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Supporting Information

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Supporting Information

for

A Quantitative Comparison of Wild Type and Gatekeeper Mutant Cdk2 for Chemical Genetic Studies with ATP Analogues

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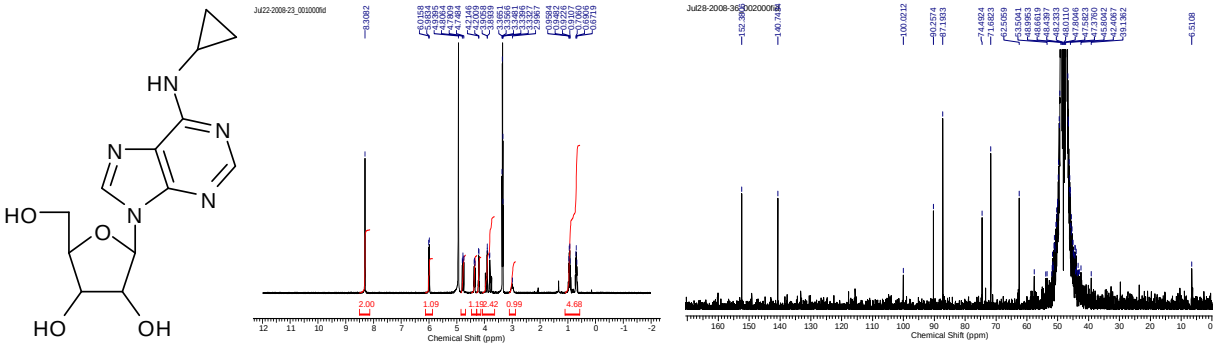
NMR Spectra were recorded on a Brucker Advance DPX200, DPX250, AV400 or AV500 machine. Chemical Shifts (δ) are reported in parts per million (ppm) with the undeuterated solvent as an internal standard. ^{31}P NMR are referenced to phosphoric acid as an external standard. ^{13}C and ^{31}P NMR spectra are proton decoupled and assignments are confirmed by COSY and HMQC spectra where required. Spetra for triphosphates are recorded with mixed triethylammonium and proton counterions. Mass spectra (*m/z*) were recorded on a micromas LCT in electrospray ionisation (ESI). Reverse phase HPLC was performed using a Dionex P680 pump with UVD-340U detector and a Phenomenex Jupiter 10µ proteo 90Å 250x21.2 mM column. Solvent A = 0.1 M triethylammonium bicarbonate (TEAB) buffer, Solvent B = 100% acetonitrile. Unless specified, all HPLC analysis and purifications were run on the following gradient: *t* = 0, 1% B, *t* = 5, 1% B, *t* = 25, 15% B, *t* = 50, 15% B, 8 mL/min. 2 M stock TEAB buffer was prepared by mixing 560 mL HPLC grade triethylamine with 1440 mL HPLC grade water. 1 L solid CO_2 was then sublimed and bubbled through the resulting mixture at room temperature. All other TEAB buffers were made by dilutions of this stock. All solvents were dried by passing through a column of activated alumina under an inert atmosphere Thin layer chromatography (tlc) was performed using Merck aluminium foil backed sheets precoated with Kieselgel 60F_{254} and visualied with a UV lamp or by staining in an iodine chamber. All reagents were purchased from Sigma Aldrich and were used without further purification, however all

non-aqueous liquids were dried over activated 3Å molecular sieves and all solids were dried over P_2O_5 prior to use. All reactions were performed under an atmosphere of Argon in pre-dried glassware. Concentrations of all adenosine phosphates were measured by UV assuming ε_{max} = 15 000, which corresponded to the average measured value for 3 of the analogue nucleosides and native adenosine (3x repeats)

Synthesis of N^6-Modified Adenosine Nucleosides

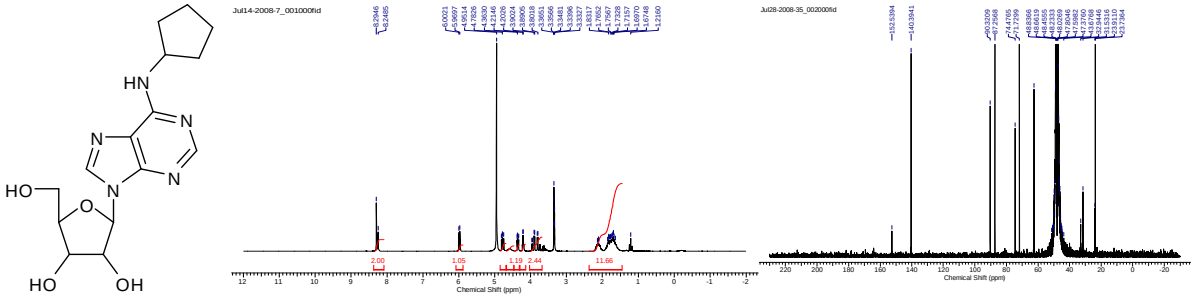
General method: N^6-chloropurine riboside was treated with 1 equiv appropriate amine and 1 equiv triethylamine in ethanol as described previously[1] with the following exception: N^6-(4-amino)piperidinyladenosine which was prepared both by direct displacement of the N^6-chloropurine riboside with 4-aminopiperidine and by displacement of the N^6-chloropurine riboside with (4-amino)-(1-benzyl)piperidine, with subsequent deprotection with H_2/Pd. Identical spectral data and biological results were obtained with both samples, and only the spectra from the direct displacement are presented here.

N^6-*(cyclopropyl)adenosine*



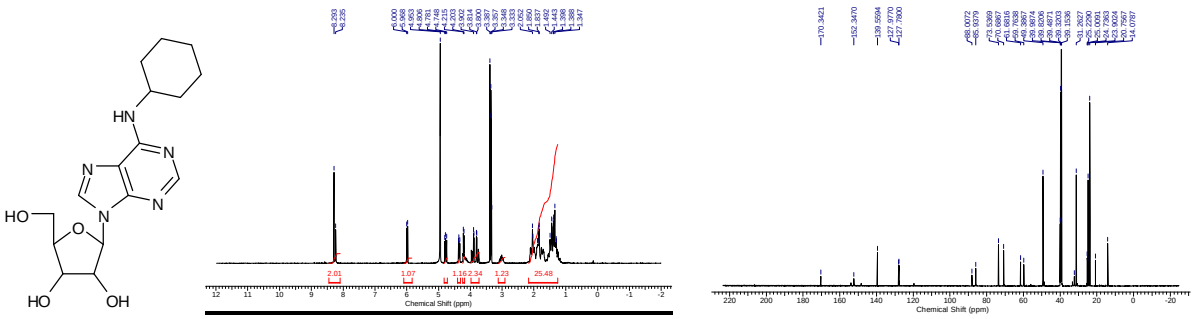
^{1}H NMR (CD_3OD) δ = 0.67 (2H, m, CH_2), 0.90 (2H, m, CH_2), 3.05 (1H, br m, CH), 3.75–3.92 (2H, 2 dd, *J* = 2.39 Hz, 12.63 Hz, 2H, 2x 5'CH), 4.20 (1H, d, *J* = 2.73 Hz, 3'CH), 4.35 (1H, dd, *J* = 2.39, 2.73 Hz, 4'CH), 4.75 (1H, ap. t, *J* = 5.80 Hz, 2'CH), 6.0 (1H, d, *J* = 6.49 Hz, 1'CH), 8.30 ppm (2H, br s, H2, H8). ^{13}C NMR (CD_3OD) ppm δ = 6.5 (2 CH_2 cyclopropane), 26.5 (CH cyclopropane), 62.5 (5'), 71.7 (3'), 74.5 (2'), 87.2 (4'), 90.3 (1'), 140.8, 152.3 (Ar); MS ESI (+ve): $[M+H]^+$ 308.13 (80%), $[M+Na]^+$ 330.11 (20%); HRMS (ESI) found 308.1173, calcd 308.1167; γ_{max} (MeOH) 267.

