Tetrahedron Letters 52 (2011) 4726–4729

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthesis and photophysical properties of novel push–pull-type solvatochromic 7-deaza-2′-deoxypurine nucleosides

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ARTICLE INFO

Article history:
Received 30 May 2011
Revised 16 June 2011
Accepted 23 June 2011
Available online 30 June 2011

Keywords:
Nucleoside
DNA
Fluorescent probe

ABSTRACT

We have synthesized two novel push–pull-type fluorescent 7-deazapurine nucleosides, ^{CNZ}**A** and ^{CNZ}**G,** and investigated their photophysical properties. In particular, ^{CNZ}**A** was found to exhibit a remarkable solvatofluorochromicity ($\Delta\lambda_{fl.max}$ = 60 nm). We incorporated ^{CNZ}**A** into oligonucleotides and found that ^{CNZ}**A** can form a stable base pair with both thymine and cytosine. Such environmentally sensitive fluorescent nucleosides have a potential as a fluorescence sensor for structural studies of nucleic acids.

Numerous fluorescent molecules have been reported for fluorescence labeling of biomolecules and widely used in bioscience.[1] However, environmentally sensitive fluorescence nucleosides in which emission spectra and quantum yields change sensitively according to solvent polarity are of greatest interest owing to their wide range of applications.[2,3] Such environmentally sensitive fluorescent nucleosides are used as fluorescence sensors in various fields, as for example, incorporation of such solvatofluorochromic nucleosides into oligonucleotides provides powerful tools for the detection of target DNA, SNP genotyping,[2] and for studying nucleic acid–protein interactions.[3]

Recently, we reported C8-substituted push–pull-type fluorescent guanosines, ^{Ac}**G** and ^{CN}**G,** which contain a covalently linked electron donor–acceptor system consisting of guanosine as electron donor and pyrene fluorophore as acceptor (Fig. 1a).[4] Although these guanosine derivatives exhibited interesting solvatofluorochromic properties, the steric bulk of the C8-substituents in both ^{Ac}**G** and ^{CN}**G** causes considerable destabilization of the DNA duplex

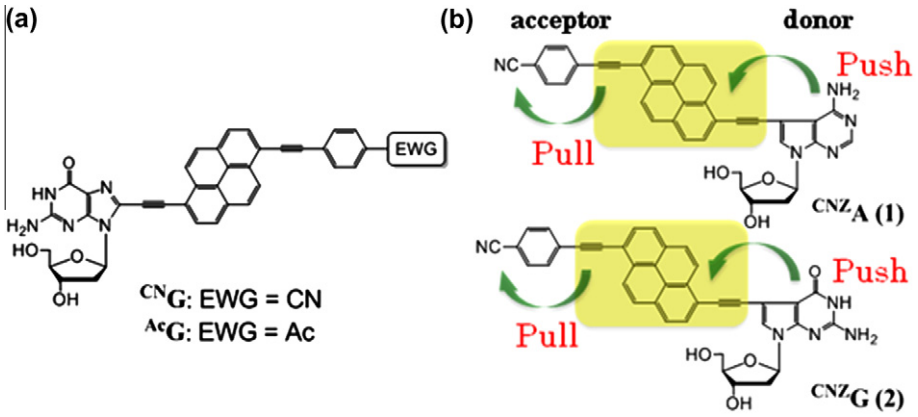

Figure 1. (a) Reported push–pull-type fluroscent nucleosides. (b) Novel push–pull-type fluroscent nucleosides.

