Structure-Based Design of Inhibitors of Purine Nucleoside Phosphorylase. 4. A Study of Phosphate Mimics

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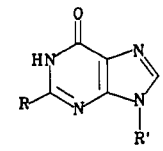
9-(3,3-Dimethyl-5-phosphonopentyl)guanine was synthesized and found to be a potent inhibitor of purine nucleoside phosphorylase (PNP) (IC_{50} = 44 nM). A number of other functional end groups were investigated as phosphate mimics attached to the 9-position of guanine by this same alkyl side chain, which provided a sensitive method for the detection of any interaction of these groups with the phosphate binding site of PNP. Both the sulfonic acid (compound **13**) and the carboxylic acid (compound **15**) end groups interact significantly with the phosphate binding site, but in different ways, as determined by X-ray crystallographic analysis of the complexes. The sulfonic acid of **13**, which binds about one-fourth as tightly as the phosphonate **12**, binds in the phosphate subsite much like the phosphonic acid. The carboxylic acid, the interaction of which is much weaker, turns away from the center of the phosphate binding site to form hydrogen bonds with Ser 200 and Met 219. Thus, the only phosphate mimics that bind like phosphate itself are themselves highly ionic, probably with limited ability to penetrate cell membranes.

Introduction

Acyclovir diphosphate (ACVdiP, **1**) is a potent inhibitor of purine nucleoside phosphorylase (PNP),[1] and its binding to the active site of the enzyme has been studied by X-ray crystallography.[2] This study showed that, as predicted,[1] ACVdiP is a bisubstrate inhibitor binding in the purine binding site and in the phosphate binding site with some interaction of the ACV side chain with the hydrophobic pocket.[2] Unfortunately, since ACVdiP is neither chemically nor enzymatically stable in plasma and cannot penetrate cells intact, it is not a drug candidate. However, the potency of its binding to PNP has led several investigators to prepare potentially useful phosphonate analogs of ACVdiP.[3-6] Most, if not all, of these compounds penetrate cells poorly, if at all, and their utility would seem to depend on devising ways, such as the preparation of prodrug forms, to increase cell permeability. Another approach would involve the attachment of a phosphate mimic to guanine by spacers of appropriate length. Previous attempts by Baker and co-workers to simulate the phosphate moiety of nucleotides were only partially successful, but no information was available on the active sites of the enzymes they tried to inhibit.[7]

Parks and his co-workers recently published an interesting paper[4] on the inhibition of PNP by 9-(phosphonoalkyl)hypoxanthines. These investigators found that the K_i of 9-(3,3-dimethyl-5-phosphonopentyl)hypoxanthine (**2**) is one-fifth that of 9-(5-phosphonopentyl)-hypoxanthine (**3**), whereas with the corresponding 9-alkylhypoxanthines **5** and **6**, the reverse is true. That is, the 3,3-dimethylpentyl derivative (**5**) is only half as potent as the pentyl compound (**6**). These data indicated that there



1: R = NH_2, R' = $CH_2O(CH_2)_2OP(O)(OH)OP(O)(OH)_2$

2: R = H, R' = $(CH_2)_2C(Me)_2(CH_2)_2P(O)(OH)_2$

3: R = H, R' = $(CH_2)_5P(O)(OH)_2$

4: R = NH_2, R' = $(CH_2)_5P(O)(OH)_2$

5: R = H, R' = $(CH_2)_2C(Me)_2CH_2CH_3$

6: R = H, R' = $(CH_2)_4CH_3$

is little difference in the 9-alkylhypoxanthine **5** and **6**, which bind significantly less tightly than hypoxanthine itself,[8] but there is a very large difference (520-fold) between the untethered 3,3-dimethylpentyl compound (**5**) and the tethered phosphonate (**2**), much larger than between the pentyl compounds **3** and **6** (47-fold). A logical explanation of these data is that the binding of the phosphonate group of **2** in the phosphate binding site orients the 3,3-dimethyl for interaction with the hydrophobic pocket of the active site without disturbing the purine binding (see below). Thus, the 3,3-dimethylpentyl side chain provides a fairly sensitive method for the measurement of interaction of a potential phosphate mimic with the phosphate binding site of PNP. The ratio of K_i or IC_{50} values at 50 and 1 mM phosphate is also a measure of interaction with the phosphate binding site, but a high ratio may only mean a bad steric interaction.[2] In order to determine whether a functional group other than phosphonate could interact favorably with the phosphate

