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Synthesis of 5-(2-Pyrenyl)-2′-deoxyuridine as a DNA Modification for Electron-Transfer Studies: The Critical Role of the Position of the Chromophore Attachment

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5-(2-Pyrenyl)-2′-deoxyuridine (2PydU, **2**) has been prepared as a new thymidine analogue in which the 2-position of the pyrene chromophore is connected covalently to the 5-position of uridine through a single C–C bond. The synthesis of **2** starts with the conversion of pyrene (**3**) into 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrene (**4**) by using an Ir catalyst that was prepared in situ from $[IrCl(cod)]_2$ and 4,4′-di-*tert*-butyl-2,2′-bipyridine (dtbpy) in the presence of NaOMe. The subsequent Suzuki–Miyaura cross-coupling of **4** with 5-iodo-2′-deoxyuridine (**5**) was performed by using 1,1′-bis[(diphenylphosphanyl)ferrocene]dichloropalladium-(II) as the catalyst in a THF/MeOH/H_2O mixture as the solvent. The modified nucleoside **2** was characterized by absorption and fluorescence spectroscopy. The results were compared with the strongly electronically coupled 5-(1-pyrenyl)-2′-deoxyuridine (1PydU, **1**). Finally, the nucleoside **2** was converted into the corresponding phosphoramidite **7** as a DNA building block. The DNA set **8a**–**8d** was synthesized to explore the optical properties of the 2PydU label in duplex DNA with respect to the counterbase opposite the 2PydX modification site and in comparison with 1PydU-modified DNA. The studies with the 2PydU nucleoside and the 2PydU-modified DNA clearly allow the conclusion to be drawn that the pyrene and the uridine moieties, as the two aromatic groups in this modified nucleoside, are only weakly electronically coupled. Accordingly, the 2PydU label behaves optically like a pyrene derivative, in contrast to the 1PydU label. The chromophore shows the ability for Watson–Crick base-pairing inside the DNA, as revealed by the absorption and fluorescence spectra. 2PydU represents an optical label for DNA that changes its absorption properties upon DNA hybridization and undergoes fluorescence quenching by charge transfer.

Introduction

The analytical problems typical of biomedicinal diagnostics and chemical bioanalysis demand powerful and versatile DNA labels for optical spectroscopy. A variety of organic dyes have been investigated and applied as artificial DNA base substitutes for fluorescent nucleic acid analysis.[1–3] Recently, the chemical detection of single nucleotide polymorphisms without the application of any enzymes was achieved by fluorescent DNA base substitutions based on, for example, ethidium,[4] thiazole orange[5] or indole.[6] Moreover, there is an increasing demand for optical DNA hybridization labels that change not only their emission but also their absorption properties as a result of duplex formation. One important way to create this kind of duplex-sensitive optical property is to attach organic chromophores covalently to DNA bases. Over the last few years, we have used this kind of modification strategy to investigate DNA-mediated electron-transfer processes[7–10] and in the development of versatile and tunable optical probes for DNA.[11,12]

Pyrenes have been widely used for nucleic acid labelling.[12–18] We used 5-(1-pyrenyl)-2′-deoxyuridine (1PydU, **1**) and other pyrene-modified nucleosides as models for understanding electron transfer in DNA.[7,8] Furthermore, in 1PydU-modified DNA duplexes, photochemically induced electron transfer from the PydU group can be studied by chemical and spectroscopic techniques.[9] Moreover, we recently described 8-(1-pyrenyl)-2′-deoxyguanosine as a duplex-sensitive probe for absorption and fluorescence spectroscopy with DNA and for the detection of base mismatches.[6,12] Pyrene-modified nucleosides have also been described as base-discriminating fluorescent probes.[14] The characteristic excimer fluorescence of two and more stacked pyrene moieties has also been applied in the diagnosis of genetic variations.[15] Helical π arrays of pyrene-modified nucleosides along DNA and RNA duplexes have been described by our group[16] and others.[17] DNA has also been used as a framework for the helical arrangement of interstrand stacked pyrenes.[18]

A critical issue for the optical properties of pyrene is the linkage between this chromophore and the oligonucleotide.

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In our previously described pyrene-modified nucleosides, such as 1PydU (**1**), the two chromophores are linked covalently by a single C–C bond.[8] According to the observed unstructured fluorescence bands with solvent-dependent maxima, strong electronic coupling exists between the aromatic parts of the molecule. These intramolecular (bonded) exciplexes exhibit both excited-state and charge-separated-state character.[19] Herein, we present the synthesis of 5-(2-pyrenyl)-2′-deoxyuridine (2PydU, **2**) as an alternative pyrene-labelled uridine and the spectroscopic evaluation of its optical properties. In the modified nucleosides, 1PydU (**1**) and 2PydU (**2**), the two aromatic groups are connected through a single C–C bond. However, in contrast to 1PydU (**1**), the uridine moiety in 2PydU (**2**) is connected to the 2-position of the pyrene chromophore which alters the optical properties of the pyrene label significantly. Based on this molecular design, 2PydU (**2**) should show optical properties typical of pyrene together with a base-pairing ability in duplex DNA due to the presence of the uridine group.

