The Synthesis and Chemical Reactivity of 6-Selenoguanosine and Certain Related Derivatives (1,1a)

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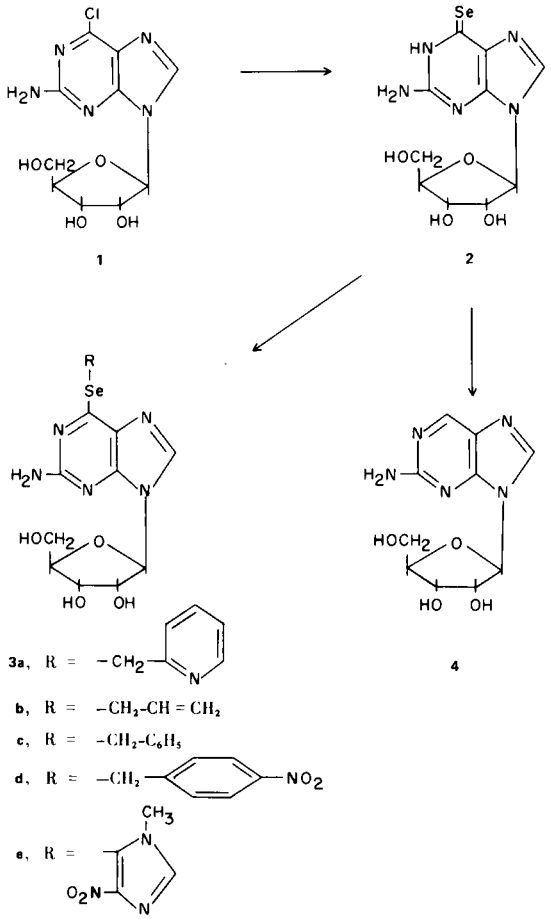
The synthesis of 6-selenoguanosine (**2**) has been accomplished by a nucleophilic displacement of the chloro group from 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine (**1**) with either selenourea or sodium hydrogen selenide. Treatment of **2** with Raney nickel has revealed that the seleno group can be removed much easier under these conditions than the corresponding mercapto group. Alkylation of **2** with several alkylating agents occurred at the exocyclic 6-seleno group to furnish several 6-alkylseleno-2-amino-9-(β-D-ribofuranosyl)purines. Nucleophilic displacement of the 6-benzylseleno group from 2-amino-6-benzylseleno-9-(β-D-ribofuranosyl)purine (**3c**) with sodium methoxide has been observed to occur at a faster rate than that observed for the corresponding 6-benzylmercapto derivative. A study on the relative stability between **2** and 6-seleno-9-(β-D-ribofuranosyl)purine toward basic conditions has revealed that the amino group at position two imparts an increase in stability.

The synthesis and subsequent antitumor activity reported for certain mercapto-substituted heterocycles (*e.g.* 6-mercaptopurine and 6-thioguanine) resulted in a tremendous research effort in this area (2,3). This was followed by the synthesis of various mercapto-containing nucleosides which also demonstrated significant biological and chemotherapeutic activity (2,3) (*e.g.*, immunosuppression (4), antitumor (5), substrates for certain enzymes (6) and inhibitors for other specific enzymes (7)). Interest in the latter area (nucleosides) has increased significantly as a result of the recent isolation and characterization of several thionucleosides from naturally occurring sources, *e.g.*, 6-(4-hydroxy-3-methyl-2-butenylamino)-2-methylthio-9-(β-D-ribofuranosyl)purine (8), 2-thiocytidine (9), 6-(3-methyl-2-butenylamino)-2-methylthio-9-(β-D-ribofuranosyl)purine (10), methyl 2-thiouridine-5-acetic acid (11,12) and various other thionucleosides from t-RNA (13) and other naturally occurring sources.

There has been an increasing accumulation of reports indicating a definite relationship between the prevalent sulfur metabolites and the corresponding selenium analogs, *e.g.*, selenomethionine. Of considerable interest in this respect is the recent report (14) that selenium-containing amino acids may act as antioxidants or "protein mis-synthesis resorters" and in this capacity function as inhibitors of premature aging. A number of disorders have been attributed to selenium deficiencies (15) and it has been found that prevention of these disorders can be accomplished much more effectively by seleno compounds



than by the sulfur amino acids, on a molar basis (16). In fact, it has been established that there is a dietary requirement for selenium, presumably as a seleno organic compound (17). In view of the tremendous advances in instrumentation and technique which were required prior to the successful isolation of the thionucleosides (minor nucleosides from t-RNA), it would be tempting to postulate that a further refinement in instrumentation and isolation techniques would result in the isolation and characterization of previously unidentified minor components as selenonucleosides. These data have prompted the present investigation into the area of selenonucleosides and the synthesis of the selenium congener of 6-thioguanosine and the naturally occurring nucleoside guanosine.

