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Synthesis and Fluorescent Properties of 5-(1-Pyrenylethynyl)-2'-deoxyuridine-containing Oligodeoxynucleotides[1]

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Abstract—Novel reagents for the fluorescent labeling of oligo- and polynucleotides have been prepared: 5-(1-pyrenylethynyl)-2'-deoxyuridine 3'-phosphoramidite and a solid support carrying this nucleoside. Oligonucleotides containing one or several modified units have been synthesized, and the fluorescence of these probes has been shown to change upon hybridization with the complementary sequence.

Key words: fluorescent detection, hybridization, modified oligonucleotide, 5-(1-pyrenylethynyl)-2'-deoxyuridine

INTRODUCTION

Over the last few years, methods of the study and homogeneous analysis of nucleic acids have been developed based on intra- and intermolecular interactions of fluorophores. The use of such methods spans the study of the structure and physicochemical properties of nucleic acids using fluorescence energy transfer (for a review, see [3]), detection of specific nucleotide sequences on the basis of molecular beacons [4–9] or energy transfer [10–14], sequencing of nucleic acids using primers labeled with a donor–acceptor pair of fluorophores [15–20], and detection of nucleic acid hybridization through the change in the ratio of the excimeric and monomeric fluorescences of pyrene labels [21–33]. In this context, the development of new methods of the site-specific introduction of fluorescent labels into oligo- and polynucleotides is an increasingly relevant problem. The pyrene fluorescence is of special interest as it can be employed in detecting various interactions involving nucleic acids even as a single label [34–42]. Here, we describe the regiospecific introduction into oligonucleotides of residues of fluorescent nucleoside 5-(1-pyrenylethynyl)-2'-deoxyuridine synthesized earlier [1] and the spectral properties of the resulting conjugates.

[1] Fluorescent Nucleosides. III. The previous communications, see [1, 2]. Prefix "d" in the oligodeoxynucleotide designations is omitted. Abbreviations: DIC, *N,N'*-diisopropylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; DMT, 4,4'-dimethoxytrityl; LCAA-CPG, long-chain aminoalkylated controlled pore glass.

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RESULTS AND DISCUSSION

The synthesis of the modified nucleoside (**VI**) was carried out via 1-ethynylpyrene (**III**) [1] as an intermediate. A number of methods for preparing 1-ethynylpyrene are known, but they did not appear satisfactory to us because of the intricacy of the procedure [43, 44], moderate yields of the product, and problems with the purification of the starting 1-bromopyrene [45, 46]. We have therefore developed a different approach to the synthesis of ethynylpyrene (**III**), underlied by the Bodendorf two-step method for a mild conversion of acetylarenes into ethynylarenes [47–50] (Scheme 1). 1-Acetylpyrene (**I**), obtained in a high yield by the Friedel–Crafts acylation of pyrene [51], was transformed using the Vilsmeier reagent ($POCl_3$/DMF) into 3-(1-pyrenyl)-3-chloro-2-propenal as a mixture of *Z* (**IIa**) and *E* (**IIb**) isomers. At this stage, 1-(1-chlorovinyl)pyrene (**IV**) was also isolated and identified.

The *E* and *Z* isomers of pyrenylacroleins (**II**) are indistinguishable upon TLC on silica gel in various solvent systems; thus, the resulting substance was characterized only by the ^1H NMR spectrum as a mixture of isomers. In this spectrum, the multiplet of nine aromatic protons in the 8.39–8.02 ppm region is accompanied by two doublets (at 10.43 and 9.18 ppm) corresponding to aldehyde protons (summary intensity 1H) and by two doublets of vinyl protons (at 6.90 and 6.64 ppm), which also correspond in total to 1H. The double resonance measurements revealed that signals at 10.43 and 6.64 ppm belong to protons of one geometric isomer, whereas signals at 9.18 and 6.90 ppm belong to protons of the second isomer. This was confirmed by the values

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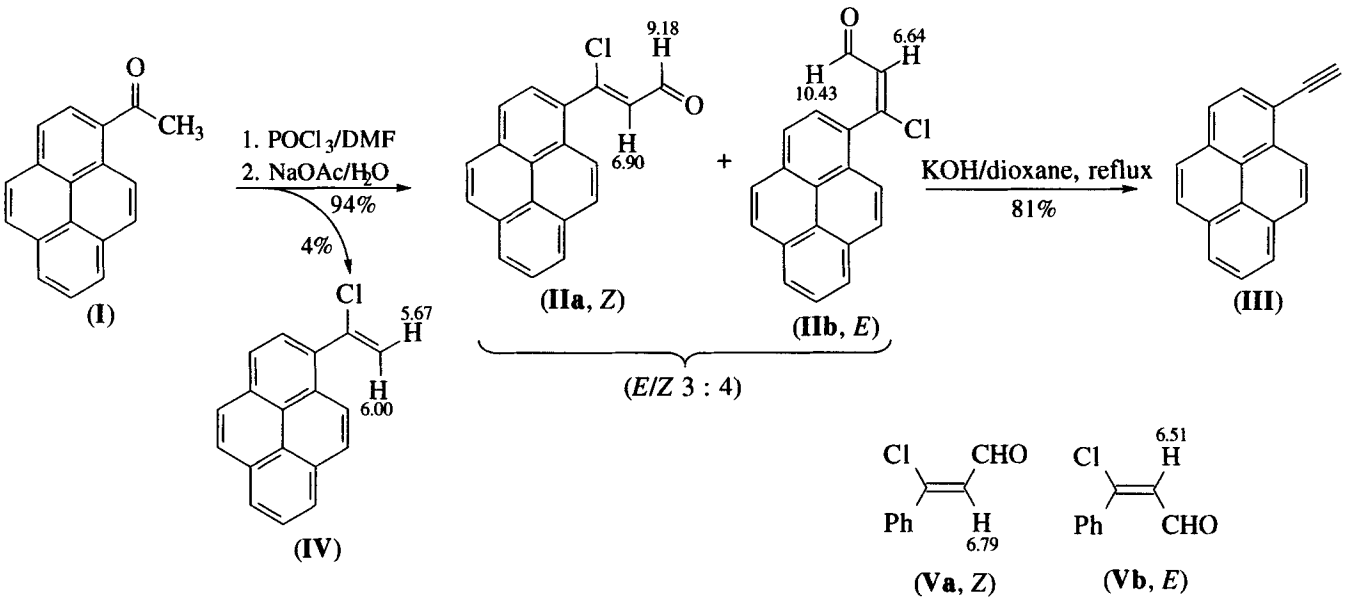


Scheme 1. Chemical shifts of the proton signals in the ^{1}H NMR spectra are given.

