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A Light-Releasable Potentially Prebiotic Nucleotide Activating Agent

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Supporting Information

ABSTRACT: Investigations into the chemical origin of life have recently benefitted from a holistic approach in which possible atmospheric, organic, and inorganic systems chemistries are taken into consideration. In this way, we now report that a selective phosphate activating agent, namely methyl isocyanide, could plausibly have been produced from simple prebiotic feedstocks. We show that methyl isocyanide drives the conversion of nucleoside monophosphates to phosphorimidazolides under potentially prebiotic conditions and in excellent yields for the first time. Importantly, this chemistry allows for repeated reactivation cycles, a property long sought in nonenzymatic oligomerization studies. Further, as the isocyanide is released upon irradiation, the possibility of spatially and temporally controlled activation chemistry is thus raised.

From the pioneering works of Orgel[1,2] and Ferris,[2,3] through to the most recent breakthroughs of Szostak and co-workers,[4,5] nucleoside 5′-phosphorimidazolides have been used as long-lived activated nucleotides for the nonenzymatic oligomerization and templated copying of RNA. Yet, a prebiotically plausible synthesis of such species remains elusive.[6,7] Isocyanides are known phosphate activating agents,[8] and we have previously described how they could have been involved in a fleeting prebiotic activation of nucleoside monophosphates by Passerini-type chemistry.[9] This chemistry requires both isocyanides and aldehydes, and while the latter species arise from the photoredox pathways that lead from HCN to amino acids, lipids, and ribonucleotides,[10,11] a prebiotic pathway to the former remains to be found. Herein, following along systems chemistry lines, we describe a potentially prebiotic synthesis of methyl isocyanide **1** and demonstrate its use in the *in situ* formation of 5′-phosphorimidazolides via interrupted Passerini chemistry in a four-component system.

Incubation of adenosine 5′-monophosphate (AMP) with imidazole **2** (Im), acetaldehyde **3**, and methyl isocyanide (pH 6) resulted in the formation of adenosine 5′-phosphorimidazolide (ImpA) in 54% yield after only 30 min (Figure 1, Table S1, and Figure S1a). The reaction could also be performed with other prebiotically relevant aldehydes, such as formaldehyde or glycolaldehyde (41 and 48% yields of ImpA were obtained after 30 min, respectively, data not shown). Screening the reaction with acetaldehyde over a pH range revealed a pH optimum of 6.5 (yield: 71%, Figure 1d and Table S1), presumably reflective of the simultaneous requirements of having imidazole as its free base (pK_a of imidazolium ≈ 7.0), dianionic AMP (pK_a of AMP monoanion ≈ 6.5), and sufficient acid to protonate acetaldehyde.[12] Mechanistically, we speculate that the transient imidoyl phosphate **4** (undetectable by NMR spectroscopy), generated from the reaction of AMP with the nitrilium ion **5**, is attacked by imidazole at phosphorus, with the consequent formation of the phosphorimidazolide (Figure 1a).

Following its initial formation, and in the absence of a primer−template complex (when polymerization can be expected),[4,5] ImpA is progressively hydrolyzed to AMP and thence formation of AMP pyrophosphate (AppA, Figure 2a,b). It is interesting to note that although these outcomes are reflective of the known reactivity of ImpA under these conditions, a connection between 5′,5′-pyrophosphate ribodinucleotides and modern cofactors has been previously suggested.[13,14] The other canonical mononucleotides, GMP, CMP, and UMP, displayed analogous behaviors, with ImpN yields ranging from 69 to 75% (Table S1 and Figure S2). Importantly, no modifications occurred to any of the nucleobases, revealing isocyanides to be selective phosphate activating agents.[9,15] The requirement for Mg^{2+} in ribonucleotide polymerization prompted us to investigate the effect of this additive on the synthesis of ImpA. Mg^{2+} catalysis, as expected, enhanced the rate of both ImpA hydrolysis and AppA production (Figure 2b), but did not affect the initial yield of imidazolide (Table S1).

