Could Purines Be Formed from Cyanamide and Cyanoacetylene in a Prebiotic Earth Environment?

Sarabjeet Kaur, Ashita Ohri, and Purshotam Sharma

Computational Biochemistry Laboratory, Department of Chemistry and Centre for Advanced Studies in Chemistry, Panjab University, Chandigarh 160014, India

ABSTRACT: Knowledge of prebiotic nucleobase formation is important for understanding the origin of contemporary genetics. Observation of nucleobase precursor radicals in previous impact laser plasma simulations of the late heavy bombardment period (Ferus et al. Proc. Natl. Acad. Sci. U.S.A. 2015, 112, 657) points toward possible nucleobase formation through free-radical pathways. However, previously explored radical routes to nucleobase formation involve a large number of reaction steps, repetitive addition of precursors, and a number of chemical transformations. The possibility of competing side reactions under such conditions questions the feasibility of such pathways. In view of these shortcomings, the present work employs density functional theory to explore purine formation pathways through reaction of cyanamide and cyanoacetylene with radicals via a five-membered intermediate, 4-cyanomidazole in the presence of ammonia. Our analysis reveals that the skeletal components of 4-cyanomidazole can be solely obtained from cyanamide and cyanoacetylene via barrierless cyclization and a small number of reaction steps. In addition, the proposed mechanisms are characterized by a small number of precursors and low energy barriers and are thus likely feasible under extreme conditions on the prebiotic earth such as meteoritic impact during late heavy bombardment period. Overall, the present study underscores the importance of cyanamide and cyanoacetylene precursors in kinetically accessible routes to purine formation.

1. INTRODUCTION

Despite inklings of the presence of nucleobases in simulations of early earth environment1−2 and extensive exploration of their possible formation from a myriad of prebiotic precursors,3−6 consensus on the prebiotic origin of these vital components of genetics is still not achieved. Nevertheless, continuous input of intense UV7 and cosmic rays, coupled with bombardment of extraterrestrial material on primitive earth during late heavy bombardment period8 suggests that nucleobases might have formed from simple precursors through radical pathways.9 Indeed, radicals such as ·NH2, ·CN, and ·NH have been detected when high-powered laser was used to induce 4500 K plasma of the reaction products during high-energy synthesis of nucleobases from formamide.1 Further, free-radical nucleobase formation routes have been theoretically proposed, which involve a variety of prebiotic molecules (e.g., hydrogen cyanide (HCN), acetylene, urea, and formamide) and radicals and construct the nucleobase skeleton using a large number of tandem reaction steps.13−17

In contrast, an alternate hypothesis involves the utilization of a small number of precursors containing ready-made nucleobase skeletal components to form nucleobases in a relatively smaller number of steps. Such scenarios will automatically optimize the atom economy and reduce the possibility of competing side reactions. Cyanamide and cyanoacetylene are two such precursors relevant to prebiotic earth.18−21 Cyanamide can be formed from an irradiated mixture of simple, prebiotically relevant molecules such as methane, ammonia, water, and HCN22 and is considered as an effective prebiotic condensing agent.23,24 Similarly, the presence of cyanoacetylene in the prebiotic environment and its involvement in prebiotic reactions have been advocated.1,25−27 For instance, cyanoacetylene is formed as a major product from the action of an electric discharge on gaseous mixtures mimicking an early earth atmosphere.28 Under prebiotic aqueous conditions, cyanoacetylene reacts not only with cyanamide to give cytidine and its derivatives26 but also with cyamate26 or urea23 to give cytosine and uracil.

Given the previously suggested possibility of the formation of radicals in an high-energy impact scenario on prebiotic earth1,28 and evidence of ammonia as a component of the early earth atmosphere,29 we expect the evolution of feasible ammonia-assisted pathways to nucleobase formation through reaction of cyanamide and cyanoacetylene under such conditions. On these lines, to evaluate the hypothesis on the coexistence and possible reactions of these precursors in a...
prebiotic scenario, we employ density functional theory to initiate preliminary investigations for exploring plausible radical routes to the formation of purines (i.e., adenine, guanine, hypoxanthine, isoguanine, purine, and xanthine) starting from cyanamide and cyanoacetylene and involving precursors such as HCN,30 H,31 and ·OH,32–34 NH2·35 formed photochemically during high-energy impact events,32 as well as urea formed from acidic hydrolysis of cyanamide.32 In addition to being unique in suggesting the role of cyanamide and cyanoacetylene in the formation of purines in the prebiotic scenario, our work will hopefully inspire further investigations to develop a better understanding of the prebiotic reactions that led to the formation of purines.

