Preparation of Pyrenyl-Modified Nucleosides via Suzuki–Miyaura Cross-Coupling Reactions

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Abstract: The modified nucleosides 5-pyrenyl-2′-deoxyuridine (**1**) and 8-pyrenyl-2′-deoxyguanosine (**2**) were synthesized via palladium-catalyzed Suzuki–Miyaura cross-coupling reactions of pyren-1-yl boronic acid (**3**) to either 5-iodo-2′-deoxyuridine (**4**), or 8-bromo-2′-deoxyguanosine (**7**), respectively. No protecting groups for the hydroxy and amino functions of the nucleoside are needed during the preparation. Both pyrene derivatives are suitable nucleoside models for the spectrosopic investigation of reductive electron transfer (in **1**), or oxidative hole transfer (in **2**).

Key words: charge transfer, nucleoside, cross-coupling, palladium, pyrene

Charge migration processes through DNA have been a subject of an intense scientific debate over the last 10 to 15 years.[1–4] The motivation for this research is mainly focused on the biological consequences of these processes, such as DNA damage, mutations, and cancer.[5] Charge transfer reactions through DNA have been initiated by photoexcitation of suitable charge donors and have been probed by various different methods, including fluorescence quenching,[1] transient absorption spectroscopy,[2] electron spin resonance spectroscopy,[3] and gel electrophoretic or HPLC analysis of lesions of the nucleic acids.[4] It is important to emphasize that in most of these experiments oxidative hole transfer processes have been observed resulting in oxidative guanine damage at distant sites on the nucleic acid. Spectroscopic efforts have focused on hole transport over short distances (10–20 Å)[1,2] while biochemical measurements have explored oxidative guanine damage at more distant sites (30–200 Å).[6]

On the other hand, reductive electron transfer through DNA duplexes is currently used extensively in DNA chip technology and DNA nanotechnology without understanding the mechanism of this type of charge migration.[7] Only a few publications deal with a reductive electron transfer through DNA. In the past, Barton et al. investigated charge transfer reactions using intercalated and covalently tethered metal complexes as charge donors and acceptors which can be interpreted in parts as electron transfer reactions.[8] More recently, Carell et al. described the repair of thymine-thymine dimers through DNA-mediated electron transfer from a distant flavine derivative as an artificial base which was synthetically incorporated into the oligonucleotide.[9] Despite the fact that spectroscopic measurements with this system 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 based mainly on the redox properties of the flavin intercalator in its reduced and deprotonated state and on the absence of a typical DNA base sequence dependence, which should be observed in case of an oxidative hole hopping mechanism.[10]

Until now suitable DNA assays for the time-resolved spectroscopic investigation of reductive electron transfer through DNA are elusive. Herein we want to present preliminary results regarding the synthesis of 5-pyrenyl-2′-deoxyuridine (**1**) and 8-pyrenyl-2′-deoxyguanosine (**2**) as nucleoside model systems for the time-resolved spectroscopic investigation of the two different types of charge transfer (electron transfer vs. hole transfer). In principle, both compounds allow the later incorporation into DNA duplexes via phosphoramidite chemistry. Pyrene derivatives have been used previously as artificial DNA bases by Kool et al.[11] With respect to the relative redox properties we chose to attach a pyrenyl group (Py) to the nucleobases thymine (resp. uracil) and guanine (Figure 1). In case of the uridine derivative **1**, excitation of the pyrene moiety leads to an intramolecular reductive electron transfer yielding the corresponding uracil radical anion [E^0 ($Py^{\bullet+}$/Py) = 1.28 V[12] and E^0 (dT/$dT^{\bullet-}$) = –1.4 V].[13] In contrast, excitation of the pyrenyl group in the guanosine derivative **2** results in formation of the guanine radical cation as the product of an oxidative hole transfer [E^0 (Py/$Py^{\bullet-}$] = –2.1 V[12] and E^0 ($dG^{\bullet+}$/dG) = 1.3 V.[14] Both charge transfer assignments are proven by ps transient absorption experiments using 5-(1-pyrenoyl)-2′-deoxyuridin[15] and using benzo{*a*}pyrenyl-2′-deoxygu-anosine conjugates.[16]

Only for the preparation of nucleoside **1** exists a published procedure by Netzel et al.[11] using a palladium-catalyzed Stille-type cross coupling of 1-pyrenyl-(tributyl)-stannane with a fully protected 5-iodo-2′-deoxyuridine derivative. According to the authors, a glove box is needed during this procedure in order to ensure that moisture and air are excluded from the preparation. Our new approach to synthesize the pyrene-modified nucleosides **1** and **2** was to apply the palladium-catalyzed Suzuki–Miyaura-type cross coupling of pyren-1-yl boronic acid (**3**) to the halogenated nucleosides. In general, Suzuki–Miyaura-

