Is Water Necessary for the Origin and Evolution of an RNA World?

A Self-Assembly Approach to RNA: The Challenges and Advantages of Water

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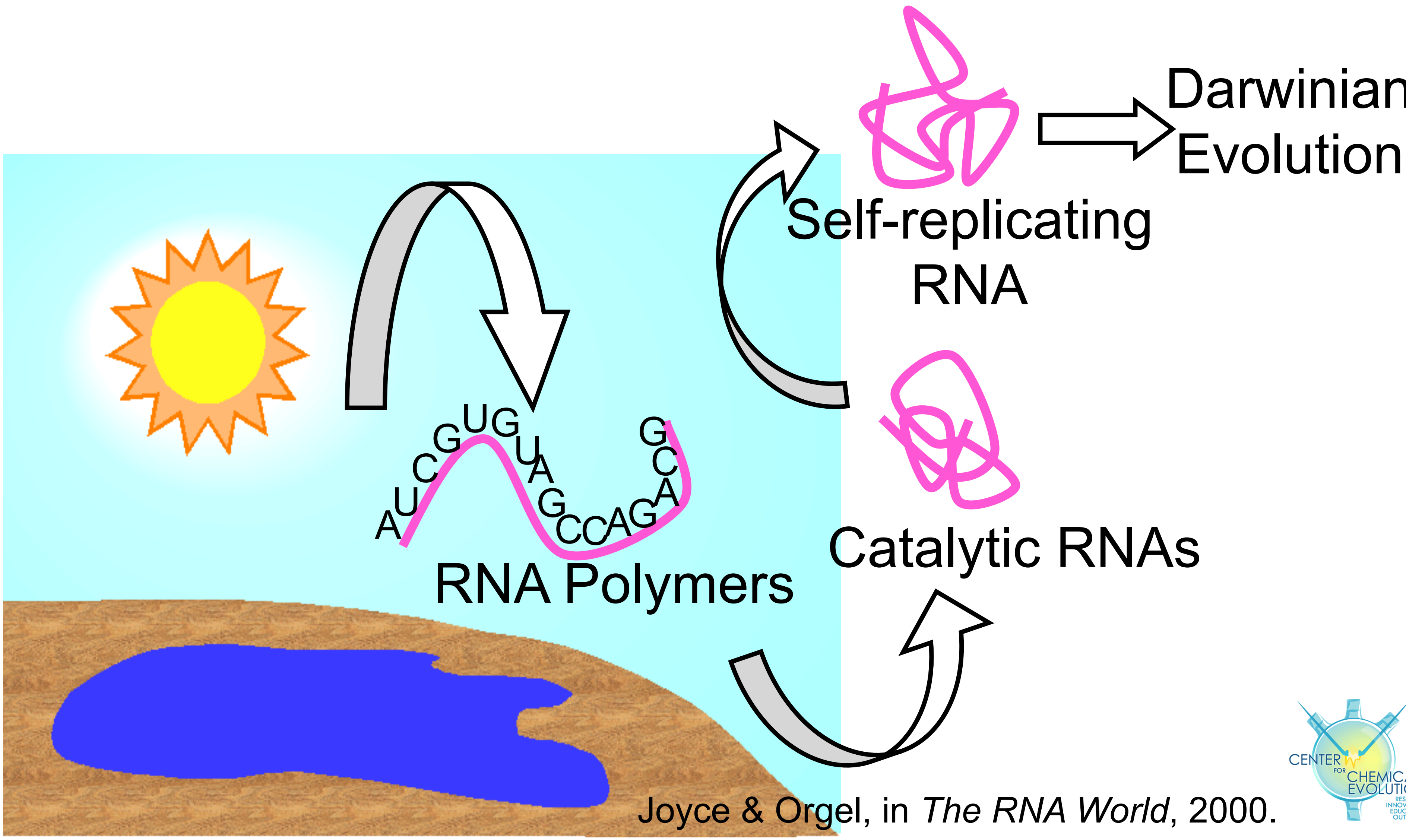
An (extreme) view of the RNA World

"The Molecular Biologist's Dream"



Joyce & Orgel, in *The RNA World*, 2000.

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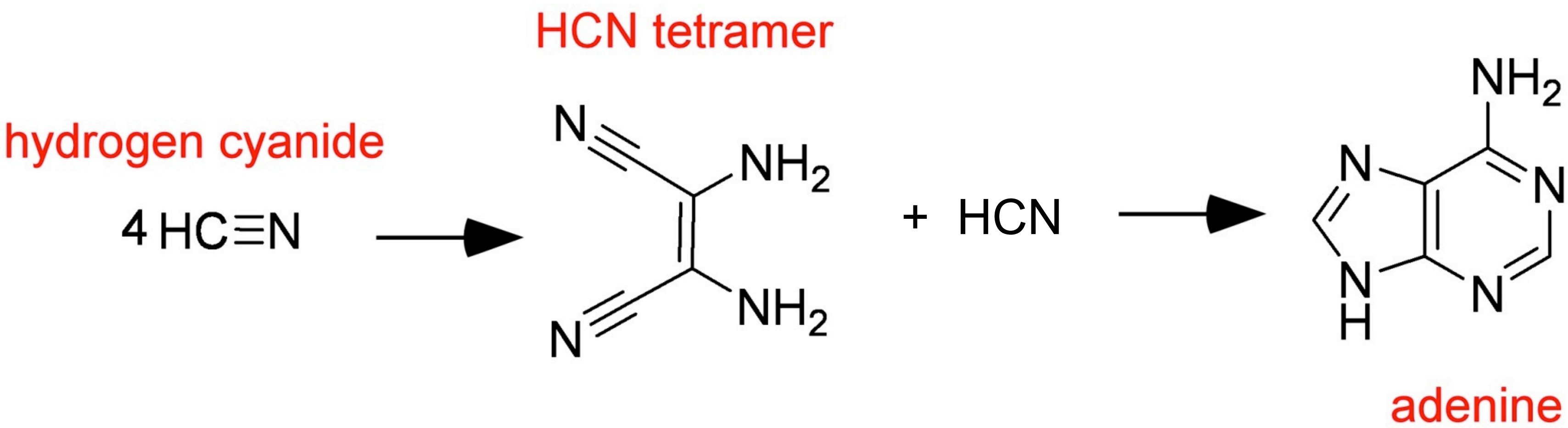
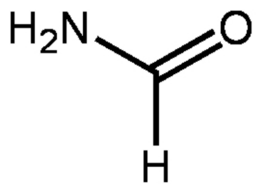
Jon Oro's discovery of abiotic adenine synthesis



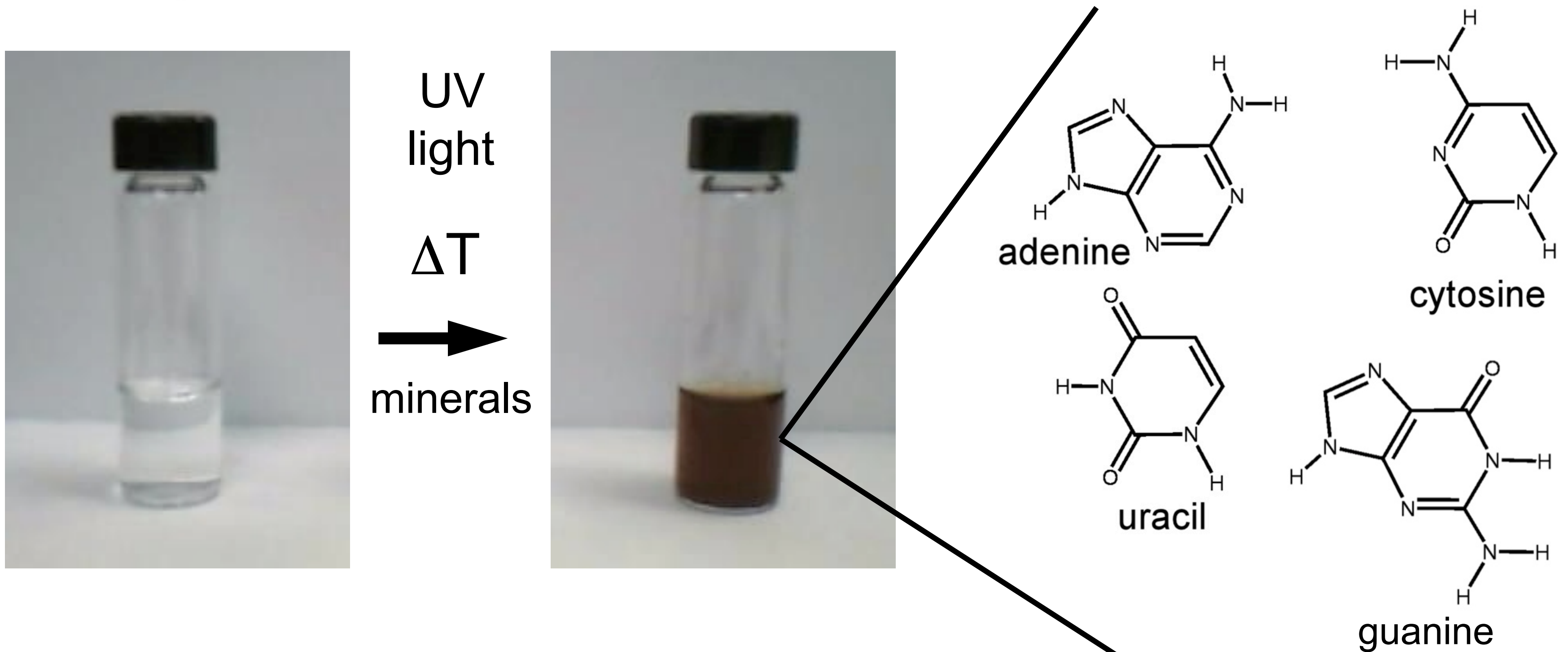
Oro, *BBRC. 2*, 1960, 407-412.; *Nature 191*, 1961, 1193-1194.

Ferris and Orgel, *J. Am. Chem. Soc.* 87, 1965, 4976-4977.

Formamide also provides an abiotic route to heterocycles, including the canonical nucleobases.



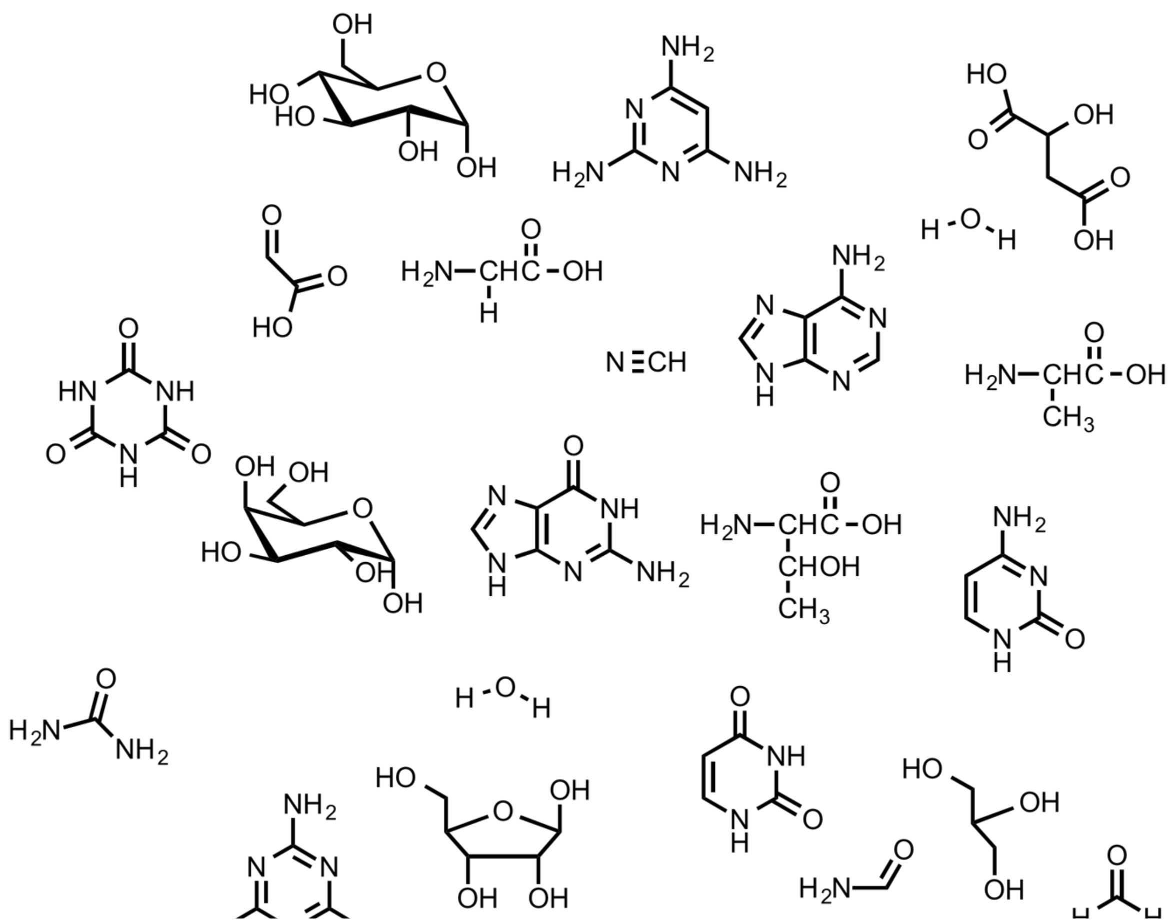
Melting point: 2°C
Boiling point: 210°C



Plus many other pyrimidine and purines.

Saladino et al., Di Mauro, *Bioorg. Med. Chem.* 2001, 9, 1249.
Saladino, *et al*. *Chem. Biodiv.* 4, 2007, 694.
Barks, *et al*. *ChemBioChem* 11, 2010, 1240.

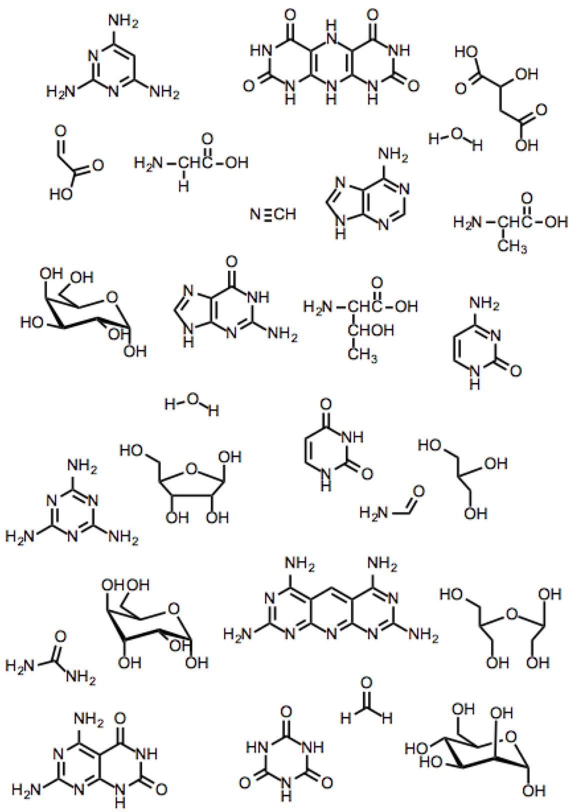
Virtually all the building blocks of extant biopolymers can be synthesized in model prebiotic reactions, as well as *many closely-related molecules*.



