Technische Universität München
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# Synthesis of Nitrogen-containing Biomolecules under Abiotic Hydrothermal Conditions

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*Ich bin nur ein großer Träumer,*
*doch mit Träumen fängt es an!*

Frank Schindel, *Der größte Träumer*
(Opening of *Digimon Tamers*)

# Zusammenfassung

Der Ursprung des Lebens, wie wir es heute kennen, bleibt trotz intensiver Forschung ein umfassendes Geheimnis. Im Laufe der Zeit, und vor allem dadurch das immer neue Erkenntnisse aus der Forschung zu frühen Erdzeitaltern erlangt werden, entwickelten sich zahlreiche Theorien, wie das Leben entstanden sein könnte.

Das Hauptaugenmerk dieser Arbeit lag darauf, die Hypothese eines möglichen Urmetabolismus unter präbiotischen, hydrothermalen Bedingungen weiterzuentwickeln. Hierfür wurden anorganische Moleküle wie CO, $CO_2$, $H_2O$ und Acetylen ($C_2H_2$) durch Metallsulfid-Katalyse zur Reaktion gebracht. Dabei wurden Bedingungen simuliert, die einer hydrothermalen, vulkanischen Umgebungen gleichen sollen.

Unter diesen Bedingungen konnten in der Vergangenheit bereits Intermediate des Citratzyklus, kurzkettige Fettsäuren und Thiophen als mögliche Biomarker synthetisiert werden. Ziel dieser Arbeit ist es nun, das bestehende Reaktionsnetzwerk um Stickstoff in Form von Ammoniumchlorid zu erweitern. Durch den Einsatz von Ammoniumchlorid konnten Vorläufer wichtiger Biomoleküle synthetisiert werden: Aminosäuren und Fettsäureamide als Vorläufer von Peptiden und schließlich Proteinen, Nukleobasen welche ein essentieller Bestandteil der Informationsübertragung in der modernen Biochemie sind, da sie in Form der DNA Informationen speichern und in Form der RNA die Baupläne für die Bildung von Proteinen darstellen und Pyrrol, welches in Form der Tetrapyrrole wie zum Beispiel Porphyrin eine wichtige Rolle als Cofaktor spielt. Hierbei ist es vor allem die Fähigkeit der Tetrapyrrole, Metallionen in ihrer Mitte zu binden, die bei diversen enzymatisch katalysierten Vorgängen und Reaktionen von Bedeutung ist. Zahlreiche Beispiele von modernen Metall-Porphyrin-Komplexen lassen zudem Rückschlüsse auf präbiotische Vorläufer zu, da man die für diese Arbeit verwenden Metalle in solchen wichtigen Komplexen wiederfinden kann.

Das Hauptwerkzeug dieser Arbeit war die Gaschromatographie gekoppelt mit Massenspektrometrie (GCMS). Sie ermöglichte es, Gemische aus kleineren Molekülen effizient aufzutrennen und zu analysieren. Größere Moleküle, deren Siedepunkt ein Verdampfen in der GCMS nicht zulässt, wurden mit einer *tert*-Butyldimethylsilyl (TBDMS)-Gruppe behandelt, um niedersiedende Derivate zu erhalten. Durch die Stabilisotopen-Markierung der Edukte konnten die erhaltenen Produkte zudem verifiziert werden, da sich die Erhöhung der Masse direkt im Massenspektrum widerspiegelt und somit auch Aussagen über die Zusammensetzung der Produkte aus den verschiedenen Edukten und die Bildung an sich zulässt. Dadurch konnten auch Rückschlüsse auf mögliche Kontrollmechanismen gezogen werden. Beispielsweise konnte durch die Nutzung von $^{13}C_2H_2$ gezeigt werden, dass Alanin in der Synthese aus anderthalb Molekülen Acetylen gebildet wurde. Im

Gegensatz dazu wurde β-Alanin aus je einem Molekül Acetylen und CO synthetisiert. Nun ist β-Alanin als Anti-Markownikow-Produkt leichter zu bilden als das Markownikow-Produkt α-Alanin, was sich auch in den erhaltenen Mengen der beiden Aminosäuren in der Reaktionslösung zeigt. Wie wir aus der modernen Biochemie wissen, ist nun α-Alanin und nicht β-Alanin die Aminosäure, die sich durchgesetzt hat. Die Natur hat also das schwerer zugängliche Alanin gewählt, um Leben aufzubauen. Zusätzlich konnte festgestellt werden, dass die verschiedenen Übergangsmetalle als Katalysatoren bei der Bildung der Produkte unterschiedlich erfolgreich eingesetzt werden. Auch die Verwendung von Gemischen der Metalle zeigt bei einigen Reaktionen eine außerordentliche Wirksamkeit. Dies ist ein weiterer Hinweis auf einen möglichen frühen Kontrollmechanismus, da die Natur möglicherweise auf eine Vielzahl von Metallen gesetzt hat, anstatt die Entstehung des Lebens an ein einziges Metall zu koppeln.

Insgesamt konnten fünf Aminosäuren, 13 Amide, Pyrrol, Dimethylpyrrol und Uracil als Vertreter der Nukleobasen in dieser Arbeit gefunden, und durch oben beschriebene Versuche mit Stabilisotopen-Markierung verifiziert werden. Zusätzlich wurde der Einfluss von verschiedenen Reaktionsparametern getestet.

# Abstract

Despite intensive research, the origin of life as we know it today remains a mystery. Over time, and especially as new knowledge from early geological eras have been discovered, numerous theories have emerged as to how life might have originated.

The focus of this work was to expand the hypothesis of a possible primordial metabolism under prebiotic, hydrothermal conditions. For this purpose, inorganic molecules such as CO, $CO_2$, $H_2O$ and acetylene ($C_2H_2$) were made to react using metal sulphide-based catalysis. The simulated conditions are supposed to resemble a hydrothermal, volcanic environment.

Under these conditions, intermediates of the citrate cycle, short-chain fatty acids and thiophene as possible biomarker have already been synthesised in the past. The objective of this work is to expand the existing reaction network to include nitrogen in the form of ammonium chloride. By using ammonium chloride, precursors of important biomolecules could be synthesised in this work: amino acids and fatty acid amides as precursors of peptides and finally proteins, nucleobases, which are an important part of information transmission in modern biochemistry, as they store information in the form of DNA and represent the blueprints for the formation of proteins in the form of RNA, and finally, pyrrole, which plays an important role as a cofactor in the form of tetrapyrroles, such as porphyrin and nucleobases. It is primarily the ability of tetrapyrroles to bind metal ions in their centre that is important in various enzymatically catalysed processes and reactions. Numerous examples of modern metal-porphyrin complexes also allow conclusions about prebiotic precursors, as the metals used for this work can be found in such important complexes.

The most important tool for this work was gas chromatography coupled with mass spectrometry (GCMS). This allowed mixtures of smaller molecules to be efficiently separated and analysed. Molecules, whose boiling points do not allow evaporation in GCMS, were treated with a *tert*-butyldimethylsilyl (TBDMS) group to produce lower boiling derivatives. The products obtained could also be verified by stable isotope labelling of the educts, as the increase in mass is directly reflected in the mass spectrum, and thus allows conclusions to be drawn about the composition of the products from the different educts. This also allowed conclusions about possible control mechanisms. For example, by using $^{13}C_2H_2$ it has been shown that alanine is synthesised from one and a half molecules of acetylene. In contrast, β-alanine was synthesised from one molecule each of acetylene and CO. Now, β-alanine is easier to form as an anti-Markownikow product, which is also reflected in the amounts of the two amino acids obtained in the reaction solution. As we know from modern biochemistry, α-alanine and not β-alanine is the amino acid that has prevailed. So nature chose the more difficult way to access α-alanine to build life. It has also been found that the

different transition metals are used with varying degrees of success as catalysts in the formation of different classes of substances. The use of mixtures of metals also shows extraordinary effectiveness in some reactions. This is also an indication of a possible early control mechanism, as nature may have relied on a variety of metals rather than tying the emergence of life to just one.

In total, five amino acids, 13 amides, pyrrole, dimethylpyrrole and uracil, as a representatives of the nucleobases could be synthesised in this work and verified by stable isotope labelling experiments described above. In addition, the influence of various reaction parameters was tested.

# List of Abbreviations

Molecules

| | |
|---|---|
| $C_2H_2$ | acetylene |
| $NH_2$ | ammonia |
| $CO_2$ | carbon dioxide |
| CO | carbon monoxide |
| COS | carbonyl sulphide |
| HCN | cyanide |
| $H_2S$ | hydrogen sulphide |
| $H_2$ | hydrogen |
| $CH_4$ | methane |
| $N_2$ | nitrogen |
| $P_4O_{10}$ | phosphorus pentoxide |
| $H_2O$ | water |

Definitions

| | |
|---|---|
| ala | alanine |
| 2-KG | alpha-ketoglutarate |
| bya | billion years ago |
| Cit | citrate |
| CoA | coenzyme A |
| DNA | deoxyribonucleic acid |
| Fum | fumarate |
| GC/MS | gas chromatography-mass spectrometry |
| GNA | glycol nucleic acid |
| Isocit | isocitrate |
| LUCA | last universal common ancestor |
| Mal | malate |
| Oxac | oxaloacetate |
| PNA | peptide nucleic acid |
| rTCA | reductive citric acid cycle |
| RNA | ribonucleic acid |
| TNA | threofuranosyl nucleic acid |

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# 1 INTRODUCTION

## 1.1 Abiogenesis or The Many Ways of Getting Life Started

Abiogenesis describes the process from non-living, abiotic molecules to the first organism. In the beginning, only simple gases and molecules existed on the early Earth. The first biomolecules, such as amino acids, fatty acids and nucleobases, were formed by simple chemical reactions. The next step was polymerization. The simple compounds must form larger chains, such as RNA, DNA, or peptides, which are necessary to perform specific life functions. To avoid drifting apart and maintaining chemical imbalances, these biopolymers must be separated from the environment to create their own chemical habitat. Ultimately, these protocells can develop further through evolution and thus create the diversity of life. This process, from early environments to the diversity of life, is shown schematically in Figure 1.

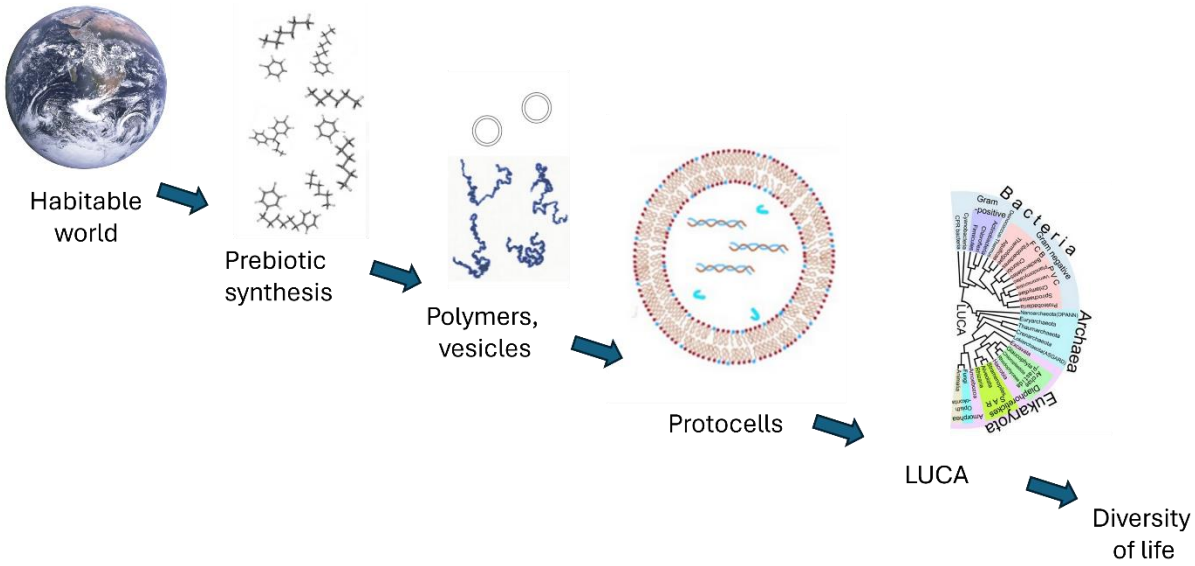



**Figure 1:** Schematic sequence of abiogenesis from the formation of the first biomolecules to the diversity of life. Adapted from Walker *et al.* 2017 (CC BY-SA 4.0)

It is likely that abiogenesis already started at the late stages of the first eon of early Earth, the Hadean eon. The first evidence of the formation of Earth is from a time 4.5 bya (Dalrymple 2001). The first fossils of living species found, however, are from ~3.8 bya (Dodd *et al.* 2017; Nutman *et al.* 2016). Therefore, life must have arisen at some point in the period between these two points in time. This period is also known as the Hadean era (4.5–4.0 bya).

### 1.1.1 The World around 4.3 bya, the Hadean Eon

The Hadean eon began with the formation of the Earth from the solar nebula about 4.5 bya (Dalrymple 2001). This process involved the accumulation of dust and gas, leading to the formation of planetesimals and, ultimately, Earth. In the beginning, Earth was a harsh place with conditions unsuitable for life. Therefore, the name of this eon

was taken from the greek “Hades”, the name of the underworld. The early Earth was searing, and temperatures were high enough to initially prevent the formation of a solid crust. The heat was caused by residual heat from accretion, radioactive decay, and frequent collisions with other celestial bodies. In particular, the late heavy bombardment, when the early Earth was exposed to a disproportionate number of asteroid collisions, which contributed to the persistence of these hostile conditions by breaking up any crust that had formed (Marchi *et al.* 2014). The heat also forced all existing water, which was brought by comets or by the reaction of minerals with primary $H_2$ (Abe *et al.* 2000), into the gas phase and converted all early accreted carbon into atmospheric carbon dioxide ($CO_2$) (Zahnle *et al.* 2007). With the end of the heavy bombardment, the Earth had the opportunity to cool, and water vapor condensed to form the first oceans (Mojzsis *et al.* 2001). The presence of liquid water was a critical factor in the subsequent emergence of life, since it provided a suitable environment for aqueous chemical reactions. As the Earth cooled, its atmosphere also changed. The so-called first atmosphere, consisting mainly of vapor and simple hydrides, changed into the second — or prebiotic — atmosphere (Zahnle *et al.* 2010). There is no consensus on the nature of this second atmosphere that prevailed during the Hadean period. It could have been of a reducing nature, i.e., consisting of gas mixtures like $CH_4/N_2$ or $CO_2/H_2/N_2$ (Zahnle *et al.* 2020). Due to prebiotic volcanic activity, it could also have been more neutral, consisting of $CO_2/N_2/H_2O$. After the formation of the first ocean and the primordial atmosphere, there was an abundance of possible niches on the early Earth that could have been conducive to the formation of early life. Various forms of energy are also being considered that could have enabled the first chemical reactions to produce biomolecules from the gases present.

### 1.1.2 The Question of Where and How

Since the evidence of the origin of life is lost, the investigation of the origin of life is only possible from a certain distance. There are two possible approaches: The so-called “top-down” approach and the so-called “bottom-up” approach (Peters & Williams 2012). The “top-down” approach tries to investigate the origin of life from a modern-day point of view. It assumes that specific properties such as metabolites (Benner *et al* 1989), biochemical systems such as translation (Fox 2010; Hury *et al.* 2006; Woese 2000), or metalloproteins (Russel 2003; McGlynn *et al.* 2009; Wächtershäuser 2006) have left traces in modern biochemistry over time, allowing us to trace them back to an initial biotic or even prebiotic chemistry. The “bottom-up” approach takes a different view. Here, conditions similar to those on the early Earth are simulated through a more experimental approach, and an attempt is made to recreate the reactions that led to the formation of life. This approach corresponds to the presumed course of abiogenesis as presented above. There are two central questions regarding the designs of these experiments:

1. What was the original energy source that made prebiotic reactions possible?
2. What possible environment could have enabled the emergence of life?

### Different Types of Energy to Start the Engine of Life

Louis Pasteur established the beginnings of the modern-day origins of life research by breaking the prevailing dogma of his time that life could arise spontaneously under everyday conditions. His experiments showed that boiled yeast water kept in an airtight container remained sterile for weeks. However, as soon as he restored the air supply, a patch of mold soon formed. From this and other experiments, Pasteur concluded that life cannot arise spontaneously, but can only emerge from other life (Ligon 2002).

One of the first and probably most important experiments that recreated the conditions of the early Earth and led to the formation of biomolecules was the so-called Miller-Urey experiment (Miller 1953). An electrical discharge was used as the energy source, leading to the formation of, e.g., alanine and glycine in a simulated primordial atmosphere consisting of $CH_4$, $H_2$ and $H_2O$. Over time, there have been experiments with many different energy sources leading to the formation of simple biomolecules, including UV (Bell *et al.* 2020; Harkiss *et al.* 2019; Sagan & Khare 1971; Steinman *et al.* 1968; Sutherland 2016), X-ray (Takahashi *et al.* 1999; Utsumi & Takahashi 1998) and proton irradiation (Kobayashi *et al.* 1990; Kobayashi *et al.* 1999) as well as shock heating from 90 °C (Lowe *et al.* 1963) to over 200 °C (Islam 2001; Marshall 1994; Yanagawa & Kobayashi 1992).