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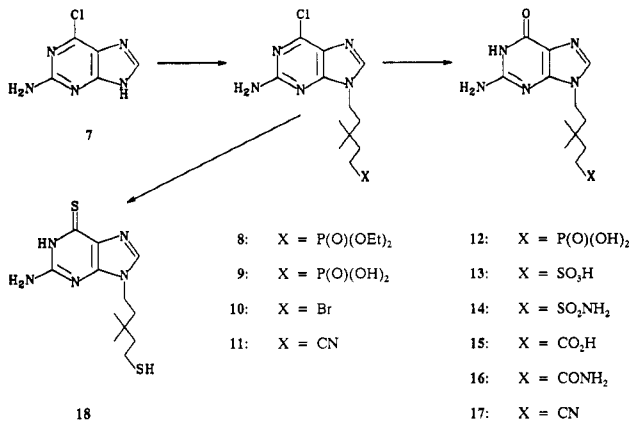
binding site, we prepared a number of 9-substituted guanine analogs containing the 3,3-dimethylpentyl chain with various end groups.

Chemistry

The synthesis of **12** was modeled after a procedure described by Nakamura *et al.*[3] for the synthesis of **3**. A Michaelis–Arbuzov reaction of 1,5-dibromo-3,3-dimethylpentane[9] with triethyl phosphite gave a 34% yield of diethyl (5-bromo-3,3-dimethylpentyl)phosphonate. Alkylation of 2-amino-6-chloropurine (**7**) with this phosphonate in the presence of potassium carbonate gave a 60% yield of the 9-substituted purine **8**. Cleavage of the phosphonate ester **8** with bromotrimethylsilane gave the phosphonic acid **9** which was hydrolyzed in normal sodium hydroxide to give the target (phosphonoalkyl)guanine **12**. Substitution of the guanine at the 9-position was confirmed by its UV spectrum which was similar to those of known 9-substituted guanines.



The analogs **13**–**17** and the thioguanine analog **18** were prepared from 2-amino-9-(5-bromo-3,3-dimethylpentyl)-6-chloropurine (**10**) which was obtained in 62% yield by alkylation of **7** with 1,5-dibromo-3,3-dimethylpentane. The sulfonic acid analog **13** was obtained in low yield by alkylation of sodium sulfite with **10** in a Strecker synthesis followed by hydrolysis of the 6-chloro group in aqueous sodium hydroxide. In a second run, the crude unpurified sulfonic acid (**13**) was treated with dichlorotriphenylphosphorane in *N,N*-dimethylacetamide to give the corresponding sulfonyl chloride[10] which was then converted *in situ*, in 23% overall yield, to the sulfonamide **14** by treatment with ammonium hydroxide.

Reaction of **10** with potassium cyanide in 2-propanol at 85 °C gave a 76% yield of the nitrile intermediate **11**, which was hydrolyzed in normal sodium hydroxide at 85 °C to give a 74% yield of the target carboxylic acid **15**. Partial hydrolysis of **11** by treatment with concentrated sulfuric acid followed by aqueous base (pH 13) gave the target carboxamide **16** in 87% yield. Hydrolysis of the 6-chloro group of **11** without conversion of the cyano group to the carboxamide was difficult but was finally accomplished by slow hydrolysis in 0.01 N sodium hydroxide to give **17**. Substitution of the guanine at the 9-position in target analogs **13**–**17** was confirmed by comparison of their UV spectra with **12** and other 9-substituted guanines.

2-Amino-9-(3,3-dimethyl-5-mercaptopentyl)purine-6(1*H*)-thione (**18**) was prepared in 43% yield by reaction of **10** with thiourea and hydrolysis of the resulting bis-isothiouronium salt in base.

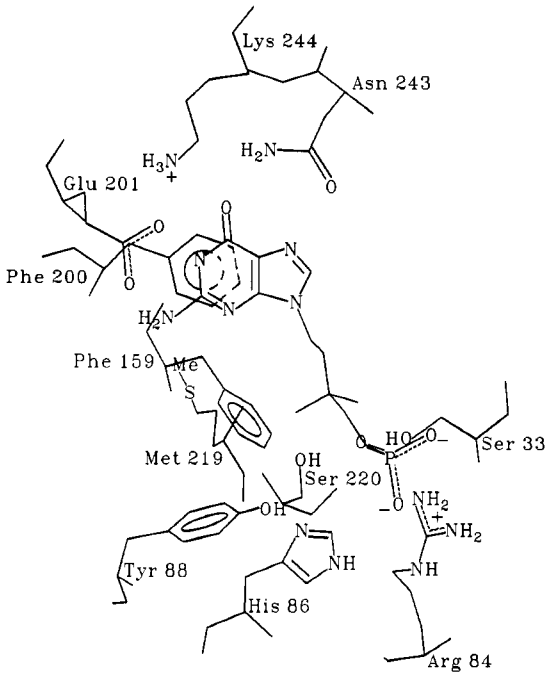


Figure 1. The binding of 9-(3,3-dimethyl-5-phosphonopentyl)-guanine (**12**) in the active site of PNP as determined by X-ray crystallographic analysis.

