Nucleosides. 5.[1] Synthesis of Guanine and Formycin B Derivatives as Potential Inhibitors of Purine Nucleoside Phosphorylase

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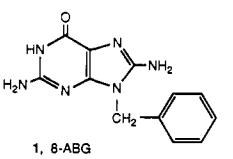
Received August 19, 1992

In an effort to develop potent human purine nucleoside phosphorylase (PNP) inhibitors as immunosuppressive and chemotherapeutic agents, several 8-aminoguanine derivatives were synthesized and evaluated as potential PNP inhibitors. These studies were designed to investigate the hydrophobic effect of a substituent on the N-9 of the purine heterocycle and/or the C-5′ positions. Compounds such as 8-aminoguanosine, guanosine, formycin B, and 8-aminoacyclovir containing a *p*-(fluorosulfonyl)benzoyl moiety were synthesized. The affinity of these compounds to erythrocytic PNP was determined and none of these compounds showed a better affinity than those of the parent compounds. However, we found that the effect of hydrophobicity at the N-9 and the C-5′ positions might play an important role in binding to the active site of PNP. Thus, 8-amino-5′-deoxy-5′-(phenylthio)guanosine (**19**) was found to be the best inhibitor in this series of compounds with a K_i = 0.45 μM.

Introduction

Purine nucleoside phosphorylase (PNP; orthophosphate ribosyltransferase; EC 2.4.2.1)[2] is considered an essential enzyme in the purine salvage pathway, catalyzing the phosphorolysis of guanosine, inosine and their 2′-deoxyribonucleoside derivatives to the respective purine bases. The discovery of T-cell-related immunodeficiency diseases associated with a lack of PNP[3,4] has prompted considerable interest in developing inhibitors of this enzyme as a selective approach to cancer chemotherapy.[5] It is well-documented that in a PNP-related immunodeficiency state, 2′-deoxyguanosine accumulates and this nucleoside is phosphorylated preferentially by immature T cells to the toxic 5′-triphosphate derivative, which results in inhibition of DNA synthesis.[6-9] It has recently been reported[10] that human PNP deficiency was caused by a single base change in the DNA genome.[10] T-cell lymphopenia is associated with a PNP-deficient patient; therefore, it was suggested that a PNP inhibitor might be used to treat T-cell leukemias, to counter autoimmune diseases without destroying the patient's humoral immunity and to treat tissue rejection after organ transplantation.[11] Furthermore, PNP inhibitors might provide some potential use for antimalarial treatment because the intraerythrocytic malaria parasite requires hypoxanthine and guanine formed by PNP as a source of nucleic acid synthesis and energy metabolism.[12]

The importance of PNP inhibitory activity for lymphocyte development and function in chemotherapy has generated considerable interest in a systematic examination of the structure–activity relationships of substrates for human erythrocytic PNP.[13,14] To date, several inhibitors of PNP have been identified and the majority of these compounds resemble purine bases or nucleosides.[5,15,16] It has been established that halogen at the C-5′ position of specific PNP substrates results in improved affinities but greatly decreased substrate activity, for example, 5′-deoxy-5′-iodoformycin (K_i = 7 μM) illustrated that the binding to PNP by iodination at C-5′ of the parent compound[17] increased the activity by 10-fold. X-ray crystallography revealed that the 5′-iodo atom is juxtaposed over the phenylalanine residue of the active site of PNP, indicative of the enhancement of binding to PNP probably due to the charge–charge interaction of the halogen atom with the phenylalanine residue in the active site of PNP.[18] In a previous communication, we assumed that 8-aminoguanine plays an important role in recognizing and binding to the active site of PNP, and through an attempt to investigate the bulky tolerance at the N-9 position of 8-aminoguanine, we found that 8-amino-9-benzylguanine (8-ABG) is a potent inhibitor of both human



1, 8-ABG

erythrocyte and parasite PNP.[12,19] The intact cell experiments showed that 8-ABG acted as a competitive (with inosine) inhibitor with a K_i value 0.22 μM and could potentiate the toxicity of 2′-deoxyguanosine to MOLT-4 T-lymphoblasts in culture. We reasoned that the increased affinity of 8-ABG to PNP, more than 10-fold by a benzyl group on the N-9 position of 8-aminoguanine, is probably due to this hydrophobic interaction between the benzyl group and the phenylalanine residue (Phe[200]) of the active site of PNP. Such a hydrophobic region adjacent to the active site of an enzyme has been observed on dihydrofolic reductase,[20] guanase,[21] and thymidine phosphorylase,[22] and the exploitation of the hydrophobic regions in the active site for the design of reversible and irreversible inhibitors have been previously studied.

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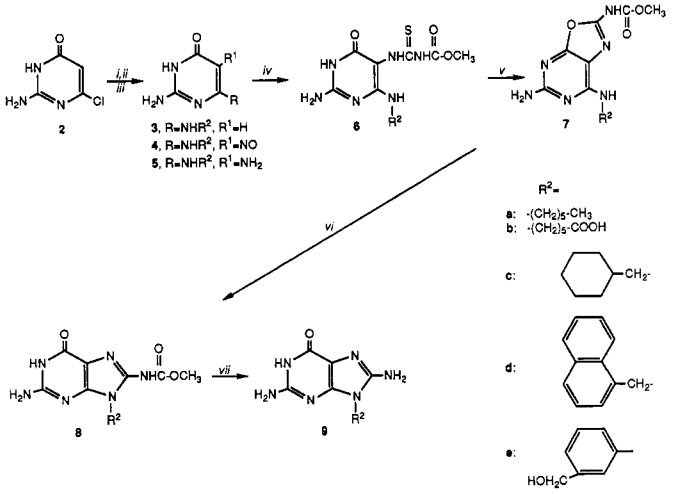
0022-2623/93/1836-1024$04.00/0

Scheme I [a]



[a] (i) Amines, ethanol; (ii) $NaNO_2$; (iii) dithionite; (iv) $SCNC(=O)OCH_3$, CH_3CN; (v) DCC, DMF, room temperature; (vi) K_2CO_3, MeOH, reflux; (vii) 10% aqueous NaOH.

Accordingly, we selected 8-aminoguanine and the corresponding nucleoside as our "lead compounds" for studies to investigate the hydrophobic effect of substituents on the N-9 of the purine heterocycle and C-5′ positions of the carbohydrate moiety.

