Structure-Based Design of Inhibitors of Purine Nucleoside Phosphorylase. 3. 9-Arylmethyl Derivatives of 9-Deazaguanine Substituted on the Methylene Group

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X-ray crystallography and computer-assisted molecular modeling (CAMM) studies aided in the design of a potent series of mammalian purine nucleoside phosphorylase (PNP) inhibitors. Enhanced potency was achieved by designing substituted 9-(arylmethyl)-9-deazaguanine analogs that interact favorably with all three of the binding subsites of the PNP active site, namely the purine binding site, the hydrophobic pocket, and the phosphate binding site. The most potent PNP inhibitor prepared during our investigation, (*S*)-9-[1-(3-chlorophenyl)-2-carboxyethyl]-9-deazaguanine (**18b**), was shown to have an IC_{50} of 6 nM, whereas the corresponding (*R*)-isomer was 30-fold less potent.

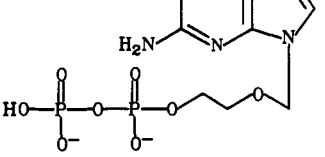
Introduction

Previous studies using X-ray crystallography and computer-assisted molecular modeling (CAMM) have proven valuable in the discovery of purine nucleoside phosphorylase (PNP) inhibitors.[1-3] In these studies, 9-substituted 9-deazaguanines[4] were found to be particularly potent inhibitors of PNP due, at least in part, to a hydrogen bond made between the 7-NH of the 9-deazaguanine base and the side-chain carbonyl of Asn-243.[1] Equally important was the 9-substituent, which was found to interact with a hydrophobic pocket formed by the side chains of the active-site residues Phe-159 (from the adjacent subunit), Phe-200, Tyr-88, His-257, and Met-219.

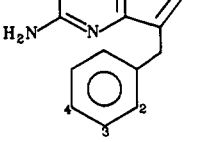
In addition to the purine binding site and the hydrophobic pocket, the PNP crystal structure[3] revealed the location of a third possible binding domain, namely the phosphate binding site. Compounds that interact favorably with this site were anticipated to be even more potent and selective inhibitors. One compound already shown by X-ray crystallography to interact with all three sites[3] is acyclovir diphosphate (K_i = 8.7 nM).[5] The pyrophosphate group, however, limits its therapeutic utility, since it is highly charged and therefore does not cross cell membranes and since it is expected to be rapidly dephosphorylated *in vivo*.

Results

Inhibitor Design: Molecular Modeling and X-ray Crystallography. The X-ray coordinates obtained for native PNP were used to generate a computer model of the 9-benzyl-9-deazaguanine–PNP complex.[1] Positioning of the inhibitor in the PNP active site was based upon difference fourier maps (*F*(complex) – *F*(native)). Visual inspection of the complex revealed which sites on the phenyl ring had the best geometrical orientation to enable interaction with the phosphate binding site. One edge of the phenyl ring (C2–C3) was found to be close to the phosphate binding site, whereas the 4-position appeared less favorable. On the basis of the distance and geometrical orientation of the phosphate relative to the aryl ring, a four-atom spacer between the phosphorus atom and the 2-position appeared to be optimal. Consequently, we studied the four-carbon analog of phosphonate **4h** (Scheme I) by molecular modeling by docking it into the active site of the model in a conformation similar to the one observed crystallographically for acyclovir diphosphate. The sulfate ion that is found in the phosphate site in the native structure (PNP is crystallized in the presence of 60% ammonium sulfate)[6] was deleted and replaced with the phosphonate group. The complex was then subjected to energy minimization using the techniques employed in our previous work on 9-substituted 9-deazaguanines.[1-3] Analysis of the molecular mechanics energy components indicated that no significant strain had been introduced into the inhibitor nor were there any unfavorable steric contacts between the inhibitor and the enzyme. These initial molecular modeling studies led us to erroneously conclude that phosphonate **4h** would be a potent inhibitor. In fact, **4h** was found to be no better an inhibitor (see Table I) than the 9-(phosphonoalkyl)hypoxanthines previously described.[7] These results conflicted with our expectation that the aryl analogs would be more potent due to favorable aryl interactions with the hydrophobic pocket aryl groups and due to the fewer degrees of conformational freedom in the 9-arylmethyl analogs. Subsequent X-ray crystallographic analysis of the PNP/**4h** complex revealed that although the inhibitor occupied



Acyclovir Diphosphate



9-Benzyl-9-deazaguanine

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Scheme I

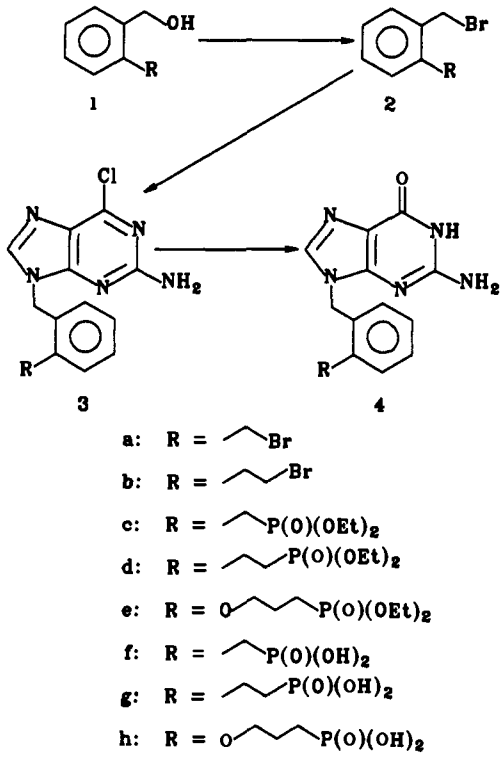
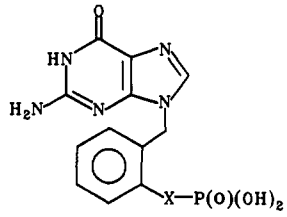


Table I. Inhibition of PNP[a]



		IC_{50} (nM)		
no.	X	1 mM PO_4	50 mM PO_4	ratio[b]
4f	$-(CH_2)_2-$	35	450	13
4g	$-(CH_2)_3-$	620	12500	20
4h	$-O(CH_2)_3-$	1000	42000	42

[a] Calf spleen. [b] Ratio = IC_{50} 50 mM PO_4/IC_{50} 1 mM PO_4.

all three subsites, the greater than optimum length of the spacer (see below) caused rotation of the phenyl ring by almost 90° from the optimal position observed in the 9-benzyl-9-deazaguanine complex.[1] Also, displacement of the guanine base had occurred. Presumably, these movements were required in order to accommodate the inhibitor and therefore provide an explanation for the lack of enhanced binding of **4h** (Figure 1).

The inaccuracy of these results was puzzling until the X-ray crystal structure for the PNP–guanine complex was solved. Analysis of this complex relative to the native structure showed that the binding of guanine induced significant movements within a loop made up of residues 241–260. As a result of this movement, the phosphate binding site moves closer to the purine and hydrophobic binding sites, thereby accounting for the inappropriate spacer length. To determine the appropriate spacer length, we undertook a molecular modeling study of 9-benzyl-9-deazaguanine analogs substituted at the 2-position with phosphonoalkyl substituents. In this study, we used the coordinates for the PNP–guanine complex and the MC/EM conformational searching techniques[8,9] described in the previous papers of this series.[1-3] The study was designed to predict plausible binding modes for the 9-benzylguanine analogs containing alkyl phosphonate side chains attached to the 2-position of the benzene ring with one to four carbon atom spacers. Inorganic sulfate bound in the phosphate binding site was deleted and replaced with the phosphonate group of the inhibitor. The same restraining potentials were used for the amino acid residues comprising the phosphate binding site as used in previous calculations in which phosphate was present. Several hundred MC/EM steps were employed.[10] The association energies (i.e., the sum of the van der Waals and electrostatic energies associated with the interaction between the inhibitor and the enzyme binding site plus the strain energy induced in the ligand upon binding) for these four compounds and the PNP binding site indicated that the compound with two spacer atoms would be the most potent PNP inhibitor. Figure 2 shows a plot of association energy as a function of the number of intervening carbon atoms. In agreement with these calculations, the inhibitor with two spacer atoms is indeed the most potent PNP inhibitor in this series (Table I). For synthetic convenience, all of the inhibitors shown in Table I were prepared in the guanine series as opposed to the 9-deazaguanine series. Nonetheless, using the coordinates for the PNP/guanine complex and more sophisticated molecular modeling studies based on the MC/EM procedure, we were able to predict *in advance of chemical synthesis* and testing which of the alkyl phosphonates would be the most potent PNP inhibitor. Furthermore, in agreement with the crystallographic analyses, the modeling results for the phosphonate inhibitors clearly showed that as the chain length increases, a change in the tilt of the benzene ring must occur to accommodate the longer chain between the 2-position of the aryl group and the phosphonate that is positioned in the phosphate binding site (see Figure 3). X-ray analysis of the complexes showed that in comparison to **4h**, the phenyl ring of **4f** more closely matches the orientation of the 9-benzyl-9-deazaguanine phenyl group, although it is still not optimal (Figure 1). These results indicated that compounds capable of occupying all three binding sites can be potent PNP inhibitors. Although potency could potentially be further optimized by using the 9-deazaguanine base or by using a different spacer,[11] we decided to turn our attention toward groups that avoided the major limitations of phosphonic acids; namely, poor cell penetration and poor oral activity.

An alternative site on 9-benzyl-9-deazaguanine for a group that could interact with the phosphate binding site is the benzylic methylene. Visual analysis of the 9-benzyl-9-deazaguanine–PNP complex showed that of the two benzylic positions, one pointed into a sterically congested area within the active site, whereas the other position pointed off into a relatively empty volume adjacent to the phosphate binding site (Figure 4). This analysis and the synthetic accessibility of this series led to the preparation of racemic 9-[1-(3-chlorophenyl)-2-cyanoethyl]-9-deazaguanine (**15b**) for studies by enzyme kinetics, X-ray crystallography, and CAMM.

Conversion of the cyano group of **15b** to a carboxyl was of considerable interest, since we expected the negatively-charged carboxyl (of **18b**) to provide some additional binding affinity through its interaction with the positively-charged phosphate binding site. Synthesis of racemic 9-[1-

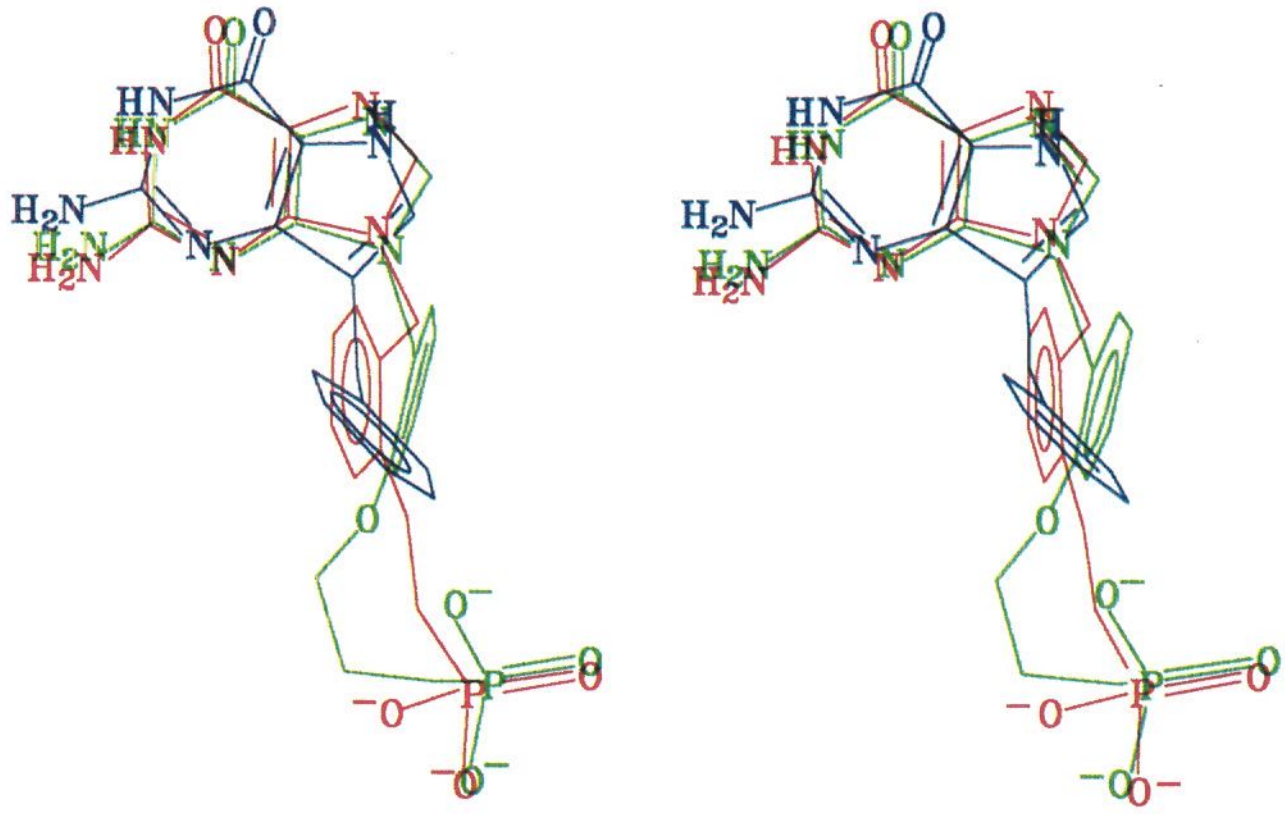


Figure 1. Stereo comparison of the binding of **4f** (blue) and **4h** (green) with that of 9-benzyl-9-deazaguanine (red) in the active site of PNP as determined by X-ray crystallography.

