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Synthesis and Biological Activity of Some 8-Substituted Selenoguanosine Cyclic 3',5'-Phosphates and Related Compounds

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8-Bromoguanosine cyclic 3',5'-monophosphate, 8-bromoguanosine 5'-monophosphate, and 8-bromoguanosine served as intermediates for the chemical synthesis of a series of 8-substituted seleno cyclic nucleotides, nucleotides, and their nucleosides. Selenourea was found to be a useful reagent in synthesizing these seleno-substituted nucleoside, nucleotide, and cyclic nucleotide. A nucleic acid analyzer was used to study the hydrolysis of these cyclic nucleotides by phosphodiesterase. It was found that all of the 8-substituted selenoguanosine cyclic 3',5'-phosphates synthesized, except 8-MeSe-cGMP, were resistant to hydrolyze by phosphodiesterase. These 8-substituted seleno cyclic GMP derivatives showed some antitumor activities against murine leukemic cells (L5178Y) in vitro and in vivo.

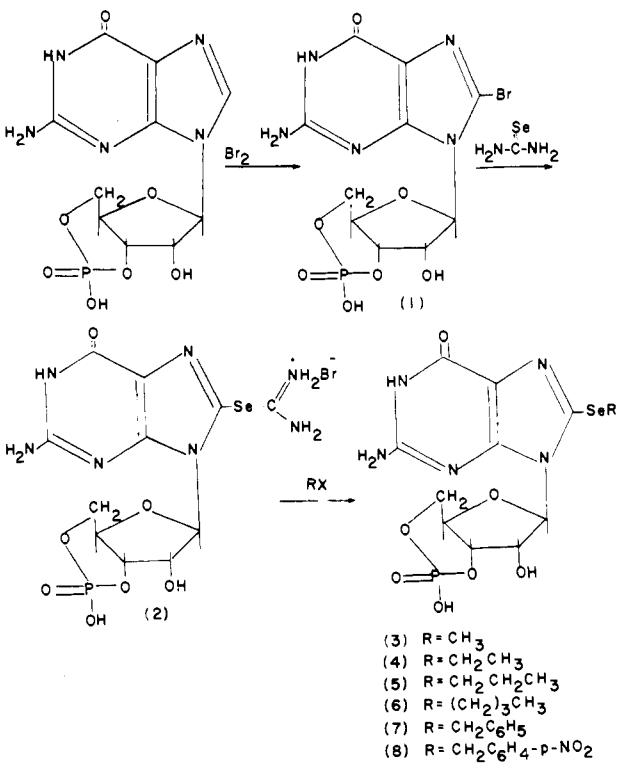
Guanosine cyclic 3',5'-phosphate (cGMP), like cAMP, has been well established as a mediator of many hormonal effects in biological regulating processes.[1] George et al.[2] first found that perfusion of acetylcholine resulted in elevation of cGMP concentration in the rat heart. Subsequently, a number of investigators have found the same results in calf heart,[3] rabbit cerebral cortex,[3] mouse cerebellum,[4] and human lymphocytes.[5] Goldberg[6] also found that the administration of oxytocin or serotonin promoted cGMP accumulation 2.5- and 3-fold in uteri (in vitro) from diethylstilbestrol-treated rats while no change in cAMP levels; clonal proliferation of lymphocytes resulted in 10–50-fold increase in cGMP levels, while cAMP levels were unaffected.[7] Recently, Miller et al.[8] have synthesized a number of 8-substituted derivatives of cGMP, cIMP, and cXMP. It was found that 8-bromo, 8-hydroxy, 8-methylthio, 8-benzylthio, and 8-*p*-chlorophenylthio analogs were more effective than cGMP in activating lobster muscle cGMP-dependent protein kinase and retained their specificity for this kinase.[8] In continuation of our interest in synthesized cyclic nucleotide analogs and study of their biological activity,[9] we have synthesized a series of 8-seleno-cGMP analogs.

Direct bromination of cGMP gave 8-bromo-cGMP (1)[8,10] in good yield. Treatment of the free acid of 8-bromo-cGMP with selenourea in refluxing methanol gave cGMP 8-isoselenouronium hydrobromide 2 as an intermediate.

Alkylation of compound 2 in situ with methyl iodide, ethyl bromide, *n*-propyl bromide, *n*-butyl bromide, benzyl bromide, and *p*-nitrobenzyl bromide yielded 8-methylseleno- (3), 8-ethylseleno- (4), 8-*n*-propylseleno- (5), 8-*n*-butylseleno- (6), 8-benzylseleno- (7), and 8-*p*-nitrobenzylseleno-cGMP (8), respectively (Scheme I). The 8-substituted guanosine cyclic 3',5'-phosphates were readily purified by crystallization from water at pH 2 with or without the aid of methanol or from preparative Avicel plates (see the Experimental Section). The physical properties of the nucleotides are shown in Table I. A comparison of the ultraviolet spectra of these 8-substituted seleno cyclic nucleotides with known 8-substituted thio analogs[8] confirmed the position of substitution. Furthermore, the cyclic structures of these nucleotides were verified by enzymatic studies (see later).

