Electronic Supporting Information for

Phenothiazine-linked Nucleosides and Nucleotides for Redox Labelling of DNA

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Contents:

1. Kinetics of incorporation of modified dNTPs

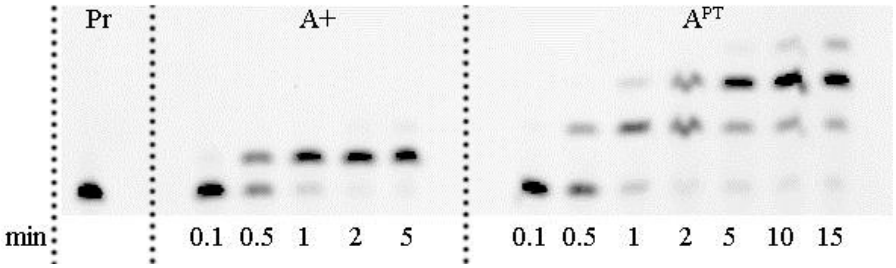


Figure S1 PAGE analyses of kinetic single nucleotide extension experiments with temp^{tempA} using KOD XL DNA polymerases **dA^{PT}TP** (dA^{PT}) in comparison with natural dATP (+). Time intervals are given in minutes.

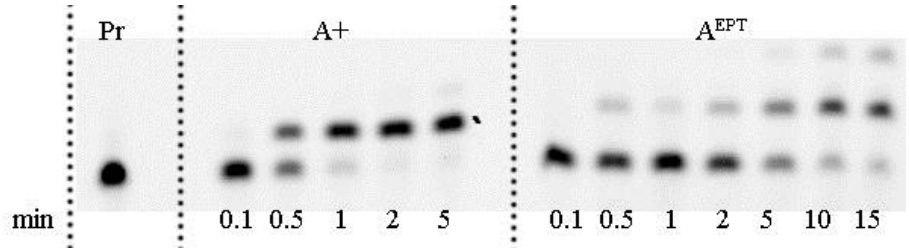


Figure S2 PAGE analyses of kinetic single nucleotide extension experiments with temp^{tempA} using KOD XL DNA polymerases **dA^{EPT}TP** (dA^{EPT}) in comparison with natural dATP (+). Time intervals are given in minutes.

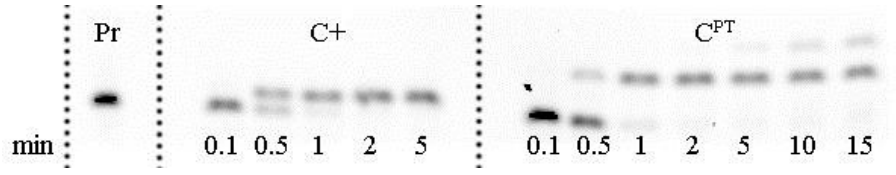


Figure S3 PAGE analyses of kinetic single nucleotide extension experiments with temp^{tempC} using KOD XL DNA polymerases **dC^{PT}TP** (dC^{PT}) in comparison with natural dCTP (+). Time intervals are given in minutes.

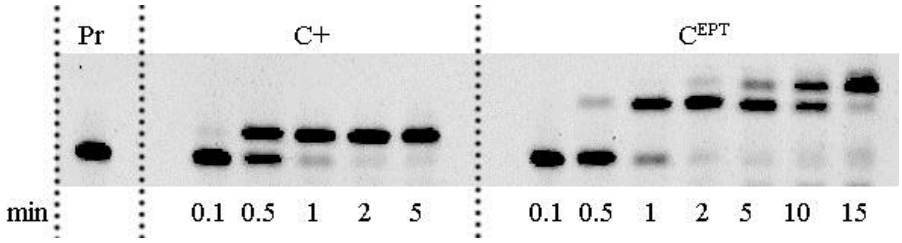


Figure S4 PAGE analyses of kinetic single nucleotide extension experiments with temp^{tempC} using KOD XL DNA polymerases **dC^{EPT}TP** (dC^{EPT}) in comparison with natural dATP (+). Time intervals are given in minutes.

2. NEAR

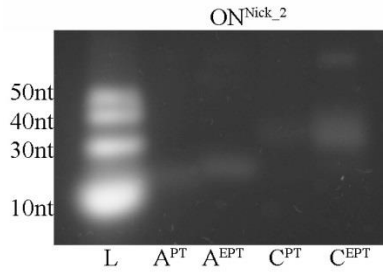


Figure S5 Incorporation of modified dNTPs in NEAR. L: DNA ladder; A^{PT}: product of NEAR with **dA^{PT}TP**, dCTP, dGTP, dTTP; A^{EPT}: product of NEAR with **dA^{EPT}TP**, dCTP, dGTP, dTTP; C^{PT}: product of NEAR with **dC^{PT}TP**, dATP, dGTP, dTTP; C^{EPT}: product of NEAR with **dC^{EPT}TP**, dATP,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

3. PCR

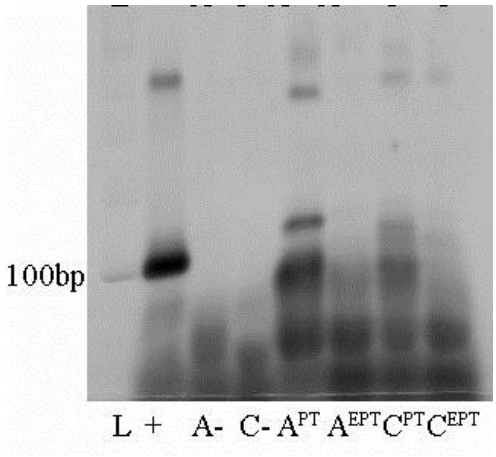


Figure S6 PCR synthesis of 98-mer by KOD XL polymerase L: DNA ladder; +: product of PCR with natural dNTPs; A-: product of PCR with dCTP, dGTP, dTTP; C-**:** product of PCR with dATP, dGTP, dTTP; A^{PT}: product of PCR with **dA^{PT}TP**, dCTP, dGTP, dTTP; A^{EPT}: product of PCR with **dA^{EPT}TP**, dCTP, dGTP, dTTP; C^{PT}: product of PCR with **dC^{PT}TP**, dATP, dGTP, dTTP; C^{EPT}: product of PCR with **dC^{EPT}TP**, dATP, dGTP, dTTP.

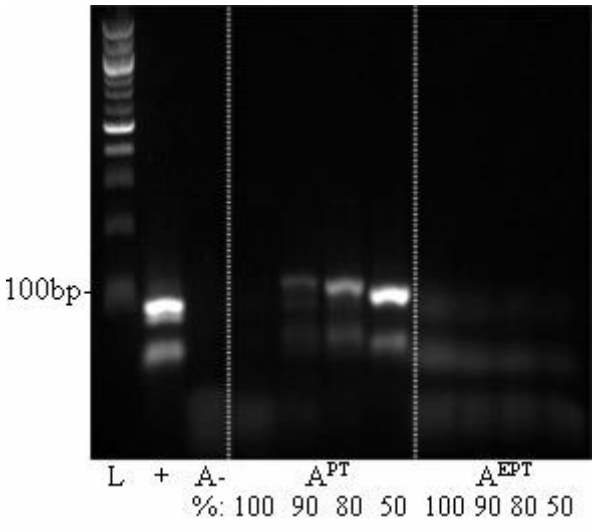


Figure S7 PCR synthesis of 98-mer by KOD XL polymerase. L: DNA ladder; +: product of PCR with natural dNTPs; A-: product of PCR with dCTP, dGTP, dTTP; C-: product of PCR with dATP, dGTP, dTTP; A^{PT}: product of PCR with **$dA^{PT}TP$**, dCTP, dGTP, dTTP; A^{EPT}: product of PCR with **$dA^{EPT}TP$**,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

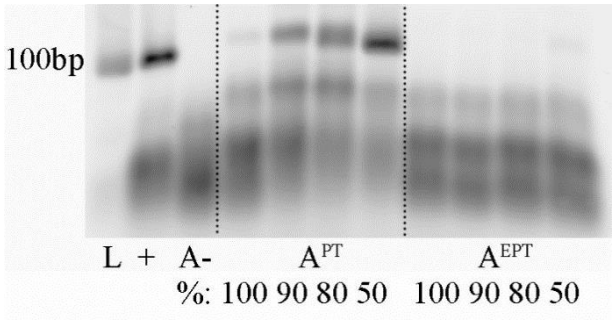


Figure S8 PCR synthesis of 98-mer by KOD XL polymerase with FAM-lableled primers. L: DNA ladder; +: product of PCR with natural dNTPs; A-: product of PCR with dCTP, dGTP, dTTP; C-: product of PCR with dATP, dGTP, dTTP; A^{PT}: product of PCR with **$dA^{PT}TP$**, dCTP, dGTP, dTTP; A^{EPT}: product of PCR with **$dA^{EPT}TP$**, dCTP, dGTP, dTTP. The percentage corresponds to the proportion of modified triphosphates (**$dA^{PT}TP$** or **$dA^{EPT}TP$**) in combination with natural dATP in the PCR reaction mixture.

4. TdT (Full gels)

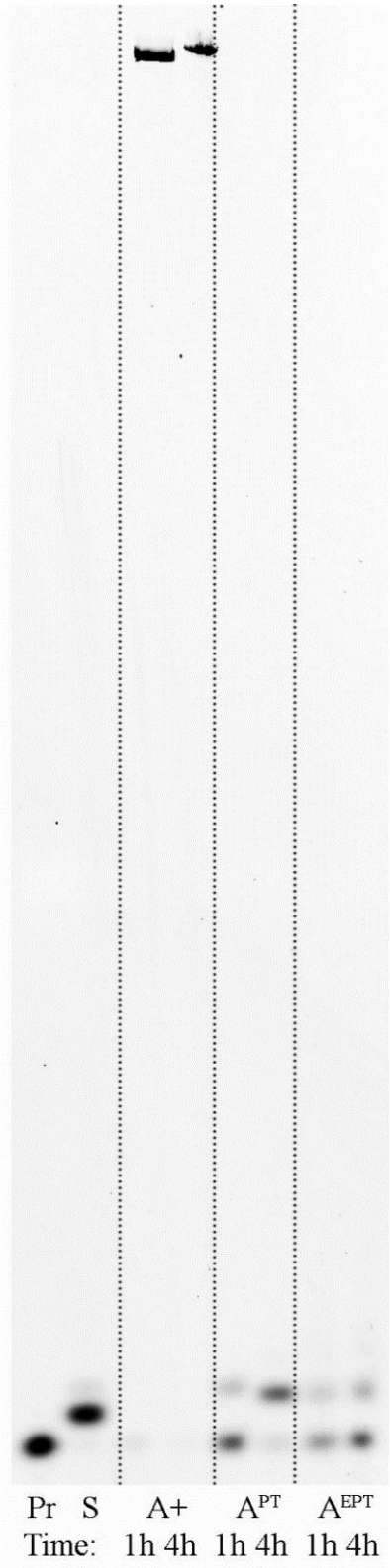


Figure S9 TdT-catalyzed DNA chain elongation. Pr: primer; S-standard (PEX product of temp^{termA} with dATP); A+, A^{PT} and A^{EPT}: products of primer^{rnd} elongation using terminal transferase and either dATP, **$dA^{PT}TP$** or **$dA^{EPT}TP$** respectively. Time intervals are given in hours.

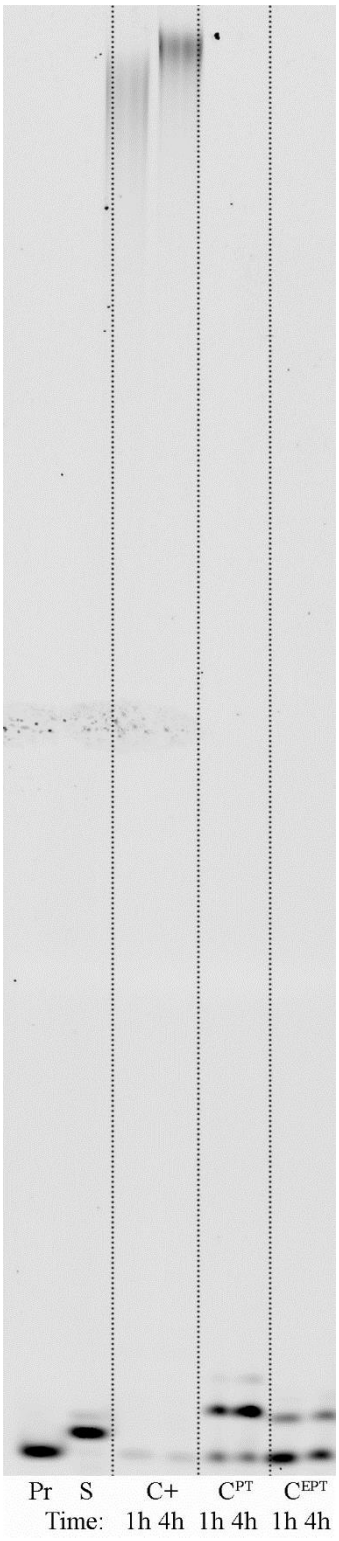


Figure S10 TdT-catalyzed DNA chain elongation. Pr: primer; S-standard (PEX product of temp^{termC} with dCTP); C+, C^{PT} and C^{EPT}: products of primer^{rnd} elongation using terminal transferase and either dCTP, **dC^{PT}TP** or **dC^{EPT}TP** respectively. Time intervals are given in hours.

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

$$A = C.l.\varepsilon$$

where ε 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 equiped 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 (Φ_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]

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

where Φ_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.

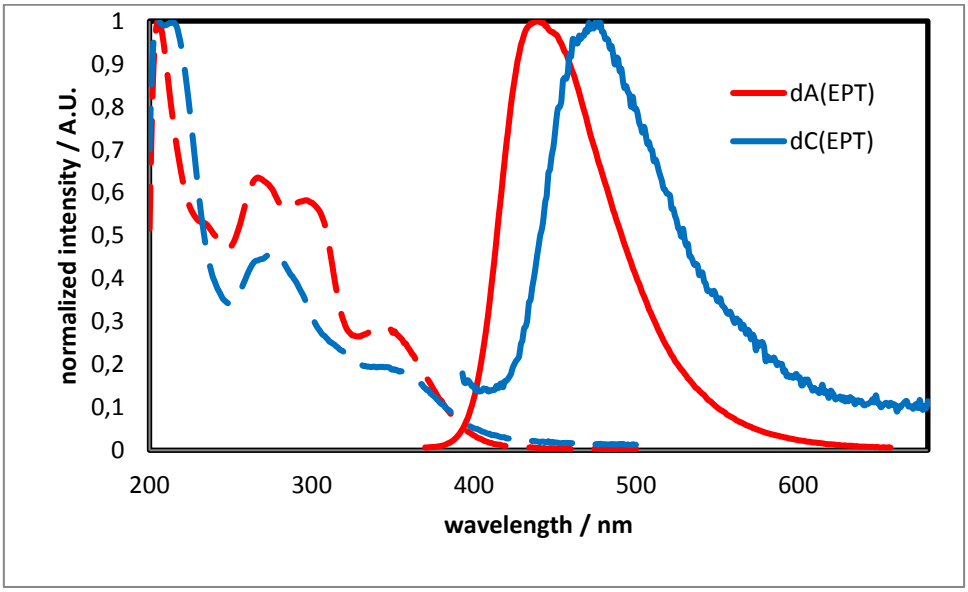


Figure S11 Normalized absorption (dashed lines) and fluorescence (solid lines) of compounds **dA^{EPT}** and **dC^{EPT}** in EtOH

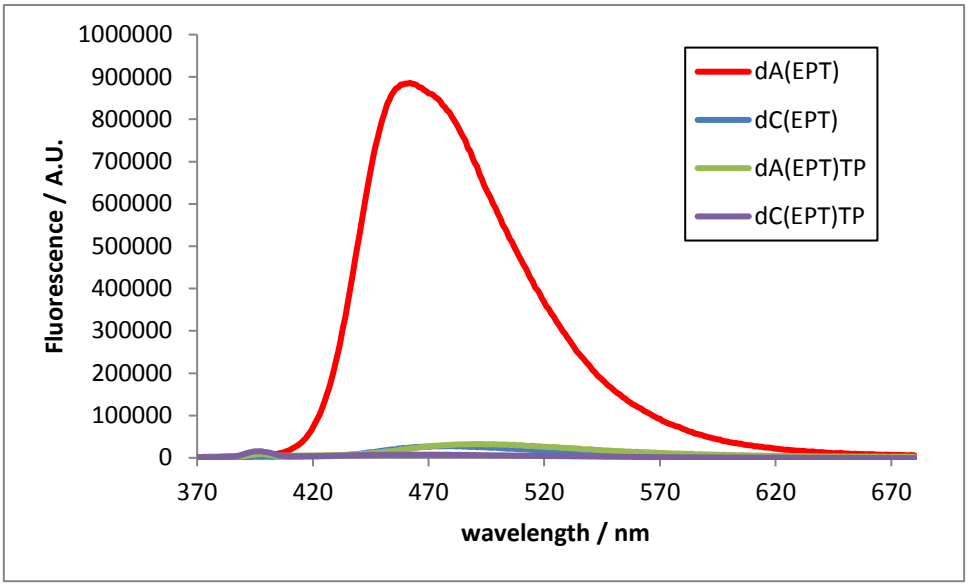


Figure S12 Fluorescence spectra of modified **dA^{EPT}** and **dC^{EPT}** in EtOH; **dA^{EPT}TP** and **dC^{EPT}TP** in aqueous solutions