N^6-*(cyclopentyl)adenosine*



^{1}H NMR (CD_3OD) ppm δ= 1.5-1.9 (6H, m, 3 CH_2) 1.9–2.3 (2H, m, CH_2), 3.70–3.95 (2H, 2 dd, *J* = 2.39 Hz, 12.63 Hz, 2 x 5'CH), 4.20 (1H, ap d, *J* = 2.39, 4'CH), 4.35 (1H, dd, *J* = 2.4 Hz, 5.12 Hz, 3'CH), 4.60 (1H, br s, CH), 4.78 (1H, ap t, *J* = 5.8 Hz, 2'CH), 6.0 (1H, d, *J* = 6.5 Hz, 1H, 1'CH), 8.25, 8.29 (2 H, 2 s, H2, H8); ^{13}C NMR (CD_3OD) ppm δ = 23.7, 31.5, 32.9 (CH_2), 42.5 (CH), 62.5 (5'C), 71.7 (3'), 74.5 (2'), 87.2 (4'), 90.3 (1'), 140.4 (C8), 152.5 (2 Ar); MS ESI (+ve) [*M*+H]$^+$ 336.25 (100%); MS ESI (-ve) [*M*-H]- 334.03 (100%); MS ESI (HRMS) found 336.1666 calcd 336.1659; γ_{max} (MeOH) 267.

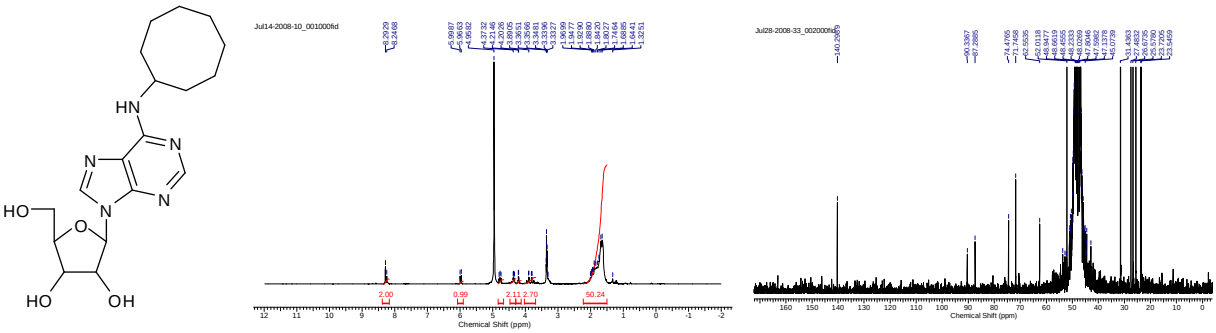
N^6-*(cyclohexyl)adenosine*



^{1}H NMR ([D_6]DMSO) δ = 1.2–2.0 (10 H, m, 5 CH_2), 3.5 – 3.75 (2H, m, 2x 5'CH), 3.95 (1H, ap d, *J* = 3.1 Hz, 4'CH), 4.15 (1H, dd, *J* = 4.6 Hz, 3 Hz, 3'CH), 4.51 (1H, m, CH), 4.6-4.7 (1H, dd, *J* = 6.1 Hz, 5.2 Hz, 2'CH), 5.2 (1H, d, *J* = 4.6 Hz, 3'OH), 5.4-5.5 (2H, dd, *J* = 6.0 Hz, 6.2 Hz, 2'OH, 5'OH), 5.95 (1H, d, *J* = 6.2 Hz, 1'CH), 7.7 (1H, br. d, *J* = 8.2 Hz, NH), 8.2 (1H, s, H2), 8.35 ppm (1H, s, H8); ^{13}C NMR (D6-DMSO) δ = 25.37 (CH_2), 25.59 (CH_2), 32.67 (2 CH_2), 48.78 (CH), 62.06 (5'C), 71.04 (3'), 73.81 (2'), 86.28 (4'), 88.83 (1'), 139.95 (C8), 152.69 (Ar) 159 ppm (2 Ar); MS ESI (+ve) [*M*+H]$^+$

350.06 (100%), [*M*+K]$^+$ 389 (10%); MS ESI (-ve) [*M*-H]- 348.05 (75%); γ_{max} (MeOH) 269.

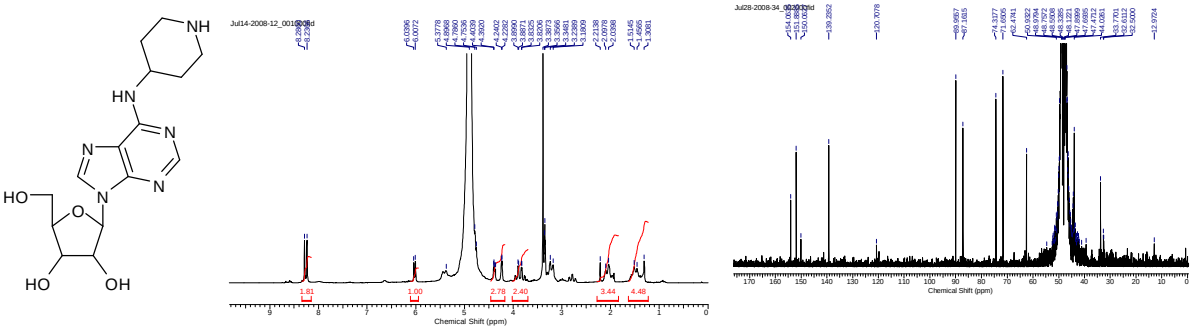
N^6-*(cyclooctyl)adenosine.*



^{1}H NMR (CD_3OD) δ = 1.45–2.10 (14H, m, 7 CH_2), 3.70–3.95 (2H, 2 dd, *J* = 2.05 Hz, 12.63 Hz, 2x 5'CH), 4.20 (1H, d, *J* = 2.05 Hz, 4'CH), 4.34–4.50 (2H, m, CH, 3'CH), 4.75 (1H, ap. t, *J* = 5.8 Hz, 1H, 2'CH), 6.0 (1H, d, *J* = 6.5 Hz, 1H, 1'CH), 8.20, 8.30 ppm (2H, 2 s, H2, H8); ^{13}C NMR (CD_3OD) δ = 23.5, 23.7, 25.5, 26.7, 27.5, 31.4 (Ch, 7x CH_2), 62.6 (5'), 71.7 (3'), 74.5 (2'), 87.3 (4'), 90.3 (1'), 140.3 ppm (CH-8); MS ESI (+ve): [*M*+H]$^+$ 378.20 (100%); MS ESI (-ve): [*M*-H]- 376.21 (10%), [*M*+Cl-H]- 412.18 (100%), [*M*-HCOO-]- 422.21 (75%); HRMS (ESI) found 378.2136, calcd 378.2131.

γmax (MeOH) 268.

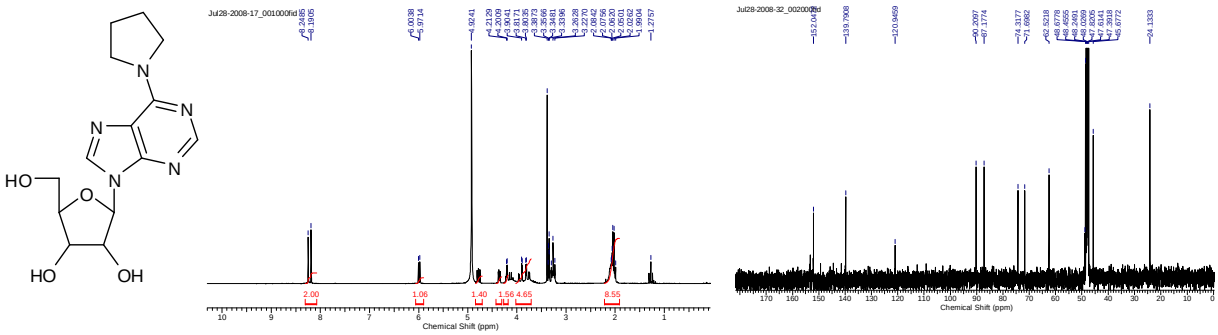
N^6-*[(4-amino)piperidinyl]adenosine*



^{1}H NMR (CD_3OD) δ = 1.50 (4H, m, 2 CH_2), 2.03 (4H, m, 2 CH_2), 3.21 (1H, d, *J* = 11.6 Hz, CHN) 3.81 (2H, 2x d, *J* = 2.39 Hz, 12.6 Hz, 5'CH), 4.23 (1H, d, *J* = 2.39 Hz, 4'CH), 4.38 (1H, m, 3'CH), 6.00 (1H, d, *J* = 6.49 Hz, 1'CH), 8.24, 8.29 ppm (2H, s, H8, H2); ^{13}C NMR (CD_3OD) δ = 32.6, 33.8, 44.0, 47.4 (CH, 4 CH_2), 62.5 (5') 71.7 (3'),

74.3 (2'), 87.2 (4'), 90.0 (1'), 139.2, 150.1 151.9, 154.1 ppm (5 Ar); MS ESI (+ve): $[M+H]^+$ 351.11 (100%); MS ESI (HRMS) found 351.1775, calcd 351.1767; γ_{max} (MeOH) 269.

