

# Photochemical Generation of C4'-Oxidized Abasic Site Containing Oligodeoxynucleotide and Its Efficient Amine Modification

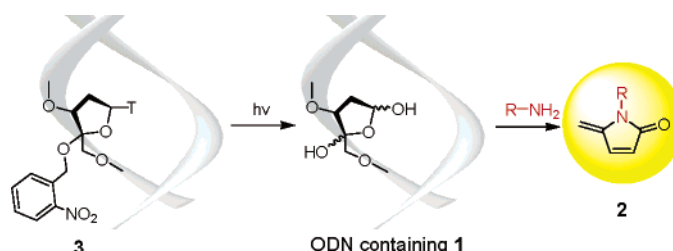
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## ABSTRACT

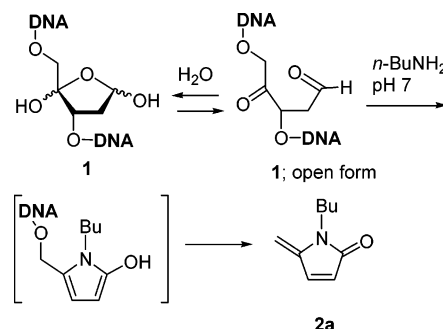


We synthesized oligodeoxynucleotide (ODN, 3), which contains 4'-*o*-nitrobenzyloxymethylthymidine (4) as a caged precursor of C4'-oxidized abasic site (1). Photoirradiation of 3 at 365 nm followed by amine treatment under neutral conditions afforded the lactam (2) efficiently. Duplexed ODN 3 was converted to 1 faster and more efficiently than single stranded 3, whereas amine treatment of 1 formed from single stranded 3 resulted in slightly faster lactam formation than with the duplex.

Bleomycin-induced oxidative damage of DNA under limiting oxygen conditions results in the formation of alkali-labile C4'-oxidized abasic site (1) (Scheme 1).<sup>1</sup> The studies of the properties and reactivities of 1 are essential because of their relation to toxicity and the effects on mutagenesis of 1. Under equilibrium, the 1,4-dihydroxytetrahydrofuran structure of 1 can exist in the open form with the 1,4-dicarbonyl structure. Reactive aldehydes, especially bifunctional ones, can be toxic as a result of the reaction of a DNA base and a protein.<sup>2</sup> We found that unsaturated lactam (2a) and a DNA fragment were

formed in the reaction of 1 with amine under mild conditions (room temperature, pH 7).<sup>3</sup> This result indicated the possibility of modifying amine-containing biomolecules by an

**Scheme 1.** Reaction of 1 with Amine to Form Lactam



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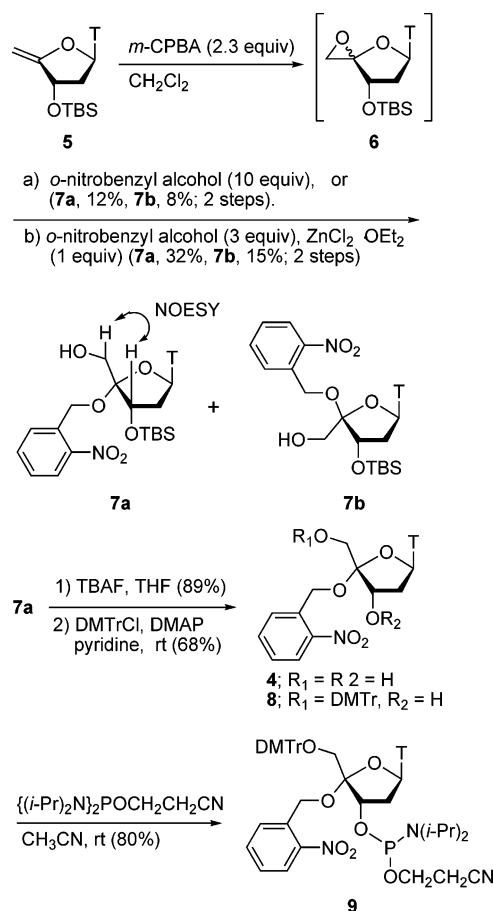
oligodeoxynucleotide (ODN) containing **1**. Lactam formation was also studied with a C4'-selenated nucleotide, which generates **1** by treatment with NBS; in this case the lactam was formed under neutral conditions in good yield.<sup>4</sup> However, quantitative studies on lactam formation from **1** in an ODN have not been done because formation of **1** in an ODN by bleomycin is not efficient.<sup>5</sup> To study reactivities of **1** in an ODN, efficient formation of **1** is necessary. We planned to prepare **1** in an ODN from a caged precursor, which might be applicable to experiments under physiological conditions.

