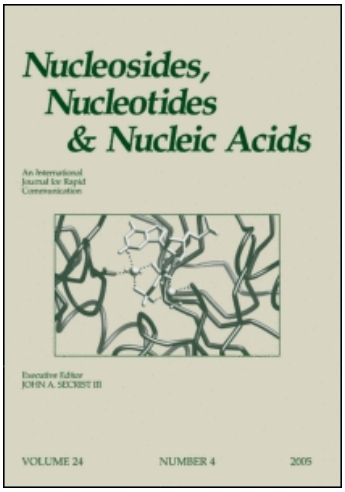

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A Pyrene *Seco*-Pseudonucleoside in Constructing Interaction-Sensitive Fluorescent DNA Probes

Vladimir A. Korshun[a]; Konstantin V. Balakin[a]; Tatyana S. Proskurina[a]; Ilya I. Mikhalev[a]; Andrei D. Malakhov[a]; Yuri A. Berlin[a]
[a] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, GSP-7 Moscow, Russia

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A PYRENE *SECO*-PSEUDONUCLEOSIDE IN CONSTRUCTING INTERACTION-SENSITIVE FLUORESCENT DNA PROBES

Vladimir A. Korshun, Konstantin V. Balakin, Tatyana S. Proskurina, Ilya I. Mikhalev, Andrei D. Malakhov and Yuri A. Berlin*

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, GSP-7 Moscow, 117871, Russia

(Fax: +7 095 335 7103; E-mail: yuber@ibch.siobc.ras.ru)

ABSTRACT: Based on 4-(1-pyrenyl)-1,3-butanediol, a pyrene *seco*-pseudonucleoside, we prepared a nonnucleotide phosphoramidite reagent and a pyrene-modified LCAA-CPG-support and employed them in solid-phase synthesis of pyrene-labeled oligonucleotides with varying numbers and positions of the pseudonucleoside residues. Changes in the fluorescence intensity and the spectral curve shape upon hybridization were observed that depended on the position(s) of the modified unit(s) in the probe.

Introduction

Covalent pyrene–nucleic acids conjugates were first synthesized more than twenty years ago,[1] and a variety of techniques for their preparation have been developed ever since. Several nucleoside-based[2–6] and non-nucleoside[7-12] phosphite reagents were designed for automated synthesis of pyrene-modified oligonucleotides. Reactive pyrene derivatives were used for covalent binding to aliphatic aldehyde-,[1,13,14] amino-,[15–18] and thiol-[19–21] modified nucleic acids or to terminal phosphorothioate[13] and phosphate[22] groups. A polyamide moiety carrying up to five pyrene residues was introduced into the 3′ end of an oligonucleotide.[23]

Noncarcinogenic pyrene, owing to its structural relation to benzo[*a*]pyrene, a potent mutagen and carcinogen, was used as a model for the nucleoside and oligonucleotide modification.[4] The hydrophobic pyrene residue was postulated to be a promising oligonucleotide modification for the antisense application.[7] Pyrene nucleoside analogues in dangling positions were shown considerably to stabilize DNA duplexes,[24] and 5-methyl-N^4-(1-pyrenylmethyl)-dC as an oligonucleotide constituent also can stabilize duplexes, triplexes, and three-way junctions.[6]

The interactions of nucleic acids with pyrene,[25,26] pyrene derivatives,[27] and covalently bound pyrene residues[2,3,7,11-14,16,23] were studied by various spectroscopic techniques. It is noteworthy that pyrene is able not only to intercalate into double-stranded DNA but also to have external binding sites and, apparently, to stack with terminal nucleic bases. Nevertheless, the mode of interaction of pyrene residues with ds- and ss-DNA is far from being completely understood.

The pyrene fluorophore has a rather long lifetime of the excited state and can form excimers,[28] which makes this probe sensitive to changes in microenvironment accompanied with an alteration in the spatial accessibility of the fluorophore for the quenchers present in the solution. The changes in the fluorescence spectrum of a single pyrene label were used for the homogeneous monitoring of nucleic acid hybridization[11,15,23,29-32] or RNA–enzyme interactions.[33] Two or more pyrene residues attached to oligonucleotides exhibited excimeric fluorescence and enabled changes in the intensity and excimer-to-monomer ratio to be used in detecting duplex formation.[11,13,14,16,23,34]

Pyrene-polylabeled oligonucleotides exhibited an increased fluorescence emission intensity in visible region relative to the monolabeled compounds[23] and can be used in place of DNA probes bearing common fluorescent dyes (e.g., fluorescein) prone to self-quenching.[35] The hybridization properties of the oligonucleotides were not significantly impaired upon their conversion to the polylabeled conjugates.[23] At the same time, the technique described for their synthesis[23] is rather intricate, combining the modified peptide and oligonucleotide syntheses, and allows only 3′ terminal labeling.