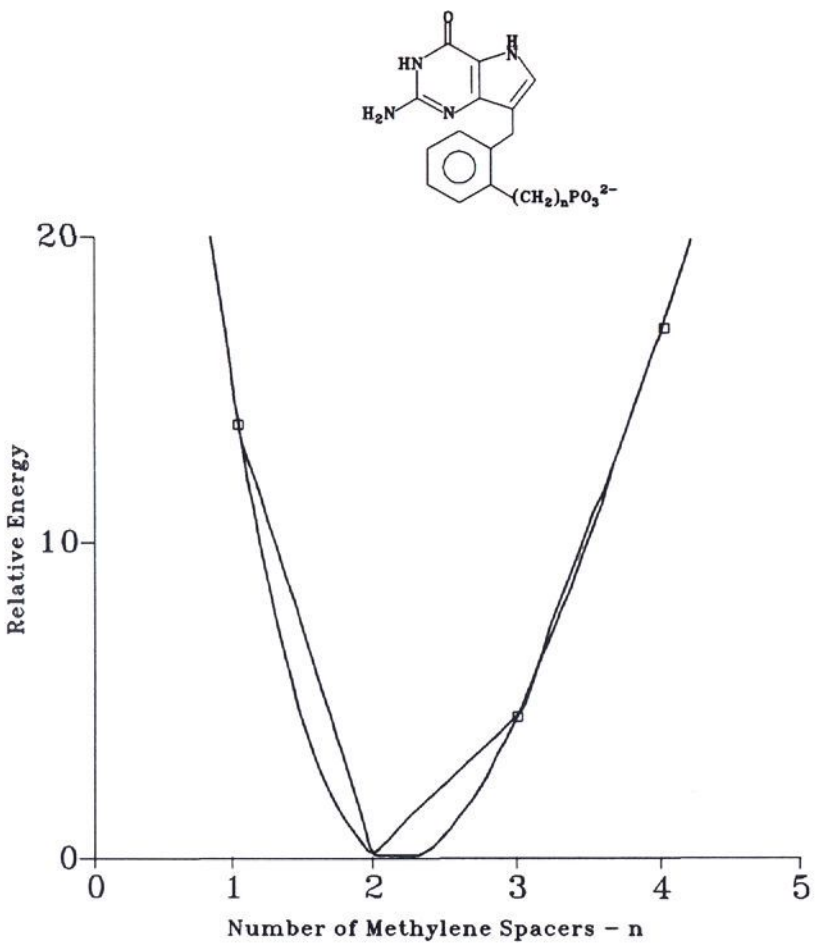


Figure 2. Plot of the energy of association between PNP and 9-benzyl-9-deazaguanine inhibitors substituted at the ortho position by phosphonate vs chain length. The association energy was computed as the sum of the van der Waals and electrostatic energy of interaction between the inhibitor and enzyme binding site residues plus the strain energy induced in the inhibitor upon binding (computed relative to the global minimum of the uncomplexed ligand).

(3-chlorophenyl)-2-carboxyethyl]-9-deazaguanine (**18b**) followed by resolution into the optically active antipodes showed that the (*S*)-isomer is about 30-fold more potent than the (*R*)-isomer with an IC_{50} of 5.9 and 160 nM, respectively (see Table II). The enantiomeric preference is in agreement with the docking experiments and the crystallographic results obtained for each PNP complex (Figure 5). The 3-chlorophenyl ring of the (*S*)-isomer is oriented almost identically to that of 9-(3-chlorophenyl)-9-deazaguanine itself, and the carboxyl group is oriented

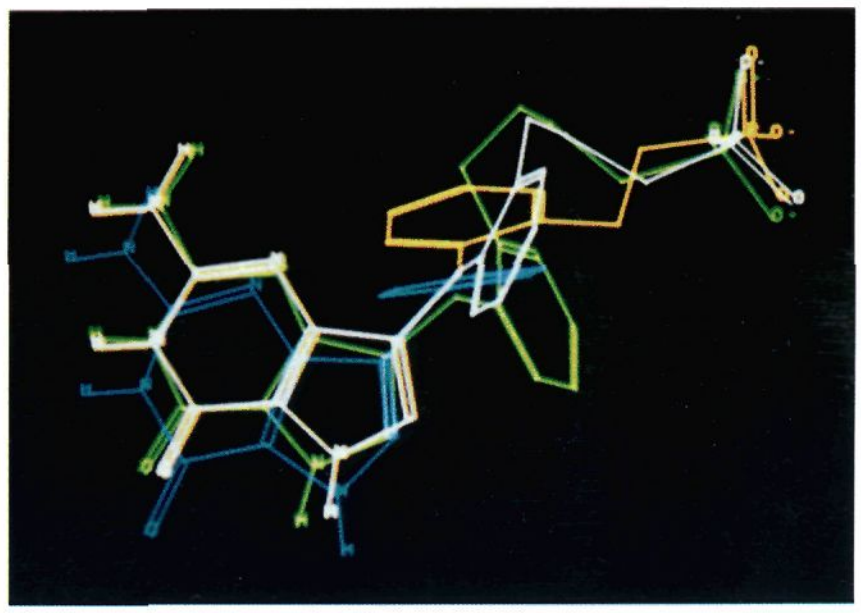


Figure 3. Comparison of the computed global minimum-energy structures obtained from MC/EM conformational searches in the PNP binding site for 9-benzyl-9-deazaguanine (blue) and its ortho-substituted alkyl phosphonates with two (orange), three (white), and four (green) intervening methylene groups (enzyme not shown).

toward the phosphate binding site (Figure 5A). In contrast, the (*R*)-isomer is bound in a different conformation with neither the 3-chlorophenyl ring nor the carboxyl group in proper orientation with the enzyme for maximal interaction (Figure 5B). The conformation of the carboxyl side chain and its potential interaction with the phosphate binding site is difficult to ascertain from crystallography, since the crystals were grown in saturated ammonium sulfate and at pH 5.5. Under these conditions, the phosphate binding site is completely occupied by sulfate and the carboxylate could exist in the protonated form. Possibly for these reasons, the crystal structure of the (*S*)-**18b**:PNP complex showed no direct contact of the carboxylate of (*S*)-**18b** with any residues of the phosphate binding site. The dependence of the IC_{50} on phosphate concentration, however, indicates either a direct interaction or a ligand-induced conformational change that affects phosphate binding. Since current crystallization conditions do not permit X-ray analysis of the inhibitor under physiological conditions (low phosphate concentration and pH 7.4), we evaluated several of the inhibitors by CAMM

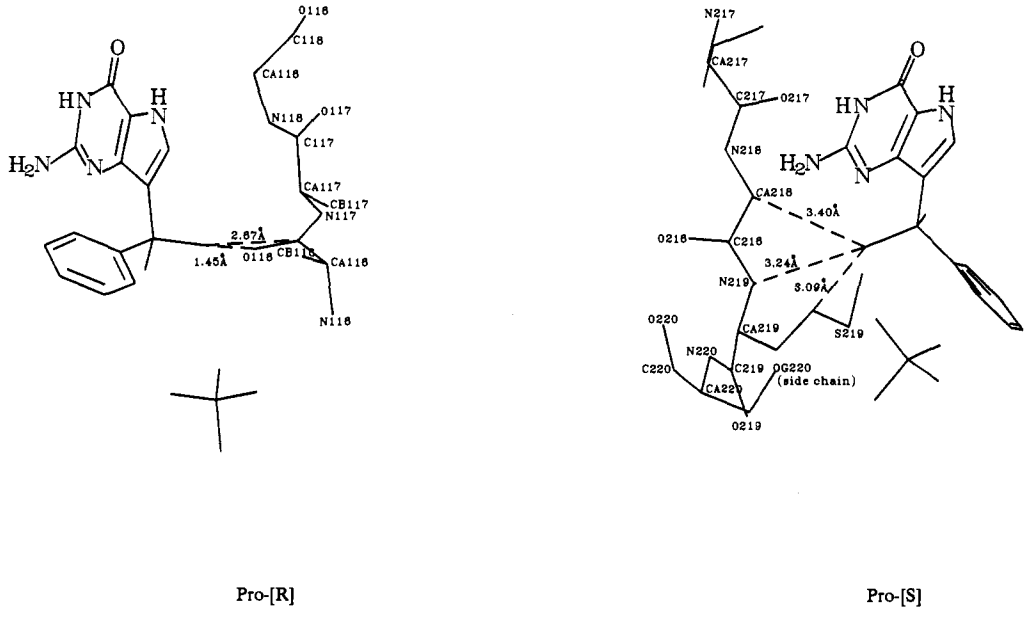
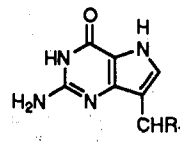


Figure 4. Docking experiment on 9-benzyl-9-deazaguanine showing the accessibility of the two positions on the benzylic carbon.

Table II. Inhibition of PNP[a]



no.	R_1	R_2	IC_{50} (nM)[b] 1 mM PO_4	IC_{50} (nM)[b] 50 mM PO_4	ratio[c]
	3-chlorophenyl[d]	H[e]	20 ± 7	150 ± 2	7.5
(*S*)-18b	3-chlorophenyl[d]	CH_2CO_2H[e]	5.9	31	5
15b	3-chlorophenyl[d]	CH_2CN[e]	11 ± 2	1500 ± 650	136
22	3-chlorophenyl[d]	CH_2CH_2OH	25	1400	56
19	3-chlorophenyl[d]	CH_2CO_2Me	85	80000	940
(*R*)-18b	3-chlorophenyl[d]	CH_2CO_2H[e]	160	900	6
	phenyl[d]	H[e]	51 ± 12	210 ± 30	4
18a	phenyl[d]	CH_2CO_2H[e]	13 ± 1	187 ± 4	14
16	phenyl[d]	CH_2CN	23 ± 3	4700 ± 3300	204
17	phenyl[d]	CH_2CONH_2	200	6600	33
	cyclohexyl[f]	H[e]	47 ± 14	2100 ± 30	45
21	cyclohexyl[f]	CH_2CO_2H[e]	97	1000	10
23	cyclohexyl[f]	CH_2CH_2OH	6000[g]	90000[g]	15

[a] Calf spleen. [b] All values are averages of two determinations except where standard deviations are shown. [c] Ratio = IC_{50} 50 mM PO_4/IC_{50} 1 mM PO_4. [d] Reference 1. [e] Structure of complex with PNP determined. [f] Reference 2. [g] Assay was performed by measuring ^3H release from [^3H]inosine via [^3H]hypoxanthine (Chang, C.-H.; Bennett, L. L., Jr.; Brockman, R. W. *Anal. Biochem.* 1989, *18*, 279–282).

in an attempt to understand the effect of phosphate on the inhibitor binding conformation. The modeling studies were performed using a computer model generated from the coordinates of the PNP–guanine complex in which the base portion of 9-(1-phenyl-2-carboxyethyl)-9-deazaguanine (**18a**) was positioned in the active site like guanine and the sulfate was replaced by phosphate. The carboxylic acid was modeled in the anionic form. Each enantiomer was subjected to the MC/EM conformational search procedure.[9] In each case, one of the low-energy conformers for both the (*S*)- and the (*R*)-isomers compared favorably with the conformations determined via X-ray diffraction studies (Figures 6 and 7). Furthermore, the difference in association energies calculated for each enantiomer is 2.8 kcal/mol in favor of the (*S*)-isomer.

A similar modeling study was conducted with phosphate replaced by water molecules. Analysis of the phosphate binding site indicated that two and possibly three water molecules could occupy the site[15] in the absence of phosphate. In this case, the MC/EM procedure was conducted so that two water molecules as well as the inhibitor (initially placed in concordance with molecular graphics analysis) were allowed to move, via translation/rotation, during the MC phase of the procedure. The inhibitor and the water molecules were also unrestrained during the energy-minimization phase. The results showed the carboxylate of **18a** was in close proximity to the NH of the positively charged His-86 and the OH of Ser-220 in the low-energy conformations.

CAMM studies on the cyanomethyl compound **16** in the presence of phosphate gave low-energy conformations similar to the results found by X-ray crystallography. In the absence of phosphate, the cyano group was capable of interacting with at least one of the water molecules that occupied the phosphate binding site (Figure 8). In view of these results, we determined the kinetics of the inhibition of PNP with respect to inorganic phosphate by the cyano compound **15b** and found that the inhibition was competitive with a K_i of 5 nM, providing an explanation for the larger IC_{50} values (Table II) in 50 mM phosphate.

Reduction of the phenyl ring of **18b** gave the cyclohexyl compound **21**, which is a weaker inhibitor than both **18b** and the parent 9-(cyclohexylmethyl)-9-deazaguanine. The loss in potency appears to arise from the way the cyclohexyl group is forced to interact with the hydrophobic pocket and from the effect of the cyclohexyl group on the side-chain carboxyl. Interestingly, when a crystal of PNP is soaked in a solution of the racemate, it is the (*R*)- not the (*S*)-isomer that binds in the active site. As can be seen from Figure 9, the binding of (*R*)-**21** forces the 9-deazaguanine moiety out of its normal position in the purine binding site, thereby weakening that normally strong interaction and probably accounts, at least in part, for the decreased potency of the cyclohexyl series relative to the aryl series. Furthermore, modeling studies indicate that, in the case of (*S*)-**21** isomer, the hydrogens of the side-chain methylene group interfere with those of the cyclohexyl ring when the carboxyl group is oriented to interact with the phosphate binding site. As a result, (*R*)-**21** is more potent than (*S*)-**21** but less potent than 9-(cyclohexylmethyl)-9-deazaguanine (see Table II).

