

Interstrand photocrosslinking of DNA via *p*-carbamoylvinyl phenol nucleoside

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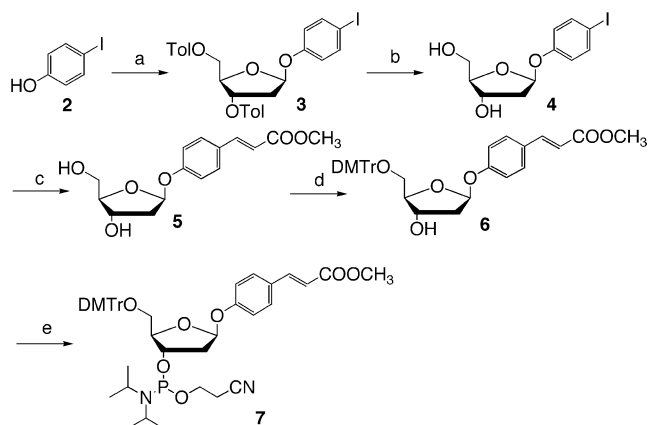
Abstract—We report a novel interstrand photocrosslinking of oligodeoxynucleotides (ODNs). In this system, a modified ODN containing *p*-carbamoylvinyl phenol nucleoside reacts by photoirradiation at 366 nm with adenine residue of a complementary template ODN to yield a crosslinked ODN in 97% yield.

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The linkage of DNA binding agents to ODNs has become a popular strategy to enhance the efficiency of antisense and triplex inhibition of cellular processes.¹ Psoralens have been used for many years as interstrand photocrosslinker of nucleic acids.² Psoralens form a reversible intercalative complex with DNA and can undergo subsequent photoactivation with near-UV light and then crosslink through a [2 + 2] cycloaddition with thymine residues. However, interstrand photocrosslink occurs between thymine residues at the duplex site 5'-TA-3' preferentially.³ To overcome such a sequence dependence, the study of photocrosslinker has been developed. The photochemical [2 + 2] cycloaddition of cinnamic acid and its methyl esters with alkenes is one of the most extensively investigated and synthetically useful of photochemical reactions.⁴ These photoreactions of cinnamic acid are expected to use as photocrosslinker for the study of interstrand photocrosslink of nucleic acids. Template directed reversible DNA photoligation via 5-vinyldeoxyuridine has already reported as a tool for DNA engineering and nanotechnology.⁵

Here we report the development of a novel interstrand photocrosslink via *p*-carbamoylvinyl phenol nucleoside (*p*-CV_P) in duplex DNA.⁶ We also demonstrate that the modified ODN containing *p*-CV_P was photocrosslinked with adjacent adenine residue by UV irradiation.

The synthesis of the phosphoramidite of methyl ester of *p*-CV_P is outlined in Scheme 1. Compound **3** was synthesized from 4-iodophenol and Hoffer's α -chlorosugar. Deprotection of **3** with sodium methoxide afforded **4**. Compound **4** was coupled with methylacrylate to afford **5**.⁷ Compound **5** was dimethoxytritylated, and converted into the nucleoside phosphoramidite **7**. The assignments of β -stereochemistry at C1' for **5** was based on COSY and NOESY spectra, which showed the cross-peak between H1' and H4'. The modified ODN



Scheme 1. Reagents and conditions: (a) NaH, chlorosugar, THF, room temperature, 19 h, 62%; (b) NaOCH₃, THF, room temperature, 15 h, 49%; (c) methyl acrylate, Pd(OAc)₂, PPh₃, Et₃N, dioxane, 115 °C, 4 h, 41%; (d) 4,4'-dimethoxytrityl chloride, DMAP, pyridine, room temperature, 16 h, 47%; (e) (iPr₂N)₂PO(CH₂)₂CN, 1*H*-tetrazole, acetonitrile, room temperature, 1 h, quant.

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containing p -CVP was prepared according to the standard phosphoramidite chemistry on a DNA synthesizer using phosphoramidite **7**. After HPLC purification, 5'-d(p -CVPGCGTG)-3' (ODN **1**) was characterized by the nucleoside composition and MALDI-TOF MS [calcd 1858.3528 for (M-H)⁻, found 1858.3695]. The modified ODN 5'-d(TGTGCC p -CVPGCGTG)-3' (ODN **2**) was characterized by the nucleoside composition and MALDI-TOF MS [calcd 3706.49 for (M+H)⁺, found 3706.34] (Fig. 1).

