Electron Injection into DNA: Synthesis and Spectroscopic Properties of Pyrenyl-Modified Oligonucleotides

Nicole Amann,[a] Evgeni Pandurski,[b] Torsten Fiebig,[b] and Hans-Achim Wagenknecht*[a]

Abstract: The nucleoside 5-(1-pyrenyl)-2′-deoxyuridine (**1**) was prepared by a Suzuki–Miyaura cross-coupling reaction and subsequently used as a DNA building block in order to prepare a range of modified oligonucleotides using phosphoramidite chemistry. The DNA duplexes contain a pyrenyl group covalently attached to the nucleobase uracil. Upon excitation at 340 nm an intramolecular electron transfer from the pyrenyl group to the uracil moiety takes place which represents an injection of an excess electron into the DNA base stack. Based on the results obtained by steady-state fluorescence and time-resolved pump-probe laser spectroscopy it was possible to show that base-to-base electron transfer can occur from the Py-dU group only to adjacent thymines.

Keywords: charge transfer · DNA structures · electron transfer · pyrenes

Introduction

Charge migration processes through DNA have been discussed very controversely by different research groups over the last decades.[1–4] With respect to the important biological consequences such as DNA damaging, mutagenesis, and carcinogenesis,[5] in most of the past experiments only *oxidative* hole transfer processes have been observed. Such hole transfer results in oxidative guanine damage at remote sites on the nucleic acids. Hole transfer through DNA has been initiated by photochemical methods and investigated both by spectroscopic techniques, for example by fluorescence,[1] transient absorption,[2] and EPR spectroscopy,[3] and by analytical experiments of oxidative lesions, for example by HPLC or gel electrophoretic analysis of irradiated samples.[5] By now, a lot of questions about these hole transfer processes have been worked out and a detailed picture has been emerged including important aspects such as mechanisms, distance dependence, and DNA base sequence dependence.

[a] Dr. H.-A. Wagenknecht, N. Amann
Institute for Organic Chemistry and Biochemistry
Technical University of Munich
Lichtenbergstrasse 4, 85747 Garching (Germany)
Fax: (+49)-89-289-13210
E-mail: wagenknecht@ch.tum.de

[b] E. Pandurski, Dr. T. Fiebig
Institute for Physical Chemistry
and Theoretical Chemistry
Technical University of Munich
Lichtenbergstrasse 4, 85747 Garching (Germany)
Fax: (+49)-89-289-13244
E-mail: fiebig@ch.tum.de

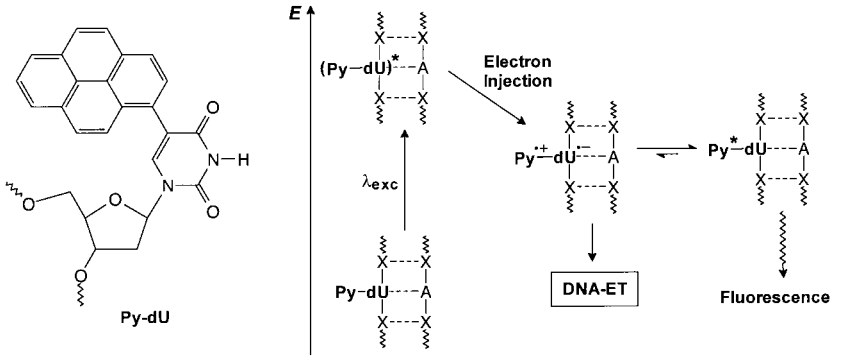
On the other hand, *reductive* electron transfer processes in DNA duplexes are currently used very extensively in DNA chip technology[6] and DNA nanotechnology.[7] Despite the broad knowledge about this bioanalytical and biomedical applications, almost nothing is known about the behaviour of excess electrons in DNA. In the past, Barton et al. investigated charge-transfer reactions through DNA using tethered and intercalated transition metal complexes as redoxactive probes[8] which in parts can be interpreted in terms of electron transfer reactions due to the MLCT character of the chosen transition metal absorption band.[9] More recently, Carell et al. published experiments describing the repair of thymine–thymine dimers from a distant flavine derivative which was synthetically incorporated as an artificial nucleobase into oligonucleotides.[10] Despite the fact that spectroscopic measurements with this systems have not been published, the thymine–thymine dimer splitting was interpreted as the chemical result of a reductive electron transfer through the DNA base stack. This interpretation is mainly based i) on the known redox properties of the flavin intercalator in its reduced and deprotonated state,[11] and ii) on the absence of a typical DNA base sequence dependence which would be observed in case of a hole hopping process.[12] Most recently, Zewail et al. reported femtosecond time-resolved studies on the reduction of thymine by photoexcited 2-aminopurine in DNA duplexes.[13]

Until now, suitable assays for the time-resolved spectroscopic investigation of reductive electron transfer in DNA are elusive. Herein, we want to present the design, preparation and preliminary spectroscopic investigations of pyrenyl-modified DNA duplexes which allow the injection of excess electrons into DNA.

  0947-6539/02/0821-4877 $ 20.00+.50/0

Results and Discussion

Design of the assay: Pyrene derivatives have been used previously as artificial DNA bases by Kool et al.,[14] Berlin et al.,[15] and a few other research groups.[16] In most of these experiments, the pyrene moiety was intercalated between the nucleobases of DNA duplexes. Based on the relative redox properties, intercalated pyrene derivatives could initiate both, oxidative hole transfer to guanines, and reductive electron transfer to thymines. Both charge transfer assignments are proven by picosecond transient absorption experiments using 5-(1-pyrenoyl)-2′-deoxyuridine,[17] or benzo[a]pyrenyl-2′-deoxyguanosine conjugates,[18] respectively. In order to avoid this dual charge transfer behaviour of intercalated pyrene derivatives, we chose to attach the pyrenyl group to the nucleobase thymine (or uracil) in order to place it outside the DNA base stack (Scheme 1).



