Electronic Supporting Information for

Phenothiazine-linked Nucleosides and Nucleotides for Redox Labelling of DNA

Anna Simonova, a Luděk Havran, b Radek Pohl, a Miroslav Fojta, *b,c Michal Hocek *a,d

a Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo namesti 2, CZ-16610 Prague 6, Czech Republic

b Institute of Biophysics, Czech Academy of Sciences, Kralovopolska 135, 612 65 Brno, Czech Republic

c Central European Institute of Technology, Masaryk University, Kamenice 753/5, CZ-625 00 Brno, Czech Republic

d Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, Prague-2 12843, Czech Republic

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1. Kinetics of incorporation of modified dNTPs

\[ 5\text{'-CATGGGCGGCATGGG-3'} \rightarrow 5\text{'-CATGGGCGGCATGGGA-3'} \]

**Pr** | **A+** | **A^PT**
---|---|---
0.1 0.5 1 2 5 | 0.1 0.5 1 2 5 | 0.1 0.5 1 2 5

**Figure S1** PAGE analyses of kinetic single nucleotide extension experiments with temp^{tempA} using KOD XL DNA polymerases dA^{PT}TP (dA^{PT}) in comparison with natural dATP (+). Time intervals are given in minutes.

\[ 5\text{'-CATGGGCGGCATGGG-3'} \rightarrow 5\text{'-CATGGGCGGCATGGGC-3'} \]

**Pr** | **C+** | **C^PT**
---|---|---
0.1 0.5 1 2 5 | 0.1 0.5 1 2 5 | 0.1 0.5 1 2 5

**Figure S2** PAGE analyses of kinetic single nucleotide extension experiments with temp^{tempA} using KOD XL DNA polymerases dA^{EPT}TP (dA^{EPT}) in comparison with natural dATP (+). Time intervals are given in minutes.

\[ 5\text{'-CATGGGCGGCATGGG-3'} \rightarrow 5\text{'-CATGGGCGGCATGGGC-3'} \]

**Pr** | **C+** | **C^PT**
---|---|---
0.1 0.5 1 2 5 | 0.1 0.5 1 2 5 | 0.1 0.5 1 2 5

**Figure S3** PAGE analyses of kinetic single nucleotide extension experiments with temp^{tempC} using KOD XL DNA polymerases dC^{PT}TP (dC^{PT}) in comparison with natural dCTP (+). Time intervals are given in minutes.
Figure S4  PAGE analyses of kinetic single nucleotide extension experiments with tempC using KOD XL DNA polymerases dC\textsuperscript{EPT}TP (dC\textsuperscript{EPT}) in comparison with natural dATP (+). Time intervals are given in minutes.

2. NEAR

Figure S5  Incorporation of modified dNTPs in NEAR. L: DNA ladder; A\textsuperscript{PT}: product of NEAR with dA\textsuperscript{PT}TP, dCTP, dGTP, dTTP; A\textsuperscript{EPT}: product of NEAR with dA\textsuperscript{EPT}TP, dCTP, dGTP, dTTP; C\textsuperscript{PT}: product of NEAR with dC\textsuperscript{PT}TP, dATP, dGTP, dTTP; C\textsuperscript{EPT}: product of NEAR with dC\textsuperscript{EPT}TP, dATP, dGTP, dTTP

