A Convenient One-Step Synthesis of 6-Selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate and Related Compounds

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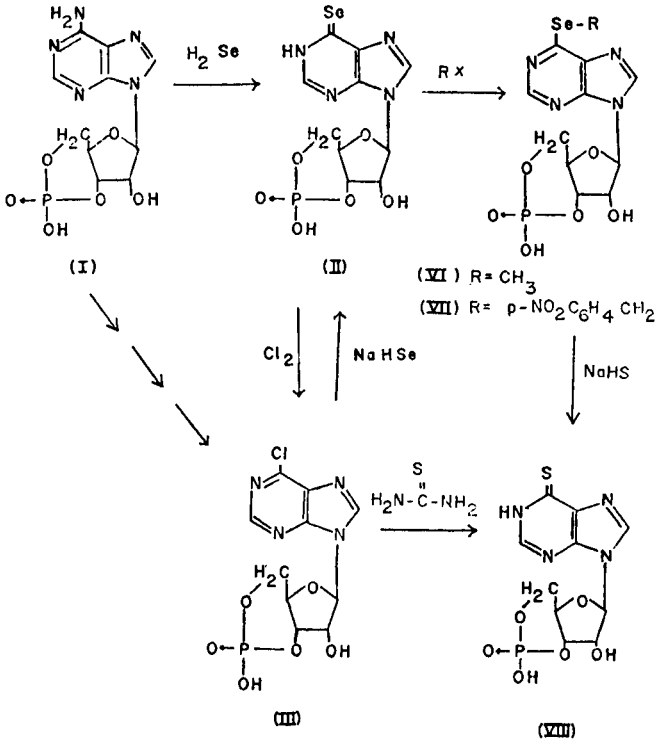
A useful, facile procedure for preparing seleno-heterocyclic compounds is reported. Treatment of cAMP, AMP, adenosine, 2-aminoadenosine, adenine arabinoside and formycin with hydrogen selenide in aqueous pyridine at 65° for 1.5-5 days gave the corresponding seleno compounds in good yield, while these compounds were relatively inert to hydrogen sulfide. A reaction mechanism is proposed.

Adenosine 3′,5′-cyclic phosphate (cAMP) has been indicated as a "second messenger" and mediator of hormone action in a variety of biological systems and reviewed in a number of books and articles (1-5). It stimulates the conversion of inactive glycogen phosphorylase into the active form in liver preparations. It also plays an important role in the regulation of RNA synthesis. Therefore, cAMP may lead the way to the control of diseases related to the disruption of the hormone system of the body (6). However, cAMP is usually quite inert when added to isolated tissues. Thus, a large number of new cAMP analogs have been synthesized with modification of the purine (7-25), carbohydrate (26-34), and phosphate moieties (35-38) with the hope that they may have either a better lipid solubility or a greater resistance to the action of the phosphodiesterase. Some of the analogs were indeed more active than the parent nucleotide in several biological systems. For example, 6-methylthiopurine riboside 3′,5′-cyclic phosphate activated the cAMP-dependent protein kinase while deactivated the cGMP-dependent kinase from lobster muscle (17).

As part of a continuing program to investigate structure-activity relationships in cAMP analogs, we were interested in the effects of replacement of the amino group by a selenium atom in the 6 position of cAMP. Seleno-heterocycles were usually synthesized from the corresponding halo-heterocycles with either sodium hydrogen selenide or selenourea (39). However, the chlorination of the carbonyl function in nucleobases is tedious. Recently we found that a certain number of seleno-heterocycles can be prepared by direct displacement of the amino group of the corresponding heterocycles with hydrogen selenide in aqueous pyridine (40a,b). We now report the full experimental details for the synthesis of 6-selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate and related compounds. In a single, simple chemical reaction, compounds such as cAMP can be converted to the corresponding selenium analog. Furthermore, these selenium analogs can be readily converted to the two most important intermediates in synthetic purine chemistry, *i.e.* 6-chloropurine and 6-methylselenopurine analogs. Thus a variety of 6-substituted purine analogs can be readily prepared by nucleophilic displacement of



