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Synthesis of Purine Antiviral Agents, Hypoxanthine and 6-Mercaptopurine*

R. Sariri and G. Khalili

Departament of Chemistry, Guilan University, Rasht, Iran

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Abstract—Some potentially biologically active 6-substituted purine derivatives have been synthesized from simple organic reagents. The reaction of urea with ethyl cyanoacetate gave 6-aminopyrimidine-2,4-dione which was converted in two steps into purine derivative, xanthine. The latter was treated with formamide at 200°C to obtain hypoxanthine. The chlorination of hypoxanthine with $POCl_3$ gave 6-chloropurine which was converted into 6-mercaptopurine via reaction with thiourea in acetonitrile, followed by treatment with boiling ethanol.

Despite intense efforts to discover drugs that may be of value in the systematic treatment of human viral infections, such infections have been resistant to chemotherapy. Intracellular and intimate relationships between viral and host functions and metabolism make it difficult to destroy viruses without damage to the host cell. Therefore, there are only a few agents effective against viruses and having an acceptable therapeutic index, i.e., the ratio of 50% cytotoxic dose (IC_{50}) to 50% antiviral dose (EC_{50}).

Following the identification of retrovirus referred to as human immunodeficiency virus (HIV), an etiological agent of acquired immunodeficiency syndrome (AIDS) [1–3], much efforts were made to search for drugs capable of treating or preventing this lethal disease. Several nucleosides were shown to exhibit *in vitro* an anti-HIV activity commensurate with development of clinical trials [4]. It was also found that a variety of purine derivatives are potential antiviral agents. Hypoxanthine (**V**, 6-hydroxypurine) possesses a strong antitumor activity with minimal side effects. Simultaneously, this compound is an intermediate product in the synthesis of other 6-substituted purines, e.g., 6-mercaptopurine.

Two main methods for building up purine ring system are known. The first way includes synthesis of pyrimidine nuclei with subsequent introduction of appropriate substituents for closure of the second purine ring [5, 6]. The second route (it is not considered in the present article) begins with the synthesis of imidazole ring, followed by closure of pyrimidine ring.

6-Mercaptopurine (**VIII**) containing a water molecule is an antiviral agent of the purine series, which was synthesized for the first time in 1951. It is a yellow solid with a molecular weight of 170.19, which decomposes on heating above 308°C. Compound **VIII** is insoluble in water, acetone, and ether but readily soluble in dilute alkalies and poorly soluble in dilute sulfuric acid [6]. 6-Mercaptopurine is used as an oral medicine in the treatment of acute lymphoblastic leukemia (ALL), acute myoblastic leukemia (AML), and chronic myelocystic leukemia (CML). Its oral absorption is relatively low, and about 50% of the drug appears in the plasma in 2 h [7]. However, due to minor side effects and the ability to pass the blood–brain barrier, 6-mercaptopurine is now known as a potent anticancer agent with a reasonable anti-HIV activity. Despite its good ability to pass the blood–brain barrier, its concentration in central spinal fluid is insufficient for treatment of leukemia [8].

We required a concise, efficient, and economic synthetic procedure allowing large-scale preparation of the target compounds. Starting from small molecules, we targeted hypoxanthine as the first product which may be starting compound for the synthesis of 6-mercaptopurine. The synthesis is based on a new approach involving closure of pyrimidine ring and subsequent formation of imidazole ring *in situ*, which should lead to purine system already containing appropriate substituents [9–12]. The total synthesis of hypoxanthine (**V**) is shown in Scheme 1, and Scheme 2 illustrates its subsequent transformation into 6-mercaptopurine (**VIII**).