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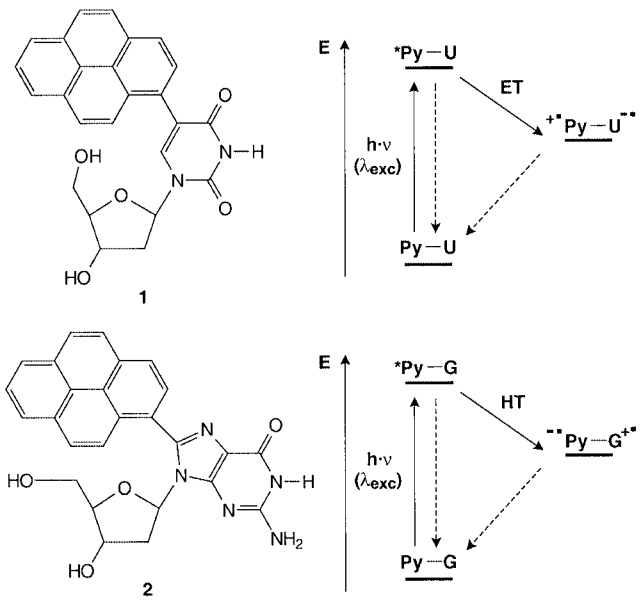
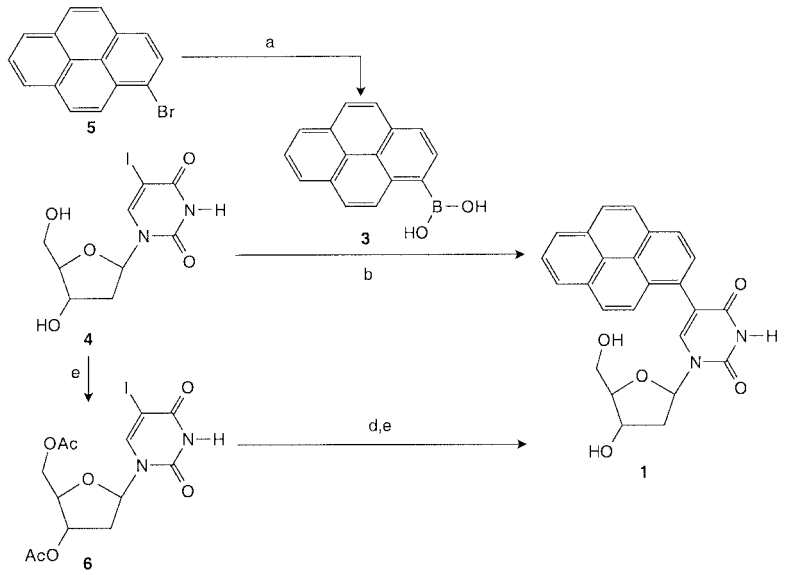


Figure 1 Pyrene-modified nucleosides **1** and **2**. Excitation of the pyrene moiety at λ = 340 nm results in either reductive electron transfer (ET) yielding the uracil radical anion (in **1**) or oxidative hole transfer (HT) yielding the guanine radical cation (in **2**).

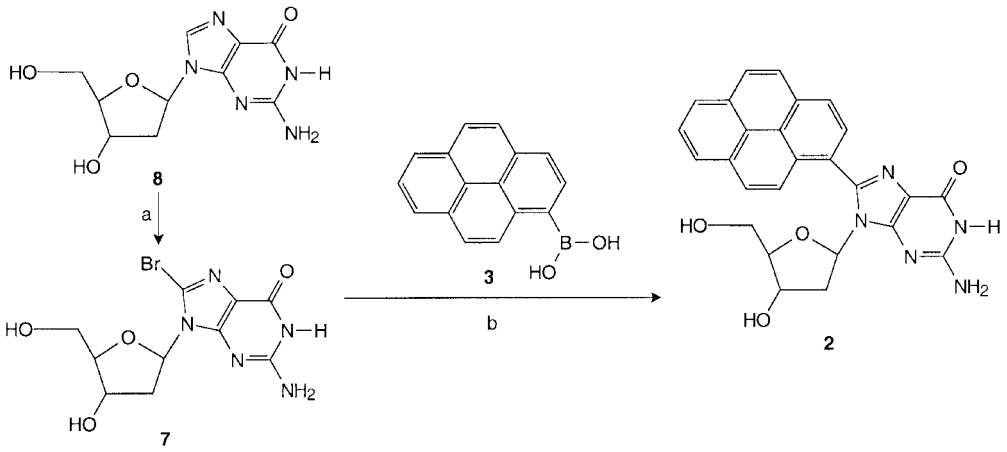
type couplings are easier to handle because they work in moist or even aqueous solutions and under air, too. Many boronic acids are commercially available and inexpensive, they are non-toxic and they tolerate the presence of some unprotected functional groups. Suzuki–Miyaura-type couplings have been performed for the preparation of arylated and alkenylated purines[17] but have not yet been used for the direct synthesis of aryl-modified nucleosides.

