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Pyrene-labeled deoxyuridine and deoxyadenosine: fluorescent discriminating phenomena in their oligonucleotides

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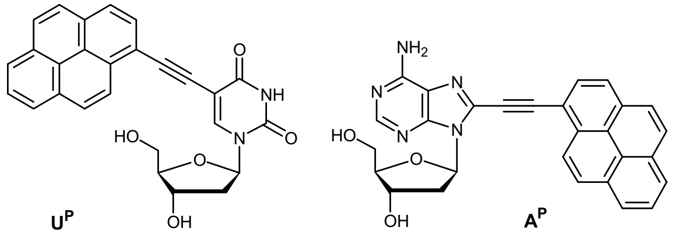
Abstract—Pyrene-labeled deoxyuridine (**U^{P}**) and deoxyadenosine (**A^{P}**) units, which are substituted at the C-5 and C-8 positions, respectively, are fluorescent unnatural nucleosides. When duplexes are formed, these nucleobases feature strong and stable interstrand stacking interactions between the two pyrene units, which compensate for the loss of hydrogen bonding and induce characteristic pyrene excimer emissions.

Hydrogen bonding and π-stacking between pairs and sequences, respectively, of nucleotides are the major interactions that stabilize the DNA double helix, and they play a critical role in affecting the conformational diversity of DNA, which is essential for biological processes to occur in cells.[1] Consequently, much attention has been devoted to the synthesis of artificial oligodeoxyribonucleotides (ODNs) possessing unnatural nucleobases that can form more stable, higher-order structures and expand the range of genetic systems; such artificial ODNs include those that are stabilized by (a) non-hydrogen-bonded and hydrophobic base pairing,[2] (b) unnatural hydrogen bonded base pairing,[3] and (c) metal-mediated artificial DNA base pairing.[4] Although research has been directed toward preparing thermally stable and selective base pairs between unnatural nucleobases mediated either by hydrogen bonding or by interstrand stacking interactions, there are no examples of such base pairs in which complementary hydrogen bonding and stacking interactions occur simultaneously. We posed the following question: If two pyrene-labeled ODNs form a duplex, which kind of noncovalent interaction—hydrogen bonding or interstrand π–π stacking—will be dominant?

To address this issue, we synthesized pyrene-labeled deoxyuridine and deoxyadenosine derivatives (Fig. 1).



1: 5'd-TGGACA **CU^{P}C** GGAATG-3'
2: 5'd-CATTCC **GA^{P}G** TGTCCA-3'
3: 5'd-CATTCC **GU^{P}G** TGTCCA-3'
4: 5'd-TGGACA **CA^{P}C** GGAATG-3'
5: 5'd-CATTCC **CU^{P}C** TGTCCA-3'
6: 5'd-CATTCC **AU^{P}A** TGTCCA-3'

Figure 1. Designed fluorescent unnatural nucleosides.

Keywords: Fluorescence; Excimer; Interstrand stacking; Nucleotides.

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Pyrene is one of the most attractive fluorophores for the development of fluorescent oligonucleotide probes because it forms a stable bimolecular excimer complex.[5] We used palladium-catalyzed Sonogashira coupling to incorporate the rigid ethynylpyrene moieties into the oligonucleotides.[6,7]

The syntheses of the pyrene-labeled deoxyuridines and their corresponding phosphoramidites were conducted as reported.[8] The pyrene-labeled deoxyadenosine phosphoramidite was synthesized from the corresponding 8-bromo-5′-*O*-dimethoxytrityl-2′-deoxyadenosine.[9]

Incorporation of these pyrene-labeled nucleosides into the central positions of the ODNs was effected by standard protocols in automated DNA synthesis.[10] The modified oligonucleotides were characterized by MALDI-TOF mass spectrometry. The absorption and emission maxima of ODNs **2** and **4**, which contain the more extensively π-conjugated $\mathbf{A^P}$ unit, are red-shifted relative to those of the ODNs **1**, **3**, **5**, and **6**, which contain the $\mathbf{U^P}$ unit. The quantum efficiencies of the former compounds are higher than those of the latter ones because deoxyuridine units serve as efficient quenchers for pyrene fluorescence in nucleosides and in single-stranded oligonucleotides.[8a,11]

