Reductive Electron Transfer in Phenothiazine-Modified DNA Is Dependent on the Base Sequence

Clemens Wagner and Hans-Achim Wagenknecht*[a]

Abstract: A new DNA assay has been designed, prepared and applied for the chemical investigation of reductive electron transfer through the DNA. It consists of 5-(10-methyl-phenothiazin-3-yl)-2′-deoxyuridine (Ptz-dU, **1**) as the photoexcitable electron injector and 5-bromo-2′-deoxyuridine (Br-dU) as the electron trap. The Ptz-dU-modified oligonucleotides were synthesised by means of a Suzuki–Miyaura cross-coupling protocol and subsequent automated phosphoramidite chemistry. Br-dU represents a kinetic electron trap, since it undergoes a chemical modification after its one-electron reduction that can be analysed by piperidine-induced strand cleavage. The quantification of the strand cleavage yields from irradiation experiments reveals important information about the electron-transfer efficiency. The performed DNA studies focused on the base sequence dependence of the electron-transfer efficiency with respect to the proposal that $C^{\bullet-}$ and $T^{\bullet-}$ act as intermediate electron carriers during electron hopping. From our observations it became evident that excess-electron transfer is highly sequence dependent and occurs more efficiently over T–A base pairs than over C–G base pairs.

Keywords: DNA · electron transfer · electron transport · oligonucleotides · phenothiazine

Introduction

In principal, DNA-mediated charge-transfer processes can be regarded as either *oxidative hole transfer* or *reductive electron transfer* reactions.[1] Both processes are in fact electron-transfer (ET) reactions; however, the differentiation is necessary with respect to the molecular orbitals that are involved. Since the interest in these processes has been driven by its relevance to oxidative damage, which causes mutagenesis and carcinogenesis,[2] the photochemically induced oxidation of DNA has attracted enormous research efforts over the last two decades.[3] Several mechanisms have been elucidated, such as the superexchange and the hopping mechanism, and at least in part, have been experimentally verified.[3] On the other hand, reductive ET processes are currently used in DNA chip technology[4] and DNA nanotechnology.[5] Injection of an extra electron into DNA initiates a type of charge transfer that is complementary to hole transfer and entails migration of this electron. This topic has remained considerably underdeveloped relative to the understanding of hole-transfer processes. As a result, the mechanisms of hole transfer have been simply transferred to the mechanistic description of reductive ET in DNA.[1] Accordingly, a hopping mechanism was proposed over long distances involving the pyrimidine radical ions $C^{\bullet-}$ and $T^{\bullet-}$ as intermediate electron carriers.[6]

Until five years ago, most knowledge about the behaviour of excess electrons in DNA came from γ-pulse radiolysis studies with randomly DNA-bound electron traps and suggesting a thermally activated, electron-hopping process above 150 K.[7] The major disadvantage of this experimental setup is that the electron injection and trapping does not occur site-selectively. Most of the more recent photochemical studies have been analysed by chemical means by using two different kinetic electron traps, which are a specially designed T–T dimer or 5-bromo-2′-deoxyuridine (Br-dU). By using a DNA assay consisting of an artificial DNA base with a flavine structure as the photoexcitable electron donor and a special T–T dimer as the electron trap, Carell et al. could show that the amount of T–T dimer cleavage depends rather weakly on the distance to the flavine group, indicating an electron-hopping process.[8] Giese et al. investigated ET in DNA using a special T derivative that undergoes Norrish I cleavage after irradiation, yielding an electron injection process onto the thymine.[9] Remarkably, they could show that a single injected electron can cleave more than

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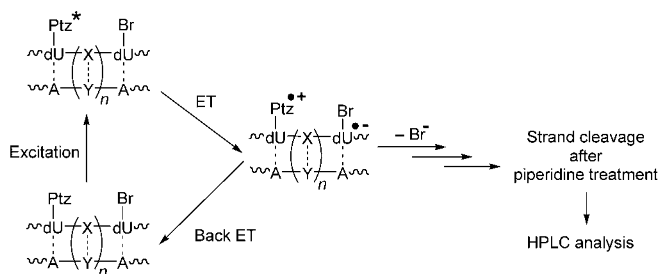
one T–T dimer. Rokita et al. analysed the ET efficiencies using a diaminonaphthalene derivative as the photoexcitable charge donor and Br-dU as the electron trap.[10] Br-dU induces a piperidine-dependent strand cleavage upon a one-electron reduction.[11] It is important to point out that a significant dependence of the ET efficiency on the intervening DNA base sequence was reported in the latter studies. This observation stands clearly in contrast to the previously mentioned T–T dimer experiments and could reflect differences of the kinetic behaviour of the two different electron traps. Only Lewis et al.[12] and our group[13] have focused on the study of the dynamics of ET processes using either DNA hairpins that have been capped by a stilbene diether derivative or pyrene-modified DNA duplexes, respectively.

