I.A.U
Karaj branch

Journal of
A p p l ied
C hemical
R esearch
jacr.kiau.ac.ir

Journal of Applied Chemical Research, 11, 3, 84-96 (2017)

A Synthetic Approach for Evaluation of Cytosinediazonium Susceptibility

Kamahldin Haghbeen[*1], Shima Rezaeian[2], Mehdi Bakavoli[2], Fatemeh Mirazizi[3]

[1]*Faculty of Basic Sciences of Biotechnology, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran*

[2]*Department of Chemistry, Ferdowsi University of Mashhad, Mashhad, Iran*

[3]*Department of Chemistry, Islamic Azad University, Mashhad branch, Mashhad, Iran*

(Received 11 Dec. 2016; Final version received 18 Mar. 2017)

Abstract

Reaction of a nucleobase with nitrosonium ion leads to its diazotization. Due to the instability, the nucleobase-diazonium assumingly falls in dediazotization and successive ring opening reactions. The resulting intermediate of the ring opening reaction is able to covalently cross-link the opposite strands of DNA. To examine this assumption for cytosine, some 4-amino-5-cyano pyrimidines were synthesized via [3+3] condensation of amidines with ethoxymethylenemalonitrile and successively converted to the corresponding 4-amino-5-carboxyamido derivatives. These compounds were then exposed to nitrosonium ion under different conditions. Results of this study indicate that the diazonium of a 4-aminopyrimidine prefers deamination over the dediazotization. Therefore, the dediazotization and its successive ring-opening reactions of the pyrimidine cation would happen under some exceptional conditions which deserve to be explored in more detailed studies. In view of the cytosine chemistry and cytotoxicity of NO, the results are presented and discussed.

Keywords: *4-Amino-pyrimidine; Cytosine; Deamination; Dediazotization; v-Triazine.*

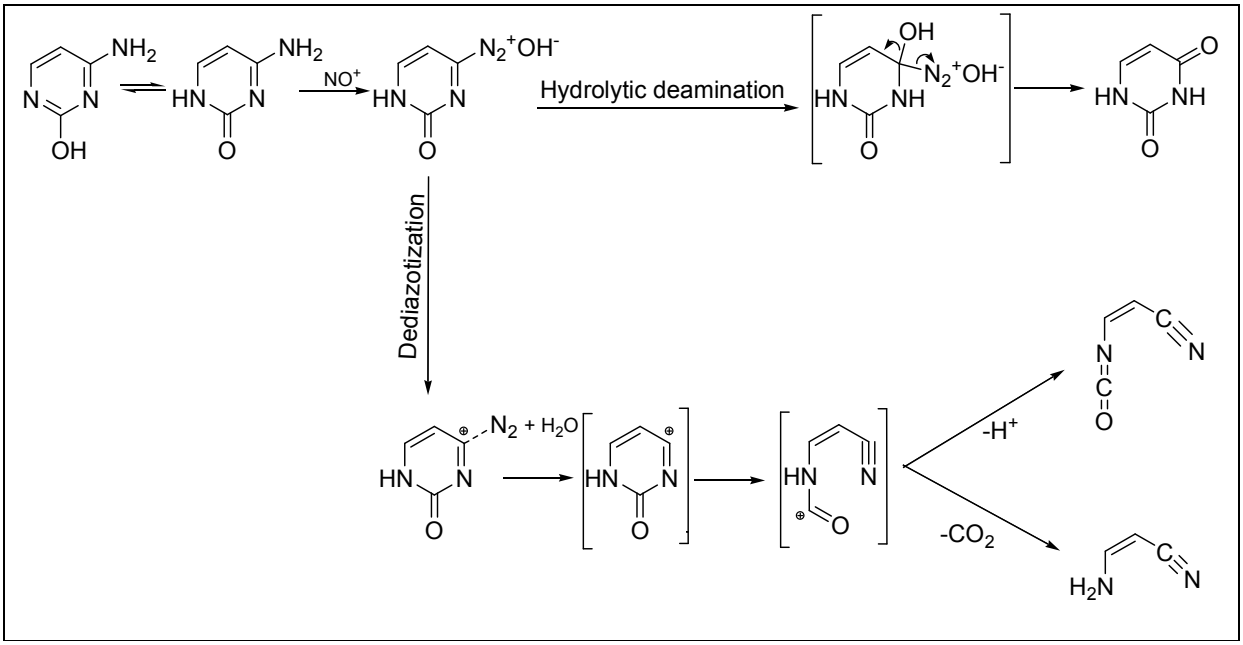
***Corresponding author**: Kamahldin Haghbeen, Faculty of Basic Sciences of Biotechnology, National Institute for Genetic Engineering and Biotechnology, P.O.Box:14165/161, Tehran, Iran. E-mail: Kamahl@nigeb.ac.ir, Tel: +98 21 44580372, Fax: +98 21 44580399.*