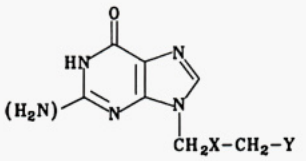
Discussion of Results

9-(3,3-Dimethyl-5-phosphonopentyl)guanine (**12**), with an IC_{50} of 44 nM, is a more potent inhibitor of PNP than is the corresponding hypoxanthine (**2**)[11] in keeping with the results with other guanine–hypoxanthine pairs. An X-ray analysis[2,12–14] of its complex with PNP shows that, as expected, the phosphonate moiety binds in the phosphate binding site, displacing the sulfate that occupies this site in the crystal of the apoenzyme (see Figure 1). As a result of this tether, the 3,3-dimethyl groups are oriented for favorable interaction with the hydrophobic pocket of the active site, explaining why the (dimethylpentyl)-phosphonates bind 4–5 times as tightly as the pentylphosphonates (see Table 1). In fact, **12** binds about as tightly as 9-[2-(2-phosphonoethyl)benzyl]guanine.[14]

In our previous studies,[2,12–14] we used computer-assisted molecular modeling techniques to evaluate the potential binding geometry of proposed inhibitors in advance of their synthesis. This approach was useful in determining whether a particular potential inhibitor was worthy of synthesis. Thus, prior to its synthesis, we conducted MC/EM conformation searches, as previously described,[2,12] on **12** docked in the PNP binding site. As before, coordinates for the PNP/guanine complex derived from X-ray crystallography were employed. In all of the low-energy conformers, the phosphonate substituent was found in the phosphate binding site in a position similar to that observed crystallographically for sulfate. In addition, in these conformers, the *gem*-dimethyl group occupied the hydrophobic binding site. Figure 2 shows the global minimum-energy conformer compared with the structure derived via X-ray studies.

The data obtained with the various phosphate mimics (see Table 1) show that the sulfonic acid (**13**) interacts quite well with the phosphate binding site, the carboxyl group (**15**), moderately well, and the sulfonamide, the carboxamide, and the nitrile (**14**, **16**, and **17**) probably not at all (compare with **5**, Table 1). These results led us to

Table 1. Inhibition of PNP



compd no.	base	X	Y	K_i (μM)[a]	IC_{50} (μM)[b] 1 mM PO_4	IC_{50} (μM)[b] 50 mM PO_4	ratio
2	H	$CH_2CMe_2CH_2$	PO_2H_2	0.21			
3	H	$(CH_2)_3$	PO_3H_2	1.1			
4	G	$(CH_2)_3$	PO_3H_2	0.17			
5	H	$CH_2CMe_2CH_2$	H	110			
12[c]	G	$CH_2CMe_2CH_2$	PO_3H_2		0.044	2.0	44
13[c]	G	$CH_2CMe_2CH_2$	SO_3H		0.18	9.0	50
14	G	$CH_2CMe_2CH_2$	SO_2NH_2		100	>300	–
15[c]	G	$CH_2CMe_2CH_2$	CO_2H		8.0	290	36
16	G	$CH_2CMe_2CH_2$	$CONH_2$		200	>300	–
17	G	$CH_2CMe_2CH_2$	CN		260	>300	–
18	G(O=S)	$CH_2CMe_2CH_2$	SH		50	95	2

[a] Human erythrocytic PNP in 1 mM PO_4, refs 3 and 4. [b] Calf spleen PNP. [c] Structure of the complex with PNP determined by X-ray crystallography.