How did small molecules become the first biopolymers?

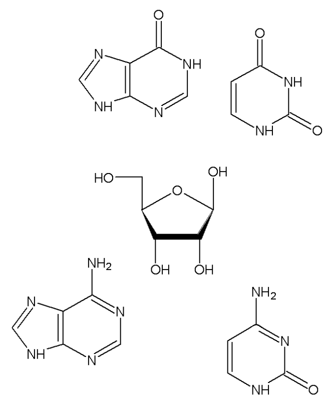
The prebiotic origin of nucleic acids

"The Chemists' Nightmare"

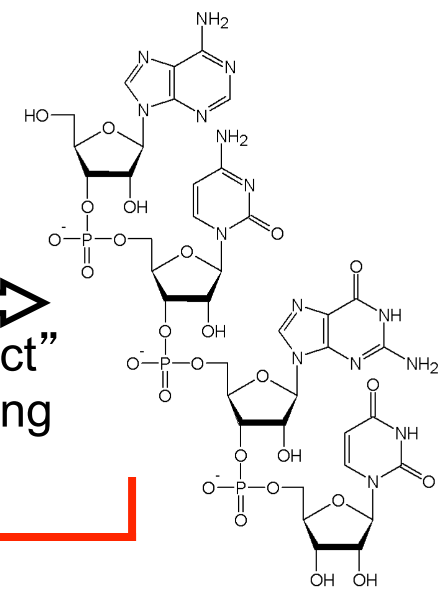


Prebiotic Chemical Inventory

molecule selection



"correct" coupling



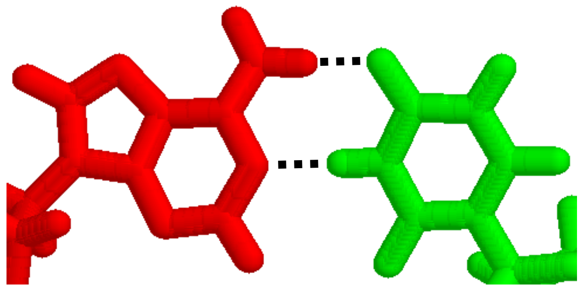
RNA, DNA

Unresolved

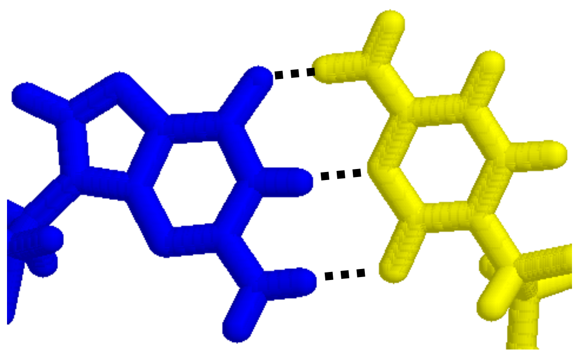
Joyce & Orgel, in *The RNA World*, 2000.

How and why were the first bases selected?

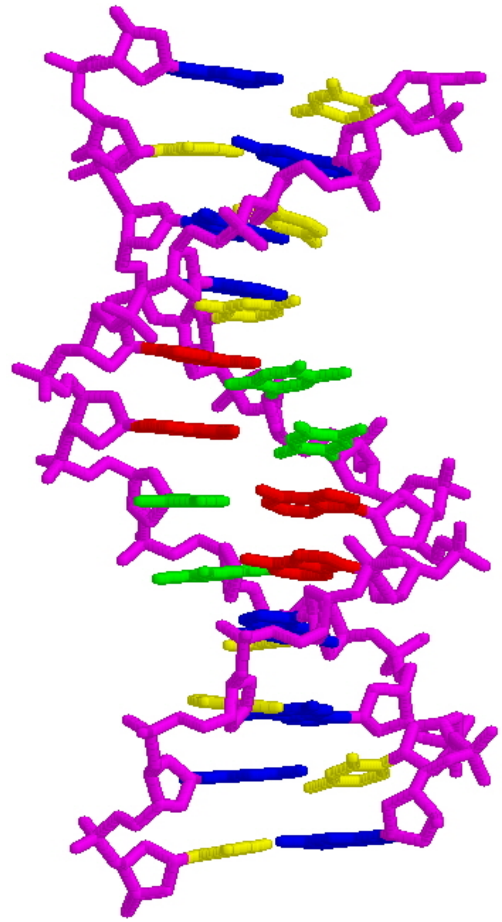
In life, Watson-Crick base pairing is essential for information storage and information transfer.



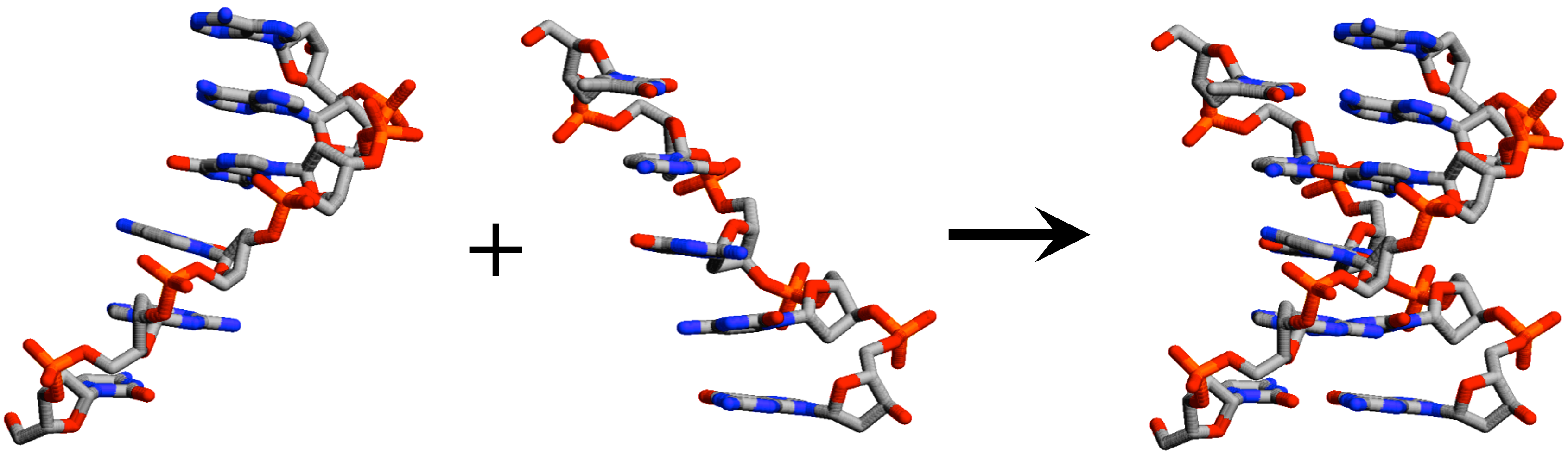
Adenine (A) Uracil (U)

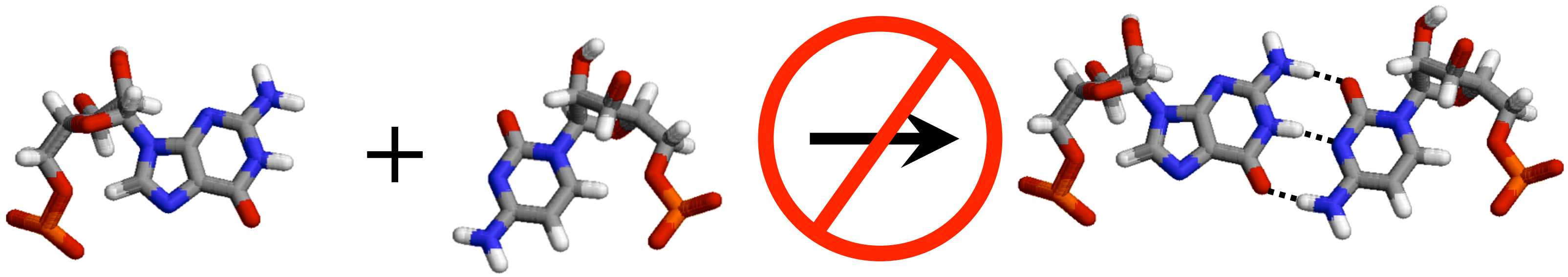


Guanine (G) Cytosine (C)



"The Paradox of Base Pairing"





The nucleobases of present-day RNA only pair as oligomers, not as monomers.

Hud and Anet, *J. Theo. Biol.*, 2000.
Engelhart and Hud, *In* Origins of Cellular Life, *Cold Spring Harb Perspect Biol*, 2010

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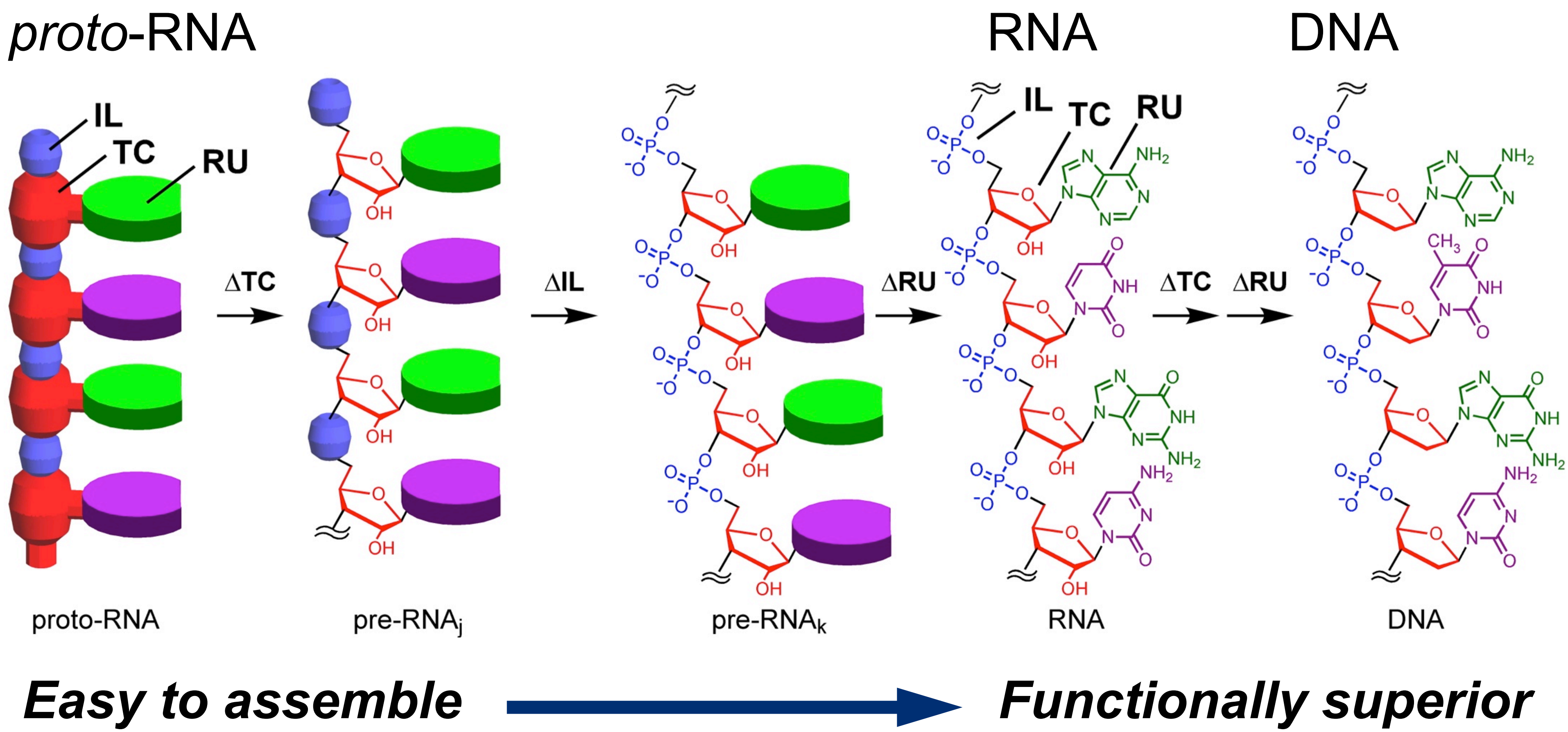
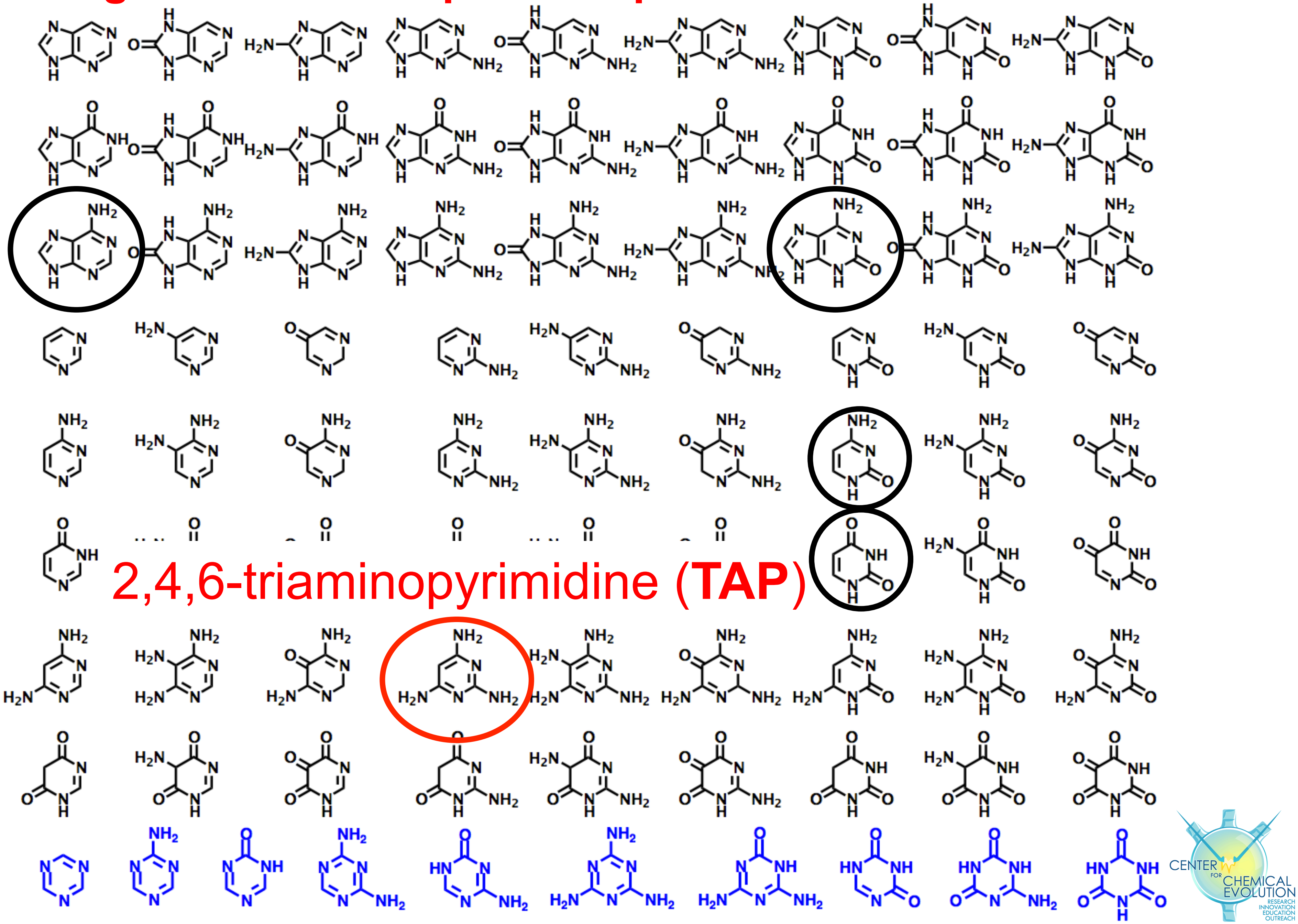
The hypothesis of nucleic acid evolution

Supported by the existence of DNA…

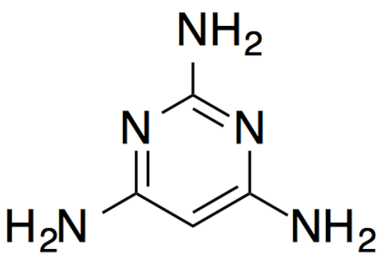


Hud, Cafferty, Krishnamurthy, and Williams, *Chem. & Biol.* 20, 466-474, 2013.

