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Synthesis of Oligonucleotides Containing a New Azobenzene Fragment with Efficient Photoisomerizability

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Abstract—The azobenzene derivatives possessing substituents of $ROCH_2CH_2O$- and-CH_2CH_2OR' or -$CONHCH_2CH_2OR'$ at *p,p′*-positions, where *R* and *R′* are 4,4′-dimethoxytrityl and 2-cyanoethyl-*N,N′*-diisopropylphophoramidite, have been synthesized for linking two oligonucelotide segments. It has been found that the azobenzene linkers efficiently undergo *trans–cis* isomerization by exposing to UV light. The conversion efficiency showed slight dependence on structure or conformation of oligonucleotides attached to the azobenzene chromophore. The *cis*-form of the azobenzene in oligonucleotides was sufficiently stable at low temperature under dark. The present findings would open the way for light switch of nucleic acid structures.

Introduction

Azobenzene derivatives have been attracting current interest as photo-responsive triggers for control of macroscopic properties in biological systems. There have been a number of papers concerning photo-regulation of enzyme activity,[1,2] receptor binding,[3] membrane permeability,[4–6] electron transfer,[7,8] and peptide conformation.[9] However, little attempt has been made to develop photo-responsive systems in relation to nucleic acids. Komiyama et al. have recently reported that oligonucleotides containing an azobenzene as a side chain fragment could be used for photo-regulation of nucleic acid binding.[10] We have synthesized an azobenzene fragment for introduction into the main chain of oligonucleotide strands.[11,12] The oligonucleotides connected by an azobenzene fragment appear attractive, since two oligonucleotide strands attached to an azobenzene fragment would be in different proximity imposed by the photoisomerization. It is expected that duplexes, triplexes, and ribozymes involving azobenzene-linked oligonucleotides would change their conformation as a result of photo-illumination. Although several non-nucleotide linkers have been shown to be used as regulators for cooperative binding of oligonucleotides to a structural RNA,[13,14] hairpin duplex and triplex formation,[15–17] and ribozyme activity,[18] little attention has been paid for addition of photo-regulatory function to these linker arms.

Our investigation was initiated by design of the azobenzene-4,4′-diamide derivatives that can be incorporated between the terminal hydroxyl functions of two oligonucleotide segments.[11,12] However, it has been found that azobenzene diamide underwent poor *trans-cis* isomerization by exposure to UV light.[12] In order to overcome this problem, we reconsidered the structural element of the azobenzene fragment. It is well known that azobenzene derivatives with a combination of substituents such as *O*-alkyl and alkyl, or *O*-alkyl and amide, at the *p,p′*-positions can undergo *trans–cis* isomerization in a conversion of over 90% under certain photo-illumination conditions.[19,20] We have, therefore, designed a new azobenzene fragment with appropriate substituents that is useful for incorporation of an azobenzene chromophore into oligonucleotide main chains. The present report describes the details of the synthesis of oligoncucleotides containing the new azobenzene fragment.

Results and Discussion

We have designed two azobenzene fragments whose structures are shown in Scheme 1. Both azobenzene derivatives possess *O*-alkyl and alkyl, or *O*-alkyl and amide substituents at the *p,p′*-positions. The synthesis began with diazo-coupling of 4-hydroxyethylaniline or 4-amino-*N*-(hydroxyethyl)benzamide with phenol. The azobenzene derivatives **1a** and **1b** thus obtained were allowed to react with *O*-dimethoxytrityl bromoethanol in DMF in the

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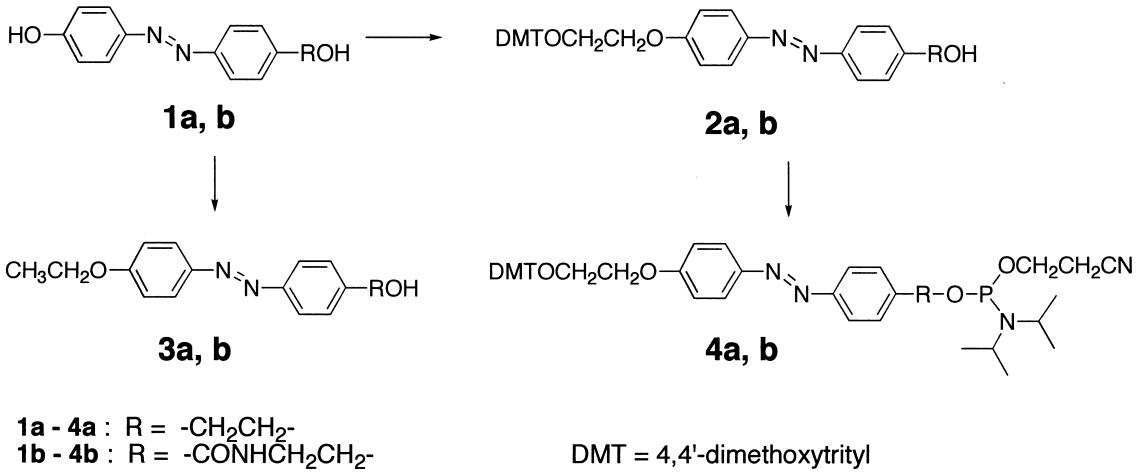