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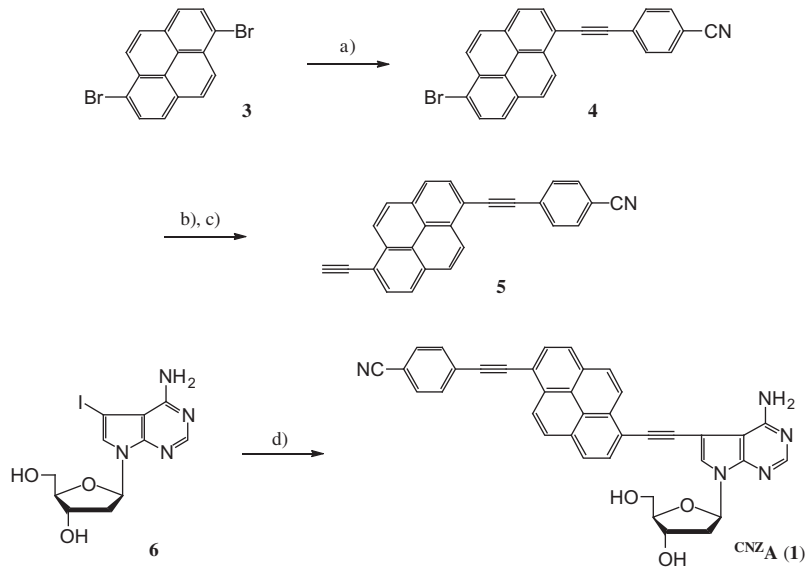
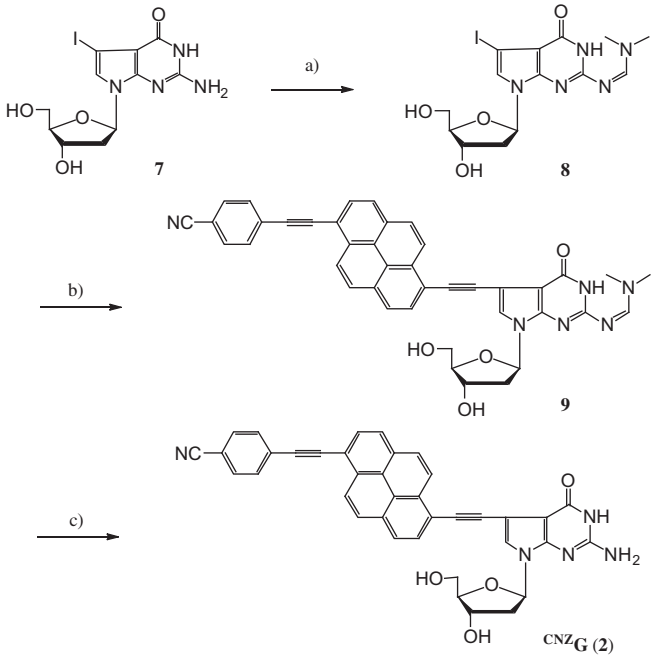
doi:10.1016/j.tetlet.2011.06.089



Scheme 1. Reagents and conditions: (a) 4-ethynylbenzonitrile, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 80 °C, overnight, 52%; (b) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, Et_3N, toluene, 90 °C, overnight; (c) K_2Co_3, MeOH/THF, rt, 2 h, 66% in two steps; (d) 5, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 80 °C, 2 h, 73%.



Scheme 2. Reagents and conditions: (a) *N,N*-dimethylformamide diethyl acetal, DMF, 60 °C, 2 h, 70%; (b) 5, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 80 °C, 2 h, 52%; (c) NH_4OH, MeOH, 60 °C, overnight, 41%.

structure due to their *syn*-conformation, and thus, these molecules may not be suitable for the use as fluorescent DNA probes. We have thus designed novel push–pull-type 7-deazapurine nucleosides. While 8-substituents on purines cause steric repulsion that drives the molecule into the *syn*-conformation, 7-substituents on 7-deazapurine do not sterically interfere with the sugar-phosphate backbone and should fit into the major groove of the B-form of DNA.[5] In addition, the electron-donating ability of 7-deazapurine seems to be similar to that of natural guanine bases, because the oxidation potentials of 7-deazaadenine and 7-deazaguanine are closer to and lower than those of natural guanine bases, respectively.[6] On the basis of these concepts, an electron-withdrawing 4-cyanophenyl group (acceptor) and 7-deazapurine nucleoside (donor) are directly attached to pyrene chromophore via triple bonds in order to construct an intramolecular donor–acceptor system. We report herein the synthesis and photophysical properties of novel push–pull-type 7-deaza-2′-deoxyadenosine (**^{CNZ}A**) and 7-deaza-2′-deoxyguanosine (**^{CNZ}G**) containing a 1,6-disubstituted pyrene chromophore (Fig. 1b).

The synthetic route of pyrene-labeled push–pull-type 7-deaza-2′-deoxyadenosine (**^{CNZ}A**) is outlined in Scheme 1. 1,6-dibromopyrene **3**[7] was coupled with 4-ethynylbenzonitrile using $Pd(PPh_3)_4$ to afford compound **4**. Sonogashira cross-coupling reaction[8] of **4** with TMS-acetylene followed by deprotection with K_2CO_3 yielded **5**. Compound **5** was then coupled with 7-iodo-7-deaza-2′-deoxyadenosine **6** prepared according to the protocol of Seela et al.[9] to form **^{CNZ}A**.[10]

