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Prebiotic Synthesis of Aminooxazoline-5'-Phosphates in Water by Oxidative Phosphorylation

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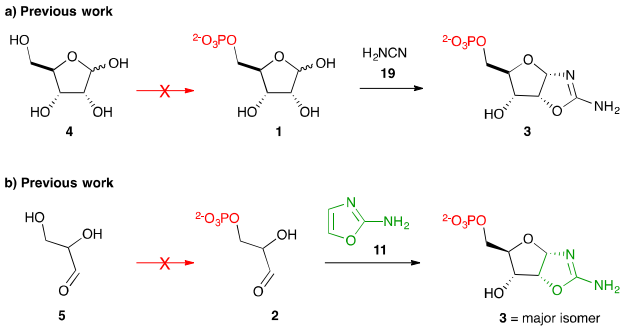
RNA is essential to all life on Earth and is the leading candidate for the first biopolymer of life. Aminooxazolines have recently emerged as key prebiotic ribonucleotide precursors, and here we develop a novel strategy for aminooxazoline-5'-phosphate synthesis in water from prebiotic feedstocks. Oxidation of acrolein delivers glycidaldehyde (90%), which directs a regioselective phosphorylation in water and specifically affords 5'-phosphorylated nucleotide precursors in upto 36% yield. We also demonstrated a generational link between proteinogenic amino acids (Met, Glu, Gln) and nucleotide synthesis.

Phosphorylation is a key step in nucleotide synthesis, however, elucidating the chemical origins of biological phosphorylation remains an unmet challenge.[1] Biology universally requires nucleotide-5'-phosphates but known prebiotic nucleotide syntheses yield 2',3'-cyclic phosphates.[2] This dichotomy between prebiotic synthesis and biochemical structure warrants investigation of prebiotic nucleotide-5'-phosphate synthesis.

Stepwise nucleotide synthesis from ribose-5-phosphate (**1**) or glyceraldehyde-3-phosphate (**2**) furnishes the key intermediate ribose aminooxazoline-5'-phosphate (**3**) (Scheme 1),[3] which can be converted into the corresponding pyrimidine β-ribonucleotides.[3,4] However, neither **1** nor **2** are prebiotically plausible substrates.[5-7] Ribose (**4**) is notoriously unstable and is only synthesised as a minor component of complex mixtures.[8] Additionally, prebiotic phosphorylations of **4** afford 1-, 2-, or 3-phosphates but not 5-phosphate **1**.[5] Equally, the highly facile (E1cB) elimination of phosphate hinders the prebiotic synthesis and the utility of **2**,[6,9] and prebiotic phosphorylation of glyceraldehyde (**5**) yields the 2-phosphate isomer rather than the desired 3-phosphate isomer **2**.[5,7] Accordingly, the synthesis of **3** remains a key unmet goal for origins of life research. Therefore, we set out to develop a selective prebiotic synthesis of **3** in water.

We recently developed a prebiotically plausible reaction network to access all the key intermediates of the core metabolic pathway of triose glycolysis.[7] Importantly our strategy bypassed the unstable aldehyde-3-phosphate **2**, which suggested that prebiotic nucleotide-5'-phosphate syntheses employing related strategies should be investigated. Accordingly, we turned our attention to oxidative phosphorylation and hypothesised that the constitutional simplicity and prebiotic versatility of acrolein (**6**)[10,11] presented a novel solution to the phosphorylation of nucleotide precursor **3**.



Scheme 1. a) Synthesis of ribose aminooxazoline-5'-phosphate (**3**) from prebiotically inaccessible (red arrow) pentose-5-phosphate (**1**).[3a] **b)** Synthesis of **3** from prebiotically inaccessible (red arrow) glyceraldehyde-3-phosphate (**2**).[3b]

Acrolein (**6**) is readily oxidised to glycidaldehyde (**7**) by treatment with hydrogen peroxide[12]—a prebiotically simple and robust oxidant.[7,13] Hydrolysis of **7** yields glyceraldehyde (**5**) and provides a direct generational link to sugars, but we suspected that the reaction of **7** in phosphate solution would lead to direct nucleophilic phosphorylation and transient access to **2** (Scheme 2). Eschenmoser and co-workers previously reported that oxirane **8** reacts with 2M phosphate (pH 10.5) to afford glycolaldehyde-2-phosphate cyanohydrin (**9**) in 70% yield.[14] Consequently, we suspected that **7**—which, unlike **8**, can be facilely prepared under prebiotically plausible

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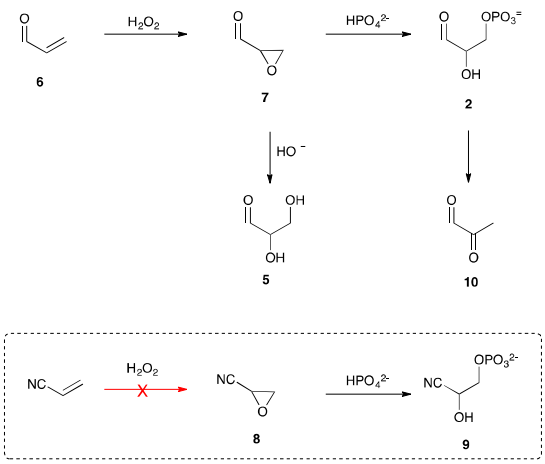
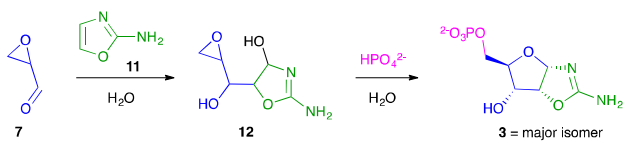
conditions—would be an ideal prebiotic substrate to direct phosphorylation in water.



