1-(Phenylethynyl)pyrene and 9,10-Bis(phenylethynyl)anthracene, Useful Fluorescent Dyes for DNA Labeling: Excimer Formation and Energy Transfer

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A series of novel modifying reagents, including phosphoramidites and solid supports, have been synthesized, and used for the introduction of 1-(phenylethynyl)pyrene (PEPy) and 9,10-bis(phenylethynyl)anthracene (BPEA) fluorescent dyes into predetermined positions of synthetic oligonucleotides. These two fluorophores have been shown to constitute an energy donor–acceptor pair, and can be used as such in fluorescent oligonucleotide probes, designed for the detection and structural studies of nucleic acids. The sensitivity of the probe to duplex formation is demonstrated. The formation of the PEPy and BPEA excimers is reported for the first time on nucleic acids.

Introduction

Fluorescent techniques are used extensively in nucleic acid research. Fluorescent labels give a direct, simple-to-register signal, and the processes involving such groups can be easily monitored in real time, repeatedly, in biomolecule-friendly conditions, and even in vivo.[1]

Currently, methods based on the detection of changes in the emission of interacting pairs of fluorophores (identical or not) make up a growing field, both in terms of the development of these methods and of their application. An example from this field is the formation of excimers (exciplexes) from two spatially proximal planar fluorophores linked to a biomolecule. Excimer fluorescence has been observed in aqueous solutions of pyrene-linked nucleic acids and their analogues.[2] Changes in the excimer/monomer fluorescence intensity ratio allowed the detection of complementary complexes, including antiparallel duplexes,[2c,2d,2f,2h,2i,2m–2o,2r,2t–2v,2x,2y] parallel duplexes,[2a,2b,2e,2k] and triplexes.[2g] Excimer emission from oligonucleotides containing 5-(1-pyrenylethynyl)uracil,[3] *trans*-stilbene,[4] and perylene[5] has also been reported.

In the cases where two different dyes were used, a relative proximity of the two moieties and an overlap of the wavelengths of one dye's emission with those of the other dye's excitation may result in fluorescence resonance energy transfer (FRET). FRET is useful in the homogeneous detection of biomolecular interactions.[1a,6] Recent examples of the application of FRET in nucleic acid chemistry include structural studies of the complexes of nucleic acids[7] and of ribozymes,[8] detection of specific sequences based on energy transfer,[9] and FRET on DNA with multiple[10] and "tunable"[11] fluorophores.

It is interesting, therefore, to broaden the range of fluorescent dyes suitable for nucleic acid labeling, and to develop new methods for their site-specific introduction into oligonucleotides. Herein we describe the synthesis of functionalized derivatives of two fluorescent aromatic hydrocarbons, 1-(phenylethynyl)pyrene (PEPy) and 9,10-bis(phenylethynyl)anthracene (BPEA), and the fluorescence properties of single- and double-stranded labeled oligonucleotides. Preliminary communications on the PEPy and BPEA reagents have been published.[12]

Results and Discussion

9,10-Bis(phenylethynyl)anthracene (BPEA) is one of the most efficient fluorescent compounds known.[1b,13] Several substituted bis(phenylethynyl)anthracenes have been reported,[1b,14] but to the best of our knowledge, this dye has been

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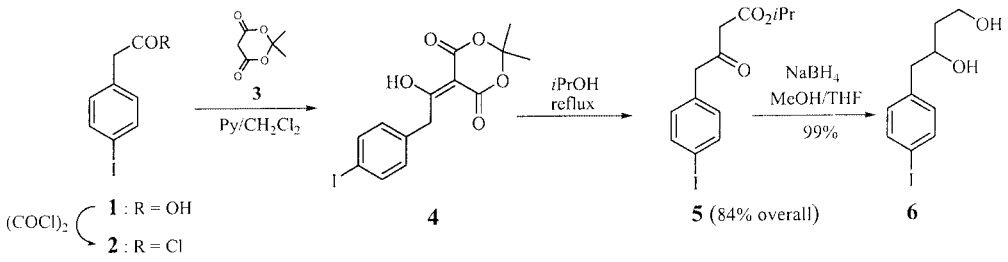


Scheme 1. Preparation of (iodophenyl)-diol **6**

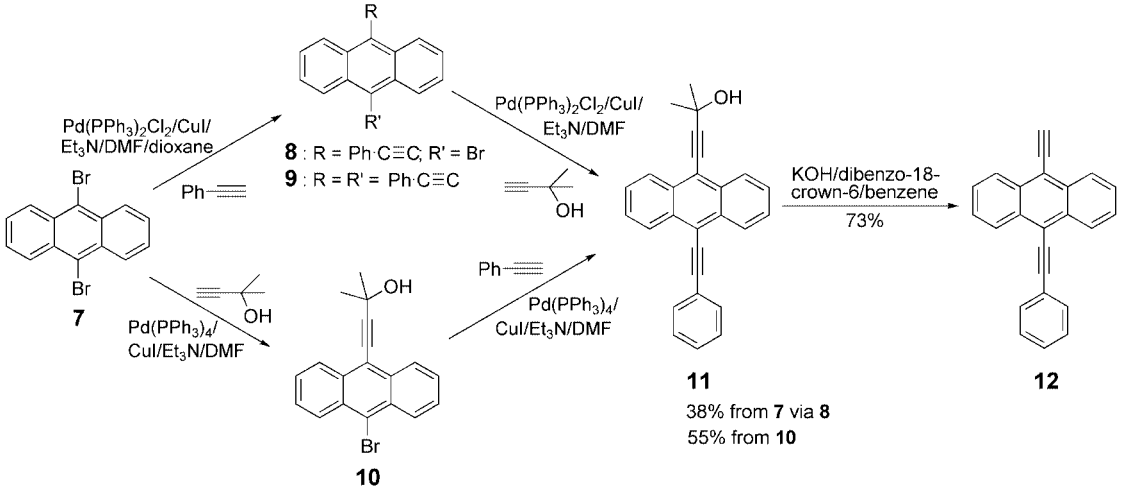
never used for the covalent labeling of biomolecules. Another parent dye, 1-(phenylethynyl)pyrene (PEPy), is a considerably less-studied fluorophore.[3] The introduction of the phenylethynyl group into a fluorescent molecule usually leads to a bathochromic shift in absorbance and emission, as well as an increase in the quantum yield of emission. This has been shown for naphthalene,[15] anthracene and tetracene,[16] pyrene,[17] perylene,[18] 2-phenylbenzoxazole,[19] bimanes,[20] Eu^{III} chelates,[21] coumarins,[22] BODIPY,[23] and fluorescein.[24]

Suitably functionalized phenylethynyl derivatives of pyrene and anthracene could be promising tools for the development of fluorescent probes for structural studies of nucleic acids. These compounds exhibit fluorescence within a range of 400−550 nm, have high quantum yields, and are chemically stable and capable of various non-covalent interactions. In addition, their spectral characteristics suggest that they can potentially be used as a donor−acceptor pair in non-radiative energy transfer. We therefore embarked on the synthesis of functionalized derivatives of PEPy and BPEA. Such derivatives could be used to prepare specifically modified oligonucleotides, or to study the spectral properties of these dyes' conjugates. We tried to mimic the 3′- and 5′-hydroxy groups of natural nucleosides by introducing a 1,3-butanediol group into these fluorophores (compounds **14** and **17**). 4-(4-Iodophenyl)-1,3-butanediol (**6**) was a key compound in the synthesis (Scheme 1).

