Synthesis, Structure, and Antiproliferative Activity of Selenophenfurin, an Inosine 5′-Monophosphate Dehydrogenase Inhibitor Analogue of Selenazofurin

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The synthesis and biological activity of selenophenfurin (5-β-D-ribofuranosylselenophene-3-carboxamide, **1**), the selenophene analogue of selenazofurin, are described. Glycosylation of ethyl selenophene-3-carboxylate (**6**) under stannic chloride-catalyzed conditions gave 2- and 5-glycosylated regioisomers, as a mixture of α- and β-anomers, and the β-2,5-diglycosylated derivative. Deprotected ethyl 5-β-D-ribofuranosylselenophene-3-carboxylate (**12**β) was converted into selenophenfurin by ammonolysis. The structure of **12**β was determined by ^{1}H- and ^{13}C-NMR, crystallographic, and computational studies. Selenophenfurin proved to be antiproliferative against a number of leukemia, lymphoma, and solid tumor cell lines at concentrations similar to those of selenazofurin but was more potent than the thiophene and thiazole analogues thiophenfurin and tiazofurin. Incubation of K562 cells with selenophenfurin resulted in inhibition of IMP dehydrogenase (IMPDH) (76%) and an increase in IMP pools (14.5-fold) with a concurrent decrease in GTP levels (58%). The results obtained confirm the hypothesis that the presence of heteroatoms such as S or Se in the heterocycle in position 2 with respect to the glycosidic bond is essential for both cytotoxicity and IMP dehydrogenase inhibitory activity in this type of C-nucleosides.

Inosine 5′-monophosphate dehydrogenase (EC 1.1.1.205, IMPDH) catalyzes the conversion of IMP to XMP utilizing NAD as a proton acceptor and is the rate-limiting enzyme in the *de novo* purine biosynthetic pathway leading to the formation of guanine nucleotides. The activity of IMPDH increases markedly in proliferating cells,[1,2] and inhibitors of the enzyme may be useful as antitumor and immunosuppressive agents. IMPDH inhibitors lead to an accumulation of the intracellular levels of IMP, which can serve as a phosphate donor for the phosphorylation of 2′,3′-dideoxynucleosides. Thus, these inhibitors are able to potentiate the anti-HIV activity of retroviral drugs such as 2′,3′-dideoxyinosine (ddI).[3,4] It has recently been reported that IMPDH exists as two isoforms, type I and type II, encoded by distinct genes.[5a,b] Type I is the prevalent species expressed in normal cells, whereas the type II isoform is upregulated and predominates in neoplastic and fast replicating cells.[6−8] Thus, selective inhibition of type II IMPDH may provide improved selectivity against cancer cells.

Selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide, NSC 340847), the selenium analogue of tiazofurin, is a widely studied agent with a diverse array of biological effects. These include potent antitumor and antiviral activity, as well as efficacy as a maturation-inducing agent.[9a−d] Selenazofurin is 5−10-fold more potent than tiazofurin in several antitumor screens and *in vitro* studies.[10] Both the antiproliferative and maturation-inducing effects of these nucleoside analogues appear to be due to inhibition of IMPDH, which induces the shutdown of guanine nucleotide synthesis.[11] In sensitive cells, tiazo- and selenazofurin are converted into analogues of the cofactor nicotinamide adenine dinucleotide (NAD). These analogues, called TAD and SAD, respectively, are excellent inhibitors of IMPDH.[11]

Crystal structures of tiazo- and selenazofurin demonstrate close intramolecular contact between the thiazole S or selenazole Se heteroatom and the furanose ring oxygen O1′.[12] Molecular orbital calculations suggest that these close contacts result from an attractive electrostatic interaction between the positively charged sulfur or selenium and the lone pair of electrons on the furanose oxygen. This interaction would be expected to constrain rotation about the C-glycosidic bond in the active anabolites TAD and SAD, influencing the binding of these dinucleotide inhibitors to the target enzyme. Observation of close S/Se···O contacts in analogues TAD and SAD bound to alcohol dehydrogenase supports this hypothesis.[13]

Recently we synthesized thiophenfurin (5-β-D-ribofuranosylthiophene-3-carboxamide, TPF), a C-nucleoside isostere of tiazofurin, in which the thiazole ring is replaced by a thiophene heterocycle.[14] Like tiazofurin, thiophenfurin was found to be active as an antitumor agent both *in vitro* and *in vivo* and to inhibit IMPDH. These studies supported the hypothesis that the presence of S in the heterocycle in position 2 with respect to the glycosidic bond is essential for cytotoxicity and IMPDH inhibition, whereas the N atom of the thiazole

