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

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Supporting Information

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Materials and Methods. Chemicals were purchased from Aldrich, Alfa Aesar and Merck. Diisopropylethylamine was dried over KOH, distilled and stored over molecular sieve (4 Å) under argon. Solvents were distilled prior to use. T.l.c. was performed on Fluka silica gel 60 F_{254} coated aluminum foil. Flash chromatography was carried out with Silica Gel 60 from Aldrich (60-43 μm). Spectroscopic measurements were recorded in Na-P_i- buffer solution (10 mM) using quartz glass cuvettes (10 mm). ESI mass spectra were measured on a Finnigan LCQ or a Finnigan TSQ7000 (for ss-**DNA1**) in negative mode. MALDI-TOF MS of ss-**DNA2** were measured on a Bruker Biflex III spectrometer using 3-hydroxypicolinic acid:diammonium citrate=9:1, 50 mg/mL in MeCN:H_2O=1:1 as a matrix. NMR spectra were recorded on a Bruker AC250 and DMX500 spectrometer in deuterated solvents. Chemical shifts are given in ppm relative to TMS. Absorption spectra and the melting temperatures (2.5 μM DNA, 10-90 °C, 0.7 °C/min, step width 0.5 °C) were recorded on a Varian Cary 100 spectrometer equipped with a 6x6 cell changer unit. Fluorescence was measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation and emission bandpass of 5 nm and are corrected for Raman emission from the buffer solution. The CD spectroscopy (2.5 μM duplex, 185-400 nm) was performed on a Jasco J-715 spectropolarimeter. Cyclic voltammetry was performed using a three-electrode system. The potential was controlled by a EG&G 683 potentiostat. Spectroelectrochemistry was performed on a Perkin-Elmer Lambda 9 spectrometer that was equipped with a electrochemical cell consisting of working electrode (gold-plated nickel grid, 600 lines/cm), counter electrode (V2A steel, nickel-plated, gold-plated) and a pseudo reference electrode (silver-coated platin), see: J. Salbeck, *Anal. Chem*., 1993, **65**, 2169. The potential was controlled by a AMEL 2053 potentiostat.

Synthesis of compound 3. 150 mg (0.47 mmol. 1 equiv) **1** and 370 mg (0.94 mmol, 2 equiv) **2** were dissolved in 8 mL dry DMF. 0.32 mL (1.88 mmol, 4 equiv) Diispropylethylamine were added. The solution was stirred at r.t. for 10 d. The solvent was

evaporated and the crude product was purified by flash chromatography (EtOAC + 1 % pyridine, then CH_2Cl_2/MeOH 10:3 + 1 % pyridine). 161 mg (0.25 mmol) of a pale yellow solid were yielded (54 %). Tlc: R_f~0.86 (CH_2Cl_2:MeOH=10:3). MS (ESI): m/z= 633 (20) [M^+], 303 (100) [DMT^+]. HRMS (ESI-FTICR): $C_{39}H_{41}N_2O_4S$ [$M+H^+$] calc. 633.27815, found. 633.27814. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 1.76 (m, 2H, C$\underline{H}_2$), 2.52 (m, 2H, PzN-C$\underline{H}_2$), 2.88 (m, 2H, OC$\underline{H}_2$-CHOH), 3.55 (m, 2H, NC$\underline{H}_2$), 3.67 (m, 1H, C$\underline{H}$OH), 3.70 (s, 3H, O-Me), 3.72 (s, 3H, O-Me), 3.86 (m, 2H, N-C$\underline{H}_2$-CHOH), 4.66 (br, 1H, O$\underline{H}$), 6.70-6.79 (m, 4H, Ph-OMe-3,5), 6.80 (m, 2H, Pz), 6.86 (m, 2H, Pz), 6.99 (m, 1H, Ph-4), 7.02 (m, 2H, Pz), 7.07 (m, 2H, Pz), 7.12 (m, 4H, Ph-OMe-2,6), 7.18 (m, 2H, Ph-3,5), 7.27 (m, 2H, Ph-2,6). ^{13}C-NMR (125.8 MHz, DMSO-d_6): δ= 27.46 ($\underline{C}H_2$), 45.61 (N-$\underline{C}H_2$-CHOH), 53.52 (PzN-$\underline{C}H_2$), 55.86 (O-Me), 67.06 (O$\underline{C}H_2$-CHOH), 67.24 (N$\underline{C}H_2$), 69.77 ($\underline{C}$HOH), 86.26 (DMT), 113.62 (Ph-OMe-3,5), 115.82, 116.16, 116.30, 123.02, 127.22 (tz), 127.75 (Ph-4), 128.27 (Pz), 128.30 (Ph-3,5), 128.34 (Ph-2,6), 130.58 (Ph-OMe-2,6), 136.11 (Ph-OMe-1), 141.89 (Pz), 145.47 (Ph-1), 159.64 (Ph-OMe-4).

