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Design of environmentally sensitive fluorescent 2′-deoxyguanosine containing arylethynyl moieties: Distinction of thymine base by base-discriminating fluorescent (BDF) probe

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ABSTRACT

We have synthesized various substituted 8-arylethynylated 2′-deoxyguanosine derivatives. Among them, acetyl substituted deoxyguanosine analogue **4c** showed a remarkable solvent dependent fluorescence property, that is, an intense fluorescence in non-polar solvents but extremely weak fluorescence in polar solvents like methanol. By using solvatofluorochromic deoxyguanosine analogue **4c**, we have developed highly thymine (T) selective fluorescent DNA probes that can sense T opposite **4c** in a target DNA regardless of the flanking sequences. We were able to demonstrate that **4c** can be used as a T specific base-discriminating fluorescent (BDF) nucleoside in homogeneous fluorescence assay.

Fluorescent nucleosides and oligonucleotides are powerful tools for structural studies of nucleic acids, sequencing, molecular diagnostics, and other applications relating genomics.[1] Particularly, the development of fluorescent nucleosides that can monitor local microenvironmental change around nucleic acids is extremely important for understanding biological events accompanying interbiomolecular interactions such as replication, transcription, expression activation, and inactivation. The number of reported fluorescent nucleosides has increased greatly in recent years, and numerous efforts to impart useful photophysical features upon natural nucleobases have been reported.[1,2]

In our continuous studies directed toward the development of practically useful fluorescent oligonucleotide probes, we reported a new concept for the design of important fluorescent nucleosides such as base-discriminating fluorescent (BDF) nucleosides[3,4] and microenvironment sensitive fluorescent nucleosides.[5]

In order to devise sensitive fluorescent nucleosides to their local environment, molecular design of highly solvent dependent fluorescent nucleobase is critically important. While 8-arylethynylated adenosine[6] and guanosine[7] are known, solvatochromic nature of such fluorescent ribonucleosides has not been reported. Through extensive studies of the substituent effects on 8-arylethynylated 2′-deoxyguanisine derivatives, we found that acetyl group on the aryl moiety induced a remarkable solvatofluorochromic property of the deoxyguanosine analogues. Unlike unsubstituted 8-arylethynylated deoxyguanosine, acetyl substituted deoxyguanosine analogue **4c** showed a remarkable solvent dependent fluorescence property. We report herein unique fluorescent 2′-deoxyguanosine analogues that are highly sensitive to their local environment. By using such fluorescent deoxyguanosine analogues, we have developed highly thymine (T) selective BDF probes that can sense T opposite BDF base in a target DNA regardless of the flanking sequences (Fig. 1).

We already reported pyrene labeled BDF nucleosides ^{py}U and ^{py}C that can distinguish A and G, respectively, opposite the BDF bases.[4] In contrast, adenosine type T specific BDF base previously reported[3d] was only effective in AT rich sequences due to the quenching by neighboring Gs. Environment sensitive fluorescent deoxyguanosine derivatives usable as a fluorescence sensor regardless of the flanking sequences are highly desirable for structural studies of nucleic acids and molecular diagnostics such as SNP genotyping.

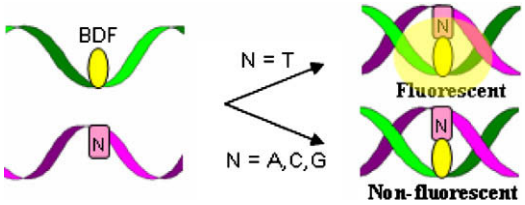

Figure 1. Schematic illustration of DNA detection using BDF nucleoside.