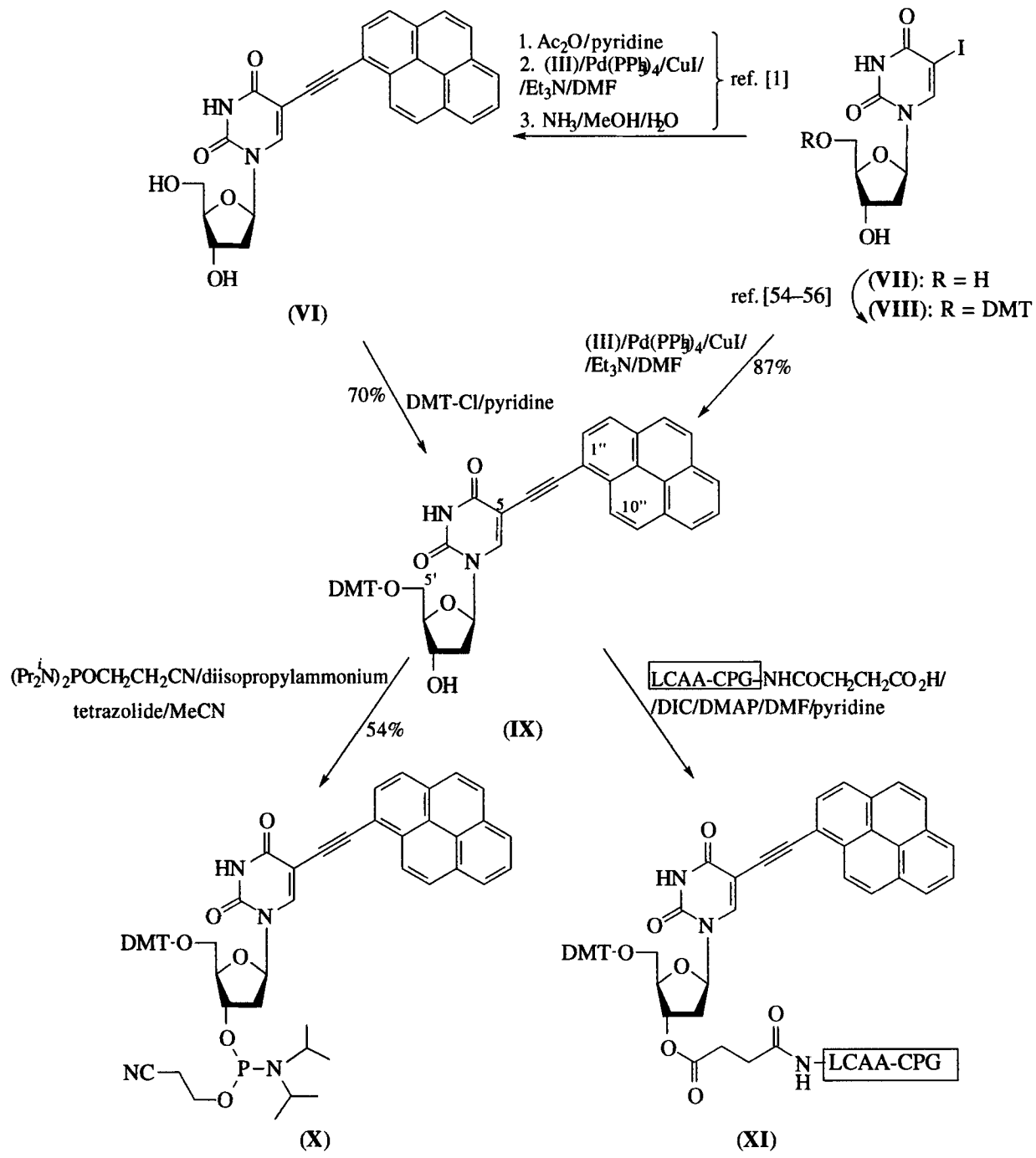
of the spin-coupling constants and integral intensities of the signals.

To unambiguously assign the signals, we calculated the chemical shifts of vinyl protons for model compounds—*E* and *Z* isomers of 3-phenyl-3-chloro-2-propenal (**V**; Scheme 1)—using formula $\delta = 5.28 + \sigma_{gem} + \sigma_{cis} + \sigma_{trans}$, where δ is the chemical shift of the vinyl proton whereas σ_{gem} (1.03 for CHO), σ_{cis} (0.14 for Cl and 0.39 for Ph), and σ_{trans} (0.09 for Cl and 0.06 for Ph) are empirical shielding constants for the *gem*, *cis*, and *trans*-substituents at the double bond relative to this proton [52]. For the *Z* isomer (**Va**), the calculated value for the vinyl proton signal (6.79 ppm) exceeds by 0.28 ppm that for the *E* isomer (**Vb**) (6.51 ppm). This implies that a similar relationship can be expected for pyrenylacroleins (**IIa**, **IIb**), which display a 0.26-ppm difference in the vinyl protons shifts. Thus, the low-field signal at 6.90 ppm should be assigned to the *Z* isomer (**IIa**), and the higher-field signal (6.64 ppm), to the *E* isomer (**IIb**). From these data, shielding constants for the pyrene-1-yl substituent at the double bond can be calculated: $\sigma_{cis} = 0.50$ and $\sigma_{trans} = 0.19$.[3] Apparently, the closer the vinyl proton to the pyrene polycycle, the larger is the low-field shift of this proton caused by the pyrenyl substituent. The effect of the pyrenyl substituent is stronger than that of the phenyl substituent, for which $\sigma_{cis} = 0.39$ and $\sigma_{trans} = 0.06$ [52].

[3] For the pyrenyl substituent in alkene (**IV**) (Scheme 1), values of $\sigma_{cis} = 0.63$ and $\sigma_{trans} = 0.25$ were obtained; the spin coupling of the vinyl protons observed thereby was insignificant ($^2J < 1$ Hz).

