DNA Manipulation

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Straightforward and Reversible Photoregulation of Hybridization by Using a Photochromic Nucleoside

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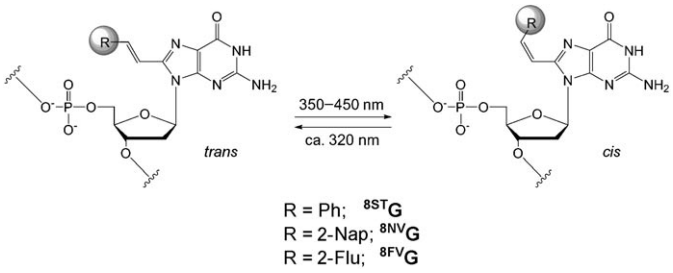
Hybridization of nucleic acids through Watson–Crick base pairing is a fundamental phenomenon in many biological events, such as gene regulation[1] by antisense agents,[2] small interfering RNA (siRNA),[3] or microRNA (miRNA).[4] In addition, the ability to form duplexes and other secondary structures through predictable hybridization has been used in constructing programmable devices and architectures on the nanometer-length scale.[5] Therefore, the necessity for methods that control hybridization by external stimuli is clear. The most promising external trigger is photoirradiation because it allows accurate and easy control of the location, dosage, and time when an event occurs. One common approach for photoregulation of hybridization involves the installation of a photoprotecting group that can be completely removed by photoirradiation.[6] This strategy, termed caging,[7] allows regulation only once and in only one direction, whereas the method employing *cis–trans* photoisomerization of azobenzene inserted as a base-pair (bp) replacement allows reversible control.[8] Although a significant melting-temperature difference (ΔT_m) is obtained upon *cis–trans* isomerization of azobenzene, this approach requires the introduction of multiple azobenzene moieties in the side chain. For example, 9 azobenzenes are required for the photoregulation of a 20-bp DNA duplex; these cause the structure of the duplex to deviate far from the B form and prevent its interaction with proteins.[9]

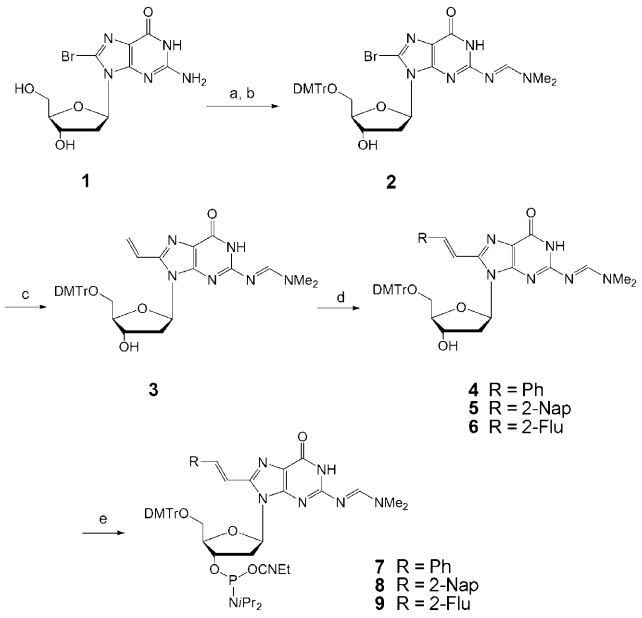
Additional efforts are, therefore, needed to create more broadly applicable photoregulation methods that promise straightforward and reversible control without harm to the native B-form structure.

Herein, we report a new strategy for the photoregulation of hybridization by using *cis–trans* photoisomerization of a photochromic nucleoside (PCN) that reversibly changes its photochemical and physical properties, such as fluorescence intensity, upon photoisomerization by an external light stimulus.[10] Our strategy successfully allowed extremely straightforward and reversible duplex regulation of a 20-bp DNA, even at room temperature. We designed three C8-substituted 2′-deoxyguanosine PCNs, ^{8ST}**G**, ^{8NV}**G**, and ^{8FV}**G**, so the stability of the duplex should change due to alteration in the steric hindrance to the backbone upon *cis–trans* isomerization (Scheme 1). The synthesis of the PCNs was achieved



Scheme 1. *cis–trans* Photoisomerization of the photochromic nucleosides. Nap = naphthyl, Flu = fluorenyl.