All of the recent studies support conclusively the proposal of a thermally activated, electron-hopping mechanism over long distances involving $C^{\bullet-}$ and $T^{\bullet-}$ as intermediate electron carriers. However, recently, using pyrene-modified nucleosides as model compounds, we showed that proton transfer interferes with ET, indicating that $C^{\bullet-}$ cannot play a major role as an intermediate electron carrier in DNA.[14] Herein, we want to follow these results and elucidate the sequence dependence of DNA-mediated ET. We present the synthesis of 5-(10-methyl-phenothiazin-3-yl)-2′-deoxyuridine (Ptz-dU, **1**) as a photoexcitable electron injector and the corresponding phenothiazine-modified oligonucleotides. Together with 5-bromo-2′-deoxyuridine (Br-dU) as the kinetic electron acceptor in irradiation experiments, we focused on the ET efficiency with respect to $C^{\bullet-}$ and $T^{\bullet-}$ as intermediate electron carriers during electron hopping (Scheme 1).



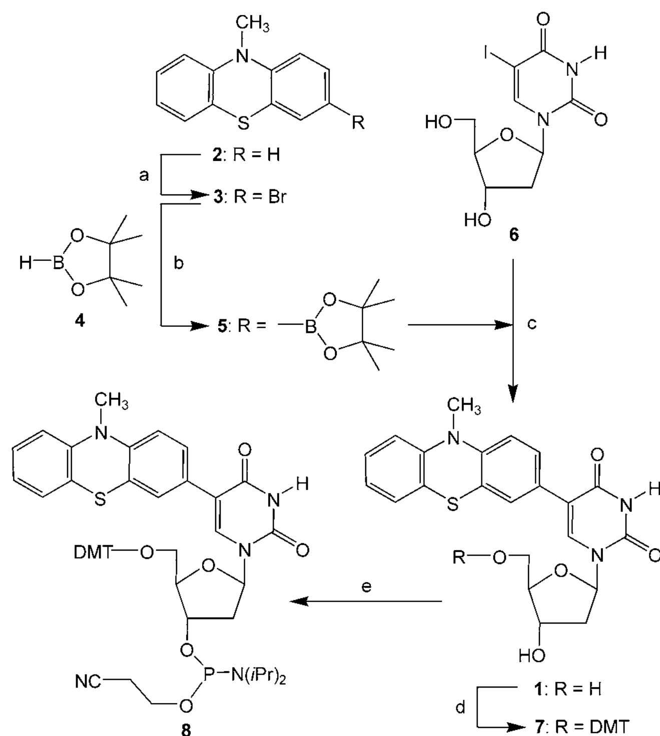
Scheme 1. Electron transfer in Ptz-dU-modified DNA: The electron is trapped kinetically on the Br-dU group yielding a DNA lesion which can be analysed by HPLC after treatment with piperidine (X–Y = T–A or C–G, n = 1–3).

Results and Discussion

Synthesis and ET properties of 5-(10-methyl-phenothiazin-3-yl)-2′-deoxyuridine: Until now our group has focused mainly on pyrene (Py) as the photoexcitable electron donor for the investigation of reductive electron transfer in DNA.[13–15] According to our studies, Py in the excited state (Py*) is a significantly weaker electron donor than calculated and, therefore, than expected. Combining the potentials $E(\mathrm{Py}^{\bullet+}/\mathrm{Py}^*) = -1.8$ V (vs NHE)[16] and $E(\mathrm{dU}/\mathrm{dU}^{\bullet-}) = -1.1$ V,[17] the driving force ΔG for the ET process in Py–dU could be −0.6 eV at most. However, our studies revealed a driving force $\Delta G \sim 0$ eV,[14,15] which requires the potential $E(\mathrm{dU}/\mathrm{dU}^{\bullet-})$ to be ~−1.8 V. In this context, the measured value $E(\mathrm{dU}/\mathrm{dU}^{\bullet-}) = -1.1$ V provided by Steenken et al.[17] is difficult to understand and could reflect the result of a proton-coupled ET. Thus it is likely, that the −1.1 V potential corresponds to $E(\mathrm{dU}/\mathrm{dU(H)}^{\bullet})$.