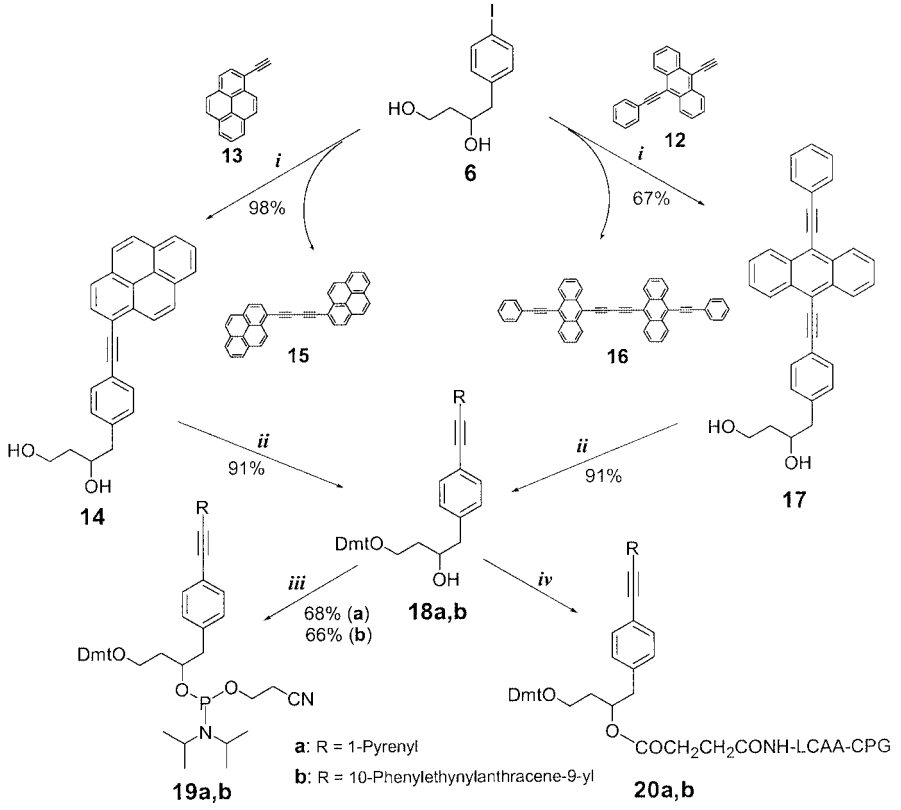
(4-Iodophenyl)acetyl chloride (**2**), obtained from the acid **1** by treatment with oxalyl chloride, was used to acylate 2,2-dimethyl-1,3-dioxane-4,6-dione (**3**) (Meldrum's acid),[25] resulting in the (4-iodophenyl)acetyl derivative **4**. Alcoholysis of the cyclic acylal **4** with 2-propanol, accompanied by decarboxylation, led to ester **5**, which crystallized as an individual keto tautomer (only two-proton singlets at δ = 3.62 and 3.84 ppm, and no lower-field one-proton singlets, were present in the ^{1}H NMR spectrum). Reduction with $NaBH_4$ in a boiling MeOH/THF mixture[26] gave the desired 4-(4-iodophenyl)-1,3-butanediol (**6**) in 99% yield.

We envisaged that the iodine atom could easily be displaced by ethynyl derivatives of polyaromatic hydrocarbons, such as 9-ethynyl-10-(phenylethynyl)anthracene (**12**) (prepared as shown in Scheme 2) or 1-ethynylpyrene (**13**) (commercially available), under Sonogashira reaction conditions.[27] The resulting pseudonucleosides, after routine dimethoxytritylation and phosphitylation (Scheme 3), may then serve as monomers in automated oligonucleotide synthesis by the phosphoramidite method.

1-Ethynylpyrene (**13**) was coupled with 4-(4-iodophenyl)-1,3-butanediol (**6**) in the presence of tetrakis(triphenylphosphane)palladium, copper(I) iodide, and triethylamine in DMF, affording 4-[4-(1-pyrenylethynyl)phenyl]-1,3-butanediol (**14**) in high yield. For all of the diol **6** to react, a moderate excess of **13** was required, as the alkyne was also consumed in a side-reaction: the Glaser oxidative dimerization, leading to 1,4-bis(1-pyrenyl)buta-1,3-diyne (**15**). Although the reaction was carried out under argon, the formation of 5−10% of this diacetylene, which is sparingly soluble in



Scheme 2. Preparation of alkyne derivative **12**



Scheme 3. Preparation of PEPy and BPEA phosphoramidites and solid supports; reagents: i: $[Pd(PPh_3)_4]$/CuI/Et_3N/DMF; ii: DmtCl/Py; iii: $(iPr_2N)_2POCH_2CH_2CN$/diisopropylammonium tetrazolide/MeCN; iv: LCAA-CPG-$NHCOCH_2CH_2CO_2H$/*N*,*N*′-diisopropylcarbodiimide/DMAP/DMF

common solvents, could not be avoided. Using standard methods of nucleoside chemistry, pseudonucleoside **14** was converted into phosphoramidite **19a** and support **20a**. These materials were then used in automated DNA synthesis to incorporate the pseudonucleoside into a number of predetermined positions in oligonucleotide chains. We describe here how these labeled oligonucleotides can be used as fluorescent probes, with the label moiety being potentially sensitive to changes in its microenvironment; for example, the interaction of such a labeled probe with an oligonucleotide with the complementary nucleic acid sequence.

We were tempted to synthesize a fluorescent pseudonucleoside which could serve as an energy acceptor for the substituted pyrene **14**. We chose to prepare BPEA derivatives, compounds that emit in the visible region and exhibit high quantum yields.[1b] The starting material, 9,10-dibromoanthracene (**7**), was allowed to react with phenylacetylene in the presence of bis(triphenylphosphane)palladium dichloride, copper(I) iodide, and triethylamine, in a 1,4-dioxane/DMF mixture under argon (Scheme 2), resulting in a mixture of 9-bromo-10-(phenylethynyl)anthracene (**8**), unchanged **7**, and the parent BPEA (**9**). Whereas **9** was easily removed by flash chromatography, dibromide **7** proved to be difficult to separate completely. Therefore, the mixture of mono- and dibromides **7** and **8** was used directly in the next coupling with 2-methyl-3-butyn-2-ol, from which the disubstituted anthracene **11** was obtained in 38% yield over two steps. Compound **11** was also obtained by an alternative method, starting from 9-bromo-10-(3-hydroxy-3-methylbutyn-1-yl)anthracene (**10**). Precursor **10** reacted with phenylacetylene under Sonogashira conditions to give, after chromatographic purification, a 55% yield of **11**. Deprotection of the ethynyl residue and formation of 9-ethynyl-10-(phenylethynyl)anthracene (**12**) were achieved by heating with powdered KOH in benzene in the presence of dibenzo-18-crown-6. After flash chromatography, compound **12** was used in the next Sonogashira reaction with aryl iodide **6** to give the BPEA-substituted 1,3-butanediol **17** in 67% yield. The diol **17** was converted into phosphoramidite **19b** and support **20b**, similarly to the PEPy derivatives (Scheme 3).