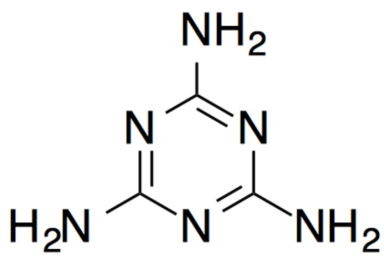
Taking a wide look at possible proto-RNA bases.



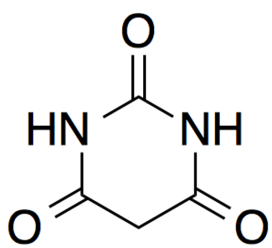
TAP and Melamine have been shown to strongly associate with Cyanuric Acid and Barbituric Acid, *but almost exclusively in organic solvents*.



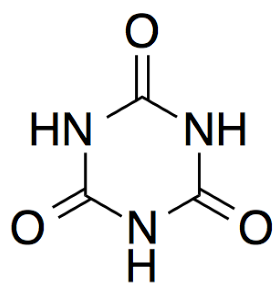
TAP



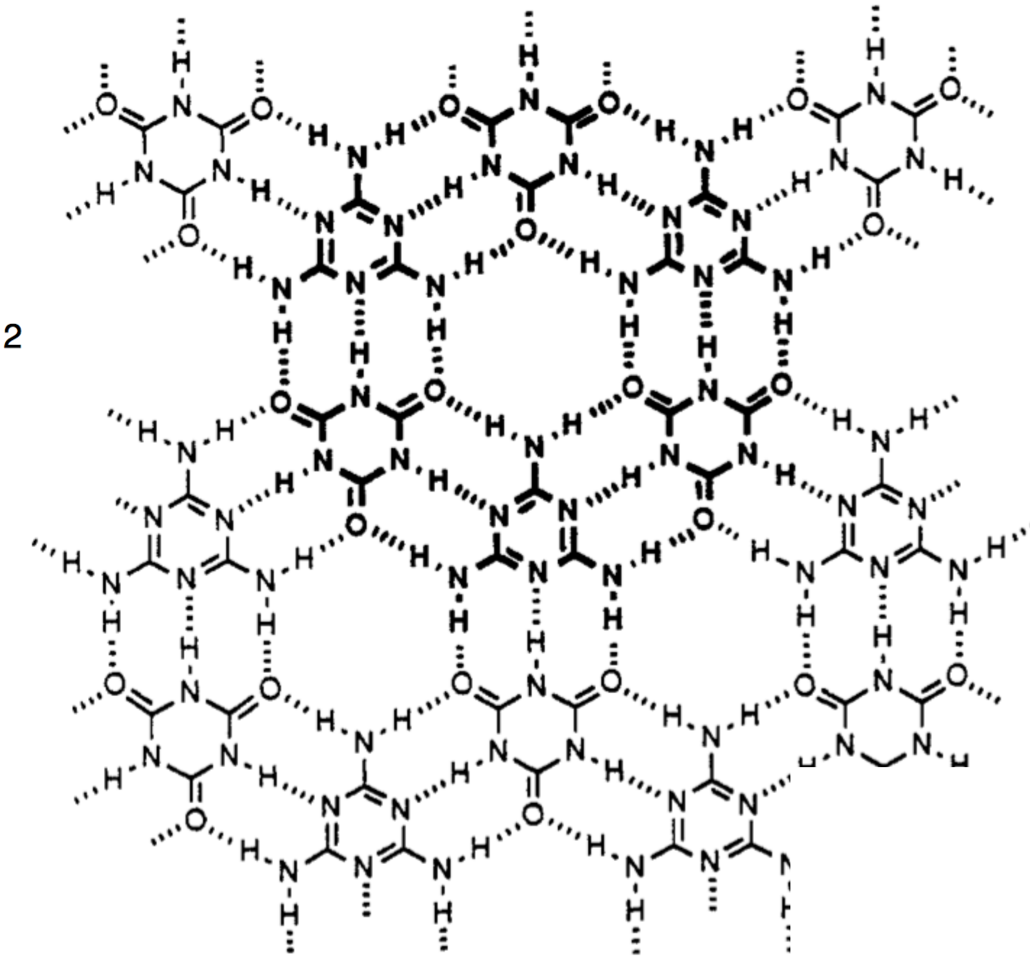
melamine



barbituric acid



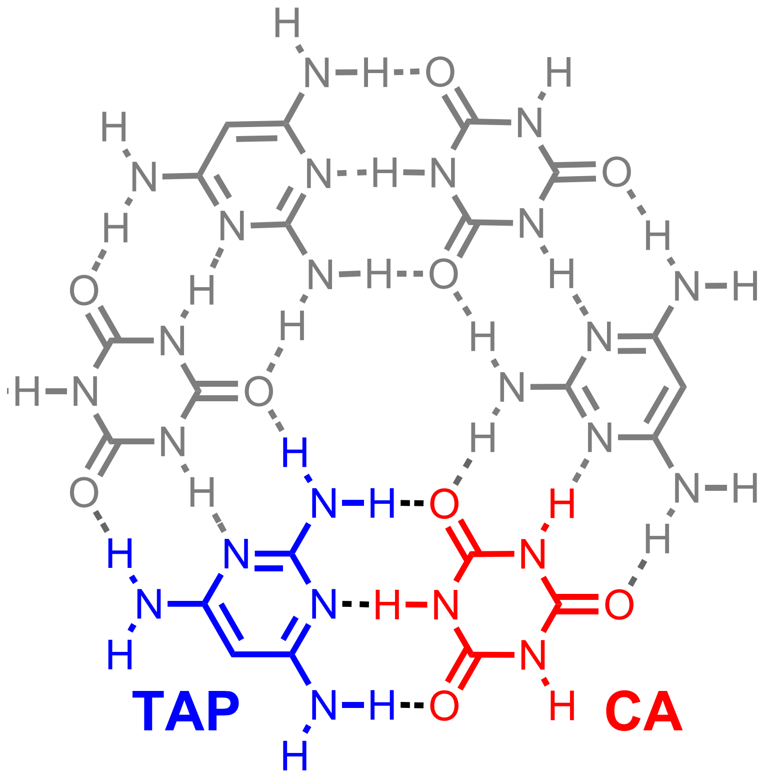
cyanuric acid (**CA**)



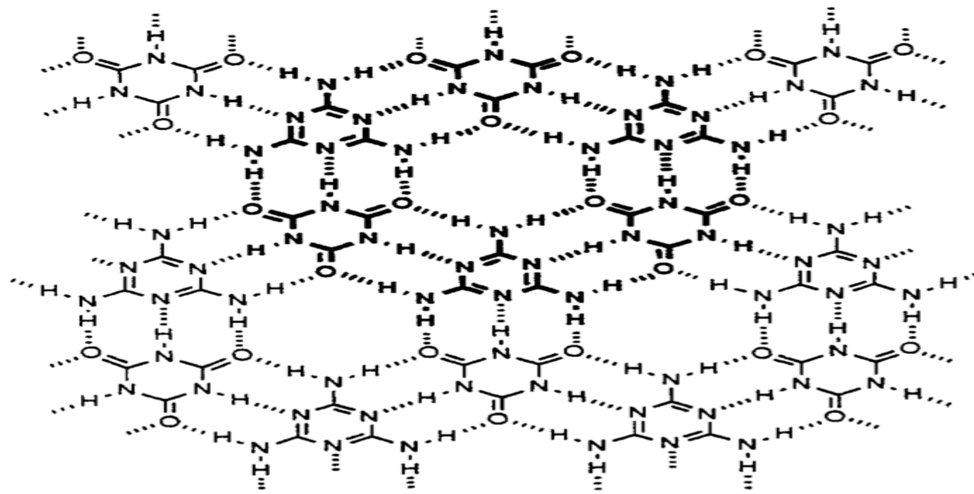
T. Seto and G. M. Whitesides, *J. Am. Chem. Soc.* 112, 1990.

J.-M. Lehn, M. Mascal, A. DeCian, and J. Fischer, *Chem. Comm.* 1990.

Exploration of **TAP** and **CA** hexad formation in water.

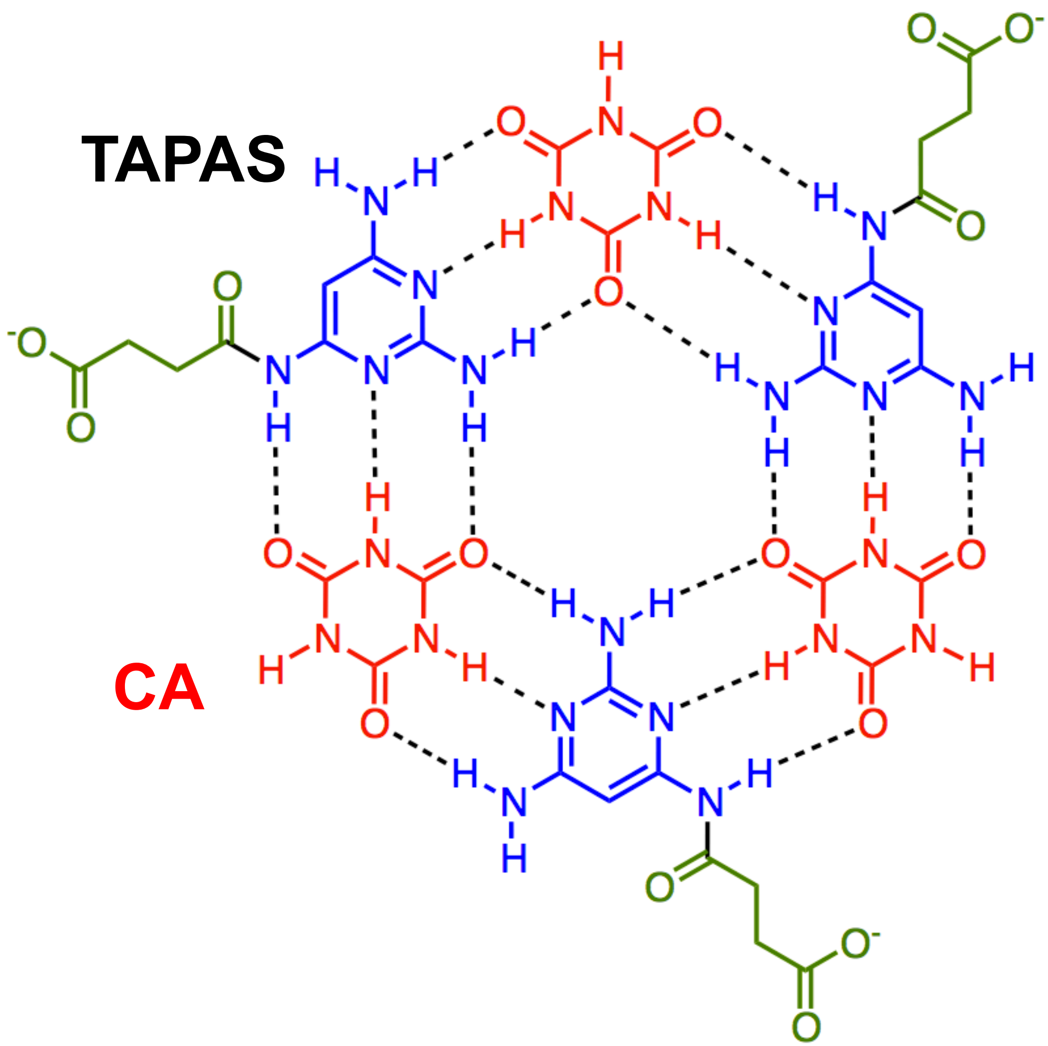


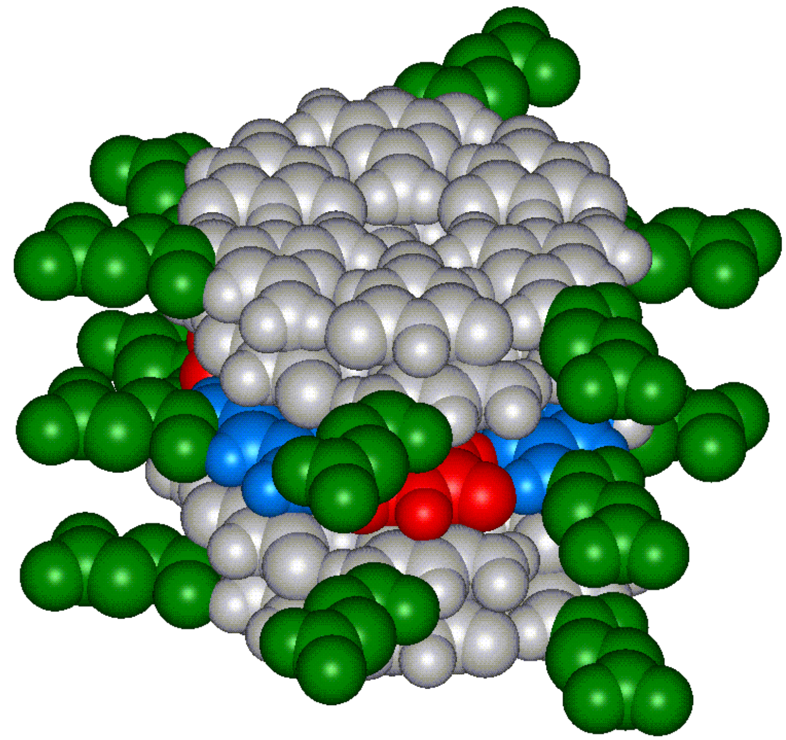
proto-Watson-Crick base pairs?



