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# Photochemistry of Benzophenone Immobilized in a Major Groove of DNA: Formation of Thermally Reversible Interstrand Cross-link

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The excited triplet state of benzophenone undergoes [2+2] cycloaddition to alkenes producing oxetanes (Paterno−Büchi reaction)[1] and hydrogen abstraction from accessible H donors eventually forming a covalent bond between the resulting geminate alkyl and ketyl radicals.[2] These photoreactions of benzophenone are used in photoaffinity labeling studies of protein−protein and protein−ligand interactions.[3] Benzophenone-mediated interstrand cross-link efficiently proceeds when the reactant is present near the benzophenone chromophore with a required geometry within a lifetime of benzophenone triplet. Otherwise, triplet benzophenone readily relaxes to the ground state without any reactions. Such cage and proximity effects in fact played important roles in one-electron oxidation of the nearest guanine from the 4-cyanobenzophenone-substituted uridine ($^{CNBP}$U) in duplex DNA.[4,5] We have synthesized a different type of benzophenone-substituted uridine ($^{BP}$U) in which the chromophore is tethered to uridine at C5 by a flexible linker rather than the structurally rigid ethynyl linker as used for $^{CNBP}$U. The benzophenone chromophore was located in a major groove of DNA upon duplex formation. The linker structure connecting C5 of uridine and benzophenone was designed so that the carbonyl group of benzophenone did not protrude out of the major groove but pointed toward the sugar−phosphate backbone of the opposite strand. We here report a highly site and sequence selective formation of an interstrand cross-link of $^{BP}$U-containing oligomer duplexes.[6] The cross-link was found spontaneously reverted to the original oligomers upon heating, providing a new method for the temporary connection of two DNA strands.[7]

Synthesis of 5′-dimethoxytrityl-$^{BP}$U **1** was accomplished as shown in Schemes 1 and S1. The modified base was incorporated into 12-mer 5′-d(GCA TA$^{BP}$U AAT TCG)-3′ (**$^{BP}$UAA**) (Table 1) by automated DNA synthesis using phosphoramidite **4**. Complementary strands are 5′-d(CGA ATT ATA TGC)-3′ (**TTA**), 5′-d(CGA AUT ATA TGC)-3′ (**UTA**), 5′-d(CGA ATU ATA TGC)-3′ (**TUA**), and 5′-d(CGA A$^{d3}$TU ATA TGC)-3′ (**$^{d3}$TUA**) containing 5-triduteriomethyluridine **$^{d3}$T**.[8] CD spectra of duplex **$^{BP}$UAA/TTA** showed a B-form structure. The melting temperature of the duplex was 37.8 °C (2.1 mM duplex, NaCl 100 mM, Na cacodylate 10 mM, pH 7.0), 3.4 °C lower than that of the unmodified duplex containing thymine.

Duplexes (83 $\mu$M for each strand) in 30 mM Na cacodylate (pH 7.0) were irradiated at room temperature with a 312 nm light obtained from a monochromator, and the reaction was monitored by HPLC. Irradiation of duplex **$^{BP}$UAA/TTA** produced two products (Figure 1a). A minor product eluted at 23.5 min was identified as an oligomer 5′-d(CGA AT=T ATA TGC)-3′ (**T=TA**) containing a thymine dimer (T=T) by both ESI-TOF MS (obsd 3642.66, calcd 3642.66) and a nucleoside analysis. The formation of **T=TA** is likely due to an energy transfer from the triplet benzophenone to neighboring thymines.[9] The major product eluted