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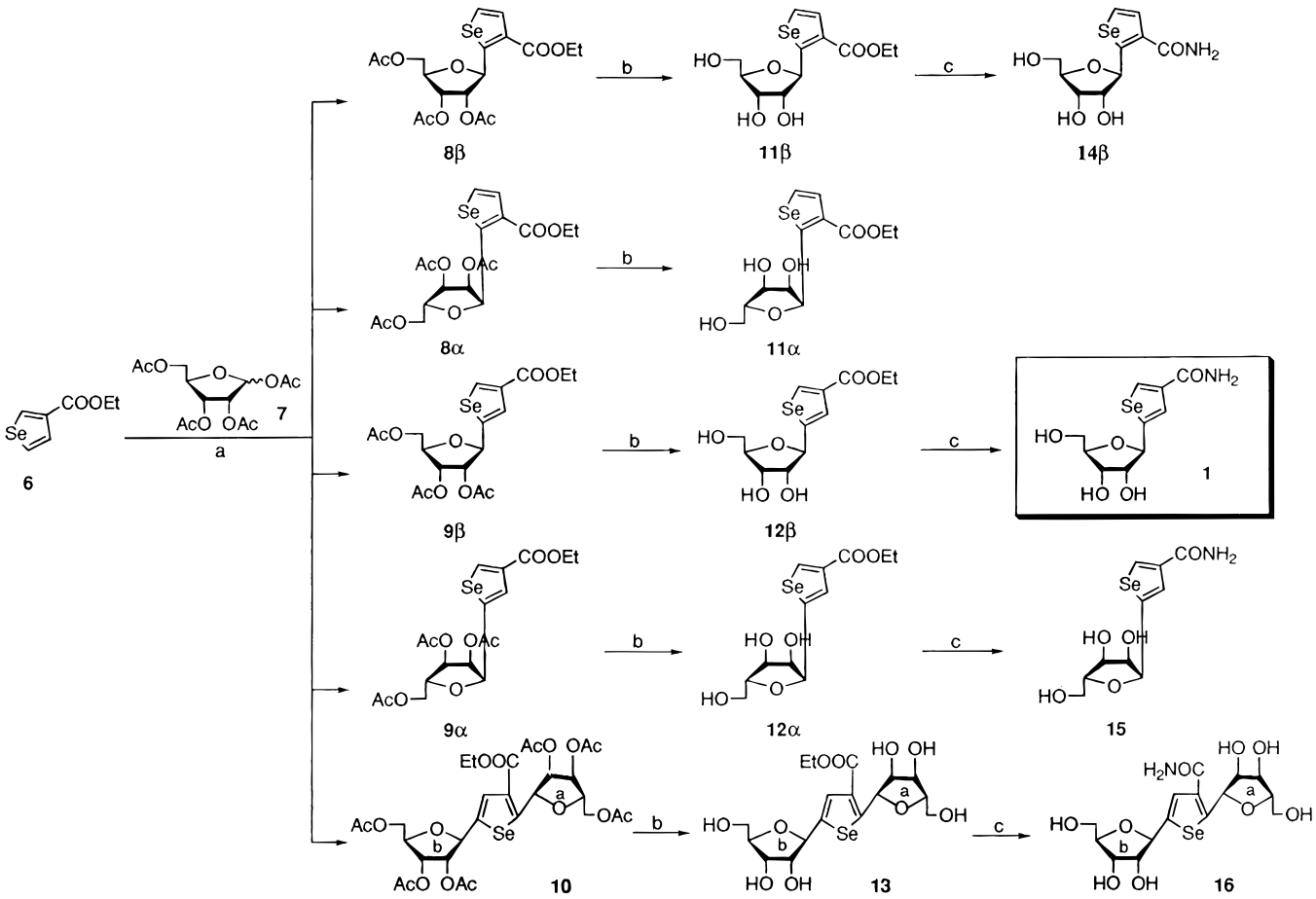
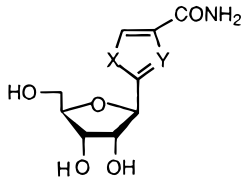
Scheme 1[a]



[a] (a) $SnCl_4/ClCH_2CH_2Cl$; (b) EtONa, EtOH; (c) 30% NH_4OH.

ring of tiazofurin is not. *Ab initio* computations suggested that, as found in tiazofurin, an electrostatic interaction between the positively charged thiophene sulfur and the furanose oxygen stabilizes the conformation in which the thiophene sulfur is *cis* to the furanose oxygen, with a marginally close nonbonded S···O contact of 3.04 Å.[14]



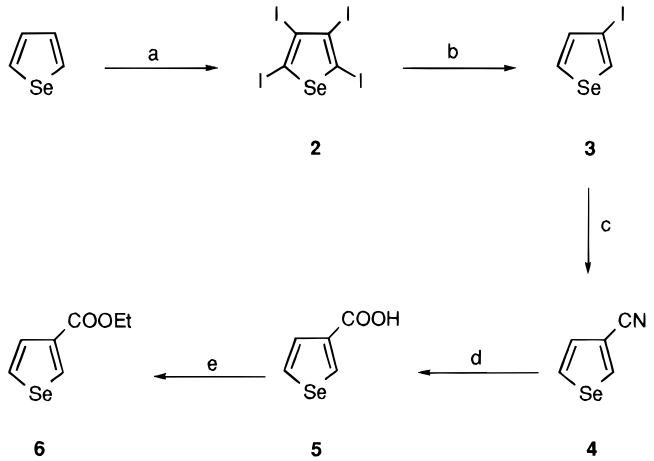
X = S, Y = N Tiazofurin
X = Se, Y = N Selenazofurin
X = S, Y = CH Thiophenfurin
X = Se, Y = CH Selenophenfurin (**1**)

This finding prompted us to extend the study of structure−activity relationships in this type of C-nucleosides to selenophenfurin (5-β-D-ribofuranosylselenophene-3-carboxamide, **1**), the selenophene analogue of selenazofurin.

Chemistry

The synthesis of selenophenfurin (**1**) was carried out as outlined in Scheme 1, by direct C-glycosylation of ethyl selenophene-3-carboxylate (**6**) under Friedel−Crafts conditions, as described for thiophenfurin.[14]

Scheme 2[a]



[a] (a) $Hg(OCOCH_3)_2$, H^+, I_2; (b) Zn/AcOH; (c) $(CH_3)_3SiCN$, $(Ph_3P)_4Pd(0)$, Et_3N; (d) HCl; (e) $SOCl_2$, EtOH.

The ethyl selenophene-3-carboxylate (**6**) was obtained by reacting the 3-carboxylic acid **5** with $SOCl_2$ and ethanol. Acid **5** is a known compound which has been prepared in different ways;[15a,b] however, we found it more convenient to carry out its synthesis by the method reported in Scheme 2. Tetraiodoselenophene (**2**), prepared by reaction with mercuric acetate in glacial acetic acid and then with iodine,[16] was treated with zinc powder and acetic acid (80%) under reflux to give 3-iodoselenophene (**3**). Compound **3** was converted into nitrile **4** by cyanation with trimethylsilyl cyanide in

anhydrous triethylamine in the presence of tetrakis-(triphenylphosphine)palladium(0); hydrolysis of **4** with hydrochloric acid under reflux gave the acid **5**.

The reaction of **6** with 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose (**7**) in 1,2-dichloroethane in the presence of $SnCl_4$ gave 2- and 5-glycosylated regioisomers as a mixture of α- and *β*-anomers (**8**α,*β*, **9**α,*β*, 42%) and the 2,5-bis(*β*-D-ribofuranosyl) derivative **10** (11%). The mixture of anomers **8**α,*β* and isomer **9***β* was separated from **9**α and **10** by chromatography.

