9-[(Phosphonoalkyl)benzyl]guanines. Multisubstrate Analogue Inhibitors of Human Erythrocyte Purine Nucleoside Phosphorylase

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A series of 9-[(phosphonoalkyl)benzyl]guanines was synthesized and tested for inhibition of human erythrocyte purine nucleoside phosphorylase (PNPase). Inhibitors of PNPase should be T-cell selective, immunosuppressive agents with potential clinical utility in the treatment of a wide variety of disorders in which T-lymphocytes are pathogenic. An initial set of six analogues of the weak PNPase inhibitor 9-benzylguanine (**2**) contained a phosphonic acid group linked to the *ortho*, *meta*, or *para* position of the aryl moiety via two- or three-atom spacers. These compounds allowed us to probe for a favorable interaction with the phosphate-binding domain. Several additional *meta* phosphonoalkyl substituents were examined in an effort to optimize the spacer. The two most potent compounds, [[3-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]benzyl]oxy]-methylphosphonic acid (**3f**) and [[3-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]benzyl]-thio]methylphosphonic acid (**3j**), were inhibitors of PNPase with K_i's of 5.8 and 1.1 nM, respectively. These inhibitors displayed competitive kinetics with respect to inosine and inorganic phosphate, which showed that these compounds possess binding determinants for both the purine- and phosphate-binding domains of the enzyme, characteristics that are consistent with **3f** and **3j** being multisubstrate analogue inhibitors of PNPase. The potency of 9-benzylguanine (**2**) was enhanced more than 6000-fold by linking a phosphonic acid residue with a (methylthio)methyl spacer to the *meta* position of **2** to give **3j**, which illustrates the potent enzyme inhibitory properties available to multisubstrate analogue inhibitors.

Introduction

Purine nucleoside phosphorylase (purine nucleoside: orthophosphate ribosyltransferase, EC 2.4.2.1, PNPase) catalyzes the reversible phosphorolysis of purine nucleosides such as inosine, 2′-deoxyinosine, guanosine,and 2′-deoxyguanosine to the purine and ribose- or 2-deoxyribose-α-1-phosphate.[1,2] Individuals who are genetically deficient in PNPase suffer from impairment of the T-cell component of their immune system but have normal B-cell function.[1] These observations led to the proposal that an inhibitor of PNPase should be a T-cell selective, immunosuppressive agent with potential clinical utility in the treatment of a wide variety of disorders in which T-lymphocytes are pathogenic.[3-6] Consequently, extensive drug-discovery research has been devoted to the design and synthesis of inhibitors of PNPase.

Compounds of diverse structure have been synthesized and tested as candidate inhibitors of PNPase.[5,6] Examples of potent, nonionic inhibitors include 8-aminoguanine,[7] 8-amino-9-benzylguanine,[8] 8-amino-9-(2-thienylmethyl)-guanine,[9] and 9-substituted 9-deazaguanines.[10,11] Several phosphonic acids have been reported to be inhibitors of PNPase. These include 9-(phosphonoalkyl) derivatives of hypoxanthine[12] and guanine,[13] and 9-[(difluorophospho-no)alkyl]guanines.[14] The diphosphates of acyclovir (**1**)[15,16] and ganciclovir[17] are potent inhibitors of PNPase with K_i's of 8.7 and 9.0 nM, respectively. However, nucleoside diphosphates generally exhibit poor cellular permeability and have short plasma half-lives, so there was little expectation that the diphosphate **1** would be active *in vivo*.[16]

Ealick et al. recently reported on the application of crystallographic and modeling methods to the design of some potent inhibitors of PNPase with IC_{50}'s in the 6–30 nM range.[10] These compounds were designed in an iterative process using X-ray data on the native enzyme and on enzyme-inhibitor complexes, computer modeling of the latter data, and organic synthesis. To take full advantage of the binding properties of all three subsites of the enzyme active-site, they designed some 9-benzylguanines that contain a substituent with affinity for the phosphate-binding site, linked to the benzyl moiety by a spacer of appropriate length. Ealick et al. reported two *ortho*-substituted alkylphosphonic acids, 9-[2-(2-phosphonoethyl)benzyl]guanine and 9-[2-(3-phosphono-propoxy)benzyl]guanine, with IC_{50}'s of 1000 and 35 nM, respectively. Their most active inhibitor (IC_{50} of 5.9 nM) contained a carboxylic acid moiety linked to the benzylic methylene, which allowed for a favorable interaction with the phosphate-binding site. The compounds in this series are claimed to be the most potent membrane-permeable inhibitors of PNPase yet reported.[10]

These laboratories have had a longstanding interest in inhibition of PNPase,[15,16,18] but until recently we had only a preliminary understanding of the structure of the enzyme active site.[5,19] Ealick's X-ray crystallography studies revealed that the phosphate-binding domain is located adjacent to the 1′ carbon of the ribose moiety and that the 9-benzyl moiety of 8-amino-9-benzylguanine, a PNPase inhibitor (K_i = 200 nM),[8] binds to the residues that form the hydrophobic side of the ribose-binding domain.[19,20] From analysis of these earlier data and prior to Ealick's recent report,[10] we reasoned that since the phosphate-binding domain is near the aryl moiety, analogues of 9-benzylguanine containing an appropriately spaced aryl substituent with affinity for the phosphate-binding site

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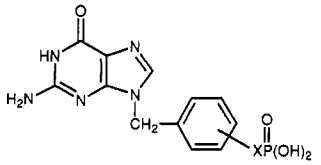
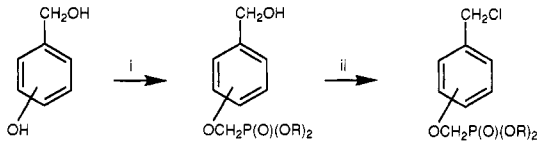
Table I. Inhibition of Human Erythrocyte Purine Nucleoside Phosphorylase by 9-[(Phosphonoalkyl)benzyl]guanines[a]



no.	X	$K_i' \pm SE$ (n), nM[b]
2[c]	H	7000 ± 900 (2)
3a	4-OCH_2	270000 ± 50000 (3)
3b	4-CH_2OCH_2	3500 ± 200 (1)
3c	2-OCH_2	8.3 ± 0.3 (1)
3d	2-CH_2OCH_2	150 ± 6 (1)
3e	3-OCH_2	600 ± 20 (1)
3f	3-CH_2OCH_2	5.8 ± 0.4 (5)
3g	3-$CH_2OCH_2CH_2$	69 ± 3 (1)
3h	3-CH=$CHCH_2$	60 ± 5 (1)
3i	3-$CH_2CH_2CH_2$	13 ± 2 (2)
3j	3-CH_2SCH_2	1.1 ± 0.1 (4)
1	acyclovir diphosphate	5.2 ± 0.4 (2)

[a] The enzyme was purified from human erythrocytes and assayed spectrophotometrically via a xanthine oxidase-coupled assay as described in the Enzyme Assays section. [b] Apparent inhibition constants (K_i') of these compounds were determined using inosine as the variable substrate as previously described.[16] The kinetic data for all inhibitors were consistent with competitive inhibition. When the number of determinations (n) was two or more, the mean K_i' value and the standard error of the mean (SE) are tabulated. When one determination was made the standard error of the fit of the data points to the competitive model is listed. [c] 9-Benzylguanine[33] was purchased from Vega Biochemicals.

Scheme I[a]



4a ; para
4c ; ortho
4e ; meta

5a ; para [b]
5c ; ortho [c]
5e ; meta [b]

6a ; para [b]
6c ; ortho [c]
6e ; meta [b]

[a] (i) $R'S(O)_2OCH_2P(O)(OR)_2$, NaH or Cs_2CO_3, DMF; (ii) $SOCl_2$, CH_2Cl_2. [b] R = Et. [c] R = *i*-Pr.

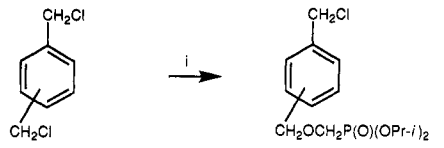
should be multisubstrate analogue inhibitors[21,22] with potent inhibition properties. To investigate this hypothesis, we initially prepared six analogues of 9-benzylguanine in which a phosphonic acid group was linked to the *ortho*, *meta*, or *para* position of the aryl moiety via two- or three-atom spacers. This set of compounds allowed us to probe for a favorable interaction with the phosphate-binding domain.[23]

Chemistry

The phosphonic acids **3a–h** (Table I) were prepared by alkylation of 2-amino-6-chloro-9*H*-purine with an appropriately functionalized benzyl chloride followed by phosphonate ester deprotection and hydrolysis of the 6-chloropurine (Schemes I-V). The intermediate [[(chloromethyl)phenoxy]methyl]phosphonates (Scheme I) were prepared by alkylation of the monosodium salt of the appropriate hydroxybenzyl alcohol **4** with a phosphonomethyl sulfonate to give compounds **5**, which were chlorinated with thionyl chloride in dichloromethane to provide **6a**, **6c**, and **6e**.

The requisite [[[(chloromethyl)benzyl]oxy]methyl]-phosphonates **8** (Scheme II) were prepared from the appropriate α,α'-dichloroxylenes **7** and diisopropyl (hydroxymethyl)phosphonate (**23**).[24–26] The anion of **23** was

Scheme II[a]

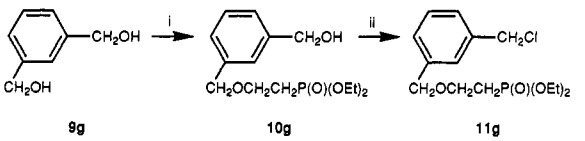


7b ; para
7d ; ortho
7f ; meta

8b ; para
8d ; ortho
8f ; meta

[a] (i) $HOCH_2P(O)(OPr\text{-}i)_2$ (**23**), NaH, DMF.

Scheme III[a]



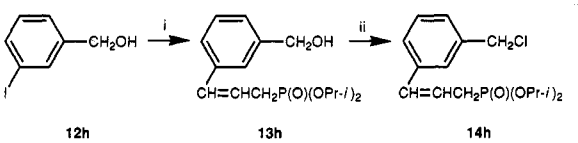
9g

10g

11g

[a] (i) CH_2=$CHP(O)(OEt)_2$, K_2CO_3; (ii) $SOCl_2$, CH_2Cl_2.

