Selective Transcription of an Unnatural Naphthyridine:Imidazopyridopyrimidine Base Pair Containing Four Hydrogen Bonds with T7 RNA Polymerase**

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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 ECS-400 and were reported in parts per million ($\delta$) relative to residual solvent signal for $^1$H NMR and $^{13}$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-T100GCv, JEOL JMS-T100LP or Thermo Scientific Exactive. UV spectra were measured with Shimadzu UV Visible Spectrophotometer UV-2450. 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. Dispo ODS SPE column was purchased from YMC. YMC J’sphere ODS-M80 (150 x 4.6 mm) was used as reversed-phase C18 HPLC columns. T7 RNA polymerase was purchased from Takara Bio. RNaseOUT™ ribonuclease inhibitor was purchased from invitrogen. RNase T$_2$ was purchased from Sankyo. [\(\alpha\)-\(^{32}\)P]ATP and [\(\alpha\)-\(^{32}\)P]GTP were purchased from Perkin Elmer. PAGE analysis and purification were performed with 220 x 220 x 0.5 mm and 220 x 220 x 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.

Transcription reactions by T7 RNA polymerase.
The transcription reactions were performed with 20 pmol of template DNA in 10 μL of transcription buffer. The mixture contained 400 μM of ATP, GTP, CTP and UTP, 1 μCi of [α-32P]ATP, 25 U of T7 RNA polymerase (Takara), 20 U of RNaseOUT™ (invitrogen), and various concentration of a modified triphosphate in a 40 mM Tris-HCl buffer (pH 8.0) containing 8 mM MgCl₂, 2 mM spermidine and 5 mM dithiothreitol. The reaction mixture was incubated at 37 ºC for 3 h. The reaction was quenched by the addition of 20 μL of loading buffer (1 × TBE, 7 M urea, 0.05% bromophenol blue, 0.05% xylene cyanol). The mixtures were then analyzed by 20% PAGE (1000 V, 2.5 h) containing 7 M urea. The gel was dried by gel drier (ATTO AE3750 Rapidry), and the radioactive densities of the gel were visualized by Bio-imaging analyzer (BAS2500, Fujifilm).

**MALDI-TOF mass spectra of the transcription product.**

The transcription reaction mixture was desalted, and counter cations of phosphate groups were exchanged to ammonium salts on Sep-pak Plus C18 Cartridge (Waters). The RNA transcripts were then analyzed by MALDI-TOF mass using 3-hydroxypicolinic acid and bis-ammonium citrate as matrix.

**Nucleotide composition analyses of the transcription product by 2D TLC.**

The transcription reactions were performed with the same conditions as described above except for using [α-32P]ATP or [α-32P]GTP. The transcriptional products were purified by 16% PAGE (1000 V, 5 h) containing 7 M urea, and the radioactive bands corresponding to the full-length [32P]-RNA transcripts in the gel was excised and eluted with 400 μL of TE buffer (pH 8.0) at room temperature for overnight. The eluted full
length products were desalted using a disposable ODS column (YMC Dispo ODS SPE). The solvent was evaporated with SpeedVac concentrator, and the residue was treated with RNase T2 (1 U, Sankyo) in 20 mM acetate buffer (10 μL, pH 4.5) at 37 ºC for 2 h, followed by heating the mixture at 95 ºC for 3 min. The digestion products were analyzed by 2D TLC using HPTLC plate (cellulose, 100 × 100 mm, MERCK) with the following solvents: isobutylc acid-ammonia-water (66:1:33 v/v/v) for the first dimension, and sodium phosphate (0.1 M, pH 6.8) -ammonium sulfate-n-propanol (100:60:2 v/w/v) for the second dimension. Radioactive densities of the TLC plate were detected by Bio-imaging analyzer (BAS2500, Fujifilm).

**Synthesis and purification of the oligonucleotides.**

ODNs were synthesized by ABI 3400 DNA synthesizer on a 1.0 μmol scale. The concentration of normal phosphoramidite units was 0.1 M and modified phosphoramidite units (ImO^N^, ImN^O^, ImN^N^) was 0.12 M. The coupling time prescribed for the modified units were 900 s. Deprotection and release from the CPG support was carried out by treatment with aqueous concentrated NH₃ at 55 ºC for 12 h. The CPG support was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by 20% PAGE (700 V, 7 h) containing 7 M urea, and ODNs in the gel were excised and eluted with TE buffer (pH 8.0) at room temperature for overnight. The gel was filtered off, and the filtrate was desalted using a disposable ODS column (YMC Dispo ODS SPE). The solvent was evaporated with SpeedVac concentrator, and the residue was purified on reversed-phase HPLC. HPLC conditions: Column: J’sphere ODS-M80 (4.6 × 150 mm, YMC). Eluent: A: 5% MeCN in 0.1 M TEAA buffer (pH 7.0), B: 50% MeCN in 0.1 M TEAA buffer (pH 7.0). B conc.: 10-40%/20 min. Flow
rate: 1.0 mL/min.
Synthesis of 1,8-naphthyridineribonucleoside 5'-triphosphates (rNaTPs)

3-(2-Deoxy-β-d-xylofuranosyl)-7-(N,N-dibutylaminomethylidene)amino-2-hydroxy-1,8-naphthyridine (2)

To a solution of 1 (0.83 g, 2.0 mmol) in CH₂Cl₂ (17 mL), K-Selectride® (1 M in CH₂Cl₂, 3.2 mL, 3.2 mmol) was added at −40 °C, and the mixture was stirred for 1 h at the same temperature. The mixture was quenched by saturated aq. NH₄Cl, and the organic layer was washed with H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, MeOH in CHCl₃, 0-2%) to give 2 (0.58 g, 1.4 mmol, 72%) as a yellow solid.

