Supporting Information for

Transition State Analogue Discrimination by Related Purine Nucleoside Phosphorylases

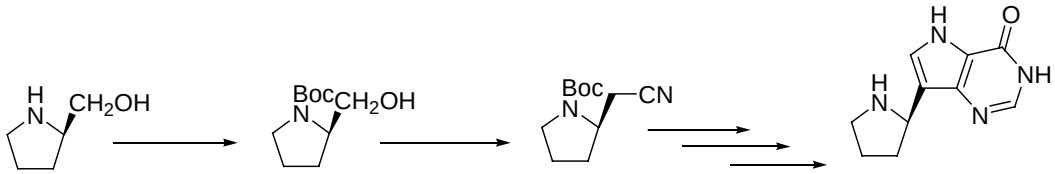
Erika A. Taylor Ringia[a], Peter C. Tyler[b], Gary B. Evans[b], Richard H. Furneaux[b], Andrew S. Murkin[a] and Vern L. Schramm[a]*

[a]*Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461 and* [b]*Carbohydrate Chemistry Team, Industrial Research Ltd., Lower Hutt, New Zealand.*

General. All chemicals were analytical grade or the highest quality commercially available. NMR spectra were recorded on a Bruker AC-300 instrument at 300 MHz (^{1}H) or 75 MHz (^{13}C) when D_2O was the solvent, acetone (δ ^{1}H, 2.20; ^{13}C, 33.2) was used as an internal reference. High-resolution accurate mass determinations were performed by Hort Research Ltd., Palmerston North, N. Z., on a VG70-250S double focusing, magnetic sector mass spectrometer under chemical ionization conditions using isobutane or ammonia as the ionizing gas, or under high-resolution FAB conditions in a glycerol or nitrobenzyl alcohol matrix. Melting points were determined on a Reichert hot stage microscope and are uncorrected. BtPNP was obtained from Sigma (St. Louis, MO) and HsPNP was prepared as a His_6-N-terminal fusion protein as previously published.[1] Protein concentrations were determined by UV absorbance using the extinction coefficients 27550 M^{-1} cm^{-1} and 28830 M^{-1} cm^{-1} at 280 nm for BtPNP and HsPNP,

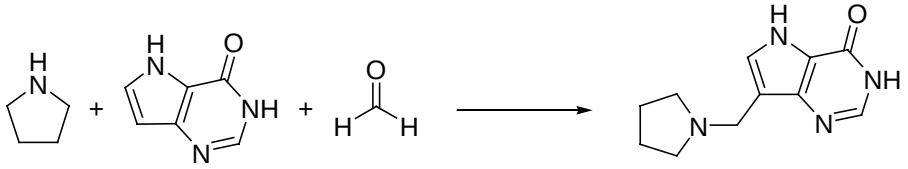
respectively.[1] Inhibitor concentrations were also determined by UV absorbance using the published extinction coefficient of 9540 M^{-1} cm^{-1} at 261 nm.[2] Kinetic parameters used in the determination of inhibition constants were determined independently to be K_M = 34 μM for BtPNP and K_M = 40 μM for HsPNP, which are consistent with published values.[2-4]

7-(pyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (7).



Di-*tert*-butyldicarbonate (2.8 g) was added to a solution of (*R*)-2-pyrrolidinemethanol (1.0 g) in dichloromethane (10 mL). After 1 h, the solution was concentrated and flash chromatography of the residue afforded the *N*-Boc derivative (2.0 g). A solution of this material in pyridine (20 mL) was treated with methanesulfonyl chloride (0.5 mL). After 1 h, chloroform was added and the solution was washed with water (x2), aq HCl, and aq $NaHCO_3$. Rotary evaporation gave a syrup which was dissolved in DMSO (20 mL), potassium cyanide (3.2 g) was added and the mixture was heated at 80 ^oC for 3 h. Chloroform was added and the mixture was washed with water (x2), dried and concentrated to a syrup (1.35 g) of nitrile adduct. This material was converted to 7-(pyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one by the general method described previously.[5] ^1H NMR (D_2O): δ 7.98 (1H, s), 7.62 (1H, s), 4.92 (1H, m), 3.44 (2H, m), 2.54-2.07 (4H, m); ^{13}C NMR (D_2O): δ 155.5, 143.6, 142.9, 128.7, 118.3, 111.0, 55.1, 45.5, 29.9, 23.9. $C_{10}H_{13}N_4O$: HRMS (MH^+): calc.: 205.1089; found: 205.1089.

