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Template directed photochemical synthesis of branched oligodeoxynucleotides via 5-carboxyvinyldeoxyuridine[†]

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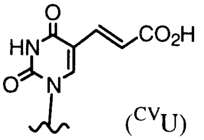
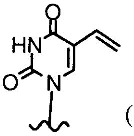
Abstract

An efficient template directed DNA photoligation using 5-carboxyvinyldeoxyuridine (^{CV}U) is described. By the use of this photoligation, a new efficient method for the synthesis of branched oligonucleotides is reported. © 2000 Elsevier Science Ltd. All rights reserved.

Branched oligodeoxynucleotides (ODNs) are useful molecules for the quantification of DNA and mRNAs as a signal amplification technology[1] and for the synthesis of polyfunctional starburst DNA oligomers.[1] Most branched ODNs were chemically synthesized by standard phophoroamidite chemistry on a solid support using phosphoroamidites containing a branched structural unit.[2,3] There are also a few reports on the photochemical introduction of a branched segment by crosslinking to the complementary strand using photosensitizer-containing ODN.[4] Recently, we reported an efficient and reversible template-directed photoligation using 5-vinyldeoxyuridine (^{V}U)-containing ODN at 5′-terminal.[5] However, in most cases, a long photo-irradiation time at 366 nm was required to complete photoligation. We now report the improvement of DNA photoligation by the use of 5-caroboxyvinyldeoxyuridine (^{CV}U) instead of ^{V}U and demonstrate a convenient and versatile method for the synthesis of branched ODNs.



(^{CV}U)



(^{V}U)

It was considered that the low photoreactivity of previous ^{V}U-containing ODNs at 366 nm irradiation was due to the very small extinction coefficient of ^{V}U at 366 nm. We therefore examined the photoligation of ^{CV}U with an extinction coefficient ca. eight times larger for the

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[†] Dedicated to Professor Harry H. Wasserman on the occasion of his 80th birthday.

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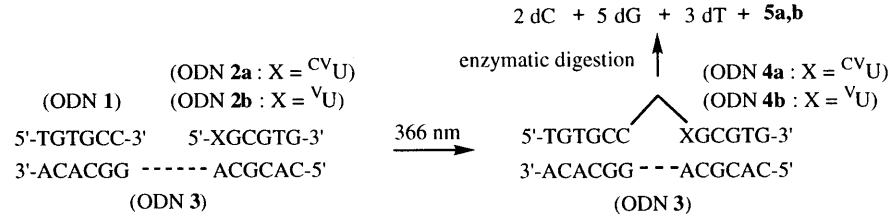
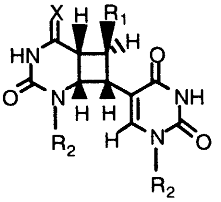
improvement of the photoreactivity.[6] ^{CV}U-containing ODNs were synthesized according to the standard phosphoramidite chemistry on a DNA synthesizer using phosphoroamidite of methyl ester of ^{CV}U[6] coupled with the post-modification procedure by treatment with conc. ammonia after ODNs synthesis.[7] The incorporation of ^{CV}U into oligonucleotides was confirmed by enzymatic digestion and ESI-TOF MS analysis.[8]

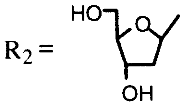
When ^{32}P-5′-end-labeled d(TGTGCC) (ODN **1**) and d(^{CV}UGCGTG) (ODN **2a**) were irradiated at 366 nm for 1 h in the presence of template ODN **3** (Scheme 1), the expected ligated 12-mer ODN **4a** was produced in 93% yield as determined by densitometric assay of PAGE (Fig. 1, lane 4).[9] From the comparison with the ^{V}U-mediated photoligation (Fig. 1, lane 2) where the ligated 12-mer ODN **4b** was produced in 31% yield, it was apparent that ^{CV}U-containing ODNs are far more useful for the photoligation with ODNs containing cytosine at 3′-terminal. ESI-TOF MS indicated that the isolated ODN **4a** obtained by HPLC purification is a ligated product of ODN **1** and ODN **2a**.[10] Enzymatic digestion of isolated ODN **4a** showed the formation of dG, dT and dC in a ratio of 5:3:2 together with dU–^{CV}U photoadduct **5a** that was a deaminated product of dC–^{CV}U adduct **5c** during enzymatic digestion process.[11] The structure of **5a** was assigned as a *cis–syn* [2+2] adduct on the basis of spectroscopic data including ^{1}H–^{1}H COSY and NOESY, as reported previously for **5b**.[5a,12]



Scheme 1.



