ORGANIC LETTERS

2012
Vol. 14, No. 23
5852–5855

Difluoro-C4′-oxidized Abasic Site for Efficient Amine Modification in Biological Systems

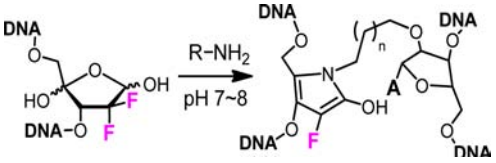
Bo Yang, Akiko Jinnouchi, Hiroshi Suemune, and Mariko Aso*

Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

aso@phar.kyushu-u.ac.jp

Received September 30, 2012

ABSTRACT

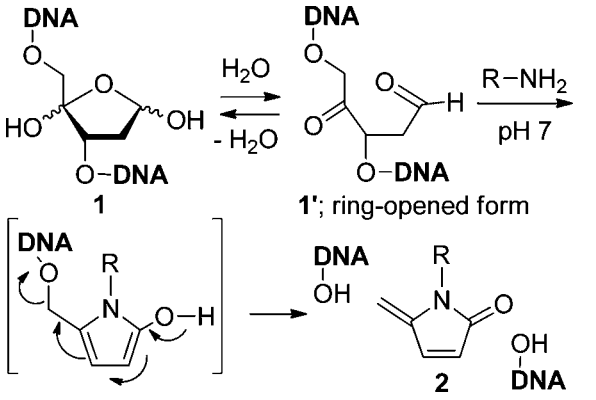


An oligodeoxynucleotide (ODN) containing a 2′,2′-difluorinated analogue of a C4′-oxidized abasic site (C4′-OAS) was designed for the amine modification of biomolecules that interact with nucleic acids. In contrast to the parent C4′-OAS, which yielded amine-modified products accompanied by DNA strand scission, the ODN containing the difluoro C4′-OAS efficiently yielded products carrying ODNs. The amine modification proceeded without additional reagents being required and might be applicable to reactions in biological systems.

DNA that carries a carbonyl group, which can be synthesized artificially or is formed when DNA is damaged owing to the actions of various DNA-damaging agents, can react with nitrogen nucleophiles such as hydrazine and hydroxylamine under physiological conditions, thereby leading to the chemical modification of DNA.[1−4] The reactions of the carbonyl groups in DNA with the amino groups in DNA-interacting proteins and nucleic acids can form Schiff bases or carbinolamines, which might affect the structures and functions of these biomolecules. Modifications resulting from the formation of irreversible covalent bonds may be promising, although the required reducing conditions may not be available in biological systems.[5]

The C4′-oxidized abasic site (**1**; C4′-OAS) is a type of oxidatively damaged DNA lesion that is produced by antitumor bleomycins.[6] We found that an oligodeoxynucleotide (ODN) containing **1** reacted with a primary amine under mild conditions that were close to those of biological systems and yielded lactam **2** efficiently by eliminating DNA fragments (Scheme 1).[7−9] Since ODN that contains **1** reacts effectively with amines and its structure is close to that of

Scheme 1. Reaction of **1** with an Amine



(1) Dutta, S.; Chowdhury, G.; Gates, K. S. *J. Am. Chem. Soc.* **2007**, *129*, 1852–1853.
(2) Dey, S.; Sheppard, T. L. *Org. Lett.* **2001**, *3*, 3983–3986.
(3) Manoharan, M.; Andrade, L. K.; Cook, P. D. *Org. Lett.* **1999**, *1*, 311–314.
(4) Angelov, T.; Guaninazzi, A.; Scharer, O. D. *Org. Lett.* **2009**, *11*, 661–664.
(5) Sczepanski, J. T.; Wong, R. S.; McKnight, J. N.; Bowman, G. D.; Greenberg, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 22475–22480.
(6) Sugiyama, H.; Kilkuskie, R. E.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 7765–7767.
(7) Aso, M.; Kondo, M.; Suemune, H.; Hecht, S. M. *J. Am. Chem. Soc.* **1999**, *121*, 9023–9033.
(8) Aso, M.; Usui, K.; Fukuda, M.; Kakihara, Y.; Goromaru, T.; Suemune, H. *Org. Lett.* **2006**, *8*, 3183–3186.

