Phenothiazine as a redox-active DNA base substitute: comparison with phenothiazine-modified uridine†

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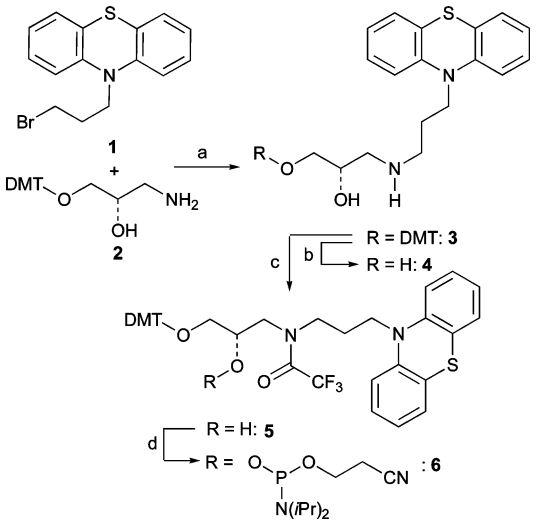
Phenothiazine can be incorporated as a redox-active probe into DNA in two conceptually different ways: the non-nucleosidic DNA base surrogate exhibits similar properties to 10-methylphenothiazine but with no preferential base-pairing properties, whereas the phenothiazine-modified uridine has different optical and electrochemical properties, but exhibits preferred Watson–Crick base pairing with adenine.

Phenothiazine (Pz) is a low-potential reductant ($Pz^{+\bullet}/Pz = 0.7$–0.8 *vs.* SCE) that possesses a spectroscopically well-characterized one-electron oxidized radical ($Pz^{+\bullet}$ at 510 nm).[1] Thus, Pz represents a promising redox-active probe for DNA. In fact, Kawai, Majima and coworkers applied Pz covalently attached to the 5′-end of oligonucleotides as a charge acceptor for time-resolved measurements of hole transfer in DNA.[2] Grinstaff and coworkers incorporated Pz at the 5′-terminal position,[3] as a C-nucleoside,[4] or attached to the 8-position of guanine.[5] Recently, we used 5-(10-methylphenothiazin-3-yl)-2′-deoxyuridine (Pz-dU) as a photoinducable charge donor in order to investigate DNA-mediated electron transfer processes.[6] Herein we describe the facile synthesis of a novel Pz DNA base substitute, its synthetic incorporation into oligonucleotides and the comparison of its opto-electronic properties with Pz-modified uridine (Pz-dU).

The 2′-deoxyribofuranoside was replaced by (*S*)-2-amino-1,3-propanediol as a flexible acyclic linker system (Scheme 1) that provides a high chemical stability during the preparation and allows the chromophore to intercalate perfectly. It has been applied similarly for DNA base substitution by other chromophores, *e.g.* ethidium,[7] indole[8] and perylene bisimide.[9] The Pz derivative **1** can be synthesized as a starting material according to published procedures[10] and used subsequently for a nucleophilic substitution with the dimethoxytrityl (DMT)-protected (*S*)-3-amino-1,2-propanediol (**2**),[7] yielding the Pz derivative **3**. For the electrochemical characterization of **4**, the DMT group of **3** was cleaved off an analytical sample. After protection of the NH function by a trifluoroacetyl group in **5**, the phosphoramidite **6** can be applied for the automated preparation of modified oligonucleotides.

We characterized the nucleoside substitute **4** by optical spectroscopy and electrochemistry methods, each in comparison with the commercially available 10-methylphenothiazine (Me-Pz) and the previously synthesized Pz-dU. Not surprisingly, the absorption and fluorescence properties of **4** are similar to Me-Pz (see Fig. S1†). In contrast, Pz-dU exhibits an exciplex-type fluorescence due



