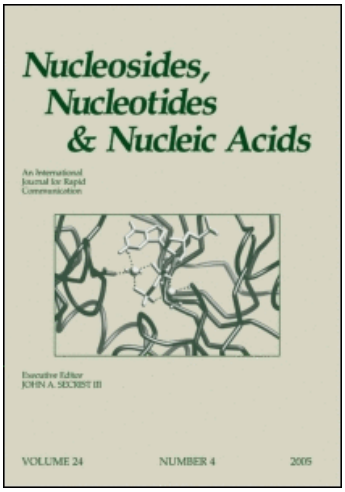

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Synthesis and Biological Activity of a Novel Class of Purine Nucleoside Phosphorylase Inhibitors

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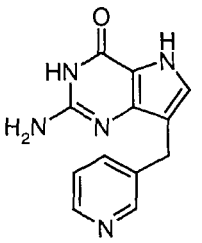
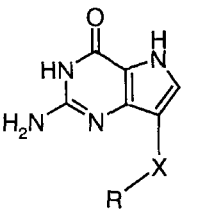
Dedicated to the memory of Gertrude B. Elion

Introduction

Purine nucleoside phosphorylase (PNP, EC. 2.4.2.1) functions as a salvage enzyme in the purine pathway and is important in the T-cell portion of the immune system. As such, PNP is an important therapeutic target for diseases which are T-cell mediated. The biochemical basis for using PNP inhibitors as well as the various classes of inhibitors developed has been recently reviewed.[1] Using X-ray crystallography we have designed and synthesized a series of 7-substituted 2-amino-1,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-ones which are potent PNP inhibitors.[2-5] The 3-(pyridinylmethyl) derivative **1** (BCX-34, peldesine, IC_{50} = 0.036 ± 0.003 μM) is in clinical trials for the treatment of cutaneous T-cell lymphoma, acute lymphoblastic leukemia and HIV infections.



1



2

2a:	X = NH	R = phenyl	**2f:**	X = O	R = cyclohexyl
2b:	X = NH	R = cyclohexyl	**2g:**	X = CH_2	R = phenyl
2c:	X = O	R = phenyl	**2h:**	X = SO_2	R = phenyl
2d:	X = O	R = 3-methylphenyl	**2i:**	X = CO	R = phenyl
2e:	X = S	R = phenyl	**2j:**	X = O	R = 3-pyridinyl

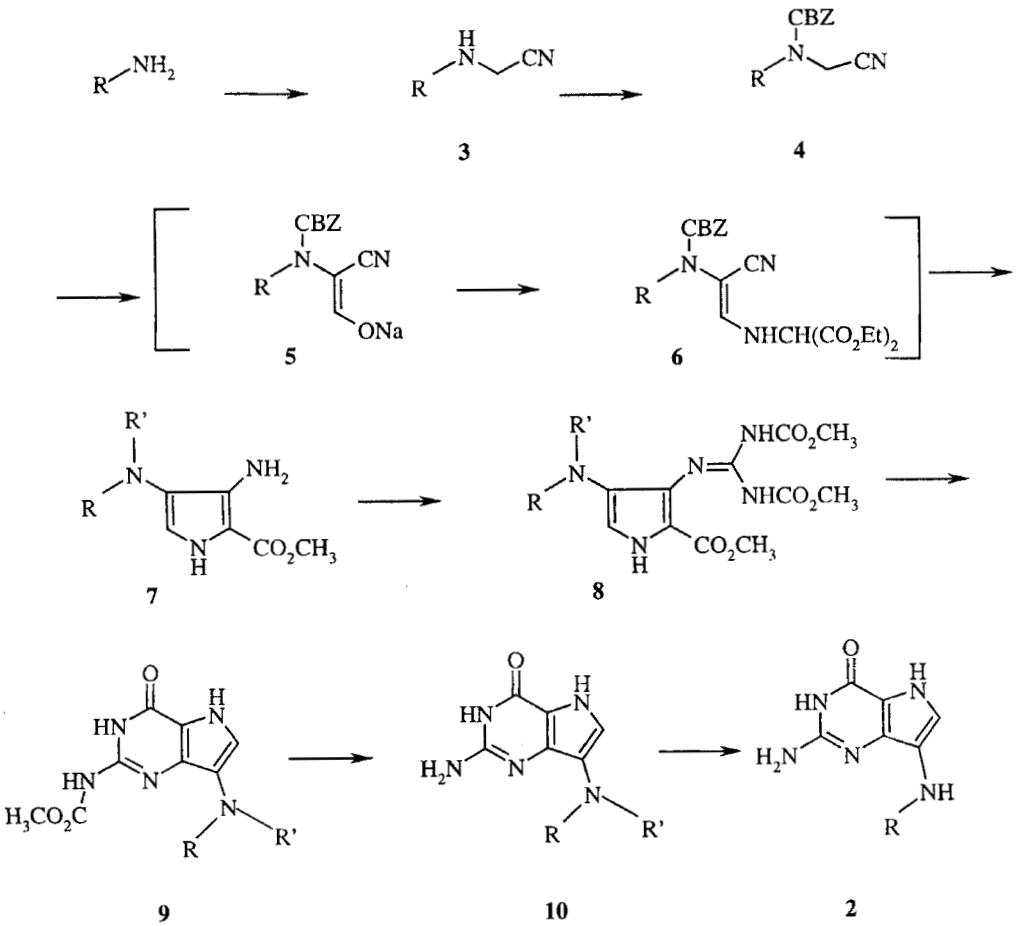
Structurally, BCX-34 is comprised of a 9-deazaguanine ring, which is linked through a methylene group to a 3-pyridinyl ring. Many analogs of BCX-34 have been prepared and tested for their ability to inhibit PNP and from these studies a great deal is known about the binding properties of these inhibitors. Most of the previous synthetic studies were aimed at modifying the heterocycle itself or the substituent attached via the methylene linker. In addition, a class of compounds in which the methylene linker arm is also substituted has been described.[4] In this paper, we wish to describe the synthesis and active site binding properties for a novel class of PNP inhibitors corresponding to the general structure **2** in which a heteroatom has been substituted for the methylene linker.

Results and Discussion

Chemistry

The first compound synthesized in this series was **2a** employing the synthetic pathway given in Scheme 1. Cyanomethylation of aniline with bromoacetonitrile gave **3a** in 43% yield. While this is a modest yield, it is an improvement over the reported yield of 22% with the less reactive chloroacetonitrile.[6] Protection of the *NH* of 3 with benzyloxycarbonyl (CBZ) chloride afforded **4a** which underwent formylation (NaH/ethyl formate) to give the stable sodium enolate **5a** which was conveniently isolated by precipitation with hexane. We were surprised to find that this formylation reaction was very facile and vigorous compared to its carbon analog.[2] Enolate **5a** was condensed with diethyl aminomalonate to give enamine **6a** which cyclized with NaOMe/MeOH to the pyrrole **7a**. Unexpectedly, during this cyclization, the CBZ group was exchanged with an ethyoxycarbonyl group. Presumably, this carbamate exchange is intramolecular and derived from the diethyl group of the enamine since it occurs in the presence of MeOH as solvent containing NaOMe. Guanylation of **7a** was accomplished with 1,3-dimethoxycarbonyl-2-methyl-2-thiopseudourea[7] under mild acid catalysis to give the adduct **8a**. Treatment of **8a** with NaOMe gave the annulated product **9a**. Reaction of **9a** with NaOH selectively cleaved the 2-amino carbamate group affording **10a**. Suprisingly, the 9-amino carbamate group remained intact, even under forcing conditions. However, final deprotection was accomplished by refluxing **10a** with hydrazine hydrate and Pearlman's catalyst for several days giving **2a** which was conveniently isolated as the hydrochloride.

The cylcohexylamino analog **2b** was synthesized using a similar reaction sequence. Despite the similarity of the schemes, dramatic differences were observed in the chemistry. Condensation of commercially available cyclohexylamine with chloroacetonitrile in benzene gave **3b** in 80% isolated yield which was protected as the CBZ derivative **4b**. Formylation under the same conditions as for **4a** gave the enolate salt **5b** with an assumed quantitative yield. Conversion of the enolate to the enamine **6b** followed by cyclization with NaOMe afforded the pyrrole **7b** in 18% overall yield from the nitrile **4b**. Unlike the cyclization of **6a** to **7a** no carbamate exchange was observed. Attempted guanylation of **7b** using the same reaction conditions for the conversion of **7a** to **8a** was unsuccessful. However, generating the highly reactive carbodiimide[8,9] derived from 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea and $HgCl_2$ gave **8b**, albeit in poor yield (15%). Treatment of **8b** with NaOMe in MeOH gave the ring annulated product as anticipated, however, unexpected cleavage of the carbamate protecting group to the free 2-NH_2

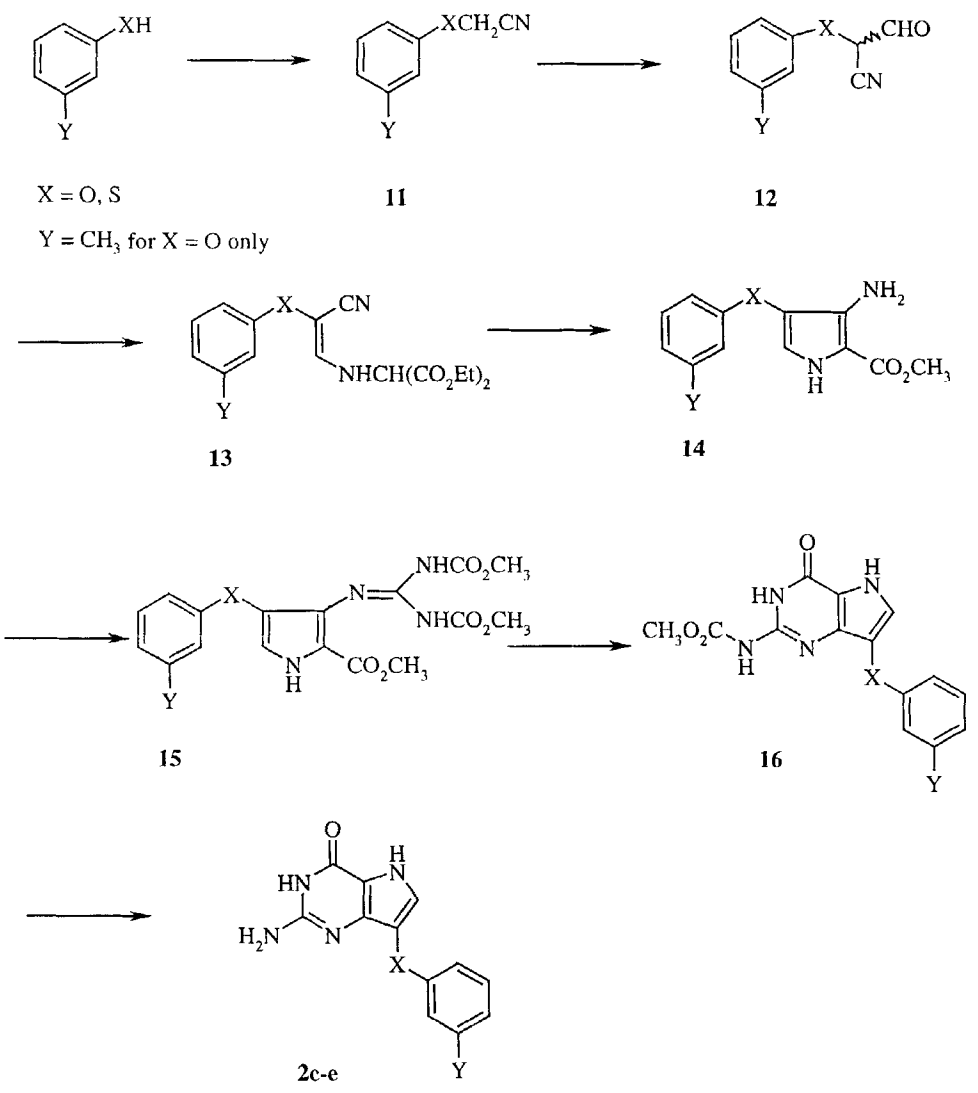


a: R = Phenyl, R' = CO_2Et
b: R = Cyclohexyl, R' = CBZ

Scheme 1.

group giving **10b** directly also occurred. Catalytic hydrogenolysis of the CBZ group afforded **2b**, which was isolated as the maleate since the base is unstable.

