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Synthesis of solvatofluorochromic 7-arylethynylated 7-deaza-2′-deoxyadenosine derivatives: application to the design of environmentally sensitive fluorescent probes forming stable DNA duplexes

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ABSTRACT

We synthesized environmentally sensitive fluorescent (ESF) 7-deaza-2′-deoxyadenosine derivatives including ethynylanthracene substituted ^{atz}**A** (**1**) and ethynylnaphthalene substituted ^{nz}**A** (**2a**), ^{cnz}**A** (**2b**), ^{anz}**A** (**2c**), and ^{dnz}**A** (**2d**), and investigated their photophysical properties. Among them, only push–pull type cyano- and acetyl-substituted naphthylethynylated 7-deaza-2′-deoxyadenosine, ^{cnz}**A** (**2b**) and ^{anz}**A** (**2c**), exhibited remarkable solvatofluorochromic properties ($\Delta\lambda$ = 71 and 63 nm, respectively). We incorporated non-solvatofluorochromic ^{atz}**A** (**1**) and solvatofluorochromic ^{cnz}**A** (**2b**) into oligodeoxynucleotides and found that ^{cnz}**A** (**2b**) forms a stable base pair with both thymine and cytosine, being accompanied by a change of fluorescence intensity. Such ESF nucleosides can be used for studying structures and functions of nucleic acids.

Fluorescent molecules that report the differences in local environments such as polarity, viscosity, and pH by a change of fluorescence wavelengths and intensities are very attractive for a wide range of applications in chemistry and chemical biology. In particular, incorporation of such environmentally sensitive fluorescent (ESF) molecules into various biomolecules provides powerful tools for investigating function and interaction of biomolecules such as proteins and nucleic acids,[1] for sensing viscosity or pH,[2] for detecting genes,[3] for diagnosis,[4] drug delivery,[5] and bioimaging.[6] Although various ESF molecules have been developed, only a limited number of ESF nucleosides have been reported.[7] In particular, there are only a few reports of DNA probes containing base-modified ESF nucleosides that can monitor microenvironmental change by a large shift in emission wavelength.[7f,i] We recently reported C8-substituted 2′-deoxyadenosine ^{2,6cn}**A** containing an electron donor–acceptor system within a molecule as a base-modified ESF nucleoside.[8] Although C8-substituted ^{2,6cn}**A** exhibited interesting solvatofluorochromic properties, its incorporation into a DNA duplex resulted in a considerable destabilization of the DNA structure due to the steric bulkiness of the C8-substituent,[8] and consequently was not appropriate for the general use as a fluorescent DNA probe.

When considering base modification of DNA duplexes, C5-position of pyrimidines and C7-position of 7-deazapurines are most appropriate, since the substituents at these positions are accommodated in the major groove of DNA. Especially, when modifying purine base, 7-deazapurine derivatives are very attractive because modifications of the C7-position of 7-deazapurine do not interfere with the sugar–phosphate backbone and retain the nucleoside in a favorable *anti* conformation, and therefore do not cause the destabilization of DNA duplexes.[9] Extensive works of Seela et al., on the synthesis and properties of a wide range of 7-deazapurine nucleoside derivatives are well known.[9,10] Seela et al. reported various 7-deazapurine nucleosides, including C7-hexynylated 7-deaza-2′-deoxyadenosine.[10c] They indicated that 7-substituted 7-deazapurine considerably stabilizes a DNA duplex when incorporated in DNA due to the bulky hydrophobic purine base in the favorable *anti* conformation required for hybridization. This means 7-deaza-2′-deoxyadenosine derivatives containing hydrophobic substituents at the C7-position may be a good candidate for DNA-stabilizing ESF nucleosides. In addition, insertion of an intramolecular donor–acceptor system into 7-deazaadenine derivatives seems to be a suitable approach for attaining remarkable solvatofluorochromic properties. The electron-donating ability of 7-deazaadenine skeleton is higher than that of natural adenine base because the oxidation potential of 7-deazaadenine is as low as that of guanine, which has the lowest oxidation potential among DNA bases.[11] Therefore, in our research program directed toward the design of base-modified ESF purine nucleosides, we synthesized various substituted C7-arylethynylated 7-deaza-2′-deoxyadenosines having base-pairing properties with canonical DNA bases (Fig. 1). Photophysical properties of oligodeoxynucleotides (ODNs) containing newly synthesized ESF nucleosides are also reported.