When 5'-d(p -CVPGCGTG)-3' (ODN **1**) was irradiated at 366 nm for 30 min in the presence of template ODN **3** (Scheme 2), ODN **4** was produced in 96% yield as determined by HPLC analysis (Fig. 2).⁸ The reaction mixture (total volume 60 μ L) containing ODN **1** (30 μ M, strand concn) in the presence of template ODN **3** (20 μ M, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 °C for 30 min.⁹ MALDI-TOF MS indicated that isolated ODN **4** obtained from HPLC purification was a crosslinked prod-

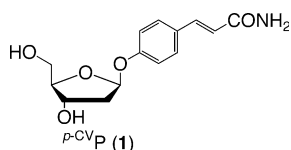
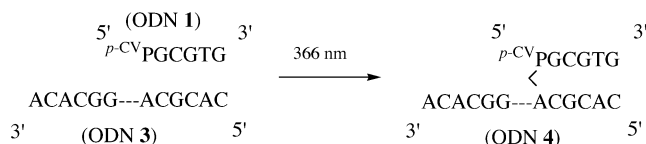


Figure 1. Structure of p -carbamoylvinyl phenol nucleoside (p -CVP).



Scheme 2. Interstrand photocrosslink of ODN **1** in the presence of template ODN **3**.

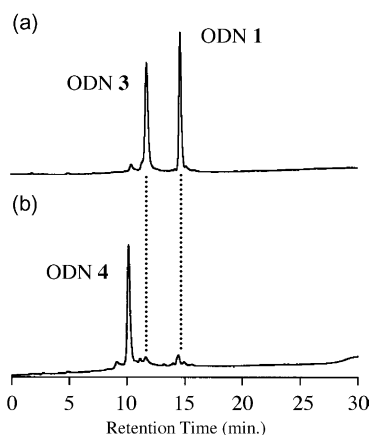


Figure 2. HPLC analysis of 366 nm irradiated ODN **1** and template ODN **3**; (a) before photoirradiation, (b) irradiation at 366 nm for 30 min, 96% yield. The progress of photoreaction was monitored by HPLC on a 5-ODS-H column (4.6 $\times$ 150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0, linear gradient over 30 min from 3% to 20% acetonitrile at a flow rate 1.0 mL/min).

uct of ODN **1** and ODN **3** [calcd 5485.74 for (M+H)⁺, found 5485.98]. The isolated ODN **4** was digested with AP, svPDE, and P1 nuclease at 37 °C for 4 h. Enzymatic digestion of isolated ODN **4** showed the formation of dC, dG, dI, and dT in a ratio of 6:6:3:1 together with dI- p -CVP photoadduct that was a deaminated product of dA- p -CVP adduct during enzymatic digestion process.¹⁰ The dI- p -CVP adduct was confirmed by MALDI-TOF MS [calcd 532.2043 for (M+H)⁺, found 532.1096]. We examined molecular modeling studies of the duplex **1** · **3**. As shown in Figure 3, the vinyl group of p -CVP is stacked on N7–C8 double bond of adjacent adenine residue of ODN **3**. It is known that photoreaction of adenine-thymine dinucleotide analog proceeds via [2 + 2] cycloaddition between the C5–C6 double bond of thymine and the N7–C8 double bond of adenine.¹¹ As judged from the molecular modeling, it is strongly suggested that the photocrosslink reaction proceed via [2 + 2] cycloaddition between the double bond of p -CVP and the N7–C8 double bond of adenine giving rise to the formation of aza-cyclobutane structure.

To demonstrate the feasibility of this photocrosslink, we examined two photoreactions of interstrand crosslink of the modified ODN containing p -CVP. When ODN **1** was irradiated at 366 nm for 30 min in the presence of template ODN **3** together with ODN **5**, ODN **4** was produced in 96% yield as determined by HPLC analysis (Scheme 3).⁸ The reaction mixture (total volume 60 μ L) containing ODN **1** (30 μ M, strand concn) and ODN **5** (20 μ M, strand concn) in the presence of template ODN **3** (20 μ M, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 °C for 30 min. When ODN **2** was irradiated at 366 nm for 30 min in the presence of template ODN **3**, ODN **6** was produced in 97% yield as determined by HPLC analysis (Scheme 4).⁸ The reaction mixture (total volume 60 μ L) containing ODN **2** (34 μ M, strand concn) in the