In comparison to Py as the electron donor, the reduction potential of phenothiazine (Ptz) in the excited state $E(\mathrm{Ptz}^{\bullet+}/\mathrm{Ptz}^*) = -2.0$ V[18] should be more efficient for the photoreduction of T and C within DNA. Ptz has only been used as a charge acceptor to investigate the DNA-mediated oxidative hole transfer.[19,20] In order to use Ptz as a charge donor for reductive electron transfer, we synthesised Ptz-dU (**1**) and incorporated it into oligonucleotides. By this synthetic approach we are able to exclusively photoinitiate a reductive electron transfer, since the intramolecular electron transfer in the Ptz-dU moiety can be regarded as an electron injection into the DNA preceeding the electron hopping.

Palladium-assisted routes to modified nucleosides have been explored extensively.[21] For the preparation of Ptz-dU (**1**), we applied a Suzuki–Miyaura cross-coupling protocol that we developed recently for the synthesis of pyrene-modified nucleosides (Scheme 2).[22] In general, this type of palladium-catalysed coupling has the advantage that it works



Scheme 2. Synthesis of Ptz-dU (**1**) and the corresponding DNA building block **8**: a) Br_2 (1 equiv), AcOH/NaOAc/CH_2Cl_2, 5 °C, 30 min; 67 %; b) **4** (2 equiv), Et_3N (3 equiv), [$PdCl_2$(dppf)] (0.03 equiv), dioxane, reflux, 16 h; 57 %; c) **5** (1.2 equiv), $PdCl_2$(dppf) (0.1 equiv), NaOH (20 equiv), THF/H_2O/MeOH, reflux, 44 h; 34 %; d) DMT-Cl (2.0 equiv), pyridine, RT, 16 h; 52 %; e) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (2.6 equiv), Et_3N (2.9 equiv), CH_2Cl_2, RT, 45 min; 95 %.

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even in aqueous solutions and it tolerates the presence of some unprotected functional groups.[23] The synthetic procedure starts with the bromination of 10-methyl-phenothiazine (**2**) by one equivalent of Br_2 and subsequent Pd^0-catalysed coupling of the 3-bromo-10-methyl-phenothiazine (**3**) with tetramethyl oxoborolane (**4**) according to the procedures by Ebdrup and Müller et al.[24] The palladium-catalysed Suzuki–Miyaura coupling of the boronic acid ester **5** with commercially available 5-iodo-2′-deoxyuridine (**6**) gives Ptz-dU (**1**) in reasonable yield (34 %).

The structure of Ptz-dU (**1**) was confirmed by different spectroscopic techniques, including high-resolution mass spectrometry and 2D NMR spectroscopy. Furthermore, the Ptz-modified nucleoside **1** was characterised by UV-visible absorption and steady-state fluorescence spectroscopy in order to study its potential as a charge donor for ET in DNA. The UV-visible spectrum of Ptz-dU (Figure 1, top)

