High selective detection of 5-methylcytosine using photochemical ligation

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General method and materials.

Tetrazole was purchased from GLEN RESEARCH. The reagents for the DNA synthesizer such as I_2 solution (I_2/H_2O/pyridine/tetrahydrofuran, 3:2:19:76), A-, G-, C-, and T-β-cyanoethyl phosphoroamidites were purchased from GLEN RESEARCH. Other regents were purchased at highest commercial quality and used without further purification unless otherwise stated. Calf intestine alkaline phosphstase (AP) (1500 units) was purchased from Promega and Roch. Nuclease P1 (500 units) was purchased from Yamasa. Reactions were monitored TLC plates precoated with Merck silica gel 60 F_{254}. Kanto Chemical Silica Gel 60 N was used for silica gel column chromatography. ODNs were synthesized on an Applied Biosystems 3400 DNA Synthesizer. Reverse phase HPLC was performed on a Cosmosil 5C_{18}AR-II (nacalai tesque) column (4.6 x 150 mm) or a CHEMCOBOND 5-ODS-H (Chemco) column (4.6 x 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 spectrometer at 260 nm. Irradiation was performed by UV-LED (OMURON, 366 nm) and transilluminator (Funakoshi TR-366 nm). Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems.

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

^{C}U-containing ODN was synthesized by automated solid-phase phosphoramidite method as reported. [S1] After automated synthesis, the oligomer was deprotected by incubation with 28% ammonia for 4 h at 65 °C and was purified on a Chemcobond 5-ODS-H column (4.6 x 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% acetonitrile, linear gradient (30 min) at a flow rate of 1.0 mL/min, 30 ˚C. Preparation of oligonucleotides was confirmed by MALDI-TOF-MS analysis.

MALDI-TOF MS: calcd. 1860.34 for ODN1(^{C}U) [$(M + H)^+$], found 1860.74)

MALDI-TOF MS: calcd. 1835.35 for ODN1(^{V}U) [$(M + H)^+$], found 1835.61)

MALDI-TOF MS: calcd. 7136.52 for ODN2(^{C}U) [$(M + H)^+$], found 7136.57)

MALDI-TOF MS: calcd. 7111.52 for ODN2(^{V}U) [$(M + H)^+$], found 7111.99)

Experimental procedure for DNA photoligation

Photoligation of DNA oligomer as monitored by HPLC.

The reaction mixture (total volume 100 μl) containing ODN1(^{C}U) (20 μM, strand conc.), ODN1(^{m}C) (20 μM, strand conc.), and cODN1 (22 μM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with a UV-LED (366 nm ± 15 nm light at 1600 mW/cm^2) at a distance of 1.5 cm at 0 °C for 15 min. After irradiation, the progress of photorection was moniterd

HPLC. The yield was calculated on the basis of ODN1(^{m}C). Reaction mixture was taken up and subjected to HPLC analysis. The photoligated ODN1(^{m}C-^{C}U) was purified by HPLC and subjected enzymatic digestion with s. v. PDE (0.25 unit/mL), P-1 nuclease (2.5 unit/mL) and AP (10 unit/mL) for 4 h to decompose to mononucleosides. Reaction mixture was taken up and subjected to HPLC analysis. Analysis was carried out on a COSMOSIL 5C_{18}-AR-II column (4.6 x 150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–20% acetonitrile, linear gradient (30 min) at a flow rate of 1.0 ml/ min, 30 °C. Enzymatic digestion of ODN(^{m}C-^{C}U) provided dC, dG, dT and dA in a ratio of 1:6:1:7 together with a slower eluting photoadduct d(^{m}C-^{C}U). Photoadduct d(^{m}C-^{C}U)was confirmed by MALDI-TOF-MS analysis.

Photochemical ligation between cytosine and 5-cyanovinyl-2´-deoxyuridine.

In the absence of the methyl group, photocrosslinking is promoted by the intercalation manner of the cyanovinyl group and the opposite site bases of the template DNA (Figure S1).

