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Enantioselective Incorporation of Azobenzenes into Oligodeoxyribonucleotide for Effective Photoregulation of Duplex Formation**

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Various organic molecules have been introduced into oligodeoxyribonucleotides (ODNs) by means of linkers to provide new functionalities.[1] We already reported that the *cis* → *trans* isomerization of an azobenzene moiety in the side chain of ODNs could reversibly photoregulate the formation and dissociation of its duplex: a *trans*-azobenzene moiety in the ODN stabilizes the duplex, whereas the *cis* form destabilizes it.[2] By using this modified ODN as a modulator, a T7 DNA polymerase reaction could also be photoregulated.[3] For even more effective photoregulation, the change in melting temperature ΔT_m induced by the *trans* → *cis* isomerization should be enhanced. A promising strategy for this purpose is to introduce multiple azobenzene groups into the ODN. However, the modified ODN was previously synthesized from the corresponding racemic mixture of phosphoramidite monomers, which were obtained from a prochiral diol as starting material (Scheme 1 A). Thus, two diastereomers were inevitably produced.[4] Since their photoregulation capabilities are significantly different,[2a] the azobenzene moieties should be enantioselectively incorporated into the ODN for more effective photoregulation. With these racemic phosphoramidite monomers, it is practically impossible to synthesize diastereochemically pure ODNs containing multiple azobenzene groups, and hence the optically pure phosphoramidite monomer is essential.

We chose threoninol as the linker (Scheme 1 B) for two reasons: 1) optically pure diols can be synthesized from the corresponding D- or L-threonine, and 2) perturbation of the framework of our previous prochiral linker (Scheme 1 A) is minimized.[5] Both L- and D-threoninol were used as optically pure linkers, and two azobenzene moieties were enantioselectively introduced. It was shown that a D-threoninol-tethered azobenzene moiety induces much larger ΔT_m on photoisomerization than does an L-threoninol-tethered one.

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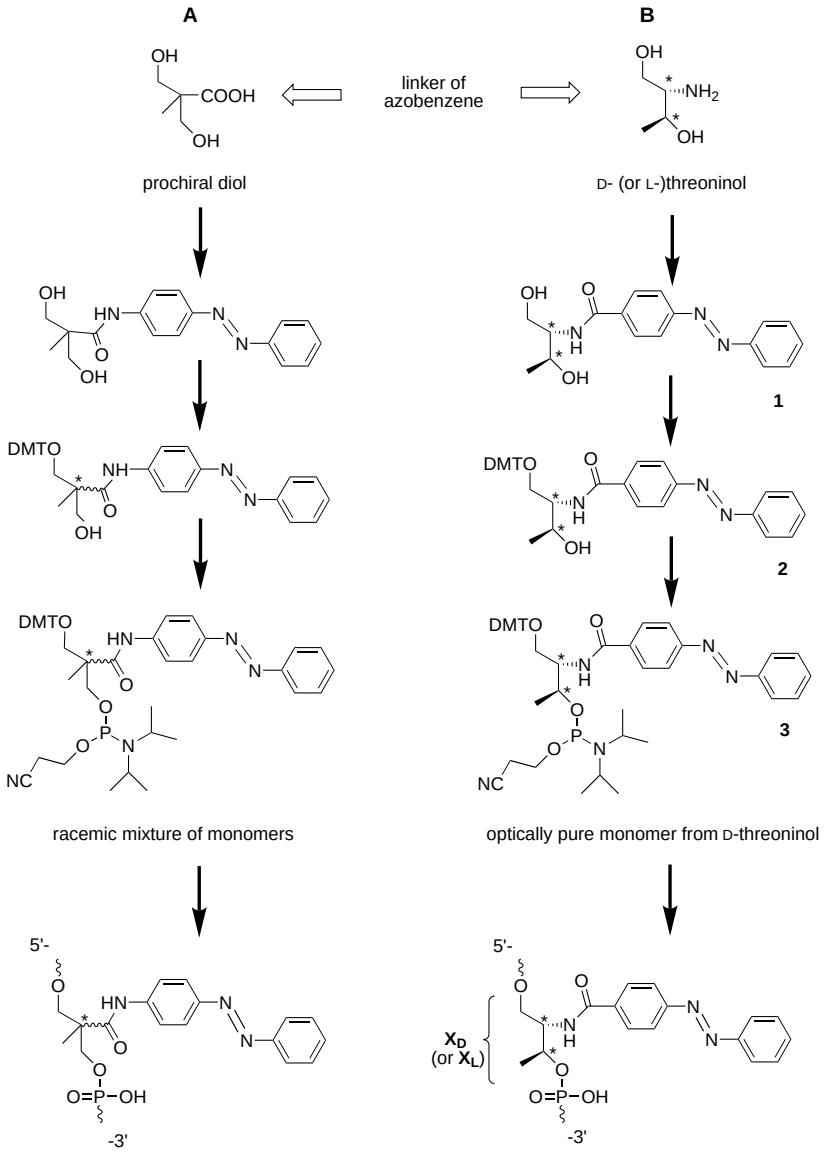