In addition to *N* analogs, *O* and *S* examples were also prepared as described in Scheme 2. Phenol was converted to its potassium salt and condensed with chloroacetonitrile in DMF to give **11c**. The reaction of **11c** with NaH/ethyl formate gave an enolate that did not precipitate from the reaction mixture (even on adding hexane) unlike the two *N* analogs above. In this case, the reaction mixture was concentrated and the residue suspended in water. The pH was adjusted to *ca.* 3 with HOAc and extracted to give the free aldehyde **12c** in essentially quantitative yield. Using our standard conditions, **12c** was converted to the corresponding enamine **13c** which was cyclized with NaOMe to the pyrrole **14c**. Guanylation with 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea/HOAc gave **15c**, which was cyclized to **16c**. Final deprotection with aq. NaOH gave **2c**. We had previously shown that **2g** could be reduced



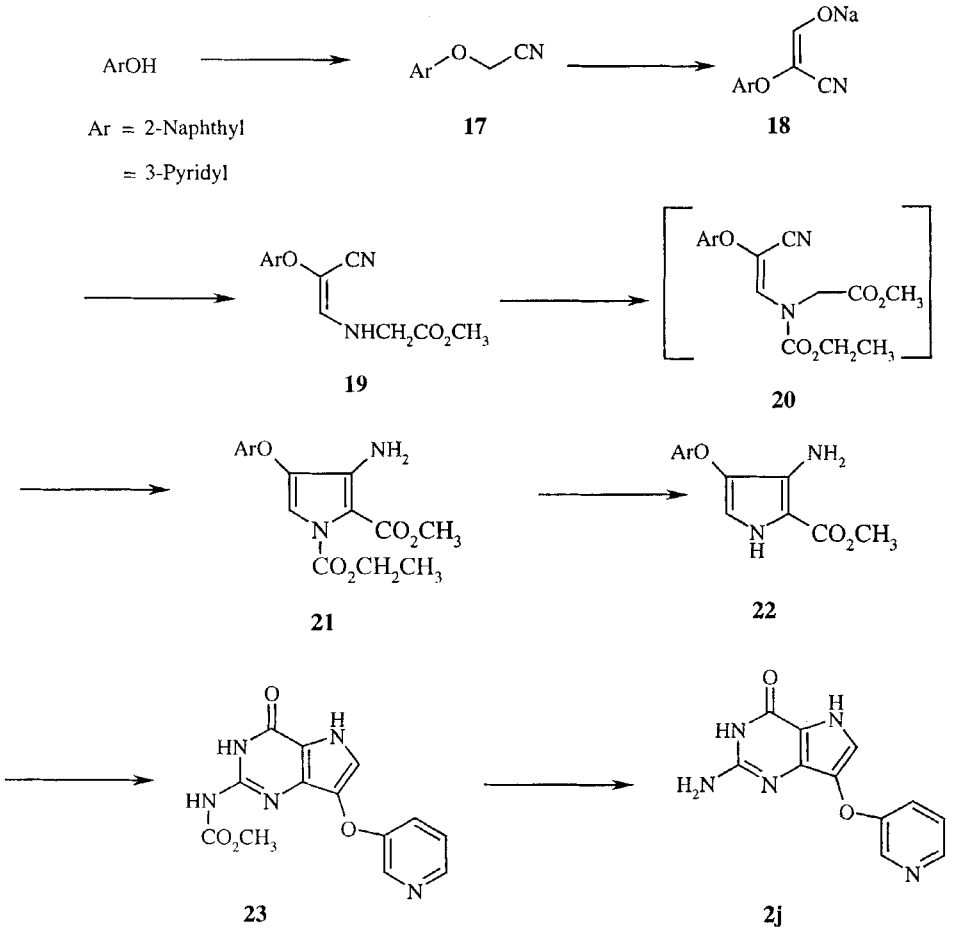
c: X = O, Y = H
d: X = O, Y = CH_3
e: X = S, Y = H

Scheme 2.

catalytically to the cyclohexyl analog in TFA with PtO_2.[10] Using these conditions, **2c** was similarly reduced to **2f** in 27% yield. The poor yield was due at least in part to the cleavage of the cyclohexyl group completely to give the highly unstable 9-*OH* compound (tentative identification made by MS). Using the same chemistry as for phenol, 3-methylphenol was converted to the corresponding protected guanidino pyrrole **15d**. Treatment of **15d** with NaOH in MeOH afforded the desired product **2d** in a single step in 28% yield. Likewise, thiophenol was converted to **15e** and, in a single step converted to **2e** (78% from **15e**). The sulfone analog **2h** was prepared by H_2O_2 oxidation of **2e** in HOAc. Attempts to

prepare the sulfoxide analog by oxidation with IO_4^- gave a mixture of the sulfone and the sulfoxide, which could not be separated. We were able to prepare the *CO* analog **2i**, however, by oxidation of **2g** with CrO_3/HOAc.

During the course of our study, we wanted to prepare a heteroatom analog of BCX-34. For ease of synthesis, we chose the 3-pyridinyloxy derivative **2j,** which we envisioned could be prepared using the same scheme, which was successful for phenol. Thus 3-hydroxypyridine was converted to **17j** which was formylated under standard conditions (Scheme 3). In this case, the sodium enolate was much less soluble than the unsubstituted phenol and precipitated from the reaction mixture to give **18j**. We envisioned that



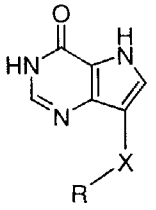
j: Ar = 3-Pyridinyl
k: Ar = 2-Naphthyl

Scheme 3.

the pyrrole **22j** could be prepared using our standard diethyl aminomalonate chemistry to give the enamine followed by cyclization to the pyrrole. Indeed, **18j** did form the corresponding enamine, but all attempts to cyclize the enamine to the pyrrole using NaOMe/MeOH failed; dark intractable mixtures with

multiple spots by TLC being obtained. We were forced to abandon this technique in favor of our previously published method[2] of cyclization utilizing ethyl glycinate to form the enamine and DBN as the base.[11-14] Thus **18j** was condensed with ethyl glycinate to form enamine **19j**. Prior to cyclization, it was necessary to protect the *NH* of the enamine with ethyl chloroformate/DBN[14] to give **20j** which was then cyclized *in situ* with additional DBN to the protected pyrrole **21j** in quantitative yield. The pyrrole *N* was deprotected by treatment with $NaHCO_3$ to give **22j**. Condensation of **22j** with 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea/HOAc at ambient temperature failed and when heated to 50 °C for 72 h directly gave the protected 9-deazaguanine **23j**. Deprotection with NaOH gave **2j**. Following this same scheme, 2-naphthol was converted to **17k** and then to the enolate **18k**. Enamine formation with ethyl glycinate/DBN gave the protected pyrrole **21k** directly. No evidence (TLC) for the formation of the protected enamine prior to cyclization was noted suggesting that it was rapidly cyclized to **21k**. Deprotection of the pyrrole *N* with $NaHCO_3$ gave **22k**. We were unable to condense **22k** with 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea under AcOH catalysis with heat (reflux for 72 h) or through the carbodiimide ($HgCl_2$) at ambient temperature or with heat.

Hypoxanthines **24** - **31** were synthesized by condensing the appropriate pyrrole with 3 eq of formamidine acetate in refluxing EtOH.[13] Hypoxanthine **27** was obtained by the H_2O_2/HOAc oxidation of **26**. The successful condensation of **22k** with formamidine acetate to form hypoxanthine **30** is noteworthy since **22k** failed to condense in a similar manner with the guanylating reagent 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea to provide the 9-deazaguanine.



24: X = NH R = phenyl
25: X = O R = phenyl
26: X = S R = phenyl
27: X = SO_2 R = phenyl
28: X = O R = 3-pyridinyl,
29: X = O R = 3-Me-phenyl
30: X = O R = 2-naphthyl
31: R = NH R = cyclohexyl

Crystallography and Biology

The PNP active site has been described previously and is comprised of three sub-sites; namely the purine binding site, the sugar binding site and the phosphate binding site.[15] Of these three sites, the purine binding pocket is the most deeply buried and, in human PNP, is specific for 6-oxopurines. This specificity for 6-oxopurines is driven in part by hydrogen bond formation from *N*-1, 2-NH_2, *O*-6 and *N*-7 with the residues Glu-201, Lys-244 and Asn-243. The other residues that make up the purine binding site are Ala-116, Phe-200, Val-217, Met-219, Gly-218 and Thr-242 and are largely hydrophobic in nature. These interactions are clearly shown in Figure 1, which shows **2g**,[2] bound to the active site of human erythrocytic PNP and this map served as the starting point for our study.

The phosphate binding site is composed of Ser-33, Arg-84, His-86 and Ser-220 and is located near the glycine-rich loop (residues 32-37). In addition, a H_2O molecule (313) is located in this site and, in concert with the hydroxyls of Ser-33 and Ser-220, accepts hydrogen bonds from the phosphate. Sulfate

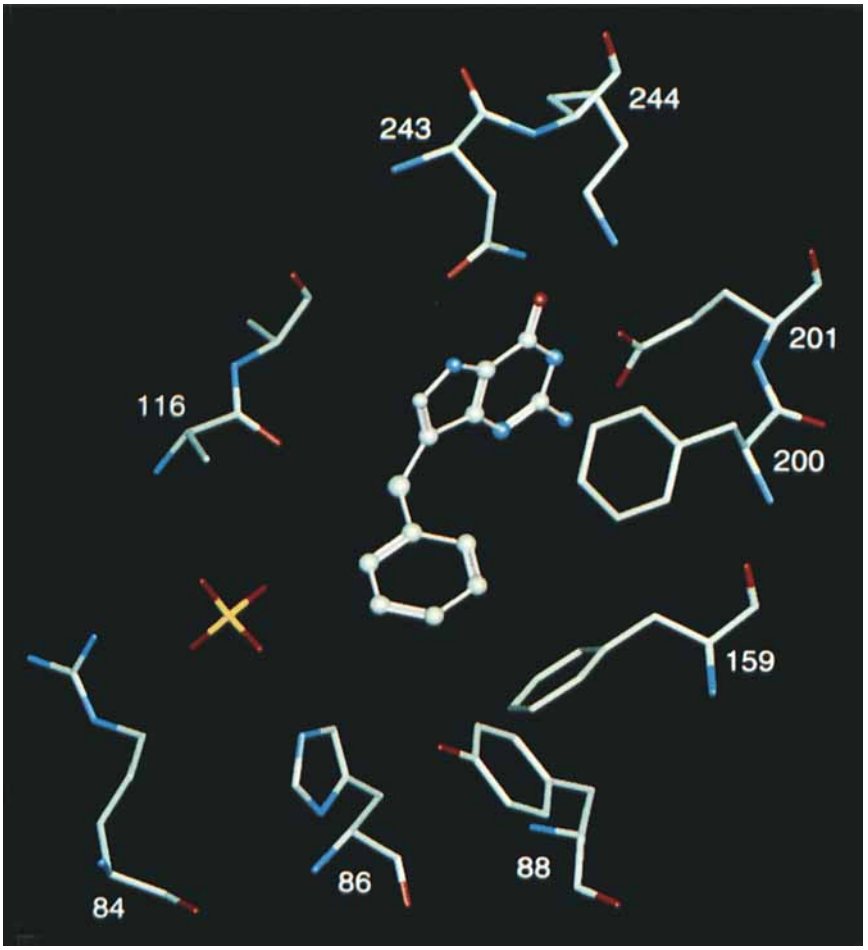


Figure 1. Compound **2g** bound in the active site of PNP as determined by x-ray crystallographic analysis. The purine ring binds mostly through hydrogen bonds to Asn-243, Lys-244, and Glu-201. The hydrophobic phenyl ring optimizes its interaction with Phe-159 and Phe-200 by adopting a "herringbone" arrangement. Sulfate anion which occupies the phosphate binding site is shown in yellow and is from the buffer.

