difference NOE spectrum of the 3′-*O*-(*tert*-butyldimethylsilyl) derivative of **37** yielded enhancements at 6-H and 5-CH_3 when 5′-H was irradiated and at 1′-H when 4′-OCH_3 was irradiated.

Biological Methods. The CD^{4+} T cell lines A3.01, H9, and MT-2, as well as the lab virus strain HIV-1 LAV and the clinical isolates G762-3 (pre-AZT treatment), G691-2 (post AZT treatment), H112-2 (pre-AZT treatment), and G910-6p2 (post-AZT treatment), were obtained from the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases (NIAID), NIH. PBL were obtained from the Stanford Blood Bank, Stanford University, Stanford, CA. The cells were infected with the virus for 3 h at 37 °C in 5% CO_2 in air. Cells were mock-infected at the same time to be used to detect cytotoxicity and to serve as cell controls. After the 3-h infection incubation, the cells were washed to remove unadsorbed virus. Test compounds were serially diluted 3-fold in 96-well plates. AZT was diluted 5-fold. The infected and uninfected cells were added to appropriate wells of the plate. For A3.01, H9, and PBL cells, the plates were incubated for 7 days with a change of medium and compound on day 4. At the end of the 7-day incubation period the cells were evaluated for cytotoxicity by visual inspection. Cytotoxicity was graded subjectively using cell morphology and cell death as criteria. For MT-2 cells, the plates were incubated for 4 days and cytotoxicity was evaluated by trypan blue dye exclusion with a hemocytometer. CC_{25} indicates the concentration with which about 25% cell destruction was observed. CC_{100} accordingly is the concentration at which complete destruction of the cell layer was observed. Virus levels were determined by two methods: For A3.01, H9, and PBL cells, a reverse transcriptase assay[54] was carried out on the cell supernatants. For MT-2 cell, p24 core antigen levels were determined with the Du Pont p24 antigen test kit. IC_{50} is the concentration of the test substance which reduced virus levels by 50% compared with control cultures. SI, the selectivity index, is the ratio of CC_{25}/IC_{50}.

Acknowledgment. Thanks are due to J. Nelson, Dr. M. Maddox, and L. Kurz for assistance in obtaining and interpreting the NMR spectra and to Nicole Grinder for expert manuscript preparation.

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Inhibitors of Human Purine Nucleoside Phosphorylase. Synthesis and Biological Activities of 8-Amino-3-benzylhypoxanthine and Related Analogues

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A series of 3-substituted hypoxanthines (**6–10, 14–17**) and related analogues (**22, 23**) have been synthesized as inhibitors of purine nucleoside phosphorylase (PNP), which may conceivably act as T-cell-selective immunosuppressive agents with potential utility in autoimmune disorders such as rheumatoid arthritis, in organ transplantations, and in T-cell leukemias. The compounds were evaluated for their PNP activity by a radiochemical assay and also for their cytotoxic effects on a T-lymphoblastoid cell line (MOLT-4). Appropriate substitutions on 3-benzylhypoxanthine (**7a**) (IC_{50} in PNP assay, 112 μM; IC_{50} in MOLT-4 assay, 204.2 μM) increase potency: 8-amino (**17a**; 42.6, 65.2), 2-hydroxy (**9a**; 13.4, 28.6), 2-amino (**10a**; 11.4, 29.1), and 2,8-diamino (**16a**; 5.0, 11.9). Variation of the 3-aryl substituents of **16a** as in **16b–d** has thus far failed to further increase potency. Replacement of the 6-oxygen function in **7a** with the analoguous nitrogen or sulfur functions, as in **22a** and **23a**, resulted in little change in activity. Other variations including the increase of the 3-aliphatic chain length as in **6h** and **7h** (n = 2), the substitution of the phenyl ring with electron-withdrawing groups as in **7e–g**, and replacement of the 2-hydrogen with methylthio as in **8a** and **14a** resulted in decrease of activity. The values for **16a–d** represent moderate but significant activities, as compared to the most active inhibitor presently known, 8-amino-9-thienylguanine (**1c**; 0.17, 0.82). 2,8-Diamino-3-substituted hypoxanthines (**16a–d**) represent a novel structural type hitherto unreported in the literature, and efficient methodologies for their synthesis were developed in the present studies. The formation of the aminoimidazole moiety occurred through a base-catalyzed 1,5-(*O*→*N*)-carbamimidoyl rearrangement (**13** to **14**, **20** to **16**).

Introduction

Human purine nucleoside phosphorylase (PNP) (EC 2.4.2.1), an essential enzyme of the purine salvage pathway, catalyzes a reversible, phosphorolytic cleavage of ribo- and deoxyribonucleosides of guanine and hypoxanthine.[1] Genetic deficiency of PNP is generally associated with severe selective impairment in T-, but not B-lymphocyte function.[2] Conceivably, inhibitors of PNP should similarly induce a selective suppression of T-cell-mediated immunity.[3-5]

T-cell-selective immunosuppressive agents are potentially useful in the treatment of autoimmune disorders such as rheumatoid arthritis (RA), in the prevention of rejection in bone marrow or organ transplants, and in the treatment of T-cell leukemias.[3-6] One of these, cyclosporine, is now an accepted prophylactic medication in allogenic organ transplants[6a] and has demonstrated efficacy in clinical studies for the treatment of RA.[6b,c] The implication of T cells in the pathogenesis of RA is also supported by the success of lymphoid irradiation,[7a] lymphapheresis,[7b] or

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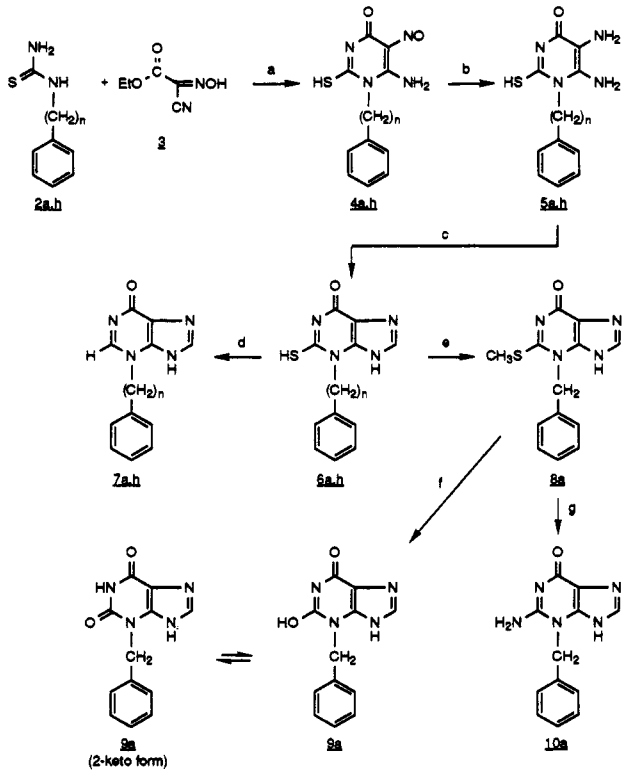
Scheme I.[a] Synthesis of 3-Benzylhypoxanthine (**7a**), 3-Phenethylhypoxanthine (**7h**), and Related Analogues **4–6** (**a**,**h**), **8–10** (**a**)



