



Synthesis of novel push–pull-type solvatochromic 2'-deoxyguanosine derivatives with longer wavelength emission

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ABSTRACT

We synthesized novel push–pull-type fluorescent guanosine derivatives, ^{CN}G and ^{Ac}G containing 1,6- and 2,7-disubstituted pyrene chromophores. 1,6-Disubstituted pyrene derivatives, 1,6-^{CN}G (**3b**) and 1,6-^{Ac}G (**3c**), exhibited highly solvatochromic fluorescence emission at longer wavelength (~540 nm). The environmentally sensitive fluorescent deoxyguanosines such as **3b** and **3c** can be used as powerful tools for structural studies of nucleic acids and molecular diagnostics.

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Fluorescent nucleosides are widely used as reporter probes for investigating chemical, biochemical, and biological phenomena,¹ and numerous fluorescent nucleosides have been reported.² Among these, modified fluorescent guanosines are of special importance due to their unique properties of forming higher ordered self-assembled structures by base pairing.³ The design of environmentally sensitive fluorescent guanosine derivatives is, therefore, very important not only as a DNA reporter probe or fluorescence sensor but also as a fluorescent building block in supramolecular chemistry. In our continuous studies directed toward the development of practically useful fluorescent nucleosides, we have reported various types of pyrene-labeled fluorescent nucleosides such as base-discriminating fluorescent nucleoside ^{Py}U,⁴ photochromic vinylpyrene-substituted guanosine **1**,⁵ and pyrene-substituted 8-ethynyl guanosine **2**.⁶ While these pyrene-contain-

ing nucleosides are useful as fluorescent reporter molecules,⁷ there is still great demand for further development of solvatochromic guanosine derivatives possessing stronger fluorescence emission at a longer wavelength greater than 500 nm. We now wish to report an intriguing concept for the molecular design of novel push–pull-type solvatofluorochromic guanosine derivatives **3** and **4** that emit strong fluorescence at ca. 540 nm. In order to construct an intramolecular donor–acceptor system, an electron-withdrawing aromatic group (acceptor) and C8 of guanosine (donor) are directly attached to pyrene chromophore via triple bonds. We report herein the synthesis and photophysical properties of novel push–pull-type fluorescent guanosines which contain 1,6- and 2,7-disubstituted pyrene chromophores (Fig. 1).

The synthetic route of pyrene-labeled 2'-deoxyguanosines, 1,6-^{HG}G (**3a**), 1,6-^{CN}G (**3b**) and 1,6-^{Ac}G (**3c**), is outlined in Scheme

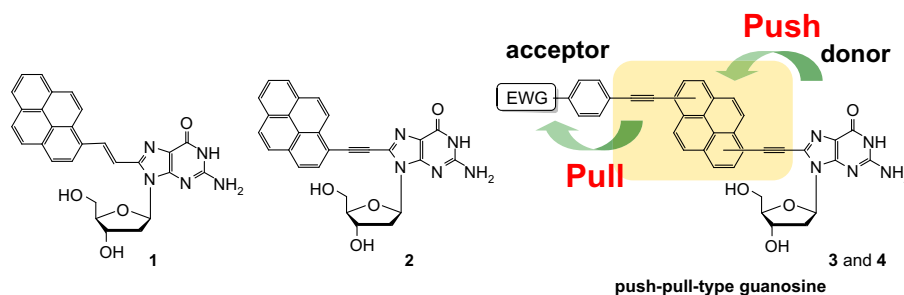
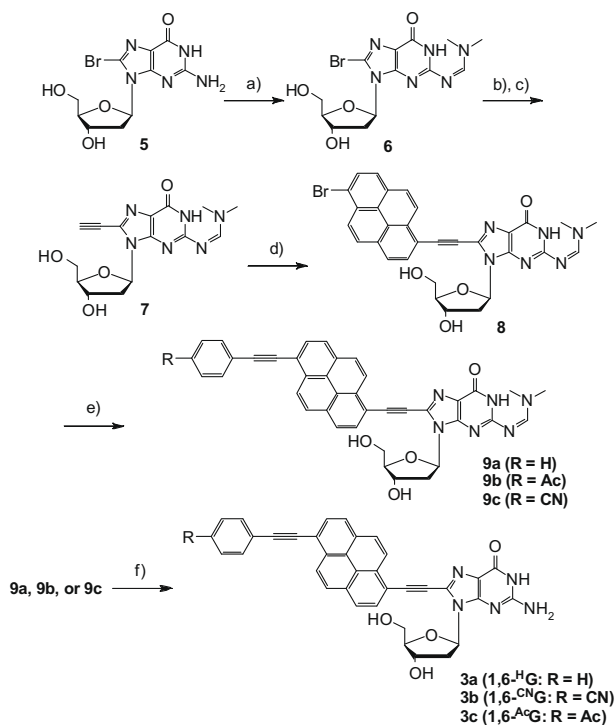


