Synthesis and Cytotoxicity of 6-Selenopurine Arabinoside and Related Compounds

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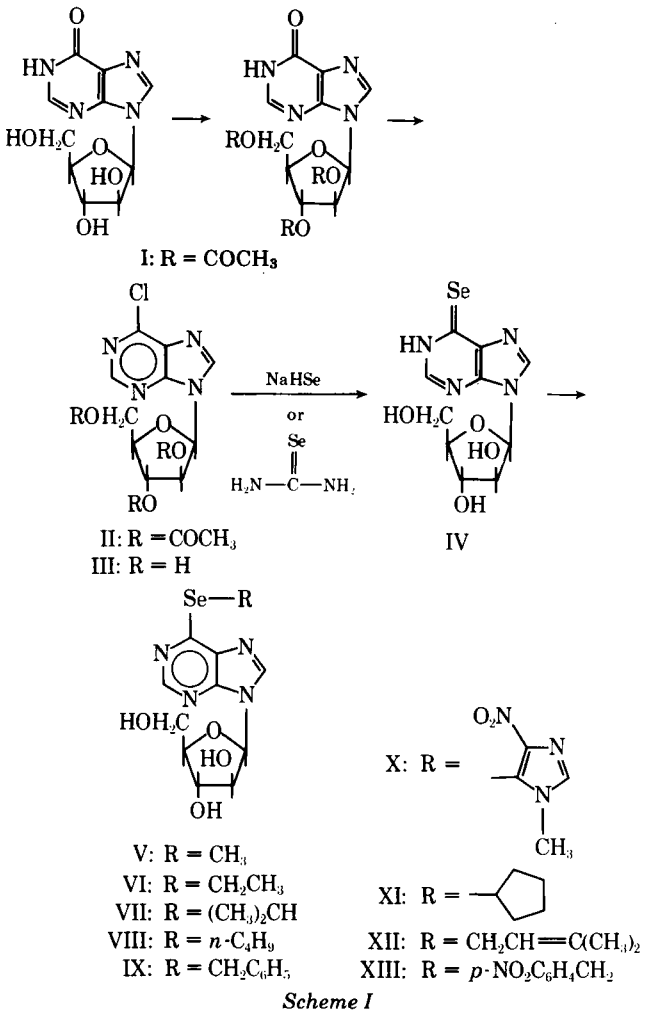
Abstract □ 6-Chloro-9-(β-D-arabinofuranosyl)purine served as an intermediate for the chemical synthesis of a series of 6-substituted selenopurine arabinosides. In an *in vitro* test using murine leukemic cells (L-5178Y) these 6-substituted selenopurine arabinosides showed some cytotoxicity. Lengthening the side chain had no effect on their cytotoxicity. Selenourea was a useful reagent for synthesizing selenopurines, selenopurine nucleosides, and selenopurine arabinoside under mild conditions.

Keyphrases □ 6-Selenopurine arabinoside and related compounds—synthesis and cytotoxicity □ Selenium analogs—synthesis and cytotoxicity of 6-selenopurine arabinoside and related compounds □ Cytotoxicity—6-selenopurine arabinoside and related compounds

Mercaptopurine and thioguanine have been employed widely in the clinical treatment of various types of leukemia (1–5). The selenium isostere, 6-selenopurine (6), was found (7) to inhibit mouse leukemia L-1210 as effectively as mercaptopurine. Unfortunately, it is not a useful compound due to its instability *in vivo*. Subsequently, selenoguanine, an analog of thioguanine, was synthesized (8, 9). It is as effective an inhibitor as thioguanine on the growth of several experimental tumors, is less toxic to the host, and has a superior therapeutic index (9).

Numerous selenium analogs were synthesized to reexplore their biochemical behavior including 6-selenopurine riboside (10), 6-seleno-*N*-alkyl purines (11), ethyl 6-selenoguanine-9-carboxylate (12), 6-selenoguanosine (13, 14), (α,β)-2′-deoxy-6-selenoguanosine (15–17), alkyl-selenopurine ribosides (14, 18, 19), 6-selenoguanosine 5′-monophosphate (20), and 8-substituted selenoadenosines and their cyclic nucleotides (21). 6-Selenoguanosine and β-2′-deoxy-6-selenoguanosine were active inhibitors of several experimental tumors *in vitro* (14, 15) and *in vivo* (17, 20).

