Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supporting Information

for

Synthesis of nucleoside mono- and triphosphates bearing oligopyridine ligands, their incorporation into DNA and complexation with transition metals

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General

Sonogashira cross-coupling reaction were performed under argon atmosphere. Oligopyridinyl[1] acetylene, halogenated mohophosphates[2] and halogenated triphosphates[3] were prepared according to the literature procedures. Other chemicals were purchased from commercial suppliers and were used as received. 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 500 or Bruker 600 (500 or 600 MHz for ^{1}H, 125.7 or 150.9 MHz for ^{13}C and 202.3 for ^{31}P) in D_2O (referenced to dioxane as internal standard, $_{\delta}$H = 3.75 ppm, $_{\delta}$C = 69.3 ppm, standard for ^{31}P NMR was external H_3PO_4) or in CD_3OD (referenced to TMS as an internal 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. NMR spectra of dNTPs were measured in phosphate buffer at pH 7.1. 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.

General Procedure for Sonogashira cross-coupling – synthesis of modified dN^{R}MPs

Mixture CH_3CN/H_2O (1:2) (1.5 ml) and Et(*i*-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing halogenated nucleoside monophosphate **dC^{I}MP** or **dA^{I}MP** (60 mg), an alkyne **1a-c** (1.5 equiv.) and CuI (10 mol%). In a separate flask, $Pd(OAc)_2$ (5 mol%) and TPPTS (5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of CH_3CN/H_2O (1:2) (0.5 ml). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 80°C for 1.5 h. The solvent was evaporated in vacuo. Products were 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 and conversion to sodium salt form (Dowex 50 in Na^+ cycle) followed by freeze-drying from water, gave the products as brownish or yellowish powder.

5-[(2', 2'''-bipyridin-5''-yl)ethynyl]-2'-deoxycytidine 5'-O-monophosphate (dC^{5bpy}MP)

This compound was prepared according to the general procedure from 5-iodo-2'-

deoxyadenosine monophosphate **dC^{I}MP** and **1b** in the yield of 89%.

^{1}H NMR (499.8 MHz, CD_3OD): 2.24 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.3, H-2'b); 2.41 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.9, $J_{2'a,3'}$ = 3.1, H-2'a); 4.11 (m, 3H, H-4',5'); 4.53 (m, 1H, H-3'); 6.30 (dd, 1H, $J_{1',2'}$ = 7.1, 5.9, H-1'); 7.44 (dd, 1H, $J_{5''',4'''}$ = 6.9, $J_{5''',6'''}$ = 4.2, H-5'''); 7.94 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 6.9, $J_{4''',6'''}$ = 1.3, H-4'''); 8.18 (dd, 1H, $J_{4'',3''}$ = 8.3, $J_{4'',6''}$ = 1.6, H-4''); 8.33 (d, 1H, $J_{3'',4''}$ = 8.3, H-3''); 8.36 (d, 1H, $J_{3''',4'''}$ = 7.9, H-3'''); 8.42 (s, 1H, H-6); 8.66 (bd, 1H, $J_{6''',5'''}$ = 4.2, H-6'''); 8.89 (bs, 1H, H-6''); ^{13}C NMR (125.7 MHz, CD_3OD): 42.00 (CH_2-2'); 65.64 (d, $J_{C,P}$ = 4.6, CH_2-5'); 72.50 (CH-3'); 85.73 (bpy-C≡**C**); 88.01 (CH-1'); 88.11 (d, $J_{C,P}$ = 8.7, CH-4'); 92.51 (C-5); 92.79 (bpy-**C**≡C); 121.63 (CH-3''); 121.70 (C-5''); 122.78 (CH-3'''); 125.45 (CH-5'''); 138.72 (CH-4'''); 141.00 (CH-4''); 146.59 (CH-6); 150.36 (CH-6'''); 152.66 (CH-6''); 155.69 (C-2''); 156.55 (C-2'''); 156.69 (C-2); 165.94 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.67.

5-[4''-(2'',2''':6'',2'''-terpyridin-1''-yl)ethynyl]-2'-deoxycytidine 5'-O-monophosphate (dC^{tpy}MP)

This compound was prepared according to the general procedure from 5-iodo-2'-deoxyadenosine monophosphate **dC^{I}MP** and **1c** in the yield of 47%.

^{1}H NMR (500.0 MHz, CD_3OD): 2.26 (ddd, 1H, Jgem = 13.6, J2'b,1' = 7.3, J2'b,3' = 6.1, H-2'b); 2.44 (ddd, 1H, Jgem = 13.6, J2'a,1' = 5.8, J2'a,3' = 3.0, H-2'a); 4.14 (m, 3H, H-4',5'); 4.54 (dt, 1H, J3',2' = 6.1, 3.0, J3',4' = 3.0, H-3'); 6.29 (dd, 1H, J1',2' = 7.3, 5.8, H-1'); 7.50 (ddd, 2H, J5',4' = 7.5, J5',6' = 4.9, J5',3' = 0.9, H-5'-terpy); 8.02 (ddd, 2H, J4',3' = 8.0, J4',5' = 7.5, J4',6' = 1.8, H-4'-terpy); 8.46 (s, 1H, H-6); 8.54 (s, 2H, H-3,5-terpy); 8.59 (bd, 2H, J3',4' = 8.0, H-3'-terpy); 8.70 (bd, 2H, J6',5' = 4.9, H-6'-terpy); ^{13}C NMR (125.7 MHz, CD3OD): 42.03 (CH2-2'); 65.78 (d, JC,P = 5.1, CH2-5'); 72.58 (CH-3'); 86.54 (terpy-C≡C); 88.17 (d, JC,P = 8.8, CH-4'); 88.26 (CH-1'); 91.91 (C-5); 93.81 (terpy-C≡C); 123.09 (CH-3'-terpy); 123.84 (CH-3,5-terpy); 125.71 (CH-5'-terpy); 134.54 (C-4-terpy); 139.07 (CH-4'-terpy); 147.38 (CH-6); 150.11 (CH-6'-terpy); 156.51 (C-2); 156.54 (C-2'-terpy); 156.75 (C-2-terpy); 165.88 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.44. MS (ES-): found m/z: 561.2(M), 562.2 (M+H), 563.2 (M+2H); HRMS (ES-+): m/z calcd for $C_{26}H_{22}O_7N_6P$: 561.129; found: 561.1292.

7-[(2'',2'''-bipyridin-6''-yl)ethynyl]-7-deaza-2'-deoxycytidine 5'-O-monophosphate (dA^{6bpy}MP)

This compound was prepared according to the general procedure from 5-iodo-2'-deoxyadenosine monophosphate **dA^{I}MP** and **1a** in the yield of 52%.

