Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 4617–4626

Oligonucleotides containing new fluorescent 1-phenylethynylpyrene and 9,10-bis(phenylethynyl)anthracene uridine-2′-carbamates: synthesis and properties☆

Natalia N. Dioubankova, Andrei D. Malakhov, Zakhar O. Shenkarev and Vladimir A. Korshun*

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya 16/10, Moscow 117997, Russia

Received 19 December 2003; revised 1 March 2004; accepted 25 March 2004

Abstract—The synthesis of two novel fluorescent uridine-2′-carbamate phosphoramidites is described. The reagents carrying fluorescent polyaromatic hydrocarbons 1-phenylethynylpyrene (PEPy) or 9,10-bis(phenylethynyl)anthracene (BPEA) are suitable for oligonucleotide synthesis. Prepared oligonucleotide conjugates show strong dye emissions at 401 and 485 nm, but low FRET rate when located in the oligonucleotide duplex. The dyes show considerable compensation of the usual carbamate duplex destabilization. The possible explanation of both effects is binding of PEPy and BPEA to the minor groove of the DNA duplex.

1. Introduction

Oligonucleotides conjugates with fluorescent dyes have numerous and diverse applications in molecular biology, bioorganic chemistry, and medicine. The sugar part of nucleosides, particularly the 2′-position, is an attractive site for modification, for example, for potential antisense applications.[1] Examples of the introduction of hetero- and polyaromatic residues at the 2′ position of nucleosides include dansyl,[2] anthracene,[3] anthraquinone,[4] fluorescein,[5] nalidixic acid,[6] porphyrins,[7] 1,8-naphthalimide,[8] and pyrene.[9] Aromatic ligands attached to the 2′-positions of ribonucleotides in oligonucleotides are placed in the minor groove and can affect duplex stability. Pyrene, a fluorescent tetracyclic aromatic hydrocarbon, is of particular interest because of its abilities to give a response in the fluorescent spectrum upon hybridization of pyrene-labeled oligonucleotides[9b,e–i,k,n] and to form excimers.[9n,o,q] Recently, we developed a convenient method of 2′-carbamate modification of uridine with pyrene,[9n] and non-nucleotide modifying reagents for introducing new fluorescent polyaromatic hydrocarbons 1-phenylethynylpyrene (PEPy) and 9,10-bis(phenylethynyl)anthracene (BPEA) into oligonucleotides.[10] PEPy and BPEA were shown to constitute an energy donor–acceptor pair, and were also able to form excimers. Here, we report the introduction of PEPy and BPEA in the 2′-position of uridine as well as the fluorescent and thermal denaturation properties of modified oligonucleotides.

2. Results and discussion

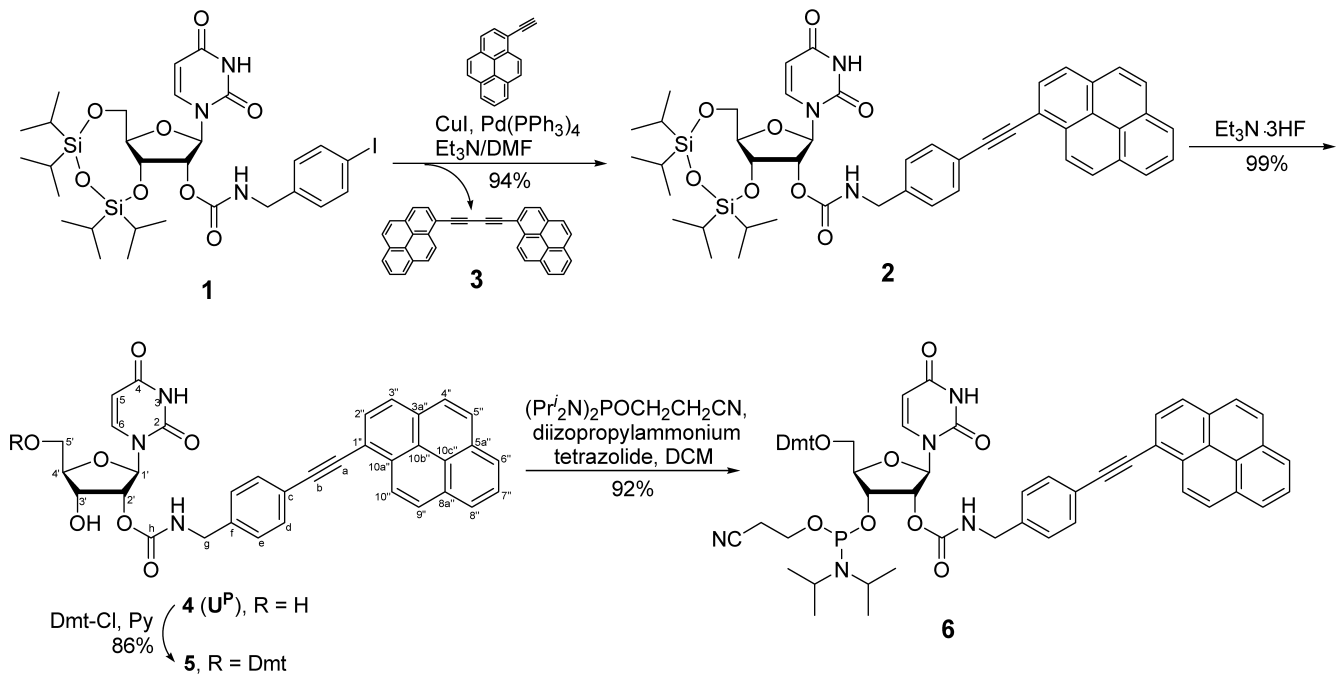
2.1. Syntheses of phosphoramidites

The first step in the preparation of PEPy-modified uridine-2′-carbamate phosphoramidite **6** is alkynylation of the starting 2′-*O*-(4-iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine **1**[9n] with 1-ethynylpyrene in the conditions of Sonogashira coupling ($Pd(PPh_3)_4$/CuI in DMF in the presence of triethylamine)[11] (Scheme 1). The reaction was performed under Ar to reduce the quantity of side product **3** from Glaser acetylene coupling. It is worthy of note, that the reacton mixture should be washed with water many times upon workup to remove the solvent (DMF), otherwise traces of **3** can accompany the main product in the course of column purification on silica gel. The Markiewicz' 3′,5′-*O*-silyl group from **2** was removed with triethylamine trihydrofluoride in THF.[9n] Crystalline nucleoside **4** ($\mathbf{U^P}$) was 5′-*O*-dimethoxytritylated, and then 3′-*O*-phosphitylated using standard methods of nucleoside chemistry.[12] Pyridine (not triethylamine) should be added to the eluent for neutralization of silica gel in chromatographic purification of 5′-*O*-Dmt derivative **5**, because more basic conditions can lead to

☆ Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2004.03.076

Keywords: Modified oligonucleotides; Polycyclic aromatic hydrocarbons; Fluorescence; Duplex stability.
* Corresponding author. Tel./fax: +7-95-3306738;
e-mail address: korshun@mail.ibch.ru