A challenge to assembly in water: Precipitates are rapidly formed upon mixing and **TAP** with **CA**, probably into graphite-like structures.

TAPAS: **TAP-A**cid-of-**S**uccinate





Cafferty, et al., *J. Am. Chem. Soc*. 135, 2447 (2013).

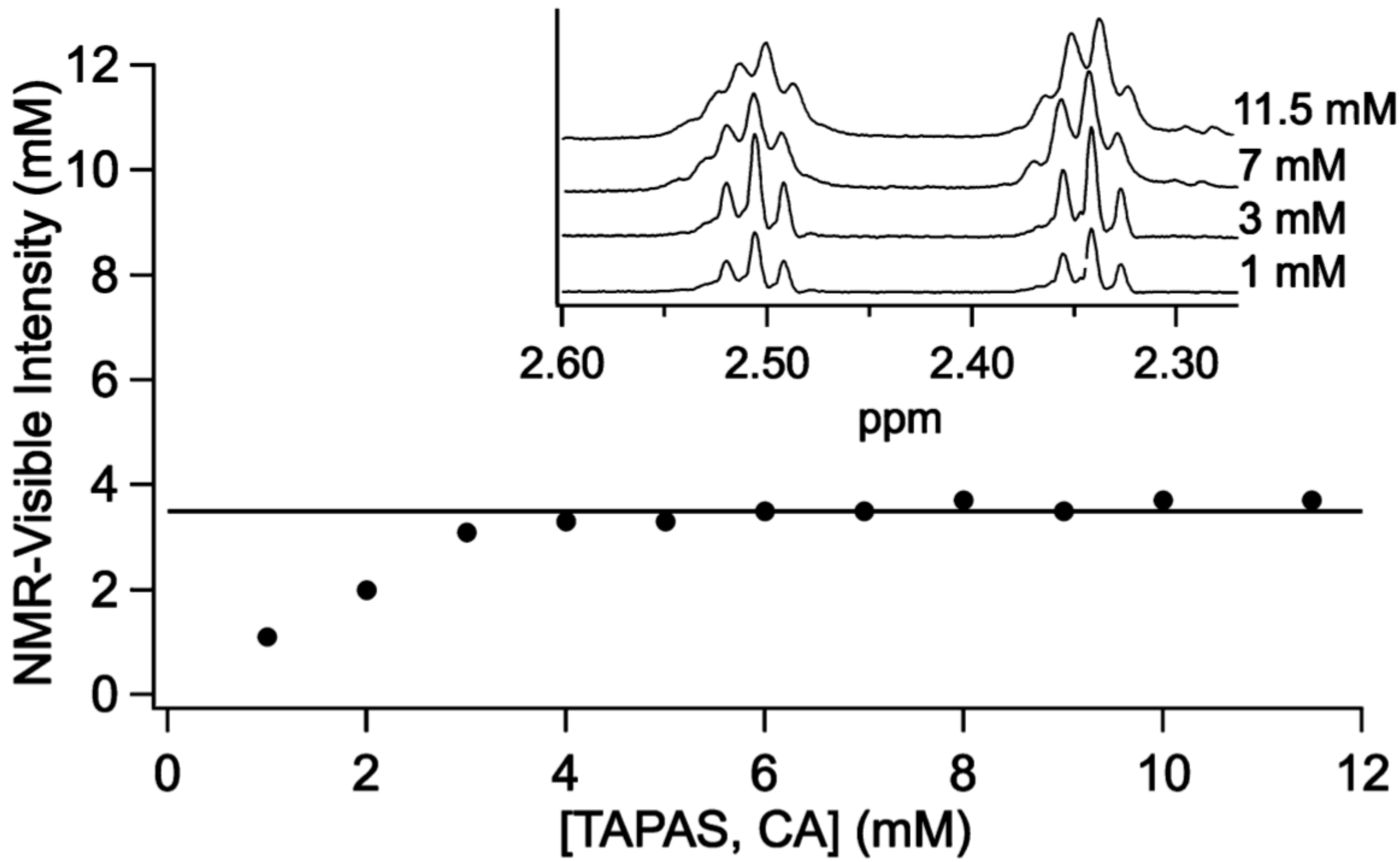
Gelation – Indicative of very long, noncovalent polymers formation in solution.

3 mM 5 mM 15 mM

[TAPAS],[CA]

Cafferty, et al., *J. Am. Chem. Soc*. 135, 2447 (2013).

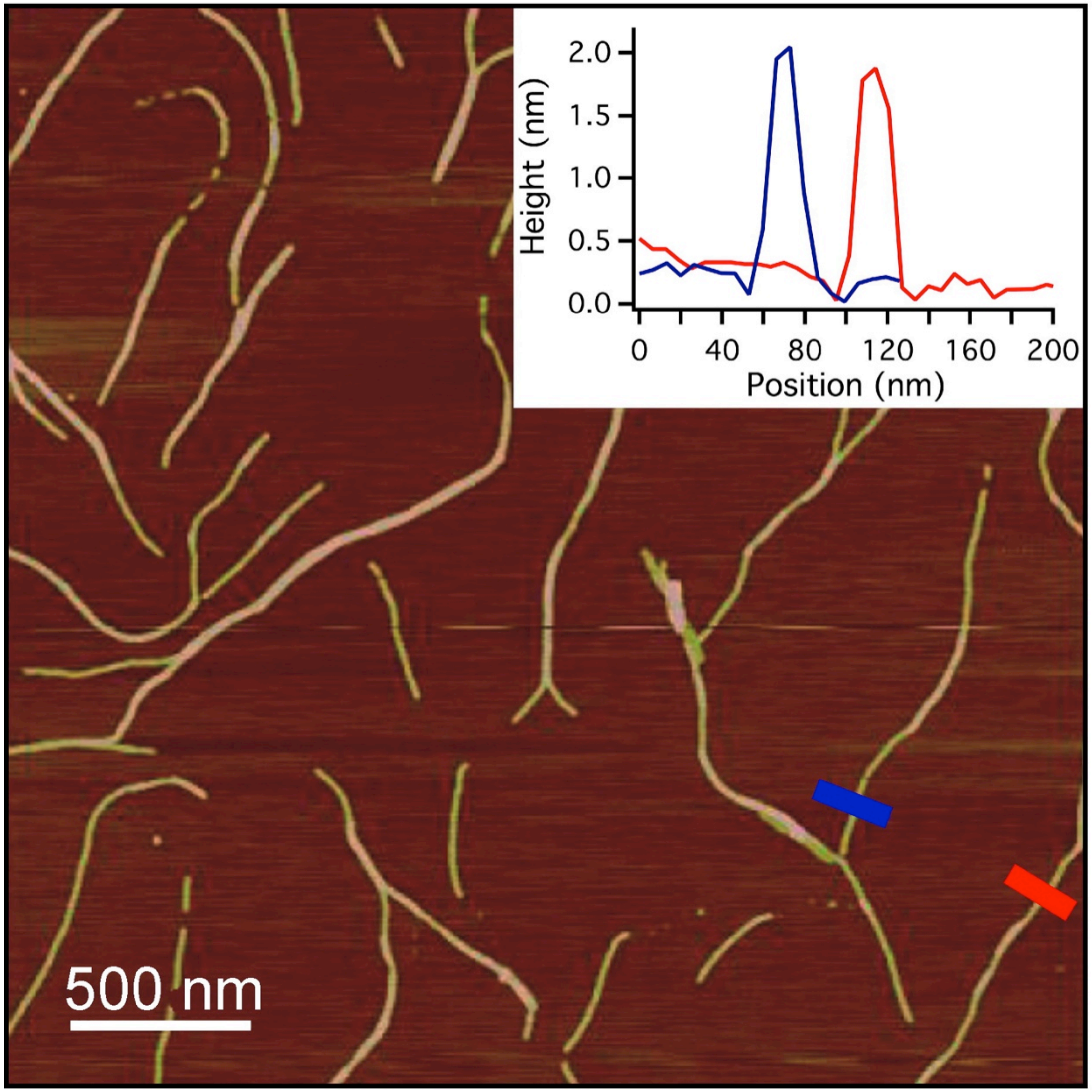
"Invisible" NMR signals – A sign of assembly into large structures that tumble too slowly to be seen by solution state NMR.



A phase transition at 3.5 mM

Cafferty, et al., *J. Am. Chem. Soc*. 135, 2447 (2013).

AFM imaging of **TAPAS** and **CA** self-assembled in water



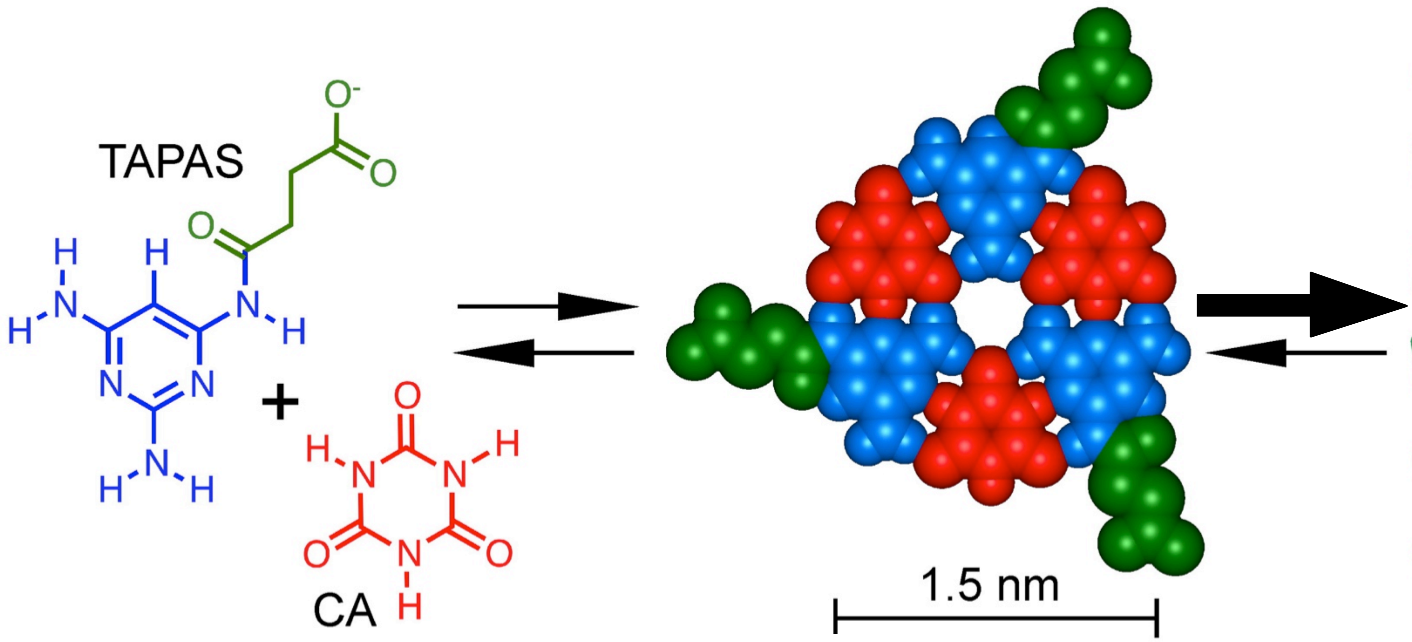
Stacked pi systems are separated by 0.34 nm.

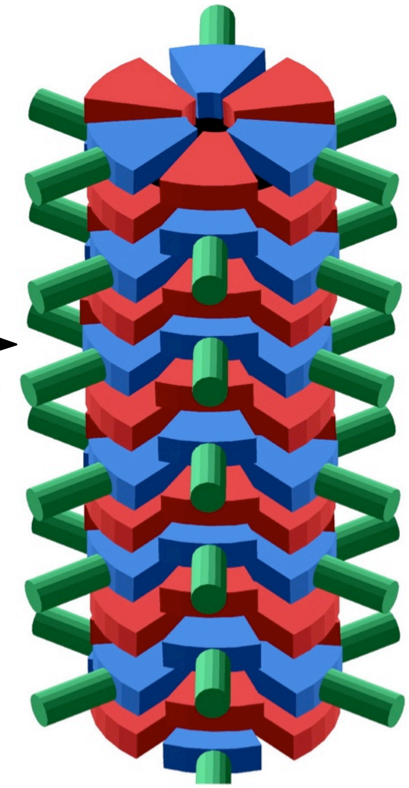
Thus, each 1 μm fiber contains 3000 stacked hexads, with 18,000 paired bases.

Gene-length noncovalent assemblies!

Cafferty, et al., *J. Am. Chem. Soc*. 135, 2447 (2013).

Estimated ΔGs of hydration and monomer association predict highly cooperative assembly.





Solvation free energy
ca. 2 kcal/mol

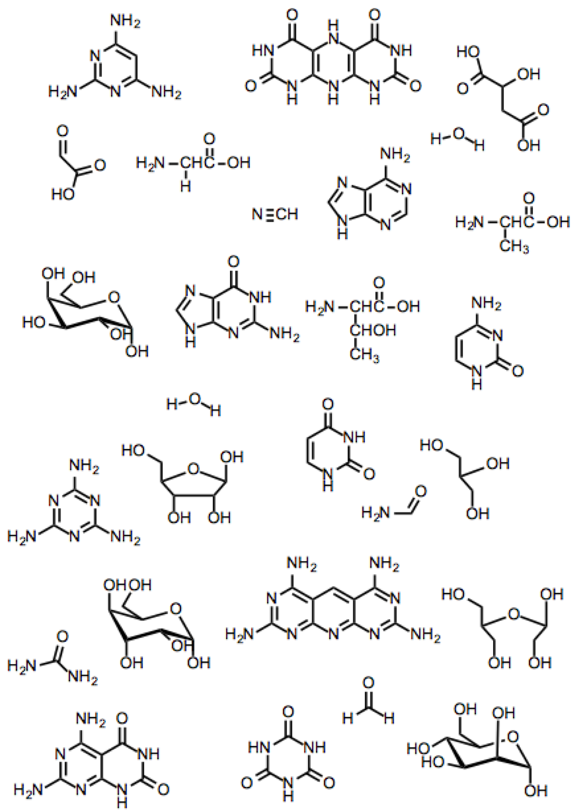
Solvation free energy
ca. 27 kcal/mol

An enormous free energy penalty for exposure of any hexads to water is predicted to drive assembly to very long polymers.

Cafferty, et al., *J. Am. Chem. Soc*. 135, 2447 (2013).