Scheme 1.

presence of K_2CO_3, giving the azobenzene (**2a** and **2b**) possessing a hydroxyl group at one end and an *O*-dimethoxytrityl at the other. These compounds were converted to the phosphoramidites **4a** and **4b** by using a standard procedure. The azobenzene derivatives **3a** and **3b** as model compounds were prepared by the reaction of **1a** and **1b**, respectively, with ethyl iodide.

The synthesis of oligonucleotides containing the azobenzene fragments **5**–**8** were carried out by a phosphoramidite chemistry on an automated DNA synthesizer. The sequence of each oligomer is shown in Table 1. The standard protocol (25 equiv of amidite, 2 min) was used in the coupling of normal deoxyribonucleoside amidites, while an excess amount (60 equiv) of the reagent was employed for 10 min in the coupling of azobenzene amidites **4**. With this protocol, the azobenzene amidites can be coupled to the hydroxyl group of oligonucleotides on a CPG support. After the usual deprotection, the azobenzene containing oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis. The oligonucleotides thus purified were characterized by chromatographic behavior and ion spray mass spectroscopy as indicated in Table 1. The oligonucleotide integrity was verified by the observation that the oligonucleotide exhibited single peak in HPLC and expected molecular mass. Without UV illumination, the synthesized oligonucleotides consisted of only the *trans*-form of the azobenzene because it is thermodynamically more stable than the *cis*-form.[21]

Figure 1 shows the UV–vis spectral changes for azobenzene containing oligonucleotide **5** and its model compound **3a** due to photoisomerization. Before irradiation, the oligonucleotide **5** exhibits two major absorption bands which appeared at 265 and 350 nm. Based on the spectrum for compound **3a**, the former band is reasonably assigned to nucleic acid bases and the latter to the azobenzene. Immediately after the illumination, it was observed that the absorption at 350 nm (due to the *trans*-form) decreased with increase in the absorption at 440 nm (attributable to the *cis*-form). After 2 min, no further spectral changes were observed. The extent of the absorption changes were found to be almost the same for both the oligomer and the model compound, in which photoisomerization from *trans* to *cis* of the azobenzene was ca. 80%. Similar efficient photoisomerization (conversion efficiency 80%) was observed for oligomer **6** and its model **3b**, indicating that both the azo compounds synthesized here have identical photoisomerizability by exposing to UV light.

The incorporated oligonucleotide segments whose sequence is not likely to form a specific complex did not affect the degree of photoisomerization of the azobenzene unit. In order to see whether photoisomerization of the azobenzene fragment is affected by structure or conformation of attached oligonucleotide segments, two oligonucleotides **7** and **8** were examined. It is noted that all binding experiments were carried out without photo-illumination or in the *trans* form of the azobenzene chromophore. The UV melting curves for oligomer **7** are shown in Figure 2(A). The oligonucleotide solution of **7** at different concentrations showed identical Tm (60°C), which is dramatically higher than that

Table 1. HPLC and ion spray mass data for oligonucleotides containing an azobenzene linker

Oligomer		RT (min)	Ion spray mass (negative) obs. (calcd.)
5:	5′-dTTTTTTTT-L1-TTTTTTTT[a]	32.5	5153.1 (5154.4)
6:	5′-dTTTTTTTT-L2-TTTTTTTT[a]	—	5197.3 (5197.5)
7:	5′-dTTTTTTTT-L1-AAAAAAAA[a]	31.1	5225.2 (5226.6)
8:	5′-dCCCTAGGAA-L1-AATCGGATCTCGG[a]	29.9	7093.6 (7093.7)

[a] L1 and L2 indicate the azobenzene linkers derived from the amidites **4a** and **4b**, respectively. Reversed HPLC (cosmosil 5C18-AR-300, 4.6×150 mm) was carried out by (i) 5% CH_3CN (5 min), (ii) a linear gradient of CH_3CN (5–25% in 30 min), and then (iii) a linear gradient of CH_3CN (25–70% in 45 min) each at a flow rate of 1.0 mL/min in 50 mM triethylammmonium acetate (pH 7).

