Synthesis and Biological Studies of Unsaturated Acyclonucleoside Analogues of *S*-Adenosyl-L-homocysteine Hydrolase Inhibitors

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The design, synthesis, and biological evaluation of several unsaturated acyclonucleosides related to augustmycin A are described. The (propargyloxy)methyl acyclonucleoside analogues of 6-chloropurine, adenine, 6-methoxypurine, hypoxanthine, 6-mercaptopurine, and azathioprine have been prepared. The 9-[(propargyloxy)methyl]adenine (**5**) and 9-[(propargyloxy)methyl]hypoxanthine (**12**) analogues were converted to the corresponding 5′-tributylstannyl intermediates (**9** and **13**), respectively, which gave 9-[[[(*Z*)-5-iodo-5-propenyl]oxy]methyl]adenine (**10**) and 9-[[[(*Z*)-5-iodo-5-propenyl]oxy]methyl]hypoxanthine (**14**), respectively, after iododestannylation. The [^{125}I]-radiolabeled congeners of **10** and **14** were prepared as potential metabolic markers. Among the unsaturated acyclonucleosides tested, 9-[(propargyloxy)methyl]-6-chloropurine (**3**), 9-[(propargyloxy)methyl]-6-mercaptopurine (**15**), 9-[(propargyloxy)methyl]azathioprine (**17**), and angustmycin A analogue **10** showed inhibition of cancer cell growth, but only at a minimal level, and **17** also showed 14% cancer cell death in vitro. Compound **10** provided ~50% protection against HIV at 10^{-4} M concentrations. Biodistribution results of [^{125}I]-**10** in mice indicate that compound **10** is readily metabolized via deiodination in vivo, possibly by serving as a substrate for the enzyme *S*-adenosyl-L-homocysteine hydrolase.

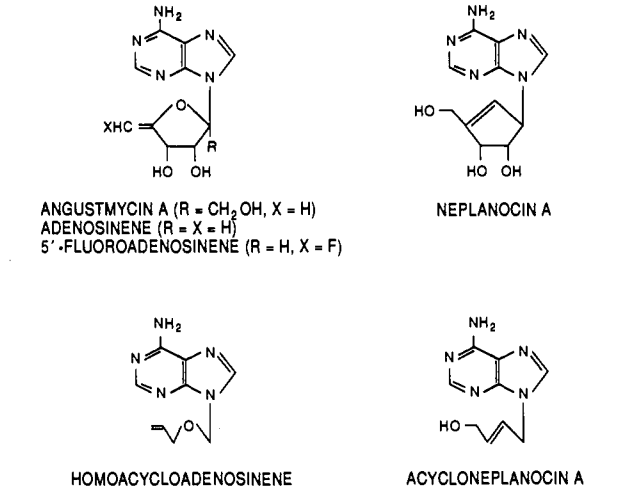
Introduction

The nucleoside analogues, by modification of the activity of the enzymes of nucleic acid biosynthesis, provide some of the most powerful and useful inhibitors of viral replication and neoplastic tissue growth.[1] Recently, the enzyme *S*-adenosyl-L-homocysteine (SAH) hydrolase has been noted as a potential target for antiviral chemotherapy.[2] This enzyme may also play a crucial role in *S*-adenosylmethionine (SAM) dependent carboxymethyltransferase methylation step during the posttranslational processing of the *ras* oncogene products required for membrane localization and neoplastic properties.[3]

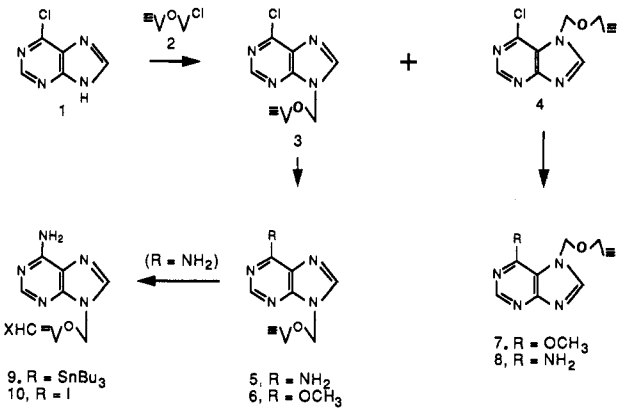
The enzyme SAH hydrolase[4] catalyses NAD ⇄ NADH mediated reversible hydrolysis of SAH to adenosine and homocysteine.[5] SAH hydrolase, by regulating the intracellular SAH/SAM ratio, interferes with biological transmethylations required for efficient translation of mRNA into proteins.[4] Recently, it has been observed that several unsaturated adenine nucleosides, including angustmycin A[6] analogues, 5′-fluoro-4′,5′-didehydro-5′-deoxyadenosine[7] (5′-fluoroadenosinene), and neplanocin A[8] (Chart I), exhibit antiviral activity. The antiviral activity of the nucleosides appears to correlate with the ability of the nucleosides to inhibit SAH hydrolase.[2,7-9]

