Based on these preliminary observations, it would appear that macromolecule methyltransferases offer excellent targets for the design of some new and novel chemotherapeutic agents. The interest in these enzymes by medicinal chemists should rapidly develop in the next several years as the biologists, biochemists, and pharmacologists continue to unravel the role of these macromolecule methylations in regulating important cellular processes.

Articles

Synthesis of Potential Inhibitors of Hypoxanthine–Guanine Phosphoribosyltransferase for Testing as Antiprotozoal Agents. 1. 7-Substituted 6-Oxopurines

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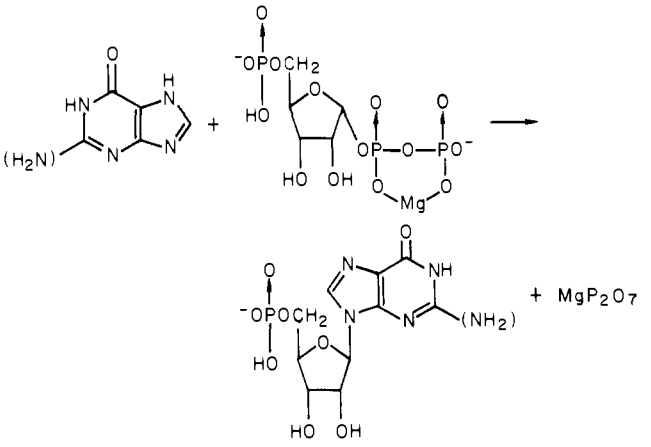
Biological evidence indicates that the enzyme hypoxanthine–guanine phosphoribosyltransferase (EC 2.4.2.8) is vital for cell proliferation in malarial parasites but nonessential for mammalian cells. 7-Substituted guanines and hypoxanthines in which the 7 substituent bears functional or hydrophobic groups were prepared with the aim of finding a suitably constituted compound whose resemblance to the normal substrate allows it to compete for the reversible purine binding site of HGPRTase while allowing a substituent group of the inhibitor molecule to form a covalent bond or strong hydrophobic bond with appropriate sites on the enzyme. Multistep syntheses that began with hydroxyalkylations and alkylations of guanosine led to four key guanines substituted at the 7 position by the following chains: 2-aminoethyl, 3-amino-2-hydroxypropyl, 3-aminobenzyl, and 4-aminobenzyl. Similarly, 7-(4-aminobenzyl)hypoxanthine was prepared. Reactions at the side-chain amino groups with bromoacetic anhydride (or, alternatively, 4-nitrophenyl bromoacetate) and 3- and 4-(fluorosulfonyl)benzoyl chlorides afforded derivatives bearing functional groups capable of forming covalent bonds with enzymes through displacement reactions. 4-Chlorobenzyl derivatives were similarly prepared as potential inhibitors that might act through hydrophobic bonding. Three 7-substituted guanines whose side chains bear other functions (7-guanine-3-propanesulfonic acid, guanine-7-acetaldehyde, and the ethyl ester of 7-guanine-4-crotonic acid) were prepared as potential inhibitors and for possible use as intermediates. None of these compounds extended the life span of *P. berghei* infected mice or showed significant in vitro inhibition of HGPRTase from H.Ep.-2 cells.

Work on purine metabolism in malarial parasites[1-12] indicates that these parasites are unable to biosynthesize purine nucleotides de novo and must depend on preformed purines of the host for the synthesis of nucleic acids necessary for multiplication, providing a firm basis for the concept that sufficiently potent inhibitors of hypoxanthine–guanine phosphoribosyltransferase (HGPRTase) should possess in vivo antimalarial activity. Adenosine is rapidly deaminated to inosine and then deribosylated to hypoxanthine on the surface or outside the parasite, while conversion of hypoxanthine to inosinic acid and, subsequently, to adenosine triphosphate occurs most probably inside the parasite.[12] Thus, hypoxanthine, a normal exit metabolite in mammalian purine metabolism (via oxidation to uric acid), is apparently the building block of the

Scheme I. Function of Hypoxanthine–Guanine Phosphoribosyltransferase



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(3) Büngener, W.; Nielsen, G. *Z. Tropenmen. Parasitol.* **1969**, *20*, 67.
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(12) Manandhar, M. S. P.; Van Dyke, K. *Exp. Parasitol.* **1975**, *37*, 138.

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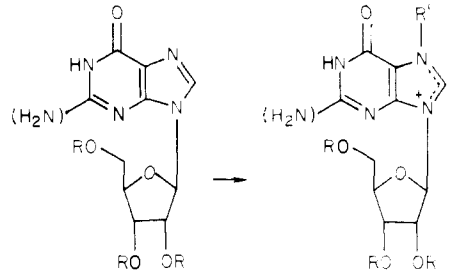
nucleotides for the parasite. In keeping with these observations, allopurinol, which is a potent inhibitor of xanthine oxidase, has been found to stimulate the multiplication of rodent plasmodia in mice and rats.[13] This effect is presumably due to host-purine dependence of the parasites; hypoxanthine concentration in the host can probably be a limiting factor for parasite multiplication. On the other hand, preformed pyrimidines, with the exception of orotic acid, are not utilized by parasites,[6] which do incorporate labeled bicarbonate into pyrimidine nucleotides, showing the existence of the pyrimidine biosynthetic pathway.[2] Thus, it would appear that excellent biochemical evidence is now available in several species of the malarial parasite to support the contention that effective inhibition of HGPRTase should cause unbalanced growth in the parasites, resulting from the purine, but not pyrimidine, nucleotide deficiency. At the same time, this inhibition should have no effect on normal, nondividing host cells and little, if any, effect on the relatively small number of dividing host cells, which can synthesize their required purines de novo and use HGPRTase primarily to salvage purine bases from degraded nucleic acids and nucleotides (stable mutant cell lines devoid of HGPRTase have been obtained from mammalian cells in culture and from certain leukemias in rodents, indicating the nonessential nature of this enzyme for mammalian cells). The result should be effective prevention of parasite proliferation without significant toxicity.

In the absence of accurate information about the active site of the enzyme, the design of inhibitors must be based on the structures of the substrates, cofactor, and products, on what is known about the details of the enzymatic reaction and on what little is known about nucleophilic groups in the active site. Thus, potential inhibitors of HGPRTase should resemble hypoxanthine and guanine or 5-phosphoribosyl 1-pyrophosphate (PRPP) and fit the active site of the enzyme. At least three studies have been carried out on the inhibition of this enzyme (isolated from human erythrocytes,[14] adenocarcinoma 755,[15] and *E. coli*[16]) by various compounds. Two conclusions can be drawn from these studies: (1) the substrate specificities vary with the enzyme source and (2) none of the compounds studied had a sufficiently low K_i/K_m ratio to be an effective in vivo inhibitor of the enzyme.[15] A more recent study on the enzyme from *Plasmodium chabaudi*[17] indicates that its substrate specificity closely resembles that of the enzyme from a mammalian source. This resemblance suggests that the enzyme from a mammalian source would serve as a valid model for evaluating the inhibitory effect of candidate compounds.

Initially it appeared that it would be more profitable to investigate substitution of hypoxanthine or guanine at positions other than N-9, since that is precisely where the enzymatic reaction occurs. The proximity of N-3 to N-9 mitigated against that choice also. In addition, the enzyme accepts hypoxanthine–guanine as substrates but not other purines such as adenine and xanthine, suggesting that substitution of the pyrimidine portion of the molecule might interfere with binding to the active site. These considerations led to the choice of N-7 as the first position to attach groups that might convert the normal substrates into inhibitors of the enzyme.

