Bioorganic & Medicinal Chemistry Letters 23 (2013) 886–892

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Naphthalene-based environmentally sensitive fluorescent 8-substituted 2′-deoxyadenosines: Application to DNA detection

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ARTICLE INFO

*Article history:*
Received 3 October 2012
Revised 7 November 2012
Accepted 8 November 2012
Available online 6 December 2012

*Keywords:*
Nucleoside
DNA
Fluorescent probe

ABSTRACT

We synthesized various C8-naphthylethynylated 2′-deoxyadenosine derivatives and investigated their photophysical properties. Among them, cyano- and *N,N*-dimethylamino-substituted 8-naphthylethynylated 2′-deoxyadenosine derivatives ($^{cn}$**A** and $^{dn}$**A**) showed strong fluorescence with high quantum yields and a remarkable solvatofuorochromicity. In particular, fluorescence of *N,N*-dimethylamino-substituted $^{2,6dn}$**A** was not quenched by neighboring guanines (Gs) when incorporated in DNA duplexes, in contrast to $^{cn}$**A**. We developed a new fluorescent probe containing $^{2,6dn}$**A** that can be used for the detection of target DNA via a bulge formation regardless of the neighboring sequences.



Fluorescent molecules are powerful research tools since they are simple, inexpensive, highly sensitive, and non-invasive. They are used for bioimaging,[1] drug delivery,[2] sensing,[3] monitoring of chemical interactions of biomolecules,[4] gene detection,[5] diagnosis[6] and targeted therapeutics.[7] Thus, there is still a growing interest in the development of newly designed fluorescent molecules, particularly, environmentally sensitive fluorophores of high quantum yield. The design of such fluorophores that changes their emission wavelength and quantum yield in response to the change of local environments such as dielectric properties, structural variation, surrounding pH and viscosity is of special interest owing to their wide range of applications. Although numerous environmentally sensitive fluorescent molecules are known, only limited number of environmentally sensitive fluorescent (ESF) nucleosides have been reported recently.[8] Incorporation of fluorescent nucleosides that are sensitive to their local environment into oligonucleotide can provide useful tools for structural study of nucleic acids as well as for the detection of target gene and for single nucleotide polymorphism (SNP) typing.

In our research program directed toward the design of environmentally sensitive fluorescent (ESF) nucleoside,[9] we have recently reported various C8-arylethynylated 2′-deoxyguanosines.[9a,b,e] Among them, acetyl-substituted 8-arylethynylated deoxyguanosine $^{ac}$**G**, which contains an electron donor–acceptor system within a molecule, exhibited a remarkable solvent-dependent fluorescence property.[9a,b] While acetyl-substituted $^{ac}$**G** derivatives exhibited interesting solvatofluorochromic properties, their emission wavelengths and quantum yields are not satisfactory and remained to be improved. Further development of more sensitive solvatofluorochromic nucleosides of high quantum yields is apparently necessary. We report herein that C8-naphthylethynylated 2′-deoxyadenosines $^{cn}$**A** and $^{dn}$**A** possessing an intramolecular donor–acceptor system can serve as an excellent ESF nucleobase of remarkably high quantum yield. Owing to the differences in the redox potentials of adenine and guanine, adenine can act as either as an electron donor or as an acceptor, depending on the substituent on the naphthalene ring unlike C8-arylethynylated guanine derivatives (Fig. 1). The newly synthesized ESF nucleosides were incorporated into oligodeoxynucleotides (ODNs). Using these ESF nucleosides, a novel fluorescent probe that can detect target DNA via a bulge formation was developed.

Various substituted C8-naphthylethynylated 2′-deoxyadenosines **1a–c** were prepared via the palladium(0)-mediated Sonogashira cross-coupling reaction[10] (Scheme 1). 8-Ethynyl-2′-deoxyadenosine **3** prepared by a previous method[11] was coupled with 2-bromo-6-cyanonaphthalene **4a** and 2-bromonaphthalene **4c** to afford the corresponding C8-substituted 2′-deoxyadenosines $^{2,6cn}$**A** (**1a**) and $^{2n}$**A** (**1c**), respectively.[12] Because of the low reactivity of bromonaphthalene containing electron-donor substituents toward cross-coupling reactions, bromonaphthalene **4b** was converted to the corresponding iodide **5b**, which was then used in the Pd(0)-mediated cross-coupling reaction to afford $^{2,6dn}$**A** (**1b**).[12]

C8-substituted-2′-deoxyadenosines **2a–c** with 1,4-disubstituted naphthalenes were also synthesized by a similar route. 8-Ethynyl-2′-deoxyadenosine **3** was coupled with 1-bromo-4-cyanonaphthalene **6a** and commercially available 1-iodonaphthalene **7c** to afford $^{1,4cn}$**A** (**2a**) and $^{1n}$**A** (**2c**), respectively. Iodonaphthalene

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http://dx.doi.org/10.1016/j.bmcl.2012.11.029