The fluorinated nucleosides have also proven to be useful as precursors to study the mechanism of SAH hydrolase inactivation by measurement of fluoride ion, apparently resulting via NAD ⇄ NADH mediated oxidation and binding of the precursor to a nucleophilic site on SAH hydrolase.[7] Various acyclic analogues of acycloneplanocin A and adenosine have been synthesized for biological evaluations.[10,11] In this paper, we describe the synthesis and biological evaluation of homoacyclonucleoside analogues of 4′,5′-didehydro-5′-deoxyadenosine (adenosinene), a substrate of SAH hydrolase,[5] related to fluorinated adenosinenes and acycloneplanocin A (Chart I).

Chart I



Scheme I



Chemistry

The preparation of the acyclic alkylating reagent (propargyloxy)methyl chloride (**2**, Scheme I) is inadequately described in the literature,[12] and our attempts to prepare the propargyl acyclonucleoside analogues via alkylation of an aglycon, adenine or 6-chloropurine (**1**), using crude **2**, following literature procedures,[13] were futile. A systematic

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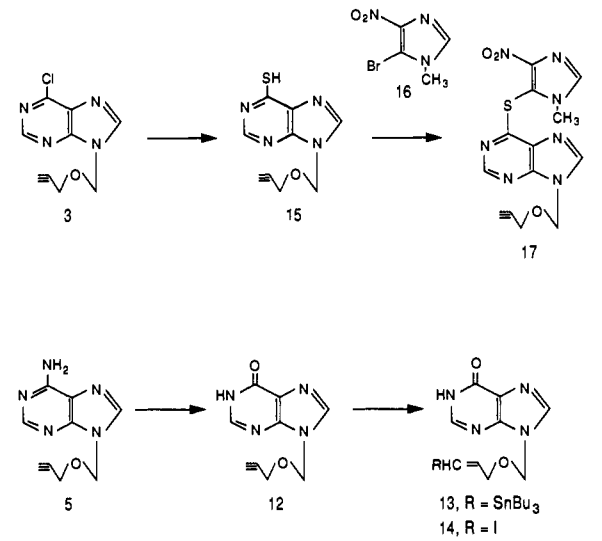
investigation of the synthetic strategy revealed that reagent **2** could be generated simply by saturating a mixture of propargyl alcohol and paraformaldehyde with hydrogen chloride and separated in the pure form by distillation of the reaction mixture under vacuum at ambient temperature. The residual mixture contained a viscous oil byproduct suspected to be the bis-[(propargyloxy)methyl]-ether ($HC{\equiv}CCH_2OCH_2OCH_2OCH_2C{\equiv}CH$) which decomposed gradually with a change in color. If not separated from **2**, the byproduct severely hindered the subsequent alkylation and isolation of the acyclic nucleoside analogues. Pure **2** can be stored in a refrigerator under anhydrous conditions for months without appreciable decomposition.

Scheme II



For the synthesis of the acyclic nucleoside analogues, the alkylation of trimethylsilylated adenine[14] with **2** was initially evaluated. This procedure provided 9-[(propargyloxy)methyl]adenine (**5**) in less than 5% yield and led us to investigate an alternative route outlined in Scheme I. The sodium hydride treatment of **1** in acetonitrile followed by alkylation with **2** provided both 9-[(propargyloxy)-methyl]-6-chloropurine (**3**) and 7-[(propargyloxy)-methyl]-6-chloropurine (**4**) in an approximately 10:1 ratio, respectively. These results were in accordance with a previous literature report[15] describing deoxyribosylation of similar aglycons including **1**. Compound **3**, on treatment with methanolic ammonia, provided a mixture from which both 9-[(propargyloxy)methyl]adenine (**5**) and 9-[(propargyloxy)methyl]-6-methoxypurine (**6**) were isolated. The congeners 7-[(propargyloxy)methyl]-6-methoxypurine (**7**) and 7-[(propargyloxy)methyl]adenine (**8**) were similarly prepared from **4**.

