[11] Stability of the resin was investigated under strongly basic (10% NaOH) as well as strongly acidic reaction conditions (37% HCl) and at 220°C (DMF, microwave irradiation) without observing any modification of the resin structure and morphology.
[12] Swelling volumes were determined by compressing a swollen resin sample in a 5-mL syringe with a weight of 2 kg and measuring the resin volume after pressure release.
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[15] Loading of hydroxy resins were determined by coupling Fmoc-Gly-OH employing the method described in ref. [14], followed by Fmoc-cleavage and spectrophotometric quantification at 267, 289, and 301 nm.
[16] The efficiency of the acylation steps on the ULTRA resins was highlighted by the structure of the main by-product (6%), which was not a deletion product, but **15** + *tert*-butyl, an incompletely deprotected 13mer-peptide.
[17] Microwave-assisted syntheses were performed on a SmithSynthesizer from Personal Chemistry AB, Uppsala, Sweden.

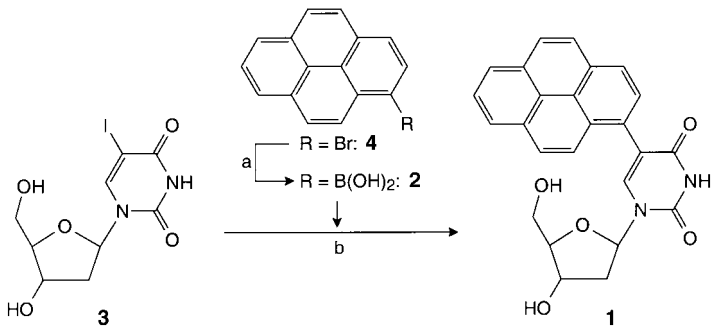
A Model Nucleoside for Electron Injection into DNA: 5-Pyrenyl-2′-Deoxyribose**

Nicole Amann, Evgeni Pandurski, Torsten Fiebig,* and Hans-Achim Wagenknecht*

Charge-transfer processes through DNA have been studied intensively in the past 15 years.[1] It is important to emphasize, that in most of these experiments oxidative hole transfer has been observed. On the other hand, reductive electron transfer (ET) is currently used extensively in DNA chip technology[2] and DNA nanotechnology[3] without an understanding of the mechanism of this type of charge-transfer. Recently, Carell and co-workers described the repair of a thymine–thymine dimer by a distant flavine derivative, which was incorporated into the DNA as an artificial base.[4] Despite the fact that spectroscopic measurements with this system have not been published, the cleavage of the thymine–thymine dimer was interpreted as the chemical result of a reductive ET through the DNA base stack. To date, suitable DNA assays for the time-resolved spectroscopic investigation of reductive ET through DNA are elusive.

We present herein the synthesis and pH-dependent spectroscopic investigation of the ET in the model nucleoside 5-pyrenyl-2′-deoxyuridine (Py-dU, **1**). Pyrene derivatives have been used previously as artificial DNA bases by Kool et al.[5] We chose a different approach and attached the pyrenyl group covalently to the uracil or thymine nucleobases. Excitation of the pyrene moiety at 340 nm leads to an intramolecular ET, which yields the corresponding uracil radical anion and the pyrenyl radical cation ($Py^{\bullet+}$-$dU^{\bullet-}$). This charge-transfer assignment has been proven previously by Netzel et al.[6] Based on the reduction potential for the $Py^{\bullet+}$/Py redox couple of 1.52 V (vs. normal hydrogen electrode)[7] and $E_{00} = 3.25$ eV,[6] the driving force ΔG of this ET process has a maximum value of −0.5 eV based on the potential of −1.2 V for the $dU/dU^{\bullet-}$ redox couple.[8] However, this value of $|\Delta G|$ seems to be too large with respect to a recent femtosecond time-resolved study on the reduction of thymine, which suggests a potential of approximately −1.8 V for the $dT/dT^{\bullet-}$ redox couple.[9] Based on steady-state fluorescence spectroscopy and nanosecond fluorescence lifetime measurements, it was proposed that ET from the photoexcited pyrene to the uracil moiety should be more favorable in MeOH than in MeCN because of a proton-coupled ET process.[6] However, this hypothesis is in disagreement with the proposed pK_a value of 6.9 for the protonated uracil radical anion $dU(H)^{\bullet}$ reported by Steenken.[10] Therefore, the prononated radical $dU(H)^{\bullet}$ represents a stronger acid than MeOH and cannot be protonated by this solvent. To elucidate the possibility of a proton-coupled ET in more detail, we chose water at different pH values and measured the steady-state fluorescence and time-resolved transient absorption spectra. An understanding of the protonation dynamics of radical anions of DNA bases is crucial to understand both ET and hole transfer through DNA. Moreover, to investigate the competition between ET to adjacent DNA bases and protonation by surrounding water molecules and/or hydrogen-bonded bases, it is particularly important to evaluate the applicability of **1** as an electron injector into DNA.