SCHEME I

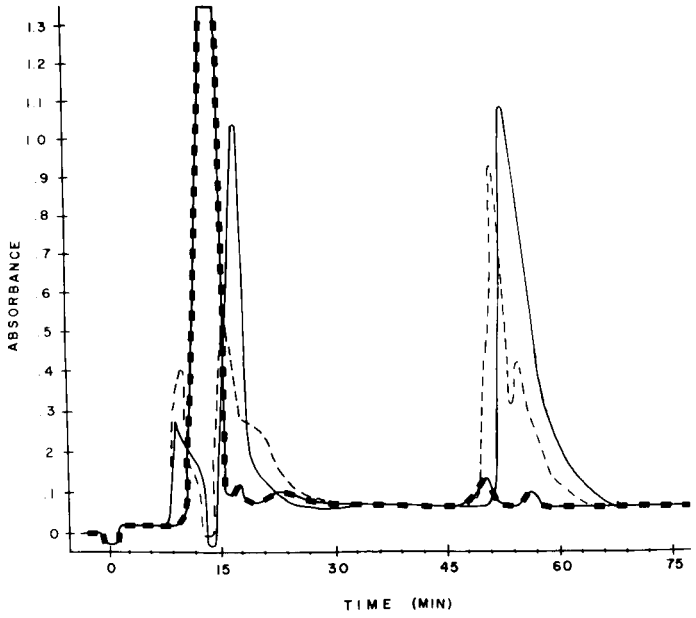


Figure 1. High pressure liquid chromatographs of 6-methyseleno-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (6-MeSe-cIMP), phosphodiesterase treated 6-MeSe-cIMP and 5′-nucleotidase-treated 6-MeSe-IMP. (—) 6-MeSe-cIMP untreated; (- - -) the reaction products of 6-MeSe-cIMP after treatment with phosphodiesterase; and (-■-■-) the reaction products of 6-MeSe-IMP after treatment with 5′-nucleotidase.

the 6-chloro or 6-methylseleno group with suitable nucleophiles.

6-Selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (II) was synthesized through three routes: (a) from cAMP, (b) from 6-chloro-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate and (c) from Dimroth rearrangement.

Treatment of cAMP (I) with hydrogen selenide in aqueous pyridine at 65° for 4 days afforded 6-selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (II) in 36% yield. Compound II was synthesized alternately from multisteps synthesized 6-chloro-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (III) (17) with hydrogen selenide in 8.7% yield. Oxidation of cAMP (I) with *m*-chloroperbenzoic acid (MCPBA) in a buffered two-phase system gave cAMP 1-oxide (IV) (20) in 91% yield. Methylation of IV with methyl iodide in DMSO gave 1-methoxyadenosine 3′,5′-cyclic phosphate (V) in 75% yield. Treatment of V with excess hydrogen selenide in the solution of water and pyridine at 65° for 2 days afforded 6-selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (II) in 53% yield. Chlorination of II with chlorine in acetonitrile gave 6-chloro-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (III). Alkylation of compound II with methyl iodide and *p*-nitrobenzyl bromide yielded 6-methylseleno (VI) and 6-*p*-nitrobenzylseleno (VII)-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate respectively (Scheme I). Treatment of compound VI with hydrogen sulfide in aqueous basic solution gave 6-thio-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (VIII) (17). The 6-substituted adenosine 3′,5′-cyclic phosphates were purified through a Dowex 50 (H^+) column and/or from preparative Avicel plates. The physical properties of the nucleotides are shown in Table I. The structures of these cyclic nucleotides were determined by ultraviolet spectra, nuclear magnetic resonance spectra and enzymatic studies.

Methylation of cAMP with methyl iodide in DMSO gave 1-methyladenosine 3′,5′-cyclic phosphate (IX). In contrast to 1-methoxyadenosine 3′,5′-cyclic phosphate (V), treatment of IX with hydrogen selenide gave 1-methyl-6-selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (X) and only trace amount of compound II. The formation of compound II may arise from the Dimroth rearrangement of compound IX to give N^6-methylamino-cAMP followed by reacting with hydrogen selenide to give II. The different behavior of compounds V and IX may be due to the different migratory aptitudes of methyl and methoxyl. In a similar manner, treatment of IX with hydrogen sulfide gave 1-methyl-6-thio-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (XI).