### The Environment: Hot, Cold, Wet, Dry?

One possible area for life to emerge on early Earth is the ocean itself. In fact, it plays a crucial role in the so-called “primordial soup theory”. Miller’s experiments, for example, are based on this theory. It can be used to explain the formation of many early biomolecules, but it also has a major drawback: the products formed are washed away and distributed throughout the more abundant ocean, so higher concentrations can hardly be achieved. This has led to other theories about where life originated that circumvent this negative concentration factor.

### Impact Craters

The impact crater of a meteorite provides a good place for the origin of life for several reasons. First, organic precursors can be delivered by the meteorite; for example, amino acids have been detected in carbonaceous meteorites of various types from C1 (Botta *et al.* 2007; Burton *et al.* 2014; Glavin *et al.* 2010) over C2 (Cronin & Pizzarello 1983; Kvenvolden *et al.* 1970; Oró *et al.* 1971) to C3 (Burton *et al.* 2013; Glavin *et al.* 2010; Pizzarello *et al.* 2012; Poulos 2014). In addition, the energy generated by the impact itself, can cause the formation of simple organic molecules (McKay & Borucki 1997). Impacts in the ocean or on newly formed thin landmasses could also lead to the

emergence of hydrothermal vents (Cockell 2006), creating an aqueous environment for the origin of life. These hydrothermal vents, unlike those at the seabed, are not further heated and therefore have the possibility to cool down completely (Osinski *et al.* 2001). This allows for the formation of more complex structures because their stability in an aqueous environment is increased at colder temperatures (Shock & Schulte 1998).

### Warm and Cold Ponds

> "It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh what a big if) we could conceive in some warm little pond with all sorts of ammonia and phosphoric salts, light, heat, electricity etc. present, that a protein compound was chemically formed, ready to undergo still more complex changes at the present such matter would be instantly devoured, which would not have been the case before living creatures were formed."

Charles Darwin wrote this in a conversation with his friend Joseph D. Hooker in 1871 (as cited in Peretó *et al.* 2009). Initially just a thought among friends, the idea of the warm little pond evolved over time into a plausible environment for the origin of life. Warm (or cold) ponds are smaller bodies of water that, in the absence of continents, have formed on smaller islands that arise around so-called "hotspot" volcanoes above sea level (Sleep 1990). This volcanic environment ensures a constant supply of reducing gases from the volcano's plume and energy from discharges in the ash column above the volcano's vent. Through ash eruptions, the reagents for the formation of the first biomolecules can now be introduced into the pond through fallout from eruptions or washed down from the flanks of the volcano (Bada & Korenaga 2018; Scheu *et al.* 2017). The water that accumulates in these ponds evaporates over time and is then replenished. These so-called wet-dry cycles could drive prebiotic reactions to greater complexity (Bada & Korenaga 2018; Becker *et al.* 2018).

### Hydrothermal Systems

Hydrothermal vents are hot springs, found on mid-ocean ridges. At a hydrothermal vent, cold seawater seeps into hot basaltic magma chambers, heats up and flows out through the vent (Colín-García 2016). The hot seawater that flows back into the ocean can reach temperatures of up to 400 °C, creating a high temperature gradient when it hits cold seawater. It is also rich in dissolved minerals, which deposit around the vent and thus form the typical chimney structure and the precipitated plume. Since hydrothermal vents are located at a depth of 2000–3000 meters below metric sea level, the water column above them generates a pressure of several hundred bar (Colín-García 2016).

They can now be found in areas where plate tectonics have induced volcanic activity. They can exist for hundreds of years or just a few decades. In general, hydrothermal

vents are divided into two types, based on the distance to the magma chamber and the typical colour of the vent cloud:

“Black smokers” (Figure 2) emit water rich in dissolved minerals from the crust, mostly iron and other metal sulphides. Several minerals precipitate when they come into contact with cold ocean water, creating a black, chimney-like structure around each vent. The water reaches temperatures of up to 400 °C, and the pH is comparatively low (Colín-García 2016).

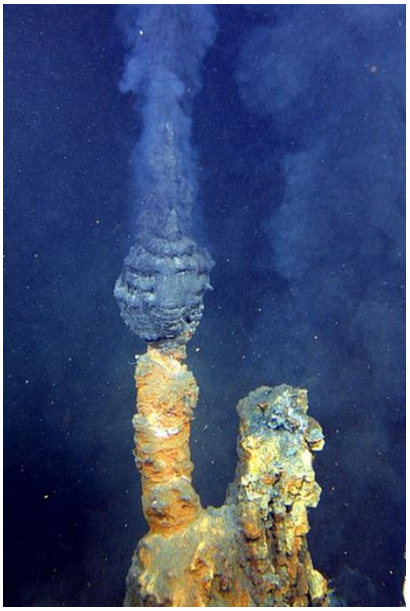


**Figure 2:** Black smokers known as “the Brothers”. (National Oceanic and Atmospheric Administration, public domain)

The water flowing out of “white smokers” has a fundamentally different composition, consisting mainly of sulphates and calcium, which is responsible for the white colour (Figure 3). White smokers vent more slowly and, due to the greater distance to the magma chamber, discharge water with slightly lower temperatures (220–330 °C) than “black smokers.” For these reasons, a substantial portion of the dissolved metals already precipitates in the chimney and is not released into the plume, resulting in its white colour (Schwander *et al.* 2023).

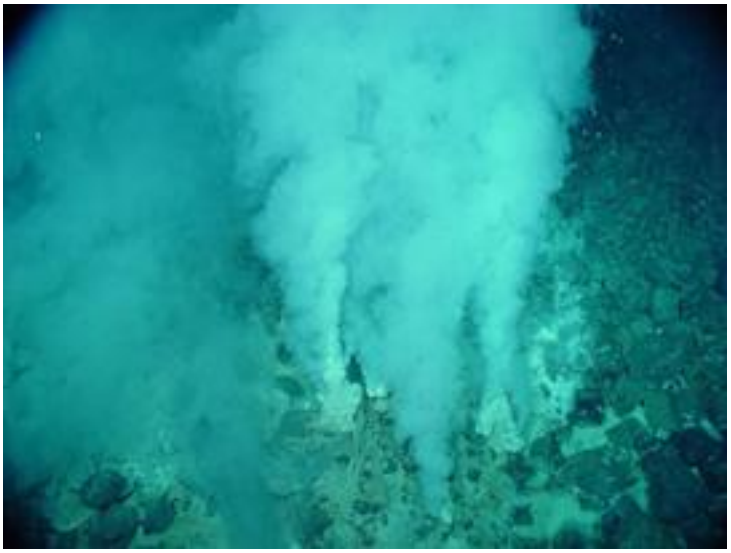


**Figure 3:** White smokers at Champagne Vent. (National Oceanic and Atmospheric Administration, public domain)

In 2000, a special type of deep-sea vents was discovered and named “lost city” (Figure 4). Unlike their smoker counterparts, “lost city” vents do not emit smoke. Their very low temperature allows all dissolved ingredients to precipitate immediately, forming chimneys of extraordinary height up to 60 meters (Kelley *et al.* 2001).

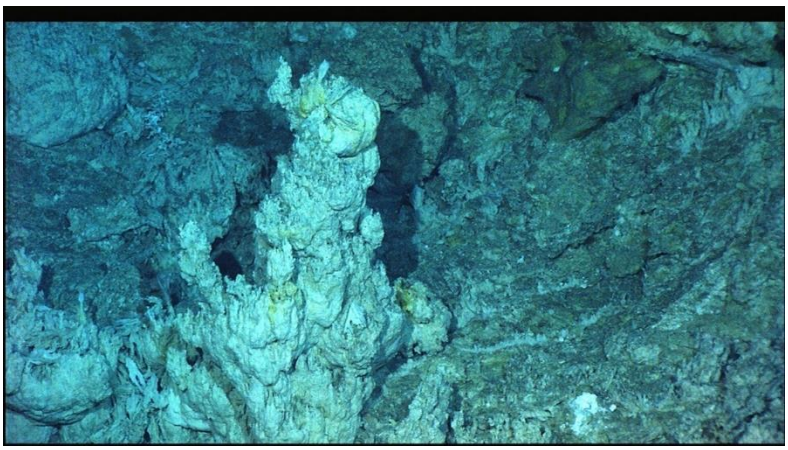


**Figure 4:** A white carbonate spire in the Lost City vent field. (NOAA Photo Library: expl2224, public domain)

Hydrothermal vents provide habitats for a wide variety of life forms, all of which are characterized by the fact that they obtain the energy they need to live from chemical sources (Gold 1992). Because this circumstance forms a possible analogy to the origin of life, soon after their discovery, hydrothermal vent systems were the subject of numerous considerations of a possible geological location for the emergence of life (Corliss *et al.* 1981; Holm & Charlou 2001; Lonsdale 1977). In 1988, the problems associated with the so-called “prebiotic soup theory” led Günther Wächtershäuser to develop a new thermophilic and chemoautotrophic theory based on pyrite-pulled

surface metabolism (Wächtershäuser 1988a, 1988b, 1992). Hydrothermal vents play an important role here, but their origin is volcanic rather than plate tectonic. It is possible that intense volcanic activity during the formation of the early Earth, when the crust was still much thinner, created these ancient volcanic hydrothermal vents.

### 1.1.3 Iron Sulphur World Theory

Wächtershäuser developed his theory further and came up with the concept of the "iron-sulphur world," which suggests that life originated chemoautotrophically (Wächtershäuser 1988a, 1990, 1992). He proposes that a pioneer organism generated by surface catalysis, preferably at transition metal sulphides, developed at the sites of volcanic hydrothermal vents.

#### The Pioneer Organism

In the entire abiogenesis process, the pioneer organism is located between the formation of the first biomolecules and the formation of low-molecular organic compounds (Figure 5). Therefore, it can be seen as a transition from primarily abiotic, inorganic chemistry to the first biomolecules and thus the biotic world.

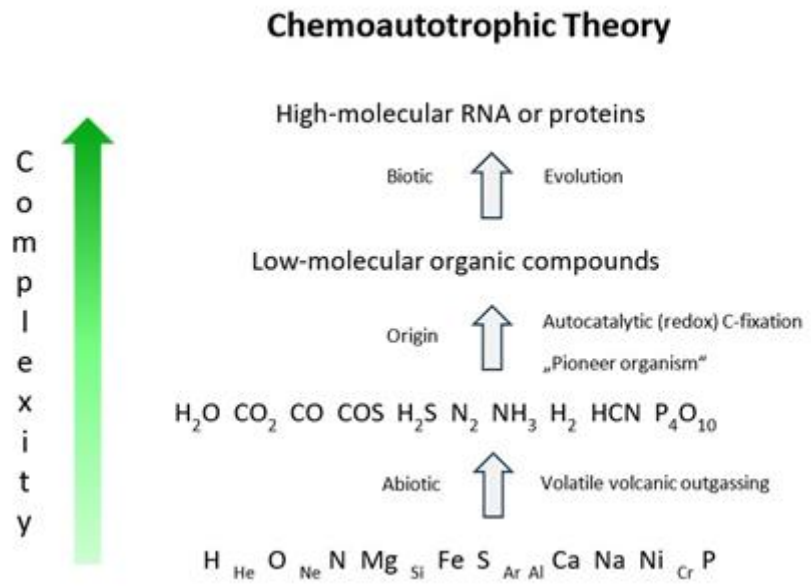



**Figure 5:** Chemoautotrophic theory by Günther Wächtershäuser. Volcanic gases deliver the resources necessary for the formation of the "pioneer organism". Low-molecular organic compound formation is the first step in biotic evolution and subsequent polymerization events. Adapted from Sobotta 2018.

Wächtershäuser's idea was that some properties based on modern biochemistry could be projected back to the origin. In a tree-like diagram (Figure 6b), the complexity of the hypothetical organisms decreases with each generation until they ultimately become a chemical entity that can reproduce and evolve: the "pioneer organism" (Wächtershäuser 2014). In stark contrast to this is the concept of "backwards

projection" (Lipmann 1965). Here, extant genetic features are directed to several precursors, resulting in a "one-to-one" projection and a community of ancestral cells that form the primordial broth (Figure 6a).

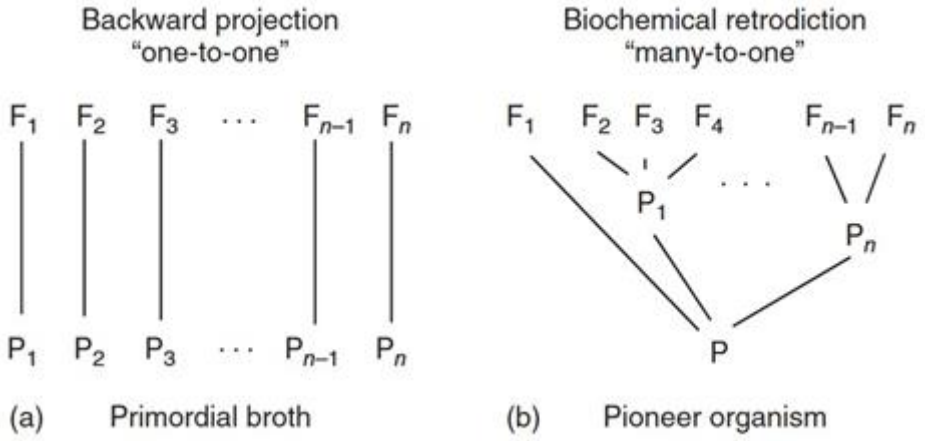



Figure 6: Schematic representation of (a) backward projection and (b) biochemical retrodiction. The left section (a) leads to a group of primitive cells with various genomes, and the right section (b) leads to a single ancestor, the "pioneer organism." P stands for precursor feature and F for current biochemical feature. Adapted from Wächtershäuser 2014.

With his iron-sulphur world theory, Wächtershäuser not only incorporated the principle of horizontal gene transfer through his many to one approach and thus created a reasonable construct based on modern biology, he also anchored it in geochemically plausible circumstances conducive to the emergence of life (Sobotta 2018). He postulates that the pioneer organism is based on the non-metal elements H, C, N, O, Se and S and at the same time on the catalytically active transition metals Fe, Co, Ni and W (Wächtershäuser 2014).

**What Does the Pioneer Organism Look Like?**

The pioneer organism should not be mistakenly thought of as a kind of first cell. Rather, it is organised as a mineral substructure-superstructure. Gases and water can pass through the (Fe/Ni)S-mineral-containing substructure. Over time, a carpet of organic material forms on this substructure, which is created due to reactions on the mineral surface (Wächtershäuser 2006), promoted by the steady replenishment of gases. This organic phase, called the superstructure, consists of molecules created by the fixation of carbon from the gases. Together, the substructure and the superstructure form the pioneer organism. A cross-sectional view of the organisation of the pioneer organism is shown in Figure 7.

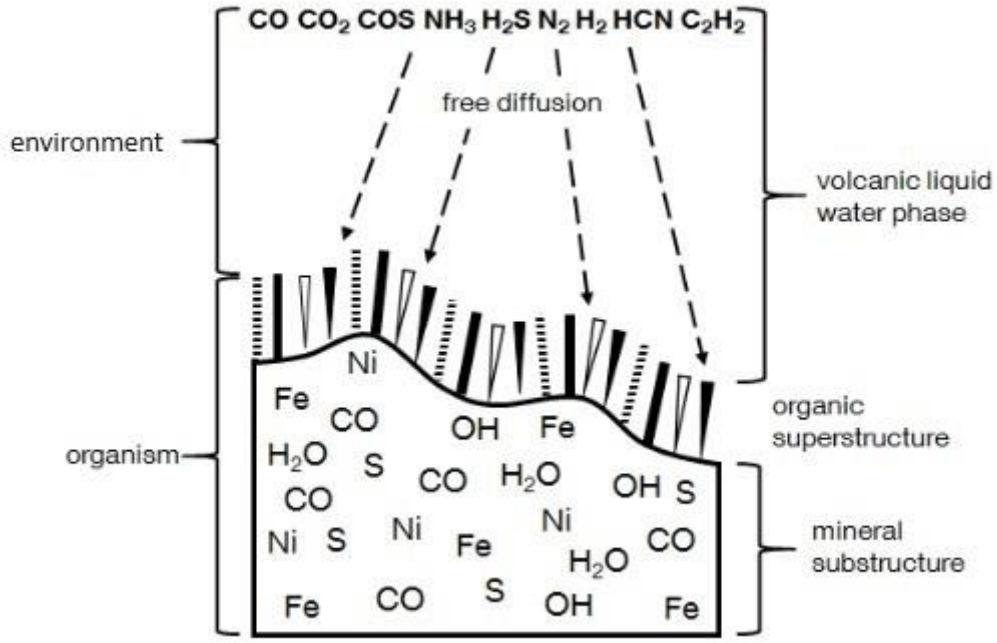



**Figure 7:** Illustration of the pioneer organism in cross-section. Together, the inorganic mineral substrate and the biological superstructure, which is produced via C-fixation, form the organism. The organism remains distinct from its surroundings, in this case the liquid water phase. There is a constant supply of educts through the water phase and the volcanic exhalations to guarantee the organism's growth. The porous surface becomes lipophilized as a result of persistent growth. Adapted from Wächtershäuser 1992.