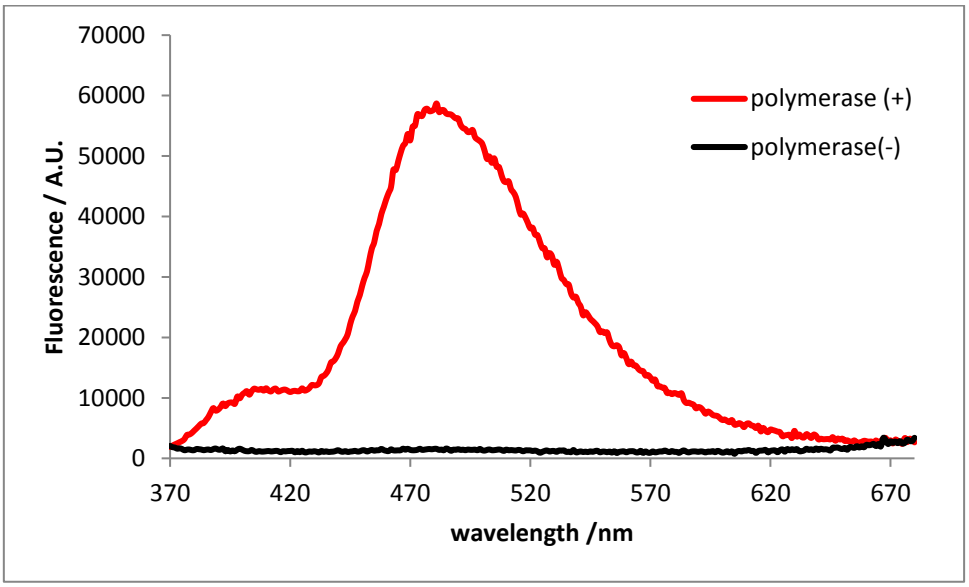


Figure S13 fluorescence spectra (λex=486 nm) of ON^{rnd16} **A^{EPT}** obtained after incubation of PEX reaction mixtures containing **dA^{EPT}TP**, dCTP, dTTP, dGTP, primer^{rnd} and biotinylated temp^{rnd16} either with (red line) or without (black line) KOD XL DNA polymerase
The reaction mixture (100 µL) contained biotinylated template^{rnd16} (100 µM, 3.2 µL), primer^{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*^{rnd} 3’-GGGTACGGCGGGTAC-5’
temp^{A} 5‘-CCCTCCCATGCCGCCCATG-3‘

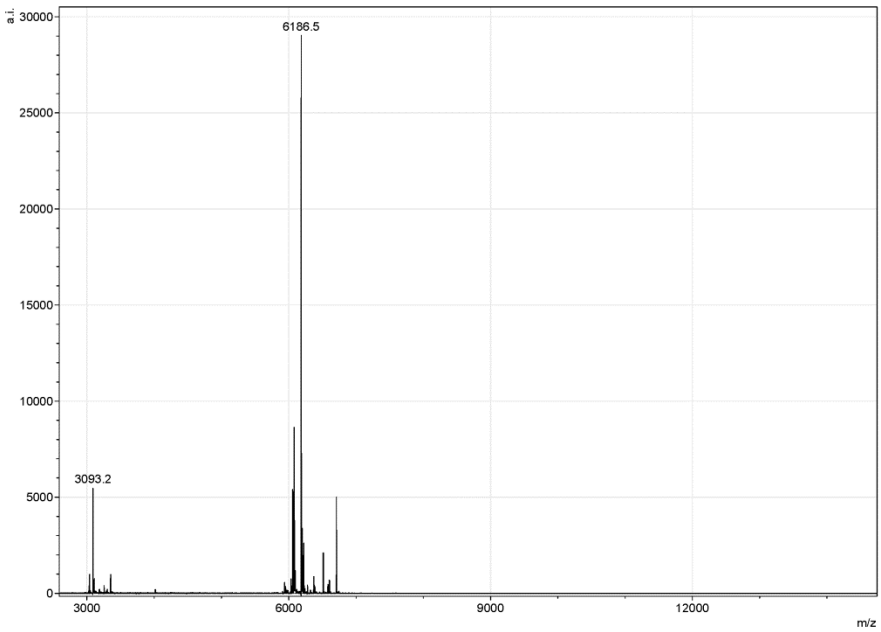


Figure S14 MALDI-TOF spectrum of ON^{A} A^{PT}. Calculated: 6185.2 Da; found: 6186.5 Da; Δ = 1.30

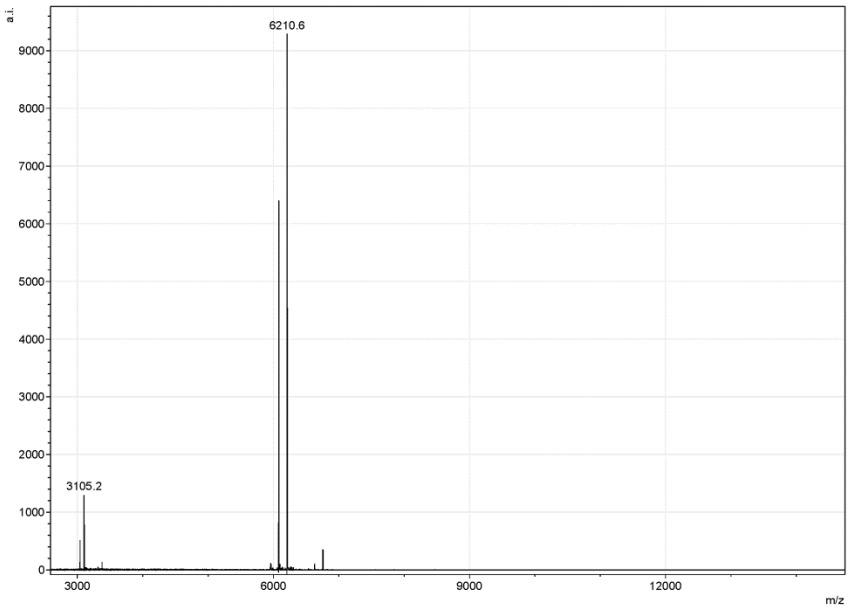


Figure S15 MALDI-TOF spectrum of ON^{A} A^{EPT}. Calculated: 6209.2 Da; found: 6210.6 Da; Δ = 1.40

b) *prim*^{rnd} 3’-GGGTACGGCGGGTAC-5’
temp^{C} 5‘-CCCGCCCATGCCGCCCATG-3‘

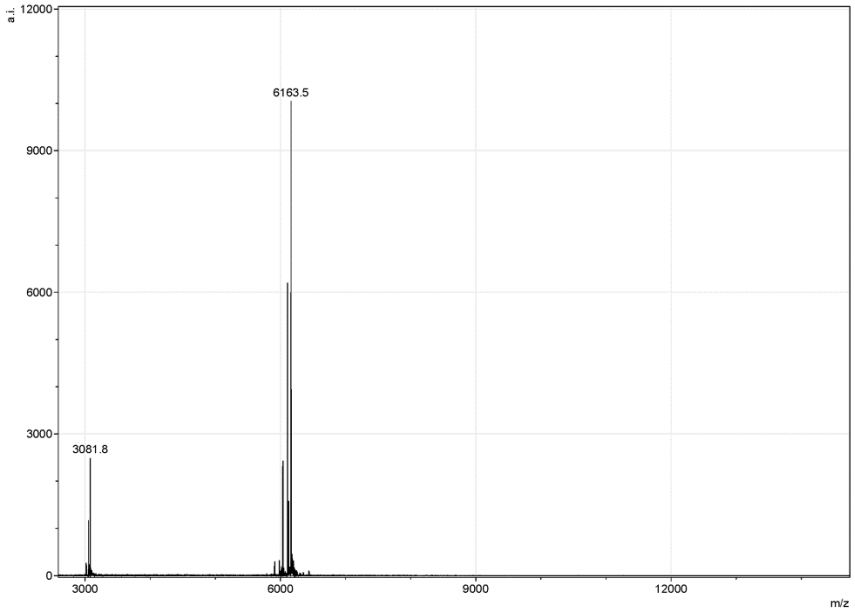


Figure S16 MALDI-TOF spectrum of ON^{C} C^{PT}. Calculated: 6162.2 Da; found: 6163.5 Da; Δ = 1.30

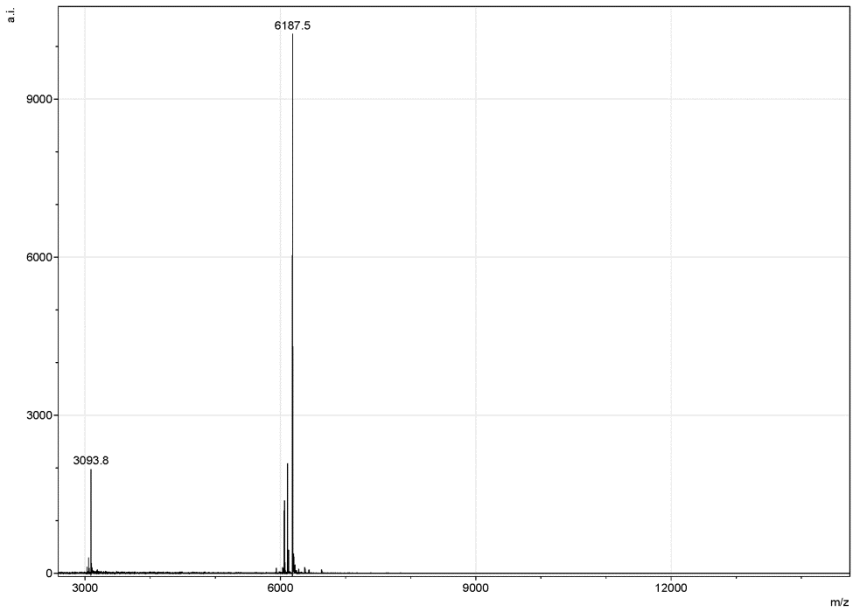


Figure S17 MALDI-TOF spectrum of ON^{C} C^{EPT}. Calculated: 6186.2 Da; found: 6187.5 Da; Δ = 1.30

c) *prim*^{rnd} 3’-GGGTACGGCGGGTAC-5’
temp^{rnd} 5‘-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3‘

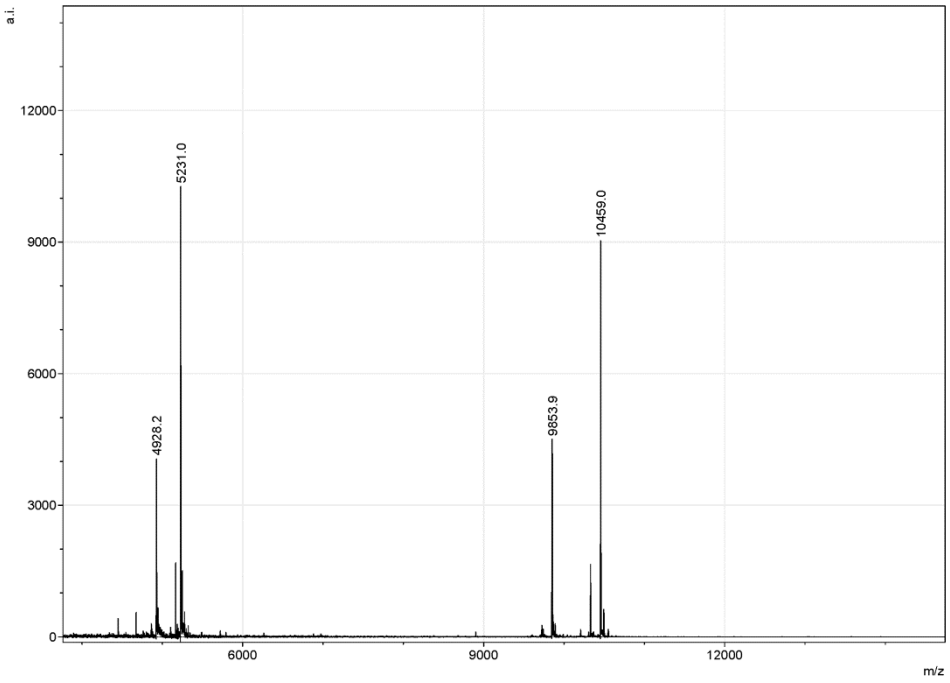


Figure S18 MALDI-TOF spectrum of ON^{rnd16} A^{PT}. Calculated: 10458.46 Da; found: 10459.00 Da; Δ = 0.54

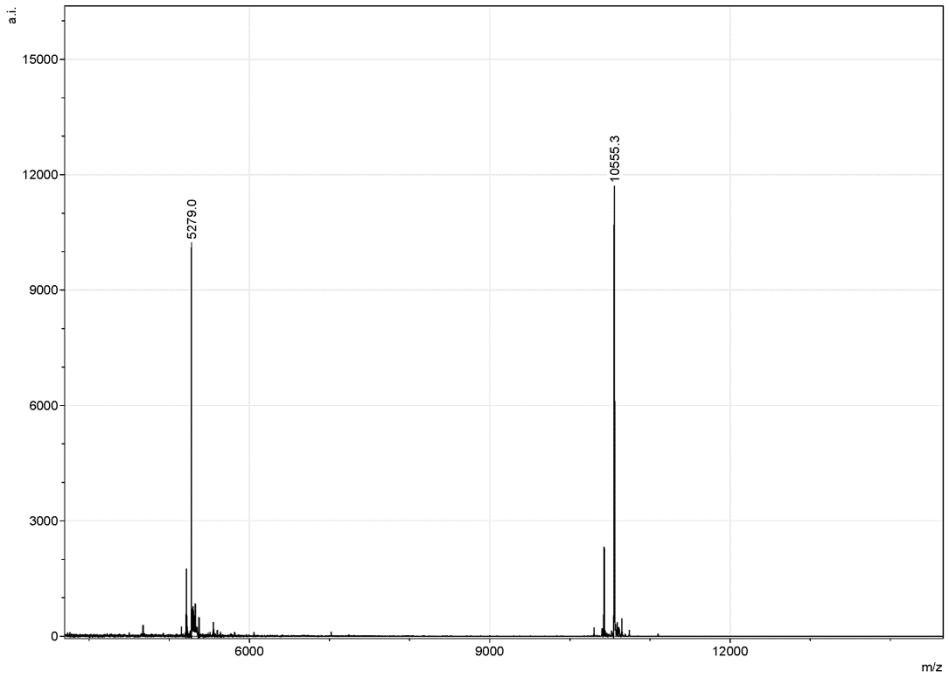


Figure S19 MALDI-TOF spectrum of ON^{rnd16} A^{EPT}. Calculated: 10554.54 Da; found: 10555.3 Da; Δ = 0.76

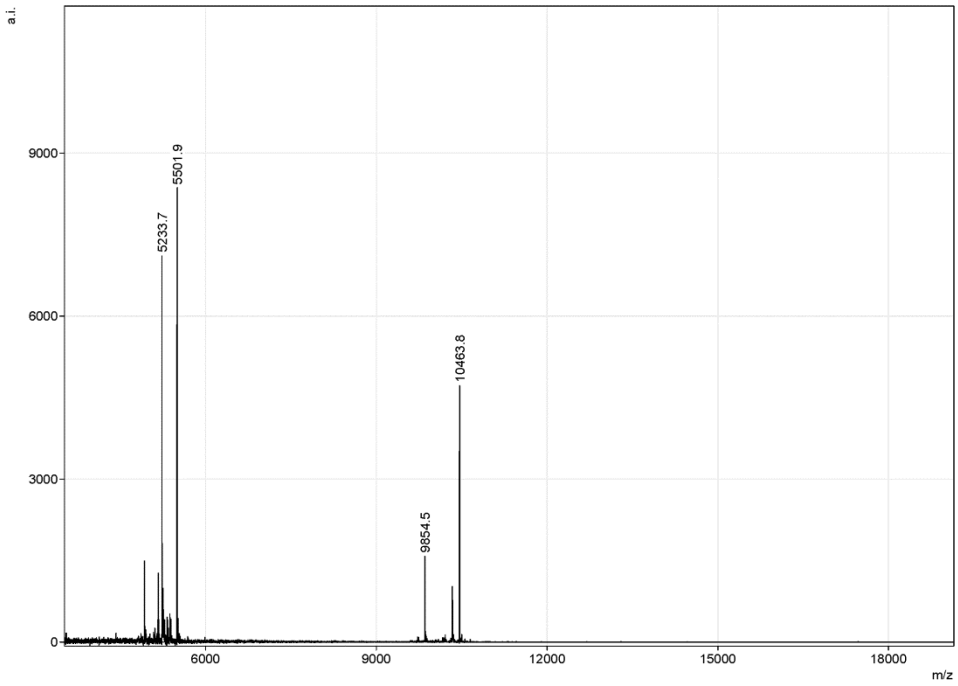


Figure S20 MALDI-TOF spectrum of ON^{rnd16} C^{PT}. Calculated: 10462.46 Da; found: 10463.8 Da; Δ = 1.34

