Supplementary Information

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Contents:

General. ^{1}H, ^{13}C and ^{31}P NMR spectra were obtained on a JEOL ECX-400P, JEOL ECA-500 or JEOL AL-400 and were reported in parts per million (δ) relative to residual solvent signal for ^{1}H NMR spectra, 1,4-dioxane signal (67.2 ppm) as internal standard for ^{13}C NMR spectra, and 85% phosphoric acid (0.0 ppm) as external standard for ^{31}P NMR spectra. Coupling constants (*J*) were reported in Hertz (Hz). Abbreviations of multiplicity were as follow: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Data were presented as follows: chemical shift (multiplicity, integration, coupling constant). LR- and HR-MS spectra were obtained on a JEOL JMS-HX110, JEOL JMS-700TZ or JEOL JMS-T100LP. UV spectra were measured with Shimadzu UV Visible Spectrophotometer UV-2450. pH was measured with Beckman Coulter F360 pH meter. DNA oligomers were prepared on an Applied Biosystems 3400 DNA Synthesizer. HPLC was performed with Shimadzu LC-10AD-VP or LC-20AB (pump), Shimadzu SPD-M10A-VP or SPD-M20A (UV-visible detector), Shimadzu CTO-10AS-VP or CTO-20A (column oven), CLASS-VP system, LabSolutions (system controller). Sep-pak Plus C18 Cartridge was purchased from Waters. YMC J'sphere ODS-M80 (150 × 4.6 mm) was used as reversed-phase C18 HPLC columns. DNA polymerases were purchased from Takara Shuzo (Klenow (exo−), exTaq), Promega (Tfl, Pfu), New England Biolabs (Vent (exo−), Deep Vent (exo−), Therminator, Therminator II) and Toyobo (KOD Dash). PAGE analysis and purification were performed with 220 × 220 × 0.5 mm and 220 × 220 × 1.5 mm sized gel, respectively. MALDI-TOF mass spectrum was measured with Bruker Daltonics Ultraflex TOF/TOF. Incubation of enzymatic reactions was performed with EYELA MG-1200.

Optimized methods of the primer extension experiments (20mer primer and 27mer

template). The following mixture was used: 0.8 μM duplex consisting of a 5'-FAM labeled primer and a DNA template, Therminator DNA polymerase (0.2 U/μL, 1.7 μM), 200 μM apioNTPs in the ThermoPol buffer [20 mM Tris-HCl (pH 8.8) containing 10 mM KCl, 10 mM $(NH_4)_2SO_4$ and 2 mM $MgSO_4$ and 0.1% Triton X-100] containing 1.25 mM $MnCl_2$ in a final volume of 10 μL. The reaction was performed at 44 °C for 1 h and quenched with the addition of 10 μL loading buffer [1 × TBE buffer (89 mM Tris/89 mM boric acid/2 mM EDTA), 7 M urea, 0.05% xylene cyanol and bromophenol blue]. The reaction mixture (8 μL) was resolved by electrophoresis at 800 V for 5 h using a 20% (19:1) denaturing polyacrylamide gel containing 7 M urea, and the gels were quantified with FLA-2000 (FUJIFILM).

MALDI-TOF mass spectrum of the elongated product (20mer primer and 27mer template). The following mixture was used: 0.8 μM duplex consisting of a primer and a DNA template, Therminator DNA polymerase (0.2 U/μL, 1.7 μM), 200 μM apioNTPs in the ThermoPol buffer containing 1.25 mM $MnCl_2$ in a final volume of 40 μL. The reaction was performed at 44 °C for 1 h and quenched with the addition of 40 μL of 10 M urea. The mixture was desalted, and counter cations of phosphate groups were exchanged to ammonium salts on ODS column (YMC disposable SPE). The oligonucleotide was then analyzed by MALDI-TOF mass using 3-hydroxypicolinic acid and bis-ammonium citrate as matrix.

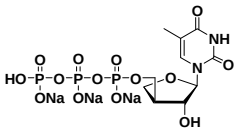
Single nucleotide insertion reactions using the steady-state method (20mer primer and 27mer template). Insertion reactions were initiated by adding 1 μL of dNTP or apioNTP solution (0.001-1 μM) to a reaction mixture containing a mixture of the 0.8

μM duplex consisting of a 5'-FAM labeled primer and a DNA template, and Therminator (0.005 U/μL, 42.5 nM) in the ThermoPol buffer [and 1.25 mM $MnCl_2$ for apioNTP (Mn (+))] in a final volume of 10 μL. The amount of dNTP or apioNTP was adjusted to 25% maximum insertion reaction. The reaction was performed at 74 °C for 3 min and quenched by adding 10 μL of the stop buffer. The reaction mixture (8 μL) was resolved by electrophoresis at 800 V for 5 h using a 20% (19:1) denaturing polyacrylamide gel containing 7 M urea, and the gels were quantified with FLA-2000 (FUJIFILM). Reaction velocities were calculated as the yield of reaction divided by reaction time. Kinetic parameters (K_m and V_{max}) were determined by linear regression analysis of a Hanes-Woolf plot[1] with an average of three independent experiments.

Synthesis and purification of the oligonucleotides. Oligonucleotides were synthesized with a DNA Synthesizer (Applied Biosystem Model 3400) by using 3'-deoxyapionucleoside phosphoramidites or commercially available 2'-deoxyribonucleoside phosphoramidite units at 1 μmol scale following the standard procedure described. Each of 3'-deoxyapionucleoside phosphoramidites was used at concentration of 0.1 M in dry MeCN, and the coupling time was extended to 15 min. After completion of the synthesis, the CPG support was treated with concentrated NH_4OH (55 °C, 12 h) and filtered off, and the filtrate was concentrated. The residue was dissolved in 500 μL of 90% formamide and purified by electrophoresis at 400 V for 8 h using a 20% (29 : 1) denaturing polyacrylamide gel (220 × 220 × 1.5 mm). The desired band was cut and extracted in TE buffer at room temperature for overnight. The extract was desalted on Sep-pak C18 column and eluted with aqueous 50% CH_3CN to obtain the desired oligonucleotide.