Results and Discussion

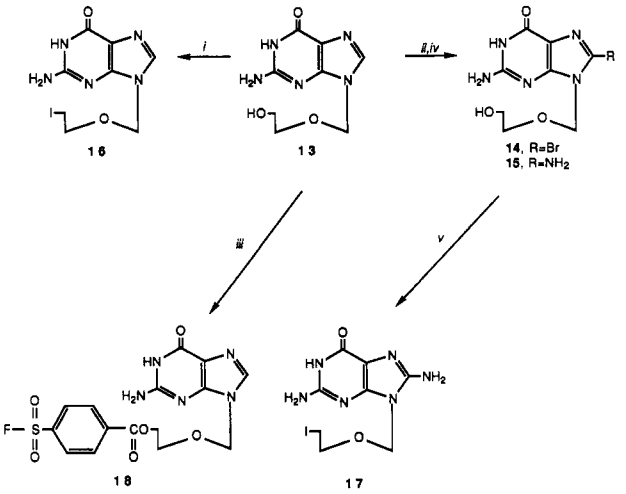
Chemistry. 8-Amino-9-substituted-guanines (**9a–e**) were synthesized (Scheme I) by a method previously published[23] from our laboratory. This involves the initial synthesis of the key intermediates, 2-amino-4-(substituted-amino)pyrimidin-6-ones (**3a–e**) which were subjected to nitrosation with sodium nitrite and subsequent reduction with sodium dithionite to give the 2,5-diamino-4-(substituted-amino)pyrimidin-6-ones (**5a–e**). Compounds **5a–e** were readily oxidized in the air; therefore, they were condensed with methoxycarbonyl isothiocyanate at once without further purification. Then cyclodesulfurization and ring annulation of the resulting thiourea derivatives **6–e** with dicyclohexylcarbodiimide, followed by a ring transformation of the oxazolo[5,4-*d*]pyrimidines **7a–e** led to the formation of the imidazo[4,5-*d*]pyrimidines **8a–e**. Subsequent hydrolysis of **8a–e** with a 10% sodium hydroxide solution afforded the target compounds.

In the 8-aminoacyclovir derivatives series (Scheme II), 8-aminoacyclovir (**15**) was synthesized by a previous method.[24] Bromination of acyclovir (**13**) was effected using saturated bromine water to give 8-bromoacyclovir (**14**) in 84% yield. Treatment of the bromo compound with 70% aqueous hydrazine at reflux afforded compound **15**. The iodination of acyclovir and 8-aminoacyclovir, with triphenylphosphine and iodine in *N*,*N*-dimethylformamide using Moffat's procedure,[25] furnished 9-[(2-iodoethoxy)methyl]guanine (**16**) and 8-amino-9-[(2-iodoethoxy)methyl]guanine (**17**), respectively.

8-Amino-5′-deoxy-5′-(phenylthio)guanosine (**19**) was prepared (Scheme III) in 59% yield by a simple treatment of 8-aminoguanosine (**12**)[26] with diphenyl disulfide in the presence of tributylphosphine.[27]

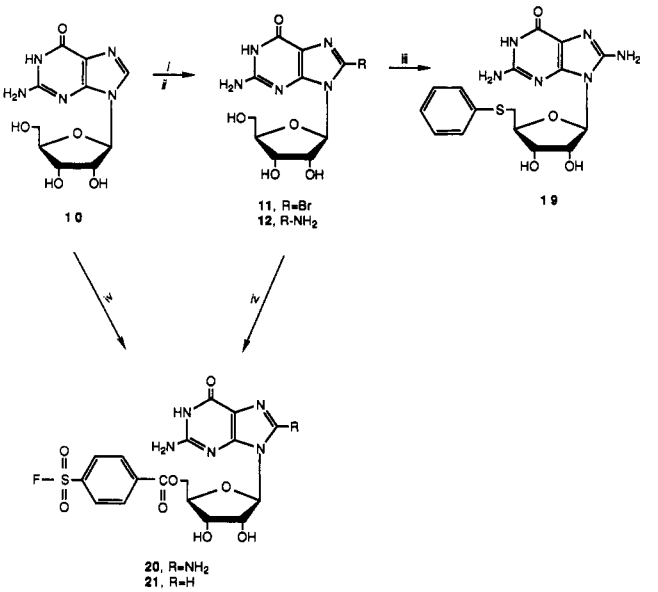
5′-[*p*-(Fluorosulfonyl)benzoyl]guanosine (**21**)[28] was previously prepared for a ligand study by a direct acylation of the 5′-OH group of guanosine hydrochloride with *p*-(fluorosulfonyl)benzoyl chloride in HMPA. For our studies, **21** was synthesized by a modification of this

Scheme II [a]



[a] (i) $(C_6H_5)_3P,I_2$, DMF, room temperature, 39%; (ii) Br_2, H_2O; (iii) *p*-(fluorosulfonyl)benzoyl chloride, HMPA, −10 °C, 82%; (iv) 50% hydrazine, 48 h; (v) $(C_6H_5)_3P$, DMF, I_2, room temperature, 23%.

Scheme III [a]

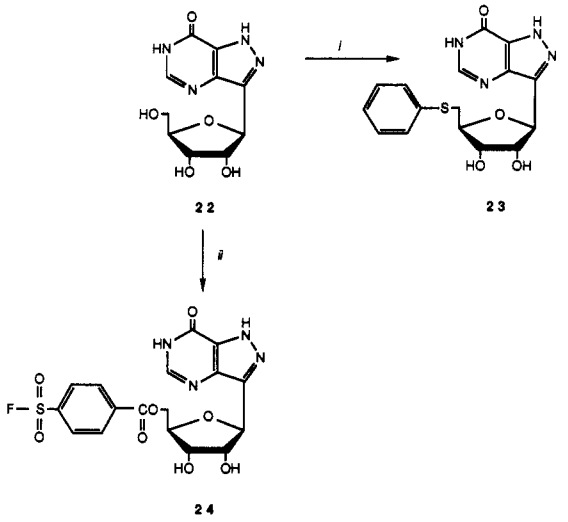


[a] (i) Bromine water, room temperature; (ii) 50% hydrazine, reflux; (iii) diphenyl disulfide, $(C_6H_5)_3P$, pyridine, room temperature, 60 h, 59%; (iv) *p*-(fluorosulfonyl)benzoyl chloride, HMPA, room temperature 37%.

procedure by a direct acylation of the 5′-OH of guanosine with *p*-(fluorosulfonyl)benzoyl chloride, instead of the hydrochloride salt. The structure of this compound was determined on the basis of its ^{1}H-NMR spectrum.

However, the treatment of 8-aminoguanosine with *p*-(fluorosulfonyl)benzoyl chloride in HMPA resulted in a nonselective acylation. The treatment of 8-aminoguanosine dihydrochloride with *p*-(fluorosulfonyl)benzoyl chloride in HMPA afforded the dihydrochloride salt of the desired compound. The neutralization of 8-amino-5′-[*p*-(fluorosulfonyl)benzoyl]guanosine dihydrochloride with triethylamine in ethanol was unsuccessful. The fluorosulfonyl moiety was found to be very unstable in aqueous basic solution. However, treatment of this salt with triethylamine in anhydrous DMF furnished 8-amino-5′-[(*p*-fluorosulfonyl)benzoyl]guanosine (**20**) as established by ^{1}H-NMR spectroscopy and elemental analysis. The structurally related compounds, 9-[[2-[*p*-(fluorosulfonyl)-

Scheme IV [a]



[a] (i) $(C_6H_5)_3P$, diphenyl disulfide, pyridine, room temperature, 89%; (ii) *p*-(fluorosulfonyl)benzoyl chloride, HMPA, room temperature, 12 h, 71%.

