Phenylselenenyl- and Phenylthio-Substituted Pyrimidines as Inhibitors of Dihydrouracil Dehydrogenase and Uridine Phosphorylase

Naganna M. Goudgaon,† Fardos N. M. Naguib,‡ Mahmoud H. el Kouni,‡ and Raymond F. Schinazi*,†

Veterans Affairs Medical Center (Atlanta), Decatur, Georgia 30033, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322, and Department of Pharmacology and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, Alabama 35294

Received September 9, 1993•

Lithiation of 5-bromo-2,4-bis(benzyloxy)pyrimidine (3) with *n*-BuLi at −80 °C followed by the addition of diphenyl diselenide or diphenyl disulfide as an electrophile furnished the corresponding 5-(phenylhetera)-2,4-bis(benzyloxy)pyrimidine, which on exposure to trimethylsilyl iodide in CH_2Cl_2 at room temperature yielded the 5-(phenylhetera)uracils in 70–75% yield. Similarly, the 6-(phenylhetera)uracils were prepared from 6-bromo-2,4-bis(benzyloxy)pyrimidine (10). 1-[(2-Hydroxyethoxy)methyl]-5-(phenylselenenyl)uracil (PSAU, 18) and 1-(ethoxymethyl)-5-(phenylselenenyl)uracil (17) were synthesized by the electrophilic addition of benzeneselenenyl chloride to the acyclic uracils under basic conditions. These compounds were evaluated for their ability to inhibit dihydrouracil dehydrogenase (DHUDase, E.C. 1.3.1.2), orotate phosphoribosyltransferase (OPRTase, E.C. 2.4.2.10), uridine phosphorylase (UrdPase, E.C. 2.4.2.3), and thymidine phosphorylase (dThdPase, E.C. 2.4.2.4). 5-(Phenylselenenyl)uracil (PSU, 6) and 5-(phenylthio)uracil (PTU, 7) inhibited DHUDase with apparent K_i values of 4.8 and 5.4 μM, respectively. The corresponding 6-analogues, compounds 13 and 14, demonstrated inhibitory activity against OPRTase. PTU as well as PSU and its riboside, 2′-deoxyriboside, and acyclonucleosides were inhibitors of UrdPase, with PSAU (18) being the most potent with an apparent K_i value of 3.8 μM. None of the compounds evaluated had any effect on dThdPase. Interestingly, most of the compounds showed modest selective anti-human-immunodeficiency-virus activity in acutely infected primary human lymphocytes.

Introduction

5-Alkyl- or 5-alkylaryl-substituted pyrimidine derivatives are useful intermediates in the synthesis of antiviral nucleosides. Schinazi et al. reported the synthesis and the biological activity of several 5-(phenylselenenyl)-pyrimidine nucleosides as potential antiviral agents.[1] More recently, a variety of newly synthesized 6-phenylselenenyl acyclic pyrimidines was found to have potent anti-human-immunodeficiency-virus-type-1 (HIV-1) activity.[2,3] Another rationale for the synthesis of a variety of selenium-containing pyrimidines is that they may act as inhibitors of the enzymes involved in pyrimidine metabolism, such as dihydrouracil dehydrogenase (DHUDase, E.C. 1.3.1.2), orotate phosphoribosyltransferase (OPRTase, E.C. 2.4.2.10), thymidine phosphorylase (dThdPase, E.C. 2.4.2.4), and uridine phosphorylase (UrdPase, E.C. 2.4.2.3). It has been demonstrated that various 5-substituted uracils are inhibitors of DHUDase[4] and pyrimidine nucleoside phosphorylases.[5-10] In addition, acyclic pyrimidine nucleosides were shown to be potent inhibitors of UrdPase.[5-13] For example, 5-benzylacyclouridine, 5-benzylbarbituric acid, acyclic nucleosides, and related compounds are potent inhibitors of UrdPase from various sources.[5-13] The selenenyl derivatives are more lipophilic than the presently available inhibitors, and it is anticipated that their effects could be directed mainly to the metabolism in the liver which is the main site for pyrimidine metabolism in the body. The new compounds were also evaluated for antiviral activity against HIV-1 in acutely infected primary human lymphocytes. Their cytotoxicity was also ascertained in human peripheral blood mononuclear (PBM) cells, T-lymphoblastoid (CEM-CCRF) cells, and African green monkey kidney (Vero) cells. Toxicity of the most promising compound, 1-[(2-hydroxyethoxy)methyl]-5-(phenylselenenyl)uracil (5-(phenylselenenyl)acylouridine or PSAU, 18), as a UrdPase inhibitor was assessed in mice.

