Electronic Supporting Information

for

Synthesis of nucleosides and dNTPs bearing oligopyridine ligands linked through octadiyne tether, their incorporation into DNA and complexation with transition metal ions

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Experimental

All reactions were performed under argon atmosphere. $POCl_3$ and $PO(OMe)_3$ used for phosphorylation of nucleoside were distilled before using. Other chemicals were purchased from commercial suppliers and were used as received. Preparative flash chromatography on reverse phase was performed on Biotage SP1 flash purification system. Semi-preparative HPLC separations were performed on column packed with 10 μm C18 reversed phase (Phenomenex, Luna C18(2)). NMR spectra were measured on a Bruker Avance 500 (500.0 MHz for ^{1}H, 125.7 MHz for ^{13}C and 202.3 for ^{31}P) or Bruker Avance II 600 (600.1 MHz for ^{1}H and 150.9 MHz for ^{13}C) in $CDCl_3$ (^{1}H referenced to TMS as an internal standard (δ = 0 ppm); ^{13}C referenced to the solvent signal (δ = 77.0 ppm)), in DMSO-d_6 (^{1}H referenced to the residual solvent signal (δ = 2.50 ppm); ^{13}C referenced to the solvent signal (δ = 39.7 ppm)), or in CD_3OD (^{1}H referenced to the residual solvent signal (δ = 3.31 ppm); ^{13}C referenced to the solvent signal (δ = 49.0 ppm); ^{31}P referenced to H_3PO_4 (δ = 0 ppm) as an external standard). Chemical shifts are given in ppm (δ scale), coupling constants (J) in Hz. Complete assignment of all NMR signals was achieved by use of 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). Mass spectra of functionalized DNA were measured by Maldi-TOF, Reflex IV (Bruker) with nitrogen laser. UV/Vis spectra were measured on Varian CARY 100 Bio spectrophotometer at room temperature.

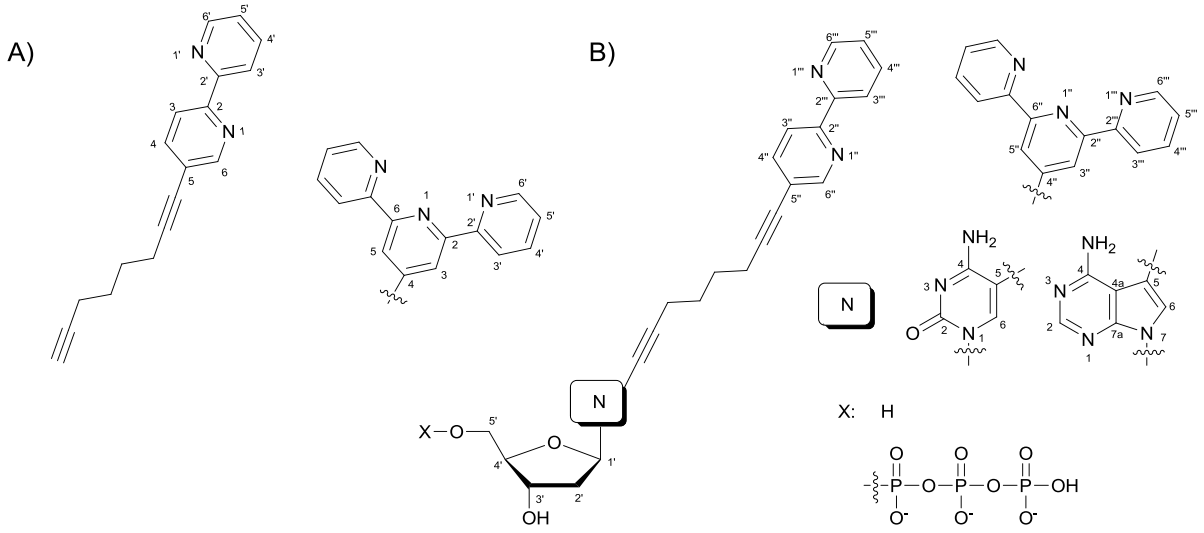

Figure S1. Numbering scheme for NMR assignment for: A) ligand building blocks; B) modified nucleosides and dNTPs.

General Procedure A: Synthesis of Ligand Building Blocks:

To an argone-purged flask containing 5-bromo-2,2'-bipyridine (**1a**) or (2,2':6',2''-terpyridine-4'-yl) trifluoromethanesulfonate (**1b**) (500mg), $Pd(PPh_3)_2Cl_2$ (5 mol%) and CuI (5 mol%) were added THF (10 mL), Et_3N (10 equiv.) and 1,7-octadiyne (3 equiv.). Reaction mixture was heated at 75 °C for 3 h. After evaporation of solvent under reduced pressure, the residue was extracted with three 100 mL portion of $CHCl_3$. Organic phases were combined and dried over $MgSO_4$. The residue was then purified by silica gel chromatography using hexane/ethyl acetate (0 % - 9 %).

5-(octa-1'',7''-diyn-1''-yl)-2,2'-bipyridine (3a)

Product was prepared according to general procedure A from 5-bromo-2,2'-bipyridine (**1a**). It was isolated as orange oil in the yield 66% (365.3 mg).

Mp 36.0 – 38.5 °C

IR: 3288, 2941, 1585, 1542, 1455, 1432, 1365 cm^{-1}.

^{1}H NMR (500.0 MHz, $CDCl_3$): 1.72 (m, 2H, HC≡C-CH_2C**H$_2$**CH_2CH_2-C≡C-bpy); 1.78 (m, 2H, HC≡C-CH_2CH_2C**H$_2$**CH_2-C≡C-bpy); 1.98 (t, 1H, ^4J = 2.7, HC≡C-); 2.27 (td, 2H, J_{vic} = 6.8, ^4J = 2.7, HC≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-bpy); 2.50 (t, 2H, J_{vic} = 6.9, HC≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-bpy); 7.40 (ddd, 1H, $J_{5',4'}$ = 7.5, $J_{5',6'}$ = 5.0, $J_{5',3'}$ = 1.2, H-5'); 7.84 (dd, 1H, $J_{4,3}$ = 8.3, $J_{4,6}$ = 2.1, H-4); 7.93 (ddd, 1H, $J_{4',3'}$ = 8.1, $J_{4',5'}$ = 7.5, $J_{4',6'}$ = 1.8, H-4'); 8.49 (m,

2H, H-3,3'); 8.69 (dd, 1H, $J_{6,4}$ = 2.1, $J_{6,3}$ = 0.9, H-6); 8.72 (ddd, 1H, $J_{6',5'}$ = 5.0, $J_{6',4'}$ = 1.8, $J_{6',3'}$ = 0.9, H-6').

^{13}C NMR (125.7 MHz, $CDCl_3$): 17.97 (HC≡C-**C**$H_2$$CH_2$$CH_2$$CH_2$-C≡C-bpy); 19.12 (HC≡C-$CH_2$$CH_2$$CH_2$**C**$H_2$-C≡C-bpy); 27.41, 27.56 (HC≡C-CH_2**C**H_2**C**$H_2$$CH_2$-C≡C-bpy); 68.64 (H**C**≡C); 77.79 (bpy-**C**≡C); 83.96 (HC≡**C**); 95.15 (bpy-C≡**C**); 120.95 (CH-3); 121.70 (C-5); 122.05 (CH-3'); 124.16 (CH-5'); 138.50 (CH-4'); 139.80 (CH-4); 147.88 (CH-6'); 151.62 (CH-6); 152.27 (C-2); 154.32 (C-2').

MS (ESI): *m/z* (%) = 261.1 (100) [M^+ + H], 283.1 (30) [M^+ + Na]

HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{18}H_{17}N_2$: 261.13863; found: 261.13813

Anal. Calcd for $C_{18}H_{16}N_2$·1/6MeOH: C, 82.13; H, 6.32; N, 10.54. Found: C, 82.36; H, 6.09; N, 10.23.