A constant supply of gases from the volcanic water phase allows the pioneer organism to grow steadily. In addition, under the anaerobic conditions of pyrite metabolism the carbon chains are chemically inert and have a strong tendency to bind to the mineral surface, which leads to lipophilization, coating the entire mineral substructure with generated lipids (Wächtershäuser 1992). This lipid film creates the prerequisites for the first membranes. Due to the nature of lipids, which have a hydrophilic head and a lipophilic tail, two separate environments form over time. One is the relatively hydrophilic metal sulphide surface to which the hydrophilic lipid heads adhere, and the other is the lipophilic region which is formed by the tails of the fatty acids (Figure 8a). In addition, this change in polarity at the surrounding of the pioneer organism further promotes reactions that cannot occur under strictly aqueous conditions. At the same time, lipophilic fatty acids attach to the lipophilic environment of the protocell (Figure 8ii, iii). The hydrophilic heads of these fatty acids must now be uncharged, otherwise they would stick to the pyrite surface. This conversion probably occurs either by protonation of the carboxyl group or by reduction of the carboxyl group into the corresponding alcohol. There are now two types to distinguish, depending on whether the lipids have polar groups at both ends (still one of them uncharged) or only at one end. If the polar groups are at both ends, a monolayer membrane is formed (Figure 8i). If the polar groups are only at one end, a bilayer membrane is formed (Figure 8ii, iii).

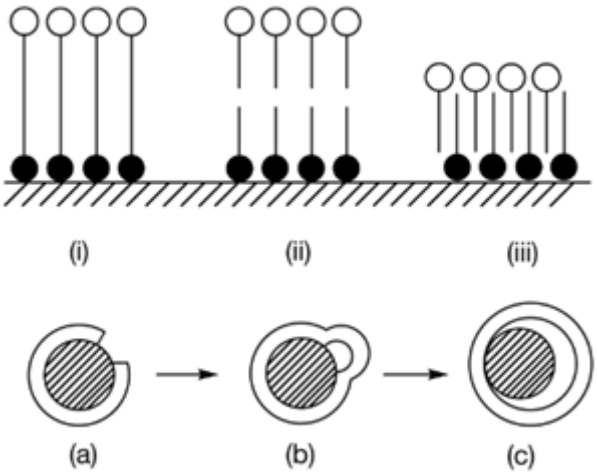



**Figure 8:** Upper part: formation of membranes depending on the constitution of the corresponding lipid. i: monolayer from lipids with hydrophilic groups on both ends, ii and iii: bilayer membranes from lipids with hydrophilic groups on one end. Whether ii or iii is formed depends on the size of the head and tail parts. If these are the same size, ii is formed, if the size of the tail parts is smaller than that of the head parts, iii is formed. Here black circles stand for anionic head groups, white circles for non-ionic head groups. – Lower part: self-cellularisation through circumferential detachment of a membrane from a pyrite grain. Adapted from Wächtershäuser 1992.

Throughout the evolutionary process, the anionic head groups that bind to the pyrite surface can now be converted into non-ionic groups through protonation or reduction. As a result, the membrane separates from the pyrite surface piece by piece. This creates a hydrophilic, metabolically active pyrite core that is surrounded by a membrane (Figure 8a–c), resulting in a semi-cellular structure (Wächtershäuser 1992).

### Energy Source and Catalytic Surfaces of the Pioneer Organism

Methanogenic archaea can be found in extreme environments in modern times. Because they are able to obtain all of their energy from the reduction of $CO_2$ and $H_2$ to methane ($CH_4$), they are independent of oxygen and can be found near hydrothermal vents, for example. This lifestyle suggests a possible early energy metabolism, although the reduction of $CO_2$ to $CH_4$ is not considered a precursor of modern energy production due to an endergonic barrier (Wächtershäuser 1988a). Inspired by this, however, Wächtershäuser discovered that the formation of $FeS_2$ (pyrite) from FeS and $H_2S$ is an exergonic reaction and can therefore provide a constant supply of energy for the fixation of $CO_2$ (Wächtershäuser 1988b). With a Gibbs energy of −38.4 kJ/mol (pH = 0; T = 25 °C), the exergonic pyrite synthesis can support the primordial organism by providing it with reducing power (Wächtershäuser 1990). The calculated exergonic formation is shown in the following reaction equation (1):

$$FeS + H_2S \longrightarrow FeS_2 + H_2 + 2e^- \quad \Delta G° = -38.4\ kJ/mol \quad (1)$$

Other than iron and pyrite, there are other possible transition metals that can lead to the same amount of energy. The similarly reactive nickel sulphide should be mentioned here, as both FeS and NiS are abundant on the early Earth (Allègre *et al.* 1995). Besides the reduction of $CO_2$, FeS and $H_2S$ have also been shown to be the reducing agents in the conversion of alkynes to alkanes, $NO_3^-$ to $NH_3$ (Blöchl *et al.* 1992) or even $N_2$ to $NH_3$ (Dörr *et al.* 2003).

According to Wächtershäuser's chemoautotrophic theory, the biomass for the pioneer organism consists of some elements that were present at the time of the early Earth (H, O, C, N, Mg, Fe, S, Ca, Na, Ni and P), which were able to form simple compounds such as $H_2O$, $CO_2$, CO, $C_2H_2$, $H_2S$, $N_2$, $NH_3$, $H_2$, HCN and $P_4O_{10}$, to name a few (Igari 2000; Oremland & Voytek 2008; Wächtershäuser 2007; Wächtershäuser 2014) **(**Figure 5). These dissolved substances now flow out of the opening of the hydrothermal vents, come into contact with the surface of the transition metal sulphides and are able to form simple organic molecules. The products of these catalytic reactions further promote their own formation reaction and thus an autocatalytic C-fixation can be established. As evolution continues, the pioneer organism can now form more complex molecules and polymers through chemoautotrophic reactions. As mentioned above, in addition to catalysis, the positively charged transition metal surface also has a strong affinity for the negatively charged ions that are formed during these reactions, namely $COO^-$, $PO_3^{2-}$ and $S^-$ (Wächtershäuser 1992), keeping them close to the catalytic surface and thus further promoting subsequent reactions. These functionalised organic molecules give rise to the cell-free pioneer organism. Under primordial conditions, CO and $CH_3SH$ can react with metal sulphide surfaces to form acetic acid, thioacetic acid or methylthioacetate.

Experiments, that deal with the possible reactions of the pioneer organism were carried out by Huber and Wächtershäuser (Huber & Wächtershäuser 1997), and Figure 9 shows a possible mechanism of the experiments (Cody 2004).

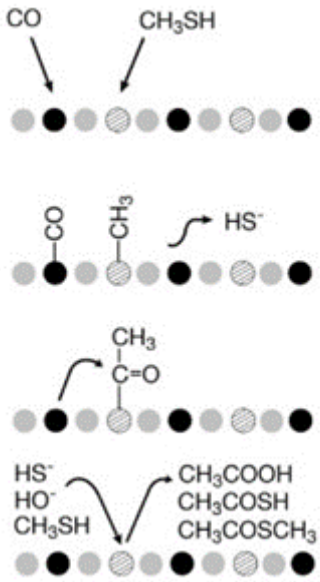



**Figure 9:** The hypothesised mechanism of a surface-catalysed reaction between CO and $CH_3SH$ provided by Wächtershäuser and Huber (Huber & Wächtershäuser 1997). Carbon monoxide is transferred to a nearby iron atom and a methyl group produced from methyl mercaptan is transferred to a nickel atom. By carbonyl insertion, an acetyl group with nickel boundaries is created. Acetic acid, thioacetic acid or methyl thioacetate are the products of nucleophilic attack by either hydoxyl, bisulfide or methane thiol. Adapted from Cody 2004.

An indication of the importance and correctness of Wächtershauser's iron-sulphur world theory can be seen in extant biochemistry (Berg *et al.* 2010; Span *et al.* 2012). There are numerous examples of enzymes that catalyse through metal-sulphur clusters. In particular, such enzymes play an essential role in reactions involving gases such as $H_2$, CO, $CO_2$ and $CH_4$ (Fontecilla-Camps *et al.* 2009; Volbeda & Fontecilla-Camps 2006). Examples are carbon monoxide dehydrogenase (Ragsdale 1995), [NiFe]-hydrogenase (Thauer 2010) and acetyl-coenzyme A (CoA) synthase (Darnault *et al.* 2003). They are also critical for non-redox processes such as the hydration of acetylene to acetaldehyde by acetylene hydratase (Seiffert *et al.* 2007; tenBrink *et al.* 2011).

### Autocatalytic approaches

In order to survive in the course of evolution, the pioneer organism must have developed its own type of metabolism, otherwise it would always be bound to the metal surface. A crucial question is whether this early metabolism was autotrophic or heterotrophic, since both types of nutrition are found in modern biology, even in higher organisms. Autotrophic organisms are now able to build up their entire materials exclusively from inorganic substances, whereas heterotrophic organisms obtain their nutrients from existing organic molecules. Wächtershäuser's theory is based on an autotrophic metabolism based on a reductive citric acid cycle (rTCA) (Wächtershäuser 1990). A one-to-one translation of the modern rTCA into an ancient one is not possible because the modern one is fully enzymatic and therefore requires a number of

cofactors. Therefore, Wächtershäuser assumes a series of historical adaptions, always mentioning first the contemporary equivalent and then its ancient adaptions (Wächtershäuser 1992):

1. Reductive metabolism → reductive/oxidative metabolism
2. (Semi)cellular containment → pyrite surface retainment
3. Anchoring to membrane and protein surfaces → anchoring to pyrite surface
4. Catalytic function of enzymes → catalytic function of pyrite surface
5. Modular synthesis in the cytosol → piecemeal growth and rearrangement on the pyrite surface
6. Lower pressure conditions requiring energy coupling for condensations → high-pressure conditions favouring condensations
7. Enzyme-induced asymmetric synthesis → pyrite-induced asymmetric synthesis
8. Thioacid structures  thioester structures
9. Reducing function of cofactors → reducing function of $FeS/H_2S$

With these adjustments, the ancient rTCA was able to obtain one molecule of succinate from four molecules of $CO_2$ by completing the cycle which starts from succinate. This process would not only be autotrophic, in that it could obtain succinate from $CO_2$, but also autocatalytic, in that it could reproduce itself (Wächtershäuser 1990). The complete process is shown in Figure 10, along with five critical vital reactions.

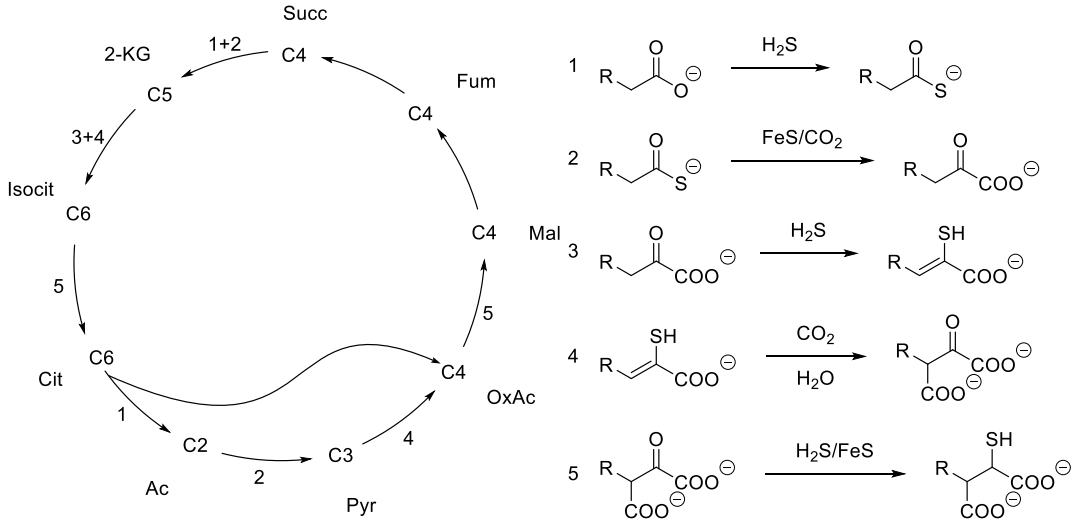



**Figure 10:** The hypothetical, simplified rTCA, beginning with succinate (Succ), is shown on the left. The corresponding ideal reaction occurring in the Hadean atmosphere is indicated by the numbers at the reaction arrows. Adapted from Wächtershäuser 1990, 1992.

With the rTCA, the pioneer organism is able to fix $CO_2$ and to use it to build more complex molecules. This is perhaps the first metabolic pathway and is being widely discussed in the scientific world (Berg et al. 2010; Keller et al. 2017; Keller et al. 2019;

Kitadai et al. 2017). However, in order to be able to transit into more complex life forms (or life forms at all), the pioneer organism must be able to perform reactions of increasing complexity. Claudia Huber was able to show, that some of those reactions are possible. A brief overview of the reactions that Huber has observed are given below.

*Activated Acetic Acid (C2)*

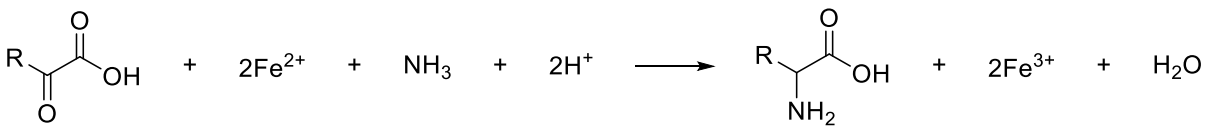
Acetyl-CoA represents the smallest metabolic entity in modern biochemistry. This C2 body is on the one hand at the end of numerous catabolic processes such as the degradation of alanine and fatty acids and on the other hand an anabolic starting material for numerous biosynthesises. Especially, the possibility of feeding it into the rTCA is of central importance. Accordingly, its importance for prebiotic metabolic pathways is high. Huber and Wächtershäuser were able to show that under NiS and FeS catalysis, a reaction temperature of 100 °C, neutral pH and atmospheric pressure, the activated acetyl thioester can be formed from CO and $CH_3SH$, similar to the reductive acetyl-CoA pathway (Huber & Wächtershäuser 1997).

*Pyruvate (C3)*

In 2000, Cody *et al.* demonstrated the formation of pyruvate, another important metabolic building block (Cody *et al.* 2000). However, the conditions — FeS, formic acid and nonylthiol at 2000 bar pressure and 250 °C — were somewhat harsh, which is difficult to reconcile with a hydrothermal environment in the Hadean eon (Wächtershäuser 2000). In fact, the formation of pyruvate could be demonstrated under milder conditions, using acetylene. The system used here consisted of NiS/β-$Ni(OH)_2$/$C_2H_2$/Co at 1 bar and 105 °C (Sobotta *et al.* 2020), which suggests a fundamental role of acetylenes in the origin of life.

*α-Hydroxy and α-Amino Acids*

Amino acids are essential components of the metabolism, so it is not surprising that Huber and Wächtershäuser took a closer look at the ability of pioneer organisms to produce them. Their first approach was to synthesise them from the corresponding alpha-keto acids by reductive amination (Huber & Wächtershäuser 2003). The corresponding reaction equation is shown in Scheme 1.




**Scheme 1:** Reaction equation for the pyrite catalysed reductive amination

In a subsequent approach, they attempted to synthesise amino acids directly from simpler molecules in a Hadean hydrothermal environment. They used CO, KCN and

$CH_3SH$ as carbon sources and Ni or Fe/Ni precipitates as transition metal catalysts. The temperature of the experiments was varied from 80 °C to 120 °C and a CO pressure of 1 bar, the reaction time was 10 days. $(Ca,Mg)(OH)_2$ was added as a buffer to keep the pH basic (Huber & Wächtershäuser 2006). As a result, a variety of α-hydroxy- and α-amino acids with a maximum chain length of C5 were identified. More specifically, it was shown that pyruvate, lactate, glycolate, glycerate and 2-hydroxy butanoic acid were produced. With the addition of KCN, the amino analogues of the previously mentioned molecules glycine, alanine, serine and aminobutanoic acid, appeared. In addition, glycinamide was discovered in these reactions, suggesting that CN ligands might produce amino acids through their corresponding carboxamides. Later, Huber *et al.* found that an increase of temperature leads to an increase of production, with a maximum at 160–180 °C (Huber *et al.* 2010). Through further research, they were able to hypothesise a mechanism that leads to the formation of lactate, alanine, glycolate, glycine, glycerate, serine and isoserine (Huber *et al.* 2012). Starting from a hydrated Ni(CN) complex, the corresponding products could be formed through several insertion and oxidation steps (Figure 11).