The mixture of **8**α,*β* and **9***β* was treated with a catalytic amount of sodium ethoxide in ethanol to give deblocked ethyl esters **11**α,*β* and **12***β* which were separated by column chromatography. Starting from **9**α and **10**, the esters **12**α and **13** were obtained in a similar way. The glycosylation position was determined by ^{1}H-NMR and proton−proton nuclear Overhauser effect (NOE) difference spectroscopy. The ^{1}H-NMR spectrum of **11***β* showed that the signal of the H-2 proton of selenophene had disappeared, indicating that the glycosylation position was at C-2. On the other hand, the ^{1}H-NMR spectra of compounds **12**α,*β* lacked the signal of the H-5 proton, supporting C-5 glycosylation. The structures of compounds **12**α,*β* were further supported by NOE experiments. In fact, when the H-1′ signal of these compounds was irradiated, a NOE effect was observed at H-4, confirming that the ribosyl moiety resides at C-5. The anomeric configuration was also assigned on the basis of NOE experiments. In fact, selective irradiation of the anomeric signal of **11***β* and **12***β* increased the intensity of the H-4′ signal (1.5% and 2.3%, respectively), while no intensity enhancement of the H-3′ signal was observed; this indicates that H-1′ and H-4′ are located on the same face of the ribosyl ring.[17] The anomeric configuration was further supported by studies of ^{13}C-NMR data. Thus, the finding that C-1′ in **12***β* resonates upfield of C-1′ in **12**α is in accord with the previous observation of anomeric chemical shifts in thiophenfurin.[14]

Treatment of **12***β* with ammonium hydroxide (30%) gave selenophenfurin (**1**). In a similar way, ethyl esters **11***β*, **12**α, and **13** were converted to the amides **14***β*, **15**, and **16**. The glycosylation position on compound **13** was established to be at C-2 and C-5 on the basis of the ^{1}H-NMR spectrum, which showed that the H-2 and H-5 signals were lacking. The anomeric configuration was established to be *β*, based on the observation that, in the ^{1}H- and ^{13}C-NMR spectra, the chemical shift patterns were similar to those reported for the corresponding thiophene derivative.[14]

Crystallographic and Computational Studies

The crystal structure of the selenophenfurin carboxylate intermediate **12***β* (ethyl 5-*β*-D-ribofuranosylselenophene-3-carboxylate) was determined, as crystals of the parent compound could not be obtained. The molecular structure of **12***β* is isomorphous to that of the corresponding thiophene analogue[14] and also shares several features observed in the structures of related thiazole and selenazole nucleosides[12] (Figure 1). The selenophene ring is planar, with the ethyl carboxylate substituent at C-3 coplanar to the heterocycle. The C-glycosidic torsion angle is 46.3°, close to that seen for thiophenfurin (46.5°) but higher than that observed in selenazofurin (30.5°).[12] However, the selenophene selenium remains *cis* to the furanose oxygen, with a nonbonded close Se···O contact of 3.12 Å.

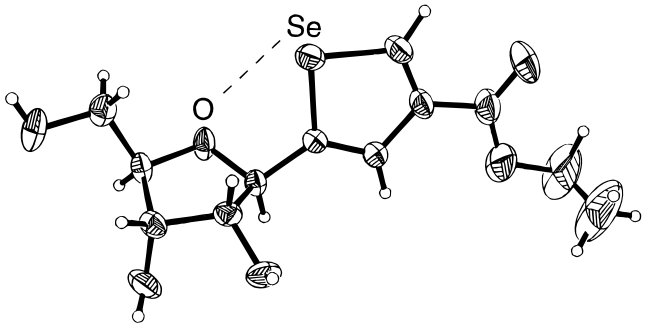


Figure 1. Molecular structure of **12***β*. Non-hydrogen atoms are represented by thermal ellipsoids at the 50% probability level. The dotted line indicates the 3.12 Å nonbonded Se···O contact.

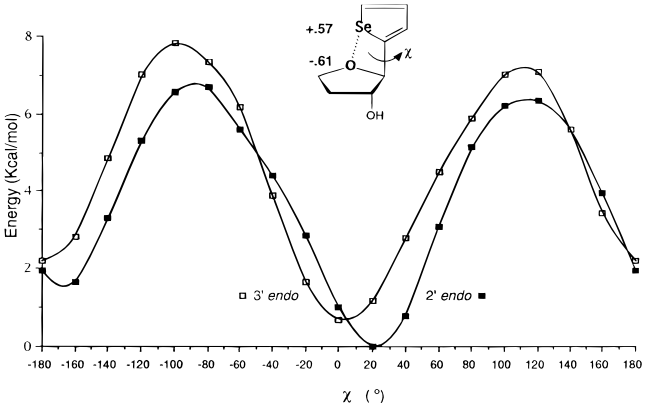


Figure 2. Energy vs C-glycosidic torsion angle *χ* in a selenophenfurin model fragment. The fragment is illustrated at top *χ* = 0° conformation. Numbers adjacent to the selenium and oxygen are natural bond orbital point charges. Rotation about *χ* is indicated by the arrow. Curves were obtained with the furanose moiety constrained in either a 3′-*endo* or 2′-*endo* pucker. Points on each curve were obtained at the RHF/3-21G(*)//3-21G(*) level.

Ab initio computations at the RHF/3-21G(*)//3-21G(*) level suggest that the Se···O contact observed in selenophenfurin is stabilized by an electrostatic interaction between a positively charged selenophene selenium and negatively charged furanose oxygen. Donation of mobile selenium valence electrons to the conjugated system of the selenophene ring results in a positive charge on the heteroatom. This effect is similar to that identified for thiophenfurin, tiazofurin, selenazofurin, and their analogues.[12,14] Calculation of energy vs C-glycosidic torsion angle *χ* for a selenophenfurin model fragment (Figure 2) yields a similar curve to that obtained for thiophenfurin.[14] The energy shows a global minimum at a C-glycosidic torsion angle of ∼20° for the 2′-*endo* sugar conformation, with a slightly higher minimum at *χ* ∼ 0° for the 3′-*endo* sugar conformation. As with thiophenfurin,[14] this result suggests that the minimum energy value of *χ* for selenophenfurin is lower than that observed in the crystal structure of the carboxylate intermediate.

