Guanine, Pyrazolo[3,4-*d*]pyrimidine, and Triazolo[4,5-*d*]pyrimidine (8-Azaguanine) Phosphonate Acyclic Derivatives as Inhibitors of Purine Nucleoside Phosphorylase

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Phosphonate acyclic derivatives of guanines, pyrazolo[3,4-*d*]pyrimidines, and triazolo[4,5-*d*]-pyrimidines (8-azaguanines) are inhibitors of the enzyme purine nucleoside phosphorylase (PNPase) with K_i' values ranging from 0.05 to 1.6 μM. These compounds are enzymatically stable congeners of the potent PNPase inhibitor acyclovir diphosphate (**53**).

Introduction

Purine nucleoside phosphorylase (PNPase) is an important enzyme in the purine salvage pathway. It catalyzes the phosphorolysis of guanosine, inosine, and the corresponding 2′-deoxyribose analogs to their purine bases in a reversible reaction.

Studies on children born with a PNP immunodeficiency have suggested utility of PNPase inhibitors for the treatment of a range of diseases. These include T-cell leukemia, organ transplant rejections, and autoimmune dysfunctions such as rheumatoid arthritis and psoriasis.[1,2] As a result of the interest in clinical applications of PNPase inhibitors, over the years a considerable number of structure−activity relationship investigations have identified several potent inhibitors.[3−7] Nanomolar potency is necessary for a clinically useful inhibitor because of the abundance of the enzyme in human organisms.

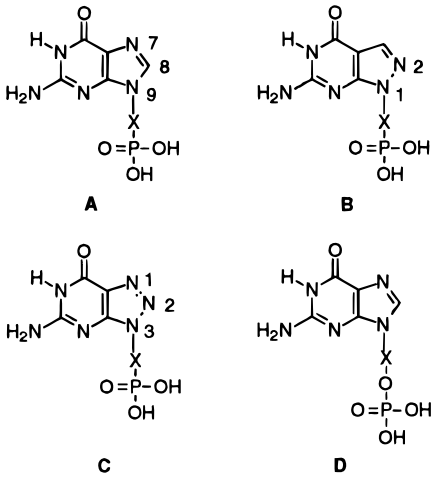
One such inhibitor, acyclovir diphosphate (**53**, ACVDP, Table 1), has been extensively studied and found to have K_i' values ranging from 4 to 10 nM.[8−10] X-ray studies of PNPase have established three discrete binding sites in the active site of the enzyme: a guanine binding region, a hydrophobic pocket, and a phosphate binding site.[11] The potency of inhibition by acyclovir diphosphate (**53**) is the result of binding to all three of these regions. However, because phosphate groups are susceptible to degradation *in vivo*, ACVDP is not a drug candidate.

Attempts to overcome the instability of the phosphate terminus have focused on evaluation of phosphonate derivatives of nucleosides, since in these phosphate mimics the phosphorus−carbon bond is not enzymatically vulnerable to cleavage by phosphatases.

As part of a program on acyclic nucleosides in these laboratories, we have explored the SAR of a series of phosphonate derivatives as PNPase inhibitors. We were interested in the effect that variations of the nitrogen in the imidazole ring of the guanine molecule would cause on K_i' and, implicitly, binding to the guanine site.

In this paper we report the results of the synthesis and biological testing of a group of guanines, pyrazolo[3,4-*d*]pyrimidines, and triazolo[4,5-*d*]pyrimidines (8-azaguanines) for comparative purposes.

Table 1. Inhibition of Human Erythrocyte Purine Nucleoside Phosphorylase



no.	X	*m*[a]	$K_i' \pm$ SE (*N*),[b] (μM)
	Guanine Phosphonates (Structure A)		
18	$(CH_2)_6CF_2$	7	0.057 ± 0.002 (2)
24a	$(CH_2)_7$	7	0.53 ± 0.01 (3)
24b	$(CH_2)_8$	8	0.39 ± 0.01 (2)
28	$(CH_2)_{10}$	10	1.6 ± 0.03 (2)
32a	$CH_2O(CH_2)_4$	6	0.056 ± 0.005 (3)
32b	$CH_2O(CH_2)_5$	7	0.16 ± 0.01 (2)
32c	$CH_2O(CH_2)_6$	8	0.22 ± 0.01 (2)
	Pyrazolo[3,4-*d*]pyrimidine Phosphonates (Structure B)		
21	$(CH_2)_6CF_2$	7	0.049 ± 0.003 (2)
26a	$(CH_2)_7$	7	0.58 ± 0.01 (2)
26b	$(CH_2)_8$	8	1.1 ± 0.01 (2)
36a	$CH_2O(CH_2)_5$	7	0.23 ± 0.01 (3)
36b	$CH_2O(CH_2)_6$	8	0.63 ± 0.0076 (2)
	Triazolo[4,5-*d*]pyrimidine Phosphonates (8-Azaguanine Phosphonates) (Structure C)		
42a	$(CH_2)_4$	4	7.3 ± 0.1 (2)
42b	$(CH_2)_5$	5	0.065 ± 0.006 (3)
42c	$(CH_2)_6$	6	0.36 ± 0.03 (3)
47a	$CH_2O(CH_2)_4$	6	0.048 ± 0.002 (3)
51	$CH_2O(CH_2)_5$[c]	7	0.55 ± 0.01 (2)
47b	$CH_2O(CH_2)_5$	7	0.096 ± 0.006 (3)
47c	$CH_2O(CH_2)_6$	8	0.19 ± 0.01 (3)
	Guanine Phosphates (Structure D)		
52	$CH_2OCH_2CH_2CH_2CH_2$	7	0.051 ± 0.002 (4)
53 (ACVDP)	$CH_2OCH_2CH_2OPO(OH)$	7	0.010 ± 0.003 (20)

[a] *m* indicates the units of spacer length. [b] Number of determinations. [c] 87% 2-isomer, 13% 3-isomer, prepared from alkylation of silylated 8-azapurine.

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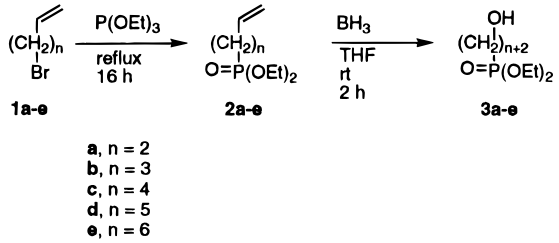
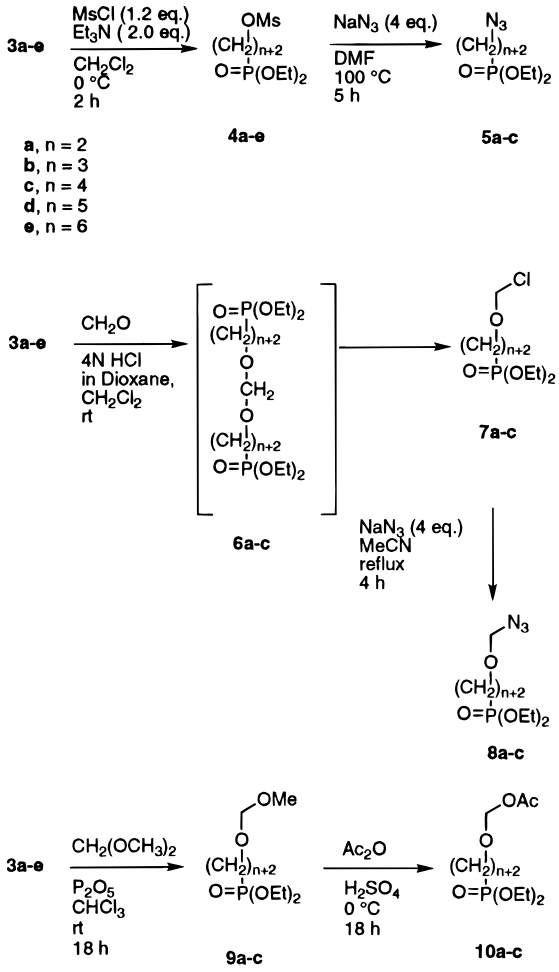
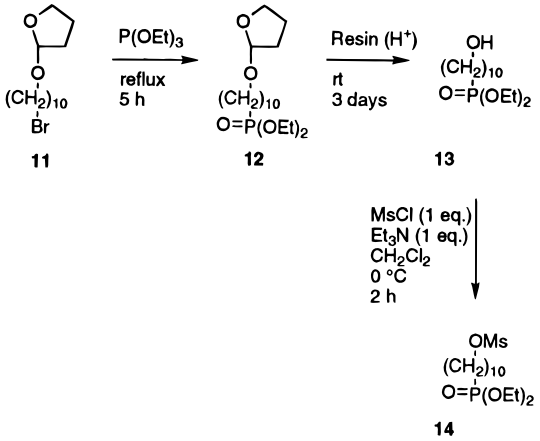
Scheme 1