The nucleoside **1** was prepared by using the palladium-catalyzed Suzuki–Miyaura type cross coupling[11] of pyren-1-ylboronic acid (**2**) to 5-iodo-2′-deoxyuridine (**3**) in a good yield of 79% (Scheme 1). Suzuki–Miyaura type couplings



Scheme 1. Synthesis of **1**: 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 room temperature, 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%.

[*] Dr. T. Fiebig, E. Pandurski
Institut für Physikalische und Theoretische Chemie
Technische Universität München
Lichtenbergstrasse 4, 85747 Garching (Germany)
Fax: (+49) 89-289-13244
E-mail: fiebig@ch.tum.de
Dr. H.-A. Wagenknecht, N. Amann
Institut für Organische Chemie und Biochemie
Technische Universität München
Lichtenbergstrasse 4, 85747 Garching (Germany)
Fax: (+49) 89-289-13210
E-mail: wagenknecht@ch.tum.de

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have been used for the preparation of arylated and alkenylated purines,[12] but have not yet been used for the direct synthesis of aryl-modified nucleosides. In contrast to the published procedure of a Stille-type coupling of pyren-1-yl-(tributyl)-stannane to a fully protected 5-iodo-2′-deoxyuridine derivative in a glovebox,[6] our synthetic approach has the advantage that the starting material **1** does not need to be protected. Furthermore, the Suzuki-type coupling works in aqueous solutions, which facilitates the synthetic procedure. Pyren-1-ylboronic acid (**2**) was synthesized by lithiation of 1-bromopyrene (**4**) and subsequent treatment with trimethyl borate and acidic workup.[13] The structure of the nucleoside **1** was confirmed by mass spectrometry and by 2D NMR spectroscopy (e.g. DQF-COSY and HMQC).

In the nucleoside **1**, the two chromophores are linked covalently by a single C–C bond, thus resulting in strong electronic coupling between them. Such systems are generally characterized by intense, unstructured fluorescence bands with solvent-dependent spectral maxima. Such emissions indicates that efficient charge transfer (CT) takes place.[14] As a result of this specific mode of interaction between pyrene and the attached chromophore, one can expect the formation of strongly fluorescent intramolecular CT states, which may be considered as intramolecular exciplexes. These exciplex states contain locally excited (LE, Py*-dU) and CT ($Py^{\bullet+}$-$dU^{\bullet-}$) contributions. In fact, exciplex emission of **1** has been observed in organic solvents such as THF, MeCN, and MeOH.[15]

Interestingly, the steady-state fluorescence spectra of **1** in water (ca. pH 8) displays mostly LE character (Figure 1 A, $\lambda_{exc} = 340$ nm). Furthermore, the observed emission is quenched completely at about pH 4. To evaluate the complete pH dependence, we measured the emission intensity of **1** at different pH values between 2 and 12 (Figure 1 B). The typical sigmoidal curve represents a pK_a value of approximately 5.5 for the protonated $Py^{\bullet+}$-dU(H)$^{\bullet}$ biradical. This value lower than the pK_a value of 6.9 given by Steenken[10] and possibly reflects the electronic influence of the directly attached pyrene moiety in **1**. The small loss of emission at pH values above 10 is probably a result of the deprotonation of the uracil moiety. Consequently, the redox properties of the model nucleoside are changed already in the ground state.

