

Nucleosides and Nucleotides

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/Incn19>

New Pyrene Derivatives for Fluorescent Labeling of Oligonucleotides

V. A. Korshun^a, I. A. Prokhorenko^a, S. V. Gontarev^b, M. V. Skorobogatyi^b, K. V. Balakin^a, E. V. Manasova^a, A. D. Malakhov^a & Yu. A. Berlin^a

^a Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, GSP-7, 117871, Russia

^b Research Institute of Epidemiology and Microbiology, Byelorussian Ministry of Health, ul. K.Zetkin 4, Minsk, 220050, Byelarus

Available online: 16 Aug 2006

To cite this article: V. A. Korshun, I. A. Prokhorenko, S. V. Gontarev, M. V. Skorobogatyi, K. V. Balakin, E. V. Manasova, A. D. Malakhov & Yu. A. Berlin (1997): New Pyrene Derivatives for Fluorescent Labeling of Oligonucleotides, Nucleosides and Nucleotides, 16:7-9, 1461-1464

To link to this article: <http://dx.doi.org/10.1080/07328319708006206>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NEW PYRENE DERIVATIVES FOR FLUORESCENT LABELING OF OLIGONUCLEOTIDES

V. A. Korshun, I. A. Prokhorenko, S. V. Gontarev[#], M. V. Skorobogaty[#],
K. V. Balakin, E. V. Manasova, A. D. Malakhov, and Yu. A. Berlin^{*}

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, GSP-7, 117871 Russia

[#]Research Institute of Epidemiology and Microbiology, Byelarusian Ministry of Health, ul. K.Zetkin 4, Minsk, 220050 Byelarus

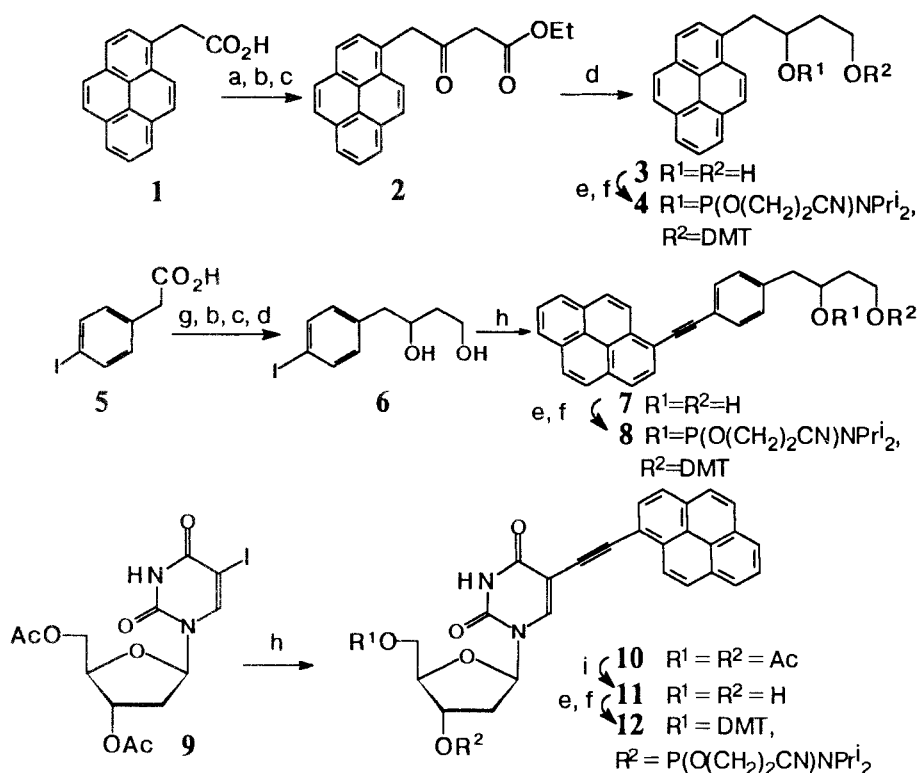
ABSTRACT. A series of pyrene-containing reagents have been synthesized and used for the fluorescent labeling of oligonucleotides.

Fluorescent labels are efficient tools for structural and functional investigations of nucleic acids¹. Of particular interest are fluorophores capable of DNA binding and exerting microenvironment-dependent emission. Pyrene derivatives are convenient in modelling and studying properties of fluorescent conjugates, since pyrene has an elevated lifetime of the excited state, is capable of intercalating into duplex DNA, forms excimers, and can be easily derivatized. In addition, pyrene derivatives are stable and fully compatible with chemistry of DNA synthesis.

Earlier we have described reagents for the synthesis of pyrene-oligonucleotide conjugates based on 3-amino-1,2-propanediol² and *trans*-4-hydroxyprolinol.³ Here we report the synthesis of three new pyrene-containing phosphoramidite reagents (SCHEME 1).

