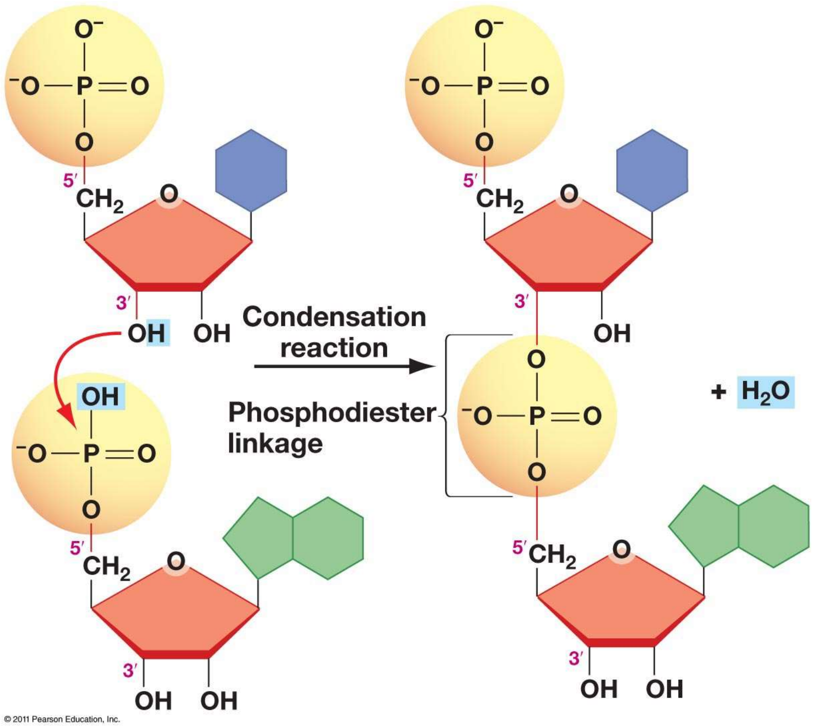
Nucleotide polymerization

Regioselective formation of 3’-5’ phosphodiester bonds between nucleotides



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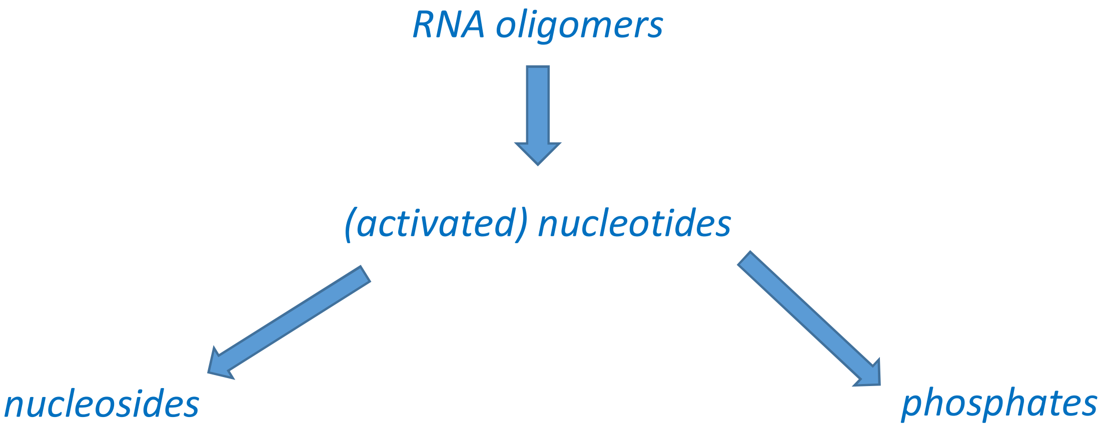
RNA oligomers – prebiotic disconnections



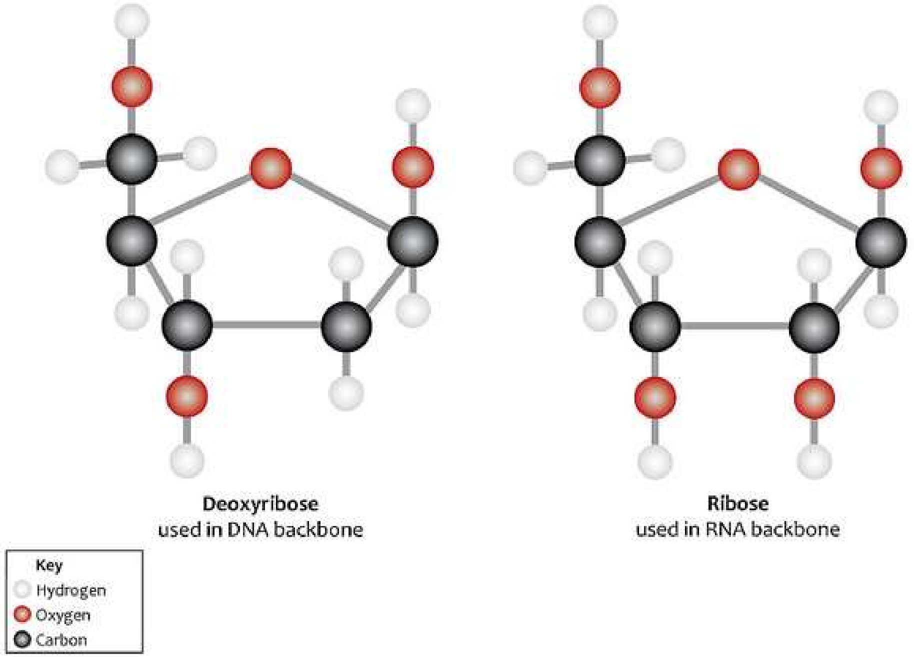
RNA oligomers

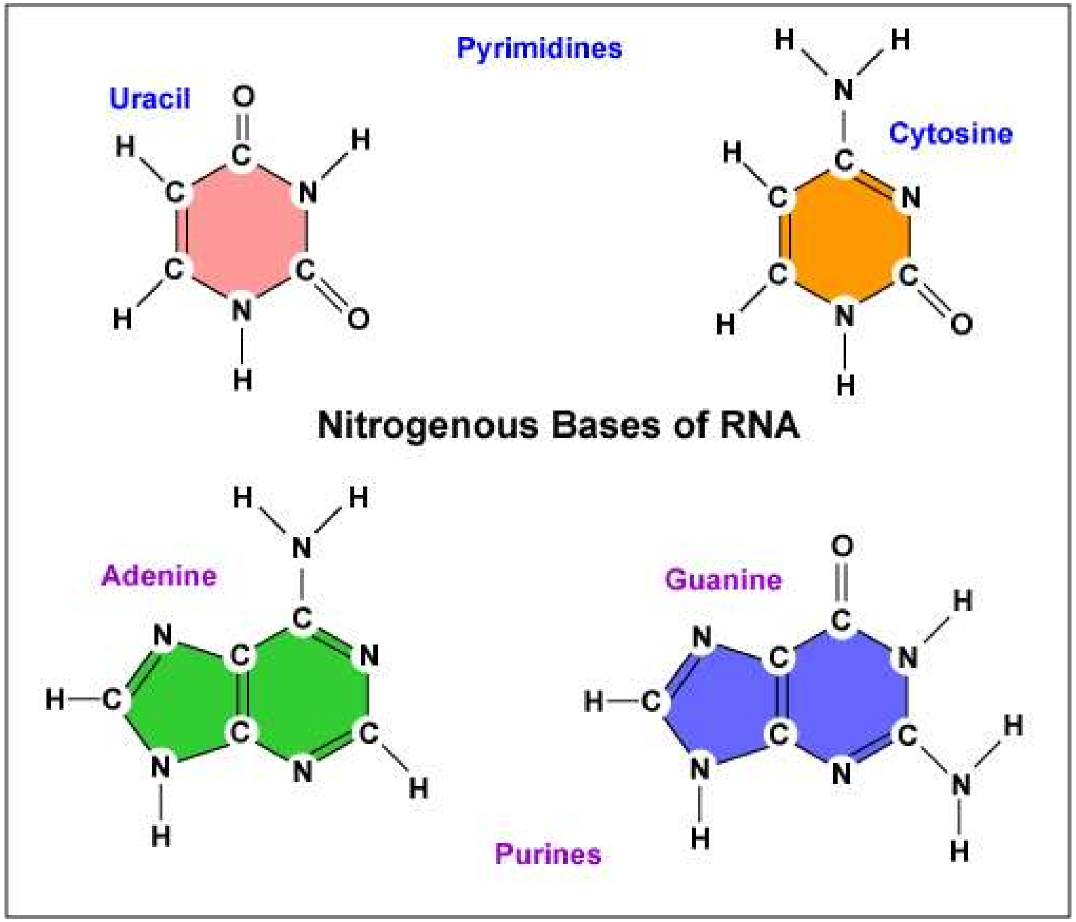
(activated) nucleotides

nucleosides

phosphates

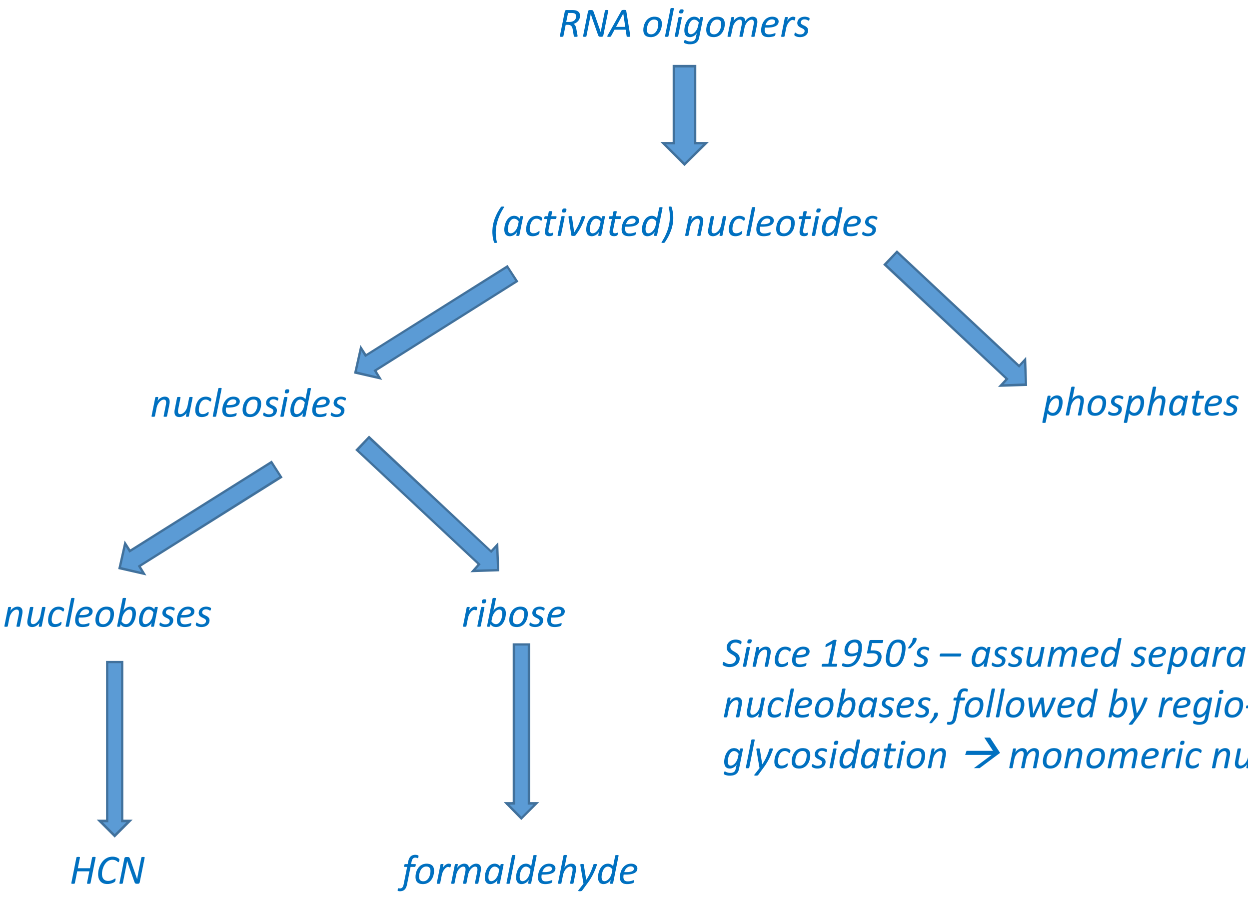
Nucleosides - nucleobases + sugars





Since 1950's – assumed separate synthesis of sugars and nucleobases, followed by regio- and diastereoselective glycosidation → monomeric nucleosides

RNA oligomers – prebiotic disconnections



RNA oligomers

(activated) nucleotides

nucleosides

phosphates

nucleobases

ribose

HCN

formaldehyde

Since 1950’s – assumed separate synthesis of sugars and nucleobases, followed by regio- and diastereoselective glycosidation → monomeric nucleotides

Literature sources

J. Oro, *Biochem. Biophys. Res. Commun.* **1960**, *2*, 407–412.

J. D. Sutherland, *Cold Spring Harbor Perspect. Biol.* **2010**, 2, a005439.

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J. D. Sutherland, *Angew. Chem. Int. Ed.* **2016**, *55*, 104-121.

B. H. Patel, C. Percivalle, D. J. Ritson, C. D. Duffy, J. D. Sutherland, *Nat. Chem*. **2015**, *7*, 301–307.

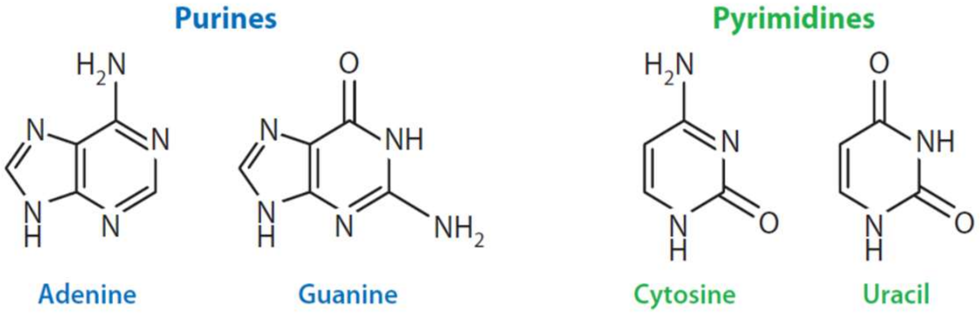
J. D. Sutherland, *et al. Nat. Chem*. **2013**, *5*, 383–389.

