

The Synthesis of 3-Deaza-6-thioguanine and Certain Related Derivatives (1)

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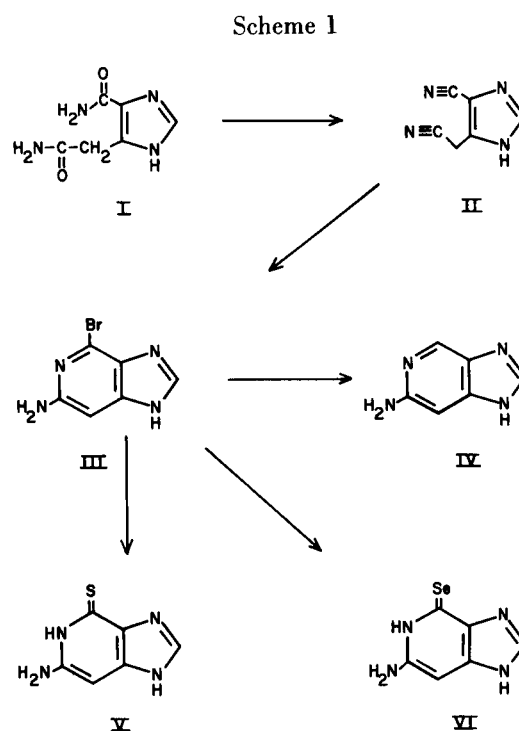
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2-Amino-6-thiopurine (6-thioguanine) has shown strong growth-inhibitory activity against transplanted tumors in animals and neoplastics cells in humans (2). In order to exert this activity, thioguanine must first be converted into its active metabolite 6-thioguanylic acid by guanine-hypoxanthine phosphoribosyl transferase (3). 6-Thioguanine, as 6-thioguanylic acid, has been shown to be incorporated into RNA and DNA and to inhibit (4-7) a number of enzymic reactions.

The closely related derivative 6-selenoguanine has shown antitumor activity on implants of lymphomas L1210 and L5178Y and sarcoma 180 (8) with a higher therapeutic index than that reported for thioguanine. It was recently reported that 6-selenoguanine was converted to selenoguanosine by a highly purified guanine-hypoxanthine phosphoribosyl transferase but failed to act as a substrate for the guanase isolated from sarcoma 180 cells (9).

This prompted us to initiate an investigation designed to afford deaza analogs of 6-thio- and 6-selenoguanine which might be converted to the corresponding active nucleotide. It would appear that replacement of one of the nitrogen atoms in the pyrimidine moiety with a methine group would exert a minimal effect on the ability of this type of analog to function as a substrate for the specific enzyme. We now wish to report on the synthesis of 3-deaza-6-thioguanine and 3-deaza-6-selenoguanine.

The reaction of imidazole-4-acetic acid-5-carboxylic acid dimethyl ester (10) with liquid ammonia for five days gave the diamide, imidazole-5(4)acetamide-4(5)carboxamide (I) and I was readily dehydrated by treatment with phosphorous oxychloride to give 4(5)acetonitrile-5(4)cianoimidazole (II) in 80% yield. Treatment of an ethereal suspension of the dinitrile II with anhydrous hydrogen bromide (11) gave the cyclized product, 6-amino-4-bromoimidazo[4,5-c]pyridine (III). While the reaction of II with hydrogen bromide readily lead to cyclization, attempts to achieve this ring closure with other similar acids were unsuccessful. The structure of III was established unequivocally by dehalogenation to afford 6-aminoimidazo[4,5-c]pyridine (IV), the physical constants of which were in excellent agreement with the published values (12). It is interesting to note that the cyclization of II occurs in one specific direction



as shown in the reaction scheme, with no formation of the isomeric 4-amino-6-bromoimidazo[4,5-c]pyridine.

Reaction of an aqueous suspension of III with thiourea in the presence of a catalytic amount of formic acid gave 6-aminoimidazo[4,5-c]pyridine-4-thione (V, 3-deaza-6-thioguanine). The reaction of a methanolic solution of III with sodium hydrogen selenide gave 6-amino-4-selenoimidazo[4,5-c]pyridine (VI, 3-deaza-6-selenoguanine) in very good yield. Treatment of III with selenourea also gave VI, however, lower yields were obtained as well as a problem in isolating the desired product due to an incomplete reaction.

