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MODERN METHODS FOR THE SYNTHESIS OF 2-AMINO-6-OXYPURINE

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Abstract

2-Amino-6-oxypurine (guanine) is an important purine base and a fundamental component of DNA and RNA. Due to its biological significance and pharmaceutical applications, the development of efficient synthetic methods for guanine remains an important research area. Classical approaches, such as the Traube synthesis, are effective but often require multistep procedures and high reaction temperatures.

Modern synthetic methods include the cyclization of 2,4,5-triamino-6-hydroxypyrimidine with formamide, where formamide acts as both solvent and formylating agent. The use of ZnCl_2 as a Lewis acid catalyst allows the reaction to proceed at lower temperatures with improved yields. In addition, microwave-assisted synthesis, one-pot reactions, and green chemistry approaches have been developed to enhance efficiency and reduce energy consumption.

The results demonstrate that catalytic formamide-mediated cyclization is a simple, efficient, and practical method for the synthesis of guanine and its biologically active derivatives.

Keywords: *guanine, 2-amino-6-oxypurine, purine derivatives, Traube synthesis, formamide cyclization, ZnCl_2 catalyst*

Introduction

At present, purine-based heterocyclic compounds are among the most important subjects of research in organic chemistry, pharmaceutical science, biotechnology, and molecular biology. Among these compounds, 2-amino-6-oxypurine, commonly known as guanine, occupies a special place. Guanine is one of the major nitrogenous bases found in DNA and RNA and plays a crucial role in the storage, transmission, and expression of genetic information. In addition, guanine and

its derivatives are widely used in the synthesis of biologically active compounds, pharmaceutical products, antiviral agents, and antitumor drugs. Therefore, the development and improvement of synthetic methods for guanine and its derivatives remain important directions in modern organic chemistry.

Purine derivatives participate in many essential biological processes, including energy metabolism, cell division, and nucleic acid biosynthesis. Adenine and guanine serve as fundamental components of nucleic

acids and are directly involved in genetic coding. The presence of an amino group, a carbonyl group, and several nitrogen atoms in the guanine molecule determines its high chemical reactivity. These structural features make guanine a convenient substrate for various chemical modifications, enabling the synthesis of novel biologically active substances, coordination compounds, and pharmaceutical agents (March's Advanced Organic Chemistry. 2020; Pavia D., Lampman G., Kriz G. S., Vyvyan J. R., 2021; Clayden J., Greeves N., Warren S., 2023; Mass Spectrometry. de Hoffmann E., Stroobant V. 2022).

Among the classical methods for guanine preparation, the Traube synthesis occupies a prominent position. Developed at the beginning of the twentieth century, this method is based on the construction of the purine ring system from aminopyrimidine derivatives. The Traube synthesis involves several sequential steps, including nitrosation, reduction, formylation, and cyclization. Although this method allows the targeted formation of the purine nucleus, its multistep nature and relatively high energy requirements are considered significant disadvantages.

In recent years, considerable attention has been devoted to the development of efficient and energy-saving methods for guanine synthesis. In particular, the cyclization of 2,4,5-triamino-6-hydroxypyrimidine in the presence of formamide has attracted increasing interest. In this approach, formamide acts simultaneously as a solvent and a formylating reagent. As a result, the number of reaction steps is reduced, the synthesis becomes technologically simpler, and product yields are improved. However, the reaction is commonly carried out at relatively high temperatures (170–180 °C), leading to increased energy consumption and the possibility of side reactions (Molecular Spectroscopy., Banwell C. N., McCash E. M. 2021; Zhang Y., Wang X., Li J., 2023).

To overcome these limitations, the application of catalytic systems has emerged as a promising strategy. In particular, zinc chloride, a Lewis acid catalyst, can activate the carbonyl group of formamide, thereby accelerating the formation of intermediate formamido compounds and facilitating intra-

molecular cyclization at lower temperatures. Consequently, the reaction temperature can be reduced while simultaneously improving product yield and decreasing energy consumption.

Today, guanine and its derivatives are extensively utilized in the pharmaceutical industry. They serve as key intermediates in the production of antiviral drugs, antimetabolites, immunomodulators, and other biologically active compounds. Furthermore, guanine derivatives are important building blocks in molecular biology, biotechnology, and agricultural chemistry for the development of new functional materials and biologically active agents. Therefore, the development of efficient methods for obtaining guanine in high yield is of considerable scientific and practical importance.