Scheme IV[a]



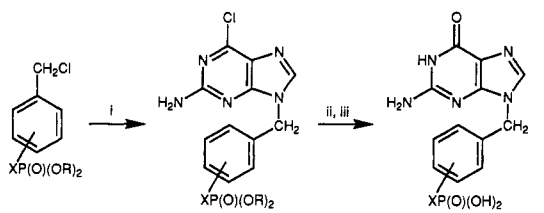
12h

13h

14h

[a] (i) CH_2=$CHCH_2P(O)(OPr\text{-}i)_2$ (**24**), $Pd(OAc)_2$, P(o-tolyl)$_3$, Et_3N, CH_3CN; (ii) $SOCl_2$, CH_2Cl_2.

Scheme V[a]



6, 8, 11, 14

15a - 15h

3a - 3h

[a] (i) 2-NH_2-6-Cl-purine, Cs_2CO_3, DMF; (ii) TMS-bromide, DMF; (iii) 1N HCl, reflux, ion exchange.

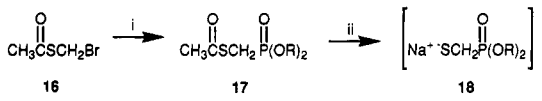
formed with sodium hydride in dimethylformamide and then alkylated with **7** to provide **8b**, **8d**, and **8f**.

The intermediate [2-(benzyloxy)ethyl]phosphonate **11g** (Scheme III) was prepared from 1,3-bis(hydroxymethyl)-benzene (**9g**). Diethyl vinylphosphonate was reacted with **9g** and potassium carbonate to give alcohol **10g**, which was chlorinated with thionyl chloride to provide **11g**.

The intermediate benzyl chloride **14h** was constructed in two steps from iodobenzyl alcohol **12h** and 2-propenylphosphonate **24**[27] (Scheme IV). The propene **24** and alcohol **12h** were coupled under Heck[28,29] conditions to give (3-phenyl-2-propenyl)phosphonate **13h**, which was chlorinated with thionyl chloride to give **14h**. The benzyl chlorides **6**, **8**, **11**, and **14** were utilized to alkylate 2-amino-6-chloro-9*H*-purine in dimethylformamide with cesium carbonate[30] to provide purines **15** as oils after column chromatography to separate the 7-isomers.

The dialkyl phosphonate moiety of **15** was deprotected with trimethylsilyl bromide in dimethylformamide, and the volatiles were removed in vacuo to give the intermediate silyl-protected phosphonic acids as foams. The latter were refluxed with 1 N hydrochloric acid to hydrolyze the 6-chloro group and silylphosphonate esters, and the crude products were purified by ion-exchange chromatography

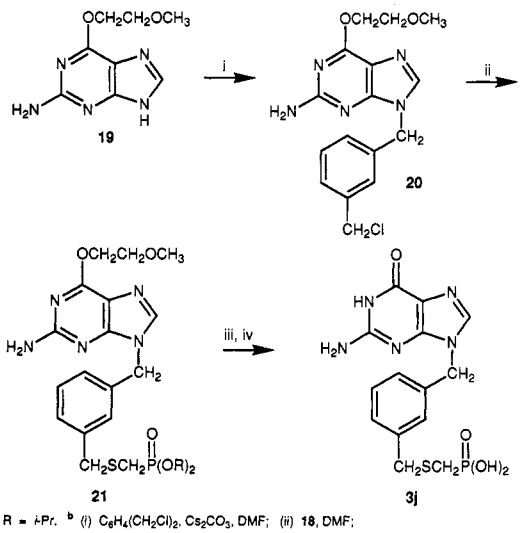
Scheme VI[a,b]



16 17 18

[a] R = *i*-Pr. [b] (i) $P(OR)_3$, 130 °C; (ii) NaOEt, 2-PrOH.

Scheme VII[a,b]



19 20 21 3j

[a] R = *i*-Pr. [b] (i) $C_6H_4(CH_2Cl)_2$, Cs_2CO_3, DMF; (ii) **18**, DMF; (iii) TMS-bromide, DMF, -75 to 25 °C; (iv) 1 N HCl, reflux, ion exchange.

(DEAE-Sephadex/ammonium bicarbonate gradient) to give the phosphonic acids **3a–h** as the ammonium salts.

The (phenylpropyl)phosphonic acid **3i** was prepared from **3h** by catalytic hydrogenation in ethanol with platinum oxide.

[(Benzylthio)methyl]phosphonic acid **3j** was prepared via a six-step convergent route from 2-amino-6-(2-methoxyethoxy)-9*H*-purine (**19**)[31] (Schemes VI and VII). The latter was alkylated with α,α'-dichloro-*m*-xylene in dimethylformamide to give **20** in 23% yield after column chromatography. The [(acetylthio)methyl]phosphonate **17** was prepared from bromomethyl sulfide **16**[32] and triisopropyl phosphite in 58% yield. The thiolate **18** was generated from **17** with sodium ethoxide in 2-propanol and reacted with **20** in dimethylformamide to give **21** in 21% yield after column chromatography. Compound **21** was deprotected and the product purified in an analogous manner to that of **3a–h** to give **3j** in 49% yield as the ammonium salt.

Biological Results and Discussion

The compounds in Table I were tested for inhibition of human erythrocyte purine nucleoside phosphorylase (PNPase) via a xanthine oxidase-coupled assay as previously described.[15,16] The apparent inhibition constants (K_i') were determined at 1 mM phosphate, which is the approximate intracellular concentration, because inhibition of PNPase by phosphates and phosphonic acids is inversely proportional to the concentration of inorganic phosphate.[12,15,17] All of the compounds in Table I displayed competitive kinetics with respect to inosine.

9-Benzyl- and 9-(heteroarylmethyl)guanines have been reported to be relatively potent inhibitors of PNPase.[8,9] The parent compound, 9-benzylguanine (**2**)[33] is a weak inhibitor with a K_i' of 7 μM (Table I). Substitution on the *para* position of **2** with the phosphonomethoxy and (phosphonomethoxy)methyl moieties to give **3a** and **3b** resulted in a decrease or no significant enhancement in activity. However, placement of the phosphonomethoxy and (phosphonomethoxy)methyl moieties in the *ortho* position to give **3c** and **3d** resulted in 800- and 40-fold increases in inhibitory activity with K_i's of 8.3 and 150 nM, respectively. Substitution on the *meta* position of **2** also yielded a potent inhibitor of PNPase. [[(Phosphonomethoxy)methyl]benzyl]guanine **3f** had a K_i' of 5.8 nM, whereas **3e**, with the shorter methoxy spacer, was 100-fold weaker with a K_i' of 600 nM. Thus, potent inhibition of PNPase is observed in phosphonomethoxy derivatives of 9-benzylguanine when the phosphonic acid is linked via a two-atom spacer to the *ortho* position (**3c**) or a three-atom spacer to the *meta* position (**3f**).

A few additional *meta* phosphonoalkyl substituents were examined in an effort to optimize the spacer. The four-atom spacer of (phosphonoethoxy)methyl **3g** is 12-fold less active than **3f** with a K_i' of 69 nM. The propyl analogue **3i** is half as active as **3f**, but changing the ether oxygen of **3f** to a sulfur, as with **3i**, led to a further enhancement in inhibitory activity. The [[[(phosphonomethyl)thio]methyl]benzyl]guanine **3j** has a K_i' of 1.1 nM when measured under conditions where acyclovir diphosphate has a K_i' of 5.2 nM.

The two most potent inhibitors, **3f** and **3j**, were studied in more detail. If the concentration of inorganic phosphate was increased from 1 to 50 mM, the K_i' values for **3f** and **3j** were increased 40- and 20-fold, respectively. When inorganic phosphate was varied from 0.1 to 8 mM at a constant concentration of inosine (0.1 mM), **3f** and **3j** displayed competitive kinetics. The K_i' values were similar to those obtained with inosine as the variable substrate. The effects of inosine and phosphate on inhibitor K_i' of **3f** and **3j** show that these compounds possess binding determinants for both the purine- and phosphate-binding domains of the enzyme. These characteristics are consistent with **3f** and **3j** being multisubstrate analogue inhibitors of purine nucleoside phosphorylase.[21,22]

Conclusion

We have combined a preliminary understanding of the three-dimensional structure of human erythrocyte PNPase with an intuitive, systematic approach to structure variation to develop potent inhibitors of PNPase. The potency of 9-benzylguanine (**2**) has been enhanced more than 6000-fold by linking a phosphonic acid residue with a (methylthio)methyl spacer to the *meta* position of **2** to give **3j**, which has a K_i' of 1.1 nM. This arrangement of structural features allows [[[(phosphonomethyl)thio]methyl]benzyl]guanine **3j** to bind very favorably to all three subsites of the enzyme active site. Compound **3j** is 5-fold more active than the best inhibitor to emerge from recent X-ray crystallographic, computer-assisted modeling studies.[20] This series of phosphonic acids, which illustrates the potent enzyme inhibitor properties available to multisubstrate analogue inhibitors, are among the most potent inhibitors of PNPase yet reported.[23]

Experimental Section

NMR spectra were recorded on a Varian XL-200 or Varian XL-300 (^{1}H NMR, 200 or 300 MHz; ^{13}C NMR, 50.31 or 75.43 MHz) spectrometer. Chemical shift values are reported in parts per million. UV spectra were recorded on a Hewlett-Packard 8452A diode-array or Beckman DU-70 spectrophotometer. UV data from the former was analyzed by an IBM PC-AT. Mass spectra (~50 MA/s) were obtained from Oneida Research Services, Whitesboro, NY, using a Finnegan 4500 TFQ mass spectrometer. FAB$^+$ mass spectra were obtained on a VG70 SQ mass spectrometer (VG Ltd., Manchester England) using a cesium

ion source and a glycerol/HCl matrix. Elemental microanalyses were determined by Atlantic Microlabs, Atlanta, GA, and gave combustion values for C, H, N, Cl and S within 0.4% of the theoretical values. Compounds analyzed for fractional amounts of solvent showed the appropriate solvent impurity signals in the ^{1}H NMR spectra. Preparative flash chromatography[34] was performed using silica gel 60 (40–63 μm, E.M. Science 9385-9). Analytical thin-layer chromatography was done using silica gel 60A (250 μm) MKGF (Whatman) plates. Melting points were determined with a Thomas-Hoover or Mel-Temp capillary melting point apparatus and are uncorrected.