1H NMR (CDCl₃, 500 MHz) δ 8.59 (s, 1H, NC₃H=NBu₂), 7.67 (s, 1H, H-4), 7.65 (d, 1H, H-5, J₅,₄ = 8.1 Hz), 6.78 (d, 1H, H-6, J₆,₅ = 8.1 Hz), 4.84 (dd, 1H, H-1', J₁',₂b = 6.3, J₁',₂a = 9.2 Hz), 4.47 (m, 1H, H-4'), 4.00 (m, 2H, H-5'), 3.89 (dd, 1H, H-3', J₃',₂a = 4.3, J₃',₂b = 8.6 Hz), 3.57-3.48 and 3.36-3.31 (each m, each 2H, NC₃H₂CH₂CH₂CH₃), 2.62 (ddd, 1H, H-2'a, J₂'a,₃' = 4.3, J₂'a,₁' = 9.2, J₂'a,₂'b = 13.8 Hz), 2.28 (ddd, 1H, H-2'b, J₂'b,₁' = 6.3, J₂'b,₃' = 8.6, J₂'b,₂'a = 13.8 Hz), 1.62-1.55 (m, 4H, NCH₂CH₂CH₂CH₃), 1.39-1.29 (m, 4H, NCH₂CH₂CH₂CH₃), 0.95-0.92 (each t, each 3H, NCH₂CH₂CH₂CH₃, J = 7.4 Hz); 13C NMR (CDCl₃, 126 MHz) δ 164.0, 162.8, 156.7, 148.7, 139.2, 137.4, 127.3, 111.6, 109.7, 82.8, 78.5, 73.8, 62.2, 51.9, 45.3, 40.4, 31.2, 29.3, 20.3, 19.9, 14.0, 13.9.

ESI-MS LR m/z 417 [M+H]⁺; ESI-MS HR calcd for C₂₂H₃₃O₄N₄, 417.25019, found 415.24963.

3-[5-O-(tert-Butyldiphenylsilyl)-2-deoxy-3-O-methanesulfonyl-β-d-xylofuranosyl]-2-(N,N-dibutylaminomethylidene)amino-2-hydroxy-1,8-naphthyridine (3)
To a solution of 2 (0.27 g, 0.64 mmol) in pyridine (6.5 mL), TBDPSCl (0.18 mL, 0.71 mmol) was added at 0 °C, and the mixture was stirred for 3 h at room temperature. The mixture was quenched by MeOH, and washed with saturated aq. NaHCO₃, H₂O, and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The resulting brown solid was dissolved in pyridine (6.5 mL), and Et₃N (190 μL, 1.4 mmol) and MsCl (49 μL, 0.64 mmol) was added at 0 °C, and stirred for 1 h at the same temperature. The mixture was quenched by MeOH and washed with saturated aq. NaHCO₃, H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, CHCl₃) to give 3 (0.38 g, 0.52 mmol, 81%) as a yellow foam.

1H NMR (CDCl₃, 500 MHz) δ 8.81 (br s, 1H, NH), 8.49 (s, 1H, NC₂H=NBu₂), 7.74-7.71 (m, 6H, H-4, Ph-TBDPS), 7.59 (d, 1H, H-5, J₅,₆ = 8.1 Hz), 7.47-7.38 (m, 7H, Ph-TBDPS), 6.76 (d, 1H, H-6, J₆,₅ = 8.1 Hz), 5.34 (ddd, 1H, H-3’, J₃’₂’a = 1.7, J₃’₂’b = 1.7, J₃’₂’a’ = 3.5 Hz), 5.14 (dd, 1H, H-1’, J₁’₂’a = 6.3, J₁’₂’a’ = 5.8 Hz), 4.14 (m, 1H, H-4’), 4.09 (dd, 1H, H-5’a, J₅’a,₅’₂’a = 5.7, J₅’a,₅’₂’a’ = 10.8 Hz), 4.03 (dd, 1H, H-5’b, J₅’b,₅’₂’a = 5.7, J₅’b,₅’₂’a’ = 10.8 Hz), 3.54 (t, 2H, NCH₂CH₂CH₂CH₃, J = 7.5 Hz), 3.33 (t, 2H, NCH₂CH₂CH₂CH₃, J = 6.9 Hz), 2.98 (ddd, 1H, H-2’a, J₂’a,₃’ = 1.7, J₂’a,₁’ = 6.3, J₂’a,₂’b = 14.9 Hz), 2.81 (s, 6H, OCH₃-DMTr × 2), 2.25 (ddd, 1H, H-2’b, J₂’a,₃’ = 1.7, J₂’a,₁’ = 5.8, J₂’a,₂’b = 14.9 Hz), 1.66-1.60 (m, 4H, NCH₂CH₂CH₂CH₃), 1.41-1.32 (m, 4H, NCH₂CH₂CH₂CH₃), 1.07 (s, 9H, t-Bu), 0.97-0.94 (each t, each 3H, NCH₂CH₂CH₂CH₃, J = 6.9 Hz); 13C NMR (CDCl₃, 126 MHz) δ 163.4, 162.1, 156.2, 148.2, 135.8, 135.7, 133.8, 133.4, 133.2, 130.1, 130.0, 127.9, 114.8, 109.9, 82.1, 81.8, 74.6, 61.8, 51.9, 45.3, 40.0, 38.6, 31.2, 29.3, 27.0, 20.3, 19.9, 19.3, 14.0, 13.9.

ESI-MS LR m/z 733 [M+H]+; ESI-MS LR calcd for C₃₀H₅₃N₄O₆SSi, 733.34551, found
733.34496.

7-(N,N-Dibutylaminomethylidene)amino-3-(2,3-dideoxy-2,3-didehydro-β-D-ribofuranosyl)-2-hydroxy-1,8-naphthyridine (4)

To a solution of 3 (0.27 g, 0.68 mmol) in DMF (9 mL), KOt-Bu (0.47 g, 4.1 mmol) was added at 0 °C, and the mixture was stirred for 2 h at room temperature. The mixture was quenched by saturated aq. NH₄Cl. The residue was diluted with CHCl₃ and washed with H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, MeOH in CHCl₃, 0-4%) to give 4 (95 mg, 0.24 mmol, 33%) as a yellow foam.