Cytarabine (1β-D-arabinofuranosylcytosine) is a potent inhibitor of the growth of tumor cells in culture and also causes striking regression of tumors in mice (22). 6-Mercapto-9-(β-D-arabinofuranosyl)purine was found to have both antitumor (23) and immunosuppressive (24) properties. 6-Mercapto-9-(β-D-arabinofuranosyl)purine also was a competitive inhibitor of L-1210 adenosine deaminase when 9-β-D-arabinofuranosyladenine was used as the substrate. The phosphorylation of adenosine, 9-β-D-arabinofuranosyladenine, and 6-methylthio-9-(β-D-ribofuranosyl)purine did not appear to be affected by 6-mercapto-9-(β-D-arabinofuranosyl)purine (25). These results prompted the synthesis of 6-selenopurine arabinoside and certain related compounds and the evaluation of their cytotoxicity.



Scheme I

RESULTS AND DISCUSSION

Chemical—Acetylation of 9-(β-D-arabinofuranosyl)hypoxanthine with acetic anhydride in pyridine gave 9-(2′,3′,5′-tri-*O*-acetyl-β-D-arabinofuranosyl)hypoxanthine (I) (26). Chlorination of I with phosphorus oxychloride–*N*,*N*-diethylaniline at refluxing temperature gave 6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (II) (27). Deacetylation of II with methanolic ammonia at room temperature gave 6-chloro-9-(β-D-arabinofuranosyl)purine (III). Compound III was used for further transformation without purification. Treatment of III with sodium hydrogen selenide in refluxing methanol gave 6-selenoxo-9-(β-D-arabinofuranosyl)purine (IV) in 52% yield.

Compound IV can be synthesized alternatively by the reaction of III with selenourea. This method was found to be a convenient way to synthesize various seleno compounds. Compound IV was relatively unstable; its half-life was 3 days in water at room temperature.

Kikugawa *et al.* (28) reported that lengthening the side chain of 2-thioadenosine increases its activity as an inhibitor of platelet aggregation; therefore, several derivatives of IV were synthesized. Treatment of IV with methyl iodide, ethyl bromide, isopropyl bromide, *n*-butyl bromide, benzyl bromide, 5-chloro-1-methyl-4-nitroimidazole, cyclopentyl bromide, 3,3′-dimethylallyl bromide, and

Table I—Physical Properties of 6-Selenopurine Arabinoside and Related Compounds

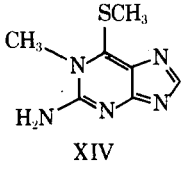
Compound	λ_{max}, nm ($\epsilon \times 10^{-3}$) pH 1	pH 11	H_2O	R_f[a]
IV	231 (8.9)	240 (11.2)	234 (10.1)	0.52
	350 (15.7)	328 (13.7)	347 (15.6)	
V	231 (12.0)	230 (10.5)	228 (8.8)	0.71
	315 (14.9)	302 (13.8)	302 (13.1)	
VI	312 (11.5)	229 (8.1)	229 (8.2)	0.71
		303 (11.4)	304 (11.7)	
VII	310 (10.8)	229 (10.6)	228 (10.5)	0.62
		304 (13.7)	304 (15.1)	
VIII	313 (12.6)	229 (10.9)	229 (8.7)	0.45
		305 (15.1)	305 (15.0)	
IX	307[b]	233 (16.2)	—	0.21
		305 (14.4)	305 (14.3)	
X	289 (17.2)	288 (15.4)	288 (15.3)	0.73
XI	237[b]	231 (8.9)	231 (8.1)	0.42
	320	306 (11.7)	306 (11.4)	
XII	314 (12.6)	221 (14.7)	306 (11.7)	0.32
		306 (13.4)		
XIII	299 (22.8)	301 (21.0)	300 (21.4)	0.11
XIV	228 (9.5)	233 (16.1)[c]	230 (17.6)	0.3
	253 (5.2)	284 (3.9)	283 (3.8)	
	336 (8.1)	340 (6.0)	340 (8.0)	
XX	266 (7.6)	238 (17.0)	228 (14.6)	0.32
	370 (17.8)	354 (17.2)	360 (17.6)	
XXI	273 (3.1)	230 (19.3)	—	0.84
	379 (15.6)	306 (11.4)	349 (13.7)	

[a] TLC was run on a polygram CEL 300 PEI and developed with water. [b] The solubilities of these compounds are low. [c] The half-life from the height of the 340-nm peak in pH 11 at room temperature was about 1 hr.