For the identification of the cyclic structures of 6-substituted seleno cyclic nucleotides several 6-substituted selenoadenosine 5′-monophosphates and selenoadenosines were synthesized. Treatment of 6-chloro-9-(β-D-ribofuranosyl)purine 5′-monophosphate with hydrogen selenide in methanol gave 6-selenoxo-9-(β-D-ribofuranosyl)purine 5′-monophosphate (XII). Compound XII was synthesized alternately from AMP and hydrogen selenide. Methylation of compound XII with methyl iodide gave 6-methylseleno-9-(β-D-ribofuranosyl)purine 5′-monophosphate (XII). Methylation of AMP with methyl iodide in DMSO gave 1-methyladenosine 5′-monophosphate (XIV). Treatment of XIV with hydrogen selenide gave 1-methyl-6-selenoxo-9-(β-D-ribofuranosyl)purine 5′-monophosphate (XV). In a similar manner, treatment of 6-selenopurine riboside with methyl iodide and *p*-nitrobenzyl bromide gave 6-methylseleno purine riboside (XVI) (41) and 6-*p*-nitrobenzylselenopurine riboside (XVII) (42) respectively. The physical properties of these compounds are listed in Table 1.

The structure assignment of compounds II and X are based on the following evidences: differences in Rf, uv, retention time in high pressure liquid chromatography,

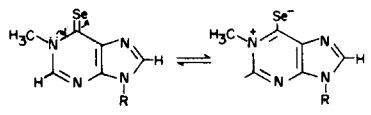
Table 1

Physical Properties of the 6-Substituted Seleno Cyclic Nucleotides and Related Compounds

Compound	Max mμ (ε x 10^{-3})			Chemical Shifts (δ) in DMSO-d_6			Rf (c)
	*p*H 1	*p*H 11	Water	H-2	H-8	Methyl	
II	351 (21.4)	328 (17.1)	350 (21.8)	8.37	8.70		0.43
VI	229 (8.6)	226 (9.2)	228 (9.3)				
	307 (12.3)	302 (14.1)	302 (15.2)	8.70 (8.51) (d)	8.77 (8.73) (d)	(2.19) (d)	0.65
VII	297 (a)	299 (a)	300 (a)				
			295 (22.5) (b)				0.05
VIII	225 (10.7)	227 (16.9)	225 (12.5)				
	322 (19.1)	312 (20.0)	322 (24.9)	8.23	8.52		0.52
IX	320 (min)	231 (min)	230 (min)				
	257 (14.11)	258 (14.18) 266 (sh)	256 (13.36)	8.43 (d)	8.65 (dL	4.08 (d)	0.76
X	235 (12.9)	232 (12.2)	235 (11.2)				
	347 (22.8)	345 (21.6)	345 (23.2)	8.88	8.60	3.83	0.56
XI	230 (20.6	230 (10.9)	230 (10.1)				
	320 (17.8)	320 (20.1)	320 (21.3)	8.80	8.47	3.90	0.67
XII	230	240	228				
							0.21
	358	324	353				
XIII	232	230	233				
							0.45
	310	303	305				
XIV	233 (min)	234 (min)	253 (min)				
							0.76
	261	261	262				
XV	237 (10.5)	238 (8.4)	235 (11.8)				
							0.42
	348 (19.0)	344 (20.1)	344 (24.4)	8.85	8.58	4.01	
XVII	(a)	(a)	296 (22.05) (b)				
							0.91
XIX	236 (11.2)	235 (13.9)	235 (12.2)				
							0.66
	350 (19.7)	345 (24.4)	344 (24.0)				
XX			235 (7.7)	8.30	8.63		
							0.53
			345 (11.2)				

(a) Compounds only partially dissolved in the solvent. (b) In methanol. (c) Thin-layer chromatography was run on a polygram CEL 300 PEI and developed with 1 *M* lithium chloride. (d) In perdeuteriomethanol.

and most important, the difference in nmr spectra. Besides a singlet at δ 3.83 for methyl group in compound X, the C-2 proton in compound X was deshielded from δ 8.37 (for compound II) to δ8.88. This substantial deshielding of H-2 in 1-methyl derivative X suggests a more extensive degree of quaterization is occuring at N-1 in compound X as compared with compound II; the electron releasing inductive effect of the methyl group will tend to stabilize the positive charge on the N-1 nitrogen.

