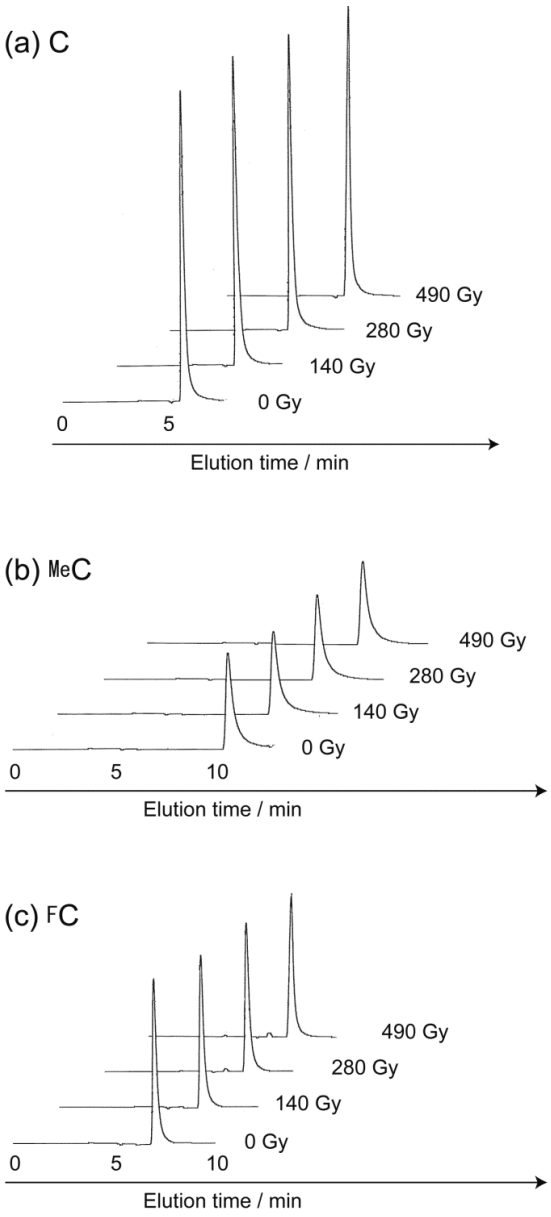


Figure S5. HPLC profiles of the aqueous solutions of ^{x}C (0.2 mM) containing isopropyl alcohol (20 mM) before and after X-ray irradiation (0-490 Gy) under an Ar-atmosphere. Aliquots of the sample solutions were delivered with 0.1 M triethylamine acetate buffer (pH 7.0) at a flow rate of 0.6 mL min^{-1}.

Photolysis and Polyacrylamide Gel Electrophoresis. ODNs (50 pmol strand concentration) were radiolabeled by phosphorylation with [γ-^{32}P]-ATP (Perkin Elmer, 370 MBq/mL) and T_4 Polynucleotide Kinase (Nippon Gene). The labeled mixtures were subsequently centrifuged through a MicroBio-Spin 6 column (Biorad) to remove excess unincorporated nucleotides. Cold and 5′-^{32}P-end-labeled ODNs (1.0 μM strand concentration) were hybridized with their complementary ODNs possessing PTZ (1.3 μM) in buffer containing 10 mM sodium phosphate and 90 mM sodium chloride (pH 7.0). Hybridization was achieved by heating the samples at 90 °C for 8 min and slowly cooling to room temperature under N_2-atmosphere. The solutions in 1.5-mL Eppendorf tubes were exposed to UV-light (365 ± 15 nm) produced from a FTI-20L transilluminator (tube lamps with a filter, Funakoshi, Tokyo) at 4 °C under N_2-atomosphere. After irradiation, all reaction mixtures were precipitated by addition of 10 μL of herring sperm DNA (1 mg/mL), 10 μL of 3 M sodium acetate (pH 5.2), and 400 μL of ethanol. The precipitated DNA was washed with 100 μL of 80% cold ethanol and then dried under reduced pressure. The precipitated DNA was dissolved in 50 μL of 10 vol% piperidine, heated at 90 °C for 20 min, and then dried under reduced pressure. The radioactivity of the samples was assayed using an Aloka 1000 liquid scintillation counter and the dried DNA pellets were resuspended in loading buffer (8 M urea, 40% sucrose, 0.025% xylene cyanol, 0.025% bromophenol blue). The samples (1.0 μL, [1–10] × 10^3 cpm) were loaded onto 20% polyacrylamide (acrylamide-bisacrylamide 19:1) gels containing 7 M urea, electrophoresed at 2000 V for approximately 90 min, transferred to cassettes, and exposed at –80 °C to Fuji X-ray films (RX-U). Each experiment was repeated at least 3 times.