a n = 1; **h** n = 2

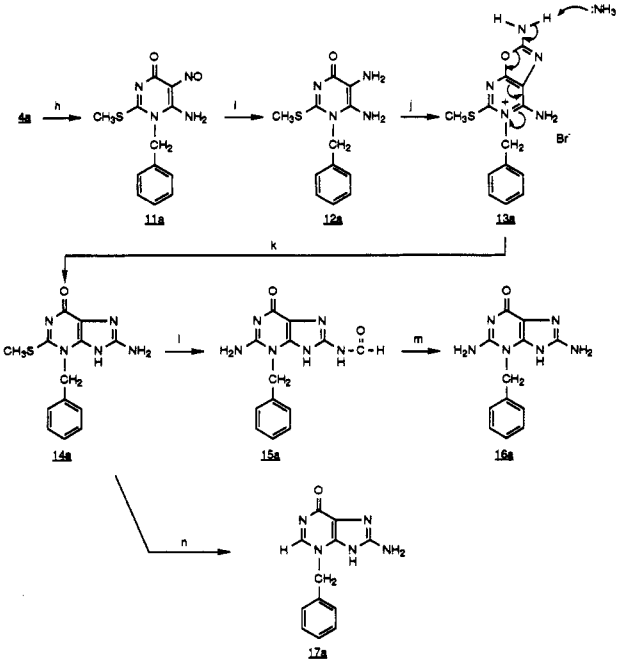
[a] Reagents, yields for series **a**, and yields for series **h**: (a) 3 NaOEt, 65%, 89%; (b) $Na_2S_2O_4$, 73%, 95%; (c) formamide, 160 °C, 90%, 92%; (d) Ni (Raney), 75%, 57%; (e) CH_3I, 96%; (f) H_2O_2, HOAc, 84%; (g) formamide, 195 °C, 58%.

thoracic duct drainage[7c] in the heroic treatment of refractory RA patients.

8-Aminoguanosine (**1a**), a potent PNP inhibitor, has served as a standard for investigation of the effects of PNP inhibitors on lymphocytes and lymphoblasts.[5] Recently, the replacement of the ribosyl moiety at N-9 by acyclosugars[3c] or other substituents such as the 2-thienylmethyl and benzyl groups (as in **1c** and **1b**, respectively; Table I)[3a,b] resulted in enhanced activity.

Our continuing interest in PNP inhibitors has led to the investigation of the structurally related 3-benzylhypoxanthines (**6–10, 14–17**) and other analogues (**22** and **23**), particularly those with 2,8-diamino substitution (**16a–d**). The synthesis and biological activities of these compounds are hereby presented.

Scheme II.[a] Syntheses of 8-Amino-3-benzylguanine (**16a**) and Some 3-Benzylhypoxanthine Analogues (**14a**,**15a**,**17a**)



[a] Reagents and yields: (h) CH_3I, 73%; (i) $Na_2S_2O_4$, 83%; (j) BrCN, 93%; (k) NH_3, 89%; (l) formamide, 200 °C, 89%; (m) 1 N NaOH, 65 °C, 87%; (n) NaOH, Ni (Raney), 70%.

Chemistry

Synthetically, the prime targets in the present study were the N-3-substituted 8-aminoguanines (**16a–d**) (Schemes II and III), a new structural type hitherto unreported in the literature. For this purpose, efficient methodologies designed for the incorporation of the 8-amino and the 3-amino groups were developed as shown in Schemes I–III.

For structure–activity correlation, the present approach to the synthesis of 3-benzylguanine (**16a**; Scheme II) was envisioned as highly versatile in providing substituent variations at many different positions of the parent molecule 3-benzylhypoxanthine (**7a**; Scheme I). Thus the analogues would contain: at C-8, amino or acylated amino (**14–17**); at C-2, amino (**10, 15,** and **16**), hydrogen (as in **17**), or other substituents (as in **6, 8, 10,** and **14**); and at N-3, varying chain length (as in **6h** and **7h**, where $n = 2$) and aromatic substituents (methoxy as in **16d**, halogen, alkyl, etc.) and other heterocycles like thiophene and furan (as in **16b** and **16c**).

As points of synthetic interest, the transformation of **2** to **4** (Scheme I) required 3 equiv of base; no reaction occurred when 1 equiv was used.[8] The transformation of

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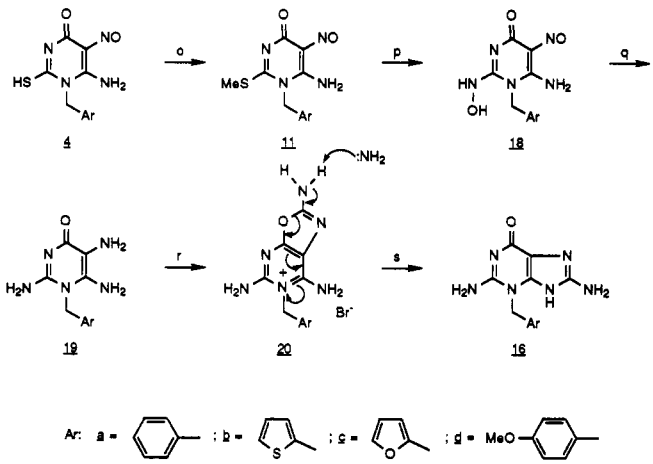
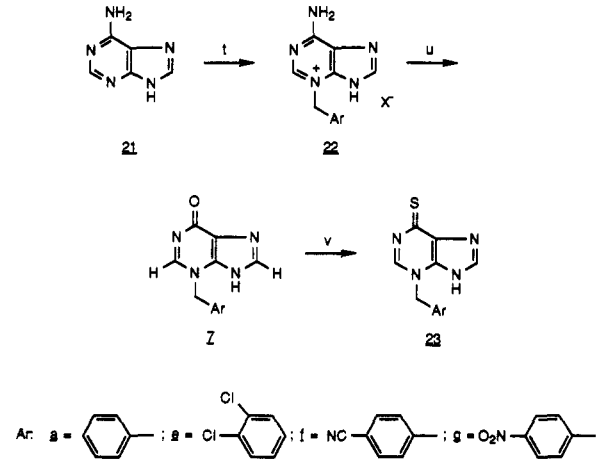
Scheme III.[a] Syntheses of 8-Amino-3-benzylguanine (**16a**) and Analogues **16b–d**



[a] Reagents: (o) CH_3I, NaOH; (p) NH_2OH·HCl, NaOH, H_2O; (q) $Na_2S_2O_4$/NaOH or Zn/HCl; (r) BrCN; (s) NH_3/DMF.

Scheme IV.[a] Syntheses of Some 3-Substituted Purine Analogues (**7,22,23**) (**a,e,f,g**)



[a] Reagents: (t) $ArCH_2X$, DMA, X = Br, Cl; (u) NaOCl, Ac_2O, pyridine; (v) P_2S_5, pyridine.