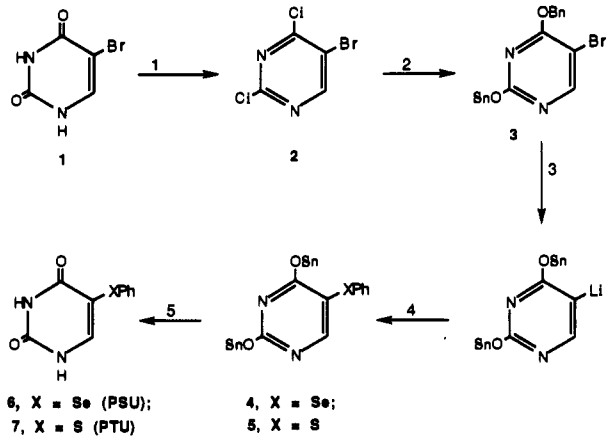
Chemistry

5-(Phenylselenenyl)uracil (PSU, 6) and 5-(phenylthio)-uracil (PTU, 7) were prepared starting from 5-bromouracil in 5 steps (Scheme I). Reaction of 5-bromouracil with excess $POCl_3$ generated 2,4-dichloro-5-bromopyrimidine (2), which on treatment with sodium benzylate in toluene at room temperature yielded 5-bromo-2,4-bis(benzyloxy)-pyrimidine (3). Lithiation of the 5-bromopyrimidine derivative below −80 °C in dry THF with *n*-BuLi (1.1 equiv) generated a C-5-lithiated species, which was subsequently reacted with either diphenyl diselenide or diphenyl disulfide (2 equiv, −75 °C, 0.5 h). Quenching the reaction with glacial AcOH followed by silica gel column chromatography provided the corresponding 5-(phenylhetera)-2,4-bis(benzyloxy)pyrimidines in 70–75% yield. As anticipated, debenzylation by catalytic hydrogenation (Pd/C) of the 5-phenylhetera-substituted compounds resulted in the isolation of starting material. However, the protecting benzyl groups were readily cleaved by exposure to trimethylsilyl iodide (2.4 equiv) in dry CH_2Cl_2 at room temperature to give the desired 5-(phenylhetera)uracils in 78–80% yield. The direct electrophilic addition of benzeneselenenyl chloride to the uracil in dry pyridine and dimethyl formamide, as reported previously,[1] failed to yield the desired PSU (6) under a variety of reaction conditions.

* To whom correspondence should be addressed. Phone: 404-728-7711; Fax: 404-728-7726.
† Veterans Affairs Medical Center and Emory University School of Medicine.
‡ University of Alabama at Birmingham.
• Abstract published in *Advance ACS Abstracts*, November 15, 1993.

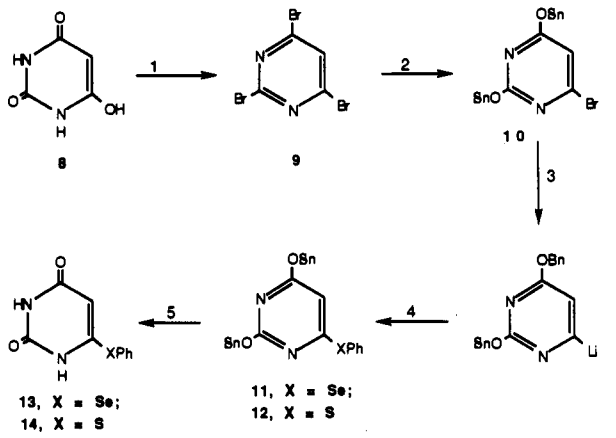
0022-2623/93/1836-4250$04.00/0

Scheme I[a]



[a] (1) $POCl_3$, 110 °C; (2) NaOBn, toluene; (3) *n*-BuLi, THF, −80 °C; (4) $(PhSe)_2$ or $(PhS)_2$, THF; (5) TMSI, CH_2Cl_2.

Scheme II[a]



[a] (1) $POBr_3$, toluene, Δ; (2) NaOBn, toluene; (3) *n*-BuLi, THF, −80 °C; (4) $(PhSe)_2$ or $(PhS)_2$, THF; (5) TMSI, CH_2Cl_2.

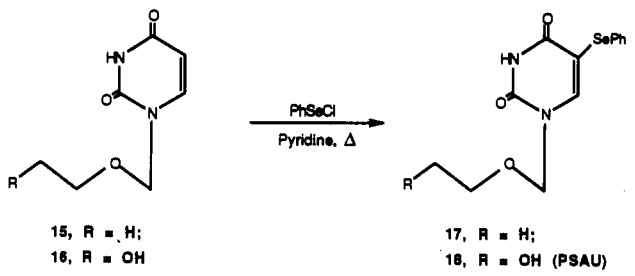
Regiospecific C-6 lithiation of 2,4-bis(benzyloxy)pyrimidine with LDA at −75 to −80 °C followed by the addition of diphenyl diselenide failed to give 6-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (11). The starting material was isolated after similar workup and used for lithiation reactions. It is likely that the C-6 proton is not sufficiently acidic for lithiation due to the aromatization of the pyrimidine ring. However, 6-(phenylselenenyl)uracils and 6-(phenylthio)uracils were successfully obtained from 6-bromo-2,4-bis(benzyloxy)pyrimidine (10) by adopting the lithiation methodology described above with *n*-BuLi, electrophilic addition, and debenzylation (Scheme II). 6-Bromo-2,4-bis(benzyloxy)pyrimidine (10) was obtained from barbituric acid as described previously.[1]

5-Phenylselenenyl acyclic nucleosides 17 and 18 were conveniently prepared by the direct electrophilic addition of benzeneselenenyl chloride to the acyclic nucleosides in dry pyridine at 60 °C as described previously by Schinazi et al.[1] (Scheme III). The products, obtained as white crystalline compounds, are readily purified by column chromatography. The structure of these compounds was confirmed by standard analytical methods. Other compounds described in Tables I and II were synthesized according to the previously reported procedure.[1-3,14]

Discussion and Conclusions

Inhibitors of DHUDase, OPRTase, dThdPase, and UrdPase may have a wide application in treating various

Scheme III



pathological and physiological conditions. For example, several of the anticancer drugs, especially the 5-fluorinated pyrimidines, lose their efficacy because of degradation by UrdPase[12,15] and DHUDase.[16] Inhibitors of these enzymes can enhance the efficacy of these drugs by preventing their catabolism. Furthermore, UrdPase inhibitors can increase the concentration and the duration of plasma uridine levels.[17-20] As a result, the toxicity of anticancer (e.g., 5-fluorouracil)[17,18] and anti-HIV drugs (e.g., 3′-azido-3′-deoxythymidine)[21-24] can be prevented by elevating the levels of plasma uridine. Therefore, the novel enzyme inhibitors may be useful when combined with certain anticancer or anti-HIV agents. They can also be used for physiological conditions where uridine can support cellular or tissue metabolic functions (e.g., CNS disorders).[25-36] Furthermore, abnormalities resulting from excess uracil catabolism (e.g., hyper-β-alanemia) or toxicity from 5-fluorouracil (e.g., neuro- and cardiotoxicity) may be prevented by the use of these inhibitors.