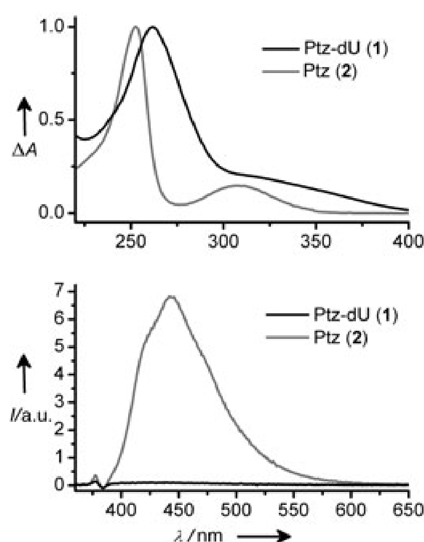
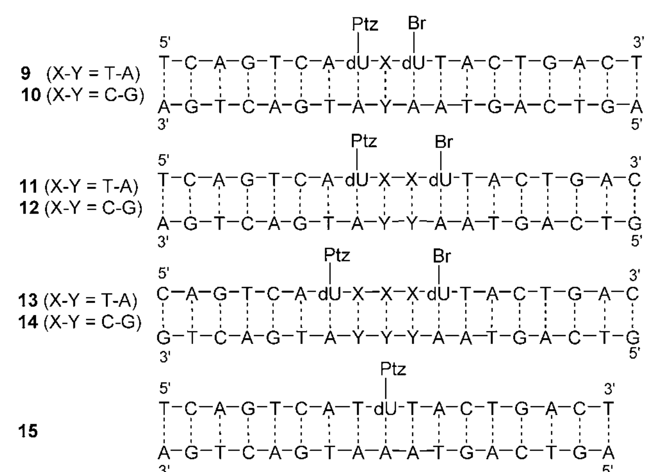


Figure 1. Absorption and fluorescence spectra of Ptz-dU (**1**) and 10-methyl phenothiazine (**2**) in $MeOH/H_2O=1:1$. The emission was recorded at equal optical density (0.3) of **1** and **2** at the excitation wavelength (340 nm).

represents a combination of the absorption of the uridine nucleoside (peak at ~260 nm) and the absorption of the Ptz chromophore as a broad shoulder between 300 and 400 nm. For the fluorescence spectroscopy measurements we chose an excitation wavelength of 340 nm, a value which is clearly in the absorption region of the Ptz moiety and avoids any partial excitation of the uridine system. By using samples of 10-methyl-phenothiazine (**2**) and Ptz-dU (**1**) that were adjusted to the same optical density of 0.3 at 340 nm, a significant difference in the emission quantum yield could be detected (Figure 1, bottom). Remarkably, the Ptz-typical emission with its maximum at 445 nm is quenched completely in case of Ptz-dU (**1**). This observation indicates an intramolecular ET process, which together with back ET into the ground state represents a nonradiative decay pathway for the excited state. This result stands in contrast to our recently published experiments using Py-modified nucleosides in which back ET occurs partially into the Py excited state.

Preparation of Ptz-dU-modified oligonucleotides: For the synthetic incorporation into oligonucleotides, the Ptz-modified uridine **1** was converted into the DMT-protected compound **7** and then to the completely protected nucleoside **8** bearing the phosphoramidite group in the 3′-position (Scheme 2). Standard procedures were applied for these two synthetic steps. From the DNA building block **8**, the Ptz-modified oligonucleotides ss**9**–ss**15** (ss = single strand) were prepared by automated solid-phase synthesis on an Expedite 8909 DNA synthesiser. Nearly quantitative coupling of the monomer **8** was achieved with the standard coupling time of 1.6 min. Additional to the Ptz-dU-modification, Br-dU was inserted into the oligonucleotides by using the corresponding commercially available phosphoramidite. The HPLC-purified oligonucleotides were identified by MALDI-TOF mass spectrometry and quantified by their UV-visible absorption.

Using the Ptz-modified oligonucleotides ss**9**–ss**15**, we prepared the corresponding DNA duplexes **9**–**15** by slow cool-



ing together with 1.2 equivalents of the unmodified complementary strands. The synthesised Ptz-modified DNA duplexes **9**–**15** were subsequently characterised by their melting temperatures T_m (Table 1) and their UV-visible absorption spectra, which show clearly the presence of the Ptz chromophore as a broad shoulder between 300 nm and 400 nm (Figure 2).

As already mentioned, the focus in this manuscript was to study the distance and base-sequence dependence of the ET efficiency in Ptz-dU-modified DNA. Thus the sequences of

Table 1. Melting temperature (T_m) of the Ptz-modified DNA duplexes **9**–**15** (2.5 μM duplex, 10 mM Na-P_i-buffer, 250 mM NaCl, pH 7.0).

