Supplementary Material for Organic & Biomolecular Chemistry
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Electronic Supporting Information

for

Synthesis of 8-bromo-, 8-methyl- and 8-phenyl-dATP and their polymerase incorporation to DNA

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Experimental section

General

NMR spectra were measured on a Bruker Avance 600 (600 MHz for ^{1}H and 151 MHz for ^{13}C nuclei) and a Bruker 500 (500 MHz for ^{1}H, 125.7 MHz for ^{13}C and 202.3 for ^{31}P) in D_2O (referenced to dioxane as internal standard, δ_H = 3.75 ppm, δ_C = 69.3 ppm, standard for ^{31}P NMR was external H_3PO_4). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Complete assignment of all NMR signals was performed using a combination of H,H-COSY, H,C-HSQC and H,C-HMBC experiments. Mass spectra were measured on LCQ classic (Thermo-Finnigan) spectrometer using ESI or Q-Tof Micro (Waters, ESI source, internal calibration with lockspray). MALDI-TOF spectra were measured on Reflex IV (Bruker Daltonics, Germany).Preparative HPLC separations were performed on a column packed with 10 μm C18 reversed phase (Phenomenex, Luna C18(2)).

Synthesis of 8-Br-dATP

Synthesis and characterization data for 9-(2-Deoxy-β-D-erythro-pentofuranosyl)-8-bromoadenine 5'-O-triphosphate were reported previously:

L. S. Gordeeva, Yu, L. Kaminskii, N. L. Rumyantseva, N. A. Patokina, N. A. Korsakova, L. F. Chernysheva, V. K. Dedova, V. L. Efimova, A. G. Neopikhanova, Khim. Prir. Soedin. **1984**, 6, 771-776.

Characterization of modified dNTPs

8-methyl-2'-deoxyadenosine 5'-*O*-triphosphate

Yield 40%. MS(ESI-): 504,1 (100, M-1), 526,1 (M +Na), HRMS: for C11 H17 N5 O12 P3 calculated 504.0087 found 504.0098. ^{1}H NMR (500 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pH = 7.1): 2.40 (ddd, 1H, J_{gem} = 14.1, $J_{2'b,1'}$ = 7.0, $J_{2'b,3'}$ = 3.7, H-2′b); 2.69 (s, 3H, CH_3); 3.10 (dt, 1H, J_{gem} = 14.1, $J_{2'a,1'}$ = $J_{2'a,3'}$ = 7.6, H-2′a); 4.19-4.32 (m, 3H, H-4′ and H-5′); 4.84 (bm, 1H, H-3′); 6.48 (dd, 1H, $J_{1'2'}$ = 7.6, 7.0, H-1′); 8.19 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pH = 7.1): 17.37 (CH_3); 39.49 (CH_2-2′); 67.84 (d, $J_{C,P}$ = 6, CH_2-5′); 73.02 (CH-3′); 86.16 (CH-1′); 87.58 (d, $J_{C,P}$ = 9, CH-4′); 120.05 (C-5); 152.77 (C-4); 154.60 (CH-2); 154.64 (C-8);

157.11 (C-6). ^{31}P (^{1}H dec.) NMR (202.3 MHz, D_2O, ref_{H3PO4} = 0 ppm, pH = 7.1): -21.28 (dd, *J* = 19.4, 19.2, P_{β}); -10.32 (d, *J* = 19.2, P_{α}); -6.73 (d, *J* = 19.4, P_{γ}).

8-phenyl-2'-deoxyadenosine 5'-*O*-triphosphate

Yield 65%. MS(ESI-): 566.4 (25, M-1), 486.4 (100, M – PO_3H_2 – 1), 588,4 (25, M+Na) HRMS: for C16 H19 N5 O12 P3 calculated 566.0243 found 566.0245 . ^{1}H NMR (500 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pH = 7.1): 2.23 (ddd, 1H, J_{gem} = 14.1, $J_{2'b,1'}$ = 7.2, $J_{2'b,3'}$ = 4.1, H-2′b); 3.26 (dt, 1H, J_{gem} = 14.1, $J_{2'a,1'}$ = $J_{2'a,3'}$ = 7.5, H-2′a); 4.12-4.22 (m, 2H, H-4′ and H-5′b); 4.29 (dt, 1H, J_{gem} = 10.5, $J_{5'a,4'}$ = $J_{H,P}$ = 4.0, H-5′a); 4.64 (ddd, 1H, $J_{3',2'}$ = 7.5, 4.1, $J_{3',4'}$ = 3.8, H-3′); 6.33 (dd, 1H, $J_{1'2'}$ = 7.5, 7.2, H-1′); 7.57-7.66 (m, 3H, H-*m,p*-Ph); 7.69 (m, 2H, H-*o*-Ph); 8.26 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pH = 7.1): 38.55 (CH_2-2′); 68.13 (d, $J_{C,P}$ = 6, CH_2-5′); 73.38 (CH-3′); 87.31 (CH-1′); 87.55 (d, $J_{C,P}$ = 8, CH-4′); 121.27 (C-5); 131.02 (C-*i*-Ph); 131.73 (CH-*m*-Ph); 132.27 (CH-*o*-Ph); 133.73 (CH-*p*-Ph); 152.82 (C-4); 155.05 (CH-2); 155.65 (C-8); 157.77 (C-6). ^{31}P (^{1}H dec.) NMR (202.3 MHz, D_2O, ref_{H3PO4} = 0 ppm, pH = 7.1): -21.41 (dd, *J* = 19.1, 17.9, P_{β}); -10.22 (d, *J* = 19.1, P_{α}); -7.46 (d, *J* = 17.9, P_{γ}).