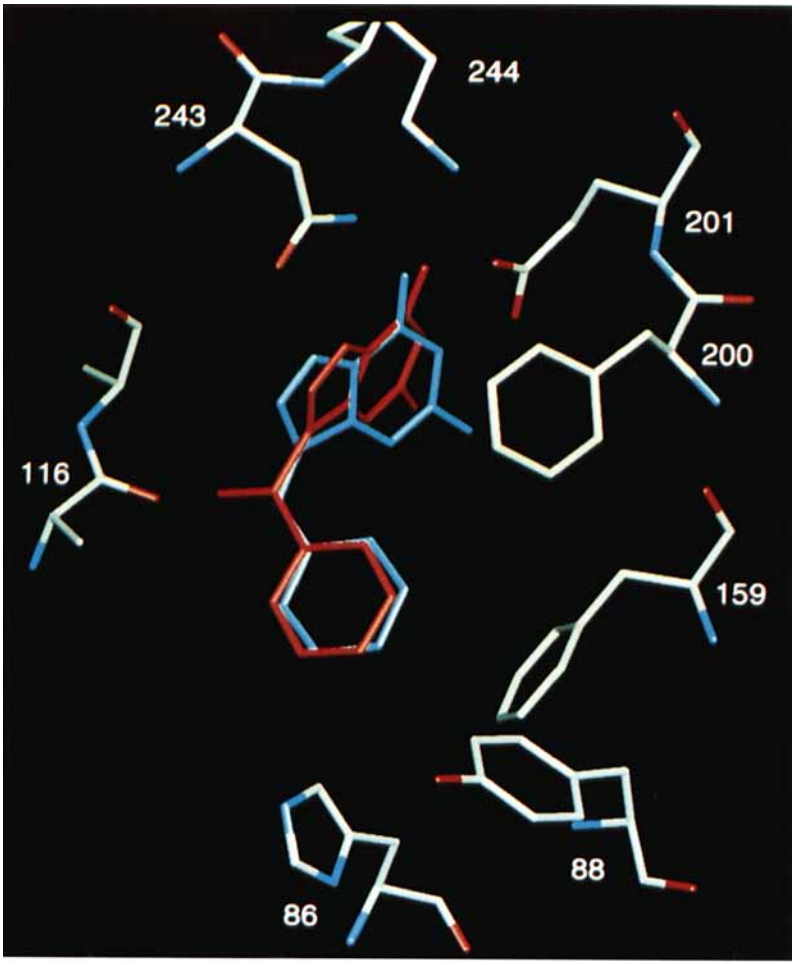


Figure 2. Comparison of the binding of the most potent compound prepared in this series **2a** (blue) and the least potent compound **2i** (red) in the active site of PNP as determined by x-ray crystallographic analysis. Hydrogen bond formation between the NH of **2a** and the CO of Ala-116 improved the activity of **2a** over its CH2 analog **2g**. Steric and electronic repulsion of the CO of **2i** and the CO of Ala-116 resulted in weak binding.

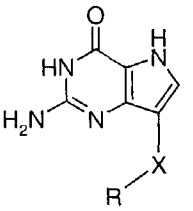
anion (an artifact of the soak study which was carried out in $(NH_4)_2SO_4$) can also occupy this site as shown yellow and red in Figure 1.

The sugar binding site is largely hydrophobic in nature; however, Tyr-88 contains a hydrogen-bonding group which interacts with *O*-3 of β-D-ribofuranose of the natural substrates. Additional hydrogen bonds are formed from the peptide *N* atom of Met-219 and the NH_2 of His-86 with the other ribofuranose hydroxyls. One side of the ribose binding site is composed of several aromatic amino acids, including Phe-159 (from an adjacent subunit), Phe-200, His-86 and Tyr-88. This hydrophobic pocket serves to orient the sugar to facilitate nucleophilic attack by phosphate and subsequent inversion at the anomeric center. The hydrophobic residues in the active site allow the sugar to be replaced with a number of aryl and alicyclic groups that are optimally attached to the purine ring through a single methylene linker group. These aromatic groups form particularly strong interactions due to the formation of the classic herringbone (i.e. edge-to-face) arrangement[16] with the phenyl rings of Phe-159 and Phe-200 in the active site and is clearly shown (Figure 1) with the phenyl group of **2g**.

Our molecular modeling studies suggested that replacing the methylene carbon with a hydrogen bond donor group such as *NH* would enhance binding through an additional interaction between the *NH* and the carbonyl of Ala-116, an assumption now supported by experimental results. In our assay, **2a** has an IC_{50} = 0.012 μM (Table 1) and is about 4-fold more potent than its CH_2 analog **2g** with an IC_{50} = 0.042 μM. The amine **2a** was also soaked into the active site and the result showing the inhibitor/PNP complex is shown in Figure 2 (**2a** in blue). The geometry and distance between the *NH* and carbonyl of Ala-116 are also consistent with hydrogen bond formation and is responsible for the increased activity of **2a**. The cyclohexylamino derivative **2b** (IC_{50} = 0.099 μM) was equi-potent with **2g,** but was 8-fold less potent than the phenyl analog **2a**. Substitution with *O* (**2c**, IC_{50} = 0.052 μM) gave a compound that was equi-potent with **2g**, an observation that was not unexpected since we had not identified any additional interactions. Replacement of the substituent attached to the *O* atom to cyclohexyl (**2f**), 3-methylphenyl (**2d**) or 3-pyridinyl (**2j**) also did not affect activity. No difference in activity was observed for the *S* analog **2e** (IC_{50} = 0.030 μM) when compared to **2g**. The soak study of **2e** shows that it binds in exactly the same manner as for **2g** and like the *O* analogs, it showed no additional interactions. However, the sulfonyl derivative **2h** was 5-fold less active than **2e**. The activity of **2h** can be rationalized from the results of the soak study that clearly shows that *O* atom of the Ala-116 carbonyl points in between the two *O* atoms of the SO_2 group to relieve both steric and electronic crowding.

The poorest inhibitor prepared in this series was **2i** with and IC_{50} = 0.876 μM which is 16-fold less potent than **2g**. Again, cystallographic analysis provides the explanation (Figure 2, **2i** in red). In this example, the *O* atom of *CO* and the carbonyl of Ala-116 are clearly oriented towards one another, which diminishes its binding capacity. Unlike the case of the SO_2 analog **2h**, the sp^2 hybridized *C* atom is rigid and is unable to relieve the unfavorable situation without a concomitant detrimental movement of the 9-deazaguanine and phenyl ring.

Table 1. IC_{50} Against Human Erythrocytic PNP



2

Compound Number	R	X	IC_{50} (μM)[1]
2a	phenyl	NH	0.012 ± 0.003
2b	cyclohexyl	NH	0.099 ± 0.006
2c	phenyl	O	0.052 ± 0.008
2d	3-Me-phenyl	O	0.046 ± 0.004
2e	phenyl	S	0.030 ± 0.003
2f	cyclohexyl	O	0.040 ± 0.005
2g	phenyl	CH_2	0.055 ± 0.002
2h	phenyl	SO_2	0.160 ± 0.034
2i	phenyl	CO	0.876 ± 0.096
2j	3-pyridinyl	O	0.086 ± 0.009

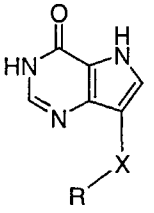
1. Mean ± SEM, n = 3.

Hypoxanthine analogs also bind in the PNP active site, but are relatively poor inhibitors when compared to their 2-NH_2 derivatives. The hypoxanthine analogs lack the 2-NH_2 group which plays an important role in binding the ring in the active site with a hydrogen bond to Glu-201. As a result, hypoxanthines are generally 5-10 times less potent than the corresponding 2-NH_2 compounds when assayed against the human erythrocytic PNP enzyme. The IC_{50}'s were measured for the hypoxanthines and are given in Table 2. Soak studies were not done with the hypoxanthines, since they were expected to bind similarly to the 9-deazagaunines.

Experimental

Thin layer chromatography was performed on Kieselgel aluminum backed silica gel 60 F_{254} plates (0.2 mm) obtained from E. Merck unless otherwise noted and were visualized using an ultraviolet light

Table 2. IC_{50} Against Human Erythrocytic PNP



24 - 31

Compound Number	R	X	IC_{50} (μM)[1]	Ratio[2]
24	phenyl	NH	0.083 ± 0.021	7
25	phenyl	O	0.450 ± 0.080	9
26	phenyl	S	0.113 ± 0.009	4
27	phenyl	SO_2	0.623 ± 0.092	4
28	3-pyridinyl	O	0.980 ± 0.017	11
29	3-Me-phenyl	O	0.500 ± 0.047	11
30	2-naphthyl	O	>250	
31	cyclohexyl	NH	2.0 ± 0.55	20

1. Mean ± SEM, n = 3
2. Ratio of the IC_{50} of the hypoxanthine/IC_{50} of the corresponding 9-deazaguanine

(254 nm) and I_2. For column chromatography, samples were typically loaded onto a column containing a 20-30:1 ratio of silica gel to compound. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. All ^1H NMR spectra were recorded with a Bruker AM spectrometer at either 360 or 400 MHz unless noted otherwise. Resonances are reported downfield from internal tetramethylsilane and spectra were recorded in the solvent indicated. Infrared spectra were recorded at on a Bio-Rad FTS-7 spectrophotometer and mass spectra were recorded using a Fisions VG Trio 2000 mass spectrometer operating in the positive electrospray mode. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. For PNP assays, purines, nucleosides, nucleotides, and human red blood cell (RBC) PNP enzymes were obtained from Sigma Co. (St. Louis, MO). [8-^{14}C]-Inosine was obtained from Moravek Chemicals, Brea California.

2-Amino-1,5-dihydro-7-(phenylamino)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2a). A sample of **8a** (50 g, 0.1 mol) was dissolved in MeOH (500 mL) and cooled to 4 °C in an ice water bath. To the

chilled solution was added 25% NaOMe in MeOH (137 mL, 0.6 mol) dropwise over 30 min after which time the solution was stirred for 2 h at ambient temperature. The solution was cooled in an ice water bath and carefully neutralized with glacial HOAc during which time a thick, white precipitate formed. The solid was collected by vacuum filtration and air dried to give 32.4 g (0.087 mol. 87%) of the cyclized fully-protected deazaguanine **9a** which was used directly in the next step.

The cyclized deazaguanine **9a** (30 g, 0.08 mol) was suspended in water to which was added sodium hydroxide (19.2 g, 0.48 mol). The mixture was then placed in a pre-heated oil bath at 50 °C for 1 h during which time the solution became homogeneous. Thin-layer chromatographic analysis (SiO_2, $CHCl_3$-CH_3OH-NH_4OH, 80:18:2) indicated complete conversion of **9a** to the monoprotected 2-amino deazaguanine **10a**. The mixture was cooled in an ice water bath and neutralized by the careful addition of glacial HOAc. The thick precipitate which formed was collected by vacuum filtration and air dried to give 24.2 g (0.077 mol. 97%) of **10a** which was used directly in the next step.

Compound **10a** (22 g 0.07 mol) was dissolved in absolute ethanol (300 mL) containing hydrazine hydrate (20 mL) and Pearlman's catalyst [$Pd(OH)_2$, 0.5 g] and the mixture was heated at reflux. After refluxing for two days, the catalyst was removed by filtration and replaced with an equal volume of fresh catalyst. Reflux was continued for an additional three days, additional hydrazine hydrate (10 mL) added, and the reaction heated at reflux for one additional day. Thin-layer chromatographic analysis (cf. system above) indicated complete conversion of the starting material to the completely deprotected material. The solution was filtered hot through Celite and concentrated to give a residue, which was purified by passing through a small pad of silica gel (5% MeOH-EtOAc). The appropriate fractions were concentrated to give 12.8 g (0.053 mol. 75.8%) of **2a** as an olive-colored powder. The material was converted to the hydrochloride, in MeOH with ethereal-HCl and recrystallized from MeOH to give tan needles; mp > 275 °C. IR (KBr) 3400, 1641 cm^{-1}; MS (*m/z*, ES+) 242.4 (100%); ^1H NMR (DMSO-d_6) δ 11.29 (bs, D_2O exchangeable, 1H), 11.28 (bs, D_2O exchangeable, 1H), 7.07 (d, J = 3.0 Hz, 1H), 7.03 (t, J = 7.3 Hz, 2H), 6.97 (bs, D_2O exchangeable, 1H), 6.60 (d, J = 7.6 Hz, 2H), 6.51 (t, J = 7.3 Hz, 1H), 5.80 (bs, D_2O exchangeable, 2H). Anal. Calcd for $C_{12}H_{11}N_5O \cdot HCl$ 0.5 CH_3OH: C, 51.11; H, 4.80; N, 23.84. Found: C, 50.94 H, 4.85; N, 23.74

2-Amino-1,5-dihydro-7-[(*N*-cyclohexyl)amino]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2b). The protected guanidine **8b** (2.2 g, 4.2 mmol) was dissolved in MeOH (50 mL) containing NaOMe (0.2 g, 4 mmol) and heated at reflux for 8 h. The volatiles were removed *in vacuo*, the residue taken up in water (50 mL), and the pH adjusted to 6 (pH paper) with HOAc. The tan precipitate was collected by filtration which was air dried to give **10b** which was used directly in the next step with no additional purification.