5a : X = O, R$_1$ = CO$_2$H
5b : X = O, R$_1$ = H
5c : X = NH, R$_1$ = CO$_2$H



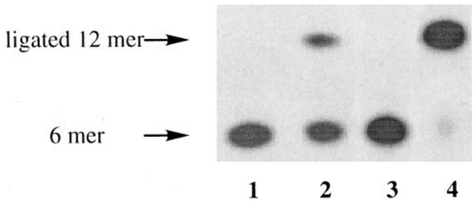
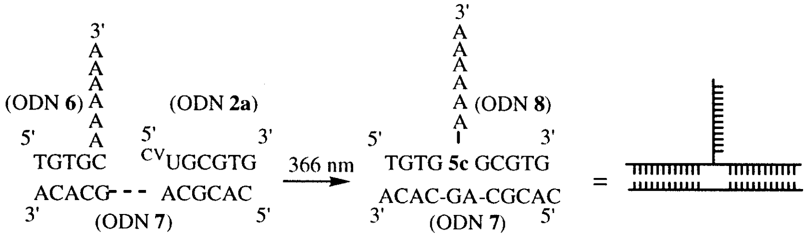


Figure 1. Autoradiogram of a denaturing polyacrylamide gel electrophoresis of photoreaction of ODN **2a**,**b** and ^{32}P-5′-end-labeled ODN **1** in the presence of template ODN **3**. Lane 1, ODN **1**+ODN **2b**/ODN **3**, without photoirradiation; lane 2, irradiation of lane 1 at 366 nm, 1 h (31%); lane 3, ODN **1**+ODN **2a**/ODN **3**, without photoirradiation; lane 4, irradiation of lane 3 at 366 nm, 1 h (93%)

The template-directed introduction of a branch structure via ^{CV}U was next examined (Scheme 2). Fig. 2(b) shows an HPLC profile of the photoirradiated mixture of d(TGTGCAAAAAA)

(ODN **6**) and d(^{CV}UGCGTG) (ODN **2a**) in the presence of template ODN **7**, showing a clean and efficient formation of the expected ligated 17-mer ODN **8** with a complete disappearance of ODN **6** and ODN **2a**.[13] MALDI-TOF MS indicated that ODN **8** obtained by HPLC purification is a ligated product of ODN **6** and ODN **2a**.[14] Enzymatic digestion of isolated ODN **8** showed the formation of dA, dG, dT and dC in a ratio of 6:5:3:1 together with dU–^{CV}U photoadduct **5a**. No formation of ODN **8** was observed in the photoirradiation of ODN **6** and ODN **2a** in the absence of template ODN **7**. These results clearly indicated that ODN **8** is a branched ODN formed by template directed crosslinking between cytosine of ODN **6** and 5-carboxyvinyluracil (^{CV}U) of ODN **2a**.



Scheme 2.