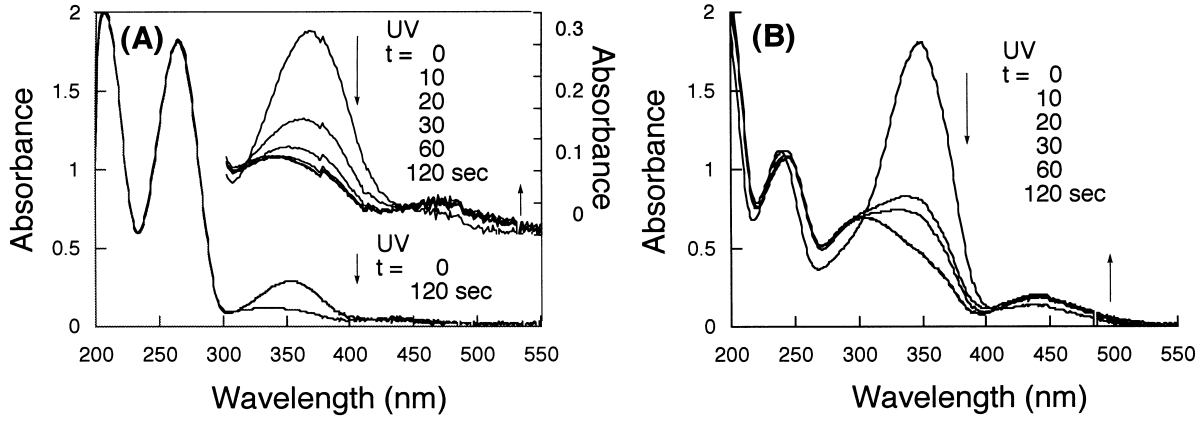

Figure 1. Changes in the UV–vis spectra for azobenzene containing oligonucleotide **5** (A) in a phosphate buffer and its model compound **3a** in CH_3CN (B) due to photoisomerization. The solution of the azobenzene derivative in a Pyrex test tube was irradiated in a cooled water bath (5°C) by a high-pressure Hg lamp using band-path filter to transmit light at around 350 nm. UV–vis spectra were obtained at the same temperature at the indicated time. After 120 s, no further spectral changes were observed for both derivatives.

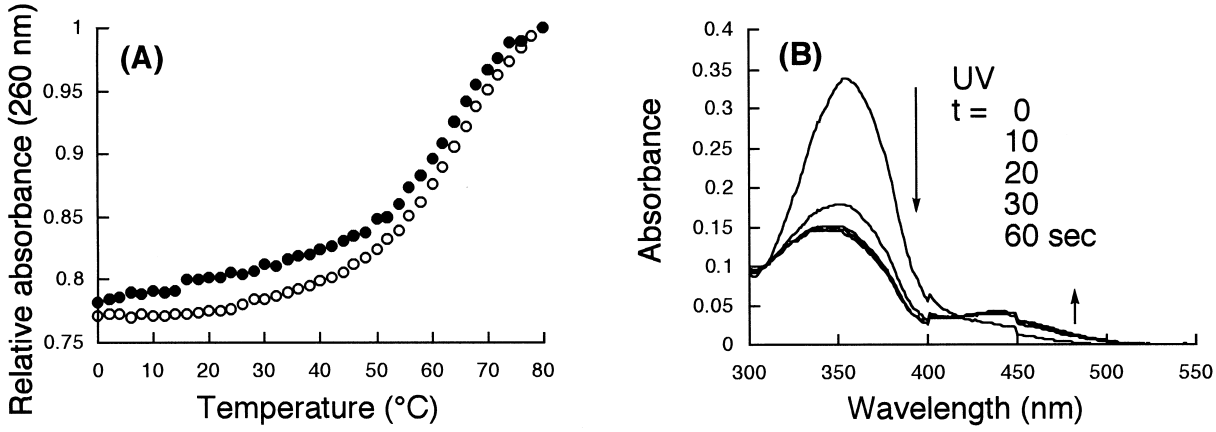

Figure 2. (A) UV melting curves for azobenzene containing oligonucleotide **7** at different strand concentrations (○:5×10^{-5} M, ●:1×10^{-5} M) in 0.1 M NaCl and 0.01 M sodium phosphate, adjusted to pH 7. The melting experiments were carried out without photo-illumination. (B) Changes in the UV–vis spectra for azobenzene containing oligonucleotide **7** in the phosphate buffer due to photoisomerization. The photoirradiation was done in the same way as described in Figure 1. After 60 s, no further spectral changes were observed.