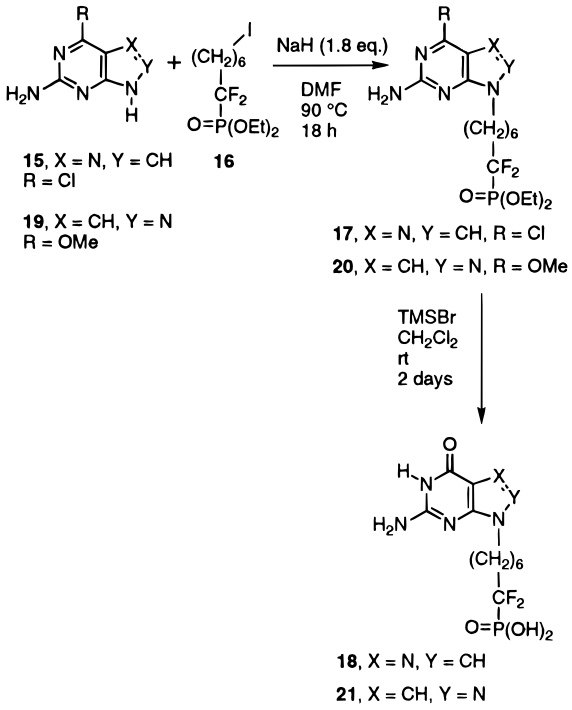
Scheme 2



Scheme 3



Scheme 4



Chemistry

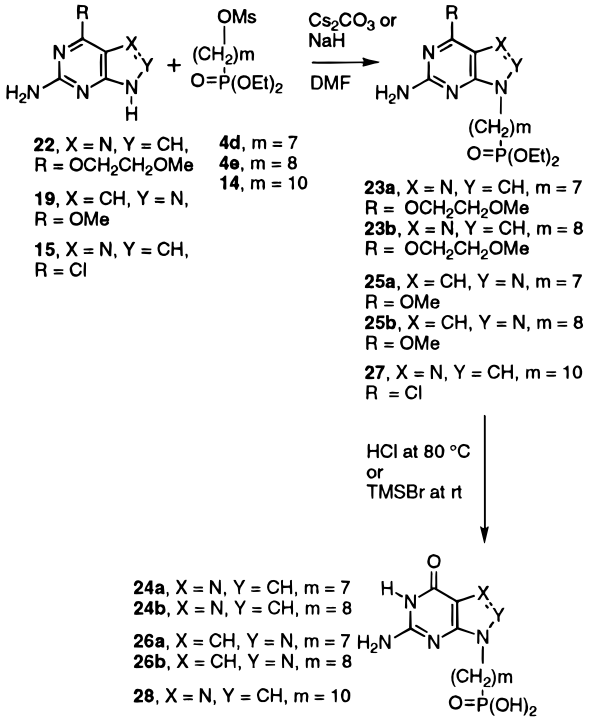
Previous PNPase studies with acyclic purine nucleotides had demonstrated the optimal length of the acyclic chain to be seven or eight methylene or oxygen atom units between the terminal phosphorous atom and N-9 nitrogen of the purine.[12] Therefore, we chose in each group to additionally synthesize shorter and longer members in each acyclic chain to further probe the binding requirements. A few α,α-difluoro phosphonate derivatives were also prepared for use in investigating reports of more potent inhibition of similar analogues in comparison to their nonfluorinated congeners.[13] Syntheses of the acyclic chains are shown in Schemes 1−3.

The *N*-alkyl phosphonates derivatives of guanine and 6-amino-4-hydroxypyrazolo[3,4-*d*]pyrimidine (Schemes 4 and 5) were prepared by direct alkylation of the appropriate heterocyclic base (**15**, **19**, or **22**) in the presence of a base (NaH or Cs_2CO_3) with the corresponding alkyl phosphonates having a good leaving group such as mesylate (**4d**, **4e**, and **14** in Scheme 5) or iodo (**16** in Scheme 4). Although ^{1}H-NMR of the original reaction mixtures showed what could possibly be minor amounts of the undesired isomers, only the proper ones were isolated after column chromatography. Hydrolysis of the phosphonate esters was achieved using either TMSBr at room temperature or heating with HCl.

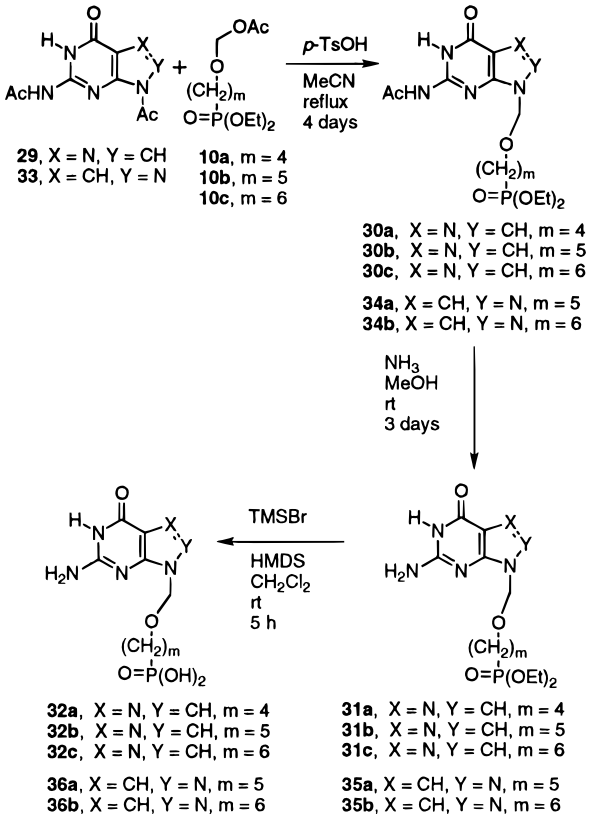
The glycosidic acyclic derivatives of guanine and 6-amino-4-hydroxypyrazolo[3,4-*d*]pyrimidine (Scheme 6) were prepared from the diacetylated base (**29** or **33**) and the corresponding [(acetoxymethoxy)alkyl]phosphonate chain (**10a**, **10b**, or **10c**) in the presence of *p*-TsOH as a catalyst at high temperatures. Removal of the acetyl group with ammonia and hydrolysis of the phosphonate esters with TMSBr afforded the final products in reasonable yields.

Syntheses of the 5-amino-7-hydroxytriazolo[4,5-*d*]-pyrimidine (8-azaguanine) acyclic derivatives were initially attempted by alkylation of silylated 8-azaguanine with the appropriate acyclic chain reagents. These reactions produced mixtures of the three isomeric products which were difficult to separate in a pure state.

Scheme 5

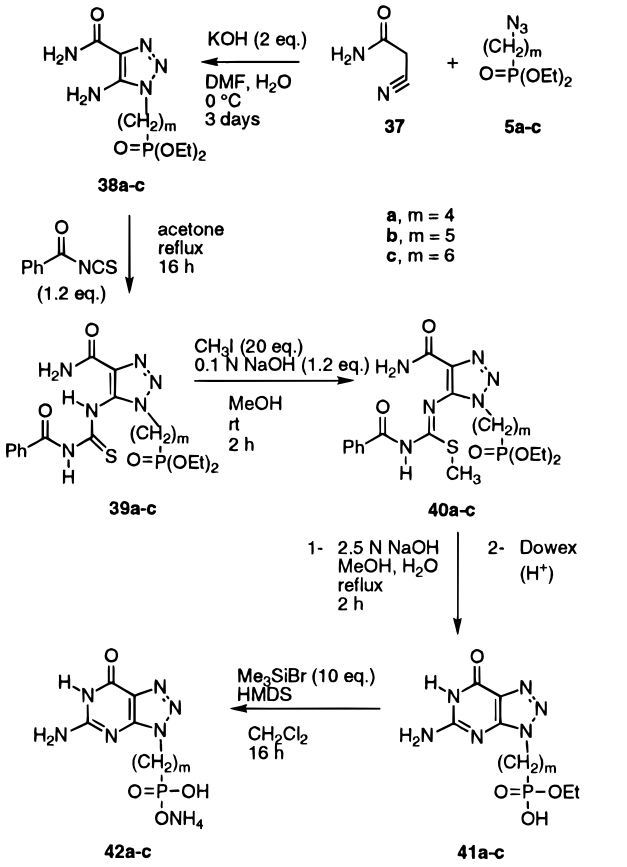


Scheme 6



Interestingly, the predominant isomer often was the N-2 positional analogue. To obtain the desired N-3 isomers exclusively, the alternative route shown in Schemes 7 and 8 was used. This route is a modification of one described in the literature for the synthesis of 9-substituted guanines[14] and was previously explored in our laboratories with simple alkyl chains.[15] This is the first reported synthesis of 3-substituted triazolo[4,5-*d*]pyrimidines (9-substituted 8-azaguanines) in a highly regio-

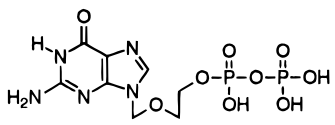
Scheme 7



specific and efficient way. The N-2 isomer (**51**) was obtained (contaminated with 13% of the N-3 isomer) by direct alkylation of the diacetylated base **48** and the phosphonate chain **10b** (Scheme 9) in a similar manner as described for the pyrazolo[3,4-*d*]pyrimidines and the guanine acyclic derivatives (Scheme 6).