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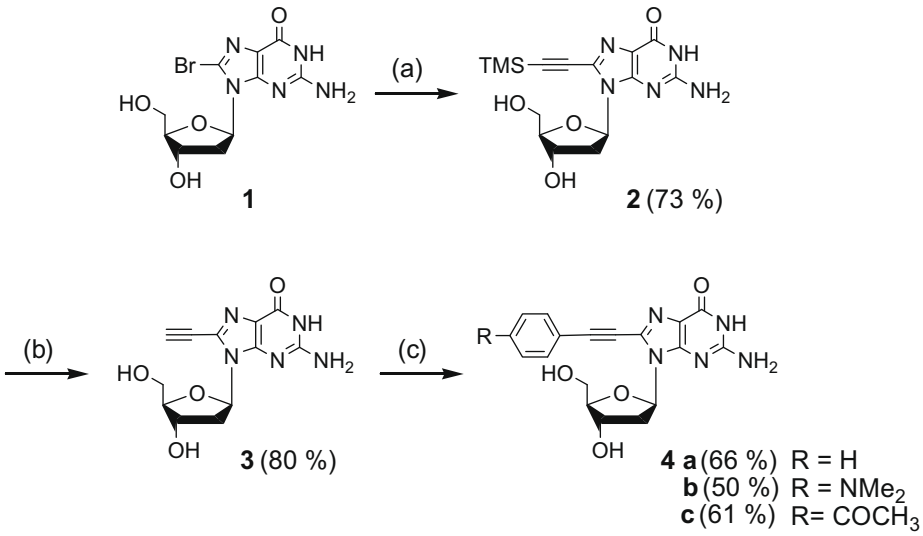


Scheme 1. Reagents and conditions: (a) trimethylsilyl acetylene, $Pd(PPh_3)_4$, TEA, DMF, 50 °C, 3 h; (b) TBAF, THF, rt, 2 h; (c) 4-iodobenzene, 4-dimethylaminoiodobenzene or 4-bromoacetophenone, $Pd(PPh_3)_4$, TEA, DMF, rt, 3 h.

8-Arylethynylated 2′-deoxyguanosine derivatives **4** were prepared via palladium (0) mediated Sonogashira cross-coupling (Scheme 1).[8] 8-Bromo-2′-deoxyguanosine **1** was coupled with trimethylsilyl acetylene to yield compound **2**. Silyl group of **2** was deprotected by tetrabutylammonium fluoride to give 8-ethynyl-2′-deoxyguanosine **3**. Compound **3** was coupled with substituted aryl halides to afford corresponding nucleosides **4a, 4b**, and **4c** in high yields.

We first examined the photophysical properties of 8-arylethynylated deoxyguanosine derivatives in various solvents. All these deoxyguanosines (**4a**, **4b**, **4c**) showed strong fluorescence at around 475 nm in non-polar solvents like ethyl acetate but relatively weak fluorescence in polar solvents like methanol and acetonitrile. Interestingly, fluorescence intensity of acetyl analogue **4c** showed a strong solvent dependency, that is, an intense fluorescence in non-polar solvent at 475 nm but extremely weak fluorescence in methanol, showing that introduction of acetyl group on the aromatic moiety induces a solvatochromic fluorescence property (Fig. 2c). Thus, we thought that solvatofluorochromic deoxyguanosine **4c** may be used as a fluorescence sensor for SNP genotyping.

Deoxyguanosine **4c** was then incorporated into oligodeoxynucleotides (ODNs) via automated DNA synthesis. After protection of amino group with *N*,*N*-dimethylformamide diethylacetal, **4c** was reacted with DMTrCl in the presence of catalytic amount of DMAP in dry pyridine to give **6**. Protected **6** was converted to phosphoramidite **7**, which was used for ODN synthesis by automated DNA/RNA synthesizer without further purification (Scheme 2). Two ODNs containing **4c** in AT rich site (ODN **1**) and GC rich site (ODN **2**) were prepared (Table 1).

Fluorescence spectra of ODN **1** and ODN **2** were measured in the absence and presence of complimentary strands. Fluorescence intensities of single stranded ODN **1** and ODN **2** and of the duplexes with complimentary strands ODN **3** and ODN **4** (N = A, G, C), respectively, were very weak. In contrast, the fluorescence spectrum of duplex ODN **1**/ODN **3** (N = T) showed an intense fluorescence at 510 nm in AT rich sequence. In GC rich sequence a similar T specific fluorescence emission was observed for duplex ODN **2**/ODN **4** (N = T), although the fluorescence intensity in GC rich sequence was negatively low due to the quench by neighboring Gs (Fig. 3a, b). The fluorescence emission was visible with naked eye only for T opposite BDF base **4c** under illumination with 365 nm transilluminator (Fig. 3c). In contrast, such T specific recognition opposite BDF base was not observed for ODNs containing **4a** and **4b**. Thus, acetyl substituted fluorescent deoxyguanosine **4c** is exceptionally useful as a T specific BDF base regardless of the