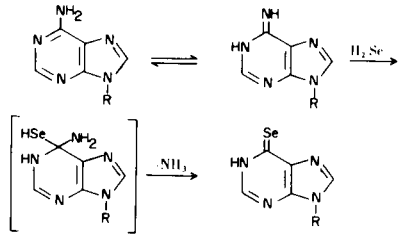


R = 3′,5′-cyclic phosphoribosyl

Furthermore, when each of the 6-substituted seleno cyclic nucleotides was incubated with 3′,5′-cyclic nucleotide phosphodiesterase in Tris buffer (*p*H 7.5), the corresponding 5′-nucleotide was obtained. When the enzymatically prepared 6-substituted seleno 5′-nucleotides were incubated with 5′-nucleotidase, 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 6-substituted selenopurine riboside 5′-monophosphates and selenopurine ribosides. For example, Figure 1 shows the behavior of 6-methylseleno-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (VI) on high pressure liquid chromatography on a Varian LCS-1000. A single peak with retention time of 53 minutes was obtained. When compound VI was treated with phosphodiesterase and chromatographed on the LCS-1000 a new peak with retention time of 52 minutes was observed. Subsequently when enzymatically prepared 6-methylseleno-9-(β-D-ribofuranosyl)purine 5′-monophosphate was treated with 5′-nucleotidase a new peak with retention time of 12 minutes was observed. Thus the cyclic structures of these 6-substituted seleno cyclic nucleotides were verified.

In order to explore the limitation of this new synthetic procedure, several seleno purine nucleosides were synthesized. Adenosine and adenine arabinoside reacted with hydrogen selenide to give 6-selenoxo-9-(β-D-ribofuranosyl)-purine (XX) (44) and 6-selenoxo-9-(β-D-arabinofuranosyl)-purine (XXI) (45) respectively. Formycin, an analog of adenosine, reacted with hydrogen selenide to give 7-selenoxo-3-(β-D-ribofuranosyl)pyrazalo[4,3-*d*]pyrimidine (XXII) (46) as expected. 2-Aminoadenosine (47) reacted with hydrogen selenide to give 6-selenoguanosine (XXIII) (41, 42). However, guanosine was relatively inert to hydrogen selenide even under more vigorous conditions. It is worthy to note that hydrogen selenide reacted with adenosine, 2-aminoadenosine, adenine arabinoside, formycin, AMP and cAMP to give good yield of the corresponding seleno compounds while these compounds were relatively inert to hydrogen sulfide even under more vigorous conditions.

The mechanism of this reaction may be proposed as follows: tautomerization of amino-imino (48) followed by addition of hydrogen selenide and then elimination of ammonia to give the final product (49).



The advantages of this procedure are that it is simpler and the yields are higher than with the chlorination method, especially in synthesizing the 6-seleno cyclic nucleotide. Therefore, this new synthetic procedure should greatly diminish the cost of biochemical research in the field of purine metabolism.

It is also interesting to note that X is more stable than II and in turn more stable than 6-selenoxo-9-(β-D-ribofuranosyl)purine in aqueous solution at room temperature. The half-life from the 343 nm band maxima was about 67 hours for II while it was 21 hours for 6-selenoxo-9-(β-D-ribofuranosyl)purine. This increased stability of X and II may offer further improvement in biological activity.

EXPERIMENTAL (50)

6-Selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate (II).

Method I.

The solution of 400 mg. (1.22 mmoles) of cAMP in 3 ml. of pyridine, 6 ml. of water and excess of hydrogen selenide in a sealed tube was kept at 65° for 4 days. The tube was cooled to room temperature and the solvent was evaporated to dryness. The residue was dissolved in a small amount of water and passed through a Dowex 50 (H^+) column. The compound was eluted with water. Evaporation of the appropriate fractions and dried *in vacuo* to give 182 mg. (36.5%) of II.

Anal. Calcd. for $C_{10}H_{11}N_4O_6PSe \cdot H_2O$: C, 29.28; H, 3.18; N, 13.62; P, 7.53. Found: C, 29.46; H, 3.18; N, 13.69; P, 7.94.

Method II.