For the identification of the cyclic structure of 8-substituted seleno cyclic nucleotides several 8-substituted seleno-

Scheme I



guanosine 5'-monophosphates and selenoguanosines were synthesized. Bromination of 5'-GMP gave 8-bromo-5'-GMP (9) as a sodium salt. Treatment of the free acid of 8-bromo-5'-GMP with selenourea in refluxing methanol gave 5'-GMP 8-isoselenouronium hydrobromide (10) as an intermediate. Alkylation of compound 10 in situ with methyl iodide yielded 8-methylseleno-GMP (11). Treatment of 8-bromoguanosine with selenourea in refluxing ethanol gave 8-selenoguanosine (12). Likewise, treatment of 8-bromo-2',3',5'-tri-*O*-acetylguanosine with selenourea in refluxing ethanol gave 8-seleno-2',3',5'-tri-*O*-acetylguanosine (13) as an intermediate. Alkylation of either compound 12 or 13 with alkyl halides gave the corresponding alkylated nu-

Table I. Physical Properties of the 8-Substituted Seleno Nucleotides and Related Compounds

Compound	λ_{max}, mμ (ε × 10^{-3}) pH 1	pH 11	H_2O	R_f[a]
cGMP				0.52
8-Br-cGMP (1)	264	273	264	0.32
8-Isoselenouronium cGMP HBr (2)	245 (12.6), 285 (14.3), 315 (13.2)	295 (22.8)	250 (21.2), 282 (15.7), 315 (11.5)	0.61
8-MeSe-cGMP (3)	275 (15.9), 295 (sh)	217 (17.3), 290 (12.2)	278 (14.6)	0.40
8-EtSe-cGMP (4)	273 (13.0), 300 (sh)	292 (14.5)	280 (16.7)	0.45
8-*n*-PrSe-cGMP (5)	272 (12.1), 300 (sh)	217 (17.1), 291 (11.2)	278 (13.9)	0.47
8-*n*-BuSe-cGMP (6)	273 (11.6), 300 (sh)	293 (16.1)	280 (13.3)	0.35
8-BzlSe-cGMP (7)	270 (15.3)	270 (9.3), 300 (10.8)	272 (15.7)	0.17
8-*p*-NO_2BzlSe-cGMP (8)	275 (21.8)	220 (23.5), 285 (18.1)	275 (21.8)	0.12
8-Br-GMP (9)	264	275	261	0.21
8-Isoselenouronium GMP HBr (10)	245,[b] 285, 315	295 (21.5)	250 (12.6), 282 (11.6), 315 (10.5)	0.61
8-MeSe-GMP (11)	272 (13.4), 295 (sh)	289 (12.2)	278 (13.2)	0.27
8-Se-GR (12)	287 (17.7), 315 (19.0)	295 (21.7)	287 (14.0), 315 (15.1)	0.30
8-MeSe-GR (14)	274 (15.3)	287 (13.5)	276 (16.8)	0.40
8-EtSe-GR (15)	275 (17.8)	290 (15.5)	278 (17.5)	0.46
8-*n*-PrSe-GR (16)	272 (13.6)	217 (21.2), 290 (13.3)	278 (15.7)	0.47
8-*n*-BuSe-GR (17)	278 (16.2)	292 (16.8)	278 (15.8)	0.36
8-MNISe-GR (18)	275 (19.6)	292 (18.1)	275 (20.0)	0.47
8-BzlSe-GR (19)	267 (11.5), 300 (8.5)	268 (10.5), 297 (11.5)	267 (16.2)	0.28
8-*p*-NO_2BzlSe-GR (20)	275 (27.5)	285 (21.6)	277 (27.6)	0.22

[a] Thin-layer chromatography was run on a polygram CEL 300 PEI and developed with 1 *M* LiCl. [b] Compound 10 only slightly dissolved in pH 1.

cleosides 14–20, respectively. The physical properties of these nucleotides and nucleosides are shown in Table I.

It is interesting to note that 8-seleno-cGMP and 8-seleno-guanosine could not be synthesized from the corresponding bromo compounds with sodium hydrogen selenide, which is in contrast to 8-seleno-cAMP.[9] Furthermore, 8-bromoadenosine reacts with selenourea to give high yield of 8-selenoadenosine (21).[9] Therefore, selenourea is a useful reagent in synthesizing seleno heterocyclic compounds from corresponding halo compounds.[11,12]

When each of the 8-substituted seleno cyclic nucleotides was incubated with cyclic 3′,5′-nucleotide phosphodiesterase in Tris buffer (pH 7.5), the corresponding 5′-nucleotide (if any) was obtained. When the enzymatically prepared 8-substituted seleno 5′-nucleotides were incubated with 5′-nucleotidase, partially purified from snake venom (Sigma product), the corresponding nucleosides were formed. The nucleotides and nucleosides released after phosphodiesterase and 5′-nucleotidase treatments migrated on PEI-cellulose (1 *M* LiCl) and high-pressure liquid chromatography identically with those of authentic 8-substituted selenoguanosine 5′-monophosphates and selenoguanosines (see Tables I and II). For example, Figure 1 shows the behavior of 8-MeSe-cGMP on high-pressure liquid chromatography on