Primer extension experiment: The reaction mixture (20 µl) contained Klenow(exo-) (New England Biolabs, 0.125 unit), Dynazyme (Finnzymes, 0.1 unit), Vent(exo-) (New England Biolabs, 0.1 unit) or Pwo DNA polymerase (PeqLab, 0.1 unit), natural dNTPs (Fetmentas, 0.1 mM), 8-modified dATP (1 mM), primer (sequence see Table 1, VBC genomics, 0.15 µM), template (sequence see table 1, VBC genomics, 0.225 µM) in polymerase reaction buffer supplied by the manufacturer. Primer was labeled by use of [γ^{32}P]-ATP according to standard techniques. Reaction mixtures were incubated for 30 min at 37 °C (using Klenow(exo-)) or 60 °C (using the rest of polymerases) in a thermal cycler and were stopped by addition of stop solution (40 µL, 80% [v/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol). Reaction mixtures were separated by use of a 12.5 % denaturing PAGE. Visualization was performed by phosphoimaging.

Supplementary results-PAGE of PEX:

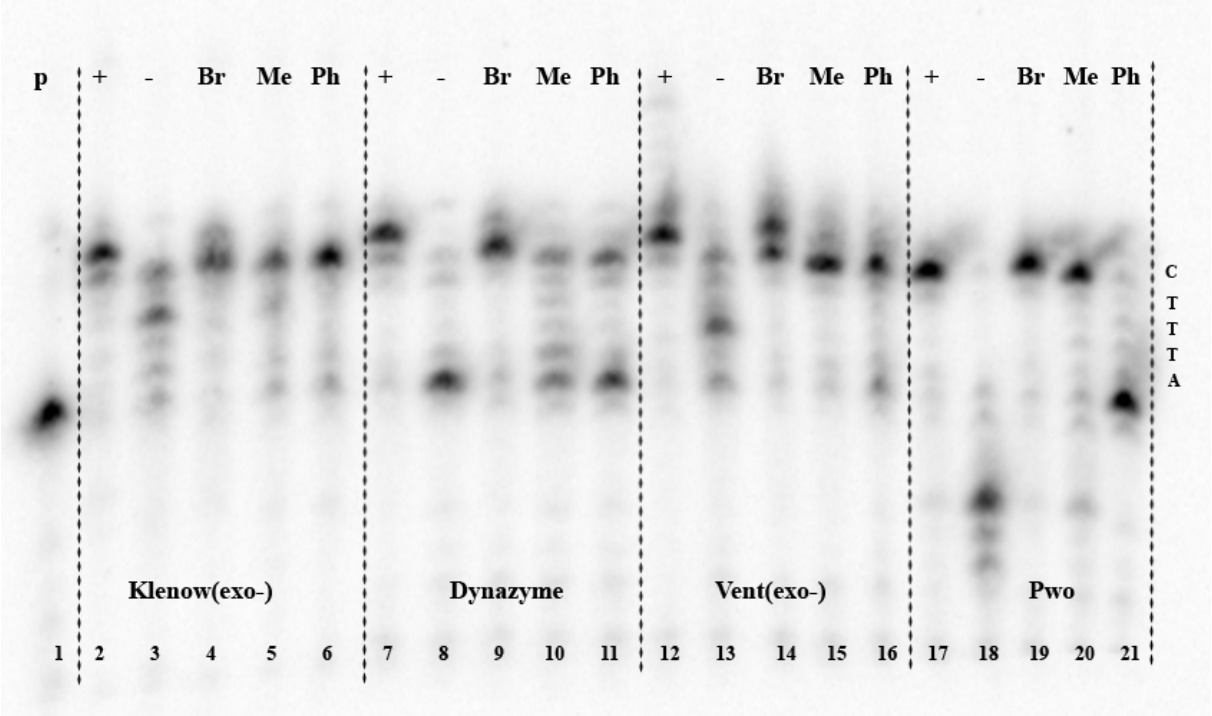


Figure S 1: PEX with temp^{A} dCTP, dTTP, dATP (lines 2, 7, 12, 17); dCTP and dTTP (lines 3, 8, 13, 18), dCTP, dTTP and 8-mod. dATP (lines 4, 5, 6, 9, 10, 11, 14, 15, 16, 19, 20, 21), line 1-^{32}P radiolabeled primer.

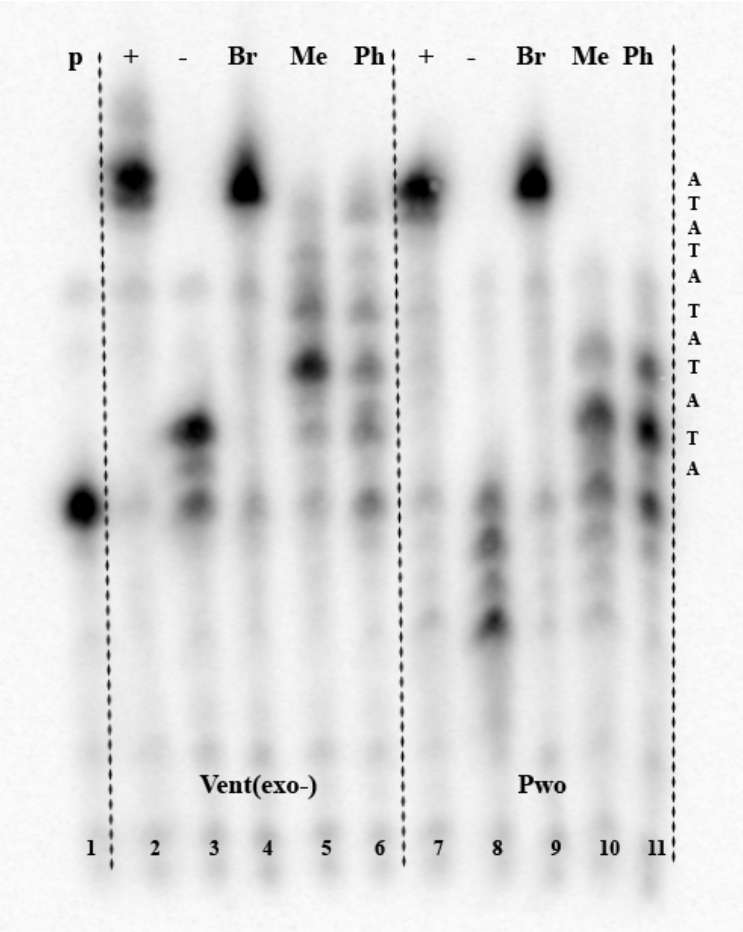


Figure S 2: PEX with temp^{ATr} dTTP, dATP (lines 2, 7); dTTP (lines 3, 8), dTTP and 8-mod. dATP (lines 4, 5, 6, 9, 10, 11), line 1-^{32}P radiolabeled primer.