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.03.076

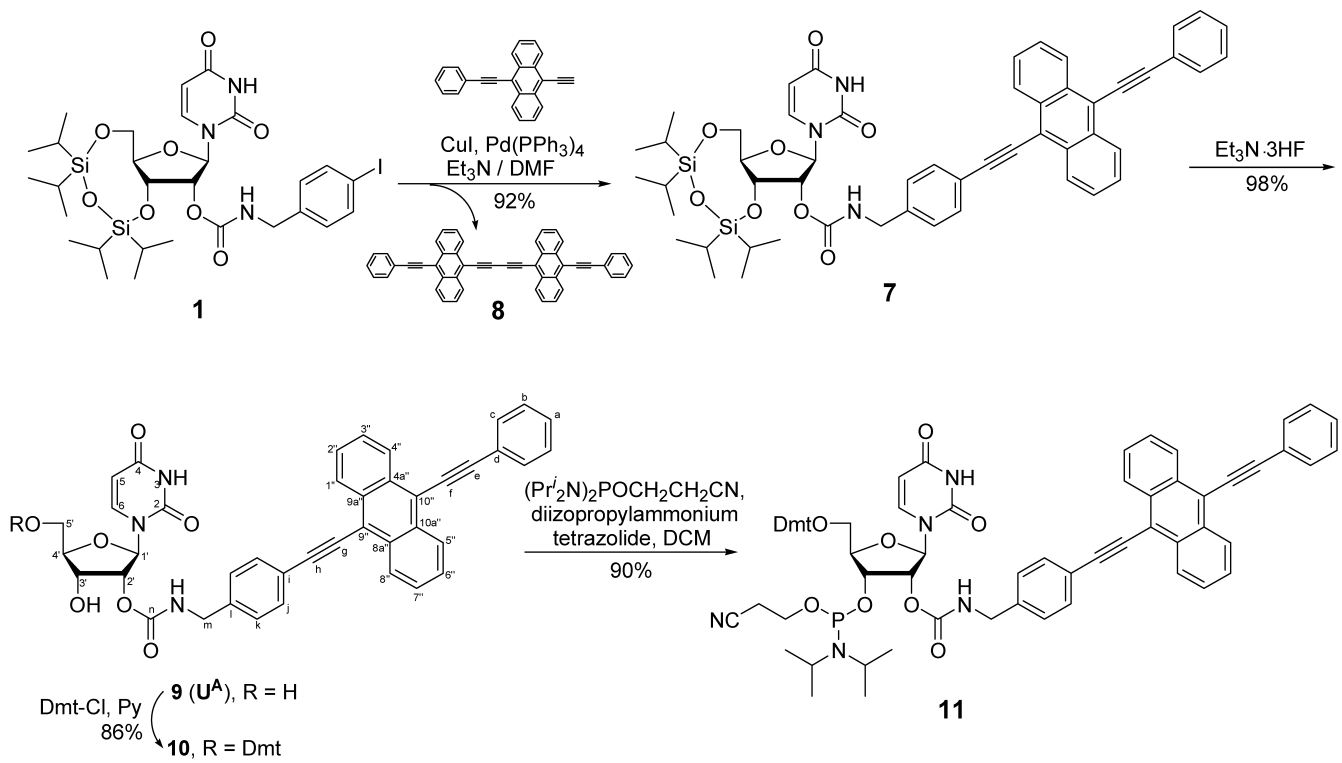


Scheme 1. Synthesis of PEPy uridine-2′-carbamate phosphoramidite **6**.

2′→3′ migration of the carbamoyl group.[9n] A small amount of bis-3′,5′-*O*-Dmt nucleoside **5a** was also isolated. Phosphoramidite **6** was isolated as a solid suitable for prolonged storage (2 years).

It was also tempting to synthesize a similar fluorescent nucleoside which could serve as an energy acceptor for the PEPy nucleoside $\mathbf{U^P}$. BPEA nucleoside $\mathbf{U^A}$ and its 5′-*O*-Dmt-3′-*O*-phosphoramidite derivative **11** were prepared similar to PEPy compounds (Scheme 2). 9-Ethynyl-10-phenylethynylanthracene[10] was used for the assembly of a dye molecule.

The structures of modified nucleosides $\mathbf{U^P}$ and $\mathbf{U^A}$ were confirmed by $^1H-^{13}C$ NMR correlations. The first step in the assignment of ^{13}C NMR spectra was distinguishing the proton-bound carbon atoms taking into account cross-peaks on HMQC spectra. The signals of non-protonated carbons



Scheme 2. Synthesis of BPEA uridine-2′-carbamate phosphoramidite **11**.

Table 1. Properties of modified and unmodified oligodeoxyribonucleotides

#	Sequence, 5′→3′	MALDI MS	Calculated mass $[M+H]^+$
ON01	CTCCCAGGCTCAAAT	4490.4	4496.0
ON02	ATTTGAGCCTGGGAG	4643.8	4643.0
ON03	CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	4930.4	4930.9
ON04	C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	4927.8	4930.9
ON05	CTCCCAGGCTCAAA**U**$^{\mathbf{A}}$p[a]	5013.4	5013.4
ON06	CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	4853.8	4854.9
ON07	C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	4855.2	4854.9
ON08	C**U**$^{\mathbf{P}}$CCCAGGC**U**$^{\mathbf{P}}$CAAAT	5214.2	5214.0
ON09	CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$p[a]	4935.8	4934.9
ON10	CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGGp[a]	6185.1	4186.1
ON11	A**U**$^{\mathbf{P}}$TTGAGCCTGGGAG	5005.9	5005.9
ON12	AT**U**$^{\mathbf{P}}$TGAGCCTGGGAG	5002.7	5005.9
ON13	ATT**U**$^{\mathbf{P}}$GAGCCTGGGAG	5007.5	5005.9
ON14	CCAGA**U**$^{\mathbf{P}}$TTGAGCCTGGGAG	6229.7	6229.2
ON15	CCAGAT**U**$^{\mathbf{P}}$TGAGCCTGGGAG	6228.7	6229.2
ON16	CCAGATT**U**$^{\mathbf{P}}$GAGCCTGGGAG	6228.2	6229.2

[a] 3′-phosphate.

were assigned using $^1H-^{13}C$ interactions through two, three and four bonds visualized as cross-peaks on HMBC spectra.[†]

2.2. Oligonucleotide synthesis

Fluorescent uridine-2′-carbamate phosphoramidites **6** and **11** were used in solid-phase oligodeoxyribonucleotide synthesis. The coupling time for the modifying reagents was increased to 10 min; the coupling yield was >95%. The sequence chosen was a 15-mer complementary to the 22–36 sequence (**ON02**) of the *trans*-activation responsive region of the human immunodeficiency virus type 1 (HIV-1) TAR RNA (**ON01**).[13] The sequences are given in Table 1. Fluorescent uridine-2′-carbamates were placed instead of internal thymidine (**ON03**, **ON06**, **ON14**-**ON16**), the thymidine located closely to the 5′-end (**ON04**, **ON07**), or at the both sites (**ON08**). Complementary unmodified (**ON02**) and modified (**ON11**-**ON16**) oligodeoxyribonucleotides were also prepared. The integrity and purity of modified oligonucleotides was analyzed by MALDI mass-spectrometry (Fig. 1, Table 1) and HPLC (Fig. 2). An insertion of such bulky moieties into oligonucleotides reduces their mobility on polyacrylamide gel (data not shown) and increases the retention time of conjugates on reverse-phase column (Fig. 2); BPEA containing oligonucleotide **ON03** is considerably more hydrophobic than PEPy labelled oligomer **ON06**.

2.3. Absorbance properties of PEPy and BPEA nucleosides

1-Phenylethynylpyrene[14] and 9,10-bisphenylethynylanthracene[15] have characteristic absorbance spectra. Figure 3 presents the comparison of UV spectra of fluorescent nucleosides $\mathbf{U}^{\mathbf{P}}$ and $\mathbf{U}^{\mathbf{A}}$ in polar solvent (water–ethanol) and Markiewicz-protected nucleosides **2** and **7** in non-polar solvent (DCM). The spectra of both chromophores in DCM have negligible long-wavelength shifts (ca. 2.5–3 nm) in comparison with their spectra in water–ethanol.

The presence of a fluorescent label in the oligonucleotides can be confirmed by UV spectra. Modified oligonucleotides, in addition to the inherent oligonucleotide absorption maximum around 260 nm, show characteristic maxima of the fluorophore, in the region of 350–400 nm (for PEPy) or 420–500 nm (for BPEA) (Fig. 4).

The absorption maxima of the fluorophore are shifted upon changing of the solvent to less polar one. An even greater bathochromic shift was observed for a dye with drastically changed microenvironment. The ‘oligonucleotide-bound’

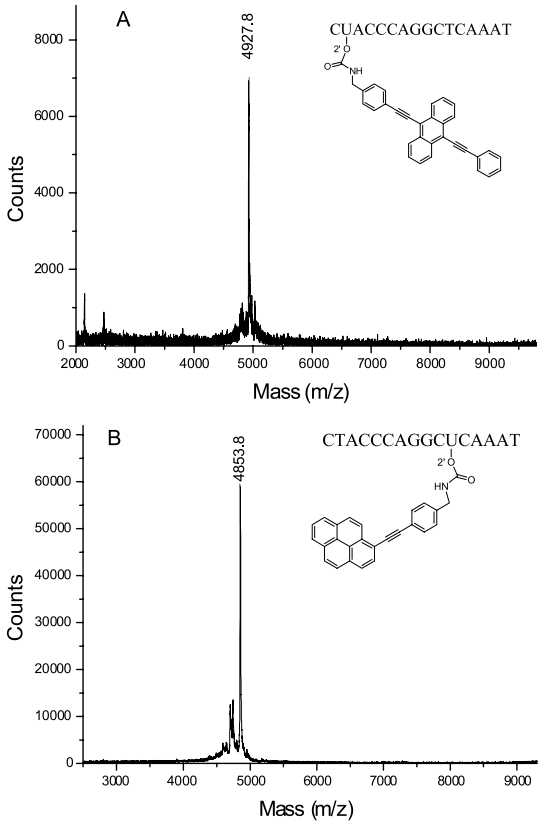


Figure 1. Examples of MALDI-TOF mass spectra: BPEA-modified oligomer **ON04** (A) and PEPy-modified oligomer **ON06** (B).

[†] See Supplementary DataApplication 1.

