Synthesis of Activated Pyrimidine Ribonucleotides in Prebiotically Plausible Conditions

Matthew W. Powner, Beatrice Gerland & John D. Sutherland,
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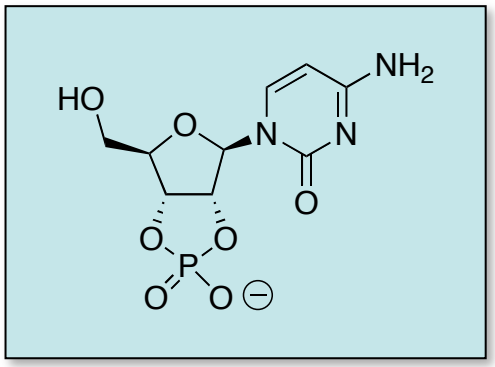
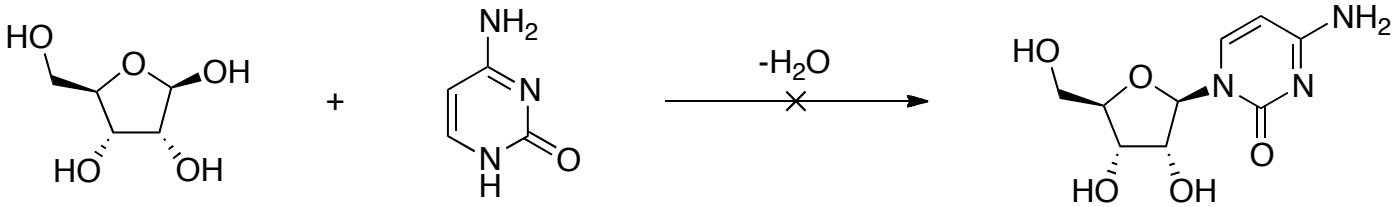


Chris Jones
Literature Presentation
15-11-11

Origin of Life - Fundamental Issues

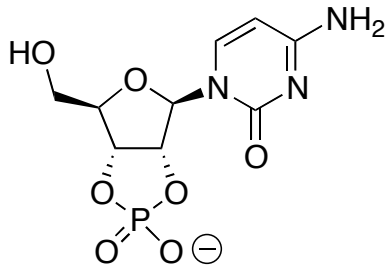
- Informational polymer must have arisen by purely chemical means – RNA?
- ‘RNA World’ hypothesis – no solid experimental support
- Formation of ribonucleotides from constituent parts?

- ribose difficult to form selectively
- addition of purines to ribose is inefficient
- addition of pyrimidines to ribose does not occur at all



Prebiotic Feedstock Molecules

- How could ribonucleotides be assembled from plausible prebiotic feedstocks?
- Reaction conditions must be consistent with early-Earth geochemical models



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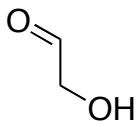
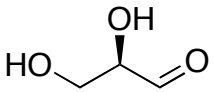
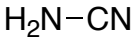


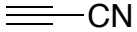
Glycoaldehyde



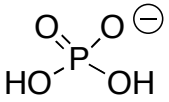
Glyceraldehyde



Cyanamide



Cyanoacetylene



Phosphate

Bypass free ribose and nucleobases altogether?

Pyrimidine Nucleotide Assembly

- Same small molecule building blocks
- Proceeds *via* arabinose amino-oxazoline intermediate **12**
- Conditions consistent with geochemical models
- Inorganic phosphate essential – functions as general acid/base catalyst, nucleophilic catalyst and pH buffer in earlier steps as well as being incorporated late on