[[4-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]phenoxy]methyl]phosphonic Acid (3a). This compound was prepared in an analogous manner to that of **3c**. The appropriate fractions from the ion-exchange column were combined and lyophilized for 96 h to give 0.645 g (79%) of **3a** as a monoammonium salt. Mp: 280–285 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 254 (ε 9600), λ_{min} 238 (ε 6900), sh 278 (ε 6700); (pH 7 buffer) λ_{max} 252 (ε 9900), λ_{min} 241 (ε 8200), sh 271 (ε 8000); (0.1 N sodium hydroxide) λ_{max} 270 (ε 9100), λ_{min} 242 (ε 6000), sh 259 (ε 8200). ^{1}H NMR (D_2O): δ 7.80 (br s, 1H, purine H-8), 7.10 (AB q, 4H, ArH), 5.14 (s, 2H, CH_2Ar), 4.10 (d, 2H, J = 9.9 Hz, CH_2P). ^{13}C NMR (D_2O): δ 158.90/158.64/153.73 (C-6/C-4/C-2), 135.15 (C-8 and C-5′), 128.87 (C-4′ and C-6′), 128.51 (C-2′), 114.87 (C-5 and C-3′ and C-7′), 64.15 (d, J = 158 Hz, (C-9′)-P), 46.37 (C-1′). ^{31}P NMR (D_2O): δ 15.081. MS: m/e 352 (MH^+), 257 ($MH - CH_4O_3P)^+$. Anal. ($C_{13}H_{14}N_5O_5P\cdot NH_3\cdot 2.1H_2O$) C, H, N.

[[[4-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]benzyl]oxy]methyl]phosphonic Acid (3b). This compound was prepared in an analogous manner to that of **3c**. The appropriate fractions from the ion-exchange column were combined and lyophilized for 48 h and dried under vacuum at 70 °C for 18 h to give 0.329 g (15%) of **3b** as a partial ammonium salt. Mp: 160 °C (softens), >260 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 253.5 (ε 11 800), 278 (ε 7700); λ_{min} 230.5 (ε 4000); (pH 7 buffer) λ_{max} 252 (ε 12 800), λ_{min} 229.5 (ε 4600), sh 273 (ε 9200); (0.1 N sodium hydroxide) λ_{max} 267 (ε 11 400), λ_{min} 227.5 (ε 2100), sh 256 (ε 10 500). ^{1}H NMR (D_2O + NaOD): δ 7.73 (s, 1H, purine H-8), 7.21 (AB q, 4H, ArH), 5.10 (s, 2H, NCH_2), 4.55 (s, 2H, CH_2Ar), 3.54 (d, 2H, J = 8.6 Hz, CH_2P). ^{13}C NMR (D_2O + NaOD): δ 158.88 (C-6), 153.85 (C-4), 151.12 (C-2), 139.58 (C-8), 137.79/134.98 (C-2′ and C-5′), 128.42/127.17 (C-3′/C-4′, C-6′/C-7′), 115.81 (C-5), 73.94 (d, ^3J = 11.5 Hz, (C-8′)-P), 68.43 (d, ^1J = 151 Hz, (C-10′)-P), 46.43 (C-1′). ^{31}P NMR (D_2O + NaOD): δ 14.11. MS: m/e 366 (MH^+). Anal. ($C_{14}H_{16}N_5O_5P\cdot 0.25NH_3\cdot 0.85H_2O$) C, H, N.

[[2-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]phenoxy]methyl]phosphonic Acid (3c). Bromotrimethylsilane (2.55 mL, 19.3 mmol) was added dropwise to a stirred solution of **15c** (1.46 g, 3.22 mmol) in dimethylformamide (20 mL) cooled to −75 °C under a nitrogen atmosphere. The reaction mixture was warmed to ambient temperature and stirred for an additional 20 h. The volatiles were removed using aspirator pressure and then with high vacuum pressure (~0.1 mmHg) (water bath, T = 75 °C) for 2 h to obtain a foam. Hydrochloric acid (1 N, 50 mL, 50 mmol) was added to the foam, and the mixture was refluxed for 18 h. The volatiles were removed by spin evaporation under reduced pressure to give a beige solid. Treatment of the solid with 1 M ammonium bicarbonate (200 mL) followed by spin evaporation *in vacuo* gave an off-white solid, which was dissolved in deionized water (250 mL), and the solution was applied to an ion-exchange column of Sephadex-DEAE (bicarbonate form). The column was eluted with a linear gradient (0 to 1 M) of ammonium bicarbonate. Fractions were collected (22 mL each) and analyzed by HPLC for purity [Hamilton PRP-1, 5 μm column eluted with 0.1 N ammonium dihydrogen phosphate/methanol (9:1)]. The clean fractions corresponding to the major component isolated from the ion-exchange column were combined, and the volatiles were spin evaporated *in vacuo*. The solid residue was treated with acetone (250 mL), and the solids were collected by suction filtration and dried under vacuum at 60 °C to give 1.04 g (81%) of **3c** monoammonium salt, contaminated with water (4.5%) and ammonium bicarbonate (3.0%). Mp: 220 °C (softens); >300 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 256 (ε 9900), 279 (ε 8500); λ_{min} 233 (ε 4000), 268 (ε 8000); (pH 7 buffer) λ_{max} 255 (ε 10 900), 273 (ε 9900); λ_{min} 235 (ε 6000), 266 (ε 9500); (0.1 N sodium hydroxide) λ_{max} 272 (ε 11 100), λ_{min} 237 (ε 5100). ^{1}H NMR (D_2O): δ 7.94 (s, 1H, purine H-8), 7.4–6.8 (m, 4H, ArH), 5.28 (s, 2H, CH_2), 4.12 (d, 2H, J = 9.8 Hz, CH_2P). ^{13}C NMR (D_2O): δ 158.68 (C-6), 156.74 (d, ^3J = 13.3 Hz, (C-3′)-P), 153.50 (C-4), 151.25 (C-2), 140.52 (C-8), 129.63/128.54 (C-5′/C-7′), 123.72/120.67 (C-2′/C-4′), 115.47 (C-5), 111.54 (C-6′), 64.78 (d, ^1J = 156 Hz, (C-8′)-P), 42.05 (C-1′). ^{31}P NMR (D_2O): δ 13.935. MS: m/e 352 (MH^+), 241 ($M - CH_3O_4P)^+$, 202 ($MH - C_5H_4N_5O)^+$, 185 ($202 - OH)^+$. Anal. ($C_{13}H_{14}N_5O_5P\cdot NH_3\cdot H_2O\cdot 0.15CH_5NO_3$) C, H, N.

[[[2-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]benzyl]oxy]methyl]phosphonic Acid (3d). This compound was prepared in an analogous manner to that of **3c**. The appropriate fractions from the ion-exchange column were combined and lyophilized for 72 h to give 1.80 g (46%) of **3d** as the monoammonium salt. Mp: 160–165 °C (softens), ≥275 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 256 (ε 12 200), λ_{min} 230 (ε 4100), sh 280 (ε 8200); (pH 7 buffer) λ_{max} 254 (ε 13 100), λ_{min} 229 (ε 4800), sh 272 (ε 9800); (0.1 N sodium hydroxide) λ_{max} 271 (ε 11 500), λ_{min} 233 (ε 5300). ^{1}H NMR (D_2O + NaOD): δ 7.67 (s, 1H, purine H-8), 7.55–7.20 (m, 3H, ArH), 6.79–6.76 (m, 1H, ArH), 5.38 (s, 2H, NCH_2), 4.70 (s, 2H, OCH_2Ar), 3.53 (d, 2H, J = 8.8 Hz, CH_2P). ^{13}C NMR (D_2O + NaOD): δ 168.34, 161.29 (C-6 and C-4), 151.67 (C-2), 138.76 (C-8), 135.39/135.14 (C-3′ and C-2′), 130.08/128.80/127.96/126.78 (C-4′/C-5′/C-6′/C-7′), 117.49 (C-5), 72.26 (d, ^3J = 11.5 Hz, (C-8′)-P), 68.65 (d, ^1J = 150 Hz, (C-10′)-P), 43.87 (C-1′). ^{31}P NMR (D_2O + NaOD): δ 14.10. MS: m/e 366 (MH^+), 202 ($MH - C_6H_6N_5O)^+$, 185 ($C_8H_{10}O_3P)^+$, 152 ($C_5H_6N_5O)^+$, 110 ($CH_3O_4P)^+$. Anal. ($C_{14}H_{16}N_5O_5P\cdot NH_3\cdot H_2O$) C, H, N.

[[3-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]phenoxy]methyl]phosphonic Acid (3e). This compound was prepared in an analogous manner to that of **3c**. The appropriate fractions from the ion-exchange column were combined and lyophilized for 48 h to give 0.410 g (40%) of **3e** as a partial ammonium salt. Mp: 295–300 °C dec. UV: (0.1 N hydrochloric acid): λ_{max} 255 (ε 11 500), λ_{min} 234 (ε 5900), sh 279 (ε 9100); (pH 7 buffer) λ_{max} 254 (ε 11 600), λ_{min} 236 (ε 7200), sh 273 (ε 10 100); (0.1 N sodium hydroxide) λ_{max} 272 (ε 11 300), λ_{min} 238 (ε 5700), sh 259 (ε 9800). ^{1}H NMR (D_2O): δ 7.81 (br s, 1H, purine H-8), 7.30–6.78 (m, 4H, ArH), 5.18 (s, 2H, CH_2Ar), 4.06 (d, 2H, ^1J = 9.6 Hz, CH_2P). ^{13}C NMR (D_2O): δ 159.31/159.14/158.84 (C-6/C-4/C-4′), 153.65 (C-2), 137.32 (C-8), 130.06 (C-3′ and C-5′), 119.84 (C-2′ and C-6′), 113.99/113.58 (C-5/C-7′), 64.04 (d, ^1J = 158 Hz, (C-8′)-P), 46.58 (C-1′). ^{31}P NMR (D_2O): δ 14.94. MS: m/e 352 (MH^+). Anal. ($C_{13}H_{14}N_5O_5P\cdot 0.75NH_3\cdot 2H_2O$) C, H, N.