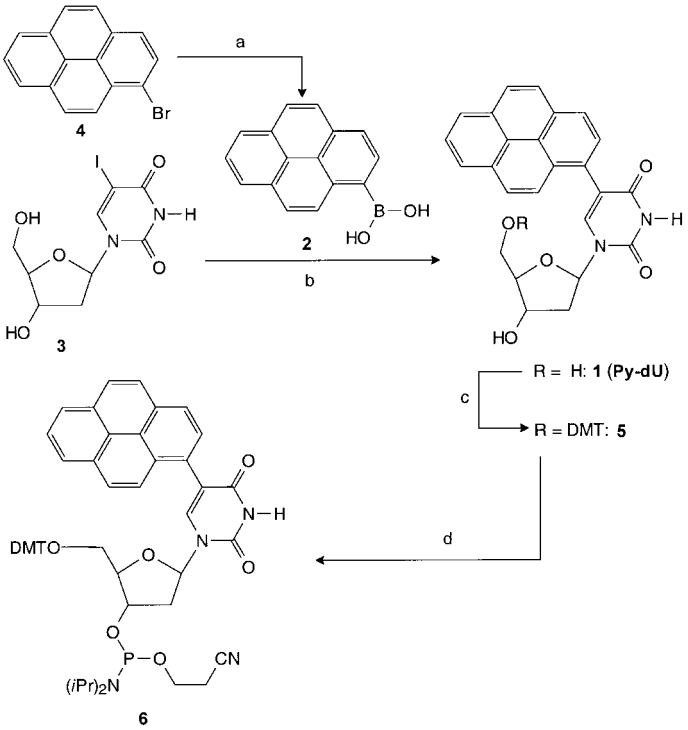
Scheme 1. Design of the assay for the spectroscopic investigation of electron injection into DNA: Excitation of the pyrenyl-modified nucleic acids at 340 nm results in an *electron transfer* representing an electron injection into DNA. This process yields the pyrenyl radical cation and the uracil radical anion. Subsequently, an intramolecular fluorescent exciplex is formed. If electron transfer to the adjacent bases occurs as an alternative pathway, quenching of the exciplex emission is observed.

Photoexcitation of the pyrenyl group results in an intramolecular electron transfer yielding the corresponding uracil radical anion and the pyrenyl radical cation ($Py^{\bullet+}$-$dU^{\bullet-}$). This charge transfer assignment has been proven by Netzel et al. based on nanosecond fluorescence lifetime measurements.[17, 19] Based on the reduction potential for $Py^{\bullet+}$/Py of 1.52 V (vs. NHE)[20] and $E_{00} = 3.25$ eV,[17, 19] the driving force ΔG of this ET process could be maximal -0.5 eV using the potential of -1.2 V for the dU/$dU^{\bullet-}$ couple given by Steenken et al.[21] However, this value of $|\Delta G|$ seems too large with respect to a recent femtosecond time-resolved study on the reduction of thymine; this suggests a potential of approximately -1.8 V for the dT/$dT^{\bullet-}$ couple.[22] Furthermore, we have characterized the properties and dynamics of the intramolecular electron transfer in the nucleoside **1** in organic solvents[23] and in water at different pH values[24] by steady-state fluorescence spectroscopy and femtosecond transient absorption spectroscopy. In organic solvents, such as THF, MeCN or MeOH, excitation of **1** at 340 nm yields the charge-separated species of **1** ($Py^{\bullet+}$-$dU^{\bullet-}$) which is converted to a fluorescent intramolecular exciplex ($Py^{\bullet+}$-$dU^{\bullet-}$)* subsequently. In MeOH, this intramolecular exciplex is stabilized by hydrogen bonding resulting in a bathochromic shift of the emission maximum to 475 nm.[23] In water at pH 8, the charge separated species $Py^{\bullet+}$-$dU^{\bullet-}$ is formed after a few ps, which is not fluorescent itself but equilibrates with the fluorescent, locally excited form of **1** (Py*-dU). No intramolecular exciplexes are formed in water. Most importantly, we could show that the lifetime of the charge-separated species $Py^{\bullet+}$-$dU^{\bullet-}$ is in the range of a few nanoseconds and that the reductive electron transfer is not coupled to protonation of the uracil radical anion at pH > 7.[24] With respect to these properties, Py-dU should be a suitable electron injector since reductive electron transfer could occur from the stacked uracil moiety of Py-dU to adjacent DNA bases (Scheme 1), once the nucleoside **1** is incorporated into DNA duplexes.

Synthesis and characterization of 5-(1-pyrenyl)-2′-deoxyuridine (1): For the preparation of the nucleoside **1**, we chose to use the Suzuki–Miyaura-type cross coupling reaction[25] (Scheme 2) which we have preliminarily described previously.[26] In general, this type of palladium-catalyzed couplings have the advantage that they work in wet or even aqueous solutions and tolerate the presence of some unprotected functional groups.[25] Such reactions have been performed previously for the preparation of arylated and alkenylated purines[27] but have not yet been used for the direct synthesis of aryl-modified nucleosides. The palladium-catalyzed reaction of pyren-1-yl boronic acid (**2**) with 5-iodo-2′-deoxyuridine (**3**) gave **1** in good yield (79%). A strong base (NaOH) was required in order to get the desired sterically hindered coupling product **1**. No protecting groups were needed for the hydroxy groups of the 2′-deoxyribose moiety in **3**. The starting material **3** is commercially available and the boronic acid **2** was synthesized according to a combination of literature procedures[28] by lithiation of 1-bromopyrene (**4**) at 0 °C, treatment with trimethyl borate at -78 °C and subsequent acidic workup at room temperature.