***Scheme 1***

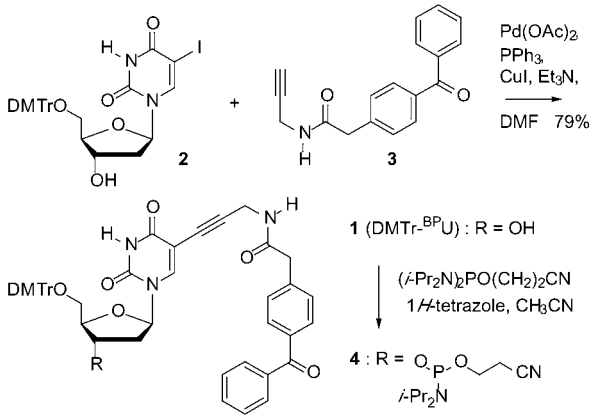



***Table 1.*** Oligomers Used for the Photoreactions

| | |
|---|---|
| **$^{BP}$UAA** | 5'-d(GCA TA$^{BP}$U AAT TCG)-3' |
| **TTA** | 5'-d(CGA ATT ATA TGC)-3' |
| **UTA** | 5'-d(CGA AUT ATA TGC)-3' |
| **TUA** | 5'-d(CGA ATU ATA TGC)-3' |
| **$^{d3}$TUA** | 5'-d(CGA A$^{d3}$TU ATA TGC)-3' |

at 29.0 min was identified as a cross-link of two oligomers (**$^{BP}$UAA-TTA**) (18% yield, conversion yield 51%) as evidenced from MALDI-TOF MS (obsd 7548.04, calcd 7549.17). To eliminate the formation of the undesired thymine dimer, oligomers **UTA** and **TUA** containing uridine in place of thymine in the 5′-TTA-3′ sequence were used for the analysis. While neither cross-link formation nor destruction of the duplex was observed for **$^{BP}$UAA/UTA**, cross-link **$^{BP}$UAA-TUA** was efficiently produced from **$^{BP}$UAA/TUA** (Figure 1b). The quantum yield for the formation of **$^{BP}$UAA-TUA** was 0.0012 at 312 nm using phenylglyoxylic acid as an actinometer.[10] A considerable difference in the cross-link formation between **UTA** and **TUA** suggests that T in the 5′-TUA-3′ of duplex **$^{BP}$UAA/TUA** is the site of a covalent bond formation. Only a small amount of cross-link was produced from the oligomer duplex containing the $^{BP}$UTA/TAA sequence, probably due to the steric repulsion between the methyl group of the 3′ side T of $^{BP}$U and the linker connecting the chromophore.[11]

The quantum yield for the formation of the cross-link from duplex **$^{BP}$UAA/$^{d3}$TUA** containing **$^{d3}$T** at the cross-linking site was virtually the same as that obtained for the photoreaction of **$^{BP}$UAA/TUA**, showing no first-order kinetic isotope effect on the cross-link formation by incorporating deuterium at the T methyl group. This strongly suggested that a direct H abstraction of methyl hydrogens of T by triplet benzophenone was not involved in the cross-link formation. Both cross-linked oligomers **$^{BP}$UAA-TTA** and **$^{BP}$UAA-TUA** were thermally reverted to original oligomers. Thus,



10.1021/ja017611r CCC: $22.00 

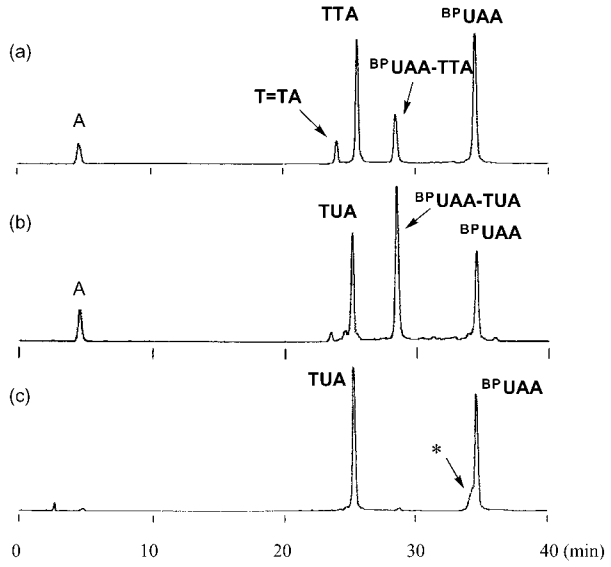
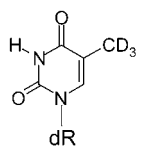
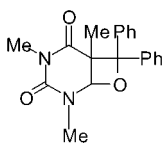