Results and Discussion

Synthesis of 5-(2-Pyrenyl)-2′-deoxyuridine (2PydU, 2): To understand the electronic differences between 1PydU (**1**) and 2PydU (**2**) we have studied the topology of the relevant molecular orbitals that are involved in the intramolecular electron-transfer processes in these nucleosides. The locally excited state of pyrene Py* is a good electron donor and can induce electron transfer yielding the charge-separated state that consists of the pyrenyl radical cation $Py^{\bullet+}$ and the corresponding uridine radical anion $dU^{\bullet-}$. This assumption is based on the redox potential (1.5 V vs. NHE) and the singlet energy of pyrene (E_{00} = 3.25 eV).[20] The driving force of this intramolecular electron transfer is a maximum 0.5–0.6 eV on the basis of the reported reduction potentials of –1.1 to –1.2 V for uracil.[21] According to this simple one-electron picture the LUMO of the uridine moiety has to accept an electron from an occupied pyrene π orbital. The LUMOs of 1PydU (**1**) and 2PydU (**2**) were calculated with the semi-empirical AM1 method (Figure 1). They were obtained after full geometry optimization. The results show clearly that 1PydU (**1**) has a large coefficient on the carbon at the 1-position that connects the pyrene to the uridine moiety. This leads to a largely delocalized orbital and, as already pointed out in the Introduction, to exciplex states that show unstructured, solvent-dependent fluorescent bands. In contrast, the LUMO of 2PydU (**2**) is strictly localized on the aromatic system of the pyrene with a low coefficient on the carbon atom (at the 2-position) connecting the uridine moiety. The overlap of the uridine and pyrene donor orbital is negligible and no significant electronic coupling takes place between the aromatic moieties. Hence, in 2PydU (**2**) the typical optical properties of pyrene and the base-pairing ability of the uridine group should reflect that the pyrene is connected covalently but not coupled electronically.

Our approach for the synthesis of the pyrene-modified nucleosides such as **1** and **2** was to apply the palladium-catalyzed Suzuki–Miyaura-type cross-coupling of pyrenylboronic acids or acid esters to the corresponding halogenated nucleosides. In general, Suzuki–Miyaura-type couplings are highly suitable for nucleoside synthesis because they work in moist or even aqueous solutions, and they tolerate the presence of unprotected hydroxy and amino groups which are the characteristic functional groups of nucleosides.[22] Hence, Suzuki–Miyaura-type couplings have been applied for the preparation of a broad variety of arylated and alkenylated nucleosides.[23,24] We have developed a special Suzuki–Miyaura coupling protocol to synthesize all four 1-pyrenyl-modified nucleosides.[8] In order to apply this protocol to the synthesis of **2** the bromination of pyrene (**3**) in the 2-position is required as the first step towards the

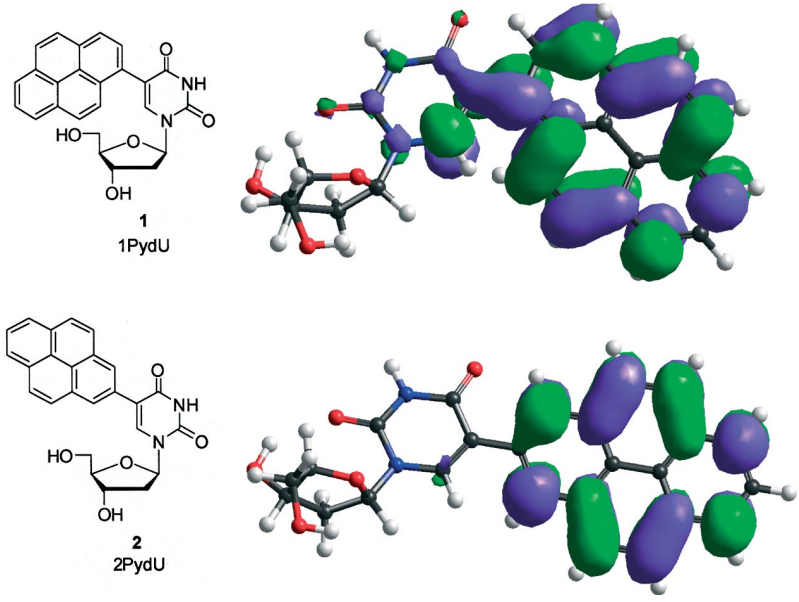
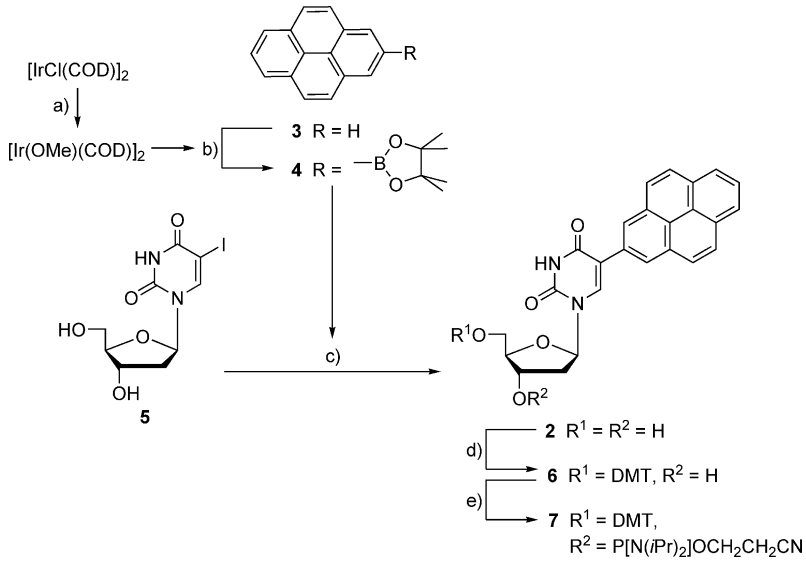


Figure 1. Calculated LUMOs of 1PydU (**1**) and 2PydU (**2**).

corresponding pyrene-2-boronic acid or acid ester. Standard electrophilic substitution of pyrene (**3**) produces mainly derivatizations in the 1-position and subsequently 1,3-, 1,6- and 1,8-disubstituted products.[25] Pyrenes that are substituted in ring position 2 are difficult to obtain. The most convenient synthetic strategy for the introduction of functional groups in the 2-position involves the conversion of pyrene (**3**) into 4,5,9,10-tetrahydropyrene by catalytic hydrogenation.[26] This biphenyl aromatic system can be brominated in the 2-position and subsequently reoxidized to the fully aromatic 2-bromopyrene.[27]