The structure of the nucleoside **1** was confirmed by different spectroscopic techniques, including ESI mass spectrometry and 2D-NMR experiments (DQF-COSY and HMQC). A critical fact about the subsequent incorporation of **1** into DNA duplexes is the assumption that the conjugate **1** forms Watson–Crick base pairs between the uracil moiety of **1** and adenine as a part of the complementary strand. Our concern was, that the large pyrene moiety may force the nucleoside **1** into a *syn*-conformation. This conformation would ensure that pyrene residue is stacked within the duplex and displace the base of the complementary strand. To rule out this structural incertainty, we performed NOESY experiments of **1** in MeOH. These spectra clearly showed a significant NOE between H-6 of the uracil part and H-2′ of

0947-6539/02/0821-4878 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 21



Scheme 2. Synthesis of the DNA building block **6**: a) 1) *n*BuLi (1.1 equiv), Et_2O, 0 °C, 30 min; 2) $B(OCH_3)_3$ (5.0 equiv), −78 °C, 6 h, then RT, 20 h; 3) H_3O^+, RT, 3 h (73 %); b) **2** (1.0 equiv), $[Pd(PPh_3)_4]$ (0.1 equiv), NaOH (20 equiv), THF/MeOH/H_2O 2:1:2, reflux, 20 h (79 %); c) 4,4′-dimethoxytriphenyl chloride (2.0 equiv), pyridine, RT, overnight (65 %); d) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (1.1 equiv), Hünig's base (3.0 equiv), CH_2Cl_2, RT, 12 h (95 %).

the 2′-deoxyribose moiety. The NOESY cross peak was comparable strong just as the cross peaks between H-2′ and H-1′, or H-3′, respectively. These NMR results can only be explained with the preferred *anti*-conformation of the nucleoside **1**.

Preparation and characterization of pyrenyl-modified nucleic acids: For subsequent incorporation into oligonucleotides, nucleoside **1** was converted into the DMT-protected compound **5** and then to the completely protected DNA building block **6** bearing the phosphoramidite group in the 3′ position (Scheme 2). Using monomer **6**, the oligonucleotides **7**–**12** were prepared by automated solid-phase synthesis using a DNA synthesizer. Quantitative coupling of the monomer **6** was achieved with a coupling time of 1.6 min using phosphoramidite chemistry and standard workup conditions. The HPLC-purified oligoucleotides were identified by MALDI-TOF mass spectrometry (Table 1). Furthermore, the UV/Vis absorption spectra (Figure 1) clearly showed the presence of the pyrenyl group with a typical absorbance at 350 nm.

Table 1. Pyrenyl-modified oligonucleotides **7**–**12**.

	Sequence	Calculated mass	MALDI-TOF mass
7	5′-AGT CAG TA(**PyU**) ATG ACT GA-3′	5419	5417
8	5′-AGT CAG TG(**PyU**) GTG ACT GA-3′	5451	5448
9	5′-TCA GTC AC(**PyU**) CAC TGA CT-3′	5291	5280
10	5′-TCA GTC AC(**PyU**) TAC TGA CT-3′	5306	5306
11	5′-TCA GTC AT(**PyU**) CAC TGA CT-3′	5306	5296
12	5′-TCA GTC AT(**PyU**) TAC TGA CT-3′	5321	5320

Using the pyrenyl-modified oligonucleotides **7**–**10**, we prepared the duplexes **Py1**–**Py6** with the corresponding unmodified complementary strands (Scheme 3). The adjacent bases of the Py-dU nucleoside were chosen to be either adenine, guanine, cytosine, or thymine on both sides (**Py1**–**Py3**, **Py6**). Despite the uncertainty related to irreversible electrochemistry, the following trend for the reducibility of the nucleobases was established: thymine > cytosine > adenine > guanine.[22] With respect to this trend, we expect that the uracil radical anion, which is formed upon photo-

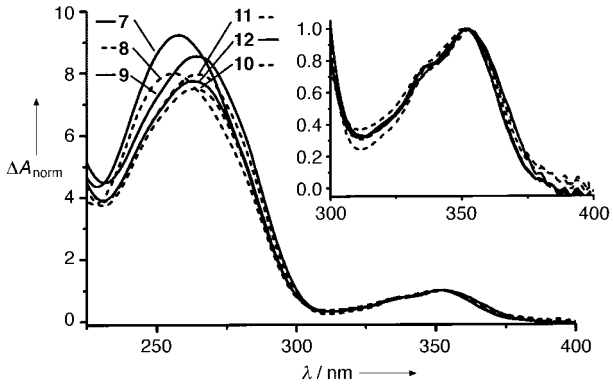
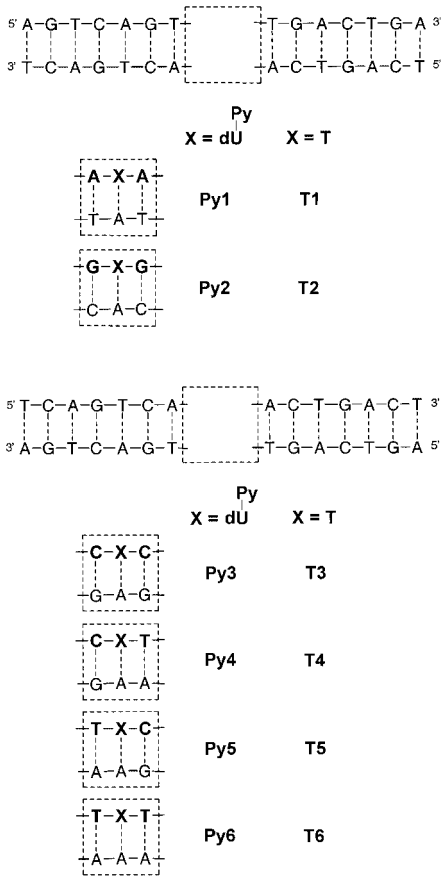


Figure 1. UV/Vis absorbance spectra of the pyrene-modified oligonucleotides **7**–**12**.



Scheme 3. DNA duplexes **Py1**–**Py6** and **T1**–**T6**.

  0947-6539/02/0821-4879 $ 20.00+.50/0

excitation and subsequent intramolecular electron transfer in Py-dU, should only be able to reduce adjacent pyrimidine bases, preferrably thymine due to its low reduction potential. According to this assumption, we decided to synthesize additionally the duplexes **Py4** and **Py5** bearing a thymine on one side of the Py-dU group and cytosine on the other. Furthermore, in order to elucidate the overall structural effects of the attached pyrenyl groups in **Py1**–**Py6**, the corresponding duplexes **T1**–**T6** with a regular thymine in the position of the Py-dU nucleotide were also prepared.