Pentafluorophenyl 1-pyrenylacetate³ **1** was used in C-acylation of Meldrum's acid (isopropylidene malonate) followed by ethanolysis⁴ to give β -ketoester **2**. Its borohydride reduction⁵ afforded compound **3** in an 82% overall yield. This compound contains a primary and a secondary hydroxyl group separated by a three-carbon-atom chain, which mimicks the nucleoside 5',3'-diol system. Diol **3** was dimethoxytritylated and then phosphitylated to give the pyrene phosphoramidite **4**.

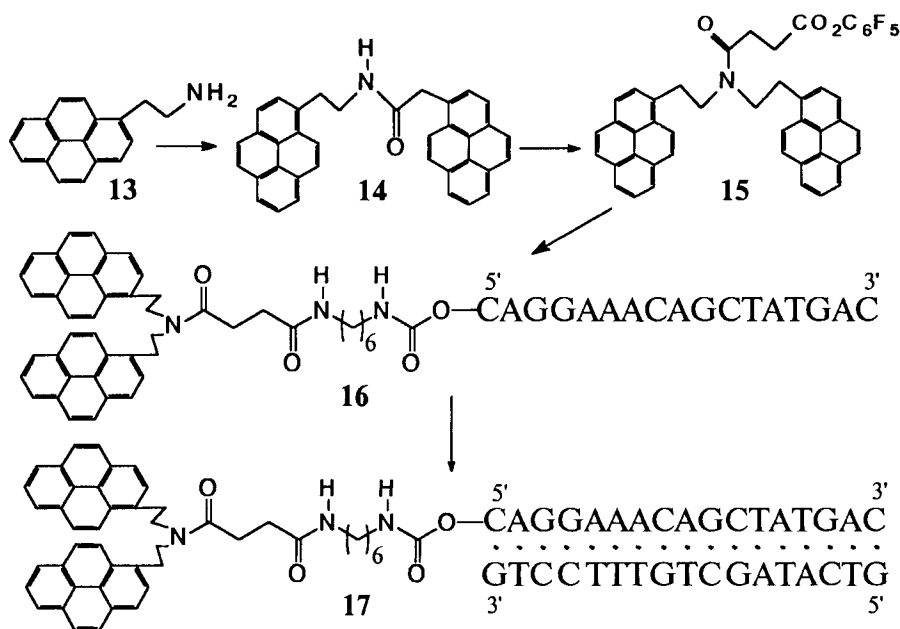
In the synthesis of the second reagent, the starting 4-iodophenylacetic acid **5** was transformed, in a 57% overall yield, into the key diol intermediate **6** (similarly to the previous synthesis, except for acid chloride rather than pentafluorophenyl ester used in acylation, and isopropanolysis instead of ethanolysis). The Pd-catalyzed [e.g., Pd(PPh₃)₄/CuI] Heck-Sonogashira coupling⁶ of iodide **6** with 1-ethynylpyrene⁷ gave a



SCHEME 1

functionalized luminescent dye derivative **7** (98%), which also was converted into the DMT-protected phosphoramidite reagent **8**.

Still another pyrene-bearing reagent was of the nucleoside nature. 5-Iodo-3',5'-O-diacetyl-2'-deoxyuridine⁸ **9** by a Pd(0)-catalyzed coupling with 1-ethynylpyrene under conditions adapted for nucleosides^{8,9} was converted into compound **10** (83%), which was quantitatively ammonolyzed to yield the unprotected nucleoside **11**. This conjugate can be conventionally transformed into the phosphitylated synthon **12** and thus introduced into oligo- and polynucleotides. It is known that attaching an alkyn-1-yl group at the uracil C5 atom does not essentially distort the substrate properties of dUTP in the DNA polymerase reaction¹⁰ and even may additionally stabilize the nucleic acid complexes.¹¹ The two-atomic spacer containing a triple bond is suitable to conjugate π -



SCHEME 2

electron systems of a fluorophore and a nucleic base, thus allowing variations in spectral properties of the conjugate and the polymer into which it is incorporated.

Attachment of an ethynyl, a substituted phenylethynyl, or a nucleoside-ethynyl group to pyrene proved to produce a considerable red shift of the absorption maxima (e. g. position of the long-wavelength maximum in methanol is 335 nm for pyrene and 392 nm for 5-(1-pyrenylethynyl)-2'-deoxyuridine) accompanied by a slight increase in absorbance. Interestingly, in the case of 5-(1-pyrenyl)-2'-deoxyuridine, where both chromophores are linked directly, the position of the pyrene long-wavelength maximum was not affected but the absorbance decreased by several times.¹² Intermediates **7** and **11** were spectroscopically characterized as models. They showed strong fluorescence in methanolic solution with $\lambda_{\text{em}}^{\text{max}}$ 389 and 410 nm (**7**), 400 and 424 nm (**11**). The substance **11** fluoresced an order of magnitude more intensively in a dioxane solution than in methanol.