Results and Discussion

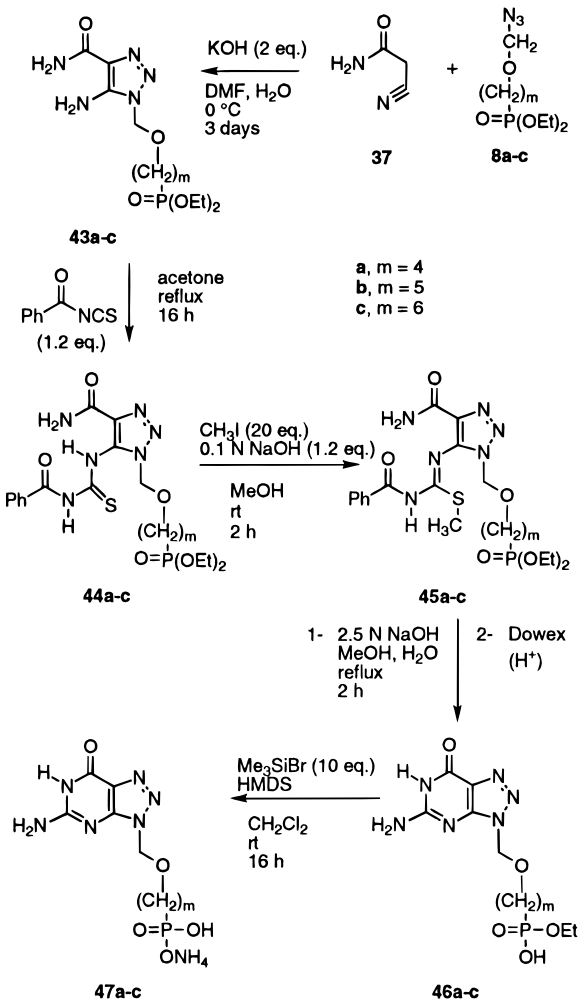
Enzyme assays and K_i' determinations were performed as described in the Experimental Section. The results obtained on the three heterocyclic series are listed in Table 1 along with reference ACVDP (**53**) and the acyclic nucleotide **52**.[12]



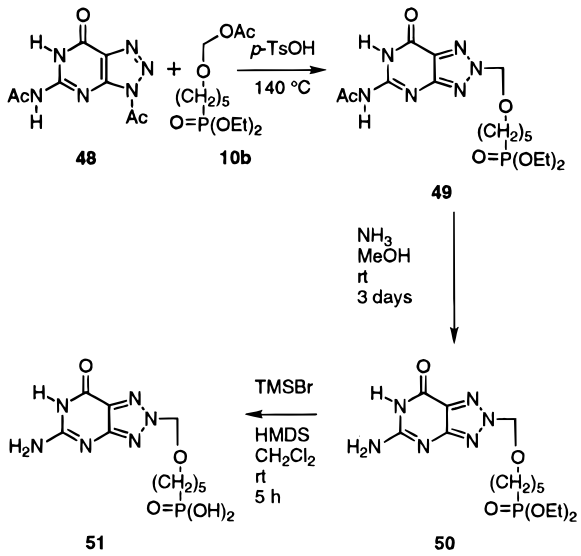
53 ACVDP

In the guanine series (structures A) the phosphonate derivatives which contained nonfluorinated acyclic chains with seven or eight atoms units, **24a**, **24b**, **32b**, and **32c**, had similar inhibitory activity. A decrease in the chain length from seven to six atom units (**32a**) increased the inhibitory potency, whereas an increase to 10 atom units (**32c**) decreased the inhibitory potency. The glycosidic compounds were slightly better inhibitors than their alkyl counterparts with equal chain lengths. This may result from the enhanced potential for hydrogen bonding and rotational freedom that the insertion of the oxygen atom produces. The insertion of the fluoro atoms on the phosphonate α carbon unit of the seven-carbon alkyl-

Scheme 8



Scheme 9



guanine **24a** to produce **18** increased the inhibitory potency approximately 10-fold. The lower K_i' may result from enhanced binding at the phosphonate binding site. This could result from an increase in the acidity of the phosphonate group (pK_{a2}), due to the electronegative fluorine atoms, coupled with altered steric effects.

The pyrazolo[3,4-*d*]pyrimidine phosphonates (structure B) were also inhibitors, with similar K_i' values as their purine counterparts. The glycosidic compounds were slightly better inhibitors than their alkyl counterparts with equal chain lengths as was the case for the guanine series described above. The increase in inhibitory potency resulting from the α-fluoro substitutions was also similar to that observed in the guanine series. These results are an indication of compatibility of the pyrazole moiety of the bicyclic ring with the guanine binding region.

In the 8-azaguanine series (structure C) the phosphonate analogues which contained a glycosidic chain (**47a**, **47b**, and **47c**) had K_i' values similar to those of their guanine counterparts. Compound **42b** which had an alkyl chain length of five atom units had a K_i' value lower than that of its four and six atom units analogues (**42a** and **42c**, respectively). An optimum acyclic chain length of five atom units is shorter than the optimum length of seven or eight atom units found previously for acyclovir monophosphate analogues.[12] The reasons for this difference is unclear.

In summary, the introduction or rearrangement of the nitrogen atoms in the five-membered ring of guanine does not appear to significantly alter the PNPase inhibition of its purine congeners. There are, however, some differences in the optimal length of the acyclic chain in each series. The availability of X-ray coordinates of the enzyme would be an instructive tool for determining conformational and binding properties of these analogues. Pyrrolo[2,3-*d*]pyrimidines have also been shown to exhibit comparable inhibitory potency as purine analogues,[16] indicating a considerable range of tolerance in this guanine binding region unrelated to differences in the p*K* of the various heteocyclic rings.

Experimental Section

General Procedures. Melting points were determined in open glass capillaries with a Thomas-Hoover apparatus, and are uncorrected. ^{1}H-NMR spectra were recorded at 300 MHz and ^{31}P-NMR at 121.4 MHz on a Varian XL-300 spectrometer. CIMS were recorded on a platform mass spectrometer (Fison Instrument) operated in a APcI (atmospheric pressure chemical ionization) mode. Ultraviolet spectra were recorded on a Hewlett-Packard 8452A spectrometer. Evaporations were performed under diminished pressure in a Buchi rotatory evaporator at 40 °C unless otherwise indicated. Solutions were dried over anhydrous Na_2SO_4. TLCs were run on precoated glass plates (0.25 mm) with silica gel $60F_{254}$ (E. Merck, Darmstad). Flash column chromatography was performed with silica gel 60 (230−400 mesh, E. Merck, Darmstad). Elemental analyses were done by Atlantic Microlabs (Atlanta, GA). For ion exchange column chromatography, 300 mg of material were dissolved in 150 mL of deionized water in a typical example. Any solids were removed by filtration. The clean solution was applied to a lobar column (2 × 30 cm) packed with DEAE Sephadex A-25 (HCO_3^- form). The column was successively eluted with H_2O (200 mL), 0.025 M NH_4HCO_3 (500 mL), 0.050 M NH_4HCO_3 (500 mL), and finally a linear gradient (0−0.5 M NH_4HCO_3). For a series of similar compounds only the ^{1}H-NMR data for the first member of the series is given.

Diethyl 3-Butenylphosphonate (2a) and 2b−e. A mixture of **1** (**a**−**e**) (122 mmol) and $P(OEt)_3$ (81 g, 84 mL, 486 mmol) was refluxed for 16 h. Excess $P(OEt)_3$ was removed at 60 °C under reduced pressure to afford pure **2** (**a**−**e**) as a syrup.

2a: 76%; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, J = 7.0, 6H, OCH_2CH_3), 1.80 (m, 2H, CH_2), 2.18 (m, 2H, CH_2), 4.08 (m, 4H, OCH_2CH_3), 5.00 (m, 2H, vinyl CH_2), 5.80 (m, 1H, vinyl CH).

2b: 60%. **2c**: 72%. **2d**: 71%. **2e**: 92%.

Diethyl (4-Hydroxybutyl)phosphonate (3a) and 3b−e. BH_3 (1.0 M) in THF (85 mL, 85 mmol) was slowly added to a well-stirred solution of **2** (**a**−**e**) (80 mmol) in dry THF (120

mL) at 0 °C. The solution was stirred for an additional 0.5 h at 0 °C and then for 2 h at room temperature. After cooling to 0−5 °C, H_2O (100 mL) was first added, followed by solid $NaBO_3 \cdot 4H_2O$ (36 g, 230 mmol). The mixture was stirred for additional 2 h at room temperature and then extracted with CH_2Cl_2. The organic solution was dried, filtered, and evaporated to afford pure **3** (**a**−**e**) as a syrup.

3a: 30%; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, $J = 7.0$, 6H, OCH_2CH_3), 1.58−1.82 (m, 6H, $(CH_2)_3$), 3.62 (m, 2H, CH_2OH), 4.04 (m, 4H, OCH_2CH_3).

3b: 83%. **3c**: 89%. **3d**: 94%. **3e**: 91%.

Diethyl [4-(Mesyloxy)butyl]phosphonate (4a) and 4b−e. To a well-stirred solution of **3** (**a**−**e**) (50 mmol) and Et_3N (10.1 g, 13.9 mL, 100 mmol) in dry CH_2Cl_2 (250 mL) at 0−5 °C was slowly added $MeSO_2Cl$ (6.85 g, 4.62 mL, 60 mmol) under nitrogen. The solution was stirred at 0 °C for 2 h and then slowly warmed up to room temperature over a period of 1 h. The solution was washed with H_2O (3 × 200 mL), dried, filtered, and evaporated to afford pure **4** (**a**−**e**) as a syrup.

4a: 76%; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, $J = 7.0$, 6H, OCH_2CH_3), 1.60−1.85 (m, 6H, $(CH_2)_3$), 2.98 (s, 3H, CH_3SO_2), 4.04 (m, 4H, OCH_2CH_3), 4.20 (t, $J = 6.1$, 2H, CH_2OMs).

4b: 99%. **4c**: 90%. **4d**: 95%. **4e**: 95%.

Diethyl (4-Azidobutyl)phosphonate (5a) and 5b,c. A suspension of **4** (**a**−**c**) (40 mmol) and NaN_3 (7.82 g, 120 mmol) in dry DMF (80 mL) was stirred at 100 °C for 5 h and then allowed to cool to room temperature. It was diluted with CH_2Cl_2 (400 mL), washed with H_2O (4 × 400 mL), dried, filtered, and evaporated to afford pure **4** (**a**−**c**) as a syrup.

5a: 64%; ^{1}H-NMR ($CDCl_3$) δ 1.31 (t, $J = 6.9$, 6H, OCH_2CH_3), 1.60−1.85 (m, 6H, $(CH_2)_3$), 3.25 (t, $J = 6.7$, 2H, CH_2N_3), 4.04 (m, 4H, OCH_2CH_3).

