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Fluorescence switching of photochromic vinylpyrene-substituted 2′-deoxyguanosine

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ABSTRACT

We synthesized C8-vinylpyrene-substituted 2′-deoxyguanosine ^{VPy}**G** and studied the photoregulated reversible *E*–*Z* isomerization. When *E*-isomer was irradiated with visible light (>420 nm), *E- to Z*-isomerization took place very rapidly, while upon irradiation with UV-light (~365 nm), *Z*-isomer was converted to *E*-isomer. When *Z*-isomer was illuminated with 365–400 nm light, no fluorescence was observed, while *E*-isomer showed a very strong fluorescence emission, indicating that ^{VPy}**G** could be a useful fluorescence switching molecule.

The design and synthesis of photoswitching molecules have attracted currently much attention for devising molecular devices such as molecular switches and sensors.[1] Particularly, fluorescence switch is of special importance due to its high sensitivity and selectivity for the application to optical devices, fluorometric assay of biomolecules as well as bioimaging.[2] We now wish to report a unique 'on–off' fluorescence switching of vinylpyrene-substituted 2′-deoxyguanosine, which would find widespread application as a photochromic nucleobase for fluorometric sensing, bioimaging, optical devices and photoregulation of nucleic acid structures (Fig. 1).

Our long-term interest in designing base-discriminating fluorescent (BDF)[3] nucleosides has led us to develop a novel vinylpyrene-substituted guanosine derivative ^{VPy}**G**, which reversibly photoisomerized on illuminating at $\lambda > 350$ nm where no UV damage occurred on DNA. This molecule showed a fluorescence emission that can be 'on–off' modulated with high sensitivity owing to its rapid photoisomerization between fluorescent (*E*-form) and non-fluorescent states (*Z*-form). ^{VPy}**G** can therefore be used as a fluorescence switching molecule.

We have designed the photoswitchable nucleoside, C8-vinylpyrene-substituted 2′-deoxyguanosine **1** (^{VPy}**G**). Pyrene was chosen because its absorption maximum lies around 350 nm where no natural nucleotides absorb light. The photochromic compound **1** was synthesized according to Scheme 1. Thus, 8-bromo-2′-deoxyguanosine **3** was treated with *N,N*-dimethylformamide diethylacetal in methanol at 60 °C to afford N^2-protected nucleoside **4**, which was then converted to C8-vinyl derivative **5** under Stille coupling conditions.[4] A second round of Pd(0)-mediated coupling of nucleoside **5** with 1-bromopyrene in the presence of sodium acetate gave compound **6** in a moderate yield, which upon treatment with NH_4OH afforded the desired photochromic nucleoside **1**. During isolation process under room light, initially formed *Z*-isomer was gradually photoisomerized to give a mixture of *E*- and *Z*-isomers. Both *E*- and *Z*-isomers were separated in a pure form using HPLC with 100% methanol as an eluting solvent.[5]

Next, we examined the photoisomerization in methanol. Upon illumination of *E*-^{VPy}**G** with visible light ($\lambda > 420$ nm), a photostationary state containing *Z*-form in a major quantity (92%) was obtained as determined by HPLC. *E*-form was regenerated by irradiation of *Z*-form with UV light (~365 nm), and it was obtained in 82% yield. The *E*/*Z* ratios were calculated at a photostationary state by measuring HPLC peak area as detected at the wavelength of isosbestic point (368 nm). These results indicate that highly reversible *E* to *Z* photoisomerization of ^{VPy}**G** can be accomplished by illumination at 365 nm and 420 nm (Fig. 2).[6] Thus, the *E*–*Z* isomerization of photoresponsive ^{VPy}**G** was conducted by 420 nm light separated from a 100 W Xenon lamp by a filter solution and

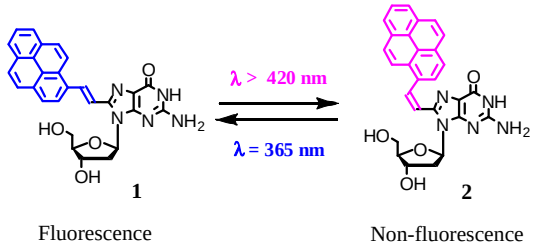

Figure 1. Fluorescence 'on–off' switching induced by *E*–*Z* photoisomerization of photochromic base ^{VPy}**G**.