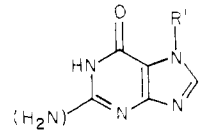
Consideration of the composition of enzyme active sites dictated the strategy used in selecting groups for attachment to N-7. Any particular part of an enzyme active site must contain either areas that might lend themselves to hydrophobic bonding (aliphatic chains or aromatic groups) or functional groups nucleophilic in nature (NH_2, SH, or OH) and capable of covalent bonding. Thus, if substitution at a particular position of hypoxanthine or guanine does not cause steric interference with binding, a group capable either of hydrophobic interaction or reaction, under physiologic conditions, with a nucleophilic center should enhance binding and lead to an effective inhibitor. This logic led us to select both aliphatic and aromatic side chains of various lengths bearing hydrophobic groups, such as *p*-chlorophenyl, or reactive groups, such as the fluorosulfonyl or bromoacetamido groups.[18]

Synthesis. Work toward the synthesis of 7-(2-aminoethyl)guanine (**15**) began with the preparation of 7-(2-hydroxyethyl)guanine (**5a**) by a reported method involving



1, R = H
2, R = Ac

3, R = H
4, R = Ac



5, R′ = $(CH_2)_2OH$
6, R′ = $CH_2CHOHCH_2Cl$
7, R′ = $CH_2CHOHCH_2NPhth$
8, R′ = $CH_2CHOHCH_2OH$
9, R′ = 4-$O_2NC_6H_4CH_2$-
10, R′ = 3-$O_2NC_6H_4CH_2$-
11, R′ = $(CH_2)_3SO_3H$

a, guanine series; b, hypoxanthine series

treatment of guanosine (**1a**) with ethylene oxide in AcOH to give the 7-substituted intermediate **3a** [R′ = $(CH_2)_2OH$], which was converted to **5a** by acid hydrolysis.[19,20] Unsuccessful efforts to convert **5a** to an appropriate sulfonate ester (mesyl or tosyl) led to attempts to develop conditions suitable for the conversion of **5a** to 7-(2-chloroethyl)-guanine (**12**) on a scale large enough to provide this key intermediate in ample quantity. These efforts were not entirely successful because of the formation of 7-(2-chloroethyl)-8-chloroguanine (**13**) under conditions stringent enough to cause complete reaction of **5a**. Reaction of **12** with NaN_3 gave 7-(2-azidoethyl)guanine (**14**), which was reduced catalytically to 7-(2-aminoethyl)guanine (**15**).

The primary target compounds 7-[2-[3-(fluorosulfonyl)benzamido]ethyl]guanine (**16a**), the 4-(fluorosulfonyl) isomer **16b**, and 7-[2-(4-chlorobenzamido)-

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(14) Lau, K. F.; Henderson, J. F. *Cancer Chemother. Rep., Part 2* **1972**, *3*, 87.
(15) Hill, D. L. *Biochem. Pharmacol.* **1970**, *19*, 545.
(16) Miller, R. L.; Ramsey, G. A.; Krenitsky, T. A.; Elion, G. B. *Biochemistry* **1972**, *11*, 4523.
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(18) Baker, B. R. "Design of Active-Site-Directed Irreversible Enzyme Inhibitors", Wiley: New York, 1967.
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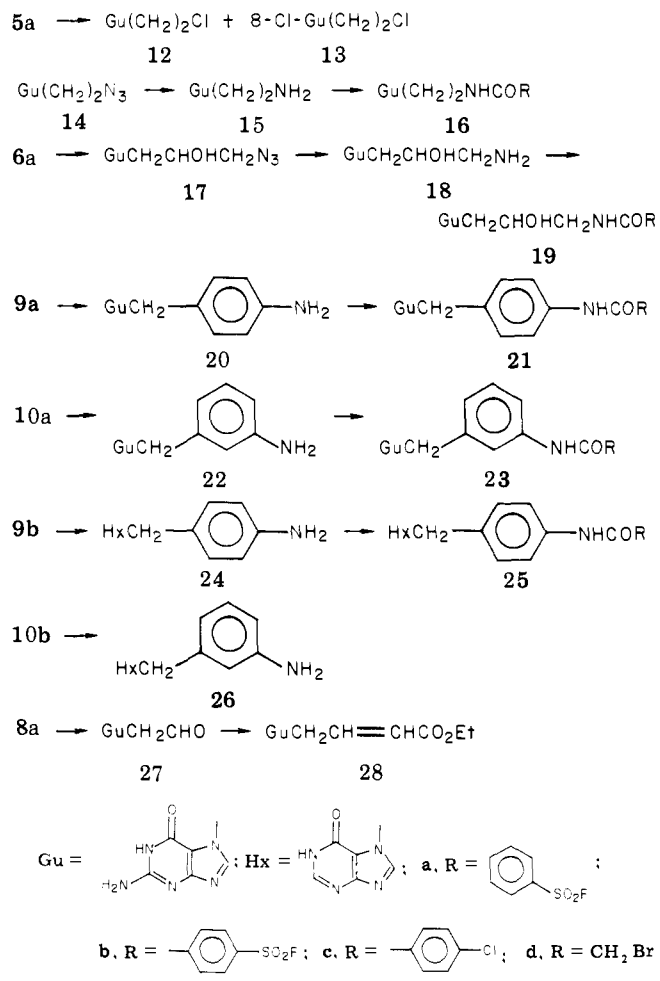
(20) Roe, R., Jr.; Paul, J. S.; Montgomery, P. O'B., Jr. *J. Heterocycl. Chem.* **1973**, *10*, 849; *Ibid.* **1973**, *10*, 859.

Scheme II



ethyl]guanine (**16c**) were prepared from the reaction of **15** with the corresponding benzoyl chlorides.

Treatment of **15** with bromoacetic anhydride[21] in glacial AcOH containing diisopropylethylamine resulted in complete reaction of **15**, but the **16d** thus formed was contaminated by 10–15% of the acetyl analogue (^{1}H NMR) that was not easily removed. This problem was overcome by the use of 4-nitrophenyl bromoacetate[22] as the bromoacetylating agent.

Treatment of guanosine (**1a**) with *N*-(2,3-epoxypropyl)phthalimide, followed by removal of the ribosyl function by acid hydrolysis, afforded 7-(2-hydroxy-3-phthalimidopropyl)guanine (**7a**). Efforts to convert **7a** to the desired amino compound **18** by dephthaloylation in boiling 6 N HCl gave mixtures of **18** and a second product that apparently resulted from cleavage of the purine ring.

Attention was then directed to finding a better approach to **18**, and the alternative route via 7-(3-azido-2-hydroxypropyl)guanine (**17**) was found to be quite effective. The azido alcohol **17** was prepared from **6a** and NaN_3 in DMF, and reduction gave **18**. Each step along this route gave good yields (Scheme II).

The use of AcOH as a medium for the conversion of **18** to target derivatives **19a–c** was avoided after it was found that partial esterification of the hydroxyl group occurred. When the aroylations were carried out heterogeneously in DMF containing diisopropylethylamine, unchanged **18** remained in each of the mixtures and had to be removed by extraction of the crude materials with 0.1 N HCl in which both **19a** and **19b** have some solubility. Losses during the extraction process lowered the yields of pure **19a** and **19b** to 32 and 20%, after the material that remained undissolved in 0.1 N HCl was recrystallized from DMF–H_2O. Pure **19c**, which appeared to be less soluble in 0.1 N HCl than **19a** or **19b** and did not require recrystallization, was obtained in 56% yield.

