

Synthesis and reversible photoisomerization of photoswitchable nucleoside, 8-styryl-2'-deoxyguanosine

Shinzi Ogasawara^{a,*}, Isao Saito^b, Mizuo Maeda^a

^a Bioengineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

^b School of Engineering, NEWCAT Institute, Nihon University, Tamura, Koriyama, Fukushima 963-8642, Japan

Received 29 November 2007; revised 21 January 2008; accepted 22 January 2008

Available online 1 February 2008

Abstract

A novel guanosine derivative with a photoswitching property, 8-styryl-2'-deoxyguanosine was synthesized, and showed very rapid and efficient reversible *E*–*Z* photoisomerization upon illumination at specific wavelength. In addition, *E*–*Z* photoisomerization can be iteratively performed by alternate illumination with monochroic 254 nm and 370 nm light without any side reactions.

© 2008 Elsevier Ltd. All rights reserved.

In the past decade there has been great interest in photochromic compounds including stilbene¹ and azobenzene² derivatives, which reversibly change the photochemical and physical properties upon *E*–*Z* photoisomerization by the external light stimuli. This photoswitching property has found numerous applications in bionanotechnology because light-triggered methods allow accurate and easy control of the location, dose, and time when an event occurs. For example, azobenzene derivatives have been incorporated into peptide,³ protein,⁴ double-stranded DNA,⁵ single-stranded RNA,⁶ RNAzyme,⁷ and DNAzyme⁸ to control activities by light illumination. Although such photoactivation systems have shown reversibility, the active/inactive ratio was not sufficiently high, due to the installation of the bulky azobenzene chromophore which may influence the active-state conformation. Therefore, if the photoisomerization function was directly installed to a biomolecule instead of attaching a photochromic compound, it would lead to a more powerful photoswitching method. Majima and co-workers have reported (deoxy)ribofuranosyl 2-phenylazoimidazole and observed that these compounds undertake *E*–*Z* photoisomerization.⁹ We describe herein the synthesis and photochemical pro-

erties of a novel guanosine derivative, 8-styryl-2'-deoxyguanosine (^{8ST}G), which reversibly photoisomerized by illuminating at specific wavelength (Fig. 1). Compared with azobenzene derivatives, stilbene derivatives have several advantages such as thermal stability and fluorescence generation. Such photoswitchable guanine base would be very useful, since guanine base is concerned with unique conformation of nucleic acid including *Z*-form,¹⁰ G-quartet,¹¹ and quadruplex found in triplet repeat sequence.¹²

The *E*-isomer ^{8ST}G(*E*) (**2**) and the *Z*-isomer ^{8ST}G(*Z*) (**4**) were prepared individually from 8-bromo-2'-deoxyguanosine (**1**),¹³ as described in Scheme 1. For ^{8ST}G(*E*), Suzuki–Miyaura cross-coupling of (*E*)-2-phenylvinylboronic acid and **1** was performed in DMF at 110 °C for 16 h, by using tetrakis(triphenylphosphine)palladium(0) as the catalyst. After purification by column chromatography on silica

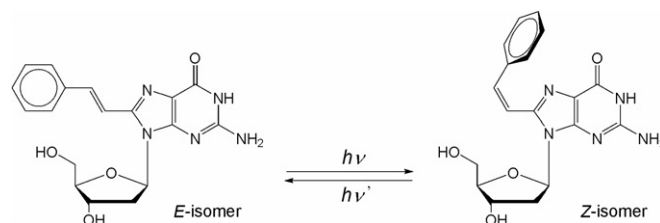
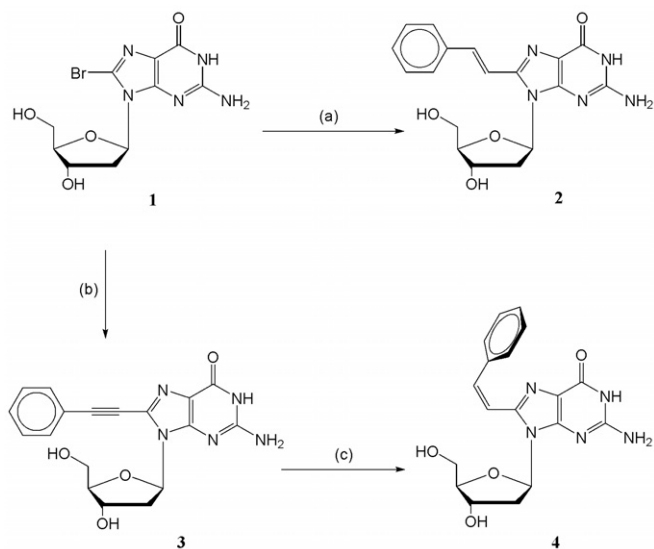


Fig. 1. Schematic illustration of *E*–*Z* photoisomerization of the 8-styryl-2'-deoxyguanosine (^{8ST}G).