The difference in distance from the pyrene residue in compounds (**IIa**) and (**IIb**) also seems to define the character of the disposition of the aldehyde protons in the ^{1}H NMR spectrum (see Scheme 1). If one assumes that the *trans*-conformations of the substituted acroleins depicted in this scheme (the mutual disposition of the C=C and C=O bonds is implied) are thermodynamically preferable, the aldehyde proton proves thereby to be proximal to the pyrene nucleus in the *E* isomer (**IIb**) and, on the contrary, quite distant from it in the *Z* isomer (**IIa**). This is consistent with the large difference in the chemical shifts: the signal of the *E* isomer aldehyde proton is shifted low-field by 1.25 ppm as compared with the signal of this proton in the *Z* isomer. The structures (**IIa**) and (**IIb**) also show that the difference between the distances from the pyrene residue for vinyl protons is much smaller than for aldehyde ones, the vinyl proton being closer to the pyrene in the *Z* isomer. This is in accordance with the smaller difference in the shifts of the vinyl protons (0.26 ppm) relative to the aldehyde protons and with the reverse disposition of their signals for the two geometrical isomers (the *Z* isomer vinyl proton gives a lower-field signal).

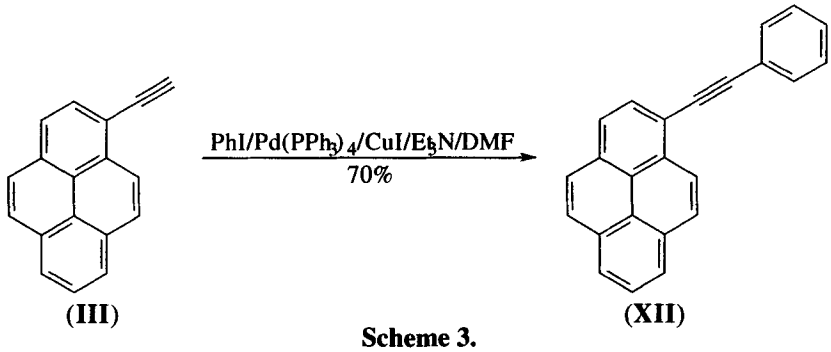
An alkaline treatment of aldehyde (**II**) led to 1-ethynylpyrene (**III**) (Scheme 1). The optimization of this step included variations in the solvent (dioxane, diglyme, DMSO) and the base (aqueous or solid NaOH or KOH, Pr^{i}ONa) and in the aldehyde–base ratio. The best results were gained upon refluxing the aldehyde in the dry dioxane with 2.5 mol of finely powered KOH; an addition of dibenzo-18-crown-6 did not enhance the reaction rate and the alkyne yield. The reaction should be carried out under argon; otherwise, the desired (**III**)



Scheme 2.

may contain an admixture of the oxidative dimerization product, 1,4-bis(1-pyrenyl)butadyyne-1,3 (cf. [2]). Thus, 1-ethynylpyrene (**III**) was obtained from 1-acetylpyrene (**I**) in two steps in 76% overall yield.

5'-*O*-(4,4-Dimethoxytrityl)-5-(1-pyrenylethynyl)-2'-deoxyuridine (**IX**), the key compound in the synthesis of the modifying reagents, namely, phosphoramidite (**X**) and solid support (**XI**) (Scheme 2), was prepared in two fashions underlied by the coupling of ethynylpyrene (**III**) with 5-iodo-2'-deoxyuridine (**VII**) or its dimethoxytrityl derivative (**VIII**). The interaction of compounds (**VII**) and (**III**) under the Heck–Sonogashira reaction conditions [53], which directly leads to nucleoside (**VI**), proved unsuitable for the preparative scale because of the poor solubility of the pyrenylethynyl derivative (**VI**) and the ensuing problems of its isolation from the reaction mixture. This can be overcome by the preliminary acetylation, in compound (**VII**), of



(III) (XII)

Scheme 3.

the hydroxyl groups, which are deblocked after the coupling [1]. The dimethoxytritylation of nucleoside (**VI**) by DMT-Cl in dry pyridine led to the 5'-monosubstituted product (**IX**) in a 70% yield. The bulky pyrene substituent impedes the tritylation: the reaction proceeds longer and the yield is lower than when conventional nucleosides are tritylated. When this way is used, compound (**IX**) is produced from 5-iodo-2'-deoxyuridine (**VII**) in four steps. The second way differs in the sequence of the tritylation and coupling reactions, which allows 5-iodo-2'-deoxyuridine (**VII**) to be converted into compound (**IX**) in two steps. At first, the dimethoxytritylation of nucleoside (**VII**) [54–56] led to the 5'-monosubstituted nucleoside (**VIII**) in a high yield; its solubility is sufficient for the coupling reaction to be performed. Then, nucleoside (**VIII**) was alkynylated by pyrenylacetylene (**III**) in standard conditions (see review [53])—in DMF in the presence of $Pd(PPh_3)_4$, CuI, and triethylamine (Scheme 2) at room temperature for 16 h. The reaction product (**IX**), similar to compound (**VIII**), is well soluble in organic solvents and can easily be isolated by column chromatography.

To prepare the modifying reagent (**X**), the one-atomic alcohol (**IX**) was phosphitylated by 2-cyanoethoxybisdiisopropylaminophosphine in the presence of diisopropylammonium tetrazolide as a catalyst [57] to obtain the desired compound in a 54% yield. After chromatography on silica gel, the substance was precipitated by hexane from toluene, lyophilized from a benzene solution, and stored at –20°C under argon; in these conditions, it is stable at least for two years. Based on the monoprotected nucleoside (**IX**), support (**XI**) for the solid phase DNA synthesis was also prepared according to [58] with the anchored nucleoside loading of 56.5 μmol/g.

As a model compound, 1-(phenylethynyl)pyrene (**XII**), an analogue of compound (**VI**) containing a phenyl in place of the nucleoside residue, was synthesized by the coupling of 1-ethynylpyrene (**III**) and iodobenzene (Scheme 3).

Phosphoramidite (**X**) and solid support (**XI**) were used in the synthesis of the modified oligonucleotides (**XIV**) and (**XVI**)–(**XIX**), containing one, two, or three substitutions of 5-pyrenylethynyl-2'-deoxyuridine (**VI**) for the thymidine residue and corresponding to the fragment 5'ACGAGGAAAGCGTAA (**XIII**) of the gene for the putative transcription factor *fet5*$^+$ of *Schizosaccharomyces pombe* [59] or to the complementary sequence (**XV**) (table).

The synthone (**X**) was employed in the same concentration (0.1 M in acetonitrile) and in the same synthetic cycle as the standard nucleosides. Under these conditions, the condensation involving the modified reagent and the first of the subsequent condensations were a little less efficient (90–95%, determined by the optical absorption of the DMT^+ cation [60]) than for the standard nucleotides (99–99.9%). If the number of the modified units introduced into an oligonucleotide is low (1–3), the conditions of the synthesis need not be altered; however, in the case of sequences with a high content of such units, the yield at the condensation step may be increased using a more concentrated solution of the modifying reagent and a longer condensation time.