We have previously studied in some detail the synthesis of vinyl iodides from the corresponding alkynyl substrates via hydrometalation followed by iododemetalation.[16-18] The optimum condition determined for the synthesis of 9-[[(5-iodo-5-propenyl)oxy]methyl]adenine (**10**) was via the formation of 9-[[[5-(tributylstannyl)-5-propenyl]oxy]-methyl]adenine (**9**) by treatment of **5** with excess tributylstannyl hydride[19] with subsequent iododestannylation

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of **9** (Scheme I). Iododestannylation predominantly provided **10** as the *Z*-isomer. The *Z*-isomeric configuration for compound **10** was assigned on the basis of NMR spectrometry. In the ^{1}H NMR spectrum of compound **10**, the signal for vinyl protons appeared as a triplet (J = 3.0 Hz) and a doublet (J = 3.5 Hz) in contrast to a set of doublets with significantly higher coupling constants ($J \geq 12$ Hz) observed normally for vinyl protons of an *E*-isomer.[20] In the ^{13}C NMR spectrum of compound **10**, the observed chemical shifts for 4′(α to the terminal position)- and 5′(terminal position)-carbons were 141.9 and 81.1 ppm, respectively, consistent with the literature reports for a *Z*-isomer.[20] Apparently, the attack by stannane on the terminal (C_5') alkynyl carbon of **5** occurs to form the energetically more favorable *Z*-isomer as compared to the corresponding *E*-isomer.

The inosine analogue 9-[(propargyloxy)methyl]hypoxanthine (**12**) was prepared for similar structural modifications and biologic and metabolic studies (Scheme II). The chloride displacement of **3** with hydroxide proceeded at a very low rate and provided **12** in poor (20%) yield. Alternatively, direct alkylation of silylated hypoxanthine (**11**) with **2** provided **12** as a mixture of N-7- and N-9-isomers which were found to be difficult to separate. Finally, **12** was conveniently prepared via diazotization of **5**. Compound **12** when stannylated provided predominately 9-[[[(*Z*)-5-(tributylstannyl)-5-propenyl]oxy]-methyl]hypoxanthine (**13**), which on iododestannylation readily yielded 9-[[[(*Z*)-5-iodo-5-propenyl]oxy]methyl]-hypoxanthine (**14**). The formation of the *Z*-isomer in the hypoxanthine series is similar to the formation of the *Z*-isomer observed in the adenine series (vide ante).

The 6-mercaptopurine riboside analogue 9-[(propargyloxy)methyl]-6-mercaptopurine (**15**) was prepared via the chloride displacement of **3** with thiourea. The alkylation of 6-mercaptopurine analogue **15** with 1-methyl-4-nitro-5-bromoimidazole[21] (**16**) yielded 6-azathioprine analogue **17** (Scheme II). The treatment of both **15** and **17** with tributylstannyl hydride provided an intractable mixture from which the desired vinylstannanes were not isolated.

The iodine-125 (^{125}I) radiolabeled analogues of **10** and **14** were also prepared as potential metabolic markers and for subsequent studies as potential substrates for adenosine deaminase and SAH hydrolase.

Biological Evaluation. The in vivo screening of the unsaturated acyclonucleosides against 60 human tumor cell lines, derived from seven cancer types (lung, colon, melanoma, renal, ovarian, brain, and leukemia), was performed under the auspices of the National Cancer Institute (NCI), National Institutes of Health.[22] The tumor cells growing in culture for 24 h after inoculation onto microtiter plates were incubated for 48 h with the test compounds at concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. The effect of test compounds on cell growth (percent inhibition or kill) was determined spectrophotometrically using the sulforhodamine B (SRB) protein assay technique.[23,24] Compound **4**, **5**, and **8** were devoid of any significant biological activity (<50% inhibition of cell growth) at the highest concentration tested. The data for compounds which showed minimal cytotoxicity or inhibitory activity are given in Table I. Compound **3** demonstrated 60–80% inhibition of cell growth against leukemia, lung, and melanoma cell lines at a concentration of 10^{-4} M. Compound **10** inhibited cell growth by 50–80% against leukemia, lung, and brain (CNS) cancer cell lines. In comparison with **10**, the reference compound[7] (*Z*)-5′-fluoroadenosinene (isomerism with respect to ribofuranose oxygen) (Chart I) shows relatively higher inhibition of cell growth[26] of leukemia cells, lower inhibition of lung/HOP-18, and similar inhibition of CNS/SNB-75. Both 6-mercaptopurine analogue **15** and 6-azathioprine analogue **17** showed 100% inhibitory activity (0% cell growth) and 14% cell kill, respectively, against the SNB-75 CNS cancer cell line at 10^{-4} M concentrations. Compound **17** also showed inhibitory activity (50–70%) against leukemia, lung, and renal cancer cell lines.

