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]

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]).

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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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. Orió’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 B12 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 intramolecularly,[13] or those in which a functional group in a molecule displays out of the ordinary reactivity due to its particular molecular context. Sequential occurrence 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

\[
\begin{align*}
NH_3 &\rightarrow NH_2 \\
HCN &\rightarrow NH_3
\end{align*}
\]

Figure 2. Orió’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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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, although aldopentoses 6 or their derivatives can be preferentially formed by formose reaction variants starting with preformed C3- and C4-sugars. 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-hydroxylaldehydes, 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 C3- (and C4-) 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, 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 Price[96]—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.[33] 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 recurrently 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 RNA 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 oxyanogen 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 I 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 aminoazoline 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 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 aminoazolines ribo- and arabino-12, and cyanoacetylene 18 (Figure 5).\[^{[27]}\] The reaction between ribo-12 and 18 gave α-cytidine 19 and a chromatographically
“faster moving product” which we subsequently showed was the cyanoethylidene acetal. Reaction in the arabino-series was to give arabino-cytidine and we also found the 3’-Z-cyanovinylether thereof. The reactions involve initial cyanovinilation of the endocyclic N-atom of the aminooxazoline, followed by closure to the anhydronucleosides. Hydroxide (generated from water through the protonation of the conjugate bases of arabino) then partly deprotonates the 3’-OH group allowing further cyanovinilation by excess cyanoacetylene. The resultant adducts, along with residual anhydronucleosides, 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. If the cyanovinilation reaction is carried out under conventional, non-prebiotic conditions in N,N-dimethylacetamide it gives the conjugate bases of the anhydronucleosides, which can then be converted to various salts of arabino by addition of acids.

Orgel found that α-cytidine could be photoanomerised to the β-isomer (Figure 6a), but the yield was too low and destroyed most of these nucleotides. The canonical pyrimidines undergo various photoisomerisations including hydration of the 5,6-double bond (Figure 6b). 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 deconstructive photochemistry we had observed for most cytidine nucleotides occurs from the unhydrated state.

There was, however, one nucleotide, β-cytidine-2’,3’-cyclic phosphate (Figure 7), that was remarkably stable to irradiation, suffering only from partial conversion to the corresponding uridine nucleotide (Figure 6c). The cis-fused five-membered ring connecting C-2’ and C-3’ on the α-face of uridine nucleotide 28 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, but less time for other, destructive photochemistry. After the irradiation ceases, final elimination of the 5’-OH group of 29 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 — that is, if we could find a synthesis of 27!

Nagyvary had showed in a seminal paper that, the 3’-phosphate of the anhydronucleoside arabino-23, as prepared by conventional synthesis, underwent smooth isomerisation to 27 (Figure 7) 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, so we subjected arabino-23 to conditions. On the basis of the generally (~10-fold)
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.\textsuperscript{[44]} It transpires that this is due to a stereoelectronic effect whereby a lone pair of the 5'-OH group of \textit{arabino-23} interacts with an antibonding orbital at C-2 of the nucleobase.\textsuperscript{[53]} 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 cyanovinylolation of \textit{arabino-12} in water at the stage of the anhydronucleoside \textit{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 cyanovinylolation stage. We were, though, slightly trepidacious because of the known propensity for cyanoacetylene 18 to react with phosphate giving Z-cyanovinylphosphate—\textsuperscript{[52]} if this reaction was more favourable than the cyanovinylolation of \textit{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 aminoazoline \textit{arabino-12}, but before its 3'-OH group. Along with the pH buffering it affords, this chemical buffering by phosphate makes the conversion of \textit{arabino-12} to the anhydronucleoside \textit{arabino-23} extremely clean, and the latter compound was produced in >90% yield.\textsuperscript{[44]}

