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Fluorometric detection of adenine in target DNA by exciplex formation with fluorescent 8-arylethynylated deoxyguanosine

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ABSTRACT

We demonstrated an intriguing method to discriminate adenine by incident appearance of an intense new emission via exciplex formation in hybridization of target DNA with newly designed fluorescent 8-arylethynylated deoxyguanosine derivatives. We described the synthesis of such highly electron donating fluorescent guanosine derivatives and their incorporation into DNA oligomers which may be used for the structural study and the fluorometric analysis of nucleic acids.

Fluorescent nucleosides and oligonucleotides are powerful tools for structural studies on nucleic acids, sequencing, molecular diagnostics, and other applications relating to genomics. Numerous efforts to impart useful photophysical features upon non-emissive native nucleobases have been reported.[1] Most of the previous approaches involve the linking of nucleobases to fluorescent aromatic or heteroaromatic chromophores via an ethynyl linker[2] or the direct attachment of fluorescent chromophores on the native nucleobases.[1,3] Particularly, the fluorescent nucleosides possessing fluorescence 'off-on' property are of special importance for the application to fluorometric assays of nucleic acids and in fluorescence imaging. The concepts of previous fluorescence 'off-on' assay of nucleic acids usually rely either on the attachment of fluorophore and quencher at both ends of a oligodeoxynucleotide (ODN) probe as exemplified by molecular beacon[4] or on the PET-based fluorescent probe.[5] We previously reported a different type of fluorescent nucleosides, base-discriminating fluorescent (BDF) nucleosides,[6] which when incorporated in a probe ODN showed an enhancement or quenching of emission intensities at a fixed wavelength depending on a base in a target DNA opposite to the BDF of the probe ODN. Recently, we reported environmentally sensitive fluorescent nucleosides such as 8-substituted deoxyadenosine and deoxyguanosine derivatives which indicate the difference in the local environment by a significant change of the emission wavelength as well as its intensity.[7] We now wish to report herein a unique approach to discriminate specific base such as adenine in a target DNA by incident appearance of a strong new emission at a long wavelength via exciplex formation with newly designed fluorescent electron donating 8-arylethynylated deoxyguanosine derivatives.[8]

Guanine is the most electron donating base among DNA bases but non-emissive. The attachment of aromatic chromophores such as phenyl or naphthyl group to the 8-position of non-emissive guanosine through a triple bond induced a strong fluorescence to provide easily accessible fluorescent guanine dreivatives.[2,7] Methoxy-substituted fluorescent deoxyguanosine derivatives **1b–d** have an intriguing property, that is, they possess lower oxidation potentials than native guanosine and strong fluorescence at around 400 nm. Oxidation potentials E_{ox}, (V vs SCE) obtained from cyclic voltammetry in acetonitrile were 1.17 (**1b**), 1.07 (**1c**) and 1.14 (**1d**) that are lower than that of 2′-deoxyguanosine (1.23 V). We expected that the stacking interaction between highly electron donating fluorescent deoxyguanosines **1b–d** and adenine base having a moderately electron accepting property in a DNA duplex may form an exciplex upon photoexcitation of the fluorescent deoxyguanosine. We report herein the synthesis of such electron donating fluorescent deoxyguanosine derivatives and their incorporation into DNA oligomers which were used as a BDF probe for the detection of adenine base fluorometrically.

8-Arylethynylated 2′-deoxyguanosine derivatives were prepared by Pd(0)-mediated cross-coupling as reported previously (Scheme 1).[7a] 8-Ethynyl-2′-deoxyguanosine **2**[7a] was coupled with various aryl iodides **3a–d** under Pd(0)-mediated cross-coupling conditions to provide corresponding fluorescent deoxyguanosine derivatives (**1a–d**). Deoxyguanosine analog **1a** was then incorporated into ODN via automated DNA synthesizer. After protection of amino group with *N,N*-dimethylformamide diethylacetal, **4a** was reacted with DMTrCl in the presence of catalytic amount of