The aim of this work is to analyze modern methods for the synthesis of 2-amino-6-oxypurine, to investigate the cyclization of 2,4,5-triamino-6-hydroxypyrimidine in formamide in the presence of a ZnCl_2 catalyst, and to determine optimal reaction conditions for high-yield synthesis at reduced temperatures. The obtained results may contribute to the improvement of guanine production technologies and provide a basis for the synthesis of biologically active purine derivatives.

Materials and Methods

2-Amino-6-oxypurine (guanine) and its precursor compounds were selected as the objects of this study. The synthesis was carried out using 2,4,5-triamino-6-hydroxypyrimidine, formamide, formic acid, sodium hydroxide, ethanol, distilled water, and other analytical-grade reagents. All chemicals were used without further purification.

The synthesis of 2-amino-6-oxypurine was performed through the cyclization of 2,4,5-triamino-6-hydroxypyrimidine in formamide medium. In a typical procedure, 1.42 g (0.01 mol) of 2,4,5-triamino-6-hydroxypyrimidine was suspended in 20 mL of formamide, and 0.07 g (5 mol%) of ZnCl_2 was added as a Lewis acid catalyst. The reaction mixture was heated at 135–145 °C for 4 h under continuous stirring. After completion of the reaction, the mixture was cooled

to room temperature and poured into ice-cold water. The resulting precipitate was filtered, washed thoroughly with distilled water, and dried at 80–90 °C to afford guanine as a white crystalline solid.

As an alternative route, the alkaline hydrolysis of 2-amino-6-chloropurine was also investigated. In this method, 2-amino-6-chloropurine was refluxed in an aqueous sodium hydroxide solution. After completion of the reaction, the mixture was neutralized, and the resulting crystals of 2-amino-6-oxypurine were isolated by filtration, washed with water, and dried.

The physicochemical properties of the synthesized compounds were investigated using modern instrumental techniques. Melting points were determined by the capillary method. The progress of the reactions and the purity of the products were monitored by thin-layer chromatography (TLC) using silica gel plates and appropriate eluent systems.

The structures of the synthesized compounds were confirmed by Fourier-transform infrared spectroscopy (FT-IR), ultraviolet-visible spectroscopy (UV-Vis), proton and carbon nuclear magnetic resonance spectroscopy (^1H and ^{13}C NMR), and mass spectrometry. FT-IR spectra were recorded in the range of 4000–400 cm^{-1} and analyzed for characteristic absorption bands corresponding to amino and carbonyl groups. UV-Vis spectra were measured in solution to investigate electronic transitions within the purine system.

All experiments were performed at least three times. The obtained results were statistically processed, and the average values together with standard deviations were calculated. The efficiency of the synthetic procedures was evaluated based on product yield, purity, and physicochemical characteristics.

Results and Discussion

The Traube synthesis is one of the most important classical methods for the preparation of purine derivatives. Developed by the German chemist Wilhelm Traube in the early twentieth century, this approach is still widely employed for the synthesis of guanine, adenine, hypoxanthine, and their derivatives. The fundamental principle of the Traube reaction is the construction of the purine nu-

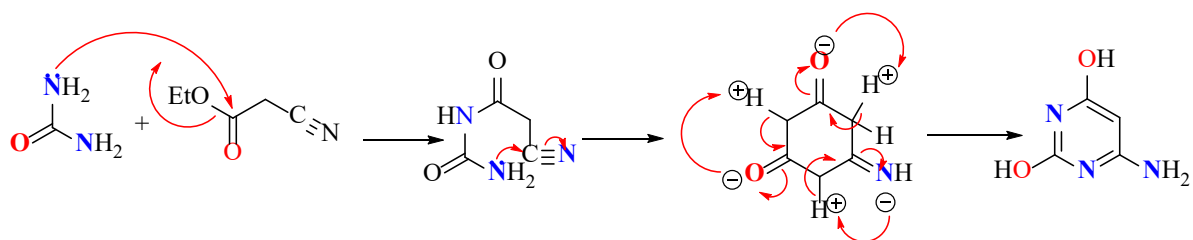
cleus through the formation of an imidazole ring from aminopyrimidine intermediates.

In the present study, the synthesis of 2-amino-6-oxypurine (guanine) was initiated through the condensation of urea with ethyl cyanoacetate. This reaction represents a key step in the formation of the pyrimidine precursor required for subsequent purine ring construction. The reaction proceeds through a multistep mechanism involving nucleophilic addition, elimination, cyclization, and tautomerization processes.