5b: 78%. **5c**: 92%.

Diethyl [4-(Chloromethoxy)butyl]phosphonate (7a) and 7b,c. To a cold (0 °C) stirred solution of **3** (**a**−**c**) (65 mmol) and paraformaldehyde (2.0 g, 66 mmol) in dry CH_2Cl_2 (130 mL) was slowly added a solution of 4 N HCl in dioxane (48.5 mL, 193 mmol). The reaction mixture was left with stirring for 24 h at room temperature. The solution was evaporated to dryness to afford a syrup that proved to be a 1:1 mixture of **7** (**a**−**c**) and the dimer **6** (**a**−**c**) by ^{1}H-NMR. The syrup was treated as before three more times until ^{1}H-NMR spectra indicated the total disappearance of the dimer. Pure **7** (**a**−**c**) was isolated as a syrup.

7a: 84%; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, $J = 7.0$, 6H, OCH_2CH_3), 1.60−1.80 (m, 6H, $(CH_2)_3$), 3.64 (m, 2H, CH_2OCH_2Cl), 4.04 (m, 4H, OCH_2CH_3), 5.45 (s, 2H, OCH_2Cl).

7b: 80%. **7c**, 66%.

Diethyl [4-(Azidomethoxy)butyl]phosphonate (8a) and 8b,c. A suspension of **7** (**a**−**c**) (57.3 mmol) and NaN_3 (18.6 g, 286 mmol) in dry MeCN (130 mL) was refluxed for 4 h. After cooling to room temperature, the solids were filtered, washed with MeCN, and evaporated to a syrup that was purified by flash column chromatography (1:1 hexane−acetone). Pure **8** (**a**−**c**) was isolated as a syrup.

8a: 30%; ^{1}H-NMR ($CDCl_3$) δ 1.31 (t, $J = 7.0$, 6H, OCH_2CH_3), 1.60−1.80 (m, 6H, $(CH_2)_3$), 3.60 (t, $J = 6.3$, 2H, $CH_2OCH_2N_3$), 4.06 (m, 4H, OCH_2CH_3), 4.61 (s, 2H, OCH_2Cl).

8b: 37%. **8c**: 25%.

Diethyl [4-(Methoxymethoxy)butyl]phosphonate (9a) and 9b,c. To a solution of compound **3** (**a**−**c**) (28.6 mmol) and dimethoxymethane (51 mL, 571 mmol) in dry $CHCl_3$ (150 mL) was added P_2O_5 (16 g, 28.6 mmol) portionwise, with vigorous stirring. After stirring at room temperature for 18 h, the reaction mixture was decanted and the solids washed with $CHCl_3$. The combined organic layers were washed with $NaHCO_3$(c) (2 × 200 mL) and H_2O (200 mL), dried, filtered, and evaporated *in vacuo* to afford **9** (**a**−**c**) as a yellow oil.

9a: 51%; ^{1}H-NMR ($CDCl_3$) δ 1.31 (t, $J = 7.1$, 6H, OCH_2CH_3), 1.60−1.78 (m, 6H, $(CH_2)_3$), 3.36 (s, 3H, $CH_2OCH_2OCH_3$), 3.51 (t, $J = 6.3$, 2H, $CH_2OCH_2OCH_3$), 4.06 (m, 4H, OCH_2CH_3), 4.60 (s, 2H, $CH_2OCH_2OCH_3$).

9b: 31%. **9c**: 56%.

Diethyl [4-(Acetoxymethoxy)butyl]phosphonate (10a) and 10b,c. To a cold solution (0 °C) of **9** (**a**−**c**) (14 mmol) in Ac_2O (11 mL, 120 mmol) was added a solution of H_2SO_4(c) (0.31 mL, 6 mmol) in Ac_2O (1 mL). The mixture was kept in the cold for 18 h and was then poured into an ice−water bath containing $NaHCO_3$(c) (100 mL). Stirring continued at room temperature for 18 h. The mixture was extracted with $CHCl_3$ (2 × 60 mL), washed with $NaHCO_3$(c) (6 × 200 mL) and H_2O (200 mL), dried, filtered, and evaporated *in vacuo* to afford **10** (**a**−**c**) as a yellow oil.

10a: 75%; ^{1}H-NMR ($CDCl_3$) δ 1.31 (t, $J = 7.1$, 6H, OCH_2CH_3), 1.60−1.73 (m, 6H, $(CH_2)_3$), 2.08 (s, 3H, $OCOCH_3$), 3.62 (t, $J = 6.3$, 2H, $CH_2OCH_2OCOCH_3$), 4.06 (m, 4H, OCH_2CH_3), 5.24 (s, 2H, $CH_2OCH_2OCOCH_3$).

10b: 80%. **10c**: 94%.

Diethyl [10-(Tetrahydropyranyloxy)decyl]phosphonate (12). A mixture of 1-bromo-10-(tetrahydropyranyloxy)decane (**11**, 18 g, 56 mmol) and $P(OEt)_3$ (19.2 mL, 111.9 mmol) was treated as described for **2** (**a**−**e**). Compound **12** was isolated as a clear oil (20.18 g, 95%): ^{1}H-NMR ($CDCl_3$) δ 1.29 (t, $J = 7.1$, 6H, OCH_2CH_3), 1.51−1.84 (m, 22H, $(CH_2)_9$ and CH_2CH_2 (tetrahydrofuranyl)), 3.31−3.74 (m, 5H, $CH_2OCHOCH_2$), 4.11 (m, 2H, OCH_2CH_3).

Diethyl (10-Hydroxydecyl)phosphonate (13). A solution of **12** (20.17 g, 53.3 mmol) in EtOH (250 mL) was treated with AG50W-X8 (H^+) ion-exchange resin (2.5 g, prewashed with methanol), and stirring was continued at room temperature for 3 days. The resin was filtered off and washed with EtOH. The solvent was evaporated *in vacuo*, and the residue was distilled, affording **13** as a clear oil (7.4 g, 47%): ^{1}H-NMR ($CDCl_3$) δ 1.32 (t, $J = 7.0$, 6H, OCH_2CH_3), 1.58−1.82 (m, 18H, $(CH_2)_9$), 3.37 (t, $J = 6.5$ Hz, 1H, OH), 3.99 (m, 2H, CH_2OH), 4.09 (m, 2H, OCH_2CH_3).

Diethyl [10-[(Methylsulfonyl)oxy]decyl]phosphonate (14). To a cold solution (0 °C) of **13** (1 g, 3.4 mmol) and Et_3N (0.47 mL, 3.4mmol) in dry CH_2Cl_2 (20 mL) was added a solution of $MeSO_2Cl$ (0.26 mL, 3.4 mmol) dropwise, under nitrogen. The mixture was stirred at 0 °C for 2 h and then poured into a beaker containing CH_2Cl_2−ice water (1:1, 100 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 60 mL). The combined organic extracts were dried, filtered, and evaporated *in vacuo* to afford **14** as a clear oil (1.05 g, 83%): ^{1}H-NMR ($CDCl_3$) δ 1.29 (t, $J = 7.1$, 6H, OCH_2CH_3), 1.38−1.71 (m, 18H, $(CH_2)_9$), 2.99 (s, 3H, CH_3SO_2), 4.11 (m, 2H, OCH_2CH_3), 4.21 (t, $J = 6.6$, 2H, CH_2OMs).

Diethyl [7-(2-Amino-1,6-dihydro-6-chloro-9*H*-purin-9-yl)-1,1-difluoroheptyl]phosphonate (17). Compound **17** was prepared in 30% yield from **15** and **16** as described in the literature.[13]

[7-(2-Amino-1,6-dihydro-6-chloro-9*H*-purin-9-yl)-1,1-difluoroheptyl]phosphonic Acid (18). Compound **18** was prepared in 44% yield from **17** as described in the literature.[13]

Diethyl [7-(6-Amino-4,5-dihydro-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-1,1-difluoroheptyl]phosphonate (20). To a cold solution (0 °C) of 6-amino-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**19**,[17] 0.7 g, 4.3 mmol) in dry DMF (50 mL) was added NaH (60% in mineral oil, 0.187 g, 7.8 mmol), under nitrogen. After the mixture was stirred at room temperature for 30 min a solution of 7-iodo-1,1-difluoro-1-(diethylphosphono)heptane[13] (**16**; 1.48 g, 3.9 mmol) in dry DMF (10 mL) was added. The reaction mixture was stirred and heated at 90 °C for 18 h under nitrogen and evaporated under reduced pressure. The residue was purified by flash column chromatography (1:1 EtOAc−EtOH). Compound **20** was isolated as a yellow oil (0.85 g, 50%): ^{1}H-NMR (DMSO-d_6) δ 1.12−1.91 (m, 16H, OCH_2CH_3, $(CH_2)_5$), 3.78 (m, 4H, OCH_2CH_3), 3.96 (s, 3H, OCH_3), 4.12 (t, $J = 5.1$, 2H, CH_2N), 6.79 (br s, 2H, NH_2), 7.79 (s, 1H, H-3).