7-(Pyrrolidin-1-ylmethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (8).



Deazahypoxanthine (3.00 g, 22.5 mmol)[6] was added portionwise to a solution of pyrrolidine (2.5 mL, 30.0 mmol) and formaldehyde (0.50 mL, 49 mmol) in water (10 mL) and heated at 95 °C for 16 h. The reaction was then cooled to room temp. and the resulting precipitate filtered and washed with acetone (25 mL) and diethyl ether (25 mL), recrystallised from 1% water in methanol to afford **8** (3.39 g, 69%) as a white solid. M.P. >300 °C. ^{1}H NMR of the HCl salt (D_2O): δ 8.64 (s, 1H), 7.49 (s, 1H), 4.14 (s, 2H), 3.15 (m, 2H), 2.76 (m, 2H), 1.70 (m, 2H), 1.54 (m, 2H). ^{13}C NMR of the HCl salt (D_2O): δ 152.2, 145.1, 133.1, 131.5, 118.3, 102.8, 53.6, 47.1, 22.7. $C_{11}H_{15}N_4$: HRMS (MH^+): calc.: 219.1246; found: 219.1240. Elemental analysis (C, H, N) was performed by Microana (Wilmington) calc.: C 60.53, H 6.47, N 25.67; found C 60.50, H 6.32, N 25.47.

Determination of K_d values for inhibitors of Table 1.

Inhibitor dissociation constants for the phosphorolysis of inosine were based on initial or equilibrium reaction rate measurements with varied inhibitor concentrations. The reactions were started by adding huPNP or *Bt*PNP to reaction mixtures containing 1 mM inosine in 50 mM $KHPO_4$, with xanthine oxidase at 60 milliunits/ml. Hypoxanthine formed by phosphorolysis of inosine was oxidized to uric acid and monitored spectrophotometrically at 293 nm (extinction coefficient for uric acid ε_{293} = 12.9 mM^{-1} cm^{-}

[1]). For compounds **1** and **2** the reactions were performed at 30 °C and pH 7.7, whereas for compounds **3** – **8** the reactions were performed at 25 °C and pH 7.4. Enzyme concentration was adjusted to give absorbance changes not exceeding 1.0 during the time required to characterize initial and final slow onset inhibition equilibria. The large excess of substrate and continuous product depletion provided extended initial rate conditions. In most cases the inhibitor concentration was >10-fold greater than the enzyme concentration as required for simple analysis of two-state slow onset tight binding inhibition. In the cases of the most tightly bound inhibitors it was not possible to maintain this condition and corrections were made to compensate for the concentration of bound inhibitor. The inhibition constant K_i describes the reversible equilibrium between enzyme and inhibitor for the initial inhibitor binding step. K_i was determined by fitting the initial rates at different inhibitor concentration to the equation for competitive inhibition: $v_i = (k_{cat} \times S)/(K_m(1 + I/K_i) + S)$, where v_i is initial reaction rate, k_{cat} is the catalytic turnover number, K_m is the Michaelis constant, K_i is the dissociation constant of enzyme-inhibitor complex (*E*I), I is inhibitor concentration, and S is substrate concentration. The dissociation constant for the complex formed after slow onset equilibrium (K_i^*) was determined by $v = (k_{cat} \times S)/(K_m(1 + I/K_i^*) + S)$, where v is the steady state reaction rate and the other variables are the same as above. The reported K_d values represent the final equilibrium dissociation constants for each enzyme-inhibitor combination.

Figure 1 ^{1}H-NMR of compound **7**.