Biological Activity

Effect of Selenophenfurin on the Proliferation of Murine and Human Tumor Cell Lines. The antiproliferative activity of selenophenfurin was tested *in vitro* against a panel of leukemia, lymphoma, and

Table 1. Antiproliferative Activity of Selenophenfurin, Selenazofurin, Thiophenfurin, and Tiazofurin

cell lines	IC_{50}[a] (μM) selenophenfurin	selenazofurin	thiophenfurin	tiazofurin
Leukemia/Lymphoma				
L1210[b]	0.3	0.5	1.7	2.9
K562[c]	0.45	0.5	2.3	2.5
Wil2-NS[d]	0.7	1.2	5.8	5.7
CCRF-SB[e]	0.9	1.5	6.4	7.5
Raji[f]	0.9	1.6	8.7	8.2
CCRF-CEM[g]	0.3	1.2	3.5	10
MOLT-4[g]	0.3	0.6	2.9	5.2
Carcinoma				
CHO-K1[h]	2.9	0.6	55	6.4
HT-29[i]	3.5	7.7	40	100
HeLa[j]	6.4	7.9	90	100
ACHN[k]	9.9	13.8	79	>100
5637[l]	1.2	2.3	17	24

[a] Compound concentration required to reduce cell viability by 50%. [b] L1210, murine lymphocytic leukemia. [c] K562, human erythroid leukemia. [d] Wil2-NS, human splenic lymphoblastoid cells. [e] CCRF-SB, human acute B-lymphoblastic leukemia. [f] Raji, human Burkitt lymphoma. [g] CCRF-CEM and MOLT-4, human acute T-lymphoblastic leukemias. [h] CHO-K1, Chinese hamster ovary. [i] HT-29, human colon adenocarcinoma. [j] HeLa, human cervix carcinoma. [k] ACHN, human renal adenocarcinoma. [l] 5637, human bladder carcinoma.

solid tumors cell lines, mostly derived from human tumors. Selenazofurin, tiazofurin, and thiophenfurin were used as reference drugs (Table 1).

Against liquid and solid tumor cell lines, no significant differences could be seen in both potency and spectrum of antiproliferative activity between selenophenfurin and selenazofurin. However, the test compound showed a potency superior to that of thiophenfurin and tiazofurin, particularly against solid tumor cell lines.

As observed for thiophenfurin, structure−activity relationships show that the glycosylation position and the anomeric configuration are determinants for the antiproliferative activity of selenophenfurin. In fact, the 2-glycosylated isomers **14**β and the α-anomer **15** were inactive (data not shown).

Effects of Selenophenfurin on IMPDH Activity and Intracellular Nucleotide Levels. Selenophenfurin and its parent compounds were also tested for inhibitory properties against IMPDH from human myelogenous leukemia K562 cells in culture, as previously reported.[14] IMPDH activity was performed by enzyme kinetic studies and is expressed as nmol of XMP formed/mg of protein/h. For these studies, K562 cells were exposed to 10 μM each of the inhibitors or saline for 2 h at 37 °C. The results indicate that IMPDH activity was strongly inhibited (76%) by the action of selenophenfurin with a potency similar to that of selenazofurin (80% inhibition) and greater than that of thiophenfurin and tiazofurin (Table 2).

Since inhibition of IMPDH results in perturbation of nucleotide pools, we examined the influence of selenophenfurin on the ribonucleotide concentration in K562 cells. The results provided in Table 3 show an increase in IMP levels (14.6-fold) with a concurrent decrease in guanylate concentration. Among the four compounds, selenophenfurin was found to have the highest activity in enhancing the IMP level and decreasing the guanine nucleotide pools. Except for the guanylates, no other purine and pyrimidine nucleotide concentrations were perturbed.

Table 2. Effects of Selenophenfurin, Selenazofurin, Thiophenfurin, and Tiazofurin (10 μM) on IMPDH Activity of K562 Cells in Culture

compd	IMPDH activity (nmol of XMP formed/h/mg of protein)	inhibition (%)[a]
control	5.04 ± 0.14	0
selenophenfurin	1.06 ± 0.28	76
selenazofurin	1.01 ± 0.12	80
thiophenfurin	2.62 ± 0.19	52
tiazofurin	2.07 ± 0.15	59

[a] Significantly different from control values ($p < 0.05$).

Table 3. Effects of Selenophenfurin, Selenazofurin, Thiophenfurin, and Tiazofurin (10 μM) on Ribonucleotide Levels of K562 Cells in Culture

nucleotide	control (nmol/g)	% of control[a] selenophenfurin	selenazofurin	thiophenfurin	tiazofurin
IMP	40.5 ± 5.6	1464	1043	703	1033
GMP	51.1 ± 2.4	0	0	0	0
GDP	58.1 ± 4.4	61	59	66	89
GTP	442.9 ± 12.5	42	50	60	55

[a] Significantly different from saline control ($p < 0.05$). K562 cells in culture (1×10^7) were incubated with saline (control), selenophenfurin, selenazofurin, thiophenfurin, or tiazofurin for 2 h at 37 °C. Cells were extracted with trichloroacetic acid and neutralized with tri-*n*-octylamine, and an aliquot was analyzed by HPLC.

Conclusions

This research shows that selenophenfurin is a C-nucleoside endowed with remarkable activity against a variety of tumor cell lines in culture. Its potency against human leukemic and solid tumor cell lines suggests that this agent might be useful against tumors in man. Replacement of the nitrogen atom by the CH group in the selenazole ring of selenazofurin does not affect the biological activity. This confirms the finding for the sulfur analogue tiazofurin that the nitrogen atom in the heterocycle is not essential for activity.

Overall, these and previous results[14,18] suggest that the major determinant for the antitumor and IMPDH inhibitory activity of this type of C-nucleosides is the presence of a sulfur or selenium heteroatom in position 2 on the base. This placement permits an attractive electrostatic interaction between the heteroatom and the furanose oxygen, potentially constraining rotation about the C-glycosidic bond. This constraint may enhance anabolism of the parent compound to the NAD analogue or stabilize binding of the NAD analogue to the target enzyme.