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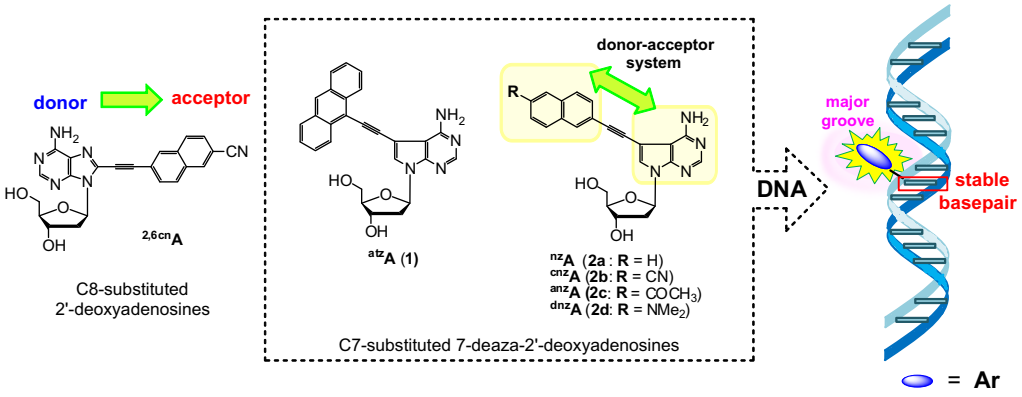


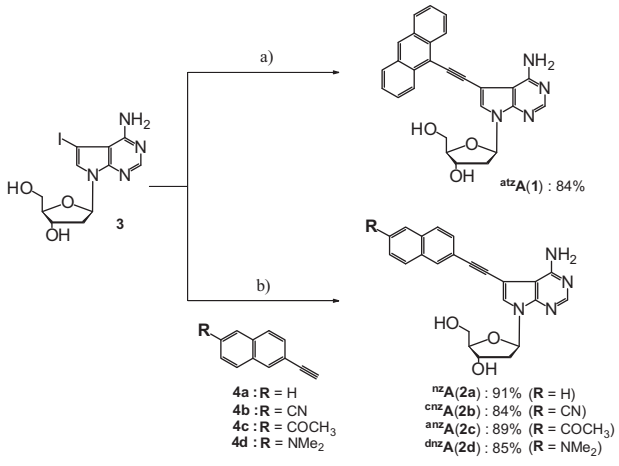
Figure 1. Design of C7-arylethynylated 7-deaza-2′-deoxyadenosines.

The synthesis of 7-deaza-2′-deoxyadenosine derivatives including ethynylanthracene substituted ^{atz}**A** (**1**) and various ethynylnaphthalene substituted ^{nz}**A** (**2a**), ^{cnz}**A** (**2b**), ^{anz}**A** (**2c**), and ^{dnz}**A** (**2d**) is shown in Scheme 1. 7-Iodo-7-deaza-2′-deoxyadenosine **3**, which was prepared according to the protocol reported by Seela et al.,[9,10b] was coupled with 9-ethynylanthracene using $Pd(PPh_3)_4$ to afford **1**.[12] According to a similar protocol, **3** was also coupled with previously reported 2-ethynylnaphthalene derivatives using $Pd(PPh_3)_4$ to afford **2a**–**d** in high yields. All the newly synthesized compounds were characterized by ^{1}H and ^{13}C NMR as well as HRESI MS and their photophysical properties were evaluated.[13]

Initially, we measured the fluorescence spectra of unsubstituted arylethynylated 7-deaza-2′-deoxyadenosine derivatives, anthracene ^{atz}**A** (**1**) and naphthalene ^{nz}**A** (**2a**), in solvents of different polarities. As shown in Figure 2, 9-ethynylanthracene conjugated 7-deaza-2′-deoxyadenosine ^{atz}**A** (**1**) exhibited a strong fluorescence emission at ca. 440–468 nm in both non-polar and polar solvents. The fluorescence quantum yields of ^{atz}**A** (**1**) in non-polar solvents such as chloroform and ethyl acetate were 0.71 and 0.52, respectively. In polar solvents such as acetonitrile and methanol, the quantum yields were decreased to 0.40 and 0.41, respectively. Although the fluorescence quantum yields of ^{atz}**A** (**1**) were significantly large, the red shift of fluorescence emission by increasing solvent polarity was not so large ($\Delta\lambda$ = 28 nm). On the other hand, ethynylnaphthalene substituted ^{nz}**A** (**2a**) exhibited a very weak fluorescence at ca. 371–400 nm in either polar or non-polar solvents, and the red shift of fluorescence emission by increasing solvent polarity was less evident as shown in Figure 2B. These results clearly indicate that unsubstituted arylethynylated 7-deaza-2′-deoxyadenosines did not show notable solvatofluorochromicity.