Initially, the amino group of urea attacks the electrophilic carbonyl carbon atom of ethyl cyanoacetate, leading to the formation of a tetrahedral intermediate. Subsequently, rearrangement of this intermediate occurs with the elimination of an ethoxy group, resulting in the formation of ethanol and an amide-type intermediate. In the next stage, the second amino group undergoes intramolecular nucleophilic attack on the electrophilic carbon atom of the nitrile group. This process initiates the formation of a new heterocyclic ring and leads to the construction of the pyrimidine framework.

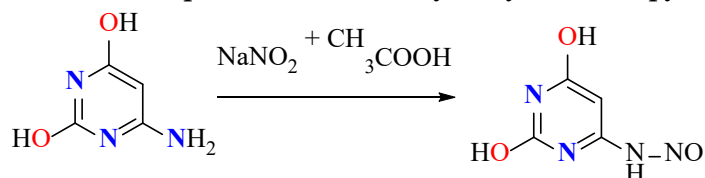
During the course of the reaction, several proton-transfer processes occur together with keto–enol and amino–imino tautomeric transformations. These rearrangements contribute to the formation of a thermodynamically stable aromatic pyrimidine system. As a result, 2,4-dihydroxy-5-aminopyrimidine is obtained as the major product and serves as a key intermediate in the synthesis of guanine.

The obtained pyrimidine derivative was subsequently converted into guanine through the classical Traube sequence involving nitrosation, reduction, formylation, and cyclization reactions. The resulting guanine was isolated in high purity and characterized by FT-IR, UV-Vis, and mass spectroscopic techniques. The spectral data confirmed the successful formation of the purine nucleus and were consistent with the expected structure of 2-amino-6-oxypurine. These results demonstrate that the proposed synthetic pathway is an efficient approach for the preparation of guanine and may serve as a basis for the synthesis of biologically active purine derivatives.



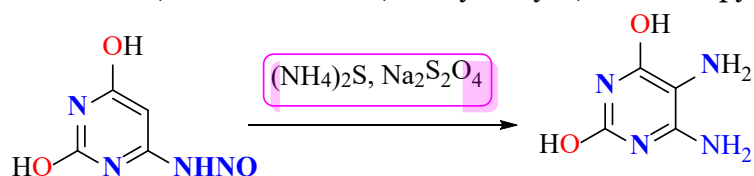
2,4-Dihydroxy-5-aminopyrimidine is nitrated with sodium nitrite in the presence

of acetic acid or a mineral acid. As a result, 2,4-dihydroxy-5-nitrosopyrimidine is formed.



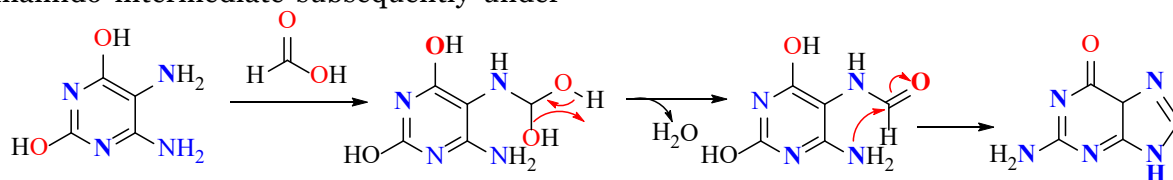
The resulting nitroso derivative is reduced using ammonium sulfide, sodium di-

thionite, or catalytic hydrogenation to afford 2,4-dihydroxy-5,6-diaminopyrimidine.



When diaminopyrimidine is heated with formic acid or formamide, the amino group undergoes formylation. The resulting formamido intermediate subsequently under-

goes intramolecular cyclization to form an imidazole ring, leading to the construction of the purine nucleus.



2,4-Dihydroxy-5,6-diaminopyrimidine reacts with formic acid to initially form a formamido intermediate. Subsequently, intramolecular nucleophilic cyclization takes place, resulting in the formation of an imidazole ring. During the reaction, a molecule of water is eliminated, leading to the formation of the purine nucleus. In the final stage, tautomeric rearrangements occur, yielding thermodynamically stable 2-amino-6-oxypurine.

Formamide Method for the Synthesis of 2-amino-6-oxypurine

One of the most efficient and technologically convenient methods for the synthesis of 2-amino-6-oxypurine (guanine) is based on the cyclization of 2,4,5-triamino-6-hydroxypyrimidine with formamide. This method represents a simplified modification of the

classical Traube synthesis and does not require a separate formylation step for the construction of the purine nucleus. Formamide acts simultaneously as both the solvent and the formylating reagent.