According to this new synthetic strategy, the nucleoside **1** was prepared via the cross coupling of pyren-1-yl boronic acid (**3**) to 5-iodo-2′-deoxyuridine (**4**) using $Pd(PPh_3)_4$ as the catalyst and THF/MeOH/H_2O = 2:1:2 as the solvent (Scheme 1). The starting material **4** is commercially available. The two hydroxy groups of the 2′-deoxyribose moiety of **4** were not protected. A strong base (NaOH) was required in order to get the desired sterically hindered coupling product **1** in sufficient yield (70%). The pyren-1-yl boronic acid (**3**) was synthesized according to a combination of different literature procedures by lithiation of 1-bromopyrene (**5**) at 0 °C and treatment with trimethyl borate at –78 °C and subsequent acidic workup at room temperature.[18] Interestingly, when the lithiation of 1-bromopyrene (**5**) was performed at –78 °C, a rather low yield of the desired boronic acid **3** (40%) was obtained whereas the lithiation of **5** at 0 °C results in an increased yield of **3** (73%). We also prepared 3′,5′-di-*O*-acetyl-2′-deoxy-5-iodo-uridine (**6**)[19] and used it for the palladium-catalyzed cross coupling with the boronic acid **3** in dry THF and Et_3N as the base. The overall yield of **1** after deprotection of the intermediate acetylated pyrene-modified uridine using NaOMe/MeOH was slightly lower than the direct cross coupling of the unprotected starting material **4** with the boronic acid **3** (55%). The structure of the nucleoside **1** was confirmed by different spectroscopic measurements, including ESI mass spectrometry and 2D-NMR experiments, such as DQF-COSY and HMQC.[20]

The second modified nucleoside, 8-pyrenyl-2′-deoxyguanosine (**2**) was prepared in a similar fashion (Scheme 2). The starting material 8-bromo-2′-deoxyguanosine (**7**) was obtained in good yield (85%) via bromination of 2′-deoxyguanosine (**8**) using *N*-bromo-



Scheme 1 Synthesis of 5-Pyrenyl-2′-deoxyuridin (**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 r.t., 20 h. 3. H_3O^+, r.t., 3 h; 73%, (b) **3** (1.0 equiv), $Pd(PPh_3)_4$ (0.1 equiv), NaOH (20 equiv), THF/MeOH/H_2O = 2:1:2, reflux, 20 h; 70%; (c) Ac_2O (2.5 equiv), pyridine, r.t., 20 h; (d) **3** (1.0 equiv), $Pd(PPh_3)_4$ (0.1 equiv), Et_3N (4.2 equiv), THF, reflux, 20 h; (e) NaOMe (1.0 equiv), MeOH, r.t., 18 h; 55% (d + e).



Scheme 2 Synthesis of 8-Pyrenyl-2′-desoxyguanosin (**2**): (a) *N*-bromosuccinimide (1.1 equiv), H_2O, r.t., 2 h; 85%; (b) **3** (1.0 equiv), $Pd(PPh_3)_4$ (0.1 equiv), NaOH (20 equiv), THF/MeOH/H_2O = 2:1:2, reflux, 20 h; 65%.

succinimide in H_2O.[21] The subsequent coupling of **7** to pyren-1-yl boronic acid (**3**) was performed in THF/MeOH/H_2O = 2:1:2 and using $Pd(PPh_3)_4$ as the catalyst. Both, the hydroxy groups of the 2′-deoxyribose part of **7**, and the exocyclic amino function in position 2 of the guanine moiety of **7** were not protected during the coupling. After purification (65% yield), the pyrene-modified nucleoside **2** has been characterized by different spectroscopic measurements, including ESI mass spetrometry and 2D-NMR experiments.[22]

