Structure-Based Design of Inhibitors of Purine Nucleoside Phosphorylase. 2. 9-Alicyclic and 9-Heteroalicyclic Derivatives of 9-Deazaguanine

John A. Secrist III,[‡] Shri Niwas,[‡] Jerry D. Rose,[‡] Y. Sudhakar Babu,[†] Charles E. Bugg,[§] Mark D. Erion,[∥,⊥] Wayne C. Guida,[∥] Steven E. Ealick,[§,#] and John A. Montgomery*,[†,‡]

BioCryst Pharmaceuticals, Inc., 2190 Parkway Lake Drive, Birmingham, Alabama 35244, Southern Research Institute, P.O. Box 55305, Birmingham, Alabama 35255-5305, Center for Macromolecular Crystallography, University of Alabama at Birmingham, Universiy Station, Birmingham, Alabama 35294-2010, and CIBA-GEIGY Corporation, 556 Morris Avenue, Summit, New Jersey 07901

Received January 25, 1993

Alicyclic and heteroalicyclic derivatives of 9-deazaguanine (2-amino-1,5-dihydro-4*H*-pyrrolo[3,2-*d*][pyrimidin-4-one) are, with one exception, potent inhibitors of purine nucleoside phosphorylase (PNP) equaling the corresponding 9-arylmethyl derivatives previously investigated. The mode of binding of these compounds to PNP was determined by X-ray crystallography.

Introduction

Inhibitors of purine nucleoside phosphorylase (PNP, EC 2.4.2.1) may have therapeutic value in the treatment of T-cell proliferative diseases such as T-cell leukemias or lymphomas, in the suppression of host-vs-graft response in organ transplants, and in the treatment of T-cell-mediated autoimmune diseases such as rheumatoid arthritis and lupus. They may also have value in protecting chemotherapeutic nucleosides from degradation.[1,2] Recently, we have demonstrated the value of X-ray crystallographic analysis and computer modeling in the design of potent inhibitors of PNP—the 9-(arylmethyl)-9-deazaguanines.[2] We now report an extension of this work to 9-alicyclic and 9-heteroalicyclic derivatives of 9-deazaguanine.

Chemistry

9-Deaza-9-substituted-guanines (2-amino-1,5-dihydro-7-substituted-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-ones, **1a**-h and 8)[3] were prepared by the procedures described in our earlier publication.[2] 3-Substituted propanenitriles, starting materials for these 9-deaza-9-substituted-guanines, are known and were prepared by methods reported in the literature.[2,4-8] By far the most difficult operation in the synthetic sequence was the first step, i.e., formylation of the propanenitrile using NaH and ethyl formate. The reaction is not stoichiometric. Several additions of the two reagents were necessary, and heating the reaction mixture at reflux was required to effect significant conversion. The rate and extent of conversion depends upon the nature of the 3-substituent. Substituents that increase the acidity of the proton adjacent to the cyano group facilitate the reaction. Thus, the aromatic propanenitriles prepared previously[2] are more reactive than cycloalkyl or heterocycloalkyl compounds described herein.

Compound **3** was obtained in 65% overall yield from 9-deaza-9-(cyclohexylmethyl)guanine (**1b**) by nitration followed by diimide reduction of the resulting nitro intermediate in ethanol. The remarkable resistance of the 9-deazaguanine moieties of **1e,f,h** to catalytic hydrogenation under acidic conditions allowed the direct conversion of the substituted phenyl and furanyl groups of these analogs to the corresponding 3-methylcyclohexyl analog **2a**, 3-(trifluoromethyl)cyclohexyl analog **2b**, and tetrahydrofuranyl analog **2d** in excellent yields (see Scheme I). Likewise, the piperidin-3-yl compound **2c** was obtained in 85% isolated yield in one step by catalytic hydrogenation of the pyridin-3-yl analog **1g** in 0.1 N HCl using PtO_2 catalyst. However, several attempts to convert the 9-deaza-9-(2-thienylmethyl)guanine to its corresponding tetrahydro analog by direct reduction were unsuccessful. Reduction methods which were tried include ionic hydrogenation[9] and catalytic hydrogenation with a variety of catalysts and conditions including Re_2S_7[10] at 190 °C and 1300 lb/in.^{2} hydrogen pressure.

An indirect method for the preparation of **6** was adopted in which the tetrahydrofuran analog **2d** was utilized as starting material. Treatment with 2 N HBr gave a mixture of isomeric bromo alcohols **5a** and **b**, consistent with reported results in which the ring opening of 2-methyltetrahydrofuran yielded mixtures of isomeric halo alcohols.[11,12] The crude product **5** was converted directly to the dibromo derivative **4** by reaction with PBr_3. The product **4** was treated with Na_2S to give the desired tetrahydrofuranyl analog **6** and a small amount of byproduct, the 1,2-dithian-3-yl analog **7**. The formation of **7** can be explained by the presence of Na_2S_2 in the Na_2S reagent, since Na_2S_2 has been used to convert 1,4-dibromobutanes to 1,2-dithianes.[13] Compounds **6** and **7** were fully characterized by mass spectral, ^{1}H NMR, and elemental analyses (see Table III).

Compound **9**, an analog in which a cycloalkyl group is directly attached to the 9-position of 9-deazaguanine, was prepared by catalytic hydrogenation of the cyclohexenyl analog **8** which, in turn, was prepared by the procedure described for **1b** starting from commercially available 1-cyclohexenylacetonitrile.

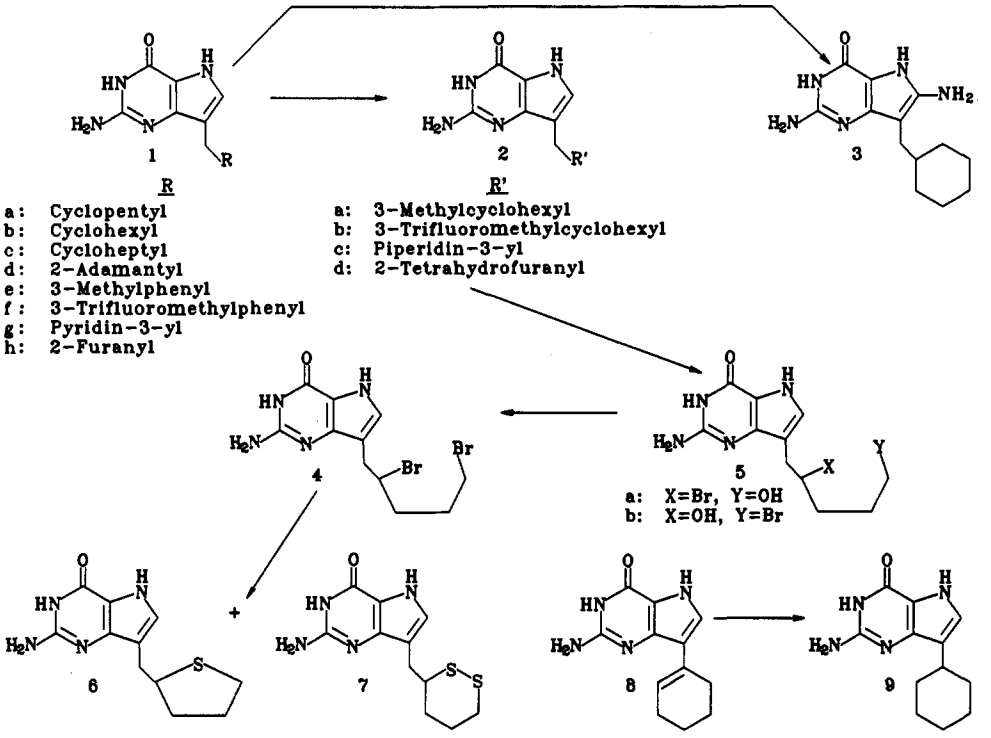
Discussion of Results

Previously we reported the potent inhibition of PNP by the 9-(arylmethyl)-9-deazaguanines. X-ray analysis and computer modeling studies indicated that the enhanced binding of these compounds to PNP was due primarily to two interactions: the donation of a hydrogen bond from the NH at position 7 (purine numbering) to Asn-243 and

* To whom correspondence should be addressed.
† BioCryst Pharmaceuticals, Inc.
‡ Southern Research Institute.
§ University of Alabama at Birmingham.
∥ CIBA-GEIGY Corporation.
⊥ Present address: Gensia Pharmaceuticals, Inc., 4575 Eastgate Mall, San Diego, CA 92121.
Present address: Department of Biochemistry, Cornell University, 207 Biotechnology Building, Ithaca, NY 14853.