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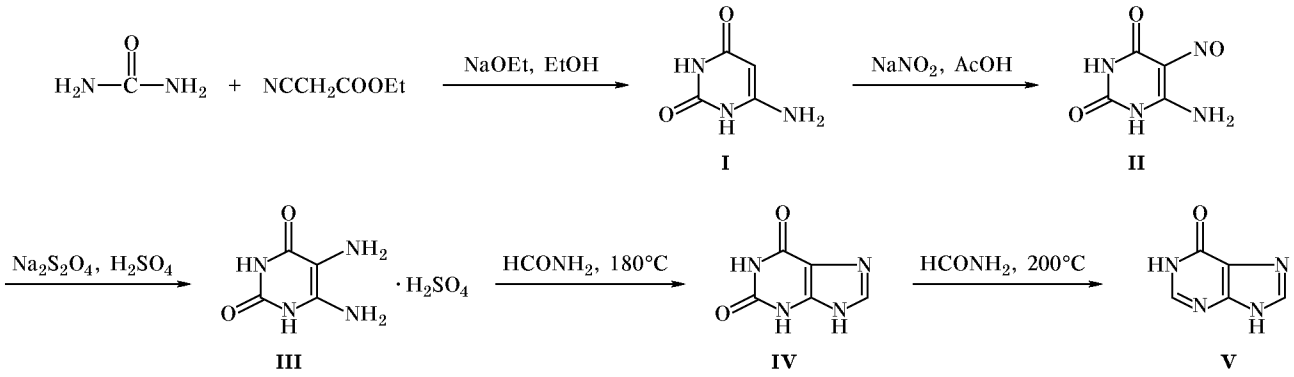
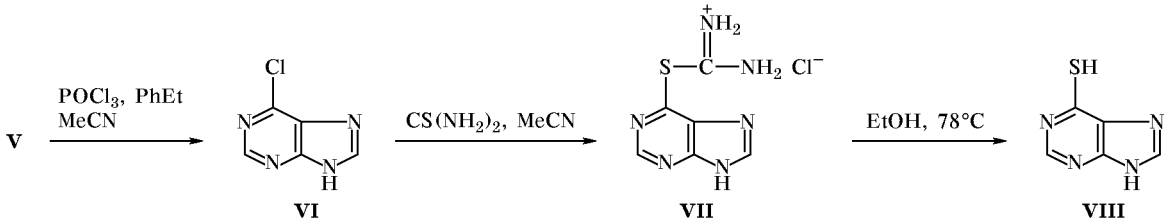
Scheme 1.



Scheme 2.



Although various methods of synthesis of purine derivatives are known, the procedure proposed by us is quite efficient. In most steps, the yield was greater than 80%. The initial compounds were of reagent grade and had low costs. We are now continuing studies in the field of synthesis of possible antagonists for the purine and pyrimidine moieties of nucleic acids. In the framework of these studies, some other purine derivatives have been prepared, which may be used as intermediate products in other synthetic methods [9].

EXPERIMENTAL

The IR spectra of compounds pelleted with KBr (0.2% dispersions) were obtained on a Perkin–Elmer 457 spectrometer.

6-Aminopyrimidine-2,4(1*H*,3*H*)-dione (I). Ethyl cyanoacetate, 212 ml, was added from a dropping funnel under vigorous stirring to a solution of sodium ethoxide prepared from 92 g of metallic sodium and 2000 ml of ethanol. A white solid separated from the solution. After the addition of ethyl cyanoacetate was complete, the mixture was stirred for 20 min at room temperature. Urea, 120 g, was added, and the mixture was heated for 3 h under reflux on a water bath. The white precipitate was filtered off, washed with ethanol, and dissolved in 1000 ml of water. The solution was adjusted to pH 7 by adding glacial acetic acid, and the mixture was stirred for 2 h to complete crystallization of the product. Yield of pyrimidine **I** 214 g (95%), mp >300°C. IR spectrum, ν, cm^{-1}: 3408, 3150, 1711, 1631, 1544, 1400, 631, 552, 526.

6-Amino-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (II). A solution of 150 g of sodium nitrite in 400 ml of water was added to a mixture of 241 g of 5-amino-2,4-dihydroxypyrimidine (**I**) and 120 ml of water. Glacial acetic acid, 170 g, was then added dropwise under vigorous stirring. A red solid precipitated, the mixture was stirred for 6 h at room temperature, and the precipitate was filtered off and washed with ethanol and water. Yield 86%. IR spectrum, ν, cm^{-1}: 3255, 1693, 1551, 1288, 1146, 1093, 787, 717, 562.