^{1}H NMR (600.1 MHz, CD_3OD): 2.37 (ddd, 1H, Jgem = 13.5, J2'b,1' = 6.0, J2'b,3' = 2.6, H-2'b); 2.65 (ddd, 1H, Jgem = 13.5, J2'a,1' = 8.2, J2'a,3' = 5.6, H-2'a); 4.03 (dt, 1H, Jgem = 11.0, JH,P = J5'b,4' = 4.6, H-5'b); 4.09 (ddd, 1H, Jgem = 11.0, JH,P = 5.8, J5'a,4' = 3.3, H-5'a); 4.13 (m, 1H, H-4'); 4.66 (dt, 1H, J3',2' = 5.6, 2.6, J3',4' = 2.6, H-3'); 6.70 (dd, 1H, J1',2' = 8.2, 6.0, H-1'); 7.47 (ddd, 1H, J5''',4''' = 7.6, J5''',6''' = 4.8, J5''',3''' = 0.8, H-5'''); 7.68 (d, 1H, J5'',4'' = 7.6, H-5''); 7.96 (dd, 1H, J4'',3'' = 7.9, J4'',5'' = 7.6, H-4''); 7.98 (ddd, 1H, J4''',3''' = 7.9, J4''',5''' = 7.6, J4''',6''' = 1.8, H-4'''); 8.05 (s, 1H, H-6); 8.16 (s, 1H, H-2); 8.26 (d, 1H, J3'',4'' = 7.9, H-3''); 8.34 (d, 1H, J3''',4''' = 7.9, H-3'''); 8.67 (bd, 1H, J6''',5''' = 4.8, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 41.48 (CH2-2'); 66.11 (d, JC,P = 4.9, CH2-5'); 73.29 (CH-3'); 84.35 (bpy-C≡C); 84.92 (CH-1□); 87.79 (d, JC,P = 8.8, CH-4□); 92.03 (bpy-C≡C); 96.63 (C-5); 103.91 (C-4a); 121.68 (CH-3''); 122.87 (CH-3'''); 125.61 (CH-5'''); 128.11 (CH-5''); 129.26 (CH-6); 138.88 (CH-4'''); 139.10 (CH-4''); 144.23 (C-6''); 150.36 (CH-6'''); 150.87 (C-7a); 153.71 (CH-2); 156.57 (C-2'''); 157.60 (C-2''); 159.08 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.99; MS (ES-): found m/z: 507.2(M), 508.2 (M+H); HRMS (ES-+): m/z calcd for $C_{23}H_{20}O_6N_6P$: 507.1187; found: 507.1189.

7-[(2'',2'''-bipyridin-5''-yl)ethynyl]-7-deaza-2'-deoxycytidine 5'-O-monophosphate (dA^{5bpy}MP)

This compound was prepared according to the general procedure from 5-iodo-2'-deoxyadenosine monophosphate **dA^{I}MP** and **1b** in the yield 70%.

^{1}H NMR (500.0 MHz, CD_3OD): 2.37 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.1, $J_{2'b,3'}$ = 2.9, H-2'b); 2.65 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 7.8, $J_{2'a,3'}$ = 5.9, H-2'a); 3.99 (dt, 1H, J_{gem} = 10.8, $J_{H,P}$ = $J_{5'b,4'}$ = 4.8, H-5'b); 4.06 (ddd, 1H, J_{gem} = 10.8, $J_{H,P}$ = 5.4, $J_{5'a,4'}$ = 4.0, H-5'a); 4.11 (m, 1H, H-4'); 4.69 (dt, 1H, $J_{3',2'}$ = 5.9, 2.9, $J_{3',4'}$ = 2.9, H-3'); 6.67 (dd, 1H, $J_{1',2'}$ = 7.8, 6.1, H-1'); 7.44 (dd, 1H, $J_{5''',4'''}$ = 6.9, $J_{5''',6'''}$ = 4.2, H-5'''); 7.94 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 6.9, $J_{4''',6'''}$ = 1.5, H-4'''); 7.99 (s, 1H, H-6); 8.07 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 1.8, H-4''); 8.15 (s, 1H, H-2); 8.35 (d, 1H, $J_{3'',4''}$ = 8.2, H-3''); 8.38 (d, 1H, $J_{3''',4'''}$ = 8.0, H-3'''); 8.66 (d, 1H, $J_{6''',5'''}$ = 4.2, H-6'''); 8.81 (bs, 1H, H-6''); ^{13}C NMR (125.7 MHz, CD_3OD): 41.15 (CH_2-2'); 65.70 (d, $J_{C,P}$ = 3.7, CH_2-5'); 73.27 (CH-3'); 84.72 (CH-1'); 87.97 (d, $J_{C,P}$ = 8.3, CH-4'); 88.18 (bpy-C≡**C**); 89.38

(bpy-**C**≡C); 96.78 (C-5); 103.88 (C-4a); 121.75 (CH-3''); 121.98 (C-5''); 122.76 (CH-3'''); 125.47 (CH-5'''); 128.81 (CH-6); 138.72 (CH-4'''); 140.48 (CH-4''); 150.36 (CH-6'''); 150.76 (C-7a); 152.29 (CH-6''); 153.61 (CH-2); 155.66 (C-2''); 156.50 (C-2'''); 159.08 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 5.04; MS (ES-): found m/z: 507.2(M), 508.2 (M+H).

7-[4''-(2'',2''':6'',2'''-terpyridin-1''-yl)ethynyl]-7-deaza-2'-deoxycytidine 5'-O-monophosphate (dA^{tpy}MP)

This compound was prepared according to the general procedure from 5-iodo-2'-deoxyadenosine monophosphate **dA^{I}MP** and **1c** in the yield 57%.

^{1}H NMR (600.1 MHz, CD_3OD): 2.37 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 5.9, $J_{2'b,3'}$ = 2.3, H-2'b); 2.65 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 8.5, $J_{2'a,3'}$ = 5.7, H-2'a); 4.05 (dt, 1H, J_{gem} = 10.8, $J_{H,P}$ = $J_{5'b,4'}$ = 4.8, H-5'b); 4.10 (ddd, 1H, J_{gem} = 10.8, $J_{H,P}$ = 5.3, $J_{5'a,4'}$ = 3.2, H-5'a); 4.14 (m, 1H, H-4'); 4.66 (dt, 1H, $J_{3',2'}$ = 5.7, 2.3, $J_{3',4'}$ = 2.3, H-3'); 6.68 (dd, 1H, $J_{1',2'}$ = 8.5, 5.9, H-1'); 7.45 (dd, 2H, $J_{5',4'}$ = 7.3, $J_{5',6'}$ = 4.6, H-5'-terpy); 7.97 (ddd, 2H, $J_{4',3'}$ = 7.9, $J_{4',5'}$ = 7.3, $J_{4',6'}$ = 1.4, H-4'-terpy); 8.03 (s, 1H, H-6); 8.16 (s, 1H, H-2); 8.43 (s, 2H, H-3,5-terpy); 8.59 (d, 2H, $J_{3',4'}$ = 7.9, H-3'-terpy); 8.66 (d, 2H, $J_{6',5'}$ = 4.6, H-6'-terpy); ^{13}C NMR (150.9 MHz, CD_3OD): 41.44 (CH_2-2'); 66.23 (d, $J_{C,P}$ = 4.8, CH_2-5'); 73.29 (CH-3'); 85.00 (CH-1'); 87.73 (d, $J_{C,P}$ = 8.8, CH-4'); 88.63 (terpy-C≡**C**); 90.81 (terpy-**C**≡C); 96.66 (C-5); 103.92 (C-4a); 122.78 (CH-3'-terpy); 123.15 (CH-3,5-terpy); 125.63 (CH-5'-terpy); 129.46 (CH-6); 134.58 (C-4-terpy); 138.72 (CH-4'-terpy); 150.23 (CH-6'-terpy); 150.85 (C-7a); 153.57 (CH-2); 156.60 (C-2'-terpy); 156.97 (C-2-terpy); 158.97 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.44. MS (ES-): found m/z: 584.2(M), 585.2 (M+H), 586.2 (M+2H); HRMS (ES-+): m/z calcd for C28H23O6N7P: 584.1453; found: 584.1454.