by employing two consecutive palladium-catalyzed cross-coupling reactions, as shown in Scheme 2. 8-Bromo-2′-deoxyguanosine (**1**) was converted into **2** by stepwise protections of



Scheme 2. Synthesis of the PCNs. Reagents and conditions: a) DMF–dimethylacetal, DMF, 55 °C, 30 min; b) DMTrCl, DMAP, pyridine, room temperature, 2 h, 80%; c) $[Pd(PPh_3)_4]$, tributyl(vinyl)tin, *N*-methylpyrrolidone, 110 °C, 45 min, 85%; d) brominated substituent, PPh_3, $Pd(OAc)_2$, triethylamine, DMF, 115 °C, 1 h, **4**: 45%, **5**: 51%, **6**: 49%; e) $(iPr_2N)_2PO(CH_2)_2CN$, 5-ethylthio-1*H*-tetrazole, dichloromethane, room temperature, 1.5 h, quant. DMTr = 4,4′-dimethoxytriphenylmethyl, DMF = *N*,*N*-dimethylformamide, DMAP = 4-dimethylaminopyridine.

the amino group with DMF–dimethylacetal and of the 5′-hydroxy group with 4,4′-dimethoxytritylchloride. Compound **2** was then subjected to cross-coupling with tributyl-

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(vinyl)tin under Stille conditions. The vinyl derivative **3** was, in turn, subjected to a Herrmann palladacycle-assisted Heck olefination with the appropriate brominated substitutents to afford *trans*-PCN derivatives **4**–**6**. After conversion into the corresponding cyanoethylphosphoramidites, PCNs **7**–**9** were incorporated into oligonucleotides (ODNs) by standard automated DNA synthesis. The ODNs used in this study are listed in Table 1.

Table 1: The oligodeoxynucleotides used in this study.

ODN	Sequence
10	5′-d(CATGTACGTGCA)-3′
11	5′-d(TGCACGTACATG)-3′
12	5′-d(TGCAC^{8ST}**G**TACATG)-3′
13	5′-d(TGCAC^{8NV}**G**TACATG)-3′
14	5′-d(TGCAC^{8FV}**G**TACATG)-3′
15	5′-d(TATGCACGTGCATACGCGTA)-3′
16	5′-d(TACGCGTATGCACGTGCATA)-3′
17	5′-d(TACGCGTAT^{8ST}**G**CACGTGCATA)-3′
18	5′-d(TACGC^{8ST}**G**TATGCAC^{8ST}**G**TGCATA)-3′
19	5′-d(TAC^{8ST}**G**CGTAT^{8ST}**G**CACGT^{8ST}**G**CATA)-3′

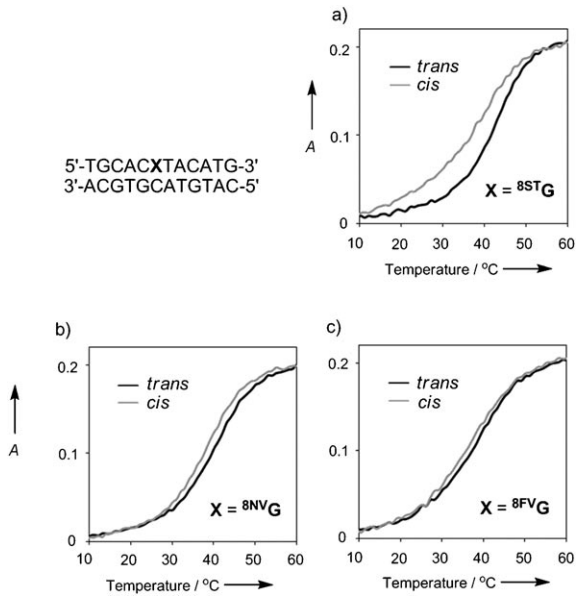

Figure 1. Melting curves for the duplexes of a) **10**/**12**, b) **10**/**13**, and c) **10**/**14**.