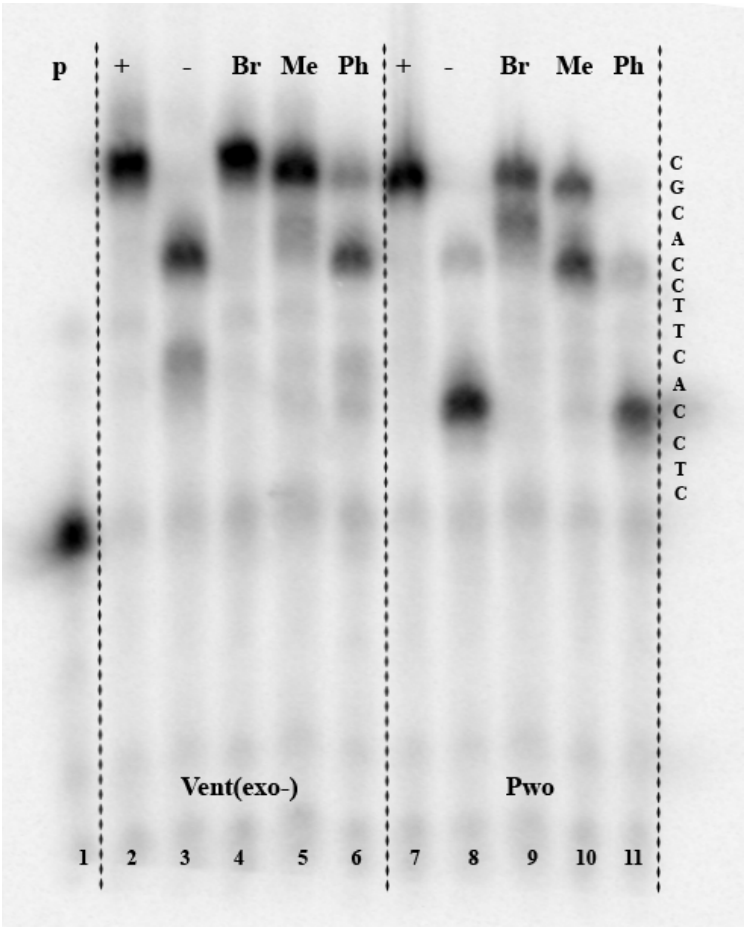


Figure S 3: PEX with temp[2A] dCTP, dGTP, dTTP, dATP (lines 2, 7); dCTP, dGTP and dTTP (lines 3, 8), dCTP, dGTP, dTTP and 8-mod. dATP (lines 4, 5, 6, 9, 10, 11), line 1-^{32}P radiolabeled primer.

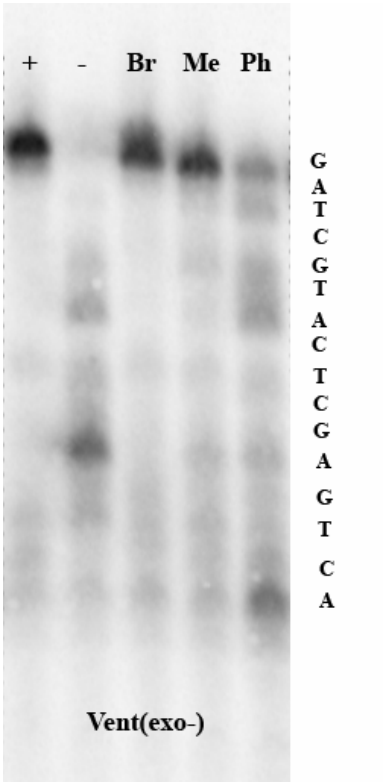


Figure S 4: Preparative PEX with temp^{4A} and Vent(exo-) polymerase. dCTP, dGTP, dTTP, dATP (+); dCTP, dGTP and dTTP (-), dCTP, dGTP, dTTP and 8-mod. dATP (Br, Me, Ph), line 1-32P radiolabeled primer.

CD spectroscopy and thermal denaturation studies: CD spectra and melting temperatures were determined for a functionalized DNA duplex from PEX in large scale. DNA duplexes containing all natural nucleotides served as control. For preparative purposes a total volume of 500 µl PEX was run and purification was carried out with a QIAquick Nucleotide Removal Kit (Qiagen). Samples were eluted with 100 µl H_2O (pH 7.5) and then freeze-dried. DNA duplexes were first dissolved in 160 µl of phosphate buffer (10mM) and 1M NaCl (pH 7) and further diluted with the buffer to optimum concentration-OD_{260} between 0.08 and 0.1. Thermal denaturation studies were performed on Cary 100 Bio (UV/Visible spectrometer with temperature controller, Varian). Data were obtained from six individual cooling/ heating cycles. Melting temperatures (T_m values in °C) were obtained by plotting temperature versus absorbance and by applying a sigmoidal curve fit. The samples were the same as for CD spectra. CD spectra were recorded on a Jasco 815 spectropolarimeter (Japan) at room temperature. The optical path length was 0.1 cm and CD signal was monitored from 190 nm to 350 nm. For each experiment the data is average of 10 scans taken with the time constant 4 s with blank subtracted. The CD spectra are expressed in ellipticities (mdeg).

Preparation of samples for measurement of melting temperatures and CD spectra:

Primer extension experiment in large scale

The reaction mixture (500 µl) contained Vent(exo-) (New England Biolabs, 2.5 unit), natural dNTPs (Fermentas, 0.1 mM), 8-modified dATP (1 mM), primer (sequence see table1, VBC genomics, 2 µM), template (sequence see table 1, VBC genomics, 2 µM) in polymerase reaction buffer supplied by the manufacturer. Reaction mixtures were incubated for 30 min 60 °C in a thermal cycler then purified by QIAquick Nucleotide Removal Kit (Qiagen), eluted with 100 µl H_2O (pH 7.5). Products were after purification freeze-dried.