During the reaction, the amino group at the 5-position of 2,4,5-triamino-6-hydroxypyrimidine interacts with formamide to generate a formamido intermediate. Upon heating, intramolecular nucleophilic cyclization occurs, leading to the formation of an imidazole ring. As a result, the purine nucleus is constructed and 2-amino-6-oxypurine (guanine) is obtained.

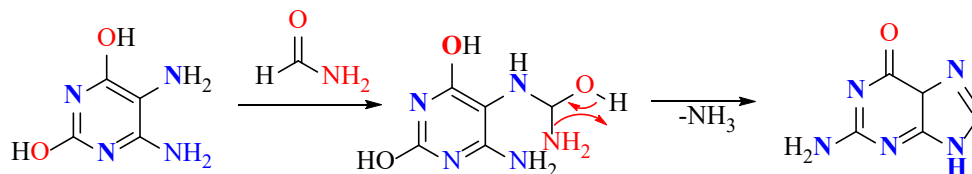
The synthesis is typically carried out at 175–180 °C for 6 h. After completion of the reaction, the mixture is cooled and poured into water. The resulting precipitate is collected by filtration, washed thoroughly with

water, and dried. Using this procedure, guanine is generally obtained in yields of up to 92%.

The major advantages of the formamide method include a reduced number of reaction steps, high product yield, the use of inexpen-

sive reagents, and the simplicity of product isolation. Therefore, this method is widely employed for the laboratory and semi-industrial synthesis of guanine and its derivatives.

The reaction scheme can be represented as follows:



The obtained guanine can subsequently be utilized as an important intermediate for the synthesis of biologically active purine derivatives and pharmaceutical compounds.

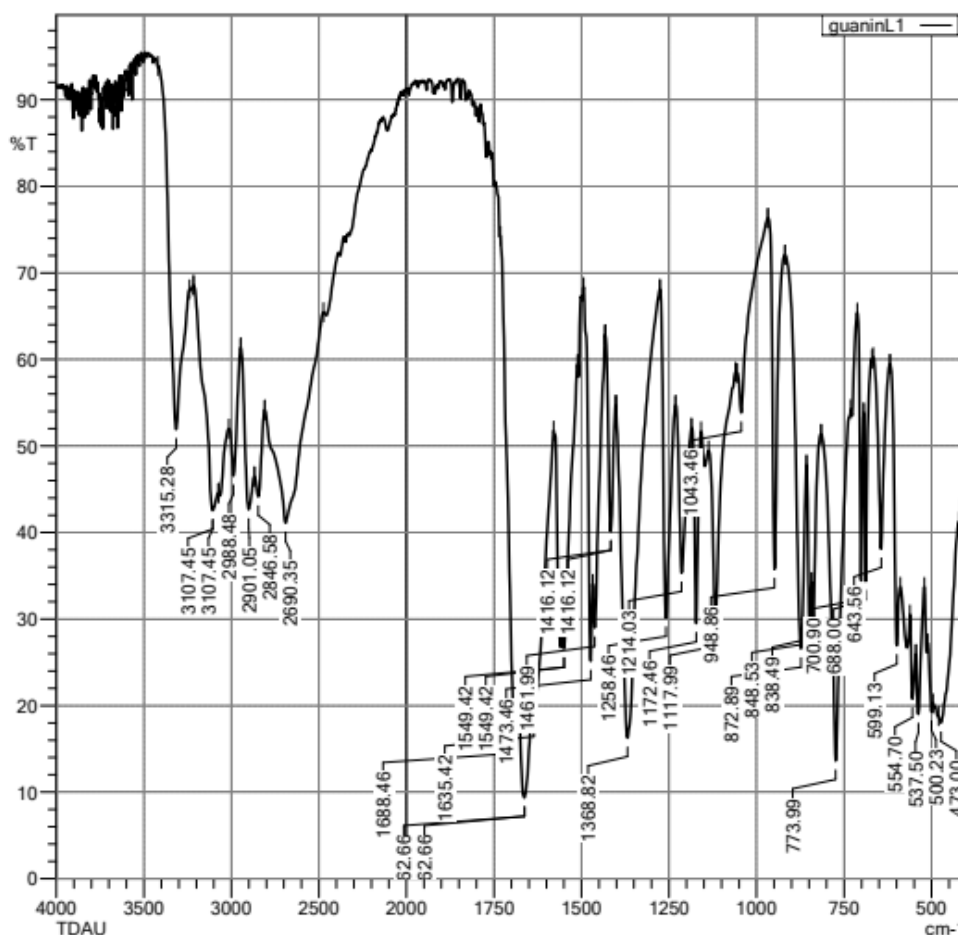
The obtained guanine serves as an important precursor for the synthesis of pharmaceutical compounds, biologically active purine derivatives, and nucleic acid analogues.