The excitation and emission spectra of pseudonucleosides **14** and **17** are shown in Figure 1. The compounds exhibit strong fluorescence in MeOH, with $^{max}\lambda_{em}$ = 389 and 410 nm for the pyrene derivative **14**, and 471 and 501 nm for the anthracene derivative **17**. It is noteworthy that the emission band of **14** overlaps with the excitation band of **17**, and in fact, these dyes constitute a potential donor−acceptor pair in non-radiative energy transfer. Therefore, this pair may be useful in the construction of probes for FRET-based determination of intra- and intermolecular distances on biomolecules.[1a]

Starting from pyrene-diol **14**, we prepared dimethoxytrityl derivative **18a**, phosphoramidite **19a**, and solid support **20a** (Scheme 3), using conventional methods of nucleoside chemistry.[28] Similar BPEA reagents **19b** and **20b** were ob-

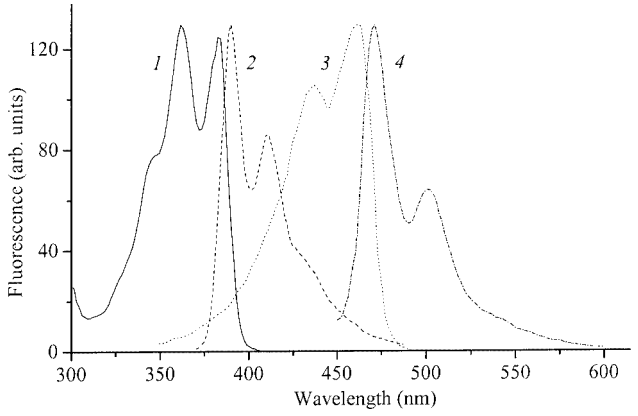


Figure 1. Normalized excitation spectra of diols **14** (*1*, λ_{em} = 440 nm) and **17** (*3*, λ_{em} = 520 nm), and fluorescence spectra of **14** (*2*, λ_{ex} = 320 nm) and **17** (*4*, λ_{ex} = 400 nm) in MeOH (10^{-6} M)

tained from the anthracene pseudonucleoside **17**. These reagents were used for introduction of single or multiple PEPy or BPEA residues into predetermined positions of oligonucleotides.

We synthesized a series of modified oligonucleotides **21**−**34** corresponding to a fragment of the gene for the hypothetical eukaryotic transcription factor *fet5*$^+$ of *Schizosaccharomyces pombe*[29] (Table 1). Between one and three substitutions of the PEPy or BPEA pseudonucleosides were made for dA or dT units. An identical coupling cycle was used for all of the phosphoramidites, which were added as 0.1 M solutions in MeCN. Under these conditions, the efficiency of the coupling of a modified phosphoramidite to the next standard nucleoside phosphoramidite, as determined by the acid-catalyzed release of Dmt$^+$ cation,[30] was ca. 99% for **19a** and 95% for the somewhat bulkier **19b**.

After standard ammonia-mediated deprotection, all the synthetic oligonucleotides **21**−**34** were isolated by 20% denaturing PAGE. A control treatment of compounds **14** and **17** with ammonia did not reveal (TLC) any transformation products. The modifications introduced into the oligomers reduced their gel mobility. Within a set of equally long oligonucleotides (i.e. **21**, **23**, **24**, and **25**; or **21**, **29**, **30**, and **31**), mobility decreased with an increase in the number of modified units. The contributions of the PEPy and BPEA moieties were almost equal, and roughly corresponded to one nucleotide increase in length.

The oligonucleotides were then analysed by MALDI-TOF mass spectrometry and reverse-phase HPLC. Not surprisingly, the highly hydrophobic modifications considerably increased the retention time values of the conjugates (Table 1).

We showed that the modified pseudonucleosides also affected the thermal stability of oligonucleotide duplexes. A PEPy or BPEA residue dangling in the terminal position of a duplex produced a stabilizing effect, as estimated from the melting curves for duplexes **22·26** and **22·32** (Table 2). Similar results were obtained upon studying pyrene-containing complementary heptanucleotides,[31] where the stabilizing effect was ascribed to stacking of an aromatic polycyclic system on the adjacent nucleobases.

The lower degree of stabilization in our case may be due to the shorter linker arm, and the fact that only one terminus is stabilized, which may be less favourable for stack-

Table 2. Duplex stability of modified oligonucleotides

Duplex	Sequence (**Y** − PEPy, **Z** − BPEA)[a]	T_m [°C]	ΔT_m [°C]
21·22	(5′) ACGAGGAAAGCGTAA (3′) TGCTCCTTTCGCATT	57.4	
22·26	(5′) ACGAGGAAAGCGTAA**Y** (3′) TGCTCCTTTCGCATT	61.9	+4.5
22·23	(5′) ACGAGG**Y**AAGCGTAA (3′) TGCTCCTTTCGCATT	56.0	−1.4
22·32	(5′) ACGAGGAAAGCGTAA**Z** (3′) TGCTCCTTTCGCATT	59.0	+1.6
22·29	(5′) ACGAGG**Z**AAGCGTAA (3′) TGCTCCTTTCGCATT	50.6	−6.8

[a] For conditions, see Exp. Sect.

Table 1. Oligonucleotides synthesized for fluorescence measurements

Compound	Sequence (**Y** − PEPy, **Z** − BPEA)	R_t[a]	MALDI-TOF mass spectra, $[M + H]^+$, found/calcd.
21	(5′) ACGAGGAAAGCGTAA	10.2	4658.8/4660.1
22	(3′) TGCTCCTTTCGCATT	11.8	4483.5/4485.9
23	(5′) ACGAGG**Y**AAGCGTAA	19.9	4797.3/4799.3
24	(5′) ACGAGG**YY**AGCGTAA	32.8, 34.3[b]	4936.0/4938.5
25	(5′) ACGAGG**YYY**GCGTAA	44.1, 44.5[b]	5078.2/5077.8
26	(5′) ACGAGGAAAGCGTAA**Y**	25.8	5112.1/5112.5
27	(3′) TGCTCC**Y**TTCGCATT	19.7	4633.1/4634.2
28	(3′) TGCTCC**YY**TCGCATT	29.9	4780.0/4782.4
29	(5′) ACGAGG**Z**AAGCGTAA	26.9	4876.7/4875.4
30	(5′) ACGAGG**ZZ**AGCGTAA	32.5, 33.1, 33.6[b]	5089.4/5090.7
31	(5′) ACGAGG**ZZZ**GCGTAA	37.1, 37.7, 38.3[b]	5304.9/5306.1
32	(5′) ACGAGGAAAGCGTAA**Z**	39.5	5189.9/5188.6
33	(3′) TGCTCC**Z**TTCGCATT	23.5	4709.8/4710.3
34	(3′) TGCTCC**ZZ**TCGCATT	30.9	4933.1/4934.6

[a] For HPLC conditions, see Exp. Sect. [b] Peaks of diastereomers.

ing. At the same time, the dye residues in the middle of the strand somewhat destabilize the duplexes, with this effect being much more pronounced in the case of BPEA (Table 2). The PEPy pseudonucleoside can effectively replace deoxyadenosine (duplex **22·23**). However, the melting temperatures remained sufficiently high for any spectral studies to be performed.

The introduction of both fluorescent labels into the oligonucleotides was confirmed by the appearance, in addition to the inherent oligonucleotide absorption maximum around 260 nm, of maxima due to the dyes, in the region of 350−400 nm for PEPy, and 420−500 nm for BPEA.

Next, we studied the luminescent characteristics of the fluorescently labeled oligonucleotides and duplexes. The fluorescence spectra were registered in aqueous phosphate buffer (pH = 7.0) at an excitation wavelength of 370 nm for PEPy- and 430 nm for BPEA-containing oligonucleotides.

As Figure 2 shows, the introduction of a single PEPy residue (oligomer **23**) results in a characteristic monomeric fluorescence spectrum, with maxima at 401 and 423 nm (curve *1*). When a second, adjacent, PEPy residue appears (i.e. in **24**), the monomeric fluorescence decreases approximately sixfold, and an intense excimer fluorescence with a maximum at 507 nm (curve *2*) can be seen. Moving to the case of three adjacent PEPy residues (i.e. in **25**) results in a complete disappearance of the monomeric fluorescence, a slight hypsochromic shift of the excimer maximum to 505 nm (curve *3*), and some decrease in its intensity, apparently because of self-quenching.

