SUPPLEMENTAL INFORMATION

## CHEMICAL SYNTHESIS INFORMATION

**General Chemical Synthesis for $N^6$-substituted purine analogues.** Nucleosides were synthesized as described previously(4). $^{31}$P-NMR sprectra were recorded at 121.5 MHz on a Bruker DRX300 instrument using $D_2O$ as the NMR solvent. The $^{31}$P-NMR spectra was calibrated using 85% $H_3PO_4$ as a reference sample. Mass spectra were recorded on an Applied Biosystems Voyager-DE PRO MALDI-TOF spectrometer using 2,5-dihydroxybenzoic acid as matrix.

**General procedure A for the synthesis of nucleoside-5'-triphosphates.** To an ice-cold solution of purine riboside (1 eq.) and proton sponge (1.5 eq) in trimethyl phosphate (6 mL/mmol) was added phosphoryl chloride (1.2 eq.) and the solution stirred at 0°C for 5 hours. To this was added simultaneously tributylamine (1.5 mL) and tetrabutylammonium pyrophosphate solution (0.5 M in DMF, 2 eq.), and the solution stirred for a further 30 minutes. The reaction was then quenched by the addition of 0.5 M triethylammonium bicarbonate (TEAB) buffer (10 mL), and stored at 4°C overnight. The solution was evaporated to dryness and re-dissolved in water (20 mL) and applied to a Sephadex A25 column in 0.05 M TEAB buffer. The column was eluted with a linear gradient of 0.05-1.0 M TEAB. Appropriate fractions were pooled and evaporated to dryness to give desired product. HPLC (Phenomenex Luna 10μ C-18 reverse phase

column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 25% to 100% buffer B over 45 minutes at 8 mL/min.) showed the product to be pure.

**2-Amino-6-chloropurine riboside-5'-triphosphate**. Prepared by general procedure A using 2-amino-6-chloropurine riboside (0.481 g, 1.59 mmol), proton sponge (0.511 g, 2.38 mmol) and phosphoryl chloride (178 μL, 1.91 mmol) in trimethyl phosphate (8 mL). Reaction mixture was stirred at 0°C for 5 hours. Tributylamine (1.5 mL) and tetrabutylammonium pyrophosphate solution (0.5 M in DMF, 6.36 mL) was then added simultaneously, and the solution stirred for a further 30 minutes. The reaction was then quenched and purified as described above to give desired product as a white solid (yield 27%). $\delta_P$ ($D_2O$) γ-P –9.1 (d); α-P –10.3, (d); β-P -22.1, (t).

**6-Chloropurine riboside-5'-triphosphate**. Prepared by general procedure A using 6-chloropurine riboside (0.503 g, 1.75 mmol), proton sponge (0.562 g, 2.62 mmol) and phosphoryl chloride (196 μL, 2.10 mmol) in trimethyl phosphate (9 mL). Reaction mixture was stirred at 0°C for 5 hours. Tributylamine (1.7mL) and tetrabutylammonium pyrophosphate solution (0.5 M in DMF, 7 mL) was then added simultaneously, and the solution stirred for a further 30 minutes. The reaction was then quenched and purified as described above to give desired product as a white solid (yield 26%). $\delta_P$ ($D_2O$) γ-P –5.4, (d); α-P –10.3, (d); β-P -21.5, (t).

***N*$^6$-Hydroxyadenosine-5'-triphosphate (JA44).** To a solution of 6-chloropurine riboside-5'-triphosphate (90.6 μmol) in water (2 mL), hydroxylamine (50% w/v in water,

100 μL) was added and resulting mixture was heated at 40°C for 3 h. The crude product was then purified by HPLC. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 58%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) γ-P –8.0, (d); α-P –10.0, (d); β-P -21.3, (t). Mass ($C_{10}H_{12}N_5O_{14}P_3 \cdot 4Na$): 610.54; Calculated, 611.

***N*$^6$-methoxyadenosine-5'-triphosphate (JA45).** To a solution of 6-chloropurine riboside-5'-triphosphate (45.3 μmol) in water (1 mL), methoxylamine (100 μL) was added and resulting mixture was heated at 40°C for 16 h. The crude product was then purified by HPLC. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 26%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) γ-P –7.3, (d); α-P –11.8, (d); β-P -23.1, (t). Mass ($C_{11}H_{14}N_5O_{14}P_3 \cdot 4Na$): 624.39; Calculated, 624.

**N$^6$-Amino-N$^6$-methyladenosine-5'-triphosphate (JA46).** To a solution of 6-chloropurine riboside-5'-triphosphate (94.0 μmol) in water (2 mL), methylhydrazine (100 μL) was added and resulting mixture was heated at 40°C for 16 h. The crude product was then purified by HPLC. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 48%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) γ-P

–7.1, (d); α-P –9.9, (d); β-P -21.0, (t). Mass ($C_{11}H_{15}N_6O_{13}P_3$·4Na): 555.43; Calculated, 555.

**2-Amino-*N*$^6$-hydroxyadenosine-5'-triphosphate (JA47).** To a solution of 2-amino-6-chloropurine riboside-5'-triphosphate (85.2 μmol) in water (3 mL), hydroxylamine (50% w/v in water, 100 μL) was added and resulting mixture was heated at 40$^o$C for 3 h. The crude product was then purified by HPLC. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 52%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) γ-P –8.7, (d); α-P –10.1, (d); β-P -21.6, (t). Mass ($C_{10}H_{15}N_6O_{10}P_3$·4Na): 629.62; Calculated, 628.

**2-Amino-*N*$^6$-methoxyadenosine-5'-triphosphate (JA48).** To a solution of 2-amino-6-chloropurine riboside-5'-triphosphate (86.4 μmol) in water (2 mL), methoxylamine (100 μL) was added and resulting mixture was heated at 40 $^o$C for 16 h. The crude product was then purified by HPLC. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 32%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) γ-P –6.2, (d); α-P –9.8, (d); β-P -20.8, (t). Mass ($C_{11}H_{17}N_6O_{14}P_3$·4Na): 641.56; Calculated, 641.

**2-Amino-N$^6$-amino-adenosine-5'-triphosphate (JA49).** To a solution of 2-amino 6-chloropurine riboside-5'-triphosphate (85.2 μmol) in water (3 mL), hydrazine

monohydrate (100 μL) was added and resulting mixture was heated at 40 $^{o}$C for 3 h. The crude product was then purified by HPLC. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 35%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) γ-P –8.8, (d); α-P –10.0, (d); β-P -21.6, (t). Mass ($C_{10}H_{14}N_7O_{13}P_3 \cdot 4Na$): 626.08; Calculated, 625.

**2-Amino-N$^6$-amino-N$^6$-methyladenosine -5'-triphosphate (JA50).** To a solution of 2-amino-6-chloropurine riboside-5'-triphosphate (85.2 μmol) in water (3 mL), methylhydrazine (100 μL) was added and resulting mixture was heated at 40$^{o}$C for 16 h. The crude product was then purified by HPLC. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 83%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) γ-P –7.1, (d); α-P –9.9, (d); β-P –21.0, (t). Mass ($C_{11}H_{16}N_7O_{13}P_3 \cdot 2Na$): 592.56; Calculated, 593.

**General procedure B for synthesis of nucleoside-5'-monophosphates.** To an ice-cold solution of purine riboside (1 eq.) and proton sponge (1.5 eq) in trimethyl phosphate (6 mL/mmol) was added phosphoryl chloride (1.2 eq.) and the solution stirred at 0°C until TLC showed disappearance of starting material. Water (3 mL) was then added, and the solution was neutralized by adding triethylamine dropwise. The solution was evaporated to dryness and re-dissolved in water (20 mL) and applied to a Sephadex A25 column in 0.05 M TEAB buffer. The column was eluted with a linear gradient of 0.05-1.0 M TEAB.

Appropriate fractions were pooled and evaporated to dryness to give desired crude product, which was subject to a second purification by HPLC (Phenomenex Luna 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 25% to 100% buffer B over 45 minutes at 8 mL/min.) to afford the desired product.

**6-Chloropurine riboside-5'-monophosphate**. Prepared by general procedure B using 6-chloropurine riboside (0.265 g, 0.92 mmol), proton sponge (0.297 g, 1.39 mmol) and phosphoryl chloride (103 μL, 2.10 mmol) in trimethyl phosphate (5 mL). Reaction mixture was stirred at 0°C for 5 hours. The reaction was then quenched and purified as described above to give desired product as a white solid (0.13 mmol, 14%). $\delta_P$ ($D_2O$) 4.27 (s).

**2-Amino-6-Chloropurine riboside-5'-monophosphate**. Prepared by general procedure B using 2-amino-6-chloropurine riboside (0.27 g, 0.90 mmol), proton sponge (0.289 g, 1.39 mmol) and phosphoryl chloride (117 μL, 1.26 mmol) in trimethyl phosphate (10 mL). Reaction mixture was stirred at 0°C for 5 hours. The reaction was then quenched and purified as described above to give desired product as a white solid (0.34 mmol, 38%). $\delta_P$ ($D_2O$) 4.25 (s).

**General procedure C for synthesis of nucleoside-5'-diphosphates.** To the nucleoside-5'-monophosphate, dissolved in 1 mL of anhydrous DMF, were added tri-*n*-butylamine (1 eq.). The solution was stirred for 10 min at room temperature and then evaporated to dryness. After resuspension in 3 mL of anhydrous DMF, 1-1'-dicarbonyldiimidazole

(CDI) (5.0 eq.) was added and the mixture was allowed to stir at room temperature for a further 3 hours. Methanol (8 eq.) was then added (to decompose unreacted CDI) and left to stir for a another 30 min. Tri-*n*-butylammonium phosphate (0.5M in DMF, 20 eq.) was then added and the resulting mixture was left to stir for 14 h at room temperature. The solvent was removed *in vacuo* and the mixture was re-dissolved in water (10 mL) and applied to a Sephadex A25 column in 0.05 M TEAB buffer. The column was eluted with a linear gradient of 0.05-1.0 M TEAB. Appropriate fractions were pooled and evaporated to dryness to give desired crude product, which was subject to a second purification by HPLC (Phenomenex Luna 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 25% to 100% buffer B over 45 minutes at 8 mL/min.) to afford the desired product.

**6-Chloropurine riboside-5'-diphosphate**. Prepared by general procedure C using 6-chloropurine riboside-5'-monophosphate (0.099 mmol), tri-*n*-butylamine (24 μL, 0.099 mmol) in anhydrous DMF (3 mL). After evaporation to dryness and resuspension in anhydrous DMF (4 mL), 1-1'-dicarbonyldiimidazole (0.080 g, 0.495 mmol) was added and the mixture was allowed to stir at room temperature for a further 3 hours. Methanol (32 μL) was then added and left to stir for another 30 min. Tri-*n*-butylammonium phosphate (0.5M in DMF, 4 mL.) was then added and the resulting mixture was left to stir for 14 h at room temperature The reaction was then purified as described above to give desired product as a white solid (0.087 mmol, 88%). $\delta_P$ ($D_2O$) β-P –5.9, (d); α-P –10.4, (d).

**2-Amino-6-chloropurine riboside-5'-diphosphate**. Prepared by general procedure C using 2-amino-6-chloropurine riboside-5'-monophosphate (0.239 mmol), tri-*n*-butylamine (57 μL, 0.239 mmol) in anhydrous DMF (2 mL). After evaporation to dryness and resuspension in anhydrous DMF (2 mL), 1-1'-dicarbonyldiimidazole (0.194 g, 1.19 mmol) was added and the mixture was allowed to stir at room temperature for a further 3 hours. Methanol (78 μL) was then added and left to stir for another 30 min. Tri-*n*-butylammonium phosphate (0.5M in DMF, 9.6 mL) was then added and the resulting mixture was left to stir for 14 h at room temperature. The reaction was then purified as described above to give desired product as a white solid (0.099 mmol, 41%). $\delta_P$ ($D_2O$) β-P –5.4, (d); α-P –9.9, (d).

***N*$^6$-Hydroxyadenosine-5'-diphosphate (JA51).** To a solution of 6-chloropurine riboside-5'-diphosphate (21.7 μmol) in water (1 mL), hydroxylamine (50% w/v in water, 14 μL) was added and resulting mixture was heated at 40$^o$C for 3 h. The crude product was then purified by HPLC (Phenomenex Gemini 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0% to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (5.2 μmol, 24%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) β-P –5.8, (d); α-P –9.7, (d).

**2-Amino-*N*$^6$-hydroxyadenosine-5'-diphosphate (JA52).** To a solution of 2-amino-6-chloropurine riboside-5'-diphosphate (29.7 μmol) in water (3 mL), hydroxylamine (50%

w/v in water, 300 μL) was added and resulting mixture was heated at 40°C for 3 h. The crude product was then purified by HPLC (Phenomenex Gemini 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0% to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (12.1 μmol, 41%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) β-P –5.8, (d); α-P –10.1, (d).

**2-Amino-*N*$^6$-amino-adenosine-5'-diphosphate (JA53).** To a solution of 2-amino-6-chloropurine riboside-5'-diphosphate (29.7 μmol) in water (3 mL), hydrazine monohydrate (300 μL) was added and resulting mixture was heated at 40°C for 3 h. The crude product was then purified by HPLC (Phenomenex Gemini 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0% to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (14.8 μmol, 50%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin ($Na^+$ form). $\delta_P$ ($D_2O$) β-P –6.1, (d); α-P –10.2, (d).

**General chemical synthesis for ribavirin 5'-diphosphate.** Pyridine was distilled from calcium hydride ($CaH_2$) and stored over 4 Å molecular sieves under nitrogen. HPLC purification was performed on an Agilent 1100 series instrument (preparative scale) equipped with a PRP-1 preparative column (21.5 x 250 mm, 7 μm; Hamilton Company) running the following gradient eluant (20 mL/min flow rate): 0.1% to 99% MeCN in

aqueous triethylammonium bicarbonate (TEAB, 0.1 M, pH = 7.4-7.6) over 21 mins. Nuclear magnetic resonance (NMR) spectroscopy employed Bruker AMX-360 and DRX-400 MHz spectrometers. The internal solvent peak was referenced for $^{1}H$ NMR. Chemical shifts for $^{13}C$ NMR and $^{31}P$ NMR were indirectly referenced to 10% acetone in $D_2O$ ($CH_3$ set to 30.89 ppm)**(3)** and 85% $H_3PO_4$ (0 ppm), respectively. $^{13}C$ chemical shifts denoted with a (*) fail to resolve into clean singlets due to apparent conformational restrictions. Mass spectral data was obtained from The University of Texas at Austin Mass Spectrometry Facility.

**Ribavirin 5'-diphosphate (sodium salt).** This compound was prepared by modification of the known procedure for the synthesis of ribavirin 5'-diphosphate lithium salt(2). Ribavirin 5'-phosphoromorpholidate triethylammonium salt (700.2 mg; ~2.3 molar equivalents of salt per mole of phosphormorpholidate), synthesized as previously described(2), was triturated with pyridine, then redissolved in pyridine (9 mL). Separately, $Bu_3N$ (anhydrous, 0.98 mL, 4.1 mmoles) was added to a suspension of $H_3PO_4$ (98% crystalline, 401.7 mg, 4.1 mmoles) in pyridine (3 mL), yielding a nearly solubilized solution. An aliquot of this phosphate solution (2.6 mL) was added to the ribavirin 5'-phosphoromorpholidate solution and the reaction was stirred for 72 hours at 23 °C. Distilled water (5 mL) was added and the reaction was concentrated in vacuo. The crude diphosphate was redissolved in aqueous TEAB (1 M, 1 mL) and purified by preparative HPLC ($t_R$ 9 – 17 mins). Lyophilization of the purified material yielded ribavirin 5'-diphosphate triethylammonium salt (270 mg) as a white solid. Conversion to the sodium salt was performed via a previously reported procedure(1). To a solution of the

triethylammonium salt (~267 mg) in distilled and deionized water ($ddH_2O$, 10 mL) was added $NaClO_4$ (1.336 g, 10.91 mmoles). The mixture was stirred for 2 h then precipitated by the addition of acetone (30 mL). The material was concentrated in vacuo yielding a colorless oil, then precipitated again by the addition of excess acetone. The resulting white solid was filtered, washed with excess acetone, and frozen in $ddH_2O$ (10 mL). Lyophilization yielded ribavirin 5'-diphosphate sodium salt (102.5 mg, ~19% yield from the phosphormorpholidate) as a white solid. $^1H$ NMR ($D_2O$, 400.1 MHz): δ 8.85 (s, 1H), 6.07 (d, $J$ = 3.7 Hz, 1H), 4.71 (m, 1H), 4.64 (m, 1H), 4.40 (m, 1H), 4.23 (m, 2H). $^{13}C$ NMR ($D_2O$, 100.6 MHz): δ 163.5*, 156.9*, 146.4*, 92.7*, 84.4*, 75.3*, 70.4, 70.3, 65.0*. $^{31}P$ NMR ($D_2O$, 145.8 MHz): δ -6.00 (m), -9.90 (m). HRMS ($CI^+$) calcd. for $C_8H_{14}N_4O_{11}NaP_2$ $[M-2Na+3H]^+$ 427.0032, found 427.0044.

FIGURE LEGENDS

**Figure S-1: Chemical structures of nucleoside and nucleotide analogues.** The structures of synthesized nucleoside triphosphate (A), and nucleoside diphosphate (B) analogues are illustrated.

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