N^6-*(pyyrolidinyl)adenosine*



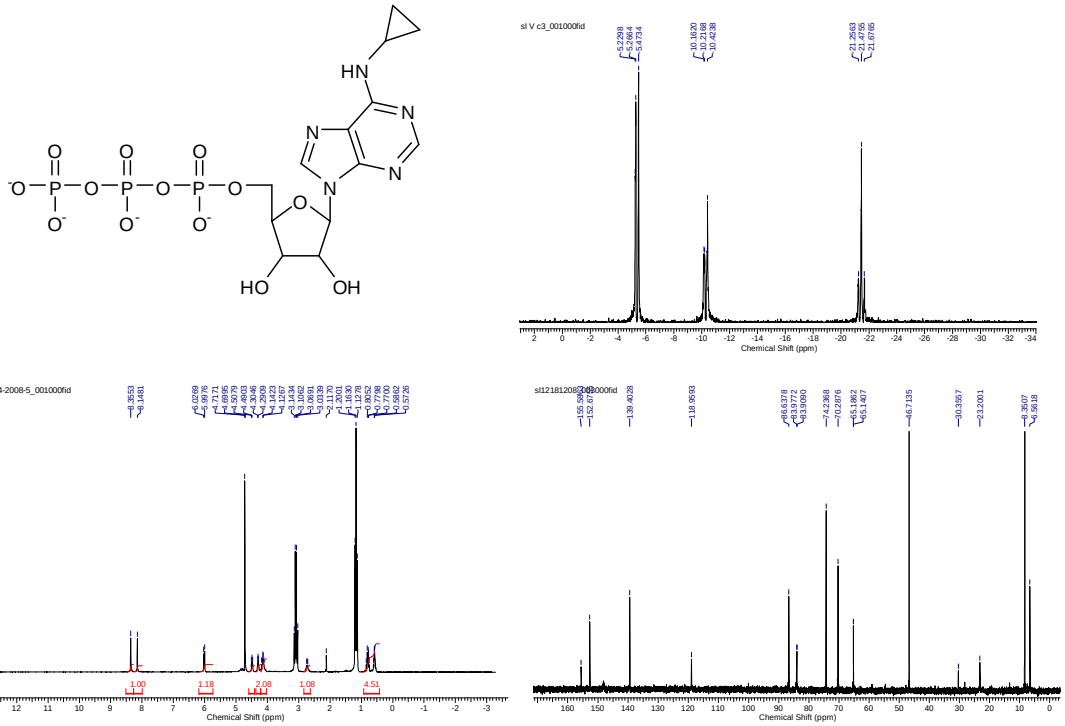
^{1}H NMR ([D_6]DMSO) δ = 1.8–2.05 (br m, 4H, 2 CH_2), 3.5–3.75 (m, 4H, 2 CH, 2x 5'CH), 3.95 (m/dd, *J* = 3.2, 6.5 Hz, 1H, 4'CH), 4.0-4.1 (br s, 2H, 2 CH), 4.15 (m/dd *J* = 3.2, 4.6 Hz, 1H, 3'CH), 4.6 (m/d, *J* = 6.1 Hz, 1H, 2'CH), 5.15 (d, *J* = 4.7 Hz, 1H, 3'OH), 5.45 (br m 2H, 2'OH, 5'OH), 5.9 (d, *J* = 6.1 Hz, 1H, 1'CH), 8.18 (br. s,1H, H2), 8.35 ppm (s, 1H, H8); ^{13}C NMR ([D_6]DMSO) δ = 19.41 (CH2), 24.5 (CH2), 26.5 (CH2), 56.87 (CH2), 62.45 (5'), 71.43 (3'), 74.30 (2'), 86.65 (4'), 88.65 (1'), 120.82 (CH6), 139.97 (CH8), 150.34, 152.91, 153.39 ppm (3 Ar); Ms ESI (+ve): $[M+H]^+$ 322.03 (100%); MS ESI (-ve): $[M-H]^-$ 320.02 (25%), $[M$-HCOO-$]^-$ 380.16 (100%); γ_{max} (MeOH) 269.

Synthesis of N^6-Modified Adenosine 5'-Triphosphates

General method: 1 equiv N^6-modified adenosine (0.8 mM) was dissolved in PO-$(OMe)_3$ (0.5 mL). 0.1 eq proton sponge (0.016 g) was added and the mixture cooled to 0^{o}C on ice. 1.1 equiv $POCl_3$ (75 uL) was added dropwise. The reaction mix was stirred at 0 ℃ for 2 h. 4 equiv 0.5 M pyrophosphate solution in DMF was then mixed with 8 equiv tributylamine and the resulting solution added to the reaction mixture. The resulting mixture was then stirred at room temperature for 2 h before being quenched by the addition of excess 0.1 M triethylammonium bicarbonate solution (2 mL). The resulting solution was warmed to ambient temperature and then stirred

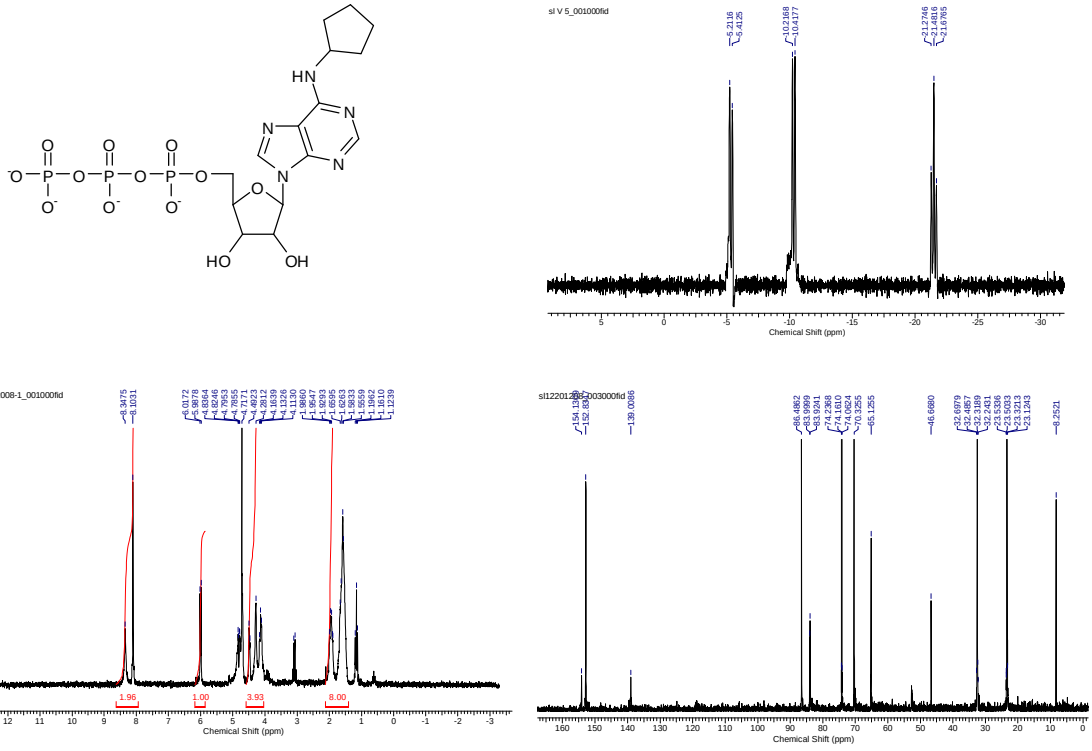
overnight. The resulting solution was filtered and purified by ion exchange chromatography on DEAE sephadex, followed by RP HPLC to give the desired product.