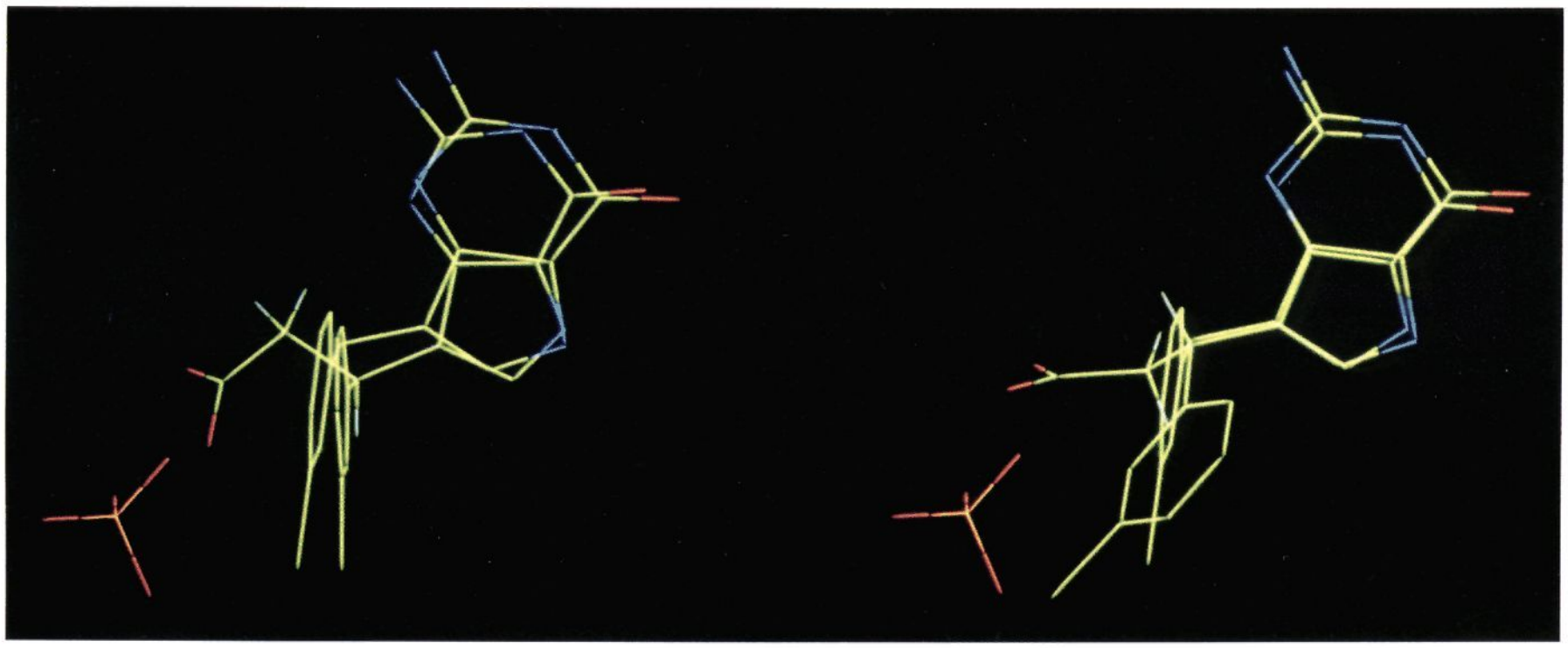


Figure 5. Comparison of the binding of (A, left) (*S*)-**18b** and (B, right) (*R*)-**18b** with that of 9-benzyl-9-deazaguanine in the active site of PNP as determined by X-ray crystallography.

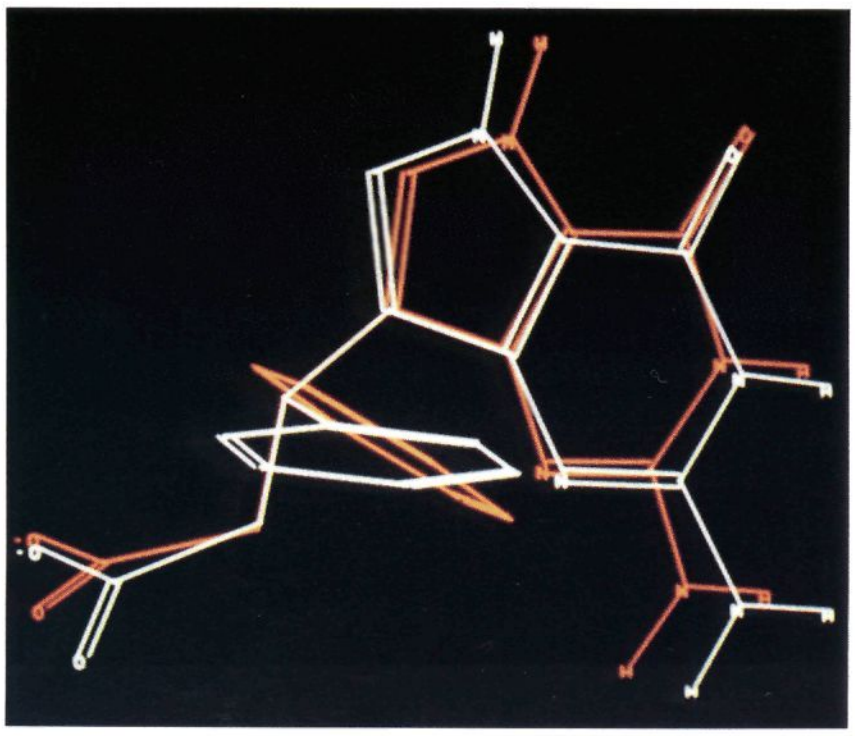


Figure 6. Superimposition of the computed (white) vs X-ray structure (red) for PNP–(*S*)-**18a** complex (enzyme not shown). The computed structure is the third lowest energy conformation obtained from an MC/EM conformational search for low-energy conformers of (*S*)-**18a** in the PNP binding site.

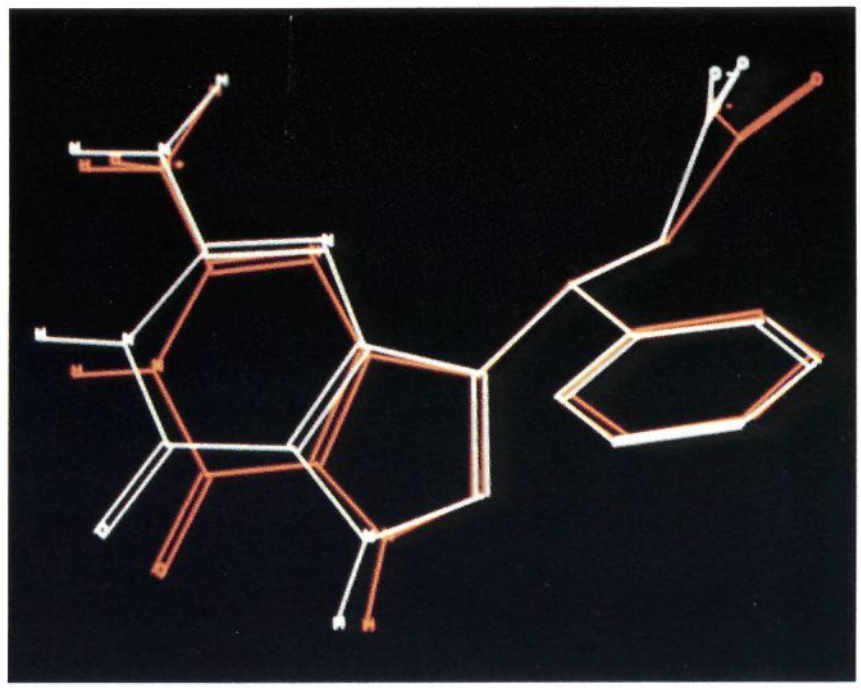


Figure 7. Superimposition of the computed (white) vs X-ray structure (red) for the PNP–(*R*)-**18a** complex (enzyme not shown). The computed structure is the twelfth lowest energy conformation obtained from an MC/EM conformational search for low-energy conformers of (*R*)-**18a** in the PNP binding site. The fourth lowest energy conformer agreed well with the X-ray structure, but not as well as the conformer shown.

Discussion

The crystal structure of PNP revealed the relative location of three separate binding sites within the active site of PNP, namely the purine, hydrophobic, and phosphate binding sites. Our previous studies[1,2] focused on compounds that optimally occupy the purine and hydrophobic binding sites. In this study, we attempted to design compounds that interact with these sites as well as the phosphate binding site (either directly or via electrostatic interactions). Starting with a model of the 9-benzyl-9-deazaguanine–PNP complex, we concluded that two of the positions on the 9-benzyl group, namely the 2-position and one of the benzylic sites, appeared to be oriented so that a group attached to either one of these positions could interact favorably with the phosphate binding site. In the initial design, we used a four-atom spacer between the phenyl ring and a phosphonate group to generate a phosphonate that would interact with residues in the phosphate binding site. This spacer length was based on the distance between the hydrophobic site and the phosphate binding site as modeled using the coordinates obtained for the native enzyme. Once the structure of the PNP–guanine complex was solved, it became apparent that binding of a purine causes a conformational change in part of the protein so that the distance between the purine and phosphate binding sites is considerably shorter than in native PNP. Consequently, a two-atom spacer was predicted in advance of chemical synthesis to be the optimal length. Synthesis of the target molecule **4f** gave a 35 nM inhibitor of PNP. These results point clearly to the importance of using coordinates generated from an enzyme inhibitor complex for conducting modeling studies. Furthermore, the X-ray crystallographic results obtained for the phosphonates indicate the importance of the aromatic ring orientation, since the major change in position in the weakly active phosphonate **4h** involved a 90° rotation of the phenyl ring. The potency of phosphonate **4f** (prepared only in the guanine series) was in

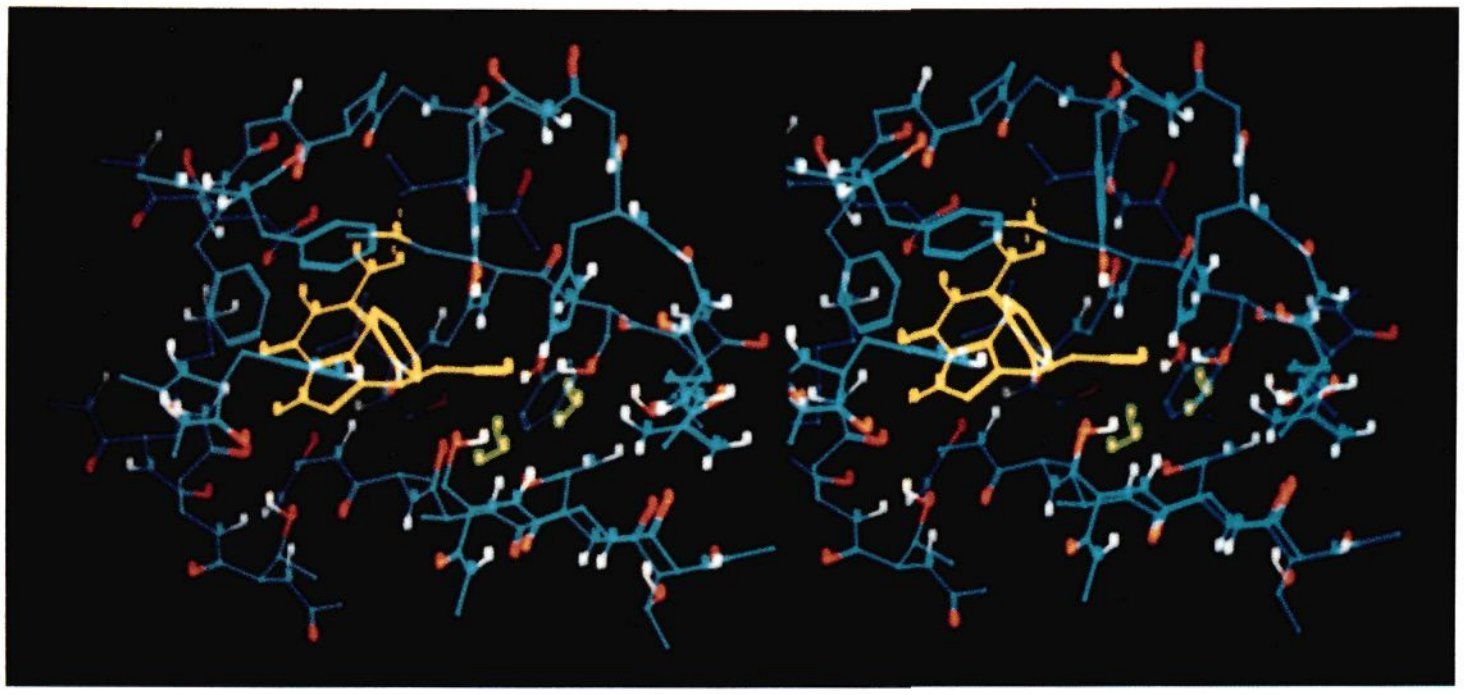


Figure 8. Stereo representation of the global minimum-energy structure of the PNP–(*S*)-**16** complex obtained from an MC/EM conformational search for the low-energy conformations of (*S*)-**16** in the PNP binding site in which phosphate had been replaced by two water molecules.