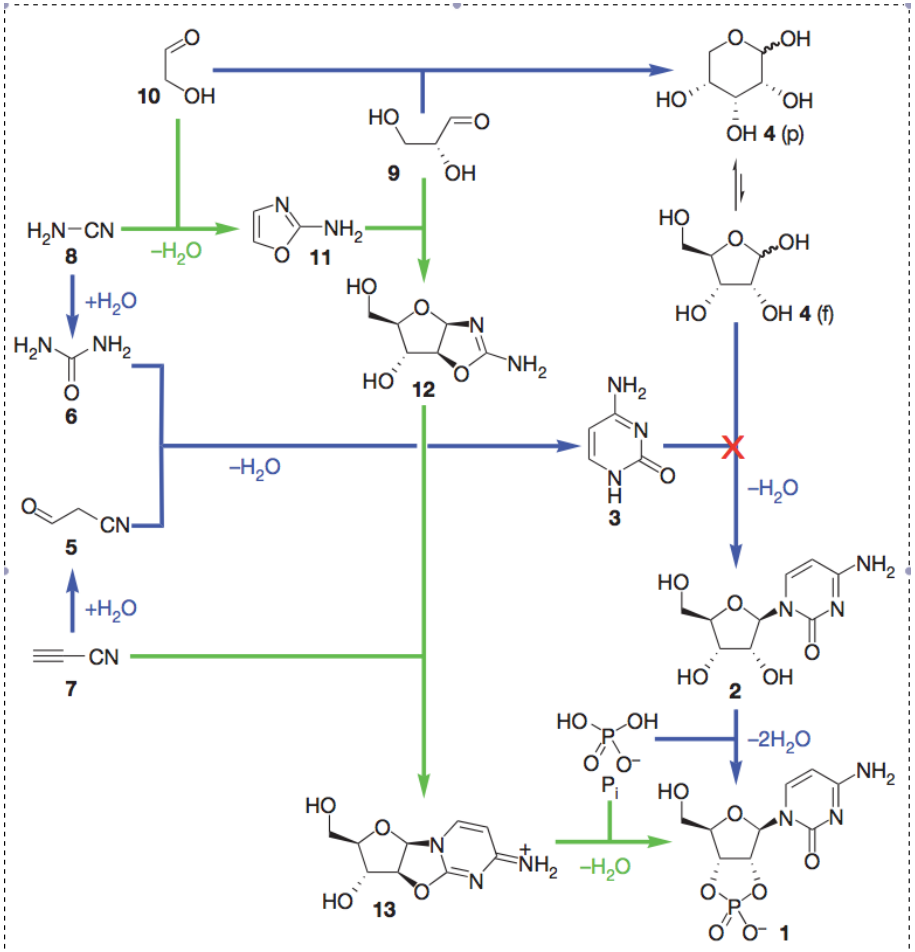
