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SYNTHESIS OF AN ANTI–VIRAL PURINE DERIVATIVE FROM A PYRIMIDINE COMPOUND

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ABSTRACT

The main purpose of the research was to synthesise guanine, a purine derivative via simple intermediates to be used for large–scale production of gancyclovir and other synthetic purine anti–viral derivatives. The synthetic pathway involved formation of the pyrimidine heterocycle (2, 4–diamino–6–hydroxy pyrimidine) (**1**) from the reaction of ethyl cyanoacetate with guanidine nitrate as the first step. Subsequent reactions on the pyrimidine ring in three more steps, eventually produced guanine (**4**) with high yield and good purity.

Key words: Guanine, Purine derivative, Anti–viral drug, Imidazole

INTRODUCTION

Most of purine derivatives are found to posses some anti–viral and anti–tumor activity. Gancyclovir, synthesized in 1982 for the first time[1–4], is an anti–viral drug that is best known for the treatment of cytomegalovirus (CMV) in AIDS and is also used in the gene therapy of cancer. Many reactions can lead to the synthesis of this compound with different degree of ease and yield. It is known that the mechanism of action of this purinic drug is mostly via phosphorylation by cellular enzymes in infected cells.

Gancyclovir has been synthesized in our laboratory from some purine derivatives in different ways[5]. The total synthesis of gancyclovir involves the formation of purine structure followed by attachment of the side chain[6–8]. In this piece of research, a simple and economical pathway has been proposed for production of guanine, one of the starting materials for large–scale production of gancyclovir, from a guanidine precursor.

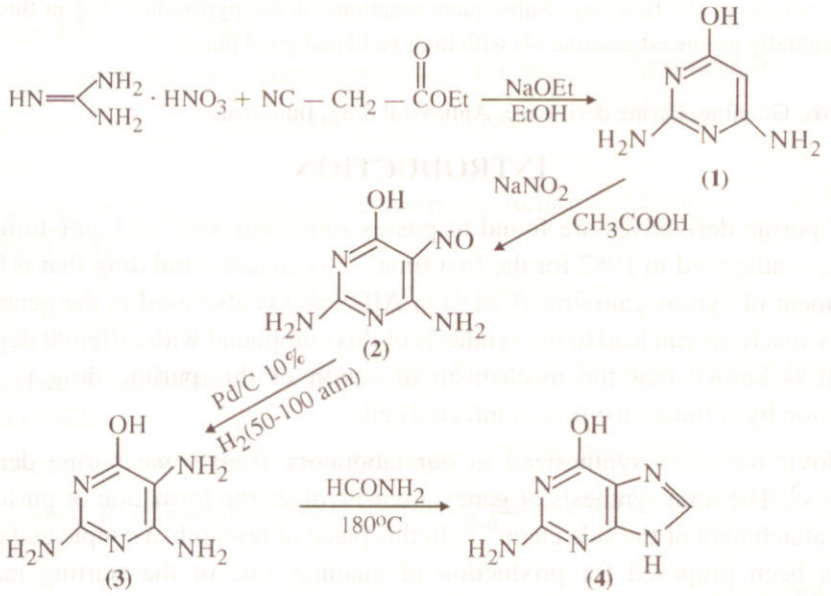
In general, the purine derivatives can be synthesized in two ways. The first stategy is synthesis of pyrimidine ring, followed by the formation of imidazole ring. The second pathway uses formation of imidazole ring as the main structural unit and attachment of pyrimidine ring in a subsequent stage.

In this research, the first strategy for synthesis of guanine has been used, i.e. the pyrimidine structure was first synthesized from simple starting materials. Guanine (**4**), the purine derivative

was synthesized from guanidine nitrate and ethyl cyanoacetate as the starting materials in four steps (Scheme 1). In the first step, a reaction between guanidine nitrate and ethyl cyanoacetate formed a pyrimidine ring (2, 4–diamino–6–hydroxy pyrimidine) (**1**), in a very high yield. In the second step, the reaction of sodium nitrate with (**1**) in an acidic medium lead to the synthesis of 2,4–di amino–6–hydroxy–5–nitroso pyrimidine (**2**) The yield of this step was also high enough for its product to be used for the next step. Hydrogenation of (**2**) in the presence of carbon–palladium produced 2, 4, 5–triamino–6–hydroxy pyrimidine sulphate (**3**) Finally, the reaction of (**3**) with formamide at a relatively high temperature caused a ring closure and the synthesis of guanine (**4**), the final product with a good yield.

