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Photochromic Nucleobase Photoisomerized by Visible Light

Shinzi Ogasawara,*[1,2] Syoji Ito,[1,3] Hiroshi Miyasaka,[3] and Mizuo Maeda[2]
[1]*PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012*
[2]*Bioengineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198*
[3]*Division of Frontier Materials Science, Graduate School of Engineering Science and Center for Quantum Science and Technology under Extreme Conditions, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka 560-8531*

(Received June 22, 2010; CL-100578; E-mail: o_shinji@riken.jp)

The compound 8-phenylazoguanosine (^{8PA}**G**), a new type of photochromic nucleobase (PCN) that can be photoisomerized by visible light irradiation, was developed. ^{8PA}**G** shows rapid and efficient reversible *cis–trans* isomerization and can be iteratively isomerized by alternate irradiation with 420 and 550 nm without photolysis.

For reversible photoregulation of nucleic acid structure and self-assembly of nucleoside derivatives,[1] we previously developed photochromic nucleobases (PCNs) that can reversibly change their photochemical and physical properties, such as absorption and fluorescence, upon photoisomerization by external light stimuli.[2] We have shown their application in the photoregulation of duplex[2] and G-quadruplex[3] formation. Recently, Spada et al. demonstrated the potential of our molecules for photoregulation of supramolecular architectures by controlling PCN self-assembly.[4] Although PCNs may be applied to photoregulation of important biological events such as RNA interference,[5] blood clotting,[6] cellular senescence,[7] transcription,[8] and translation[9] through duplex or G-quadruplex regulation, there are disadvantages in that the photoisomerization requires ultraviolet light, which causes critical damage to nucleic acids including formation of cyclobutane dimer and (6–4) photoproducts.[10] Therefore, PCNs that can be photoisomerized by longer wavelengths are needed for biological applications. Herein, we report the synthesis and photoisomerization properties of a new type of PCN, 8-phenylazoguanosine (^{8PA}**G**), which can be reversibly photoisomerized using visible light (Figure 1).

For synthesis of ^{8PA}**G** we employed the stepwise procedure shown in Scheme 1 because the direct reaction of guanosine and benzenediazonium ion yielded 8-phenylguanosine.[11] Guanine (**1**) reacted with the benzenediazonium ion rapidly in cold aqueous solution at pH 10 to give 8-phenylazoguanine (**2**) in 98% yield. A suspension of **2** in dry DMA and isobutyric anhydride was stirred at 150 °C for 2 h. The crystalline ocher product of this reaction was filtered and washed twice with EtOH to yield 8-phenylazo-2-*N*-isobutylguanine (**3**) in 72% yield. Treatment of a suspension of **3** in 1,2-dichloroethane with bis(trimethylsilyl)-acetamide (BSA) at 80 °C for 1 h gave trimethylsilyl-protected **3**, which was then coupled with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in anhydrous toluene at 80 °C for 2 h in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst. Purification by flash column chromatography give the product **4** as an orange foam in 66% yield. Deprotection of **4** with NH_3/H_2O/MeOH at 60 °C for 4 h gave a 79% yield of ^{8PA}**G** (**5**). As is typical for purine ribonucleoside synthesis, a mixture of N7 and N9 isomers was expected. However, the main product was only the N9 isomer, the structure of which was confirmed by 2D NMR (HMBC). In the HMBC analysis, we observed a correlation between H1′ and C4 (see Supporting Information).[13]

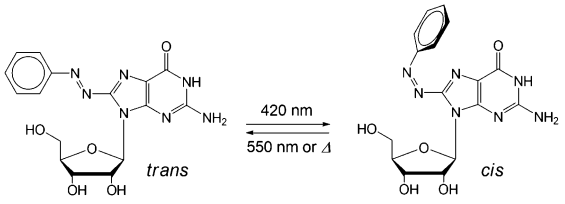
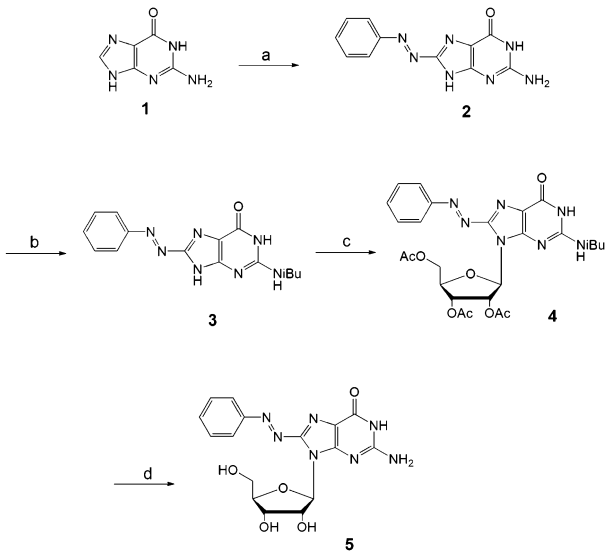


Figure 1. Schematic illustration of *cis–trans* photoisomerization of 8-phenylazoguanosine (^{8PA}**G**).