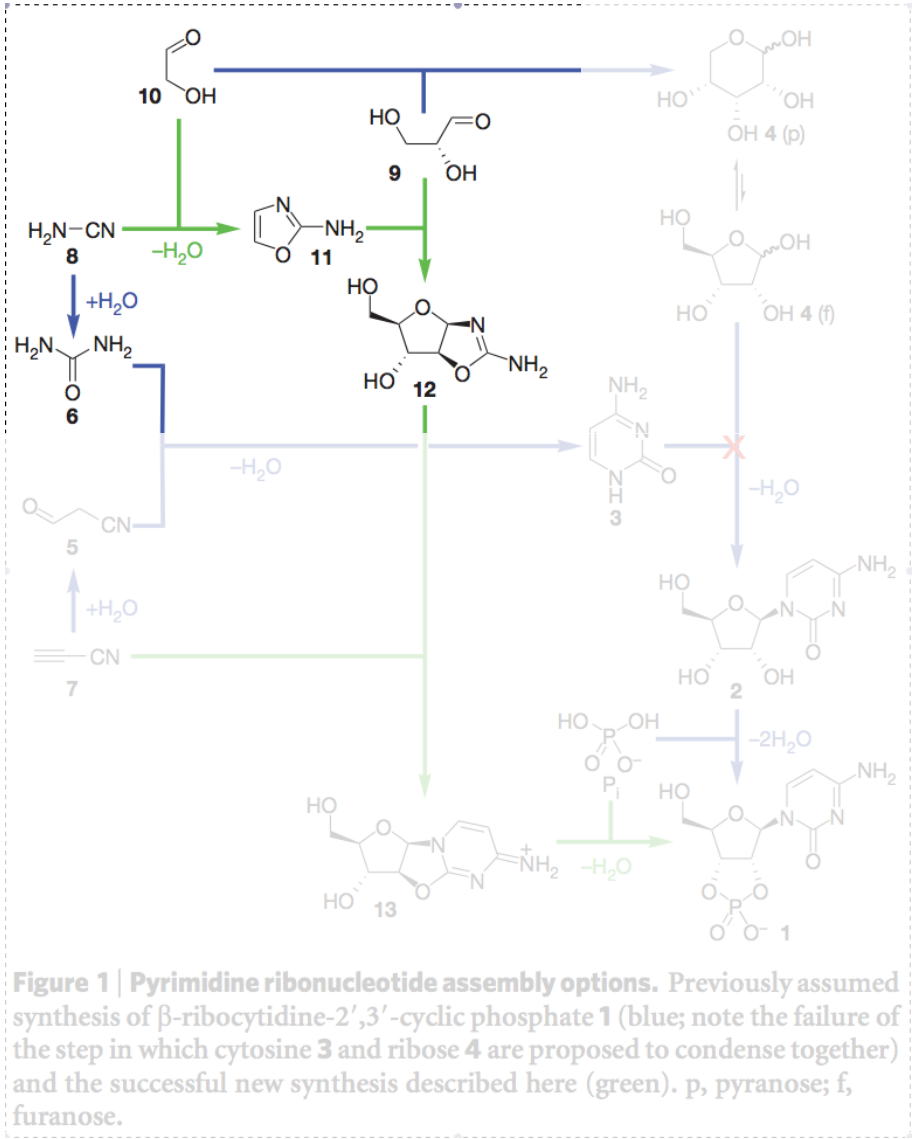