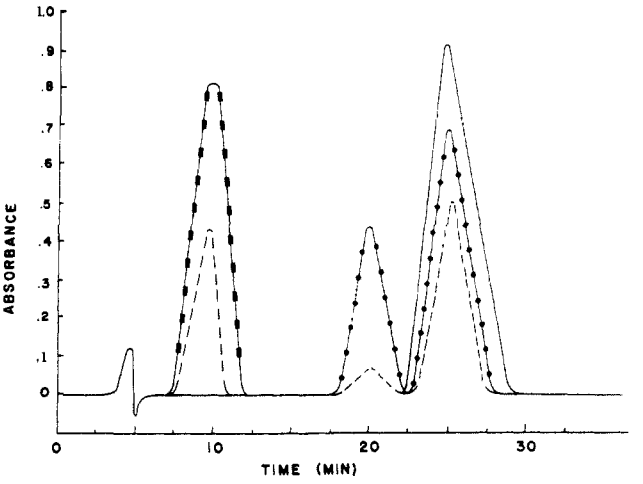


Figure 1. High-pressure liquid chromatographs of 8-MeSe-cGMP, 8-MeSe-guanosine, phosphodiesterase-treated 8-MeSe-cGMP, and 5′-nucleotidase-treated 8-MeSe-GMP: (——) 8-MeSe-cGMP untreated; (■–■–■) authentic 8-MeSe-guanosine; (- - - -) the reaction products of 8-MeSe-GMP after treatment with 5′-nucleotidase; and (●–●–●) the reaction products of 8-MeSe-cGMP after treatment with phosphodiesterase.

a Varian LCS-1000 under the conditions described by Brown.[13] A single peak with retention time of 30 min was obtained. When 8-MeSe-cGMP was treated with phosphodiesterase and chromatographed on the LCS-1000, a new peak with retention of 20 min was observed. Subsequently, when enzymatically prepared 8-MeSe-GMP was treated with 5'-nucleotidase, a new peak with retention time of 10 min was observed. The extent of the hydrolysis of these seleno cyclic nucleotides by phosphodiesterase was calculated from the peak area and shown in Table II.

For the comparison of the relative rates of hydrolysis of these 8-substituted seleno cAMP and cGMP toward phosphodiesterase, the relative rates of hydrolysis of 8-substituted seleno cAMP were also included in Table II. Table II shows that modification of purine nucleus substituents in the 8 position considerably increases resistance to hydrolysis which is in agreement with those of Miller et al.[8] It also shows that lengthening the side chain increases resistance to hydrolysis. Furthermore, it indicates cGMP derivatives are more resistant to phosphodiesterase hydrolysis than cAMP derivatives.

Experimental Section†

8-Bromoguanosine Cyclic 3',5'-Phosphate (1). 8-Bromoguanosine cyclic 3',5'-phosphate was prepared by the method of Mian et al.[10] with guanosine cyclic 3',5'-phosphate (4.39 g, 10 mmol) and Br_2 (2 g, 25 mmol) in aqueous solution and then neutralized with 2 *N* NaOH in 94% yield. The compound was isolated as sodium salt. The free acid 1 was obtained by passing an aqueous solution of the Na^+ salt of 1 through a Dowex 50 X12 (H^+) column.

Guanosine Cyclic 3',5'-Phosphate 8-Isoselenouronium Hydrobromide (2). The solution of 760 mg (1.81 mmol) of 8-bromoguanosine cyclic 3',5'-phosphate (1) and 600 mg (5.50 mmol) of selenourea[14] in 60 ml of absolute MeOH was refluxed for 20 min. The mixture was filtered and the filtrate was cooled in an ice bath. The yellow precipitate was filtered, washed with MeOH, and dried in vacuo to give 670 mg (63.5%) of **2**. Anal. ($C_{11}H_{15}N_7O_7PSeBr\cdot 2H_2O$) C, H, N, P.

8-Methylselenoguanosine Cyclic 3',5'-Phosphate (3). The solution of 370 mg (0.87 mmol) of 8-bromo-cGMP and 290 mg (2.64 mmol) of selenourea in 40 ml of absolute MeOH was refluxed for 8 min. The solution was cooled and then 60 mg (2.52 mmol) of Na in 5 ml of absolute MeOH was added. Into the red solution, 0.2 ml (2.8 mmol) of MeI in 2 ml of absolute MeOH was added and the solution was stirred at room temperature overnight. After evaporation of the solvent, the residue was suspended in 10 ml of EtOH, filtered, and then dissolved in 1 ml of H_2O. The solution was acidified with concentrated HCl to pH 2. MeOH was added to the solution which was then cooled to cause crystallization of 190 mg (49%) of **3**. Anal. ($C_{11}H_{13}N_5O_7PSe\cdot 1.25HCl\cdot 1.25H_2O$) C, H, N, Cl.

8-Ethylselenoguanosine Cyclic 3',5'-Phosphate (4). The solution of 370 mg (0.87 mmol) of 8-bromo-cGMP and 350 mg (2.85 mmol) of selenourea in 40 ml of absolute MeOH was refluxed for 40 min. The solution was cooled in an ice bath and then 60 mg (2.52 mmol) of Na in 10 ml of absolute MeOH was added. Into the red solution, 0.8 ml (10.7 mmol) of EtBr was added and the solution was stirred at room temperature for 2 days. The solvent was evaporated; the residue was dissolved in H_2O and passed through a Dowex 50 (H^+) column (2.5 × 10 cm). The compound was eluted with H_2O, evaporated, and then purified twice with preparative Avicel plates (1000 μ). The plates were developed with 94% aqueous *n*-BuOH–44% aqueous propionic acid (v/v 1:1). The spots with R_f 0.43 were scraped, dissolved in MeOH, filtered, and evaporated to dryness to give 150 mg (35.3%) of **4**. Anal. ($C_{12}H_{16}N_5O_7PSe\cdot 2H_2O$) C, H, N, P.

†Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Ultraviolet spectra were determined on a Perkin-Elmer Model 402 spectrophotometer. Elemental analyses were performed by Midwest Microlab, Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values. Thin-layer chromatography was run on a polygram CEL 300 PEI and developed with 1 *M* LiCl. High-pressure liquid chromatography was run on a Varian LCS-1000 nucleic acid analyzer. Evaporations were accomplished using a Buchler flash evaporator under reduced pressure with a bath temperature of 40°.