We have observed, as expected, an increased resistance of the bromo group towards nucleophilic displacement in comparison to the corresponding purine analog. We are studying ways to increase the ease of nucleophilic displacement of the bromo group by either acylation of the exocyclic amino group or alkylation of an imidazole nitrogen.

EXPERIMENTAL (13)

Imidazole-5(4)acetamide-4(5)carboxamide (I).

Twenty g. of finely powdered imidazole-4-acetic acid-5-carboxylic acid dimethyl ester (10) was added to 200 ml. of liquid ammonia and the solution heated in a steel reaction vessel at 110° for 5 days. The ammonia was removed at room temperature and a vacuum was applied to the glass liner to remove excess ammonia. The amber residue was powdered and added to 250 ml. of ethanol, neutralized to pH 7 with concentrated aqueous hydrochloric acid, heated to boiling and the solid collected by filtration to furnish 16 g. of product. Recrystallization from water gave 12.5 g. (average yield) of colorless crystals, m.p. 248° dec. (an additional 2.5 g. can be obtained by concentrating the filtrate); uv: λ max (pH 11) 243 (ϵ 9,700); λ max (methanol) 239 nm (ϵ 10,500).

Anal. Calcd. for $C_6H_8N_4O_2$: C, 42.86; H, 4.76; N, 33.33. Found: C, 43.00; H, 5.20; N, 33.20.

4(5)Acetonitrile-5(4)cianoimidazole (II).

To finely powdered imidazole-4(5)acetamido-5(4)carboxamide (I) (10.4 g.) was added 200 ml. of phosphorus oxychloride and the mixture was heated at reflux temperature for 2.5 hours. The excess phosphorus oxychloride was removed *in vacuo* utilizing a water bath at 80° for a source of heat. The syrup was triturated with methylene chloride (6 x 100 ml.) and these extracts were discarded. The semisolid residue was dissolved in water (150 ml.) which was kept below 10°, with acetone-dry ice bath, then neutralized with concentrated ammonium hydroxide to pH 7 (total volume 180 ml.). The mixture was allowed to stand at 4° for 18 hours, the solid was then collected by filtration to yield 6.3 g. of II, (air dried) (80%). The product was recrystallized from ethanol to give light, yellow platelets, m.p. 199-200°; uv: λ max (pH 1) 221 (ϵ 10,800), λ max (methanol) 230 nm (ϵ 9,600), λ max (pH 11) 239 nm (ϵ 13,900); pmr (DMSO- d_6): δ 4.25 (S, 2, CH_2CN), 7.96 (S, 1, H2).

Anal. Calcd. for $C_6H_4N_4$: C, 54.6; H, 3.03; N, 42.4. Found: 54.5; H, 3.23; N, 42.1.

6-Amino-4-bromoimidazo[4,5-c]pyridine (III).

4(5)Acetonitrile-5(4)cianoimidazole (II) (6.0 g.) was stirred in dry ether (150 ml.), dry hydrogen bromide gas was added while the flask was immersed in an acetone-dry ice bath and the temperature of the mixture was kept at -30° until saturated (indicating drierite turned green). The mixture was then stirred at room temperature for 5 hours and allowed to stand for 18 hours in the refrigerator at 4°. The mixture was evaporated to dryness *in vacuo*, the colorless residue (14.9 g.) was added to 50 ml. of water, the pH was adjusted with 6*N* sodium hydroxide to pH 6 and then allowed to stand at 5° for 18 hours. The colorless product was collected by filtration and washed with ice water (30 ml.) to yield 7.5 g. (71.5% of III). Recrystallization of the product from a minimal amount of water afforded an analytical sample, m.p. 241-242°; uv: λ max (pH 1) 325 nm (ϵ 5,500), 246 nm (ϵ 7,300); λ max (pH 11) 309 nm (ϵ 6,200); λ max (ethanol) 312 nm (ϵ 6,000), 255 nm (ϵ 6,600); pmr (DMSO- d_6): δ 5.58 (broad singlet, 2, 6-NH₂), 6.49 (S, 1, H7), 8.08 (S, 1, H2).