N^6-*(cyclopropyl)adenosine-5'-triphosphate*



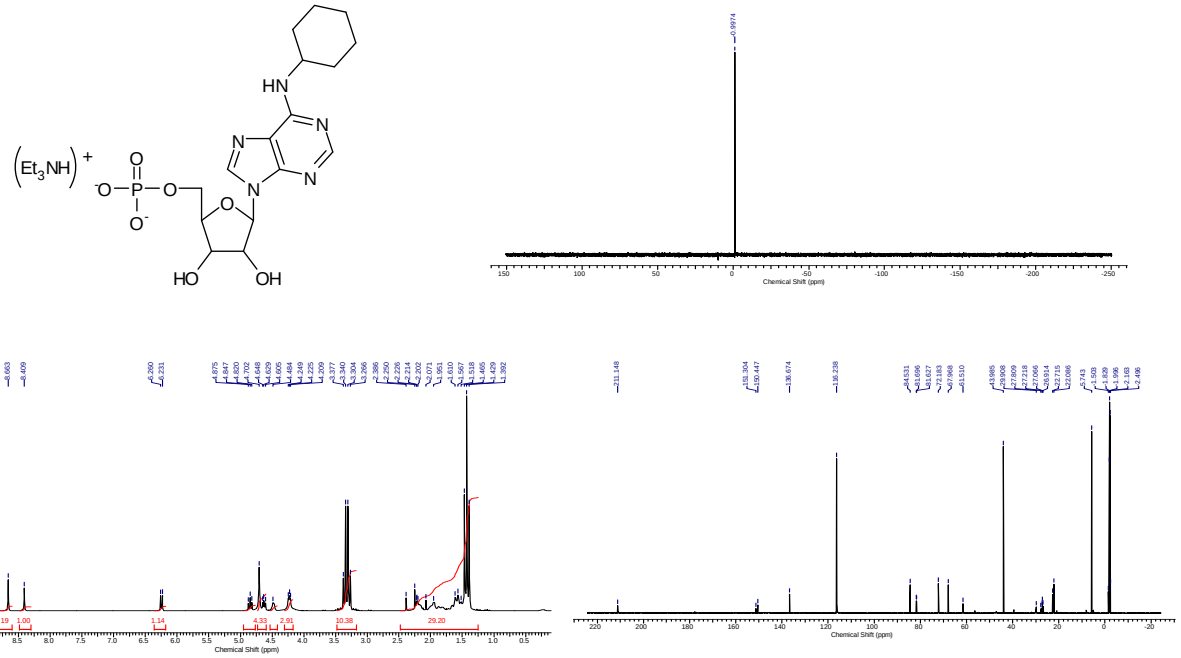
^{1}H NMR (D_2O) ppm δ = 0.60 (2H, m, CH_2), 0.85 (2H, m, CH_2), 2.80 (1H, br. m, CH), 4.10-4.20 (2H, br. m, 2x 5'CH), 4.32 (1H, m, 3'CH), 4.53 (1H, m, 4'CH), 6.0 (1H, d, *J* = 6.49 Hz, 1'CH), 8.17, 8.35 (2H, 2 br s, H2, H8); ^{13}C NMR (D_2O) δ = 6.5 (2 CH_2 cyclopropane), 23.1 (CH cyclopropane), 65.2 (5'), 70.3 (4'), 74.2 (3'), 84.1 (2'), 86.7 (1'), 118.9, 139.4, 148.2, 152.7, 155.6 ppm (5 Ar); ^{31}P NMR (D_2O pH 8–9) –5.36 (d, *J* = 20 Hz, γ), -10.29 (d, *J* = 22 Hz, α), -21.45 (ap t, *J* = 21 Hz, β); elution from Sephadex:- 0.6 – 0.8 M TEAB pH8; γ_{max} (MeOH/H_2O, pH 8–9) 267; ESI MS (-ve) [*M*-H]- 546.01 (100%), [*M*-PO_3]- 466.05 (65%); ESI MS (HRMS) found 546.0198 calcd 546.0195; t_R RP HPLC 23.8 min.

N^6-*(cyclopentyl)adenosine-5'-triphosphate.*



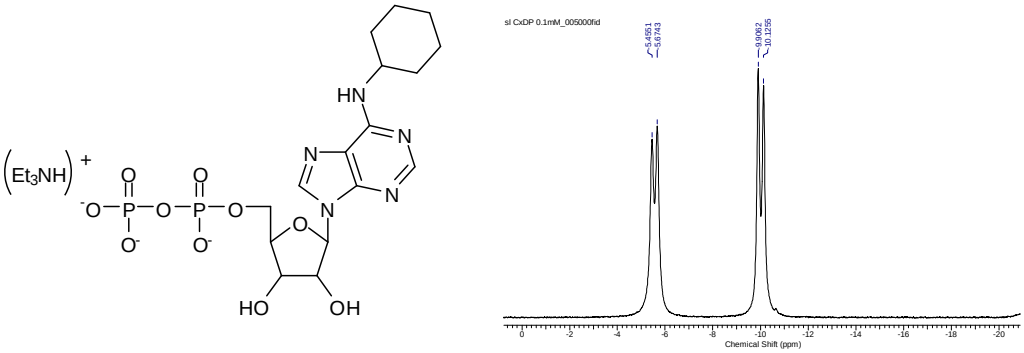
^{1}H NMR (D_2O) δ = 1.5-1.7 (6H, m, 3 CH_2) 1.8-1.9 (2H, m, 1x CH_2), 4.10 – 4.20 (2H, 2x m, 2x 5'CH), 4.30 (2H, m, CH, 4'CH), 4.55 (1H, m, 3'CH), 6.0 (1H, d, *J* = 6.5 Hz, 1H, 1'CH), 8.10, 8.40 ppm (2x 1H, 2x s, H2, H8); ^{13}C NMR (D_2O) ppm δ = 8.2, 23.2, 32.5 (3 CH_2), 46.6 (CH), 52.7 (CH_2), 65.1 (5'C), 70.3 (3'), 74.1 (2'), 84.0 (4'), 86.5 (1'), 139.0 (C8), 152.8, 154.1 (2 Ar); ^{31}P NMR (D_2O in H_2O, pH 8–9) –5.3 (d *J* = 20 Hz, γ), -10.3 (d, *J* = 22 Hz, α), -21.5 (ap t, *J* = 21 Hz, β); elution from Sephadex:- 0.5 – 0.8 M TEAB pH 8; γ_{max} (H_2O, pH 8–9) 268; ESI MS (-ve) [*M*-H]- 574.08 (20%), [*M*-PO_3]- 494.11 (50%), [*M*-2xPO_3]- 414.14 (10%); ESI MS (HRMS) found 574.0475 calcd 574.0509; t_R RP HPLC 32.2 min.

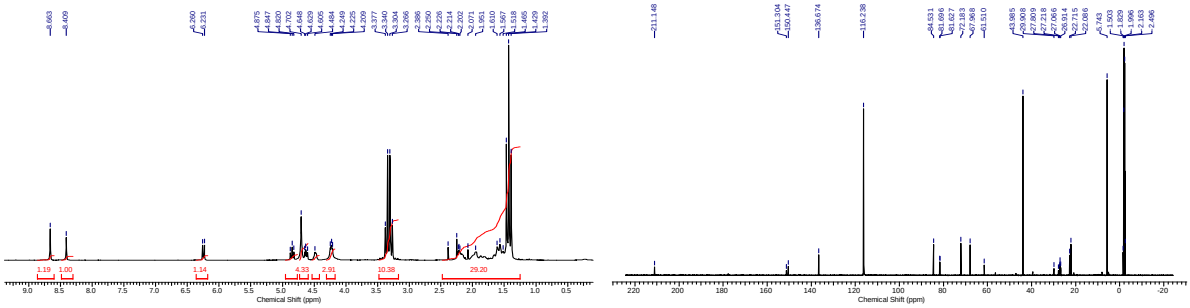
N^6-*(cyclohexyl)adenosine-5'-monophosphate (triethylammonium salt)*: 1 equiv *N*^{6}-(cyclohexyl)adenosine (0.29 g, 0.8 mM) was dissolved in $PO(OMe)_3$ (0.5 mL). 0.1 equiv proton sponge (0.016 g) was added and the mixture cooled to 0^{o}C on ice. 1.1 equiv $POCl_3$ (75 µL) was added dropwise. The reaction mix was stirred at 0^{o}C for 2 h and then quenched by the addition of excess 0.1 M triethylammonium bicarbonate solution (2 mL). The resulting solution was warmed to ambient temperature and then stirred overnight. The resulting solution was filtered and purified by RP HPLC to give the desired product in 62% yield.