General Procedure B: Sonogashira Cross-Coupling Reaction - Synthesis of Modified Deoxynucleosides:

DMF (1 mL) and Et(*i*-Pr)$_2$N (0.25 mL, 10 equiv) were added to an argon-purged flask containing nucleoside 5-iodo-2`-deoxycytidine (**dC$^I$, 5**) or 7-iodo-7deaza-2`-deoxyadenosine (**dA^I, 4**) (50 mg), an octadiyne modified oligopyridine **3a-b** (1.5 equiv) and CuI (10 mol%). In a separate flask, Pd(OAc)$_2$ (5 mol%) and P(Ph-SO_3Na)$_3$ (2.5 equiv to Pd) were combined, evacuated and purged with argon followed by addition of DMF (0.5 mL). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 75 °C for 2 h. The solvent was then evaporated in vacuo. Products were directly purified by flash chromotagraphy on reverse phase using H_2O/MeOH (0% to 100%) as an eluent. Products were recrystallized from the mixture MeOH/H_2O.

7-[8''''-(2'',2'''-bipyridin-5''-yl)octa-1'''',7''''-diyn-1''''-yl)-7-deaza-2'-deoxyadenosine (dA^{Obpy}, 6a)

Product **6a** was prepared according to general procedure B from **dA^I** (**4**) and **3a**. It was isolated as a brownish powder in the yield of 46% (31.1 mg).

Mp 157 – 163 °C

IR: 3398, 3324. 2934, 1632, 1572, 1456, 1295, 1200, 1087, 1036 cm^{-1}.

^{1}H NMR (600.1 MHz, DMSO-d_6): 1.74 (m, 4H, dapur-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-bpy); 2.16 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.7, H-2′b); 2.46 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 8.2, $J_{2'a,3'}$ = 5.7, H-2′a); 2.56 (m, 2H, dapur-C≡C-C**H$_2$**$CH_2$$CH_2$$CH_2$-C≡C-bpy); 2.57 (m, 2H,

dapur-C≡C-$CH_2CH_2CH_2C\mathbf{H_2}$-C≡C-bpy); 3.50 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 5.8, $J_{5'b,4'}$ = 4.4, H-5′b); 3.75 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,OH}$ = 5.4, $J_{5'a,4'}$ = 4.4, H-5′a); 3.81 (td, 1H, $J_{4',5'}$ = 4.4, $J_{4',3'}$ = 2.5, H-4′); 4.30 (m, 1H, $J_{3',2'}$ = 5.7, 2.7, $J_{3',OH}$ = 4.1, $J_{3',4'}$ = 2.5, H-3′); 5.05 (dd, 1H, $J_{OH,5'}$ = 5.8, 5.4, OH-5′); 5.24 (d, 1H, $J_{OH,3'}$ = 4.1, OH-3′); 6.47 (dd, 1H, $J_{1',2'}$ = 8.2, 6.0, H-1′); 6.63 (bs, 2H, NH_2); 7.46 (ddd, 1H, $J_{5''',4'''}$ = 7.4, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5‴); 7.66 (s, 1H, H-6); 7.94 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.2, H-4″); 7.95 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4‴); 8.10 (s, 1H, H-2); 8.35 (dd, 1H, $J_{3'',4''}$ = 8.2, $J_{3'',6''}$ = 0.8, H-3″); 8.36 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.8, H-3‴); 8.690 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.8, H-6‴); 8.691 (dd, 1H, $J_{6'',4''}$ = 2.2, $J_{6'',3''}$ = 0.8, H-6″).

^{13}C NMR (150.9 MHz, DMSO-d_6): 18.56 (dapur-C≡C-$CH_2CH_2CH_2\mathbf{C}H_2$-C≡C-bpy); 18.66 (dapur-C≡C-$\mathbf{C}H_2CH_2CH_2CH_2$-C≡C-bpy); 27.49, 27.67 (dapur-C≡C-$CH_2\mathbf{C}H_2\mathbf{C}H_2CH_2$-C≡C-bpy); 39.95 ($CH_2$-2′); 62.07 ($CH_2$-5′); 71.14 (CH-3′); 74.03 (dapur-**C**≡C); 77.97 (bpy-**C**≡C); 83.27 (CH-1′); 87.64 (CH-4′); 95.56 (dapur-C≡**C**); 95.27 (bpy-C≡**C**); 95.63 (C-5); 102.48 (C-4a); 120.10 (CH-3″); 120.54 (C-5″); 120.82 (CH-3‴); 124.55 (CH-5‴); 125.67 (CH-6); 137.56 (CH-4‴); 139.77 (CH-4″); 149.21 (C-7a); 149.58 (CH-6‴); 151.46 (CH-6″); 152.74 (CH-2); 153.89 (C-2″); 154.66 (C-2‴); 157.74 (C-4).

MS (ESI): *m/z* (%) = 509.1 (52) [M^++H], 531.1 (100) [M^+ + Na]

HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{29}H_{29}O_3N_6$: 509.22957; found: 509.22965.

5-[8''''-(2'',2'''-bipyridin-5''-yl)-octa-1'''',7''''-diyn-1''''-yl)-2'-deoxycytidine (dC^{Obpy}, 7a)

Product **7a** was prepared according to the general precedure B from **dC^I** (**5**) and **3a**. It was isolated as a brownish powder in the yield of 38% (26.1 mg).

Mp 187 – 193 °C

IR: 3414, 3185, 3092, 2935, 1632, 1456, 1106 cm^{-1}.

^{1}H NMR (600.1 MHz, DMSO-d_6): 1.70 (m, 4H, cyt-C≡C-$CH_2C\mathbf{H_2}C\mathbf{H_2}CH_2$-C≡C-bpy); 1.98 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.2, $J_{2'b,3'}$ = 6.1, H-2′b); 2.12 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.0, $J_{2'a,3'}$ = 3.4, H-2′a); 2.48 (m, 2H, cyt-C≡C-$C\mathbf{H_2}CH_2CH_2CH_2$-C≡C-bpy); 2.55 (m, 2H, cyt-C≡C-$CH_2CH_2CH_2C\mathbf{H_2}$-C≡C-bpy); 3.55, 3.61 (2 × ddd, 2 × 1H, J_{gem} = 11.9, $J_{5',OH}$ = 5.1, $J_{5',4'}$ = 3.7, H-5′); 3.78 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 3.4, H-4′); 4.20 (ddt, 1H, $J_{3',2'}$ = 6.1, 3.4, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.4, H-3′); 5.07 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 5.21 (d, 1H, $J_{OH,3'}$ = 4.3, OH-3′); 6.11 (dd, 1H, $J_{1',2'}$ = 7.2, 6.0, H-1′); 6.77 (bs, 1H, $NH_a\mathbf{H_b}$); 7.46 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5‴); 7.70 (bs, 1H, $N\mathbf{H_a}H_b$); 7.946 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 1.8, H-4″); 7.753

(ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4‴); 8.09 (s, 1H, H-6); 8.36 (dd, 1H, $J_{3'',4''}$ = 8.2, $J_{3'',6''}$ = 0.8, H-3″); 8.37 (ddd, 1H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.8, H-3‴); 8.690 (dd, 1H, $J_{6'',4''}$ = 1.8, $J_{6'',3''}$ = 0.8, H-6″); 8.693 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.8, H-6‴).