[[[3-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]benzyl]oxy]methyl]phosphonic Acid (3f). This compound was prepared in an analogous manner to that of **3c** with replacement of **15c** with **15f**. The appropriate fractions from the ion-exchange column were combined and spin evaporated *in vacuo*. The residual solid was collected by suction filtration using acetone and dried to give 2.40 g (90%) of **3f** as the monoammonium salt. Mp: 170 °C (softens), ≥235 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 255 (ε 11 300), 279 (ε 7600); λ_{min} 231 (ε 3800), 274 (ε 7500); (pH 7 buffer) λ_{max} 255 (ε 12 100), λ_{min} 229 (ε 4300), sh 272 (ε 9200); (0.1 N sodium hydroxide) λ_{max} 270 (ε 10 600), λ_{min} 234 (ε 4800). ^{1}H NMR (D_2O): δ 7.79 (s, 1H, purine H-8), 7.4–7.1 (m, 4H, ArH), 5.16 (s, 2H, NCH_2), 4.57 (s, 2H, OCH_2Ar), 3.61 (d, 2H, J = 8.6 Hz, CH_2P). ^{13}C NMR (D_2O): δ 158.54 (C-6), 153.49 (C-4), 151.13 (C-2), 139.63 (C-8), 138.01 (C-4′), 135.74 (C-2′), 128.89/127.75/127.03/126.64 (C-3′/C-5′/C-6′/C-7′), 115.70 (C-5), 74.06 (d, ^3J = 11.9 Hz, (C-8′)-P), 66.55 (d, ^1J = 156 Hz, (C-10′)-P), 46.54 (C-1′). ^{31}P NMR (D_2O): δ 16.29. MS: m/e 366 (MH^+), 255 ($M - CH_3O_4P)^+$, 202 ($MH - C_6H_6N_5O)^+$, 185 ($C_8H_{10}O_3P)^+$, 152 ($C_5H_6N_5O)^+$, 110 ($CH_3O_4P)^+$. Anal. ($C_{14}H_{16}N_5O_5P\cdot NH_3\cdot 0.4H_2O$) C, H, N.

[2-[[3-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]benzyl]oxy]ethyl]phosphonic Acid (3g). This compound was prepared in an analogous manner to that of **3c**. The appropriate fractions from the ion-exchange column were combined using an acetone rinse and spin evaporated *in vacuo*. The residual solid was dissolved in deionized water (150 mL), and concentrated ammonium hydroxide (1 mL) was added to the mixture. The solution was lyophilized to give 1.34 g (77%) of **3g** as the monoammonium salt. Mp: 100 °C (softens), >160 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 255 (ε 13 200), sh 278 (ε 8900); (pH

7 buffer) λ_{max} 254 (ε 13 500), sh 270 (ε 10 500); (0.1 N sodium hydroxide) λ_{max} 269 (ε 12 600), sh 257 (ε 11 700). ^{1}H NMR (DMSO-d_6): δ 7.78 (s, 1H, purine H-8), 7.4-7.0 (m, 4H, ArH), 6.73 (br s, 2H, NH_2), 5.18 (s, 2H, CH_2), 4.40 (s, 2H, OCH_2), 3.59 (m, 2H, OCH_2), 1.75 (m, 2H, J = 9 Hz, CH_2P). ^{13}C NMR (DMSO-d_6): δ 157.10 (C-6), 153.94 (C-4), 151.26 (C-2), 139.13 (C-4'), 137.40 (C-8), 137.21 (C-2'), 128.46/126.60/126.15/125.82 (C-3'/C-5'/C-6'/C-7'), 116.36 (C-5), 71.16 (C-8'), 66.60 (C-10'), 45.66 (C-1'), 30.54 (d, ^1J = 126 Hz, (C-11')-P). ^{31}P NMR (DMSO-d_6): δ 17.51. MS: m/e 380 (MH^+), 254 ($MH - C_2H_6O_4P$)$^+$. Anal. ($C_{15}H_{18}N_5O_5P \cdot NH_3 \cdot 1.5H_2O$) C, H, N.

(*E*)-[3-[3-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]phenyl]-2-propenyl]phosphonic Acid (3h). This compound was prepared in an analogous manner to that of **3c**. The appropriate fractions from the ion-exchange column were combined and spin evaporated *in vacuo*. The residual solid was dissolved in deionized water (150 mL), and 1 mL of concentrated ammonium hydroxide was added to the mixture to effect dissolution. The solution was lyophilized for 72 h to give 1.08 g (62%) of **3h** as the monoammonium salt. Mp: 180 °C (softens), >200 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 254 (ε 31 100), sh 283 (ε 10 600); (pH 7 buffer) λ_{max} 255 (ε 32 700); (0.1 N sodium hydroxide) λ_{max} 259 (ε 31 400). ^{1}H NMR (DMSO-d_6): δ 7.77 (s, 1H, purine H-8), 7.4-6.9 (m, 4H, ArH), 6.74 (br s, 2H, NH_2), 6.29 (m, 2H, 2 × vinyl H), 5.11 (s, 2H, NCH_2), 2.40 (m, 2H, CH_2P). ^{13}C NMR (DMSO-d_6): δ 157.16 (C-6), 153.92 (C-4), 151.21 (C-2), 137.95 (C-2' or C-4'), 137.37 (C-8), 137.26 (C-4' or C-2'), 129.85 (d, ^3J = 13 Hz, (C-8')-P), 126.46 (d, ^2J = 10 Hz, (C-9')-P), 128.65/125.13/124.86/124.59 (C-3'/C-5'/C-6'/C-7'), 116.33 (C-5), 45.70 (C-1'), 35.02 (d, ^1J = 132 Hz, (C-10')-P). ^{31}P NMR (DMSO-d_6): δ 17.85. MS: m/e 362 (MH^+), 282 ($MH - HO_3P$)$^+$. Anal. ($C_{15}H_{16}N_5O_4P \cdot NH_3 \cdot 1.5H_2O$) C, H, N.

[3-[3-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]phenyl]propyl]phosphonic Acid (3i). A mixture of **3h** monoammonium salt (0.488 g, 1.35 mmol), platinum oxide hydrate (0.200 g), and 50% aqueous ethanol (50 mL) was shaken in the presence of 3-4 atm of hydrogen gas for 20 h. The reaction mixture was filtered through a 2.4-cm pad of filter-aid (Celite) and rinsed with absolute ethanol (50 mL). Concentrated ammonium hydroxide (0.1 mL) was added to the combined filtrates, and the solution was spin evaporated *in vacuo*. The residue was coevaporated with absolute ethanol (1 × 25 mL) to give a white solid which was dissolved in deionized water (50 mL). Concentrated ammonium hydroxide (0.1 mL) was added to the mixture, and the mixture was warmed to effect dissolution. The solution was filtered through fluted filter paper, and the filtrate was lyophilized for 18 h to give 0.353 g (72%) of **3i** as a partial ammonium salt hydrate. Mp 165-175 °C (softens), >240 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 255 (ε 12 000); (pH 7 buffer) λ_{max} 254 (ε 12 200); (0.1 N sodium hydroxide) λ_{max} 269 (ε 10 900). ^{1}H NMR (DMSO-d_6): δ 7.75 (s, 1H, purine H-8), 7.2-6.9 (m, 4H, ArH), 6.75 (br s, 2H, NH_2), 5.11 (s, 2H, NCH_2), 2.54 (t, 2H, CH_2Ar), 1.70 (m, 2H, CH_2P), 1.35 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 157.49 (C-6), 154.29 (C-4), 151.65 (C-2), 142.96 (C-2' or C-4'), 137.72 (C-8), 137.34 (C-4' or C-2'), 128.77/127.95/127.82/124.62 (C-3'/C-5'/C-6'/C-7'), 116.72 (C-5), 46.06 (C-1'), 36.40 (d, ^3J = 15 Hz, (C-8')-P), 28.80 (d, ^1J = 136 Hz, (C-10')-P), 25.77 (d, ^2J = 2.4 Hz, (C-9')-P). ^{31}P NMR (DMSO-d_6): δ 22.843. MS: m/e 364 (MH^+). Anal. ($C_{15}H_{18}N_5O_4P \cdot 0.5NH_3 \cdot 1.95H_2O$) C, H, N.

[[[3-[(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methyl]benzyl]thio]methyl]phosphonic Acid (3j). This compound was prepared in an analogous manner to that of **3c**. The appropriate fractions from the ion-exchange column were combined using an acetone rinse and spin evaporated *in vacuo*. The white solid was dissolved in deionized water (50 mL), and the solution was lyophilized. The solid was dried under vacuum at 90 °C for 6 h to give 0.141 g (49%) of **3j** as a partial ammonium salt monohydrate. Mp: 154-160 °C (softens), ≥200 °C dec. UV: (0.1 N hydrochloric acid) λ_{max} 254 (ε 12 500), sh 279 (ε 8300); (pH 7 buffer) λ_{max} 255 (ε 11 900), sh 271 (ε 9700); (0.1 N sodium hydroxide) λ_{max} 269 (ε 11 800), sh 256 (ε 11 100). ^{1}H NMR (DMSO-d_6): δ 10.8 (br s, 1H, NH), 7.80 (s, 1H, purine H-8), 7.4-7.0 (m, 4H, ArH), 6.65 (br s, 2H NH_2), 5.17 (s, 2H, NCH_2), 3.82 (s, 2H, SCH_2), 2.39 (d, J = 13 Hz, 2H, CH_2P). ^{13}C NMR (DMSO-d_6): δ 157.27 (C-6), 154.12 (C-4), 151.58 (C-2), 139.41 (C-2' or C-4'), 137.79 (C-8), 137.57 (C-4' or C-2'), 128.92/128.58/128.30/125.65 (C-3'/C-5'/C-6'/C-7'), 116.75 (C-5), 45.98 (C-1'), 36.02 (C-8'), 27.26 (d, ^1J = 138 Hz, (C-10')-P). ^{31}P NMR (DMSO-d_6): δ 17.74. MS: m/e 382 (MH^+), 255 ($MH - CH_4O_3PS$)$^+$. Anal. ($C_{14}H_{16}N_5O_4PS \cdot 0.5NH_3 \cdot H_2O$) C, H, N, S.