The CBZ-protected amine **10b** was dissolved in MeOH (250 mL), and hydrogenated (50 psig) with 10% Pd-C (250 mg) for 18 h. Maleic acid (0.6 g, 5 mmol) was added and the catalyst removed by filtration through Celite and the filtrate concentrated to dryness. The residue was triturated using EtOAc, collected by filtration and dried *in vacuo* (acetone reflux) to give **2b** as a tan solid, mp 178-180 °C. IR

(KBr) 3141, 2943 and 1691 cm^{-1}; MS (*m/z*, ES+) 248.5 (100%); ^1H NMR (DMSO-d_6) δ 11.99 (s, D_2O exchangeable, 1H), 10.75 (s, D_2O exchangeable, 1H), 7.23 (s, 1H), 6.22 (s, D_2O exchangeable, 2H), 6.13 (s, 3H), 3.36 (bm, 1H), 2.49 (m, 3H), 1.97 – 1.12 (bm, 9H). Anal Calcd for $C_{12}H_{17}N_5O \cdot 2\ C_4H_4O_4 \cdot 0.25\ H_2O$: C, 49.63; H, 5.31; N, 14.47. Found: C, 49.52; H, 5.32; N, 14.47.

2-Amino-1,5-dihydro-7-(phenyloxy)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2c). A sample of **15c** (0.64 g, 1.65 mmol) was suspended in MeOH (10 mL) and 25% NaOMe (2.25 mL, 9.90 mmol). The suspension was stirred 3 h at room temperature and then neutralized with HOAc. The resulting precipitate was collected by filtration and then washed thoroughly with MeOH and then water affording **16c** which was used with no further purification in the next step.

The solid from above was dissolved in water (7.83 mL) containing NaOH (0.39 g, 9.9 mmol) and the mixture heated at 50 °C overnight. The reaction was then neutralized with HOAc. The resulting precipitate was collected by filtration and then washed thoroughly with water. The pale yellow solid was recrystallized from ethanol, collected by filtration and dried *in vacuo* at toluene reflux to give 0.12 g (30%) of **2c**, mp 335 °C (dec). IR (KBr) 3419, 3330, 3192 and 3135 cm^{-1}; MS (*m / z*, ES+) 243.3 (100%); ^1H NMR (DMSO-d_6) δ 11.45 (s, D_2O exchangeable, 1H), 10.49 (s, D_2O exchangeable, 1H), 7.25 (t, J = 7.4 Hz, 2H), 7.15 (d, J = 3.2 Hz, 1H), 6.94 (t, J = 7.4 Hz, 1H), 6.87 (d, J = 7.8 Hz, 2H), 5.88 (bs, D_2O exchangeable, 2H). Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.31; H, 4.22; N, 22.98.

2-Amino-1,5-dihydro-7-[(3-methyl)phenyloxy]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2d). A sample of **15d** (3.76 g, 9.31 mmol) was dissolved in MeOH (85 mL) containing sodium hydroxide (1.86 g, 46.53 mmol) and the mixture heated at reflux overnight. The reaction was cooled and then neutralized with glacial HOAc. The resulting solution was cooled and then both hexane (75 mL) and EtOAc (enough to dissolve the two layers) were added to precipitate the product. The solution was cooled for 1 h and the precipitate collected by filtration and washed thoroughly with water. The solid was dried *in vacuo* at 110 °C for 24 h to give 1.0 g (28%) of **2d**, mp 275.8-276.8 °C (dec). IR (KBr) 3408, 3170, 1674, 1653 and 1627 cm^{-1}; MS (*m / z*, ES+) 257.3 (100%); ^1H NMR (DMSO-d_6) δ 11.44 (bs, D_2O exchangeable, 1H), 10.47 (D_2O exchangeable, 1H), 7.14 (m, 2H), 6.77 (d, J = 6.8 Hz, 1H), 6.68 (t, J = 8.7 Hz, 2H), 5.90 (bs, D_2O exchangeable, 2H), 2.24 (s, 3H). Anal. Calculated for $C_{13}H_{12}N_4O_2$: C. 60.93; H, 4.72; N. 21.86. Found: C, 60.66; H, 4.74; N, 21.64.

2-Amino-1,5-dihydro-7-(phenylthio)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2e). A sample of **15e** (4.0 g, 9.85 mmol) was dissolved in MeOH (100 mL) containing NaOH (1.97 g, 49.26 mmol) and the mixture heated at reflux overnight. The reaction was cooled and then neutralized with glacial HOAc. The resulting precipitate was collected by filtration and washed thoroughly with water and then MeOH. The solid was dried *in vacuo* at toluene reflux for 8 h to give 1.98 g (78%) of **2e**, mp 293.5-294.5 °C (dec). IR (KBr) 3475, 3289, 3143 and 1646 cm^{-1}; MS (*m / z*, ES+) 259.3 (100%); ^1H NMR (DMSO-d_6) δ 12.06 (bs, D_2O exchangeable, 1H), 10.59 (bs, D_2O exchangeable, 1H), 7.45 (s, 1H), 7.20 (t, J = 7.8 Hz, 2H), 7.04

(m, 3H), 6.06 (s, D_2O exchangeable, 2H). Anal. Calcd for $C_{12}H_{10}N_4OS \cdot 0.75\ H_2O$: C, 53.04; H, 4.24; N, 20.63. Found C, 52.91; H, 4.30; N, 20.66.

2-Amino-7-(cyclohexyloxy)-1,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2f). A sample of **2c** (0.72g, 3 mmol) was dissolved in TFA (25 mL) containing PtO_2 (0.2 g). The mixture was hydrogenated at ambient temperature for 18 h, diluted with ethanol and filtered through Celite. The filtrate was concentrated to give **2f** contaminated with significant amounts of the 9-hydroxy product. Purification by column chromatography (SiO_2, $CHCl_3$-CH_3OH-NH_4OH 80:18:2) gave **2f** (0.2 g, 27%) as an off-white powder, mp >200 °C. IR (KBr) 3347, 3183, 1681 and 1657 cm^{-1}; MS (*m / z*, ES+) 249.4 (100%); ^1H NMR (DMSO-d_6) δ 10.91 (bs, D_2O exchangeable, 1H), 10.36 (bs, D_2O exchangeable, 1H), 6.83 (s, 1H), 5.79 (bs, D_2O exchangeable, 2H), 4.08 (m, 1H), 2.00 – 1.00 (bm, 10H). Anal. Calcd for $C_{12}H_{16}N_4O_2$: C, 58.05; H, 6.49; N, 22.56. Found: C, 58.13: H, 6.53; N, 22.63.

2-Amino-1,5-dihydro-7-(phenylsulfonyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2h). A sample of **2e** (0.55 g, 2.1 mmol) was suspended in HOAc (2.5 mL) to which a 30% H_2O_2 solution (1.20 mL, 10.7 mmol) was added and the mixture heated at reflux for 75 min. After cooling, the precipitate was collected by filtration and washed thoroughly with water to give 0.30 g (48%) of a white solid. An analytical sample was prepared by recrystallizing from MeOH. The solid was collected by filtration and then dried *in vacuo* at toluene reflux to give **2h**, as a white solid, mp 346-348 °C. IR (KBr) 3475, 3122, 2992 and 1654 cm^{-1}; MS (*m / z*, ES+) 291.3 (100%); ^1H NMR (DMSO-d_6) δ 12.60 (bs, D_2O exchangeable, 1H), 10.75 (bs, D_2O exchangeable, 1H), 8.05 (m, 2H), 7.71 (d, *J* = 3.7 Hz, 1H), 7.60 (m, 3H), 6.25 (bs, D_2O exchangeable, 2H). Anal. Calcd for $C_{12}H_{10}N_4O_3S$: C, 49.65; H, 3.47; N, 19.29. Found: C, 49.55; H, 3.48; N, 19.20.

2-Amino-1,5-dihydro-7-(phenylcarbonyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2i). A sample of **2g** (1.2 g, 5 mmol) was dissolved with heating in HOAc (125 mL) and allowed to cool to ambient temperature. CrO_3 (1.5 g, 15 mmol) was added portion-wise over 1 h after which time the mixture was allowed to stand overnight. During this time, a bright yellow precipitate formed which was collected by vacuum filtration. TLC (SiO_2, $CHCl_3$-CH_3OH-NH_4OH 80:18:2) indicated an equal mixture of starting material and the desired ketone, which ran at a slightly lower R_f. The ketone was isolated by column chromatography (same system as for TLC) to give 0.31 g (24%) of **2i**, which was recrystallized from HOAc to give **2i** as a yellow powder, mp > 250 °C. IR (KBr) 3269, 1680 and 1623 cm^{-1}; MS (*m / z*, ES+) 255.0 (100%); ^1H NMR (DMSO-d_6) δ 12.32 (bs, D_2O exchangeable, 1H), 10.69 (bs, D_2O exchangeable, 1H), 7.79 (d, *J* = 7.3 Hz, 2H), 7.65-7.3 (m, 3H), 6.12 (bs, D_2O exchangeable, 3H). Anal. Calcd for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.03. Found: C, 61.14: H, 4.29; N, 21.81.

2-Amino-1,5-dihydro-7-(3-pyridinyloxy)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2j). A sample of **23j** (0.58 g, 1.93 mmol) was dissolved in a mixture of MeOH (18 mL) and water (1 mL) containing sodium hydroxide (0.39 g, 9.63 mmol) and heated at reflux overnight. The reaction was cooled and then neutralized with glacial HOAc. The solution was cooled for 1 h and the precipitate collected by filtration and washed thoroughly with water. The solid was dried *in vacuo* at 110 °C for 24 h to give 0.22 g (47%)

of **2j** as a white solid, mp 308-309 °C (dec). IR (KBr) 3463, 3344 and 1629^{-1}; MS (*m / z*, ES+) 244.3%; ^{1}H NMR (DMSO-d_6) δ 11.55 (bs, D_2O exchange, 1H), 10.51 (bs, D_2O exchange, 1H), 8.31 (s, 1H), 8.20 (s, 1H), 7.31 (m, 1H), 7.25 (s, 2H), 5.94 (s, D_2O exchangeable, 2H). Anal. Calcd for $C_{11}H_9N_5O_2 \cdot 0.4$ H_2O: C, 52.76; H, 3.94; N, 27.97. Found: C, 52.69; H, 4.03; N, 27.89.

***N*-(Cyanomethyl)phenylamine (3a).** Aniline (47.1 g, 0.5 mol) was dissolved in ethanol (300 mL) containing triethylamine (70.2 mL, 0.5 mol) and bromoacetonitrile (35.5 mL, 1.0 mol). The solution was then heated at reflux for 18 h in an oil bath. The mixture was cooled to ambient temperature and concentrated to give a viscous syrup. The syrup was dissolved in EtOAc - during which time the salts precipitated and were removed by filtration. The EtOAc was then washed with water, dried (Na_2SO_4) and concentrated to give a viscous oil which was purified by vacuum distillation (0.2 mm Hg, 110 °C) to give 28.5 g (0.22 mol, 43%) of **3a** which crystallized during distillation. An analytical sample was prepared by recrystallization from EtOAc – hexane, mp 40-41 °C (lit. bp 150°C/0.6 mm Hg). IR (KBr) 3390 and 2251 cm^{-1}; MS (*m / z*, ES+) 133.2 (100%); ^{1}H-NMR ($CDCl_3$) δ 7.27 (t, *J* = 8.6 Hz, 2H), 6.68 (t, *J* = 7.4 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 2H), 4.09 (d, *J* = 6.9 Hz, 2H) 3.96 (bs, D_2O exchangeable, 1H). Anal. Calcd for $C_8H_8N_2$: C, 72.70; H, 6.10; N, 21.19. Found: C, 72.52; H, 6.14; N, 21.13.

