hydrolysis products were separated by thin-layer chromatography of the supernatants. We used two different eluent systems (ethanol/water (8:2, v/v) for the separation of Ala from Boc-Pro-Ala; chloroform/methanol/acetic acid (85:10:5, v/v) for the separation of Boc-Pro from Boc-Pro-Ala). Ala was revealed by ninhydrin and Boc-Pro or Boc-Pro-Ala by chlorine reagent. In parallel, the release of Ala was quantitatively measured by amino acid analysis using an automatic amino acid analyzer (Beckman Model 120 C).

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Registry No. Boc-D-Pro-L-Ala, 101653-95-6; Boc-L-Pro-L-Ala, 36301-70-9; hydrolase, 9027-41-2; carboxypeptidase, 9031-98-5; amidase, 9012-56-0; penicillin, 61-33-6.

Synthesis and Biological Activity of 5-Phenylselenenyl-Substituted Pyrimidine Nucleosides

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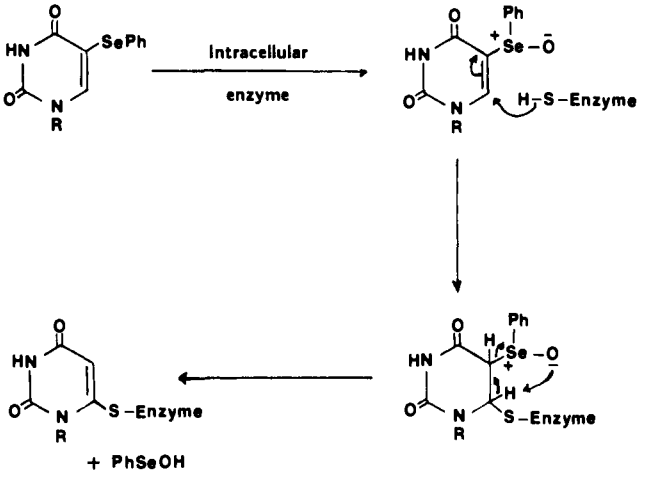
Several 5-phenylselenenyl derivatives of pyrimidine nucleosides were synthesized by electrophilic addition of phenylselenenyl chloride to the nucleosides under basic conditions. With use of this route, 5-(phenylselenenyl)-6-azauracil (**6**) was also prepared. These compounds may serve as inhibitors of thymidylate synthase, as potential antiviral and anticancer agents, and as versatile intermediates for the synthesis of 5- or 6-substituted nucleosides. 5-(Phenylselenenyl)arabinosyluracil (PSAU, **4**) and the corresponding cytosine analogue (PSAC, **5**) were poor inhibitors of a promyelocytic leukemia cell line that was arabinosylcytosine-resistant. PSAU and PSAC were significantly less active than *ara*-C against L1210 cells and were found to selectively interfere with the cellular uptake and/or phosphorylation of 2′-deoxycytidine and 2′-deoxyuridine in intact L1210 cells.

Much effort in the development of antiviral and anticancer drugs has been concentrated on the inhibition of enzymes necessary for purine or pyrimidine nucleoside synthesis.[1,2] Most of these compounds exert their effect on replicating cells and viruses either by blocking the salvage or the de novo pathways of nucleotide biosynthesis, nucleic acid biosynthesis, or nucleic acid function after incorporation into the macromolecule. This paper describes the synthesis and biological activity of several new pyrimidine nucleosides substituted at the C-5 position with a phenylselenenyl group.