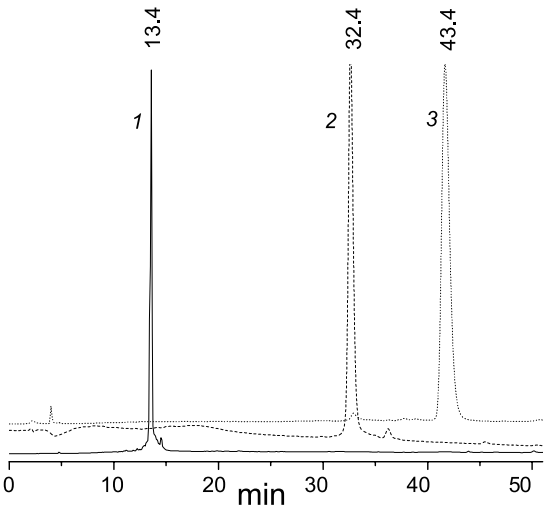


Figure 2. Examples of HPLC profiles of crude oligonucleotides: unmodified 15-mer **ON01** (1), PEPy-modified oligomer **ON06** (2) and BPEA-modified oligomer **ON03** (3). For HPLC conditions see Section 4.

PEPy has a 15 nm shift as compared to the PEPy nucleoside (plot 2 in Fig. 5). This may indicate significant hydrophobic environment of the dye and the decrease in the number of degrees of freedom for the rotational movement, for example, location in the minor groove.

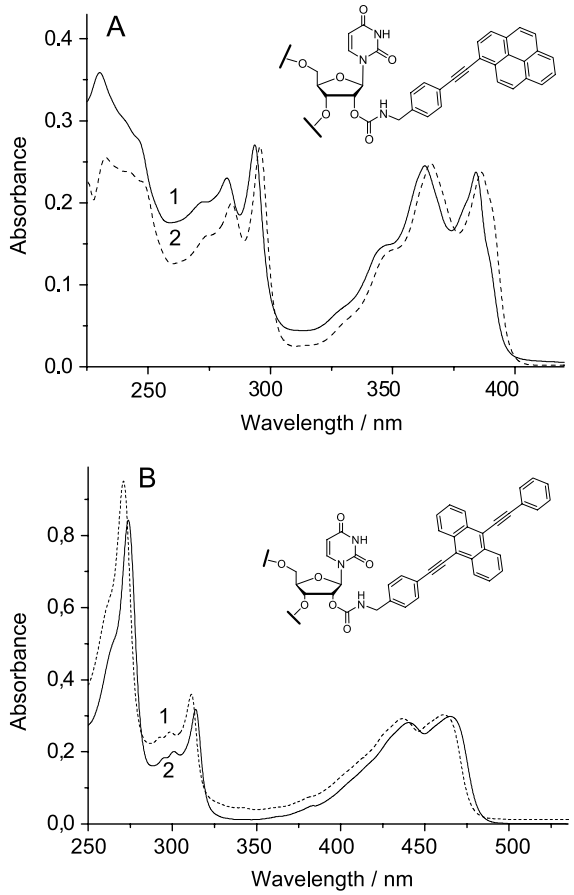


Figure 3. UV spectra of PEPy derivatives (A), normalized at dye absorbance around 360 nm and BPEA derivatives (B), normalized at dye absorbance around 460 nm. Conditions: solution $\mathbf{U}^{\mathbf{P}}$ (A, 1) or $\mathbf{U}^{\mathbf{A}}$ (B, 1) in water/ethanol 1:1 (v/v), solution of **2** (A, 2) or **7** (B, 2) in DCM.

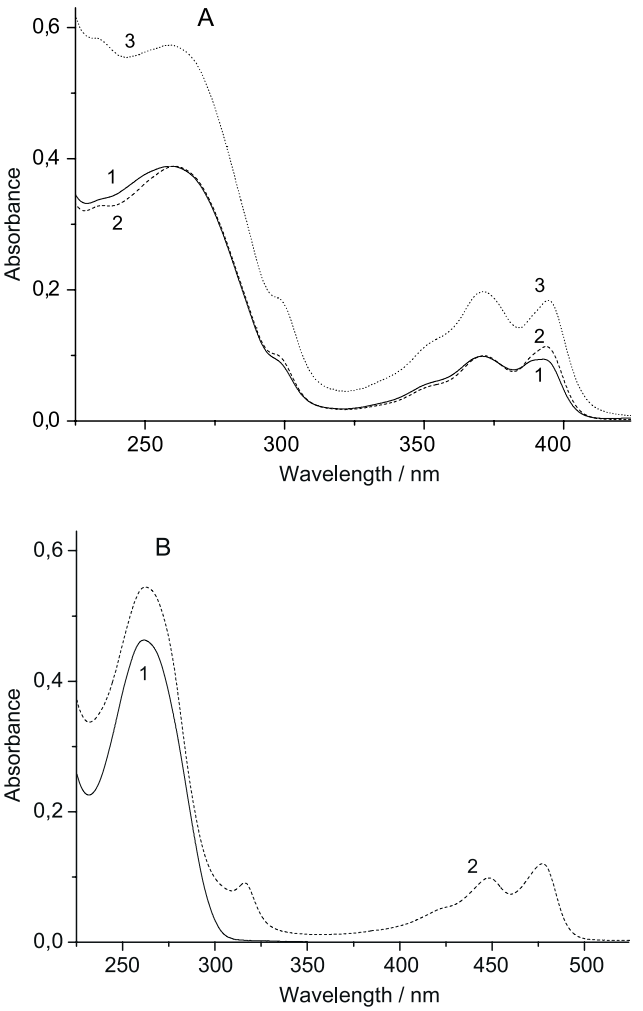


Figure 4. UV spectra of (A) PEPy-modified oligonucleotides **ON06** (1), **ON07** (2), **ON07** (3) in water, normalized by pyrene absorbance, and (B) unmodified **ON01** and BPEA-modified oligonucleotide **ON03**, normalized by oligonucleotide absorbance at 260 nm; ε_{260} for BPEA is approx. 22,000.[16]

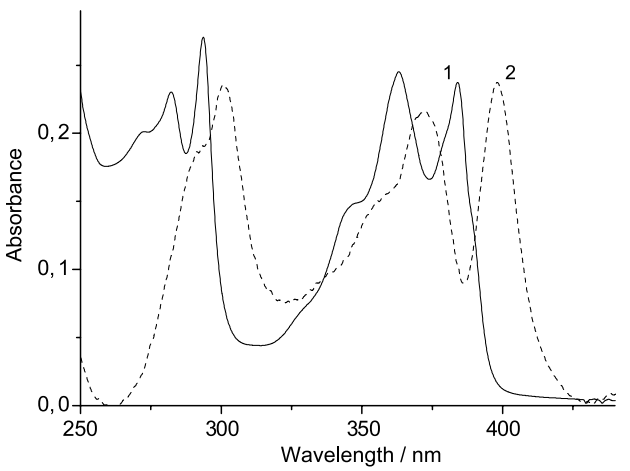


Figure 5. Comparison of the UV spectrum of PEPy-modified nucleoside $\mathbf{U}^{\mathbf{P}}$ in water/ethanol 1:1 (v/v) (1) and the difference between UV spectra of oligonucleotides **ON08** and **ON06** (2) in water, normalized by PEPy absorbance.

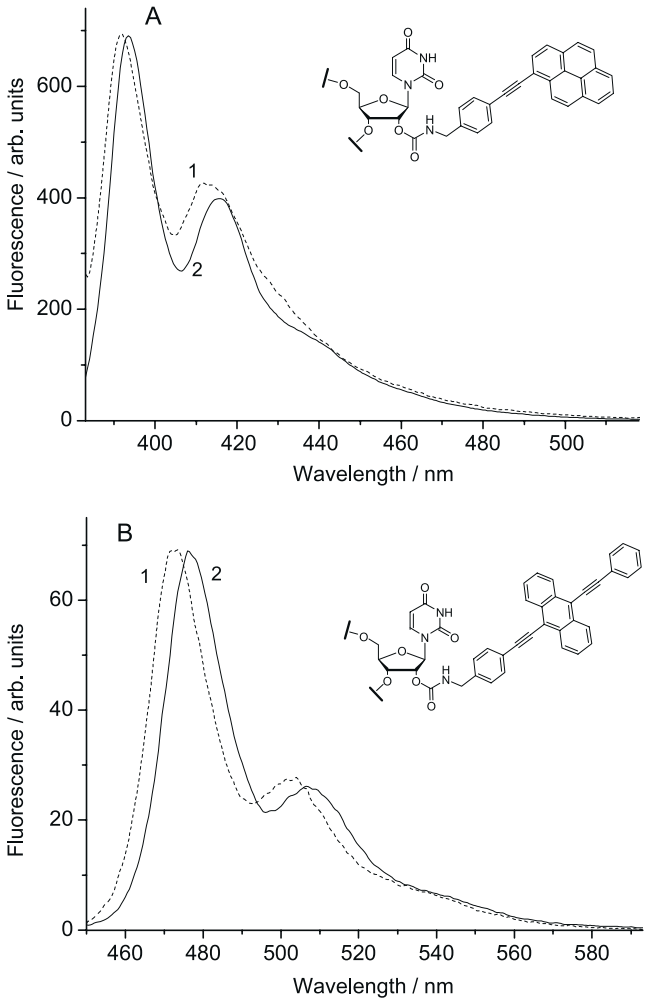


Figure 6. Fluorescence spectra of PEPy (A), and BPEA derivatives (B), normalized at fluorescence maximum. Conditions: solution $\mathbf{U}^{\mathbf{P}}$ (A, 1) or $\mathbf{U}^{\mathbf{A}}$ (B, 1) in water/ethanol 1:1 (v/v), solution **2** (A, 2) or **7** (B, 2) in DCM, hybridization buffer (Section 4).

