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Template-Directed DNA Photoligation via α-5-Cyanovinyldeoxyuridine

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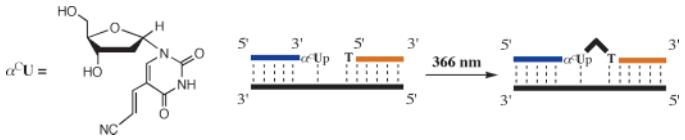
ABSTRACT



We describe an efficient template-directed photoligation of oligodeoxynucleotides (ODNs) using α-5-cyanovinyldeoxyuridine (α^{C}U). An efficient photoligation was produced by photoirradiation of an ODN containing α^{C}U at the 3′ end with an ODN containing thymine at the 5′ end in the presence of a template ODN. This photoligation method is a new and efficient way to synthesize branched ODNs.

Nonenzymatic template-directed DNA has recently attracted considerable interest because of its potential application in genetic regulation and use as a tool for molecular biology research, as well as for its role in prebiotic nucleic acid synthesis.[1] Many template-directed chemical ligation approaches have been reported that join the ends of two DNA strands by a native phosphodiester bond or by nonnatural linkages.[2] Although Fujimoto et al. reported the template-directed reversible photoligation of DNA oligomer via 5-vinyldeoxyuridine derivative at the 5′ end, they did not observe photoligation via 5-vinyldeoxyuridine at the 3′ end (Figure 1).[3] We used molecular modeling to study the duplexes of oligodeoxynucleotides (ODNs) containing β-5-cyanovinyldeoxyuridine (β^{C}U) (Figure 2) as usual at the 3′ end. Our molecular modeling study suggested that there is less stacking between the cyanovinyl group and the C5−C6 double bond of the 5′-terminal dT (Figure 3a). We examined an α-anomer as an alternative probe. A molecular modeling study suggested base stacking between the cyanovinyl group in α-5-cyanovinyldeoxyuridine (α^{C}U) (Figure 2) and the C5−C6 double bond. Our molecular modeling study predicted that the cyanovinyl group of α^{C}U is effectively stacked

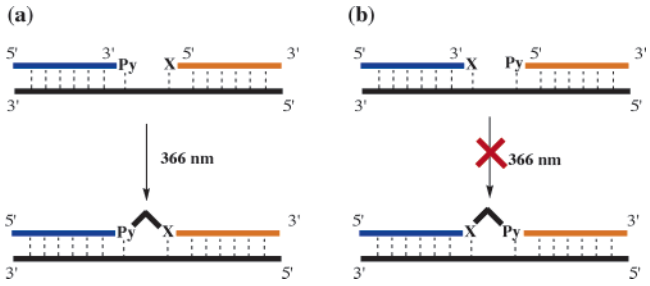


Figure 1. Contrasting photoreactions of template-directed DNA photoligation (a) for an ODN containing β^{C}U at the 5′ end and (b) for an ODN containing β^{C}U at the 3′ end (X, modified base).

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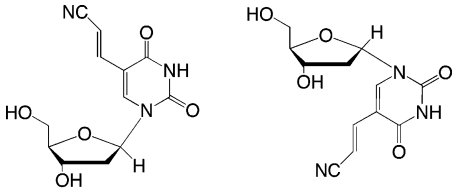


β ^{C}U (1) α ^{C}U (2)

Figure 2. Structures of β-5-cyanovinyldeoxyuridine (β^{C}U) and α-5-cyanovinyldeoxyuridine (α^{C}U).

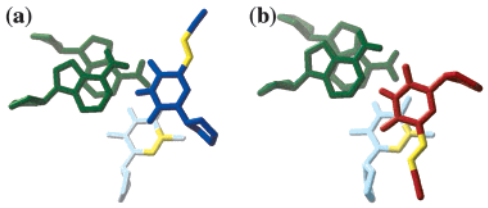


Figure 3. Molecular modeling of the stacking geometry in B-form DNA. The model was optimized by AMBER* force field in water using MacroModel version 8.1. Blue, red, green, and water blue molecules are β^{C}U, α^{C}U, dA, and dT, respectively. The vinyl group and C5−C6 double bond of the 5′-terminal dT are shown in yellow: (a) 3′-terminal β^{C}U and 5′-terminal dT, (b) 3′-terminal α^{C}U and 5′-terminal dT.

on the C5−C6 double bond of the 5′-terminal dT (Figure 3b). We here report our development of a novel, nonenzymatic, template-directed photoligation of ODN via the α^{C}U contained at the 3′ end. We used melting temperature (T_m) experiments to study the hybridization affinity of the ODN for complementary and mismatched ODNs in detail.