7-Deaza-2′-deoxyguanosine derivative (**^{CNZ}G**) was synthesized according to Scheme 2 via a similar route. The amino group of 7-iodo-7-deaza-2′-deoxyguanosine **7**[11] was reacted with *N,N*-dimethylformamide diethyl acetal to give protected amine **8** in 70% yield. Compound **8** was then coupled with **5** under Sonogashira conditions using $Pd(PPh_3)_4$ to afford **9**. After deprotection of DMF group with 28% aq NH_4OH-methanol, 7-deaza-2′-deoxyguanosine derivative (**^{CNZ}G**) containing an electron-withdrawing substituent was obtained.[10]

The photophysical properties of newly synthesized push–pull-type 7-deazapurine derivatives, **^{CNZ}A** and **^{CNZ}G,** were examined. Initially, we measured the fluorescence spectra of **^{CNZ}A** in various solvents of different polarity. Upon excitation of **^{CNZ}A** at 416 nm in chloroform, strong fluorescence emission was observed at 470 nm as shown in Figure 2a (Φ_{fl} = 0.448).[12] Upon excitation of ^{CNZ}A in THF, we observed moderate emission at 510 nm (Φ_{fl} = 0.305). In contrast, very weak fluorescence emission was observed at 530 nm in a polar solvent such as DMF (Φ_{fl} = 0.089). As expected, push–pull-type 7-deaza-2′-deoxyadenosine derivative **^{CNZ}A** exhibited a considerable solvatofluorochromicity ($\Delta\lambda_{fl,max}$ = 60 nm), as also demonstrated by the fluorescence color image depicted in Figure 2c.

The photophysical properties of 7-deaza-2′-deoxyguanosine derivative **^{CNZ}G** were also examined. While the fluorescence intensity of **^{CNZ}G** is strong in low-polarity solvents such as chloroform, weak fluorescence was observed in polar solvents as is presented in Figure 2b. In the case of **^{CNZ}G,** no remarkable redshift of fluorescence emission was observed by changing solvent polarity, unlike

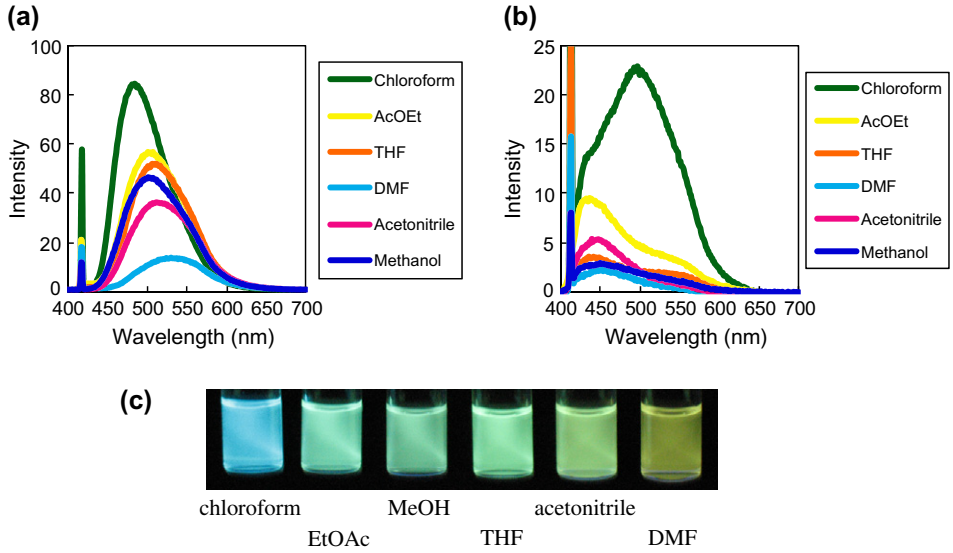


Figure 2. Fluorescence spectra of (a) ^{CNZ}**A** (**1**, 2.5 μM) and (b) ^{CNZ}**G** (**2**, 2.5 μM) in various solvents, (c) Fluorescence color image of ^{CNZ}**A** (**1**) in different solvents. The sample solutions were illuminated with 365 nm transilluminator.