In order to know the effect of electron donor and acceptor substituents on the naphthalene moiety, the photophysical properties of C7-substituted-7-deaza-2′-deoxyadenosines with 2,6-disubstituted naphthalenes, cyano-substituted ^{cnz}**A** (**2b**), acetylated ^{acz}**A** (**2c**), and *N*,*N*-dimethylamino substituted ^{dnz}**A** (**2d**) were investigated in various solvents. With excitation of 2,6-disubstituted naphthalene derivative ^{cnz}**A** (**2b**) containing an electron-withdrawing cyano substituent at 345 nm, a strong fluorescence emission (Φ_{fl} = 0.10) was observed at 423 nm in chloroform as shown in Figure 3A. Upon excitation of ^{cnz}**A** (**2b**) in ethyl acetate, we observed a medium emission at 458 nm. In contrast, the fluorescence emission of ^{cnz}**A** (**2b**) in more polar solvents like methanol and acetonitrile was weak and emitted at longer wavelengths of 478 and 493 nm, respectively. As expected, ^{cnz}**A** (**2b**) showed a high sensitivity to solvent polarity, indicating a remarkably large solvatofluorochromicity ($\Delta\lambda$ = 71 nm). It is noteworthy that the wavelength of the emission is about 20–40 nm longer than that previously reported for C8-substituted 2′-deoxyadenosine derivative ^{2,6cn}**A**.[8] It is supposed that the high electron-donating ability of the 7-deazaadenine skeleton gives rise to the formation of an intramolecular donor–acceptor system between the 7-deazaadenine moiety and the electron-withdrawing cyanonaphthalene ring to result in a remarkably large solvatofluorochromicity. A similar solvatofluorochromicity was also observed with electron-withdrawing acetyl-substituted ^{anz}**A** (**2c**) (Fig. 3B, $\Delta\lambda$ = 63 nm). The photophysical properties of ^{dnz}**A** (**2d**) bearing an electron-donating *N*,*N*-dimethylamino group were next investigated. As shown in Figure 3C and Table 1, the fluorescence quantum yields of ^{dnz}**A** (**2d**) were much more larger than those of ^{nz}**A** (**2a**) and ^{cnz}**A** (**2b**). However, when solvent polarity was increased, the red shift of the fluorescence emission was not observed in contrast to electron-withdrawing cyano group substituted ^{cnz}**A** (**2b**), and ^{dnz}**A** (**2d**) did not show any solvatofluorochromic properties (Fig. 3C). In the case of electron-withdrawing cyano- or acetyl-substituted naphthalenes ^{cnz}**A** (**2b**) or ^{anz}**A** (**2c**), the 7-deazaadenine moiety acted as an electron donor to result in a formation of an intramolecular donor–acceptor system. In contrast, the 7-deazaadenine moiety is unable to act as an electron acceptor in ^{dnz}**A** (**2d**) containing an electron-donating *N*,*N*-dimethylamino group. It is confirmed that an intramolecular donor–acceptor system such as those in ^{cnz}**A** (**2b**) and ^{anz}**A** (**2c**) is very important for creating solvatofluorochromic properties.

To study the thermal stability and photophysical properties of oligodeoxynucleotides (ODN) containing these newly synthesized fluorescent nucleosides, non-solvatofluorochromic anthracene



Scheme 1. Reagents and conditions: (a) 9-ethynylanthracene, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 80 °C, 2 h; (b) **4a**, **4b**, **4c**, or **4d**, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 60 °C, 1 h.