Condensed hydrogen selenide (51) (1 ml.) was bubbled through a solution of 0.2 g. (8.7 mmoles) of sodium in 30 ml. of absolute methanol. 6-Chloro-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate triethylammonium salt (III) (17) (synthesized from 2.0 g. of cIMP) in 30 ml. of absolute methanol was added. The mixture was stirred under nitrogen at room temperature for 2 hours. The dark mixture was evaporated to dryness *in vacuo* and then dissolved in 20 ml. of water. The mixture was filtered through celite, the clear yellow filtrate was acidified (*p*H 3.5) with acetic acid and then evaporated to dryness. The yellow residue was dissolved in a small amount of water, passed through 100 ml. of Dowex 50 (triethylammonium form) and eluted with water. The residue, after evaporation, was chromatographed on a column containing 40 g. of silica gel. The column was eluted with chloroform-methanol (v/v 4:1). The organic solvent was evaporated from yellow fractions. The yellow compound was then taken up in water. The product, after evaporation was washed with a small amount of water and then chloroform. The yellow solid was dried at room temperature (phosphorus pentoxide) *in vacuo* to yield 200 mg. (8.7%) of II.

Method III.

The sealed tube containing 600 mg. (1.52 mmoles) of 1-methoxy-cAMP (V) in 2.5 ml. of water, 1.3 ml. of pyridine and 2 ml. of hydrogen selenide was heated at 65° for 2 days. The solvent was evaporated to dryness. The residue was dissolved in 20 ml. of water and filtered. The filtrate was passed through a

Dowex 50 (H^+) column. The compound was eluted with water and dried to give 330 mg. (53%) of yellow solid which was identified by uv and tlc to be identical with II prepared by Method I.

6-Chloro-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate (III).

Method I.

Compound III was prepared according to the elegant procedure (17) from inosine 3′,5′-cyclic phosphate and phosphorus oxychloride.

Method II.

Chlorine gas was bubbled through a suspension of 200 mg. (0.49 mmole) of 6-selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (II) in 15 ml. of acetonitrile (52, 53) for 10 minutes, The solution was stirred at room temperature for an additional 20 minutes and then evaporated to dryness. The residue was washed with ethanol several times and then dried *in vacuo* to give 75 mg. of III. The filtrate was reacted with 200 mg. of thiourea to give 20 mg. of 6-thio-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (VIII). Total yield of III was 56%.

Adenosine 3′,5′-Cyclic Phosphate 1-Oxide (IV).

Compound IV was synthesized by the method of Meyer *et al.* (20) with adenosine 3′,5′-cyclic phosphate (2 g., 6.075 mmoles) and *m*-chloroperbenzoic acid (3.6 g.) in a biphasic mixture of ethyl acetate and sodium acetate buffer solution to give 91% yield.

1-Methoxyadenosine 3′,5′-Cyclic Phosphate (V).

Compound V was synthesized by the method of Meyer *et al.* (20) with adenosine 3′,5′-cyclic phosphate 1-oxide (IV) (1.94 g., 5.09 mmoles), and methyl iodide (1 ml.) in 1,5-diazabicyclo-[5.4.0]-5-undecene (DBU) and DMSO to give a 72% yield.

6-Methylseleno-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate (VI).

The solution of 617 mg. (1.5 mmoles) of 6-selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-cyclic phosphate (II), 320 mg. (2 mmoles) of sodium carbonate in 20 ml. of water and 1.5 ml. of methyl iodide in 10 ml. of methanol was stirred at room temperature for 2 hours. The solution was evaporated to dryness. The residue was dissolved in 5 ml. of water and passed through a Dowex 50 (H^+) column (1.25 x 12 cm.). The compound was eluted with water. Evaporation of the appropriate fractions gave 260 mg. (42.5%) of VI. The analytical sample was recrystallized from ethanol and purified further with Avicel plates (1000 microns) developed with butanol:acetic acid:water (v/v 4:1:1).

Anal. Calcd. for $C_{11}H_{13}N_4O_6PSe \cdot O \cdot 4C_2H_5OH$: C, 33.23; H, 4.21; N, 13.13. Found: C, 33.00; H, 3.87; N, 12.98.

6-*p*-Nitrobenzylseleno-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate (VII).