0022-2623/93/1836-1847$04.00/0

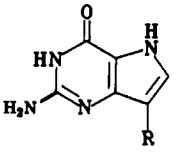
Scheme I



the interaction between the 9-arylmethyl groups and the hydrophobic pocket made up of the aromatic group from Phe-159 (B Subunit),[1] Phe-200, and Tyr-88. The X-ray structures of PNP complexed to several of these inhibitors showed that the binding conformation of all of these inhibitors was similar. In each case, the aromatic ring orientation was edge to face with Phe-159 (B Subunit) and face to edge with Phe-200 in the classical "herringbone" type interaction.[2,14] Substitution at the 2-position of the phenyl ring is detrimental to binding, and X-ray analysis of 9-(2-chlorobenzyl)-9-deazaguanine/PNP complex showed that the 2-chlorophenyl group is deeper in the hydrophobic pocket[2] of the active site resulting in a stronger hydrophobic interaction, but also resulting in displacement of the 9-deazapurine ring from its optimal position in the purine binding site, thus weakening the hydrogen-bonding interactions. This latter effect must be the cause of the decreased binding of the 2-chlorobenzyl compound relative to the 3- and 4-chlorobenzyl compounds, which bind more like the unsubstituted benzyl compound. Other aryl groups such as thienyl, furanyl, and pyridyl also provided potent inhibitors.

It occurred to us that reduction of the benzene ring in 9-benzyl-9-deazaguanine to afford 9-(cyclohexylmethyl)-9-deazaguanine might result in a substituent that would occupy a greater volume of the hydrophobic binding site and thus bind with higher affinity. Inspection of the binding site (using molecular graphics) suggested that the cyclohexyl group could indeed fit within the hydrophobic binding cavity. Nonetheless, reduction of the aromatic substituents, in general, to the corresponding alicyclic and heteroalicyclic analogs would disrupt the "herringbone" interaction observed for the aryl analogs. In addition, the alicyclic compounds differ from the corresponding aryl analogs in molecular volume, geometry, and rigidity, all of which could dramatically influence the binding conformation and affinity. To determine the effect of this replacement on binding affinity, a series of such compounds were prepared and evaluated as PNP inhibitors and studied by X-ray crystallography and computer

Table I. Inhibition of PNP[a]



no.	R	IC_{50} (μM)[b] 1 mM PO_4	IC_{50} (μM)[b] 50 mM PO_4	ratio[c]
6	2-tetrahydrothienyl-methyl[d]	0.011	0.22	20
7	1,2-dithian-3-ylmethyl	0.018	0.20	11
2b	[3-(trifluoromethyl)-cyclohexyl]methyl	0.025 ± 0.006	0.80 ± 0.08	32
2a	(3-methylcyclohexyl)-methyl	0.025	0.82	33
1a	cyclopentylmethyl[d]	0.029	1.8	62
1c	cycloheptylmethyl	0.030	0.86	29
1b	cyclohexylmethyl[d]	0.047 ± 0.014	2.1 ± 0.03	45
2d	(2-tetrahydrofuranyl)-methyl	0.070	0.30	4
1d	2-adamantylmethyl	0.090	2.5	28
2c	piperidin-3-ylmethyl[e]	1.0	1.0	1
9	cyclohexyl[d]	1.3	150	110
8	cyclohexenyl[d]	1.9	19	10
3	cyclohexylmethyl (8-amino)[d]	6.6 ± 0.07	150 ± 120	23

[a] Calf spleen. [b] All values are averages of two determinations except where standard deviations are shown. [c] IC_{50} 50 mM PO_4/IC_{50} 1 mM PO_4. [d] Structure of complex with PNP determined by X-ray crystallography. [e] This compound, which is hygroscopic, was weighed in a nitrogen atmosphere.

modeling. Reduction of the aryl and heterocyclic rings of our previously-prepared compounds provides, with one exception, PNP inhibitors of equal potency when assayed in 1 mM phosphate (see Table I). In 50 mM phosphate, some of the reduced compounds are 3–11 times less potent. The inhibition by 9-(cyclohexylmethyl)-9-deazaguanine (**1b**) is competitive with respect to inosine with a K_i of 37 nM.

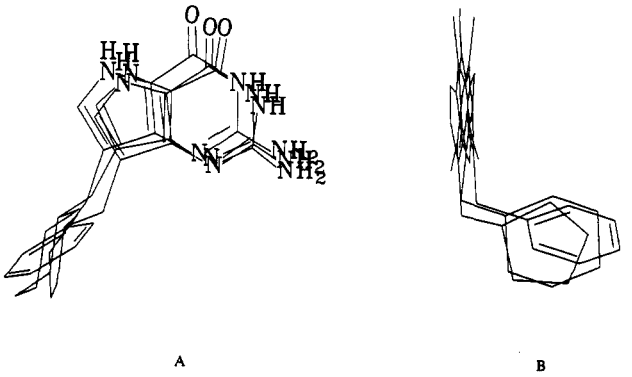


Figure 1. Comparison of the binding of 9-(cyclopentylmethyl)- and 9-(cyclohexylmethyl)-9-deazaguanine (1a and 1b) with 9-benzyl-9-deazaguanine in the active site of PNP, as determined by X-ray crystallography.

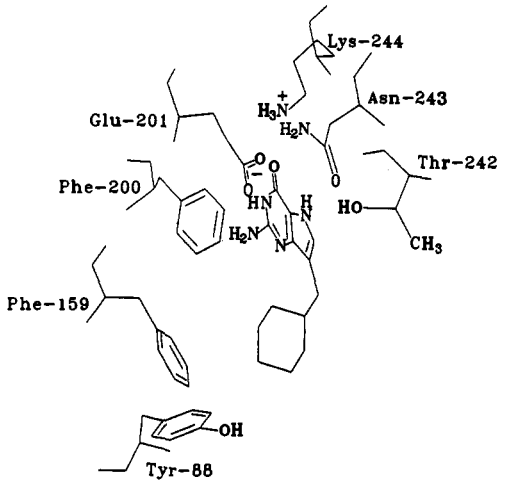


Figure 2. The interaction of the cyclohexyl ring of 9-(cyclohexylmethyl)-9-deazaguanine (1b) with Phe-159 and Phe-200 of the active site of PNP, as determined by X-ray crystallography.

A comparison of the binding in the active site of PNP of the 9-(cyclopentylmethyl)- and 9-(cyclohexylmethyl)-9-deazaguanine (1a and 1b) with that of 9-benzyl-9-deazaguanine shows that they all bind in a similar manner: the 9-deazaguanine interactions are essentially the same as enumerated previously,[2] and the cycloaliphatic rings occupy the same space as the phenyl ring (see Figure 1). It is clear that there are strong hydrophobic interactions in all three cases, although the mode of the interactions is somewhat different. Instead of the "herringbone" interaction peculiar to aromatic rings, the face of the cycloaliphatic rings interacts with the phenyl rings making up the hydrophobic pocket (Figure 2). The heteroalicyclics 2c and d interact in a similar manner. Since 9-alkylhypoxanthines are notoriously poor inhibitors of PNP,[15] with K_i's 3–6-fold greater than hypoxanthine itself,[16] the semirigid character of these alicyclic and heteroalicyclic rings must be important to the strong interactions observed.

There is little information in the literature concerning comparisons of the hydrophobic interactions of alicyclic or heteroalicyclic rings with those of the corresponding aromatic ring. One study does conclude that the affinity of α-chymotrypsin for acetyl-L-phenylalaninamide is no greater and is probably less than for acetyl-L-hexahydrophenylalaninamide.[17]

Docking 9-(2-adamantylmethyl)-9-deazaguanine (1d) in the active site of PNP with the 9-deazaguanine moiety binding normally showed that even the bulky adamantyl group could position itself reasonably relative to the hydrophobic pocket and the phosphate binding site (see below also). Despite its poor solubility, which prevented determination of the structure of its complex with PNP, 1d has an IC_{50} of 0.09 μM. It is about $^1/_3$ to $^1/_2$ as potent as the cycloaliphatics 1a–c, in keeping with the docking experiment.