Introduction

Nitric oxide (NO) is a well-known natural messenger that plays key roles in some important physiological processes such as inhibition of platelet aggregation, blood vessel relaxation, and neurotransmission. Nevertheless, the NO concentration is a matter of concern [1]. Higher concentration of NO is observed in inflamed cells. Upon infection, NO works as a secondary messenger that enhances the expression of inflammatory genes including TNFα and NF-κB [2]. Higher concentrations of NO could be cytotoxic, too. In the presence of oxygen, NO forms N_2O_3, a notorious nitrosating agent. It has been demonstrated that N_2O_3 can attack pyrimidine and purine bases susceptible to diazotization such as cytosine and guanine [3]. This is similar to the reaction of nitrous acid with aromatic amines. In both reactions, an aromatic diazonium is produced as an intermediate [4]. Nitrous acid is the product of N_2O_3 hydrolysis and also a byproduct of nitrates used as meat preservatives.

Spontaneous deamination of nucleobases such as cytosine requires direct reaction of water with the amino group of the base. This reaction is thermodynamically unfavorable with a half-life of 30,000 years for cytosine in double-stranded DNA [5]. Diazotization changes the amino group to a very good leaving group. Aromatic diazoniums are more stable at temperatures lower than 4°C but their activities increase as the temperature rises [6]. Aromatic diazoniums are susceptible to water molecules, Scheme 1.

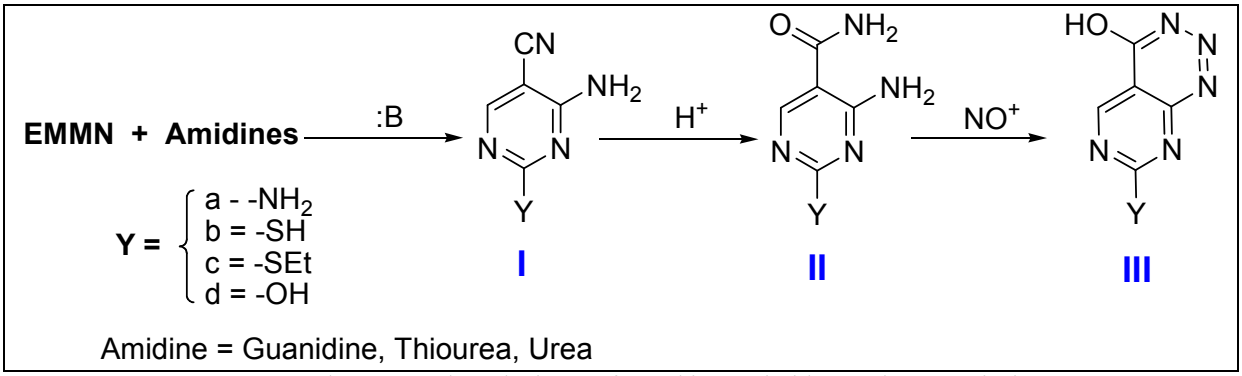
This reaction is extremely important to DNA bases as it paves the way for structural modifications of the DNA. Hydrolytic deamination of cytosine, adenine, guanine, and 5-methylcytosine results in the formation of uracil, hypoxanthine, xanthine, and thymine, respectively [3]. Any modification of DNA structure is potentially cytotoxic as it could result in the advent of errors into the genetic codes leading to mutagenicity [7]. Most of the mutagenic lesions are processed by the DNA repair enzymes. However, the number of the known lesions is expanding and the research has unveiled that the repair system is not completely efficient or free of errors [8].



Scheme 1. Cytosinediazonium can take part in either dediazotization or hydrolytic deamination.

Cytosine deamination is in the core of attention [9]. Despite the invaluable reports on this subject, recent studies on guanine diazotization indicate that the diazotization chemistry of cytosine also deserves more examination [10]; especially, when theoretical calculations seriously question the sustainability of cytosinediazonium ion [11]. This is why the availability of the free electrons of the amino group, at position 4 of pyrimidines, to nitrosonium and the stability of the resulting diazonium ion are two important objectives of this study.