[7-(6-Amino-4,5-dihydro-4-oxo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-1,1-difluoroheptyl]phosphonic Acid (21). TMSBr (1 mL, 7.81 mmol) was added slowly to a solution of **20** (0.82 g, 1.95 mmol) in dry CH_2Cl_2 (11 mL) at 0 °C under nitrogen. The reaction mixture was stirred at room temperature for 2 days and evaporated *in vacuo* to dryness. The residue was dissolved in dry MeCN and then treated with H_2O (0.3 mL). The milkish solution was diluted with MeOH (5 mL), and the solids that precipitated were collected by filtration, suspended in 1 N HCl (7 mL), and heated at 95 °C for 18 h. The solvent was evaporated *in vacuo*, and the resulting residue

was purified by ion exchange column chromatography to afford compound **21** as a white solid (0.35 g, 44%): ^{1}H-NMR (D_2O) δ 1.15−1.89 (m, 10H, $(CH_2)_5$), 3.99 (t, J = 5.1, 2H, CH_2N), 7.69 (s, 1H, H-3). Anal. ($C_{12}H_{18}N_5O_4PF_2 \cdot 0.1\ NH_4OH \cdot 1.0NH_4HCO_3$) C, H, N.

Diethyl [7-(2-Amino-1,6-dihydro-6-(2-methoxyethoxy)-9*H*-purin-9-yl)heptyl]phosphonate (23a) and 23b. Anhydrous cesium carbonate (2.8 g, 8.6 mmol) was added to a stirred suspension of 2-amino-6-(2-methoxyethoxy)-9*H*-purine (**22**,[18] 1.0 g, 4.78 mmol) and **4** (**d**,**e**) (5.26 mmol) in dry DMF (40 mL) at room temperature under nitrogen. The reaction mixture was stirred and heated at 80 °C for 18 h. The solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography (5:2 Et_2O−EtOH), affording **23** (**a**,**b**) as a yellow oil.

23a: 50%; ^{1}H-NMR (DMSO-d_6) δ 1.15−2.06 (m, 18H, $(CH_2)_6$ and OCH_2CH_3), 3.33 (s, 3H, OCH_3), 3.71 (t, J = 4.6, 2H, OCH_2CH_2O), 3.93−4.04 (m, 4H, OCH_2CH_3), 4.38 (t, J = 5.1, 2H, CH_2N), 4.53 (t, J = 4.5, 2H, OCH_2CH_2O), 6.41 (br s, 2H, NH_2), 7.88 (s, 1H, H-8).

23b: 47%.

[7-(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)heptyl]phosphonic Acid (24a) and 24b. A solution of **23** (**a**,**b**) (2.6 mmol) in HCl(c) (25 mL) was stirred and heated at 80 °C for 18 h. The solvent was evaporated *in vacuo*, and the resulting residue was purified by ion exchange column chromatography to afford compound **24** (**a**,**b**) as a white solid.

24a: 44%; ^{1}H-NMR (DMSO-d_6) δ 1.11−1.85 (m, 12H, $(CH_2)_6$), 3.92 (t, J = 7, 2H, CH_2N), 6.47 (br s, 2H, NH_2), 7.69 (s, 1H, H-8), 10.55 (br s, 1H, NH); UV λ_{max} nm ($\epsilon \times 10^3$) pH 1 = 253 (10.88), 278 (7.3), pH 7 = 253 (11.2), 270 (8.6), pH 13 = 255 (9.2), 268 (9.9). Anal. ($C_{12}H_{20}N_5O_4P \cdot 0.3H_2O \cdot 0.5NH_3$) C, H, N.

24b: 58%. Anal. ($C_{13}H_{22}N_5O_4P \cdot 0.7H_2O$) C, H, N.

Diethyl [7-(6-Amino-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)heptyl]phosphonate (25a) and 25b. To a cold solution (0 °C) of 6-amino-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**19**,[17] 0.8 g, 4.88 mmol) in dry DMF (50 mL), was added NaH (80% in mineral oil, 0.14 g, 5.8 mmol) at room temperature, under nitrogen. After stirring the mixture for 30 min, a solution of **4** (**d** or **e**) (5.36 mmol) in dry DMF (10 mL) was added. The reaction mixture was heated at 80 °C for 18 h, under nitrogen. The solvent was evaporated under reduced pressure and the residue purified by flash column chromatography (2:1 EtOAc−EtOH) to afford **25** (**a** or **b**) as a yellow oil.

25a: 50%; ^{1}H-NMR (DMSO-d_6) δ 1.15−1.98 (m, 18H, $(CH_2)_6$, and OCH_2CH_3), 3.93−4.04 (m, 9H, OCH_2CH_3, CH_2N, OCH_3), 6.76 (br s, 2H, NH_2), 7.81 (s, 1H, H-3).

25b: 60%.

[7-(6-Amino-4,5-dihydro-4-oxo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)heptyl]phosphonic Acid (26a) and 26b. A solution of **25** (**a**,**b**) (1.42 mmol) in dry DMF (25 mL) was treated with TMSBr (1.88 mL, 14.2 mmol) as described for **21**. Purification by ion exchange column chromatography afforded compound **26** (**a**,**b**) as a white solid.

26a: 54%; ^{1}H-NMR (D_2O) δ 1.11−1.85 (m, 12H, $(CH_2)_6$), 4.09 (t, J = 7, 2H, CH_2N), 7.88 (s, 1H, H-3); ^{31}P-NMR (D_2O) δ 28.09; UV λ_{max} nm ($\epsilon \times 10^3$) pH 1 = 252 (12.5), pH 7 = 254 (12.9), pH 13 = 256 (10.8). Anal. ($C_{12}H_{20}N_5O_4P \cdot 0.7H_2O \cdot 0.2NH_3 \cdot 0.2NH_4HCO_3$) C, H, N.

26b: 52%. Anal. ($C_{13}H_{22}N_5O_4P \cdot 0.5H_2O \cdot 0.5NH_3$) C, H, N.

Diethyl [10-(2-Amino-1,6-dihydro-6-chloro-9*H*-purin-9-yl)decyl]phosphonate (27). To a cold solution (0 °C) of **15** (1.75 g, 10.5 mmol) in dry DMF (12 mL) was added NaH (60% in mineral oil, 0.31 g, 7.6 mmol) at room temperature, under nitrogen. After stirring for 30 min, a solution of **14** (2.0 g, 5.2 mmol) in dry DMF (8 mL) was added. The reaction mixture was stirred at room temperature for additional 18 h, under nitrogen. The solvent was evaporated under reduced pressure and the residue purified by flash column chromatography (95:5 EtOAc−EtOH) to afford **27** as a yellow oil (1.2 g, 52%): ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, J = 7.0, 6H, OCH_2CH_3), 1.41−1.91 (m, 18H, $(CH_2)_9$), 3.98−4.15 (m, 6H, CH_2N and OCH_2CH_3), 7.74 (s, 1H, H-8).

[10-(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)decyl]phosphonic Acid (28). Compound **27** (6.33 g, 14.2 mmol) was treated with TMSBr (18.8 mL, 142 mmoL) in dry DMF (250 mL) as described for **21**. Evaporation of the solvent gave a brown oil (1.2 g), which was used in the next step without any further purification. A solution of this material in 1 N HCl (40 mL) was refluxed for 18 h. The solvent was evaporated *in vacuo*, and the resulting residue was purified by ion exchange column chromatography to afford compound **28** as a white solid (0.60 g, 13%): ^{1}H-NMR (D_2O) δ 1.02−1.63 (m, 18H, $(CH_2)_9$), 3.88 (s, 2H, CH_2N), 7.55 (s 1H, H-8); UV λ_{max} nm ($\epsilon \times 10^3$) pH 1 = 253 (11.7), pH 7 = 252 (11.9), pH 13 = 254 (9.6). Anal. ($C_{15}H_{36}N_5O_4P \cdot 0.5H_2O$) C, H, N.

Diethyl [4-((2-Acetamido-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy)butyl]phosphonate (30a) and 30b,c. A suspension of *p*-TsOH·H_2O (0.257 g, 10 mmol), diacetylguanine (**29**,[19] 1.6 g, 6.76 mmol), and **10** (**a**−**c**) (10 mmol) in anhydrous MeCN (65 mL) was refluxed for 4 days, under nitrogen. The mixture was cooled to room temperature and concentrated *in vacuo* and the residue purified by flash column chromatography (95:5 $CHCl_3$−MeOH) to afford compound **30** (**a**,**b**) as a beige solid or **30c** as a clear oil.

30a: 12%; ^{1}H-NMR (DMSO-d_6) δ 1.18 (t, J = 7.0, 6H, OCH_2CH_3), 1.21−1.71 (m, 6H, $(CH_2)_3$), 2.18 (s, 3H, CH_3CO), 3.42 (t, J = 4.6, 2H, NCH_2OCH_2), 3.98 (m, 4H, OCH_2CH_3), 5.43 (s, 2H, NCH_2O), 8.12 (s, 1H, H-8), 11.91 (br s, 1H, NH).

30b: 31%. **30c**: 14%.

Diethyl [4-((2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy)butyl]phosphonate (31a) and 31b,c. A mixture of **30** (**a**−**c**) (0.72 mmol) in methanolic ammonia (20 mL) was stoppered and allowed to stand at room temperature for 3 days. The solvent was evaporated *in vacuo*, and the white residue was purified by flash column chromatography (8:2 $CHCl_3$−MeOH) to afford **31** (**a**−**c**) as a white solid.

31a: 100%; ^{1}H-NMR (DMSO-d_6) δ 1.18 (t, J = 7.0, 6H, OCH_2CH_3), 1.21−1.71 (m, 6H, $(CH_2)_3$), 3.42 (t, J = 4.6, 2H, NCH_2OCH_2), 3.93 (m, 4H, OCH_2CH_3), 5.33 (s, 2H, NCH_2O), 6.52 (br s, 2H, NH_2), 7.85 (s, 1H, H-8), 10.61 (br s, 1H, NH).

31b: 90%. **31c**: 81%.