An oligonucleotide-attached pyrene fluorophore was also used as a donor or quencher for other fluorescent molecules[36] and as sensitizer for the complementarily addressed DNA modification with an aryl azide–oligonucleotide conjugate.[22]

In this paper we describe a facile synthesis of a new pyrene non-nucleotide phosphoramidite and a pyrene-modified support for solid-phase oligonucleotide synthesis, and properties of several conjugates containing one or more pyrene residues attached to the 3′ or 5′ ends of oligonucleotides. Some hybridization-dependent fluorescent properties of pyrene-labeled oligonucleotides were reported recently.[37] For preliminary communications, see.[38,39]

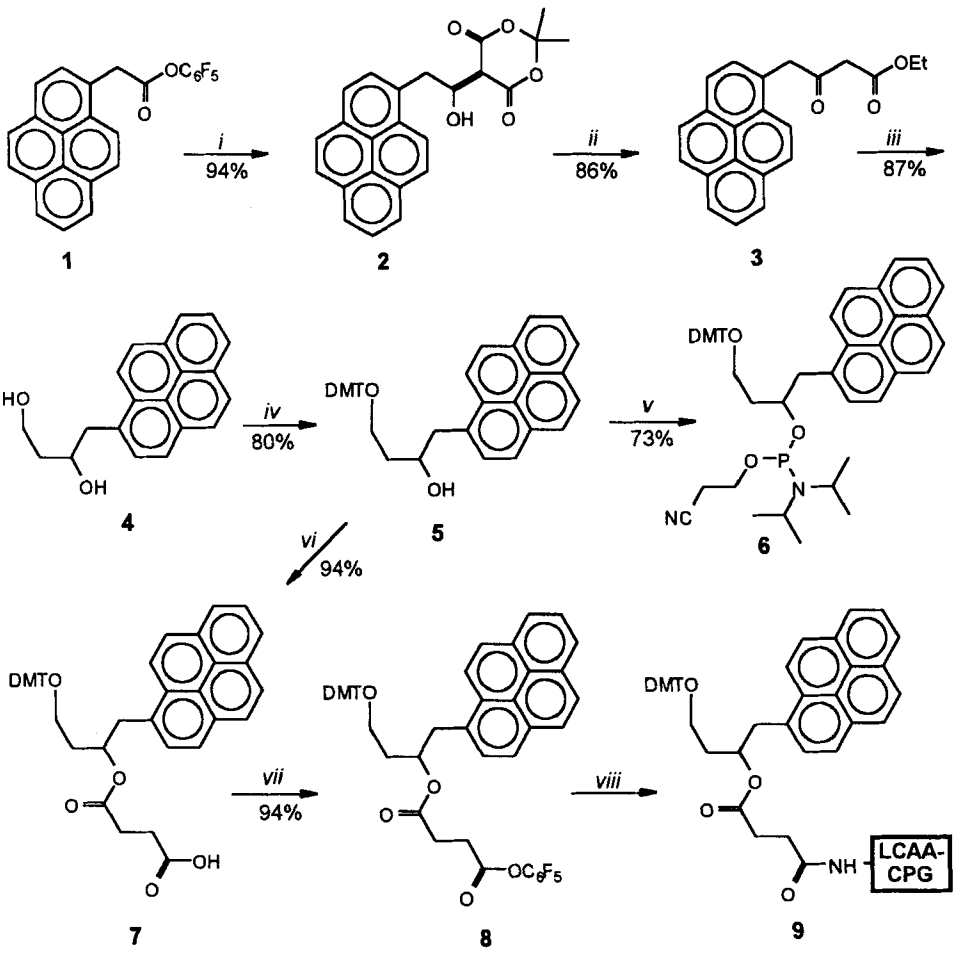
Results

The starting pentafluorophenyl 1-pyreneacetate **1**[16] was employed as an activated component in the Yonemitsu method of the β-ketoester synthesis[43] (**Scheme**). The acylation of isopropylidene malonate (Meldrum's acid) gave enol **2**, which was ethanolized in the presence of trifluoroacetic acid to yield ester **3** (crystallization afforded an individual keto form as follows from the presence of two two-proton singlets at 4.54 and 3.43 ppm in the ^{1}H NMR spectrum). Its reduction with $NaBH_4$ in THF/MeOH[44] gave the key pseudonucleoside **4** in 87% yield. Diol **4** was mono-dimethoxytritylated with DMTCl in pyridine at the primary hydroxyl group, and the resulting alcohol **5** was phosphitylated with bis(N,N-diisopropylamino)-2-cyanoethoxyphosphine to give phosphoramidite **6** as a mixture of diastereomers. Alternatively, alcohol **5** was acylated by succinic anhydride to give the acid succinate **7**, which was transformed into the activated ester **8**. This compound was used to attach a pyrene-containing synthon to aminoalkylated CPG to give solid support **9**.

A series of modified oligodeoxynucleotides (**10–20**) containing one to five pyrene pseudonucleotide units were synthesized using the conventional phosphoramidite approach. For reagent **6**, the coupling yields were higher than 98% (determined by the released trityl cation). The crude oligomers were purified by PAGE. One major fluorescent band was observed for each oligomer under UV light (data not shown).

The reverse-phase HPLC analysis showed an anticipated increase in the retention time values of the modified oligomers as the number of the pyrene residues grew: thus, for the unmodified oligomer **21** and mono-, di-, and tripyrenylated conjugates **10**, **11**, and **7**, the respective retention time values are 8.5, 12.8, 18.7, and 25.6 min (Fig. 1). The chromatographic mobility of the pentapyrenylated conjugate **12** (rt 55 min, data not shown) was lower than expected, which may be due to conformational effects resulting, for example, from an increased aggregation of highly hydrophobic pyrenyl moieties.