One of the interesting groups of triazines is fused v-triazines. An established synthetic route to this type of heterocyclic compounds is the cyclisation of a diazotized amino group ortho-substituted to a nucleophilic function. Through this method, successful synthesis of pyrido[3,2-d][1,2,3]triazines, pyrimido[5,4-d][1,2,3]triazines, pyrolo[2,3-d][1,2,3]triazines were reported [12-14].



Scheme 2. The proposed synthetic rout for making pyrimido[4,5-d][1,2,3]triazine.

To address the objectives of this research, compounds with the general structure of **II** were synthesized from the corresponding cyano derivatives, **I**, as illustrated in Scheme 2. Then, compounds **II** were exposed to nitrosonium at different conditions. It was assumed that upon diazotization of compounds **II**, the resulting diazonium derivatives would enter intramolecular cyclisation producing pyrimido[4,5-d][1,2,3]triazines (**III**), if they were stable enough, Scheme 2. Electron donating group at position 2 was expected to facilitate diazotization and increase the stability of the anticipated final product. In view of the cytosine deamination chemistry, results of these experiments are presented and discussed.

Experimental

The chemicals used in this work were taken from the authentic samples mainly purchased from the Merck distributor. NMR (^{1}H, ^{13}C) spectra were recorded at ambient temperature on a Brucker (Ultrashield, 300 MHz) spectrometer. Proton and carbon chemical shifts are relative to TMS. FT-IR (Res: 0.025 cm^{-1}) spectra were obtained using a Brucker (Tensor 27) spectrometer. Mass spectra were recorded by a JEOL HX110 double focusing mass spectrometer using Electron Impact ionization. Melting points were recorded with a Gallenhamp capillary melting point apparatus. Elemental analyses of the desired samples were performed in Microanalytical Laboratory of Iran's Research Institute of Petroleum Industry. It is important to mention that a small part of compounds **I** and **II** remained protonated even after repeated recrystalisation because of their basicity and the preparation conditions. This phenomenon affected the results of the elemental analyses. However, the structures of compounds **I** and **II** could be precisely elucidated by the applied spectroscopic methods.

Synthesis of 4-amino-5-cyano pyrimidines

4-Amino-5-cyano pyrimidine derivatives (**I**) were synthesized via [3+3] condensation reactions between ethoxymethylene malonitrile (EMMN) and the desired amidines. However, because of different activities of the amidines toward EMMN, different conditions were applied. Thus, compounds **Ia**, **Ib**, and **Id** were synthesized by mixing 0.05 mole sodium methoxide in the appropriate solvent with 0.05 mole of the desired amidine. To this mixture, 0.05 mole EMMN was added and the reaction was followed under the conditions showed in Table 1. The product precipitated gradually in the reaction medium which was collected and recrystallized from hot water. The structures of the products were elucidated by Mass, FT-IR and FT-NMR. Exchangeable protons were tested by adding D_2O into the DMSO-d^6 solution of the sample under ^{1}HNMR recording conditions.

Table 1. Applied conditions for the reactions of various amidines with EMMN.

Y	Amidine	Solvent	Conditions	Product	Yield (%)
$-NH_2$	Guanidine .HCl	Methanol	Stirring for 8 h at 25°C	Ia	72
-SH	Thio urea	Absolute ethanol	12 h reflux	Ib	75
-OH	Urea	Propanol	24 h reflux	Id	40

*Compound **Ia** (2,4-diamino-5-cyano pyrimidine)*

Yellow powder, m.p. decomposed above 320 °C, m/e = 135, IR (KBr): pyrimidine ring (1591, 1550, 1056, 788 cm^{-1}), amine groups (3320, 3435, 1656, 545 cm^{-1}), cyan group (2204 cm^{-1}), ^{1}HNMR in DMSO-d^6: δ 8.14 (s, 1H, aromatic proton), 6.90 and 7.07 (br, 2H, amine groups at 2 and 4 position, respectively). ^{13}CNMR in DMSO-d^6: δ 165.27 (C_2), 165.86 (C_4), 108.21 (C_5), 162.25 (C_6), 118.98 (CN). *Anal*. Calcd for $C_5H_5N_5$: C (44.44), H (3.73), and N (51.83); found: C (44.17), H (3.65), and N (49.60).