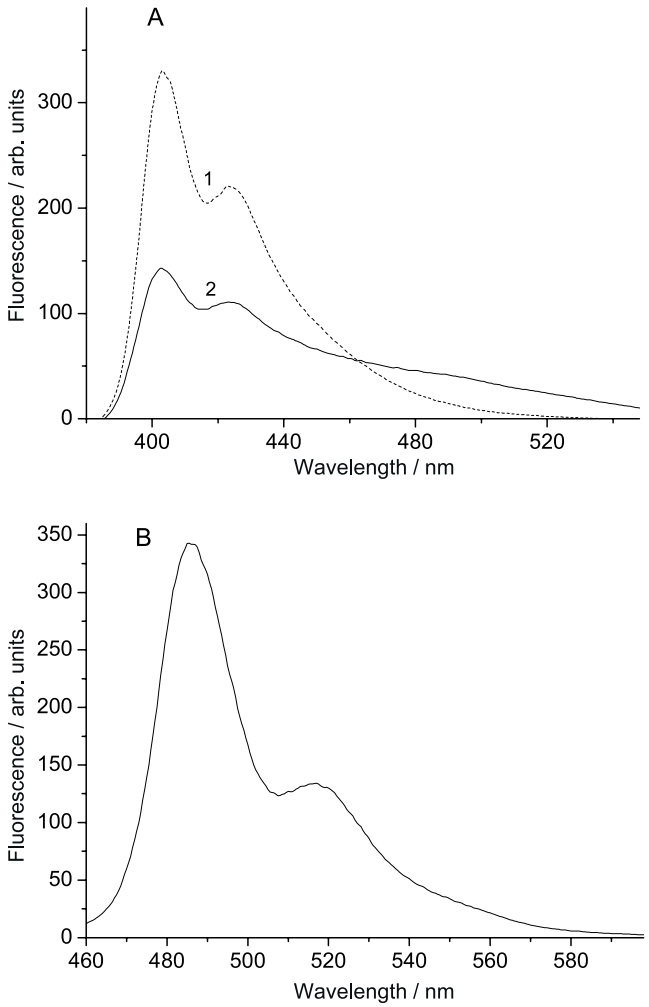


Figure 7. Fluorescence spectra of single stranded (A) PEPy-modified oligonucleotides **ON07** (mono-labeled, 1) and **ON08** (double-labeled, 2), (B) BPEA-modified oligonucleotide **ON04**.

2.4. Fluorescent measurements

The changes in fluorescent spectra of the dyes upon replacement of the solvent are similar to those for absorption. There are negligible long-wave shifts of the fluorescent bands in DCM compared to those in ethanol–water 1:1 solution for both PEPy (about 1.5 nm) and BPEA (about 4 nm) derivatives (Fig. 6).

We studied the luminescent characteristics of the labelled oligonucleotides and their duplexes. The fluorescence spectra were registered in aqueous phosphate buffer (pH 7.0) at the excitation wavelength of 375 nm for PEPy-containing oligonucleotides and 440 nm for BPEA-containing oligonucleotides. As Figure 7 shows, the introduction of a dye residue results in a characteristic monomeric fluorescence with maxima at 401, 423 nm (PEPy, A), and at 485, 516 nm (BPEA, B).

A study of the spectral properties of duplex **ON03×ON11** revealed an irradiative energy transfer. It turned out that BPEA is an energy acceptor for PEPy: hybridization of the above sequence, containing these residues, results in a fluorescence resonance energy transfer (Fig. 8A, plot 3). The effect slightly depends on the distance between fluorophores within duplexes (Fig. 8B).

2.5. Thermal denaturation studies

The negative effect of 2′-carbamate function on the thermal stability of DNA–DNA and DNA–RNA duplexes is well known.[1,9n] Because of the aromatic nature of dyes (PEPy, BPEA) the ‘carbamate’ destabilization is reduced and the melting temperatures remained sufficiently high for spectral studies to be performed (Table 2). This is caused presumably by fluorophore interaction with grooves of DNA duplexes. In cases of near-terminal positions of chromophores duplexes are more stabilized (**ON04×ON11** and **ON07×ON11**). On the other hand, a close proximity of two fluorophores placed in different strands leads to an increase in the melting temperature (cf. **ON03×ON13** and **ON03×ON11** or **ON07×ON13** and **ON07×ON11**). Moreover, the combination of BPEA and PEPy results in better stabilization than two PEPy residues (compare **ON03×ON13** and **ON07×ON13** or **ON03×ON12** and **ON07×ON12** or **ON03×ON11** and **ON07×ON11**).

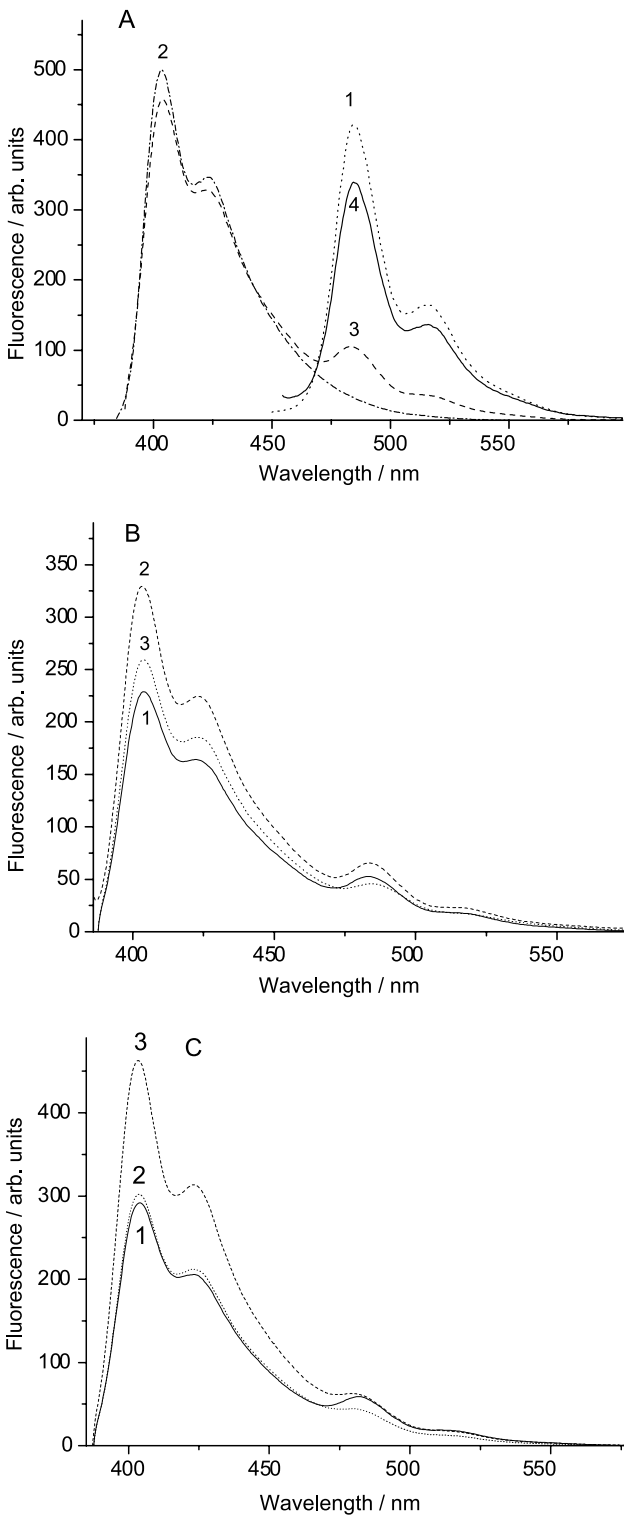


Figure 8. Fluorescence spectra of (A) single stranded BPEA-modified oligonucleotide **ON03** λ_{ex} 440 nm (1), PEPy-modified oligonucleotide **ON11** λ_{ex} 375 nm (2), and their duplex λ_{ex} 375 nm (3), λ_{ex} 440 nm (4); (B) duplexes **ON03×ON11** (1), **ON03×ON12** (2), **ON03×ON13** (3), λ_{ex} 375 nm; (C) duplexes **ON04×ON11** (1), **ON04×ON12** (2), **ON04×ON13** (3), λ_{ex} 375 nm. Conditions: hybridization buffer, concentration of each oligo 10^{-7} M.