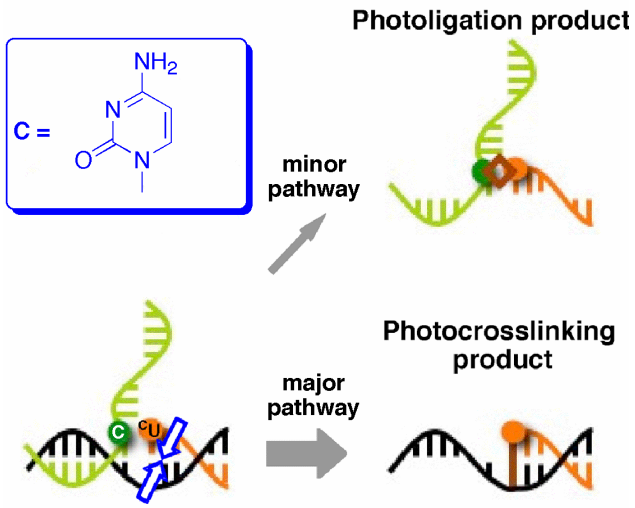
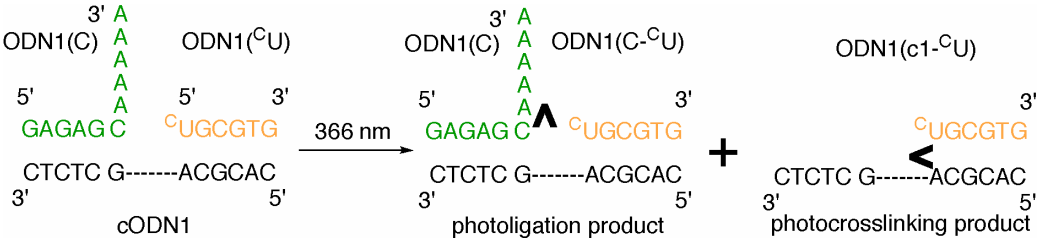


Figure S1. Conceptual scheme of the photochemical reaction between cytosine and 5-cyanovinyl-2´-deoxyuridine.

We examined photoirradiation by using ODN1(C) (5'-GAGAGCAAAAA-3') as shown in Scheme S1. We observed the appearance of the two new peaks at a retention time of 16 and 17 min by HPLC (Figure S2). As a result of mass analysis, we confirmed that ODN1(C-^{C}U) at a retention time of 17 min was a ligated product of ODN1(^{C}U) and ODN1(C) (MALDI-TOF MS: calcd. 5268.53 for ODN1(C-^{C}U) [(M + H)$^{+}$], found 5268.57.). Enzymatic digestion of isolated ODN1(C-^{C}U) showed the formation of dC, dG, T and dA in a ratio of 1:6:1:7, together with a d(C-^{C}U) photoadduct (MALDI-TOF MS: (calcd. 507.183 for

d(C-^{C}U) [(M + H)$^{+}$], found 507.444.). MALDI-TOF MS determined the product with a retention time of 16 min corresponded to ODN(c1-^{C}U) photocrosslinked product of cODN1 and ODN1(^{C}U) (MALDI-TOF MS: calcd. for 5427.59 ODN(c1-^{C}U) [(M + H)$^{+}$], found 5427.55.). Enzymatic digestion of isolated ODN(c1-^{C}U) showed the formation of dC, dG, T and dA in a ratio of 7:5:3:1, together with a new product corresponded to d(A-^{C}U) by MALDI-TOF MS (MALDI-TOF MS: (calcd. 531.195 for d(A-^{C}U) [(M + H)$^{+}$], found 531.535.).



Scheme S1. Template-directed DNA photoligation of ODNs with ^{C}U.

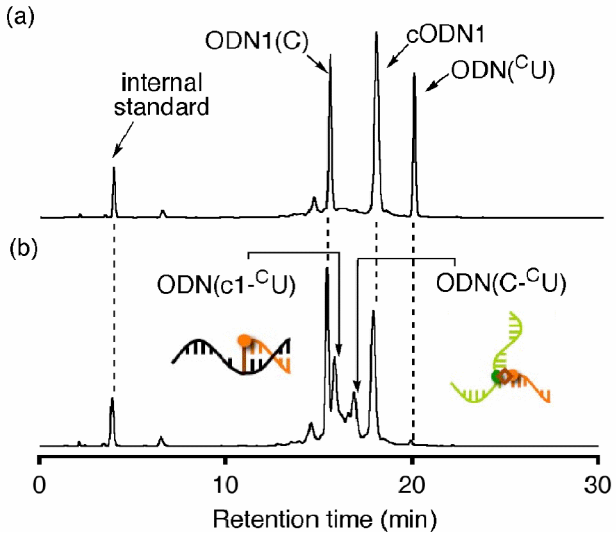


Figure S2. HPLC analysis of the ODN1(^{C}U) and ODN1(C) in the presence of template cODN1 with irradiation at 366 nm. (a) Before photoirradiation. (b) Irradiation at 366 nm for 15 min. 2′-Deoxycytidine (dC) was used as an internal standard.