Table I. Kinetic Constants for Human PNP

compounds	K_i (μM)
8-aminoguanine	0.8
8-aminoguanosine (**12**)	7.0
8-amino-9-benzylguanine (**1**)	0.2
8-amino-9-hexylguanine (**9a**)	12.4
8-amino-9-(carboxypentyl)guanine (**9b**)	10.0
8-amino-9-(cyclohexylmethyl)guanine (**9c**)	*a*
8-amino-9-(naphthylmethyl)guanine (**9d**)	*a*
8-amino-9-[*m*-(hydroxymethyl)phenyl]guanine (**9e**)	*b*
acyclovir (**13**)	180.0
8-bromoacyclovir (**14**)	18.7
8-aminoacyclovir (**15**)	13.4
9-[(2-iodoethoxy)methyl]guanine (**16**)	15.0
8-amino-9-[(2-iodoethoxy)methyl]guanine (**17**)	23.0
8-amino-5′-deoxy-5′-(phenylthio)guanosine (**19**)	0.45
5′-[*p*-(fluorosulfonyl)benzoyl]guanosine (**21**)	93.0
8-amino-5′-[*p*-(fluorosulfonyl)benzoyl]guanosine (**20**)	37.0
9-[[2-[*p*-(fluorosulfonyl)benzoyl]ethoxy]methyl]-guanine (**18**)	*c*
5′-[*p*-(fluorosulfonyl)benzoyl]formycin B (**24**)	>300
5′-deoxy-5′-(phenylthio)formycin B (**23**)	26.0

[a] Insoluble. [b] No inhibition at 1 mM. [c] 95% inhibition at 1 mM.

benzoyl]ethoxy]methyl]guanine (**18**) and 5′-[*p*-(fluorosulfonyl)benzoyl]formycin B (**24**) were synthesized in 82 and 71% yields, respectively. 5′-Deoxy-5′-(phenylthio)-formycin B (**23**) was obtained in 89% from formycin B (**22**) by the same approach as that used for the synthesis of **19**.

Biological Aspects

PNP activity was assayed in dialyzed extracts from MOLT-4 T-lymphoblasts or human erythrocytes using a previously described radiochemical assay.[11] Kinetic constants were estimated from double reciprocal plots of initial velocity versus substrate (inosine) concentration. All inhibitors were competitive with inosine. The results are summarized in Table I.

Several aspects of the stereoelectronic requirements of substrates for human erythrocytic PNP have been studied[15,16] and it has been demonstrated that groups on the C-8 position larger than NH_2, such as $NHCH_3$ and $N(CH_3)_2$ can not be accommodated by PNP. Also, purine analogues with OH, SCH_3, and propyl groups substituted at C-8 show little or no PNP inhibitory activity.[29] In a previous communication, we assumed that an electron-donating group at the C-8 position, without producing excessive steric hindrance, would increase the electron density at N-7 and thus increase the affinity of the compound toward the enzyme. On the basis of this assumption, we found 8-amino-9-benzylguanine was a potent PNP inhibitor and concluded that the length of the hydrophobic substituent cannot exceed a benzyl group.[19] In order to investigate the hydrophobic character on both sides of the benzyl group, 9-cyclohexyl-, and 9-naphthyl-8-aminoguanine were synthesized and evaluated. Unfortunately, due to the poor solubility of these compounds, the data on their PNP inhibitory activity were not obtained. The PNP inhibitory activity of compounds **13**–**17** and 9-hexyl-8-aminoguanine are not better than those obtained for **9c**–**e**. However, we found that by adding an amino group at the 8 position of acyclovir, to obtain compound **15** led to an increase of more than a 10-fold affinity to PNP.

A recent report on a metabolite of the antiherpetic drug acyclovir, acycolvir diphosphate, revealed this compound was the most potent inhibitor of PNP to date with a K_i = 0.0087 μM (determined at a low inorganic phosphate concentration.[30]) The K_i value increased to 0.51 μM when the phosphate concentration was increased to 50 μM, suggesting that there is a positive charge adjacent to the active site which is binding to an anionic charge on the terminal phosphate ester moiety of acyclovir diphosphate. Accordingly, metabolically stable "multisubstrate" acyclic nucleotide analogues containing a purine and phosphate-like moiety such as 9-(phosphonoalkyl)hypoxanthines and guanines have been synthesized and evaluated as potential purine nucleoside phosphorylase inhibitors.[31-33] In this series, 9-(5-phosphonopentyl)guanine was considered as the most potent with a K_i = 170 nM. 8-Amino-9-(carboxypentyl)guanine (**9b**), containing a carboxylate ion at the terminal end, which is considered as an isostere of phosphono or phosphate, is a multisubstrate analogue. However, compound **9b** demonstrated a weak PNP inhibitory activity with a K_i = 10.0 μM.

Modifications at C-5′ of the ribose moiety in a purine nucleoside is considered to be a promising approach to develop PNP inhibitors. It is generally agreed that a halogen atom at the C-5′ position provides a better affinity for the nucleoside to PNP.[12,16,17] Similarly, the PNP inhibitory activity of acyclovir was increased 10-fold by replacing the 2-hydroxy group with an iodo atom to give 9-[(2-iodoethoxy)methyl]guanine (**16**). However, a similar replacement of the 2-hydroxy group of 8-aminoacyclovir with an iodo atom did not enhance the affinity to PNP. We found that compound **19**, having a phenylthio group at the C-5′ position, with a K_i = 0.45 μM was 40-fold better than that of 8-aminoguanosine and possessed potency equal to 8-amino-5′-deoxy-5′-chloroguanosine.[12] However, compound **23**, with the same substituent at the C-5′ position of formycin B, showed poor inhibitory activity with a K_i = 26 μM which is weaker than that of **19**. Although compound **23** is not as potent as 5′-deoxy-5′-iodoformycin (K_i = 7.0 μM), it is still better than the parent compound formycin B (K_i = 100 μM). The results obtained from these studies coincide with reports that halogens or a thiomethyl group at the C-5′ position of specific PNP substrates resulted in unchanged or improved affinities but greatly decreased substrate activity.[17] The increased affinity of 8-amino-5′-deoxy-5′-(phenylthio)guanosine to PNP was probably due to an interaction of the 5′-

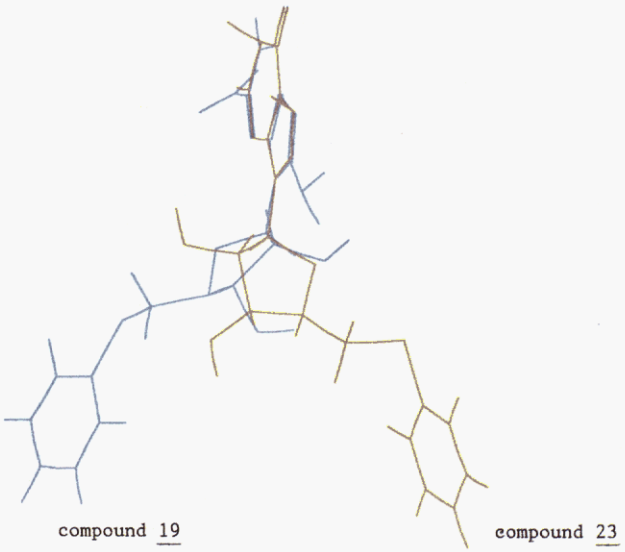


Figure 1. Superimposition of compound **19** and **23** in energy-minimized conformation state.

phenylthio group with the phenylalanine residue and is indicative of a hydrophobic region adjacent to the active site of PNP.