10.1021/ol302703m
Published on Web 11/14/2012

unmodified natural DNA, it could modify the lysine residues of DNA-interacting proteins[10] and could be used to map lysine residues in DNA-binding proteins as a part of structure–function investigations. The 1,4-dicarbonyl structure in the ring-opened form **1′**, which exists in equilibrium with **1**, might be a key to effective amine modification.[11] However, the ODN containing **1** undergoes β-elimination from the ring-opened form **1′** under basic and heating conditions, and the resultant cleavage of the binding sequence might lead to a decrease in the affinity for the target molecules, and thus a decrease in the modification efficiency.[12] Reaction of the ODN containing **1** with amine-containing molecules yields molecules bearing lactam but does not result in conjugation, which is also useful to introduce various functions into DNA[13] and to study DNA interacting molecules. We designed an ODN containing **3**, the 2,2-difluorinated analogue of **1** (Figure 1). The introduction of the difluoro groups was intended to prevent DNA strand cleavage by β-elimination. In addition, an increase in the reactivity of the aldehyde in the ring-opened form **3′** was expected owing to the electron-withdrawing effect of the fluoride. Herein, we report the synthesis of ODN containing **3** that can carry out amine modifications to yield ODN products under conditions that are similar to those of biological systems.

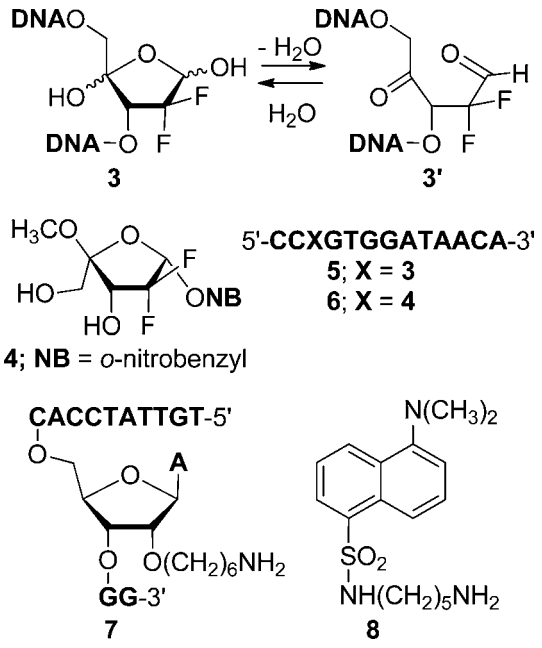


Figure 1. ODNs **5** and **6** containing **3** and caged sugar **4** and amines **7** and **8**.

The preparation of ODN containing **3** was carried out by photoirradiating a caged ODN containing **4**, which was similar to generating the ODN containing **1** from its corresponding caged precursor ODN.[9] In this study, ODN **5** was prepared by photoirradiating **6**. The synthesis of caged sugar **4** was carried out using the 4,5-unsaturated sugar **14** as a key intermediate (Scheme 2). The reaction of 2-deoxy-2,2-difluororibose[14] with *o*-nitrobenzyl bromide in the presence of DBU resulted in nitrobenzyl ether **9** (30%) and its anomer (40%). After the hydrolysis of benzoyl groups of **9**, **10** was obtained, and its 5-hydroxyl group was iodinated to yield **11**. The attempted conversion of **11** into **14** by treatment with DBU only led to the decomposition of **11**. On the other hand, the 3-acetylated **12** was successfully converted into **13** by a similar treatment. After hydrolysis of the 3-acetyl group, treatment of the obtained **14** with *m*-CPBA in the presence of methanol resulted in the introduction of a methoxy group at the 4-position, from which **4** was obtained as a major product along with its C4-epimer. Compound **4** was converted into phosphoramidite **15**, and ODN **6** was synthesized using the conventional phosphoramidite method by employing an automated DNA synthesizer: MALDI-TOF MS data: m/z = 4051 (MW of **6** = 4050). It was anticipated that the photoirradiation of **6** yielded the ring-opened form **5′**, which existed in equilibrium with **5**. ODN **5** might be composed of an equilibrating mixture of isomers at the anomeric and C4-positions.[15]

The photoirradiation of **6** was carried out using a 10 μM solution of **6** in H_2O at 365 nm and 0 °C. After 4 h, HPLC analysis of the reaction mixture revealed the clear conversion of **6** into a new product (13 min; Figure 2). The result of the MALDI-TOF MS of the photolyzed sample indicated the formation of **5** (m/z = 3904: MW of **5** = 3902). The yield of **5** was estimated (ca. 80%) on the basis of a comparison of the area of its peak with that of the peak of the internal standard. For the amine modification of a target molecule, **3** must be accessible to the amino group in the complex. In order to evaluate the reactivity of **3** with a proximal amine, ODN **5** was subjected to a reaction with the complementary ODN **7**,[16] which carried a hexylamine that could approach **3** in the duplex (Figure 1). ODN **6** (25.4 μM in 50 mM phosphate buffer; pH values of 7 and 8) was hybridized with ODN **7** (10 μM; half the concentration of **5** after photoirradiation). The decaging of **6** in the duplex yielded duplex **5**:**7**. The resultant duplex was incubated at 37 °C in a phosphate buffer and was analyzed

(9) Usui, K.; Aso, M.; Fukuda, M.; Suemune, H. *J. Org. Chem.* **2008**, *8*, 241–248.