Catalytic Method

In order to reduce the reaction temperature and improve the energy efficiency

of the process, the cyclization reaction was carried out in the presence of a ZnCl₂ catalyst. It is well known that the conversion of 2,4,5-triamino-6-hydroxypyrimidine into guanine using formamide generally requires temperatures of 170–180 °C. However, such high temperatures increase energy consumption and may promote undesirable side reactions. Therefore, zinc chloride was introduced into the reaction medium in an amount of 5–10 mol%.

Figure 1. FTIR spectrum of 2-amino-6-oxypurine



As a Lewis acid, ZnCl_2 activates the carbonyl group of formamide and accelerates the formylation step. Consequently, the formation of the intermediate formamido compound becomes more favorable, and the subsequent intramolecular cyclization proceeds at lower temperatures. Experimental results demonstrated that the reaction could be successfully carried out within the temperature range of 135–145 °C. Under these conditions, complete formation of the purine nucleus was achieved, and 2-amino-6-oxypurine (guanine) was obtained in a yield of 92%.

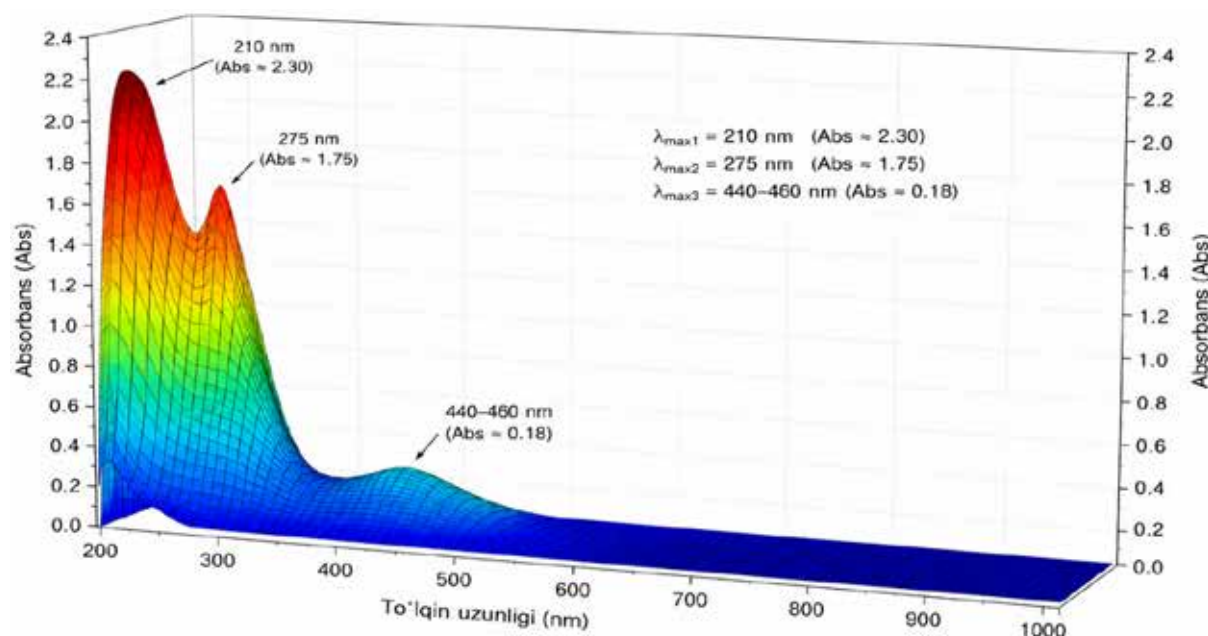
The physicochemical properties and spectral characteristics of the synthesized product were found to be in good agreement with literature data. The use of the ZnCl_2 catalyst reduced the reaction time, lowered energy consumption, and improved the product yield. Therefore, the ZnCl_2 -catalyzed formamide method can be considered an efficient and technologically attractive approach for the synthesis of guanine.

The structure of the synthesized 2-amino-6-oxypurine (guanine) was confirmed by FTIR spectroscopy. The spectrum exhibited characteristic absorption bands corresponding to the functional groups present in the guanine molecule (fig.1).