The active site of purine nucleoside phosphorylase has been recently characterized using X-ray diffraction data.[18a] This study revealed that guanine, hypoxanthine, and certain analogues donate a hydrogen bond at N-1 and accept a hydrogen bond at O-6. Asn-243 and Lys-244 are near O-6 while Glu-201 is near N-1. Futhermore, the amino group at C-2 of guanine is in position to form an additional hydrogen bond with Glu-201. According to this description of the purine binding site of PNP, we assumed that the 8-aminoguanine moiety and the pyrazolopyrimidine moiety of formycin B could enter the active site of PNP with the ribose moiety being outside of the binding site. On the basis of this assumption, Figure 1 shows energy-minimized conformations of **19** and **23** (superimposed), generated using the ALCHEMY II molecular modeling program.[18b] These 8-aminoguanine derivatives and formycin B analogues provide the same arrangement of hydrogen bond donors and receptors in the active site of PNP while the ribose and the 5′-phenylthio group of **19** and **23** are distorted in different directions. However, the 5′-phenylthio moiety of **19** is anti to the 8-amino group while the 5′-phenylthio group of **23** is syn to the 8-position. The latter conformation is very similar to that reported for 5′-iodoformycin B binding to the active site of PNP (in the X-ray diffraction data). The introduction of a phenylthio group at the 5′ position leads to a 40-fold improvement in the 8-aminoguanosine series, but only a 4-fold improvement in the formycin B series, and it is probably due to the phenylthio groups selecting a different hydrophobic site. It should be noted that one side of the "ribose pocket" has been characterized as hydrophobic and includes at least 4 aromatic residues (Phe-200, Tyr-88, and His-257 from one subunit, along with Phe-159 from the neighboring subunit). Accordingly, we propose that two hydrophobic binding sites might exist in the pocket as illustrated in Figure 2. The hydrophobic area A is in or near the active site and plays a major role while the other area is in the neighboring subunit and most likely plays a minor role.

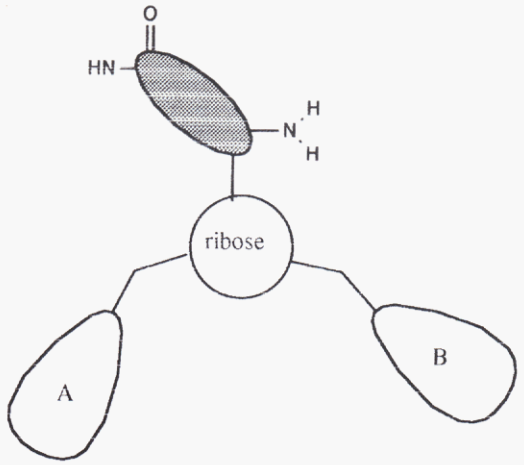


Figure 2. Proposed three binding sites for purine nucleoside phosphorylase inhibitors. Shaded part is purine binding site; A and B represent two hydrophobic areas.

At the outset, we presumed that the 8-aminoguanine nucleus might be responsible for the recognition and binding of 8-aminoguanosine to the active site of PNP. This prompted us to make the assumption that perhaps there is another pocket for the C-5′ position of the ribose which would allow us to use a chemically reactive functional group such as *p*-(fluorosulfonyl)benzoyl group on the C-5′ position of the ribose for irreversible binding to PNP. However, nucleosides containing this sulfonyl fluoride moiety have been previously synthesized and evaluated as affinity labeling agents for adenosine nucleotide sites in glutamate dehydrogenase,[34] pyruvate kinase,[35] RNA polymerase,[36] phosphofructokinase,[37,38] and the mitochondrial F1-ATPase.[39] We expected this sulfonyl fluoride moiety to be capable of functioning as an electrophilic functional group in covalent reactions with several classes of amino acids such as lysine, tyrosine, histidine, and serine in the active site of PNP.[40] Thus, the synthesis of 8-aminoguanosine with several chemically reactive functions attached to the C-5′ position became one of our primary goals in the design of PNP inhibitors, e.g., 9-[[2-[*p*-(fluorosulfonyl)benzoyl]ethoxy]methyl]guanine (**18**), 8-amino-5′-[*p*-(fluorosulfonyl)benzoyl]guanosine (**20**), 5′-[*p*-(fluorosulfonyl)benzoyl]guanosine (**21**), and 5′-[*p*-(fluorosulfonyl)benzoyl]formycin B (**24**) were synthesized and evaluated. As can be seen from Table I, none of these compounds showed irreversible inhibitory activity to PNP. In fact, the inhibitory activities of these compounds are even weaker than those of the parent compounds. This might be because there is no nucleophilic residue at the active site of PNP and/or the sulfonyl fluoride moiety is too large to fit into the active site of PNP. However, the enzymatic inhibitory activity of compound **20** is still better than that of **21**, indicative of the importance of having an amino group at the 8-position.

In summation, the influence of an amino substituent at the 8-position of guanosine was consistent with that of the previous assumption that 8-aminoguanine plays an important role in recognition and binding to the active site of purine nucleoside phosphorylase. The area around the 9-position of 8-aminoguanine is most likely a pocket which can accomodate a larger group and could provide a labeled and chemically reactive functional group. The same phenomena was also observed at the C-5′ position of the ribose moiety of 8-aminoguanosine. However, a chemically reactive functional group at the C-5′ position did not

provide an irreversible inhibitor and would suggest that nucleophilic residues (if they are in that area) are not in the proper juxtaposition for a reaction to occur.

Experimental Section

Melting points were obtained on a Electrothermal apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 983 G spectrophotometer; ^1H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker Model AM-300 spectrometer using Me_2SO-d_6 as the solvent and internal standard. UV spectra were recorded on a Shimadzu UV-210 A spectrophotometer. AICA hydrochloride and AICA-riboside were purchased from Sigma Chemical Co. Elemental analysis were carried out in Cheng-Kung University, Tainan, Taiwan.