3. PCR

Figure S6  PCR synthesis of 98-mer by KOD XL polymerase L: DNA ladder; +: product of PCR with natural dNTPs; A-: product of PCR with dCTP, dGTP, dTTP; C-: product of PCR with dATP, dGTP, dTTP; A\textsuperscript{PT}: product of PCR with dA\textsuperscript{PT}TP, dCTP, dGTP, dTTP; A\textsuperscript{EPT}: product of PCR with dA\textsuperscript{EPT}TP, dCTP, dGTP, dTTP; C\textsuperscript{PT}: product of PCR with dC\textsuperscript{PT}TP, dATP, dGTP, dTTP; C\textsuperscript{EPT}: product of PCR with dC\textsuperscript{EPT}TP, dATP, dGTP, dTTP.
**Figure S7** PCR synthesis of 98-mer by KOD XL polymerase. L: DNA ladder; +: product of PCR with natural dNTPs; A-: product of PCR with dCTP, dGTP, dTTP; C-: product of PCR with dATP, dGTP, dTTP; A<sup>PT</sup>: product of PCR with dA<sup>PT</sup>TP, dCTP, dGTP, dTTP; A<sup>EPT</sup>: product of PCR with dA<sup>EPT</sup>TP, dCTP, dGTP, dTTP. The percentage corresponds to the proportion of modified triphosphates (dA<sup>PT</sup>TP or dA<sup>EPT</sup>TP) in combination with natural dATP in the PCR reaction mixture.

**Figure S8** PCR synthesis of 98-mer by KOD XL polymerase with FAM-labeled primers. L: DNA ladder; +: product of PCR with natural dNTPs; A-: product of PCR with dCTP, dGTP, dTTP; C-: product of PCR with dATP, dGTP, dTTP; A<sup>PT</sup>: product of PCR with dA<sup>PT</sup>TP, dCTP, dGTP, dTTP; A<sup>EPT</sup>: product of PCR with dA<sup>EPT</sup>TP, dCTP, dGTP, dTTP. The percentage corresponds to the proportion of modified triphosphates (dA<sup>PT</sup>TP or dA<sup>EPT</sup>TP) in combination with natural dATP in the PCR reaction mixture.
4. TdT (Full gels)

**Figure S9** TdT-catalyzed DNA chain elongation. Pr: primer; S-standard (PEX product of temp<sup>termA</sup> with dATP); A+, A<sup>PT</sup> and A<sup>EPT</sup>: products of primer<sup>mpl</sup> elongation using terminal transferase and either dATP, dA<sup>PT</sup>TP or dA<sup>EPT</sup>TP respectively. Time intervals are given in hours.
Figure S10 TdT-catalyzed DNA chain elongation. Pr: primer; S-standard (PEX product of temp\textsuperscript{termC} with dCTP); C+, C\textsuperscript{PT} and C\textsuperscript{EPT}: products of primer\textsuperscript{rd} elongation using terminal transferase and either dCTP, dC\textsuperscript{PT}TP or dC\textsuperscript{EPT}TP respectively. Time intervals are given in hours.
5. Fluorescence

**Determination of extinction coefficients**

Extinction coefficients were measured using 1 ml quartz cuvettes on a Cary 100 UV-VIS spectrometer (Agilent Technologies). The absorption coefficients were calculated according to the following The Beer-Lambert Law equation

\[ A = C \cdot l \cdot \varepsilon \]

where \( \varepsilon \) is the extinction coefficient, \( C \) is the exact concentration of the sample in the cuvette, \( l \) is the length of the path that the light travels through the cuvette and \( A \) is the absorbance of the sample. Measurements were triplicated.

**Determination of fluorescence quantum yields**

Fluorescence spectra were measured on a Fluoromax 4 spectrofluorimeter equipped with a thermostated cuvette holder at 25 °C. (HORIBA Scientific). The excitation wavelength was 350 nm and the recorded spectral range was 370 – 680 nm. Relative determination of the fluorescence quantum yields was performed using quinine sulfate in 0.5 M H_2SO_4 (\( \Phi_f = 0.546 \) at 25 °C) as a standard.\(^{S1}\) The absorbance of sample solutions at the excitation wavelength were kept below 0.08 to avoid inner filter effect. The quantum yields were calculated using following equation\(^{S2}\)

\[
\Phi_{f,x} = \Phi_{f,st} \frac{F_x}{F_{st}} \frac{1 - 10^{-Abs_{st}} n_x^2}{1 - 10^{-Abs_x} n_{st}^2}
\]

where \( \Phi_f \) is the quantum yield, \( F \) is the integrated fluorescence intensity, \( Abs \) is the absorbance of solution at the excitation wavelength, \( n \) is the refractive index of the solvent. The subscripts \( x \) and \( st \) stand for the sample and standard, respectively. Measurements were triplicated.