(8°C) for the duplex of oligothymidylate (T_8) and oligodeoxyadenylate (dA_8). The observed melting temperature is almost identical to that for the hairpin duplex capped by the azobenezene diamide derivative.[12] These observations clearly indicate that the oligomer **7** containing self-complementary sequence formed a remarkably stable hairpin duplex. By analogy of the stilbene-linked hairpin duplex,[22] the azobenzene chromophore is suggested to be stacked with the base-pair of the duplex. As shown in Figure 2(B), the UV–vis spectral changes of **7** upon UV illumination were observed similarly for oligonucleotide **5**. In this oligomer, however, the azobenzene fragment was isomerized in slightly less efficiency to afford *cis*-isomer (ca. 60%) under the photo-illumination conditions.

A DNA bulge structure would be formed by the mixing of the oligonucleotide **8** with the sequence of 5′-dCCGAGATCCGACC TAGGG (18-mer target), since there are excess residues of-AA-L1-AA- on one side of the duplex.[23] The UV melting profiles for duplexes of oligomer **8**, 5′-dCCCTAGGAAAAAATCGGATCTCGG (24-mer), and 5′-dCCCTAGGTCGGATCTCGG (18-mer) formed with 18-mer target are shown in Figure 3(A). Each melting profile exhibited a typical sigmoidal curve as seen for a DNA duplex. The duplex of the 24-mer and the 18-mer target, which is known to form a bulge structure,[23] showed a relatively lower Tm than that for the full-match 18-mer duplex. Similarly, the azobenzene containing duplex exhibited the lowered Tm. These observations suggested that the azobenzene containing duplex forms a bulge structure. The azo chromophore, which may be looped out from the duplex, was isomerized by exposing of UV light in ca. 50% conversion from *trans* to *cis* form as shown in Figure 3(B). The single-stranded azobenzene-oligonucleotide (**8**) was found to be photoisomerized in a similar conversion (80%) for oligomer **5** under the same conditions.

We next examined the thermal stability of *cis*-form of the azo chromophore in the oligonucleotides produced by the UV illumination. Figure 4(A) showed the time-course of the absorbance changes at 350 nm for the photoisomerized oligomer **5** at the different temperatures. After a prolonged time, the absorbance at

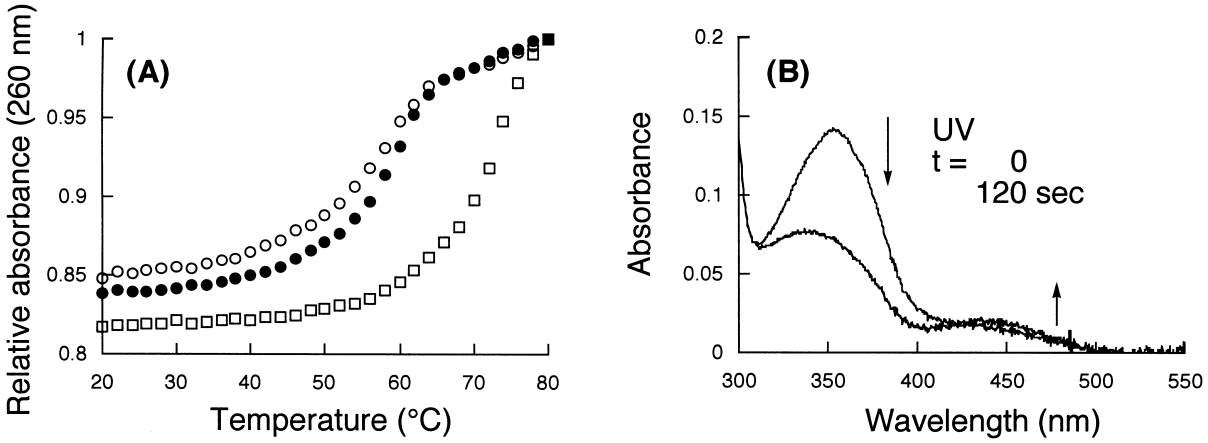

Figure 3. (A) UV melting profiles for duplexes of (●) oligomer **8**, (○) 5′-dCCCTAGGAAAAAATCGGATCTCGG (24-mer), and (□) 5′-dCCCTAGGTCGGATCTCGG (18-mer) formed with 18-mer target (5′-dCCGAGATCCGACCTAGGG) at a strand concentration of 5×10^{-5} M in 1.0 M NaCl and 0.01 M sodium phosphate, adjusted to pH 7. The melting experiments were carried out without photo-illumination. (B) Changes in the UV–vis spectra for azobenzene containing duplex **8** in the phosphate buffer due to photoisomerization. The photoirradiation was done in the same way as described in Figure 1. After 120 sec, no further spectral changes were observed.