11a to **12a** (Scheme II) gave impure product with a low yield of 12% at first. However, with the observation that **11a** was highly sensitive to base, the dithionite reduction was carried out in neutral conditions, and the yield was increased to 93%. Desulfurization by Raney nickel in DMF (**6h** to **7h**; Scheme I) resulted in contamination of the product by nickel ions, which were difficult to remove. However, the problem was effectively eliminated by the use of methoxyethanol–aqueous sodium hydroxide as solvents (**6a** to **7a**; Scheme I) (**14a** to **17a**; Scheme II).

The introduction of an amino group to C-2 by azide displacement of the methylthio group in **8a** (Scheme I) did not proceed cleanly. On the other hand, the 2-methylsulfonyl group appeared to be highly susceptibile to nucleophilic displacement, as only the displacement product **9a**, bearing a hydroxyl group at C-2, was obtained during the oxidation of the methylthio group in **8**. However, the use of boiling formamide led to the desired product 2-amino-3-benzylhypoxanthine (**10a**).

Direct functionalization of C-8 of **7a** (Scheme I), for the purpose of subsequent amination, was unsuccessful with *N*-bromosuccinimide in acetic acid or with *p*-chlorodiazonium salt. Attempted aminoimidazole ring formation from **12a** (Scheme II) using cyanamide at 130 °C gave no reaction, and attempted thioimidazole formation using thiophosgene led to a mixture of products.

Reaction of **12a** (Scheme II) with cyanogen bromide led to the formation of a fused oxazole ring involving the 5-amino and the 6-oxo functions, giving the pyrimidinium salt **13a** in 38% yield. The yield was subsequently raised to 93% by an improved procedure. Upon treatment of **13a** with ammonia, a 1,5-(*O*→*N*)-carbamimidoyl rearrangement occurred to give the desired product **14a** in 89% yield. The rearrangement was apparently initiated by deprotonation of the amino group of the oxazole ring, followed by reaction of the resulting carbodiimide with the amino group on the pyrimidine ring to give the desired aminoimidazole in **14a**.

Reaction of **14a** (Scheme II) with formamide at 200 °C, analogous to conditions previously used for the 3-methyl analogue[9] gave **15a** in 89% yield, and hydrolysis of the formyl group in **15a** gave **16a** in 87% yield. However, the application of this method to the synthesis of 3-(4-methoxybenzyl)guanine (**16d**; Scheme III) was unsuccessful, as the displacement of the 3-thiomethyl group from the analogous precursor **14d** (**14a**, benzyl = 4-methoxybenzyl; Scheme II) failed.

As an improved, alternate methodology generally applicable to the synthesis of the various 3-arylmethyl analogues of **16a** (Scheme III), the 3-amino function was first introduced to **11** before the construction of the 8-aminoimidazole moiety. It has been reported that the 2-methylthio group is more easily displaced at the pyrimidine stage, particularly if there is an electron-withdrawing group at the 5-position.[10]

Accordingly, **11a–d** (Scheme III) were treated with aqueous ammonia, heat was required, and several products were formed. However, the 2-methylthio group could be easily displaced with hydroxylamine at room temperature under neutral conditions, giving the 2-hydroxyamino derivatives **18a–d**. Treatment of **18a–d** with either zinc/hydrochloric acid or sodium dithionite/sodium hydroxide reduced both the 5-nitroso and the 3-hydroxyamine group to give the triamines **19a–d**. Reaction of **19a–d** with cyanogen bromide again gave the fused (aminooxazo)pyrimidinium salts **20a–d**, which rearranged (in an analogous manner as with **13a** above) to give the target compounds **16a–d**.

The syntheses of **7a**, **22a**, and **23a** were accomplished by methods previously reported in literature[11] as shown in Scheme IV. The new analogues **7e–g** and **22e–g** were synthesized by these methods.

The sample of 3-benzylhypoxanthine (**7a**) prepared as shown in Scheme I was identical to that from Scheme IV,[11] thereby confirming the course of reaction between **2a** and **3**, leading to N-3 substitution, as depicted in Scheme I, instead of N-1 substitution, which would have resulted from an alternative pairing of the reactive centers in the two compounds. As further confirmation, the samples of **7a** prepared by both methods were clearly distinct from a sample of 1-benzylhypoxanthine prepared from the re-

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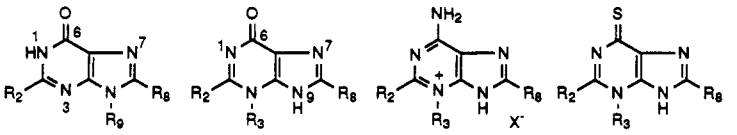
Table I. Activities of Selected Standards (**1a–e**), 3-Substituted Hypoxanthines (**6–10, 14–17**), and Related Analogues (**22, 23**)



1 6-10, 14-17 22 23

compd	R_2	R_8	R_3	R_9	PNP-4:[b] IC_{50}, μM (% inhibition)	HTBA-1; T-cell + dGuo (10 μM): IC_{50}, μM
1a	NH_2	NH_2		Rib	1.40	3.25
1b	NH_2	NH_2		$C_6H_5CH_2$	0.47	0.77
1c	NH_2	NH_2		2-$ThCH_2$	0.17	0.82
1d	NH_2	NH_2		2-$FuCH_2$	0.25[c]	1.47[c]
1e	NH_2	H		$C_6H_5CH_2$	29.6[d]	35.0[d]
6a	SH	H	$C_6H_5CH_2$		<300 (78.3%)	
6b	SH	H	$C_6H_5CH_2CH_2$		>300 (10.9%)	
7a	H	H	$C_6H_5CH_2$		112; <300 (81.0%)	204.2
7e	H	H	3,4-diCl-$C_6H_3CH_2$		>300 (36.9%)	
7f	H	H	4-CN-$C_6H_4CH_2$		>300 (22.2%)	
7g	H	H	4-NO_2-$C_6H_4CH_2$		>300 (13.1%)	
7h	H	H	$C_6H_5CH_2CH_2$		>150 (26.8%)	
8a	SCH_3	H	$C_6H_5CH_2$		>150 (23.3%)	
9a	OH	H	$C_6H_5CH_2$		13.4	28.6
10a	NH_2	H	$C_6H_5CH_2$		11.4	29.1
14a	SCH_3	NH_2	$C_6H_5CH_2$		>75	
15a	NH_2	NHCHO	$C_6H_5CH_2$		>150	
16a	NH_2	NH_2	$C_6H_5CH_2$		5.0	11.9
16b	NH_2	NH_2	2-$ThCH_2$		4.6	>25
16c	NH_2	NH_2	2-$FuCH_2$		5.2	42.5
16d	NH_2	NH_2	4-MeO-$C_6H_4CH_2$		8.9	
17a	H	NH_2	$C_6H_5CH_2$		42.6	65.2
22a (Cl^-)	H	H	$C_6H_5CH_2$		95	
22g (Br^-)	H	H	4-NO_2-$C_6H_4CH_2$		*e*	
23a	H	H	$C_6H_5CH_2$		131	