Photochemical detection of 5-methylcytosine on a DNA chip by using 5-vinyl-2´-deoxyuridine (^{V}U).

To demonstrate that template-directed photoligation by using ODN2(^{V}U) could be incorporated into platforms suitable for DNA chip technologies, we constructed the DNA chip by attaching amino-labeled ODN containing ^{V}U, onto the aldehyde-modified glass surface. We determined the feasibility of the template-directed photoligation through ODN2(^{V}U) on a DNA chip A glass chip spotted with 2 μM target ODN2(C) or ODN2(^{m}C) and template cODN2, was irradiated at 366 nm for 240 min in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride. After the chip had been washed with deionized water at 98 °C for 5 min, Cy3-containing ODN(Cy3) conjugate was added to the surface, and the chip was washed twice in PBS. Fluorescence signals were detected on a microarray scanner. As shown in Figure S4 and Figure S5, we measured the fluorescence signal of the photoligated product with the ^{m}C case. To investigate the generality of sequence discrimination, we constructed C-containing ODN.

ODN2(^{V}U): 5 ´-^{V}UGACGTGTATCGCATTGGSSSSNH$_2$-3 ´

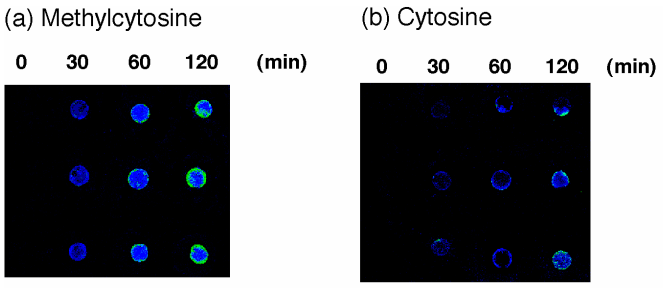


Figure S3. Fluorecence images acquired on microarray scanner for the product of photoligation on methylcytosine (a) and cytosine (b) target DNAs by using ^{V}U.

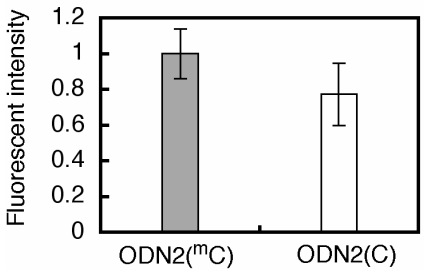


Figure S4. Selectivity for different photosensitive nucleotides condition. The data points of represent the average of three experimental runs by irradiation at 366 nm for 240 min.

Photochemical detection of 5-methylcytosine on a DNA chip by using 5-cyanovinyl-2´-deoxyuridine (^{C}U).

To demonstrate that template-directed photoligation by using ODN2(^{C}U) could be incorporated into platforms suitable for DNA chip technologies, we constructed the DNA chip by attaching amino-labeled ODN containing ^{C}U, onto the aldehyde-modified glass surface. We determined the feasibility of the template-directed photoligation through ODN2(^{C}U) on a DNA chip A glass chip spotted with 2 μM target ODN2(C) or ODN2(^{m}C) and template cODN2, was irradiated at 366 nm for 90 min in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride. After the chip had been washed with deionized water at 98 °C for 5 min, Cy3-containing ODN(Cy3) conjugate was added to the surface, and the chip was washed twice in PBS. Fluorescence signals were detected on a microarray scanner. As shown in Figure S5, we measured the strong fluorescence signal of the photoligated product with the ^{m}C case. To investigate the generality of sequence discrimination, we constructed C-containing ODN.

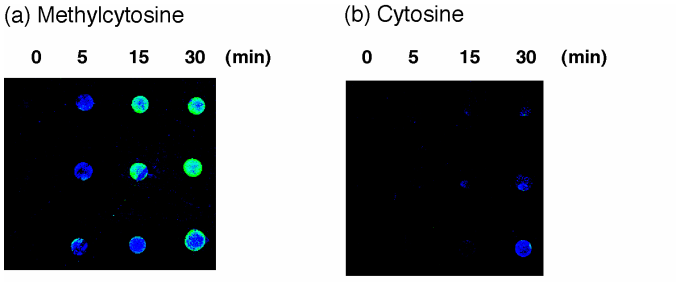


Figure S5. Fluorecence images acquired on microarray scanner for the product of photoligation on methylcytosine (a) and cytosine (b) target DNAs by using ^{C}U.