[4-((2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy)butyl]phosphonic Acid (32a) and 32b,c. A solution of **31** (**a**−**c**) (0.91 mmol) in a 1:1 mixture of CH_2Cl_2−HMDS (20 mL) was treated with TMSBr (1.2 mL, 9.11 mmol) at room temperature. The resulting mixture was stirred at room temperature for 5 h. The solvent was evaporated *in vacuo*, and the resulting residue was purified by ion exchange column chromatography to afford compound **32** (**a**−**c**) as a white solid.

32a: 55%; ^{1}H-NMR (D_2O) δ 0.93−1.06 (m, 6H, $(CH_2)_3$), 3.08 (m, 2H, NCH_2OCH_2), 4.97 (s, 2H, NCH_2O), 7.37 (s, 1H, H-8); UV λ_{max} nm ($\epsilon \times 10^3$) pH 1 = 255 (17.8), sh 278 (11.7), pH 7 = 252 (20.2), sh 273 (14.1), pH 13 = 261 (16.9). Anal. ($C_{10}H_{16}N_5O_5P \cdot 0.2H_2O \cdot 0.3NH_3 \cdot 4.5NH_4HCO_3$) C, H, N.

32b: 62%. Anal. ($C_{11}H_{18}N_5O_5P \cdot 0.4H_2O \cdot 0.5NH_3 \cdot 3.0NH_4HCO_3$) C, H, N.

32c: 60%. Anal. ($C_{12}H_{20}N_5O_5P \cdot 0.1H_2O \cdot 2.6NH_4HCO_3$) C, H, N.

Diethyl [5-((6-Acetamido-4,5-dihydro-4-oxo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)methoxy)pentyl]phosphonate (34a) and 34b. A suspension of *p*-TsOH·H_2O (257 mg, 10 mmol), **33**[20] (3.33 mmol), and **10** (**b**,**c**) (10 mmol) in dry MeCN (65 mL) was treated as described for **30** (**a**−**c**). After flash column chromatography (97:3 $CHCl_3$−MeOH) compound **34a** was isolated as a clear oil and compound **34b** as a white solid.

34a: 62%; ^{1}H-NMR (DMSO-d_6) δ 1.18 (t, J = 7.0, 6H, OCH_2CH_3), 1.21−1.71 (m, 8H, $(CH_2)_4$), 2.21 (s, 3H, CH_3CO), 3.42 (t, J = 4.6, 2H, NCH_2OCH_2), 3.99 (m, 4H, OCH_2CH_3), 5.52 (s, 1H, NCH_2O), 8.1 (s, 1H, H-3), 11.81 (br s, 1H, NH).

34b: 65%.

Diethyl [5-((6-Amino-4,5-dihydro-4-oxo-1*H*-pyrazolo-[3,4-*d*]pyrimidin-1-yl)methoxy)pentyl]phosphonate (35a) and 35b. A mixture of **34** (**a**,**b**) (0.72 mmol) in methanolic ammonia (20 mL) was treated as described for **31** (**a**−**c**) affording, after flash column chromatography (8:2 $CHCl_3$−MeOH), compound **35a** as a clear oil and **35b** as a white solid.

35a: 86%; ^{1}H-NMR (DMSO-d_6) δ 1.18 (t, J = 7.0, 6H, OCH_2CH_3), 1.21−1.71 (m, 8H, $(CH_2)_4$), 3.42 (t, J = 4.6, 2H,

NCH_2OCH_2), 3.89 (m, 4H, OCH_2CH_3), 5.37 (s, 1H, NCH_2O), 6.71 (br s, 2H, NH_2), 7.8 (s, 1H, H-3), 10.58 (br s, 1H, NH).

35b: 100%.

[5-((6-Amino-4,5-dihydro-4-oxo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)methoxy)pentyl]phosphonic Acid (36a) and 36b. A solution of **35** (**a**,**b**) (0.91 mmol) in a 1:1 mixture of CH_2Cl_2−HMDS (20 mL) was treated with TMSBr (1.2 mL, 9.11 mmol) at room temperature as described for **32** (**a**−**c**). After ion-exchange chromatography, compounds **36** (**a**,**b**) were isolated as white solids.

36a: 91%; ^{1}H-NMR (D_2O) δ 1.0−1.41 (m, 8H, $(CH_2)_4$), 3.39 (t, J = 4.6, 2H, NCH_2OCH_2), 5.35 (s, 2H, NCH_2O), 7.72 (s, 1H, H-3); UV λ_{max} nm ($\epsilon \times 10^3$) pH 1 = 252 (19.4), pH 7 = 252 (19.8), pH 13 = 264 (15.1). Anal. ($C_{11}H_{18}N_5O_5P\cdot 0.1H_2O\cdot 0.2NH_3\cdot 2.2NH_4HCO_3$) C, H, N.

36b: 88%. Anal. ($C_{12}H_{20}N_5O_5P\cdot 1.0H_2O\cdot 0.9NH_4HCO_3$) C, H, N.

Diethyl [4-(5-Amino-4-carbamoyl-1-*H*-1,2,3-triazol-1-yl)butyl]phosphonate (38a) and 38b,c. A solution of DMF (180 mL), KOH (5.04 g, 90 mmol) and water (45 mL) was stirred at 0 °C for 10 min. Cyanoacetamide (**37**, 7.56 g, 90 mmol) was added, and the mixture was stirred at 0 °C. After all the solid materials had dissolved (5 min) a solution of the side chain **5** (**a**−**c**) (45 mmol) in dry DMF (45 mL) was added in one portion and the reaction mixture stirred for 3 days at 0−5 °C. The resulting red solution was neutralized with 1 N HCl (90 mL) and evaporated at 60−65 °C *in vacuo*. The red syrup was partially dissolved in CH_2Cl_2 (300 mL). The solids that separated from the solution were filtered and washed with more CH_2Cl_2. The organic solution was evaporated to a red syrup and was purified by flash chromatography (9:1 $CHCl_3$−MeOH) to afford **38** (**a**−**c**) as a yellow solid. This material was used in the next step without any further purification. An analytical sample was prepared by crystallization from hexane−EtOAc to afford pure **38** (**a**−**c**) as a white solid.

38a: 55%; mp 100−102 °C; ^{1}H-NMR (DMSO-d_6) δ 1.19 (t, J = 7.0, 6H, OCH_2CH_3), 1.38−1.82 (m, 6H, $(CH_2)_3$), 3.93 (m, 4H, OCH_2CH_3), 4.11 (t, J = 6.8, 2H, NCH_2), 6.27 (s, 2H, NH_2), 7.03 (br s, 1H, $CONH_2$), 7.39 (br s, 1H, $CONH_2$); CIMS m/z 320 (M + 1)$^+$. Anal. ($C_{11}H_{22}N_5O_4P$) C, H, N.

38b: 69%; mp 127−129 °C; CIMS m/z 334 (M + 1)$^+$. Anal. ($C_{12}H_{24}N_5O_4P$) C, H, N.

38c: 52%; mp 101−103 °C; CIMS m/z 348 (M + 1)$^+$. Anal. ($C_{13}H_{26}N_5O_4P$) C, H, N.

Diethyl [4-((5-Amino-4-carbamoyl-1-*H*-1,2,3-triazol-1-yl)methoxy)butyl]phosphonate (43a) and 43b,c. Compounds **43** (**a**−**c**) were prepared from **8** (**a**−**c**) following the same procedure for the synthesis of **38** (**a**−**c**).

43a: 53%; mp 98−100 °C; ^{1}H-NMR (DMSO-d_6) δ 1.19 (t, J = 7.1, 6H, OCH_2CH_3), 1.40−1.70 (m, 6H, $(CH_2)_3$), 3.45 (t, J = 5.7, 2H, NCH_2OCH_2), 3.92 (m, 4H, OCH_2CH_3), 5.51 (s, 2H, NCH_2O), 6.45 (s, 2H, NH_2), 7.07 (br s, 1H, $CONH_2$), 7.42 (br s, 1H, $CONH_2$); CIMS m/z 372 (M + 23)$^+$. Anal. ($C_{12}H_{24}N_5O_5P$) C, H, N.

43b: 60%; mp 88−90 °C; CIMS m/z 364 (M + 1)$^+$. Anal. ($C_{13}H_{26}N_5O_5P$) C, H, N.

43c: 50%; mp 101−103 °C; CIMS m/z 378 (M + 1)$^+$. Anal. ($C_{14}H_{28}N_5O_5P$) C, H, N.

Diethyl [4-(5-((*N*′-Benzoylthiocarbamoyl)amino)-4-carbamoyl-1-*H*-1,2,3-triazol-1-yl)butyl]phosphonate (39a) and 39b,c. To a stirred suspension of **38** (**a**−**c**) (20 mmol) in dry acetone (40 mL) was slowly added benzoyl isothiocyanate (3.92 g, 3.20 mL, 24 mmol), and the reaction mixture was refluxed for 16 h. The bright orange solution was evaporated under vacuum to afford a yellow syrup. Purification by flash column chromatography (9:1 $CHCl_3$−MeOH) yielded compounds **39** (**a**−**c**) as analytically pure yellow foams.

39a: 92%; ^{1}H-NMR (DMSO-d_6) δ 1.21 (t, J = 7.0, 6H, OCH_2CH_3), 1.40−2.00 (m, 6H, $(CH_2)_3$), 3.96 (m, 4H, OCH_2CH_3), 4.26 (t, J = 6.8, 2H, NCH_2), 7.46 (br s, 1H, $CONH_2$), 7.84 (br s, 1H, $CONH_2$), 7.55−8.06 (m, 5H, ArH), 12.08 (br s, 2H, PhCONHCSNH); CIMS m/z 483 (M + 1)$^+$. Anal. ($C_{19}H_{27}N_6O_5PS$) C, H, N, S.

39b: 90%; CIMS m/z 497 (M + 1)$^+$. Anal. ($C_{20}H_{29}N_6O_5PS$) C, H, N, S.