The solution of 432 mg. (2 mmoles) of *p*-nitrobenzyl bromide in 50 ml. of methanol was added to a solution of 617 mg. (1.5 mmoles) of compound II and 320 mg. (3 mmoles) of sodium carbonate in 25 ml. of water. The solution was stirred at room temperature for 5 days. The precipitate was filtered and dried to give 280 mg. of solid. The filtrate was evaporated to dryness. The residue was washed with ether (3 x 70 ml.) and then passed through a Dowex 50 (H^+) column (2.5 x 12 cm.). The compound was eluted with water. Evaporation of the appropriate fractions followed by drying gave 110 mg. of VII. The analytical sample was purified with Avicel Plates (1000 microns) developed with butanol:acetic acid:water (v/v 4:1:1).

Anal. Calcd. for $C_{17}H_{16}N_5O_8PSe$: C, 38.65; H, 3.05; N, 13.26. Found: C, 38.41; H, 3.34; N, 13.17.

6-Thio-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate (VIII).

The solution of 100 mg. (0.25 mmole) of VI, 150 mg. of potassium bicarbonate and excess hydrogen sulfide in 10 ml. of water was stirred at room temperature overnight. The solution was evaporated to dryness. The residue was dissolved in 10 ml. of water and passed through a Dowex 50 (H^+) column (1.25 x 10 cm.). The compound was eluted with water. Evaporation of the appropriate fractions gave 90 mg. of crude VIII. The compound was washed with ether, ethanol and dried *in vacuo* to give 48 mg. of pure VIII.

Anal. Calcd. for C H N O PS·1.75H O: C, 31.79; H, 3.87; N, 14.82. Found: C, 31.77; H, 3.71; N, 14.50.

1-Methyladenosine 3′,5′-Cyclic Phosphate (IX).

Compound IX was synthesized by the method of Meyer *et al.* (20) with adenosine 3′,5′-cyclic phosphate (0.97 g., 2.95 mmoles) and methyl iodide (0.5 ml.) in DBU and DMSO in 41% yield.

1-Methyl-6-selenoxo-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate (X).

The solution of 100 mg. (0.29 mmole) of IX in 1 ml. of pyridine, 2 ml. of water and excess of hydrogen selenide in a sealed tube was kept at 65° for 2 days. The solvent was evaporated to dryness. The residue was dissolved in water and filtered. The filtrate was passed through a Dowex 50 (H^+) column. The compound was eluted with water, evaporated and dried *in vacuo* to give 90 mg. (69.7%) of X and a trace amount of II. The analytical sample was purified with Avicel plates (1000 microns) developed with 94% aqueous 1-butanol:44% propionic acid (v/v 1:1).

Anal. Calcd. for $C_{11}H_{13}N_4O_6PSe \cdot 2H_2O$: C, 29.81; H, 3.87; N, 12.64; P, 6.99. Found: C, 30.05; H, 3.74; N, 12.54; P, 7.43.

1-Methyl-6-thio-9-(β-D-ribofuranosyl)purine 3′,5′-Cyclic Phosphate (XI).

The solution of 200 mg. (0.58 mmole) of IX in 1 ml. of pyridine, 2 ml. of water and excess of hydrogen sulfide in a sealed tube was kept at 65° for 3 days. The solvent was evaporated to dryness. The residue was dissolved in water and passed through a Dowex 50 (H^+) column. The compound was eluted with water. Evaporation of the appropriate fractions followed by drying *in vacuo* gave 50 mg. (23%) of XI.

Anal. Calcd. for $C_{11}H_{13}N_4O_6PS \cdot H_2O$: C, 34.93; H, 4.00; M. 14.81. Found: C, 35.12; H, 4.40; N, 14.48.

6-Selenoxo-9-(β-D-ribofuranosyl)purine 5′-Monophosphate (XII).

Method I.

Condensed hydrogen selenide (0.4 ml.) was bubbled through a solution of 0.04 g. (1.80 mmoles) of sodium in 30 ml. of absolute methanol and then 0.4 g. (1.1 mmoles) of 6-chloropurine riboside 5′-monophosphate was added. The solution was stirred at room temperature for 16 hours. The mixture was evaporated to dryness. The residue was dissolved in 10 ml. of water and passed through a Dowex 50 (H^+) column (2,5 x 10 cm.). The compound was eluted with water and dried to give 370 mg. of XII.

Method II.