L. E. Orgel, *Crit. Rev. Biochem. Mol. Biol.* **2004**, *39*, 99-123.

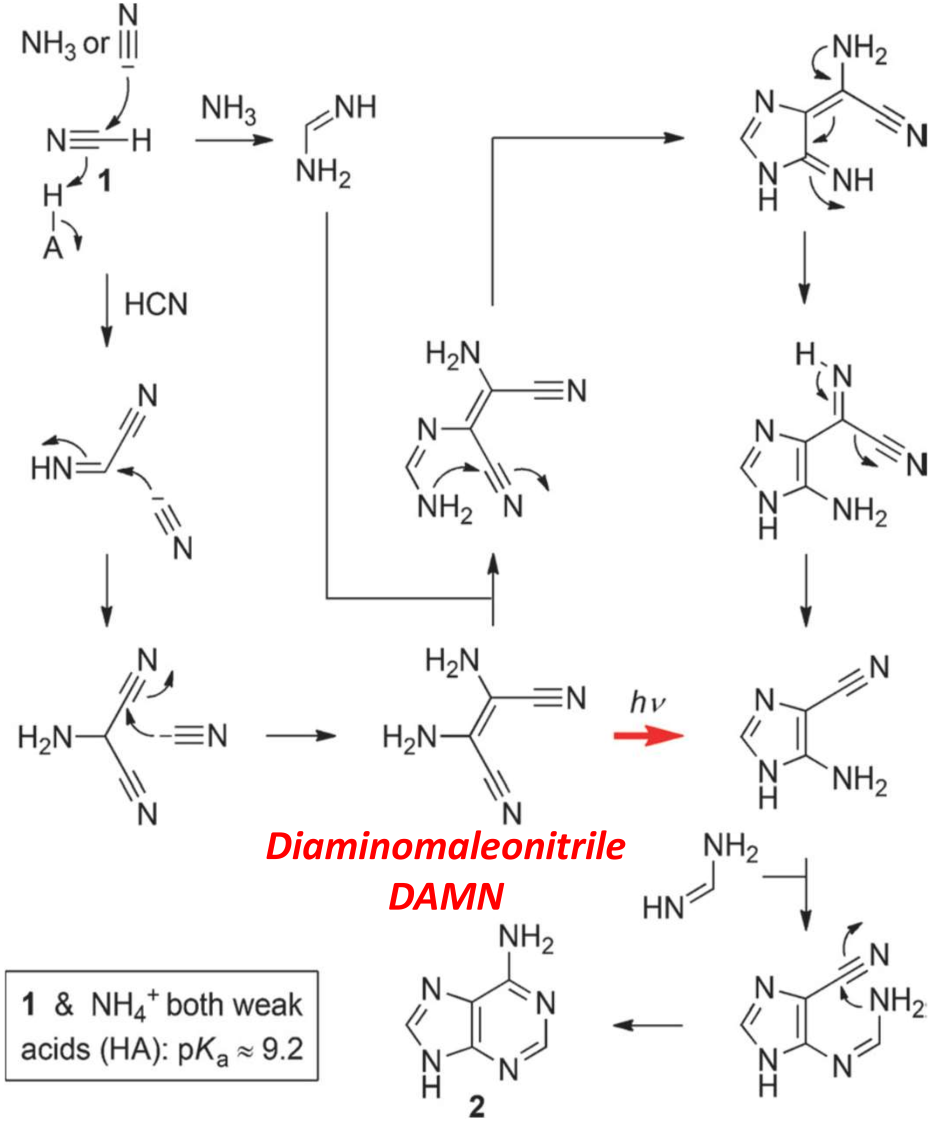
Powner, M. W.; Sutherland, J. D.; Szostak, J. W. *J. Am. Chem. Soc.* **2010**, *132*, 16677

T. Carell, *Nature* **2016**, *352(6287)*, 833-836

Prebiotic synthesis of nucleobases



Prebiotic synthesis of adenine – the pentamer of HCN!



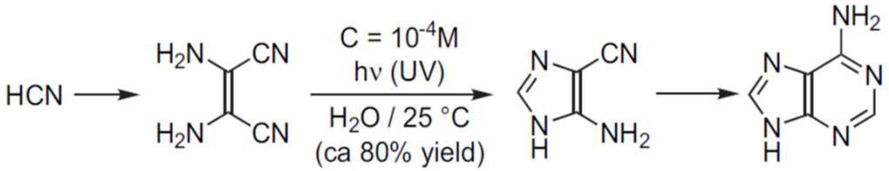
Diaminomaleonitrile
DAMN

1 & NH_4^+ both weak acids (HA): pK_a ≈ 9.2

1960 - Oró’s synthesis of adenine **2** from hydrogen cyanide **1** and ammonia (general acid–base catalysis, presumed to operate in most steps, is only shown once).
Heating ammonium cyanide at 70⁰C for a few days
→ 0.5% adenine
Heating HCN with liquid ammonia in a sealed tube → 20% adenine

The photochemical shortcut discovered by Ferris and Orgel is shown by the red arrow.
Optimized yields – up to 20% for adenine, 3% for guanine

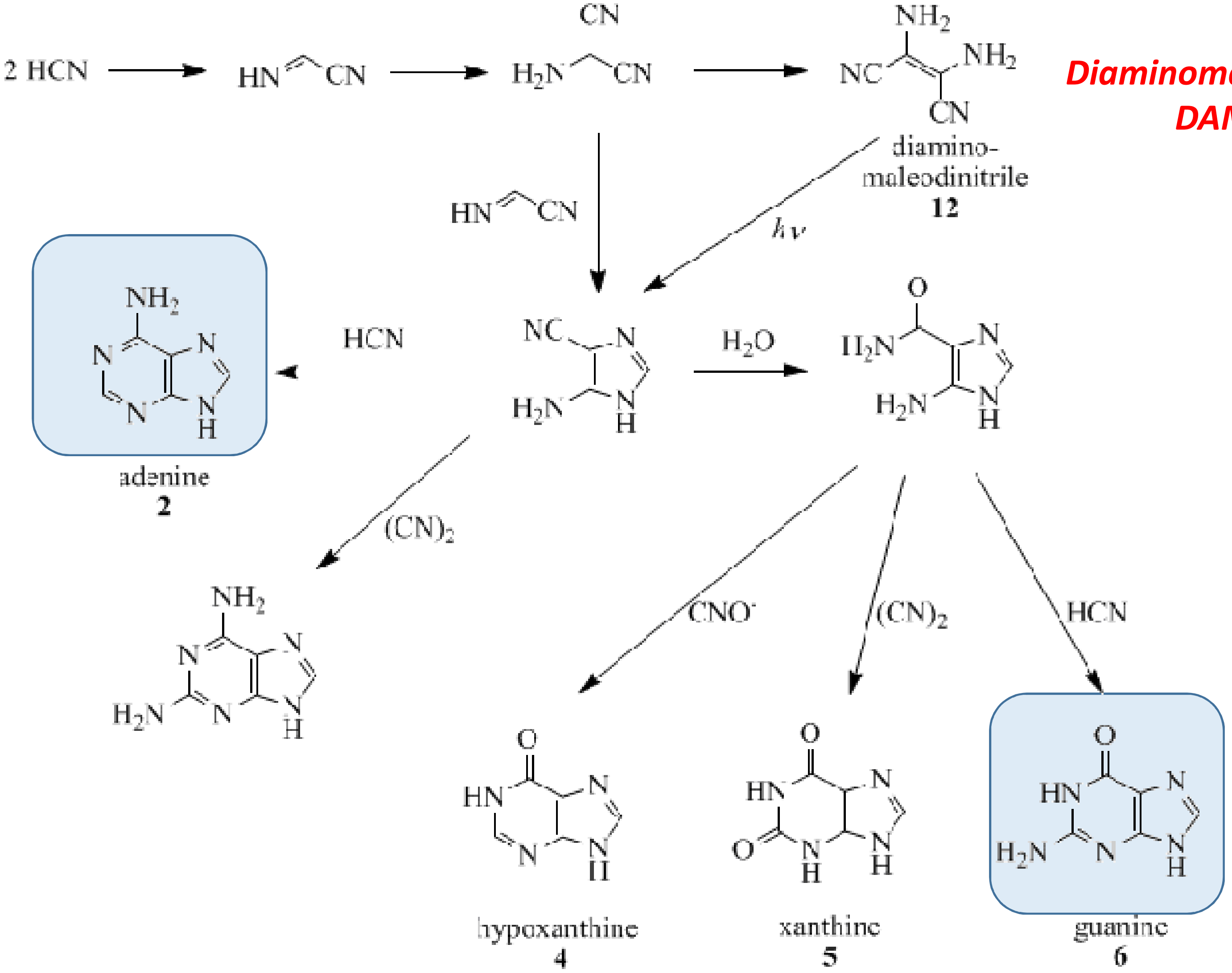
Eutectic freezing (-20⁰C) increases the yield of DAMN formation by concentrating HCN between pure ice crystals



J. Oro *Biochem. Biophys. Res. Commun.* **1960**, *2*, 407.

J. P. Ferris, L. E. Orgel, *J. Am. Chem. Soc.* **1966**, *88*, 1074

Prebiotic synthesis of purines

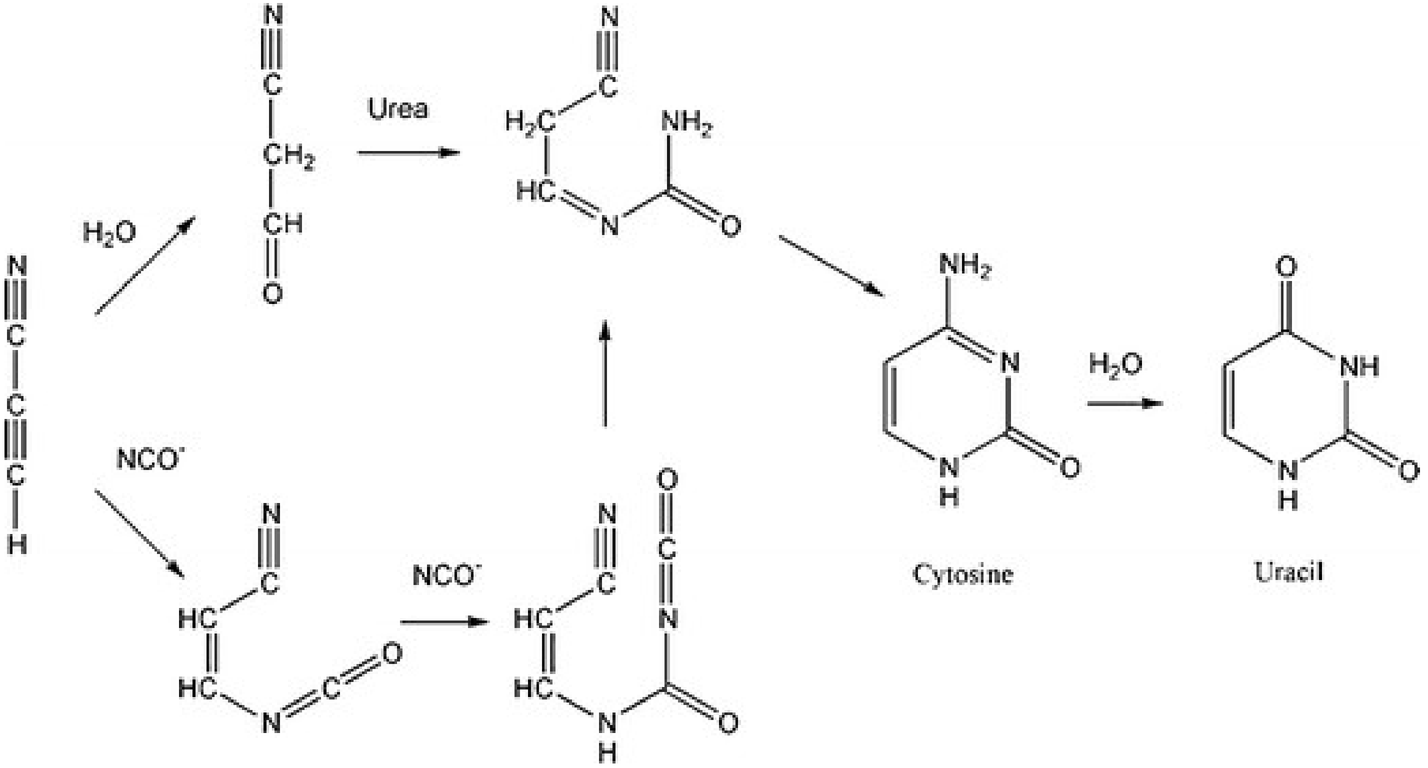


Diaminomaleonitrile
DAMN

Prebiotic synthesis of pyrimidines

Cyanoacetylene is a major product of electric discharges in the mixture of nitrogen and methane

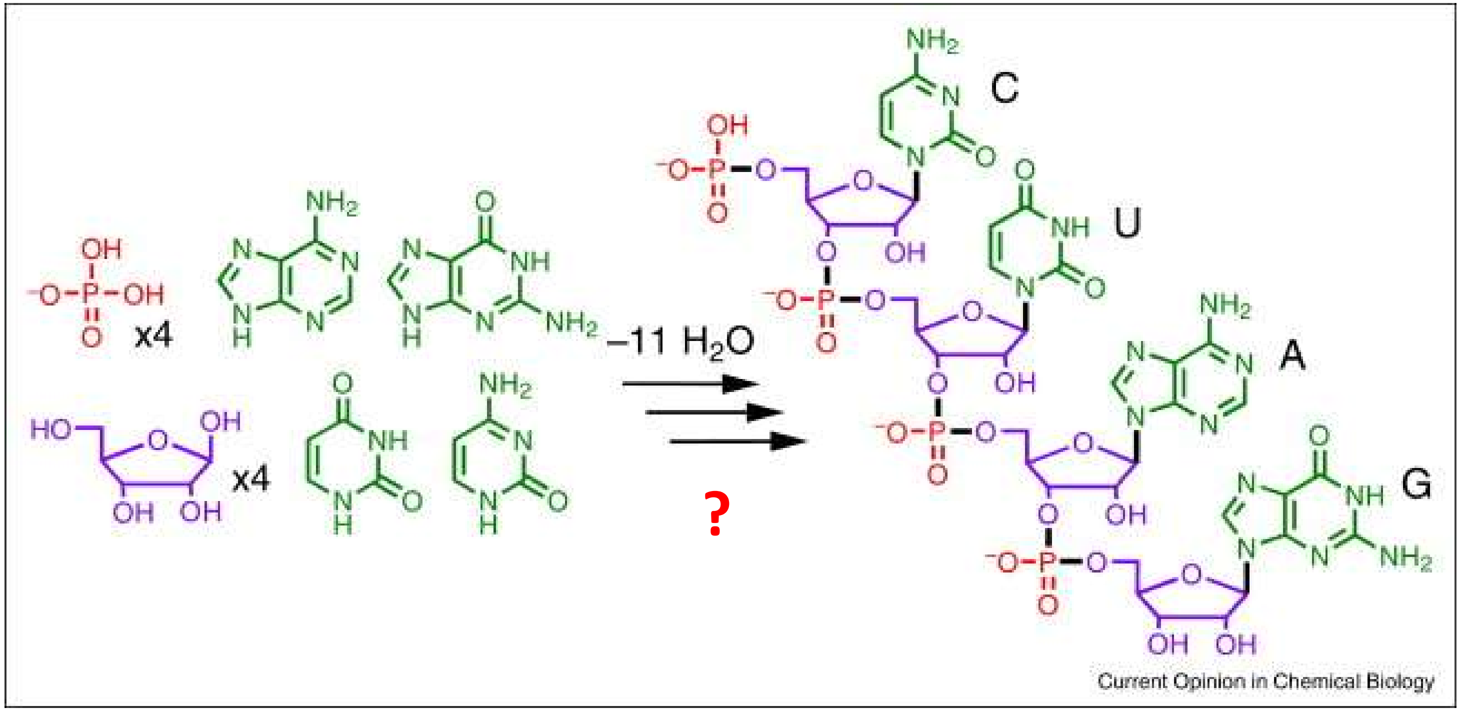
Cyanoacetylene can be hydrolysed to cyanoacetaldehyde. That compound can condense with urea to form cytosine



Cyanoacetylene incubated with saturated solution of urea yields up to 50% **cytosine**.
Other methods typically yield up to 5% cytosine.It is further converted to uracil by hydrolysis.