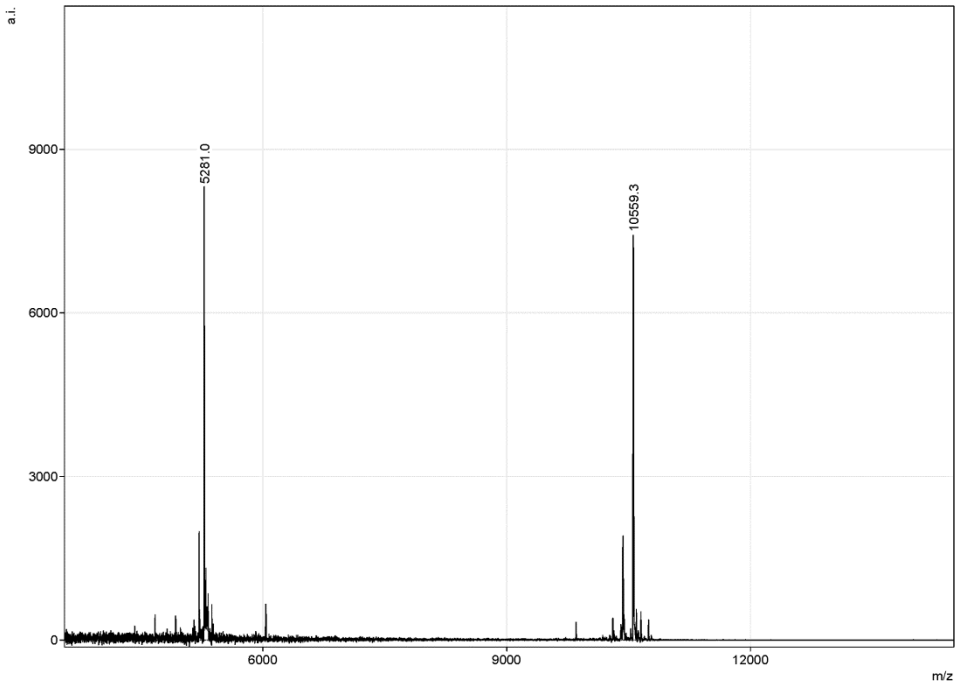


Figure S21 MALDI-TOF spectrum of ON^{rnd16} C^{EPT}. Calculated: 10558.54 Da; found: 10559.3 Da; Δ = 0.76

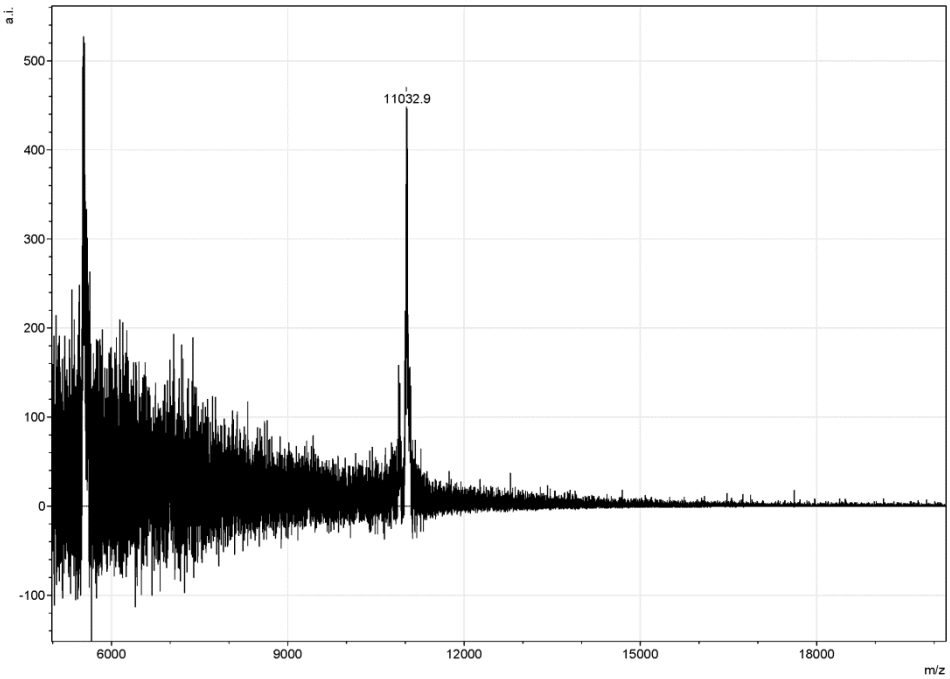


Figure S22 MALDI-TOF spectrum of ON^{rnd16} A^{PT}C^{EBF}. Calculated: 11031,10 Da; found: 11032.90 Da; Δ = 1.8

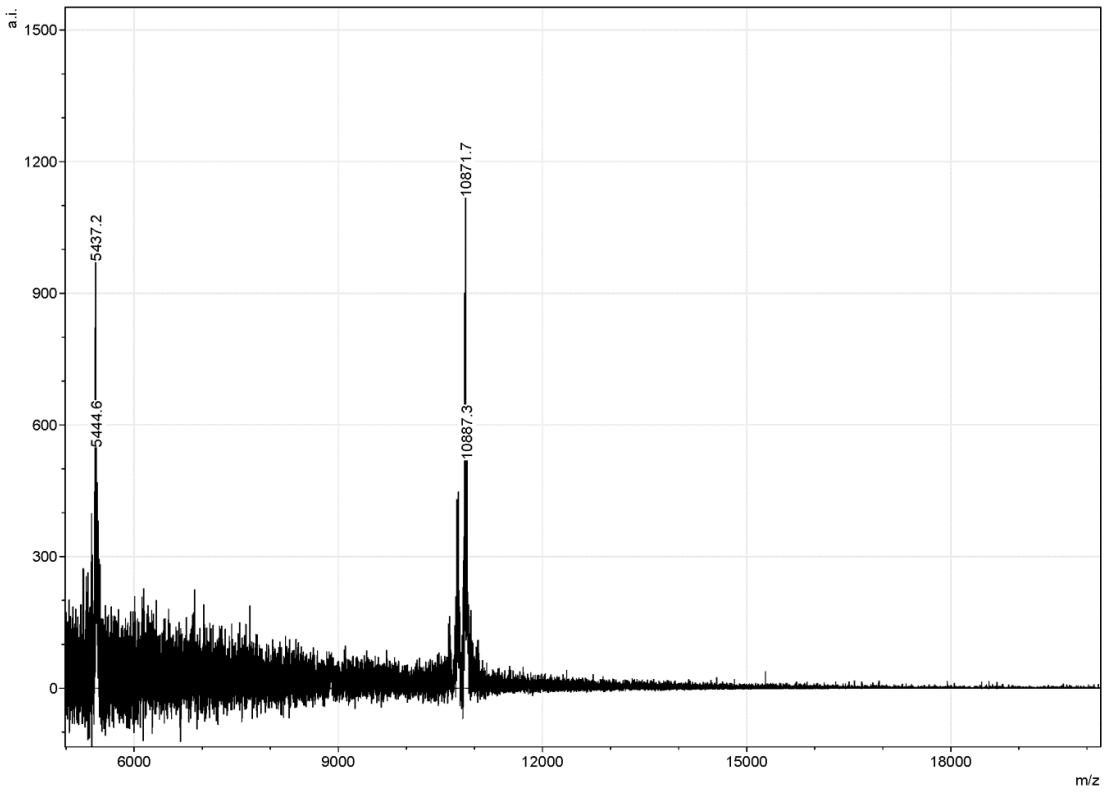


Figure S23 MALDI-TOF spectrum of ON^{rnd16} A^{PT}U^{NO2}. Calculated: 10886.94 Da; found: 10887.3 Da; Δ = 0.36

* Found mass 10871,7 Da corresponds to the absence of one N-Me group from phenothiazine label in the sample $[M\text{-}CH_3]^+$

Nick_1A 5′-P-GTCGTG**A**GTG-3′

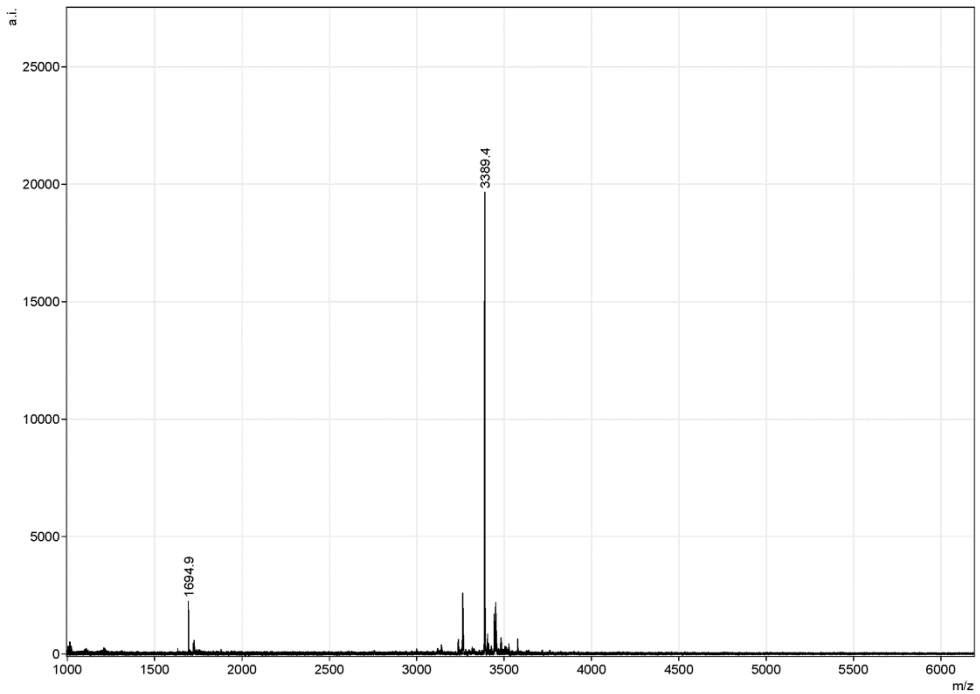


Figure S24 MALDI-TOF spectrum of ON^{Nick_1A} A^{PT}. Calculated: 3388.29 Da; found: 3389.4 Da; Δ = 1.11

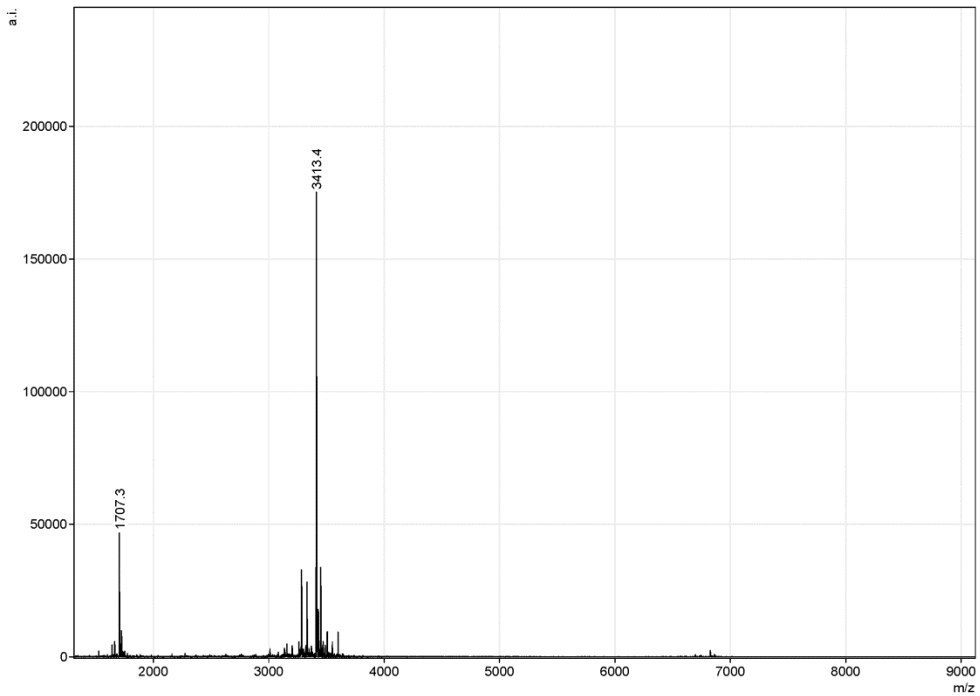


Figure S25 MALDI-TOF spectrum of ON^{Nick_1A} A^{EPT}. Calculated: 3412.31 Da; found: 3413.4 Da; Δ = 1.09

Nick_10mer_1C 5′-P-GT**C**ATGAGTG-3′

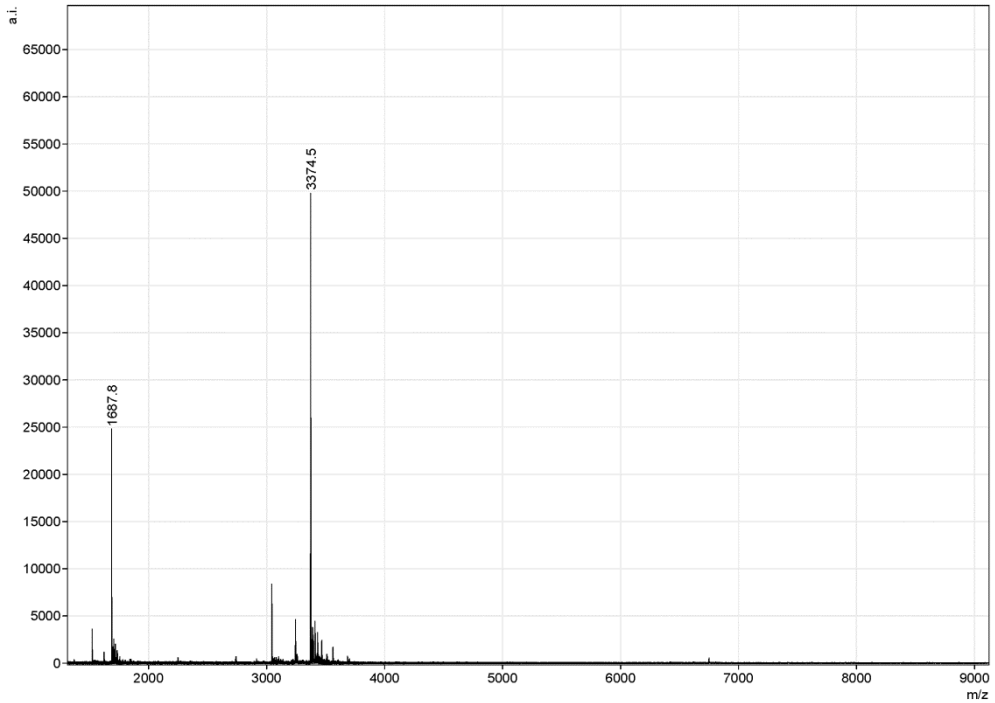


Figure S26 MALDI-TOF spectrum of ON^{Nick_1C} C^{PT}. Calculated: 3373.69 Da; found: 3374.5 Da; Δ = 0.81

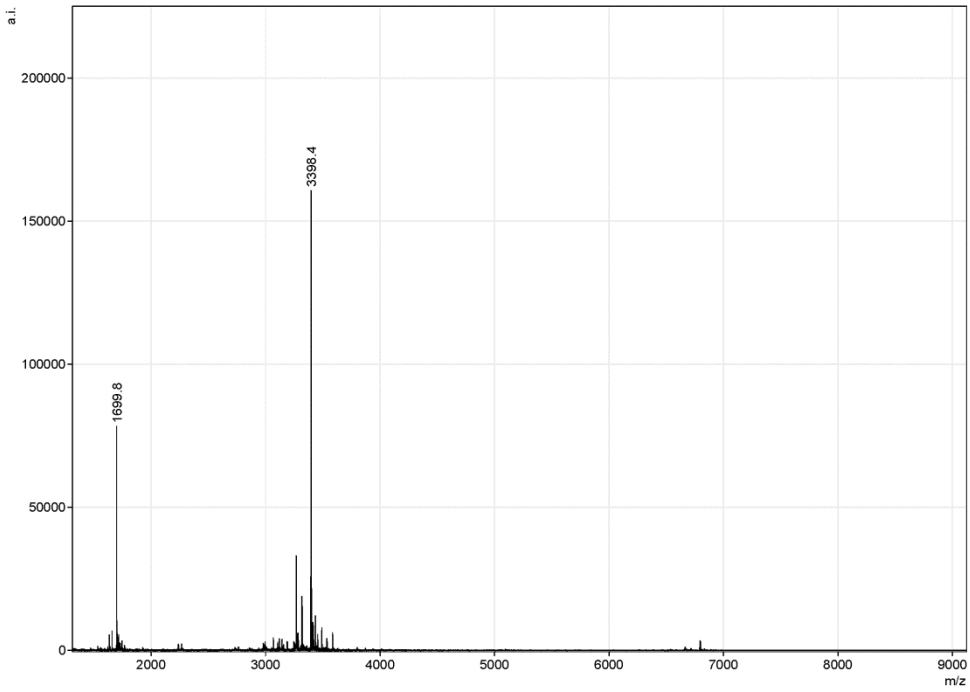


Figure S27 MALDI-TOF spectrum of ON^{Nick_1C} C^{EPT}. Calculated: 3397.41 Da; found: 3398.4 Da; Δ = 0.99

5′-P-TTC**A**TG**A**CTG-3′

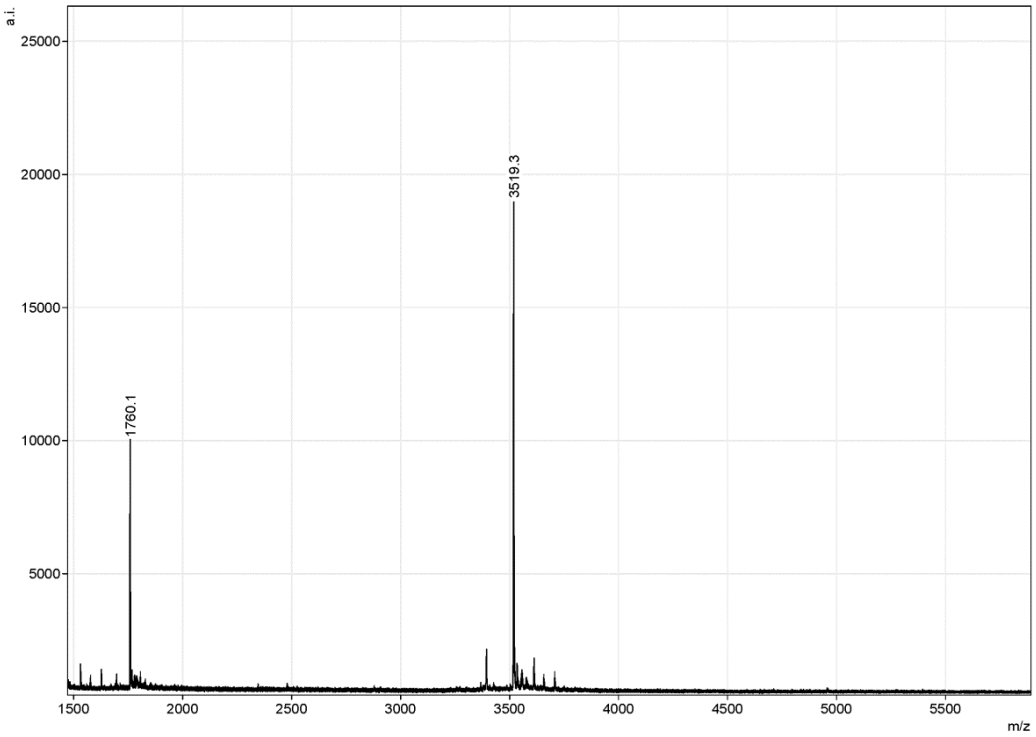


Figure S28 MALDI-TOF spectrum of ON^{Nick_2A} A^{PT}. Calculated: 3517.58 Da; found: 3519.3 Da; Δ= 1.72