The CD spectra measured for all duplexes confirmed the B-DNA structure. A small CD effect is observed in the range of the pyrene absorption (≈350 nm) of the Py-dU modified duplexes. This CD effect represents the interaction of the pyrene moiety with its chiral environment and has also been observed for the free nucleoside. Therefore, the CD spectra do not provide direct structural information about the pyrene site. Additionally, the melting temperature T_m of all synthesized duplexes were also determined (Table 2). In most cases,

Table 2. Melting temperatures (T_m) of the pyrenyl-modified duplexes **Py1**–**Py6** and the unmodified duplexes **T1**–**T6** (2.5 μM duplex, 10 mM Na-P_i-buffer, 1M NaCl).

	Central sequence	T_m		Central sequence	T_m
Py1	5′-A-(**PyU**)-A-3′	57 °C	**T1**	5′-A-T-A-3′	64 °C
Py2	5′-G-(**PyU**)-G-3′	64 °C	**T2**	5′-G-T-G-3′	71 °C
Py3	5′-C-(**PyU**)-C-3′	61 °C	**T3**	5′-C-T-C-3′	70 °C
Py4	5′-C-(**PyU**)-T-3′	64 °C	**T4**	5′-C-T-T-3′	71 °C
Py5	5′-T-(**PyU**)-C-3′	65 °C	**T5**	5′-T-T-C-3′	64 °C
Py6	5′-T-(**PyU**)-T-3′	64 °C	**T6**	5′-T-T-T-3′	66 °C

a significant decrease of the melting temperature could be observed by comparing the unmodified duplexes **T1**–**T6** and pyrenyl-modified duplexes **Py1**–**Py6**. Only in case of the duplexes **Py5** and **Py6** was the melting temperature nearly the same as for the corresponding unmodified duplexes **T5** and **T6**. These results represent a structural pertubation of the covalently attached pyrenyl groups in the major groove of the duplexes and are in agreement with the NOESY experiments with the nucleoside **1** showing the preferred *anti*-conformation. Based on these results, we assume that the modified Py-dU nucleoside forms a regular Watson–Crick base pair between the uracil moiety and the adenine of the complementary strand within duplex DNA.

Spectroscopic results: We measured the emission of the pyrenyl-modified duplexes **Py1**–**Py6** under steady-state conditions (Figure 2). Equal optical densities were chosen for all samples at the excitation wavelength of 340 nm. The emission maxima of all six duplexes are located between 490 and 500 nm. Note that these fluorescence spectra are similar to the one observed for the nucleoside **1** in MeOH,[17, 19] but significantly different from the one obtained for PydU in water which was recently reported.[24]

The time-resolved transient absorption spectroscopy of **1** in MeOH revealed an intramolecular exciplex which is formed

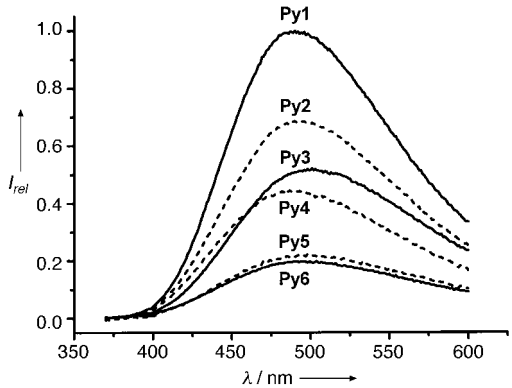


Figure 2. Relative emission of the duplexes **Py1**–**Py6** during excitation at 340 nm (2.5 μM duplex, 10 mM Na-P_i-buffer).

subsequently from an intramolecular ion pair state. Formally, the formation of the exciplex is a partial back electron transfer from the charge-separated state ($Py^{\bullet+}$-$dU^{\bullet-}$) into a locally excited (LE) state of the Py-dU conjugate. Previously, we observed a bathochromic shift in the emission wavelength maximum of **1** in MeOH (475 nm) in comparision to MeCN (422 nm); this suggests a strong stabilization of the fluorescent exciplex in MeOH through hydrogen bonding. According to these previous results, we assume that the observed emission of the pyrenyl-modified DNA duplexes **Py1**–**Py6** originates from an exciplex of the Py-dU group which is partially intercalated in the base stack through the uracil moiety and well fixed in an array of hydrogen bonds to the adenine of the complementary strand.

Interestingly, at equal optical densities of the duplexes **Py1**–**Py6** at 340 nm the emission quantum yield differs significantly depending on the neighboring bases of the Py-dU group. As described above, the trend for the reducibility of the nucleobases is thymine > cytosine > adenine > guanine.[22] The redox potential of uracil is similar to that of thymine,[22] so that we expect that the uracil radical anion which is formed upon photoexcitation and subsequent intramolecular electron transfer in the Py-dU group should only be able to reduce adjacent pyrimidine bases, preferably thymine due to its low reduction potential. According to this assumption and the previous results about the formation of exciplexes of **1**, we expect a quenching of fluorescence when an electron transfer from the uracil radical anion to the adjacent bases occurs (Scheme 4). In fact, a significant quenching of the exciplex emission can be observed, when thymines are placed adjacent to the Py-dU group, as it is the case in **Py6**. As expected from the reduction potentials, the fraction quenched is highest for **Py6** (5′-T-(PyU)-T-3′) in comparison with all other pyrenyl-modified duplexes containing equal bases on both sides of the Py-dU group (**Py1**–**Py3** and **Py6**). Interestingly, there is a significant difference in the fraction quenched of the "mixed-sequence" DNA duplexes **Py4** (5′-C-(PyU)-T-3′) and **Py5** (5′-T-(PyU)-C-3′). The duplex **Py4** bearing a cytosine on the 5′-side of the Py-dU group shows a similar amount of emission as **Py3**, whereas the emission of **Py5**, bearing a thymine on the 5′-side, is quenched nearly as efficient as in case of **Py6**. In conclusion, an effective fluorescence quenching occurs only when a thymine is located

0947-6539/02/0821-4880 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 21

on the 5′-side next to the Py-dU unit. This observation may reflect the fact that the pyrenyl group in the major groove is located in a non-symmetric way, which leads to a preferred electron transfer to the nucleobase on the 5′-side. The question what causes the quenching in the DNA duplexes **Py2**–**Py4** with either guanine or cytosine on the 5′-side of the Py-dU group remains unclear and is currently under investigation. Given the fact, that pyrene can undergo both oxidative and reductive charge transfer with DNA, the fluorescence quenching observed in **Py2** can be due to a hole transfer to the adjacent guanine yielding the guanine radical cation and the pyrenyl radical anion.