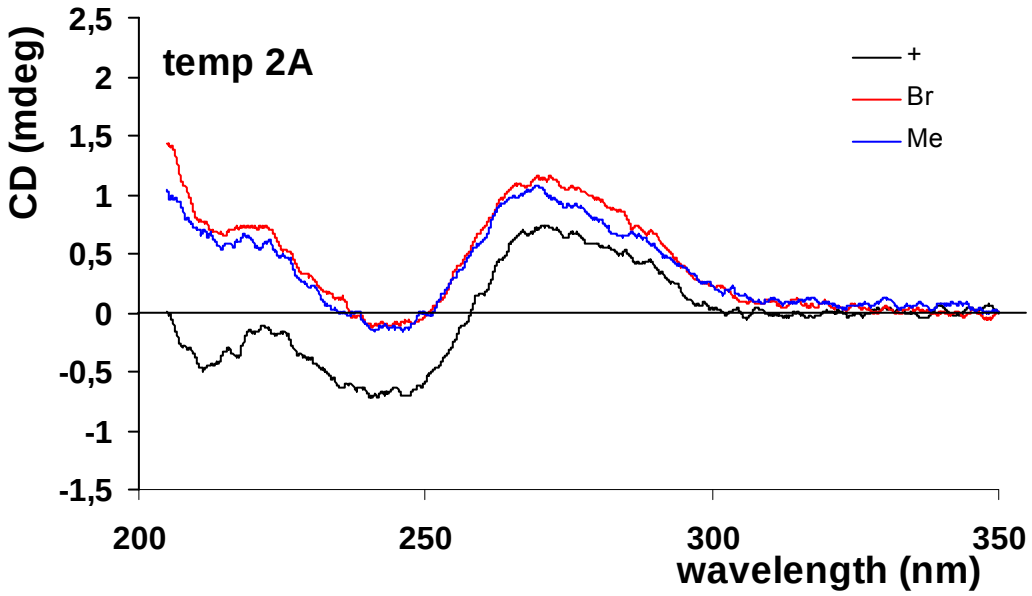


Figure S5: CD spectra of PEX products with temp[2A] measured 4 days after isolation (+: Natural DNA, Br: DNA bearing one bromine on A, Me: DNA bearing one methyl on A)

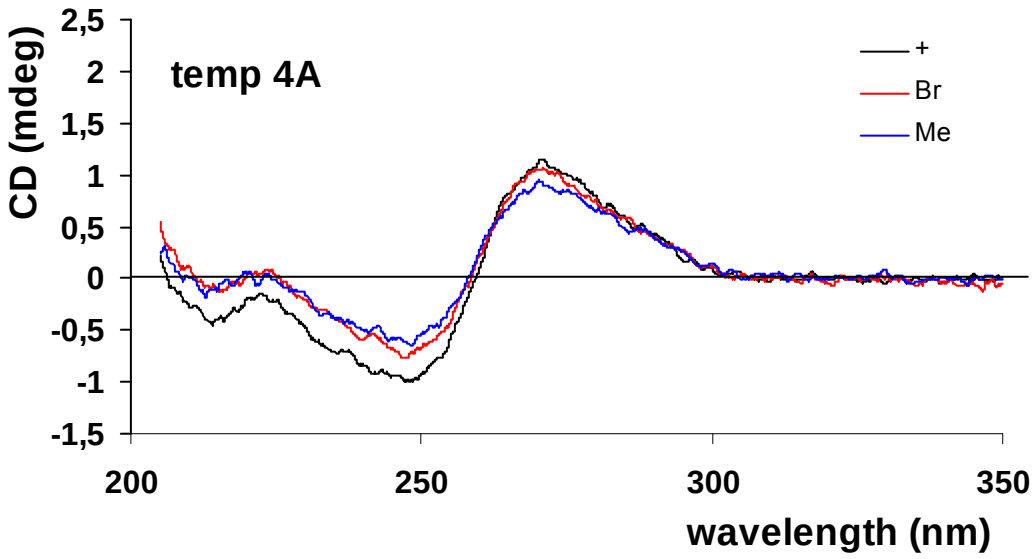


Figure S6: CD spectra of PEX products with temp^{4A} measured 4 days after isolation (+: Natural DNA, Br: DNA bearing one brome on A, Me: DNA bearing one methyl on A)

Preparation of samples for MALDI-TOF analysis:

The reaction mixture (100 µl) contained Vent(exo-) (New England Biolabs, 0.5 unit), natural dNTPs (Fermentas, 0.1 mM), 8-modified dATP (1 mM), primer (sequence see table1, VBC genomics, 2 µM), 5-biotinylated template (sequence see table 1, VBC genomics, 2 µM) in polymerase reaction buffer supplied by the manufacturer. Reaction mixtures were incubated for 30 min 60 °C in a thermal cycler and then captured at DB_{Stv} via biotin tags tethered to the 5'-ends of the template strands. The PEX reaction mixtures were added to the DB_{Stv} [50 µl of the stock suspension washed twice by 100 µl of 0.3 M NaCl, 10 mM Tris-HCl, pH 7.4 (buffer H)]. The mixtures were incubated on a shaker for 30 min at 20 °C. Then the beads were subsequently washed three times by 400 µl of PBS (0.14 M NaCl, 3 mM KCl, 4 mM sodium phosphate pH 7.4), three times by 400 µl of the buffer H, after two times by water and resuspended in 100 µl of deionized water. The extended primer strands were released by heating at 75 °C for 2 min. Each medium exchange was performed using a magnetoseparator (Dynal, Norway). Products were after purification freeze-dried.