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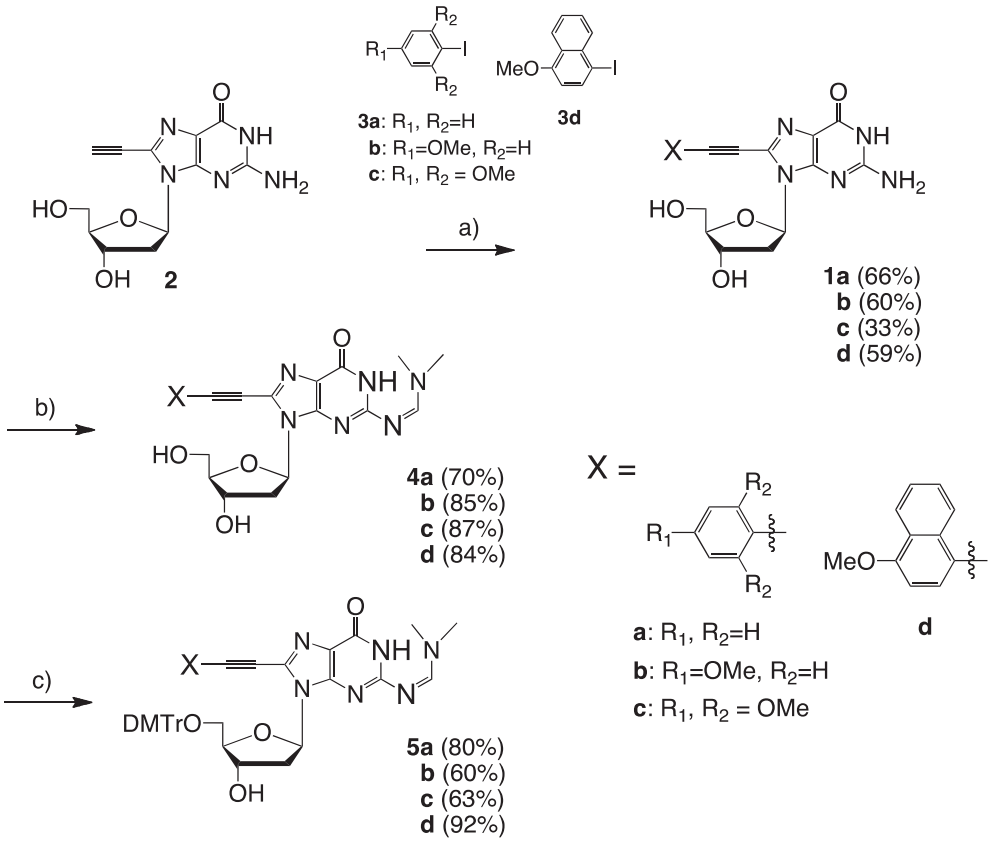


Scheme 1. Reagents and conditions: (a) Compounds **3a–d**, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 50 °C, 3 h; (b) *N*,*N*-dimethylformamide diethylacetal, DMF, rt, 3 h; (c) DMTrCl, DMAP, pyridine, rt, 17 h.

DMAP in dry pyridine to give **5a**. Phosphoramidite obtained from **5a** was used to prepare modified ODNs via automated DNA synthesizer. Other 8-arylethynylated guanosine derivatives **1b–d** were also incorporated into ODNs in a similar way. ODNs thus obtained were purified by HPLC and characterized by MALDI-TOF mass spectrometry.

After getting all the nucleosides in hand, we first examine their photophysical properties in organic solvents of varying polarity. The fluorescence spectra of monomer 8-arylethylated deoxyguanosine derivatives **1a** and **1b** in different solvents were shown in Figure 1. These deoxyguanosine derivatives exhibited strong fluorescence at around 390 nm but did not show solvatochromic behavior. Next, UV–visible absorption and fluorescence spectra of ODNs containing **1a–d** were examined. Fluorescence emission of single stranded ODN **I** (X = **1b**) containing **1b** in the middle of the strand was found to be relatively weak and appeared at ca. 390 nm. When the opposite base of a complementary strand is adenine (N = A) in duplex ODN **I** (X = **1b**)/ODN **II** (N = A), very strong new emission was observed at longer wavelength around 435 nm (Fig. 2).[10] The emission at 435 nm was not observed when the opposite bases are G, C and T, suggesting that the new emission is an exciplex emission from a complex formed between **1b** and adenine (A). In support of this hypothesis, a weak new absorption band in a longer wavelength region (ca. 360 nm) was observed in the UV–visible spectra of duplex ODN **I** (X = **1b**)/ODN **II** (N = A), indicating the formation of ground state complex between **1b** and A in the

