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Photo-Responsive Oligonucleotides Carrying Azobenzene in the Side-Chains

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Abstract The title oligonucleotides were prepared by using a new phosphoramidite monomer. The *cis-trans* isomers with respect to the stereochemistry of the azobenzene residue, obtained on photo-irradiation, were completely resolved by reversed-phase HPLC. The physicochemical properties of these oligonucleotides were significantly changed by the photo-induced isomerization. © 1998 Elsevier Science Ltd. All rights reserved.

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Chemical modification of oligonucleotides has been widely attempted.[1] One main goal of these studies is to provide useful tools for artificial regulation of cell functions. Incorporation of photo-responsive groups is an especially attractive methodology, since the interactions between the oligonucleotides and other molecules could then be regulated by photo-irradiation. Recently, azobenzene was introduced into the main chains of oligonucleotides.[2] In order to extend the scope of molecular design, however, introduction of photo-responsive residues into the side chains should be also important. The interactions between the oligonucleotides and other molecules (e.g., DNA and DNA-binding proteins) can be modulated by photo-induced conformational change of the oligonucleotides. The present chemical modification causes less perturbation in the primary structure of oligonucleotides than does the introduction of the photo-responsive residues into the main chains.

This communication reports on the incorporation of azobenzene residues to the side chains of oligonucleotides. A new phosphoramidite monomer (**2** in **Scheme 1**) is prepared for the purpose. The diastereomers of the oligonucleotides are separated by HPLC, and the photo-induced *cis-trans* isomerization of the azobenzene residues is described. Notable effects of the isomerization on the properties of oligonucleotides are evidenced, indicating a potential for reversible photo-regulation of their functions.

The phosphoramidite monomer **2** carrying an azobenzene residue was synthesized according to **Scheme 1**.[3] First, 2,2-bis(hydroxymethyl)propionic acid was coupled with 4-aminoazobenzene. After one of the hydroxyl groups was protected by 4,4'-dimethoxytrityl (DMT) residue, **1** was converted to the phosphoramidite monomer **2**. All the intermediates and the product were purified by either recrystallization or silica-gel column chromatography, and characterized by NMR spectroscopy.[4] The two diastereomers of **2** were used as the

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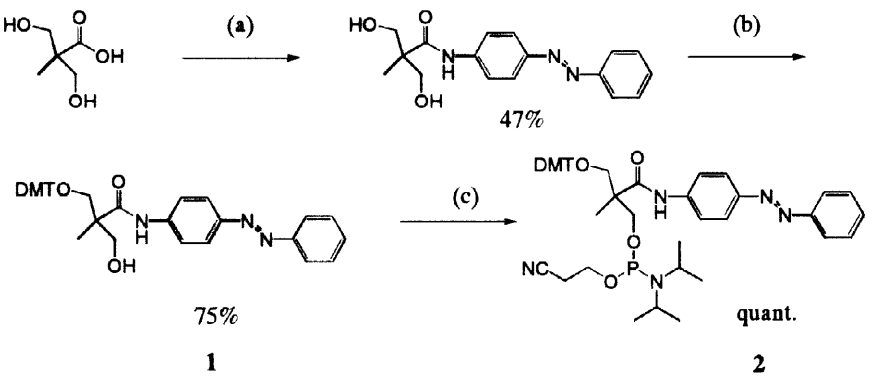

Scheme 1. Synthesis of the phosphoramidite monomer **2**.
(a) 4-aminoazobenzene, dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, DMF
(b) 4,4'-dimethoxytrityl (DMT) chloride, 4-dimethylaminopyridine, pyridine, CH_2Cl_2
(c) 2-cyanoethyl N,N,N',N',-tetraisopropylphosphorodiamidite, 1*H*-tetrazole, CH_3CN

mixture for DNA synthesis. However, they were completely separated, after being incorporated into oligonucleotides (*vide infra*).

By using **2** and the conventional phosphoramidite monomers, oligonucleotides were prepared on an automated synthesizer. The reversed-phase HPLC pattern of the 8-mer nucleotide (5'-AAAXAAAA-3') is presented in **Fig. 1**.[5] Here, X denotes the residue arising from **2**. Two major peaks and two minor ones are observed. The two major products (**C** and **D**) were isolated and analyzed. According to TOF-MS spectroscopy (negative-mode), the molecular weights of both of the fractions fairly coincide with the expected value for the target 8-mer nucleotide (Obsd: 2505±2 (for **C**) and 2504±2 (for **D**); Calcd for [AAAXAAAA - H^+]: 2504).