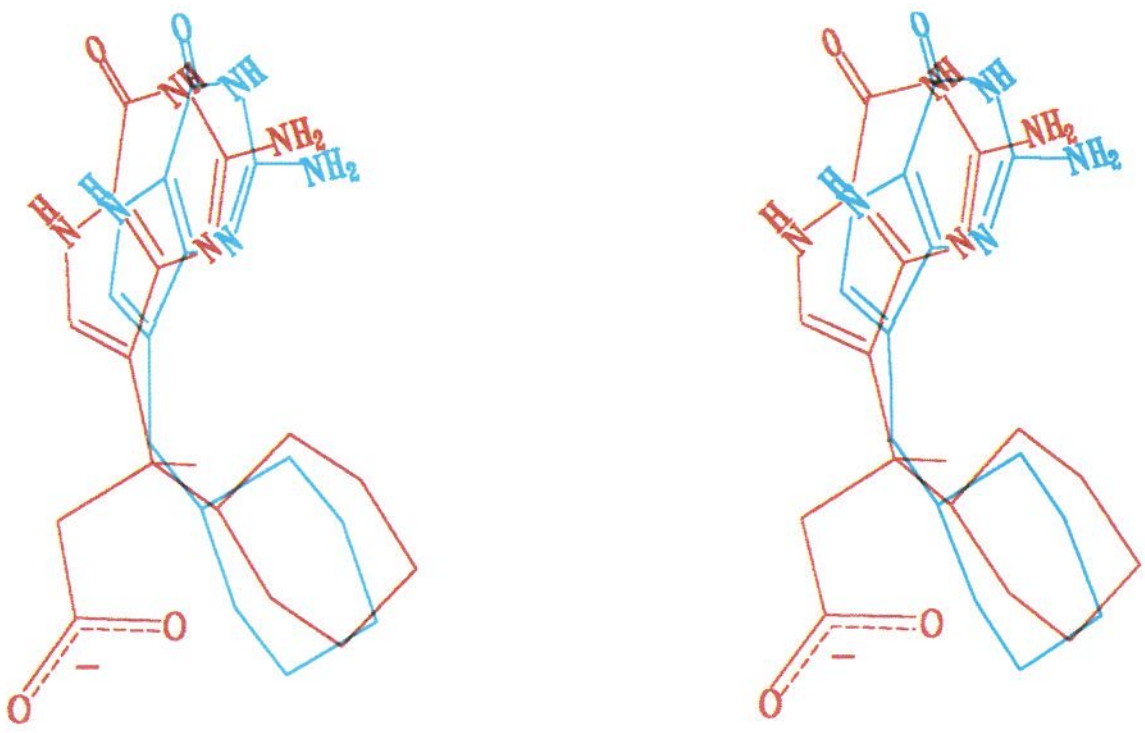


Figure 9. A stereo comparison of the binding to PNP of (*R*)-**21** (red) with that of 9-(cyclohexylmethyl)-9-deazaguanine (blue) as determined by X-ray crystallography.

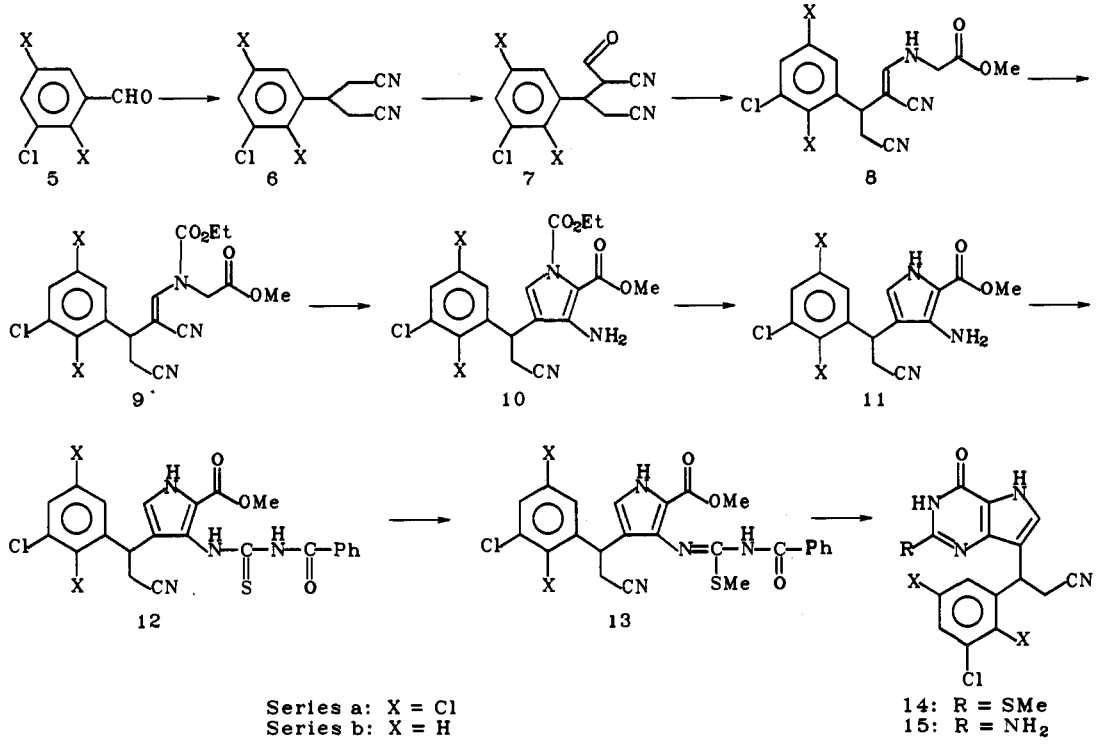
the range of the potencies of the previously described 9-(arylmethyl)-9-deazaguanine series.[1-3] Since increased potency has been observed[1] for PNP inhibitors in the 9-deazaguanine series relative to those in the guanine series, it could well be that a compound like phosphonate **4f** that bears the 9-deazaguanine would be a very potent PNP inhibitor. Nonetheless, for the reasons mentioned above, we have not pursued this line of investigation.

The other site on the 9-benzyl group of 9-benzyl-9-deazaguanine that modeling studies indicated points directly toward the phosphate binding site was the *pro-S* hydrogen of the benzylic methylene group. Accordingly, the (*S*)-isomer of the acid **18b** was prepared and found to be a 30-fold more potent inhibitor of PNP than the (*R*)-isomer, but only a 2–4-fold more potent inhibitor than the parent 9-(3-chlorobenzyl)-9-deazaguanine. X-ray crystallographic results indicated that the carboxylate made few, if any, of the same interactions with the protein as phosphate. These results, however, were generated in the presence of high sulfate at pH 5.5, and thus the phosphate site was fully occupied by sulfate. X-ray analysis of inhibitor binding without sulfate in the phosphate binding site has thus far been impossible due to the inability to grow quality crystals under more physiologic conditions. CAMM studies, on the other hand, allow replacement of the sulfate with water, thereby providing a method for analyzing inhibitors that can interact with the phosphate binding site. No significant changes were found in the inhibitor binding conformations, but potential interactions with water molecules localized in the phosphate binding site were noted. Although extension of the spacer group might sterically allow the interacting group (ie., carboxyl or cyano) to reside within the phosphate binding site, we have evidence that suggests that such a change would not greatly enhance the potency of **15b** or **18b**.[16]

Interestingly, when the aryl ring was reduced to the cyclohexyl analog, a significant portion of the binding affinity was lost and the (*R*)-isomer was favored over the (*S*)-isomer. CAMM studies indicated that the side-chain orientation could not be maintained identically to the analogous aryl example due to unfavorable interactions between the side chain and the hydrogen atoms of the cyclohexyl ring.

In summary, X-ray crystallography and molecular modeling studies proved useful in identifying positions on 9-benzyl-9-deazaguanine that properly substituted

Scheme II. 9-[2-Cyano-1-(3-chlorophenyl)ethyl]-9-deazaguanine



could interact with the phosphate binding site. One potent PNP inhibitor discovered during the course of this work was phosphonate **4f** which occupied all three binding sites as shown by X-ray crystallography (Figure 1). A second series of compounds was realized from substitution for the *pro-S* hydrogen of the benzylic methylene. The most potent compounds found in this series of 9-benzyl-9-deazaguanine analogs were competitive inhibitors with IC_{50} values around 5 nM. Although these compounds did not occupy the phosphate binding site, they did have groups positioned nearby and therefore likely benefited from the hydrophilic nature of this region of the PNP active site. To the best of our knowledge, the cyanomethyl compound **15b** is the most potent membrane-permeable PNP inhibitor prepared to date.

Chemistry

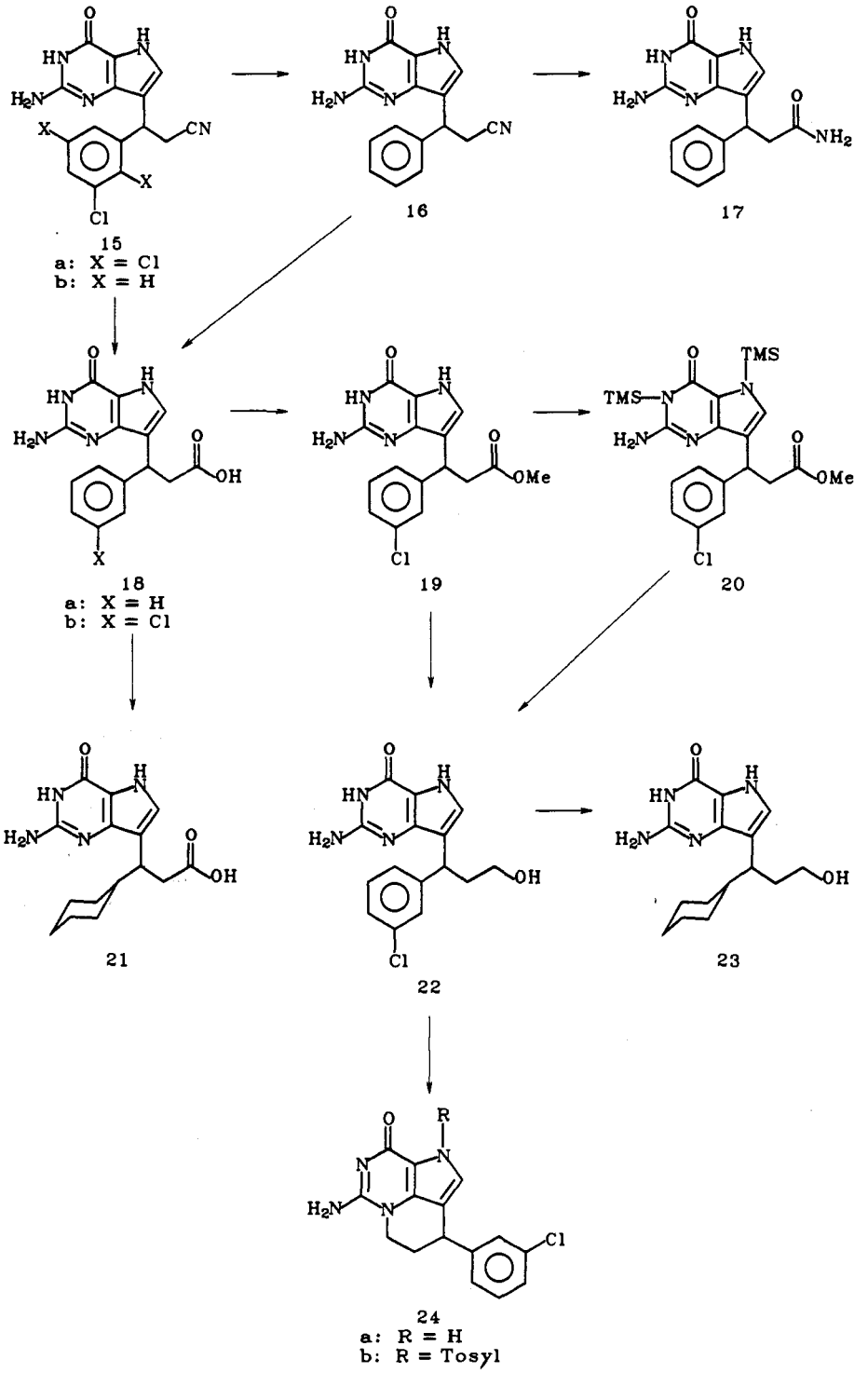
Two synthetic sequences were used to prepare the aryl phosphonate analogs of acyclovir diphosphate (Scheme I). In the first approach, the whole side chain (**2e**) was assembled and used to alkylate 2-amino-6-chloropurine to give **3e**. In the second sequence, the (bromoalkyl)benzyl bromides **2a** and **2b** were prepared[17] and used to alkylate 2-amino-6-chloropurine to give **3a** and **3b**, which were allowed to react with triethyl phosphite to give **3c** and **3d**. The diethyl phosphonates **3c–e** were treated with trimethylsilyl bromide to convert them to the phosphonic acids (**3f–h**), which were hydrolyzed in aqueous base to give the desired guanine derivatives (**4f–h**).

The 9-deaza-9-substituted-guanines **15a** and **15b** were prepared by the procedures reported in our earlier publications (Scheme II).[1,2] The 3-substituted pentanedinitriles (**6a** and **6b**), starting materials for these 9-deaza-9-substituted-guanines, were prepared by the treatment of the corresponding benzaldehydes (**5**) with a several-fold excess of cyanoacetic acid in the presence of sodium acetate as the catalyst in a solution of toluene and pyridine (2.5:1). However, superior results were obtained when pyridine was used as a solvent along with a catalytic amount of piperidine consistent with reported results.[18] These 3-arylpentanedinitriles (**6a** and **6b**) were characterized by MS, IR, ^{1}H NMR, and elemental analyses. Their infrared spectra exhibited a single band at 2257 (**6a**) and two bands at 2260 and 2240 cm^{-1}, consistent with bands for nitriles.

Compound **16** was prepared in 88% yield by catalytic hydrogenation of **15a** with Pd–C in the presence of triethylamine (Scheme III). The acetonitrile **16** was converted to the corresponding amide **17** by treatment with concentrated H_2SO_4.