*Compound **Ib** (2-thio-4-amino-5-cyano pyrimidine)*

Bright yellow powder, m.p. 280-283 °C, m/e = 152, IR (KBr): pyrimidine ring (1608, 1545, 1056, 769 cm^{-1}), amine group (3333, 3225, 1641, 555 cm^{-1}), cyan group (2229 cm^{-1}), ^{1}HNMR in

DMSO-d^6: δ 8.41 (br, 1H, thiol proton), 8.24 (s, 1H, aromatic proton), 7.88 (br, 2H, amine protons). ^{13}CNMR in DMSO-d^6: δ 180.01 (C_2), 158.94 (C_4), 81.34 (C_5), 150.55 (C_6), 114.47 (CN). *Anal*. Calcd for $C_5H_4N_4S$: C (39.46), H (2.65), N (36.82), and S (21.07); found: C (38.54), H (2.51), N (35.85), and S (20.11).

*Compound **Ic** (2-ethylthio-4-amino-5-cyano pyrimidine)*

Pale yellow powder, m.p. 210-212 °C, m/e = 180, IR (KBr): pyrimidine ring (1617, 1574, 956, 806 cm^{-1}), amine group (3344, 3200, 1640, 540 cm^{-1}), cyan group (2224 cm^{-1}), alkyl group (2930, 2874, 1373 cm^{-1}), ^{1}HNMR in $CDCl_3$: δ 8.33 (s, 1H, aromatic proton), 5.17 (br, 2H, amine group), 3.18 (q, 2H, methylen group), 1.32 (t, 3H, methyl group). ^{13}CNMR in DMSO-d^6: δ 175.95 (C_2), 161.03 (C_4), 88.27 (C_5), 152.60 (C_6), 116.42 (CN), 22.71 (-CH_2), 15.10 (-CH_3). *Anal*. Calcd for $C_7H_8N_4S$: C (46.65), H (4.47), N (31.09), and S (17.79); found: C (46.34), H (4.43), N (30.99), and S (17.68).

The synthesis of **Ic** was achieved through ethylation of **Ib** by ethyl iodide. However, because of the thiol-thione tautomery in the starting material [15], the reaction resulted in formation of two isomers. Stirring C_2H_5I in aqueous solution of the sodium salt of **Ib** for 3 hours at ambient temperature produced a precipitate which contained two isomers. Opposite to the N-ethyl isomer, 2-ethylthio isomer was soluble in ethanol which after evaporization of the solvent and recrystallization from hot water produced a bright yellow solid with a melting point of 210-2°C.

Compound **Id** (2-hydroxy-4-amino-5-cyano pyrimidine): Brown powder, decomposed above 310 °C, m/e = 136, IR (KBr): pyrimidine ring (1640, 1580, 1024, 798 cm^{-1}), amine group (3420, 3236, 1660, 565 cm^{-1}), cyan group (2222 cm^{-1}), ^{1}HNMR in DMSO-d^6: δ 8.30 (s, 1H, aromatic proton), 7.09 (br, 2H, amine group). ^{13}CNMR in DMSO-d^6: δ 156.81 (C_2), 169.15 (C_4), 93.19 (C_5), 162.17 (C_6), 118.10 (CN). *Anal*. Calcd for $C_5H_4N_4O$: C (44.12), H (2.96), N (41.16); found: C (43.02), H (2.87), and N (39.83).

Synthesis of 4-amino-5-carboxamido pyrimidines

Hydrolysis of the cyano group of compounds (**I**) either in acidic or basic medium produced the corresponding 5-carboxyamide derivatives with the general structure of (**II**). However, the yield of the hydrolysis in acidic medium was almost quantitative. Therefore, 0.01 mole of each of the

compounds (**I**) was dissolved in 10 ml of concentrated H_2SO_4 (or concentrated HCl) and left stirring overnight. Then, the reaction mixture was transferred onto the crashed ice and neutralized by ammonia solution (25%). The product was recrystallized from hot water and the structure was determined by means of different spectroscopic methods.

*Compound **IIa** (2,4-diamino-5-carboxamide pyrimidine)*

Yellow powder, decomposed above 250 °C, m/e = 153.1, IR (KBr): cyan group (disappeared), -$CONH_2$ (1675 cm^{-1}), ^{1}HNMR in DMSO-d^6: δ 8.34 (s, 1H, H_6), 7.91 (br, 2H, $CONH_2$), 7.66 (br, 2H, 2-NH_2), 7.45 (br, 2H, 4-NH_2). ^{13}CNMR in DMSO-d^6: δ 154.38 (C_2), 163.04 (C_4), 101.62 (C_5), 145.60 (C_6), 168.07 ($CONH_2$). *Anal*. Calcd for $C_5H_7N_5O$: C (39.21), H (4.61), and N (45.73); found: C (38.34), H (4.49), and N (44.38).