Scheme 1. Synthesis of ^{8PA}**G**. Reagents and conditions: (a) aniline, sodium nitrite, water, 0 °C, 2 h, 98%; (b) isobutyric anhydride, dry DMA, 150 °C, 2 h, 72%; (c) i: bis(trimethylsilyl)acetamide, 1,2-dichloroethane, 80 °C, 1 h, ii: 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, trimethylsilyl trifluoromethanesulfonate, anhydrous toluene, 80 °C, 2 h, 66%; (d) NH_3/H_2O/MeOH, 60 °C, 4 h, 79%.

The absorption spectrum of *trans*-^{8PA}**G** showed a peak at 420 nm and a shoulder around 470 nm. By analogy with the absorption spectra of azobenzene derivatives such as 4-*N*,*N*-dimethylaminoazobenzene,[12] it is likely that the large absorption band at 420 nm corresponds to the $\pi\pi^*$ transition, while the shoulder corresponds to the $n\pi^*$ transition. We demonstrated the reversible photoisomerization of ^{8PA}**G** in ethanol using a 300 W

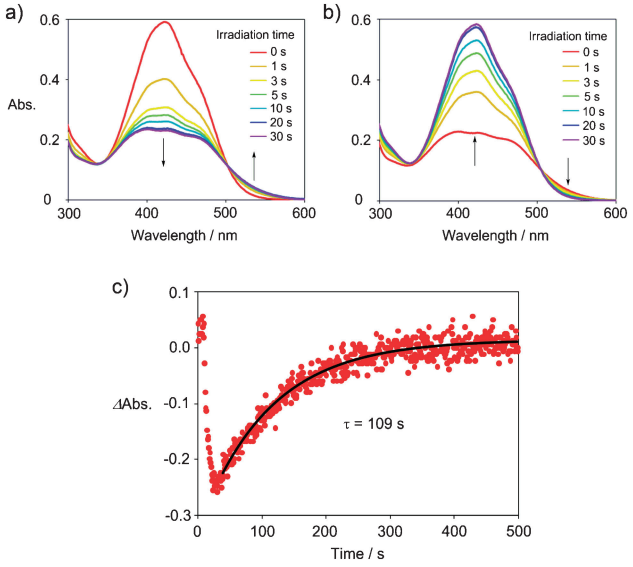


Figure 2. (a) Absorption spectra of ^{8PA}**G** for *trans*-to-*cis* photoisomerization with illumination at 420 nm, and (b) *cis*-to-*trans* photoisomerization with illumination at 550 nm. Arrows indicate increases and decreases of the absorption peaks. (c) Time profile of transient absorbance for thermal *cis*-to-*trans* isomerization of ^{8PA}**G** monitored at 430 nm. Solid black line is the best calculated curve.

xenon lamp (MAX-302, Asahi Spectra Co., Ltd.) which can provide a specific wavelength with a 10-nm peak width at half height by employing an adequate bandpass filter. As shown in Figures 2a and 2b, upon irradiation of a solution of ^{8PA}**G** with light at 420 nm (corresponding to the $\pi\pi^*$ absorption), the absorption spectra changes in a way similar to that observed for the *trans–cis* photoisomerization of azobenzene derivatives. The intense $\pi\pi^*$ peak decreases and is accompanied by a slight increase at the tail portion (500–600 nm) of the $n\pi^*$ absorption until a photostationary state is reached. Subsequent irradiation at the longer 550 nm wavelength reverses the course of the reaction and the original spectrum is completely recovered in 30 s.

The thermal *cis*-to-*trans* isomerization rate of ^{8PA}**G** was measured in ethanol at 21 °C. Figure 2c shows the time profiles of transient absorbance monitored at 430 nm after excitation with a femtosecond 400 nm laser pulse with an output energy of 8.2 mW for 30 s. Thermal *cis*-to-*trans* isomerizaiton of ^{8PA}**G** was observed with a time constant of 109 s. Finally, the reversible switching was repeated a dozen times by alternate illumination with 420 and 550 nm light, and good reversibility of the *cis–trans* photoisomerization was observed without any side reactions (Figure 3).

In summary, we have successfully developed a new type of photochromic nucleobase, 8-phenylazoguanosine (^{8PA}**G**), that can be photoisomerized by visible light irradiation. This compound shows a very rapid and highly efficient reversible *cis–trans* photoisomerization upon illumination at specific wavelengths. In addition, photoisomerization can be iteratively performed by alternate illumination with monochroic 420 and 550 nm light without any side reactions. ^{8PA}**G** can be widely used for the reversible photoregulation of nucleic acid structures and supramolecular architectures. An application to in vivo photoregulation of important biological events is opened by this type of PCN and is now in progress.

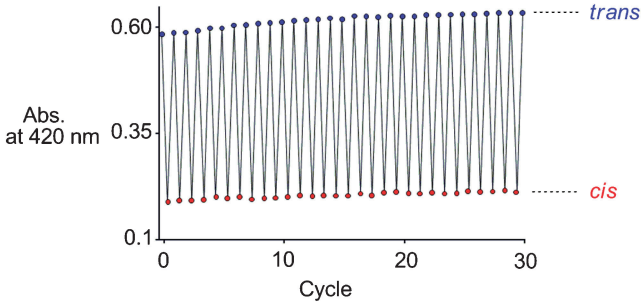


Figure 3. Switching cycles between *trans* and *cis* by alternate illumination with 420 and 550 nm light. The illumination periods are 20 s for both wavelengths.

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13 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