Synthesis of compound 4. 30 mg (0.05 mmol) **3** were dissolved in 5 mL CH_2Cl_2 and 1 mL $Cl_2CHCOOH$. After 30 min of stirring at r.t., the reaction was neutralized with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The solvent was evaporated and the crude product was purified by flash chromatography (EtOAc:MeOH=10:1, then MeOH). 15 mg (0.05 mmol) of a white solid were yielded (95 %). MS (ESI): m/z=330 (100) [M^+]. ^{1}H-NMR (250 MHz, $CDCl_3$): δ= 1.73 (m, 2H, C$\underline{H}_2$), 2.55 (m, 2H, PzN-C$\underline{H}_2$), 2.80 (m, 2H, OC$\underline{H}_2$-CHOH), 3.30 (m, 2H, NC$\underline{H}_2$), 3.75 (m, 1H, C$\underline{H}$OH), 3.81 (m, 2H, N-C$\underline{H}_2$-CHOH), 6.81-6.87 (m, 4H, Pz), 7.09-7.17 (m, 4H, Pz).

Synthesis of compound 5. 298 mg (0.47 mmol) **3** were dissolved in 10 mL CH_2Cl_2. 0.23 mL (2.8 mmol, 6 equiv) pyridine and 59.6 μL (0.47 mmol, 1 equiv) Me_3SiCl were added. The solution was stirred at 0 °C for 3 h. 130.9 μL (0.94 mmol, 2 equiv) Trifluoroacetic acid anhydride were added. The solution was stirred at 0 °C for 20 min and at r.t. for another

10 min, washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. Subsequently, 1 mL (1 mmol, 2 equiv) 1 M TBAF in THF were added. The solution was stirred at r.t. for 30 min, washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. 325 mg (0.45 mmol) of a pale yellow oil were yielded (95 %). Tlc: R_f ~0.79 (CH_2Cl_2:MeOH 20:3). MS(ESI): m/z=729 (20) [$M+H^+$], 303 (100) [DMT^+]. ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 1.74 (m, 2H, C$\underline{H}_2$), 2.59 (m, 2H, PzN-C$\underline{H}_2$), 2.86 (m, 2H, OC$\underline{H}_2$-CHOH), 3.50 (m, 2H, NC$\underline{H}_2$), 3.71 (m, 1H, C$\underline{H}$OH), 3.73 (s, 3H, O-Me), 3.74 (s, 3H, O-Me), 3.81 (m, 2H, N-C$\underline{H}_2$-CHOH), 4.74 (br, 1H, O$\underline{H}$), 6.70-6.80 (m, 6H, Ph-OMe-3,5, Ptz), 6.89 (m, 2H, Pz), 6.99-7.12 (m, 9H), 7.20 (m, 2H, Ph-3,5), 7.29 (m, 2H, Ph-2,6).

Synthesis of DNA building block 6. Under nitrogen 168 mg (0.23 mmol, 1 equiv) **5** were dissolved in 10 mL dry CH_2Cl_2. 177 µL (1.04 mmol, 4.5 equiv) dry diisopropylethylamine and 92 µL (0.42 mmol, 1.8 equiv) 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidit were added. The mixture was stirred for 45 min. The mixture was quenched with 0.1 mL EtOH (abs.), washed by freshly prepared sat. aq. $NaHCO_3$, dried over Na_2SO_4, and evaporated to dryness. 203 mg (0.22 mmol) of a pale yellow oil were yielded (95 %). Due to the instability of the trifluoroacetyl and the phosphoramidite groups, **6** was only characterized by MS and directly used for the preparation of oligonucleotides. Tlc: R_f ~0.68 (CH_2Cl_2:MeOH 20:3). MS(ESI): 928 (100) [M^+], 303 (70) [DMT^+].