The solution of 300 mg. (0.86 mmole) of AMP in 1 ml. of pyridine, 2 ml. of water and excess hydrogen selenide was kept at 65° for 4.5 days. The solvent was evaporated to dryness. The

residue was dissolved in 7 ml. of water and passed through a Dowex 50 (H^+) column. The compound was eluted with water. Evaporation of the appropriate fractions followed by drying *in vacuo* gave 240 mg. of XII.

6-Methylseleno-9-(β-D-ribofuranosyl)purine 5′-Monophosphate (XIII).

The solution of 200 mg. of XII, 240 mg. (1.5 mmoles) of sodium carbonate in 10 ml. of water and 0.5 ml. of methyl iodide in 5 ml. of methanol was stirred at room temperature for 2.5 hours. The solution was evaporated to dryness. The residue was dissolved in 10 ml. of water and passed through a Dowex 50 (H^+) column (1.25 x 7 cm.). The compound was eluted with water. Evaporation of the appropriate fractions followed by drying *in vacuo* gave 70 mg. of XIII.

1-Methyladenosine 5′-Monophosphate (XIV).

To a solution of 970 mg. (2.79 mmoles) of AMP and 0.40g. of DBU in 4 ml. of DMSO, 0.5 ml. of methyl iodide was added. The solution was stirred at room temperature for 1 hour. The solution had jelled. The mixture was stirred for an additional hour and then 35 ml. of ehtanol was added. The precipitate was homogenized and filtered. The solid was suspended in 40 ml. of ethanol, filtered and then dried *in vacuo* to give 920 mg. (91%) of XIV.

1-Methyl-6-selenoxo-9-(β-D-ribofuranosyl)purine 5′-Monophosphate (XV).

The solution of 300 mg. (0.83 mmole) of 1-methyl-9-(β-D-ribofuranosyl)purine 5′-monophsophate (XIV) in 2 ml. of water, 1 ml. of pyridine and excess hydrogen selenide was kept at 65° for 2 days. Work up as described for X to give 180 mg. (49%) of XV with a trace amount of 6-selenopurine riboside and compound XII. The analytical sample was purified with Avicel plates (1000 microns) developed with 94% 1-butanol:44% propionic acid (v/v 1:1).

Anal. Calcd. for $C_{11}H_{15}N_4O_7PSe \cdot H_2O$: C, 29.81; H, 3.87; N, 12.64; P, 6.99. Found: C, 30.06; H, 4.08; N, 12.35; P, 6.84.

6-Methylseleno-9-(β-D-ribofuranosyl)purine (XVI).

Compound XVI was synthesized from the known procedure (42) with 6-selenopurine riboside and methyl iodide.

6-*p*-Nitorbenzylseleno-9-(β-D-ribofuranosyl)purine (XVII).

Into a solution of 580 mg. (1.64 mmoles) of 6-selenoxo-9-(β-D-ribofuranosyl)purine in 4.4 ml. of 0.4 *N* sodium hydroxide, 37.5 mg. (1.76 mmoles) of *p*-nitrobenzyl bromide in 20 ml. of methanol was added. The solution was stirred at room temperature. After 10 minutes the white crystals precipitated out. The mixture was stirred for an additional hour. The crystals were filtered by suction, washed with ether, water and then recrystallized from methanol-water to give 730 mg. of XVII, m.p. 179-180° (lit. (43) m.p. 178-180°). The filtrate was evaporated to dryness. The residue was washed with ether, water and then recrystallized from methanol-water to give additional 50 mg. of of XVII. Total yield of XVII was 780 mg. (98%).

1-Methyladenosine Hydroiodide (XVIII).

Compound XVIII was synthesized in 65% yield by the method of Robins, *et al.* (54) with adenosine (5.4 mmoles) and methyl iodide (2 ml.) in DMF.

1-Methyl-6-selenoxo-9-(β-D-ribofuranosyl)purine (XIX).

The solution of 400 mg. (0.98 mmole) of 1-methyladenosine (XVIII) in 1 ml. of pyridine, 2 ml. of water and excess hydrogen selenide was kept at 70° for 2.5 days. The solution was evaporated to dryness. The residue was dissolved in 7 ml. of 3% sodium carbonate solution and filtered. The filtrate was acidified with acetic acid and cooled. The precipitates were collected, washed with a small amount of water and dried to give 60 mg. (17%) of XIX with a small amount of 6-selenopurine riboside. The analytical sample was purified with Avicel plates (1000 microns) and developed with water. The bands containing the compound were scraped out, dissolved in water and filtered. The filtrate was evaporated to dryness, ocevaporated with ethanol and dried *in vacuo*.