2. RESULTS

Previous experimental8,33–35 and theoretical17,36 studies have highlighted the significance of imidazole derivatives in the formation of purines. Thus, we envision the prebiotic purine synthesis using cyanamide and cyanoacetylene through the involvement of the 4-cyanoimidazole intermediate, which subsequently reacts with secondary precursors to add a six-membered heterocyclic ring characteristic of various purines (Figure 1).

![Figure 1](image)

Figure 1. Reaction pathways leading to purine skeleton formations from cyanamide (P1) and cyanoacetylene (P2) proposed in the present work.

2.1. Formation of 4-cyanoimidazole Intermediate. We envision the formation of 4-cyanoimidazole (Figure 2) through a barrierless attack of ·OH on one equivalent of cyanamide (P1; Figure 2) to generate the radical intermediate 2 (Table S2 and Figures 2 and 11). The next step involves the formation of intermediate 4 through 1,3-hydrogen shift from the amino group to the C·a barrierless attack of the radical center on the carbon−nitrogen double bond to form a five-membered ring, which is concomitant with the shift of radical center from the carbon bearing the cyano group to the carbon bearing the hydroxyl group. The subsequent step involves the formation of neutral species 8 from the radical intermediate 7 through a modest energy barrier (15.6 kcal mol$^{-1}$; Figure 2) step involving an ammonia molecule.

Intermediate 8 differs from 4-cyanoimidazole by a single carbon−nitrogen double bond. Due to a significantly high (50.6 kcal mol$^{-1}$) barrier, the concerted water elimination is disfavored (Figure S2). Alternatively, the small concentration of ·NH$_2$ available in the vicinity can barrierlessly abstract hydrogen from the ring nitrogen of 8 to generate ammonia and radical intermediate 9. Intermediate 9 crosses a modest (21.2 kcal mol$^{-1}$) barrier to release ·OH consumed in the first step of the mechanism to form the carbon−nitrogen bond characteristic of 4-cyanoimidazole 10.

2.2. Formation of Guanine and Hypoxanthine. The first step involves the modest-barrier (3.0 kcal mol$^{-1}$) activation of 10 by ·OH to form enol radical 11 (Table S3 and Figures 3 and 11). 11 undergoes low-barrier (9.0 kcal mol$^{-1}$) abstraction of hydrogen from ammonia to form the neutral species 12, which barrierlessly tautomerizes to its keto form 13 through the assistance of ammonia. Despite the possibility of side reactions (e.g., ·NH$_2$ recombination), the α-amino group of 13 is then attacked by·NH$_2$ along an energy barrier of 2.9 kcal mol$^{-1}$ to generate the keto tautomer form 14 of 11 (Figure 3). Both the enol 11 and keto 14 tautomers are capable of forming guanine or hypoxanthine through a series of steps described below.

2.2.1. Guanine. The formation of guanine through the enol radical 11 proceeds via the attack of 11 on the electron-rich (sp) carbon of cyanamide P1 after crossing a barrier of 5.2 kcal mol$^{-1}$ to form 15a that bears the radical on nitrogen (Figure 4). The two −OH rotamers of 15a (15a1 and 15a2; Figure S3) undergo intramolecular free-radical cyclization along 16.0 and 11.9 kcal mol$^{-1}$ barriers (Figure 4) to form 16a1 and 16a2, respectively (Figure S4), which bear the radical at C5. The carbon−carbon double bond bridging the five-membered and six-membered rings of guanine is then created by removal of hydrogen from C4 of 16a1 or 16a2 through a barrier of 17.8 or 18.1 kcal mol$^{-1}$ to form 17a1 or 17a2 (Figure S5), respectively, which represent the enol tautomer of guanine (Figure 4). However, only 17a1 can convert to the canonical (keto) tautomer of guanine 18 through a barrierless ammonia-assisted transformation (Figure 4).

In contrast, the formation of guanine through the keto radical 14 proceeds through the attack of 14 on the electron-rich C≡N center of cyanamide through a 5.1 kcal mol$^{-1}$ barrier to form 15b with the nitrogen-centered radical (Figure 4). 15b further undergoes modest-barrier (8.0 kcal mol$^{-1}$) intramolecular cyclization to form intermediate 16b, which bears a radical at C5. The bridging C≡C is then created by
the loss of hydrogen from C4 of 16b through a 23.8 kcal mol$^{-1}$ barrier to form guanine.

2.2.2. Hypoxanthine. In contrast to guanine, hypoxanthine formation via the enol radical 11 occurs through the attack of a secondary precursor (HCN, P3) on 11 along a barrier of 14.6 or 9.7 kcal mol$^{-1}$ to form 19a1 or 19a2 –OH rotamers (Figure S6), respectively, that bear a radical on nitrogen (Figure 5). Intramolecular radical attack on the double bond of the imidazole moiety of 19a1 or 19a2 through a 16.5 or 10.0 kcal mol$^{-1}$ barrier forms 20a1 or 20a2 (Figure S7), respectively, which further releases -OH via a 17.7 kcal mol$^{-1}$ barrier to form the bridging double bond of 21a1 or 21a2, respectively (Figure 5 and Figure S8). However, only 21a1 is capable of undergoing barrierless ammonia-assisted keto–enol tautomerism to give canonical hypoxanthine 22.