2-Amino-6-chloro-9-(β-D-ribofuranosyl)purine (18) (**1**) was treated with a methanolic solution of sodium hydrogen selenide (sodium hydroselenide) for 15 minutes to furnish a good yield of nucleoside material which was characterized by uv and nmr spectral data as 2-amino-6-seleno-9-(β-D-ribofuranosyl)purine (**2**, 6-selenoguanosine); uv λ max (*p*H 1) 260 nm (ϵ = 5,500) and 366 nm (ϵ = 18,700); λ max (water) 359 nm (ϵ = 21,200); λ max (*p*H 11) 256 nm (ϵ = 12,250) and 330 nm (ϵ = 18,100); pmr, low broad peak in the δ 12-13 region (N1 proton), a sharp singlet at δ 6.8 (exocyclic 2-amino group) and the characteristic pattern for the ribose moiety including a doublet ($J_{1',2'}$ = 6Hz) for the anomeric proton. The preparation of **2** was also accomplished by treatment of **1** with selenourea in absolute ethanol at reflux temperature. The nucleoside prepared by the latter method was proved to be identical [nmr, thin layer chromatography in 4 solvent systems, mixture melting point and superimposable uv spectra] to the 6-selenoguanosine (**2**) prepared by Method 1. The uv spectral data observed for **2** was very similar to that reported (19) previously for the heterocyclic moiety (6-selenoguanine). 6-Selenoguanine has also demonstrated (19) significant activity against L-1210 Lymphoma and L-5178 Y Lymphoma.

It has been reported (20) that the lack of antitumor activity for 6-seleno-9-(β-D-ribofuranosyl)purine was probably a direct result of the extremely short biological half-life of this nucleoside. This half-life was determined by monitoring a phosphate-citrate buffer (*p*H 7.4) containing the above nucleoside at 37° by uv spectroscopy. This prompted us to determine the relative stability between the above selenonucleoside and **2** on exposure to basic conditions. Treatment of 6-selenoguanosine (**2**) with aqueous 1*N* sodium hydroxide at reflux temperature for 30 minutes resulted in no appreciable decomposition as determined by uv absorbance at 330 nm. There was observed a decrease in the ϵ max at 330 nm of ~ 15% after 1 hour and the appearance of a small amount of red selenium metal. Exposure of 6-seleno-9-(β-D-ribofuranosyl)purine to aqueous 1*N* sodium hydroxide at reflux temperature resulted in an immediate change in color (to yellow) of solution, the appearance of a strong stench and within 15 minutes there was observed a considerable quantity of selenium metal. Only a very small amount of starting material was detected after 1 hour. Therefore, the presence of an exocyclic amino group at position two has resulted in a significant increase in stability toward basic conditions which indicates that **2** can be used as starting material for certain reactions involving basic conditions.

We have also studied the removal of the exocyclic seleno group by Raney nickel. The reaction conditions were essentially the same as those reported (21) for the dethiation of 6-thioguanosine. The rate of deseleniation was followed by a frequent uv absorption spectrum survey of the reaction mixture. The absorption peak at 350 nm disappeared very rapidly (3-5 minutes) with the concomitant appearance of an absorption peak at 303 nm which is the reported (21) λ max for 2-amino-9-(β-D-ribofuranosyl)purine (**4**). The nucleoside material obtained (97%) by this procedure was found to be identical (uv spectral data and tlc in 4 solvent systems) to authentic **4** which was prepared (21) by dethiation of 6-thioguanosine. This was of considerable interest since it demonstrates that in a situation where very mild reaction conditions are essential, it may be advantageous to use a seleno derivative rather than the thio derivative.