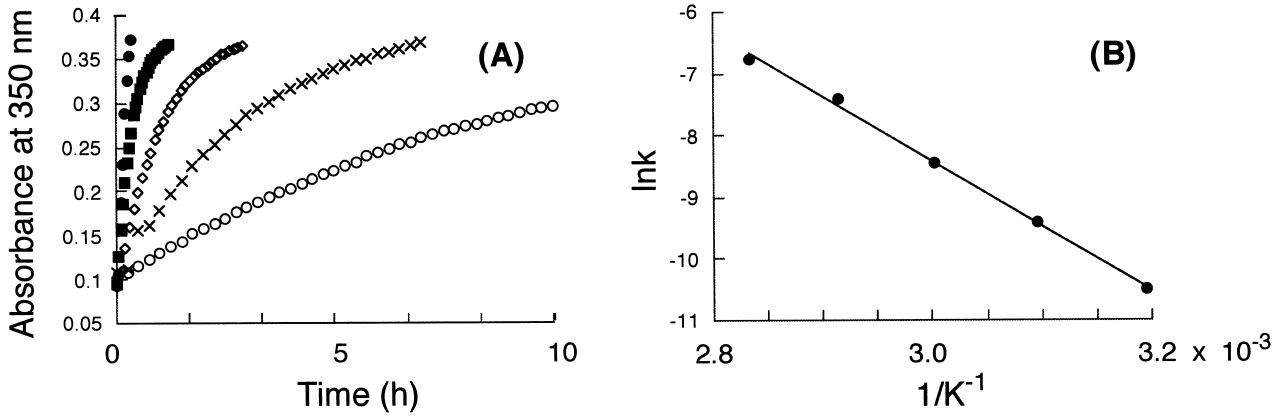

Figure 4. (A) Time-dependent changes in absorption at 350 nm for photoisomerized oligomer 5 at different temperatures: (○) 80°C, (■) 70°C, (◇) 60°C, (x) 50°C, (○) 40°C. (B) Arrhenius plot for the *cis-trans* isomerization of oligomer **5**.

350 nm was returned to the original obtained before illumination. Based on these absorbance changes, the apparent half-life for the *cis* azo chromophore at each temperature can be estimated to yield the first order kinetic constant for the *cis–trans* isomerization. The analysis of these data from the Arrhenius plot (Fig. 4(B)) provided the activation energy to be 20 kcal/mol. Similar kinetic parameters were obtained for the *cis-trans* isomerization in the duplexes of oligomers **7** and **8**. These results indicated that the *cis* form of the azo chromophore incorporated into oligonucleotides or duplexes is sufficiently stable at low temperature. We, therefore, anticipate that photo-induced structural or conformational changes that occurred in azobenzene-oligonucleotide conjugates would be locked for a considerable period under the dark conditions.

Conclusion

We have accomplished the synthesis of a novel photoisomerizable linker for linking oligonucleotide segments. It has been found that the azobenzene linker efficiently undergoes *trans–cis* isomerization by exposing to UV light. The conversion efficiency showed slight dependence on structure or conformation of oligonucleotides attached to the azobenzene chromophore. The *cis* form of the azobenzene in oligonucleotides was stable at low temperature in the dark. The present findings would open the way for light switch of nucleic acid structures.

Experimental

General methods. ^{31}P NMR spectra were obtained on a JEOL GX-400 spectrometer using 85% H_3PO_4 as an external standard. ^{1}H NMR spectra were measured on a JEOL EX-270 spectrometer, in which chemical shifts (δ ppm) were determined on the basis of a residual peak of solvent (2.49 for DMSO-d_6 or 7.26 for $CDCl_3$). High-performance liquid chromatography (HPLC) was performed on a Waters 600E model apparatus with a Hitachi LC 4200 UV–VIS detector at 345 nm

using a reversed phase Cosmosil 5C18-AR-300 column (4.6×150 mm). Column chromatography and thin-layer chromatography (TLC) were carried out on Merck silica gel 60 and Merck 60 PF_{254}, respectively. Ultraviolet (UV) spectra were recorded with a Hitachi U-3000 spectrophotometer equipped with a thermoelectrically controlled cell holder (Hitachi SPR-10).