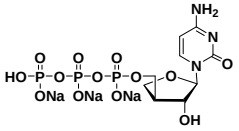
Single nucletide insertion reactions using the steady-state method (24mer primer and 27mer template). Insertion reactions were initiated by adding 1 μL of apioATP solution (0.001-1 μM) to a reaction mixture containing a mixture of the 0.8 μM duplex consisting of a 5'-FAM labeled primer and a DNA template, and Therminator (0.2 U/μL, 1.7 μM) in the ThermoPol buffer [and 1.25 mM $MnCl_2$ for apioATP (Mn (+))] in a final volume of 10 μL. The amount of apioATP was adjusted to 25% maximum insertion reaction. The reaction was performed at 74 °C for 3 min and quenched by adding 10 μL of the stop buffer. The reaction mixture (8 μL) was resolved by electrophoresis at 800 V for 4 h using a 20% (29:1) denaturing polyacrylamide gel containing 7 M urea, and the gels were quantified with FLA-2000 (FUJIFILM). Reaction velocities were calculated as the yield of reaction divided by reaction time. Kinetic parameters (K_m and V_{max}) were determined by linear regression analysis of a Hanes-Woolf plot[1] with an average of three independent experiments.

The synthesis of 3'-deoxyapionucleoside 3''-triphosphates (apioNTPs)

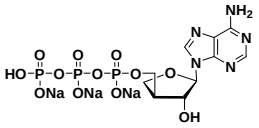


1-{(2*R*,3*R*,4*R*)-3-Hydroxy-4-[(triphosphoryl)methyl]tetrahydrofuran-2-yl}thymine sodium salt (**apioTTP**): A solution of 2'-*O*-acetyl-3'-deoxy-D-apiothymidine[2] (69 mg, 0.24 mmol) in pyridine/1,4-dioxane (1/3, 960 μL) was treated with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (73 mg, 0.36 mmol) in 1,4-dioxane (480 μL) at room temperature for 10 min. The reaction mixture was treated with 0.5 M solution of bis(tri-*n*-butylammonium)pyrophosphate in dry DMF (960 μL, 0.48 mmol) and tri-*n*-butylamine (320 μL, 1.3 mmol) at room temperature for 10 min. The reaction mixture was treated with 1% iodine in pyridine/water (98/2) (ca. 3 mL) for 5 min, and which was treated with 5% aqueous solution of $NaHSO_3$ (ca. 2 mL) for additional 30 min. The reaction mixture was concentrated *in vacuo*, and the residue was treated with saturated aqueous ammonia solution (20 mL) at room temperature overnight. The reaction mixture was concentrated *in vacuo,* and the residue was dissolved in H_2O (300 mL), and the solution applied to a DEAE Sephadex column, which was eluted with a linear gradient of 750 mL each of H_2O and 1.0 M TEAB (pH 8.0). Fractions containing desired product were concentrated *in vacuo* and coevaporated with H_2O/EtOH (1/1). The residue was dissolved in H_2O (10 mL), and the solution was applied to a column of DIAION PK 212 (H^+ form), which was eluted with H_2O. The eluate was applied to a DIAION WK 40 (Na^+ form), which was eluted with H_2O. Fractions containing apioTTP were concentrated *in vacuo* to give **apioTTP** (51%) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 7□55 (1H, s, H-6), 5.84 (1H, d, H-1', $J_{1',2'}$ = 6.4 Hz), 4.45 (1H, dd, H-2' $J_{2',1'}$ = 6.4, $J_{2',3'}$ = 6.8 Hz), 4.30 (1H, dd, H-4'a□□J_{gem} = 8.6, $J_{4'a,3'}$ = 8.6 Hz), 4.15 (1H, dd,

H-4'b□□J_{gem} = 8.6, $J_{4'b,3'}$ = 9.7 Hz), 4.13 (2H, dd, H-3'', J_{gem} = 9.7, $J_{3'',3'}$ = 7.4 Hz), 2.74 (1H, m, H-3'), 1.89 (3H, s, Me). ^{3}□P NMR (D_2O, 202 MHz) δ –10.45, –10.67, –22.79. ESIMS-LR *m/z* 481 [M-H]; ESIMS-HR calcd for $C_{10}H_{16}N_2O_{14}P_3$ 480.9814, found 480.9802.

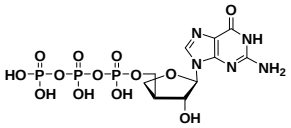


1-{(2*R*,3*R*,4*R*)-3-Hydroxy-4-[(triphosphoryl)methyl]tetrahydrofuran-2-yl}cytosine sodium salt (**apioCTP**, 54%, as a white solid) was obtained from 2'-*O*-acetyl-N^4-benzoyl-3'-deoxy-D-apiocytidine[2] (129 mg, 0.34 mmol) as described for the synthesis of **apioTTP**. ^{1}H NMR (D_2O, 500 MHz) δ 7□73 (1H, d, H-6, $J_{6,5}$ = 7.4 Hz), 6.05 (1H, d, H-5, $J_{5,6}$ = 7.4 Hz), 5.85 (1H, d, H-1', $J_{1',2'}$ = 5.7 Hz), 4.41 (1H, dd, H-2', $J_{2',1'}$ = 5.7, $J_{2',3'}$ = 6.3 Hz), 4.29 (1H, dd, H-4'a□□J_{gem} = 8.6, $J_{4'a,3'}$ = 8.0 Hz), 4.12 (1H, dd, H-4'b□□J_{gem} = 8.6, $J_{4'b,3'}$ = 8.0 Hz), 4.06 (2H, dd, H-3'', J_{gem} = 5.7, $J_{3'',3'}$ = 5.2 Hz), 2.70 (1H, m, H-3'). ^{3}□P NMR (D_2O, 202 MHz) δ□–6.33, –10.03, –21.91. ESIMS-LR *m/z* 466 [M-H]; ESIMS-HR calcd for $C_9H_{15}N_3O_{13}P_3$ 465.9818, found 465.9817.



9-{(2*R*,3*R*,4*R*)-3-Hydroxy-4-[(triphosphoryl)methyl]tetrahydrofuran-2-yl}adenine sodium salt (**apioATP**, 55%, as a white solid) was obtained from 2'-*O*-acetyl-N^6-benzoyl-3'-deoxy-D-apioadenosine[2] (78 mg, 0.20 mmol) as described for the synthesis of **apioTTP**. ^{1}H NMR (D_2O, 500 MHz) δ 8□35□□1H, s, H-2), 8.11 (1H, s, H-8), 5.94 (1H, d, H-1', $J_{1',2'}$ = 5.4 Hz), 4.84 (1H, d, H-2', $J_{2',1'}$ = 5.4, $J_{2',3'}$ = 6.9

Hz), 4.36 (1H, dd, H-4'a□□J_{gem} = 9.2, $J_{4'a,3'}$ = 8.7 Hz), 4.19 (1H, dd, H-4'b□□J_{gem} = 9.2, $J_{4'b,3'}$ = 8.7 Hz), 4.18 (2H, dd, H-3'', J_{gem} = 8.6, $J_{3'',3'}$ = 7.4 Hz), 2.86 (1H, m, H-3'). ^{3}□P NMR (D_2O, 202 MHz) δ –8.43, –10.17, –21.68. ESIMS-LR *m/z* 490 [M-H]; ESIMS-HR calcd for $C_{10}H_{15}N_5O_{12}P_3$ 489.9930, found 489.9913.