Table I. Effect of Unsaturated Acyclonucleosides (10^{-4} M) on Cancer Cell Growth in Vitro[a]

acyclonucleoside (NSC No.)	cancer/cell line	% cell growth (% inhibn)
3 (633128-Q/1)	leukemia/K-562	34.8 (65.2)
	leukemia/Molt-4	40.7 (59.3)
	leukemia/SR	38.4 (61.6)
	lung/DMS 114	21 (79)
	melanoma/LOX IMUI	33.5 (66.5)
10 (633124-M/1)	leukemia/CCRF-CEM	47.4 (52.6)
	lung/HOP-18	21.2 (78.8)
	CNS/SNB-75	25.2 (74.8)
15 (633667-A/2)	CNS/SNB-75	0 (100)
17 (633668-C/2)	leukemia/CCRF-CEM	50 (50)
	lung/NCS-H522	32 (68)
	lung/LXFL529	37 (63)
	CNS/SNB-75	−14 (14% cell death)
	renal/UO-31	48 (52)

[a] The acyclonucleosides were tested against 60 human cancer cell lines in vitro[22,23] and were found to be inactive at concentrations lower than 10^{-4} M.

Compounds **3–5**, **8**, **10**, **12**, **15**, and **17** were also evaluated at the National Cancer Institute for anti-HIV activity at 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M concentrations.[25] Most of these compounds were judged to be inactive in this screen (less than 50% protection), except for compound **10**, which provided 50% protection against infection at 2.06×10^{-7} M concentration.

Biodistribution results of [^{125}I]-**10** in Balb-C mice indicate initial uptake in tumor and other organs with subsequent washout (24 h) of the radioactivity from all the

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Table II. Mean Biodistribution and Elimination Data for [^{125}I]-10 in Tumor-Bearing Female Balb-C Mice following Intravenous Administration[a,b]

time ater injection, h	tumor	blood	kidney	liver	lung	thyroid[c]	urine[d]
1	1.50	3.15	3.68	3.40	3.41	1.29	
4	0.60	1.33	1.00	1.34	1.30	3.61	
24	0.06	0.34	0.20	0.33	0.35	3.49	94.36

[a] Four animals per group were used. [b] ID = 0.28 μCi/1 mg in 0.1 mL of saline. [c] Values as %ID/thyroid. [d] Values as %ID/animal.

organs except thyroid (this organ traps free iodine) (Table II). Approximately 94% of the radioactivity was excreted in the urine (24 h). The TLC analysis (solvent *E*) of the urine by UV and radioscanning indicated the absence of any detectable amounts of either [^{125}I]-10 (control **10**, R_f 0.71) or the corresponding deaminase metabolite [^{125}I]-14 (control **14**, R_f 0.60). In addition to the base-line impurities, three major spots on TLC were detected: one radioactive spot (R_f 0.87) was attributed to an iodide. The two other spots (R_f 0.19 and R_f 0.15), visible under UV light but nonradioactive, could potentially be attributed to homoacyclonucleoside metabolites arising via enzymatic dehalogenation[7,8] of substrate **10**.

Conclusions

Among the various unsaturated acyclonucleosides tested, only **10** showed activity against both tumor and virus screens in vitro. Studies with [^{125}I]-**10** indicate that this compound is readily metabolized in vivo to a nonradioactive (deiodinated) compound, possibly suggesting a dehalogenation mechanism similar to that involving the attack of enzyme hydrolase nucleophile on the nucleoside substrate. Both neplanocin A and fluorinated unsaturated nucleosides are potent inhibitors of SAH hydrolase and exhibit chemotherapeutic activity.[2,7,8] The results from these studies further point out the potential utility of halogenated unsaturated adenine nucleosides to study enzymes of nucleotide metabolism as targets for biological activity. The halogen-radiolabeled congeners could also be useful as markers to study intermediary metabolism.

Experimental Section

General Methods. Chemical. All solvents, chemicals, and reagents were analytical grade and were used without further purification unless otherwise indicated. The melting points (mp) were determined on a Thomas-Hoover apparatus in open capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 spectrometer and are reported in ppm downfield from the internal tetramethylsilane (TMS = δ 0) standard or an appropriate deuterated solvent ($CDCl_3$ = δ 77.0, CD_3OD = δ 49.0 and DMSO-d_6 = δ 39.5), respectively. The thin-layer chromatographic (TLC) analyses were performed using precoated 250-nm layers of SiO_2 glass plates (Analtech, Inc.). Solvent *E* for TLC consisted of ethyl acetate/2-propanol/water, 7:1:2, v/v (top layer). The R_f values provided relate to specific experiments and the absolute number may vary depending upon the experimental conditions. The mass spectral analyses (MS) were determined by the Organic Spectroscopy Group, Analytical Chemistry Division at the Oak Ridge National Laboratory (ORNL), by laser ionization FT mass spectrometry. The elemental analyses were determined by Galbraith Laboratories (Knoxville, TN). Sodium [^{125}I]iodide was purchased from New England Nuclear, Inc. (North Billerica, MA).