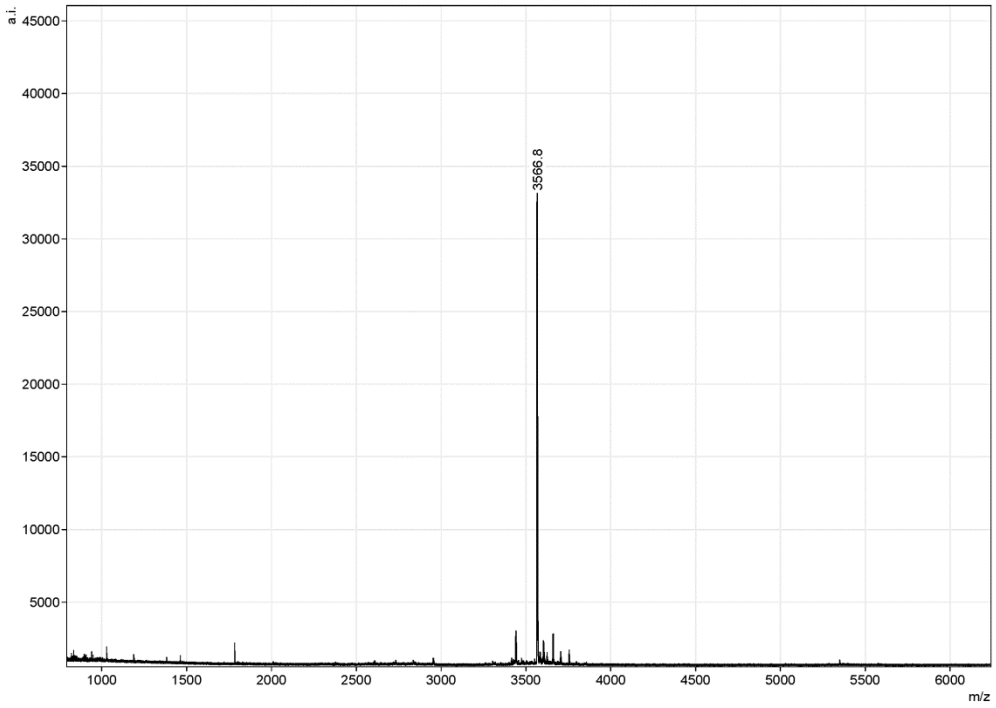


Figure S29 MALDI-TOF spectrum of ON^{Nick_2A} A^{EPT}. Calculated: 3565.62 Da; found: 3566.8 Da; Δ = 1.18

5′-P-TT**C**ATGA**C**TG-3′

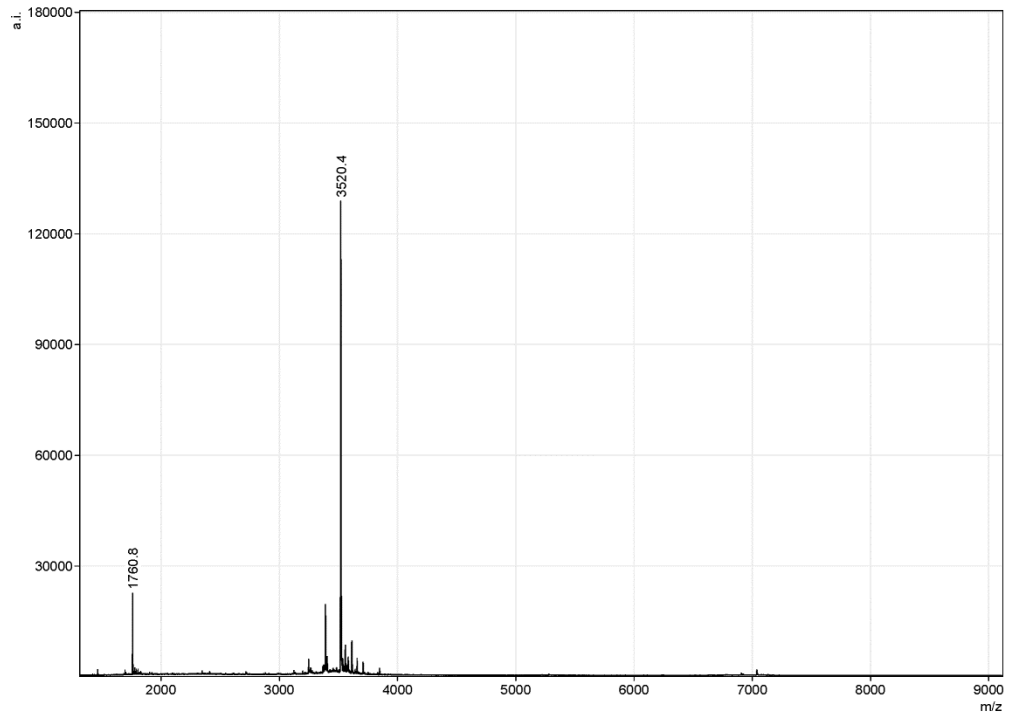


Figure S30 MALDI-TOF spectrum of ON^{Nick_2C} C^{PT}. Calculated: 3519.58 Da; found: 3520.4 Da; Δ = 1.34

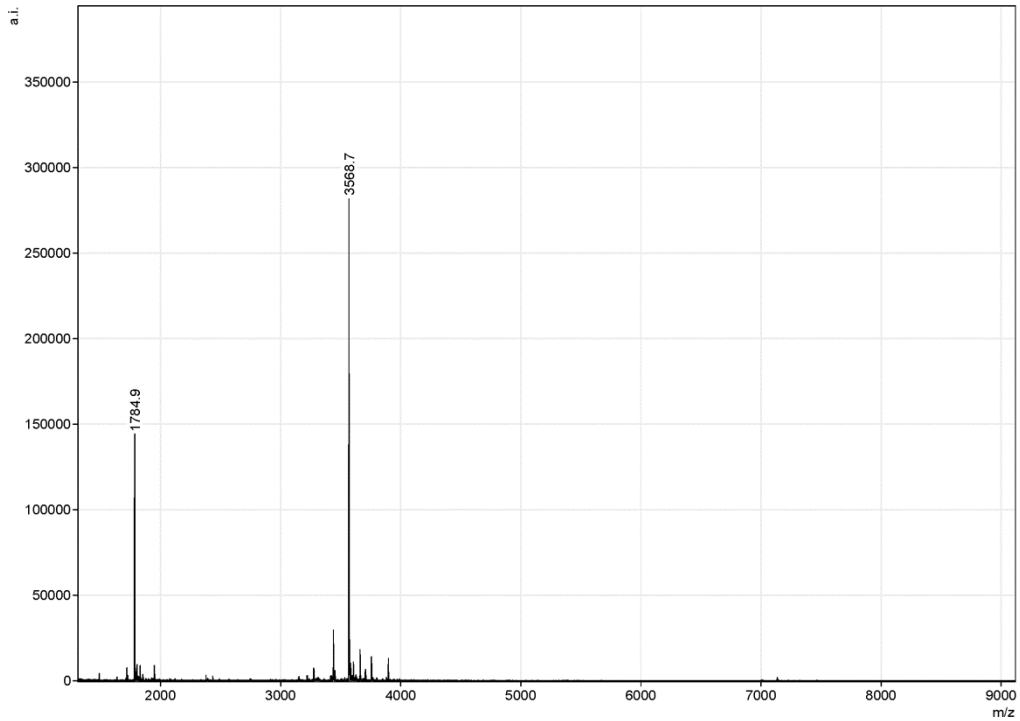


Figure S31 MALDI-TOF spectrum of ON^{Nick_2C} C^{EPT}. Calculated: 3567.62 Da; found: 3568.7 Da; Δ = 1.34

5′-P-T**A**GC**A**TGCT**A**CGTC**A**G-3′

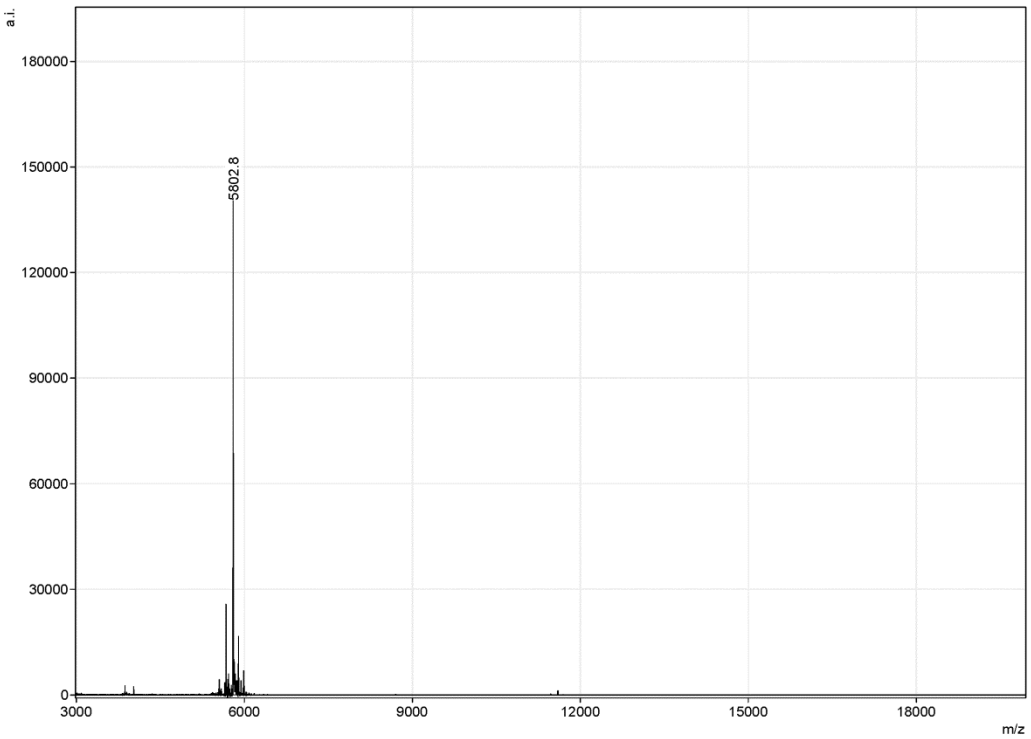


Figure S32 MALDI-TOF spectrum of ON^{Nick_4A} A^{PT}. Calculated: 5801.36 Da; found: 5802.8 Da; Δ = 1.44

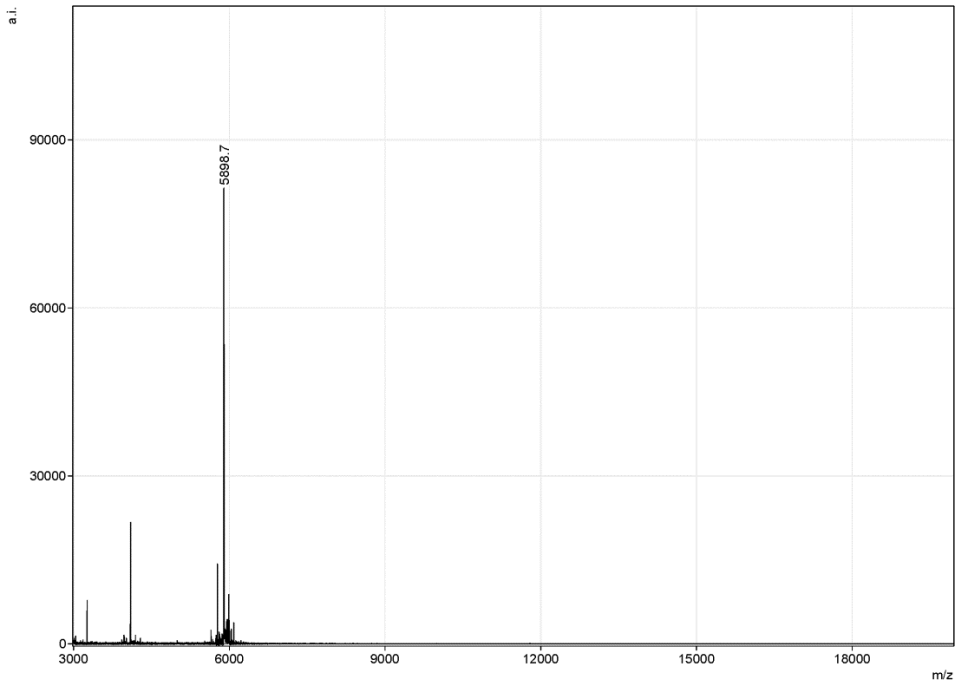


Figure S33 MALDI-TOF spectrum of ON^{Nick_4A} A^{EPT}. Calculated: 5897.44 Da; found: 5898.7 Da; Δ = 1.26

5′-P-TAG**C**ATG**C**TA**C**GT**C**AG-3

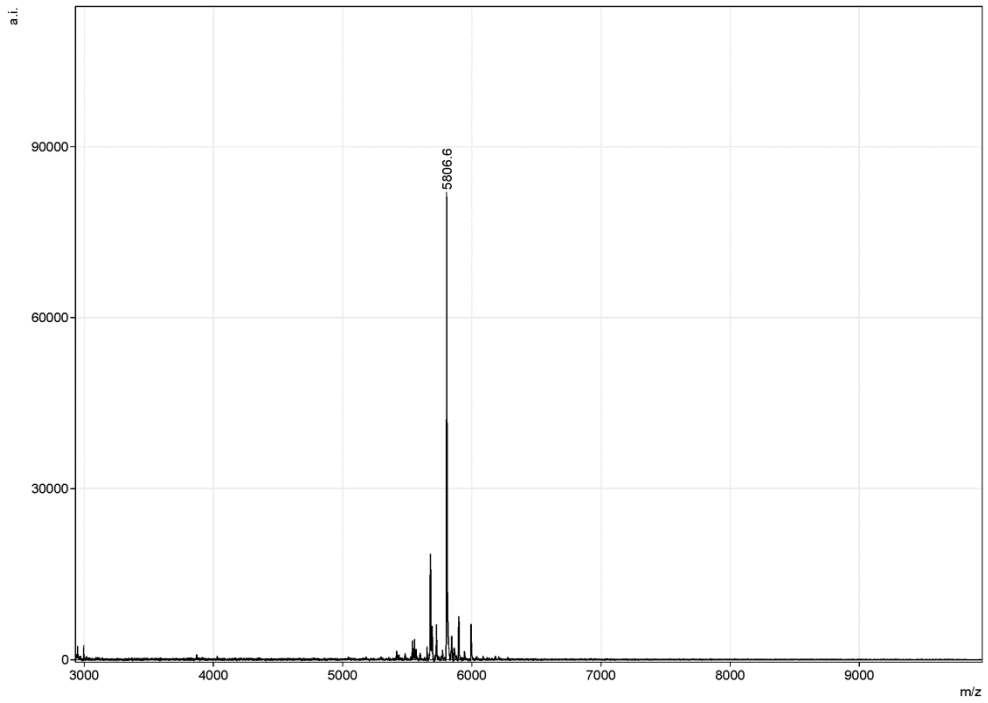


Figure S34 MALDI-TOF spectrum of ON^{Nick_4C} C^{PT}. Calculated: 5805.36 Da; found: 5806.6 Da; Δ = 1.24

