Prebiotic Systems Chemistry

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The Origin of Life—Out of the Blue

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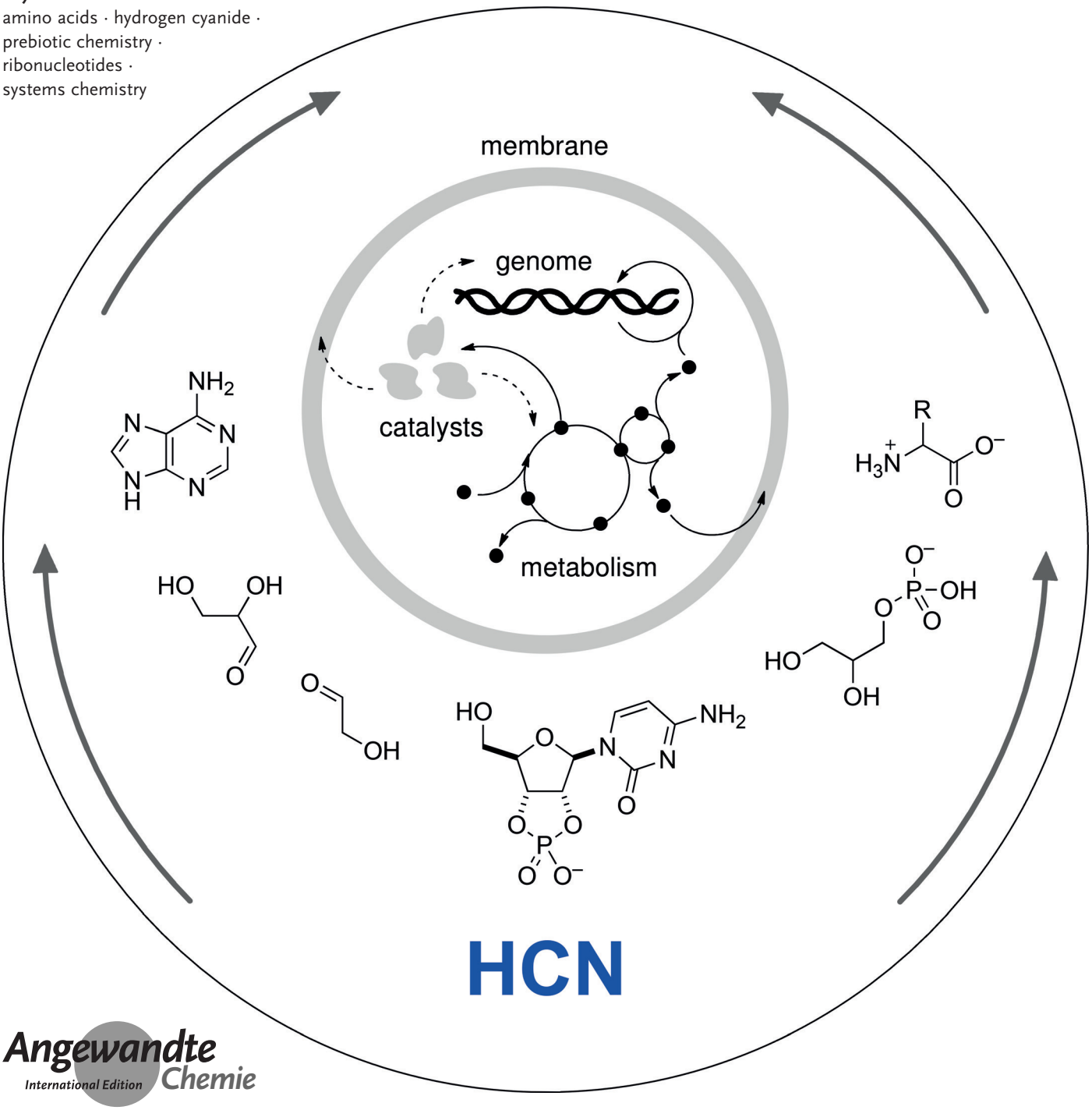

Either to sustain autotrophy, or as a prelude to heterotrophy, organic synthesis from an environmentally available C_1 feedstock molecule is crucial to the origin of life. Recent findings augment key literature results and suggest that hydrogen cyanide—"Blausäure"—was that feedstock.

"*The answer has to come from revisiting the chemistry of HCN*"
Albert Eschenmoser.[1]

From the Contents

1. Introduction: How to Study the Origin of Life?

In principle, the origin of life can be studied from geochemistry up, or from biology down, but in practice, there are problems with both approaches.

Starting from geochemistry, planetary science suggests that the early Earth could have offered a wide range of environments and conditions. A huge amount of chemistry is potentially possible in a submarine vent, or a drying lagoon, or an impact crater, or a reduced atmosphere subject to lightning, or whatever other scenario one can imagine. However, there is insufficient constraint from geochemistry per se to settle on one particular scenario, and thence to systematically explore its chemistry with a view to uncovering intrinsically favoured syntheses of biomolecules, or their precursors, from simple feedstock molecules.

Starting from extant biology, phylogeny can only go so far down, and biology before the speciation thresholds that gave rise to the three kingdoms of life cannot be so usefully plumbed in this way.[2] Conceptual and experimental reduction of cells to the simplest (imaginable), minimal cell still leaves a dauntingly complex system comprising seamlessly integrated informational, metabolic, catalytic and compartment-forming subsystems (Figure 1). But, imagining the abiotic assembly of such an overall system places huge demands on hypothetical prebiotic chemistry—surely completely different chemistries are needed to make the various subsystems and surely these different chemistries would interfere with each other. Therefore, it is not surprising that in the past, most in the field assumed that one or other subsystem came first and then "invented" the others, with the primal subsystem being designated according to personal prejudice ("Darwinian evolution needs informational molecules, so RNA must have come first."[3] "You can't get by without building blocks and energy, so metabolism must have come first."[4] "Genetics and metabolism without catalysis is hard to imagine, so proteins must have come first."[5] "The development of Darwinian selection is hard to imagine without compartments, so membranes must have been there at the outset."[6]).

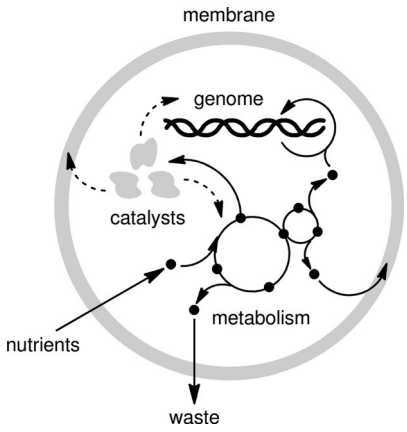

Figure 1. A minimal cell with emphasis on its subsystems.

Several years ago, we realised that this (quadru)polarisation of the field was severely hindering progress, and we planned a more holistic approach. We set out to use experimental chemistry to address two questions, the previously *assumed* answers to which had led to the polarisation of the field: "Are completely different chemistries needed to make the various subsystems?" "Would these chemistries be compatible with each other?"[7] Our approach was to take the following steps:

i) experimentally evaluate the prebiotic chemistry of the various subsystems;

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ii) use any commonality of intermediates and/or (by-)products to link the subsystems;
iii) use the results to infer rough geochemical scenarios;
iv) assess other chemical consequences of the inferred scenario.

We envisaged that using chemical results to constrain geochemical scenarios, and then letting the inferred scenario back-inform the chemistry would be an iterative process, continued application of which would lead to refinements in both the scenario and the chemistry.

2. The Informational Subsystem

We initially set out to explore the assembly chemistry of an informational subsystem based on RNA because there is so much to indicate its antiquity in biology. The traditional retrosynthetic disconnection of RNA proceeds thus: RNA ⇒ (activated) nucleotides ⇒ nucleosides plus phosphate; nucleosides ⇒ ribose plus nucleobases; ribose ⇒ formaldehyde, and nucleobases ⇒ hydrogen cyanide and other nitrogenous precursors.[8] Although this disconnection has not led to a corresponding synthesis under prebiotically plausible conditions, despite many groups' efforts over many decades, its pursuit has uncovered much fascinating chemistry. Some of this chemistry and a couple of the problems encountered are reviewed briefly here because they provide a backdrop for what follows.

We start out by touching on nucleobase synthesis. Oró's demonstration that simply mixing hydrogen cyanide **1** and ammonia in solution gives rise to adenine **2**[9] has been described as the "rock of faith" of prebiotic chemistry.[10] As modified by Ferris and Orgel,[11] this synthesis produces adenine **2** in reasonable yield from five molecules of hydrogen cyanide **1** (Figure 2).

To many in the field, the fact that pentamerisation of something as fundamental as hydrogen cyanide **1** can give rise to a heterocycle that is so pervasive in biology is surely no coincidence: "*The example of adenine refutes the opinion that an inquiry into the origin of cofactor structures is futile since it would be a priori impossible to draw conclusions or to study the problem experimentally. Were cofactors of prebiotic (or of otherwise nonenzymic) origin, then structural complexity of such molecules would be an apparent complexity; straightforward pathways of structural self-assembly of these structures would have to exist, and these pathways would be detectable experimentally.*"[12] In other words, if, through experiment, one were to discover efficient synthetic pathways to other natural products along inherently favoured routes, it would be reasonable to conclude that said products originated this way, and any complexity would only be in the eye of the beholder. Because of his extraordinary success (along with Woodward) in synthesising vitamin B_{12} by conventional means, it was natural for Eschenmoser to first write about potentially favoured reactions in the prebiotic synthesis of cofactors,[12] but the concept would obviously apply to other molecules crucial to biology at the dawn of life. Indeed, the author as a PhD student in Oxford in the mid-1980s, on hearing Eschenmoser talk about this concept in a lecture, determined to investigate it in relation to RNA. Inherently favoured reactions might include those that are catalysed by another component of the system, those which are part of an autocatalytic cycle, those which are catalysed through induced intramolecularity,[13] or those in which a functional group in a molecule displays out of the ordinary reactivity due to its particular molecular context. Sequential occurence of several such reactions might lead to dramatic syntheses of a few products (destined to play a role in the advent of biology) from mixtures that prophets of gloom would have us believe are prone only to become tar.[14]

We continue by reflecting on problems associated with sugar synthesis. From the outset, prebiotic chemists relied on Butlerow's synthesis of formose[15] as a source of sugars. In

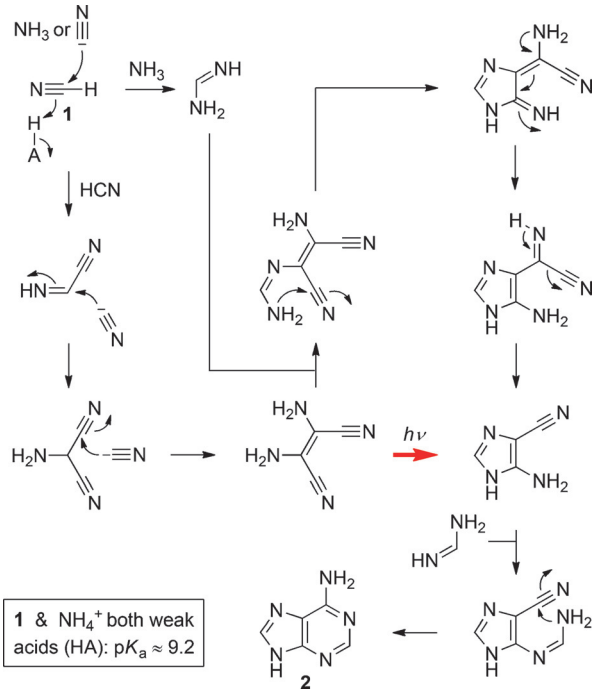

Figure 2. Oró's synthesis of adenine **2** from hydrogen cyanide **1** and ammonia (general acid–base catalysis, presumed to operate in most steps, is only shown once). The photochemical shortcut discovered by Ferris and Orgel is shown by the red arrow.