The alcohol **22** was prepared in 67% overall yield in four steps from **15b** by the hydrolysis of the nitrile group with 6 N HCl to the corresponding carboxylic acid **18b** which, in turn, was converted to the methyl ester **19** in 81% yield by treatment with methanol and thionyl chloride. Direct reduction of the ester **19** to the target alcohol **22** was only partially successful due to its limited solubility in solvents like Et_2O or THF that are compatible with $LiAlH_4$. Silylation of **19** by refluxing with hexamethyldisilazane and a catalytic amount of $(NH_4)_2SO_4$ overcame this difficulty, providing improved solubility and rapid completion of reduction. Stirring the crude product from **20** for a few minutes with 0.1 N HCl easily desilylated the alcohol to give **22**. The remarkable resistance of the 9-deazaguanine moieties of the acid **18b** and the alcohol **22** to catalytic hydrogenation under acidic conditions allowed direct conversion of the chlorophenyl groups of these analogs to the corresponding cyclohexyl analogs **21** and **23**, respectively. An attempt to prepare the tosyl derivative of **22** by treating it with *p*-toluenesulfonyl chloride in pyridine with a catalytic amount of 4-(dimethylamino)pyridine gave compound **24a**, which was identified by mass spectral analysis. Another attempt to obtain the tosyl derivative involved reaction of **22** with NaH in DMAC followed by addition of *p*-toluenesulfonyl chloride. The

Scheme III



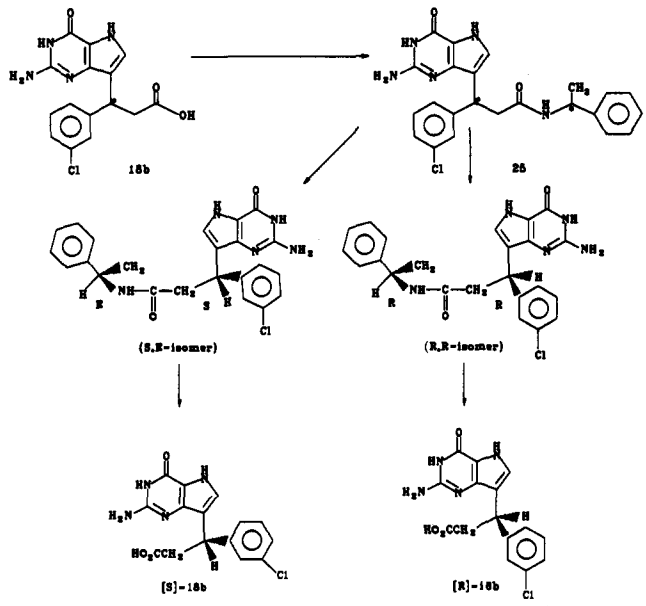
product of this reaction was identified as compound **24b** by mass spectral and ^1H NMR data.

Compound **18b**, a racemic mixture of our most potent PNP inhibitor, was resolved as shown in Scheme IV. Treatment of **18b** with (*R*)-(+)-α-methylbenzylamine, *N*-methylmorpholine, and diphenylphosphoryl azide in dry DMF gave the desired amide **25** as a mixture of diastereomers, which were separated by repeated flash column chromatography on silica gel using acetonitrile and 1 M ammonium hydroxide (98:2) as the eluent to give the (*S*,*R*)-isomer followed by the (*R*,*R*)-isomer. These pure (*S*,*R*)- and (*R*,*R*)-amides were hydrolyzed by treatment with 6 N HCl to the corresponding (*S*)- and (*R*)-acids.

Experimental Section

Chemistry. All evaporations were carried out *in vacuo* with a rotary evaporator or by short-path distillation into a dry ice/acetone-cooled receiver under high vacuum. Analytical samples were normally dried *in vacuo* over P_2O_5 at room temperature for 16 h; high-melting compounds were dried at 110 °C. Analtech precoated (250 μm) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and absorption of iodine vapor. All analytical samples gave a single spot on TLC. Melting points were determined by the capillary method with a Mel-Temp apparatus unless otherwise specified and are uncorrected. Purifications by gravity column and by flash chromatography were carried out on Merck silica gel 60 (230–400 mesh) using the slurry method of column packing.

Scheme IV



The UV absorption spectra were determined in 0.1 N HCl (pH 1), pH 7 buffer, and 0.1 N NaOH (pH 13) with a Cary 17 spectrophotometer and a Perkin-Elmer ultraviolet–visible near-infrared spectrophotometer Model Lambda 9: the maxima are reported in nanometers ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}). The ^1H NMR spectra of all compounds were determined with a Nicolet/GE NT 300NB spectrometer operating at 300.635 MHz with tetramethylsilane as an internal reference. Chemical shifts (δ, ppm) quoted in the case of multiplets are measured from the approximate center. The mass spectra were obtained with a Varian-MAT 311A mass spectrometer in the fast-atom-bombardment (FAB) mode or the electron-impact (EI) mode. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Infrared spectra were determined in pressed KBr disks with a Nicolet Model 10DX spectrophotometer; values are reported in reciprocal centimeters (cm^{-1}).

2-[3-(Diethylphosphono)propoxy]benzyl Alcohol (1e). A mixture of 2-hydroxybenzyl alcohol (1.24 g, 0.01 mol), diethyl-(bromopropyl)phosphonate (2.59 g, 0.01 mol), and anhydrous potassium carbonate (3.04 g, 0.022 mol) in anhydrous dimethylformamide (10 mL) was stirred in an oil bath at 84 °C for 7 h. The mixture was concentrated under reduced pressure, and the residue was partitioned between $CHCl_3$ (50 mL) and 5% aqueous NaOH (50 mL). The $CHCl_3$ layer was washed with water (2 × 50 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by chromatography over a column of silica using 2% MeOH in EtOAc as the eluent to obtain 1.72 g (57%) of **1e** as a colorless oil: TLC 98:2 EtOAc–MeOH, R_f 0.26; MS m/z 302 (M^+); ^1H NMR ($CDCl_3$) δ 1.32 (6H, t, CH_2CH_3), 1.96 (2H, m, CH_2P), 2.14 (2H, m, $CH_2CH_2CH_2$), 2.72 (1H, br s, OH), 4.0–4.2 (6H, m, CH_2CH_3 and CH_2CH_2O), 4.7 (2H, s, CH_2OH), 6.8–7.35 (4H, aryl-H).

2-[3-(Diethylphosphono)propoxy]benzyl Bromide (2e). A stirred suspension of **1e** (0.605 g, 2.0 mmol) and $NaHCO_3$ (0.84 g, 10 mmol) in CH_2Cl_2 (10 mL) was treated dropwise with a solution of PBr_3 (1.272 g, 4.7 mmol) in CH_2Cl_2 (10 mL). After the exothermic reaction and evolution of CO_2 subsided, the mixture was stirred at room temperature for 4 h and then poured on an ice water mixture. The layers were separated, and the organic layer was washed with water (20 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure yielded 0.686 g (94%) of **2e** as a colorless oil: TLC EtOAc, R_f 0.32; FAB MS m/z 365 $(M + 1)^+$. The product thus obtained was used in the next step without further purification.

2-Amino-6-chloro-9-[2-(2-bromoethyl)benzyl]purine (3a). To a suspension of 2-amino-6-chloropurine (5.0 g, 29.65 mmol) in anhydrous DMF (80 mL) was added the benzyl bromide (**2a**) (7.5 g, 26.95 mmol) and anhydrous potassium carbonate (7.45 g, 53.9 mmol). The mixture was stirred at room temperature for 4 h and stored at 5 °C for 64 h before filtering and evaporating the filtrate under reduced pressure. Portions of toluene and then methanol were added and evaporated to remove all traces of DMF. The residue was taken up in dichloromethane and filtered, and the filtrate was applied to a pad (6 × 9 cm) of silica gel (70–230 mesh), eluting first with dichloromethane and then with 2% methanol in dichloromethane. Fractions containing homogeneous product were combined, and the solvent was removed by evaporation under reduced pressure to provide the chloropurine in 63% yield: mp 156–8 °C; TLC EtOAc, R_f 0.56; ^1H NMR ($CDCl_3$) δ 7.65 (s, 1H), 7.4–7.1 (m, 4H), 5.25 (s, 2H), 3.5 (t, 2H), 3.25 (t, 2H). Anal. ($C_{14}H_{13}N_5BrCl$) C, H, N, Br, Cl.

2-Amino-6-chloro-9-[2-(3-bromopropyl)benzyl]purine (3b). A mixture of the benzyl bromide **2b** (3.1 g, 10.6 mmol), 2-amino-6-chloropurine (2.0 g, 11.8 mmol), and anhydrous potassium carbonate (2.95 g, 21.2 mmol) in anhydrous DMF (32 mL) was stirred, with the exclusion of moisture, for 4 h at room temperature, and then stored for 16 h at 5 °C. The mixture was filtered and the filtrate evaporated to dryness (portions of toluene and methanol were added and repeatedly evaporated). The residual syrup was dissolved in dichloromethane and filtered, and the filtrate was applied to a pad (6 × 9 cm) of silica gel (70–230 mesh), eluting successively with dichloromethane and 2% methanol in dichloromethane. Fractions containing homogeneous product were combined and evaporated to provide the benzylpurine in 20.6% yield: TLC EtOAc, R_f 0.48; ^1H NMR ($CDCl_3$) δ 7.6 (s, 1H), 7.3–7.05 (m, 4H), 5.3 (s, 2H), 5.2 (broad s, 2H), 3.35 (t, 2H), 2.8 (t, 2H), 2.1–2.0 (m, 2H). Anal. ($C_{15}H_{15}N_5ClBr$) C, H, N, Cl, Br.

2-Amino-6-chloro-9-[2-[2-(diethylphosphono)ethyl]benzyl]purine (3c). A mixture of the chloropurine **3a** (2.0 g, 5.4 mmol) in triethyl phosphite (10.0 mL, 56.2 mmol) was heated to 150 °C and stirred at this temperature for 3.5 h. Excess reagent was removed by evaporation under reduced pressure at 100 °C, and the residue was pumped to dryness. The residue was dissolved in ethyl acetate and filtered, and the filtrate was evaporated *in vacuo*. The residue was redissolved in ethyl acetate and applied to a column (23 × 4 cm) of silica gel (0.04–0.063 mm), eluting first with ethyl acetate followed by 3% and 4% solutions of methanol in ethyl acetate. Fractions containing homogeneous material were pooled and evaporated to dryness under reduced pressure to yield the required product in 20.7% yield: mp 129–131 °C; TLC 95:5 EtOAc–MeOH, R_f 0.22; ^1H NMR ($CDCl_3$) δ 7.75 (s, 1H), 7.35–7.1 (m, 4H), 5.6 (s, 2H), 5.3 (s, 2H), 4.2–4.0 (m, 6H), 3.2–3.05 (q, 2H), 2.2–2.0 (m, 2H), 1.3 (t, 3H). Anal. ($C_{18}H_{23}N_5O_3ClP$) C, H, N, Cl, P.

2-Amino-6-chloro-9-[2-[3-(diethylphosphono)propyl]benzyl]purine (3d). A suspension of the (bromobenzyl)purine **3b** (1.5 g, 3.94 mmol) in triethyl phosphite (8.0 mL, 45.0 mmol) was heated to 150 °C and stirred at this temperature for 3.5 h under continuous nitrogen bubbling. The solution was evaporated under reduced pressure, the residue was dissolved in ethyl acetate and filtered, and the filtrate was applied to a column (14 × 4 cm) of silica gel (0.04–0.063 mm), eluting with ethyl acetate followed by 3% and 4% methanol in ethyl acetate. Fractions containing homogeneous product were pooled and evaporated to dryness under reduced pressure to yield the diethyl phosphonate in 29.1% yield: TLC 95:5 EtOAc–MeOH, R_f 0.18; ^1H NMR ($CDCl_3$) δ 7.75 (s, 1H), 7.3–7.0 (m, 4H), 5.3 (s, 2H), 4.15–4.0 (m, 4H), 2.74 (t, 2H), 2.1–1.9 (m, 2H), 1.85–1.7 (m, 2H), 1.3 (t, 6H). Anal. ($C_{18}H_{26}N_5O_3ClP$) C, H, N, Cl.

2-Amino-6-chloro-9-[2-[3-(diethylphosphono)propoxy]benzyl]purine (3e). A mixture of **2e** (0.686 g, 1.88 mmol), 2-amino-6-chloropurine (0.35 g, 2.07 mmol), and anhydrous K_2CO_3 (0.52 g, 3.76 mmol) in anhydrous dimethylformamide (5.5 mL) was stirred at room temperature for 4 h and then allowed to stand in the refrigerator overnight. The mixture was filtered, the inorganic residue was washed with DMF (1 mL), and the combined filtrate was evaporated under reduced pressure. The residue was chromatographed over a column of silica using 5% MeOH in $CHCl_3$ as the eluent to obtain 0.447 g (52.4%) of colorless crystals: TLC 95:5 EtOAc–MeOH, R_f 0.2; FAB MS m/z 454 $(M + 1)^+$; UV (EtOH) λ_{max} 221 nM (ϵ 34 740), 248 (sh), 280 (4260), 309 (8650); ^1H NMR (DMSO-d_6) δ 1.20 (6H, t, CH_2CH_3), 1.8–2.0 (4H, m, CH_2CH_2P), 3.9–4.1 (6H, m, OCH_2CH_2 and CH_2CH_3),

5.22 (2H, s, CH_2N), 6.82–7.02 (5H, m, NH_2 and 3′,5′,6′-aryl H), 7.28 (1H, m, 4′-H), 8.08 (1H, s, H-8).

2-Amino-6-chloro-9-[2-(2-phosphonoethyl)benzyl]purine (3f). The purine diethyl phosphonate **3c** (175 mg, 0.41 mmol) was dissolved in dichloromethane (4 mL), cooled to 0 °C, and treated with trimethylsilyl bromide (0.23 mL, 1.7 mmol). The solution was allowed to warm to room temperature and stirred for 6 h before evaporating to dryness and triturating the residue with water. The solid that was obtained was collected by filtration, washed with a little cold water, and dried to provide the phosphonic acid in 76.2% yield: mp 296–298 °C; TLC concentrated NH_4OH–EtOH, R_f 0.11; ^1H NMR (MeOD) δ 8.05 (s, 1H), 7.3–7.0 (m, 4H), 5.4 (s, 2H), 3.1–3.0 (m, 2H), 2.5–1.95 (m, 2H). Anal. ($C_{14}H_{15}N_5O_3ClP·H_2O$) C, H, N.

2-Amino-6-chloro-9-[2-(3-phosphonopropyl)benzyl]purine (3g). A solution of the diethyl phosphonate **3d** (0.2 g, 0.46 mmol) in anhydrous dichloromethane (4.5 mL) was cooled to 0 °C and treated, under an atmosphere of nitrogen, with trimethylsilyl bromide (0.27 mL, 2.0 mmol). The mixture was allowed to warm to room temperature and stirred for 6 h under nitrogen before evaporating to dryness under reduced pressure and triturating the residue with water. The resultant solid was collected by filtration, washed with cold water, and dried *in vacuo* to give the propyl phosphonate in 93% yield: TLC concentrated NH_4OH–EtOH, R_f 9.56; ^1H NMR (DMSO-d_6) δ 8.2 (s, 1H), 7.3–6.7 (m, 4H), 5.3 (s, 2H), 2.8 (m, 2H), 1.9–1.75 (m, 2H), 1.7–1.5 (m, 2H).