Experimental Section

Melting points were determined on a Buchi apparatus and are uncorrected. Elemental analyses were determined on a EA 1108 CHNS-O (Fisons Instruments) analyzer. Ultraviolet spectra were recorded on an HP 8452 A diode array spectrophotometer driven by an Olivetti M 24. Thin layer chromatography (TLC) was run on silica gel 60 F_{254} and RP-18 plates; silica gel 60 (70−230 and 230−400 mesh; Merck) for column chromatography was used. Nuclear magnetic resonance ^1H and ^{13}C spectra were determined at 300 and 75 MHz, respectively, with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Stationary NOE experiments were run on degassed solutions at 25 °C. A presaturation delay of 1 s was used, during which the decoupler low power was set at 20 dB attenuation.

Tiazofurin and selenazofurin were obtained through the Drug Synthesis & Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD).

Tetraiodoselenophene (2). To a mixture of selenophene (48 g, 0.366 mol), acetic acid (1.8 L), and mercury acetate (466.5 g, 1.464 mol) heated at 95 °C was added iodine (373.08 g, 1.47 mol) portionwise under stirring. The heating was continued for 30 min. After cooling at room temperature, the mixture was diluted with water (2.7 L), stirred for 20 min, and then stored for 15 h at 4 °C. The solid formed was filtered, washed with water, and added to a water solution (3.6 L) of KI (358.8 g). The mixture was stirred for 2 h at room temperature, and the solid was filtered, washed with water, and recrystallized from DMF/H_2O to give **2** (226.4 g, 97.4%) as a white solid: mp 205−210 °C (lit.[16] mp 208 °C). ^{13}C-NMR (Me_2SO-d_6): δ 92.5 (C-3, C-4), 110.58 (C-2, C-5). Anal. (C_4I_4Se) C.

3-Iodoselenophene (3). To a stirred mixture of **2** (226 g, 0.356 mol) and acetic acid (80% solution in water, 4.5 L) was added portionwise zinc powder (122.4 g, 1.87 mol). The mixture was heated under reflux for 2 h, cooled at room temperature, and filtered. The aqueous phase was neutralized with a saturated solution of $NaHCO_3$ and extracted with ethyl ether (400 mL × 3). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated to dryness to give an oily residue which was purified by distillation: bp 83 °C/1.5 mmHg (lit.[16] bp 56 °C/1 mmHg); 50.3 g, 55%.

Selenophene-3-carbonitrile (4). Tetrakis(triphenylphosphine)palladium(0) (4.39 g, 3.8 mmol) was added under nitrogen atmosphere to a stirred solution containing **3** (50 g, 0.195 mol) and trimethylsilyl cyanide (38.9 mL, 0.292 mol) in anhydrous triethylamine (388 mL, 2.78 mol). The reaction mixture was heated under reflux for 30 min. After cooling to room temperature, the mixture was partitioned between water and benzene. The organic phase was separated, dried over anhydrous Na_2SO_4, and evaporated to dryness under vacuum to give an oily residue which was purified by distillation: bp 80 °C/1 mmHg (lit.[15] bp 90−91 °C/10 mmHg); 21.55 g, 71%.

Selenophene-3-carboxylic Acid (5).[15] A mixture of **4** (21 g, 0.134 mol) and concentrated HCl (500 mL) was heated under reflux for 1 h. After cooling at room temperature the precipitated solid was collected and recrystallized from water to give **5** (15.8 g, 67%) as a white solid.

Ethyl Selenophene-3-carboxylate (6). A mixture of **5** (15 g, 85.69 mmol) and $SOCl_2$ (30 mL, 0.41 mol) was heated at 100 °C for 45 min. After evaporation, to the oily residue cooled at 0 °C was added anhydrous ethanol (20 mL), and the mixture was stirred at room temperature for 5 h. After evaporation in vacuo, the ester **6** was obtained as a yellow oil (17.4 g, 100%). TLC ($CHCl_3$): R_f = 0.72. ^{1}H-NMR (Me_2SO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 7.7 (dd, J = 1.3, 5.4 Hz, 1H, H-4), 8.22 (dd, J = 2.5, 5.5 Hz, 1H, H-5), 9.05 (dd, J = 1.3, 2.5 Hz, 1H, H-2). Anal. ($C_7H_8O_2Se$) C, H.

Ethyl 2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)selenophene-3-carboxylate (8β), Ethyl 2-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyl)selenophene-3-carboxylate (8α), Ethyl 5-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)selenophene-3-carboxylate (9β), Ethyl 5-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyl)selenophene-3-carboxylate (9α), and Ethyl 2,5-Bis(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)selenophene-3-carboxylate (10). To a stirred solution of ethyl selenophene-3-carboxylate (**6**) (17 g, 83.7 mmol) in dry 1,2-dichloroethane (160 mL) were added 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**7**) (26.6 g, 83.7 mmol) and $SnCl_4$ (8.3 mL), and the mixture reacted at room temperature for 18 h. The reaction mixture was diluted with H_2O, neutralized with $NaHCO_3$, and extracted with CH_2Cl_2 (100 mL × 3). The combined CH_2Cl_2 extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The obtained residue was chromatographed on a silica gel column eluting with $CHCl_3$−*n*-hexane (80:20). Three main fractions were separated. From the fast eluate, a mixture of **8**β,α and **9**β was obtained as a colorless oil (13.9 g, 36%). TLC ($CHCl_3$−*n*-hexane, 80:20): R_f = 0.36.

Evaporation of the following fraction gave **9**α as an oil (2.32 g, 6%). TLC ($CHCl_3$): R_f = 0.43. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 2.00−2.10 (3s, 9H, $OCOCH_3$), 4.10−4.35 (m, 5H, H-5′, H-4′, OCH_2CH_3), 5.38 (dd, J = 4.5, 7.0 Hz, 1H, H-3′), 5.50 (t, J = 3.8 Hz, 1H, H-2′), 5.65 (d, J = 3.0 Hz, 1H, H-1′), 7.62 (s, 1H, H-4), 9.03 (s, 1H, H-2). Anal. ($C_{18}H_{22}O_9Se$) C, H.

