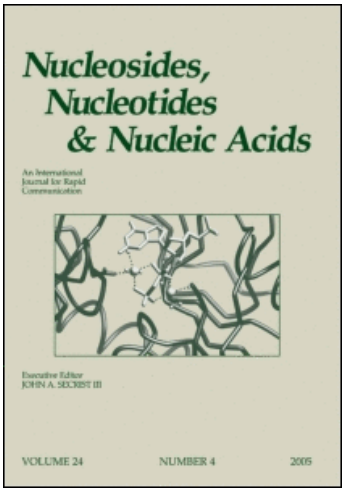

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2'-Bis-Pyrene Modified Oligonucleotides: Sensitive Fluorescent Probes of Nucleic Acids Structure

D. S. Novopashina[a]; D. A. Stetsenko[b]; O. S. Totskaya[a]; M. N. Repkova[a]; A. G. Venyaminova[a]
[a] Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia [b] MRC Laboratory of Molecular Biology, Cambridge, UK

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2′-BIS-PYRENE MODIFIED OLIGONUCLEOTIDES: SENSITIVE FLUORESCENT PROBES OF NUCLEIC ACIDS STRUCTURE

D. S. Novopashina ▫ *Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia*

D. A. Stetsenko ▫ *MRC Laboratory of Molecular Biology, Cambridge, UK*

O. S. Totskaya, M. N. Repkova, and A. G. Venyaminova ▫ *Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia*

▫ *A new type of fluorescent nucleic acid probes, 2′-bis-pyrene-modified oligonucleotides, is described. Preparation of these conjugates involves attachment of two pyrene moieties to the 2′-phosphate group introduced into any position within a sequence by solid-phase phosphoramidite synthesis. Good hybridization properties of the 2′-bis-pyrene probes, their nuclease resistance and sensitivity of fluorescence to the type of complementary nucleic acid have been demonstrated.*

Keywords 2′-Modified Oligonucleotides, 2′-Bis-Pyrene Modification, Nuclease Resistance, Fluorescent Probes, Nucleic Acids Detection

INTRODUCTION

Design and synthesis of fluorescently labeled oligonucleotides that display an enhanced signal upon hybridization with their complementary DNA or RNA target has been the subject of intense research over the last two decades. These oligonucleotide derivatives may be used for DNA sequencing, hybridization analysis, RNA structure determination, studies of biopolymers interactions, and in aptamer-based biosensors. Amongst a number of fluorophores, pyrene is one of the most attractive dyes for development of highly sensitive probes of DNA and RNA structure. Excimer formation in bis-pyrene conjugates upon their binding to NA is affected by nucleotide sequence and local environment.[1–5] A new type of

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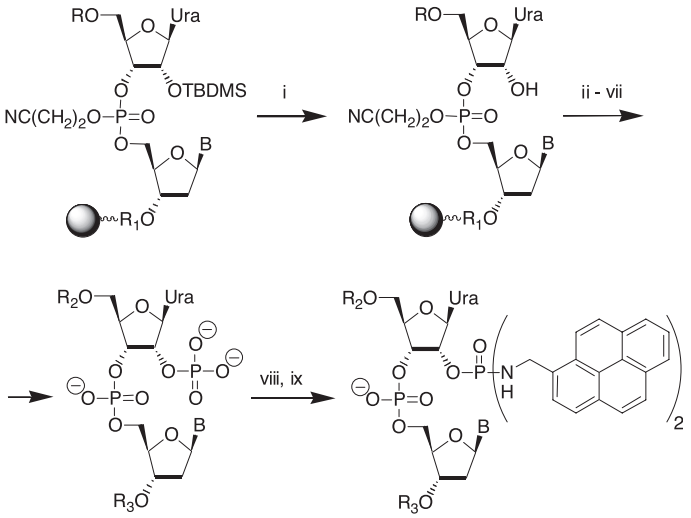
Address correspondence to D. S. Novopashina, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk 630090, Russia; Fax: +7-3832-33-36-77; E-mail: danov@niboch.nsc.ru



SCHEME 1 Synthesis of the 2′-phosphate and 2′-bis-pyrenyl phosphorodiamidate containing oligonucleotides (R, R_1 = protected oligodeoxyribonucleotide chain; R_2, R_3 = unprotected chain). Reagents: i) $Et_3N \cdot 3HF$; ii) $DMTrO(CH_2)_2SO_2(CH_2)_2OP(NPr^i_2)(OEtCN)$, 5-ethylthio-1*H*-tetrazole; iii) Ac_2O/N-MeIm; iv) 0.02M I_2; v) 2% $CHCl_2COOH$; vi). NH_4OH conc.; vii) HPLC purification; viii) Ph_3P, Py_2S_2, DMAP, DMSO; ix) 1-pyrenemethylamine hydrochloride, Et_3N, DMSO.

bis-pyrene fluorescent oligonucleotide probes is proposed, and a method of its synthesis is developed.