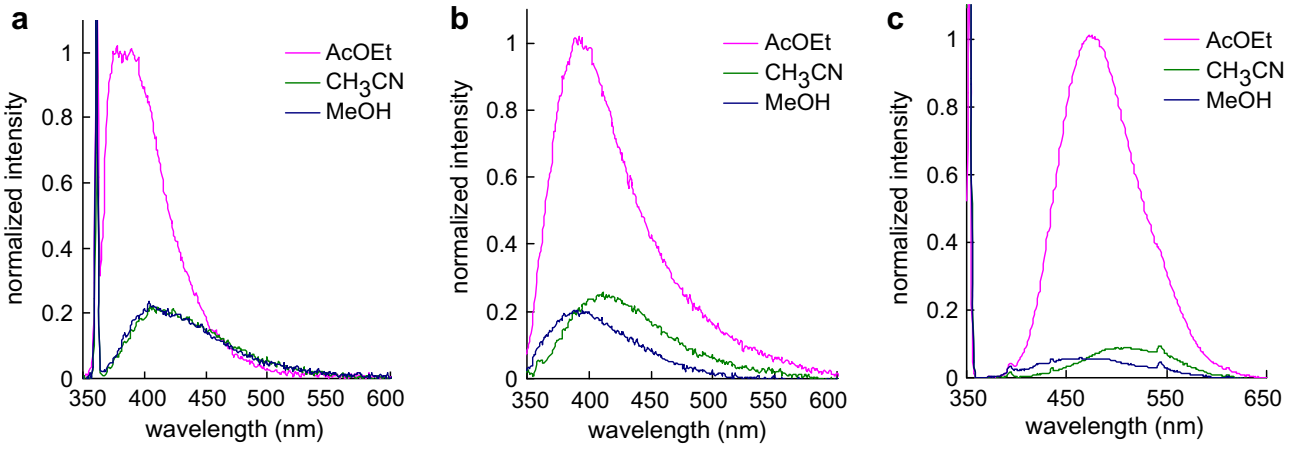
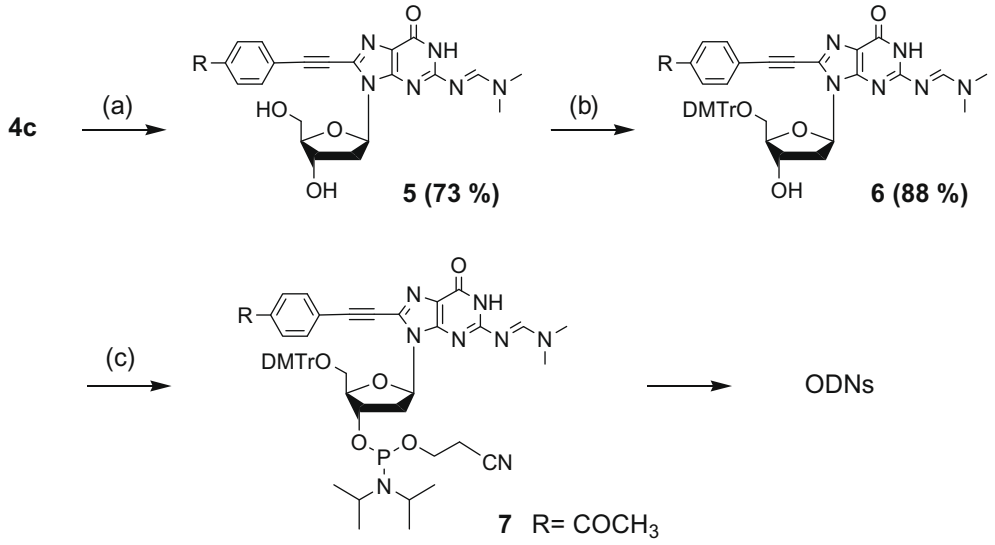


Figure 2. Fluorescence spectra of **4a** (a), **4b** (b) and **4c** (c) in various solvent (each 10 μM conc.). Excitation wavelength was 355 nm (a), 345 nm (b), and 350 nm (c).