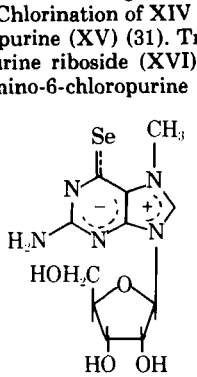
p-nitrobenzyl bromide gave the corresponding purine arabinoside derivatives of 6-(methylseleno) (V), 6-(ethylseleno) (VI), 6-(isopropylseleno) (VII), 6-(*n*-butylseleno) (VIII), 6-(benzylseleno) (IX), 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)seleno] (X), 6-(cyclopentylseleno) (XI), 6-[(3-methyl-2-butenyl)seleno] (XII), and 6-(*p*-nitrobenzylseleno) (XIII), respectively (Scheme I).

Alkylations with primary halides were performed in aqueous basic solution, but alkylations with secondary halides or relatively active halides were carried out in anhydrous media. Alkylation of IV with secondary halides in aqueous basic solution gave low yields of products. The low yields may arise from the hydrolysis of these secondary halides into the corresponding alcohols. The 6-selenopurine arabinosides were purified by continuous extraction with ether or by recrystallization from water. The physical properties of the 6-selenopurine arabinosides are shown in Table I. The products were identified by elemental analysis and by a comparison of their UV spectra with known thio analogs (26).

Selenourea has been reported to react with 6-chloropurine, ethyl 2-amino-6-chloropurine-9-carboxylate, and 7-chloro-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine to give 6-selenopurine, ethyl 2-amino-6-selenopurine-9-carboxylate, and 7-selenoxo-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (6, 12, 29), respectively. To test the limitation of this procedure, several substituted purines were synthesized. Methylation of 1-methyl-6-thioguanine (30) with methyl iodide in sodium hydroxide solution gave 2-amino-1-methyl-6-(methylthio)purine (XIV). Chlorination of XIV in methanol gave 2-amino-6-chloro-1-methylpurine (XV) (31). Treatment of XV, 2-amino-6-chloro-7-methylpurine riboside (XVI) (32), 6-chloropurine riboside (XVII), 2-amino-6-chloropurine riboside (XVIII), and 2-amino-6-chloropurine (XIX) with selenourea in refluxing methanol or at room temperature gave the corresponding seleno compounds (XX–XXIV) in good yield. The advantages of this procedure are that handling of hydrogen selenide gas is avoided and reaction conditions are milder than in the conventional sodium hydrogen selenide method.



XIV



XXI

Biological Effects—The analogs were evaluated in two studies for their cytotoxicity, using L-5178Y murine leukemic cells grown in culture.

Long-Term Studies—These studies were conducted for 72 hr over a wide range of concentration (10^{-4}–10^{-7} *M*). As shown in Table II, 6-thioguanosine, IV, and X exhibit 50% inhibition at concentrations of 5.6×10^{-6}, 6.6×10^{-5}, and 1.9×10^{-5} *M*, respectively. Compounds V–IX, XI, and XII caused less than 50% inhibition at the highest concentration (10^{-4} *M*) studied, so percent inhibition at 10^{-5} or 10^{-4} *M* was determined as shown in columns 3 and 4 of Table II.

Short-Term Studies—As shown in Table III, L-5178Y cells in the exponential phase of growth were treated with the inhibitory compounds (10^{-4}–10^{-7} *M*) for 2 hr and cell viability was determined by colony formation in dilute agar (33). The cloning efficiency of the untreated cells was approximately 75%. All values were normalized to 100% acute cell kill after 2 hr of incubation.

Compound X, which was shown to be active by long-term and short-term studies, was also evaluated for its ability to cause acute cell death by exposure of cells for 1–5 hr at two concentrations (10^{-4} and 10^{-5} *M*); cell viability was determined by colony formation in dilute agar (33). The results (Table IV) demonstrated that the logarithmic cell kill by X was 29%/hr at 1×10^{-4} *M* and 15%/hr at 1×10^{-5} *M*.

EXPERIMENTAL[1]

9 - (2′,3′,5′ - Tri-*O*- acetyl-β-D- arabinofuranosyl)hypoxanthine (I)—This compound was prepared by the method of Reist *et al.* (26) with 9-(β-D-arabinofuranosyl)hypoxanthine (1.5 g, 0.57 mmole) and acetic anhydride (2.1 ml, 2.25 mmoles) in 90 ml of pyridine in 60% yield.