After deprotection with ammonia (nucleoside (**VI**) in these conditions seems to be stable as the control treatment with ammonia did not give any TLC-detectable products of its transformation), the modified oligonucleotides (**XIV**) and (**XVI**)–(**XIX**) were isolated by electrophoresis in 20% denaturing PAG. As exemplified by conjugate (**XVIII**), it was shown that UV irradiation (λ 254 nm) produces fluorescence detectable both in gel (ca. 5 pmol/band) and aqueous solution (ca. 0.5 nmol/ml). Modifications reduce the electrophoretic mobility of oligonucleotides as illustrated by a series of oligomers of the same length with an increasing number of the modified units: (**XV**) > (**XVII**) > (**XVIII**) > (**XIX**). This effect is unambiguously to be accounted for by the sheer steric factors determining the interaction of a migrating polyanion with the crosslinked polyacrylamide matrix.

As expected, the introduction of the hydrophobic pyrene residue increases, owing to its strong affinity to the reversed phase, the retention time of the conjugates at the reverse-phase HPLC (table). This characteristic for an oligonucleotide with a single modified unit only slightly depends on the position of the unit in the chain

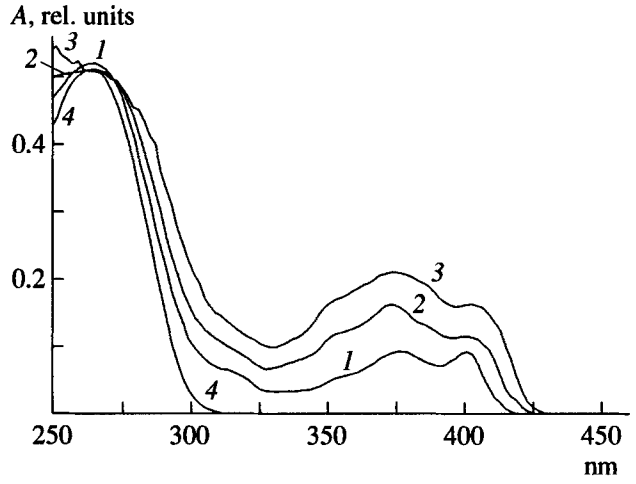


Fig. 1. UV spectra of modified oligonucleotides (**XVII**) (*1*), (**XVIII**) (*2*), (**XIX**) (*3*), and unmodified (**XV**) (*4*) in water (normalized at 264 nm).

(conjugates (**XVI**) and (**XVII**)), but is strongly dependent upon the nucleotide composition of the conjugate ((**XIV**) and (**XVI**)). It may well be that the contribution of a pyrene residue to the mobility of a conjugate depends on its shielding by the oligonucleotide moiety, that is, on the spatial structure of the conjugate, which is in turn defined by its nucleotide sequence. An increase in the number of the pyrene residues in the molecule (conjugates (**XVII**)–(**XVIII**)–(**XIX**)) results in a dramatic increase in the retention time, which was earlier observed for polypyrenylated oligonucleotides [61].

Figure 1 presents UV spectra of conjugates (**XVII**)–(**XIX**), containing a varying number of the modified nucleosides. The increase in the intensity of the long-wave absorption, characteristic for the fluorophore, as compared with the absorption of the oligonucleotide moiety in the region of 260–265 nm, confirms the respective presence of one, two, and three modified units in these oligonucleotides. The pyrenyl-substituted nucleoside contributes to the absorption of an oligonucleotide near 260 nm, which can be inferred from the shape of the spectral curves: the classical maximum in this region, characteristic for oligo- and polunucleotides and still preserved in the case of the monopyrenyl derivative (**XVII**) (curve *1*), is essentially smoothed out upon introduction of two and three modified units because of an increase in the contribution of the short-wave pyrene component (curves *2* and *3*). Interestingly, a growth in the number of the pyrenylated nucleoside residues is also accompanied by a change in the ratio of intensities of absorption at two maxima of the fluorophore in the spectra of the conjugates: the absorption around 375 nm rises stronger than around 400 nm.

As the sterically rigid 1-alkyne-1-yl substituents at position 5 of pyrimidines are directed into the large groove of the duplex, they do not destabilize nucleic acid complexes (see reviews [62, 63]). It was tempting to find out whether fluorophores at the adjacent positions of the nucleotide chain interact with each other and whether the fluorescence spectra of the conjugates are changed upon hybridization with the complementary sequence.

In the fluorescence spectrum of oligonucleotide (**XVII**) in aqueous buffer (curve *1* in Fig. 2a), a broad band with a poorly pronounced maximum of about 436 nm corresponds to a 5-(1-pyrenylethynyl)-2'-deoxyuridine residue. On the contrary, in organic solvents, the spectra of nucleoside (**VI**) are rather structured (two pronounced maxima) [1]. The spectra presented in Fig. 2a show that the introduction of a second pyrenylated unit next to the first one sharply increases the fluorescence (curve *2*): the emission intensity for conjugate (**XVIII**) is approximately three times higher than for the monomodified oligomer (**XVII**), although the concentration is thereby reduced by 20 times (in the concentration range used, the concentration quenching is low so that emission is approximately proportional to the concentration; the excitation spectra of the modified oligonucleotides differ scantily (data not shown)). A considerable bathochromic shift of the emission maximum (to 472 nm) also occurs. The introduction of a third modified residue (conjugate (**XIX**)) additionally

Primary structures and HPLC retention time values of the oligonucleotides synthesized

Number	Sequence*	Retention time**, min
(XIII)	(5')ACGAGGAAAGCGTAA	10.2
(XIV)	(5')ACGAGGAAAGCGU^{P}AA	14.6
(XV)	(3')TGCTCCTTTCGCATT	11.8
(XVI)	(3')U^{P}GCTCCTTTCGCATT	16.2
(XVII)	(3')TGCTCCU^{P}TTCGCATT	16.0
(XVIII)	(3')TGCTCCU^{P}U^{P}TCGCATT	20.6
(XIX)	(3')TGCTCCU^{P}U^{P}U^{P}CGCATT	27.6

*U^{P}, 5-(1-pyrenylethynyl)-2'-deoxyuridine.