In contrast, the mechanism involving the keto intermediate 14 proceeds through the barrierless attack of HCN on 14 to form 19b (Figure 5). The nitrogen-centered radical in 19b attacks the double bond of the imidazole moiety along a barrier of 8.6 kcal mol$^{-1}$ to form 20b. The loss of hydrogen attached to C4 of 20b via a 31.6 kcal mol$^{-1}$ barrier forms the bridging double bond of hypoxanthine.

2.3. Formation of Adenine. Adenine formation involves the attack of -NH$_2$ on the cyano group of 10 through a 7.2 kcal mol$^{-1}$ barrier to form 23 (Table S3 and Figures 6 and 11). Intermediate 23 reacts with P4 (generated from the attack of -
The formation of guanine (18) from 4-cyanoimidazole (10) involves the reaction of adduct 24 with radical center from N9 to C5 to form intermediate 25. Intermediate 25 loses H from tetrahedral C4 to form the double bond of 26 by crossing a barrier of 24.2 kcal mol$^{-1}$. Although the direct loss of NH$_3$ from 26 to form adenine 28 involves a high (30.9 kcal mol$^{-1}$) barrier, inclusion of the NH$_3$/·NH$_2$ catalytic pair results in barrierless abstraction of N9 hydrogen by ·NH$_2$ of 26 to generate 27, which subsequently loses ·NH$_2$ through a 16.6 kcal mol$^{-1}$ barrier to form adenine 28 (Figure S9).

2.4. Formation of Isoguanine. The first step in isoguanine formation involves the generation of urea radical from urea P5 through the attack of ·NH$_2$ along the barrier of 4.8 kcal mol$^{-1}$ (Table S3 and Figure 8). Urea radical 29 then undergoes attack on the cyano group of 10 through a barrier of 7.7 kcal mol$^{-1}$ to form 30. The radical center on 30 then undergoes 1,5-rearrangement in two tandem steps involving the NH$_3$/·NH$_2$ pair. Intermediate 31 then undergoes barrierless abstraction of N9 hydrogen by ·NH$_2$ of 26 to generate 27, which subsequently loses ·NH$_2$ through a 16.6 kcal mol$^{-1}$ barrier to form adenine 28 (Figure S9).

2.5. Formation of Xanthine and Purine. Xanthine formation proceeds through the attack of urea radical 29 on neutral intermediate 13 generated in previous steps (Figure 3) through a 11.6 kcal mol$^{-1}$ barrier to form 40, which barrierlessly releases ·NH$_2$ to form 41 (Table S3 and Figures 9 and 12). This is followed by barrierless abstraction of hydrogen from the amino group of 41 by ·NH$_2$ to form 42. Intermediate 42 undergoes low-barrier (2.4 kcal mol$^{-1}$) cyclization to form 43 that subsequently loses ·H through a 29.9 kcal mol$^{-1}$ barrier to form xanthine 44.

In contrast, purine formation proceeds through the attack of ·H on 10 through a 4.9 kcal mol$^{-1}$ barrier to form 45 (Table S5 and Figures 10 and 12). This is followed by the attack of HCN (P3) on 45 through a 12.3 kcal mol$^{-1}$ barrier to form 46. Intermediate 46 then undergoes 14.0 kcal mol$^{-1}$ barrier to form

---

Figure 4. Stepwise proposed radical mechanism along with corresponding transition states (TSs) for the formation of guanine (18) from 4-cyanoimidazole (10). Gas-phase B3LYP/6-311G(d,p) relative electronic energies (kcal mol$^{-1}$) of the TSs (barriers) with respect to reactants of each step are provided in parentheses. Reactants are in red color, and the product is in blue. Each reactant is considered in its free optimized state while calculating the electronic energies. As a result, some of the complexed transition states possess negative energies relative to the reactants.
The release of hydrogen attached to C4 of 47 through an 18.3 kcal mol$^{-1}$ barrier gives purine 48. Although ·H is highly reactive and can combine with other species once it is formed, it only plays a catalytic role in the formation of purine, that is, it is regenerated in the end.