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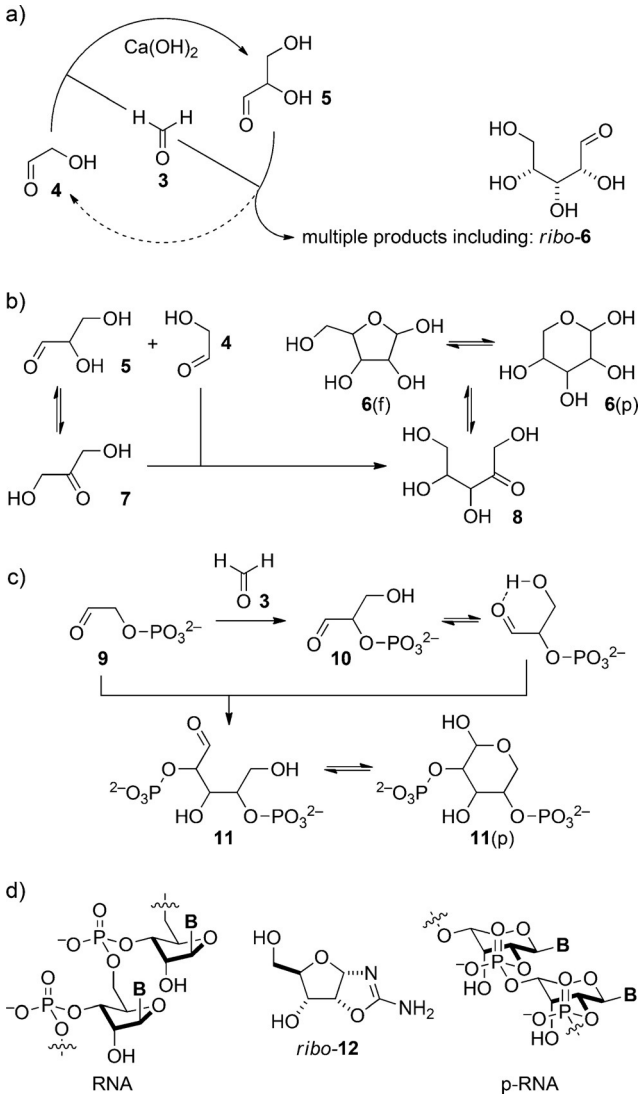

Figure 3. Sugars and the informational subsystem. a) Ribose as an unfavoured product of the formose reaction proper, b) inherently favoured aldolisation of C_2- and C_3-sugars, c) aldolisation favoured through restriction of other chemistries by α-hydroxyaldehyde phosphorylation d) (stabilised) ribose derivatives. **B** = canonical nucleobase.

this reaction (Figure 3a), formaldehyde **3**—containing traces of glycolaldehyde **4**—is heated in calcium hydroxide solution.

Aldol hydroxymethylation of **4** by **3** gives glyceraldehyde **5** and then myriad aldol, Cannizzaro, and Lobry de Bruyn–Alberda van Ekenstein reactions ensue giving a whole slew of products. The resultant mixture is sweet-tasting, but not rich in ribose *ribo*-**6** (< 1% of total sugars), the route to which is not favoured relative to other products.[16] However, part of the formose reaction network is (thought to be) an autocatalytic cycle,[17] which amplifies the input glycolaldehyde **4**, and this would be attractive if flux through the cycle could somehow be diverted towards *ribo*-**6**. Accordingly there have been several attempts at streamlining the formose reaction by changing the catalyst[18] or by adding borate[19]—the choice of this additive prompted by the hope that it might chaperone intermediates through complexation in such a way as to favour the production of ribose *ribo*-**6**. The reaction proper starting with formaldehyde **3** (and traces of glycolaldehyde **4**) has resisted such attempts, however,[19] although aldopentoses **6** or their derivatives can be preferentially formed by formose reaction variants starting with preformed C_2- (and C_3-) sugars.[20,21] These preferred routes to pentoses can either be inherently favoured, or made favoured by substrate modification (Figure 3b,c).

The former is the case in the calcium hydroxide catalysed reaction of glycolaldehyde **4** and glyceraldehyde **5** in dilute aqueous solution, which gives excellent yields of aldopentoses, including ribose (Figure 3b).[20] When enolates form in this system, they tend to reprotonate faster than adding to aldehyde groups because the system is dilute and both starting materials are extensively hydrated. Reversible enolisation of glycolaldehyde **4** is futile, but enolisation of glyceraldehyde **5** followed by ketonisation leads to dihydroxyacetone **7**. The reduced hydration of **7**, relative to **4** and **5**, means that it undergoes enolisation faster, and crossed aldolisation is then preferred because glycolaldehyde **4** is a better aldol electrophile than **7** (despite the former's hydration). The resultant ketopentose crossed aldol products **8** then isomerise with the corresponding aldopentoses **6**, and the latter are favoured at equilibrium because of the increased stability of their cyclic furanose **6**(f) and pyranose **6**(p) hemiacetal forms.[22]

Modifying glycolaldehyde **4** by phosphorylation allows favoured aldolisation to pentose phosphate derivatives in the presence of formaldehyde by shutting down Lobry de Bruyn–Alberda van Ekenstein reactions of α-hydroxyaldehydes, because enolates formed therefrom are prevented from undergoing protonation to give the corresponding α-hydroxyketones by their phosphate shackles (Figure 3c).[21] Glycolaldehyde phosphate **9** could undergo aldol reaction with itself or with formaldehyde **3**, and reaction with the latter is preferred because **3** is such a good aldol electrophile. The resultant glyceraldehyde-2-phosphate **10** is a worse aldol nucleophile than glycolaldehyde phosphate **9** (for steric reasons) but a better electrophile (potentially due to an intramolecular H-bond between the hydroxy and carbonyl groups, Figure 3c) and thus crossed aldolisation to give pentose-2,4-diphosphates **11** ensues. Adoption of the cyclic hemiacetal form **11**(p) then effectively stops further aldolisation chemistry.

Although these favoured routes to aldopentoses **6** and their 2,4-diphosphates **11** are beautiful from a purely synthetic point of view, they raised questions in our minds when we first saw them as plausible routes to the ribose in prebiotic RNA. Both schemes suffered from their requirement for C_2- (and C_3-) sugar (derivative) starting materials. Although it was known that formaldehyde **3** can be formed through UV irradiation of atmospheres containing carbon dioxide/carbon monoxide and water vapour,[23] glycolaldehyde **4** and glyceraldehyde **5** had not been formed in anything other than traces by similar chemistry.[24,25] Product stability was also an issue. The ribose *ribo*-**6** formed in the inherently favoured reaction of glycolaldehyde **4** and glyceraldehyde **5** is unstable,[26] especially under prolonged conditions of its formation. On the other hand, the phosphate ester bonds of ribose-2,4-

diphosphate *ribo*-**11**, appeared, to us at least, too stable to allow easy isomerisation to the 3,5-phosphorylation pattern needed to make RNA. In the case of free *ribo*-**6**, stabilisation by borate complexation was a possibility—as first suggested by Prieur[26]—although this complexation would presumably have to be undone in order to progress to nucleosides. More intriguing was the possible stabilisation of *ribo*-**6** as the cyanamide adduct *ribo*-**12** (Figure 3d)[27–29] as this might not need to revert to the free sugar to progress to nucleosides, more of which later. In the case of ribose-2,4-diphosphate *ribo*-**11**, Eschenmoser reasoned that the generation of a pyranosyl isomer of RNA was potentially favoured.[30] His demonstration—mainly through assessing duplex stability—that such an isomer (p-RNA, Figure 3d) is a functioning informational polymer[31,32] led to his discovery of a dizzying array of other such polymeric systems with varying degrees of (potential) informational function, and some non-functional ones.[33] This provided potential support to earlier suggestions that inherently favoured chemistry first presented nature with an informational polymer—let us call it XNA—functionally inferior to RNA, and then biology based on XNA sampled other informational molecules through catalysed syntheses, and chose RNA for functional reasons.[34] Another possibility, and the one we preferred, was that there is an inherently favoured route to ribonucleotides and RNA, but it had thus far eluded discovery. According to this second possibility, RNA was not a biotic invention, but a prebiotic product,[35] and it just so happened that it turned out to function well enough as an informational polymer for life to start. But for how long and how hard should one study potentially prebiotic RNA synthesis in search of an efficient route that should be detectable experimentally, and at what point should one give up? We need to digress for a while.

3. A Digression

For many years, we (unsuccessfully) pursued multifarious approaches to ribonucleotides in the belief that we had not yet systematically investigated their synthesis under prebiotic conditions.[35] In particular we sought to follow non-classical retrosynthetic disconnections hoping to find an inherently favoured, straightforward, albeit non-obvious, route. In contrast, by the mid-2000s, Eschenmoser had gravitated towards the view that RNA originated not as a consequence of synthetic contingency, but as a result of synthetic variation and functional selection: "*In fact, our returns from a great many conceptual excursions into the unvarnished chemical and physical details of conceivable scenarios of a prebiotic assembly of oligonucleotide systems, together with some of our findings in cautious experimental explorations of selected problems of a potentially prebiotic oligonucleotide chemistry, have recurringly reinforced the doubts we share with others concerning the postulate of an abiotic origin of RNA.*"[36] Our continuous exploration of potential RNA assembly chemistry was motivated by the fear that the search for a biotic origin of RNA might be even harder. We were wary of being beguiled by the prospect that Darwinian evolution might solve all the problems. Darwinism still resonates in the natural sciences—functional selection from a diverse population is an enormously powerful driver of evolution. This leads to the impression that when something is good in biology, it is the result of natural selection and thus readily achievable, not a bad impression to have most of the time, but what about biology in the beginning? Consider what would be required for the origin of RNA to have been biotic. Firstly, the synthesis of XNA would have to proceed along favoured lines. This is fine, at least conceptually, as is the next requirement, which is that a biology built around XNA would have to emerge, and progress to a level of sophistication that enabled it to catalyse chemical reactions.[37] The problems start when one considers the presumed biosynthesis of RNA in an XNA-based system. Unless an inherently favoured prebiotic synthesis of RNA had stalled at a very late stage, any biosynthetic path to RNA from an environmentally available substance would have to go through several intermediates. This struck us as problematic because evolution proceeds in small steps, each of which must confer an advantage to the unit of selection, and yet the functional superiority of RNA over XNA is only manifest at the polymeric level many steps down the synthetic line. Furthermore, why should the XNA then (altruistically) vanish from the scene leaving RNA centre stage? Nature has a habit of changing the function of newly redundant entities rather than disposing of them—wouldn't there then be some traces of XNA in extant biology? To top all this, the list of possible XNA candidates is almost endless. We preferred to continue our search for an inherently favoured route to ribonucleotides and RNA.