PSU (6) and PTU (7) reported herein inhibited DHUDase with apparent K_i values in the range of 4.8–5.4 μM (Table I). PSU also inhibited UrdPase with an apparent K_i value of 205 μM. Attachment of an "acyclo tail" to PSU enhanced binding by over 50-fold. PSAU inhibited UrdPase from mouse liver with an apparent K_i value of 3.8 μM. 6-Phenylselenenyl-substituted acyclic uridines showed modest inhibitory activity at 20–35 μM. None of the compounds evaluated had any effect on dThdPase. Mice given PSAU (18) intraperitoneally (i.p.) at doses up to 50 mg/kg/day for 5 days and monitored for 30 days showed no sign of toxicity as measured by weight loss or failure to gain weight. Furthermore, PSAU at 30 mg/kg, i.p., raised the plasma uridine level and half-life by 3-fold (O. M. Ashour and M. H. el Kouni, unpublished data).

All the compounds were evaluated in human PBM cells acutely infected with HIV-1_{LAI}. Virus production was monitored by measuring the level of HIV-1 RT present in disrupted virions obtained from the supernatant from cells exposed to the compounds. In general, phenylselenenyl-substituted pyrimidines and uracils were found to be more potent against HIV-1 than the corresponding phenylthio counterparts (Table II). The median effective concentrations (EC_{50}) for selenium-containing compounds were in the range of 0.1–28.8 μM and for sulfur-containing compounds were 6.4–100 μM. All the compounds exhibited no cytotoxicity in human PBM cells or in CEM cells, although some cytotoxicity was noted in Vero cells. Whereas phenylselenenyl-substituted uracils were not cytotoxic when tested in Vero cells up to 100 μM, the phenylthio-substituted pyrimidines and uracils exhibited cytotoxicity in the range of 13–71 μM.

Table I. Apparent Inhibition Constants of Different Compounds with Enzymes Isolated from Mouse Liver

compound	enzyme (apparent K_i, μM ± SD)			
	UrdPase	dThdPase	DHUDase	OPRTase
5-(phenylselenenyl)uracil (PSU, 6)	205 ± 35	a	4.8 ± 0.6	b
5-(phenylselenenyl)-6-azauracil	a	a	a	a
5-(phenylselenenyl)uridine	4.0 ± 0.2	a	b	b
5-(phenylselenenyl)-2'-deoxyuridine	5.5 ± 0.6	a	b	b
5-(phenylselenenyl)acyclouridine (PSAU, 18)	3.8 ± 0.8	a	a	a
5-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (4)	a	a	a	b
1-(ethoxymethyl)-5-(phenylselenenyl)uracil (17)	313 ± 32	a	a	a
5-(phenylthio)uracil (PTU, 7)	744 ± 85	a	5.4 ± 0.6	a
5-(phenylthio)-2,4-bis(benzyloxy)pyrimidine (5)	a	a	a	a
6-(phenylselenenyl)uracil (13)	a	a	a	a
6-(phenylselenenyl)accyclouridine	19.3 ± 1.5	a	b	b
6-(phenylselenenyl)acyclothymidine	a	a	b	b
6-(phenylselenenyl)acyclo-5-BrUrd	a	a	b	b
6-(phenylselenenyl)acyclo-5-FUrd	35.0 ± 5.6	a	b	b
6-(phenylselenenyl)acyclo-5-ClUrd	a	a	b	b
6-(phenylselenenyl)acyclo-2-thiouridine	a	a	b	b
1-(ethoxymethyl)-6-(phenylselenenyl)uracil	a	a	a	a
1-(ethoxymethyl)-6-(phenylselenenyl)-5-ethylUra	a	a	a	b
6-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (11)	a	a	a	a
6-(phenylthio)uracil (14)	a	a	a	2280 ± 1600
1-(ethoxymethyl)-6-(phenylthio)thymine	a	a	a	a
6-(phenylthio)-2,4-bis(benzyloxy)pyrimidine (12)	a	a	a	a
5-ethyl-1-(ethoxymethyl)-6-(phenylthio)uracil	a	a	a	a
6-(dihydroxyboryl)-2,4-bis(benzyloxy)pyrimidine	a	a	a	1660 ± 700
5-benzylacyclouridine (BAU)[c]	3.1 ± 0.22	a	a	b

[a] No inhibition when tested up to 1.0 mM. [b] Not tested. [c] See ref 9.