***N*-(Cyanomethyl)cyclohexylamine (3b).** Chloroacetonitrile (7.6 g, 0.10 mol. 6.4 mL, 1.0 eq) and cyclohexylamine (20 g, 0.20 mol) were combined in benzene (100 mL) and stirred for 20 h at room temperature. The mixture was filtered to remove cyclohexylamine hydrochloride and the filtrate distilled to give 11.1 g (0.80 mol. 80%) of **3b** as a white solid, mp 23-25 °C. IR (neat) 3055, 2932, 2306 and 1265 cm^{-1}; MS (*m/z*, ES+) 139.1 (20%); ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 2H), 2.70 (m, 1H), 1.84 (m, 2H), 1.75 (m, 2H), 1.64 (m, 1H), 1.21 (m, 6H). Anal. Calcd for $C_8H_{14}N_2$: C. 69.52: H, 10.21; N, 20.27. Found C, 69.27; H, 10.25; N, 20.06.

***N*-(Benzyloxycarbonyl)anilinoacetonitrile (4a).** A solution of benzyl chloroformate (65.7 mL, 0.46 mol) in dichloromethane (100 mL) was added over 0.5 h to a dichloromethane (200 mL) solution of **3a** (30.4 g, 0.23 mol). After the last addition, a solution of 1 *N* $NaHCO_3$ (200 mL) was added in one portion and the mixture stirred overnight. The dichloromethane layer was separated and washed with water, pyridine (18 mL. 0.23 mol) added to react with excess benzyl chloroformate and the solution again washed with water. The solution was dried (Na_2SO_4) and concentrated to give a syrup, which was dried overnight *in vacuo* to give 48.8 g (0.183 mol. 80%) of **4a**. An analytical sample was prepared by column chromatography (SiO_2, hexane-EtOAc, 8:2) to give **4a** as light brown oil which was dried overnight *in vacuo* at acetone reflux. IR (KBr) 1712 cm^{-1}; MS (*m/z*, ES+) 267.4 (100%); ^{1}H NMR ($CDCl_3$) δ 7.50 – 7.10 (bm, 10H), 5.18 (s, 2H), 4.51 (s, 2H). Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.29; N, 10.51. Found C, 72.40; H, 5.37; N, 10.27.

Methyl 3-amino-4-[[*N*-(ethoxycarbonyl)-*N*-phenyl]amino]-1*H*-pyrrole-2-carboxylate (7a). Compound **4a** (532 g, 2 mol) was dissolved in dry THF (1 L) and added dropwise to a stirred suspension of sodium hydride (120 g, 3 mol. 60% pre-washed with hexane) in THF (500 mL) over 30 min. After the

last addition, ethyl formate (968 mL, 12 mol) was added to the reaction by adding first 100 mL, stirring for 30 min. and then adding the remaining ethyl formate over 1.5 h with ice cooling. The mixture was allowed to stir overnight. The mixture was vacuum filtered and the cake washed thoroughly with hexane. The cake was dried *in vacuo* to near dryness to give 563 g (1.78 mol. 89%) of the formylated product **5a** in the sodium enolate form. This product was used directly in the next step with no further purification.

The enolate **5a** (563 g, 1.78 mol) was dissolved carefully in MeOH (2 L) and water (500 mL) and to this was added diethyl aminomalonate hydrochloride (414 g, 1.96 mol). The mixture was then warmed to ca. 45 °C and stirred for 48 h during which time the pH was maintained at 5-5.5 by addition of HCl or sodium acetate as required. The mixture was concentrated and the residue partitioned between EtOAc (1.2L) and water (600 mL). The EtOAc layer was separated and dried thoroughly ($MgSO_4$). The solution was filtered and concentrated to give the enamine **6a** (283 g, 0.67 mol) in 38% yield as a syrup which was used directly in the next step.

The enamine **6a** (84.6 g, 0.2 mol) was dissolved in anhydrous MeOH (300 mL) and cooled in an ice water-NaCl bath for 10 min with stirring. To the cooled, stirred solution was added NaOMe (25% in MeOH, 68.6mL. 0.3 mol) dropwise over 0.5 h and the solution stirred for 1 h followed by reflux for 0.5 h. The solution was cooled to ambient temperature, neutralized with HOAc and concentrated to give a semi-solid material which was partitioned between EtOAc (600 mL) and water (300 mL). The EtOAc layer was separated, dried (Na_2SO_4) and concentrated to give the pyrrole as a powder (54 g, 0.178 mol. 89%). During the enamine to pyrrole step, the benzyl group was intramolecularly exchanged for an ethyl group. An analytical sample was prepared by column chromatography (SiO_2, EtOAc-hexane 1:1) to afford **7a** as a white powder, mp 150-153 °C. IR (KBr) 3362, 3282, 1706, 1654 and 1626 cm^{-1}; MS (*m/z*, ES+) 304.4 (100%); ^1H NMR ($CDCl_3$) δ 8.3 (bs, D_2O exchangeable, 1H), 7.4-7.0 (m, 5H), 6.62 (bs, 1H), 4.27 (q, J = 7.0 Hz, 2H and 3-NH_2 overlapping), 3.83 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H). Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.39; H, 5.65; N, 13.85. Found: C, 59.77; H, 5.69; N, 13.79.

Methyl 3-amino-4[[*N*-cyclohexyl-*N*-(phenylmethyloxycarbonyl]amino]-1*H*-pyrrole-2-carboxylate (7b). The nitrile **3b** (81.4 g, 0.59 mol) was dissolved in dichloromethane (1 L). Benzyl chloroformate (182 g, 1.06 mol) was added over 1 h followed by sodium bicarbonate solution (109 g, 1.30 mol in 1 L water). The reaction was stirred for 2 h, then the dichloromethane layer was separated and stirred with pyridine (37 g, 0.47 mol) for 30 min before being washed with water and concentrated to give a yellow oil which was purified by passing through a short pad of silica (dichloromethane) to give **4b**.

The nitrile **4b** (27.2 g, 0.10 mol) was added in one portion to NaH (3.7 g, 0.15 mol) in THF (100mL) followed by addition of ethyl formate (44.4 g, 0.60 mol) over 2 h. The slurry was stirred for 20 h, then the sodium enolate **5b** was collected by vacuum filtration. The yield for **5b** was assumed to be quantitative and was used in the next step with no further purification.

This enolate **5b** was dissolved in MeOH (280 mL) and water (70 mL) to which diethyl aminomalonate hydrochloride (20.2 g, 0.11 mol) was added. The pH was adjusted to 5-5.5 (HCl) and the solution heated at 46 °C for 48 h. The MeOH was removed *in vacuo* and the resulting gum extracted with dichloromethane (2 X 200 mL). The extracts were combined and concentrated to give the enamine **6b** as a yellow syrup.

The enamine **6b** was dissolved in MeOH (100 mL) and NaOMe (6.6 g, 0.12 mol. 28 mL of 25% solution) was added. The solution was stirred at room temperature for 4 h. The MeOH was removed *in vacuo* and the resulting gum stirred in water (200 mL) for 1 h during which time the product precipitated. The precipitate was collected by filtration and recrystallized from MeOH to give **7b** (6.7 g, 18%) as a white solid, mp 180-181 °C. IR (KBr) 3312 and 1662 cm^{-1}; MS (*m/z*, ES+) 372.5 (100%); ^1H NMR ($CDCl_3$) δ 8.46 (bs, D_2O exchangeable, 1H), 7.27 (m, 5H), 6.57 (bs, 1H), 5.14 (bs, 2H), 4.13 (bs, 1H), 3.84 (s, 3H), 3.57 (bs, D_2O exchangeable, 2H), 0.8 – 2.0 (bm, 10H). Anal. Calcd for $C_{20}H_{25}N_3O_4$: C, 64.67; H, 6.78; N, 11.31. Found: C, 64.57; H, 6.80; N, 11.29.

Methyl 3-[[[(methoxycarbonyl)amino][methoxycarbonyl)imino]methyl]amino]-4-[[*N*-(ethoxycarbonyl)-*N*-phenyl]amino]-1*H*-pyrrole-2-carboxylate (8a). A sample of **7a** (77.5 g, 0.21 mol) was suspended in MeOH (1 L) containing HOAc (61 mL 1.0 mol) and 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea (48.2 g. 0.23 mol) and the mixture allowed to stir overnight. The reaction mixture was concentrated to *ca* one-half of its volume and the solid that formed was collected by vacuum filtration and washed thoroughly with MeOH. The solid was air-dried to give 55.7 g (0.10 mol. 58%) of the adduct **8a**. An analytical sample was prepared by recrystallization twice from EtOAc-hexane and the solid dried *in vacuo* at acetone reflux for 18 h to give **8a** as a white solid, mp 181-183 °C. IR (KBr) 3282, 1697 and 1625 cm^{-1}; MS (*m/z*, ES+) 462.6 (100%); ^1H NMR (DMSO-d_6) δ 12.08 (bs, D_2O exchange, 1H), 11.41 (bs, D_2O exchange, 1H), 9.46 (bs, D_2O exchange, 1H), 7.5-6.8 (m, 6H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.71 (s, 6H), 3.31 (s, 3H), 1.13 (t, J = 7.1 Hz, 3H). Anal. Calcd for $C_{20}H_{23}N_5O_8$: C, 52.06; H, 5.02; N, 15.17. Found: C, 52.02; H, 4.98; N, 15.15.

Methyl 3-[[[(methoxycarbonyl)amino][methoxycarbonyl)imino]methyl]amino]-4-[[*N*-cyclohexyl-*N*-[(phenylmethyl)oxy]carbonyl]amino]-1*H*-pyrrole-2-carboxylate (8b). The pyrrole **7b** (10.0 g, 26.8 mmol) was dissolved in DMF (500 mL) containing Et_3N (0.81 mmol), 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea (6.1 g, 30 mmol) and $HgCl_2$ (8.0 g, 30 mmol) and the reaction stirred for 130 h at room temperature. The volatiles were removed *in vacuo* and the resulting gum stirred in water (1 L) for 16 h. The solid was collected by filtration and extracted with hot MeOH (1 L). The extracts were concentrated *in vacuo* and purified by column chromatography (hexane – EtOAc 1:1) to give **8b** (2.2 g, 15% yield). An analytical sample was recrystallized from 2-propanol to give a white solid, mp 146-148 °C. IR (KBr) 3389, 3267, 2929 and 1728 cm^{-1}; MS (*m/z*, ES+) 530.5 (100%); ^1H NMR (DMSO-d_6) δ 12.04 (s, D_2O exchangeable, 1H), 11.44 (s, D_2O exchangeable, 1H), 9.40 (s, D_2O exchangeable, 1H), 7.26 (s, 3H), 7.19 (s, 2H), 6.94 (s, 1H), 5.01 (bs, 2H), 3.91 (m, 1H), 3.79 (s, 3H), 3.67 (s, 3H), 3.48 (s, 3H),

0.87 – 1.73 (bm, 10H). Anal. Calcd for $C_{25}H_{31}N_5O_8$: C, 56.70; H, 5.90; N, 13.23. Found: C, 56.55; H, 5.88; N, 13.24.

3-[(Methylphenyl)oxy]acetonitrile (11d). Potassium hydroxide (195.8 g, 3.0 mol) was dissolved in water (750 mL) and then added to a solution of *m*-cresol (310 mL, 3.0 mol) in MeOH (1.20 L). The reaction was concentrated and then co-evaporated with toluene (4 X 500 mL) and then with EtOH (2 X 250 mL) in order to remove all traces of water. The potassium salt was dried *in vacuo* at ambient temperature for 2 h and then dissolved in DMF (2.0 L) to which chloroacetonitrile (190 mL, 3.1 mol) was added dropwise under a nitrogen stream. The reaction was stirred overnight at ambient temperature and then filtered to remove KCl. After the filtrate was concentrated, the residue was suspended in water (500 mL) and extracted with EtOAc (4 X 750 mL). The combined organic layers were washed once with water, dried (Na_2SO_4), filtered, and concentrated. The resulting brown oil was purified by short path distillation at 85 °C to give 381.05 g (86%) of **11d** as a pale yellow oil. IR (KBr) 3378, 3049 and 2921 cm^{-1}; MS (*m / z*, ES+) 147.2 (100%); ^1H NMR (DMSO-d_6) δ 7.24 (t, *J* = 7.1 Hz, 1H), 6.89 (m, 3H), 5.15 (s, 2H), 2.31 (s, 3H). Anal. Calcd for C_9H_9NO C, 73.47: H, 6.12; N, 9.52. Found C, 73.51; H, 6.20; N, 9.45.