Figure 1 | Pyrimidine ribonucleotide assembly options. Previously assumed synthesis of β-ribocytidine-2′,3′-cyclic phosphate **1** (blue; note the failure of the step in which cytosine **3** and ribose **4** are proposed to condense together) and the successful new synthesis described here (green). p, pyranose; f, furanose.

2-Amino-oxazole Synthesis

- Constitutionally arises from condensation of cyanamide **8** and glycoaldehyde **10**
- Typically requires strongly alkaline conditions
- Urea **6** produced when excess **8**

Glyceraldehyde needs neutral-pH reaction conditions



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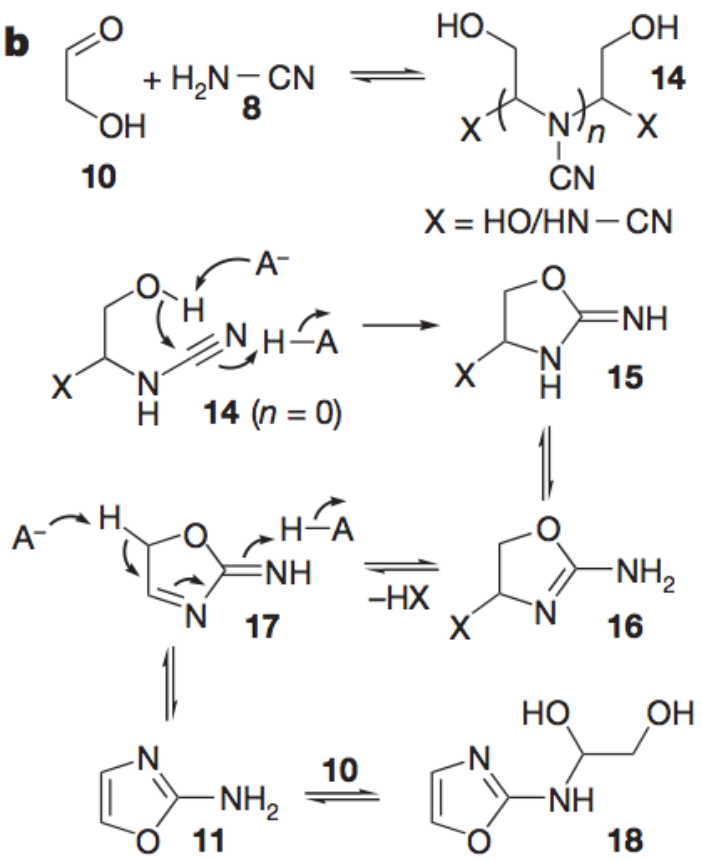
2-Amino-oxazole Synthesis - Mechanism

