doi:10.1246/cl.2010.1254

Published on the web October 30, 2010

Editor's Choice

Modified OligoDNA Having Two Consecutive Silylated-pyrene Moieties in Minor Groove Exhibiting an Excimer Fluorescent Signal upon Binding to Fully Complementary DNA Strand

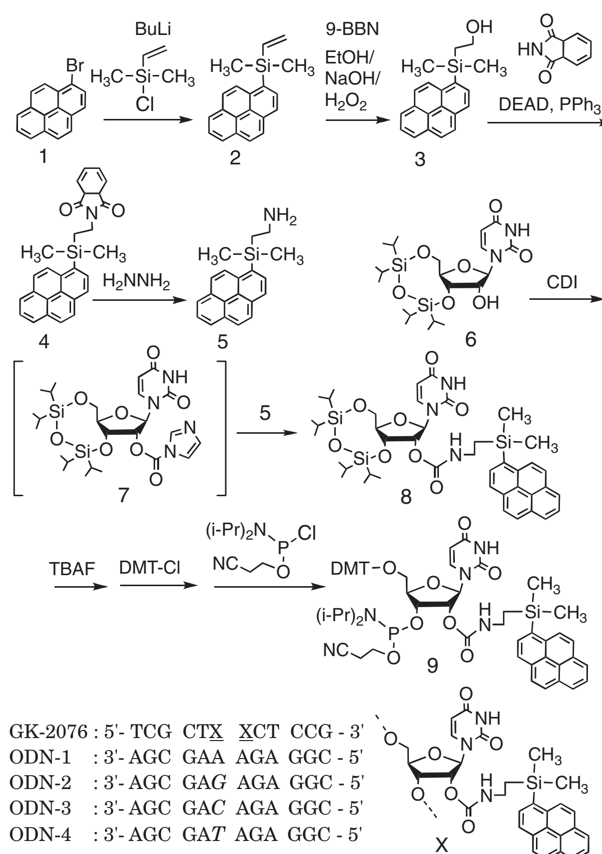
Mika Mogi,[1] Md. Gias Uddin,[1] Mayumi Ichimura,[1] Tomohisa Moriguchi,[1,2] and Kazuo Shinozuka*[1,2]
[1]*Department of Chemistry and Chemical Biology, Graduate School of Engineering, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515*
[2]*International Education and Research Center for Silicon Science, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515*

(Received September 6, 2010; CL-100758; E-mail: sinozuka@chem-bio.gunma-u.ac.jp)

OligoDNA possessing consecutive modified nucleotide residues bearing silylated pyrene at C-2′ position was synthesized. The fluorescent oligoDNA exhibited marked excimer fluorescent signal upon binding to a fully matched complementary DNA strand, however, the signal was effectively quenched in the single-stranded form as well as in the mismatched base pair duplex.

Sensitive and sequence specific detection of certain gene fragments are key technology for diagnostics and genetic studies. Fluorescent oligonucleotide probes have been intensively studied for the past decades as a tool to meet these demands and substituting conventional radio-labeled probes.[1] As a feasible gene-detecting tool, however, it is highly desirable that the probe exhibits a specific fluorescent signal only when it binds to the target oligonucleotide. To avoid false results, the probe should also have the capability of recognizing uncomplementary dissimilarities of the target, including single-nucleotide mismatches (SNPs). A probe possessing such ability would greatly simplify the detection process because of the abridgment of tedious separation and washing steps after the binding of the probe to the sample to be examined. Meanwhile, a common fluorescent material pyrene is known to form a characteristic excimer in an appropriate condition to emit bright fluorescence at around 460 nm. Several modified oligonucleotide probes utilizing pyrene to exhibit excimer fluorescence upon hybridization to their complementary oligonucleotides have been reported.[2] On the other hand, silylated pyrene bearing a modifiable functional group is a recently developed new derivative of pyrene capable of introduction into biological molecules such as nucleic acids and lipids.[3,4] The compound exhibits enhanced fluorescent quantum yield along with a bathochromic shift in absorption and emission, due to the specific σ–π interaction.[5,6] Thus, the compound is more advantageous as a fluorescent labeling agent compared to original pyrene. During the course of our study to investigate the utilities of silylated pyrene derivatives, we have found that a modified oligonucleotide having silylated pyrene moieties at the C-2′ position of neighboring uridine residues exhibits bright excimer-based fluorescent signal in the presence of fully complementary oligoDNA strand.[7] The signal was, however, strongly quenched in the single-stranded form and in the duplexes having mismatched base pairs.

