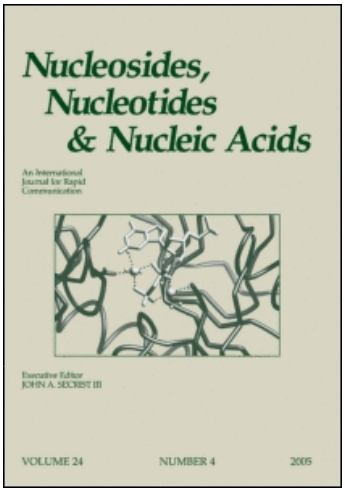

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PERYLENE ATTACHED TO DNA THROUGH STIFF OR FLEXIBLE LINKER: DUPLEX STABILITY AND FRET

Mikhail V. Skorobogatyj, Anna A. Pchelintseva, Alexei V. Ustinov, Vladimir A. Korshun, and Andrei D. Malakhov ▫ *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

▫ *Two fluorescent nucleosides, 5-(perylen-3-ylethynyl)-2′-deoxyuridine and 5-[(perylen-3-yl)methoxypropyn-1-yl]-2′-deoxyuridine, were incorporated into synthetic oligodeoxyribonucleotides and spectral properties of the conjugates and their duplexes were studied.*

INTRODUCTION

Fluorescent nucleoside analogues are important tools in biological studies and DNA technology. 5-(Pyren-1-ylethynyl)-2′-deoxyuridine **1** was the first modified nucleoside where nucleobase is conjugated with fluorescent dye through the ethynyl linker. Spectral properties of **1** differ substantially from those of parent pyrene.[1] The fluorescence of **1** in oligonucleotides changes upon formation of complementary complexes.[2] Recently, 5-(pyren-1-ylethynyl)-2′-deoxyuridine-containing probe was shown to discriminate between perfect and one-base-mismatched base pairing by changes in its fluorescence intensity.[3] The residue of **1** is also able to inject an electron into the base stack and simultaneously act as a spectroscopic label to observe this process.[4] Pyrenylethynyl derivatives of other nucleosides are important fluorescent labels with different spectroscopic properties in single and double strands.[5] When a flexible linker is inserted between ethynyl group and pyrene, the resulting pyrene-modified dU is also very useful for point mutation detection.[6]

Perylene is a fluorescent polycyclic aromatic hydrocarbon resembling pyrene in its chemical behavior, but superior in fluorescence quantum yield.[7,8] We decided to investigate fluorescence of 5-(perylen-3-ylethynyl)-2′-deoxyuridine **2**, prepared by us earlier,[9,10] within oligonucleotide probes. As an example of a

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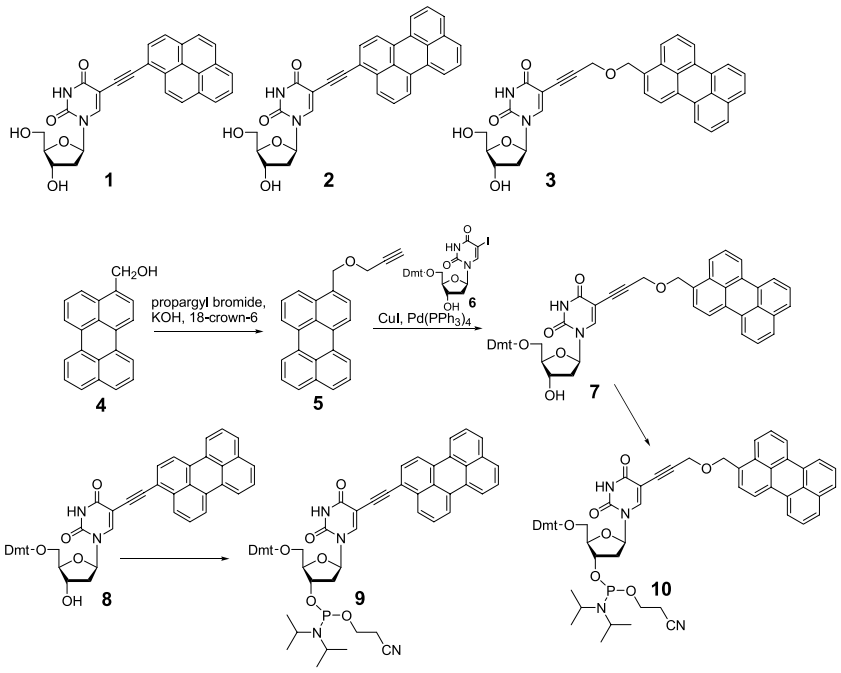

SCHEME 1 Synthesis of phosphoramidite reagents.

fluorescent nucleoside, containing non-conjugated perylene, 5-[(perylen-3-yl)methoxypropyn-1-yl]-2′-deoxyuridine **3** was prepared.