^{13}C NMR (150.9 MHz, DMSO-d_6): 18.59 (cyt-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-bpy); 18.85 (cyt-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-bpy); 27.49, 27.53 (cyt-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-bpy); 40.93 (CH_2-2′); 61.24 (CH_2-5′); 73.36 (CH-3′); 72.47 (cyt-**C**≡C); 77.98 (bpy-**C**≡C); 85.43 (CH-1′); 87.60 (CH-4′); 90.57 (C-5); 95.41 (bpy-C≡**C**); 95.56 (cyt-C≡**C**); 120.16 (CH-3″); 120.60 (C-5″); 120.86 (CH-3‴); 124.63 (CH-5‴); 137.64 (CH-4‴); 139.83 (CH-4″); 143.77 (CH-6); 149.64 (CH-6″); 151.51 (CH-6‴); 153.71 (C-2); 153.92 (C-2″); 154.68 (C-2‴); 164.62 (C-4).

MS (ESI): *m/z* (%) = 485 (10) [M^+ + H], 508 (100) [M^+ + Na]

HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{27}H_{28}O_4N_5$: 486.21358; found: 486.21341

Anal. Calcd for $C_{27}H_{27}O_4N_5$: C, 66.79; H, 5.61; N, 14.42. Found: C, 66.71; H, 5.52; N, 14.13.

5-[8'''''-(2'',2''':6''',2''''-terpyridin-4'''-yl)octa-1''''',7''''-diyn-1''''-yl] -2'-deoxcytidine (dC^{Otpy}, 7b)

Product **7b** was prepared according to general procedure B from **dC^I** (**5**) and **3b**. It was isolated as a brownish powder in the yield of 72% (57.4 mg).

Mp 155 - 169 °C

IR: 3438, 3320, 2940, 1643, 1582, 1467, 1048 cm^{-1}.

^{1}H NMR (600.1 MHz, DMSO-d_6): 1.73 (m, 4H, cyt-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-tpy); 1.97 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.2, $J_{2'b,3'}$ = 6.2, H-2′b); 2.11 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.4, H-2′a); 2.50 (m, 2H, cyt-C≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-tpy); 2.60 (m, 2H, cyt-C≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-tpy); 3.54, 3.60 (2 × ddd, 2 × 1H, J_{gem} = 11.9, $J_{5',OH}$ = 5.1, $J_{5',4'}$ = 3.7, H-5′); 3.77 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 3.4, H-4′); 4.19 (ddt, 1H, $J_{3',2'}$ = 6.2, 3.4, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 3.4, H-3′); 5.06 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 5.20 (d, 1H, $J_{OH,3'}$ = 4.2, OH-3′); 6.11 (dd, 1H, $J_{1',2'}$ = 7.2, 6.1, H-1′); 6.78 (bs, 1H, NH_a**H$_b$**); 7.52 (ddd, 2H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5‴); 7.70 (bs, 1H, N**H$_a$**H_b); 8.02 (ddd, 2H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4‴); 8.09 (s, 1H, H-6); 8.34 (s, 2H, H-3″,5″); 8.61 (ddd, 2H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 1.0, H-3‴); 8.72 (ddd, 2H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 1.0, H-6‴).

^{13}C NMR (150.9 MHz, DMSO-d_6): 18.52 (cyt-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-tpy); 18.85 (cyt-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-tpy); 27.37, 27.48 (cyt-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-tpy); 40.91

(CH_2-2′); 61.25 (CH_2-5′); 70.37 (CH-3′); 72.50 (cyt-**C**≡C); 79.03 (tpy-**C**≡C); 85.43 (CH-1′); 87.59 (CH-4′); 90.58 (C-5); 95.55 (cyt-C≡**C**); 96.50 (tpy-C≡**C**); 121.06 (CH-3‴); 122.25 (CH-3″,5″); 124.98 (CH-5‴); 133.56 (C-4″); 137.77 (CH-4‴); 143.81 (CH-6); 149.65 (CH-6‴); 153.72 (C-2); 154.51 (C-2″,6″); 155.48 (C-2‴); 164.62 (C-4).MS (ESI): *m/z* (%) = 563 (5) [M^+ + H], 585 (100) [M^+ + Na]

HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{32}H_{31}O_4N_6$: 563.24013; found: 563.23995.

General Procerude C: Phosphorylation of Oligopyridine Modified Nucleosides (dN^R) – Synthesis of Modified dN^{R}TPs:

Dry trimethyl phosphate (1 mL) was added to an argon-purged flask containing nucleoside analogue **dN^R** (**6a-b** or **7a-b**, 50 mg), cooled to 0 °C on ice followed by the addition of $POCl_3$ (1.5 equiv.). A solution of $(NHBu_3)_2H_2P_2O_7$ (5 equiv.,1 mL) in dry DMF with an addition of Bu_3N (4.5 equiv.) was prepared in separate flask and cooled down to 0 °C. Like this prepared solution was then added to the reaction mixture and stirred for 1.5 h and quenched by 2 M TEAB buffer (2 mL). The product was isolated on DEAE Sephadex column (150 mL) eluting with a gradient 0 to 1.2 M TEAB, evaporated, co-distilled with water (3 times) and re-purified by semi-preparative HPLC on C18 column using linear gradient of 0.1 M TEAB (triethylamonium bicarbonate) in H_2O to 0.1 M TEAB in H_2O/MeOH (1:1) as an eluent. Several co-distillations with water followed by freeze-drying from water, gave the products as brownish powder.

7-[8''''-(2'',2'''-bipyridin-5''-yl)octa-1'''',7''''-diyn-1''''-yl)-7-deaza-2'-deoxadenosine-5'-O-triphosphate (dA^{Obpy}TP, 8a).

This compound was prepared according the general procedure C from **dA^{Obpy}** (**6a**) in the yield of 35% (36.2 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.29 (t, 27H, J_{vic} = 7.3, C**H$_3$**CH_2N); 1.81 (m, 4H, dapur-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-bpy); 2.32 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.1, $J_{2'b,3'}$ = 3.1, H-2′b); 2.54 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 7.8, $J_{2'a,3'}$ = 6.0, H-2′a); 2.26 (t, 2H, J_{vic} = 6.6, dapur-C≡C-C**H$_2$**$CH_2$$CH_2$$CH_2$-C≡C-bpy); 2.28 (t, 2H, J_{vic} = 6.6, dapur-C≡C-$CH_2$$CH_2$$CH_2$C**H$_2$**-C≡C-bpy); 3.17 (q, 18H, J_{vic} = 7.3, CH_3C**H$_2$**N); 4.12 (tdd, 1H, $J_{4',5'}$ = 4.4, $J_{4',3'}$ = 3.1, $J_{H,P}$ = 1.0, H-4′); 4.21 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 5.4, $J_{5'b,4'}$ = 4.4, H-5′b); 4.26 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.8, $J_{5'a,4'}$ = 4.4, H-5′a); 4.65 (dt, 1H, $J_{3',2'}$ = 6.0, 3.1, $J_{3',4'}$ = 3.1, H-3′); 6.61 (dd, 1H, $J_{1',2'}$ = 7.8, 6.1, H-1′); 7.41 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5‴); 7.68 (s, 1H, H-6);

7.88 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.1, H-4″); 7.92 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4‴); 8.20 (s, 1H, H-2); 8.26 (dd, 1H, $J_{3'',4''}$ = 8.3, $J_{3'',6''}$ = 0.8, H-3″); 8.32 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.9, H-3‴); 8.62 (dd, 1H, $J_{6'',4''}$ = 2.1, $J_{6'',3''}$ = 0.8, H-6″); 8.63 (ddd, 1H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6‴).