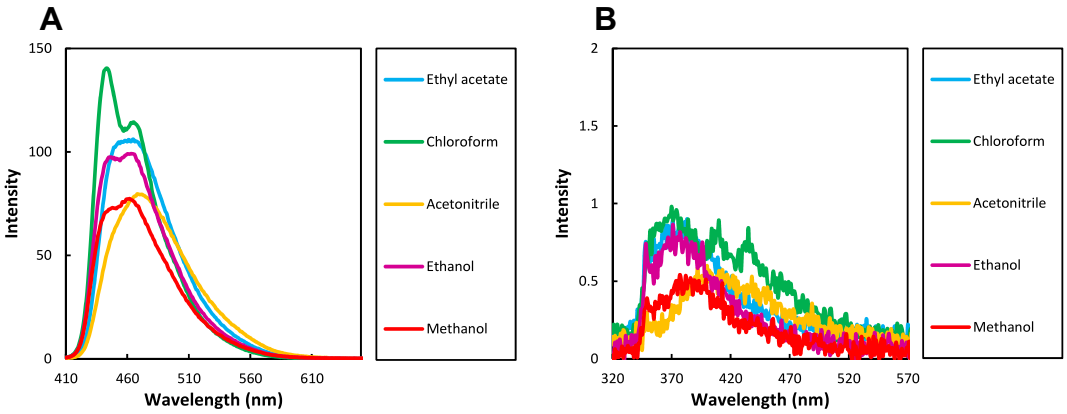


Figure 2. Fluorescence spectra of (A) ^{atz}**A** (**1**, 10 μM) and (B) ^{nz}**A** (**2a**, 10 μM) in various solvents. The slit width was 1.5 nm