General Procedure for Sonogashira cross-coupling – synthesis of modified dN^{R}TPs

Mixture CH_3CN/H_2O (1:2) (1.5 ml) and Et(*i*-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing halogenated nucleoside triphosphate **dC^{I}TP** or **dA^{I}TP** (60 mg), an alkyne **1a-c** (1.5 equiv. for **dC^{I}TP** and 2 equiv. for **dA^{I}TP**) and CuI (10 mol%). In a separate flask, $Pd(OAc)_2$ (5 mol%) and TPPTS (5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of CH_3CN/H_2O (1:2) (0.5 ml). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 80°C for 1 h. The solvent was evaporated in vacuo. Products were 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 and conversion to sodium salt form (Dowex 50 in Na^+ cycle) followed by freeze-drying from water, gave the products as white or yellow powder.

5-[(2', 2'''-bipyridin-5''-yl)ethynyl]-2'-deoxycytidine 5'-O-triphosphate (dC^{5bpy}TP)

This compound was prepared according to the general procedure from 5-iodo-2'-deoxyadenosine triphosphate **dC^{I}TP** and **1b** in the yield 59%.

^{1}H NMR (499.8 MHz, D_2O, ref$_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.25 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.4, $J_{2'b,3'}$ = 6.3, H-2′b); 2.44 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 4.0, H-2′a); 4.25 (m, 3H, H-4′,5′); 4.61 (dt, 1H, $J_{3',2'}$ = 6.3, 4.0, $J_{3',4'}$ = 4.0, H-3′); 6.10 (dd, 1H, $J_{1',2'}$ = 7.4, 6.1, H-1′); 7.28 (dd, 1H, $J_{5''',4'''}$ = 7.0, $J_{5''',6'''}$ = 4.6, H-5‴); 7.78 (dd, 1H, $J_{4''',3'''}$ = 7.5, $J_{4''',5'''}$ = 7.0, H-4‴); 7.82 (d, 1H, $J_{4'',3''}$ = 7.9, H-4″); 7.84 (s, 1H, H-6); 7.93 (d, 1H, $J_{3''',4'''}$ = 7.5, H-3‴); 7.95 (d, 1H, $J_{3'',4''}$ = 7.9, H-3″); 8.39 (bs, 1H, H-6″); 8.42 (d, 1H, $J_{6''',5'''}$ = 4.6, H-6‴); ^{13}C NMR (125.7 MHz, D_2O, ref$_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.88 (CH_2-2′); 68.04 (d, $J_{C,P}$ = 5.3, CH_2-5′); 73.03 (CH-3′); 86.72 (bpy-C≡**C**); 88.17 (d, $J_{C,P}$ = 8.6, CH-4′); 89.05 (CH-1′); 94.42 (C-5); 94.75 (bpy-**C**≡C); 122.21 (C-5″); 124.04 (CH-3″); 124.75 (CH-3‴); 127.04 (CH-5‴); 141.09 (CH-4‴); 142.81 (CH-4″); 147.31 (CH-6); 151.61 (CH-6″); 153.30 (CH-6‴); 156.06 (C-2″); 156.46 (C-2‴); 158.04 (C-2); 166.39 (C-4); ^{31}P NMR (202.3 MHz, D_2O, ref$_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.78 (t, J = 19.2, P_β); -10.01 (d, J = 19.2, P_α); -6.51 (d, J = 19.2, P_γ); MS (ES$^-$): found *m/z*: 644.0 (M-1), 666.0 (M+Na); HRMS (ES$^-$): *m/z* calcd for $C_{21}H_{21}O_{13}N_5P_3$: 644.0354; found: 644.0354.

5-[4''-(2'',2''':6'',2'''-terpyridin-1''-yl)ethynyl]-2'-deoxycytidine 5'-O-triphosphate (dC^{tpy}TP)

This compound was prepared according to the general procedure from 5-iodo-2'-deoxyadenosine triphosphate **dC^{I}TP** and **1c** in the yield 69%.

^{1}H NMR (499.8 MHz, D_2O, ref$_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.31, 2.48 (2 × bm, 2 × 1H, H-2′); 4.24 (bm, 2H, H-5′); 4.27 (bm, 1H, H-4′); 4.61 (bm, 1H, H-3′); 6.09 (bm, 1H, H-1′); 7.12 (bm, 2H, H-5′-terpy); 7.35 (bm, 2H, H-3′-terpy); 7.66 (bs, 1H, H-6); 7.68 (bm, 2H, H-4′-terpy); 8.02 (bs, 2H, H-3,5-terpy); 8.07 (bm, 2H, H-6′-terpy); ^{13}C NMR (125.7 MHz, D_2O, ref$_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.47 (CH_2-2′); 68.28 (CH_2-5′);

73.33 (CH-3′); 87.58 (terpy-C≡**C**); 88.08 (d, $J_{C,P}$ = 9.2, CH-4′); 89.44 (CH-1′); 93.66 (C-5); 95.91 (terpy-**C**≡C); 124.26 (CH-3′-terpy); 124.76 (CH-3,5-terpy); 127.33 (CH-5′-terpy); 134.80 (C-4-terpy); 141.40 (CH-4′-terpy); 148.33 (CH-6); 150.87 (CH-6′-terpy); 155.34 (C-2′-terpy); 156.43 (C-2,6-terpy); 157.89 (C-2); 166.14 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.78 (bdd, *J* = 20.1, 19.3, P_β); -8.91 (d, *J* = 19.3, P_α); -6.88 (bd, *J* = 20.1, P_γ); MS (ES^-): found *m/z*: 721.0 (M-1), 743.0 (M+Na); HRMS (ES^-): *m/z* calcd for $C_{26}H_{24}O_{13}N_6P_3$: 721.0620; found: 721.0595.

7-[(2'',2'''-bipyridin-6''-yl)ethynyl]-7-deaza-2'-deoxycytidine 5'-O-triphosphate (dA^{6bpy}TP)

This compound was prepared according to the general procedure from 7-iodo-7-deaza-2'-deoxyadenosine triphosphate **dA^{I}TP** and **1a** in the yield 42%.