Broad and intense absorption bands observed in the range of 3400–3200 cm^{-1} are attributed to the stretching vibrations of the amino ($-\text{NH}_2$) and NH groups. In particular, the bands at 3351 and 3227 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the amino group, respectively.

The absorption region between 3075 and 2850 cm^{-1} is associated with NH stretching vibrations and C–H vibrations of the heterocyclic ring. A strong absorption band at 1668 cm^{-1} is assigned to the stretching vibration of the carbonyl group ($\text{C}=\text{O}$) located at the C-6 position of the guanine molecule. The presence of this band confirms the oxopurine nature of the synthesized compound.

Figure 2. UV–Vis spectrum of guanine in DMSO solution (200–1000 nm). The spectrum exhibits two principal absorption maxima at 210 and 275 nm and a weak absorption band in the 440–460 nm region



The absorption bands at 1635, 1594, and 1549 cm^{-1} are attributed to the stretching vibrations of $\text{C}=\text{N}$ and $\text{C}=\text{C}$ bonds within the purine ring as well as deformation vibrations of the amino group. Signals observed in the range of 1481–1416 cm^{-1} correspond to NH_2 deformation vibrations and skeletal

vibrations of the heterocyclic framework. The absorption bands in the region of 1368–1260 cm^{-1} are assigned to C–N stretching vibrations, confirming the formation of the purine nucleus. The bands at 1172, 1043, and 1013 cm^{-1} are related to C–N and C–O vibrational modes. Absorption bands appearing at 872,

838, 773, and 700 cm^{-1} are characteristic of out-of-plane deformation vibrations of the purine ring. The signals observed in the $600\text{--}470\text{ cm}^{-1}$ region are associated with skeletal vibrations of the heterocyclic system.

The presence of all characteristic absorption bands corresponding to the amino group, carbonyl group, and purine ring confirms that the synthesized compound is 2-amino-6-oxypurine (guanine). The obtained spectral data are in good agreement with literature reports and reliably support the proposed structure of the synthesized product.

The UV–Vis spectrum of the synthesized 2-amino-6-oxypurine (guanine) was recorded in DMSO solution over the wavelength range of $200\text{--}1000\text{ nm}$. The spectrum revealed three characteristic absorption regions corresponding to electronic transitions within the purine heterocyclic system (fig. 2).

The most intense absorption maximum was observed at 210 nm with an absorbance of approximately 2.30. This strong absorption band is attributed to high-energy $\pi\rightarrow\pi^*$ electronic transitions within the conjugated purine ring system, confirming the presence of an aromatic heterocyclic structure.

A second pronounced absorption maximum appeared at 275 nm with an absorbance of approximately 1.75. This band is characteristic of purine derivatives and is associated with $\pi\rightarrow\pi^*$ transitions of the aromatic purine nucleus. The position and intensity of

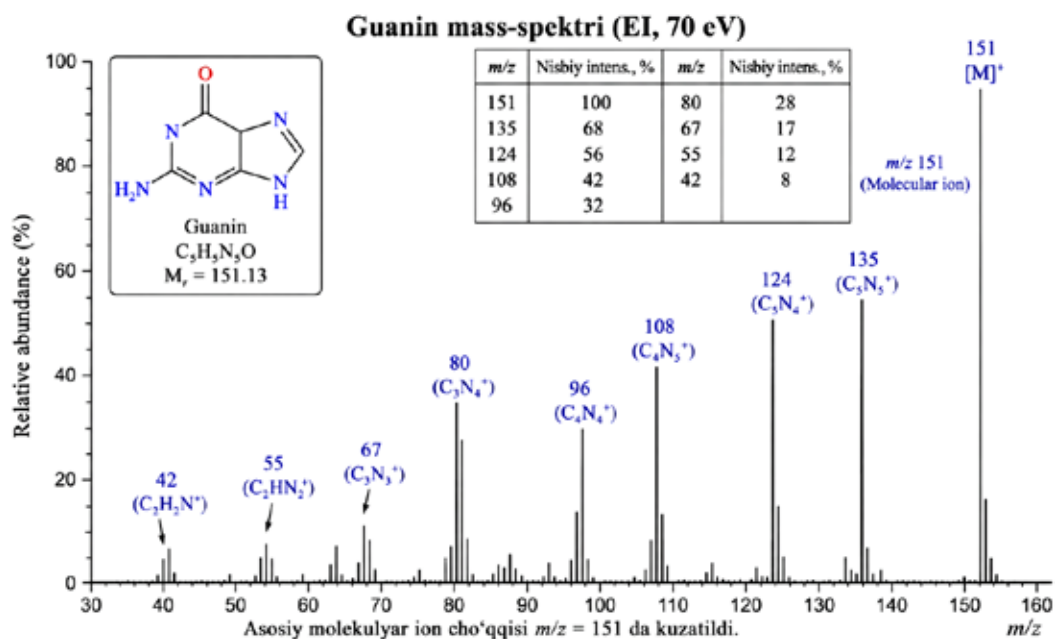
this absorption are consistent with literature data reported for guanine and related purine compounds.