Table II. Biological Evaluation of Various Substituted Pyrimidines against HIV-1 and for Cytotoxicity in PBM, CEM, and Vero Cells

compound	anti-HIV-1 in PBMC, EC_{50} (μM)	toxicity, IC_{50} (μM)		
		in PBM cells	in CEM cells	in Vero cells
5-(phenylthio)-2,4-bis(benzyloxy)pyrimidine (5)	7.2	>100	>100	42.8
5-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (4)	3.5	>100	>100	65.0
6-(phenylthio)-2,4-bis(benzyloxy)pyrimidine (12)	59.3	>100	>100	71.4
6-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (11)	0.1	>100	>100	27.4
5-(phenylthio)uracil (PTU, 7)	>100	>100	>100	21.3
5-(phenylselenenyl)uracil (PSU, 6)	29.5	>100	>100	>100
6-(phenylthio)uracil (14)	6.4	>100	>100	12.8
6-(phenylselenenyl)uracil (13)	28.8	>100	>100	>100
1-(ethoxymethyl)-5-(phenylselenenyl)uracil (17)	9.8	>100	>100	69.3
5-(phenylselenenyl)acyclouridine (PSAU, 18)	>100	>100	>100	>100
6-(dihydroxyboryl)-2,4-bis(benzyloxy)pyrimidine	21.6	>100	>100	24.8
3'-azido-3'-deoxythymidine (AZT)	0.004	>100	13.0	26.0

Experimental Section

Materials. Melting points were determined on an Electrothermal IA 8100 digital melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a General Electric QE-300 (300-MHz) spectrometer. Experiments were monitored using TLC analysis performed on Kodak chromatogram sheets precoated with silica gel and a fluorescent indicator, while column chromatography, employing silica gel (60–200 mesh; Fisher Scientific, Fair Lawn, NJ), was used for the purification of products. Tetrahydrofuran (THF) was freshly distilled from the sodium benzophenone salt. LDA (2.0 M), *n*-BuLi (1.6 M), diphenyl diselenide, diphenyl disulfide, trimethylsilyl iodide, and other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). Microanalyses were performed at Atlantic Microlabs (Atlanta, GA). [2-^{14}C]Uridine (56 Ci/mol), [2-^{14}C]-thymidine (56 Ci/mol), and [6-^{14}C]uracil (56 Ci/mol) were obtained from Moravek Biochemicals Inc., Brea, CA; [6-^{14}C]-orotate (46.9 Ci/mol) from New England Nuclear Research Products, Du Pont Co., Boston, MA; silica gel G/UV$_{254}$ polygram and polyethyleneimine cellulose 300 PEI/UV$_{254}$ and cellulose CEL 300 UV polygram thin-layer chromatography plates from Brinkmann, Westbury, NJ; and a prote in assay kit from Bio-Rad Laboratories, Richmond, CA. All other chemicals were obtained from Sigma Co., St. Louis, MO.

Chemistry. 5-(Phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (4). To a solution of 5-bromo-2,4-bis(benzyloxy)-pyrimidine (3)[14] (742 mg, 2 mmol) in dry THF (10 mL) at −80 °C was added dropwise *n*-BuLi (1.6 M, 1.5 mL, 2.4 mmol) with stirring under an argon atmosphere. After the mixture was stirred for 15 min, diphenyl diselenide (1.25 g, 4 mmol) dissolved in THF (10 mL) was added and the temperature was maintained below −70 °C. After 1 h at that temperature, the reaction was quenched with glacial AcOH (0.5 mL) and the solution was allowed to warm to room temperature. The solution was concentrated to dryness *in vacuo*, and the residue was purified by silica gel column chromatography using hexane:CH_2Cl_2 (6:4) as the eluent to yield a white solid which was crystallized from EtOH to give white needles of 5-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (778 mg, 87%): mp 66–68 °C; ^{1}H NMR ($CDCl_3$) δ 5.38 and 5.42 (2 s, 4H, CH_2), 7.23–7.49 (m, 15H, 2 Ph and SePh), 8.26 (s, 1H, 6-H). Anal. ($C_{24}H_{20}N_2O_2Se$) C, H, N.

5-(Phenylselenenyl)uracil (PSU, 6). To a solution of 5-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (447 mg, 1 mmol) in dry CH_2Cl_2 (10 mL) was added trimethylsilyl iodide (520 mg, 2.6 mmol) under anhydrous conditions at room temperature. The yellow solution was stirred for 1 h. The excess trimethylsilyl iodide was destroyed, and the intermediate trimethylsilyl ethers formed during the reaction were hydrolyzed by the addition of MeOH. The precipitate was filtered and the solid crystallized from EtOH to give pure PSU (210 mg, 78%): mp 249–251 °C; ^{1}H NMR (DMSO-d_6) δ 7.16–7.37 (m, 5H, SePh), 7.93 (s, 1H, 6-H), 11.28 and 11.39 (2 s, 2H, 2 NH, D_2O exchangeable). Anal. ($C_{10}H_8N_2O_2Se$) C, H, N.

5-(Phenylthio)-2,4-bis(benzyloxy)pyrimidine (5). Reaction of 5-bromo-2,4-bis(benzyloxy)pyrimidine (742 mg, 2 mmol) sequentially with *n*-BuLi (1.6 M, 1.5 mL, 2.4 mmol) and diphenyl

disulfide (872 mg, 4 mmol), as described for the preparation of the 5-phenylselenenyl-substituted pyrimidine derivative, yielded the title compound (630 mg, 79%): mp 61–63 °C; ^{1}H NMR ($CDCl_3$) δ 5.41 and 5.45 (2 s, 4H, CH_2), 7.06–7.48 (m, 15H, 2 Ph and SPh), 8.37 (s, 1H, 6-H). Anal. ($C_{24}H_{20}N_2O_2S$) C, H, N.