RESULTS AND DISCUSSION

A new and general procedure has been developed for preparation of oligonucleotide derivatives bearing two pyrene residues at the 2′-position of ribose. It involves the incorporation of a single 2′-O-*tert*-butyldimethylsilyl (TBDMS)-protected uridine residue into an oligodeoxyribonucleotide by solid-phase phosphoramidite synthesis, its 2′-deprotection and phosphorylation on solid phase. The deprotected oligonucleotide was then isolated, and the 2′-phosphate group was

TABLE 1 Structure and Properties of Oligonucleotides Used in this Study

No	Oligonucleotide sequence,[a] 5′→3′	MALDI-TOF, calc./found	T_m,°C[b]
(**I**)	GCA TCA AGC AGC TCC AGG C	5783.8/5784.2	70
(**II**)	GCA TCA AGC AGC $\boldsymbol{U^{P}}$CC AGG C	5865.7/5864.2	68
(**III**)	GCA TCA AGC AGC $\boldsymbol{U^{Pyr}}$CC AGG C	6292.3/6294.9	71
(**IV**)	GCA $\boldsymbol{U^{P}}$CA AGC AGC TCC AGG C	5865.7/5863.8	69
(**V**)	GCA $\boldsymbol{U^{Pyr}}$CA AGC AGC TCC AGG C	6292.3/6294.2	74

[a] $\boldsymbol{U^{P}}$, uridine 2′-phosphate; $\boldsymbol{U^{Pyr}}$, uridine 2′-bis-pyrenyl phosphorodiamidate.

[b] Conditions: buffer 0.1 M NaCl, 10 mM Na cacodylate, pH 7.4, 1 mM Na_2EDTA; [oligonucleotide] = [target] = 1.3×10^{-5} M, target = d(TGCCTGGAGCTGCTTGATGC) (**VI**).

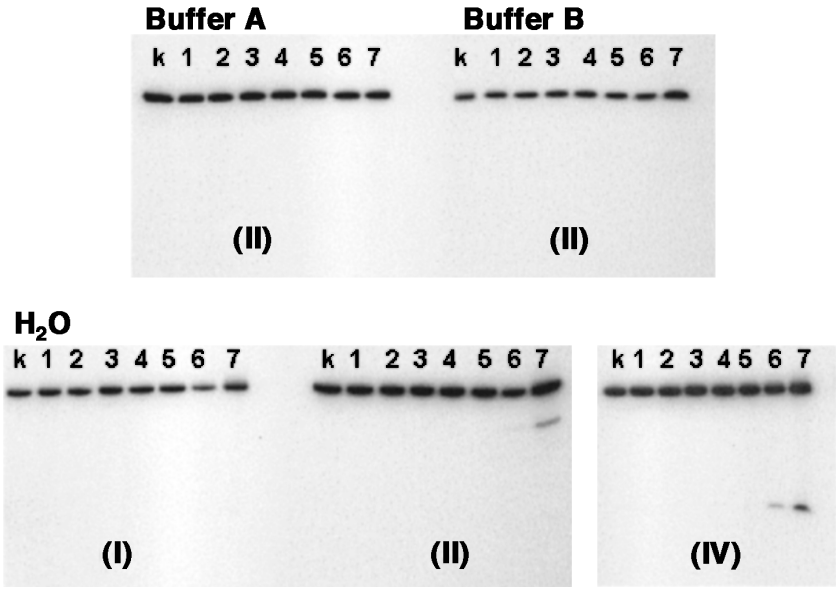


FIGURE 1 Stability of 2′-modified oligonucleotides in different pH conditions. Oligonucleotides **I**, **II**, and **IV** were incubated in buffer A: 0.05 M HEPES, pH 9.0, or buffer B: 0.05 M MES, pH 6.0, or in distilled water at 37°C. [Oligonucleotide] = $2 \cdot 10^{-7}$ M. Aliquots were taken after 15, 30, 60, 180, 360 min, and 1 and 3 days (lanes 1–7); k, initial oligomer.

converted into bis-pyrene phosphorodiamidate using Mukaiyama reagents[6] and nucleophilic catalysis[7] (Scheme 1).