CE analysis revealed a distinct retention time value for each of the conjugates. It turned out that every pyrene pseudonucleotide unit contributes to the electrophoretic



Scheme. *Reagents and conditions*: *i*, isopropylidene malonate/Et_3N/THF; *ii*, CF_3CO_2H/EtOH, reflux; *iii*, $NaBH_4$/MeOH/THF, reflux; *iv*, DMTCl/pyridine, 0°C; *v*, $(Pr^i_2N)_2POCH_2CH_2CN$/diisopropylammonium tetrazolide/MeCN; *vi*, succinic anhydride/DMAP/pyridine; C_6F_5OH/DCC/EtOAc/pyridine; *vii*, LCAA-CPG/Et_3N/$CHCl_3$.

retardation of an oligonucleotide approximately as much as a natural nucleotide unit does (Fig. 2).

The UV spectra of the pyrenylated conjugates are presented in Fig. 3. Monopyrenylated conjugate **10** has three absorption maxima at 260, 335, and 350 nm. As the number of pyrenes in the conjugate increases, a fourth peak at 247 nm becomes significant. The presence of pyrene residues in the conjugate does not substantially distort

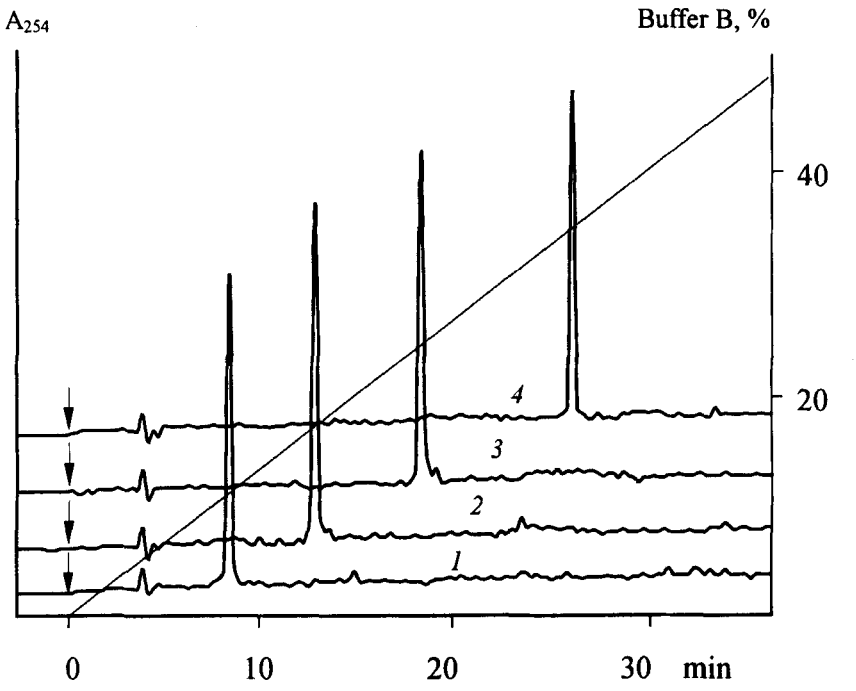


Figure. 1. Analytical reverse-phase HPLC of PAGE-purified unmodified olgonucleotide **22** (*1*) and labeled conjugates: monopyrenylated **10** (*2*), dipyrenylated **11** (*3*), and tripyrenylated **17** (*4*). Arrows indicate the moments of injection.

the UV spectrum around 260 nm so that these oligonucleotides can be quantitated spectrophotometrically (cf. ref. 23).

The incorporation of one or two pyrene moieties into a terminal dangling position of a duplex increased its T_m by 2.5÷3.5°C (duplexes and their T_m, °C: **(21)·(22)**, 48.5±0.5; **(10)·(22)**, 51±0.5;. **(23)·(24)**, 63±0.5; **(23)·(16)**, 66.5±0.5; **(15)·(24)**, 66.2±0.5). These data show that two such moieties exhibit essentially equal stabilizing effects on the duplex regardless of to which end (5′ or 3′) of the strand they are attached (duplexes **(15)·(24)** and **(23)·(16)**).

The fluorescence excitation spectra of the conjugates described in this paper are very similar, with maxima at 243 and 350 nm (data not shown). In the emission spectra of mono- and dipyrenylated conjugates **10** and **11**, only two peaks, at 380 and 400 nm, were observed, corresponding to pyrene monomer emission (Fig. 4). In the tripyrenylated conjugate **17**, excimer fluorescence at 460 nm became significant (data not shown). As

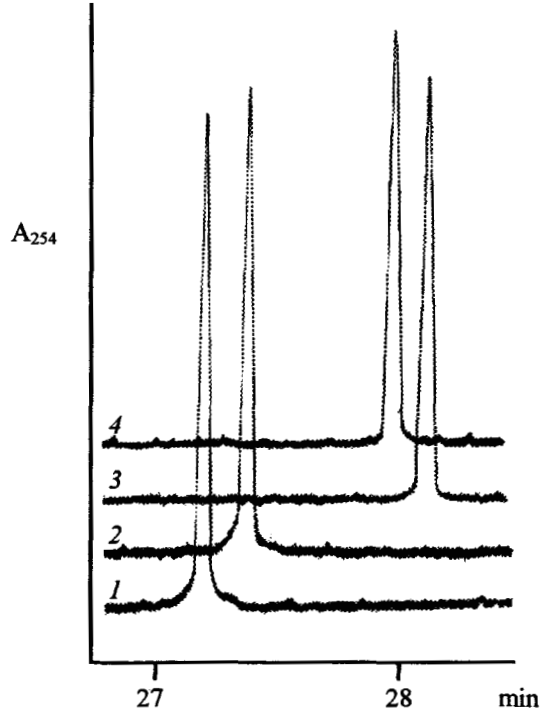


Figure. 2. Capillary electrophoresis profiles of modified oligonucleotides: *(1)* 16-mer **10**, *(2)* 17-mer **11**, *(3)* 22-mer **15**, *(4)* 23-mer **17**.