The lactam formation reaction of **1** does not require extra reagents such as NaBH<sub>4</sub> as in the lysine modification via a Schiff base. In addition, the structure of the ODN containing **1** should be similar to the unmodified ODN. Based on these ideas, this lactam formation reaction of **1** may be applicable to in situ lysine modification and mapping of lysine residues of DNA binding proteins for their structure–function studies. Recently, Greenberg et al. reported generation of **1** by photochemical cleavage of caged sugars, which carry 3,4-dimethoxy-6-nitrobenzyloxy groups at the C1' and C4' positions of 2'-deoxyribose. 5'-O-Silylated O-methyl phosphoramidites prepared from the caged sugars were incorporated into an ODN. They studied chemical stabilities and biological effects of the ODN containing **1**.<sup>6</sup> In contrast, we designed a caged nucleoside that retained the base moiety at the C1'-position and carried a *o*-nitrobenzyloxy group at the C4'-position. We expect that the use of the caged nucleoside that we have designed will be advantageous for a site-specific lysine modification, because irradiation of a caged ODN–protein complex was possible, in which the interaction of the retained base moiety and the target protein could be maintained. Thus, we prepared ODN **3** containing 4'-*o*-nitrobenzyloxythymidine (**4**). The sequence of the unmodified parent ODN of **3**, **3-nat**, contains the binding site of the DnaA protein that is involved in the initiation of replication in *Escherichia coli*. Recently, the X-ray crystal structure of the complex of DNA binding domain (domain IV) of DnaA protein and duplex ODN (**3-nat**) was reported.<sup>7</sup> The results of X-ray crystallography suggested that one of the lysine residues in domain IV (Lys-415) is located near the phosphate group between C<sub>2</sub> and T<sub>3</sub>. The open form of **1** might react with Lys-415 to modify it if the lysine residue can come close to carbonyl functions during the molecular vibrations of the protein conformation in solution. Presently, we describe the synthesis of **3** and its photochemical reaction

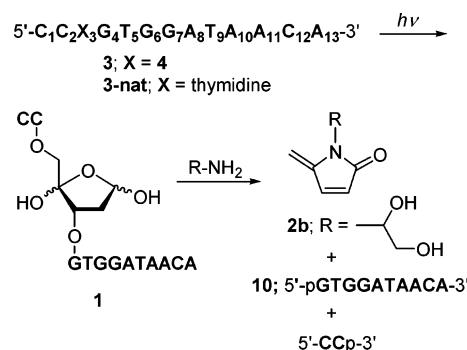
to form the ODN containing **1** followed by amine treatment, which results in efficient lactam formation.

Reaction of 4',5'-unsaturated thymidine (**5**)<sup>8</sup> with *m*-CPBA in the presence of *o*-nitrobenzyl alcohol (10 equiv) gave **7a** and **7b**, in 12% and 8%, respectively (Scheme 2). Stereo-

**Scheme 2.** Synthesis of 4'-*o*-Nitrobenzyloxythymidine Derivatives



**Scheme 3.** Photoreaction of **3** Followed by Treatment with Amine



chemistry of **7a** was determined by NOESY measurements. Epoxide **6** could be an intermediate though it was not isolated.<sup>9</sup> Addition of Lewis acid improved the yields of the