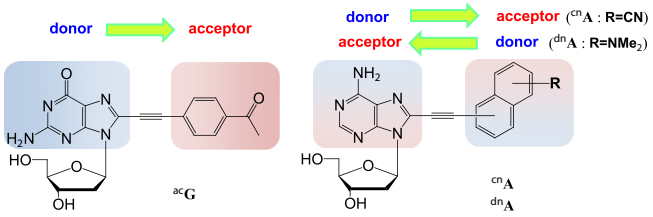


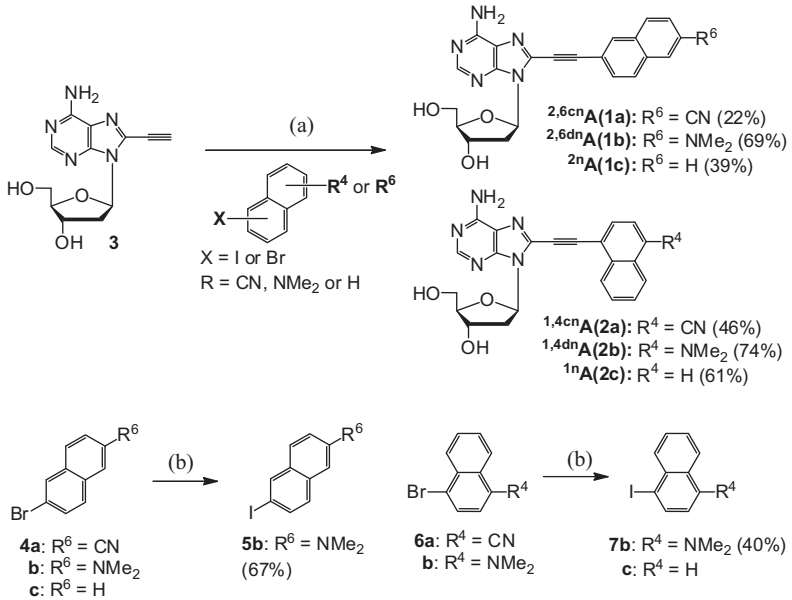

**Figure 1.** Structures of solvatofluorochromic nucleosides with donor–acceptor system.

**7b** prepared from **6b** was coupled with **3** using $Pd(PPh_3)_4$ to afford $^{1,4dn}$**A** (**2b**).

The photophysical properties of newly synthesized 2′-deoxyadenosine derivatives **1a–c** and **2a–c** were examined. Initially, we measured the fluorescence spectra of 2,6-disubstituted naphthalene derivative $^{2,6cn}$**A** (**1a**) containing an electron-withdrawing cyano substituent in various solvents of different polarities. By excitation of $^{2,6cn}$**A** (**1a**) at 340 nm in 1,4-dioxane, strong fluorescence emission was observed at 424 nm with a high quantum yield (0.82) as shown in Figure 2a. Fluorescence emission of $^{2,6cn}$**A** (**1a**) was observed at 454 nm in acetonitrile. In contrast, in polar solvents such as methanol (462 nm), DMF (478 nm), and DMSO (490 nm), weaker fluorescence emissions were observed for $^{2,6cn}$**A** (**1a**) at longer wavelengths. Thus, $^{2,6cn}$**A** (**1a**) exhibited a typical solvatofluorochromocic behavior with a considerable large solvatofluorochromicity ($\Delta\lambda$ = 66 nm). Interestingly, a similar but much larger solvatofluorochromicity ($\Delta\lambda$ = 71 nm) was observed with $^{2,6dn}$**A** (**1b**) bearing an electron donor substituent (Fig. 2b). While C8-naphthylethynylated 2′-deoxyadenosine derivatives $^{2,6cn}$**A** (**1a**) and $^{2,6dn}$**A** (**1b**) exhibited highly solvatofluorochromic properties, non-substituted naphthalene derivative $^{2n}$**A** (**1c**) did not show a clear solvatofluorochromic behavior, indicating the requirement of an intramolecular donor–acceptor system within a molecule for the solvatofluorochromicity (Fig. 2c, Table 1). In case of electron-withdrawing cyano-substituted naphthalene $^{2,6cn}$**A** (**1a**), adenine moiety acts as an electron donor. In contrast, adenine moiety can act as an electron acceptor in $^{2,6dn}$**A** (**1b**), which contains an electron-donating *N,N*-dimethylamino group. It is assumed that such an intramolecular donor–acceptor system is the origin for the solvatofluorochromic effects.

The photophysical properties of C8-substituted-2′-deoxyadenosines with 1,4-disubstituted $^{1,4cn}$**A** (**2a**) and $^{1,4dn}$**A (2b)** and non-substituted $^{1n}$**A** (**2c**)-naphthalenes were also investigated. As shown in Figure 3, very similar results to those observed for 2,6-disubstituted naphthalene derivatives were obtained. The fluorescence wavelengths of non-substituted $^{1n}$**A** (**2c**) were not changed remarkably by changing solvent polarity, showing almost no solvatochromocity. In contrast, $^{1,4cn}$**A** (**2a**) and $^{1,4dn}$**A** (**2b**) exhibited very strong fluorescence emissions in non-polar solvents but only weak red-shifted fluorescence emission in polar solvents, indicating that both $^{1,4cn}$**A** (**2a**) and $^{1,4dn}$**A** (**2b**) are effective ESF nucleosides of a considerable solvatofluorochromicity (**2a**: $\Delta\lambda$ = 71 nm; **2b**: $\Delta\lambda$ = 36 nm). Interestingly, each of 2,6- and 1,4-disubstituted naphthalene derivatives $^{2,6cn}$**A** (**1a**), $^{2,6dn}$**A** (**1b**), $^{1,4cn}$**A** (**2a**), and $^{1,4dn}$**A** (**2b**) exhibited high solvatofluorochromic properties. Among them, $^{2,6dn}$**A** (**1b**) bearing an electron donating *N,N*-dimethylamino substituent is a very attractive ESF nucleoside that has a high quantum yield (0.88 in dioxane) and may probably be usable regardless of the flanking sequences, because unlike cyano-substituted $^{2,6cn}$**A** (**1a**), electron donor substituted naphthalene $^{2,6dn}$**A** (**1b**) may not be a quenchable fluorophore by neighboring Gs when incorporated in duplex DNA. It should be reminded here that *N,N*-dimethylamino substituted $^{2,6dn}$**A** (**1b**) exhibit strong fluorescence at nonpolar environments but only weak fluorescence in polar aqueous solvents at longer wavelength (Fig. 2b).