Biological. Tumor-bearing female Balb-C mice implanted with Line-1 spontaneous lung carcinoma cells were obtained from ORNL Biology Division (courtesy of Dr. Stephen J. Kennel) and were used 1 week after tumor inoculation of 10^6 cells. Iodine-125-labeled **10** was formulated in normal saline. The solution was filtered through a 0.22-μm Millipore filter and injected via a lateral tail vein into the ether-anesthetized animals. At a given time interval after the injection, the animals were reanesthetized with ether and killed by cervical fracture. The tumor and other desired organs were excised, rinsed with saline solution, blotted dry, placed in tared vials, and weighed. The samples were counted for radioactive contents in a Packard Minaxi 5000 sodium iodide auto γ counter. Results are expressed as percent injected dose/gram (ID/g) tissue, except for thyroid and urine, for which the results are expressed as ID/tissue.

(Propargyloxy)methyl Chloride (2). A mixture of propargyl alcohol (11.2 g, 200.0 mmol) and paraformaldehyde (6.0 g) was saturated with anhydrous HCl gas at −25 to −20 °C until a clear solution was obtained (~45 min). The reaction mixture was distilled under vacuum at ambient temperature to provide pure (propargyloxy)methyl chloride as an oil: yield, 12.4 g, (60%); ^{1}H NMR ($CDCl_3$) δ 5.55 (s, 2 H, H-5), 4.32 (d, $J_{1,3}$ = 2.0 Hz, 2 H, H-3), 2.5 (t, $J_{1,3}$ = 0.5 Hz, 1 H, H-1). The residual oil [^{1}H NMR ($CDCl_3$) δ 5.0 (s, 4 H, O-CH_2-O), 4.35 (s, 4 H, CH_2O), 2.5 (s, 2 H, ≡CH)] was assumed to be the bis[(propargyloxy)methyl] ether.

6-Chloro-9-[(propargyloxy)methyl]purine (3) and 6-Chloro-7-[(propargyloxy)methyl]purine (4). Sodium hydride (60% oil dispersion, 280 mg, 7.0 mmol) was added to 6-chloropurine (1, 1.0 g, 6.5 mmol) in anhydrous acetonitrile (45 mL) and the mixture was stirred at room temperature under an argon atmosphere for 1 h. (Propargyloxy)methyl chloride (900 mg) was then added slowly at room temperature and the mixture was heated (65 °C) with stirring (argon) overnight. The mixture was cooled to room temperature and the insoluble material removed by filtration. Solvent was evaporated and the crude mixture was purified by silica gel column chromatography (petroleum ether). The column was eluted with 25–50% chloroform in petroleum ether. Fractions 13–66 (18–20 mL each) contained 6-chloro-9-[(propargyloxy)methyl]purine (**3**): yield, 830 mg (59%); R_f 0.66 (ethyl acetate); mp 95–96 °C ($CHCl_3$); UV λ_{max} (MeOH) 263.5 nm, (0.1 N HCl) 264 nm, (0.1 N NaOH) 264 nm; ^{1}H NMR ($CDCl_3$) δ 9.0 (s, 1 H, H-8), 8.5 (s, 1 H, H-2), 6.0 (s, 2 H, H-1′), 4.35 (d, J = 3.0 Hz, 2 H, H-3′), 2.60 (t, J = 2.0 Hz, 1 H, H-5′); ^{13}C NMR ($CDCl_3$) δ 152.4, 151.2, 145.0, 144.9, 131.4, 76.4, 76.3, 71.5, 56.9. Anal. ($C_9H_7ClN_4O$) C, H, N, Cl. Fractions 69–77 (18–20 mL each) contained 6-chloro-7-[(propargyloxy)methyl]purine (**4**): yield, 88 mg (6.3%); R_f 0.35 (ethyl acetate); mp 116–117 °C ($CHCl_3$); UV λ_{max} (MeOH) 283 nm, (0.1 N HCl) 264 and 282.5 nm (shoulder), (0.1 N NaOH) 283 nm; ^{1}H NMR ($CDCl_3$) δ 9.1 (s, 1 H, H-8), 8.6 (s, 1 H, H-2), 6.1 (s, 2 H, H-1′), 4.35 (d, J = 3.0 Hz, 2 H, H-3′), 2.65 (t, J = 1.8 Hz, 1 H, H-5′); ^{13}C NMR ($CDCl_3$) δ 162.2, 152.8, 149.2, 143.5, 122.1, 77.0, 76.9, 73.2, 55.8. Anal. ($C_9H_7ClN_4O$) C, H, N, Cl.