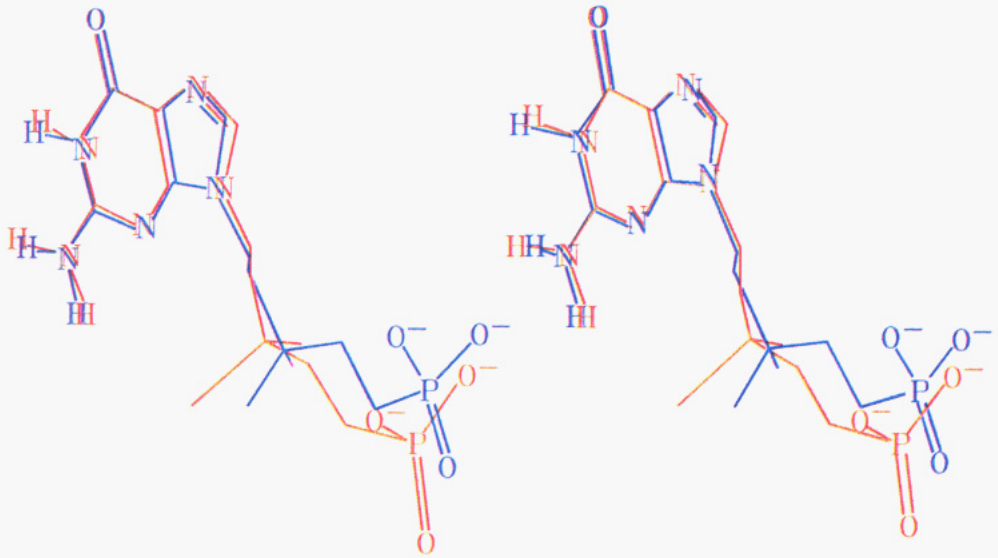


Figure 2. Stereocomparison of the calculated global minimum energy conformer (blue) of **12** bound in the active site of PNP with the actual binding (red) as determined by X-ray crystallographic analysis.

Table 2. Summary of X-ray Crystallographic Data for PNP/Inhibitor Complexes[a]

compd no.	no. of crystals	no. of observations	no. of reflections	R_{merge}[b]	R_{frac}[c]
12	1	17453	8251	0.076	0.15
13	1	19592	9701	0.101	0.17
15	1	19768	9213	0.106	0.21

[a] 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. The resolution of the data sets is 3 Å. [b] R_{merge} is the R factor on intensities for merging symmetry-related reflections. [c] R_{frac} is the percent change between native and scaled PNP/inhibitor structure factor data.

examine, by X-ray crystallography, the PNP/inhibitor complexes for **13** and **15** (see Table 2). The conformation of these inhibitors in the complexes are compared in Figure 3. The binding of **13** is almost identical with that of **12**, with the sulfonic acid group binding in the phosphate binding site, in keeping with IC_{50} values. The carboxy group of **15**, on the other hand, turns away from the center of the phosphate binding site to form hydrogen bonds with the OH of Ser 220 (2.6 and 2.9 Å) and the backbone nitrogen of Met 219 (2.8 Å). These interactions pull the guanine moiety out of the position it occupies in the purine binding site in the complexes of PNP with **12**, **13**, and guanine itself, weakening that interaction. However, the hydrogen bonds with Ser 220 and Met 219 more than compensate in the overall binding of **15**, as can be seen by comparing the IC_{50} value of **15** with the K_i of **5** (the guanine analog of **5** is not available for a direct comparison). It is likely that the poor binding of **5** relative to hypoxanthine itself (K_i = 17 μM)[8] similarly results from interaction of the 3,3-dimethylpentyl side chain with the hydrophobic pocket pulling the hypoxanthine moiety out of the purine binding site. We have observed this effect in other cases also.[12,13] The mercapto group of the 6-thioguanine (**18**) may interact with some of the residues of the phosphate binding site, but the interaction is weak at best. However, 6-thioguanine itself is 5-fold less potent than guanine itself.[15] Thus, the only phosphate mimics that interact significantly with the phosphate binding site in this study are themselves ionic (**12** and **13**), probably with limited ability to penetrate cell membranes.

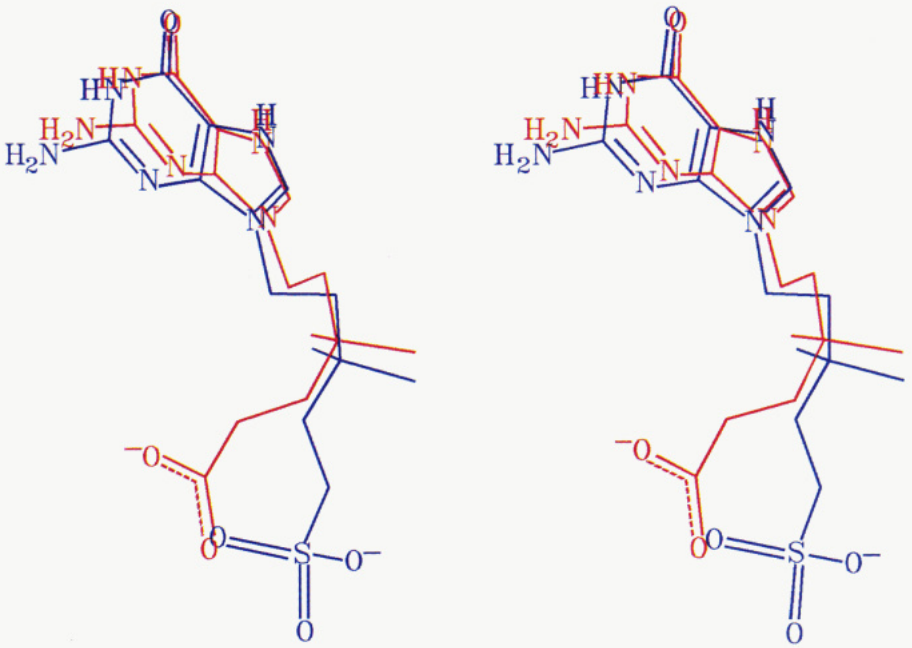


Figure 3. Stereocomparison of the binding of the sulfonic acid **13** (blue) with that of the carboxylic acid **15** (red) in the active site of PNP as determined by X-ray crystallographic analysis.