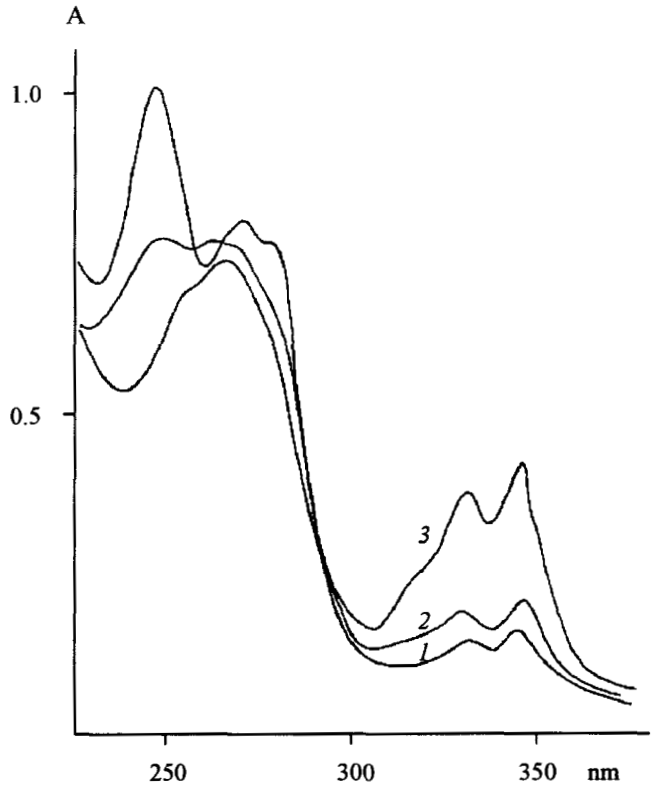


Figure. 3. UV spectra of PAGE-purified modified oligonucleotides in aqueous solution: *(1)* monopyrenylated oligo **10**, *(2)* dipyrenylated oligo **11**, *(3)* pentapyrenylated oligo **12**. Concentrations 10^{-6} M.

expected, the excimer-to-monomer fluorescence ratio rose considerably with an increasing number of the pyrene residues in the conjugate. A dramatic increase in excimer fluorescence was observed for the pentapyrenylated conjugate **12** (Fig. 4). The hybridization-dependent emission of the modified conjugates was also evaluated upon examination of some schemes of homogeneous hybridization analysis: while no considerable response was observed in complexes **15·16, (10+13)·25,** and **(10+14)·25,** and a fourfold rise in the monomer emission, albeit without excimer effect, was detected for the lateral loop-containing complex **20·25,** an intense excimer emission was revealed upon hybridization of internally bipyrenylated oligonucleotides **18** with the nonmodified complementary oligonucleotide **22** or its internally bipyrenylated analogue **19**.[37]

The emission spectrum of pentapyrenylated conjugate **12** was found to be strongly influenced by the methanol concentration: when it increased from 20 to 50%, the excimer fluorescence intensity rose by an order of magnitude and the excimer-to-monomer fluorescence ratio from 1 to 5 (Fig. 5).

Discussion

The detection of nucleic acids by nonradioactively labeled probes can be performed with immobilized analytes on blots, *in situ*, or in solution. The last way allows one to skip the analyte or probe immobilization: not only is this step time-consuming but, more important, the specifity of the molecular recognition on a mixed phase (in heterogeneous conditions) may drop. Recently, several formats of homogeneous analysis were developed predominantly based on the use of the resonance energy transfer.[45,46] Other principles involve the microenvironment-sensitive fluorophores such as pyrene.[11,13-16,23,29-32] The key problem in all the above cases is the preparation of fluorescently labeled oligonucleotides.

In the course of our studies on the DNA–pyrene interactions, we needed and had to design a technique for introducing a pyrene moiety into synthetic oligonucleotides. The current methods were not fully satisfactory because they did not provide a reliable way to oligonucleotides containing various numbers of pyrenes in predetermined positions.

