Electron Transfer in DNA

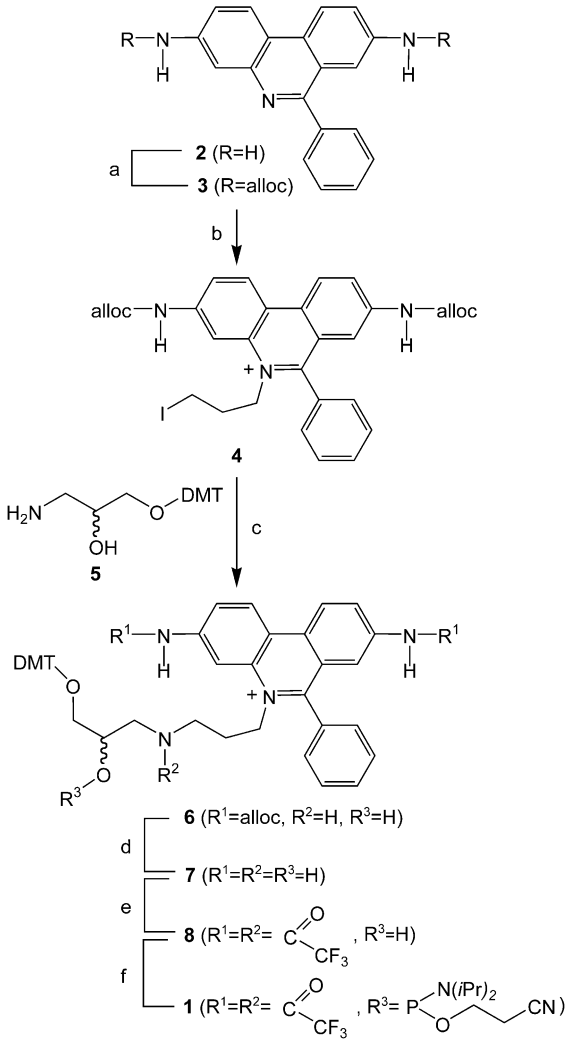
Phenanthridinium as an Artificial Base and Charge Donor in DNA**

*Nicole Amann, Robert Huber, and Hans-Achim Wagenknecht**

3,8-Diamino-5-ethyl-6-phenylphenanthridinium, known as "ethidium", has been widely used in fluorescence assays with nucleic acids.[1] Ethidium and its derivatives are also potent trypanocidal drugs.[2] In addition, ethidium represents an important donor for photoinduced charge transfer processes in DNA.[3–6] Relative redox potentials indicate that ethidium in the photoexcited state (Et^{*+}) is not able to oxidize or reduce DNA to initiate hole or electron hopping, respectively.[7] Hence, a suitable charge acceptor has to be provided. 7-Deazaguanine quenches the emission of ethidium in DNA[8] and has been applied as the acceptor in hole-transfer studies.[5,6] Remarkably, investigations of DNA duplexes with ethidium covalently attached to the 5′-end through an alkyl linker found no dependence of the rate of DNA-mediated oxidative hole transfer on the distance,[6] although the hopping model[9] cannot be applied in this case.

Site-specific intercalation of ethidium into DNA is crucial for a detailed study of its binding interactions and charge donor properties. We incorporated the phenanthridinium heterocycle of ethidium as an artificial base at specific sites in duplex DNA. The hydrolytic lability of the corresponding ethidium 2′-deoxyribofuranoside[10] made it necessary to replace the sugar moiety with an acyclic linker system tethered to the N-5 position of the phenanthridinium heterocycle (Scheme 1).

To synthesize the corresponding DNA building block **1**, we started with the protection of the two exocyclic amino functions of 3,8-diamino-6-phenylphenanthridine (**2**) by treatment with allyl chloroformate. The bisalloc-protected phenanthridine derivative **3** was then alkylated with 1,3-diiodopropane. THF is the best solvent for this reaction because the starting material **3** is soluble in THF, whereas the alkylation product **4** is not. Hence, **4** can be collected simply by filtration. The phenanthridinium **4** was linked to DMT-protected 3-amino-1,3-propanediol (**5**) under the typical conditions used for a nucleophilic substitution. Compound **5** was synthesized according to literature procedures and carries the DMT protecting group necessary for automated oligonucleotide coupling at a later stage.[11] After attachment of **5**, the alloc protecting groups were exchanged by trifluoroacetyl groups. This procedure is necessary since trifluoroacetyl groups are not stable enough to be used in the alkylation of the phenanthridine heterocycle at N-5[12] but can be cleaved under typical DNA workup conditions. This protecting-group strategy has the additional advantage that the secondary amino function of the alkyl linker is also protected. The preparation of the phosphoramidite **1** was completed by using standard procedures, and the product was used for the automated preparation of phenanthridinium-modified oligonucleotides. An extended coupling time (1 h instead of the 1.5 min used for standard couplings), a higher phosphoramidite concentration (0.2 M instead of 0.067 M), and three