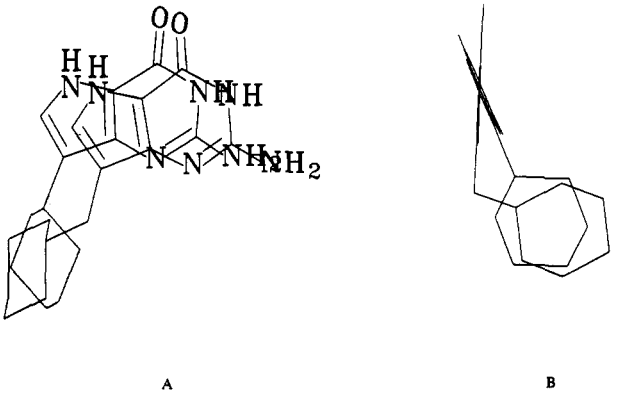


Figure 3. The binding of 9-(cyclohexylmethyl)-9-deazaguanine (1b) and 9-cyclohexyl-9-deazaguanine (9) in the active site of PNP, as determined by X-ray crystallography.

Compound 3, the 8-amino derivative of 9-(cyclohexylmethyl)-9-deazaguanine (1c), is a relatively poor inhibitor of PNP having an IC_{50} in 1 mM PO_4 of 150 times that of 1c itself. This observation is consistent with that previously observed with the 8-amino-9-(arylmethyl)-9-deazaguanines and the explanation is the same.[2] That is to say the shift in the position of Thr-242, caused by the hydrogen bond between the NH of the 9-deazaguanine and Asn-243, positions the methyl group of Thr-242 close to the 8-amino group causing a bad interaction.

To study the importance of a spacer atom between the 9-deazapurine ring and the alicyclic group, we prepared 9-cyclohexyl-9-deazaguanine (9) and evaluated it against PNP. The IC_{50} values were dramatically increased, being 30-fold higher in 1 mM PO_4 and 70-fold higher in 50 mM PO_4 (Table I). A comparison of the complex of 9 with that of 1b showed that the cyclohexyl group of 9 and of 1b occupy almost the same space in the complexes with the enzyme (Figure 3). In order for this to occur with 9, the 9-deazaguanine moiety is pulled out of its normal position in the purine binding site weakening the hydrogen bonding interactions, as observed previously[2] with 9-(2-chlorobenzyl)-9-deazaguanine. In the case of 9, however, the hydrophobic interaction appears to be no greater than it is with 1b and the displacement of the 9-deazaguanine ring is greater. As a result, the decrease in binding is more than four times greater (based on IC_{50} values). The binding of 9 relative to 1b is yet another demonstration of the power of hydrophobic forces in the binding of small molecules to proteins. The poor binding of 9-phenyl-8-aminoguanine relative to that of 9-benzyl-8-aminoguanine has been reported.[18] These results emphasize the importance of spacing of the parts of an inhibitor that bind to different parts of an enzyme active site and with different types of interactions. To predict the relative magnitudes of the forces involved would be difficult indeed.

The one exception to the general observation that replacement of the aromatic rings of 9-(arylmethyl)-9-deazaguanine analogs with its fully reduced equivalent was found with the pyridin-3-yl compound (1g). Piperidin-

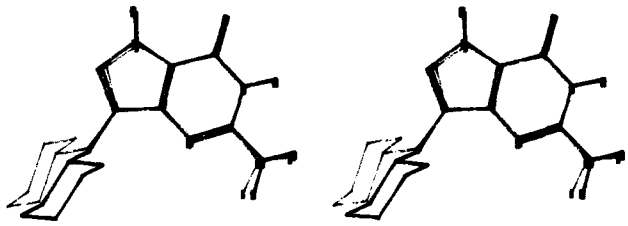


Figure 4. Comparison of the computed structure (thick like) vs X-ray structure (thin line) for 9-(cyclohexylmethyl)-9-deazaguanine. In this particular case, the bound conformer corresponding to the global energy minimum compares favorably with the structure of the bound inhibitor deduced from Fourier difference maps.

3-yl (**2c**) was found to be a relatively poor inhibitor with an IC_{50} of 1 μM in both 1 and 50 mM PO_4. The basic nature of piperidine (pK_a = 11.1), which is protonated at physiologic pH, may be responsible for the poor binding of **2c**.

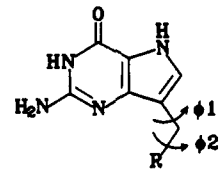
Molecular Modeling Studies

Our previous work on 9-(arylmethyl)-9-deazaguanines[1,2] demonstrated the utility of Monte Carlo/energy minimization (MC/EM) techniques[2,19,20] for computing the binding conformation of these PNP inhibitors. In this procedure, inhibitors are docked into the binding site of the PNP/guanine complex (determined via protein crystallographic analysis) by overlapping the purine ring of the inhibitor with that of guanine. After superimposing the inhibitor, the guanine molecule is removed from the binding site and the inhibitor is subjected to the MC/EM conformational search procedure. During the Monte Carlo phase of the MC/EM procedure, bonds of the inhibitor that can undergo free rotation are subjected to torsion angle variation. During the energy-minimization phase, the enzyme residues and phosphate are constrained by parabolic restraining potentials whereas the inhibitor is free to move within the enzyme binding site as previously described.[19,20] Qualitative evaluation[21] of the low-energy structures obtained via this conformational search procedure has allowed us to prioritize potential inhibitors for chemical synthesis.

The first conformational search on the present series of inhibitors was conducted on 9-(cyclohexylmethyl)-9-deazaguanine. After 2000 MC/EM steps, a total of 10 unique low-energy conformers (within 5 kcal/mol of the global minimum) was discovered. Inspection of the lowest energy conformers indicated that the cyclohexane ring was equatorially substituted and that it occupied the hydrophobic binding site in approximately the same manner as had been determined previously (via protein crystallographic analysis) for the phenyl substituent in 9-benzyl-9-deazaguanine. Hence, we anticipated that this compound would be a potent inhibitor of PNP. This assumption was confirmed upon testing this species in the enzyme inhibition assay. The global minimum-energy conformer found during the MC/EM conformational search procedure compared favorably with the X-ray structure (Figure 4). Figure 5 shows six of the lowest energy equatorially substituted conformers discovered. Inspection of the low-energy conformers in the enzyme binding site demonstrated that in *each case* the cyclohexyl substituent is embedded within the hydrophobic binding cavity.

Prior to their synthesis, MC/EM conformational searches were also conducted on 9-(cyclopentylmethyl)-, 9-(cycloheptylmethyl)-, and 9-(cyclohexylmethyl)-9-deazaguanine. The results of these conformational searches are depicted in Table II. In general, one of the lowest energy conformers compared favorably to the one observed crystallographically.[22] In all cases, inspection of the lowest energy conformers leads one to conclude that the cycloalkyl moiety occupies the hydrophobic binding site in a manner similar to that observed for the phenyl substituent of 9-benzyl-9-deazaguanine.

Table II. Monte Carlo/Energy Minimization of Inhibitors in the PNP Binding Site[a]



no.	R	no. of unique structures[b]	ΔE[c]	calculated[d] $\phi1$	calculated[d] $\phi2$	observed[e] $\phi1$	observed[e] $\phi2$
1a	cyclopentyl	11	0.4	−69	−61	−59	−48
1b	cyclohexyl	12	0.0	−72	−58	−58	−36
1c	cycloheptyl	73[f]	0.0	−75	−47		
1d	2-adamantyl	2	0.0	−48	−57		
9	cyclohexyl[g]	5	0.0	−90		−100	

[a] Generally 1000–2000 MC/EM steps were performed; the AMBER force field was employed with a distance dependent diecectric constant further attenuated by a factor of 2.0. [b] Number of conformers within a 5 kcal/mol energy window. [c] Energy difference of the conformer most closely resembling the one observed crystallographically and the global minimum. [d] Torsion angles for the low-energy conformation that most closely resembled the one observed crystallographically. [e] Torsion angles derived from Fourier difference maps. [f] Different ring conformations were also considered. [g] Cyclohexyl group attached directly to the 9-deazaguanine.