The synthesis of phosphoramidite reagents is given on Scheme 1. 3-Perylenemethanol **4**[11] was alkylated with propargyl bromide to give acetylene **5**. The latter was coupled with 5′-*O*-dimethoxytrityl-5-iodo-2′-deoxyuridine[12] in conditions optimized for nucleosides.[13] 5′-*O*-protected compounds **7** and **8**[10] were phosphitylated in standard conditions[14] yielding corresponding phosphoramidites **10** and **9** suitable for automated solid phase oligonucleotide synthesis.*

A number of modified oligonucleotides containing nucleosides **2** and **3** have been prepared. The thermal denaturation studies showed that rigid perylene analogue **2** slightly destabilizes DNA duplexes, whereas flexible perylene nucleoside has considerable stabilizing effect.

The fluorescence intensity of oligonucleotide probes, containing one or two nucleosides **2** and **3** changes upon hybridization with complementary DNA target. The phenomenon will be investigated in detail.

Fluorescent perylene nucleoside **2** proved to be very good energy acceptor for pyrene derivative **1**. Fluorescence resonance energy transfer (FRET) experiment is shown in Figure 1. The fluorescence of the acceptor dye **2** is observed almost exclusively upon excitation at pyrene nucleoside **1** absorption wavelength. FRET

*Detailed procedures and characterization of compounds will be published elsewhere.

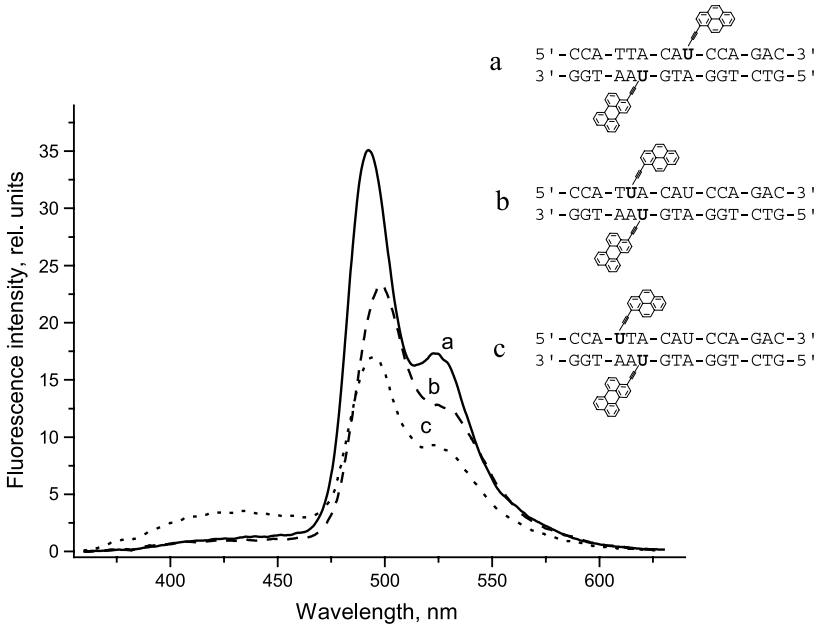

FIGURE 1 Fluorescence spectra of the duplexes **a**, **b**, and **c**. Excitation wavelength 340 nm.

efficiency is very high in case of duplexes **a** and **b**. Interestingly, the exchange of dye' positions (duplexes **b** and **c**) led to different emission shape.

To conclude, two new perylene nucleoside analogues were prepared and used in fluorescent DNA probes. These nucleosides are useful as individual labels as well as for FRET applications.