We have also investigated the susceptibility of **2** toward nucleophilic displacement of the exocyclic 6-seleno group. Treatment of **2** with sodium methoxide (250 mg.) in methanol (25 ml.) at reflux temperature for 24 hours revealed essentially no displacement. It has been demonstrated that an alkythio group can be displaced by a nucleophile under forcing conditions and prompted the synthesis of a 6-alkylseleno-2-amino-9-(β-D-ribofuranosyl)purine for use in the above reaction. 6-Selenoguanosine was suspended in methanol, a sodium methoxide solution added to effect a clear solution which was then treated with α-bromotoluene to afford a good yield of 6-benzylseleno-2-amino-9-(β-D-ribofuranosyl)purine (**3c**). The structure of **3c** was established unequivocally by treatment with Raney nickel which furnished a reaction mixture with a uv spectrum identical (λ max) with that observed for **4**. Reaction of **3c** with methanolic sodium methoxide at reflux temperature resulted in the appearance of another compound within 15 minutes as determined by thin layer chromatography. The reaction was complete after 18 hours and furnished 2-amino-6-methoxy-9-(β-D-ribofuranosyl)purine as characterized by thin layer chromatography (disappearance of all starting material) and uv absorbance at 278 nm (22). It was found that the corresponding 6-benzylthio compound [2-amino-6-benzylthio-9-(β-D-ribofuranosyl)purine] under the same reaction conditions re-

sulted in a much slower displacement. A small amount of the 6-methoxy nucleoside was observed after 1 hour (tlc) but complete displacement had not occurred even after 24 hours since starting material was still present as determined by tlc and uv spectral data. This indicated that although the alkylseleno group was not extremely reactive toward displacement by a nucleophile it was qualitatively better than the corresponding alkylthio group.

Treatment of **2** with α-picolyl chloride, allyl bromide, *p*-nitrobenzyl bromide and 5-chloro-1-methyl-4-nitroimidazole under reaction conditions similar to those used for the preparation of **3c** furnished a good yield of **3a**, **3b**, **3d** and **3e**, respectively.

EXPERIMENTAL

Melting points were observed on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet absorption spectra were obtained with a Beckman DK-2 spectrophotometer. Spectra were obtained on a Varian A-60 instrument using tetramethylsilane as an internal standard. Elemental Analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Missouri. Thin layer chromatography utilizing 0.25 mm thick Mallinkrodt SilicAR 7 GF plates and a solvent system consisting of ethyl acetate 4 parts to methanol 1 part was utilized for chromatographic separations unless specified otherwise.

2-Amino-6-seleno-9-(β-D-ribofuranosyl)purine (**2**, 6-selenoguanosine).

Method I.

2-Amino-6-chloro-9-(β-D-ribofuranosyl)purine (18) (**1**, 1.5 g.) and selenourea (650 mg.) were added to absolute ethanol (50 ml.). This mixture was stirred and heated at reflux temperature for 1 hour, the yellow reaction mixture cooled to 10° and the solid collected by filtration. The solid was washed with ethanol (25 ml.) at room temperature and dried in a vacuum dessicator over Drierite to yield 1.20 g. of product. The solid was recrystallized from water to yield 800 mg. of a bright yellow solid, m.p. 204-206° dec. A small sample was recrystallized from either water or absolute methanol (as with Method II) to afford a sample with a m.p. of 206-208° dec. This nucleoside was shown to be identical in every respect [mixture melting point, nmr, uv and thin layer chromatography in 4 solvent systems] to the nucleoside prepared by method II.

Method II.

2-Amino-6-chloro-9-(β-D-ribofuranosyl)purine (18) (**1**, 1.0 g.) was dissolved in absolute methanol (25 ml.) and to this stirred solution at reflux temperature was added a sodium hydrogen selenide solution [prepared by saturating a solution of absolute methanol (8 ml.) containing 470 mg. of sodium methoxide with hydrogen selenide]. The yellow solution was stirred and heated at reflux temperature for 15 minutes (or until a yellow solid begins to separate) and then allowed to stand at 5° for 30 minutes. The solid was collected by filtration, washed with absolute methanol (25 ml.) and dried in a vacuum dessicator over Drierite to yield 1.0 g. of product. The bright yellow solid was recrystallized twice from absolute methanol to yield 600 mg. of an analytical sample, m.p. 206-208° dec.; pmr low broad peak in the δ 12-13 region (N1 proton), a sharp singlet at δ 8.3 (C8 proton), a singlet δ 6.8 (exocyclic amino group) and the characteristic pattern for the ribose moiety.