**For conditions, see the Experimental section.

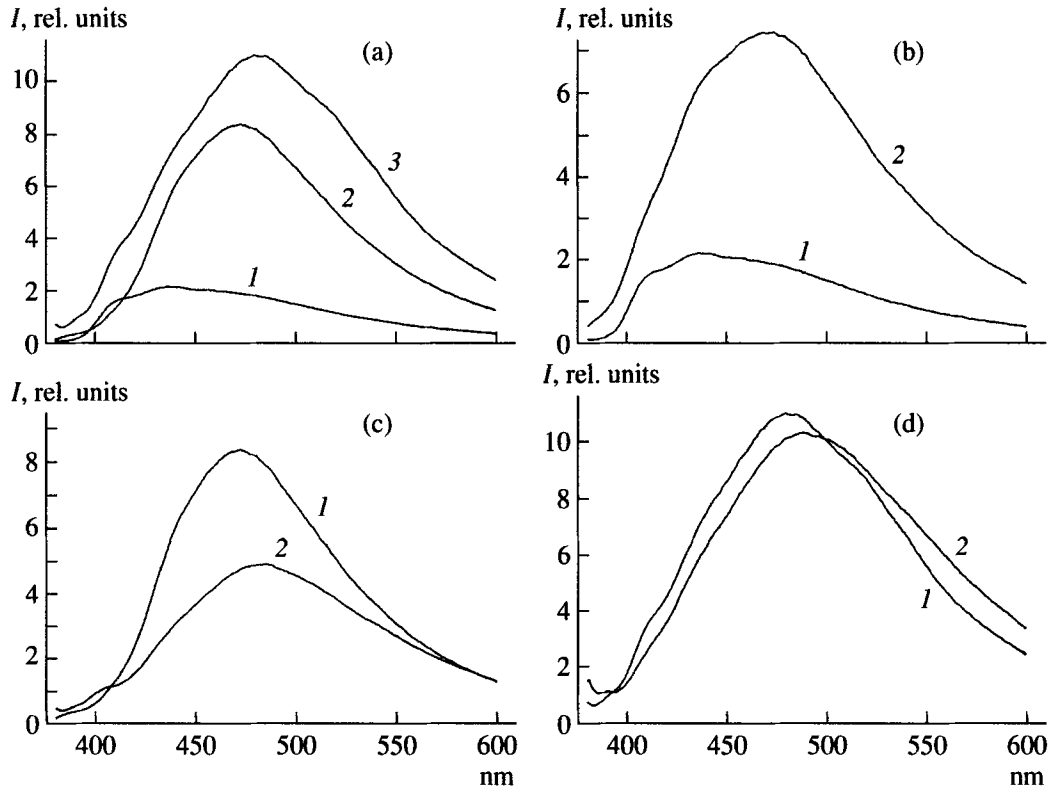


Fig. 2. Fluorescence spectra of modified oligonucleotides and duplexes in buffer containing 0.1 M NaCl, 0.01 M KH_2PO_4/K_2HPO_4, pH 7.0; λ_{ex} 370 nm. (a): **(XVII)** (*1*), **(XVIII)** (*2*), **(XIX)** (*3*); (b): **(XVII)** (*1*), **(XVII)**–**(XIII)** (*2*); (c): **(XVIII)** (*1*), **(XVIII)**–**(XIII)** (*2*); (d): **(XIX)** (*1*), **(XIX)**–**(XIII)** (*2*). Concentrations of oligonucleotides **(XVIII)** and **(XIX)** and duplexes **(XVIII)**–**(XIII)** and **(XIX)**–**(XIII)** 5×10^{-8} M, oligonucleotide **(XVII)** and duplex **(XVII)**–**(XIII)** 1×10^{-6} M.

intensifies the fluorescence and shifts the emission maximum to 479 nm (curve *3*).

Figures 2b–2d illustrate changes in the fluorescence spectra of the labeled oligomers after hybridization with the complementary oligonucleotide **(XIII)**. The most pronounced changes were observed for the monomodified oligonucleotide **(XVII)**: upon duplex formation, the emission intensity increases three to four times and the fluorescence maximum shifts from 436 to 469–473 nm (Fig. 2b). In contrast, for twice-modified oligomer **(XVIII)**, the fluorescence intensity upon hybridization even somewhat diminished and the bathochromic shift of the emission maximum was not as considerable—from 472 to 484 nm (Fig. 2c). The fluorescence intensity of the triple-modified oligonucleotide **(XIX)** upon hybridization practically did not change, and the bathochromic shift of the maximum was only 9 nm (from 479 to 488 nm) (Fig. 2d).

Thus, the maximum response of the fluorescence spectrum to hybridization is observed for the single label in oligonucleotide **(XVII)**—an intensity increase by several times and a bathochromic shift of the maximum by approximately 35 nm (Fig. 2b, curve *2*). Such sensitivity of the 5-(1-pyrenylethynyl)-2'-deoxyuridine residue to the structural surroundings makes this nucleoside potentially useful in probing various interactions of nucleic acids, such as formation of complementary complexes and binding to enzymes and other proteins.

Noteworthy is also the capacity of the 5-(1-pyrenylethynyl)uracil fluorophore to the fluorescence increase when several such residues are in proximity. The nature of the fluorescence of the double- and triple-modified DNA oligomers remains obscure. On the one hand, broad bands in the range of 470–480 nm in the fluorescence spectra of conjugates **(XVIII)** and **(XIX)** resemble the excimer fluorescence of pyrene (or alkylpyrenes) [64]. However, as the emission maximum for the duplex of the monomodified oligomer **(XVII)** is located in the same region, the excimer formation is not the only plausible explanation for the considerable bathochromic shift. A similar phenomenon (strong bathochromic shift of the emission maximum upon hybridization of the monopyrenylated oligonucleotide with the complementary sequence) was earlier accounted for by the formation of an exciplex of pyrene with nucleic bases [42]. This hypothesis is fairly acceptable in our case for, on the one hand, in duplex, (and sometimes in a single-stranded oligomer) the neighboring bases are stacked and, on the other hand, the fluorophore in 5-(1-pyrenylethynyl)-2'-deoxyuridine is the conjugated pyrenylethynyluracil system, which is confirmed by the UV and fluorescence spectra

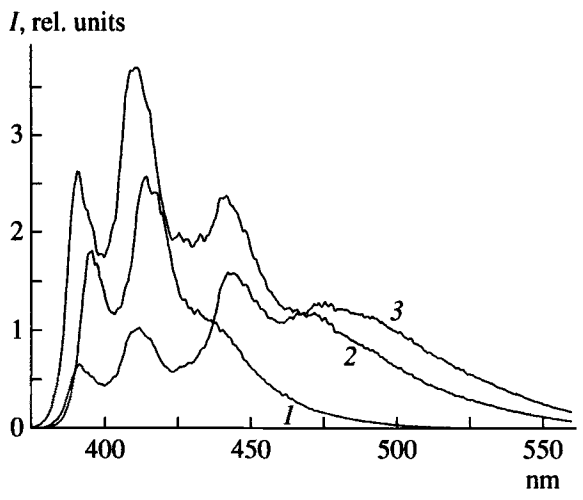


Fig. 3. Fluorescence spectra of 1-phenylethynylpyrene (**XII**) in THF; concentration 1×10^{-4} (*1*), 1×10^{-2} (*2*), and 5×10^{-2} M (*3*); λ_{ex} 337 nm.