39c: 99%; CIMS m/z 511 (M + 1)$^+$. Anal. ($C_{21}H_{31}N_6O_5PS$) C, H, N, S.

Diethyl [4-((5-((*N*′-Benzoylthiocarbamoyl)amino)-4-carbamoyl-1-*H*-1,2,3-triazol-1-yl)methoxy)butyl]phosphonate (44a) and 44b,c. Compounds **44** (**a**−**c**) were prepared from **43** (**a**−**c**) following the same procedure for the synthesis of **39** (**a**−**c**).

44a: 97%; ^{1}H-NMR (DMSO-d_6) δ 1.20 (t, J = 7.1, 6H, OCH_2CH_3), 1.40−1.80 (m, 6H, $(CH_2)_3$), 3.54 (t, J = 5.5, NCH_2OCH_2), 3.95 (m, 4H, OCH_2CH_3), 5.67 (s, 2H, NCH_2O), 7.54 (br s, 1H, $CONH_2$), 7.91 (br s, 1H, $CONH_2$), 7.58−8.06 (m, 5H, ArH), 12.20 (br s, 2H, PhCONHCSNH); CIMS m/z 535 (M + 23)$^+$. Anal. ($C_{20}H_{29}N_6O_6PS$) C, H, N, S.

44b: 89%; CIMS m/z 549 (M + 23)$^+$. Anal. ($C_{21}H_{31}N_6O_6PS$) C, H, N, S.

44c: 96%; CIMS m/z 535 (M + 23)$^+$. Anal. ($C_{22}H_{33}N_6O_6PS$) C, H, N, S.

Diethyl [4-(5-((*N*′-benzoyl-*S*-methylisothiocarbamoyl)amino)-4-carbamoyl-1*H*-1,2,3-triazol-1-yl)butyl]phosphonate (40a) and 40b,c. To a well stirred solution of **39** (**a**−**c**) (8 mmol) in MeOH (40 mL) containing 0.1 N NaOH (100 mL, 10 mmol) was added MeI (18.2 g, 8 mL, 128 mmol). The reaction mixture was stirred at room temperature for 2 h and then neutralized with AcOH (a turbidity appeared). The residue was partitioned between H_2O (200 mL) and CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried, filtered, evaporated to a syrup, and purified by flash column chromatography (95:5 $CHCl_3$−MeOH). Compounds **40** (**a**−**c**) were obtained as white foams and were analytically pure.

40a: 87%; ^{1}H-NMR (DMSO-d_6) δ 1.16 (t, J = 6.8, 6H, OCH_2CH_3), 1.40−1.90 (m, 6H, $(CH_2)_3$), 2.45 (s, 3H, SCH_3), 3.92 (m, 4H, OCH_2CH_3), 4.17 (t, J = 6.9, 2H, NCH_2), 7.42 (br s, 1H, $CONH_2$), 7.79 (br s, 1H, $CONH_2$), 7.40−7.70 (m, 5H, ArH), 11.29 (br s, 1H, PhCON=C(SCH_3)NH); CIMS m/z 497 (M + 1)$^+$. Anal. ($C_{20}H_{29}N_6O_5PS$) C, H, N, S.

40b: 91%; CIMS m/z 511 (M + 1)$^+$. Anal. ($C_{21}H_{31}N_6O_5PS$) C, H, N, S.

40c: 78%; CIMS m/z 525 (M + 1)$^+$. Anal. ($C_{22}H_{33}N_6O_5PS$) C, H, N, S.

Diethyl [4-((5-((*N*′-benzoyl-*S*-methylisothiocarbamoyl)amino)-4-carbamoyl-1*H*-1,2,3-triazol-1-yl)methoxy)butyl]phosphonate (45a) and 45b,c. Compounds **45** (**a**−**c**) were prepared from **44** (**a**−**c**) following the same procedure for the synthesis of **40** (**a**−**c**).

45a: 80%; ^{1}H-NMR (DMSO-d_6) δ 1.17 (t, J = 7.1, 6H, OCH_2CH_3), 1.40−1.70 (m, 6H, $(CH_2)_3$), 2.46 (s, 3H, SCH_3), 3.54 (t, J = 5.80, 2H, NCH_2OCH_2), 3.93 (m, 4H, OCH_2CH_3), 5.51 (s, 2H, NCH_2O), 7.42 (br s, 1H, $CONH_2$), 7.85 (br s, 1H, $CONH_2$), 7.40−7.70 (m, 5H, ArH), 11.29 (br s, 1H, PhCON=C(SCH_3)NH); CIMS m/z 527 (M + 1)$^+$. Anal. ($C_{21}H_{31}N_6O_6PS$) C, H, N, S.

45b: 88%; CIMS m/z 541 (M + 1)$^+$. Anal. ($C_{22}H_{33}N_6O_6PS$) C, H, N, S.

45c: 87%; CIMS m/z 555 (M + 1)$^+$. Anal. ($C_{23}H_{35}N_6O_6PS$) C, H, N, S.

Ethyl [4-(5-Amino-6,7-dihydro-7-oxo-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)butyl]phosphonate (41a) and 41b,c. A suspension of **40** (**a**−**c**) (4.3 mmol) in MeOH (4.3 mL) and 2.5 N NaOH (43 mL) was refluxed for 2 h. After cooling to room temperature it was neutralized with Dowex 50W (H^+). The resin was filtered, washed with water, and extracted with CH_2Cl_2 (5 × 100 mL). The organic phase was discarded, and the aqueous solution containing the final product was evaporated *in vacuo* at 65 °C. Compounds **41** (**a**−**c**) were obtained as a white solids. This material was used in the next step without any further purification.

41a: 53%; ^{1}H-NMR (DMSO-d_6) δ 1.07 (t, J = 7.1, 3H, OCH_2CH_3), 1.30−1.80 (m, 6H, $(CH_2)_3$), 3.70 (m, 2H, OCH_2CH_3), 4.20 (t, J = 7.1, NCH_2), 7.27 (br s, 2H, NH_2), 11.80 (br s, 1H, NH); CIMS m/z 317 (M + 1)$^+$.

41b: 77%; CIMS m/z 331 (M + 1)$^+$.

41c: 88%; CIMS m/z 345 (M + 1)$^+$.

Ethyl [4-((5-Amino-6,7-dihydro-7-oxo-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)methoxy)butyl]phosphonate (46a) and 46b,c. Compounds **46** (**a**−**c**) were prepared from **45** (**a**−**c**) following the same procedure for the synthesis of **41** (**a**−**c**).

46a: 99%; ^{1}H-NMR (DMSO-d_6) δ 1.07 (t, J = 7.1, 3H, OCH_2CH_3), 1.20−1.60 (m, 6H, $(CH_2)_3$), 3.44 (t, J = 6.1, 2H, NCH_2OCH_2), 3.66 (m, 2H, OCH_2CH_3), 5.47 (s, 2H, NCH_2O), 7.80 (br s, 2H, NH_2), 12.95 (br s, 1H, NH); CIMS m/z 369 $(M + 23)^+$.

46b: 83%; CIMS m/z 361 $(M + 1)^+$.

46c: 79%; CIMS m/z 375 $(M + 1)^+$.

[4-(5-Amino-6,7-dihydro-7-oxo-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidin-3-yl)butyl]phosphonic Acid Ammonium Salt (42a) and 42b,c. To a suspension of **41** (**a**−**c**) (1.5 mmol) and HMDS (15 mL) in dry CH_2Cl_2 (15 mL) was added TMSBr (2.29 g, 1.98 mL, 15 mmol). The mixture was stirred overnight at room temperature. After cooling to 0−5 °C, MeOH was slowly added. The solvent was evaporated *in vacuo*, and the resulting residue was purified by ion exchange column chromatography to afford compounds **42** (**a**−**c**) as analytically pure white solids.

42a: 81%; ^{1}H-NMR (D_2O) δ 1.30−1.50 (m, 4H, $(CH_2)_2$), 1.83 (m, 2H, PCH_2), 4.21 (t, J = 7.1, 2H, NCH_2); ^{31}P-NMR (D_2O) δ 25.67; UV λ_{max} nm ($\epsilon \times 10^3$) (pH 1) 252 (10.5), (pH 7) 252 (9.9), (pH 13) 277 (9.4); CIMS m/z 289 ($M - NH_3 + 1$). Anal. ($C_8H_{16}N_7O_4P$) C, H, N.

42b: 60%; CIMS m/z 303 $(M - NH_3 + 1)^+$. Anal. ($C_9H_{18}N_7O_4P$) C, H, N.

42c: 66%; CIMS m/z 317 $(M - NH_3 + 1)^+$. Anal. ($C_{10}H_{20}N_7O_4P$) C, H, N.

[4-((5-Amino-6,7-dihydro-7-oxo-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidin-3-yl)methoxy)butyl]phosphonic Acid Ammonium Salt (47a) and 47b,c. Compounds **47** (**a**−**c**) were prepared from **46** (**a**−**c**) following the same procedure for the synthesis of **42** (**a**−**c**).

47a: 73%; ^{1}H-NMR (D_2O) δ 1.20−1.50 (m, 6H, $(CH_2)_3$), 3.36 (t, J = 6.2, 2H, NCH_2OCH_2), 5.49 (s, 2H, NCH_2O); ^{31}P-NMR (D_2O) δ 27.21; UV λ_{max} nm ($\epsilon \times 10^3$) (pH 1) 254 (7.5), (pH 7) 254 (7.5), (pH 13) 278 (6.6); CIMS m/z 321 ($M - NH_3 + 1$). Anal. ($C_9H_{18}N_7O_5P$) C, H, N.