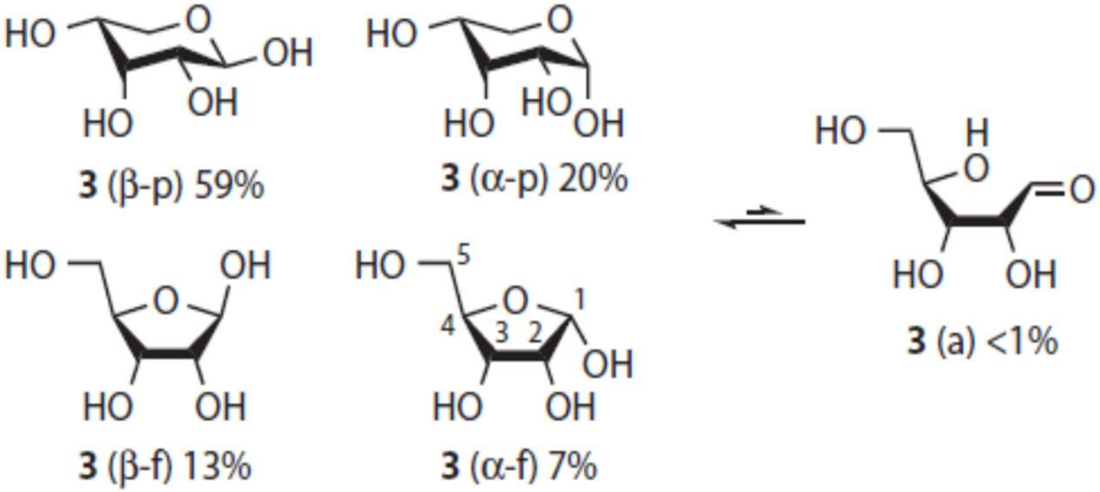
R. Shapiro PNAS **1999**, *96,* 4396-4401

Prebiotic synthesis of nucleosides, nucleotides and RNA



Current Opinion in Chemical Biology

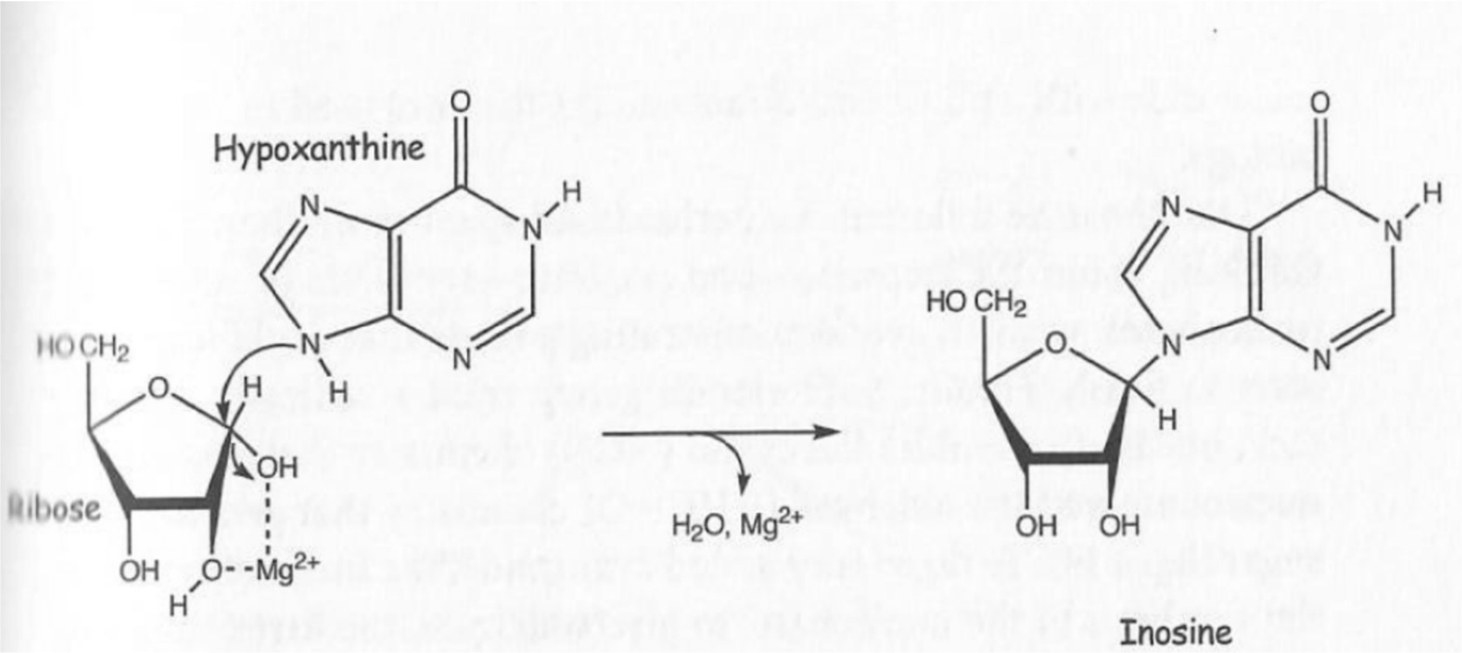
Prebiotic synthesis of nucleosides



The difficulties of assembling beta-ribonucleosides by nucleobase ribosylation:

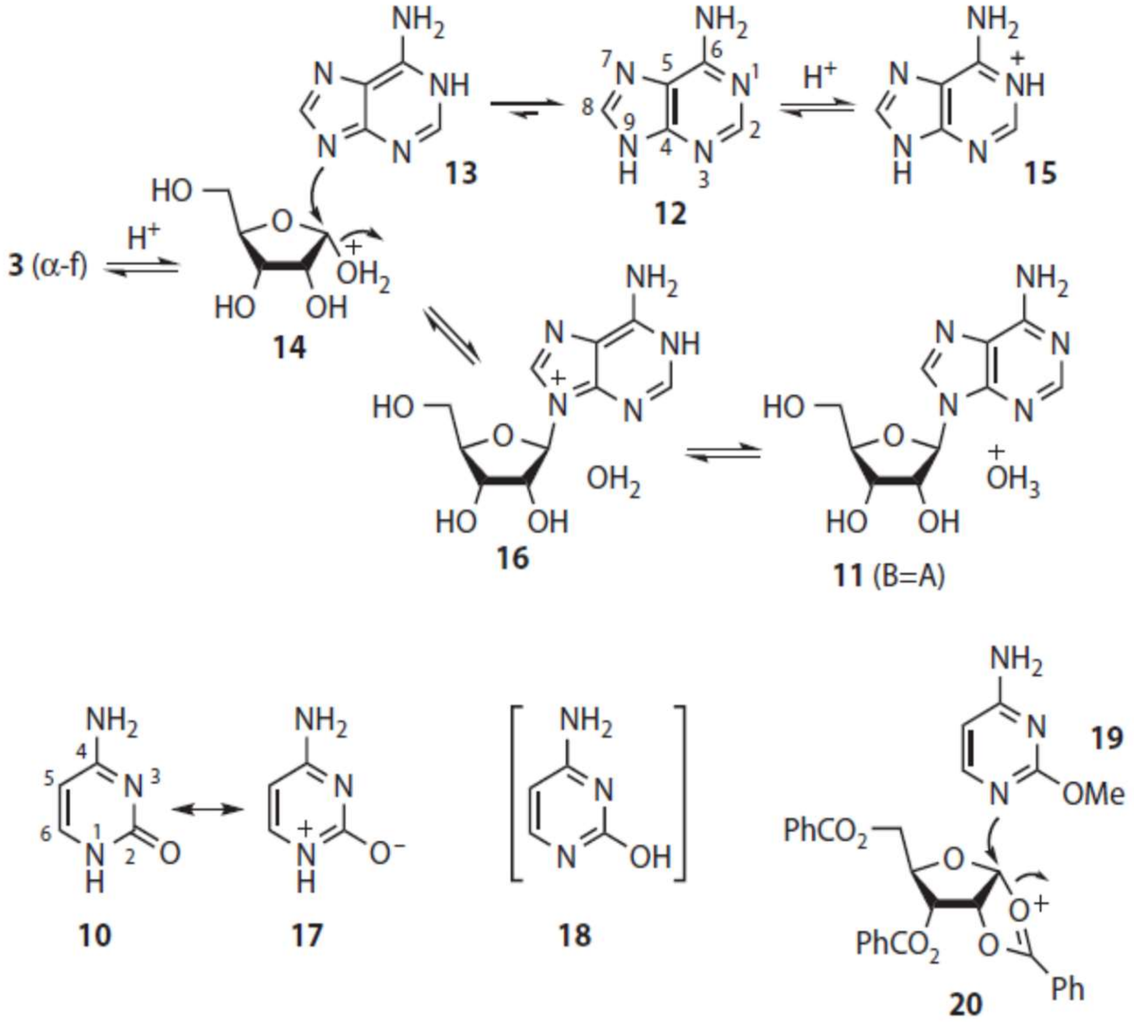
The many different forms of ribose **3** adopted in aqueous solution. The pyranose (p) and furanose (f) forms interconvert via the open-chain aldehyde (a), which is also in equilibrium with an open-chain aldehyde hydrate (not shown).

Prebiotic synthesis of nucleosides



Heating purines with ribose and magnesium salts yields beta-nucleosides, although with low yields
β-inosine – 8%, β-adenosine – 4%, β-guanosine – 9%
Other isomers (e.g. alpha-glycosides) also present.

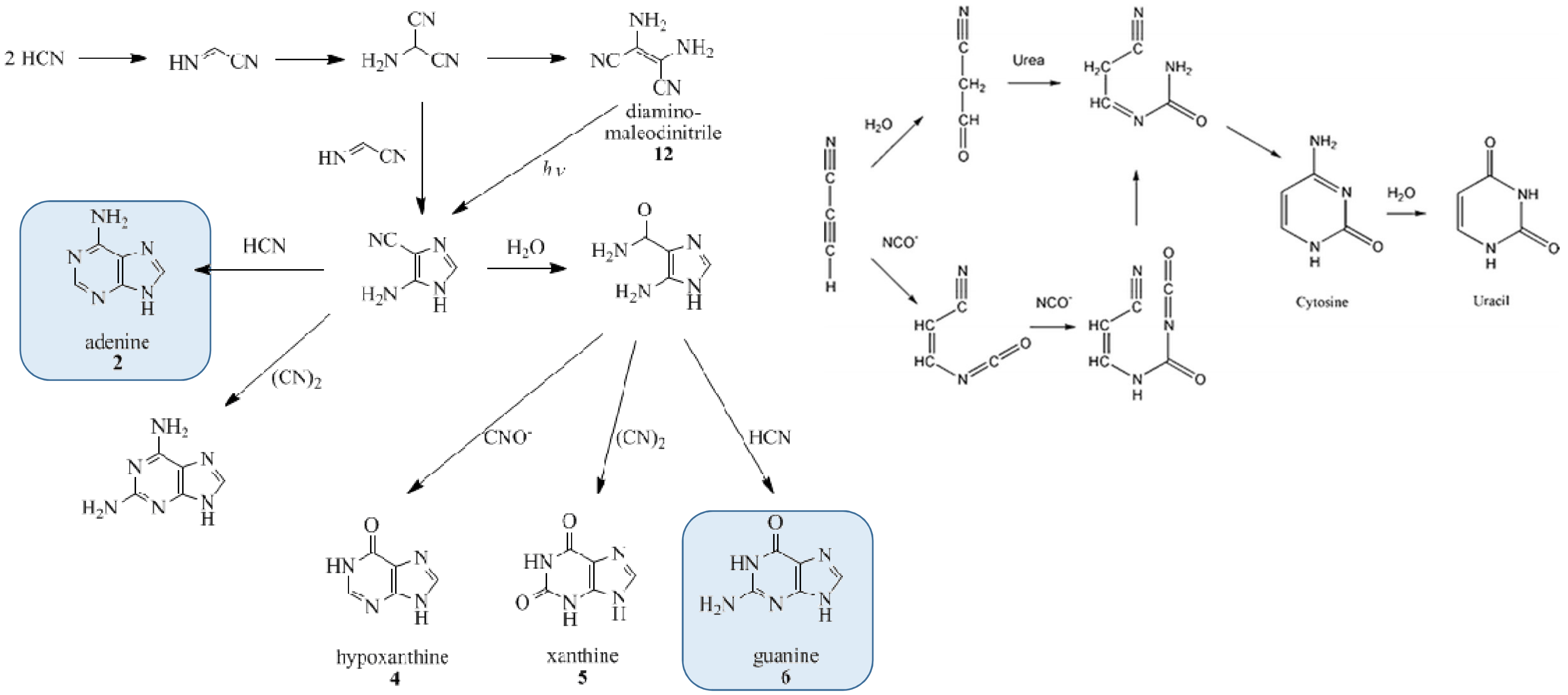
W. Fuller, R. Sanchez, L. Orgel *J. Mol. Biol.* **1972**, *67*, *25-33*



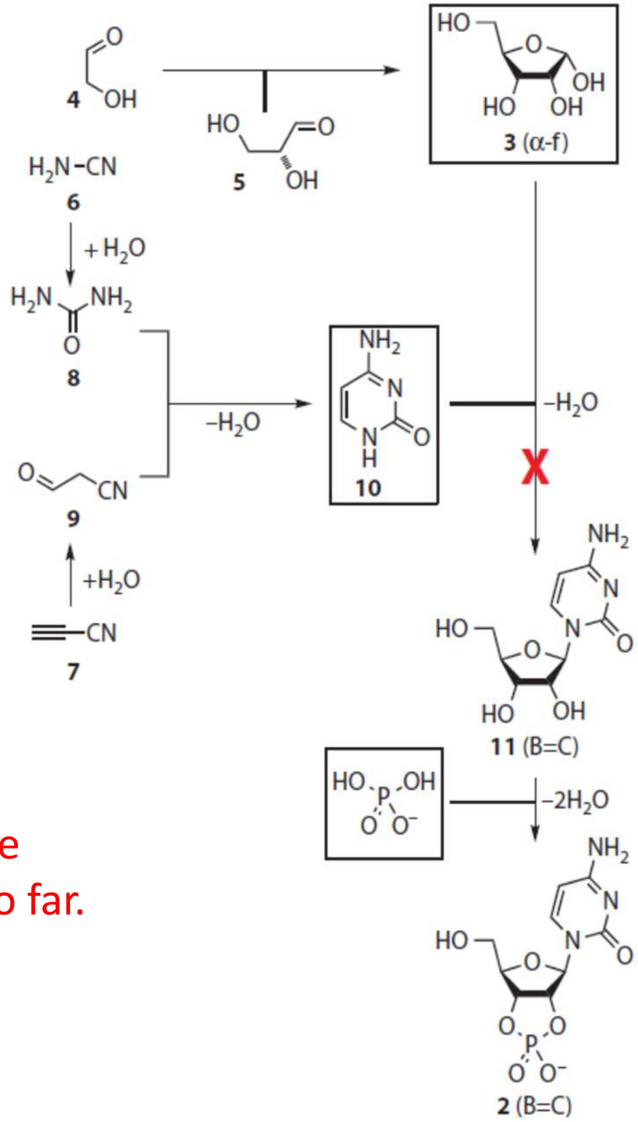
Adenine tautomerism and the ribosylation step necessary to make the adenosine **11** thought to be needed for RNA assembly. The low abundance of the reactive entities **13** and **14** is partly responsible for the low yield of **11**. The reason for the lower nucleophilicity of *N1* of the pyrimidines, and the conventional synthetic chemist's solution to the problems of ribosylation.