4. The Informational Subsystem—Resumed

Our digression over, we return to our exploration of RNA assembly chemistry and conclude our brief review of chemistry associated with the classic disconnection by considering perceived problems with mixed ribose and nucleobase assembly chemistries, and real problems with nucleobase ribosylation.

The chemistry of sugar synthesis is very different to that of nucleobase synthesis, and a dogma of prebiotic chemistry has been that never the twain shall meet: "*One of the persistent weaknesses of the conventional scenario for the constitutional self-assembly of a prebiotic oligonucleotide base-pairing system is the necessity of assuming a spatial and temporal separation between the nitrogenous chemistry producing the nucleobases and the oxygenous chemistry supposed to give rise to carbohydrates. Drastically enhanced chemical complications would be expected for a scenario without that separation.*"[38] Thus, for example, the hydrogen cyanide **1** needed for the synthesis of adenine **2** would react with the aldehydes needed for sugar synthesis giving cyanohydrins, and it had been assumed that this, and other incompatibilities, would prevent both syntheses operating at the same time and place.[39] This had led to the suggestion of scenarios in which the two nucleoside components were synthesised separately—by the two very different chemistries—and then brought together. Although such scenarios could not be denied, they had an air of desperation about them and this

desperation increased when the issue of conjoining the components was investigated. Without the protecting and controlling groups of conventional synthetic chemistry, joining ribose to the canonical nucleobases is notoriously difficult for kinetic and thermodynamic reasons. Under prebiotically plausible conditions, the reaction of ribose with the pyrimidines does not occur and with the purines it only proceeds in low yield and with little selectivity.[40]

Against this backdrop, we continued exploring RNA syntheses suggested by non-classical disconnections. Whilst making aldopentose aminooxazoline derivatives **12** (for want of anything better to do), we made a strange observation.[41] Although three of the aldopentoses gave only furanose derivatives, lyxose *lyxo*-**6** gave both a furanose, *lyxo*-**12**(f), and a pyranose derivative, *lyxo*-**12**(p), and these interconverted (Figure 4a). The simplest mechanism for this interconversion appeared to involve sugar ring opening and closure via iminium ion *lyxo*-**13**. On pondering this mechanism, we realised that **13** might also be the initial product of an intermolecular reaction between glyceraldehyde **5** and 2-aminooxazole **14** (Figure 4b). Synthesis of **14** by reaction of glycolaldehyde **4** with cyanamide **15** under strongly alkaline conditions is reported in the literature,[42] and material prepared by this method indeed turned out to undergo reaction with glyceraldehyde **5**.[43] The products were the pentose aminooxazolines **12** in excellent overall yield and with a strong *ribo*- plus *arabino*- selectivity (Figure 4b).

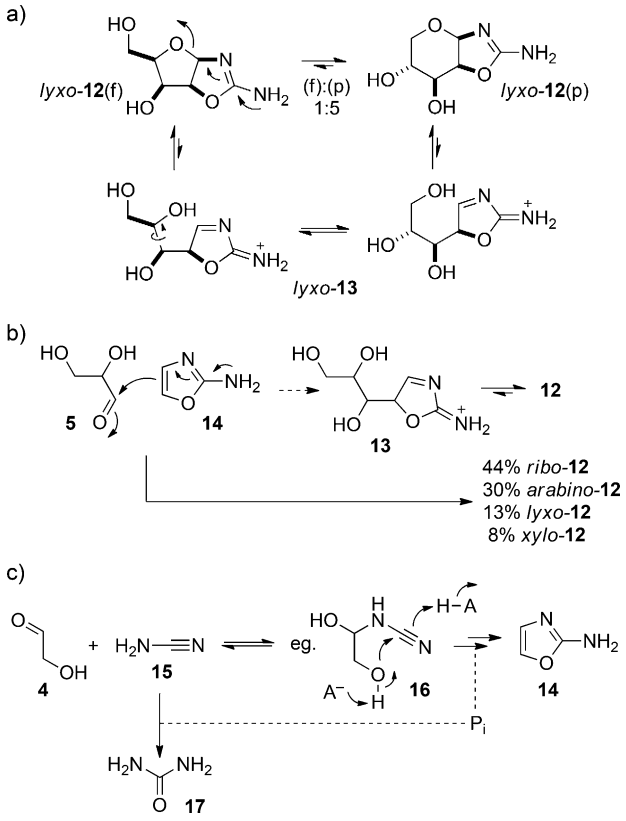

Figure 4. Behaviour and synthesis of pentose aminooxazolines **12**. a) The interconversion of furanose and pyranose forms of *lyxo*-**12**, b) reaction of 2-aminooxazole **14** and glyceraldehyde **5**, c) phosphate as a catalyst in the reactions of cyanamide; catalysis indicated by dashed lines. P_i = inorganic phosphate, HA = general acid.

We immediately wondered if this inherently favoured reaction under prebiotically plausible conditions might be part of a sequence of such reactions leading to ribonucleotides and thence to RNA. The literature conditions for the formation of 2-aminooxazole **14** were not prebiotically plausible to us at the time, however, so we first investigated the reaction of glycolaldehyde **4** and cyanamide **15** at neutral pH and were somewhat depressed to find that **14** was only formed in low yield with most of the material tied up in various (oligomeric) carbonyl addition adducts, e.g. **16** (Figure 4c). Reasoning that the reaction might be stalled at intermediate stages due to sluggish protonation–deprotonation, we sought a general acid–base catalyst. The choice of phosphate as a potential catalyst was made on the basis of systems chemistry considerations—if phosphate is ultimately required to assemble RNA, then why should it not be present in the system from an early stage? Although not yet needed as a reagent, phosphate might have no effect on the reaction or it might catalyse it—or it might catalyse another (undesired) reaction. In the event, the inclusion of phosphate turned the reaction between glycolaldehyde **4** and cyanamide **15** at neutral pH into a really good one, and 2-aminooxazole **14** was now formed in around 90% yield.[44] When cyanamide **15** is in excess, phosphate first catalyses the production of **14**, and then catalyses the hydration of surplus **15** to urea **17**—the latter catalysed reaction having first been noted by Orgel[45]—and **17** comes in useful later.

Another reaction first noted by Orgel was that between the pentose aminooxazolines *ribo*- and *arabino*-**12**, and cyanoacetylene **18** (Figure 5).[27] The reaction between *ribo*-**12** and **18** gave α-cytidine **19** and a chromatographically

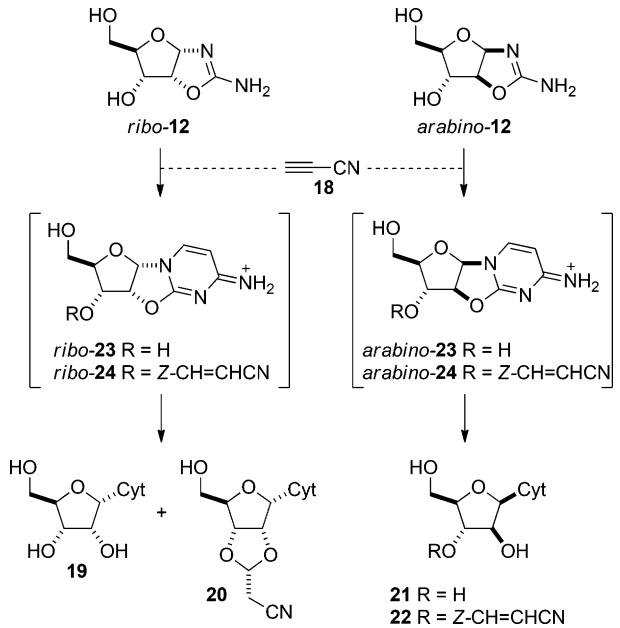

Figure 5. Cyanovinylation of pentose aminooxazolines **12**. Cyt = N-1-linked cytosinyl.

"faster moving product" which we subsequently showed was the cyanoethylidene acetal **20**.[44] Reaction in the *arabino*-series was reported to give *arabino*-cytidine **21** and we also found **22**, the 3′-*Z*-cyanovinylether thereof. The reactions involve initial cyanovinylation of the endocyclic N-atom of the aminooxazoline, followed by closure to the anhydronucleosides **23**. Hydroxide (generated from water through the protonation of the conjugate bases of **23**) then partly deprotonates the 3′-OH group allowing further cyanovinylation by excess cyanoacetylene **18**. The resultant adducts **24**, along with residual anhydronucleosides **23**, are then hydrolysed under the transiently basic conditions to cytidines, and in the *ribo*-series, the 3′-*Z*-cyanovinylether undergoes 5-*exo*-*trig* cyclisation to give the acetal **20**. If the cyanovinylation reaction is carried out under conventional, non-prebiotic conditions in *N,N*-dimethylacetamide it gives the conjugate bases of the anhydronucleosides **23**, which can then be converted to various salts of **23** by addition of acids.

Orgel found that α-cytidine **19** could be photoanomerised to the β-isomer **25** (Figure 6a), but the yield was too low and

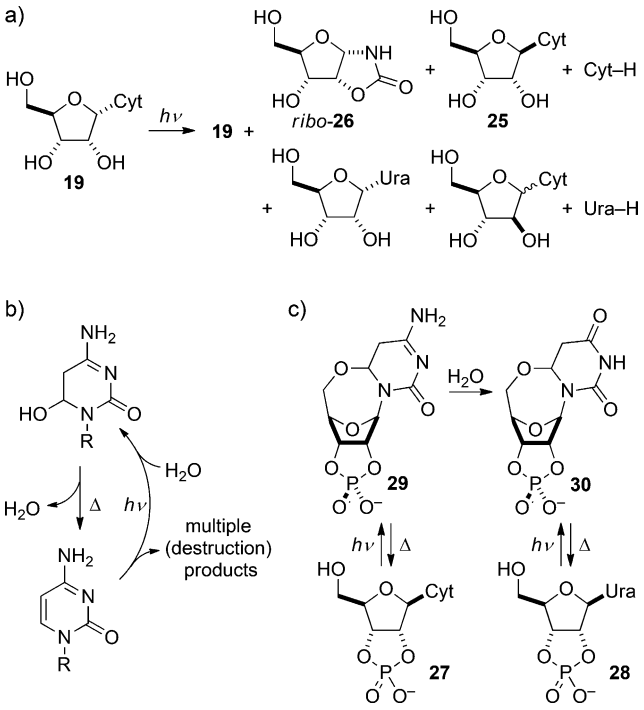

Figure 6. Photochemistry of cytidine nucleosides and nucleotides. Ura = N-1-linked uracilyl.

there were too many by-products for this to constitute a prebiotically plausible synthesis.[27] We reinvestigated this photochemistry and discovered that the main product, oxazolidinone *ribo*-**26**, was one of nucleobase destruction.[46] We were running out of ideas and entered another of those phases where one does things in an unfocussed way for want of anything better to do. Systematically looking at the photochemistry of phosphorylated cytidine derivatives, in the vague hope that we would find a good photoanomerisation reaction, we instead found that UV irradiation eventually destroyed most of these nucleotides. The canonical pyrimidines undergo various photoreactions including hydration of the 5,6-double bond (Figure 6b).[47] The resultant photohydrates (thermally) lose water, and thus cycling between the pyrimidine and its photohydrate occurs upon prolonged irradiation. The photohydrates of cytidines undergo hydrolysis to the photohydrates of uridines, but photohydrates of both pyrimidines are relatively stable to irradiation. Thus, the destructive photochemistry we had observed for most cytidine nucleotides occurs from the unhydrated state.

