

## Expanded Fluorescent Nucleoside Analog as Hybridization Probe<sup>†</sup>

Young Jun Seo, Sankarprasad Bhuniya, Subhasish Tapadar, Jeong Wu Yi, and Byeang Hyeon Kim\*

Department of Chemistry, BK School of Molecular Science, Pohang University of Science and Technology, Pohang 790-784, Korea. \*E-mail: bhkim@postech.ac.kr

Received June 17, 2007

**Key Words :** Fluorescence, Nucleoside, Oligonucleotide, Hybridization probe

The design and synthesis of fluorescent nucleosides has been the subject of intensive research because these nucleoside derivatives can be used as tools in molecular biology and diagnostics.<sup>1</sup> Fluorescent nucleoside analogs that are sensitive to the local environment in DNA duplexes are attractive candidate probes for DNA hybridization and for investigating nucleic acid structure. They display a strong signal change upon hybridization with a target DNA,<sup>2</sup> and structural changes in DNA such as the formation of G-quadruplexes and i-motifs.<sup>3</sup> Although a broad range of substituted fluorescent dyes are suitable for labelling nucleic acids, rapid growth in this area requires new and efficient fluorescent nucleoside analogs.<sup>4</sup>

Our strategy to synthesize an efficient fluorescent analog is based on the use of expanded nucleobase analogs, which have good fluorescent properties such as high quantum efficiency and high sensitivity to the microenvironment. This system does not change the conformation of stable B-DNA and also yields higher duplex stability owing to the bulky hydrophobic planar structure.<sup>5</sup> In this context, we synthesized a new fluorescent nucleoside analog, 1-(2-Deoxy- $\beta$ -D-erythro-pentofuranosyl) benzothieno[3,2,-d]-pyrimidine 2,4(3H)-dione (**BTU**).

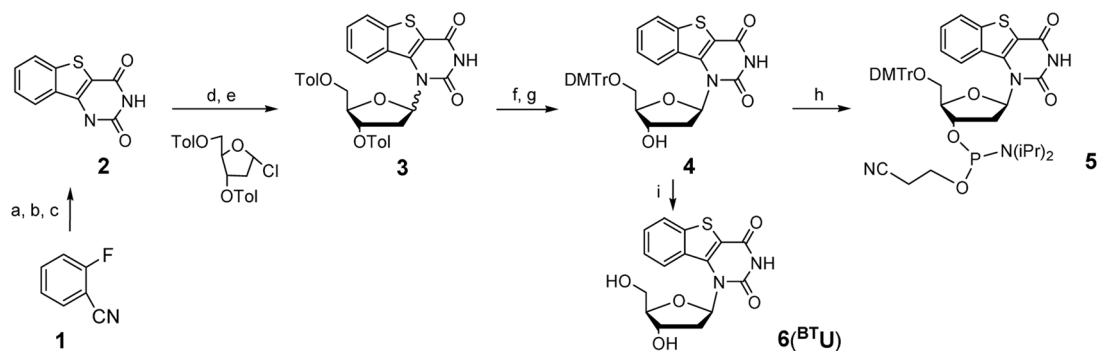
Scheme 1 shows our synthetic strategy to prepare the expanded fluorescent nucleoside **BTU**. Compound **2** was prepared from **1** in three steps, as shown in Scheme 1. The initial step is nucleophilic displacement of *o*-halobenzonitrile (2-fluorobenzonitrile) **1** with thioglycolate anion, followed by spontaneous base-induced aldol cyclization

with ethyl carboxyisocyanate and base treatment<sup>6</sup> to produce **2** as a white solid in an overall yield of 40% starting from compound **1**. Compound **2** was coupled to a sugar moiety to afford an unresolved anomeric mixture of compound **3** in 72%.<sup>7</sup> After detoluoylation, attempts to protect the 5'-position of the  $\alpha/\beta$  anomeric mixture with DMT-Cl failed. Thus, instead of DMT-Cl, 4,4'-dimethoxytrityl tetrafluoroborate (DMTBF<sub>4</sub>) in pyridine was employed for selective protection of the 5'-hydroxyl group of the nucleoside<sup>8</sup> to obtain the protected product. The 5'-ODMT anomeric mixture was separated on a flash silica gel column. A small amount of each anomer was deprotected using aqueous acetic acid to afford the free nucleosides, which were subjected to extensive spectroscopic analysis to determine the anomeric configuration unambiguously and to measure the quantum yield.