To synthesize a modified oligonucleotide, we have applied C2′-functionalization via a carbamate function[8] utilizing 1,1′-carbonyldiimidazole (CDI) and a silylated pyrene derivative bearing primary amine function. The outline of the synthesis of the key compound, a modified uridine phosphoramidite bearing silylated pyrene at 2′-position through carbamate function (**9**), is summarized in Scheme 1.



Scheme 1. Synthesis of uridine phosphoramidite **9** bearing silylated pyrene at 2′-position.

1-Bromopyrene (**1**) was treated with *n*-BuLi in dry THF (−78 °C) under argon atmosphere followed by the addition of vinyldimethylsilyl chloride. The mixture was allowed to reach room temperature to give the corresponding silylated pyrene **2**. The obtained compound **2** was treated with 9-borabicyclo-[3.3.1]nonane (9-BBN) followed by the mixture of $EtOH/H_2O_2/NaOH/H_2O$ to give an hydroxyethyl derivative **3**. The reaction of **3** with phthalimide under the presence of diethyl azodicarboxylate and triphenylphosphine in dioxane gave the corre-

© 2010 The Chemical Society of Japan

sponding phthalimide derivative **4** and subsequent reaction of **4** with excess of hydrazine hydrate in EtOH afforded the desirable dimethylsilylated pyrene derivative bearing a primary amine function (**5**). It is worth noting that the fluorescent quantum yield of silylated pyrene **5** increased to 0.64 from 0.32 of unmodified pyrene, as it was reported previously.[3] This can be attributed to the Si-associated σ–π interaction.[5,6] Meanwhile, 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**6**) was treated with CDI in CH_2Cl_2 and resulting 2′-*O*-(imidazol-1-ylcarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**7**) was further reacted with compound **5** without isolation. The obtained compound **8** was subjected to the deprotection of 3′- and 5′-hydroxy groups, dimethoxytritylation of the 5′-hydroxy group and phosphoramidation of the 3′-hydroxy group to afford silylated pyrene-bearing uridine phosphoramidite **9**.[9] Incorporation of compound **9** into an oligoDNA was accomplished using an automated DNA synthesizer with a standard protocol with longer coupling period (360 min) compared with usual nucleoside phosphoramidite derivatives to achieve satisfactory coupling yield (ca. 92%). After the assembly, the support-bound oligoDNA was treated with concd ammonium hydroxide (60 °C, 12 h) followed by reversed-phase HPLC purification. The yield of the fluorescent oligoDNA bearing modified uridine residue **X** in two consecutive positions in the middle of the sequence (**GK-2076**) thus obtained was about 14%.[10] The sequence of **GK-2076** and its complementary DNA (**ODN-1**) along with the partial complementary strands (**ODN-2**, **-3**, and **-4**) having one-base substitution at the counter-position of **X** in **GK-2076** are shown in Scheme 1.

Fluorescent properties of **GK-2076** (1.5 µM) were examined in both a single-stranded form and a double-stranded form in buffer solution (10 mM sodium phosphate containing 100 mM NaCl) using excitation wavelength of 314 nm. Interestingly, fluorescence of silylated pyrene molecules in **GK-2076** is effectively quenched while the DNA stays as a single-stranded form. The fluorescent quantum yield of **GK-2076** alone was only about 0.04 under these conditions. In addition, the fluorescent signal we could observe in this form was mainly a monomer-state signal around 380 nm and a excimer-state signal around 480 nm was essentially absent. The excimer-to-monomer fluorescence intensity ratio,[2d] I_{ex}/I_m, in this form was 0.17. These are depicted in Figure 1a. The presence of the complementary DNA (**ODN-1**) in the solution containing **GK-2076**, however, brought about substantial change to the fluorescent signal. Thus, unlike the analogous-modified probe bearing unmodified-pyrene molecules at C-2′ position of neighboring uridine residues,[2c,2d] the addition of an equal amount of **ODN-1** to **GK-2076** at room temperature (20 °C) caused increase of excimer-state signal within 5 min up to about 5 times compared to that of the single-stranded form (Figure 1a). On the other hand, the monomer-state fluorescent signal was almost unchanged (I_{ex}/I_m, in this form was 0.68). It should be noted that these characteristic changes in the fluorescent signal can be easily recognized by the naked eye as shown in Figure 1b.