There was, however, one nucleotide, β-cytidine-2′,3′-cyclic phosphate **27**, that was remarkably stable to irradiation, suffering only from partial conversion to the corresponding uridine nucleotide **28** (Figure 6c).[44] The *cis*-fused five-membered ring connecting C-2′ and C-3′ on the α-face of **27**, allows the sugar to access conformations (East and West), that similarly unconstrained nucleotides cannot access (leaving the latter preferring North and South conformations). The result of this conformational switching is that the 5′-OH group of **27** spends time in the vicinity of C-6 of the nucleobase on the β-face, and under conditions of irradiation, can add to it in place of water. The intermediate thus produced, **29**, is apparently more stable to elimination than a cytidine photohydrate and thus has a longer lifetime. There is thus more time for hydrolysis to the corresponding uridine derivative **30**, but less time for other, destructive photochemistry. After the irradiation ceases, final elimination of the 5′-OH group of **29** and **30** ensues leaving a mixture of the two canonical pyrimidine nucleoside-2′,3′-cyclic phosphates **27** and **28**. Thwarted in our efforts to find a good photoanomerisation reaction, we now had an alternative use for UV irradiation: we could use it to destroy any unwanted isomers formed during the synthesis of β-cytidine-2′,3′-cyclic phosphate **27**—that is, if we could find a synthesis of **27**!

Nagyvary had showed in a seminal paper that **31**, the 3′-phosphate of the anhydronucleoside *arabino*-**23**, as prepared by conventional synthesis, underwent smooth isomerisation to **27** (Figure 7)[48] and so we sought a prebiotically plausible synthesis of **27** via **31**. Partially reversible nucleoside phosphorylations in urea melts or formamide had been described by Orgel and Schoffstall,[49,50] so we subjected *arabino*-**23** to these conditions. On the basis of the generally (≈10-fold)

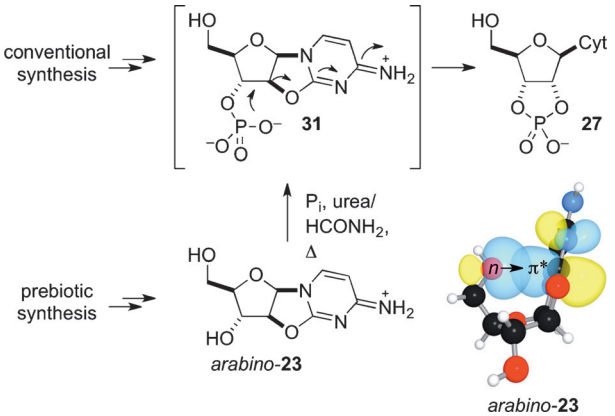

Figure 7. Ribonucleotides by C-2′ stereoinversion.

increased nucleophilicity of a primary alcohol over a secondary one, 5′-OH group phosphorylation was expected to dominate (at least in the early stages of reaction), but we still hoped that there would be some 3′-OH group phosphorylation giving **31** and then **27**.

In the event there was far more **27** formed than we could possibly have hoped for—the production of **31** over the corresponding 5′-phosphate is clearly inherently favoured![44] It transpires that this is due to a stereoelectronic effect whereby a lone pair of the 5′-OH group of *arabino*-**23** interacts with an antibonding orbital at C-2 of the nucleobase.[51] This n→π* overlap has the effect of reducing the electron density of the 5′-OH group and increasing its steric encumbrance, thus making the 3′-OH group the better nucleophile. So, if we could somehow stop the cyanovinylation of *arabino*-**12** in water at the stage of the anhydronucleoside *arabino*-**23** (Figure 5), we now had a synthesis of the pyrimidine ribonucleotides. Inclusion of phosphate in the reaction once again turned out to be the key. We tried it because it had proven to be beneficial at the beginning of the synthesis, and was needed in the phosphorylation step, and so, according to our increased systems level thinking, it should also be in the reaction mix at the cyanovinylation stage. We were, though, slightly trepidacious because of the known propensity for cyanoacetylene **18** to react with phosphate giving *Z*-cyanovinylphosphate[52]—if this reaction was more favourable than the cyanovinylation of *arabino*-**12**, we would have been in trouble. It actually turned out that phosphate has just the right reactivity with **18**, and reacts with it after the endocyclic N-atom of the aminooxazoline *arabino*-**12**, but before its 3′-OH group. Along with the pH buffering it affords, this chemical buffering by phosphate makes the conversion of *arabino*-**12** to the anhydronucleoside *arabino*-**23** extremely clean, and the latter compound was produced in > 90 % yield.[44]

Before summarising the synthesis we had thus far achieved, the question of absolute stereochemistry needs addressing. *Ribo*-**12** is the major aminooxazoline product in the reaction of 2-aminooxazole **14** with glyceraldehyde **5** (Figure 4b), and yet we had now found a route from the less abundant *arabino*-**12** to the pyrimidine ribonucleotides **27** and **28** involving C-2′ stereoinversion. On cooling, *ribo*-**12** selectively crystallises from the solution of products,[43] making *arabino*-**12** the most abundant product in the mother liquor, and for a while we were happy to view this phase separation as a way of enriching for the latter aminooxazoline stereoisomer. However, there was a troubling aspect to this as we found that if the input glyceraldehyde **5** is non-racemic, the *ribo*-**12** that crystallises has an enhanced enantiomeric excess (*ee*), and above a certain threshold input **5** *ee*, is enantiomerically pure.[43] This is the behaviour of a true conglomerate—although *ribo*-**12** actually forms an enantiomorphously twinned one[29]—and it would clearly be expedient if it could contribute to the formation of enantiopure ribonucleotides **27** and **28**. But, as things stood, with us invoking further synthetic steps from the *arabino*-**12** that was left in solution, we were missing out. Accordingly, we wondered if we could find conditions under which *ribo*-**12**, enantioenriched through crystallisation, would convert to, or interconvert with, *arabino*-**12**. A potential mechanism whereby the aminooxazolines *ribo*- and *arabino*-**12** might interconvert sprang to mind based on our earlier pondering about the interconversion of the furanose and pyranose forms of *lyxo*-**12** (Figure 4a,b). If the iminium ion *ribo*-**13** derived by ring opening of *ribo*-**12** could be deprotonated at C-2′, then a substituted 2-aminooxazole **32** would result (Figure 8), and if this underwent C-2′

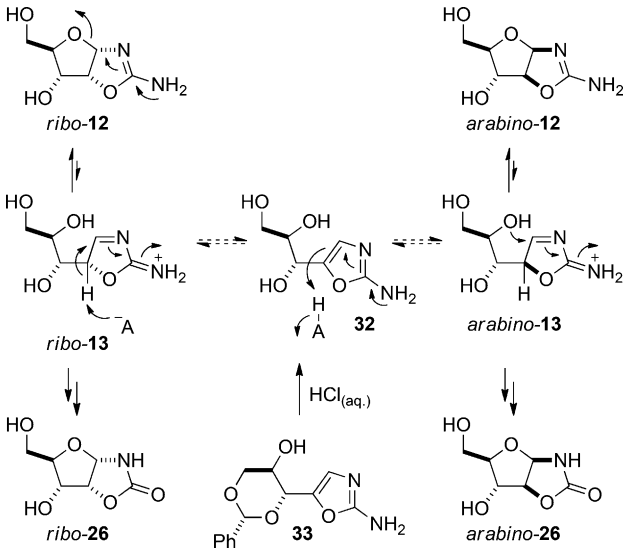

Figure 8. Interconversion of pentose aminooxazoline stereoisomers.

protonation, equilibration with *arabino*-**13**, and thence *arabino*-**12**, ought to be possible.

The reprotonation and deprotonation taking place at carbon, general acid–base catalysis would be needed and so we incubated *ribo*-**12** in phosphate buffer for a prolonged period. Interconversion with *arabino*-**12** was indeed observed, though there was also some hydrolysis to the corresponding oxazolidinones *ribo*- and *arabino*-**26**.[53] We could also detect what we thought was the intermediate substituted 2-aminooxazole **32**, and we proved this through conventional synthesis of a standard. The last step of this synthesis was the *specific* acid catalysed hydrolysis of the acetal **33**, and the stability of **32**, so formed, towards equilibration with *ribo*- and *arabino*-**12** supported our assumption that interconversion of these two aminooxazolines would require *general* acid–base catalysis.

By this stage, we felt that we had a route to the activated pyrimidine ribonucleotides **27** and **28** that comprised enough inherently favoured reaction steps for the overall synthesis to merit the same epithet. There were detractors from this opinion—of course—some whose criticisms we took note of[33] and others that we viewed as naysayers. In the latter category were those who cited earlier criticism of multistep prebiotic synthesis per se—"*Consider a golfer who, having played a ball through an 18-hole course, then assumes that the ball could also play itself around the course in his absence*"[54]—to criticise our work selectively.[55] The golf analogy draws one in because of the similarity between a golf course and a fairly flat free energy surface—why should

a reaction sequence follow one particular coordinate when several others appear equally favourable? But, if the free energy surface is sloped, then one particular coordinate might become sufficiently favoured for the corresponding multistep reaction sequence to proceed without help from an experimenter. That the putative synthesis of ribonucleosides based on the traditional disconnection suffered energetically has been (presciently) noted: "*A common feature of the metabolic pathways functioning in living organisms is that they are either energetically downhill or are coupled to a reaction that acts as an energy source. In addition, the first and last steps of the reaction must be markedly exothermic to initiate and complete a multistep reaction path. The internal steps are usually accompanied by small energy changes, and might be even endothermic. By applying the above principle to the synthesis of nucleosides, it seems likely that the synthetic route through ribose and nucleobases is prebiotically less relevant, because this reaction (which is indeed the final step of the pathway) is known to be endothermic.*"[56] On the other hand, calculations suggested that our experimentally demonstrated route (Figure 9) had the right free energy profile to be prebiotically relevant.[56]

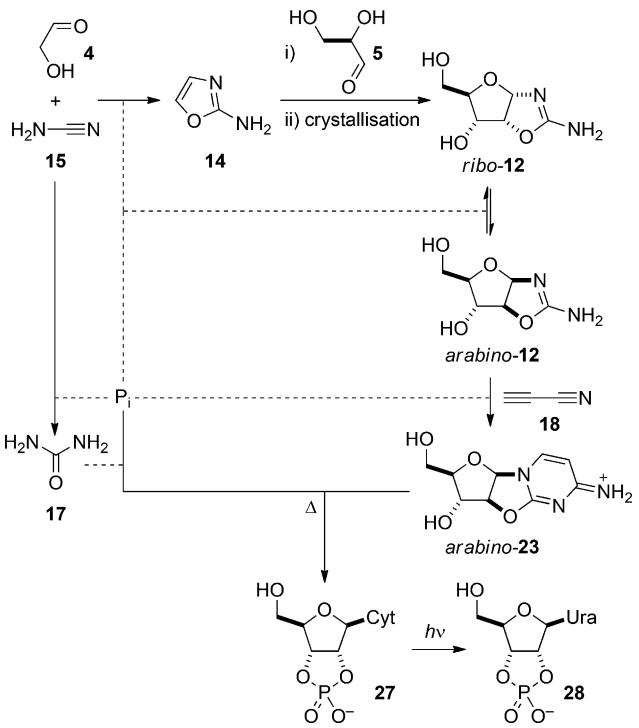

Figure 9. Potentially prebiotic synthesis of activated pyrimidine ribonucleotides. Catalysis, and reaction control through pH and chemical buffering, is indicated by dashed lines.