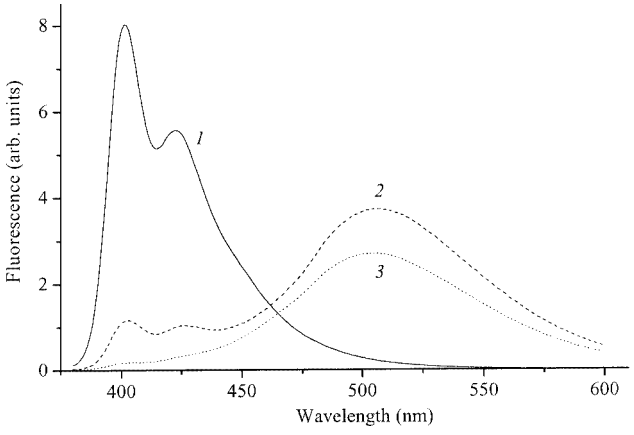


Figure 2. Fluorescence spectra of modified oligonucleotides **23** (*1*), **24** (*2*), and **25** (*3*); λ_{ex} = 370 nm

Since a single-stranded oligonucleotide **24** containing two adjacent PEPy residues showed an intense excimer fluorescence, we then checked whether an excimer would be formed in the case of a duplex in which two dye residues, one in each of the complementary strands, were positioned opposite one another (duplex **23·27**). It can be seen from Figure 3 that formation of a duplex (curve *2*) does not substantially affect the intrinsic luminescent properties of the PEPy residues (curve *1*), nor does any major change occur in the case of duplex **24·28**, which has two adjacent PEPy residues in each strand (curve *3*). Duplex formation only leads to the complete disappearance of the residual monomeric fluorescence of a single-stranded doubly-modified oligomer (curve *4*; cf. curve *3*).

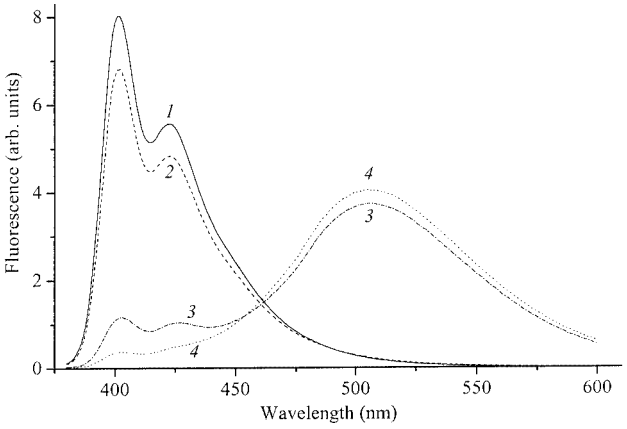


Figure 3. Fluorescence spectra of modified oligonucleotides **23** (*1*) and **24** (*3*), and duplexes **23·27** (*2*) and **24·28** (*4*); λ_{ex} = 370 nm

On the other hand, a dramatic change in the emission spectrum of the bis(modified) oligonucleotide **24** occurred upon formation of a duplex with unmodified oligonucleotide **22**: the excimer fluorescence that was seen in a spectrum of **24** alone (Figure 4, curve *1*) almost completely disappeared, giving way to intense monomer fluorescence (Figure 4, curve *2*). This change may be caused by the fact that duplex formation impedes the interaction of the two PEPy residues, and, as a consequence, diminishes the probability of the formation of an excited dimer. Hence, oligonucleotides bearing two adjacent fluorophore residues may be promising tools in constructing sensitive and specific probes for nucleic acids. It should be noted that in the case of the similar oligonucleotide **25**, which contains three neighboring dye residues, duplex formation with **22** is not sufficient to decouple all the interactions of the dye residues: excimer fluorescence of **25** remains virtually unchanged, with no monomeric fluorescence detectable (Figure 4, curve *3*).

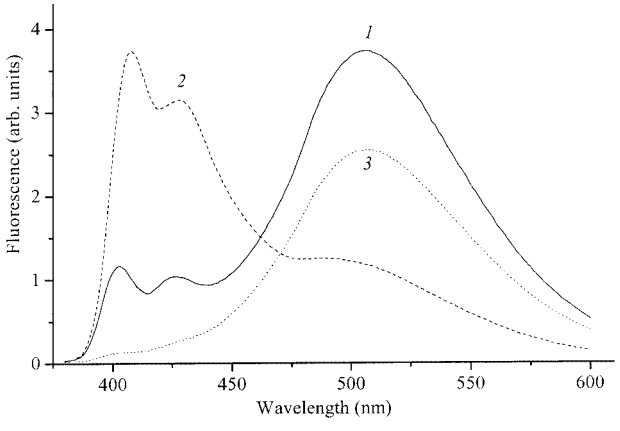


Figure 4. Fluorescence spectra of modified oligonucleotide **24** (*1*) and duplexes **22·24** (*2*) and **22·25** (*3*); λ_{ex} = 370 nm

In contrast to the PEPy derivatives, the fluorescence spectra of the BPEA conjugates **27**−**29** are essentially indepen-

dent of the number of dye residues in the molecule (they all display maxima at 487 and 517 nm),[12b] and are not affected by hybridization with complementary oligonucleotides.

At the same time, modification of both strands of a duplex (duplex **29·33**), leads to a strong quenching of fluorescence and the appearance of a new band in the region of 530−630 nm (Figure 5, curve *1*), probably due to excimer formation. Excimer emission has previously been detected for some fluorophores whose excited state has a nanosecond lifetime (which is characteristic of BPEA derivatives),[14c,32] namely, *trans*-stilbene[4b,4c] and perylene[5] residues that were attached to nucleic acids and were spatially close due to duplex formation. In duplex **30·34**, where two pairs of adjacent fluorophores are in the opposite strands, the excimer band is absent (Figure 5, curve *2*). Apparently, the formation of an excimer, which is possible in the case of duplex **29·33**, is precluded in duplex **30·34**, possibly because of increased steric hindrance.

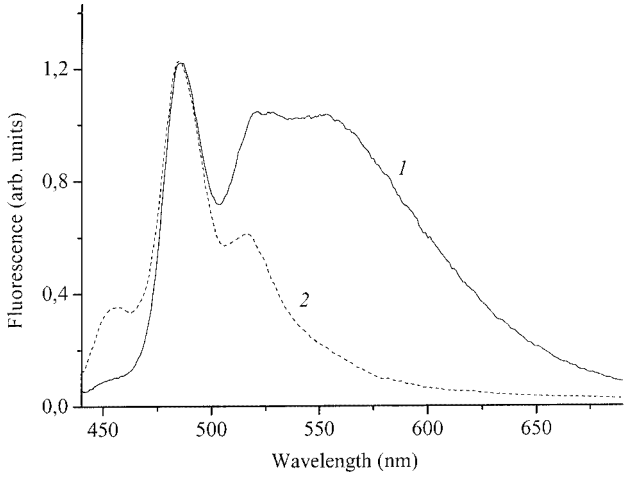


Figure 5. Fluorescence spectra of modified duplexes **29·33** (*1*) and **30·34** (*2*); λ_{ex} = 430 nm

A study of the spectral properties of duplexes **23·33**, **26·33**, and **24·34** (Figures 6 and 7) revealed an non-radiative energy transfer. Apparently, BPEA is an efficient energy acceptor for PEPy. Hybridization of the sequences containing these dyes results in FRET (Figure 6, curves *2*−*4*); excitation in the wavelength range in which PEPy absorbs leads to distinct BPEA fluorescence. Such a donor−acceptor pair could allow the efficient detection of duplex formation, and could therefore become a useful tool in structural studies. It should be noted that when the donor and acceptor are placed opposite each other in complementary strands (duplex **23·33**), the energy transfer between two residues is less effective (curve *2*) than when the donor is at the end of one oligonucleotide (duplex **26·33**), separated from acceptor by ca. 25 Å (curve *3*). This may be due to a higher mobility of the donor moiety in the terminal position, which in turn increases the κ^2 factor value (i.e. the possibility of attaining the most favorable orientation of dipole moments of the donor and acceptor within the lifetime of the donor's excited state). It is noteworthy that this effect leads to significant changes in the characteristic structure of the emission spectrum of BPEA; the ratio of fluorescence intensity at 490 nm versus 540 nm gradually decreases from 5 (curve *3*) to 2.2 (curve *2*) to 1.5 (curve *4*). The appearance of emission in the region of 530−580 nm may reflect the contribution of the PEPy−BPEA exciplex to the overall fluorescence.