Figure 1. Structure of pyrene-labeled fluorescent guanosine derivatives.

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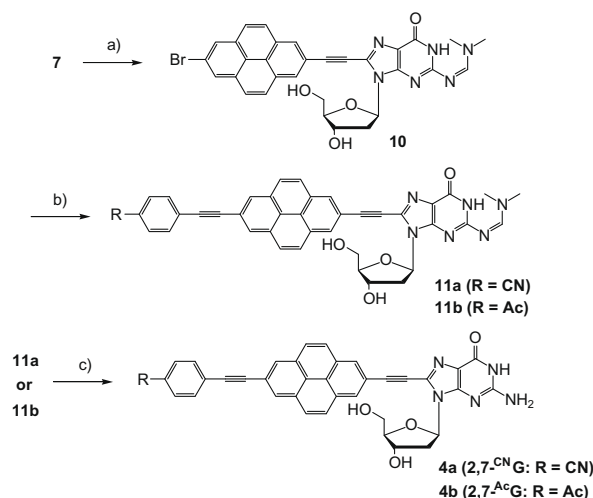
E-mail address: saitoy@chem.ce.nihon-u.ac.jp (Y. Saito).



1. Sonogashira cross-coupling reaction⁸ of 8-bromo-2'-deoxyguanosine derivative **6** with TMS-acetylene followed by deprotection with K₂CO₃ yielded compound **7**, which was then coupled with 1,6-dibromopyrene⁹ using Pd(PPh₃)₄ to afford **8**. The third Sonogashira coupling reaction of **8** with ethynylbenzene, 4-ethynylbenzonitrile, and 4-ethynylacetophenone afforded corresponding coupling products **9a–c**, respectively. Deprotection of **9a–c** with 28% aq NH₄OH–MeOH afforded **3a–c**¹⁰, which were then evaluated for photochemical properties.

2,7-Disubstituted pyrene derivatives, 2,7-^{CN}G (**4a**) and 2,7-^{Ac}G (**4b**), were also synthesized according to Scheme 2 through a similar reaction route. Compound **10** prepared from 2,7-dibromopyrene¹¹ and **7** was coupled with ethynylaryl derivatives using Pd(PPh₃)₄ to afford **11a** and **11b**. After deprotection of DMF acetal group, two 2,7-disubstituted pyrene derivatives, 2,7-^{CN}G (**4a**) and 2,7-^{Ac}G (**4b**), were obtained. The photophysical properties of all newly synthesized guanosine derivatives **3a–c** and **4a–b** were examined.