9-{(2*R*,3*R*,4*R*)-3-Hydroxy-4-[(triphosphoryl)methyl]teterahydrofuran-2-yl}guanine sodium salt (**apioGTP**, 44%, as a white solid) was obtained from 2'-*O*-acetyl-N^2-benzoyl-3'-deoxy-D-apioguanosine[2] (37 mg, 0.09 mmol) as described for the synthesis of **apioTTP**. ^{1}H NMR (D_2O, 500 MHz) δ 8□□□ (1H, s, H-8), 5.78 (1H, d, H-1', $J_{1',2'}$ = 5.7 Hz), 4.91 (1H, dd, H-2', $J_{2',1'}$ = 5.7, $J_{2',3'}$ = 7.4 Hz), 4.31 (1H, dd, H-4'a□□J_{gem} = 8.6, $J_{4'a,3'}$ = 8.6 Hz), 4.21 (2H, dd, H-3'', J_{gem} = 8.6, $J_{3'',3'}$ = 8.6 Hz), 4.19 (1H, dd, H-4'b□□J_{gem} = 8.6, $J_{4'b,3'}$ = 8.6 Hz), 2.86 (1H, m, H-3'). ^{3}□P NMR (D_2O, 202 MHz) δ□–5.28, –10.00, –20.97. ESIMS-LR *m/z* 506 [M-H]; ESIMS-HR calcd for $C_{10}H_{15}N_5O_{13}P_3$ 505.9879, found 505.9874.

A PAGE experiment for the elongation reaction using all four apioNTPs under various concentrations of Therminator DNA polymerase (20mer primer and 27mer template).

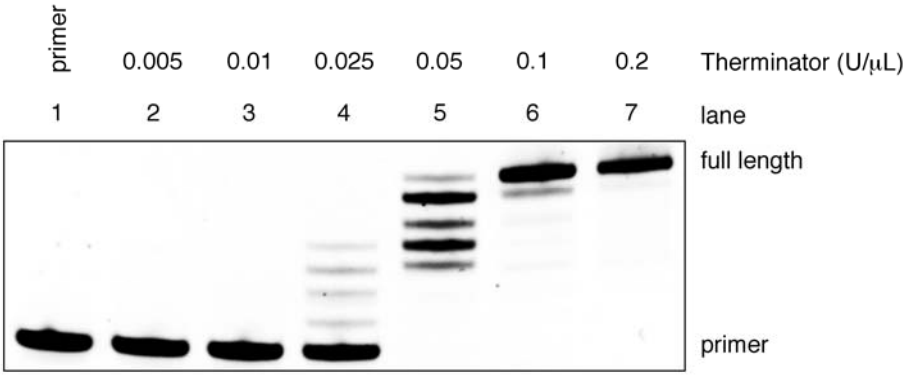


Figure S1. A PAGE experiment for the elongation reaction in the presence of 1.25 mM $MnCl_2$ using all four apioNTPs at 44 °C for 1 h under various concentrations of Therminator DNA polymerase. Lane 1; primer, lanes 2-7; the elongation product by using apioNTPs under various concentrations of Therminator DNA polymerase. Sequence of the primer-template complex and apioNA elongation product were shown in Figure 2A.

MALDI-TOF mass spectrum of elongated product (20mer primer and 27mer template).

m/z [M]⁻ calculated	measured	oligonucleotide
8401.43	8401.83	GACGGAATATAAGCTGGTGG**ACGTCGT**

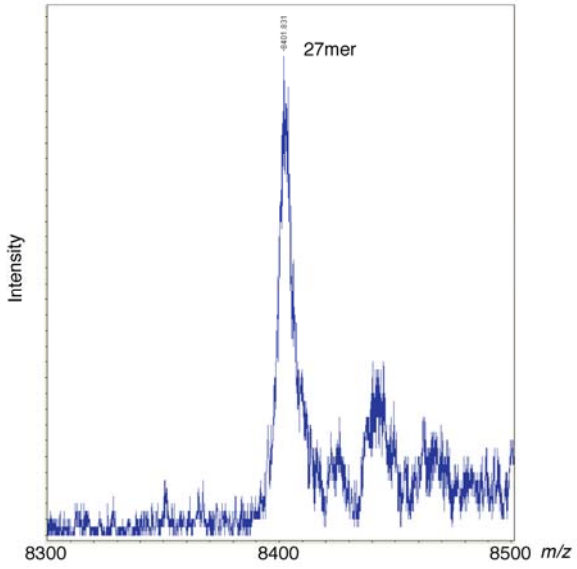


Figure S2. MALDI-TOF mass spectrum of the elongation product by using apioNTPs. The Spectrum was obtained from elongation reaction followed by ODS column purification.

A PAGE experiment for the longer elongation reaction using all four apioNTPs (21mer primer and 43mer template).