In an effort to prepare 7-[3-(bromoacetamido)-2-hydroxypropyl]guanine (**19d**), 7-(3-amino-2-hydroxypropyl)guanine (**18**) was treated with 4-nitrophenyl bromoacetate in AcOH. The progress of the reaction was followed by TLC (*n*-BuOH–AcOH–H_2O, 5:2:3) with detection of unchanged **18** by the ninhydrin reagent and the bromoacetylated product(s) by the active-halogen test with 4-(4-nitrobenzyl)pyridine (NBP).[23] The disappearance of **18** was complete after 5 days at about 25 °C, but two narrowly separated NBP-positive spots were present on the day-5 chromatogram and on that produced by the isolated material, which was precipitated from the reaction solution by addition of Et_2O. Both of these spots were detectable in the reaction solution after 1 day. The upper spot remained smaller and less intense than the other. No other contaminant was detectable by UV absorption or NBP in the isolated material. Although bromoacetylation of **18** with 4-nitrophenyl bromoacetate in AcOH did not lead to a homogeneous product, it was the best procedure of the many tried. A scaled-up preparation led to a product in 96% yield which produced the same thin-layer chromatographic results as that of the earlier run described above, but in a different TLC solvent system ($CHCl_3$–MeOH, 1:1) it appeared homogeneous. The ^{1}H NMR spectrum of this sample supports the finding by TLC that it is slightly contaminated, as evidenced by a barely detectable signal near δ 8.85, just at the base of the stronger signal at δ 8.90 due to the C_8 H of **19d**. The remainder of the spectrum is as expected for **19d**. A portion of this sample of **19d**, judged to be at least 90% pure on the basis of TLC, ^{1}H NMR spectral data, and elemental analyses, was stirred with pyridine until a portion of the reaction mixture no longer gave a positive test for active halogen. The pyridine-insoluble product was then recrystallized to give pure pyridinium salt.

An early effort to prepare 7-(4-nitrobenzyl)guanine (**9a**) using a molar ratio of 4-nitrobenzyl bromide to guanosine (**1a**) of 3:1 in Me_2NAc at 35–38 °C, modeled after a literature procedure for the preparation of 7-benzylguanine,[24] gave the 7,9-disubstituted salt, which crystallized from the reaction solution as a monosolvate with Me_2NAc and was identified on the basis of elemental analyses and its ^{1}H NMR spectrum. The use of equimolar amounts of guanosine (**1a**) and 4-nitrobenzyl bromide led to two successful preparations of **9a**. The more convenient of the two involved a 5-h reaction period at 85–90 °C and led to a 22% yield of pure **9a**; the other, carried out at room temperature for 11 days, gave pure **9a** in 40% yield. Loss of the ribose moiety occurred in Me_2NAc in the run carried out at 85–90 °C; crystalline **9a**·HBr separated from the cooled solution. Approximately one-third of the total amount of **9a** obtained from the run at room temperature also crystallized from the reaction solution as **9a**·HBr. The remaining portion was obtained after hydrolytic treatment (1 N HCl) of material obtained from the filtrate. The salts of **9a** were obtained as white, crystalline materials, and simple contact with H_2O caused them to undergo dissociation to H_2O-

(21) Schaeffer, H. J.; Johnson, R. N. *J. Pharm. Sci.* **1966**, *55*, 929.
(22) Russell, D. W. *Biochem. J.* **1961**, *78*, 696.
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(24) Brookes, P.; Dipple, A.; Lawley, P. D. *J. Chem. Soc. C* **1968**, 2026.

insoluble, yellow **9a**. Later it was found that treatment of the tri-*O*-acetyl derivative **2a** with 4-nitrobenzyl bromide (molar ratio 1:1) in Me_2NAc at 50–60 °C for 16 h, followed by appropriate workup, led to **9a**·HCl in 66% yield.

7-(4-Aminobenzyl)guanine (**20**) was obtained by catalytic (5% Pd on C) reduction of **9a** in glacial AcOH at atmospheric pressure. The reduction was later carried out more conveniently in 0.3 N HCl, which was neutralized after clarification, giving a precipitate of pure **20**.

The bromoacetyl derivative **21d** was first prepared by treatment of suspended **9a** with bromoacetic anhydride in DMF. Unchanged **9a** was removed by treatment with 0.1 N HCl in which **21d** is insoluble. The ^1H NMR spectrum of the material obtained was as expected for **21d**, but elemental analysis results were not quite satisfactory. Subsequently, pure **21b** was obtained by treatment of **9a** with bromoacetic anhydride in glacial AcOH, in which **9a** is more soluble than in DMF.

These observations led to consideration of the use of glacial AcOH as the medium for aroylation reactions planned for **9a**. A model experiment consisting of treatment of aniline with benzoyl chloride in AcOH containing diisopropylethylamine, followed by addition of 0.1 N HCl to the reaction mixture, gave benzanilide in 86% yield. These conditions were then employed in successful preparations of the 3- and 4-(fluorosulfonyl)benzoyl derivatives **21a** and **21b** and the 4-chlorobenzoyl derivative **21c**.

Treatment of **1a** with excess 3-nitrobenzyl chloride (molar ratio 1:2.5) in Me_2NAc at about 80 °C led to pure 7-(3-nitrobenzyl)guanine (**10a**) in 30% yield after recrystallization from 1 N HCl and trituration with H_2O. The material that crystallized directly from the 1 N HCl proved to be **10a**·HCl, which, like the 4-nitro isomer described above, underwent dissociation to give the base form in the treatment with H_2O. Catalytic reduction of **10a** in 0.3 N HCl as described for **20** afforded 7-(3-aminobenzyl)guanine (**22**) in good yield. The derivatives **23a–d** were prepared essentially as described for the analogous **21** series.

Treatment of **2b** with 4-nitrobenzyl bromide in Me_2NAc at 25 °C for several days appeared (TLC) to produce a complex mixture of products, but after removal of the ribose function, pure **9b** was indeed easily obtained in 42% yield after recrystallization.

Catalytic (5% Pd on C) reduction of **9b** to 7-(4-aminobenzyl)hypoxanthine (**24**) was best carried out in glacial AcOH at 3.0–3.5 atm. A sample of pure **24** was obtained in 56% yield by recrystallization, but **24** obtained after removal of the catalyst, evaporation of the solvent, and trituration with 5% $NaHCO_3$ was of satisfactory purity for conversion to derivatives.

Treatment of **24** with 3- and 4-(fluorosulfonyl)benzoyl chloride in AcOH containing diisopropylethylamine afforded the (fluorosulfonyl)benzoyl derivatives **25a** and **25b**, which crystallized from the reaction solutions. The 4-chlorobenzoyl derivative **25c** was similarly obtained from **24** and 4-chlorobenzoyl chloride. Treatment of **24** with bromoacetic anhydride in AcOH afforded the bromoacetyl derivative **25d**, which precipitated when the reaction solution was treated with Et_2O.

The best conditions found for the preparation of 7-(3-nitrobenzyl)hypoxanthine (**10b**) involved treatment of 2′,3′,5′-tri-*O*-acetylinosine (**2b**) with excess 3-nitrobenzyl chloride in Me_2NAc at 25 °C for 10 days, followed by removal of the sugar moiety in the usual manner. Although the overall yields of **10b** from two runs were only 15 and 19%, this process is preferable to the only published alternative route.[21] Catalytic reduction of **10b** as described for the preparation of the 4-aminobenzyl isomer afforded 7-(3-aminobenzyl)hypoxanthine (**26**) in 40% overall yield.

Treatment of **1a** with 1,3-propane sultone in Me_2SO as described by B. L. Goldschmidt et al.[25] afforded a product whose ^1H NMR spectrum is consistent with that expected for the inner salt **3a** [R′ = $(CH_2)_3SO_3^-$]. The published listing of major IR bands for this compound includes five closely grouped bands at 1720, 1700, 1672, 1642, and 1612 cm^{-1}. The spectrum of our sample has only three bands in the pertinent region: strong bands of nearly equal intensity at 1700, 1635, and 1600 cm^{-1}. These three bands could correspond to the second, fourth, and fifth bands of the five reported in the order listed above. Since 7-guanine-3-propanesulfonic acid (**11**) is reported to have bands occurring at 1721 and 1670 cm^{-1}, two of the reported bands for **3a** [R′ = $(CH_2)_3SO_3^-$] appear to be due to the presence of **11**. An attempt to convert the inner salt to **11** by heating it with 2 N NaOH at 90 °C for 5 min[25] gave a product whose UV spectra are not characteristic of a 7-substituted guanine. In contrast, treatment of the inner salt with 1 N HCl at 100 °C gave pure **11**, which crystallized from the hot reaction solution in 89% yield. The UV spectral data from this sample agrees with that reported.[25]

Guanine-7-acetaldehyde (**27**) was prepared for use as an intermediate in chain-extending conversions with Wittig reagents. Several samples of **27** were easily prepared just prior to use by treatment of **8** with $NaIO_4$ in dilute HCl solution. The material that separated directly in about 70% yield from the neutralized reaction solution was of high purity. Elemental analyses agreed with values calculated for hydrated **27**. The mass spectrum features a molecular ion (M^+) of *m/e* 193 and another peak of *m/e* 165 (M − CO), and ^1H NMR spectra in Me_2SO-d_6 and CF_3CO_2D offer evidence suggesting covalent hydration.[26] Treatment of the aldehyde **27** with excess ethoxycarbonylmethylenetriphenylphosphorane in Me_2SO readily afforded ethyl 7-guanine-4-crotonate (**28**). In contrast, two attempts to prepare 7-(5-bromo-2-pentenyl)guanine by reaction of **27** with the appropriate Wittig reagent were unsuccessful.