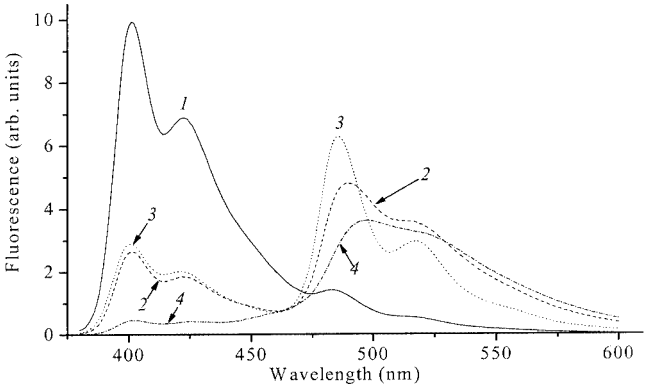


Figure 6. Fluorescence spectra of a mixture of non-complementary modified oligonucleotides **23** and **29** (*1*), and duplexes **23·33** (*2*), **26·33** (*3*), and **24·34** (*4*); λ_{ex} = 370 nm

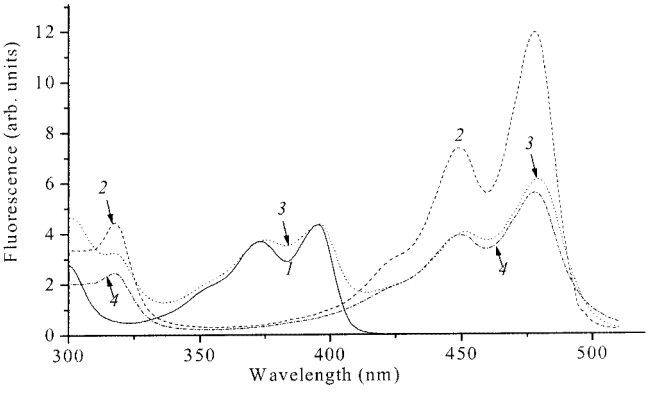


Figure 7. Excitation spectra of modified oligonucleotides **23** (*1*), λ_{em} 440 nm, **29** (*2*), and duplexes **23·33** (*3*), **29·33** (*4*), λ_{em} = 520 nm

In the case of duplex **24·34** (Figure 6, curve *4*), which contains two donors vis-à-vis two acceptors in the complementary strands, excitation with wavelength corresponding to the PEPy absorption maximum leads only to fluorescence corresponding to BPEA. This illustrates an especially effective energy transfer between two fluorophores.

The occurrence of non-radiative energy transfer was also confirmed by recording the excitation spectra (Figure 7). Curves *1* and *2* display spectral characteristics of the PEPy oligonucleotide **23** and the BPEA oligonucleotide **29**, respectively. At the same time, the excitation spectrum of duplex **23·33**, whose fluorescence occurs outside the emission region of PEPy (λ_{em} = 520 nm), clearly contains two peaks corresponding to the excitation maxima of the parent hydrocarbon (curve *3*).

Conclusion

We have described the chemical synthesis of reagents suitable for the introduction of 1-(phenylethynyl)pyrene (PEPy)

and 9,10-bis(phenylethynyl)anthracene (BPEA) residues into any predetermined position of synthetic oligonucleotides. The properties of the conjugates thus obtained might be useful in the construction of new fluorescent probes for the detection of nucleic acids or for structural studies. Duplex formation can be monitored either by observing changes in the monomer/excimer emission ratio, or by observing FRET between PEPy and BPEA.

Experimental Section

General Remarks: (4-Iodophenyl)acetic acid[33] (now available from Lancaster and Aldrich), Meldrum's acid[34] (also available from all major companies), 1-ethynylpyrene[3] (the compound available from Lancaster should be purified by chromatography using 15% $CHCl_3$ in hexanes), tetrakis(triphenylphosphane)palladium(0)[35] (the quality of commercially available batches varies), diisopropylammonium tetrazolide,[28] and (2-cyanoethoxy)bis(diisopropylamino)phosphane[36] (good quality compound is available from Fluka) were prepared as described. Other chemicals and solvents were obtained from Fluka and Aldrich. LCAA-CPG 500 Å pore size was obtained from Pierce. DMF was distilled under reduced pressure. MeCN, pyridine, and CH_2Cl_2 were distilled from CaH_2. Other solvents were used as received. TLC was performed on the Kieselgel 60 F_{254} pre-coated aluminium plates (Merck), spots were visualized under UV light (254 and 366 nm) and, in the case of dimethoxytritylated compounds, by exposing the plate to TFA vapours. Column chromatography was carried out using Kieselgel 60 (0.040−0.063 mm, Merck). 500 MHz ^{1}H and 125.7 MHz ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer and referenced to [D$_6$]DMSO (δ =2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C) and $CDCl_3$ (δ =7.25 ppm for ^{1}H). 161.9 MHz ^{31}P spectra were recorded with a Varian XR-400 spectrometer with chemical shifts (δ) referenced to 85% aq. H_3PO_4 as external standard. ^{1}H NMR coupling constants are reported in Hz and refer to the apparent multiplicities. Full ^{1}H and ^{13}C signal assignment was obtained by a combination of standard homonuclear and heteronuclear techniques (^{1}H double resonance, ^{1}H NOESY, ^{1}H-^{13}C gradient-selected HMQC and HMBC). Melting points were determined using a Boetius heating table and are uncorrected. MALDI-TOF mass spectra were recorded with a VISION 2000 spectrometer (Thermo Bioanalysis Corp). MALDI-TOF mass spectra of oligonucleotides and amidites **19a**,**b** were recorded with a Voyager-DE BioSpectrometry Workstation (PerSeptive Biosystems) in positive ion mode, using a mixture (1:1, v/v) of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH) and aqueous diammonium hydrogen citrate (80 mg/mL), mixed just before loading the samples onto a plate, as the matrix. Thermal denaturation experiments with oligonucleotide duplexes were performed with a Gilford 2400-2 spectrometer with a thermostatted cell (200 μL) in a buffer containing 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH = 7.0. Double-stranded DNA concentrations were $5\cdot10^{-6}$ M, heating rate 1 °C·min^{-1}. Fluorescence spectra were obtained using a Hitachi F-4000 spectrofluorometer at 20 °C.