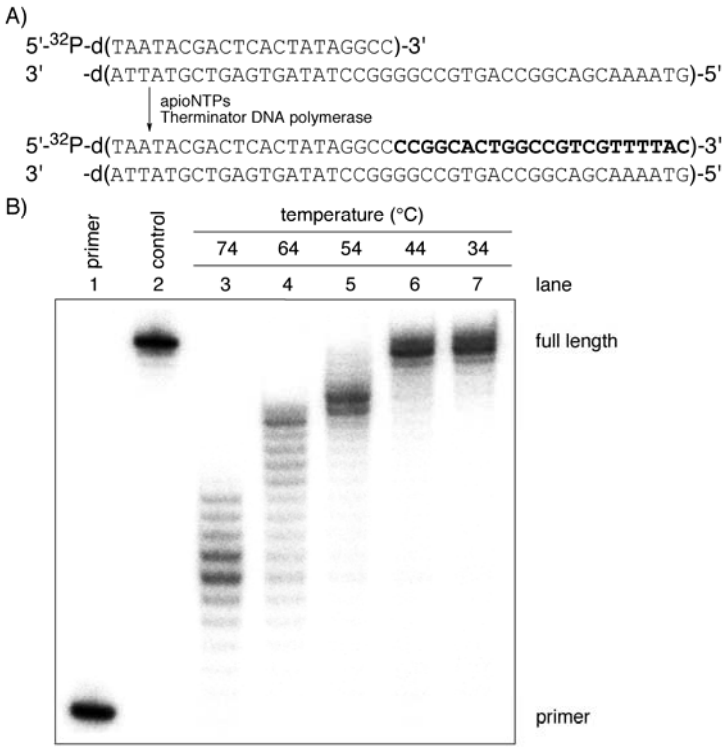
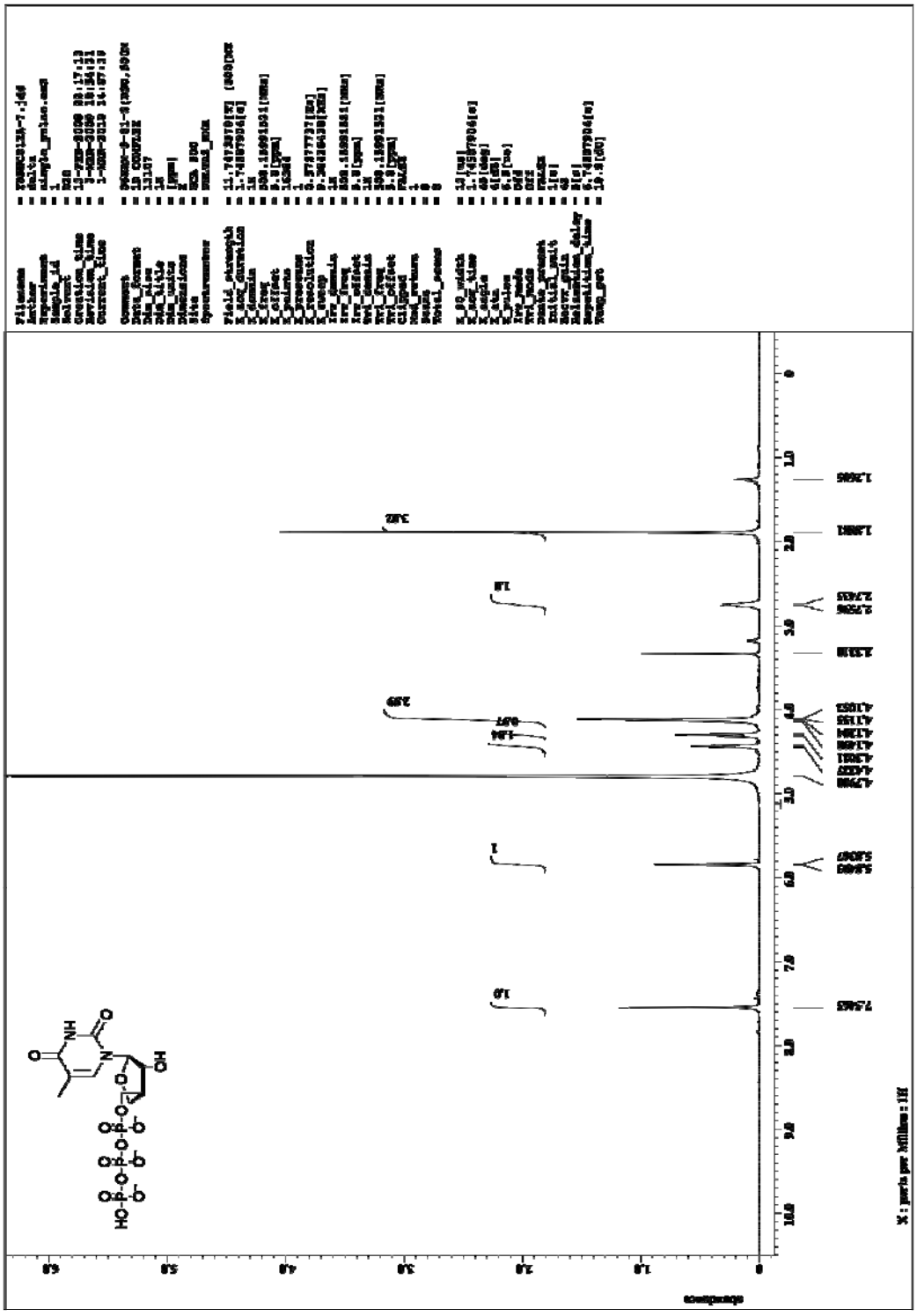
