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Design and synthesis of highly solvatochromic fluorescent 2′-deoxyguanosine and 2′-deoxyadenosine analogs

Katsuhiko Matsumoto [b], Naoya Takahashi [a], Azusa Suzuki [a], Takashi Morii [b], Yoshio Saito [a,*], Isao Saito [a,*]

[a] Department of Chemical Biology and Applied Chemistry, School of Engineering, Nihon University, Koriyama, Fukushima 963-8642, Japan
[b] Institute of Advanced Energy, Kyoto University, Uji, Kyoto 611-0011, Japan

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ABSTRACT

We synthesized various substituted 8-styryl-2′-deoxyguanosine and 8-styryl-2′-deoxyadenosine derivatives. Among them only acetyl substituted 8-styryl-2′-deoxyguanosine analog **5b** showed a remarkable solvatochromicity ($\Delta\lambda_{max}^{em}$ = 91 nm),that is, strong fluorescence at 477 nm in 1,4-dioxane, but in methanol the fluorescence was red shifted to 558 nm with very low intensity. Such environmentally sensitive solvatochromic fluorescent guanosine analogs may be useful as a sensor for investigating interactions of DNA with DNA binding proteins.

Numerous efforts to impart useful photophysical features (e.g., long emission wavelength and high quantum efficiency) upon non-emissive natural nucleobases have been reported.[1] Most of the previous approaches involve the linking of natural nucleobases to fluorescent aromatic or heteroaromatic chromophores via an ethynyl linker[1] or the direct attachment of fluorescent chromophores on the natural nucleobases.[2] Although numerous 8-arylethynylated adenine[3] and guanine[4] derivatives have been reported, guanine derivatives that are π-conjugated with aromatics at the 8-position via a double bond are few.[5] In our research program directed toward the design of environmentally sensitive fluorescent nucleosides, we have successfully designed for the first time highly solvatochromic fluorescent 2′-deoxyguanosine and 2′-deoxyadenosine analogs that are π-conjugated with aromatic chromophores through a double bond. We now report herein that acetyl substituted 8-styryl-2′-deoxyguanosine (**5b**) showed a highly solvent polarity sensitive fluorescence emission with a large solvatochromicity ($\Delta\lambda_{max}^{em}$ = 91 nm) that may be useful for investigating nucleic acid structure, dynamics and recognition (Fig. 1). In contrast to our previously reported base-discriminating fluorescent (BDF) nucleosides,[1c,6] which show increasing or decreasing emission intensity at fixed wavelength, solvatochromic fluorescent 2′-deoxyguanosine and 2′-deoxyadenosine analogs described here may indicate the change in their local environments by a drastic change of emission wavelength together with intensity change.

We synthesized 2′-deoxyguanosine analog **5b** according to the synthetic route shown in Scheme 1. Amino group of 8-bromo-2′-deoxyguanosine **1** was protected by *N,N*-dimethylformamide diethylacetal to yield **2**. Protected **2** was reacted with tetravinyltin (IV) under palladium (0) mediated Stille coupling conditions to

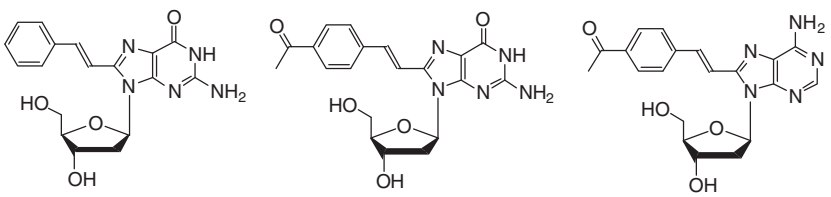

Figure 1. Chemical structures of aromatic fluorophore substituted purine nucleosides.

* Corresponding authors.
E-mail address: saitoy@chem.ce.nihon-u.ac.jp (Y. Saito).