2′-Bis-pyrenyl conjugates as well as their parent oligonucleotide 2′-phosphates prepared by this method (Table 1) were purified by PAGE, their structures were confirmed by MALDI-TOF mass spectrometry and UV and fluorescence spectra.

Hydrolytic stability of the 3′,5′-phosphodiester linkage neighboring the 2′-phosphate group and its resistance to nucleases have been investigated. We found it stable for 3 days at both pH 6.0 and 9.0, while in distilled water some cleavage was detected (Figure 1).

Out of the nucleases tested, snake venom phosphodiesterase produced digestion of all oligomers, whereas the cleavage of 2′-bis-pyrene conjugates by nuclease P1 has been largely inhibited, probably due to steric interference (Figure 2).

It was demonstrated that the presence of the 2′-phosphate residue accelerated oligonucleotide degradation in cultural medium, the 2′-bis-pyrene conjugates again being more stable than the parent 2′-phosphorylated oligonucleotides (Figure 3).

The introduction of the 2′-bis-pyrenyl phosphorodiamidate group increases stability of a DNA duplex compared to an unmodified one, while the presence of the 2′-phosphate group decreases melting temperature slightly (Table 1).

2′-Bis-pyrene-labeled oligonucleotide probes exhibit sharp changes in fluorescence upon binding to their complementary target (Figure 4). A considerable difference in wavelength, intensity and ratio of monomeric and excimeric emission

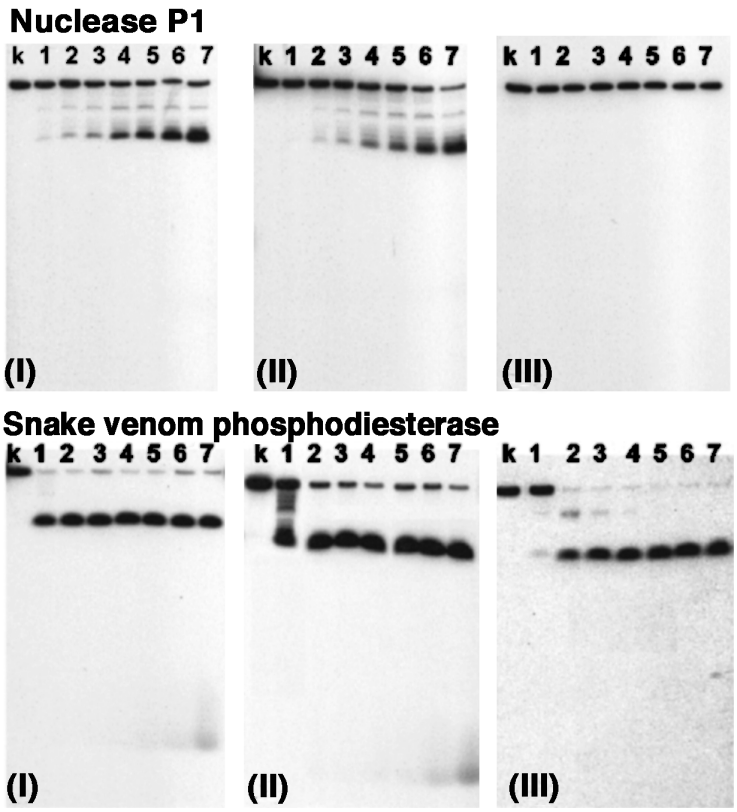


FIGURE 2 Nuclease stability of 2′-modified oligonucleotides. Oligonucleotides **I**–**III** were incubated at 37°C with nuclease P1 (0.01 units/mL) in buffer 0.03 M NaOAc, 1 mM $ZnSO_4$, pH 5.2, or snake venom phosphodiesterase (0.01 units/mL) in buffer 10 mM Tris-HCl, pH 7.8, 0.5 mM $MgCl_2$; [oligonucleotide] = $2 \cdot 10^{-7}$ M. Aliquots were taken after 1, 3, 5, 10, 15, 30, and 60 min of incubation (lanes 1–7).

has been recorded for the duplexes of 2′-bis-pyrene-labeled oligomers with RNA or DNA. It should be emphasized that hybridization to RNA resulted in a moderate increase in monomer fluorescence and prominent peak of excimer, while the duplex with DNA showed high intensity monomer signal and disappearance of excimer emission (Figure 4). The 2′-bis-pyrenyl group is also sensitive to any mismatch in a duplex near the site of modification (data not shown).