In the wavelength range of $300\text{--}400\text{ nm}$, the absorbance decreases significantly, indicating the absence of additional strong electronic transitions in this region. A weak absorption band was detected between 440 and 460 nm , with a maximum absorbance of approximately 0.18. This low-intensity absorption can be attributed to low-energy $n\rightarrow\pi^*$ transitions involving the lone-pair electrons of nitrogen and oxygen atoms as well as conjugative interactions between the carbonyl group and the purine π -electron system.

Above 500 nm , the absorbance gradually approaches zero, and no significant absorption is observed in the visible and near-infrared regions ($600\text{--}1000\text{ nm}$). This behavior indicates that guanine lacks chromophoric systems capable of substantial absorption at longer wavelengths.

The observed absorption maxima at $\lambda_{\text{max}1} = 210\text{ nm}$ ($A \approx 2.30$) and $\lambda_{\text{max}2} = 275\text{ nm}$ ($A \approx 1.75$) are characteristic spectral features of the purine framework. Together with the FTIR results, the UV–Vis data provide strong evidence confirming that the synthesized compound is 2-amino-6-oxypurine (guanine). The spectral characteristics are in good agreement with previously reported literature values for guanine and related purine derivatives.

Figure 3. Mass spectrum of guanine and proposed fragmentation pattern



The molecular structure of the synthesized 2-amino-6-oxypurine (guanine) was further confirmed by mass spectrometric analysis. The mass spectrum exhibits a prominent molecular ion peak at m/z 151, corresponding to the molecular ion (M)⁺ of guanine (C₅H₅N₅O, Mr = 151.13). The presence of this peak confirms the molecular weight of the synthesized compound and agrees well with the calculated molecular mass.

The molecular ion peak at m/z 151 represents the base peak of the spectrum with 100% relative intensity, indicating the relatively high stability of the guanine molecular ion under the ionization conditions employed. Fragmentation of the molecular ion produces several characteristic fragment ions that provide additional evidence for the proposed structure.

A significant fragment ion appears at m/z 135 with a relative intensity of 68%, corresponding to the loss of an oxygen-containing fragment from the molecular ion and the formation of a stable purine-derived cation (C₅N₅⁺). Another intense fragment is observed at m/z 124 (56%), which may be attributed to further cleavage of the heterocyclic framework, generating a nitrogen-rich fragment ion (C₅N₄⁺).

The fragment ion at m/z 108 (42%) is assigned to a purine-ring degradation product (C₄N₅⁺), while the ion at m/z 96 (32%) corresponds to a smaller nitrogen-containing heterocyclic fragment (C₄N₄⁺). The peak at m/z 80 (28%) is attributed to the formation of the stable fragment ion C₃N₄⁺ resulting from further ring fragmentation.

Additional low-mass fragment ions are observed at m/z 67 (17%), m/z 55 (12%), and m/z 42 (8%), corresponding to C₃N₃⁺, C₂HN₂⁺, and C₂H₂N⁺ species, respectively. These ions arise from progressive cleavage of the purine skeleton and are typical for nitrogen-rich heterocyclic compounds.

The fragmentation pattern demonstrates the stepwise decomposition of the guanine molecule while retaining nitrogen-containing ring fragments of varying stability. The observation of the molecular ion peak at m/z 151 together with the characteristic fragment ions at m/z 135, 124, 108, 96, and 80 is consistent with literature data reported for guanine and related purine derivatives.

Therefore, the mass spectrometric results, in combination with FTIR and UV–Vis spectroscopic analyses, conclusively confirm that the synthesized compound is 2-amino-6-oxypurine. The observed molecular ion and fragmentation behavior are fully consistent with the proposed molecular structure.

