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) (IC50 = 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 phosphate 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 (ACVdp, 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
ACVdp 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
cavity.2 Unfortunately, since ACVdp is neither chemi-

cally 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 ACVdp.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
paper4 on the inhibition of PNP by 9-(phosphono-
alkyl)hypoxantines. These investigators found that the
Ki of 9-(3,3-dimethyl-5-phosphonopentyl)hypoxan-
tine (2) is one-fifth that of 9-(5-phosphonopentyl)-
hypoxanthine (3), whereas with the corresponding 9-alky-
lhypoxanthines 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

\[
\begin{align*}
1: R &= \text{NH}_2, R' = (\text{CH}_3)(\text{CH}_2)\text{O}(\text{OP(O)(OH)})\text{OP(O)(OH)} \\
2: R &= \text{H}, R' = (\text{CH}_3)(\text{CH}_2)\text{O}(\text{OP(O)(OH)}) \\
3: R &= \text{H}, R' = (\text{CH}_3)_2\text{P(O)(OH)} \\
4: R &= \text{NH}_2, R' = (\text{CH}_3)_2\text{P(O)(OH)} \\
5: R &= \text{H}, R' = (\text{CH}_3)(\text{CH}_2)_2\text{CH}_2\text{CH}_3 \\
6: R &= \text{H}, R' = (\text{CH}_3)_2\text{CH}_2\text{CH}_3
\end{align*}
\]

is little difference in the 9-alkylhypoxanthines 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 hydro-
phobic 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
Ki or IC50 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. for the synthesis of 3. A Michaelis–Arbuzov reaction of 1,5-dibromo-3,3-dimethylpentane with triethyl phosphate 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. Cleavage of the phosphonate was hydrolyzed in normal sodium hydroxide to give the target (phosphonooalkyl)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.

![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.](image)

**Discussion of Results**

9-(3,3-Dimethyl-5-phosphonopentyl)guanine (12), with an IC₅₀ of 44 nM, is a more potent inhibitor of PNP than is the corresponding hypoxanthine (2) in keeping with the results with other guanine-hypoxanthine pairs. An X-ray analysis of its complex with PNP shows that, as expected, the phosphate 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 tightly as 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.

In our previous studies, 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, 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
### Inhibitors of Purine Nucleoside Phosphorylase

**Table 1. Inhibition of PNP**

<table>
<thead>
<tr>
<th>compd no.</th>
<th>base</th>
<th>X</th>
<th>Y</th>
<th>$K_i (\mu M)^a$</th>
<th>IC$_{50}$ ($\mu M$)$^b$</th>
<th>1 mM PO$_4$</th>
<th>50 mM PO$_4$</th>
<th>ratio</th>
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<tr>
<td>2</td>
<td>H</td>
<td>CH$_2$CM$_2$CH$_2$</td>
<td>PO$_4$H$_2$</td>
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<td>0.44</td>
<td>2.0</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>(CH$_2$)$_2$</td>
<td>PO$_4$H$_2$</td>
<td>1.1</td>
<td>9.0</td>
<td>9.0</td>
<td>50</td>
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<tr>
<td>4</td>
<td>G</td>
<td>(CH$_2$)$_2$</td>
<td>PO$_4$H$_2$</td>
<td>0.17</td>
<td>8.0</td>
<td>290</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>H</td>
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<td>H</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>G</td>
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<td>36</td>
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<td>G</td>
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<td>100</td>
<td>200</td>
<td>&gt;300</td>
<td>&gt;300</td>
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<tr>
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<td>CO$_2$H</td>
<td>8.0</td>
<td>8.0</td>
<td>290</td>
<td>36</td>
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<td>16</td>
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<td>CH$_2$CM$_2$CH$_2$</td>
<td>CONH$_2$</td>
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<td>&gt;300</td>
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<td>CN</td>
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<td>260</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td></td>
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<tr>
<td>18</td>
<td>G(O=S)</td>
<td>CH$_2$CM$_2$CH$_2$</td>
<td>SH</td>
<td>50</td>
<td>50</td>
<td>95</td>
<td>95</td>
<td>2</td>
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$^a$ 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**

