Supplementary Information

Mayumi Kataoka, Yasuo Kouda, Kousuke Sato, Noriaki Minakawa and Akira Matsuda*

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

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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 (\(\delta\)) 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 \(\times\) 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 \(\times\) 220 \(\times\) 0.5 mm and 220 \(\times\) 220 \(\times\) 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 (NH₄)₂SO₄ and 2 mM MgSO₄ and 0.1% Triton X-100] containing 1.25 mM MnCl₂ 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 MnCl₂ 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 MnCl₂ 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 (Kₘ and Vₘₐₓ) 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 NH₄OH (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% CH₃CN to obtain the desired oligonucleotide.
Single nucleotide 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 MnCl₂ 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-{(2R,3R,4R)-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 \(\mu\)L) was treated with 2-chloro-4\(H\)-1,3,2-benzodioxaphosphorin-4-one (73 mg, 0.36 mmol) in 1,4-dioxane (480 \(\mu\)L) 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 \(\mu\)L, 0.48 mmol) and tri-n-butylamine (320 \(\mu\)L, 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 \textit{in vacuo}, and the residue was treated with saturated aqueous ammonia solution (20 mL) at room temperature overnight. The reaction mixture was concentrated \textit{in vacuo}, and the residue was dissolved in \(\text{H}_2\text{O}\) (300 mL), and the solution applied to a DEAE Sephadex column, which was eluted with a linear gradient of 750 mL each of \(\text{H}_2\text{O}\) and 1.0 M TEAB (pH 8.0). Fractions containing desired product were concentrated \textit{in vacuo} and coevaporated with \(\text{H}_2\text{O}/\text{EtOH}\) (1/1). The residue was dissolved in \(\text{H}_2\text{O}\) (10 mL), and the solution was applied to a column of DIAION PK 212 (\(\text{H}^+\) form), which was eluted with \(\text{H}_2\text{O}\). The eluate was applied to a DIAION WK 40 (\(\text{Na}^+\) form), which was eluted with \(\text{H}_2\text{O}\). Fractions containing apioTTP were concentrated \textit{in vacuo} to give apioTTP (51%) as a white solid. \(^1\text{H} \text{NMR} (\text{D}_2\text{O}, 500 \text{MHz}) \delta 7.55 (1\text{H}, \text{s}, \text{H}-6), 5.84 (1\text{H}, \text{d}, \text{H}-1', J_{1',2'} = 6.4 \text{ Hz}), 4.45 (1\text{H}, \text{dd}, \text{H}-2', J_{2',1'} = 6.4, J_{2',3'} = 6.8 \text{ Hz}), 4.30 (1\text{H}, \text{dd}, \text{H}-4'a \quad \text{J}_{\text{gem}} = 8.6, J_{4'a,3'} = 8.6 \text{ Hz}), 4.15 (1\text{H}, \text{dd},
H-4'b  \( J_{\text{gem}} = 8.6, J_{4'b,3'} = 9.7 \) Hz), 4.13 (2H, dd, H-3'', \( J_{\text{gem}} = 9.7, J_{3'',3'} = 7.4 \) Hz), 2.74 (1H, m, H-3'), 1.89 (3H, s, Me).\(^3\) P NMR (D\(_2\)O, 202 MHz) \( \delta -10.45, -10.67, -22.79. \) ESIMS-LR \( m/z \) 481 [M-H]; ESIMS-HR calcd for \( \text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_{14}\text{P}_3 \) 480.9814, found 480.9802.

1-\{(2\(R,3\)R,4\(R\))-3-Hydroxy-4-[(triphosphoryl)methyl]tetrahydrofuran-2-yl\}cytosine sodium salt (apio\textit{CTP}, 54\%, as a white solid) was obtained from 2'-\(O\)-acetyl-\(N^6\)-benzoyl-3'-deoxy-\(\delta\)-apiocytidine\(^2\) (129 mg, 0.34 mmol) as described for the synthesis of apio\textit{TTP}. \(^1\)H NMR (D\(_2\)O, 500 MHz) \( \delta \) 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 \( J_{\text{gem}} = 8.6, J_{4'a,3'} = 8.0 \) Hz), 4.12 (1H, dd, H-4'b \( J_{\text{gem}} = 8.6, J_{4'b,3'} = 8.0 \) Hz), 4.06 (2H, dd, H-3'', \( J_{\text{gem}} = 5.7, J_{3'',3'} = 5.2 \) Hz), 2.70 (1H, m, H-3').\(^3\) P NMR (D\(_2\)O, 202 MHz) \( \delta -6.33, -10.03, -21.91. \) ESIMS-LR \( m/z \) 466 [M-H]; ESIMS-HR calcd for \( \text{C}_{9}\text{H}_{15}\text{N}_3\text{O}_{13}\text{P}_3 \) 465.9818, found 465.9817.

9-\{(2\(R,3\)R,4\(R\))-3-Hydroxy-4-[(triphosphoryl)methyl]tetrahydrofuran-2-yl\}adenine sodium salt (apio\textit{ATP}, 55\%, as a white solid) was obtained from 2'-\(O\)-acetyl-\(N^6\)-benzoyl-3'-deoxy-\(\delta\)-apioadenosine\(^2\) (78 mg, 0.20 mmol) as described for the synthesis of apio\textit{TTP}. \(^1\)H NMR (D\(_2\)O, 500 MHz) \( \delta \) 8.35 (1H, s, H-2), 8.11 (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), 4.73 (1H, dd, H-4'a \( J_{\text{gem}} = 8.6, J_{4'a,3'} = 8.0 \) Hz), 4.64 (1H, dd, H-4'b \( J_{\text{gem}} = 8.6, J_{4'b,3'} = 8.0 \) Hz), 4.06 (2H, dd, H-3'', \( J_{\text{gem}} = 5.7, J_{3'',3'} = 5.2 \) Hz), 2.70 (1H, m, H-3').\(^3\) P NMR (D\(_2\)O, 202 MHz) \( \delta -6.33, -10.03, -21.91. \) ESIMS-LR \( m/z \) 466 [M-H]; ESIMS-HR calcd for \( \text{C}_{9}\text{H}_{15}\text{N}_3\text{O}_{13}\text{P}_3 \) 465.9818, found 465.9817.
9-{(2R,3R,4R)-3-Hydroxy-4-[(triphosphoryl)methyl]teterahydrofuran-2-yl}guanine sodium salt (apioGTP, 44%, as a white solid) was obtained from 2'-O-acetyl-N2-benzoyl-3'-deoxy-D-apioguanosine[2] (37 mg, 0.09 mmol) as described for the synthesis of apioTTP. 1H NMR (D2O, 500 MHz) δ 8 (1H, s, H-8), 5.78 (1H, d, H-1', J1',2' = 5.7 Hz), 4.91 (1H, dd, H-2', J2',1' = 5.7, J2',3' = 7.4 Hz), 4.31 (1H, dd, H-4'a Jgem = 8.6, J4'a,3' = 8.6 Hz), 4.21 (2H, dd, H-3'', Jgem = 8.6, J3'',3' = 8.6 Hz), 4.19 (1H, dd, H-4'b Jgem = 8.6, J4'b,3' = 8.6 Hz), 2.86 (1H, m, H-3'). P NMR (D2O, 202 MHz) δ −5.28, −10.00, −20.97. ESIMS-LR m/z 506 [M-H]; ESIMS-HR calcd for C10H15N5O13P3 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 MnCl₂ 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.
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