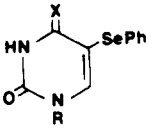
The rationale for adding a selenium moiety on the C-5 position of pyrimidine nucleosides was twofold. First, some of the uracil derivatives may inactivate thymidylate synthase (EC 2.1.1.45), an enzyme that undergoes conjugate addition with the 5,6-unsaturated portion of pyrimidine nucleotides,[3] by the oxidative mechanism shown in Scheme I. Although at present there is no evidence that oxidation of selenium can take place intracellularly, one can presume that by analogy with sulfur that this process may take place in vivo. For example, the antiinflammatory drug sulindac can undergo oxidation in animals and humans.[4] Further support for this oxidation is provided by the facile preparation at room temperature of the stable 5-(phenylseleninyl)-2′-deoxyuridine by the action of hydrogen peroxide on 5-(phenylselenenyl)-2′-deoxyuridine (Chart I, **3**) in tetrahydrofuran (unpublished results). Several other nucleosides containing selenium were previously synthesized and some were found to have biological activity.[5–11] For example, Choi et al.[5] recently found that 5-hydroseleno-2′-deoxyuridylate is a potent inhibitor of *Lactobacillus casei* thymidylate synthase. This compound was synthesized as an analogue of 5-mercapto-2′-deoxyuridylate, a known inhibitor of this enzyme.[12]

Scheme I

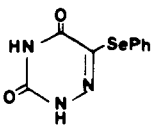


R = 5-Phospho-2-deoxyribosyl

Chart I



1 PSU: X = O; R = ribosyl
2 PSC: X = NH; R = ribosyl
3 PSDU: X = O; R = 2-deoxyribosyl
4 PSAU: X = O; R = arabinosyl
5 PSAC: X = NH; R = arabinosyl



6 PSAZ

Secondly, 5-selenopyrimidines and their corresponding nucleoside or nucleotide derivatives could serve as versatile

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Table I. Characterization of 5-(Phenylselenenyl)pyrimidines

compd	yield, %	mp,[a] °C	formula[b]	^{1}H NMR (J, Hz)[c] 6-H	5-Ph	1'-H
PSU	44	210–212	$C_{15}H_{16}N_2O_6Se$	8.45 (s)	7.31 (m)	5.77 (d, 4.7)
PSDU	38	186–189	$C_{15}H_{16}N_2O_5Se$	8.31 (s)	7.31 (m)	6.15 (t, 6.5)
PSC	49	217–220	$C_{15}H_{17}N_3O_5Se$	8.57 (s)	7.29 (m)	5.76 (d, 2.9)
PSAU	40	213–214	$C_{15}H_{16}N_2O_6Se$	8.06 (s)	7.32 (m)	6.07 (d, 4.0)
PSAC	79	161–163	$C_{15}H_{17}N_3O_5Se \cdot 1.5H_2O$	8.28 (s)	7.32 (m)	6.06 (d, 4.0)
PSAZ	43	181–183	$C_9H_7N_3O_2Se \cdot 0.25H_2O$		7.52 (m)	

UV,[d] nm λ_{max} (ϵ)	λ_{min} (ϵ)
261 sh (10540), 243 (11850)	230 (9000)
260 sh (10530), 244 (11940)	230 (8780)
270 sh (6040), 239 (13310)	233 (1280)
260 sh (9450), 246 (10800), 255 sh (10300)	230 (7770)
270 sh (5590), 238 (12400)	232 (11920)
255 sh (1770), 230 (7520)	

[a] Uncorrected. [b] Analytical results (C, H, N) are within ±0.4%. [c] NMR spectra were recorded on a Brucker 270 HX spectrometer in Me_2SO-d_6; chemical shifts δ (ppm); s = singlet, d = doublet, t = triplet, m = multiplet. [d] UV spectra were recorded on a Beckman 25 UV spectrophotometer in EtOH; sh = shoulder.

intermediates in the synthesis of 6-substituted uracil and cytosine derivatives by using the well-known selenide β-elimination reaction (also known as the selenoxide fragmentation reaction).[13] For example, Zima and colleagues[14,15] demonstrated both a convenient method for the generation of phenylseleno enones from α,β-unsaturated carbonyl compounds and the use of phenylseleno enones in the synthesis of α,β-disubstituted enones through a conjugate addition–elimination sequence.