Anal. Calcd. for $C_{11}H_{14}N_4O_4Se \cdot 0.6EtOH$: C, 39.30; H, 4.16; N, 15.02. Found: C, 38.95; H, 4.68; N, 14.77.

6-Selenoxo-9-(β-D-ribofuranosyl)purine (XX).

The solution of 400 mg. (1.50 mmoles) of adenosine in 2 ml. of pyridine, 4 ml. of water and excess of hydrogen selenide was kept at 65° for 5 days. The solution was evaporated to dryness. The residue was dissolved in 16 ml. of 3% sodium carbonate solution and filtered. The filtrate was acidified with acetic acid to *p*H 4 and cooled. The precipitate was filtered by suction, washed with a small amount of water and dried *in vacuo* to give 278 mg. (56%) of XX (44).

6-Selenoxo-9-(β-D-arabinofuranosyl)purine (XXI).

The solution of 300 mg. (1.1 mmoles) of adenine arabinoside in 2 ml. of pyridine, 4 ml. of water and excess of hydrogen selenide was kept at 65° for 3.5 days. The solution was evaporated to dryness, the residue was dissolved in 7 ml. of 3% sodium carbonate solution and filtered. The filtrate was acidified with acetic acid and cooled. The precipitate was filtered with suction, washed with a small amount of water and dried *in vacuo* to give 60 mg. of XXI (45). The combined filtrate was adjusted to *p*H 8 and methyl iodide added. The solution was stirred at room temperature overnight. The precipitates were filtered with suction and recrystallized from water to give 26 mg. of 6-methylseleno-9-(β-D-arabinofuranosyl)purine (45). Total yield of XXI is 22%.

7-Selenoxo-3-(β-D-ribofuranosyl)pyrazalo[4,3-*d*]pyrimidine(Selenoformycin B) (XXII).

The solution of 200 mg. (0.75 mmole) of formycin (55) in 1 ml. of pyridine, 2 ml. of water and excess hydrogen selenide was kept at 65° for 1.5 days. The solution was evaporated to dryness, the residue was dissolved in 4 ml. of 3% sodium carbonate solution and filtered. The filtrate was acidified with acetic acid to *p*H 4 and cooled. The precipitates were filtered with suction, washed with a small amount of water and dried *in vacuo* to give 80 mg. (32%) of XXII (46).

Anal. Calcd. for $C_{10}H_{12}N_4O_4Se$: C, 36.26; H, 3.62; N, 16.92. Found: C, 35.91; H, 3.67; N, 16.76.

2-Amino-6-selenoxo-9-(β-D-ribofuranosyl)purine (6-Selenogaunosine) (XXIII).

The mixture of 300 mg. (1.07 mmoles) of 2-aminoadenosine (47) in 4 ml. of water, 2 ml. of pyridine and excess of hydrogen selenide was kept at 65° for 5 days. The solution was evaporated to dryness. The residue was dissolved in 7 ml. of 3% sodium carbonate solution and filtered. The filtrate was acidified with acetic acid to *p*H 4 and cooled in an ice-bath. The precipitate was filtered by suction, washed with a small amount of water and dried *in vacuo* to give 76 mg. (21%) of XXIII (41, 42).

Enzymatic Studies (56).

Each 3′,5′-cyclic nucleotide (1 μmole) was added to tubes

containing Tris buffer (*p*H 7.5, 50 μmole), magnesium sulfate hydrate (1 μmole), 3′,5′-cyclic nucleotide phosphodiesterase (1 μmole) in a final volume of 1 ml. The mixtures were incubated at 30° for 30 minutes, and the reaction was stopped by boiling in water-bath for 3 minutes. An aliquot of each incabation mixture was subjected to tlc and to high pressure liquid chromatography on a Varian LCS-1000. Peak areas were determined by multiplying the height of the peak by the width at half-height.

After release of 5′-nucleotides, 200 μg. of 5′-nucleotidase was added to each tube and incubated at 37° for 1 hour. The reaction was stopped by boiling in a water-bath for 15 minutes. The supernatants were subjected to tlc and to high pressure liquid chromatography.

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