Finally, we want to present preliminary results which can be concluded from the time-resolved pump-probe laser spectroscopy measurements. Representatively, we want to show here the transient absorption spectrum of the modified duplexes **Py1** (5′-A-(PyU)-A-3′) and **Py6** (5′-T-(PyU)-T-3′). According to the relative reduction potentials and to the emission spectra of the two duplexes, as described above, electron transfer occurs only in the duplex **Py6** but not in the duplex **Py1**. This result can be supported by the transient absorption spectrum 10 ps after excitation (Figure 3): The spectra of both duplexes,

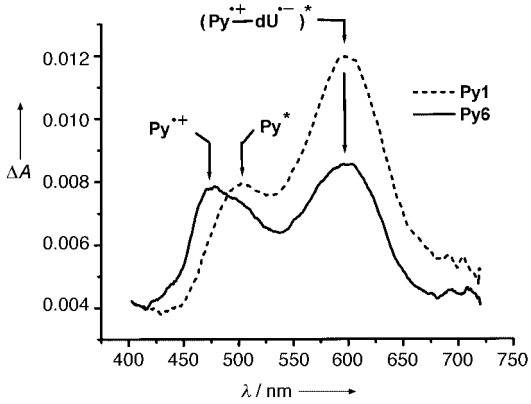


Figure 3. Transient absorption spectra of **Py1** and **Py6** obtained 10 ps after excitation (350 μM duplex, 10 mM Na-P_i-buffer).

Py1 and **Py6**, show a strong absorption peak at 600 nm representing the exciplex state of the Py-dU group. Such intramolecular exciplexes are formed due to a strong electronic coupling between the two chromophores in Py-dU and have been previously observed upon photoexcitation of the nucleoside in organic solvents, such as MeCN, THF, or MeOH.[17] Additionally, both spectra show a peak around 500 nm which is characteristic for Py*.[29] These transients explain why emission is observed in the steady-state fluorescence spectrum of both duplexes, **Py1** or **Py6**, during excitation at 340 nm. Most importantly, the transient absorption spectrum of **Py6** shows an additional peak at 475 nm which gives evidence for the presence of $Py^{\bullet+}$.[24] This absorption is not observed upon excitation of **Py1**. The presence of $Py^{\bullet+}$ in the transient absorption spectrum together with the observed quenching of the emission of **Py6** in comparison to **Py1** clearly indicates that electron transfer occurs only in **Py6**, but not in **Py1**.

Conclusion

5-(1-Pyrenyl)-2′-deoxyuridine (**1**) was prepared by a palladium-catalyzed Suzuki–Miyaura cross-coupling reaction. Using this nucleoside **1**, a range of modified oligonucleotides was prepared bearing a covalently attached pyrenyl group located outside the DNA base stack. Upon excitation at 340 nm an electron transfer from the pyrenyl group to the uracil base takes place which represents an injection of an excess electron into the DNA base stack. Based on the results obtained by steady-state fluorescence spectra and time-resolved transient absorption spectra we conclude that electron transfer can only occur from the Py-dU group to adjacent thymines. Work is currently in progress i) to investigate the dynamics of this electron injection processes more detail, and ii) to synthetically modify DNA duplexes with suitable electron acceptors in order to perform investigations concerning the base sequence dependence and the rate of base-to-base reductive electron transfer.

Experimental Section

Materials and methods: ^{1}H, ^{13}C, ^{31}P and the two-dimensional NMR spectra were recorded on a Bruker AC250 or DMX500 spectrometer. NMR signals were assigned based on 2D NMR measurements (DQF-COSY, HMQC). ESI mass spectra were measured in the analytical facility of the institute on a Finnigan LQC-ESI spectrometer. MALDI-TOF was performed in the analytical facility of the institute on a Bruker Biflex III spectrometer using 3-hydroxypicolinic acid in aq. ammonium citrate as the matrix. 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 semipreparative HPLC columns (300 Å) were purchased from Supelco. Solvents were dried according to standard procedures. All reactions were carried out under argon. Chemicals were purchased from Fluka and used without further purification. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm, pump-probe laser spectroscopy: 1 mm) and using Na-P_i-buffer (10 mM). Absorption spectra and the melting temperature (2.5 μM duplex, 1 M NaCl, 260 nm, 10–80 °C, interval 1 °C) were recorded on a Varian Cary 100 spectrometer. The B-DNA conformation of all duplexes was confirmed by CD spectroscopy (2.5 μM duplex, 185–310 nm) performed on Jasco J-715 spectropolarimeter. The fluorescence spectra (2.5 μM duplex) were recorded on a Fluoromax-3 fluorimeter (Jobin-Yvon) and corrected for Raman emission from the buffer solution. All emission spectra were recorded with a bandpass of 2 nm for both excitation and emission and are intensity corrected.