Anal. Calcd. for $C_6H_5BrN_4$: C, 33.80; H, 2.34; N, 26.29; Found: C, 34.04; H, 2.22; N, 26.34.

6-Aminoimidazo[4,5-c]pyridine (IV).

6-Amino-4-bromoimidazo[4,5-c]pyridine (III, 639 mg.) was dissolved in 120 ml. of water to which 3.0 ml. of 1*N* sodium hydroxide was added followed by 250 mg. of 5% Pd/C. This mixture was

hydrogenated at 45 psi of hydrogen for 2 hours, filtered through a celite pad, washed with water (20 ml.) and the filtrate evaporated to a small volume (5 ml.). The resulting solid was collected by filtration, washed with water (5 ml.) and dried giving 320 mg. of crude product. Recrystallization from water with charcoal treatment gave 245 mg. (61%) of IV, m.p. 199-200° [lit. (12) m.p. 200°]; uv: λ max (pH 1) 324 nm (ϵ 4,800), 252 nm (ϵ 5,500); λ max (water) 297 nm (ϵ 4,100), 246 nm (ϵ 5,500); λ max (pH 13) 303 nm (ϵ 4,500), 249 sh (4,300) [lit. (12): λ max (pH 1) 324 nm (ϵ 4,200), 252 nm (ϵ 5,000); λ max (pH 13) 303 nm (ϵ 3,800), 255 nm (2,700)]; pmr (DMSO- d_6): δ 5.45 (broad singlet, 2, 6-NH₂), 6.58 (d, 1, H7), 8.03 (S, 1, H2), 8.40 (d, 1, H4) [lit. (12) (DMSO- d_6): δ 6.2-6.5 (6-NH₂), 6.65 (d, H7), 8.13 (S, H2), 8.50 (d, H4)].

6-Aminoimidazo[4,5-c]pyridine-4-thione (3-deaza-6-thioguanine) (V).

A mixture of thiourea (2.0 g.) and III (2.13 g.) was heated at reflux temperature for 6 hours in 30 ml. of water containing two drops of 98% formic acid. The solution was neutralized to pH 7 with concentrated ammonium hydroxide, the yellow precipitate was collected by filtration, washed with water (20 ml.) and then acetone (20 ml.) to give 1.5 g. of product. Recrystallization from a water-methanol mixture gave an analytical sample, m.p. >300° dec; uv: λ max (pH 1) 374 nm (ϵ 16,250) 281 nm (ϵ 6,400); λ max (water) 355 nm (ϵ 15,200), 257 nm (ϵ 6,750); λ max (pH 11) 334 (ϵ 11,100); pmr: δ 6.44 (S, 1, H7), 7.78 (broad singlet, 2, 6-NH₂), 7.98 (S, 1, H2).

Anal. Calcd. for $C_6H_6N_4S \cdot 1.5H_2O$: C, 37.30; H, 4.66; N, 29.02. Found: C, 37.22; H, 4.39; N, 29.26.

6-Amino-4-selenoimidazo[4,5-c]pyridine (3-deaza-6-selenoguanine) (VI).

A solution of III (2.13 g.) in 60 ml. of dry methanol was slowly added to a solution of sodium hydrogen selenide (prepared by condensing 3 ml. of anhydrous hydrogen selenide into 80 ml. of methanol containing 350 mg. of sodium) and stirred at room temperature for 8 hours. The mixture was filtered, the greenish solid was washed with methanol and then dissolved in 1% aqueous sodium hydroxide and filtered. The filtrate was acidified with acetic acid, the yellow solid collected, washed with cold water (20 ml.) and air dried, giving 2.05 g. of crude product. Recrystallization from water gave 1.51 g. (71%) of VI, m.p. 258-261° dec. uv: λ max (pH 1) 389 nm (ϵ 5,100); λ max (pH 11) 322 nm (ϵ 4,000); pmr (DMSO- d_6): δ 6.04 (broad singlet, 2, 6-NH₂), 6.27 (S, 1, H7), 8.20 (S, 1, H2), 12.35 (broad singlet, 2, H1 and H5).

Anal. Calcd. for $C_6H_6N_4Se$: C, 33.80; H, 2.81; N, 26.29. Found: C, 33.79; H, 2.84; N, 26.11.

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