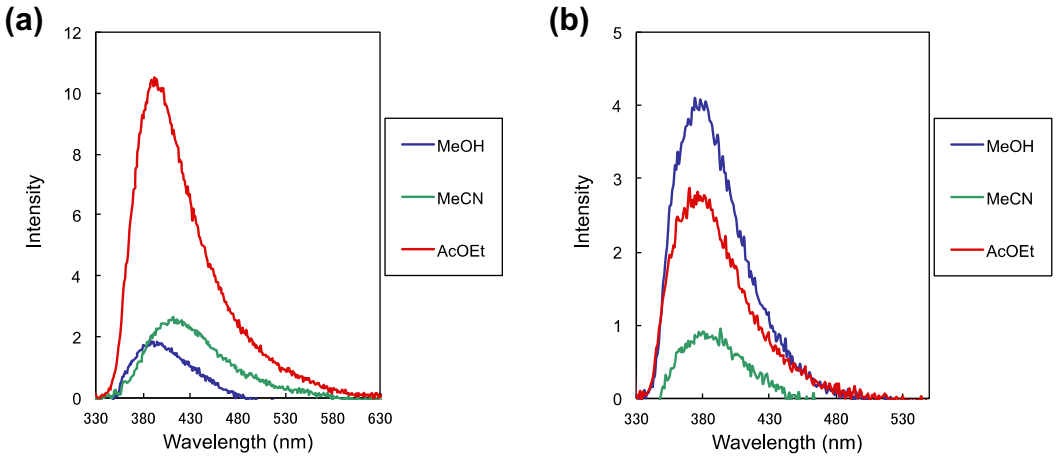
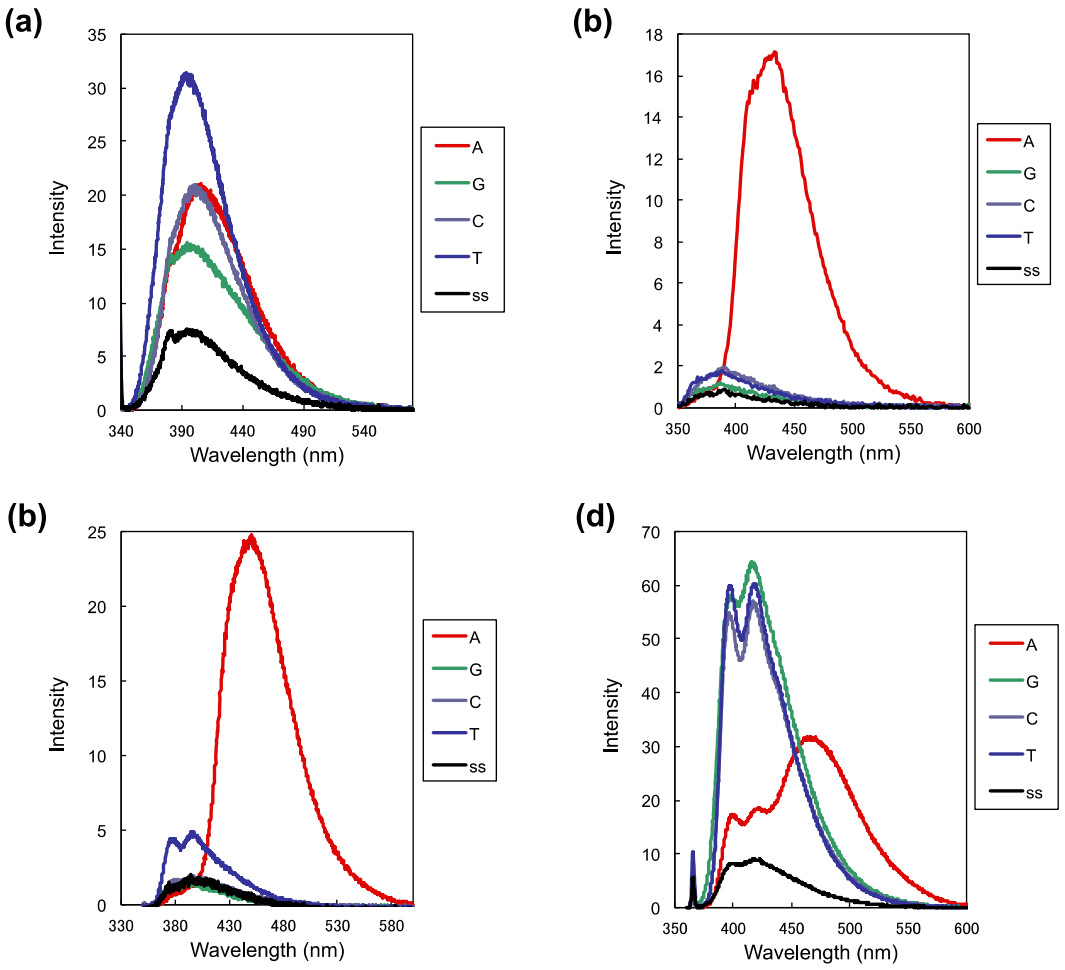


Figure 1. Fluorescence spectra of (a) **1a** (2.5 μM) and (b) **1b** (2.5 μM) in various solvents. Excitation wavelength was 325 nm.