There was a big problem, however, and that was the provenance of the starting materials. Our palette of organic starting materials—glycolaldehyde **4**, glyceraldehyde **5**, cyanamide **15** and cyanoacetylene **18**—appeared too rich and unstable to have been forged by atom (re)combination chemistry in a protoplanetary disk, and then survived delivery to Earth during late stage accretion. There were even problems with simple, inorganic phosphate because of its insolubility in the form of many salts. It was time to use our chemistry, and that of others, to try and formulate a compatible geochemical scenario. If we were on the right track, then the hope was that this scenario would furnish all of our starting materials.

5. First Hints at an Impact Scenario

The first clue was the source of phosphate. Pasek and Kee suggested that phosphate, along with phosphite and hypophosphite, might have been produced on the early Earth by corrosion of phosphide inclusions in meteorites,[57,58] and this got us thinking about impacts. After the collison that formed the Moon, Earth and its new satellite took a pounding from meteorite and comet impacts as evidenced most graphically by the current appearance of the Moon (Figure 10).

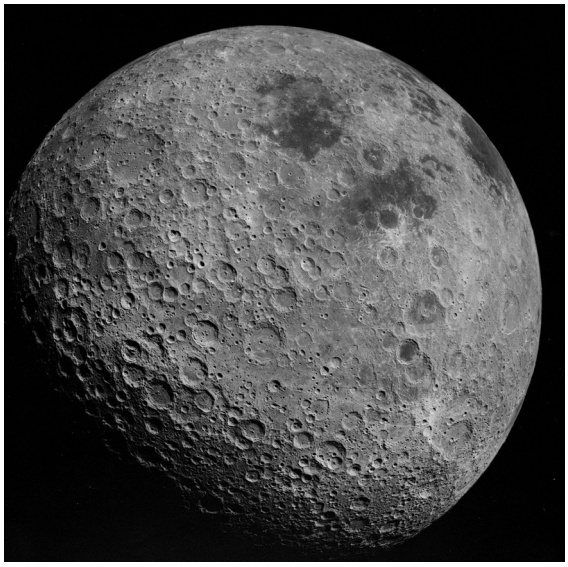

Figure 10. Far side of the Moon (NASA Apollo 16 photograph AS16-3021).

It is thought that this barrage spiked 3.9 to 3.8 billion years ago, an event known as the late heavy bombardment.[59] The details of what this did to Earth are not clear because tectonics and weathering have erased the evidence, but some aspects can be gleaned by studying the more readily discernible consequences of later impacts. We consider two sorts: large energetic impacts of meteorites or comets in general, and smaller impacts, specifically of iron–nickel meteorites.

Large, fast moving objects tend to vapourise on impact, and deliver sufficient kinetic energy to melt the Earth's crust and create heated fracture zones. Subsequent solidification and hydrothermal alteration can result in the formation and concentration of metal sulfides.[60] The Sudbury Impact Crater in Ontario[61,62] is a now deformed crater, 60 km long and 30 km wide, that was created 1.85 billion years ago by the impact of a body, thought to be a comet around 15 km in

diameter, with an impact velocity of 40–50 km s^{-1}. The crater margin regions, known as the Sudbury Igneous Complex, are so rich in copper and nickel sulfides that they have been extensively mined. More widespread impact metalogenesis on the Hadean Earth might, therefore, have resulted in significant enrichment of these metal sulfides at many locations at or near the Earth's surface.

Impact of small iron–nickel meteorites does not result in their complete destruction, and various sized fragments tend to end up scattered in and around the site of impact. Meteor Crater in Arizona was formed 50 thousand years ago by the impact of the Canyon Diablo meteorite, which is estimated to have been 40 m in diameter, with an impact velocity of 12 km s^{-1}.[[63,64]] By studying the remaining fragments of this meteorite, its bulk composition can be inferred to have been 90% kamacite (a very iron-rich iron–nickel alloy), 1–4% taenite (another iron–nickel alloy containing more nickel) and up to 8.5% inclusions of graphite and iron and nickel sulfides, with these inclusions typically rimmed by the phosphide mineral schreibersite, $(Fe,Ni)_3P$. It is corrosion of the latter mineral that Pasek and Kee had suggested as the source of phosphate for prebiotic chemistry.[57,58] Impact fragments that ended up in groundwater would have corroded anoxically through local electrochemical cells, formed due to bulk heterogeneity, with the most electropositive regions undergoing preferential oxidation. Because of its high iron content, kamacite would have dissolved first, and as the inclusions tend to be swathed by this alloy, they would have become detached from the fragment.[65] Those inclusions that fell and became electrically separated from the fragment would then have undergone corrosion themselves, and this is when phosphate would have been produced—largely as its ferrous salt, vivianite, $Fe_3(PO_4)_2{\cdot}8H_2O$.[66] The insolubility of this salt initially concerned us, but the great affinity of cyanide for certain transition metal ions suggested a way in which soluble phosphate could have been produced in an impact scenario. Hydrogen cyanide **1** might have been both delivered to the surface of Earth during late stage accretion,[67] and produced by reaction of carbonaceous meteoritic material with atmospheric nitrogen.[68] Hydrogen cyanide **1** that dissolved in groundwater containing ferrous and other transition metal ions would have produced cyanometallate salts,[69] and we reasoned that vivianite might have ended up being dissolved in this way. The resultant soluble phosphate would have been available as a catalyst and buffer for the early reactions in our scheme, and for the conversion of anhydronucleoside *arabino*-**23** into the activated pyrimidine ribonucleotides **27** and **28** in due course, but what of the cyanometallates? We needed to study their chemistry in general, but given that we had an indication that irradiation was important in the conversion of **27** to **28**, we first decided to study the photochemistry of cyanometallates. Although we thought that cyanoferrate(II) would have been the most abundant of such species, we also considered the cyanide complexes of other transition metal ions that might have been plentiful.

6. Chemical Implications of an Impact Scenario

Having just achieved a synthesis of the ribonucleotides **27** and **28**, we were on the look out for chemistry that might lead to the corresponding purine derivatives. Cyanogen **34** is known to catalyse the oligomerisation of hydrogen cyanide **1** to purine precursors and we were thus intrigued by literature reports that **34** can be produced, along with hydrated electrons, by irradiation of cyanocuprates(I) through the operation of a photoredox cycle (Figure 11 a).[70,71] Tricyanocuprate(I) **35** first undergoes photooxidation to tricyanocuprate(II) **36**, reversible dimerisation of which provides access to hexacyanodicuprate(II) **37**. Reductive elimination of cyanogen **34** from **37** then gives dicyanocuprate(I) **38**, and, finally, cyanation of **38** regenerates **35**. This cycle had been studied for its intrinsic interest by

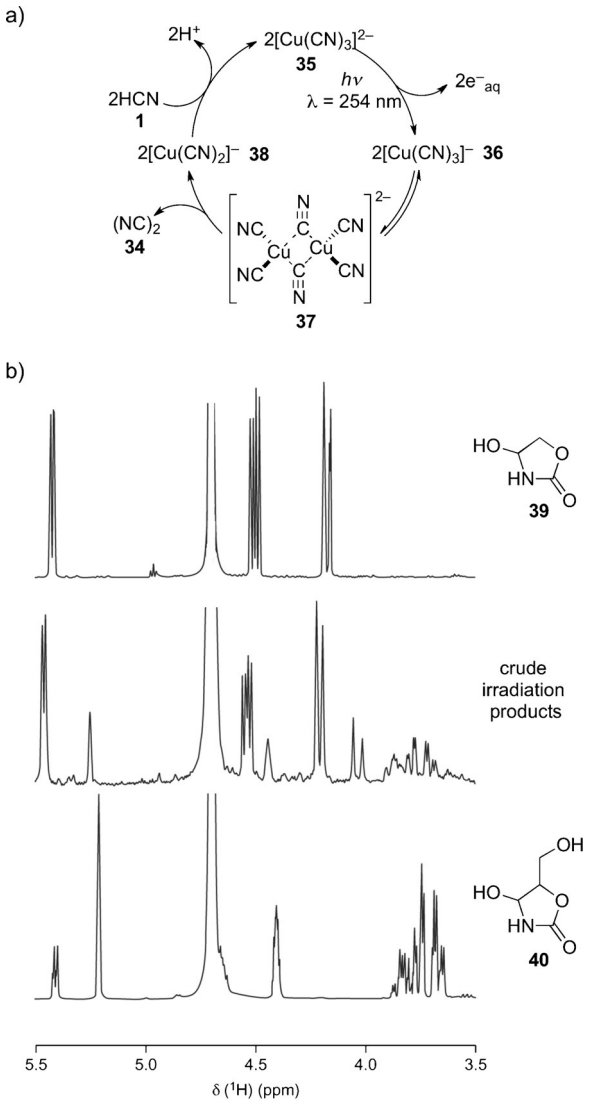

Figure 11. Hydrogen cyanide–cyanocuprate photoredox chemistry. a) Cyanocuprate photoredox cycle, b) ^{1}H NMR spectrum of the crude products of irradiation of hydrogen cyanide **1** and copper(I) cyanide along with spectra of **39** and **40**, the isocyanate adducts of glycolaldehyde **4** and glyceraldehyde **5** respectively.

inorganic and physical chemists, but it attracted us as organic chemists because of its synthetic potential. It was not just that the production of cyanogen **34** appeared conducive to synthesis of purine precursors, we also had high hopes of the hydrated electrons. Accordingly, we added copper(I) cyanide and potassium cyanide to an H_2O/D_2O mixture, neutralised the resultant solution and irradiated it. As it happened, purines were not produced but reductive synthetic chemistry took place.[72] The ^1H NMR spectrum of the reaction products (Figure 11 b) initially confounded us as it clearly revealed the presence of compounds containing contiguous protonated carbon atoms. After a while, we started to suspect that the products were **39** and **40**, the isocyanate adducts of glycolaldehyde **4** and glyceraldehyde **5** respectively, and we proved this by comparison with authentic samples. So, by some wonderful combination of oxygenous and nitrogenous chemistries, the very sugars, **4** and **5**, that we needed for ribonucleotide synthesis were being served up to us—before being snatched away by similarly mixed chemistry that struck us as anything but wonderful. We were not deterred, however, and felt that a better understanding of the systems (photo)-chemistry and its underpinning scenario might lead to a way of making the free sugars.