In accord with a recent related report,[28] we chose the direct approach to the synthesis of a pyrene-2-boronic acid ester as potential precursor for the subsequent Suzuki–Miyaura cross-coupling (Scheme 1). This approach is based on studies by Hartwig and Miyaura who have shown that



Scheme 1. Synthesis of 2PydU (**2**) and the corresponding DNA building block **7** for automated oligonucleotide synthesis: Reagents and conditions: a) NaOMe (3.0 equiv.), cyclohexane, 60 min, room temp.; b) **3** (1.0 equiv.), bis(pinacolato)diborane (1.1 equiv.), dtbpy (0.1 equiv.), $[Ir(OMe)(cod)]_2$ (0.05 equiv.), cyclohexane, 48 h, 80 °C (37%, a+b); c) **4** (1.2 equiv.), $[Pd(dppf)_2Cl_2]$ (0.11 equiv.), THF/H_2O/MeOH = 2:1:1, NaOH (19.5 equiv.), 60 h, 65 °C (62%); d) DMTCl (1.0 equiv.), pyridine, 48 h, room temp. (78%); e) cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (1.0 equiv.), $EtN(iPr)_2$ (3.6 equiv.), CH_2Cl_2, 1 h, room temp. (95%).

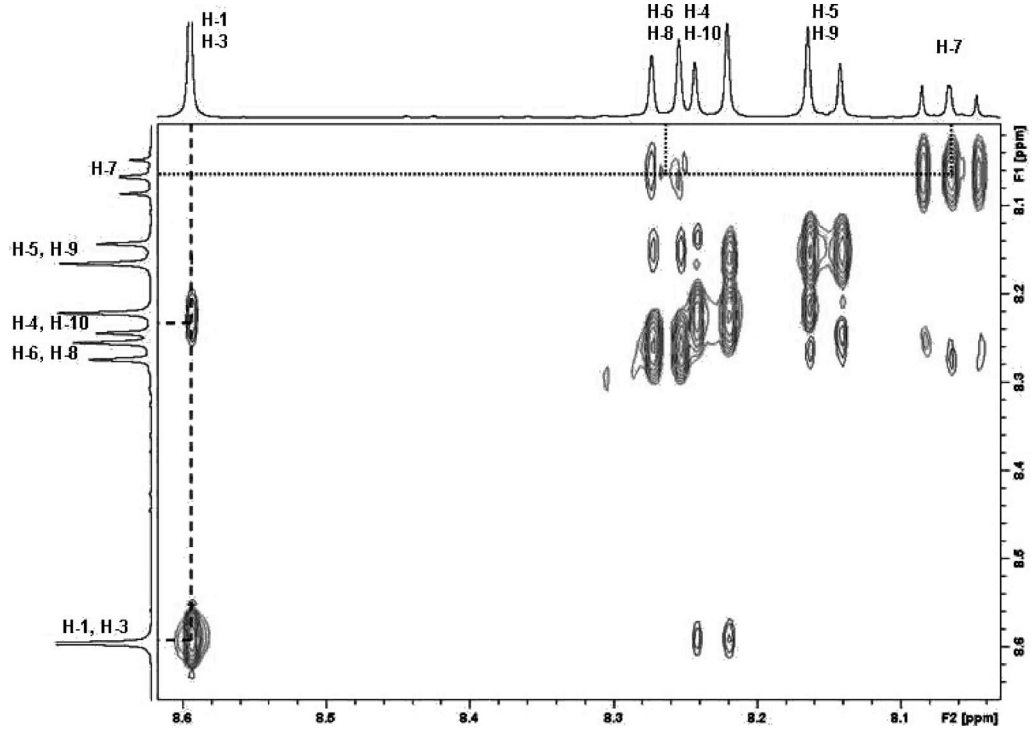


Figure 2. Assigned NOESY spectrum of the aromatic region of **4**.

iridium catalysts are particularly efficient for the direct borylation of aromatic compounds.[29] We started the synthesis of **2** by converting pyrene (**3**) into 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrene (**4**) using the catalyst that was prepared in situ from $[IrCl(cod)]_2$ and 4,4′-di-*tert*-butyl-2,2′-bipyridine (dtbpy) in the presence of NaOMe. The product **4** was obtained in 37% yield by using 5 mol-% of the precursor for the active Ir catalyst in cyclohexane. In order to prove the successful derivatization at the 2-position of the pyrene chromophore spectroscopically we first assigned all pyrene protons by 2D NMR spectroscopy. As expected, the corresponding spectrum shows one singlet for the protons 1-H and 3-H which are adjacent to the modification site. Subsequently, a NOESY spectrum of **4** was recorded to support this result. This spectrum shows the characteristic NOE pattern of a 2-substituted pyrene (Figure 2). Accordingly, the protons 1-H and 3-H show a strong NOE to each other and a weak NOE to 4-H and 10-H. On the other side, 7-H exhibits a NOE with 6-H and 8-H.

The subsequent Suzuki–Miyaura cross-coupling of **4** with 5-iodo-2′-deoxyuridine (**5**) was performed using our previously described protocol: 1,1′-bis[(diphenylphosphanyl)ferrocene]dichloropalladium(II) $[Pd(dppf)_2Cl_2]$ (0.11 equiv.) was used as the catalyst in a THF/MeOH/H_2O mixture as solvent. After 60 h at 65 °C the product **2** was isolated in 62% yield.

Spectroscopic and Electrochemical Characterization of 2PydU (2): The synthesized nucleoside **2** was characterized by optical spectroscopy. All measurements were compared with the pyrene nucleoside **1**, which was synthesized according to the literature,[8] and commercially available pyrene (**3**). The steady-state optical characterization of 2PydU (**2**) was performed in MeOH and MeCN as representative solvents with and without hydrogen-bonding capabilities (Figure 3). We have previously shown that the dynamics of intramolecular electron transfer in 1PydU (**1**) in a non-protic solvent (MeCN) and in a protic one (MeOH) are significantly different, although both solvents have similar dielectric properties.[8] The UV/Vis spectra of both modified compounds, **1** and **2**, in both solvents exhibit shapes typical of pyrene derivatives (Figure 3). Compared with pyrene (**3**), the absorption spectra of 1PydU (**1**) are significantly more redshifted than those of 2PydU (**2**), indicating the stronger electronic coupling between the two aromatic parts in **1** compared with **2**.