^{1}H NMR (200 MHz, 1:1 D_2O:CD_3CN) 1.40-2.25 (11H, m, Cx; 3H, m, TEAH) 3.30 (3H, m, CH, TEAH; 1H, m, Cx), 4.22 (2H, m, 2x5'), 4.48 (1H, m, 4'), 4.64 (1H, dd, *J* = 3.8 Hz, 4.8 Hz, 3') 4.70 (1H, br. S, TEAH), 4.85 (1H, ap. t, *J* = 5.31, 2') 6.25 (1H, d, *J* = 5.6 Hz, 1') 8.41, 8.66 (2H, 2 s, 2 Ar); ^{31}P NMR (400 MHz, 1:1 D_2O:CD_3CN) -1.0 (s, α); ^{13}C NMR (400 MHz, 1:1 D_2O:CD_3CN) ca. 0 (TEA + MeCN) 22.1, 22.7, 26.9, 27.2, 27.8, 29.9 (Cx) 44.3 (TEA) 61.5 (5') 68.0 (3') 72.2 (2') 81.7 (4') 84.5 (1') 116.6 (MeCN) 136.7, 150.4, 151.3, 211.1 (5 Ar); γ_{max} = 270 nM; t_R HPLC = 41.5 min; HRMS (ESI, -ve) expected 428.1330, found 428.1330 ($C_{16}H_{23}N_5O_7P_1$).

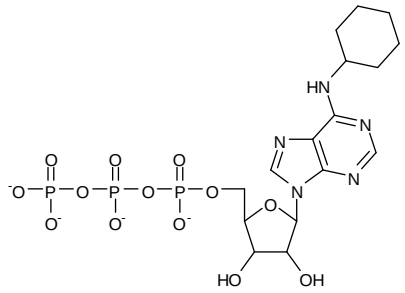
N^6-*(cyclohexyl)adenosine-5'-diphosphate*: *N*^{6}-(cyclohexyl)-adenosine-5'-diphosphate was synthesised from *N*^{6}-(cyclohexyl)-adenosine-5'-monophosphate by the procedure described by Hoard and Ott.[2]

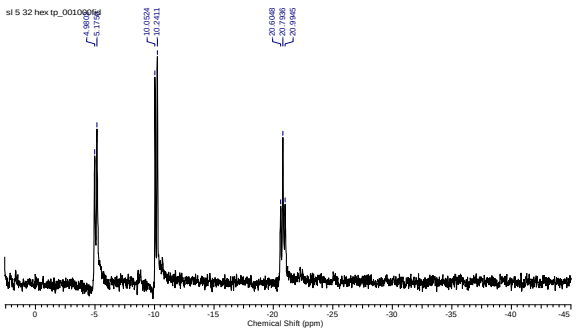


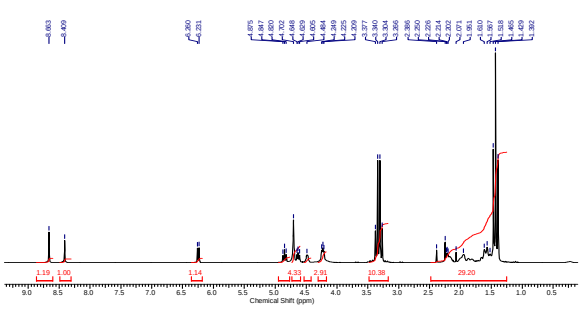


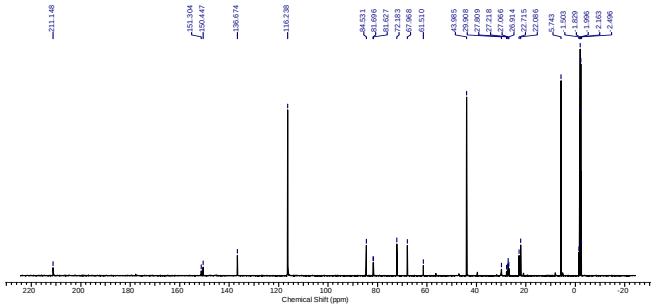
^{1}H NMR (200 MHz, 1:1 D_2O:CD_3CN) 1.40-2.25 (11H, m, Cx; 3H, m, TEAH) 3.30 (3H, m, CH, TEAH), 4.22 (2H, m, 2 x 5'CH), 4.48 (1H, m, 4'CH), 4.64 (1H, dd, *J* = 3.8 Hz, 4.8 Hz 3'CH) 4.70 (1H, br. S, TEAH; 1H, br s, Cx), 4.85 (1H, ap. t, *J* = 5.31, 2'CH) 6.25 (1H, d, *J* = 5.6 Hz, 1'CH) 8.41, 8.66 (2 H, 2 s, 2 Ar); ^{13}C NMR (400 MHz, 1:1 D_2O/CD_3CN) ca. 0 (TEA + MeCN) 22.1, 22.7, 26.9, 27.2, 27.8, 29.9 (Cx) 44.3 (TEA) 61.5 (5') 68.0 (3') 72.2 (2') 81.7 (4') 84.5 (1') 116.6 (MeCN) 136.7, 150.4, 151.3, 211.1 (5 Ar); ^{31}P NMR (D_2O in H_2O, pH 8–9) –5.52 (d, *J* = 21 Hz, β phosphate), -10.01 (d, *J* = 22 Hz, α phosphate); γ_{max} (H_2O, pH 8–9) 267; ESI MS (-ve) [*M*-H]- 508.2 (100%), [*M*-PO_3]- 428.2 (45%); t_R RP HPLC 30.5 min.

N^{6}-*(cyclohexyl)adenosine-5'-triphosphate*



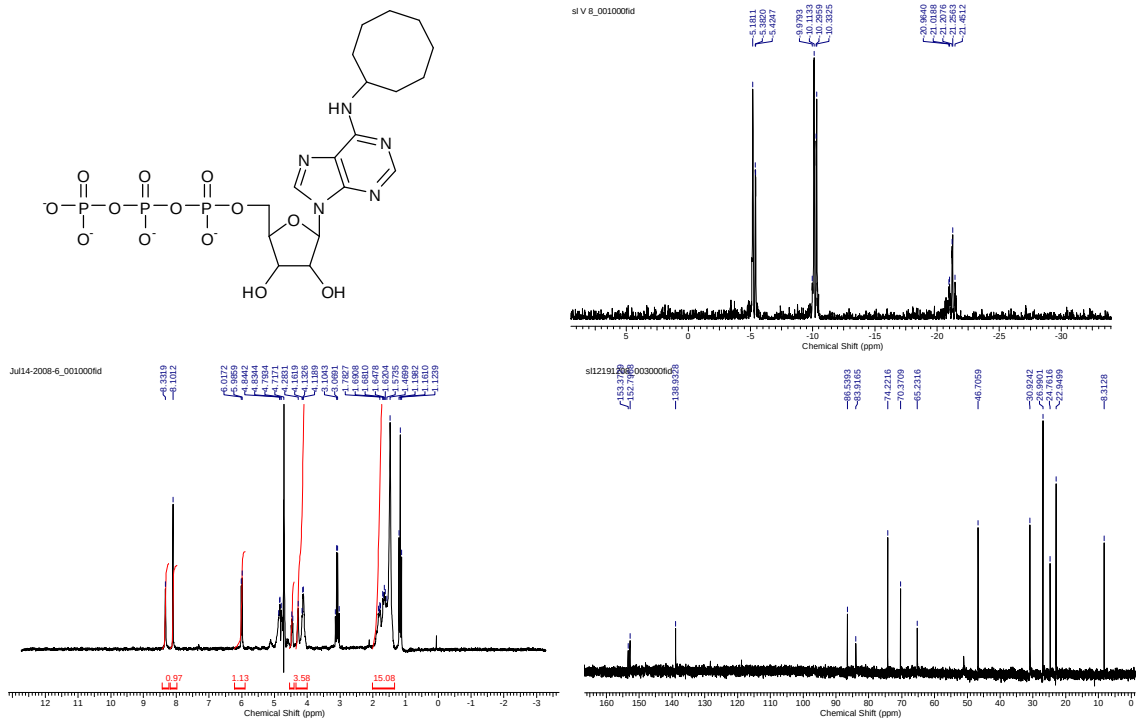






^{1}H NMR (200 MHz, 1:1 D_2O:CD_3CN) 1.40-2.25 (11H, m, Cx; 3H, m, TEAH) 3.30 (3H, m, CH, TEAH), 4.22 (2H, m, 2 x 5'CH), 4.48 (1H, m, 4'CH), 4.64 (1H, dd, *J* = 3.8 Hz, 4.8 Hz 3'CH) 4.70 (1H, br. S, TEAH; 1H, br s, Cx), 4.85 (1H, ap. t, *J* = 5.31, 2'CH) 6.25 (1H, d, *J* = 5.6 Hz, 1'CH) 8.41, 8.66 (2 H, 2 s, 2 Ar); ^{13}C NMR (400 MHz, 1:1 D_2O/CD_3CN) ca. 0 (TEA + MeCN) 22.1, 22.7, 26.9, 27.2, 27.8, 29.9 (Cx) 44.3 (TEA) 61.5 (5') 68.0 (3') 72.2 (2') 81.7 (4') 84.5 (1') 116.6 (MeCN) 136.7, 150.4, 151.3, 211.1 (5 Ar); ^{31}P NMR (D_2O, pH 8–9) –5.50 (d, *J* = 20 Hz, γ), -10.34 (d, *J* = 22 Hz, α), -21.51 (ap t, *J* = 21 Hz, β); elution from Sephadex: 0.45 – 0.75 M TEAB pH 8; γ_{max} (H_2O, pH 8–9) 270.5; ESI MS (-ve) [*M*-H]- 588.2 (50%), [*M*-PO_3]- 508.2 (100%); t_R RP HPLC 33 min.