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. Analtech precoated (250 μM) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated aqueous $(NH_4)_2SO_4$. All analytical samples were homogeneous by TLC. Melting points were determined with a Mel-Temp apparatus unless otherwise specified. Purifications by "gravity column" and by flash chromatography[16] 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 NMR spectra of all compounds were determined with a Nicolet/GE NT 300NB spectrometer operating at 300.35 MHz for ^1H NMR 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 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.

Diethyl (5-Bromo-3,3-dimethylpentyl)phosphonate. Magnetically stirred 1,5-dibromo-3,3-dimethylpentane[9] (6.00 g, 23.3 mmol) in a two-neck flask equipped with dropping funnel and a short-path distillation head was heated in an oil bath at 150 °C and treated dropwise with triethyl phosphite (2.66 mL, 15.5 mmol) over a period of 22 min. The mixture was heated for an additional hour and placed under high vacuum to remove excess dibromide. The residual oil was purified on a flash column of 250 g of silica gel using 1:1 cyclohexane–ethyl acetate as eluting solvent. The product fraction was evaporated to give 1.66 g (34% yield) of pure title compound as an oil: MS (FAB) *m/z* 315 (M + H)$^+$. Anal. ($C_{11}H_{24}BrO_3P$) C, H.

2-Amino-6-chloro-9-[5-(diethoxyphosphinyl)-3,3-dimethylpentyl]purine (8). A solution of diethyl (5-bromo-3,3-dimethylpentyl)phosphonate (186 mg, 0.590 mmol) and 2-amino-6-chloropurine (**7**, 100 mg, 0.590 mmol) in anhydrous Me_2NAc (2 mL) containing anhydrous K_2CO_3 (163 mg, 1.18 mmol) was stirred in a stoppered flask for 18 h at 25 °C, 19 h at 5.0 °C, and 3 h at 80 °C. The reaction mixture was filtered and evaporated to dryness under high vacuum. The residue in 95:5 $CHCl_3$–MeOH was applied to a flash column of 25 g of silica gel and eluted with the same solvent. The product fraction which contained an impurity was purified on a second flash column using 98:2 $CHCl_3$–MeOH as solvent. The product fraction was evaporated to give 145 mg (60%) of **8** as a colorless, viscous syrup: MS (FAB) *m/z* 404 (M + H)$^+$; ^1H NMR (Me_2SO-d_6) δ 8.20 (s, 1, H_8), 6.87 (s, 2, NH_2), 3.98 (m, 6, CH_2O, CH_2N), 1.70 (m, 4, CH_2CMe_2, CH_2P), 1.42 (m, 2, CH_2P), 1.42 (m, 2, CH_2CMe_2), 1.22 (t, 6, CH_3CH_2), 0.91 (s, 6, Me_2C). Anal. ($C_{16}H_{27}ClN_5O_3P$·0.33H_2O) C, H, N.

2-Amino-9-(5-bromo-3,3-dimethylpentyl)-6-chloropurine (10). A stirred mixture of **7** (500 mg, 2.95 mmol), 1,5-dibromo-3,3-dimethylpentane (996 mL, 5.90 mmol), and powdered anhydrous K_2CO_3 (814 mg, 5.90 mmol) in anhydrous Me_2NAc (10 mL) in a stoppered flask was heated in an oil bath at 50 °C for 17 h. The reaction mixture was filtered and the precipitate rinsed with Me_2NAc. Evaporation of the filtrate and wash under high vacuum gave a solid which was purified on a flash column of 25 g of silica gel using 99:1 $CHCl_3$–MeOH as solvent. The product fraction was evaporated to give pure **10** as a white crystalline solid: yield 630 mg (62%); mp 165 °C; MS (FAB) *m/z* 346 (M + H)$^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 220 (27.8), 241 (5.47), 315 (7.12); pH 7, 224 (27.6), 245 (4.30), 309 (7.48); pH 13, 223 (27.3), 245 (4.56), 308 (7.39); ^1H NMR (Me_2SO-d_6) δ 8.20 (s, 1, H_8), 6.87 (s, 2, NH_2), 4.06 (m, 2, NCH_2), 3.55 (m, 2, CH_2Br), 1.86 (t, 2, CH_2CH_2Br), 1.74 (m, 2, NCH_2CH_2), 0.96 (s, 6, CH_3). Anal. ($C_{12}H_{17}BrClN_5$) C, H, N.