Table 2. Thermal denaturation studies of modified oligonucleotides

#	Sequence	T_m (°C)	ΔT_m (°C)
ON01	5′-CTCCCAGGCTCAAAT	57.6	—
ON02	3′-GAGGGTCCGAGTTTA		
ON03	5′-CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	57.2	−0.4
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON03	5′-CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	57.5	−0.1
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON03	5′-CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	59.2	+1.6
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	56.0	−1.6
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	56.1	−1.5
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	57.5	−0.1
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON04	5′-C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	62.1	+4.5
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON04	5′-C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	58.0	+0.4
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON04	5′-C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	60.7	+3.1
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON07	5′-C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	62.0	+4.4
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON07	5′-C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	60.8	+3.2
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON07	5′-C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	59.5	+1.9
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	55.0	−2.6
ON14	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$AGACC		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	54.7	−2.9
ON15	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TAGACC		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	55.5	−2.1
ON16	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTAGACC		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	63.3	+5.7
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	58.7	+1.1
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	58.8	+1.2
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	62.2	+4.6
ON14	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$AGACC		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	59.0	+1.4
ON15	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TAGACC		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	58.7	+1.1
ON16	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTAGACC		

3. Summary

The results presented indicate that fluorescent hydrocarbons 1-phenylethynylpyrene (PEPy) and 9,10-bis(phenylethynyl)anthracene (PEPy), when attached through a carbamate spacer to the 2′-position of uridine and directed to the minor groove of DNA duplex, show weak FRET signal in comparison with PEPy–BPEA pair from non-nucleotide reagents. PEPy and BPEA covalently bound to duplexes give increase in T_m thus suggesting the

polyaromatics are interacting with DNA double helix. The possible explanation of the results is that PEPy and BPEA hydrocarbons are bound to the minor groove of DNA and have unfavourable spatial orientation for resonance energy transfer. Dye–DNA interaction in the minor groove can also increase DNA strands affinity and thus reduce the negative effect of 2′-carbamate modification.

4. Experimental

4.1. General

4-Iodobenzylamine, 1-ethynylpyrene were from Lancaster Synthesis; triethylamine trihydrofluoride, triethylamine, were from Aldrich; *N*,*N*-carbonyldiimidazole (CDI) was from Sigma, 4,4′-dimethoxytritylchloride (DmtCl) was from Avocado; bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine was from Fluka. 2′-*O*-(4-Iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine,[9n] 9-ethynyl-10-phenylethynyl-anthracene,[10] and diisopropylammonium tetrazolide[12] were prepared as described. DCM was always used freshly distilled over CaH_2. Anhydrous pyridine was from Aldrich; THF was freshly distilled over powdered $LiAlH_4$ and stored over 4 Å molecular sieves under nitrogen. Other solvents were used as received.

500 MHz ^{1}H, 125.7 MHz ^{13}C and 202.4 MHz ^{31}P NMR spectra were measured on a Bruker AC-500 spectrometer. Chemical shifts (δ) for ^{1}H, ^{13}C and ^{31}P are referenced to internal solvent resonances and reported relative to $SiMe_4$ (^{1}H and ^{13}C) and 85% aq. H_3PO_4 (^{31}P). ^{1}H NMR coupling constants are reported in Hz and refer to apparent multiplicities. MALDI-TOF mass spectra were measured on a Voyager-DE BioSpectrometry Workstation (PerSeptive Biosystems) in positive ion mode. Elemental analysis was performed on CHNS-analyser/EA1112 'ThermoFinnigan'. TLC and column chromatographies were carried out with Macherey-Nagel silica gel (ALUGRAM® SIL G/UV$_{254}$ and Kieselgel 60 0.040–0.063 mm). Thermal denaturation experiments with oligonucleotide duplexes were performed on a Perkin–Elmer Lambda 40 UV/VIS Spectrometer with PTP 6 (Peltier Temperature Programmer). Fluorescence spectra were obtained using a Perkin–Elmer LS 50B Luminescence Spectrometer. Thermal denaturation studies for DNA duplexes were done in a buffer containing 100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0.

4.1.1. 2′-*O*-[4-(Pyren-1-ylethynyl)phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (2). To a solution of 2′-*O*-(4-iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine[9n] (2.24 g, 3.0 mmol) in DMF (25 mL), 1-ethynylpyrene (792 mg, 3.5 mmol), CuI (130 mg, 0.68 mmol), $Pd(PPh_3)_4$ (390 mg, 0.34 mmol) and Et_3N (1.0 mL, 7.1 mmol) were added with stirring under argon. After 24 h at room temperature, TLC showed that the reaction is complete. The reaction mixture was diluted with DCM (300 mL) and washed with 0.3 M EDTA–$(NH_4)_2$ (2×200 mL), water (10×200 mL) and 5% citric acid (200 mL), the organic layer was dried and evaporated, and the residue was chromatographed on silica gel in step gradient 0⟶50% EtOAc in $CHCl_3$ (v/v). Yield 2.38 g (94.1%), yellow foam. R_f 0.31 ($CHCl_3$/EtOAc, 1:1). MALDI-TOF MS (matrix 2,4,6-trihydroxyacetophenone (2,4,6-THAP)) 846.87, 868.28. Calcd 844.34 $[M+H]^+$, 866.33 $[M+Na]^+$. ^{1}H NMR ([D$_6$]DMSO): δ 11.41 (s, 1H, H-3), 8.61 (d, 1H, $J_{9'',10''}$=9.1 Hz, H-10″), 8.41–8.21 (m, 7H, H-2″–6″,8″,9″), 8.12 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}$=7.7 Hz, H-7″), 8.05 (br t, J=6.1 Hz, OCON*H*), 7.70 (m, 3H, H-6, Hd), 7.39 (d, 2H, $J_{d,e}$=8.0 Hz, He), 5.71 (d, 1H, $J_{1',2'}$=1.5 Hz, H-1′), 5.62 (d, 1H, $J_{5,6}$=8.1 Hz, H-5), 5.41 (dd, 1H, $J_{1',2'}$=1.5 Hz, $J_{2',3'}$=5.7 Hz, H-2′), 4.51 (dd, $J_{2',3'}$=5.7 Hz, $J_{3',4'}$=8.1 Hz, 1H, H-3′), 4.27 (m, 2H, J_{NHCH}=6.1 Hz, ^2J=15.8 Hz, NCH_2), 4.11–3.88 (m, 3H, $J_{5'a,5'b}$=12.9 Hz, $J_{4,5'a}$=4.0 Hz, H-4′, H-5′), 1.07–0.97 (m, 28H, Pr^i). Anal. Calcd for $C_{47}H_{53}N_3O_8Si_2$: C, 66.88; H, 6.33; N, 4.98. Found: C, 66.96; H, 6.21; N, 5.01.

4.1.2. 2′-*O*-[4-(9-Phenylethynylanthracen-10-ylethynyl)-phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (7). To a solution of 2′-*O*-(4-iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (0.863 g, 1.157 mmol) in DMF (25 mL), 9-ethynyl-10-(phenylethynyl)anthracene[10] (350 mg, 1.157 mmol), CuI (44 mg, 0.231 mmol), $Pd(PPh_3)_4$ (134 mg, 0.157 mmol) and Et_3N (0.403 mL, 2.89 mmol) were added with stirring under argon for 24 h at room temperature. The workup was as above; the compound was isolated by chromatography on silica gel in step gradient of 0⟶35% EtOAc in $CHCl_3$ (v/v). Yield 0.98 g (92.1%), orange foam. R_f 0.31 ($CHCl_3$/EtOAc, 1:1). MALDI-TOF MS (matrix 2,4,6-THAP) 921.77, 944.23, 960.48. Calcd 921.21 $[M+H]^+$, 943.20 $[M+Na]^+$, 959.30 $[M+K]^+$. ^{1}H NMR ([D$_6$]DMSO): δ 11.41 (s, 1H, H-3), 8.67 (m, 4H, H-1″,4″,5″,8″), 8.07 (br t, 1H, J=6.1 Hz, OCON*H*), 7.89 (m, 2H, Hc), 7.78 (m, 6H, Hj, H-2″,3″,6″,7″), 7.70 (d, 1H, $J_{5,6}$=8.1 Hz, H-6), 7.53 (m, 3H, Ha,b), 7.41 (d, 2H, $J_{j,k}$=8.1 Hz, Hk), 5.71 (br s, 1H, H-1′), 5.60 (m, 1H, H-5), 5.40 (m, 1H, H-2′), 4.52 (dd, 1H, $J_{2',3'}$=5.8 Hz, $J_{3',4'}$= 8.1 Hz, H-3′), 4.27 (m, 2H, J_{NHCH}=6.1 Hz, ^2J=15.8 Hz, NCH_2), 4.11–3.88 (m, 3H, $J_{5'a,5'b}$=12.9 Hz, $J_{4,5'a}$=3.7 Hz, H-4′, H-5′), 1.04–0.98 (m, 28H, Pr^i). Anal. Calcd for $C_{53}H_{57}N_3O_8Si_2$: C, 69.18; H, 6.24; N, 4.57. Found: C, 69.04; H, 6.30; N, 4.62.