(10) Jacobs, A. C.; Kreller, C. R.; Greenberg, M. M. *Biochemistry* **2011**, *50*, 136–143.

(11) (a) Op de Beeck, M.; Madder, A. *J. Am. Chem. Soc.* **2011**, *133*, 796–807. (b) Op de Beeck, M.; Madder, A. *J. Am. Chem. Soc.* **2012**, *134*, 10737–10740.

(12) Formation of DNA interstrand cross-link involving cleavage of the strand containing the C4′-OAS was reported by Greenberg. Fragments (15-mers) formed by cleavage might still have enough affinity to the complementary 31-mer for cross-link formation. Sczepanski, J. T.; Jacobs, A. C.; Greenberg, M. M. *J. Am. Chem. Soc.* **2008**, *130*, 9646–9647. Sczepanski, J. T.; Jacobs, A. C.; Majumdar, A.; Greenberg, M. M. *J. Am. Chem. Soc.* **2009**, *131*, 11132–11139.

(13) Lucas, R.; V.-Climent, E.; G.-Pinto, I.; Avino, A.; Eritja, R.; Gonzalez, C.; Morales, J. C. *Chem. Commun.* **2012**, *48*, 2991–2993.

(14) Chou, T. S.; Heath, P. C.; Patterson, L. E.; Poteet, L. M.; Lakin, R. E.; Hunt, A. H. *Synthesis* **1992**, 565–570.

(15) The structure of the C4′-oxidized abasic site in the duplex was studied by Stubbe et al.: Chen, J.; Dupradeau, F.-Y.; Case, D. A.; Turner, C. J.; Stubbe, J. *Biochemistry* **2007**, *46*, 3096–3107.

(16) Manoharan, M.; Inamati, G.; Tivel, K. L.; Conklin, B.; Ross, B. S.; Cook, P. D. *Nucleosides Nucleotides* **1997**, *16*, 1141–1143.

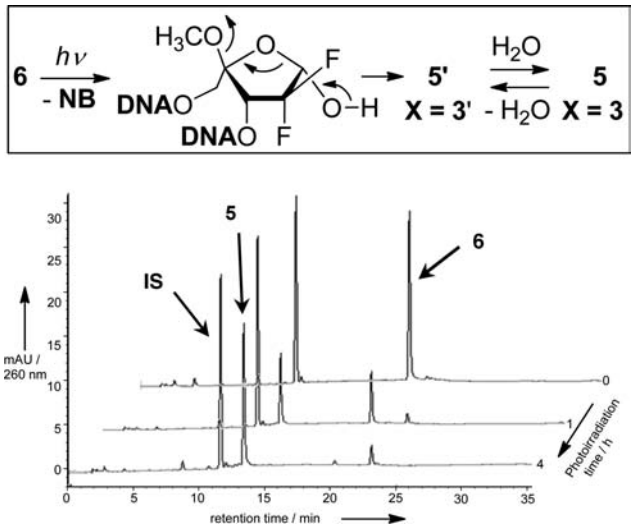


Figure 2. HPLC analysis of the photoreaction of **6**. ODNs **6** and **5** were eluted at retention times of 20 and 13 min, respectively. 5′-d(ATAC)-3′ was added as an internal standard (IS) (retention time of 11 min).

by HPLC and denaturing polyacrylaminde gel electrophoresis (PAGE) (Figure 3a,b; reaction at pH 8). The formation of two cross-linked products at the expense of the starting duplex was observed. The major cross-linked product was eluted at 19 min by HPLC analysis and corresponded to the slower migrating band seen in the results of the PAGE analysis. After being isolated, its structure was judged to be **17** (R-NH_2 = **7** at *O*-hexylamine; MW = 7911) from its MALDI-TOF MS spectrum (m/z = 7910). This product might have formed from the five-membered-ring amine intermediate **18** (MW = 7949) by the elimination of H_2O (MW = 18) and HF (MW = 20; Scheme 3). The structure of the minor cross-linked product was also judged to be **19** from its MALDI-TOF MS spectrum (m/z = 7331, MW of **19** = 7333). The yields of **17** and **19** in the reaction at pH 7, which were 27% and 17%, respectively, at 24 h, were determined by comparing the areas of their peaks with that of internal standard. The yields increased significantly when the reaction was carried out at pH 8 (55% and 43%, respectively). The cross-link formation by **3** contrasted with the lactam formation by **1**, although both reactions proceeded without the need for additional reagents.