ROESY showed a correlation spot for H-1' and H-4', as expected for the  $\beta$  configuration of **6**. The  $\beta$  anomer of **4** was treated with standard phosphoramidite reagent<sup>9</sup> to yield the nucleoside 3'-*O*-phosphoramidite derivative, **5**.

To evaluate the sensitivity of **BTU** to its microenvironment, fluorescence spectra of **BTU** were measured in different solvents (Figure 1A). The emission intensity of **BTU** was markedly affected by solvent polarity. **BTU** showed higher quantum efficiency in polar than in non-polar solvents (Table 1).

We also measured the emission change for **BTU** at different pH values to evaluate its sensitivity. **BTU** exhibited different signals, depending on the pH (acidic, neutral, basic) (Figure 1B). This difference may arise from O-4 protonation in



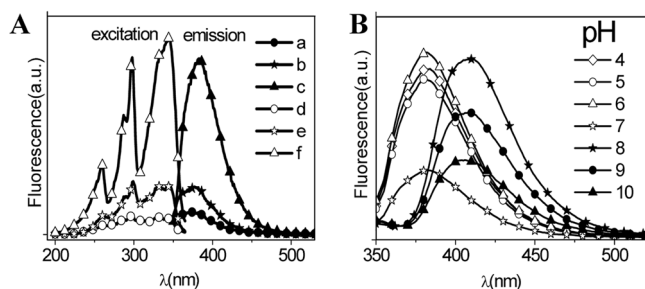
**Scheme 1.** Synthesis of the expanded fluorescent nucleoside **6** (**BTU**). Reagents: a) SHCH<sub>2</sub>COOC<sub>2</sub>H<sub>5</sub>, triethylamine, DMSO, 100 °C, 3 h, 70%; b) OCNCO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>, benzene, 0 °C to reflux, 3 h, 84%; c) 6% NaOH solution, 90 °C, 6 h, 67%; d) (CH<sub>3</sub>)<sub>3</sub>SiNHSi(CH<sub>3</sub>)<sub>3</sub>, CH<sub>3</sub>CONH<sub>2</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, reflux, 2 days; e) SnCl<sub>4</sub>, 1,2-dichloroethane, 0 °C, 1 h, 72%; f) NaOMe, MeOH, 3 h, rt, 85%; g) DMTrBF<sub>4</sub>, pyridine, rt, 5 h, 51%; h) 4-methoxypiperidine, MC, 2-cyanoethyl-*N,N'*-diisopropyl-chorophosphor amidite, 25 °C, 1 h, 90% yield; i) 80% AcOH, rt, 30 min, 77%.

<sup>†</sup>This paper is dedicated to Professor Sang Chul Shim in commemoration of his distinguished academic achievements.

**Table 1.** Photophysical data of <sup>BT</sup>U(6)<sup>a</sup>

nucleoside	Φ in Et <sub>2</sub> O	Φ in CH <sub>2</sub> Cl <sub>2</sub>	Φ in MeOH	λ <sub>ab</sub> MeOH (nm)	λ <sub>em</sub> MeOH (nm)
<sup>BT</sup> U	0.13	0.30	0.48	333,344	383

<sup>a</sup>Quantum efficiencies were determined using 9,10-diphenylanthracene as a standard; λ<sub>ex</sub> = 366 nm, 1 × 10<sup>-5</sup> M.<sup>10</sup>