Preparation of DNA. Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry. Reagents and CPG (1 µmol) were purchased from ABI. The synthesis of Pz-modified oligonucleotides was performed using a modified protocol with an extended coupling time of 15 min. After preparation, the trityl-off oligonucleotide was cleaved from the solid support and deprotected by treatment with conc. NH_4OH at 55 °C for 10 hours. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 A, Supelco)

using the following conditions: A= NH_4OAc buffer (50 mM), pH = 6.5; B= acetonitrile, gradient 0- 30 % B over 45 min., flow rate 2.5 mL/min, UV/Vis detection at 290 and 315 nm. The oligonuclotides were lyophilized and quantified by their absorbance at 260 nm on a Varian Cary 100 spectrometer, see: J. D. Puglisi, I. Tinoco, *Meth. Enzymol*,. 1989, **180**, 304, and ε_{260}=7.200 $M^{-1}cm^{-1}$ for Pz and ε_{260}=53.200 $M^{-1}cm^{-1}$ for Pz-dU. The Pz-dU building block and the synthesis of the corresponding modified oligonucleotide was performed according to the literature, see: C. Wagner, H.-A. Wagenknecht, *Chem. Eur. J.*, 2005, **11**, 1871. Duplexes were formed by heating of the modified oligonucleotides to 90 °C (15 min) in the presence of 1.0 equiv. of the corresponding complementary unmodified oligonucleotide strand, followed by slow cooling to r. t. UV/Vis: Pz-modified strand ss-**DNA1**: ε_{260}=157.500 $M^{-1}cm^{-1}$; : Pz-dU-modified strand ss-**DNA2**: ε_{260}=203.500 $M^{-1}cm^{-1}$; counterstrands: ss-**DNAa**: ε_{260}=184.00 $M^{-1}cm^{-1}$; ss-**DNAb**: ε_{260}=176.600 $M^{-1}cm^{-1}$; ss-**DNAc**: ε_{260}=180.600 $M^{-1}cm^{-1}$; ss-**DNAd**: ε_{260}=178.000 $M^{-1}cm^{-1}$; ss-**DNAe**: ε_{260}=170.100 $M^{-1}cm^{-1}$. MS (ESI): Pz-modified strand ss-**DNA1**: calc. 5221, found m/z = 1304.9 [M^{4-}]. MS (MALDI-TOF): Pz-dU-modified strand ss-**DNA2**: calc. 5330, found m/z = 5324.

Figure S1

Normalized UV/Vis absorption and fluorescence spectra of Me-Pz (0.1 M in MeOH), Pz-dU (36 μM in H_2O:MeOH=1:1) and **4** (0.1 M in MeOH), λ_{exc}=315 nm.

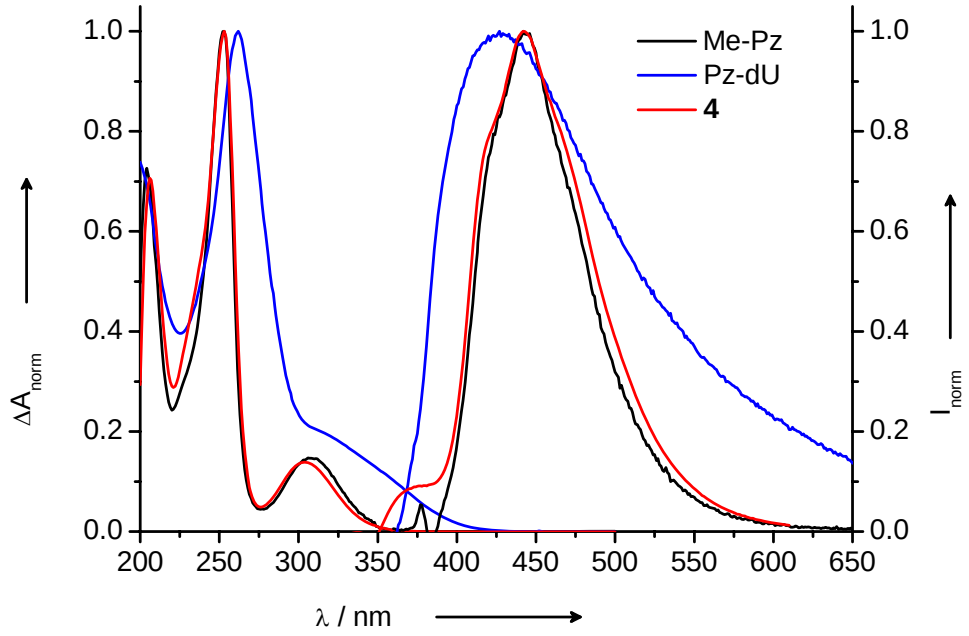

Figure S2

UV/Vis absorption spectra of **DNA1a** (2.5 μM) and **DNA2a** (2.5 μM) in 10 mM Na-P_i buffer, pH 7.