^{1}H NMR (499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.35 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 3.2, H-2′b); 2.49 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.1, H-2′a); 4.14 (m, 2H, H-5′); 4.19 (m, 1H, H-4′); 4.68 (dt, 1H, $J_{3',2'}$ = 6.1, 3.2, $J_{3',4'}$ = 3.2, H-3′); 6.19 (dd, 1H, $J_{1',2'}$ = 7.9, 6.1, H-1′); 7.25 (bdd, 1H, $J_{5''',4'''}$ = 7.7, $J_{5''',6'''}$ = 4.2, H-5‴); 7.29 (bd, 1H, $J_{5'',4''}$ = 7.7, H-5″); 7.52 (s, 1H, H-6); 7.57 (bd, 1H, $J_{3'',4''}$ = 7.7, H-3″); 7.65 (bt, 1H, $J_{4''',3'''}$ = $J_{4''',5'''}$ = 7.7, H-4‴); 7.71 (bt, 1H, $J_{4'',3''}$ = $J_{4'',5''}$ = 7.7, H-4″); 7.75 (bd, 1H, $J_{3''',4'''}$ = 7.7, H-3‴); 7.81 (s, 1H, H-2); 8.30 (bd, 1H, $J_{6''',5'''}$ = 4.2, H-6‴); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.20 (CH_2-2′); 68.32 (d, $J_{C,P}$ = 5.6, CH_2-5′); 73.72 (CH-3′); 85.53 (bpy-C≡**C**); 85.56 (CH-1′); 87.81 (d, $J_{C,P}$ = 8.7, CH-4′); 93.87 (bpy-**C**≡C); 98.48 (C-5); 104.92 (C-4a); 122.92 (CH-3″); 124.26 (CH-3‴); 127.25 (CH-5‴); 130.16 (CH-5″); 130.26 (CH-6); 140.76 (CH-4″); 141.02 (CH-4‴); 144.49 (C-6″); 150.52 (C-7a); 150.76 (CH-6‴); 153.73 (CH-2); 155.78 (C-2‴); 156.86 (C-2″); 158.75 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.86 (bdd, *J* = 19.2, 18.9, P_β); -9.60 (d, *J* = 19.2, P_α); -7.40 (bd, *J* = 18.9, P_γ); MS (ES^-): found *m/z*: 647.0 (M-1), 587.1 (M–PO_3H_2–1); HRMS (ES^-): *m/z* calcd for $C_{23}H_{22}O_{12}N_6P_3$: 667.0514; found: 667.0504.

7-[(2',2'''-bipyridin-5''-yl)ethynyl]-7-deaza-2'-deoxycytidine 5'-O-triphosphate (dA^{5bpy}TP)

This compound was prepared according to the general procedure from 7-iodo-7-deaza-2'-

deoxyadenosine triphosphate **dA^{I}TP** and **1b** in the yield 48%.

^{1}H NMR 499.8 MHz, D_2O, ref$_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.42 (ddd, 1H, J_{gem} = 13.7, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.9, H-2′b); 2.59 (ddd, 1H, J_{gem} = 13.7, $J_{2'a,1'}$ = 7.8, $J_{2'a,3'}$ = 6.4, H-2′a); 4.17 (m, 2H, H-5′); 4.22 (m, 1H, H-4′); 4.72 (dt, 1H, $J_{3',2'}$ = 6.4, 2.9, $J_{3',4'}$ = 2.9, H-3′); 6.15 (dd, 1H, $J_{1',2'}$ = 7.8, 6.0, H-1′); 7.48 (dd, 1H, $J_{5''',4'''}$ = 7.2, $J_{5''',6'''}$ = 4.6, H-5‴); 7.41 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.2, $J_{4''',6'''}$ = 1.2, H-4‴); 7.45 (s, 1H, H-6); 7.52 (d, 1H, $J_{3''',4'''}$ = 8.0, H-3‴); 7.62 (dd, 1H, $J_{4'',3''}$ = 8.3, $J_{4'',6''}$ = 1.7, H-4″); 7.67 (d, 1H, $J_{3'',4''}$ = 8.3, H-3″); 7.78 (s, 1H, H-2); 8.03 (d, 1H, $J_{6''',5'''}$ = 4.6, H-6‴); 8.16 (bs, 1H, H-6″); ^{13}C NMR (125.7 MHz, D_2O, ref$_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.25 (CH_2-2′); 68.42 (d, $J_{C,P}$ = 6.0, CH_2-5′); 73.83 (CH-3′); 85.50 (CH-1′); 87.83 (d, $J_{C,P}$ = 8.7, CH-4′); 89.25 (bpy-C≡**C**); 91.61 (bpy-**C**≡C); 98.74 (C-5); 104.82 (C-4a); 122.62 (C-5″); 123.31 (CH-3″); 123.71 (CH-3‴); 126.72 (CH-5‴); 129.11 (CH-6); 140.39 (CH-4‴); 142.02 (CH-4″); 150.45 (C-7a); 150.52 (CH-6‴); 152.62 (CH-6″); 153.97 (CH-2); 154.67 (C-2″); 155.18 (C-2‴); 159.01 (C-4); ^{31}P NMR (202.3 MHz, D_2O, ref$_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.84 (dd, J = 19.2, 18.9, P$_\beta$); -9.61 (d, J = 19.2, P$_\alpha$); -7.29 (d, J = 18.9, P$_\gamma$); MS (ES$^-$): found *m/z*: 647.0 (M-1), 587.2 (M–PO_3H_2–1); HRMS (ES$^-$): *m/z* calcd for $C_{23}H_{22}O_{12}N_6P_3$: 667.0514; found: 667.0512.

7-[4''-(2'',2''':6'',2'''-terpyridin-1''-yl)ethynyl]-7-deaza-2'-deoxycytidine 5'-O-triphosphate (dA^{tpy}TP)

This compound was prepared according to the general procedure from 7-iodo-7-deaza-2'-deoxyadenosine triphosphate **dA^{I}TP** and **1c** in the yield 40%.

^{1}H NMR (499.8 MHz, D_2O, ref$_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.34, 2.44 (2 × bm, 2 × 1H, H-2′); 4.10 (bm, 2H, H-5′); 4.20 (bm, 1H, H-4′); 4.61 (bm, 1H, H-3′); 6.06 (bm, 1H, H-1′); 6.77 (bm, 2H, H-5′-terpy); 7.06 (bm, 2H, H-3′-terpy); 7.18 (bs, 1H, H-6); 7.32 (bm, 2H, H-4′-terpy); 7.72 (bs, 4H, H-3,5-terpy, H-6′-terpy); 7.76 (bs, 1H, H-2); ^{13}C NMR (125.7 MHz, D_2O, ref$_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 40.72 (CH_2-2′); 68.47 (d, $J_{C,P}$ = 4.7, CH_2-5′); 73.66 (CH-3′); 85.38 (CH-1′); 87.65 (d, $J_{C,P}$ = 8.6, CH-4′); 89.66 (terpy-C≡**C**); 92.89 (terpy-**C**≡C); 97.94 (C-5); 104.84 (C-4a); 123.36 (CH-3′-terpy); 124.08 (CH-3,5-terpy); 126.96 (CH-5′-terpy); 129.67 (CH-6); 134.80 (C-4-terpy); 140.73 (CH-4′-terpy); 150.08 (CH-6′-terpy); 150.37 (C-7a); 154.14 (CH-2); 154.74 (C-2′-terpy); 155.68 (C-2,6-terpy); 158.77 (C-4); ^{31}P NMR (202.3 MHz, D_2O, ref$_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.65 (bt, J = 18.7, 19.3, P$_\beta$); -9.82 (d, J = 18.7, P$_\alpha$); -6.49 (b, P$_\gamma$); MS (ES$^-$): found *m/z*: 744.0

(M-1), 664.1 (M–PO_3H_2–1); HRMS (ES^-): *m/z* calcd for $C_{28}H_{25}O_{12}N_7P_3$: 744.0780; found: 744.0767.