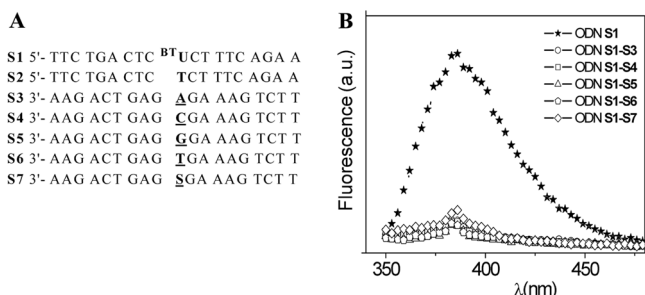
**Figure 1.** (A) Steady-state emission and excitation spectra of <sup>BT</sup>U in different solvents: (a, d) Et<sub>2</sub>O, (b, e) CH<sub>2</sub>Cl<sub>2</sub>, and (c, f) MeOH. (B) pH-dependent emission spectra of 1 × 10<sup>-5</sup> M solutions in distilled water of <sup>BT</sup>U recorded at 20 °C, λ<sub>ex</sub> = 340 nm, pH value is adjusted by 0.1 N HCl or NaOH aqueous solution.

acidic conditions<sup>11a</sup> and NH-3 deprotonation in basic conditions of the uracil moiety in <sup>BT</sup>U.<sup>11b</sup> These results confirm that <sup>BT</sup>U is very sensitive to changes in its microenvironment.

Next, the ODNs S1-S7 were designed and synthesized<sup>12</sup> to study the signaling properties of <sup>BT</sup>U in the hybridization state and its effect on duplex stability (Figure 2A). When ODN S1 was hybridized either to its perfectly matched complementary sequence (ODN S3) or to sequences with one mismatched base (ODN S4-ODN S7), the emission intensity was dramatically quenched (Figure 2B).

Interestingly, thermal denaturation curves of these <sup>BT</sup>U-containing oligonucleotide duplexes showed higher thermal stability compared to the duplex of unmodified ODN S2 (Table 2). We believe that the higher stability of <sup>BT</sup>U-containing duplexes can be attributed to intercalation of <sup>BT</sup>U in the duplex. Intercalation can lead to high stability and induce quenching of <sup>BT</sup>U in the duplex.<sup>13</sup>

In summary, we have designed and synthesized a novel nucleoside building block, <sup>BT</sup>U, which is very sensitive to



**Figure 2.** (A) ODNs synthesized, S: abasic site. (B) Emission spectra of ODN S1, ODN S1-S3, ODN S1-S4, ODN S1-S5, ODN S1-S6, and ODN S1-S7. were recorded using 1.5 μM solutions in buffer (10 mM Trizma HCl, 10 mM MgCl<sub>2</sub>, 100 mM NaCl, pH 7.2) at 20 °C, with λ<sub>ex</sub> = 340 nm.

**Table 2.** Melting temperature (T<sub>m</sub>) of ODNs<sup>a</sup>

	S3	S4	S5	S6	S7
S1	50 °C	49 °C	52 °C	51 °C	52 °C
S2	49 °C	43 °C	47 °C	46 °C	44 °C

<sup>a</sup>Recorded using 1.5 mM solutions in buffer (10 mM Trizma HCl, 10 mM MgCl<sub>2</sub>, 100 mM NaCl, pH 7.2) at 20 °C and 260 nm.

changes in microenvironment, such as polarity and pH. It also easily intercalates into duplex DNA and shows quenching on hybridization. These experimental observations can be utilized to design ODN probes to detect structural changes in DNA and in various biosensor applications.

**Acknowledgement.** We are grateful to KOSEF for financial support through the National Research Laboratory Program (Laboratory for Modified Nucleic Acid Systems), the Gene Therapy R&D program (M10534000011-05N3400-01110) and the KNRRC program.

