(21) R. A. Long, G. L. Szekeres, T. A. Khwaja, R. W. Sidwell, L. N. Simon, and R. K. Robins, *J. Med. Chem.*, **15**, 1215 (1972).
(22) W. W. Lee, L. V. Fisher, and L. Goodman, *J. Heterocycl. Chem.*, **8**, 179 (1971).
(23) T. A. Khwaja, R. Harris, and R. K. Robins, *Tetrahedron Lett.*, 4681 (1972).
(24) R. E. Holmes and R. K. Robins, *J. Amer. Chem. Soc.*, **86**, 1242 (1964).
(25) M. Ikehara and K. Muneyama, *J. Org. Chem.*, **32**, 3039 (1967).
(26) M. Ikehara, M. Kaneko, and Y. Ogiso, *Tetrahedron Lett.*, 4673 (1970).
(27) J. P. Miller, D. A. Shuman, M. B. Scholten, M. K. Dimmitt, C. M. Stewart, T. A. Khwaja, R. K. Robins, and L. N. Simon, *Biochemistry*, **12**, 1010 (1973).
(28) G. A. LePage and E. M. Hersh, *Biochem. Biophys. Res. Commun.*, **46**, 1918 (1972).
(29) R. G. Hughes, Jr., and A. P. Kimball, *Cancer Res.*, **32**, 1791 (1972).
(30) M. Ikehara and S. Uesugi, *Chem. Pharm. Bull.*, **17**, 348 (1969).

Synthesis and Antitumor Activity of α- and β-2'-Deoxy-6-selenoguanosine and Certain Related Derivatives†

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Synthesis of the α and β anomers of 2'-deoxy-6-selenoguanosine (**2** and **4**) has been accomplished in good yield. Treatment of **2** and **4** with several alkyl halides under basic conditions has furnished the corresponding 2'-deoxy-6-alkylselenoguanosines. Treatment of the α and β anomers of 2-acetamido-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl)purine (**5** and **1**) with sodium methoxide has provided the α and β anomers of 2-amino-6-methoxy-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine (**10** and **6**). Treatment of **5** and **1** with hydrogen in the presence of a Pd-on-C catalyst was followed by treatment with sodium methoxide to provide the α and β anomers of 2-amino-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine (**8** and **9**). Compounds **10** and **6** as well as **8** and **9** have also been prepared by an alternate route. The antitumor activity against leukemia L-1210 for the above compounds as well as certain related selenonucleosides prepared previously in this laboratory is discussed.

The antitumor activity[1,2] of 6-thioguanine (TG) is suggested to result from incorporation into DNA with ultimate cell death. It is of interest that TG has been recently isolated[3] from a fermentation broth of *pseudomonas* sp. C, H (HLR 186 B). Several tumor cell lines have developed[4] a resistance toward the chemotherapeutic effect of TG. A better therapeutic index and comparable antitumor inhibition have been reported[5] for 6-selenoguanine (SeG) and this prompted the first synthesis of 6-selenoguanosine (SeGR) in our laboratory.[6] A comparative study[7,8] (thio *vs.* seleno) has subsequently established that SeG and SeGR inhibit the growth of Sarcoma 180 ascites cells more effectively than the corresponding thionucleosides. The first synthesis of α- and β-2'-deoxy-6-selenoguanosine was accomplished in our laboratory[9] on the basis that both α- and β-2'-deoxy-6-thioguanosine had demonstrated[10] sufficient antitumor activity to be considered as candidates for clinical trials.

The initial route we envisaged for synthesizing selenonucleosides[9] in the deoxynucleoside area [2-amino-6-seleno-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**4**, β-2'-deoxy-6-selenoguanosine) and 2-amino-6-seleno-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (**2**, α-2'-deoxy-6-selenoguanosine)] was based on our successful preparation of SeGR by treatment of 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine with sodium hydrogen selenide in methanol at reflux temperature. However, synthesis of the starting materials [the α and β anomers of 2-amino-6-chloro-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine] presented some difficulties since treatment of the individual anomers (α and β) of 2-acetamido-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl)purine[11] (**5** and **1**, respectively) with methanolic sodium methoxide at reflux temperature, which was required for a complete removal of the acyl group from the exocyclic 2-amino group, furnished a mixture of nucleosides in each instance. These nucleosides were separated by tlc and assigned the structures 2-amino-6-chloro-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine (minor component) and 2-amino-6-methoxy-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine on the basis of a comparison of uv spectra with model compounds.[12,13] Since the desired nucleoside was found to be the minor component, this prompted us to initiate an alternate route for the synthesis of **2** and **4**.