*Compound **IIb** (2-thio-4-amino-5-carboxamide pyrimidine)*

Pale yellow precipitate, decomposed above 240 °C, m/e = 170.1, IR (KBr): cyan group (disappeared), -$CONH_2$ (1662 cm^{-1}), ^{1}HNMR in DMSO-d^6: δ 8.34 (s, 1H, H_6), 7.91 (br, 2H, $CONH_2$), 7.66 (br, 2H, 2-NH_2), 7.45 (br, 2H, 4-NH_2). ^{13}CNMR in DMSO-d^6: δ 176.33 (C_2), 145.36 (C_4), 99.99 (C_5), 158.54 (C_6), 166.11 ($CONH_2$). *Anal*. Calcd for $C_5H_6N_4OS$: C (35.29), H (3.55), N (32.92), and S (18.84); found: C (34.14), H (3.38), N (31.31), and S (17.91).

*Compound **IIc** (2-ethylthio-4-amino-5-carboxamide pyrimidine)*

Yellow powder, decomposed above 240 °C, m/e = 198.2, IR (KBr): cyan group (disappeared), -$CONH_2$ (1643 cm^{-1}), ^{1}HNMR in DMSO-d^6: δ 8.34 (s, 1H, H_6), 7.91 (br, 2H, $CONH_2$), 7.66 (br, 2H, 2-NH_2), 7.45 (br, 2H, 4-NH_2). ^{13}CNMR in DMSO-d^6: δ 172.78 (C_2), 156.06 (C_4), 103.72 (C_5), 161.93 (C_6), 168.61 ($CONH_2$), 24.55 (-CH_2), 15.08 (-CH_3). *Anal*. Calcd for $C_7H_{10}N_4OS$: C (42.41), H (5.07), N (28.26), and S (16.17); found: C (42.27), H (5.03), N (28.07), and S (16.08).

*Compound **Iid** (2-hydroxy-4-amino-5-carboxamide pyrimidine)*

Bright cream powder, decomposed above 250 °C, m/e = 154.1, IR (KBr): cyan group (disappeared), -$CONH_2$ (1674 cm^{-1}), ^{1}HNMR in DMSO-d^6: δ 8.34 (s, 1H, H_6), 7.91 (br, 2H, $CONH_2$), 7.66 (br, 2H, 2-NH_2), 7.45 (br, 2H, 4-NH_2). ^{13}CNMR in DMSO-d^6: δ 156.91 (C_2),

165.83 (C_4), 105.45 (C_5), 162.38 (C_6), 169.27 ($CONH_2$). *Anal*. Calcd for $C_5H_6N_4O_2$: C (38.96), H (3.92), and N (36.35); found: C (37.69), H (3.80), and N (35.48).

Diazotization of 4-amino-5-carboxyamido pyrimidines

Diazotization of compounds **II** was studied under four different conditions introduced in Table 2.

Table 2. Various diazotization conditions.

Sign	Description of theConditions
A	glacial acetic acid (GAA) as solvent and inorganic acid/$NaNO_2$ as reagent
B	absolute ethanol as solvent and inorganic acid/$NaNO_2$ as reagent
C	GAA as solvent and trifluoroacetic acid (TFAA)/ isoamyl nitrite (iAN) as reagent
D	absolute ethanol as solvent and TFAA/iAN as reagent

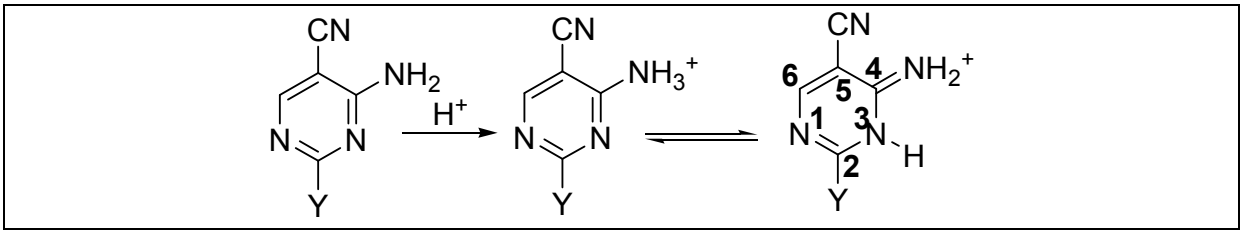
Results and discussion

Availability of 4-amino-pyrimidines for diazotization

NO produces N_2O_3 in the presence of a proper flow of oxygen [3]. In the old literature, N_2O_3 was known as nitrous anhydride. Although an anhydride structure, (O=N-O-N=O) was never found for this molecule, there is little doubt about the formation of nitrous acid after N_2O_3 reaction with water:

$$N_2O_3 + H_2O \rightleftharpoons 2HNO_2 \dashrightarrow [NO^+]$$

In fact, the above mentioned equation explains the cytotoxicity of nitric oxide as the resulting nitrosonium intermediate can attack all the nucleophilic functional groups including amines and thiols [16]. Amino group at position 2, 4, and 6 of a pyrimidine ring can participate in an amine-imine tautomery, Scheme 3, which shifts toward imine tautomer in low pH(s) [17]. Does this phenomenon affect the availability of the lone-pair electrons of the amino group for diazotization?



Scheme 3. Amine-imine tautomery in 4-aminopyrimidines.

Experiments of this research showed that **Ia** (Scheme 2) reacted with diazotization reagent at higher temperatures. Diazotization of **Ia** by conventional method using nitrous acid in water at cold temperature (0 – 5°C) produced no changes in the starting material. Diazotization of **Ia** was also examined in GAA in the presence of TFAA and iAN [18]. No changes in the starting material was observed, when the reaction was carried out at 0 to 25°C. However, elevating temperature to 90ºC resulted quantitatively in formation of a product showing m/e = 137 in its Mass spectrum.

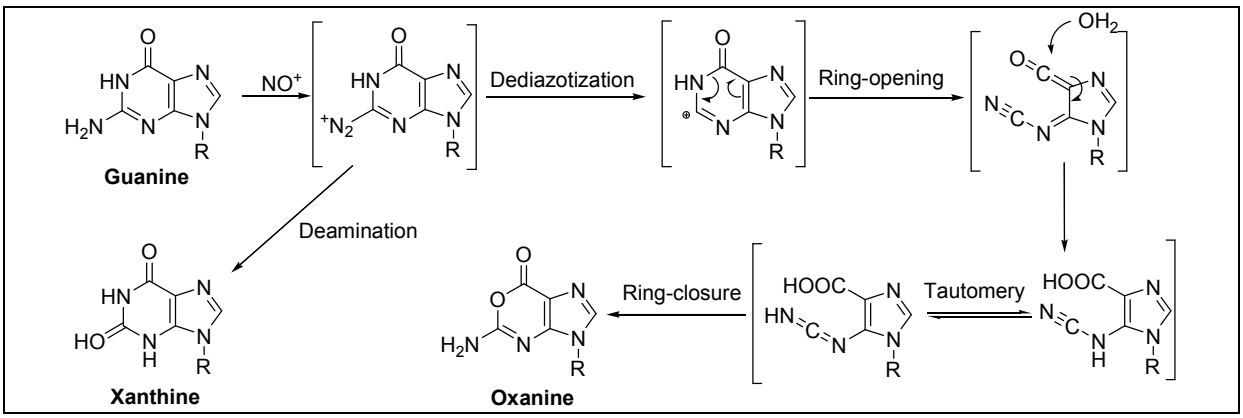
Spectroscopic evidence indicated that **Ia** was converted to 5-cyano-uracil because amine protons were disappeared from its both ^{1}HNMR and FT-IR spectra. ^{1}HNMR showed two equal signals at δ = 8.3 and 11.6 for H^6 and H^3, respectively, and its FT-IR spectrum had bands at 1710 and 2224 cm^{-1} belonged to keto and cyano groups, respectively. Elemental analysis of this compound showed 43.52% carbon, 2.14% hydrogen and 29.85% nitrogen. All these evidence supported the idea that, in spite of hard accessibility of the amino groups, the diazotization of 2,4-diamino-5-cyano pyrimidine had been carried out and the successive hydrolysis of the diazotized compound produced 5-cyano uracil.