Diethyl [[4-(Hydroxymethyl)phenoxy]methyl]phosphonate (5a). This compound was prepared in an analogous manner to that of **5c** with the replacement of **22** with (diethylphosphono)methyl 4-toluenesulfonate,[35] and the reaction was stirred at ambient temperature instead of at 40 °C. The chromatography fractions were spin evaporated *in vacuo* to give 2.18 g (28%) of **5a** as a light oil, contaminated with 16% of (diethylphosphono)methyl 4-toluenesulfonate. TLC: dichloromethane/methanol (19:1), R_f = 0.31. ^{1}H NMR ($CDCl_3$): δ 7.10 (AB q, 4H, ArH), 4.62 (s, 2H, CH_2Ar), 4.35-4.15 (overlaping m, 7H, $CH_2P/2 \times OCH_2/OH$), 1.32 (t, 6H, $2 \times CH_3$). MS: m/e 275 (MH^+), 257 ($M - OH$)$^+$, 245 ($M - Et$)$^+$. Anal. ($C_{12}H_{19}O_5P \cdot 0.17C_{12}H_{19}O_6PS$) C, H.

Diisopropyl [[2-(Hydroxymethyl)phenoxy]methyl]phosphonate (5c). To a stirred dispersion of pentane-washed sodium hydride (60% dispersion in mineral oil) (1.06 g, 26.6 mmol) in dry dimethylformamide (20 mL) chilled to 0 °C was added a solution of 2-hydroxybenzyl alcohol (**4c**) (3.00 g, 24.2 mmol) in dry dimethylformamide (20 mL) under nitrogen. The reaction was stirred for 1.5 h at ambient temperature and then transferred with a syringe to an addition funnel. The sodium phenoxide/dimethylformamide solution was added dropwise over the course of 0.5 h to a solution of (diisopropylphosphono)methyl 4-toluenesulfonate (**22**) (8.47 g, 24.2 mmol) in dry dimethylformamide (30 mL) cooled to 0 °C. The reaction mixture was stirred at 40 °C for 18 h. Water (25 mL) was added slowly to the cooled reaction mixture, and the volatiles were spin evaporated *in vacuo* under high vacuum. The residue was treated with ethyl acetate (300 mL) and the solids removed by suction filtration. The solids were rinsed with ethyl acetate (25 mL), and the combined filtrates were washed with saturated sodium bicarbonate solution (2 × 20 mL) and dried over sodium sulfate. The mixture was filtered, and the volatiles removed under reduced pressure by rotary evaporation and dried further under high vacuum to give 6.65 g of an oil. The oil was applied to a column of silica gel 60 wetted with ethyl acetate/hexane (1:1). The column was eluted with ethyl acetate/hexane (1:1) (2.5 L), with collection of 100-mL fractions. The fractions containing product (lowest R_f spot) were combined and spin evaporated *in vacuo* to give 1.92 g (25%) of **5c** as a light yellow oil, which was shown by NMR to be 95% product and 5% ethyl acetate. TLC: dichloromethane/methanol (19:1), R_f = 0.44. ^{1}H NMR ($CDCl_3$): δ 7.31-6.87 (m, 4H, ArH), 4.82 (m, 2H, 2 × CH), 4.68 (s, 2H, CH_2O), 4.26 (d, 2H, J = 9.8 Hz, CH_2P), 2.98 (br s, 1H, OH), 1.34 (pseudotriplet, 12H, 4 × CH_3). MS: m/e 303 (MH^+), 285 ($M - OH$)$^+$, 243 ($M - OC_3H_7$)$^+$. Anal. ($C_{14}H_{23}O_5P \cdot 0.15C_4H_8O_2 \cdot 0.2H_2O$) C, H.

Diethyl [[3-(Hydroxymethyl)phenoxy]methyl]phosphonate (5e). A solution of (diethylphosphono)methyl trifluoromethanesulfonate[36] (7.26 g, 24.2 mmol) and dimethylformamide (5 mL) was added to a stirring mixture of 3-hydroxybenzyl alcohol (**4e**) (2.00 g, 16.1 mmol), cesium carbonate (7.88 g, 24.2 mmol), and dimethylformamide (20 mL). After stirring at ambient temperature for 1 h, the reaction mixture was poured into water (50 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic phase was washed with water (50 mL) and saturated brine (50 mL) and dried with sodium sulfate for several hours. The organic phase was spin evaporated *in vacuo* to give a light amber oil. Additional product was obtained by concentrating the aqueous phase to near dryness and extracting the residue with ethyl acetate (100 mL). The mixture was filtered and the filtrate concentrated to dryness. The semisolid residue was extracted with dichloromethane (50 mL), and the mixture was filtered. The filtrate was combined with the oil obtained from the ether extraction, and the mixture was concentrated to an amber oil (5.45 g) under reduced pressure. The oil was dissolved in dichloromethane (25 mL), and the solution was added to a flash column (50-mm diameter) of silica gel 60 (40-63 μm) wetted with dichloromethane. The column was eluted first with dichloromethane (500 mL) and then with methanol/dichloromethane (1:99) (500 mL), with methanol/dichloromethane (2:98) (500 mL), and with methanol/dichloromethane (3:97) (500

mL), with collection of 25-mL fractions. The intermediate R_f material was collected as a two-component mixture. This mixture was combined with material which was prepared similarly to give a combined yield of 4.19 g of an amber oil. This oil was dissolved in dichloromethane (50 mL) and rechromatographed as before using dichloromethane (500 mL) and then methanol/dichloromethane (2:98) (500 mL) and finally methanol/dichloromethane (5:95) (500 mL) as the eluant. The highest R_f material was collected, and the combined fractions were spin evaporated *in vacuo* to give 1.42 g (17%) **5e** as a lightly colored oil, containing 1.6% water. TLC: dichloromethane/methanol (19:1), R_f = 0.36. ^{1}H NMR ($CDCl_3$): δ 7.32–6.85 (m, 4H, ArH), 4.67 (s, 2H, CH_2Ar), 4.30–4.15 (m, 6H, CH_2P + 2 × CH_2), 1.36 (t, 6H, 2 × CH_3). MS: *m/e* 275 (MH^+), 257 ($M - OH)^+$, 107 ($C_7H_7O)^+$. Anal. ($C_{12}H_{19}O_5P$·0.25H_2O) C, H.

Diethyl [[4-(Chloromethyl)phenoxy]methyl]phosphonate (6a). This compound was prepared in an analogous manner to that of **6c**. After workup and coevaporation with dichloromethane (2 × 50 mL), 2.49 g (104%) of **6a** was obtained as a light yellow oil, with 14% (diethylphosphono)methyl 4-toluenesulfonate carried through as a contaminant. TLC: dichloromethane/methanol (19:1), R_f = 0.74. ^{1}H NMR ($CDCl_3$): δ 7.12 (AB q, 4H, ArH), 4.55 (s, 2H, CH_2Ar), 4.3–4.1 (overlapping m, 6H, CH_2P/2 × OCH_2), 1.32 (t, 6H, 2 × CH_3). MS: *m/e* 293 (MH^+), 257 ($M - Cl)^+$, 107 ($C_7H_7O)^+$. Anal. ($C_{12}H_{18}ClO_4P$·0.04$C_{12}H_{19}O_6PS$) C, H, Cl.

Diisopropyl [[2-(Chloromethyl)phenoxy]methyl]phosphonate (6c). Thionyl chloride (0.438 mL, 6.00 mmol) in dichloromethane (5 mL) was added dropwise to an ice-cold solution of **5c** (1.65 g, 5.46 mmol) in dichloromethane (25 mL). The reaction was refluxed with stirring for 1.5 h and stirred at ambient temperature for 18 h. The volatiles were removed by spin evaporation *in vacuo*, and the residual oil was coevaporated with dichloromethane (2 × 50 mL) to give 1.56 g (89%) of **6c** as a light yellow oil. TLC: dichloromethane, R_f = 0.24. ^{1}H NMR ($CDCl_3$): δ 7.37–6.92 (m, 4H, ArH), 4.84 (m 2H, 2 × CH), 4.68 (s, 2H, CH_2Cl), 4.28 (d, 2H, J = 10.1 Hz, CH_2P), 1.37 (pseudotriplet, 12H, 4 × CH_3). MS: *m/e* 321 (MH^+), 285 ($M - Cl)^+$, 243 [M − [Cl + (C_3H_6)]]$^+$, 201 [M − [Cl + 2(C_3H_6)]]$^+$. Anal. ($C_{14}H_{22}ClO_4P$) C, H, Cl.

Diethyl [[3-(Chloromethyl)phenoxy]methyl]phosphonate (6e). This compound was prepared in an analogous manner to that of **6c**. After workup and coevaporation with dichloromethane, 1.30 g (100%) of **6e** was obtained as a light golden oil. TLC: dichloromethane/methanol (19:1), R_f = 0.51. ^{1}H NMR ($CDCl_3$): δ 7.35–6.88 (m, 4H, ArH), 4.55 (s, 2H, CH_2Cl), 4.33–4.15 (m, 6H, CH_2P + 2 × CH_2), 1.36 (t, 6H, 2 × CH_3). MS: *m/e*: 293 (MH^+), 257 ($M - Cl)^+$, 107 ($C_7H_7O)^+$. Anal. ($C_{12}H_{18}ClO_4P$) C, H, Cl.

Diisopropyl [[[4-(Chloromethyl)benzyl]oxy]methyl]phosphonate (8b). This compound was prepared in an analogous manner to that of **8f** with the replacement of α,α'-dichloro-*m*-xylene (**7f**) with α,α'-dichloro-*p*-xylene (**7b**). The appropriate fractions from column chromatography were combined and spin evaporated *in vacuo* to give 4.06 g (39%) of **8b** as a fluorescent yellow oil, contaminated with 17% 1,4-bis[[diisopropylphosphono)methoxy]methyl]benzene. TLC: dichloromethane/methanol (19:1), R_f = 0.63; ^{1}H NMR ($CDCl_3$): δ 7.4–7.3 (AB q, 4H, ArH), 4.77 (m, 2H, 2 × CH), 4.64 (s, 2H, OCH_2), 4.57 (s, 2H, CH_2Cl), 3.70 (d, 2H, J = 8.7 Hz, CH_2P), 1.33 (d, 6H, 2 × CH_3), 1.31 (d, 6H, 2 × CH_3). Anal. ($C_{15}H_{24}ClO_4P$·0.14$C_{22}H_{40}P_2O_8$) C, H, Cl.