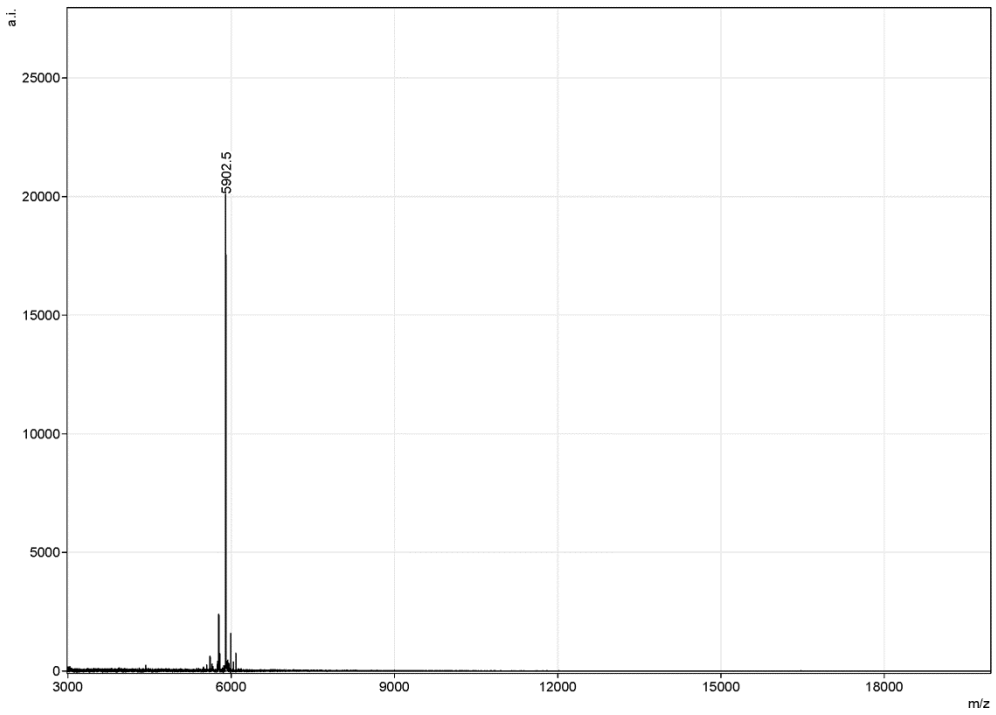
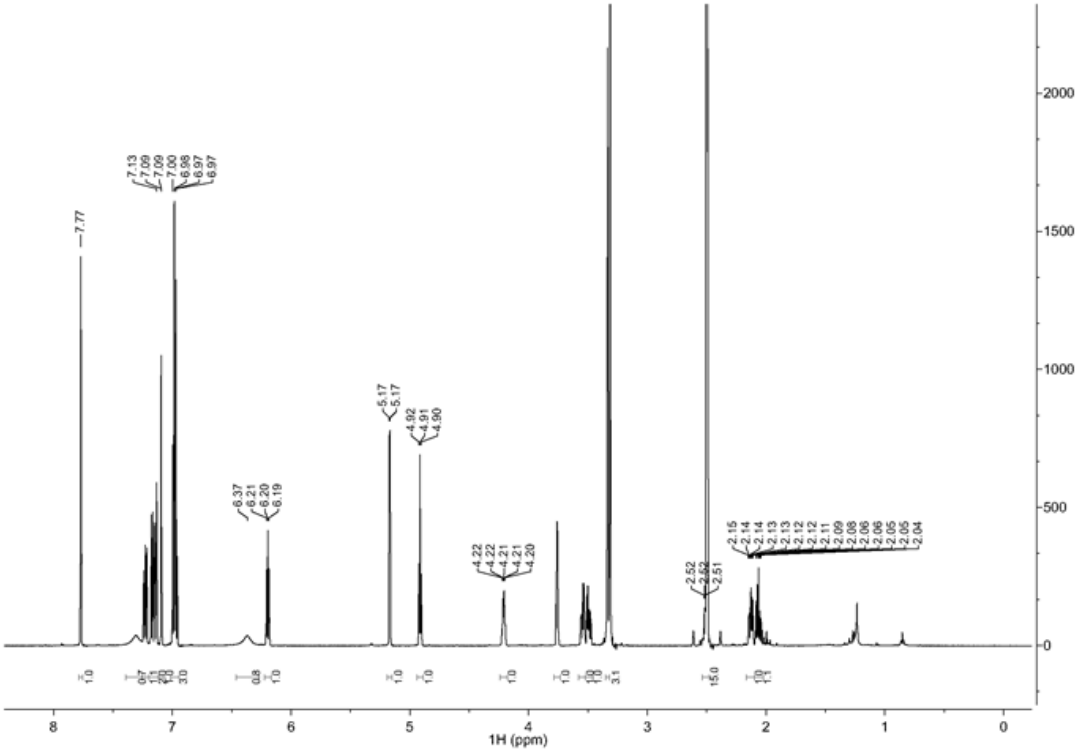
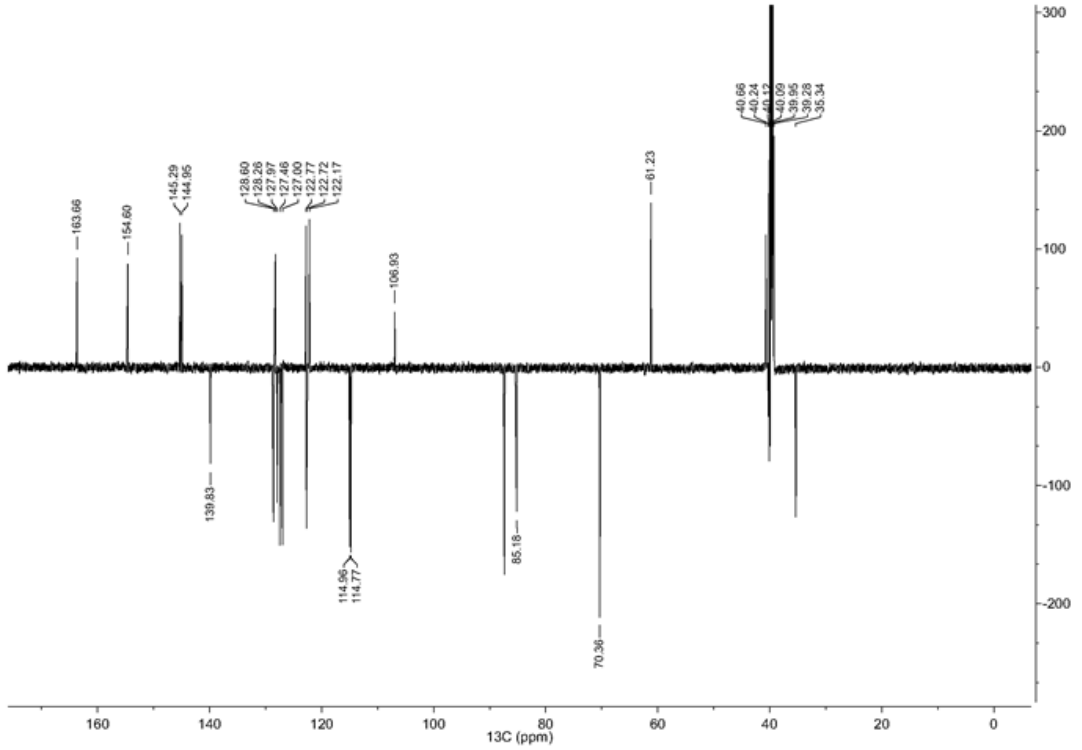


Figure S35 MALDI-TOF spectrum of ON^{Nick_4C} C^{EPT}. Calculated: 5901.44 Da; found: 5902.5 Da; Δ = 1.06

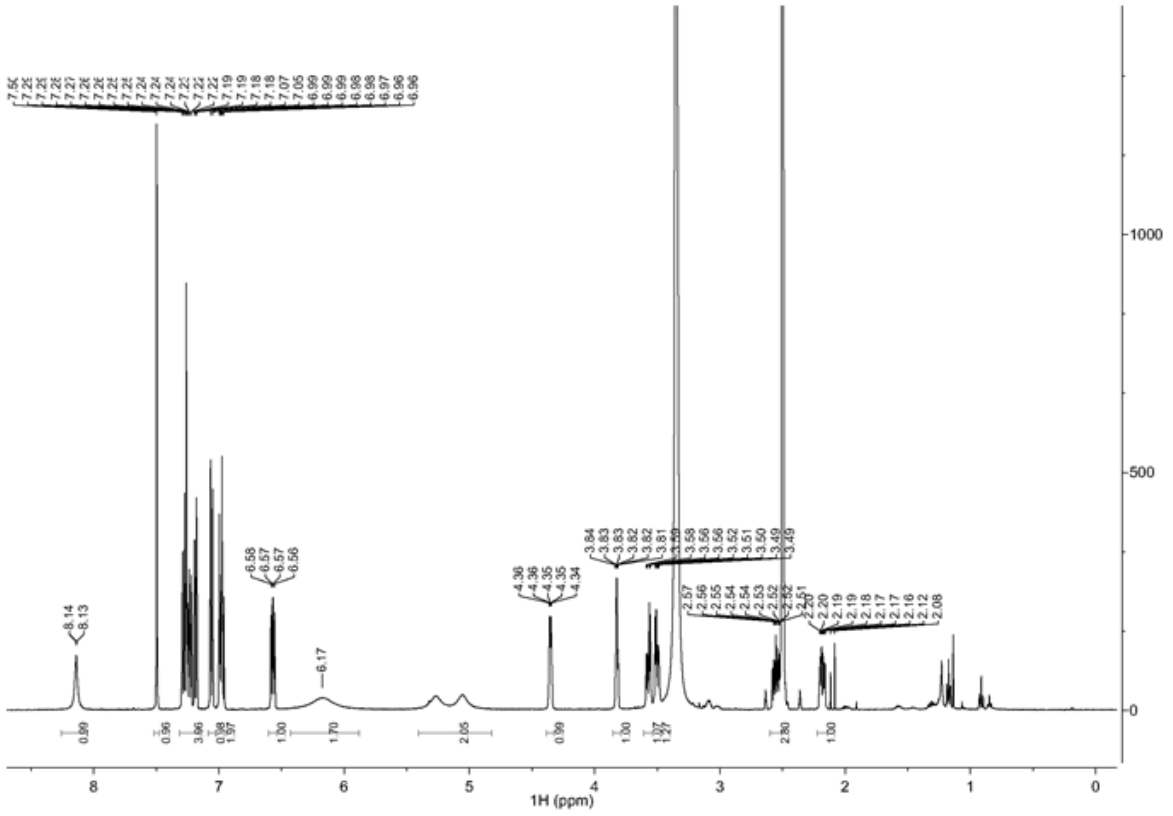
7. Copies of NMR spectra

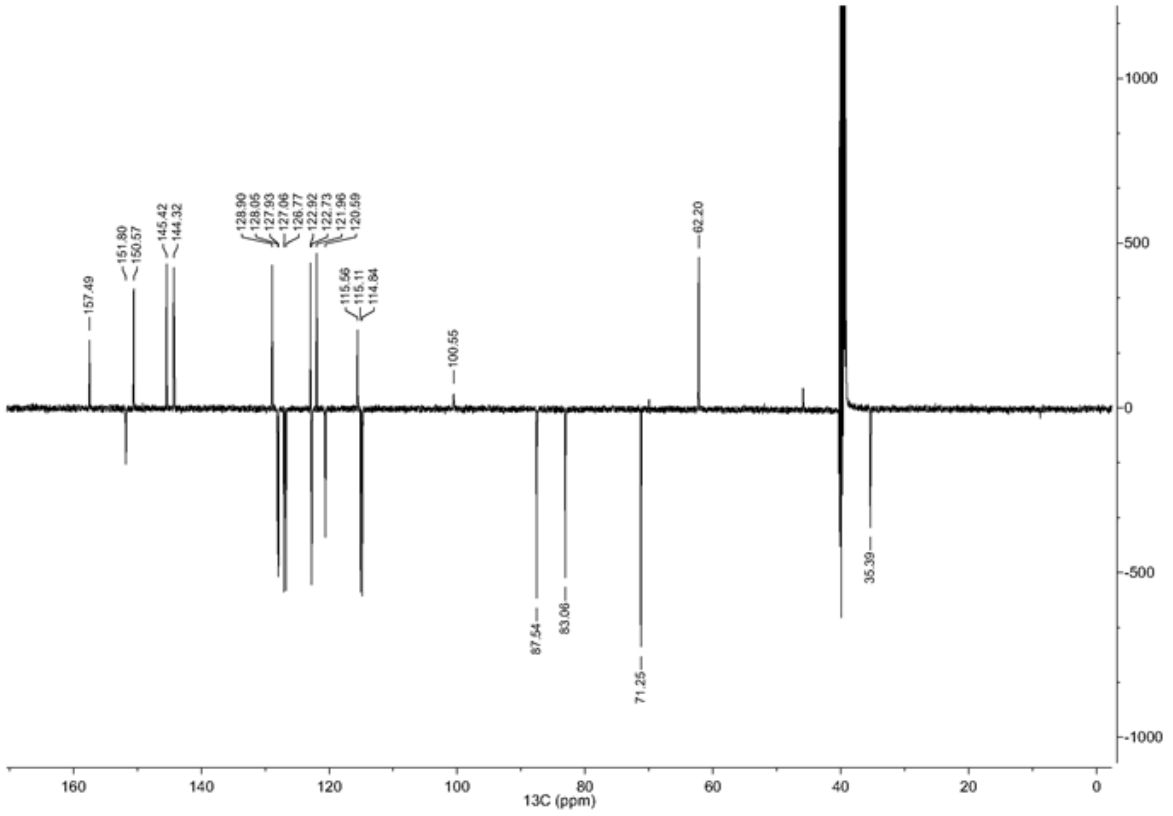
^{1}H NMR and ^{13}C spectra of **dC^{PT}.**



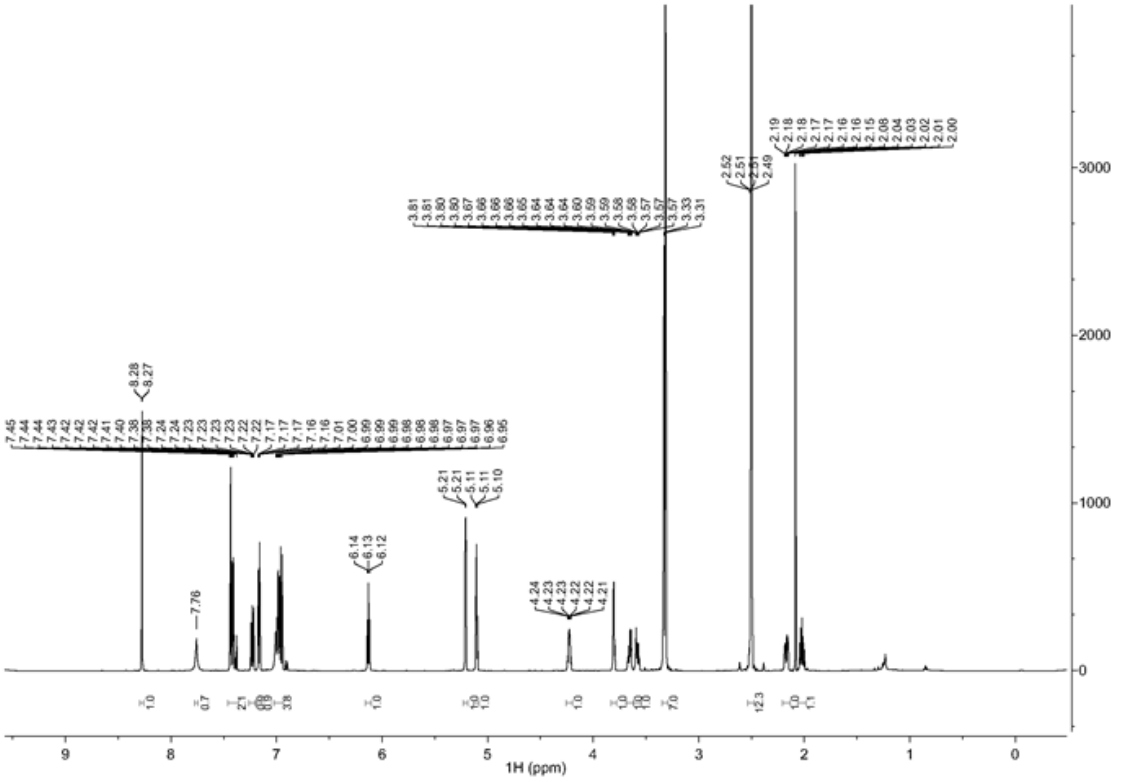


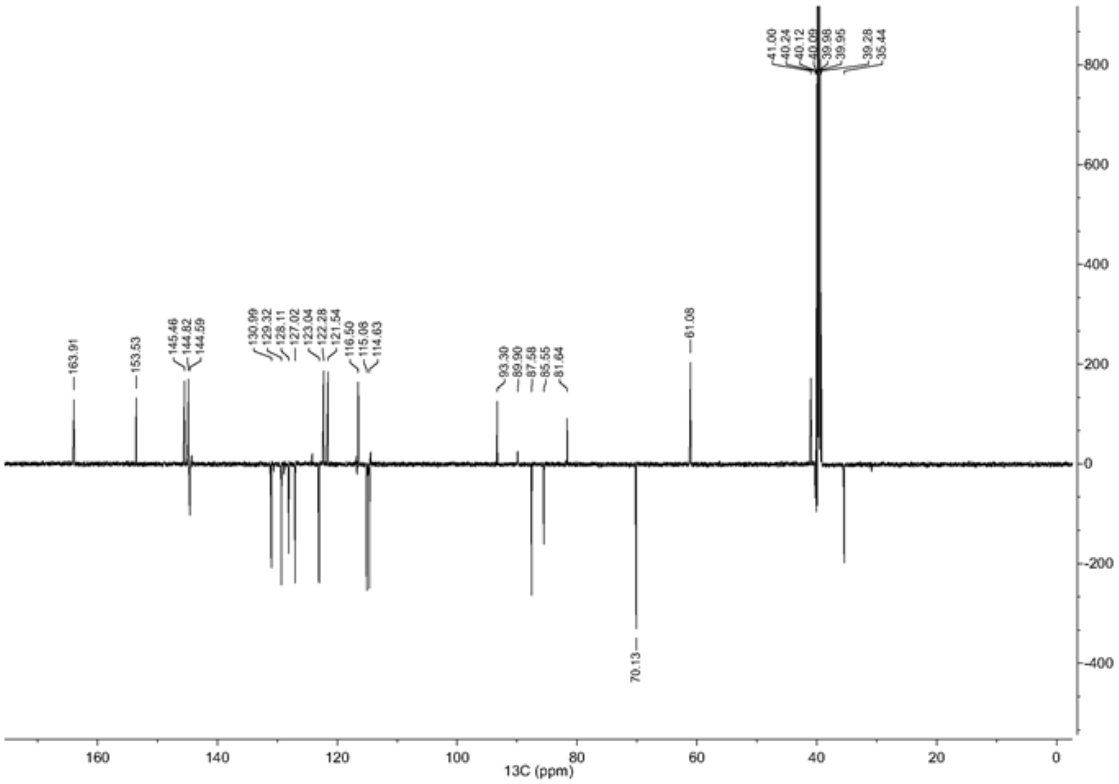
^{1}H NMR and ^{13}C spectra of **dA^{PT}.**