* Corresponding author. Tel.: +81 48 467 9312; fax: +81 48 462 4658.
E-mail address: o_shinzi@riken.jp (S. Ogasawara).



Scheme 1. Synthesis of 8-styryl-2'-deoxyguanosine. Reagents and conditions: (a) (*E*)-2-phenylvinylboronic acid (1.2 equiv), Pd(PPh₃)₄ (0.05 equiv), Et₃N (4.0 equiv), 110 °C, DMF, 64%; (b) ethynylbenzene (1.2 equiv), Pd(PPh₃)₄ (0.025 equiv), CuI (0.05 equiv), Et₃N (3.0 equiv), 110 °C, DMF, 59%; (c) 5% Pd/C, H₂, EtOH, 40%.

gel, **2** was obtained in 64% yield.¹⁴ Sonogashira cross-coupling reaction of **1** and ethynylbenzene was employed to give **3**. Hydrogenation of **3** on 5% Pd/C at room temperature for 16 h gave **4** in 40% yield.¹⁵ Both purified ⁸STG(*E*) and ⁸STG(*Z*) were characterized by different spectroscopic measurements, including FAB mass spectrometry and NMR experiments. It is known that substitution at C8 of 2'-deoxyguanosine causes an *anti* to *syn* conformational change with respect to the relevant N9–C1' glycosidic bond, which can be confirmed by NMR spectroscopy. An *anti* to *syn* conformational change results in a downfield shift of the C1', C3', C4', and H2' signals, but an upfield shift of the C2' signals as compared to that of 2'-deoxyguanosine.¹⁶ Such shifts were not observed for ⁸STG(*E*), while downfield shift of the C1' and H2', and upfield shift of the C2' signals were observed for ⁸STG(*Z*). Furthermore, NOE between 2-amino proton and the 5'-hydroxy proton, and H1' and vinyl proton have been observed only in ⁸STG(*Z*). These results indicate that ⁸STG(*Z*) prefers *syn* conformation relative to ⁸STG(*E*).

The photoisomerization of ⁸STG was examined in aqueous solution containing 2.0% acetonitrile.¹⁷ The wavelength dependence of the *E*:*Z* ratio in the photostationary state (PSS) was investigated within the range of 240 nm to 390 nm at 10 nm intervals by illuminating with a monochromic light. The PSS was obtained after 2 h irradiation. As seen in Figure 2, the photostationary equilibrium tends to be *E* < *Z* by illumination at more than 300 nm, whereas illumination at below 300 nm tends to be *E* > *Z*. The most lopsided *E*:*Z* ratio was 6:94 at 370 nm and 80:20 at 250 nm. These results indicate that highly reversible *E*–*Z* photoswitching of ⁸STG can be realized by alternate illumination with 250 nm and 370 nm light.

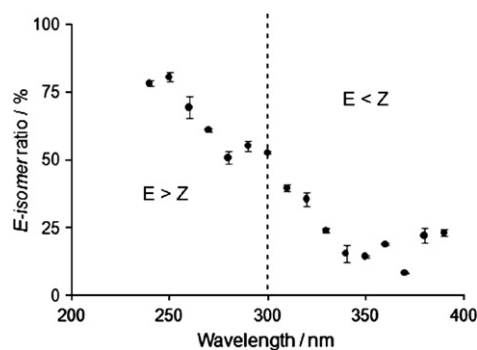


Fig. 2. Wavelength-dependence of the *E*-isomer ratio in the photostationary state (PSS) as determined by the HPLC profiles.