47b: 57%; CIMS m/z 333 $(M - NH_3 + 1)^+$. Anal. ($C_{10}H_{20}N_7O_5P$) C, H, N.

47c: 36%; CIMS m/z 347 $(M - NH_3 + 1)^+$. Anal. ($C_{11}H_{19}N_6O_5P \cdot 0.6NH_3$) C, H, N.

Diethyl [5-(5-Acetamido-6,7-dihydro-7-oxo-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-2-yl)pentyl]phosphonate (49). A mixture of **48** (0.422 g, 1.79 mmol), *p*-TsOH·H_2O (0.034 g, 0.18 mmol), and **10b** (2.12 g, 7.16 mmol) was heated at 140 °C under vacuum for 20 min. The oil was taken in methanol and purified by flash column chromatography (95:5 $CHCl_3$−MeOH) to give **49** as a clear oil (0.350 g, 45%): ^{1}H-NMR (DMSO-d_6) δ 1.21 (t, J = 7.0, OCH_2CH_3), 2.22 (s, 3H, CH_3CO), 1.24−1.90 (m, 8H, $(CH_2)_4$), 3.53 (m, 2H, NCH_2OCH_2), 3.95 (m, 4H, OCH_2-CH_3), 5.77 (s, 2H, NCH_2O).

[5-(5-Amino-6,7-dihydro-7-oxo-2*H*-1,2,3-triazolo[4,5-*d*]-pyrimidin-2-yl)pentyl]phosphonic Acid (51). Compound **49** (0.300 g, 0.69 mmol) was deacetylated as described for **31** (**a**−**c**) and purified by flash column chromatography (95:5 $CHCl_3$−MeOH) to give **50** as a white residue, which was immediately treated with TMSBr (0.442 mL, 3.3 mmol) in a 1:1 mixture of CH_2Cl_2−HMDS (16 mL). The mixture was stirred at room temperature for 5 h, worked up as described for **32** (**a**−**c**), and purified by ion exchange chromatography to give **51** as a white solid (0.08 g, 72%): ^{1}H-NMR (D_2O) δ 0.91−1.46 (m, 4H, $(CH_2)_2$), 3.33 (m, 2H, NCH_2OCH_2), 5.51 (s, 2H, NCH_2O); ^{31}P-NMR (DMSO-d_6) δ 23.68; UV λ_{max} nm ($\epsilon \times 10^3$) pH 1 = 271 (9.5), pH 7 = 239 (8.4), 294 (6.6), pH 13 = 256 (6.4), 299 (8.5). Anal. ($C_{10}H_{17}N_6O_5P \cdot 0.3H_2O \cdot 0.6NH_3 \cdot 0.5NH_4HCO_3$) C, H, N.

Enzyme Assays. PNPase was purified from human erythrocytes and assayed using a xanthine oxidase-coupled spectrophotometric assay as described previously.[8,12] In addition to enzyme the assay mixtures contained inhibitor, 0.1 mM inosine, 1.0 mM potassium phosphate buffer, 100 mM Tris-hydrochloride buffer, 0.1 mM ethylenediaminetetraacetic acid disodium salt, and 0.2 IU/mL of xanthine oxidase at pH 7.4. The apparent inhibition constant (K_i') of a compound was determined from its ability to inhibit the phosphorolysis of inosine at 1 mM phosphate and was calculated from fractional inhibition as described previously.[21]

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References

(1) Parks, R. E., Jr.; Stoeckler, J. D.; Cambor, C.; Savarese, T. M.; Crabtree, G. W.; Chu, S.-H. Purine Nucleoside Phosphorylase and 5′-Methylthioadenosine Phosphorylase: Targets of Chemotherapy. In *Molecular Actions and Targets for Cancer Chemotherapeutic Agents;* Sartorelli, A. C., Lazo, J. S., Bertino, J. R., Eds.; Academic Press: New York, 1981; pp 229−252.

(2) Sircar, J. C.; Gilbertsen, R. B. Purine Nucleoside Phosphorylase (PNP) Inhibitors: Potential Selective Immunosuppressive Agents. *Drugs Future* **1988**, *13*, 653-668.

(3) Shewach, D. S.; Chern, J.-W.; Pillote, K. E.; Townsend, L. B.; Daddona, P. E. Potentiation of 2′-Deoxyguanosine cytotoxicity by a novel inhibitor of purine nucleoside phosphorylase, 8-amino-9-benzylguanine. *Cancer Res.* **1986**, *46*, 519-523.

(4) Stein, J. M.; Stoeckler, J. D.; Li, S.-Y.; Tolman, R. L.; MacCoss, M.; Chen, A.; Karkas, J. D.; Ashton, W. T.; Parks, R. E., Jr. Inhibition of Human Purine Nucleoside Phosphorylase by Acyclic Nucleosides and Nucleotides. *Biochem. Pharmacol.* **1987**, *36*, 1237−1244.

(5) Sircar, J. C.; Kostlan, C. R.; Pinter, G. W.; Suto, M. J.; Bobovski, T. P.; Capiris, T.; Schwender, C. F.; Dong, M. K.; Scott, M. E.; Bennett, M. K.; Kossarek, L. M.; Gilbertsen, R. B. 8-Amino-9-substituted Guanines: Potent Purine Nucleoside Phosphorylase (PNP) Inhibitors. *Agents Actions* **1987**, *21*, 253−256.

(6) Halazy, S.; Eggenspiller, A.; Ehrhard, A.; Danzin, C. Phosphonate Derivatives of N^9-Benzylguanine: A New Class of Potent Purine Nucleoside Phosphorylase Inhibitors. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 407−410.

(7) Kelley, J. L.; Linn, J. A.; McLean, E. W.; Tuttle, J. V. 9-(Phosphono)alkyl)benzyl)guanines. Multisubstrate Analogue Inhibitors of Human Erythrocyte Purine Nucleoside Phosphorylase. *J. Med. Chem.* **1993**, *36*, 3455−3463.

(8) Tuttle, J. V.; Krenitsky, T. A. Effects of Acyclovir and its Metabolites on Purine Nucleoside Phosphorylase. *J. Biol. Chem.* **1984**, *259*, 4085−4069.

(9) Gilbertsen, R. B.; Sircar, J. C. Enzyme Cascades: Purine Metabolism and Immunosuppression. In *Comprehensive Medicinal Chemistry;* Sammes, P. G., Ed.; Pergamon Press: Oxford, 1990; Vol. 2, pp 443−480.

(10) Martin, D. W.; Gelfand, E. W. Biochemistry of Diseases of Immunodevelopment. *Annu. Rev. Biochem.* **1981**, *50*, 845−877.

(11) Gilbertsen, R.B.; Ealick, S. E.; Rule, S. A.; Carter, D. C.; Greenbough, T. J.; Babu, Y. S.; Cook, W. J.; Habash, J.; Halliwell, J. R.; Stoeckler, J. D.; Parks, R. E. Jr.; Chen, S.-F.; Bugg, C. E. Three Dimensional Structure of Human Eyrthrocytic Purine Nucleoside Phosphorylase at 3.2 Å Resolution. *J. Biol. Chem.* **1990**, *265*, 1812-820.

(12) Krenitsky, T. A.; Tuttle, J. V.; Miller, W. H.; Moorman, A. R.; Orr, G. F.; Beauchamp, L. Nucleotide Analogue Inhibitors of Purine Nucleoside Phosphorylase. *J. Biol. Chem.* **1990**, 265, 3066−3069.

(13) Halazy, S.; Ehrhard, A.; Danzin, C. 9-(Difluorophosphonoalkyl) Guanines as a New Class of Multisubstrate Analogue Inhibitos of Purine Nucleoside Phosphorylase. *J. Am. Chem. Soc.* **1991**, *11*, 315−317.

(14) Yamazaki, A.; Okutsu, M.; Yamada, Y. Synthesis of Guanosine and its Derivatives from 5-Amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide. IV. A new route to Guanosine via Cyanamide derivative. *Nucleic Acids Res.* **1976**, *3*, 251−259.

(15) Sznaidman, M.; Beauchamp, L. Unpublished results.

(16) Bzowska, A.; Kulikowska, E.; Shugar, D.; Chen, B. Y.; Lindborg, B.; Johansson, N. G. Acyclonucleoside Analogue Inhibitors of Mammalian Purine Nucleoside Phosphorylase. *Biochem. Pharmacol.* **1991**, *41*, 1791−803.

(17) Seela, F.; Steker, H. Synthesis of the β-D-Deoxyribofuranoside of 6-Amino-1*H*-Pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one. A new isoster of 2′-Deoxyguanosine. *Heterocycles* **1985**, *23*, 2521−2525.

(18) Kjellberg, J.; Liljenberg, M.; Johansson, N. G. Regioselective alkylation of 6-(β-Methoxy)Guanine to give the 9-Alkylguanine derivative. *Tetrahedron Lett.* **1986**, *27*, 877−880.

(19) Ishido, I.; Hosono, A.; Isome, S.; Maruyama, A.; Sato, T. Improved fusion method for the synthesis of Purine Nucleosides. Acid-catalyzed reactions of N^7- or N^9-acylpurines with acetylated sugars. *Bull. Chem. Soc. Jpn.* **1964**, *37*, 1389−1390.

(20) Beauchamp, L. M.; Dolmatch, B. L.; Schaeffer, H. J.; Collins, P.; Bauer, D. J.; Keller, P. M.; Fyfe, J. A. Modifications on the Heterocyclic Base Acyclovir: Synthesis and Antiviral Properties. *J. Med. Chem.* **1985**, *28*, 982−987.

(21) Kelley, J. L.; McLean, E. W.; Crouch, R. C.; Averett, D. R.; Tuttle, J. V. *J. Med. Chem.* **1995**, *38*, 1005−1014.

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