DNA	T_m [°C]	DNA	T_m [°C]
9	60	**10**	61
11	58	**12**	61
13	58	**14**	62
15	56		

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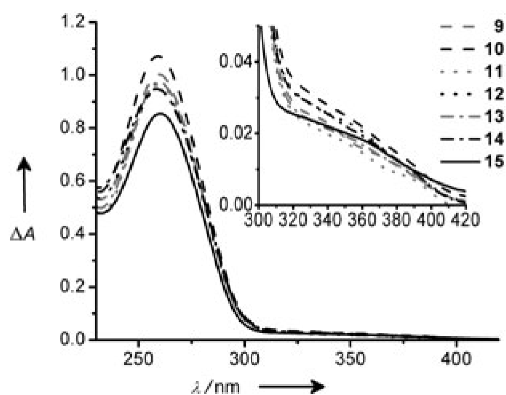

Figure 2. UV-visible absorbance spectra of the Ptz-modified DNA duplexes **9**–**15** (2.5 μM) in buffer (10 mM Na-P_i, 250 mM NaCl, pH 7).

duplexes **9**–**14** have been designed in such a way that the Br-dU group as the electron acceptor is placed either two, three or four base pairs away from the Br-dU group as the photoexcitable electron donor. With respect to $C^{\bullet-}$ and $T^{\bullet-}$ as the potential intermediate electron carriers during electron hopping, the intervening base pairs X–Y were chosen to be either T–A or C–G. If proton transfer interferes with electron transfer, we should observe significant differences in the ET efficiencies between DNA set **9**, **11** and **13** versus DNA set **10**, **12** and **14**. The DNA **15** was prepared as a control duplex lacking the Br-dU group as a chemically reactive electron trap.

Irradiation experiments with Ptz-dU-modified DNA duplexes: As mentioned previously, Br-dU undergoes a chemical modification after its one-electron reduction that can be analysed by piperidine-induced strand cleavage (Scheme 1).[11] Hence, the quantification of the strand cleavage yields important information about the ET efficiency.[10] Theoretical studies showed that the electron affinity of Br-dU is significantly higher than that of T.[25] However, based on reduction potentials, Br-dU seems to be not a significantly better electron acceptor.[26] In conclusion, Br-dU represents more characteristics of a kinetic than a thermodynamic electron trap.

The irradiation experiments of DNA duplexes **9**–**15** have been performed in such a way that after the start of the experiment, aliquots were collected every 5 min that were subsequently treated with piperidine at elevated temperature and finally analysed by HPLC. Each experiment had to be repeated at least three times. A 75 W Xe lamp with a cut-off filter (>305 nm) was used for these experiments in order to selectively photoexcite the Ptz chromophore and to avoid any degradation of the oligonucleotides by irradiation at smaller wavelengths. No strand cleavages were observed during the irradiation of DNA duplex **15**. This experiment represents an important control that any observed strand cleavage in the DNA duplexes **9**–**14** can be assigned to the presence of Br-dU as the electron acceptor. Indeed, strand degradation can be observed during the irradiation of all DNA duplexes **9**–**14**, but the efficiencies of the strand cleavage show significant differences (Figure 3). These duplexes can be divided into three groups: the DNA duplexes **9** and **11** show comparable and high cleavage efficiency, the DNA duplexes **13** and **10** are in the middle and the DNA duplexes **12** and **14** show the lowest cleavage efficiency. Interestingly, the DNA duplexes with the intervening T–A base pairs (**9**, **11** and **13**) show significantly higher cleavage efficiency relative to the DNA duplexes with the intervening C–G base pairs (**10**, **12** and **14**). It should be noted that the cleavage efficiency of DNA **13** is comparable to that of DNA **10**. Thus considering the fact that strand degradation represents the chemical result of the DNA-mediated ET process, it is remarkable that just one intervening C–G base pair exhibits a similar ET efficiency as three intervening T–A base pairs. It is evident that in our assay T–A base pairs transport an electron more efficiently than C–G base pairs.