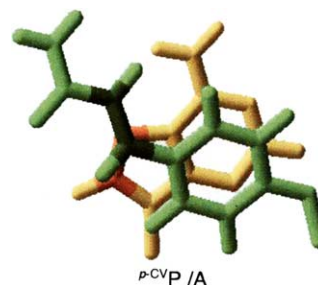
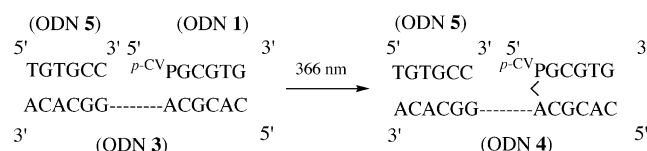
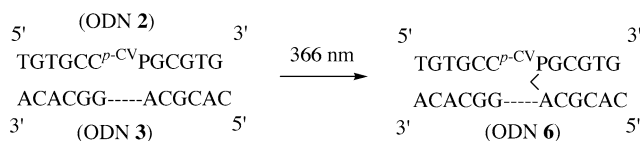


Figure 3. Molecular modeling of stacked geometry in B-form DNA. The model was optimized by AMBER* force field in water by using MacroModel version 8.1.



Scheme 3. Interstrand photocrosslink using ODN **1**.



Scheme 4. Interstrand photocrosslink using ODN 2.

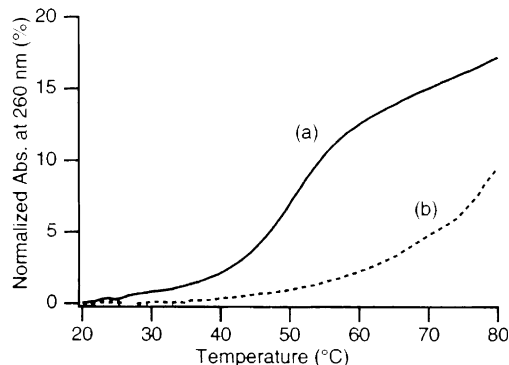


Figure 4. Melting curves of the 2·3 duplex (a) and ODN 6 (b). T_m values of the 2·3 duplex (5.0 μ M) and ODN 6 (5.0 μ M) were measured in 50 mM sodium cacodylate and 100 mM sodium chloride, pH 7.0.

presence of template ODN 3 (20 μ M, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 °C for 30 min. MALDI-TOF MS indicated that isolated ODN 6 obtained from HPLC purification was a crosslinked product of ODN 2 and ODN 3 [calcd 7330.92 for (M+H)⁺, found 7331.57].

To examine the influence of photocrosslink reaction on the thermal stability, the melting temperature (T_m) of the duplex 2·3 or ODN 6 was determined by UV-monitored thermal denaturation. As shown in Figure 4, the duplex 2·3 showed a melting temperature of 50.5 °C, whereas ODN 6 melted at over 80 °C. Example of this behavior has been seen for crosslinked ODNs by the introduction of thiol groups.¹² Thus, photocrosslinking increased T_m of ODN, a dramatic stabilization of the duplex form.

In conclusion, we demonstrate here that a modified ODN containing ^{p-CV}P can be crosslinked by irradiating at 366 nm with adjacent adenine residue in a [2 + 2] manner. This feature of the photoreactivity may provide the intriguing methodology that is applicable to anti-sense and antigene.

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- Spectroscopic data for selected compounds are as follows. Compound 5: ¹H NMR (CDCl₃, 300 MHz) δ 2.32 (dt, 1H, J = 14.2 Hz, 5.7 Hz), 2.55 (ddd, 1H, J = 14.2 Hz, 6.9 Hz, 1.7 Hz), 3.61–3.75 (m, 2H), 3.77 (s, 3H), 4.07–4.11 (m, 1H), 4.62–4.68 (m, 1H), 5.90 (dd, 1H, J = 5.7 Hz, 1.7 Hz), 6.30 (d, 1H, J = 16.2 Hz), 7.05 (d, 2H, J = 8.6 Hz), 7.44 (d, 2H, J = 8.6 Hz), 7.62 (d, 1H, J = 16.2 Hz). HRMS (MALDI): calcd for C₁₅H₁₈O₆Na [(M+Na)⁺] 317.0996, found 317.0938, UV (H₂O/CH₃OH = 1:1) λ_{max} (ϵ) 306 nm (14,300 M⁻¹ cm⁻¹), ϵ_{366} = 155.
- The yield was calculated on the basis of ODN 3.
- Isolation of each interstrand crosslinked photoproducts that were produced in two photoreactions of ODN 1 in the presence of template ODN 5'-d(CACGCTGGCACA)-3' or 5'-d(CACGCCGGCACA)-3' was not successful due to furnish lower conversions of photo-induced crosslinking reactions.
- dI-^{p-CV}P adduct: UV (H₂O) λ_{max} (ϵ) 256 nm (7540 M⁻¹ cm⁻¹).
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