Biological Data. All of these compounds were tested for activity against *P. berghei* in mice[27] and for their ability to inhibit hypoxanthine–guanine phosphoribosyltransferase (HGPRTase) in crude extracts of H.Ep.-2 cells.[15] None extended significantly the life span of mice with *P. berghei* or inhibited HGPRTase to any extent. The similarity of the substrate specificity of the enzyme from *P. chabaudi* to that of the enzyme from a mammalian source[17] (see above) makes it reasonable to suggest that the *P. berghei* enzyme is probably not inhibited in the in vivo tests. This failure to inhibit may result from a lack of bulk tolerance in the region adjacent to N-7 and C-8 of the purine ring of these hypoxanthine–guanine analogues. Subsequent papers will describe the synthesis and activity of 6-oxopurines substituted at other positions in an effort to avoid this problem.

Experimental Section

Melting points were observed on a Mel-Temp apparatus; since several of the compounds reported lack definite melting points,

(25) Goldschmidt, B. L.; Frenkel, K.; Van Duren, B. L. *J. Heterocycl. Chem.* **1974**, *11*, 719.
(26) Kamiya, T.; Saito, Y.; Hashimoto, M.; Sek, H. *Tetrahedron* **1972**, *28*, 899.
(27) The antimalarial test results were provided by the Walter Reed Army Institute of Research. The method of Rane and co-workers was employed (ref 28).
(28) Osdene, T. S.; Russell, P. B.; Rane, L. *J. Med. Chem.* **1967**, *10*, 431.

Table I. 7-(Hydroxyalkyl)guanines

no.	mp, °C dec	yield, %	molecular formula[a]
5a	>330	73	$C_7H_9N_5O_2 \cdot H_2O$
6a	>250	87	$C_8H_{10}ClN_5O_2$
7a	>350	65	$C_{16}H_{14}N_8O_4$
8a	~310	53	$C_8H_{11}N_5O_3 \cdot H_2O$

[a] Anal. C, H, N.

the figure given represents the onset of visible decomposition. Analytical results indicated by element symbols were within ±0.4% the theoretical values. Microanalyses were performed, for the most part, by Galbraith Laboratories, Knoxville, Tenn. Spectral determinations and some of the elemental analyses were performed at Southern Research Institute (Molecular Spectroscopy Section) under the direction of Dr. William C. Coburn, Jr. The ^1H NMR spectra were determined with a Varian XL-100-15 or Varian T-60A spectrometer in the solvent indicated using Me_4Si as internal reference. Chemical shifts (δ, in ppm) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assignments indicated. Addition of D_2O produced the expected simplifications in the spectra. The UV (Cary Model 17 spectrometer) and IR spectra (Perkin-Elmer Models 521 and 621 spectrometers) were consistent with those to be expected from the assigned structures. Thin-layer chromatographic analyses [Analtech precoated (250 μm) silica gel G(F) plates] were used to follow the course of reactions and examine products for homogeneity. Solutions were clarified, when so indicated, by treatment with Norit and filtration through Celite. Unless other conditions are specified, evaporations were performed with a rotary evaporator and a water aspirator, and products were dried in vacuo (oil pump) over P_2O_5 at room temperature.

7-(Hydroxyalkyl)guanines 5a, 6a, and 8a were prepared via the corresponding guanosinium acetates (**3a**), which were converted to the desired products by treatment with 1 N HCl as reported for the preparation of **5a**.[19,20] Results are listed in Table I.

7-(2-Hydroxy-3-phthalimidopropyl)guanine (7a). A mixture of **1a** (2.83 g, 10.0 mmol), *N*-(2,3-epoxypropyl)phthalimide (4.06 g, 20.0 mmol), and glacial AcOH (50 mL) was stirred at 25 °C for 96 h. The solution that formed was evaporated, and the residue was stirred overnight with Me_2CO before it was collected and dried. The solid was dissolved in 1 N HCl (100 mL), and the solution was kept at 100 °C for 5.5 h. Crystalline material (2.6 g of **7a**·HCl) that separated from the cooled solution was collected and then stirred for 0.5 h with H_2O (mL) containing $NaHCO_3$ (0.65 g) to give pure **7a**. Additional data are given in Table I.

7,9-Bis(4-nitrobenzyl)guaninium Bromide. A mixture of **1a** (8.50 g, 30.0 mmol) and 4-nitrobenzyl bromide (19.4 g, 90.0 mmol) in Me_2NAc (170 mL) was stirred at 35–38 °C for 5 days. Solution occurred during the first day, and a precipitate formed after 3 days. The precipitate was collected, washed with Et_2O, and dried to give 6.60 g of white solid. Elemental analysis and and ^1H NMR spectral results showed this product to be a 1:1 solvate with Me_2NAc (37% yield): ^1H NMR (CF_3CO_2D) δ 2.64 (s, 3, CH_3CO), 3.41 (s, 6, CH_3N), 5.72 (s, 2, CH_2), 5.94 (s, 2, CH_2), 7.7 (q, 4, $C_{2'}$ H and $C_{6'}$ H), 8.3 (q, 4, $C_{3'}$ H and $C_{5'}$ H), 9.26 (s, 1, C_8 H). Anal. ($C_{19}H_{16}BrN_7O_5 \cdot C_4H_9NO$) H, N; C: calcd, 46.87; found, 47.30.

7-(4-Nitrobenzyl)guanine (9a). Method A. A stirred mixture of 0.150 mol each of **1a** (42.5 g) and 4-nitrobenzyl bromide (32.4 g) in Me_2NAc (1.5 L) was heated during 1 h to 85 °C and kept at 85–90 °C for 5 h. White crystalline material, presumably **9a**·HBr, separated while the mixture was left overnight at 25 °C. Following refrigeration, the solid was collected and washed with Me_2CO and Et_2O. The air-dried product was then stirred with H_2O, which changed it instantly to yellow, finely divided solid. The collected solid was washed with Me_2CO, followed by Et_2O, and dried, yield 10.4 g (24%). Recrystallization from hot 1 N HCl (~1 L; Norit, Celite) gave short white needles (**9a**·HCl), which were collected with the aid of 1 N HCl, then removed from the funnel, and stirred with H_2O. Yellow **9a** formed instantly and, after a stirring period, was collected: yield 9.4 g (22%); mp ~375 °C dec; homogeneous by TLC (*n*-BuOH–AcOH–H_2O, 5:2:3; detection by UV absorption). This sample was identical (mp, UV spectra, and TLC) with an analytical sample described below under method B.

Method B. A mixture of 20.0 mmol each of **1a** (5.67 g) and 4-nitrobenzyl bromide (4.32 g) in Me_2NAc (150 mL) was stirred at 25 °C for 11 days. Solution occurred on day 8, and a crystalline precipitate began forming on day 9. The precipitate (0.95 g) was removed by filtration and recrystallized from 1 N HCl (~100 mL) to give pure **9a**·HCl·0.75H_2O (0.81 g, 12% yield). Anal. ($C_{12}H_{10}N_6O_3 \cdot HCl \cdot 0.75H_2O$) C, H, N. This sample gave the same UV absorption spectra as the analytical sample of **9a** described in the following paragraph.