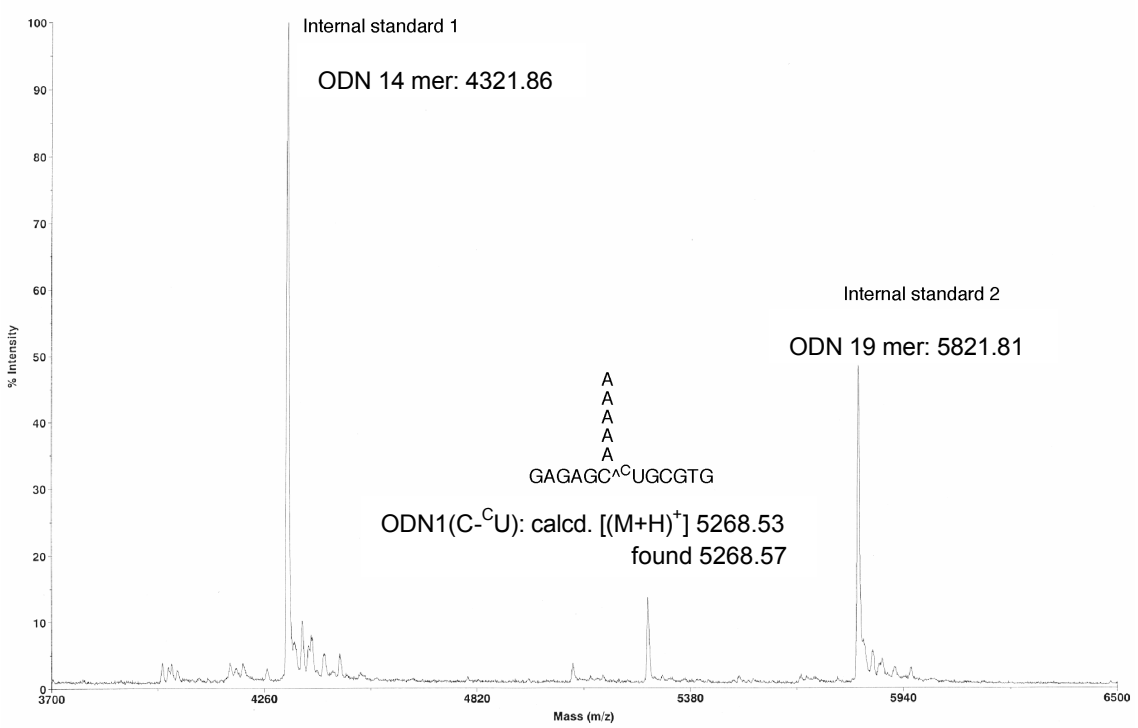


Figure S6. MALDI-TOF MS spectrum of ODN1(C-^{C}U) using a 3-hydroxypicolinic acid matrix.

