This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Photoinduced changes in hydrogen bonding patterns of 8-thiopurine nucleobase analogues in a DNA strand

Kunihiko Morihiro, a,b Tetsuya Kodama, c Shohei Mori a and Satoshi Obika* a,b

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Hydrogen bonds (H-bonds) formed between nucleobases play an important role in the construction of various nucleic acid structures. The H-donor and H-acceptor pattern of a nucleobase is responsible for selective and correct base pair formation. Herein, we describe an 8-thioadenine nucleobase analogue and an 8-thiohypoxanthine nucleobase analogue with a photolabile 6-nitroveratryl (NV) group on the sulfur atom (SA NV and SH NV, respectively). Light-triggered removal of the NV group causes tautomerization and a change in the H-bonding pattern of SA NV and SH NV. This change in H-bonding pattern has a strong effect on base recognition by 8-thiopurine nucleobase analogues. In particular, base recognition by SH NV is clearly shifted from guanine to adenine upon photoirradiation. These results show that a photoinduced change in H-bonding pattern is a unique strategy for manipulating nucleic acids assembly with spatiotemporal control.

Introduction

The complementarity of natural A-T and G-C base pairs in DNA is the principal mechanism for the preservation and flow of genetic information. The hydrogen-bonding (H-bonding) patterns of the four natural nucleobases play an important role in the selective and correct formation of base pairs. These H-bonding interactions can result in the formation of higher order complexes of nucleic acids, depending on the sequence. Therefore, the control of H-bonding interactions using external stimuli is important for regulating biological processes, and for the possibility of developing unique DNA-based molecular machines. Various external stimuli have been used to this end; light is an ideal trigger because the timing, location, intensity of the irradiation can be easily controlled. Among such strategies, nucleobase caging strategies involving the installation of a photolabile group are very important. Photolabile caging groups perturb the H-bonding capabilities of the nucleobases. Photoirradiation reinstates the H-bonding capabilities and allows nucleobase interaction in the “OFF to ON” direction.

Nucleobase-caged nucleosides can be widely used for the photoregulation of antisense oligodeoxynucleotides (ODNs), siRNAs, aptamers, ribozymes and deoxyribozymes, diagnostic ODNs, DNA architectures, and DNA logic gates. Recently, we reported the synthesis and properties of a unique light-responsive nucleobase analogue derived from 2-mercaptobenzimidazole (SB NV) (Fig. 1a).14 SB NV is modified with a photolabile 6-nitroveratryl (NV) group, and the nitrogen at the 3-position serves as an H-acceptor (A). SB NV can selectively form a base pair with guanine even before photoirradiation, unlike conventional caged nucleobases. Light-triggered removal of the NV group causes tautomerization of the nucleobase, and changes the role of the 3-nitrogen atom from H-A to H-donor (D). Following this change in H-bonding pattern, base recognition by SB NV can be shifted from guanine to adenine. We also demonstrated that a light-triggered strand exchange reaction targeting different mRNA fragment sequences could be achieved using ODNs containing SB NV. These results indicate...
Results and discussion

The synthesis of the phosphoramidites bearing \( \text{SA}^{\text{NV}} \) and \( \text{SH}^{\text{NV}} \) as a nucleobase are summarized in Scheme 1. 6-Nitroveratrylthiol (2) was prepared from 6-nitroveratryl bromide (1). (Scheme 1) and subjected to reaction with 8-bromo-2'-deoxyadenosine derivative (3)\(^{18}\) to afford 4 (Scheme 2). Phosphitylation at the 3'-hydroxyl group provided \( \text{SA}^{\text{NV}} \)-phosphoramidite 5. For the preparation of \( \text{SH}^{\text{NV}} \)-phosphoramidite 9, 8-bromoinosine (6)\(^{19}\) was treated with 2 to give 7 (Scheme 3). Tritylation of the primary hydroxyl group in 7 and phosphitylation of the secondary hydroxyl group provided phosphoramidite 9. Amide blocks 5 and 9 were applied to an automated DNA synthesizer to incorporate \( \text{SA}^{\text{NV}} \) and \( \text{SH}^{\text{NV}} \) into ODNs. \( \text{SA}^{\text{NV}} \) and \( \text{SH}^{\text{NV}} \) were incorporated in the middle of the pyrimidine (T) strand of ODN 10 and ODN 11.