Scheme 2. Reagents and conditions: (a) *N*,*N*-dimethylformamide diethylacetal, DMF, rt, 3 h; (b) DMTrCl, DMAP, pyridine, rt, 17 h; (c) *N*,*N*-diisopropylchlorophosphoramidite, TEA, CH_2Cl_2, rt, 15 min.

Table 1
Sequences of oligonucleotides

ODNs	Sequence
1	5′-d(CGCAATXTAACGC)-3′ (X = **4c**)
2	5′-d(CGCAACXCAACGC)-3′ (X = **4c**)
3	5′-d(GCGTTANATTGCG)-3′ (N = A, G, C, T)
4	5′-d(GCGTTGNGTTGCG)-3′ (N = A, G, C, T)

flanking sequences in a fluorescence hybridization assay. The reason for the T specific fluorescence emission is not clear at present. However, it seems likely that in base pairing of **4c** with T the chromophore of **4c** is located at a hydrophobic site inside the groove as suggested by the fact that intense fluorescence of **4c** appeared only in non-polar environment. Thus the microenvironment around **4c** when base paired with T is slightly different from those for other

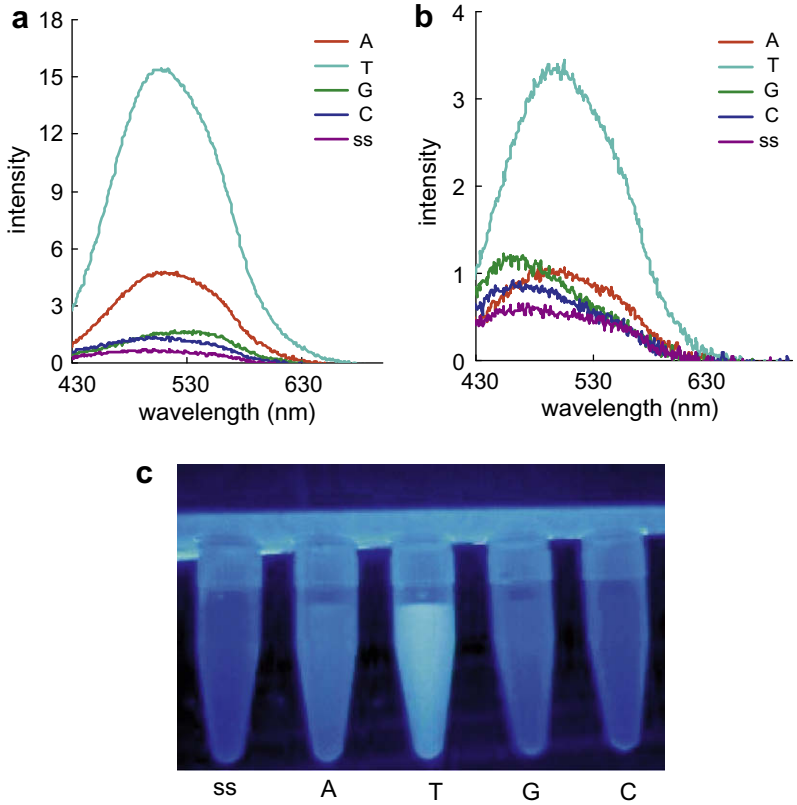


Figure 3. Fluorescence spectra of ODN **1** (a) and ODN **2** (b) together with those of the duplexes formed by hybridization with ODN **3** and **4** (N = A, T, G, C), respectively (2.5 μM ODNs, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). (c) Fluorescence image of ODN **1** (ss) and the duplexes formed with ODN **3** (N = A, T, G, C) under illumination with transilluminator (365 nm). 'ss' denotes single stranded ODN.

Table 2
Thermal melting behavior of ODNs[a]

X/N	ODN **1**/ODN **3** (°C)	ODN **2**/ODN **4** (°C)
4c/A	49.6	58.0
4c/G	51.5	58.1
4c/T	48.7	55.4
4c/C	52.5	59.3
G/C	58.7	63.8

[a] All T_ms of ODNs (2.5 μM conc.) were measured in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride.

bases (A, G) on the opposite site, not inconsistent with melting temperature experiments (Table 2).