6-Amino-9-[(propargyloxy)methyl]purine (5) and 6-Methoxy-9-[(propargyloxy)methyl]purine (6). A solution of **3** (800 mg, 3.6 mmol) in methanolic ammonia (40 mL, saturated at 0 °C) was heated at 100 °C for 6 h in a steel bomb. The bomb was cooled (ice bath) and opened and the solvent was evaporated. The crude mixture, after purification by silica gel column chromatography (25% ethyl acetate in chloroform), yielded (85 mg, 11.6%) the faster moving minor product **6**: R_f 0.86 (solvent E); mp 133–134 °C (MeOH); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.5 (s, 1 H, H-8), 8.3 (s, 1 H, H-2), 5.85 (s, 2 H, H-1′), 4.22 (d, J = 2.5 Hz, 5 H, OMe and H-3′), 2.65 (t, J = 2.8 Hz, 1 H, H-5′). Anal. ($C_{10}H_{10}N_4O_2$) C, H, N. Further elution yielded (400 mg, 55%) **5** as the slower moving product: R_f 0.72 (solvent *E*); mp 179–180 °C (H_2O); ^{1}H NMR (DMSO-d_6) δ 8.4 (s, 1 H, H-8), 8.3 (s, 1 H, H-2), 7.4 (bs, 2 H, NH_2, exchangeable with D_2O), 5.7 (s, 2 H, H-1′), 4.35 (d, J = 3.0 Hz, 2 H, H-3′), 3.5 (t, J = 2.0 Hz, 1 H, H-5′); ^{13}C NMR (DMSO-d_6) δ 155.82, 152.76, 148.9, 140.97, 118.5, 79.0, 77.59, 71.02, 56.18. Anal. ($C_9H_9N_5O$) C, H, N.

6-Methoxy-7-[(propargyloxy)methyl]purine (7) and 6-Amino-7-[(propargyloxy)methyl]purine (8). A solution of **4** (800 mg) in methanolic ammonia (50 mL, saturated at 0 °C) was heated overnight in a steel bomb at 100 °C. The bomb was cooled (ice bath) and opened and the solvent was evaporated under

vacuum. The residue obtained was purified by silica gel column chromatography (EtOAc/MeOH) to yield 80 mg (10%) of 7: mp 98–99 °C ($CHCl_3$/ether); ^1H NMR ($CDCl_3$) δ 8.8 (s, 1 H, H-8), 8.3 (s, 1 H, H-2), 5.9 (s, 2 H, H-1′), 4.25 (d, J = 4.0 Hz, 5 H, OCH_3 and H-3′), 2.6 (t, J = 3.0 Hz, 1 H, H-5′). Anal. ($C_{10}H_{10}N_4O_2$) C, H, N. Further elution provided 400 mg (55%) of 8: mp 194–195 °C (MeOH); ^1H NMR (DMSO-d_6) δ 8.6 (s, 1 H, H-8), 8.4 (s, 1 H, H-2), 7.0–6.8 (bs, 2 H, NH_2, exchangeable with D_2O), 5.95 (s, 2 H, H-1′), 4.35 (d, J = 3.0 Hz, 2 H, H-3′), 3.6 (t, J = 2.5 Hz, 1 H, H-5′). Anal. ($C_9H_9N_5O$) C, H, N.

6-Amino-9-[[[(Z)-5-(tributylstannyl)-5-propenyl]oxy]methyl]purine (9). Tributylstannyl hydride (3.37 g, 12 mmol) was added to a solution of 6-amino-9-[(propargyloxy)methyl]-purine (5, 609 mg, 3 mmol) and azobis(2-methylpropionate) (80 mg, 0.5 mmol) in anhydrous THF (20 mL). The mixture was refluxed for 4 h after which more tributylstannyl hydride (1.5 g) was added and the refluxing was continued for an additional 2 h. The solvent was evaporated in vacuo with exclusion of moisture. The residue was triturated with petroleum ether. The crystalline product which formed was collected by filtration under an argon atmosphere and dried under vacuum at room temperature to yield 900 mg (62%) of 9: mp 163–167 °C; ^1H NMR ($CDCl_3$) δ 8.5 (s, 1 H, H-8), 7.9 (s, 1 H, H-2), 6.2 (m, 1 H, HC=CHSnBu_3), 5.8 (m, 1 H, HC=CHSnBu_3), 5.6 (s, 2 H, H-1′), 4.08 (d, J = 4.2 Hz, 2 H, H-3′), 1.4–0.7 (m, 9 H, Bu_3Sn).