Scheme 2. Acrolein (**6**) as a three-carbon-atom precursor of glyceraldehyde (**5**) and glyceraldehyde-3-phosphate (**2**). **Box:** Reaction of prebiotically inaccessible (red arrow) oxirane nitrile (**8**) with 2M phosphate (pH 10.5) to yield glycolaldehyde-2-phosphate cyanohydrin (**9**).

To test our hypothesis, we incubated **6** with hydrogen peroxide (140mM, pH 8.5) and obtained **7** in 90% yield. We then incubated **7** (250mM) in 1M phosphate (room temperature, pH 8.5) and were pleased to observe the formation of **2** (61% by NMR). However, as anticipated, only transient access to **2** was observed followed by rapid elimination to methyl glyoxal (**10**).

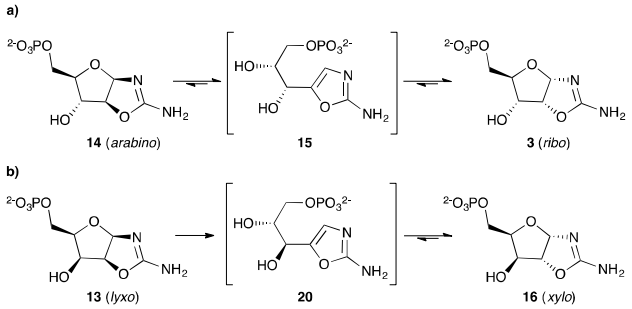
Intrigued by the predisposed chemical activation of **7** towards terminal phosphorylation in water and its simple prebiotically plausible synthesis, we set out to resolve the elimination problem encountered during synthesis of **2**. Pleasingly, we found that incubation of **7** (86mM) and 2-aminooxazole (**11**; 103mM)[15,16] for 18 h yielded **12** (78%) (Scheme 3).



Scheme 3. Synthesis of pentose aminooxazoline **3** from prebiotically accessible glycidaldehyde (**7**) and 2-aminooxazole (**11**), exploiting the epoxide moiety and inorganic phosphate to deliver highly regioselective 5'-phosphation in water.

Compound **12** retains its epoxide moiety, and therefore is an appropriate substrate for 5'-phosphorylation. Indeed, incubation of epoxide **12** in 1M phosphate (pH 7.0) gave pentose aminooxazoline-5'-phosphate (36% over two steps from **7**). This method bypasses both ribose **1** and aldehyde-phosphate **2**, and provides a regio- and diastero-selective prebiotic synthesis of oxazoline **3**. This phosphorylation strategy exploiting the intrinsic nucleophilicity of inorganic phosphate to achieve 5'-phosphorylation in water.

The diastereoselectivity of the reaction of **7** and **11** was observed to favour the *ribo*-stereochemistry of **3** (*ribo*/*arabino* 2.8:1) more strongly than the reaction of **5** with **11** (*ribo*/*arabino* 1.1:1).[15] Additionally, the *lyxo*-**13** was not observed during the synthesis of **3** from **7**. The propensity of **7** to yield the *ribo*-configuration was observed upon both alkaline hydrolysis of epoxide **12** at pH 12.0 (ESI Fig. S6) and nucleophilic phosphorylation of epoxide **12** at pH 7.0 (ESI Fig. S7). However the kinetic *ribo* selectivity, observed during alkaline hydrolysis, is further augmented by phosphate-catalysed stereochemical inversion of *arabino*-**14** → *ribo*-**3** (Scheme 4) during nucleophilic phosphorylation.[17]



Scheme 4. *Ribo/xylo*-selective C2'-epimerisation of pentose aminooxazoline-5'-phosphates. **a)** C2'-Epimerisation of arabinose aminooxazoline-5'-phosphate **14** and ribose aminooxazoline-5'-phosphate **3** **b)** C2'-Epimerisation of lyxose aminooxazoline-5'-phosphate **13** and xylose aminooxazoline-5'-phosphate **16**.

Incubation of **14** in 1M phosphate (40°C, pH 7.0, 5 d) gave **3** (58%) as the major aminooxazoline product, whereas incubation of **3** returned only a moderate amount of **14** (10%) after 5 d. Furthermore, at room temperature, incubation of **14** yielded **3** (50% after 20 d), whereas under comparable conditions incubation of **3** established an equilibrium with oxazole **15**, but not **14**, which allowed selective transformation of *arabino* → *ribo* stereochemistry.