Methyl 3-amino-4-(phenyloxy)-1*H*-pyrrole-2-carboxylate (14c). Potassium hydroxide (32.6 g, 0.51 mol) was dissolved in water (125 mL) and then added to a solution of phenol (44.0 mL, 0.50 mol) in MeOH (200 mL). The reaction was concentrated and then co-evaporated five times with toluene in order to remove all traces of water. The potassium salt was dried *in vacuo* at ambient temperature for 1.5 h and then suspended in DMF (500 mL). Chloroacetonitrile (32.3 mL, 0.51 mol) was added dropwise to the suspension under a nitrogen atmosphere. The reaction was stirred at 80 °C overnight and then cooled to ambient temperature. KCl that formed was removed by filtration and washed thoroughly with EtOAc. The EtOAc washings and the filtrate were concentrated and the residue suspended in water (400 mL) and extracted with EtOAc (4 X 250 mL). The combined extracts were dried (Na_2SO_4) and concentrated to give a brown oil which was purified by short path distillation (120 °C). The resulting oil was contaminated with a trace of phenol which was removed by washing with 5% NaOH. Final workup afforded **11c** (38 g, 57%) as a clear colorless oil which was used directly in the next step.

Phenyloxyacetonitrile **11c** (26.6 g, 0.2 mol) in THF (70 mL) was added dropwise over a ten-minute period to a suspension of NaH (7.51 g, 0.300 mol) and THF (100 mL) under a nitrogen stream. Ethyl formate (9.70 mL, 0.120 mol) was added dropwise to initiate the reaction. Heavy evolution of hydrogen started. After thirty minutes, the remaining portion of ethyl formate (87.2 mL, 1.08 mol) was added over a half-hour period. The reaction was stirred overnight at ambient temperature. The reaction was then concentrated and the residue suspended in water (200 mL). The pH was adjusted to 3 with glacial HOAc and extracted with EtOAc (3 X 300 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated. The orange oil was dried *in vacuo* at ambient temperature to give ≈32.2 g (100%) of crude **12c**.

Water (100 mL), MeOH (300 mL), and diethyl aminomalonate hydrochloride (52.91 g. 0.250 mol) were added to a sample of **12c** (32.2 g, 0.20 mol). The pH was adjusted to 5 with NaOAc and the mixture allowed to stir for 48 h at 50 °C. The mixture was concentrated and the residue partitioned between EtOAc (300 mL) and water (150 mL). The organic layer was separated and dried thoroughly ($MgSO_4$). The mixture was filtered and the filtrate concentrated to give the crude enamine **13c** as a semi-crystalline material which was used without further purification.

The above crude enamine **13c** was dissolved in absolute MeOH (100 mL) to which was added 25% NaOMe (68.3 mL, 0.30 mol) in one portion. The mixture was stirred overnight at ambient temperature and then concentrated *in vacuo*. The residue was suspended in water (200 mL) and extracted with EtOAc (3 X 250 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated to give a viscous, brown mass. The brown mass was purified by column chromatography using 7:3 hexane/EtOAc as the eluent. Relevant fractions were collected and concentrated to give 1.75 g (4%) of **14c** as a brown oil. An analytical sample was prepared by recrystallizing from ether. The solid was collected by filtration and dried *in vacuo* at acetone reflux to give **14c** as a tan solid, mp 105.5-106.5 °C. IR (KBr) 3291 and 1675 cm^{-1}; MS (*m / z*, ES+) 233.4 (100%); ^1H NMR (DMSO-d_6) δ 10.8 (s, 1H), 8.20 (t, *J* = 8.2 Hz, 2H), 7.00 (t, *J* = 7.3 Hz, 1H), 6.90 (d, *J* = 7.9 Hz, 2H), 6.70 (s, 1H), 4.70 (bs, D_2O exchangeable, 2H), 3.90 (s, 3H). Anal. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.21; H, 5.27; N. 11.84.

Methyl 3-amino-4-[(3-methylphenyl)oxy]-1*H*-pyrrole-2-carboxylate (14d). The nitrile **11d** (220.5 g, 1.5 mol) in THF (100 mL) was added dropwise over a ten-minute period to a suspension of 95% NaH (90.0 g, 1.5 mol) and THF (1.4 L). Ethyl formate (77 mL, 0.95 mol) was added dropwise over 0.25 h to the reaction. Vigorous hydrogen evolution occurred and the flask had to be cooled for 15 min. The reaction was then allowed to stir for 1 h at ambient temperature. The remaining portion of ethyl formate (650 mL, 8.1 mol) was added over 1 h and the reaction stirred overnight at ambient temperature. The reaction was then concentrated and the residue suspended in water (1.25 L). The pH was adjusted to 3 with glacial HOAc and extracted with EtOAc (3 X 1 L). The combined extracts were dried (Na_2SO_4), filtered, and concentrated. The orange oil was dried *in vacuo* at ambient temperature to give about 262.5 g (100%) of crude **12d**.

Water (0.5 L), MeOH (1.5 L), and diethyl aminomalonate hydrochloride (396.84 g, 1.88 mol) were added to the crude **12d** from above and the pH adjusted to 5 with NaOAc. The mixture was allowed to stir at ambient temperature for 25 h. The MeOH was removed by concentration and the residue extracted with EtOAc (3 X 400 mL). The organic layer was separated and dried thoroughly over Na_2SO_4. The mixture was filtered and the filtrate concentrated to give the crude enamine **13d** as a semi-crystalline material which was used without further purification.

The enamine **13d** was dissolved in absolute MeOH (930 mL) to which was added 25% NaOMe (260 mL, 1.13 mol) in one portion. The mixture was stirred overnight at ambient temperature and then

additional 25% NaOMe (260 mL, 1.13 mol) was added in two portions over a 1 h period. After 1 h, the reaction was neutralized with glacial HOAc and concentrated *in vacuo*. Water (500 mL) was added to the residue and then extracted with EtOAc (3 X 600 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give a black oil. The black oil was purified several times by column chromatography using 7:3 or 4:1 hexane/EtOAc as the eluent. Relevant fractions were collected and concentrated to give two batches: 10 g of a crude sample of methyl and ethyl pyrrole and 10 g of pure methyl pyrrole. The crude mixture (containing both methyl and ethyl esters) was dissolved in MeOH (100 mL) and 25% NaOMe (4 mL) and heated at reflux for 1 h and then stirred overnight at ambient temperature. More 25% NaOMe (4 mL) was added and the reaction heated at reflux. The reaction was neutralized with glacial HOAc and then concentrated. Water (50 mL) was added to the residue and then extracted with EtOAc (2 X 100 mL). The organic extracts were combined and dried (Na_2SO_4), filtered, and concentrated to give crude methyl pyrrole. An analytical sample was prepared by recrystallization from toluene-hexane. The solid was collected by filtration and dried *in vacuo* at ambient temperature to give **14d** as a tan solid, mp 87.5-88.5 °C. IR (KBr) 3393, 3301, 3130 and 1688 cm^{-1}; MS (*m / z*, ES+) 247.4 (100%); ^1H NMR (DMSO-d_6) δ 10.74 (s, D_2O exchangeable, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 6.7 Hz, 1H), 6.74 (d, *J* = 3.6 Hz, 3H), 4.64 (s, D_2O exchangeable, 2H), 3.75 (s, 3H), 2.27 (s, 3H). Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.54; H, 5.83, N, 11.28.

Methyl 3-amino-4-(phenylthio)-1*H*-pyrrole-2-carboxylate (14e). Sodium (11.5 g, 0.5 mol) was added over 1 h to a solution of thiophenol (51.3 mL, 0.5 mol) in ethanol (500 mL) under a nitrogen atmosphere. The solution was heated at reflux and then chloroacetonitrile (31.64 g, 0.5 mol) was added dropwise over 0.5 h. After refluxing for an additional 2 h, the reaction was cooled to ambient temperature and NaCl removed by filtration. The filtrate was concentrated and the residue purified by short path distillation at 80-105 °C to give 58.99 g (79%) of the nitrile **11e** as a colorless oil which was used directly in the next step.

Phenylthioacetonitrile **11e** (49.66 g, 0.333 mol) in THF (100 mL) was added dropwise over a 10-min. period to a suspension of 95% sodium hydride (25.25 g, 1.0 mol) and THF (150 mL). Vigorous hydrogen evolution occurred and the flask had to be cooled for 5 min. After 30 min of stirring at ambient temperature, ethyl formate (16.47 mL, 0.2 mol) was added dropwise over 0.25 h to the reaction. Vigorous hydrogen evolution occurred again and the flask had to be cooled for 5 min. The reaction was then allowed to stir for 1.25 h at ambient temperature. The remaining portion of ethyl formate (145 mL, 1.8 mol) was added over 0.5 h and the reaction stirred overnight at ambient temperature. The reaction was then concentrated and the residue suspended in water (250 mL). The pH was adjusted to 3 with glacial HOAc and extracted with EtOAc (3 X 200 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated. The orange oil was dried *in vacuo* at ambient temperature to give about 58.99 g (100%) of crude **12e**.

Water (150 mL), MeOH (450 mL), and diethyl aminomalonate hydrochloride (88.07 g, 0.416 mol) were added to a crude sample of **12e** (58.99 g, 0.33 mol) and the pH adjusted to 5 with sodium

acetate and the mixture allowed to stir at 40 °C for 48 h. The mixture was concentrated and the residue partitioned between EtOAc (400 mL) and water (150 mL). The organic layer was separated and dried (Na_2SO_4). The mixture was filtered and the filtrate concentrated to give the crude enamine **13e** as a semi-crystalline material, which was used without further purification.

The enamine **13e** was dissolved in absolute MeOH (100 mL) to which was added 25% NaOMe (89.58 mL, 0.393 mol) in one portion. The mixture was stirred overnight at ambient temperature and then concentrated *in vacuo*. Water (150 mL) was added to the residue and then extracted with EtOAc (3 X 300 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give a black oil. The black oil was purified twice by column chromatography using 7:3 hexane/EtOAc as the eluent. Relevant fractions were collected and concentrated to give 14 g of a crude sample of methyl and ethyl pyrrole. The residue was dissolved in MeOH (300 mL) and 25% NaOMe (5.5 mL), and stirred overnight at ambient temperature. After 16 h, the reaction was heated at reflux and portions of 25% NaOMe (5.5 mL) were added at 0 h, 3 h, and 6 h. The reaction was neutralized with glacial HOAc and concentrated. Water (200 mL) was added to the residue and then extracted twice with 150-mL portions of EtOAc. The organic extracts were combined and dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved in MeOH (300 mL) and then treated with carbon twice. The carbon was removed by filtration and the filtrate concentrated to give 9.25 g (14%) of an orange solid. An analytical sample was prepared by washing the solid with water and then MeOH. The solid was collected by filtration and dried *in vacuo* at ambient temperature to give **14e** as a tan solid, mp 128-129 °C. IR (KBr) 3281 and 1658 cm^{-1}; MS (*m / z*, ES+) 249.2 (100%); ^1H NMR (DMSO-d_6) 11.39 (s, D_2O exchangeable, 1H), 7.30 (t, J = 7.3 Hz, 2H), 7.00 (m, 4H), 5.01 (bs, D_2O exchangeable, 2H), 3.80 (s, 3H). Anal. Calcd for $C_{12}H_{12}N_2O_2S$: C, 58.06; H, 4.84; N, 11.29. Found: C, 58.02; H, 4.90; N, 11.22.

Methyl 3-[[[(methoxycarbonyl)amino][methoxycarbonyl)imino]methyl]amino]-4-(phenyl oxy)-1*H*-pyrrole-2-carboxylate (15c). A sample of **14c** (1.25 g, 5.28 mmol) was suspended in MeOH (20 mL) containing HOAc (1.54 mL, 26.9 mmol) and 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea (1.22 g, 5.92 mmol) was added to this mixture and the reaction allowed to stir overnight. The precipitate was collected by filtration and air dried to give 0.84 g (40%) of the adduct as a white solid. An analytical sample was prepared by recrystallization from MeOH and drying *in vacuo* at acetone reflux to give **15c**, mp 146-148 °C. IR (KBr) 3277, 1647, 1622 and 1601 cm^{-1}; MS (*m / z*, ES+) 391.2 (100%); ^1H NMR (DMSO-d_6) δ 11.90 (s, D_2O exchangeable, 1H), 11.30 (s, D_2O exchangeable, 1H), 9.50 (s, D_2O exchangeable, 1H), 7.30 (t, J = 7.5 Hz, 2H), 6.93 (m, 4H), 3.72 (s, 6H), 3.54 (s, 3H). Anal. Calcd for $C_{17}H_{18}N_4O_7$: C, 52.31; H. 4.65; N, 14.35. Found: C, 52.15: H, 4.69; N. 14.24.