2-Amino-4-(*n*-hexylamino)pyrimidin-6-one (3a). A mixture of 2-amino-4-chloropyrimidin-6-one (**2**) (5.5 g, 37.67 mmol) and *n*-hexylamine (15 mL, 0.11 mol) in anhydrous ethanol (100 mL) was heated at reflux in an oil bath for 2 days. The mixture was evaporated in vacuo (water pump) at 70 °C to obtain an oily residue. A solid was formed by the addition of methanol (50 mL) to this oily residue. The solid was collected by filtration and recrystallized from methanol to give 5.1 g (71%) of **3a**: mp 179–180 °C; ^1H NMR (270 MHz, DMSO-d_6): δ 0.85 (t, 3 H, CH_3), 1.24 (s, 4 H, $2CH_2$), 1.43 (m, 2 H, CH_2), 3.00 (m, 2 H, CH_2), 4.38 (s, 1 H, =CH), 6.08 (s, 2 H, NH_2, D_2O exchangeable), 6.30 (br s, 1 H, NH, D_2O exhangeable), 9.63 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{10}H_{18}N_4O$). C, H, N.

2-Amino-4-[(cyclohexylmethyl)amino]pyrimidin-6-one (3c). Compound **3c** was prepared in 45% yield using a procedure similar to that which afforded **3a**. An analytical sample was prepared by recrystallization from a mixture of ethanol and water: mp 226 °C dec; ^1H NMR (100 MHz, DMSO-d_6) δ 0.78–1.75 (m, 11 H), 2.87 (t, 2 H, CH_2), 4.40 (s, 1 H, =CH), 6.07 (s, 2 H, NH_2, D_2O exchangeable), 6.35 (t, 1 H, NH, D_2O exchangeable), 9.60 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{11}H_{18}N_4O$) C, H, N.

2-Amino-4-[(naphthylmethyl)amino]pyrimidin-6-one (3d). Compound **3d** was prepared in 34% yield using a procedure similar to that which afforded **3a**: mp 260 °C dec; ^1H NMR (100 MHz, DMSO-d_6) δ 4.48 (s, 1 H, =CH), 4.76 (d, 2 H, CH_2), 6.17 (s, 2 H, NH_2, D_2O exchangeable), 6.93 (t, 1 H, NH, D_2O exchangeable), 7.42–8.15 (m, 7 H, ArH), 9.64 (br s, 1 H, NH, D_2O exchangeable). Anal. ($C_{15}H_{14}N_4O \cdot {}^1/_5H_2O$) C, H, N.

2-Amino-4-(*n*-hexylamino)-5-[1-[3-(methoxycarbonyl)-thioureido]]pyrimidin-6-one (6a). Sodium nitrite (4.0 g, 57.97 mmol) in water (10 mL) was added to a mixture of **3a** (4.0 g, 19.02 mmol), glacial acetic acid (5 mL), and water (150 mL) with stirring at room temperature. After 12 h, the red solid was collected by filtration and washed with water (50 mL). The red solid was then suspended in water (150 mL) and heated on a hot plate with stirring. Sodium dithionite was added to the hot stirring mixture in small portions over a period of 1 hour until the red color changed to a light yellow color. The solid was collected by filtration and was subsequently mixed with 3 equiv of methoxycarbonyl isothiocyanate in acetonitrile (100 mL). The mixture was heated at reflux for 5 h. After the mixture was cooled to room temperature, the solid was collected by filtration and washed with ether (50 mL). The solid was recrystallized from a mixture of DMF and water to afford 4.21 g (65%) of **6a**: mp 224–225 °C. Anal. ($C_{13}H_{22}N_6O_3$) C, H, N.

2-Amino-4-[(carboxypentyl)amino]-5-[1-[3-(methoxycarbonyl)thioureido]]pyrimidin-6-one (6b). A mixture of 2-amino-4-chloropyrimidin-6-one (1.5 g, 10 mmol) and 6-aminohexanoic acid (1.5 mL 12 mmol) in anhydrous ethanol (100 mL) was heated at reflux in an oil bath for 2 days. The mixture was evaporated in vacuo (water pump) at 70 °C to obtain **3b** (1.9 g, 7.92 mmol) as an oily residue. Sodium nitrite (1.6 g, 23.2 mmol) in water (5 mL) was subsequently added to a mixture of this oily residue (**3b**, 1.9 g, 7.92 mmol) in glacial acetic acid (3 mL) and water (50 mL). After stirring at room temperature for 5 h, the red solid was collected by filtration and washed with water (100 mL). The solid was suspended in water (200 mL) and heated on a hot plate with stirring. Sodium dithionite was added to the hot stirring mixture in small portions over a period of 1 hour until the color had changed to a light yellow color. After the mixture was cooled to room temperature, and the solid was collected by filtration and mixed with 3 equiv of methoxycarbonyl isothiocyanate in acetonitrile (50 mL). The mixture was heated at reflux in an oil bath for 6 h. The white solid was collected by filtration and recrystallized from a mixture of ethanol and water to give 1.78 g (60% from **3b**): mp 221–222 °C; ^1H NMR (270 MHz, DMSO-d_6) δ 1.26 (q, 2H, CH_2), 1.5–1.3 (m, 4H, CH_2), 2.17 (t, 2H, CH_2), 3.19 (q, 2H, CH_2), 3.70 (s, 3H, CH_3), 6.17 (t, 1H, NH, D_2O exchangeable), 6.27 (s, 2H, NH_2, D_2O exchangeable), 9.93 (br s, 1H, NH, D_2O exchangeable), 10.07 (s, 1H, NH, D_2O exchangeable), 11.11 (s, 1H, NH, D_2O exchangeable), 11.95 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{20}N_6O_5S$). C, H, N.

2-Amino-4-[(cyclohexylmethyl)amino]-5-[1-[3-(methoxycarbonyl)thioureido]]pyrimidin-6-one (6c). Compound **6c** was prepared in 79% yield using a procedure similar to that which afforded **6a**: mp 222–224 °C; ^1H NMR (100 MHz, DMSO-d_6) δ 0.86–1.78 (m, 11 H), 3.08 (t, 2 H, CH_2), 3.71 (s, 3 H, CH_3), 6.13 (t, 1 H, NH), 6.26 (s, 2 H, NH_2, D_2O exchangeable), 9.91 (s, 1 H, NH, D_2O exchangeable), 10.08 (s, 1 H, NH, D_2O exchangeable), 11.08 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{14}H_{22}N_6O_3S$) C, H, N.

2-Amino-4-[(naphthylmethyl)amino]-5-[1-[3-(methoxycarbonyl)thioureido]]pyrimidin-6-one (6d). Compound **6d** was prepared in 58% yield using a procedure similar to that which afforded **6a**: mp 233 °C dec; ^1H NMR (100 MHz, DMSO-d_6) δ 3.70 (s, 3 H, CH_3), 4.97 (d, 2 H, CH_2), 6.29 (s, 2 H, NH_2, D_2O exchangeable), 6.9 (t, 1 H, NH, D_2O exchangeable), 7.34–8.14 (m, 7 H, ArH), 10.01 (s, 1 H, NH, D_2O exchangeable), 10.18 (s, 1 H, NH, D_2O exchangeable), 11.12 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{18}H_{18}N_6O_3S \cdot {}^1/_4H_2O$) C, H, N.