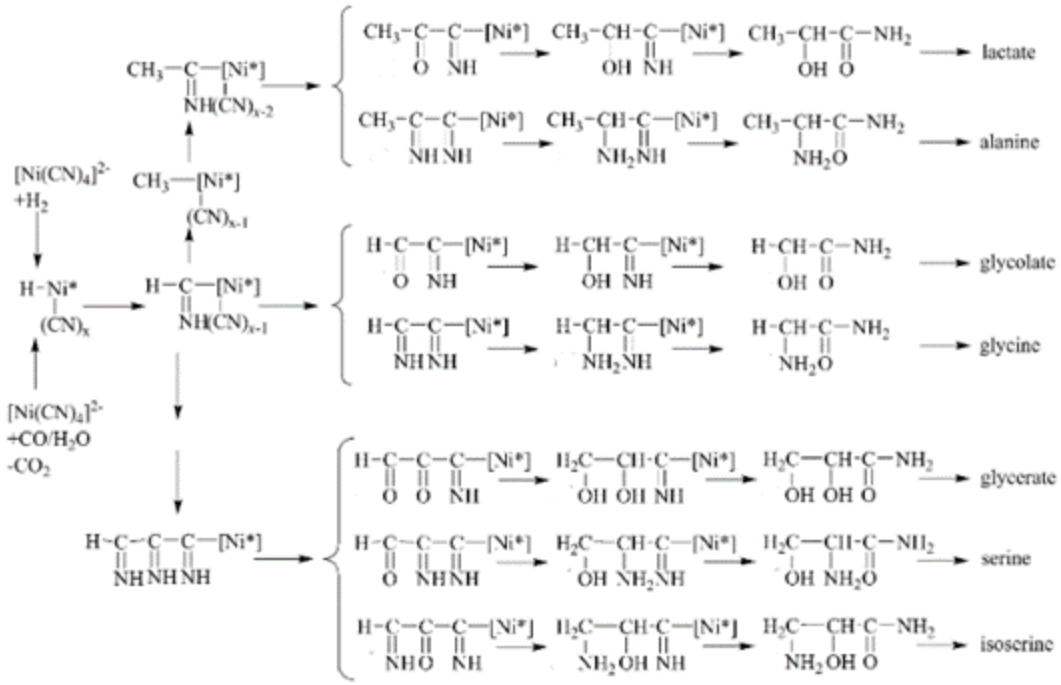



**Figure 11:** Theoretical mechanism by which tetracyanonickelate reacts to alanine/lactate, glycolate/glycine and glycerate/serine/isoserine through numerous cyanide insertions. Adapted from Huber *et al.* 2012.

*Peptides*

As a consequence of the synthesis of amino acids, Huber and Wächtershäuser turned to the next logical step, the formation of peptides. They started to mix amino acids with

$H_2S$ or $CH_3SH$ and were able to show the formation of dipeptides of the amino acids glycine, phenylalanine and tyrosine (Huber & Wächtershäuser 1998).

In further studies, the authors proposed a peptide cycle for the formation of dipeptides (Huber *et al.* 2003).

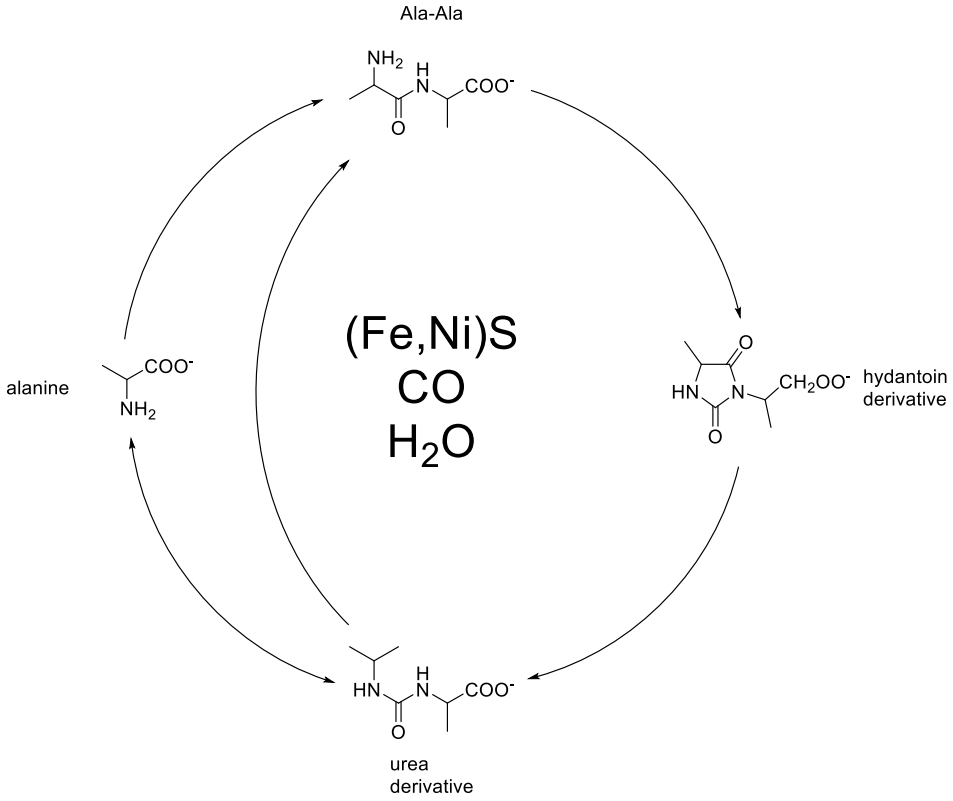



**Figure 12:** Reduced alanine peptide cycle driven by CO in an aqueous (Fe/Ni)S system. Creation of the dipeptide ala-ala using urea and hydantoin derivatives. Adapted from Huber 2013; Wächtershäuser 2006.

Figure 12 shows a condensed form of a CO-driven alanine-to-alanylalanine cyclic process (Huber *et al.* 2003; Huber 2013). COS activates the amino acid to form its aminoacyl-N-carboxanhydride. The dipeptide is formed by the nucleophilic action of a second ala molecule. The dipeptide is converted to the hydantoin derivative upon activation by COS. The urea derivative is created by hydrolysis of the intramolecular amide bond. The dipeptide is recovered by cleavage of a carboxyl group and rearrangement of the molecule. Tripeptides or even tetrapeptides can be formed by repeating the cycle. The reaction is favoured by increasing the pressure with CO gas (Huber *et al.* 2003; Wächtershäuser 2006). There are similarities between the mechanism of the newly hypothesized peptide cycle and the modern nickel enzyme urease. In addition, the continually produced hydantoin can be thought of as a precursor of the purine pathway (Huber *et al.* 2003).

*The acetyleno reaction network*

Inspired by the ability of certain tungsten-iron-sulphur enzymes to convert acetylene into acetaldehydes (Seiffert *et al.* 2007), Wächtershäuser incorporated acetylene into his iron-sulphur world theory. On early Earth, acetylene can be formed by volcanic processes (Mukhin 1976) and/or from $CaC_2$ in the reaction with water. An extraterrestrial origin of acetylene, as well as several smaller related molecules, has also been proven (Ehrenfreund *et al.* 2011; McCarthy *et al.* 2019). Acetylene represents a C2 body, similar to acetyl-CoA in today's biochemistry. Due to its unsaturated character, it also has a high reactivity. Indeed, several essential biomolecules or precursor functionalities could be synthesised in prebiotic reaction settings, using acetylene as the main or one of the primary carbon sources: fatty acids (Scheidler *et al.* 2016), several intermediates of TCA (Sobotta *et al.* 2020), the heterocycles thiophene (Geisberger *et al.* 2021) and pyrrole (Seitz *et al.* 2021), acetaldehyde and other functionalities based on it (Diederich *et al.* 2023), vesicular structures derived from fatty acids Geisberger *et al.* 2023) and amino acids and short chain fatty acid amides (Seitz *et al.* 2024) (Figure 13).

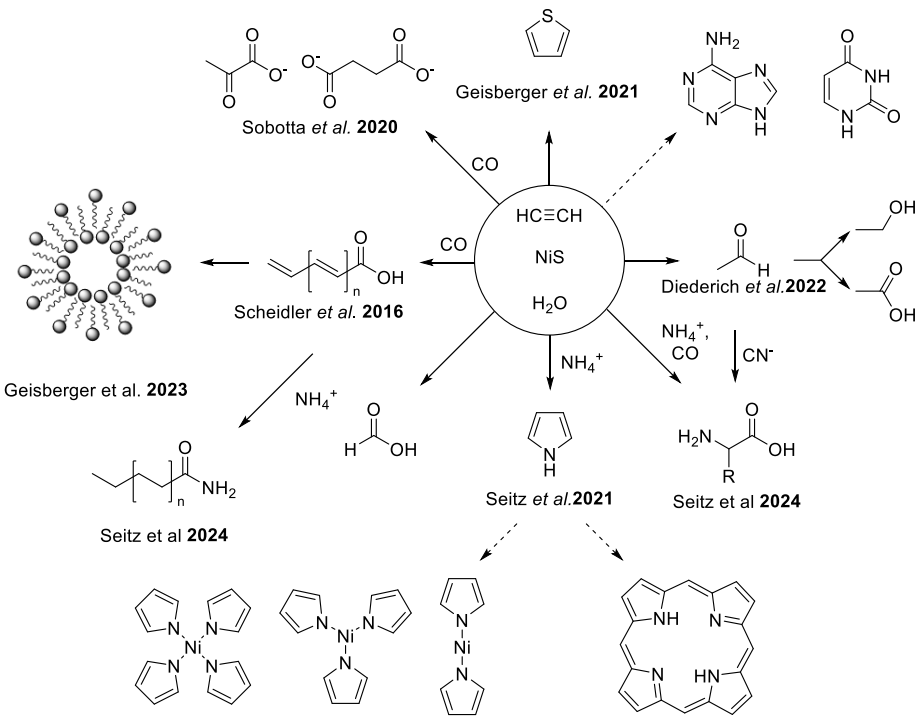



**Figure 13:** Achievements of the acetylene network so far. Dashed arrows show reactions that may be possible in theory but have not yet been proven.

## 1.2 The Next Step: Polymerization and Encapsulation

### 1.2.1 The Indispensability of Polymers

What is life? The answer to this question is rarely simple and never precise. According to his own statement, Daniel E. Koshland, Jr. is trying to formulate things cautiously.

In 2002, he described the characteristics that make up life with a model of seven pillars (Koshland 2002). These are program, improvisation, compartmentalization, energy, regeneration, adaptability and seclusion. This already quite advanced list can hardly be applied to representatives of the first organism, which is why it is concerted into three indispensable properties, which were found in all living systems (Eigen 1997):

I. Metabolism — external energy and material supply is necessary; without this, an equilibrium state would be reached.
II. Self-reproduction — implies the information transfer; without it, information gets lost after every generation.
III. Mutation — implies the development of a living system; without it, information would be invariant.

In today's biochemistry, these requirements are met by polymers. Proteins, the polymerization product of amino acids, are responsible for metabolism. Through specific folds, they form enzymes that can act as catalysts. Through them, reactions that do not take place under normal conditions can be accelerated and the organism can be prevented from reaching a state of chemical equilibrium. DNA and RNA are both polymeric structures made up of nucleobases. DNA is responsible for storing information. This happens because of the sequence of nucleobases in which information can be stored. Through transcription, a complementary RNA is formed, which now serves as a blueprint for proteins and other molecules.

### 1.2.2 Metabolism First vs. Transcription First

After the formation of the first biomolecules, there are now several possible paths that lead to known life. These are usually based on which biopolymer was formed first and therefore, which of the aforementioned characteristics of life developed first. On the one hand, we have "metabolism first" or the assembly of proteins. This hypothesis suggests that metabolic networks, rather than genetic information, were the first systems to emerge and drive the development of life. Here, through the development of simple reactions, the first metabolic series and cycles are formed, which ensure energy gain and thus growth and division. The disadvantage of this theory is that the information that a possible ancestral organism might have received cannot be passed on to daughter organisms. On the other hand, there is the "transcription first" hypothesis, which initially focused on the idea that the processes related to transcription and the interplay between genetic information and catalytic functions might have emerged early in the evolution of life.

### 1.2.3 The RNA World Theory

The question of what was there first — metabolism or reproduction — divides the scientific community. There are good arguments for both sides of the debate. In

modern biochemistry, there are two different polymer molecules for catalysis (proteins) and information storage (DNA). The problem now is that both are interdependent to each other (Crick 1970): proteins are formed and folded based on the information stored in DNA; at the same time, proteins, in the form of enzymes, are necessary to catalyse the reactions that form DNA. Assuming that the two did not evolve at the same time and independently, a chicken-and-egg problem arises. RNA now offers an elegant solution to this problem: as a polynucleotide, RNA, like DNA, is able to store information. At the same time, it has been known since the discovery of ribosomes that RNA has catalytic properties (Guerrier-Takada *et al.* 1983; Kruger *et al.* 1982). This finding argues for RNA as candidate for a first replicator and forms the foundation of "RNA world" hypothesis (Gilbert 1986), because it makes RNA a potential candidate to initially carry out both tasks, before it is replaced or supplemented in the course of evolution by the more stable DNA on the one hand and by the more flexible proteins on the other. As RNA is already a very complex molecule, it was also discussed whether there were precursors to it in an earlier state of evolution. The main topics addressed were theories that suggested the replacement of the sugar-phosphate backbone by, for example, peptides (PNA) (Nelson *et al.* 2000), threofuranose (TNA) (Schöning *et al.* 2000) or glycol (GNA) (Zhang *et al.* 2005).

### 1.2.4 The Formation of Protocells

Independent of metabolism, transcription and self-reproduction, an early organism must have been able to develop cellular structures to separate itself from its environment. In today's biochemistry, cells maintain their function by forming several reaction spaces, so called compartments, in which metabolic processes take place separately and undesirable reactions can be avoided. At the same time, concentration gradients can be built up. In 1960, Sydney Fox proved that the protein-like products that arise when dry amino acid mixtures are heated can also form self-aggregating droplets known as microspheres. They separate themselves from the environment by a semi-permeable membrane and absorb more protein-like material from the environment. This causes them to grow further and break up into smaller droplets again. Fox also found that these systems have enzymatic properties, breaking down glucose or behaving like esterases or peroxidases, without the addition of enzymes from outside (Fox *et al.* 1974; Fox & Harada 1960). In 2008, Jack Szostak showed in a model experiment that vesicles composed of fatty acids, fatty alcohols and fatty acid glycerol esters showed strong temperature independence and could exist in the range from 0° to 100 °C, which further strengthened the possible origin of life near hydrothermal vents (Mansy & Szostak 2008). Actually, the ability of the pioneer organism to form prebiotic cellular structures was recently demonstrated by Geisberger *et al.* (Geisberger *et al.* 2023).

## 1.3 When Chemistry Becomes Biology or The Beginning of Everything

At this point in prebiotic evolution, all the prerequisites are present to speak of life: transcription is fulfilled with an early version of RNA/DNA which enables the organism to pass on information to the next generation, early forms of metabolism are developed to enable the organism to produce its own energy, and everything is brought together and encapsulated in a vesicular structure, to be able to obtain chemical gradients.

### 1.3.1 The Last Universal Common Ancestor: This is LUCA

All known living creatures can be classified into one of the three major domains of biology: bacteria, archaea or eukarya. The fact that they share common properties, such as the (essentially identical) genetic code (Kubyshkin & Budisa 2017) or amino acid chirality (Fujii & Saito 2004), suggests that these properties have been inherited over time and that the domains trace back to a common ancestor. This hypothetical common ancestor is called LUCA (last universal common ancestor). Based on LUCA, the three domains of biology can now be separated, as shown in the so-called phylogenetic tree (Figure 14) derived from Darwinian evolution. This tree originally consisted of only two domains, the eukarya and the bacteria, but was extended to include the archaea after the discovery of those unusual bacteria (Woese 1981; Woese *et al.* 1990) (Figure 14 A). It is now even assumed that eukarya evolved from the archaea, which in turn would result in two domains again, but archaea instead of eukarya (Raymann *et al.* 2015) (Figure 14 B).

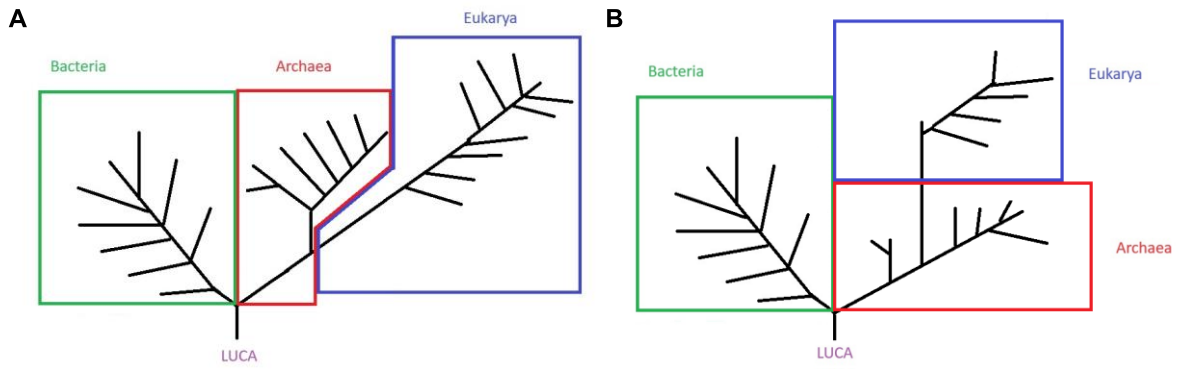



**Figure 14:** Phylogenetic tree of life showing the three domains of life, bacteria (green), archaea (red) and eukarya (blue). A shows a three-domain system, B shows a two-domain system where it is assumed that eukarya developed from archaea. Adapted from Doolittle 1999; Raymann *et al.* 2015; Woese *et al.* 1990.

