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Author(s)	Fujimoto, Kenzo; Yoshimura, Yoshinaga; Ikemoto, Tadayoshi; Nakazawa, Akio; Hayashi, Masayuki; Saito, Isao
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# Photoinduced DNA end capping via N<sup>3</sup>-methyl-5-cyanovinyl-2'-deoxyuridine

Kenzo Fujimoto,<sup>\*ab</sup> Yoshinaga Yoshimura,<sup>b</sup> Tadayoshi Ikemoto,<sup>a</sup> Akio Nakazawa,<sup>c</sup> Masayuki Hayashi<sup>c</sup> and Isao Saito<sup>c</sup>

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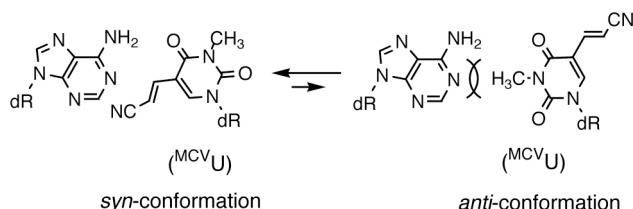
DOI: 10.1039/b000000x [DO NOT ALTER/DELETE THIS TEXT]

A modified oligodeoxynucleotide (ODN) containing N<sup>3</sup>-methyl-5-cyanovinyl-2'-deoxyuridine reacts by photoirradiation at 366 nm with an adenine residue of a complementary template ODN to yield an end-capped ODN in 87% yield.

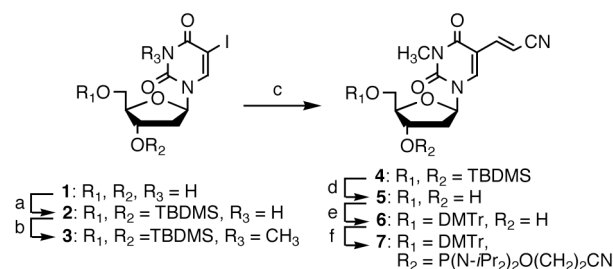
Since the double helical structure of DNA was first described by Watson and Crick in 1953, a wide variability of DNA conformations has been observed as non-ground state structures, such as hairpin-DNA, cruciform, Z-DNA and triple helix in nucleic acid.<sup>1</sup> It has been difficult to study such unusual DNA conformations by biophysical analysis because of the narrow range of limited conditions under which they exist. Among these structures, the hairpin stem-loop structure has attracted interest because of its generality in palindromic sequences associated with the regulation of transcription and other biological functions.<sup>2</sup> To overcome these problems, chemical probes for the trapping and stabilization of such hairpin structures have been developed to explore DNA conformations, dynamics and their biological roles.<sup>3</sup> Recently, we have reported efficient and reversible template-directed photoligations with ODNs containing 3'-terminal cytosine using 5'-vinyl-2'-deoxyuridine (<sup>V</sup>U) containing ODN at the 5'-terminal.<sup>4</sup> A remarkable stacking between a vinyl residue of <sup>V</sup>U and 5'-pyrimidine within the same strand will be responsible for the efficient photoreaction in our template-directed DNA photoligation system via <sup>V</sup>U. We have now examined photochemical end capping, using N<sup>3</sup>-methyl-5-cyanovinyl-2'-deoxyuridine (<sup>MCV</sup>U) instead of <sup>V</sup>U, in which the more photoreactive vinyl group was incorporated. The photoreactive cyanovinyl group in <sup>MCV</sup>U was designed to stack effectively with a base in the opposite strand by an N<sup>3</sup>-methyl group substitution that allows stabilization of the *syn* orientation of <sup>MCV</sup>U and release from the Watson–Crick base pair (Figure 1). Herein we report the photochemical DNA end capping via <sup>MCV</sup>U instead of <sup>V</sup>U to generate the stabilized hairpin analogue at its end.