6-Amino-9-[[[(Z)-5-iodo-5-propenyl]oxy]methyl]purine (10). A solution of sodium iodide (75 mg, 0.5 mmol) in 50% aqueous THF (0.2 mL) was added to a solution of 9 (247 mg, 0.5 mmol) in THF (1 mL). The mixture was stirred in the dark at room temperature for 30 min. The solvent was evaporated under vacuum, the residue was dissolved in aqueous $CHCl_3$ (15 mL, 1:1, v/v) and the mixture was treated with an aqueous solution (1 mL) saturated with sodium thiosulfate. The $CHCl_3$ portion was separated, washed with H_2O (5 mL), and dried (Na_2SO_4). The solvent was evaporated under vacuum to provide the crude product, which was purified by silica gel column chromatography (25% EtOAc in $CHCl_3$) to yield 120 mg (73%) of 10: mp 195–196 °C (MeOH); MS m/z 332 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 8.4 (s, 1 H, H-8), 8.25 (s, 1 H, H-2), 7.5–7.2 (s, 2 H, NH_2, exchangeable with D_2O), 6.55 (t, J = 3.0 Hz, 1 H, HC=CH), 5.6 (d, J = 3.5 Hz, 3 H, H-1′ and —CH=CH—), 4.1 (m, 2 H, H-3′); ^{13}C NMR (DMSO-d_6) δ 155.8, 152.7, 149.5, 141.9, 140.7, 118.4, 81.1, 71.49, 70.1. Anal. ($C_9H_{10}N_5IO$) C, H, N.

9-[(Propargyloxy)methyl]hypoxanthine (12). A solution of 5 (609 mg, 3 mmol) in glacial acetic acid (15 mL) was diazotized by adding a solution of sodium nitrite (1.8 g) in water (4.0 mL) at 0 °C. The vessel was sealed and the reaction was allowed to proceed for 18 h at 40 °C. The solvent was evaporated under vacuum and coevaporated with H_2O to provide 12 as the crude product which was purified by silica gel column chromatography. Elution of the column with methanol in ethyl acetate (7–10%) yielded (560 mg, 92%) 12: mp 197–198 °C; ^1H NMR (DMSO-d_6) δ 12.19 (bs, 1 H, NH exchangeable with D_2O), 8.2 (s, 1 H, H-8), 8.08 (s, 1 H, H-2), 5.59 (s, 2 H, H-1′), 4.25 (d, J = 2.4 Hz, 2 H, H-3′), 3.22 (t, J = 2.04 Hz, 1 H, H-5′). Anal. ($C_9H_8N_4O_2$) C, H, N.

9-[[[(Z)-5-Iodo-5-propenyl]oxy]methyl]hypoxanthine (14) via Synthesis of 9-[[[(Z)-5-(Tributylstannyl)-5-propenyl]oxy]methyl]hypoxanthine (13). A mixture of 12 (204 mg, 1 mmol), tributylstannyl hydride (1.6 mL), and a catalytic amount of AIBN in anhydrous THF (10 mL) was refluxed under argon for 2 d. The reaction mixture was cooled and the solvent was evaporated under vacuum. The residue was triturated with petroleum ether, filtered, and purified by preparative TLC (ethyl acetate). The major band (R_f 0.2) was scraped and eluted with ethyl acetate to yield 178 mg (36%) of the stannyl intermediate 13: mp 175–176 °C; ^1H NMR ($CDCl_3$) δ 8.37 (s, 1 H, H-8), 7.98 (s, 1 H, H-2), 6.33–6.23 (d, J = 19 Hz, 1 H, —HC=CH—), 6.10–5.9 (m, 1 H, —HC=CH—), 5.61 (s, 2 H, H-1′), 4.08 (d, J = 5.13 Hz, 2 H, H-3′), 1.6–1.1, 0.9–0.7 (m, 27 H, 3 × Bu); ^{13}C NMR ($CDCl_3$) δ 159.71, 149.97, 146.33, 142.66, 140.82, 134.38, 124.97, 73.70, 72.79, 29.86, 29.68, 27.86, 14.31, 10.28. The stannyl intermediate (63 mg, 0.13 mmol) was dissolved in THF (2 mL) and a solution of NaI (57 mg, 0.38 mmol) in 50% aqueous THF (0.5 mL) was added, followed by the addition of NCS (51 mg, 0.38 mmol) in THF (0.5 mL). The reaction mixture was stirred at room temperature while protected from light for 20 min. A saturated aqueous solution of sodium thiosulfate (0.5 mL) was added and the solvent evaporated under vacuum. The residue was passed through a small column (2 × 10 cm) of SiO_2 (slurry in ethyl acetate). Elution of the column with 5% methanol in ethyl acetate afforded (29 mg, 69%) of 14: mp 151–152 °C; ^1H NMR (CD_3OD) δ 8.22 (s, 1 H, H-8), 8.13 (s, 1 H, H-2), 6.52 (t, J = 1.2 Hz, 1 H, —HC=CH—), 5.69 (d, J = 1.7 Hz, 1 H, —HC=CH—), 5.65 (s, 2 H, H-1′), 4.06 (t, J = 1.6 Hz, 2 H, H-3′); ^{13}C NMR (CD_3OD) δ 155.8, 150.2, 147.1, 142.3, 142.1, 125.1, 80.1, 73.7, 72.2. Anal. ($C_9H_9IN_4O_2$) C, H, I.