Isopropyl 4-(4-Iodophenyl)-3-oxobutanoate (5): Oxalyl chloride (8.1 mL, 94.6 mmol) was added to a suspension of (4-iodophenyl)-acetic acid (12.4 g, 47.3 mmol) in benzene (20 mL). The acid dissolved within a few hours to give a greenish solution, which was kept at room temperature for 24 h, after which time the solvents were evaporated in vacuo. The resulting (4-iodophenyl)acetyl chloride was added dropwise within 30 min to a stirred solution of Meldrum's acid (**3**) (6.29 g, 43.7 mmol) and pyridine (6.8 mL, 87.3 mmol) in dry CH_2Cl_2 (35 mL), cooled in an ice bath. The bath was removed, and stirring was continued for 1 h. The reaction mixture was diluted with $CHCl_3$ (100 mL), washed with 1 M HCl (100 mL) and H_2O (100 mL), dried with Na_2SO_4, and the solvents were evaporated. The resulting crude isopropylidene 2-[2-(4-iodophenyl)ethylidene]malonate (**4**) was mixed with 2-propanol (100 mL) and refluxed for 4 h. After concentration, the residue was purified by chromatography on silica gel using 1% (v/v) EtOAc in benzene to afford ester **5** (13.76 g, 84%) as a colorless oil, which crystallized on storage, m.p. 54 °C (benzene/hexane), R_f = 0.16 (benzene). MALDI TOF MS: obsd. 346.8 [M + H]$^+$, calcd. for $C_{13}H_{15}IO_3$ 346.2. ^{1}H NMR ([D$_6$]DMSO): δ = 1.18 (d, J = 6.2 Hz, 6 H, CH_3), 3.62 (s, 2 H, COCH_2CO), 3.84 (s, 2 H, ArCH_2), 4.91 [sept, J = 6.2 Hz, 1 H, (CH$_3$)$_2$CH], 6.99 (d, J = 8.2 Hz, 2 H, ArH), 7.67 (d, J = 8.2 Hz, 2 H, ArH) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 21.62 (2 C), 48.22, 48.93, 68.20, 92.83, 132.36 (2 C), 134.04, 137.16 (2 C), 166.71, 201.00 ppm.

4-(4-Iodophenyl)-1,3-butanediol (6): Sodium borohydride (1.22 g; 30 mmol) was added to a solution of isopropyl 4-(4-iodophenyl)-3-oxobutanoate (**5**) (2.08 g; 6.0 mmol) in THF (25 mL). The mixture was refluxed for 30 min and then, after dropwise addition of MeOH (30 mL), for a further 30 min. After cooling, the reaction mixture was diluted with an equal volume of $CHCl_3$, washed with 1 M HCl (2 × 50 mL) and H_2O (2 × 50 mL), dried with Na_2SO_4, and the solvents were evaporated to dryness. The residue was purified by chromatography on a silica gel column eluted with 1% (v/v) MeOH in $CHCl_3$ to give diol **6** as a colorless crystalline solid, yield 1.74 g (99%), R_f = 0.33 (5% MeOH in $CHCl_3$), m.p. 84 °C (benzene/hexane). MALDI-TOF MS: obsd. 292.9 [M + H]$^+$; calcd. for $C_{10}H_{13}IO_2$ 292.1. ^{1}H NMR ([D$_6$]DMSO): δ = 1.48−1.54 (m, 2 H, CH_2CH$_2$O), 2.60 (m, 2 H, ArCH_2), 3.49 (m, 2 H, CH_2O), 3.74 (m, 1 H, CHO), 4.35 (t, J = 5.1 Hz, 1 H, CH$_2$OH), 4.49 (d, J = 5.5 Hz, 2 H, CHOH), 7.02 (d, J = 8.2 Hz, 2 H, ArH), 7.61 (d, J = 8.2 Hz, 2 H, ArH), ppm.

9-(3-Hydroxy-3-methylbutyn-1-yl)-10-(phenylethynyl)anthracene (11). A: Phenylacetylene (1.02 g, 10 mmol), [Pd(PPh$_3$)$_2$Cl$_2$] (140 mg, 0.2 mmol), CuI (95 mg, 0.5 mmol), and Et$_3$N (2.09 mL, 15 mmol) were added to a solution of 9,10-dibromoanthracene (**7**) (3.36 g, 10 mmol) in DMF (50 mL) and 1,4-dioxane (20 mL) under argon. The reaction mixture was heated at 80 °C for 24 h, then cooled to room temperature. The mixture was diluted with CH_2Cl_2 (150 mL) and washed with H_2O (100 mL), 0.3 M diammonium EDTA (100 mL), and H_2O (5 × 100 mL), was dried with Na_2SO_4, and then the solvents were evaporated. Chromatography on a short silica gel column, eluting with benzene, gave 10-bromo-9-(phenylethynyl)anthracene (**8**) containing a considerable amount of the starting material, 9,10-dibromoanthracene (**7**) (4.9 g). This mixture was dissolved in DMF (60 mL), and 2-methyl-3-butyn-2-ol (1.5 g, 17.8 mmol), [Pd(PPh$_3$)$_2$Cl$_2$] (140 mg, 0.2 mmol), CuI (57 mg, 0.3 mmol), and Et$_3$N (2.02 g, 20 mmol) were added. The reaction mixture was stirred under argon at 80 °C for 48 h and then treated as above. After chromatography eluting with benzene, the yield of **11** was 1.40 g (38%), R_f = 0.66 (benzene/MeOH, 9:1, v/v), R_f = 0.45 (benzene/EtOAc, 17:3, v/v), m.p. 136−139 °C (benzene/hexane). MALDI-TOF MS: obsd. 360.2 [M + H]$^+$, calcd. for $C_{27}H_{20}O$ 360.5. ^{1}H NMR ([D$_6$]DMSO): δ = 1.70 (s, 6 H, CH_3), 5.82 (br.s., 1 H, OH), 7.54 (m, 3 H, ArH), 7.77 (m, 4 H, ArH), 7.88 (m, 2 H, ArH), 8.55 (m, 2 H, ArH), 8.66 (m, 2 H, ArH) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 31.84 (2 C), 64.47, 76.89, 85.86, 102.53, 110.05, 117.10, 118.24, 122.39, 126.84 (2 C), 126.95 (2 C), 127.61 (2 C), 127.84 (2 C), 129.09 (2 C), 129.49, 131.39 (2 C), 131.44 (2 C),

131.79 (2 C) ppm. **B:** 9-Bromo-10-(3-hydroxy-3-methylbutyn-1-yl)-anthracene (**10**) (203 mg, 0.6 mmol), prepared by a modified method[14a] [^{13}C NMR ([D$_6$]DMSO): δ = 31.78 (2 C), 64.44, 76.46, 109.40, 117.96, 122.77, 126.96 (2 C), 127.64 (2 C), 127.70 (2 C), 128.46 (2 C), 129.64 (2 C), 132.32 (2 C) ppm] was treated in 20 mL of DMF with phenylacetylene (73 mg, 0.72 mmol), [Pd(PPh$_3$)$_4$] (69 mg, 0.06 mmol), CuI (29 mg, 0.12 mmol), and Et$_3$N (167 μL, 1.2 mmol) at 60 °C for 7 h to give 118 mg (55%) of **10**, identical by melting point and UV spectrum to the compound obtained by method **A**.

9-Ethynyl-10-(phenylethynyl)anthracene (12): Finely powdered KOH (300 mg, 5.36 mmol) and dibenzo-18-crown-6 (200 mg, 0.56 mmol) were added under argon to a solution of 9-(3-hydroxy-3-methylbutyn-1-yl)-10-(phenylethynyl)anthracene (**11**) (800 mg, 2.2 mmol) in benzene (60 mL). The mixture was heated at 60 °C for 3 h, then cooled and diluted with benzene (150 mL), then washed with H$_2$O (100 mL), 5% citric acid (100 mL), and H$_2$O (100 mL). The organic layer was dried (Na$_2$SO$_4$) and the solvents were evaporated. The residue was purified by chromatography on silica gel eluted with benzene to give alkyne **12** (490 mg, 73%), R_f = 0.68 (hexane/benzene, 1:4, v/v), m.p. > 300 °C (benzene/hexane). MALDI-TOF MS: obsd. 303.3 [M + H]$^+$, calcd. for C$_{24}$H$_{14}$ 302.4. ^{1}H NMR (CDCl$_3$): δ = 4.07 (s, 1 H, ≡C*H*), 7.44 (m, 3 H, Ar*H*), 7.62 (m, 4 H, Ar*H*), 7.77 (m, 2 H, Ar*H*), 8.62 (m, 2 H, Ar*H*), 8.68 (m, 2 H, Ar*H*) ppm.