Scheme 1. Synthesis of DNA building block **1**: a) allyl chloroformate (10 equiv), CH_2Cl_2, RT, 24 h, 98%; b) 1,3-diiodopropane, THF, 65 °C, 9 days, 82%; c) **5** (1.5 equiv), *N*,*N*-diisopropylethylamine (3 equiv), dimethylformamide, RT, 55 °C, 91%; d) Bu_3SnH (3.2 equiv), $[Pd(PPh_3)_4]$ (0.02 equiv), PPh_3 (0.2 equiv), CH_2Cl_2/H_2O (300:1), RT, 90 min, 97%; e) $(CF_3CO)_2O$ (6 equiv), CH_2Cl_2/pyridine (5:1), 0 °C, 10 min, RT, 10 min, 59%; f) 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite (1.5 equiv), Et_3N (3 equiv), CH_2Cl_2, RT, 2 h. alloc = allyloxycarbonyl; THF = tetrahydrofuran; DMT = dimethoxytrityl.

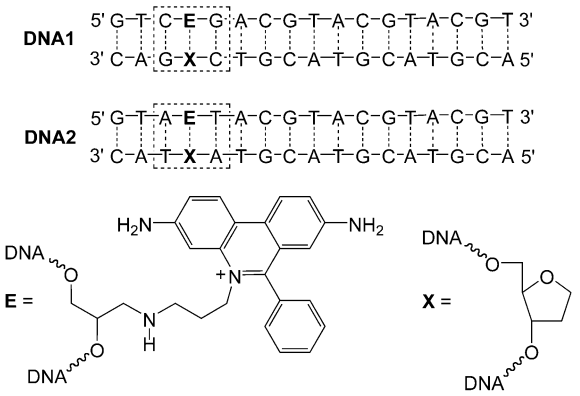
[*] Dipl.-Chem. N. Amann, MSc. R. Huber, Dr. H.-A. Wagenknecht
Institute for Organic Chemistry and Biochemistry
Technical University of Munich
Lichtenbergstrasse 4, 85747 Garching (Germany)
Fax: (+49) 89-289-13210
E-mail: wagenknecht@ch.tum.de

[**] This work was supported by the Deutsche Forschungsgemeinschaft (Grant no. Wa 1386/7-1), the Volkswagen Stiftung, and the Fonds der Chemischen Industrie. We are grateful to Professor Horst Kessler, Technical University of Munich, for his generous support.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200353153

coupling cycles interrupted by washing steps were necessary to achieve nearly quantitative coupling. The phenanthridinium-containing oligonucleotides in **DNA1** and **DNA2** (Scheme 2) were identified by MALDI-TOF mass spectrometry, purified by semi-preparative HPLC, and quantified by UV/Vis absorption spectroscopy.[13] The DNA duplexes **DNA1** and **DNA2** were prepared by slow cooling of the appropriate phenanthridinium-modified oligonucleotide in the presence of an excess (1.2 equiv) of the corresponding complementary unmodified oligonucleotide strand to ensure the quantitative formation of the modified duplex. An abasic site analogue (**X**)[14] was incorporated into the counterstrands to allow optimal intercalation of the phenanthridinium heterocycle (**E**).



Scheme 2. Duplexes **DNA1** and **DNA2**.

The sequences of **DNA1** and **DNA2** are identical except for the bases adjacent to the phenanthridinium site (**E**). The overall B-DNA conformation of the modified DNA duplexes was confirmed by CD spectroscopy (see the Supporting Information). Absorption and steady-state fluorescence measurements were performed to verify that intercalation of the phenanthridinium moiety had occurred and to show the similarity of the structure to that of intercalated ethidium. At 10 °C, the UV/Vis absorption spectra of **DNA1** and **DNA2** have maxima at 530 and 535 nm, respectively, peaks typical of intercalated ethidium (Figure 1).[15,16] The absorption spectrum of "free" ethidium in aqueous solution has its maximum at approximately 480 nm.[17] The absorption by **DNA1** and **DNA2** increases slightly at higher temperatures and the maxima shift to 503 and 515 nm, respectively, as a result of dehybridization and interruption of the stacking interactions between the phenanthridinium and the adjacent DNA bases. Excitation of **DNA1** and **DNA2** at 520 nm results in emission spectra with maxima at 626 and 623 nm (at 10 °C), which are again typical peaks for intercalated ethidium (Figure 2).[15,16] The emission of "free" ethidium in water has a maximum at around 635 nm[17] and is significantly quenched by protonation of the excited state.[16] Temperature-dependent fluorescence measurements with **DNA1** and **DNA2** show the same ethidium-type behavior. As the temperature is increased, the excited-state phenanthridinium moiety becomes more and more accessible to water because of dehybridization and interruption of base stacking. As a result, the maxima are shifted to 637 and 635 nm (at 80°) and the emission is quenched to 20–25 % of the duplex quantum yield at RT. In conclusion, characterization of **DNA1** and **DNA2** by optical spectroscopy shows clearly that the phenanthridinium heterocycle of the artificial DNA base is intercalated in the duplex DNA and exhibits similar properties to those of noncovalently bound, intercalated ethidium. Interestingly, the difference in the base pairs in the duplex environment immediately surrounding the artificial base (A-T in **DNA1**, G-C in **DNA2**)