Me_2NAc was removed from the filtered reaction mixture by evaporation in vacuo (bath at 25–30 °C, pressure <1 mm) and the yellow oil that remained solidified when stirred with Me_2CO. The collected solid (6.6 g) gave a nearly clear solution when stirred with boiling 1 N HCl (300 mL). The solution was refluxed for 2 h and clarified. The white crystalline material (2.81 g) that separated from the cooled filtrate was recrystallized from 1 N HCl, and the collected solid was washed finally with H_2O, which changed it instantly to yellow **9a**: yield 1.58 g (28%); mp ~375 °C dec. The total yield was 40%. This sample was identical (mp, UV spectra, and TLC as described under method A) with an analytical sample obtained from a pilot run: ^1H NMR (CF_3CO_2D) δ 5.90 (s, 2, CH_2), 7.7 (d, 2, $C_{2'}$ H and $C_{6'}$ H), 8.3 (d, 2, $C_{3'}$ H and $C_{5'}$ H), 8.86 (s, 1, C_8 H). Anal. ($C_{12}H_{10}N_6O_3$) C, H, N.

7-(4-Nitrobenzyl)hypoxanthine (9b). A solution of **2b** (7.88 g, 20.0 mmol) and 4-nitrobenzyl bromide (5.46 g, 25.0 mmol) in Me_2NAc (100 mL) was kept at 25 °C for 10 days. The solvent was removed by evaporation in vacuo (< 1 mm, bath at 25 °C), and the residue was washed with three portions of Et_2O (1.5 L total), each of which was removed by decantation. The last of the Et_2O was removed by evaporation under reduced pressure. The residue was then dissolved in 1 N HCl (150 mL), and the solution was heated at 100 °C for 1 h, clarified while hot, and allowed to cool. The solid that separated amounted to 1.83 g. Neutralization of the filtrate from this crop by addition of NaOH solution gave another crop of 1.52 g. The two crops were combined and recrystallized from H_2O (1.5 L) to give **9b** in 42% yield (2.34 g): mp 295 °C dec; ^1H NMR (Me_2SO-d_6) δ 5.72 (s, CH_2), 7.5 (d, $C_{2'}$ H and $C_{6'}$ H), 8.2 (d, $C_{3'}$ H and $C_{5'}$ H), 8.00 (s, C_8 H or C_2 H), C_2 H), 8.44 (s, C_8 H or C_2 H). Anal. ($C_{12}H_9N_5O_3 \cdot 0.5H_2O$) C, H, N.

7-(3-Nitrobenzyl)guanine (10a). A mixture of **1a** (10.0 g, 35.4 mmol) and 3-nitrobenzyl chloride (15.2 g, 88.4 mmol) in Me_2NAc (100 mL) was stirred at 75–85 °C (oil bath temperature, mainly at 75 °C) for 16 h. The mixture, which thickened markedly, was cooled and mixed with Me_2CO (~300 mL) to facilitate filtration. The white solid was collected, washed successively with Me_2CO, H_2O, and Me_2CO again, and then suction dried for a time before it was dissolved in boiling 1 N HCl (~100 mL required). Granular crystalline solid separated from the cooled solution and was recrystallized a second time from 1 N HCl. The twice-recrystallized material was stirred thoroughly with H_2O, which caused collapse of the crystals and gave a finely divided, pale-yellow solid. Several washes with H_2O preceded final washes with Me_2CO and Et_2O, and the product was then dried in vacuo (78 °C, P_2O_5) to give pure **10a**, mp ~380 °C dec, in 30% yield (3.05 g). Anal. ($C_{12}H_{10}N_6O_3$) C, H, N.

The preparation was repeated as described above but on a scale four times that given, and the treatment of the twice-recrystallized product with H_2O was omitted. The material filtered from the 1 N HCl was washed with Me_2CO and Et_2O and then dried in vacuo (78 °C, P_2O_5) to give pure **10a**·HCl, but the yield was only 16% (7.43 g). Anal. ($C_{12}H_{10}N_6O_3 \cdot HCl$) C, H, N.

7-(3-Nitrobenzyl)hypoxanthine (10b). A solution of **2b** (3.94 g, 10.0 mmol) and 3-nitrobenzyl chloride (4.29 g, 25.0 mmol) in Me_2NAc (50 mL) was stirred at 75–85 °C (bath temperature) for 72 h. The solvent was removed by evaporation in vacuo, and the residual oil was stirred with Et_2O–Me_2CO (4:1 v/v, 125 mL) to give a solid, which was collected and washed with Et_2O. The air-dried solid was then suspended in 1 N HCl (50 mL), and the stirred mixture was heated at 100 °C for 1.5 h. The resulting solution was chilled (ice bath) and treated with 50% NaOH solution to produce pH 6.0. Crude **10b** precipitated and was

purified by recrystallization from H_2O, yield 19% (528 mg). Anal. ($C_{12}H_9N_5O_3 \cdot 0.25H_2O$) C, H, N.

7-Guanine-3-propanesulfonic Acid (11). A solution of **1a** (7.08 g, 25.0 mmol) in Me_2SO (175 mL) was treated dropwise with 1,3-propane sultone (17.5 mL, 210 mmol), and the resulting solution was stirred at 35–40 °C for 48 h. The cooled solution was combined with EtOH (1.5 L) to cause precipitation of the crude inner salt, which was collected, washed with EtOH, and dried, yield 7.56 g. This material was twice reprecipitated from H_2O solution by the addition of EtOH, washed with Et_2O, and dried to give pure **3a**·H_2O [R′ = $(CH_2)_3SO_3^-$] in 33% yield (3.58 g). Anal. ($C_{13}H_{19}N_5O_8S \cdot H_2O$) C, H, N. A solution of this material (2.40 g, 5.65 mmol) in 1 N HCl (15 mL) was heated at 100 °C (bath temperature). Crystalline **11** began separating from the hot solution after 15 min. After 30 min, the solution was allowed to cool, and the solid was collected, washed with EtOH, and dried to give pure **11**, mp >350 dec, in 91% yield (1.34 g). Anal. ($C_8H_{11}N_5O_4S$) C, H, N.

7-(2-Chloroethyl)guanine (12). A suspension of **5a**·H_2O (500 mg, 2.35 mmol) in $SOCl_2$ (7.5 mL) containing pyridine (2 drops) was refluxed for 1 h. The cooled mixture was diluted with Et_2O (75 mL), and the solid was collected by filtration, washed with Et_2O, and dissolved in H_2O (30 mL). The filtered solution was chilled in an ice bath and carefully neutralized to pH 6 with KOH solution. The solid that formed was collected by centrifugation and washed successively with H_2O, Me_2CO–H_2O (1:1), and Me_2CO, yield 249 mg (50% crude yield). Recrystallization from H_2O–AcOH (2:1) gave a white solid which was analytically pure after being dried in vacuo (140 °C, P_2O_5 and NaOH pellets): yield 138 mg (28%); mp > 300 °C; ^1H NMR (CF_3CO_2D) δ 4.0 (t, 2, CH_2Cl), 4.9 (t, 2, NCH_2), 8.8 (s, 1, C_8H); MS *m/e* 213 (M^+). Anal. ($C_7H_8ClN_5O$) C, H, N.

Attempted preparation of **12** on a 47-mmol scale by the above procedure gave mostly **13** (see procedure in the following paragraph). The most effective scaled-up preparation of **12** was as follows. A suspension of **5a**·H_2O (5.33 g, 25.0 mmol) in anhydrous dioxane (150 mL) containing freshly distilled $SOCl_2$ (7.25 mL, 100 mmol) was refluxed with rapid stirring for 1 h. Dioxane and excess $SOCl_2$ were removed in vacuo, and the solid that remained was dissolved in 1 N HCl (300 mL). The clarified solution was treated with NaOH solution to pH 6. The crude product (5.31 g) was recrystallized from H_2O–AcOH (2:1) and dried in vacuo (140 °C, P_2O_5 and NaOH pellets) to give **12** in 62% yield (3.31 g). This sample produced UV and mass spectra identical with those of the analytical sample described above.

