Supplementary Material (ESI) for Chemical Communications

Site-Specific Transition of Cytosine to Uracil *via* Reversible DNA Photoligation

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Supporting Information Available. Experiment details, characterization data, and PAGE chart (4 pages). See any current masthead for ordering information and Web access instructions.

Gneneral. ^{1}H NMR spectra were measured on Varian GEMINI-200 (200 MHz) or JEOL JNM A-400 (400 MHz) spectrometers. Coupling constants (*J* values) are reported in Hz. The chemical shifts are expressed in ppm downfield from residual chloroform (δ = 7.24 in ^{1}H NMR) and water (δ = 4.65 in ^{1}H NMR) as an internal standard. Mass spectra were recorded on a JEOL JMS DX-300. JASCO V-550 UV/VIS spectrophotometer was used for absorption spectra measurements. Irradiation was performed by VILBER LOURMAT transilluminator (TFP-35L, 366 nm, 180 W, 4000 J/cm^2) or FUNAKOSHI transilluminator (FTI-36M, 302 nm, 180 W). HPLC was performed on a Chemcobond 5-ODS-H column (10 × 150 mm, 4.6 × 150 mm) or a Chemcosorb 5-ODS-H column (4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 254 and 230 nm. Photodiode array HPLC was performed on a Cosmosil 5C18AR column (4.6 × 150 mm) with a Tosoh CCPE-II system equipped with a Photal MCPD-3600 system.

Experimental procedure for site-specific transition via reversible DNA Photoligation.

The oligonucleotides (ODNs, 400 pmol, strand concentration) were 5'-end-labeled by phosphorylation with 4 μL of [γ-^{32}P]ATP and 4 μL of T4 polynucleotide kinase using standard procedures.[S1] The 5'-end-labeled ODNs were recovered by ethanol precipitation and further purified by 15% denaturing gel electrophoresis and isolated by the crush and soak method.[S2] A solution (total volume 10 μL) containing ^{32}P-5'-end-labeled ODN **1** (ca. 2 × 10^4 cpm, 1 μM) and ODN **2** (7 μM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM NaCl was irradiated with a transilluminator at 366 nm at a distance of 3-5 cm at 0 °C for 3 h. The reaction mixture was heated at 90 °C for 2 h and followed by irradiation at 302 nm for 1 h at 40 °C. To the reaction mixture was next added Uracil DNA glycosylase (UDG, 1 unit) and incubated at 37 °C for 1 h. The reaction mixture was treated with 1 M piperidine at 90 °C for 20 min and then evaporated under vacuum followed by coevaporation with water twice. To the reaction mixture were added 10 μL of loading buffer (a solution of 80% v/v formamide 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue) and 10 μL of water and the samples (1-2 μL, ca. 2-4 × 10^3 cpm) were loaded onto 20% (19 : 1) polyacrylamide and 7 M urea denatured gel and electrophoresed at 700 V for 30 min. The gel was dried and exposed to X-ray film with intensifying sheets at –80 °C.

[S1] J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor Laboratory Press: New York, **1989**.
[S2] A. M. Maxam, W. Gilbert, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 560; T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning*; Cold Spring Harbor Laboratory Press: Plainview, New York, **1982**.

Synthesis and characterization of ^{CV}U-containing ODN.

^{CV}U-containing ODN was synthesized by automated solid-phase phosphoramidaite method as reported.[S3] After automated synthesis, the oligomers were cleaved from the support and deprotected by 0.4 M methanolic sodium hydroxide (methanol : water = 4 : 1) for 17 h at room temperature and purified by reverse phase HPLC. The purity and concentration of all oligodeoxynucleotides were determined by complete digestion with s.v. PDE, AP, and Nuclease P1 to 2'-deoxymononucleosides at 37 °C for 1 h. Incorporation of ^{CV}U into oligonucleotides was confirmed by enzymatic digestion and MALDI-TOF-MS analysis. MALDI-TOF-MS: calcd. for ODN **2** ($C_{206}H_{259}N_{75}O_{128}P_{20}$) [$(M–H)^-$] 6452.25; found 6452.60.