ODN **I** : 5'-CGC AAT X TAA CGC-3' (X = **1a**, **1b**, **1c**, **1d**)

ODN **II** : 3'-GCG TTA N ATT GCG-5' (N = A, G, C, T)

Figure 2. Fluorescence spectra of (a) ODN **I** (X = **1a**), (b) ODN **I** (X = **1b**), (c) ODN **I** (X = **1c**) and (d) ODN **I** (X = **1d**) together with those of duplexes ODN **I**/ODN **II** formed by hybridization with complementary ODN **II** (N = A, T, G, C), respectively, (2.5 μM ODNs, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt).

duplex form (Supplementary data, Fig. S1-b). When ODN **I** (X = **1a**) containing unsubstituted phenyl group instead of **1b** was used, such exciplex emission has not been observed in hybridization with ODN **II** (N = A) (Fig. 2a), indicating a requirement of the electron donor substituent for the exciplex formation. A similar strong exciplex emission was also observed at ca. 450 nm with duplex ODN **I** (X = **1c**)/ODN **II** (N = A) containing trimethoxy-substituted **1c** (Fig. 2c). A more prominent example is the emission from methoxy-substituted naphthalene derivative **1d**. A characteristic fluorescence from the naphthalene chromophore appeared at 390–440 nm for single stranded ODN **I** (X = **1d**). However, in a duplex form [ODN **I** (X = **1d**)/ODN **II** (N = A)], a new strong exciplex emission at 470 nm emerged together with the appearance of original naphthalene fluorescence, suggesting a partial formation of excited state complex (Fig. 2d). The formation of ground state complex between the naphthalene chromophore and A was also indicated by the appearance of a new absorption band in the UV–visible spectrum of the duplex ODN **I** (X = **1d**)/ODN **II** (N = A) (Supplementary data, Fig. S1-d). The fluorescence color change depending upon the opposite base was readily observable by naked eye under illumination with 365 nm transilluminator (Fig. 3).

To evaluate the formation of exciplex in detail, we measured fluorescence lifetimes of single and double stranded ODN **I**

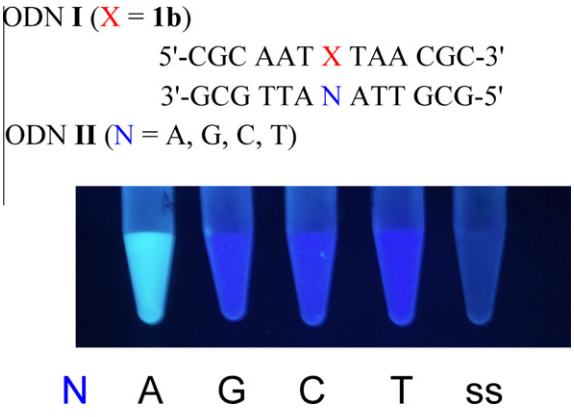


Figure 3. Comparison of the fluorescence colour change upon the bases opposite to **1b** in duplex ODN **I** (X = **1b**)/ODN **II** (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). 'ss' denotes as a single-stranded ODN **I** (X = **1b**). The sample solutions were illuminated with a 365 nm transilluminator.

(X = **1d**) and ODN **I** (X = **1d**)/ODN **II** (N = A), respectively. As shown in Table 1, longer lifetime component (τ = 2.2 ns) was ascribable to the formation of an exciplex between **1d** and A, whereas the shorter

Table 1
Fluorescence lifetimes of single stranded ODN **I** (X = **1d**) and duplex ODN **I**/ODN **II**[a]

Sample	τ_f (ns)
ODN **I** (X = **1d**)	0.90 (420 nm)
ODN **I** (X = **1d**)/ODN **II** (X = A)	1.1 (420 nm), 2.2 (470 nm)

[a] Experimental conditions: [ssODN or duplex ODN] = 3.0 μM in deaerated 50 mM sodium phosphate buffer, pH 7.0, 100 mM NaCl, λ_{ex} = 365 nm.

lifetime component (τ = 1.1 ns) was resulted from the naphthalene chromophore of **1d**. The study of fluorescence lifetime clearly indicated the existence of two excited species that are responsible for fluorescence emission and strongly suggests that the longer lifetime component with a strong emission at 470 nm is from the exciplex between **1d** and A. Actually, molecular modeling study of duplex ODN **I** (X = **1d**)/ODN **II** (N = A) by using MacroModel ver. 9.0 indicated a strong π-stacking between methoxynaphthalene moiety and adenine (Supplementary data, Fig. S2).

In conclusion, we have devised an unprecedented type of fluorescent probe containing highly electron donating fluorescent deoxyguanosine analogs which gave rise to the appearance of a strong new emission at longer wavelength via exciplex formation with adenine in hybridization with target DNA. Such electron donating deoxyguanosine analogs **1b,c,d** capable of forming excited state complexes specifically with adenine may be widely used as a fluoresce probe for structural study of DNA and RNA and for the fluorometric analysis of nucleic acids and fluorescent imaging.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.011.

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10. It should be noted that such adenine specific exciplex formation is sequence dependent. When the flanking base pair of fluorescent deoxyguanosine **1b** was G–C base pair, such A specific exciplex emission was not observed.