Chlorosugar **3** was coupled with 5-iodouracil in the presence of CuI. The two resulting isomers were deprotected with concentrated aqueous ammonia to yield 5-iodouridine **5**. Compound **5** was transferred to **2** by the Heck reaction. α,β-Anomers were isolated by silica gel column chromatography. **2** was dimethoxytritylated and converted to the corresponding cyanoethyl phosphoramidite using a conventional method (Scheme 1). The α^{C}U-containing ODN, 5′-d(TGTGC α^{C}Up)-3′ (ODN **1**), was synthesized on an ABI 3400 DNA synthesizer using 3′-phosphate CPG.[4] Incorporation of α^{C}U into the ODN was confirmed by enzymatic digestion and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS).[5] The ODNs used in this study are summarized in Table 1.

We next determined the feasibility of photoligation of the α^{C}U-containing ODN (Scheme 2). ODN **1** and 5′-d(TGCGTG)-3′ (ODN **2**) were irradiated at 366 nm for 30 min

Scheme 1

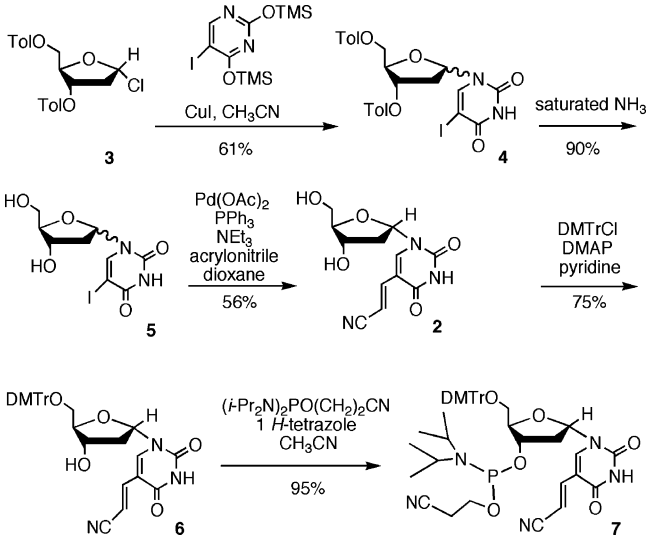
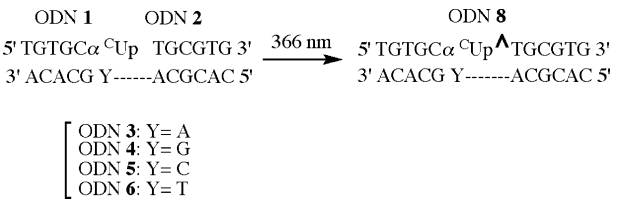


Table 1. ODNs Used in This Study

	sequences
ODN **1**	5′-d(TGTGCα^{C}Up)-3′
ODN **2**	5′-d(TGCGTG)-3′
ODN **3**	5′-d(CACGCAAGCACA)-3′
ODN **4**	5′-d(CACGCAGGCACA)-3′
ODN **5**	5′-d(CACGCACGCACA)-3′
ODN **6**	5′-d(CACGCATGCACA)-3′
ODN **7**	5′-d(AAAAAATGCGTG)-3′

Scheme 2



at 0 °C in the presence of template ODN **3**.[6] Capillary gel electrophoresis analysis of a mixture of ODN **1** and ODN **2** photoirradiated with template ODN **3** indicated a clean and efficient formation of ligated ODN **8** and the concomitant disappearance of ODN **1** and ODN **2** (Figure 4). MALDI-TOF MS indicated that ODN **8** was a ligated product of ODN **1** and ODN **2**.[7] Enzymatic digestion of isolated ODN **8** showed the formation of dC, dG, and dT in a ratio of 2:5:3, together with a new product. Spectroscopic data, including MALDI-TOF MS, indicated that this new product was the

(4) Walton, T. A.; Lyttle, M. H.; Dick, D. J.; Cook, R. M. *Bioconjugate Chem.* **2002**, *13*, 1155−1158.

(5) MALDI-TOF MS: calculated for ODN **1** ($C_{61}H_{76}N_{20}O_{40}P_6$) [M − H]$^-$ 1913.2876, found 1913.5459.

(6) When an ODN with the phosphate group of ODN **1** at the 3′ end removed and ODN **2** were irradiated at 366 nm for 1 h at 0 °C in the presence of template ODN **3**, the product was obtained in 10% yield.

(7) MALDI-TOF MS: calculated for ODN **8** ($C_{120}H_{151}N_{42}O_{76}P_{11}$) [M − H]$^-$ 3735.6277, found 3735.8486.

