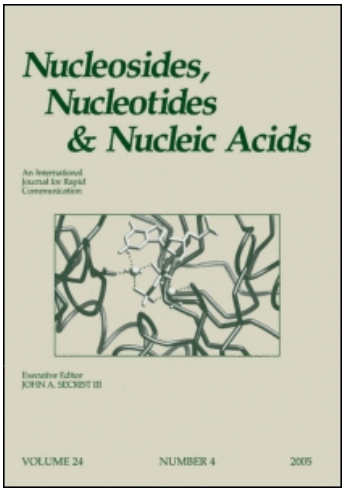

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Perylene-Labeled Oligonucleotide as a Probe in Homogeneous Hybridization Assay

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PERYLENE-LABELED OLIGONUCLEOTIDE AS A PROBE IN HOMOGENEOUS HYBRIDIZATION ASSAY

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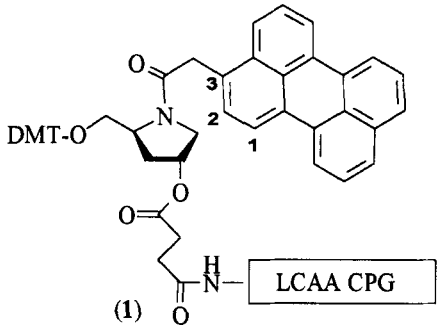
ABSTRACT: An oligodeoxynucleotide bearing a 3′-terminal perylene-containing pseudonucleoside unit was synthesized and used as a probe in homogeneous hybridization. The fluorescence anisotropy of the perylene dye rose upon hybridization of the modified conjugate with the complementary nucleotide sequence. These results provide for designing efficacious hybridization probes.

Unlike a variety of common fluorophores, little experimental data are available on the employment of the perylene dye in the structural studies of nucleic acids,[1,2] apparently, because of problems with its regiospecific derivatization and introduction into DNA and strongly hydrophobic character of its molecule. On the other hand, the low lifetime of the perylene excited state provides for an elevated quantum yield and favors the registration of the effects associated with the fluorescence anisotropy.

To introduce a perylene residue into a synthetic oligonucleotide, we synthesized a perylene-containing pseudonucleoside unit using the acylation of a hydroxyprolinol ((3*R*,5*S*)-3-hydroxy-5-hydroxymethylpyrrolidine)[3] with pentafluorophenyl 3-perylene-acetate (cf. the synthesis of an analogous pyrene compound[4]). This nucleoside analogue was coupled with a succinylated aminoalkyl CPG to give the corresponding support **1** (**FIG. 1**). Reagent **1** ensures the effective introduction of a perylene residue into the 3′-terminal position of oligonucleotides in the course of the automated nucleic acid synthesis (we also described the introduction of a hexamethylenediamine-tethered 3-peryleneacetyl residue into the oligonucleotide 5′ terminus[2]).

We showed that such conjugates can be used in the homogeneous hybridization detection. Thus, the fluorescence anisotropy of the oligomer **2** carrying a 3′-terminal perylene-containing unit increased when the nonmodified complementary oligomer **3** was added and the corresponding duplex formed (**FIG. 2**, curve 1) but did not change if the noncomplementary nucleotide **4** was added (curve 2). Apparently, in this case the hybridization makes the rotational mobility of the perylene residue lower, thus diminishing the depolarization of its fluorescence.



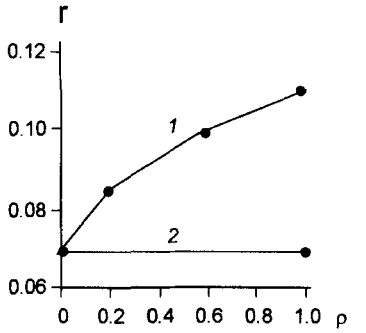


FIG. 1. Perylene-containing CPG-support (1). DMT, 4,4′-dimethoxytrityl; LCAA CPG, long chain aminoalkyl controlled pore glass. Left

FIG. 2. Changes in the fluorescence anisotropy (r) of conjugate 5′TTACGCTTTCCT-**Prl** (**2**) upon addition of either complementary 15-mer 5′ACGAGGAAAGCGTAA (**3**) (curve 1) or noncomplementary 30-mer 5′GTGTCCAACCTTGGCGGGGATCCTGGACAT (**4**) in water; λ_{ex} 420 nm, λ_{em} 475 nm; concentration of (**2**) was 10^{-7} M. **Prl** is the hydroxyprolinol-based pseudonucleoside unit; ρ is the nonmodified to modified oligomer molar ratio. Right

Based on these results, convenient oligo- and polynucleotide probes may be designed suitable for the specific detection of nucleic-nucleic and protein-nucleic interactions in solution.

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