- At low [OH-], **14** to **15** and **17** to **11** are very slow… *but cannot use OH- as specific base (?)*

- Inorganic phosphate (P_i) can act as general base (i.e. 2nd pK_a is close to neutrality, 7.21 in H_2O)

- In 1.0 M phosphate buffer at pH 7.0, **11** produced in >80 % yield



Mixed chemical systems – reactants for a particular reaction step can control other steps

Arabinose Amino-oxazoline Formation

- In absence of P_i - **12** and **21** major products (Fig a)
- In presence of P_i – arabinose **12**, ribose **21**, xylose **22** and lyxose **23** derivatives all reversibly form mixture of SM and either **24** or **25** (Fig b)
- In 'mixed chemical system' - **12** and **21** major products (**21** can be selectively removed from solution by crystallisation, Fig c)

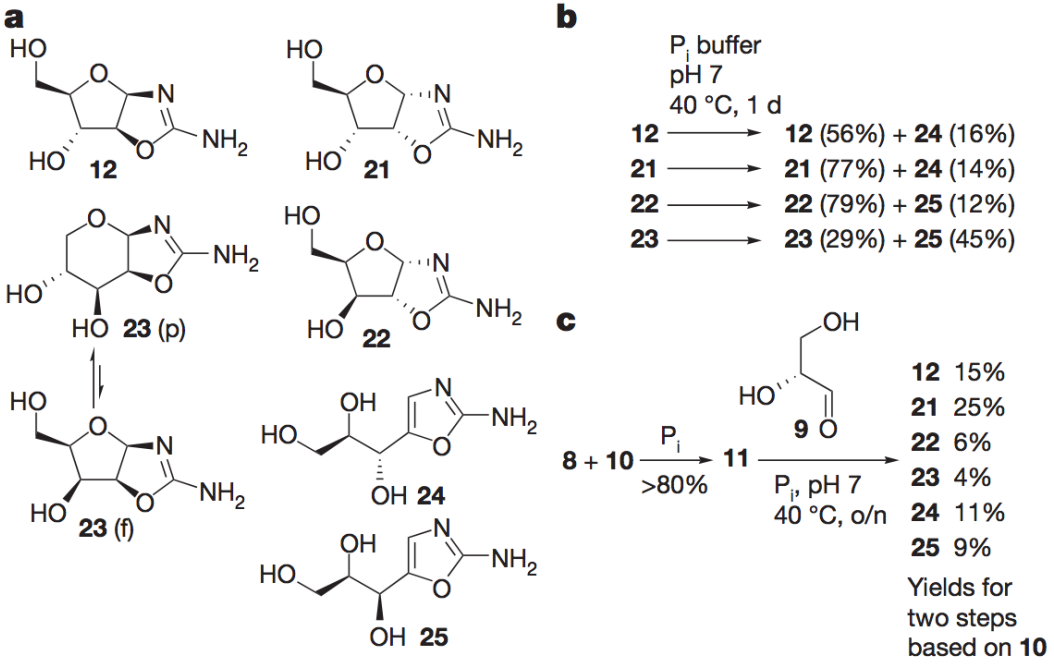


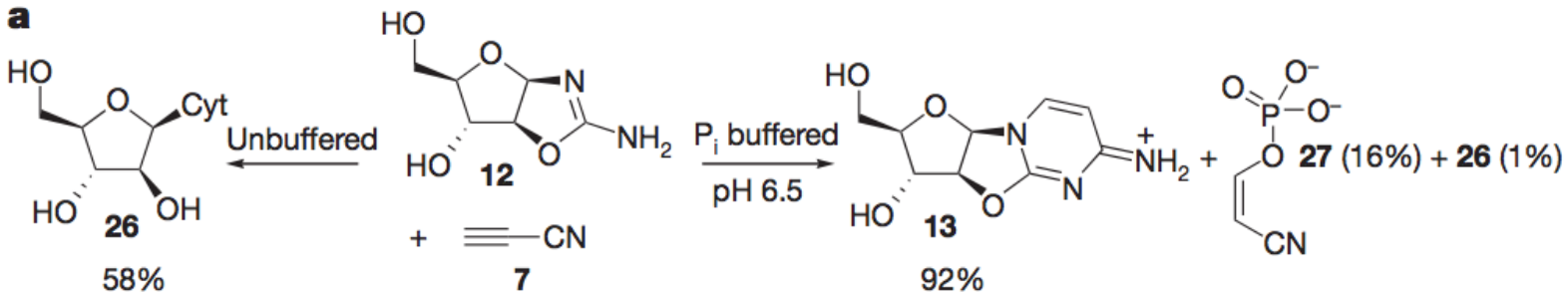
Figure 3 | Pentose amino-oxazoline stability, and assembly chemistry. **a**, Structures of the arabinose (**12**), ribose (**21**), xylose (**22**) and lyxose (**23**) amino-oxazolines and their elimination products **24** and **25**. **b**, Relative stabilities of the amino-oxazolines in the presence of phosphate. **c**, Formation of amino-oxazolines by addition of glyceraldehyde **9** to a solution of 2-amino-oxazole **11**, with the latter freshly formed *in situ* from cyanamide **8** and glycolaldehyde **10**. P_i, inorganic phosphate; o/n, overnight.

Desired arabinose amino-oxazoline **12** major product in solution

Arabinose Anhydronucleoside Formation

- Unbuffered – pH rises during reaction, causing hydrolysis of **13** and subsequent reaction of hydroxyl groups with **7** (thus relatively low yield of **26**)