Based on the recorded absorbance spectra, the excitation wavelength for the steady-state fluorescence measurements was chosen to be 338 nm. It is important to point out that the samples were adjusted to an identical optical density at this excitation wavelength in order to elucidate exclusively the influence of hydrogen bonding on the emission. The corresponding fluorescence spectra of **1**–**3** show remarkable differences (Figure 4). The emission of pyrene (**3**) is nearly identical in MeCN and MeOH; no influence of the solvent was observed. The fluorescence spectra of 2PydU (**2**) exhibit the typical pyrene band structure, although significantly redshifted by approximately 30 nm. The emission maxima of **2** in MeCN and MeOH are nearly the same (double band

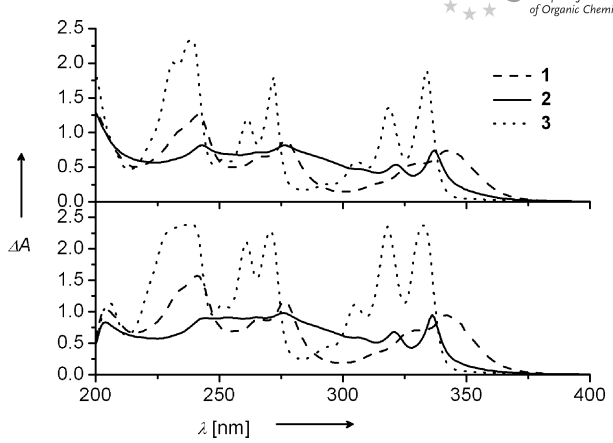


Figure 3. UV/Vis absorption spectra of 2PydU (**2**) in comparison with 1PydU (**1**) and pyrene (**3**), measured in MeCN (top) and MeOH (bottom). The samples were adjusted to an identical optical density at the excitation wavelength of 338 nm (in MeCN: **1**: 30 μM; **2**: 35 μM; **3**: 55 μM; in MeOH: **1**: 40 μM; **2**: 45 μM; **3**: 100 μM).

with maxima at 399 and 421 nm). In contrast, 1PydU (**1**) shows a broad fluorescence band in MeCN (425 nm) that is redshifted in MeOH (447 nm) which is typical of a strongly coupled bonded exciplex state, as discussed above. In MeCN, some of the locally excited state of the pyrene group in **1** persists (side-band at 388 nm). The relative fluorescence intensities of both nucleosides **1** and **2** are significantly reduced in MeOH compared with MeCN. The measured quantum yields reflect the fluorescence observations quantitatively (Table 1). The quantum yield of **1** is significantly reduced from 24% in MeCN to 2.5% in MeOH. This is also observed in the case of **2**, however, not to such an extent. As previously shown by time-resolved studies, the electron-transfer yield in **1** was greatly enhanced in MeOH.[8] Clearly, this effect is much weaker in **2** as a result of the weaker electronic coupling in this nucleoside. This result emphasizes the critical role of hydrogen bonding in electron transfer involving DNA bases.

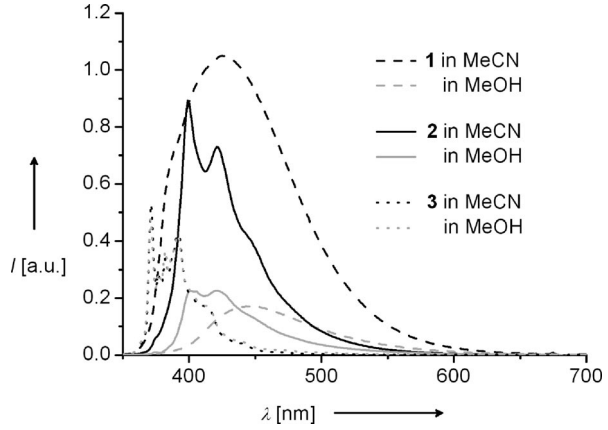


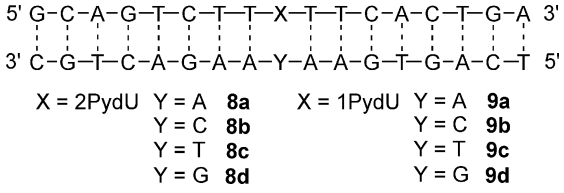
Figure 4. Fluorescence spectra of 2PydU (**2**) in comparison with 1PydU (**1**) and pyrene (**3**), measured in MeCN (black) and MeOH (grey). The samples were adjusted to an identical optical density at the excitation wavelength of 338 nm (in MeCN: **1**: 30 μM; **2**: 35 μM; **3**: 55 μM; in MeOH: **1**: 40 μM, **2**: 45 μM; **3**: 100 μM).

Table 1. Quantum yields of 2PydU (**2**) in comparison with 1PydU (**1**) and pyrene (**3**), measured at 338 nm.

Compound	Φ (MeCN)	Φ (MeOH)
1	0.235	0.025
2	0.067	0.027
3	0.019	0.018

It is remarkable to note that the optical properties of 2PydU (**2**) reflect clearly the electronic properties that were expected from its design. This interpretation is based mainly on three observations: (i) only a small redshift of the absorption compared with pyrene (**3**), (ii) an emission that exhibits the structure of the locally excited state of the pyrene chromophore, and (iii) no solvent-dependence of the fluorescence maxima and a smaller effect of the solvent on the fluorescence intensity. Hence, in contrast to 1PydU (**1**), the two aromatic moieties in 2PydU (**2**) are only weakly electronically coupled. Hence, the absorption and fluorescence properties of **2** are similar to those of pyrene (**3**).