Further study revealed what was going on, at least in outline (Figure 12). Hydrated electrons are potent reducing agents, adding with greatest ease to those organic substances that are thereby converted into stabilised radical anions, or—if the addition is general acid catalysed—free radicals. Iminyl radicals are relatively stable entities,[73] and hydrogen cyanide **1** is a general acid with $pK_a = 9.2$, thus the addition of hydrated electrons to **1** to give the methaniminyl radical **41** is

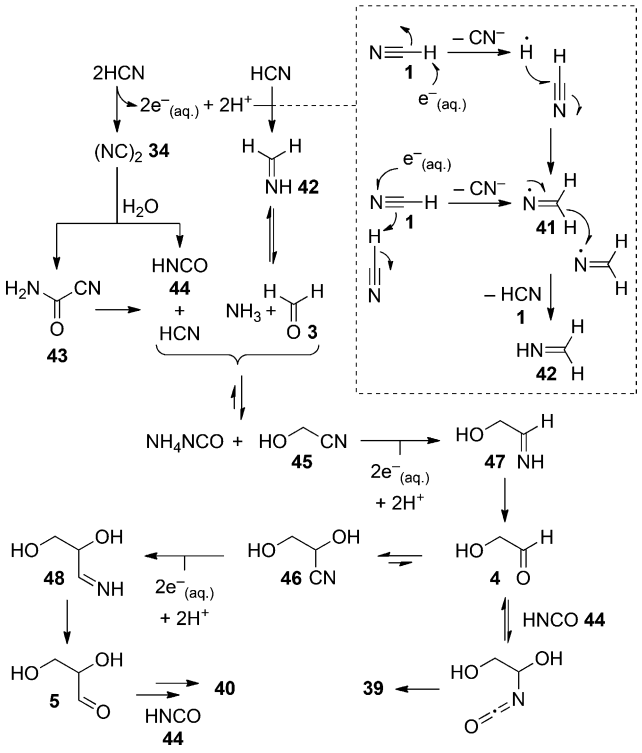

Figure 12. Hydrogen cyanide–cyanocuprate systems photochemistry.

inherently favoured in aqueous solutions near pH neutrality (Figure 12, box). Disproportionation of this radical then regenerates **1** and generates methanimine **42**, which is in equilibrium with formaldehyde **3** and ammonia. Meanwhile, the other product of the disproportionation of hydrogen cyanide **1**, cyanogen **34**, undergoes direct and indirect (via cyanoformamide **43**) hydrolysis to isocyanic acid **44** and **1**.[74] Isocyanic acid **44** ($pK_a = 3.7$) protonates ammonia displacing the equilibrium between **42** and **3** in favour of the latter, which then forms the cyanohydrin, glycolonitrile **45**, by reaction with additional hydrogen cyanide **1**.

Iteration of this reductive homologation then converts **45** to glyceronitrile **46** by way of the imine **47** and glycolaldehyde **4**. Some of the glyceronitrile **46** is then reduced to the imine **48**, which undergoes hydrolysis to glyceraldehyde **5**, but depletion of hydrogen cyanide **1** (as reductant) limits this. The shortage of **1** allows overall aldehyde levels to creep up, and undesirable chemistry (as far as we were concerned) kicks in with the addition of isocyanate to the aldehydes **4** and **5** giving the adducts **39** and **40**. Thus, at the heart of this hydrogen cyanide–cyanocuprate systems photochemistry there was a beautiful synthesis of sugars, but it was marred by the presence of isocyanate. We tried in vain for a long time to get around this through addition to the system of other components, which had the potential to react preferentially with isocyanic acid **44**. Even phosphate, which had come to our rescue on so many occasions, failed to solve this problem—the known equilibrium reaction between phosphate plus isocyanic acid **44** and carbamyl phosphate not outdoing aldehyde–isocyanate adduct formation.[75] Our only hope was to find an alternative stoichiometric reductant. We were also concerned that we had deviated somewhat from studying the photochemistry of cyanometallates as suggested by our outline (post-)impact scenario. This was because the scenario invoked cyanometallate accumulation in solution due to the favourabilitiy of complexation equilibria, and the formation of tricyanocuprate(I) **35** from the corresponding dicyanocuprate **38** is not very favourable. Thus, a high concentration of hydrogen cyanide **1** is needed in the chemistry we had uncovered and accumulation of free **1** to high levels in solution would not be expected because of unfavourable buffering with atmospheric **1**. That was the scenario as it stood, though, was there a plausible extension that would lead to high concentrations of cyanide in solution and maybe, even, an alternative stoichiometric reductant?

7. Refinements to the Impact Scenario

The literature on cyanide and cyanometallate chemistry is vast, dispersed across several disciplines, and extends back into the depths of time. It took us a while to sift through it, but what we were able to piece together really excited us.

The first finding was that thermal decomposition of cyanoferrate(II) salts gives products that depend on the nature of the cation(s) with sodium and potassium cyanoferrate(II) giving sodium and potassium cyanide.[76]

This suggested a means of obtaining concentrated cyanide solutions from a solution of cyanoferrates(II) produced by

$(Na,K)_4[Fe(CN)_6] \xrightarrow{700^oC} 4(\mathbf{Na/K})\mathbf{CN} + Fe(CN)_2$

$3Fe(CN)_2 \xrightarrow{700^oC} Fe_3C + 5C + 3N_2$

$Fe(CN)_2 \xrightarrow{700^oC} Fe + 2C + N_2$

complexation of ferrous ions with hydrogen cyanide **1** absorbed from the atmosphere. If such a solution containing sodium and potassium counterions evaporated, and the resultant evaporite layer underwent heating due to impact or geothermal activity, metamorphosis to a solid containing sodium and potassium cyanide would have occurred. Limited rainfall, or the inflow of a stream, could then produce a concentrated cyanide solution. But it was better than this, thermal metamorphosis of calcium and magnesium cyanoferrates(II) produces calcium cyanamide and magnesium nitride.[77,78]

$Ca_2[Fe(CN)_6] \xrightarrow{660^oC} 2Ca(CN)_2 + Fe(CN)_2$

$Ca(CN)_2 \xrightarrow{660^oC} \mathbf{CaNCN} + C \underset{}{\overset{1000^oC}{\rightleftharpoons}} \mathbf{CaC_2} + N_2$

$Mg_2[Fe(CN)_6] \xrightarrow{315^oC} 2Mg(CN)_2 + Fe(CN)_2$

$Mg(CN)_2 \xrightarrow{395^oC} MgCN_2 + C$

$3MgCN_2 \xrightarrow{420^oC} \mathbf{Mg_3N_2} + 3C + 2N_2$

These salts, upon hydration, would give the cyanamide **15** needed for ribonucleotide synthesis and the ammonia needed for synthesis of the purines, inter alia. Furthermore, calcium cyanamide and carbon equilibrate at high temperature with calcium carbide,[79] which we hoped might somehow furnish the acetylenic moiety of cyanoacetylene **18**. Thus, we had the first suggestion that all the building blocks for ribonucleotide assembly might be produced through the thermal metamorphosis of cyanoferrate(II) salts.

Our second finding in the cyanide literature was that the sulfides of certain metals, including iron and copper, dissolve in cyanide solution with the production of cyanometallates.[80,81] This was initially interesting because it suggested that cyanocuprates(I) might have been produced on the early Earth if cyanide containing streams ran over ground enriched, through impact metalogenesis, in copper sulfide. But then the penny dropped: the co-product of this dissolution process is hydrosulfide (HS^-, the conjugate base of hydrogen sulfide; $pK_a = 7.2$), which is a potent reductant—could it function as the stoichiometric reductant in our photoredox chemistry?

8. Chemical Implications of the Refined Impact Scenario

We quickly tested whether hydrosulfide could function as our "dream" reductant by irradiating a neutral aqueous system containing glycolonitrile **45**, copper(I) cyanide, phosphate (as pH buffer) and hydrosulfide/hydrogen sulfide. To our delight, free glycolaldehyde **4** was produced in good yield along with a few other compounds, most notably what we first branded an over-reduction product, acetaldehyde **49** (Figure 13a).[82]

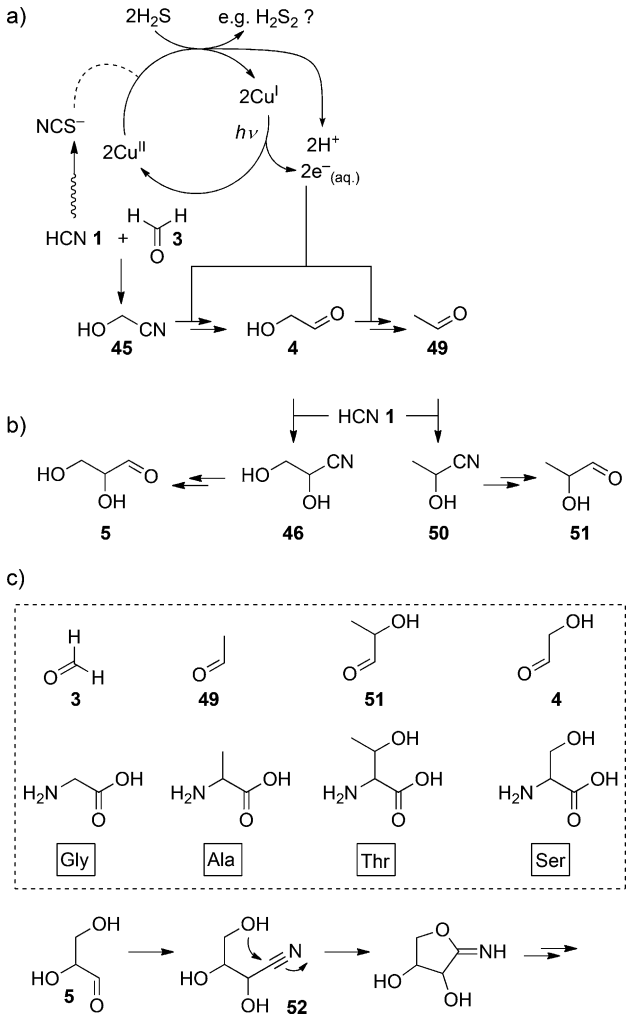

Figure 13. Photoredox systems chemistry with hydrosulfide as the stoichiometric reductant. a) (Over-)reduction of glycolonitrile **45** to glycolaldehyde **4** (and acetaldehyde **49**), b) reductive homologation of **4** (and **49**) to **5** (and **51**), c) most of the aldehydes produced by this chemistry as Strecker amino acid precursors (boxed) and the self-destruction (as regards potential Strecker chemistry) of the cyanohydrin **52**.