The presence of selenium in a molecule does not preclude unacceptable toxicity as has been noted with inorganic selenium.[16] It is now well appreciated that selenium is an essential micronutrient for mammals, including humans.[17,18] For example, selenium deprivation increases the incidence of fatal myocarditis in mice infected with Coxsackie B virus.[19] Selenium can incorporate into growing *Escherichia coli* tRNA and is a natural component of several bacterial and probably mammalian tRNAs.[20,21] It is also a component of glutathione oxidase and other selenium-dependent enzymes.[17,18]

Chemistry. Choi et al.[5] synthesized 5-substituted selenium derivatives of uracil with biological activity by a multistep procedure. Our synthetic approach was to synthesize the 5-substituted phenylselenenyl nucleosides directly by electrophilic addition of phenylselenenyl chloride to the nucleosides in dry pyridine. Although the reaction can proceed at room temperature, the addition was more efficient at higher temperature (about 60 °C). After removal of the solvent, the product was easily separated from starting material and byproducts by column chromatography.

This synthetic approach was adopted for the synthesis of 5-phenylselenenyl analogues of uridine (PSU, 1), 2'-deoxyuridine (PSDU, 3), cytidine (PSC, 2), arabinosyluracil (PSAU, 4), arabinosylcytosine (PSAC, 5), and 6-azauracil (PSAZ, 6). The ^{1}H NMR of the compounds confirmed that the phenylselenenyl function was attached to the 5-position of the pyrimidine ring and that the structural assignment for the compounds was correct. In accordance with a previous report,[5] a significant deshielding effect of 6-H was caused by the presence of a 5-selenium substituent in the nucleoside derivatives.

Although the precise mechanism of the electrophilic addition is unknown, based on the work of Zima and Liotta,[14] it is possible that the reaction for the *uracil* derivatives proceeds via (1) nucleophilic attack of C-6 by pyridine, (2) electrophilic attack on the resulting enolate by the pyridinium phenyl selenide, and (3) elimination of pyridinium hydrochloride. For the *cytosine* analogues (PSC and PSAC) or as an alternative mechanism for the uracil derivatives, the reaction may proceed via direct addition of phenylselenenyl chloride to the 5,6-double bond followed by elimination of HCl by pyridine. However, for the 6-azauracil analogue (PSAZ), the addition may take place by abstraction of a proton from the 1-position of 6-azauracil, followed by addition of the electrophile to the resulting 4,5-enolate.

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Biological Activity. The compounds synthesized were tested for anticancer activity in two different cell lines. With the exception of PSAC, L1210 and S-180 cells were essentially refractory to the drugs when tested up to 300 μM. Because of the concern that PSAC (ED_{50} = 25 μM for L1210 cells) may contain traces of arabinosylcytosine (*ara*-C) (ED_{50} = 0.03 μM for L1210 cells), this compound and its deaminated product, PSAU, were tested in an *ara*-C-resistant promyelocytic leukemia cell line that is also dCyd-kinase deficient (HL-60-HGPRT$^-$/dCK$^-$). At 400 μM, PSAC inhibited this cell line by 51% (ED_{50} = 375 μM). In contrast, 400 μM *ara*-C inhibited these cells by 93% (ED_{50} = 30 μM; the ED_{50} of *ara*-C for the parent cell line, HL-60-GHPRT$^+$/dCK$^+$, was 0.047 μM). These results suggest that PSAC may have been either contaminated with about 10% *ara*-C, which is unlikely on the basis of TLC, HPLC, and elemental analysis, or PSAC may have been deaminated to PSAU. The latter compound produced an inhibition of 88% at a concentration of 400 μM (ED_{50} = 150 μM). This prompted us to test in this system arabinosyluracil (*ara*-U), the precursor of PSAU. The results indicate that *ara*-U is a very poor inhibitor of the replication of HL-60, HGPRT$^-$/HL60, and HGPRT$^-$/dCK$^-$HL-60 cells with ED_{50} values of about 5 mM.

None of the compounds inhibited herpes simplex virus type 1 (HSV-1, F strain) in plaque reduction assay in Vero cells at concentrations ≤ 180 μM. However, in a yield reduction assay, PSC, PSU, and PSAU inhibited HSV-1 (KOS strain) by 45%, 63%, and 99.9% at a concentration of 60, 285, and 390 μM, respectively. The compounds were not toxic to uninfected Vero cells when tested up to 300 μM.