4-[4-(1-Pyrenylethynyl)phenyl]-1,3-butanediol (14): 1-Ethynylpyrene (**13**) (250 mg, 1.1 mmol), Et$_3$N (280 μL, 2 mmol), CuI (38 mg, 0.2 mmol), and [Pd(PPh$_3$)$_4$] (116 mg, 0.1 mmol) were added to a stirred solution of 4-(4-iodophenyl)-1,3-butanediol (**6**) (292 mg, 1 mmol) in DMF (20 mL) under argon. After 24 h at room temperature, TLC showed that the reaction had gone to completion. The precipitate of butadiyne **15** was filtered off, and the reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL) and washed with 0.3 M diammonium EDTA (2 × 50 mL) and H$_2$O (5 × 50 mL). The organic layer was dried (Na$_2$SO$_4$) and the solvents were evaporated. The residue was purified by chromatography on a silica gel column eluted with EtOAc/benzene (3:2, v/v) to give diol **13** (422 mg, 98%), m.p. 120−122 °C (benzene), R_f = 0.53 (THF), 0.22 (EtOAc). MALDI-TOF MS: obsd. 391.9 [M + H]$^+$, calcd. for C$_{28}$H$_{22}$O$_2$ 390.5. ^{1}H NMR ([D$_6$]DMSO): δ = 1.45−1.62 (m, 2 H, C*H*$_2$CH$_2$O), 2.74 (m, 2 H, ArC*H*$_2$), 3.54 (m, 2 H, C*H*$_2$O), 3.85 (m, 1 H, C*H*O), 4.35 (t, *J* = 5.0 Hz, 1 H, CH$_2$O*H*), 4.57 (d, *J* = 5.5 Hz, 2 H, CHO*H*), 7.35 (d, *J* = 8.1 Hz, 2 H, C$_6$H$_4$), 7.68 (d, *J* = 8.1 Hz, 2 H, C$_6$H$_4$), 8.10−8.42 [m, 8 H, Ar*H* (pyrene)], 8.61 (d, *J* = 8.8 Hz, 1 H, pyrene 10-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = ca. 40, 43.95, 58.26, 68.62, 87.86, 95.71, 117.14, 119.92, 123.59, 123.85, 125.01, 125.09, 126.09 (2 C), 126.91, 127.39, 128.47, 128.96, 129.68, 130.12 (2 C), 130.68, 131.01 (2 C), 131.17, 131.35 (2 C) ppm.

4-{4-[10-(Phenylethynyl)anthracen-9-ylethynyl]phenyl}-1,3-butanediol (17): This compound was prepared in a similar manner, from 4-(4-iodophenyl)-1,3-butanediol (**6**) (292 mg, 1.0 mmol), [Pd(PPh$_3$)$_4$] (23 mg, 0.02 mmol), CuI (10 mg, 0.05 mmol), Et$_3$N (1.4 mL, 10 mmol), and alkyne **12** (a concentrated benzene solution after the deprotection of 1.3 mmol of compound **11**) in DMF (20 mL), except that the reaction was carried out at 25 °C for 12 h. Side product **16** was filtered off, and the subsequent workup was similar to that for compound **14**. After chromatography with a gradient from 0 to 50% Et$_2$O in benzene, the yield of diol **17** was 410 mg (67%), R_f = 0.46 (benzene/MeOH, 4:1, v/v), m.p. 195−197 °C (benzene). MALDI-TOF MS: obsd. 466.1 [M + H]$^+$, calcd. for C$_{34}$H$_{26}$O$_2$ 466.6. ^{1}H NMR ([D$_6$]DMSO): δ = 1.45−1.62 (m, 2 H, C*H*$_2$CH$_2$O), 2.76 (m, 2 H, ArC*H*$_2$), 3.54 (m, 2 H, C*H*$_2$O), 3.86 (m, 1 H, C*H*O), 4.35 (t, *J* = 5.0 Hz, 1 H, CH$_2$O*H*), 4.57 (d, *J* = 5.5 Hz, 2 H, CHO*H*), 7.39 (d, 2 H, *J* = 8.2 Hz, C$_6$H$_4$), 7.54 (m, 3 H, Ph), 7.80 (m, 6 H, anthracene, C$_6$H$_4$), 7.90 (m, 2 H, Ph), 8.68 (m, 4 H, anthracene) ppm.

***O*^{1}-(4,4′-Dimethoxytrityl)-4-[4-(1-pyrenylethynyl)phenyl]-1,3-butanediol (18a):** 4-[4-(1-Pyrenylethynyl)phenyl]-1,3-butanediol (**14**) (290 mg, 0.743 mmol) was co-evaporated with dry pyridine (2 × 20 mL), dissolved in dry pyridine (30 mL), and the solvents evaporated to two thirds of their volume. 4,4′-Dimethoxytrityl chloride (266 mg, 0.785 mmol) was added with vigorous stirring, and the reaction mixture was stirred at room temperature. After 24 h, the reaction was complete according to TLC. The mixture was diluted with CH$_2$Cl$_2$ (100 mL) and washed with a saturated Na$_2$CO$_3$ solution (2 × 50 mL). The organic layer was dried with Na$_2$SO$_4$ and the solvents were evaporated. The residue was purified by chromatography on a silica gel column (3.5 × 15 cm) eluted with 0−5% of EtOAc in benzene containing 0.1% Et$_3$N. Yield of compound **18a** as a yellow amorphous solid was 471 mg (91%), R_f = 0.32 (benzene/EtOAc, 95:5, v/v). MALDI-TOF MS: obsd. 694.0 [M + H]$^+$, calcd. for C$_{34}$H$_{26}$O$_2$ 692.8. ^{1}H NMR ([D$_6$]DMSO): δ = 1.66 (m, 2 H, C*H*$_2$CH$_2$O), 2.70 (m, 2 H, ArC*H*$_2$), 3.09 (m, 2 H, C*H*$_2$O), 3.73 (s, 6 H, OC*H*$_3$), 3.86 (m, 1 H, C*H*O), 4.58 (d, *J* = 5.5 Hz, 1 H, CHO*H*), 6.88 [d, *J* = 8.6 Hz, 4 H, Ar*H* (Dmt)], 7.22 [d, *J* = 8.6 Hz, 4 H, Ar*H* (Dmt)], 7.28−7.39 [m, 7 H, Ar*H* (Ph, C$_6$H$_4$)], 7.68 [d, *J* = 8.0 Hz, 2 H, Ar*H* (C$_6$H$_4$)], 8.10−8.42 [m, 8 H, Ar*H* (pyrene)], 8.63 [d, *J* = 9.2 Hz, 1 H, (pyrene 10-H)] ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 37.02, 43.92, 55.16 (2 C), 60.55, 68.66, 85.41, 87.93, 95.70, 113.27 (4 C), 117.13, 119.99, 123.60, 123.85, 125.01, 125.11, 126.11 (2 C), 126.69, 126.92, 127.40, 127.83 (2 C), 127.90 (2 C), 128.48, 128.97, 129.74 (4 C), 130.06 (2 C), 130.68, 130.96, 131.03, 131.18, 131.40 (2 C), 136.25 (2 C), 140.91, 145.41, 158.12 (2 C) ppm.