Treatment of **1** with sodium hydrogen selenide in methanol effected a facile nucleophilic displacement of the 6-chloro group as evidenced by tlc and uv spectrum. The protecting groups were then removed with sodium methoxide in methanol at reflux temperature to furnish a bright yellow crystalline nucleoside which was characterized as β-2'-deoxy-6-selenoguanosine (**4**): pmr (DMSO-d_6) sharp singlet at δ 6.8 (exocyclic amino group), a triplet centered at δ 6.20 (peak width 14 Hz) (anomeric proton), and the characteristic pattern usually observed for the remaining protons of a 2-deoxyribofuranose moiety.[14] A similar procedure using **5** furnished a 46% yield of α-2'-deoxy-6-selenoguanosine (**2**). The pmr data obtained for **2** were found to be essentially the same as that observed for the β anomer **4**, except for the quartet at δ 6.2 (peak width 10.5 Hz) instead of a triplet which is generally characteristic[14] for the anomeric proton of a α-2'-deoxyribofuranoside. The synthesis of both anomers (α and β) of 2'-deoxy-6-selenoguanosine is of interest in view of a report[15] on the phosphorylation of both anomers of 2'-deoxy-6-thioguanosine. The β anomer of 2'-deoxy-6-thioguanosine has been converted to a nucleotide derivative by certain neoplastic and normal cells while the α anomer was more specific and although several neoplasms did afford the nucleotide derivative there was observed no phosphorylation in the normal tissues studied. A more recent

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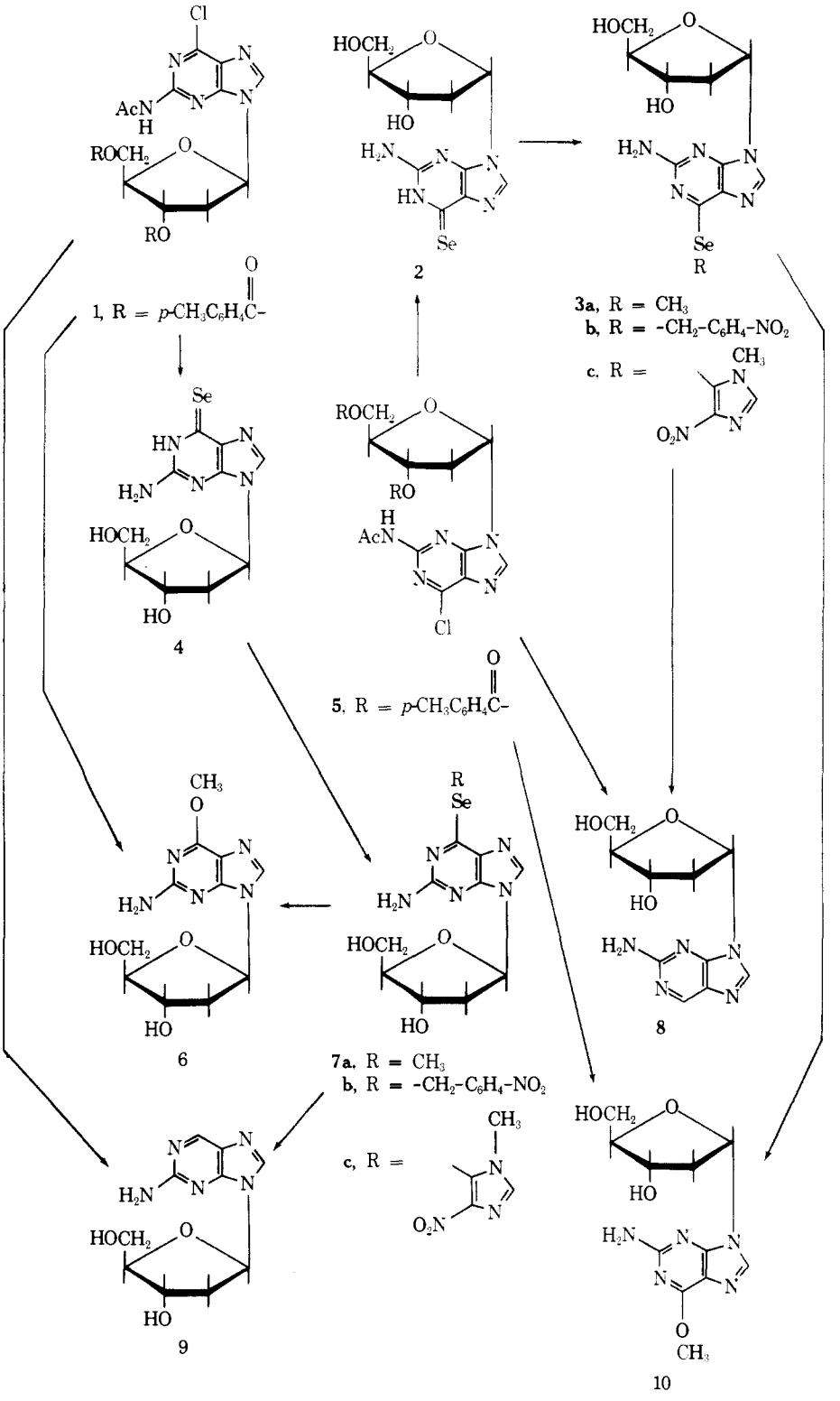
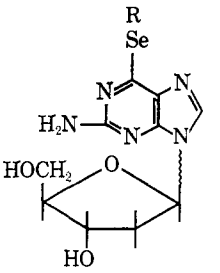
Scheme I

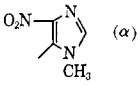
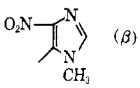


study[16] has revealed that the β anomer of 2′-deoxy-6-thioguanosine can be phosphorylated by a purified deoxycytidine-deoxyguanosine kinase although this purified enzyme had now lost all ability to phosphorylate the α anomer. Apparently, there is another kinase presumably removed during the purification process, which is capable of phosphorylating both the α and β anomers or perhaps may even be specific for the α anomer. These studies are of considerable interest since it is the phosphorylation of β-2′-deoxy-6-thioguanosine with subsequent incorporation into DNA[17] which effectively circumvents the resistant mechanism observed to develop toward the use of 6-thioguanine, *per se*.