The pyrene-modified nucleosides **1** and **2** were characterized by UV/VIS absorbance and steady-state fluorescence spectroscopy.[23] The measurements were performed in MeCN and MeOH as typical examples of organic solvents without (MeCN) or with hydrogen bonding capabilities (MeOH). In case of the uridine derivative **1**, the previously published absorbance and emission spectra[15] could be reproduced. As expected, the UV/Vis spectrum of both compounds, **1** and **2**, exhibit a similar shape of absorbance in both solvents. Based on this absorbance spectra, the excitation wavelength for both nucleosides was chosen to be λ = 340 nm. In the steady-state fluorescence spectra, both compounds display an emission profile, which is different from pure pyrene indicating a strong electronic interaction between the nucleoside moiety and the covalently attached pyrene. In fact, preliminary femtosecond-resolved transient absorption data indicates charge transfer processes in both pyrene-modified nucleosides.[24] Interestingly, when excited at λ = 340 nm, the uridine derivative **1** shows a remarkable bathochromic shift of the emission wavelength in comparison between MeCN (λ_{em} = 422 nm) and MeOH (λ_{em} = 482 nm) as a solvent, which has also been observed previously.[15] This observation was interpreted by protonation of the uracil radical anion. But, according to Steenken et al., protonation of the uracil radical anion by MeOH is rather unlikely since the pK_a value of the protonated uracil radical is 6.9.[24] Another explanation could be the solvation of the charge-separated species of **1** in MeOH via hydrogen bonding.[25] Interestingly, the guanosine derivative fluorescence does not display this

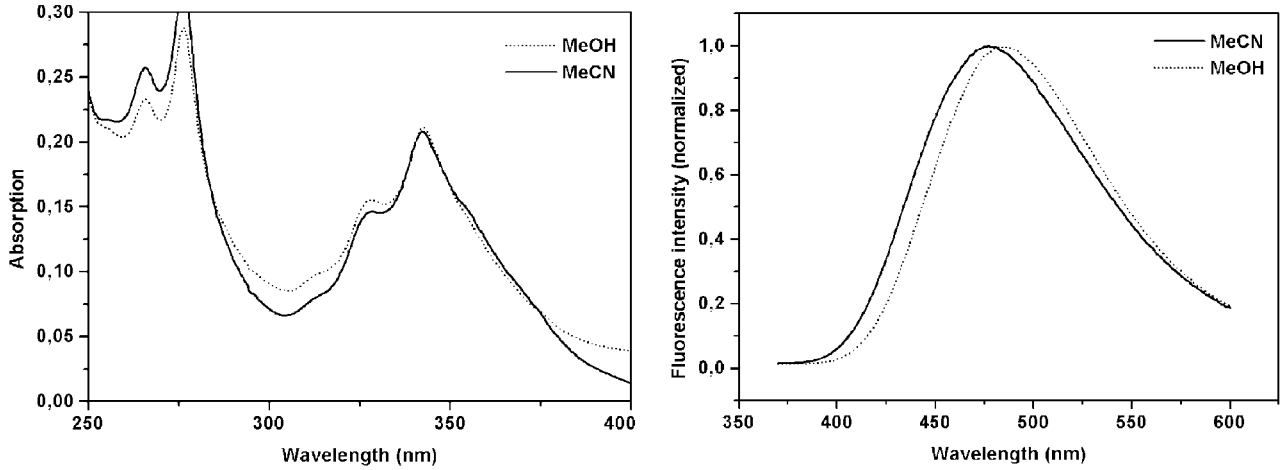


Figure 2 UV/Vis absorbance spectra (left) and steady-state fluorescence spectra (right) of the pyrene-modified nucleoside **2** (ΔA = 0.2 at λ_{exc} = 340 nm). The corresponding absorption and emission spectra of **1** have been published previously by Netzel et al.[15]

difference between MeCN (λ_{em} = 477 nm) and MeOH (λ_{em} = 485; Figure 2). Currently, time-resolved spectroscopic measurements on the femtosecond time scale are conducted in order to detect the intermediates by transient absorption, to characterize the nature of the charge-separated species, and, finally, to explore the detailed mechanisms of the charge transfer processes observed in the pyrene-modified nucleosides **1** and **2**.[26]

In this communication, we present a new approach for the synthesis of pyrene-modified nucleosides via palladium-catalyzed Suzuki–Miyaura-type cross couplings. This protocol is very versatile and can be extended to other nucleosides as well as to other aryl boronic acids. The two synthesized pyrene-modified nucleosides **1** and **2** are suitable as nucleoside models for electron vs. hole transfer.

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