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Synthesis of A New Photoisomerizable Linker for Connecting Two Oligonucleotide Segments

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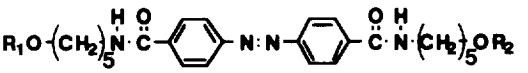
ABSTRACT: Synthesis of a photoisomerizable linker containing an azobenzene unit for connecting oligonucleotide segments has been described. 4,4'-Azobenzene dicarbonyl chloride was converted to the fully protected diol by treatment with the O-silyl protected aminopentanol. Partial deprotection of the silyl group afforded the monoprotected diol, which was protected by a dimethoxytrityl group. Then the remaining silyl protecting group was removed to afford the alcohol. The hydroxyl function was converted to the phosphoramidite. The amidite can be used for joining two oligonucleotides via the 5'-terminal hydroxyl group of one oligomer and the 3'-terminal oxygen of the other.

Recent reports have demonstrated the utilities of oligonucleotide segments connected with a non-nucleotide linker in several nucleic acids recognition systems. Two oligonucleotide segments joined by such linker have been shown to exhibit cooperative binding to appropriately positioned sequences in a single stranded RNA, which can, in principle, serve as a structural probe for a natural RNA.[1] Enhanced stability has been observed in the triple-helix formation of two covalently linked oligopyrimidines with oligopurine.[2,3] Ribozyme acitivity can be regulated by the linker length between the substrate and the enzyme oligoribonucleotide segments.[4] The stilbene linker can serve as an effective cap for strongly binding of two oligonucleitde segments.[5]

We have designed a new non-nucleotide linker containing an azobenzene unit that would be useful for incorporating into oligonucleotide chains. An attractive feature of this type of linker is that the azobenzene undergoes photochemically-induced *trans-cis* isomerization.[6] Two oligonucleotides attached on the 4,4'-positions of the azobenzene would be *reversibly* in different proximity of two strands imposed by photo-illumination. It is thus expected that the complexes such as duplexes, triplexes, and ribozymes involving azobenzene-linked oligonucleotides would reversibly change their conformation as a result of photo-illumination. This novel light switch should find several interesting biological applications in relation to nucleic acids.[7] Described herein is the synthesis and characterization of oligonucleotide derivatives containing the azobenzene linker.

Incorporation of the azobenzene linker between the 5'-terminal hydroxyl function of one oligomer and the 3'-terminal group of the other would be possible by use of the azobenzene which is substituted at 4,4'-positions through an appropriate oligomethylene by dimethoxytrityl group on terminal oxygen of one end and 2-cyanoethyl phosphordiisopropylamidite on another terminal oxygen. The structure of this linker is shown in Chart 1. 4,4'-Azobenzene dicarbonyl chloride[8] was treated with an excess of *tert*-butyldimethylsilyl protected aminopentanol[9] in the presence of triethylamine in DMF (r.t., overnight), affording the fully

Chart 1



1: R_1=R_2= *tert*-butyldimethylsilyl

2: R_1= H, R_2= *tert*-butyldimethylsilyl

3: R_1= 4,4'-dimethoxytrityl, R_2= H

4: R_1= 4,4'-dimethoxytrityl, R_2= $P(OCH_2CH_2CN)N(i\text{-}Pr)_2$

protected diol, **1** (54 %).[10] Partial deprotection of the silyl group was conducted by use of a limited amount of tetrabutylammonium fluoride (0.75 equiv. to **1**) in THF (r.t., 70 min). After usual work-up, the monoprotected diol, **2**, was obtained by recrystallization from CH_2Cl_2 in a yield of 29 %.[11] The alcohol function was protected by a dimethoxytrityl group in the usual way and then the silyl protecting group was removed to afford the alcohol, **3**.[12] The hydroxyl function was converted to the phosphoramidite by a standard phosphitilation procedure.[13]

With a dimethoxytrityl at one end and a reactive phosphorus function at the other, the amidite, **4**, could readily be used in the automated synthesis of oligonucleotides. The oligonucleotides, 5'TTTTCTTTTCCCCCCT - L - TCCCCCCTTTTCTTTT3' (**5**), 5'TTTTCTTTTCCCCCCT - L - T (**6**), and T - L - TCCCCCCTTTTCTTTT3' (**7**) (L indicates the azobenzene linker), were synthesized by use of a slightly modified standard protocol on a DNA synthesizer.[14] With this protocol, the activated amidite was coupled in an efficiency of 90% with the hydroxyl function of oligonucleotides on a CPG support. After the usual deprotection, the oligomers were purified by denaturing polyacrylamide gel (20 %) electrophoresis.

Table 1. HPLC and UV data for the oligonucleotides containing azobenzene linker, **5-7**.