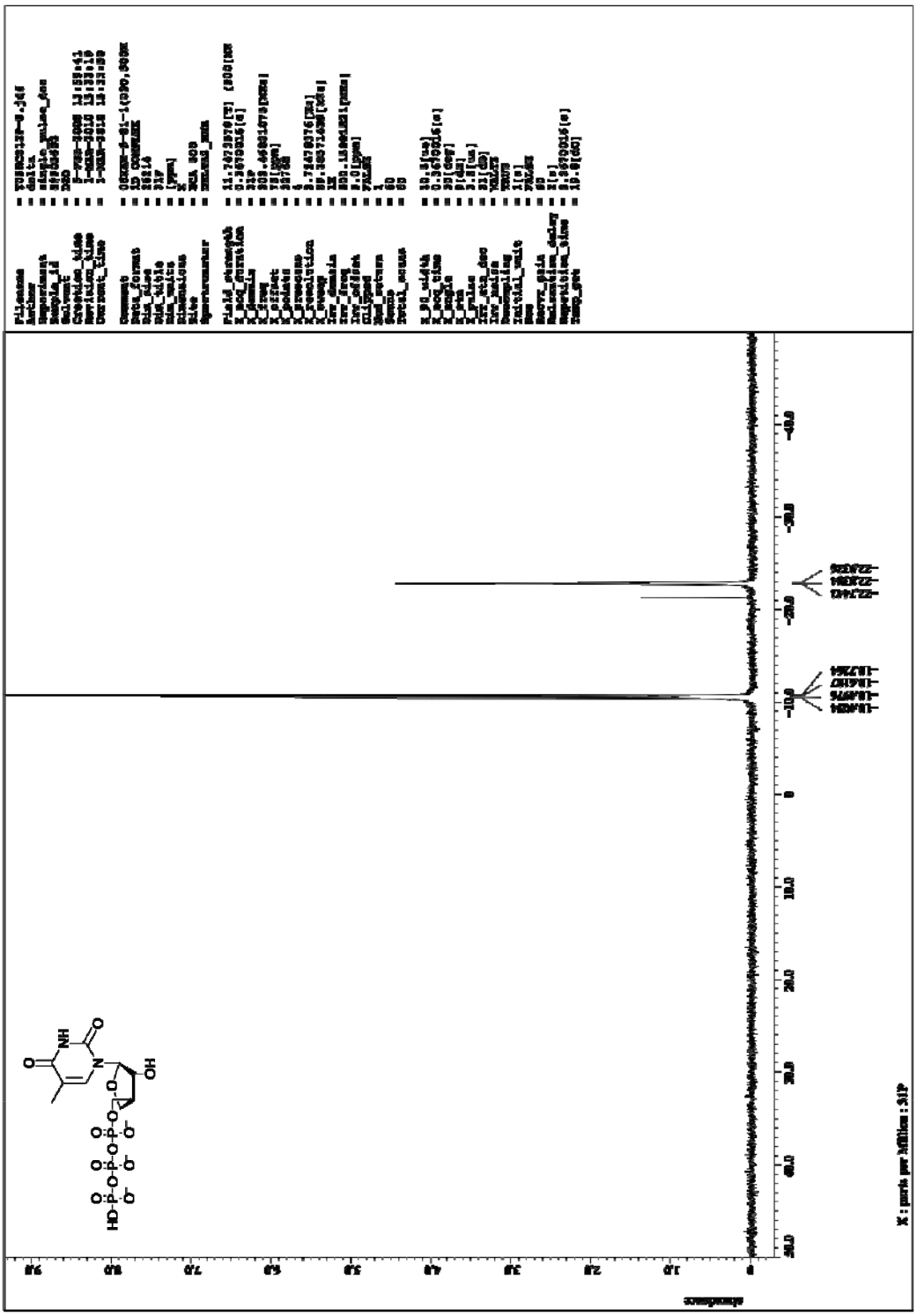
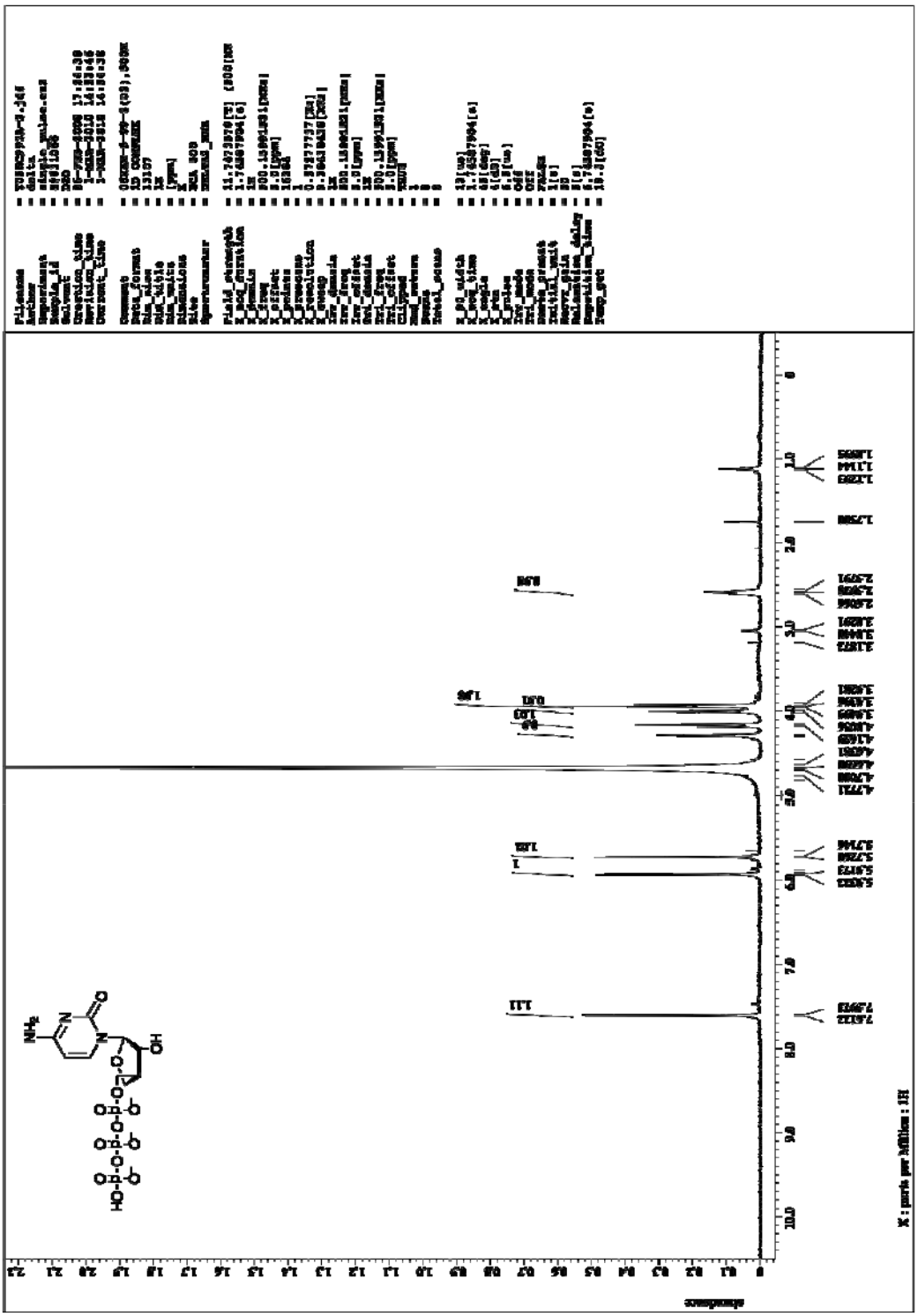