^{13}C NMR (150.9 MHz, CD_3OD): 9.09 (**C**H_3CH_2N); 19.75 (dapur-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-bpy); 19.96 (dapur-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-bpy); 28.98 (dapur-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-bpy); 41.31 (CH_2-2′); 47.32 (CH_3**C**H_2N); 66.97 (d, $J_{C,P}$ = 5.7, CH_2-5′); 72.63 (CH-3′); 73.57 (dapur-**C**≡C); 78.59 (bpy-**C**≡C); 84.68 (CH-1′); 87.46 (d, $J_{C,P}$ = 8.8, CH-4′); 94.37 (dapur-C≡**C**); 95.93 (bpy-C≡**C**); 99.27 (C-5); 103.48 (C-4a); 121.72 (CH-3″); 122.66 (CH-3‴); 122.73 (C-5″); 125.35 (CH-5‴); 127.47 (CH-6); 138.68 (CH-4‴); 140.82 (CH-4″); 149.21 (C-7a); 149.49 (CH-2); 150.30 (CH-6″); 152.61 (CH-6‴); 155.28 (C-2″); 156.00 (C-4); 156.56 (C-2‴).

^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): -22.23 (t, J = 20.7, P_β); -9.70 (d, J = 20.7, P_α); -8.91 (d, J = 20.7, P_γ).

MS (ES^-): found *m/z*: 747.3 (M-1), 667.3 (M-PO_3H_2-1)

HRMS (ES): *m/z* calcd for $C_{29}H_{30}O_{12}N_6P_3$: 747.11400; found: 747.11335.

5-[8''''-(2'',2'''-bipyridin-5''-yl)-octa-1'''',7''''-diyn-1''''-yl)-2'-deoxycytidine-5'-O-triphosphate (dC^{Obpy}TP, 9a)

This compound was prepared according the general procedure C from **dC^{Obpy}** (**7a**) in the yield of 39% (41.2 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.30 (t, 27H, J_{vic} = 7.3, C**H**$_3$$CH_2N$); 1.80 (m, 4H, cyt-C≡C-$CH_2$C**H**$_2$C**H**$_2$$CH_2$-C≡C-bpy); 2.18 (ddd, 1H, J_{gem} = 13.7, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.6, H-2′b); 2.34 (ddd, 1H, J_{gem} = 13.7, $J_{2'a,1'}$ = 6.0, $J_{2'a,3'}$ = 3.6, H-2′a); 2.55 (t, 2H, J_{vic} = 6.8, cyt-C≡C-C**H**$_2$$CH_2CH_2CH_2$-C≡C-bpy); 2.57 (t, 2H, J_{vic} = 6.7, cyt-C≡C-$CH_2CH_2CH_2$C**H**$_2$-C≡C-bpy); 3.19 (q, 18H, J_{vic} = 7.3, CH_3C**H**$_2$N); 4.08 (ddd, 1H, $J_{4',5'}$ = 4.8, 3.8, $J_{4',3'}$ = 3.6, H-4′); 4.20 (ddd, 1H, J_{gem} = 11.1, $J_{H,P}$ = 5.3, $J_{5'b,4'}$ = 4.8, H-5′b); 4.28 (ddd, 1H, J_{gem} = 11.1, $J_{H,P}$ = 6.9, $J_{5'a,4'}$ = 3.8, H-5′a); 4.55 (dt, 1H, $J_{3',2'}$ = 6.6, 3.6, $J_{3',4'}$ = 3.6, H-3′); 6.23 (dd, 1H, $J_{1',2'}$ = 7.3, 6.0, H-1′); 7.44 (ddd, 1H, $J_{5''',4'''}$ = 7.4, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.0, H-5‴); 7.90 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.1, H-4″); 7.95 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.4, $J_{4''',6'''}$ = 1.7, H-4‴); 8.04 (s, 1H, H-6); 8.29 (d, 1H, $J_{3'',4''}$ = 8.2, H-3″); 8.35 (d, 1H, $J_{3''',4'''}$ = 7.9, H-3‴); 8.63 (d, 1H, $J_{6'',4''}$ = 2.1, H-6″); 8.65 (d, 1H, $J_{6''',5'''}$ = 4.8, H-6‴).

^{13}C NMR (150.9 MHz, CD_3OD): 9.09 (**C**H_3CH_2N); 19.73 (cyt-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-bpy); 19.94 (cyt-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-bpy); 28.81, 28.98 (cyt-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-bpy); 41.23 (CH_2-2′); 47.33 (CH_3**C**H_2N); 66.60 (d, $J_{C,P}$ = 5.7, CH_2-5′); 71.71 (cyt-**C**≡C); 71.93 (CH-3′); 78.49 (bpy-**C**≡C); 87.51 (CH-1′); 87.60 (d, $J_{C,P}$ = 8.8, CH-4′); 93.81 (C-5); 96.10 (bpy-C≡**C**); 97.95 (cyt-C≡**C**); 121.79 (CH-3″); 122.72 (CH-3‴); 122.84 (C-5″); 125.42 (CH-5‴); 138.86 (CH-4‴); 140.87 (CH-4″); 144.82 (CH-6); 150.21 (CH-6″); 152.61 (CH-6‴); 155.11 (C-2″); 155.52 (C-2); 156.46 (C-2‴); 165.70 (C-4).

^{31}P{^{1}H} NMR (162.0 MHz, CD_3OD): -22.52 (t, J = 21, P_β); -9.97 (d, J = 21, P_α); -9.21 (d, J = 21.0, P_γ).

MS (ES^-): found *m/z*: 724.1 (M-1), 644.1 (M-PO_3H_2-1)

HRMS (ES): *m/z* calcd for $C_{27}H_{29}O_{13}N_5P_3$: 724.09802; found: 724.09741.

5-[8'''''-(2'',2''':6''',2''''-terpyridin-4'''-yl)octa-1'''''',7'''''-diyn-1'''''-yl] -2'-deoxcytidine-5'-O-triphosphate (dC^{Otpy}TP, 9b)

This compound was prepared according the general procedure C from **dC^{Otpy}** (**7b**) in the yield of 31% (30.0 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.30 (t, 27H, J_{vic} = 7.3, C**H$_3$**CH_2N); 1.85 (m, 4H, cyt-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-tpy); 2.16 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.6, H-2′b); 2.33 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.9, $J_{2'a,3'}$ = 3.5, H-2′a); 2.57 (t, 2H, J_{vic} = 6.8, cyt-C≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-tpy); 2.61 (t, 2H, J_{vic} = 6.8, cyt-C≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-tpy); 3.18 (q, 18H, J_{vic} = 7.3, CH_3C**H$_2$**N); 4.07 (ddd, 1H, $J_{4',5'}$ = 4.4, 4.0, $J_{4',3'}$ = 3.5, H-4′); 4.19 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 5.5, $J_{5'b,4'}$ = 4.4, H-5′b); 4.27 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 7.2, $J_{5'a,4'}$ = 4.0, H-5′a); 4.54 (dt, 1H, $J_{3',2'}$ = 6.6, 3.5, $J_{3',4'}$ = 3.5, H-3′); 6.20 (dd, 1H, $J_{1',2'}$ = 7.3, 5.9, H-1′); 7.47 (bdd, 2H, $J_{5''',4'''}$ = 7.0, $J_{5''',6'''}$ = 4.7, H-5‴); 7.98 (ddd, 2H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.0, $J_{4''',6'''}$ = 1.6, H-4‴); 8.02 (s, 1H, H-6); 8.31 (s, 2H, H-3″,5″); 8.59 (bd, 2H, $J_{3''',4'''}$ = 8.0, H-3‴); 8.68 (bd, 2H, $J_{6''',5'''}$ = 4.7, H-6‴).