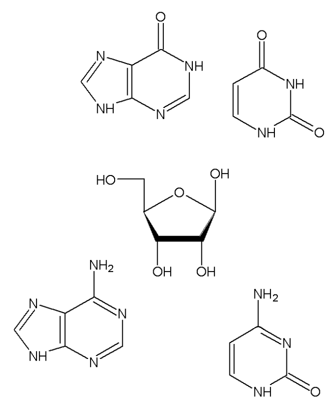
The prebiotic origin of nucleic acids

“The Chemists’ Nightmare”

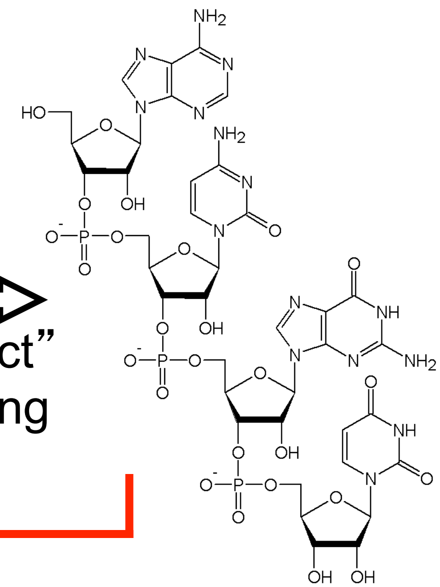


Prebiotic Chemical Inventory

molecule selection



“correct” coupling

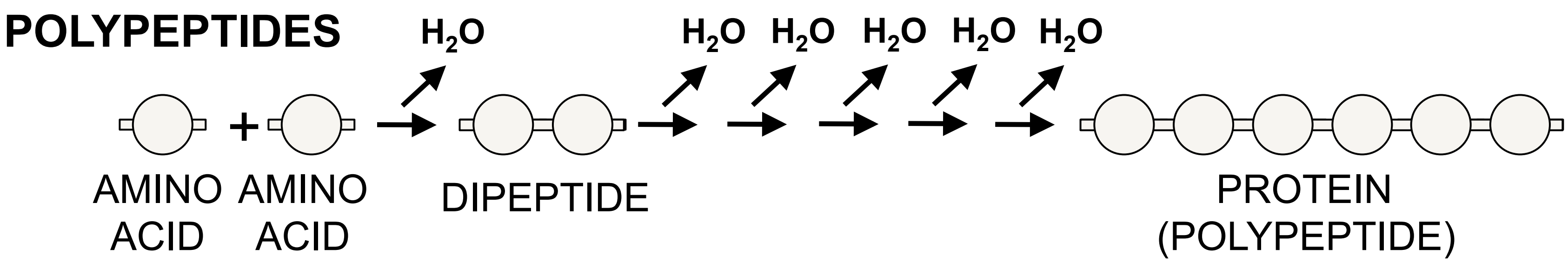


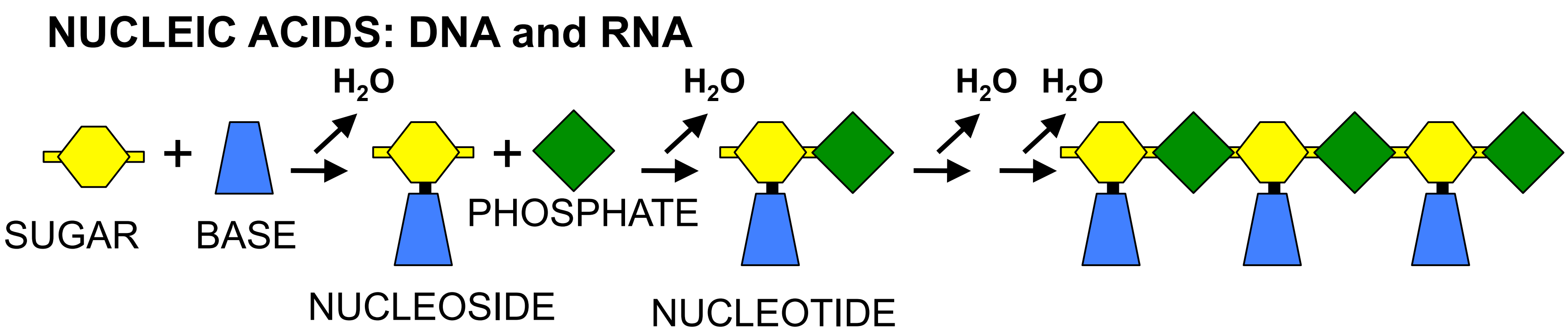
RNA, DNA

Unresolved

Joyce & Orgel, in *The RNA World*, 2000.

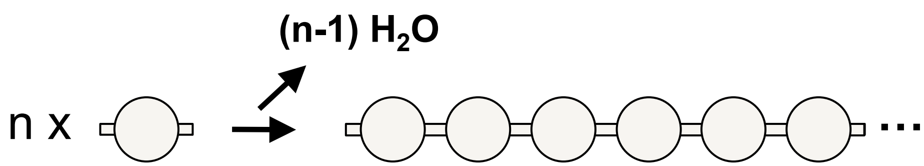
Biopolymer structures provide clues to their origins





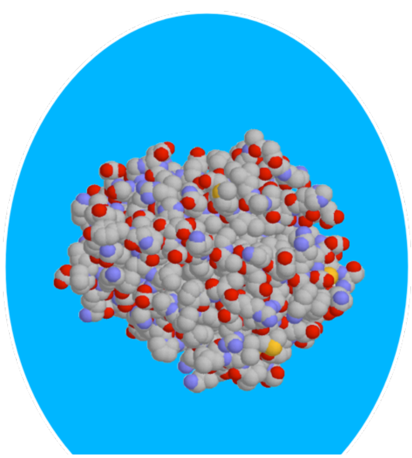
Each bond is different, but all are formed by "condensation-dehydration" reactions.

The best place to promote condensation-dehydration reactions is in a hot-dry environment.



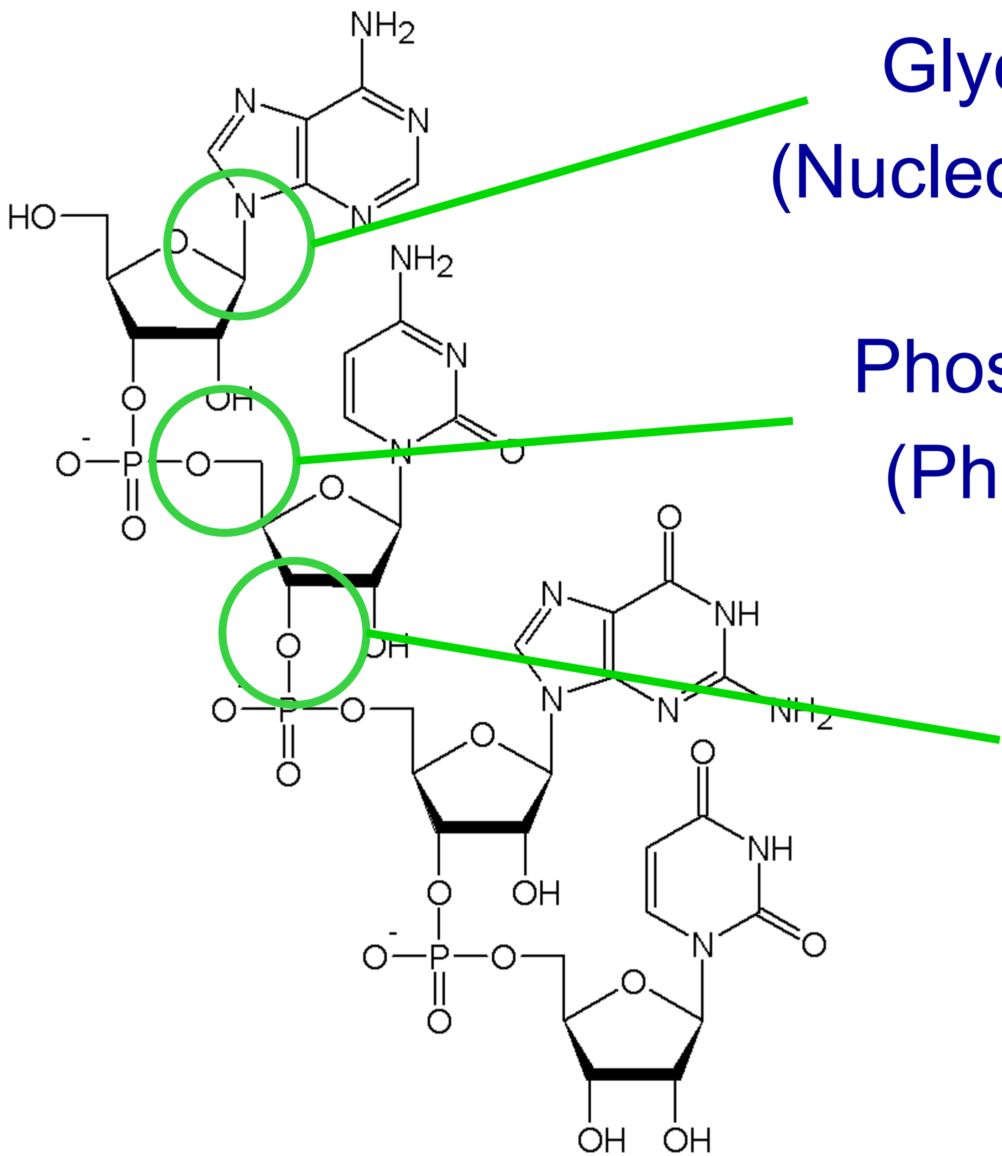
Biopolymer synthesis

…but life is believed to have started in water because the polymers of life functions in water.



Folded, active biopolymer

Covalent bonds that must be made to from nucleic acids from pre-formed nucleobases, ribose and phosphate.



Glycosidic bond
(Nucleoside formation)

Phosphoester bond
(Phosphorylation)

Phosphodiester bond
(Polymerization)