Scheme 1. Modified ODN carrying an azobenzene moiety attached by a prochiral diol linker (A) and by a chiral diol linker (B). DMT = 4,4-dimethoxytrityl.

Furthermore, an even larger ΔT_m is obtained by introducing two D-threoninol-tethered azobenzene groups.

The azobenzene moieties of all the modified ODNs (Table 1) overwhelmingly adopted the *trans* form before UV irradiation. On UV irradiation ($300 < \lambda < 400$ nm), they promptly isomerized to the *cis* form. The isomerization was reversible: the *cis*-azobenzene moiety was isomerized to the *trans* form by irradiation with visible light ($\lambda > 420$ nm).[6] The melting temperatures of the duplexes formed by the modified DNAs and their complementary counterpart in the *trans* or *cis* form are summarized in Table 1.[7] The ΔT_m significantly depended on the chirality of the linker. The melting temperature of the duplex formed by **L1**, containing one azobenzene moiety linked through L-threoninol, and its complementary counterpart **C** decreased from 45.1 (*trans*-azobenzene) to 40.8 °C on *trans* → *cis* isomerization.[8] The ΔT_m induced by the photoisomerization was 4.3 °C. In contrast, a much larger ΔT_m was induced when the azobenzene moiety was tethered by D-threoninol: the T_m of the *trans*- and *cis*-**D1**/**C** duplexes were 50.9 and 36.6 °C, respectively ($\Delta T_m = 14.3$ °C). The duplex was more strongly stabilized by *trans*-azobenzene that was tethered by D-threoninol as opposed to the L-form, whereas it was strongly destabilized by D-threoninol-tethered *cis*-azobenzene. As a result, a larger ΔT_m was accomplished by the D-threoninol linker.

By using the present optically pure linkers, two azobenzene moieties can be enantioselectively incorporated into ODNs, and it was found that the chirality of the linker then affects ΔT_m to an even greater extent. When two azobenzene moieties were introduced into the ODN through two L-threoninol residues (**LL2**), the melting curve of the *trans*-**LL2**/**C** duplex was almost superimposed on that of *cis*-**LL2**/**C** (Figure 1a; $\Delta T_m \approx 0$ °C).[9] With two azobenzene moieties on two D-threoninol residues (**DD2**), however, the two curves are widely separated from each other (Figure 1b). The ΔT_m of **DD2**/**C** was as large as 21.5 °C, which fairly exceeded the ΔT_m of **D1**/**C** duplex (14.3 °C) involving one azobenzene moiety. The order of the ΔT_m for all the possible diastereomers was as follows: **DD2** > **LD2** > **DL2** >

Table 1. Melting temperature T_m of the duplexes between the modified ODN and its complementary counterpart.[a]

ODN[b]	Sequence	T_m [°C]		ΔT_m [°C][c]
		trans	*cis*	
L1	5′-GCGA$\mathbf{X_L}$GTCG-3′	45.1	40.8	4.3
D1	5′-GCGA$\mathbf{X_D}$GTCG-3′	50.9	36.6	14.3
LL2	5′-GC$\mathbf{X_L}$GAGT$\mathbf{X_L}$CG-3′	25.4	25.5	−0.1
DL2	5′-GC$\mathbf{X_D}$GAGT$\mathbf{X_L}$CG-3′	31.9	22.2	9.7
LD2	5′-GC$\mathbf{X_L}$GAGT$\mathbf{X_D}$CG-3′	38.7	22.9	15.8
DD2	5′-GC$\mathbf{X_D}$GAGT$\mathbf{X_D}$CG-3′	43.9	22.4	21.5