After cleavage from the resin and purification by reversed-phase (RP) HPLC, the structure of each ODN was confirmed by MALDI-TOF MS analysis. The sequence of each ODN used in this study is shown in Fig. 2.

The photoreactivity of \( \text{SA}^{\text{NV}} \) and \( \text{SH}^{\text{NV}} \) in a DNA strand was investigated by RP-HPLC analysis using ODN 10 and ODN 11. When irradiated at 365 nm at 37 °C, ODN 10 and ODN 11 gradually disappeared. MALDI-TOF MS showed that the resulting ODNs were \( \text{SA}/\text{SH}-\text{ODNs} \) and confirmed that the NV analogues have a pronounced effect on base recognition abilities.

![Fig. 2 ODN sequences used in this study.](image)

![Fig. 3 Time course conversion of (a) \( \text{SA}^{\text{NV}} \) to \( \text{SA} \) in ODN 10 and (b) \( \text{SH}^{\text{NV}} \) to \( \text{SH} \) in ODN 11 by photoirradiation. Conditions: each ODN (0.1 nmol, 10 µM), sodium phosphate buffer (pH 7.2, 25 mM). Irradiation (365 nm) was performed at rt. Error bars indicate standard deviation (n = 3).](image)
group of SA\textsuperscript{NV} and SH\textsuperscript{NV} was efficiently removed. Fig. 3 shows the percentage of the remaining SA\textsuperscript{NV}/SH\textsuperscript{NV}- and resulting SA-/SH-ODNs at several irradiation time points. The photoreaction was complete within 60 sec for both ODNs, and the yield of NV-removed ODNs was estimated from the HPLC peak area to be about 80%.

The effects of photoinduced changes in H-bonding pattern of SA\textsuperscript{NV} and SH\textsuperscript{NV} on their base recognition ability were examined by measuring the $T_m$ values of DNA duplexes containing ODN 10 and ODN 11 (Table 1). ODN 10 and ODN 11 were individually hybridized to four ODNs, generating eight distinct duplexes in which each nucleobase analogue was paired with all possible natural nucleobases. For comparison, naturally matched duplexes containing the A:T and G:C base pairs in the same position were also examined. The duplex containing the SA\textsuperscript{NV}:G pair showed the highest $T_m$ value of all the combinations of SA\textsuperscript{NV} with other nucleobases ($\Delta T_m \geq 3 \, ^\circ C$). The SA\textsuperscript{NV}:G pair was slightly less stable than natural base pairs, and similar to the stability of the G:T wobble base pair. After photoradiation at 365 nm for 5 min, SA showed the highest affinity towards thymine ($\Delta T_m \geq 4 \, ^\circ C$); however, the $T_m$ values of duplexes containing SA are, on the whole, low ($T_m \leq 32 \, ^\circ C$). SH\textsuperscript{NV} in ODN 11 showed the highest affinity towards guanine, similar to SA\textsuperscript{NV} ($\Delta T_m \geq 4 \, ^\circ C$). The $T_m$ value of the duplex containing the SH\textsuperscript{NV}:G base pair was also slightly lower than that of natural duplexes. In contrast, after irradiation, the preferred base-pairing partner for SH\textsuperscript{NV} clearly changed to adenine ($\Delta T_m \geq 8 \, ^\circ C$). Notably, the stability of SH:A was comparable to that of the natural A:T base pair. These results suggest that photoradiation induces a change in base recognition by SH\textsuperscript{NV} from guanine to adenine. Fig. 4 illustrates the changes in the UV melting profiles of ODN-11-formed DNA duplexes and clearly indicates that the change in base recognition by SH\textsuperscript{NV} is triggered by photoradiation.