5-(Phenylthio)uracil (PTU, 7). Reaction of 5-(phenylthio)-2,4-bis(benzyloxy)pyrimidine (5) (400 mg, 1 mmol) with trimethylsilyl iodide (520 mg, 2.6 mmol) in CH_2Cl_2 (15 mL), as described for the preparation of 5-(phenylselenenyl)uracil, gave 5-(phenylthio)uracil (160 mg, 72%): mp 269–271 °C (lit.[37] mp 272 °C); ^{1}H NMR (DMSO-d_6) δ 7.04–7.25 (m, 5H, SPh), 7.86 (s, 1H, 6-H), 11.32 and 11.41 (2 s, 2H, 2 NH, D_2O exchangeable).

6-(Phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (11). Reaction of 6-bromo-2,4-bis(benzyloxy)pyrimidine[14] (742 mg, 2 mmol) sequentially with *n*-BuLi (1.6 M, 1.5 mL, 2.4 mmol) and diphenyl diselenide (1.25 g, 4 mmol), as described for the preparation of the 5-(phenylselenenyl)pyrimidine analogue, yielded the title compound (590 mg, 66%): mp 97–99 °C; ^{1}H NMR ($CDCl_3$) δ 5.28 and 5.39 (2 s, 4H, CH_2), 6.00 (s, 1H, 5-H), 7.26–7.74 (m, 15H, 2 Ph and SePh). Anal. ($C_{24}H_{20}N_2O_2Se$) C, H, N.

6-(Phenylselenenyl)uracil (13). Reaction of 6-(phenylselenenyl)-2,4-bis(benzyloxy)pyrimidine (11) (447 mg, 1 mmol) with trimethylsilyl iodide (520 mg, 2.6 mmol) in CH_2Cl_2 (15 mL), as described for the preparation of the 5-phenylselenenyl analogue, gave the desired product (215 mg, 80%): mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ 4.66 (s, 1H, 5-H), 7.43–7.70 (m, 5H, SePh), 11.16 and 11.28 (2 s, 2H, 2 NH, D_2O exchangeable). Anal. ($C_{10}H_8N_2O_2Se$) C, H, N.

6-(Phenylthio)-2,4-bis(benzyloxy)pyrimidine (12). Reaction of 6-bromo-2,4-bis(benzyloxy)pyrimidine (742 mg, 2 mmol) sequentially with *n*-BuLi (1.6 M, 1.5 mL, 2.4 mmol) and diphenyl disulfide (872 mg, 4 mmol), as described for the preparation of the 5-(phenylselenenyl)pyrimidine analogue, yielded the title compound (610 mg, 76%): mp 102–104 °C; ^{1}H NMR ($CDCl_3$) δ 5.32 and 5.40 (2 s, 4H, CH_2), 5.83 (s, 1H, 5-H), 7.28–7.60 (m, 15H, 2 Ph and SPh). Anal. ($C_{24}H_{20}N_2O_2S$) C, H, N.

6-(Phenylthio)uracil (14). Reaction of 6-(phenylthio)-2,4-bis(benzyloxy)pyrimidine (12) (400 mg, 1 mmol) with trimethylsilyl iodide (520 mg, 2.6 mmol) in CH_2Cl_2 (15 mL), as described for the preparation of the 5-phenylselenenyl analogue, gave 6-(phenylthio)uracil (190 mg, 86%): mp 266–267 °C; ^{1}H NMR (DMSO-d_6) δ 7.04–7.25 (m, 5H, SPh), 7.86 (s, 1H, 6-H), 11.32 and 11.41 (2 s, 2H, 2 NH, D_2O exchangeable). Anal. ($C_{10}H_8N_2O_2S$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-5-(phenylselenenyl)uracil (PSAU, 18). Benzeneselenenyl chloride (1.14 g, 6 mmol) was dissolved in dry pyridine (15 mL), and then, 1-[(2-hydroxyethoxy)methyl]uracil (16)[2] (1.0 g, 5.37 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h. The mixture was allowed to cool to room temperature and then concentrated *in vacuo* to remove pyridine. The residue was coevaporated with benzene (2 × 10 mL) and then with absolute EtOH (10 mL). The residue was loaded onto a silica gel column and eluted first with $CHCl_3$ to remove residual diphenyl diselenide. The product was then obtained by elution with $CHCl_3$:MeOH (95:5), and the TLC pure fractions were pooled and concentrated. The solid residue was recrystallized from absolute EtOH to yield the desired product as a white crystalline solid (1.4 g, 76%): mp 118–120 °C; ^{1}H NMR (DMSO-d_6) δ 1.60 (s, 1H, OH, D_2O exchangeable), 3.68–3.74 (m, 4H, OCH_2CH_2O), 5.17 (s, 2H, NCH_2O), 7.26–7.57 (m, 6H, SePh and 6-H), 8.37 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{14}N_2O_4Se$) C, H, N.

1-(Ethoxymethyl)-5-(phenylselenenyl)uracil (17). Reaction of benzeneselenenyl chloride (1.15 g, 6 mmol) with 1-(ethoxymethyl)uracil (15)[3] (850 mg, 5 mmol) in pyridine (25 mL), as described above for the synthesis of 1-[(2-hydroxyethoxy)methyl]-5-(phenylselenenyl)uracil, yielded the title compound (1.20 g, 74%): mp 143–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3CH_2O), 3.57 (q, 2H, CH_3CH_2O), 5.08 (s, 2H, NCH_2O), 7.22–7.53 (m, 5H, SePh and 6-H), 8.94 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{14}N_2O_3Se$) C, H, N.

Biological Evaluation. 1. Enzyme Studies. Preparation of Enzyme Extracts. Mouse livers were obtained from female Swiss albino (CD1) mice weighing 20–24 g (Charles River Laboratories, Boston MA). Mice were sacrificed by cervical dislocation and the livers removed, weighed, minced, and homogenized in ice-cold (3:1, v/w) buffer (20 mM potassium phosphate, pH 8.0, 1 mM dithiothreitol (DTT), and 1 mM EDTA) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NJ). The homogenates were centrifuged at 105 000*g* for 1 h at 4 °C. The supernatant fluids (cytosol) were collected and used as the enzyme source.