Methyl 3-[[[(methoxycarbonyl)amino][methoxycarbonyl)imino]methyl]amino]-4-[(3-methyl phenyl)oxy]-1*H*-pyrrole-2-carboxylate (15d). A sample of **14d** (8.33 g, 33.86 mmol) was suspended in MeOH (100 mL) containing HOAc (9.68 mL, 169.3 mmol) and 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea (7.67 g, 37.3 mmol) and the reaction allowed to stir overnight. The reaction was concentrated to one-half volume and 50 mL of hexane was added. Sufficient EtOAc was added to afford

one layer. The reaction was cooled for 1 h and then the precipitate collected by filtration and dried at ambient temperature to give 4.0 g (29%) of the adduct as a white solid. An analytical sample was washed with ether and dried *in vacuo* at ambient temperature for 24 h to give **15d**, mp 130.5-131.5 °C. IR (KBr) 3277, 1734, 1709, 1645 and 1615 cm^{-1}; MS (*m / z*, ES+) 405.3 (100%); ^1H NMR (DMSO-d_6) δ 12.00 (bs, D_2O exchangeable, 1H), 11.34 (D_2O exchangeable, 1H), 9.46 (bs, D_2O exchangeable, 1H), 7.13 (t, *J* = 7.9 Hz, 1H), 6.90 (d, *J* = 3.6 Hz, 1H), 6.80 (d, *J* = 7.8 Hz, 3H), 6.76 (d, *J* = 9.07, 1H), 3.75 (s, 6H), 3.51 (s, 3H), 2.23 (s, 3H). Anal. Calcd for $C_{18}H_{20}N_4O_7$: C, 53.46; H, 4.98; N, 13.86. Found: C, 53.40; H, 4.96; N, 13.82.

Methyl 3-[[[(methoxycarbonyl)amino][methoxycarbonyl)imino]methyl]amino]-4-(phenyl thio)-1*H*-pyrrole-2-carboxylate (15e). A sample of **14e** (6.0 g, 24.19 mmol) was suspended in MeOH (75 mL) containing HOAc (6.93 mL, 121 mmol) and 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea (5.48 g, 26.6 mmol) and the reaction allowed to stir overnight. The precipitate was collected by filtration and dried at ambient temperature to give 4.20 g (42.9%) of the adduct as a white solid. An analytical sample was dried *in vacuo* at toluene reflux for 8 h to give **15e**, mp 164-165.5 °C. IR (KBr) 3263, 1737, 1707 cm^{-1}; MS (*m / z*, ES+) 407.3 (100%); ^1H NMR (DMSO-d_6) δ 12.49 (bs, D_2O exchangeable, 1H), 11.44 (bs, D_2O exchangeable, 1H), 9.57 (bs, D_2O exchangeable, 1H), 7.28 (d, *J* = 3.3 Hz, 1H), 7.21 (m, 2H), 7.10 (m, 3H), 3.75 (bs, 6H), 3.49 (bs, 3H). Anal. Calcd for $C_{17}H_{18}N_4O_6S \cdot 0.5\ H_2O$: C, 49.16; H, 4.58; N, 13.49. Found: C, 48.99; H, 4.36; N, 13.42.

3-[(Pyridinyl)oxy]acetonitrile (17j). A sample of 3-hydroxypyridine (95.1 g, 1 mol) was dissolved in MeOH (500 mL) to which was added a solution of potassium hydroxide (56.11 g. 1 mol) in water (400 mL) in one portion. The mixture was stirred for 0.5 h and then concentrated *in vacuo* to give a brown syrup which was dried thoroughly by azeotropic removal of water with toluene several times. The resulting oil was crystallized by addition of ethyl ether and swirling vigorously. The solid was collected by vacuum filtration and washed with ethyl ether, crushed to a fine powder with a mortar and pestle and the brown powder dried overnight *in vacuo* at ambient temperature to give 133 g (1 mol., quant.) of the potassium salt of 3-hydroxpyridine which was used immediately in the next step.

The salt (133 g, 1 mol) was partially dissolved in dry DMF (500 mL) under a nitrogen atmosphere with mechanical stirring. To the stirred suspension was added chloroacetonitrile (63.3 mL, 1 mol) over 0.5 h during which time the reaction became homogenous and a slight exotherm was observed. The mixture was then allowed to stir overnight at 40 °C. The mixture was filtered to remove precipitated salts and the filtrate concentrated to give a dark solid. The solid was distilled (Kugelrohr) *in vacuo* to give a white solid which thin-layer chromatographic analysis (SiO_2, EtOAc-hexane 1:1) indicated was contaminated with 3-hydroxpyridine. The solid was then dissolved in EtOAc (500 mL) and extracted with 1 *N* NaOH (1 X 125 mL). The EtOAc layer was dried over Na_2SO_4 and concentrated to give 33.2 g (0.25 mol. 25%) of **17j** as a white solid, mp 38-40 °C. IR (KBr) 3448, 3069, 2917 and 1575 cm^{-1}; MS (*m / z*, ES+) 135.1 (100%); ^1H NMR ($CDCl_3$) δ 8.41 (m, 2H), 7.32 (m, 2H), 4.83 (s, 2H), 1.77 (s, 1H). Anal. Calcd for $C_7H_6N_2O$: C, 62.68; H, 4.50; N, 20.88. Found: C, 62.66: H, 4.48; N, 20.83.

Methyl 3-amino-4-[(3-pyridinyl)oxy]-1*H*-pyrrole-2-carboxylate (21j). A sample of **17j** (47.99 g. 0.358 mol) in THF (200 mL) was added dropwise over a 10-min period to a suspension of 95% sodium hydride (13.57 g. 0.54 mol) and THF (700 mL). Ethyl formate (19 mL, 0.22 mol) was added dropwise over 0.5 h to the reaction which was then allowed to stir for 1.5 h at ambient temperature. The remaining portion of ethyl formate (154 mL, 1.98 mol) was added over 0.5 h and the reaction stirred 2 h at ambient temperature and the resulting slurry stirred for 0.2 h. The sodium enolate **18j** precipitate was collected by filtration and used without further purification.

Water (200 mL), MeOH (600 mL), and glycine methyl ester hydrochloride (67.45 g. 0.54 mol) were added to a crude sample of the sodium enolate **18j** (67.89 g, 0.358 mol). The mixture now at pH 7, was allowed to stir at ambient temperature for 24 h and at 40 °C for 2 h. The MeOH was removed by concentration and the residue extracted with EtOAc (3 X 200 mL). The combined organic layers were separated and dried thoroughly (Na_2SO_4). The mixture was filtered and the filtrate concentrated to give the crude enamine **19j** as a semi-crystalline material which was used without further purification.

The enamine **19j** was dissolved in dry dichloromethane (500 mL) and the solution cooled to 0 °C. 1,5-diazabicyclo[4.3.0]non-5-ene (DBN, 88.95 g. 0.716 mol) was added dropwise over a 5-min period. Ethyl chloroformate (51.1 mL, 0.54 mol) was added dropwise so that the temperature of the reaction stayed around 4 °C. The reaction was refrigerated for 24 h. Additional DBN (16 mL) and ethyl chloroformate (8 mL) were added to complete the amine protection (**20j**). The reaction was then refrigerated for a further 24 h after which DBN (44.25 mL, 0.358 mol) was added to cyclize the intermediate to the protected pyrrole. The reaction was again refrigerated for 24 h and then concentrated to give a black oil. The black oil was purified by column chromatography using EtOAc as the eluent. Relevant fractions were collected and concentrated to give protected pyrrole **21j**.

The pyrrole **21j** (109.19 g, 0.358 mol) was deprotected by dissolving the compound in MeOH (750 mL) and reacting it with sodium carbonate (18.98 g, 0.179 mol) at ambient temperature for 1.5 h. Excess base was removed by filtration and the filtrate concentrated. Dichloromethane (500 mL) was added to the residue and the solution was washed with H_2O (2 X 200 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to give crude methyl pyrrole. The dark oil was purified twice by column chromatography using EtOAc as the eluent. Relevant fractions were collected and concentrated to give 17.23 g (21%) of pyrrole. An analytical sample was prepared by column chromatography using EtOAc as the eluent. Relevant fractions were pooled and concentrated. The residue was triturated with ether and the solid collected by filtration and dried *in vacuo* at ambient temperature to give **22j** as a yellow solid, mp 92.5-93.5 °C. IR (KBr) 1735 cm^{-1}; MS (*m / z*, ES+) 234.2 (100%); ^1H NMR (DMSO-d_6) δ 8.41 (bs, 1H), 8.33 (bs, 1H), 7.45 (m, 2H), 7.27 (m, D_2O exchangeable, 1H), 7.17 (bs, 1H), 3.93 (d, J = 6.4 Hz, 2H), 3.65 (bs, 3H). Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02. Found: C, 56.38; H, 4.76; N, 17.94.

Methyl 3-amino-4-[(2-naphthyl)oxy]-1*H*-pyrrole-2-carboxylate (22k). A sample of **17k**[17] (36.6 g, 0.2 mol) was added to a stirred suspension of NaH (12 g. 0.3 mol) in THF (500 mL) under a

nitrogen atmosphere. After the last addition, ethyl formate (44.4 g, 0.6 mol) was added over a 1 h period and the mixture stirred at ambient temperature for 3 h. An additional 1.6 g of NaH was added and the mixture allowed to stir overnight. The mixture was concentrated *in vacuo* and the residue treated with hexane (800 mL) which caused the sodium enolate **18k** to precipitate. The solid was collected by vacuum filtration, washed with additional hexane, and used with no further purification.

The enolate salt **18k** (35.7 g, 0.153 mol) was dissolved in MeOH (600 mL) and water (100 mL) and to the solution was added glycine methyl ester hydrochloride (28.9 g, 0.23 mol) in one portion. The pH of the solution was about 7. The mixture was allowed to stir overnight, at which time thin-layer chromatographic analysis (10% MeOH - chloroform) showed disappearance of the lower R_f aldehyde with formation of the higher R_f enamine. The mixture was concentrated and the residue partitioned between EtOAc (500 mL) and water (300 mL). The EtOAc layer was separated, dried over Na_2SO_4, and concentrated to give the enamine **19k** as a thick syrup (36.2 g, 0.135 mol, 88%) which was used directly in the next step with no further purification.

The enamine **19k** (36.2 g, 0.135 mol) was dissolved in dry dichloromethane (300 mL) and cooled in an ice-water-NaCl bath under a nitrogen atmosphere until the solution temperature was about 0 °C. To the stirred cooled solution was added DBN (33.5 mL, 0.27 mol) over 5 min with the solution temperature maintained at < 2 °C. Ethyl chloroformate was added over a 30 min period and after the last addition was placed in the refrigerator for 18 h. An additional equivalent of DBN was added and the solution refrigerated for three days. Protected pyrrole **21k** was isolated directly by concentration of the solution and was used directly in the next step with an assumed quantitative yield (47.8 g, 0.135 mol).

The above carbamate protected pyrrole **21k** was dissolved in MeOH (300 mL) to which was added sodium carbonate (22.4 g, 0.162 mol). The mixture was stirred at ambient temperature for 1 h at which time thin-layer chromatographic analysis (SiO_2, EtOAc-hexane 1: 1) indicated formation of the lower R_f deprotected pyrrole. The mixture was purified by column chromatography and the appropriate fractions concentrated to give 4.66 g (0.0165 mol, 12%) of **22k** as colorless cubes, mp 165-167 °C. IR (KBr) 3353, 3065 and 1732 cm^{-1}; MS (*m / z*, ES+) 283.4%; ^1H NMR (DMSO-d_6) δ 7.96 (m, 3H), 7.55 (t, J = 7.1 Hz, 1H), 7.44 (m, 2H), 7.32 (s, 1H), 7.13 (s, D_2O exchangeable, 2H), 3.92 (s, 2H), 3.65 (s, 3H). Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 68.08; H, 5.02; N, 9.99.