To characterize the products that are formed upon excitation of **1**, we measured the transient absorption spectrum in water at two different pH values (8 and 4), in both cases 13 ps after excitation (Figure 2). The spectra at both pH values show a strong absorption peak at around 475 nm, which is characteristic of $Py^{\bullet+}$.[16] In addition, a shoulder is observed in the spectrum (at pH 8) at around 505 nm, which provides evidence for Py*.[17] This shoulder is absent in the spectrum recorded at pH 4. Additional absorption bands at longer wavelength can be assigned to the radical product states involving the uracil moiety. Depending on the pH value, one can see clear differences in the intensity and spectral position of the bands which strongly suggests that the final products involve different uracil species.

Based on the steady-state fluorescence spectra and the femtosecond time-resolved pump-probe data, we conclude that ultrafast intramolecular ET occurs upon photoexcitation

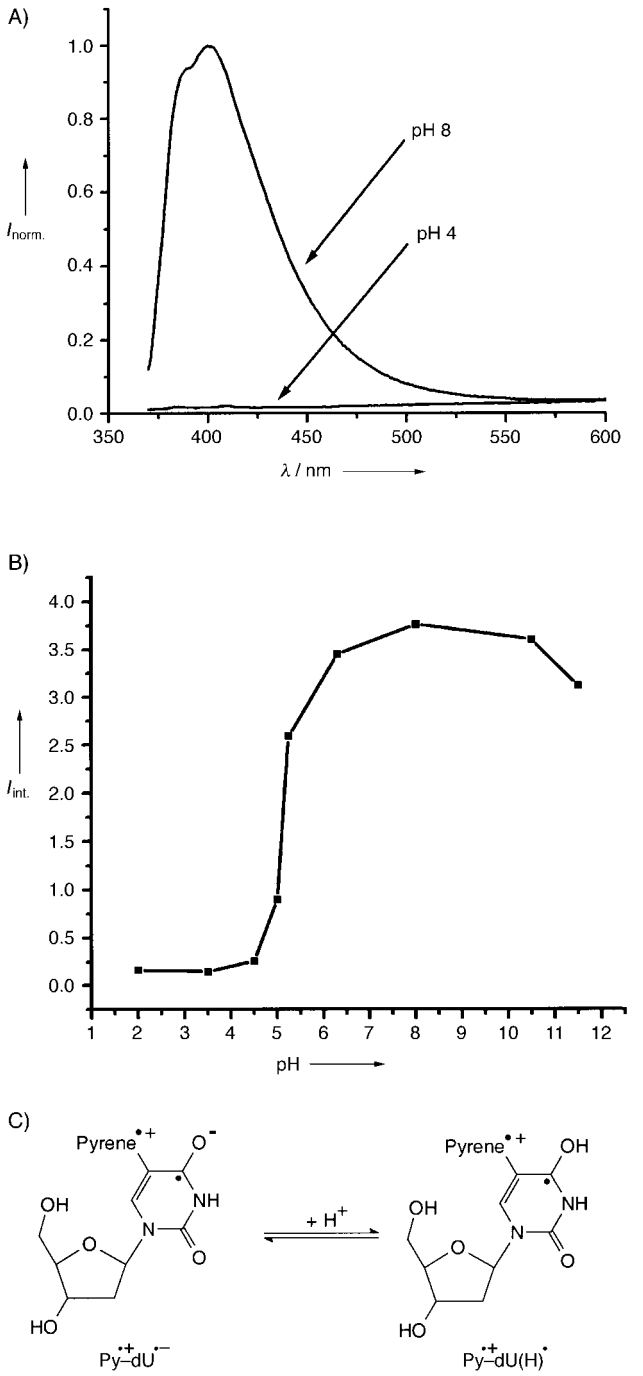

Figure 1. A) Steady-state fluorescence spectra of **1** (40 μM; $\lambda_{exc} = 340$ nm) in water at pH 8 and pH 4; B) pH dependence of the fluorescence intensity; C) the emission is quenched completely at pH < 5 as a result of the protonation of the uracil radical anion.

of **1**, independent of the pH value. Moreover, the charge-separated species that is initially formed ($Py^{\bullet+}$-$dU^{\bullet-}$) is not fluorescent and equilibrates with the fluorescent LE form Py*-dU (Scheme 2). The $dU^{\bullet-}$ moiety can only be protonated in the presence of excess protons. This important observation excludes a proton-coupled ET mechanism, which means that protonation is not a prerequisite for ET. However, the transient absorption spectra show significant differences in the spectral range between 500 and 700 nm at pH 4 and pH 8, which suggests that a proton-transfer step occurs after ET.