[S3] K. Fujimoto, N. Ogawa, M. Hayashi, S. Matsuda, I. Saito, *Tetrahedron Lett.* **2000**, *41*, 9437.

Experimental procedure for time course of the deamination reaction.

A solution (total volume 10 μL) containing ^{32}P-5'-end-labeled ODN **1** (ca. 2×10^4 cpm, 1 μM) and ODN **2** (7 μM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM NaCl was irradiated with a transilluminator at 366 nm at a distance of 3-5 cm at 0 °C for 3 h or 4 h. The reaction mixture was heated at 90 °C for each time. The reaction samples were irradiated at 302 nm and treated with UDG and piperidine as described above. The reaction samples were loaded onto 20% (19 : 1) polyacrylamide and 7 M urea denatured gel and electrophoresed at 700 V for 30 min. The gel was dried and exposed to X-ray film with intensifying sheets at –80 °C. The gels were analyzed by autoradiography with a densitometer and BIORAD Molecular Analyst software (version 2.1). The intensities of the cleaved bands were determined by volume integration (Figure S1). The rate constant (k) for deamination of C-^{CV}U photoadduct in the ligation site can be calculated from the equation (1)

$$k = -\ln(1-x)/t \qquad (1)$$

where x is the fraction of cytosine converted into uracil which is determined from the intensities of the cleaved bands and t is time of incubation.

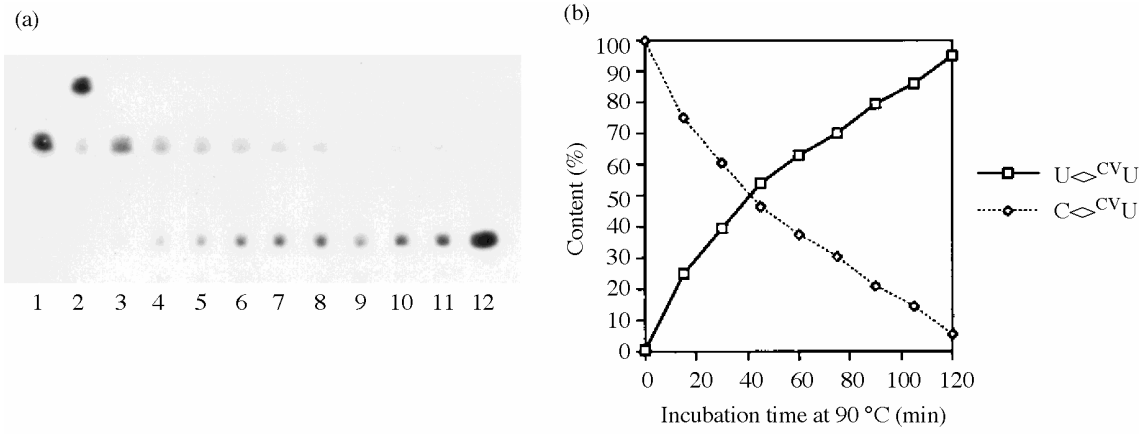

Figure S1. (a) PAGE analysis for time course of deamination. Lane 1, before photoligation; lane 2, ODN **1**+ ODN **2**, irradiation at 366 nm, 1 h on ice; lane 3, lane 2 + 90 °C, 0 min + 302 nm, 1 h, r.t. + UDG (37 °C, 1 h) + piperidine (90 °C, 30 min) ; lane 4, 5, 6, 7, 8, 9, 10, 11, under the same condition of lane 3 except for 90 °C, 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min, 120 min respectively ; lane 12, treatment of ODN **4** with UDG and piperidine treatment under the same condition of lane 3. (b) Time course for the conversion of C<>^{cv}U adduct and the formation of U-^{cv}U adduct in ligated site based on the intensities of the cleaved bands were determined by volume integration.