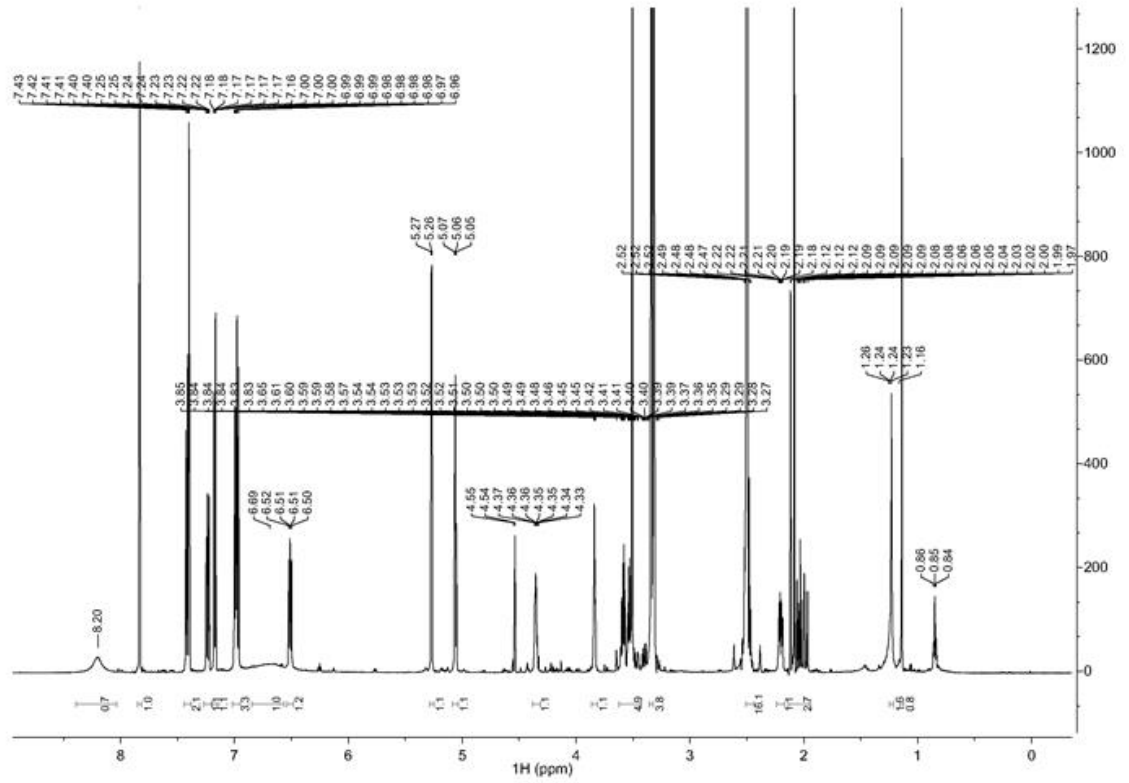


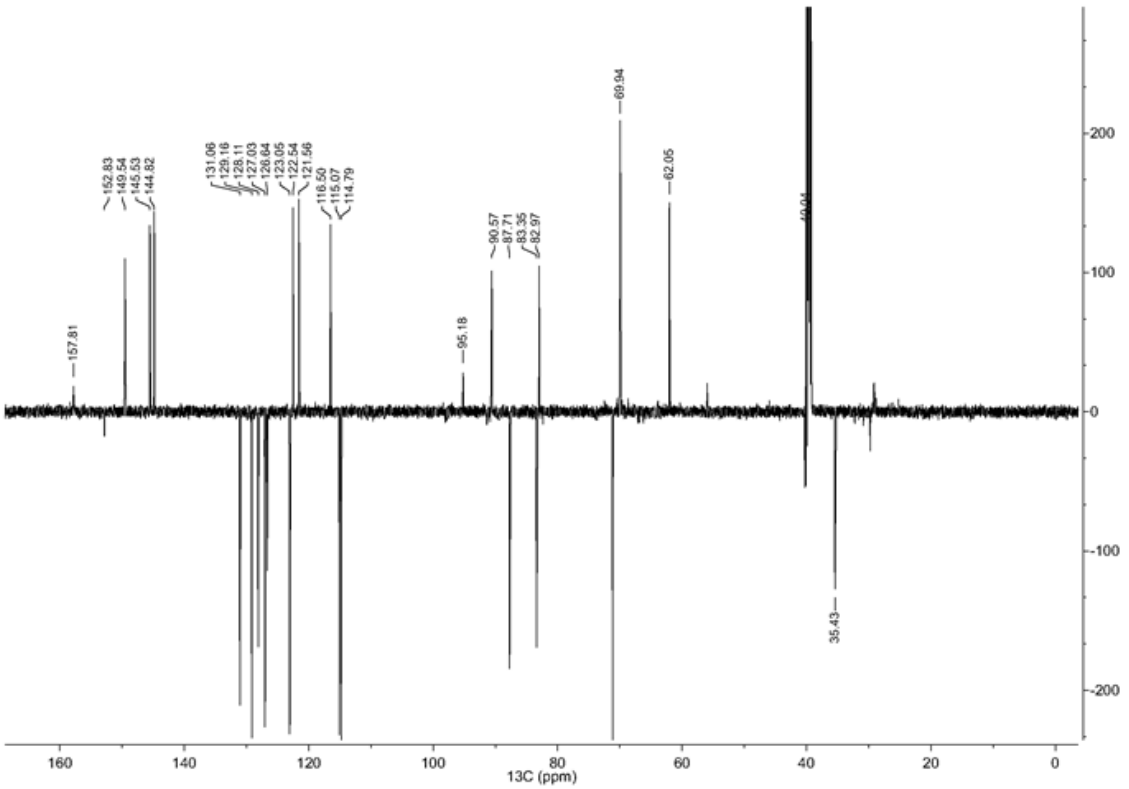
^{1}H NMR and ^{13}C spectra of **dC^{EPT}.**



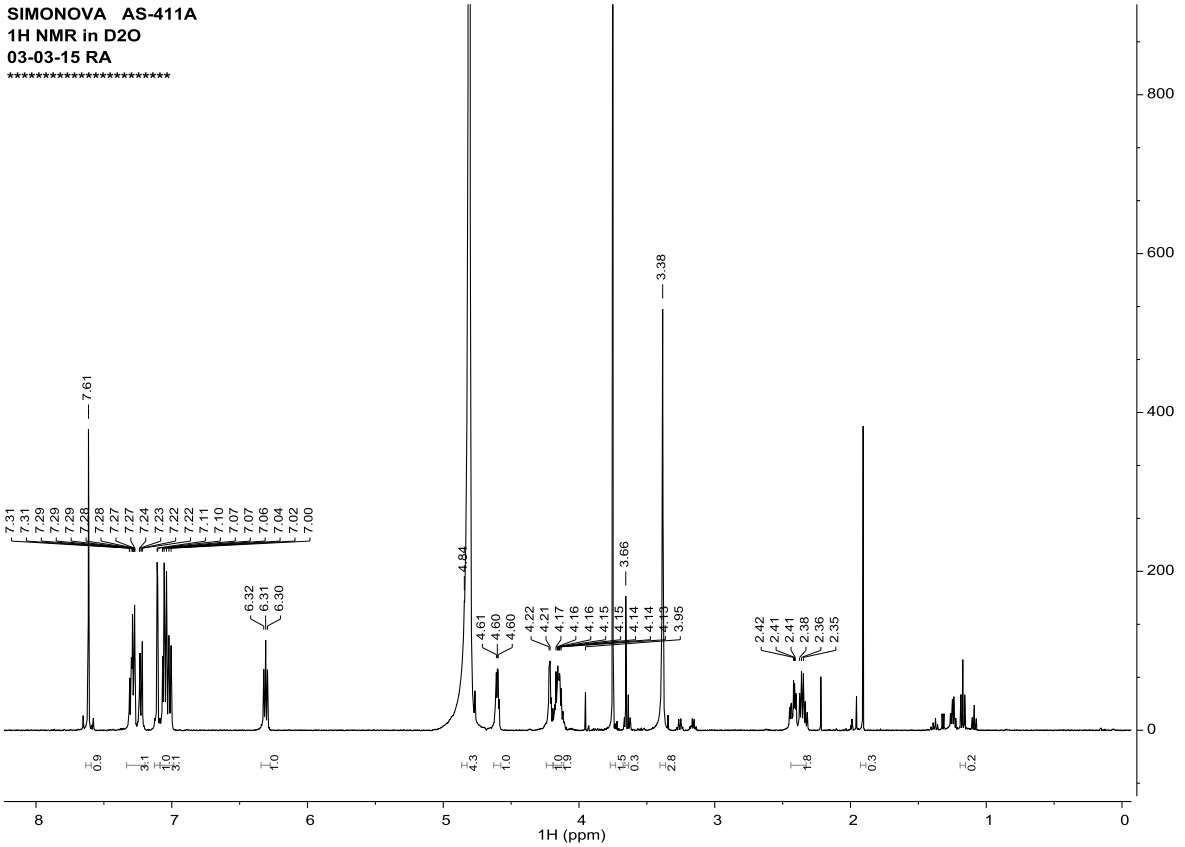


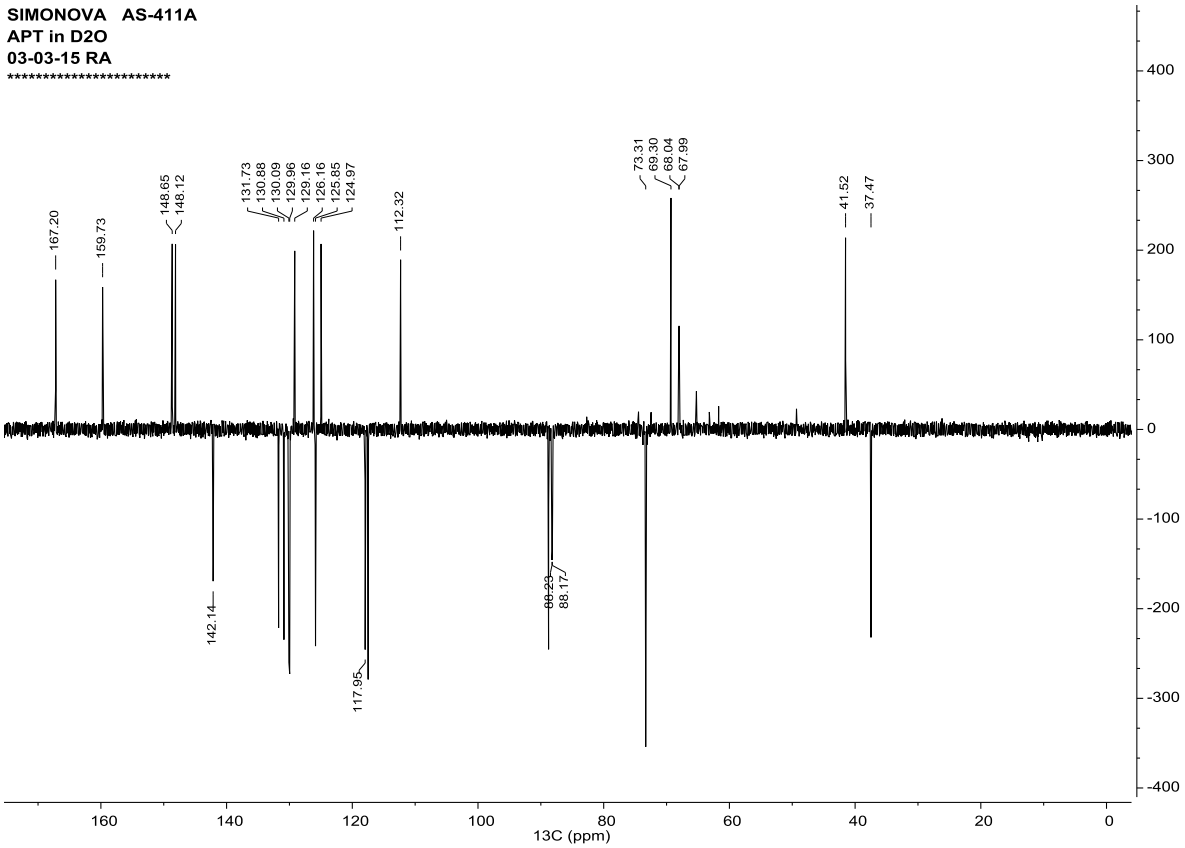
^{1}H NMR and ^{13}C spectra of **dA^{EPT}.**

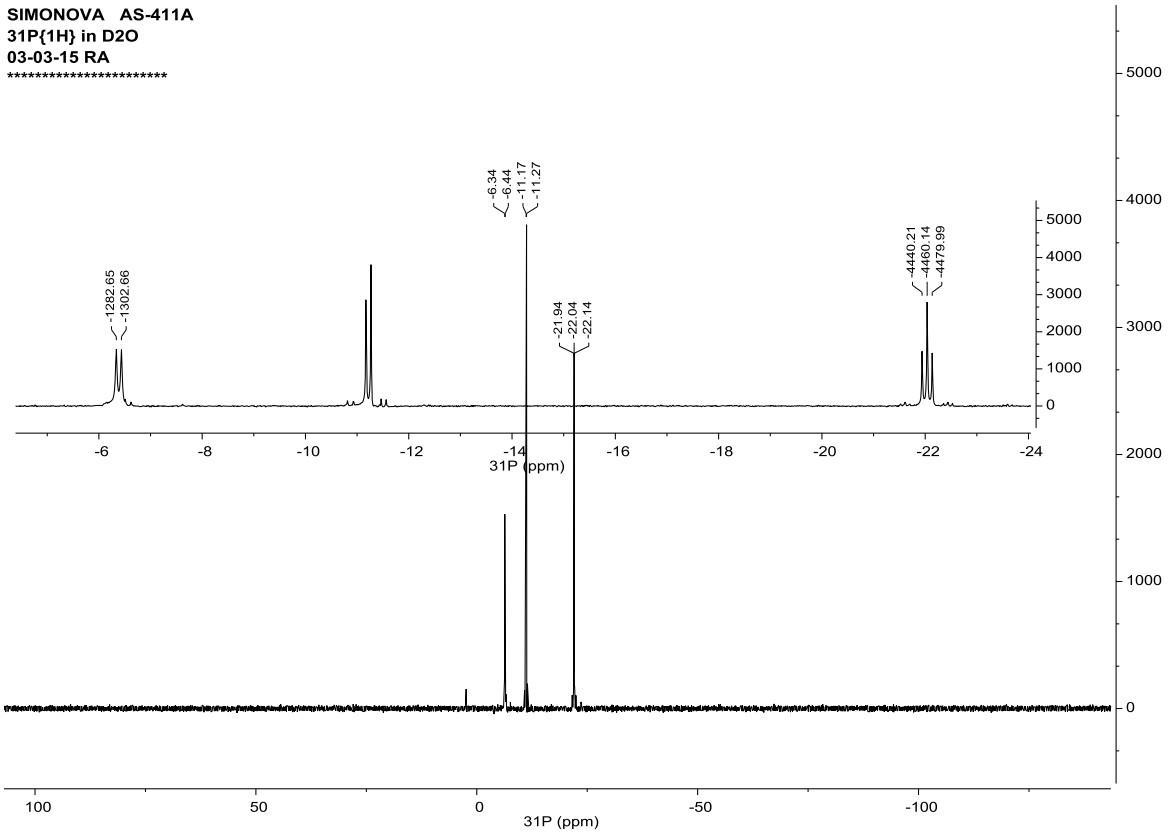




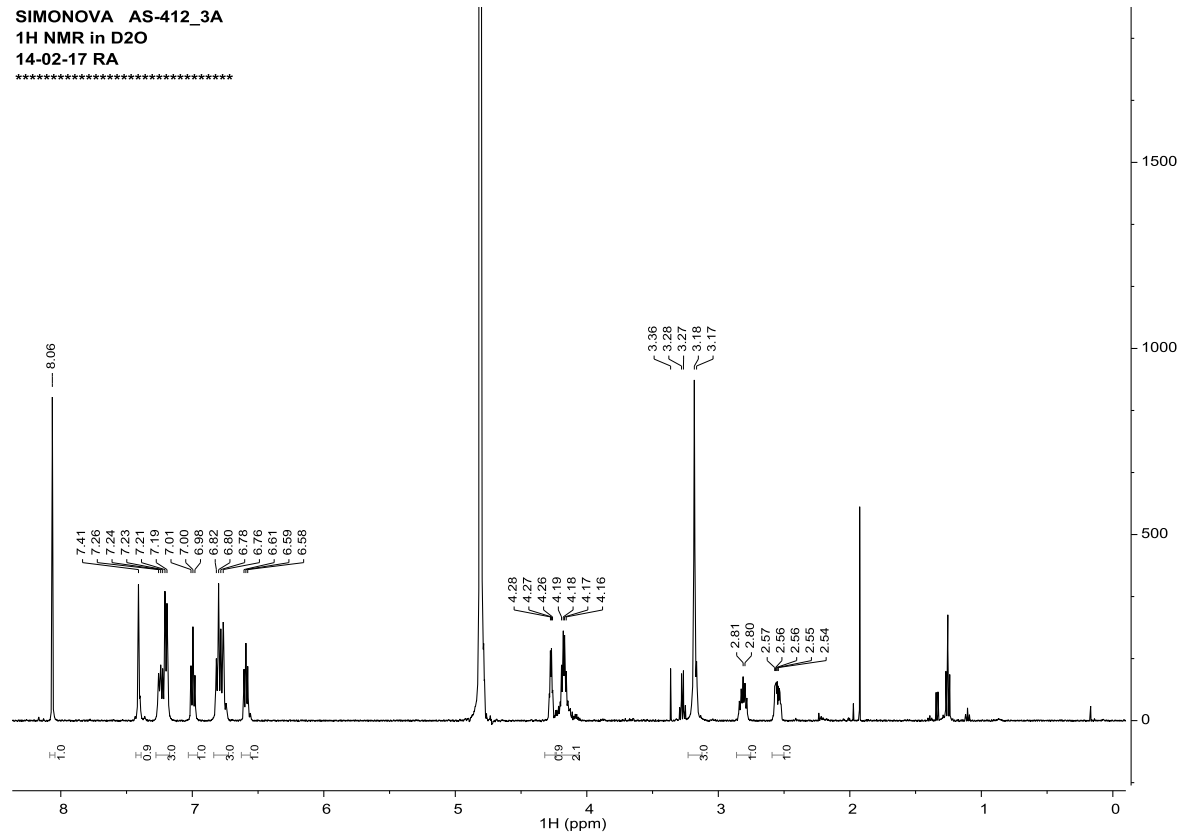
^{1}H NMR, ^{13}C and ^{31}P spectra of **dC^{PT}TP.**