4.1.3. 2′-*O*-[4-(Pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (4). To a solution of 2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (1.688 g, 2 mmol) in THF (5 mL) in a teflon flask (Nalgene, screw-top) was added triethylamine trihydrofluoride (0.814 mL, 5 mmol) and the mixture was left overnight at room temperature (the completion of deprotection was checked by TLC (15% MeOH in $CHCl_3$, v/v), then diluted with hexane (25 mL). The upper layer was discarded, and the residue (oil) was washed with 1:1 (v/v) toluene–hexane mixture (3×25 mL) by decantation, triturated in absolute ethanol (5 mL), and the crystalline product was filtered off, washed with ethanol (5 mL), diethyl ether (10 mL), and dried in vacuo. Yield 1.193 g (99.1%), yellow crystals. R_f 0.4 ($CHCl_3$/MeOH, 17:3 (v/v)), mp 144–145.5 °C. MALDI-TOF MS (matrix 2,4,6-THAP) 602.78, 625.80. Calcd 602.19 $[M+H]^+$, 624.17 $[M+Na]^+$. ^{1}H NMR ([D$_6$]DMSO): δ 11.38 (s, 1H, H-3, exchangeable with D_2O), 8.62 (d, 1H, $J_{9'',10''}$=9.1 Hz,

H-10″), 8.39–8.21 (m, 7H, H-2″–6″,8″,9″), 8.13 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}=7.6$ Hz, H-7″), 7.98–7.93 (m, 2H, OCONH, H-6), 7.73 (d, 2H, $J_{e,d}=8.0$ Hz, Hd), 7.37 (d, 2H, $J_{e,d}=8.0$ Hz, He), 6.05 (d, 1H, $J_{1',2'}=5.7$ Hz, H-1′), 5.69 (d, 1H, $J_{5,6}=8.1$ Hz, H-5), 5.56 (d, 1H, $J_{3',OH}=5.1$ Hz, 3′-OH), 5.20 (t, 1H, $J=5.7$ Hz, 5′-OH) (both exchangeable with D_2O), 5.13 (apparent t, 1H, $J_{1'',2''}=J_{2',3'}=5.7$ Hz, H-2′), 4.25 (m 3H, H-3′, NCH_2), 3.94 (m, 1H, H-4′), 3.64 (m, 2H, H-5′). ^{13}C NMR ([D_6]DMSO), δ 43.6 (Cg), 60.8 (C5′), 68.9 (C3′), 74.8 (C2′), 85.4 (C1′), 85.7 (C4′), 87.9 (Ca), 95.2 (Cb), 102.1 (C5), 117.0 (C1″), 120.7 (Cc), 123.3 (C10c″), 123.6 (C10b″), 124.7 (C3″), 124.8 (C10″), 125.9 (2C, C6″, C8″), 126.6 (C4″), 127.1 (C7″), 127.2 (2C, Ce), 128.3 (C5″), 128.7 (C9″), 129.5 (C2″), 130.4, 130.7, 130.8, 130.9 (C3a″, C5a″, C8a″, C10a″), 131.4 (2C, Cd), 140.6 (2C, C6, Cf), 150.5 (C2), 155.4 (Ch), 162.9 (C4). Anal. Calcd for $C_{35}H_{27}N_3O_7$: C, 69.88; H, 4.52; N, 6.98. Found: C, 69.91; H, 4.49; N, 6.92.

4.1.4. 2′-*O*-[4-(9-Phenylethynylanthracen-10-ylethynyl)-phenylmethylaminocarbonyl]uridine (9). Prepared from 2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (0.98 g, 1.1 mmol) and triethylamine trihydrofluoride (0.5 mL, 3 mmol) in similar manner to compound **4**. Yield 0.707 g (98%), orange crystals. R_f 0.4 ($CHCl_3$/MeOH, 17:3 (v/v)), mp 165–166 °C. MALDI-TOF MS (matrix 2,4,6-THAP) 679.17, 701.61, 717.64. Calcd 678.71 $[M+H]^+$, 700.69 $[M+Na]^+$, 716.80 $[M+K]^+$. ^1H NMR ([D_6]DMSO): δ 11.38 (s, 1H, H-3, exchangeable with D_2O), 8.69 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.5$ Hz, H-1″,4″,5″,8″), 7.99 (br t, 1H, OCONH), 7.93 (d, 1H, $J_{5,6}=8.1$ Hz, H-6), 7.91 (m, 2H, Hc), 7.85 (d, 2H, $J_{j,k}=8.1$ Hz, Hj), 7.80 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.5$ Hz, H-2″,3″,6″,7″), 7.54 (m, 3H, Ha, Hb), 7.40 (d, 2H, $J_{j,k}=8.1$ Hz, Hk), 6.05 (d, 1H, $J_{1',2'}=5.7$ Hz, H-1′), 5.69 (d, 1H, $J_{5,6}=8.1$ Hz, H-5), 5.61 (d, 1H, $J_{3',OH}=5.1$ Hz, 3′-OH), 5.19 (m, 1H, 5′-OH) (both exchangeable with D_2O), 5.12 (apparent t, 1H, $J_{1',2'}=J_{2',3'}=5.7$ Hz, H-2′), 4.26 (m, 3H, H-3′, NCH_2), 3.93 (m, 1H, H-4′), 3.63 (m, 2H, H-5′). ^{13}C NMR ([D_6]DMSO), δ 43.6 (Cm), 60.8 (C5′), 68.8 (C3′), 74.8 (C2′), 85.4 (2C, C1′, Cg), 85.7 (2C, C4′, Cf), 102.1 (C5), 102.7 (Ce), 102.8 (Ch), 117.3, 117.6 (2C, C9″, C10″), 120.4 (Ci), 122.1 (Cd), 126.7 (4C, C1″, C4″, C5″, C8″), 127.3 (2C, Ck), 127.7 (4C, C2″, C3″, C6″, C7″), 128.8 (2C, Cb), 129.3 (Ca), 131.2 (4C, 4a″, C8a″, C9a″, 10a″), 131.6 (4C, Cj, Cc), 140.6 (C6), 141.0 (Cl), 150.5 (C2), 155.4 (Cn), 162.9 (C4). Anal. Calcd for $C_{41}H_{31}N_3O_7$: C, 72.66; H, 4.61; N, 6.20. Found: C, 72.61; H, 4.55; N, 6.24.