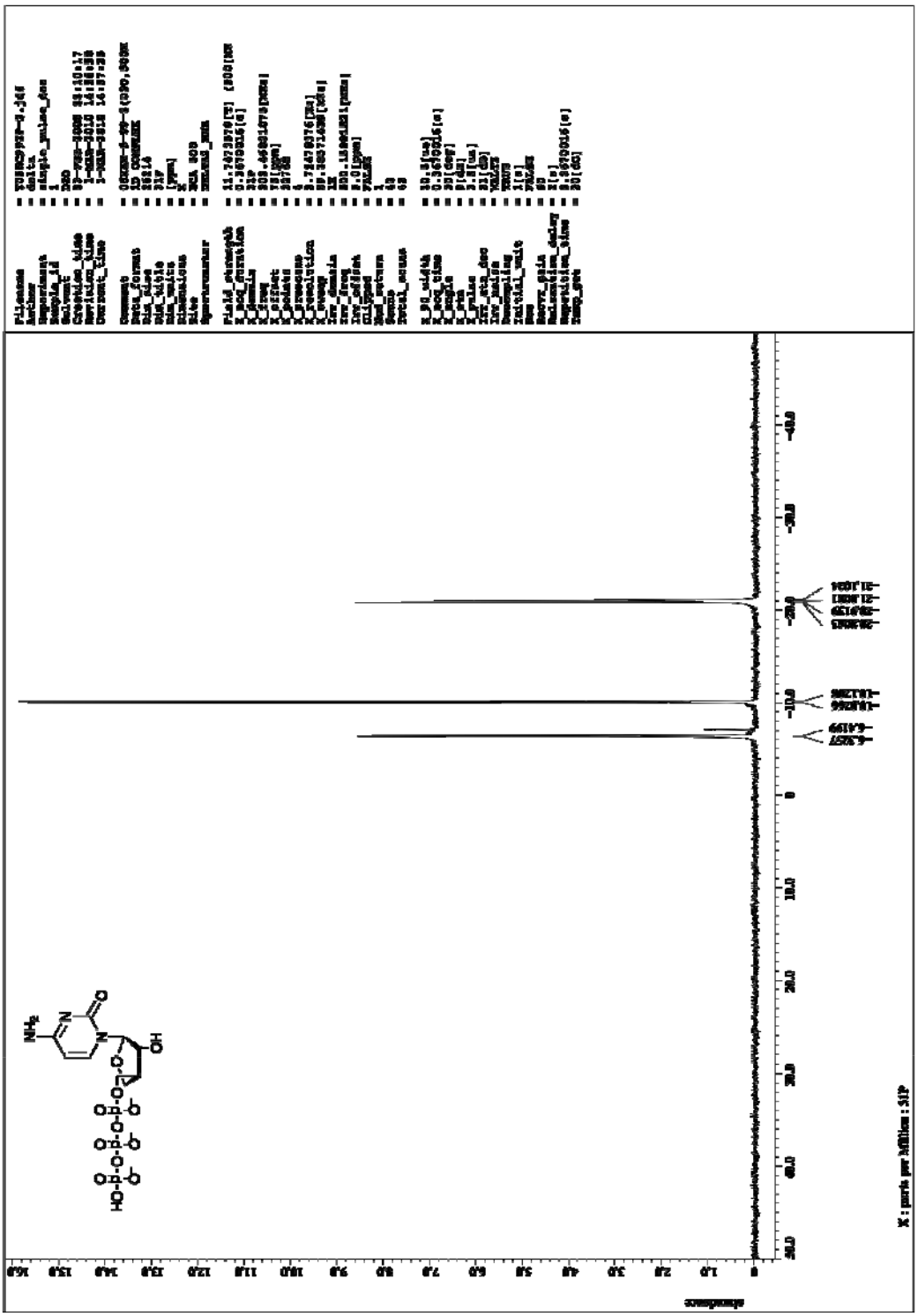
Figure S3. A) Sequence of the primer (21mer)-template (43mer) complex and the apioNA elongation product. Elongated apioNA is shown in bold letters. B) Primer extension experiments: The reactions were performed with same reaction conditions in general method (see supporting information) except for reaction temperature; 74, 64, 54, 44, and 34 °C (lanes 3-7), respectively. Lane 1; primer, lane 2; control.