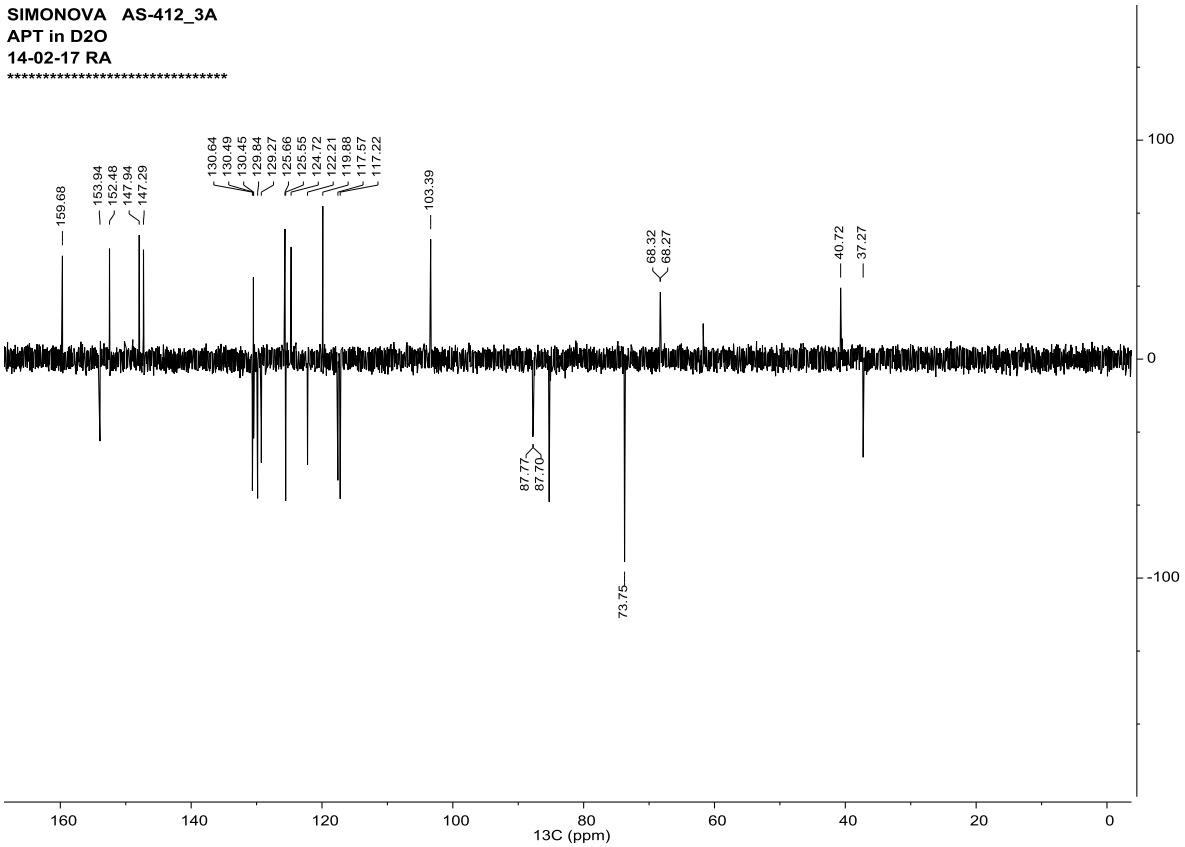


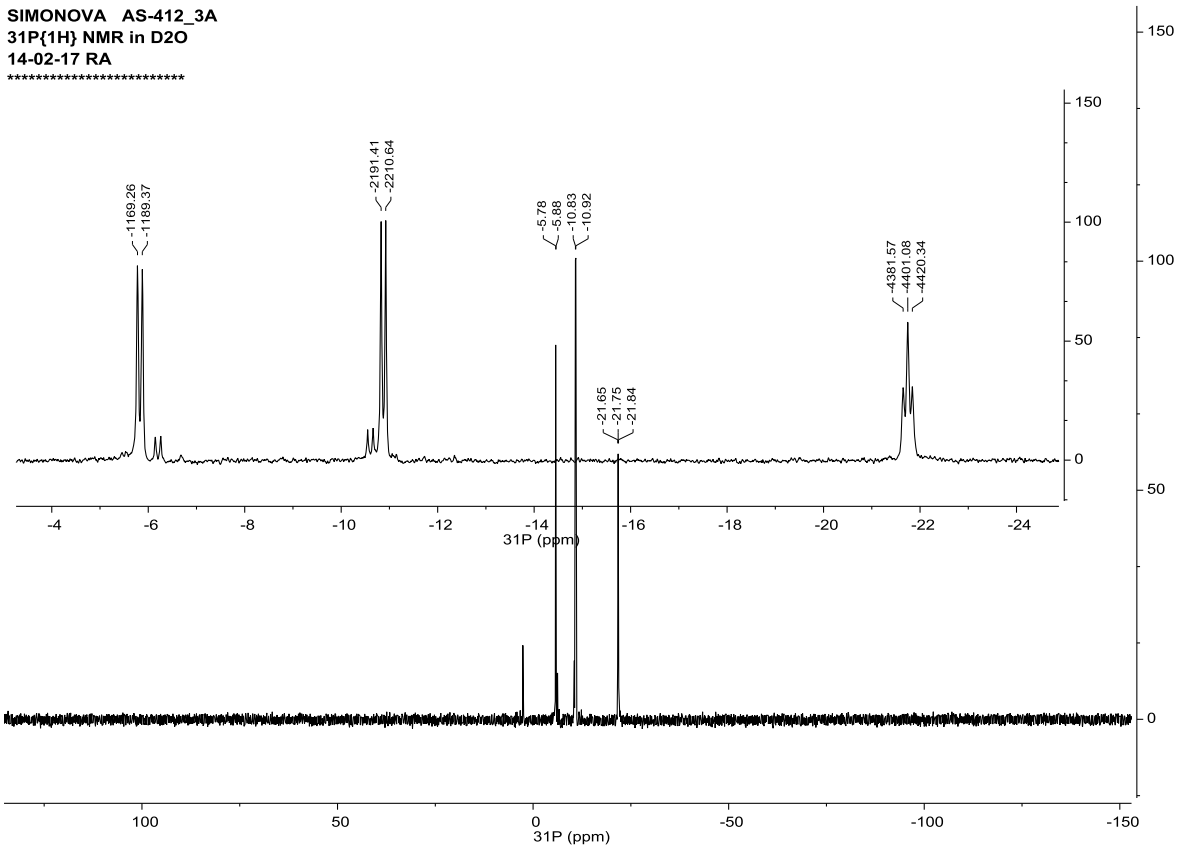




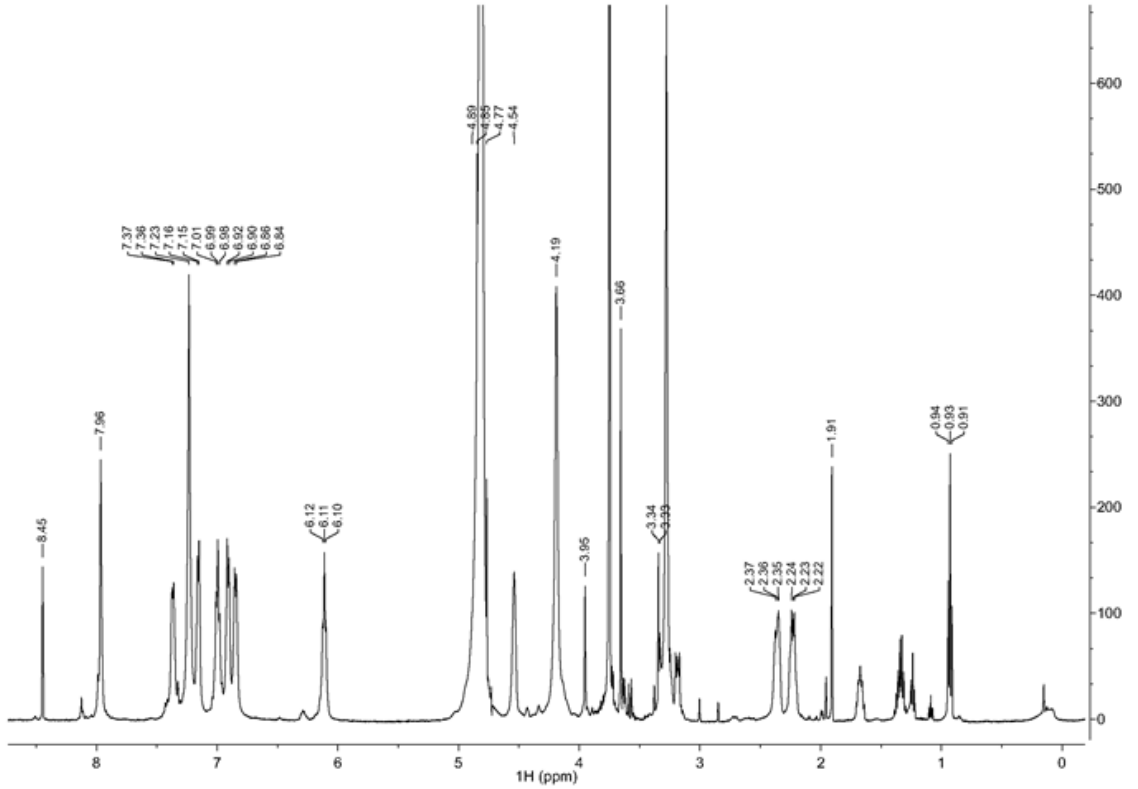
^{1}H NMR, ^{13}C and ^{31}P spectra of **dA^{PT}TP.**

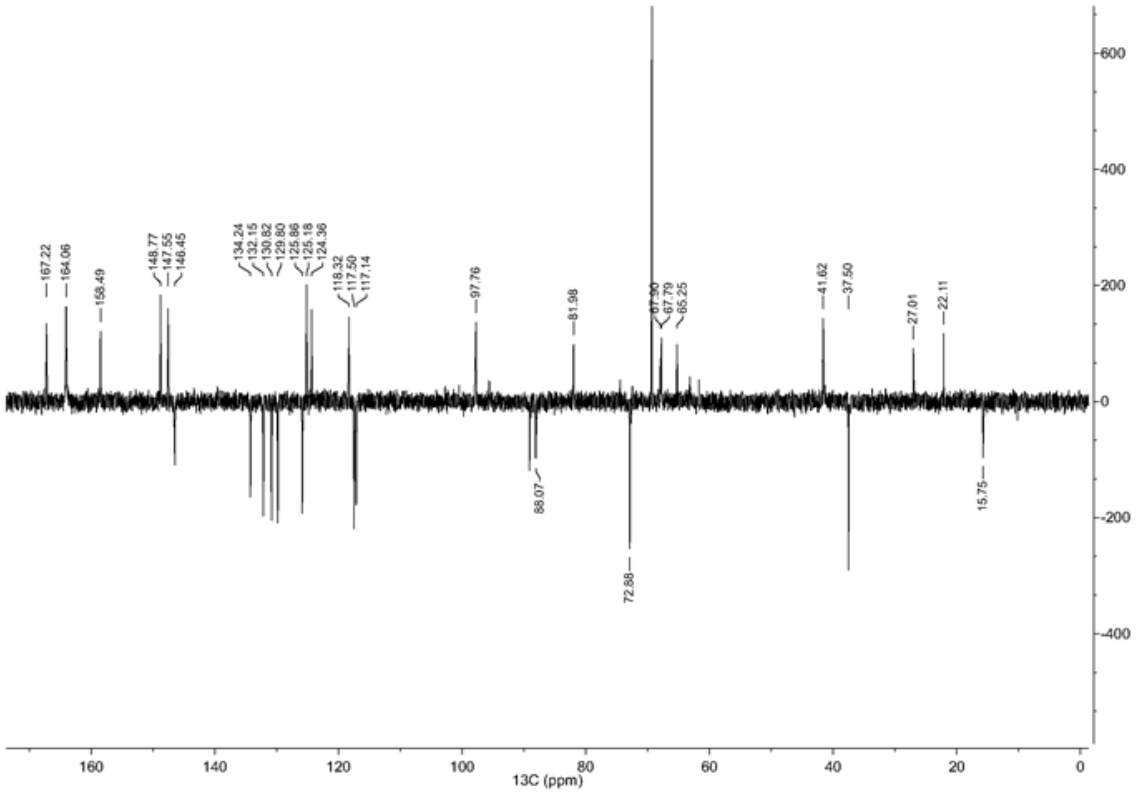


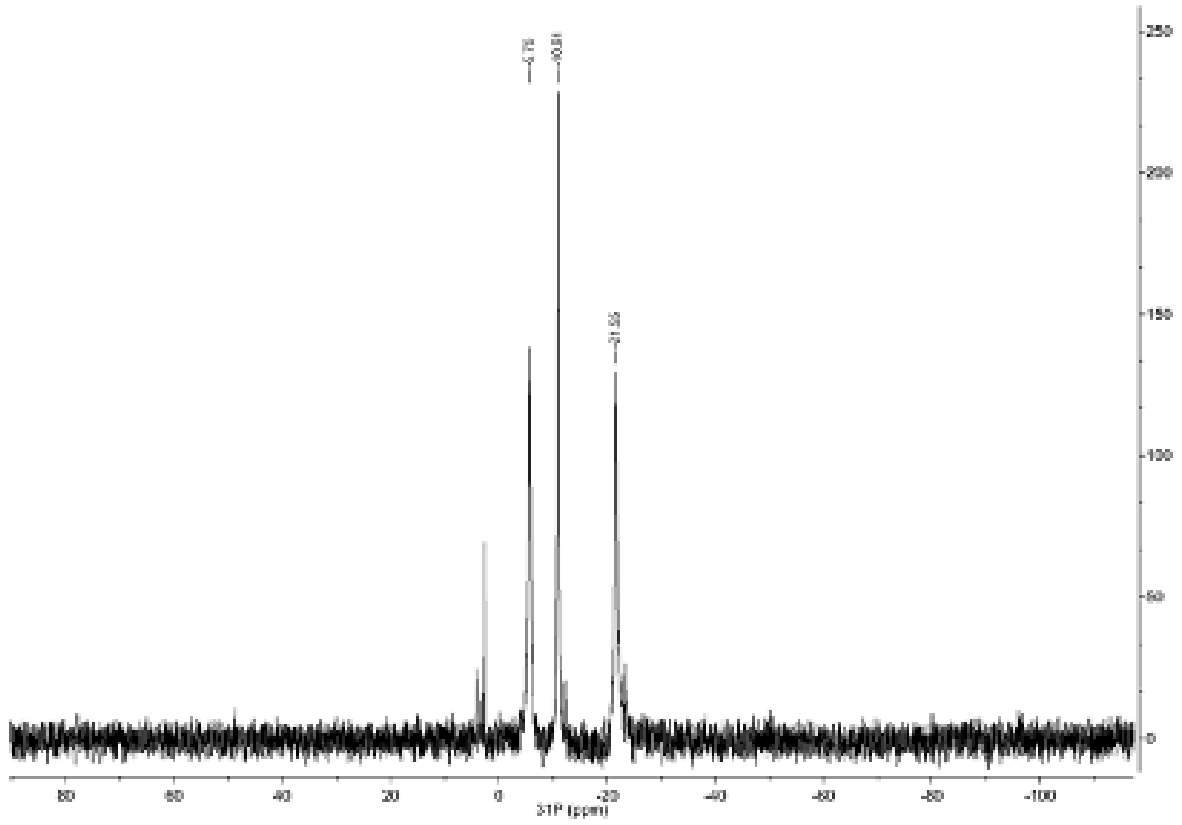

SIMONOVA AS-412_3A
APT in D2O
14-02-17 RA
****
159.68
153.94
152.48
147.94
147.29
130.64
130.49
130.45
129.84
129.27
125.66
125.55
124.72
122.21
119.88
117.57
117.22
103.39
87.77
87.70
73.75
68.32
68.27
40.72
37.27
100
0
-100
160
140
120
100
80
60
40
20
0
13C (ppm)


SIMONOVA AS-412_3A
31P{1H} NMR in D2O
14-02-17 RA
****
-1169.26
-1189.37
-2191.41
-2210.64
-5.78
-5.88
-10.83
-10.92
-21.65
-21.75
-21.84
-4381.57
-4401.08
-4420.34
150
100
50
0
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
-24
31P (ppm)
100
50
0
-50
-100
-150
31P (ppm)