<table>
<thead>
<tr>
<th>compd no.</th>
<th>no. of crystals</th>
<th>no. of observations</th>
<th>no. of reflections</th>
<th>$R_{merge}^b$</th>
<th>$R_{free}^c$</th>
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<tr>
<td>12</td>
<td>1</td>
<td>17453</td>
<td>8051</td>
<td>0.076</td>
<td>0.15</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>19592</td>
<td>9701</td>
<td>0.101</td>
<td>0.17</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>19768</td>
<td>9913</td>
<td>0.106</td>
<td>0.21</td>
</tr>
</tbody>
</table>

$^a$ 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. $^c$ $R_{free}$ 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 ($K_i = 17 \mu M$) 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.
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 P₂O₅ at room temperature for 16 h. Analytical precocured (250 μM) silica gel GF(F) plates were used for TLC analyses; the spots were detected by irradiation with a Minelight and by charring after spraying with saturated aqueous (NH₄)₂SO₄. All analytical samples were homogeneous by TLC. Melting points were determined with a Mel-Temp apparatus unless otherwise specified. Purifications by “gravity fractionation” 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 with a Cary 17 spectrophotometer and a Perkin-Elmer ultraviolet-visible near-infrared spectrophotometer Model Lambda 9: the maxima and minima were within ±0.4% of the theoretical values.

Diethyl (5-bromo-3,3-dimethylpentyl)phosphonate. Magnetically stirred 1,5-dibromo-3,3-dimethylpentane (8.00 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 a further 5 h. The filtered reaction mixture was evaporated to give 1.66 g (34% yield) of pure title compound as an oil: MS (FAB) m/z 315 (M + H)+; lH NMR (Me₂SO-d₆) δ 8.20 (s, 1, H₈), 6.87 (s, 2, NH₂), 4.06 (m, 2, NCH₂), 3.55 (m, 2, CH₂Br), 2.49 (m, 2, CHZCH₂), 1.61 (t, 2, CH₂CH₂Br), 1.74 (m, 2, CH₂CH₂), 0.91 (s, 6, Me₂). Anal. (C₁₁H₁₄BrO₃P) C, H, N.

2-Amino-6-chloro-9-(5-hydroxyphosphinoyl)-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 Me₂NAC (2 mL) containing anhydrous K₂CO₃ (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 1 g CHCl₃–MeOH was applied to a flash column of 25 g 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 CHCl₃–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 (MeSO-d₆) δ 8.20 (s, 1, H₈), 6.87 (s, 2, NH₂), 3.98 (m, 6, CH₂O, CHN), 1.70 (m, 4, CH₂CMe₂, CH₂P), 1.42 (m, 2, CH₂P), 1.42 (m, 2, CH₂CMe₂), 1.22 (t, 6, CH₂CH₂), 0.91 (s, 6, Me₂). Anal. (C₁₃H₁₆ClN₅O₇P) 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 K₂CO₃ (814 mg, 5.90 mmol) in anhydrous Me₂NAC (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 Me₂NAC. 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 CHCl₃–MeOH as eluent. The product fraction was evaporated to give 145 mg (60%) of 8 as a white crystalline solid: yield 630 mg (62%); mp 165 °C; MS (FAB) m/z 346 (M + H)+; UV (10-3) pH 1, 220 (27.8), 241 (5.35), 315 (7.12); pH 7, 224 (27.6), 245 (4.30), 308 (7.48); pH 13, 223 (27.3), 245 (4.50), 308 (7.39); 1H NMR (MeSO-d₆) δ 8.20 (s, 1, H₈), 6.87 (s, 2, NH₂), 4.06 (m, 2, CH₂), 3.55 (m, 2, CH₂Br), 1.86 (t, 2, CH₂CH₂Br), 1.74 (m, 2, CH₂CH₂), 0.96 (s, 6, CH₃). Anal. (C₁₃H₁₇BrCIN₅P) 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 H₂O (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 silica gel using 98:2 CHCl₃–MeOH for elution. The product fraction (Rₚ 0.4 in 95:5 CHCl₃–MeOH) was evaporated to give 223 mg (76%) of pure 11: mp 145 °C; MS (FAB) m/z 238 (M + H)+; UV (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 (MeSO-d₆) δ 8.19 (s, 1, H₈), 6.89 (s, 2, NH₂), 4.04 (m, 2, CH₂), 2.49 (m, 2, CH₂CH₂CN), 1.89 (m, 2, CH₂CH₂), 1.61 (t, 2, CH₂CN), 0.93 (s, 6, CH₃). Anal. (C₁₃H₁₇ClN₅P) C, H, N.