Based on the above results, we demonstrated the reversible photoisomerization of ⁸STG using monochromic 370 nm and 254 nm light for *E*→*Z* and *Z*→*E* photoisomerization, respectively.¹⁸ Illumination of ⁸STG(*E*) at 370 nm using a 100 W Xenon lamp generated ⁸STG(*Z*), which led to a rapid decrease in the absorption at λ_{max} = 340 nm and an increase in the absorption at 249 nm (Fig. 3a). In this reaction, the PSS was achieved within 5 s at 96% conversion, as determined by the peak area in HPLC analysis (Fig. 3c). In contrast, when ⁸STG(*Z*) was illuminated at 254 nm, the intensity of the peak at λ_{max} = 249 nm decreased, while the intensity of the peak at 340 nm increased, indicating *Z*→*E* photoisomerization (Fig. 3b). This reaction reached the PSS within 40 s and had 67% conversion (Fig. 3d). Conversion for *E*→*Z* and *Z*→*E* photoisomerization at the PSS correspond to the ratio of absorbance coefficient at 370 nm and 254 nm, respectively.

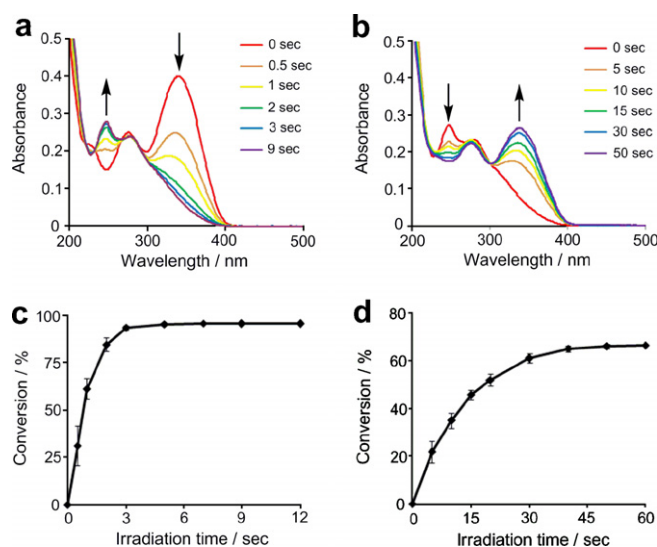


Fig. 3. (a) Absorption spectra for photoisomerization of ⁸STG(*E*) with illumination at 370 nm, and (b) photoisomerization of ⁸STG(*Z*) with illumination at 254 nm. Arrows indicate increase and decrease of the absorption peaks. (c) Time course for photoisomerization of ⁸STG(*E*) and (d) ⁸STG(*Z*).

Table 1
Photophysical properties^a

Entry	Φ_{iso}	λ_{abs} (nm)	λ_{em} (nm)
⁸ STG(<i>E</i>)	0.35	340	450
⁸ STG(<i>Z</i>)	0.15	249	450

^a In nitrogen-saturated aqueous solutions (concentration: 5 μM). Φ_{iso} represents $\Phi_{E \rightarrow Z}$ and $\Phi_{Z \rightarrow E}$ for ⁸STG(*E*) and ⁸STG(*Z*), respectively.

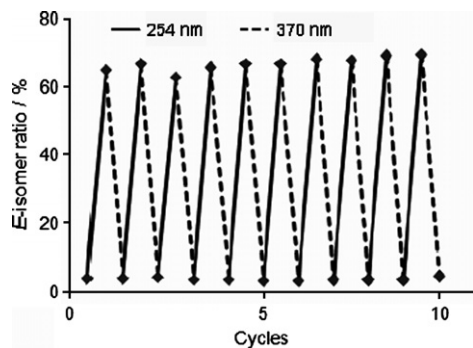


Fig. 4. Switching cycles between *E* and *Z* by alternate illumination with 254 nm and 370 nm light. The illumination periods are 50 s and 7 s for 254 nm and 370 nm, respectively.

We next investigated the quantum yield for *E*–*Z* photoisomerization and the fluorescence spectrum in an aqueous solution at room temperature. As summarized in Table 1, the quantum yields were $\Phi_{E \rightarrow Z} = 0.35$ and $\Phi_{Z \rightarrow E} = 0.15$, which were obtained by comparing the initial rates of photoisomerization to that of *E*-stilbene.¹⁹ Fluorescence emission was observed for both ⁸STG(*E*) and ⁸STG(*Z*), which had a similar fluorescence maximum at 450 nm, but the intensity for ⁸STG(*Z*) was only one-sixth of ⁸STG(*E*). Finally, the reversible switching was repeated several times by alternate illumination with 254 nm and 370 nm light, and good reversibility of *E*–*Z* photoisomerization was observed without any side reactions, as shown in Figure 4.