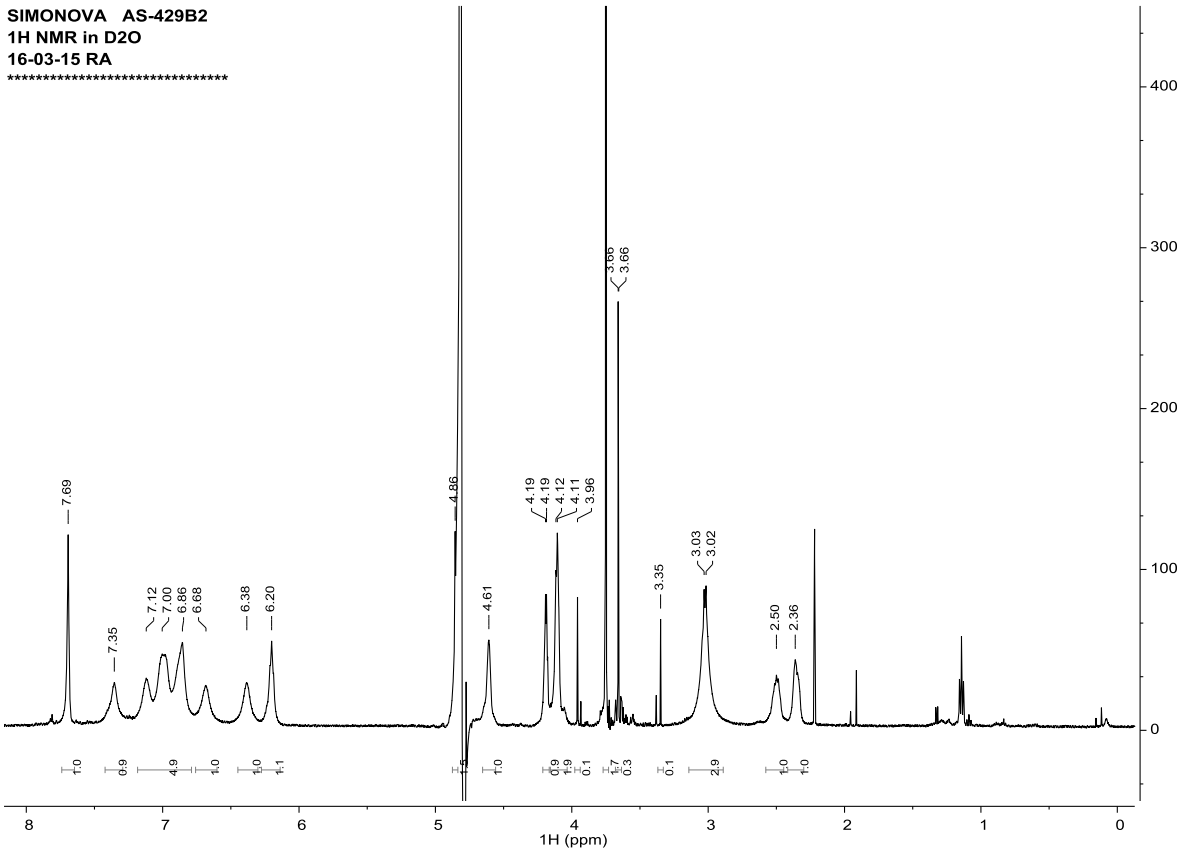
^{1}H NMR, ^{13}C and ^{31}P spectra of **dC^{EPT}TP.**

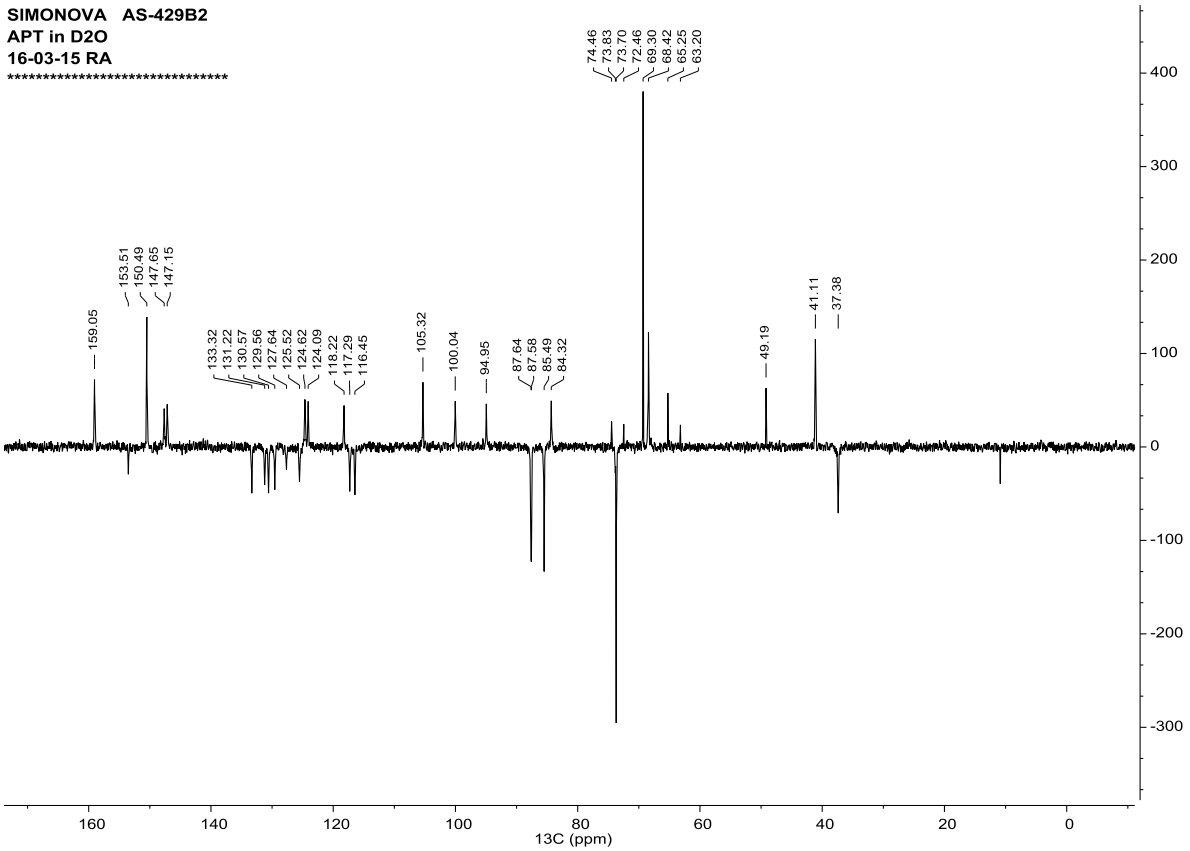


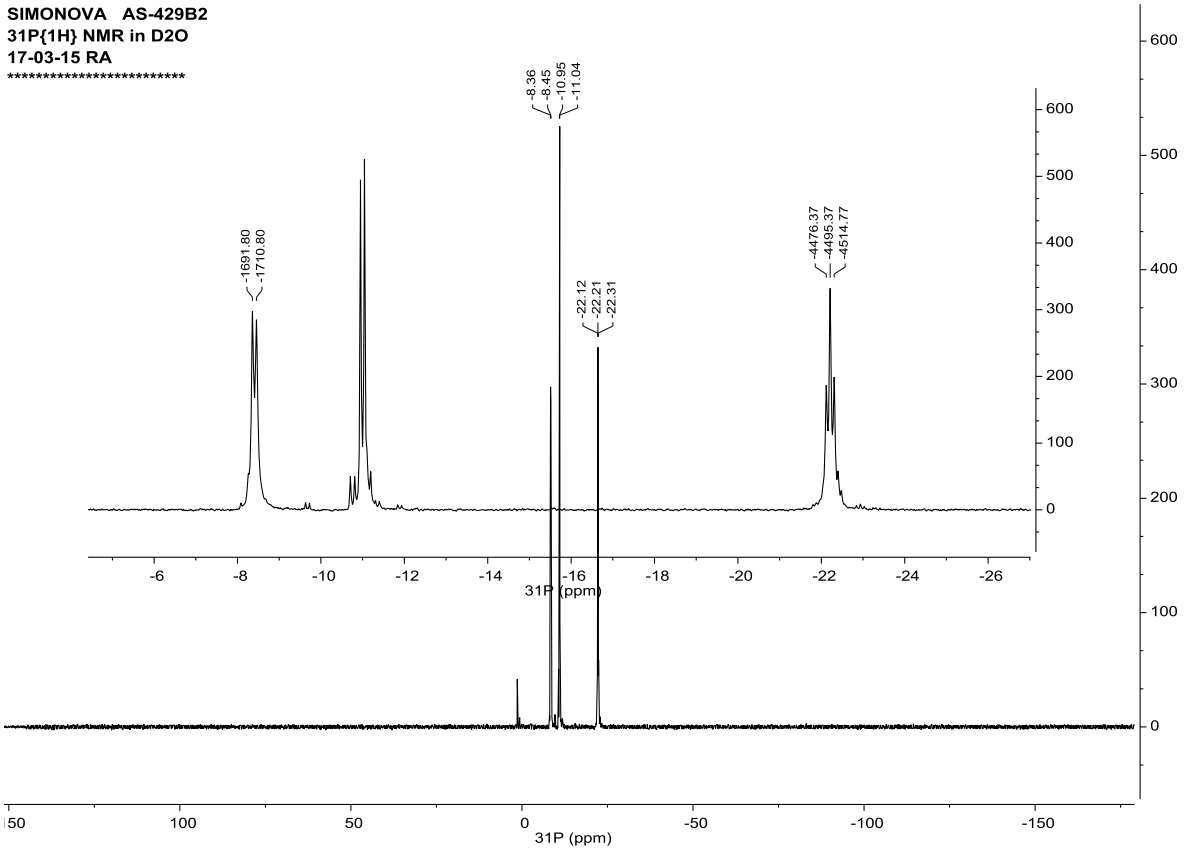




^{1}H NMR, ^{13}C and ^{31}P spectra of **dA^{EPT}TP.**




SIMONOVA AS-429B2
APT in D2O
16-03-15 RA
****
74.46
73.83
73.70
72.46
69.30
68.42
65.25
63.20
153.51
150.49
147.65
147.15
159.05
133.32
131.22
130.57
129.56
127.64
125.52
124.62
124.09
118.22
117.29
116.45
105.32
100.04
94.95
87.64
87.58
85.49
84.32
49.19
41.11
37.38
13C (ppm)


SIMONOVA AS-429B2
31P{1H} NMR in D2O
17-03-15 RA
****
-8.36
-8.45
-10.95
-11.04
-1691.80
-1710.80
-22.12
-22.21
-22.31
-4476.37
-4495.37
-4514.77
31P (ppm)

8. Additional electrochemistry data

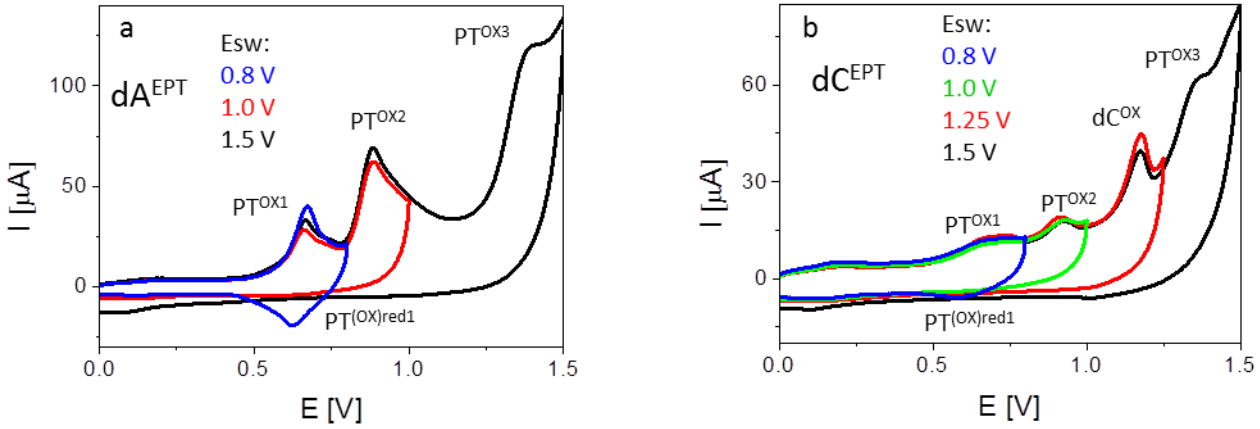


Figure S36 CV responses of **dA^{EPT}** (a), **dC^{EPT}** (b) at PGE. C = 40 µM, electrolyte 0.2 M acetate buffer (pH 5,0). CV parameters: scan rate 1V/s, Ei = 0.0 V, Esw see legend in the Figure.

(Supplement to Fig. 6 that shows CVs of PT and **dN^{PT}** conjugates)

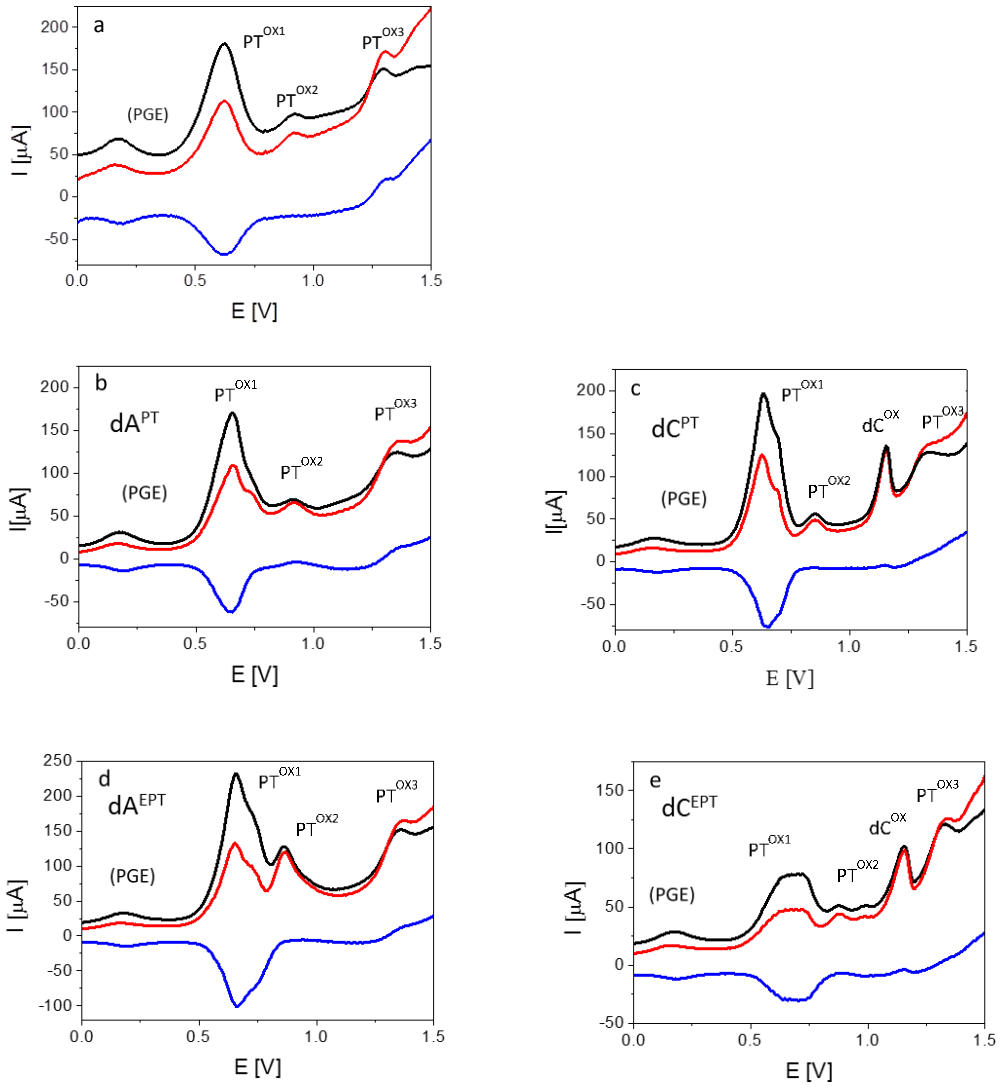


Figure S37 Components of the SWV current of phenothiazine (a) , **dA^{PT}** (b), **dC^{PT}** (c), **dA^{EPT}** (d), **dC^{EPT}** (e) at PGE. C = 40 µM, electrolyte 0.2 M acetate buffer (pH 5,0). parameters: frequency 200 Hz, amplitude 50 mV, Ei = 0.0 V. net current (black), forward current (red), backward 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

	PT^{ox1}/mV	PT^{ox2}/mV	PT^{ox3}/mV	dC/mV
PT	625	920	1290	-
dA^{PT}	655*	920	1355	-
dA^{EPT}	655*	860	1360	-
dC^{PT}	630*	855	1330	1160
dC^{EPT}	640/720#	880/985	1330	1160

	PT^{ox1}/mV	PT^{ox2}/mV	PT^{ox3}/mV	PT^{ox2}/$G(A^*)^{ox}$/ mV	$G(A^*)^{ox}$/ mV	A^{ox}/mV
ON^{nick_4A} **A^{PT}**	460	790	1285	-	1060	-
ON^{nick_4A}**A^{EPT}**	510	-	1320	995	-	-
ON^{nick} natural	-	-	-	-	1080	1360

	PT^{ox1}/mV	PT^{ox2}/mV	G^{ox}/mV	A^{ox}/mV
ON^{rnd16} **A^{PT}**	550	815	1090	1360
ON^{rnd16} natural	-	-	1080	1360

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

S1 W. H. Melhuish, W. H. *J. Phys. Chem.*, 1961, **65**, 229–235

S2 C. Würth, M. Grabolle, J. Pauli, M. Spieles, U. Resch-Genger, *Nature Protocols* , 2013, **8**, 1535–1550.