Before summarising the synthesis we had thus far achieved, the question of absolute stereochemistry needs addressing. \textit{Ribo-12} is the major aminoazoline 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 \textit{arabino-12} to the pyrimidine ribonucleotides 27 and 28 involving C-2' stereoinversion. On cooling, \textit{ribo-12} selectively crystallises from the solution of products,\textsuperscript{[49]} making \textit{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 aminoazoline stereoisomer. However, there was a troubling aspect to this as we found that if the input glyceraldehyde 5 is non-racemic, the \textit{ribo-12} that crystallises has an enhanced enantiomeric excess (ee), and above a certain threshold input 5 ee, is enantiomerically pure.\textsuperscript{[49]} This is the behaviour of a true conglomerate—although \textit{ribo-12} actually forms an enantiomorphously twinned one—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 \textit{arabino-12} that was left in solution, we were missing out. Accordingly, we wondered if we could find conditions under which \textit{ribo-12}, enantioenriched through crystallisation, would convert to, or interconvert with, \textit{arabino-12}. A potential mechanism whereby the aminoazolines \textit{ribo-} and \textit{arabino-12} might interconvert sprang to mind based on our earlier pondering about the interconversion of the furanose and pyranose forms of \textit{lyxo-12} (Figure 4a,b). If the iminium ion \textit{ribo-13} derived by ring opening of \textit{ribo-12} could be deprotonated at C-2', then a substituted 2-aminoazolone 32 would result (Figure 8), and if this underwent C-2' protonation, equilibration with \textit{arabino-13}, and thence \textit{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 \textit{ribo-12} in phosphate buffer for a prolonged period. Interconversion with \textit{arabino-12} was indeed observed, though there was also some hydrolysis to the corresponding oxazolidinones \textit{ribo-} and \textit{arabino-26}.\textsuperscript{[53]} We could also detect what we thought was the intermediate substituted 2-aminoazolone 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 \textit{ribo-} and \textit{arabino-12} supported our assumption that interconversion of these two aminoazolines 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 and others that we viewed as naysayers. In the latter category were those who cited earlier criticism of multistep prebiotic synthesis per se—\textit{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}—to criticise our work selectively.\textsuperscript{[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.” On the other hand, calculations suggested that our experimentally demonstrated route (Figure 9) had the right free energy profile to be prebiotically relevant.

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 phosphate inclusions in meteorites, and this got us thinking about impacts. After the collision 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).

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.

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

Figure 10. Far side of the Moon (NASA Apollo 16 photograph AS16-3021).
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, \((\text{Fe}_3\text{Ni})_3\text{P}\). 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, \(\text{Fe}_3(\text{PO}_4)_2\cdot8\text{H}_2\text{O}\). [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 anhydro-nucleoside \textit{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 lookout 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 11a). [30,71] Tricyanocuprate(I) 35 first undergoes photoreduction 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) 138, and, finally, cyanation of 38 regenerates 35. This cycle had been studied for its intrinsic interest by
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$_2$O/D$_2$O mixture, neutralised the resultant solution and irradiated it. As it happened, purines were not produced but reductive synthetic chemistry took place.\[72\] The $^1$H NMR spectrum of the reaction products (Figure 11b) 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 oxogenous 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 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 cyanoformamidate 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, glycononitrile 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 undoing 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 favourability 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 cyanoferate(II) salts gives products that depend on the nature of the cation(s) with sodium and potassium cyanoferate(II) giving sodium and potassium cyanide.\[76\] This suggested a means of obtaining concentrated cyanide solutions from a solution of cyanoferates(II) produced by
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.\cite{77, 78}

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\cite{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.\cite{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 glycoaldehyde 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).\cite{82}

$$\text{Ca}_2[\text{Fe(CN)}_6] \xrightarrow{660^\circ C} 2\text{Ca} \text{(CN)}_2 + \text{Fe(CN)}_2$$

$$\text{Ca} \text{(CN)}_2 \xrightarrow{660^\circ C} \text{CaCN} + \text{C} \xrightarrow{1000^\circ C} \text{CaC}_2 + \text{N}_2$$

$$\text{Mg}_2[\text{Fe(CN)}_6] \xrightarrow{315^\circ C} 2\text{Mg} \text{(CN)}_2 + \text{Fe(CN)}_2$$

$$\text{Mg} \text{(CN)}_2 \xrightarrow{395^\circ C} \text{MgCN} + \text{C}$$

$$3\text{Mg} \text{(CN)}_2 \xrightarrow{420^\circ C} \text{Mg}_2 \text{N}_2 + 3\text{C} + 2\text{N}_2$$