In summary, we have successfully developed a photochromic 2'-deoxyguanosine, 8-styryl-2'-deoxyguanosine (⁸STG). This compound shows a very rapid and highly efficient reversible *E*–*Z* photoisomerization upon illumination at specific wavelength. In addition, *E*–*Z* photoisomerization can be iteratively performed by alternate illumination with monochromic 254 nm and 370 nm light without any side reactions.²⁰ Therefore, ⁸STG may be widely used for the photochemical control of nucleic acid structure. To the best of our knowledge, this is the first example for the nucleobase carrying photoswitching property.

Supplementary data

Detailed synthesis and fluorescence spectra for both of ⁸STG(*E*) and ⁸STG(*Z*), ¹H and ¹³C NMR spectra of compounds 2, 3, and 4. Supplementary data associated with

this article can be found in the online version, at doi:10.1016/j.tetlet.2008.01.124.

References and notes

- (a) Meier, H. *Angew. Chem., Int. Ed.* **2001**, *40*, 1851–1853; (b) Guaten, E.; Klasinc, L. *Tetrahedron Lett.* **1968**, *26*, 3097–3101; (c) Gegiou, D.; Muszkat, K. A.; Fischer, E. *J. Am. Chem. Soc.* **1968**, *90*, 12–18; (d) Malkin, S.; Fischer, E. *J. Phys. Chem.* **1964**, *68*, 1153–1163.
- (a) Diau, E. W.-G. *J. Phys. Chem. A* **2004**, *108*, 950–956; (b) Hirose, Y.; Yui, H.; Sawada, T. *J. Phys. Chem. A* **2002**, *106*, 3067–3071; (c) Lednev, I. K.; Ye, T.-Q.; Hester, R. E.; Moore, J. N. *J. Phys. Chem.* **1996**, *100*, 13338–13341; (d) Rau, H.; Luddecke, E. *J. Am. Chem. Soc.* **1982**, *104*, 1616–1620.
- (a) Hayashi, G.; Hagihara, M.; Dohno, C.; Nakatani, K. *J. Am. Chem. Soc.* **2007**, *129*, 8678–8679; (b) Zhang, Z. H.; Burns, D. C.; Kumita, J. R.; Smart, O. S.; Woolley, G. A. *Bioconjugate Chem.* **2003**, *14*, 824–829; (c) Ulysse, L.; Cubillos, J.; Chmielewski, J. *Chem. Commun.* **1995**, 8466–8467.
- (a) Volgraf, M.; Gorostiza, P.; Numano, R.; Kramer, R. H.; Isacoff, E. Y.; Trauner, D. *Nat. Chem. Biol.* **2006**, *2*, 47–52; (b) Loudwing, S.; Bayley, H. *J. Am. Chem. Soc.* **2006**, *128*, 12404–12405; (c) Nakayama, K.; Endo, M.; Majima, T. *Chem. Commun.* **2004**, 2386–2387.
- Liu, M. Z.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2006**, *128*, 1009–1015.
- Roy, S.; Nagarajan, R.; Wu, P. C.; Yang, K.; Bruno, F. F.; Parmer, V. S.; Tripathy, S. K.; Kumar, J.; Samuelson, L. A. *J. Macromol. Sci., Pre Appl. Chem.* **2001**, *38*, 1383–1392.
- Liu, Y.; Sen, D. *J. Mol. Biol.* **2004**, *341*, 887–892.
- Keiper, S.; Vyle, J. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 3306–3309.
- Endo, M.; Nakayama, K.; Kaida, Y.; Majima, T. *Tetrahedron Lett.* **2003**, *44*, 6903.
- Ha, S. C.; Lowenhaupt, K.; Rich, A.; Kim, Y.-G.; Kim, K. K. *Nature* **2005**, *437*, 1183–1186.
- (a) Bertrand, H.; Monchaud, D.; Cian, A. D.; Guillot, R.; Mergny, J.-L.; Fichou, M.-P. T. *Org. Biol. Chem.* **2007**, *5*, 2555–2559; (b) Paeschke, K.; Simonsson, T.; Postberg, J.; Rhodes, D.; Lipps, H. J. *Nat. Struct. Mol. Biol.* **2005**, *12*, 847–854; (c) Parkinson, G. N.; Lee, M. P. H.; Neidle, S. *Nature* **2002**, *417*, 876–880; (d) Arnal-Herault, C.; Banu, A.; Barboiu, M.; Michau, M.; Lee, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4268–4272; (e) Wong, A.; Ida, R.; Spindler, L.; Wu, G. *J. Am. Chem. Soc.* **2005**, *127*, 6990–6998.
- Sotoya, H.; Matsugami, A.; Ikeda, T.; Ouhashi, K.; Uesugi, S.; Katahira, M. *Nucleic Acids Res.* **2004**, *32*, 5113–5118.
- Gannett, P. M.; Sura, T. P. *Synth. Commun.* **1993**, *23*, 1611–1615.
- ¹H NMR (DMSO-*d*₆) δ : 10.7 (s, 1H), 7.68 (d, *J* = 7.3, 2H), 7.53 (d, *J* = 16.1, 1H), 7.47 (d, *J* = 16.1, 1H), 7.37 (m, 2H), 7.29 (m, 1H), 6.47 (s, 2H), 6.37 (dd, *J* = 8.6, 6.2, 1H), 5.26 (d, *J* = 4.2, 1H), 5.17 (t, *J* = 5.1, 1H), 4.45 (m, 1H), 3.82 (m, 1H), 3.71 (m, 1H), 3.65 (m, 1H), 2.60 (m, 1H), 2.09 (m, 1H). ¹³C NMR (DMSO-*d*₆) δ : 156.2, 153.1, 151.5, 144.1, 136.0, 132.8, 128.6, 128.2, 127.0, 116.4, 115.7, 87.1, 82.3, 70.2, 61.2. HR-FAB (M+H)⁺ for C₁₈H₁₉N₅O₄. Calcd: 370.1515; found: 370.1571.
- ¹H NMR (DMSO-*d*₆) δ : 10.8 (s, 1H), 7.56 (m, 2H), 7.29 (m, 3H), 6.83 (d, *J* = 13.0, 1H), 6.64 (d, *J* = 13.0, 1H), 6.47 (s, 2H), 6.15 (dd, *J* = 8.5, 6.6, 1H), 5.18 (s, 1H), 5.00 (s, 1H), 4.33 (s, 1H), 3.76 (dd, *J* = 7.8, 4.6, 1H), 3.61 (m, 1H), 3.53 (m, 1H), 2.71 (m, 1H), 1.83 (m, 1H). ¹³C NMR (DMSO-*d*₆) δ : 156.4, 153.3, 151.2, 142.6, 135.4, 135.3, 129.0, 128.1, 127.9, 117.4, 116.3, 87.3, 83.1, 70.6, 61.7, 38.0. HR-FAB (M+H)⁺ for C₁₈H₁₉N₅O₄. Calcd: 370.1515; found: 370.1564.
- (a) Greene, K. L.; Wang, Y.; Live, D. *J. Biomol. NMR* **1995**, *5*, 333–338; (b) Uesugi, S.; Ikehara, M. *J. Am. Chem. Soc.* **1977**, *99*, 3250–3253.
- A photostationary state (PSS) was achieved by illumination for 2 h at room temperature using a spectrofluorometer (FP-6500, JASCO), which can modulate monochromic light with a 1 nm peak width at half height.