Prebiotic synthesis of nucleobases

Diaminomaleonitrile
DAMN

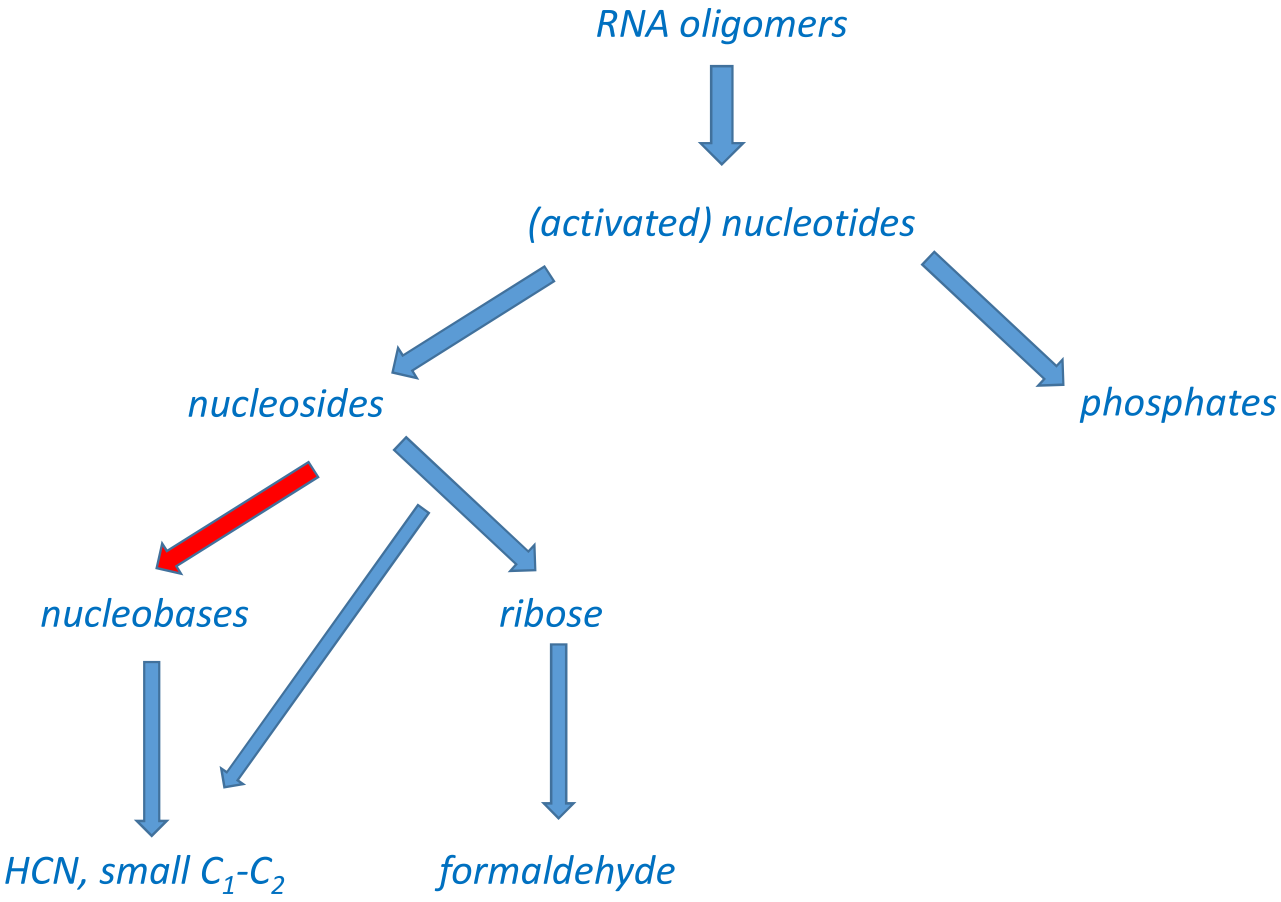


Prebiotic synthesis of pyrimidine nucleosides



No direct synthesis of pyrimidine nucleosides from ribose reported so far.

RNA oligomers – prebiotic disconnections



RNA oligomers

(activated) nucleotides

nucleosides

phosphates

nucleobases

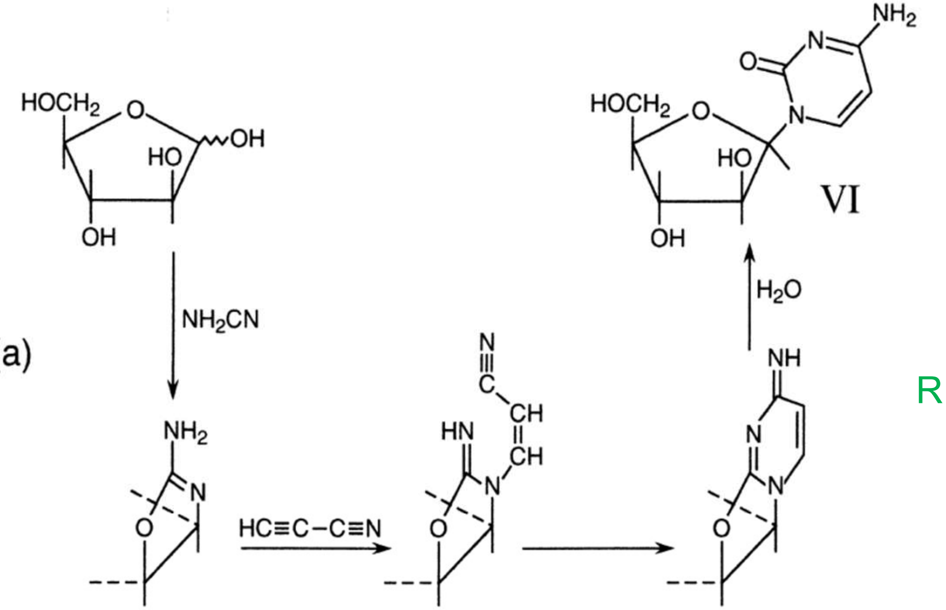
ribose

HCN, small C_1-C_2

formaldehyde

Prebiotic synthesis of nucleosides

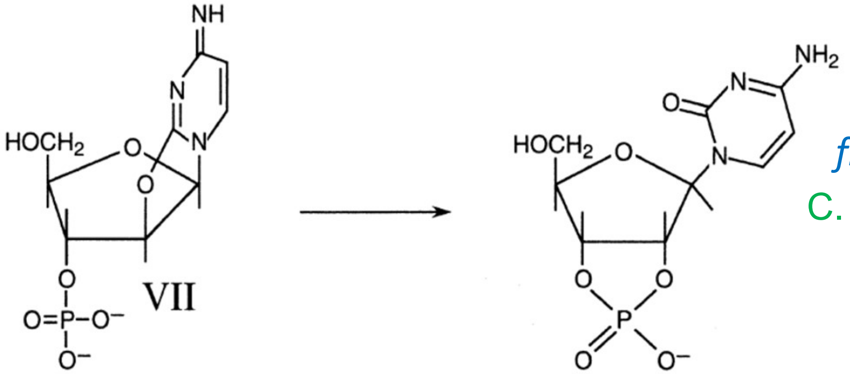
(a)



cytosine arabinoside synthesis

R. Sanchez, L. Orgel *J. Mol. Biol.* **1970**, *47, 531-543*

(b)



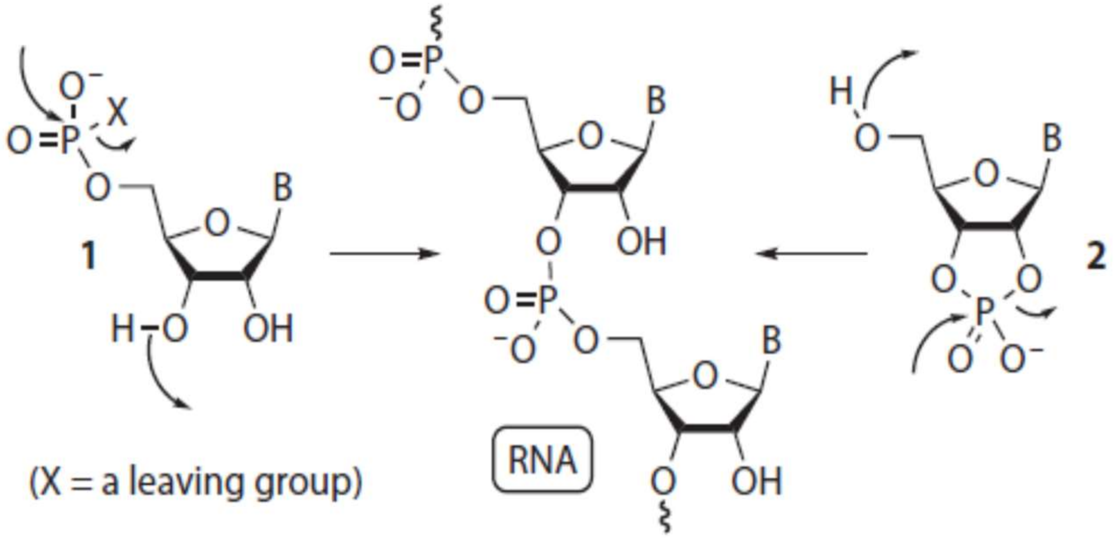
cytosine riboside (as a cyclic phosphate) is obtained from a phosphorylated substrate

C. M. Tapiero, J. Nagyvary *Nature* **1971**, *231, 42-43*

Review: L. E. Orgel *Crit. Rev. Biochem. Mol. Biol.* **2004**, *39, 99123*

Ara-3'P + NH_2CN + HCC-CN → Cyt-2',3'cP: A. Ingar, R. W. A. Luke, B. R. Hayter, J. D. Sutherland *ChemBioChem* **2003**, *4*, 504-507

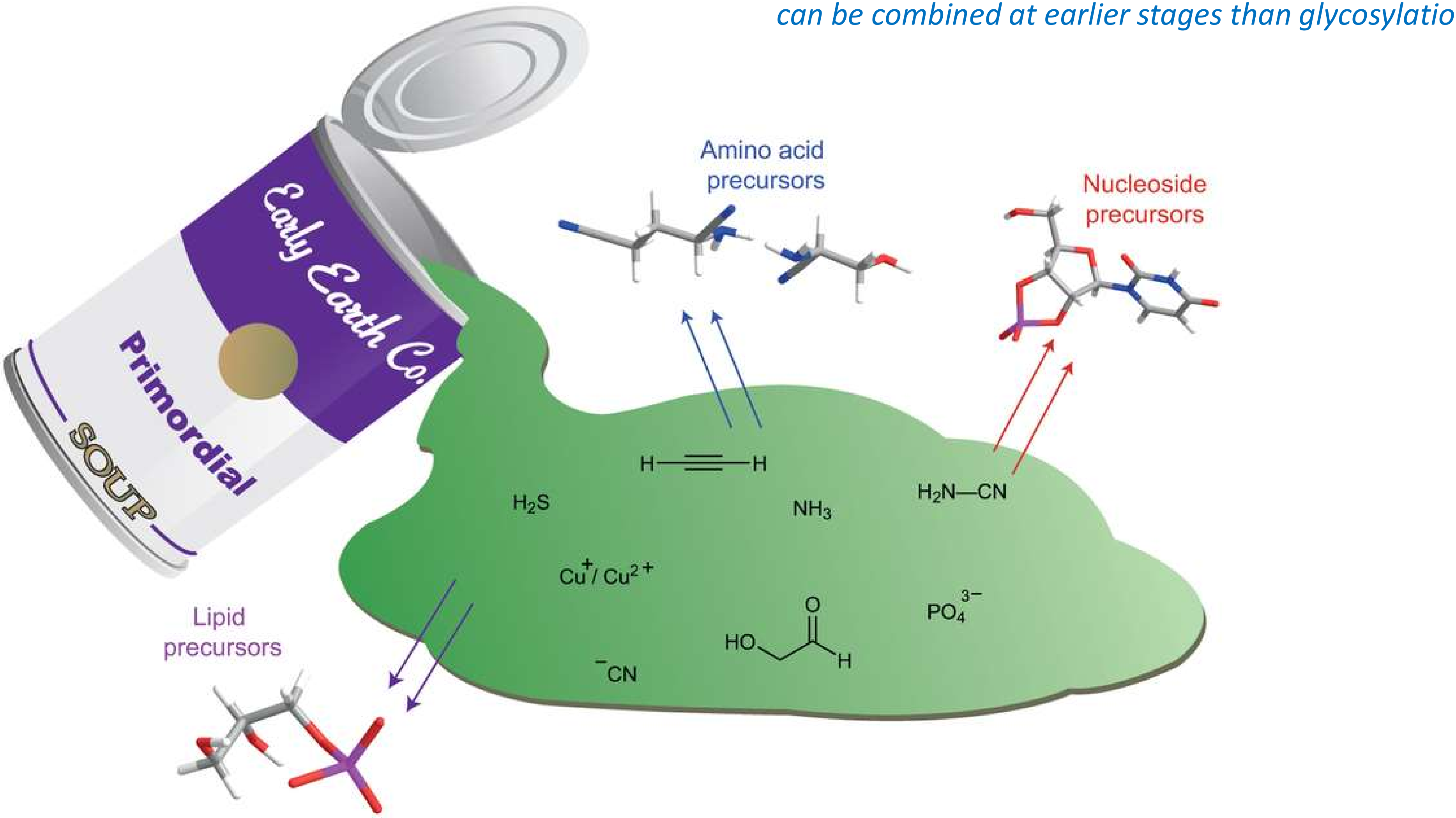
Prebiotic synthesis of oligonucleotides



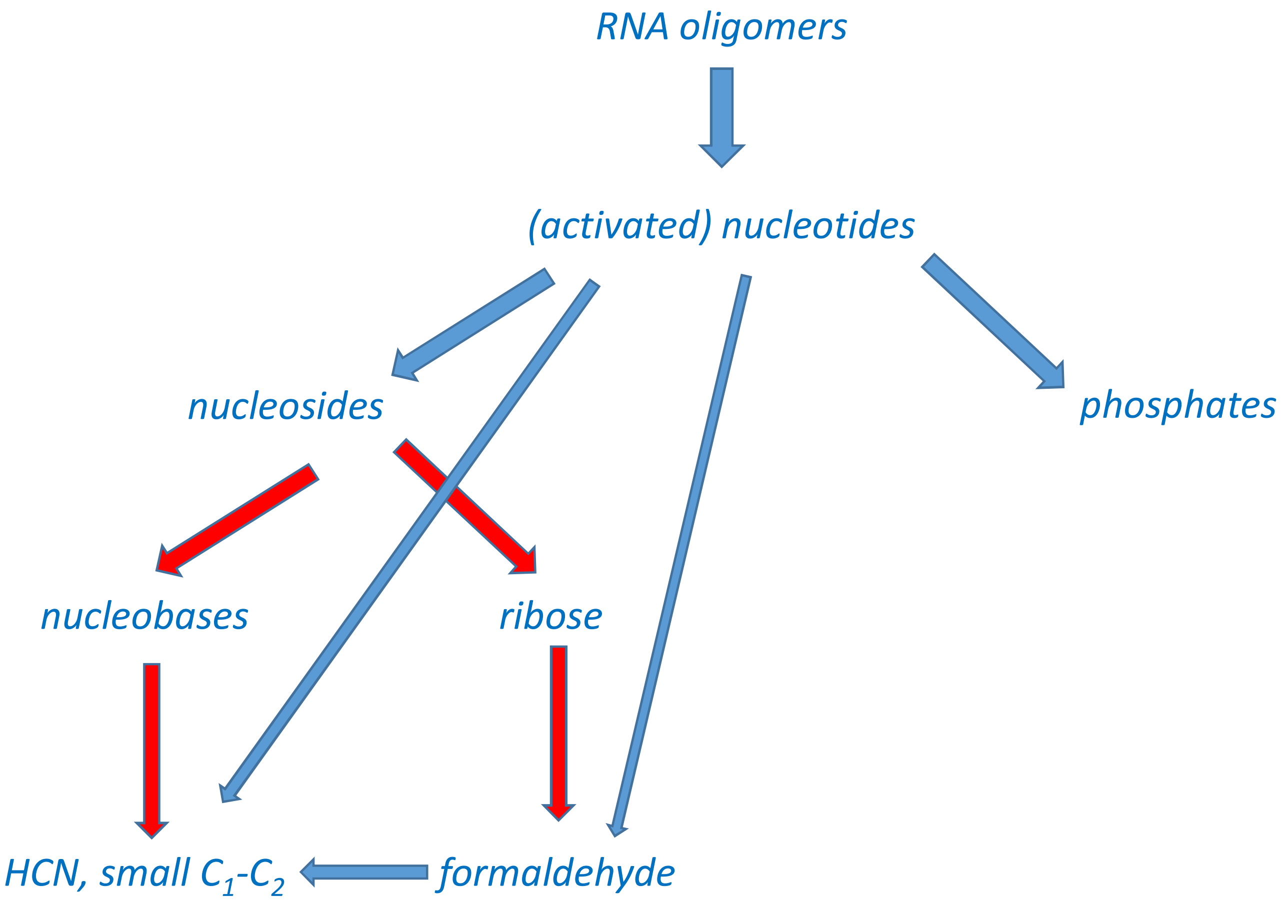
Activated ribonucleotides in the potentially prebiotic assembly of RNA. Potential P–O bond forming polymerization chemistry is indicated by the curved arrows.