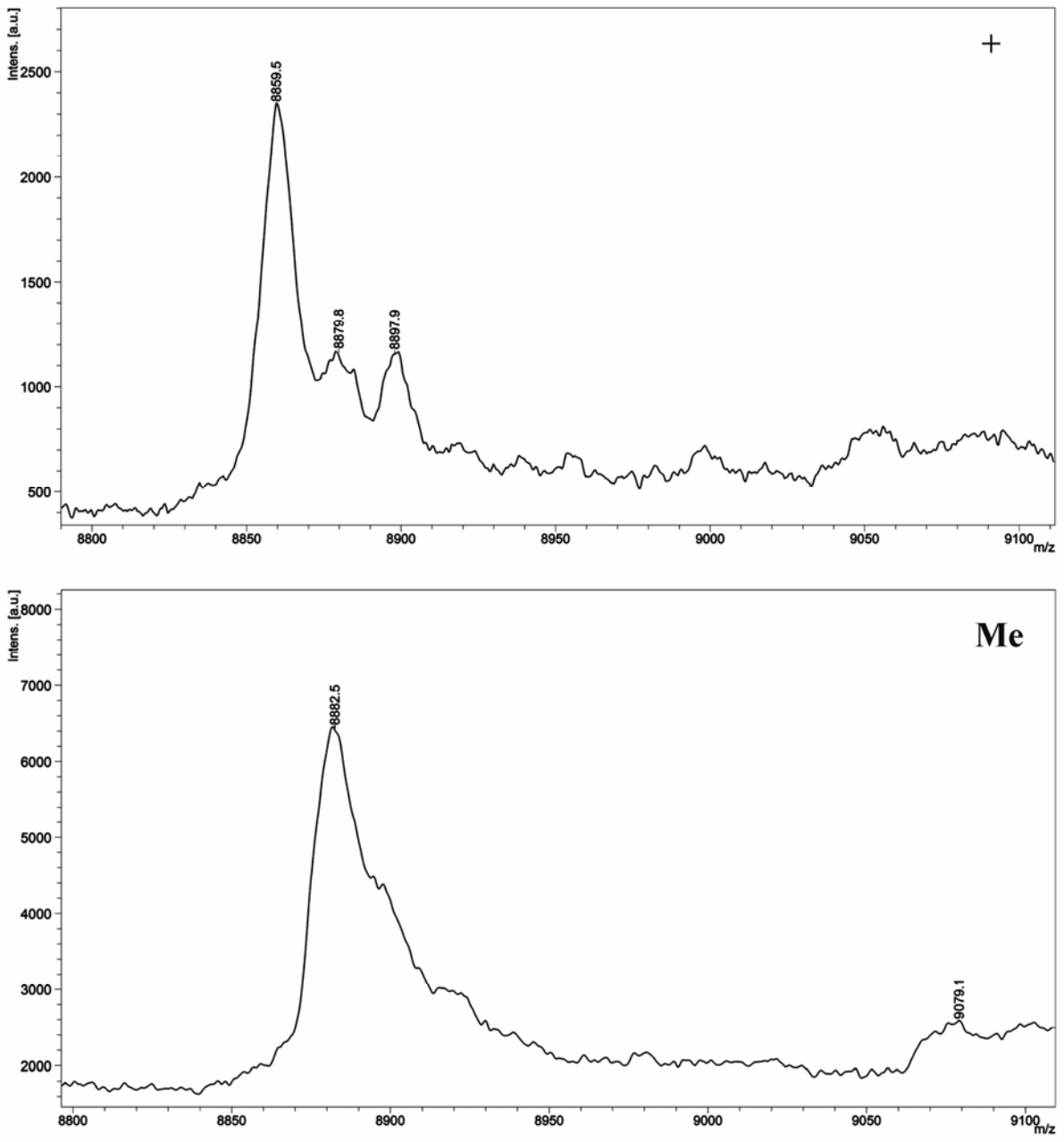
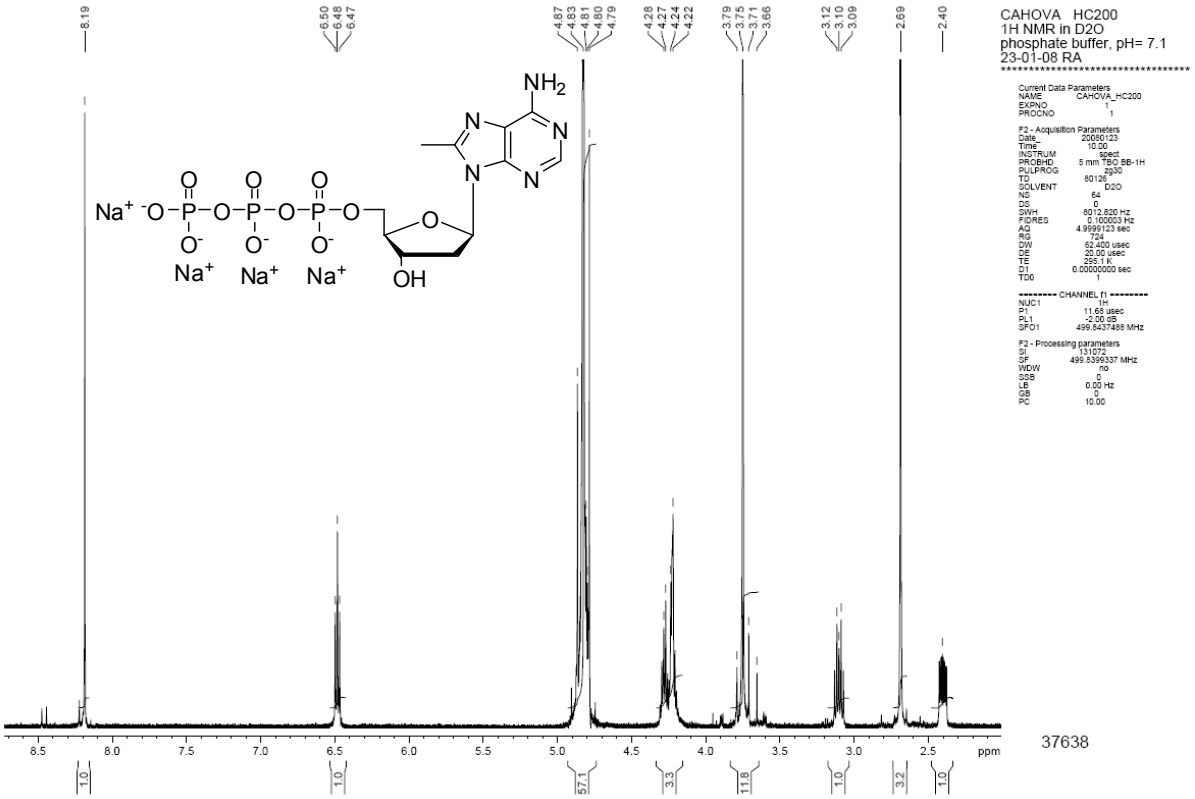
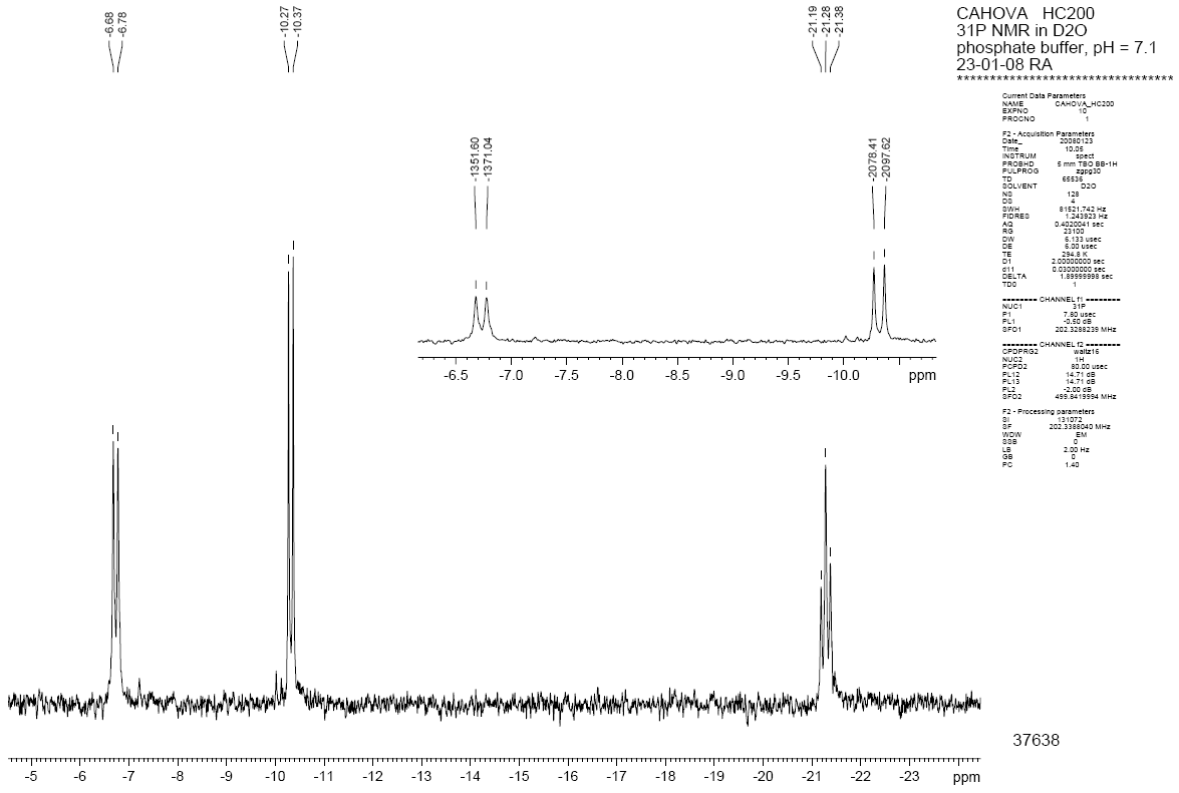