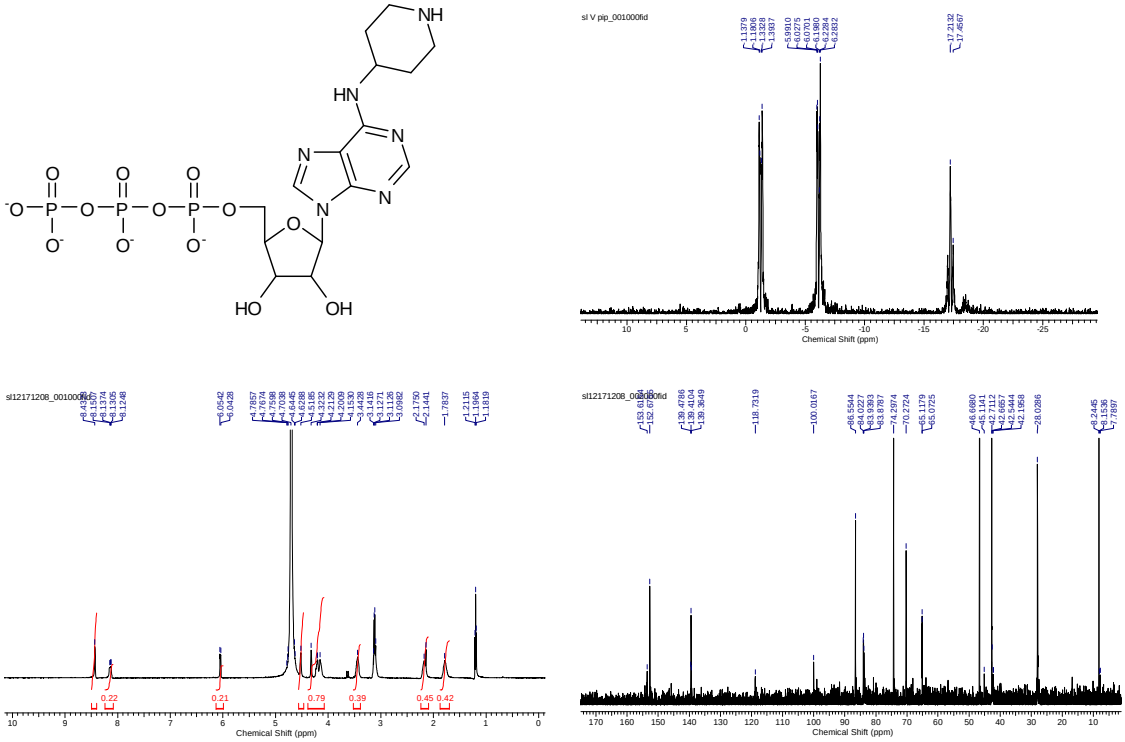
N^6-*(cyclooctyl)adenosine-5'-triphosphate*



^{1}H NMR (D_2O) δ = 1.40–1.90 (14H, m, 7 CH_2), 4.10 – 4.22 (3H, m, CH, 2 x 5'CH), 4.33 (1H, m, 4'CH), 4.50 (1H, m, 3'CH), 6.0 (1H, d, *J* = 6.5 Hz, 1H, 1'CH), 8.13, 8.35 ppm (2 H, 2 br s, H2, H8); ^{13}C NMR (D_2O) δ = 22.9, 24.7, 27.0, 30.9 (CH, 7 CH_2), 65.2 (5'), 70.4 (3'), 74.2 (2'), 84.0 (4'), 86.5 (1'), 138.9, 152.8, 153.3 ppm (Ar); ^{31}P NMR (D_2O, pH 8–9) –5.3 (d, *J* = 20 Hz, γ), -10.3 (d, *J* = 22 Hz, α), -21.2 (ap. t, *J* = 21 Hz, β); elution from Sephadex:- 0.55 – 0.8 M TEAB pH 8; γ_{max} (MeOH/H_2O, pH 8–9)

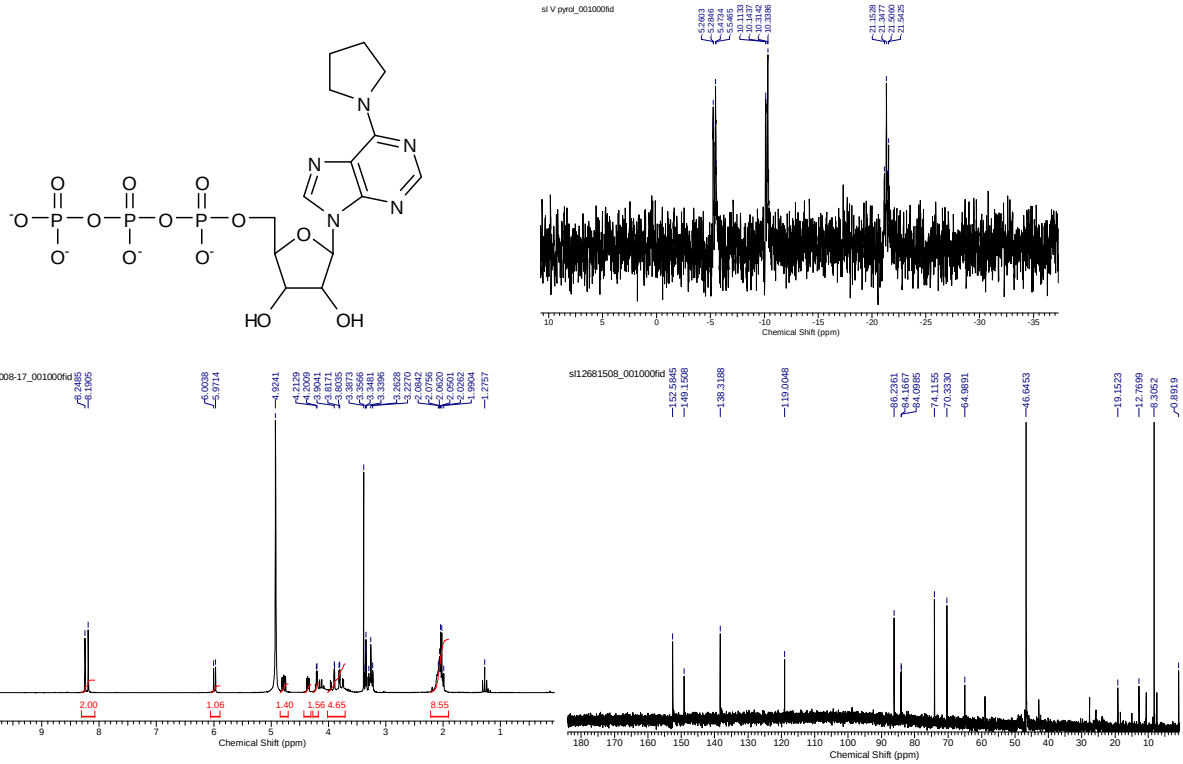
267; ESI MS (-ve) [*M*-H]- 619.09 (100%), [*M*-PO_3]- 536.14 (20% or 100%); ESI MS (HRMS) found 616.0980 calcd 616.0972; t_R RP HPLC 37.8 min.