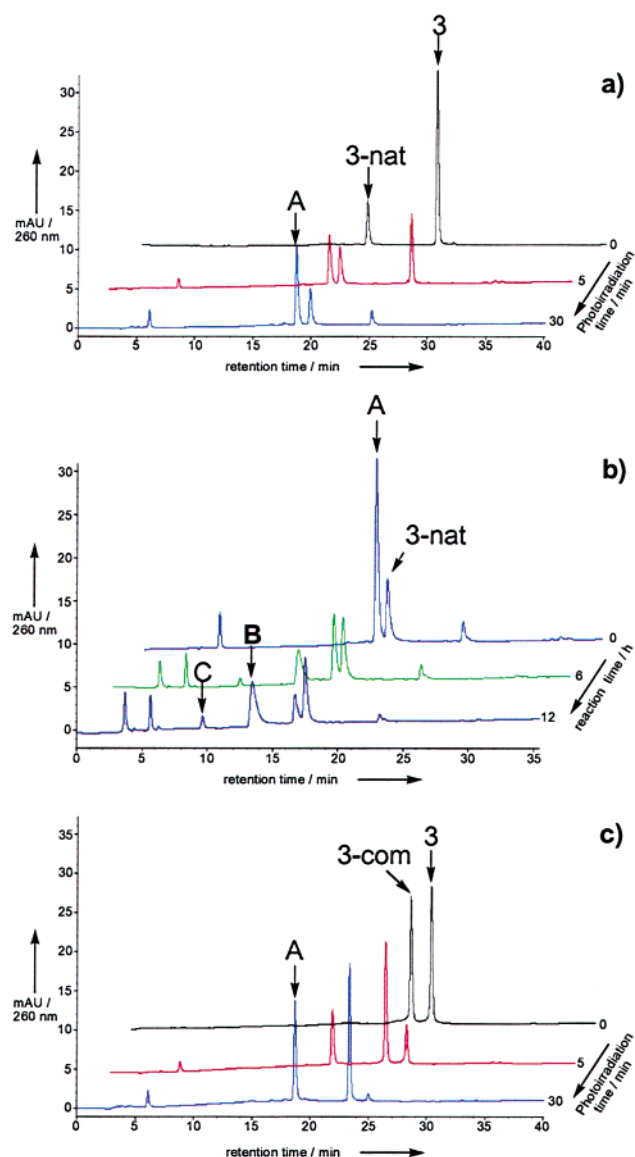
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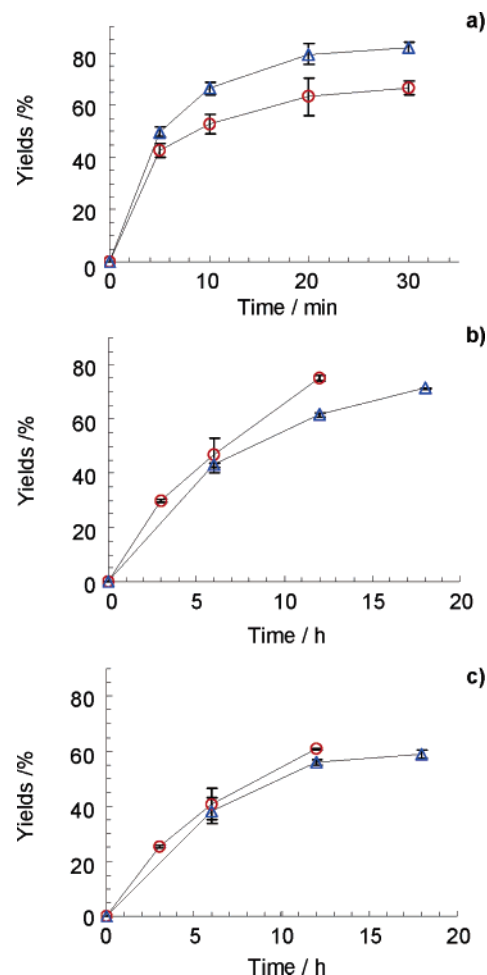
**Figure 1.** HPLC analysis. (a) Photoirradiation of **3** (retention time = 27 min) in H<sub>2</sub>O containing 100 mM NaCl. **3-nat** (retention time = 20 min) was added as an internal standard. Product **A** eluted at 18 min. (b) Reaction mixture of the photolyzed **3** with 3-amino-1,2-propanediol. Products **B** and **C** eluted at 9 and 13 min, respectively. (c) Photoirradiation of **ds 3**. Complementary strand of **3** (**3-com**) eluted at 24 min.

products. To a mixture of **5** and *o*-nitrobenzyl alcohol (**3** equiv) was added *m*-CPBA at 0 °C. After consumption of **5**, 1 equiv of ZnCl<sub>2</sub>·OEt<sub>2</sub> was added at –78 °C. Diastereomers **7a** and **7b** were isolated in 32% and 15%, respectively. Desilylation of **7a** afforded C4'-substituted thymidine **4**, which was further converted to phosphoramidite **9** via **8**.

The phosphoramidite **9** was incorporated into the 13-mer by the conventional phosphoramidite method with an auto-

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**Figure 2.** Time course of (a) formation of **1** from **3** (O) and **ds 3** (Δ) and (b) formation of **2b** and (c) **10** from **1** prepared from **3** (O) and **ds 3** (Δ).

mated DNA synthesizer. After the synthesis of ODN in trityl on mode, it was purified with HPLC, and the DMTr group of the ODN was removed under acidic conditions (20% AcOH, room temperature, 4 h) to give **3** (Supporting Information). The structure of the obtained ODN was confirmed by MALDI TOF MS (*m/z* 4107.44 (4109.73, calcd for **3**)). *T<sub>m</sub>* values of the duplex between **3** and the complementary 13-mer (**3-com**) were close (38.7 and 46.0 °C in H<sub>2</sub>O and phosphate buffer) to those of **3-nat** (42.4 and 47.7 °C in H<sub>2</sub>O and phosphate buffer; Supporting Information).

