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Description	

Photoinduced DNA end capping via N³-methyl-5-cyanovinyl-2'-deoxyuridine

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A modified oligodeoxynucleotide (ODN) containing N³-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.¹ 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.² 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.³ Recently, we have reported efficient and reversible template-directed photoligations with ODNs containing 3'-terminal cytosine using 5'-vinyl-2'-deoxyuridine (^VU) containing ODN at the 5'-terminal.⁴ A remarkable stacking between a vinyl residue of ^VU and 5'-pyrimidine within the same strand will be responsible for the efficient photoreaction in our template-directed DNA photoligation system via ^VU. We have now examined photochemical end capping, using N³-methyl-5-cyanovinyl-2'-deoxyuridine (^{MCV}U) instead of ^VU, in which the more photoreactive vinyl group was incorporated. The photoreactive cyanovinyl group in ^{MCV}U was designed to stack effectively with a base in the opposite strand by an N³-methyl group substitution that allows stabilization of the *syn* orientation of ^{MCV}U and release from the Watson–Crick base pair (Figure 1). Herein we report the photochemical DNA end capping via ^{MCV}U instead of ^VU to generate the stabilized hairpin analogue at its end.

^{MCV}U-containing ODN was synthesized according to the standard phosphoramidite chemistry on a DNA synthesizer. The phosphoramidite of ^{MCV}U was prepared in six steps from 5-iodo-2'-deoxyuridine as shown in Scheme 1.⁵ Incorporation of ^{MCV}U into ODN was confirmed by enzymatic digestion and MALDI-TOF-MS.⁶

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

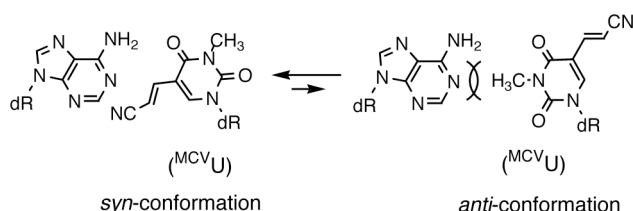
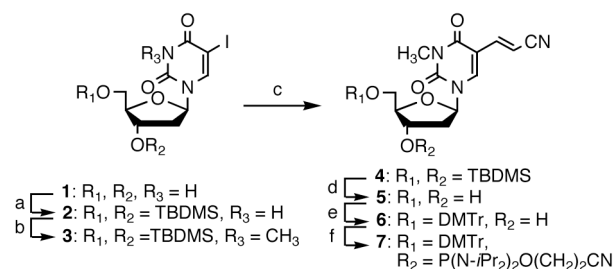
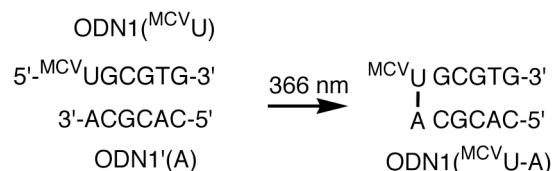


Fig. 1 Proposed two conformer about the base pair between adenine and ^{MCV}U at the terminal site.



Scheme 1 Reagents and conditions: (a) TBDMSCl, imidazole, pyridine, 3 h, 95%; (b) dimethylcarbonate, 18-crown-6, K₂CO₃, DMF, 3 h, 98%; (c) acrylonitrile, Pd(OAc)₂, PPh₃, 8 h, 70%; (d) TBAF, THF, 3 h, 85%; (e) DMTrCl, DMAP, pyridine, 75%; (f) P(N-*i*Pr)₂O(CH₂)₂CN, tetrazole, CH₃CN, 2 h, 98%.



Scheme 2 Photochemical end capping via ODN1(^{MCV}U).

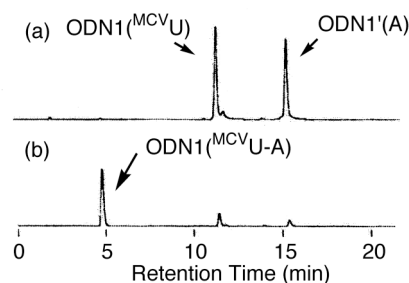


Fig. 2 HPLC profile of photoreaction of ODN1(^{MCV}U) and ODN1'(A). (a) before photoirradiation; (b) irradiation at 366 nm for 30 min, 87% yield.