In conclusion, we have succeeded in the molecular design of a solvatofluorochromic 2′-deoxyguanosine derivative for the first time. We found that acetyl substitution on the aromatic moiety of 8-arylethynylated 2′-deoxyguanosine induces a remarkable solvatofluorochromic property. We also demonstrated that fluorescent deoxyguanosine **4c** can be used as a thymine specific base-discriminating fluorescent (BDF) nucleoside in homogenous fluorescence assay. Fluorescence DNA probes containing BDF base **4c** are very useful for genetic analysis to detect point mutations and SNPs and may provide multiplexing capability.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.055.

References and notes

1. For reviews, see: (a) Hawkins, M. E. *Cell Biochem. Biophys.* **2001**, *34*, 257; (b) Rist, M. J.; Marino, J. P. *Curr. Org. Chem.* **2002**, *6*, 775; (c) Okamoto, A.; Saito, Y.; Saito, I. *J. Photochem. Photobiol. C: Photochem. Rev.* **2005**, *6*, 108; (d) Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487; (e) Venkatesan, N. Y.; Seo, J.; Kim, B. H. *Chem. Soc. Rev.* **2008**, *37*, 648.
2. Tor, Y. Ed., *Tetrahedron symposium in print*, **2007**, *63*.
3. (a) Okamoto, A.; Tainaka, K.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 4972; (b) Okamoto, A.; Tanaka, K.; Fukuda, T.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 4972; (c) Okamoto, A.; Tainaka, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820; (d) Saito, Y.; Hanawa, K.; Motegi, K.; Omoto, K.; Okamoto, A.; Saito, I. *Tetrahedron Lett.* **2005**, *46*, 7605; (e) Dohno, C.; Saito, I. *ChemBioChem* **2005**, *6*, 1075; (f) Saito, Y.; Bag, S. S.; Kusakabe, Y.; Nagai, C.; Matsumoto, K.; Kodate, S.; Suzuka, I.; Saito, I. *Chem. Commun.* **2007**, 2133; (g) Saito, Y.; Motegi, K.; Bag, S. S.; Saito, I. *Bioorg. Med. Chem.* **2008**, *16*, 107.
4. Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820.
5. (a) Tainaka, K.; Tanaka, K.; Ikeda, S.; Nishiza, K.-i.; Unzai, T.; Fujiwara, Y.; Saito, I.; Okamoto, A. *J. Am. Chem. Soc.* **2007**, *129*, 4776; (b) Saito, Y.; Miyauchi, Y.; Okamoto, A.; Saito, I. *Chem. Commun.* **2004**, 1704.
6. (a) Volpini, S.; Costanzi, S.; Lambertucci, C.; Vittori, S.; Klotz, K.-N.; Lorenzen, A.; Cristalli, G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1931; (b) Costanzi, S.; Lambertucci, C.; Vittori, S.; Volpini, R.; Cristalli, G. *J. Mol. Graphics Modell.* **2003**, *21*, 253; (c) Abou-Elkhair, R. A. I.; Netzel, T. L. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 85; (d) Seo, Y. J.; Rhee, H.; Joo, T.; Kim, B. H. *J. Am. Chem. Soc.* **2007**, *129*, 5244; (e) O'Mahony, G.; Ehrman, E.; Grøtli, M. *Tetrahedron* **2008**, *64*, 7151; (f) Abou-Elkhair, R. A. I.; Dixon, D. W.; Netzel, T. L. *J. Org. Chem.* **2009**, *74*, 4712.
7. (a) Sessler, J. L.; Sathiosatham, M.; Brown, C. T.; Rhodes, T. A.; Wiederrecht, G. *J. Am. Chem. Soc.* **2001**, *123*, 3655; (b) Mayer, E.; Valis, L.; Wagner, C.; Rist, M.; Amann, N.; Wagenknecht, H. A. *ChemBioChem* **2004**, *5*, 865; (c) Okamoto, A.; Ochi, Y.; Saito, I. *Chem. Commun.* **2005**, 1128; (d) Firth, A. G.; Fairlamb, I. J. S.; Darley, K.; Baumann, C. G. *Tetrahedron Lett.* **2006**, *47*, 3529.
8. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.