In conclusion, new 2′-bis-pyrene-labeled oligonucleotide conjugates are sensitive fluorescent probes possessing high nuclease and serum stability and good hybridization properties and may be used for RNA and DNA detection and structure determination. Oligonucleotides (deoxy, 2′-O-methyl, LNA, and other analogs) containing a single ribonucleoside residue bearing the 2′-phosphate group may serve as precursors for postsynthetic introduction of various ligands in design of nucleic acids conjugates for a wide range of applications.

The details of the synthesis of novel 2′-bis-pyrene conjugates of oligonucleotides will be described elsewhere.

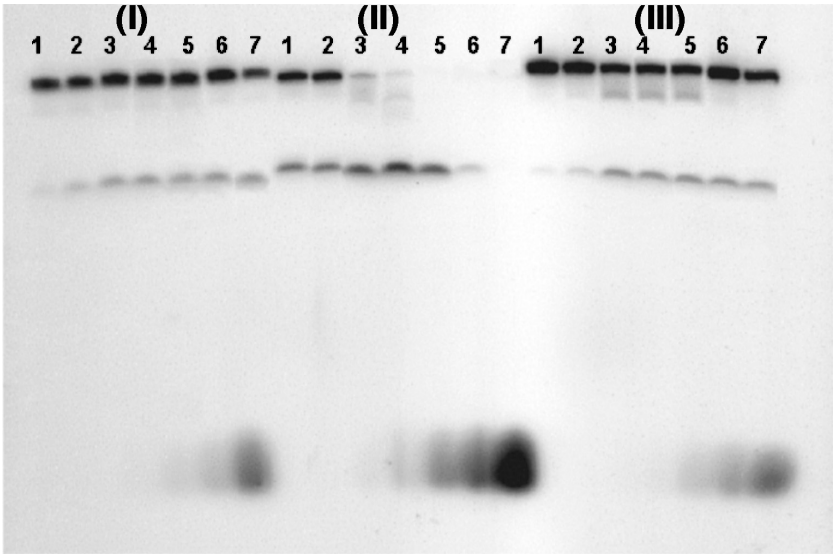


FIGURE 3 Stability of 2′-modified oligonucleotides **I–III** in a cultural medium (IMDM) containing 10% fetal calf serum at 37°C. Probes were taken after 5, 10, 30 min, and 1, 3, 6, and 24 h of incubation (lanes 1–7).

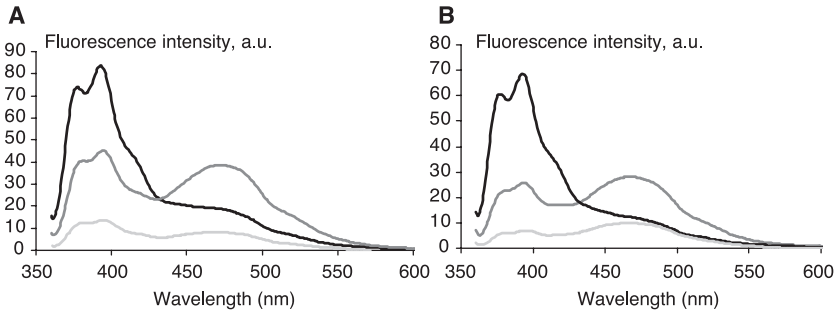


FIGURE 4 Fluorescence emission spectra of bis-pyrene conjugates **III** (**A**) and **V** (**B**) and their duplexes with complementary DNA or RNA. Spectra were recorded at 25°C at the excitation wavelength 345 nm in a buffer 0.1 M NaCl, 10 mM Na cacodylate, pH 7.4, 1 mM Na_2EDTA; [oligonucleotides] = 100 nM. Conjugate is in light grey, duplex with d(TGCCTGGAGCTGCTTGATGC) (**VI**), in black, and with RNA; r(UGCCUGGAGCUGCUUGAUGC), in dark grey.

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