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

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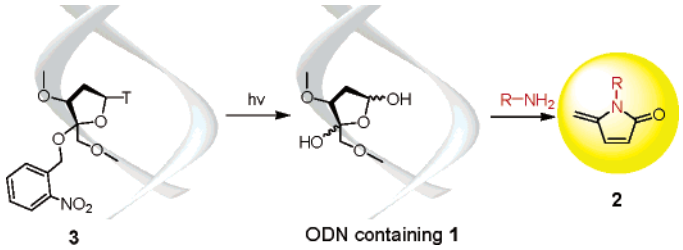
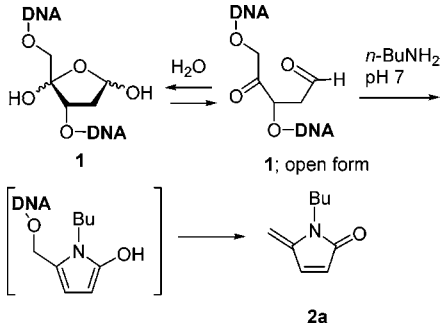
ABSTRACT



We synthesized oligodeoxynucleotide (ODN, 3), which contains 4′-*o*-nitrobenzyloxythymidine (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).[1] 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.[2] 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).[3] 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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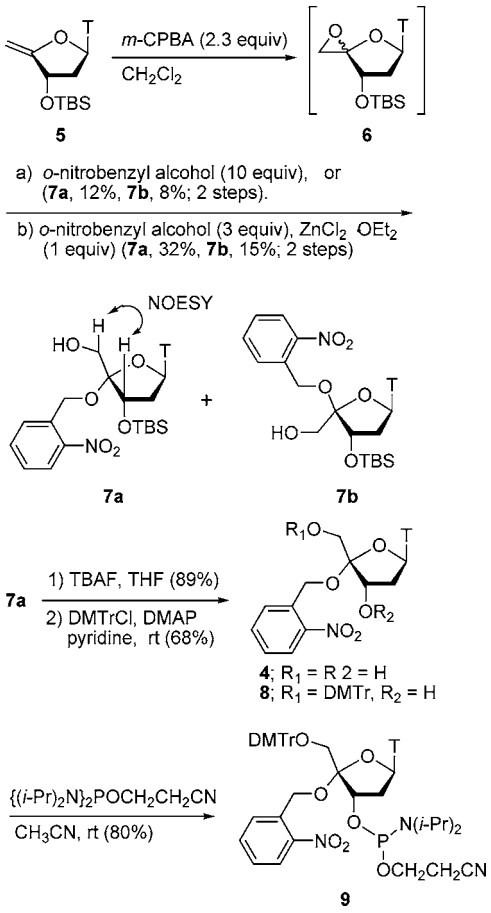
oligodeoxynucleotide (ODN) containing **1**. Lactam formation was also studied with a C4′-selenated nucleotide, which generates **1** by treatment with NBS; in this case the lactam was formed under neutral conditions in good yield.[4] However, quantitative studies on lactam formation from **1** in an ODN have not been done because formation of **1** in an ODN by bleomycin is not efficient.[5] 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.

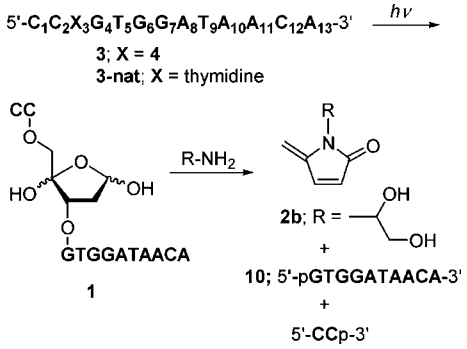
The lactam formation reaction of **1** does not require extra reagents such as $NaBH_4$ as in the lysine modification via a Schiff base. In addition, the structure of the ODN containing **1** should be similar to the unmodified ODN. Based on these ideas, this lactam formation reaction of **1** may be 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**.[6] 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.[7] 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_2 and T_3. 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**)[8] with *m*-CPBA in the presence of *o*-nitrobenzyl alcohol (10 equiv) gave **7a** and **7b**, in 12% and 8%, respectively (Scheme 2). Stereochemistry of **7a** was determined by NOESY measurements. Epoxide **6** could be an intermediate though it was not isolated.[9] Addition of Lewis acid improved the yields of the