Enzyme Assays. All assays were conducted at 37 °C under conditions where the enzyme activity was linear with respect to time and enzyme concentration. For each inhibitor, five concentrations were used ranging from 8 to 900 μM. Reactions were started by the addition of extract and stopped by boiling in a water bath for 2 min followed by freezing. Precipitated proteins were removed by centrifugation. Substrates were separated from products in the supernatant by TLC, and the radioactivity in the spots was determined on a percentage basis using a Berthold LB-2821 automatic TLC-linear analyzer.

Pyrimidine Nucleoside Phosphorylases (dThdPase and UrdPase). Nucleoside cleavage was measured isotopically by following the formation of nucleobases from their respective nucleosides, as previously described.[12] The reaction mixture contained 20 mM potassium phosphate (pH 8), 1 mM EDTA, 1 mM DTT, 1 mM [2-^{14}C]uridine or [2-^{14}C]thymidine (56 Ci/mol), and 25 μL of cytosol in a final volume of 50 μL. The incubation was terminated after 30 min. Uridine and thymidine were separated from their respective nucleobases on silica gel TLC plates developed with $CHCl_3$:MeOH:AcOH (90:5:5, v/v/v). The R_f values were uridine, 0.07; uracil, 0.43; thymidine, 0.14; and thymine, 0.62.

DHUDase. The activity of the enzyme was measured by following the formation of dihydrouracil, carbamyl-β-alanine, and β-alanine from [6-^{14}C]uracil, as previously described.[16] The reaction mixture contained 20 mM potassium phosphate (pH 8), 1.0 mM EDTA, 2 mM DTT, 5 mM $MgCl_2$, 25 μM [6-^{14}C]uracil (56 Ci/mol), 100 μM NADPH, and 25 μL of cytosol in a final volume of 50 μL. The incubation was terminated after 15 min. Uracil, dihydrouracil, carbamyl-β-alanine, and β-alanine were separated on cellulose TLC plates developed in the top phase of a mixture of *n*-BuOH:H_2O:ammonia (90:45:15, v/v/v). R_f values were dihydrouracil, 0.46; uracil, 0.23; and carbamyl-β-alanine, 0.09. DHUDase activity was determined as the sum of the products dihydrouracil, carbamyl-β-alanine, and β-alanine.

OPRTase. Enzyme activity was measured by following orotidine 5′-monophosphate (OMP), orotidine, UMP, uridine, and uracil formation from [6-^{14}C]orotate.[38] The standard assay mixture contained 20 mM potassium phosphate buffer (pH 8.0), 2.5 mM 5-phosphoribosyl-1-pyrophosphate, 1 mM EDTA, 5 mM $MgCl_2$, 2 mM DTT, 0.05 mM [6-^{14}C]orotate (46.9 Ci/mol), and 25 μL of cytosol extract in a final volume of 50 μL. The incubation was terminated after 30 min. A 10-μL aliquot of the supernatant fluid was spotted on prewashed PEI cellulose plates. The plates were developed first in distilled water to a front of 10 cm. They were then dried and redeveloped in 0.2 M LiCl. R_f values were OMP, 0.16; UMP, 0.51; orotate, 0.62; orotidine, 0.77; and uridine and uracil, 0.95. OPRTase activity was measured as the sum of OMP, orotidine, UMP, uridine, and uracil.

Kinetic Studies. The determination and significance of apparent K_i values were performed using uridine (1 mM) and five different concentrations of the inhibitor ranging from 50 to 900 μM. Apparent K_i values were estimated from Dixon's plots ($1/v$ vs [I]) of the data by a computer program with least-squares fitting. The program was written by Dr. Sungman Cha and modified to fit IBM BASIC by Dr. Fardos Naguib. Apparent K_i values are related to K_i values by the following equation:

$$\text{apparent } K_i = \frac{K_{is}(1 + [S]/K_m)}{1 + ([S]/K_m)(K_{is}/K_{ii})}$$

where K_{is} and K_{ii} are inhibition constants that would have been estimated from the replot of slope and intercept, respectively, of Lineweaver–Burk plots vs [I]. If a compound is a competitive inhibitor with respect to uridine, $K_{ii} = \infty$ and $K_{is} = K_i$. Therefore, apparent $K_i = K_i(1 + [S]/K_m)$. Thus, for UrdPase from mouse liver which has a K_m value of 66 μM for uridine,[12] the apparent K_i of a competitive inhibitor, measured at a uridine concentration of 1 mM, is approximately 16-fold higher than their respective

K_i values. It should be noted, however, that we have not characterized the compounds used in this study with regard to the type of inhibition (competitive, noncompetitive, or uncompetitive) or whether they are substrates for the enzyme.

Protein Determination. Protein concentrations were determined spectrophotometrically by the method of Bradford[39] using bovine γ-globulin as a standard.