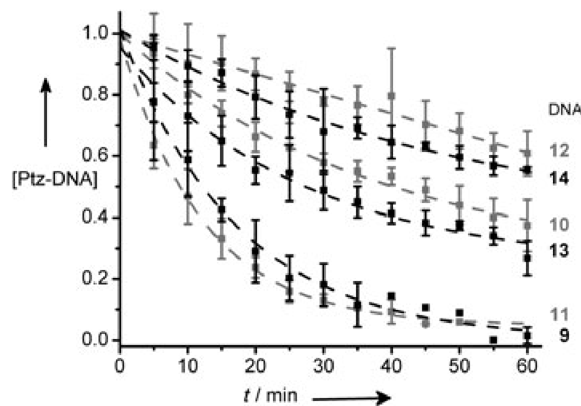

Figure 3. Analysis of the strand cleavage experiments with DNA **9**–**14** (4 μM) in buffer (10 mM Na-Pi, 250 mM NaCl, pH 7). Each experiment has been repeated at least three times.

Conclusion

The central motivation for this study was to elucidate the role of pyrimidine bases during excess-electron transfer through DNA. It was postulated that electron hopping in DNA involves all base pairs (T–A and C–G) meaning that both pyrimidine radical anions, $C^{\bullet-}$ and $T^{\bullet-}$, play the role of intermediate charge carriers,[6] although it was already known that both radical anions exhibit a significantly different basicity.[27] The DNA-mediated ET in the assay presented herein was initiated by photoexcitation of Ptz-dU as the electron donor and probed chemically with Br-dU as the electron acceptor. Remarkably, from these strand cleavage experiments it becomes clear that in our assay T–A base pairs transport electrons more efficiently than C–G base pairs (Scheme 3). This implies that $C^{\bullet-}$ is not likely to play a major role as an intermediate electron carrier.

This observation is supported by a number of recent publications:

1) As already mentioned in the introduction, Rokita applied aromatic amines as electron donors together with Br-dU as an electron trap und showed that the ET efficiency significantly depends on the intervening base se-

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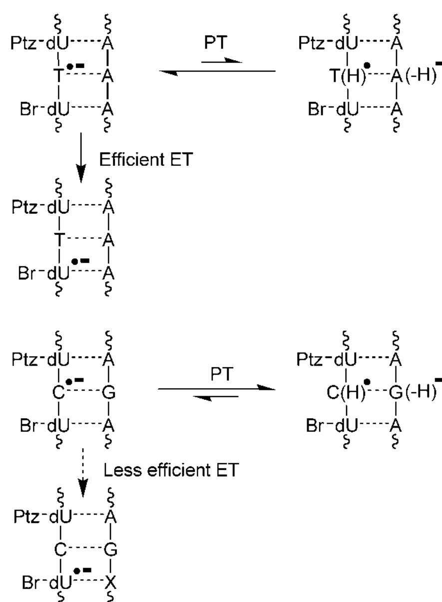

Scheme 3. Sequence dependence of the ET in Ptz-dU-modified DNA: The ET occurs more efficiently over T–A base pairs than over C–G base pairs, since proton-transfer (PT) interferes with ET.

quence.[10] The presence of C–G base pairs lowered the ET efficiency significantly.

2) Sevilla employed EPR spectroscopy and showed that proton transfer can slow down excess-electron transfer, but does not stop it.[28]

3) The result fits into the interpretation of our spectroscopic studies with Py-modified pyrimidine nucleosides as models for ET in DNA.[14] Therein, the nonprotonated radical anion of C could not be observed in aqueous solution suggesting that the protonation of $C^{\bullet-}$ by the complementary DNA base G or the surrounding water molecules will occur rapidly. Furthermore, we could show that such protonation of $C^{\bullet-}$ and deprotonation of $C(H)^{\bullet}$ can occur within several picoseconds. During this time the hydrogen-bond interface can readjust and stabilise the excess electron on the base by separating its spin from its charge. Although these processes must be microscopically reversible they may ultimately terminate electron migration in DNA due to the separation of spin and charge.

In summary, it is clear now that excess-electron transfer through hopping is highly sequence dependent and occurs faster and more efficiently over T–A base pairs than over C–G base pairs.

Experimental Section

Materials and methods: ^{1}H, ^{13}C and the 2D NMR spectra were recorded on a Bruker DMX500 spectrometer. NMR signals were assigned based on 2D NMR measurements (DQF-COSY, HSQC). ESI mass spectra were measured in the analytical facility of the institute on a Finnigan LCQ-ESI spectrometer. MALDI-TOF MS was performed in the analytical facility of the institute on a Bruker Biflex III spectrometer by using 3-hydroxypicolinic acid in aqueous 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 and protected from light. Chemicals were purchased from Fluka, Lancaster and Aldrich and were used without further purification. Spectroscopic measurements were performed in quartz glass cuvettes (1 cm) and with Na-P_i buffer (10 mM). Absorption spectra and the melting temperatures (2.5 μM duplex, 250 mM NaCl, 260 nm, 10–80 °C, interval 1 °C) were recorded on a Varian Cary 100 spectrometer.