Inspection of these lowest energy conformers of 9-(cyclohexylmethyl)-9-deazaguanine suggested that incorporation of substituents larger than cyclohexyl or cycloheptyl might be accommodated by the hydrophobic binding pocket. In fact, the low-energy conformers (Figure 5) suggested to us that an adamantyl substituent should be tolerated within this binding site. Molecular graphics analysis[21] also indicated that the hydrophobic binding cavity could accommodate substituents as large as adamantyl. MC/EM conformational searches on 9-(2-adamantylmethyl)-9-deazaguanine produced low-energy conformers in which the adamantane ring was contained within the hydrophobic binding site. The interaction energy (i.e., the sum of the van der Waals and electrostatic energy of interaction between the inhibitor and enzyme binding site) for the global minimum energy conformer of 9-(2-adamantylmethyl)-9-deazaguanine was computed and compared to the interaction energies computed for the 9-benzyl, 9-cyclohexylmethyl, 9-cyclopentylmethyl, and 9-cycloheptylmethyl analogs. Similar values (all lying within a 5 kcal/mol energy window) for these interaction energies were obtained. Although the interaction energies would be expected to be only a crude measure of binding free energies, we nonetheless postulated that if there was an exceptionally poor fit between a potential inhibitor and the PNP binding site, this would be reflected in an interaction energy that deviated significantly for that calculated for a tightly bound inhibitor such as 9-benzyl-9-deazaguanine. In fact, the interaction energy calculated for 9-(2-adamantylmethyl)-9-deazaguanine was ~3 kcal/mol *lower* in energy than that calculated for the 9-benzyl analog. Furthermore, we concluded that no significant strain had been induced in the adamantyl-substituted analog by comparing the molecular mechanics energy of

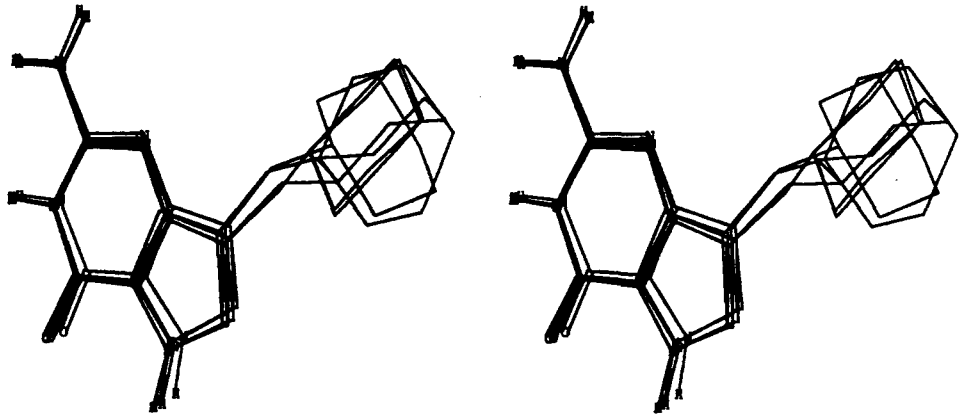


Figure 5. Superimposition of six of the lowest energy (equatorially substituted) conformers of 9-(cyclohexylmethyl)-9-deazaguanine determined using the MC/EM conformational search procedure applied to the inhibitor docked to the enzyme binding site.

isolated 9-(2-adamantylmethyl)-9-deazaguanine (in its enzyme bound conformation) with its isolated global minimum-energy conformation. Thus, it was postulated that 9-(2-adamantylmethyl)-9-deazaguanine should be a reasonably potent PNP inhibitor. This prediction was verified upon synthesis of this material and measurement of its IC_{50}.

In contrast to the crystallographically observed binding geometry for 9-cyclohexyl-9-deazaguanine, MC/EM conformational searches produced low-energy conformers in which the cyclohexyl substituent only partially occupied the hydrophobic binding site but the purine ring remained in approximately the same position that had been observed crystallographically for 9-(cyclohexylmethyl)-9-deazaguanine. In fact, protein crystallographic analysis revealed that the *cyclohexyl substituent* in the 9-cyclohexyl analog occupies approximately the same location as it does in the 9-cyclohexylmethyl analog and the *purine base* is displaced from its "normal" position. Although in this case the molecular modeling studies allow us to predict that the 9-cyclohexyl analog should have decreased potency relative to the 9-cyclohexylmethyl analog, it was for the wrong reason. This result underscores the necessity of checking molecular modeling results against those determined experimentally using protein crystallographic analysis. Furthermore, this result suggests that our calculations led to an over estimation of the hydrogen bonding interactions relative to the hydrophobic interactions. A possible explanation is that the O6–N7 face of the purine ring is positioned in a hydrophilic region of the active site, which will undergo desolvation upon binding of the inhibitor. Solvent was not considered in our calculations but would be expected to decrease the strength of these hydrogen-bond interactions relative to the binding of the cyclohexyl group to the hydrophobic binding pocket. Although molecular mechanics-based calculations are often remarkably accurate in allowing the prediction of molecular geometries, they are nonetheless only an approximation of the true molecular energetics. For example, the force fields currently in use are, in fact, a rather crude representation of the potential energy of molecular systems. Thus, it is not surprising that we were unable to accurately predict the observed binding geometry for 9-cyclohexyl-9-deazaguanine since it may have been determined by subtle differences between hydrophobic effects and hydrogen bonding interactions. It is not possible for 9-cyclohexyl-9-deazaguanine to simultaneously form optimal hydrogen bonds to the enzyme and optimize its hydrophobic association with the enzyme. Apparently, the hydrophobic forces predominate and ultimately determine the binding geometry.

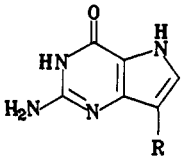
Experimental Section

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 were homogeneous by 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. 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 ^{1}H-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. Spectral and analytical data for compounds **1a–d**, **2a–d**, **3**, and **6–9** appear in Table III.

Synthesis of 2-Amino-1,5-dihydro-7-substituted-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (1). Standard Procedure. The synthesis of 2-amino-7-(cyclohexylmethyl)-1,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**1b**) is described to illustrate the standard procedure[2] by which compounds **1a-d** and **8** were prepared. Similar yields were obtained in all cases.

α-Formylcyclohexanepropanenitrile. Under an atmosphere of dry nitrogen, NaH (5.38 g, 0.224 mol) was added to a solution of the cyclohexanepropanenitrile (22.3 g, 0.16 mol) in anhydrous tetrahydrofuran (200 mL). The mixture was heated to ~55 °C on a water bath, and ethyl formate (55.4 g, 0.75 mol) was added dropwise, accompanied by vigorous H_2 evolution. After 2 h at 50–55 °C, additional portions of NaH (2.0 g) and ethyl formate (15.0 mL, 13.76 g) were added, and the reaction mixture was stirred for 3 days at 55 °C and then allowed to cool to room temperature. Volatiles were evaporated *in vacuo*, and the residual pale yellow crust was dissolved in water (75 mL) at 0 °C and adjusted to pH 6 by slow addition of cold 6 N HCl. The oily mixture was extracted with $CHCl_3$ (3 × 100 mL); the extract was washed with H_2O (100 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give an amber oil: yield 25.22 g. Thin-layer chromatography showed two significant components: nitrile starting material, >50%, and a product spot presumed to be the desired