In mice infected intracerebrally with five LD_{50} of herpes simplex virus type 2 (strain G),[22] PSAU and PSDU were ineffective in increasing survival or the mean day of death at intraperitoneal doses of 50 mg/kg per day given twice a day for 4 days.

None of the compounds inhibited thymidylate synthase activity in intact L1210 cells at 100 μM extracellular concentration, as indicated by interference with the release of tritium from both labeled substrate precursors. In the case of PSDU and PSAC, a selective inhibition of tritium release was observed. The ID_{50} value obtained for PSDU was 70 μM with use of 1 μM [5-^{3}H]dCyd for the cellular enzyme assay. For PSAC the ID_{50} value was 60 μM with use of 1 μM [5-^{3}H]dUrd. In contrast, no significant inhibition of tritium release from [5-^{3}H]dUrd by 100 μM PSDU or from [5-^{3}H]dCyd by 100 μM PSAC was observed. The results suggest that these compounds selectively interfere with the cellular uptake and phosphorylation of 2′-deoxycytidine and 2′-deoxyuridine or both. It is interesting that this inhibitory effect has an opposite base specificity.

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Experimental Section

Chemical Methods and Materials. Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Ultraviolet spectra were recorded on a Beckman 25 spectrophotometer. ^{1}H NMR spectra were recorded on a Brucker 270 HX spectrometer. Chemical shifts are reported downfield from internal Me_4Si. TLC was performed on plastic film coated with silica gel Merck F-254 (EM Laboratories, Inc., Elmsford, NY) with fluorescent indicator. The elemental analyses were carried out by Atlantic Microlabs, Inc., Atlanta, GA. *ara*-U was synthesized from uridine, as described previously.[23]

General Method for Preparation of 5-Phenylselenenyl Derivatives. Phenylselenenyl chloride (2.6 mmol) was dissolved in dry pyridine (7 mL), and then the nucleoside or pyrimidine base (2.2 mmol) was added. The reaction mixture was stirred at 60–65 °C for 24–26 h. The mixture was allowed to cool to room temperature and then concentrated in high vacuo to remove pyridine. The residue was coevaporated with benzene (4 × 10 mL) and then with absolute EtOH (10 mL). In some experiments, a hexane extraction was also used to remove diphenyl diselenide prior to column chromatography. The residue was loaded onto a silica column (10 × 2.5 cm), eluting first with $CHCl_3$ to remove residual yellow diphenyl diselenide. The product was then obtained by elution with $CHCl_3$/EtOH (9:1 to 4:1). Fractions containing the product, as determined by TLC, were pooled and evaporated in vacuo to yield an oil, which solidified upon addition of a trace of EtOH. The solid was then filtered and recrystallized twice from EtOH.

Biological Evaluation. Compounds reported in this paper were screened for activity against L1210 and S-180 cells and HSV-1 (strain F) and HSV-1 (strain KOS) by using the methodologies described previously.[22,24] Some of the compounds were also tested against several HL-60 derived cell lines, including an *ara*-C-resistant promyelocytic leukemia cell line that is also dCyd-kinase deficient (HL-60-HGPRT$^-$/dCK$^-$). Cytotoxicity assays were carried out in rapidly dividing Vero cells, as described previously.[22] Stock solutions were prepared by first dissolving the compounds in EtOH (<0.1% final concentration), and then cell culture medium was added.

Cellular Enzyme Assays. Inhibition of thymidylate synthase in L1210 cells in situ was determined by using the tritium release assay, as previously described.[25]

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Registry No. 1, 101760-63-8; 1 (deselenated), 58-96-8; **2**, 101760-64-9; **2** (deselenated), 65-46-3; **3**, 101760-65-0; **3** (deselenated), 951-78-0; 4, 101760-66-1; **4** (deselenated), 3083-77-0; **5**, 101760-67-2; **5** (deselenated), 147-94-4; **6**, 101760-68-3; **6** (deselenated), 461-89-2; thymidylate synthase, 9031-61-2.

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