6-Chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (II)—Into a mixture of 35 ml of phosphorus oxychloride and 2 ml of *N,N*-diethylaniline, 6.0 g (15.8 mmoles) of I was added. The suspension was refluxed rapidly for 3 min. The resulting clear brown solution was evaporated *in vacuo*, and the residue was added to chloroform and ice water. The solution was extracted with chloroform (2 × 50 ml). The organic layer was extracted with 1 *N* HCl (2 × 60 ml) and then water until the aqueous layer was neutral to pH paper. The organic layer was dried and evaporated to dryness to give 3.8 g of crude II.

6-Chloro-9-(β-D-arabinofuranosyl)purine (III)—Into 70 ml of absolute methanol saturated with ammonia, 3.8 g (9.6 mmoles) of II was added. The solution was stirred at room temperature for 6 hr and then evaporated to dryness. The residue was dried *in vacuo* at 65° for 1 day to give 2.4 g (92.7%) of III.

6-Selenoxo-9-(β-D-arabinofuranosyl)purine (IV)—*Method I*—Condensed hydrogen selenide[2] (1.0 ml, 24 mmoles) was bubbled through a solution of 0.2 g (8.7 mmoles) of sodium in 50 ml of absolute methanol. Compound III (2.2 g, 7.7 mmoles) in 10 ml of absolute methanol was added, and the suspension was refluxed for 40 min. The resulting yellow precipitate was filtered by suction, dissolved in 20 ml of 3% Na_2CO_3, and filtered. The filtrate was acidified with acetic acid to pH 4 and cooled. The yellow solid was collected, washed with a small amount of cold water, and dried *in vacuo* to give 1.4 g (52%) of IV, mp 155–157° dec.

Anal.—Calc. for $C_{10}H_{12}N_4O_4Se \cdot H_2O$: C, 34.64; H, 4.04; N, 16.04. Found: C, 34.80; H, 4.07; N, 16.01.

Method II—The suspension of 100 mg (0.36 mmole) of III and 45 mg (0.36 mmole) of selenourea (34) in 5 ml of methanol was refluxed for 20 min. The yellow solution was filtered, cooled in an ice

[1] Melting points were determined on a Gallenkamp melting-point apparatus and are uncorrected. UV spectra were determined on a Perkin-Elmer model 402 spectrophotometer. Elemental analyses were performed either by Midwest Microlab, Indianapolis, Ind., or Robertson Lab, Florham Park, N.J. TLC was run on a polygram CEL 300 PEI and developed with water. Evaporations were accomplished using a Buchler flash evaporator under reduced pressure with a bath temperature of 40°.

[2] Hydrogen selenide, 98.0% minimum purity, from Matheson Co., East Rutherford, NJ 07073

bath, and then poured into 50 ml of ether. The precipitate was held in the refrigerator overnight, filtered, and then dried *in vacuo* to give 45 mg (38%) of yellow solid. The nucleoside isolated was established by UV and TLC to be identical with IV prepared by Method I.

6-(Methylseleno)-9-(β-D-arabinofuranosyl)purine (V)—To a solution of 288 mg (0.82 mmole) of IV in 2.2 ml (0.87 mmole) of 0.4 *N* NaOH, 0.11 ml of methyl iodide was added. The suspension was stirred at room temperature for 2 hr. The solid was filtered by suction, recrystallized from water, and dried *in vacuo* to give 200 mg (84%) of V, mp 171–173°.

Anal.—Calc. for $C_{11}H_{14}N_4O_4Se \cdot H_2O$: C, 36.39; H, 4.45; N, 15.42. Found: C, 36.60; H, 4.66; N, 15.45.

6-(Ethylseleno)-9-(β-D-arabinofuranosyl)purine (VI)—By following the procedure described for V, VI was synthesized from IV and ethyl bromide in 36% yield, mp 160–162°.

Anal.—Calc. for $C_{12}H_{16}N_4O_4Se$: C, 40.12; H, 4.49; N, 15.59. Found: C, 40.56; H, 4.66; N, 15.83.