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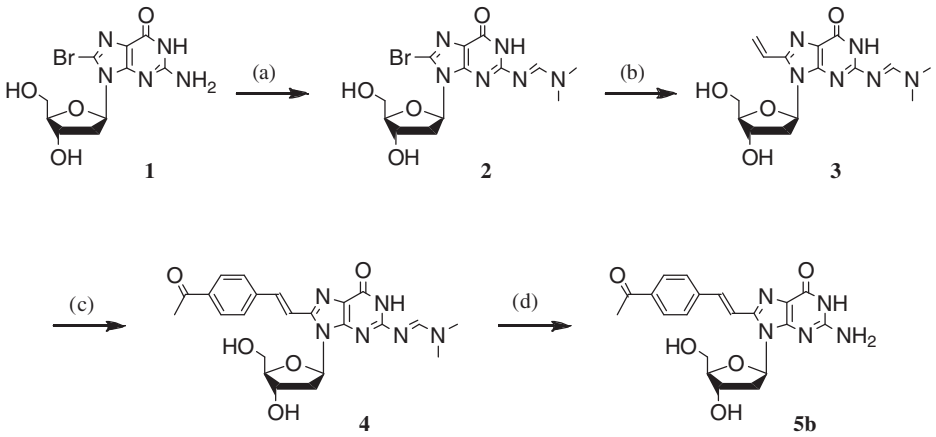
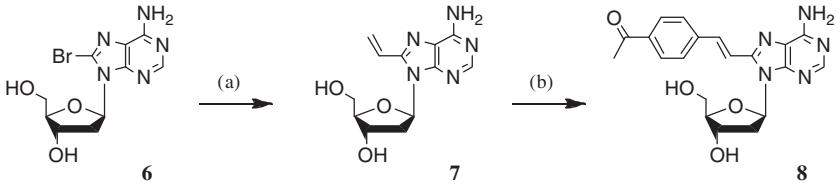


Scheme 1. Reagents and conditions: (a) *N*,*N*-dimethylformamide diethylacetal, MeOH, 60 °C, 12 h, (98%); (b) tetravinyltin (IV), $Pd(PPh_3)_4$, Et_3N, DMF, 80 °C, 12 h, (62%); (c) 4-bromoacetophenone, $Pd(PPh_3)_4$, AcONa, DMF, 100 °C, 6 h, (quant.); (d) NH_4OH, MeOH, 55 °C, 8 h, (68%).



Scheme 2. Reagents and conditions: (a) tetravinyltin (IV), $Pd(PPh_3)_4$, AcONa, DMF, 90 °C, 3 h, (82%); (b) 4-bromoacetophenone, $Pd(PPh_3)_4$, AcONa, DMF, 120 °C, 5 h, (36%).