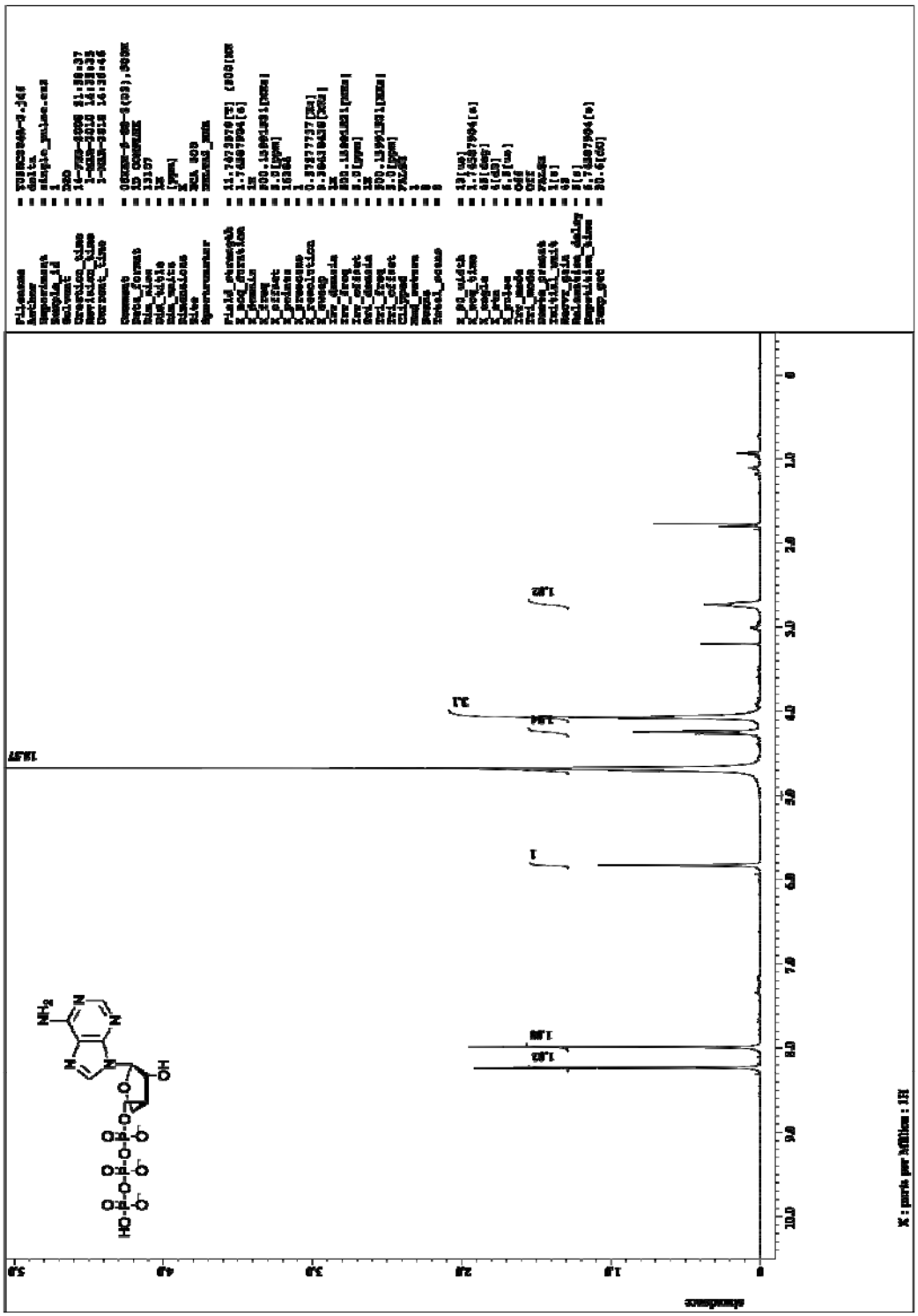
^{1}H and ^{31}P NMR spectra of apioNTPs.