Methylation of 4 with methyl iodide in methanolic sodium methoxide furnished 2-amino-6-methylseleno-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (7a) on the basis of the following data: a sharp singlet in the pmr spectra at $\delta \cong 2.5$ and a hypsochromic shift (λ_{max}) in the uv spectra which indicated that methylation had occurred on the exocyclic seleno group rather than a ring nitrogen. Additional corroboration for the actual site of methylation was provided when treatment of 7a with Raney nickel in

Table I‡



R	Dose, mg/kg	Survivors	Animal wt diff ($T - C$)	Survival days, T/C	%	Test status
H (α)	200	5/7	−3.8	13.4/9.8	136	23P
H (β)	100	6/7				22P
	50	6/6	−3.3	11.6/9.4	123	22P
	25	6/6	−1.6	14.1/9.3	151	22P
	12.5	6/6	−1.6	13.7/9.3	147	22P
 (α)	100	6/6	−1.7	11.0/11.2	98	22F
	50	6/6	−1.1	9.2/9.3	98	22F
 (β)	200	1/6	−6.4	7.0/11.2	Toxic	22
	100	3/6	−5.4	9.3/11.2	Toxic	22
	25	5/6	−1.9	13.4/9.3	144	22P
$-CH_2-C_6H_4-p-NO_2$ (β)	200	6/6	−5.0	11.8/11.2	105	22F
	100	6/6	−3.6	12.7/11.2	113	22F
$-CH_2-C_6H_4-p-NO_2$ (α)	200	5/6	−3.2	10.8/10.7	100	22F
	100	6/6	−3.3	10.8/10.7	100	22F

methanol furnished 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**9**):[18] pmr spectra (DMSO-d_6) revealed two sharp singlets at δ 8.3 and 8.7 (C-8, C-6 protons), a singlet at δ 6.50 (2-NH_2), a pseudotriplet centered at δ 6.35 (peak width 14 Hz) (anomeric proton), and the absence of a peak at δ ≅2.5 for the methylseleno group. This nucleoside (**9**) was identical with the compound obtained by hydrogenation of **1** in the presence of a Pd-on-C catalyst in ethyl acetate followed by a removal of the blocking groups from the carbohydrate moiety with methanolic sodium methoxide. A similar series of reactions was performed on **3a** which was obtained by methylation of **2**. This furnished 2-amino-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (**8**) which was identical with the compound obtained by hydrogenation of **5** with a Pd-on-C catalyst in ethyl acetate followed by the removal of blocking groups from the carbohydrate moiety with methanolic ammonia.

Treatment of **4** with *p*-nitrobenzyl bromide in methanolic sodium methoxide furnished a good yield of **7b**. Treatment of **7b** with methanolic sodium methoxide resulted in the formation of 2-amino-6-methoxy-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine[19] (**6**) by a nucleophilic displacement of the *p*-nitrobenzylseleno group. A pmr spectrum (DMSO-d_6) of **6** revealed a sharp singlet at δ 8.1 (C-8 proton), a broad singlet at δ 6.4 (2-NH_2 group), a pseudotriplet centered at δ 6.3 (peak width 16.0 Hz) (anomeric proton), and a 3-proton singlet at δ 4.0 for the 6-methoxy group. Alkylation of **2** with *p*-nitrobenzyl bromide under similar conditions furnished **3b**. Treatment of **3b** with methanolic sodium methoxide furnished a nucleoside which was identical with the nucleoside [2-amino-6-methoxy-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (**10**)] obtained by treatment of **5** with methanolic sodium methoxide. The pmr spectrum of **10** was essentially identical with that observed for **6** except for a quartet centered at δ 6.35 (peak width 10.5 Hz) (anomeric proton) instead of a triplet (Scheme I).

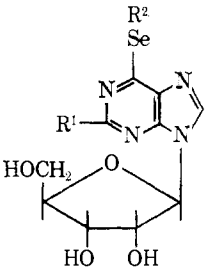
Treatment of **4** and **2** with 5-chloro-1-methyl-4-nitroimidazole under basic conditions in methanol furnished **7c** and **3c**, respectively, which were prepared primarily for their potential antitumor activity.