### 3. DISCUSSION

The present work analyses plausible free-radical pathways for the formation of purines in a prebiotic high-energy impact scenario. Specifically, during late heavy bombardment on early earth, the impact of extraterrestrial bodies would have formed radicals that could potentially react with primary precursors, that is, cyanamide and cyanoacetaldehyde, thereby initiating the succession of chemical transformations leading to purine formation. Particularly, the first step of our pathways involves the formation of 4-cyanoimidazole, a precursor to the five-membered imidazole ring of purines. This intermediate can be solely obtained from cyanamide and cyanoacetylene through a series of low-barrier radical-induced reactions involving the catalytic role of one ·OH and two NH$_3$ groups. The formation of 4-cyanoimidazole is followed by the creation of the six-membered ring of various purines. Although the formation of six-membered ring of guanine utilizes an extra cyanamide, the six-membered rings of other purines are formed from the interaction of 4-cyanoimidazole with secondary precursors (i.e., HCN, urea, ·H, ·OH, NH$_3$, and ·NH$_2$), which are either consumed or play catalytic roles. Specifically, during the formation of the six-membered ring of guanine or hypoxanthine from cyanamide or HCN, ·OH acts as the source of the carbonyl group. In contrast, during adenine synthesis, one ·NH$_2$ acts as a source of the amino group, whereas ·OH, ·H, and another ·NH$_2$ play a catalytic role. On the other hand, the formation of the six-membered ring of isoguanine involves the addition of urea and the catalytic role of three ·NH$_2$ groups. Similarly, the six-membered skeleton of xanthine forms through the addition of urea and ·OH, where ·OH acts as a source of one of the carbonyl groups. Additionally, one ·NH$_2$ acts as a catalyst, whereas one ammonia molecule consumed in this step is recovered in the form of ·NH$_2$. Finally, the formation of the six-membered ring of purine involves the addition of HCN, where ·H acts as a catalyst.

To highlight the importance of the proposed pathways, we compared the energy barriers associated with the previously proposed purine formation pathways starting from formamide. In contrast to the a 20 kcal mol$^{-1}$ barrier to the cyclization step involved in the 4-aminomidazole intermediate formation in the formamide-based mechanism, the cycliza-
tion step leading to the 4-cyanoimidazole intermediate formation in our study occurs barrierlessly. Further, all the steps leading to imidazole ring formation in our proposed mechanism observe lower energy barriers (0−21 kcal mol\(^{-1}\)) compared to the formamide-based synthesis (5−28 kcal mol\(^{-1}\)).\(^{16−18}\) In addition, the barriers involved in the formation of the six-membered ring of purines in our mechanism (up to 36 kcal mol\(^{-1}\)) are lower than the associated barriers observed in formamide-based pathways (up to 45 kcal mol\(^{-1}\)).\(^{16−18}\) This indicates that our mechanistic proposals are energetically more favorable as compared to formamide-derived mechanisms. Further, despite significant (up to 36 kcal mol\(^{-1}\)) barriers, our proposed pathways are possibly feasible in high temperature conditions induced during the impact on the environment of primitive earth. Further, in addition to our lower calculated barriers relative to formamide\(^{16−18}\) based previous pathways, the formation of purines from 4-cyanoimidazole precursor in our mechanism proceeds with a smaller number of steps (4 to 10) compared to purine formation from 4-aminoimidazole in the formamide-based mechanism (10 to 14).\(^{16−18}\)

The potential role of enolate chemistry in prebiotic reactions has been previously signposted in the literature.\(^{18}\) Particularly, Jeilani et al. described the formation of a carbonyl group of xanthine and isoguanine through keto−enol tautomerization under prebiotic conditions.\(^{18}\) In their study, the direct formation of the carbonyl group of xanthine and isoguanine is energetically deterred, and the pathway proceeds only through the enol tautomer, which eventually converts into xanthine or isoguanine. On the contrary, in our prebiotic synthesis of purines from cyanamide and cyanoacetylene, favorable keto and enol mechanisms pertaining to guanine and hypoxanthine formation from 4-cyanoimidazole have been formulated. The feasible formation of guanine and hypoxanthine from keto\(^{11}\) and enol tautomer\(^{14}\) is highlighted by the barrierless formation of carbonyl groups (characteristics of guanine and hypoxanthine) from the imidazole intermediate. Further, comparison of the cyclization barriers of our keto tautomer-based mechanism (8 kcal mol\(^{-1}\) (guanine); 9 kcal mol\(^{-1}\) (hypoxanthine)) with the enol tautomer-based mechanism (16 kcal mol\(^{-1}\)) reveals that the keto pathways are relatively more feasible. Thus, our pathways successfully lead

Figure 6. Proposed radical mechanism along with corresponding transition states (TSs) for the formation of adenine (28) from 4-cyanoimidazole (10) and cyanamide (P1). Gas-phase B3LYP/6-311G(d,p) relative electronic energies (kcal mol\(^{-1}\)) of the TSs (barriers) with respect to reactants of each step are provided in parentheses. Reactants are in red color, and the product is in blue. Each reactant is considered in its free optimized state while calculating the electronic energies. As a result, some of the complexed transition states possess negative energies relative to the reactants.