7-(2-Chloroethyl)-8-chloroguanine (13). A suspension of **5a**·H_2O (10.0 g, 47.0 mmol) in freshly distilled $SOCl_2$ (150 mL) containing pyridine (1.5 mL) was heated at reflux for 3 h. The cooled mixture was diluted with Et_2O (1.5 L), and the solid was collected by filtration and washed with Et_2O (500 mL). The solid was extracted with H_2O (600 mL), and the H_2O-insoluble part was dried in vacuo. The crude product (8.0 g, 68% yield) was washed with hot 2:1 H_2O–AcOH (three portions totaling ~1 L) and dried in vacuo (140 °C, P_2O_5 and NaOH pellets) to give pure **13**, mp >350 °C dec, in 42% yield (4.9 g): ^1H NMR (CF_3CO_2D) δ 4.0 (t, 2, CH_2Cl), 4.8 (t, 2, NCH_2); MS *m/e* 247 (M^+). Anal. ($C_7H_7Cl_2N_5O$) C, H, N.

7-(2-Azidoethyl)guanine (14). A mixture of **12** (3.0 g, 14 mmol) and NaN_3 (1.8 g, 28 mmol) in DMF (100 mL) was stirred at 100 °C for 6 h. The solid was filtered from the cooled mixture, washed with DMF and H_2O (3 × 50 mL), and dried, yield 2.9 g (95%). Two recrystallizations from H_2O (1.5 L) gave pure **14**, mp >300 °C dec, in 53% yield (1.6 g): IR, strong azido band at 2100 cm^{-1}. Anal. ($C_7H_8N_8O$) C, H, N.

7-(3-Azido-2-hydroxypropyl)guanine (17). A suspension of **6a** (8.90 g, 36.5 mmol) and NaN_3 (4.75 g, 73.0 mmol) in DMF (275 mL) was stirred at 100 °C for 6 h. Crude **17** was filtered from the cooled mixture, washed with DMF followed by H_2O (3 × 50 mL), and dried, yield 7.90 g (87%). Recrystallization from H_2O (6.5 L) gave pure **17**, mp ~250 °C dec, in 75% yield (6.87 g): IR, strong azido band at 2100 cm^{-1}. Anal. ($C_8H_{10}N_8O$) C, H, N.

A larger run (96.3 mmol of **6a**, 193 mmol of NaN_3, 600 mL of DMF) was carried out at 100–110 °C (bath temperature) for 12 h. Solid **17** filtered from the cooled mixture was washed thoroughly with DMF followed by several portions of H_2O. The product thus obtained in 83% yield (20.1 g) gave an IR spectrum identical with that of the recrystallized sample described above and was suitable for conversion to **18**.

7-(2-Aminoethyl)guanine (15) Monohydrochloride. Hydrogenation of **14** (2.72 g, 12.4 mmol) in a stirred suspension with PtO_2 (300 mg) in H_2O (500 mL) was carried out at 55–65 °C (bath temperature) at atmospheric pressure. The H_2 atmosphere was recharged after 2 and 20 h. After 22 h, the catalyst was filtered from the warm solution, and the filtrate was evaporated to dryness. The residue was recrystallized from H_2O (200 mL), yield 1.82 g (76%). Elemental analyses indicated that the free base had absorbed atmospheric CO_2. The solid was dissolved in an equimolar amount of 0.10 N HCl (93.8 mL), and the filtered solution was evaporated to dryness. The solid residue was triturated with Et_2O, collected, and dried: yield 2.0 g; mp >300 °C dec; homogeneous by TLC (*n*-BuOH–AcOH–H_2O, 5:2:3; detection by UV absorption and ninhydrin test); ^1H NMR (CF_3CO_2D) δ 4.0 and 5.1 (2 t, 2 H each, CH_2CH_2), 8.85 (s, 1, C_8H). Anal. ($C_7H_{10}N_6O \cdot HCl \cdot 0.7H_2O$) C, H, N.

7-(3-Amino-2-hydroxypropyl)guanine (18). Hydrogenation of **17** (3.00 g, 12.0 mmol) in a stirred suspension with PtO_2 (300 mg) in H_2O–EtOH (1:1, 500 mL) was carried out at 50–65 °C (bath temperature) at atmospheric pressure. The H_2 atmosphere was recharged after 2, 4, 16, and 24 h. After 28 h, the warm mixture was filtered through Celite, and the Celite mat was washed three times with hot H_2O (500-mL portions). Evaporation of the filtrate plus the washings gave a white solid which was recrystallized from H_2O (150 mL) to give pure **18**·$0.5H_2O$: yield 2.01 g (69%); mp >300 °C dec; homogeneous by TLC (*n*-BuOH–AcOH–H_2O, 5:2:3; detection by UV absorption and ninhydrin test); ^1H NMR (CF_3CO_2D) δ 3.7 (m, 2, CH_2NH_2), 4.9 (m, 3, $CH_2CHOHCH_2$ and NCH_2), 8.9 (s, 1, C_8H). Anal. ($C_8H_{12}N_6O_2 \cdot 0.5H_2O$) C, H, N.

7-(4-Aminobenzyl)guanine (20). Method A. Compound **9a** (5.00 g, 17.5 mmol) was hydrogenated during 18 h at atmospheric pressure in a rapidly stirred mixture with 5% Pd on C (1.25 g) and glacial AcOH (500 mL). About 80% of the total H_2 uptake occurred during the first 3 h. The mixture was filtered through a Celite mat, which was first washed with AcOH at 25 °C and then with boiling AcOH (60 mL). The combined filtrate was evaporated to dryness (H_2O aspirator, bath to 50 °C), and the yellow solid residue was stirred with H_2O, collected, and washed successively with EtOH and Et_2O to give crude **20** (3.27 g, 73% crude yield). The mixture of catalyst and Celite was then extracted with several portions of boiling AcOH (200 mL total), and the combined filtered solution was evaporated. The residue was worked up as above to increase the crude yield to 3.89 g (87%). A sample of the crude **20** (0.33 g) was stirred with HCl solution (15 mL of 0.33 N), and the cloudy solution that formed was clarified and evaporated to give a yellow crystalline residue. This solid was twice recrystallized by dissolving it in H_2O (5 mL) and adding EtOH (50 mL) and Et_2O (50 mL) in that order. The pale-yellow crystalline solid obtained (0.32 g) gave satisfactory elemental analysis results for hydrated **20**·2HCl. Anal. ($C_{12}H_{12}N_6O \cdot 2HCl \cdot 0.8H_2O$) C, H, N. The remaining crude **20** (3.57 g) was stirred with HCl solution (150 mL of 0.33 N), and the resulting solution, after clarification, was chilled (ice bath) and treated with NaOH solution to produce pH 7.8. The pale-yellow solid that separated was collected by centrifugation and washed in the centrifuge tube with H_2O (until the decantate was free of chloride ion) followed by EtOH. After decantation of the final EtOH wash, the solid was suspended in Et_2O and collected by filtration to give 2.98 g (67% yield) of pure **20**, mp indefinite with progressive darkening from about 250 °C. The total yield including the sample purified as the dihydrochloride was 73%. Anal. ($C_{12}H_{12}N_6O$) C, H, N.