Table III. Spectral and Analytical Data



no.	R	mp, °C	analytical/spectral data
1a	cyclopentylmethyl	324–326	MS (FAB) *m/e* 233 $(M + H)^+$, 465 $(2 M + H)^+$; UV 0.1 N HCl 236 (17.1), 274 (14.9), pH 7 buffer 232 (19.2), 273 (11.1), 0.1 N NaOH 230 (21.1), 266 (7.9), 287 (sh); ^{1}H NMR (DMSO-d_6) δ 1.17, 1.53, 2.16 (complex m, 9 H, cyclopentyl H), 2.46 (d, 2 H, CH_2R), 5.80 (s, 2 H, NH_2), 6.93 (d, J = 3.0, 1 H, H-6), 10.32 (s, 1 H, 3-N*H*), 11.09 (s, 1 H, 5-N*H*). Anal. ($C_{12}H_{16}N_4O$) C, H, N.
1b	cyclohexylmethyl	>300	MS (EI) *m/e* 246 (M^+); UV 0.1 N HCl 236 (16.9), 274 (14.9), pH 7 232 (18.7), 273 (11.2); 0.1 N NaOH 231 (19.2), 267 (7.6); IR (KBr) 3462, 3177, 3130, 2921, 2850, 1682, 1659, 1625, 1580, 1522, 1375; ^{1}H NMR (DMSO-d_6) δ 0.88, 1.12, 1.48, 1.62 (complex m, 11 H, cyclohexyl *H*), 1.32 (d, J = 7.0, 2 H, CH_2R), 5.80 (s, 2 H, NH_2), 6.9 (d, J = 3.0, 1 H, H-6), 10.30 (s, 1 H, 3-N*H*), 11.10 (s, 1 H, 5-N*H*). Anal. ($C_{13}H_{16}N_4O$) C, H, N.
1c	cycloheptylmethyl	>300	MS (FAB) *m/e* 261 $(M + H)^+$, 521 $(2 M + H)^+$; UV 0.1 N HCl 236 (17.0), 274 (14.9), pH 7 273 (11.3); 0.1 N NaOH 267 (7.6); ^{1}H NMR (DMSO-d_6) δ 1.40 (complex m, 13 H, cycloheptyl *H*), 2.34 (d, J = 6.8, 2 H, CH_2R), 5.83 (s, 2 H, NH_2) 6.91 (α, J = 3.2, 1 H, H-6), 10.35 (s, 1 H, 3-N*H*), 11.14 (s, 1 H, 5-N*H*). Anal. ($C_{14}H_{20}N_4O$) C, H, N.
1d	2-adamantylmethyl	>300	MS (FAB) *m/e* 299 $(M + H)^+$; UV 0.1 N HCl 236 (16.5), 275 (14.8), 0.1 N NaOH 268 (7.6); IR (KBr) 3324, 3259, 3185, 3177, 1670, 1626, 1561, 1524, 1411, 1372; ^{1}H NMR (DMSO-d_6) δ 1.45, 1.64, 1.80, 1.96 (m, 17 H, adamantyl *H*) 2.58 (d, J = 8.0, 2 H, CH_2-R), 5.84 (s, 2 H, NH_2), 6.90 (d, J = 3.0, 1 H, 6-*H*), 10.35 (bs, 1 H, 3-N*H*) 11.14 (d, 1 H, 5-N*H*). Anal. ($C_{17}H_{22}N_4O{\cdot}0.4H_2O$) C, H, N.
2a	(3-methylcyclohexyl)-methyl	280	MS (FAB) *m/e* 261 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 0.55, 1.20, 1.45 (m, 10 H, cyclohexyl *H*), 0.81, 0.85 (d, J = 7.5, 3 H, CH_3), 2.36 (d, J = 7.5, 2 H, CH_2R), 7.11 (d, J = 3.0, 1 H, 6-*H*), 7.36 (bs, 1 H, NH_2), 11.94 (bs, 1 H, 5-N*H*), 12.25 (very broad, seen in the integral, 1 H, 3-N*H*). Anal. ($C_{14}H_{20}N_4O$) C, H, N.
2b	[3-(trifluoromethyl)-cyclohexyl]methyl	165	MS (FAB) *m/e* 315 $(M + H)^+$; 629 $(2 M + H)^+$; UV 0.1 N HCl 236 (16.2), 274 (14.6), pH 7 232 (18.9), 272 (10.6), 0.1 N NaOH 266 (7.3); ^{1}H NMR (DMSO-d_6) δ 0.85, 1.20, 2.20, 2.40 (complex m, 10 H, cyclohexyl *H*), 2.38 (m, 2 H, CH_2R), 6.08 (bs, 2 H, NH_2), 6.98 (d, J = 3.0, 1 H, 6-*H*), 10.60 (very broad, seen in the integral, 1 H, 3-N*H*), 11.32 (bs, 1 H, 5-N*H*). Anal. ($C_{14}H_{17}F_3N_4O{\cdot}0.7H_2O$) C, H, N.
2c	piperidin-3-ylmethyl	softens from 200 °C	MS (FAB) *m/e* 248 $(M + H)^+$; UV 0.1 N HCl 234 (15.7), 273 (13.8), pH 7 buffer 232 (19.1), 271 (9.3), 0.1 N NaOH 229 (19.0), 266 (7.1), 288 (sh); ^{1}H NMR (DMSO-d_6) δ 1.19, 1.61, 1.77 (m, 4 H, H-4's and 5's of piperidine), 1.96 (m, 1 H, H-3 of piperidine), 2.53 (d, 2 H, -CH_2-), 2.69 (m, 2 H, H-2's of piperidine), 3.14 (d, 2 H, H-6's of piperidine), 7.25 (d, J = 2.8, 1 H, H-6), 7.76 (s, 2 H, NH_2), 8.81, 9.18 (br, 1 each, N*H*$^+$ exchanges with D_2O), 12.24 (s, 1 H, 5-N*H*). The spectrum confirmed presence of EtOH and H_2O. Anal. ($C_{12}H_{17}N_5O{\cdot}2.8HCl{\cdot}0.2C_2H_6O{\cdot}H_2O$) C, H, N, Cl.
2d	(2-tetrahydrofuranyl)-methyl	284–286	MS (FAB) *m/e* 235 $(M + H)^+$; UV 0.1 N HCl 235 (17.1), 273 (14.7), pH 7 buffer 232 (20.0), 271 (10.5), 0.1 N NaOH 228 (20.2), 264 (7.0), 286 (6.2); ^{1}H NMR (DMSO-d_6) δ 1.50, 1.78 (m, 4 H, H-3's and 4's of tetrahydrofuranyl), 2.52, 2.69 (2 d, 2 H, CH_2R), 3.57, 3.76 (2 m, 1 each, H-5's of tetrahydrofuranyl), 5.82 (s, 2 H, NH_2), 6.97 (d, J = 2.8, 1 H, H-6), 10.34 (s, 1 H, 3-N*H*), 11.18 (s, 1 H, 5-N*H*). Anal. ($C_{11}H_{14}N_4O_2$) C, H, N.
3	cyclohexylmethyl (8-amino)	269–71	MS (EI) *m/e* 261 (M^+); ^{1}H NMR (DMSO-d_6) δ 0.90, 1.10, 1.60 (m, 11 H, cyclohexyl *H*), 2.16 (d, J = 7.5, 2 H, CH_2R), 4.94 (bs, 2 H, NH_2), 5.74 (bs, 2 H, NH_2), 7.5–9.5 (very broad, seen in the integral, 1 H, N*H*), 9.91 (s, 1 H, *NH*). Anal. ($C_{13}H_{19}N_5O$) C, H, N.
6	(2-tetrahydrothienyl)-methyl	311–313	MS (FAB) *m/e* 251 $(M + H)^+$; UV 0.1 N HCl 234 (17.4), 274 (15.2), pH 7 buffer 232 (20.9), 272 (10.6), 0.1 N NaOH 228 (21.3), 265 (7.3), 287 (6.5); IR (KBr) 3211, 3203, 3127, 3108, 2936, 1661, 1609, 1567, 1524, 1369 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.62, 1.87, 2.01 (complex m, 4 H, H-3's and 4's of tetrahydrothienyl), 2.72 (m, 4 H, -CH_2- overlapping H-5's of tetrahydrothienyl), 3.64 (p, 1 H, H-2 of tetrahydrothienyl), 5.82 (s, 2 H, NH_2), 6.97 (d, J = 3.0, 1 H, H-6), 10.34 (s, 1, 3-N*H*), 11.17 (s, 1, 5-N*H*). Anal. ($C_{11}H_{14}N_4OS$) C, H, N.
7	1,2-dithian-3-yl-methyl	300–302	MS (FAB) *m/e* 283 $(M + H)^+$; IR (KBr) 3139, 3135, 3131, 2926, 1673, 1658, 1609, 1569, 1523, 1370 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.53, 1.67, 2.04 (complex m, 4 H, H-4's and 5's of dithianyl), 2.67 (m, 2 H, CH_2R); 2.75 (m, 2 H, H-6's of dithianyl), 3.17 (m, 1 H, H-3 of dithianyl), 5.84 (s, 2 H, NH_2), 7.00 (d, J = 3.0, 1 H, H-6), 10.34 (s, 1 H, 3-N*H*), 11.24 (s, 1 H, 5-N*H*). Anal. ($C_{11}H_{14}N_4OS_2$) C, H, N.
8	cyclohexenyl	280	MS (FAB) *m/e* 231 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.56 (m, 2 H, CH_2CH_2CH), 1.67 (m, 2 H, $CH_2CH_2C{\equiv}$), 2.11 (m, 2 H, CH_2CH), 2.28 (m, 2 H, $CH_2C{\equiv}$), 5.78 (s, 2 H, NH_2), 6.8 (m, 1 H, CH_2CH), 7.06 (d, J = 3.0, 1 H, 6-*H*), 10.38 (s, 1 H, 3-N*H*), 11.3 (d, 1 H, 5-N*H*). Anal. ($C_{12}H_{14}N_4O{\cdot}0.6H_2O$) C, H, N.
9	cyclohexyl	>300	MS (FAB) *m/e* 233 $(M + H)^+$; UV 0.1 N HCl 236 (17.5), 274 (14.9), pH 7 231 (19.5), 273 (11.0), 0.1 N NaOH 266 (7.5), 286 (6.5); ^{1}H NMR (DMSO-d_6) δ 1.28, 1.72, 1.91 (m, 10 H, cyclohexyl *H*), 2.57 (m, 1 H, CH_2CH), 5.78 (s, 2 H, NH_2), 6.88 (d, J = 2.8, 1 H, 6-*H*), 10.28 (s, 1 H, 3-N*H*), 11.08 (s, 1 H, 5-N*H*). Anal. ($C_{12}H_{16}N_4O{\cdot}0.1EtOH$) C, H, N.