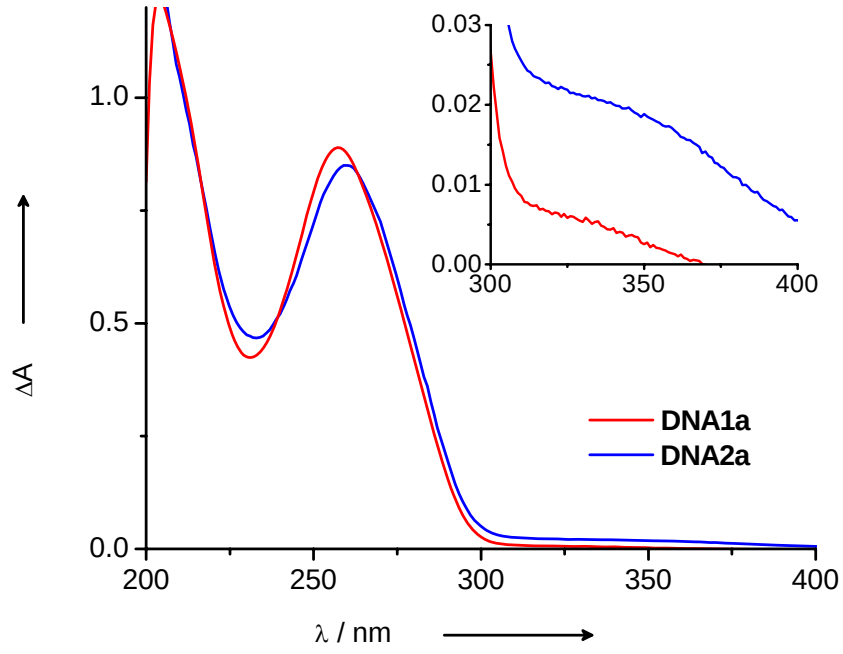

Figure S3

Fluorescence spectra of **DNA1a** (12.5 μM, λ_{exc}=315 nm) and **DNA2a** (12.5 μM, λ_{exc}=340 nm) in 10 mM Na-P_i buffer, pH 7.

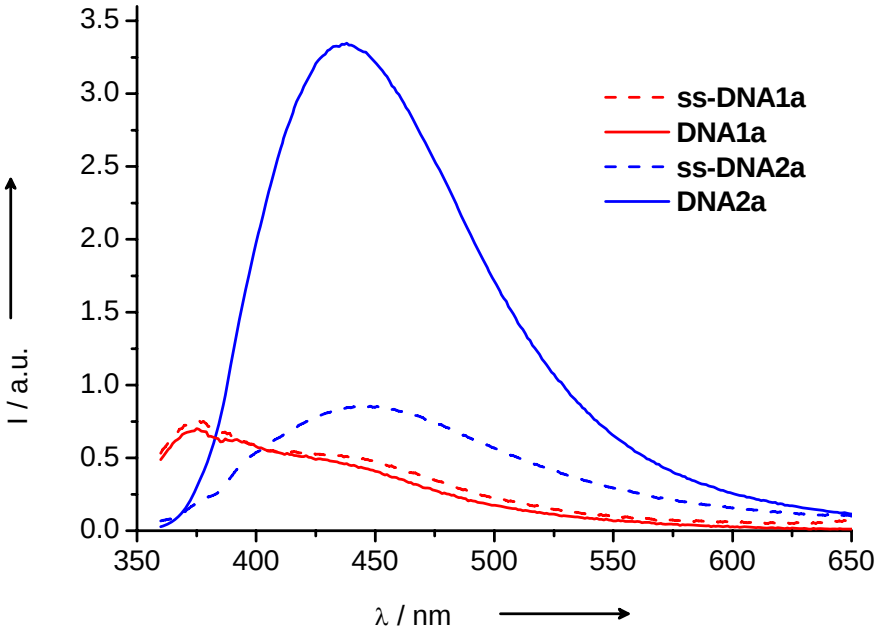

Figure S4

CD spectra of **DNA1a** (2.5 μM) and **DNA2a-DNA2e** (2.5 μM) in 10 mM Na-P_i buffer, pH 7, 250 mM NaCl