Cyanosulfidic chemistry

The aldol chemistry of sugars and cyanide chemistry of nucleobases can be combined at earlier stages than glycosylation.



RNA oligomers – prebiotic disconnections



RNA oligomers

(activated) nucleotides

nucleosides

phosphates

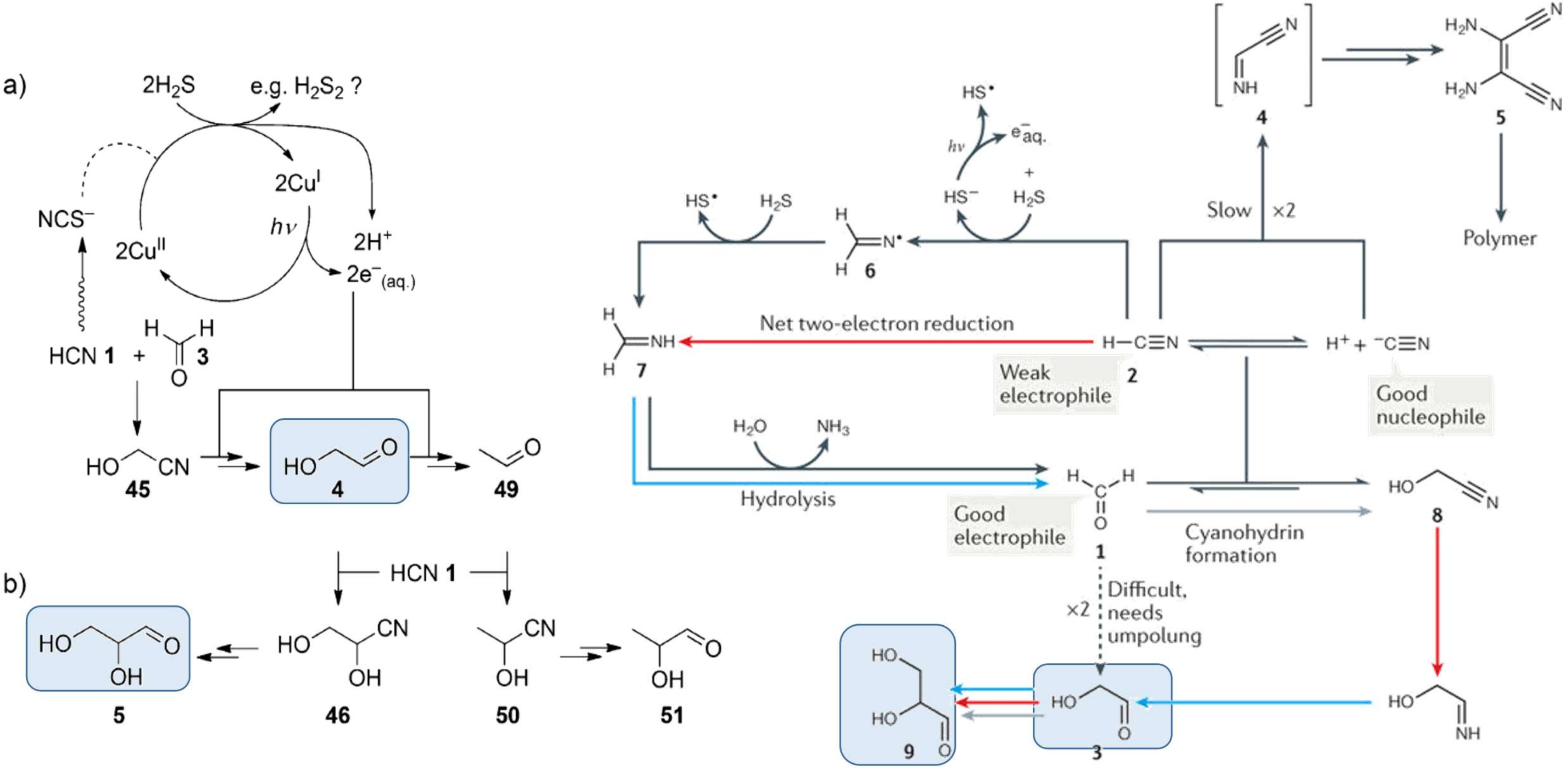
nucleobases

ribose

HCN, small C_1-C_2

formaldehyde

Cyanosulfidic chemistry for the Kiliani-Fischer homologation



J. Sutherland, *Nature Reviews Chemistry* **2017**, *1*, Article 0012, doi:10.1038/s41570-016-0012

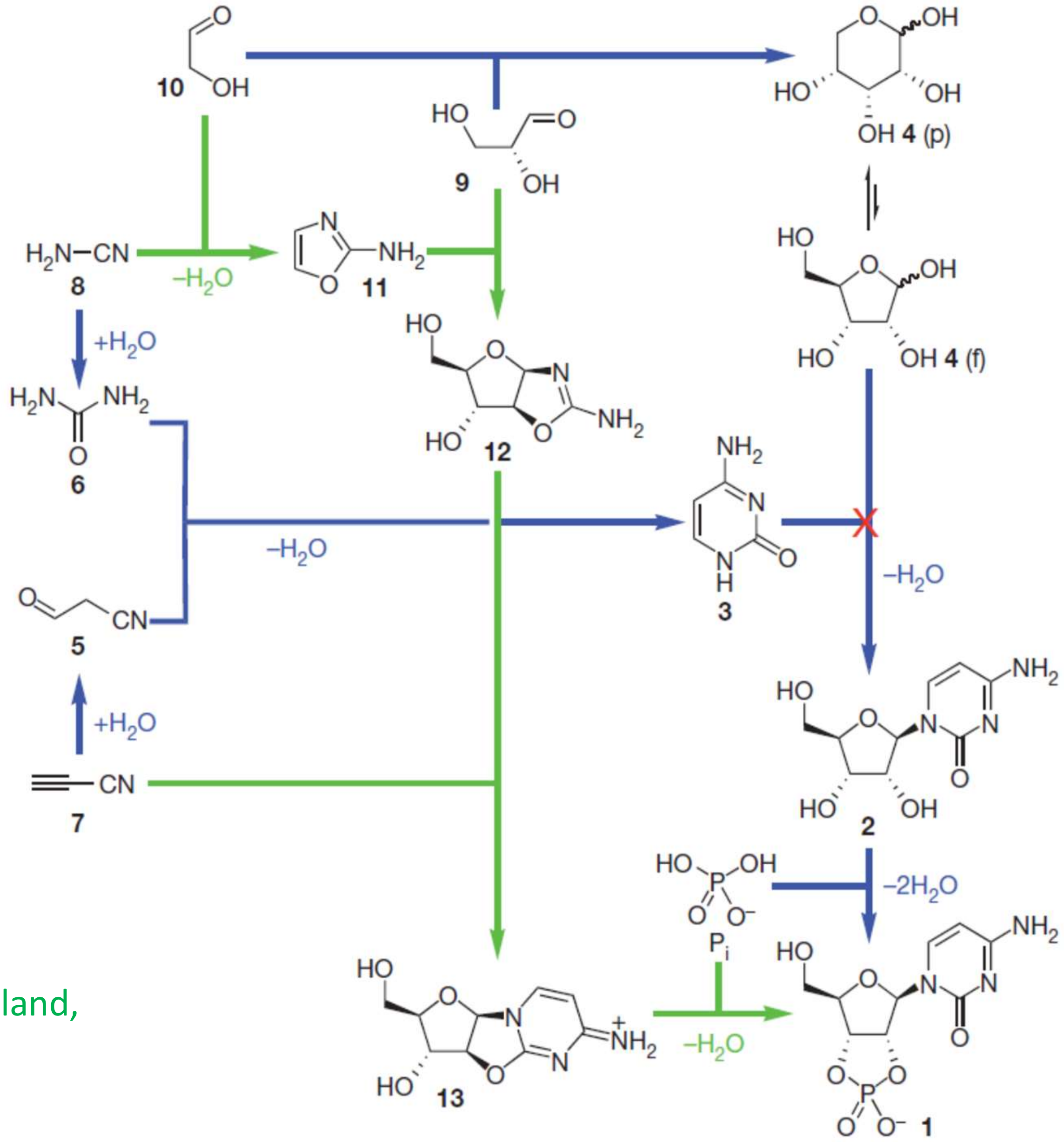
Nature Reviews | Chemistry

Cyanosulfidic chemistry

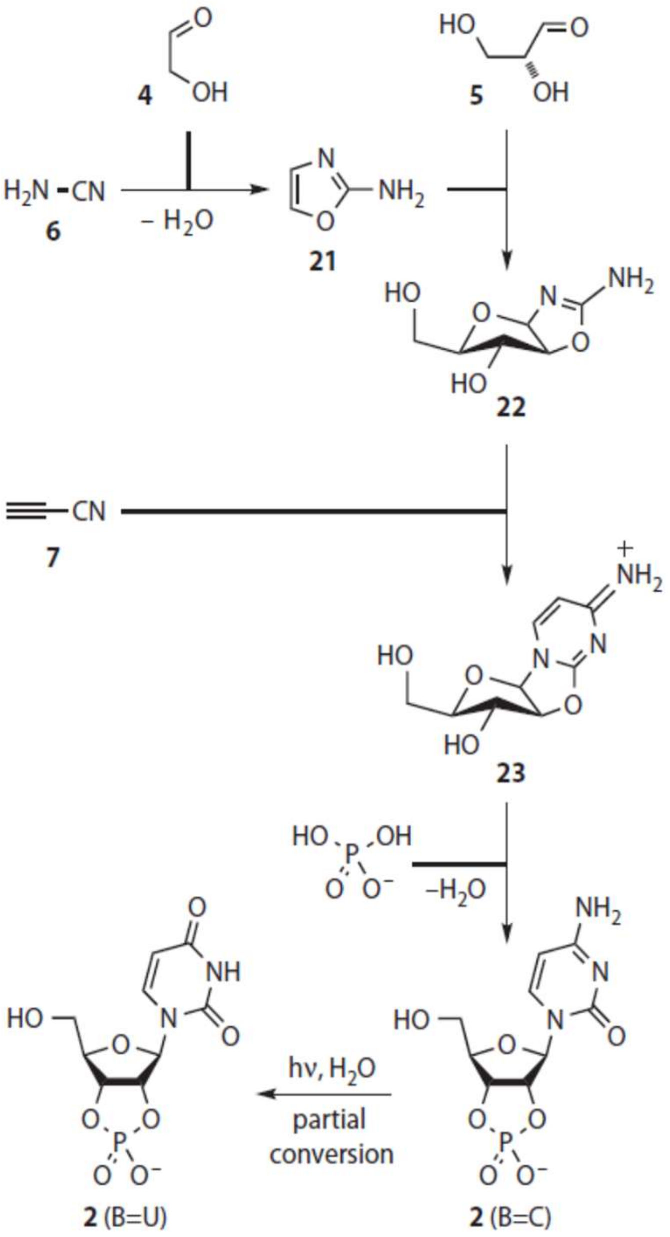
Cyanamide (8) is a fertilizer from calcium carbide and nitrogen.
The calcium carbide is formed upon heating of calcium cyanide

Cyanoacetylene (7) is a major nitrogen-containing product of the action of an electric discharge on a mixture of methane and nitrogen.

M. W. Powner, B. Gerland, J. D. Sutherland,
Nature **2009**, *459*, 239–242



Cyanosulfidic chemistry

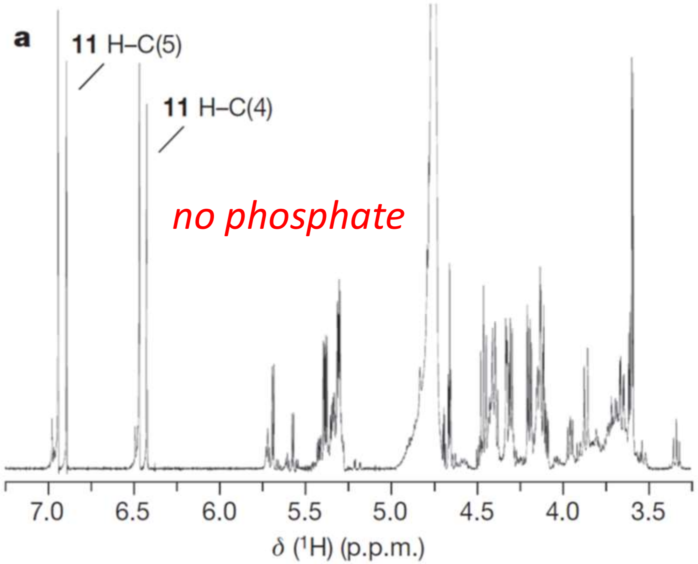


The recently uncovered route to activated pyrimidine nucleotides **2**.