PTI-523

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Job No 35580200

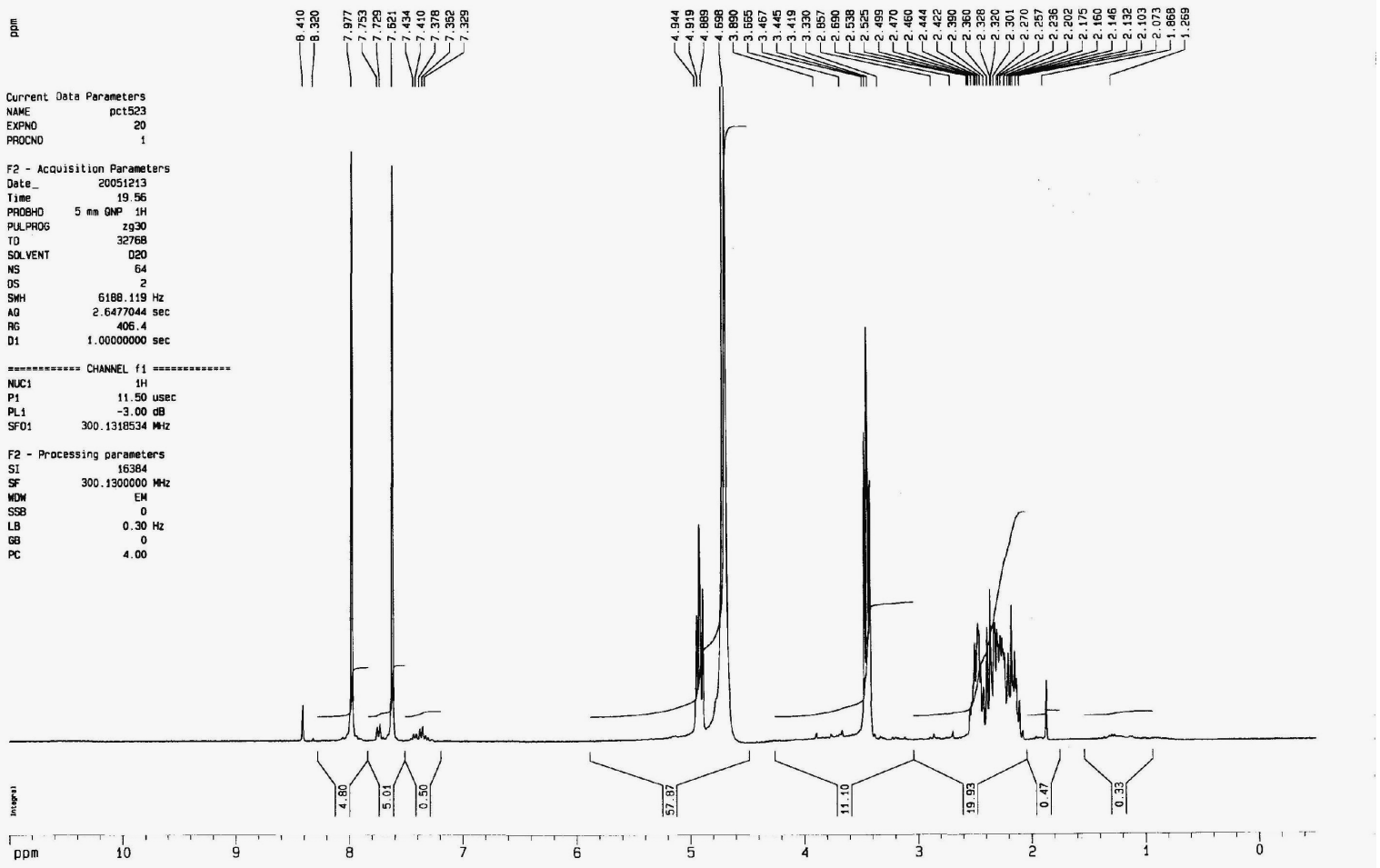
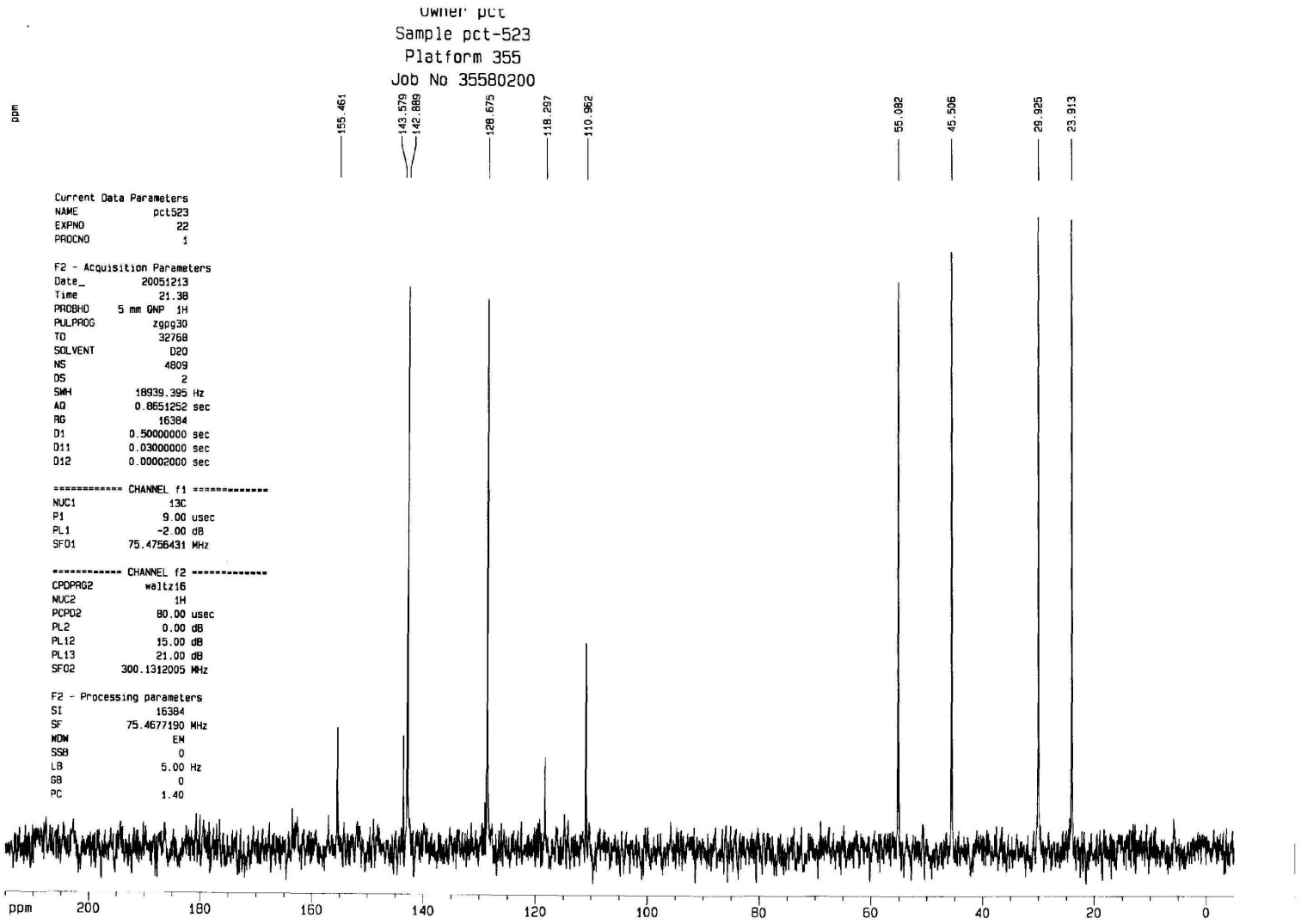


Figure 2 ^{13}C-NMR of compound **7**.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GB 0
PC 1.40
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20
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References

1. Extinction coefficients were determined using the Protparam tool (www.expasy.org/tools/protparam/html).
2. Miles, R. W.; Tyler, P.C.; Furneaux, R. H.; Bagdassarian, C. K.; Schramm, V. L. *Biochemistry*, **1998**, *37*, 8615-8621;
3. Lewandowicz, A., and Schramm, V. L. *Biochemistry*, **2004**, *43*, 1458-1468.
4. Erion, M. D.; Takabayashi, K.; Smith, H. B.; Kessi, J.; Wagner, S.; Honger, S.; Shames, S. L.; Ealick, S. E. *Biochemistry*, **1997**, *36*, 11725-11734.
5. Evans, G.B.; Furneaux, R.H.; Gainsford, G.J.; Schramm, V.L.; Tyler, P.C. *Tetrahedron* **2000**, *56*, 3053-3062.
6. Evans, G.B.; Furneaux, R.H.; Lewandowicz, A., and Schramm, V. L.; Tyler, P.C. *J. Med. Chem.*, **2003**, *46*, 5271-5276.