5-(1-Pyrenyl)-2′-deoxyuridin (1): 5-Iodo-2′-deoxyuridine (**3**) (0.28 g, 0.8 mmol) was dissolved in tetrahydrofuran/water (60 mL, 1:1). Subsequently, a solution of **2** (0.20 g, 0.8 mmol) and $[Pd(PPh_3)_4]$ (0.92 g, 0.08 mmol, 0.1 equiv) in THF (10 mL), a solution of NaOH in water (0.64 g, 16 mmol, 20 equiv), and MeOH (25 mL) were added. The solution was saturated with nitrogen at RT (10 min), refluxed for 20 h, neutralized with solid NH_4Cl and extracted with EtOAc (4 × 30 mL). The combined organic phase was dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel (CH_2Cl_2/acetone 4:1, then EtOAc/MeOH 10:1) give a pale yellow solid (79 % yield). Analytical HPLC ensured a purity of > 99.5 %. R_f = 0.65 (EtOAc/MeOH/water 10:1:0.5); NMR signals were assigned based on 2D NMR measurements (DQF-COSY, HMQC); ^{1}H NMR (500 MHz, CD_3OD): δ = 2.29 (m, J = 6.4 Hz, 2H, 2′-H), 3.50–3.60 (ddd, J = 12.0, 3.3 Hz, 2H, 5′-H), 3.84 (m, J = 3.2 Hz, 1H, 3′-H), 4.31 (m, J = 4.3 Hz, 1H, 4′-H), 6.35 (t, J = 6.6 Hz, 1H, 1′-H), 7.84–8.14 (m, 9H, Pyren-H), 8.21 (s, 1H, 5-H); additional signals in ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 4.79 (t, 1H, 5′-OH), 5.24 (d, 1H, 5′-OH), 11.64 (brs, 1H, NH); ^{13}C NMR (125.8 MHz, [D_6]DMSO+5 % CD_3OD): δ = 165.53 (C=O), 153.23 (C=O), 142.95 (C-6), 133.67, 133.54, 133.28, 132.34, 131.33, 131.24, 130.15, 129.86, 129.80, 128.83, 127.85, 127.81,

  0947-6539/02/0821-4881 $ 20.00+.50/0

127.72, 127.13, 126.85, 126.70, 116.66, 90.24 (4′-C), 87.59 (1′-C), 73.25 (3′-C), 63.74 (5′-H), 42.84 (2′-C); ESI-MS: m/z: 451 $[M+Na]^+$, 879 $[2M+Na]^+$, 1307 $[3M+Na]^+$; UV/Vis (H_2O, pH ≈ 8): $\varepsilon = 14600$ (260 nm), 23500 $M^{-1}cm^{-1}$ (343 nm); elemental analysis calcd for $C_{25}H_{20}N_2O_5 \times H_2O$ (446.5): C 67.25, H 4.97, N 6.54; found: C 67.77, H 5.25, N 5.59.

Pyren-1-yl boronic acid (2): A solution of 1-bromo pyrene (**4**; 1.0 g, 3.56 mmol) in Et_2O (50 mL) was cooled to 0 °C. A solution of *n*BuLi (4.16 mmol) in hexane (1.6 M) was added. The solution was stirred 30 min at RT and then added dropwise to a solution of $B(OCH_3)_3$ (17.8 mmol) in Et_2O (5 mL) at −78 °C. The solution was stirred at −78 °C for 3 h and then at RT overnight. 2 N HCl (20 mL) was added and stirred 3 h at RT The mixture was extracted several times with EtOAc. The combined organic phases were washed with water, dried with Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel (hexane/EtOAc 20:1, then EtOH) yielding a pale yellow solid (0.64 g, 73 %). $R_f = 0.32$ (hexane/Et_2O 3:1). All spectroscopic data of **2** were in agreement with the published data.[28]

5′-*O*-(4,4′-Dimethoxytrityl)-5-(1-pyrenyl)-2′-deoxyuridine (5): 4,4′-Dimethoxytriphenylmethyl chloride (190 mg, 0.56 mmol) was added to a solution of **1** (120 mg, 0.28 mmol) in dry pyridine (5 mL). The mixture was stirred overnight at RT, MeOH (3 mL) was added and the solution concentrated to dryness. The crude product was purified by flash chromatography (CH_2Cl_2/acetone 4:1, then EtOAc/MeOH 10:1) yielding a pale yellow solid (134 mg, 65 %). $R_f = 0.85$ (ethyl acetate/methanol/water 6:2:1); NMR signals were assigned based on 2D NMR measurements (DQF-COSY, HMQC); ^{1}H NMR (250 MHz, [D$_6$]DMSO): $\delta = 1.80-1.97$, 2.06–2.23 (m, 2H, 2′-H), 2.77–2.93 (m, 2H, 5′-H), 3.43–3.51 (m, 1H, 3′-H), 3.68–3.70 (s, 6H, Me), 4.04 (m, 1H, 4′-H), 6.32 (t,1H, 1′-H), 6.81–7.40 (m, 13H, DMT-H), 7.88–8.29 (m, 10H, Pyren-H), 11.70 (s, 1H, NH); ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): $\delta = 39.68$ (2′-C), 55.92 (3′-C), 56.38 (OMe), 63.79 (5′-C), 87.12 (4′-C), 88.14 (1′-C), 114.02, 114.37, 115.79, 125.08, 125.15, 125.76, 126.60, 126.64, 127.64, 128.28, 128.57, 128.85, 129.13, 129.35, 130.57, 130.87, 131.14, 131.25, 131.71, 132.09, 137.08, 137.14, 146.42, 151.65, 159.60, 159.66, 163.76, 181.0; ESI-MS: m/z: 753 $[M+Na]^+$.

5′-*O*-(4,4′-Dimethoxytrityl)-5-(1-pyrenyl)-2′-deoxyuridine-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidit (6): Compound **5** (134 mg, 0.18 mmol) was dissolved in dry CH_2Cl_2 (5 mL). Hünig's base (0.1 mL, 0.54 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.05 mL, 0.20 mmol) were added and the solution stirred for 12 h at RT. The mixture was poured into aq. sat. $NaHCO_3$ (50 mL) and extracted three times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated yielding the phosphoramidite **6** as a pale yellow solid (159 mg, 95 %), which was used directly for the oligonucleotide synthesis. $R_f = 0.96$ (ethyl acetate/methanol/water 6:2:1); ^{31}P NMR (101.3 MHz, CD_3CN): $\delta =$ 159.0; ESI-MS: m/z: 969 $[M+K]^+$.