<sup>MCV</sup>U-containing ODN was synthesized according to the standard phosphoramidite chemistry on a DNA synthesizer. The phosphoramidite of <sup>MCV</sup>U was prepared in six steps from 5-iodo-2'-deoxyuridine as shown in Scheme 1.<sup>5</sup> Incorporation of <sup>MCV</sup>U into ODN was confirmed by enzymatic digestion and MALDI-TOF-MS.<sup>6</sup>

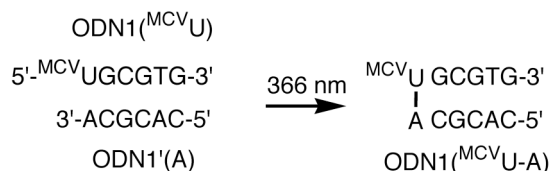
When 5'-d(<sup>MCV</sup>UGCGTG)-3' ODN1(<sup>MCV</sup>U) was irradiated at 366 nm for 30 min in the presence of 5'-d(CACGCA)-3' ODN1'(A) (Scheme 2), ODN1(<sup>MCV</sup>U-A) was produced in 87% yield, as determined by HPLC analysis (Figure 2).<sup>7,8</sup> MALDI-TOF-MS indicated that ODN1(<sup>MCV</sup>U-A) obtained by



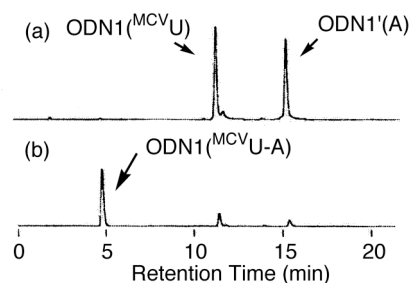
**Fig. 1** Proposed two conformer about the base pair between adenine and <sup>MCV</sup>U at the terminal site.



**Scheme 1** Reagents and conditions: (a) TBDMSCl, imidazole, pyridine, 3 h, 95%; (b) dimethylcarbonate, 18-crown-6, K<sub>2</sub>CO<sub>3</sub>, DMF, 3 h, 98%; (c) acrylonitrile, Pd(OAc)<sub>2</sub>, PPh<sub>3</sub>, 8 h, 70%; (d) TBAF, THF, 3 h, 85%; (e) DMTTrCl, DMAP, pyridine, 75%; (f) P(N-*i*Pr)<sub>2</sub>O(CH<sub>2</sub>)<sub>2</sub>CN, tetrazole, CH<sub>3</sub>CN, 2 h, 98%.



**Scheme 2** Photochemical end capping via ODN1(<sup>MCV</sup>U).



**Fig. 2** HPLC profile of photoreaction of ODN1(<sup>MCV</sup>U) and ODN1'(A). (a) before photoirradiation; (b) irradiation at 366 nm for 30 min, 87% yield.

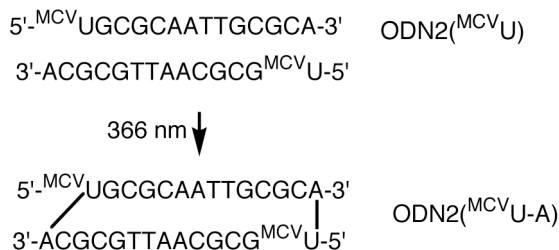
† Electronic Supplementary Information (ESI) available: Experimental details. See <http://www.rsc.org/suppdata/xx/b0/b000000x/>

\* kenzo@jaist.ac.jp

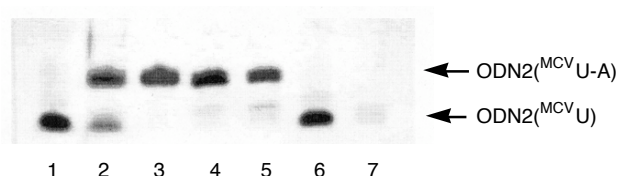
**Table 1** Melting temperature of end-capped ODN1<sup>(MCVU-A)</sup> in comparison with duplex ODN1<sup>(MCVU)</sup>/ODN1'(A) and T4 loop hairpin ODN