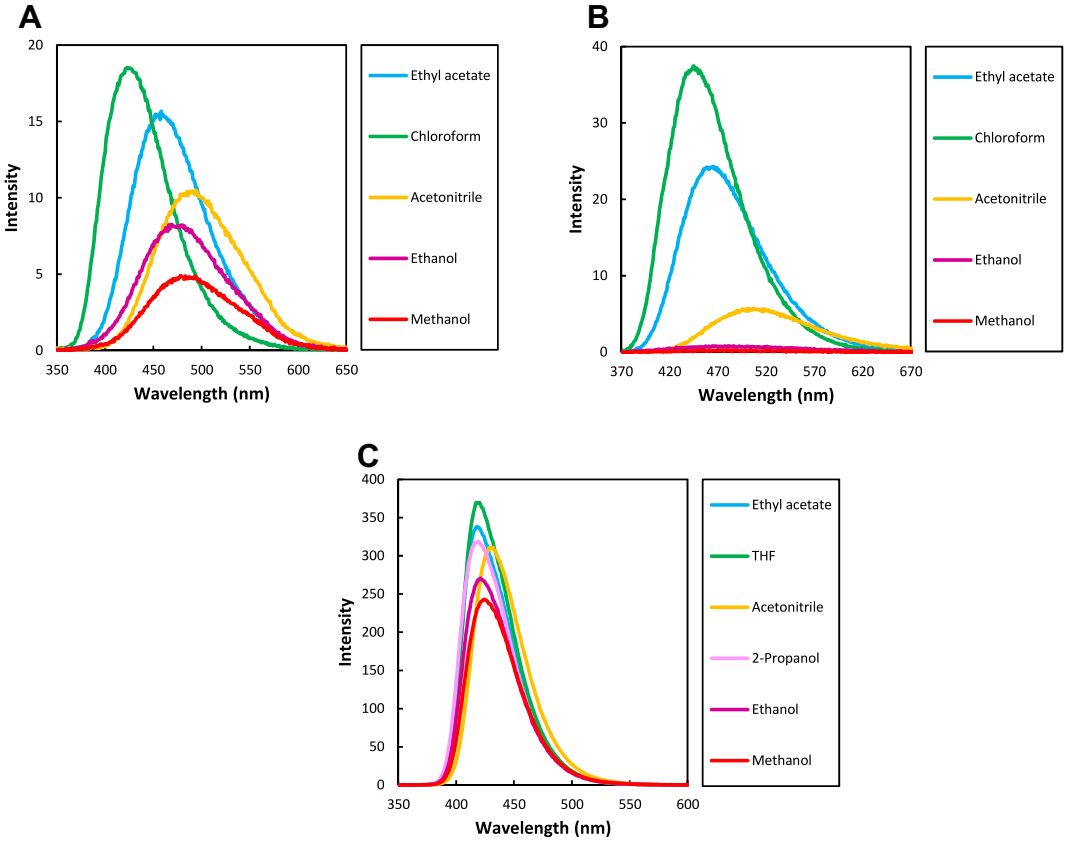


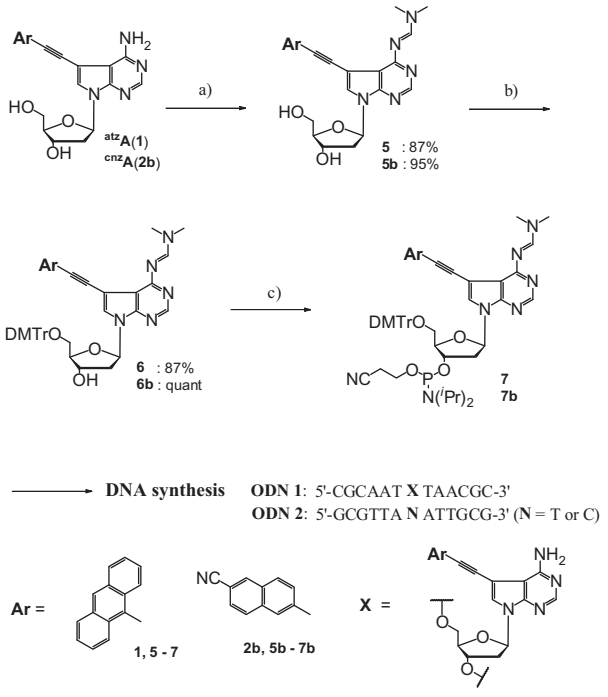
Figure 3. Fluorescence spectra of (A) ^{cnz}**A** (**2b**, 10 μM), (B) ^{anz}**A** (**2c**, 10 μM), and (C) ^{dnz}**A** (**2d**, 10 μM) in various solvents. The slit width was 1.5 nm.

substituted ^{atz}**A** (**1**) and highly solvatofluorochromic ^{cnz}**A** (**2b**) were incorporated into ODNs via automated DNA synthesis. The synthetic route of the corresponding phosphoramidites is indicated in Scheme 2. After protection of the amino group of ^{atz}**A** (**1**) with *N*,*N*-dimethylformamide diethylacetal, **5** was reacted with DMTrCl in dry pyridine to give **6**. Protected **6** was then converted to phosphoramidite **7**. The phosphoramidite of ^{atz}**A** (**1**) was used for ODN synthesis by employing an automated DNA/RNA synthesizer. The phosphoramidite of ^{cnz}**A** (**2b**) was also prepared and incorporated into ODNs via a similar protocol.

Thermal stability of DNA duplexes containing modified nucleosides is profoundly affected by glycosidic bond conformations of modified nucleosides. It is known that substituents at the C8 position of purine base destabilize the normally preferred *anti* conformation of 2′-deoxyadenosine (**A**) due to the steric repulsion to result in a *syn* conformation.[14] Incorporation of such C8 substituted adenine nucleoside into ODN resulted in a large decrease of melting temperatures due to the lack of A/T base pair formation. In contrast, it has been reported that 7-deaza-2′-deoxyadenosine derivatives preferred normal *anti* conformation even when bulky substituents were introduced at the C7 position.[10c] To understand the effect of C7-substituents on the *syn* and *anti* conformations of newly synthesized 7-deaza-2′-deoxyadenosine derivatives, their inherent glycosidic bond conformations were examined by ^{1}H and ^{13}C NMR spectroscopy. It is well established that an *anti*-to-*syn* conformational change results in a downfield shift of C1′, C3′, C4′, and H2′ signals, as well as an upfield shift of C2′ signal.[10c] As shown in Table 2, such shifts were not observed for all the C7-

Table 1
Photophysical properties of fluorescent 7-deaza-2′-deoxyadenosine derivatives