Cleavage of the labeled strand was quantified by autoradiography using the ATTO Densitograph software (version 3.0).

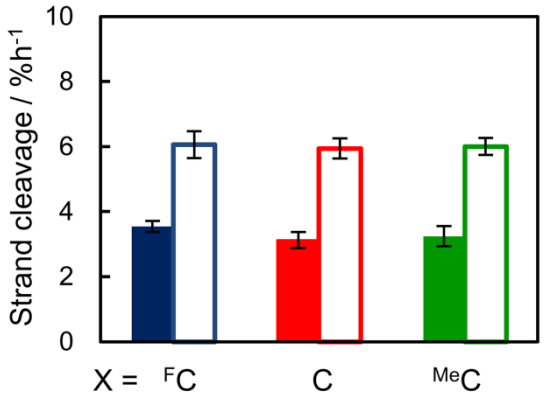


Figure S6. Photoinduced EET in DNA 3. Photo-irradiation and piperidine treatment were performed as indicated in the caption of Figure 2.

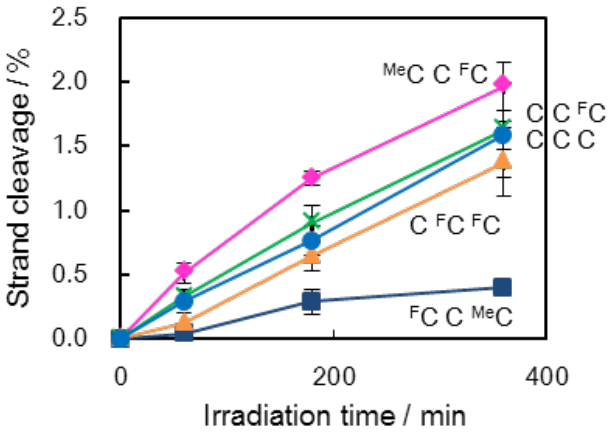
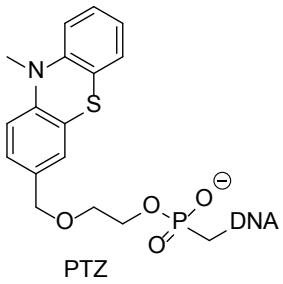


Figure S7. Time-dependent formation of the strand cleavage products after photoinduced EET in DNA 4.

Table S3. ESI MS Data for the synthesized ODNs.

Sequence	Calcd. for $[(M-3H)^{3-}]$	Observed
5'-PTZ-TGA AGC ACT GGA	1346.57	1346.56
5'-PTZ-AGA AGC ACT GGA	1349.56	1349.92
5'-PTZ-TGG GAG CAC TGG A	1461.59	1461.90



PTZ

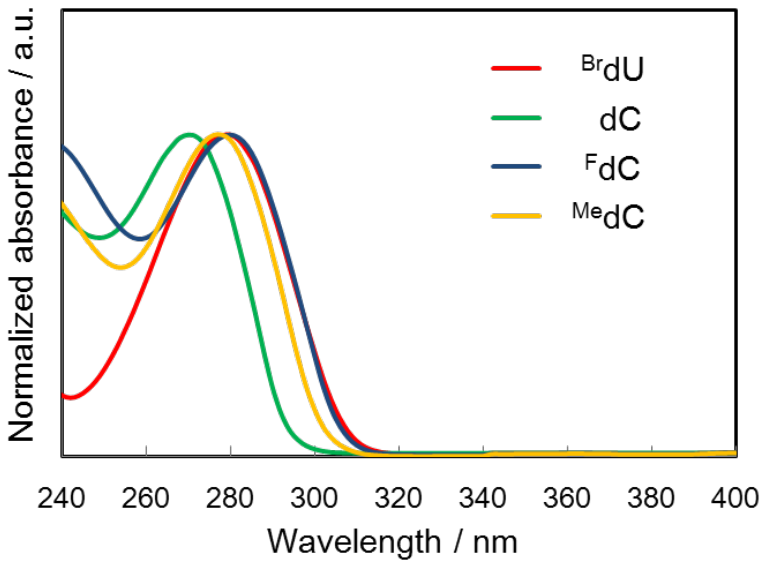


Figure S8. Normalized absorption spectra of 2'-deoxycytidine derivatives and 5-bromo-2'-deoxyuridine in 10 mM phosphate buffer solution (pH 7.0).