^{13}C NMR (150.9 MHz, CD_3OD): 9.10 (**C**H_3CH_2N); 19.71 (cyt-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-tpy); 19.95 (cyt-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-tpy); 28.79, 28.85 (cyt-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-tpy); 41.23 (CH_2-2′); 47.36 (CH_3**C**H_2N); 66.63 (d, $J_{C,P}$ = 5.7, CH_2-5′); 71.89 (cyt-**C**≡C); 71.94 (CH-3′); 80.01 (tpy-**C**≡C); 87.54 (CH-1′); 87.60 (d, $J_{C,P}$ = 8.9, CH-4′); 93.83 (C-5); 96.90 (tpy-C≡**C**); 97.89 (cyt-C≡**C**); 122.78 (CH-3‴); 123.94 (CH-3″,5″); 125.65 (CH-5‴);

135.62 (C-4′′); 138.85 (CH-4′′′y); 144.78 (CH-6); 150.20 (CH-6′′′); 155.76 (C-2); 156.60 (C-2′′,6′′); 156.79 (C-2′′′); 165.87 (C-4).

$^{31}P\{^{1}H\}$ NMR (202.3 MHz, CD_3OD): -22.48 (bdd, J = 21.2, 20.6, P_β); -10.02 (d, J = 21.2, P_α); -9.21 (d, J = 20.6, P_γ).

MS (ES^-): found *m/z*: 801.1 (M-1), 721.1 (M-PO_3H_2-1)

HRMS (ES): *m/z* calcd for $C_{32}H_{32}O_{13}N_6P_3$: 801.12457; found: 801.12398.

Primer extension, purification and analysis of the PEX products

Synthetic ONs were purchased from Sigma Aldrich (USA). For sequences of primer and templates see Table 1. Templates used in experiment involving the DBstv magnetoseparation procedure were biotinylated at their 5' ends. Streptavidine magnetic beads MagPrep P-25 Streptavidine Particles were obtained from Novagen (EMD Chemicals, USA), Pwo DNA polymerase from PeqLab (Germany), DyNAzyme II DNA polymerases from Finnzymes (Finland), KOD XL DNA polymerase from Novagen (EMD Chemicals, USA), Deep Vent DNA polymerases as well as T4 polynukleotide kinase and natural nucleoside triphosphate (dATP, dCTP, dGTP and dCTTP) from New England Biolabs (Great Britain) and γ-^{32}P-ATP from Izotop, Institute of isotopes Co, Ltd. (Hungary).

Table 1. Primer and templates used for primer extension experiment.[a]

prim^{rnd}	5'-CATGGGCGGCATGGG-3'
prim^{comp}	5'-*CATGGGCGGCATCTC*-3'
temp^{rnd16}	5'-**CTAGCATGAGCTCAGT***CCCATGCCGCCCATG*-3'
temp^{comp3gA}	5'-**CAGACCAGCCCTCCC***GAGATGCCGCCCATG*-3'
temp^{rndA}	5'-**CAGACACGAGCTACG***CCCATGCCGCCCATG*-3'
temp^{A}	5'-**CCCT***CCCATGCCGCCCATG*-3'
temp^{C}	5'-**CCCG***CCCATGCCGCCCATG*-3'
temp^{compA}	5'-**GAGT***GAGATGCCGCCCATG*-3'
temp^{A1}	5'-**T***CCCATGCCGCCCATG*-3'
temp^{C1}	5'-**G***CCCATGCCGCCCATG*-3'
temp^{compA1}	5'-**T***GAGATGCCGCCCATG*-3'

[a] In the template (temp) ONs segments that form a duplex with primer are printed in italics, the replicated segments are printed in bold.

Primer Extension Experiment: The reaction mixture (20 μl) contained DNA polymerase: DyNAzyme II polymerase (0.2 U/ μl, 1 μl), KOD XL (0.25 U/μl, 0.8 μl), Deep Vent (0.2 U/μl, 1 μl), dNTPs (either natural or modified, 4mM, 1 μl), ^{32}P-prelabelled primer at 5'-end (3 μM, 1 μl) and template (3 μM, 1.5 μl) in 2 μl of corresponding buffer suplied by manufacturer Reaction mixture was incubated for 30 min at 60 °C.

Denaturating Polyacrylamide Gel Electrophoresis: The products of the primer extension reaction were mixed with loading buffer (40 µl, 80% [w/v] formamide, 20 mM EDTA, 0.025% [w/v] bromphenole blue, 0.025% [w/v] xylene cyanol), heated 5 min at 95 °C and subjected to gel electrophoresis in 12.5% denaturating polyacrylamide gel containing 1xTBE buffer (pH 8) and 7% urea at 60 W for ~ 60 min. Gel was dried and visualized by phosphoimager.

General procedure for complexation

Complexation of dN^{R}s: Complexes of modified nucleoside **dN^{R}s** (**6a-b** or **7a-b**) with diverse transition metals were prepared by mixing of 100 µl of methanolic solution of corresponding nucleosides (100 µM) with 100 µl of methanolic solution of divalent metal ions M^{2+} (50 µM, $Cu(BF_4)_2.6H_2O$, $Ni(BF_4)_2.6H_2O$, $Zn(BF_4)_2.H_2O$, $Fe(BF_4)_2.6H_2O$) at room temperature for 10 minutes.

Complexation of ONs for recording UV-spectra: Double stranded DNAs were prepared by PEX-experiment on larger scale. The reaction mixture (100 µl) contained Deep Vent polymerase (2 U/µl, 7.5 µl) or DyNazyme II polymerase (2 U/µl, 7.5 µl), dNTP (either natural or modified, 4 mM, 15 µl), unlabeled primer (100 µM, 6 µl), and temp^{rnd16} (100 µM, 6 µl) in 10 µl of corresponding buffer supplied by manufacturer. Reaction mixture was incubated for 30 min. at 60 °C. PEX-products were purified by NucAway Spin Columns (Ambion), where 50 µl portions of each sample were applied on the top of the column. After collecting all the portions 0.5 equiv. of $Fe(BF_4)_2.6H_2O$ to number of modification (0.24 µl, 10 mM) was added to the corresponding sample and the solution was mixed overnight (25 °C, 550 rpm).

Complexation of ONs for gel electrophoresis: Double stranded DNAs were prepared by PEX-experiment. The reaction mixture (20 µl) contained DNA polymerase: Pwo (0.1 U/µl, 2 µl), DyNAzyme II (0.2 U/µl, 1 µl), dNTP (either natural or modified, 4 mM, 1 µl), ^{32}P-prelabelled primer at 5'-end primer (3 µM, 1 µl), and template (3 µM, 1.5 µl) in 2 µl of corresponding buffer supplied by manufacturer. Reaction mixture was incubated for 30 min. at 60 °C. After addition of 1 µl of $Fe(BF_4)_2 \cdot 6H_2O$ (4 mM), the solution was mixed overnight (25 °C, 550 rpm).

Non-denaturating SB Polyacrylamide Gel Electrophoresis: The products of the primer extension reaction were mixed with loading buffer (4 µl, 40% [w/v] sacchrose, 0.2% [w/v] bromphenole blue, 0.2% [w/v] xylene cyanol) subjected to gel electrophoresis in 8% non-denaturating polyacrylamide gel containing 1xSB buffer (pH 8) and at 600 V for ~ 3 h at room temperature. Gel was dried and visualized by phosphoimager.