With hydrosulfide as the stoichiometric reductant, isocyanate production was avoided and hydrogen disulfide was presumably formed, and then, through reaction with cyanide, thiocyanate. Because there was not much hydrogen cyanide **1** in the system, the chemistry was stalled at the stage of

glycolaldehyde **4**, and we reasoned that for reductive homologation to proceed, further **1** would have to be added. Recognising that this would result in the formation of cyanohydrins from all aldehydes present, we therefore had to consider the fate of lactonitrile **50** as well as glyceronitrile **46** (Figure 13b). Reduction of these two cyanohydrins using hydrosulfide as stoichiometric reductant then gave lactaldehyde **51** and glyceraldehyde **5**. So, subject to a few difficulties in fitting the chemistry and geochemical scenario together, we now at last had a synthesis of glycolaldehyde **4** and glyceraldehyde **5** in the free form needed for our ribonucleotide synthesis. And then we realised that we actually had a lot more (Figure 13c). Formaldehyde **3**, acetaldehyde **49**, and lactaldehyde **51**, which are ineluctably associated with the synthesis of **4** and **5**, just so happen to be the Strecker precursors of the amino acids glycine, alanine and (*allo*)-threonine. Furthermore, glycolaldehyde **4** is the Strecker precursor of another "natural" amino acid, serine, and although glyceraldehyde **5** is, on the face of it, the Strecker precursor of an "unnatural" amino acid, the cyanohydrin intermediate **52** potentially en route is known to undergo an inherently favoured cyclisation leading to hydrolysis and ammonolysis products.[83]

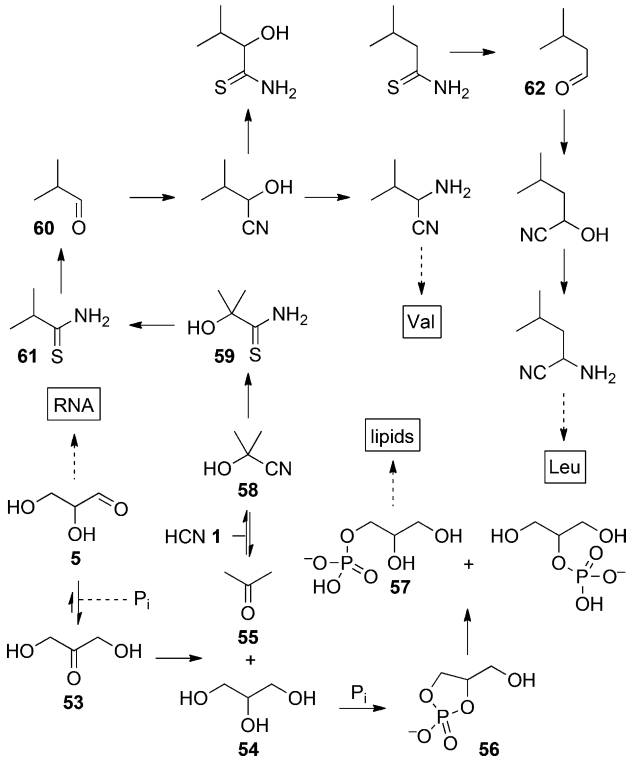

Figure 14. First signs of a linkage between all subsystems through cyanosulfidic chemistry.

9. Linkage of all Subsytems through Cyanosulfidic Chemistry

We thus had our first evidence that the informational subsystem could be linked to a peptide based catalytic subsystem, through the synthesis of amino acid precursors at the same time as ribonucleotide precursors.[84] It was also beginning to look as though some of the proteinogenic amino acids used by Nature might be inherently chemically favoured, and that non-proteinogenic amino acids might be similarly disfavoured. Buoyed by these findings, we wondered if it might also be possible to establish a link to the compartment-forming subsystem.

Whilst the informational and catalytic molecules of cells of all the three kingdoms of life are the same—RNA and proteins—the compartment-forming lipid molecules are different.[85] Bacterial and eukaryal lipids are predominantly diesters of one enantiomer of glycerol-1-phosphate, or its derivatives, with fatty acids. Archaeal lipids on the other hand are predominantly di-isoprenoid ethers of the opposite enantiomer of glycerol-1-phosphate (derivatives). This leaves the nature of the hydrophobic component of ancestral lipids uncertain, but suggests that the hydrophilic component was either glycerol-1-phosphate or a derivative thereof. Accordingly, we looked to our chemistry to try and discern a connection with this phosphorylated triol. Glyceraldehyde **5** and inorganic phosphate seemed to be the most likely precursors, and so, based on systems chemistry considerations, we incubated these two compounds together in aqueous solution. Not surprisingly, dihydroxyacetone **53** was formed in good yield by Lobry de Bruyn–Alberda van Ekenstein reaction (Figure 14).[86] Photoreduction of **53** with hydrosulfide as the stoichiometric reductant gave two major products: glycerol **54** and acetone **55**. These two products were both formed in ≈30% yield and at first we were disappointed that competing deoxygenation of **53** had lowered the yield of **54**. However, we soon saw in the geminal methyl groups of acetone **55** a possible link to other (proto)biological molecules having this structural motif. Furthermore, we were beginning to realise that what one strives for in a conventional synthetic reaction—a high yield of a single product—is not always what one wants in a systems chemistry synthesis of multiple products. Before pursuing the synthetic lead offered by acetone **55**, we turned our focus back to glycerol **54** and subjected it to the same phosphorylation conditions we had used to convert the anhydronucleoside *arabino*-**23** to the ribonucleotide **27** (Figure 7). The phosphorylation reaction gave mainly glycerol-1,2-cyclic phosphate **56** and a small amount of glycerol-1-phosphate **57**, however substantially more of the latter was formed when the cyclic phosphate underwent subsequent hydrolysis. So we now had a link between the generational chemistry of RNA, protein and lipid building blocks through cyanosulfidic chemistry—it was time to flesh out the scheme.[86]

We turned back to acetone **55** and attempted photoreduction of its cyanohydrin **58** with hydrosulfide, but encountered a problem. For some (steric?) reason, **58** is less easily reduced than the hydrogen cyanide **1** with which—along with acetone **55**—it is in equilibrium. However, when the equilibrium mixture of **1**, **55** and **58** was left in the dark with hydrosulfide, smooth conversion to the α-hydroxythioamide **59** took place. For some (electronic?) reason **58** is more susceptible to hydrosulfide addition than hydrogen cyanide **1**. Photoreduction of α-hydroxythioamides turns out to follow

a different path to photoreduction of cyanohydrins: the latter are first reduced to α-hydroxyaldehydes, which then undergo partial deoxygenation; the former are first deoxygenated to thioamides, which then undergo reduction to aldehydes. Thus photoreduction of hydroxythioamide **59** gave isobutyraldehyde **60**—the Strecker precursor of valine—by way of thioamide **61**. Reductive homologation of isobutyraldehyde **60** using the thioamide route then gave isovaleraldehyde **62**, the Strecker precursor of leucine. Given that the direct reduction of its cyanohydrin, **58**, to an α-hydroxyaldehyde did not prove possible, then homologation via the thioamide route is all that is possible for acetone **55**, and inherently favoured cyanosulfidic chemistry gives rise to valine and leucine and not their hydroxylated variants. This stands in contrast to the homologation of formaldehyde **3** (Figure 13) where direct reduction of glycolonitrile gives glycolaldehyde **4** *in addition* to the reduced and deoxygenated product acetaldehyde **49**. Alanine is therefore produced *alongside* its hydroxylated variant, serine, and its hydroxylated homologated variant, threonine. Subtle chemical reasons for the structures of the first amino acids were becoming apparent—if, indeed, nature first used amino acids on the basis of synthetic contingency.

By now, we were satisfied that our haul of nucleotides, amino acids and lipid precursors was enough to establish a strong link between the various subsystems and we decided to tie up loose ends, the most glaring of which was the source of cyanoacetylene **18**.

We hoped that acetylene **63** derived from the hydration of calcium carbide might be coupled with hydrogen cyanide **1** to give cyanoacetylene **18** (Figure 15). In our geochemical scenario, slow percolation of water through a thermally metamorphosed ferrocyanide evaporite layer might enable the production of acetylene **63** over a reasonable period of time. We had in mind that copper(II) might be an ideal coupling agent because the self-coupling of **63** to give di- and

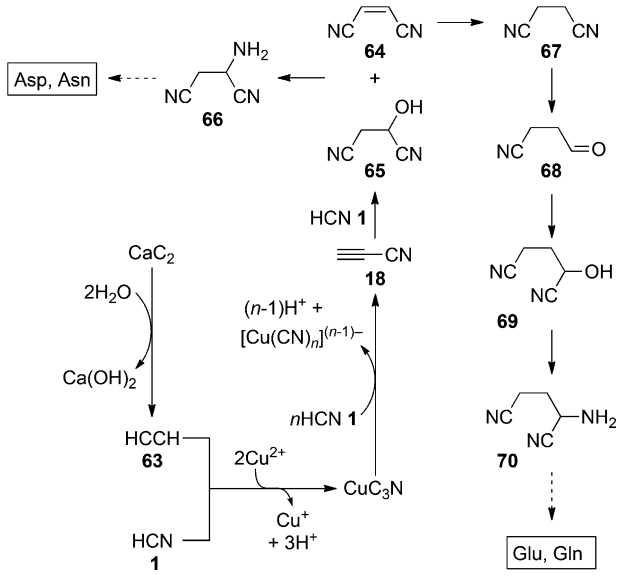

Figure 15. Synthesis of cyanoacetylene **18** and reactions leading to amino acid precursors therefrom.

oligoacetylenes is known and we were by now very aware of the corresponding oxidative self-coupling of hydrogen cyanide **1** to give cyanogen **34**. The desired cross-coupling of **18** and **1** proved possible through addition of copper(II) to a solution of copper(I) in the presence of high concentrations of chloride ions. However, we nearly missed the reaction as the cyanoacetylene **18** was not produced in free form, but in the form of an insoluble copper(I) coordination compound, CuC_3N.[87] It was only when we added further hydrogen cyanide **1** that **18** was released into solution. Despite the fact that this insoluble copper(I) derivative of cyanoacetylene **18** had nearly caused us to miss the cross-coupling reaction, we soon came to appreciate it because it assuaged concerns that we (and others)[88] had about invoking reasonable concentrations of such a reactive species as free **18** in our nucleotide synthesis. Produced as its copper(I) derivative, **18** is indefinitely stable and can be released to give highly concentrated solutions. The high salt needed to solubilise copper(I) in the cross-coupling reaction was consistent with our geochemical scenario if calcium ferrocyanide, or a similar, mixed salt, was deposited in the evaporite layer at a late stage, along with sodium and potassium chloride, prior to thermal metamorphosis. The high solubility of calcium ferrocyanide lends support to this contention.[89]

Cyanoacetylene **18** is known to react with hydrogen cyanide **1** to give maleonitrile **64** and the cyanohydrin **65**,[90] and these two compounds were produced when we added an excess of hydrogen cyanide **1** to a slurry of CuC_3N in water.[86] Reaction of this product mixture with ammonia then gave the aminonitrile **66**, a precursor of both aspartic acid and asparagine. Photoreduction of maleonitrile **64** with hydrosulfide proceeded cleanly in stages giving first succinonitrile **67** and then the semialdehyde **68**. Reaction of **68** with hydrogen cyanide **1** gave the cyanohydrin **69** which underwent reaction with ammonia to give aminonitrile **70**, the precursor of glutamic acid and glutamine. Whilst we focussed on the latter reaction sequences as routes to amino acids, we note that the dinitriles **64**, **65** and **67** might additionally be precursors of citric acid cycle intermediates.

Having found that the product of copper(II) driven oxidative cross-coupling of acetylene **63** and hydrogen cyanide **1** is a precursor of amino acids, we also investigated chemistry leading from acrylonitrile **71**, the known product of copper(I) catalysed cross-coupling of the same two substances (Figure 16). We were most interested in β-aminopropionitrile **72**, the ammonia adduct of **71**, as we saw it a potential source of lysine and arginine. A prebiotically plausible synthesis of these basic amino acids would strengthen the case for widespread peptide–RNA binding in early biology.