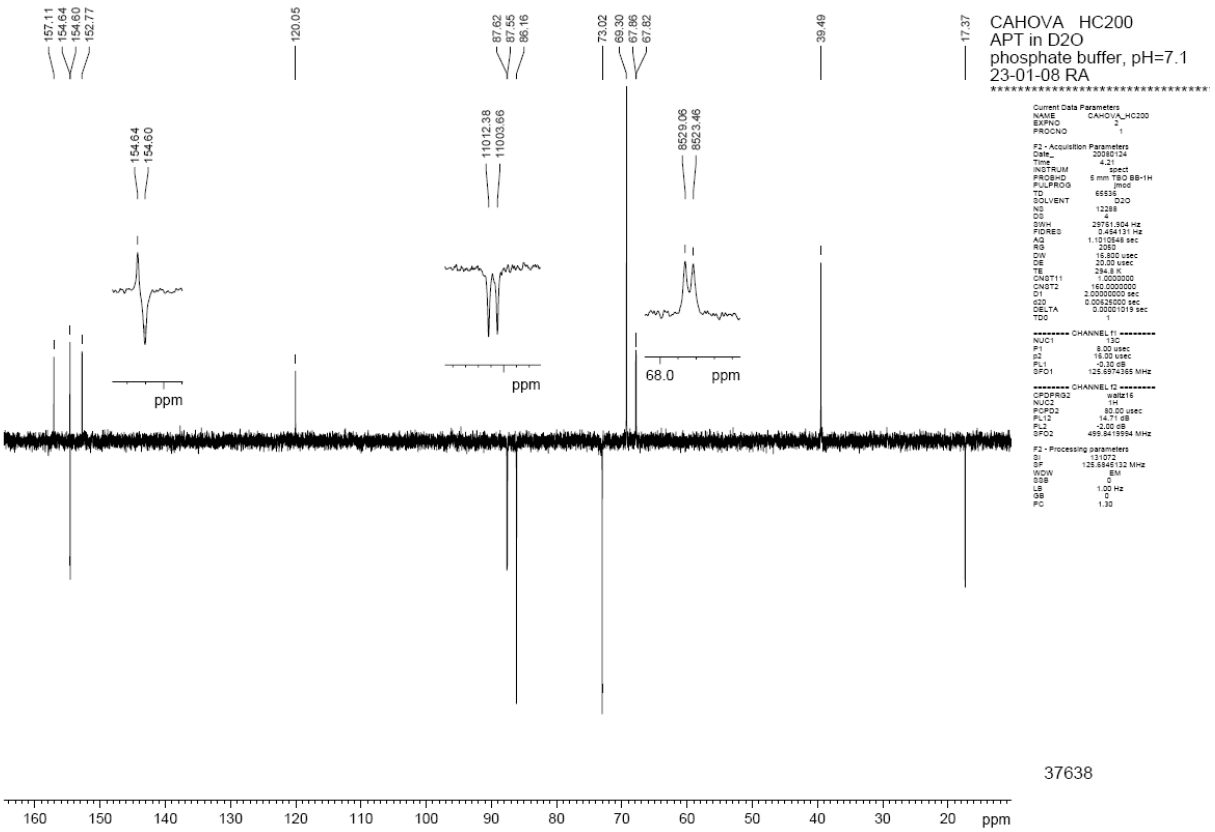
Figure S6: MALDI-TOF spectra of ssDNA products of PEX with temp^{2A} (+: Natural DNA (calculated mass: 8855.8 Da), Me: DNA bearing two methyl group on A(calculated mass: 8883.8Da))