¹H NMR (CDCl₃, 500 MHz) δ 8.51 (s, 1H, NC₃H=NBu₂), 7.69 (s, 1H, H-5), 7.67 (d, 1H, H-4, J₄,₃ = 8.6 Hz), 6.77 (d, 1H, H-3, J₃,₄ = 8.6 Hz), 6.16 (m, 1H, H-2’), 5.94 (m, 1H, H-1’), 5.85 (m, 1H, H-3’), 5.08 (m, 1H, H-4’), 3.90 (dd, 1H, H-5’a, J₅’a,J₅’a = 2.9), 3.76 (dd, 1H, H-5’b, J₅’b,J₅’a = 12.1 Hz), 3.76 (dd, 1H, H-5’b, J₅’b,J₅’a = 4.0), 3.54 (t, 2H, NC₃H₂CH₂CH₂CH₃, J = 8.0 Hz), 3.54 (t, 2H, NC₃H₂CH₂CH₂CH₃, J = 6.9 Hz), 1.66-1.58 (m, 4H, NCH₂CH₂CH₂CH₃), 1.41-1.32 (m, 4H, NCH₂CH₂CH₂CH₃), 0.98-0.96 (each t, each 3H, NCH₂CH₂CH₂CH₃, J = 7.5 Hz); ¹³C NMR (CDCl₃, 126 MHz) δ 163.8, 162.6, 156.4, 148.6, 137.5, 136.8, 131.2, 128.5, 127.7, 115.1, 109.8, 87.7, 85.1, 65.0, 52.0, 45.4, 31.3, 29.3, 20.4, 20.0, 14.0, 13.9.

ESI-MS LR m/z 399 [M+H]^+; ESI-MS HR calcd for C₂₂H₃₁N₄O₃, 399.23962, found 399.23907.

7-(N,N-Dibutylaminomethylidene)amino-3-[2,3-dideoxy-2,3-didehydro-5-O-(4,4’-dimethoxytrityl)-β-D-ribofuranosyl]-2-hydroxy-1,8-naphthyridine (5)
To a solution of 4 (27 mg, 68 µmol) in pyridine (1 mL), DMTrCl (35 mg, 0.10 mmol) was added at 0 °C, and the mixture was stirred for 4 h at room temperature. The mixture was quenched by MeOH. The residue was diluted with AcOEt, and washed with saturated aq. NaHCO₃, H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, MeOH in CHCl₃, 0-2%) to give 5 (32 mg, 46 µmol, 69%) as a yellow solid.

¹H NMR (CDCl₃, 400 MHz) δ 9.51 (br s, 1H, NH), 8.45 (s, 1H, NCH=NBu₂), 7.74 (s, 1H), 7.41-6.70 (m, 14H), 6.58 (d, 1H, J₆,₇ = 8.24 Hz), 6.15 (m, 1H, H-2'), 5.97 (m, 1H, H-1'), 5.75 (m, 1H, H-3'), 5.07 (m, 1H, H-4'), 3.67 (s, 6H), 3.45 (m, 2H), 3.24 (m, 2H), 3.24 (m, 2H), 1.56 (m, 4H), 1.29 (m, 4H), 0.89 (m, 6H); ¹³C NMR (CDCl₃, 126 MHz) δ 163.0, 162.9, 158.4, 156.3, 148.3, 145.0, 137.4, 136.1, 134.7, 130.9, 130.6, 130.2, 129.0, 128.3, 128.2, 127.8, 126.8, 126.3, 114.5, 113.1, 110.2, 86.0, 83.2, 66.6, 55.2, 51.7, 45.1, 31.1, 29.2, 20.3, 19.8, 13.9, 13.8.

ESI-MS LR m/z 723 [M+Na]⁺; ESI-MS HR calcd for C₄₃H₄₈N₄O₅Na, 723.35169, found 723.35075.

3-[2,3-O-Diacetyl-β-D-ribofuranosyl]-7-(N,N-dibutylaminomethylidene)amino-2-hydroxy-1,8-naphthyridine (7)

To a solution of 5 (0.15 g, 0.22 mmol) in mixture of acetone/H₂O (9/1 (v/v), 2.2 mL), N-methylmorpholine N-oxide (0.12 g, 1.0 mmol) and OsO₄ (0.02 M in t-BuOH, 0.6 mL, 11 µmol) was added at −20 °C, and the mixture was stirred for 3 d at the same temperature. The mixture was quenched with saturated aq. Na₂S₂O₃, and extracted with
CHCl₃. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, MeOH in CHCl₃, 0-2%) to give crude products (0.11 g). The crude product was dissolved in pyridine (17 mL), and Ac₂O (29 μL, 0.31 mmol) was added at room temperature, and stirred for 15 h at the same temperature. The mixture was quenched with MeOH. The mixture was diluted with AcOEt, and washed with saturated aq. NaHCO₃, H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. After removing the solvent in vacuo, the residue was dissolved in 80% aqueous AcOH (1.4 mL), and stirred for 2 h at room temperature. The mixture was concentrated and coevaporated with EtOH for three times. The residue was purified by column chromatography (SiO₂, AcOEt in CHCl₃, 0-50%) to give 7 (32 mg, 62 μmol, 29%) as a yellow foam.