6-(Isopropylseleno)-9-(β-D-arabinofuranosyl)purine (VII)—To a solution of 145 mg (0.41 mmole) of IV in 5 ml of dry dimethylformamide, 150 mg (1.42 mmoles) of anhydrous sodium carbonate and 65 mg (0.5 mmole) of isopropyl bromide were added. The suspension was stirred at room temperature for 4 hr and partitioned with ethyl acetate–water. The organic layer was dried over anhydrous magnesium sulfate, condensed to a small volume, and partitioned with chloroform–water. The organic layer was dried and evaporated to a small volume. This solution was added dropwise to 200 ml of vigorously stirred hexane. The resulting precipitate was filtered and dried to give 20 mg of VII. The aqueous layer was held at room temperature overnight, and the crystals were collected and dried *in vacuo* to give 40 mg of VII, mp 160–161.5°. The total yield of VII was 60 mg (35%).

Anal.—Calc. for $C_{13}H_{18}N_4O_4Se \cdot H_2O$: C, 39.91; H, 5.15; N, 14.31. Found: C, 39.60; H, 4.88; N, 13.99.

If the reaction was run in aqueous solution at room temperature overnight, the compound isolated was mostly starting material as indicated by UV and TLC.

6-(*n*-Butylseleno)-9-(β-D-arabinofuranosyl)purine (VIII)—To a solution of 288 mg (0.82 mmole) of IV in 2.2 ml of 0.4 *N* NaOH, 120 mg (0.85 mmole) of *n*-butyl bromide was added. The solution was stirred at room temperature overnight and then continuously extracted with ether to give 110 mg (34%) of VIII. The analytical sample was recrystallized from water, mp 142–144°.

Anal.—Calc. for $C_{14}H_{20}N_4O_4Se$: C, 43.42; H, 5.31; N, 14.46. Found: C, 43.02; H, 5.24; N, 14.37.

6-(Benzylseleno)-9-(β-D-arabinofuranosyl)purine (IX)—Compound IX was synthesized in 70% yield from IV and benzyl bromide by the method described for V. The analytical sample was recrystallized from methanol–water, mp 182–184°.

Anal.—Calc. for $C_{17}H_{17}N_4O_4Se \cdot H_2O$: C, 46.48; H, 4.59; N, 12.75. Found: C, 46.48; H, 4.49; N, 12.75.

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)seleno]-9-(β-D-arabinofuranosyl)purine (X)—To a solution of 290 mg (0.82 mmole) of IV in 2.2 ml of 0.4 *N* NaOH, 130 mg (0.81 mmole) of 5-chloro-1-methyl-4-nitroimidazole in 10 ml of methanol was added. The solution was stirred at room temperature for 7 hr and evaporated to dryness, and then 3 ml of water was added. The solid was collected, washed with water, and dried to give 250 mg (64%) of X. The analytical sample was recrystallized twice from water, mp 179–180.5°.

Anal.—Calc. for $C_{14}H_{15}N_7O_6Se \cdot H_2O$: C, 35.46; H, 3.61; N, 20.67. Found: C, 35.89; H, 3.32; N, 20.73.

6-(Cyclopentylseleno)-9-(β-D-arabinofuranosyl)purine (XI)—Compound XI was synthesized in 20% yield from IV and cyclopentyl bromide by the method described for V except that the reaction mixture was stirred at room temperature for 6 days. The analytical sample was recrystallized from methanol–water, mp 96–99°.

Anal.—Calc. for $C_{15}H_{20}N_4O_4Se \cdot H_2O$: C, 43.17; H, 5.31; N, 13.42. Found: C, 43.31; H, 5.27; N, 13.47.

6-[(3-Methyl-2-butenyl)seleno]-9-(β-D-arabinofuranosyl)-purine (XII)—Compound XII was synthesized in 25% yield from IV and 3,3-dimethylallyl bromide by the method described for VII. The analytical sample was recrystallized from methanol–water, mp 113–116° dec.

Anal.—Calc. for $C_{15}H_{20}N_4O_4Se$: C, 45.12; H, 5.05; N, 14.02. Found: C, 44.82; H, 5.20; N, 13.79.