***Figure 1.*** Reversed-phase HPLC profiles for the photoreactions of duplexes (a) **$^{BP}$UAA/TTA** and (b) **$^{BP}$UAA/TUA** and (c) for a heat-induced splitting of cross-links of **$^{BP}$UAA-TUA**. Photoirradiation was carried out at 312 nm for (a) 3000 and (b) 4000 counts with a monochromator (JASCO CRM-FD, 300 W Xe lamp) in the presence of adenine (A) as an internal standard. One count of photoirradiation approximately corresponds to a surface energy of 0.02 J/cm$^2$. Isolated **$^{BP}$UAA-TUA** in water was heated at 90 °C for 60 min. A shoulder peak marked with an asterisk is **$^{BP}$UAA** containing a thymine dimer.

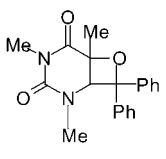
**$^{BP}$UAA-TUA** completely disappeared in HPLC with heating at 90 °C for 1 h in water accompanied by a concomitant formation of oligomers **$^{BP}$UAA** and **TUA** (Figure 1c). The half-life of **$^{BP}$UAA-TUA** in water (pH 7.0) was 38.5 min at 80 °C and considerably decreased to 8.9 min at pH 5.2. All attempts to isolate thymidine−benzophenone adduct by enzymatic digestions of **$^{BP}$UAA-TUA** were unsuccessful due to the thermal instability of the cross-linked structure, but a model photoreaction of benzophenone with 1,3-dimethylthymine in aqueous acetonitrile provided significant insights into the cross-linked structure. In addition to the formation of known oxetane **6** (27% yield),[12] we isolated without precedent oxetane **5**, which is a structural isomer of **6**, in 38% yield. The structure of **5** involving a *N*,*O*-acetal functionality was unambiguously determined by a complete assignment of the $^1$H and $^{13}$C NMR signals by HMQC and HMBC spectra (Figure S1). While oxetane **6** was stable under heating at 90 °C in chloroform, isomeric oxetane **5** was decomposed within 1 h to a one-to-one mixture of 1,3-dimethylthymine and benzophenone in an almost quantitative yield. Thermal dissociation of **5** did not proceed in refluxing rigorously dried benzene, suggesting that a proton source was essential for the dissociation process. These observations were in good agreement with the thermal dissociation of **$^{BP}$UAA-TTA** and **$^{BP}$UAA-TUA** to original oligomers and the acceleration of the reaction rate in acidic solution. On the basis of these data, it is most likely that the cross-link reaction of **$^{BP}$UAA** with **TTA** and **TUA** proceeds via Paterno−Büchi type reaction between a carbonyl group of benzophenone and a C5−C6 double bond of thymine producing the oxetane having the *N*,*O*-acetal structure.




**$^{d3}$T**




**5**




**6**

In marked contrast to a highly efficient cross-link formation from duplex **$^{BP}$UAA/TTA**, photoirradiation of single-stranded **$^{BP}$UAA** resulted in a complete destruction of the oligomer without formation of any significant detectable products. While triplet benzophenone oxidizes guanine in DNA by one electron transfer,[13] guanine oxidation was at the best a very minor reaction in the photoinduced cross-link formation of **$^{BP}$UAA/TTA**. It is likely that a restricted dynamic motion of the chromophore in the major groove of DNA effectively suppressed nonspecific reactions leading to the destruction of **$^{BP}$UAA**, but promoted the specific photocross-linking with a high site selectivity. These results described here demonstrated the significance of the cage and proximity effects attained by immobilizing a benzophenone chromophore in a major groove of the duplex DNA. Furthermore, thermally reversible interstrand cross-link may be useful for a temporary connection of two oligomers in DNA manipulations.

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**Supporting Information Available:** Experimental protocol for photoreactions of duplexes, the detailed synthesis of **4** and **$^{BP}$UAA**, and NMR assignment for **5** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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