Diisopropyl [[[2-(Chloromethyl)benzyl]oxy]methyl]phosphonate (8d). This compound was prepared in an analogous manner to that of **8f** with the replacement of α,α'-dichloro-*m*-xylene (**7f**) with α,α'-dichloro-*o*-xylene (**7d**). After chromatography the appropriate fractions were combined and spin evaporated *in vacuo* to give 5.13 g (58%) of **8d** as a light yellow oil. TLC: dichloromethane/methanol (19:1), R_f = 0.58. ^{1}H NMR ($CDCl_3$): δ 7.5–7.3 (m, 4H, ArH), 4.78 (s, 2H, OCH_2), 4.75 (m, 2H, 2 × CH), 4.72 (s, 2H, CH_2Cl), 3.73 (d, 2 H, J = 8.5 Hz, CH_2P), 1.32 (pseudotriplet, 12H, 4 × CH_3). MS: *m/e* 335 (MH^+), 299 ($M - Cl)^+$, 293 ($MH - C_3H_6)^+$, 251 ($MH - 2 \times C_3H_6)^+$. Anal. ($C_{15}H_{24}ClO_4P$) C, H, Cl.

Diisopropyl [[[3-(Chloromethyl)benzyl]oxy]methyl]phosphonate (8f). A solution of **23** (5.00 g, 25.5 mmol) in dimethylformamide (20 mL) was added over the course of 1 h to an ice-cooled mixture of pentane-washed sodium hydride (60% dispersion in mineral oil) (1.22 g, 30.6 mmol) and dimethylformamide (30 mL) with stirring under a nitrogen atmosphere. The reaction was warmed to ambient temperature and stirred for another hour, and the contents of the reaction were transferred via a syringe to an addition funnel. The solution was added in 2-mL portions over the course of 30 min to a stirred solution of α,α'-dichloro-*m*-xylene (**7f**) (13.4 g, 76.5 mmol) in dimethylformamide (50 mL) cooled to 0 °C. The reaction was warmed to ambient temperature, stirred for 20 h, and then poured into water (100 mL). The aqueous mixture was extracted with diethyl ether (4 × 100 mL). The combined organic extracts were washed with water (4 × 50 mL) and with saturated brine (50 mL) and dried over sodium sulfate. The filtered mixture was concentrated to a yellow oil by spin evaporation *in vacuo*. The oil was dissolved in dichloromethane (50 mL), and the solution was applied to a column of silica gel 60 wetted with dichloromethane. The column was eluted with dichloromethane (700 mL) and then dichloromethane/methanol (19:1) (1 L) and dichloromethane/methanol (93:7) (500 mL); 50-mL fractions were collected. The fractions containing the lower R_f material were combined and spin evaporated *in vacuo* to give 3.38 g (38%) of **8f** as a light amber oil, with 4% methanol as a contaminant. TLC: dichloromethane/methanol (19:1), R_f = 0.60. ^{1}H NMR ($CDCl_3$): δ 7.4–7.3 (m, 4H, ArH), 4.76 (m, 2H, 2 × CH), 4.65 (s, 2H, OCH_2), 4.58 (s, 2H, CH_2Cl), 3.72 (d, 2H, J = 8.6 Hz, CH_2P), 1.33 (pseudotriplet, 12H, 4 × CH_3). MS: *m/e* 335 (MH^+), 299 ($M - Cl)^+$, 293 ($MH - C_3H_6)^+$, 139 ($C_8H_8Cl)^+$. Anal. ($C_{15}H_{24}ClO_4P$·0.4CH_4O) C, H, Cl.

Diethyl [2-[[3-(Hydroxymethyl)benzyl]oxy]ethyl]phosphonate (10g). Diethyl vinylphosphonate (6.18 mL, 39.0 mmol) was added to a magnetically stirred mixture of 1,3-bis(hydroxymethyl)benzene (**9g**) (5.00 g, 35.5 mmol) and potassium carbonate (0.491 g, 3.55 mmol). The reaction mixture was heated at 110–120 °C for 20 h, cooled to ambient temperature, and extracted with diethyl ether (200 mL). The decanted liquid phase was washed with water (2 × 25 mL) and dried with sodium sulfate. The mixture was filtered, and the filtrate was concentrated by spin evaporation *in vacuo* using aspirator pressure to give an oil. The oil was dissolved in ethyl acetate (75 mL), and the solution was applied to a column of silica gel 60 wetted with ethyl acetate. The column was eluted with 1 L of ethyl acetate, 1 L of methanol/ethyl acetate (1:49), 1 L of methanol/ethyl acetate (1:24), 1 L of methanol/ethyl acetate (1:19), and 2 L of methanol/ethyl acetate (1:9), with collection of 50-mL fractions. The fractions containing clean product (highest R_f major spot) were combined and spin evaporated *in vacuo* to give 1.98 g (18%) of **10g** as a light oil, contaminated with 2.1% of water. TLC: ethyl acetate/methanol (19:1), R_f = 0.62. ^{1}H NMR ($CDCl_3$): δ 7.4–7.2 (m, 4H, ArH), 4.68 (s, 2H, OCH_2), 4.52 (s, 2H, OCH_2), 4.08 (m, 4H, 2 × CH_2), 3.72 (m, 2H, OCH_2), 2.4 (br s, 1H, OH), 2.12 (m, 2H, CH_2P), 1.29 (m, 6H, 2 × CH_3). MS: *m/e* 303 (MH^+), 285 ($M - OH)^+$, 211 ($C_{10}H_{12}O_3P)^+$. Anal. ($C_{14}H_{23}O_5P$·0.36H_2O) C, H.

Diethyl [2-[[3-(Chloromethyl)benzyl]oxy]ethyl]phosphonate (11g). This compound was prepared in an analogous manner to that of **6c**. After workup, 2.44 g (102%) of **11g** (98% purity by NMR) was obtained as an oil. TLC: dichloromethane/methanol (19:1), R_f = 0.33. ^{1}H-NMR ($CDCl_3$): δ 7.4–7.2 (m, 4H, ArH), 4.59 (s, 2H, CH_2Cl), 4.53 (s, 2H, OCH_2), 4.10 (m, 4H, 2 × CH_2), 3.76 (m, 2H, OCH_2), 2.16 (m, 2H, CH_2P), 1.31 (m, 6H, 2 × CH_3). MS: *m/e* 321 (MH^+), 285 ($M - Cl)^+$, 166 ($C_6H_{15}O_3P)^+$, 139 ($C_8H_8Cl)^+$. Anal. ($C_{14}H_{22}ClO_4P$) C, H, Cl.

(*E*)-Diisopropyl [3-[3-(Hydroxymethyl)phenyl]-2-propenyl]phosphonate (13h). This compound was prepared in an analogous manner to that described by Xu et al.[29] for the preparation of diethyl (2-arylethenyl)phosphonates from aryl bromides and diethyl vinylphosphonate. In this example, 3-iodobenzyl alcohol was coupled to 2-propenylphosphonate **24**. After the appropriate workup, the crude oil (10.8 g) was dissolved in 100 mL of dichloromethane, and the solution was applied to a column of silica gel 60 wetted with dichloromethane. The column was eluted with 500 mL of dichloromethane, 500 mL of methanol/dichloromethane (1:99), 500 mL of methanol/dichloromethane (1:49), 500 mL of methanol/dichloromethane (3:97), 500 mL of methanol/dichloromethane (1:24), and 1 L of methanol/dichloromethane (1:19), with collection of 50-mL fractions. The fractions containing the major spot were combined and spin

evaporated *in vacuo* to give 9.64 g (67%) of **13h** as a golden oil, contaminated with 7% of **24**. TLC: dichloromethane/methanol (19:1), R_f = 0.23; ^{1}H NMR ($CDCl_3$): δ 7.4–7.2 (m, 4H, ArH), 6.50 (m, 1H, J = 16 Hz, vinyl H), 6.18 (m, 1H, vinyl H), 4.68 (m, 4H, OCH_2 + 2 × OCH), 2.71 (m, 2H, CH_2P), 2.05 (br s, 1H, OH), 1.30 (m, 12H, 4 × CH_3); 16 Hz coupling parallels 18 Hz coupling in the literature,[29] and steady state NOE after irradiation at δ 6.50 showed −1.3% NOE at 6.18 and 9.4% at 2.71. MS: *m/e* 313 (MH^+), 295 ($M - OH)^+$, 271 ($MH - C_3H_6)^+$, 207 ($C_9H_{20}O_3P)^+$, 165 ($C_6H_{14}O_3P)^+$, 123 ($C_3H_8O_3P)^+$. Anal. ($C_{16}H_{25}O_4P$·0.11$C_9H_{19}O_3P$) C, H.

(*E*)-Diisopropyl [3-[3-(Chloromethyl)phenyl]-2-propenyl]phosphonate (14h). This compound was prepared in an analogous manner to that of **6c**. The crude oil from the workup was only partially purified on a column of silica gel 60 using dichloromethane/methanol as the eluting solvent. The appropriate fractions containing product were combined and spin evaporated *in vacuo*. The residual oil was dissolved in ethyl acetate (100 mL), and the solution was filtered with suction through a 2-inch pad of silica gel 60 using successive washes with ethyl acetate (3 × 250 mL). The combined ethyl acetate filtrates were spin evaporated *in vacuo* to give 2.57 g (79%) of **14h** as a light yellow oil, contaminated with 1.3% of ethyl acetate and 1.3% of water. TLC: dichloromethane/methanol (19:1), R_f = 0.52. ^{1}H NMR ($CDCl_3$): δ 7.4–7.2 (m, 4H, ArH), 6.50 (m, 1H, J = 16 Hz, vinyl H), 6.19 (m, 1H, vinyl H), 4.72 (m, 2H, 2 × OCH), 4.57 (s, 2H, CH_2Cl), 2.72 (m, 2H, CH_2P), 1.32 (m, 12H, 4 × CH_3). MS: *m/e* 331 (MH^+), 295 ($M - Cl)^+$, 289 ($MH - C_3H_6)^+$, 247 ($MH - 2 \times C_3H_6)^+$.