Anal. Calcd. for $C_{10}H_{13}N_5O_4Se$: C, 34.68; H, 3.76; N, 20.22. Found: C, 34.51; H, 3.76; N, 20.02.

2-Amino-6-(2-methylpyridylseleno)-9-(β-D-ribofuranosyl)purine (**3a**).

To 6-selenoguanosine (**2**, 1.0 g.) in 25 ml. of absolute methanol was added a sufficient amount of sodium methoxide solution (prepared by dissolving 4 g. of sodium hydroxide in 100 ml. of methanol) to effect a clear solution plus a 2.5 ml. excess. To this solution was added α-picolyl chloride (480 mg.), the resulting solution stirred at room temperature for 2 hours and then evaporated to dryness *in vacuo*. To this residue was added 50 ml. of water and the liquid then removed by decantation. This process was repeated two more times and the remaining solid air dried. This solid was dissolved in a small amount of boiling ethanol (10 ml.), cooled to room temperature, the solid collected by filtration and washed well with benzene (25 ml.). The product was recrystallized from absolute ethanol to yield a yellow crystalline solid which was dried in a vacuum dessicator over Drierite to yield 600 mg. of **3a**, m.p. 130° dec. The product was also dried under vacuum in an Abderhalden apparatus at 80° with the weight and m.p. of the product remaining unchanged.

Anal. Calcd. for $C_{16}H_{18}N_6O_4Se \cdot \frac{1}{2}C_2H_5OH$: C, 44.40; H, 4.56; N, 18.26. Found: C, 43.91; H, 4.43; N, 18.10. The ½ mole of C_2H_5OH was verified by pmr spectrum.

2-Amino-6-allylseleno-9-(β-D-ribofuranosyl)purine (**3b**).

6-Selenoguanosine (**2**, 1.0 g.) was suspended in methanol (25 ml.) and a sufficient amount of a sodium methoxide solution (prepared by dissolving 4 g. of sodium hydroxide in 100 ml. of methanol) was added to effect a clear solution. To this solution was added 350 mg. of allyl bromide and the solution stirred at room temperature for 1 hour. The *p*H of the resulting solution was adjusted to 6-7 with glacial acetic acid and then evaporated to dryness *in vacuo* to afford a yellow solid. The solid was triturated with hot benzene, cooled to room temperature (1 hour) and the solid collected by filtration. The product was triturated with water (50 ml.), collected by filtration, air dried and then recrystallized from isopropanol to yield 500 mg. of **3b**, m.p. 80° foams then decomposes. The sample was dried over Drierite in a vacuum dessicator.

Anal. Calcd. for $C_{13}H_{17}N_5O_4Se \cdot H_2O$: C, 38.60; H, 4.71; N, 17.33. Found: C, 38.48; H, 4.60; N, 17.76. One mole of water was verified by pmr spectrum.

2-Amino-6-benzylseleno-9-(β-D-ribofuranosyl)purine (**3c**).

6-Selenoguanosine (**2**, 1.0 g.) was suspended in 25 ml. of absolute methanol and a sufficient amount of a sodium methoxide solution (prepared by dissolving 4 g. of sodium hydroxide in 100 ml. of methanol) was added to effect a clear solution. α-Bromotoluene (500 mg.) was then added and the solution stirred at room temperature for 1 hour. The *p*H of the solution was then adjusted to 6-7 with glacial acetic acid and evaporated to dryness. The resulting residue was dissolved in a small amount of methanol and then added, with stirring, to 100 ml. of ice water. The liquid was decanted and the residue washed with 100 ml. of water. The residue was dissolved in ethyl acetate (100 ml.) and washed with water (2 x 50 ml.). The organic phase was dried over sodium sulfate and evaporated to a white foam *in vacuo*. This foam was suspended in benzene (50 ml.), the solid collected by filtration, washed with benzene (25 ml.) and air dried to yield 850 mg. of a white solid, softens at 60-70°. The solid was recrystallized from toluene to yield white crystals (550 mg.) of **3c**, softens at 65-70°.

Anal. Calcd. for $C_{17}H_{19}N_5O_4Se$: C, 46.80; H, 4.36; N, 16.06. Found: C, 46.69; H, 4.39; N, 16.03.