This result indicates that nitrosonium can access to the amino group at either position 2 or/and 4 of pyrimidines but not as easily as an amino group on a benzene ring. Therefore, a cytosine residue in a double-stranded DNA must be less available. Based on the results of some experiments at higher temperatures which extrapolated to the physiological temperature, the rate of the spontaneous deamination of cytosine in a double-stranded DNA at 37°C was estimated to be less than 10^{-12} s^{-1} [19]. However, deamination becomes faster under nitrosative stress. Frankel et al. showed that about 9% of cytosines of DNA was modified to uracil after 36 h treatment with nitrosonium at pH 4.1 [20]. But what makes the results of our experiment noteworthy is that the deamination of **Ia** occurred without ring-opening reaction even at 90°C.

Stability of pyrimidine diazoniums

It was known that the nitrosative stress could cause DNA lesions through modifying cytosine and guanine to uracil and xanthine, respectively [7]. Further studies disclosed that oxanine is also produced from guanine under nitrosative conditions [21]. An acceptable explanation for this outcome was the assumption of a successive ring-opening, ring-closure process after dediazotization of the guaninediazonium ion, Scheme 4 [22]. This assumption was further supported by both experimental evidence and theoretical calculations [13, 23].

Results of the Ab initio studies indicated that not only guaninediazonium but also cytosinediazonium were kinetically and thermodynamically unstable toward dediazotization [22]. Following these theoretical calculations, Rayat et al. reported that cytosinediazonium could also fall in ring-opening reactions, Scheme 1, after dediazotization. The intermediates, formed after the cytosine ring cleavage, are able to participate in cross-linking reactions and cause damage to DNA [24]. Now the question is; which reaction does proceed faster, deamination or dediazotization?



Scheme 4. Guaninediazonium can take part in either hydrolytic deamination and produce xanthine or dediazotization and produce oxanine.

As shown, **Ia** participated in diazotization reaction in the presence of nitrous acid and proceeded through deamination mechanism without involving in ring-opening reaction. To check whether the diazonium of a 4-aminopyrimidine is stable enough to take part in an interamolecular cyclization reaction, Scheme 2, 5-carboxamido derivatives of compounds **I** were synthesized and subjected to various diazotization conditions introduced in Table 2.

Polarity and the amount of the available water decrease as the conditions change, from A to D,

in Table 2. Treating each of the compounds **II** with diazotization reagents under the conditions of A and B gradually produced a white precipitate at 0 to 12ºC. Treating compounds **II** with diazotization reagents under the conditions of C and D at 0 to 25ºC produced no changes in the starting materials. Therefore, the reaction mixtures were refluxed under the conditions of C and D which resulted in formation of white precipitates during the first hour of the reactions. The FT-IR analysis of the collected precipitates from the above mentioned reactions revealed that they were all the same.

Stability of pyrimido-v-triazine

The white precipitate was soluble in water and showed no carbon either in its ^{13}CNMR spectrum or in the elemental analysis. The ^{1}HNMR spectrum of the precipitate in D_2O contained only a broad band between 6 to 7 ppm and had no similarity to the intermediates proposed by Rayat et al. for the ring-opening reaction of cytosinediazonium, Scheme 1 [24]. The elemental analysis (N = 18.5%, H = 5.4%) and the strong stretching bands of N-N and –N=O at 1117 and 1402 cm^{-1}, respectively, support the assumption that the precipitate consisted of inorganic nitrogen oxide salt(s). Formation of such a precipitate can be explained by considering a ring cleavage reaction but not through the proposed route for cytosinediazonium as illustrated in Scheme 1.

Before Rayat et al., Clark et al. had investigated the ring-opening of 4-aminopyrimidine derivatives for several years [25, 26]. He showed substituted olefins were the major products of the ring-opening reactions. Considering the literature and the results of these experiments, it is unlikely that the inorganic salt obtained from the ring cleavage of any diazoniums of compounds **II**. Furthermore, it was shown here that the diazotization of **Ia** resulted in formation of a stable organic compound, 5-cyano-uracil.

Consequently, it is assumed that in the absence of any electron withdrawing group at position 2, diazotization of compounds **II** occurred successfully and the resulting diazonium was stable enough to take part in the interamolecular cyclisation before dediazotization happens. As a result, the anticipated triazine (**III**), illustrated in Scheme 2, was formed. But, because of the strong electron deficiency in the triazine ring, this ring took part in a ring-opening reaction. Further research is required to suggest a mechanism for this process due to the uncertainties associated with the position of protonation of the v-triazine ring.

In contrast to guanine, cytosine is a mono-cyclic compound with higher aromatic stability.

Considering this fact and the results of this research, it seems unlikely that cytosine enters in ring-opening reactions under the nitrosative stress unless some exceptional conditions are established.