Diethyl [[4-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]phenoxy]methyl]phosphonate (15a). This compound was prepared in an analogous manner to that of **15c**. The appropriate chromatography fractions were combined and spin evaporated *in vacuo* to give 1.24 g (37%) of **15a** as a light oil, contaminated with 1.3% dimethylformamide, 2.5% acetone, 18% dichloromethane, and 1.3% water. TLC: dichloromethane/methanol (19:1), R_f = 0.32. ^{1}H NMR (DMSO-d_6): δ 8.18 (s, 1H, purine H-8), 7.11 (AB q, 4H, ArH), 6.92 (s, 2H, NH_2), 5.19 (s, 2H, CH_2-Ar), 4.36 (s, 2H, J = 9.8 Hz, CH_2P), 4.10 (m, 4H, 2 × CH_2), 1.21 (t, 6H, 2 × CH_3). Anal. ($C_{17}H_{21}ClN_5O_4P$·0.1C_3H_7NO·0.2C_3H_6O·0.2CH_2Cl_2·0.35H_2O) C, H, N.

Diisopropyl [[[4-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]benzyl]oxy]methyl]phosphonate (15b). This compound was prepared in an analogous manner to that of **15c**. The appropriate fractions from column chromatography were combined and spin evaporated *in vacuo* to give 3.09 g (60%) of **15b** as a greenish yellow solid. MP: 103–106 °C. TLC: dichloromethane/methanol (9:1), R_f = 0.66. ^{1}H NMR (DMSO-d_6): δ 8.21 (s, 1H, purine H-8), 7.26 (AB q, 4H, ArH), 6.92 (br s, 2H, NH_2), 5.27 (s, 2H, NCH_2), 4.54 (m, 2H, 2 × CH), 4.52 (s, 2H, CH_2Ar), 3.70 (d, 2H, J = 8.4 Hz, CH_2P), 1.21 (d, 6H, 2 × CH_3), 1.19 (d, 6H, 2 × CH_3). MS: *m/e* 468 (MH^+), 432 ($M - Cl)^+$, 426 ($MH - C_3H_6)^+$. Anal. ($C_{20}H_{27}ClN_5O_4P$) C, H, N, Cl.

Diisopropyl [[2-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]phenoxy]methyl]phosphonate (15c). A solution of **6c** (1.55 g, 4.83 mmol) in dimethylformamide (5 mL) was added to a stirred mixture of 2-amino-6-chloro-9*H*-purine (0.745 g, 4.39 mmol), cesium carbonate (1.57 g, 4.83 mmol), and dimethylformamide (10 mL). The reaction mixture was stirred at ambient temperature for 64 h and then poured into water (50 mL). The aqueous mixture was extracted with ethyl acetate (4 × 100 mL), and the combined organic extracts were washed with water (2 × 50 mL) and with saturated brine (25 mL) and dried with sodium sulfate. The mixture was filtered, and the filtrate was concentrated by spin evaporation *in vacuo* using aspirator pressure, followed by high vacuum to remove traces of dimethylformamide. The crude, amber oil was dissolved in dichloromethane (65 mL), and the solution was applied to a column of silica gel 60 wetted with dichloromethane. The column was eluted with 500 mL of dichloromethane, 1 L of methanol/dichloromethane (3:97), and 500 mL of methanol/dichloromethane (1:19), with collection of 50-mL fractions. The fractions containing clean product (highest R_f major spot) were combined and spin evaporated *in vacuo* to give 1.91 g (88%) of **15c** (92% purity) as an off-white solid, contaminated with 7.3% of **6c** and 0.89% of dimethylformamide. MP: 104-107 °C. TLC: dichloromethane/methanol (9:1), R_f = 0.63. ^{1}H NMR (DMSO-d_6): δ 8.12 (s, 1H, purine H-8), 7.4-6.9 (m, 4H, ArH), 6.90 (s, 2H, NH_2), 5.18 (s, 2H, CH_2N), 4.72 (m, 2H, 2 × CH), 4.42 (s, 2H, J = 9.92 Hz, CH_2P), 1.26 (pseudotriplet, 12H, 4 × CH_3). MS: *m/e* 454 (MH^+), 4.18 ($M - Cl)^+$, 285 ($MH - C_5H_4ClN_5)^+$, 243 ($C_{11}H_{16}O_4P)^+$, 201 ($C_8H_{10}O_4P)^+$. Anal. ($C_{19}H_{25}ClN_5O_4P$·0.12$C_{14}H_{23}O_5P$·0.06C_3H_7NO) C, H, N, Cl.

Diisopropyl [[[2-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]benzyl]oxy]methyl]phosphonate (15d). This compound was prepared in an analogous manner to that of **15c**. The chromatography fractions containing clean product were combined and spin evaporated *in vacuo* to give 5.07 g (78%) of **15d** as a light yellow oil, with 1.3% water and 0.46% dimethylformamide as minor contaminants. TLC: dichloromethane/methanol (9:1), R_f = 0.62. ^{1}H NMR (DMSO-d_6): δ 8.16 (s, 1H, purine H-8), 7.45–6.85 (m, 4H, ArH), 6.92 (br s, 2H, NH_2), 5.34 (s, 2H, NCH_2), 4.79 (s, 2H, OCH_2), 4.62 (m, 2H, 2 × CH), 3.84 (s, 2H, CH_2P), 1.26 (pseudotriplet, 12H, 4 × CH_3). MS: *m/e* 468 (MH^+), 432 ($M - Cl)^+$, 426 ($MH - C_3H_6)^+$, 299 ($M - C_5H_3ClN_5)^+$. Anal. ($C_{20}H_{27}ClN_5O_4P$·0.33H_2O·0.03C_3H_7NO) C, H, N, Cl.

Diethyl [[3-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]phenoxy]methyl]phosphonate (15e). This compound was prepared in an analogous manner to that of **15c**. The chromatography fractions containing clean product were combined and spin evaporated *in vacuo* to give 1.28 g (60%) of **15e** as a light yellow oil, with 19% bis[(diethylphosphono)methyl] ether, and 6% dimethylformamide as contaminants. TLC: dichloromethane/methanol (19:1), R_f = 0.30; ^{1}H NMR (DMSO-d_6): δ 8.24 (s, 1H, purine H-8), 7.4–6.8 (m, 4H, ArH), 6.96 (br s, 2H, NH_2), 5.25 (s, 2H, CH_2Ar), 4.40 (d, 2H, ^1J = 9.9 Hz, CH_2P), 4.10 (q, 4H, 2 × CH_2), 1.23 (t, 6H, 2 × CH_3). MS: *m/e* 426 (MH^+), 390 ($M - Cl)^+$, 275 ($MH - C_5H_{12}O_3P)^+$, 257 ($M - C_5H_3N_5Cl)^+$. Anal. ($C_{17}H_{21}ClN_5O_4P$·0.47C_3H_7NO·0.35$C_{10}H_{24}O_7P_2$) C, H, N.

Diisopropyl [[[3-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]benzyl]oxy]methyl]phosphonate (15f). This compound was prepared in an analogous manner to that of **15c**. The chromatography fractions containing clean product were combined and spin evaporated *in vacuo* to give 3.84 g (64%) of **15f** as a light yellow oil, with 5.5% methanol, 3.5% dimethylformamide, and 1% dichloromethane as contaminants. TLC: dichloromethane/methanol (9:1), R_f = 0.58. ^{1}H NMR (DMSO-d_6): δ 8.20 (s, 1H, purine H-8), 7.4–7.1 (m, 4H, ArH), 6.93 (s, 2H, NH_2), 5.28 (s, 2H, NCH_2), 4.55 (m, 2H, 2 × CH), 4.51 (s, 2H, CH_2Ar), 3.70 (d, 2H, J = 8.6 Hz, CH_2P), 1.17 (pseudotriplet, 12H, 4 × CH_3). MS: *m/e* 468 (MH^+), 299 ($M - C_5H_3ClN_5)^+$, 147 ($C_6H_5N_5)^+$. Anal. ($C_{20}H_{27}ClN_5O_4P$·0.9CH_4O·0.25C_3H_7NO·0.06CH_2Cl_2) C, H, N, Cl.

Diethyl [2-[[3-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]benzyl]oxy]ethyl]phosphonate (15g). This compound was prepared in an analogous manner to that of **15c**. The chromatography fractions containing clean product were combined and spin evaporated *in vacuo* to give 2.07 g (68%) of **15g** as an oil, contaminated with 0.9% of dimethylformamide, 0.9% of dichloromethane, and 1% of water. TLC: dichloromethane/methanol (9:1), R_f = 0.57. ^{1}H-NMR (DMSO-d_6): δ 8.24 (s, 1H, purine H-8), 7.4–7.1 (m, 4H, ArH), 6.96 (s, 2H, NH_2), 5.31 (s, 2H, CH_2), 4.46 (s, 2H, OCH_2), 3.97 (m, 4H, 2 × CH_2), 3.61 (m, 2H, OCH_2), 2.10 (m, 2H, CH_2P), 1.22 (m, 6H, 2 × CH_3). MS: *m/e* 454 (MH^+), 418 ($M - Cl)^+$, 285 ($C_{14}H_{22}O_4P)^+$. Anal. ($C_{19}H_{25}ClN_5O_4P$·0.25H_2O·0.06C_3H_7NO·0.05CH_2Cl_2) C, H, N, Cl.

(*E*)-Diisopropyl [3-[3-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]phenyl]-2-propenyl]phosphonate (15h). This compound was prepared in an analogous manner to that of **15c**. The chromatography fractions containing clean product were combined and spin evaporated at 70 °C for 0.5 h under high vacuum to give 2.18 g (68%) of **15h** as a colorless glass, contaminated with 2% of water. Mp: 35–42 °C. TLC: dichloromethane/methanol (9:1), R_f = 0.55. ^{1}H-NMR (DMSO-d_6): δ 8.26 (s, 1H, purine H-8), 7.4–7.1 (m, 4H, ArH), 6.97 (br s, 2H, NH_2), 6.53 (m, 1H, vinyl H), 6.09 (m, 1H, vinyl H), 5.31 (s, 2H, NCH_2), 4.56 (m, 2H, 2 × OCH), 2.75 (m, 2H, CH_2P), 1.22 (m, 12H, 4 × CH_3). MS: *m/e* 464 (MH^+), 428 ($M - Cl)^+$, 422 ($MH - C_3H_6)^+$. Anal. ($C_{21}H_{27}ClN_5O_3P$·0.5H_2O) C, H, N, Cl.