**Table 1.** $T_m$ values of DNA duplexes

<table>
<thead>
<tr>
<th>Duplex</th>
<th>X:Y</th>
<th>$T_m$ (°C)</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UV (−)</td>
<td>UV (+)</td>
</tr>
<tr>
<td>10:14</td>
<td>SA\textsuperscript{NV}:A</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>10:15</td>
<td>SA\textsuperscript{NV}:G</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>10:16</td>
<td>SA\textsuperscript{NV}:C</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>10:17</td>
<td>SA\textsuperscript{NV}:T</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>11:14</td>
<td>SH\textsuperscript{NV}:A</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>11:15</td>
<td>SH\textsuperscript{NV}:G</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>11:16</td>
<td>SH\textsuperscript{NV}:C</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>11:17</td>
<td>SH\textsuperscript{NV}:T</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>12:17</td>
<td>A:T</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>13:16</td>
<td>G:C</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>12:15</td>
<td>A:G (mismatch)</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>12:16</td>
<td>A:C (mismatch)</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>13:14</td>
<td>G:A (mismatch)</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>13:17</td>
<td>G:T (wobble)</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

$^a$Conditions: each ODN (4.0 µM), NaCl (20 mM), sodium phosphate buffer (10 mM, pH 7.2). $^b$ $T_m$ values of DNA duplexes after irradiation (365 nm) at 37 °C for 5 min.

Although further conclusive experiments such as NMR or X-ray structural analysis are needed to elucidate the precise base pair structures, the results of the $T_m$ measurements suggest that SA\textsuperscript{NV} and SH\textsuperscript{NV} recognize guanine via two H bonds on the Hoogsteen face, as shown in Fig. 5. The stabilities of the SA\textsuperscript{NV} and SH\textsuperscript{NV}:G base pairs were slightly lower than that of the natural base pairs. It would appear that steric repulsion between the 8-sulfur atom in SA\textsuperscript{NV} and the 2-amino group in guanine decreases the stability of the SA\textsuperscript{NV}:G pair (Fig. 5a). This observation is consistent with previous reports showing that the base pair between 2-thioracil and 2,6-diaminopurine is significantly destabilized because of steric hindrance.\textsuperscript{20,21} Also, SH\textsuperscript{NV} may form a wobble pair with guanine similar to the U:G mismatch pair commonly found in RNA\textsuperscript{22} (Fig. 5b); therefore, the SH\textsuperscript{NV}:G pair is less stable than natural base pairs. Light-induced changes in H-bonding patterns have profound effects on the base recognition abilities of 8-thiopurine nucleobase analogues. ODN containing SA showed low recognition ability toward any nucleobase; this can be explained by the fact that SA has an H-bonding [D, D] pattern on the Hoogsteen face, but no natural nucleobase has an [A, A] pattern for base pair formation with SA (Fig. 5a). SA can interact with thymine using its Watson-Crick face in the anti-conformation; however, the resulting poor base-recognition-ability indicated that SA still adopted a syn conformation after photoradiation due to the steric bulk around the 8-thio group. On the other hand, SH can interact with adenine using an [A, D] H-bonding pattern on the Hoogsteen face (Fig. S5).
believe that these unique light-responsive nucleobase analogues could be powerful tools for the spatiotemporal control of DNA assembly.

**Experimental**

**General**

Reagents and solvents were purchased from commercial suppliers and were used without purification unless otherwise specified. All experiments involving air- and/or moisture-sensitive compounds were carried out under an N	extsubscript{2} atmosphere. All reactions were monitored with analytical TLC (Merck Kieselgel 60 F254). Column chromatography was carried out using Fuji Silsys FL-100D. Physical data were measured as follows: NMR spectra were recorded on a JEOL JNM-ECS-400 spectrometer using CDCl	extsubscript{3} or DMSO-	extsubscript{d6} as the solvent with tetramethylsilane as an internal standard. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO P-2200 instrument. FAB mass spectra were measured on a JEOL JMS-700 mass spectrometer. MALDI-TOF mass spectra were recorded on a Bruker Daltonics Autoflex II TOF/TOF mass spectrometer.