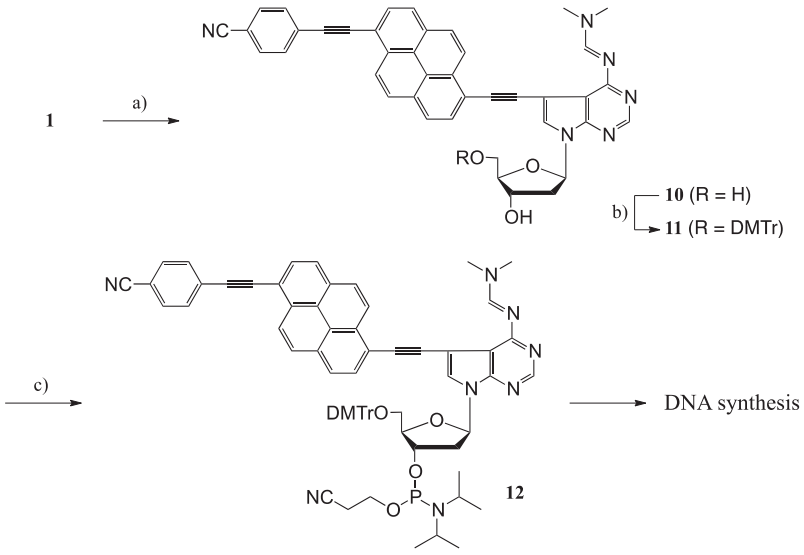
7-deazapurine derivative, ^{CNZ}**A** which exhibited a redshift with increasing solvent polarity. As shown in Figure 2a and b, the shapes of the fluorescence spectra of ^{CNZ}**A** and ^{CNZ}**G** were very different. ^{CNZ}**A** showed broad emission bands and solvent polarity-dependent fluorescence emissions at longer wavelengths, whereas the emission bands of ^{CNZ}**G** are similar to those of the vibrational structures of the parent pyrene chromophore.

To study the thermal stability and photophysical properties of the synthesized solvatofluorochromic nucleoside in DNA, ^{CNZ}**A** was incorporated into oligodeoxynucleotide (ODN) via automated DNA synthesis. The synthetic route of the corresponding phosphoramidite is indicated in Scheme 3. After protection of the amino group with *N,N*-dimethylformamide diethylacetal, **10** was reacted with DMTrCl in the presence of a catalytic amount of DMAP in dry pyridine to give **11**. Protected **11** was then converted to phosphoramidite **12**. Phosphoramidite of ^{CNZ}**A** was used for ODN synthesis by using an automated DNA/RNA synthesizer without further purification (Table 1).

Table 1
Thermal melting properties and photophysical properties ODN 1: 5′-CGCAAT X TAACGC-3′ (X = ^{CNZ}**A** or A) ODN 2: 3′-GCGTTA N ATTGCG-5′ (N = T, C, A or G)

Duplexes	T_m (°C)	λ^{Abs}_{max}	λ^{fl}_{max}	ϕ_{fl}
ODN 1 (^{CNZ}**A**)	—	424	484	0.372
ODN 1 (^{CNZ}**A**)/ODN 2 (T)	45.5	422	484	0.454
ODN 1 (^{CNZ}**A**)/ODN 2 (C)	46.7	435	483	0.466
ODN 1 (^{CNZ}**A**)/ODN 2 (A)	43.4	434	484	0.387
ODN 1 (^{CNZ}**A**)/ODN 2 (G)	43.4	421	485	0.428
ODN 1 (A)/ODN 2 (T)	52.6	—	—	—

Synthesized single-stranded oligonucleotide (ODN 1) containing push–pull-type fluorescent nucleoside ^{CNZ}**A** was hybridized with complimentary DNA strands (ODN 2) and resulting duplexes were tested for thermal stability. Seela and co-workers reported that 7-deaza-2′-deoxyadenosine (^{Z}**A**) generates a strong base pair, not only with **T** but also with **C** and **G**. In particular, replacement of the purine base by a pyrrolo[2,3-*d*]pyrimidine system causes



Scheme 3. Reagents and conditions: (a) *N,N*-dimethylformamide diethyl acetal, DMF, 60 °C, 1 h, 73%; (b) DMTrCl, DMAP, pyridine, r.t, 2 h, 88%; (c) 2-cyanoethyl *N,N*-diissopropylchlorophosphoramidite, Et_3N, acetonitrile, rt, 1 h.

strong deazapurine (^{Z}**A**)–**C** interaction.[13] As shown in Table 1, the melting temperatures of ODN 1(^{CNZ}**A**)/ODN 2(T) and ODN 1(^{CNZ}**A**)/ODN 2(C) were considerably higher than those observed in other mismatched duplexes in a sodium phosphate buffer (pH 7.0), suggesting that ^{CNZ}**A** can also form a stable base pair with both natural **T** and **C**, although incorporation of bulky ^{CNZ}**A** into duplexes resulted in a considerable destabilization of the duplex (ΔT_m = 7.1 °C) as compared with unmodified matched ODN 1(A)/ODN 2(T).