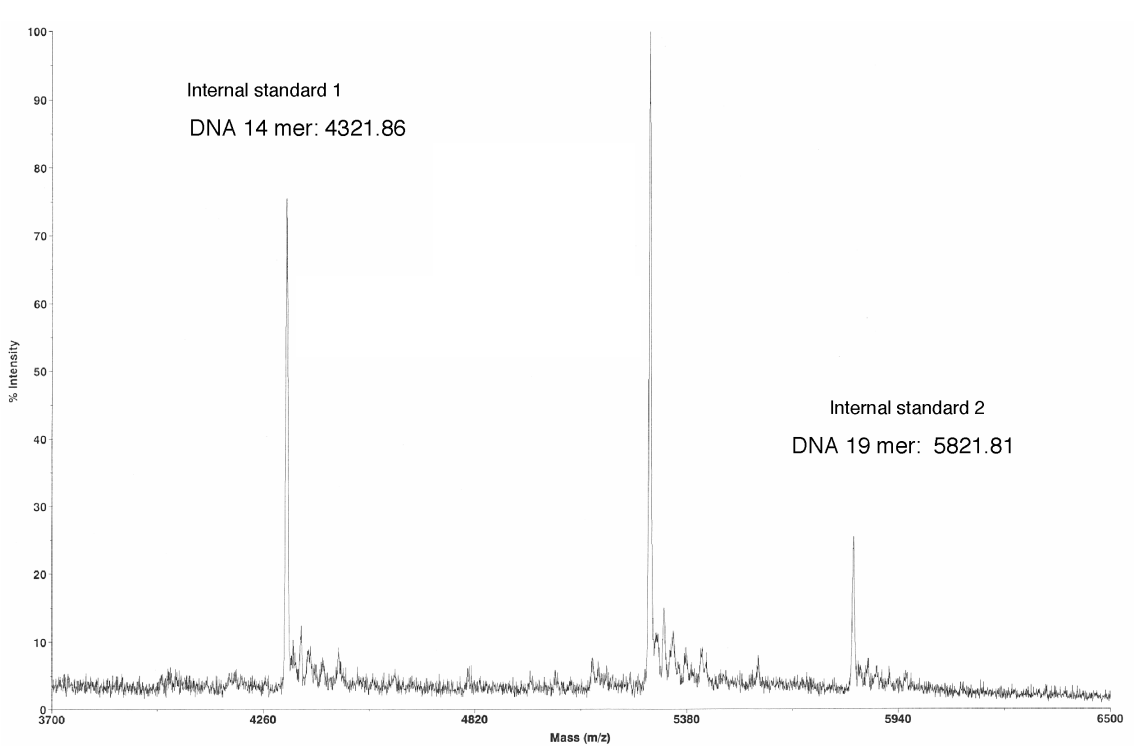


Figure S7. MALDI-TOF MS spectrum of ODN1(^{m}C-^{C}U) using a 3-hydroxypicolinic acid matrix.

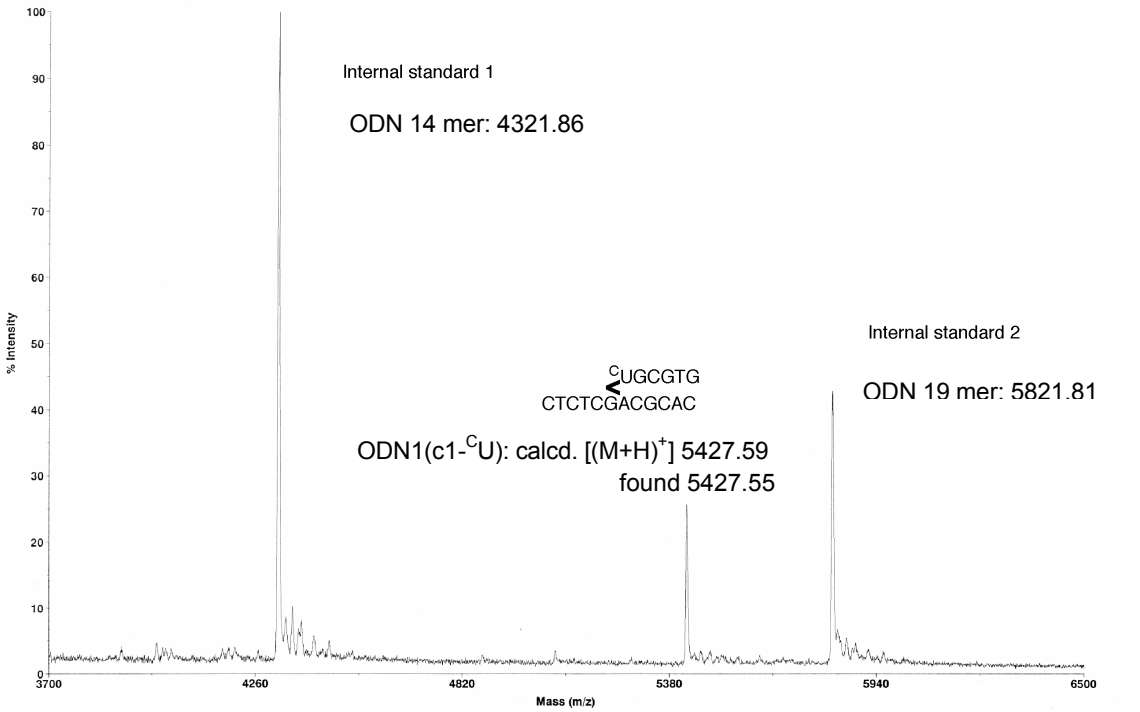


Figure S8. MALDI-TOF MS spectrum of ODN(c1-^{C}U) using a 3-hydroxypicolinic acid matrix.

