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# SYNTHESIS OF OLIGONUCLEOTIDE DERIVATIVES WITH PYRENE GROUP AT SUGAR FRAGMENT

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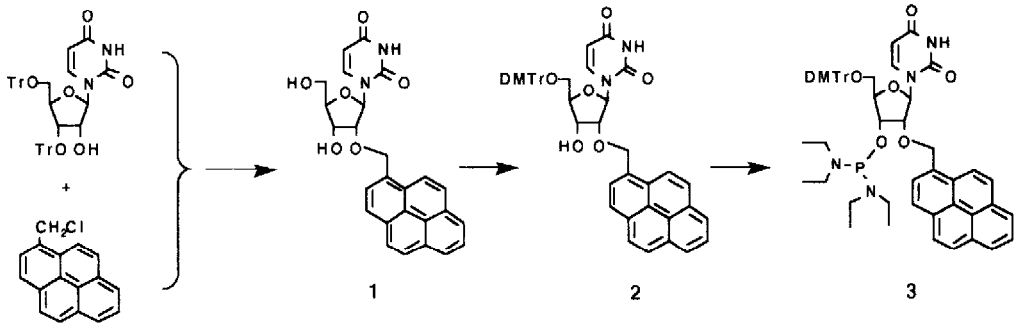
*Key Words: oligonucleotide-pyrene conjugate; specific sugar residue; 2'-(1-pyrenylmethyl)uridine; phosphorobisdiethylamidite; binding properties*

***Abstract:*** *The synthesis of oligonucleotide derivatives possessing pyrene group at a specific sugar residue has been described. The incorporation of the pyrene was accomplished by preparation of the pyrene-modified uridine, 2'-(1-pyrenylmethyl)uridine, which was then converted to the protected phosphorobisdiethylamidite. This reagent was used for the solid-phase synthesis of oligonucleotide-pyrene conjugates. The purification of the conjugates was effected by reversed-phase HPLC. The oligonucleotide-pyrene conjugates synthesized here can bind to their complementary sequences with a cooperative interaction between the pyrene group and the adjacent base-pairs.*

Oligonucleotide derivatives with an intercalating aromatic fragment have interest as gene probes[1,2] and as agents for inhibiting gene expression.[3] The synthesis of oligonucleotide-intercalator conjugates has been accomplished by linking oligonucleotides via linker arms to intercalating agents at an internal phosphorus,[1] a pyrimidine C-5[2] or a terminal position.[3] It has been shown that these conjugates have increased affinity for their complementary segments without losing specificity of recognition.[1-3] Owing to the flexible long linker, however, there are two possible base-pairs in the duplex for the intercalation of the aromatic fragment.[3a] On the other hand, we have recently developed the method for incorporating the intercalator, anthraquinone, via a relatively short linker into a specific sugar residue of oligonucleotides.[4] The advantage of our approach involves that the intercalating agent locates in between the designated base-pairs of the duplex. It is therefore expected that oligonucleotides with a fluorescent intercalator at a designated sugar residue would have some merits as non-radioactive fluorescent probes for detection of specific nucleic acid sequences. In this report, we describe the synthesis of oligonucleotides with a pyrenylmethyl substituent at a specific sugar residue.[5] Their binding properties with the complementary sequences are also elucidated in connection with nucleic acids probes.

**Scheme 1**




Tr: trityl
DMTr: 4,4'-dimethoxytrityl

The incorporation of the pyrene group into the sugar of oligonucleotides was initiated by preparation of the pyrene-modified uridine derivative, which was then converted to the protected phosphorobisdiethylamidite. This reagent was used for the solid-phase synthesis of oligonucleotide-pyrene conjugates. The synthesis of 5'-dimethoxytrityl 2'-(1-pyrenylmethyl)uridine 3'-phosphorobisdiethylamidite (**3**) is shown in Scheme 1. 3',5'-dirityluridine (730 mg, 1 mmol) was allowed to react in benzene-dioxane(7 ml-3 ml) with 1-pyrenylmethyl chloride[6] (280 mg, 1.1 mmol) in the presence of potassium hydroxide (500 mg) under refluxed condition for 2 h. It should be noted that this reaction afforded the desired 2'-modified uridine without any side product such as $N^3$-alkylated product.[7] The successive treatment with 80% acetic acid at 100°C for 30 min gave 2'-(1-pyrenylmethyl)uridine, U(pyr) (**1**)[8] (294 mg, 65 %). U(pyr) was treated with 4,4'-dimethoxytrityl chloride (DMTr-Cl) in dry pyridine at r.t. for 2 h, giving DMTr-U(pyr) (**2**)[9] in 75 % yield. Nucleoside **2** was then phosphitylated by bis(diethylamino)phosphorochloridite in a usual manner, yielding 5'-DMTr-U(pyr) phosphorobisdiethylamidite (**3**).[4, 10, 11]