Next, to study the photophysical properties of these ESF nucleosides in duplex DNA, $^{2,6cn}$**A** (**1a**), $^{2,6dn}$**A** (**1b**), $^{1,4cn}$**A** (**2a**), and $^{1,4dn}$**A** (**2b**) were incorporated into oligodeoxynucleotides (ODNs) via automated DNA synthesis. The synthetic route to the corresponding phosphoramidites of the 2,6-disubstituted naphthylethynylated 2′-deoxyadenosines is indicated in Scheme 2. After protection of the amino group with *N,N*-dimethylformamide diethylacetal, compounds of general formula **8** were reacted with DMTrCl in the presence of catalytic amount of DMAP in dry pyridine to give **9**. The protected compounds **9** were then converted to phosphoramidites **10**. Using a similar protocol, phosphoramidites of 1,4-disubstituted naphthylethynylated 2′-deoxyadenosines were also prepared (Supplementary data, Scheme S1). Each phosphoramidite




**Scheme 1.** Reagents and conditions: (a) **4a,c**, **5b**, **6a**, **7b**, or **7c**, $Pd(PPh_3)_4$ CuI, $Et_3N$, DMF, 50–80 °C, 30 min–3 h; (b) *n*-BuLi, THF, −78 °C, 30 min and then $I_2$, THF, rt, 30 min.