Synthesis and Characterization of 2PydU-Modified DNA: In order to explore the optical properties of the pyrene-modified nucleoside **2** in DNA we synthesized the duplex set **8a**–**8d** bearing a random DNA base sequence (Scheme 2). Measurements were also performed with the duplex set **9a**–**9d** which contain identical sequences except with the 1PydU modification instead of the 2PydU group. The duplexes **9a**–**9d** were prepared according to the literature.[9] In both duplex sets, **8a**–**8d** and **9a**–**9d**, the counterbase Y opposite the pyrene modification site X was varied in order to explore the Watson–Crick base-pairing properties of 1PydU and 2PydU inside duplex DNA.



Scheme 2. Sequences of DNA duplex sets **8a**–**8d** and **9a**–**9d**.

The pyrene-modified uridine **2** was converted into the DMT-protected compound **6** and then to the completely protected nucleoside **7** carrying the phosphoramidite group in the 3′-position (Scheme 1). Standard procedures were applied to these two synthetic steps. By using the DNA building block **7**, the 2PydU-modified oligonucleotide **8** was prepared by automated solid-phase synthesis using the Expedite 8909 DNA synthesizer. Nearly quantitative coupling of the monomer **7** was achieved with the standard coupling time of 1.6 min. The HPLC-purified oligonucleotide was identified by ESI mass spectrometry and quantified by its UV/Vis absorption spectrum by using ε_{260} = 18600 $M^{-1}cm^{-1}$ for the 2PydU modification. By using the 2PydU-modified oligonucleotide **8** we prepared the corresponding DNA duplexes **8a**–**8d** by slow cooling in the presence of 1.2 equiv. of the unmodified complementary strands. These DNA duplexes were subsequently characterized by CD spectroscopy (Figure 5) and by their melting temperatures T_m (Table 2). The CD spectra of the duplexes **8a**–**8d** and **9a**–**9d** confirm the normal right-handed helical B-DNA conformation. The melting temperatures of the duplexes **9a**–**9d** are all lower than those of the duplexes **8a**–**8d**, indicating that the steric demand and local perturbation of the pyrene group is higher in 1PydU than in 2PydU. The "mismatched" duplexes **8b** and **8c**, but not **8d**, exhibit increased melting temperatures which indicate a partial intercalation causing hydrophobic stabilization of the pyrene. However, the T_m data does not clearly reveal a preferred Watson–Crick base-pairing for 2PydU.

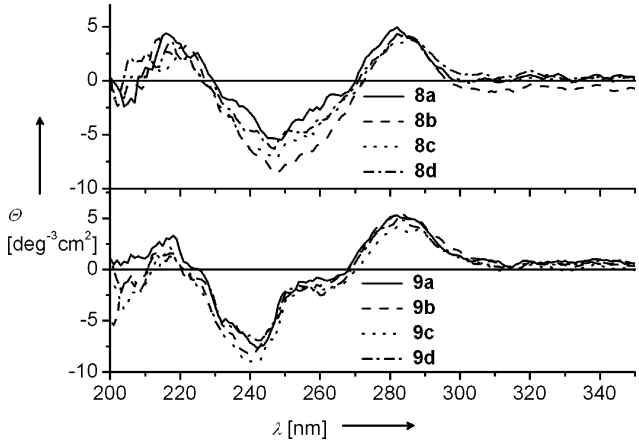


Figure 5. CD spectra of DNA **8a**–**8d** (top) and **9a**–**9d** (bottom) [2.5 μM duplex in 10 mM Na-P_i-buffer (pH 7.0), 250 mM NaCl, 20 °C].

Table 2. Melting temperatures (T_m) of DNA duplex sets **8a**–**8d** and **9a**–**9d** [2.5 μM duplex, 10 mM Na-P_i-buffer (pH 7.0), 250 mM NaCl, 260 nm, 10–90 °C, heating rate: 0.7 °C/min].

Counterbase Y	DNA	T_m [°C]	DNA	T_m [°C]
A	**8a**	55.2	**9a**	52.5
C	**8b**	58.2	**9b**	55.8
T	**8c**	56.0	**9c**	54.0
G	**8d**	54.3	**9d**	53.1

The UV/Vis absorption spectra of both duplex sets, **8a**–**8d** and **9a**–**9d**, show clearly the presence of the pyrene chromophore in the range between 300 and 400 nm (Figure 6). However, the shapes of the pyrene absorption bands are significantly different for the two chromophore-modified nucleosides. The single-stranded oligonucleotide **8** has an absorption maximum at 344 nm that is maintained after hybridization with the mismatched counterstrands yielding the DNA duplexes **8b**–**8d**. It is important to point out that only in DNA **8a**, bearing an adenine as the correct Watson–Crick counterbase opposite the 2PydU modification, is the absorption maximum blueshifted to 338 nm. Together with the melting temperatures discussed above, this blueshift can be interpreted as the result of the destacking of the pyrene chromophore out of the DNA helix as a result of the base-pairing of the uridine group of 2PydU with the adenine counterbase. This result is not observed with the duplex set

9a–**9d**. The single-stranded oligonucleotide **9** exhibits absorption maxima at 335 and 350 nm, similar to the absorption of 1PydU (**1**) in MeOH. After hybridization with the counterstrands, the four duplexes **9a**–**9d** show a similar absorption with a broad maximum at around 355 nm. The absorption differences between the duplexes **8a** and **8b**–**8d** on the one hand and the similarity of the duplexes **9a**–**9d** on the other are remarkable. In fact, the 2PydU moiety behaves like a pyrene covalently attached but not electronically coupled to the DNA base uridine. According to the absorption spectra the chromophore shows an ability for Watson–Crick base-pairing in DNA. In contrast, the strongly coupled pyrene chromophore in the 1PydU group does not exhibit this property.