3-Bromo-10-methyl-phenothiazine (3) and 10-methyl-3-(4,4,5,5-tetramethyl [1,3,2]dioxaborolan-2-yl)-phenothiazine (5): These compounds were synthesised according to procedures published by Ebdrup and Müller et al.[24] The analytical data were in agreement with the published values.

5-(10-Methyl-phenothiazin-3-yl)-2′-deoxyuridine (1): 5-Iodo-2′-deoxyuridine (**6**; 0.50 g, 1.41 mmol) was dissolved in THF/water (120 mL, 1:1). Subsequently, **5** (0.58 g, 1.69 mmol), PdCl$_2$(dppf) (1:1 complex with dichloromethane, 0.10 g, 0.14 mmol, 0.1 equiv), NaOH (1.13 g, 28.3 mmol, 20 equiv) and MeOH (50 mL) were added. The solution was saturated with nitrogen at RT (30 min), refluxed for 44 h, neutralised with HCl (1 M), filtered through silica and extracted with EtOAc (4×30 mL). The combined organic phase was dried over $MgSO_4$ 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) to give a yellow solid (34% yield). Analytical HPLC ensured a purity of >99.5%. R_f=0.69 (EtOAc/MeOH/water 12:2:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=2.18 (m, 2H; 2′-H), 3.31 (s, 3H; CH$_3$), 3.59 (m, 2H; 5′-H), 3.79 (m, 1H; 4′-H), 4.27 (m, 1H; 3′-H), 5.10 (br, 1H; 5-OH), 5.24 (br, 1H; 3-OH), 6.20 (t, 1H; 1′-H), 6.94 (m, 3H; Ptz-H), 7.14 (m, 1H; Ptz-H), 7.20 (m, 1H; Ptz-H), 7.36 (m, 2H; Ptz-H), 8.12 ppm (s, 1H; 5-H); ^{13}C NMR (125.8 MHz, [D$_6$]DMSO): δ=162.59 (4-C), 150.31 (2-C), 145.58, 144.82, 137.62 (6-C), 128.29, 127.93, 127.63, 127.29, 126.46, 123.00, 122.31, 122.15, 115.07, 114.69, 112.83 (5-C), 87.99 (4′-C), 84.95 (1′-C), 70.65 (3′-C), 61.45 (5′-C), 39.90 (2′-C), 34.90 ppm (CH$_3$); MS (ESI): m/z: 439 [M^+], 440 [M^++H], 879 [2M^++H]; HRMS (MALDI): m/z calcd for $C_{22}H_{21}N_3O_5S$: 439.11263 [M^++H$^+$]; found: 439.1196; UV/Vis (MeOH/H$_2$O 1:1, pH~6.5): ν_{max} (ε)=260 nm (53 200 M^{-1}cm^{-1}).