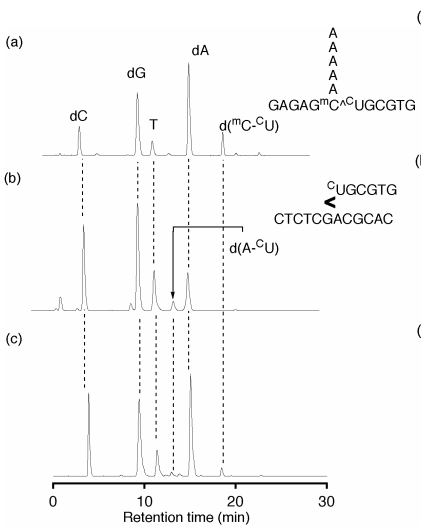


Figure S9. HPLC profiles of enzymatic digestion of photoreaction mixture by using ODN1(^{m}C). (a) Enzymatic digestion of isolated photoligated ODN1(^{m}C-^{C}U). (b) Enzymatic digestion of isolated photocrosslinking ODN(c1-^{C}U). (c) Enzymatic digestion of photoreaction mixture by using ODN1(^{m}C).

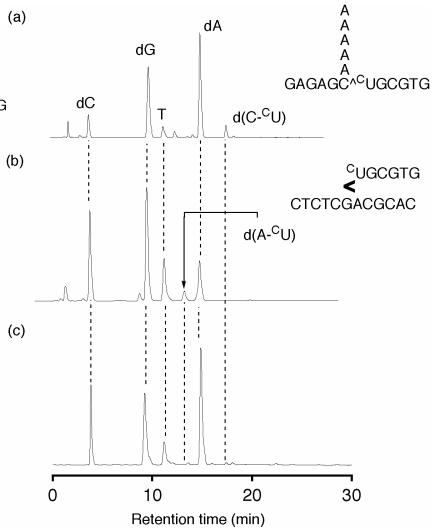


Figure S10. HPLC profiles of enzymatic digestion of photoreaction mixture by using ODN1(C). (a) Enzymatic digestion of isolated photoligated ODN1(C-^{C}U). (b) Enzymatic digestion of isolated photocrosslinking ODN(c1-^{C}U). (c) Enzymatic digestion of photoreaction mixture by using ODN1(C).

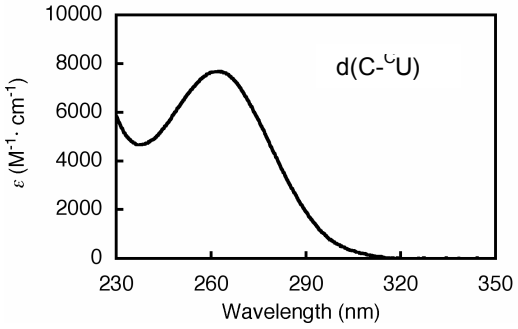


Figure S11 UV spectra of d(C-^{C}U) adducts

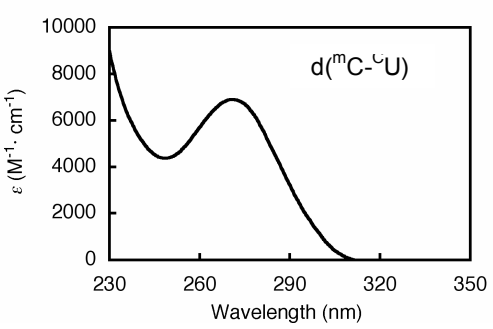


Figure S12 UV spectra of d(^{m}C-^{C}U) adducts

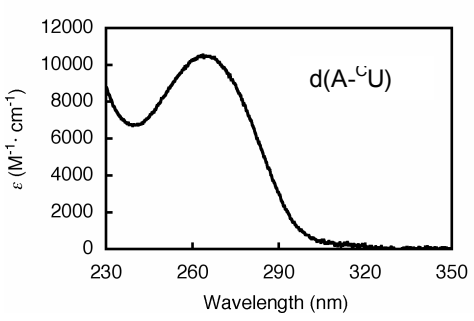


Figure S13 UV spectra of d(A-^{C}U) adducts

Reference

S1 M. Ogino and K. Fujimoto, *Angew. Chem., Int. Ed.* 2006, **45**, 7223