A Unified Mechanism for Abiotic Adenine and Purine Synthesis in Formamide**

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The synthesis of purines through dehydration and condensation of formamide is a potential abiotic source of nucleobases. It has been reported since the 1950s that heating formamide to near boiling generates purine in high yield (> 70%).[1] The scope of this formamide condensation has been broadened by the identification of multiple biologically relevant products, including nucleobases and amino acid derivatives, from both neat and mineral-doped formamide.[2–4] Mechanistic pathways to purines have been proposed,[3–8] though there are significant variations between routes leading to related products. Herein, data is presented suggesting a common pathway for the abiotic syntheses of both purine and adenine from formamide; the proposed route is also highly reminiscent of the biosynthesis of purine nucleobases (Scheme 1). This is the first evidence suggesting that a glycine derivative is a critical intermediate in purine synthesis from formamide, and that this glycine backbone forms a scaffold for purine ring production in the same orientation as in purine ring biosynthesis. Parallels between nucleobase biosynthesis and plausible abiotic syntheses may allude to the origins of this metabolic pathway.[9]

Abiotic nucleobases and their analogues have been identified in meteorites,[10] spark discharge experiments,[11] and hydrogen cyanide (HCN) condensation reactions.[12–15] Typical laboratory procedures call for one to 15 m HCN in aqueous ammonia at 25–70 °C.[12–14] Analyses of the non-photochemical, mechanistic route from HCN to adenine have been conducted by Oró, Orgel, and Ferris.[12–15] Briefly, diaminomaleonitrile (DAMN), a relatively stable HCN tetramer, has been identified as an important intermediate. It provides the ring junction scaffold that subsequently closes to an imidazole and then a bicyclic purine, through reaction with cyanide-derived formamidine (Scheme 2). In aqueous ammonia, hydrogen cyanide is partially aminated to formamidine, and hydrated to formamide and ammonium formate (Scheme 3). Reactions that replace the aqueous ammonia with formamide are also consistent with the DAMN mechanism; adenine carbons C4, C5, and C6 are derived from HCN, while formamide provides C2 and C8 (Scheme 2).[5]

The synthesis of nucleobases in neat formamide has also been investigated.[2–7] The abiotic concentration of formamide, arising from dilute aqueous mixtures by evaporation, is more plausible than that of HCN. Formamide's vapor pressure is lower than that of both HCN and water, with ammonia, hydrogen cyanide is partially aminated to formamidine, and hydrated to formamide and ammonium formate (Scheme 3). Reactions that replace the aqueous ammonia with formamide are also consistent with the DAMN mechanism; adenine carbons C4, C5, and C6 are derived from HCN, while formamide provides C2 and C8 (Scheme 2).[5]

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a wide liquid range at atmospheric pressure (2–210°C). Dehydration of formamide to cyanide, though slow,[16] yields the carbon nucleophile necessary for the synthesis of complex organic compounds.

Purine is the most abundant nucleobase derivative produced by heating neat formamide.[2] Experimental and computational data support a pyrimidine-first mechanism in reactions starting from equimolar concentrations of HCN and formamide (Scheme 4).[7] This pathway bears little resemblance to the DAMN-based adenine synthesis from HCN and is complicated by the requirement for an in situ reduction. Pathways from formamide to adenine have been theorized to progress through pyrimidine[3] or DAMN[4] intermediates, though mechanistic experiments have not been conducted.

The mechanistic pathway presented herein begins with the dehydration of formamide to cyanide (Scheme 5).[16] This reaction is slow, though it yields the carbon nucleophile necessary for the synthesis of complex organic compounds.

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The mechanistic pathway presented herein begins with the dehydration of formamide to cyanide (Scheme 5).[16] Subsequent formylation and dehydration (formiminylation) generates 2-iminoacetonitrile (I, Scheme 5), a strong electrophile capable of progressing to either purine or adenine.

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Competing with hydride for 2-iminoacetonitrile (I) is an additional cyanide nucleophile, which reacts to generate 2-aminomalononitrile (III) on the pathway to adenine. The progression of AICN to adenine proceeds by formiminylation and tautomerization to Vb, ring closing to VIa, and a final tautomerization to adenine. In our hands, 25 mg of aminomalononitrile produced adenine in 17% yield under typical reaction conditions. Hydrated isomers of III and IV (R=CN) have previously been identified in mineral-doped formamide condensation reactions.[2c]

Consistent with the proposed mechanisms are product distributions resulting from varied starting concentrations of ammonium formate and cyanide. The pathways to purine and adenine compete for 2-iminoacetonitrile, thus an increased concentration of hydride-generating formate favors the reduction pathway to purine. A reaction of 5.0 mg of KCN in 1.0 mL of formamide favors adenine production over purine production by 5.5:1 (Table 1). However, the same reaction, but with 15 mol% formate favors purine by over 6:1.
Table 1: Adenine and purine production as a function of [formate].[a]

<table>
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<tbody>
<tr>
<td>0</td>
<td>220</td>
<td>40</td>
<td>5.5:1</td>
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<tr>
<td>1</td>
<td>150</td>
<td>80</td>
<td>1.9:1</td>
</tr>
<tr>
<td>2</td>
<td>240</td>
<td>190</td>
<td>1.3:1</td>
</tr>
<tr>
<td>5</td>
<td>210</td>
<td>330</td>
<td>1:1.6</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>290</td>
<td>1:3.2</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>250</td>
<td>1:6.3</td>
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</tbody>
</table>

[a] 0–15 mg KNO in 1.0 mL of 15 mol% ammonium formate in formamide heated for 2 h at 165 °C.

Table 2: Adenine and purine production as a function of [cyanide].[b]

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>0</td>
<td>&lt; 10</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>260</td>
<td>1:26</td>
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<tr>
<td>5</td>
<td>40</td>
<td>250</td>
<td>1:6.3</td>
</tr>
<tr>
<td>9</td>
<td>110</td>
<td>230</td>
<td>1:2.1</td>
</tr>
<tr>
<td>14</td>
<td>360</td>
<td>230</td>
<td>1:6:1</td>
</tr>
<tr>
<td>30</td>
<td>1400</td>
<td>190</td>
<td>7:4:1</td>
</tr>
</tbody>
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[b] 0–30 mg KCN in 1.0 mL of 15 mol% ammonium formate in formamide heated for 2 h at 165 °C.

Cyanide is the source of a single carbon in the proposed mechanism to purine (red-label, Scheme 4); the previously described pyrimidine-based mechanism consumes two cyanide carbons (red-label, Scheme 4). Evidence supporting the proposed mechanistic pathway in formamide (with low concentrations of cyanide) was obtained by heating 14 mg of K13CN in 1.0 mL of 15 mol% ammonium formate in formamide at 165 °C for 2 h. The reaction produced a 3:2 ratio of adenine:purine, which were separated by reverse phase-HPLC (RP-HPLC) and analyzed by NMR spectroscopy and high-resolution mass spectrometry (MS). The m/z of the purine product was measured at 122.0555 (m/z 122.0548 predicted for mono-labeled purine, Figure S6). The 1H NMR spectrum of the purine fraction displays three doublets, each arising from a single 1H-13C coupling, consistent with a mono-labeled product and the proposed mechanism (Figure 1a). The 13C NMR spectrum has two isotopically enhanced peaks resulting in a pair of doublet of doublets (8 peaks, Figure 1b).

Reactions in formamide that begin with high initial concentrations of 13C-labeled HCN generate tri-labeled adenine,[3] implicating a DAMN mechanism (Scheme 2). However, in our experiments, which contain low starting cyanide concentrations (K13CN) as a better model of 150–180 °C formamide, the adenine fraction is primarily di-isotopically labeled, consistent with the proposed mechanism (Scheme 5). MS analysis of the product reveals m/z 138.0685 (m/z 138.0690 predicted for di-labeled adenine, Supporting Information, Figure S9). In the 1H NMR spectrum, two aromatic adenine protons are each split by two 13C atoms, resulting in a pair of doublet of doublets (8 peaks, Figure 1b).

The 13C NMR spectrum has two isotopically enhanced peaks consistent with C4 and C6 at δ = 149.3 and 150.6 ppm, respectively (Supporting Information, Figure S9, S10). The DAMN reaction pathway is observable as a minor secondary route; labeled carbon at C5 (δ = 113.9 ppm) split by C4 (δ = 256.3 ppm) and C6 (δ = 304.3 ppm) is consistent with the tri-labeled adenine expected for this pathway (Supporting Information, Figure S9).

In summary, we have described a unified mechanistic pathway to adenine and purine in formamide. The order and chemoselectivity of this route is reminiscent of purine biosynthesis. Observations of such similarities may help elucidate the evolution of early biotic metabolism.

Keywords: cyanides · formamide · nucleobases · prebiotic chemistry · purines

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