![Normalized absorption (dashed lines) and fluorescence (solid lines) of compounds dA\(^{EPT}\) and dC\(^{EPT}\) in EtOH](image)

**Figure S11** Normalized absorption (dashed lines) and fluorescence (solid lines) of compounds dA\(^{EPT}\) and dC\(^{EPT}\) in EtOH
Figure S12 Fluorescence spectra of modified dA^{EPT} and dC^{EPT} in EtOH; dA^{EPT}TP and dC^{EPT}TP in aqueous solutions

Figure S13 fluorescence spectra (λex=486 nm) of ON\textsuperscript{rnd16} A^{EPT} obtained after incubation of PEX reaction mixtures containing dA^{EPT}TP, dCTP, dTTP, dGTP, primer\textsuperscript{rnd} and biotinylated temp\textsuperscript{rnd16} either with (red line) or without (black line) KOD XL DNA polymerase

The reaction mixture (100 μL) contained biotinylated template\textsuperscript{rnd16} (100 μM, 3.2 μL), primer\textsuperscript{rnd} (100 μM, 3.2 μL), dNTPs (4 mM, 5.2 μL), KOD XL polymerase (1.25 U KOD XL; in the case of negative control Milli-Q water was added instead) in enzyme reaction buffer (5 μL) supplied by the manufacturer. The reaction mixture was incubated for 1h at 60 °C in a thermal cycler. The reaction was stopped by cooling to 4°C. DNA from solutions was isolated using Streptavidin Magnetic Particles (Roche, 100 μL) and purified by using QIAquick Nucleotide Removal Kit (QIAGEN). The difference between two samples indicates that dA^{EPT}TP is accepted as a substrate by DNA polymerase and does not bind unspecifically to DNA.
6. MALDI-TOF spectra

a) *prim*<sup>nd</sup> 3′-GGGTACGGCGGTAC-5′

temp<sup>λ</sup> 5′-CCCTCCCATGCGGCCCATG-3′

**Figure S14** MALDI-TOF spectrum of ON<sup>A</sup> A<sup>EPT</sup>. Calculated: 6185.2 Da; found: 6186.5 Da; Δ = 1.30

**Figure S15** MALDI-TOF spectrum of ON<sup>A</sup> A<sup>EPT</sup>. Calculated: 6209.2 Da; found: 6210.6 Da; Δ = 1.40
b) prim\textsuperscript{ind} 3'-GGTACGCGGGTAC-5'
\text{temp}\textsuperscript{C} 5'-CCCGCCCATGCCCCCAG-3'

\textbf{Figure S16} MALDI-TOF spectrum of ON\textsuperscript{C} C\textsuperscript{PT}. Calculated: 6162.2 Da; found: 6163.5 Da; \( \Delta = 1.30 \)

\textbf{Figure S17} MALDI-TOF spectrum of ON\textsuperscript{C} C\textsuperscript{EPT}. Calculated: 6186.2 Da; found: 6187.5 Da; \( \Delta = 1.30 \)
c) $\text{prim}^{\text{rnd}}$ 3'-GGGTACGGCGGGTAC-5’

$\text{temp}^{\text{rnd}}$ 5’-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3’

**Figure S18** MALDI-TOF spectrum of ON$^{\text{rnd}16}$ A$^{\text{PT}}$. Calculated: 10458.46 Da; found: 10459.00 Da; $\Delta = 0.54$