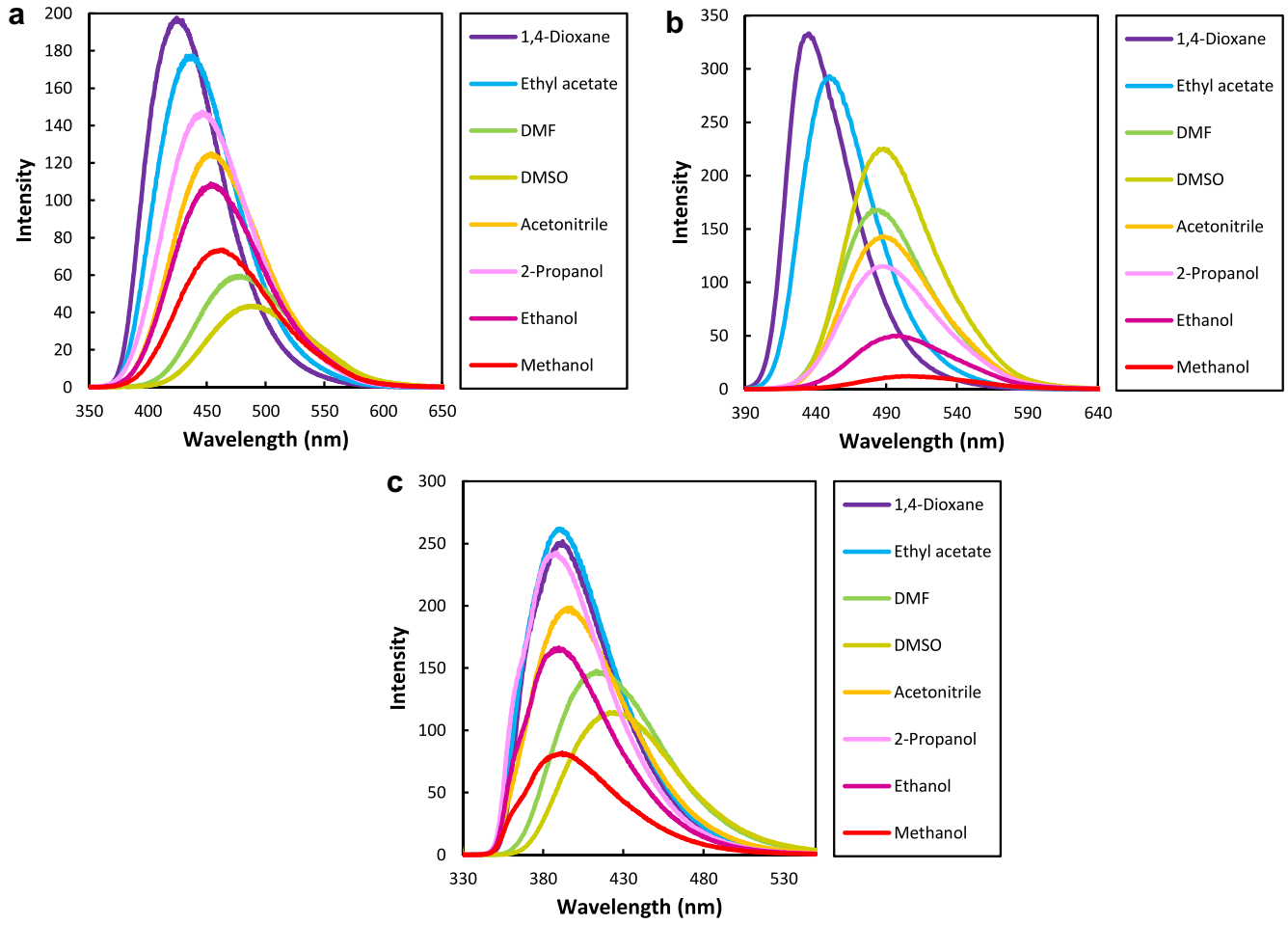



**Figure 2.** Fluorescence spectra of (a) $^{2,6cn}$**A** (**1a**, 10 μm), (b) $^{2,6dn}$**A** (**1b**,10 μm) and (c) $^{2n}$**A** (**1c**, 10 μm) in various solvents. The excitation was conducted at (a) 340 nm, (b) 382 nm and (c) 326 nm. (slit width: 1.5).

**Table 1**
Photophysical properties of fluorescent 2'-deoxyadenosine derivatives

| Compound | Solvent | $\lambda^{abs}_{max}$ (nm) | $\lambda^{fl}_{max}$ (nm) | $\Phi_{fl}$ |
|---|---|---|---|---|
| $^{2,6cn}$**A** (**1a**) | 1,4-Dioxane | 347 | 424 | 0.82 |
| | Acetonitrile | 338 | 454 | 0.58 |
| | DMF | 348 | 478 | 0.33 |
| | Methanol | 338 | 462 | 0.36 |
| $^{2,6dn}$**A** (**1b**) | 1,4-Dioxane | 360 | 435 | 0.88 |
| | Acetonitrile | 365 | 488 | 0.51 |
| | DMF | 385 | 480 | 0.62 |
| | Methanol | 385 | 506 | 0.05 |
| $^{2n}$**A** (**1c**) | 1,4-Dioxane | 330 | 392 | 0.66 |
| | Acetonitrile | 325 | 398 | 0.57 |
| | DMF | 333 | 414 | 0.51 |
| | Methanol | 328 | 392 | 0.20 |
| $^{1,4cn}$**A** (**2a**) | 1,4-Dioxane | 362 | 450 | 0.51 |
| | Acetonitrile | 357 | 482 | 0.14 |
| | DMF | 365 | 503 | 0.04 |
| | Methanol | 359 | 494 | 0.04 |
| $^{1,4dn}$**A** (**2b**) | 1,4-Dioxane | 371 | 442 | 0.41 |
| | Acetonitrile | 371 | 465 | 0.40 |
| | DMF | 375 | 464 | 0.47 |
| | Methanol | 374 | 478 | 0.05 |
| $^{1n}$**A** (**2c**) | 1,4-Dioxane | 339 | 397 | 0.52 |
| | Acetonitrile | 335 | 401 | 0.40 |
| | DMF | 340 | 424 | 0.36 |
| | Methanol | 336 | 391 | 0.20 |

was used for ODN synthesis using an automated DNA synthesizer. Two 13 mer fluorescent ODN probes containing ESF nucleoside X at AT rich site (ODN 1) and at GC rich site (ODN 2) were synthesized (Table 2).

As predicted by molecular modeling, adenine base containing large substituents such as naphthylethynyl group at C8 position cannot form Watson–Crick AT base pair when hybridized with complementary strand, because the steric bulkiness of the naphthylethynyl group is repulsive to sugar backbone and the *syn*-conformation of the adenine glycoside bond is generally preferable for adenines with large substituents at C8 position. In fact, melting temperature of full matched 13 mer duplex ODN 1(X = $^{2,6cn}$**A**)/3′-GCGTTA**T**ATTGCG-5′ (13 mer) is 9.3 °C lower than that of the native duplex (52.6 °C) containing unmodified ODN1(X = A), suggesting a lack of base pairing between $^{2,6cn}$**A** and T to result in the observed large destabilization of the duplex (Supplementary data, Table S1). Thus, 8-naphthylethynylated adenosines such as $^{2,6cn}$**A** (**1a**) and $^{2,6dn}$**A** (**1b**) cannot form AT base pair with opposite T in hybridization with a complementary strand and such ESF nucleosides are not usable as a modified adenine base opposite to T in a fluorescence hybridization assay. We, therefore, selected 12 mer blODN 1 as a target ODN that may form a bulge structure at X in hybridization with ODN1 (X). For GC rich sequence, ODN2 (X) and 12 mer blODN 2 were selected. Examples of such ODNs are summarized in Table 2.

First, the fluorescence of cyano-substituted ESF nucleoside ($^{2,6cn}$**A**)-containing 13 mer ODN was examined. The fluorescence