Table II. Action of Cyclic 3',5'-Nucleotide Phosphodiesterase on 8-Substituted Seleno Cyclic Nucleotides

Compound	Retention time, min	Rel rate[c] of hydrolysis
cGMP	18[a]	100
8-Br-cGMP (1)	29[a]	0
8-Isoselenouronium cGMP HBr (2)	46[a]	0
8-MeSe-cGMP (3)	30[a]	19
8-EtSe-cGMP (4)	23[a]	0
8-*n*-PrSe-cGMP (5)	32[a]	0
8-*n*-BuSe-cGMP (6)	43[a]	0
8-BzlSe-cGMP (7)	55[a]	0
8-*p*-NO_2BzlSe-cGMP (8)	66[a]	0
GMP	15[a]	
8-Br-GMP (9)	27[a]	
8-Isoselenouronium GMP HBr (10)	40[a]	
8-MeSe-GMP (11)	20[a]	
8-MeSe-GR (14)	10[a]	
cAMP	25[b]	100
8-Br-cAMP	43[b]	70
8-Se-cAMP	106[b]	0
8-MeSe-cAMP	48[b]	31
8-EtSe-cAMP	46[b]	19
8-BzlSe-cAMP	104[b]	6

[a]The buffer solutions are 0.002 *M* KH_2PO_4 and 0.5 *M* KH_2PO_4 in 1.0 *M* KCl. [b]The buffer solutions are 0.015 *M* KH_2PO_4 and 0.25 *M* KH_2PO_4 in 2.2 *M* KCl. [c]Relative rates of hydrolysis are determined by high-pressure liquid chromatography.

8-*n*-Propylselenoguanosine Cyclic 3',5'-Phosphate (5). The solution of 740 mg (1.74 mmol) of 8-bromo-cGMP and 580 mg (5.28 mmol) of selenourea in 40 ml of absolute MeOH was refluxed for 10 min. The yellow solution was cooled and then 120 mg (5.04 mmol) of Na in 5 ml of absolute MeOH followed by 0.6 ml of *n*-propyl bromide was added. The solution was stirred at room temperature overnight. The mixture was filtered and the filtrate was evaporated to dryness. The residue was suspended in 50 ml of EtOH, filtered, and then washed with 50 ml of ethyl ether. The solid was dissolved in 2 ml of H_2O and acidified with concentrated HCl to pH 2 and then 1 ml of MeOH was added. The solution was held in refrigerator overnight. The solution was evaporated to dryness, dissolved in 5 ml of H_2O, and passed through a Dowex 50 (H^+) column (2.5 × 10 cm). The compound was eluted with H_2O. The appropriate fractions were collected, evaporated, and dried in vacuo to give 12 mg of **5**. The mixture was purified with Avicel plates as described for 4 to give another 170 mg of **5**; the total yield of **5** is 182 mg (22.4%). Anal. ($C_{13}H_{18}N_5O_7PSe$) C, H, N, P.

8-*n*-Butylselenoguanosine Cyclic 3',5'-Phosphate (6). The solution of 200 mg (0.47 mmol) of 8-bromo-cGMP and 170 mg (1.38 mmol) of selenourea is 30 ml of absolute MeOH was refluxed for 15 min. The yellow solution was cooled and then 60 mg (2.52 mmol) of Na in 10 ml of absolute MeOH followed by 1 ml of *n*-butyl bromide was added. The solution was stirred at room temperature overnight. The solution was evaporated to dryness. The residue was washed with 50 ml of ethyl ether. The solid was dissolved in H_2O and passed through a Dowex 50 (H^+) column (2.5 × 10 cm). The compound was eluted with H_2O, evaporated, and then purified twice with preparative Avicel plates as described for **4** to give 47 mg (20%) of **6**. Anal. ($C_{14}H_{20}N_5O_7PSe\cdot H_2O$) C, H, N, P.

8-Benzylselenoguanosine Cyclic 3',5'-Phosphate (7). The solution of 370 mg (0.87 mmol) of 8-bromo-cGMP and 290 mg (2.64 mmol) of selenourea in 40 ml of absolute MeOH was refluxed for 8 min. The solution was cooled and then 60 mg (2.52 mmol) of Na in 5 ml of absolute MeOH was added. Into the red solution 0.31 ml (2.8 mmol) of BzlBr was added and the solution was stirred at room temperature overnight. The solvent was evaporated to dryness and the residue was suspended in 50 ml of EtOH and filtered. The solid was dissolved in hot H_2O, cooled, and then acidified with

Table III. Inhibition of L5178Y Cells by 8-Substituted Derivatives of Selenoguanosine Cyclic 3′,5′-Phosphate[a]

Compound	Control	% survival			
		1×10^{-7} M	1×10^{-6} M	1×10^{-5} M	1×10^{-4} M
cGMP	100	85 ± 9.2	76 ± 6.4	71.5 ± 0.71	74 ± 4.2
8-Br-cGMP (1)	100	93 ± 4.6	68 ± 3.28	72 ± 3.54	73 ± 4.6
8-Isoselenouronium cGMP HBr (2)	100	68 ± 8.49	70 ± 1.42	51 ± 6.72	3 ± 0.52
8-MeSe-cGMP (3)	100	97 ± 1.66	89 ± 4.72	75 ± 1.41	71 ± 0.94
8-EtSe-cGMP (4)	100	78 ± 4.95	79 ± 7.42	74 ± 5.66	50 ± 6.51
8-*n*-PrSe-cGMP (5)	100	97 ± 5.8	88 ± 6.6	80 ± 2.1	72 ± 6.1
8-*n*-BuSe-cGMP (6)	100	96 ± 3.18	67 ± 10.67	77.3 ± 3.89	69 ± 4.9
8-BzlSe-cGMP (7)	100	97 ± 1.91	93.3 ± 3.6	79 ± 1.4	67 ± 1.09
8-*p*-NO_2BzlSe-cGMP (8)	100	100	100	65 ± 5.3	56 ± 3.18