Initially, we measured the fluorescence spectra of 1,6-disubstituted pyrene derivatives, 1,6-^HG (**3a**), 1,6-^{CN}G (**3b**), and 1,6-^{Ac}G (**3c**), in THF. As shown in Figure 2, 1,6-^HG (**3a**) having larger π-electron system than parent **2** emitted strong fluorescence at a longer wavelength (523 nm) than that of **2**. Interestingly, we found that the introduction of electron-withdrawing substituent such as cyano (**3b**) or acetyl (**3c**) group induced ca. 60 nm red shift than parent **2**. We also measured the fluorescence spectra of these deoxy Gs in various solvents of different polarities. With excitation of 1,6-^{CN}G (**3b**) at 422 nm in chloroform, strong fluorescence emission at 493 nm was observed (Fig. 3a). Upon excitation of 1,6-^{CN}G (**3b**) at 424 nm in THF, we observed strong emission at 542 nm. In ethyl acetate, medium fluorescence emission was observed at 531 nm. In contrast, the fluorescence emission of 1,6-^{CN}G (**3b**) in polar solvents such as acetonitrile, DMF, and MeOH was very weak.



Scheme 2. Reagents and conditions: (a) 2,7-dibromopyrene, Pd(PPh₃)₄, CuI, Et₃N, 80 °C, 3 h, 45%; (b) 4-ethynylbenzonitrile (for **11a**) or 4-ethynylacetophenone (for **11b**), Pd(PPh₃)₄, CuI, Et₃N, DMF, 80 °C, 6 h, 71% (**11a**), 68% (**11b**); (c) NH₄OH, MeOH, rt, 24 h, 73% (**4a**), 81% (**4b**).

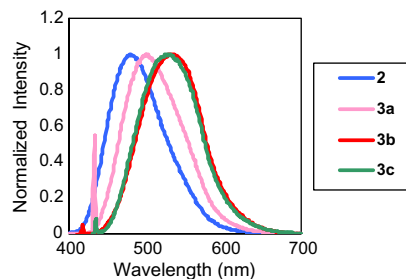


Figure 2. Normalized fluorescence emission spectra of **2**, 1,6-^HG (**3a**), 1,6-^{CN}G (**3b**), and 1,6-^{Ac}G (**3c**) in THF.

Similar results were obtained with 1,6-^{Ac}G (**3c**) (Fig. 3b). As expected, these push–pull-type deoxyguanosine derivatives **3b** and **3c** showed a remarkable solvatochromic property, as evident from the fluorescence image shown in Figure 3c.

The photophysical properties of 2,7-disubstituted pyrene derivatives were also investigated. Fluorescence spectra of 2,7-^{CN}G (**4a**) and 2,7-^{Ac}G (**4b**) measured in different solvents are shown in Figure 4a and b. While fluorescence intensities of both 2,7-^{CN}G (**4a**) and 2,7-^{Ac}G (**4b**) are strong in non-polar solvents such as chloroform and ethyl acetate, very weak fluorescence was observed in polar solvents such as DMF, acetonitrile, and MeOH. In the case of 2,7-disubstituted pyrene derivatives, red-shift of fluorescence emission was not observed by changing solvent polarity, although 1,6-disubstituted pyrene derivatives showed a remarkable red-shift with increasing solvent polarity.

The shapes of the fluorescence spectra of 1,6- and 2,7-disubstituted derivatives were also different. The sharp emission bands of 2,7-disubstituted derivatives are similar to the emission bands from the vibrational structures of parent pyrene chromophore. In this case, the fluorescence wavelength was not changed by changing solvent polarity. On the other hand, 1,6-disubstituted derivatives show a broad emission band and exhibited a solvent polarity-dependent fluorescence emission at longer wavelength. For absorption spectra, 2,7-derivatives exhibited an intense absorption band at around 340 nm, whereas the absorption bands of 1,6-derivatives were observed at around 290 and 420 nm (Table 1, Figs. S3 and S4). These results clearly indicate that both 1,6- and 2,7-isomers have quite different photophysical properties to each other.