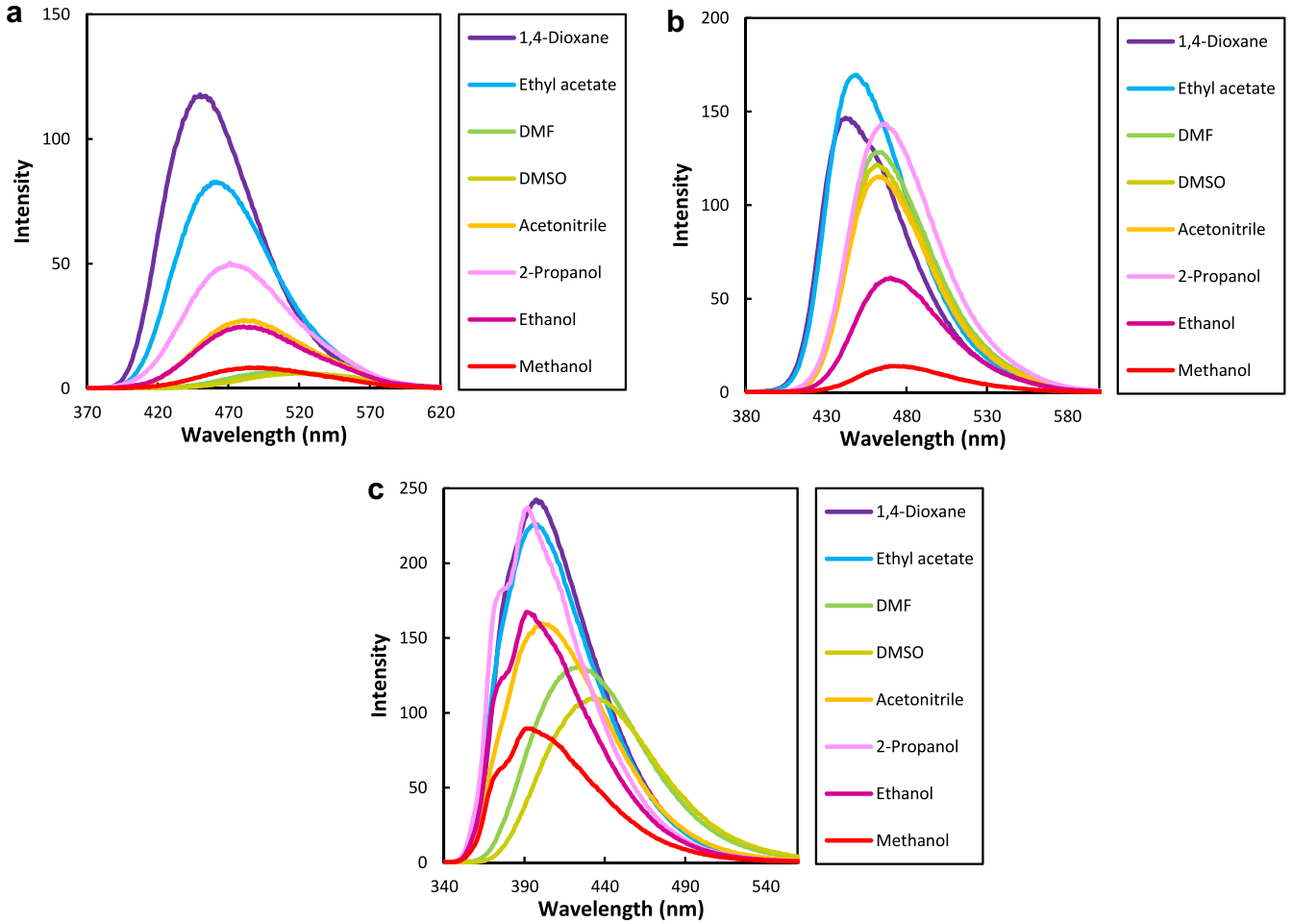
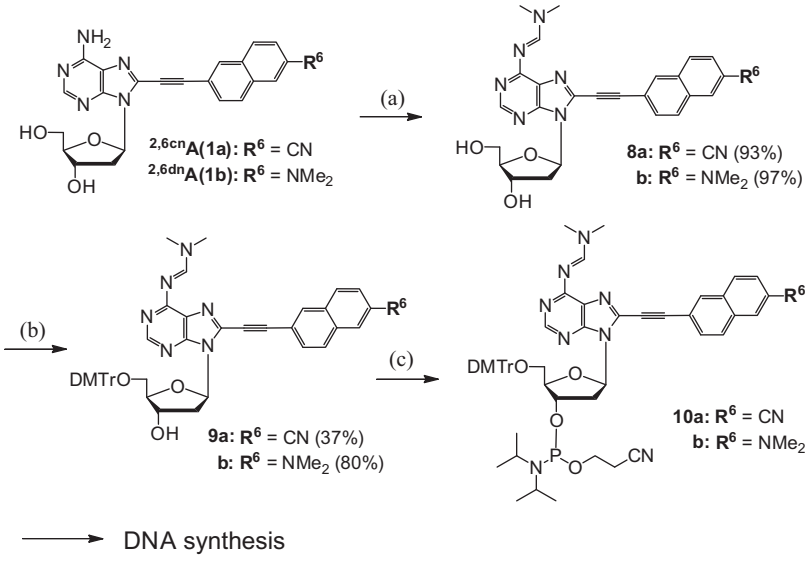



**Figure 3.** Fluorescence spectra of (a) $^{1,4cn}$**A** (**2a**, 10 μm), (b) $^{1,\ 4dn}$A (**2b**, 10 μm) and (c) $^{1n}$**A** (**2c**, 10 μm) in various solvents. The excitation was conducted at (a) 360 nm, (b) 375 nm and (c) 337 nm. (slit width: 1.5).