We initially investigated the differences in thermal stability of 12-bp duplexes containing ^{8ST}**G**, ^{8NV}**G**, or ^{8FV}**G** in the *trans* and *cis* forms by monitoring the melting temperature (T_m). These PCNs showed rapid and highly efficient reversible *cis*–*trans* photoisomerization upon irradiation with monochroic light. The *trans* forms of ^{8ST}**G**, ^{8NV}**G**, and ^{8FV}**G** contained in the ODNs were photoisomerized to the *cis* forms by irradiation for 5 min at 370, 410, and 420 nm with 86, 63, and 77% conversion, respectively, as determined by the peak area in HPLC analysis. In addition, subsequent irradiation for 2 min at 254, 290, and 310 nm yielded the *trans* forms with 94, 87, and 77% conversion, respectively (see the Supporting Information). In these PCNs, both the *cis* and *trans* isomers were thermally stable. They showed no thermal isomerization, even at 80 °C. As indicated in Table 2, the ^{8ST}**G**-containing duplex **10**/**12** showed a significant T_m difference (ΔT_m) between the *trans* and *cis* forms. The T_m value of the *trans* form was 7.9 °C higher than that of the *cis* form (Figure 1a). This is probably due to a difference in the steric hindrance of the benzene ring with its neighboring nucleobase and backbone; this idea is supported by molecular dynamics simulations (see the Supporting Information). Previous 2D-NMR studies of ^{8ST}**G** have also indicated that *cis*-^{8ST}**G** prefers to adopt the *syn* conformation with respect to the relevant N9–C1′ glycosidic bond, due to steric considerations.[10] By contrast, the ΔT_m value in the ^{8NV}**G**- and ^{8FV}**G**-containing duplexes **10**/**13** and **10**/**14** is not marked. The ΔT_m values induced by *trans* to *cis* isomerization were only 1.6 and 1.4 °C, respectively, for these duplexes (Figures 1b and c). One possible reason is destabilization of the duplex even when ^{8NV}**G** and ^{8FV}**G** adopt the *trans* form. The bulky substituents, naphthalene and fluorene in ^{8NV}**G** and ^{8FV}**G**, may cause serious steric hindrance with the backbone, even in the *trans* form; the duplex would thus be destabilized regardless of which form it adopts.

Table 2: Melting temperatures (T_m) of the 12-bp duplexes.[a]

Duplex	T_m [°C]		ΔT_m [°C][b]
	trans	*cis*	
10/**11**	49.3 ± 0.8		–
10/**12**	43.4 ± 1.0	35.5 ± 1.0	7.9
10/**13**	39.6 ± 0.9	38.0 ± 0.9	1.6
10/**14**	37.5 ± 0.8	36.1 ± 0.7	1.4

[a] All T_m values for the duplexes (5 μM) were determined in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl. The T_m values given are the average of at least three data points. [b] The change in the T_m value induced by the *cis*–*trans* photoisomerization.

On the basis of the above results, we performed photoregulation of a 20-bp duplex by using multiple ^{8ST}**G** insertions. The ΔT_m value of the duplex increased with the number of ^{8ST}**G** insertions, as shown in Table 3. Surprisingly, when three ^{8ST}**G** PCNs were introduced into a 20-mer ODN (**19**), we observed a drastic change in thermal stability upon *cis*–*trans* photoisomerization. The T_m value of the *trans* form was 60.2 °C, whereas that of the *cis* form was not determined because the typical hyperchromicity due to denaturation was

Table 3: Melting temperature T_m of the 20 bp duplexes.[a]

Duplex	T_m [°C]		ΔT_m [°C]
	trans	*cis*	
15/**16**	68.6 ± 0.7		–
15/**17**	66.8 ± 0.7	63.3 ± 0.9	3.2
15/**18**	63.3 ± 0.4	53.1 ± 0.7	10.2
15/**19**	60.2 ± 0.7	n.d.[b]	–

[a] All T_m values for the duplexes (5 μM) were determined in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl. The T_m values given are the average of at least three data points. [b] n.d. = not determined. A clear local maximal value in the first derivative did not appear.

not clearly observed, which implies that the duplex was not formed (Figure 2). However, the melting curve indicating duplex formation reappeared upon *cis* to *trans* isomerization

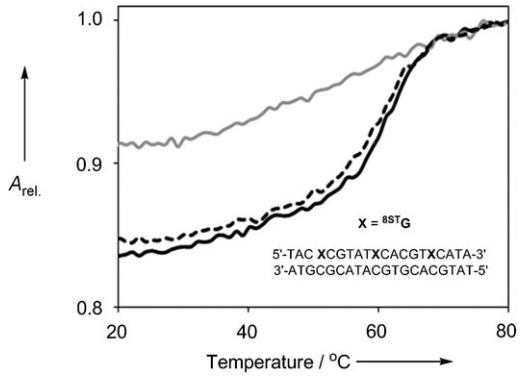

Figure 2. Melting curves for the **15**/**19** duplex involving three ^{8ST}**G** PCNs. The absorbance at 260 nm was measured in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl. Solid black line: before irradiation (*trans* form); solid gray line: after irradiation at 370 nm for 5 min (*cis* form); broken black line: after subsequent irradiation at 254 nm for 2 min (*trans* form).