[a] L5178Y cells (2×10^5/ml) in the exponential phase of growth were incubated with 8-substituted selenoguanosine cyclic 3′,5′-phosphate related compounds singly (10^{-4}–10^{-7} M) for a period of 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%.

concentrated HCl to pH 2. The precipitate was filtered, washed with ice-cold H_2O, and dried in vacuo to give 220 mg (43%) of **7**. Anal. ($C_{17}H_{18}N_5O_7PSe \cdot 2HCl$) C, H, N, Cl.

8-*p*-Nitrobenzylselenoguanosine Cyclic 3′,5′-Phosphate (8). The solution of 185 mg (0.44 mmol) of 8-bromo-cGMP and 145 mg (1.32 mmol) of selenourea in 20 ml of absolute MeOH was refluxed for 10 min. The solution was cooled and then 30 mg (1.26 mmol) of Na in 5 ml of absolute MeOH was added. Into the red solution 302 mg (1.4 mmol) of *p*-NO_2BzlBr in 15 ml of absolute MeOH was added. The solution was stirred at room temperature overnight. The mixture was evaporated to dryness. The residue was dissolved in hot H_2O, cooled, and then acidified with concentrated HCl to pH 2. The suspension was filtered, suspended in 100 ml of Et_2O, and then dried in vacuo to give 113 mg (42%) of **8**. Anal. ($C_{17}H_{17}N_6O_9PSe \cdot 1.5HCl$) C, H, N, Cl.

8-Bromoguanosine 5′-Monophosphate (9). To a suspension of 2.2 g (5 mmol) of GMP in 15 ml of H_2O, 1 g (12.5 mmol) of Br_2 in 5 ml of H_2O was added dropwise. The solution was stirred at room temperature for an additional 2 hr and then extracted with ether (2 × 50 ml). The aqueous layer was decolorized with sodium sulfate and then neutralized with 2 *N* NaOH. Two volumes of EtOH (80 ml) were added. The precipitate was held in freezer overnight, filtered, washed with EtOH and Et_2O, and then dried in vacuo to give 2.30 g (100%) of **9** as the sodium salt. The free acid of **9** was obtained by passing an aqueous solution of the Na^+ salt of **9** through a Dowex 50 X12 (H^+) column.

Guanosine 5′-Monophosphate 8-Isoselenouronium Hydrobromide (10). The solution of 185 mg (0.44 mmol) of 8-Br-GMP and 145 mg (1.18 mmol) of selenourea in 20 ml of MeOH was refluxed for 8 min. The mixture was filtered; the filtrate was cooled in an ice bath. The yellow precipitate was filtered by suction and dried in vacuo to give 160 mg (61.4%) of **10**. Anal. ($C_{11}H_{17}N_7O_8PSeBr \cdot 1.5H_2O$) C, H, N, P.

8-Methylselenoguanosine 5′-Monophosphate (11). The solution of 185 mg (0.44 mmol) of 8-Br-GMP and 145 mg (1.18 mmol) of selenourea in 20 ml of MeOH was refluxed for 8 min. The solution was cooled in ice bath and then 30 mg (1.3 mmol) of Na in 5 ml of MeOH followed by 0.1 ml of MeI was added. The mixture was stirred at room temperature overnight and evaporated to dryness. The residue was suspended in 20 ml of EtOH and filtered. The solid was dissolved in 2 ml of H_2O and filtered and then 4 ml of EtOH was added into the filtrate. The precipitate was filtered by suction, washed with EtOH and Et_2O and dried in vacuo to give 140 mg of **11** as the sodium salt. Anal. ($C_{11}H_{14}N_5O_4PSeNa_2 \cdot 0.75H_2O$) C, H, N, P.

8-Selenoguanosine (12). The suspension of 2 g (5.6 mmol) of 8-bromoguanosine and 40 mg (8 mmol) of selenourea in 120 ml of EtOH was refluxed for 40 min. The mixture was cooled in ice bath and the precipitate was filtered by suction to give 1.64 g of crude **12**. The solid was dissolved in 20 ml of 1 *N* NH_4OH and filtered. The filtrate was acidified with glacial HOAc to pH 4 and cooled in an ice bath. The solid was filtered, washed with cold water, and dried in vacuo to give 1.3 g (65%) of **12**, mp 210° dec. Anal. ($C_{10}H_{13}N_5O_6Se \cdot 2H_2O$) C, H, N.

8-Seleno-2′,3′,5′-tri-*O*-acetylguanosine (13). The mixture of 4 g of 8-bromo-2′,3′,5′-tri-*O*-acetylguanosine and 1 g of selenourea in 100 ml of EtOH was refluxed for 30 min. The solution was filtered. The filtrate was cooled in ice bath. The precipitate was filtered by suction and dried in vacuo to give 2.6 g (65%) of **13**.