- P_i buffered – clean reaction, phosphate removes excess **7**

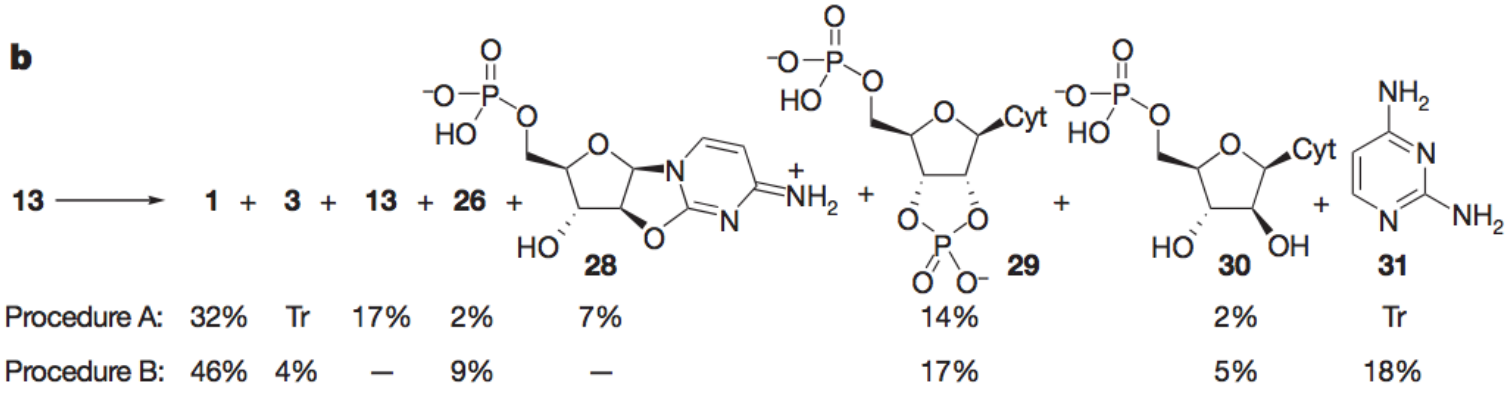


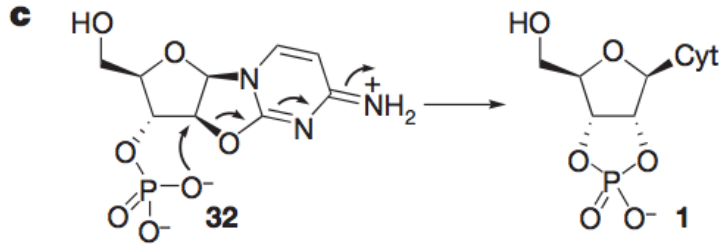
P_i performs dual role to control reaction

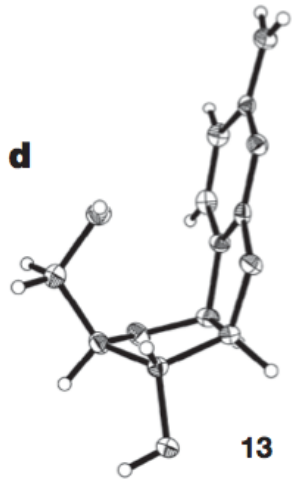
Arabinose Anhydronucleoside Phosphorylation

- Two potential procedures for phosphorylation – both employ urea (formed earlier in sequence)

- X-ray structure revealed 5’-OH abnormally sterically hindered, thus phosphorylation selective for 3’-OH







Procedure A:
pyrophosphate & urea

Procedure B: *P_i & urea*

Arabinose Anhydronucleoside Phosphorylation

- Cyclic phosphate **1** major product, but what about presence of contaminants in subsequent incorporation into RNA?

- Irradiation at 254 nm leads to destruction of all nucleotides and nucleosides except **1**

- Prolonged irradiation leads to partial hydrolysis of **1** resulting in uracil cyclic phosphate **33**