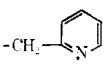
Since only preliminary data are available, definite comparisons in the selenonucleoside area cannot be made at this time. However, some general observations of interest are as follows. From the preliminary data (Table I) it is obvious that the α and β anomers of 2′-deoxy-6-selenoguanosine both possess some antitumor activity‡ with the β anomer possessing a slightly higher T/C value. Alkylation of **2** and **4** appears to effect a marked decrease in antitumor activity. SeGR and the 6-alkylselenoguanosine derivatives were found to be the most active compounds in the 6-selenoinosine[21] and SeGR area (Table II). In fact, the derivative 6-(1-methyl-4-nitroimidazol-5-yl)selenoguanosine was more active (T/C of 255 at 50-mg/kg dose with 6/6 survivors) than 6-selenoguanosine. This is of considerable interest since the reverse appears to be true for the 2-deoxynucleosides (*vide infra*) and would suggest that the mode of action for this series of nucleosides may be different unless further evaluation of the 2′-deoxynucleosides provides some very different results. The 6-alkylselenopurine ribonucleosides[21] displayed no antitumor activity and were more toxic than the aforementioned guanosine analogs. This is interesting in view of the recent finding[7] that 6-methylselenopurine ribonucleoside is an excellent inhibitor of the *de novo* pathway of purine biosynthesis and these closely related 6-alkylselenopurine ribonucleosides would be expected to inhibit the same pathway.

It has been reported[8] that 6-methylselenoguanosine is completely inactive as an inhibitor of the *de novo* pathway of purine biosynthesis and the feedback inhibition exhibited by SeGR was found to be due to its conversion by purine nucleoside phosphorylase to SeG which was then converted to SeGR 5′-phosphate *via* the salvage pathway of purine biosynthesis. This was followed by conversion of the 5′-phosphate to the triphosphate by guanylate kinase.

‡ Screening was performed under the auspices of DR & D according to the protocols described in ref 20; instruction 14 from Drug Research and Development, National Cancer Institute, March 1972.

Table II‡



R^1	R^2	Dose, mg/kg	Survivors	Animal wt diff ($T - C$)	Survival days, T/C	%	Test status
NH_2	H	50	6/6	−3.7	15.2/8.1	187	22
		25	6/6	−2.4	22.0/9.6	229	22
		12.5	6/6	−1.1	20.5/9.6	213	22
		200	1/6	−3.0	7.0/9.5		22F
H	H	100	6/6	−4.0	10.0/9.5	105	22F
		50	6/6	1.0	9.7/10.1	96	22F
NH_2		50	6/6	−0.9	24.5/9.6	255	22
		25	5/6	−2.3	18.0/9.6	189	22
		25	6/6	−1.7	14.2/8.1	175	22
		12.5	6/6	−1.6	13.5/8.1	166	22
H		200	0/6	−1.1		Toxic	
		100	0/6	−1.1		Toxic	
		25	6/6	−1.5	9.2/9.3	98	22F
		12.5	6/6	−1.2	9.7/9.3	104	22F
NH_2	$-CH_2-C_6H_4-NO_2$	100	4/6	−3.9	13.7/9.3	147	22
		50	6/6	−2.3	10.5/8.8	119	22
		25	6/6	−1.2	9.8/8.8	111	22
H	$-CH_2-C_6H_4-NO_2$	200	0/6	−1.0		Toxic	22
		100	1/6	−5.5	6.0/9.2	Toxic	22
		50	3/6	−3.1	9.7/12.1	Toxic	22F
		25	6/6	−0.5	12.2/12.1	100	22F
NH_2	$-CH_2CH=CH_2$	200	0/6			Toxic	22
		100	0/6			Toxic	22
		25	6/6	−2.0	9.3/9.3	100	22F
		12.5	6/6	0.7	9.7/9.3	104	22F
H	$-CH_2CH=CH_2$	50	6/6	−4.1	9.0/8.6	104	22F
		25	6/6	−0.7	9.5/8.6	110	22F
		12.5	6/6	+0.5	8.6/8.6	100	22F
		6.25	6/6	1.3	9.6/9.5	90	22F
H	$-CH_2-C_6H_5$	25	0/6	−1.8		Toxic	22
		12.5	6/6	−4.3	11.2/10.1	110	22
		6.25	6/6	−1.7	9.8/10.1	97	22F
		3.12	6/6	−0.4	9.5/10.1	94	22F
H	$-CH_2CH=C<^{CH_3}_{CH_3}$	50	4/6	−4.4	8.8/10.1	87	22
		25	6/6	−2.2	9.2/10.1	91	22
		12.5	6/6	−1.5	9.2/10.1	91	22
H		12.5	6/6	−2.2	9.8/8.8	111	22
		6.25	6/6	−1.3	10.0/8.8	113	22
		3.12	6/6	−2.0	9.0/8.8	102	22

It has been proposed[22] that GMP kinase inhibition by TGMP may play a role in the inhibitory mechanism of TG. It is tempting to postulate that since inhibition of GMP kinase by SeGR 5′-phosphate was observed, that the mechanism of action of SeG and Tg and the ribonucleosides SeGR and TGR may be similar in that respect. This implies that a greater inhibition of GMP kinase by SeGR 5′-phosphate in comparison to the thio analog may be responsible for the increased antitumor activity observed for SeGR in comparison to TGR.[23]

Comparative studies between these selenonucleosides and the corresponding thionucleosides as well as studies on the specific mode of action for certain selenonucleosides are currently under investigation.