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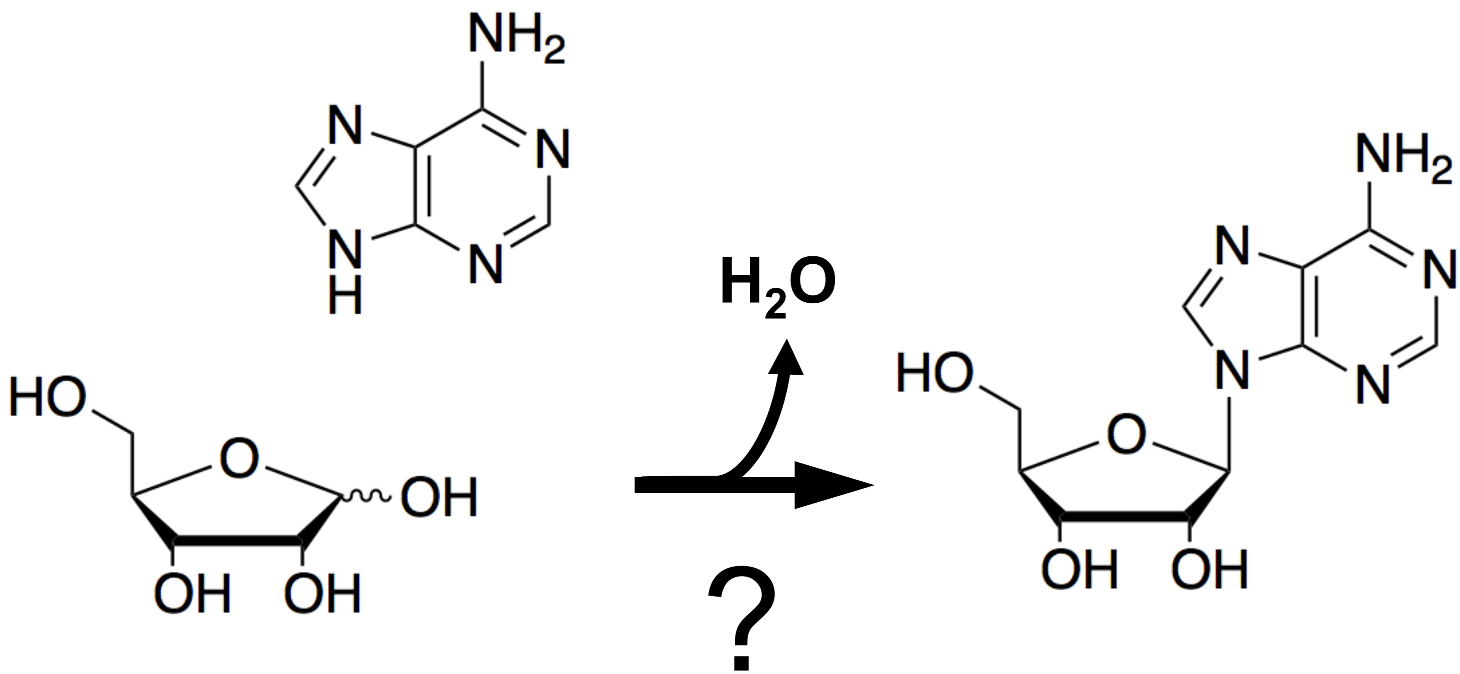
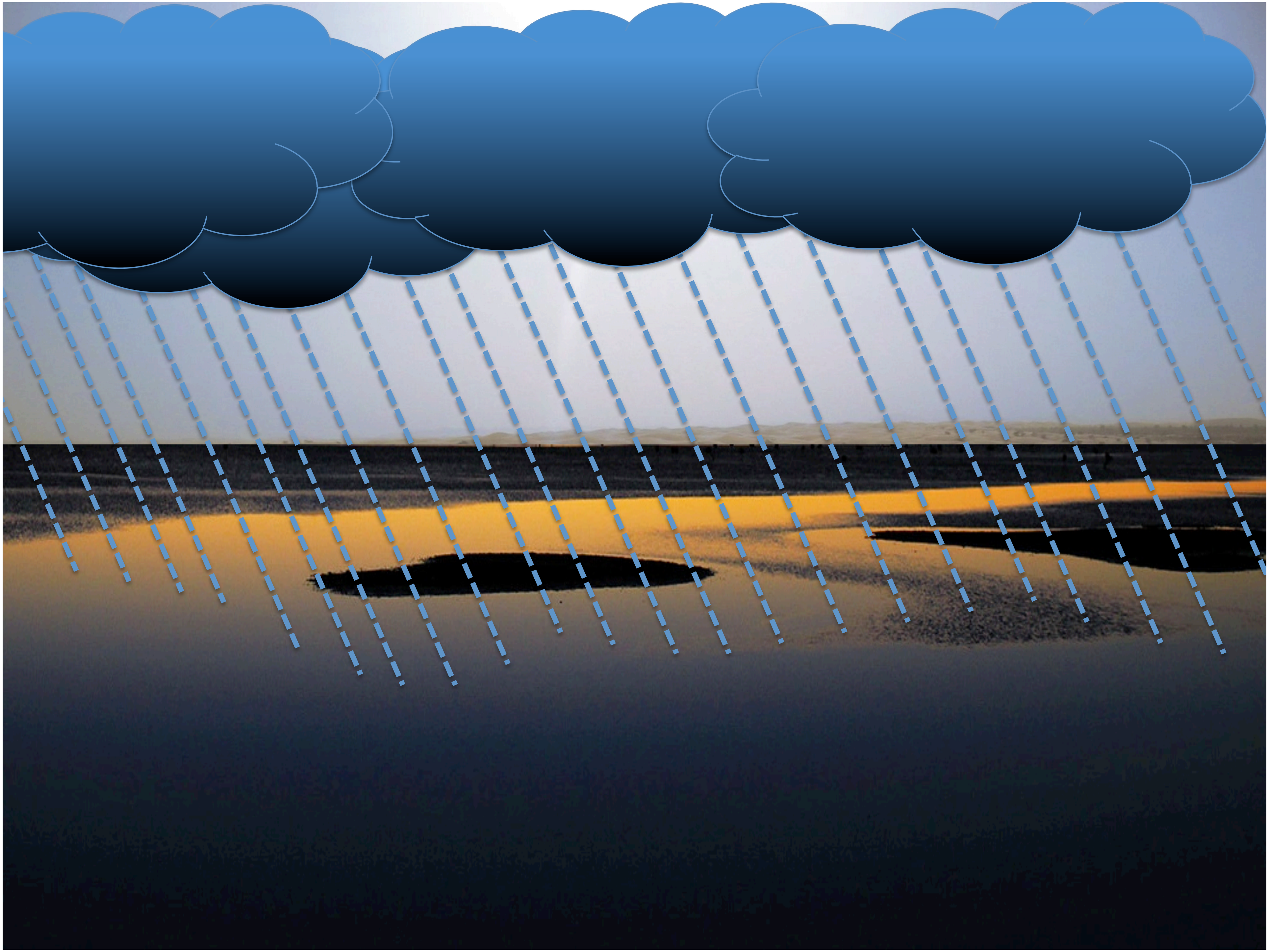
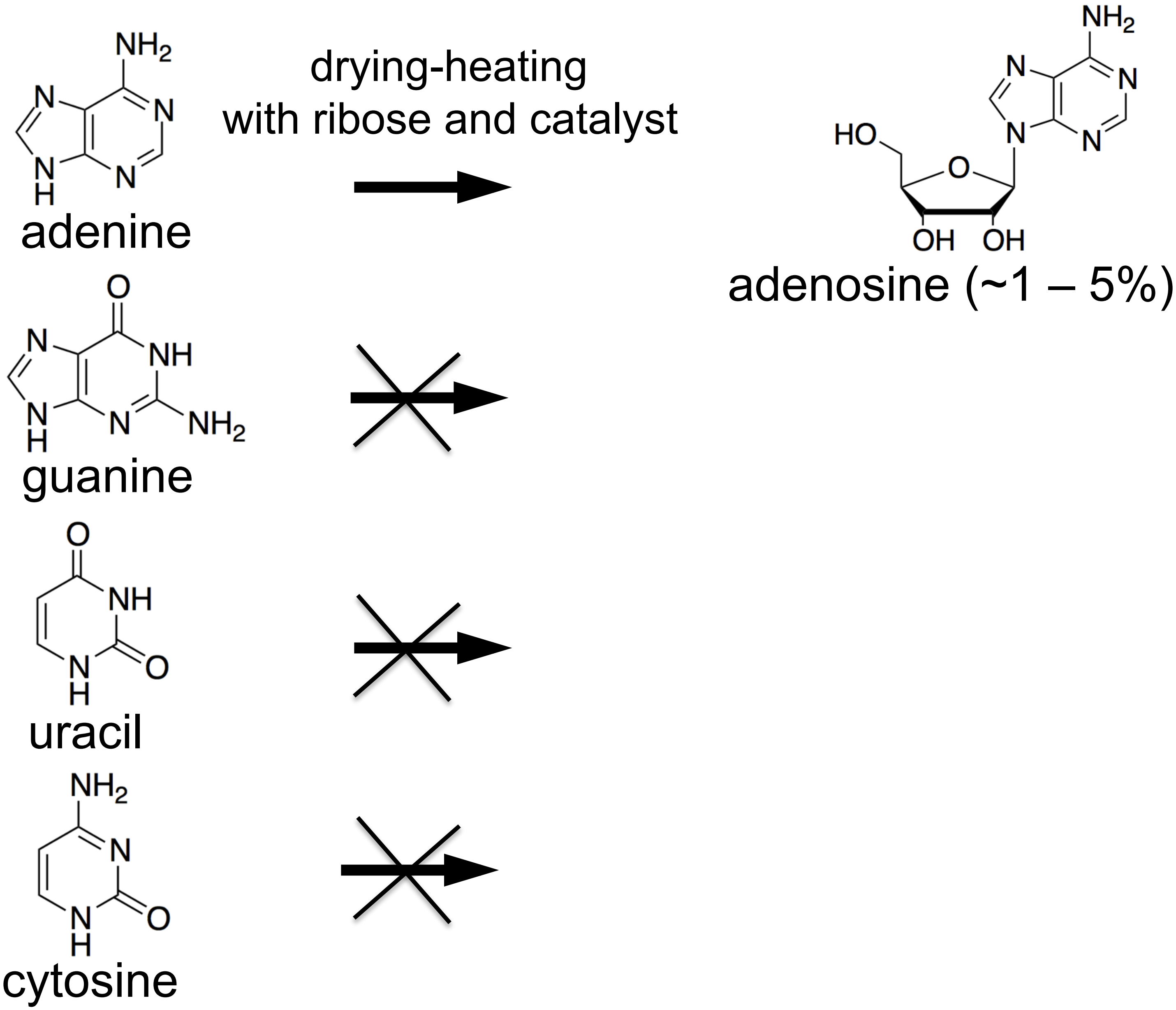
Is there a simple, prebiotic reaction for nucleoside formation from pre-existing nucleobases and ribose?



Investigated extensively by Orgel and coworkers in the 1970s.

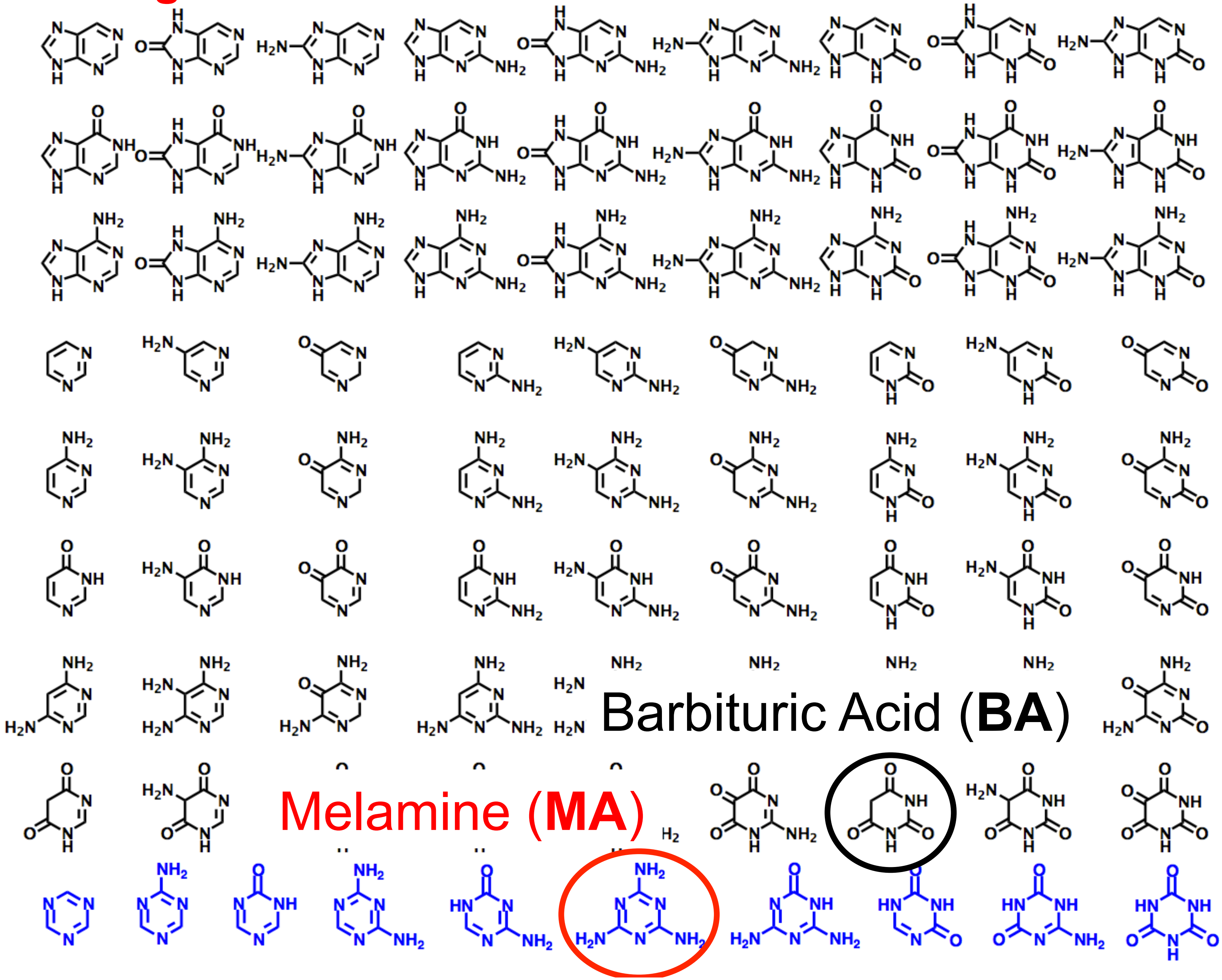


Prebiotic *canonical* nucleoside formation as of 2009.

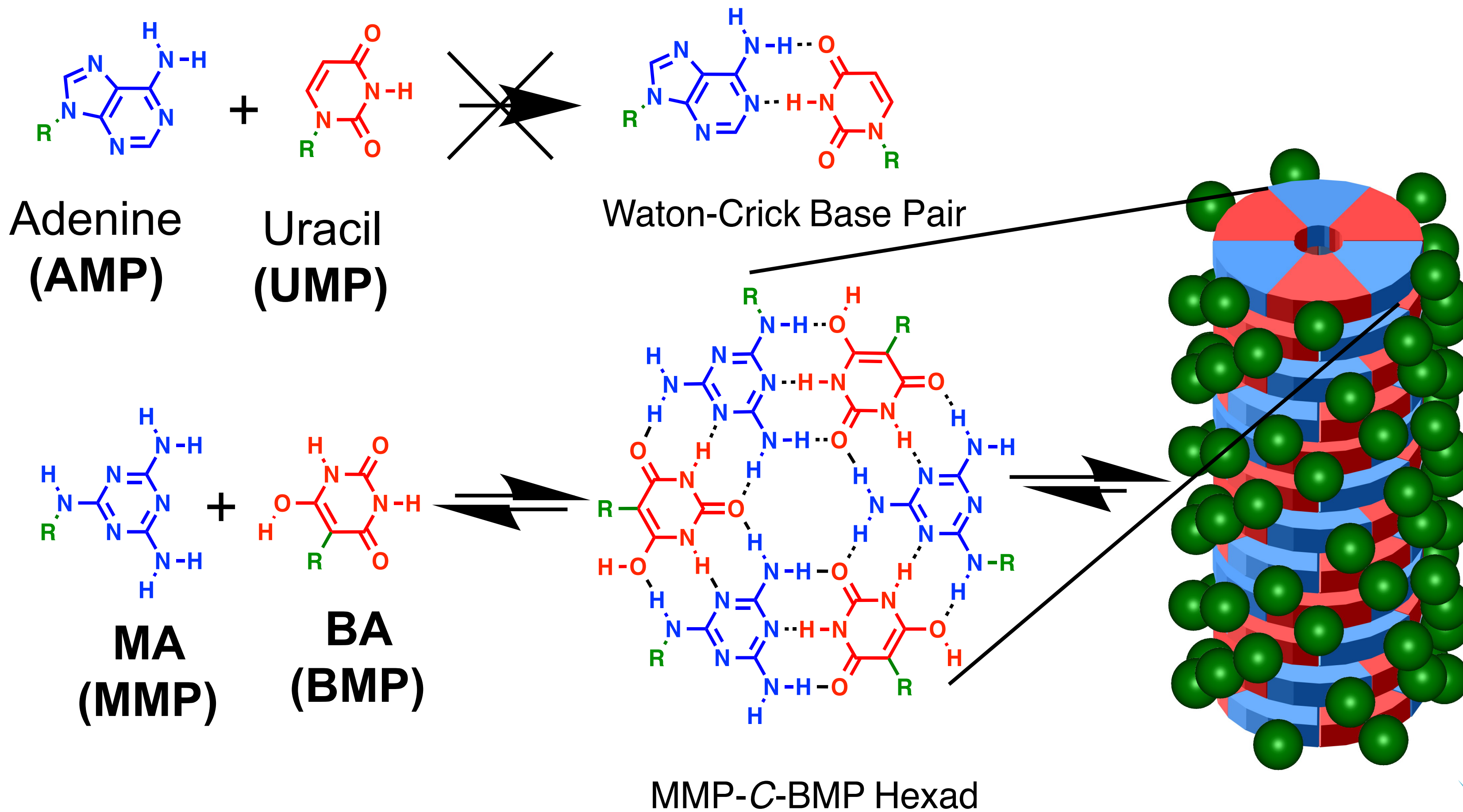


Fuller, W. D.; Sanchez, R. A.; Orgel, L. E. *J. Mol. Evol.* 1, 249 (1972).

Looking for other nucleobases that form nucleosides.

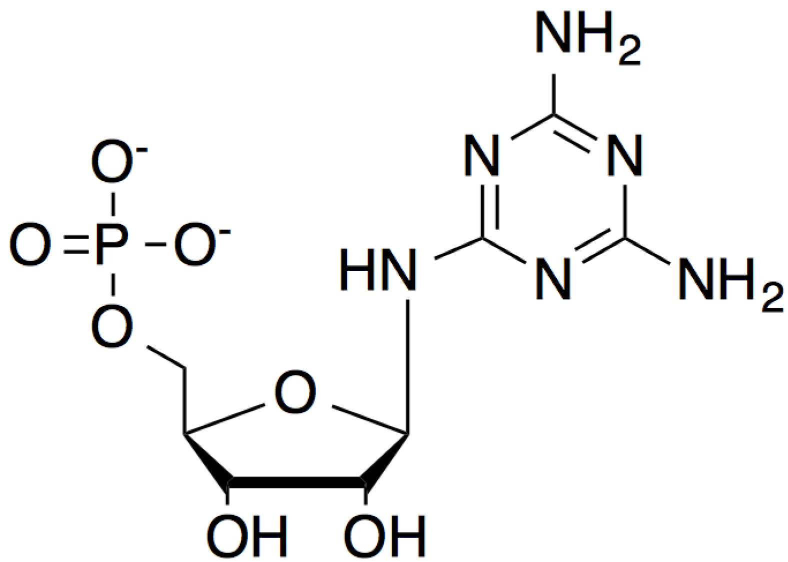


Can Melamine and Barbituric Acid solve both the selection problem and the nucleoside problem?