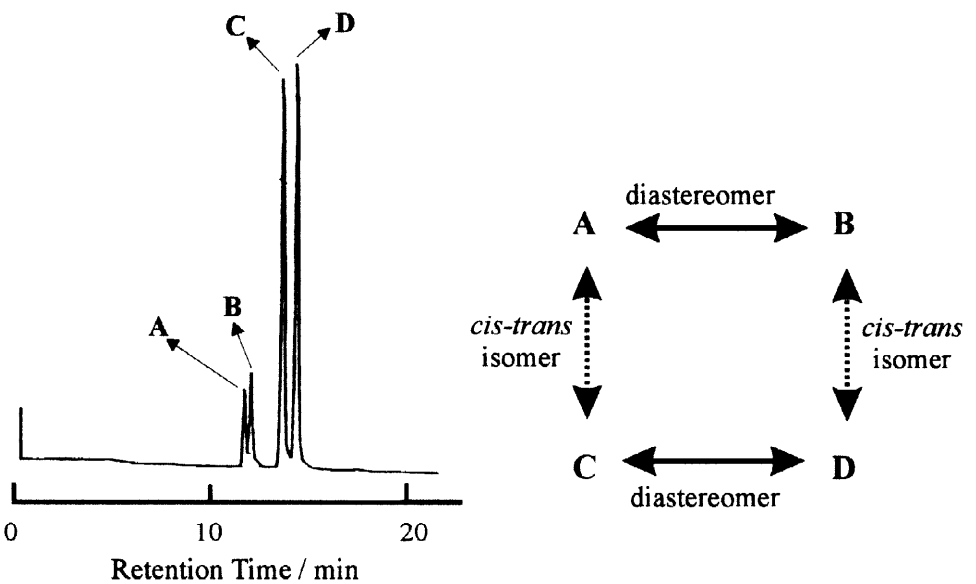

Figure 1. Reversed-phase HPLC pattern (left) of the 8-mer nucleotide (AAAXAAAA) obtained by using **2** and the conventional phosphoramidite monomer. The relationship between the four isomers is schematically illustrated on the right.

The product **D** (**Fig. 2** (**a**)) was irradiated in water with UV light (wavelength (λ) = 300-400 nm).[6] As shown in the upper part in **Fig. 2** (**b**), the intensity of the HPLC signal for the product **D** greatly diminished, and, concurrently, a strong signal appeared at the retention time corresponding to the product **B** in **Fig. 1**. In the UV-visible spectrum, the absorption band at around 350 nm (assignable to the *trans*-form of azobenzene) weakened, and a new absorption band (due to the *cis*-form) appeared around 450 nm (compare the lower parts of **Fig. 2** (**a**) and (**b**)). Apparently, the azobenzene residue in the product **D** was isomerized by the UV-irradiation from the *trans*-form to the *cis*-form.

The photo-isomerization is reversible. When the specimen in **Fig. 2** (**b**) was further irradiated with visible light ($\lambda > 400$ nm),[6] the HPLC signal corresponding to the product **B** gradually disappeared and the one for the product **D** became dominant (see **Fig. 2** (**c**)). Furthermore, the absorption band at 350 nm increased and the one at 450 nm decreased. The *cis*⇒*trans* isomerization was induced by the visible light.[7] When the sample was then irradiated with UV light, the *trans*⇒*cis* isomerization again occurred, and both the HPLC pattern and the absorption spectrum were virtually identical with those in **Fig. 2** (**b**). The photo-induced isomerization was repeated many times without apparent deterioration.