Next, we measured the fluorescence spectra of ODN 1 in the presence or absence of complementary strands. Strong fluorescence emissions of single-stranded ODN 1 and the duplexes with complementary strands ODN 2 (T, C, A, G) were observed at around 485 nm. However, the fluorescence wavelength and intensity were not changed remarkably by changing the nucleosides opposite to ^{CNZ}**A** (Table 1, Fig. S1), indicating that ^{CNZ}**A** cannot be used as a base-discriminating fluorescent nucleoside.[14]

In summary, we have synthesized novel push–pull-type fluorescent 7-deazapurine nucleosides ^{CNZ}**A** and ^{CNZ}**G**, the first highly fluorescent pyrene containing 7-deaza-2′-deoxypurine nucleosides. ^{CNZ}**A** exhibited strong fluorescence at long wavelength and a remarkable solvatofluorochromicity ($\Delta\lambda_{fl.max}$ = 60 nm). ^{CNZ}**A** was incorporated into oligonucleotides and the thermal stability of the resulting duplex was evaluated. The thermal melting data indicated that ^{CNZ}**A** can form a stable base pair with natural **T** and **C**, similar to that reported for 7-deaza-2′-deoxyadenosine (^{Z}**A**). Such types of environmentally sensitive and strongly fluorescent nucleosides may be used as a fluorescence sensor for structural studies of nucleic acids and as a building block for constructing a fluorescent DNA nanostructure.

Acknowledgment

This work was supported by a Grant-in-Aid for Scientific research of MEXT, from Japanese Government.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.06.089.

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10. Spectroscopic data for ^{CNZ}**A** (**1**): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.31 (ddd, J = 2.9, 6.0, 13.2 Hz, 1H), 2.57 (m, 1H), 3.60–3.71 (complex, 2H), 3.91 (m, 1H), 4.44 (m, 1H), 4.97 (dd, J = 5.5, 5.5 Hz, 1H), 5.20 (d, J = 4.2 Hz, 1H), 6.60 (m, 1H), 6.77 (br, 2H), 7.96–8.01 (complex, 4H), 8.11 (s, 1H), 8.28 (s, 1H), 8.36–8.45 (complex, 6H), 8.69 (d, J = 9.0 Hz, 1H), 8.70 (d, J = 9.0 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 61.7, 70.7, 78.9(×2), 83.2, 87.5, 89.5, 89.6, 91.9, 93.9, 110.9, 116.4, 118.1, 118.2, 123.1, 123.2, 125.3, 125.4, 125.7, 125.9, 127.0, 127.3, 128.3, 128.7, 129.8, 130.2, 130.3, 130.7, 131.2, 131.4, 132.1(×2), 132.4(×2), 149.5, 152.6, 157.5. C(2′) overlapped with DMSO; HRMS (ESI) m/z 622.1855 calcd for $C_{38}H_{25}N_5O_3Na$ [M+Na]$^+$, found 622.1858.
Spectroscopic data for ^{CNZ}**G** (**2**): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.21 (ddd, J = 2.8, 5.9, 13.1 Hz, 1H), 2.45 (ddd, J = 5.9, 8.1, 13.1 Hz, 1H), 3.57–3.65 (complex, 2H), 3.85 (m, 1H), 4.38 (m, 1H), 4.86 (m, 1H), 5.16 (d, J = 2.9 Hz, 1H), 6.37–6.40 (complex, 3H), 7.56 (s, 1H), 7.96–8.01 (complex, 4H), 8.22 (d, J = 8.0 Hz, 1H), 8.32–8.41 (complex, 5H), 8.66 (d, J = 9.1 Hz, 1H), 9.07 (d, J = 9.1 Hz, 1H), 10.40 (br, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 61.8, 70.7, 82.4, 87.2, 88.7, 91.2, 92.1, 93.8, 98.4, 99.6, 110.9, 116.0, 118.2, 119.4, 122.1, 123.2, 123.2, 124.9, 125.1, 125.7, 127.0, 127.1, 127.8, 128.8, 128.9, 129.8, 130.1, 131.0, 131.4, 131.5, 132.1(×2), 132.4(×2), 150.5, 153.3, 158.8. C(2′) overlapped with DMSO; HRMS (ESI) m/z 638.1804 calcd for $C_{38}H_{25}N_5O_4Na$ [M+Na]$^+$, found 638.1818.
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