[a] Th = Thieno; Fu = furan; Rib = β-D-ribofuransoyl. [b] Format for data preceded by ">" or "<": μM concentration tested (% inhibition at indicated concentration). [c] Reference 3a. [d] J. C. Sircar, et al., unpublished data. [e] Insoluble.

ported procedure.[12] An analogous course of interactive pairing has also been reported for a related reaction between ethyl cyanoacetate and *N*-methylthiourea.[13]

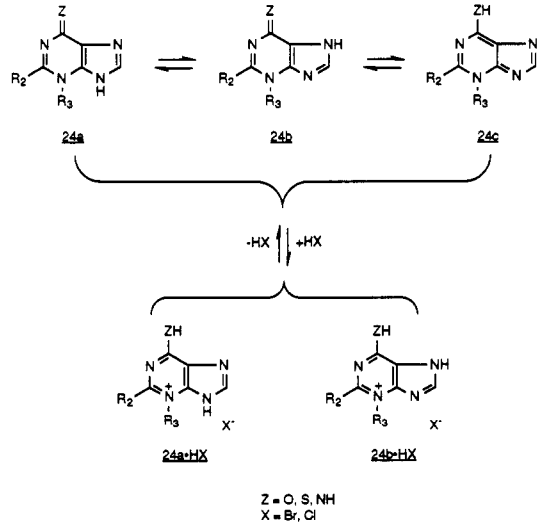
The structures of 3-benzylhypoxanthine and its related analogues in the present study have been represented as the para-quinoid form **24a** (Scheme V; Z = O or S), with the exception of the 3-(arylmethyl)adenine salts **22**, which have been represented as **24a·HX** (Z = NH). However, other tautomeric forms are possible, such as **24b** and the 6-hydroxy or thiol forms **24c**. Obviously, the free base forms of **22**, i.e., 3-(arylmethyl)adenines, should also exist in one or more of the forms **24a–c** and therefore be considered as the 6-nitrogen analogues of the other compounds bearing 6-oxygen or 6-sulfur functions.

Biological Results and Discussion

The biological activities of various compounds, determined by methods previously described,[14] are shown in Table I. The PNP inhibitory activities of the compounds were evaluated by a radiochemical assay. Their cytotoxic effects on the growth of human T- and B-lymphoblastoid cell lines (MOLT-4 and MGL-8, respectively) were evaluated by measuring the inhibition of thymidine uptake in the presence of 10 μM deoxyguanosine. In the presence of an inhibitor of PNP, exogeneously added 2′-deoxyguanosine has been found to be highly cytotoxic for T-lymphoblasts, presumably because it is phosphorylated to dGTP, a potent inhibitor of ribonucleotide reductase.

As indicated in Table I, 3-benzylhypoxanthine (**7a**) was found to be moderately active in the PNP assay and weakly active in the HTBA-1 assay. These activities were

Scheme V. Possible Tautomeric Forms of 3-Substituted Hypoxanthines (Z = O) and the Corresponding 6-Hetero Analogues (Z = S, NH), as Free Base (**24a–c**) and Salts (**24a·HX, 24b·HX**; X = Cl, Br)



24a 24b 24c

-HX +HX

24a·HX 24b·HX

Z = O, S, NH
X = Br, Cl

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modified by substituent variations, and certain trends are apparent. First, activities were enhanced by substitution of **7a** with appropriate hydrogen-bonding groups at C-2 and, to a lesser extent, at C-8, as shown by 2-hydroxyl substitution in **9a**, 2-amino substitution in **10a**, and 8-amino substitution in **17a**, respectively. Concurrent 2,8-diamino substitution (**16a**) resulted in the best activity of 5.0 μM in the PNP assay and 11.9 μM in the MOLT-4 assay. However, C-2 substitution with the larger thiol group resulted in little change or decrease in activities, as in **6a** (vs **7a**) and **6h** (vs **7h**), respectively. Secondly, substitution of the benzyl group by other heteroarylmethyl groups (as in **16b–d**) failed to improve activities. Thirdly, the replacement of the 6-hydroxyl group with an amino or mercapto group as in **22** and **23**, respectively, resulted in little change in activities. Finally, other variations including the increase of the spacer group between the aryl and purine moieties as in **7h** and **6h** (n = 2), the substitution of the phenyl ring with electron-withdrawing groups as in **7e–g**, and the replacement of 2-hydrogen with a methylthio function as in **8a** and **14a** all resulted in decrease of activities.

The comparable activities of 3-benzylhypoxanthine (**7a**) vs its corresponding 6-nitrogen analogue **22a** (3-benzyladenine; cf. Scheme V) and 6-sulfur analogue **23a**, all moderate inhibitors, as well as the comparable activities of 2-aminohypoxanthine (**10a**) vs its 2-hydroxy analogue **9a** (3-benzylxanthine), both relatively strong inhibitors, are particularly noteworthy and reflect the nature of the probable hydrogen-bonding environments of the enzyme at the proximities of the 6,1- and 2,1-positions, respectively. The moderate enhancement of activities from the 8-amino analogue **17a** over **7a** may similarly indicate a hydrogen-bonding environment at the vicinity of the 8-position. The requirement of a precise spacer, as indicated by the superior activities of the 3-arylmethyl analogues **6a** and **7a** relative to the respective 3-arylethyl analogues **6h** and **7h**, reflects the geometry of the lipophilic binding region of the enzyme near the 3-position. This region is obviously crucial for the activities of the series of compounds in the present study and could be part of the same region which binds to the aryl or heteroaryl groups of the 9-substituted guanine analogues **1b–e**.

3-Substituted hypoxanthine and guanines are generally less potent than the corresponding 9-substituted guanine analogues, and the potencies also appear to be less susceptible to substituent changes than in the 9-substituted series. A clear similarity in the two series is the enhancement effect of the C-8 amino substituent (cf. **16a** vs **10a**, **17a** vs **7a**, and **1b** vs **1e**). In contrast, the replacement of benzyl with 2-thienylmethyl or 2-furanylmethyl groups, which has a significant enhancing effect in the 9-substituted guanine series (cf. **1b–d**), did not increase activity in the 3-substituted guanine series (cf. **16a–d**).

The best compound in the 3-substituted series, 8-amino-3-benzylguanine (**16a**), shows somewhat lower activity than the standard 8-aminoguanosine (**1a**). Nevertheless, unlike 8-aminoguanosine, 8-amino-3-benzylguanine is not a substrate of PNP and is therefore more advantageous as a potential medicinal agent in this respect.

However, 8-amino-3-benzylguanine (**16a**) shows 10 times lower PNP inhibitory activity than the corresponding 9-benzyl analogue **1b** and 30 times lower activity than 8-amino-9-(2-thienylmethyl)guanine (**1c**; PD119229 or CI-950).[3a,b] Thus, for the 3-substituted series to be a viable choice for further investigation, the compounds would need to show superiority in other factors such as bioavailability, oral absorption, and toxicity. Subsequent study showed, however, that **16a** had no better in vivo effects than CI-950 (**1c**).