Oligomer	RT1 (min)[a]	RT2 (min)[b]	A330/A260[c]
5	21.6	36.8	0.12 (0.13[d])
6	23.4	39.2	0.20 (0.24[d])
7	24.2	41.8	0.20 (0.24[d])

The oligomers **5-7** exhibited single peak in two different HPLC conditions: *a)* HPLC (YMC-pak C18, 6 x 150 mm) were carried out by a linear gradient of CH_3CN (1 %/min) starting from 10 % CH_3CN in 0.1 M triethylammonium acetate buffer (pH 7.0); *b)* 5 % CH_3CN (5 min) and then a linear gradient of CH_3CN (5 % - 35 % for 30 min, 35 % - 55 % for 20 min) in the same buffer.
c) UV measurements were done in aqueous non-buffered solutions.
d) Theoretical value for the ratio of A_{330}/A_{260}. Calculations were carried out by using ε value at 330 nm of compound **2** and total ε at 260 nm of T and dC in the oligomer.

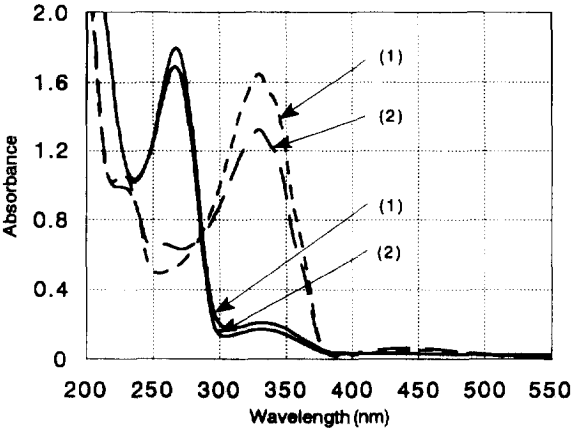


Figure 1. Absorption spectra of **2** (···) and **5** (—) : (1) before irradiation; (2) after irradiation (60 min). Experiments were done in a pH 7 phosphate buffer containing 0.1 M NaCl for the oligonucleotide and in CH_3CN for the azobenzene unit.

The purity of these oligomers was verified by reversed phase HPLC (Table 1). The enzymatic digestion analysis showed the presence of the nucleic acid bases and the azobenzene unit in the oligomers.[15] The purified oligomers exhibited characteristic absorption bands at around 265 nm, 330 nm, and 450 nm. Based on the UV spectra of compound **2**,[11] the first absorption band is attributable to the nucleic acid bases and the latter two are due to the azobenezene fragment. The observed absorbance ratio of 265 to 330 nm is coincident with the calculated value based on the expected molar ratio of the azobenzene to the nucleic acid bases to be present in each synthesized oligomer as shown in Table 1. These observations indicate that the azobenzene unit was successfully incorporated into oligonucleotide chains without modification.

The UV-vis spectra of compound **2** and the oligonucleotide containing azobenzene **5** before and after irradiation with a high-pressure Hg lamp filtered to transmit light at 313 nm are shown in Figure 1. The absorption intensity at around 330nm decreased to similar extent for both compounds.[16] Under the dark condition, the initial spectra were regenerated completely. It is noted that the incorporated oligonucleotide segments did not affect the degree of photoisomerization of the azobenzene unit. These observations strongly suggest that the azobenzene linker would be useful for light switch for the conformational change of the oligonucleotides.

We have shown that the appropriately derivatized azobenzene can be used for incorporating into two oligonucloetide segments via the 5' hydroxyl function of one oligomer and the 3' hydroxyl group of the other. Study on the binding of oligonucleotides joined by the photoisomerizable linker to appropriate sequences in DNA or RNA is now in progress.

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REFERENCES AND NOTES

1) (a) Richardson, P.L.; Schepartz, A. *J. Am. Chem. Soc.* **1991**, 113, 5109. (b) Cload, S.T.; Schepartz, *A. J. Am. Chem. Soc.* **1991**, 113, 6324.

2) (a) Giovannangeli, C.; Montenay-Garestier, T.; Rougee, M.; Chassignol, M.; Thoung, N.T.; Helene, C. *J. Am. Chem. Soc.* **1991**, 113, 7775. (b) Giovannangeli, C.; Thoung, N.T.; Helene, C. *Proc. Natl. Acad. Sci. USA.* **1993**, 90, 10013.

3) (a) Kool, E.T. *J. Am. Chem. Soc.* **1991**, 113, 6265. (b) Prakash, G.; Kool, E.T. *J. Am. Chem. Soc.* **1992**, 114, 3523. (c) Salunkhe, M.; Wu, T.; Letsinger, R.L. *J. Am. Chem. Soc.* **1992**, 114, 8768.

4) Komatsu, Y; Koizumi, M.; Makamura, H.; Ohtsuka, E. *J. Am. Chem. Soc.* **1994**, 116, 3692.