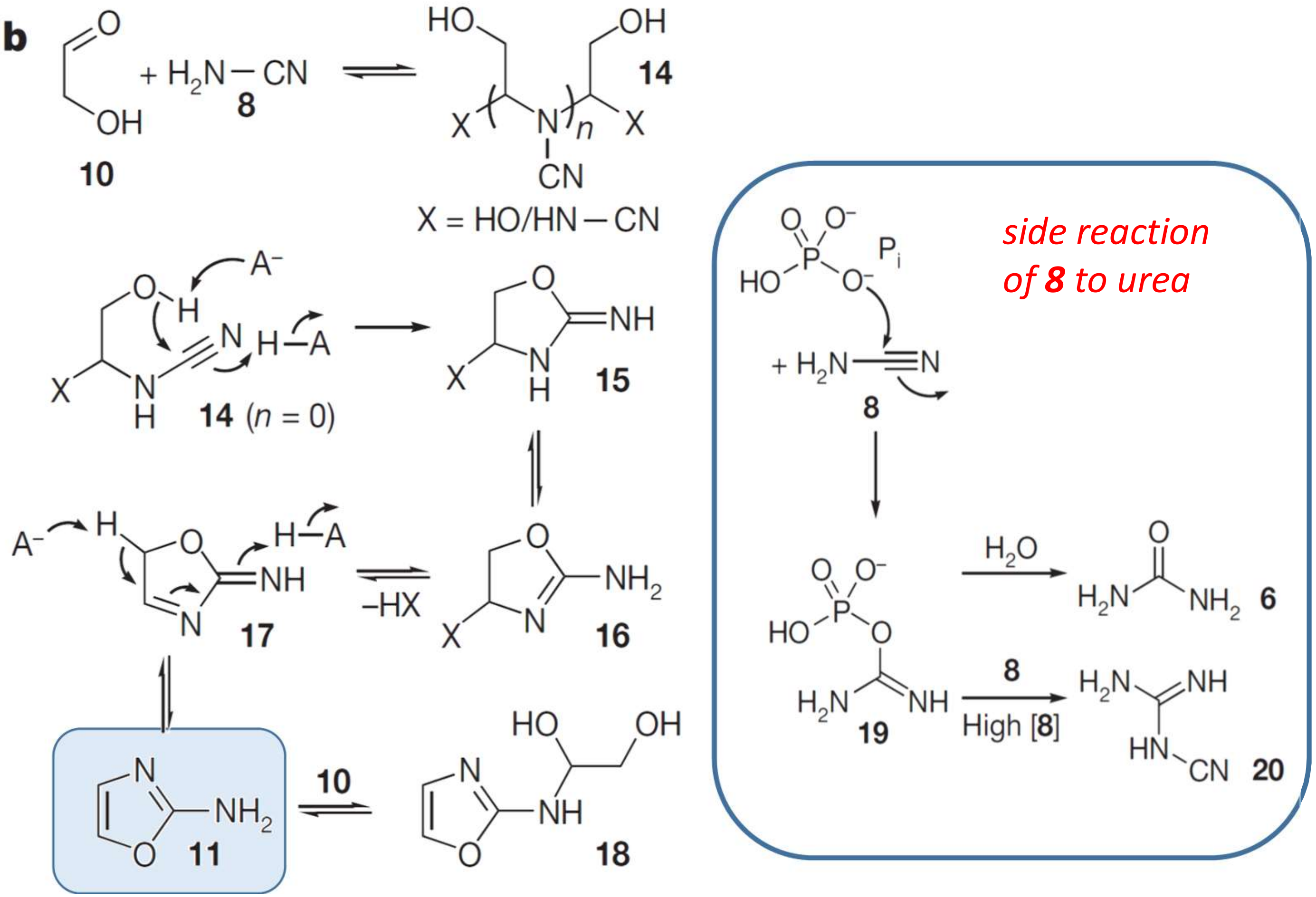
The nucleobase ribosylation problem is circumvented by the assembly proceeding through 2-aminooxazole **21**, which can be thought of as the chimera of half a pentose sugar and half a nucleobase. The second half of the pentose - glyceraldehyde **5** -and the second half of the nucleobase—cyanoacetylene **7**—are then added sequentially to give the anhydronucleoside **23**.

Phosphorylation and rearrangement of **23** then furnishes **2** (B=C), and UV irradiation effects the partial conversion of **2** (B=C) to **2** (B=U).

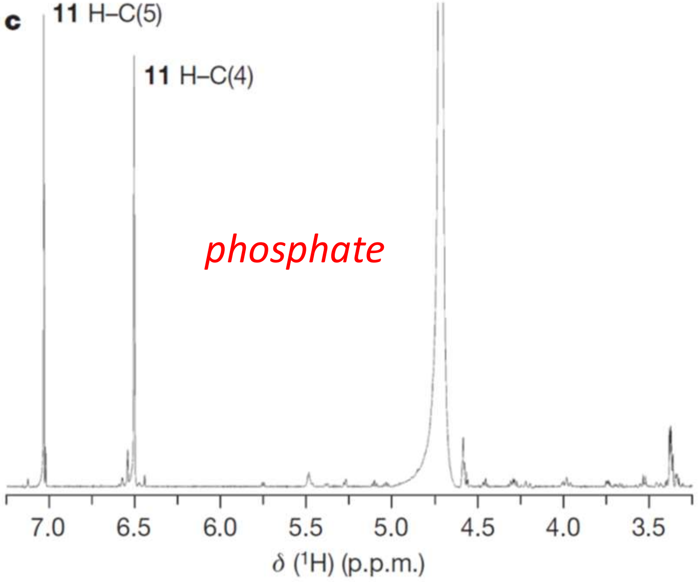
Cytosine-2',3'cP – step 1: 2-amino-oxazole



no phosphate



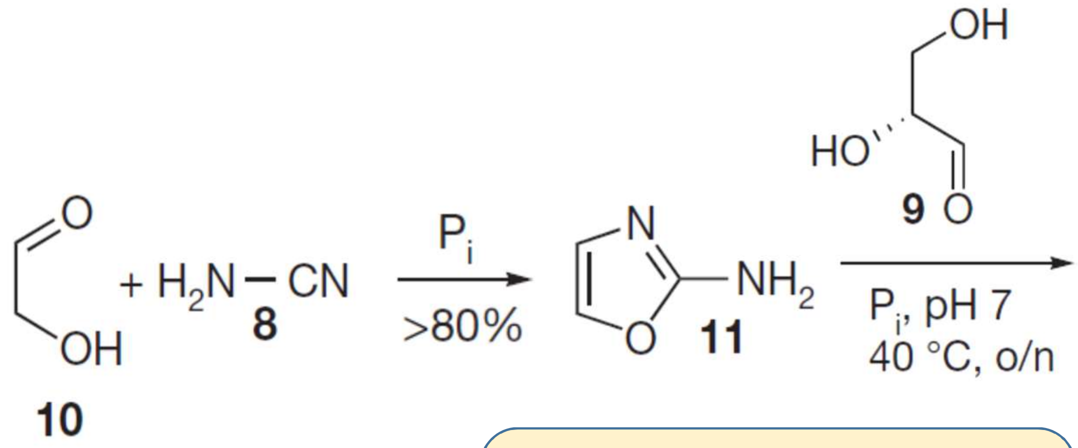
*side reaction of **8** to urea*



phosphate

M. W. Powner, B. Gerland, J. D. Sutherland,
Nature **2009**, *459*, 239–242

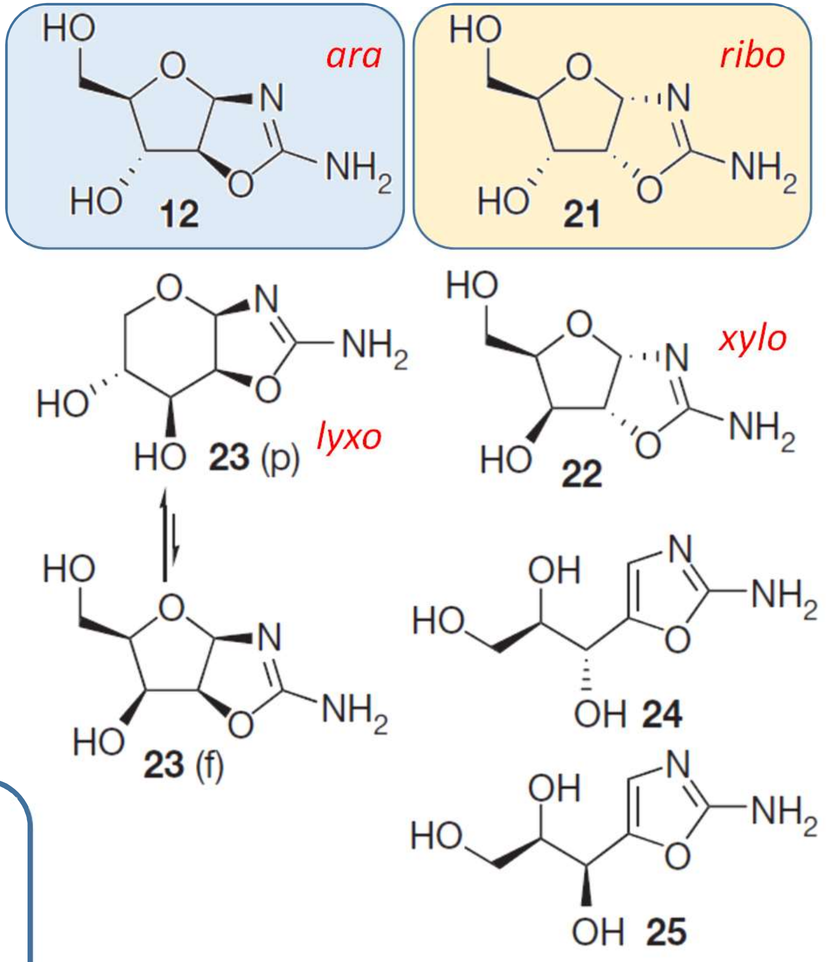
Cytosine-2’,3’cP – step 2: pentose-amino-oxazolines

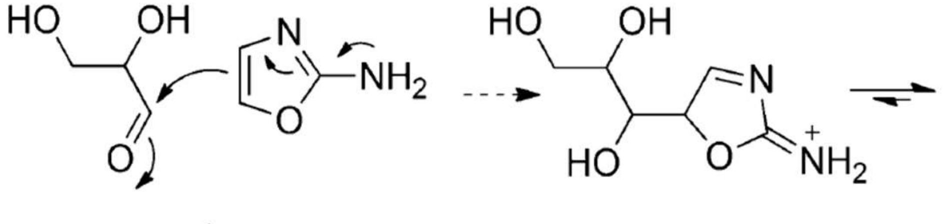


12 15%
21 25%
22 6%
23 4%
24 11%
25 9%

Yields for two steps based on **10**

*ribo-**21** – selectively crystallizes on cooling → ara-**12** becomes the main mixture component*



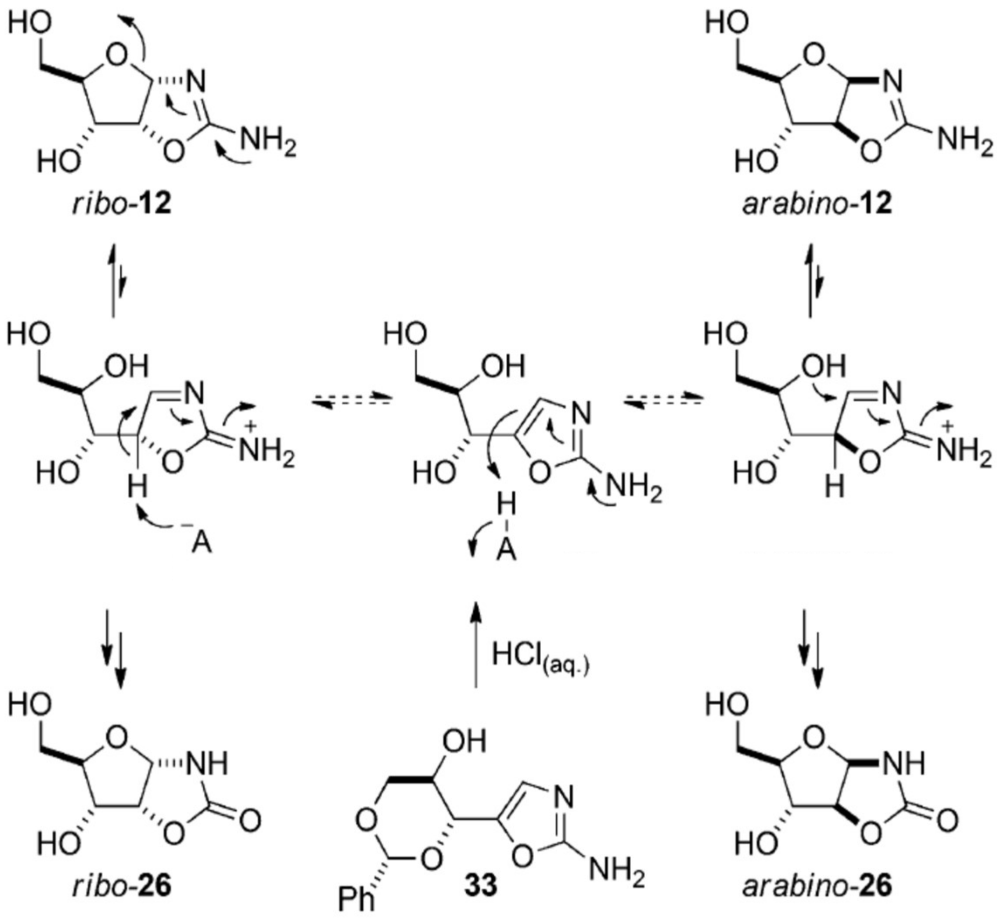


44% *ribo*-**12**
30% *arabino*- **21**
13% *lyxo*- **23**
8% *xylo*- **22**

M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **2009**, *459*, 239–242

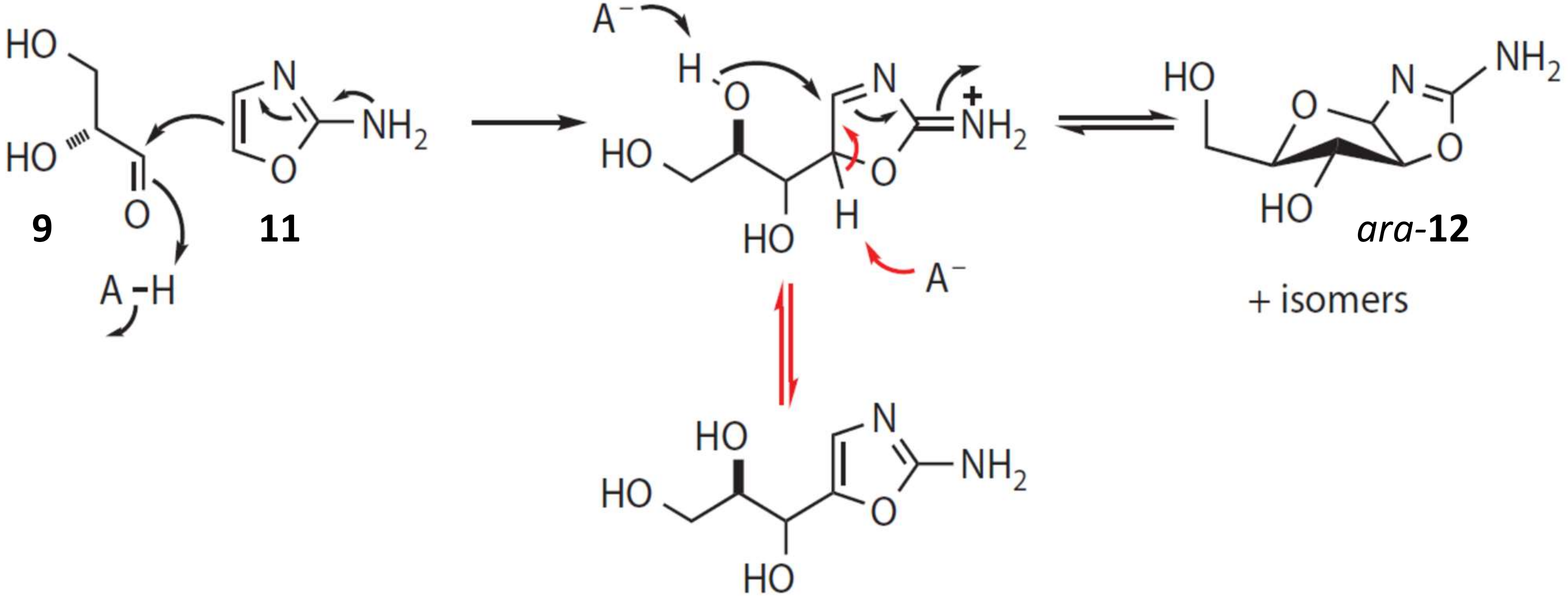
Cyanosulfidic chemistry

Interconversion of pentose aminooxazoline stereoisomers.

