Photoinduced DNA end capping *via* N^3-methyl-5-cyanovinyl-2′-deoxyuridine†

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A modified oligodeoxynucleotide (ODN) containing N^3-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 in 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 (^{V}U) containing ODN at the 5′-terminal.[4] A remarkable stacking between a vinyl residue of ^{V}U and 5′-pyrimidine within the same strand will be responsible for the efficient photoreaction in our template-directed DNA photoligation system *via* ^{V}U. We have now examined photochemical end capping, using N^3-methyl-5-cyanovinyl-2′-deoxyuridine (^{MCV}U) instead of ^{V}U, 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 N^3-methyl group substitution that allows stabilization of the *syn* orientation of ^{MCV}U and release from the Watson–Crick base pair (Fig. 1). Herein we report the photochemical DNA end capping *via* ^{MCV}U instead of ^{V}U 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 (Fig. 2).[7,8] MALDI–TOF–MS indicated that ODN1(^{MCV}U-A) obtained by HPLC purification is a cross-adduct of ODN1(^{MCV}U) and ODN1′(A).[9] Enzymatic digestion of isolated ODN1(^{MCV}U-A) showed the composition of dA, dG, dT and dC in a ratio of 1:4:1:4 together with dA-d^{MCV}U photoadduct.[10] These results clearly indicate that ODN1(^{MCV}U-A) was an end-capped ODN formed by cross-linking between an adenine of ODN1′(A) and ^{MCV}U of ODN1(^{MCV}U) at the strand end. Unfortunately, the dA-d^{MCV}U photoadduct derived from enzymatic digestion of ODN1(^{MCV}U-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^{MCV}U 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 (ΔT_m = +46 °C), indicating that this capped ODN traps the hairpin structure photochemically. It is also observed that end capping of ODN

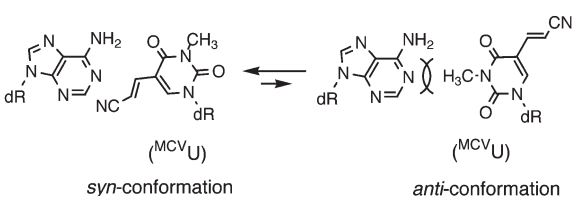
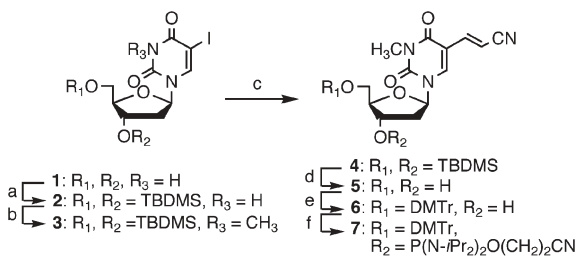


Fig. 1 Proposed two conformers about the base pair between adenine and ^{MCV}U at the terminal site.



Scheme 1 Reagents and conditions: (a) TBDMSCl, imidazole, pyridine, 3 h, 95%; (b) dimethylcarbonate, 18-crown-6, K_2CO_3, DMF, 3 h, 98%; (c) acrylonitrile, $Pd(OAc)_2$, PPh_3, 8 h, 70%; (d) TBAF, THF, 3 h, 85%; (e) DMTrCl, DMAP, pyridine, 75%; (f) $P(N\text{-}i\text{Pr}_2)_2O(CH_2)_2CN$, tetrazole, CH_3CN, 2 h, 98%.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b5/b504162g/

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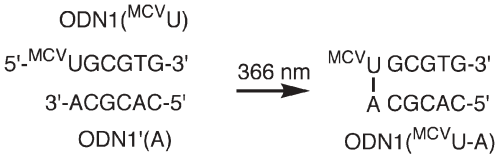


Scheme 2 Photochemical end capping *via* ODN1(^{MCV}U).