The synthetic scheme proposed may be useful for the synthesis of various polyaromatic fluorophores with a 1,3-diol group and their subsequent introduction into synthetic oligonucleotides. A similar approach was used for preparing hapten phosphoramidites.[47]

Compound **4** contains a pseudosugar backbone whose primary and secondary hydroxyls, divided by a three-carbon-atom chain, mimic the 5′,3′-dihydroxy moiety of

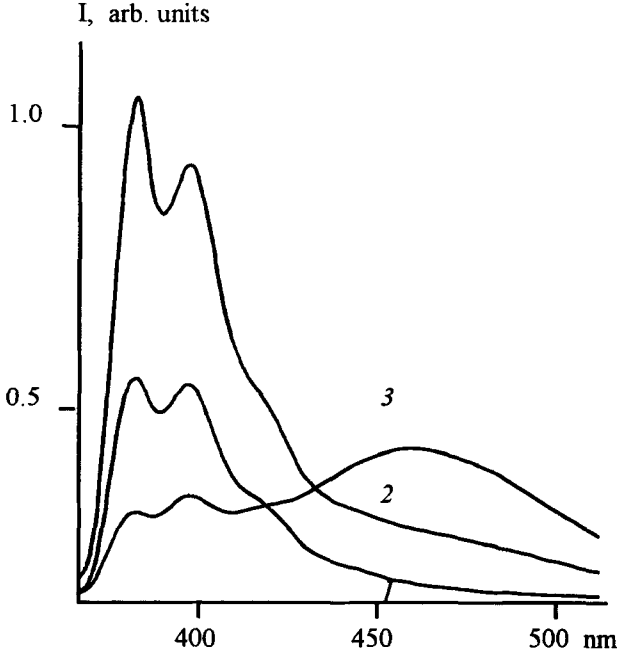


Figure. 4. Fluorescence spectra of conjugates in aqueous solution: *(1)* monopyrenylated **10**, *(2)* dipyrenylated **11**, *(3)* pentapyrenylated **12**. Concentrations $5 \cdot 10^{-7}$ M; excitation at 345 nm.

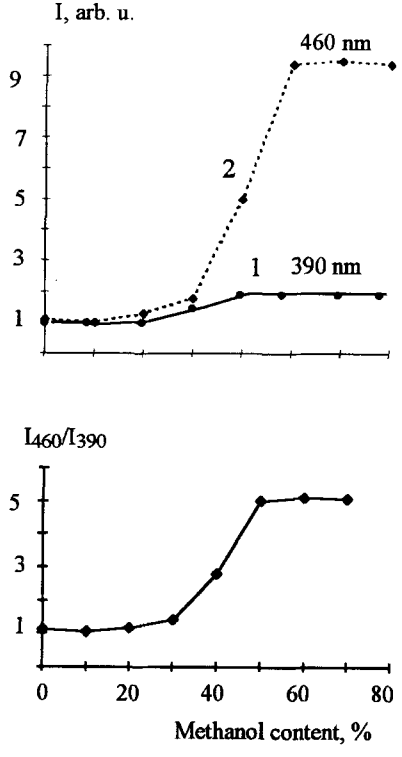


Figure. 5. Effect of solvent on the fluorescent characteristics of pentapyrenylated conjugate **12**. (a): the monomer (at 390 nm) (*1*) and excimer (at 460 nm) (*2*) fluorescence intensity vs. methanol concentration. (b): the excimer-to-monomer fluorescence intensity ratio vs. methanol concentration. Concentrations $5 \cdot 10^{-7}$ M; excitation at 345 nm.

deoxynucleosides, and the pyrene residue serves as a nucleobase analogue. It is noteworthy that the close coplanar mutual approach of two pyrene residues located in the neighboring positions of a modified oligonucleotide chain is strongly inhibited because of the small length of the methylene linker. In fact, only monomer emission was observed for the bipyrenylated modified oligomers **11**, **15**, **16**, **18**, and **19** (see, however, examples of excimer emission displayed by two adjacent pyrene residues in oligonucleotides[23]). Molecular modelling showed that the excimer formation by two adjacent pyrene residues could result in a strain in the backbone.[37] The lack of excimer fluorescence in the case of oligonucleotide harbouring two pyrene-containing units and the appearance of such fluorescence upon association with unmodified or modified complementary sequence provides additional opportunities in designing special DNA probes for homogeneous hybridization analysis.

An obvious prerequisite for hybridization analysis is the stability of duplexes of modified oligomers with the complementary strands. We found that one or two pseudonucleoside units in dangling positions increase the duplex stability by 2.5–3.5°C, thus allowing the design of duplex-stabilizing DNA probes. These results are consistent with the observations,[24] where the bulky terminal aromatics in dangling positions stabilized duplexes formed by self-complementary oligomers.

The multilabeled conjugates are infrequent in the field of fluorescent modification of nucleic acids, as the preparation of such conjugates is far from straightforward. The pentapyrenylated conjugate **12** is particularly interesting. There is a strong excimer band in its fluorescence spectrum (Fig. 4). It is noteworthy that while the monomer fluorescence of pyrene is in the UV region, the excimer fluorescence is bathochromically shifted to the visible region, which enables polypyrenylated conjugates to be used in fluorescent microscopy.

Somewhat unexpected results were gained upon studying the effect of the solvent polarity on fluorescence of pentapyrenylated conjugate **12** (Fig. 5). The tenfold increase of excimer emission with an increasing methanol content in solution may be accounted for by aggregation of the oligonucleotide molecules to form micelle-like structures. This suggestion is supported by a 10-nm red shift of the excimer maximum (cf. ref 28) in 60% methanol as compared with the aqueous solution (data not shown), and by a characteristic *S*-shaped form of the curves (Fig. 5). This implies that the multipyrenylated conjugates are potential tools in membrane studies.