Pictures of a traditional phylogenetic tree show that gene transfer occurs vertically from predecessor to successor. However, Doolittle showed that this gene transfer is not the only process which can cause the transfection of genes; he also discovered horizontal gene transfer in microorganisms (Doolittle 1999). In order to draw conclusions about

the properties of LUCA from a modern point of view, this horizontal gene transfer must be excluded. Otherwise, this gene would be found in all domains of life, but it cannot be ruled out that it could be the result of a post-LUCA gene origin and interdomain horizontal gene transfer (Kannan *et al.* 2013). Weiss *et al.* therefore examined proteins and thus genes under two criteria: first, the protein should be present in at least two higher taxa, and second, its tree should recover bacterial and archaeal monophyly (Weiss *et al.* 2016), assuming the two-domain phylogenetic tree model. They concluded that LUCA is an "anaerobic, $CO_2$-fixing, $H_2$-dependent [organism] with a Wood-Ljungdahl pathway (the reductive acetyl-CoA pathway), [and is] $N_2$-fixing and thermophilic. LUCA's biochemistry was replete with FeS clusters and radical reaction mechanisms." (Weiss *et al.* 2016), which brings LUCA close to Wächtershäuser's iron-sulphur world theory.

According to Glansdorff *et al.,* the emergence of LUCA and the three domains can be represented by the following chronology (Glansdorff *et al.* 2008): first, there was prebiotic chemistry on minerals rich in iron-sulphur clusters, which preceded the first biochemical reactions. A pregenomic epoch resulted from the gradual assembly of the reaction network into a self-replicating proto-metabolism, which, as it progressed, required the formation of vesicles around the catalytic networks that synthesised peptides and polynucleotides This phase also marks the beginning of the era of the community of ancestral cells. Furthermore, a protonucleus is formed and there is an RNA progenote in this age, which implies that the genetic code began started. As a result, numerous metabolic types evolve. The resulting organisms were metabolically and morphologically diverse and genetically redundant. This was followed by the transition from RNA to DNA, which led to diversification in three domains. In conclusion, LUCA represents the end of the so-called early evolution and simultaneously the beginning of the diversity of life as we know it today.

### 1.3.2 LUCA and the Earliest Life we know about

As mentioned above, LUCA is only a hypothetical organism. The first fossil evidence of life is now estimated to be 3.7 billion years old (Nutman *et al.* 2016). These fossils may be the remains of LUCA, but they may also be the remains of descendants of LUCA that have already gone through thousands of evolutionary stages. There is ample evidence of possible early carbon fixation or even fossils of early organisms. Geological evidence suggests that some of the very first creatures, possibly including LUCA, may have originated near hydrothermal vents. To name some of the oldest:

3.5 billion years ago: samples from the Dresser Formation in the North Pole area of the Pilbara Craton, Western Australia, may show methanogenesis (Ueno *et al.* 2006).

3.7 billion years ago: in the Isua supracrustal belt in southwestern Greenland, stromatolites with microbial structures are indicating the establishment of biotic $CO_2$ fixation (Nutman *et al.* 2016).

3.8 billion years ago: carbonaceous inclusions from the Isua supracrustal belt were found in West Greenland and on Akilia Island. Carbon isotope composition analysis indicated carbon fixation and provided evidence for the origin of life on Earth at least 3.8 billion years ago (Mojzsis *et al.* 1996).

3.8 billion years ago: fossils of microorganism were found in sedimentary rocks in the Nuvvuagittuq Greenstone Belt, Quebec, Canada. They are interpreted as hydrothermal vents (Dodd *et al.* 2017).

4.1 billion years ago: stable isotope evidence of carbon fixation has been found in zircons from the Jack Hills in Western Australia (Bell *et al.* 2015).

These geological findings support the theories of chemoautotrophic genesis and the chemical evolution of hydrothermal systems (Wächtershäuser 1992). In fact, a variety of microorganisms, including archaea, can still survive in autotrophic habitats near hot volcanic flow conditions (Berg *et al.* 2010).

## 1.4 The Importance of Nitrogen

This thesis now deals with the formation of various precursor molecules that contain nitrogen. Starting from Wächtershäuser's iron-sulphur world theory, a hydrothermal reaction environment was simulated to produce these precursor molecules from a prebiotic reaction mixture.

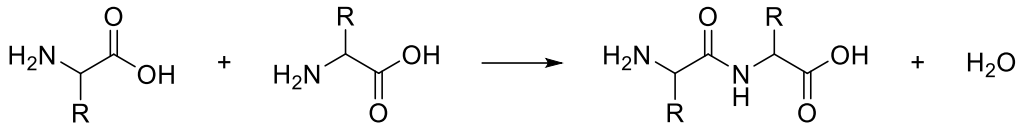
Nitrogen is an element in the second period and fifth main group of the periodic table. Due to its unique position, it has properties that make it indispensable in biochemistry. Thanks to its five valence electrons, it can form a maximum of three covalent bonds, leaving a lone pair of electrons on the nitrogen. This lone pair is responsible for the essential character of most nitrogen compounds and is ideal for forming hydrogen bonds. It is this property that makes nitrogen so valuable in biochemistry.

### 1.4.1 Nitrogen in Today's Biochemistry

Nitrogen has an important function in biochemistry and is essential for all living organisms. Here are the main aspects of nitrogen's role in biochemistry:

## Amino Acids, Proteins and the Peptide Bond Reaction

In living organisms, the so-called proteinogenic alpha-amino acids play a crucial role. They carry the amino group in the α-position to the carbonyl group and are able to form peptides through a condensation reaction with themselves.



**Scheme 2:** Chemical equation for the formation of a dipeptide from two amino acids by separation of water.

This reaction is possible for amino acids at both the carboxyl and the amino groups, allowing peptide chains to form. Through the corresponding side chains of the amino acids, the resulting peptides can link together through hydrogen bonds to form characteristic structures called proteins. This unique structures are necessary to catalyse the reactions of the respective substrates as precisely as possible using a key-lock principle.

## Nucleic Acids and DNA/RNA

The ability to form hydrogen bonds plays an important role in nucleobases. Nucleobases, together with phosphate residues and the sugar deoxyribose, are the building blocks of DNA. Four nucleobases play a crucial role: the two pyrimidines cytosine and thymine, and the two purines adenine and guanine. DNA consists of two separate strands. These are placed on top of each other so that two bases are always opposite each other. Because of their respective structures, adenine always binds to thymine, forming two hydrogen bonds, and guanine always binds to cytosine, forming three hydrogen bonds. This means that the sequence of nucleobases can be explicitly mapped onto the complementary strand. Three nucleobases always form a triplet and determine which amino acid is used in protein synthesis.

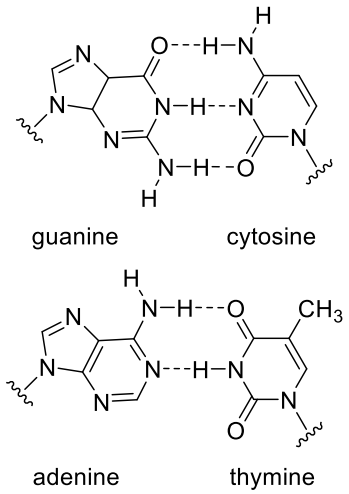



**Figure 15:** Base pairing in DNA. Guanine always pairs with cytosine, forming three hydrogen bonds; adenine always pairs with thymine, forming two hydrogen bonds.

## Pyrroles, Porphyrins and Corrins

Another important application of nitrogen in modern biochemistry are cofactors such as haem or cobalamin. The most important functional group in both is a ring made up of four pyrrole units with a metal ion bound in the middle. In the case of haem, this is iron, which can bind oxygen. This means that it plays a significant role in respiration. Haemoproteins modify the surroundings of the haem macrocycle within the protein matrix to achieve their astounding functional diversity (Poulos 2014). For instance, certain amino acid residues close to the haem molecule enable haemoglobin to efficiently carry oxygen to tissues (Thom *et al.* 2013). In the lungs, haemoglobin reversibly binds to oxygen under conditions of high pH and low carbon dioxide. Haemoglobin releases oxygen to tissues when the conditions are reversed (low pH and high carbon dioxide concentrations). The structure of haem b, one of the four main types of haem, is shown in Figure 16. The different haem molecules differ in the nature of their side chains.

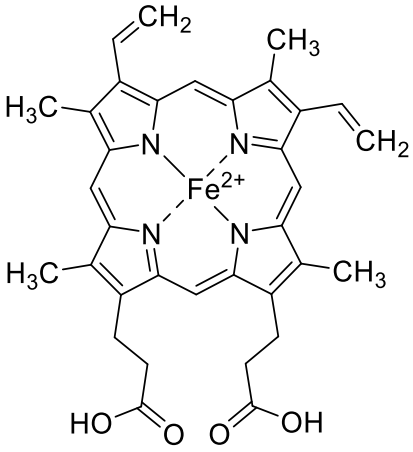



**Figure 16:** Structure of haem b.

In the case of cobalamin, the central atom is cobalt. In addition, the central tetrapyrrole unit is not a porphyrin ring, but a corrin ring. Here, one of the methylene bridges between two pyrrole subunits is missing, and the two pyrroles are directly linked together (Figure 17). Coenzyme $B_{12}$, also known as cobalamin, is involved in the synthesis of amino acids (Yamada 2013). More precisely, it is the coenzyme of three classes of enzymes: isomerases (Takahashi-Iñiguez *et al.* 2012), methyltransferases (Froese *et al.* 2019) and dehalogenases (Payne *et al.* 2015; Reinhold *et al.* 2012).

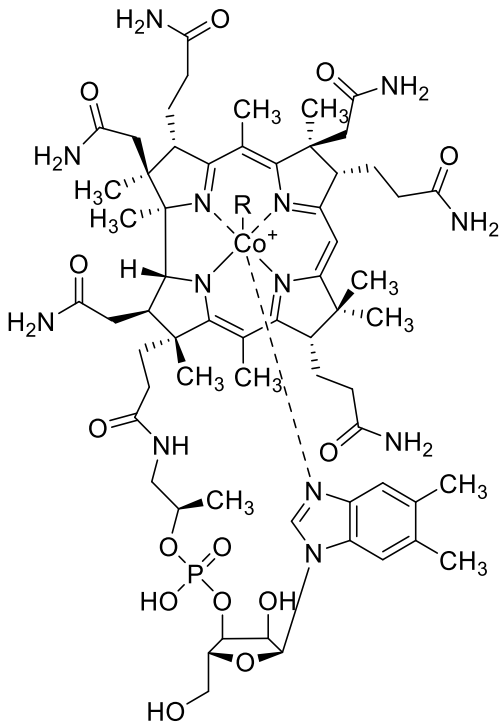



**Figure 17:** Structure of the cofactor vitamin $B_{12}$ (cobalamine).

### 1.4.2 Another Player in the Reaction Network of Acetylene: Ammonia

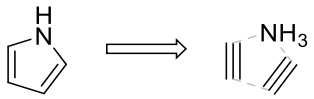
Cyanide is a plausible early molecule that can be used to synthesise various precursors of proteins and RNA (Das *et al.* 2019; Sanchez *et al.* 1967; Sutherland 2016). Cyanide is abundant on the early Earth due to photochemical reactions (Tian *et al.* 2011), high-temperature reactions during asteroid impacts (Parkos *et al.* 2018), electrical discharges (Cleaves *et al.* 2008) and cometary inputs (Matthews & Minard 2008). However, cyanide also has a major drawback that encourages critics to raise their voices: Very high concentrations are required for cyanide to polymerise. Furthermore, cyanide is unstable at higher concentrations and quickly reacts in an aqueous environment to formamide, which further decomposes to ammonia and formic acid (Miyakawa *et al.* 2002), especially at higher temperatures. Thus, the equilibrium of $HCN/NH_3$ at the temperature of a hydrothermal vent (100 °C) is almost entirely on the ammonia side. These facts imply that ammonia itself should be considered in origin-of-life research.

# 2 Motivation

The origin of life is a complex yet fascinating topic to study. Its most intriguing aspect is that every day we walk past thousands of pieces of evidence that life must have originated somehow, but the knowledge of exactly how is still hidden. In previous works, a reaction network around acetylene, CO, $H_2O$ and cyanide has been established. Using acetylene and carbon monoxide, together with metal sulphide catalysts according to the iron-sulphur world theory, it was possible to synthesise some important biomolecules in a hydrothermal reaction set. Cyanide was successfully used as a possible nitrogen source. However, as mentioned above, the problem is that cyanides rapidly hydrolyse to formamide at 100 °C, which further reacts to $NH_3$ and formic acid.

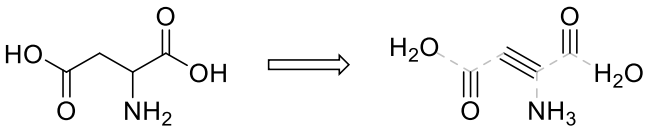
The aim of this work is to further develop the reaction network by adding ammonia. As described above, ammonia as a potent nitrogen source is very present in an origin of life scenario due to various ways of synthesis.

The first part of this work focuses on pyrrole, a precursor of tetrapyrrole structures such as haemoglobin or vitamin $B_{12}$, which are essential molecules for extant biochemistry. Pyrrole can be formed from two molecules of acetylene and one molecule of ammonia (Scheme 3).



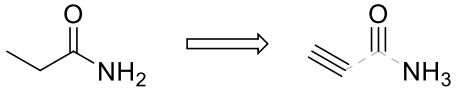

**Scheme 3:** Retrosynthesis of pyrrole formed from two molecules of acetylene and one molecule of ammonia. Adapted from Seitz *et al.* 2021.

In addition, the work of previous members has been continued to investigate the synthesis of amino acids and amides in the hydrothermal reaction setting. Two examples of how amino acids and amides can be formed without using cyanide as a nitrogen source are shown in Scheme 4 and Scheme 5. Here aspartic acid is formed from two molecules of carbon monoxide and water, and one molecule each of acetylene and ammonia.



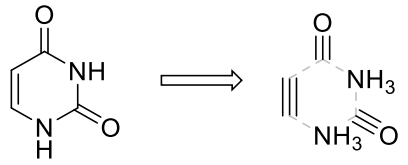

**Scheme 4:** Retrosynthesis of aspartic acid formed from two molecules of CO and $H_2O$ and one molecule each of acetylene and ammonia. Adapted from Seitz *et al.* 2024.

The formation of propionamide, as an example of a short chain fatty acid amide, is shown from one molecule each of acetylene, carbon monoxide and ammonia.



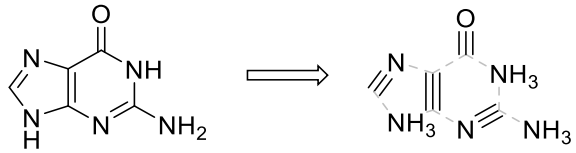

Scheme 5: Retrosynthesis of propionamide formed from one molecule each of acetylene, CO and ammonia. Adapted from Seitz *et al.* 2024.

Finally, nucleobases, another important group of nitrogen-containing biomolecules, were studied. We were able to show the formation of uracil from one molecule of acetylene and two molecules each of carbon monoxide and ammonia (Scheme 6).




**Scheme 6:** Retrosynthesis of uracil formed from two molecules of CO and ammonia and one molecule of acetylene.

Theoretically, it should be possible to form other nucleobases by combining ammonia and cyanide in the reaction. For example, guanine can be formed from one molecule each of acetylene and carbon monoxide, two molecules of cyanide and three molecules of ammonia (Scheme 7).




**Scheme 7:** Retrosynthesis of guanine formed from three molecules of ammonia, two molecules of cyanide and one molecule each of CO and acetylene.

# 3 RESULTS

The articles shown on the following pages are referenced as:

Seitz, C.; Eisenreich, W.; Huber, C. The Abiotic Formation of Pyrrole under Volcanic, Hydrothermal Conditions – An Initial Step towards Life's First Breath? Life **2021,** *11,* 980.

Seitz, C.; Geisberger, T.; West, A. R.; Fertl, J.; Eisenreich, W.; Huber, C. From Zero to Hero: The Cyanide-Free Formation of Amino Acids and Amides from Acetylene, Ammonia and Carbon Monoxide in Aqueous Environments in a Simulated Hadean Scenario. Life **2024,** *14,* 719.

## 3.1 Summary and Article: "The Abiotic Formation of Pyrrole under Volcanic, Hydrothermal Conditions – An Initial Step towards Life's First Breath?"

Tetrapyrrole structures, such as haemoglobin, play an important role in today's biochemistry. However, in order to build tetrapyrrole structures, the building block of these structures must first be studied. In this work, we show the formation of pyrrole under simulated hydrothermal conditions.

The reaction setup was designed to simulate volcanic, hydrothermal vent conditions. A mixture of acetylene and CO gas was filled in a reaction vessel with freshly precipitated metal sulphide catalyst and ammonia. The vessel was prepared at room temperature and then heated to 105 °C for one day. The supernatant was analysed by GC/MS. In order to determine the composition of the products from the starting compounds, experiments were performed with stable isotope labelled precursors such as $^{13}CO$ and $^{15}NH_4Cl$.

Pyrrole could be synthesised from two molecules of acetylene and one molecule of ammonia. Additionally, by using propyne instead of acetylene, dimethyl pyrrole could be studied. Different parameters such as pH, reaction time and the amount and composition of the metal sulphide catalyst were examined. A maximum yield based on the conversion of $NH_4Cl$ was found at pH of 9.1, equimolar amount of $Na_2S$ and $NiSO_4$, resulting in a perfect concentration of NiS catalyst, without inhibiting the reaction by pH increase due to strong overflow of $Na_2S$, and a reaction time of 24 hours. Pyrrole was detected by GC/MS and confirmed by using $^{15}NH_4Cl$ (Fig. 18).