Cross-link **17** was chemically stable under the reaction conditions, and conversion of **17** into **19** did not take place (pH 7 and 8 at 37 °C for 24 h). On heating of a solution of **17** (10 mM phosphate buffer containing 100 mM NaCl; pH 7) from 20 to 90 °C at 0.5 °C/min, its UV absorbance profile exhibited a melting transition region at 80 °C (Figure 3c). Under similar heating conditions (50 mM phosphate buffer containing 100 mM NaCl; pH 7 and 8), **17** was essentially intact (ca. 97% remained at pH 7), and

(17) At pH 8, 90% of **19** remained (Supporting Information).

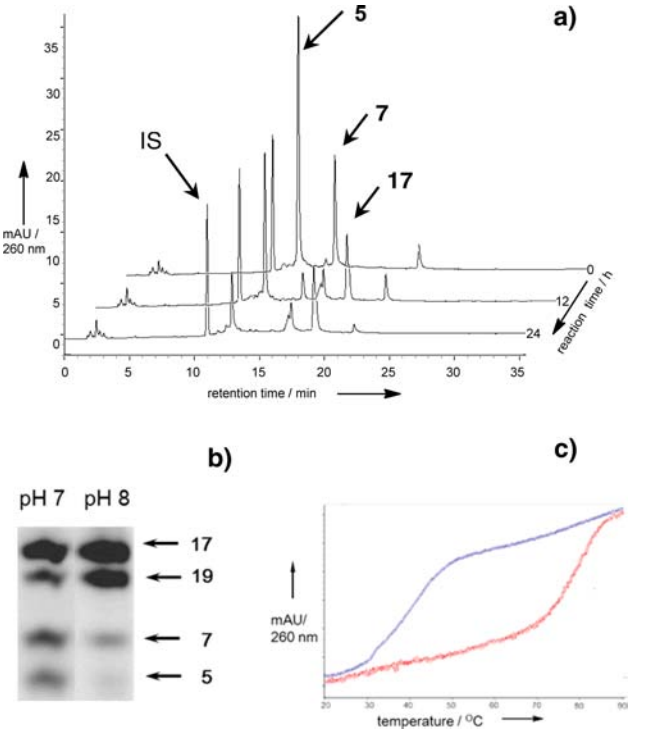


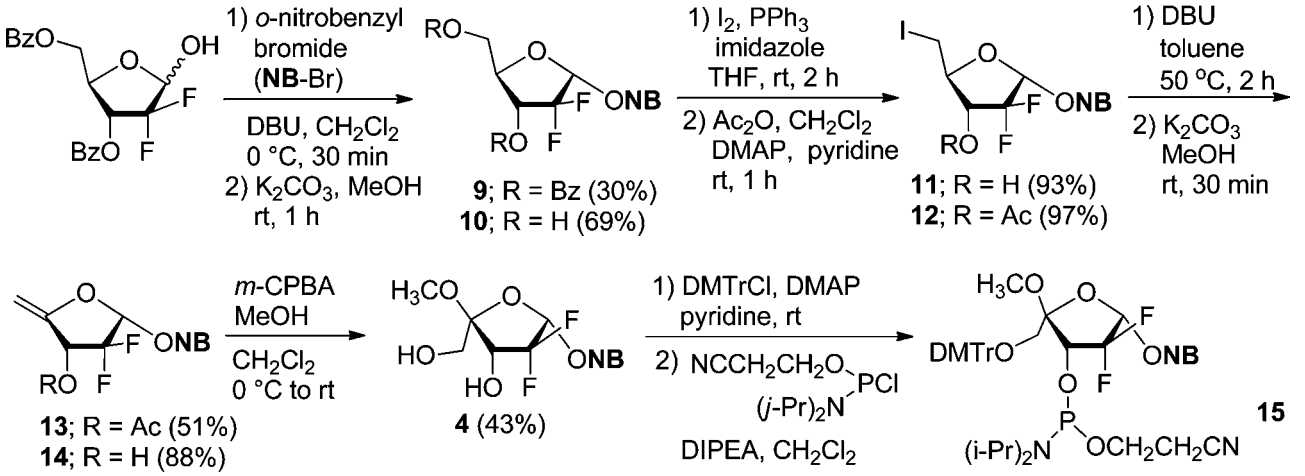
Figure 3. (a) HPLC analysis of the reaction of **5** and **7** (50 mM phosphate buffer; pH 8; 37 °C). **5**: retention time of 13 min. **7**: retention time of 16 min. 5′-d(ATAC)-3′ was added as an internal standard (IS) (retention time of 11 min). The cross-linked **17** and **19** were eluted at retention times of 19 and 17 min, respectively. (b) Monitoring of the reaction of **5** and **7** at pH 7 and 8 by denaturing PAGE analysis on a 20% polyacrylamide/8 M urea gel. (c) UV absorbance−temperature profile of **17** (red) and the duplex **5**:**7** before cross-linking (blue).