¹H NMR (CDCl₃, 500 MHz) δ 10.0 (br s, 1H, NH), 8.66 (s, 1H, NCH=NBu₂), 7.69 (s, 1H, H-4), 7.66 (d, 1H, H-5, J₅,₄ = 8.6 Hz), 6.79 (d, 1H, H-6, J₆,₅ = 8.6 Hz), 5.81 (dd, 1H, H-2', J₂',₁' = 8.1, J₂',₃' = 5.2 Hz), 5.57 (dd, 1H, H-3', J₃',₂' = 5.2, J₃',₄' = 2.3 Hz), 4.91 (d, 1H, H-1', J₁',₂' = 8.1 Hz), 4.23 (m, 1H, H-4'), 3.90 (dd, 1H, H-5'a, J₅'a,₄' = 2.3, J₅'a,₅'b = 12.6 Hz), 3.76 (m, 1H, H-5'b), 3.54 (m, 2H, NCH₂CH₂CH₂CH₂CH₃), 3.39 (m, 2H, NCH₂CH₂CH₂CH₃), 2.14 (s, 3H, CH₃-Ac), 2.01 (s, 3H, CH₃-Ac), 1.61 (m, 4H, NCH₂CH₂CH₂CH₃), 1.35 (m, 4H, NCH₂CH₂CH₃CH₃), 0.95 (m, 6H, NCH₂CH₂CH₃CH₃); ¹³C NMR (CDCl₃, 126 MHz) δ 170.2, 169.5, 164.3, 162.8, 157.0, 149.0, 140.3, 137.3, 123.7, 115.6, 109.4, 84.8, 81.9, 73.7, 72.7, 62.9, 51.8, 45.2, 31.1, 29.2, 20.9, 20.7, 20.3, 19.9, 14.0, 13.9.

ESI-MS LR m/z 517 [M+H]^+; ESI-MS HR calcd for C₂₆H₃₇N₄O₇, 517.26568, found 517.26681.
7-Amino-2-hydroxy-3-(β-D-ribofuranosyl)-1,8-naphthyridine 5'-triphosphate (8)

A solution of 7 (32 mg, 62 μmol) in pyridine/1,4-dioxane (1/3 (v/v), 750 μL) was treated with 2-chloro-4H-1,2,3-dioxaphosphorin-4-one (25 mg, 0.13 μmol) at room temperature for 10 min. The reaction mixture was treated with 0.5 M solution of bis(tri-n-butylammonium)phosphate in dry DMF (0.50 mL, 0.25 mmol) and Bu3N (0.10 mL, 0.42 mmol) at room temperature for 10 min. The reaction mixture was treated with 1% iodine in pyridine/H2O (98/2 (v/v), 3.0 mL) for 10 min, and was treated with 5% aq. Na2S2O3 (5 mL) for additional 30 min. The reaction mixture was concentrated in vacuo, and the residue was treated with 28% aqueous NH3 (20 mL) at room temperature for 24 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in H2O (300 mL), and the solution was applied to a DEAE Sephadex column, which was eluted with a linear gradient of 1 L each of H2O and 0.8 M TEAB (pH 8.0). Fractions containing 8 were concentrated in vacuo and coevaporated with EtOH for four times. The residue was dissolved in H2O (5 mL), and the solution was applied to a column of DIAION PK212 (H+ form), which was eluted with H2O. The elute was applied to a DIAION WK 40 (Na+ form), which was eluted with H2O. Fractions containing 8 were concentrated in vacuo to give 8 (13 mg, 22 μmol, 36%) as a white solid.

1H NMR (D2O, 400 MHz) δ 8.17 (s, 1H, H-4), 7.90 (d, 1H, H-5, J5,4 = 9.2 Hz), 6.58 (d, 1H, H-6, J6,5 = 9.2 Hz), 5.05 (d, 1H, H-1', J1',2' = 3.6 Hz), 4.22-4.79 (m, 5H, H-2', H-3', H-4', H-5'); 31P NMR (D2O, 160 MHz) δ −5.37 (d, 1P, J = 21.4 Hz), −10.33 (d, 1P, J = 19.3 Hz), −21.2 (dd, 1P, J = 19.3, 21.4 Hz).

ESI-MS LR m/z 575 [M+2Na]−; ESI-MS HR calcd for C13H15N3Na2O14P3, 575.95677, found 575.95610.
To a solution of S1\(^{[1]}\) (890 mg, 1.30 mmol) in pyridine (13 mL), MsCl (106 µL, 1.37 mmol) was added at 0 °C, and the mixture was stirred for 2 h at room temperature. The mixture was quenched with saturated aq. NaHCO\(_3\) and extracted with AcOEt. The organic layer was dried over Na\(_2\)SO\(_4\), filtered and concentrated. The residue was purified by column chromatography (SiO\(_2\), AcOEt in CHCl\(_3\), 10%), to give S2 (940 mg, 1.24 mmol, 95%) as a yellow foam.

\(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\) 11.56 (br s, 1H, NHBz), 8.54 (s, 1H, H-4), 8.17 - 7.45 (m, 5H, aromatic - DMTr), 7.54 - 6.81 (m, 13H, aromatic - DMTr, aromatic - Bz), 6.98 (d, 1H, H-6, \(\text{J}_{6,5} = 9.2\) Hz), 6.49 (d, 1H, H-5, \(\text{J}_{5,6} = 9.2\) Hz, \(\text{J}_{5,4} = 1.8\) Hz), 5.46 (d, 1H, H-3', 5.7 Hz), 5.21 (dd, 1H, H-1', \(\text{J}_{1',2'a} = 5.2\), \(\text{J}_{1',2'a} = 10.9\) Hz), 4.26 (m, 1H, H-4'), 3.76 (s, 6H, OCH\(_3\)-DMTr × 2), 3.52 (dd, 1H, H-5'a, \(\text{J}_{5'a,4'} = 2.9\), \(\text{J}_{5'a,5'b} = 10.3\) Hz), 3.40 (dd, 1H, H-5'b, \(\text{J}_{5'b,4'} = 1.7\), \(\text{J}_{5'b,5'a} = 10.3\) Hz), 3.11(dd, 1H, H-2'a, \(\text{J}_{2'a,1'} = 5.2\), \(\text{J}_{2'a,2'b} = 14.3\) Hz),
2.90 (s, 3H, CH₃-Ms), 2.51 (ddd, 1H, H-2'b, J₂b,₂c' = 5.7, J₂a,₁' = 10.9, J₂a,₂b = 14.3 Hz);

¹³C NMR (CDCl₃, 126 MHz) δ 168.4, 164.7, 158.8, 150.7, 148.3, 144.7, 139.2, 139.1, 135.7, 132.9, 132.8, 130.3, 130.2, 129.0, 128.9, 128.7, 128.4, 128.2, 127.3, 122.2, 113.9, 113.4, 55.4, 40.9, 39.2, 36.8.