Materials and solvent. 2-Cyanoethyl-*N,N,N′,N′*-tetraisopropyl phosphorodiamidite was obtained from Aldrich. Protected deoxynucleoside 3′-(2-cyanoethyl)-*N,N′*-diisopropylphosphoramidites and nucleoside-loaded controlled pore glass (CPG) supports were purchased from Cruachem. Oligonucleotides were prepared by a phosphoramidite chemistry on a Pharmacia LKB Gene Assembler Plus DNA/RNA synthesizer. For synthesis of the modified oligonucleotides, the X-bottle was used to supply the modified amidite solution. CH_2Cl_2, triethylamine (TEA), and diisopropylethylamine (DIEA) were refluxed over CaH_2 for 5 h, distilled, and stored over CaH_2. DMF was distilled under the reduced pressure over ninhydrin and stored over molecular sieves. All other reagents and solvents were used as received.

Synthesis of 4-aminophenetyl alcohol. To a solution of 4-nitrophenetyl alcohol (2.0 g, 12 mmol) in methanol (60 mL), hydrogen gas was passed through over Pd-C (0.2 g) for 4 h. After monitoring the completion of the reaction by TLC, the reaction mixture was filtered. The filtrate was evaporated to give a white powder (1.4 g, 87%). TLC (4:1, EtOAc:hexane, v/v) R_f 0.31; ^{1}H NMR (DMSO-d_6): δ = 2.73 (t, 2H), 3.78 (t, 2H), 6.64 (d, 2H), 7.02 (d, 2H).

Synthesis of 4-hydroxyethyl-4-hydroxyazobenzene (1a). To an ice-cooled aqueous solution (90 mL) of 4-aminophenetyl alcohol (1.4 g, 10 mmol) and $NaNO_2$ (0.7 g, 10 mmol) in 0.3 N HCl, a solution (20 mL) of phenol (1.0 g, 10 mmol) in 10% NaOH was added with stirring. After the dark-red precipitate was removed by filtration the filtrate was neutralized with 2 N HCl. The orange colored precipitate was filtered and washed well with water. The solid was dissolved in a small amount of EtOAc and then applied on a silica gel column. The elution was carried out by using a mixed solvent of EtOAC:hexane (4:1, v/v). The appropriate fractions were collected and the solvent was removed in vacuo affording a bright orange solid (0.9 g, 37%). TLC (4:1, EtOAc:hexane, v/v) R_f 0.75; ^{1}H NMR (DMSO-d_6): δ = 2.80 (t, 2H), 3.66 (m, 2H), 4.70 (br, 1H), 6.94 (d, 2H), 7.39 (d, 2H), 7.75 (dd, 4H), 10.25 (s, 1H); EIMS (positive) *m/z* 242.

Synthesis of 4-hydroxyethyl-4′-(2′-*O*-dimethoxytrityl ethyl-oxy)azobenzene (2a). 1a (0.47 g, 2 mmol) was allowed to react with *O*-dimethoxytrityl protected bromoethanol (1.3 g, 3 mmol) in the presence of K_2CO_3 (0.5 g, 3.6 mmol) in DMF (30 mL) at 40°C for 5 h. After the solvent was evaporated, the residue was separated into EtOAc and water. The organic layer was dried with Na_2SO_4 and then evaporated to nearly dryness. The residue was applied on a silica gel column (3:7, EtOAc:hexane, v/v). The appropriate fractions were collected and then the solvent was removed to give a orange solid material (0.7 g, 63%). TLC (3:7, EtOAc:hexane, v/v) R_f 0.64; ^{1}H NMR (DMSO-d_6): δ = 2.81 (t, 2H), 3.30 (t, 2H), 3.66 (m, 2H), 3.72 (s, 6H), 4.28 (t, 2H), 4.67 (t, 1H), 6.86–6.93 (m, 4H), 7.05–7.42 (m, 13H), 7.76–7.88 (m, 4H); FABMS (positive) *m/z* 589 [M + 1].