Supplementary results – PAGE of PEX:

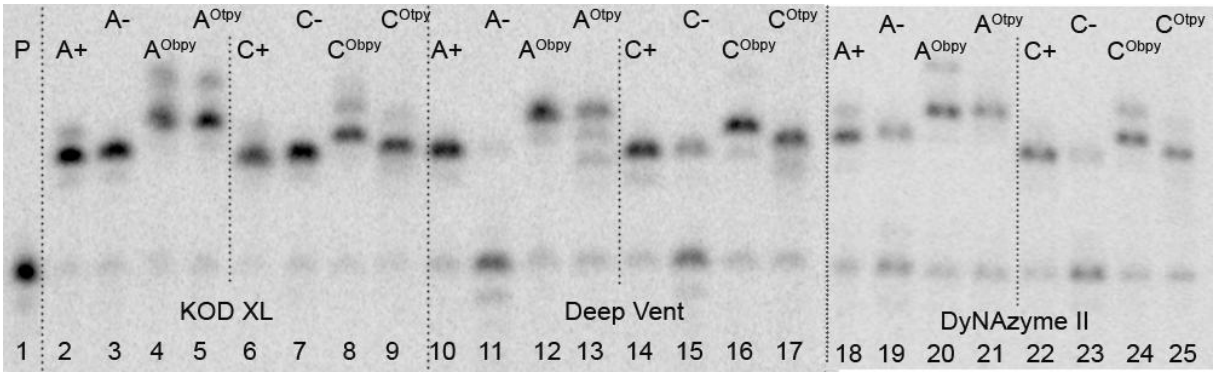

Figure S2. Denaturing PAGE analysis of PEX experiment synthetized on temp^{A} (lanes 2 – 5, 10 - 13, 18 - 21) and temp^{C} (lanes 6 – 9, 14 – 17, 22 - 25) with KOD XL (lanes 2 – 9), Deep Vent (lanes 10 - 17) and DyNAzyme II (lanes 18 – 25) polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; A+: natural dATP, dGTP; A-: dGTP; A^{Obpy}: **dA^{Obpy}TP** (**8a**), dGTP; A^{Otpy}: **dA^{Otpy}TP** (**8b**), dGTP; C+: natural dCTP, dGTP; C-: dGTP; C^{Obpy}: **dC^{Obpy}TP** (**9a**), dGTP; C^{Otpy}: **dC^{Otpy}TP** (**9b**), dGTP.

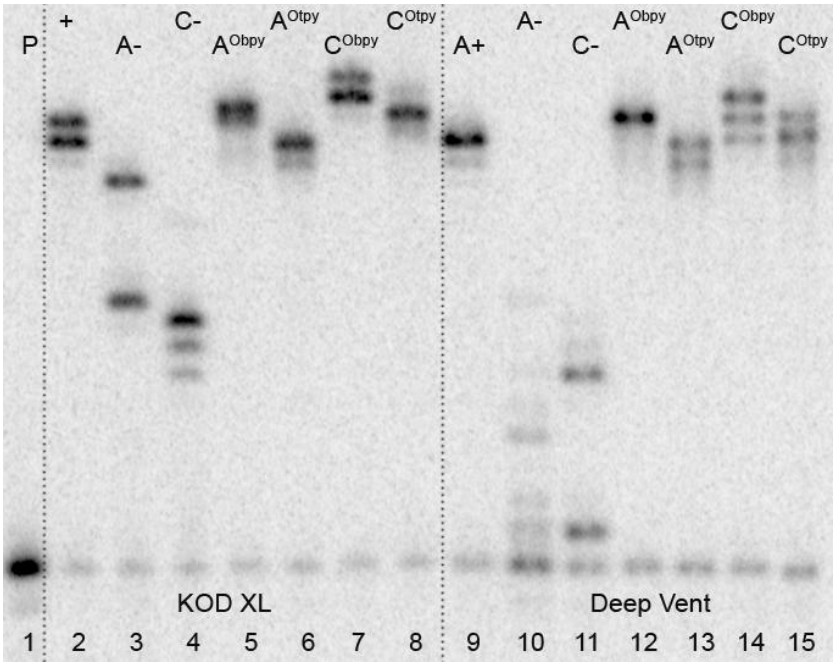

Figure S3. Denaturing PAGE analysis of PEX experiment synthetized on temp^{rnd16} with KOD XL (lanes 2-8) and Deep Vent (lanes 9-15) polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: P: Primer; +: unmodified DNA (dATP, dTTP, dCTP, dGTP); A-: unmodified DNA (dTTP, dCTP, dGTP); C-: unmodified DNA (dATP, dTTP, dGTP); A^{Obpy}: **dA^{Obpy}TP** (**8a**), dTTP, dCTP, dGTP; A^{Otpy}: **dA^{Otpy}TP** (**8b**), dTTP, dCTP, dGTP; C^{Obpy}: dATP, dTTP, **dC^{Obpy}TP** (**9a**), dGTP; C^{Otpy}: dATP, dTTP, **dC^{Otpy}TP** (**9b**), dGTP

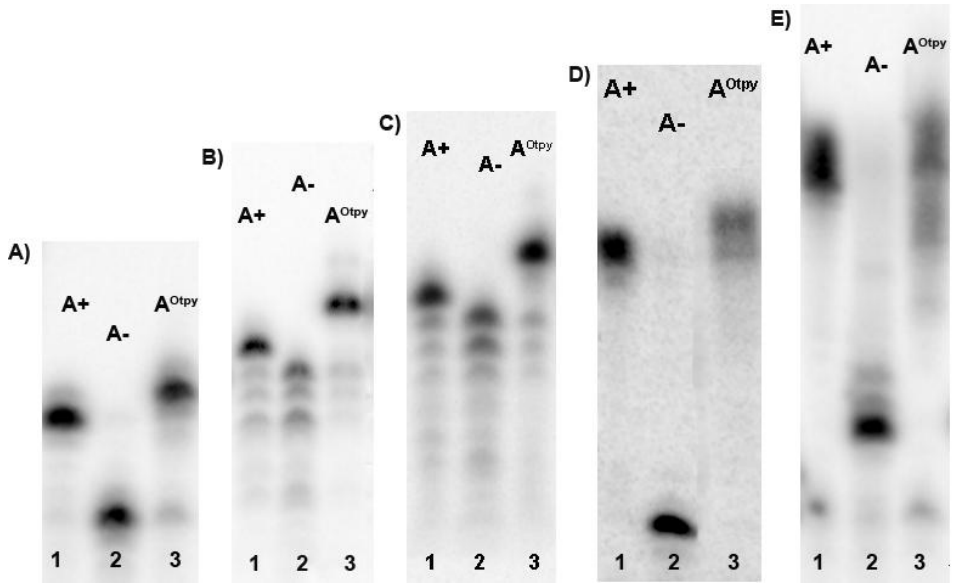

Figure S4. Denaturing PAGE analysis of PEX experiment synthetized on temp^{compA} (A), temp^{compA1} (B), temp^{A1} (C), temp^{rndA} (D), temp^{comp3gA} (E) with Pwo polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA (natural dNTPs); A-: unmodified DNA (unmodified dNTPs in the absence of dATP); A^{Otpy}: Otpy-modified DNA (**dA^{Otpy}TP** (**8b**) in combination with natural dNTPs required according to the template used for PEX).

MALDI-TOF experiment (ssDNA)

The reaction mixture (200 µl) contained DNA polymerase: Pwo (1U/µl, 10 µl), Deep Vent (2U/µl, 5µl) or DyNAzyme II (2U/µl, 5 µl), dNTPs (either natural or modified, 4mM, 10 µl), unlabeled primer prim^{rnd} (10 µM, 40 µl, 5'-CAT GGG CGG CAT GGG-3') and biotinylated template temp^{A}-bio (10 µM, 40 µl, 5'-**CCC T***CC CAT GCC GCC CAT G*-3'), temp^{C}-bio (10 µM, 40 µl, 5'-**CCC G***CC CAT GCC GCC CAT G*-3') or temp^{rnd16}-bio (10 µM, 40 µl, 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3') in 20 µl of corresponding buffer suplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C. The separation on magnetic beads (50 µl, Novagen) was caried out according to standard techniques. As matrix for MALDI-TOF measurement was used a mixture of 3-hydroxypicolinic acid (HPA)/picolinic acid (PA)/ammonium tartrate in ration 8/1/1 in 50% acetonitrile. Then 2 µl of the matrix and 1 µl of the sample were mixed on MTP 384 polished steel target by use of anchor-chip desk. The crystallized spots were washed once by 0.1% formic acid and once by water. The acceleration tension in reflectron mode was 19.5 kV and range of measurement 3 – 13 kDa. The found differences of 2-9 Da for 6 KDa DNA and 3-12 Da for 10 KDa DNA are still within the experimental error (ca 0.1%) of the low resolution machine also considering the very small amounts of DNA produced by PEX.