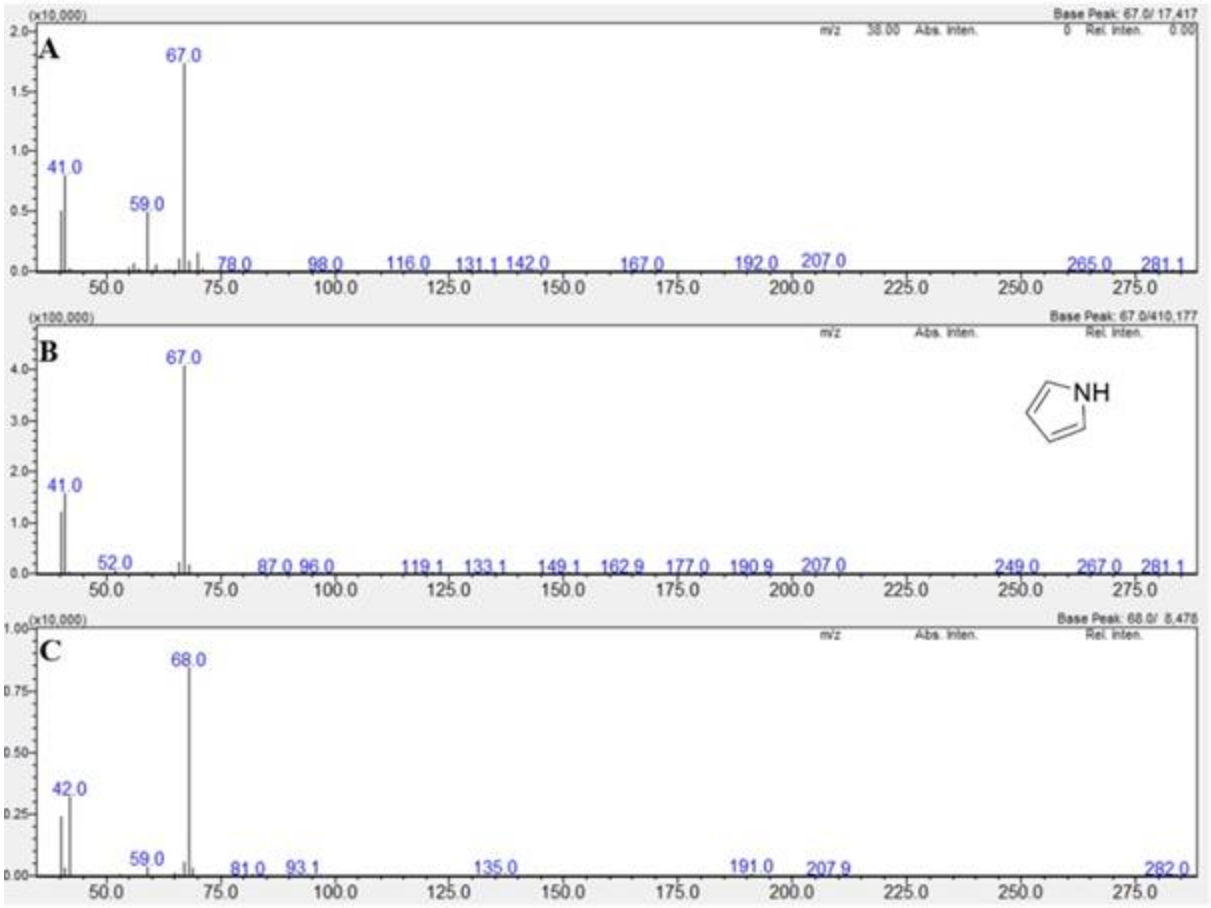



**Figure 18:** Different mass spectra from a typical experiment described above. A is the measured pyrrole spectrum, B is the spectrum from database and C is the spectrum from a run with stable isotope labelled ammonia. Adapted from Seitz *et al.* 2021.

Author contribution: My individual contribution to this work included the design and execution of the experiments. I also analysed and interpreted the data obtained and drafted the published manuscript.

life

MDPI

*Article*

# The Abiotic Formation of Pyrrole under Volcanic, Hydrothermal Conditions—An Initial Step towards Life's First Breath?

**Christian Seitz, Wolfgang Eisenreich and Claudia Huber ***

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**Abstract:** Porphyrins, corrins, and tetrapyrroles constitute macrocycles in essential biomolecules such as heme, chlorophyll, cobalamin, and cofactor F430. The chemical synthesis as well as the enzymatic synthesis of these macrocycles starts from pyrrole derivatives. We here show that pyrrole and dimethyl pyrrole can be formed under the simulated volcanic, hydrothermal conditions of Early Earth, starting from acetylene, propyne, and ammonium salts in the presence of NiS or CoS as catalysts.

**Keywords:** pyrrole; dimethylpyrrole; acetylene; propyne; transition metal sulfides; hydrothermal conditions; origin of life



**Citation:** Seitz, C.; Eisenreich, W.; Huber, C. The Abiotic Formation of Pyrrole under Volcanic, Hydrothermal Conditions—An Initial Step towards Life's First Breath? *Life* **2021**, *11*, 980. https://doi.org/10.3390/life11090980

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## 1. Introduction

Tetrapyrroles can be found in almost all modern domains of life with important functions, being mainly involved in respiration (heme), photosynthesis (chlorophyll), amino acid synthesis (cobalamin), and methane formation (cofactor F430) [1–3].

The basic structure of tetrapyrroles can be seen in Figure 1. The structures of heme, chlorophyll, cobalamin, and cofactor F430 are shown in the supplemental section (Figures S1–S4).

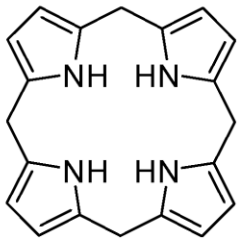


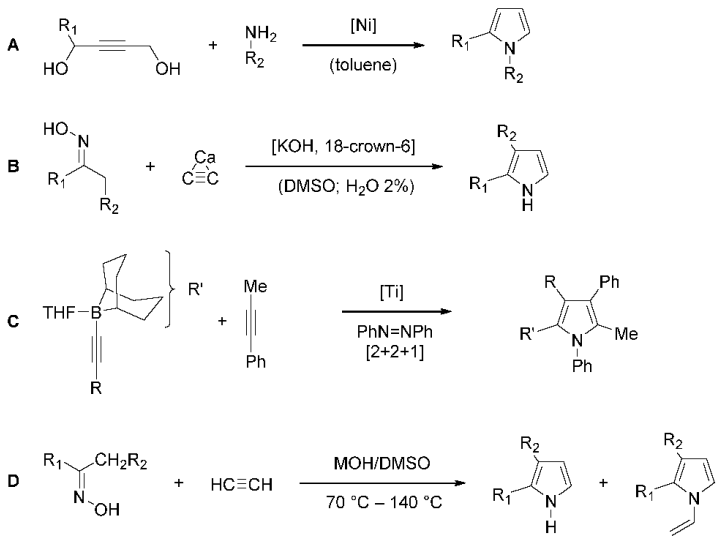

**Figure 1.** Structure of porphyrinogen, the most simple, non-aromatic tetrapyrrole.

The chemical synthesis of pyrrole and its derivatives was one of the early milestones in classical organic chemistry. *Ludwig Knorr* discovered in the 1880s that α-amino ketones (1) and a compound with an electron-withdrawing group next to a carbonyl group (2) yield the corresponding substituted pyrroles (*Knorr* pyrroles) (3) [4]. In the 1940s, *Hans Fischer* and *Emmy Fink* discovered that the *Knorr* pyrroles (3) are not the only products of this reaction. With a second carbonyl group (e.g., β-ketoesters) in the non-amino educt, a mixture of the Knorr (3) and the *Fischer–Fink* products (4) could be observed (Schemes S1–S3) [5]. *Kleinspehn* further designed the reaction parameters so that the *Fischer–Fink* product is the exclusive reaction product [6].

The *Paal–Knorr* reaction, developed in 1884, is another example of the formation of a pyrrole derivative [7,8]. Here, a 1,4-diketone (5) reacts with an amine (6) to produce

the corresponding pyrrole derivate (7) (Schemes S4 and S5). Alternatively, α-haloketones (8) can be reacted with ammonia (or primary amines) (6) and β-ketoesters (9) to afford the corresponding pyrroles (10) (Hantzsch pyrrole synthesis, Schemes S6 and S7) [9].

Next to these classical reactions, pyrroles can also be synthesized from acetylene or acetylene-like molecules as one of the starting materials [10]. In the following, four examples are shown which are, in some aspects, related to our setting. More specifically, nickel was used as a catalyst for the condensation of an acetylene derivative with an amine (Scheme 1A) [11]. The reaction can also be performed with acetylene generated in situ from calcium carbide (Scheme 1B) [12], or by using one nitrogen compound and two acetylene-like compounds (Scheme 1C) [13]. In the setting used by *Trovimov* et al. (Scheme 1D), the pyrrole ring was built from ketones (ketoximes) and acetylene in superbasic catalytic systems, finally leading to a variety of pyrrole-like structures through variations in the ketoxime side chains [10].




**Scheme 1.** Examples of modern-day pyrrole synthesis. (**A**) Pyrrole synthesis with nickel as catalyst [11]; (**B**) pyrrole synthesis with calcium carbide [12]; (**C**) pyrrole synthesis with two acetylene-like compounds [13]; (**D**) pyrrole synthesis with acetylene [10].

All examples use very specific catalysts and non-aqueous solvents to form the corresponding pyrrole derivatives. In contrast with these conditions, our reactions are performed from simple, prebiotic, relevant building blocks in an aqueous medium.

Acetylene can be either formed in volcanic processes [14], or by the reaction of $CaC_2$ with water [15]. Acetylene has also been detected in extant fumarolic gases [16] and in volcanic glasses [17]. Atmospheric acetylene has been suggested as part of a presumptive methane-rich primordial atmosphere due to photolysis of methane [18]. Acetylene is highly reactive and shows strong ligation to transition metals. It is therefore considered as a possible carbon source for the formation of biomolecules in prebiotic scenarios. Propyne (methylacetylene) can be found besides acetylene in volcanic gas samples [14], can be formed from propylene through pyrolysis [19], and is also detected in interstellar cold clouds [20].

In previous work, we showed the formation of short-chain fatty acids [21] and intermediates of extant carbon fixation cycles [22] from acetylene and carbon monoxide as carbon sources under simulated volcanic hydrothermal conditions.

By adding ammonium chloride as a nitrogen source, we now show that pyrrole is formed out of acetylene and ammonia in the presence of NiS and CoS at 105 °C after 1 day. Transition metal sulfides served as catalysts, according to *Günter Wächtershäuser's* Iron–Sulfur World theory [23]. This proof-of-principle reaction could provide the basis for the prebiotic synthesis of more complex pyrroles, including tetrapyrroles or related molecules.

## 2. Materials and Methods

All chemicals were purchased from Sigma-Aldrich GmbH (Steinheim, Germany) in the highest purity available. Acetylene 2.6 (acetone free) was purchased from Linde AG (Pullach, Germany), and CO 2.5 and argon 4.6 were purchased from Westfalen AG (Münster, Germany). In a typical run (run 1, Table S1), a 125 mL glass serum bottle was charged with 1.0 mmol $NiSO_4 \cdot 6\ H_2O$ and 1.0 mmol $NH_4Cl$, and closed with a gas-tight silicon stopper. The bottle was evacuated three times and filled with argon, finally resulting in a de-aerated state. Subsequently, the bottle was filled with 1 M $Na_2S$ solution, 1 M NaOH solution, and argon-saturated water, resulting in a total reaction volume of 5 mL. In this mixture, a precipitate of black NiS was immediately formed due to its low solubility constant of $1 \times 10^{-22}$ [15,24,25] in aqueous solution. Finally, 60 mL of acetylene gas and 60 mL of CO were added. Liquids and gases were injected by using gas-tight syringes. The freshly precipitated NiS ($NiSO_4 + Na_2S \rightarrow NiS\downarrow + Na_2SO_4$) acted as a putative transition metal catalyst for the reaction, and the molar variations of $Na_2S$ to $NiSO_4$ resulted in free sulfide ions in the solution. For the formation of dimethylpyrrole, propyne was used instead of acetylene.

Reactions were carried out at 105 °C. pH variations were achieved through the addition of $H_2SO_4$ solution (1 M), NaOH solution (1 M), and solid $Ca(OH)_2$. For safety reasons (danger of explosion) and for technical reasons, the reactions were carried out at low gas pressure. Bottles were filled with ~1 bar at room temperature, leading to a total absolute pressure of ~2.5 bar at 105 °C. After the defined reaction time, the reaction mixture was allowed to cool down and transferred into a test tube. After centrifuging at 3000 rpm for 10 min, the supernatant was extracted with 3 × 1 mL ethyl acetate. The organic phases were dried over $Na_2SO_4$ and directly analyzed with gas chromatography–mass spectrometry (GC–MS). GC–MS analysis was performed with a GC-2010, coupled with MS-QP2010 Ultra (Shimadzu GmbH, Duisburg, Germany) with a 30 m × 0.25 mm × 0.25 µm fused silica capillary column (Equity TM5, Supelco, Bellefonte, PA, USA) and an AOC-20i auto injector. Additionally, 1 mL of supernatant was freeze dried and treated with 250 µL ACN and N-(tert-butyldimethylsilyl)-N-methyltrifluoracetamide each, and heated at 70 °C for about an hour.

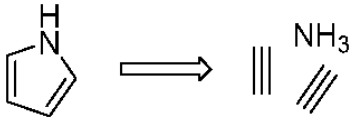
Temperature program and settings for ethyl acetate phase: 0–6 min at 40 °C; 6–25 min at 40–280 °C, 10 °C/min; injector temperature, 260 °C; interface temperature, 260 °C; column flow rate, 1 mL/min; scan interval, 0.5 s; and injection volume, 1.0 µL.

Temperature program and settings for silylated products: 0–6 min at 90 °C; 6–25 min at 90–310 °C, 10 °C/min; injector temperature, 260 °C; interface temperature, 260 °C; column flow rate, 1 mL/min; scan interval, 0.5 s; and injection volume, 0.5 µL.

Peak assignment was achieved by comparison with the retention times and mass spectra of purchased reference compounds, as well as with data from the National Institute of Standards and Technology (NIST) spectral library. Pyrrole showed a retention time of 5.4 min; dimethylpyrrole a retention time of 8.8 min. Quantification was performed by external calibration using known concentrations of pyrrole and dimethylpyrrole, respectively. Runs without a transition metal compound or with argon instead of acetylene were performed for comparison.

## 3. Results

We here show the formation of pyrrole under simulated volcanic hydrothermal conditions from simple and geochemically feasible building blocks. In a retrosynthetic view, two molecules of acetylene and one molecule of $NH_3$ forms pyrrole (Scheme 2).




**Scheme 2.** Retrosynthesis of pyrrole, formed by two molecules of acetylene (≡) and $NH_3$.

Using our GC–MS settings, pyrrole was found at a retention time of 5.6 min (65.9 °C), as shown by comparison with the reference material. The highest mass peak was observed at 67.0 u. A second characteristic peak was detected at 41.0 u, which belongs to the C-C=N$^+$ fragment. When the stable isotope labeled $^{15}NH_4Cl$ was used instead of unlabeled $^{14}NH_4Cl$, the detected product displayed a molecule mass +1 in the corresponding GC–MS analysis (Figure 2).

**Table 1.** Pyrrole formation from acetylene and ammonia using different transition metals as catalysts and different gas compositions after one day at 105 °C. Metal sulfides were formed in situ from $MeSO_4$ and $Na_2S$. pH values were adjusted with 1 mL 1 M NaOH and measured at the end of the reaction time. Yields are based on the conversion of $NH_4Cl$. Dimethylpyrrole was formed from propyne instead of acetylene.

| Run | NiS | FeS | CoS | $Ni(OH)_2$ | NaOH | $NH_4Cl$ | CO | $C_2H_2$ | Propyne | $t_{reac}$ | pH | Pyrrole (Runs 1–5) Dimethylpyrrole (Run 6) | Yield |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (mmol) | (mmol) | (mmol) | (mmol) | (mmol) | (mmol) | (mL) | (mL) | (mL) | (d) | | (µM) | (%) |
| **1** | 1 | - | - | - | 1 | 1 | 60 | 60 | - | 1 | 9.1 | 11.38 | 1.14 |
| **2** | - | 1 | - | - | 1 | 1 | 60 | 60 | - | 1 | 9.1 | <0.1 | <0.01 |
| **3** | - | - | 1 | - | 1 | 1 | 60 | 60 | - | 1 | 8.7 | 5.31 | 0.53 |
| **4** | - | - | - | - | 1 | 1 | 60 | 60 | - | 1 | 9.9 | <0.1 | <0.01 |
| **5** | - | - | - | 1 | 1 | 1 | 60 | 60 | - | 1 | 9.8 | <0.1 | <0.01 |
| **6** | 1 | - | - | - | 1 | 1 | - | 120 | - | 1 | 8.6 | 5.75 | 0.57 |
| 7 | 1 | - | - | - | 1 | 1 | 60 | | 60 | 1 | 9.3 | 132.25 | 13.2 |

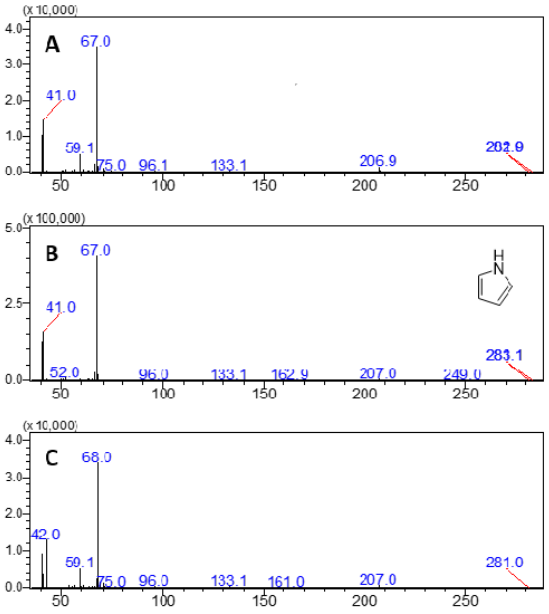



**Figure 2.** Mass spectra of pyrrole with 67.0 u as the molecule mass and 41.0 u as the mass of the characteristic fragment C-C=N$^+$. (**A**) Pyrrole spectrum from run 1 (Table 1). (**B**) Pyrrole spectrum from standard. (**C**) Pyrrole spectrum from a run performed with $^{15}NH_4Cl$.