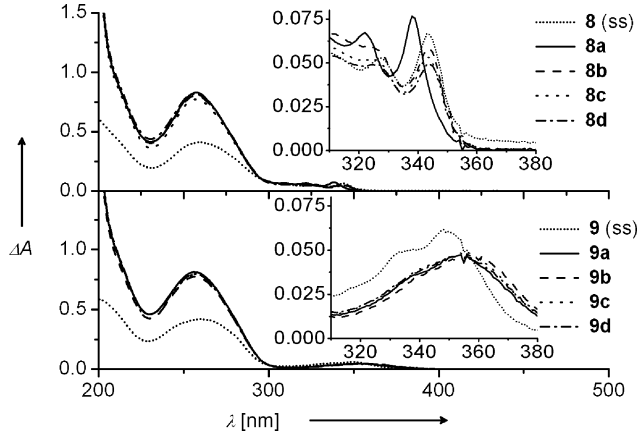


Figure 6. UV/Vis absorption spectra of DNA **8a**–**8d** (top) and DNA **9a**–**9d** (bottom) [2.5 μM duplex in 10 mM Na-P_i-buffer (pH 7.0), 250 mM NaCl, 20 °C].

The fluorescence spectra of the duplexes were recorded at an excitation wavelength of 340 nm for the 2PydU-modified DNA **8a**–**8d** and 360 nm for the 1PydU-modified DNA **9a**–**9d** (Figure 7). In both duplex sets, the emission increases significantly compared with the single-stranded oligonucleotides **8** and **9**. The emission of the 2PydU chromophore allows the right counterbase (A in **8a**) to be discriminated against the wrong ones (**8b**–**8d**); the emission of the former is greater than the latter by a factor of at least 2. This is not the case for the 1PydU-modified duplexes **9a**–**9d**. This result is similar to that already pointed out above for the absorption differences. However, one has to take into account the fact that the quantum yields of all the duplexes lie in the range between 1.0 and 1.5%. In comparison with the quantum yield for the isolated nucleoside **2** of 7% in MeCN this result indicates a significant quenching of the 2PydU fluorescence in DNA. Based on our previous experiments with 1PydU-modified duplexes,[9] we assume that this fluorescence quenching can be assigned to a photoinduced electron transfer inside the DNA, preferably to the adjacent thymines. This result makes the 2PydU label a highly promising one-electron donor for spectroscopic studies of charge transfer in DNA. The critical issue of the 1PydU label that we have applied until now to elucidate the dynamics of electron transfer in DNA was the strong electronic coupling in the charge-separated state. As a result of direct π-orbital overlap between the radical cation $Py^{\cdot+}$ and the radical anion $dU^{\cdot-}$ the transient absorption exhibits broad spectral signals that range from around 450 to around 730 nm which are difficult to assign and to correlate with the dynamics of electron transfer. Based on the steady-state studies of the nucleoside 2PydU (**2**) and the representative synthesized 2PydU-modified DNA **8a** we can conclude a weak electronic coupling between the two aromatic groups in the new label. Hence, we expect that the dynamics of electron transfer can be assigned to the transient absorption signals of the 2PydU label in DNA more clearly than was the case with the 1PydU-modified DNA.

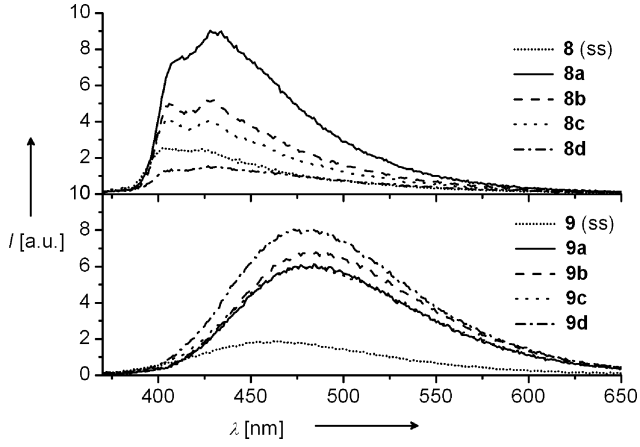


Figure 7. Fluorescence spectra of DNA **8a**–**8d** (top) and DNA **9a**–**9d** (bottom) [2.5 μM duplex, 10 mM Na-P_i-buffer (pH 7.0), 250 mM NaCl, 20 °C].

Conclusions

The critical issue for the optical properties of the 1PydU label that we have studied extensively over the last few years was the strong electronic coupling between the pyrene and the uridine as the two aromatic groups in this modified nucleoside. Hence, the 2PydU label was designed, synthesized and studied as an alternative pyrene-modified nucleoside and optical label for DNA. In both uridines, 1PydU and 2PydU, the pyrene is attached covalently through a single C–C bond. However, due to the fact that the uridine moiety in 2PydU is connected to the 2-position of the pyrene chromophore and not to the 1-position as in 1PydU the electronic coupling is significantly weaker. The absorption and fluorescence spectroscopy studies described above clearly allow this conclusion to be drawn. Accordingly, the properties of 2PydU exhibit characteristics typical of a pyrene derivative. 2PydU behaves optically and electrochemically more like a pyrene label for DNA than does 1PydU. Strong fluorescence quenching of the 2PydU label is observed in duplex DNA presumably due to a charge transfer into the DNA.

In general, the observed differences between 1PydU and 2PydU underscore the fact that the position of attachment of large aromatic chromophores is critical and determines the optical properties. Pyrene as an artificial DNA base sur-

rogate has previously been used as a fluoroside[30] and in the investigation of DNA–protein interactions.[31] The 2PydU modification represents a pyrene label that retains the unique and characteristic optical properties of the pyrene chromophore and connects it, but does not couple it electronically, to the DNA base. The chromophore shows the ability for Watson–Crick base-pairing inside DNA, as revealed by the absorption and fluorescence spectra.