Preparation and characterization of the oligonucleotides

General procedure: The oligonucleotides were prepared on a Expedite 8909 DNA synthesizer from Applied Biosystems by standard phosphoramidite protocols using chemicals and CPG (1 μmol) from Glen Research. After preparation, the trityl-off oligonucleotide was cleaved off 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 semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient: 0–15 % B over 45 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm[30] on a Varian Cary 100 spectrometer. Duplexes were formed by heating to 80 °C (10 min), followed by slow cooling.

Solid-phase synthesis of the pyrenyl-modified oligonucleotides 7–12

General procedure: The syntheses were performed on a 1 μmol scale (CPG 1000 Å, Glen Research) using standard phosphoramidite protocols. Quantitative coupling of the building block **6** was achieved using the a minimal coupling time of 1.6 min. After preparation, the trityl-off oligonucleotide was cleaved off 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 semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient: 0–15 % B over 45 min. The oligonucleotides were lyophilized, quantified by their absorbance at 260 nm[31] and using $\varepsilon =$ 14600 (260 nm) for **1**.

Femtosecond broadband pump-probe setup: The pyrenyl-modified oligonucleotides were exited by pump pulses at 340 nm. The changes in optical density were probed by a femtosecond white-light continuum (WLC) generated by tight focusing of a small fraction of the output of a commercial Ti:Sa based pump laser (CPA-2001, Clark-MXR) into a 3 mm CaF_2 plate. The obtained WLC provided a usable probe source between 370 and 720nm. The WLC was split into two beams (probe and reference) and focused into the sample using reflective optics. After passing through the sample both probe and reference were spectrally dispersed and simultaneously detected on a CCD sensor. The pump pulse (340 nm, 100–200 nJ) was generated by frequency doubling of the compressed output of a commercial NOPA system (Clark-MXR, 680 nm, 8 μJ, 30 fs). To compensate for group velocity dispersion in the UV pulse we used an additional prism compressor. Independent measurements of the chirp of the WLC were carried out to correct the pump-probe spectra for time-zero differences. The overall time resolution of the setup was obtained from the rise time of the signal (above 580 nm). Assuming a Gaussian shape cross-correlation we obtained a width of 100–120 fs (FWHM). A spectral resolution of 7–10 nm was obtained. Measurements were performed with magic angle geometry (54.7°) for the polarization of pump and probe pulses to avoid contributions from orientational relaxation. Pump energy and pump spot size (≈200–400 μm) were adjusted to minimize contributions from the solvent to the signal. Steady state absorption and fluorescence spectra of the samples measured before and after the time resolved experiments were compared with each other and no indications for degradation were found. A sample cell with 1.25 mm fused silica windows and a light path of 1 mm was used for all measurements. The sample concentration was 350 μM duplex.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Wa 1386/7-1, Fi 721/3-1,3-4) and by the Fonds der Chemischen Industrie. N.A. and H.A.W. are grateful to Professor Horst Kessler, Technical University of Munich, for his generous support. We thank Dr. Burkhard Luy, Technical University of Munich, for performing the NOESY experiments.

[1] a) M. R. Arkin, E. D. A. Stemp, R. E. Holmlin, J. K. Barton, A. Hörmann, E. J. C. Olson, P. F. Barbara, *Science* **1996**, *273*, 475; b) K. Fukui, K. Tanaka, *Angew. Chem.* **1998**, *110*, 167; *Angew. Chem. Int. Ed.* **1998**, *37*, 158; c) S. O. Kelley, J. K. Barton, *Science* **1999**, *283*, 375; d) J. M. Jean, J. M., K. B. Hall, Proc. *Natl. Acad. Sci. USA* **2001**, *98*, 37; e) M. Kawai, M. J. Lee, K. O. Evans, T. M. Nordlund, *J. Fluoresc.* **2001**, *11*, 23; f) O. F. A. Larsen, I. H. M. van Stokkum, B. Gobets, R. van Grondelle, H. van Amerongen, *Biophys. J.* **2001**, *81*, 1115.

[2] a) E. D. A. Stemp, M. R. Arkin, J. K. Barton, *J. Am. Chem. Soc.* **1997**, *119*, 2921; b) F. D. Lewis, X. Liu, J. Liu, S. E. Miller, R. T. Hayes, M. R. Wasielewski, *Nature* **2000**, *406*, 51; c) C. Wan, T. Fiebig, O. Schiemann, J. K. Barton, A. H. Zewail, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14052; d) V. Shafirovich, A. Dourandin, W. Huang, N. P. Luneva, N. E. Geacintov, *J. Phys. Chem. B* **1999**, *103*, 10924; e) H.-A. Wagenknecht, S. R. Rajski, M. Pascaly, E. D. A. Stemp, J. K. Barton, *J. Am. Chem. Soc.* **2001**, *123*, 4400; f) W. B. Davies, S. Hess, I. Naydenova, R. Haselsberger, A. Ogrodnik, M. D. Newton, M. E. Michel-Beyerle, *J. Am. Chem. Soc.* **2002**, *124*, 2422; g) F. D. Lewis, Y. Wu, *J. Photochem. Photobiol. C* **2001**, *2*, 1.

[3] O. Schiemann, N. J. Turro, J. K. Barton, *J. Phys. Chem. B* **2000**, *104*, 7214.

[4] a) M. E. Nunez, J. K. Barton, *Curr. Opin. Chem. Biol.* **2000**, *4*, 199; b) G. B. Schuster, *Acc. Chem. Res.* **2000**, *33*, 253; c) T. T. Williams, J. K. Barton, *J. Am. Chem. Soc.* **2002**, *124*, 1840; d) K. Nakatani, C. Dohno, A. Ogawa, I. Saito, *Chem. Biol.* **2002**, *9*, 361; e) B. Giese, A. Biland, *Chem. Commun.* **2002**, 667.

[5] a) P. O'Neill, M. Fielden, *Adv. Radiat. Biol.* **1993**, *17*, 53; b) D. Wang, D. A. Kreutzer, J. M. Essigmann, *Mutat. Res.* **1998**, *400*, 99.