**Scheme 2.** Reagents and conditions: (a) DMF acetal, DMF, 80 °C, 30 min; (b) DMTrCl, DMAP, pyridine, rt, 3 h; (c) 2-cyanoethyldiisopropylchlorophosphoramidite, $Et_3N$, acetonitrile, rt, 30 min.

spectra of single-stranded ODN1 ($^{2,6cn}$**A**) exhibited relatively weak fluorescence at 446 nm. Then, the fluorescence of ODN1 ($^{2,6cn}$**A**) when hybridized with a complementary 12 mer blODN1 forming a bulge structure at $^{2,6cn}$**A** was examined. Strongly enhanced fluorescence emission appeared with a maximum at 446 nm (Fig. 4a), suggesting that the naphthalene fluorophore is located at the

**Table 2**
Oligonucleotides (ODNs) used in this study

| ODNs | Sequences |
|---|---|
| ODN1 (**x**) | 5′-d(CGCAATXTAACGC)-3′ **X** = $^{cn}$**A** or $^{dn}$**A** (13 mer) |
| ODN2 (**x**) | 5′-d(CGCAACXCAACGC)-3′ **X** = $^{cn}$**A** or $^{dn}$**A** (13 mer) |
| b1ODN1 | 3′-d(GCGTTAATTGCG)-5′ (12 mer, bulge) |
| b1ODN2 | 3′-d(GCGTTGCTTGCG)-5′ (12 mer, bulge) |

hydrophobic site in the bulge structure as illustrated in Figure 4e. On the other hand, when the fluorescence probe ODN2 ($^{2,6cn}$**A**) for GC rich sequence was used, the fluorescence emission was completely quenched by the neighboring C/G base pairs in the bulge formed in duplex ODN2 ($^{2,6cn}$**A**)/blODN2 (Fig. 4c). This result showed that the fluorescence emission of $^{2,6cn}$**A** in DNA is completely quenched by neighboring guanines (Gs) in a bulged structure, indicating that there is a strict sequence limitation for the use of cyano substituted ESF nucleoside fluorescence $^{2,6cn}$**A**.

Then, the fluorescence spectrum of the electron-donating *N,N*-dimethylamino-substituted $^{2,6dn}$**A**-containing oligodeoxynucleotide ODN1($^{2,6dn}$**A**) was examined in the absence and presence of bulge-forming 12 mer blODN1. As shown in Figure 4b, the fluorescence intensity of single-stranded ODN1($^{2,6dn}$**A**) was weak at 520 nm, probably because the naphthalene fluorophore is extruded to a polar aqueous solvent. In contrast, the fluorescence of the duplex ODN1($^{2,6dn}$**A**)/blODN1 (bulge) was enhanced 3.6-folds with the fluorescence maximum being red-shifted to 537 nm