8-Methylselenoguanosine (14). The mixture of 578 mg (1.6 mmol) of 8-Se-GR (**12**), 240 mg of K_2CO_3, and 220 mg (2.8 mmol) of dimethyl sulfate in 8 ml of DMF was stirred at 75° for 3 hr. The solution was cooled to room temperature and then poured into 100 ml of acetone. The precipitate was recrystallized twice from H_2O and dried in vacuo to give 130 mg (20%) of **14**, mp 159–161°. Anal. ($C_{11}H_{15}N_5O_5Se \cdot 1.5H_2O$) C, H, N.

8-Ethylselenoguanosine (15). The mixture of 488 mg (1 mmol) of 8-seleno-2′,3′,5′-tri-*O*-acetylguanosine (**13**), 450 mg of Na_2CO_3, and 150 mg of EtBr in 10 ml of H_2O and 40 ml of MeOH was stirred at room temperature overnight. The mixture was filtered by suction. The filtrate was evaporated to dryness. The residue was recrystallized twice from H_2O and dried in vacuo to give 114 mg (26.2%) of **15**, mp 146–147°. Anal. ($C_{12}H_{17}N_5O_5Se \cdot 2.5H_2O$) C, H, N.

8-*n*-Propylselenoguanosine (16). The mixture of 361.2 mg (1 mmol) of 8-Se-GR (**12**), 166 mg of K_2CO_3, and 148 mg (1.2 mmol) of *n*-propyl bromide in 4 ml of DMF was stirred at room temperature for 2 days. The red mixture was poured into 100 ml of acetone. The precipitate was collected, recrystallized from H_2O, and dried in vacuo to give 110 mg (24.5%) of **16**, mp 150–153°. Anal. ($C_{13}H_{19}N_5O_5Se \cdot 5H_2O$) C, H, N.

8-*n*-Butylselenoguanosine (17). Compound **17** was synthesized from **13** and *n*-BuBr in 46.4% yield by the method described for **15**: mp 136–139°. Anal. ($C_{14}H_{21}N_5O_5Se \cdot 2H_2O$) C, H, N.

8-(1-Methyl-4-nitro-1*H*-imidazol-5-yl)selenoguanosine (18). Compound **18** was synthesized from **13** and 1-methyl-4-nitro-5-chloroimidazole in 38.2% yield by the method described for **15**: mp 197–201°. Anal. ($C_{14}H_{16}N_8O_7Se \cdot H_2O$) C, H, N.

8-Benzylselenoguanosine (19). Compound **19** was synthesized from **12** and BzlBr in 18% yield by the method described for **16**: mp 140–143°. Anal. ($C_{17}H_{19}N_5O_5Se \cdot 1.25H_2O$) C, H, N.

8-*p*-Nitrobenzylselenoguanosine (20). The suspension of 488 mg (1 mmol) of **13**, 450 mg of Na_2CO_3, and 250 mg (1.2 mmol) of *p*-nitrobenzyl bromide in 10 ml of H_2O and 40 ml of MeOH was stirred at room temperature overnight. The mixture was filtered by suction. The filtrate was evaporated to dryness. The residue was washed with Et_2O and then recrystallized from H_2O to give 105 mg (20%) of **20**, mp 185° dec. Anal. ($C_{17}H_{18}N_6O_7Se \cdot 1.5H_2O$) C, H, N.

8-Selenoadenosine (21). The suspension of 500 mg (1.4 mmol) of 8-bromoadenosine and 200 mg (1.63 mmol) of selenourea in 30 ml of EtOH was refluxed for 2 hr. The solution was filtered and the filtrate was cooled in an ice bath overnight. The precipitate was filtered by suction and dried in vacuo to give 408 mg (80%) of yellow solid which was identified as **21**[9] by TLC and uv.

Biological Studies. (A) Enzymatic Studies.[‡] Each cyclic

[‡] Cyclic 3′,5′-nucleotide phosphodiesterase (from beef heart) and snake venom were obtained from Sigma Chemical Co., St. Louis, Mo.

Table IV. Animal Antitumor Activity of 8-Substituted Derivatives of Selenoguanosine Cyclic 3',5'-Phosphate[a]

Compound	Control	% survival		
		20 mg/kg	40 mg/kg	100 mg/kg
cGMP	100	85 ± 11.7	77 ± 8.4	Not tested
8-Br-cGMP (1)	100	94 ± 4.6	76 ± 2.12	Not tested
8-Isoselenouronium cGMP HBr (2)	100	2 ± 0.4		
8-MeSe-cGMP (3)	100	63 ± 4.95	57 ± 4.24	38 ± 0.71
8-EtSe-cGMP (4)	100	89 ± 7.42	79 ± 11.67	70 ± 10.62
8-*n*-PrSe-cGMP (5)	100	56 ± 7.78	59 ± 10.96	48 ± 6.01
8-*n*-BuSe-cGMP (6)	100	84 ± 11.67	86 ± 7.6	39 ± 9.19
8-BzlSe-cGMP (7)	100	67 ± 2.12	61 ± 8.3	39 ± 3.89
8-*p*-NO_2BzlSe-cGMP (8)	100	59 ± 9.1	56 ± 11.67	44 ± 4.95

[a]The leukemic cells L5178Y (4×10^5 cells/mouse) were injected ip on day 0. Drugs (20, 40, and 100 mg/kg) were injected on day 5 and incubated for 2 hr. At the end of incubations, 5.0 ml of Fischer's medium containing 10% horse serum (FMS) was injected ip sterily. After mixing 1.0-ml aliquots were withdrawn and transferred to a culture tube containing 5.0 ml of FMS. Cells were centrifuged immediately, washed twice, and resuspended in 5.0 ml of FMS. 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 56%. All values were normalized to 100%.