2-Amino-4-[[*m*-(hydroxymethyl)phenyl]amino]-5-[1-[3-(methoxycarbonyl)thioureido]]pyrimidin-6-one (6e). Compound **6e** was prepared in 42% yield using a procedure similar to that which afforded **6a**: mp 239–240 °C; ^1H NMR (100 MHz, DMSO-d_6) δ 3.72 (s, 3 H, CH_3), 4.44 (s, 2 H, CH_2), 5.10 (br s, 1 H, OH, D_2O exchangeable), 6.43 (s, 2 H, NH_2, D_2O exchangeable), 6.90 (d, 1 H, ArH), 7.16 (t, 1 H, ArH), 7.37 (s, 1 H, ArH), 7.50 (d, 1 H, ArH), 8.02 (s, 1 H, NH, D_2O exchangeable), 10.35 (s, 1 H, NH, D_2O exchangeable), 11.17 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{14}H_{16}N_6O_4S \cdot {}^1/_2H_2O$) C, H, N.

8-Amino-9-hexylguanine (9a). A mixture of **7a** (1.35 g, 4.38 mmol) and anhydrous potassium carbonate (1.31 g, 9.49 mmol) in anhydrous methanol (30 mL) was heated at reflux in an oil bath. After 6 h, the solvent was removed by evaporation in vacuo (water pump) at 70 °C. Water (100 mL) was added to the residue, and the mixture was then heated at reflux in an oil bath for 24 h. The mixture was cooled to room temperature and the pH was adjusted to 5 with glacial acetic acid. The solid was collected by filtration and washed with water (50 mL). The crude product was recrystallized from 1 N sodium hydroxide solution and glacial acetic acid to afford 0.82 g (75%) of **9a**: mp 300 °C; UV λ_{max} nm ($\epsilon \times 10^4$) (MeOH–DMF, v:v, 8:2) 259 (1.5), 293 (0.9); (pH 1) 289 (1.0); (pH 11) 255 (1.7); ^1H NMR (270 MHz, DMSO-d_6) δ 0.83 (t, 3 H, CH_3), 1.23 (s, 4 H, 2 CH_2), 1.55 (m, 2 H, CH_2), 3.73 (t, 2 H, CH_2), 5.83 (s, 2 H, NH_2, D_2O exchangeable), 6.17 (s, 2 H, NH_2, D_2O exchangeable), 10.44 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{11}H_{18}N_6O$) C, H, N.

8-Amino-9-(carboxypentyl)guanine (9b). Dicyclohexylcarbodiimide (1.1 g, 5.34 mmol) was added to a mixture of **6b** (1.0 g, 2.69 mmol) and methanol (50 mL). The mixture was heated at reflux in an oil bath for 6 h. The solvent was then removed by evaporation to dryness in vacuo (water pump) at 70 °C. Boiling toluene (50 mL) was added to the residue, and the white solid was collected and washed with boiling toluene (50 mL). A mixture of this crude product and potassium carbonate (0.74 g, 5.34 mmol) in methanol (50 mL) was heated at reflux in an oil bath for 6 h. The solvent was then removed by evaporation in vacuo (water pump) at 70 °C. Water (50 mL) was added to the residue, and the mixture was heated at reflux for 12 h. The mixture was cooled to room temperature and the pH was adjusted to 5 with glacial acetic acid. The solid was collected by filtration and washed with water (100 mL). The crude product was recrystallized from 1 N sodium hydroxide solution and glacial acetic acid to afford 0.51 g (65%) of **9b**: mp 300 °C; ^1H NMR (270 MHz, DMSO-d_6) δ 1.30–1.24 (m, 2 H, CH_2), 1.88–1.4 (m, 4 H, CH_2), 2.18

(t, 2 H, CH_2), 3.74 (t, 2 H, CH_2), 5.99 (s, 2 H, NH_2, D_2O exchangeable), 6.30 (s, 2 H, NH_2, D_2O exchangeable), 10.78 (s, 1 H, NH, D_2O exchangeable), 12.06 (s, 1 H, NH, D_2O exchangeable); UV λ_{max} nm ($\epsilon \times 10^4$) (MeOH–DMF, v:v, 8:2) 258 (1.3), 293 (0.8); (pH 1) 289 (0.9); (pH11) 257 (1.2), 288 (0.8). Anal. ($C_{11}H_{16}N_6O_3$) C, H, N.

8-Amino-9-(cyclohexylmethyl)guanine (9c). Compound **9c** was prepared in 64% yield using a procedure similar to that which afforded **9a**: mp >300 °C; UV λ_{max} nm ($\epsilon \times 10^4$) (0.1 N NaOH) 253 (0.68); ^{1}H NMR (100 MHz, DMSO-d_6) δ 0.94–1.63 (m, 11 H), 3.58 (d, 2 H, CH_2), 5.79 (s, 2 H, NH_2, D_2O exchangeable), 6.12 (s, 2 H, NH_2, D_2O exchangeable), 10.30 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{12}H_{18}N_6O \cdot {}^1/_4H_2O$) C, H, N.

8-Amino-9-(naphthylmethyl)guanine (9d). Compound **9d** was prepared in 67% yield using a procedure similar to that which afforded **9a**: mp >300 °C; UV λ_{max} nm ($\epsilon \times 10^4$) (0.1 N NaOH) 282 (1.08); ^{1}H NMR (100 MHz, DMSO-d_6) δ 5.5 (s, 2 H, CH_2), 5.92 (s, 2 H, NH_2, D_2O exchangeable), 6.14 (s, 2 H, NH_2, D_2O exchangeable), 7.31–8.24 (m, 7 H, ArH), 10.4 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{16}H_{14}N_6O \cdot H_2O$) C, H, N.

8-Amino-9-[*m*-(hydroxymethyl)phenyl]guanine (9e). Compound **9e** was prepared in 73% yield using a procedure similar to that which afforded **9a**: mp >300 °C: UV λ_{max} nm ($\epsilon \times 10^4$) (0.1 N NaOH) 270 (1.2); ^{1}H NMR (100 MHz, DMSO-d_6) δ 4.55 (d, 2 H, CH_2), 5.28 (t, 1 H, OH, D_2O exchangeable), 5.54 (s, 2 H, NH_2, D_2O exchangeable), 6.14 (s, 2 H, NH_2, D_2O exchangeable), 7.50–7.21 (m, 5 H, ArH), 10.39 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{12}H_{12}N_6O_2 \cdot H_2O$) C, H, N.