9-(3,3-Dimethyl-5-phosphonopentyl)guanine (12). A solution of 8 (76 mg, 0.184 mmol) in anhydrous CH₃Cl (0.4 mL) under N₂ was treated with bromotrimethylsilane (121 μL, 0.92 mmol) and stirred in a stoppered flask for 1 h. The solution was
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evaporated to dryness in vacuo and the residue in MeCN (2 mL) treated with H$_2$O (0.2 mL) and stirred for 1 h. The white precipitate of 2-amino-6-chloro-9-(3,3-dimethyl-5-phosphono-pentyl)purine (9) was collected, washed with MeCN, and dried in vacuo: yield 30 mg; mp 212–213°C (dec from 210°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, and concentrated to 4 mL. The precipitate of 12 was collected by filtration, washed with cold H$_2$O, and dried in vacuo (P$_2$O$_5$): yield 47 mg (74%); MS (FAB) m/z 330 (M + H$^+$); neg FAB m/z 328 (M + H$^+$); UV $\lambda_{max}$ (e $\times 10^4$) pH 1, 253 (11.4), 279 (7.73); pH 7, 253 (11.9), 279 (8.94); pH 13, 256 sh (8.89), 289 (7.99). $\lambda$ NMR (Me$_2$SO-d$_6$) $\delta$ 12.05 (s, 1, OH or CO$_2$H), 10.55 (s, 1, NH or CO$_2$H), 7.73 (s, 1, H), 6.40 (s, 2, NH$_2$), 3.92 (m, 2, NCH$_2$), 2.20 (t, 2, CH$_2$CO$_2$), 1.63 (m, 2, NCH$_2$CH$_2$), 1.50 (m, 2, CH$_2$CH$_2$CO$_2$), 0.89 (s, 6, CH$_3$). Anal. (C$_{12}$H$_{17}$NO$_4$) C, H, N.

9- (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 50% W-X$_4$ (H$^+$) ion-exchange resin, filtered, and lyophilized to a white powder. A solution (some insoluble) of this solid in 6:4:0.4 CHCl$_3$-MeOH-concentrated NH$_4$OH 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$_4$OH) was evaporated to dryness and the residue triturated with H$_2$O, collected, and dried in vacuo: yield 57 mg (74%); mp ca. 215°C; MS (FAB) m/z 294 (M + H$^+$); UV $\lambda_{max}$ (e $\times 10^4$) pH 1, 253 (11.4), 279 (7.73); pH 7, 253 (11.9), 279 (8.94); pH 13; 256 sh (8.89), 289 (7.99). $\lambda$ NMR (Me$_2$SO-d$_6$) $\delta$ 12.05 (s, 1, NH or CO$_2$H), 10.55 (s, 1, NH or CO$_2$H), 7.73 (s, 1, H), 6.40 (s, 2, NH$_2$), 3.92 (m, 2, NCH$_2$), 2.20 (t, 2, CH$_2$CO$_2$), 1.63 (m, 2, NCH$_2$CH$_2$), 1.50 (m, 2, CH$_2$CH$_2$CO$_2$), 0.89 (s, 6, CH$_3$). Anal. (C$_{12}$H$_{17}$NO$_4$) C, H, N.