Figure 7. Competing pathways for the formation of P4 from P1 corresponding to the proposed mechanism leading to the synthesis of adenine (28). Gas-phase B3LYP/6-311G(d,p) relative electronic energies (kcal mol\(^{-1}\)) of the TSs (barriers) with respect to reactants of each step are provided in parentheses. Each reactant is considered in its free optimized state while calculating the electronic energies. As a result, some of the complexed transition states possess negative energies relative to the reactants.
to the introduction of requisite functionality (carbonyl group) in purines through both enol and keto tautomers.

Since the free radical reactions are not selective, the studied pathways are competitive with the alternate routes involving radical recombination and the formation of byproducts using competing pathways cannot be avoided. Nevertheless, despite lower concentrations, and in synchrony with previous pathways, involving formamide\(^{16,18}\) and HCN\(^{16}\) simple radicals can lead to the formation of various purines using cyanoacetylene and cyanamide, particularly during high-energy, extraterrestrial impact event.

4. CONCLUSIONS

In summary, the present work highlights the relevance of cyanamide and cyanoacetylene in prebiotic purine formation through the involvement of the 4-cyanoimidazole intermediate. The 4-cyanoimidazole formation pathway involving these precursors proceeds in a lesser number of steps since they are reaction-ready molecules that possess the skeletal components of imidazole and cyano side groups. The subsequent addition of secondary precursors such as HCN, •H, urea, or •OH augment the 4-cyanoimidazole intermediate with an adjacent six-membered ring and necessary functional groups to give purines. Further, such pathways are suitable to specific extreme conditions prevailing during the early earth environment where radicals were prevalent due to molecular fragmentation by UV radiation\(^{13}\) or due to the formation of high-energy impact plasma ensued by meteoritic impacts.\(^{1}\) Particularly, in the presence of ammonia in the early earth atmosphere,\(^{29}\) radicals such as -NH\(_2\) increase the feasibility of prebiotic purine formation by providing alternative lower barrier routes.

The likelihood of availability of feedstock precursors at the same prebiotic setting is an important criterion in deciding the suitability of a particular pathway in forming purines. While in many experimental studies,\(^{9,37,38}\) cyanamide has been demonstrated to react with cyanoacetylene under favorable, prebiotic physicochemical conditions, the co-existence of urea and cyanamide is highlighted from the fact that it is formed from acidic hydrolysis of cyanamide.\(^{32}\) Similarly, HCN was an...
important product of primitive atmospheric chemistry. In addition, an extreme environment such as late heavy bombardement would ensure continuous influx of radicals that drive such pathways and thus would likely provide an optimal background for purine formation. Further, precursor radicals such as ·H and ·OH might have formed photochemically from dissociation of water vapors, whereas ·NH$_2$ would have formed from ammonia in earth’s prebiotic atmosphere either under the influence of UV radiation or during high-energy impact events.

To conclude, we hope that the present work will motivate more extensive study with regard to experimental, thermodynamic, and kinetic aspects of prebiotic reactions involving cyanamide and cyanoacetylene.

5. COMPUTATIONAL DETAILS

All the quantum chemical calculations were carried out using the Gaussian 09 suite of programs. The reactants, transition states, and intermediates along the reaction coordinates associated with purine formation were subjected to gas-phase geometry optimizations and zero-point vibrational energy (ZPVE)-corrected electronic energy calculations using B3LYP/6-311G(d,p). This method was chosen in synchrony with recent computational studies on radical pathways of prebiotically important reactions. The stationary points on the reaction surfaces were characterized as minima (reactants, intermediates, and products) or first-order saddle points (transition states) using vibrational frequency calculations.

Further calculations were performed to understand the method-dependence of the derived results and confirm the feasibility of the proposed pathways. First, B3LYP/6-311G-(d,p) intrinsic reaction coordinate scans were carried out to confirm the pathways connecting the transition states to reactants and products of each step (Figures S11–S19). Second, to understand the effect of dispersion corrections, calculations were re-performed on select (i.e., imidazole formation and purine formation) mechanisms at B3LYP-D3/6-311G(d,p) level (Tables S1 and S4). The addition of dispersion correction leads to only a slight (up to 0.4 kcal mol$^{-1}$) change in energy barriers (Tables S1 and S4). Third, to further ascertain the choice of DFT functional, calculations were re-performed using three additional functionals (i.e., ωB97XD, M06-2X, and B2PLYP), which reveal deviations of 0.8–6.6 kcal mol$^{-1}$ compared to all calculated energy barriers and only up to 2.9 kcal mol$^{-1}$ in the highest barrier calculated using B3LYP (Tables S2, S3 and S5). Fourth, the influence of basis set superposition error (BSSE) corrections on barrier heights was evaluated by recalculating the electronic energies of the transition states of select (i.e., imidazole and purine)
mechanisms using the counterpoise method\(^{(60)}\) (Table S7). Further, the spin contamination in the ground state of the studied radicals was estimated in the form of expectation value of the square of the total spin operator \(\langle S^2 \rangle\), which differs from the ideal value (i.e., 0.7500 obtained from the \(s(s+1)\) value for radical species) by less than 2% after spin annihilation (Table S6). Finally, CCSD(T)/6-311G(d,p) single-point calculations performed on B3LYP/6-311G(d,p) optimized geometries reveal that CCSD(T) barriers differ from B3LYP barriers by up to 9 kcal mol\(^{-1}\) (Table 1). Regardless, the addition of dispersion corrections or recalculation at CCSD(T) does not significantly affect the conclusions derived from the present work.