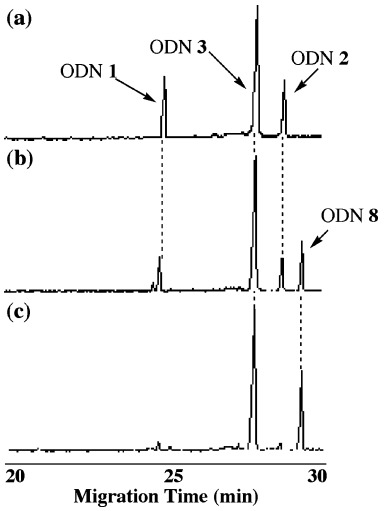


Figure 4. Capillary gel electrophoresis of photoreaction of ODN **1** (30 μM) and ODN **2** (30 μM) in the presence of template ODN **3** (33 μM) (a) before photoirradiation, (b) after irradiation at 366 nm for 5 min at 0 °C (42% yield), and (c) after irradiation at 366 nm for 30 min at 0 °C (95% yield).

Table 2. Template-Dependent Conversion of ODN **1** and ODN **2** by Photoirradiation

	conversion (%)	
	ODN **1**	ODN **2**
ODN **3** (Y = A)	95	95
ODN **4** (Y = G)	11	15
ODN **5** (Y = C)	9	12
ODN **6** (Y = T)	14	13

d(α^{C}U−T) photoadduct.[8] The molecular weight of ODN **8** was equal to the sum of the molecular weight of ODN **1** and ODN **2**. Judging from the molecular modeling (Figure 3b), it is strongly suggested that the photoligation reaction proceeded via [2 + 2] cycloaddition between the double bond of α^{C}U side chain and the C5−C6 double bond of thymine, giving rise to the formation of a cyclobutane structure as observed for ^{CV}U.[3d]

We carried out a test of selectivity of α^{C}U against the opposite-site base (Scheme 2). ODN **1** and ODN **2** were irradiated at 366 nm for 30 min at 0 °C in the presence of ODN **4**, ODN **5**, and ODN **6**, respectively. Results showed that photoligation proceeded with low conversion. Conversion after 30 min was estimated at 9−15% in the singly mismatched case (Table 2). These results suggested that this photoligation depends on sequence specificity and that α^{C}U has the ability to recognize the opposite-site dA.

We performed UV melting studies using ODN **8** and the 12-mer template ODN **3**. The thermal melting behavior exhibited a monophasic helix-coil transition characteristic of a double helix, which resulted from the cooperative dissociation of ODN **8** from ODN **3**. The T_m value (40.5 °C) of ODN **8** and ODN **3** was higher than that of two 6-mers ODN **1** and ODN **2** and template ODN **3** (T_m = 18.2 °C) (Figure 5). These results indicated that ligated ODN **8** can form a stable duplex with the complementary ODN **3**.

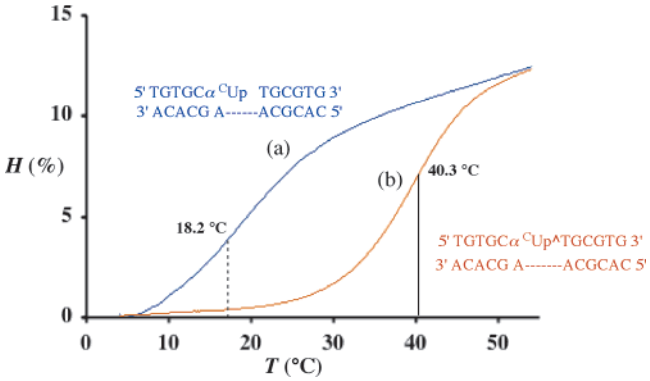


Figure 5. UV melting curves (a) before photoirradiation (T_m = 18.2 °C) and (b) after irradiation at 366 nm for 30 min (T_m = 40.5 °C) in 5.0 μM each strand, 100 mM NaCl, and 50 mM Na cacodylate buffer (pH 7.0).

We measured the thermal stability of the double helix formed between modified ODNs and complementary ODNs. We applied the same method to measure the T_m values of the duplexes of ODN **1**·ODN **2** and mismatched complementary strands ODN **4**, ODN **5**, and ODN **6**. As shown in Table 3, the T_m value of the ODN **1**·ODN **2** and complementary ODN **3** was the greatest of all combinations, and when another ODN was used, a decrease in the T_m value of 1.8−3.6 °C was observed. As a result, we considered that the photoligation reaction is promoted by thermal stability and that the cyanovinyl group and C5−C6 double bond are fixed by the A−α^{C}U base pair.[9]

Table 3. T_m Values for Duplex to Single-Strand Conversions of ODN **1** and ODN **2** in the Presence of ODNs **3**−**6**

	T_m (°C)[a]	DT_m (°C)
ODN **3** (Y = A)	18.2	
ODN **4** (Y = G)	14.6	−3.6
ODN **5** (Y = C)	16.4	−1.8
ODN **6** (Y = T)	15.9	−2.3

[a] Conditions: 100 mM NaCl, 50 mM Na cacodylate buffer (pH 7.0), 5.0 μM each strand.