Table II—Inhibition of Reproduction of L-5178Y by 6-Selenopurine Arabinoside and Related Compounds[a]

Compound	Concentration Causing 50% Inhibition	Concentration Causing <50% Inhibition: Concentration	Concentration Causing <50% Inhibition: % Inhibition
6-Thioguanosine	5.6×10^{-6} *M*		
IV	6.6×10^{-5} *M*		
X	1.9×10^{-5} *M*		
V	—[b]	10^{-5} *M*	17
VI	—[b]	10^{-4} *M*	10
VII	—[b]	10^{-5} *M*	10
VIII	—[b]	10^{-4} *M*	24
IX	—[b]	10^{-5} *M*	12
XI	—[b]	10^{-4} *M*	31
XII	—[b]	10^{-4} *M*	45

[a] The leukemic cells L-5178Y were grown from an inoculum of 4×10^3 cells/ml for 72 hr in the presence of different levels (10^{-4}–10^{-7} *M*) of 6-selenopurine arabinoside and its derivatives. The final number of cells were determined in a Coulter particle counter, model B, and the 50% inhibition of each drug was determined as the amount of drug required to cause a 50% decrease in the number of doublings undergone by the cell population when compared with the untreated controls. [b] Compounds V–IX, XI, and XII caused less than 50% inhibition at the highest concentration (10^{-4} *M*) studied.

6-*p*-Nitrobenzylseleno-9-(β-D-arabinofuranosyl)purine (XIII)—To a solution of 144 mg (0.41 mmole) of IV in 1.1 ml of 0.4 *N* NaOH, 94 mg (0.44 mmole) of *p*-nitrobenzyl bromide in 5 ml of methanol was added. The solution was stirred at room temperature for 4 hr. The mixture was evaporated to dryness, and the residue was washed with 50 ml of ether and then with 7 ml of water. The solid was recrystallized from methanol–water and dried *in vacuo* to give 115 mg (60%) of XIII, mp 118–120°.

Anal.—Calc. for $C_{17}H_{17}N_5O_6Se$: C, 43.79; H, 3.68; N, 15.01. Found: C, 43.42; H, 3.72; N, 15.26.

2-Amino-1-methyl-6-(methylthio)purine (XIV)—To a solution of 365 mg (2 mmoles) of 1-methyl-6-thioguanine in 5 ml of 0.4 *N* NaOH, 1.4 ml of methyl iodide was added. The mixture was stirred at room temperature for 30 min. The white precipitate was filtered by suction, washed with water, and dried *in vacuo* to give 290 mg (66%) of XIV, mp 242–245°.

Anal.—Calc. for $C_7H_9N_5S \cdot 1.4H_2O$: C, 38.14; H, 5.40; N, 31.77. Found: C, 37.79; H, 5.24; N, 32.19.

When XIV was heated at 140° *in vacuo*, it dehydrated to give $C_7H_9N_5S$.

Anal.—Calc. for $C_7H_9N_5S$: C, 43.07; H, 4.65; N, 35.86. Found: C, 43.34; H, 4.65; N, 35.73.

1-Methyl-6-selenoguanine (XX)—*Method I*—A mixture of 60 mg (0.27 mmole) of XIV, 300 mg (3.0 mmoles) of potassium bicarbonate, and 0.1 ml (2.6 mmoles) of hydrogen selenide in 3 ml of water was kept at 105° overnight. The solution was cooled to room temperature. The solid was filtered, washed with water, dissolved

Table III—Inhibition of L-5178Y Cells by 6-Selenopurine Arabinoside and Related Compounds[a]

Compound	Control	Survival, % at 1×10^{-7} *M*	at 1×10^{-6} *M*	at 1×10^{-5} *M*	at 1×10^{-4} *M*
IV	100	87 ± 4.08	85 ± 9.98	69 ± 2.53	68 ± 2.08
V	100	87 ± 3.74	83 ± 1.89	79 ± 5.72	73 ± 5.74
VI	100	89 ± 3.56	82 ± 1.89	75 ± 2.06	70 ± 2.63
VII	100	92 ± 7.81	82 ± 6.35	78 ± 4.04	70 ± 2.0
IX	100	92 ± 1.26	82 ± 6.85	75 ± 0.96	63 ± 1.41
X	100	87 ± 8.89	86 ± 3.61	78 ± 3.79	67 ± 1.15

[a] The L-5178Y cells (2×10^5/ml) in the exponential phase of growth were incubated with 6-selenopurine arabinoside and related compounds singly (10^{-4}–10^{-7} *M*) for 2 hr. Cell viability was determined by the dilute agar colony method. Each observation represents the mean value of three experiments with four replicates per experiment. A minimum of 200 colonies was counted for each group. The cloning efficiency of the untreated cells was approximately 75%. All values were normalized to 100%.