We further examined the effect of the one-base substitution in the complementary strand at the counter-position of **X** in **GK-2076** to the fluorescent signal. The fluorescent spectrum of the mixture containing **GK-2076** and **ODN-2**, in which an A-residue at the middle region of the sequence of **ODN-1** was substituted with G-residue, is also depicted in Figure 1a. As it is

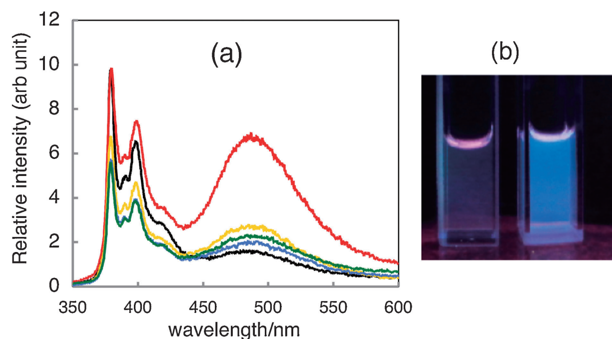


Figure 1. (a) Fluorescent spectra of **GK-2076** (black: **GK-2076** alone, red: **GK-2076 + ODN-1**, green: **GK-2076 + ODN-2**, blue: **GK-2076 + ODN-3**, orange: **GK-2076 + ODN-4**). (b) Fluorescent signal of **GK-2079** alone (left), **GK-2076 + ODN-1** (right) with the concentration of 1.5 µM.

clear from the spectrum, the increment of neither the monomer-state signal nor the excimer-state signal was observed in the mixture. The same results were obtained in mixtures containing other mismatched oligomers (**ODN-3** and **ODN-4**). At this moment, we could not obtain clear UV-melting curves of the duplexes examined in the study through usual UV-melting experiments and, therefore, we could not discuss the detailed mechanism of the observed characteristic fluorescent properties of **GK-2076**. It would be worth noting, however, that the monomer fluorescence of silylated pyrene is effectively quenched by thymine residue via photoinduced electron-transfer mechanism.[11] Thus, the current modified oligoDNA can also nicely discriminate a single-nucleotide substitution in the complementary DNA stand via its fluorescent signal and would be a practically feasible probe to detect certain gene fragments in solution by simple mixing.

References and Notes

1 L. J. Kricka, P. Fortina, *Clin. Chem.* **2009**, *55*, 670.
2 a) F. D. Lewis, Y. Zhang, R. L. Letsinger, *J. Am. Chem. Soc.* **1997**, *119*, 5451. b) E. Kostenko, M. Dobrikov, D. Pyshnyi, V. Petyuk, N. Komarova, V. Vlassov, M. Zenkova, *Nucleic Acids Res.* **2001**, *29*, 3611. c) K. Yamana, T. Iwai, Y. Ohtani, S. Sato, M. Nakamura, H. Nakano, *Bioconjugate Chem.* **2002**, *13*, 1266. d) P. J. Hrdlicka, B. R. Babu, M. D. Sørensen, N. Harrit, J. Wengel, *J. Am. Chem. Soc.* **2005**, *127*, 13293. e) I. V. Astakhova, V. A. Korshun, J. Wengel, *Chem.—Eur. J.* **2008**, *14*, 11010.
3 T. Sekiguchi, Y. Ebara, T. Moriguchi, K. Shinozuka, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6883.
4 T. Moriguchi, M. Hosaka, A. Yoshinari, A. Ozaki, T. Takeuchi, K. Shinozuka, *Chem. Lett.* **2009**, *38*, 966.
5 S. Kyushin, M. Ikarugi, M. Goto, H. Hiratsuka, H. Matsumoto, *Organometallics* **1996**, *15*, 1067.
6 H. Maeda, Y. Inoue, H. Ishida, K. Mizuno, *Chem. Lett.* **2001**, 1224.
7 A. Mahara, R. Iwase, T. Sakamoto, K. Yamana, T. Yamaoka, A. Murakami, *Angew. Chem., Int. Ed.* **2002**, *41*, 3648.
8 a) K. Seio, T. Wada, K. Sakamoto, S. Yokoyama, M. Sekine, *J. Org. Chem.* **1998**, *63*, 1429. b) I. Dubey, G. Pratviel, B. Meunier, *J. Chem. Soc., Perkin Trans. 1* **2000**, 3088. c) V. A. Korshun, D. A. Stetsenko, M. J. Gait, *J. Chem. Soc., Perkin Trans. 1* **2002**, 1092.
9 ^{31}P NMR ($CDCl_3$): 117.6, 148.3 ppm.
10 ESI-MS: 4225.9 (calcd 4226.2).
11 T. Moriguchi, M. Hattori, T. Sekiguchi, K. Shinozuka, *Nucleic Acids Symp. Ser.* **2009**, *53*, 31.

 © 2010 The Chemical Society of Japan