Evaporation of the last fraction gave **10** as a colorless oil (6.6 g, 11%). TLC ($CHCl_3$): R_f = 0.37. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 2.0−2.10 (m, 18H, $OCOCH_3$), 4.15−4.45 (m, 8H, H-4′a, H-4′b, 2H-5′a, 2H-5′b, OCH_2CH_3), 5.20 (m, 3H, H-2′a, H-3′a, H-3′b), 5.40 (m, 1H, H-2′b), 5.81 (d, J = 3.3 Hz, 1H, H-1′b), 6.01 (d, J = 3.3 Hz, 1H, H-1′a), 7.63 (s, 1H, H-4). Anal. ($C_{29}H_{36}O_{16}Se$) C, H.

Ethyl 2-β-D-Ribofuranosylselenophene-3-carboxylate (11β), Ethyl 2-α-D-Ribofuranosylselenophene-3-carboxylate (11α), and Ethyl 5-β-D-Ribofuranosylselenophene-3-carboxylate (12β). The oily mixture of **8**β,α and **9**β (13 g) was treated with sodium ethoxide in ethanol (273 mL, 15.12 mmol) for 1 h at room temperature. To the reaction mixture was added 0.55 g of Dowex 50w × 8 resin (H^+) (washed with ethanol), and the suspension was stirred for 1 h. The resin was filtered off, washed with ethanol, and discarded. The filtrate was evaporated to dryness, and the residue was chromatographed on a silica gel column with $CHCl_3$−MeOH (96:4) as eluent to give **11**β as a white solid (0.57 g, 6%): mp 65−67 °C. TLC ($CHCl_3$−MeOH, 96:4): R_f = 0.35. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.6 (m, 2H, H-5′), 3.76 (m, 1H, H-4′), 3.82 (m, 2H, H-2′, H-3′), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.82 (t, J = 5.4 Hz, 1H, OH), 4.94 (d, J = 5.5 Hz, 1H, OH), 5.02 (d, J = 5.3 Hz, OH), 5.52 (d, J = 3.5 Hz, 1H, H-1′), 7.60 (d, J = 5.8 Hz, 1H, H-4), 8.04 (d, J = 5.8 Hz, 1H, H-5). Anal. ($C_{12}H_{16}O_6Se$) C, H.

Evaporation of the following fraction gave **11**α (0.187 g, 2%) as a colorless oil. TLC ($CHCl_3$−MeOH, 96:4): R_f = 0.3. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.50, 3.60 (2m, 2H, H-5′), 3.75 (m, 1H, H-4′), 3.82 (m, 1H, H-3′), 3.96 (m, 1H, H-2′), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.85 (t, J = 5.4 Hz, 1H, OH), 5.0 (m, 1H, OH), 5.20 (d, J = 7.3 Hz, 1H, OH), 5.55 (dd, J = 5.4, 5.1 Hz, 1H, H-1′), 7.60 (d, J = 5.8 Hz, 1H, H-4), 8.02 (d, J = 5.8 Hz, 1H, H-5). Anal. ($C_{12}H_{16}O_6Se$) C, H.

Evaporation of the last fraction gave **12**β (4.7 g, 50%) as white needles (crystallized from $CHCl_3$): mp 115−116 °C. TLC ($CHCl_3$−MeOH, 96:4): R_f = 0.26. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.50 (t, J = 5.0 Hz, 2H, H-5′), 3.70 (m, 1H, H-4′), 3.82 (q, J = 4.5 Hz, 1H, H-3′), 3.92 (m, 1H, H-2′), 4.22 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.80 (d, J = 6.9 Hz, 1H, OH), 4.85 (t, J = 5.4 Hz, 1H, OH), 5.05 (d, J = 4.6 Hz, 1H, OH), 5.20 (d, J = 7.2 Hz, 1H, H-1′), 7.52 (d, J = 1.4 Hz, 1H, H-4), 8.9 (d, J = 1.4 Hz, 1H, H-5). ^{13}C-NMR (Me_2SO-d_6): δ 14.6 (CH_3CH_2), 60.8 (CH_3CH_2), 62.4 (C-5′), 72.0 (C-3′), 78.7 (C-2′), 81.1 (C-1′), 86.0 (C-4′), 125.3 (C-4), 135.0 (C-5), 140.1 (C-2), 153.9 (C-3), 163.0 (C=O). Anal. ($C_{12}H_{16}O_6Se$) C, H.

Ethyl 5-α-D-Ribofuranosylselenophene-3-carboxylate (12α). Compound **12**α was obtained from **9**α (2 g, 4.33 mmol) as described for **11**β, as a colorless oil (1.14 g, 79%), after chromatography on a silica gel column eluting with $CHCl_3$−MeOH (94:6). TLC ($CHCl_3$−MeOH, 96:4): R_f = 0.10. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.45 (m, 1H, H-5′), 3.60 (m, 1H, H-5′), 3.80 (m, 1H, H-4′), 3.97 (m, 1H, H-3′), 4.18 (m, 1H, H-2′), 4.22 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.70 (t, J = 5.7 Hz, 1H, OH), 4.95 (m, 1H, OH), 5.2 (d, J = 2.5 Hz, 1H, H-1′), 5.28 (d, J = 3.7 Hz, 1H, OH), 7.60 (s, 1H, H-4), 9.0 (s, 1H, H-2). ^{13}C-NMR (DMSO-d_6): δ 14.4 (CH_3CH_2), 60.5 (CH_3CH_2), 61.7 (C-5′), 72.7 (C-2′, C-3′), 79.2 (C-1′), 82.2 (C-4′), 127.3 (C-4), 133.7 (C-5), 142.1 (C-2), 149.4 (C-3), 163.1 (C=O). Anal. ($C_{12}H_{16}O_6Se$) C, H.