18. *E-Z* photoisomerization was performed in an aqueous solution containing 25 μM **8STG** and 2% acetonitrile at room temperature using a 100 W Xenon lamp (LAX-Cute, Asahi Spectra Co., Ltd), which can extract a specific wavelength with a 10 nm peak width at half height by employing an adequate bandpass filter (HQBP254-UV for *Z*→*E* photoisomerization and HQBP370-UV for *E*→*Z* photoisomerization, Asahi Spectra Co., Ltd). After illumination, the *E*-isomer ratio was determined by the peak area in HPLC analysis with UV detection at the isosbestic point (268 nm) in the *E-Z* photoisomerization.
19. Values of the $\Phi_{E \rightarrow Z}$ and $\Phi_{Z \rightarrow E}$ were obtained by comparing the initial rates of photoisomerization to that of *E*-stilbene during irradiation in nitrogen-saturated methanol solution ($\Phi_{E \rightarrow Z} = 0.48$); Gorner, H.; Kuhn, H. J. *Adv. Photochem.* **1995**, *19*, 1–117.
20. A reviewer comment that most of DNA especially d(TT) should undergo the dimerization to produce thymine dimer under photoirradiation of short wavelength light (254 nm). We agree with the reviewer and will effort to develop a novel photo-switchable nucleoside, which can be photoisomerized by longer wavelength.