9-[(2-Iodoethoxy)methyl]guanine (16). A mixture of acyclovir (225 mg, 1 mmol), triphenylphosphine (262 mg, 1 mmol), and iodine (254 mg, 1 mmol) in anhydrous DMF (5 mL) was allowed to stand at room temperature for 3 days. The solvent was then evaporated to dryness at 60 °C in vacuo (oil pump). The residue was purified by silica gel column chromatography using methanol–chloroform (2:5) as eluent. The proper portions of eluent were collected and evaporated to dryness to afford 9-[(2-iodoethoxy)methyl]guanine (130 mg, 39%): mp 180 °C dec; UV λ_{max} nm ($\epsilon \times 10^4$) (methanol) 254 (1.5); (pH 1) 256 (1.3); (pH 11) 264 (1.2); ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.25 (t, 2 H, CH_2), 3.71 (t, 2 H, CH_2), 5.37 (s, 2 H, CH_2), 6.50 (s, 2 H, NH_2, D_2O exchangeable), 7.83 (s, 1 H, NH, D_2O exchangeable), 10.62 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_8H_{10}N_5O_2I$) C, H, N.

8-Amino-9-[(2-iodoethoxy)methyl]guanine (17). A solution of **13** (0.3 g, 1.25 mmol), triphenylphosphine (0.33 g, 1.26 mmol) and iodine (0.32 g, 2.5 mmol) in anhydrous DMF (15 mL) was allowed to stand at room temperature for 24 h. Sodium dithionite (0.3 g) was added to the mixture and the mixture was then dissolved in a mixture of DMF and water (1:1, v:v, 20 mL). This solution was coevaporated with silica gel (2.5 g) to dryness at 60 °C in vacuo. The solid was subjected to column chromatography (silica gel) and was eluted with chloroform–methanol (7:3, v:v). The proper fraction was collected and evaporated to dryness to furnish 8-amino-9-[(2-iodoethoxy)methyl]guanine (100 mg, 23%): mp 272–273 °C; UV λ_{max} nm ($\epsilon \times 10^4$) (methanol) 257 (1.3), 293 (0.7); (pH 1) 250 (1.3), 288 (0.8); (pH 11) 259 (1.1); ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.30 (t, 2 H, CH_2), 3.71 (t, 2 H, CH_2), 5.24 (s, 2 H, CH_2), 6.03 (s, 2 H, NH_2, D_2O exchangeable), 6.26 (s, 2 H, NH_2, D_2O exchangeable), 10.53 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_8H_{11}N_6O_2I$) N.

9-[[2-[*p*-(Fluorosulfonyl)benzoyl]ethoxy]methyl]guanine (18). Acyclovir (1.0 g, 4.4 mmol) was coevaporated with DMF (2 × 10 mL) at 60 °C in vacuo and then was dissolved in HMPA (10 mL) with slight warming. The solution was immersed in an ice bath and *p*-(fluorosulfonyl)benzoyl chloride (1.2 g, 5.4 mmol) was added into the mixture in small portions over a 3-h period. The mixture was stirred in an ice bath for another 1 h and was then poured into ice–water (100 mL) to afford a precipitate. The white solid was collected by filtration and recrystallized from methanol to furnish 9-[[2-[*p*-(fluorosulfonyl)benzoyl]ethoxy]methyl]guanine (1.5 g, 82%): mp 237–238 °C; UV λ_{max} nm ($\epsilon \times 10^4$) (methanol) 255 (1.5); (pH 1) 257 (1.3); (pH 11) 265 (1.2); ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.38 (t, 2 H, CH_2), 4.41 (t, 2 H, CH_2), 5.39 (s, 2 H, CH_2), 6.48 (s, 2 H, NH_2, D_2O exchangeable), 7.84 (s, 1 H, H-8), 8.13 (d, 2 H, ArH, J = 8.4 Hz), 8.25 (d, 2 H, ArH, J = 8.4 Hz), 10.58 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{15}H_{14}N_5FS \cdot {}^1/_2H_2O$) C, H.

8-Amino-5′-deoxy-5′-(phenylthio)guanosine (19). Tributylphosphine (6.0 mL, 24.05 mmol) was added to a suspension of 8-aminoguanosine[26] (**12**, 2.08 g, 6.98 mmol) and diphenyl disulfide (3.5 g, 15.56 mmol) in pyridine (40 mL). The mixture was stirred at room temperature for 60 h and then poured slowly into a stirred mixture of water (100 mL) and diethyl ether (100 mL). The resulting solid was collected, washed with chloroform (10 mL) and diethyl ether (20 mL). The solid was recrystallized from a mixture of acetone and water (v:v, 1:1) to afford 8-amino-5′-deoxy-5′-(phenylthio)guanosine (1.6 g, 59%): mp 240–242 °C; ^{1}H-NMR (360 MHz, DMSO-d_6) δ 3.4–3.28 (m, 2 H, H-5′), 3.85 (q, 1 H, H-4′), 4.15 (q, 1 H, H-3′), 4.91 (q, 1 H, H-2′), 5.12 (d, 1 H, H-3′ OH, J = 5.4 Hz), 5.34 (d, 1 H, H-2′ OH, J = 6.08 Hz), 5.58 (d, 1 H, H-1′, J = 5.76 Hz), 5.98 (s, 2 H, NH_2, D_2O exchangeable), 6.39 (s, 2 H, NH_2, D_2O exchangeable), 7.14–7.35 (m, 5 H, ArH), 11.04 (s, 1 H, NH); UV λ_{max} nm ($\epsilon \times 10^4$) (methanol) 254 (2.3), 292 (0.83); (pH 1) 251 (2.1), 291 (0.87); (pH 11) 254 (2.0). Anal. ($C_{16}H_{18}N_6SO_4$) C, H, N.

8-Amino-5′-[*p*-(fluorosulfonyl)benzoyl]guanosine (20). Concentrated hydrochloric acid (0.7 mL) was added to a stirred suspension of 8-aminoguanosine[26] (**12**, 1.0 g, 3.36 mmol) in anhydrous DMF (10 mL). The mixture was allowed to stir at room temperature. After 15 min, THF (70 mL) was added to the mixture (clear solution) and the upper layer was decanted. Methanol (10 mL) was added to the yellowish oily residue and then anhydrous ether (50 mL) afforded a precipitate. The white solid was collected by filtration and dried in vacuo (water pump) over P_2O_5 and silica gel for 12 h to furnish 8-aminoguanosine dihydrochloride (1.10 g). The dihydrochloride salt was then dissolved in HMPA (8 mL). The solution was immersed in an oil bath and *p*-(fluorosulfonyl)benzoyl chloride (0.72 g) was added in small portions to this solution. The reaction mixture was allowed to stir at room temperature for 48 h and was then extracted with petroleum ether (70 mL) and a mixture of ethyl acetate–ether (1:1) (100 mL). The resulting yellowish oily residue was dissolved in methanol (10 mL). Ether (100 mL) was added to this solution to afford a precipitate. The white solid was collected by filtration and subsequently dissolved in anhydrous DMF (10 mL). Anhydrous triethylamine (1 mL) was added to the resulting solution to afford a precipitate. To the suspension was added ether (100 mL) slowly with stirring. The light yellow solid was collected by filtration and air-dried. The yellow solid was recrystallized from a mixture of ethanol and water (1:2) (150 mL) with activated charcoal (1 g) to afford 8-amino-5′-[*p*-(fluorosulfonyl)benzoyl]guanosine (0.6 g, 37%): mp 240 °C dec; ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.06 (q, 1 H, H-4′), 4.56–4.45 (m, 2 H, H-5′), 4.65 (q, 1 H, H-3′), 4.75 (q, 1 H, H-2′), 5.07 (d, 1 H, H-3′ OH, J = 6.3 Hz, D_2O exchangeable), 5.42 (d, 1 H, H-2′ OH, J = 5.4 Hz, D_2O exchangeable), 5.63 (d, 1 H, H-1′, J = 4.13 Hz), 5.86 (s, 2 H, NH_2, D_2O exchangeable), 6.17 (s, 2 H, NH_2, D_2O exchangeable), 8.24 (s, 4 H, ArH), 10.50 (s, 1 H, NH, D_2O exchangeable); UV λ_{max} nm ($\epsilon \times 10^4$) (methanol) 256 (1.9), 289 (1.1); (pH 1) 288 (1.2). Anal. ($C_{17}H_{17}N_6SO_8F$) C, H, N.