Conclusion

In parallel with the previous studies reported on the diazotization of 4-aminopyrimidines, the results of this research confirm that the amino group at position 4 of a pyrimidine ring has a very low tendency toward diazotization. However, polar mediums enhance the tendency. These results also indicate that the diazonium of a 4-aminopyrimidine prefers deamination over the dediazotization. So, the dediazotization and its successive ring-opening reactions of the pyrimidine cation would happen under some exceptional conditions which deserve to be explored and clarified in more detailed studies.

Acknowledgements

The valuable collaboration of both Chemistry Department and Chemical Engineering Department, especially Professor R.L. Legge's research laboratories, at Waterloo University (Ontario-Canada) is sincerely appreciated.

References

[1] S. K. Choudhari, M. Chaudhary, S. Bagde, A. R. Gadbail, V. Joshi, *World J. Surgical Oncol*., 11, 1477 (2013).

[2] M. Alemi, F. Sabouni, F. Sanjarian, K. Haghbeen, S. Ansari, *J. AAPS Pharm. Sci. Tech*., 14, 160 (2013).

[3] J. L. Caulfield, J. S. Wishnok, S. R. Tannenbaum, *J. Biol. Chem.*, 273, 12689 (2013).

[4] D. A. Wink, K. S. Kasprzak, C. M. Maragos, R. K. Elespuru, M. Misra, T. M. Dunams, T. A. Cebula, W. H. Koch, A. W. Andrews, J. S. Allen, *Science*, 254, 1001 (1991).

[5] L. A. Frederico, T. A. Kunkel, B. R. Shaw, *Biochemistry*, 29, 2532 (1990).

[6] K. Haghbeen, E. W. Tan, *J. Org. Chem.*, 63, 4503 (1990).

[7] K. S. Gates, *J. Chem. Res. Toxicol.*, 22, 1747 (2009).

[8] N. Chatterjee, W. Siede, *J. Cold Spring Harbor Perspectives in Biology*, 5, 1943 (2013).

[9] I. Losito, R. Angelico, B. Introna, A. Ceglie, F. Palmisano, *J. Mass Spect.*, 47, 1384 (2012).

[10] M. Qian, R. Glaser, *J. Am. Chem. Soc.*, 127, 880 (2005).

[11] R. Glaser, S. Rayat, M. Lewis, M. S. Son, S. Meyer, *J. Am. Chem. Soc.*, 121, 6108 (1999).

[12] J. Clark, G. Varvounis, *J. Chem. Soc. Perkin Trans, 1*, 1475 (1984).

[13] M. Debeljak-Sustar, B. Stanovnik, M. Tisler, Z. Zrimsek, *J. Org. Chem.*, 43, 393 (1978).

[14] M. T. Migawa, L. B. Townsend, *J. Org. Chem.*, 66, 4776 (2001).

[15] O. S. Jung, J. Chon, H. Chae, *J. Bull. Kore. Chem. Soc.*, 20, 648 (1999).

[16] A. Daiber, D. Frein, D. Namgaladze, V. Ullrich, *J. Biol. Chem.*, 277, 11882 (2002).

[17] A. A. Hasanein, S. A. *Int. J. Quant. Chem*., 111, 3993 (2011).

[18] B. G. Gowenlock, G. B. Richter-Addo, *J. Chem. Rev.*, 104, 3315 (2004).

[19] J. C. Shen, W. M. Rideout, P. A. Jones, *J. Nuc. Acids Res.*, 22, 972 (1994).

[20] A. D. Frankel, B. K. Duncan, P. E. Hartman, *J. Bacteriol.*, 142, 335 (1984).

[21] R. Glaser, M. S. Son, *J. Am. Chem. Soc.*, 118, 10942 (1996).

[22] R. Glaser, H. Wu, M. Lewis, *J. Am. Chem. Soc.*, 127, 7346 (2005).

[23] T. Nakano, A. Katafuchi, R. Shimizu, H. Terato, T. Suzuki, H. Tauchi, K. Makino, M. Skorvaga, B. Van Houten, H. Ide, *J. Nuc. Acids Res.*, 33, 2181 (2005).

[24] S. Rayat, M. Qian, R. Glaser, *J. Chem. Res. Toxicol.*, 18, 1211 (2005).

[25] J. Clark, I. Gelling, G. Neath, *J. Chem. Commun. (London)*, 859 (1967).

[26] J. Clark, B. Parvizi, I. W. Southon, *J. Chem. Soc. Perkin Trans. 1*, 125 (1976).