2-Amino-6-chloro-9-(5-cyano-3,3-dimethylpentyl)purine (11). A solution of **10** (347 mg, 1.00 mmol) in 2-propanol (6 mL) was mixed with a solution of KCN (143 mg, 2.20 mmol) in H_2O (0.5 mL) and stirred while heating in a sealed tube at 85 °C (oil bath) for 5 h. The filtered reaction mixture was evaporated to dryness and the residue purified on a flash column of 25 g of silica gel using 98:2 $CHCl_2$–MeOH for elution. The product fraction (R_f 0.4 in 95:5 $CHCl_3$–MeOH) was evaporated to give 223 mg (76%) of pure **11**: mp 145 °C; MS (FAB) *m/z* 293 (M + H)$^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 219 (28.1), 241 (5.4), 315 (7.17); pH 7, 223 (28.7), 245 (4.63), 308 (7.77); pH 13, 223 (28.5), 245 (4.74), 308 (7.69); ^1H NMR (Me_2SO-d_6) δ 8.19 (s, 1, H_8), 6.89 (s, 2, NH_2), 4.04 (m, 2, NCH_2), 2.49 (m, 2, CH_2CH_2CN), 1.69 (m, 2, NCH_2CH_2), 1.61 (t, 2, CH_2CN), 0.93 (s, 6, CH_3). Anal. ($C_{13}H_{17}ClN_6$) C, H, N.

9-(3,3-Dimethyl-5-phosphonopentyl)guanine (12). A solution of **8** (76 mg, 0.184 mmol) in anhydrous CH_2Cl_2 (0.4 mL) under N_2 was treated with bromotrimethylsilane (121 μL, 0.92 mmol) and stirred in a stoppered flask for 1 h. The solution was

evaporated to dryness *in vacuo* and the residue in MeCN (2 mL) treated with H_2O (0.2 mL) and stirred for 1 h. The white precipitate of 2-amino-6-chloro-9-(3,3-dimethyl-5-phosphonopentyl)purine (**9**) was collected, washed with MeCN, and dried *in vacuo*: yield 80 mg; mp ca. 220 °C dec (soft from 140 °C); MS (FAB) *m/z* 348 $(M + H)^+$. A solution of **9** (78 mg) in 1 N NaOH (4 mL) was heated in an oil bath at 85 °C for 2 h, cooled to 25 °C, acidified to pH 2–3 with 6 N HCl, and cooled in an ice bath. The precipitate of **12** was collected by filtration, washed with cold H_2O, and dried *in vacuo* (P_2O_5): yield 47 mg (74%); MS (FAB) *m/z* 330 $(M + H)^+$; neg FAB *m/z* 328 $(M - H)^-$; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 253 (11.4), 279 (7.75); pH 7, 253 (12.2), 272 sh (9.19); pH 13, 256 sh (9.65), 269 (10.6); ^1H NMR (Me_2SO-d_6) δ 10.52 (s, 1, NH), 7.72 (s, 1, H_8), 6.44 (s, 2, NH_2), 3.91 (m, 2, NCH_2), 1.61 (m, 2, NCH_2CH_2), 1.5 (m, 4, CH_2CH_2P), 0.87 (s, 6, CH_3). Anal. ($C_{12}H_{20}N_5O_4P \cdot 1.4H_2O$) C, H, N.

9-(3,3-Dimethyl-5-sulfopentyl)guanine (13). A solution of **10** (200 mg, 0.576 mmol) and Na_2SO_3 (87.2 mg, 0.692 mmol) in EtOH (12 mL) and H_2O (12 mL) was heated at reflux temperature in an oil bath for 19 h, cooled to 25 °C, filtered, and evaporated to dryness *in vacuo*. The residue was washed with $CHCl_3$ (3 mL) and heated in an oil bath at 85 °C for 2 h in a solution of 1 N NaOH (6 mL). The solution was neutralized with 6 N HCl, filtered and evaporated to dryness. A suspension of the residue in 5 mL of 2:1 $CHCl_3$–MeOH containing 5% HOAc was mixed into the upper 3 cm of a flash column of 25 g of silica gel and eluted with the same solvent. The product fraction (R_f 0.2, eluting solvent) was concentrated to 3 mL and the solution refrigerated. The crude product was collected and further purified on a second silica gel column as above. The product from this column was triturated in hot MeOH, cooled to 25 °C, collected, and dried *in vacuo*, yield 44 mg (19%). Elemental analysis indicated the presence of a small amount of sodium acetate: MS (FAB) *m/z* 330 $(M + H)^+$, neg FAB 328 $(M - H)^-$; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 253 (11.9), 280 (8.14); pH 7, 253 (12.7), 271 sh (9.50); pH 13, 256 sh (10.0), 269 (11.0); ^1H NMR (Me_2SO-d_6) δ 10.69 (s, 1, NH), 8.03 (s, 1, H_8), 6.62 (s, 2, NH_2), 3.96 (m, 2, NCH_2), 2.47 (m, CH_2SO_2), 1.91 (s, ^-OAc), 1.61 (m, 4, CH_2CMe_2), 0.88 (s, 6, CH_3). Anal. ($C_{12}H_{19}N_5O_4S \cdot 1.7H_2O \cdot 0.4NaOAc$) C, H, N.