5′-*O*-(4,4′-Dimethoxytrityl)-5-(10-methylphenothiazin-3-yl)-2′-deoxyuridine (7): 4,4′-Dimethoxy-triphenylmethyl chloride (185 mg, 0.55 mmol) was added to a solution of **1** (120 mg, 0.27 mmol) in dry pyridine (10 mL). The mixture was stirred overnight at RT. Subsequently, MeOH (1 mL) was added. After 1 h at RT, the solution was 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 (52% yield). R_f=0.85 (ethyl acetate/methanol/water 12:2:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=2.22 (m, 1H; 2′-H), 2.36 (m, 1H; 2′-H), 3.12 (m, 1H; 5′-H), 3.18 (m, 1H; 5′-H), 3.25 (s, 3H; N-Me), 3.63 (s, 3H; O-Me), 3.65 (s, 3H; OMe), 3.94 (m, 1H; 4′-H), 4.28 (m, 1H; 3′-H), 5.33 (br, 1H; 3′-OH), 6.22 (dd, J=6.7 Hz, 1H; 1′-H), 6.60 (d, J=8.5 Hz, 1H; Ptz), 6.73 (m, 4H; DMT), 6.95 (m, 2H; Ptz), 7.06–7.11 (m, 3H; Ptz), 7.14–7.22 (m, 8H; Ptz, DMT), 7.31 (m, 2H; DMT), 7.63 (s, 1H; 5-H), 11.56 ppm (s, 1H; N-H); ^{13}C NMR (125.8 MHz, [D$_6$]DMSO): δ=162.10 (4-C), 157.98, 157.97, 149.82 (2-C), 145.08, 144.68, 144.39, 136.40 (6-C), 135.43, 135.21, 129.61, 127.74, 127.52, 127.43, 127.04, 126.74, 126.56, 126.28, 122.48, 121.91, 121.62, 114.51, 113.80, 113.07, 113.04, 85.78, 85.64 (4′-C), 84.79 (1′-C), 70.52 (3′-C), 63.84 (5′-C), 54.90 (O-CH$_3$), 54.88 (O-CH$_3$), 39.65 (2′-C), 34.90 ppm (N-CH$_3$); ESI-MS: m/z: 741 [M^+], 764 [M^++Na].

5′-*O*-(4,4′-Dimethoxy)trityl-5-(10-methylphenothiazin-3-yl)-2′-deoxyuridine-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (8): Compound **7** (150 mg, 0.2 mmol) was dissolved in dry CH_2Cl_2 (14 mL). Triethylamine (0.08 mL, 0.6 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.08 mL, 0.35 mmol) were added and the solution stirred for 1 h at RT. Ethanol (0.1 mL) was added and the mixture was

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poured into aqueous saturated $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated yielding the phosphoramidite **8** as a yellow solid (95%), which was used directly for the oligonucleotide synthesis. ESI-MS: m/z: 941 [M^+].

Preparation and characterisation of the oligonucleotides—general procedure: The oligonucleotides were prepared on a Expedite 8909 DNA synthesiser from Applied Biosystems (ABI) by standard phosphoramidite protocols with chemicals and CPG (1 mol) from ABI and Glen Research. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with concentrated 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) by 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 lyophilised and quantified by their absorbance at 260 nm[29] on a Varian Cary 100 spectrometer. Duplexes were formed by heating to 80°C (10 min), followed by slow cooling.

General procedure for the solid-phase synthesis of the phenothiazine-modified oligonucleotides 9–15: The syntheses were performed on a 1 mol scale (CPG 1000 Å, Glen Research) by using standard phosphoramidite protocols. Quantitative coupling of the building block **8** was achieved by using the minimal coupling time of 1.6 min. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with concentrated NH_4OH at RT for 36 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 = 0–30 B over 45 min. The oligonucleotides were lyophilised, quantified by their absorbance at 260 nm[29] and using $\varepsilon = 53200$ (260 nm) for **1**. MS (MALDI): m/z calcd for ss**9**: 5700 [M^+]; found: 5711; m/z calcd for ss**10**: 5685 [M^+]; found: 5704; m/z calcd for ss**11** 5700 [M^+]; found: 5702; m/z calcd for ss**12**: 5670 [M^+]; found: 5675; m/z calcd for ss**13** 5700 [M^+]; found: 5705; m/z calcd for ss**14** 5655 [M^+]; found: 5663; m/z calcd for ss**15**: 5346 [M^+]; found: 5345.

Strand cleaving experiments: Duplexes (4 μM DNA, 10 mM Na-P_i buffer, 250 mM NaCl) were prepared by heating equimolar solutions of the single strands to 80°C for 10 min in the dark and subsequent slow cooling. The measurements were performed in quartz glass cuvettes (1 cm). The freshly prepared duplexes were irradiated by an Xe lamp (75 W Xe lamp, Oriel Instruments) and a cut-off filter (Andover Corporation, >305 nm). Every 5 min aliquots (30 μL) of the sample solution (1000 μL) were taken. Piperidine (3 μL) was added and the samples were heated to 90°C (30 min), lyophilised, dissolved in water (15 μL) and analysed by HPLC (RP-C18, Supelco) under the following conditions: A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient = 0–30% B over 45 min. The obtained peaks were processed to give a ratio between unmodified and modified ssDNA.

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