4.1.5. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (5). 2′-*O*-[4-(Pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (1.004 g, 1.67 mmol) was coevaporated with toluene (3×20 mL), pyridine (3×20 mL), dissolved in dry pyridine (15 mL), cooled in an ice bath, and DmtCl (623 mg, 1.84 mmol) was added in one portion. After completion of the reaction, the excess of DmtCl was quenched with MeOH (1 mL), and after 10 min the mixture was diluted with $CHCl_3$ (100 mL), washed with water (100 mL), 5% $NaHCO_3$ (100 mL), and water (100 mL), then dried (Na_2SO_4), evaporated, coevaporated with toluene (3×25 mL) and the residue was chromatographed on silica gel column in step gradient of 0.5→1→1.5→2% MeOH in $CHCl_3$/EtOAc 2:1+0.5% pyridine (v/v/v/v). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford **5** (1.3 g, 86.2%) as a yellow foam. R_f 0.31 ($CHCl_3$/EtOAc 1:1+1% Et_3N (v/v/v)). MALDI-TOF MS (matrix 2,4,6-THAP) 905.81, 928.04, 944.55. Calcd 904.32 $[M+H]^+$, 926.31 $[M+Na]^+$, 942.28 $[M+K]^+$. ^1H NMR ([D_6]DMSO): δ 11.41 (s, 1H, H-3) (exchangeable with D_2O), 8.62 (d, 1H, $J_{9'',10''}=9.0$ Hz, H-10″), 8.41–8.22 (m, 7H, H-2″–6″,8″,9″), 8.14 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}=7.5$ Hz, H-7″), 8.00 (br t, 1H, $J=6.1$ Hz, OCONH), 7.73 (m, 3H, H-6,d), 7.43–7.22 (m, 9H, ArH (Dmt)), 6.89 (d, 4H, $J_{d,e}=8.6$ Hz, He), 5.97 (d, 1H, $J_{1',2'}=4.9$ Hz, H-1′), 5.61 (d, 1H, $J_{3',OH}=5.6$ Hz, OH) (exchangeable with D_2O), 5.41 (1H, $J_{5,6}=8.1$ Hz, H-5), 5.23 (apparent t, 1H, $J_{1'2'}=J_{2'3'}=5.2$ Hz, H-2′), 4.37 (m, 1H, H-3′), 4.29 (m, 2H, $NHCH_2$), 4.01 (m, 1H, H-4′), 3.74 (s, 6H, CH_3), 3.32–3.22 (m, 2H, H-5′). Anal. Calcd for $C_{56}H_{45}N_3O_9$: C, 74.40; H, 5.02; N, 4.65. Found: C, 74.62; H, 4.93; N, 4.37. A byproduct, 5′,3′-*O*-bis-(4,4′-dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine, was also isolated as a foam (30 mg, 1.5%). ^1H NMR ([D_6]DMSO): δ 11.46 (s, 1H, H-3) (exchangeable with D_2O), 8.63 (d, 1H, $J_{9'',10''}=9.0$ Hz, H-10″), 8.41–8.21 (m, 8H, H-2″–6″,8″,9″, OCONH), 8.14 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}=7.5$ Hz, H-7″), 7.72 (d, 2H, $J_{a,b}=7.9$ Hz, H-b), 7.52 (d, 1H, $J_{5,6}=8.0$ Hz, H-6), 7.41–7.11 (m, 18H, ArH (Dmt)), 6.86–6.74 (m, 8H, ArH (Dmt)), 6.16 (d, 1H, $J_{1',2'}=6.0$ Hz, H-1′), 5.39 (1H, $J_{5,6}=8.0$ Hz, H-5), 5.10 (apparent t, 1H, $J_{1'2'}=J_{2'3'}=6.0$ Hz, H-2′), 4.34–4.26 (m, 4H, H-3′, H-4′, $NHCH_2$), 3.73 (s, 6H), 3.69 (s, 6H) (CH_3), 3.30 (m, 2H,‡ H-5′).

4.1.6. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (10). Prepared from 2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (0.68 g, 1.00 mmol) in similar manner to compound **5**. Yield 0.839 g (85.6%), orange foam. R_f 0.4 ($CHCl_3$–EtOAc 1:1+0.5% pyridine (v/v/v)). ^1H NMR ([D_6]DMSO): δ 11.42 (s, 1H, H-3, exchangeable with D_2O), 8.69 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.4$ Hz, H-1″,4″,5″,8″), 8.03 (br t, $J=6.1$ Hz, 1H, OCONH), 7.91 (d, 2H, $J=6.4$ Hz), 7.85 (d, 2H, $J=8.0$ Hz) (Hc,j), 7.80 (m, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.4$ Hz, H-2″,3″,6″,7″), 7.73 (d, 1H, $J_{5,6}=8.0$ Hz, H-6), 7.57–7.26 (m, 14H, Ha,b,k, ArH (Dmt)), 6.90 (d, $J=8.5$ Hz, 4H, ArH (Dmt)), 5.98 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1′), 5.63 (d, 1H, $J_{3',OH}=5.0$ Hz, 3′-OH), 5.41 (d, 1H, $J_{5,6}=8.0$ Hz, H-5), 5.11 (apparent t, 1H, $J_{1',2'}=J_{2',3'}=4.5$ Hz, H-2′), 4.40–4.29 (m, 3H, H-3′, NCH_2), 4.02 (m, 1H, H-4′), 3.74 (s, 6H, CH_3), 3.31 (m, 2H,‡ H-5′). Anal. Calcd for $C_{62}H_{49}N_3O_9$: C, 75.98; H, 5.04; N, 4.29. Found: C, 76.21; H, 4.93; N, 4.36. Bis-Dmt-derivative, 5′,3′-*O*-bis-(4,4′-dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine, was isolated as a minor byproduct (28 mg, 2.6%). ^1H NMR ([D_6]DMSO): δ 11.45 (s, 0.7H), 11.42 (m, 0.2H), 11.39 (m, 0.1H) (H-3), 8.69 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.5$ Hz, H-1″,4″,5″,8″), 8.03 (br t, 1H, $J=6.1$ Hz, OCONH), 7.91–7.60 (m, 32H, H-2″,3″,6″,7″, H-6, Ha,b,c,j,k, ArH (Dmt)), 6.86–6.74 (m, 8H, ArH (Dmt)),

‡ Calculated value; the signal of water is also present in the region.

6.16 (d, 0.7H, $J_{1',2'}$=6.5 Hz), 6.13 (d, 0.2H, $J_{1',2'}$=6.5 Hz), 6.09 (d, 0.1H, $J_{1',2'}$=6.5 Hz) (H-1′), 5.39 (m, 1H, H-5), 5.11 (apparent t, 1H, $J_{1',2'}=J_{2',3'}$=4.5 Hz, H-2′), 4.36–4.24 (m, 4H, H-3′, H-4′, NCH_2), 3.72 (s, 6H), 3.69 (s, 6H) (CH_3), 3.30 (m, 2H,‡ H-5′).

4.1.7. 3′-*O*-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (6). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)-phenylmethylaminocarbonyl]uridine (1.00 g, 1.10 mmol) was coevaporated with dry DCM (2×20 mL), dissolved in dry DCM, diisopropylammonium tetrazolide (285 mg, 1.66 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.527 mL, 1.66 mmol) were added, and the mixture was stirred under nitrogen for 2 h. After conversion of the starting compound was complete (monitoring by TLC) the mixture was diluted with $CHCl_3$, washed with 5% $NaHCO_3$ (100 mL), 20% NaCl (100 mL), dried over Na_2SO_4, evaporated to dryness, and the residue was chromatographed on silica gel column in stepwise gradient 5→10→20→25% acetone in $CHCl_3$+1% Et_3N (v/v/v). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford **6** (1.3 g, 92%) as a yellow foam. R_f 0.17, 0.23 ($CHCl_3$/EtOAc 1:1+1%Et_3N (v/v/v)). ^{1}H NMR (5% $CDCl_3$ in [D_3]MeCN): δ 8.66 (d, 1H, $J_{9'',10''}$=8.8 Hz, H-10″), 8.33–8.08 (m, 8H, H-2″–9″), 7.74–7.24 (m, 15H, H-6, Ar*H* (Dmt, He,d), OCON*H*), 6.87 (m, 4H, Ar*H* (Dmt)), 6.08 (m, 1H, H-1′), 5.49–5.37 (m, 2H, H-2′,5), 4.64 (m, 1H, H-3′), 4.42–4.33 (m, 2H, NHCH_2), 4.01–4.29 (m, 1H, H-4′), 3.78 (s, 6H, CH_3), 3.75–3.37 (m, 6H,‡ POCH_2, PNC*H*, H-5′), 2.76 (t, 0.9H, *J*=5.9 Hz), 2.51 (t, 1.1H, *J*=5.9 Hz) (CH_2CN, diastereomers), 1.26–1.05 (m, 12H, CHCH_3). ^{31}P NMR (5% $CDCl_3$ in [D_3]MeCN): δ 151.13, 150.99 (diastereomers, ~10:8).

4.1.8. 3′-*O*-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (11). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (0.8 g, 0.82 mmol) was phosphitylated as above with diisopropylammonium tetrazolide (21 mg, 1.23 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.39 mL, 1.23 mmol). The compound was chromatographed on silica gel column in stepwise gradient 5→10→20→25% acetone in $CHCl_3$+1% Et_3N (v/v/v). Yield 1.069 g (90.5%). R_f 0.17, 0.23 ($CHCl_3$/EtOAc 1:1+1%Et_3N (v/v/v)). ^{1}H NMR ([D_3]MeCN): δ 8.70 (m, 4H, H-1″,4″,5″,8″), 7.84 (m, 2H), 7.78 (m, 2H) (Hc,j), 7.74 (m, H-2″,3″,6″,7″), 7.67 (m, 1H, H-6), 7.53–7.24 (m, 14H, Ha,b,k, Ar*H* (Dmt)), 6.88 (d, *J*=8.5 Hz, 4H, Ar*H* (Dmt)), 6.08–6.06 (m, 1H, H-1′), 5.44–5.36 (m, 2H, H-5), 4.69–4.59 (m, 1H, H-2′), 4.42–4.01 (m, 4H, H-3′, NCH_2, H-4′), 3.84–3.37 (m, 12H,‡ OCH_3, POCH_2, PNC*H*, H-5′), 2.77 (t, 2H, *J*=5.8 Hz, CH_2CN), 1.28–1.01 (m, 12H, CHCH_3). ^{31}P NMR ([D_3]MeCN): δ 151.00, 150.89 (diastereomers, ~2:1).

