



# Phenylazonaphthalene as a superb photo-regulator for DNA-triplex formation

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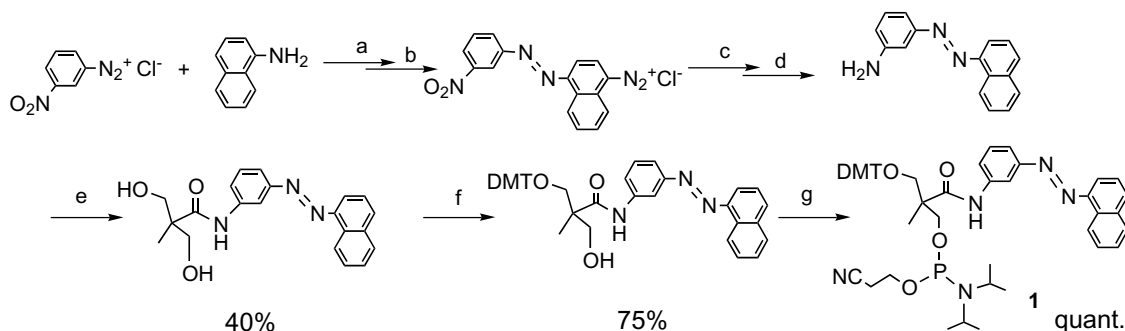
**Abstract**—Phenylazonaphthalene was tethered to the 5'-end of oligo(T) as photo-responsive moiety for the photo-regulation of DNA-triplex formation. The melting temperature of DNA-triplex changed as greatly as 40.5°C on *trans*→*cis* isomerization of phenylazonaphthalene. This change was much greater than that achieved by azobenzene. © 2001 Elsevier Science Ltd. All rights reserved.

DNA-triplex formation has been regarded as one of the most powerful techniques for the control of gene expression.<sup>1</sup> If this process is regulated by outer stimuli, it undoubtedly widens the scope of application. Previously,<sup>2,3</sup> we reported that triplex formation is photo-regulated by *cis*–*trans* isomerization of the azobenzene incorporated into the third strand. Planar *trans*-azobenzene stabilizes the triplex by stacking interaction, whereas non-planar *cis*-azobenzene destabilizes it by steric hindrance. It is indicated that more efficient photo-regulation is plausible by using a photo-responsive molecule which is larger than azobenzene. In this paper, we show that a phenylazonaphthalene regulates DNA-triplex formation far more efficiently than does

an azobenzene. The change of melting temperature ( $T_m$ ) on the *cis*–*trans* isomerization exceeds 40°C.

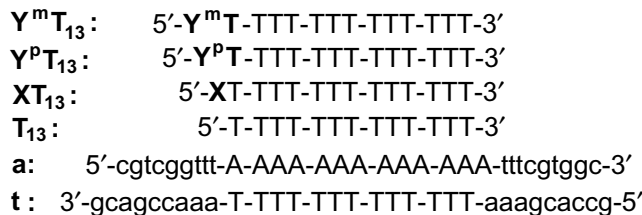
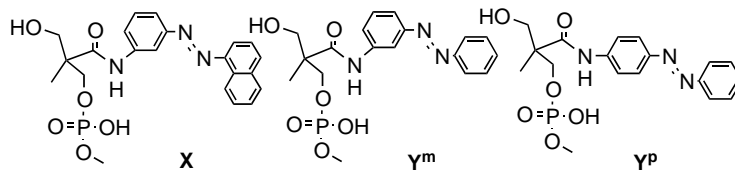
The phosphoramidite monomer **1** carrying a phenylazonaphthalene moiety was synthesized according to Scheme 1, and incorporated into oligonucleotides on an automated synthesizer.<sup>4,5</sup> The target for triple-helix formation with **XT**<sub>13</sub> is the **T**<sub>13</sub>/(**dA**)<sub>13</sub> portion in the [**a/t**] duplex, formed from two complementary 31-mer oligonucleotides (see Scheme 2).