Conclusion

In this paper we described new reagents based on 4-(1-pyrenyl)-1,3-butanediol allowing the introduction of this nucleoside substitute into any predetemined position(s) of oligonucleotides. Varying the combination of the pyrene residues involved makes it possible to control the hydrophobicity and fluorescent properties of the resulting conjugates. The changes in fluorescence emission intensity and spectral curve shape (the excimer-to-monomer emission intensity ratio) can be used for monitoring the conjugate interactions, for example, hybridization with a complementary target. These results pave the way for constructing useful DNA hybridization probes.

Experimental

Materials and Methods: Pentafluorophenyl 1-pyreneacetate,[16] Meldrum's acid,[40] diisopropylammonium tetrazolide, and bis(diisopropylamino)-2-cyanoethoxyphosphine[41] were synthesized according to the published procedures. 4-Dimethylaminopyridine (DMAP), succinic anhydride, triethylamine, trifluoroacetic acid, and 1,3-dicyclohexylcarbodiimide (DCC) were from Fluka; pentafluorophenol and 4,4'-dimethoxytrityl chloride (DMTCl) were from Aldrich; long chain alkyl amino controlled pore glass support (LCAA-CPG, 500 Å pore size) was from Pierce; the solvents were from Merck. Acetonitrile, pyridine, and methylene chloride were distilled over CaH_2; THF was distilled over sodium benzophenone ketyl before use. TLC was run on Kieselgel 60 F_{254} precoated plates (Merck) with the aluminium backing; the spots were visualized under UV light (254 and 366 nm) or (in the case of dimethoxytrityl-containing compounds) by exposing the plate to the trifluoroacetic acid vapour. Column chromatography was carried out using Kieselgel 60 (40–63 μm, Merck).

^1H NMR spectra were obtained on a Bruker AC-500 spectrometer in $CDCl_3$ (δ in ppm relative to solvent line). Mass spectral data were obtained on mass specrometers Varian MAT-44S (electron impact, EI), Kratos MS-50TC (fast atom bombardment, FAB), and ^{252}Cf plasma desorption time-of-flight instrument (Electron, Sumy, Ukraine) (plasma desorption, PD). Melting points were determined on a Boetius instrument and are uncorrected.UV spectra of oligonucleotides were recorded on a Beckman DU-65 spectrophotometer at room temperature. Melting curves were taken in 150 mM NaCl and 10 mM TBE (pH 8.3) on a Gilford 2400-2 spectrophotometer with a thermostated cell (200 μl); dsDNA concentrations were 5×10^{-6} M, heating rate was 1°C min^{-1}.Fluorescent spectra were measured on a Hitachi F-4000 spectrofluorometer in double distilled water or 0–70% aqueous methanol at 20°C. Concentrations of the conjugates were 5×10^{-7} M. Excitation wavelength was 340 nm.

Synthesis of the pyrenylbutanediol phosphoramidite and pyrene-modified LCAA-CPG solid support : 2,2-Dimethyl-5-(1-pyreneacetyl)-1,3-dioxane-4,6-dione (2). To a solution of isopropylidene malonate (586 mg, 4.07 mmol) in THF (15 ml) at 20°C were consecutively added Et_3N (1.13 ml, 8.12 mmol) and pentafluorophenyl 1-pyreneacetate **1** (1.580 g, 3.70 mmol) with vigorous stirring; and the reaction mixture was stirred for 1 h, diluted with benzene (50 ml), and washed with a 1 N HCl-saturated brine 1:1 mixture (2×40 ml) and brine (40 ml). The organic layer was dried over Na_2SO_4 and evaporated, and the residue (off-white foam) was crystallized from dry EtOH to give pure enol **2** (1.350 g, 94%) as colorless crystalls, mp 95–96°C. R_f 0.41 (CH_2Cl_2–MeOH 19:1).

Ethyl 4-(1-pyrenyl)-3-oxobutanoate (3). A solution of enol **2** (1.100 g, 2.85 mmol) and trifluoroacetic acid (330 µl, 4.3 mmol) in dry ethanol (50 ml) was refluxed for 30 min, then evaporated to one fourth, and kept for 16 h at −18°C. The crystalline precipitate formed was filtered off, washed with cold ethanol (10 ml), and dried in vacuum to give 590 mg of pure β-ketoester **3**. The mother liquor and washing were combined and evaporated; the residue was taken in benzene (30 ml), washed with 5% aq $NaHCO_3$ (30 ml) and water (30 ml), dried (Na_2SO_4), and evaporated to dryness; and the residue was chromatographed (benzene) and then crystallized from ethanol to give an additional portion of pure **3** (220 mg). The total yield was 810 mg (86%), mp 83–84°C. R_f 0.48 (CH_2Cl_2). MS (EI), m/z^+: 330, calculated 330.38 ($C_{22}H_{18}O_3$). ^{1}H NMR ($CDCl_3$): 8.22–7.89 (m, 9H, Ar*H*), 4.54 (s, 2H, COCH_2CO), 4.13 (q, 2H, J = 6.9 Hz, CH_2CH$_3$), 3.43 (s, 2H, ArCH_2CO), 1.23 (t, 3H, J = 6.9 Hz, CH$_2$CH_3).