**Synthesis of the phosphoramidite-bearing SA	extsuperscript{NV} and SH	extsuperscript{NN} nucleobase analogues**

**6-Nitroveratrylthiol (2).** To a solution of 6-nitroveratryl bromide (1.47 g, 5.36 mmol) in dry THF (54.0 mL) was added potassium thiocetate (734 mg, 6.43 mmol) and the reaction mixture was stirred for 5 h at room temperature. The solvent was removed in vacuo and the residue was partitioned between AcOEt and H	extsubscript{2}O. The separated organic layer was washed with brine, then dried (Na	extsubscript{2}SO	extsubscript{4}) and concentrated in vacuo. The resulting residue (1.52 g) was dissolved in MeOH (51.0 mL) and 35% aqueous HCl (3.20 mL) was added. After being stirred for 12 h at 60 °C, the solvent was removed in vacuo. The residue was purified on a silica gel column eluted with hexane/AcOEt (4:1 to 1:1) to give 2 (1.15 g, 94%) as a yellow solid; Mp 86-87 °C; 	extsuperscript{1}H NMR (400 MHz, CDCl	extsubscript{3}) δ 7.66 (1H, s), 6.86 (1H, s, H-2 or H-5), 4.03 (2H, d, J = 8.5 Hz, SCH	extsubscript{2}Ar), 3.99 (3H, s, Ar-CH	extsubscript{3}), 3.95 (3H, s, Ar-CH	extsubscript{3}), 2.23 (1H, t, J = 8.5 Hz, SH); 	extsuperscript{13}C NMR (100 MHz, CDCl	extsubscript{3}) δ 153.3, 147.8, 139.6, 132.0, 112.5, 108.2, 56.3, 56.2, 27.0; IR (KBr) 2577, 1520, 1273 cm	extsuperscript{-1}; FAB-MS m/z = 252 (M	extsuperscript{+}).

**6-N-Benzoyl-5’-O-(4,4’-dimethoxytrityl)-8-(6-nitroveratrylthio)-2’-deoxyadenosine (4).** To a solution of 3 (300 mg, 0.408 mmol) in dry DMF (4.10 mL) was added potassium carbonate (679 mg, 4.80 mmol) and 2 (103 mg, 0.449 mmol) at room temperature. After being stirred for 1 h at room temperature, the resulting mixture was partitioned between EtO	extsubscript{2} and H	extsubscript{2}O. The separated organic layer was washed with brine, dried (Na	extsubscript{2}SO	extsubscript{4}) and concentrated in vacuo. The resulting residue was purified on a silica gel column eluted with hexane/AcOEt (2:3 to 1:2 with 0.5% Et	extsubscript{3}N) to give 4 (187 mg, 52%) as a yellow foam; 	extsuperscript{1}H NMR (400 MHz, CDCl	extsubscript{3}) δ 8.93 (1H, brs, NH), 8.43 (1H, s, H-2), 8.04 (2H, d, J = 7.5 Hz), 7.64-7.16 (14H, m), 6.77-6.73 (4H, m), 6.27 (1H, t, J = 7.0 Hz, H-1’), 4.99-4.85 (3H, m, H-3’ and SCH	extsubscript{2}Ar), 4.06 (1H, dd, J = 10.0 and 6.0 Hz, H-4’), 3.88 (3H, s, Ar-CH	extsubscript{3}).

**6-N-Benzoyl-5’-O-(4,4’-dimethoxytrityl)-3’-(N,N-disopropyl-β-cyanoethylophosphoramidyl)-8-(6-nitroveratrylthio)-2’-deoxyadenosine (5).** To a suspension of 4 (150 mg, 0.17 mmol) in dry MeCN (1.7 mL) was added N,N-disopropylethylamine (0.089 mL, 0.51 mmol) and 2- (cyanomethyl)thymine (829 mg, 6.00 mmol) at room temperature. After being stirred for 30 min, the resultant mixture was partitioned between AcOEt and H	extsubscript{2}O. The separated organic layer was washed with saturated aqueous NaHCO	extsubscript{3}, followed by brine, then dried (Na	extsubscript{2}SO	extsubscript{4}) and concentrated in vacuo.

The residue was purified on a silica gel column eluted with hexane/AcOEt (3:2 with 0.5% Et	extsubscript{3}N) to give 5 (142 mg, 77%) as a yellow foam; 	extsuperscript{13}P NMR δ 141.4, 141.1; FAB-MS m/z = 885 (MH	extsuperscript{+}); FAB-HRMS calcd for C	extsubscript{83}H	extsubscript{64}N	extsubscript{12}O	extsubscript{12}S	extsubscript{8} 885.2918, found 885.2928.