Entry	Oligomer	$T_m$ / °C <sup>a</sup>
1	ODN1 <sup>(MCVU)</sup> /ODN1'(A)	28.1
2	ODN1 <sup>(MCVU-A)</sup>	74.5
3	5'-d(CACGCATTTTTCGTG)-3'	42.6

<sup>a</sup> UV melting curves were obtained in a 50 mM sodium cacodylate buffer (pH 7.0) containing 100 mM NaCl at a strand concentration of 5.0 μM.



**Scheme 3** Photochemical end capping via ODN2<sup>(MCVU)</sup>.



**Fig. 3** Time-dependent phosphodiesterase-mediated degradation of the end-capped ODN. Lane 1: ODN2<sup>(MCVU)</sup>; lane 2: 366 nm irradiation of lane 1 for 3 h; lane 3: isolated ODN2<sup>(MCVU-A)</sup>; lane 4: phosphodiesterase treatment of lane 3 for 30 min; lane 5: phosphodiesterase treatment of lane 3 for 24 h; lane 6: ODN2<sup>(MCVU)</sup>; lane 7: phosphodiesterase treatment of lane 6 for 30 min. Bands were visualized by silver staining method.

HPLC purification is a cross-adduct of ODN1<sup>(MCVU)</sup> and ODN1'(A).<sup>9</sup> Enzymatic digestion of isolated ODN1<sup>(MCVU-A)</sup> showed the composition of dA, dG, dT and dC in a ratio of 1:4:1:4 together with dA-d<sup>MCVU</sup> photoadduct.<sup>10</sup> These results clearly indicate that ODN1<sup>(MCVU-A)</sup> was an end-capped ODN formed by crosslinking between an adenine of ODN1'(A) and <sup>MCVU</sup> of ODN1<sup>(MCVU)</sup> at the strand end. Unfortunately, the dA-d<sup>MCVU</sup> photoadduct derived from enzymatic digestion of ODN1<sup>(MCVU-A)</sup> was too labile to be isolated because of its thermal instability in water. However, its inability to be photoreversed by 254 nm irradiation suggests that the dA-d<sup>MCVU</sup> photoadduct was the [2+2] cycloadduct between the vinyl group and 1,6-double bonds of an adenine-like major photoadduct in the TpA sequence.<sup>11,12</sup>

To evaluate the stability of end-capped ODN, thermal denaturation experiments were examined (Table 1). From entries 1 and 2, it can be seen that end capping of ODN produced a significantly increased melting temperature ( $\Delta T_m = +46$  °C), indicating that this capped ODN traps the hairpin structure photochemically. It is also observed that end capping of ODN resulted in an increase in thermal stability by 32 °C as compared with the T4 loop hairpin ODN, reflecting the effect of the linker conformationally restricting the hairpin conformation. Thus, the photochemical end capping effectively stabilizes the hairpin

structure with a minimum unit constructed from the base analogue. We also investigated the resistance of the end-capped ODN to nucleolytic digestion by snake venom phosphodiesterase. After photoirradiation of self-complemental d<sup>(MCVU)</sup>UGCGCAATTGCGCA<sub>2</sub> ODN2<sup>(MCVU)</sup>, doubly end-capped ODN ODN2<sup>(MCVU-A)</sup> was isolated<sup>13</sup> and used in nucleolytic digestion for 30 min compared with quantitative degradation of starting ODN2<sup>(MCVU)</sup> (Figure 3, lane 4 and lane 7).<sup>14,15</sup> No degradation of ODN2<sup>(MCVU-A)</sup> was observed in phosphodiesterase treatment for 24 h (Figure 3, lane 5). These results show that the end-capped ODN2<sup>(MCVU-A)</sup> increases significantly its stability in the biological medium and its possibility as a decoy DNA for directly targeting transcription factors and for globally controlling the expression of genes.<sup>16</sup>

In conclusion, we have synthesized <sup>MCVU</sup>-containing ODN as a probe for trapping and stabilizing the hairpin structure and demonstrated the photochemical end capping of ODN via <sup>MCVU</sup>. This <sup>MCVU</sup>-mediated photochemical end capping may find application in the investigation of nucleic acid structure and function.