Experimental Section

Materials and Methods: ^{1}H, ^{13}C, ^{31}P and 2D NMR spectra were recorded at 300 K with a Bruker Avance 300 or 400 MHz spectrometer. NMR signals were assigned on the basis of 2D NMR measurements (HSQC, HMBC, NOESY). ESI, EI and HR-EI mass spectra were recorded in the analytical facility of the institute. The HR-ESI mass spectra (ESI-FTICR) were recorded by Coring System Diagnosticx GmbH. Analytical chromatography was performed on Merck silica gel 60 F254 plates. Flash chromatography was performed on Merck silica gel (40–63 μm). C18-RP analytical and semi-preparative HPLC columns (300 Å) were purchased from Supelco. Solvents were dried according to standard procedures. All reactions were carried out under dry nitrogen. Commercial chemicals were purchased by Fluka, Sigma–Aldrich and Alpha Aesar and were used without further purification. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm) using Na-P_i-buffer (10 mM). Absorption spectra and the melting temperatures (2.5 μM duplex, 250 mM NaCl, 260 nm, 10–90 °C, interval 0.7 °C) were recorded with a Varian Cary 100 spectrometer. The B-DNA conformation of all the duplexes was confirmed by CD spectroscopy (2.5 μM duplex, 200–350 nm) performed with a Jasco J-715 spectropolarimeter. The fluorescence spectra (2.5 μM duplex) were recorded with a Fluoromax-3 fluorimeter (Jobin–Yvon) and corrected for Raman emission from the buffer solution. All emission spectra were recorded with a band-pass of 2 nm for both excitation and emission and are intensity-corrected. The fluorescence quantum yields were determined according to literature methods using quinine sulfate as the standard.[32]

2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)pyrene (4):[28] Bis(1,5-cyclooctadiene)diiridium(I) dichloride (132 mg, 0.197 mmol, 0.05 equiv.) was suspended in dry cyclohexane (10 mL) under nitrogen. NaOMe (33 mg, 0.591 mmol, 3 equiv.) was added and the suspension was stirred at room temp. for 60 min. Pyrene (**3**) (797 mg, 3.94 mmol, 1.0 equiv.), bis(pinacolato)diborane (1.10 g, 4.33 mmol, 1.1 equiv.) and dtbpy (106 mg, 0.394 mmol, 0.1 equiv.) were dissolved in dry cyclohexane. The prepared catalyst solution was added through a cannula and the solution turned dark immediately. After stirring at 80 °C for 48 h the solvent was removed under vacuum. The crude product was purified by flash chromatography (SiO_2, hexane/CH_2Cl_2 = 1:1) to give 486 mg (37%) of a pale yellow solid. TLC (hexane/CH_2Cl_2 = 1:1): R_f = 0.60. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.59 (s, 2 H, 1-H, 3-H), 8.31 (d, $^3J_{HH}$ = 7.7 Hz, 2 H, 6-H, 8-H), 8.27 (d, $^3J_{HH}$ = 9.3 Hz, 2 H, 4-H, 10-H), 8.20 (d, $^3J_{HH}$ = 8.8 Hz, 2 H, 5-H, 9-H), 8.11 (dd, $^3J_{HH}$ = 7.1, $^3J_{HH}$ = 7.9 Hz, 1 H, 7-H), 1.40 (s, 12 H, CH_3) ppm. ^{13}C NMR (75.4 MHz, [D$_6$]-DMSO): δ = 131.0, 130.9 (C-1, C-3), 129.9, 127.6 (C-4, C-10), 127.3 (C-5, C-9), 126.7 (C-7), 125.4, 125.0 (C-6, C-8), 123.6, 84.0 (CO), 24.8 (CH_3) ppm. EI-MS: *m*/*z* (%) = 328.1(100) [M]$^+$. HR-MS (EI): calcd. for $C_{22}H_{21}BO_2$ [M]$^+$: 328.1635; found 328.1629 [M]$^+$.

5-(2-Pyrenyl)-2′-deoxyuridine (2): Pyrene derivative **4** (130 mg, 0.396 mmol, 1.2 equiv.), 5-iodo-2′-deoxyuridine (**5**) (117 mg, 0.330 mmol, 1.0 equiv.) and 1,1′-bis[(diphenylphosphanyl)ferrocene]dichloropalladium(II) (29.4 mg, 0.036 mmol, 0.11 equiv.) were dissolved in degassed THF/H_2O (2:1, 40 mL) under nitrogen. After addition of MeOH (10 mL) and NaOH (257 mg, 6.44 mmol, 19.5 equiv.) the mixture was stirred at 65 °C for 60 h, neutralized with 2 N HCl and extracted with EtOAc (150 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/acetone = 4:1, eluent: EtOAc/MeOH = 10:3) to give 89 mg (62%) of a pale brown solid. TLC (EtOAc/MeOH/H_2O = 10:1:0.5): R_f = 0.56. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.67 (br. s, 1 H, NH), 8.53 [s, 1 H, 6-H (dU)], 8.48 [s, 2 H, 1-H, 3-H (pyrene)], 8.29 [d, $^3J_{HH}$ = 7.7 Hz, 2 H, 6-H, 8-H (pyrene)], 8.20 [s, 4 H, 4-H, 5-H, 9-H, 10-H (pyrene)], 8.06 [dd, $^3J_{HH}$ = 7.1, $^3J_{HH}$ = 7.9 Hz, 1 H, 7-H (pyrene)], 6.30 (t, $^3J_{HH}$ = 6.6 Hz, 1 H, 1′-H), 5.30 (m, 1 H, 3′-OH), 5.24 (m, 1 H, 5′-OH), 4.36 (m, 1 H, 4′-H), 3.86 (m, 1 H, 3′-H), 3.66 (m, 2 H, 5′-H), 2.25 (m, 2 H, 2′-H) ppm. ^{13}C NMR (75.4 MHz, [D$_6$]DMSO): δ = 162.3, 150.0, 139.2 [C-5 (dU)], 131.2, 130.6, 130.4, 127.5 [C-4, C-5, C-9, C-10 (pyrene)], 126.2 [C-7 (pyrene)], 125.1 [C-6, C-8 (pyrene)], 124.5 [C-1, C-3 (pyrene)], 123.6, 122.8, 113.5, 87.5 (C-3′), 84.7 (C-1′), 70.0 (C-4′), 60.8 (C-5′), 40.2 (C-2′) ppm. ESI-MS: *m*/*z* (%) = 427.2 (100) [M – H$^+$]$^-$, 463.2 [M + Cl]$^-$, 487.2 [M + CH_3COO]$^-$. HR-MS (ESI-FTICR): calcd. for $C_{25}H_{20}N_2O_5$ 428.1372; found 427.1301.