Synthesis of 4-hydroxyethyl-4′-ethoxyazobenzene (3a). 1a (0.47 g, 2 mmol) was allowed to react with ethyl iodide (0.5 g, 3 mmol) in the presence of K_2CO_3 (0.5 g, 3.6 mmol) in DMF (30 mL) at 40°C for 5 h. The reaction mixture was evaporated to nearly dryness. The residue was dissolved in EtOAc which was washed with water. After the organic layer was dried with Na_2SO_4, the solvent was removed in vacuo. The residual material was applied on a silica gel column which was eluted by EtOAc:hexane (4:1, v/v). The appropriate fractions were collected and then the solvent was removed in vacuo to give a orange crystalline (0.48 g, 86%). TLC (4:1, EtOAc:hexane, v/v) R_f 0.61; ^{1}H NMR (DMSO-d_6): δ = 1.36 (t, 3H), 2.80 (t, 2H), 3.65 (m, 2H), 4.13 (q, 2H), 4.68 (t, 1H), 7.10 (d, 2H), 7.40 (d, 2H), 7.75 (d, 2H), 7.85 (d, 2H); EIMS (positive) *m/z* 270.

Synthesis of 4-(2′-*O*-dimethoxytrityl ethyl-oxy)azobenzene-4′-ethyl (2-cyanoethyl)-*N,N′*-diisopropylphosporamidite (4a). To the solution of **2a** (0.28 g, 0.5 mmol) and 1*H*-tetrazole (0.039 g, 1.1 equiv) and diisopropylamine (0.078 mL) in 2.5 mL of dry CH_2Cl_2 was added 2-cyanoethyl-*N,N,N′,N′*-tetraisopropyl phosphorodiamidite (0.35 mL, 2.0 equiv). The solution was continued to stir at room temperature for 2 h. MeOH (2 mL) was added to terminate the reaction and then the solution was diluted with 3.0 mL of EtOAc containing a small amount of DIEA. The solution was washed in 10% $NaHCO_3$ and the organic phase was dried by Na_2SO_4 and concentrated in vacuo. The residual solution was applied to silica gel column chromatography (30:60:10, EtOAc:hexane:TEA, v/v). The appropriate fractions were collected and evaporated in vacuo to give the phosphoramidite (0.38 g, 92%). TLC (30:60:10, EtOAc:hexane:TEA, v/v) R_f 0.70; ^{31}P NMR (CH_3CN): 148.0 ppm; FABMS (positive) *m/z* 789 [M + 1].

Synthesis of 4-amino-*N*-hydroxyethylbenzamide. 2-Aminoethanol (4.5 mL, 75 mmol) was allowed to react with 4-nitrobenzoyl chloride (9.3 g, 50 mmol) in CH_2Cl_2 (75 mL) containing TEA (15 mL) at 0°C for 30 min. The precipitate was filtered and then dried in vacuo over P_2O_5. The solid material obtained was treated with H_2 over Pd-C in a similar manner described for 4-aminophenetyl alcohol, affording a white solid (7.3 g, 82%). TLC (9:1, CH_2Cl_2:MeOH, v/v) R_f 0.18; ^{1}H NMR ($CDCl_3$): δ = 3.60 (t, 2H), 3.81 (t, 2H), 6.68 (d, 2H), 7.61 (d, 2H).

Synthesis of 4-hydroxy-4′-(*N*-hydroxyethyl)azobenzamide (1b). The synthesis and purification of **1b** was carried out by essentially the same procedures described for **1a** to give a bright orange crystalline (46%). TLC (9:1, CH_2Cl_2:MeOH, v/v) R_f 0.32; ^{1}H NMR ($CDCl_3$):

δ = 3.35 (t, 2H), 3.51 (t, 2H), 4.75 (t, 1H), 6.99 (d, 2H), 7.82 (m, 4H), 8.01 (d, 2H), 8.58 (t, 1H); FABMS (positive) *m*/*z* 286 [M + 1].

Synthesis of 4-(2′-*O*-dimethoxytrityl ethyl-oxy)-4′-(*N*-hydroxyethyl)azobenzamide (2b). The synthesis and purification of **2b** was carried out by essentially the same procedures described for **2a** to give an orange crystalline (67%). TLC (9:1, CH_2Cl_2:MeOH, v/v) R_f 0.62; ^{1}H NMR ($CDCl_3$): δ = 3.27–3.55 (m, total 6H), 3.72 (s, 6H), 4.30 (m, 2H), 4.74 (t, 1H), 6.86–6.93 (m, total 5H), 7.19–7.42 (m, total 12H), 7.89–8.04 (m, 4H), 8.58 (t, 1H); FABMS (positive) *m*/*z* 632 [M + 1].