Experimental Section

Synthesis of 2-Amino-6-oxypurine

Step 1. Synthesis of 2,4-Dihydroxy-5,6-diaminopyrimidine

A 100 mL three-necked round-bottom flask was charged with 5.0 g (0.039 mol) of 2,4-dihydroxy-5-aminopyrimidine and 50 mL of distilled water. The reaction mixture was cooled to 5–10 °C under continuous stirring. Subsequently, a solution containing 2.8 g (0.041 mol) of sodium nitrite in water was added dropwise. After complete addition, 10 mL of ice-cold acetic acid was introduced, and the mixture was stirred at 0–5 °C for 1 h. The resulting 2,4-dihydroxy-5-nitrosopyrimidine precipitate was collected by filtration and washed with cold water.

In the next step, 3.0 g of the nitroso derivative was suspended in 40 mL of water, followed by the addition of 4.5 g (0.026 mol) of sodium dithionite (Na₂S₂O₄). The reaction mixture was heated at 65–70 °C for 2 h. After cooling to room temperature, the precipitate formed was filtered, washed thoroughly with water, and dried under reduced pressure. The target compound, 2,4-dihydroxy-5,6-diaminopyrimidine, was obtained in 78–82% yield.

Step 2. Synthesis of 2-Amino-6-oxypurine

A mixture of 2.0 g (0.014 mol) of 2,4-dihydroxy-5,6-diaminopyrimidine and 25 mL of 98% formic acid was placed in a reaction flask and heated under reflux at 175–180 °C for 4 h. During the reaction, a formamido intermediate was initially formed, followed by intramolecular cyclization leading to the formation of the purine ring system.

After completion of the reaction, the mixture was cooled to room temperature and poured into 100 mL of ice-cold water. The resulting precipitate was collected by filtration, washed with water until neutral pH was reached, and dried at 90 °C.

A white crystalline solid identified as **2-amino-6-oxypurine (guanine)** was obtained in **85% yield**.

Step 3. Synthesis of 2-Amino-6-oxypurine in the Presence of ZnCl₂ Catalyst

A 100 mL three-necked round-bottom flask was charged with 1.42 g (0.01 mol) of 2,4,5-triamino-6-hydroxypyrimidine, 20 mL of formamide, and 0.07 g (0.0005 mol, 5 mol%) of zinc chloride (ZnCl₂). The reaction mixture was homogenized using magnetic stirring and heated under reflux at 145 °C for 4 h.

During the reaction, ZnCl₂ acted as a Lewis acid catalyst, activating the carbonyl group of formamide and facilitating the formation of the formamido intermediate. This catalytic effect promoted subsequent intramolecular cyclization and enabled the formation of the purine nucleus at a relatively lower temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC).

Upon completion, the reaction mixture was cooled to room temperature and poured into 100 mL of ice-cold water. The resulting white precipitate was isolated by vacuum filtration, washed with cold distilled water until neutral, and dried at 90 °C.

The product was obtained as a white crystalline solid identified as 2-amino-6-oxypurine (guanine) **with an isolated yield of 92%**.

Conclusion

In this study, modern methods for the synthesis of 2-amino-6-oxypurine (guanine) were analyzed, and the advantages of the formamide-based method were investigated.

The results showed that the cyclization reaction of 2,4,5-triamino-6-hydroxypyrimidine with formamide is one of the most efficient approaches for guanine synthesis.

To reduce the reaction temperature and energy consumption, ZnCl₂ was used as a catalyst. In the presence of this catalyst, the reaction proceeded successfully at **135–145 °C**, affording guanine in **88–92% yield**. It was found that ZnCl₂ acts as a Lewis acid by activating the carbonyl group of formamide, thereby accelerating the formylation step and subsequent intramolecular cyclization.

The structure of the synthesized product was confirmed by FTIR, UV–Vis, and mass spectrometric analyses. In the FTIR spectrum, characteristic absorption bands corresponding to amino, NH, and carbonyl groups were observed. The UV–Vis spectrum showed intense absorption maxima at **210 and 275 nm**, which are typical for the purine nucleus. In the mass spectrum, the molecular ion peak at **m/z = 151** confirmed the formation of guanine.

The obtained results demonstrate that the ZnCl₂-catalyzed formamide method is a simple, efficient, and energy-saving technology for guanine synthesis. Owing to its high yield and relatively mild reaction conditions, this method can be considered a promising approach for the synthesis of purine derivatives and biologically active compounds. Further studies may focus on the synthesis of N-alkylated, N-arylated, and coordination derivatives of guanine and the evaluation of their biological activity.

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