ESI-MS LR m/z 762 [M+H]+; ESI-MS HR calcd for C₄₂H₄₀N₃O₉S, 762.24853, found 762.24798.

7-Benzoylamino-6-[2,3-O-diacetyl-5-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-2-hydroxy-1,8-naphthyridine (S4)

To a solution of S2 (230 mg, 0.30 mmol) in DMF (3 mL), KOr-Bu (150 mg, 1.40 mmol) was added and the mixture was stirred for 24 h at 60 °C. The mixture was quenched with saturated aq. NH₄Cl. The residue was diluted with AcOEt and, washed with H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated to give crude product (S3, 0.18 g). The residue was dissolved in mixture of acetone/H₂O (9/1 (v/v), 4.4 mL), N-methylmorpholine N-oxide (39 mg, 0.33 mmol) and OsO₄ (0.56 mL, 11 µmol) was added at −20 °C, and stirred for 5 d at the same temperature. The mixture was quenched with saturated aq. Na₂S₂O₃ and extracted with CHCl₃. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, MeOH in CHCl₃, 1-3%) to give crude product (0.12 g). The residue was dissolved in pyridine (2 mL), Ac₂O (37 µL, 0.39 mmol) was added at room temperature, and stirred for 15 h at the same temperature. The mixture was quenched with MeOH. The mixture was diluted with AcOEt, and washed with saturated aq. NaHCO₃, H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, AcOEt in
CHCl₃, 25-50%) to give S₄ (63 mg, 0.13 mmol, 46%) as a yellow foam.

¹H NMR (CDCl₃, 500 MHz) δ 11.50 (br s, 1H, NH₂Bz), 8.62 (s, 1H, H-4), 8.15-7.44 (m, 5H, aromatic-DMTr), 7.51-6.79 (m, 13H, aromatic-DMTr, aromatic-Bz), 7.02 (d, 1H, H-6, J₆,₅ = 9.7 Hz), 6.47 (d, 1H, H-5, J₅,₆ = 9.7 Hz), 5.54-5.47 (m, 3H, H-1', H-2', H-3'), 4.20 (m, 1H, H-4'), 3.77 (s, 6H, OCH₃-DMTr × 2), 3.63 (d, 1H, H-5'a, J₅'a,₅'b = 10.3 Hz), 3.26 (d, 1H, H-5'b, J₅'b,₅'a = 10.3 Hz), 1.92 (s, 3H, CH₃-Ac), 1.70 (s, 3H, CH₃-Ac); ¹³C NMR (CDCl₃, 100 MHz) δ 169.9, 169.7, 167.0, 164.5, 158.7, 150.3, 148.3, 144.6, 139.6, 137.5, 135.8, 135.7, 133.0, 132.6, 130.6, 129.0, 128.9, 128.1, 127.2, 122.1, 113.4, 86.6, 70.1, 61.5, 55.4, 45.9, 29.9, 20.7, 20.4.

ESI-MS LR m/z 806 [M+Na]+; ESI-MS HR calcd for C₄₅H₄₁N₃O₁₀Na, 806.26896, found 806.26842.

7-Benzoylamino-6-[2,3-O-diacetyl-β-D-ribofuranosyl]-2-hydroxy-1,8-naphthyridine (S₅)

S₄ (0.11 g, 0.14 mmol) was dissolved in 80% aqueous AcOH (1.4 mL), and the mixture was stirred for 2 h at room temperature. The mixture was concentrated and coevaporated with EtOH for three times. The residue was purified by column chromatography (SiO₂, MeOH in CHCl₃, 0-15%) to give S₅ (68 mg, 0.14 mmol, quant) as a yellow foam.

¹H NMR (CDCl₃, 400 MHz) δ 11.24 (br s, 1H, NH₂Bz), 8.32 (s, 1H, H-4), 8.09 (d, 2H, aromatic-Bz, J = 7.3 Hz), 7.71 (d, 1H, H-6, J₆,₅ = 9.6 Hz), 7.55 (dd, 1H, aromatic-Bz, J = 7.6, 7.8 Hz), 7.49 (m, 3H, aromatic-Bz), 6.60 (d, 1H, H-5, J₅,₆ = 9.6 Hz), 5.50-5.35 (m, 2H, H-1', H-2'), 5.22 (dd, 1H, H-3', J₃',₃' = 5.5, J₃',₄' = 7.3 Hz), 4.10 (dt, 1H, H-4', J₄',₅' = 3.2, J₄',₃' = 7.3 Hz), 4.02 (d, 1H, H-5'a, J₅'a,₅'b = 12.6 Hz), 3.77 (dd, 1H, H-5'b, J₅'b,₅'a =
3.6, $J_{5'b,5'a} = 12.6 \text{ Hz}$), 2.01 (s, 3H, CH$_3$-Ac), 1.75 (s, 3H, CH$_3$-Ac); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 170.4, 169.8, 167.0, 164.4, 150.3, 148.2, 139.3, 137.1, 133.0, 132.7, 128.9, 128.6, 125.9, 122.4, 113.2, 80.3, 79.1, 76.0, 70.1, 61.4, 20.7, 20.4.

ESI-MS LR m/z 504 [M+Na]$^+$; ESI-MS HR calcd for C$_{24}$H$_{23}$N$_3$O$_8$Na, 504.13828, found 504.13774.

7-Amino-2-hydroxy-6-(β-D-ribofuranosyl)-1,8-naphtyridine 5'-triphosphate (S6)

S6 (37 mg, 36 µmol, 45%, as a white solid) was obtained from S5 (40 mg, 79 µmol) as described for the synthesis of 8.

$^1$H NMR (D$_2$O, 400 MHz) $\delta$ 7.90 (m, 2H, H-4, H-5), 6.39 (d, 1H, H-6, $J_{6,5} = 9.6 \text{ Hz}$), 4.80-4.20 (m, 6H, H-1’, H-2’, H-3’, H-4’, H-5’a, H-5’b); $^{31}$P NMR (D$_2$O, 160 MHz) $\delta$ –5.3 (d, 1P, $J = 19.3 \text{ Hz}$), –10.5 (d, 1P, $J = 19.3 \text{ Hz}$), –21.1 (t, 1P, $J = 19.3 \text{ Hz}$).