**Kenzo Fujimoto,<sup>a,b</sup> Yoshinaga Yoshimura,<sup>b</sup> Tadayoshi Ikemoto,<sup>a</sup> Akio Nakazawa,<sup>c</sup> Masayuki Hayashi<sup>c</sup> and Isao Saito<sup>c</sup>**

<sup>a</sup>The School of Materials Science, Japan Advanced Institute of Science and Technology, Ishikawa 923-1292, Japan. Fax: +81 761 51 1671; Tel: +81 761 51 1671; E-mail: kenzo@jaist.ac.jp

<sup>b</sup>PRESTO, Japan Science and Technology Agency, Ishikawa 923-1292, Japan.

<sup>c</sup>Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606-8501, Japan.

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- 5 <sup>MCVU</sup>:  $\lambda_{\max}$  (water) 299 nm,  $\epsilon$  12,500 ( $\epsilon$  at 366 nm, 85).
- 6 MALDI-TOF-MS: calcd. for ODN1<sup>(MCVU)</sup> [(M-H)]<sup>+</sup> 1873.30; found 1873.47.
- 7 The yield was calculated based on ODN1'(A).
- 8 Each of the reaction mixtures containing ODN1<sup>(MCVU)</sup> (20 μM, strand concn) and ODN1'(A) (20 μM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride in a Pyrex tube was irradiated with a 25 W transilluminator (366 nm,

- 5,700  $\mu\text{W}/\text{cm}^2$ ) at 0 °C for 30 min. After irradiation, the progress of photoreaction was monitored by HPLC on a Chemcobond 5C18 ODS column (4.6  $\times$  150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0, linear gradient over 40 min from 3% to 10% acetonitrile at a flow rate 1.0 mL/min).
- 9 MALDI-TOF-MS: calcd. for  $\text{ODN1}^{(\text{MCV})}\text{U-A}$  [(M-H)<sup>-</sup>] 3633.52; found 3633.87.
  - 10 MALDI-TOF-MS: calcd. for dA-d<sup>(MCV)</sup>U photoadduct [(M+H)<sup>+</sup>] 545.52; found 545.26.
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  - 13 The reaction mixture containing  $\text{ODN2}^{(\text{MCV})}\text{U}$  (20  $\mu\text{M}$ , strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride in a Pyrex tube was irradiated with a 25 W transilluminator (366 nm, 5,700  $\mu\text{W}/\text{cm}^2$ ) at 0 °C for 3 h. Then, end-capped  $\text{ODN2}^{(\text{MCV})}\text{U-A}$  was obtained from the isolated peak at 13.5 min from HPLC analysis. The progress of photoreaction was monitored by HPLC on a Chemcobond 5C18 ODS column (4.6  $\times$  150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0, linear gradient over 40 min from 3% to 12% acetonitrile at a flow rate 1.0 mL/min).
  - 14 To a solution (0.5 mL) containing HPLC purified  $\text{ODN2}^{(\text{MCV})}\text{U}$  (40  $\mu\text{M}$ , strand concn) or  $\text{ODN2}^{(\text{MCV})}\text{U-A}$  (40  $\mu\text{M}$ , strand concn), snake venom phosphodiesterase (0.2 mL, 0.3 units/mL) was added and incubated at 37 °C.
  - 15 PAGE analysis was carried out on 20% polyacrylamide gel and eletrophoresis at 280 V for 30 min.
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