The transition melting temperatures and photophysical data were obtained for each duplex: the matched pairs **1·2**, **3·4**, **1·3**, and **2·4**, and the mismatched pairs **4·5**, **4·6**, **1·5**, and **1·6** (see SI). We observe increases in the values of T_m for all of the modified duplexes relative to their unmodified duplexes; this finding suggests that each pyrene unit interacts with either its opposing pyrene unit or its neighboring bases and that these interactions stabilize the duplex.

As presented in Figure 2a, the hybridization of the matched duplexes **1·2**, **3·4**, and **2·4** have red-shifted excimer emissions because of π–π interactions occurring between the two pyrene units. A characteristic feature of these excimer bands is that they contain no monomer emission band, presumably because each pyrene unit is linked to its base through an ethynyl unit, which induces rigidity, and so the stacking interactions are very strong. Surprisingly, this strong and stable interstrand stacking compensates for the loss of hydrogen bonding in duplexes **1·2** and **3·4**. The temperature-dependent excimer bands of duplexes **1·2**, **3·4**, and **2·4** disappear and the monomer emission bands reappear at temperatures above the melting points of the duplexes; these findings suggest that the stabilizing π–π interactions are maintained until the duplexes melt. In addition, the excitation spectra recorded at possible monomer and excimer wavelengths of duplexes **1·2**, **3·4**, **1·3**, and **2·4** are almost identical. Finally the shapes and positions of these bands are similar to those reported previously for pyrene excimer emissions.[12] Figure 2b indicates that duplexes **3·4**, **1·3**, and **2·4**, which all feature interstrand stacking interactions, exhibit strikingly different fluorescence properties. When the $\mathbf{U^P}$ unit bonds with the $\mathbf{A^P}$ unit, such as in the duplexes **1·2** and **3·4**, a moderately intense fluorescence is emitted (quantum yields are listed in SI). When $\mathbf{A^P}$ forms the duplexes with $\mathbf{A^P}$, as in the case of duplex **2·4**, the fluorescence intensity is increased, but the $\mathbf{U^P}$–$\mathbf{U^P}$ couple causes fluorescence emission to be quenched (Fig. 3). For the mismatched duplexes **4·5**, **4·6**, **1·5**, and **1·6**, we observe two distinct monomer bands. We assume that if no hydrogen bonding interactions exist between the bases neighboring the $\mathbf{U^P}$ and $\mathbf{A^P}$ units, as is the case in duplexes **4·5**, **4·6**, **1·5**, and **1·6**, such interstrand stacking interactions are not allowed. To elucidate the detailed three-dimensional structures of these duplexes, we calculated possible structures for duplexes **1·2**, **1·3**, and **2·4**. Figure 4a indicates that the pyrene moieties in duplex **1·3** reside in the major groove, that is, the pyrene units are located in a highly polar environment. In contrast, the pyrene units in duplexes **1·2** (see SI) and **2·4** (Fig. 4b) are exposed to hydrophobic environment in the minor groove. These observations suggest that $\mathbf{U^P}$ experiences environments of differing polarity in duplexes **1·2** and **1·3** and that the polar microenvironment of the $\mathbf{U^P}$–$\mathbf{U^P}$ pair in duplex **1·3** causes the fluorescence intensity to be quenched.[11d,e,13] The two pyrene units in duplexes **1·2**, **1·3**, and **2·4** are positioned nearly parallelly and are overlapped almost ideally—they inter-