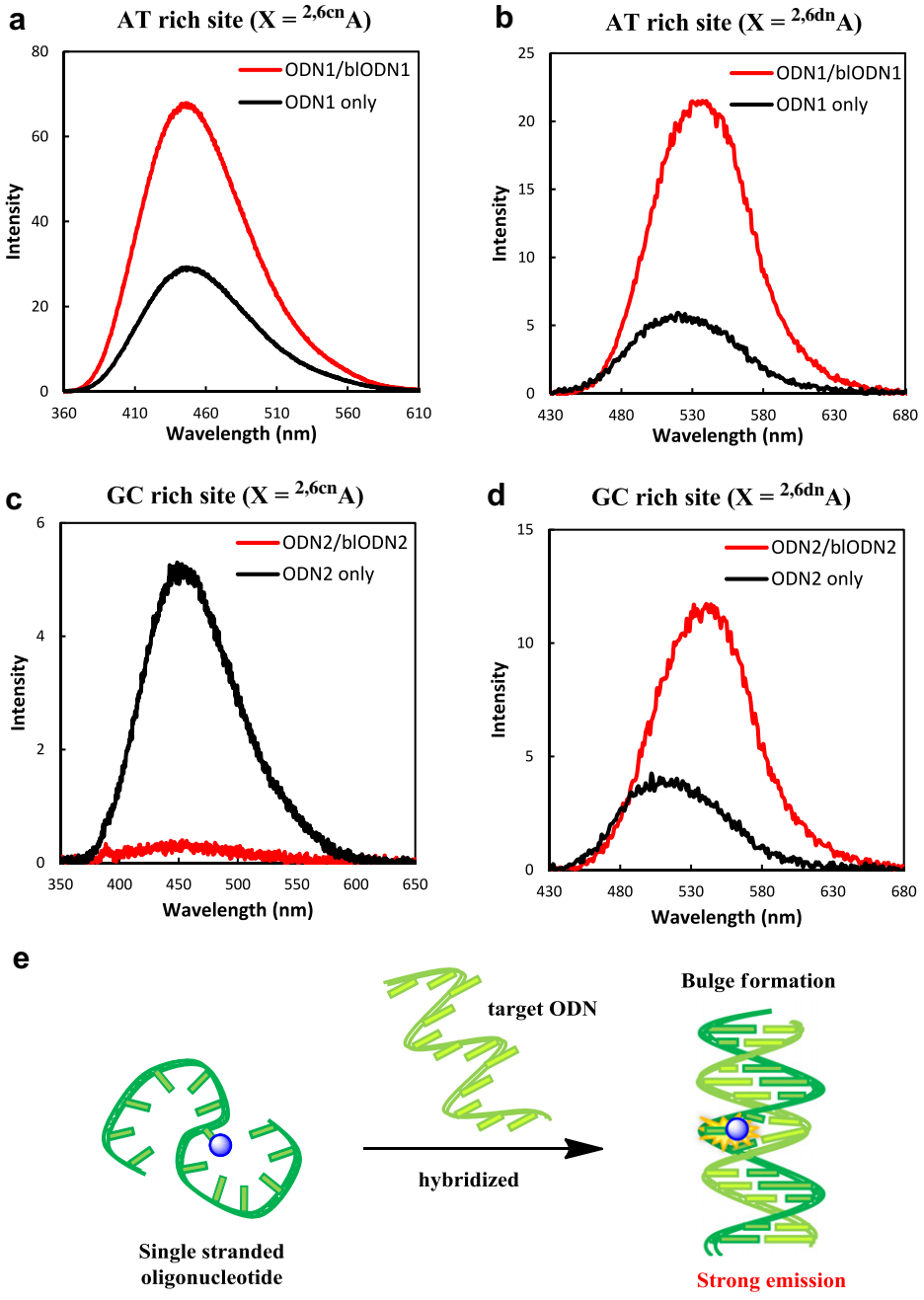



**Figure 4.** Fluorescence spectra of ODN1 [**X** = $^{2,6cn}$**A** (a) and $^{2,6dn}$**A** (b)] hybridized with b1ODN1 and of ODN2 [**X** = $^{2,6cn}$**A** (c) and $^{2,6dn}$**A** (d)] hybridized with blODN2 (2.5 μm ODNs, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). The excitation wavelength was (a) 349 nm, (b) 420 nm, (c) 342 nm, and (d) 420 nm; (e) schematic illustration of the target DNA detection by $^{2,6dn}$**A**-containing ODN probe via a bulge formation. (slit width: 1.5).

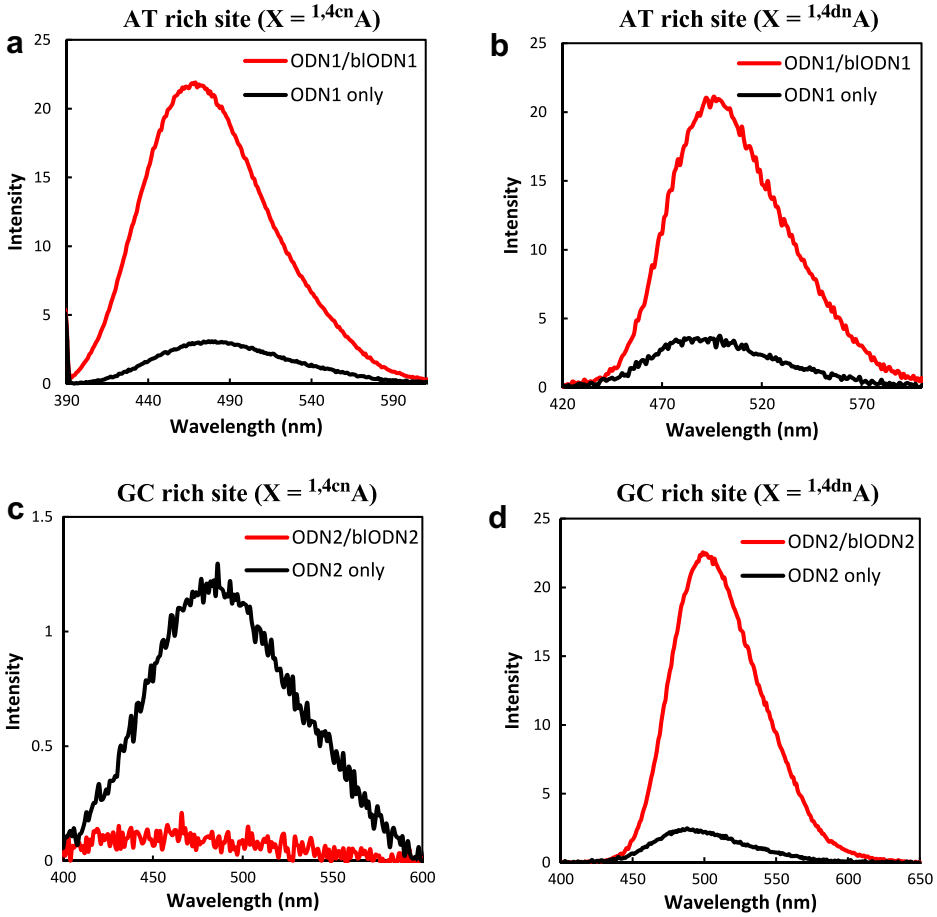



**Figure 5.** Fluorescence spectra of ODN1 [**X** = $^{1,4cn}$**A** (a) and $^{1,4dn}$**A** (b)] hybridized with blODN1 and of ODN2 [**X** = $^{1,4cn}$**A** (c) and $^{1,4dn}$**A** (d)] hybridized with blODN2 (2.5 μm ODNs, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). The excitation wavelength was (a) 368 nm, (b) 380 nm, (c) 365 nm and (d) 380 nm. (slit width:1.5).