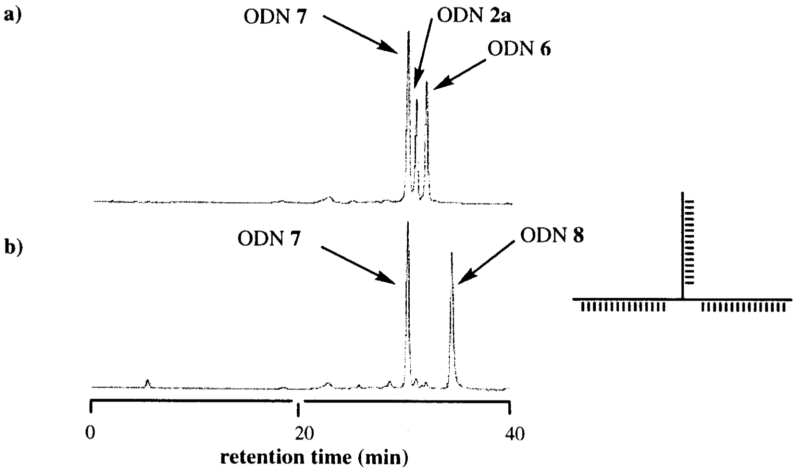


Figure 2. HPLC analysis of 366 nm irradiated ODN **2a** and ODN **6** in the presence of template ODN **7**. (a) Before photoirradiation, (b) irradiation at 366 nm for 1 h, 97% yield

In conclusion, we disclose a very effective photoligation using ^{CV}U. By using this novel photoligation method, we demonstrate a convergent and versatile synthesis of branched ODNs which would be particularly useful in DNA nanotechnology.

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6. ^{V}U: λ_{max} (water) 288 nm, ε 7,300 (ε at 366 nm, 10). ^{CV}U: λ_{max} (water) 297 nm, ε 12,500 (ε at 366 nm, 76).
7. Deprotection was conducted by heating with conc. aqueous ammonia for 6 h at 65°C. Aqueous ammonia was then removed by evaporation, and the ODN **2a** was isolated as a major product by HPLC from the mixture contained amide analogue. Deprotection with 0.4 M NaOH in 80% MeOH for 17 h at room temperature was also available as indicated by Glen Research for the synthesis of ^{CV}U-containing ODN. Phosphoroamidite of ^{CV}U analogue (Carboxy–dT) was available from Glen Research.
8. ESI-TOF MS: calcd for ODN **2a** ($C_{61}H_{74}N_{22}O_{38}P_5$) (M–H$^-$) 1877.322; found 1877.364.
9. The reaction mixture (total volume 10 μL) containing ODN **2a** or **2b** (each 7 μM, strand conc.) and ODN **1** (ca. 2×10^4 cpm, 0.1–0.3 μM) in the presence of template ODN **3** (9 μM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) was irradiated with transilluminator (366 nm) at 0°C for 1 h and then subjected to 15% polyacrylamide–7 M urea gel electrophoresis.
10. ESI-TOF MS: calcd for ODN **4a** ($C_{119}H_{149}N_{42}O_{74}P_{10}$) (M–H$^-$) 3659.656; found 3659.600.
11. Photoligated ODN **4b** was shown to be stable at pH 5.0–9.0 but deaminated by adding alkaline phosphatase to produce dU–^{V}U adduct containing ODN **5b**,[5a] suggesting that **5a** was produced similarly from deaminaion of dC–^{CV}U adduct **5c** during enzymatic digestion.
12. Molecular modeling studies of the duplex consisting of ODN **1**, ODN **2a** and template ODN **3** suggested that the C–C double bond of 5′-terminal ^{CV}U of ODN **2a** in its *s-trans* conformation is stacked with the 5,6-double bond of 3′-terminal dC of ODN **1** to be able to produce a *cis–syn* [2+2] adduct.
13. Each of the reaction mixtures containing ODN **2a** (20 μM, strand conc.) and ODN **6** (20 μM, strand conc.) in the presence of ODN **7** (22 μM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a Pyrex tube was irradiated with transilluminator (366 nm) at 0°C for 1 h. After irradiation, the reaction mixture was subjected to HPLC analysis. HPLC conditions; Chemcobond 5C$_{18}$ ODS column; 0.05 M ammonium formate containing 3–10% acetonitrile, linear gradient, 40 min; flow rate of 1.0 mL/min.
14. MALDI-TOF MS: calcd for ODN **8** ($C_{170}H_{209}N_{69}O_{98}P_{15}$) (M–H$^-$) 5248.95; found 5249.12.