**Figure S19** MALDI-TOF spectrum of ON$^{\text{rnd}16}$ A$^{\text{EPT}}$. Calculated: 10554.54 Da; found: 10555.3 Da; $\Delta = 0.76$
Figure S20 MALDI-TOF spectrum of ON\textsuperscript{rnd16} \textsuperscript{C\textsubscript{PT}}. Calculated: 10462.46 Da; found: 10463.8 Da; $\Delta = 1.34$

Figure S21 MALDI-TOF spectrum of ON\textsuperscript{rnd16} \textsuperscript{C\textsubscript{EPT}}. Calculated: 10558.54 Da; found: 10559.3 Da; $\Delta = 0.76$
Figure S22 MALDI-TOF spectrum of ON\textsuperscript{rnd16} A\textsuperscript{PT}C\textsuperscript{EBF}. Calculated: 11031.10 Da; found: 11032.90 Da; Δ = 1.8

Figure S23 MALDI-TOF spectrum of ON\textsuperscript{rnd16} A\textsuperscript{PT}U\textsuperscript{NO2}. Calculated: 10886.94 Da; found: 10887.3 Da; Δ = 0.36

* Found mass 10871.7 Da corresponds to the absence of one N-Me group from phenothiazine label in the sample [M-CH\textsubscript{3}]\textsuperscript{+}
Nick_1A 5'-P-GTCGTGAGTG-3'

Figure S24 MALDI-TOF spectrum of ON^{Nick, A}\textsuperscript{PT}. Calculated: 3388.29 Da; found: 3389.4 Da; Δ = 1.11

Figure S25 MALDI-TOF spectrum of ON^{Nick, A}\textsuperscript{EPT}. Calculated: 3412.31 Da; found: 3413.4 Da; Δ = 1.09
**Nick_10mer_1C**  5′-P-GT\text{CATGAGTG}-3′

**Figure S26** MALDI-TOF spectrum of ON\textsuperscript{Nick,1C} \textsuperscript{CPT}. Calculated: 3373.69 Da; found: 3374.5 Da; \( \Delta = 0.81 \)

**Figure S27** MALDI-TOF spectrum of ON\textsuperscript{Nick,1C} \textsuperscript{EPT}. Calculated: 3397.41 Da; found: 3398.4 Da; \( \Delta = 0.99 \)
5′-P-TTCATGACTG-3′

Figure S28 MALDI-TOF spectrum of ON\textsuperscript{Nick}_{24} A\textsuperscript{PT}. Calculated: 3517.58 Da; found: 3519.3 Da; \( \Delta = 1.72 \)

Figure S29 MALDI-TOF spectrum of ON\textsuperscript{Nick}_{24} E\textsuperscript{PT}. Calculated: 3565.62 Da; found: 3566.8 Da; \( \Delta = 1.18 \)
5′-P-TT\text{CATGA\text{CTG}}-3′

Figure S30 MALDI-TOF spectrum of ON^{\text{Nick}_2C\text{C}^\text{pt}}. Calculated: 3519.58 Da; found: 3520.4 Da; $\Delta = 1.34$

Figure S31 MALDI-TOF spectrum of ON^{\text{Nick}_2C\text{C}^\text{EPT}}. Calculated: 3567.62 Da; found: 3568.7 Da; $\Delta = 1.34$
5′-P-TAGCAGTCGTCGAG-3′

**Figure S32** MALDI-TOF spectrum of \(\text{ON}^{\text{Nick,44}} \text{A}^{\text{PT}}\). Calculated: 5801.36 Da; found: 5802.8 Da; \(\Delta = 1.44\)

**Figure S33** MALDI-TOF spectrum of \(\text{ON}^{\text{Nick,44}} \text{A}^{\text{EPT}}\). Calculated: 5897.44 Da; found: 5898.7 Da; \(\Delta = 1.26\)
5'-P-TAGCATGCTACGTCA-3