product based on a positive rosaniline spray test. Since the nitrile is inert in the next reaction, the mixture was used without further purification.

α-[[(2-Methoxy-2-oxoethyl)amino]methylene]cyclohexanepropanenitrile. To a solution of crude α-formylcyclohexanepropanenitrile (25.22 g) in a mixture of MeOH/H_2O (4:1, 500 mL) was added glycine methyl ester hydrochloride (30.60 g, 0.24 mol) and sodium acetate (19.99 g, 0.24 mol). After the solution was stirred at room temperature for 24 h, the methanol was evaporated on a rotary evaporator at room temperature. The turbid mixture of residual oil and water was extracted with $CHCl_3$ (3 × 100 mL). The extract was washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated to give an amber oil. Chromatography on a silica gel column (230-400 mesh) with $CHCl_3$

gave 13.38 g of cyclohexanepropanenitrile (60% of the weight used in the previous reaction) and the desired enamine as a viscous light yellow gum: yield 5.23 g; MS (EI) m/z 236 $(M)^+$, 177 (236 – $CO_2CH_3)^+$; ^1H NMR (DMSO-d_6): δ 0.9, 1.15, 1.36 (m, 11 H, cyclohexyl *H*), 1.83 (d, J = 7.5 Hz, 0.67 H, CH_2 of *trans*-isomer), 1.87 (d, J = 7.0 Hz, 0.33 H, CH_2 of *cis*-isomer), 3.64 (s, 3 H, OCH_3), 3.87 (d, 2 H, CH_2-NH), 6.52 (m, 0.33 H, N*H* of *cis*-isomer), 6.58–6.74 (m, 1 H, N*H* of *trans*- and CH=C of *cis*-isomers), 6.80 (d, J = 13.5 Hz, 0.67 H, CH=C of *trans*-isomer).

1-Ethyl 2-Methyl 3-Amino-4-(cyclohexylmethyl)-1*H*-pyrrole-1,2-dicarboxylate. Under a nitrogen atmosphere, a solution of enamine from the previous step (2.0 g, 8.46 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C and treated with 1,5-diazabicyclo-[4.3.0]non-5-ene (DBN, 2.1 g, 16.9 mmol, 2.0 equiv) followed by dropwise addition of ethyl chloroformate (1.38 g, 12.69 mmol). After stirring at 0 °C for 1 h at room temperature overnight, TLC showed only a trace of enamine starting material, but there was considerable uncyclized blocked enamine. Additional DBN (1.5 mL, 12.7 mmol) and $ClCO_2Et$ (0.5 mL) were added, and the reaction mixture was allowed to stand overnight. Volatiles were evaporated *in vacuo*; a solution of the viscous residue in $CHCl_3$ was applied to a short silica gel column to remove the DBN. Evaporation of product-containing fractions gave 2.60 g (100%) of the desired N-protected pyrrole that was used in the next step without further purification.

Methyl 3-Amino-4-(cyclohexylmethyl)-1*H*-pyrrole-2-carboxylate. Anhydrous Na_2CO_3 (2.23 g, 21.04 mmol) was added to a solution of the N-blocked pyrrole (2.6 g, 8.43 mmol) in MeOH (100 mL), and the mixture was stirred at room temperature for 48 h. The MeOH was evaporated, H_2O (100 mL) was added to dissolve the inorganics, and the mixture was extracted with $CHCl_3$ (3 × 100 mL). The extract was dried (Na_2SO_4) and evaporated *in vacuo* to give a viscous gum, which was purified by column chromatography on silica gel with $CHCl_3$ as eluent to give the desired pyrrole: yield 1.67 g (84%); mp 73–74 °C; MS (EI) m/z 236 $(M)^+$, 205 (236 – $OCH_3)^+$; IR (KBr) 3395, 3310, 3150, 3068, 2922, 2909, 1678, 1592, 1515; ^1H NMR (DMSO-d_6) δ 0.86, 1.14, 1.37, 1.62 (m, 11 H, cyclohexyl *H*), 2.15 (d, J = 7.0 Hz, 2 H, CH_2), 3.68 (s, 3 H, OCH_3), 4.76 (bs, 2 H, D_2O exchangeable, NH_2), 6.47 (d, J = 3.3 Hz, 1 H, pyrrole ring proton), 10.36 (bs, 1 H, N*H*). Anal. ($C_{13}H_{20}N_2O_2$) C, H, N.

Methyl 3-[[(Benzoylamino)thioxomethyl]amino]-4-(cyclohexylmethyl)-1*H*-pyrrole-2-carboxylate. Under an atmosphere of dry N_2, benzoyl isothiocyanate (0.74 g, 4.53 mmol) was added at room temperature to a stirred solution of the pyrrole (0.95 g, 4.02 mmol) in dry CH_2Cl_2 (20 mL). After 1 h at room temperature, the solvent was evaporated and the gummy residue was triturated with Et_2O (50 mL) with almost immediate separation of crystalline solid which was collected by filtration. The Et_2O filtrate was heated to boiling and diluted with an equal volume of warm cyclohexane. On cooling slowly the solution gave additional product as a crystalline solid that was dried *in vacuo* over P_2O_5 for 2 h at 80 °C: total yield 1.41 g (88%); mp 156–157 °C; MS (EI) m/z 399 $(M)^+$; 278 (399 – $NH_2C(O)$ – $Ph)^+$; IR (KBr) 3254, 2925, 2919, 1670, 1535, 1498, 1445; ^1H NMR (DMSO-d_6) δ 0.85, 1.14, 1.46, 1.60 (m, 11 H, cyclohexyl *H*), 2.30 (d, J = 7.0 Hz, 2 H, CH_2), 3.72 (s, 3 H, OCH_3), 6.82 (d, J = 3.2 Hz, 1 H, pyrrole C*H*), 7.54, 7.67, 8.0 (t, t, d, 2 H, 1 H, 2 H, benzoyl C*H*), 11.59 (s, 1 H, N*H*), 11.78 (s, 1 H, pyrrole N*H*), 12.12 (s, 1 H, NH). Anal. ($C_{21}H_{25}N_3O_3S$) C, H, N.