Experimental Section

Pharmacology. Human erythrocyte PNP activity was assayed by measuring the conversion of [8-^{14}C]inosine to [8-^{14}C]hypoxanthine according to a chromatographic method previously described.[14] For assay of toxicity in the human MOLT-4 T-lymphoblast tissue culture system, compounds were tested for their ability to potentiate the cytotoxicity of 2′-deoxyguanosine in the presence or absence of 10 μM 2′-deoxyguanosine, as quantitated by measurement of [^{3}H]thymidine incorporation.[14]

Chemistry. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were determined on a Varian EM 390 or Brucker XL200 spectrometer using tetramethylsilane as internal standard. MS data were obtained with a Finnigan 1015 quadrupole spectrometer. Where not reported, spectral data agreed with the proposed structures. TLC analysis was carried out on silica gel 60 F_{254} plates, 0.25 mm (Merck, Darmstadt), using the following solvent systems: A. toluene–THF–89% formic acid–*tert*-butyl alcohol–water, 55:34:10:0.5:0.5; B. toluene–methanol–triethylamine, 14:7:1; C. chloroform–methanol–15 N ammonia, 79:20:1. Analytical results are within 0.4% unless otherwise indicated.

6-Amino-2-mercapto-5-nitroso-1-(phenylmethyl)-4(1*H*)-pyrimidinone (4a). To a solution of sodium ethoxide, prepared from 1.11 g of sodium (48.3 mmol) and 30 mL of ethanol, was added 2.35 g (16.0 mmol) of 97% ethyl cyanoglyoxylate 2-oxime (**3**). The mixture was stirred, and a solution of 2.66 g (16.0 mmol) of 1-benzyl-2-thiourea (**2a**) in 21 mL of 2-methoxyethanol was added. The resulting orange solution was heated under reflux for 5.5 h, cooled to 0 °C, and treated with 48 mL of water. The deep red solution, pH 13.4, was acidified with stirring to pH 2.0 with 46 mL of 1 N HCl. The solid was filtered, washed three times with 3-mL portions of water, and dried in vacuo overnight to give 2.73 g (65%) of deep blue product: mp 190 °C (dec with a puff of smoke); TLC, R_f 0.36 (solvent system A). Anal. ($C_{11}H_{10}N_4O_2S$) C, H, N, S.

6-Amino-2-mercapto-5-nitroso-1-(2-phenylethyl)-4(1*H*)-pyrimidinone (4h) was similarly prepared as for **4a**: yield 88.7%; mp 193 °C. Anal. ($C_{12}H_{12}N_4O_2S\cdot0.8H_2O$) C, H, N, S.

5,6-Diamino-2-mercapto-1-(phenylmethyl)-4(1*H*)-pyrimidinone (5a). To a solution of 1.60 g of **4a** in 22.5 mL of 1 N sodium hydroxide, a solution of 3.44 g of sodium dithionite in 30 mL of water was added with magnetic stirring in 3.5 min during which time the temperature rose from 23 to 35 °C. After 50 min, the precipitate was filtered, washed with water, and dried in vacuo overnight to 1.108 g (73.1%) of **5a**: mp 229–231 °C; TLC R_f 0.12 (solvent A; R_f of **6a** 0.30). Anal. ($C_{11}H_{12}N_4OS$) C, H, N, S.

5,6-Diamino-2-mercapto-1-(2-phenylethyl)-4(1*H*)-pyrimidinone (5h) was similarly prepared as for **5a**: yield 95.0%, mp 245–247 °C. Anal. ($C_{12}H_{14}N_4OS$) C, H, N, S.

3,9-Dihydro-2-mercapto-3-(phenylmethyl)-6*H*-purin-6-one (6a). A mixture of 615 mg (2.48 mmol) of **5a** and 2.8 mL of formamide was heated for 32 min in an oil bath at 160–163 °C. Complete solution occurred after 2 min, and bubbling occurred. The crystals which formed rapidly upon cooling were filtered, washed with water, and dried in vacuo overnight to give 570 mg (90%) of **6a**; mp 216–218 °C. Anal. ($C_{12}H_{10}N_4OS$) H, N, S; C: calcd, 55.80; found, 55.22.

3,9-Dihydro-2-mercapto-3-(2-phenylethyl)-6*H*-purin-6-one (6h). A mixture of 6.27 g (23.9 mmol) of **5h** and 34 mL of formamide was heated with stirring at 160 °C for 45 min and cooled. The crystalline precipitate was filtered, washed with formamide, ethanol, and ether, and dried in vacuo to give 5.97 g of product, mp 320–328 °C. Anal. ($C_{13}H_{12}N_4OS$) C, H, N, S.

3,9-Dihydro-3-(phenylmethyl)-6*H*-purin-6-one (3-Benzylhypoxanthine; 7a). (i) Method of Scheme I. Raney 3100, active molybdenum-promoted nickel catalyst in water, from Davison Chemical, was washed four times with 2-methoxyethanol, and 0.45 mL of the centrifuged, wet preparation (0.98 g) was added to a solution of 104.5 mg of **6a** in 0.5 mL of 1 N sodium hydroxide and 2.35 mL of 2-methoxyethanol. The mixture was heated at 63–68 °C for 1 h, at which time TLC (solvent system A; R_f of **7a** 0.24; R_f of **6a** 0.67) showed that reaction was complete. After cooling to room temperature, the mixture was filtered through

Supercel, and the solid was washed two times with 1-mL portions of 2-methoxyethanol. The clear filtrate, pH 12.9, was neutralized to pH 6.8 with glacial acetic acid, and evaporated to give 384 mg of a clear yellow oil. The crystals obtained by treatment with 0.55 mL of water were filtered, washed with water, ethanol, and ether, and dried to give 59.8 mg of **7a**, mp 269–272 °C dec (lit. 245–247 °C[11c]). The filtrate and washings were evaporated and worked up in a similar manner to give an additional 9.1 mg of product; total yield from the two crops was 75%.

(ii) Method of Scheme IV. Compound **7a** was also prepared from deamination of the free base from **22a** with nitrosyl chloride in acetic anhydride according the procedure of Montgomery and Thomas,[11c] mp 258–263 °C dec, mixed mp with sample from (a) 261–266 °C. Anal. ($C_{12}H_{10}N_4O$) C, H, N.

(iii) Differentiation from 1-Benzylhypoxanthine. 1-Benzylhypoxanthine was prepared by benzylation of hypoxanthine under the conditions described by Shaw,[12] and the syrupy crude product was purified by crystallization from methanol. Attempts to prepare the initial crystal seed by the reported procedure failed. It was, however, obtained by preparative TLC using solvent system C. The crystalline 1-benzylinosine was converted to 1-benzylhypoxanthine hydrochloride by hydrolysis in 6 N ethanolic hydrogen chloride (67 °C, 37 min) followed by evaporation and crystallization from ethanol. A methanolic solution of the hydrochloride was treated with 1.28 equiv of 1 N sodium hydroxide and evaporated to dryness, and the residue was triturated with water to give 1-benzylhypoxanthine free base, mp 269–275 °C (lit. mp 268–270 °C[12]).