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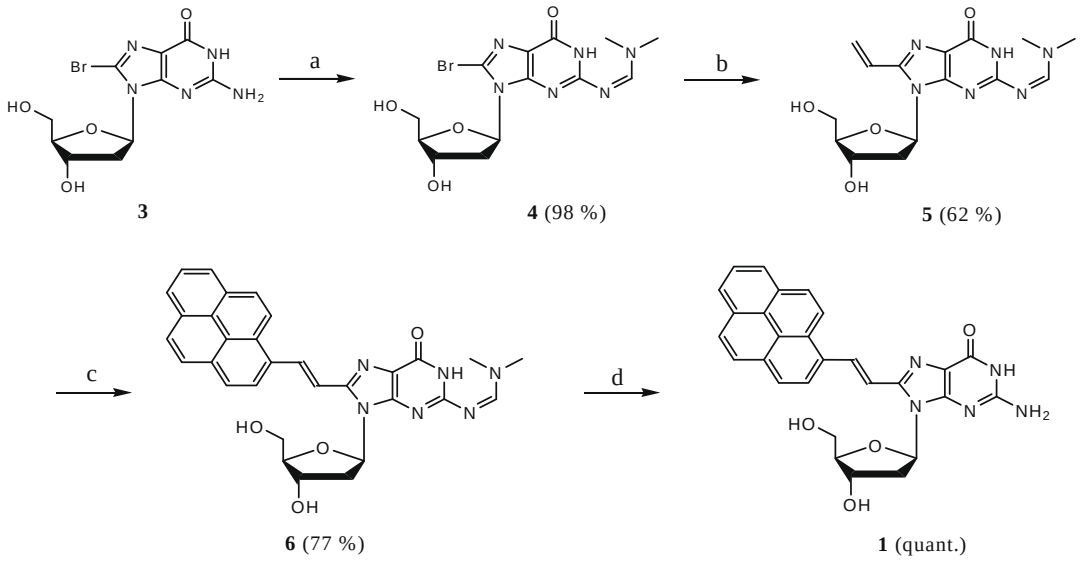

Scheme 1. Synthesis of photochromic nucleoside ^{VPy}G. Reagent and conditions: (a) DMF diethylacetal, methanol, 60 °C, 3 h; (b) $Pd(PPh_3)_4$, $Sn(CHCH_2)_4$, Et_3N, DMF, 60 °C, 12 h; (c) 1-bromopyrene, $Pd(PPh_3)_4$, CH_3COONa, DMF, 80 °C, 12 h; (d) NH_4OH/methanol, rt, 8 h.