Diisopropyl [(Acetylthio)methyl]phosphonate (17). A mixture of triisopropyl phosphite (15.7 mL, 60.3 mmol) and

bromomethyl acetyl sulfide (16)[32] (10.2 g, 60.3 mmol) was heated with stirring at 130 °C for 3 h using a Dean–Stark trap to remove the 2-bromopropane byproduct. The reaction mixture was distilled in several fractions, and the two cleanest fractions were combined to give 8.9 g of 17. The higher boiling fraction gave 2.7 g (18%) of 17 as a colorless liquid. Bp: 115–125 °C (0.65 mmHg). ^{1}H-NMR ($CDCl_3$): δ 4.71 (m, 2H, 2 × CH), 3.18 (d, 2H, CH_2P), 2.37 (s, 3H, CH_3), 1.31 (m, 12H, 4 × CH_3). MS: *m/e* 255 (MH^+), 213 ($MH - C_3H_6)^+$, 171 ($MH - 2 \times C_3H_6)^+$. Anal. ($C_9H_{19}O_4PS$) C, H, S.

2-Amino-9-[3-(chloromethyl)benzyl]-6-(2-methoxyethoxy)-9*H*-purine (20). α,α′-Dichloro-*m*-xylene (0.250 g, 1.43 mmol) was added to a magnetically stirred mixture of 2-amino-6-(2-methoxyethoxy)-9*H*-purine (19)[31] (0.100 g, 0.478 mmol), anhydrous cesium carbonate (0.171 g, 0.526 mmol), and dry dimethylformamide (2 mL) at ambient temperature under nitrogen for 18 h. The reaction mixture was filtered through a pad of silica gel 60, and the pad was rinsed with dichloromethane (2 × 75 mL). The combined filtrates were spin evaporated *in vacuo* under aspirator pressure and then under high vacuum to give an amber oil. The oil was dissolved in dichloromethane (5 mL), and the solution was applied to a column of silica gel 60 wetted with dichloromethane. The column was eluted with 100 mL of dichloromethane and 150 mL of methanol/dichloromethane (1:19), with collection of 20-mL fractions. The fractions containing clean product (intermediate R_f spot) were combined and spin evaporated *in vacuo* to give 0.041 g (23%) of **20** as a glasslike, white solid, contaminated with 10% of α,α′-dichloro-*m*-xylene and 3% of methanol. Mp: 123–125 °C. TLC: dichloromethane/methanol (19:1), R_f = 0.25. ^{1}H NMR (DMSO-d_6): δ 7.98 (s, 1H, purine H-8), 7.4–7.1 (m, 4H, ArH), 6.45 (br s, 2H, NH_2), 5.27 (s, 2H, NCH_2), 4.73 (s, 2H, CH_2Cl), 4.53 (m, 2H, OCH_2), 3.70 (m, 2H, OCH_2), 3.31 (s, 3H, OCH_3). MS: *m/e* 348 (MH^+), 312 $(M - Cl)^+$, 139 $(C_8H_8Cl)^+$. Anal. ($C_{16}H_{18}ClN_5O_2 \cdot 0.36CH_4O \cdot 0.06C_8H_8Cl_2$) C, H, N, Cl.

Diisopropyl [[[3-[[2-Amino-6-(2-methoxyethoxy)9*H*-purin-9-yl]methyl]benzyl]thio]methyl]phosphonate (21). Sodium metal (0.089 g, 3.9 mmol) was dissolved with warming in a mixture of 2-propanol/ethanol (2 mL/0.5 mL). To this solution was added 17 (0.989 g, 3.89 mmol) dropwise. After 15 min, the solution was added to a solution of **20** (1.23 g, 3.01 mmol) and dry dimethylformamide (20 mL) at ambient temperature. The mixture was magnetically stirred for 18 h at ambient temperature and poured into water (100 mL). The aqueous mixture was extracted with dichloromethane (3 × 125 mL), using saturated brine to break up the emulsion. The combined organic extract was washed with water (2 × 50 mL) and then with brine and dried with sodium sulfate. The filtered mixture was concentrated to a yellow oil by spin evaporation *in vacuo* under aspirator pressure and then under high vacuum. The oil was dissolved in 25 mL of methanol/dichloromethane (1:19), and the solution was applied to a column of silica gel 60 wetted with methanol/dichloromethane (1:19). The column was eluted with 500 mL of methanol/dichloromethane (1:19), 500 mL of methanol/dichloromethane (7:93), and 500 mL of methanol/dichloromethane (1:9), with collection of 50-mL fractions. The fractions containing the highest R_f major spot were combined and spin evaporated *in vacuo* at 65 °C under high vacuum for 0.5 h to give 0.36 g (21%) of **21** as a light yellow oil, contaminated with 6% of methanol. TLC: dichloromethane/methanol (9:1), R_f = 0.50. UV (CH_3OH): λ_{max} 283, 248; λ_{min} 262, 233. ^{1}H NMR (DMSO-d_6): δ 7.96 (s, 1H, purine H-8), 7.4–7.1 (m, 4H, ArH), 6.44 (br s, NH_2), 5.26 (s, 2H, NCH_2), 4.56 (m, 4H, 2CH + OCH_2), 3.88 (s, 2H, SCH_2), 3.70 (m, 2H, OCH_2), 2.67 (d, 2H, CH_2P), 1.24 (m, 12H, 4 × CH_3). MS: *m/e* 524 (MH^+), 492 $(M - OCH_2)^+$, 213 $(C_7H_{18}O_3PS)^+$, 171 $(C_4H_{12}O_3PS)^+$, 129 $(CH_6O_3PS)^+$. Anal. ($C_{23}H_{34}N_5O_5PS \cdot CH_4O$) C, H, N, S.

(Diisopropylphosphono)methyl 4-Toluenesulfonate (22). This compound was prepared in an analogous manner for the preparation of (diethylphosphono)methyl 4-toluenesulfonate.[35] Purified[37] *p*-toluenesulfonyl chloride (13.1 g, 68.7 mmol) was dissolved in diethyl ether (100 mL). The resultant solution was added slowly over the course of 1 h to a stirring mixture of diisopropyl (hydroxymethyl)phosphonate (13.2 g, 67.3 mmol), triethylamine (9.60 mL, 68.9 mmol), and diethyl ether (100 mL) cooled to −70 °C under a nitrogen atmosphere. The reaction was allowed to warm to ambient temperature and stirred for 18 h. The mixture was suction filtered, and the solids were rinsed with diethyl ether (200 mL). The combined filtrates were concentrated under reduced pressure to an oil which was dissolved in dichloromethane (20 mL). The solution was suction filtered through silica gel 60 (5 cm) in a sintered glass funnel, and the pad was rinsed with dichloromethane (600 mL). The combined filtrates were concentrated under reduced pressure to a yellow oil which was applied to a column of silica gel 60 wetted with ethyl acetate/hexane (1:1). The column was eluted with ethyl acetate/hexane (1:1) (2 L), and the fractions that contained product (lowest R_f spot) were combined and spin evaporated *in vacuo* to give 13.1 g (56%) of **22**.[25] TLC: dichloromethane/methanol (19:1). ^{1}H NMR ($CDCl_3$): δ 7.56 (AB q, 4H, ArH), 4.73 (m, 2H, 2 × CH), 4.10 (d, 2H, J = 10.1 Hz, CH_2P), 2.44 (s, 3H, $ArCH_3$), 1.29 (pseudotriplet, 12H, 4 × CH_3). MS: *m/e* 351 (MH^+), 309 $(MH - C_3H_6)^+$, 267 $(MH - 2(C_3H_6))^+$, 197 $(MH - C_7H_7SO_2)^+$. Anal. ($C_{14}H_{23}O_6PS$) C, H, S.

Diisopropyl (Hydroxymethyl)phosphonate (23). This compound was prepared in an analogous manner to the preparation of diethyl (hydroxymethyl)phosphonate.[26] A mixture of paraformaldehyde (15.0 g, 0.500 mol), diisopropyl phosphite (83.1 g, 0.500 mol), and triethylamine (5.06 g, 0.050 mol) was stirred under a nitrogen atmosphere and heated to 125–130 °C for 3 h. The reaction was cooled, and the volatiles were removed by rotary evaporation under reduced pressure. The residual liquid was distilled in a horizontal molecular still under high vacuum. A liquid fraction was collected between 90 and 100 °C. Upon cooling to ambient temperature, 64.9 g (65%) of **23** was obtained as deliquescent crystals. Mp: 22.5–24.5 °C (lit.[25] bp: 105–113 °C at 0.005 mmHg). ^{1}H NMR ($CDCl_3$): δ 4.74 (m, 2H, 2 × CH), 3.82 (d, 2H, J = 6.08 Hz, CH_2P), 3.78 (br s, 1H, OH), 1.32 (d, 12H, 4 × CH_3). MS: *m/e* 197 (MH^+), 155 $(MH - C_3H_6)^+$, 113 $(MH - 2(C_3H_6))^+$. Anal. ($C_7H_{17}O_4P \cdot 0.25H_2O$) C, H.

Diisopropyl 2-Propenylphosphonate (24). A magnetically stirred mixture of allyl bromide (10.0 mL, 0.116 mol) and triisopropyl phosphite (30.1 mL, 0.116 mol) was heated at 120 °C for 3 h. The 2-bromopropane byproduct was collected in a Dean–Stark trap. The reaction mixture was distilled under high vacuum to give several fractions. Only the final fraction collected was free of triisopropyl phosphite, which gave 8.6 g (36%) of **24** as a colorless liquid. Bp: 51–56 °C at 0.5–0.6 mmHg (lit.[27] bp: 63 °C at 2 mmHg). ^{1}H NMR ($CDCl_3$) (lit.[38] ^{1}H NMR is identical): δ 5.78 (m, 1H, =CH), 5.17 (m, 2H, $=CH_2$), 4.68 (m, 2H, 2 × CH), 2.55 (m 2H, CH_2P), 1.30 (m, 12H, 4 × CH_3).

Enzyme Assays. Purine nucleoside phosphorylase (PNPase) was purified from human erythrocytes and assayed as described previously,[15,16] except that the reactions also contained 0.1 mM Na_2EDTA and 100 mM sodium HEPES buffer (pH 7.4), which was used instead of Tris-HCl. The compounds tested as PNPase inhibitors did not interfere with the xanthine oxidase-coupled PNPase assay since xanthine oxidase was not rate limiting in the presence of any of the compounds. In addition, none of the compounds (1.0 mM) were detectably oxidized by bovine milk xanthine oxidase (<0.1% of the rate with 0.1 mM xanthine) when assayed at pH 7.4 using the ferricyanide assay.[39]

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