The sample of **7a** prepared from methods of Scheme I was identical to that from Scheme IV, according to HPLC and TLC (R_f 0.31, system C). They were clearly different from the sample of 1-benzylhypoxanthine prepared as described above (R_f 0.51, system C).

3-[(3,4-Dichlorophenyl)methyl]-3,9-dihydro-6*H*-purin-6-one (7e) was prepared by deamination of **22e** with nitrosyl chloride in acetic anhydride–acetic acid–pyridine according to "Method of Scheme IV" as described for **7a** above,[11c] mp 307–310 °C. Anal. ($C_{12}H_8Cl_2N_4O\cdot0.25H_2O$) C, H, N; Cl: calcd 23.67; found, 24.38.

3-[(4-Cyanophenyl)methyl]-3,9-dihydro-6*H*-purin-6-one (7f) was prepared by the same procedure as for **7e**, mp 264–266 °C. Anal. ($C_{13}H_8N_5\cdot0.4H_2O$) C, H, N.

3,9-Dihydro-3-[(4-nitrophenyl)methyl]-6*H*-purin-6-one (7g) was prepared by the same procedure as for **7e**, mp 303–305 °C (Fisher–Johns). Anal. ($C_{12}H_9N_5O_3\cdot0.15H_2O$) C, H, N.

3,9-Dihydro-3-(2-phenylethyl)-6*H*-purin-6-one (7h). A solution of 1.72 g of **6h** in 50 mL of DMF was treated with 14.5 g of DMF-washed Raney Nickel (Davison) and heated with stirring at 70 °C for 3 h. The mixture was filtered through Celite, and the solid was washed with two 10-mL portions of hot DMF. The dark green filtrate was evaporated in vacuo to a glassy residue, which was then triturated with water to give 1.35 g of a light green solid containing nickel as complex or salt. Anal. [$(C_{13}H_{11}N_4O)_2\cdot Ni\cdot0.2Ni(OH)_2\cdot3.2H_2O$], i.e., [$(C_{13}H_{12}N_4O\cdot0.6Ni(OH)_2\cdot0.6H_2O$] C, H, N. The nickel ions caused severe NMR spectral perturbations and were qualitatively detected by the formation of a red complex when a small amount of the test substance was added to a solution containing 1 or 2 drops of 11.7% dimethylglyoxime in DMF and 1 drop of 15 N ammonia. A portion of the product, 509 mg, was dissolved in 1.5 mL of warm DMF, cooled in ice, and treated with 0.5 mL of 12 N HCl followed by 1 mL of 1 N hydrochloric acid. The solid which gradually formed was collected, dried (419 mg), and found to give a negative test for nickel ions. A suspension of 379 mg of the solid in 1 mL of water was treated with 2.8 mL of 1 N sodium hydroxide. The resulting solution was clarified by filtration, then acidified with 1 mL of 2 N hydrochloric acid. The solid was filtered, stirred with 2.5 mL of 0.13 M aqueous ammonia, filtered, washed with ethanol and ether, and dried to give 302 mg of product, mp 264–274 °C. Anal. ($C_{13}H_{12}N_4O\cdot0.08HCl\cdot0.08H_2O$) C, H, N, Cl.

3,9-Dihydro-2-(methylthio)-3-(phenylmethyl)-6*H*-purin-6-one (8a). A solution of 2.59 g of **6a** in 58 mL of water and 17 mL of 1 N NaOH was stirred with 0.9 mL of methyl iodide (14.56 mmol) until TLC indicated the absence of starting material (6 h). The solid which formed was filtered, washed with water, ethanol, and ether giving 1.02 g (37.9%) of product. The filtrate, pH 10.5, was acidified to pH 2.56 with 3 mL of 2 N HCl, and the precipitate was isolated as above to give an additional 1.6 g (59.5%) of product: mp 278–285 °C; NMR (D_2O + NaOD), SCH_3 peak at δ 2.557; TLC, R_f 0.5 (system A). Anal. ($C_{13}H_{12}N_4OS\cdot0.1H_2O$) C, H, N, S.

3,9-Dihydro-3-(phenylmethyl)-1*H*-purine-2,6-dione (9a). A solution of 136 mg of **8a** in 4.0 mL of glacial acetic acid and 0.30 mL of 30% hydrogen peroxide was heated at 60–62 °C for 20 min and then evaporated in vacuo to give 324 mg of residue. The solid which formed upon treatment with 1.4 mL of water was filtered, washed with water, and dried in vacuo to give 107 mg of **9a** (84%): mp 280–305 °C dec; MS 242.2 (25.61%), 91.1 (100%). Anal. ($C_{12}H_{10}N_4O_2\cdot0.25H_2O$) C, H, N.

2-Amino-3,9-dihydro-3-(phenylmethyl)-6*H*-purin-6-one (3-Benzylguanine; 10a). A mixture of 367 mg (1.35 mmol) of **8a** and 3.7 mL of formamide was heated in an oil bath at 190–192 °C. Complete solution occurred after 4 min, and a stream of gas was evolved. After 1 h the temperature range was increased by 5 °C, and heating was continued for another 0.5 h. Upon cooling, the precipitate was filtered and washed with 0.5 mL of formamide, followed by ether to give a black solid, which was dried in vacuo to 259 mg of crude product. A 46-mg sample of the crude product was heated with 0.24 mL of DMSO at 65 °C, then cooled, and filtered. The solid was washed with ethanol, which removed much of the black color, followed by ether to give 38.4 mg of grayish white product: mp 345–352 °C dec; TLC, R_f 0.1 (solvent A). Anal. ($C_{12}H_{11}N_5O\cdot0.15H_2O$) C, H, N.

6-Amino-2-(methylthio)-5-nitroso-1-(phenylmethyl)-4-(1*H*)-pyrimidinone (11a). To a solution of **4a** 5.2 g (20 mmol) in 100 mL of water and 40 mL of 1 N NaOH was added 1.6 mL of methyl iodide. The mixture was stirred for 15 h, at which time TLC showed that reaction was complete. The solution was clarified by filtration to remove some brown solid (0.53 g after drying). The filtrate, pH 8.67, was neutralized with 6.9 mL of 2 N HCl with stirring to pH 6.9. The precipitate was filtered, washed with water, and dried in vacuo for 2 days to give 4.0 g (73%) of a dark green solid: mp 140 °C dec; R_f 0.1 (solvent system A; R_f of **4a**, 0.45); NMR (DMSO-d_6 + NaOD) δ 2.379 (SCH_3). Anal. ($C_{12}H_{12}N_4O_2S\cdot0.5H_2O$) C, H, N; S: calcd, 11.24; found, 10.80.