by irradiation at 254 nm. We further investigated the effect of photoisomerization of three ^{8ST}**G** PCNs on the duplex by circular dichroism. The **15**/**19** duplex gave the characteristic CD signature expected for a B-form duplex, with a maximum at 275 nm and a minimum at 252 nm, when the three ^{8ST}**G** PCNs were in the *trans* form (Figure 3). After isomerization

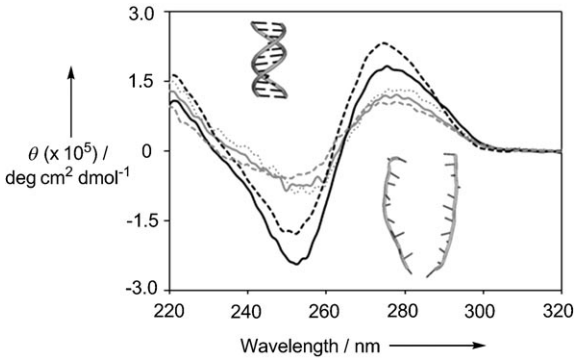

Figure 3. CD spectra of the **15**/**19** duplex. Solid black line: before irradiation (*trans* form); solid gray line: after irradiation at 370 nm for 5 min (*cis* form); broken gray line: after irradiation at 370 nm for 5 min, measured at 70 °C; broken black line: **15**/**16** duplex (native B-form duplex); dotted gray line: superposition of the spectra of *cis*-form single strands of **15** and **19**. The CD data were obtained with a 10 μM total strand concentration in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl at 25 °C (except for the measurement at 70 °C).

to the *cis* form by irradiation at 370 nm for 5 min, the intensity of those peaks decreased, which indicated the presence of unstructured single strands; a similar CD spectrum was obtained from measurement at high temperature (70 °C). Moreover, these spectra corresponded to the control experiment, which was a superposition of the spectra of *cis*-form single strands of **15** and **19**. These observations strongly suggest that ODNs **15** and **19** cannot form a duplex when the three ^{8ST}**G** PCNs adopt the *cis* form.

Interestingly, PCNs allow monitoring of the conformational state, the *trans* or *cis* form (that is, duplex or single strands), by their fluorescence without extra labeling because the fluorescence intensity of the PCNs dramatically changes upon *cis–trans* photoisomerization. For example, fluorescence emission was observed for both *trans*-^{8ST}**G** and *cis*-^{8ST}**G**, which had a similar fluorescence maximum at 450 nm, but the intensity for *cis*-^{8ST}**G** was only one-sixth that of *trans*-^{8ST}**G** (see the Supporting Information). By using this photochromic property, we traced the reversible photoswitching of hybridization induced by alternate illumination with 254 and 370 nm light. When the reaction mixture was irradiated at 370 nm for 5 min, slight fluorescence was observed, which indicated that the ^{8ST}**G** PCNs were isomerized to the *cis* form and the duplex was denatured. By subsequent irradiation at 254 nm for 2 min, fluorescence was recovered, which indicated that the ^{8ST}**G** PCNs had isomerized to the *trans* form and hybridization had occurred. This switching was performed for two cycles and showed good reversibility (Figure 4).

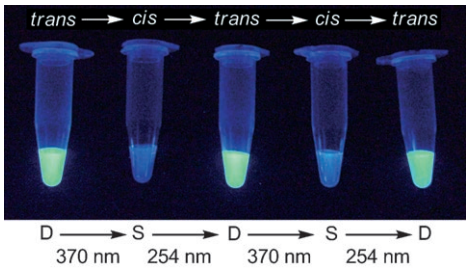

Figure 4. Fluorescence change upon *cis–trans* photoisomerization of ^{8ST}**G** PCNs incorporated into ODN **19**. The reaction mixture was alternately illuminated at 370 nm for 5 min and 254 nm for 2 min at room temperature. The solutions were irradiated with a transilluminator (365 nm). D = duplex formation between **15** and **19**, S = single strands.