9-(3,3-Dimethyl-5-sulfamylpentyl)guanine (14). A solution of **10** (100 mg, 0.288 mmol) and Na_2SO_3 (47.2 mg, 0.374 mmol) in EtOH (6 mL) and H_2O (6 mL) was refluxed under N_2 for 22 h, cooled to 25 °C, filtered, and evaporated to dryness *in vacuo*. The residue was washed with $CHCl_3$ (2 × 2 mL), dried, and heated with 1 N NaOH (1 mL) in an oil bath at 85 °C for 2 h. The solution was adjusted to pH 7 with 1 N HCl and lyophilized to a powder which was further dried at 78 °C under high vacuum for 18 h. The residue of crude **13** in anhydrous Me_2NAc (4 mL) was treated with 85% dichlorotriphenylphosphorane (338 mg, 0.864 mmol) and stirred for 2 h. The solution containing the sulfonyl chloride was treated in one portion with concentrated NH_4OH (20 mL), stirred rapidly in a stoppered flask for 20 h, and evaporated to dryness *in vacuo*. A solution of the residue in MeOH was deposited on 3 g of silica gel by evaporation under high vacuum. This solid was deposited on top of a flash column of 25 g of silica gel prepared in 6:1 $CHCl_3$–MeOH containing 5% HOAc. The column was eluted with the same solvent and the product fraction (R_f 0.75 in 2:1 $CHCl_3$–MeOH containing 5% HOAc) evaporated to dryness and evaporated from MeOH to give a white solid which was boiled with MeOH (2 mL) and cooled, and the precipitate of **14** was collected, boiled again with MeOH (1 mL), cooled, and collected: yield 22 mg (23%); MS (FAB) *m/z* 329 $(M + H)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 253 (11.4), 279 (7.73); pH 7, 253 (12.1), 270 sh (9.09); pH 13, 256 sh (9.43), 269 (10.3); ^1H NMR (Me_2SO-d_6) δ 7.69 (s, 1, H_8), 6.77, 6.71 (s, s, 4, NH_2), 3.93 (m, 2, NCH_2), 3.01 (m, z, CH_2SO_2), 1.68 (m, CH_2, CMe_2), 0.93 (s, 6, CH_3). Anal. ($C_{12}H_{20}N_6O_3S \cdot 0.6H_2O$) C, H, N.

9-(5-Carboxy-3,3-dimethylpentyl)guanine (15). A suspension of **11** (50 mg, 0.171 mmol) in 1 N NaOH (3 mL, 3.00 mmol) was stirred in an oil bath at 85 °C for 3 h and 45 min. The reaction solution was adjusted to pH 9.6 by slow addition of 50W-X4 (H^+) ion-exchange resin, filtered, and lyophilized to a white powder. A solution (some insoluble) of this solid in 5:4:0.4 $CHCl_3$–MeOH–concentrated NH_4OH was applied to a flash column of 25 g of silica gel and the column developed with the same solvent. The product fraction (R_f 0.4 in 5:4:0.4 $CHCl_3$–MeOH–NH_4OH) was evaporated to dryness and the residue triturated with H_2O, collected, and dried *in vacuo*: yield 37 mg (74%); mp ca. 315 °C; MS (FAB) *m/z* 294 $(M + H)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 253 (11.4), 279 (7.72); pH 7, 253 (11.9), 271 sh (8.94); pH 13; 256 sh (8.83), 269 (9.79). ^1H NMR (Me_2SO-d_6) δ 12.05 (s, 1, NH or CO_2H), 10.53 (s, 1, NH or CO_2H), 7.73 (s, 1, H_8), 6.40 (s, 2, NH_2), 3.92 (m, 2, NCH_2), 2.20 (t, 2, CH_2CO_2), 1.63 (m, 2, NCH_2CH_2), 1.50 (m, 2, $CH_2CH_2CO_2$), 0.89 (s, 6, CH_3). Anal. ($C_{13}H_{19}N_5O_3$) C, H, N.