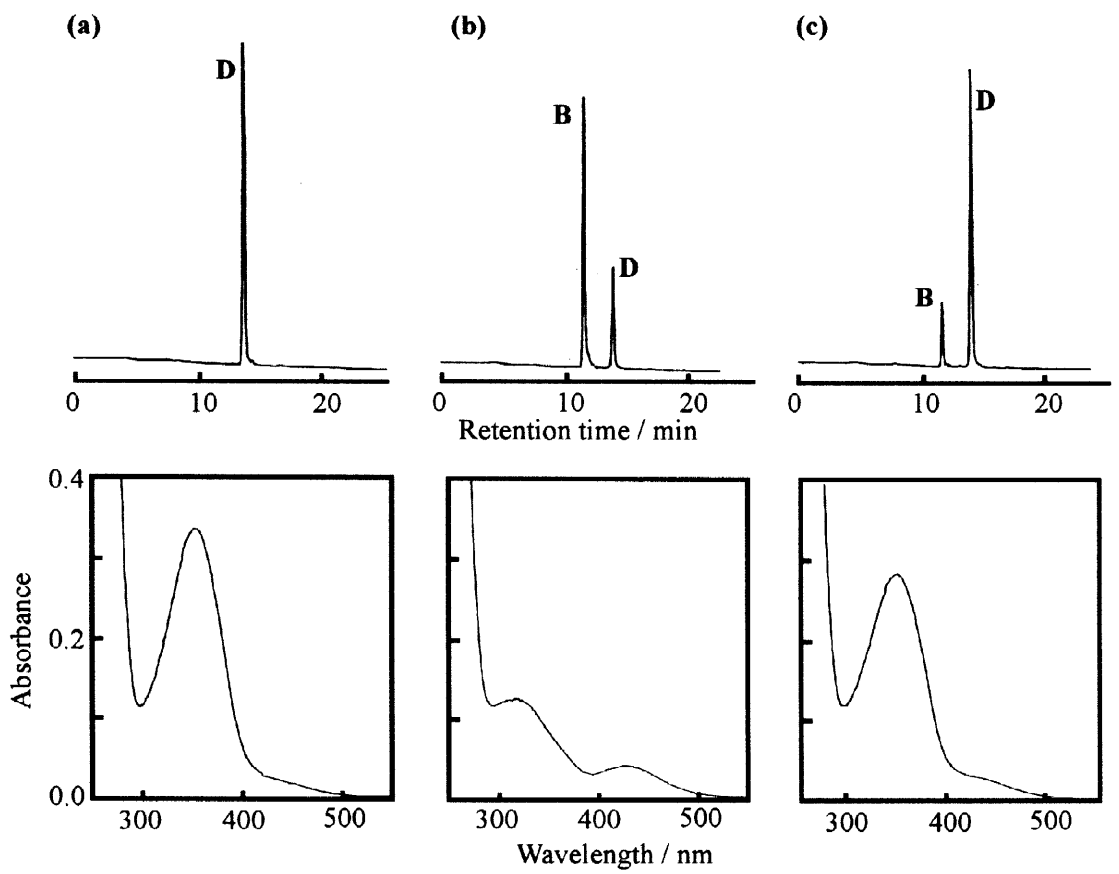

Figure 2. The HPLC patterns (top) and UV-visible absorption spectra (below) of the aqueous solution of the product **D**: (**a**) before and (**b**) after the irradiation of UV light (300 nm < λ < 400 nm). The results of the irradiation of visible light ($\lambda > 400$ nm) on the sample (**b**) are shown in (**c**). The photo-irradiation was carried out for 30 min at room temperature.

Similarly, the product **C** was converted to the product **A** by the irradiation with UV light (300 nm < λ < 400 nm). The resultant product **A** was then transformed to the **C** by irradiating visible light (λ > 400 nm). Thus, the products **B** and **D** (as well as the products **A** and **C**) are *cis-trans* isomers with respect to the stereochemistry of the azobenzene. It is noteworthy that these isomers can be completely resolved by the reversed-phase HPLC (**Figs. 1** and **2**), although they differ only in the stereochemistry of one azobenzene residue. The hydrophobicities (and other properties) of the oligonucleotides are greatly changed on the *cis-trans* isomerization. Assumedly, the stereochemistry of the azobenzene residue affects the conformations of the whole molecules of oligonucleotides, resulting in considerable difference in their apolar characters. The clear resolution of the diastereomers of oligonucleotides by HPLC would be also associated with conformational differences. Consistently, the diastereomers of 3-mer (5'-AXA-3') could not be separated under any HPLC conditions investigated. The potential of the present oligonucleotides as photo-responsive agents is strongly indicated.

Neither the product **B** nor **D** was detected in the mixtures from the product **C**, whereas the products **A** and **C** were absent in the mixtures from the product **D**. The products **A** and **B** (as well as the products **C** and **D**) are diastereomers to each other (see the right part in **Fig. 1**).

In conclusion, photo-responsive oligonucleotides carrying azobenzene residues in the side chains have been prepared in diastereochemically pure forms. The photo-induced *cis-trans* isomerization of the azobenzene exerts notable effects on the physicochemical properties of the oligonucleotides. Study on the interactions of these modified oligonucleotides with either DNA or proteins is currently underway.

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4. **1**: ^{1}H-NMR [$CDCl_3$(TMS), 270 MHz] δ 9.56(s, 1H, -NHCO-), 7.91-6.80(m, 22H, aromatic protons of DMT and azobenzene), 3.80(s, 6H, -OCH_3), 3.67 and 3.63 (d, 2H, J=5.3 Hz, -C$\underline{H}_2$OH), 3.45 and 3.38 (d, 2H, J=9.6 Hz, DMT-OCH_2-), 1.35(s, 3H, -CH_3). **2**: ^{1}H-NMR [$CDCl_3$] δ 9.25(s, 1H, -NHCO-), 7.90-6.83(m, 22H, aromatic protons of DMT and azobenzene), 3.90-3.67(m, 10H, -OCH_3, -CH_2OP, -CH_2-OP), 3.65-3.47(m, 2H, -C$\underline{H}$(CH_3)$_2$), 3.45-3.40(m, 2H, DMT-OCH_2-), 2.68-2.48(m, 2H, -CH_2CN), 1.31-1.07(m, 15H, -CH(C$\underline{H}_3$)$_2$, -CH_3); ^{31}P-NMR [$CDCl_3$, 109.4 MHz] δ 149.8, 149.3.
5. The HPLC conditions: a Merck LiChrospher 100 RP-18(e) column, 260 nm, 1.0 ml/min, a linear gradient 5-25 % (25 min) acetonitrile/water containing 50 *mM* ammonium formate.
6. The light from 150 W Xenon lamp was irradiated for 30 min through an appropriate filter. Infrared light was cut off by using water filter.
7. The *cis*⇒*trans* conversion slowly proceeded even in dark (it took about 5 h to convert half of the oligonucleotide **A** to the **C** at 25°C).