Experimental Section

Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet absorption spectra were obtained with a Beckman DK-2 spectrophotometer and pmr spectra on Varian 56/60 instrument using tetramethylsilane as an internal standard. Thin layer chromatography used 0.25-mm thick Mallinckrodt SilicAR 7 GF plates unless otherwise specified and for dry column chromatography Baker silica gel powder and Du Pont No. 609 phosphor were used. All products were dried under vacuum with an oil pump unless otherwise specified.

2-Amino-6-seleno-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (4). A three-necked flask containing 2.50 g of 1[11] (0.0044 mol) in 250 ml of dry MeOH was fitted with a gas inlet tube and a reflux condenser protected with a drying tube, and the stirred suspension was then saturated with dry N_2 gas for 5 min. The mixture was heated to reflux temperature and saturated with dry H_2Se gas for 1 min, and then 15 ml of a 1 *M* NaSeH solution in MeOH was added with the passage of dry H_2Se being continued for another 10 min. The flow of H_2Se was then terminated and the mixture stirred and heated at reflux temperature for an additional 45 min. To this solution was added a 1 *M* $NaOCH_3$ solution in MeOH (7.5 ml) and the solution then stirred and heated at reflux temperature for another hour. The solution was exposed to the atmosphere, allowed to cool to room temperature, and then gravity filtered (two or three times) to remove all the selenium

metal. The light yellow filtrate was evaporated to dryness *in vacuo* and the residue then triturated with 125 ml of $CHCl_3$ and 125 ml of H_2O. The aqueous layer was washed with $CHCl_3$ (2 × 100 ml); the pH of the solution was adjusted to 6–7 with glacial HOAc and immediately lyophylized. The fluffy yellow solid was added to boiling water (30–40 ml), the elemental selenium removed by filtration, and the solution allowed to stand at 5° for 4 hr. The yellow solid was collected by filtration, washed with ice water (20 ml), and dried over $CaCl_2$ at 100° for 1 hr *in vacuo* to furnish 1.1 g of product (75% yield): mp softens ≃180° with dec >186°. A small sample was recrystallized from H_2O and dried for 2 hr *in vacuo* over Drierite at the temperature of refluxing toluene: mp ≃180° with dec >186° (lit.§ mp 166–167°); uv λ_{max} (pH 1) 265 nm (ε 6260), 368 (18,640); uv λ_{max} (MeOH) 366 nm (ε 22,580); uv λ_{max} (pH 11) 254 nm (ε 13,650), 330 (19,200). *Anal.* ($C_{10}H_{13}N_5O_3Se$) C, H, N.

2-Amino-6-seleno-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (2). The procedure is the same as that used for the synthesis of 4 except that 3.0 g (0.0053 mol) of **5** and 18 ml of a 1 *M* NaSeH solution in MeOH were used, followed by 9 ml of 1 *M* $NaOCH_3$ solution. After lyophylization, 20 ml of H_2O was added to the solid, the elemental selenium was removed by gravity filtration, and the solution was allowed to stand at 5° for 24 hr. The tan solid was collected by filtration, washed with 10 ml of ice water, and dried over $CaCl_2$ for 2 hr at 100° to furnish 800 mg (46%) of a light tan solid, mp 203–204° dec (lit.§ mp 176°). A sample was recrystallized from H_2O and dried *in vacuo* over Drierite at the temperature of refluxing toluene for 2 hr: mp unchanged; uv λ_{max} (pH 1) 265 nm (ε 6260), 368 (18,640); uv λ_{max} (MeOH) 366 nm (ε 22,580); uv λ_{max} (pH 11) 254 nm (ε 13,650), 330 (19,200). *Anal.* ($C_{10}H_{13}N_5O_3Se$) C, H, N.

2-Amino-6-methylseleno-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (7a). To 500 mg (0.0015 mol) of **4** in 25 ml of MeOH containing 100 mg of $NaOCH_3$ was added 250 mg of methyl iodide. The solution was stirred at room temperature for 10 min and evaporated *in vacuo* to a foam which was dissolved in 50 ml of MeOH containing 15 ml of J. T. Baker silica gel. The mixture was evaporated to dryness *in vacuo* and the resulting solid was placed on the top of a nylon dry column (3.75 × 24.5 cm) packed with Baker silica gel + 0.4% of a phosphor. The column was eluted with EtOAc–MeOH (8:1); the uv-absorbing fractions containing nucleoside material were determined by tlc and evaporated *in vacuo* to afford a residue which was dissolved in 20 ml of H_2O and lyophylized. The solid was then placed in a Soxhlet extraction thimble and extracted with diethyl ether (150 ml) at reflux temperature for 48 hr. The ether solution was evaporated to dryness *in vacuo*, the white solid was dissolved in EtOAc (5 ml), and sufficient cyclohexane was then added to produce a permanent cloud point at the boiling point of the mixture. The solution was allowed to stand at 5° for 18 hr; the white solid was collected by filtration, washed with 10 ml of cyclohexane, and dried at room temperature for 24 hr *in vacuo* to yield 250 mg of product (48%): mp slowly softens to a glass >70°; uv λ_{max} (pH 1) 336 nm (ε 13,600); uv λ_{max} (MeOH) 312 nm (ε 14,400); uv λ_{max} (pH 11) 316 nm (ε 14,400). *Anal.* ($C_{11}N_{15}N_5O_3Se \cdot 0.5H_2O$) (verified by pmr spectrum) C, H, N.