[6] a) N. M. Jackson, M. G. Hill, *Curr. Opin. Chem. Biol.* **2001**, *5*, 209; b) E. M. Boon, J. E. Salas, J. K. Barton, *Nat. Biotechnol.* **2002**, *20*, 282; c) M. C. Pirrung, *Angew. Chem.* **2002**, *114*, 1326; *Angew. Chem. Int. Ed.* **2002**, *41*, 1276.

[7] a) C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144; b) H.-W. Fink, C. Schönenberger, *Nature* **1999**, *398*, 407; c) D. Porath, A. Bezryadin, S. de Vries, C. Dekker, *Nature* **2000**, *403*, 635; d) C. M. Niemeyer, *Angew. Chem.* **2001**, *113*, 4254; *Angew. Chem. Int. Ed.* **2001**, *40*, 4128.
[8] R. E. Holmlin, P. J. Dandliker, J. K. Barton, *Angew. Chem.* **1997**, *109*, 2830; *Angew. Chem. Int. Ed.* **1997**, *36*, 2714.
[9] E. J. C. Olson, D. Hu, A. Hörmann, A. M. Jonkman, M. R. Arkin, E. D. A. Stemp, J. K. Barton, P. F. Barbara, *J. Am. Chem. Soc.* **1997**, *119*, 11458.
[10] a) A. Schwögler, L. T. Burgdorf, T. Carell, *Angew. Chem.* **2000**, *112*, 4082; *Angew. Chem. Int. Ed.* **2000**, *39*, 3918; b) C. Behrens, L. T. Burgdorf, A. Schwögler, T. Carell, *Angew. Chem.* **2002**, *114*, 1841; *Angew. Chem. Int. Ed.* **2002**, *41*, 1763.
[11] T. Carell, L. T. Burgdorf, L. M. Kundu, M. Cichon, *Curr. Opin. Chem. Biol.* **2001**, *5*, 491.
[12] a) B. Giese, M. Spichty, *ChemPhysChem* **2000**, *1*, 195; b) B. Giese, J. Amaudrut, A.-K. Köhler, M. Spormann, S. Wessely, *Nature* **2001**, *412*, 318.
[13] T. Fiebig, C. Wan, A. H. Zewail, *ChemPhysChem* **2002**, *3*, in press.
[14] E. T. Kool, J. C. Morales, K. M. Guckian, *Angew. Chem.* **2000**, *112*, 1046; *Angew. Chem. Int. Ed.* **2000**, *39*, 990.
[15] a) V. A. Korshun, N. B. Pestov, K. R. Birikh, Y. A. Berlin, *Bioconjugate Chem.* **1992**, *3*, 559; b) I. A. Prokhorenko, V. A. Korshun, A. A. Petrov, S. V. Gontarev, Y. A. Berlin, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2081; c) K. V. Balakin, V. A. Korshun, I. I. Mikhalev, G. V. Maleev, A. D. Malakhov, I. A. Prokhorenko, Y. A. Berlin, *Biosens. Bioelectron.* **1998**, *13*, 771.
[16] a) R. Kierzek, Y. Li, D. H. Turner, P. C. Bevilacqua, *J. Am. Chem. Soc.* **1993**, *115*, 4985; b) M. Manoharan, K. L. Tivel, M. Zhao, K. Nafisi, T. L. Netzel, *J. Phys. Chem.* **1995**, *99*, 17461; c) F. D. Lewis, Y. Zhang, R. L. Letsinger, *J. Am. Chem. Soc.* **1997**, *119*, 5451; d) K. Yamana, M. Takei, H. Nakano, *Tetrahedron Lett.* **1997**, *38*, 6051; e) J. D. Frazer, S. M. Horner, S. A. Woski, *Tetrahedron Lett.* **1998**, *39*, 1279.
[17] T. L. Netzel, M.Zhao, K. Nafisi, J. Headrick, M. S. Sigman, B. E. Eaton, *J. Am. Chem. Soc.* **1995**, *117*, 9119.
[18] V. Y. Shafirovich, S. H. Courtney, N. Ya, N. E. Geacintov, *J. Am. Chem. Soc.* **1995**, *117*, 4920.
[19] T. L. Netzel, K. Nafisi, J. Headrick, B. E. Eaton, *J. Phys. Chem.* **1995**, *99*, 17948.
[20] T. Kubota, J. Kano, B. Uno, T. Konse, *Bull. Chem. Soc. Jpn.* ***1987***, *60*, 3865.
[21] S. Steenken, J. P. Telo, H. M. Novais, L. P. Candeias, *J. Am. Chem. Soc.* **1992**, *114*, 4701.
[22] C. A. M. Seidel, A. Schulz, M. H. M. Sauer, *J. Phys. Chem.* **1996**, *100*, 5541.
[23] E. Pandurski, N. Amann, H.-A. Wagenknecht, T. Fiebig, unpublished results.
[24] N. Amann, E. Pandurski, T. Fiebig, H.-A. Wagenknecht, *Angew. Chem.* **2002**, *114*, 3084; *Angew. Chem. Int. Ed.* **2002**, *41*, 2978.
[25] N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457.
[26] N. Amann, H.-A. Wagenknecht, *Synlett* **2002**, 687.
[27] a) M. Havelková, D. Dvořák, M. Hocek, *Synthesis* **2001**, 1704–1710; b) M. Hocek, A. Holý, H. Dvořáková, *Collect. Czech. Chem. Commun.* **2002**, *67*, 325–335.
[28] a) H. Suenaga, K. Nakashima, T. Mizuno, M. Takeuchi, I. Hamachi, S. Shinkai, *J. Chem. Soc. Perkin Trans. 1* **1998**, 1263; b) M. Beinhoff, W. Weigel, M. Jurczok, W. Rettig, C. Modrakowski, I. Brüdgam, H. Hartl, A. D. Schlüter, *Eur. J. Org. Chem.* **2001**, 3819.
[29] P. Foggi, L. Pettini, I. Sànta, R. Righini, S. Califano, *J. Phys. Chem.* **1995**, *99*, 7439.
[30] J. D. Puglisi, I. Tinoco, *Meth. Enzymol.* **1989**, *180*, 304.

Received: May 13, 2002 [F4087]

  0947-6539/02/0821-4883 $ 20.00+.50/0