5) (a) Letsinger, R.L.; Wu, T. *J. Am. Chem. Soc.* **1994**, 116, 811. (b) Letsinger, R.L.; Wu, T. *J. Am. Chem. Soc.* **1995**, 117, 7323.

6) Turro, N.J. "*Modern Molecular Photochemistry*" **1978**, 473, Benjamin/Cumming Publishing Co., Inc., CA, USA.

7) For recent example of light switch in the designed peptide, see: Ulysse, L.; Cubillos, J.; Chmielewski, J. *J. Am. Chem. Soc.* **1995**, 117, 8466.

8) Tomlinson, M.L. *J. Chem. Soc.* **1946**, 756.

9) By using a similar procedure, the synthesis of 4,4'-azobenzene di(*tert*-butyldimethylsiloxyethylamide) was also successful.

10) ^{1}H nmr (270 MHz in $CDCl_3$) of **1**: δ (ppm) = 0.05 (12H, s, Me_2Si-), 0.89 (18H, s, t-BuSi-), 1.46-1.71(12H, m, -$(CH_2)_3$-), 3.50 (4H, m, -CH_2N-), 3.64 (4H, t, -CH_2O-), 6.23 (2H, t, -NHCO-), 7.92-7.96 (total 8H, d, aromatic).

11) ^{1}H nmr (270 MHz in $CDCl_3$) of **2**: δ (ppm) = 0.03 (6H, s, Me_2Si), 0.87 (9H, s, t-BuSi), 1.47-1.71 (12H, m, -$(CH_2)_3$-), 3.44-3.49 (4H, m, -CH_2N-), 3.59-3.66 (total 4H, m, -CH_2OH & -CH_2OSi), 6.21 (2H, t, -NHCO-), 7.87-7.97 (total 8H, d, aromatic). λ_{max} (MeOH) of **2**: 330 nm ($\varepsilon = 33.2 \times 10^3$), 450 nm ($\varepsilon = 1.11 \times 10^3$).

This reaction also gave the diol derivative **2'** (R_1=R_2=H) in an isolated yield of 15 %. ^{1}H nmr (270 MHz in DMSO-d_6) of **2'**: δ (ppm) = 1.24-1.57 (12H, m, -$(CH_2)_3$-), 3.24-3.41 (total 8H, m, CH_2-OH & CH_2N), 7.97 (4H, d, aromatic), 8.01 (4H, d, aromatic), 8.64 (2H, t, -NHCO-).

12) ^{1}H nmr (270 MHz in $CDCl_3$) of **3**: δ (ppm) = 1.33-1.72 (12H, m, -$(CH_2)_3$-), 3.08 (2H, t, -CH_2ODMT), 3.49 (4H, m, -CH_2N-), 3.67 (2H, m, -CH_2OH), 3.77 (6H, s, CH_3O-), 6.34 (1H, t, -NHCO-), 6.51 (1H, t, -NHCO-), 6.81 7.19-7.44 (total 13H, m, aromatic of dimethoxytrityl), 7.85-7.89 (total 8H, d, aromatic of azobenzene)

13) Atkinson, T.; Smith, M. "*Oligonucleotide Synthesis: A Practical Approach*" **1984**, edited by Gait, M.J., 35, IRL Press, Oxford, UK.

14) The 0.2 mmol scale synthesis of oligonucleotide derivatives was carried out on a Pharmacia Gene Assembler Plus by using the standard protocol (0.2 mmol scale coupling) for normal deoxyribonucleoside phosphoramidites and the 1.3 mmol scale synthesis protocol for the amidite, **4**.

15) The modified-oligonucleotides (0.5 O.D.) were digested at 37°C for 2 h by Nuclease P1 and alkaline phosphatase in a buffer (pH 8.8) containing 50 mM tris-acetate (40 mL). The products were analyzed by reversed-phase HPLC by using the elution conditions described in the footnote of Table 1, b). In all the hydrolyzed products, the original oligomers **5-7** were not found. The nucleosides, dT (4.1 min) and dC (6.5 min), and the azobenzene diol **2'** (52.7 min) were observed as major products together with one minor product (48.5 min). Snake venom phosphodiesterase and alkaline phosphatase digestions of the oligomer **5-7** gave dT, dC, and the product appeared at a retention time of 48.5 min. In this case, the azobenzene diol was not observed. Based on these observations, we tentatively assign the peak at 48.5 min to be 5'T-L in which L indicates the azobenzene linker. We did not attempt to find an appropriate condition for complete hydrolysis of 5'T-L by using Nuclease P1.

16) Based on the assumption that the absorbance of the *cis* isomer at the employed wavelength (330 nm) is negligible, *tans/cis* ratio in the azobenzene **2** and the oligomer **5** was about 4:1 under the present photoirradiation conditions.

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