Ethyl 2,5-Di-β-D-ribofuranosylselenophene-3-carboxylate (13). Compound **13** was prepared from **10** (3.8 g, 5.55 mmol) by the above method. The reaction residue was purified by flash chromatography using $CHCl_3$−MeOH (90:10) to give **13** as a foam (2.17 g, 56%). TLC ($CHCl_3$−MeOH, 90:10): R_f = 0.15. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.48 (t, J = 5.1 Hz, 2H, H-5′b), 3.58 (m, 2H, H-5′a), 3.70−3.90 (m, 6H, H-4′a, H-4′b, H-3′a, H-3′b, H-2′a, H-2′b), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.70 (d, J = 7.2 Hz, 1H, H-1′b), 4.82 (t, J = 5.3 Hz, 1H, OH), 4.95 (d, J = 4.7 Hz, 1H,

OH), 5.0 (d, J = 4.8 Hz, 1H, OH), 5.16 (d, J = 7.2, 1H, OH), 5.54 (d, J = 3.7 Hz, 1H, H-1′a), 7.46 (s, 1H, H-4). Anal. ($C_{17}H_{24}O_{10}Se$) C, H.

2-β-D-Ribofuranosylselenophene-3-carboxamide (14β). Treatment of compound **11**β (0.5 g, 1.08 mmol) with 30% ammonium hydroxide (15 mL) for 8 h at room temperature and evaporation to dryness afforded a product which was purified by chromatography on a silica gel column eluting with $CHCl_3$−MeOH (80:20). Evaporation of the homogeneous fractions gave 90 mg (27.5%) of **14**β as a white foam. TLC ($CHCl_3$−MeOH, 80:20): R_f = 0.46. ^{1}H-NMR (DMSO-d_6): δ 3.5 (t, J = 4.9 Hz, 2H, H-5′), 3.68 (t, J = 5.9 Hz, 1H, H-4′), 3.82 (dd, J = 4.7 Hz, 8.1 Hz, 1H, H-3′), 3.9 (br s, 1H, H-2′), 4.8 (t, J = 5.2 Hz, 1H, OH), 4.9 (br s, 1H, OH), 5.38 (d, J = 7.0 Hz, 1H, H-1′), 5.9 (br s, 1H, OH), 7.48 (br s, 1H, NH), 7.52 (d, J = 5.8 Hz, 1H, H-4), 7.82 (br s, 1H, NH), 8.0 (d, J = 5.8 Hz, 1H, H-5). Anal. ($C_{10}H_{13}NO_5Se$) C, H. N.

5-β-D-Ribofuranosylselenophene-3-carboxamide (1). The title compound was obtained from **12**β (4 g, 11.93 mmol) (reaction time 9 h) by the above method. After chromatographic purification of the reaction mixture ($CHCl_3$−MeOH−NH_4OH, 70:29:1), **1** was obtained as a white foam (2.15 g, 58%). TLC ($CHCl_3$−MeOH, 70:30): R_f = 0.31. UV (MeOH) λ_{max} 210 nm (ε 35 300), 240 (ε 10 500). ^{1}H-NMR (Me_2SO-d_6): δ 3.50 (t, J = 5.1 Hz, 2H, H-5′), 3.72 (m, 1H, H-4′), 3.80−3.95 (m, 2H, H-3′, H-2′), 4.76 (d, J = 6.6 Hz, 1H, H-1′), 4.8 (t, J = 5.5 Hz, 1H, OH), 5.0 (d, J = 3.5 Hz, 1H, OH), 5.18 (d, J = 7.0 Hz, 1H, OH), 7.12 (br s, 1H, NH), 7.58 (s, 1H, H-4), 7.70 (br s, 1H, NH), 8.70 (s, 1H, H-2). Anal. ($C_{10}H_{13}NO_5Se$) C, H, N.

5-α-D-Ribofuranosylselenophene-3-carboxamide (15). Compound **15** was prepared from **12**α (1 g, 2.98 mmol) (reaction time 9 h) by the above method. The reaction residue was chromatographed on a silica gel column with $CHCl_3$−MeOH (90:10) to give **15** as a foam (0.486 g, 53%). TLC ($CHCl_3$−MeOH, 90:10): R_f = 0.07. ^{1}H-NMR (Me_2SO-d_6): δ 3.46 (m, 2H, H-5′), 3.65 (t, J = 6.0 Hz, 1H, H-4′), 3.81 (m, 1H, H-3′), 3.90 (m, 1H, H-2′), 4.75 (d, J = 7.0 Hz, 1H, H-1′), 4.80−5.30 (br s, 3H, OH), 7.1 (br s, 1H, NH), 7.57 (s, 1H, H-4), 7.70 (br s, 1H, NH), 8.68 (s, 1H, H-2). Anal. ($C_{10}H_{13}NO_5Se$) C, H, N.

2,5-Di-β-D-ribofuranosylselenophene-3-carboxamide (16). Compound **16** was obtained from **13** (1.7 g, 3.65 mmol) by the above method and purified by chromatography on a silica gel column ($CHCl_3$−MeOH, 80:20) as a foam (0.8 g, 50%). TLC ($CHCl_3$−MeOH−NH_4OH, 60:30:10): R_f = 0.29. ^{1}H-NMR (Me_2SO-d_6): δ 3.40 (m, 4H, 2H-5′a, 2H-5′b), 3.60−3.90 (3m, 6H, H-4′a, H-4′b, H-3′a, H-3′b, H-2′a, H-2′b), 4.65 (d, J = 7.0, 1H, H-1′b), 4.70−5.12 (3 br s, 6H, OH), 5.33 (d, J = 7.3 Hz, 1H, H-1′a), 7.37 (s, 1H, H-4), 7.40 (br s, 1H, NH), 7.80 (br s, 1H, NH). Anal. ($C_{15}H_{21}NO_9Se$) C, H, N.