5′-[*p*-(Fluorosulfonyl)benzoyl]guanosine (21). Guanosine (1.0 g, 3.5 mmol) was coevaporated with anhydrous DMF (2 × 10 mL) at 60 °C in vacuo and then was dissolved in hexamethylphosphoric triamide (8 mL) with slight warming. The solution was immersed in an ice bath and to this solution was added *p*-(fluorosulfonyl)benzoyl chloride (1.5 g, 6.7 mmol) in small portions. The mixture was allowed to stir at room temperature for 4 h and was then extracted with petroleum ether (50 mL). The upper layer was discarded and the lower layer became a yellowish oil by the addition of ethyl acetate–ether (1:1) (100 mL). The resulting yellowish oil was further washed twice with ethyl acetate–ether (1:1) (50 mL). The resulting solid was crystallized from a mixture of methanol and water to afford 5′-[*p*-(fluorosulfonyl)benzoyl]guanosine (0.26 g, 26%): ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.17 (q, 1 H, H-4′), 4.32 (t, 1 H, H-3′), 4.50 (m, 2 H, H-5′), 4.61 (q, 1 H, H-2′), 5.35 (br s, 1 H, H-3′ OH, D_2O exchangeable), 5.59 (brs, 1 H, H-2′ OH, D_2O exchangeable), 5.73 (d, 1 H, H-1′, J = 4.7 Hz), 6.48 (s, 2 H, NH_2, D_2O exchangeable), 7.87 (s, 1 H, =CH), 8.26 (s, 4 H, ArH), 10.64 (s, 1 H, NH, D_2O exchangeable).

5′-Deoxy-5′-(phenylthio)formycin B (23). To a suspension of formycin B (1.0 g, 3.7 mmol) and diphenyl disulfide (1.84 g, 8.42 mmol) in pyridine (30 mL) was added tributylphosphine

(3.0 mL). The mixture was allowed to stir at room temperature for 3 h and then concentrated in vacuo at 60 °C to 10 mL. To the concentrated mixture was added ether (50 mL), with shaking, to afford a white solid. The solid was collected by filtration and washed with ether (100 mL) to afford 1.0 g of the crude product. The filtrate was evaporated to dryness and then to this oil residue was added water (20 mL), methanol (5 mL), and ether (100 mL) to obtain another 0.4 g of crude product. The total crude product (1.4 g) was recrystallized from acetone and water to furnish 5′-deoxy-5′-(phenylthio)formycin B (1.2 g, 89%): mp 207–209 °C; UV λ_{max} nm ($\epsilon \times 10^4$) (methanol) 255 (1.5); (pH 11) 252 (1.2), 280 (0.9). ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.30 (m, 2 H, H-5′), 3.92 (q, 1 H, H-4′), 4.14 (q, 1 H, H-3′), 4.56 (q, 1 H, H-2′), 4.92 (d, 1 H, H-1′, J = 5.53 Hz), 5.09 (d, 1 H, H-2′ OH, J = 5.8 Hz, D_2O exchangeable), 7.30 (m, 5 H, ArH), 7.89 (s, 1 H, =CH), 12.28 (s, 1 H, NH, D_2O exchangeable), 14.07 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{16}H_{16}N_4O_4S$) C, H, N.

5′-[*p*-(Fluorosulfonyl)benzoyl]formycin B (24). To a suspension of formycin B (0.5 g, 1.87 mmol) in HMPA (10 mL) was added *p*-(fluorosulfonyl)benzoyl chloride (0.5 g). The mixture was allowed to stir at room temperature for 12 h. The mixture was then poured into ice–water to furnish a white solid precipitate. The solid was collected by filtration and recrystallization from methanol to afford 5′-[*p*-(fluorosulfonyl)benzoyl]formycin B (0.6 g, 71%): mp 244–246 °C; ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.15 (t, 1 H, H-4′), 4.61–4.24 (m, 4 H, H-5′, H-2′ and H-3′), 5.04 (d, 1 H, H-1′, J = 4.35 Hz), 5.15 (t, 2 H, H-2′ OH, D_2O exchangeable), 7.81 (d, 1 H, =CH, J = 3.2 Hz), 8.30 (s, 4 H, ArH), 12.25 (s, 1 H, NH, D_2O exchangeable), 14.12 (s, 1 H, NH, D_2O exchangeable). Anal. ($C_{17}H_{15}N_4O_8SF \cdot {}^1/_4H_2O$) C, H, N.

PNP Inhibitory Assay.[19] Substrate, [8-^{14}C]inosine, is used at a specific activity of 22.5 mCi/mmol. For K_m determinations, a fixed amount of diluted cell extract is incubated with variable amounts of radiolabeled substrate (12.5–100 mM) and inorganic phosphate (50 mM). K_i determinations were performed with variable radiolabled inosine concentrations (12.5–100 mM), fixed inorganic phosphate (50 μM) and variable inhibitor concentrations (0.03 mM to 1.0 μM). All reactions are incubated for 10 min at 37 °C. Substrate and product (inosine and hypoxanthine, respectively) are separated by high voltage paper electrophoresis. The radiolabeled product of the reaction is visualized by UV light (290 nm), cut from the paper, and counted in a toluene-based scintillation fluid in a Packard Tri-carb liquid scintillation spectrometer. Enzyme-free blank reactions are used as controls for all reactions. In all initial velocity determinations, with or without inhibitor, not more than 15% of the substrate is converted to product. Double reciprocal plots of the initial velocity values versus the substrate concentrations are linear.

Acknowledgment. This investigation was supported by research grants (No. NSC75-0412-B016-64 and NSC76-0208-M016-01 to J.-W.C.) from National Science Council of the Republic of China and by the UNDP/World Bank/WHO Special Programs for Research and Training in Tropical Diseases and Scientific Work Group of Filariasis (I.D. 840398 and 870387 to L.B.T.).

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