5,6-Diamino-2-(methylthio)-1-(phenylmethyl)-4(1*H*)-pyrimidinone (12a). A mixture of **11a** (418 mg, 1.40 mmol), $Na_2S_2O_4$ (926.4 mg, 5.32 mmol), 5 mL of 2-methoxyethanol, and 2.5 mL of water was prepared with ice-cooling. With temperature maintained at 23–26 °C by cooling, 0.89 mL of 1 N sodium hydroxide was added with stirring at such a rate that the pH did not exceed 7.2. After 20 min, the solution, pH 6.7, was filtered to remove insoluble material, which was washed with 0.75 mL of aqueous 2-methoxyethanol. The filtrate was evaporated in vacuo to 1.07 g of solid, which was triturated with about 1.3 mL of water. The product was filtered, washed with 1.8 mL of water, 1 mL of THF–ether (1:4), and ether, and dried in vacuo to 299 mg of **12a** (83% yield): mp 222–223 °C dec; R_f 0.51 (solvent B). Anal. ($C_{12}H_{14}N_4OS\cdot0.3H_2O$) C, H, N, S.

2,7-Diamino-5-(methylthio)-6-(phenylmethyl)oxazolo-[5,4-*d*]pyrimidinium Bromide (13a). To a solution of 745 mg of **12a** in 2.7 mL of 2-methoxyethanol was added 1.62 g of cyanogen bromide (97%). The reaction was slightly exothermic and was cooled to room temperature. After 1 day, the solution was evaporated in vacuo to 1.86 g of dark red oil, which was triturated twice with ether, once with ethanol, filtered, and dried in vacuo overnight to 414 mg of crude product, R_f 0.52 (solvent A).

A portion of the crude product, 388 mg, was heated with 18 mL of ethanol, and a small amount of insoluble solid was filtered. The filtrate was concentrated to 1.73 g, and the solid which formed was filtered, washed with ethanol, and dried in vacuo to 144 mg. This was triturated with 0.2 mL of water, filtered, and dried at 56 °C in vacuo for 4 h and at room temperature for 12 h to give 134 mg (14%) of **13a**: mp 220–230 °C; MS (EI), 287.2 (21.5%). Anal. ($C_{13}H_{13}N_5OS\cdot0.92HBr\cdot0.3C_2H_5OH$) C, H, N, Br, S. Workup of various fractions gave an additional 101 mg of product, giving a total of 235 mg (24.6%).

As an improved procedure, 7.5 mL of 2-methoxyethanol was added to a mixture of 2.61 g (9.96 mmol) of **12a** and 4.58 g (41.9 mmol) of cyanogen bromide in a 100-mL round-bottom flask. The mixture was immediately swirled and cooled slightly with water (room temperature). As soon as most of the solid went into

solution (3.0 min), the product started to crystallize rapidly. After stirring for 1 day, the mixture was filtered, washed with 0.5- and 0.2-mL portions of 2-methoxyethanol, 1 mL of ethanol, then ether, and dried to 2.58 g of **13a**. It expanded to an orange glass at 220–226 °C and melted with decomposition at 248–270 °C. Anal. ($C_{13}H_{13}N_5OS$·HBr) C, H, N, Br, S. The mother liquor was evaporated to a residue, which was triturated with 1 mL of 2-methoxyethanol, filtered, washed, and dried to give an additional 0.85 g of product. Total yield was 3.43 g (93%).

8-Amino-3,9-dihydro-2-(methylthio)-3-(phenylmethyl)-6*H*-purin-6-one (14a). Anhydrous ammonia (73 mg) was bubbled into a solution of 464 mg of **13a** in 7.5 mL of DMF. Precipitate started to form and, after 1 day, was collected by filtration, washed with ethanol followed by ether, and dried at 56 °C in vacuo for 8 h to give 268 mg of **14a**: mp 342–344 °C dec; NMR (TFA) δ 4.104 (s), δ 4.096 (s); TLC, R_f 0.54 (system A). Anal. ($C_{13}H_{13}N_5OS$) C, H, N, S.

***N*-[2-Amino-6,9-dihydro-6-oxo-3-(phenylmethyl)-3*H*-purin-8-yl]formamide (15a).** A mixture of 73.8 mg of **14a** and 1.5 mL of formamide was heated with stirring at 193–195 °C for 36 min and then at 200 °C for 40 min. After the mixture was cooled and filtered, and the solid was washed with ethanol and ether and dried in vacuo at 56 °C for 5.5 h, 60 mg of **15a** was obtained: no mp up to 360 °C, darkening at about 327 °C; R_f 0.05 (solvent A). Anal. Calcd. for $C_{13}H_{12}N_6O_2$: C, 54.92; H, 4.26; N, 29.57. Found: C, 54.33; H, 4.02; N, 30.12.

Similarly, 1.58 g of **14a** was treated with 35 mL of formamide at 205 °C for 75 min to give 1.40 g of **15a** (89.7% yield).

2,8-Diamino-3,9-dihydro-3-(phenylmethyl)-6*H*-purin-6-one (8-Amino-3-benzylguanine; 16a). (i) Method of Scheme II. A mixture of 1.39 g of **15a** in 25.2 mL of 1 N sodium hydroxide was heated with stirring at 64–66 °C for 4 h to give a black solution. After removal of a small amount of black precipitate by filtration, the solution was neutralized by dropwise addition of 1 N hydrochloric acid to pH 6 with stirring. The solid was filtered, washed with water, ethanol, and ether, and dried at 56 °C in vacuo for 5 h to give 1.17 g of crude product. UV_{max} 287 $E^{1\%}_{1cm}$, 553, MeOH); R_f 0.05 (solvent A). The crude product (1.04 g) was stirred 1 h with 14 mL of water, filtered, washed with ethanol and ether, and dried in vacuo for 38 h to give 1.03 g of **16a**: mp, charring at 352–358 °C; MS (EI) 256 (53.2%), 91 (100%). Anal. ($C_{12}H_{12}N_6O$·0.85H_2O): C; H: calcd, 5.08; found 4.67; N: calcd, 30.95; found, 31.64.

A 0.032 M solution of **16a** in 1 N HCl was diluted 1 to 500 with methanol, UV_{max} 289.5 (ϵ 1.27 × 10^4), UV_{min} 238 (ϵ 2.8 × 10^3).

(ii) Method of Scheme III. Compound **18a** (18.65 g, 67 mol) was added to a solution of 1 N NaOH (140 mL), water (700 mL), and methoxyethanol (60 mL) at 80 °C. Sodium dithionite (60 g) was added in portions, and the reaction mixture was heated at 85–90 °C for 30 min. The reaction mixture was filtered, cooled, and then concentrated to 300 mL under vacuum. Crude **19a** (10.37 g, 65%) was collected by filtration and dried over P_2O_5. The compound is unstable to air and was used immediately in the next reaction. Thus, crude **19a** (3.32 g, 14.3 mmol) was suspended in methoxyethanol (15 mL). Cyanogen bromide (6.05 g, 55.4 mmol) was added, and the reaction mixture was stirred for 2 days at room temperature. Acetonitrile (20 mL) was added, and the precipitate was rinsed with acetonitrile and then dichloromethane. The intermediate salt **20a** (1.92 g) was taken up in DMF (30 mL), and ammonia gas was bubbled into the resulting suspension for 30 min. The reaction mixture was stirred at room temperature overnight, and the solid was collected by filtration. The crude **16a** was purified by repeated reprecipitation from 1 N HCl by neutralizing a solution of **16a** with aqueous ammonium hydroxide. The product was dried over P_2O_5 under vacuum: yield (0.71 g, 18%); mp >300 °C dec. The compound is identical to that prepared by Scheme II according to NMR, IR, and MS. Anal. ($C_{12}H_{12}N_6O$·0.75H_2O) C, H, N.