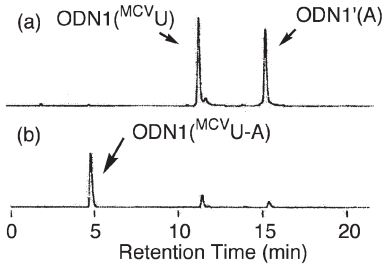


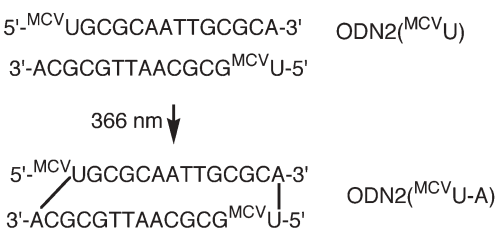
Fig. 2 HPLC profile of photoreaction of ODN1(^{MCV}U) and ODN1′(A). (a) before photoirradiation; (b) irradiation at 366 nm for 30 min, 87% yield.

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

Entry	Oligomer	T_m/°C[a]
1	ODN1(^{MCV}U)/ODN1′(A)	28.1
2	ODN1(^{MCV}U-A)	74.5
3	5′-d(CACGCATTTTTGCGTG)-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.

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(^{MCV}UGCGCAATTGCGCA)$_2$ ODN2(^{MCV}U) as shown in Scheme 3, doubly end-capped ODN ODN2(^{MCV}U-A) was isolated[13] and used in nucleolytic digestion for 30 min compared with quantitative degradation of starting ODN2(^{MCV}U) (Fig. 3, lane 4 and lane 7).[14,15] No degradation of ODN2(^{MCV}U-A) was observed in phosphodiesterase treatment for 24 h (Fig. 3, lane 5). These results show that the end-capped ODN2(^{MCV}U-A) increases significantly its stability in the biological medium and its possibility



Scheme 3 Photochemical end capping *via* ODN2(^{MCV}U).

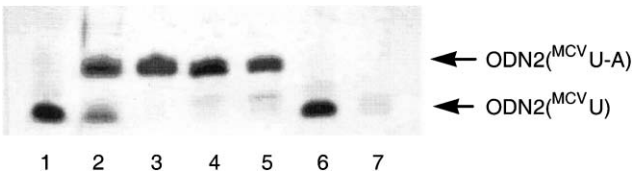


Fig. 3 Time-dependent phosphodiesterase-mediated degradation of the end-capped ODN. Lane 1: ODN2(^{MCV}U); lane 2: 366 nm irradiation of lane 1 for 3 h; lane 3: isolated ODN2(^{MCV}U-A); lane 4: phosphodiesterase treatment of lane 3 for 30 min; lane 5: phophodiesterase treatment of lane 3 for 24 h; lane 6: ODN2(^{MCV}U); lane 7: phosphodiesterase treatment of lane 6 for 30 min. Bands were visualized by silver staining method.

as a decoy DNA for directly targeting transcription factors and for globally controlling the expression of genes.[16]

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

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5 ^{MCV}U: λ_{max} (water) 299 nm, ε 12,500 (ε at 366 nm, 85).
6 MALDI–TOF–MS: calcd. for ODN1(^{MCV}U) [(M–H)$^-$] 1873.30; found 1873.47.
7 The yield was calculated based on ODN1′(A).
8 Each of the reaction mixtures containing ODN1(^{MCV}U) (20 μM, strand concentration) and ODN1′(A) (20 μM, strand concentration) 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 μW 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^{-1}).

9 MALDI–TOF–MS: calcd. for ODN1(^{MCV}U-A) [$(M - H)^-$] 3633.52; found 3633.87.

10 MALDI–TOF–MS: calcd. for dA-d^{MCV}U photoadduct [$(M + H)^+$] 545.52; found 545.26.

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13 The reaction mixture containing ODN2(^{MCV}U) (20 μM, strand concentration) 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 μW/cm^2) at 0 °C for 3 h. Then, end-capped ODN2(^{MCV}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 × 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^{-1}).

14 To a solution (0.5 mL) containing HPLC purified ODN2(^{MCV}U) (40 μM, strand concn) or ODN2(^{MCV}U-A) (40 μM, strand concentration), snake venom phosphodiesterase (0.2 mL, 0.3 units mL^{-1}) 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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