Photoreduction of β-aminopropionitrile **72** gave β-aminopropionaldehyde **73**, the amino group of which underwent an inherently favoured reaction with cyanamide **15** because of induced intramolecularity, through formation of the carbonyl addition product **74**. The carbinolamine tether of the resultant product, **75**, was then cleaved by reaction with hydrogen cyanide **1** giving the cyanohydrin **76**. Homologation of **76** using hydrosulfide driven reduction gave cyanohydrin **77** which underwent reaction with ammonia giving **78**, the aminonitrile precursor of arginine. Direct homologation of

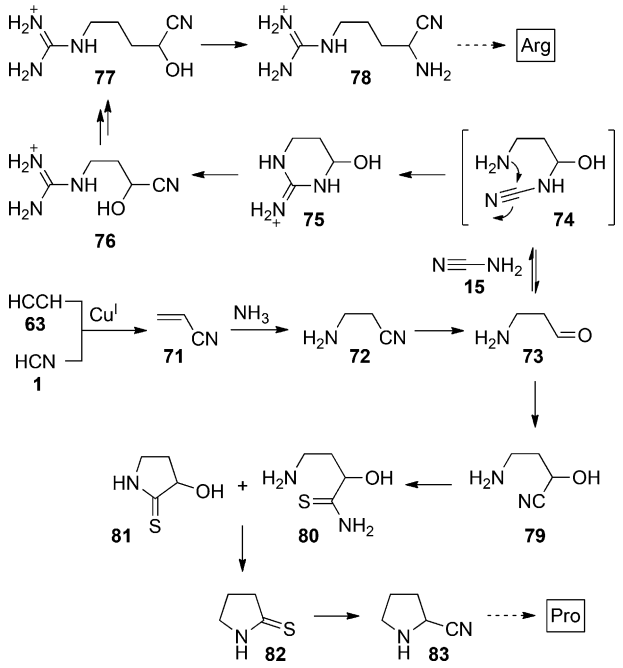

Figure 16. Synthesis of acrylonitrile **71** and reactions leading to amino acid precursors therefrom.

β-aminopropionaldehyde **73** was seen as a potential route to lysine, but cyclisation of intermediates prevented the formation of precursors to this amino acid. Thus, addition of hydrosulfide to **79**, the cyanohydrin of **73**, gave both the open chain α-hydroxythioamide **80** and the α-hydroxythiolactam **81**, and reduction of this mixture gave just the thiolactam **82**. Further reduction and addition of hydrogen cyanide **1** resulted in **83**, the aminonitrile precursor of proline.

Ordinarily, our inability to demonstrate a reductive homologation route to any particular amino acid might indicate that such a route does not exist, or simply that we have not (yet) found it. In the case of lysine, however, the cyclisation of intermediates such that a proline precursor is produced instead is a chemical indication that lysine might be a later addition to biology, first accessed by biosynthesis. Consistent with this, lysine is the one amino acid that violates the "class rule"—that each amino acid be activated by a class I *or* a class II aminoacyl-tRNA synthetase—being acted on by enzymes of subclass b from class I and class II in different organisms.[91,92]

10. Onwards and Upwards

With twelve amino acids, two ribonucleotides and the hydrophilic moiety of lipids synthesised by common chemistry, we feel that we have gone a good way to answering the first of the questions we posed at the outset. "Are completely different chemistries needed to make the various subsystems?"—we would argue no! We need to find ways of making the purine ribonucleotides, but hydrogen cyanide **1** is already strongly implicated as a starting material. We also need to find ways of making the hydrophobic chains of lipids, and maybe a few other amino acids, but there is hope in reductive homologation chemistry or what we have called "cyanosulfidic protometabolism".[86] Some have worried that the differences between the synthetic pathways we have uncovered and the biosynthetic pathways now used in biology mean that biology would have had to overwrite almost the entire reaction network. However, we would argue that the underlying chemistry now used by biology has almost zero chance of operating across the board efficiently enough to sustain life through the generation of all these products without enzyme catalysis. By synthesising compounds needed to initiate biology and sustain it in its earliest stages, the chemistry we have discovered could have provided the evolutionary incentive for biology to learn biosynthetic routes to the same products. Our results thus point towards the heterotrophic nature of the first living systems, and suggest that autotrophy evolved later.

The answer to the second question—"Would these chemistries be compatible with each other?"—is a bit more vague (thus far). The chemistries associated with the different subsystems are variations on a theme, but to operate most efficiently some sort of separation would seem to be needed. Because a late stage of our scenario has small streams or rivulets flowing over ground sequentially leaching salts and other compounds as they are encountered (Figure 17), it provides a very simple way in which variants of the chemistry could play out separately before all the products became mixed.[86]

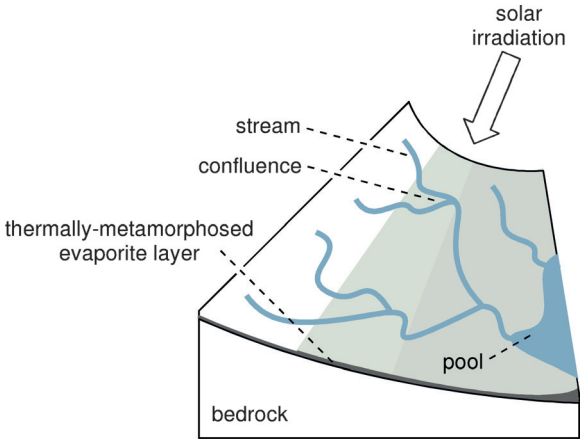

Figure 17. Late stage of the geochemical scenario.

Separate streams might encounter salts and other compounds in different orders and be exposed to solar radiation differently. Furthermore streams might dry out and the residues become heated through geothermal activity before fresh inflow of water. If streams with different flow chemistry histories then merged, convergent synthesis might occur at the confluence and downstream thereof, or products might simply mix. It would be most plausible if only a few streams were necessary for the various strands of the chemistry to operate efficiently before merger. Our current working model divides the reaction network up such that the following groups of building blocks would be made separately: ribonu-

cleotides; alanine, threonine, serine and glycine; glycerol phosphates, valine and leucine; aspartic acid, asparagine, glutamic acid and glutamine; and arginine and proline. Because the homologation of all intermediates uses hydrogen cyanide **1**, products of reductive homologation of **1**—especially glycine—could be omnipresent.

Life is more than a collection of building blocks and we need to understand how further synthesis could progress the system to the biopolymer stage and beyond. We have found it useful to consider this in the context of a graph with time (or system complexity) as the abscissa and degree of "aliveness" as the ordinate (Figure 18).[93]

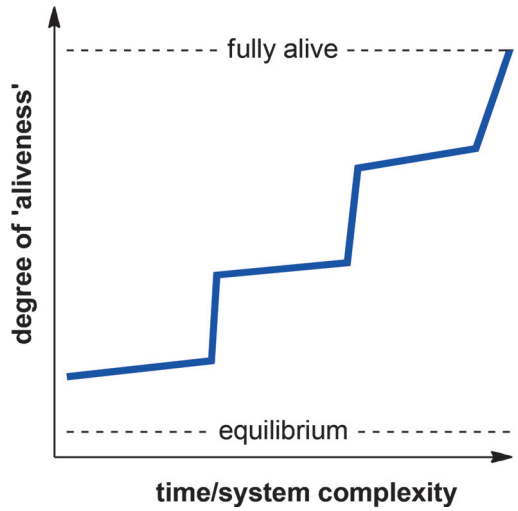

Figure 18. Transition of a system from the inanimate to the animate state.

There is little consensus on what constitutes a rigorous definition of life and this is accommodated in such a graph by having "aliveness" as a variable. The equilibrium state is undoubtedly inanimate and the end state animate, but what of intermediate states and the trajectory to life? A smooth increase in aliveness over time seems unlikely to us, as does a single transition from inanimate to animate, so we (and others)[94] prefer a series of steps. The steep increases might correspond to major innovations such as RNA replication, vesicle division, or translation, whilst the shallow increases correspond to combinations of optimisation and drift that set the stage for the next innovation. Optimisation might be through the process of mutation and selection, or occur by another mechanism, but we think that all upwards progress must be accompanied by energy dissipation to avoid the degradation of the system towards an equilibrium state.

The scenario and chemistry we have outlined suggest a few clues regarding the synthesis of biopolymers, which we hope might be productively followed up on. Firstly, the separation of the groups of building blocks allows that subsequent (partial) polymerisation chemistry might occur before or after mixing. Polymerisation before mixing could reduce the number of different peptide sequences made through compositional restriction. According to our current model, this would result in four groups of useful, compositionally restricted peptides with different bulk properties: polar (composed predominantly of alanine, threonine, serine and glycine); non-polar (valine and leucine derived), acidic (aspartic acid, asparagine, glutamic acid and glutamine derived) and basic (arginine and proline derived). The non-polar peptides would be produced alongside lipid precursors and might preferentially become incorporated into vesicles. The basic peptides would be equipped to interact with RNA, and the acidic peptides to bind metal ions. Conversion of the ribonucleotides to short oligonucleotides might be followed after mixing by ligation to enable replication, and some sort of (coded) aminoacylation and aminoacyl-transfer chemistry to synthesise more of any useful compositionally restricted peptides. We have long been fascinated by the prospect of replicating RNA by ligation of triplets and having simultaneous coded peptide synthesis take place.[28,95] Given that the subunit interface of the ribosome is apparently more recent than the peptidyl transferase core domain,[96] then separate evolution of the two ribosomal subunits is suggested. Synthesis of compositionally restricted (but not sequence coded) peptides could have been the driver for evolution of the large ribosomal subunit, and template-directed trinucleotide ligation the driver for evolution of the small subunit (the latter's movement along mRNA in triplet steps then making sense).[97]

Clearly there is a lot more to do before we can understand how life originated,[98,99] but the way in which the building blocks of biology correspond to products of hydrogen cyanide chemistry surely suggests that life emerged "out of the blue".

Acknowledgements

It is a pleasure to acknowledge the practical skills and intellectual input of the members of my research group, past and present. I thank them wholeheartedly for their dedication and good humour, particularly when faced with the difficult challenges I set them—seeing them progress has been my greatest professional pleasure.

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I owe a final debt of gratitude to Albert Eschenmoser and not just for (inadvertently) stimulating me to move into the origin of life field. His chemical accomplishments are awe-inspiring and penetrating insight radiates from his beautifully written papers—he set the standards that we should all aspire to. Over the years, conversations with him have been hugely enjoyable and his wise counsel has been much appreciated. This Review is accordingly dedicated to him with thanks on the occasion of his 90th birthday.

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[98] This Review is based on a lecture that I gave at the Bürgenstock Conference in 2014, and, as such, focuses on work from my group. For an excellent survey of the field in general, see: K. Ruiz-Mirazo, C. Briones, A. de La Escosura, *Chem. Rev.* **2014**, *114*, 285 – 366.
[99] Understanding how life originated historically on Earth may assist efforts to create synthetic life (P. Strazewski, *Isr. J. Chem.* **2015**, *55*, 851 – 864) and should aid our understanding of the prospects for life elsewhere.

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