[a] The complementary counterpart is ODN **C** (3′-CGCTCAGC-5′) for all the ODNs. [b] $\mathbf{X_L}$ and $\mathbf{X_D}$ denote the azobenzene moieties (see Scheme 1B) tethered on L- and D-threoninol, respectively. T_m of 5′-GCGAGTCG-3′/3′-CGCTCAGC-5′ duplex without $\mathbf{X_D}$ or $\mathbf{X_L}$ is 46.6 °C. [c] Change in T_m induced by *cis* → *trans* isomerization.

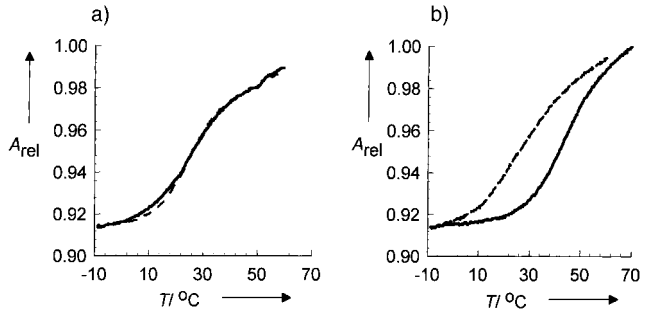

Figure 1. Melting curves for the duplex formation of **LL2**/**C** (a) and **DD2**/**C** (b) in the *trans* (solid lines) and *cis* forms (dashed line). The T_m values obtained from these curves are listed in Table 1. A_{rel} = relative absorbance.

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LL2 $\approx 0\,^{\circ}C$, that is, D-threoninol is advantageous as a linker for azobenzene moieties.

According to a molecular model, the azobenzene moiety on a D-threoninol residue protrudes towards the minor groove, and that on an L-threoninol residue towards the major groove (see Supporting Information). In the narrow, minor groove, a structural change of azobenzene would significantly affect the stability of the duplex, whereas the effect should be smaller in the wide, major groove.[10] Therefore, the D-threoninol-tethered azobenzene induces larger ΔT_m values.

In conclusion, azobenzene moieties were enantioselectively introduced into ODNs by using optically pure linkers. D-Threoninol is an excellent linker of azobenzene for the effective photoregulation of duplex formation.[11]

Experimental Section

Synthesis of the azobenzene-containing phosphoramidite monomer:[12] D- or L-threoninol was obtained by the reduction of the corresponding D- or L-threonine methyl ester with $LiAlH_4$ in dry THF.[13] Then the D-threoninol was coupled with 4-phenylazobenzoic acid in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in DMF (yield 80%). **1**: ^{1}H NMR: (500 MHz, $CDCl_3$): $\delta = 7.96-7.38$ (m, 9H, ArH of azobenzene), 7.12 (d, $^3J(H,H) = 8.0$ Hz, 1H, N*H*CO), 4.33 (m, 1H, C*H*(OH)CH_3), 4.09 (m, 1H, HOCH_2C*H*(NHCO)), 3.98 (d, $^3J(H,H) = 4.5$ Hz, 2H, CH_2OH), 1.29 (d, $^3J(H,H) = 6.5$ Hz, 3H, CH(OH)CH_3); ESI-MS: *m*/*z*: 313.7; calcd for [**1**+H$^+$]: 314.1.

Tritylation of **1**: 4,4-dimethoxytrityl chloride (DMT-Cl) in CH_2Cl_2 was added to a dry pyridine solution containing **1** and dimethylaminopyridine (yield of **2**: 63%). **2**: ^{1}H NMR for **2** (500 MHz, $CDCl_3$): $\delta = 8.00-6.78$ (m, 23H, ArH of DMT, azobenzene; N*H*CO), 4.25 (m, 1H, C*H*(OH)CH_3), 4.17 (m, 1H, OCH_2C*H*(NHCO)), 3.77, 3.76 (s, 6H, $C_6H_4OCH_3$), 3.60, 3.42 (dd, $^2J(H,H) = 10.0$, $^3J(H,H) = 4.0$ Hz, 2H, CH_2ODMT), 1.23 (d, $^3J(H,H) = 6.5$, 3H, CH(OH)CH_3). ESI-MS for **2**: *m*/*z*: 638.1; calcd for [**2**+Na$^+$]: 638.3.