9-(5-Carbamoyl-3,3-dimethylpentyl)guanine (16). A solution of **11** (60 mg, 0.205 mmol) in concentrated H_2SO_4 (1 mL) remained at 25 °C for 18 h and was added dropwise with stirring to crushed ice (5 cm^3). The solution was adjusted to pH 13 with 50% NaOH, allowed to stand at 25 °C for 1 h, and adjusted to pH 4.5 with 6 N HCl. The precipitate of **15** was collected by filtration, washed with H_2O, and dried *in vacuo* (P_2O_5): yield 53 mg (87%); mp 285–287 °C; MS (FAB) *m/z* 293 $(M + H)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1 253 (11.8), 279 (7.85); pH 7 253 (12.5), 271 sh (9.17); pH 13, 257 sh (9.74), 269 (10.6); ^1H NMR (Me_2SO-d_6) δ 10.52 (s, 1, NH), 7.72 (s, 1, H_8), 7.27, 6.73 (s, s, 2, $CONH_2$), 6.41 (s, 2, 2-NH_2), 3.93 (m, 2, NCH_2), 2.05 (m, 2, CH_2CO), 1.62 (m, 2, NCH_2CH_2), 1.49 (m, 2, CH_2CH_2CO), 0.90 (s, 6, CH_3). Anal. ($C_{13}H_{20}N_6O_2 \cdot 0.3H_2O$) C, H, N.

9-(5-Cyano-3,3-dimethylpentyl)guanine (17). A solution of 69 mg (0.24 mmol) of **1** in 144 mL of 0.01 N NaOH was heated at 80 °C for 48 h. An HPLC aliquot indicated 70% **2**, 17% **1**, 9% amide, 2% acid, and 1% of an unknown component. The solution was introduced directly on a bio-bead column (Bio Rad, 5 M-4, 20–50 mesh, 1 cm × 14 cm). Water elution removed the NaOH as well as the carboxylic acid. Water–methanol (3:1) elution gave 26 mg of product **2** as well as some **1** and amide. A solution of this material in methanol was purified by flash chromatography (50 g of silica gel) using $CHCl_3$–MeOH (17:3) as the eluting solution. The product **1** was obtained as an impure solid (18 mg). A solution of this material in H_2O was treated with 2 or 3 drops of concentrated acetic acid and recolumned on the bio-bead column, eluting first with water and then with methanol to give 14 mg of product that was still impure. A solution of this material in methanol was put on a column of silica gel (50 g). The column was eluted first with CH_2Cl_2, then CH_2Cl_2–iPrOH (3:1), and then 100% iPrOH, gradually changing to MeOH when the product was obtained. Evaporation gave **1** as a white solid: yield 12 mg (16%); mp 234–235 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) 278.8 (7.20) and 253 (11.01) at pH 1; 252.5 (11.69) at pH 7; 269 (9.97) at pH 13; TLC, $CHCl_3$–MeOH (17:3), R_f 0.53; MS *m/z* 275 $(M + 1)^+$, 297 $(M + Na)^+$, 571 $(2M + Na)^+$; ^1H NMR (DMSO-d_6) δ 10.86 (br s, N^1H), 7.68 (s, H-8), 6.51 (br s, NH_2), 3.92 (m, NCH_2), 3.32 (s, H_2O), 2.5 (m, $NCCH_2$), 1.63 (m, $CH_2C(Me)_2CH_2$), 1.05 (d, $(CH_3)_2CH$), 0.92 (s, CH_3).

2-Amino-9-(3,3-dimethyl-5-mercaptopentyl)purine-6(1*H*)-thione (18). A solution of **10** (100 mg, 0.288 mmol) and thiourea (43.9 mg, 0.576 mmol) in 95% EtOH (5 mL) was heated at reflux temperature for 5 h and evaporated to dryness. The residue in 2 N NaOH (2 mL) was stirred in a hot water bath to give a solution which was filtered and adjusted to pH 7 with 6 N HCl. The resulting precipitate was collected, washed with a minimum of water, redissolved in hot 2 N NaOH (3 mL), filtered, and adjusted to pH 7 with 1 N HCl. The mixture was cooled in an ice bath and the product collected, washed with H_2O, and dried *in vacuo* (P_2O_5): yield 37 mg (43%); MS (FAB) *m/z* 298 $(M + H)^+$; ^1H NMR (Me_2SO-d_6) δ 7.92 (s, 1, H_8), 6.67 (s, 2, NH_2), 3.95 (m, NCH_2), 2.45 (m, CH_2S), 1.67 (m, NCH_2CH_2), 1.55 (m, CH_2CH_2S), 0.91 (s, CH_3). Anal. ($C_{12}H_{19}N_5S_2$) C, H, N.

Compound Evaluations. The X-ray crystallographic analysis (Table 2), computer modeling studies, and the *in vitro* enzyme inhibition studies were carried out as previously described.[12]

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