Modifying phosphoramidites **4**, **8**, and **12** allow introduction of conformationally restricted pyrene labels into any predetermined site of an oligonucleotide. To study the interaction of less restricted pyrene residues with DNA, we synthesized a compound which contains a pyrene bifluorophore capable of excimeric fluorescence, attached it to an oligonucleotide, and monitored changes in the fluorescent properties of the resultant conjugate upon its hybridization with the complementary nucleotide sequence.

Amine **13** (prepared from 1-pyrenylacetic acid by the borane reduction of its amide) was reacted with pentafluorophenyl pyrenylacetate to give amide **14**. The latter was converted into activated derivative **15** by sequential reduction with $\text{BH}_3 \cdot \text{THF}$, acylation with succinic anhydride, and esterification with $\text{C}_6\text{F}_5\text{OH}/\text{DCC}$. The activated pyrene bichromophore derivative **15** was used in the synthesis of a labeled oligonucleotide **16** (SCHEME 2). The conjugate obtained was hybridized with the complementary oligonucleotide to give duplex **17**.

The attachment of the pyrene bifluorophore to an oligonucleotide lead to a considerable increase in the label's monomeric fluorescence and disappearance of excimeric fluorescence but did not affect the overall intensity of fluorescence. Of special interest is the absence of excimeric fluorescence, which evidences that the interaction of at least one of the pyrene residues with a single-stranded DNA oligomer prevents stacking of the planar pyrene systems. Upon formation of duplex **17**, not only monomeric fluorescence additionally increased but also excimeric fluorescence reappeared, although less intensive than for the free label. Thus, the pyrene bifluorophore can be practical as an environment-sensitive excimer-forming probe in structure-functional studies of biomolecules.

Acknowledgment. This work was supported by the Russian Foundation for Basic Research, grant no. 94-03-08828.

REFERENCES

1. For reviews see: Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165-187; Beaucage, S.L.; Iyer, R.P. *Tetrahedron* **1993**, *49*, 1925-1963; Korshun, V.A.; Berlin, Yu.A. *Bioorg. Khim.* **1994**, *20*, 565-616; Turner, D.H.; Li, Y.; Fountain, M.; Profenno, L.; Bevilacqua, P.C. in *Catalytic RNA* (Nucleic Acids and Molecular Biology, Vol. 10), Eckstein, F.; Lilley, D.M.J., eds.; Springer: Berlin, **1996**, pp. 19-32.
2. Korshun, V.A.; Pestov, N.B.; Birikh, K.R.; Berlin, Yu.A. *Bioconjugate Chem.* **1992**, *3*, 559-562.
3. Prokhorenko, I.A.; Korshun, V.A.; Petrov, A.V.; Gontarev, S.V.; Berlin, Yu.A. *Bioorg. & Med. Chem. Lett.* **1995**, *5*, 2081-2084.
4. Oikawa, Y.; Sugano, K.; Yonemitsu, O. *J. Org. Chem.* **1978**, *43*, 2087-2088.
5. Soai, K.; Oyamada, H. *Synthesis* **1984**, 605-607.
6. Rossi R.; Carpita, A.; Bellina, F. *Org. Prep. Proc. Int.* **1995**, *27*, 127-160.
7. Gan, L.-S. L.; Acebo, A. L.; Alworth, W. L. *Biochemistry* **1984**, *23*, 3827-3836.
8. Robins, M.J.; Barr, P.J. *J. Org. Chem.* **1983**, *48*, 1854-1862.
9. Robins, M.J.; Vinayak, R.S.; Wood, S.G. *Tetrahedron Lett.* **1990**, *31*, 3731-3734.
10. Confalone, P. *J. Heterocycl. Chem.* **1990**, *27*, 31-46.
11. Froehler, B.C.; Wadwani, S.; Terhorst, T.J.; Gerrard, S.R. *Tetrahedron Lett.* **1992**, *33*, 5307-5310; Moulds, G.; Lewis, J.G.; Froehler, B.C.; Grant, D.; Huang, T.; Milligan, J.F.; Matteucci, M.D.; Wagner, R.W. *Biochemistry* **1995**, *34*, 5044-5053.
12. Netzel, T.L.; Zhao, M.; Nafisi, K.; Headrick, J.; Sigman, M.S.; Eaton, B.E. *J. Am. Chem. Soc.* **1995**, *117*, 9119-9128.