Methyl 3-[[[[(Benzoylamino)methyl]thio]methylene]-amino]-4-(cyclohexylmethyl)-1*H*-pyrrole-2-carboxylate. Under a nitrogen atmosphere, a solution of the thioureido compound obtained in the previous step (0.96 g, 2.40 mmol) and DBN (0.33 g; 2.64 mmol) in dry CH_2Cl_2 (20 mL) was cooled to 0 °C and treated with methyl iodide (1.1 g, 7.74 mmol). The light yellow solution was stirred at 0 °C for 15 min and then allowed to warm to room temperature and stirred for an additional hour. Solvent was evaporated *in vacuo*, and the residue was extracted with $CHCl_3$, washed with H_2O (2 × 20 mL), dried (Na_2SO_4), and evaporated to give a thick oil: yield 0.92 g (92%) used in the next step without further purification.

2-Amino-7-(cyclohexylmethyl)-1,5-dihydro-4*H*-pyrrolo-[3,2-*d*]pyrimidin-4-one (1b). A solution of the methylthio compound from the previous step (0.8 g, 1.93 mmol) in MeOH saturated with NH_3 at 0 °C (59 mL), placed into a glass-lined stainless steel bomb, was submerged in an oil bath preheated to 95 °C and heated for 24 h. The contents of the cooled vessel were evaporated to dryness *in vacuo*, and the solid residue was stirred with Et_2O (40 mL) for a few minutes. The insoluble white solid was collected by filtration and washed with Et_2O. The filtrate contained most of the benzamide and 2-methylthio analog of 9-deaza-9-substituted-guanine. A solution of the ether-insoluble solid (0.45 g) in MeOH was evaporated to dryness with ~10 g of silica gel. The dry material was layered onto a silica gel column, which was then eluted with $CHCl_3$/MeOH/HOAc (95:5:1) to give 7-(cyclohexylmethyl)-1,5-dihydro-2-(methylthio)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one: yield 55 mg; mp 194 °C; MS (EI) m/e 277 (M^+); ^1H NMR (DMSO-d_6) δ 0.82, 1.13, 1.62 (complex m, 11 H, cyclohexyl *H*), 2.46 (d, J = 6.6, 2 H, -CH_2R), 2.52 (s, 3 H, SCH_3), 7.80 (d, J = 2.9 Hz, 1 H, H-6), 11.65 (s, 1 H, 5-N*H*), 12.0 (s, 1 H, 3-N*H*). Anal. ($C_{14}H_{19}N_3OS$) C, H, N. Further elution of the column gave the desired 2-amino product **1b**: yield 230 mg (49%).

2-Amino-1,5-dihydro-7-[(3-methylcyclohexyl)methyl]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2a). A solution of **1e** (0.2 g, 0.78 mmol) in TFA (20 mL) was hydrogenated with Pt (from 140 mg of PtO_2) at 60 lb/in.^{2} for 24 h. The catalyst was filtered off through Celite, and the filtrate was evaporated to dryness. The residue was triturated with methanol and left in the refrigerator overnight. The trifluoroacetate salt of **2a** crystallized from the solution and was collected by filtration. The salt was suspended in 8 mL of H_2O, adjusted to pH 8 by concentrated NH_4OH, and sonicated. Pure **2a** was collected, washed with H_2O, and dried: yield 165 mg (81%).

2-Amino-1,5-dihydro-7-[[(trifluoromethyl)cyclohexyl]methyl]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2b). The procedure described for **2a** was also used to prepare compound **2b** from compound **1f**: yield 145 mg (69%).

2-Amino-1,5-dihydro-7-(piperidin-3-yl)-4*H*-pyrrolo[3,2-*d*]-pyrimidin-4-one Hydrochloride (2c). A solution of the compound **1g** (45 mg, 0.18 mmol) in H_2O (45 mL) and 1 N HCl (5 mL) was shaken with Pt (from 150 mg of PtO_2) for 24 h in a Parr apparatus at an initial hydrogen pressure of 60 lb/in.^{2}. The catalyst was filtered off under N_2 pressure and washed with H_2O. The filtrate was evaporated *in vacuo*; the residue was evaporated again with EtOH (50 mL) and redissolved in dry EtOH (25 mL), and the solution was treated dropwise with Et_2O (125 mL) to precipitate the product as a hygroscopic white solid that was dried *in vacuo* over P_2O_5 at 80 °C for 6 h: yield 60 mg (85%).

2-Amino-1,5-dihydro-7-(2-tetrahydrofuranyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (2d). A solution of **1h** (3.04 g, 13.2 mmol) in MeOH (600 mL) was hydrogenated with 30% Pd/C (1.0 g) for 48 h at 50 lb/in.^{2} in a Parr shaker. The mixture was diluted with MeOH to a volume of 1000 mL and warmed on a water bath under N_2 to dissolve the solid that had deposited. The solution was filtered under N_2 pressure, and the catalyst was washed with several portions of warm MeOH. The colorless filtrate was concentrated to ~300 mL, heated to boiling, and cooled slowly to give a deposit of the desired material as white crystalline solid. Further concentration to ~100 mL gave a small second crop. The combined product was dried *in vacuo* over P_2O_5 at 110 °C for 18 h; yield 2.49 g (80%).

2,6-Diamino-1,5-dihydro-7-(cyclohexylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (3). To a solution of **1b** (0.2 g, 0.81 mmol) in concentrated H_2SO_4 (3 mL) was added a mixture of concentrated HNO_3 (0.061 mL) and concentrated H_2SO_4 (0.3 mL) at 0 °C with stirring. The reaction mixture was allowed to stir at 0–5 °C for an additional hour and then poured onto crushed ice. The precipitated light yellow solid was collected by filtration, washed with cold water (3 × 15 mL) and $NaHCO_3$ solution (2 × 15 mL), and dried over P_2O_5: yield of 2-amino-1,5-dihydro-7-(cyclohexylmethyl)-6-nitro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one, 221 mg (92.5%); MS (EI) 291 $(M)^+$, 274 (291 – $OH)^+$. To a light yellow solution of nitro compound (0.2 g, 0.69 mmol) in MeOH (60 mL) was added at reflux 30% Pd/C (100 mg) followed by the portionwise addition of a solution of hydrazine hydrate (1.0 mL) in MeOH (2.0 mL). The reaction mixture was refluxed for an additional 30 min, during which time it became colorless. The catalyst was removed by filtration through Celite, and the filtrate was filtered through No. 42 Whatman filter paper. The solvent was evaporated *in vacuo* at room temperature to give the desired 8-amino compound **3** as a white solid: yield, 137.3 mg (76.5%).

Table IV. Summary of X-ray Crystallographic Data for PNP/Inhibitor Complexes

no.	no. of crystals	no. of observations	no. of reflections	R_{merge}	R_{frac}
1a	1	19262	10619	0.130	0.20
1b	1	16900	9703	0.060	0.17
3	1	15388	8720	0.064	0.20
6	1	17124	10868	0.065	0.19
8	1	17774	7712	0.086	0.16
9	1	19541	10118	0.097	0.19

[a] R_{merge} is the R factor on intensities for merging symmetry-related reflections. [b] R_{frac} is the percent change between native and scaled PNP/inhibitor structure factor data.

2-Amino-1,5-dihydro-7-(2-tetrahydrothienyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (6) and 2-Amino-1,5-dihydro-7-(1,2-dithian-3-yl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (7). A solution of the tetrahydrofuranyl compound **2d** (0.96 g, 4.1 mmol) in 2 N HBr (100 mL), to which a crystal of phenol had been added, was warmed in an oil bath at 40 °C for 20 h and then stirred at room temperature for 24 h before it was evaporated to dryness *in vacuo* at room temperature. The residue was triturated thoroughly with Et_2O, which was decanted, and then several small portions of benzene were added and evaporated to aid removal of water. The crude isomeric bromo alcohols **5** so obtained were used in the next step without further purification. A solution of crude **5** in anhydrous DMAc (20 mL) was cooled in an ice bath and treated with PBr_3 (2.22 g, 8.2 mmol). The yellow solution was stirred at 0 °C for 4 h and then allowed to warm to room temperature before it was evaporated *in vacuo* at room temperature. The residue was washed thoroughly with toluene by trituration and decantation and dried *in vacuo* over NaOH to give 2-amino-1,5-dihydro-7-(2,5-dibromopentyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**4**) as crude material which was used in the next step without further purification.