3: In dry acetonitrile under nitrogen, **2** and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite were treated with 1*H*-tetrazole according to the recommended procedure.[2b] ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.00-6.79$ (m, 22H, ArH of DMT, azobenzene), 6.62 (d, $^3J(H,H) = 8.5$ Hz, 1H, N*H*CO), 4.48 (m, 1H, C*H*(CH_3)OP), 4.39 (m, 1H, OCH_2C*H*(NHCO)), 4.21–4.10 (m, 2H, CH_2OP), 3.77 and 3.76 (s, 6H, $C_6H_4OCH_3$), 3.57–3.34 (m, 4H, C*H*(CH_3)$_2$, CH_2ODMT), 2.76–2.72 (m, 2H, CH_2CN), 1.30–1.25 (m, 15H, CH(CH_3)$_2$, CH(OP)CH_3). ESI-MS: *m*/*z*: 838.1; calcd for [**3**+Na$^+$]: 838.4.

The phosphoramidite monomer of the L-isomer was synthesized from L-threoninol by the same procedure as the D-isomer.

Synthesis of azobenzene-containing modified ODNs: All the modified oligonucleotides in Table 1 were synthesized on an automated DNA synthesizer by using the phosphoramidite monomer **3** and conventional monomer. The coupling efficiency of the monomer **3** was as high as those of conventional monomers, as judged from the coloration of released trityl cation. After the recommended workup, they were purified by reversed-phase HPLC.

MALDI-TOF MS: *m*/*z*: **L1**: 2824, **D1**: 2824 (calcd for [**L1** – H$^+$]: 2823); **LL2**: 3198, **DD2**: 3195, **LD2**: 3198, **DL2**: 3197 (calcd for [**LL2** – H$^+$]: 3197).

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[7] The concentration of each ODN was 5 μM in pH 7.0 phosphate buffer (10 mM) in the presence of 0.1M NaCl. The T_m value was determined from the maximum in the first derivative of the melting curve, which was obtained by measuring the absorbance at 260 nm as a function of temperature. The heating rate was $1.0\,^{\circ}C\,min^{-1}$.[2a] Throughout the T_m measurement, the fractions of *cis* and *trans* isomers remained almost constant, as monitored by UV/Vis spectroscopy.

[8] For **L1** and **D1**, the azobenzene moiety adopted the *trans* form to an extent of greater than 98% in the dark, and the *cis* form amounted to 76% after UV irradiation, as determined by reversed-phase HPLC and UV spectroscopy.

[9] In the dark, greater than 98% of the azobenzene moieties adopted the *trans* form in all four diastereomers. Both of the *trans*-azobenzene moieties were promptly isomerized to *cis* form on UV irradiation (*cis* fraction 62%).

[10] The photoregulation of duplex formation is based on the stabilization of the duplex by the planar *trans*-azobenzene and destabilization by the nonplanar *cis*-azobenzene.[2a, d] Intercalation of *trans*-azobenzene into the base pairs was revealed in both diastereomers (**D1**/**C** and **L1**/**C**) by the bathochromic shift of the peak maximum of azobenzene resulting from the duplex formation (see Supporting Information). Weak but explicit circular dichroism was also negatively induced for both *trans*-**D1**/**C** and *trans*-**L1**/**C** at 330 nm, where the peak maximum of azobenzene exists (see Supporting Information). This fact also supports the intercalation of *trans*-azobenzene (R. Lyng, A. Rodger, B. Nordén, *Biopolymers* **1992**, *32*, 1201–1214).

[11] The present results are applicable, for example, to the photoregulation of the RNA polymerase reaction or gene expression by antisense strategy, since these processes involve the dissociation of DNA duplexes into single strands prior to chemical reaction.

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