N^6-*[(4-amino)piperdinyl]adenosine-5'-triphosphate*



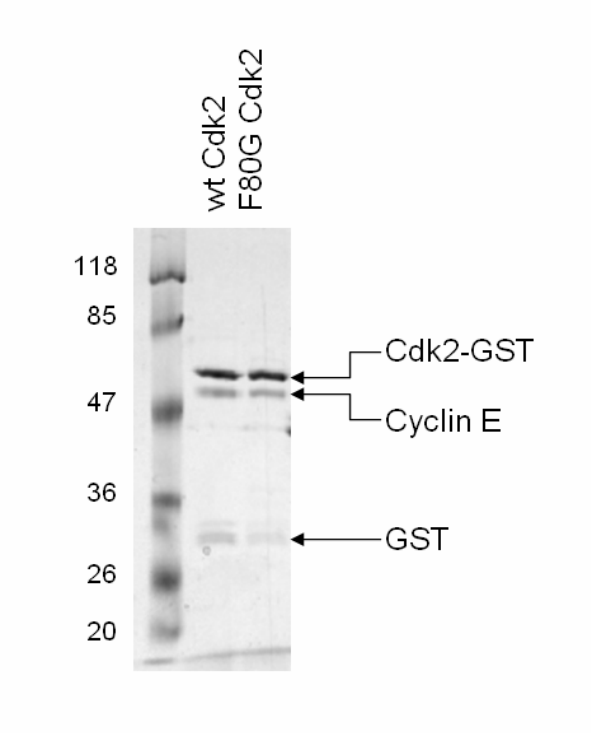
^{1}H NMR (D_2O) δ = 1.75 (4H, m, 2 CH_2), 2.18 (4H, m, 2 CH_2), 3.45 (1H, m, CHN) 4.15 (2H, 2 d, *J* = 2.39 Hz, 12.6 Hz, 5'CH), 4.30 (1H, m, 4'CH), 4.55 (1H, m, 3'CH), 6.05 (1H, m, 1'CH), 8.12, 8.41 ppm (2H, s, H8, H2); ^{13}C NMR (D_2O) δ = 28.0 (4 CH_2), 45.1 (CH_2), 65.1 (5') 70.3 (3'), 74.3 (2'), 84.0 (4'), 86.6 (1'), 139.4, 152.7 ppm (5 Ar); ^{31}P NMR (D_2O, pH 6.5) –1.3 (d, *J* = 22 Hz, γ), -6.1 (d, *J* = 22 Hz, α), -17.2 (ap t, *J* = 21 Hz, β); elution from Sephadex:- 0.5 – 0.8 M TEAB pH 8; γ_{max} (H_2O, pH 8–9) 268; ESI MS (HRMS) found 589.0620 calcd 589.0615; t_R RP HPLC 22.2 min.

N^6-*(pyrrolidinyl)adenosine-5'-triphosphate*



^{1}H NMR (D_2O) δ = 1.8–2.05 (br m, 4H, 2 CH_2), 4.15 (2H, 2 d, J = 2.39 Hz, 12.6 Hz, 5'CH), 4.30 (1H, m, 4'CH), 4.55 (1H, m, 3'CH), 6.05 (1H, m, 1'CH), 8.12, 8.41 ppm (2H, s, H8, H2); ^{13}C NMR (D_2O) δ = 8.31, 12.77, 19.15, 46.64 (4 CH_2), 64.99 (5'), 70.33 (3'), 74.11 (2'), 84.10 (4'), 86.24 (1'), 119.00 (CH6), 138.31 (CH8), 149.15, 152.58 ppm (3 Ar); ionic strength of elution from Sephadex:- 0.5 – 0.8 M TEAB pH 8; ^{31}P NMR (D_2O, pH 8–9); γ_{max} (H_2O, pH 8–9) 268; ESI MS (HRMS) found 589.0620 calcd 589.0615; t_R RP HPLC 22.2 min.

GST-purified cyclin E/cdk2: Cyclin/cdk complexes were purified as described in the methods. Samples were resolved by SDS-PAGE and stained with Coomassie Brilliant Blue. Molecular weight markers are indicated on the left hand side of the figure.

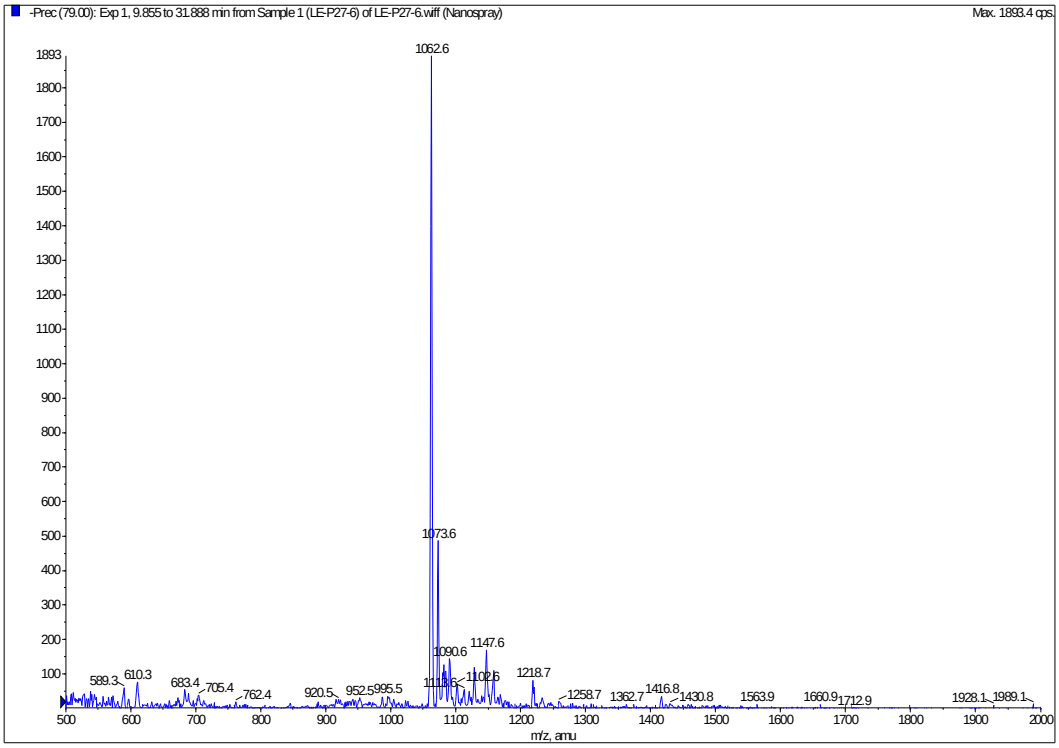


Mass spec analysis of p27-GST fragments: The clone used for phosphorylation and subsequent mass spec analysis was the GST tagged N-terminal of human p27, amino acids 105-198.

Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPY
YIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDF
ETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLD
AFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPG
ISGGGGGILDSMDVSGSRPAAPLIGAPANSEDTHLVDPKTDPSDSQTGLAEQCAGI
RKRPATDDSSTQNKRANRTEENVSDGSPNAGSVEQTPKKPGLRRRQT

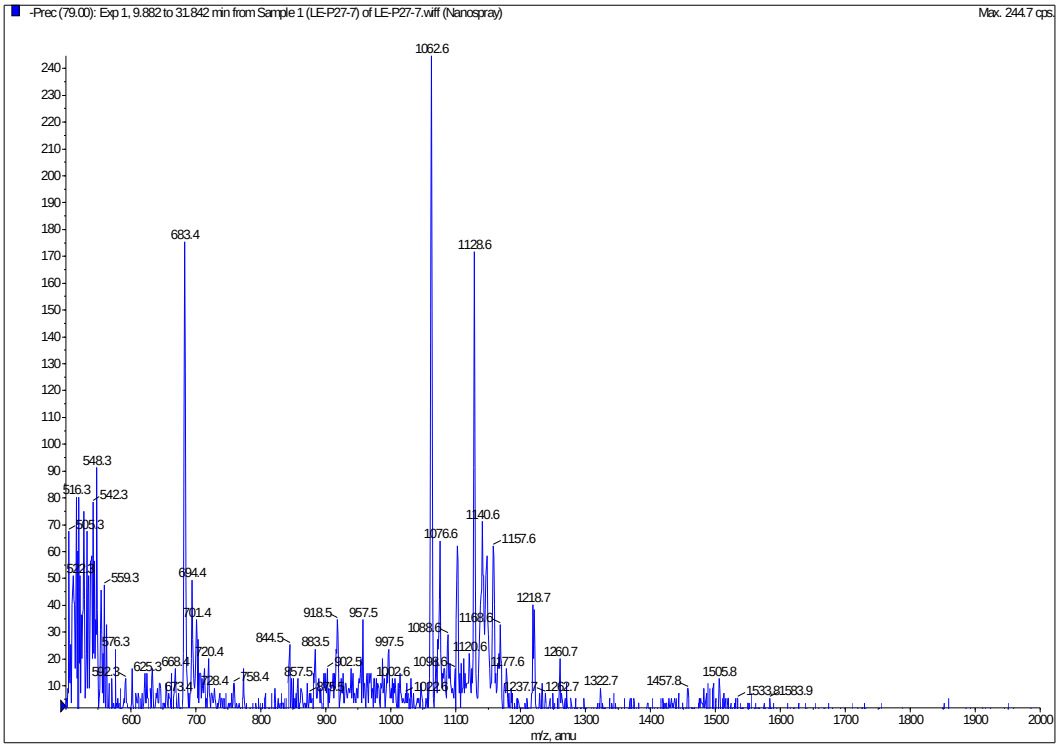
Pre79 ion spectrum for p27-GST phosphorylated with wt cdk2 + ATP.