[1]. Thus, in the modified duplexes, the fluorophore is definitely involved in the stacking (that is, preorganized state suitable for the exciplex formation) with the neighboring bases.

These data give evidence that the 5-(1-pyrenylethynyl)uracil fluorophores in conjugates interact both with each other and with the neighboring nucleosides; when they are located adjacently, this may result in the mutual shielding of the pyrene residues from the solvent and ion-quenchers in aqueous solution.

Little is known about the spectral properties of arylethynylpyrenes: the quantum-chemical calculation of the luminescent properties of 1-phenylethynylpyrene (**XII**) was made [65] and its fluorescent metabolites were mentioned [66]; recently, excimeric fluorescence of 1,6-(bis)alkylethynyl derivatives of pyrene was described [67]. We were interested in revealing whether the model 1-phenylethynylpyrene (**XII**) can form excimers in solution. The emission spectra of compound (**XII**) are independent of the concentration (data not shown). Figure 3 presents the fluorescence spectra of this compound in THF (this solvent is suitable for preparing solutions of pyrene derivatives at high concentrations). At low concentrations (10^{-4} M and lower), the spectrum is similar to that of pyrene somewhat bathochromically shifted (curve *1*). At a concentration of 10^{-2} M, a characteristic peak at 440–444 nm appears, whose analogue is absent from the pyrene spectrum, and a broad maximum at 475 nm emerges resembling the pyrene excimeric signal (curve *2*). A further fivefold increase in the concentration (curve *3*) makes the pattern even more pronounced (the overall fluorescence intensity somewhat decreases because of the internal filter effect). Thus, phenylethynylpyrene (**XII**) displays fluorescence of the concentration excimer although it requires concentrations by one to two orders of magnitude higher than for pyrene itself. This may be due to the effect of the phenylethynyl substituent, whose phenyl group in the ground state must be normal to the pyrene polycycle and hinder the approach to the excited molecule. The nature of the peak at 440–444 nm is not clear; its appearance may be related to the existence of variously oriented excited dimers or to the extent of the overlapping of the pyrene planes. Thus, arylethynyl derivatives of pyrene are capable of forming excimers so that fluorescence of 5-(1-pyrenylethynyl)-2'-deoxyuridine within oligonucleotides in favorable structural situations may also contain a contribution of the excimeric emission.

To conclude, the 5-(1-pyrenylethynyl)-2'-deoxyuridine (**VI**) residue within oligonucleotides is a promising label for studying interactions involving nucleic acids.

EXPERIMENTAL

The following chemicals were used: DIC, DMAP, 5-iodo-2'-deoxyuridine, $POCl_3$ (Fluka); 4,4'-dimethoxytrityl chloride, CuI, $PdCl_2$, $Pd(PPh_3)_4$ (Aldrich); other reagents and solvents were of domestic production. Acetonitrile (special purity grade) was distilled over P_4O_{10} and then refluxed and distilled over CaH_2; pyridine (reagent grade) was stored over KOH and distilled successively over ninhydrin and CaH_2; DMF (analytical grade) was dried by the azeotropic distillation of the water with benzene and then distilled in a vacuum; triethylamine (reagent grade) was stored over KOH and distilled successively over phthalic anhydride, KOH, and CaH_2; dioxane (bathochromic shift) was stored over KOH and distilled over sodium; petroleum ether (reagent grade, bp 70–100°C) and hexane (reagent grade) were filtered through neutral (here and henceforth) aluminum oxide (Merck, activity II; particle size 40–100 μm) and distilled; ethyl acetate, methylene chloride, and chloroform (all of analytical grade) were filtered through a layer of aluminum oxide and distilled; benzene (analytical grade) was shaken with conc. H_2SO_4, filtered through a layer of aluminum oxide, and distilled; methanol (special purity grade) was used without purification. Triphenylphosphine (analytical grade) was crystallized from petroleum ether. 1-Acetylpyrene [51], 5'-*O*-dimethoxytrytyl-5-iodo-2'deoxyuridine [53–55], 2-cyanoethoxybisdiisopropylaminophosphine, and diisopropylammonium tetrazolide [57] were synthesized as described. The reactions were monitored by TLC on the Kieselgel 60 F_{254} plates (Merck); the spots were visualized in UV light at 256 nm. For column chromatography, Kieselgel 60 (particle size 40–63 μm; Merck) was used. The solutions were dried over Na_2SO_4 and rotary evaporated in the water-jet pump vacuum at a bath temperature of 30–50°C.

^{1}H NMR spectra were recorded on a Bruker AC-500 instrument (δ scale; residual protons in deuterated sol-

vents served as an internal standard; spin-coupling constants are given in Hz). The ^{31}P NMR spectrum was measured on a Varian XR-400 instrument, working frequency for ^{31}P 161.9 MHz; chemical shifts are given relative to 85% H_3PO_4 as an external standard. Mass spectra were measured on Varian-MAT-44S (electron impact ionization (EI)) and VISION 2000 (Thermo Bioanalysis Corp.; time-of-flight mass spectrometer with matrix-assisted laser desorption ionization (MALDI-TOF)) instruments. Melting points were determined on a Boetius heating table (uncorrected). UV spectra were registered on a Shimadzu OPC-65 spectrophotometer. Oligonucleotide synthesis was performed on an automated synthesizer ASM-102U (BIOSAN, Novosibirsk). Oligonucleotides were purified by SDS-PAGE and analyzed on a Beckman 153 (Altex) chromatograph. Fluorescence spectra were measured on a Hitachi F-4000 spectrofluorometer and (for 1-phenylethynylpyrene (**XII**)) on an optical multichannel analyzer Princeton Applied Research OMA-2 with a Jobin–Yvon HR-320 monochromator and nitrogen laser LG-21 (337 nm) for excitation.