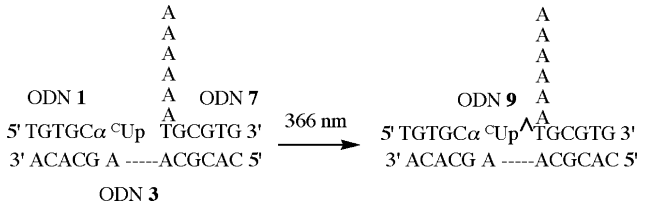
There is a great potential for using DNA as a generic material instead of a genetic material. Branched DNA molecules have various uses in signal amplification technol-

(8) MALDI-TOF MS: calculated for d(α^{C}U−T) ($C_{22}H_{27}N_5O_{10}$) [M + H]$^+$ 522.1836, found 522.1866.

(9) We examined molecular modeling of ODNs **1**−**3**. As a result, We expected a weak hydrogen bond between the lone pair of 1N in dA and 3NH of α^{C}U. When ODNs **4**−**6** were used as the template ODN, the interaction was considered to be not effective on the basis of the result of molecular modeling.

ogy,[10] nanotechnology applications such as DNA computing,[11] DNA nanostructures using self-assembled branched units,[12] DNA sensors,[13] and nanoelectronic devices.[14] The past 10 years have seen remarkable success in the construction of DNA nanoarchitectures. For example, one- and two-dimensional DNA lattices[15] and dendrimerlike DNA[16] have been constructed from a rich set of branched DNA. Various autonomous DNA walker devices based on DNA cleavage and the ligation of branched DNA were explored experimentally. Various template-directed enzymes and the introduction of a branch structure via $\alpha^{C}U$ have also been explored theoretically (Scheme 3). Figure 6 shows the results

Scheme 3



of capillary gel electrophoresis of the photoirradiated mixture of 5′-d(AAAAAATGCGTG)-3′ (ODN **7**) and ODN **1** in the presence of template ODN **3**, with the clean and efficient formation of the expected ligated 18-mer ODN **9** and the disappearance of ODN **7** and ODN **1**. MALDI-TOF MS indicated that ODN **9**, purified by HPLC, was the ligated product of ODN **7** and ODN **1**.[17] Enzymatic digestion of isolated ODN **9** showed the formation of dA, dG, dT, and dC in a ratio of 6:5:3:2, together with a d(α^{C}U−T) photoadduct. We observed no formation of ODN **9** after photoirradiation of ODN **7** and ODN **1** in the absence of template ODN **3**. These results clearly indicated that ODN **9** was a branched ODN formed by template-directed cross-linking between the thymine of ODN **7** and the α^{C}U of ODN **1**.

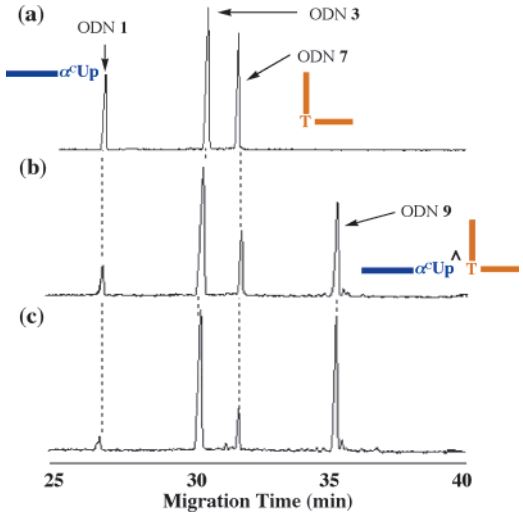


Figure 6. Capillary gel electrophoresis of photoreaction of ODN **1** (30 μM) and ODN **7** (30 μM) in the presence of template ODN **3** (33 μM) (a) before photoirradiation, (b) after irradiation at 366 nm for 2 h at 0 °C (30% yield), and (c) after irradiation at 366 nm for 6 h at 0 °C (82% yield).

In conclusion, we have demonstrated that an ODN containing α^{C}U at the 3′ end can be employed in the photoligation of DNA by irradiation at 366 nm in the presence of a template DNA, with no side reaction. This method has the same photosensitivity as the usual method using β^{C}U. Using this novel photoligation method, we have demonstrated a convenient and versatile method of generating branched oligonucleotides, which should be particularly useful in DNA nanotechnology.

Supporting Information Available: Experimental procedures for the synthesis, purification, and analysis of ODNs **2**−**7**, oligonucleotide digestion, and melting studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) MALDI-TOF MS: calculated for ODN **9** ($C_{180}H_{223}N_{72}O_{106}P_{17}$) [M + H]$^{+}$ 5618.7373, found 5618.7555.