2-Amino-6-methylseleno-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (3a). The procedure was the same as that used for the synthesis of **7a** except that 500 mg (0.0015 mol) of **2** was used and the yield from the EtOAc–cyclohexane mixture was 200 mg (39%) of product, mp 167–170°. For analysis the sample was dried *in vacuo* over Drierite for 1 hr at the temperature of refluxing toluene: mp unchanged; uv λ_{max} (pH 1) 334 nm (ε 14,400); uv λ_{max} (MeOH) 313 nm (ε 15,800); uv λ_{max} (pH 11) 316 nm (ε 15,500). *Anal.* ($C_{11}H_{15}N_5O_3Se$) C, H, N.

2-Amino-6-*p*-nitrobenzylseleno-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (7b). β-2'-Deoxy-6-selenoguanosine (4, 1.0 g, 0.003 mol) was added, with stirring, to 50 ml of MeOH containing 200 mg of $NaOCH_3$. To this mixture was added *p*-nitrobenzyl bromide (650 mg) and the resulting solution was stirred at room temperature for 15 min. The mixture was allowed to stand at 0° for 2 hr; the solid was collected by filtration, washed with cold (−20°) MeOH (20 ml), and air-dried. The solid was recrystallized from absolute MeOH and then air-dried to yield 900 mg of product (65%), mp 204–206° with presoftening at ≃200°. A small sample was recrystallized from MeOH and then dried *in vacuo* over Drierite for 1 hr at the temperature of refluxing toluene: mp unchanged; uv λ_{max} (pH 1) 282 nm (ε 15,700), 324 (15,700); uv λ_{max} (MeOH) 313 nm (ε 19,000), 352 (19,300); uv λ_{max} (pH 11) 252 nm (ε 15,300), 313 (19,500). *Anal.* ($C_{17}H_{18}N_6O_5Se$) C, H, N.

§ The synthesis of this compound has also been reported by Chu and Davidson.[24]

2-Amino-6-*p*-nitrobenzylseleno-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (3b). The procedure was the same as that for **7b** except that 800 mg (0.0024 mol) of **2**, 160 mg of $NaOCH_3$, and 520 mg of *p*-nitrobenzyl bromide were used. The yield after recrystallization from MeOH was 950 mg (83%) of **3b**, mp 110° with presoftening at 100°. A sample was dried *in vacuo* over Drierite at the temperature of refluxing EtOH for 2 hr: mp unchanged. *Anal.* ($C_{17}H_{18}N_6O_5Se \cdot 0.5H_2O$) (verified by pmr spectrum) C, H, N.

2-Amino-6-(1-methyl-4-nitroimidazol-5-yl)seleno-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (7c). β-2'-Deoxy-6-selenoguanosine (4, 1.0 g, 0.003 mol) was added to 50 ml of MeOH containing 200 mg of $NaOCH_3$. To this mixture was added 490 mg of 5-chloro-1-methyl-4-nitroimidazole and the solution stirred at room temperature 0.5 hr. After being adjusted to pH 6–7 with glacial HOAc, the resulting solution was evaporated *in vacuo* to afford an oily residue. The residue was triturated with 50 ml of acetone and evaporated *in vacuo* to dryness. This residue was triturated with 20 ml of H_2O and then MeOH was added to effect a solution at the boiling point of the mixture. This solution was allowed to stand at 5° for 24 hr; the solid was collected by filtration, washed with 20 ml of ice water, and dried over $CaCl_2$ *in vacuo* at 100° for 2 hr to yield 1.0 g (73%) of product: mp softens at 150° with dec 178° (foams up); uv λ_{max} (pH 1) 315 nm (ε 14,500); uv λ_{max} (MeOH) 246 nm (ε 14,100), 310 (14,400); uv λ_{max} (pH 11) 310 nm (ε 15,500). *Anal.* ($C_{14}H_{16}N_8O_5Se$) C, H, N.

2-Amino-6-(1-methyl-4-nitroimidazol-5-yl)seleno-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (3c). The procedure was the same as that for **7c** except that 800 mg (0.0024 mol) of **2**, 160 mg of $NaOCH_3$, and 400 mg of 5-chloro-1-methyl-4-nitroimidazole were used. The product obtained by crystallization from MeOH–H_2O was dried over Drierite *in vacuo* at the temperature of refluxing toluene for 2 hr to yield 1.0 g (91%) of **3c**: mp 148° softens to a glass. *Anal.* ($C_{14}H_{16}N_8O_5Se \cdot 0.5H_2O$) (verified by pmr spectrum) C, H, N.