2. Antiviral and Cytotoxicity Assays. The methods for determining the anti-HIV-1 activity of the compounds in human peripheral blood mononuclear cells have been described previously.[40] Stock solutions (5–10 mM) of the new compounds were prepared in pure DMSO and then diluted to the desired concentration. Other compounds such as AZT were dissolved in water. HIV-1_{LAI} was obtained from Dr. Paul Feorino (Emory University). Virus obtained from the cell supernatant was quantitated on day 6 after infection by a reverse transcriptase assay using $(rA)_n \cdot (dT)_{12-18}$ as the template primer. The DMSO present in the diluted solution (<0.1%) had no effect on the virus yield. The toxicity of the compounds was assessed in human PBM, CEM, and Vero cells, as described previously.[40] The EC_{50} and median inhibitory concentration (IC_{50}) were obtained from the concentration-response curve using the median effective method described by Chou and Talalay.[41]

Acknowledgment. We thank A. McMillan, R. Mathis, and Brian Kopicki for excellent technical support. This work was supported in part by the National Institutes of Health (CA-53892), by the Department of Veterans Affairs, and by the Georgia VA Research Center for AIDS and HIV Infections.

References

(1) Schinazi, R.; Arbiser, J.; Lee, J.; Kalman, T.; Prusoff, W. Synthesis and biological activity of 5-phenylselenenyl substituted pyrimidine nucleosides. *J. Med. Chem.* **1986**, *29*, 1293–1295.
(2) Goudgaon, N. M.; Schinazi, R. F. Activity of acyclic 6-phenylselenenyl pyrimidine nucleosides against human immunodeficiency viruses in primary lymphocytes. *J. Med. Chem.* **1991**, *34*, 3305–3309.
(3) Goudgaon, N. M.; McMillan, A.; Schinazi, R. F. 1-(Ethoxymethyl)-6-(phenylselenyl)pyrimidines with activity against human immunodeficiency virus types 1 and 2. *Antiviral Chem. Chemother.* **1992**, *3*, 263–266.
(4) Naguib, F. N. M.; el Kouni, M. H.; Cha, S. Structure-activity relationship of ligands of dihydrouracil dehydrogenase from mouse liver. *Biochem. Pharmacol.* **1989**, *38*, 1471–1480.
(5) Niedzwick, J. G.; el Kouni, M. H.; Chu, S. H.; Cha, S. Pyrimidine acyclonucleosides, inhibitors of uridine phosphorylase. *Biochem. Pharmacol.* **1981**, *30*, 2097–2101.
(6) Niedzwicki, J. G.; Chu, S. H.; el Kouni, M. H.; Rowe, E. C.; Cha, S. 5-Benzylacylouridine and 5-benzyloxybenzylacyclouridine, potent inhibitors of uridine phosphorylase. *Biochem. Pharmacol.* **1982**, *31*, 1857–1861.
(7) Niedzwicki, J. G.; el Kouni, M. H.; Chu, S. H.; Cha, S. Structure–activity relationship of ligands of the pyrimidine nucleoside phosphorylases. *Biochem. Pharmacol.* **1983**, *32*, 399–415.
(8) el Kouni, M. H.; Naguib, F. N. M.; Chu, S. H.; Cha, S.; Ueda, t.; Gosselin, G.; Imbach, J.-L.; Shealy, Y. F.; Otter, B. A. Effect of the *N*-glycosidic bond conformation and modifications in the pentose moiety on the binding of nucleoside ligands to uridine phosphorylase. *Mol. Pharmacol.* **1988**, *34*, 104–110.
(9) Naguib, F. N. M.; el Kouni, M. H.; Chu, S. H.; Cha, S. New analogues of benzylacyclouridines, specific and potent inhibitors of uridine phosphorylase from human and mouse livers. *Biochem. Pharmacol.* **1987**, *36*, 2195–2201.
(10) Naguib, F. N. M.; Levesque, D. L.; Wang, E.-C.; Panzica, R. P.; el Kouni, M. H. 5-Benzylbarbituric acid derivatives, potent and specific inhibitors of uridine phosphorylase *Biochem. Pharmacol.* **1993**, *46*, 1273–1283.
(11) el Kouni, M. H.; Naguib, F. N. M.; Niedzwicki, J. G.; Iltzsch, M. H.; Cha, S. Uridine phosphorylase from *Schistosome mansoni*. *J. Biol. Chem.* **1988**, *263*, 6081–6086.
(12) el Kouni, M. H.; el Kouni, M. M.; Naguib, F. N. M. Differences in activities and substrate specificity of human and murine pyrimidine nucleoside phosphorylases: Implications for chemotherapy with 5-fluoropyrimidines. *Cancer Res.* **1993**, *53*, 3687–3693.
(13) Park, K. S.; el Kouni, M. H.; Krenitsky, T. A.; Chu, S. H.; Cha, S. Inhibition of uridine phosphorylase from *Escherichia coli* by benzylacylouridines. *Biochem. Pharmacol.* **1986**, *35*, 3853–3855.
(14) Schinazi, R. F.; Prusoff, W. H. Synthesis of 5-dihydroxyboryl-2′-deoxyuridine and related boron-containing pyrimidines. *J. Org. Chem.* **1985**, *50*, 841–847.
(15) Chu, M. Y. W.; Naguib, F. N. M.; Iltzsch, M. H.; el Kouni, M. H., Chu, S. H.; Cha, S.; Calabresi, P. Potentiation of 5-fluoro-2′-deoxyuridine antineoplastic activity by the uridine phosphorylase inhibitors benzylacyclouridine and benzyloxybenzylacyclouridine. *Cancer Res.* **1984**, *44*, 1852–1856.
(16) Naguib, F. N. M.; el Kouni, M. H.; Cha, S. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res.* **1985**, *45*, 5405–5412.