Primer extension, purification and analysis of the PEX products

Synthetic ONs were purchased from Sigma Aldrich (USA). Primer: 5'-CAT GGG CGG CAT GGG-3'; templates: 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3'(temp^{rnd16}) segments forming duplex with the primer are in italics, the replicated segments are in bold). Templates used in experiment involving the DBstv magnetoseparation procedure were biotinylated at their 5' ends. Streptavidine magnetic beads MagSelect were obtained from Sigma Aldrich (USA), Pwo DNA polymerase from PeqLab (Germany), DyNAzyme II and Phusion DNA polymerases from Finnzymes (Finland), KOD XL DNA polymerase from Novagen, Vent (exo^-), Deep Vent, Deep Vent (exo^-) and Therminator 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)

Primer Extension experiment: The reaction mixture (20 μl) contained DNA polymerase: Vent (exo-) (0.2U/μl, 1 μl), Phusion (0.2U/μl, 1 μl), KOD (0.25U/μl, 0.8 μl), Therminator (0.2U/μl, 1 μl), Deep Vent (0.2U/μl, 1 μl), Deep Vent (exo-) (0.2U/μl, 1 μl), dNTPs (either natural or modified, 4mM, 1 μl), ^{32}P-prelabelled primer at 5'-end (3 μM, 1 μl) and template temp^{rnd16} (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}MPs: Complexes of modified nucleoside monophosphates **dN^{R}MPs** with diverse transition metals were prepared by mixing 100 μl of corresponding monophosphate (100 μM) with 100 μl 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.

Supplementary results – PAGE of PEX:

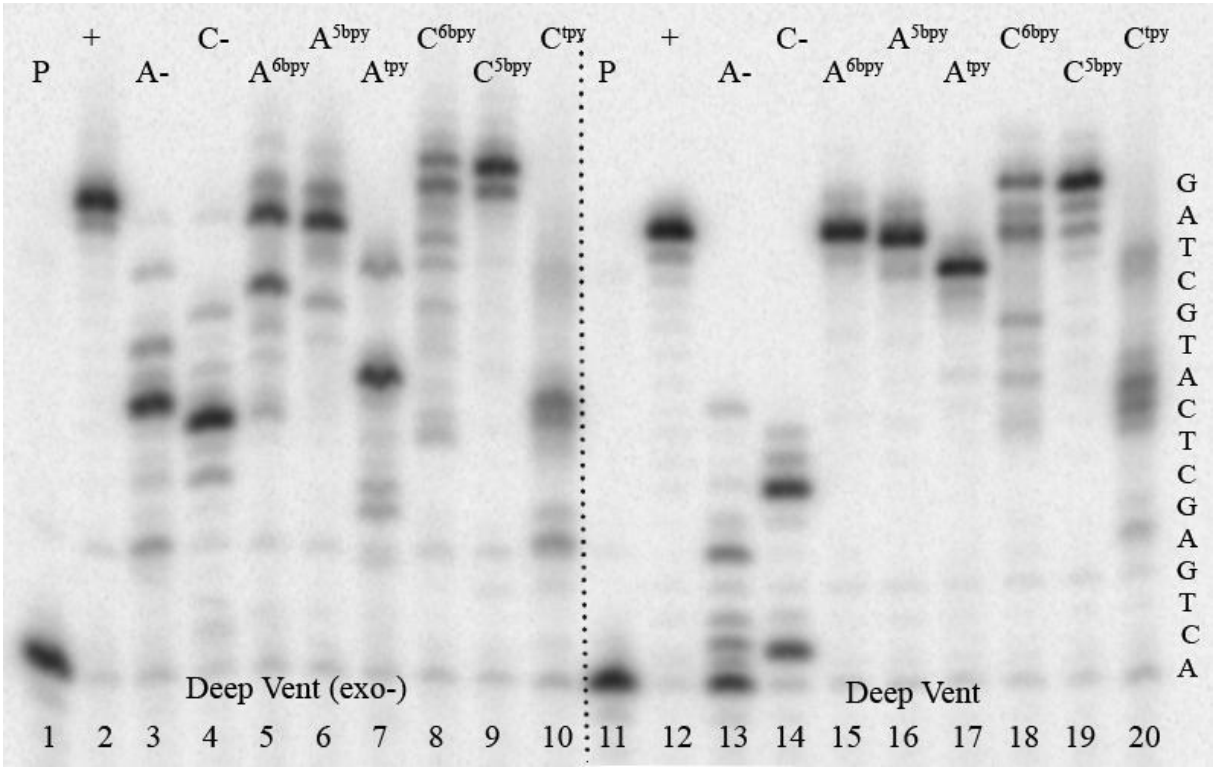

Fig. S1. Denaturing PAGE analysis of PEX experiment synthetized on temp^{rnd16} with Deep Vent (exo-) and Deep Vent polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; +: natural dNTPs; A-: dTTP, dCTP, dGTP; C-:dATP, dTTP, dGTP; A^{6bpy}: **dA^{6bpy}TP**, dTTP, dCTP, dGTP; A^{5bpy}: **dA^{5bpy}TP**, dTTP, dCTP, dGTP; A^{tpy}: **dA^{tpy}P**, dTTP, dCTP, dGTP; C^{6bpy}: **dC^{6bpy}TP**, dATP, dTTP, dGTP; C^{5bpy}: **dC^{5bpy}TP**, dATP, dTTP, dGTP; C^{tpy}: **dC^{tpy}TP**, dATP, dTTP, dGTP.