2-Amino-6-methoxy-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine[21] (6). Method I. The nucleoside 1 (1.0 g, 0.0018 mol) was suspended in 100 ml of dry MeOH containing 500 mg of $NaOCH_3$ and heated at reflux temperature for 1 hr. After standing at 0° for 1 hr, the solution was adjusted to pH 7 with glacial HOAc and evaporated to dryness *in vacuo*. The residue was redissolved in 50 ml of MeOH, 10 ml of Baker silica gel was added, and the mixture evaporated to dryness *in vacuo*. The solid was placed on top of a nylon dry column (1.5 × 10 in.) packed with Baker silica gel + 0.4% phosphor and the first nucleoside band as determined by tlc was eluted with EtOAc–MeOH (4:1 v/v). The eluent was evaporated to a solid foam *in vacuo*. The foam was dissolved in a minimum amount of hot 2-propanol and then allowed to stand at 0° for 48 hr. The white crystalline solid was collected by filtration, washed with 15 ml of cold 2-propanol, and air-dried to yield 225 mg (45%) of a white solid: mp 129–131° melts to a foamy glass. The product was recrystallized from 2-propanol and dried over Drierite at the temp of refluxing toluene for 2 hr *in vacuo*: mp remained unchanged; uv λ_{max} (pH 1) 285 nm (ε 11,500); uv λ_{max} (MeOH) 247 nm (ε 10,000), 281 (9550); uv λ_{max} (pH 11) 247 nm (ε 9550), 279 (9250). *Anal.* ($C_{11}H_{25}N_5O_4$) C, H, N.

Method II. The nucleoside **7b** (100 mg) and $NaOCH_3$ (100 mg) were added to 25 ml of MeOH and the mixture was stirred and heated at reflux temperature for 24 hr while the reaction was monitored by uv and tlc. At the end of 24 hr, all trace of **7b** had disappeared and only **6** remained. The mixture was filtered and a small portion of the filtrate was applied to 0.25-mm SilicAR 7GF plates. These plates were developed using EtOAc–MeOH (10:1 v/v) and the first uv-absorbing band was eluted with MeOH–EtOAc (1:2 v/v). The nucleoside material obtained from the eluent was established by uv and tlc comparisons in four different solvent systems to be identical in every respect with **6** prepared by method I.

2-Amino-6-methoxy-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (10). Method I. The nucleoside **5** (500 mg, 0.0009 mol) was dissolved in 50 ml of dry MeOH containing 250 mg of $NaOCH_3$ and the solution heated at reflux temperature with stirring for 1 hr. The solution was allowed to stand at 0° for 2 hr and the pH adjusted to 6–7 with glacial AcOH. The solution was evaporated to dryness *in vacuo*; the residue was suspended in 20 ml of acetone with the solid being collected by filtration, then washed with 10 ml of acetone. The solid was retained and the filtrate was evaporated to dryness. To the resulting residue was added 25 ml of benzene and the mixture stirred for 18 hr at room

temperature. The solid was collected by filtration and washed with 10 ml of benzene, and the remaining solid was combined with that obtained above. The combined solids were dissolved in 25 ml of MeOH, 10 ml of Baker silica gel was added, the mixture was evaporated to dryness *in vacuo*, and the solid was placed on top of a nylon dry column (1.5 × 10 in.) packed with Baker silica gel + 0.4% phosphor. The first uv-absorbing fractions (as determined by tlc) eluted with EtOAc–MeOH (4:1 v/v) were collected and evaporated *in vacuo* to afford a white solid which was crystallized from EtOAc and air-dried to yield 185 mg (71%) of **10**: mp 170° slowly softens to a solid melt or glass. For analysis a sample was dried over Drierite *in vacuo* over refluxing toluene for 1 hr: mp unchanged; uv λ_{max} (pH 1) 285 nm (ϵ 12,600); uv λ_{max} (MeOH) 247 nm (ϵ 11,600), 281 (10,600); λ_{max} (pH 11) 247 nm (ϵ 11,300), 280 (10,400). *Anal.* ($C_{11}H_{15}N_5O_4 \cdot 0.5H_2O$) (verified by pmr spectrum) C, H, N.

Method II. The procedure was the same as that used for the preparation of **6** (method II) except that 100 mg of **3b** was used and the nucleoside product was established by uv and tlc comparisons to be identical in every respect with **10** prepared by method I.

2-Amino-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (8). Method I. The nucleoside **5** (1.0 g, 0.0018 mol) was dissolved in ethyl acetate (100 ml) containing 200 mg of triethylamine and 500 mg of a 5% Pd/C catalyst. The mixture was stirred on an atmospheric hydrogenator for 2 hr, at which time 44–45 ml of H_2 had been adsorbed. The mixture was filtered through a Celite pad and washed with 100 ml of boiling EtOAc, and the filtrate was evaporated *in vacuo* to a white solid. The solid was dissolved in dry MeOH (100 ml) containing 200 mg of $NaOCH_3$ and this solution was heated at reflux temperature for 1 hr. The solution was then allowed to stand at 0° for 24 hr, adjusted to pH 7 with glacial HOAc, and evaporated to a solid foam *in vacuo*. To the foam was added 50 ml of $CHCl_3$ and the mixture stirred at room temperature for 4–5 hr. The solid was collected by filtration, washed with 25 ml of $CHCl_3$, and then suspended in 50 ml of acetone. This mixture was stirred at room temperature for 18 hr; the solid was collected by filtration, resuspended in 50 ml of acetone, and again stirred at room temperature for 18 hr. The solid was collected by filtration and the combined filtrates were then evaporated to a white solid *in vacuo*. The solid was recrystallized from 2-propanol and the product dried *in vacuo* for 2 hr over Drierite at the temperature of refluxing toluene to yield 175 mg (39%) of **8**: mp 190–192°; uv λ_{max} (pH 1) 314 nm (ϵ 4420); uv λ_{max} (MeOH) 245 nm (ϵ 7500), 309 (8000); uv λ_{max} (pH 11) 244 nm (ϵ 7100), 304 (7400). *Anal.* ($C_{10}H_{13}N_5O_3$) C, H, N.