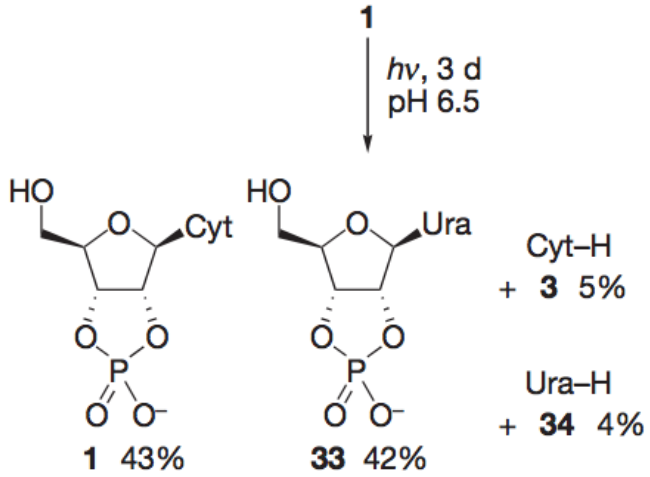
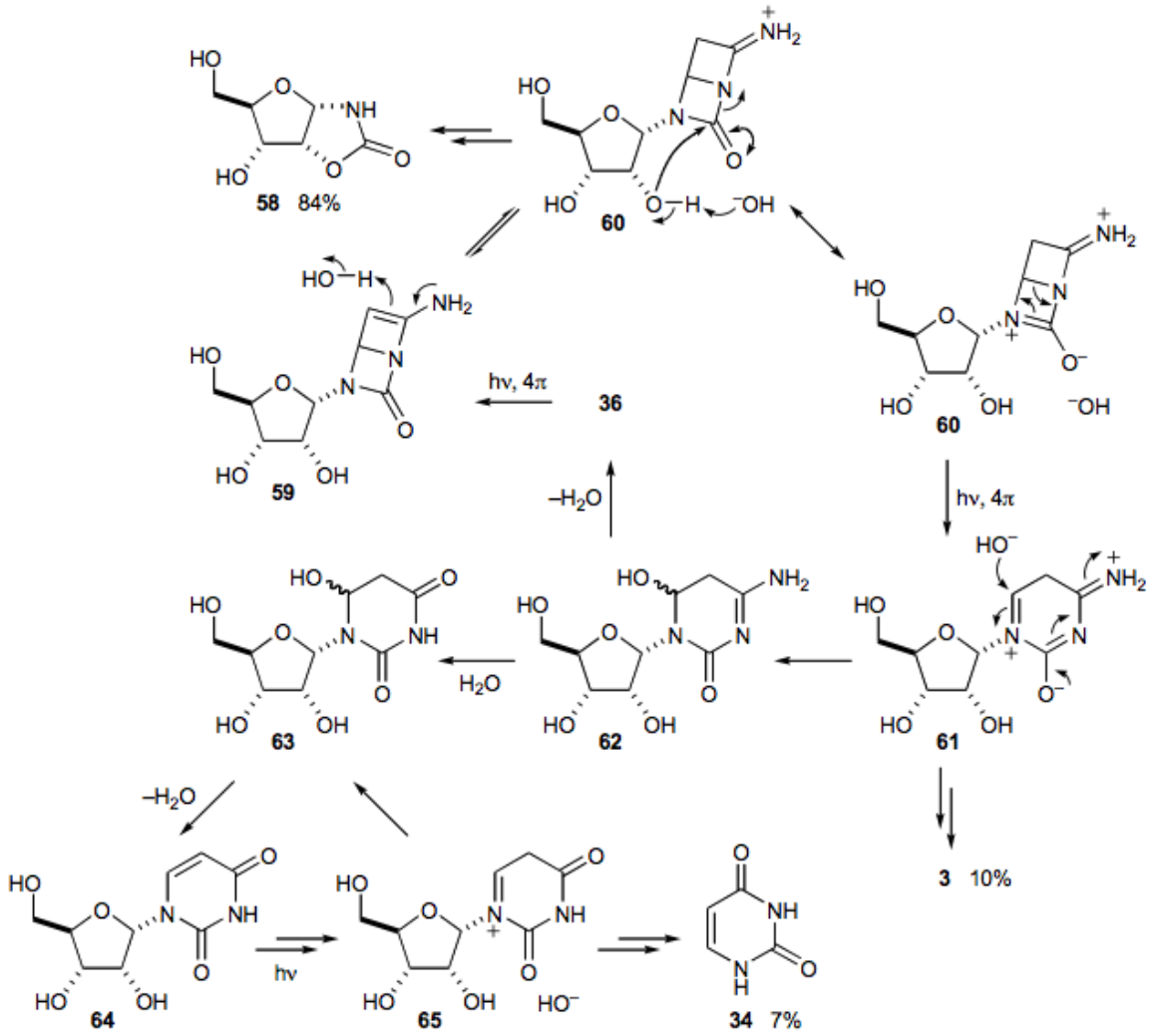


Figure 5 | Photochemistry of β-ribocytidine-2′,3′-cyclic phosphate 1. Under conditions of irradiation that destroy most other pyrimidine nucleosides and nucleotides (Supplementary Information), **1** undergoes partial hydrolysis and slight nucleobase loss. Ura, N1-linked uracil; Cyt–H, cytosine; Ura–H, uracil.

Conclusion

Prebiotic synthesis of activated pyrimidine nucleotides should be viewed as predisposed

Photodestruction – Possible Mechanism?



Photodestruction – Possible Mechanism?

Figure S13: *Potential mechanism to account for the stability of* ***1*** *and* ***33*** *towards irradiation*