Copies of NMR spectra

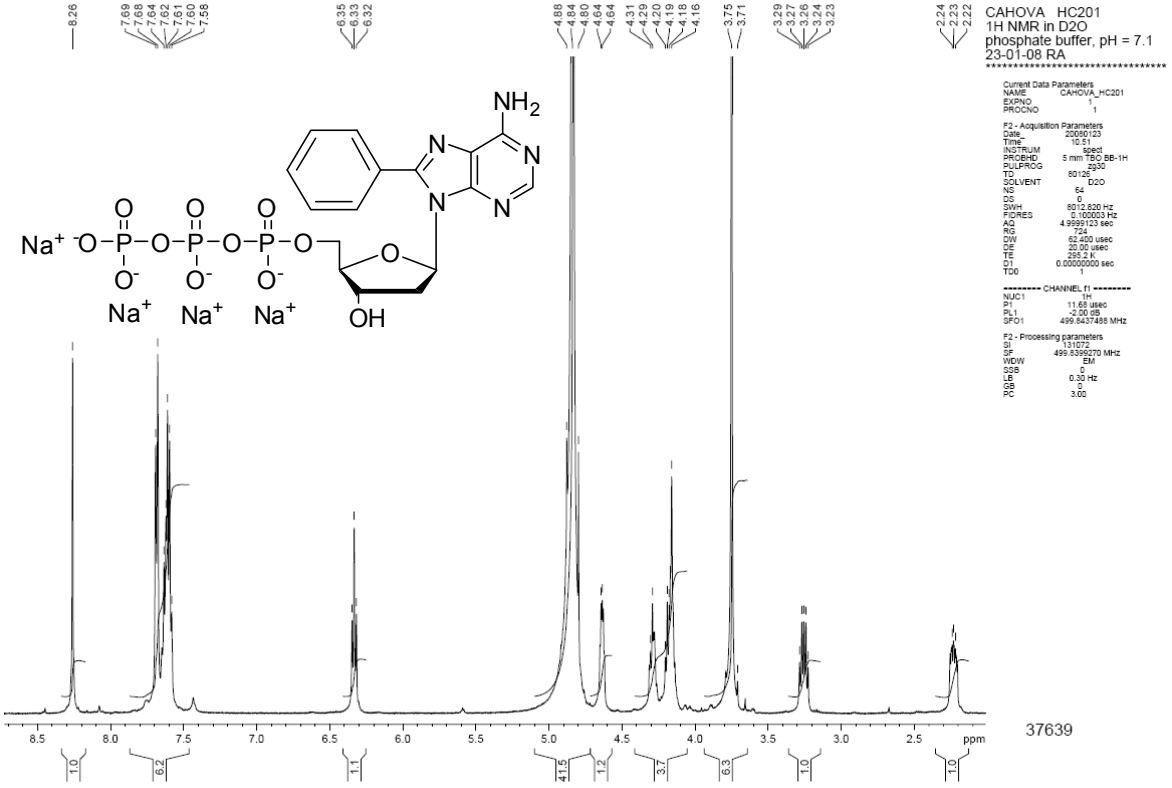
8-Me-dATP

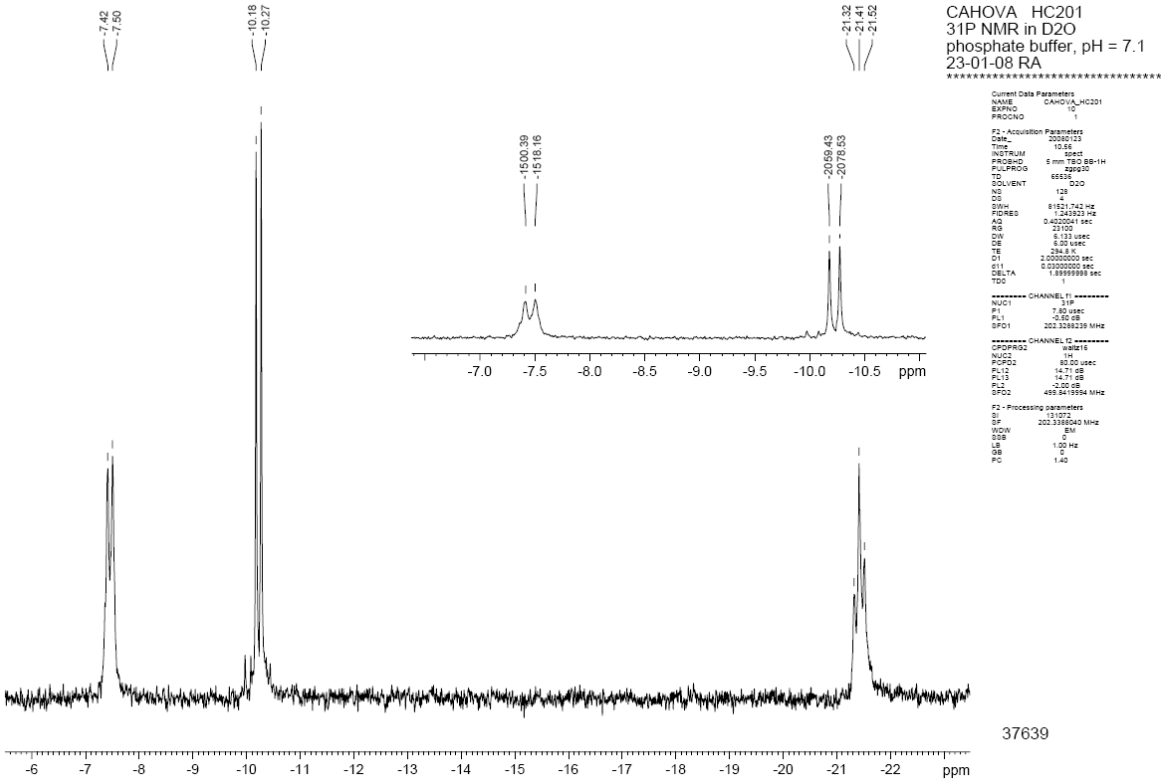


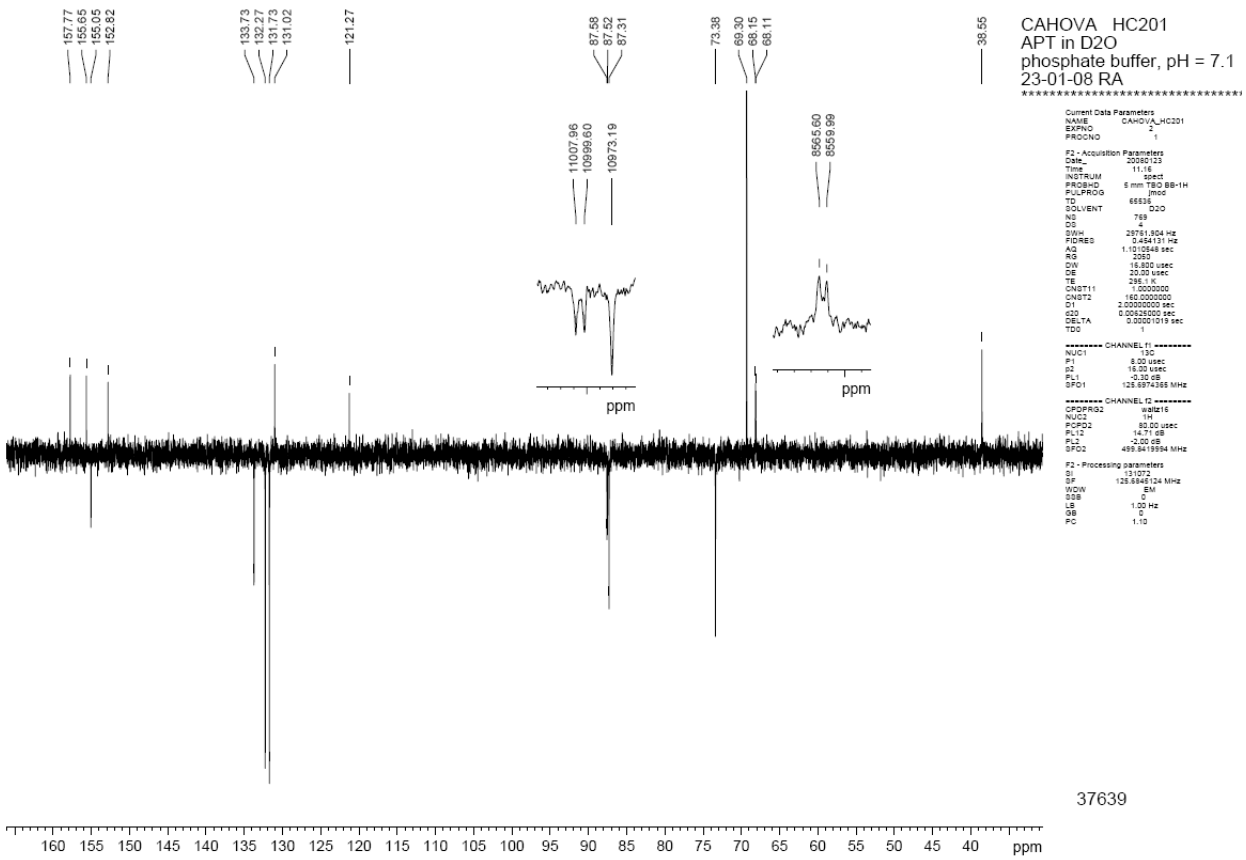



157.11
154.64
154.60
152.77
120.05
87.62
87.55
86.16
73.02
69.30
67.86
67.82
39.49
17.37
154.64
154.60
110 12.38
110 03.66
85 29.06
85 23.46
ppm
ppm
68.0
ppm
CAHOVA HC200
APT in D2O
phosphate buffer, pH=7.1
23-01-08 RA
37638
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

8-Ph-dATP


CAHOVA HC201
1H NMR in D2O
phosphate buffer, pH = 7.1
23-01-08 RA
37639
ppm


CAHOVA HC201
31P NMR in D2O
phosphate buffer, pH = 7.1
23-01-08 RA
37639
ppm


157.77
155.65
155.05
152.82
133.73
132.27
131.73
131.02
121.27
87.58
87.52
87.31
73.38
69.30
68.15
68.11
38.55
11007.96
10999.60
10973.19
8565.60
8559.99
ppm
ppm
CAHOVA HC201
APT in D2O
phosphate buffer, pH = 7.1
23-01-08 RA
37639
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 ppm