METHODS

^{1}H NMR spectra were recoded on a Bruker AMX 400 spectrometer at 400 MHz with DMSO–d_6 as the solvent using tetramethylsilane (TMS) as an internal standard (chemical shifts in ppm). The IR spectra were recoded on a Perkin - Elmer Model 457 infrared spectrophotometer.



Scheme 1 The total reaction pathway used to synthesize guanine (**4**) from 2,4–di–amino–6–hydroxy pyrimidine (**1**).

Synthesis of 2,4–diamino–6–hydroxy pyrimidine (1). A 2000 mL flask was placed in an ice bath, equipped with a condenser and a calcium tube to prevent any moisture entry. Ethanol (1000 mL) was added drop–wise from a funnel to the flask containing small portions of cleaned sodium (30 g) in order to dissolve the metal and to form sodium ethoxide. Ethyl cyanoacetate (72.8 g) was added slowly to the vigorously stirred sodium ethoxide solution. A white

precipitate was formed to which guanidine nitrate (78.4 g) was added and refluxed for 3 hr in an oil bath. The excess ethanol was distilled off in reduced pressure. The precipitate was completely dissolved in distilled water (500 mL). To dissolve the impurities and to form the product, acetic acid was added until pH 5.0 was reached. The precipitated 2,4–diamino–6–hydroxy pyrimidine, was filtered and dried.

Yield : 95%

IR (KBr, cm^{-1}): 3390, 1721.4, 1617, 1539, 1257.4, 1031.1, 971.5, 840.8, 621.5

Synthesis of 2,4–diamino–6–hydroxy–5 nitroso pyrimidine (2). In a three liter beaker containing 1200 mL water, 2,4–diamino–6–hydroxy pyrimidine was added (60 g). A solution of sodium nitrite (34 g) in distilled water (100 mL) was then added to the beaker and while stirred with magnetic stirrer, glacial acetic acid (40 mL) wad intorduced drop–wise. The colour of the solution was changed to red as a result of acetic acid addition. The reaction mixture was stirred at room temperature for 16 hr. The precipitate was filtered, washed with distilled water and ethanol and finally dried.

Yield: 59 g (80%)

IR (KBr, cm^{-1}): 3200, 1700, 1625, 1310, 1260, 1150, 790, 690, 535, 410.

Synthesis of 2, 4, 5–triamino–6–hydroxy pyrimidine sulphate (3). To a beaker containing hot (80°C) distilled water (440 mL), 2,4–diamino–6–hydroxy–5–nitroso pyrimidine (52 g) was added. The beaker was placed in a water bath (90°C) and the suspension was stirred with a magnetic stirrer for at least 1 hr. A solution of 20% sodium hydroxide (46 mL) was added to the mixture in order to increase the solubility of the suspension. Sodium dithionite (15 g) was then added in 20 g portions and 20 minutes intervals. The suspension was vigorously stirred at 80°C until the addition was completed in order to prevent the mixture to become too viscose. The gradual reduction of the nitroso compound gradually faded the red colour of the suspension. The beaker contents were remained at 80°C after all of sodium dithionite was added to ensure the complete reduction of the nitroso compound. The product was boiled and the hot mixture was filtered through buchner funnel while hot. The supernatant was cooled, filtered and washed with cold water. Sulphuric acid (56 mL concentrated in 500 mL water) was added to the precipitate and its solution and the mixture was heated in a boiling water bath for 90 minutes. The precipitate 2, 4, 5–triamino–6–hydroxy pyrimidine sulphate was washed with distilled water and ethanol followed by drying at 80°C.

Yield 70%, mp > 300°C

IR (KBr, cm^{-1}): 3321.8, 3093.6, 2728, 2510.2, 1669.7, 1586, 1129.9, 1078.8, 541.1.

Synthesis of guanine (4). In a one liter flask equipped with a condenser, 2,4,5–tri amino–6–hydroxy pyrimidine sulphate (50 g) and formamide (250 mL) were placed. The flask was heated in an oil bath at about 170–180°C for 2 hr. Guanine was formed during the time and

filtered after being cooled. The product was purified by dissolving in sodium hydroxide to which active carbon was also added and stirred at 60°C for 1 hr. The solution was filtered and formic acid was added to the supernatant drop–wise until the pH reached 9.5. Guanine, which was precipitated at this pH, was then filtered. The product was washed with distilled water and ethanol and dried at 80°C. The purified product was 31.8 g.

Yield 94%, mp > 200°C

IR (KBr, cm^{-1}): 3320.9, 3118.4, 2695, 1674, 1565, 1417.5, 1262.3, 1174, 778.5

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