NiS was used as a transition metal catalyst for the formation of pyrrole from acetylene and ammonia. Run 1 (composition: 1 mmol $NiSO_4$, 1 mmol $Na_2S$, 1 mmol $NH_4Cl$, 1 mL 1 M NaOH, 60 mL $C_2H_2$, 60 mL CO, 105 °C) (see Table 1) was set as the standard run with a maximum yield of 1.14% pyrrole, based on the conversion of $NH_4Cl$. In runs without a transition metal, acetylene, or nitrogen source, but otherwise identical conditions to run 1, no pyrrole formation was observed.

FeS and CoS were tested as further transition metal catalysts for pyrrole formation. In these cases, $FeSO_4$ and $CoSO_4$ were used instead of $NiSO_4$. The pyrrole formation in experiments using cobalt was about 50% compared to experiments with nickel. In the case of iron, no formation of pyrrole could be observed (Table 1).

In the above-described setup, acetylene was present with a small molar excess compared to $NH_4Cl$ (2.57 mmol to 1.00 mmol). When the amount of acetylene was increased to 120 mL (5.14 mmol), the yield of pyrrole decreased to 50% as compared to run 1, indicating that pyrrole formation was not limited by $C_2H_2$ in our setting.

The yield of pyrrole had its optimum around pH 9.1 (Figure 3; Table S1), which corresponds to the pKa of ammonia of 9.25. At acidic pH values, pyrrole was not detected. At higher pH values than the pKa of ammonia, the yields decreased rapidly (Figure 3). This observation could be explained by higher concentrations of $OH^-$ and therefore a blockage of catalytic nickel sites. This is supported by runs, with $Ni(OH)_2$ instead of $NiSO_4$, under otherwise identical parameters, which show no formation of pyrrole (Table 1).

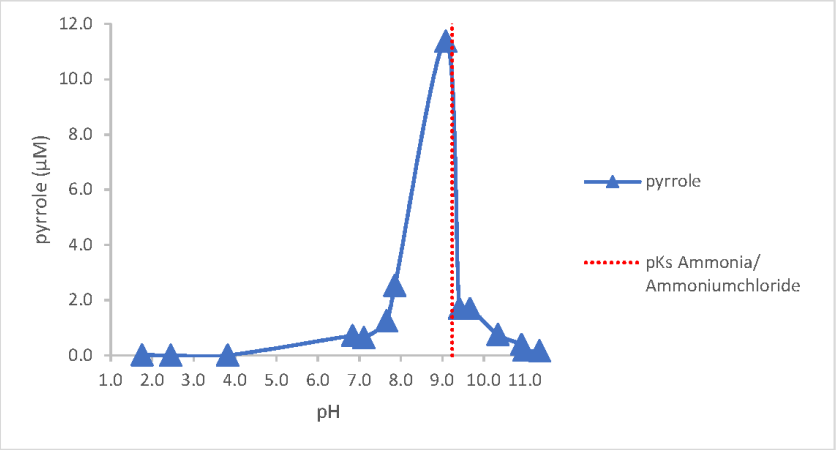



**Figure 3.** Formation of pyrrole at different pH values. pH values were measured at the end of the reaction time. The red dotted line shows the pKa of $NH_3/NH_4Cl$.

Pyrrole formation was observed in reaction times from 0 min to 7 days (168 h). Notably, pyrrole formation could already be observed after the short reaction time of one hour, and showed its maximum after one day (24 h) (Figure 4; Table S2). A longer reaction period led to continuously declining yields of pyrrole. This observation indicates follow-up reactions of pyrrole, which, under optimized conditions, combined with suitable reactants, could lead to the required oligomerization of pyrrole to biologically important cyclic tetrapyrroles (see Figures S1–S4).

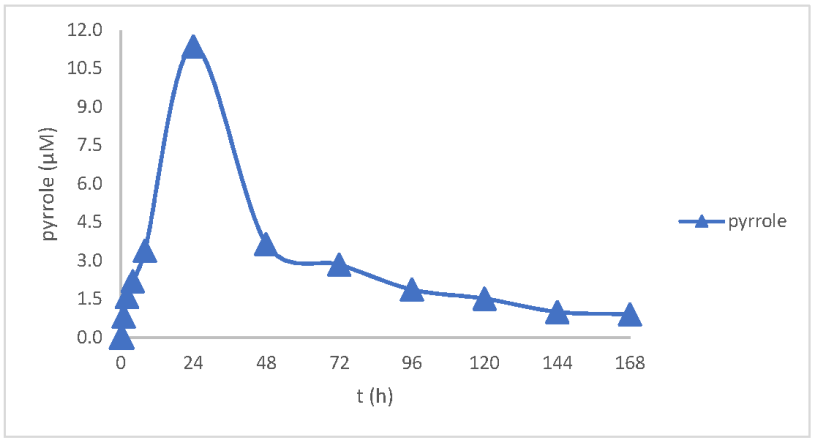



**Figure 4.** Time-dependent NiS-catalyzed formation of pyrrole from acetylene and ammonia.

The concentration of catalysts also played an important role. Out of the possible nickel compounds in our experimental setup, $NiSO_4$, NiOH, and NiS, only NiS supported pyrrole formation (Table 1, Table S3). Therefore, we changed the amount of $Na_2S$ in some runs to achieve different concentrations of NiS. The maximum yield of pyrrole was found at an equimolar concentration to the transition metal (Figure 5, Table S3). As mentioned earlier, a high pH inhibited the formation of pyrrole. Therefore, high concentrations of $Na_2S$ hindered the formation of pyrrole by increasing the pH of the solution. On the other hand, with $NiSO_4$ not catalytically active, a low concentration of $Na_2S$ showed lower yields of pyrrole, since NiS could not be formed.

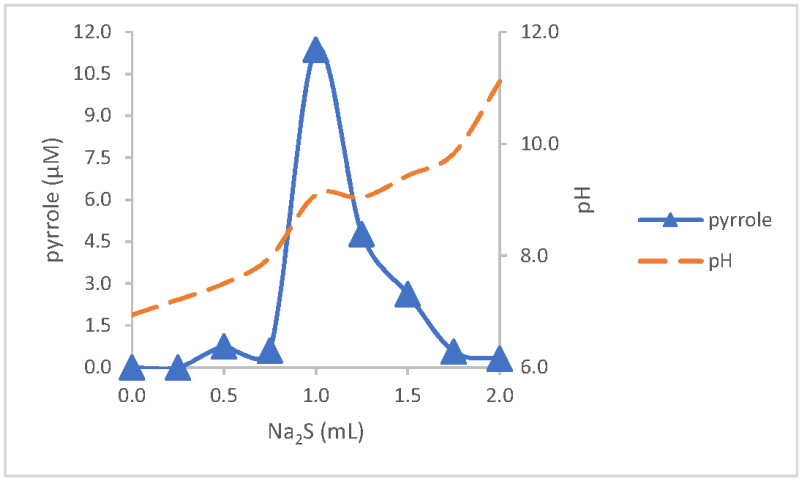
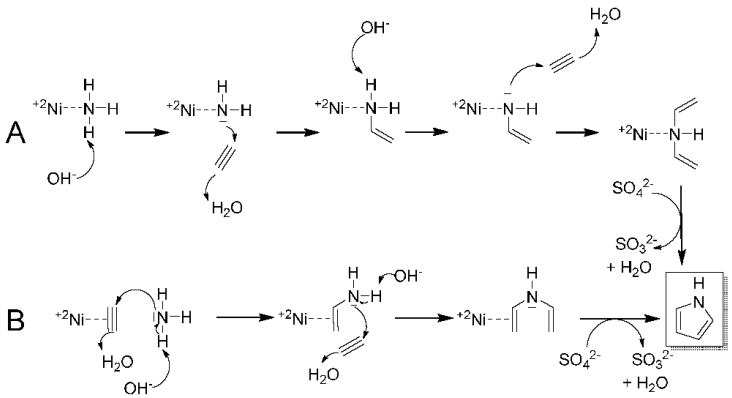



**Figure 5.** Formation of pyrrole with different concentrations of $Na_2S$. The orange line indicates the pH of the corresponding experiment.

The classical pyrrole syntheses mentioned above involve the nucleophilic attack of nitrogen at a carbonyl group, resulting in the elimination of water. In our experimental setting, this mechanism would also be possible via the formation of carbonyl groups from CO. However, the formation of pyrrole was also observed in the absence of CO. Due to this result, two mechanisms for the formation of pyrrole can be suggested. The first one would be similar to the possible mechanism for the formation of thiophene, which was published earlier [26,27]. Here, $NH_3$ displaces the sulfide ion from the transition metal and therefore acts as the reactant (Scheme 3A). After two steps of deprotonation and binding to two acetylene molecules, the corresponding divinyl amine undergoes an oxidative ring closure. At this stage, $SO_4^{2-}$ is possibly reduced to $SO_3^{2-}$, which can be found in the freeze dried liquid after silylation (Figure S5). Another possibility of an oxidizing agent in our oxygen-free reaction mixture would be the reduction of $Ni^{2+}$ to $Ni^0$. The formation of $Ni^0$ from $Ni^{2+}$ was shown earlier in similar experiments [28].




**Scheme 3.** Proposed mechanism of the Ni-catalyzed formation of pyrrole. (**A**) starts with the binding of ammonia to the Ni-ion, (**B**) starts with an acetylene–nickel bond.

Another possible mechanism starts with acetylene binding to the Ni ion (Scheme 3B). Notably, acetylene shows strong coordination tendency towards Ni ions [29,30]. The following reaction steps would be identical; namely, two deprotonations, binding to two molecules of acetylene, and ring closure.

The oligomerization of pyrrole to porphin rings would require an additional CH group. As a proof of principle towards the formation of these derivatives, we tested propyne (methylacetylene) instead of acetylene under experimental conditions, which led to the formation of pyrrole. Indeed, one of the products in this reaction was dimethylpyrrole. Dimethylpyrrole can be found at a retention time of 8.8 min. The typical masses were 95 u, which is the molecular peak, 94 u as M-1, and 80.0 u, which results from the loss of one methyl group (Figure 6). According to our mechanistic view, we assume that the position of the two methyl groups was at C2 and C5. However, products with other positions are possible. The dimethylpyrrole yield, based on the conversion of $NH_4Cl$, was 13.2%, which is about ten times higher compared to the yield of pyrrole from acetylene.

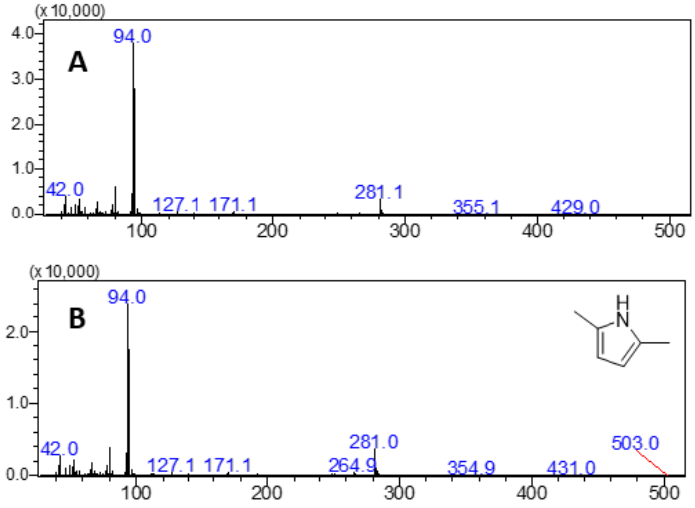



**Figure 6.** Mass spectra of dimethylpyrrole with 94.0 u as the molecule, and 80.0 u as the mass resulting of the loss of a methyl group. (**A**) First spectrum is of run 7 (Table 1). (**B**) Dimethylpyrrole spectrum from standard.

In addition to pyrrole and dimethylpyrrole, further nitrogen-containing molecules were detected in trace amounts in single runs; for example, methylthiazole and pyridinol (Figures S6 and S7).

## 4. Discussion

We here showed that pyrrole and dimethyl pyrrole can be abiotically formed from acetylene or methylacetylene, ammonium chloride, and metal sulfides under aqueous conditions at 105 °C. The best yields for pyrrole were achieved at a pH of 9.1, an equimolar concentration of $Na_2S$ and $NiSO_4$, and after one day of reaction time. The conditions of our experiments were chosen to fit a scenario where life possibly emerged near volcanic exhalations [31] or hydrothermal vents at hadean times [32]. Nowadays, for the synthesis of pyrrole derivatives, very specific educts, solvents, and catalysts are used, in an attempt to optimize yields. In contrast, the currently shown synthesis of pyrrole is based on simple starting materials under aqueous and oxygen-free reaction conditions, in order to simulate prebiotic environments. As mentioned above, iron, nickel, and cobalt are still part of important biomolecules in modern biochemistry. It is therefore tempting to speculate that they were also involved in the prebiotic synthesis of pyrrole and its polymers. In support of this hypothesis is the fact that iron, nickel, and cobalt are commonly found in the crust of the Earth [33,34]. In our experiments, NiS and CoS showed their potential for catalytic activity in the formation of pyrrole from acetylene and ammonia.

The most common belief regarding the atmosphere on early Earth is that it mostly consisted of $CO_2$ and water vapor [35,36], with only small traces of $N_2$ and CO. Ammonia, which does not fit in this highly oxidized atmosphere, could be formed out of $NO_3^-$ due to reduction driven by $FeS/H_2S$ [37]. Nitrate, in this scenario, could be formed from atmospheric $N_2$ and $CO_2$ by electric discharges under oxygen-free conditions [38] and subsequently be dissolved in the ancient ocean.

The yields of pyrrole and dimethylpyrrole in our experiments are comparatively low. For safety reasons, due to the danger of explosion, experiments were performed at low pressure. At sub-seafloor level (>1000 bar), yields would be increased [39,40].

The synthesis of pyrrole can be seen as a first step in the formation of larger porphyrin systems. A first achievement in this direction could represent the synthesis of dimethylpyrrole in our experiments when starting from propyne instead of acetylene. Different side chains could then be achieved by using the corresponding starting materials, either to enhance the reactivity of the molecule for polymerization, or to match the side chains of biological tetrapyrrole structures. As transition metal sulfides first served as catalysts in this reaction system, a later substitution of the sulfide through the newly formed pyrrole structures could be imagined for the evolution of the potent catalyst factor F430, cobalamin, and also at a later stage, when oxygen was available, hemoglobin and chlorophyll.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/life11090980/s1, Figure S1: Structural formula of heme b, Figure S2: Structural formula of chlorophyll b, Figure S3: Structural formula of cobalamin (vitamin B12), Figure S4: Structural formula of coenzyme F430, Figure S5. Mass spectra of bis(tert-butyldimethylsilyl)-sulfite, Figure S6. Mass spectra of 2-methylthiazol, Figure S7. Mass spectra of 3-tert-butyldimethylsilyloxypyridine, Scheme S1: Knorr pyrrole synthesis, Scheme S2: Mechanism of Knorr pyrrole synthesis, Scheme S3: Mechanism of Fischer–Fink pyrrole synthesis, Scheme S6: Paal–Knorr reaction, Scheme S7: Mechanism of Paal–Knorr pyrrole synthesis, Scheme S4: Pyrrole synthesis according to Hantzsch, Scheme S5: Mechanism of Hantzsch pyrrole synthesis, Table S1: Formation of pyrrole depending on pH, Table S2: Formation of pyrrole depending on reaction time, Table S3: Formation of pyrrole depending on concentration of catalyst.

**Author Contributions:** Conceptualization, C.H. and W.E.; data curation, C.S.; funding acquisition, C.H.; methodology, C.S.; supervision, C.H. and W.E.; visualization, C.S.; writing—original draft, C.S. and C.H.; writing—review and editing, C.S., C.H. and W.E. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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## 3.2 Summary and Article: „ From Zero to Hero: The Cyanide-Free Formation of Amino Acids and Amides from Acetylene, Ammonia and Carbon Monoxide in Aqueous Environments in a Simulated Hadean Scenario”

It has already been shown by Huber et al. that amino acids can be synthesised by using CO and $CN^-$ as carbon sources. Here we show an alternative to the Strecker scheme for the formation of amino acids, which is thought to be a likely reaction to form amino acids in prebiotic chemistry. In addition, we show the formation of amides under aqueous conditions. The condensation reaction used in extant biochemistry to form peptide bonds is unlikely to occur in water, and therefore amino acids must first be activated to perform this reaction. In a prebiotic scenario an aqueous environment is essential, concentrations are low and activating agents are not available. Amides could now play a key role in the emergence of life, as it is possible to form the peptide functionality directly from the starting substances, and thus bypass the hindered condensation reaction.