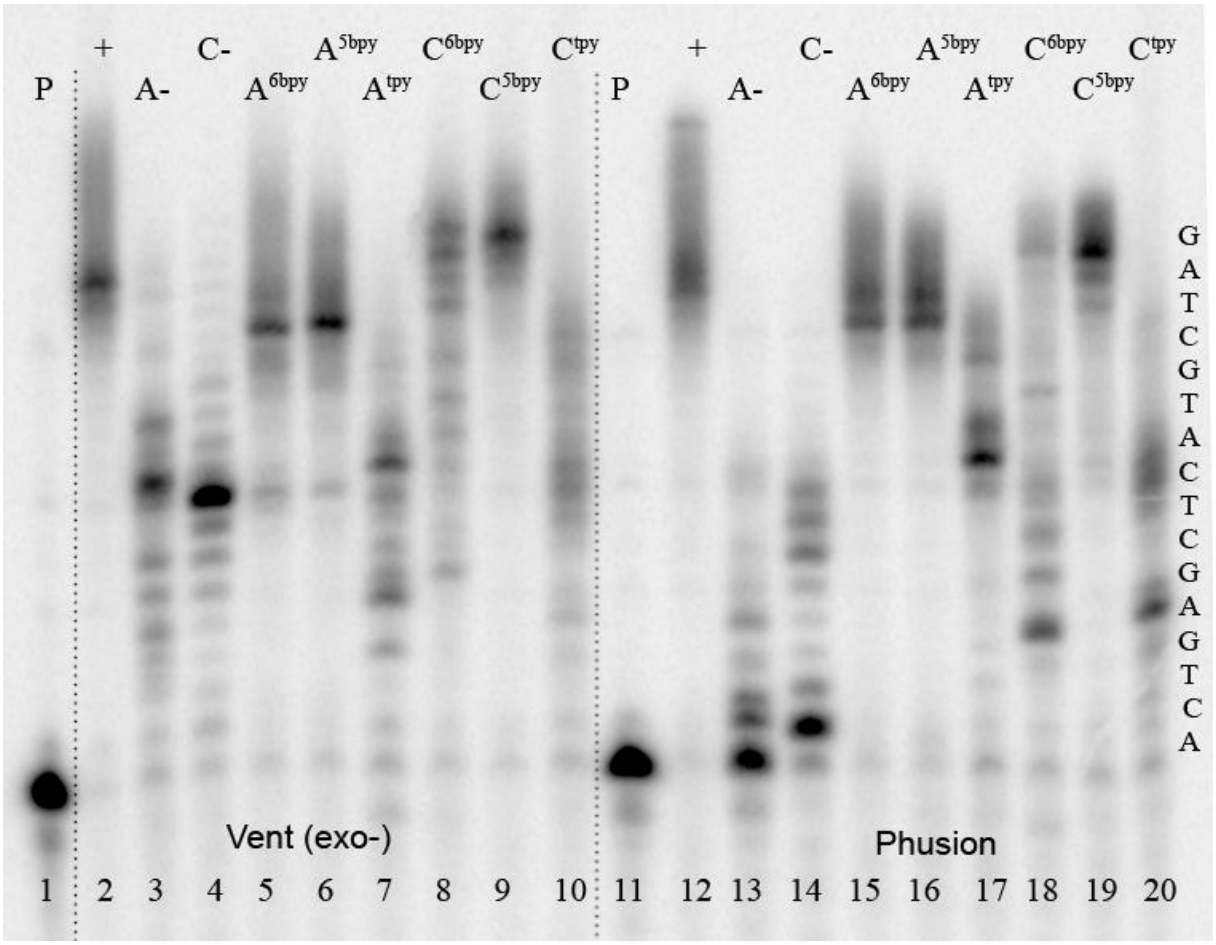

Fig. S2. Denaturing PAGE analysis of PEX experiment synthetized on temp^{rnd16} with Vent (exo-) and Phusion polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; +: natural dNTPs; A-: dTTP, dCTP, dGTP; C-:dATP, dTTP, dGTP; A^{6bpy}: **dA^{6bpy}TP**, dTTP, dCTP, dGTP; A^{5bpy}: **dA^{5bpy}TP**, dTTP, dCTP, dGTP; A^{tpy}: **dA^{tpy}P**, dTTP, dCTP, dGTP; C^{6bpy}: **dC^{6bpy}TP**, dATP, dTTP, dGTP; C^{5bpy}: **dC^{5bpy}TP**, dATP, dTTP, dGTP; C^{tpy}: **dC^{tpy}TP**, dATP, dTTP, dGTP.

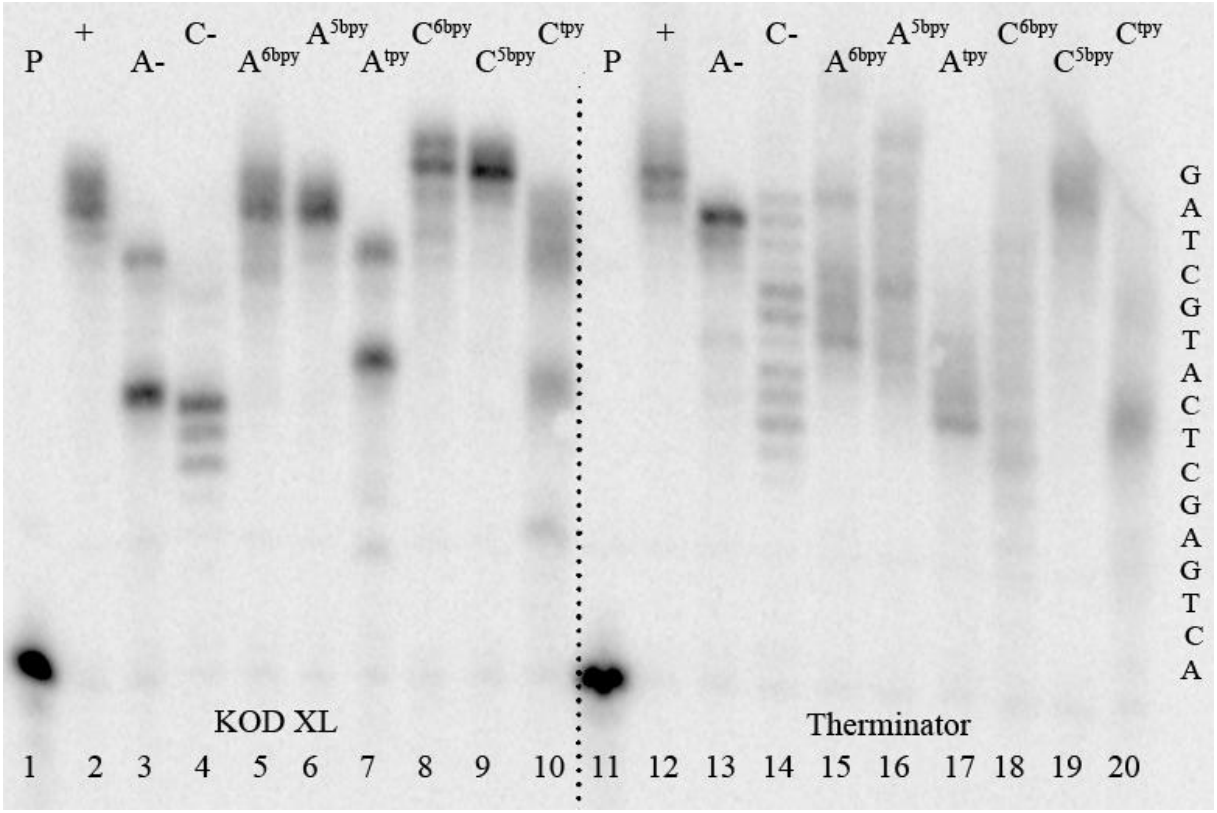

Fig. S3. Denaturing PAGE analysis of PEX experiment synthetized on temp^{rnd16} with KOD XL and Therminator polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; +: natural dNTPs; A-: dTTP, dCTP, dGTP; C-:dATP, dTTP, dGTP; A^{6bpy}: **dA^{6bpy}TP**, dTTP, dCTP, dGTP; A^{5bpy}: **dA^{5bpy}TP**, dTTP, dCTP, dGTP; A^{tpy}: **dA^{tpy}P**, dTTP, dCTP, dGTP; C^{6bpy}: **dC^{6bpy}TP**, dATP, dTTP, dGTP; C^{5bpy}: **dC^{5bpy}TP**, dATP, dTTP, dGTP; C^{tpy}: **dC^{tpy}TP**, dATP, dTTP, dGTP.