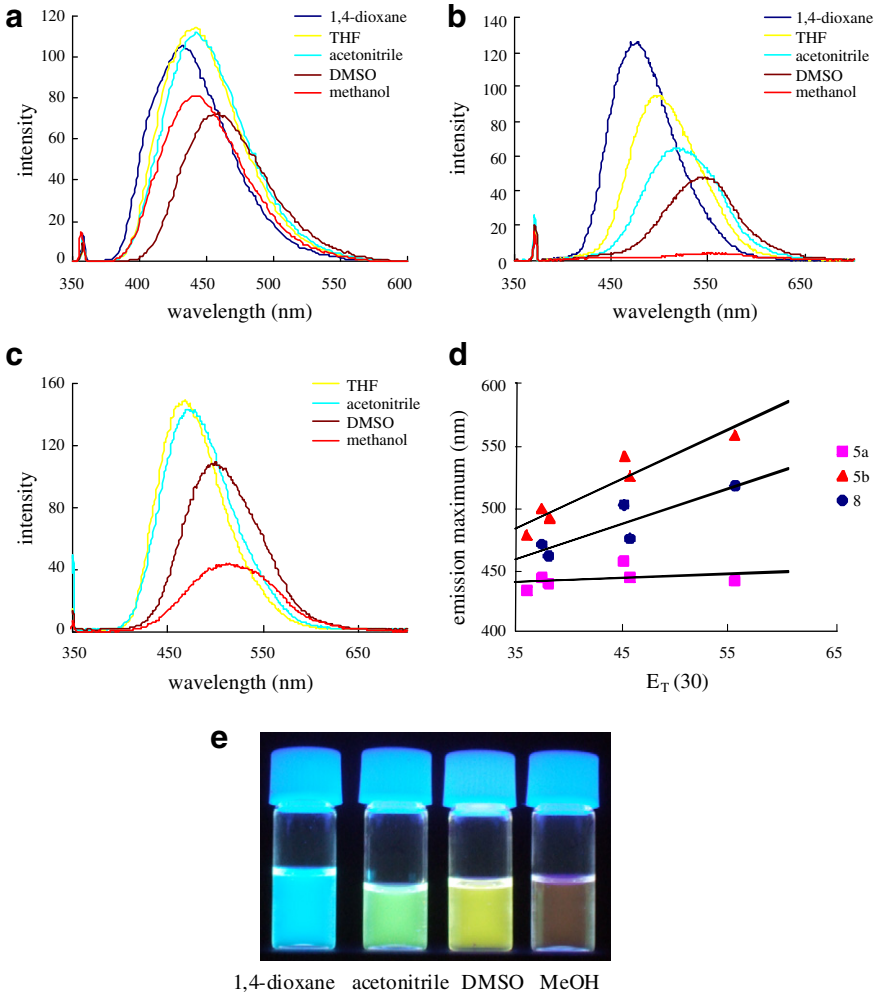


Figure 2. Fluorescence spectra of **5a** (a), **5b** (b) and **8** (c) in various solvents (each 10 μM concn). Excitation wavelength was 355 nm (a), 370 nm (b), and 350 nm (c). (d) Plots of emission maximum of **5a** (■), **5b** (▲) and **8** (●) as a function of E_T (30). (e) Fluorescence image of **5b** under illumination with transilluminator (365 nm).

Table 1
Photophysical properties of fluorescent 2′-deoxypurine nucleosides **5a**, **5b** and **8**

Solvent	E_T (30)[8]	**5a**		**5b**		**8**	
		λ^{em} (nm)	Φ_F	λ^{em} (nm)	Φ_F	λ^{em} (nm)	Φ_F
1,4-Dioxane	36.0	432	0.26	477	0.62	—	—
Elhylacelale	38.1	438	0.19	491	0.48	460	0.39
THF	37.4	442	0.34	499	0.49	470	0.37
DMSO	45.1	456	0.16	541	0.34	502	0.27
Acelonitrile	45.6	442	0.25	525	0.29	474	0.33
Methanol	55.4	440	0.12	558	0.02	517	0.10

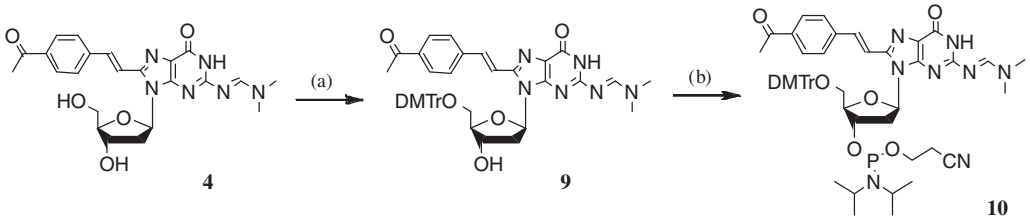
produce **3**. Compound **3** was coupled with 4-bromoacetophenone under Heck reaction conditions and the treatment with ammonia afforded acetylated (*E*)-8-styryl-deoxyguanosine **5b**.

Acetyl substituted 8-styryl-2′-deoxyadenosine **8** was also synthesized by a similar route shown in Scheme 2. 8-Bromo-2′-deoxyadenosine **6** was reacted with tetravinyltin (IV) in the presence of catalytic amount of Pd(0) to yield 8-vinyl-2′-deoxyadenosine **7**. Compound **7** was coupled with 4-bromoacetophenone under Heck reaction conditions to give acetylated (*E*)-8-styryl deoxyadenosine **8**. Acetyl substituted purine nucleosides **5b** and **8** were characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopy.[7] (*E*)-8-Styryl-2′-deoxyguanosine **5a** was also synthesized according to the reported protocol.[5]

We first examined the fluorescence behavior of phenyl substituted **5a** and acetophenone conjugated **5b** in various solvents. Fluorescence wavelength and intensity of non-substituted **5a** were not changed remarkably by changing solvent polarity (Fig. 2a). In contrast, acetyl substituted **5b** showed strong fluorescence emission in non-polar solvents but very weak red shifted fluorescence emission in polar solvents, indicating that **5b** is an effective environmentally sensitive nucleoside (Fig. 2b, e, Table 1). Interestingly, **5b** showed almost no fluorescence in methanol. We thought that such phenomenon may also occur with acetylated 2′-deoxyadenosine **8**. In fact, **8** showed strong fluorescence in non-polar solvents and the fluorescence in polar solvents was red shifted with low intensity (Fig. 2c). These fluorescence emission maxima in various solvents were plotted against E_T (30) values, a solvent polarity parameter.[8] The slopes obtained from the linear plots indicated that **5b** is most solvatofluorochromic (Fig. 2d). It should be noteworthy that during fluorescence measurement acetyl substituted **5b** and **8** were not photoisomerized, whereas **5a** gradually gave a mixture of (*E*) and (*Z*) isomers during fluorescence measurement.

Solvatochromic fluorescent deoxyguanosine **5b** was incorporated into 16mer oligodeoxynucleotide (ODN **1**, 5′-GTATCCA**5b**AG ATTGAA-3′). 5′-Hydroxyl group of **4** was protected by dimethoxytrityl group to afford **9**. The compound **9** was reacted with *N*,*N*-diisopropylchlorophosphoramidite to give amidite **10**, which was incorporated into oligonucleotide by automated DNA/RNA synthesizer without further purification (Scheme 3). The synthesized ODN **1** was purified by reverse phase HPLC.

Fluorescence spectrum of ODN **1** was measured in the absence and presence of complementary 15mer ODN **2** (5′-TTCAATCTT GGATAC-3′) forming a bulge structure at **5b**. Fluorescence intensity of single stranded ODN **1** was very weak and the fluorescence maximum was observed at 550 nm. In contrast, the fluorescence intensity of duplex ODN **1**/ODN **2** containing bulge was enhanced and the fluorescence maximum was blue shifted to 526 nm (Fig. 3a). The fluorescence color change was readily observable by naked eye under illumination with 365 nm transilluminator (Fig. 3b, T_m = 50.9 °C). The fluorescence change between single stranded and double stranded structures may be ascribable to the local environmental change around **5b**.



Scheme 3. Reagents and conditions: (a) DMTrCl, 4,4-dimethylaminopyridine, pyridine, rt, 17 h, (54%); (b) *N*,*N*-diisopropylchlorophosphoramidite, triethylamine, acetonitrile, rt, 5 min.

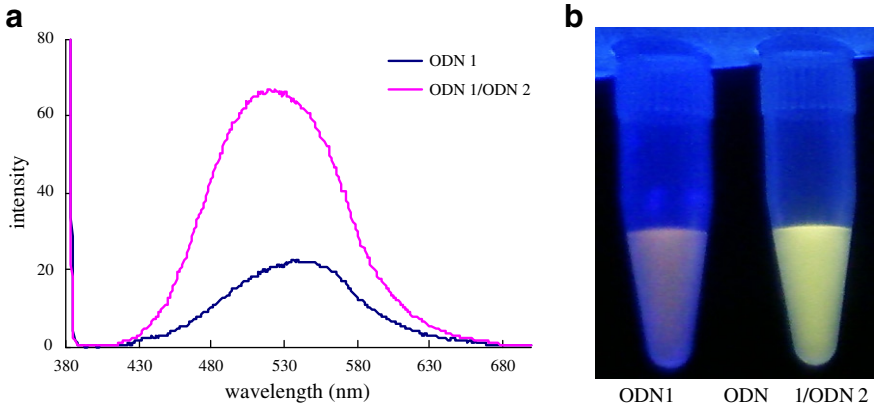


Figure 3. (a) Fluorescence spectra of single stranded ODN 1 and duplex formed by hybridization with ODN 2. (2.5 μM ODNs, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt) Excitation wavelength was 380 nm. (b) Fluorescence image of ODN 1 and the duplexes formed with ODN 2 under illumination with transilluminator (365 nm).

In conclusion, we have succeeded in the design and synthesis of solvatofluorochromic 2′-deoxyguanosine and adenosine analogs. Interestingly, acetyl substituted 2′-deoxypurine derivatives showed a remarkable high solvatofluorochromicity. Particularly, acetyl substituted 2′-deoxyguanosine **5b** showed a large solvatochromicity ($\Delta\lambda_{max}^{em} = 91$ nm). Highly fluorescent solvatochromic guanosine analog **5b** may be used as a fluorescence sensor in various fields.

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7. 8-(4-Acetylstyryl)-2′-deoxyguanosine (**5b**). ^{1}H NMR (DMSO-d_6, 400 MHz) δ; 2.12 (m, 1H), 2.51–2.59 (complex, 4H), 3.69–3.73 (complex, 2H), 3.85 (m, 1H), 4.47 (m, 1H), 5.27 (dd, 1H, *J* = 5.0, 5.2 Hz), 5.31 (d, 1H, *J* = 4.0 Hz), 6.40 (dd, 1H, *J* = 6.2, 8.8 Hz), 6.56 (br s, 2H), 7.60 (d, 1H, *J* = 15.8 Hz), 7.69 (d, 1H, *J* = 15.8 Hz), 7.85 (d, 2H, *J* = 8.4 Hz), 7.95 (d, 2H, *J* = 8.4 Hz), 10.72 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ; 26.7, 44.0, 61.5, 70.6, 83.4, 87.7, 117.6, 119.1, 127.6 (2C), 128.6 (2C), 134.5, 136.4, 140.0, 147.3, 149.8, 152.1, 155.6, 197.2; HRMS (ESI) *m*/*z* 434.1440 calcd for $C_{20}H_{21}N_5O_5Na$ [M+Na]$^+$, found 434.1418. 8-(4-Acetylstyryl)-2′-deoxyadenosine (**8**). ^{1}H NMR (DMSO-d_6, 400 MHz) δ; 2.21 (m, 1H), 2.61 (s, 3H), 2.91 (m, 1H), 3.62 (m, 1H), 3.72 (m, 1H), 3.91 (m, 1H), 4.53 (m, 1H), 5.35 (d, 1H, *J* = 4.3 Hz), 5.50 (dd, 1H, *J* = 4.3, 6.9 Hz), 6.65 (dd, 1H, *J* = 6.6, 8.2 Hz), 7.41 (s, 2H), 7.78 (d, 1H, *J* = 15.8 Hz), 7.82 (d, 1H, *J* = 15.8 Hz), 7.91 (d, 2H, *J* = 8.6 Hz), 7.99 (d, 2H, *J* = 8.6 Hz), 8.12 (s, 1H) ^{13}C NMR (DMSO-d_6, 100 MHz) δ; 26.6, 39.6, 61.1, 70.1, 82.4, 87.2, 116.7, 118.5, 127.2(2C), 128.6(2C), 131.4, 135.8, 140.7, 143.7, 151.7, 153.4, 156.4, 197.1; HRMS (ESI) *m*/*z* 418.1491 calcd for $C_{20}H_{21}N_5O_4Na$ [M+Na]$^+$, found 418.1462.
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