Compound	Solvent	λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_{fl}
^{atz}**A** (**1**)	Ethyl acetate	405	465	0.52
	Chloroform	407	443, 462	0.71
	Acetonitrile	405	468	0.40
	Ethanol	405	441, 463	0.50
	Methanol	403	440, 462	0.41
^{nz}**A** (**2a**)	Ethyl acetate	318	383	<0.01
	Chloroform	319	374	<0.01
	Acetonitrile	318	400	<0.01
	Ethanol	318	371	<0.01
	Methanol	315	377	<0.01
^{cnz}**A** (**2b**)	Ethyl acetate	345	458	0.08
	Chloroform	345	423	0.10
	Acetonitrile	345	494	0.05
	Ethanol	345	469	0.04
	Methanol	345	478	0.02
^{anz}**A** (**2c**)	Ethyl acetate	347	461	0.08
	Chloroform	345	445	0.12
	Acetonitrile	344	508	0.02
	Ethanol	346	475	<0.01
	Methanol	344	464	<0.01
^{dnz}**A** (**2d**)	Ethyl acetate	333	418	0.54
	THF	335	418	0.58
	Acetonitrile	336	432	0.51
	2-Propanol	333	419	0.57
	Ethanol	334	421	0.49
	Methanol	336	424	0.39



Scheme 2. Reagents and conditions: (a) *N*,*N*-dimethylformamide diethylacetal, DMF, 60–80 °C, 2–5 h; (b) DMTrCl, pyridine, rt, 3–6 h; (c) 2-cyanoethyldiisopropylphosphoramidite, Et_3N, acetonitrile, rt, 30 min.

substituted 7-deaza-2′-deoxyadenosine derivatives synthesized as compared to previously reported ^{2,6cn}**A**, indicating their preference of *anti* conformation.

Single-stranded (ss) ODNs containing ^{atz}**A** (**1**) and ^{cnz}**A** (**2b**) were hybridized with complementary ODNs, and the resulting duplexes were examined for thermal stability. As for the base recognition of 7-deazaadenosine derivatives toward four canonical

Table 2
^{1}H and ^{13}C NMR chemical shifts of 2′-deoxyadenosine and 7-deaza-2′-deoxyadenosine derivatives in DMSO-d_6

Compound	H (2′)	C (1′)	C (2′)	C (3′)	C (4′)	C (5′)
A (*anti*)	2.72	84.0	38.9	71.0	88.0	61.9
Br-A[a] (*syn*)	3.25	86.4	37.0	71.1	88.3	62.1
^{2,6cn}**A**[8] (*syn*)	3.20	85.1	37.8	71.2	88.4	62.2
^{z}**A**[10c] (*anti*)	2.50	83.3	b	71.1	87.3	62.1
^{atz}**A** (**1**)	2.60	83.2	b	70.9	87.6	61.9
^{nz}**A** (**2a**)	2.54	83.3	b	71.0	87.6	61.9
^{cnz}**A** (**2b**)	2.54	83.3	b	71.0	87.6	61.9
^{anz}**A** (**2c**)	2.50	83.3	b	71.0	87.6	61.9
^{dnz}**A** (**2d**)	2.53	83.3	b	70.9	87.6	61.8

[a] 8-Bromo-2′-deoxyadenosine.
[b] Overlapped with DMSO (39.5 ppm).

Table 3
Thermal melting temperatures (T_m) and photophysical properties of duplexes ODN 1/ODN 2; ODN 1: 5′-CGCAAT **X** TAACGC-3′ (**X** = ^{cnz}**A**, ^{atz}**A** or **A**), ODN 2: 3′-GCGTTA **N** ATTGCG-5′ (**N** = T or C)

Duplex	T_m (°C)	λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)
ODN 1 (^{cnz}**A**)	—	351	461
ODN 1 (^{cnz}**A**)/ODN 2 (T)	52.8	350	468
ODN 1 (^{cnz}**A**)/ODN 2 (C)	50.1	336, 351	446
ODN 1 (^{atz}**A**)	—	410, 430	447, 469
ODN 1 (^{atz}**A**)/ODN 2 (T)	52.2	410, 435	447, 472
ODN 1 (^{atz}**A**)/ODN 2 (C)	53.1	410, 435	448, 472
ODN 1 (A)/ODN 2 (T)	52.6	—	—
ODN 1 (A)/ODN 2 (C)	45.4	—	—