Method II. The procedure was the same as that used for the synthesis of **9** (method II) except that 50 mg of **3a** was used. The product (~15 mg) was found by uv, tlc (in four different solvent systems), and mixture melting point to be identical in every respect with the product obtained from method I.

2-Amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (9). Method I. The procedure was the same as that for **8** except that 1 (1.0 g, 0.0018 mol) was used and a reaction time of 3 hr was required for hydrogenation. The yield of product obtained by crystallization from 2-propanol was 200 mg (44%): mp slowly shrinks >105°, melts >148° to a glass. For analysis the sample was dried *in vacuo* over Drierite for 2 hr at the temperature of refluxing 2-propanol: mp unchanged; uv λ_{max} (pH 1) 314 nm (ϵ 4500); uv λ_{max} (MeOH) 245 nm (ϵ 7500), 309 (8200); uv λ_{max} (pH 11) 244 nm (ϵ 7800), 304 (8000). *Anal.* ($C_{10}H_{13}N_5O_3 \cdot 0.5H_2O$) (verified by pmr spectrum) C, H, N.

Method II. The nucleoside **7a** was dissolved in MeOH (25 ml) containing ~500 mg (wet weight) of Raney nickel[=] with the mixture then being stirred and heated at reflux temperature for 30 min. The Raney nickel was removed by filtration and washed with 25 ml of boiling MeOH, and the filtrate was evaporated *in vacuo* to afford a white solid. The solid was crystallized from 2-propanol to yield ~20 mg of a white solid which was established by mixture melting point, uv, and tlc comparison in four different solvent systems to be identical in every respect with the product obtained by method I above.

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References

(1) G. A. LePage and N. Howard, *Cancer Res.*, **23**, 622 (1963).
(2) G. A. LePage, *Cancer Res.*, **20**, 403 (1960).
(3) J. P. Scannell, D. L. Pruess, M. Kellett, T. C. Demny, and A. Stempel, *J. Antibiot., Ser. A*, **24**, 328 (1971).
(4) G. A. LePage, I. G. Junga, and B. Bowman, *Cancer Res.*, **24**, 835 (1964).
(5) H. G. Mautner, S.-H. Chu, J. J. Jaffe, and A. C. Sartorelli, *J. Med. Chem.*, **6**, 36 (1963).
(6) L. B. Townsend and G. A. Milne, *J. Heterocycl. Chem.*, **7**, 753 (1970).
(7) G. W. Crabtree, E. M. Scholar, S.-H. Chu, and R. E. Parks, Jr., *Biochem. Pharmacol.*, **22**, 155 (1973).
(8) A. F. Ross, K. C. Agarwal, S.-H. Chu, and R. E. Parks, Jr., *Biochem. Pharmacol.*, **22**, 141 (1973).
(9) G. H. Milne and L. B. Townsend, *Biochim. Biophys. Acta*, **269**, 344 (1972).
(10) (a) R. R. Engle, 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971, MEDI 12; (b) M. C. Henry, R. K. Morrison, D. E. Brown, M. Marlow, R. David, and D. A. Cooney, *Cancer Chemother. Rep., Suppl.*, **4** (3), 41 (1973).
(11) R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963).
(12) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, **28**, 945 (1963).
(13) G. H. Milne and L. B. Townsend, *J. Heterocycl. Chem.*, **8**, 379 (1971).
(14) L. B. Townsend in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 2, W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N. Y., 1973, Chapter 7.
(15) G. A. LePage, *Advan. Enzyme Regul.*, **8**, 323 (1970).
(16) Y. Nakai and G. A. LePage, *Cancer Res.*, **32**, 2445 (1972).
(17) A. Peery and G. A. LePage, *Cancer Res.*, **29**, 617 (1969).
(18) Japanese Patent (to Takeda Chem. Industries, Ltd. Fr.) 1,402,909 (CI Co7d) (June 18, 1965); S. Frederiksen, *Arch. Biochem. Biophys.*, **113**, 383 (1966).
(19) O. M. Friedman, G. N. Mahapatra, and R. Stevensen, *Biochim. Biophys. Acta*, **68**, 144 (1963).
(20) *Cancer Chemother. Rep.*, **25**, 3 (1962).
(21) G. H. Milne and L. B. Townsend, *J. Chem. Soc., Perkin Trans. 1*, 2677 (1972).
(22) R. P. Mirch, R. E. Parks, Jr., J. H. Anderson, Jr., and A. C. Sartorelli, *Biochem. Pharmacol.*, **16**, 2222 (1967).
(23) A. Goldin, H. B. Wood, Jr., and R. R. Engle, *Cancer Chemother. Rep., Suppl.*, 1 (2), 207 (1968).
(24) S.-H. Chu and D. D. Davidson, *J. Med. Chem.*, **15**, 1088 (1972).

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