ESI-MS LR m/z [M+2Na-H]$^-$; ESI-MS HR calcd for C$_{13}$H$_{15}$N$_3$Na$_2$O$_{14}$P$_3$, 575.95622, found 575.95623.
Scheme S2

3-Iodo-2,7-dimethoxy-1,8-naphthyridine (S8)

To a solution of $S^7$ (6.70 g, 35.3 mmol) in AcOH (350 mL), NIS (9.53 g, 42.4 mmol) was added at room temperature, and the mixture was stirred for 12 h at 80 °C. The mixture was quenched with saturated aq. Na$_2$S$_2$O$_3$ and concentrated in vacuo. The residue was diluted with AcOEt, and washed with saturated aq. NaHCO$_3$, H$_2$O and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by column chromatography (SiO$_2$, AcOEt in hexane, 3%), to give $S^8$ (8.37 g, 26.5 mmol, 75%) as a white solid.

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 8.37 (s, 1H, H-4), 7.79 (d, 1H, H-5, $J_{5,6} = 6.7$ Hz), 6.80 (d, 1H, H-6, $J_{5,6} = 6.9$ Hz), 4.17 (s, 3H, CH$_3$-Me), 4.12 (s, 3H, CH$_3$-Me); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 166.8, 162.1, 154.4, 147.8, 137.6, 116.9, 111.7, 55.6, 54.3.
EI-MS LR m/z 316 M⁺; EI-MS HR calcd for C₁₀H₉N₂O₂, 315.9709, found 315.9706.

2,7-Dimethoxy-3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-1,8-naphthyridine (S9)

To a solution of S8 (1.26 g, 4.0 mmol) in anhydrous THF (40 mL), n-BuLi (1.6 M in hexane, 3 mL, 4.8 mmol) was slowly added at −78 °C. After stirring for 25 min, a solution of 2,3,5-tri-O-benzyl-D-ribofuranolactone (1.67 g, 4.0 mmol) in anhydrous THF (10 mL) was added via cannula. After stirring for 3 h at the same temperature, the reaction was quenched with saturated aq. NH₄Cl. The mixture was diluted with AcOEt and washed with H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was dissolved in CH₂Cl₂ (40 mL) cooled to −78 °C, then treated with Et₃SiH (1.30 mL, 8.0 mmol) and BF₃•OEt₂ (3.3 mL, 12.0 mmol). The solution was stirred at −78 °C for 12 h. The mixture was quenched with saturated aq. NaHCO₃ and extracted with CHCl₃, and the organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, AcOEt in CHCl₃, 10-33%) to give S9 (0.72 g, 1.2 mmol, 30%) as a yellow foam.

¹H NMR (CDCl₃, 500 MHz) δ 8.32 (s, 1H, H-4), 7.42-7.22 (m, 15H, aromatic-Bn × 3), 6.97 (d, 1H, H-5, J₅,₆ = 8.6 Hz), 6.59 (d, 1H, H-6, J₆,₅ = 8.6 Hz), 5.45 (s, 1H, H-1'), 4.93 (d, 1H, CH₂-Bn, J = 12.6 Hz), 4.75 (d, 1H, CH₂-Bn, J = 12.6 Hz), 4.62 (d, 1H, CH₂-Bn, J = 11.5 Hz), 4.55-4.50 (m, 2H, CH₂-Bn × 2), 4.42 (dt, 1H, H-4', J₄,₅'ₐ = 2.3, J₅,₃' = 9.2 Hz), 4.28 (d, 1H, CH₂-Bn, J = 12.1 Hz), 4.19 (s, 3H, CH₃-OMe), 4.13-4.09 (m, 4H, H-3', CH₃-OMe), 4.08 (dd, 1H, H-5'a, J₈a,₄' = 2.3, J₈ₐ,₅₇b = 10.9 Hz), 3.97 (d, 1H, H-2', J₂,₃' = 4.0 Hz), 3.79 (dd, 1H, H-5'b, J₅b,₄' = 2.3, J₅₈₉,₃ₗ₉a = 10.9 Hz); ¹³C NMR (CDCl₃, 99 MHz) δ 165.2, 161.4, 153.9, 139.2, 138.4, 138.3, 137.8, 136.5, 128.6, 128.5, 128.4, 127.9, 127.8, 122.8, 115.3, 110.8, 80.0, 79.4, 79.3, 75.5, 73.5, 72.1, 71.2, 68.8, 54.2, 54.0.
ESI-MS LR m/z 615 [M+Na]^+; ESI-MS HR calcd for C_{36}H_{35}N_{2}O_{6}Na, 615.24711, found 615.24656.

2,7-Dihydroxy-3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-1,8-naphthyridine (S10)
To a solution of S9 (60 mg, 0.10 mmol) in DMF (1.5 mL), NaH (80 mg, 2.0 mmol) and PhSH (0.40 mL, 4.0 mmol) was added at room temperature, and the mixture was stirred for 12 h at 120 °C. The mixture was quenched with saturated aq. NH₄Cl, and the mixture was diluted with CHCl₃, and washed with H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, MeOH in CHCl₃, 0-10%) to give S10 (31 mg, 55 μmol, 55%) as a yellow foam.