nucleosides (A, T, G, C), Seela et al. reported that 7-deaza-2′-deoxyadenosine (^{z}**A**) can form a strong base pair not only with T but also with C.[10f] As shown in Table 3, the melting temperatures of ODN 1 (X = ^{atz}**A** or ^{cnz}**A**)/ODN 2 (T) and ODN 1 (X = ^{atz}**A** or ^{cnz}**A**)/ODN 2 (C) were considerably higher than those of unmodified mismatched duplex ODN 1 (A)/ODN 2 (C) and other mismatched duplexes (Supplementary data, Table S2), suggesting that newly synthesized 7-deazaadenosine derivatives ^{atz}**A** (**1**) and ^{cnz}**A** (**2b**) can form stable base pairs with both T and C. This is consistent with the previous report that 7-substituted 7-deazapurines generally form a protonated base pair with cytosine.[10f] In the CD spectra of ODN 1 (^{cnz}**A**)/ODN 2 (N = T or C), a negative peak at 250 nm and a positive peak at 280 nm were observed (Supplementary data, Fig. S4). These spectral data also indicate that ^{cnz}**A**-containing DNA duplexes maintain a normal B-DNA structure.

UV–visible absorption and fluorescence spectra of ODNs containing ^{atz}**A** (**1**) and ^{cnz}**A** (**2b**) were then examined. As shown in Figure 4A, the fluorescence emission of ss ODN 1 (^{cnz}**A**) was relatively weak and appeared at 461 nm. When complementary strands were added, the fluorescence intensity of the duplex ODN was considerably enhanced. When the opposite base of the complementary strand is thymine (T) in duplex ODN 1 (^{cnz}**A**)/ODN 2 (T), a strong emission was observed at a slightly red shifted wavelength (468 nm). Interestingly, when the opposite base of the complementary strand is cytosine (C) in duplex ODN 1 (^{cnz}**A**)/ODN 2 (C), the fluorescence maximum was blue-shifted to 446 nm by 22 nm with a stronger emission intensity (Fig. 4A). The fluorescence change caused by the difference in the opposite base of a complementary strand may be ascribable to the local environmental change near the solvatofluorochromic ^{cnz}**A** (**2b**) moiety.[15] On the other hand, non-solvatofluorochromic ^{atz}**A**-labeled ODN 1 showed an environmentally insensitive nature. Even when the opposite base of complementary strand is changed from thymine to cytosine, a similar but slightly enhanced fluorescence was observed in both cases (Fig. 4B). Although ODN containing

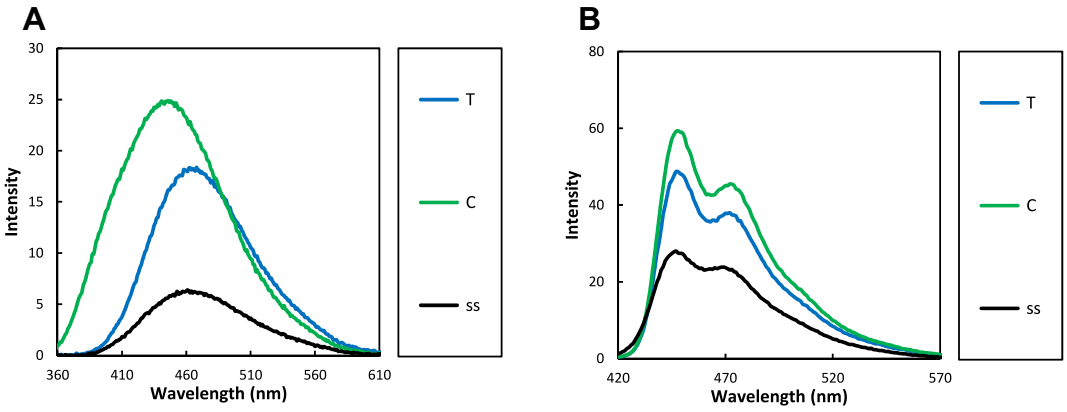


Figure 4. Fluorescence spectra of ODN 1 [**X** = ^{cnz}**A** (**A**) and ^{atz}**A** (**B**)] hydridized with ODN 2 (**N** = T or C); 'ss' denotes a single-stranded ODN 1 (2.5 μM ODNs, 0.1 M sodium chloride, 50 mM sodium phosphate buffer, pH 7.0, rt). The slit width was 1.5 nm.

solvatofluorochromic ^{cnz}**A** (**2b**) showed an environmentally sensitive fluorescence emission when hybridized with complementary ODNs, such a drastic change in fluorescent intensity and wavelength was not observed in non-solvatofluorochromic ^{atz}**A**-containing ODN (Fig. 4, Table 3). Thus, it is clear that non-solvatofluorochromic anthracene-containing ^{atz}**A** (**1**) can be used as a fluorescent probe of high intensity but never used as an environmentally sensitive fluorescent ODN probe.