The photoreaction of **3** was studied with a 10 μM solution in water or phosphate buffer (10 mM, pH 7) containing 100 mM NaCl and **3-nat** (3 μM), which was added to the reaction mixture as an internal standard.<sup>10</sup> Irradiation at 365 nm with a handy UV lamp led to smooth conversion of **3** to a new product **A** in 30 min (Figure 1a). Liberation of thymine was also confirmed by co-injection of the reaction mixture and

(10) HPLC analysis of the reaction mixture suggested that the peak areas of **3-nat** and **3-com** were not changed during reactions.

thymine (Supporting Information). The reaction mixture of photolyzed **3** containing **A** was subjected to the reaction with an aqueous solution of 3-amino-1,2-propanediol<sup>11</sup> (1000 equiv, pH was adjusted to 7 with AcOH in phosphate buffer). Heating the reaction mixture at 37 °C resulted in conversion of **A** to the new products **B** and **C** (Figure 1b). Peaks **B** and **C** comigrated with 10-mer **10** and the synthesized authentic lactam **2b**, respectively. Peak **C** and **2b** were next analyzed with LC–MS. The mass spectra of **C** and **2b** were identical with a quasi-molecular ion peak (MH<sup>+</sup>) at 170 and fragment peaks at 96 and 138, indicating **C** was identical with **2b** (Supporting Information). The formation of **10** and **2b** suggested **A** was the ODN containing **1**. The yields of ODN containing **1**, **2b**, and **10** were determined on the basis of comparison of peak areas with that of **3-nat** (ODN containing **1**; 67% from **3**, **2b**, and **10**; 75% and 61% from ODN containing **1**, respectively; Figure 2a–c).

Next, we studied photoreaction of duplexed **3** (**ds 3**) under similar conditions. The formation of the ODN containing **1** occurred faster in duplexed form than it did in the single-stranded form. The yield of **1** from **ds 3** was higher than that from **3** (82% based on peak area of **3-com**<sup>10</sup>). Treatment of ODN containing **1** from **ds 3** with amine resulted in slightly slower lactam formation compared to the reaction of that from **3**. However, the efficiencies of lactam formation were comparable (Figure 2b).

The *o*-nitrobenzyl group at the C4' position of 2'-deoxyribose might be located in the minor groove of the duplex. The position of the *o*-nitrobenzyl group in **ds 3** was

(11) Preliminary results showed that the reaction of lysine can give a mixture of  $\alpha$ - and  $\epsilon$ -lactams under conditions similar to those in the formation of **2b**. 3-Amino-1,2-propanediol and **2b** are soluble in water, which was appropriate for the reaction and analysis.

advantageous in the decaging reaction. In regard to the lactam formation, the duplex containing **1** was more stable than the single strand, based on HPLC analysis. Thus, the structure of the ODN affected efficiencies of the photoreaction of **3** and the following lactam formation.

Here, we synthesized ODN (**3**) containing 4'-*o*-nitrobenzyloxythymidine by a conventional phosphoramidite method. ODN **3** was photolyzed efficiently to give ODN containing **1**. Efficient lactam formation of ODN containing **1** under neutral conditions is a good indication of possibilities of protein modification.

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**Supporting Information Available:** Experimental procedures and characterization for compounds **2b**, **4**, **7a**, **7b**, **8**, **9**; <sup>1</sup>H NMR spectra of compounds **2b**, **4**, **7a**, **7b**, **8**, **9**, 1-[2-deoxy-3,5-*O*-methylphosphoryl-4-*o*-nitrobenzyloxy- $\beta$ -D-ribofuranosyl] thymine (synthesized for determination of the extinction coefficient of **3**) and NOESY spectra of **7a** and **7b**; HPLC analyses of crude ODN with DMTr group after cleavage from solid support and crude **3** after treatment under acidic conditions; MALDI TOF MS spectrum of **3**; UV-melting curves of duplexes of **3** and **3-nat**; HPLC traces of co-injection of the photolyzed **3** and thymine and co-injection of the reaction mixture of photolyzed **3** and **2b**, and **10**; LC–MS spectra of the reaction mixture containing **2b** and the authentic **2b**; and time course of formation of **1** and **2b** from **3** and **ds 3**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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