4.1.9. Synthesis of oligodeoxynucleotides. The polymer-supported synthesis of oligonucleotides was performed on a ABI 380B DNA/RNA synthesizer, with commercially available 2′-deoxynucleoside phosphoramidites (Cruachem). Oligonucleotides were synthesized starting on a 1 μmol scale. Oligonucleotides were isolated using electrophoresis in 20% denaturing (7 M urea) PAGE in Tris–borate buffer, pH 8.3. The mass of each oligonucleotide was checked by MALDI-TOF mass spectrometry on a Perseptive Biosystems Voyager DE mass spectrometer in positive ion mode using a 1:1 v/v mixture of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH) and aq. diammonium hydrogen citrate (80 mg/mL) as a matrix premixed just before loading the samples onto a plate.

4.2. Supplementary Data

Original ^{1}H, ^{13}C, ^{1}H–^{13}C HMQC and HMBC NMR spectra of nucleosides **4** (**U^P**) and **9** (**U^A**).

Acknowledgements

This research was partially supported by RFBR grants (00-03-32701, 02-03-32376 and 03-03-32196). V.A.K. was also supported by Ribozyme Pharmaceutical (1999–2001), A.D.M. was supported by Astra-Zeneca (2002–2003); N.N.D. thanks the MRC for the support during her visit to Cambridge in 2002. The authors are grateful to Donna Williams (MRC Laboratory of Molecular Biology) for oligonucleotide synthesis and to the elemental analysis facility of Moscow Institute of Fine Chemical Technology for elemental analysis data. We thank Professor Vladimir A. Efimov, Igor A. Prokhorenko (Shemyakin-Ovchinnikov Institute), Dr. Dmitry A. Stetsenko and Dr. Michael J. Gait (MRC LMB) for their encouraging interest and generous support.

References and notes

1. (a) Freier, S. M.; Altmann, K.-H. *Nucleic Acids Res.* **1997**, *25*, 4429–4443. (b) Zatsepin, T. S.; Stetsenko, D. S.; Arzumanov, A. A.; Romanova, E. A.; Gait, M. J.; Oretskaya, T. S. *Bioconjugate Chem.* **2002**, *13*, 822–830.
2. Yamana, K.; Mitsui, T.; Hayashi, H.; Nakano, H. *Tetrahedron Lett.* **1997**, *38*, 5815–5818.
3. Yamana, K.; Aota, R.; Nakano, H. *Tetrahedron Lett.* **1995**, *36*, 8427–8430.
4. (a) Yamana, K.; Nishijima, Y.; Ikeda, T.; Gokota, T.; Ozaki, H.; Nakano, H.; Sangen, O.; Shimidzu, T. *Bioconjugate Chem.* **1990**, *1*, 319–324. (b) Deshmukh, H. M.; Joglekar, S. P.; Broom, A. D. *Bioconjugate Chem.* **1995**, *6*, 578–586. (c) Ly, D.; Sanii, L.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *121*, 9400–9410. (d) Connors, W. H.; Narayanan, S.; Kryatova, O. P.; Richert, C. *Org. Lett.* **2003**, *5*, 247–250.
5. Yamana, K.; Mitsui, T.; Nakano, H. *Tetrahedron* **1999**, *55*, 9143–9150.
6. Kryatova, O. P.; Connors, W. H.; Bleczinski, C. F.; Mokhir, A. A.; Richert, C. *Org. Lett.* **2001**, *3*, 987–990.
7. Dubey, I.; Prativel, G.; Meunier, B. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3088–3095.
8. Kawai, K.; Kawabata, K.; Tojo, S.; Majima, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2363–2366.
9. (a) Yamana, K.; Ohashi, Y.; Nunota, K.; Kitamura, M.;

Nakano, H.; Sangen, O.; Shimidzu, T. *Tetrahedron Lett.* **1991**, *32*, 6347–6350. (b) Yamana, K.; Gokota, T.; Ozaki, H.; Nakano, H.; Sangen, O.; Shimidzu, T. *Nucleosides Nucleotides* **1992**, *11*, 383–390. (c) Manoharan, M.; Johnson, L. K.; Tivel, K. L.; Springer, R. H.; Cook, P. D. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2765–2770. (d) Manoharan, M.; Tivel, K. L.; Andrade, L. K.; Cook, P. D. *Tetrahedron Lett.* **1995**, *36*, 3647–3650. (e) Manoharan, M.; Tivel, K. L.; Zhao, M.; Nafisi, K.; Netzel, T. L. *J. Phys. Chem.* **1995**, *99*, 17461–17472. (f) Iwase, R.; Furutani, S.; Yamaoka, T.; Yamana, K.; Murakami, A. *Nucleic Acids Symp. Ser.* **1996**, *35*, 117–118. (g) Iwase, R.; Tsuchida, H.; Yamaoka, T.; Yamana, K.; Murakami, A. *Nucleic Acids Symp. Ser.* **1997**, *37*, 205–206. (h) Yamana, K.; Asanuma, K.; Nakano, H. *Nucleic Acids Symp. Ser.* **1999**, *42*, 113–114. (i) Yamana, K.; Iwase, R.; Furutani, S.; Tsuchida, H.; Zako, H.; Yamaoka, T.; Murakami, A. *Nucleic Acids Res.* **1999**, *27*, 2387–2392. (j) Hwang, J.-T.; Greenberg, M. M. *Org. Lett.* **1999**, *1*, 2021–2024. (k) Silverman, S. K.; Cech, T. R. *Biochemistry* **1999**, *38*, 14224–14237. (l) Hwang, J.-T.; Greenberg, M. M. *J. Org. Chem.* **2001**, *66*, 363–369. (m) Yamana, K.; Zako, H.; Asazuma, K.; Iwase, R.; Nakano, H.; Murakami, A. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 1104–1106. (n) Korshun, V. A.; Stetsenko, D. A.; Gait, M. J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1092–1104. (o) Mahara, A.; Iwase, R.; Sakamoto, T.; Yamana, K.; Yamaoka, T.; Murakami, A. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 3648–3650. (p) Takada, T.; Kawai, K.; Tojo, S.; Majima, T. *Tetrahedron Lett.* **2003**, *44*, 3851–3854. (q) Dioubankova, N. N.; Malakhov, A. D.; Stetsenko, D. A.; Gait, M. J.; Volynsky, P. E.; Efremov, R. G.; Korshun, V. A. *ChemBioChem* **2003**, *4*, 841–847.

10. Malakhov, A. D.; Skorobogatyi, M. V.; Prokhorenko, I. A.; Gontarev, S. V.; Stetsenko, D. A.; Stepanova, I. A.; Shenkarev, Z. O.; Berlin, Y. A.; Korshun, V. A. *Eur. J. Org. Chem.* **2004**, 1298–1307.
11. (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467–4470. (b) Sonogashira, K. In *Comprehensive organic synthesis. Comprehensive organic synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 3, pp 521–549. (c) Rossi, R.; Carpita, A.; Bellina, F. *Org. Prep. Proc. Int.* **1995**, *27*, 127–160. (d) Sonogashira, K. In *Handbook of organopalladium chemistry for organic synthesis*; Negishi, E., de Meijere, A., Eds.; Wiley-Interscience: New York, 2002; Vol. 1, pp 493–529.
12. Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J.-Y. *Methods Enzymol.* **1987**, *154*, 287–313.
13. (a) Arzumanov, A.; Godde, F.; Moreau, S.; Toulmé, J.-J.; Weeds, A.; Gait, M. J. *Helv. Chim. Acta* **2000**, *83*, 1424–1436. (b) Arzumanov, A.; Walsh, A. P.; Rajwanshi, V. K.; Kumar, R.; Wengel, J.; Gait, M. J. *Biochemistry* **2001**, *40*, 14645–14654.
14. Malakhov, A. D.; Malakhova, E. V.; Kuznitsova, S. V.; Grechishnikova, I. V.; Prokhorenko, I. A.; Skorobogatyi, M. V.; Korshun, V. A.; Berlin, Y. A. *Bioorg. Khim.* **2000**, *26*, 39–50, *Russ. J. Bioorg. Chem.* (*Engl.*), **2000**, 26, 34–44.
15. Haugland, R. P. *Handbook of fluorescent probes and research products*; Molecular Probes: Eugene, OR, 2002.
16. Rio, M. G. *Compt. Rend.* **1952**, *235*, 1308–1310.