3-(1-Pyrenyl)-3-chloro-2-propenal (IIa, IIb). The Vilsmeier reagent prepared from DMF (10 ml) and $POCl_3$ (4.5 ml, 48 mmol) upon cooling with a water bath (10°C) was added dropwise under argon to a stirred solution of 1-acetylpyrene (2.44 g, 10.0 mmol) in DMF (15 ml) within 20 min. The stirring continued for 20 h, the reaction mixture was poured on ice (ca. 400 g), pH was adjusted to 6 by adding AcONa · $3H_2O$ and extracted with CH_2Cl_2 (150 ml). The organic layer was separated, washed with water (6 × 500 ml), dried, and evaporated; the residue was chromatographed on a silica gel column (4.5 × 10 cm) upon successive elution with 25, 35, and 50% CH_2Cl_2 in petroleum ether. Fractions containing the final product were pooled and evaporated, and the residue was dried in vacuum to afford 2.75 g (94%) of (**IIa, IIb**); R_f 0.49 (CH_2Cl_2). Mass-spectrum (**EI**), $(m/z)^+$: 290 (100), 273 (32), 255 (98), 224 (94), 202 (81); calc. for $C_{19}H_{11}ClO$ 290.75. ^1H NMR ($CDCl_3$): 10.43 (0.43H, d, *J* 6.7, (*E*) C*H*O), 9.18 (0.57H, d, *J* 7.5, (*Z*) C*H*O), 8.39–8.02 (9H, m, ArH), 6.90 (0.57H, d, *J* 7.5, (*Z*) C*H*CHO), 6.64 (0.43H, d, *J* 6.7, (*E*) C*H*CHO).

As a by-product, 1-(1-chlorovinyl)pyrene (**IV**) was isolated in a yield of 110 mg (4%); R_f 0.61 (petroleum ether–CH_2Cl_2 1 : 1); mp 65–66°C (mp 73–74°C [68]). Mass-spectrum (*EI*), $(m/z)^+$: 262 (100), 226 (90), 202 (76); calc. for $C_{18}H_{11}Cl$ 262.74. ^1H NMR ($CDCl_3$): 8.47 (1H, d, $J_{9,10}$ 9.2, H-10), 8.24–8.00 (8H, m, H-2 to H-9), 6.00 (1H, s, (*E*) =CH), 5.67 (1H, s, (*Z*) =CH).

1-Ethynylpyrene (III). Finely powdered KOH (582 mg, 2.0 mmol) was added to a solution of 3-(1-pyrenyl)-3-chloro-2-propenal (**IIa, IIb**) in dioxane (10 ml) under argon, and the reaction mixture was refluxed for 2 h (the solution grows dark), cooled to 20°C, diluted with 5% aqueous solution of citric acid (1.5 ml), and evaporated to dryness. The residue was distributed between water (15 ml) and CH_2Cl_2 (50 ml); the organic phase was washed with water (10 ml), dried, evaporated, and chromatographed on a silica gel column (2 × 10 cm) in 15% CH_2Cl_2 in petroleum ether. The corresponding fractions were pooled and evaporated, and the residue was dried in vacuum to afford (**III**) (369 mg (81%) as colorless crystals), R_f 0.56 (petroleum ether–CH_2Cl_2 1 : 1); mp 117.5–118.5°C (hexane) (mp 105–106°C [45], 112–114°C [44], 113–114°C [43], 116–117.5°C [46]). Mass-spectrum (EI), $(m/z)^+$: 226 (100), 198 (17); calc. for $C_{18}H_{10}$ 226.28. ^1H NMR ($CDCl_3$): 8.60 (1H, d, $J_{9,10}$ 8.8, H-10), 8.25–8.02 (8H, m, H-2 to H-9), 3.61 (1H, s, ≡CH).

The scaling-up does not affect the yield: interaction of 5.815 g (20 mmol) of 3-(1-pyrenyl)-3-chloro-2-propenal (**IIa, IIb**) and 2.806 g (50 mmol) of KOH gave 3.674 g (81%) of alkyne (**III**).

5'-*O*-(4,4'-Dimethoxytrityl)-5-(1-pyrenylethynyl)-2'-deoxyuridine (IX). A. 5-(1-Pyrenylethynyl)-2'-deoxyuridine (**VI**) (263 mg, 0.58 mmol) was twice evaporated with dry pyridine (2 × 30 ml), then dissolved in dry pyridine (70 ml), and evaporated by a third. To the resulting solution under vigorous stirring was added 4,4'-dimethoxytrityl chloride (354 mg, 1.045 mmol), and the reaction mixture was left for 12 h at room temperature. After evaporation, the residue was dissolved in ethyl acetate (150 ml), washed with saturated $NaHCO_3$ (100 ml) and water (2 × 100 ml), dried, and evaporated. The product was purified by chromatography on silica gel (column 3.5 × 10 cm, 0 ⟶ 5% gradient of methanol in benzene + 1% triethylamine) and dried in vacuum to afford 307 mg (70%; light-yellow foam). R_f 0.31 (benzene–methanol 9 : 1). ^1H NMR ($CDCl_3$): 8.46 (1H, d, $J_{9'',10''}$ 9.0, H-10"), 8.32 (1H, s, H-6), 8.19–8.14 (2H, m, H-6", H-8"), 8.08–7.98 (2H, m, H-4", H-5", H-7"), 7.93 (1H, d, $J_{2'',3''}$ 7.9, H-2"), 7.91 (1H, d, $J_{9'',10''}$ 9.0, H-9"), 7.62 (1H, d, $J_{2'',3''}$ 7.9, H-3"), 7.49 (2H, m, ArH (DMT)), 7.38 (4H, m, ArH (DMT)), 7.25 (2H, m, ArH (DMT)), 7.09 (1H, m, ArH (DMT)), 6.75 (4H, m, ArH (DMT)), 6.42 (1H, dd, $J_{1',2'\alpha}$ 5.6, $J_{1',2'\beta}$ 7.2, H-1'), 4.59 (1H, m, H-3'), 4.11–4.19 (2H, m, H-4', H-5'a), 3.52 (1H, s, OCH_3), 3.49 (1H, s, OCH_3), 3.37 (1H, dd, $^2J_{5'a,2'b}$ 10.7, $J_{5'b,4'}$ 3.3, H-5'b), 2.60 (1H, ddd, $^2J_{2'\alpha,2'\beta}$ 14.0, $J_{1',2'\alpha}$ 5.6, $J_{2'\alpha,3'}$ 2.2, H-2'α), 2.40 (1H, m, H-2'H2'β).