2-Amino-6-chloro-9-[2-(3-phosphonopropoxy)benzyl]purine (3h). Under an atmosphere of argon, a stirred suspension of **3e** (0.447 g, 0.985 mmol) in CH_2Cl_2 (10 mL) was cooled in an ice water bath and treated with trimethylsilyl bromide (0.612 g, 4.0 mmol). The cooling bath was then removed, and the mixture was allowed to warm to room temperature and stirred for 6 h. Volatile components were removed under reduced pressure, and the residue was stirred with water (15 mL) and evaporated to dryness under reduced pressure. The residue was triturated with water (10 mL) and the product was collected by filtration and dried in air to obtain 0.39 g (100%) as a colorless solid. TLC as solution in EtOH containing a few drops of aqueous NH_4OH, 95:5 EtOH–concentrated NH_4OH, R_f 0.5; FAB MS *m/z* 398 (M + 1)$^+$; ^1H NMR (DMSO-d_6) δ 1.76 (2H, m, CH_2P), 1.98 (2H, $CH_2CH_2CH_2$), 4.10 (2H, t, OCH_2), 5.40 (2H, s, CH_2N), 6.8–7.34 (4H, m, aryl-H), 8.10 (1H, s, H-8). The product thus obtained was used in the next step without further purification.

9-[2-(2-Phosphonoethyl)benzyl]guanine (4f). A suspension of the chloropurine **3f** (91 mg, 0.25 mmol) in 10% aqueous NaOH solution (6.6 mL) was heated to 80 °C and stirred for 5 h. The solution was filtered, and the filtrate was cooled to 0 °C and acidified (pH 1) with 2 M HCl. The precipitated product was collected by filtration, washed with a little cold water, and dried (11.5 mg, 13.3%). The filtrate and washings were combined, cooled to 0 °C, and neutralized with 1 M NaOH before evaporating to dryness and redissolving in water (3 mL). Reacidification of the solution gave a second batch of product which was collected by filtration and dried (19.0 mg, 22.0%). The combined batches were dissolved in 1 M NH_4OH (1.0 mL) and applied to a column (7.5 × 2 cm) of Sephadex LH-20 gel filtration resin (5 g of resin previously swollen overnight in 50 mL water) and eluted with water (flow rate ca. 0.2 mL min^{-1}). Fractions containing homogeneous product were combined and evaporated to dryness under reduced pressure, redissolved in water, filtered, and lyophilized to provide **4f** in 18% yield: TLC 3:2 MeCN–1 M NH_4OH, R_f 0.49; ^1H NMR (D_2O) δ 7.6 (s, 1H), 7.25 (m, 2H), 7.1 (m, 1H), 6.8 (d, 1H), 5.2 (s, 2H), 2.8 (m, 2H), 1.85–1.65 (m, 2H). Anal. ($C_{14}H_{16}N_5O_4P·0.5H_2O$) C, H, N.

9-[2-(3-Phosphonopropyl)benzyl]guanine (4g). A suspension of the chloropurine **3g** (0.14 g, 0.36 mmol) in 10% aqueous NaOH (9.7 mL) was stirred for 5 h at 80 °C, cooled to 0 °C in ice, and acidified (pH 1) with 2 M HCl (12.5 mL). The precipitate that formed was collected by filtration, washed with a little cold water, and dried *in vacuo* to yield the crude guanine (35.1 mg, 26.3%). The filtrate was stored at 5 °C for 16 h and provided a second crop of crude product (40.5 mg, 30.4%) following filtration and drying *in vacuo*. The first crop of crude product was dissolved in 1 M NH_4OH (1 mL) and applied to a column (7 × 2.5 cm) of Sephadex LH-20 gel filtration resin, eluting with water. Fractions containing homogeneous product were combined, diluted with water, filtered, and lyophilized to provide **4g** (22.1 mg, 16.6% overall): TLC 3:2 MeCN–1 M NH_4OH, R_f 0.42; ^1H NMR (D_2O) δ 7.65 (s, 1H), 7.3–6.9 (m, 4H), 5.2 (s, 2H), 2.7 (m, 2H), 1.8–1.5 (m, 4H). Anal. ($C_{15}H_{18}N_5O_4P$) C, H, N.

9-[2-(3-Phosphonopropoxy)benzyl]guanine (4h). A solution of **3h** (0.38 g, 0.96 mmol) in 10% aqueous NaOH (25 mL) was stirred in an oil bath at 80 °C for 5 h, cooled, and acidified to pH 1 with 1 N aqueous HCl. The solid that separated was collected by filtration, washed with water, and dried in air. The crude product was dissolved in a minimum quantity of 1 N aqueous NH_4OH, and the solution was applied to a column of Sephadex LH-20 in water. The product was eluted with water. The fractions containing the product were pooled and concentrated under reduced pressure to a volume of about 5 mL. The concentrated solution was cooled and acidified to pH 1 with 0.1 N aqueous HCl. The product obtained was collected by filtration, washed with water, and dried in air to yield 0.072 g (63%) as a colorless solid: mp 254–256 °C dec; TLC 3:2 CH_3CN–1 N aqueous NH_4OH, R_f 0.45; FAB MS *m/z* 380 (M + 1)$^+$; UV (H_2O) λ_{max} 254 nM (ε 12 230), 272 (10 950); IR (KBr) 3490, 3455, 3360, 1676, 1640, 1251, 1159, 979 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.78 (2H, m, CH_2P), 1.98 (2H, m, $CH_2CH_2CH_2$), 4.08 (2H, t, OCH_2), 5.14 (2H, s, CH_2N), 6.48 (2H, br s, NH_2), 6.76–6.92 (2H, m, H-5′,6′), 7.0 (1H, d, H-3′), 7.28 (1H, m, H-4′), 7.70 (1H, s, H-8), 10.56 (1H, br s, NH). Anal. ($C_{15}H_{18}N_5O_5·0.5H_2O$) C, H, N.

3-(2,3,5-Trichlorophenyl)pentanedinitrile (6a). A mixture of 2,3,5-trichlorobenzaldehyde (**5a**) (25 g, 119.35 mmol), cyanoacetic acid (25.38 g, 29.83 mmol), pyridine (130 mL), and piperidine (4 mL) was heated at reflux for 8 h. Most of the solvents were removed *in vacuo*, the residue was taken up in toluene (250 mL), and the solution was washed with aqueous sodium bisulfite, water, aqueous sodium carbonate, and again with H_2O (2 × 100 mL). The organic layer was dried (Na_2SO_4) and evaporated to give the crude product. A solution of the crude product in $CHCl_3$ was evaporated to dryness with ~50 g of silica gel. The dried material was layered onto a silica gel column, which was eluted with hexane/ethyl acetate (9:1) to give 4.71 g (17%) acrylonitrile byproduct. Further elution of the column (20% EtOAc/hexane) gave the desired pentane dinitrile **6a**: yield 23.83 g (73%); mp 90–91 °C; MS (EI) *m/z* 272 (M$^+$), 232 (272 − CH_2CN)$^+$; IR (KBr) 2257, 1560, 1429, 1418, 1411, 1395; ^1H NMR (DMSO-d_6) 3.1 (m, 4H, CH_2's), 4.02 (m, 1H, $CHCH_2$), 7.81 (d, 1H, aromatic CH), 7.90 (α, 1H, aromatic H). Anal. ($C_{11}H_7Cl_3N_2$) C, H, N.

3-(3-Chlorophenyl)pentanedinitrile (6b). The experimental procedure is exactly the same as described for **6a**, starting from 3-chlorobenzaldehyde (**5b**): yield 25.1 g (69%); mp 62 °C; MS (EI) *m/z* 204 (M$^+$), 164 (204 − CH_2CN)$^+$; IR (KBr) 2260, 2240, 1598, 1573, 1480, 1436, 1420. Anal. ($C_{11}H_9ClN_2$) C, H, N.

2-Formyl-3-(2,3,5-trichlorophenyl)pentanedinitrile (7a). A 60% suspension of sodium hydride in mineral oil (1.56 g, 65.05 mmol) was added to dry THF (100 mL) and cooled to 5 °C. To this suspension were added ethyl formate (14.78 g, 199.51 mmol) and a solution of the pentanedinitrile **6a** (10.17 g, 37.17 mmol) in THF (50 mL) dropwise under nitrogen. The solution was stirred at 5–10 °C for 2 h and at room temperature overnight. Volatiles were evaporated *in vacuo*. The residual thick paste was dissolved in H_2O (100 mL) at 5–10 °C and the solution adjusted to pH 6.0 with 20% (v/v) concentrated HCl. The heavy oil that separated was dissolved in ethyl acetate, and the solution was washed with H_2O (100 mL) and dried (Na_2SO_4) before evaporation to give a red-brown oil (11 g), which was used in the next step without further purification.

2-Formyl-3-(3-chlorophenyl)pentanedinitrile (7b). The experimental procedure is essentially the same as that described for **7a** starting from 3-(3-chlorophenyl)pentanedinitrile (**6b**): crude yield 50 g (100%). The crude **7b** was sufficiently pure for use in the next step.

Methyl *N*-[2,4-Dicyano-3-(2,3,5-trichlorophenyl)-1-butenyl]glycine (8a). To a solution of the crude formyl compound **7a** (11 g) in a mixture of MeOH (80 mL) and H_2O (20 mL) was added glycine methyl ester hydrochloride (8.17 g, 65.06 mmol) and sodium acetate (5.33 g, 65.06 mmol), and the reaction mixture was stirred at room temperature for 22 h. The solution was evaporated *in vacuo* at low temperature (~15 °C) until most of

the methanol was removed. The mixture of heavy oil and water was extracted with ethyl acetate (3 × 100 mL), and the resulting solution was washed with H_2O (100 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give the desired enamine, which was purified on a silica gel column (2% MeOH in $CHCl_3$) to give the analytically pure enamine **8a** as a mixture of *cis–trans* isomers. Recrystallization from methanol gave 10.41 g (75%): mp 142–143 °C; MS (FAB) *m/z* 372 $(M + H)^+$; IR (KBr) 3394, 2188, 1745, 1637, 1435, 1419; ^1H NMR (DMSO-d_6) δ 3.16 (dd, *J* = 17 Hz, *J* = 8 Hz, 1H, CH*H*), 3.20 (dd, *J* = 17 Hz, *J* = 8 Hz, 1H, C*H*H), 3.97 (m, CH_2CO), 4.18 (t, *J* = 8 Hz, 1H, CHC=), 7.15 (m, 2H, =C*H*N*H*CH_2), 7.60 (d, *J* = 2.2 Hz, 1H, aromatic CH), 7.85 (d, *J* = 2.2 Hz, 1H, aromatic CH). Anal. ($C_{15}H_{12}Cl_3N_3O_2$) C, H, N.

Methyl *N*-[3-(3-Chlorophenyl)-2,4-dicyano-1-butenyl]glycine (8b). Compound **8b** was prepared from **7b** by the procedure described for **8a**: yield 44.08 g (60.5%); mp 63 °C; MS (FAB) *m/z* 304 $(M + H)^+$; IR (KBr) 3365, 2150, 2190, 1746, 1644, 1596.

1-Ethyl 2-Methyl 3-Amino-4-[2-cyano-1-(2,3,5-trichlorophenyl)ethyl]-1*H*-pyrrole-1,2-dicarboxylate (10a). A solution of the enamine **8a** (10.0 g, 26.84 mmol) in dry CH_2Cl_2 (100 mL) was cooled to 0 °C and treated with DBN (10.53 g, 84.79 mmol) followed by the dropwise addition of ethyl chloroformate (6.90 g, 63.57 mmol) under nitrogen. The solution was stirred at 0 °C for 1 h and then at room temperature for 48 h. Volatiles were evaporated *in vacuo* to give a viscous dark gum, which was purified by flash column chromatography (silica gel) using $CHCl_3$ as the eluent. The $CHCl_3$ solution was evaporated to give a pale yellow foam, which was stirred in MeOH (100 mL) to give **10a** as a crystalline solid. Recrystallization from $CHCl_3$–MeOH gave 8.92 g (74.7%): mp 160–161 °C; MS (EI) *m/z* 443 (M^+), 412 (443 – $OCH_3)^+$; IR (KBr) 3470, 3375, 2248, 1722, 1664, 1466, 1396, 1377, 1352; ^1H NMR (DMSO-d_6) δ 1.25 (t, 3H, CH_2CH_3), 3.17–3.40 (m, 2H, CH_2CN), 3.68 (s, 3H, OCH_3), 4.25 (q, 2H, CH_2CH_3), 4.76 (t, 1H, C*H*CH_2), 5.76 (s, 2H, NH_2), 7.06 (s, 1H, pyrrole CH), 7.63 (d, *J* = 4 Hz, 1H, aromatic CH), 7.85 (d, *J* = 4 Hz, 1H, aromatic CH). Anal. ($C_{18}H_{16}Cl_3N_3O_4$·0.2EtOH) C, H, N.

1-Ethyl 2-Methyl 3-Amino-4-[2-cyano-1-(3-chlorophenyl)ethyl]-1*H*-pyrrole-1,2-dicarboxylate (10b). Compound **10b** was prepared from **8b** by the procedure described for **10a**: crude yield 53.1 g (71.5%).