Method B. Pulverized **9a** (1.57 g, 5.50 mmol) was hydrogenated during 2 h at atmospheric pressure in a rapidly stirred mixture with 5% Pd on C (1.50 g) and 0.3 N HCl (75 mL). The resulting solution was filtered from the catalyst, and the chilled filtrate was treated with NH_4OH solution to produce pH 7.4. The white precipitate that formed was collected and washed with H_2O, Me_2CO, and Et_2O to give 0.95 g (67% yield) of **20**. In a scaled-up run (10.66 g, 37.2 mmol, of **9a**: 10 g of 5% Pd on C, 550 mL of 0.3 N HCl), the reduction was complete after about 4 h, and the yield of **20** was 67% (6.40 g). The samples of **20** prepared in this manner produced IR and UV spectra in good agreement with those

of the analytical sample described under method A.

7-(3-Aminobenzyl)guanine (22). Catalytic reduction of **10a** (1.90 g, 6.64 mmol) at atmospheric pressure in rapidly stirred 0.3 N HCl (90 mL) containing 5% Pd on C (0.50 g) was complete after 3 h. The catalyst was removed by filtration, and the filtrate, which had a bluish-purple tinge, was treated with Norit and filtered through Celite. The colorless filtrate was treated with dilute NH_4OH solution to produce pH 7.5. White solid separated readily, and, after a refrigeration period (2 h), was collected and washed successively with H_2O, Me_2CO, and Et_2O to give 1.19 g (70% yield) of pure **22**, mp >360 °C dec. Anal. ($C_{12}H_{12}N_6O$) C, H, N.

A larger run using the sample of **10a**·HCl described above (7.43 g, 23.0 mmol) gave pure **22** in 84% yield (5.40 g). This sample gave UV spectra identical with those of the analytical sample.

7-(4-Aminobenzyl)hypoxanthine (24). A suspension of **9b** (2.52 g, 9.00 mmol) and 5% Pd on C (1.5 g) in glacial AcOH (300 mL) was hydrogenated at 3–3.5 atm (Parr shaker) at 25 °C for 4 h. Following removal of the catalyst, the solvent was removed by evaporation, and the residue was suspended in 5% $NaHCO_3$ solution (25 mL), collected, washed with H_2O, and dried to give **24** in 83% yield (1.80 g). Recrystallization from EtOH (750 mL) afforded pure **24**, mp 215–217 °C dec, in 56% yield (1.22 g) (dried in vacuo at 100 °C), homogeneous by TLC ($CHCl_3$–MeOH, 5:1). Anal. ($C_{12}H_{11}N_5O$) C, H, N.

7-(3-Aminobenzyl)hypoxanthine (26). The nitro compound **10b** (1.3 g) was reduced in two runs in the manner described for the conversion of **9b** to **24**. After the treatment with $NaHCO_3$ solution, the crude samples were combined (720 mg) and recrystallized from MeCN to give **26**, mp 240–242 °C dec, in 40% overall yield (460 mg). Anal. ($C_{12}H_{11}N_5O$) H, N; C: calcd, 59.74; found, 59.10.

Aroylation of the Amino Compounds. The aroyl derivatives listed in Table II were prepared from the amines and aroyl chlorides in the presence of $(i\text{-Pr})_2NEt$. Compounds **16a**,b and **19a–c** were prepared in DMF; all others were prepared in glacial AcOH. In preparations of **16a–c**, the molar ratio of amine to aroyl chloride was 1:2, but in all others smaller excesses (5–10%) were used. $(i\text{-Pr})_2NEt$ was equimolar with aroyl chloride. The reaction procedure involved treatment of a stirred mixture of the amine and $(i\text{-Pr})_2NEt$ in the appropriate solvent at 25 °C with a solution of the aroyl chloride in the same solvent. Mixtures were typically stirred for 1–2 h at 25 °C. Crude products were isolated by one of the methods listed in Table II and then treated as indicated in the table before being dried.

7-[2-(Bromoacetamido)ethyl]guanine (16d). A solution of **15** (100 mg, 0.52 mmol) and 4-nitrophenyl bromoacetate (296 mg, 1.14 mmol) in glacial AcOH (10 mL) was stirred at 25 °C for 20 h. More 4-nitrophenyl bromoacetate (140 mg, 0.57 mmol) was added, and stirring was continued for 96 h. The solution was poured into Et_2O (100 mL), and the solid that formed was collected, suction dried, washed with H_2O, and dried to give pure **16d**, mp 280 °C dec, in 59% yield (97 mg). Anal. ($C_9H_{11}BrN_6O_2$) C, H, N.

7-[3-(Bromoacetamido)-2-hydroxypropyl]guanine (19d) was prepared from **18** (1.01 g, 4.5 mmol) as described above for **16d**: yield 96% (1.49 g); mp 265 °C dec; ^1H NMR (CF_3CO_2D) δ 3.8 (m, aliphatic CH), 4.07 (s, CH_2Br), 4.5–5.2 (br m, aliphatic CH), 8.93 (s, C_8 H). Since satisfactory analytical data could not be obtained for **19b**, a sample (69 mg) of it was stirred with dry pyridine for 30 h at 25 °C. The solid pyridinium derivative was collected, washed with benzene, and dried, yield 86% (73 mg). The material was dissolved in 4:1 EtOH–H_2O (10 mL), and the solution was mixed with Et_2O (100 mL) to cause separation of crystalline product. Anal. ($C_{15}H_{18}BrN_7O_3 \cdot 2H_2O$) C, H, N.

7-[4-(Bromoacetamido)benzyl]guanine (21d). Bromoacetic anhydride (1.37 g, 5.26 mmol) was added in one portion to a rapidly stirred suspension of **20** (0.90 g, 3.5 mmol) in AcOH (100 mL). The mixture was stirred rapidly for 30 min while it increased in thickness. The solid was then collected and washed with Me_2CO and Et_2O. The air-dried solid was then removed from the funnel and stirred in a centrifuge tube with 0.1 N HCl. The mixture was then centrifuged, and the supernatant was removed by decantation. The precipitate was treated with 0.1 N HCl in this manner three more times and was then treated similarly with Me_2CO until it could be collected by filtration. The collected solid was washed with Et_2O and dried: yield 50% (0.69 g); mp indefinite with progressive darkening from about 250 °C. Anal. ($C_{14}H_{13}BrN_6O_2 \cdot H_2O$) C, H, N.

Table II. Aroyl Derivatives

no.	crude product isolation[a]	purification method[b]	mp, °C dec	yield %	molecular formula[e]
16a	I	A, B[c]	275	51	$C_{14}H_{13}FN_6O_4S \cdot 0.9H_2O$
16b	I	A, C, B	270	41	$C_{14}H_{13}FN_6O_4S \cdot H_2O$
16c	I	A, B[d]	300	37	$C_{14}H_{13}ClN_6O_3 \cdot H_2O$
19a	II	B, C, B[d]	330	32	$C_{15}H_{15}FN_6O_5S \cdot H_2O$[f]
19b	II	A, C, B[d]	330	20	$C_{15}H_{15}FN_6O_4S \cdot H_2O$[f]
19c	I	A, C, B	315	56	$C_{15}H_{15}ClN_6O_3 \cdot H_2O$
21a	III	D, A, C, B[c]	300	49	$C_{19}H_{15}FN_6O_4S \cdot 0.9H_2O$
21b	III	D, A, C, B[c]	310	50	$C_{19}H_{15}FN_6O_4S \cdot 0.75H_2O$
21c	III	E, A, C, B	>375	90	$C_{19}H_{15}ClN_6O_2$
23a	I	C, B	300	81	$C_{19}H_{15}FN_6O_4S \cdot H_2O$
23b	III	C, B	300	73	$C_{19}H_{15}FN_6O_4S \cdot 0.5H_2O$
23c	I	C, B, A	360	86	$C_{19}H_{15}ClN_6O_2$
25a	III	A, F, B	235	70	$C_{19}H_{14}FN_5O_4S \cdot H_2O$
25b	III	A, F, B	245	74	$C_{19}H_{14}FN_5O_4S \cdot 0.75H_2O$
25c	III	A, C, B	322	66	$C_{19}H_{14}ClN_5O_2 \cdot 0.25H_2O$

[a] Crude products isolated as follows: I, reaction mixture evaporated in vacuo; II, reaction mixture added to H_2O to cause precipitation of product; III, product filtered directly from reaction mixture. [b] Crude product was washed with the following solvents or solutions in the order given: A, Et_2O; B, H_2O; C, 0.1 N HCl; D, AcOH; E, Me_2CO; F, 1% $NaHCO_3$. [c] Then recrystallized from DMF. [d] Then reprecipitated from DMF solution by the addition to H_2O. [e] Anal. C, H, N. [f] Kjeldahl nitrogen determination.