Scheme 1 Synthesis of DNA building block **6**. *Reagents and conditions*: a) **2** (2 equiv.), iPr$_2$NEt (4 equiv.), DMF, r.t., 10 d; 54%; b) $Cl_2CHCOOH$, CH_2Cl_2, r.t., 30 min; 95%; c) Me_3SiCl (1 equiv.), pyridine (6 equiv.), CH_2Cl_2, 0 °C, 3 h; $(F_3CCO)_2O$ (2 equiv.), 0 °C, 20 min, r.t., 10 min; 1 M (nBu)$_4$NF in THF (1 equiv.), 30 min, r.t.; 95%; d) iPr$_2$NEt (4 equiv.), 2-(cyanoethyl)diisopropylchlorophosphoramidite (2 equiv.), CH_2Cl_2, r.t., 45 min; 95%.

to the strong electronic coupling between the uridine and Pz *via* a single C–C bond.[13,14] The electrochemical potentials were measured by cyclic voltammetry *vs.* ferrocene ($Fc^{+\bullet}/Fc$) and transferred into potentials *vs.* NHE using a conversion constant of +0.63 V (Fig. 1).[11] The Pz derivative **4** as a non-nucleosidic DNA base substitute has a potential of $E_{1/2} = 0.81$ V that is

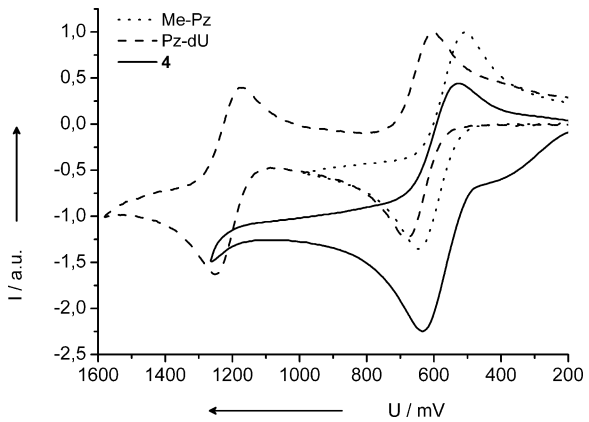


Fig. 1 Cyclic voltammetry with **4**, Me-Pz and Pz-dU (0.5 mM) in MeCN, 25 °C, *vs.* ferrocene ($Fc^{+\bullet}/Fc$).

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identical to that of Me-Pz. The corresponding potential of Pz-dU is shifted by 60 mV to 0.87 V, indicating the small electron-withdrawing effect of the covalently attached uridine moiety. The second potential at 1.44 V can be assigned to the oxidation of the uridine moiety. Comparison with the potential of the structurally similar nucleoside thymine ($T^{+\bullet}/T$) at 1.90 V[12] shows the strong electron-donating character of the Pz chromophore, which shifts the potential by −0.56 V.

Spectroelectrochemical characterization under oxidizing conditions revealed that the radical cation of **4** absorbs at 508 nm, similar to that of Me-Pz at 512 nm (Fig. 2).[1] In contrast, the absorption of the radical cation of Pz-dU is significantly red-shifted to 620 nm. The covalent attachment of the Pz chromophore to uridine changes not only the redox potentials of the Pz moiety slightly, but also the spectro-optical properties significantly.

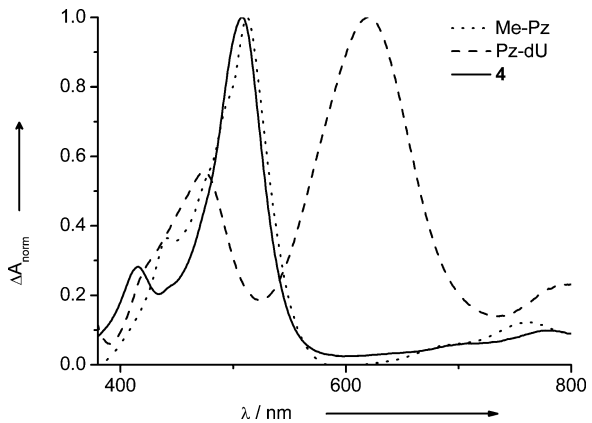


Fig. 2 Spectroelectrochemistry with **4**, Me-Pz and Pz-dU (0.5 mM in MeCN), 25 °C, Δ*U ca.* 800 mV.

