Supplementary Information

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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 (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 follows: 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$_4$)$_2$SO$_4$ 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 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-[\text{(triphosphoryl)methyl} \text{tetrahydrofuran-2-yl}] \text{thymine sodium salt (apioTTP): A solution of 2'O-acetyl-3'-deoxy-D-apiothymidine[2] (69 mg, 0.24 mmol) in pyridine/1,4-dioxide (1/3, 960 \mu\text{L}) was treated with 2-chloro-4\text{H}-1,3,2-benzodioxaphosphorin-4-one (73 mg, 0.36 mmol) in 1,4-dioxide (480 \mu\text{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\text{L}, 0.48 mmol) and tri-n-butylamine (320 \mu\text{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 NaHSO₃ (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 H₂O (300 mL), and the solution applied to a DEAE Sephadex column, which was eluted with a linear gradient of 750 mL each of H₂O and 1.0 M TEAB (pH 8.0). Fractions containing desired product were concentrated \textit{in vacuo} and coevaporated with H₂O/EtOH (1/1). The residue was dissolved in H₂O (10 mL), and the solution was applied to a column of DIAION PK 212 (H⁺ form), which was eluted with H₂O. The eluate was applied to a DIAION WK 40 (Na⁺ form), which was eluted with H₂O. Fractions containing apioTTP were concentrated \textit{in vacuo} to give apioTTP (51%) as a white solid. ¹H NMR (D₂O, 500 MHz) δ 7.55 (1H, s, H-6), 5.84 (1H, d, H-1', J₁₁₂ = 6.4 Hz), 4.45 (1H, dd, H-2', J₂₂₂ = 6.4, J₂₂₂ = 6.8 Hz), 4.30 (1H, dd, H-4'a J₉₉ = 8.6, J₉₉ = 8.6 Hz), 4.15 (1H, 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 C\(_{10}H_{16}N_2O_{14}P_3\) 480.9814, found 480.9802.

1-\{(2R,3R,4R)-3-Hydroxy-4-[(triphosphoryl)methyl]tetrahydrofuran-2-yl\}cytosine sodium salt (apioCTP, 54%, as a white solid) was obtained from 2'-O-acetyl-N\(^{\alpha}\)-benzoyl-3'-deoxy-D-apiocytidine\(^{[2]}\) (129 mg, 0.34 mmol) as described for the synthesis of apioTTP. \(^1\)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 C\(_9\)H\(_{15}\)N\(_3\)O\(_{13}\)P\(_3\) 465.9818, found 465.9817.

9-\{(2R,3R,4R)-3-Hydroxy-4-[(triphosphoryl)methyl]tetrahydrofuran-2-yl\}adenine sodium salt (apioATP, 55%, as a white solid) was obtained from 2'-O-acetyl-N\(^{\alpha}\)-benzoyl-3'-deoxy-D-apioadenosine\(^{[2]}\) (78 mg, 0.20 mmol) as described for the synthesis of apioTTP. \(^1\)H NMR (D\(^2\)O, 500 MHz) \( \delta \) 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), 4.06 (2H, d, H-3**, \( J_{\text{gem}} = 5.4, J_{3'',3'} = 5.2 \) 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 C\(_9\)H\(_{15}\)N\(_3\)O\(_{13}\)P\(_3\) 465.9818, found 465.9817.
Hz), 4.36 (1H, dd, H-4'a $J_{\text{gem}} = 9.2, J_{4'a,3'} = 8.7$ Hz), 4.19 (1H, dd, H-4'b $J_{\text{gem}} = 9.2, J_{4'b,3'} = 8.7$ Hz), 4.18 (2H, dd, H-3'', $J_{\text{gem}} = 8.6, J_{3'',3'} = 7.4$ Hz), 2.86 (1H, m, H-3').

$^3$P NMR (D$_2$O, 202 MHz) $\delta$ –8.43, –10.17, –21.68. ESIMS-LR $m/z$ 490 [M-H]; ESIMS-HR calcd for C$_{10}$H$_{15}$N$_5$O$_{12}$P$_3$ 489.9930, found 489.9913.

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-N$_2$-benzoyl-3'-deoxy-D-apioguanosine$^{[2]}$ (37 mg, 0.09 mmol) as described for the synthesis of apioTTP. $^1$H NMR (D$_2$O, 500 MHz) $\delta$ 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 $J_{\text{gem}} = 8.6, J_{4'a,3'} = 8.6$ Hz), 4.21 (2H, dd, H-3'', $J_{\text{gem}} = 8.6, J_{3'',3'} = 8.6$ Hz), 4.19 (1H, dd, H-4'b $J_{\text{gem}} = 8.6, J_{4'b,3'} = 8.6$ Hz), 2.86 (1H, m, H-3').

$^3$P NMR (D$_2$O, 202 MHz) $\delta$ –5.28, –10.00, –20.97. ESIMS-LR $m/z$ 506 [M-H]; ESIMS-HR calcd for C$_{10}$H$_{15}$N$_5$O$_{13}$P$_3$ 505.9879, found 505.9874.
A PAGE experiment for the elongation reaction using all four apiO-NTPs 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 apiO-NTPs at 44 °C for 1 h under various concentrations of Therminator DNA polymerase. Lane 1; primer, lanes 2-7; the elongation product by using apiO-NTPs under various concentrations of Therminator DNA polymerase. Sequence of the primer-template complex and apiO-NA elongation product were shown in Figure 2A.
MALDI-TOF mass spectrum of elongated product (20mer primer and 27mer template).

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**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).

**A)** Sequence of the primer (21mer)-template (43mer) complex and the apiON elongation product. Elongated apiON is shown in bold letters.

\[
5' - 32P-d(TAATACTGAGTGCATATCCGACGTTTTAC)-3' \\
3' - d(ATTATGCTGAGTGCATATCCGACGTTTTAC)-5' \\
\]

**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.

**Figure S3.** A) Sequence of the primer (21mer)-template (43mer) complex and the apiON elongation product. Elongated apiON 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