Methyl 3-Amino-4-[2-cyano-1-(2,3,5-trichlorophenyl)ethyl]-1*H*-pyrrole-2-carboxylate (11a). A suspension of the *N*-protected pyrrole **10a** (8.6 g, 19.34 mmol) in MeOH (300 mL) was treated with Na_2CO_3 (5.12 g, 48.34 mmol), and the reaction mixture was stirred at room temperature for 17 h. The solid sodium carbonate was removed by filtration and washed well with methanol (100 mL). The filtrate was reduced to a volume of ~25 mL and kept in a refrigerator for 1 h to give 5.23 g of the desired product as a crystalline solid. Further concentration of the mother liquor gave an additional 0.14 g of the pure product: total yield 6.45 g (89.5%); mp 178–181 °C; MS (EI) *m/z* 371 (M^+), 331 (371 – $CH_2CN)^+$, 3457, 3360, 2253, 1675, 1606, 1575; ^1H NMR (DMSO-d_6) δ 3.13–3.40 (m, 2H, CH_2CN), 3.70 (s, 3H, OCH_3), 4.70 (t, 1H, C*H*CH_2CN), 4.94 (s, 2H, NH_2), 6.63 (d, *J* = 2.8 Hz, 1H, pyrrole CH), 7.49 (d, *J* = 1.0 Hz, 1H, aromatic CH), 7.78 (d, *J* = 1 Hz, 1H, aromatic CH), 10.85 (s, 1H, pyrrole NH). Anal. ($C_{15}H_{12}N_3O_2$·0.1MeOH) C, H, N.

Methyl 3-Amino-4-[2-cyano-1-(3-chlorophenyl)ethyl]-1*H*-pyrrole-2-carboxylate (11b). Compound **11b** was prepared from **10b** by the procedure described for **11a**: overall yield from enamine **8b**, 16.28 g (54.3%); mp 159–160 °C; MS (FAB) *m/z* 304 $(M + H)^+$, 303 (M^+), 272 (303 – $OCH_3)^+$.

***N*-Benzoyl-*N'*-[4-cyano-1-(2,3,5-trichlorophenyl)ethyl]-2-(methoxycarbonyl)-1*H*-pyrrol-3-yl]thiourea (12a).** To a stirred suspension of pyrrole **11a** (5.83 g, 15.64 mmol) in anhydrous CH_2Cl_2 (100 mL) was added benzoyl isothiocyanate (2.88 g, 17.64 mmol) at room temperature under nitrogen. Stirring for 30 min resulted in the separation of the desired thioureido compound. Additional benzoyl isothiocyanate (0.5 mL) was added, and the reaction mixture was stirred for another 30 min. The solvent was evaporated to dryness, and the light yellow solid residue was triturated with methanol (50 mL). The resulting white crystalline solid was collected by filtration and recrystallized from $CHCl_3$–ether to give pure **12a**: yield 7.71 g (92%); mp 210–211 °C; MS (EI) *m/z* 534 (M^+), 499 (534 – $Cl)^+$, 413 (534 – $NH_2COPh)^+$; IR (KBr) 3175, 2300, 1678, 1589, 1530, 1502, 1450, 1405, 1392; ^1H NMR (DMSO-d_6) δ 3.26–3.44 (m, 2H, CH_2CN), 3.71 (s, 3H, OCH_3), 4.90 (t, 1H, C*H*CH_2CN), 7.27 (d, *J* = 6.5 Hz, 1H, aromatic CH), 7.41 (d, *J* = 4 Hz, 1H, aromatic CH), 7.56 (t, 2H, aromatic CH), 7.69 (m, 2H, pyrrole CH and aromatic CH), 7.95 (dd, 2H, aromatic CH), 11.68 (s, 1H, NH), 11.83 (s, 1H, NH), 12.17 (d, 1H, pyrrole N*H*). Anal. ($C_{23}H_{17}Cl_3N_4O_3S$) C, H, N.

***N*-Benzoyl-*N'*-[4-[2-cyano-1-(3-chlorophenyl)ethyl]-2-(methoxycarbonyl)-1*H*-pyrrol-3-yl]thiourea (12b).** Compound **12b** was prepared from **11b** by the procedure described for **12a**: yield 19.54 g (94%); mp 215–216 °C; MS (FAB) *m/z* 467 $(M + H)^+$.

***N*-Benzoyl-*N'*-[4-[2-cyano-1-(2,3,5-trichlorophenyl)ethyl]-2-methoxycarbonyl-1*H*-pyrrol-3-yl]-*S*-methylthiourea (13a).** A solution of the thioureido **12a** (6.75 g, 12.6 mmol) and DBN (176 g, 14.20 mmol) in anhydrous CH_2Cl_2 (200 mL) under nitrogen was cooled to 0 °C and treated with methyl iodide (5.20 g, 36.65 mmol). The reaction mixture was stirred at 0 °C for 10 min and at room temperature for 1 h. The solvent was evaporated *in vacuo* at room temperature, and the residual gum was dissolved in $CHCl_3$ (3 × 100 mL). The solution was washed with H_2O (2 × 50 mL), dried (Na_2SO_4), and evaporated to give **13a** as a foam: crude yield 6.95 g (100%); MS (EI) *m/z* 548 (M^+), 501 (548 – $SMe)^+$. Crude **13a** was used in the next step without further purification.

***N*-Benzoyl-*N'*-[4-[2-cyano-1-(3-chlorophenyl)ethyl]-2-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-*S*-methylthiourea (13b).** Compound **13b** was prepared by the procedure described for **13a**. The foam obtained in quantitative yield was used in the next step without further purification.

3-[2-(Methylthio)-4-oxo-3*H*,5H-pyrrolo[3,2-*d*]pyrimidin-7-yl]-3-(2,3,5-trichlorophenyl)propanenitrile (14a) and 3-(2-Amino-4-oxo-3*H*,5H-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(2,3,5-trichlorophenyl)propanenitrile (15a). A solution of **13a** (6.90 g, 12.54 mmol) in MeOH (200 mL) saturated with ammonia at 0 °C was heated at 100 °C for 20 h in a glass-lined stainless steel bomb. The reaction mixture was cooled to room temperature, and the solvent was evaporated to dryness *in vacuo*. A solution of the solid residue in $CHCl_3$–MeOH was evaporated to dryness with ~60 g of silica gel. The dry mixture was layered carefully onto a silica gel flash column which was eluted with $CHCl_3$ to give the methylthio compound **14a**: yield 1.1 g (21%); mp 290–291 °C; MS (EI) *m/z* 412 (M^+), 372 (412 – $CH_2CN)^+$; IR (KBr) 3440, 3063, 2245, 1556, 1421, 1396; ^1H NMR (DMSO-d_6) δ 2.50 (s, 3H, SCH_3), 3.40–3.56 (m, 2H, CH_2CN), 5.0 (t, 1H, $CHCH_2CN$), 7.44 (d, *J* = 3 Hz, 1H, pyrrole ring CH), 7.71 (d, *J* = 1.5 Hz, 1H, aromatic CH), 7.77 (d, *J* = 1.5 Hz, 1H, aromatic CH), 12.60 (s, 1H, NHCO), 12.22 (s, 1H, pyrrole NH). Anal. ($C_{16}H_{11}Cl_3N_4OS$·0.3EtOH) C, H, N.

Further elution of the column with 5% MeOH in $CHCl_3$ gave the desired compound **15a** as a white solid: yield 2.76 g (57.5%); mp 284–285 °C; MS (EI) 381 (M^+), 346 (381 – $Cl)^+$, 341 (381 – $CH_2CN)^+$; IR (KBr) 3323, 3180, 3162, 2255, 1685, 1630, 1604, 1558, 1525, 1414, 1393; ^1H NMR (DMSO-d_6) δ 3.27–3.50 (m, 2H, CH_2CN), 4.86 (t, 1H, $CHCH_2CN$), 5.90 (s, 2H, NH_2), 7.15 (d, *J* = 2 Hz, 1H, pyrrole ring CH), 7.57 (d, *J* = 1.5 Hz, aromatic CH), 7.79 (d, *J* = 1.7 Hz, 1H, aromatic CH), 10.50 (s, 1H, NHCO), 11.58 (s, 1H, pyrrole ring NH). Anal. ($C_{15}H_{10}Cl_3N_5O$·0.45H_2O) C, H, N.

3-(3-Chlorophenyl)-3-[2-(methylthio)-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]propanenitrile (14b) and 3-(3-Chlorophenyl)-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-*d*]pyrimidin-7-yl)propanenitrile (15b). The cyclization of the methylthio intermediate **13b** with methanolic ammonia as described for the preparation of **14a** and **15a** afforded a mixture of **14b** and **15b** which was chromatographed on a silica gel column using $CHCl_3$ as the eluent to give 2.8 g (19.5%) of **14b**: mp 198–199 °C; MS (FAB) *m/z* 345 $(M + H)^+$; IR (KBr) 3178, 3171, 3141, 3097, 2250, 1676, 1578, 1537, 1432, 1416; ^1H NMR (DMSO-d_6) δ 3.36–3.54 (m, 2H, CH_2CN), 4.54 (t, 1H, $CHCH_2CN$), 7.22–7.63 (m, 5H, aromatic CH and pyrrole ring CH), 11.95 (s, 1H, pyrrole NH), 12.20 (s, 1H, NHCO). Anal. ($C_{16}H_{13}ClN_4OS$) C, H, N.

Further elution of the column with 3% MeOH in $CHCl_3$ gave **15b**: yield 6.5 g (49.8%); mp 157–158 °C; MS (FAB) *m/z* 314 $(M + H)^+$, 273 (313 – $CH_2CN)^+$; IR (KBr) 3313, 3166, 3148, 2920,

2240, 1682, 1625, 1596, 1573, 1525; ^{1}H NMR (DMSO-d_6) δ 3.24–3.48 (m, 2H, CH_2CN), 4.41 (t, 1H, $CHCH_2CN$), 5.88 (s, 2H, NH_2), 7.13 (d, J = 3.5 Hz, 1H, pyrrole ring CH), 7.20–7.50 (m, 4H, aromatic CH), 10.44 (s, 1H, NHCO), 11.45 (d, 1H, pyrrole ring NH). Anal. ($C_{15}H_{12}ClN_5O$·0.2MeOH) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-phenylpropanenitrile (16). A solution of the 2,3,5-trichlorophenyl compound **15a** (2.0 g, 5.22 mmol) in warm ethanol (250 mL) and DMF (100 mL) was hydrogenated with 30% Pd–C catalyst (1.0 g) in the presence of triethylamine (2.64 g, 5.0 equiv) at atmospheric pressure. After 5 h, the dehalogenation was complete (TLC; 10% MeOH–$CHCl_3$, R_f 0.4), and the catalyst was filtered off under nitrogen. The solid obtained by evaporation of the filtrate under reduced pressure was triturated and sonicated with H_2O. After filtration, the solid was collected and dried over P_2O_5: yield 1.28 g (88%); mp 168–170 °C; MS (FAB) m/z 280 (M + H)$^+$, 239 (280 – CH_2CN)$^+$; ^{1}H NMR (DMSO-d_6) δ 3.20–3.46 (m, 2H, CH_2CN), 4.37 (t, 1H, $CHCH_2CN$), 5.86 (s, 2H, NH_2), 7.05 (d, J = 1.5 Hz, 1H, pyrrole ring CH), 7.21 (t, 1H, aromatic CH), 7.30 (t, 2H, aromatic CH), 7.39 (d, J = 7.5 Hz, 2H, aromatic CH), 10.41 (s, 1H, NHCO), 11.40 (s, 1H, pyrrole NH). Anal. ($C_{15}H_{13}N_5O$) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-phenylpropanamide (17). A solution of the phenylpropanenitrile **16** (0.2 g, 0.72 mmol) in concentrated H_2SO_4 (0.5 mL) was stirred at room temperature for 20 h. The H_2SO_4 solution was poured over crushed ice and adjusted to pH 6.8 by concentrated NH_4OH. The precipitated solid was collected by filtration, washed well with H_2O, and dried to give the desired material as a white solid: yield 0.18 g (84.5%); mp 199–201 °C; MS (FAB) m/z 298 (M + H)$^+$; IR (KBr) 3324, 3259, 3185, 3178, 1670, 1626, 1561, 1524, 1411; ^{1}H NMR (DMSO-d_6) δ 2.60 (dd, 1H, CH*H*), 2.86 (dd, 1H, C*H*H), 4.52 (t, 1H, $CHCH_2$), 5.98 (bs, 2H, NH_2), 6.67 (s, 2H, $CONH_2$), 6.99 (d, J = 2.5 Hz, 1H, pyrrole ring CH), 7.06–7.31 (m, 5H, aromatic CH), 10.60 (very broad, 1H, NHCO), 11.37 (bs, 1H, pyrrole ring NH). Anal. ($C_{15}H_{15}N_5O_2$·0.8H_2O) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-phenylpropanoic Acid (18a). A solution of the nitrile **16** in 6 N HCl (3.0 mL) was heated at reflux for 18 h. The solvent was evaporated *in vacuo*, and the light yellow residue was dissolved in H_2O (6 mL) and adjusted to pH 10 by NH_4OH solution. Insoluble impurities were removed by filtration, and the pH of the filtrate was lowered to ~6.8 to give the desired product as a white solid which was collected by filtration, washed with H_2O, and dried: yield 0.19 g (89%); mp 290 °C dec; MS (FAB) m/z 299 (M + H)$^+$; IR (KBr) 3187, 3181, 1688, 1636, 1557, 1524, 1495, 1398, 1376; UV 0.1 N HCl 234 (17.15), 273 (15.05); pH 7 231 (20.05), 272 (10.72); 0.1 N NaOH 227 (22.3), 264 (7.29), 288 (6.66); ^{1}H NMR (DMSO-d_6) δ 2.86–3.0 (dd, 1H, CH*H*), 3.02–3.14 (dd, 1H, C*H*H), 4.47 (t, 1H, $CHCH_2CN$), 5.85 (s, 2H, NH_2), 6.95 (d, J = 3 Hz, 1H, pyrrole ring CH), 7.14 (t, 1H, aromatic CH), 7.24 (t, 2H, aromatic CH), 7.33 (d, J = 7.0 Hz, 2H, aromatic CH), 10.38 (bs, 1H, NHCO), 11.25 (bs, 1H, pyrrole ring NH), 11.1–12.1 (very broad, seen in the integral, 1H, COO*H*). Anal. ($C_{15}H_{14}N_4O_3$·0.8H_2O) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(3-chlorophenyl)propanoic Acid (18b). 3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(3-chlorophenyl)propanenitrile (**15b**; 2.0 g, 63.75 mmol) was hydrolyzed to its corresponding acid **18b** by the procedure described for the preparation of **18a**: yield 1.8 g (84.9%); mp 295–296 °C; MS (FAB) m/z 333 (M + H)$^+$; IR (KBr) 3182, 3170, 1686, 1639, 1596, 1572, 1525, 1477, 1402, 1377; UV 0.1 N HCl 235 (17.2), 273 (15.42); pH 7 233 (20.2), 272 (10.58), 0.1 N NaOH 231 (20.9), 263 (7.2), 288, (6.67); ^{1}H NMR (DMSO-d_6) δ 2.96 (m, 2H, CH_2CN), 4.48 (t, 1H, $CHCH_2CN$), 6.06 (bs, 2H, NH_2), 7.11 (d, J = 2.6 Hz, 1H, pyrrole ring CH), 7.21 (d, J = 8 Hz, 1H, aromatic CH), 7.25–7.46 (m, 3H, aromatic CH), 10.3–11.0 (very broad, seen in the integral, 1H, NH or COOH), 10.42 (bs, 1H, pyrrole ring NH), 11.7–12.3 (very broad, seen in the integral, 1H, NH or COOH). Anal. ($C_{15}H_{13}ClN_4O_3$·0.7H_2O) C, H, N.