6-N-Benzoyl-5’-O-(4,4’-dimethoxytrityl)-3’-(N,N-disopropyl-β-cyanoethylophosphoramidyl)-8-(6-nitroveratrylthio)-2’-deoxyadenosine (5).

6-N-Benzoyl-5’-O-(4,4’-dimethoxytrityl)-2’-deoxyinosine (7). To a solution of 6 (1.65 g, 5.00 mmol) in dry DMF (50.0 mL) was added K	extsubscript{2}CO	extsubscript{3} (829 mg, 6.00 mmol) and 2 (1.37 g, 6.00 mmol) at room temperature. After being stirred for 24 h at room temperature, the solvent was removed in vacuo. The residue was purified on a silica gel column eluted with AcOEt to AcOEt/MeOH (10:1) to give 7 (596 mg, 25%) as a yellow powder; 	extsuperscript{1}H NMR (400 MHz, DMSO-	extsubscript{d6}) δ 12.5 (1H, brs, NH), 8.02 (1H, s), 7.68 (1H, s), 7.47 (1H, s), 6.14 (1H, t, J = 6.5 Hz, H-1’), 5.33 (1H, brs, OH), 4.91 (1H, brs, OH), 4.79 and 4.75 (each 1H, each J = 13.5 Hz, SCH	extsubscript{2}Ar), 4.37 (1H, brs, H-3’), 3.86 (3H, s, Ar-CH	extsubscript{3}), 3.85 (3H, s, Ar-CH	extsubscript{3}), 3.81-3.77 (1H, m, H-4’), 3.60-3.57 (1H, m, H-5’a),
Organic & Biomolecular Chemistry

Oligonucleotides synthesis

Solid-phase oligonucleotide synthesis was performed on an nS-8 Oligonucleotide Synthesizer (GeneDesign, Inc.) using commercially available reagents and phosphoramidites. The modified phosphoramidite was incorporated into the oligonucleotide with a coupling efficiency comparable to that of commercially available phosphoramidites without any modifications to the coupling conditions. Oligonucleotides were synthesized (trityl-off) on a 500 Å CPG solid support column (0.2 μmol scale) using 5-bis-3,5-trifluoromethylphenyl-1H-tetrazole (0.25 M in MeCN) as the activator. Cleavage from the solid support and deprotection were accomplished with concentrated ammonium hydroxide solution at 55 °C for 12 h.

The crude oligonucleotides were purified on a NAP 10 column (GE Healthcare) followed by RP-HPLC on a XBridge™ C18 column, 2.5 μm, 10 x 50 mm (Waters) using MeCN in 0.1 M triethylammonium acetate buffer (pH 7.0). The purified oligonucleotides were quantified by UV absorbance at 260 nm and confirmed by MALDI-TOF mass spectrometry.

UV melting experiments

Melt temperatures (Tm) of the oligonucleotides were determined by measuring the change in absorbance at 260 nm as a function of temperature using a SHIMADZU UV-Vis spectrophotometer UV-1650PC equipped with a TMSPC-8 Tm analysis accessory. The samples were denatured at 100 °C and annealed slowly to room temperature. Absorbance was recorded in the forward and reverse direction between 5 and 90 °C at a rate of 0.5 °C/min. Tm values of duplexes after photoirradiation were measured using samples irradiated (365 nm) at 37 °C.

Photoirradiation reaction

Photoradiation of oligonucleotides was performed in sodium phosphate buffer (pH 7.2) at 37 °C for 5 minutes using an OMRON UV-LED lamp ZUV-C30H as the light source (365 nm) and a ZUV-L10H as the lens unit (760 mW/cm²). Analyses of the photoproducts were carried out without further purification.

Acknowledgement

This work was supported by the Japan Society for the Promotion of Science (JSPS), the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and the Advanced Research for Medical Products Development Programme of the National Institute of Biomedical Innovation (NIBIO), Japan.

Notes and references

Table of contents entry

Photoinduced change in hydrogen bonding pattern has a strong effect on base recognition by nucleobase analogues.