5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-5-(2-pyrenyl)-2′-deoxyuridine (6): Compound **2** (60 mg, 0.140 mmol, 1.0 equiv.) was dissolved in dry pyridine (5 mL) under nitrogen. After addition of 4,4′-dimethoxytriphenylmethyl chloride (47 mg, 0.140 mmol, 1.0 equiv.) the solution was stirred at room temp. for 48 h. The reaction was quenched with MeOH (2 mL) and stirred for 1 h at. room temp. The solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/acetone = 4:1) to give 80 mg (78%) of a brown solid. TLC (EtOAc/MeOH/H_2O = 10:1:0.5): R_f = 0.68. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 11.67 (br. s, 1 H, NH), 8.25 [d, $^3J_{HH}$ = 7.7 Hz, 2 H, 6-H, 8-H (pyrene)], 8.19 [s, 2 H, 1-H, 3-H (pyrene)], 8.08 [d, $^3J_{HH}$ = 9.3 Hz, 2 H, 4-H, 10-H (pyrene)], 8.04 [dd, $^3J_{HH}$ = 7.9, $^3J_{HH}$ = 7.7 Hz, 1 H, 7-H (pyrene)], 7.98 [s, 1 H, 6-H (dU)], 7.80 [d, $^3J_{HH}$ = 9.1 Hz, 2 H, 5-H, 9-H (pyrene)], 7.29–7.07 [m, 9 H, arom. (DMT)], 6.53 [m, 4 H, arom. (DMT)], 6.30 (t, $^3J_{HH}$ = 6.6 Hz, 1 H, 1′-H), 5.33 (m, 1 H, 3′-OH), 4.28 (m, 1 H, 4′-H), 3.95 (m, 1 H, 3′-H), 3.44 (s, 3 H, OCH_3), 3.39 (s, 3 H, OCH_3), 3.17 (m, 2 H, 5′-H), 2.41 (m, 1 H, 2′-H), 2.27 (m, 1 H, 2′-H) ppm. ^{13}C NMR (150 MHz, [D$_6$]-DMSO): δ = 162.3, 157.8, 157.7, 150.0, 144.5, 138.2 [C-6 (dU)], 135.3, 135.2, 130.7, 130.5, 130.2, 129.5, 149.4, 127.6 [arom. (DMT)], 127.5 [arom. (DMT)], 127.2 [C-5, C-9 (pyrene)], 126.4 (DMT), 126.1 [C-7 (pyrene)], 125.0 [C-6, C-8 (pyrene)], 124.9 [C-1, C-3 (pyrene)], 123.5, 122.9, 114.4, 112.9 [arom. (DMT)], 85.9, 85.6 (C-3′), 85.0 (C-1′), 70.6 (C-4′), 63.7 (C-5′), 54.6 (OCH_3), 45.7, 40.0 (C-2′), 30.6 ppm. ESI-MS: *m*/*z* (%) = 729.4 (100) [M – H$^+$]$^-$, 765.4 [M + Cl]$^-$, 789.4 [M + CH_3COO]$^-$.

5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-5-(2-pyrenyl)-2′-deoxyuridin-3′-yl 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (7): Compound **6** (80 mg, 0.109 mmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (3.3 mL) under nitrogen. Dry EtN(*i*Pr)$_2$ (55 μL, 0.394 mmol, 3.6 equiv.) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (24 μL, 0.109 mmol) were added. The solution was stirred at room temp. for 1 h. The reaction was quenched with dry EtOH (100 μL) and quickly washed with freshly prepared aq. $NaHCO_3$ solution. The organic phase was dried with Na_2SO_4 and the solvent was removed under vacuum to yield 96 mg (95%) of a brown, viscous liquid. TLC (CH_2Cl_2/acetone = 6:1): R_f = 0.56. ^{31}P NMR (121.5 MHz, [D$_6$]DMSO): δ = 148.66, 148.31 ppm.

Preparation and Characterization of the Oligonucleotides: The oligonucleotides were prepared on a Expedite 8909 DNA synthesizer from Applied Biosystems by standard phosphoramidite protocols using chemicals and CPG (1 μmol) from Applied Biosystems and Proligo. Quantitative coupling of the building block **7** was achieved by using the standard coupling time of 1.6 min. After preparation, the trityl of the oligonucleotide was cleaved from the resin and was deprotected by treatment with conc. NH_4OH at 60 °C for 10 h. The oligonucleotide was dried and purified by HPLC on a semi-preparative RP-C18 column (300 Å, Supelco) by using the following conditions: (i) unmodified oligonucleotides A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient: 0–15% B over 45 min; (ii) 2PydU-modified oligonucleotides A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient: 0–30% B over 55 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm by using ε_{260} = 18600 $M^{-1}cm^{-1}$ (MeOH) for **2** and ε_{260} = 17000 $M^{-1}cm^{-1}$ (MeOH) for **1** using a Varian Cary 100 spectrometer. Duplexes were formed by heating to 90 °C (10 min) followed by slow cooling.

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