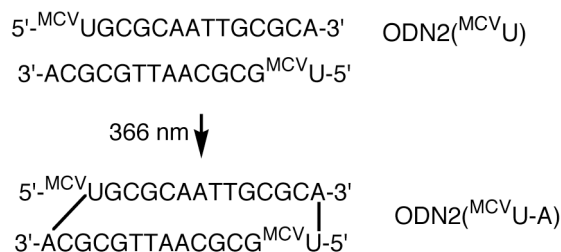
† Electronic Supplementary Information (ESI) available: Experimental details. See <http://www.rsc.org/suppdata/xx/b0/b000000x/>

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Table 1 Melting temperature of end-capped ODN1^(MCVU-A) in comparison with duplex ODN1^(MCVU)/ODN1^{'(A)} and T4 loop hairpin ODN

Entry	Oligomer	T _m / °C ^a
1	ODN1 ^(MCVU) /ODN1 ^{'(A)}	28.1
2	ODN1 ^(MCVU-A)	74.5
3	5'-d(CACGCATTTTTCGCGT)-3'	42.6

^a 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^(MCVU).

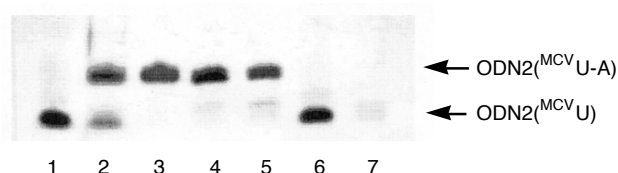


Fig. 3 Time-dependent phosphodiesterase-mediated degradation of the end-capped ODN. Lane 1: ODN2^(MCVU); lane 2: 366 nm irradiation of lane 1 for 3 h; lane 3: isolated ODN2^(MCVU-A); lane 4: phosphodiesterase treatment of lane 3 for 30 min; lane 5: phosphodiesterase treatment of lane 3 for 24 h; lane 6: ODN2^(MCVU); 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^(MCVU) and ODN1^{'(A)}.⁹ Enzymatic digestion of isolated ODN1^(MCVU-A) showed the composition of dA, dG, dT and dC in a ratio of 1.4:1.4 together with dA-d^{MCVU} photoadduct.¹⁰ These results clearly indicate that ODN1^(MCVU-A) was an end-capped ODN formed by crosslinking between an adenine of ODN1^{'(A)} and ^{MCVU} of ODN1^(MCVU) at the strand end. Unfortunately, the dA-d^{MCVU} photoadduct derived from enzymatic digestion of ODN1^(MCVU-A) 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^{MCVU} 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.^{11,12}

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^(MCVU)UGCGCAATTGCGCA₂ ODN2^(MCVU), doubly end-capped ODN ODN2^(MCVU-A) was isolated¹³ and used in nucleolytic digestion for 30 min compared with quantitative degradation of starting ODN2^(MCVU) (Figure 3, lane 4 and lane 7).^{14,15} No degradation of ODN2^(MCVU-A) was observed in phosphodiesterase treatment for 24 h (Figure 3, lane 5). These results show that the end-capped ODN2^(MCVU-A) 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.¹⁶

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

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- 5 ^{MCVU}: λ_{max} (water) 299 nm, ε 12,500 (ε at 366 nm, 85).
- 6 MALDI-TOF-MS: calcd. for ODN1^(MCVU) [(M-H)]⁻ 1873.30; found 1873.47.
- 7 The yield was calculated based on ODN1^{'(A)}.
- 8 Each of the reaction mixtures containing ODN1^(MCVU) (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 × 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 $\text{ODN1}^{(\text{M}^{\text{CV}}\text{U}-\text{A})} [(\text{M}-\text{H})^-]$ 3633.52; found 3633.87.
 - 10 MALDI-TOF-MS: calcd. for $\text{dA-d}^{(\text{M}^{\text{CV}}\text{U})}$ photoadduct $[(\text{M}+\text{H})^+]$ 545.52; found 545.26.
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 - 13 The reaction mixture containing $\text{ODN2}^{(\text{M}^{\text{CV}}\text{U})}$ (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 3 h. Then, end-capped $\text{ODN2}^{(\text{M}^{\text{CV}}\text{U}-\text{A})}$ 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 × 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 $\text{ODN2}^{(\text{M}^{\text{CV}}\text{U})}$ (40 μM , strand concn) or $\text{ODN2}^{(\text{M}^{\text{CV}}\text{U}-\text{A})}$ (40 μM , 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 electrophoresis at 280 V for 30 min.
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