2,8-Diamino-3,9-dihydro-3-(2-thienylmethyl)-6*H*-purin-6-one (16b) was prepared by method (b) (Scheme III) as described for **16a**. The product was treated with 1 N HCl in aqueous methanol to give the hydrochloride salt: mp, slowly decomposing above 155 °C without melting. Anal. ($C_{10}H_{10}N_6OS$·HCl·0.8H_2O) C, H, N, Cl, S.

2,8-Diamino-3,9-dihydro-3-(2-furanylmethyl)-6*H*-purin-6-one (16c) was prepared and isolated as the hydrochloride salt as described for **16b**: mp >130 °C dec. Anal. ($C_{10}H_{10}N_6O_2$·HCl·1.5H_2O) C, H, N, Cl.

2,8-Diamino-3,9-dihydro-3-(4-methoxybenzyl)-6*H*-purin-6-one (16d) was prepared and isolated as the hydrochloride salt as described for **16b**: mp >190 °C dec. Anal. ($C_{13}H_{14}N_6O_2$·HCl·0.5H_2O) C, H, N, Cl.

8-Amino-3,9-dihydro-3-(phenylmethyl)-6*H*-purin-6-one (17a). To a mixture of 2.22 g of **14a**, 48 mL of 2-methoxyethanol, and 12 mL of water was added with stirring 1 N sodium hydroxide until a clear solution was obtained (9.4 mL used). Raney nickel, prepared in ethanol according to the procedure of Burgstahler,[15] was centrifuged to give a wet-packed solid weighing 1.83 g/mL, 6 mL of which was then washed with 2-methoxyethanol and added to the reaction mixture. The mixture was heated with stirring at 65 °C for 1 h, and the product was detected by TLC (R_f 0.11, solvent A).

The mixture was heated for another 3.3 h during which 1.3-, 1.7-, and 1.1-mL portions of the wet-packed Raney nickel, similarly processed, was added. The reaction mixture containing **17a** and traces of two emerging side products was filtered hot through a Celite pad, and the solid was washed with 20 mL of 2-methoxyethanol at 50–60 °C. The filtrate was neutralized with 1 N acetic acid (8.5 mL), evaporated to about one-third of its original volume, and then coevaporated with 10 mL of water to 11 g of thick white slurry. The slurry was treated with 9 mL of water and filtered, and the solid was washed with water, ethanol, and ether, and dried in vacuo for 21 h to give 1.38 g of **17a** (70% yield); mp, decomposing rapidly within a 1 °C range between 310 to 320 °C. Anal. ($C_{12}H_{11}N_5O$·0.7H_2O) C, H; N: calcd, 27.59; found, 26.98.

6-Amino-5-nitroso-2-oximino-1-(phenylmethyl)-4(1*H*)-pyrimidinone (18a). A suspension of **11a** (23.4 g, 0.084 mol) in a solution of hydroxylamine hydrochloride (36.5 g, 0.52 mol) and sodium hydroxide (18.25 g, 0.46 mol) in water (500 mL) was stirred at room temperature overnight. The resulting orange solid was collected by filtration and dried over P_2O_5 under vacuum to give **18a** (16.2 g, 68%). Anal. ($C_{11}H_{11}N_5O_3$·H_2O) C, H; N: calcd, 25.07; found, 24.23.

6-Amino-3-(phenylmethyl)-9*H*-purinium chloride (3-benzyladenine hydrochloride; 22a, X = Cl) was prepared by treatment of 30 g of adenine with 81.4 mL of benzyl chloride in DMA at 116–118 °C according to the procedure of Thomas and Montgomery:[11c] mp 262–265 °C; yield 73%. Anal. ($C_{12}H_{11}N_5$·HCl) C, H, N, Cl.

6-Amino-3-[(3,4-dichlorophenyl)methyl]-9*H*-purinium chloride (22e, X = Cl) was prepared by a procedure similar to that for **22a**: yield 70%; mp 271–277 °C dec. Anal. ($C_{12}H_9Cl_2N_5$·HCl) C, H, N, Cl.

6-Amino-3-[(4-cyanophenyl)methyl]-9*H*-purinium bromide (22f, X = Br) was similarly prepared as for **22a**; yield 65.7%; mp 267–269 °C. Anal. ($C_{13}H_{10}N_6$·HBr) C, H, N, Br.

6-Amino-3-[4-nitrophenyl)methyl)]-9*H*-purinium bromide (22g, X = Br) was similarly prepared as for **22a**: yield 64%; mp 275 °C dec. Anal. ($C_{12}H_{10}N_6O_2$·HBr) C, H, N, Br.

3,9-Dihydro-3-(phenylmethyl)-6*H*-purine-6-thione (23a) was prepared by the treatment of **7a** with phosphorus pentasulfide in dry pyridine heated under reflux according to the procedure of Thomas and Montgomery,[11c] mp 282–284 °C. Anal. ($C_{12}H_{10}N_4S$·0.2H_2O) C, H, N, S.

Registry No. **2a**, 621-83-0; **2h**, 6815-00-5; **3**, 3849-21-6; **4a**, 139460-79-0; **4h**, 139460-80-3; **5a**, 59008-19-4; **5h**, 139460-81-4; **6a**, 28741-76-6; **6h**, 139460-82-5; **7a**, 3649-39-6; **7e**, 139460-83-6; **7b**, 139493-18-8; **7g**, 139460-84-7; **7h**, 139460-85-8; **8a**, 28741-77-7; **9a**, 19844-93-0; **10a**, 28741-78-8; **11a**, 139460-86-9; **12a**, 139460-87-0; **13a**, 139493-19-9; **14a**, 139460-88-1; **15a**, 139460-89-2; **16a**, 139460-90-5; **16b**, 139461-01-1; **16b**·HCl, 139460-93-8; **16c**, 139461-02-2; **16c**·HCl, 139460-94-9; **16d**, 139493-20-2; **16d**·HCl, 139460-95-0; **17a**, 139460-96-1; **18a**, 139460-97-2; **19a**, 139460-91-6; **20a**, 139460-92-7; **22a**, 95196-69-3; **22e**, 139460-98-3; **22f**, 139460-99-4; **22g**, 139461-00-0; **23a**, 7151-30-6; formamide, 75-12-7; adenine, 73-24-5; purine nucleoside phosphorylase, 9030-21-1.

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