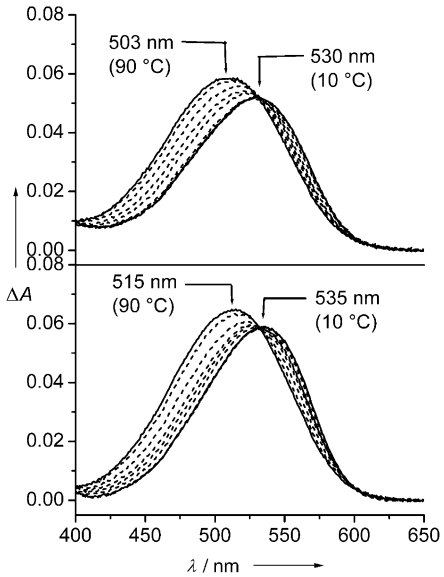


Figure 1. Temperature-dependent UV/Vis absorption spectra of **DNA1** (top) and **DNA2** (bottom). 12.5 μM duplex, 10 mM Na/phosphate buffer, pH 7, $\Delta T = 10$ °C.

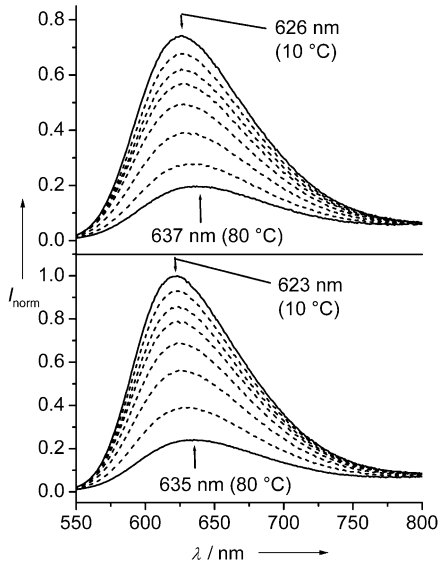


Figure 2. Temperature-dependent steady-state fluorescence spectra of **DNA1** (top) and **DNA2** (bottom). 12.5 μM duplex, 10 mM Na/phosphate buffer, pH 7, $\Delta T = 10$ °C.

does not significantly influence the absorption and emission properties of the molecules.

Finally, the electron-donor properties of the artificial phenanthridinium DNA base were elucidated by using methyl viologen (**MV**) as a noncovalently bound electron acceptor.[4] The redox potentials indicate that an electron can be transferred from the excited state of ethidium to methyl viologen.[18] The emission of **DNA1** and **DNA2** was quenched significantly by **MV** as a result of this electron-transfer process (Figure 3). This result shows clearly that the synthesized

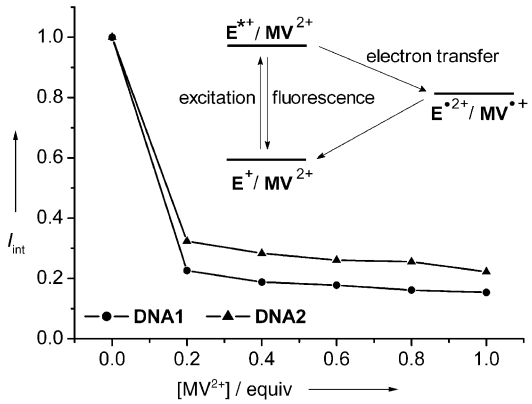


Figure 3. Electron transfer experiments with **DNA1** and **DNA2**. 12.5 μM duplex, 10 mM Na/phosphate buffer, pH 7. Methyl viologen was added in increasing amounts.

phenanthridinium–DNA has the potential to allow spectroscopic investigation of electron *transfer* (not electron *hopping*) in DNA. Use of this molecule will also make it possible to compare the rate of *reductive electron transfer* with that of *oxidative hole transfer* by using either methyl viologen or 7-deazaguanine as the electron or hole acceptor, respectively.

Experimental Section

The details of the synthesis of DNA building block **1** will be published separately.[19] Experimental details of the preparation and spectroscopic characterization of **DNA1** and **DNA2** are described in the Supporting Information.

Received: October 27, 2003 [Z53153]

Keywords: DNA · electron transfer · ethidium ion · fluorescence spectroscopy · oligonucleotides

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