Cafferty et al., submitted.

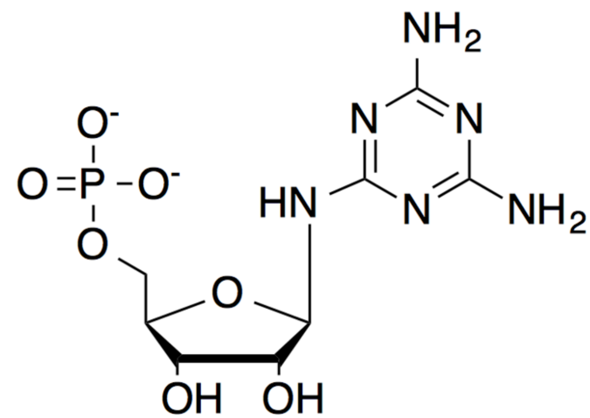
Does Melamine (**MA**) make nucleosides when dried and heated with ribose or ribose-5-phosphate?

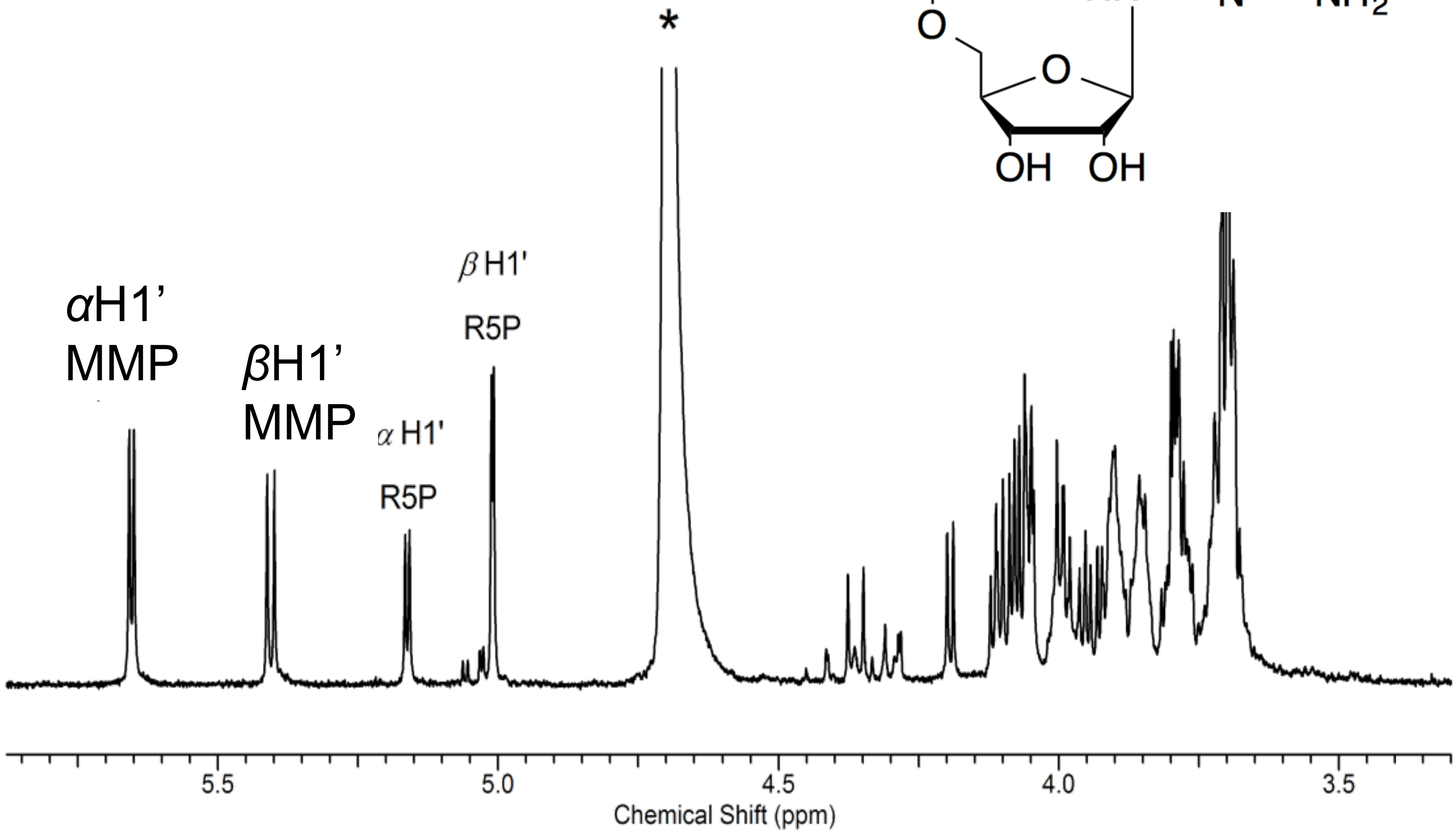


“MMP” (melamine monophosphate)

Yes!

…in 55% yield!

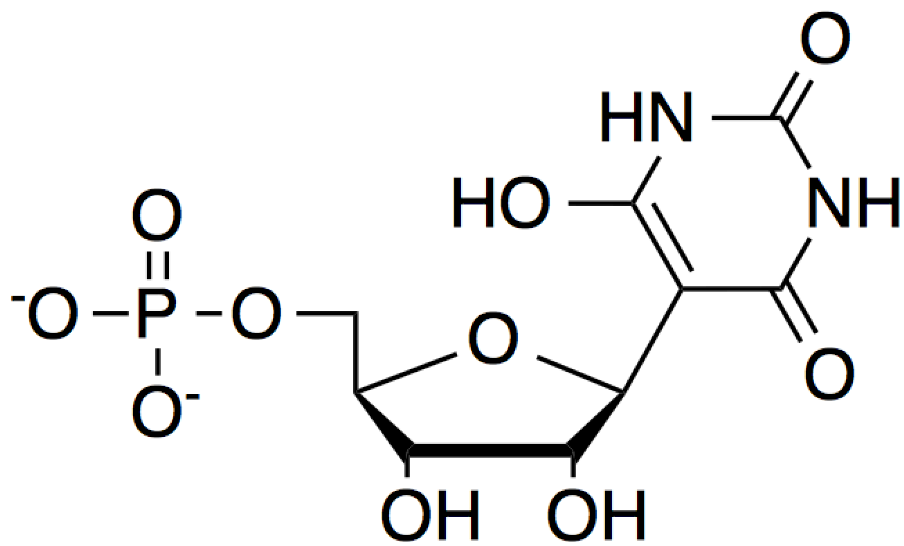




[1]H NMR of melamine+ribose-5-phosphate reaction at 65 °C for X hours (see Materials and Methods for synthesis) acquired at 15 °C. Yields of total nucleotide production were found to be 55% (*α*-MMP 52%, *β*-MMP 48%).

Cafferty et al., submitted.

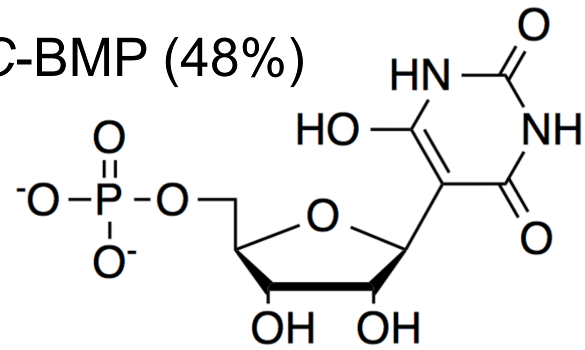
Does Barbituric Acid (**bA**) make nucleosides when dried and heated with ribose or ribose-5-phosphate?



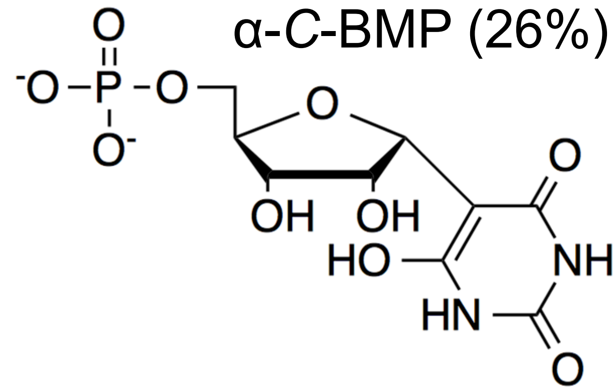
“*C*-BMP”
(C-nucleoside barbituric acid monophosphate)

Yes!

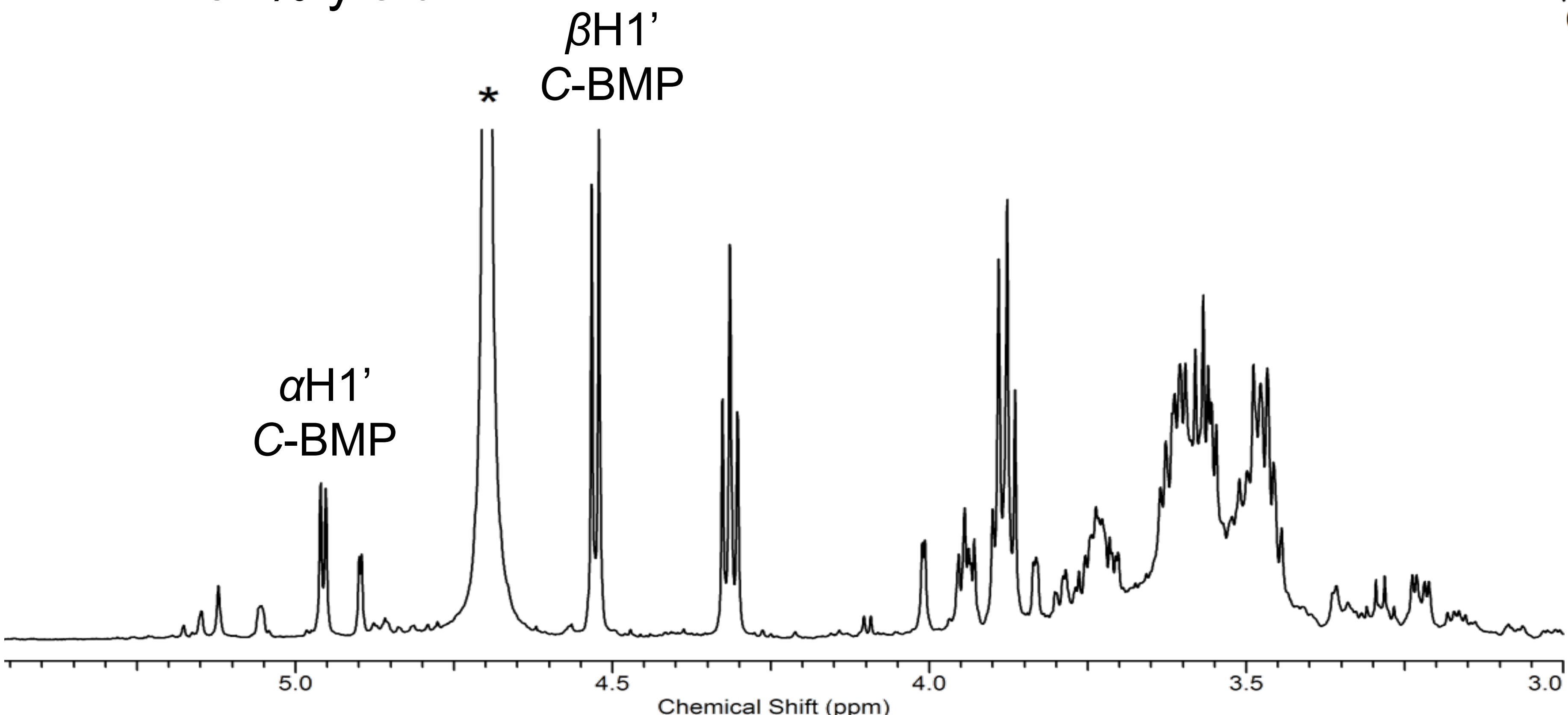
β-*C*-BMP (48%)



α-*C*-BMP (26%)



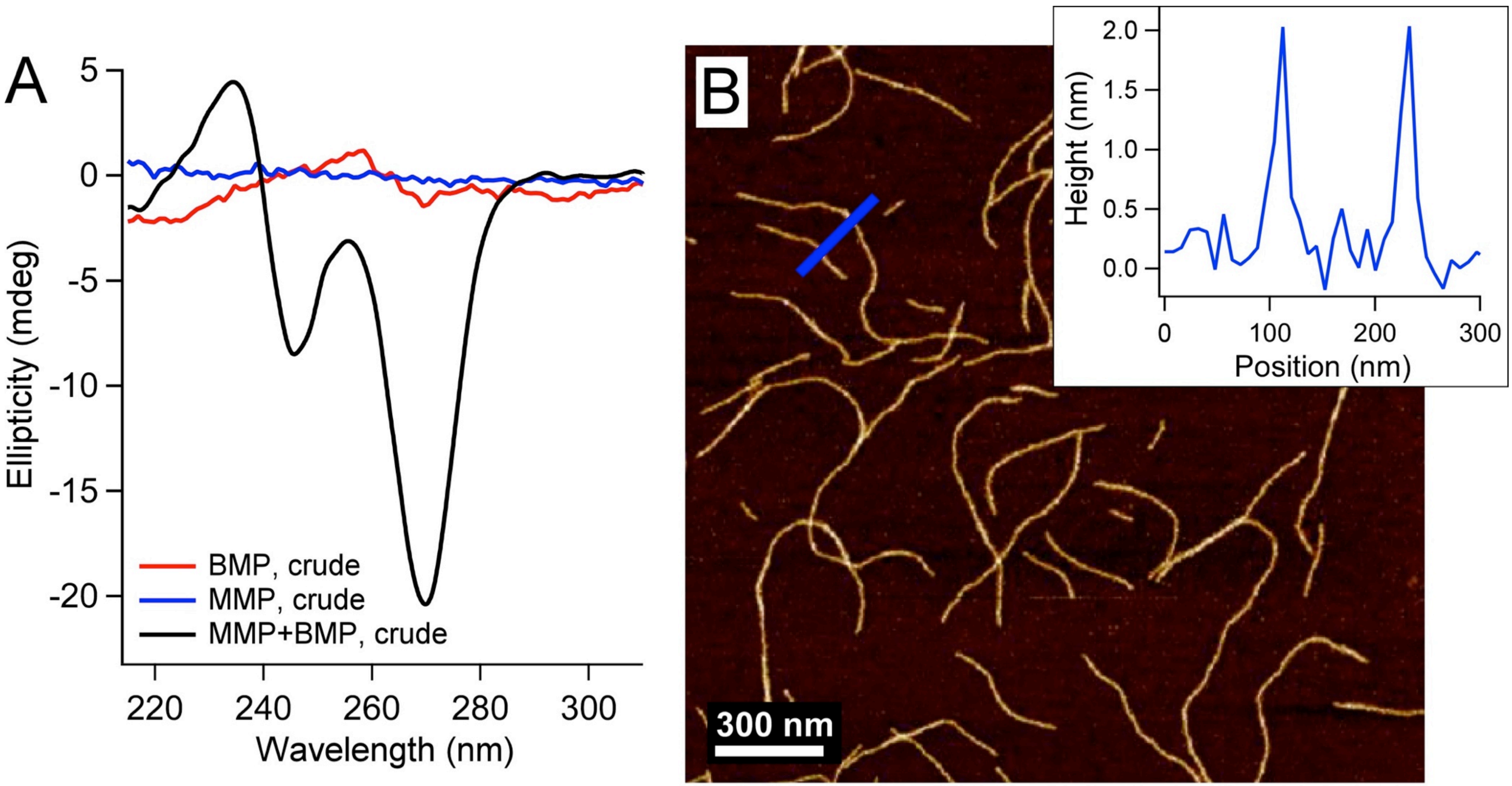
…in 82% yield!