Mass - pex^{A} (dATP, dGTP): calculated: 5973.0 Da; found: 5976.3 Da

Mass - pex^{A} (**dA^{Obpy}TP**, dGTP): calculated: 6230.3 Da; found: 6231.6 Da

Mass - pex^{A} (**dA^{Otpy}TP**, dGTP): calculated: 6307.4 Da; found: 6310.0 Da

Mass – pex^{C}(dCTP, dGTP): calculated: 5949.0 Da; found: 5948.2 Da

Mass – pex^{C} (**dC^{Obpy}TP**, dGTP): calculated: 6207.3 Da; found: 6208.8 Da

Mass – pex^{C} (**dC^{Otpy}TP**, dGTP): calculated: 6284.4 Da; found: 6286.0 Da

Mass - pex^{rnd16} (dATP, dCTP, dTTP, dGTP): calculated: 9617.3 Da; found: 9616.5

Mass - pex^{rnd16} (**dA^{Obpy}TP**, dCTP, dTTP, dGTP): calculated: 10646.6 Da; found: 10648.6 Da

Mass - pex^{rnd16} (**dA^{Otpy}TP**, dCTP, dTTP, dGTP): calculated: 10954.9 Da; found: 10956.1 Da

Mass - pex^{rnd16} (dCTP, dATP, dTTP, dGTP): calculated: 9617.3 Da; found: 9618.3 Da

Mass - pex^{rnd16} (**dC^{Obpy}TP**, dATP, dTTP, dGTP): calculated: 10650.6 Da; found: 10651.7 Da

Mass - pex^{rnd16} (**dC^{Otpy}TP**, dATP, dTTP, dGTP): calculated: 10.958.9 Da; found: 10959.8Da

Supplementary results – UV/Vis spectra of dN^{R}s with divalent metals:

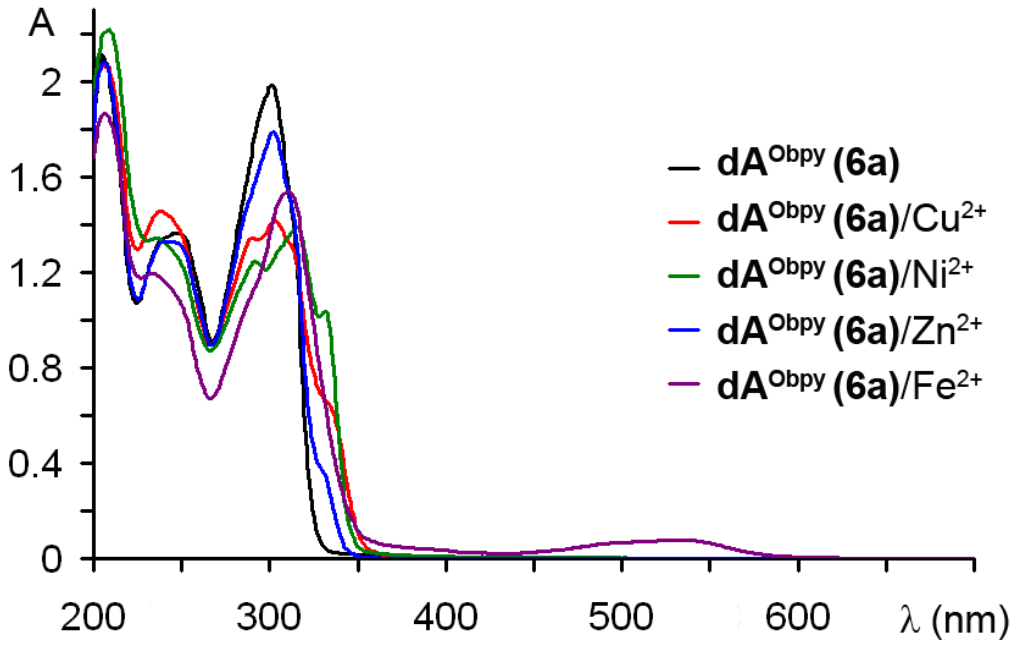

Figure S5. UV/Vis spectra of **dA^{Obpy}** (**6a**) with divalent metals.

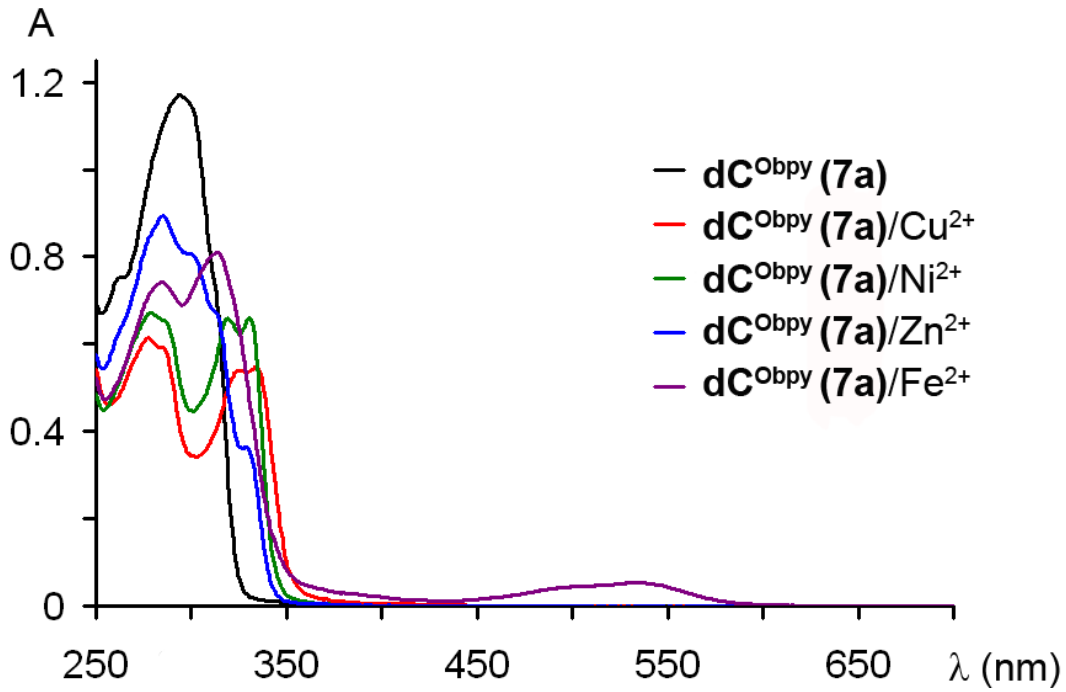

Figure S6. UV/Vis spectra of **dC^{Obpy}** (**7a**) with divalent metals.