Table V. Inhibition of Reproduction of L5178Y by 8-Isoselenouronium cGMP HBr and Related Compounds[a]

Compound	Concn (*M*) causing 50% inhibn
6-Thioguanosine	5.6×10^{-6}
8-Isoselenouronium cGMP HBr (2)	7.2×10^{-6}
8-Isoselenouronium GMP HBr (10)	2.4×10^{-5}
8-Se-GR (12)	No inhibn at 10^{-4}

[a]The leukemic cells L5178Y were grown from an inoculum of 4×10^3 cells per ml for a period of 72 hr in the presence of different levels (10^{-4}–10^{-7} *M*) of 8-isoselenouronium cGMP HBr and related compounds. The final number of cells was determined in a Coulter 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 of the cell population undergone when compared with the untreated controls.

3',5'-nucleotide (1 μmol) was added to tubes containing Tris buffer (pH 7.5, 50 μmol), magnesium sulfate hydrate (1 μmol), and cyclic 3',5'-nucleotide phosphodiesterase (0.42 mg) in a final volume of 1 ml. The mixtures were incubated at 30° for 30 min, and the reaction was stopped by boiling in a water bath for 3 min. An aliquot of each incubation mixture was subjected to TLC and to high-pressure liquid chromatography on a Varian LCS-1000 under the conditions described by Brown.[13] Peak areas were determined by multiplying the height of the peak by the width at half-height.

After the release of 5'-nucleotides, 200 μg of 5'-nucleotidase was added to each tube and incubated at 37° for 4 hr. The reaction was stopped by boiling in a water bath for 15 min. The supernatants were subjected to TLC and to high-pressure liquid chromatography. The results are shown in Tables I and II.

(B) **Effects on Cultured Mouse Leukemia Cells.** The 8-substituted selenoguanosine cyclic 3',5'-phosphates were evaluated in two studies for their cytotoxicity using L5178Y murine leukemic cells grown in culture and in animal.

(1) **Short-Term Studies.** (a) In culture studies over a wide range of concentration. L5178Y cells in the experimental phase of growth were treated with the analogs (10^{-4}–10^{-7} *M*) for a period of 2 hr and cell viability was determined by colony formation in dilute agar.[15] As shown in Table III, all 8-selenoguanosine cyclic 3',5'-phosphate analogs show inhibitory activity with a short period of incubation (2 hr). 8-Isoselenouronium cGMP HBr (2) exhibits the most acute cell kill, the percent survival being 70, 51, and 3 at 10^{-6}, 10^{-5}, and 10^{-4} *M*, respectively.

(b) In animal studies for antitumor effects. The overall objective of animal tumor studies is to accurately predict the chemotherapeutic effectiveness of novel regimens in human patients and provide information relevant to pharmacology and toxicology and the relationship between chemical structures and molecular mode of action of novel chemotherapeutic anticancer regimens. Investigations in animal species are essential in the evaluation of novel agents, for example, host activation (e.g., cyclophosphamide). Useful anticancer agents in man are uniformly active in a properly selected spectrum of animal systems. In order to evaluate new agents and drug combinations in animals in shorter periods of time, a novel method has been developed by Chu and Fischer.[16] L5178Y murine leukemic cells (4×10^5/BDF, mouse) were injected ip on day 0. Drugs, singly, were injected on day 5 and incubated for the periods of time required. At the end of incubations, 5.0 ml of Fischer's medium containing 10% horse serum (FMS) was injected ip sterily. After mixing, 1.9-ml aliquots were withdrawn and transferred immediately, washed twice, and resuspended in 50 ml of FMS. Cell viability was determined by the dilute agar colony method.

Results in Table IV demonstrate that 8-MeSe-cGMP, 8-*n*-PrSe-cGMP, 8-BzlSe-cGMP, and 8-*p*-NO_2-BzlSe-cGMP exhibit antitumor activity at 20 mg/kg, while 8-Br-cGMP, 8-EtSe-cGMP, and 8-*n*-BuSe-cGMP are less active. The analog 8-isoselenouronium cGMP HBr (2) causes 98% acute cell kill or 2% survival and cells were unable to clone at higher dosages (40 and 100 mg/kg) indicating its potential as an antitumor agent.

(2) **Long-Term Studies.** In culture studies (72 hr) over a wide range of concentration (10^{-4}–10^{-7} *M*). As shown in Table V, 6-thioguanosine, 8-isoselenouronium cGMP HBr (2), and 8-isoselenouronium GMP HBr (10) exhibit 50% inhibition at a concentration of 5.6×10^{-6}, 7.2×10^{-6}, and 2.4×10^{-5} *M*, respectively. Table V also points out that 8-Se-GR (12) displayed no inhibitory activity at 10^{-4} *M*. It is of interest that 8-isoselenouronium cGMP HBr (2) has activity approximately equal to thioguanosine. 8-Isoselenouronium GMP HBr (10) was less active than its corresponding cGMP derivative 2.