![Figure 11. Potential energy curves depicting the B3LYP/6-311G(d,p) relative energies (kcal mol\(^{-1}\)) of the various species associated with proposed mechanism for the formation of (a) 4-cyanoimidazole, (b) guanine (black for enol mechanism and blue for keto mechanism), (c) hypoxanthine (black for enol mechanism and blue for keto mechanism), and (d) adenine. Energies of transition states and products are reported relative to the energies of the reactants of each step using the free reactant state as the reference state.](image)

![Figure 12. Potential energy curves depicting the B3LYP/6-311G(d,p) relative electronic energies (kcal mol\(^{-1}\)) of the various species associated with proposed mechanism of (a) isoguanine, (b) xanthine, and (c) purine. Energies of transition states and products are reported relative to the energies of the reactants of each step using the free reactant state as the reference state.](image)

**Table 1. Comparison of Gas-Phase CCSD(T)/6-311G(d,p) Relative Electronic Energies (kcal mol\(^{-1}\)) and Gas-Phase B3LYP/6-311G(d,p) Relative Electronic Energies of Transition States and the Corresponding Products Associated with the Selected Steps of the Proposed Mechanisms of Imidazole\(^{(60)}\)**

| reactant | Relative energy for imidazole formation |
|----------|-----------------------------------------|
|          | CCSD(T)/6-311G(d,p) | B3LYP/6-311G(d,p) |
|          | TS | product | TS | product |
| P1 → 2   | 6.3 | −10.1  | −2.7 | −31.8  |
| 2 → 3    | 3.4 | 6.4    | 6.7 | 5.6    |
| 3 → 4    | 7.6 | 0.9    | 4.1 |−2.4    |
| 4 → P5 → 6 | −4.7 | −9.9 | 5.7 | −30.4 |
| 6 → 7    | 13.5 | −12.2 | 0.1 | −8.1  |
| 7 → 8    | 17.8 | 11.9  | 15.6 | 17.6  |
| 8 → 9    | −2.5 | −26.4 | −5.5 | −29.9 |
| 9 → 10   | 24.2 | 18.9  | 21.2 | 25.2  |

\(^{(60)}\)Electronic energies are calculated using the gas-phase B3LYP/6-311G(d,p) optimized geometries and are reported relative to the energies of the reactants. Each reactant is considered in its free optimized state while calculating the electronic energies. As a result, some of the complexed transition states have negative energies relative to the reactants.
Competing pathways involved in imidazole and adenine mechanism, structures of competing formers involved in guanine and hypoxanthine mechanisms; IRC scans of TSs associated with 4-cyanoimidazole, final tautomer, guanine, hypoxanthine, adenine, isoguanine, xanthine, and purine mechanism; tables of relative electronic energies of structures of 4-cyanoimidazole, guanine, hypoxanthine, adenine, isoguanine, xanthine, and purine in the gas phase and at different levels of theory; spin contamination data for radicals associated with various mechanisms; and Cartesian coordinates of structures associated with mechanisms (PDF).

**AUTHOR INFORMATION**

**Corresponding Author**
E-mail: psharma@pu.ac.in.

**ORCID**
Sarabjeet Kaur: 0000-0003-1234-4161
Purshotam Sharma: 0000-0002-5164-9833

**Funding**
P.S. thanks the Department of Science and Technology (DST) and University Grants Commission (UGC), New Delhi for financial support through the DST INSPIRE (IFA14-CH1162) and the UGC FRP (F.4-5(176-FRP/2015(BSR))) programs, respectively. S.K. thanks the Department of Science and Technology (DST) for financial support through WOS-A (SR/WOS-A/CS-99/2018) program.

**Notes**
The authors declare no competing financial interest.

**REFERENCES**
(1) Ferus, M.; Nesvorný, D.; Šponer, J.; Kubelík, P.; Micháčková, R.; Shestivská, V.; Šponer, J.E.; Civiš, S. High-energy chemistry of formamide: A unified mechanism of nucleobase formation. Proc. Natl. Acad. Sci. U.S.A. 2015, 112, 657–662.
(2) Barks, H. L.; Buckley, R.; Grieves, G. A.; Di Mauro, E.; Hud, N. V.; Orlando, T. M. Guanine, adenine, and hypoxanthine production in UV-irradiated formamide solutions: relaxation of the requirements for prebiotic purine nucleobase formation. ChemBioChem 2010, 11, 1240–1243.
(3) Ferus, M.; Pietrucci, F.; Saitta, A. M.; Knížek, A.; Kubelík, P.; Ivaněk, O.; Shestivska, V.; Civiš, S. Formation of nucleobases in a miller-urey reducing atmosphere. Proc. Natl. Acad. Sci. U.S.A. 2017, 114, 4306–4311.
(4) Cleaves, H. J., II; Nelson, K. E.; Miller, S. L. The prebiotic synthesis of pyrimidines in frozen solution. Naturwissenschaften 2006, 93, 228–231.
(5) Borquez, E.; Cleaves, H. J.; Lazcano, A.; Miller, S. L. An investigation of prebiotic purine synthesis from the hydrolysis of HCN polymers. Origins Life Evol. Biospheres 2005, 35, 79–90.
(6) Boulanger, E.; Aノーap, A.; Nachitgallova, D.; Thiel, W.; Barbatti, M. Photochemical steps in the prebiotic synthesis of purine precursors from HCN. Angew. Chem., Int. Ed. 2013, 125, 8138–8161.
(7) Menor-Salván, C.; Ruiz-Bermejo, M.; Guzmán, M. L.; Osuna-Esteban, S.; Veintemillas-Verdaguer, S. Synthesis of pyrimidines and triazines in ice: implications for the prebiotic chemistry of nucleobases. Chem. – Eur. J. 2009, 15, 4411–4418.
(8) Oroó, J.; Kimball, A. P. Synthesis of purines under possible primitive earth conditions. I. Adenine from hydrogen cyanide. Arch. Biochem. Biophys. 1961, 94, 217–227.
(9) Powner, M. W.; Gerland, B.; Sutherland, J. D. Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. Nature 2009, 459, 239–242.
(10) Shapiro, R. Prebiotic cysteine synthesis: a critical analysis and implications for the origin of life. Proc. Natl. Acad. Sci. U. S. A. 1999, 96, 4396–4401.
(11) Wakamatsu, H.; Yamada, Y.; Saito, T.; Kumashiro, I.; Takenishi, T. Synthesis of adenine by oligomerization of hydrogen cyanide. J. Org. Chem. 1966, 31, 2035–2036.
(12) Ponnamperuma, C.; Lemmon, R. M.; Mariner, R.; Calvin, M. Formation of adenine by electron irradiation of methane, ammonia, and water. Proc. Natl. Acad. Sci. U. S. A. 1963, 49, 737–740.
(13) Cockell, C. S.; Horné, G. The history of the UV radiation climate of the earth—theoretical and space-based observations. Photochem. Photobiol. 2001, 73, 447–451.
(14) Lunine, J. I. Physical conditions on the early earth. Philos. Trans. R. Soc. B 2006, 361, 1721–1731.
(15) Nguyen, H. T.; Jeilani, Y. A.; Hung, H. M.; Nguyen, M. T. Radical Pathways for the Prebiotic Formation of Pyrimidine Bases from Formamide. J. Phys. Chem. A 2015, 119, 8871–8883.
(16) Menor-Salván, C.; Marín-Yaseli, M. R. A new route for the prebiotic synthesis of nucleobases and hydantoin solutions involving the photochemistry of acetylene. Chem. – Eur. J. 2013, 19, 6488–6497.
(17) Jeilani, Y. A.; Williams, P. N.; Walton, S.; Nguyen, M. T. Unified reaction pathways for the prebiotic formation of RNA and DNA nucleobases. Phys. Chem. Chem. Phys. 2016, 18, 20177–20188.
(18) Jeilani, Y. A.; Nguyen, M. T.; Newallo, D.; Dimanda, J.-M.; Nguyen, M. T. Free radical routes for prebiotic formation of DNA nucleobases from formamide. Phys. Chem. Chem. Phys. 2013, 15, 21084–21093.
(19) Jeilani, Y. A.; Nguyen, H. T.; Cardelino, B. H.; Nguyen, M. T. Free radical pathways for the prebiotic formation of xanthine and isoguanine from formamide. Chem. Phys. Lett. 2014, 598, 58–64.
(20) Duverney, F.; Chiavassa, T.; Borget, F.; Ayard, J. P. Experimental study of water-ice catalyzed thermal isomerization of cyanamide into carbodiimide: implication for prebiotic chemistry. J. Am. Chem. Soc. 2004, 126, 7772–7773.
(21) Steinman, G.; Lemmon, R. M.; Calvin, M. Cyanamide: a possible key compound in chemical evolution. Proc. Natl. Acad. Sci. U. S. A. 1964, 52, 27–30.
(22) Orgel, L. E. Is cyanoacetylene prebiotic? Origins Life Evol. Biospheres 2002, 32, 279–281.
(23) Sanchez, R.; Ferris, J.; Orgel, L. Cyanacetylene in prebiotic synthesis. Science 1966, 154, 784–785.
(24) Schimpl, A.; Lemmon, R. M.; Calvin, M. Cyanamide Formation under Primitive Earth Conditions. Science 1965, 147, 149–150.
(25) Lohrmann, R.; Orgel, L. E. Prebiotic synthesis: phosphorylation in aqueous solution. Science 1968, 161, 64–66.
(26) Sanchez, R. A.; Orgel, L. E. Studies in prebiotic synthesis. V. Synthesis and photoamination of pyrimidine nucleosides. J. Mol. Biol. 1970, 47, 531–543.
(27) Ferris, J. P.; Sanchez, R. A.; Orgel, L. E. Studies in prebiotic synthesis: III. Synthesis of pyrimidines from cyanoacetylene and cyanate. J. Mol. Biol. 1968, 33, 693–704.
(28) Civiš, S.; Ferus, M.; Knížek, A.; Kubelík, P.; Kamas, M.; Špané, P.; Dryahina, K.; Shestivska, V.; Juha, L.; Skřehot, P.; Latil, V.; Civiš, S. Spectroscopic investigations of high-energy-density plasma transformations in a simulated early reducing atmosphere containing methane, nitrogen and water. Phys. Chem. Chem. Phys. 2016, 18, 27317–27325.
(29) Kasting, J. F. Earth’s early atmosphere. Science 1993, 259, 920–926.
(30) Zahle, K. J. Photochemistry of methane and the formation of hydrocyanic acid (HCN) in the earth’s early atmosphere. J. Geophys. Res.: Atmos. 1986, 91, 2819–2834.
(31) Vander Wood, T. B.; Thiemens, M. H. The fate of the hydroxyl radical in the earth’s primitive atmosphere and implications for the production of molecular oxygen. *J. Geophys. Res.: Oceans* **1980**, *85*, 1605−1610.

(32) Kilpatrick, M. L. A mechanism for the hydrolysis of cyanamide in acid solution. *J. Am. Chem. Soc.* **1947**, *69*, 40−46.

(33) Sanchez, R. A.; Ferris, J. P.; Orgel, L. E. Studies in prebiotic synthesis: II. synthesis of purine precursors and amino acids from aqueous hydrogen cyanide. *J. Mol. Biol.* **1967**, *30*, 223−253.

(34) Sanchez, R. A.; Ferris, J. P.; Orgel, L. E. Studies in prebiotic synthesis. IV. Conversion of 4-aminimidazole-5-carbonitrile derivatives to purines. *J. Mol. Biol.* **1968**, *38*, 121−128.

(35) Ferris, J. P.; Orgel, L. Studies in Prebiotic Synthesis. I. Aminomalonalonitrile and 4-Amino-5-cyanoimidazole. *J. Am. Chem. Soc.* **1966**, *88*, 3829−3831.

(36) Hudson, J. S.; Eberle, J. F.; Vachhani, R. H.; Rogers, L. C.; Wade, J. H.; Krishnamurthy, R.; Springsteen, G. A unified mechanism for abiotic adenine and purine synthesis in formamide. *Angew. Chem., Int. Ed.* **2012**, *51*, 5134−5137.

(37) Ingar, A. A.; Luke, R. W. A.; Hayter, B. R.; Sutherland, J. D. Synthesis of cytidine ribonucleotides by stepwise assembly of the heterocycle on a sugar phosphate. *ChemBioChem* **2003**, *4*, 504−507.

(38) Anastasi, C.; Buchet, F. F.; Crowe, M. A.; Helliwell, M.; Raftery, J.; Sutherland, J. D. The search for a potentially prebiotic synthesis of nucleotides via arabinose-3-phosphate and its cyanamide derivative. *Chem. − Eur. J.* **2008**, *14*, 2375−2388.

(39) Frisch, M.; Trucks, G.; Schlegel, H.; Scuseria, G.; Robb, M.; Cheeseman, J.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. *Gaussian*, Wallingford, CT (USA) 2009.

(40) Boys, S. F.; Bernardi, F. The calculation of small molecular interactions by the differences of separate total energies. Some procedures with reduced errors. *Mol. Phys.* **1970**, *19*, 553−566.