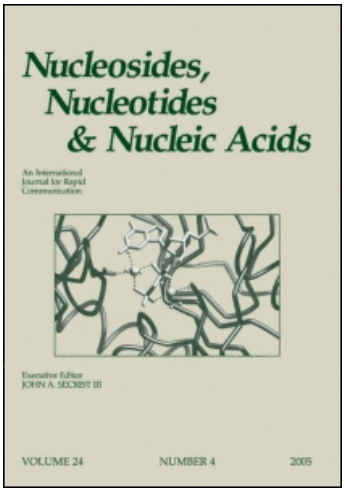

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Synthesis and Properties of Oligonucleotides Involving a Perylene Unit Linked to a 2'-Deoxyribose Residue

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Synthesis and Properties of Oligonucleotides Involving a Perylene Unit Linked to a 2′-Deoxyribose Residue

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ABSTRACT

We report here the synthesis and binding properties of oligonucleotides involving a perylene unit linked to the anomeric position of a 2′-deoxyribose residue. Both anomers were separated and incorporated separately at either the 5′-end or the internal position of a pyrimidine sequence. In any case the presence of the perylene unit stabilizes the complexes formed with either the single or the double-stranded target.

Key Words: Oligonucleotide-perylene conjugates; Perylene-2′-deoxyribose unit; Synthesis; Binding properties.

Modifications developed to increase the affinity of ''antisense'' and ''antigene'' oligonucleotides for their targets include the covalent coupling of intercalating agents. We have previously reported that a perylene derivative involving five fused rings linked to the 5′- or the 3′-end of a pyrimidine decamer stabilizes duplex and triplex structures.[1] In order to further explore the possibility of increasing the stability of the triplex and duplex structures we decided to incorporate the perylene

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residue at the internal position of the oligonucleotide sequence. The covalent attachment of a ligand to an internucleotidic phosphate induces the formation of two stereoisomers with different properties. Following this method, linking the ligand to any position of the sequence requires the preparation of sixteen pairs of dinucleosides and the separation of the isomers before their incorporation inside the sequence. In order to reduce the chemical work, we used another strategy based on the linkage of the perylene moiety to the anomeric position of a 2′-deoxyribose residue.

The synthesis of the sugar-perylene unit was achieved as follows: first, the perylene was monobrominated by reaction with N-bromosuccinimide,[2,3] then a Sonogashira[4] reaction between the brominated perylene and propargyl alcohol gave the perylene with a hydroxylated linker. The latter was then glycosylated by reaction with 2′-deoxy-3,5-di-O-(4-toluoyl)-α-D-erythro-pentofuranosyl chloride to give the perylene sugar unit as the anomer mixture. The α- and β-anomers were separated by chromatography and after removal of the *p*–toluoyl groups and dimethoxytritylation of the 5′-hydroxyl function they were transformed into their H-phosphonate derivatives by using the 2-chloro-5,6 benzo 1,3,2-dioxaphosphorin-4-one.[5] Monoincorporation of each anomer was then performed at either the 5′-end or internal position of a pyrimidine sequence (Fig. 1). The chain assembly was carried out at a 1 μmole scale and the perylene-sugar unit was incorporated manually. After the chain assembly (trityl-off mode), deprotection was performed by treatment of the modified oligonucleotides bound to the support with concentrated NH_4OH solution overnight at room temperature. After removal of the ammonia, extraction of the organic impurities and filtration the crude modified oligonucleotides were analyzed and purified by reversed-phase chromatography and characterized by electrospray mass spectrometry.

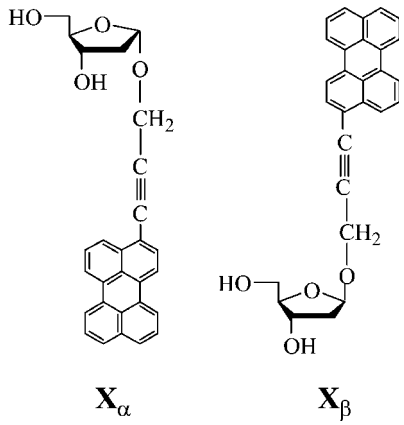
The binding properties of the modified oligonucleotides with the double-stranded DNA target $^{5'}$TAGTTTCTCTTCTTTTTCTTCTCTT$^{3'}$/$^{3'}$ATCAAAGAG-AAGAAAAAGAAGAGAA$^{5'}$ (circularized by using two hexaethylene linkers[6] in order to increase its stability) and the single-stranded complementary DNA target

$^{5'}$TTCTTTTTCT**X**$_\alpha$TCTCT$^{3'}$ **1**

$^{5'}$TTCTTTTTCT**X**$_\beta$TCTCT$^{3'}$ **2**

$^{5'}$**X**$_\alpha$TTCTTTTTCTTCTCT$^{3'}$ **3**

$^{5'}$**X**$_\beta$TTCTTTTTCTTCTCT$^{3'}$ **4**



X$_\alpha$ **X**$_\beta$

Figure 1. Structures of the modified oligonucleotides.

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$^{5'}$CTCAGAGAAGAAAAAGAACTC$^{3'}$ were studied by absorption spectroscopy. Molar extinction coefficients of the oligonucleotide-perylene conjugates **1**, **2**, **3** and **4** were determined by titration of conjugate solutions at 3°C with a solution of the single-stranded complementary sequence. Molar extinction coefficients of the targets and unmodified oligonucleotide $^{5'}$TTCTTTTTCTTCTCT$^{3'}$used as reference were determined according to the literature.[7] In the case of the triplexes, the experiments were performed with a 1 μM concentration in the circularized double-stranded target and a 1.5 μM concentration in the third oligonucleotide in a 10 mM cacodylate buffer, pH 7, containing 140 mM KCl and 5 mM $MgCl_2$. In the case of the duplexes, a 1 μM concentration in oligonucleotides (each strand) was used in a 10 mM sodium cacodylate buffer, pH 7, containing 100 mM NaCl. Duplex and triplex stabilities were determined by thermal denaturation. One transition was observed in the melting profile of each duplex while two transitions were observed in the melting of each triplex. The transition with the higher Tm corresponds to the melting of the target duplex (around 77°C for all complexes) and the transition with the lower Tm to the dissociation of the third strand. The stabilization observed for the duplexes was nearly equivalent when the incorporation was performed at the internal position (ODNs **1** and **2**) or at the 5′-end (ODNs **3** and **4**) of the sequence and no important difference was observed with either of the anomers (Δ Tm = + 6-8°C). In the case of the triplexes, the strongest stabilization was observed when the perylene unit was attached to the 5′-end of the third strand (ODNs **3** and **4**) (Δ Tm = + 12 and 11°C, respectively). When the incorporation was performed at the internal position (ODNs **1** and **2**), the stabilizing effect was weaker (Δ Tm = + 8°C with each anomer).

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