Before photo-irradiation, the phenylazonaphthalene in **XT**<sub>13</sub> mostly (>90%) takes the *trans*-form. The melting curve (at 280 nm) of *trans*-**XT**<sub>13</sub>/**a/t** mixture is presented



**Scheme 1.** Synthesis of the phosphoramidite monomer **1** involving phenylazonaphthalene residue. (a) HCl(aq); (b) H<sub>2</sub>SO<sub>4</sub>, CH<sub>3</sub>COOH, NaNO<sub>2</sub>; (c) H<sub>3</sub>PO<sub>2</sub>(aq); (d) NaSH(aq), ethanol/H<sub>2</sub>O; (e) 2,2-bis(hydroxymethyl)propionic acid, dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, DMF; (f) 4,4'-dimethoxytrityl (DMT) chloride, 4-dimethylaminopyridine, pyridine, CH<sub>2</sub>Cl<sub>2</sub>; (g) 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite, 1*H*-tetrazole, CH<sub>3</sub>CN.

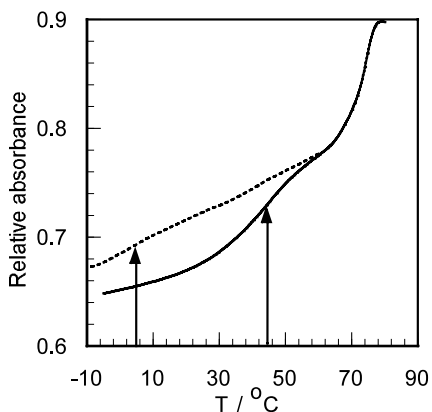
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**Scheme 2.** Sequences of the oligonucleotides used in this study.

by the solid line in Fig. 1.<sup>6</sup> The  $T_m$  for the triplex formation is 45.5°C. Upon irradiating the solution with UV light (375–400 nm),<sup>7</sup> the phenylazobenzene is rapidly isomerized to the *cis*-form (>70%), as confirmed by UV–visible spectroscopy and HPLC analysis (Supplementary Material available). Concurrently, the melting curve drastically shifts towards the lower temperature side, with respect to that of the *trans*-form (compare the broken line with the solid one). The  $T_m$  value is 5.0°C. Thus, the change of  $T_m$  ( $\Delta T_m$ ), induced by the *cis*–*trans* isomerization of the phenylazobenzene, is as large as 40.5°C. When the *cis*-isomer is further isomerized back to the *trans*-form by visible light irradiation (>420 nm),<sup>7</sup> the melting curve is almost superimposed with that before the UV irradiation. The present photo-regulation is reversible.

For the purpose of comparison, the results for the azobenzene-tethered oligonucleotides  $\mathbf{Y}^P\mathbf{T}_{13}$  and  $\mathbf{Y}^m\mathbf{T}_{13}$  (see Scheme 2 for the structures) are listed in Table 1. The  $\Delta T_m$  values (16.0°C for  $\mathbf{Y}^P\mathbf{T}_{13}$  and 19.5°C for  $\mathbf{Y}^m\mathbf{T}_{13}$ ) are notably smaller than the corresponding value for  $\mathbf{X}\mathbf{T}_{13}$ . Superiority of phenylazobenzene as a photo-regulator of triplex formation is conclusive. Because of the



**Figure 1.** Melting curves for *trans*- $\mathbf{X}\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  triplex (solid line) and *cis*- $\mathbf{X}\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  triplex (dotted line): pH 7.0 (HEPES), 0.2 M  $\text{MgCl}_2$ ,  $[\mathbf{a}] = 2.0 \mu\text{M}$ ,  $[\mathbf{t}] = 2.4 \mu\text{M}$ ,  $[\mathbf{X}\mathbf{T}_{13}] = 2.0 \mu\text{M}$ . The arrows in the Figure indicate the  $T_m$ s.