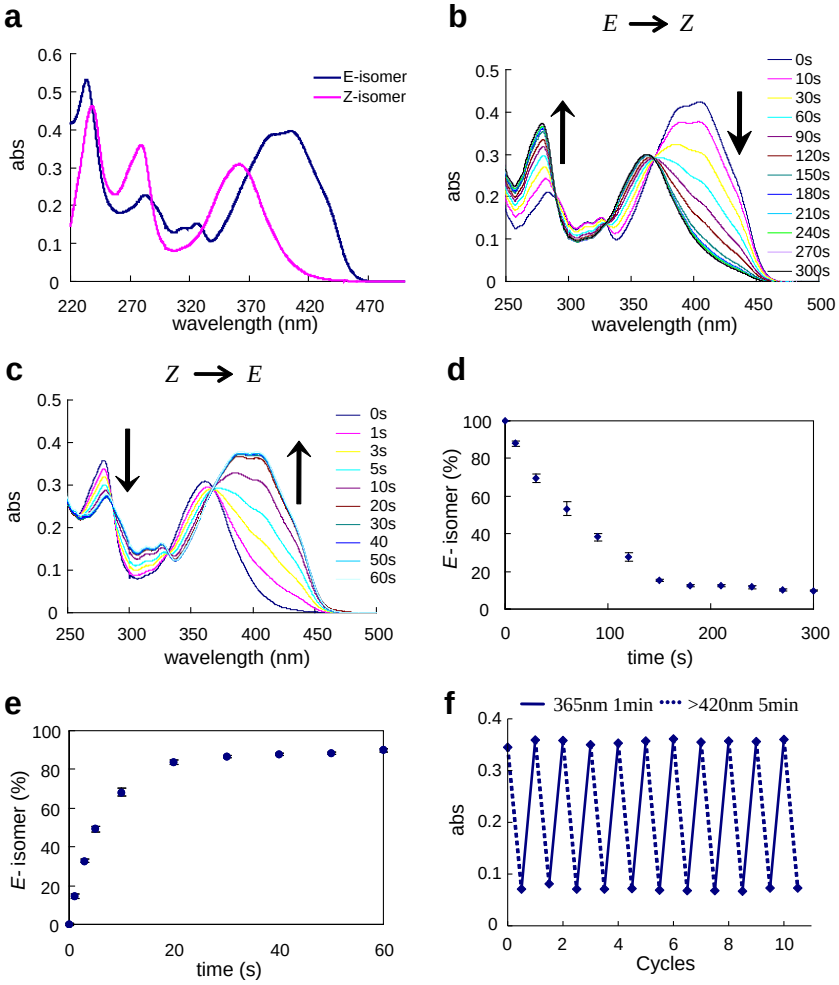

Figure 2. (a) Absorption spectra of *E*- and *Z*-VPyG. Spectral change for photoirradiation of (b) *Z*- to *E*-isomer and (c) *E* to *Z*-isomer. Time course for the photoisomerization of (d) *Z* to *E* and (e) *E* to *Z*. (f) Switching cycles between *E*- and *Z*-isomer. It was monitored at 410 nm UV–vis absorbance.

by 365 nm light from a UV-transilluminator. Photoirradiation of *E*-^{VPy}**G** at 420 nm resulted in a rapid decrease in the absorption at 405 nm with a blue shift of ca. 40 nm and in an increase in the absorption at 280 nm with a blue shift of ca. 2 nm, indicating a

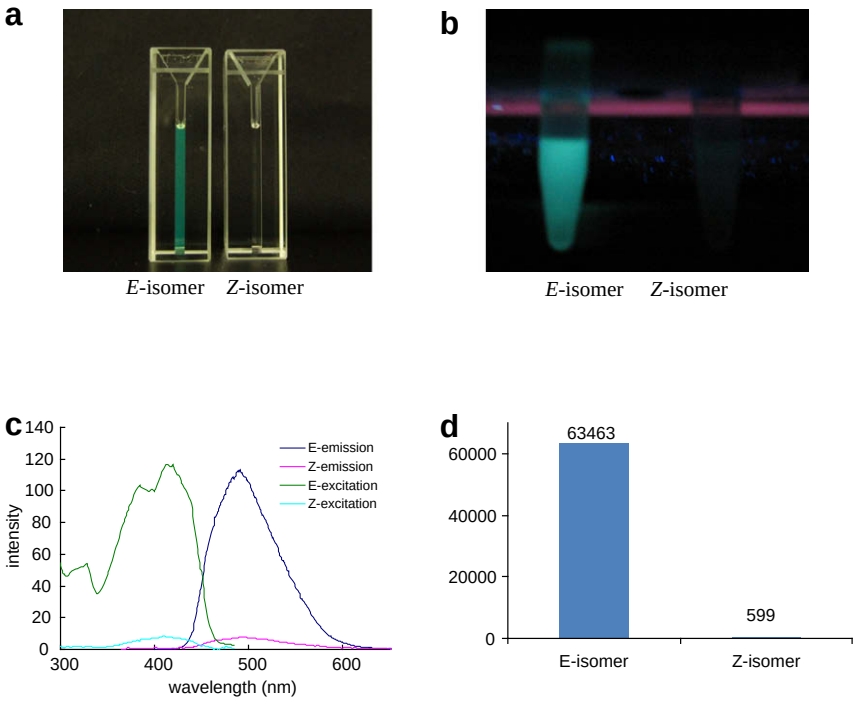

Figure 3. (a) Visible color of *Z*- and *E*-isomers in methanol. (b) Fluorescence image of *E*- and *Z*-isomers illuminated at 365 nm. (c) Fluorescence spectra and fluorescence excitation spectra of *E*- and *Z*-isomers. (d) Fluorescence intensity of *E*- and *Z*-isomers as determined by fluorescence plate reader.