4-(1-Pyrenyl)-1,3-butanediol (4). To a solution of β-ketoester **3** (170 mg, 0.51 mmol) in THF (10 ml) was added $NaBH_4$ (43 mg, 1.14 mmol) in one portion, and the reaction mixture was heated to reflux. Dry methanol (300 µl) was added to the refluxing mixture over a period of 20 min. The heating was removed, and the reaction mixture was left for 2 h at room temperature, quenched with water (1.5 ml), and evaporated to dryness. The residue was dissolved in EtOAc (15 ml), washed with 1 N HCl (2×10 ml) and water (10 ml), dried (Na_2SO_4), evaporated, and chromatographed using gradient elution with THF (0→10%) in CH_2Cl_2. After crystallization from CH_2Cl_2–hexanes, diol **4** was obtained (130 mg, 87%) as colorless crystals, mp 80-81°C. R_f 0.34 (CH_2Cl_2–MeOH 19:1). MS (EI), m/z^+: 290, calculated 290.36 ($C_{20}H_{18}O_2$). ^{1}H NMR ($CDCl_3$): 8.29–7.83 (m, 9H, Ar*H*), 4.32 (m, 1H, CH$_2$C*H*OH), 3.79 and 3.88 (two m, 2H, CH_2OH), 3.50 (d, 2H, J = 7.0 Hz, ArCH_2), 1.76 (m, 2H, CH_2CH$_2$OH).

O^1-(4,4'-Dimethoxytrityl)-4-(1-pyrenyl)-1,3-butanediol (5). To a solution of diol **4** (184 mg, 0.63 mmol) in dry pyridine (2 ml) cooled on ice to 0°C was added DMTCl (226 mg, 0.66

mmol) in dry pyridine (2 ml) within 15 min under argon. The cooling was removed, and the reaction mixture was allowed to warm to room temperature for 1 h. After quenching with methanol (100 μl), the mixture was diluted with CH_2Cl_2 (15 ml), washed with water (3×15 ml), dried (Na_2SO_4), and evaporated to dryness; the residue was chromatographed (CH_2Cl_2–hexanes 1:1 + 1% Et_3N as eluent) to yield pure monoprotected diol **5** (300 mg, 80%) as an amorphous yellowish solid. R_f 0.38 (CH_2Cl_2). MS (FAB), m/z^+: 593, calculated 592.73 ($C_{41}H_{36}O_4$). ^{1}H NMR ($CDCl_3$): 8.30–7.78 (m, 9H, Ar*H* (pyrene)), 7.42–7.19 (m, 9H, Ar*H* (DMT)), 6.80 (m, 4H, Ar*H* (DMT)), 4.29 (m, 1H, CH_2CHOH), 3.72 (s, 6H, $2CH_3O$), 3.45 (m, 2H, $ArCH_2$), 3.36 and 3.18 (two m, CH_2O), 1.92 and 1.80 (two m, 2H, CH_2CH_2O).

O^1-(4,4'-Dimethoxytrityl)-O^3-(N,N-diisopropylamino-2-cyanoethoxyphosphinyl)-4-(1-pyrenyl)-1,3-butanediol (6). The mono-DMT-protected diol **5** (103 mg, 0.174 mmol) was coevaporated with acetonitrile (2×1.5 ml) and dissolved in dry acetonitrile (2 ml), and diisopropylammonium tetrazolide (16.4 mg, 0.096 mmol) and bis(N,N-diisopropylamine)-2-cyanoethoxyphosphine (65 μl, 0.20 mmol) were added with stirring under argon. The mixture was half evaporated (bath temperature below 30°C) and then allowed to react for 1.5–2 h with stirring. The disappearance of the starting compound was monitored by TLC (1% MeOH and 0.5% Et_3N in benzene). After evaporation to dryness, the residue was dissolved in ethyl acetate (15 ml), washed with brine (2×15 ml), dried (Na_2SO_4), evaporated, and flash chromatographed (1% Et_3N in benzene) to give 100 mg (73%) of the product as a white foam. This was dissolved in benzene (2 ml), cooled at -18°C, and lyophilized at 0.04 mm Hg before use in DNA synthesizer. R_f 0.37 (1% MeOH in benzene). Spectral characteristics are given in [37].

O^3-(3-Carboxypropionyl)-O^1-(4,4'-dimethoxytrityl)-4-(1-pyrenyl)-1,3-butanediol (7). To a stirred solution of compound **5** (200 mg, 0.34 mmol) in pyridine (2 ml) were added succinic anhydride (34 mg, 0.34 mmol) and DMAP (9.90 mg, 0.081 mmol). The mixture was left for five days at room temperature, diluted with ethyl acetate (15 ml), washed with water (3×15 ml), dried (Na_2SO_4), evaporated, and chromatographed using gradient elution (0→50% THF in $CHCl_3$). Yield 220 mg (94%) as an off-white foam. R_f 0.44 (4% MeOH in benzene). MS (PD), m/z^+: 693.3, calculated 692.81 ($C_{45}H_{40}O_7$). ^{1}H NMR ($CDCl_3$): 8.20-7.89 (m, 9H, Ar*H* (pyrene)), 7.34-7.16 (m, 9H, Ar*H* (DMT)), 6.73 (m, 4H, Ar*H* (DMT)), 5.62 (m, 1H, $CHOCO$), 3.82 (m, 1H, ArCH*H'*), 3.74 (s, 6H, $2CH_3$), 3.41 (m, 1H, ArCH'*H*), 3.14 (m, 2H, CH_2ODMT), 2.54 (m, 4H, $CH_2CH_2CO_2H$), 1.96 and 1.87 (two m, 2H, CH_2CH_2ODMT).