2-Amino-6-(*p*-nitrobenzylseleno)-9-(β-D-ribofuranosyl)purine (**3d**).

6-Selenoguanosine (**2**, 1.0 g.) was suspended in 25 ml. of absolute methanol and just enough 1*N* sodium methoxide solution was added to effect a clear solution. To this solution was added 650 mg. of *p*-nitrobenzyl bromide and the solution stirred at room temperature for 2 hours. The solid was collected by filtration and washed with 25 ml. of methanol. The product was recrystallized from absolute methanol to yield 750 mg. of a crystalline solid, m.p. 200-202°. The product was recrystallized from methanol to afford an analytical sample which was dried under vacuum in an Alderhalden apparatus over toluene at reflux temperature, m.p. 201-202°.

Anal. Calcd. for $C_{17}H_{18}N_6O_6Se$: C, 42.45; H, 3.74; N, 17.48. Found: C, 42.65; H, 3.42; N, 17.25.

2-Amino-6-(1-methyl-4-nitro-5-imidazolylseleno)-9-(β-D-ribofuranosyl)purine (**3e**).

6-Selenoguanosine (**2**, 1.0 g.) was suspended in methanol (25 ml.) and a sufficient amount of a sodium methoxide solution (prepared by dissolving 4 g. of sodium hydroxide in 100 ml. of methanol) added to effect a clear solution. 1-Methyl-5-chloro-4-nitroimidazole (450 mg.) was then added and the solution stirred at room temperature for two hours. The solid was collected by filtration, washed with water (50 ml.), methanol (25 ml.) and then air dried at room temperature to yield a solid. This solid was recrystallized first from aqueous methanol and then absolute methanol to yield 750 mg. of a crystalline solid which softens at 150° and then melts at 155-157° dec. (foams up). The product was dried under vacuum in an Abderhalden apparatus at 65° to yield an analytical sample.

Anal. Calcd. for $C_{14}H_{16}N_8O_6Se \cdot 2H_2O$: C, 33.20; H, 3.95; N, 22.10. Found: C, 33.18; H, 3.95; N, 22.01. Two moles of water were verified by pmr spectrum.

2-Amino-9-(β-D-ribofuranosyl)purine (**4**).

6-Selenoguanosine (**2**, 500 mg.) was added to 50 ml. of boiling water and 4 g. (*wet wt.*) of Raney nickel (23) was then added to the well stirred solution. The reaction mixture was stirred and heated at reflux temperature for 10 minutes, the Raney nickel was then removed by filtration and washed with 50 ml. of hot water. The filtrate was evaporated *in vacuo* to afford a solid (375 mg., 97%) which was recrystallized from absolute ethanol to yield a yellow to cream colored solid, m.p. 110°, effervesces 138°, clear liquid 166°. This nucleoside was found to be identical [uv spectra, mixture melting point and tlc chromatography in 4 solvent systems] with **4** prepared by the literature procedure (21).

Treatment of **3c** and 6-Benzylthioguanosine with Sodium Methoxide.

6-Benzylthioguanosine (24) (250 mg.) of sodium methoxide in 25 ml. of absolute methanol were heated at reflux temperature for 24 hours. 6-Benzylselenoguanosine (**3c**, 250 mg.) was treated under the same conditions, the reaction mixtures were monitored by thin layer chromatography using ethyl acetate/methanol (6:1/v:v) as the solvent system. Ultraviolet spectroscopy (after 18 hours) showed a disappearance of the peak at 315 nm (for **3c**) and the appearance of a peak at 280 nm. The 6-benzylthioguanosine reaction retained a peak at 305-310 nm even after 24 hours which indicated that a complete displacement had not occurred.

Stability of 6-Seleno-9-(β-D-ribofuranosyl)purine and 6-Selenoguanosine (**2**) Toward Basic Conditions.

6-Selenoguanosine (**2**, 40 mg.) was dissolved in 10 ml. of aqueous 1*N* sodium hydroxide and refluxed for 1 hour. 6-Seleno-9-(β-D-ribofuranosyl)purine was also subjected to the same reaction conditions. The reaction mixtures were monitored by thin layer chromatography and ultraviolet absorption spectroscopy. At the end of 1 hour, **2** was found to be essentially unchanged while 6-seleno-9-(β-D-ribofuranosyl)purine was completely changed.

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