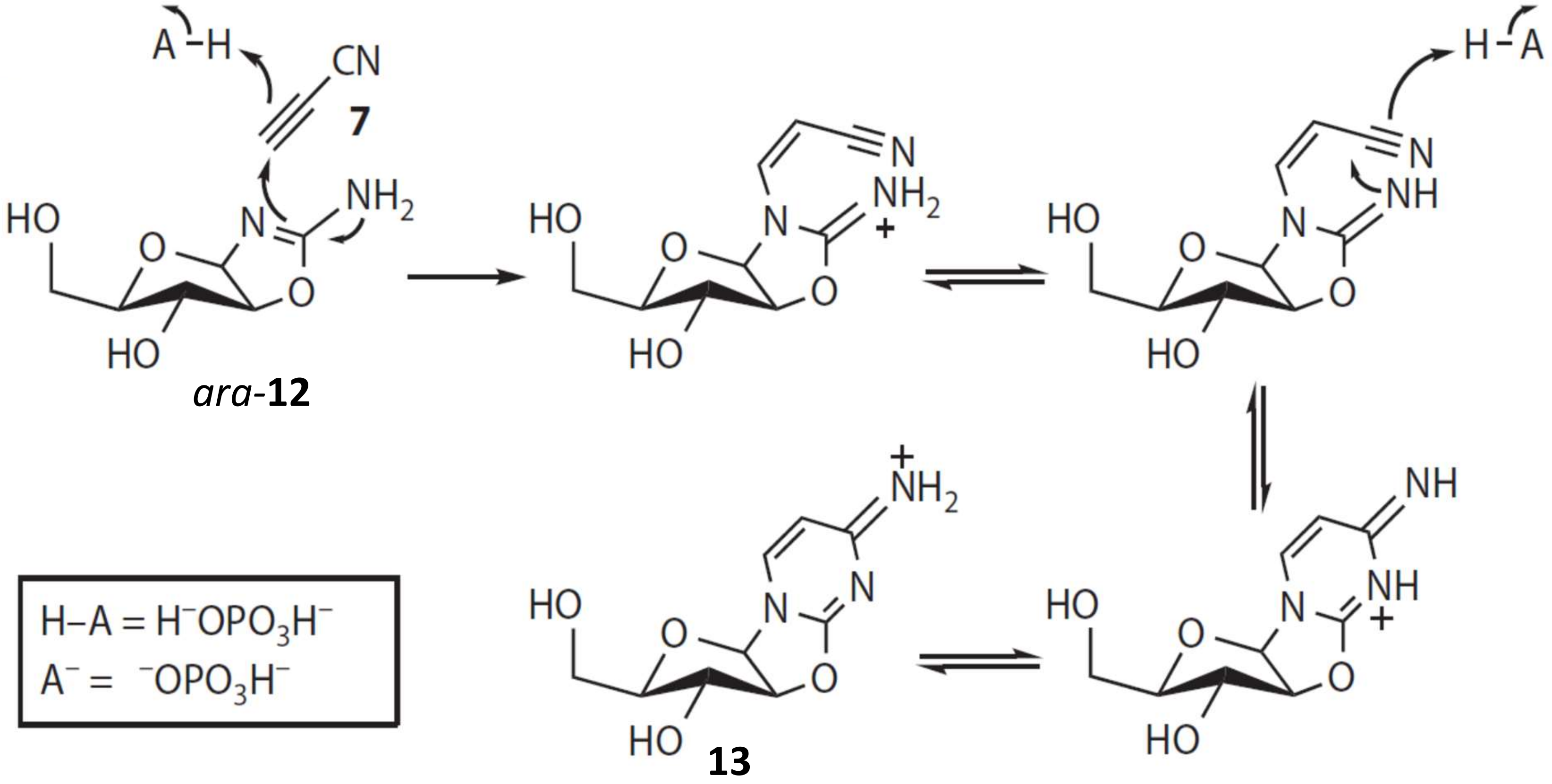


The „side product" *ribo*-**21** can be converted to the expected *arabino*-**12** by general acid-base catalysis in phosphate buffer, although with some decomposition to **26**

Cytosine-2’,3’cP – step 2: pentose-amino-oxazolines



Cytosine-2’,3’cP – step 3: arabinose-anhydronucleosides



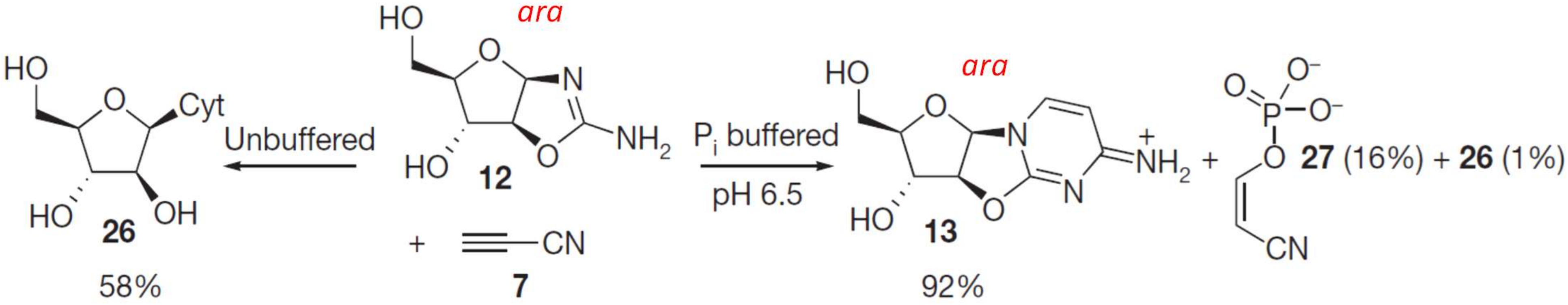
ara-**12**

13

H–A = $H^{-}OPO_3H^{-}$

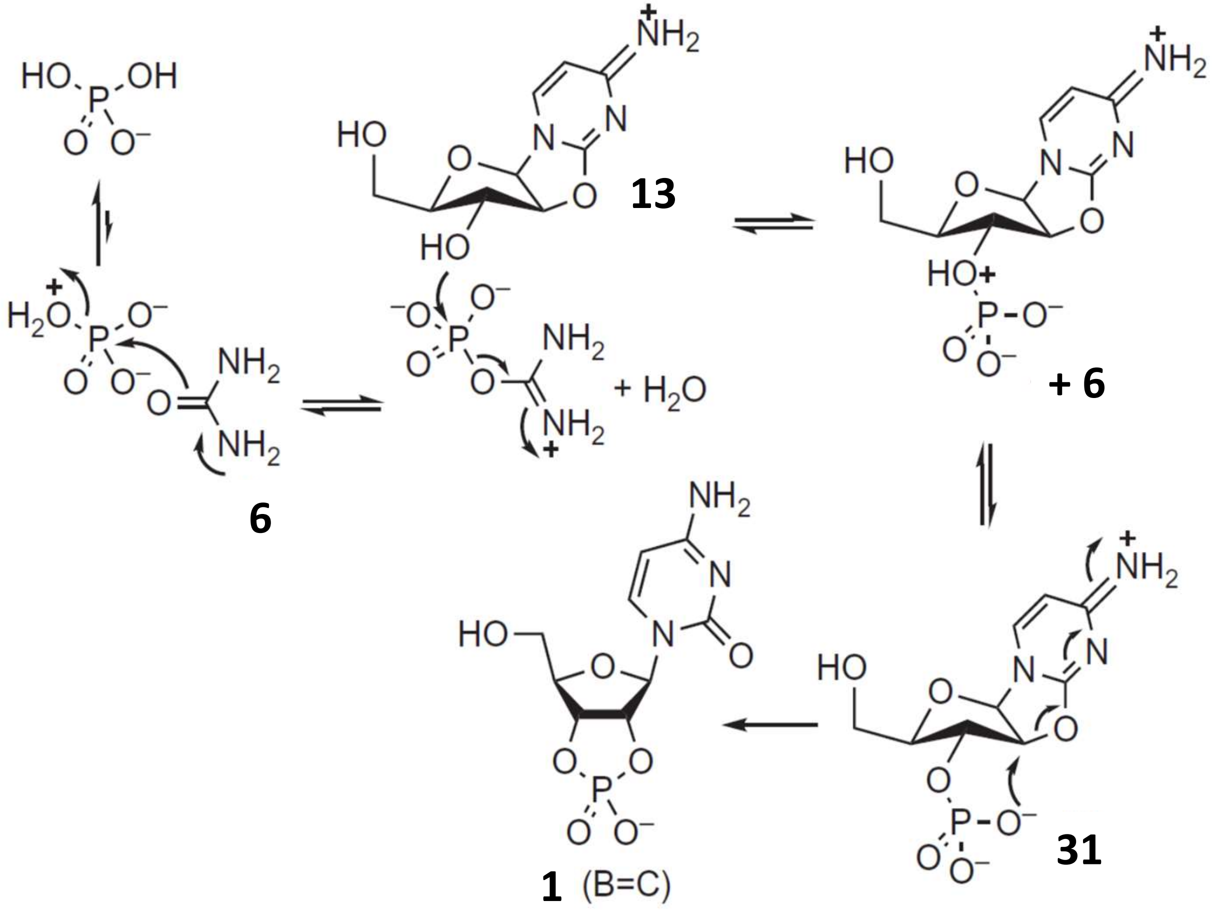
A^{-} = $^{-}OPO_3H^{-}$

Cytosine-2’,3’cP – step 3: arabinose-anhydronucleosides



M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **2009**, *459*, 239–242

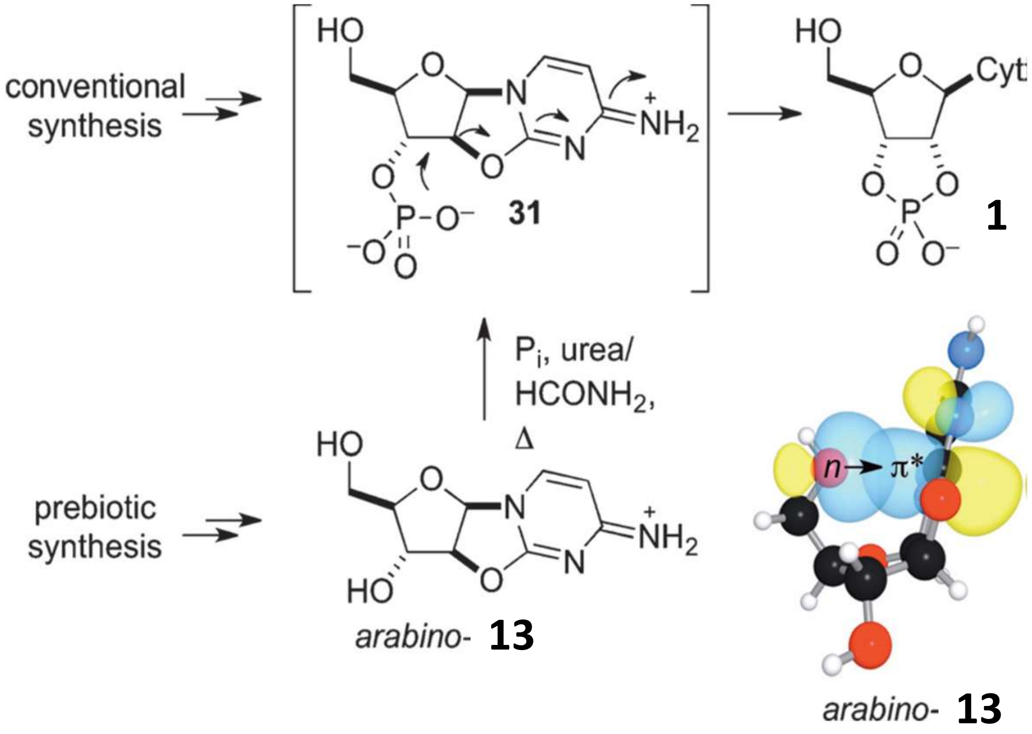
Cytosine-2′,3′cP – step 4: β-ribocytidine-2′,3′-cyclic phosphate

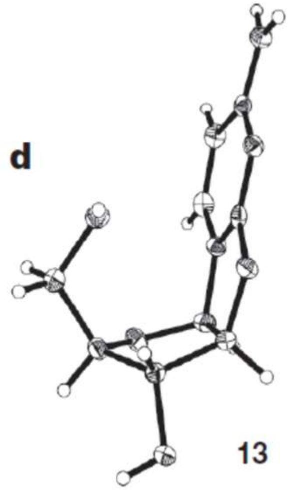


M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **2009**, *459*, 239–242

Cytosine-2′,3′cP – step 4: β-ribocytidine-2′,3′-cyclic phosphate

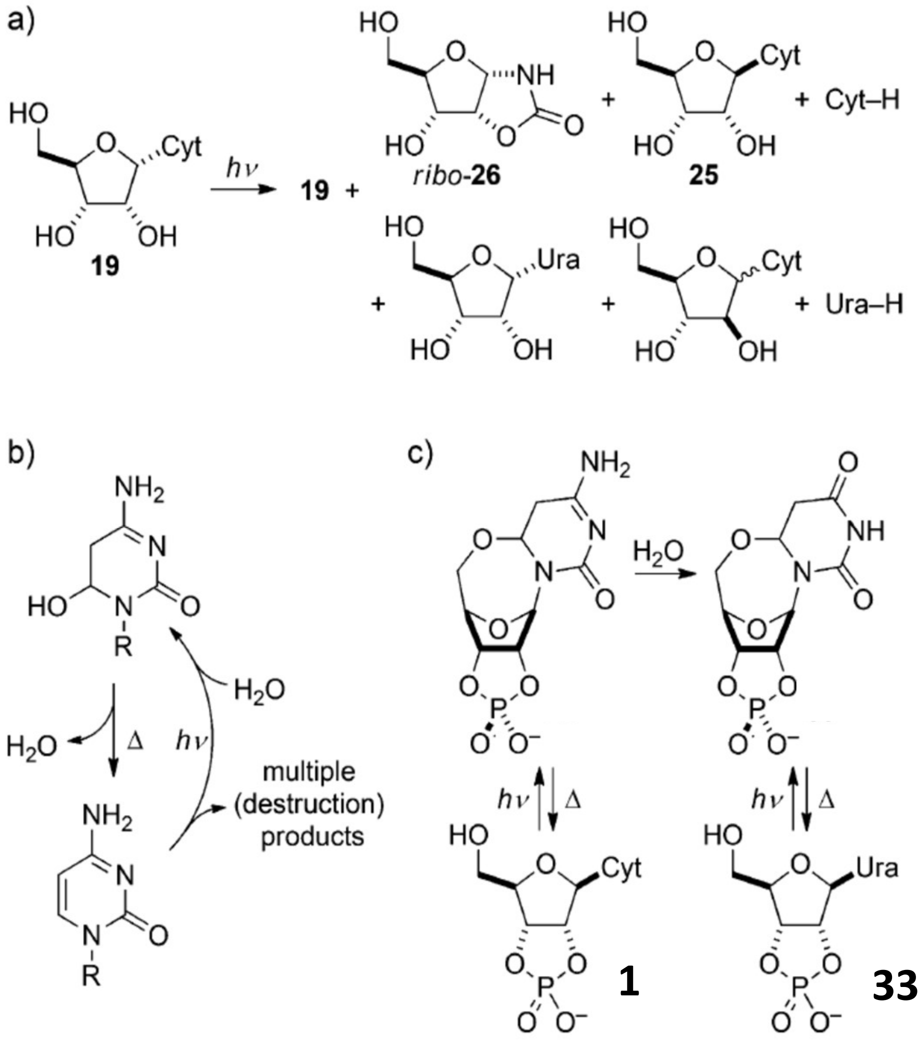
Rearrangement of **31**, the 3’-phosphate of *arabino*-**13**, to **1** by intramolecular nucleophilic substitution.