(Fig. 4b), probably because the naphthalene fluorophore is located at the hydrophobic site in the bulge structure as proposed in case of $^{2,6cn}$**A**. Interestingly, when the fluorescence probe containing GC rich sequence ODN2 ($^{2,6dn}$**A**) was used, the quenching by flanking C/G base pairs in the bulge was not observed as shown in Figure 4d. The fluorescence intensity of duplex ODN2($^{2,6dn}$**A**)/blODN2 (bulge) was enhanced 2.9-folds compared to single-stranded ODN2($^{2,6d-n}$**A**). These results indicate that *N,N*-dimethylamino substituted ESF nucleoside $^{2,6dn}$**A** is usable for the detection of target DNA via the formation of a bulge structure regardless of the flanking bases, in contrast to $^{2,6cn}$**A** whose fluorescence was strongly quenched by neighboring Gs. Essentially the same results were obtained with ODNs containing 1,4-disubstituted naphthylethynylated 2′-deoxyadenosines, $^{1,4cn}$**A** and $^{1,4dn}$**A** as shown in Figure 5. Thus, ESF nucleoside $^{1,4dn}$**A** is also useful for the detection of target sequence regardless of the flanking sequences via the bulge formation.

In summary, we have succeeded in the molecular design of solvatofluorochromic ESF 2′-deoxyadenosine derivatives. We found that cyano- and *N,N*-dimethylamino substitution on the naphthalene ring of 8-naphthylethynylated 2′-deoxyadeosine derivatives induces a remarkable solvatofluorochromic property. In particular, *N,N*-dimethylamino containing ESF nucleosides $^{2,6dn}$**A** and $^{1,4dn}$**A** exhibited a strong fluorescence emission with high quantum yield without quenching by neighboring Gs contained in DNA duplexes. We demonstrated that $^{2,6dn}$**A**- and $^{1,4dn}$**A**-containing ESF oligonucleotides can be used as an effective fluorescent probe for the detection of target DNA via a bulge formation in a homogeneous fluorescence assay without DNA sequence limitations.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.029.

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12. *Spectroscopic data for* $^{2,6cn}$***A*** (***1a***): $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 2.32 (ddd, $J$ = 2.9, 6.6, 13.2 Hz, 1H), 3.20 (m, 1H), 3.54 (ddd, $J$ = 5.0, 7.6, 11.8 Hz, 1H), 3.71 (ddd, $J$ = 4.4, 4.5, 11.8 Hz, 1H), 3.94 (m, 1H), 4.56 (m, 1H), 5.33 (dd, $J$ = 4.5, 7.6 Hz, 1H), 5.41 (d, $J$ = 4.4 Hz, 1H), 6.60 (m, 1H), 7.71 (br, 2H), 7.88 (m, 1H), 7.91 (dd, $J$ = 1.6, 8.5 Hz, 1H), 8.18–8.24 (complex, 3H), 8.49 (m, 1H), 8.68 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 37.8, 62.2, 71.2, 80.4, 85.1, 88.4, 93.9, 110.0, 118.9, 119.8, 120.5, 127.6, 129.4, 129.4, 129.5, 131.9, 132.4, 132.5, 133.8, 134.3, 148.6, 153.6, 156.2; HRMS (ESI) $m/z$ 449.1338 calcd for $C_{23}H_{18}N_6O_3Na$ $[M+Na]^+$, found 449.1309.
*Spectroscopic data for* $^{2,6dn}$***A*** (***1b***): $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 2.30 (ddd, $J$ = 2.6, 6.5, 13.2 Hz, 1H), 3.07 (s, 6H), 3.19 (m, 1H), 3.54 (ddd, $J$ = 4.9, 7.9, 11.8 Hz, 1H), 3.70 (ddd, $J$ = 4.3, 4.4, 11.8 Hz, 1H), 3.95 (m, 1H), 4.54 (m, 1H), 5.39–5.42 (complex, 2H), 6.59 (m, 1H), 6.98 (m, 1H), 7.31 (dd, $J$ = 2.5, 9.2 Hz, 1H), 7.51 (dd, $J$ = 1.7, 8.5 Hz, 1H), 7.64 (br, 2H), 7.74 (d, $J$ = 8.5 Hz, 1H), 7.84 (d, $J$ = 9.2 Hz, 1H), 8.12 (m, 1H), 8.18 (s, 1H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 37.8, 40.0 (×2), 62.3, 71.3, 77.8, 85.2, 88.4, 96.2, 105.1, 111.8, 116.9, 119.7, 125.2, 126.6, 127.9, 129.0, 132.3, 133.4, 135.2, 148.6, 149.6, 153.2, 156.0; HRMS (ESI) $m/z$ 467.1808 calcd for $C_{24}H_{24}N_6O_3Na$ $[M+Na]^+$, found 467.1782.
*Spectroscopic data for* $^{2n}$***A*** (***1c***): $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 2.32 (ddd, $J$ = 2.8, 6.6, 13.2 Hz, 1H), 3.20 (m, 1H), 3.54 (ddd, $J$ = 4.9, 7.7, 11.8 Hz, 1H), 3.71 (ddd, $J$ = 4.4, 4.5, 11.8 Hz, 1H), 3.95 (m, 1H), 4.55 (m, 1H), 5.36 (dd, $J$ = 4.4, 7.7 Hz, 1H), 5.41 (d, $J$ = 4.4 Hz, 1H), 6.60 (m, 1H), 7.62–7.73 (complex, 5H), 8.01–8.07 (complex, 3H), 8.20 (s, 1H), 8.37 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 37.8, 62.2, 71.2, 78.9, 85.1, 88.4, 94.8, 117.2, 119.7, 127.3, 127.7, 127.9, 128.0, 128.1, 128.8, 132.4, 132.5, 132.9, 133.1, 148.6, 153.5, 156.1; HRMS (ESI) $m/z$ 424.1386 calcd for $C_{22}H_{19}N_5O_3Na$ $[M+Na]^+$, found 424.1409.