9- (Carboxy-3,3-dimethylpentyl)guanine (16). A solution of 11 (80 mg, 0.205 mmol) in concentrated H$_2$SO$_4$ (1 mL) remained at 25°C for 18 h and was added dropwise to stirring with 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$_2$O, and dried in vacuo (P$_2$O$_5$): yield 53 mg (87%); mp 285–287°C; MS (FAB) m/z 293 (M + H$^+$); UV $\lambda_{max}$ (e $\times 10^4$) pH 1 253 (11.8), 279 (7.85); pH 7 253 (12.5), 279 sh (9.17); pH 13; 257 sh (9.74), 269 (10.6). $\lambda$ NMR (Me$_2$SO-d$_6$) $\delta$ 10.52 (s, 1, NH), 7.72 (s, 1, H), 7.27, 6.73 (m, 2, CONH$_2$), 6.41 (s, 2, 2-NH$_2$), 3.93 (m, 2, NCH$_2$), 2.05 (m, 2, CH$_2$CO$_2$), 1.62 (m, 2, NCH$_2$CH$_2$), 1.49 (m, 2, CH$_2$CH$_2$CO$_2$), 0.90 (s, 6, CH$_3$). Anal. (C$_{12}$H$_{17}$NO$_4$) C, H, N.

9- (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 45 h. An HPLC eluption indicated 70% of 1, 17% 2, 16% 9, 10% amide, 2% acid, and 1% of an unknown component. The solution was introduced directly on a bio-bead column (Bio Rad, 5 M 4.20–60 mesh, 1 cm x 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$_2$O was treated with 2.5 drops of concentrated acetic acid and further purified 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$_2$Cl$_2$, then CH$_2$Cl$_2$–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 $\lambda_{max}$ (e $\times 10^4$) 278.3 (7.20) and 263 (11.01) at pH 1; 252.5 (11.09) at pH 7; 259 (9.97) at pH 13; $\lambda$ NMR (CDCl$_3$–Me$_2$SO-d$_6$) $\delta$ 2.53; MS m/z 275 (M + H$^+$), 297 (M + Na$^+$), 671 (2M + Na$^+$); $\lambda$ NMR (DMSO-d$_6$) $\delta$ 10.86 (br, s, NH$_2$), 7.68 (s, H–H), 6.51 (br, s, NH$_2$), 3.92 (m, NCH$_2$), 3.32 (m, H$_2$O), 2.5 (m, NCCCH$_2$), 1.63 (m, CH$_2$CH$_2$CH$_2$CO$_2$), 1.05 (m, CH$_2$CO$_2$), 0.92 (s, CH$_3$). 2-Amino-9-(3,3-dimethyl-5-mercaptopentyl)purine-6-(1H)-thione (18). A solution of 10 (100 mg, 0.288 mmol) and thiorurea (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 H$_2$O, and dried in vacuo (P$_2$O$_5$): yield 37 mg (43%); MS (FAB) m/z 298 (M + H$^+$); $\lambda$ NMR (Me$_2$SO-d$_6$) $\delta$ 7.92 (s, 1, H$_2$), 6.67 (s, 2, NH$_2$), 3.95 (m, NCH$_2$), 2.45 (m, CH$_2$), 1.67 (m, NCH$_2$CH$_2$), 1.65 (m, CH$_2$CH$_2$), 0.91 (s, CH$_3$). Anal. (C$_{12}$H$_{17}$NO$_4$S) C, H, N.

Compound Evaluations. The X-ray crystallographic analysis (Table 3), computer modeling studies, and the in vitro enzyme inhibition studies were carried out as previously described.12

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References


(10) This previously unreported procedure will be described in greater detail at a later date.

(11) The available data do not permit a direct comparison.