In conclusion, we have synthesized various substituted arylethynylated 7-deaza-2′-deoxyadenosine derivatives. Among them, only push–pull type cyano- and acetyl-substituted naphthylethynylated 7-deaza-2′-deoxyadenosines, ^{cnz}**A** (**2b**), and ^{anz}**A** (**2c**), exhibited remarkable solvatofluorochromic properties ($\Delta\lambda = 71$ and 63 nm, respectively). Unsubstituted and *N*,*N*-dimethylamino-substituted derivatives having no intramolecular donor–acceptor system did not show such solvatofluorochromic properties. Thermal melting data indicated that an ODN probe containing ^{cnz}**A** (**2b**) can form a stable base pair either with thymine or cytosine, and maintain a stable B-DNA structure, similar to that reported for 7-deaza-2′-deoxyadenosine (^{z}**A**). The microenvironmental change caused by the change of opposite bases resulted in a considerable change of the fluorescence intensity and emission wavelength in ^{cnz}**A**-containing ODN probe. Fluorescent DNA probes containing such a base-modified ESF nucleoside can be used as a tool for studying structures and functions of nucleic acids and for studying interactions between nucleic acids and proteins.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.02.063.

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13. Spectroscopic data for ^{atz}**A** (**1**): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.27 (ddd, J = 2.8, 6.0, 13.2 Hz, 1H), 2.60 (ddd, J = 5.8, 8.0, 13.2 Hz, 1H), 3.54–3.67 (complex, 2H), 3.88 (m, 1H), 4.41 (m, 1H), 5.12 (m, 1H), 5.34 (d, J = 4.1 Hz, 1H), 6.59 (dd, J = 6.0, 8.0 Hz, 1H), 6.84 (br, 2H), 7.63 (m, 2H), 7.70 (m, 2H), 8.17 (s, 1H), 8.19 (d, J = 8.4 Hz, 2H) 8.22 (s, 1H), 8.53 (m, 2H), 8.71 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 61.9, 70.9, 83.2, 87.4, 87.6, 94.3, 94.9, 102.2, 116.3, 125.9 (×2), 126.1 (×2), 127.4, 127.4 (×2), 127.9, 129.0 (×2), 130.8 (×2), 131.8 (×2), 149.6, 152.9, 157.7. C(2′) overlapped with DMSO; HRMS (ESI) m/z 473.1590 calcd for $C_{27}H_{22}N_4O_3Na$ [M+Na]$^+$, found 473.1596. Spectroscopic data for ^{cnz}**A** (**2b**): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.24 (ddd, J = 2.8, 6.0, 13.2 Hz, 1H), 2.54 (m, 1H, overlapped with DMSO), 3.55–3.63 (complex, 2H), 3.86 (m, 1H), 4.38 (m, 1H), 5.12 (m, 1H), 5.32 (d, J = 4.1 Hz, 1H), 6.54 (dd, J = 6.0, 7.8 Hz, 1H), 6.88 (br, 2H), 7.82–7.86 (complex, 2H), 7.98 (s, 1H), 8.09–8.14 (complex, 2H) 8.18 (s, 1H), 8.34 (m, 1H), 8.61 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 61.9, 71.0, 83.3, 85.6, 87.6, 91.0, 94.4, 102.0, 109.0, 119.1, 123.4, 127.3, 127.7, 128.9, 129.1, 129.7, 130.6, 131.1, 134.0, 134.2, 149.6, 152.9, 157.6. C(2′) overlapped with DMSO; HRMS (ESI) m/z 448.1386 calcd for $C_{24}H_{19}N_5O_3Na$ [M+Na]$^+$, found 448.1407.
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15. When ODN probes containing GC rich sequence like –C^{cnz}**A**C– were used, the fluorescence emission was strongly quenched by flanking C/G base pairs. This indicates that there is a limitation for the sequence when ODN probe containing ^{cnz}**A** is used.