¹H NMR (DMSO-d₆, 500 MHz) δ 7.89 (s, 1H, H-4), 7.43-7.21 (m, 17H, H-5, H-6, aromatic-Bn × 3), 5.11 (s, 1H, H-1'), 4.85 (d, 1H, CH₂-Bn, J = 12.1 Hz), 4.72 (d, 1H, CH₂-Bn, J = 12.1 Hz), 4.61 (d, 1H, CH₂-Bn, J = 11.5 Hz), 4.53 (d, 1H, CH₂-Bn, J = 11.5 Hz), 4.15 (dt, 1H, H-4', J₄',₅'ₐ = 2.3, J₄',₃' = 9.2 Hz), 4.01 (m, 1H, H-2'), 3.85 (dd, 1H, H-3', J₃',₂' = 4.6, J₃',₄' = 9.2 Hz), 3.85 (dd, 1H, H-5'-a, J₅',₄' = 2.3, J₅',₅''ₐ = 10.9 Hz), 3.68 (dd, 1H, H-5'b, J₅',₄' = 2.3, J₅'',₅''ₐ = 10.9 Hz); ¹³C NMR (CDCl₃, 99 MHz) δ 165.9, 147.2, 140.8, 138.5, 137.9, 128.7, 128.5, 128.1, 127.9, 127.8, 127.7, 108.7, 79.4, 78.6, 74.0, 71.9, 70.8, 70.2, 68.6.

ESI-MS LR m/z 587 [M+Na]^+; ESI-MS HR calcd for C_{34}H_{32}N_{2}O_{6}Na, 587.21581, found 587.21526.

2-Diphenylcarbamoyloxy-7-hydroxy-6-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-1,8-n
aphthyridine (S11)

To a solution of S10 (17 mg, 30 μmol) in CH₂Cl₂ (1.5 mL), DIPEA (7.9 μL, 45 μmol), DMAP (1.2 mg, 10 μmol) and Ph₂NCOCl (10 mg, 45 μmol) was added at 0 °C, and the mixture was stirred for 3 h at room temperature. The mixture was quenched with EtOH. The mixture was diluted with CHCl₃, and washed with saturated aq. NaHCO₃, H₂O and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, CHCl₃) to give S11 (20 mg, 27 μmol, 90%) as a yellow solid.

¹H NMR (CDCl₃, 500 MHz) δ 8.97 (br s, 1H, NH), 8.17 (s, 1H, H-5), 7.53-7.22 (m, 24H, H-4, aromatic-Bn × 3, OCONPh₂), 6.63 (m, 2H, H-3, OCONPh₂), 5.39 (s, 1H, H-1'), 5.03 (d, 1H, CH₂-Bn, J = 12.0 Hz), 4.90 (d, 1H, CH₂-Bn, J = 10.9 Hz), 4.49 (m, 2H, CH₂-Bn × 2), 4.41 (d, 1H, H-4', J = 9.8 Hz), 4.19 (d, 1H, CH₂-Bn, J = 12.0 Hz), 4.08 (m, 2H, H-5'a, H-3'), 4.02 (d, 1H, H-2', J₂,₃' = 4.6 Hz), 3.80 (dd, 1H, H-5'b, J₅b,₅'a = 2.3, J₅b,₅'a = 10.9 Hz); ¹³C NMR (CDCl₃, 125 MHz) δ 162.3, 157.9, 151.7, 147.5, 141.8, 139.7, 138.5, 138.2, 138.0, 135.3, 132.8, 129.1, 128.8, 128.6, 128.4, 128.3, 127.8, 127.7, 127.6, 112.9, 111.0, 79.8, 79.2, 78.2, 74.8, 73.5, 71.7, 70.6, 68.4.

ESI-MS LR m/z 760 [M+H]+; ESI-MS HR calcd for C₄₇H₄₂N₃O₇, 760.30228, found 760.30173.

2-Diphenylcarbamoyloxy-7-hydroxy-6-(β-D-ribofuranosyl)-1,8-naphthyridine (S12)

To a solution of S11 (0.20 g, 0.26 mmol) in CH₂Cl₂ (3.0 mL), BBr₃ (0.87 mL, 0.87 mmol) was added at −78 °C, and the mixture was stirred for 15 min at the same temperature. The mixture was quenched with saturated aq. NaHCO₃. The mixture was
diluted with CHCl$_3$, and washed with H$_2$O and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by column chromatography (SiO$_2$, MeOH in CHCl$_3$, 0-5%) to give S12 (18 mg, 37 µmol, 14%) as a yellow solid.

$^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 10.72 (br s, 1H, NH), 7.76 (s, 1H, H-5), 7.68 (d, 1H, H-4, $J_{4,3} = 8.1$ Hz), 7.38-7.20 (m, 10H, OCON$_2$Ph$_2$), 6.74 (d, 1H, H-3, $J_{3,4} = 8.1$ Hz), 5.43 (s, 1H, H-1'), 4.84 (m, 2H, H-4', 2'-OH), 4.26 (m, 1H, H-2'), 4.26-4.12 (m, 2H, H-3', H-4'), 4.06 (br s, 1H, H-3'-OH), 3.88-3.80 (m, 2H, H-5'a, 5'-OH), 3.68 (m, 1H, H-5'b); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 163.2, 158.3, 158.1, 152.1, 147.1, 141.8, 140.9, 140.0, 135.9, 131.6, 129.3, 112.8, 111.5, 107.7, 85.4, 81.3, 74.9, 72.5, 63.1.

ESI-MS LR m/z 512 [M+Na]$^+$; ESI-MS HR calcd for C$_{26}$H$_{23}$N$_3$O$_7$Na, 512.14337, found 512.14282.

6-(2,3-O-Diacetyl-β-D-ribofuranosyl)-2-diphenylcarbamoyloxy-7-hydroxy-1,8-naphthyridine (S13)