only a small amount of **19** was formed from **17** (Supporting Information).[17] These data indicated that the cross-linked product significantly increased the stability of the duplex compared to the duplex before the cross-link product had formed (T_m 42 °C). These data were similar to those pertaining to the cross-linking between the ODNs containing an abasic site and an alkyl amine, which was formed by reductive amination.[3] It was assumed that the cross-linked **19** might not have been formed from **17** by the elimination of the 5′-ODN fragment. The elimination of the 5′-ODN fragment from **5** was not observed during the course of the reaction.

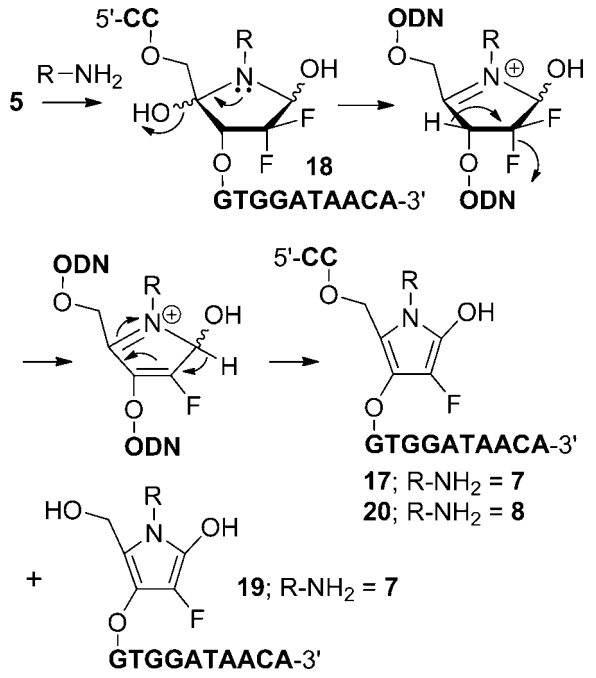
The reaction of **5** (20 μM in 50 mM phosphate buffer, pH 8) with excess *N*-6-aminohexyl dansylsulfonamide **8**[18] (2 mM) was also studied. When the reaction mixture was incubated at 37 °C and pH 8, the formation of a new product at the expense of **5** was observed by HPLC analysis (Supporting Information). The MALDI-TOFF MS data of the isolated product (m/z = 4182) indicated that its structure was that of product **20** (R-NH_2 = **8**; MW of **20** = 4182), which might have been formed from the intermediate **18** (R-NH_2 = **7**; MW = 4220) by the

(18) Narayanan, R.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1976**, *70*, 1122–1128.

Scheme 2. Synthesis of the Caged Sugar **4** and the Phosphoramidite **15**



Scheme 3. Reaction of ODN **5** with an Amine



elimination of H_2O (MW = 18) and HF (MW = 20). Although the reaction with the proximal amine was much faster, the reaction proceeded with moderate yield (66% at 24 h). During the reaction with **8**, products that were lacking the 5′-ODN fragment, such as **19**, were not observed. This reaction could be useful for synthesizing DNA conjugates of molecules that contain amines, even if the conjugates are not stable under reducing conditions.

In summary, we demonstrated effective amine modification by a ODN containing **3**. After generation of **3** by light, modification proceeds without the need for additional reagents. This method might be applicable to biological systems. Useful DNA conjugates of amine-containing molecules can be prepared under mild conditions using this technique.

Supporting Information Available. Experimental procedures and chracterization of compounds **4** and **9**–**15**; ^{1}H NMR spectra of **4** and **9**–**15**; NOESY spectra of **4** and **9**; ^{13}C NMR spectra of **4** and **15**; HPLC analyses of the photoreaction of **6**; the reaction of duplex **5**:**7**; heating of **17** to determine T_m; reaction of **5** with **8**; MALDI-TOF MS spectra of **6**, **5**, **17**, **19**, and **20**.This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.