Methyl 3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(3-chlorophenyl)propanoate (19). A solution of the acid **18a** (7.5 g, 21.4 mmol as a monohydrate) in MeOH (400 mL) was cooled below 0 °C in an ice/salt bath, and thionyl chloride (10.31 g, 86.7 mmol, 4 equiv) was added dropwise to minimize heating. With protection from atmospheric moisture, the solution was allowed to warm to ambient temperature on standing overnight. The solvent was evaporated *in vacuo*, and fresh MeOH (100 mL) and then toluene (100 mL) were added and evaporated to aid removal of acid. A suspension of the residue in cold H_2O (200 mL) was adjusted to pH 7 with dilute NaOH; the solid was collected by filtration, washed with cold H_2O (50 mL), and dried *in vacuo* over P_2O_5 at room temperature and at 110 °C for 4 h: yield 6.08 g (81%). This product was judged by TLC to be suitable for use as an intermediate.

In a small-scale pilot run, the product obtained by the procedure above was further purified by extraction into boiling MeOH in a Soxhlet apparatus to give fine white crystals that were dried *in vacuo* over P_2O_5 at 110 °C for 20 h to provide a chromatographically homogeneous sample: mp 302–303 °C dec; MS (FAB) m/z 347 (M + H)$^+$, 273 (346 – $CH_2CO_2CH_3$)$^+$; UV 0.1 N HCl 220 (sh), 232 (sh), 273 (15.1), pH 7 buffer 232 (18.1), 270 (7.4), 0.1 N NaOH 262 (6.5), 288 (5.9); ^{1}H NMR (DMSO-d_6) δ 3.15 (dd, J, 2H, $CH_2CO_2CH_3$), 3.48 (s, 3H, $-CO_2CH_3$), 4.48 (t, 1H, $CHCH_2$), 5.83 (s, 2, NH_2), 7.06 (d, J, 1H, pyrrole ring CH), 7.19, 7.26, 7.39 (complex m, 4H, chlorophenyl CH), 10.36 (s, 1H, 3-NH), 11.30 (d, J, 1H, 5-NH). Anal. ($C_{18}H_{15}ClN_4O_3$·0.3CH_3OH) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-cyclohexylpropanoic Acid (21). A solution of **18b** (83 mg, 0.28 mmol) in trifluoroacetic acid (15 mL) was hydrogenated with PtO_2 (83 mg) at 60 lb/in.^{2} for 24 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated *in vacuo*. The residue was dissolved in dilute NH_4OH (pH 8.5) and the resulting solution filtered through no. 42 Whatman filter paper. The colorless filtrate was adjusted to pH ~6.8, and the compound that precipitated was filtered, washed with H_2O, and dried: yield 65 mg (77%); mp >300 °C; MS (FAB) m/z 305 (M + H)$^+$; IR (KBr) 3330, 3194, 2926, 2852, 1683, 1639, 1557, 1522, 1401, 1377; ^{1}H NMR (DMSO-d_6) 0.85, 1.06, 1.60 (m, 11H, cyclohexyl H), 2.56 (m, 2H, CH_2CO_2H), 3.05 (m, 1H, $CHCH_2$), 5.79 (s, 2H, NH_2), 6.89 (d, J = 2.5 Hz, 1H, pyrrole ring CH), 10.31 (bs, 1H, pyrrole NH), 11.16 (s, 1H, NHCO), 11.76 (bs, 1H, pyrrole NH). Anal. ($C_{15}H_{20}N_4O$·1.75H_2O) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(3-chlorophenyl)propanol (22). A stirred suspension of the methyl ester **19** (6.00 g, 17.3 mmol) and dry $(NH_4)_2SO_4$ (100 mg) in hexamethyldisilazane (400 mL) was refluxed for 8 h with protection from atmospheric moisture. The clear solution was reduced to about half volume by distillation at atmospheric pressure, and the remaining HMDS was evaporated *in vacuo* to give trimethylsilyl derivative **20** as a viscous gum which was dried over P_2O_5. Crude **20** was used in the next step without further purification: MS (FAB) m/z 491 (M + H)$^+$.

A solution of $LiAlH_4$ in tetrahydrofuran (26 mL of 1 M, 26 mmol) was added dropwise to a solution of the trimethylsilylated ester **20** (assume 17.3 mmol) in dry THF (200 mL). After stirring for 1 h, excess hydride was destroyed by addition of EtOAc (100 mL), and all solvents were evaporated *in vacuo*. A stirred suspension of the residue in cold H_2O (200 mL) was adjusted to pH 1 with dilute HCl, and after 15 min the pH was adjusted to pH 7 with dilute NaOH. The solid was collected by filtration, washed with cold H_2O, dried superficially *in vacuo*, and then triturated by stirring with a 1:1 mixture (100 mL) of cyclohexane and Et_2O. The solid was filtered off and partially dried on the funnel under a stream of N_2. A solution of the solid in hot MeOH was evaporated to dryness with silica gel (230–400 mesh; 50 g), and the mixture was layered carefully onto a silica gel column which was eluted with $CHCl_3$–MeOH 17:3 and 4:1 to give the desired alcohol **22** (4.65 g, 84%). Two recrystallizations from H_2O–EtOH (2:1) gave white crystals that were dried *in vacuo* over P_2O_5 for 30 h at 110 °C: yield 3.36 g (61%); MS (FAB) m/z 319 (M + H)$^+$, 321 (Cl isotope peak of 319)$^+$, 301 (318 – OH)$^+$, 151 (B + 2H)$^+$; UV 0.1 NHCl 233 (17.9), 274 (15.7), pH 7 buffer 230 (22.3), 272 (10.6), 0.1 N NaOH 266 (7.3), 288 (6.7); ^{1}H NMR (DMSO-d_6) δ 2.07, 2.21 (2 sextets, 1H each, $CHCH_2CH_2OH$), 3.29 (m, 1H, CH_2OH), 4.17 (t, 1H, $CHCH_2CH_2OH$), 4.45 (t, 1H, CH_2OH), 5.79 (s, 2H, NH_2), 7.09 (d, J, 1H, pyrrole ring CH), 7.18, 7.27, 7.34 (complex m, 4H, chlorophenyl CH), 10.36 (s, 1H, 3-NH), 11.29 (d, J, 1H, 5-NH). Anal. ($C_{15}H_{15}ClN_4O_2$) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-cyclohexylpropanol (24). A solution of the alcohol **22** (100 mg, 0.314 mmol) in CF_3COOH (20 mL) was hydrogenated by shaking with platinum catalyst (from 100 mg PtO_2) in a Parr apparatus at an initial pressure of 60 lb/in.^{2}. The catalyst was removed by filtration through a thin Celite pad under N_2. The filtrate was evaporated, the residue was twice dissolved in MeOH (5.0 mL), and the solution was evaporated. A turbid solution of the residue in H_2O/concentrated NH_4OH (20 mL, 1:1) was allowed to stand for 4 h at room temperature before it was filtered and evaporated to dryness. A solution of the residue in H_2O (30 mL) containing 2 drops of 1 N NaOH was neutralized with 1 N HCl and cooled to 5 °C. The white solid that precipitated was collected by filtration, washed with cold H_2O, and dried *in vacuo* over P_2O_5 at 110 °C for 6 h: yield 63 mg (67%); mp 181–186 °C; MS (FAB) *m/z* 291 $(M + H)^+$; UV 0.1 N HCl 236 (16.2), 275 (14.9), pH 7 buffer 231 (18.8), 274 (12.1), 0.1 N NaOH 230 (19.6), 268 (7.8), 288 (sh); ^1H NMR (DMSO-d_6) δ 0.85, 1.08, 1.59 (complex multiplets, 11H, cyclohexyl CH), 1.78 (m, 2H, $CHCH_2CH_2OH$), 2.62 (m, 1H, $CHCH_2CH_2OH$), 3.14, 3.17, 3.24 (complex multiplets, 2H, CH_2OH), 4.25 (br, 1H, CH_2OH), 5.82 (s, 2H, NH_2), 6.86 (d, *J*, 1H, H-6), 10.35 (br, 1H, 3-NH), 11.17 (d, *J*, 1H, 5-NH). Anal. ($C_{15}H_{22}N_4O_2 \cdot 0.5H_2O$) C, H, N.

3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(3-chlorophenyl)-*N*-(phenylethyl)propanamide (25). A solution of diphenyl phosphorazidate (0.72 g, 2.6 mmol) in DMF (10 mL) was added dropwise during 10 min to a mechanically-stirred, cold (−5 to 0 °C) solution of **18b** (0.790 g, 2.4 mmol) and (*R*)-(+)-α-methylbenzylamine (0.32 g, 2.6 mmol) in DMF (100 mL). A solution of *N*-methylmorpholine (0.48 g, 4.75 mmol) in DMF (5 mL) was then added dropwise during 10 min, and the solution was kept near 0 °C for 5 h. It was then allowed to warm to room temperature and was stirred overnight (18 h). A second portion of diphenyl phosphorazidate (0.36 g), (*R*)-(+)-α-methylbenzylamine (0.16 g), and *N*-methylmorpholine (0.24 g) was added at 0 °C and the reaction mixture was stirred for 2 days. The solvent was removed *in vacuo*, and the residue was dissolved in an 8:2 mixture of acetonitrile and ammonium hydroxide (1 M). The crude product was adsorbed on silica gel and dried *in vacuo* to remove the last traces of solvent. Flash column chromatographic purification using acetonitrile and 1 M ammonium hydroxide (95:5) gave the desired material (**26**) as a mixture of diastereomers: yield 0.63 g (48.1%); MS (FAB) *m/z* 436 $(M + H)^+$.

The diastereomeric mixture (**25**) was separated by repeated flash column chromatography on silica gel using acetonitrile and 1 M ammonium hydroxide (98:2) as the eluent to give 0.18 g (13.7%) of the (*S*,*R*)-isomer: mp 170–175 °C dec; MS (FAB) *m/z* 402 $(M + H)^+$.

Further elution of the column gave the (*R*,*R*)-isomer: yield 0.12 g (9.2%); mp 155–160 °C; MS (FAB) *m/z* 458 $(435 + Na)^+$, 436 $(M + H)^+$, 273 $(436 - CH_2CONHCH(CH_3)Ph)^+$.

(*S*)-3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(3-chlorophenyl)propanoic Acid [(*S*)-18b]. A solution of the (*S*,*R*)-isomer (0.17 g, 0.4 mmol) in 6 N HCl (30 mL) was heated at reflux for 6 h and left at room temperature for 6 h. The solvent was evaporated *in vacuo*, and the residue was dissolved in H_2O (5 mL). The resulting solution was adjusted to pH ~10 with concentrated ammonium hydroxide solution, and the insoluble material was removed by filtration. The pH of the filtrate was lowered to ~6.8 with HCl. The white precipitate was collected, washed with H_2O, and dried to give 90 mg (97.8%) of the crude material which was purified by flash column chromatography using a 98:2 mixture of acetonitrile and ammonium hydroxide (1 M): yield 0.048 g; mp 285 °C dec; MS (FAB) *m/z* 333 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 2.96–3.10 (m, 2H, CH_2CO_2H), 4.48 (t, 1H, $CHCH_2CO_2H$), 6.00 (bs, 2H, NH_2), 7.10 (bs, 1H, pyrrole ring CH), 7.18–7.42 (complex m, 4H, aromatic CH), 10.3–10.6 (very broad, seen in the integral, 1H, NHCO), 11.41 (bs, 1H, pyrrole ring NH), 11.9–12.1 (very broad, seen in the integral, 1H, COOH). Anal. ($C_{15}H_{13}ClN_4O_3 \cdot H_2O$) C, H, N.

(*R*)-3-(2-Amino-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-(3-chlorophenyl)propanoic Acid [(*R*)-18b]. The (*R*,*R*)-isomer of compound **25** was hydrolyzed by the procedure described for **26a** to give the desired acid: yield 30.4 mg (40%); mp >280 °C dec; MS (FAB) *m/z* 333 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 2.95–3.10 (m, 2H, CH_2CO_2H), 4.46 (t, 1H, $CHCH_2CO_2H$), 5.83 (s, 2H, NH_2), 7.05 (d, *J* = 3.5 Hz, 1H, pyrrole ring CH), 7.16–7.38 (complex m, 4H, aromatic CH), 10.36 (s, 1H, NHCO), 11.29 (d, *J* = 1 Hz, 1H, pyrrole ring NH), 12.2 (bs, 1H, COOH). Anal. ($C_{15}H_{13}ClN_4O \cdot 0.7H_2O$) C, H, N.

Compound Evaluations. The X-ray crystallographic analyses, computer modeling studies, and *in vitro* enzyme inhibition studies were carried out as previously described.[1-3]

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