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



Scheme 3. Photoreaction of 3 Followed by Treatment with Amine



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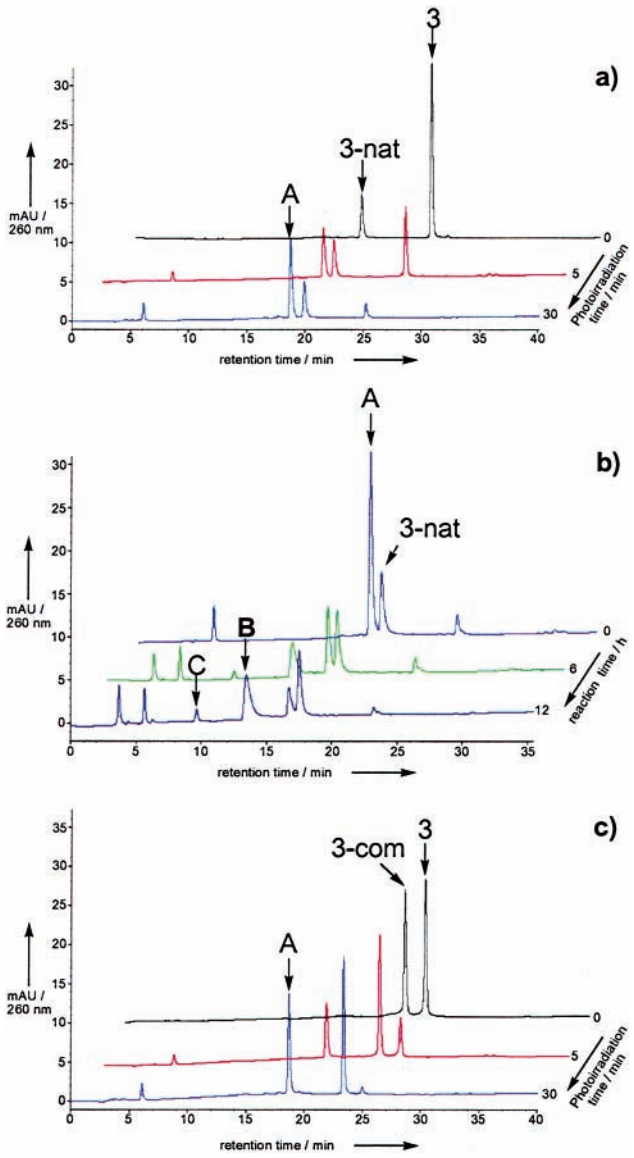

Figure 1. HPLC analysis. (a) Photoirradiation of **3** (retention time = 27 min) in H_2O containing 100 mM NaCl. **3-nat** (retention time = 20 min) was added as an internal standard. Product **A** eluted at 18 min. (b) Reaction mixture of the photolyzed **3** with 3-amino-1,2-propanediol. Products **B** and **C** eluted at 9 and 13 min, respectively. (c) Photoirradiation of **ds 3**. Complementary strand of **3** (**3-com**) eluted at 24 min.

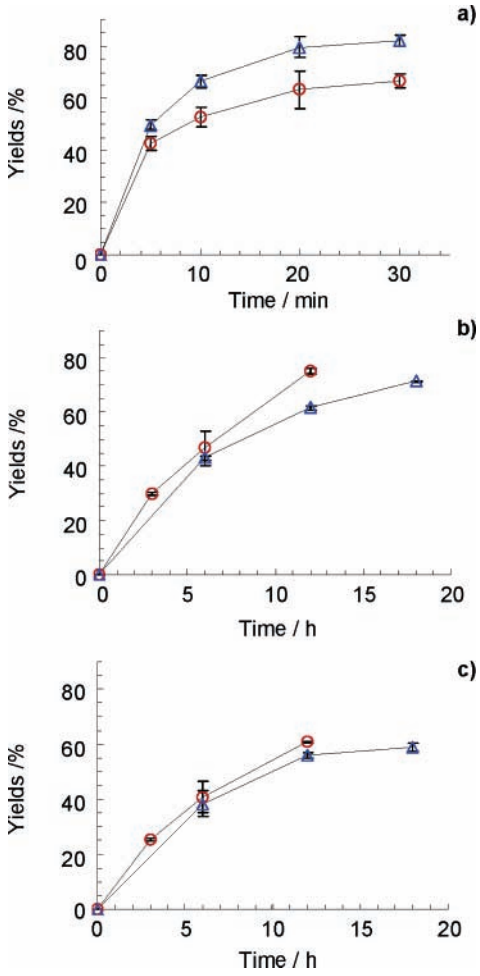

Figure 2. Time course of (a) formation of **1** from **3** (○) and **ds 3** (△) and (b) formation of **2b** and (c) **10** from **1** prepared from **3** (○) and **ds 3** (△).

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 $ZnCl_2$·OEt_2 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 automated 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_m values of the duplex between **3** and the complementary 13-mer (**3-com**) were close (38.7 and 46.0 °C in H_2O and phosphate buffer) to those of **3-nat** (42.4 and 47.7 °C in H_2O 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.[10] 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

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(10) HPLC analysis of the reaction mixture suggetsed 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[11] (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^+) 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**[10]). 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 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.

Acknowledgment. We are grateful for the financial support from Sapporo Bioscience Foundation.

Supporting Information Available: Experimental procedures and characterization for compounds **2b**, **4**, **7a**, **7b**, **8**, **9**; ^{1}H NMR spectra of compounds **2b**, **4**, **7a**, **7b**, **8**, **9**, 1-[2-deoxy-3,5-*O*-methylphosphory-4-*o*-nitrobenzyloxy-β-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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(11) Preliminary results showed that the reaction of lysine can give a mixture of α- and ϵ-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.