5,6-Diaminopyrimidine-2,4(1*H*,3*H*)-dione sulfate (III). A 3-l flask was charged with 250 g of 6-amino-2,4-dihydroxy-5-nitrosopyrimidine (**II**), and 1300 ml of hot (90°C) distilled water was added. The colored suspension was heated on a boiling water bath, and 100 g of sodium dithionite was added over a period of 15 min. The mixure lost its color, and the viscous solution was stirred with a mechanical stirrer for 20 min on heating. The precipitate of 5,6-diamino-2,4-dihydroxypyrimidine dihydrogen sulfate was filtered off, dried, and transferred into a 3-l flask. A solution of 150 ml of concentrated sulfuric acid in 1500 ml of water was added, and the mixture was heated for 1 h on a boiling water bath and cooled. The precipitate was filtered off, washed with acetone,

and dried. Yield 200 g (70%). IR spectrum, ν, cm^{-1}: 3384, 3156, 1715, 1649, 1533, 1404, 1107, 1051, 539.

Xanthine (IV). A suspension of 150 g of 5,6-diamino-2,4-dihydroxypyrimidine sulfate in 750 ml of formamide in a 3-l flask was heated for 90 min at 180°C. The mixture was cooled, and the precipitate was filtered off and thoroughly washed with water and cold ethanol. Yield 80 g (90%), mp >300°C. IR spectrum, ν, cm^{-1}: 3065, 1572, 1491, 1392, 1320, 1233, 989, 635, 605.

Hypoxanthine (V). A mixture of 100 g of xanthine in 1000 ml of formamide in a high-pressure reactor was heated for 30 min at 200°C under stirring with a magnetic stirrer. The mixture was cooled, and the precipitate was filtered off and dried at 80°C. Yield 65 g (70%), mp >300°C. IR spectrum, ν, cm^{-1}: 3050, 1670, 1580, 1421, 1214, 1137, 965, 892, 547.

6-Chloropurine (VI). A flask equipped with a reflux condenser was charged with a mixture of 22.6 g of hypoxanthine (**V**), 65 g of acetonitrile, and 64 g of ethylbenzene, and 82 ml of phosphoryl chloride was added from a dropping funnel. When the entire amount of $POCl_3$ was added, the mixture was stirred for 6 h at 65°C. It was then cooled, and the yellow precipitate was filtered off and dissolved in 5 N NaOH. Activated charcoal, 3 g, was added, and the mixture was stirred for 30 min and filtered. Acetone, 72 ml, was added to the yellow solution, and the mixture was adjusted to pH 7 by adding dilute hydrochloric acid. The precipitate was filtered off, washed with 100 ml of aqueous acetone and 50 ml of acetone, and dried. Yield 14 g (53%), mp >300°C. IR spectrum, ν, cm^{-1}: 3065, 1573, 1491, 1392, 1330, 1233, 990, 640, 605.

***S*-(Purin-6-yl)thiuronium chloride (VII).** A mixture of 10 g of 6-chloropurine, 4.6 g of thiourea, and 150 ml of acetonitrile was refluxed for 90 min. After cooling, the yellow precipitate was filtered off and washed with cold acetonitrile. Yield 14.2 g. The product was brought into the next step without additional purification. mp 246°C.

6-Mercaptopurine (VIII). A 500-ml flask was charged with 10 g of *S*-(purin-6-yl)thiuronium chloride (**VII**) and 130 ml of anhydrous ethanol, and the mixture was heated for 2 h under reflux. The mixture was cooled, and the precipitate of 6-mercaptopurine was filtered off and reprecipitated from water. Yield 6 g (81%), mp >300°C. IR spectrum, ν, cm^{-1}: 3000, 2679, 1615, 1529, 1409, 1346, 1224, 1015, 877.

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