Crystallographic Study. Small colorless needle-shaped crystals of the carboxylate precursor of selenophenfurin [ethyl 5-β-D-ribofuranosylselenophene-3-carboxylate, $C_{12}H_{16}O_6Se$ (**12**β)] were obtained from chloroform. Crystals are orthorhombic, space group $P2_12_12_1'$ with cell dimensions a = 4.934(1) Å, b = 12.760(3) Å, and c = 21.764 Å, Z = 4, and are isomorphous with those of the thiophene analogue.[14] Diffraction data were collected at 223(2) K on a Siemens SMART CCD detector system using graphite monochromatized Mo Kα radiation from a sealed-tube source. A total of 8695 reflections were measured to θ = 28.3° and averaged to yield 3262 unique reflections. The structure was solved by routine application of direct methods and refined to R = 3.8% (R_w^2 = 9.4%) for all data using the SHELXTL package.[19] The structure is isomorphous to that of the thiophene analogue.[14] All non-hydrogen atoms were refined with anisotropic temperature factors using full-matrix least-squares techniques. Thiophene and ribose methylene protons were added in idealized positions and refined using a "riding" model.[19] Initial positions of hydroxyl and methyl protons were determined from maxima in difference map values over the loci of possible hydrogen positions and their torsion angles subsequently refined.[19] Isotropic temperature factors for hydrogens were set at either 1.2 or 1.5 times the value of the equivalent isotropic factor of the bound carbon or oxygen.[19] High thermal parameters in the ethyl carbons suggested some disorder in this portion of the carboxylate substituent. Similar values were observed in the thiophene analogue.

Computational Study. Energy as a function of C-glycosidic torsion angle χ was computed for a selenophenfurin model fragment using methods similar to those described for thiophenfurin.[14] *Ab initio* calculations were performed using the Gaussian 94 system of programs and internal basis sets.[20]

The selenophenfurin model fragment contained a selenophene heterocycle with the carboxamide substituent omitted. The heterocycle was connected via a C-glycosidic bond to a 2′-hydroxyfuranose ring. By analogy to selenazofurin, χ was defined by atoms O1′−C1′−C2−Se1. A value of χ = 0° refers to the conformation in which the furanose oxygen is *cis* planar to the selenophene selenium. A positive value of χ indicates a counterclockwise rotation of the C2-O1/Se bond relative to the C1′−O1′ bond when viewed down the C-glycosidic bond from C2 to C1′.

Energy profiles for the selenophenfurin model compound were obtained with the furanose ring fixed in both 3′-*endo* and 2′-*endo* puckers. The slightly lower global energy minimum for the 2′-*endo* conformer was normalized to 0 kcal/mol. Energies for each conformer were obtained for values of χ between χ = −180° and 180°. In each case, the value of χ was incremented in 20° steps and fixed. All remaining geometry variables describing the fragment were then optimized, with the exception of the furanose torsion angles. The starting geometry at each value of χ was the optimized geometry obtained at the previous value. All geometry optimizations used the analytical gradient method.[20,21] Optimized geometries and associated SCF energies were obtained using the 3-21G* basis set. Thus, each point in Figure 2 represents a calculation at the RHF/3-21G*//3-21G* level.[20,21] Point charges were obtained from the selenophenfurin fragment using the natural population analysis method incorporated in Gaussian 94.[20,22] Charges were obtained from optimized geometries at the RHF level of theory using a 3-21G* basis set.[20,21]

Antiproliferative Assays. The cells used are as follows: L1210, murine lymphocytic leukemia cells; K562, human myelogenous leukemia cells; Wil2-NS, human splenic B-lymphoblastoid cells; CCRF-SB, human acute B-lymphoblastic cells; Raji, human lymphoblast-like cells from a Burkitt lymphoma; CCRF-CEM and MOLT-4, human acute T-lymphoblastic leukemias; CHO-K1, Chinese hamster ovary cells; HT-29, human colon adenocarcinoma; HeLa, human cervix carcinoma; ACHN, human renal adenocarcinoma; 5637, human bladder carcinoma. Cell cultures were grown in their specific media supplemented with 10% FCS and antibiotics and incubated at 37 °C in a humidified, 5% CO_2 atmosphere. Exponentially growing leukemia and lymphoma cells were resuspended at a density of 1 × 10^5 cells/mL, in growth medium, and cultured with various concentrations of the compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[23] Activity against solid tumor-derived cells was evaluated in exponentially growing cultures seeded at 5 × 10^4 cells/mL and allowed to adhere for 16 h to culture plates before addition of the drugs. Cell viability was determined by the MTT method 4 days later.

Cell growth at each drug concentration is expressed as percentage of untreated controls, and the concentration resulting in 50% growth inhibition (IC_{50}) was determined by linear regression analysis.

IMP Dehydrogenase Assay. K562 cells (1 × 10^6 cells/mL; 10 mL) in logarithmic phase of growth were incubated with saline or indicated concentrations of the agents for 2 h at 37 °C. The cells were then harvested by centrifugation, washed once with cold phosphate-buffered saline, and lyzed in buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM $MgCl_2$, 0.5% NP-40, and 2 μg/mL aprotinin. The lysate was kept for 20 min on ice and centrifuged at 13000*g* for 20 min, and the supernatant was used as a source of IMPDH.

The enzyme activity was measured according to the published methodology.[24] Briefly, 5 μL aliquots of 0.5 M KCl containing 20 mM allopurinol were dispensed into the apex of Eppendorf tubes and dried at room temperature (25 °C). For the conduct of the assay, in a total volume of 10 μL, tubes

contained 5 μL of the substrate mixture containing 286 μM [2,8-^{3}H]IMP (200 μCi/mL) and 1 mM NAD. The reaction was initiated by the addition of a 5 μL aliquot of enzyme extract. After 30 min at 37 °C, the reaction was terminated by heating for 1 min at 95 °C. Tubes were centrifuged at 13000*g* for 0.5 min, 5 μL of 100% KOH was deposited on the underside of the cap, and the tubes were closed and incubated at room temperature overnight (16 h). The caps containing the droplet were cut, and the radioactivity was determined by scintillation spectrometry. IMP dehydrogenase activity is expressed as nmol of XMP formed/mg of protein/h.

Determination of the Concentration of Intracellular Ribonucleotides. Cells in culture were treated with saline or agents for 2 h at 37 °C; cells were harvested by centrifugation and washed once with cold saline. Cells were extracted with cold 10% trichloroacetic acid and centrifuged for 0.5 min; the supernatant was immediately neutralized with 0.5 M tri-*n*-octylamine in freon. An aliquot of the neutralized extract was analyzed on HPLC using a Partisil 10-SAX column as described.[25]

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Supporting Information Available: Coordinates, thermal parameters, bond lengths and angles, and crystal and refinement data for selenophenfurin-3-carboxylate (**12**β) (6 pages); table of observed and calculated structure factors (8 pages). Ordering information is given on any current masthead page.

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