## References

- (a) Brookes, A. J. *Gene* **1999**, 234, 177. (b) Collins, F. S.; Brooks, L. D.; Chakravarti, A. *Genome Res.* **1998**, 8, 1229. (c) Wojczewski, C.; Stolze, K.; Engels, J. W. *Synlett* **1999**, 1667.
- (a) Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *J. Am. Chem. Soc.* **2003**, 125, 9296. (b) Asanuma, H.; Kashida, H.; Liang, X.; Komiyama, M. *Chem. Commun.* **2003**, 1536.
- (a) Alberti, P.; Mergny, J.-L. *Proc. Natl. Acad. Sci. USA* **2003**, 100, 1569. (b) Jianwei, J. L.; Weihong, T. *Nano Lett.* **2002**, 2, 315. (c) Nagatoishi, S.; Nojima, T.; Juskowiak, B.; Takenaka, S. *Angew. Chem. Int. Ed.* **2005**, 44, 5067. (d) Liu, D.; Balasubramanian, S. *Angew. Chem. Int. Ed.* **2003**, 42, 5734. (e) Liedl, T.; Simmel, F. C. *Nano Lett.* **2005**, 5, 1894.
- Tan, W.; Wang, K.; Drake, T. J. *Curr. Opin. Chem. Biol.* **2004**, 8, 547.
- (a) Secrist, J. A. III; Barrio, J. R.; Leonard, N. J. *Science* **1972**, 175, 646-647. (b) Holmén, A.; Albinsson, B.; Nordén, B. *J. Phys. Chem.* **1994**, 98, 13460. (c) Liu, H.; Gao, J.; Lynch, S.; Maynard, L.; Saito, D.; Kool, E. T. *Science* **2003**, 302, 868. (d) Zeng, Y.; Cao, H.; Wang, Y. *Org. Lett.* **2006**, 8, 2527.
- Venugopal, B.; Desai, P. D.; de Souza, N. J. *J. Heterocyclic Chem.* **1998**, 25, 1633.
- Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, 39, 3654.
- Bleasdale, C.; Ellwood, S. B.; Golding, B. T. *J. Chem. Soc. Perkin Trans. I* **1990**, 803.
- (a) Seo, Y. J.; Rhee, H.; Joo, T.; Kim, B. H. *J. Am. Chem. Soc.* **2007**, 129, 5244. (b) Hwang, G. T.; Seo, Y. J.; Kim, B. H. *J. Am. Chem. Soc.* **2004**, 126, 6528. (c) Seo, Y. J.; Kim, B. H. *Chem. Commun.* **2006**, 150. (d) Seo, Y. J.; Jeong, H. S.; Bang, E. K.; Hwang, G. T.; Jung, J. H.; Jang, S. K.; Kim, B. H. *Bioconjugate Chem.* **2006**, 17, 1151. (e) Venkatesan, N.; Seo, Y. J.; Bang, E. K.; Park, S. M.; Lee, Y. S.; Kim, B. H. *Bull. Korean Chem. Soc.* **2006**, 27, 613. (f) Ryu, J. H.; Seo, Y. J.; Hwang, G. T.; Lee, J. Y.; Kim, B. H. *Tetrahedron* **2007**, 63, 3538. (g) Seo, Y. J.; Hwang, G. T.; Kim, B. H. *Tetrahedron Lett.* **2006**, 47, 4037.
- Morris, J. V.; Mahaney, M. A.; Huber, J. R. *J. Phys. Chem.* **1976**, 80, 969.
- (a) Raytchev, M.; Mayer, E.; Amann, N.; Wagenknecht, H. A.; Fiebig, T. *ChemPhysChem.* **2004**, 5, 706. (b) Chandra, A. K.; Nguyen, M. T.; Huyskens, T. Z. *J. Phys. Chem. A* **1998**, 102, 6010.
- (a) Gait, M. J. *Oligonucleotide Synthesis: A Practical Approach*; IRL Press: Washington, DC, 1984. (b) Seo, Y. J.; Ryu, J. H.; Kim, B. H. *Org. Lett.* **2005**, 7, 4931. (c) Kim, S. J.; Kim, B. H. *Nucleic Acids Research* **2003**, 31, 2725.
- Krueger, A. T.; Lu, H.; Lee, A. H. F.; Kool, E. T. *Acc. Chem. Res.* **2007**, 40, 141.