**Figure S34** MALDI-TOF spectrum of ON_{Nick,AC}C_{EPT}. Calculated: 5805.36 Da; found: 5806.6 Da; Δ = 1.24

**Figure S35** MALDI-TOF spectrum of ON_{Nick,AC}C_{EPT}. Calculated: 5901.44 Da; found: 5902.5 Da; Δ = 1.06
7. Copies of NMR spectra

$^1$H NMR and $^{13}$C spectra of dC$^\text{PT}$. 
$^1$H NMR and $^{13}$C spectra of dAPT.
$\text{H NMR and }^{13}\text{C spectra of } \text{dC}^{\text{EPT}}.$
$^1$H NMR and $^{13}$C spectra of dA$^{EPT}$. 
$^1$H NMR, $^{13}$C and $^{31}$P spectra of dCPTTP.
$^1$H NMR, $^{13}$C and $^{31}$P spectra of dA<sup>PT</sup>TP.
$^1$H NMR, $^{13}$C and $^{31}$P spectra of dC$^{\text{EPT}}$TP.
$^1$H NMR, $^{13}$C and $^{31}$P spectra of dA^{EPT}TP.
8. Additional electrochemistry data

Figure S36 CV responses of dA<sub>EPT</sub> (a), dC<sub>EPT</sub> (b) at PGE. C = 40 µM, electrolyte 0.2 M acetate buffer (pH 5.0). CV parameters: scan rate 1V/s, Ei = 0.0 V, Esw see legend in the Figure.

(Supplement to Fig. 6 that shows CVs of PT and dN<sub>PT</sub> conjugates)
Figure S37 Components of the SWV current of phenothiazine (a), dA^{PT} (b), dC^{PT} (c), dA^{EPT} (d), dC^{EPT} (e) at PGE. C = 40 μM, electrolyte 0.2 M acetate buffer (pH 5.0). parameters: frequency 200 Hz, amplitude 50 mV, Ei = 0.0 V. net current (black), forward current (red), backward current (blue).

(Supplement to Fig. 7 to demonstrate reversibility of SWV peak PT^{ox1} and irreversibility of the more positive signals. See the counter peak on blue curves representing the backward current component).
Table S1 SWV peak potentials of PT, PT-modified nucleosides and ONs

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<tr>
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<th>PT_{ox1}/mV</th>
<th>PT_{ox2}/mV</th>
<th>PT_{ox3}/mV</th>
<th>dC/mV</th>
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<td>PT</td>
<td>625</td>
<td>920</td>
<td>1290</td>
<td>-</td>
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<tr>
<td>dA_{PT}</td>
<td>655*</td>
<td>920</td>
<td>1355</td>
<td>-</td>
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<tr>
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<td>1360</td>
<td>-</td>
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<td>1160</td>
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<tr>
<td>dC_{EPT}</td>
<td>640/720#</td>
<td>880/985</td>
<td>1330</td>
<td>1160</td>
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<th>PT_{ox1}/mV</th>
<th>PT_{ox2}/mV</th>
<th>PT_{ox3}/mV</th>
<th>PT_{ox3}/G(A^*){ox}/mV</th>
<th>G(A^*){ox}/mV</th>
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<td>ON^{nick}<em>{4A} A</em>{PT}</td>
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<td>790</td>
<td>1285</td>
<td>-</td>
<td>1060</td>
<td>-</td>
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<td>1320</td>
<td>995</td>
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<tr>
<td>ON^{nick}_{natural}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1080</td>
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<th>PT_{ox1}/mV</th>
<th>PT_{ox2}/mV</th>
<th>G{ox}/mV</th>
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<td>ON^{rnd16}_{natural}</td>
<td>-</td>
<td>-</td>
<td>1080</td>
<td>1360</td>
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All potentials are given against Ag|AgCl|3M KCl reference electrode; conditions of measurements as in Fig. 6 and Fig. 7.

* the major SWV peak

#range of potentials spanning the double-peak envelope, see Fig. 7

9. References