6-Mercapto-9-[(propargyloxy)methyl]purine (15). Anhydrous thiourea (228 mg, 3.0 mmol) was added to a solution of compound 3 (222.5 mg, 1.0 mmol) in absolute ethanol (5.0 mL) and mixture was heated at 70 °C for 2 h. The solution was allowed to cool to room temperature and filtered. The residue was washed with cold methanol and dried to yield 200 mg of crude 15, which was recrystallized with ethanol/water to yield (188 mg, 85%) of 15: mp 239–240 °C; ^1H NMR (DMSO-d_6) δ 8.5 (s, 1 H, H-8), 8.25–8.15 (d, J = 3.8 Hz, 2 H, H-2 and NH), 5.6 (s, 2 H, H-1′), 4.2 (d, J = 2.2 Hz, 2 H, H-3′), 3.4 (t, J = 1.5 Hz, H-5′). Anal. ($C_9H_8N_4OS$·0.25CH_3OH) C, H, N, S.

9-[(Propargyloxy)methyl]-6-[(1-methyl-4-nitroimidazolyl)thio]purine [9-[(propargyloxy)methyl]azathioprine 17]. Sodium hydride (40 mg, 60% oil dispersion) was added to a stirred suspension of compound 15 (222 mg, 1 mmol) in anhydrous DMF (2 mL) under argon at room temperature. After 30 min of stirring at room temperature, 5-bromo-1-methyl-4-nitroimidazole[21] (205 mg, 1 mmol) was added to the suspension and the reaction mixture was allowed to stir at 85 °C for 4 h. The solvent was evaporated under vacuum to provide a residue which was taken up in chloroform, washed with water, and dried (Na_2SO_4). Evaporation of chloroform under vacuum provided 340 mg of the crude product, which was passed through a column packed with silica gel slurry in $CHCl_3$. Elution of the column with 25% $CHCl_3$ in ethyl acetate afforded pure product, which was crystallized with chloroform to yield (290 mg, 84%) 17: mp 130–132 °C; ^1H NMR ($CDCl_3$ + CD_3OD) δ 8.9 (s, 1 H, H-8), 8.5 (s, 1 H, H-2), 8.15 (s, 1 H, H-2 of imidazole), 5.95 (s, 2 H, H-1′), 4.4 (d, J = 2.0 Hz, 2 H, H-2′), 3.9 (s, 3 H, CH_3), 2.6 (m, 1 H, H-5′). Anal. ($C_{13}H_{11}N_7O_3S$) C, H, S.

Radiolabeling Experiments. Synthesis of [^{125}I]-10 and [^{125}I]-14. General Method. The [^{125}I]-radiolabeled compounds 10 and 14 were prepared from the corresponding stannyl substrates 9 and 13, respectively. A mixture of NaI (1.5 mg, 0.01 mmol) and Na[^{125}I] (2.0 mCi) in aqueous THF (0.1 mL) was added to the solution of stannyl substrate (0.01 mmol) in THF (0.5 mL). A solution of NCS (3.0 mg) in THF (0.2 mL) was added and the reaction mixture was stirred in the dark for 30 min at room temperature. The solvent was evaporated under a stream of argon to remove THF and the residue (syrup) was diluted with water (0.2 mL). Sodium thiosulfate (2.0 mg) was added, and the solution was extracted with ethyl acetate (1.0 mL × 2). The ethyl acetate portion was dried (Na_2SO_4), the volume was reduced (0.1 mL) by evaporation under a stream of argon, and the residue was applied onto a 20 × 20 (250 μm) TLC plate. The plate was developed in solvent E, and the band corresponding to 10 (or 14) was then scraped and eluted with acetone. Evaporation of acetone under argon provided radiolabeled 10 and 14 in approximately 50% radiochemical yield. The radioactive compounds were divided into small portions (ca. 100 μCi) and stored in sealed vials in a freezer.

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