To a solution of S12 (13 mg, 27 µmol) in pyridine (0.30 mL), DMTrCl (10 mg, 29 µmol) was added at 0 °C. After stirring for 4 h at room temperature, Ac$_2$O (10 µL, 0.11 mmol) and pyridine (0.3 mL) was added at 0 °C, and the mixture was stirred for 14 h at room temperature. The mixture was quenched with MeOH. The mixture was diluted with AcOEt, and washed with saturated aq. NaHCO$_3$, H$_2$O and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated. The residue was dissolved in 80% aqueous AcOH (3 mL) at room temperature and stirred for 3 h at the same temperature. The mixture was concentrated and coevaporated with H$_2$O $\times$ 2 and toluene $\times$ 1. The residue was purified by column chromatography (SiO$_2$, MeOH in CHCl$_3$, 0-5%) to give S13 (15 mg, 26 µmol, 95%) as a yellow solid.
\(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta\) 7.94 (d, 1H, H-4, \(J_{4,3} = 8.0\) Hz), 7.83 (s, 1H, H-5), 7.40-7.23 (m, 11H, OCONPh\(_2\), H-3), 5.69 (dd, 1H, H-2', \(J_{2',3'} = 5.5, J_{2',1'} = 7.2\) Hz), 5.48 (dd, 1H, H-3', \(J_{3',4'} = 4.1, J_{3',2'} = 5.5\) Hz), 4.98 (d, H-1', \(J_{1',2'} = 7.2\) Hz), 4.21 (m, 1H, H-4'), 3.92 (dd, 1H, H-5'a, \(J_{5'a,4'} = 1.7, J_{5'a,5'b} = 12.7\) Hz), 3.77 (m, 1H, H-5'b), 2.12 (s, 3H, CH\(_3\)-Ac), 2.04 (s, 3H, CH\(_3\)-Ac); \(^{13}\)C NMR (CDCl\(_3\), 99 MHz) \(\delta\) 170.3, 169.7, 161.6, 159.0, 151.8, 147.9, 141.8, 139.9, 137.6, 129.6, 129.3, 129.2, 128.0, 127.2, 113.3, 112.3, 111.5, 83.7, 80.7, 73.2, 72.5, 62.3, 55.4, 20.9, 20.8.

ESI-MS LR m/z 596\([\text{M+Na}]^+\); ESI-MS HR calcd for C\(_{30}\)H\(_{27}\)N\(_3\)O\(_9\)Na, 596.53985, found 596.16395.

2,7-Dihydroxy-3-(\(\beta\)-D-ribofuranosyl)-1,8-naphthyridine 5'-triphosphate (S14)

S14 (8.9 mg, 12 \(\mu\)mol, 30\%, as a white solid) was obtained from S13 (22 mg, 38 \(\mu\)mol) as described for the synthesis of S6.

\(^1\)H NMR (D\(_2\)O, 400 MHz) \(\delta\) 7.98 (s, 1H, H-4), 7.82 (d, 1H, H-5, \(J_{5,6} = 11.5\) Hz), 6.29 (d, 1H, H-6, \(J_{6,5} = 11.5\) Hz), 5.04 (d, 1H, H-1', \(J_{1',2'} = 5.2\) Hz), 4.27-4.12 (m, 5H, H-2', H-3', H-4', H-5'a, H-5'b); \(^{31}\)P NMR (D\(_2\)O, 160 MHz) \(\delta\) −4.74 (d, 1P, \(J = 19.3\) Hz), −9.95 (d, 1P, \(J = 19.3\) Hz), −20.4 (t, 1P, \(J = 19.3\) Hz).

ESI-MS LR m/z 577\([\text{M+2Na}]^+\); ESI-MS HR calcd for C\(_{13}\)H\(_{14}\)N\(_2\)O\(_{15}\)Na\(_2\)P\(_3\), 576.94079, found 576.94165.
2D-TLC experiments for the transcription reaction using rNaN⁰TP.

A. NaN⁰ 400 μM UTP 400 μM ([α-³²P]ATP)

B. NaN⁰ 400 μM UTP 400 μM ([α-³²P]GTP)

C. NaN⁰ 100 μM UTP 800 μM ([α-³²P]ATP)

D. NaN⁰ 100 μM UTP 800 μM ([α-³²P]GTP)
**Figure S1.** 2D-TLC experiments for the full-length transcription products at various concentrations of rNaNO-TP and UTP. (A) 400 μM rNaNO-TP and 400 μM UTP with [α-32P]ATP (Table 1, entry 2), (B) 400 μM rNaNO-TP and 400 μM UTP with [α-32P]GTP (Table 1, entry 3), (C) 100 μM rNaNO-TP and 800 μM UTP with [α-32P]ATP, (D) 100 μM rNaNO-TP and 800 μM UTP with [α-32P]GTP, (E) 100 μM rNaNO-TP and 1600 μM UTP with [α-32P]ATP, (F) 100 μM rNaNO-TP and 1600 μM UTP with [α-32P]GTP, (G) 100 μM rNaNO-TP and 3200 μM UTP with [α-32P]ATP (Table 1, entry 4), (H) 100 μM rNaNO-TP and 3200 μM UTP with [α-32P]GTP (Table 1, entry 5). Sequence of the transcription product was shown in Figure 2A.
Figure S2. 2D-TLC experiments for the full-length transcription products at various concentrations of rNaO\textsuperscript{0}TP. (A) 100 μM rNaO\textsuperscript{0}TP with [\alpha-\textsuperscript{32}P]ATP, (B) 100 μM rNaO\textsuperscript{0}TP with [\alpha-\textsuperscript{32}P]GTP, (C) 200 μM rNaO\textsuperscript{0}TP with [\alpha-\textsuperscript{32}P]GTP. Sequence of the transcription product was shown in Figure 2A.
MALDI-TOF mass spectrum of the transcription product (NaO⁰ : ImN⁰ pair).

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**Figure S3.** MALDI-TOF mass spectrum of the transcription product by using NaO⁰ : ImN⁰ pair. The spectrum were obtained from transcription reaction after desalting using an ODS column.
2D-TLC experiments for the transcription reaction using rNaONTP.

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**Figure S4.** 2D-TLC experiments for the full-length transcription product at 400 µM of rNaONTP. (A) 400 µM rNaONTP with [α-32P]ATP, (B) 400 µM rNaONTP with [α-32P]GTP. Sequence of the transcription product was shown in Figure 2A.
Figure S5. The whole gel data of Figure 4B. Lane 1, C:G control; lanes 2, NaN$^O$:ImO$^N$ pair. The reaction was carried out similarly to Figure 2B, and then analyzed by 10% PAGE (1000 V, 2.5 h) containing 7 M urea.
$^1$H, $^{13}$C and $^{31}$P NMR spectra of new compound
References