REFERENCES

1. Korshun, V.A.; Manasova, E.V.; Balakin, K.V.; Prokhorenko, I.A.; Buchatskii, A.G.; Berlin, Y.A. 5-(1-Pyrenylethynyl)-2′-deoxyuridine, a novel fluorescent nucleoside analogue. Bioorg. Khim. **1996**, *22*(12), 923–925 [Engl. transl.: Russ. J. Bioorg. Chem. **1996**, *22* (12), 807–809].
2. Malakhov, A.D.; Malakhova, E.V.; Kuznitsova, S.V.; Grechishnikova, I.V.; Prokhorenko, I.A.; Skorobogatyi, M.V.; Korshun, V.A.; Berlin, Y.A. Synthesis and fluorescent properties of 5-(1-pyrenylethynyl)-2′-deoxyuridine-containing oligonucleotides. Bioorg. Khim. **2000**, *26*(1), 39–50 [Engl. transl.: Russ. J. Bioorg. Chem. **2000**, *26* (1), 34–44].
3. Hwang, G.T.; Seo, Y.J.; Kim, S.J.; Kim, B.H. Fluorescent oligonucleotide incorporating 5-(1-ethynylpyrenyl)-2′-deoxyuridine: sequence-specific fluorescence changes upon duplex formation. Tetrahedron Lett. **2004**, *45*(18), 3543–3546.
4. Rist, M.; Amann, N.; Wagenknecht, H.-A. Preparation of 1-ethynylpyrene-modified DNA via Sonogashira-type solid-phase couplings and characterization of the fluorescence properties for electron-transfer studies. Eur. J. Org. Chem. **2003**, *13*, 2498–2504.
5. Mayer, E.; Valis, L.; Wagner, C.; Rist, M.; Amann, N.; Wagenknecht, H.-A. 1-Ethynylpyrene as a tunable and versatile molecular beacon for DNA. Chem. Biochem. **2004**, *5*(6), 865–868.
6. Okamoto, A.; Kanatani, K.; Saito, I. Pyrene-labeled base-discriminating fluorescent DNA probes for homogeneous SNP typing. J. Am. Chem. Soc. **2004**, *126*(15), 4820–4827.
7. Berlman, I.B. *Handbook of Fluorescence Spectra of Aromatic Molecules*, 2nd Ed.; Academic Press: New York, 1971.

8. Masuko, M.; Ohuchi1, S.; Sode, K.; Ohtani, H.; Shimadzu, A. Fluorescence resonance energy transfer from pyrene to perylene labels for nucleic acid hybridization assays under homogeneous solution conditions. Nucleic Acids Res. **2000**, *28*(8), e34.
9. Korshun, V.A.; Manasova, E.V.; Balakin, K.V.; Malakhov, A.D.; Perepelov, A.V.; Sokolova, T.A.; Berlin, Y.A. New fluorescent nucleoside derivatives–5-alkynylated 2′-deoxyuridines. Nucleosides Nucleotides **1998**, *17*(9/11), 1809–1812.
10. Andronova, V.L.; Skorobogatyi, M.V.; Manasova, E.V.; Berlin, Y.A.; Korshun, V.A.; Galegov, G.A. Antiviral activity of some 5-arylethynyl-2′-deoxyuridines. Bioorg. Khim. **2003**, *29*(3), 290–295 [Engl. transl.: Russ. J. Bioorg. Chem. **2003**, *29* (3), 262–266].
11. Grechishnikova, I.V.; Johansson, L.B.; Molotkovsky, J.G. Synthesis of new bifluorophoric probes adapted to studies of donor-donor electronic energy transfer in lipid systems. Chem. Phys. Lipids **1996**, *81*(1), 87–98.
12. Ahmadian, M.; Zhang, P.; Bergstrom, D.E. A comparative study of the thermal stability of oligodeoxyribonucleotides containing 5-substituted 2′-deoxyuridines. Nucleic Acids Res. **1998**, *26*(13), 3127–3135.
13. Robins, M.J.; Vinayak, R.S.; Wood, S.G. Solvent, not palladium oxidation state, is the primary determinant for successful coupling of terminal alkynes with iodo-nucleosides. Tetrahedron Lett. **1990**, *31*(26), 3731–3734.
14. 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. Chemial synthesis of deoxyoligonucleotides by the phosphoramidite method. Methods Enzymol. **1987**, *154*, 287–313.