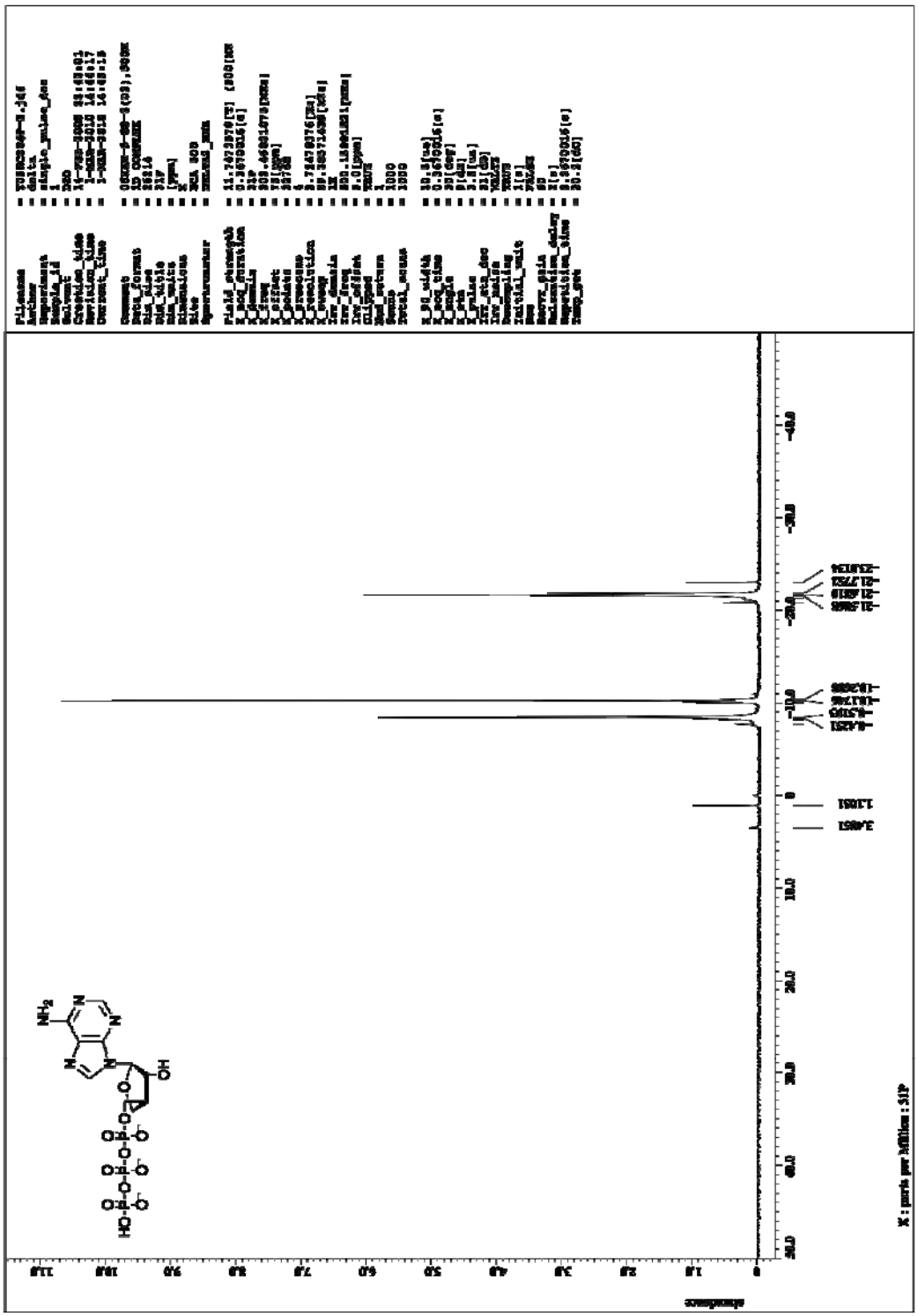


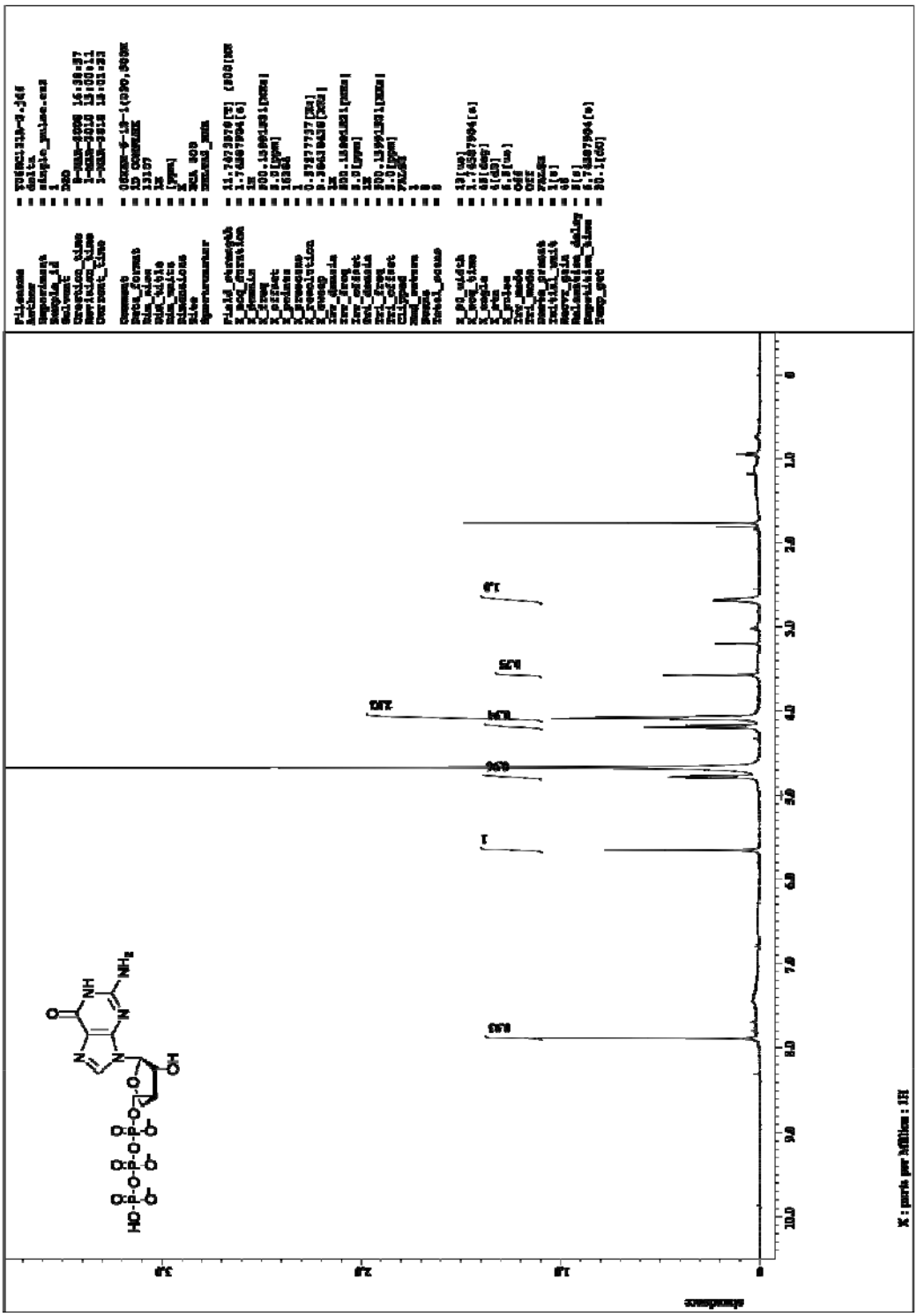


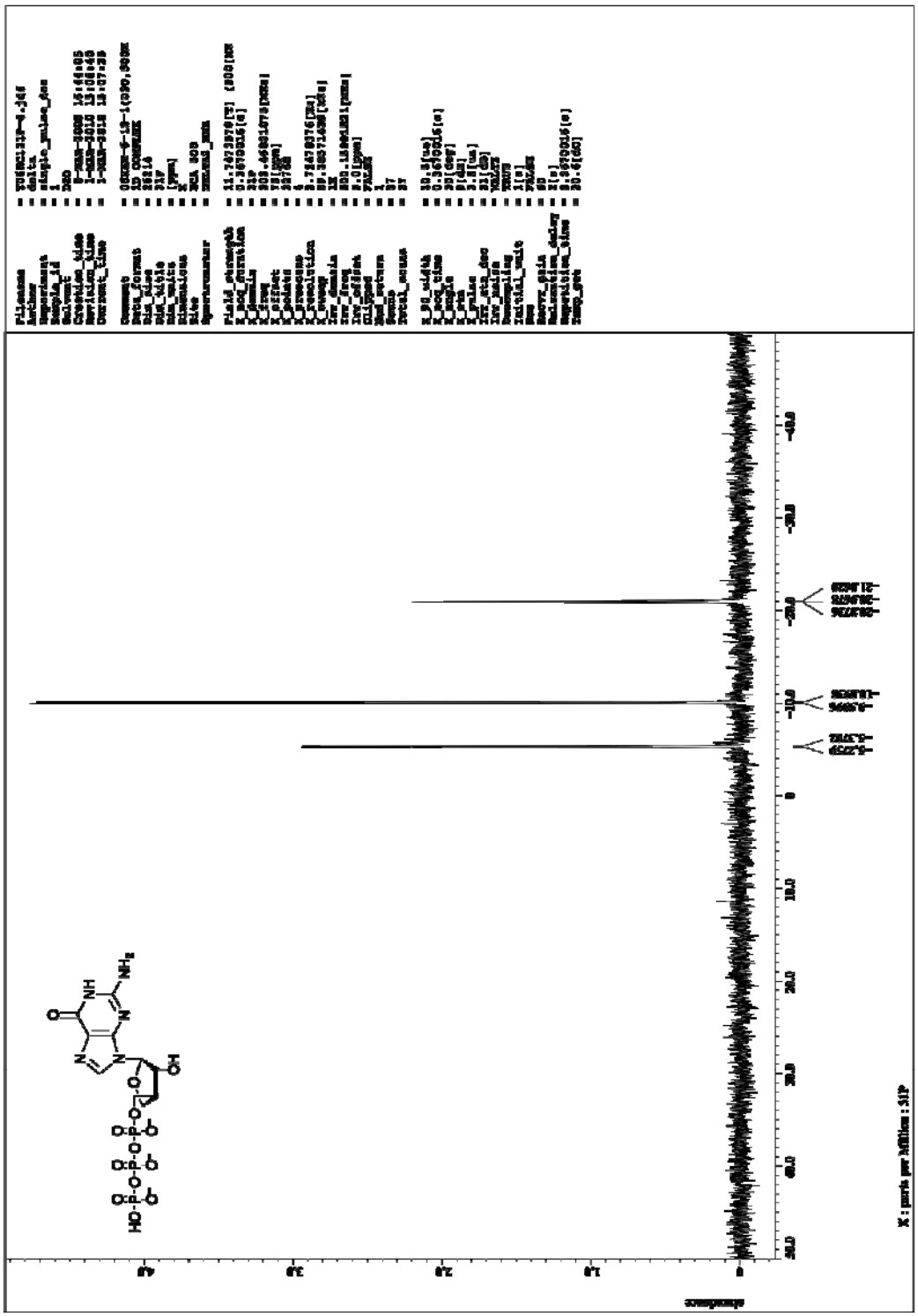












References

1 C. S. Hanes, *Biochem. J.* 1932, **26**, 1406.

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