O^1-(4,4'-Dimethoxytrityl)-O^3-(3-(pentafluorophenyloxycarbonyl)propionyl)-4-(1-pyrenyl)-1,3-butanediol (8). Pentafluorophenol (63.6 mg, 0.346 mmol) in pyridine (2 ml) was

added in one portion to a solution of acid **7** (230 mg, 0.332 mmol) in ethyl acetate (2.5 ml). The mixture was cooled to 0°C (ice bath), and a solution of DCC (71 mg, 0.34 mmol) in pyridine (1 ml) was added dropwise with stirring. After 24 h the N,N′-dicyclohexylurea formed was filtered off, and the solution was evaporated to dryness. The residue was chromatographed (1% Et_3N in benzene) to give the desired ester **8** (267 mg, 94%) as a white foam. R_f 0.73 (benzene). MS (PD), m/z^+: 858.2, calculated 858.86 ($C_{51}H_{39}F_5O_7$). ^{1}H NMR ($CDCl_3$): 8.21-7.85 (m, 9H, Ar*H* (pyrene)), 7.38-7.15 (m, 9H, Ar*H* (DMT)), 6.74 (m, 4H, Ar*H* (DMT)), 5.66 (m, 1H, C*H*OCO), 3.73 (s, 6H, 2CH_3), 3.72 and 3.47 (two m, 2H, ArCH_2), 3.16 (t, 2H, J = 6.7 Hz, CH_2CH_2O), 2.76 (m, 2H, $CH_2CO_2C_6F_5$), 2.55 (m, 2H, CH_2CO_2CH), 2.00 and 1.95 (two m, 2H, CH_2CH_2O).

Pyrene-modified CPG-support (9). LCAA-CPG (300 mg) was activated by a 3-h shaking with a 2% TFA solution in $CHCl_3$ (1.5 ml), filtered off, washed with Et_3N (2 ml), CH_2Cl_2 (3×5 ml), and ether (3×5 ml), and dried in vacuum. The activated support was treated with ester **8** (70 mg) and Et_3N (40 μl) in $CHCl_3$ (2 ml) for 24 h at room temperature, then filtered off, washed with pyridine (2 ml), chlorofom (3×5 ml), and ether (3×5 ml), dried in vacuum, and suspended in a capping solution prepared directly before use by mixing solutions of acetic anhydride (100 μl) in THF (2 ml) and DMAP (120 mg) in THF (2 ml). After shaking (2 h), the support was filtered off, washed with pyridine (2 ml), $CHCl_3$ (3×5 ml), THF (3×5 ml), and ether (3×5 ml), and dried in vacuum. The loading, determined by acid-promoted release of DMT cation (λ_{max} 502 nm), was 25 μmol/g.

Oligonucleotide synthesis. Solid-phase oligonucleotide synthesis was carried out on an ASM-2U DNA Synthesizer (Novosibirsk, Russia). Phosphoramidite **6** and support **9** were used as conventional nucleoside-based reagents, with no changes in the synthetic cycle. The coupling efficacy was determined from the trityl absorption as described (72). The oligonucleotides were cleaved off the support and deprotected with conc. NH_4OH (55°C, 6 h), precipitated from 1 M $LiClO_4$ with acetone, purified by 20% PAGE, and desalted on a Toyopearl HW 40 (Toyo Soda) column. Analytical HPLC was carried out on a Beckman 153 chromatographic unit (column SOTA C18E (5 μ) 4.5×220 mm, buffer A: 5% MeCN in 0.1 M NH_4OAc, buffer B: 5% 0.1 M NH_4OAc in MeCN, 0→45% B in A for 30 min). The purity of conjugates was also confirmed by CE on an Appled Biosystems 270A apparatus (100 mM Tris-borate supplemented with 1 mM EDTA (TBE), pH 8.3, l = 50 cm, d = 75 μm, voltage 15 kV, detection at 260 nm). The following modified and unmodified oligonucleotides were prepared (5'→3'; **Prb** stands for a pyrenylbutanediol residue): ATGTCCAGGATCCCC**Prb** **(10)**, ATGTCCAGGATCCCC(**Prb**)$_2$ **(11)**, ATGTCCAGGATCCCC(**Prb**)$_5$ **(12)**, **Prb**CCAAGGTTGGACAC **(13)**,

PrbGCCAAGGTTGGACAC **(14)**, ACACTGTGTCTGTCAAGTCT(**Prb**)$_2$ **(15)**, (**Prb**)$_2$AGACTTGACAGACACAGTGT **(16)**, (**Prb**)$_3$AGACTTGACAGACACAGTGT **(17)**, ATGTCC(**Prb**)$_2$GATCCCC **(18)**, GGGGATC(**Prb**)$_2$GGACAT **(19)**, AGGATCCCC(**Prb**)$_2$TT(**Prb**)$_2$GCCAAGGTT **(20)**, ATGTCCAGGATCCCC **(21)**, GGGGATCCTGGACAT **(22)**, ACACTGTGTCTGTCAAGTCT **(23)**, AGACTTGACAGACACAGTGT **(24)**, GTGTCCAACCTTGGCGGGGATCCTGGACAT **(25)**.

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