A solution of the **4** in $EtOH/H_2O$ (1:1, 100 mL) was warmed to 60 °C on a water bath, and solid $Na_2S{\cdot}9H_2O$ was added in portions until the solution was neutral. Additional $Na_2S{\cdot}9H_2O$ (1.1 g) was added, and the solution was refluxed for 24 h under a nitrogen atmosphere and then evaporated to dryness *in vacuo*. An aqueous solution (150 mL) of the residue was acidified (pH 3) with 6 N HCl, and the mixture was evacuated for a few minutes to remove most of the H_2S. Neutralization (1 N NaOH) gave a beige precipitate that was collected by filtration, washed with cold H_2O, and dried in vacuo; yield 0.993 g. A solution of the solid in MeOH was evaporated with silica gel (20 g), and the mixture was layered onto a silica gel column, which was eluted with $CHCl_3$/MeOH, 4:1. Further chromatography of some of the fractions containing product using $CHCl_3$/MeOH, 95:5, was required to give material that was free of a minor byproduct (**7**) that ran just slightly ahead of the main product band. Fractions containing **6** were evaporated to give a crystalline solid (117 mg) which was freed of a small amount of silica gel by continuous extraction into isopropyl acetate. The white crystalline solid was dried *in vacuo* over P_2O_5 at 110 °C for 20 h; yield of **6**, 97 mg (see Table II for physical and spectral data). 2-Amino-1,5-dihydro-7-(1,2-dithian-3-yl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**7**) was obtained from the other column fractions: yield, 19 mg.

2-Amino-7-cyclohexyl-1,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (9). A solution of cyclohexenyl derivative **8** (0.2 g, 0.86 mmol) in ethanol (50 mL) was hydrogenated with 10% Pd/C (50 mg) at 45 lb/in.^{2} for 16 h and filtered hot through Celite. The filtrate was evaporated to dryness, and the residue was crystallized from hot ethanol to give **9**: yield, 157 mg (78%).

Compound Evaluations. The X-ray crystallographic analyses, computer modeling studies, and *in vitro* enzyme inhibition studies were carried out as previously described.[2] A summary of the X-ray crystallographic data for the complexes is given in Table IV. The complexes were prepared by allowing the PNP crystals to equilibrate for 24 h in a stabilizing buffer solution containing the compound. All X-ray intensity measurements were recorded with a Nicolet/Siemens X-100 multiwire area detector on a Rigaku RU-300 rotating anode X-ray generator.

Acknowledgment. We wish to thank Dr. W. Cook for providing crystals of PNP, Dr. L. L. Bennett, Jr., and Ms. P. Allan for the IC_{50} determinations, and the Molecular Spectroscopy Section of Southern Research Institute for the spectral determinations and elemental analyses.

References

(1) Ealick, S. E.; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Montgomery, J. A.; Secrist, J. A., III. Application of Crystallographic and Modeling Methods in the Design of Purine Nucleoside Phosphorylase Inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11540–11544.

(2) Montgomery, J. A.; Niwas, S.; Rose, J. D.; Secrist, J. A., III; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Ealick, S. E. Structure-Based Design of Inhibitors of Purine Nucleoside Phosphorylase. 1. 9-Arylmethyl Derivatives of 9-Deazaguanine. *J. Med. Chem.* **1993**, *58*, 55–69.

(3) We have used the name 9-deazaguanine to emphasize the relationship of these compounds to guanine. The systematic names of the compounds are given in the Experimental Section.

(4) New Cyclic Thioimidates. Netherlands Appl. 6,610,204, 1967; *Chem. Abstr.* **1968**, *68*, 39631K.

(5) Profitt, J. A.; Watt, D. S.; Corey, E. J. A Reagent for the α,β Reduction of Conjugated Nitriles. *J. Org. Chem.* **1975**, *40*, 127–128.

(6) Zikan, V.; Semonsky, M. Ergot Alkaloids XIV. Partial Synthesis of Some ω-Cyclopentylakylamides of D-Isolysergic and D-Lysergic Acids. *Collect. Czech. Chem. Commun.* **1959**, *24*, 1274–1280.

(7) Ohno, M.; Ishizaki, K.; Eguchi, S. Synthesis of Adamantane Derivatives by Bridgehead Redical Addition to Electron-Deficient Unsaturated Bonds. *J. Org. Chem.* **1988**, *53*, 1285–1288.

(8) McKay, A. F.; Garmaise, D. L.; Paris, G. Y.; Gelblum, S.; Ranz, R. J. The Preparation of Substituted Thionocarbamates. *Can. J. Chem.* **1960**, *38*, 2042–2052.

(9) Kursanov, D. N.; Parnes, Z. N.; Bolestova, G. I.; Belen'Kii, L. I. Ionic Hydrogenation of Thiophenes. *Tetrahedron* **1975**, *31*, 311–315.

(10) Broadbent, H. S.; Slaugh, L. H.; Jarvis, N. L. Rhenium Sulfides as Liquid-Phase Hydrogenation Catalysts. A Comparison With Molybdenum Sulfide and Cobalt Polysulfide. *J. Am. Chem. Soc.* **1954**, *76*, 1519–1523.

(11) Yadav, V. K.; Fallis, A. G. Regioselective Cleavage of 2-Methyltetrahydrofuran: A Versatile Synthesis of 1-Halo-4-pentanols and 4-Halo-1-pentanols. *J. Org. Chem.* **1986**, *51*, 3372–3374.

(12) Guindon, Y.; Therien, M.; Girard, Y.; Yoakim, C. Regiocontrolled Opening of Cyclic Ethers Using Dimethylboron Bromide. *J. Org. Chem.* **1987**, *52*, 1680–1686.

(13) Cook, M. J. Six-membered Rings With More Than One Oxygen or Sulfur Atom. In *Comprehensive Heterocyclic Chemistry*; Boulton, A. J., McKillop, A., Eds.; Pergamon Press: Oxford, England, 1984; Vol. 3, p 991.

(14) Burley, S. K.; Petsko, G. A. Aromatic-Aromatic Interaction: A Mechanism of Protein Structure Stabilization. *Science* **1985**, *229*, 23–28.

(15) Nakamura, C. E.; Chu, S.-H.; Stoeckler, J. D.; Parks, R. E., Jr. Inhibition of Purine Nucleoside Phosphorylase by Phosphonoalkylpurines. *Nucleosides, Nucleotides* **1989**, *8*, 1039–1040.

(16) Stoeckler, J. D.; Cambor, C.; Kuhns, V.; Chu, S.-H.; Parks, R. E., Jr. Inhibitors of Purine Nucleoside Phosphorylase. *Biochem. Pharmacol.* **1982**, *31*, 163–171.

(17) Jennings, R. R.; Niemann, C. The Kinetics of the α-Chymotrypsin Catalyzed Hydrolysis of Acetyl-L-hexahydrophenylalaninamide in Aqueous Solutions at 25° and pH 7.9. *J. Am. Chem. Soc.* **1953**, *75*, 4687–4692.

(18) Shewach, D. S.; Chern, J.-W.; Pillote, K. E.; Townsend, L. B.; Daddona, P. E. Potentiation of 2'-Deoxyguanosine Cytotoxicity by a Novel Inhibitor of Purine Nucleoside Phosphorylase, 8-Amino-9-benzylguanine. *Cancer Res.* **1986**, *46*, 519–523.

(19) Chang, G.; Guida, W. C.; Still, W. C. An Internal Coordinate Monte Carlo Method for Searching Conformational Space. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.

(20) Guida, W. C.; Bohacek, R. S.; Erion, M. D. Probing the Conformational Space Available to Inhibitors in the Thermolysin Active Site Using Monte Carlo/Energy Minimization Techniques. *J. Comput. Chem.* **1992**, *13*, 214–228.

(21) Bohacek, R. S.; McMartin, C. M. Definition and Display of Steric, Hydrophobic, and Hydrogen-Bonding Properties of Ligand Binding Sites in Proteins Using Lee and Richards Accessible Surface: Validation of a High-Resolution Graphical Tool for Drug Design. *J. Med. Chem.* **1992**, *35*, 1671–1684.

(22) The experimental error in the observed torsion angles is estimated to be approximately 10°.