A desirable feature in prebiotic nucleotide activation chemistry is the possibility of repeatedly activating the spent monomers that derive from imidazolide hydrolysis to allow for further rounds of polymerization.[15] Although it was beyond the scope of this study to investigate polymerization, we sought to establish repeated activation chemistry. Importantly, adding methyl isocyanide in aliquots, followed by intervals in which hydrolysis and pyrophosphate formation served as a proxy for polymerization in a more complex system, resulted in cycles of AMP activation, in which every fresh portion of the activating agent triggered the regeneration of ImpA with comparable efficiency (Figure 2c).

Recently, Szostak and co-workers[4,16] have reported the superiority of nucleoside 5′-phosphoro-2-aminoimidazolides ($2NH_2$ImpN) in the nonenzymatic copying of oligoribonucleotides, as a result of more efficient formation of a transient imidazolium bridged dinucleotide. In our system, activation of

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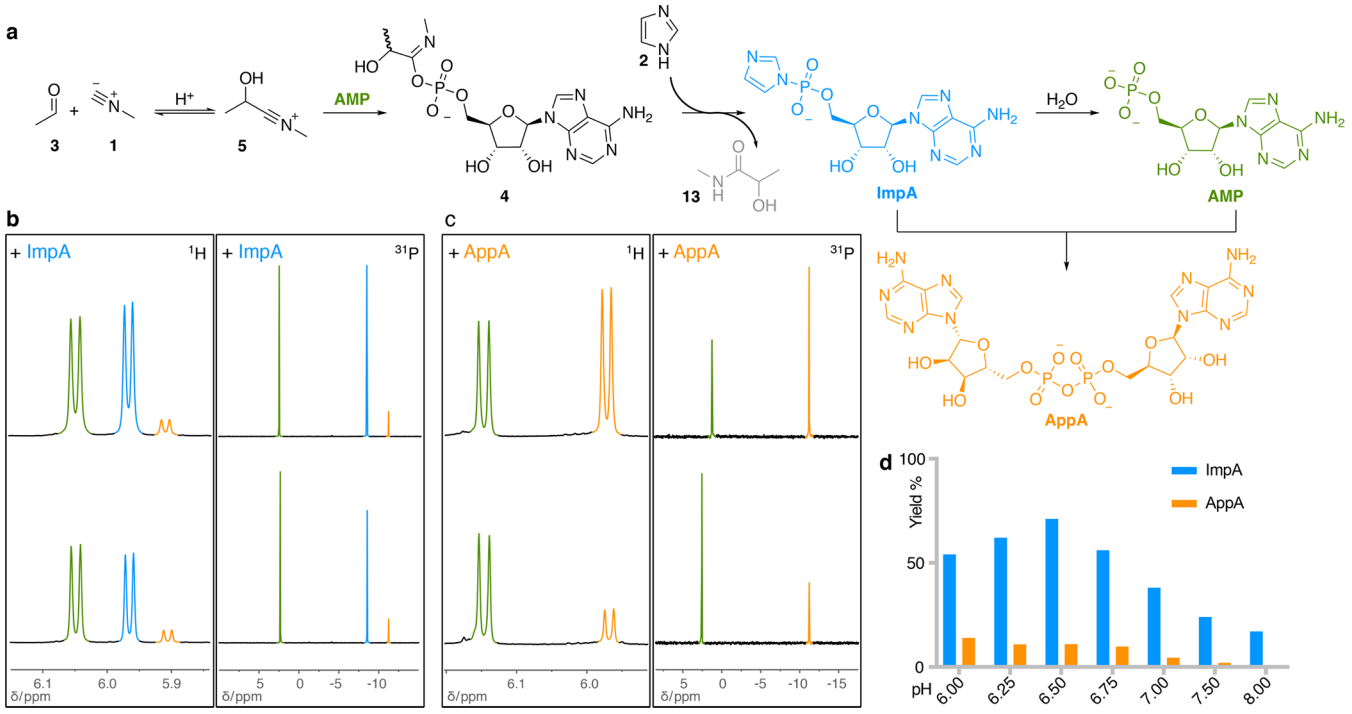

Figure 1. Synthesis of ImpA and spiking experiments. (a) Suggested mechanism for the methyl isocyanide-mediated synthesis of ImpA. ^{1}H (left, magnification showing the H−C(1′) region) and ^{31}P (right) NMR spectra confirming the formation of (b) ImpA (100 mM AMP, 400 mM **1**, 100 mM **2**, 400 mM **3**, pH 6.5, 3.5 h) and (c) AppA (100 mM AMP, 400 mM **1**, 100 mM **2**, 400 mM **3**, 20 mM Mg^{2+}, pH 6.5, 72 h; 5-fold dilution before spiking), by spiking with authentic samples (top). (d) Plot of the maximum % yield of ImpA vs pH of the reaction. (green, AMP; blue, ImpA; orange, AppA).

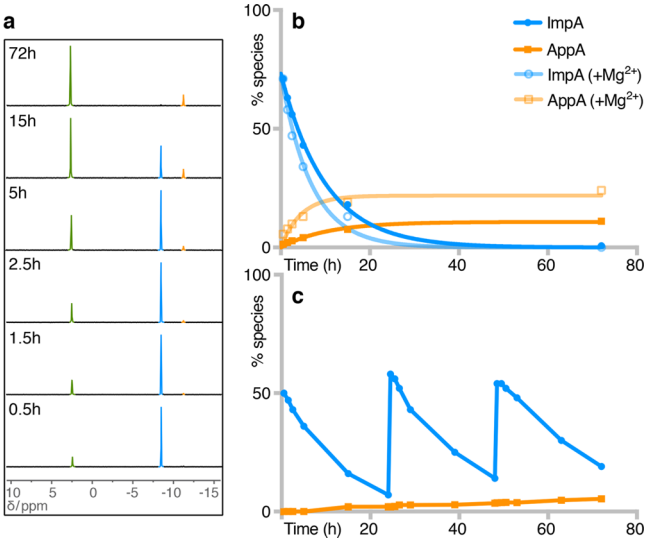

Figure 2. Synthesis and recycling of ImpA. (a) ^{31}P NMR spectra showing ImpA formation and products thereof (100 mM AMP, pH 6.5; reaction times as labeled on each spectrum). (b) Plot of % ImpA and AppA (from a) vs time and effect of Mg^{2+}. (c) Plot of % ImpA and AppA vs time for the (re)cycling of (spent) AMP, by iterative cycles of activation (green, AMP; blue, ImpA; orange, AppA).

AMP with methyl isocyanide and acetaldehyde in the presence of 2-aminoimidazole **6** resulted in its conversion to the corresponding 2NH_2ImpA at an optimum pH of 7 (Table S1 and Figures S1b and S3). Further optimization resulted in improvements to the production of both ImpA and 2NH_2ImpA, with 89% and 76% yields obtained, respectively (Table S1).

For this selective phosphate activation chemistry to have occurred on the primordial Earth, a prebiotic synthesis of methyl isocyanide would have been required. We thus considered its possible formation as a result of metallo-organic chemistry.[10,17,18] Iron, as the most abundant transition metal on Earth, probably played a fundamental role in the emergence of life's building blocks.[19] Upon exposure to a primordial atmosphere containing HCN, CO, and NO·, iron would have formed not only stable homoleptic complexes such as $[Fe(CN)_6]^{4-}$ (ferrocyanide **7**, Figure 3)[20] but also mixed ligand complexes with ligands isoelectronic with cyanide of the form $[Fe(CN)_5L]^{n-}$, where L = CO (n = 3) or NO (n = 2).[19] Continuing a study initiated by Beck,[19,21] we investigated the chemistry of the nitrosyl complex $[Fe(CN)_5NO]^{2-}$ (nitroprusside, **8**), known, *inter alia*, to mediate the diazotization of amines.[21,22] To determine whether nitroprusside could plausibly have formed under prebiotic conditions on Earth, we considered its possible formation from ferrocyanide and the nitrogen oxides NO·, NO_2^- (nitrite), and NO_3^- (nitrate). Lightning and meteorite impacts in the N_2-rich primordial atmosphere would have produced NO· (nitric oxide, Figure 3), which could have been directly absorbed into ferrocyanide containing pools, producing nitroprusside *in situ*. Alternatively, NO_2^- and NO_3^- could have accumulated in solution following disproportionation of NO·.[23]

Combining literature reports that nitroprusside[24] can be formed from NO_2^- and $[Fe(CN)_5H_2O]^{3-}$, and that the latter can be produced by photoaquation of ferrocyanide,[25−27] we found that irradiating a mixture of ferrocyanide and NO_2^- in the pH range 7−9.8 with 365 nm light afforded nitroprusside as the only new Fe^{II} complex detectable by ^{13}C NMR spectroscopy (Figure S4a). Importantly, irradiation of the same

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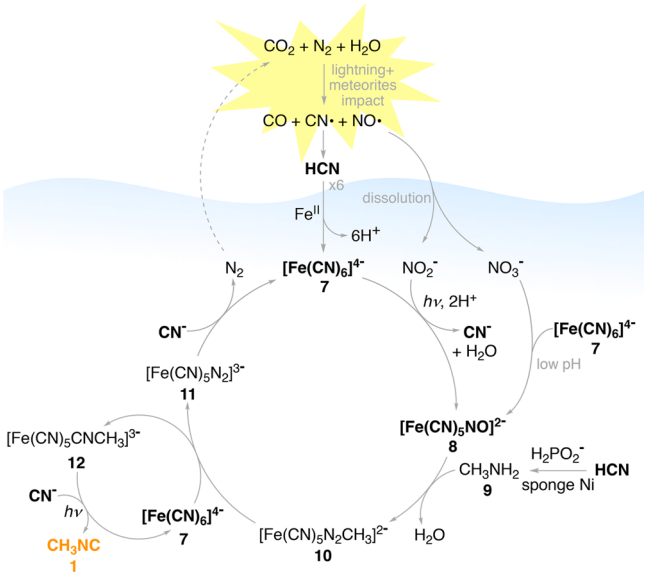

Figure 3. Schematic representation of the systems chemistry network producing methyl isocyanide (**1**).[21] High-energy atmospheric chemistry generates HCN (via CN·), CO and NO·. NO· undergoes various disproportionation reactions upon dissolution,[23] providing NO_2^- and NO_3^-. HCN is stored in solution as **7**, which reacts with NO_2^- (or NO_3^-) to provide **8**.[37] HCN is also a source of CH_3NH_2 **9**, which is diazotized by **8**. Alkylation of **7** by **10** gives **12** and **11**. Irradiation of **12** in the presence of CN^- releases **1** and returns **7**; reaction of **11** with CN^- returns **7**, releasing N_2.

mixture with 254 nm light also afforded nitroprusside, albeit less efficiently, suggesting that broad band irradiation from the young sun could have supported the formation of this complex.

We next investigated the reactions of nitroprusside with methylamine **9**, which would have been delivered to the primordial Earth in comets.[28] Alternatively, hydrogenation of HCN to methylamine would have been expected to occur in a geochemical scenario in which iron–nickel meteorites containing schreibersite $(Fe,Ni)_3P$ underwent corrosion in HCN-containing pools. In an anoxic environment, corrosion of schreibersite has been shown to give soluble Fe^{II}, $H_2PO_2^-$ (hypophosphite), HPO_3^{2-} (phosphite), and HPO_4^{2-} (phosphate), while the bulk iron–nickel alloy matrix gives insoluble porous nickel (sponge nickel) or nickel-enriched alloy.[29,30] As hypophosphite is known to decompose to H_2 and phosphite in the presence of Raney nickel,[31] we have recently investigated the hypophosphite-sponge nickel combination as a plausible prebiotic hydrogenation system in keeping with the general geochemical scenario described above. Accordingly, we found that reduction of HCN with hypophosphite and sponge nickel afforded methylamine as the sole organic product, identified by ^1H and ^{13}C NMR spectroscopy (Figure S4b,c).

The reaction of methylamine with nitroprusside in the presence of CN^- and HPO_4^{2-} (pH 9.8) proceeded with slow evolution of a gas, presumably N_2, indicative of diazotization chemistry. Analysis of the reaction mixture by ^1H and ^{31}P NMR spectroscopy confirmed the presence of products expected from the trapping of $[Fe(CN)_5N_2CH_3]^{2-}$ **10** by the nucleophiles H_2O, CN^-, and HPO_4^{2-}, namely CH_3OH (methanol), CH_3CN (acetonitrile), and $CH_3OPO_3^{2-}$ (methyl phosphate, Figure 4a,b and Figure S5a), respectively, together with an initially unidentified species. Reasoning that ferrocyanide is also produced under the above reaction

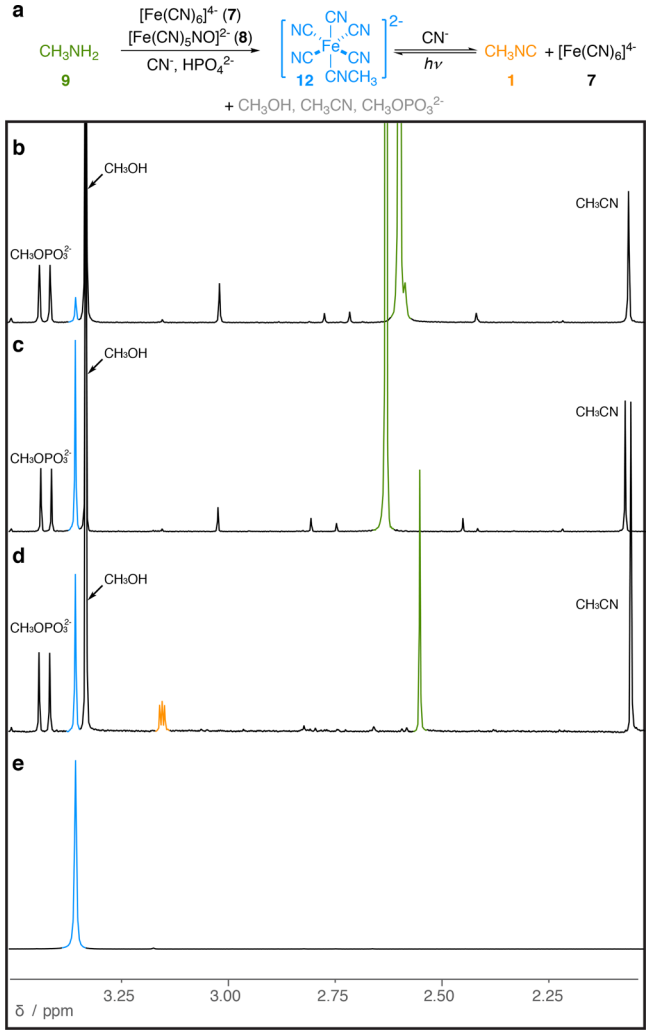

Figure 4. Prebiotic synthesis of methyl isocyanide (**1**). (a) Schematic representation of the synthesis of **1** via **12**. (b) ^1H NMR spectrum showing the synthesis of **12** without added **7** (20 h). (c) Same as (b) with **7** present from the start (20 h). (d) ^1H NMR spectrum showing the mixture of products obtained following photolysis of prebiotically synthesized **12** (2 h of irradiation). (e) ^1H NMR spectrum of synthetically prepared **12** (green, $CH_3NH_2/CH_3NH_3^+$; blue, $[Fe(CN)_5CNCH_3]^{3-}$; orange, $CNCH_3$).

conditions (by reaction of cyanide with $[Fe(CN)_5N_2]^{3-}$ **11**, Figure 3, or $[Fe(CN)_5H_2O]^{3-}$), and realizing that it could act as a nucleophile in its own right, we tentatively assigned the species as the isocyanide complex $[Fe(CN)_5CNCH_3]^{3-}$ **12**. This assignment was strengthened by repeating the above reaction with ferrocyanide (1 equiv) present from the outset, which led to an increase in the intensity of the new signal, and unambiguously confirmed by comparison with the ^1H NMR spectrum of an authentic standard prepared by addition of methyl isocyanide to a solution of $[Fe(CN)_5H_2O]^{3-}$ (Figure 4c,e and Figure S6b–d). Although initially concerned by the pH discontinuity between the activation chemistry and methyl isocyanide synthesis, we found that complex **12** could be generated in a pH range between 7 and 9.8, as the diazotization chemistry still proceeds, albeit more slowly, at neutral pH.

Mixed ligand isocyanide complexes are known to undergo isocyanide ligand exchange in coordinating organic solvents upon irradiation at 365 nm.[32] Irradiation of an aqueous

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solution of complex **12** at this wavelength, in the presence of excess CN^-, provided free methyl isocyanide (yield: 50% after 2 h, Figure S6), clearly identified by its characteristic 1:1:1 triplet at δ 3.16 in the ^{1}H NMR spectrum, and ferrocyanide.[33] The remaining materials detectable by ^{1}H NMR spectroscopy were residual complex **12** (41%), and two new complexes, tentatively assigned as *cis*-$[Fe(CN)_4(CNCH_3)_2]^{2-}$ and *trans*-$[Fe(CN)_4(CNCH_3)_2]^{2-}$ (signals unassigned, 9%). Further irradiation did not improve the yield of methyl isocyanide, which we attribute to a photostationary equilibrium having been reached.

Next, we sought to link the diazotization chemistry and the photolysis of complex **12**, in order to demonstrate that methyl isocyanide could be synthesized in a plausible geochemical setting. Accordingly, a mixture of methylamine, ferrocyanide, and nitroprusside, in the presence of CN^- and HPO_4^{2-} (pH 9.8), was allowed to react for 20 h. The mixture of products obtained was then irradiated for 2 h in the presence of excess CN^-. Analysis by ^{1}H NMR spectroscopy showed the expected mixture of products, including methyl isocyanide (Figure 4d).

Interestingly, the byproduct of the imidazolide synthesis described above is 2-hydroxy-*N*-methylpropanamide **13**, the hydrolysis of which would regenerate methylamine and thus feed back into a new cycle for the production of fresh methyl isocyanide. The other product of the hydrolysis would be lactate, a major player in extant and maybe early metabolism.

Overall these findings depict a common plausible scenario in which HCN is not only central to the synthesis of protein, lipid, and RNA building blocks but also drives chemical pathways that ultimately lead to nucleotide activation. In this scenario, methyl isocyanide could have been produced in a ferrocyanide and nitroprusside containing environment upon delivery of methylamine. Pools containing different accumulated materials (possibly at different pH), could have occasionally been linked by streams,[34] allowing the methyl isocyanide and nucleotide producing subsystems to mix, thereby enabling nucleotide activation and polymerization chemistry. The lack of high-yielding and prebiotically plausible phosphate activating agents has been a central problem in origin of life research for nearly 60 years, prompting the use of preactivated nucleotide substrates,[4,7] synthetic surrogates of ineffective prebiotic reactants,[6,35] or prebiotically questionable syntheses of desirable activating agents.[36] Here, for the first time, we describe a prebiotically plausible synthesis of methyl isocyanide, a storable and light-releasable activating agent and demonstrate its use in the efficient *in situ* activation of nucleotide monophosphates.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b05189.

Materials, methods, compound characterization, supplementary figures and tables (PDF)

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Notes

The authors declare no competing financial interest.

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