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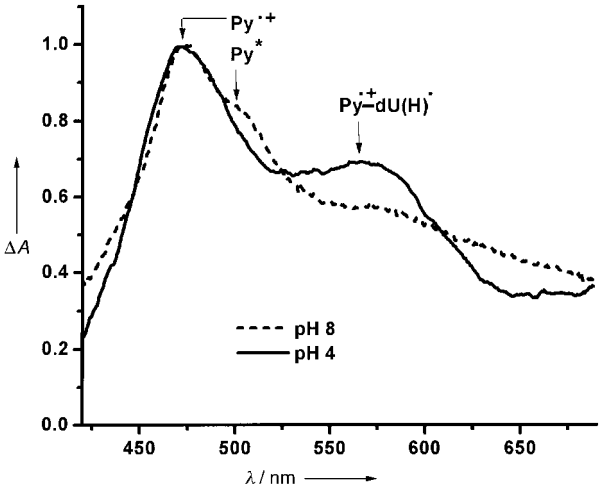
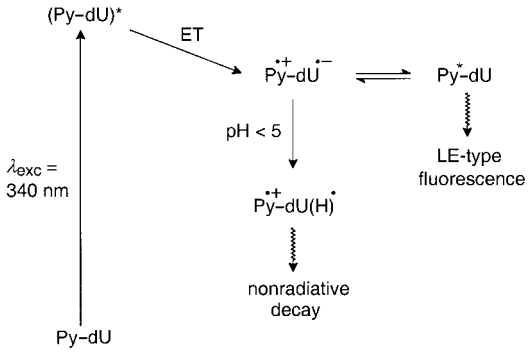


Figure 2. Transient absorption spectra of **1** in water (400 μM) at pH 8 and pH 4, obtained 13 ps after excitation.



Scheme 2. Summary of the processes that occur in **1**: Excitation of **1** (Py-dU) at 340 nm results in intramolecular ET. The charge-separated species ($Py^{\bullet+}$-$dU^{\bullet-}$) itself is not fluorescent, but equilibrates with the fluorescent Py*-dU form. At pH < 5, the fluorescence is quenched as a result of the protonation of the uracil radical anion which yields $Py^{\bullet+}$-$dU(H)^{\bullet}$.

From the temporal evolution of the spectra (data not shown) we estimate the rate of protonation to be similar to the rate of charge transfer, that is, in the order of 100 fs.

Finally, we observed strong differences in the lifetimes of $Py^{\bullet+}$-$dU^{\bullet-}$ by its transient absorption, depending on the pH value. At pH 8, the ET intermediates show a lifetime in the range of several nanoseconds, whereas at pH 4 the transient absorption has decayed almost completely after 100 ps.

In summary, we have characterized the dynamics and pH dependence of the ET in the model nucleoside **1**. The intramolecular ET is an ultrafast process, $Py^{\bullet+}$-$dU^{\bullet-}$ can already be observed a few picoseconds after excitation in water. The pK_a value of the protonated $Py^{\bullet+}$-$dU(H)^{\bullet}$ biradical has been determined by steady-state fluorescence to be about 5.5. This value shows clearly that neither water in the environment of the nucleoside nor H-bonding donors such as the complementary DNA bases are able to protonate the uracil radical anion $dU^{\bullet-}$. Furthermore, our results suggest that reductive ET through DNA is not coupled to protonation. At pH values above 7, the lifetime of $Py^{\bullet+}$-$dU^{\bullet-}$ is in the nanosecond range. Assuming only a small change in the free energy of this ET process within DNA duplexes, the nucleoside **1** should be a suitable electron injector, as ET to adjacent DNA bases probably occurs on a subnanosecond timescale.

Experimental Section

The synthesis of 5-(1-pyrenyl)-2′-deoxyuridin (**1**) and a brief description of the laser setup is provided in the Supporting Information. Steady-state fluorescence spectra were obtained with a Fluoromax-3 fluorimeter (Jobin Yvon) and were intensity-corrected.

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