Similar treatment of **20** (200 mg, 0.78 mmol) with bromoacetic anhydride (300 mg, 1.15 mmol) in DMF (6 mL) at 0–5 °C for 35 min, followed by a workup like that described above, also gave **21d**: yield 0.20 g (65%); ^1H NMR (CF_3CO_2D) δ 4.16 (s, 2, $COCH_2Br$), 5.76 (s, 2, NCH_2), 7.6 (m, 4, C_6H_4), 8.75 (s, 1, C_8 H).

7-[3-(Bromoacetamido)benzyl]guanine (23d) was prepared from **22** and bromoacetic anhydride in AcOH in the manner and scale given for isomeric **25d**. The yield of **23d** thus obtained was 79% (1.04 g). Anal. ($C_{14}H_{13}BrN_6O_2$) C, H; N: calcd, 22.28; found, 22.95. The ^1H NMR spectrum (in CF_3CO_2D) for this sample was as expected for **23d** except for a slight contaminant, possibly the corresponding acetyl compound, that produced a weak signal at δ 2.42. The signals due to **23d** and their assignments are as follows: δ 4.12 (s, 2, $COCH_2Br$), 5.76 (s, 2, NCH_2), 7.46 (m, 3, $C_{4'}$ H, $C_{5'}$ H and $C_{6'}$ H), 7.90 (s, 1, $C_{2'}$ H), 8.80 (s, 1, C_8 H). The integral produced by the spurious signal at δ 2.42 was too small to measure. Anal. Calcd for $C_{14}H_{13}BrN_6O_2$: C, 44.58; H, 3.47; N, 22.28. Found: C, 45.19; H, 3.75; N, 22.71.

7-[4-(Bromoacetamido)benzyl]hypoxanthine (25d). A stirred solution of **24** (965 mg, 4.00 mmol) in glacial AcOH (25 mL) at 25 °C was treated dropwise during 5 min with a solution of freshly distilled bromoacetic anhydride (1.6 g, 6.0 mmol) in AcOH (10 mL). The solution was kept at 25 °C for 30 min and then poured into Et_2O (700 mL) to cause separation of **25d**, which was collected, washed with Et_2O, and dried: yield 88% (1.27 g); mp >300 °C dec. Anal. ($C_{14}H_{12}BrN_5O_2$) C, H, N.

Guanine-7-acetaldehyde (27). The clear solution formed by addition of HCl (12 mL of 1 N) to a stirred suspension of **8a** (2.25 g, 10.0 mmol) in H_2O (25 mL) was treated dropwise under N_2

during 20 min with a solution of $NaIO_4$ (2.35 g, 11.0 mmol) in H_2O (25 mL). The resulting solution was stirred under N_2 for 1 h and was then treated with NaOH (11.8 mL of 1 N) to produce pH 6. The solid that formed was collected by centrifugation, washed with H_2O (three times with 50-mL portions by centrifugation), and dried to give **27**: mp 290 °C dec; 66% yield (1.46 g); homogeneous by TLC; ^1H NMR (CF_3CO_2D) δ 5.6 (s, 2, CH_2CHO), 8.6 (s, 1, C_8 H), 9.7 (br s, 1, CH_2CHO). Anal. ($C_7H_7N_5O_2 \cdot 1.5H_2O$) C, H, N.

Ethyl 7-Guanine-4-crotonate (28). A solution of 5.00 mmol each of (ethoxycarbonylmethyl)triphenylphosphonium bromide (2.15 g) and NaOMe (270 mg) in MeOH (25 mL) was stirred at 25 °C under N_2 for 1 h. The MeOH was removed by evaporation in vacuo, and the residue was suspended in Me_2SO (10 mL). The stirred mixture was treated dropwise with a solution of **27**·1.5H_2O (424 mg, 2.00 mmol) in Me_2SO (40 mL), and the solution that formed was kept at 25 °C for 3 h before the solvent was removed by evaporation in vacuo. The residue was stirred with boiling C_6H_6 (50 mL), collected, and washed on the funnel with warm C_6H_6 followed by several portions of warm hexane [to remove $(C_6H_5)_3PO$]. The solid was then washed with H_2O and recrystallized from EtOH–H_2O (7:3, v/v) to give pure **28**, mp 280 °C dec, in 50% yield (264 mg): MS *m/e* 263 (M^+), 217 (M^+ – EtOH), 190 (M^+ – CO_2Et). Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

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[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin and [1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin, Two Highly Potent Antagonists of the Vasopressor Response to Arginine-vasopressin

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As part of our studies on the design and synthesis of antagonists of the vasopressor response to arginine-vasopressin (AVP), we have synthesized [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]arginine-vasopressin [1, d(CH_2)$_5$Tyr(Me)AVP] (in duplicate) and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin [2, d(CH_2)$_5$AVP]. The required protected intermediate for 1 was synthesized by (a) a combination of solid-phase synthesis and an 8 + 1 coupling in solution and (b) entirely by solid-phase synthesis. The required protected precursor for 2 was synthesized entirely by solid-phase methods. 1 and 2 were tested for agonistic and antagonistic activities in rat vasopressor, rat antidiuretic, and rat uterus assay systems. d-(CH_2)$_5$Tyr(Me)AVP exhibits a surprisingly potent and prolonged antivasopressor effect. It has an antivasopressor pA_2 of 8.62 ± 0.03 and an antidiuretic potency of 0.31 ± 0.07 unit/mg. Material from the second synthesis of d(CH_2)$_5$Tyr(Me)AVP has a pA_2 value of 8.67 ± 0.02. d(CH_2)$_5$AVP also exhibited a potent, although less prolonged, antivasopressor effect. It has an antivasopressor pA_2 of 8.35 ± 0.09 and an antidiuretic potency of only 0.033 ± 0.005 unit/mg. These are the two most potent vasopressor antagonists reported to date. Both analogues also antagonize the actions of oxytocin on the rat uterus (a) in the absence of Mg^{2+}, (b) in the presence of 0.5 mM Mg^{2+}, and (c) in situ. They exhibit, respectively, the following pA_2 values in each of the assay systems a–c: (1) a, 8.13 ± 0.12; b, 7.24 ± 0.07; c, 6.62 ± 0.07; (2) a, 8.15 ± 0.20; b, 7.19 ± 0.08; c, 6.79 ± 0.19. With their potent ability to antagonize the vasopressor effects of AVP, d(CH_2)$_5$Tyr(Me)AVP and d(CH_2)$_5$AVP should be valuable additions to our previously reported antagonists for use as pharmacological tools with which to probe the possible role(s) of AVP in cardiovascular regulation in normal and pathophysicological states.

In attempting to design an antagonist of the antidiuretic response to arginine-vasopressin (AVP), we synthesized [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP)[1] and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$VDAVP].[2] These analogues were designed by replacing the two hydrogens on the β carbon at position 1 of the highly active and selective antidiuretic peptide [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP)[3] with two methyl groups and a cyclopentamethylene group, respectively. These substituents had previously been shown to convert the highly potent oxytocic agonist [1-deamino]oxytocin[4] (dOT) into the potent antagonists of the oxytocic response to oxytocin, [1-deaminopenicillamine]oxytocin (dPOT)[5] and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]oxytocin (d(CH_2)$_5$OT).[6] Neither dPVDAVP nor d(CH_2)$_5$VDAVP were antagonists of the antidiuretic response to AVP, although they possessed $^1/_{10}$ and only $^1/_{10\,000}$ the antidiuretic activity of

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