In conclusion, we have synthesized three C8-substituted 2′-deoxyguanosine PCNs, for a new type of photoregulation of hybridization. PCNs contained in ODNs showed very rapid and efficient reversible *cis–trans* photoisomerization upon illumination with monochroic light at the appropriate wavelength, and this isomerization induced significant changes in the thermal stability of the duplexes. Our strategy enables a switch between the duplex and single strands in an extremely straightforward and reversible manner by light stimulation, even at room temperature. Additionally, installation of PCNs into DNA had little influence on the B-form structure when the duplex was formed. Moreover, PCNs can be used as molecular trace labels for functional nucleic acids in vivo without an extra photoswitchable label[11] because the fluorescence intensity drastically changes upon *cis–trans* photoirradiation, in a similar manner to that observed with the photochromic fluorescent protein Dronpa.[12]

Experimental Section

Photoisomerization of PCN-containing ODNs was performed in a mixture containing 5 μM ODN, 10 mM phosphate buffer (pH 7.0), and 100 mM NaCl at room temperature by using a 300 W Xenon lamp (MAX-302; 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, HQBP290-UV, M.C.310, HQBP370-UV, M.C.410, M.C.420; Asahi Spectra Co., Ltd.).

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[1] Y. Dorsett, T. Tuschl, *Nat. Rev. Drug Discovery* **2004**, *3*, 318–329.
[2] S. T. Crooke, *Curr. Mol. Med.* **2004**, *4*, 465–487.
[3] S. M. Elbashir, J. Harborth, W. Lendeckel, A. Yalcin, K. Weber, T. Tuschl, *Nature* **2001**, *411*, 494–498.
[4] A. M. Denli, B. B. J. Tops, R. H. A. Plasterk, R. F. Ketting, G. J. Hannon, *Nature* **2004**, *432*, 231–235.
[5] a) T. R. Kelly, *Angew. Chem.* **2005**, *117*, 4194–4198; *Angew. Chem. Int. Ed.* **2005**, *44*, 4124–4127; b) Y. He, T. Ye, M. Su, C. Zhang, A. E. Ribbe, W. Jiang, C. Mao, *Nature* **2008**, *452*, 198–201; c) P. W. K. Rothemund, *Nature* **2006**, *440*, 297–302; d) U. Feldkamp, C. M. Niemeyer, *Angew. Chem.* **2006**, *118*, 1888–1910; *Angew. Chem. Int. Ed.* **2006**, *45*, 1856–1876.
[6] a) X. Tang, J. Swaminathan, A. M. Gewirtz, I. J. Dmochowski, *Nucleic Acids Res.* **2007**, *36*, 559–569; b) S. Shah, S. Rangarajan, S. H. Friedman, *Angew. Chem.* **2005**, *117*, 1352–1356; *Angew. Chem. Int. Ed.* **2005**, *44*, 1328–1332; c) L. Kröck, A. Heckel, *Angew. Chem.* **2005**, *117*, 475–477; *Angew. Chem. Int. Ed.* **2005**, *44*, 471–473.
[7] a) G. C. R. Ellis-Davies, *Nat. Methods* **2007**, *4*, 619–628; b) G. Mayer, A. Heckel, *Angew. Chem.* **2006**, *118*, 5020–5042; *Angew. Chem. Int. Ed.* **2006**, *45*, 4900–4921; c) H. Ando, T. Furuta, R. Y. Tsien, H. Okamoto, *Nat. Genet.* **2001**, *28*, 317–325.
[8] a) H. Asanuma, X. G. Liang, H. Nishioka, D. Matsunaga, M. Liu, M. Komiyama, *Nat. Protocols* **2007**, *2*, 203–212; b) C. Dohno, S. Uno, K. Nakatani, *J. Am. Chem. Soc.* **2007**, *129*, 11898–11899.
[9] H. Asanuma, D. Matsunaga, M. Komiyama, *Nucleic Acids Symp. Ser.* **2005**, *49*, 35–36.
[10] S. Ogasawara, I. Saito, M. Maeda, *Tetrahedron Lett.* **2008**, *49*, 2479–2482.
[11] M. Bates, B. Huang, G. T. Dempsey, X. Zhuang, *Science* **2007**, *317*, 1749–1753.
[12] R. Ando, H. Mizuno, A. Miyawaki, *Science* **2004**, *306*, 1370–1373.