MALDI-TOF experiment (ssDNA)

The reaction mixture (200 µl) contained DNA polymerase: Pwo (1U/µl, 10 µl) or DyNAzyme II (2U/µl, 10 µl), dNTPs (either natural or modified, 4mM, 10 µl), unlabeled primer (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) or temp^{rnd16} (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, Sigma -Aldrich) were 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 terget 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: 5976.5 Da
Mass - pex^{A} (**dA^{6bpy}TP**, dGTP): calculated: 6150.2 Da; found: 6154.4 Da
Mass - pex^{A} (**dA^{5bpy}TP**, dGTP): calculated: 6150.2 Da; found: 6152.2 Da
Mass - pex^{A} (**dA^{tpy}TP**, dGTP): calculated: 6227.3 Da; found: 6236.5 Da
Mass - pex^{rnd16} (dATP, dCTP, dTTP, dGTP): calculated: 9613.1 Da; found: 9616.9 Da
Mass - pex^{rnd16} (**dA^{6bpy}TP**, dCTP, dTTP, dGTP): calculated: 10321.9 Da; found: 10328.6 Da
Mass - pex^{rnd16} (**dA^{5bpy}TP**, dCTP, dTTP, dGTP): calculated: 10321.9 Da; found: 10326.5 Da
Mass - pex^{rnd16} (**dA^{tpy}TP**, dCTP, dTTP, dGTP): calculated: 10630.3 Da; found: 10637.1 Da
Mass - pex^{rnd16} (dCTP, dATP, dTTP, dGTP): calculated: 9613.1 Da; found: 9616.7 Da
Mass - pex^{rnd16} (**dC^{6bpy}TP**, dATP, dTTP, dGTP): calculated: 10325.9 Da; found: 10338.1 Da
Mass - pex^{rnd16} (**dC^{5bpy}TP**, dATP, dTTP, dGTP): calculated:10325.9 Da; found:10331.4 Da

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

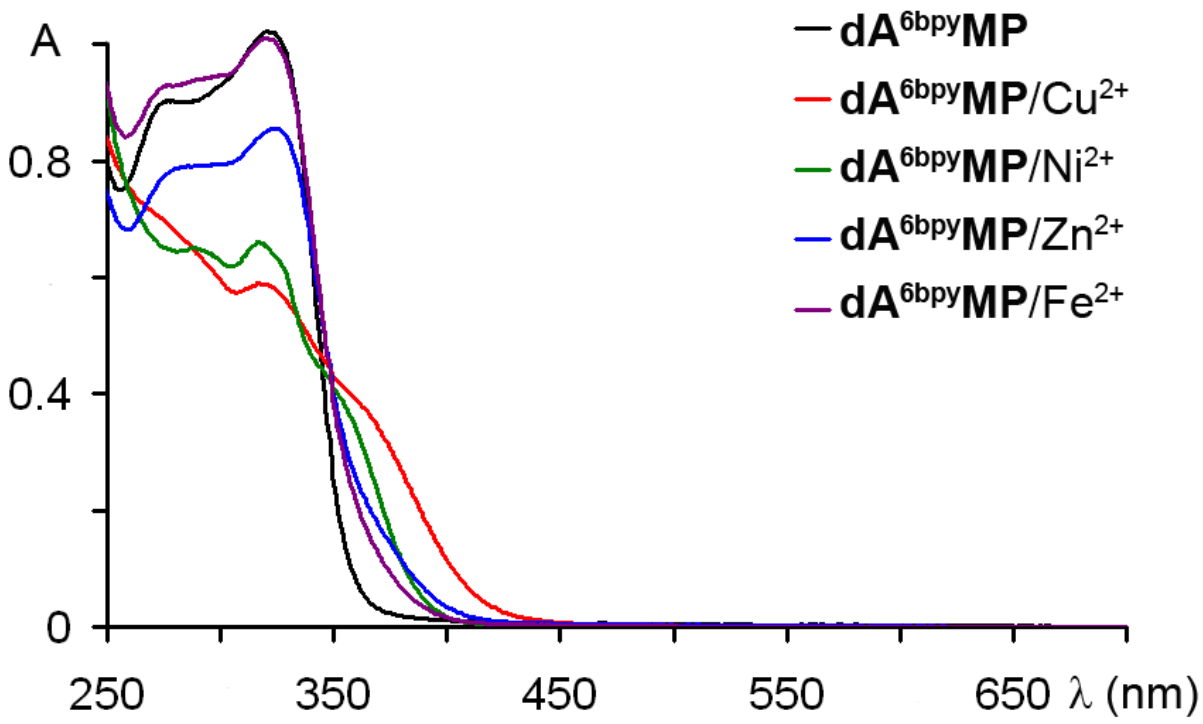

Figure S4. UV/Vis spectra of **dA^{6bpy}MP** with divalent metals.

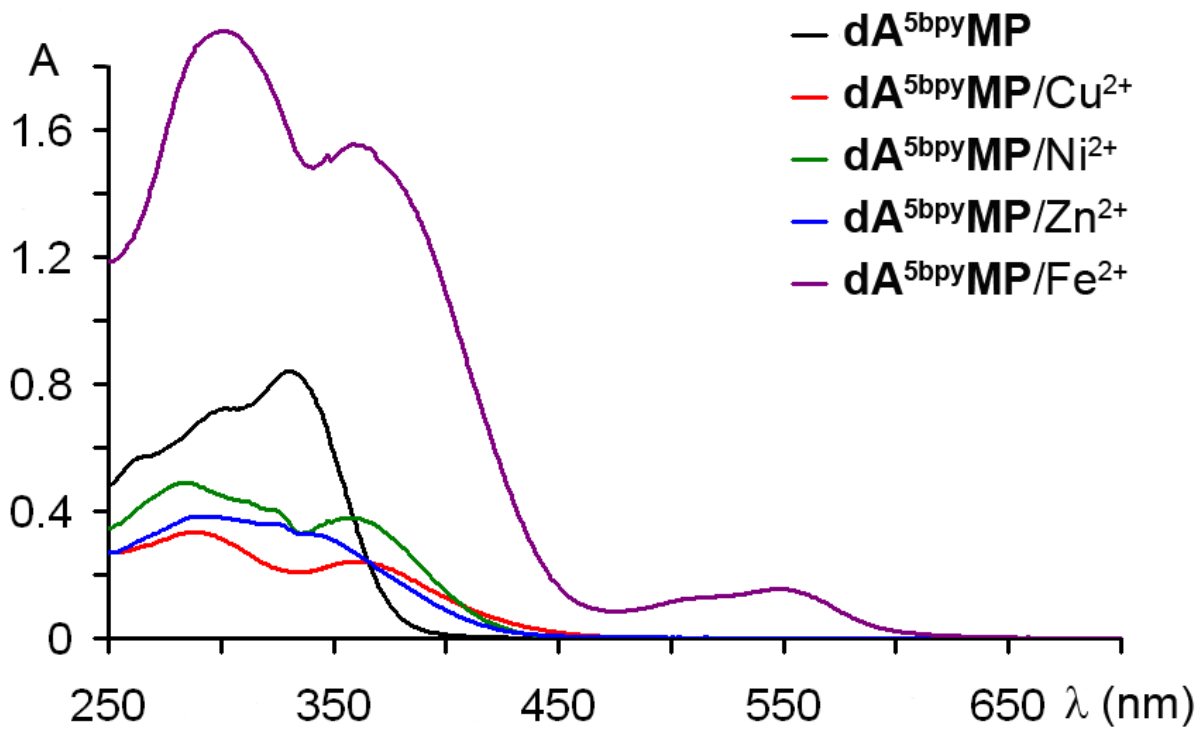

Figure S5. UV/Vis spectra of **dA^{5bpy}MP** with divalent metals.