M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **2009**, *459*, 239–242

Activated pyrimidine nucleotides – step 5: UV-light induced rearrangement C→ U + cleanup of the side products



Orgel reported photoanomerization of alpha-cytidine **19** to the beta-anomer **25**, but the reaction was very low-yielding and combined with massive decomposition

R. A. Sanchez, L. E. Orgel, *J. Mol. Biol.* **1970**, *47,* 531-543

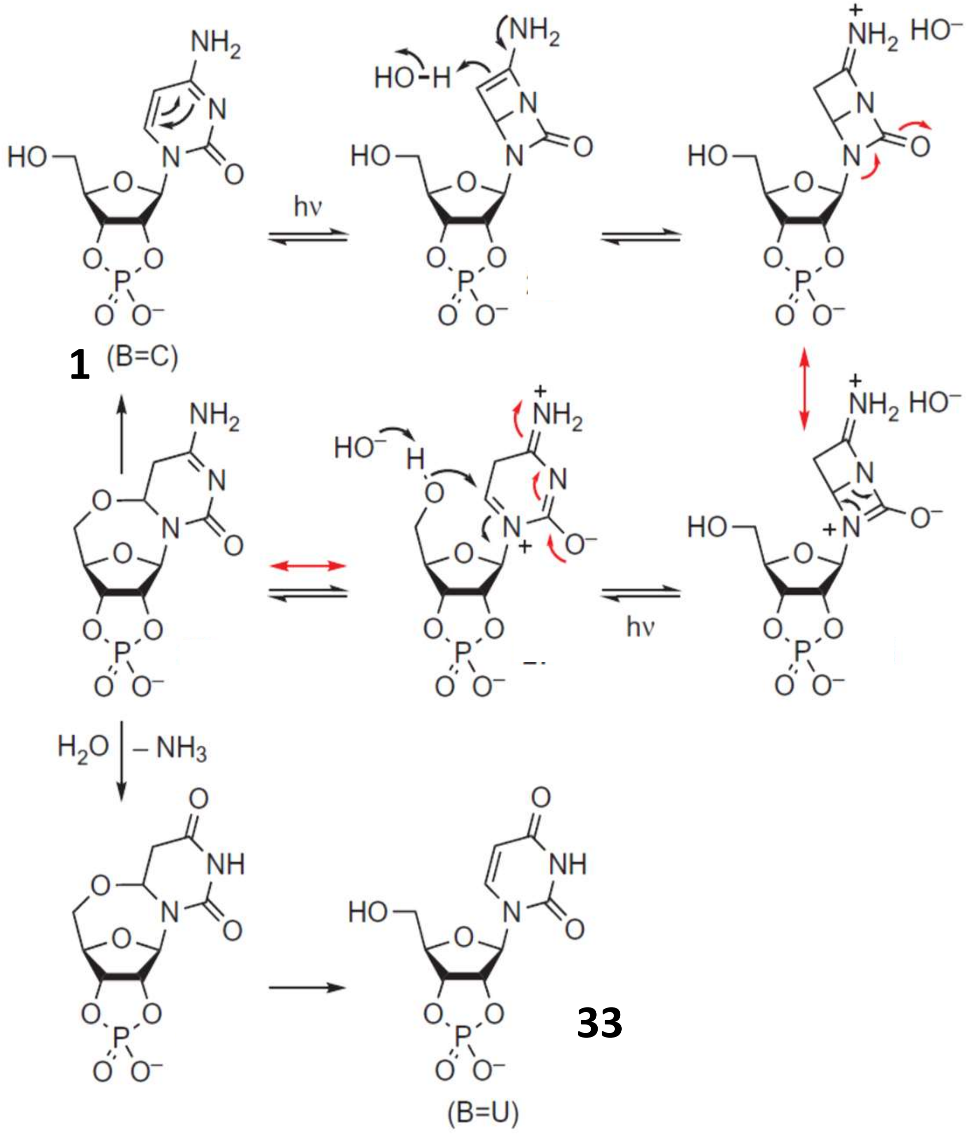
UV light tends to destroy most of phosphorylated cytidine derivatives (by photohydration and followed decomposition).

EXCEPTION: beta-cytidine-2’,3’-cyclic phosphate **1**

→ Partial conversion to the uridine nucleotide **33**, but both are stable on further irradiation

Photochemistry of cytidine nucleosides and nucleotides.

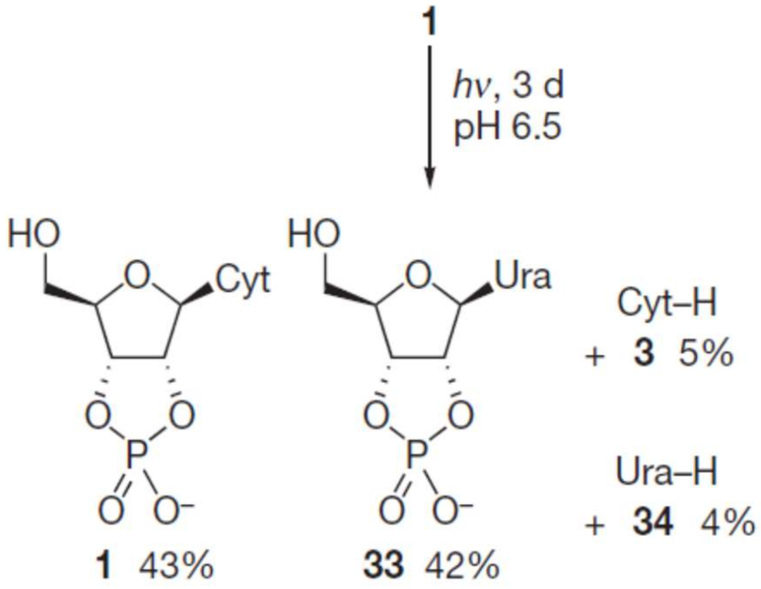
step 5: UV-light induced rearrangement C→U + cleanup of the side products



1 (B=C)

33 (B=U)

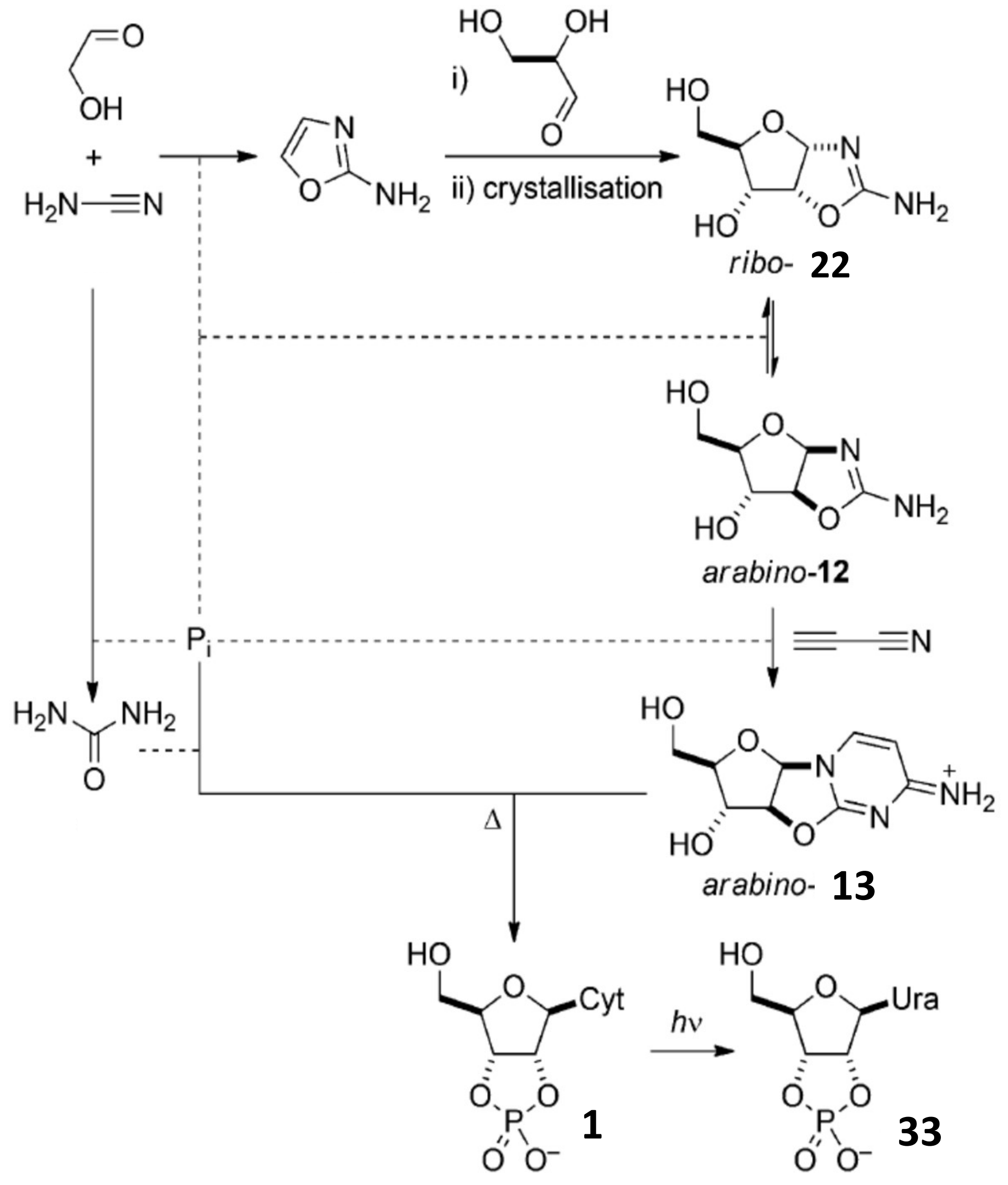
step 5: UV-light induced rearrangement C→U + cleanup of the side products



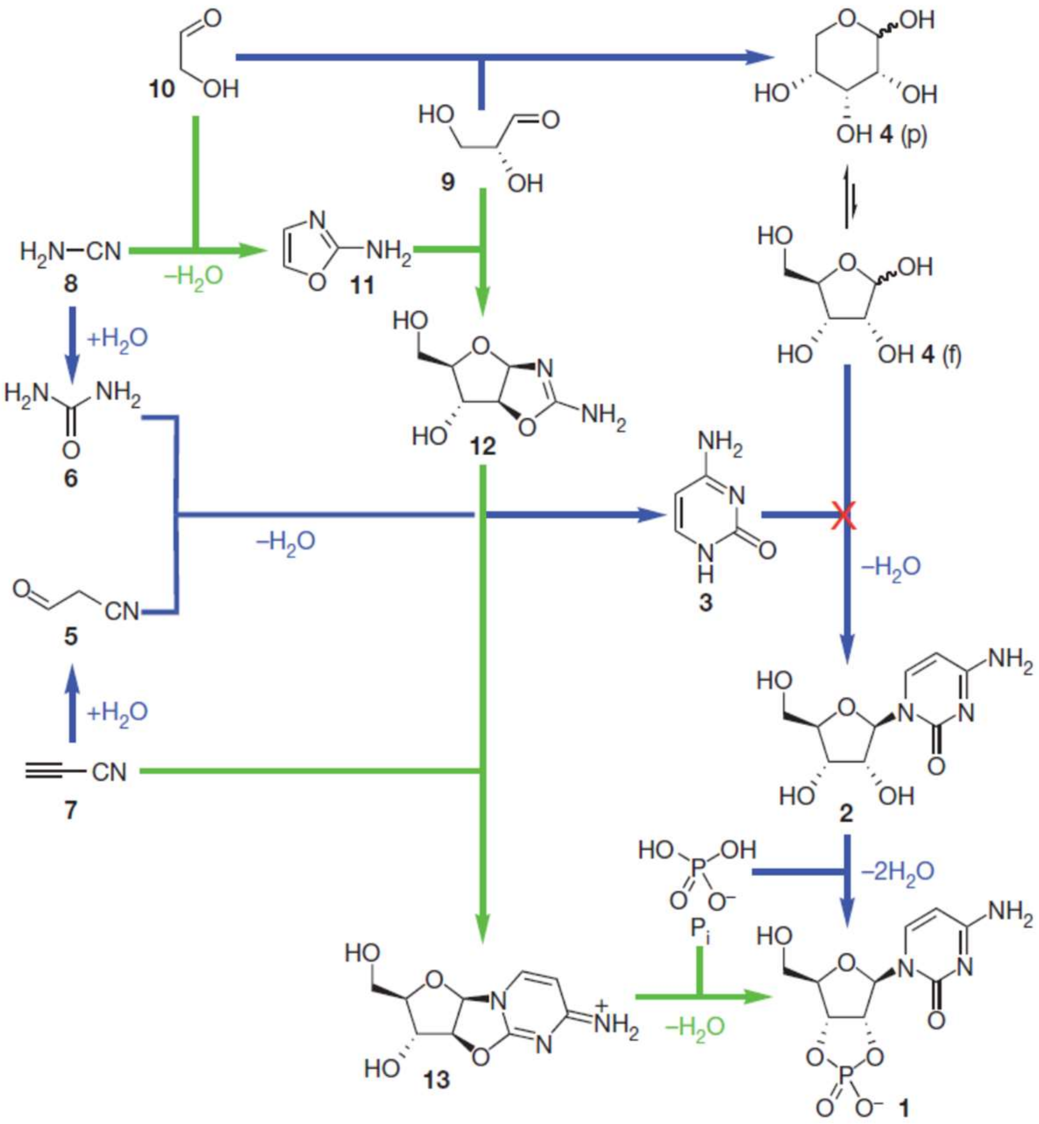
Photochemistry of *beta*-ribocytidine-2’,3’-cyclic phosphate **1**. Under conditions of irradiation that destroy most other pyrimidine nucleosides and nucleotides, **1** undergoes partial hydrolysis and slight nucleobase loss. Ura, N1-linked uracil; Cyt–H, cytosine; Ura–H, uracil.

Prebiotic synthesis of activated pyrimidine nucleotides

Catalysis, and reaction control through pH and chemical buffering, is indicated by dashed lines.



Prebiotic route to pyrimidine nucleotides



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