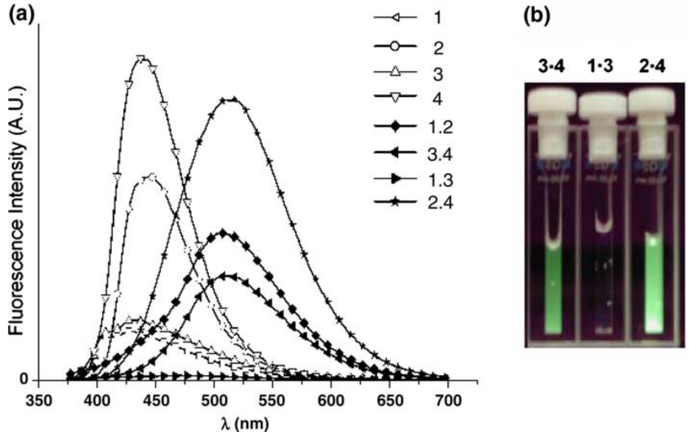
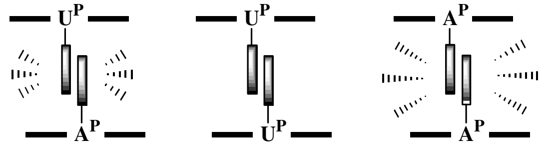


Figure 2. (a) Emission spectra recorded at 20 °C of ODNs and their duplexes (concentrations are 1.5 μM). $\lambda_{ex} = 366$ nm. (b) Photographs of the emission behavior at 25 °C of the duplexes (concentrations are 4.5 μM), $\lambda_{ex} = 365$ nm. Measured in a buffer of 100 mM NaCl, 20 mM $MgCl_2$, and 10 mM Tris–HCl (pH 7.2).



moderate fluorescence *quenched* *strong fluorescence*

Figure 3. Fluorescent base pairs based on pyrene–pyrene stacking interactions.

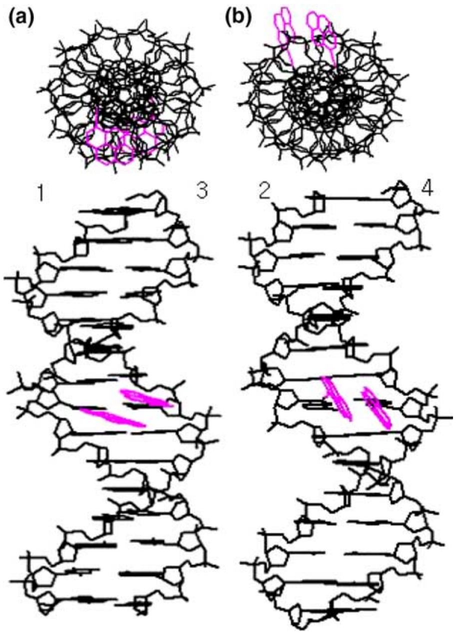


Figure 4. MM$^+$-minimized structures of duplexes (a) **1**·**3** and (b) **2**·**4**. Top views (top) and side views from the major groove (bottom) are shown. 1-Ethynylpyrene units are presented in violet. Energy minimization was followed by molecular dynamics for 50 ps at a simulation temperature of 300 K, which was followed by another energy minimization using *HyperChem* (version 7.0) software with the MM$^+$ force field.

act with one another at distances of 3.3, 3.5, and 3.4 Å for duplexes **1**·**2**, **1**·**3**, and **2**·**4**, respectively; these values fall within the characteristic range of distances for excimer formation.[5]

In conclusion, we have demonstrated that pyrene-labeled deoxyuracil and deoxyadenine units are novel unnatural nucleobases. These fluorescent nucleobase analogues allow strong interstrand stacking interactions to compensate for a loss of hydrogen bonding and exhibit a range of different emission intensities when they form duplexes with one another. These findings may provide new insights into the design of new probes and nucleobase analogues for applications in molecular biology.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.tetlet.2005.01.015.

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13. The fluorescence quantum yields of $\mathbf{U^P}$ in $CHCl_3$ and MeOH solutions are 0.81 and 0.12, respectively. Such fluorescence quenching in MeOH indicates that efficient electron injection takes place from the pyrene unit to the uracil base. See Refs. 11d and 11e.