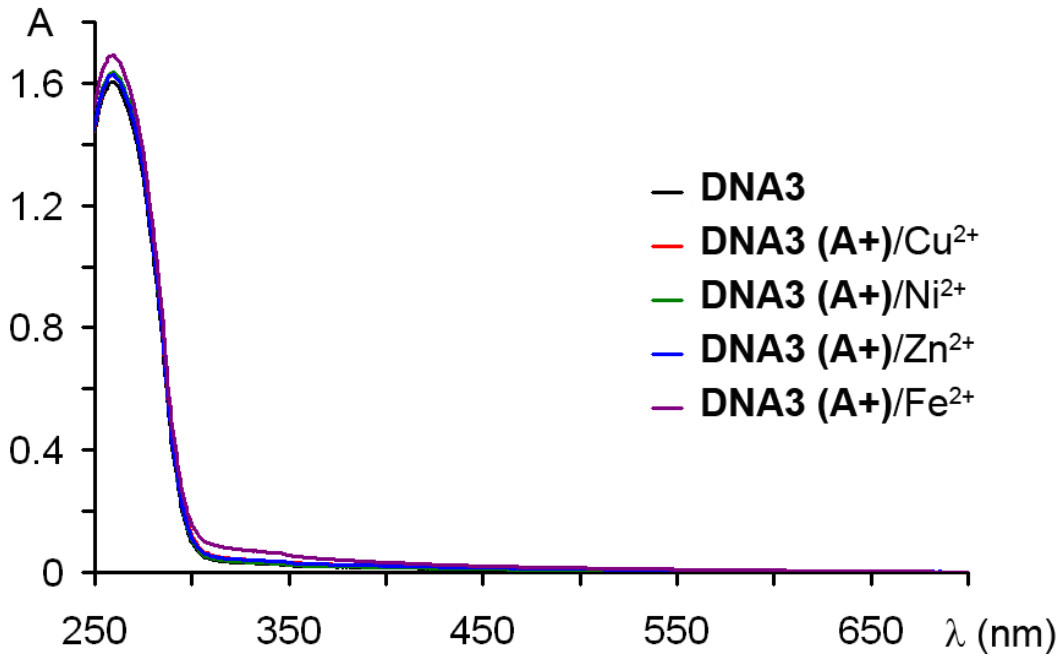

Figure S7. UV/Vis spectra of natural **DNA3 (A+)** with divalent metals.

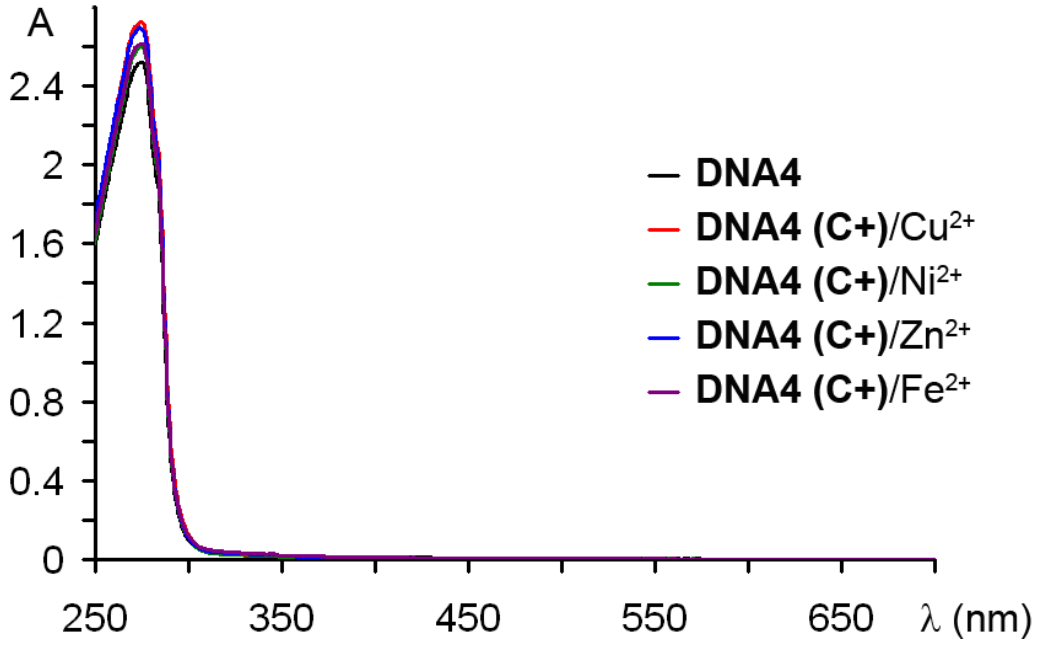

Figure S8. UV/Vis spectra of natural **DNA4 (C+)** with divalent metals.

Supplementary results – SB_PAGE

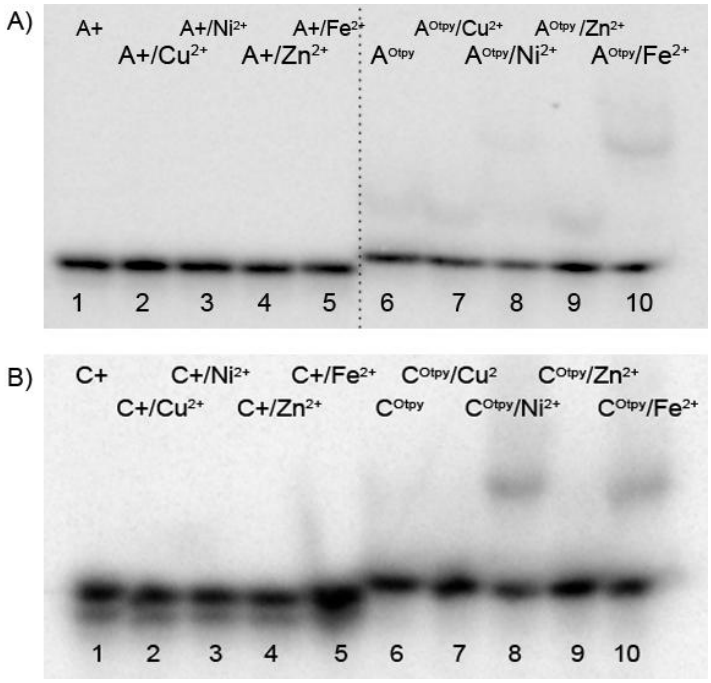

Figure S9. Non-denaturing gel electrophoresis (8% SB_PAGE) of DNA duplexes in the absence and in the presence of M^{2+} for pex^{A} (A) and pex^{C} (B). 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA (dATP, dGTP); A+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; A^{Otpy}: Otpy-modified DNA (**dA^{Otpy}TP 8b**, dGTP); A^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals; C+: unmodified DNA (dCTP, dGTP); C+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; C^{Otpy}: Otpy-modified DNA (**dC^{Otpy}TP 9b**, dGTP); C^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals.

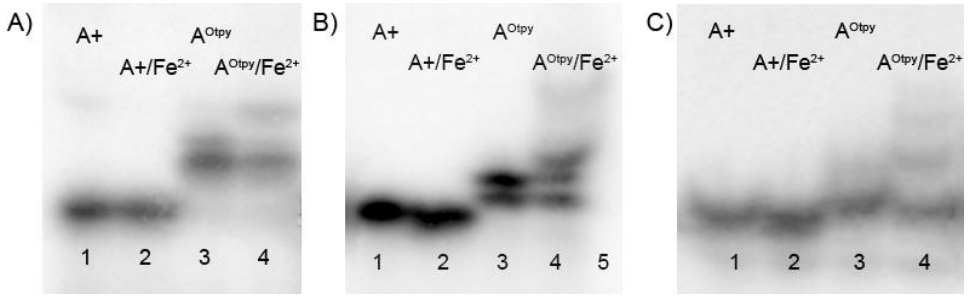

Figure S10. Non-denaturing gel electrophoresis (8% SB_PAGE) of DNA duplexes in the absence and in the presence of M^{2+} for pex^{A1} (A), pex^{compA} (B) and pex^{rndA} (C). 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA (dATP in combination with natural dNTPs required according to the template used in PEX); A+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; A^{Otpy}: Otpy-modified DNA (**dA^{Otpy}TP 8b** in combination with natural dNTPs required according according to the template used in PEX); A^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals.