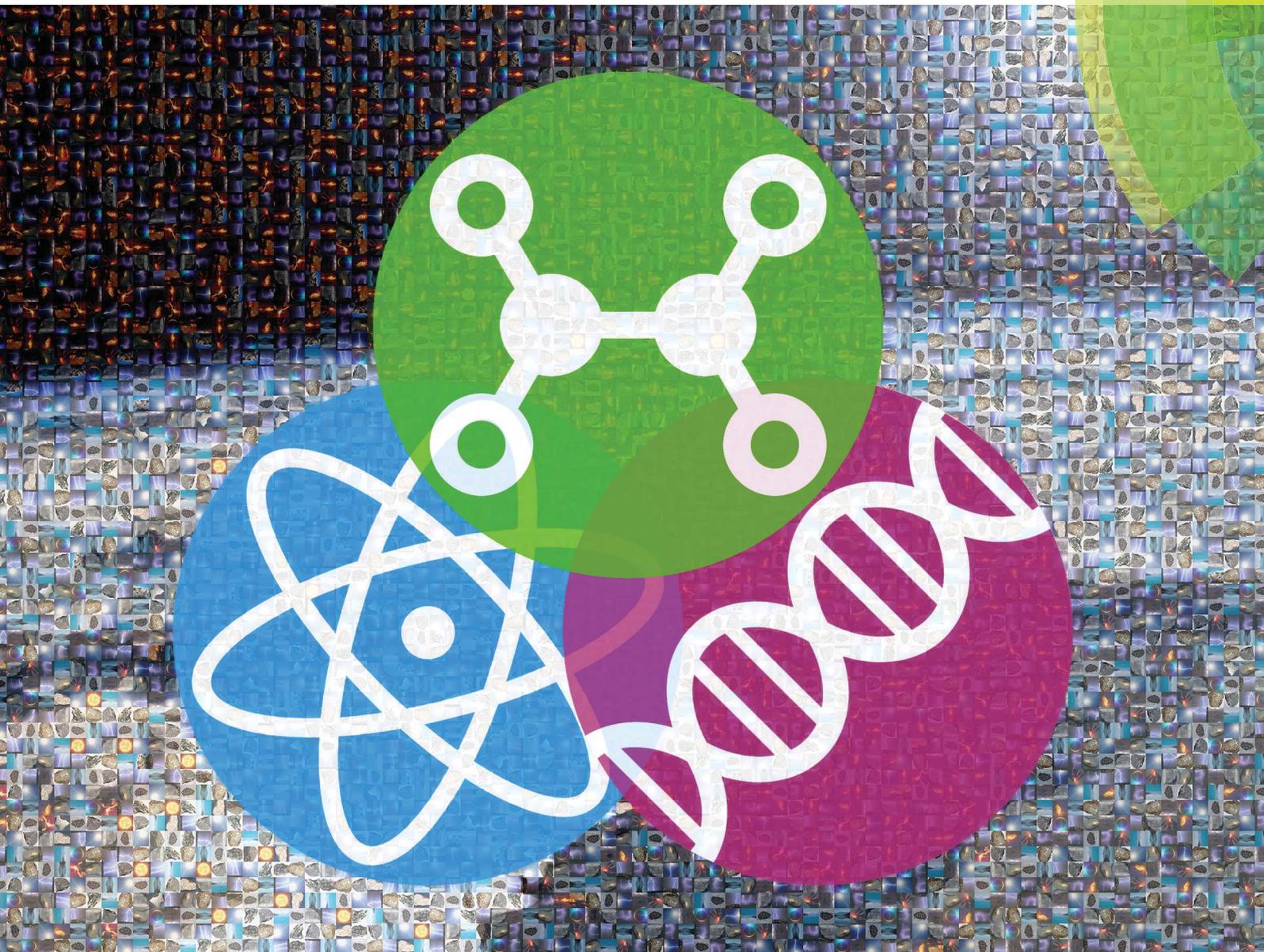


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## Tuning the reactivity of nitriles using Cu(II) catalysis – potentially prebiotic activation of nucleotides†

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During the transition from prebiotic chemistry to biology, a period of solution-phase, non-enzymatic activation of (oligo)nucleotides must have occurred, and accordingly, a mechanism for phosphate activation must have existed. Herein, we detail results of an investigation into prebiotic phosphate activation chemistry using simple, prebiotically available nitriles whose reactivity is increased by Cu<sup>2+</sup> ions. Furthermore, although Cu<sup>2+</sup> ions are known to catalyse the hydrolysis of phosphodiester bonds, we found this deleterious activity to be almost completely suppressed by inclusion of amino acids or dipeptides, which may suggest a productive relationship between protein and RNA from the outset.

### Introduction

For over half a century the search for a simple prebiotic reagent which is capable of efficiently activating inorganic/nucleoside phosphate(s) has been largely unsuccessful.<sup>1</sup> Yet, it stands to reason that an abiotic means of activating nucleotides (to allow replication by non-enzymatic polymerisation/ligation) was available on Earth in order to progress toward a more advanced chemical system. To circumvent this impasse, generally speaking, two approaches have been taken. In the first, *N,N*-dialkyl carbodiimides, not considered to have been available on early Earth but ubiquitous in synthetic chemistry, are used as phosphate activating agents, often at high concentration, in the presence or absence of other catalysts.<sup>2</sup> A parallel has been drawn between *N,N*-dialkyl carbodiimides and carbodiimide (HN=C=NH), the tautomer of cyanamide, a presumed prebiotically abundant molecule.<sup>1b</sup> However, this tautomeric equilibrium lies overwhelmingly in favour of cyanamide, meaning that only trace amounts of carbodiimide are available for reaction with phosphate, which is reflected in the vast excesses of cyanamide that have to be employed to achieve moderate yielding but sluggish reactions.<sup>3</sup> Consequently, arguments describing *N,N*-dialkyl carbodiimides as suitable prebiotic surrogates for cyanamide are not convincing. In the second approach, preformed (purified) phosphorimidazolides are used in the desired reaction, often with multiple rounds of addition thereof. Orgel found imidazole was a likely prebiotic molecule,<sup>4</sup> produced by photochemical isomerization of  $\beta$ -

aminoacrylonitrile.<sup>5</sup> Furthermore, realising that nucleoside-5'-triphosphates are kinetically stable in the absence of enzyme catalysis, Orgel suggested that nucleophilic displacement of pyrophosphate from a nucleoside 5'-triphosphate by imidazole would give the corresponding phosphorimidazolide, a molecule with a similar free energy of hydrolysis to the parent triphosphate but also kinetically labile.<sup>6</sup> Nucleoside 5'-phosphorimidazolides are known to be capable of efficiently extending a primer-template complex.<sup>7</sup> However, absent from the literature is a prebiotically plausible, high-yielding, solution-phase synthesis of these activated monomers. The obvious requirement for prebiotic phosphate activation coupled with the obstinate problem of how prebiotic phosphate activation was achieved, is likely why the use of implausible activating agents and preformed phosphorimidazolides have been 'tolerated' in origins of life research – in order that 'downstream' prebiotic chemistry can be investigated. Current studies suggest that if non-enzymatic RNA replication was achieved *via* sequential monomer addition to a primer-template complex, phosphorimidazolides were required.<sup>8</sup> If a convincing prebiotic synthesis of phosphorimidazolides cannot be found, it would suggest that non-enzymatic RNA replication was achieved *via* ligation of oligonucleotides on a template, as suggested by the work of von Kiedrowski.<sup>9</sup> Furthermore, the chemistry which leads to activated (oligo)nucleotides could provide valuable insight into the geochemical scenario in which phosphate activation, and previous prebiotic synthesis, took place.

The early phase of high energy chemistry which must have taken place on primitive Earth, would be expected to generate a significant amount of small, multiple bond-rich molecules.<sup>10</sup> If the potential energy locked in these multiple bonds could be harnessed, plentiful sources of prebiotic activating agents could have been available. This was recognized many years ago and was partly why molecules such as cyanate (NCO<sup>-</sup>), cyanogen

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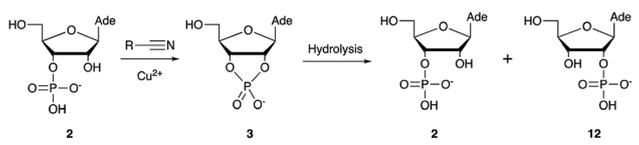
((CN)<sub>2</sub>) and cyanamide (NH<sub>2</sub>CN) were investigated as prebiotic activating agents.<sup>1</sup> It is noteworthy that a large number of (proto)biomolecules are accessible in prebiotically plausible syntheses using, or producing, these same, small, high energy molecules.<sup>10,11</sup> These chemical networks are consistent with a geochemical scenario.<sup>10b,11c,12</sup> Given that these molecules are omnipresent in our protometabolic network, we were curious if their re-evaluation, in the context of our developing geochemical model, could provide a means to overcome inherent kinetic barriers and 'switch on' their reactivity by nitrile coordination. Thus, our attention turned to Fe<sup>2+</sup>, Fe<sup>3+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup> and Zn<sup>2+</sup> ions. Intriguingly, work from the Dronskowski group had shown that a variety of transition metal cations form complexes with cyanamide **1**. Being azophilic Cu<sup>2+</sup> is expected to associate strongly with cyanamide **1**,<sup>13</sup> but this is in contrast to oxophilic metal ions such as Mg<sup>2+</sup>. We began to examine the effect of these ions on reaction of adenosine 3'-phosphate (**2**, A3'P) with **1**.

## Results and discussion

Over the past few decades, nucleoside 3'-phosphates have been employed as a model system to study prebiotic phosphate activation chemistry.<sup>1a,9,14</sup> In 1968, Orgel firstly recognized that transiently activated nucleoside 3'-phosphates would rapidly

react intramolecularly with the adjacent nucleophilic 2'-OH group, consequently yielding nucleoside 2',3'-cyclic phosphates (e.g. A > P **3**).<sup>1a</sup> More recently, we demonstrated the divergent reactivity of the transiently activated intermediate, as the 2'-OH group can attack either phosphorus or carbon, with the formation of the cyclic phosphate or a 2'-transferred product, respectively.<sup>9,14a,15</sup> Hence, we began our study by investigating the effect of different metal ions (including Fe<sup>2+</sup>, Fe<sup>3+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>) on the cyanamide-induced activation of A3'P **2**. In a typical experiment, cyanamide **1** and **2** were incubated at 40 °C with, or without the metal ion, and the reaction was monitored after 20 h. While neither Fe<sup>2+</sup> nor Fe<sup>3+</sup> displayed any detectable catalytic effect on the cyclisation reaction and only a small improvement could be found when Zn<sup>2+</sup> or Ni<sup>2+</sup> or Co<sup>2+</sup> were included in the mixture, Cu<sup>2+</sup> efficiently promoted the formation of adenosine 2',3'-cyclic phosphate (Table S1†). The catalytic effect of copper was significant even at concentrations as low as 1% relative to **1** (yield: 52% in 20 hours), whilst no competing 2'-transfer was observed under all the conditions tested. This cyanamide-Cu<sup>2+</sup> system proved to be able to activate not only 3'-, but also 5'-phosphates, as demonstrated by the formation of adenosine pyrophosphate (A5'PP5'A, yield: 9%), following incubation of adenosine 5'-phosphate (A5'P) under similar conditions. But we have been unsuccessful in attempts to form the imidazolide of A5'P by *in situ* nitrile group activation

Table 1 Cu<sup>2+</sup>-nitrile-mediated activation of A'3P and effect of Gly or GlyGly



Entry	Nitrile	R	Yield of 3 or 2'-transfer adducts <sup>a</sup> (%)					
			CuCl <sub>2</sub>		CuCl <sub>2</sub> and Gly		CuCl <sub>2</sub> and GlyGly	
			3 <sup>b</sup>	2'-adduct	3 <sup>b</sup>	2'-adduct	3 <sup>b</sup>	2'-adduct
1	<b>1</b>	-NH <sub>2</sub>	84	n.d. <sup>c</sup>	85 <sup>d</sup>	n.d.	80	n.d.
2	<b>4</b>	-CH <sub>3</sub>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	<b>5</b>	-CH <sub>2</sub> -NH <sub>2</sub>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	<b>6</b>	-CH <sub>2</sub> -OH	5	n.d.	2	n.d.	n.d.	n.d.
5	<b>7</b>	-O <sup>-</sup>	24	15	21	8	23	5.4
6	<b>8</b>	-CN	53	6	50	4	97 <sup>e</sup>	3
7	<b>10</b>	-NH <sub>2</sub>	75	n.d.	60	n.d.	5	n.d.
8	<b>11</b>	-NH <sub>2</sub>	35	n.d.	22	n.d.	34	n.d.

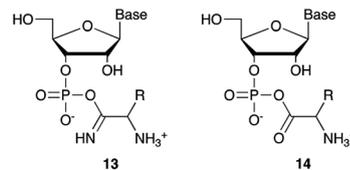
<sup>a</sup> Standard reaction conditions: nitrile (100 mM), **2** (50 mM), CuCl<sub>2</sub> (25 mM) and Gly or GlyGly (50 mM) in 90% H<sub>2</sub>O, 10% D<sub>2</sub>O at pH 4 (entries 1, 5 and 8) or pH 5.5 (entries 2, 3, 4, 6 and 7), heated at 40 °C for 20 hours. <sup>b</sup> Inferred from the amount of **3** plus adenosine 2'-phosphate A2'P **12**, assuming that when **3** hydrolyses it always gives **2** and **12** in 1.8 : 1 ratios.<sup>17</sup> <sup>c</sup> Not detected. <sup>d</sup> Initial pH 5.5. <sup>e</sup> Initial pH 4.



chemistry. Whether this is either because imidazolides are not formed or because they are formed and then hydrolysed has not been investigated.

Next, we investigated whether  $\text{Cu}^{2+}$  could catalyse phosphate activation using other prebiotically relevant nitrile-containing molecules (Table 1).<sup>11</sup> Acetonitrile **4**, 3-aminopropionitrile **5** and glycolonitrile **6** were unsuccessful in promoting  $\text{Cu}(\text{II})$ -catalysed cyclization of A3'P **2**. Cyanate **7** and cyanogen **8** displayed a dual behaviour, producing **3** together with the 2'-adduct which will be discussed later (**9**, Scheme 1). Intriguingly, cyanogen **8** reacts with A3'P **2** even in the absence of a metal catalyst; however, the relative formation of the cyclised product and the 2'-transfer product is greatly affected by including  $\text{Cu}^{2+}$  in the mixture. And the reaction of A3'P with other nitriles in the absence of  $\text{Cu}^{2+}$  is insignificant.

We then turned our attention to aminoacetonitrile **10** and 2-aminopropionitrile **11**, the Strecker precursors of glycine and alanine, respectively, previously shown to originate from the same prebiotic pathways that form ribonucleotides, amino acids and phospholipid precursors.<sup>11c</sup> Interestingly, attack of a nucleoside monophosphate onto an  $\alpha$ -aminonitrile would involve the formation of a transient imidoyl phosphate, analogous to the mixed anhydride produced by aminoacylation of nucleotides,<sup>14a,15</sup> with the only difference being an imidoyl-**13** instead of a carbonyl-**14** derivative (Scheme 2). Based on the observation by Moureu and Bongrand<sup>16</sup> that cuprous



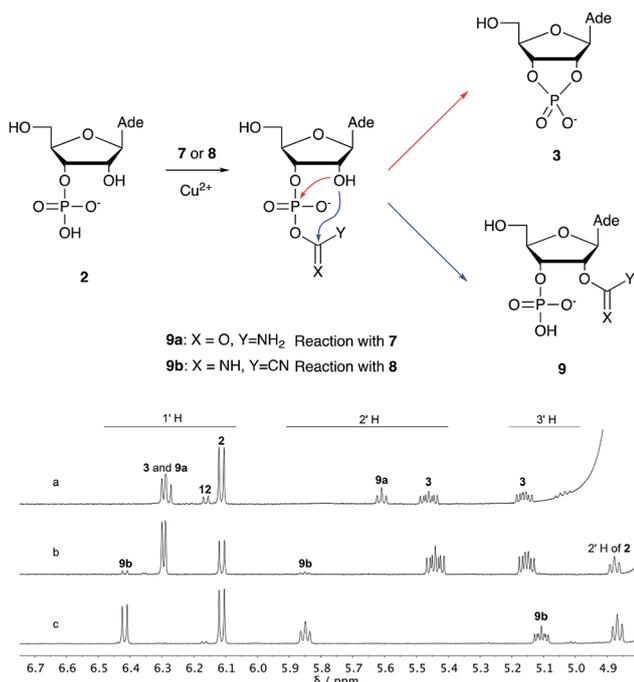
Scheme 2 Structure of nucleoside 3'-phosphate imidoyl-**13** and carbonyl-**14** mixed anhydride derivatives.

cyanooacetylde undergoes Glaser coupling<sup>17</sup> to give dicyanodiacetylene on oxidation, we did not investigate  $\text{Cu}^{2+}$ -catalysed addition of phosphates to cyanoacetylene as the related Eglinton reaction<sup>18</sup> was anticipated.

Whilst mixed anhydride **14** has been previously been shown to both cyclise and give the 2'-adduct, both  $\alpha$ -aminonitriles **10** and **11** triggered cyclisation of **2**, but the related 2'-transferred products were not detected. We speculate that the reactivity and/or the geometry of **13** could be altered by the simultaneous coordination of both the imido- and amino-nitrogen atoms to copper, somehow favouring cyclisation and hydrolysis over transfer. In this regard, we performed an experiment in which A5'P, aminoacetonitrile and  $\text{Cu}^{2+}$  were incubated in either  $\text{H}_2^{16}\text{O}$ ,  $\text{H}_2^{18}\text{O}$  or a mixture of  $\text{H}_2^{16}\text{O}/\text{H}_2^{18}\text{O}$  (1 : 2), and monitored the isotopic composition of the products by mass spectrometry. In the absence of a vicinal hydroxyl group, activation of the 5'-phosphate of A5'P would result in the attack of water either on the activated phosphate or on the imidoyl-carbon of the imidoyl phosphate, producing  $^{18}\text{O}$ -labelled A5'P or  $^{18}\text{O}$ -labelled glycineamide, respectively (Scheme S1, S2 and Fig. S1†). In our system, glycineamide was the only new labelled product detected (the ratio of unlabelled/ $^{18}\text{O}$ -labelled glycineamide was equal to the  $\text{H}_2^{16}\text{O}/\text{H}_2^{18}\text{O}$  ratio), thereby suggesting the selective attack of water on the imidoyl-carbon, and possibly a link with the aminoacyl-transfer chemistry described by Schimmel and co-workers on a minihelix.<sup>19</sup>

Optimization for cyclisation of A3'P **2** to **3** by modifying reaction conditions revealed that moderate to high yields could be obtained under slightly acidic conditions, but, alongside the expected cyclized product, we could detect the formation of adenosine 2'-phosphate (A2'P, **12**, Tables 1 and S2†).

Reasoning that the latter derived from hydrolysis of the former as the reaction progressed, we started examining the hydrolysis of **3** under these reaction conditions. As expected,  $\text{Cu}^{2+}$  catalysed the opening of the cyclic phosphate both at pH 4 and 5.5, producing **2** and **12** in 1.8 : 1 ratios.<sup>20</sup> We thus wondered if ligands able to coordinate  $\text{Cu}^{2+}$  would attenuate the metal's hydrolytic activity. In particular, we focused our attention on prebiotically plausible chelating agents able to form bi- and tri-dentate complexes with copper ions,<sup>21</sup> namely glycineamide, as the by-product of the aminoacetonitrile-mediated activation described above, its hydrolysis product glycine (Gly) and the dipeptide glycylglycine (GlyGly). The excellent coordinating properties of these ligands (Table S3†) considerably decreased the degree of cyclic phosphate hydrolysis, probably by competing with **3** for binding to the metal centre. In parallel, we examined urea and ammonium carbonate, the by-products



Scheme 1 Mechanism of cyclisation vs. transfer in the cyanate- or cyanogen-mediated activation of **2** and  $^1\text{H}$  NMR spectra of the mixtures. (a)  $^1\text{H}$  NMR spectrum after 20 h following incubation of **2** (50 mM),  $\text{CuCl}_2$  (25 mM) and cyanate **7** (100 mM) at pH 4, 40 °C, showing the formation of **3** and **9a**; (b)  $^1\text{H}$  NMR spectrum after 1 h following incubation of **2** (12.5 mM), Gly (50 mM)  $\text{CuCl}_2$  (25 mM) and cyanogen **8** (100 mM) at pH 5.5, RT, showing the formation of **3** and **9b**; (c) as (b) but without  $\text{CuCl}_2$ . N.B. conditions are different to Table 1.





glycinamide hydrolysis will feed the glycine pool (and eventually produce GlyGly under coupling conditions), thereby preventing hydrolysis and enabling the accumulation of short oligonucleotides (Scheme 3).

## Conflicts of interest

There are no conflicts to declare.

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