Table IV—Inhibition of L-5178Y Cells by X at Different Time Intervals[a]

Concentration, *M*	Survival, %				
	1 hr	2 hr	3 hr	4 hr	5 hr
0	100	100	100	100	100
1×10^{-5}	98 ± 3.9	80.2 ± 2.39	71 ± 6.69	61 ± 3.67	49 ± 1
1×10^{-4}	81 ± 5.36	60.4 ± 2.7	45 ± 5.24	31.2 ± 5.22	18.5 ± 0.71

[a]The L-5178Y cells (2×10^5/ml) in the exponential phase of growth were incubated with X at different time intervals. Cell viability was determined by the dilute agar colony method. Each observation represents the mean value of three experiments with four replicates per experiment. A minimum of 200 colonies was counted for each group. The cloning efficiency of the untreated cells was approximately 75%. All values were normalized to 100%.

in 5 ml of 3% Na_2CO_3 solution, and then acidified with acetic acid to pH 4. The precipitate was collected, washed with cold water, and dried *in vacuo* to give 42 mg (66%) of XX, mp 275–278° dec.

Anal.—Calc. for $C_6H_7N_5Se \cdot 1.1H_2O$: C, 29.07; H, 3.74; N, 28.24. Found: C, 28.81; H, 3.85; N, 28.44.

Method II—A suspension of 93 mg (0.46 mmole) of XV (31) and 61 mg (0.5 mmole) of selenourea in 5 ml of methanol was stirred at room temperature for 1 hr. The yellow precipitate was filtered by suction, washed with 5 ml of methanol, and dried *in vacuo* to give 101 mg (89%) of yellow solid, which was identified as XX by TLC and UV.

2-Amino-7-methyl-6-selenoxo-9-(β-D-ribofuranosyl)purine (7-Methyl-6-selenoguanosine) (XXI)—*Method I*—A solution of 1 g (3.02 mmoles) of 2-amino-6-chloropurine riboside and 1 g (70.4 mmoles) of methyl iodide in 6 ml of dimethylformamide was stirred at room temperature for 40 hr. Then 0.8 g (6.5 mmoles) of selenourea was added, and the solution was stirred at room temperature for an additional 2 hr. At the end of the reaction, the solution was neutralized with methanolic ammonia and diluted with 120 ml of acetone. The precipitate was collected, recrystallized from methanol, and dried *in vacuo* to give 50 mg (6%) of XXI, mp 175° dec.

Anal.—Calc. for $C_{11}H_{15}N_5O_4Se$: C, 36.68; H, 4.20; N, 19.43. Found: C, 36.39; H, 4.14; N, 19.40.

Method II—A solution of 500 mg of XVI in 2.5 ml of dimethylformamide was added to a solution of 50 mg of sodium and 0.5 ml of hydrogen selenide in 5 ml of methanol. The solution was stirred at room temperature for 1 hr and then neutralized with methanolic ammonia. The yellow precipitate was collected and recrystallized from methanol to give 50 mg (10%) of yellow solid, which was verified by UV and TLC to be identical with XXI prepared by Method I.

6-Selenoxo-9-(β-D-ribofuranosyl)purine (XXII)—A suspension of 100 mg (0.36 mmole) of XVII and 45 mg (0.36 mmole) of selenourea in 5 ml of methanol was refluxed for 20 min. The yellow solution was filtered, cooled in an ice bath, and then poured into 50 ml of ether. The precipitate was held in a refrigerator overnight, filtered, and dried *in vacuo* to give 60 mg (50%) of yellow solid, which was identified as XXII (10) by UV and TLC.

2-Amino-6-selenoxo-9-(β-D-ribofuranosyl)purine (6-Selenoguanosine) (XXIII)—A suspension of 200 mg (0.61 mmole) of XVIII and 90 mg (0.72 mmole) of selenourea in 10 ml of methanol was refluxed for 20 min. The solution was filtered, cooled in an ice bath, and poured into 100 ml of ether. The precipitate was filtered and dried *in vacuo* to give 160 mg (76%) of yellow compound, which was identified as XXIII (13, 14) by UV and TLC.

2-Amino-6-selenopurine (6-Selenoguanine) (XXIV)—A suspension of 170 mg (1 mmole) of XIX and 123 mg (1 mmole) of selenourea in 5 ml of methanol was refluxed for 30 min. The yellow precipitate was cooled, filtered, and dried *in vacuo* to give 210 mg (98%) of a yellow solid, which was identified as XXIV (9) by UV and TLC.

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