(17) Darnowski, J. W.; Handschumacher, R. E. Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine. *Cancer Res.* **1985**, *45*, 5364–5368.
(18) Martin, D. S.; Stolfi, R. L.; Sawyer, C. Use of oral uridine as a substitute for parenteral uridine rescue of 5-fluorouracil therapy, with and without the uridine phosphorylase inhibitor 5-benzylacyclouridine. *Cancer Chemother. Pharmacol.* **1989**, *24*, 9–14.
(19) Monks, A.; Ayers, O.; Cysyk, R. L. Effect of 5-benzylacyclouridine, a potent inhibitor of uridine phosphorylase, on the metabolism of circulating uridine by the isolated rat liver. *Biochem. Pharmacol.* **1983**, *32*, 2003–2009.
(20) Peters, G. J.; van Groeningen, C. J.; Laurensse, E. J.; Lankelma, J.; Leyva, A.; Pinedo, H. M. Uridine-induced hypothermia in mice and rats in relation to plasma and tissue levels of uridine and its metabolites. *Cancer Chemother. Pharmacol.* **1987**, *20*, 101–108.
(21) Calabresi, P.; Falcone, A.; St. Clair, M. H.; Wiemann, M. C.; Chu, S. H.; Darnowski, J. W. Benzacyclouridine reverses azidothymidine induced marrow suppression without impairment of anti-human immunodeficiency virus activity. *Blood* **1990**, *76*, 2210–2215.
(22) Falcone, A.; Darnowski, J. W.; Ruprecht, R. M.; Chu, S.-H.; Brunetti, I.; Calabresi, P. Differential effect of benzylacyclouridine on the toxic and therapeutic effects of azidothymidine in mice. *Blood* **1990**, *76*, 2216–2221.
(23) Sommadossi, J.-P.; Carlisle, R.; Schinazi, R. F.; Zhou, Z. Uridine reverses the toxicity of 3′-azido-3′-deoxythymidine in normal human granulocyte-macrophage progenitor cells *in vitro* without impairment of the antiretrovial activity. *Antimicrob. Agents Chemother.* **1988**, *7*, 997–1001.
(24) Cretton, E.; Schinazi, R. F.; McClure, H. M.; Anderson, D.; Sommadossi, J.-P. Pharmacokinetics of 3′-azido-3′-deoxythymidine and catabolites and interactions with probenecid in rhesus monkeys. *Antimicrob. Agents Chemother.* **1991**, *35*, 801–807.
(25) Bonavita, V.; Monaco, P.; Tripi, E. Analysis of the central effect of purine and pyrimidine nucleotides and their derivatives. III. Pharmacological Experiment. *Acta Neurol.* **1964**, *19*, 215–222.
(26) Bonavita, V.; Zito, M. Analysis of the action of pyrimidine nucleosides on the human electroencephalogram activated with megimide. *Rivista Neurol.* **1968**, *38*, 317–319.
(27) Dwivedi, C.; Harbison, R. D. Anticonvulsant activities of 8- and 9-tetrahydrocannabinol and uridine. *Toxicol. Appl. Pharmacol.* **1975**, *31*, 452–458.
(28) Jann, G.; Delzanno, G. B. Effect of cytidine and uridine in the treatment of the neuropsychic syndrome from encephalopathy of various origin. *Minerva Med.* **1969**, *60*, 2092–2108.
(29) Geiger, A.; Yamasaki, S. Cytidine and uridine requirement of the brain. *J. Neurochem.* **1956**, *1*, 93–100.
(30) Guarneri, P.; Guraneri, R.; Mocciaro, C.; Piccoli, F. Interaction of uridine with GABA binding sites in cerebellar membranes of the rat. *Neurochem. Res.* **1983**, *8*, 1537–1545.
(31) Guarneri, P.; Guraneri, R.; Mocciaro, C.; Piccoli, F. Interaction of uridine with GABA-mediated inhibitory transmission: Studies *in vivo* and *in vitro*. *Epilepsia* **1985**, *26*, 666–671.
(32) Monticone, G. F.; Bergamasco, B.; Congnazzo, A. Therapeutic use of the cytidine and uridine nucleosides for certain neurological conditions. *Minerva Med.* **1966**, *57*, 4348–4352.
(33) Roberts, C. A. Anticonvulsant effects of uridine: comparative analysis of metrazol and penicillin induced foci. *Brain Res.* **1973**, *55*, 291–308.
(34) Roberts, C. A.; Kreisman, N. R.; Waltman, M. Uridine anticonvulsant effects: Selective control of nucleoside incorporation in experimental epilepsy. *Epilepsia* **1974**, *15*, 479–500.
(35) Sanguineti, I.; Zerbi, D. The use of some non-vasoactive nucleosides (cytidine and uridine) in the therapy of cerebral vasculopathies. *Minerva Med.* **1965**, *56*, 3352–3361.
(36) Sepe, O. Efficacy of the combination of cytidine-uridine for encephalovascular infections. *Minerva Med.* **1970**, *61*, 5934–5941.
(37) Roth, B.; Bunnett, J. F. 5-Arylthiopyrimidines. V. Kinetics of the cyclization of 4-oxo derivatives to 10H- and 10-alkylpyrimido[5,4-*b*][1,4]benzothiazines (1,3-diazaphenothiazines). *J. Am. Chem. Soc.* **1965**, *87*, 340–349.
(38) el Kouni, M. H.; Naguib, F. N. M. Pyrimidine salvage pathways in adult *Schistosoma mansoni*. *Inter. J. Parasitol.* **1990**, *20*, 37–44.
(39) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(40) Schinazi, R. F.; Sommadossi, J.-P.; Saalmann, V.; Cannon, D. L.; Xie, M.-Y.; Hart, G. C.; Smith, G. A.; Hahn, E. F. Activity of 3′-azido-3′-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061–1067.
(41) Chou, T.-C.; Talalay, P. Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* **1984**, *22*, 27–55.