B. To a solution of 5'-*O*-(4,4'-dimethoxytrityl-5-iodo-2'-deoxyuridine (**VIII**) (525 mg, 0.80 mmol), 1-ethynylpyrene (**III**) (191 mg, 0.84 mmol) and triethylamine (167 μl, 1.2 mmol) in DMF (50 ml) under argon were successively added $Pd(PPh_3)_4$ (92.5 mg, 0.08 mmol) and CuI (30.5 mg, 0.16 mmol), and the reaction mixture was stirred for 16 h at room temperature. Then, the mixture was diluted with CH_2Cl_2 (200 ml), washed with 3% aqueous EDTA-$(NH_4)_2$ (5 × 200 ml) and water (5 × 200 ml), dried, and evaporated

to dryness. The residue was chromatographed on a silica gel column (4.5 × 10 cm) in a 0 → 3% gradient of methanol in benzene. The isolated substance (604 mg) was dissolved in 5 ml of CH_2Cl_2, and the solution was added dropwise to 50 ml of hexane; the precipitate formed was filtered off, washed with hexane, and dried in a vacuum to afford 523 mg (87%) of the title compound as a light-yellow amorphous powder.

3'-*O*-(Diisopropylamino-2-cyanoethoxyphosphinyl)-5'-*O*-(4,4'-dimethoxytrityl)-5-(1-pyrenylethynyl)-2'-deoxyuridine (X). 5'-*O*-(4,4'-Dimethoxytrityl)-5-(1-pyrenylethynyl)-2'-deoxyuridine (**IX**) (307 mg, 0.41 mmol) was evaporated with dry acetonitrile (2 × 30 ml) and then dissolved in 70 ml of dry acetonitrile. To the resulting solution were added diisopropylammonium tetrazolide (141 mg, 0.82 mmol) and 2-cyanoethoxybisdiisopropylaminophosphine (852 μl, 2.68 mmol), and the mixture was evaporated by a third and left for 12 h at room temperature (TLC monitoring). The reaction mixture was evaporated to dryness; the residue was dissolved in ethyl acetate (150 ml) and successively washed with saturated $NaHCO_3$ (2 × 100 ml), saturated NaCl (100 ml), and water (100 ml). The organic layer was dried and evaporated, and the product was isolated by chromatography (column 3 × 10 cm, a 0 → 20% gradient of ethyl acetate in benzene + 1% triethylamine) to afford 210 mg (54%) of the title compound. R_{f1} 0.61; R_{f2} 0.49 (ethyl acetate–benzene 1 : 1; diastereomers). ^{31}P NMR (CD_3CN): 151.910, 151.843 (diastereomers, ca. 2 : 1).

Modified support (XI). Support LCAA-CPG 500 Å (300 mg) acylated by succinic anhydride according to [58] was suspended in a DMF–pyridine mixture (1 : 1; 4 ml) containing monotritylated nucleoside (**IX**) (189 mg, 0.25 mmol), DIC (280 μl, 1.8 mmol), and DMAP (20 mg) and stored for 48 h at room temperature. Then, a solution of pentafluorophenol (100 mg) in pyridine (1 ml) was added, and the mixture was kept for a further 12 h. The support was filtered off, suspended in 5% solution of pyrrolidine in pyridine (3 ml), kept for 10 min, filtered again, and washed successively with chloroform, methanol, acetonitrile, and ether (10 ml each). The nucleoside loading of the support, determined by the dimethoxytrityl cation absorption [60], was 56.5 μmol/g.

1-Phenylethynylpyrene. Triethylamine (570 μl, 4.09 mmol), CuI (38 mg, 0.2 mmol), Ph_3P (52 mg, 0.2 mmol), and $PdCl_2$ (28 mg, 0.1 mmol) were added to a solution of 1-ethynylpyrene (**III**) (452 mg, 2.0 mmol) and iodobenzene (470 μl, 4.2 mmol) in benzene (30 ml), and the stirring continued for 16 h at room temperature. The reaction mixture was evaporated to dryness, the residue was dissolved in chloroform (100 ml) and successively washed with 100-ml portions of water, 1% citric acid, 3% EDTA-$(NH_4)_2$ (thrice), and water again. The organic phase was dried and evaporated, the residue was chromatographed on silica gel (column 36 × 95 mm) in a 25 → 55% gradient of benzene in petroleum ether to afford 422 mg (70%) of compound (**XII**), mp 119.5–120.5°C (petroleum ether–benzene; mp 120–122°C [69]); R_f 0.63 (benzene). Mass-spectrum, $(m/z)^+$: 302 (MALDI-TOF); calc. for $C_{24}H_{14}$ 302.37. UV spectrum (THF): λ_{max}, nm (ε, M^{-1} cm^{-1}): 283 (34900), 293 (49 400), 349 sh. (26700), 364 (46000), 384 (44700); λ_{min}: 287 (30000), 308 (4100), 374 (30000).

Modified oligonucleotides. The solid phase phosphoramidite oligonucleotide synthesis was performed in the standard regimen corresponding to the manufacturer's recommendations. As a support, macroporous glass CPG-500 was used with grafted 5'-*O*-protected nucleosides (Millipore) or the modified support (**XI**). The modifying reagent (**X**) lyophilized from benzene was dissolved in dry acetonitrile to a concentration of 0.1 M; the condensation step with its participation, like in the case of the conventional nucleoside phosphoramidites, took 30 s. After completion of the synthesis and elimination of the 5'-terminal DMT-group, the support with protected oligonucleotides was deblocked by treatment with ammonia (25% aqueous NH_3, 60°C, 5 h). The resulting solution was evaporated, and the residue was twice reprecipitated from 2 M $LiClO_4$ with a 5 to 10-fold volume of acetone. Then, the oligonucleotide was isolated by electrophoresis in 20% PAG, eluted from the gel with 0.5 M $LiClO_4$, reprecipitated with acetone, and desalted on a 1 × 8 cm column with Sephadex G-25 (medium) in "salt-free" buffer (100 μM Tris-HCl, 10 μM EDTA-Na_2, pH 8.0).

Analytical HPLC used a reverse-phase column (C-18 SOTA, 4.5 × 250 mm) and a linear gradient (5 → 50%) of acetonitrile in 0.1 M NH_4OAc for 45 min.

Duplexes were prepared from equimolar amounts of each of conjugates (**XVII**)–(**XIX**) and oligonucleotide (**XIII**) in buffer containing 0.1 M NaCl, 0.01 M KH_2PO_4/K_2HPO_4, pH 7.0; the mixture was kept for 5 min at 95°C and then cooled down to 20°C within 1 h.

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