1,5-Dihydro-2-(methoxycarbonyl)amino-7-(3-pyridinyloxy)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (23j). A sample of **22j** (16.31 g, 70.0 mmol) was dissolved in HOAc (100 mL) and 1,3-dicarbomethoxy-2-methyl-2-thiopseudourea (38.92 g, 188.83 mmol) added to the solution. The reaction was allowed to stir for 72 h at 50 °C. A precipitate which formed was collected by filtration, washed with water, and dried *in vacuo* at 110 °C for 24 h to give 0.32 g (2%) of **23j** as an off-white solid, mp 248.8-249.3 °C. IR (KBr) 3215, 3159, 2928 and 1713 cm^{-1}; MS (*m / z*, ES+) 302.4 (100%); ^1H NMR (DMSO-d_6) δ 12.10 (bs, D_2O exchange, 1H), 11.30 (bs, D_2O exchange, 1H), 11.07 (bs, D_2O exchange, 1H), 8.31 (s, 1H), 8.21 (s, 1H), 7.44 (s, 1H), 7.29 (m, 2H), 3.69 (s, 3H). Anal. Calcd for $C_{13}H_{11}N_5O_4$: C, 51.83; H, 3.68; N, 23.25. Found: C, 51.77; H3.73; N, 23.17.

General Procedure for the Hypoxanthines

1,5-Dihydro-7-(phenylamino)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (24). A sample of **7a** (0.150 g, 0.5 mmol) was dissolved in absolute ethanol containing formamidine acetate (0.156 g, 1.5 mmol) and the mixture heated at reflux for 18 h during which time a solid formed. The mixture was cooled to ambient temperature and the solid collected by vacuum filtration to give the hypoxanthine (0.075 g, 66%) as a gray powder, mp > 350 °C. IR (KBr) 3413 and 1677 cm^{-1}; MS (*m / z*, ES+) 227.6 (100%); ^{1}H-NMR (DMSO-d_6) δ 11.87 (bs, D_2O exchangeable, 2H), 7.74 (s, 1H), 7.38 (s, D_2O exchangeable, 1H), 7.30 (s, 1H), 7.07 (t, *J* = 6.6 Hz, 2H) 6.75 (d, *J* = 7Hz, 2H), 6.58 (t, *J* = 6.5 Hz, 1H). Anal. Calcd for $C_{12}H_{10}N_4O \cdot 0.25\ H_2O$: C, 62.46; H, 4.58; N, 24.28. Found: C, 62.85; H, 4.64; N, 23.97

1,5-Dihydro-7-(phenyloxy)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (25). 47% yield; off-white solid; mp>357-358 °C (dec). IR (KBr) 3124, 3090, 3045, and 2910 cm^{-1}; MS (*m/z*, ES+) 228.2 (100%); ^{1}H-NMR (DMSO-d_6) 12.10 (s, D_2O exchangeable, 1H), 11.95 (s, D_2O exchangeable, 1H), 7.72 (d, *J*= 3.3 Hz, 1H), 7.39 (d, *J* = 3.2 Hz, 1H), 7.27 (t, *J* = 7.5, 8.3 Hz, 2H), 6.99 (t, *J* = 7.3 Hz, 1H), 6.91 (d, *J* = 8.0 Hz, 2H). Anal. Calcd for $C_{12}H_9N_3O_2 \cdot 0.2\ H_2O$: C, 62.44; H, 4.10; N, 18.20. Found: C, 62.74; H, 4.17; N, 18.20.

1,5-Dihydro-7-(phenylthio)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (26). 66% yield; pale gray solid; mp 395-396 °C (dec). IR (KBr) 3089, and 3029 cm^{-1}; MS (*m / z*, ES+) 244.5 (100%); ^{1}H-NMR (DMSO-d_6) 12.66 (s, 1H,D_2O exchangeable), 12.09 (s, 1 H, D_2O exchangeable), 7.84 (d, *J* = 2.8 Hz, 1H), 7.73 (d, *J* = 2.8 Hz, 1H), 7.20 (t, *J* = 7.4, 8.1 Hz, 2H), 7.05 (m, 3H). Anal. Calcd for $C_{12}H_9N_3OS$: C, 59.26; H, 3.66; N, 17.28. Found: C, 59.25; H, 3.74; N, 17.31.

1,5-Dihydro-7-(phenylsulfonyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (27). Same procedure as for the conversion of **2e** to **2h**; 68% off-white solid, mp >360 °C. IR (KBr) 3146, 3029 and 1675 cm^{-1}; MS (*m / z*, ES+) 274.2 (100%); ^{1}H-NMR (DMSO-d_6) 13.25 (s, D_2O exchangeable, 1H), 12.35 (s, D_2O exchangeable, 1H), 8.25-7.50 (m, 7H). Anal. Calcd for $C_{12}H_9N_3O_3S$: C, 52.35; H, 3.29; N, 15.26. Found: C, 52.48; H, 3.39; N, 15.29.

1,5-Dihydro-7-(3-pyridinyloxy)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (28). 31% yield, brown solid, mp 313-315 °C (dec). IR (KBr) 3437, 3036, 2905, 2852 and 1667 cm^{-1}; MS (*m / z*, ES+) 229.4 (100%); ^{1}H-NMR (400 MHz, DMSO-d_6) 12.20 (s, D_2O exchangeable, 1H), 12.01 (s, D_2O exchangeable, 1H), 8.37 (s, 1H), 8.25 (s, 1H), 7.76 (d, *J* = 3.5 Hz, 1H), 7.50 (d, *J* = 3.2 Hz, 1H). 7.32 (s, 2H). Anal. Calcd for $C_{11}H_8N_4O_2 \cdot 0.3\ H_2O$: C, 56.56, H, 3.71; N, 23.98. Found: C, 56.72; H, 3.75; N, 23.62.

1,5-Dihydro-7-[(3-methylphenyl)oxy]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (29). 47% yield, as a pale-gray solid, mp 364-365 °C (dec). IR (KBr) 3415, 2923 and 1676cm^{-1}; MS (*m/z*, ES+) 242.3 (100%); ^{1}H-NMR (DMSO-d_6) 12.05 (s, D_2O exchangeable, 1H), 11.91 (s, D_2O exchangeable, 1H), 7.70 (s, 1H), 7.34 (s, 1H), 7.13 (t, *J* = 7.8.Hz, 1H), 7.90-7.60 (m, 3H), 2.22 (s, 3H). Anal. Calcd for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.78; H, 4.64; N, 17.39.

1,5-Dihydro-7-[(2-naphthyl)oxy]-4*H*-pyrrolo[3,2-*d*]pyrimidine-4-one (31). 75% yield, as a white powder, mp>250 °C. IR (KBr) 3439, 3054 and 1681cm^{-1}; MS (*m/z*, ES+) 278.0 (100%); ^{1}H-NMR (DMSO-d_6) 12.14 (s, D_2O exchangeable, 1H), 11.95 (s, D_2O exchangeable, 1H), 7.87 (t, J = 9.3 Hz, 2H), 7.70 (s, 1H), 7.47 (s, 1H), 7.34 (m, 3H), 2.22 (S, 3H). Anal. Calcd for $C_{16}H_{11}N_3O_2$: C, 69.30; H, 3.99; N, 15.15. Found: C, 69.50; H, 4.06; N, 15.31.

1,5-Dihydro-7-[(*N*-cyclohexyl)amino]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (32). 8% yield as a green solid, mp>320 °C. IR (KBr) 3424, 1680, 1527 cm^{-1}; MS (*m / z*, ES+) 233.1 (100 %); ^{1}H-NMR (DMSO-d_6) δ 12.26 (bs, D_2O exchangeable, 1H), 12.09 (bs, D_2O exchangeable, 1H), 7.90 (s, 1H), 7.32 (s, 1H), 6.11 (s, 3H), 1.95-1.33 (bm, 13H). Anal. Calcd for $C_{16}H_{20}N_4O_5 \cdot 0.1H_2O$; C, 54.88; H, 5.81; N, 16.00. Found: C, 54.62; H, 5.81; N, 15.87.

X-ray Crystallographic Analysis.

Crystals of PNP used for X-ray diffraction studies were prepared from ammonium sulfate solutions using vapor diffusion techniques as previously described.[15] The crystals were grown from 4-μL drops hanging from siliconized microscope cover slips over the wells of a Linbro cell culture plate. The droplet consisted of 2 μL of 10-20 mg/mL of protein solution in 10 mM potassium phosphate buffer (pH 7.1) plus 2 μL of 35-40% saturated ammonium sulfate solution in 20 mM citrate buffer (pH 5.3). The droplet was equilibrated at room temperature against 1 mL of 35-40% saturated ammonium sulfate solution in 50 mM citrate buffer at pH 5.3 that was contained in the well of the Linbro plate. Rhombohedral-shaped crystals with dimensions up to 0.5 mm were obtained after 3-5 days. For X-ray studies these crystals were transferred to 55% saturated ammonium sulfate solution in 50 mM citrate buffer at pH 5.4 and stored at room temperature for up to several months.

Complexes between PNP and inhibitor molecules were prepared by transferring a pre-formed PNP crystal into 2 mL of the ammonium sulfate stabilizing solution described above in which the inhibitor has been dissolved. The final concentration of inhibitor was 1 mM or sometimes less for compounds with poor solubility. For these compounds, saturating amounts were added to the solution plus a small amount of excess solid. The PNP crystal was allowed to equilibrate for 24 h and then removed from the solution and mounted in a sealed glass capillary for X-ray intensity measurements.

All X-ray intensity measurements were recorded with a Nicolet/Siemens X-100 multiwire area detector. The X-rays were produced by a Rigaku RU-300 rotating anode generator operating at 100 mA and 50 kV with a 0.3 x 3 mm focus and a Cu anode. The X-ray beam was reflected from a graphite monochromator and passed through a 0.25-mm collimator. The crystal to detector distance was 120 mm and the detector was offset by 8° relative to the direct X-ray beam. X-ray intensity data was measured on 0.25° oscillation frames at 300 s of exposure per frame. Each crystal yielded from 300-500 frames of data before radiation damage prevented further measurements. One to three crystals were used for each PNP/inhibitor complex depending on crystal size and data quality.

The X-ray intensity data were reduced using the XENGEN package of programs. The integrated intensities were then scaled and merged to produce a final data set containing only unique reflections. The R-value for different measurements of equivalent reflections ranged from 8-12%. The final data sets were reasonably complete at 3.0-Å resolution with some data present between 3.0- and 2.8-Å resolution. The percent of possible reflections observed from a single crystal ranged from about 50-90% at 3.0 Å resolution depending on the initial orientation of the crystallographic three-fold axis and the number of data frames that were measured. For difference Fourier calculations the data for the PNP complexes was scaled to the native data using relative Wilson plots. The fractional change in intensity relative to the native data set ranged from 14-20%.

Difference Fourier maps were calculated at 3.2-Å resolution using F (complex) – F (native) as the Fourier coefficient and calculated phases from 5-3.2-Å resolution and experimentally determined phases from 10-5-Å resolution. Analysis of the difference electron density was performed on an Evans and Sutherland PS300 interactive computer graphics workstation using the computer graphics program FRODO. Idealized models were constructed for each inhibitor and then manually fitted to the difference electron density by varying torsion angles and overall orientation and position. The final coordinates were stored for further comparison and computational analysis.

Human PNP Enzyme Inhibition

A radiochemical PNP assay was used following the procedures of Krenitsky[18] and Fox[19] to determine the IC_{50} of inhibitors against human PNP. Enzyme activity was measured by the conversion of [8-^{14}C]-inosine to [8-^{14}C]-hypoxanthine. The reaction mixture was composed of 70 μL of 100 mM HEPES buffer (pH = 7.4, containing 1 mM phosphate) containing various concentrations of inhibitors and 10 μL of diluted RBC PNP (0.001 unit). The inhibitor was initially dissolved at 1 mg/mL in 10% DMSO/100 mM HEPES buffer. The reaction was started by the addition of 20 μL of 8 *M* formic acid. Thirty μL of the marker solution (hypoxanthine and inosine, 0.5 mg/mL and 1.3 mg/mL of water, respectively) was added to the reaction mixture. The product, hypoxanthine, was separated from inosine by chromatography of 20 μL of the reaction mixture on TLC sheets (Kodak cellulose). The sheets were developed in 1.6 *M* lithium chloride. The spots that were identified under UV lights as inosine or hypoxanthine were cut out and counted in 8-10 mL of scintillation counting solution. Percent conversion of substrate to product was calculated as described by Walsh[20] and the IC_{50} determinations were performed in triplicate.

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