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]

9. Linkage of all Subsystems 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-hydroxaldehydes, which then undergo partial deoxygenation; the former are first deoxygenated to thioamides, which then undergo reduction to aldehydes. Thus photoreduction of hydroxothioamide 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-hydroxaldehyde did not prove possible, then homologation via the thioamide route is all that is possible for ketone 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 acetalddehyde 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 oligoacetylenes is known and we were by now very aware of the corresponding oxidative self-coupling of hydrogen cyanide 1 to give cyanoacetylene 18. 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, CuC₃N₃. 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) 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.

Cyanoacetylene 18 is known to react with hydrogen cyanide 1 to give malononitrile 64 and the cyanohydrin 65, and these two compounds were produced when we added an excess of hydrogen cyanide 1 to a slurry of CuC₃N₃ in water. Reaction of this product mixture with ammonia then gave the aminonitrile 66, a precursor of both aspartic acid and asparagine. Photoreduction of malononitrile 64 with hydroxylamine proceeded cleanly in stages giving first succinonitrile 67 and then the semialdehyde 68. Reaction of 68 with hydrogen cyanide 1 gave the cyanohydrid 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-aminoacrylonitrile 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-aminoacrylonitrile 72 gave β-aminoacrylonitrile 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 cyanohydride 76. Homologation of 76 using hydroxylamine driven reduction gave cyanhydrin 77 which underwent reaction with ammonia giving 78, the aminonitrile precursor of arginine. Direct homologation of...
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]

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).

![Figure 18](image)

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) 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. Given that the subunit interface of the ribosome is apparently more recent than the peptidyl transferase core domain 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).

Clearly there is a lot more to do before we can understand how life originated 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”.

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[22] Based on the incorrect premise that aldolisation of 4 and 5 involves addition of the enolate of 4 to the carbonyl group of 5, and on the supposition that (therefore unwanted) enolisation of 5 would be prevented by complexation with borate, Benner et al. investigated the effect of borate on the aldolisation of 4 and 5: A. Ricard, M. A. Carrigan, A. N. Olcott, S. A. Benner, Science 2004, 303, 196. Although borate stabilised the aldolopenose products, it did not much alter their initial yield suggesting either that the inherently favoured aldolisation involving 7 was still operating (in which case the supposition that borate should suppress enolisation of 5 is wrong), or that aldolisation was now involving the enolate of 4 adding to the carbonyl group of 5 as per the incorrect initial premise (in which case why suppress an inherently favoured reaction to allow a second best reaction to prevail if both give the same products? The stabilisation by borate not being worth the penalty associated with the prebiotic implications for the emergence of life on Earth must surely await the outcome of a thorough investigation of the propensity of these XNAs to be produced by constitutional self-assembly. If no synthesis along favourith lines is ultimately demonstrable, then regardless of their wonderful functions, these XNAs can be sidelined as evolutionary forerunners of RNA.
such a connection has been experimentally demonstrated before: J. Oró, S. S. Kamat, Nature 1961, 190, 442–443. The connection has also been made by Eschenmoser.[7]


[94] At the time that I conceived the “aliveness” vs. time diagram used in Ref.[93], I was unaware of its striking similarity to Kuhn’s “knowledge” vs. time diagram: H. Kuhn, Naturwissenschaften 1976, 63, 68–80. I would like to thank a referee for making me aware of this reference, and cite in my defence a comment that Eschenmoser made to me (Workshop on the Origin of Life, Stockholm, June 8–10, 2006): “Not knowing the literature is a sin, knowing it is a virtue. But who has only virtues?”


[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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