P27/wt/ATP is phosphorylated at **Thr187.**

Evidence: The Pre79 ion at 1062.6 (1063.5 in Mascot) represents the peptide R.TEENVSDGSPNAGSVEQTPK.K + Phospho (ST)

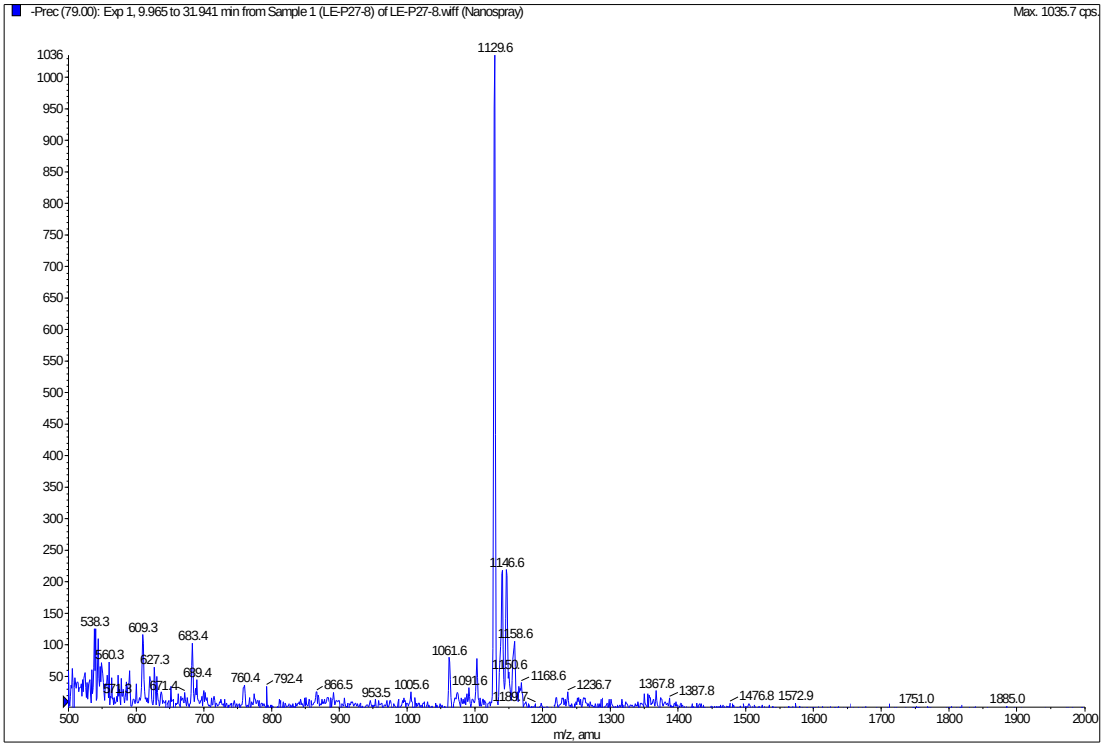
Pre79 ion spectrum for p27-GST phosphorylated with F80G cdk2 + ATP.



P27/F80G/ATP is phosphorylated at **Thr187.**

Evidence: The Pre79 ion at 1062.6 (1063.5 in Mascot) represents the peptide R.TEENVSDGSPNAGSVEQTPK.K + Phospho (ST)

Pre79 ion spectrum for p27-GST phosphorylated with F80G cdk2 + CxATP.



P27/F80G/CxATP is phosphorylated at **Thr187.**

Evidence: The Pre79 ion at 1061.6 (1063.5 in Mascot) represents the peptide R.TEENVSDGSPNAGSVEQTPK.K + Phospho (ST)

Calculation of K_m and V_{max} (Lineweaver–Burke)

In order to ensure that the observed velocity of the kinase assays were linear, ATP levels were measured at the lowest concentration (1 μM) to ensure no more than 10% of the total available ATP was used. It was then assumed that if no more than 10% of ATP was being used at the lowest concentration, this would also be applicable to higher concentrations. Aliquots of supernatant were removed from kinase reactions following a 30 min incubation and the ATP levels were measured using an ATP determination kit according to the manufacturer's instructions (Biaffin). Control samples were incubated without kinase and test samples (containing Rb, Kinase and ATP) are expressed as a percentage of ATP in a control assay lacking a kinase. No ATP was detected in GST preparations of Rb or Kinase.

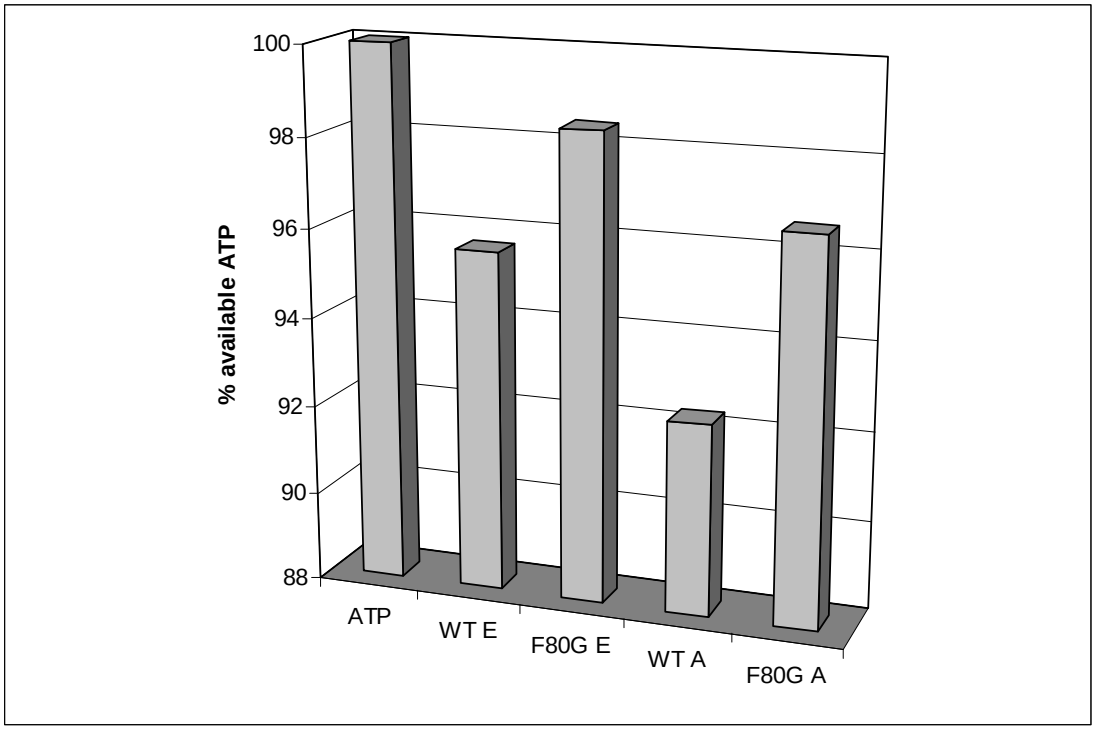


Figure S1: Average ATP consumption in 1 μM kinase reaction. Data is shown as an average (*n* = 3).

1. K. Kikugawa, K. Iizuka, M. Ichino, *J. Med. Chem.* **1973**, *16*, 358-364.

2. D. E. Hoard, D. G. Ott, *J. Am. Chem. Soc.* **1965**, *87*, 1785-1788.