quantitative *E* to *Z* photoisomerization (Fig. 2b). On the other hand, when *Z*-^{VPy}**G** was illuminated at 365 nm, the absorbance of the peak at 280 nm decreased with a red shift of 2 nm, while the intensity of the peak at 365 nm increased with a red shift of ca. 40 nm (Fig. 2c). In the case of *Z* to *E* photoisomerization, the photostationary state was attained within 60 s (Fig. 2e). To reach the photostationary state from *E*-isomer, longer irradiation time (>170 s) was required (Fig. 2d). Such reversible photoisomerization was repeated more than 10 times without any side reaction (Fig. 2f). Interestingly, we observed a visible yellow color and bright bluish green fluorescent for *E*-isomer, while no visible color and no fluorescence were observed for *Z*-isomer (Fig. 3a and b). We examined the fluorescence behavior of photochromic ^{VPy}**G** as a photoswitching fluorescent molecule in more detail. Interestingly, when excited in the 365–400 nm region, *Z*-isomer **2** showed almost no fluorescence, whereas *E*-isomer **1** showed a strong fluorescence with an emission maximum at 490 nm (Fig. 3c). Very weak fluorescence at 490 nm observed for *Z*-isomer (Fig. 3c) was ascribable to the fluorescence of a small amount of *E*-isomer formed during fluorescence measurement. This was further confirmed by the fluorescence excitation spectra of the low intensity fluorescence peak at 490 nm, which was identical with that of the fluorescence excitation spectra of *E*-isomer, not for the *Z*-isomer. We also measured the fluorescence intensity of *E*- and *Z*-isomers by using fluorescence plate reader within a time scale of 0.1 s in methanol (excited at 355 nm, detected at 535 nm) (Fig. 3d). The data clearly indicated that *Z*-isomer is non-fluorescent. The fluorescence lifetime of *E*-isomer was also measured in methanol at room temperature. These photophysical properties of *E*- and *Z*-^{VPy}**G** are summarized in Table 1.

Thus, we concluded that *E*-isomer is in the fluorescence 'on' state, while *Z*-isomer is in the "off" state. The lack of fluorescence emission for *Z*-isomer is probably due to the strong interaction of photoexcited pyrene moiety with neighboring electron-donating guanine base. We observed a good reversibility of *E*–*Z* photoisomerization without any side reaction (Fig. S1).

Table 1
Photophysical data for *E*- and *Z*- ^{VPy}**G** in methanol

Isomer	ε_{360}	ε_{410}	λ_{max} (nm)	φ_F	τ_F[a] (ns)
E	22,200	38,600	489	0.43 (λ_{ex} = 410 nm)	2.3
Z	30,800	3800	—	~0 (λ_{ex} = 410 nm)	—

[a] A single component.

In conclusion, we have successfully developed photochromic C8-vinylpyrene-substituted 2′-deoxyguanosine (^{VPy}**G**) as a photoswitching molecule.[8] The nucleoside showed a very rapid and reversible photoisomerization without any side reaction. We have also demonstrated that *E*-isomer emits a strong fluorescence, while *Z*-isomer does not. Therefore, due to the drastic fluorescence change, the photochromic guanine base ^{VPy}**G** might be a very useful fluorescence switching molecule.

Acknowledgments

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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.2009.01.029.

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5. C8-vinylpyrene-substituted 2′-deoxyguanosine (**1**). (*E*-isomer): ^{1}H NMR (DMSO, 400 MHz) δ; 2.18 (ddd, 1H, J = 2.3, 6.2, 13.1), 2.51 (m, 1H), 3.69–3.80 (complex, 2H), 3.90 (ddd, 1H, 3.6, 3.6, 3.6), 4.52 (m, 1H), 5.27 (dd, 1H, J = 5.3, 5.3), 5.34 (d, 1H, J = 4.1), 6.50 (dd, 1H, 6.2, 8.7), 6.59 (s, 2H), 7.84 (d, 1H, J = 15.5 Hz), 8.10–8.70 (complex 10H), 10.74 (br, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 40.5, 61.2, 70.3, 82.5, 87.3, 116.7, 118.2, 122.4, 123.8, 123.9, 124.2, 125.3, 125.3, 125.6, 126.4, 127.3, 127.5, 128.0, 128.0, 128.4, 130.0, 130.3, 130.7, 130.9, 144.5, 151.8, 153.3, 156.4. **2** (*Z*-isomer): ^{1}H NMR (DMSO, 400 MHz) δ; 1.82 (ddd, 1H, J = 2.8, 6.4, 13.1 Hz), 2.74–2.81 (m, 1H), 3.56–3.65 (m, 1H), 3.67–3.72 (m, 1H), 4.38–4.42 (m, 1H), 5.04 (dd, 1H, J = 5.76, 5.76 Hz), 5.20 (d, 1H, J = 4.4 Hz), 6.28 (dd, 1H, J = 6.4, 8.2 Hz), 7.09 (d, 1H, J = 12.3 Hz), 7.76 (d, 1H, J = 12.3 Hz), 7.99 –8.73 (complex, 9H), 10.58 (br, 1H); HR-ESIMS m/z calcd for $[C_{28}H_{23}N_5O_4 + Na]^+$ 516.1648 found 516.1643.
6. Quantum yields of *E*- to *Z*- and *Z*- to *E*-conversions were approximately 0.09 and 0.46, respectively, as determined by using azobenzene as an actinometer.[7]
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