It is of note that the free sugar ribose (**4**) isomerises within 13 d at 25°C, pH 7.0 to yield 75% arabinose, 6% ribulose, and only 19% ribose (**4**);[18] therefore, thermodynamic selection against the *ribose* is observed in the free sugar, whereas thermodynamic selection for *ribo*-stereochemistry is observed in aminooxazoline-5'-phosphates. Several groups have suggested mineralise stablisation of **4** under prebiotic constaints,[19] therefore it is notable that ribose aminooxazolines are (at least) 70 times more stable than ribose-borate.[16,20] These observations suggest that prebiotic nucleotide synthesis pathways that avoid the free sugars will be a key element in elucidating the chemical origins of Life.[8]

We suspected that the same C2'-epimerisation pattern would be reflected in the *lyxo*-**13** and *xylo*-**16**,[21] and was responsible for **13** not being observed during our synthesis of pentose aminooxazoline-5'-phosphates. To test this xylose-5-phosphate (**17**)[22] and lyxose-5-phosphate (**18**) were synthesised (Scheme

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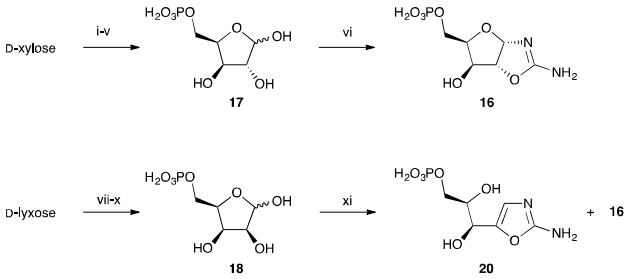
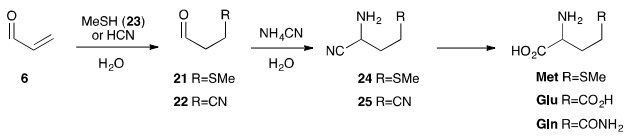
5), and their reactivity with cyanamide (**19**) was investigated. *Xylo*-**16** was readily synthesised and incubation in 1M phosphate (40 ˚C, pH 7.0) gave oxazole **20** by reversible dehydration, but lyxo-**13** was not observed. The reaction of **18** with cyanamide (**19**) gave a mixture of oxazole **20** and xylo-**16**, but again lyxo-**13** was not observed (ESI Fig. S21), which supported the observation that *lyxo*-**13** was not obtained from the reaction of epoxide **12** and phosphate.



Scheme 5. Synthesis of D-xylose-5-phosphate (**17**) and D-lyxose-5-phosphate (**18**) and their reaction with cyanamide (**19**). **D-xylose-5-phosphate 17**: (i) $CuSO_4$, H_2SO_4, $(CH_3)_2CO$, 4 d, r.t. (ii) 0.1M HCl, 3 h, r.t., 44% over two steps. (iii) $(PhO)_2POCl$, CH_2Cl_2, pyridine, 7 h, 0°C, 90%. (iv) H_2, PtO_2, MeOH, 16 h, r.t. 97%. (v) H_2O, 16 h, 50°C, quant. (vi) **19**, NH_4OH, H_2O, 64% isolated yield (>95% NMR yield). **D-lyxose-5-phosphate 18**: (vii) H_2SO_4, $(CH_3)_2CO$, 2 h, r.t. 93%. (viii) $(PhO)_2POCl$, CH_2Cl_2, pyridine, 6 h, 0°C, 92%. (ix) H_2, PtO_2, MeOH, 18 h, r.t. quant. (x) H_2O, 16 h, 50 °C, quant. (xi) **19**, NH_4OH, H_2O, (70% (**20**) and 30% (**16**) NMR yield).

Finally, given the close biochemical relatioship between amino acids and nucleotides, we turned our attention to prebiotic amino acid synthesis. Analysis of the proteinogenic amino acids suggested a clear link between glycidalehyde (**7**) and the amino acids methionine (**Met**), glutamic acid (**Glu**) and glutamine (**Gln**).

Met, **Gln** and **Glu** acid can be synthesised from 3-(methylthio)propanal (**21**) and 3-(cyano)propanal (**22**), which are in turn accessed from **6** by Michael addition of methanethiol (**23**) or cyanide, respectively (Scheme 6).[10,11] Therefore, we incubated **6** (140mM) in water at room temperature with **23** (saturated, pH 7.0) or potassium cyanide (700mM, pH 9.2). Pleasingly, we observed smooth conversion of **6** to aldehyde **21** (94%) and the cyanohydrin of aldehyde **22** (95%), respectively. Subsequently, aldehydes **21** and **22** were readily converted to aminonitriles **24** and **25** via Strecker reaction in water.



Scheme 6. Prebiotic synthesis of amino acids methionine (**Met**), glutamine (**Gln**), and glutamic acid (**Glu**) from acrolein (**6**).

These studies demonstrate the first prebiotically plausible synthesis of aminooxazoline-5'-phosphates in water by a facile nucleophilic phosphorylation. Importantly, we demonstrate that acrolein (**6**) provides a generational link between a key nucleotide precursor **3** and proteinogenic amino acids (**Met**, **Gln** and **Glu**), this new generational link supports the unified origins of these two distinct classes of metabolite.

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