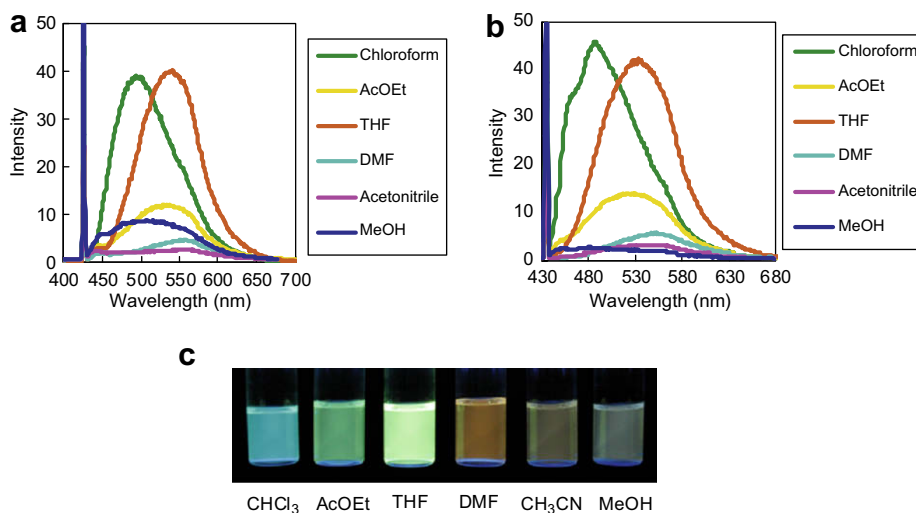


Figure 3. Fluorescence spectra of (a) 1,6-^{CN}G (**3b**) (2.5 μM) and (b) 1,6-^{Ac}G (**3c**) (2.5 μM) in various solvents. (c) Fluorescence colors of 1,6-^{CN}G (**3b**) in different solvents. The sample solutions were illuminated with a 365 nm transilluminator.

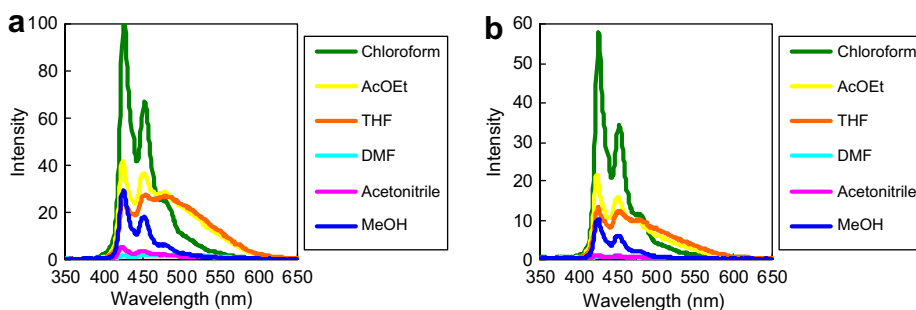


Figure 4. Fluorescence spectra of (a) 2,7-^{CN}G (**4a**) (2.5 μM) and (b) 2,7-^{Ac}G (**4b**) (2.5 μM) in various solvents.

Table 1
Photophysical properties of fluorescent guanosine derivatives and their HOMO and LUMO energy levels

Compound	Solvent ^a	$\lambda_{\text{max}}^{\text{abs}}$ ^b (nm)	$\lambda_{\text{max}}^{\text{fl}}$ ^c (nm)	ϕ ^d	HOMO ^e (eV)	LUMO ^e (eV)	$\Delta E_{\text{LUMO-HOMO}}$ ^e (eV)
2	CHCl ₃	387	455	0.467	-5.06	-1.96	3.10
	THF	392	491	0.243			
	CH ₃ CN	385	498	0.110			
1,6- ^H G (3a)	CHCl ₃	413	482	0.407	-5.01	-2.19	2.82
	THF	413	523	0.225			
	CH ₃ CN	410	536	0.091			
1,6- ^{CN} G (3b)	CHCl ₃	420	493	0.258	-5.20	-2.52	2.68
	THF	421	542	0.190			
	CH ₃ CN	416	554	0.014			
1,6- ^{Ac} G (3c)	CHCl ₃	421	487	0.237	-5.12	-2.42	2.70
	THF	422	534	0.162			
	CH ₃ CN	416	549	0.015			
2,7- ^{CN} G (4a)	CHCl ₃	341	427	0.129	-5.39	-2.20	3.19
	THF	341	454	0.076			
	CH ₃ CN	339	424	0.008			
2,7- ^{Ac} G (4b)	CHCl ₃	341	426	0.081	-5.34	-2.11	3.23
	THF	341	453	0.039			
	CH ₃ CN	339	424	0.002			

^a Solvent is used in the presence of 10% v/v of DMF because of its low solubility.

^b Absorption spectra were measured at 25 °C using 1 cm path length cell (10 μM). See Figures S1–S4.

^c Fluorescence spectra were measured at 25 °C using 1 cm path length cell (10 μM). See Figures 3, 4, S1 and S2.

^d The fluorescence quantum yields (ϕ) were calculated by using 9,10-diphenylanthracene as a reference according to Ref. 12.

^e The calculation was conducted for N⁹-methylated guanine derivatives instead of deoxyribose residue.

We next calculated the HOMO–LUMO energy levels of these guanosine derivatives. As shown in Table 1, the energy levels of the HOMO and LUMO obtained for N⁹-methylated guanine derivatives at DFT(B3LYP)/6-31G* levels were consistent with experimentally observed spectroscopic data.¹³ The fluorescence spectrum of 1,6-^HG (**3a**) is red shifted from that of parent **2**, consistent with the smaller HOMO–LUMO gap by the calculation. 1,6-^{CN}G (**3b**) and 1,6-^{Ac}G (**3c**), both of which have a larger red shift relative to 1,6-^HG (**3a**), have much smaller HOMO–LUMO energy gap than that of 1,6-^HG (**3a**). On the other hand, the HOMO–LUMO energy gaps for 2,7-^{CN}G (**4a**) and 2,7-^{Ac}G (**4b**) are larger than that of parent **2**, which are in agreement with the blue shift of their fluorescence. For N⁹-methylated 1,6-^{CN}G, the HOMO and the LUMO orbitals are broadly distributed over the π -system, whereas for N⁹-methylated 2,7-^{CN}G, the HOMO and LUMO are localized mainly at the guanine base and phenyl acetylene linker (Fig. S5). These calculations strongly support the view that only 1,6-disubstituted pyrene-labeled guanosine derivatives, not the 2,7-disubstituted pyrenes, exhibit longer fluorescence emission than **2**.

In summary, we have developed novel push–pull-type pyrene-containing guanosine derivatives. The fluorescence emission of these nucleosides, particularly, those of 1,6-^{CN}G (**3b**) and 1,6-^{Ac}G (**3c**), showed a strong solvent dependency. Such environmentally sensitive fluorescent deoxyguanosines can be used as a powerful tool for structural studies of nucleic acids and molecular diagnostics.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.012.

References and notes

- (a) Printz, M.; Richert, C. *Chemistry* **2009**, *15*, 3390; (b) Greco, N. J.; Sinkeldam, R. W.; Tor, Y. *Org. Lett.* **2009**, *11*, 1115; (c) Srivatsan, S. G.; Tor, Y. *J. Am. Chem. Soc.* **2007**, *129*, 2044; (d) Cekan, P.; Sigurdsson, S. T. *Chem. Commun.* **2008**, 3393.
- (a) Venkatesan, N.; Seo, Y. J.; Kim, B. H. *Chem. Soc. Rev.* **2008**, *37*, 648; (b) Tor, Y., Ed.; Tetrahedron Symposium in Print, 2007, 63.; (c) Wilson, J. N.; Kool, E. T. *Org. Biomol. Chem.* **2006**, *4*, 4265; (d) Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487.
- (a) Sessler, J. L.; Sathiosatham, M.; Doerr, K.; Lynch, V.; Abboud, K. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 1300; (b) Manet, I.; Francini, L.; Masiero, S.; Pieraccini, S.; Spada, G. P.; Gottarelli, G. *Helv. Chim. Acta* **2001**, *84*, 2096; (c) Shi, X.; Mullaugh, K. M.; Fettingter, J. C.; Jiang, Y.; Hofstadler, S. A.; Davis, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 10830; For examples of DNA triplexes, see: (d) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645; For examples of guanine quadruplex formation, see: (e) Davis, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668. and references therein.
- Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820.
- Saito, Y.; Matsumoto, K.; Takeuchi, Y.; Bag, S. S.; Kodate, S.; Morii, T.; Saito, I. *Tetrahedron Lett.* **2009**, *50*, 1403.
- Okamoto, A.; Ochi, Y.; Saito, I. *Chem. Commun.* **2005**, 1128.
- (a) Saito, Y.; Bag, S. S.; Kusakabe, Y.; Nagai, C.; Matsumoto, K.; Mizuno, E.; Kodate, S.; Suzuka, I.; Saito, I. *Chem. Commun.* **2007**, 2133; (b) Saito, Y.; Miyauchi, Y.; Okamoto, A.; Saito, I. *Tetrahedron Lett.* **2004**, *45*, 7827; (c) Saito, Y.; Shinohara, Y.; Bag, S. S.; Takeuchi, Y.; Matsumoto, K.; Saito, I. *Tetrahedron* **2009**, *65*, 934; (d) Matsumoto, K.; Shinohara, Y.; Bag, S. S.; Takeuchi, Y.; Morii, T.; Saito, Y.; Saito, I. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6392; (e) Okamoto, A.; Saito, Y.; Saito, I. *J. Photochem. Photobiol. C: Photochem. Rev.* **2005**, *6*, 108.
- Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.
- Grimshaw, J.; Trocha-Grimshaw, J. J. *Chem. Soc., Perkin Trans. 1* **1972**, 1622.
- Spectroscopic data for 1,6-^{CN}G (3b)*: ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.31 (m, 1H), 3.29 (m, 1H), 3.59 (m, 1H), 3.67 (m, 1H), 3.93 (m, 1H), 4.51 (m, 1H), 4.76 (m, 1H), 5.21 (m, 1H), 6.53–6.58 (complex, 3H), 7.93–7.98 (complex, 4H), 8.33–8.43 (complex, 6H), 8.58 (d, 1H, *J* = 9.1 Hz), 8.70 (d, 1H, *J* = 9.0 Hz), 10.84 (br, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 37.9, 62.7, 71.7, 84.7, 86.4, 88.5, 92.3, 92.5, 94.8, 111.7, 116.4, 117.6, 118.7, 118.9, 123.7 ($\times 2$), 126.1, 126.5, 126.5, 126.7, 127.7, 129.4, 129.6, 130.0, 130.6, 131.0, 131.7, 131.9, 132.0, 132.1, 132.8 ($\times 2$), 133.1 ($\times 2$), 151.6, 154.5, 156.6; HRMS (ESI) *m/z* 639.1736 calcd for C₃₇H₂₄N₆O₄Na [M+Na]⁺, found 639.1757. These synthesized guanosine derivatives are photostable upon UV irradiation.
- (a) Lee, H.; Harvey, R. G. *J. Org. Chem.* **1986**, *51*, 2847; (b) Connor, D. M.; Allen, S. D.; Collard, D. M.; Liotta, C. L.; Schiraldi, D. A. *J. Org. Chem.* **1999**, *64*, 6888.
- Quantum yields (Φ) were calculated according to following reference: Morris, J. V.; Mahaney, M. A.; Huber, J. R. *J. Phys. Chem.* **1976**, *80*, 969.
- The theoretical calculations were performed by density functional theory (DFT) calculations at B3LYP/6-31G* level using SPARTAN '08 for Windows (Wavefunction, Inc.).