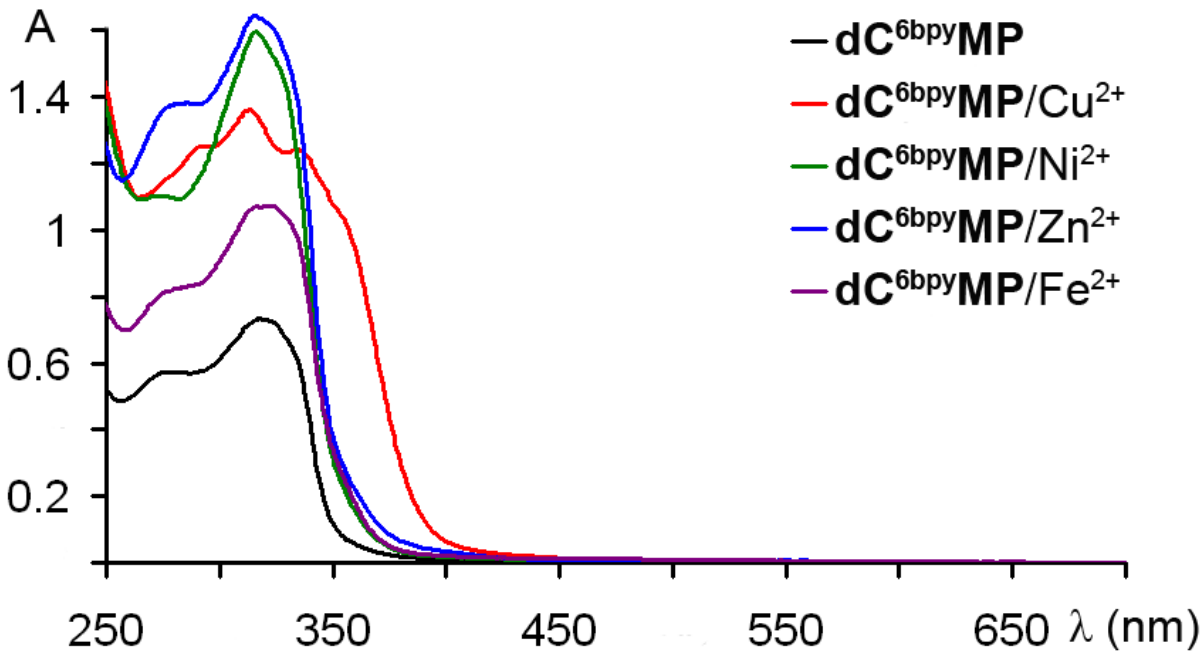

Figure S6. UV/Vis spectra of **dC^{6bpy}MP** with divalent metals.

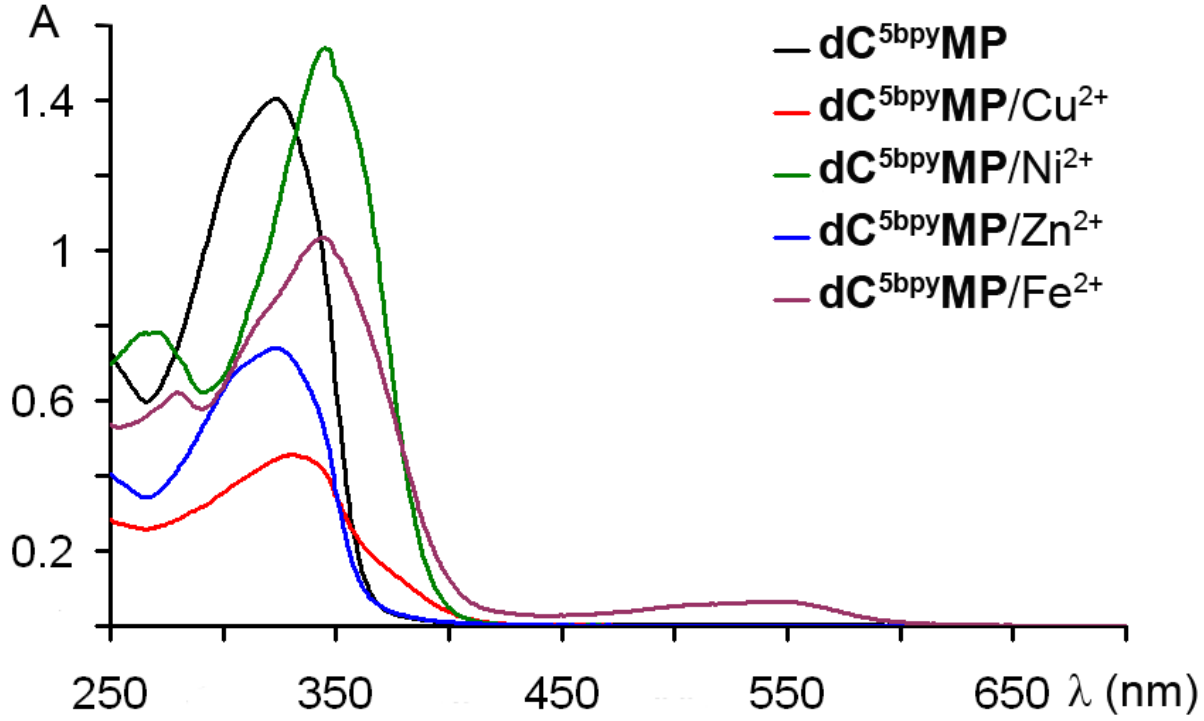

Figure S7. UV/Vis spectra of **dC^{5bpy}MP** with divalent metals.

1 V. Grosshenny, F. M. Romero and R. Ziessel, *J. Org. Chem.*, 1997, **62**, 1491.

2 (a) V. Raindlová, R. Pohl, M. Šanda and M. Hocek, *Angew. Chem. Int. Ed.*, 2010, **49**, 1064; (b) P. Kielkowski, H. Macíčková-Cahová, R. Pohl and M. Hocek, *Angew. Chem. Int. Ed.*, 2011, DOI: 10.1002/anie.201102898.

3 (a) P. Čapek, H. Cahová, R. Pohl, M. Hocek, C. Gloekner and A. Marx, *Chem. Eur. J.*, 2007, **13**, 6196; (b) H. Cahová, L. Havran, P. Brázdilová, H. Pivoňková, R. Pohl, M. Fojta and M. Hocek, *Angew. Chem. Int. Ed.*, 2008, **47**, 2059.