The utility of the 5'-DMTr-U(pyr) 3'-phosphorobisdiethylamidite **3** was demonstrated in the solid-phase synthesis of the oligonucleotides possessing pyrene group at the specific sugar residue, dU(pyr)CTAGAGG (**4**) and dU(pyr)CGAGTCTAGAGG (**5**). The synthesis began with 5'-DMTr-G (0.2 μmol) bound CPG. The fully protected dDMTr-CTAGAGG-CPG and dCGAGTCTAGAGG-CPG were synthesized by using deoxyribonucleoside 3'-(ß-cyanoethyl N-diisopropyl)phosphoramidite reagents on automated DNA synthesizer (Applied Biosystems Model 391-02). After deprotection of dimethoxytrityl group, the introduction of U(pyr) into the oligonucleotide sequences

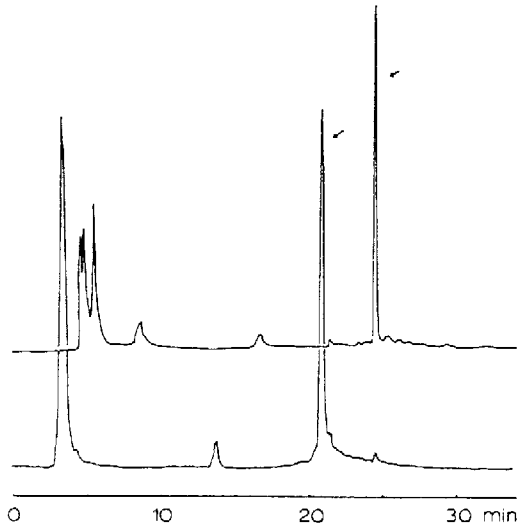



Figure 1. HPLC(YMC-pack C18, 0.6x15 cm) of crude dU(pyr)CTAGAGG (**4**) (upper) and dU(pyr)CGAGTCTAGAGG (**5**) (lower). Both elutions were carried out by a linear gradient of $CH_3CN$ from 10 % to 50 % (40 min) in 0.05 M TEAA (pH 7) at a flow rate of 1.0 ml/min. Each peak corresponding to the desired oligonucleotide is shown by an arrow.

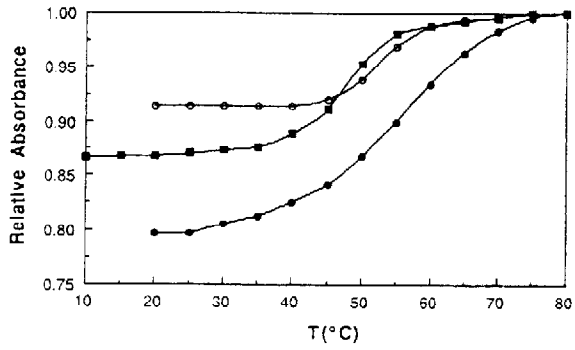



Figure 2. UV melting curves for duplexes of dU(pyr)CGAGTCTAGAGG (**5**) [○260 nm;● 345 nm] and dTCGAGTCTAGAGG [■ 260 nm] with dCCTCTAGACTCGACCT at a common total strand concentration of $6.0 \times 10^{-6}$ M. The buffer used contained 0.1 M NaCl and 0.01 M sodium phosphate, adjusted to pH 7.0.

Table 1. Melting temperatures(tms) for the duplexes of oligonucleotide-pyrene conjugates with their complementary sequences.

| duplex | tm(°C) |
|---|---|
| dU(pyr)CTAGAGG **4** + dCCTCTAGAGTCGACCT[1] | 22.5 |
| dTCTAGAGG + dCCTCTAGAGTCGACCT[1] | 22.5 |
| dU(pyr)CGAGTCTAGAGG **5**+ dCCTCTAGACTCGACCT[1] | 52.5 |
| dTCGAGTCTAGAGG + dCCTCTAGACTCGACCT[1] | 47.5 |
| dU(pyr)TTTTTTT **6** + Poly A[2] | 13.5 |
| dTTTTTTTT + Poly A[2] | 12.5 |
| dTTU(pyr)TTTTTTT **7** + Poly A[3] | 18.4 |
| dU(pyr)TTTTTTTTT **8** + Poly A[3] | 25.1 |
| dTTTTTTTTTT + Poly A[3] | 23.5 |

All measurements were carried out for oligonucleotide solutions containing 1:1 complementary bases in 0.1 M NaCl and 0.01 M sodium phosphate (pH 7). Tm values were obtained from the absorbance change at 260 nm. [1]Total strand concentration was $6.0 \times 10^{-6}$ M. [2]Total nucleotide concentration was $2.0 \times 10^{-4}$ M. [3]Total nucleotide concentration was $6.6 \times 10^{-5}$ M.

was carried out by the manual coupling (15 min) using a solution (0.3 ml) of **3** (0.4 M in $CH_2Cl_2$) activated with an equal volume of 0.2 M of 4-(4'-nitrophenyl)tetrazole in $CH_3CN$. The DMTr cation measurements showed the coupling efficiency of 80 % for U(pyr) in the both syntheses. The lower coupling efficiency probably attributes to the bulky pyrene group at the 2'-position. The CPG-bound oligonucleotide-pyrene conjugates **4** and **5** were treated with concentrated ammonium hydroxide at 55°C for 12 h. Reversed-phase HPLC profiles of the deprotected oligomers **4** and **5** are shown in Figure 1. As can be seen in the Figure, the pyrene-modified oligonucleotide can be separated very well from the failed sequences because of hydrophobicity of the pyrene group. The purification of both oligomers **4** and **5** was thus effected by the reversed-phase HPLC, affording 2.6 and 4.6 A260 units, respectively. The purified oligonucleotides were subjected to digestion with snake venom phosphodiesterase and alkaline phosphatase.[4] Reversed-phase HPLC analyses[12] of the reaction mixtures gave deoxyribonucleosides and U(pyr) in an expected molar ratio of dC:dA:T:dG:U(pyr)=1:2:1:3:1 for oligomer **4** and 2:3:2:5:1 for oligomer **5**. The synthesis of oligonucleotide-pyrene conjugates with other sequences, dU(pyr)TTTTTTT (**6**), dTTU(pyr)TTTTTTT (**7**), and dU(pyr)TTTTTTTTT (**8**), were similarly successful.

Interactions of the oligonucleotide-pyrene conjugates with their complementary sequences in aqueous solution were investigated spectrophotometorically. Figure 2 shows the UV melting curves for the duplexes of oligomer **5** and the unmodified oligodeoxyribonucleotide dTCGAGTATAGAGG formed with the complementary oligodeoxyribonucleotide 16-mer(dCCTCTAGACTCGACCT). The shapes of the profiles both at 260 nm and 350 nm for duplex **5** exhibit sigmoidal curves similar to that for the unmodified oligomer, indicating that the oligonucleotide-pyrene conjugate binds to the complementary sequence by Watson-Crick base-pairing. It is also suggested that interaction of the pyrene group between the adjacent base-pairs occurs in a cooperative manner with the base-pairing. Similar melting behaviors were observed for all the duplexes of oligonucleotide-pyrene conjugates studied here. Analyses of the melting curves provide the melting temperature (tm) values which are listed in Table1. Inspection of the tm values reveals that the introduction of the pyrene group at the 5'-end sugar residue of oligonucleotides slightly increases the duplex melting temperatures relative to the corresponding unmodified oligomers. It should be emphasized that the binding properties of the present oligonucleotide-pyrene conjugates indeed satisfy design criteria for a fluorescent nucleic acids probe.

A new and general procedure has been developed for introduction of a pyrene group into a specific sugar residue of oligonucleotide sequences. The oligonucleotide-pyrene conjugates were easily synthesized by use of the pyrene-modified uridine phosphorobisamidite on a solid-support and purified by a usual reversed-phase HPLC. The pyrene conjugates binds to their complementary sequences in aqueous solution to form duplexes with a normal thermal stability. The pyrene interacts, probably by a modified intercalation, with the adjacent base-pairs in a cooperative manner with the duplex formation. This interaction would affect the fluorescence properties of the pyrene group. Preliminary measurements showed that the fluorescence of oligonucleotide-pyrene conjugates considerably increased upon binding to the complementary segments. The details of the fluorescence properties related to the pyrene interaction will be described elsewhere.

**Acknowledgment**

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5. The incorporation of the pyrenylmethyl substituent into specific bases of oligodeoxyribonucleotides has been reported: Lee, H., Hintz, M., Stezowski, J.J., and Harvey, R.G. (1990) *Tetrahedron Lett. 31*, 6773.
6. 1-Pyrenylmethyl chloride (mp 144-145°C; Anal. Calcd for $C_{17}H_{11}Cl$: C, 81.44; H, 4.42; Cl, 14.14. Found: C, 81.55; H, 4.43; Cl, 13.62) was synthesized as follows: 1-pyrene carboxyaldehyde was treated with $NaBH_4$ in DMF, giving 1-pyrenylmethanol in a yield of 83 %. The alcohol was allowed to react with $SOCl_2$ in benzene, yielding the chloride(84 %).
7. This was confirmed by $^1H$ NMR analysis. The analogous reaction of 3',5'-dirityluridine with benzyl chloride gave only 2'-benzylated uridine derivative: Reese, C.B., and Trentham, D.R. (1966) *Tetrahedron Lett.*, 4349.
8. compound **1**: mp 133-135°C; TLC(silica $60F_{254}$, $CH_2Cl_2$-MeOH 9:1 v/v) Rf 0.5; Anal. Calcd for $C_{26}H_{22}O_6N_2 \cdot H_2O$ : C, 65.54; H, 5.08; N, 5.88. Found: C, 65.30; H, 4.88; N, 5.68. $^1H$ NMR(400 MHz, DMSO-$d_6$) δ 3.61 (m, 2H, $H_{5'}$) δ 3.97 (m, 1H, $H_{4'}$) δ 4.19 (dd, 1H, $H_{2'}$) δ 4.29 (ddd, 1H, $H_{3'}$) δ 5.38 (d, 2H, $J_{gem}$=11.72 Hz, $ArCH_2$) δ 5.46 (d, 1H, uracil $H_5$) δ 6.04 (d, 1H, $J_{1',2'}$=4.37 Hz, $H_{1'}$) δ 7.82 (d, 1H, uracil $H_6$) δ 8.1-8.4 (m, total 9H, ArH).
9. compound **2**: mp 138-139.5°C; TLC(silica $60F_{254}$, $CH_2Cl_2$-MeOH 20:1 v/v) Rf 0.65; Anal. Calcd for $C_{46}H_{40}O_8N_2$: C, 73.78; H, 5.38; N, 3.74. Found: C, 73.72; H, 5.24; N, 3.46. $^1H$ NMR(400 MHz, $CDCl_3$) δ 3.46 (m, 2H, $H_{5'}$) δ 3.76 (s, 6H, $CH_3O$ of DMT) δ 4.03 (m, 1H, $H_{4'}$) δ 4.16 (dd, 1H, $H_{2'}$) δ 4.29 (dd, 1H, $H_{3'}$) δ 5.09 (d, 1H, uracil $H_5$) δ 5.53 (d, 2H, $J_{gem}$=11.72 Hz, $ArCH_2$) δ 6.14 (d, 1H, $J_{1',2'}$= 2.44 Hz, $H_{1'}$) δ 6.8-7.3 (m, total 13H, ArH of DMT) δ 7.80 (d, 1H, uracil $H_6$) δ 8.0-8.4 (m, total 9H, ArH of pyrene).
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11. Yamana, K., Nishijima, Y., Negishi, K., Yashiki, T., Nakano, H., and Sangen, O. (1991) *Tetrahedron Lett.*, in press.
12. Analyses of deoxyribonucleosides were performed as described before (ref.4). U(pyr) was detected on a YMC-pack $C_{18}$ column(0.6x15 cm) eluting with 40 % $CH_3CN$ in 0.05 M TEAA (pH 7) at a flow rate of 1.0 ml/min. Retention time of U(pyr) was 8.5 min.

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