**Table 1.**  $T_m$  values of triplexes formed from the modified oligonucleotides and the  $[\mathbf{a}/\mathbf{t}]$  duplex<sup>a,b</sup>

Modified oligonucleotide	$T_m$ (°C)		$\Delta T_m$ (°C)
	<i>trans</i> -Form	<i>cis</i> -Form	
$\mathbf{X}\mathbf{T}_{13}$	45.5	5.0	40.5
$\mathbf{Y}^P\mathbf{T}_{13}$	29.5	13.5	16.0
$\mathbf{Y}^m\mathbf{T}_{13}$	30.2	10.7	19.5

<sup>a</sup> pH 7.0 (10 mM HEPES buffer), 0.2 M  $\text{MgCl}_2$ ,  $[\mathbf{a}] = 2.0 \mu\text{M}$ ,  $[\mathbf{t}] = 2.4 \mu\text{M}$ , and [the third strand] = 2.0  $\mu\text{M}$ .

<sup>b</sup>  $T_m$  of the native triplex  $\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  is 22.5°C.

greater conjugate system, *trans*-phenylazobenzene shows stronger stacking interaction with adjacent DNA base-pairs than does *trans*-azobenzene. Thus, the  $T_m$  of *trans*- $\mathbf{X}\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  is much higher (by 15–16°C) than those of *trans*- $\mathbf{Y}^P\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  and *trans*- $\mathbf{Y}^m\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$ . This triplex is more stable than native triplex  $\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  ( $T_m = 22.5^\circ\text{C}$ ) and is efficiently formed under physiological conditions. These arguments are supported by CD and UV–visible spectroscopy,<sup>8</sup> as well as by computer molecular modeling. Furthermore, *cis*-phenylazobenzene induces more significant steric hindrance against the target duplex, so that *cis*- $\mathbf{Y}^P\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  triplex is even less stable (by 5–7°C) than either *cis*- $\mathbf{Y}^P\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$  or *cis*- $\mathbf{Y}^m\mathbf{T}_{13}/\mathbf{a}/\mathbf{t}$ . These two factors render the phenylazobenzene highly potent for photo-regulation of DNA-triplex formation.

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4. MALDI-TOFMS of  $\text{XT}_{13}$ : calcd  $[\text{M}-\text{H}^+]$ : 4314.7; obsd.: 4315.0. All the products and reaction intermediates in Scheme 1 were sufficiently characterized by usual methods (see Supplementary Material).
5.  $\text{Y}^{\text{P}}\text{T}_{13}$  and  $\text{Y}^{\text{M}}\text{T}_{13}$  were synthesized as described previously: Asanuma, H.; Ito, T.; Komiyama, M. *Tetrahedron Lett.* **1998**, *39*, 9015; Asanuma, H.; Liang X. G.; Komiyama, M. *Tetrahedron Lett.* **2000**, *41*, 1055.
6. The absorbance at 280 nm was monitored on a JASCO model V-530 spectrophotometer, equipped with a programmed temperature controller. The rate of temperature change was  $1.0^{\circ}\text{C min}^{-1}$ . The  $T_{\text{m}}$  value was determined as the maximum of the first derivative of melting curve. The  $\text{t}:\text{a}$  ratio was kept at 1.2 in order to ensure the absence of free **a**, which would form a duplex with the third strand DNA and perturb the measurement.
7. The photo-isomerization was accomplished by irradiating light from a 150 W xenon lamp for 10 min through an appropriate filter: a P10-390-R band pass filter from Koyo Corporation for UV irradiation (375–400 nm), and a Y-43 color glass filter from Asahi Technoglass Corporation for visible-light irradiation ( $>420$  nm). The photochemical properties of phenylazonaphthalene are similar to those of azobenzene.
8. When the *trans*- $\text{XT}_{13}/\text{a}/\text{t}$  triplex is formed, circular dichromism (CD) is negatively induced at around 390 and 480 nm. This indicates that the *trans*-phenylazonaphthalene intercalates between the base pairs and stabilizes the triple helix by hydrophobic stacking, as described in Ref. 2. This argument is further certified by the bathochromic shift (16 nm) of the phenylazonaphthalene.