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Nucleosides of 2-Azapurines. 7*H*-Imidazo[4,5-*d*]-1,2,3-triazines. 2†

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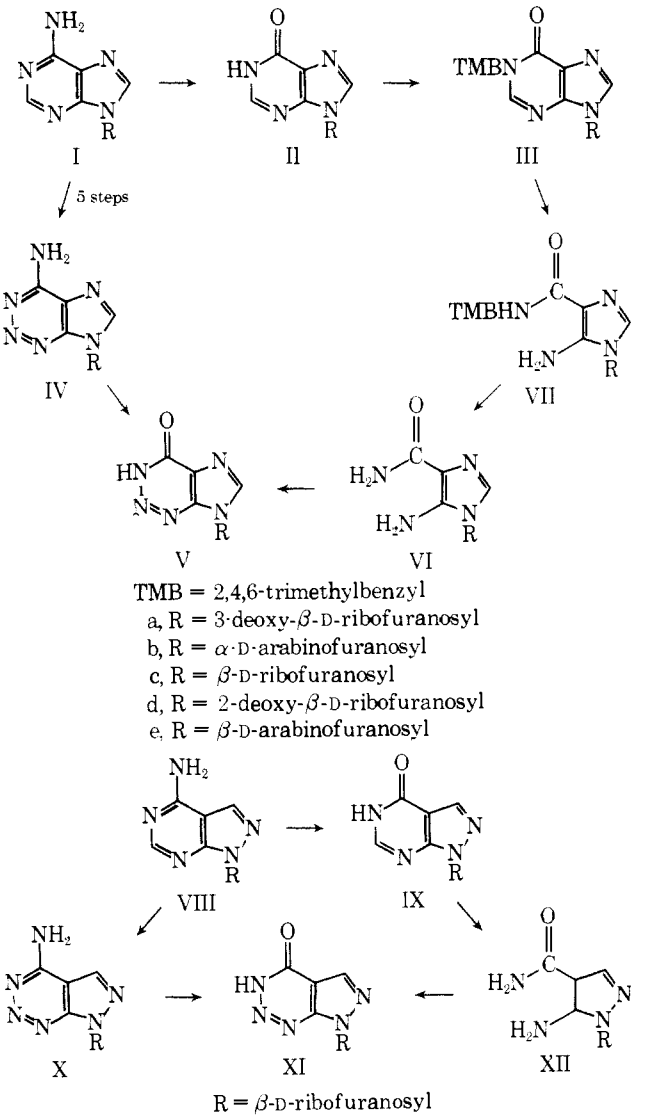
A number of nucleosides of 2-azaadenine (4-amino-7*H*-imidazo[4,5-*d*]-1,2,3-triazine) were prepared by a previously described route, and some of these were deaminated by means of adenosine deaminase. Alternatively, nucleosides of 2-azahypoxanthine (7*H*-imidazo[4,5-*d*]-1,2,3-triazin-4(3*H*)-one) were prepared from hypoxanthine nucleosides by a ring-opening and reclosure sequence. The cytotoxicity of these compounds to human epidermoid carcinoma No. 2 cells in culture and to certain resistant sublines thereof was determined. 2-Azaadenine nucleosides can be metabolized to nucleotides, the cytotoxic agents, by two pathways, but the activity of the 2-azahypoxanthine nucleosides appears to result only from cleavage back to 2-azahypoxanthine.

The biologic activity of 2-azaadenosine[1] led us to prepare other nucleosides of 2-azaadenine and also nucleosides of 2-azahypoxanthine. One of the 2-azaadenine derivatives was 2-azacordycepin (IVa) and another the α-arabinonucleoside (IVb). Interest in the latter nucleoside was based on the biologic activity of the α-arabinofuranosides of adenine[2] and 8-azaadenine.[2,3] These compounds were prepared from the adenine nucleosides by the route previously described: oxide formation at N-1, O-benzylation of the *N*-oxide, ring opening and deformylation with methanolic ammonia, and nitrosative ring closure (Scheme I).[1]

One approach to the synthesis of nucleosides of 2-azahypoxanthine is the deamination of the corresponding nucleosides of 2-azaadenine. Chemical deamination of 2-azaadenosine (IVc) failed, but deamination with adenosine deaminase was rapid and complete (uv and TLC). 2-Azainosine (Vc)[4,5] was isolated through its lead salt. This method was also successful for the conversion of 2-aza-2′-deoxyadenosine (IVd) to 2-aza-2′-deoxyinosine (Vd), but this compound was so sensitive to acidic cleavage that the procedure used for 2-azainosine had to be modified to eliminate the use of acetic acid, and, even so, the yield was low albeit deamination was complete (uv and TLC). Although the deamination of nucleosides of 2-azaadenine is quite a satisfactory procedure for substrates of adenosine deaminase, its usefulness is limited by the procedure required to prepare the 2-azaadenine nucleosides.[1] Alternatively, adenine nucleosides can be deaminated—by adenosine deaminase when feasible and chemically when the nucleosides are not substrates for the enzyme—and the resulting hypoxanthine nucleosides converted to the 2-aza derivatives. Unfortunately, however, the 1-oxide of these hypoxanthine nucleosides cannot be prepared directly,[6] and other 1-substituents have not proven completely satisfactory.[7-9] Alkylation of inosine (IIc) with α-2-chloroisodurene proceeded in good yield to give 1-(2,4,6-trimethylbenzyl)inosine (IIIc), the pyrimidine ring of which was readily cleaved by aque-

Scheme I



TMB = 2,4,6-trimethylbenzyl
a, R = 3-deoxy-β-D-ribofuranosyl
b, R = α-D-arabinofuranosyl
c, R = β-D-ribofuranosyl
d, R = 2-deoxy-β-D-ribofuranosyl
e, R = β-D-arabinofuranosyl

R = β-D-ribofuranosyl

'*Chemical Abstracts*' name: 7*H*-imidazo[4,5-*d*]-1,2,3-triazine. The trivial 2-azapurine names and the purine numbering system have been used throughout to emphasize the relationship of this ring system to the purine ring system.