Synthesis of 4-ethoxy-4′-(*N*-hydroxyethyl)azobenzamide (3b). The synthesis and purification of **3b** was carried out by essentially the same procedures described for **3a** to give an orange crystalline (70%). TLC (9:1, CH_2Cl_2:MeOH, v/v) R_f 0.45; ^{1}H NMR ($CDCl_3$): δ = 1.37 (t, 3H), 3.28–3.54 (m, total 4H), 4.15 (m, 2H), 4.73 (t, 1H), 7.13 (d, 2H), 7.87–8.04 (m, total 6H), 8.57 (t, 1H); EIMS (positive) *m*/*z* 313.

Synthesis of 4-(2′-*O*-dimethoxytrityl ethyl-oxy)-4′-{*N*-ethyl (2-cyanoethyl)-*N*,*N*′-diisopropylphosporamidite}azobenzamide (4b). The synthesis and purification of **4b** was carried out by essentially the same procedures described for **4a** to give the phosphoramidite (97%). TLC (60:30:10, EtOAc:hexane:TEA, v/v) R_f 0.81; ^{31}P NMR (CH_3CN): 148.1 ppm; FABMS (positive) *m*/*z* 832 [M + 1].

Synthesis of oligonucleotides containing an azobenzene fragment. The synthesis of azobenzene modified oligonucleotides was accomplished by a phosphoramidite chemistry beginning with 5′-DMT-nucleoside (1.3 μmol) bound to a CPG support. For the coupling of normal deoxyribonucleoside phosphoramidites, the standard protocol (120 μL of 0.1 M amidite and 120 μL of 0.1 M tetrazole in acetonitrile, 1.5 min) was used. For the coupling of azobenzene phosphoramidites, 120 μL of the 0.12 M amidite and 120 μL of 0.1 M tetrazole in acetonitorile (10 min) were used. With these conditions, the amidites **4a** and **b** can be coupled in efficiencies of 40–65% with the hydroxyl functions of oligonucleotide chains attached to CPG. The CPG bound oligonucleotides were treated with concentrated ammonium hydroxide at 55°C for 12 h. Purification of the modified oligonucleotides was performed with 20% denaturing polyacrylamide gel electrophoresis as described.[12] The integrity of oligonucleotide structure was verified with spray mass analysis.

Preparation of oligonucleotide solution for UV melting measurements. Oligonucleotide solutions were prepared using a buffer containing 0.1 or 1.0 M NaCl and 0.01 M sodium phosphate, adjusted to pH 7.0. Oligonucleotide concentrations were determined by absorbance at 260 nm and the calculated single-strand extinction coefficients based on a nearest neighbor model.[12] Before melting experiments, the solutions were heated to 80°C, kept there for 5 min, and then gradually cooled down to room temperature. All duplex melting curves by UV spectra were measured with an increase in temperature from 0 to 80°C at a rate of 0.5°C/min.

Analysis of photoisomerization of azobenzene. The solutions of azobenzene containing oligonucleotides in a phosphate buffer and model compounds **3a** and **b** in CH_3CN were irradiated in a cooled water bath (5°C) by a high-pressure Hg lamp using band-path filter to transmit light at around 350 nm. The aliquot of the solution was analyzed by UV–vis spectra at 5°C after an appropriate time interval.

The calculations of the *cis*% in the oligonucleotides after the irradiation were carried out by using the absorbance changes at 350 nm based on the assumption that the absorbance of the *cis*-isomer at the employed wavelength is negligible. The model compound **3a** was analyzed by ^{1}H NMR in $CDCl_3$ before and after the UV illumination. Before irradiation, the compound exhibited four doublets (7.02, 7.36, 7.84 and 7.91 ppm) at aromatic region, indicating only the *trans* of the azo chromophore. Four new doublets (6.77, 6.93, 7.04 and 7.18 ppm) attributable to the *cis* form of the azobenzene appeared after exposing of UV light. The integration of these peaks provided the content of *cis*-form (60%). The UV–vis spectral measurements were done for the same solution, giving the *cis* content to be 50% based on the absorbance changes at 350 nm. It is therefore noted that the calculated *cis*% based on the absorbance changes may be slightly lower than expected.

The thermal back reaction (in dark) from *cis* to *trans* was monitored by UV–vis spectra at different temperatures. After a prolonged time, the absorbance at 350 nm was returned to the original obtained before illumination. Based on these absorbance changes, the apparent half-life for the *cis* azo chromophore at each temperature can be estimated, yielding the first order kinetic constants. The analysis of these data from the Arrhenius plot (Fig. 4(B)) provided the activation energy for the *cis*–*trans* isomerization.

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