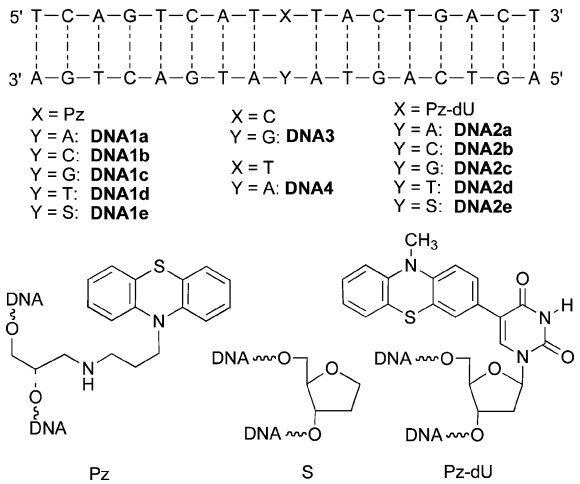
Using the DNA building block **5**, we synthesized a range of duplexes, **DNA1a–DNA1e** (Scheme 2). Using our previously published synthetic protocol,[6] a second set was synthesized, **DNA2a–DNA2e**, bearing the Pz-modified uridine (Pz-dU). In both DNA sets, the base opposite to Pz modification site was varied, including the abasic site analog S. Representatively for each set, the matched duplexes **DNA1a** and **DNA2a** were investigated by absorption (Fig. S2), fluorescence (Fig. S3), and CD spectroscopy (Fig. S4). In these DNA duplexes, the fluorescence of Pz and Pz-dU is quenched significantly. The quantum yields are remarkably low, $\Phi = 0.1\%$ (**DNA1a**) and $\Phi = 0.3\%$ (**DNA2a**). This result underscores the redox-activity of the Pz chromophore in DNA. As we know from our previous studies, photoexcitation of Pz in DNA initiates very efficient electron hopping *via* thymines and cytosines as electron carriers.

Thermal dehybridization experiments were performed with all duplexes (Table 1). The T_m values of the two duplex sets exhibit a remarkable difference between the non-nucleosidic base surrogate Pz and the modified nucleosides Pz-dU. In the Pz-duplex set **DNA1a–DNA1d** the T_m values do not reveal any preferential base pairing. In contrast, the correctly matched Pz-dU-modified duplex **DNA1a** shows a higher T_m value compared to the others (**DNA2b–DNA2d**), indicating the preferential base pairing with the correct counterbase adenine. Only the presence of the abasic site analog S seems to enhance the hydrophobic interactions of the Pz chromophore inside the DNA, leading to a stabilization of 6 °C.

Table 1 Melting temperatures (T_m) for **DNA1a–DNA1e** and **DNA2a–DNA2e** (260 nm, 0.5 °C min^{-1}, 2.5 μM DNA in 10 mM Na–P_i buffer, pH 7, 250 mM NaCl)

Duplex	T_m/°C	Duplex	T_m/°C
DNA1a	54, 50[a]	**DNA2a**	60
DNA1b	50[a]	**DNA2b**	56
DNA1c	50[a]	**DNA2c**	55
DNA1d	49[a]	**DNA2d**	55
DNA1e	56[a]	**DNA2e**	63
DNA3	60	**DNA4**	63

[a] **DNA1b–DNA1e** had to be measured without the addition of 250 mM NaCl.



Scheme 2 Sequences of **DNA1a–DNA1e** and **DNA2a–DNA2e**.

In conclusion, both Pz modifications presented herein can be used as redox-active probes in DNA for electrochemical analytics or the investigation of charge transfer in DNA. The non-nucleosidic Pz derivative **4** as a DNA base surrogate behaves similarly to the Me-Pz chromophore, but shows no selective base-pairing in DNA, whereas Pz-dU has altered optical and electrochemical properties, but exhibits preferred Watson–Crick base pairing with adenine.

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