CHEMICAL SYNTHESIS INFORMATION

General Chemical Synthesis for N6-substituted purine analogues. Nucleosides were synthesized as described previously(4). 31P-NMR spectra were recorded at 121.5 MHz on a Bruker DRX300 instrument using D2O as the NMR solvent. The 31P-NMR spectra was calibrated using 85% H3PO4 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/mmole) 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%). $\delta_P (D_2O) \gamma-P –9.1 (d); \alpha-P –10.3, (d); \beta-P -22.1, (t)$.

**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, 6 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%). $\delta_P (D_2O) \gamma-P –5.4, (d); \alpha-P –10.3, (d); \beta-P -21.5, (t)$.

**$N^6$-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). δP (D₂O) γ-P –8.0, (d); α-P –10.0, (d); β-P -21.3, (t). Mass (C₁₀H₁₂N₅O₁₄P₃·4Na): 610.54; Calculated, 611.

**N⁶-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). δP (D₂O) γ-P –7.3, (d); α-P –11.8, (d); β-P -23.1, (t). Mass (C₁₁H₁₄N₅O₁₄P₃·4Na): 624.39; Calculated, 624.

**N⁶-Amino-N⁶-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). δP (D₂O) γ-P
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°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\(_2\)O) \(\gamma\)-P –8.7, (d); \(\alpha\)-P –10.1, (d); \(\beta\)-P -21.6, (t). Mass (C\(_{10}\)H\(_{15}\)N\(_6\)O\(_{10}\)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 °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\(_2\)O) \(\gamma\)-P –6.2, (d); \(\alpha\)-P –9.8, (d); \(\beta\)-P -20.8, (t). Mass (C\(_{11}\)H\(_{17}\)N\(_6\)O\(_{14}\)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 °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). δₚ (D₂O) γ-P –8.8, (d); α-P –10.0, (d); β-P -21.6, (t). Mass (C₁₀H₁₄N₇O₁₃P₃·4Na): 626.08; Calculated, 625.

2-Amino-N⁶-amino-N⁶-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°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). δₚ (D₂O) γ-P –7.1, (d); α-P –9.9, (d); β-P –21.0, (t). Mass (C₁₁H₁₆N₇O₁₃P₃·2Na): 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%). $\delta_P$ (D2O) 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%). $\delta_P$ (D2O) 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' dicarboxyldiimidazole
(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 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$_2$O) $\beta$-P –5.9, (d); $\alpha$-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 \(\mu\)L, 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 \(\mu\)L) 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\(_2\)O) \(\beta\)-P –5.4, (d); \(\alpha\)-P –9.9, (d).

\(N^6\)-Hydroxyadenosine-5'-diphosphate (JA51). To a solution of 6-chloropurine riboside-5'-diphosphate (21.7 \(\mu\)mol) in water (1 mL), hydroxylamine (50% w/v in water, 14 \(\mu\)L) was added and resulting mixture was heated at 40\(^\circ\)C for 3 h. The crude product was then purified by HPLC (Phenomenex Gemini 10\(\mu\) 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 \(\mu\)mol, 24%). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na\(^+\) form). \(\delta_p\) (D\(_2\)O) \(\beta\)-P –5.8, (d); \(\alpha\)-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 \(\mu\)mol) 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). \(\delta_P (D_2O)\) \(\beta-P\) −5.8, (d); \(\alpha-P\) −10.1, (d).

**2-Amino-N6-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). \(\delta_P (D_2O)\) \(\beta-P\) −6.1, (d); \(\alpha-P\) −10.2, (d).

**General chemical synthesis for ribavirin 5'-diphosphate.** Pyridine was distilled from calcium hydride (CaH₂) 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$_2$O (CH$_3$ set to 30.89 ppm)$^{(3)}$ and 85% H$_3$PO$_4$ (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$_3$N (anhydrous, 0.98 mL, 4.1 mmoles) was added to a suspension of H$_3$PO$_4$ (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$_2$O, 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$_2$O (10 mL). Lyophilization yielded ribavirin 5'-diphosphate sodium salt (102.5 mg, ~19% yield from the phosphormorpholidate) as a white solid. $^1$H NMR (D$_2$O, 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$_2$O, 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$_2$O, 145.8 MHz): δ -6.00 (m), -9.90 (m). HRMS (CI$^+$) calcd. for C$_8$H$_{14}$N$_4$O$_{11}$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.

REFERENCES