^{1}H NMR of barbiturate+ribose-5-phosphate reaction at 20°C. Yields of total nucleotide production were found to be 82% (α-BMP 26%, β-BMP 48%).

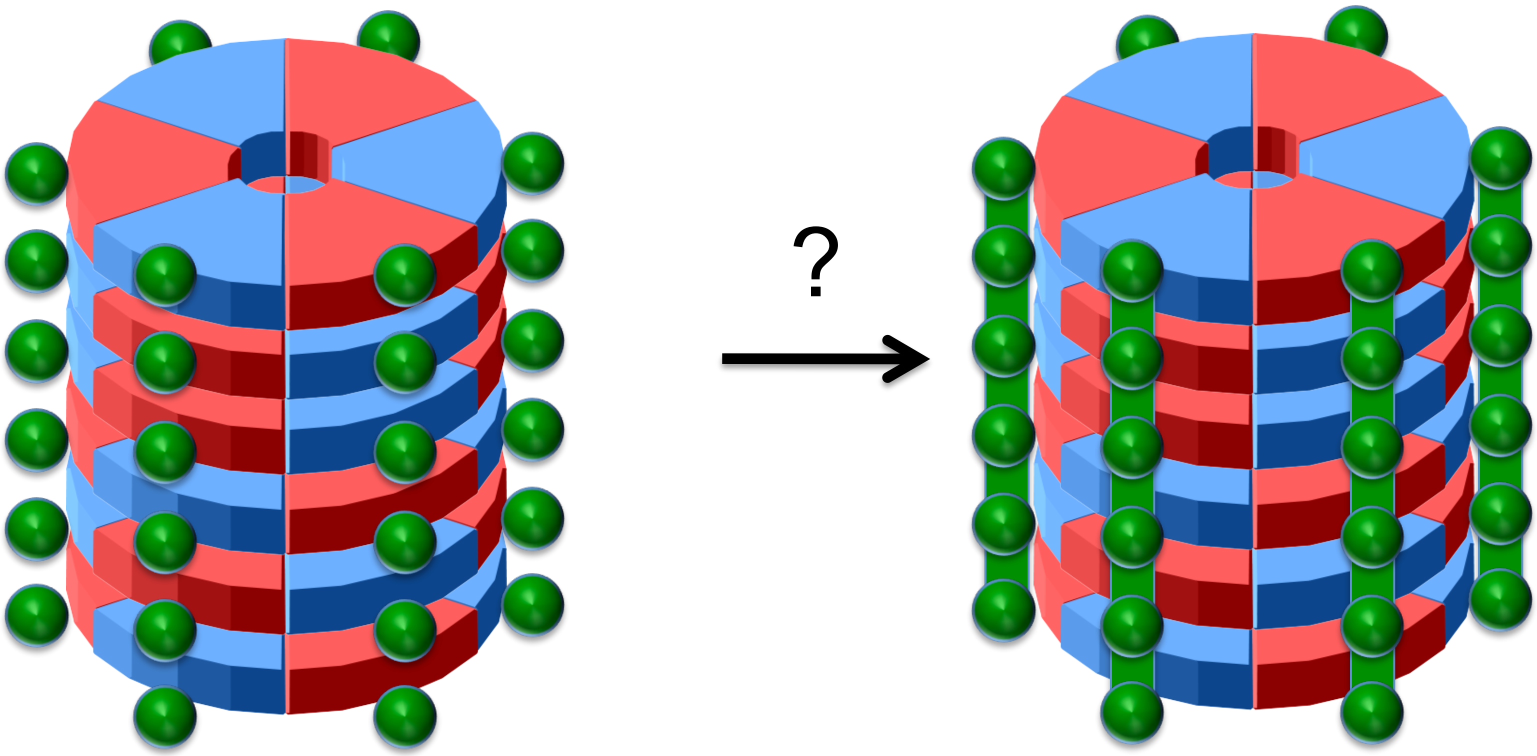
Cafferty et al., submitted.

Crude product mixtures of **MA** and **BA** reactions with ribose assemble into "gene-length" non-covalent polymers.



Cafferty et al., submitted.

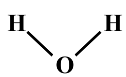
One bond away from a proto-RNA?



Covalently linking assembled nucleotides in a plausible prebiotic reaction: *Out current goal.*

Biopolymer synthesis in the absence of water might be easier, but can biopolymers fold/function without water?

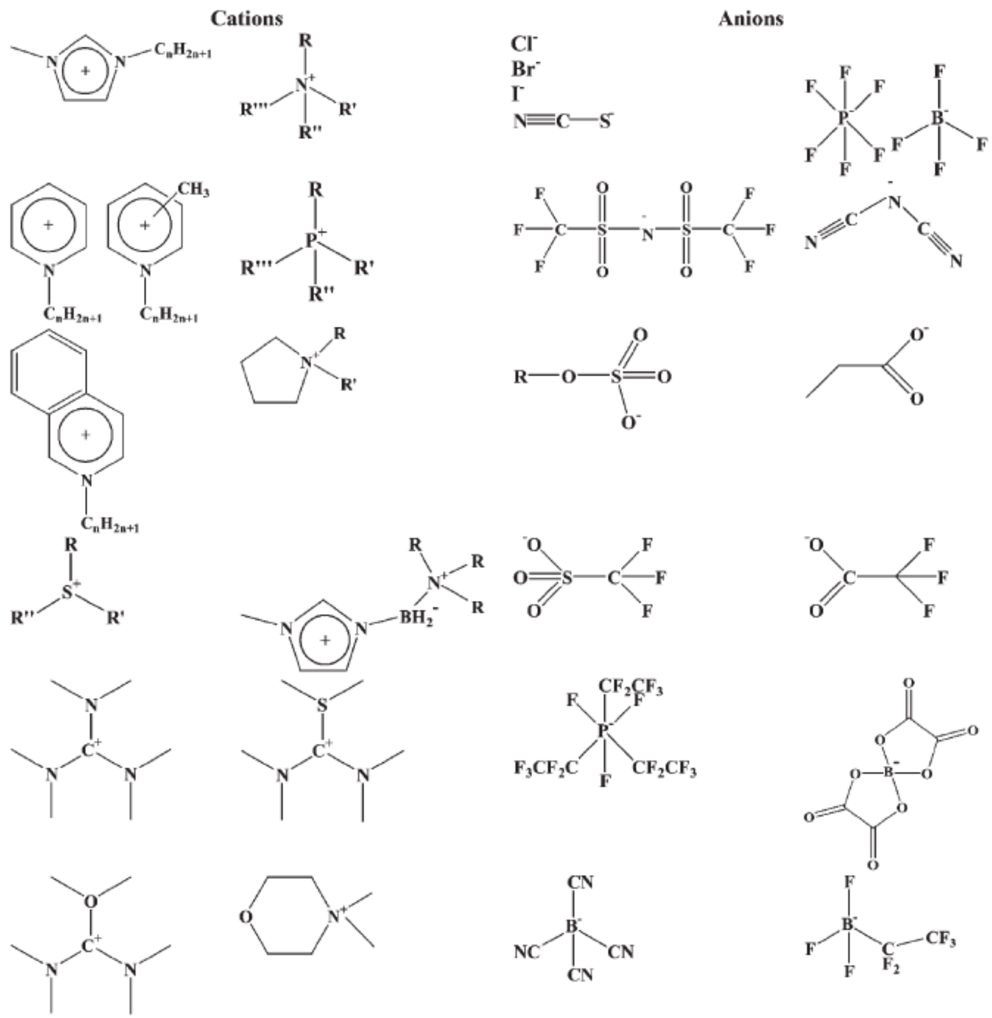
Water



Ionic Liquids

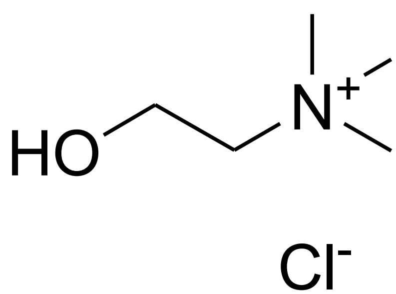
Cations

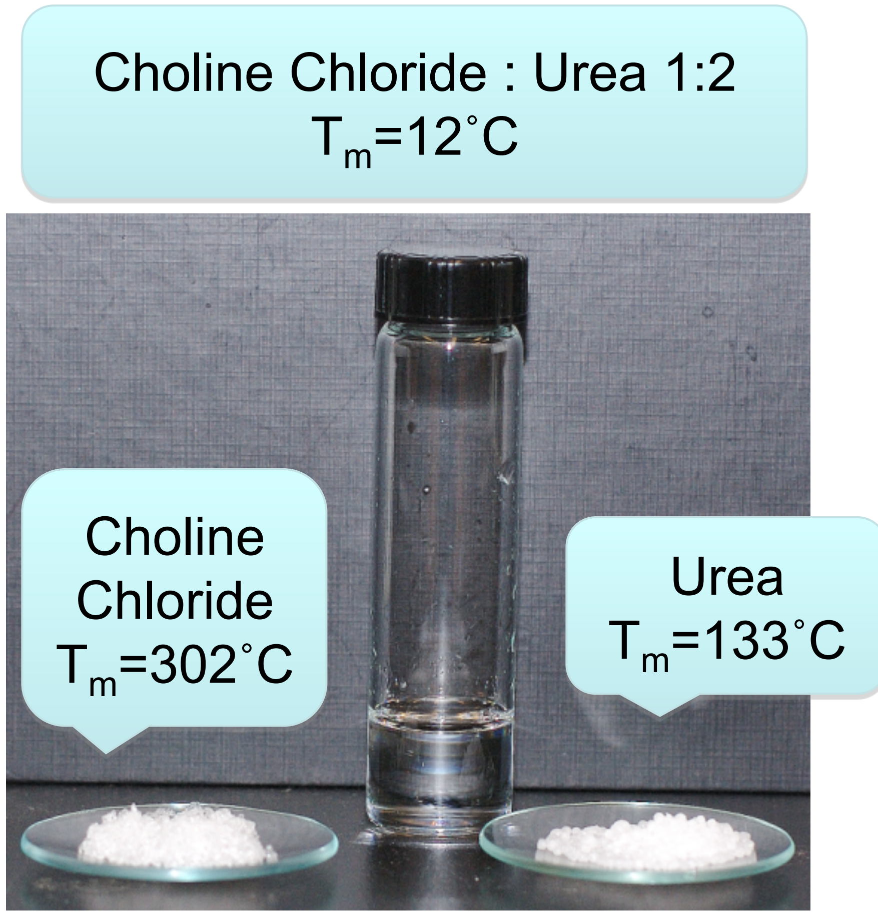
Anions

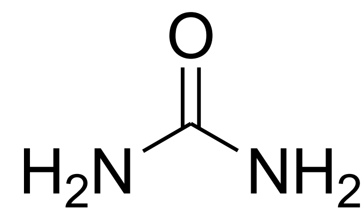


Reichert, *et al.*, *Chem. Commun.* 2006, 4767-4779

A Deep Eutectic Solvent (DES) is formed by a choline chloride/urea mixture, with solvent properties similar to Ionic Liquids, *i.e.* terrific solvents with “no” vapor pressure.







Abbott, et al., *Chem. Comm.* 2003, 70-71.

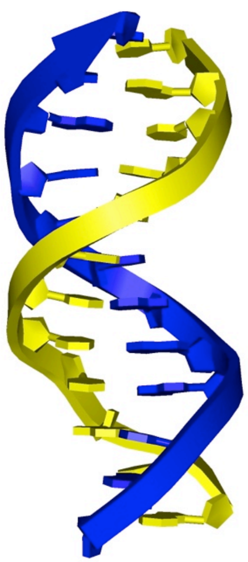
DNA and RNA duplexes can exist without water.

T_M (°C) of DNA and RNA duplexes in various solvents.

Nucleic Acid	100 m*m* NaCl	DES	3.7 *m* NaCl	3.7 *m* ChCl	NaCl* Urea
32-bp DNA	75	39	85	83	65
12-bp RNA	≥95	44	>95	69	83
$d(CG)_8$	≥85	44	nd	72	76
$d(AT)_{16}$	57	29	68	77	38
$d(A_4T_4)_4$	57	26	73	79	52

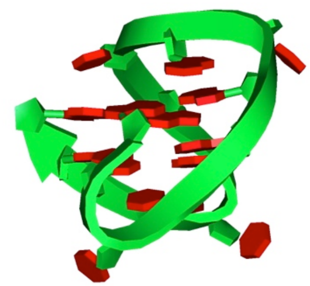
Mamajanov et al., *Angew. Chem. Int. Ed. Eng.* 49, 6310–6314 (2010).

All three nucleic acid secondary structures were found to be stable in the water-free choline Cl-urea DES.



Duplex
Aqueous T_m = 75°C
DES T_m = 39°C

Triplex
Aqueous T_m = 28°C
DES T_m = 66°C



G-quadruplex
Aqueous T_m = 50°C
DES T_m = 59 °C

Ability to form secondary structures in the absence of water opens the possibility of thermodynamically-driven polymerization.

Structure–dependent differential water vs. DES stability was unexpected.

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Is Water Necessary for the Origin and Evolution of an RNA World?

The RNA World is a model. It is possible that polypeptides, polysaccharides, and lipid assemblies also played a major role in the origin of life, and emerged along with RNA (or a pre-RNA).

Q: Is Water Necessary for the Origin and Evolution of Life as We Know It?

A: Some reactions/processes would be easier without water, but some would be more difficult without water.
In any case, life would likely be very different without water.

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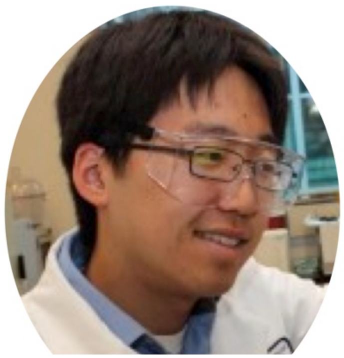

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