***O*^{1}-(4,4′-Dimethoxytrityl)-4-{4-[10-(phenylethynyl)anthracen-9-ylethynyl]phenyl}-1,3-butanediol (18b)** was prepared from the bis-(phenylethynyl)anthracene diol **17** (500 mg, 1.07 mmol) and 4,4′-dimethoxytrityl chloride (360 mg, 1.07 mmol) in pyridine. The title compound was purified by chromatography on silica gel in 0−10% MeOH in benzene. The yield was 750 mg (91%) of an amorphous orange solid, R_f = 0.41 (benzene/EtOAc, 95:5, v/v). MALDI-TOF MS: obsd. 770.1 [M + H]$^+$, calcd. for C$_{55}$H$_{44}$O$_4$ 768.9. ^{1}H NMR (CDCl$_3$): δ = 1.75−1.85 (m, 2 H, C*H*$_2$CH$_2$O), 2.20−2.30 (m, 1 H, C*H*HO), 2.80−3.00 (m, 2 H, ArC*H*$_2$), 3.40−3.45 (m, 1 H, CH*H*O), 4.04−4.10 (m, 1 H, C*H*O), 6.86 (d, 4 H, Ar*H*), 7.20−7.40 (m, 9 H, Ar*H*), 7.45−7.54 (m, 5 H, Ar*H*), 7.60−7.80 (m, 8 H, Ar*H*), 8.60−8.80 (m, 4 H, Ar*H*) ppm.

***O*^{3}-[(2-Cyanoethoxy)(diisopropylamino)phosphanyl]-*O*^{1}-(4,4′-dimethoxytrityl)-4-[4-(1-pyrenylethynyl)phenyl]-1,3-butanediol (19a):** *O*^{1}-(4,4′-Dimethoxytrityl)-4-[4-(1-pyrenylethynyl)phenyl]-1,3-butanediol (**18a**) (346 mg, 0.5 mmol) was co-evaporated with dry MeCN (2 × 25 mL) and dissolved in dry MeCN (150 mL). Diisopropylammonium tetrazolide (47 mg) and (2-cyanoethoxy)bis(diisopropylamino)phosphane (166 mg, 175 μL, 0.55 mmol) were added. The reaction mixture was concentrated to half its volume (bath temperature 30−35 °C) and stirred for a further 5 h, after which time the solvents were evaporated. The residue was dissolved in EtOAc (120 mL) and washed successively with brine (2 × 100 mL), 5% NaHCO$_3$ (100 mL), and H$_2$O (2 × 100 mL); the organic layer was dried with Na$_2$SO$_4$ and the solvents were evaporated. The residue was purified by chromatography on a silica gel column, eluting with CH$_2$Cl$_2$ containing 1% (v/v) Et$_3$N, to yield amidite **19a** (295 mg, 68%) as a yellow amorphous solid, R_f = 0.78 (CH$_2$Cl$_2$/hexane/Et$_3$N, 9:9:2, v/v/v). MALDI-TOF MS: obsd. 894.4

$[M + H]^+$, calcd. for $C_{58}H_{57}N_2O_5P$ 893.1. ^{31}P NMR (CD_3CN): δ = 150.349 and 151.362 ppm (1:1 mixture of diastereomers).

***O*³-[(2-Cyanoethoxy)(diisopropylamino)phosphanyl]-*O*¹-(4,4′-dimethoxytrytyl)-4-{4-[10-(phenylethynyl)anthracen-9-ylethynyl]-phenyl}-1,3-butanediol (19b):** This compound was prepared from dimethoxytritylated diol **18b** (700 mg, 0.91 mmol), diisopropylammonium tetrazolide (156 mg, 0.91 mmol), and (2-cyanoethoxy)bis-(diisopropylamino)phosphane (318 μL, 1 mmol) as described above. After chromatography on silica gel, eluting with 0−5% EtOAc in benzene containing 1% Et_3N, the yield of phosphoramidite **19b** was 700 mg (66%) of an amorphous orange solid, R_f = 0.51 (benzene/EtOAc, 93:7, v/v). MALDI-TOF MS: obsd. 970.1 $[M + H]^+$, calcd. for $C_{64}H_{61}N_2O_5P$ 969.2. ^{31}P NMR (CD_3CN): δ = 150.31 and 151.34 ppm (1:1 mixture of diastereomers).

Solid Supports 20a,b: These were prepared by a modification of a published method.[37] LCAA CPG-500 Å (300 mg) was suspended in a 3% (v/v) TFA solution in CH_2Cl_2 (10 mL), stirred at room temperature for 4 h, filtered, washed successively with 10-mL portions of an MeCN/Et_3N (9:1, v/v) mixture, CH_2Cl_2, and Et_2O, and dried in vacuo over P_4O_{10}. A solution of succinic anhydride (300 mg, 3 mmol) and DMAP (80 mg, 0.66 mmol) in dry pyridine (10 mL) was added to this support under argon, and the mixture was left at room temperature for 24 h with occasional swirling. After filtration, successive washes with 10-mL portions of pyridine, CH_2Cl_2, and Et_2O, and drying over P_4O_{10}, the succinylated LCAA CPG was suspended in DMF/pyridine (4 mL, 1:1, v/v); monotritylated pseudonucleoside **18a** (173 mg, 0.25 mmol) (or 192 mg of **18b**), 1,3-diisopropylcarbodiimide (280 μL, 1.8 mmol), and DMAP (20 mg) were added; and the suspension was left at room temperature for 48 h. To block the remaining carboxylic groups, a solution of pentafluorophenol (100 mg) in pyridine (1 mL) was then added, and the mixture was kept at room temperature for a further 12 h. The support was filtered, resuspended in a 5% (v/v) solution of pyrrolidine in pyridine (3 mL), and allowed to react for 10 min. The support was filtered again, washed successively with 10-mL portions of $CHCl_3$, MeOH, MeCN, and Et_2O, and dried in vacuo. Loadings of the pseudonucleoside were determined by treating a portion (5−7 mg) of the support with 1 mL of 3% (w/v) CCl_3CO_2H in 1,2-dichloroethane and measuring the absorbance of the resulting dimethoxytrityl cation at 504 nm (ε = 75 mL·cm^{-1}·μmol^{-1});[30] these were found to be 44 μmol/g (**20a**) and 35 μmol/g (**20b**).

Modified Oligonucleotides: The solid-phase phosphoramidite oligonucleotide synthesis was performed with an automated synthesizer ASM-102 U (BIOSSET, Russia) according to the manufacturer's recommendations. LCAA-CPG-500 charged with 5′-*O*-Dmt-protected nucleosides (Millipore), or modified supports **20a**,**b**, were used as carriers. Dye phosphoramidites **19a**,**b** were lyophilized from benzene prior to use, and dissolved in dry acetonitrile to a concentration of 0.1 M. A coupling step for these monomers lasted 30 s, as with the conventional nucleoside amidites. After completion of the synthesis and removal of the 5′-terminal Dmt group, the oligonucleotides were cleaved from the support and deprotected by 25% aq. NH_3 (60 °C, 5 h). The supernatant fluid was evaporated, and the residue was precipitated twice from 1 M $LiClO_4$ with a 5−10-fold excess of acetone. The resulting oligonucleotides were isolated by 20% denaturing (7 M urea) PAGE in Tris-borate buffer, pH = 8.3. The bands, visualized by illumination at 260 nm against a fluorescent background, were cut out of the gel, and the oligonucleotides were eluted with 0.5 M $LiClO_4$, precipitated with acetone, and desalted on Sephadex G-25 in a "saltless" buffer (100 μM Tris/HCl, 10 μM disodium EDTA, pH = 8.0). Further purification was achieved by RP-HPLC on a SOTA C_{18} column 4.5 × 250 mm, linear gradient from 5 to 50% MeCN in 0.1 M NH_4OAc for 60 min, or linear gradient from 5 to 45% MeCN in 0.1 M NH_4OAc for 40 min. Analytical HPLC was performed under the same conditions. Duplexes were prepared from the equimolar amounts of corresponding oligonucleotides in 10 mM potassium phosphate buffer (pH = 7.0) supplemented with 100 mM NaCl and 0.1 mM EDTA by heating for 5 min at 95 °C and cooling down to 20 °C within 1 h. Fluorescence spectra were recorded in this buffer at the resulting concentrations of oligonucleotides and duplexes, which was $1 \cdot 10^{-7}$ M.

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