A typical experiment is the same as in 3.1. We could detect a total of five amino acids and 13 amides in our reaction network. The influence of reaction parameters has been discussed in detail for alanine, aspartic acid, propionamide and succinamic acid. All show maximum yields in runs with $Na_2S$ concentration equimolar to the metal ion in the reaction, a pH around 8.5 and a reaction time of 7 days. As for the transition metal sulphide, we used nickel, cobalt, iron and mixtures thereof. We observed the best formation of alanine with the Co/Fe mixture and of aspartate with the Ni/Co mixture. The best formation of propionamide was obtained with Co and Co/Fe, and the best formation of succinamic acid was obtained with Co and Ni/Co. This shows a broad catalytic basis and large variability regarding further evolution towards peptide formation.

**Table 1:** Amino acids and amides formed from acetylene, ammonia and carbon monoxide in the presence of NiS in 5 ml $H_2O$ at 105 °C after 7 days. All substances were detected as their corresponding TBDMS derivatives. The number of carbon and nitrogen atoms derived from the reactants is identified by stable isotope labelling as indicated. Adapted from Seitz *et al.* 2024.

| Compound | # C | # N | $^{13}C_2H_2$ | $^{15}NH_4Cl$ | $^{13}CO$ |
|---|---|---|---|---|---|
| Amino acids | | | | | |
| Glycine | 2 | 1 | 2 | 1 | - |
| Alanine | 3 | 1 | 3 | 1 | - |
| β-Alanine | 3 | 1 | 2 | 1 | 1 |
| Aspartic acid | 4 | 1 | 2 | 1 | 2 |
| β-Homoserine | 4 | 1 | 2 | 1 | 2 |
| Amides | | | | | |
| Formamide | 1 | 1 | 1 | 1 | - |
| Urea | 1 | 2 | - | 2 | 1 |
| Acetamide | 2 | 1 | 2 | 1 | - |
| Acrylamide | 3 | 1 | 2 | 1 | 1 |
| Propionamide | 3 | 1 | 2 | 1 | 1 |
| β-Alanine amide | 3 | 1 | 2 | 2 | 1 |
| Succinamic acid | 4 | 1 | 2 | 1 | 2 |
| Fumaramic acid | 4 | 1 | 2 | 1 | 2 |
| Pentenoic amides | 5 | 1 | 4 | 1 | 1 |
| Pentanoic amide | 5 | 1 | 4 | 1 | 1 |
| 2-Aminobenzamide | 7 | 2 | 6 | 2 | 1 |
| 2,4-Heptadienoic amide | 7 | 1 | 6 | 1 | 1 |
| Benzamide | 7 | 1 | 6 | 1 | 1 |

Author contribution: My individual contribution to this work included the interpretation of the available data and the conception and performance of additional necessary experiments. I was also involved in the preparation of the published research paper.

*life*

MDPI

*Article*

# From Zero to Hero: The Cyanide-Free Formation of Amino Acids and Amides from Acetylene, Ammonia and Carbon Monoxide in Aqueous Environments in a Simulated Hadean Scenario

**Christian Seitz, Thomas Geisberger, Alexander Richard West, Jessica Fertl, Wolfgang Eisenreich and Claudia Huber ***

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**Abstract:** Amino acids are one of the most important building blocks of life. During the biochemical process of translation, cells sequentially connect amino acids via amide bonds to synthesize proteins, using the genetic information in messenger RNA (mRNA) as a template. From a prebiotic perspective (i.e., without enzymatic catalysis), joining amino acids to peptides via amide bonds is difficult due to the highly endergonic nature of the condensation reaction. We show here that amides can be formed in reactions catalyzed by the transition metal sulfides from acetylene, carbon monoxide and ammonia under aqueous conditions. Some α- and β-amino acids were also formed under the same conditions, demonstrating an alternative cyanide-free path for the formation of amino acids in prebiotic environments. Experiments performed with stable isotope labeled precursors, like $^{15}NH_4Cl$ and $^{13}C$-acetylene, enabled the accurate mass spectroscopic identification of the products formed from the starting materials and their composition. Reactions catalyzed using the transition metal sulfides seem to offer a promising alternative pathway for the formation of amides and amino acids in prebiotic environments, bypassing the challenges posed by the highly endergonic condensation reaction. These findings shed light on the potential mechanisms by which the building blocks of life could have originated on early Earth.

**Keywords:** amino acids; amide; peptide bond; acetylene; transition metal sulfides; hydrothermal conditions; origin of life



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## 1. Introduction

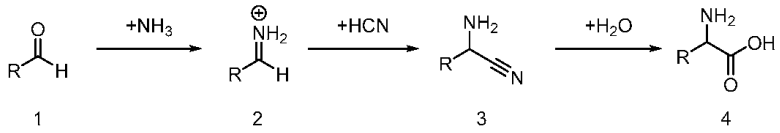
The role of amino acids as the fundamental building blocks of life is paramount in both modern biochemistry and theories of the origin of life. For modern biochemistry, α-amino acids are essential building blocks for peptides and proteins. In the context of the origin of life, various abiotic conditions, whether terrestrial or extraterrestrial in nature, have been proposed to explain their formation. One of the first experiments, which synthesized organic compounds such as amino acids from an inorganic starting material, were the experiments by Miller and Urey [1,2]. These pioneers of research on the origin of life used electric discharges as an energy form in a reducing gas atmosphere consisting of $NH_3$, $H_2$ and $CH_4$. Additionally, later, there were also experiments using a more neutral atmosphere consisting of $CO_2$, CO, $N_2$ and $H_2O$; however, the yields were not as high as the ones in a reducing gas atmosphere [3].

In the meantime, many other types of energy sources were considered to form organic compounds from simple precursors on the early Earth. There have been attempts using UV [4–8], X-ray [9,10] and proton irradiation [11,12], shock heating from 90 °C [13] to over 200 °C [14–16] or using volcanism-induced electric discharges as other possible energy sources [17,18]. In the iron–sulfur theory of the origin of metabolism [19], chemical

energy acts in the formation of amino acids [20] and peptides [21] using FeS/NiS catalysts. Herrera, another pioneer in the origin of life field, simply mixed ammonium thiocyanate with formaline and discovered amino acids and further organic molecules [22].

However, amino acids could also have originated from extra-terrestrial sources. Amino acids were detected in carbonaceous meteorites of various types from C1 [23–25], through to C2 [26–28] and C3 [24,29,30]. Recently, glycine oligopeptides were synthesized from C, CO and $NH_3$ under simulated stellar conditions [31].

The prebiotic synthesis of amino acids likely follows a Strecker reaction [32], starting from an aldehyde (Scheme 1). With the addition of ammonia, an imine is built, which reacts with cyanide to form α-aminonitrile which is finally hydrolyzed to an α-amino acid. Depending on the structure of R in the starting aldehyde, different amino acids can be synthesized by this type of reaction [33].




**Scheme 1.** Basic process of the Strecker reaction. The reaction proceeds via the nucleophilic addition of ammonia to the aldehyde (1). This generally extends to the iminium ion (2). The cyanide adds to this electrophilic species, resulting in an α-aminonitrile (3). The amino acid (4) is ultimately formed through hydrolysis.

In nature, amino acids are linked together via a peptide bond to form larger molecules. In this process, the carboxy group of one amino acid reacts with the amino group of another amino acid in a condensation reaction. However, an aqueous solution is unfavorable for the formation of amide bonds [34] and the reaction can, therefore, not be performed under normal conditions without catalysis. An alternative to the formation of peptides by stringing together individual amino acids via peptide bonds could be the formation of amino acids using simple molecules directly from the previous amino acid. Amides could play a central role in this scenario, where a functional peptide can be formed without being dependent on the unfavorable condensation reaction. In the context of the origin of life, the formation of amides has been shown in wet–dry cycles [35,36] or in reactions coupled to pyrite formation [37].

In previous works, we demonstrated the formation of fatty acids [38,39] and intermediates of existing carbon fixation cycles [40] using acetylene and carbon monoxide as carbon sources under simulated volcanic hydrothermal conditions. Nitrogen was successfully introduced into the system via ammonia, as shown by the formation of pyrrole, another essential unit in the biochemistry of modern life [41]. In these reactions, transition metal sulfides served as catalysts, in accordance with the iron–sulfur world theory of Günter Wächtershäuser [19]. In contrast to the typical cyanide-based Strecker scheme, we now show the simultaneous metal-catalyzed formation of amino acids from acetylene, carbon monoxide and ammonia under simulated hydrothermal conditions, again demonstrating the synthetic importance of acetylene for prebiotic chemistry. Moreover, we detected various amides under these aqueous conditions underlining the putative role of amide functionalities in the evolution of peptides and proteins.

## 2. Materials and Methods

All chemicals were purchased from Sigma Aldrich GmbH (Steinheim, Germany) in the highest purity available. Acetylene 2.6 (acetone free) was purchased from Linde AG (Pullach, Germany), and CO 2.5 and argon 4.6 were purchased from Westfalen AG (Münster, Germany).

Experiments were performed as published previously [41]. Briefly, in a typical run (run 1, Table S2), a 125 mL glass serum bottle was charged with 1.0 mmol $NiSO_4 \cdot 6\ H_2O$ and 1.0 mmol $NH_4Cl$ and closed with a gas tight silicon stopper.

The bottle was evacuated three times and filled with argon, finally resulting in a de-aerated state. Subsequently, the bottle was filled with argon-saturated water, 1 M $Na_2S$ solution and 1 M NaOH solution, resulting in a total reaction volume of 5 mL. In this mixture, a precipitate of black NiS was immediately formed due to its low solubility constant of $1 \times 10^{-22}$ [42,43] in aqueous solution. Finally, 60 mL of acetylene gas and 60 mL of CO were added. Reactions were carried out at 105 °C using reaction times up to seven days. Variations were achieved through the addition of different volumes of NaOH or $Na_2S$ solution and the use of $FeSO_4$ or $CoSO_4$ instead of or additionally to $NiSO_4$. After the defined reaction time, 1 mL of the reaction mixture was freeze dried and derivatized with 0.5 mL acetonitrile and 0.5 mL *N-tert*-butyldimethylsilyl-*N*-methyltrifluoracetamide (MTBSTFA) at 70 °C for one hour.

Stable isotope precursors ($^{13}CO$, $^{13}C_2$-Acetylene and $^{15}\ NH_4Cl$) were used to elucidate the composition of the products. $^{13}CO$ and $^{15}\ NH_4Cl$ were directly added instead of their analogs. $^{13}C_2$-Acetylene gas was obtained by adding tetra-*n*-butylammonium fluoride (TBAF) to solid $^{13}C_2$-(trimethylsilyl)acetylene in an evacuated serum bottle via a syringe. The resulting $^{13}C_2$-acetylene gas was then used for experiments.

The TBDMS derivatives of the products were analyzed by GC-MS using a GC-2010, coupled with MS-QP2010 Ultra (Shimadzu GmbH, Duisburg, Germany) with a 30 m × 0.25 mm × 0.25 µm fused silica capillary column (Equity TM5, Supelco, Bellefonte, PA, USA) and an AOC-20i auto injector.

The applied temperature of the column oven was as follows: 0–6 min at 90 °C; 6–25 min at 90–310 °C, 10 °C/min; injector and transfer temperature were kept at 260 °C.

Identification was performed by comparison of retention times and mass spectra of purchased reference compounds, as well as with data from the National Institute of Standards and Technology (NIST) spectral library. Retention times are given in Table S1.

Quantification was performed by external calibration using a solution of alanine with different concentrations.

For comparison, blank runs with argon instead of acetylene and runs without a transition metal compound were performed.

## 3. Results

We reacted acetylene, carbon monoxide and ammonia under demanding anaerobic, aqueous conditions at 105 °C for up to 7 days. As transition metal catalysts, FeS, NiS, CoS and mixtures of them were used, which were freshly prepared in situ from metal sulfates and sodium sulfide.

Under similar conditions, mainly unsaturated, odd numbered carboxylic acids from formic acid up to nonadecenoic acids were detected [38,39]. We now show the formation of carboxylic acid amides up to a chain length of $C_5$ and the simultaneous formation of amino acids (Table 1). Runs with $^{13}CO$, $H^{13}C{\equiv}^{13}CH$ and $^{15}NH_4Cl$ obtained these products as genuine reaction products and it could be seen that they were composed from the starting materials. In the absence of NiS, FeS or CoS, these amides and amino acids were not formed.

Specifically, we detected α-alanine, β-alanine, glycine, aspartic acid and β-homoserine and 13 amides including formamide, propionamide and succinamic acid (Table 1). All substances were analyzed as their corresponding *tert*-butyldimethylsilyl (TBDMS) derivatives by GC-MS.

Further condensation to detectable amounts of peptides under otherwise similar conditions would probably require higher concentrations [44,45]. In an origin of life scenario, this could be achieved through surface bonding on the catalyzing mineral [46], thermal concentration in hydrothermal rock pores [47] and dehydration–hydration cycles [48]. Nevertheless, the detection of surrogate amides provides evidence for the one-pot formation of amides and amino acids from acetylene, CO and ammonia in a hydrothermal environment.

It is tempting to speculate that this scenario could support further evolution into peptides on the early Earth, avoiding the unfavorable condensation of amino acids.

**Table 1.** Amino acids and amides formed from acetylene (2.71 mmol), ammonia (1.00 mmol) and carbon monoxide (2.68 mmol) in the presence of NiS (1.00 mmol) in 5 mL $H_2O$ at 105 °C after 7 days. All substances were analyzed as their corresponding TBDMS derivatives. Number of carbon and nitrogen atoms derived from the reactants are identified by stable isotope labelling as indicated.

| Compound | # C | # N | $^{13}C_2H_2$ | $^{15}NH_4Cl$ | $^{13}CO$ |
|---|---|---|---|---|---|
| Amino acids | | | | | |
| Glycine | 2 | 1 | 2 | 1 | - |
| Alanine | 3 | 1 | 3 | 1 | - |
| β-Alanine | 3 | 1 | 2 | 1 | 1 |
| Aspartic acid | 4 | 1 | 2 | 1 | 2 |
| β-Homoserine | 4 | 1 | 2 | 1 | 2 |
| Amides | | | | | |
| Formamide | 1 | 1 | 1 | 1 | - |
| Urea | 1 | 2 | - | 2 | 1 |
| Acetamide | 2 | 1 | 2 | 1 | - |
| Acrylamide | 3 | 1 | 2 | 1 | 1 |
| Propionamide | 3 | 1 | 2 | 1 | 1 |
| β-Alanine amide | 3 | 1 | 2 | 2 | 1 |
| Succinamic acid | 4 | 1 | 2 | 1 | 2 |
| Fumaramic acid | 4 | 1 | 2 | 1 | 2 |
| Pentenoic amides | 5 | 1 | 4 | 1 | 1 |
| Pentanoic amide | 5 | 1 | 4 | 1 | 1 |
| 2-Aminobenzamide | 7 | 2 | 6 | 2 | 1 |
| 2,4-Heptadienoic amide | 7 | 1 | 6 | 1 | 1 |
| Benzamide | 7 | 1 | 6 | 1 | 1 |

For more a more specific study, two amino acids and two amides were chosen as representatives. In Figures 1–4 the mass spectra of differently labelled propionamide (Figure 1), succinamic acid (Figure 2), alanine (Figure 3) and aspartic acid (Figure 4) are shown. In each Figure A shows the corresponding spectra of the unlabeled compounds; B shows the spectra of the reaction products in which $^{13}$C-acetylene was used; C, in which $^{13}$CO was used, and D, in which $^{15}$N-labelled ammonia was used. Propionamide, succinamic acid and aspartic acid, which were used in high amounts, were measured in SCAN mode. Due to the comparatively low yield of alanine, we used single-ion monitoring (SIM) to show the labelling patterns of alanine. Here, only the typical masses of TBDMS-alanine, which are $m/z$ = 260, $m/z$ = 232 and $m/z$ = 158, were measured (Figure S1). The most intensive mass peak usually represents the fragment lacking a t-butyl group (M-57$^+$). In the case of propionamide, succinamic acid and aspartic acid these are $m/z$ = 130, $m/z$ = 288 and $m/z$ = 418, respectively. It should be noted that peptides or peptide-like assemblies could not be observed.

Based on the specific mass data for the reaction products obtained from the experiments with different stable isotope labelled precursors, the starting materials from which the individual compounds were synthesized could be identified. It turned out that propionamide was formed from one molecule of acetylene, carbon monoxide and ammonia, succinamic acid from one molecule of acetylene, two molecules of CO and one molecule of ammonia, alanine from one and a half molecule of acetylene, two molecules of water and one molecule of ammonia, and aspartic acid from one molecule of acetylene, two molecules of carbon monoxide, two molecules of water and one molecule of ammonia (Scheme 2). Interestingly, all of the carbon atoms in alanine came from acetylene. In contrast, β-alanine was formed from one molecule of acetylene and one molecule of carbon monoxide (Table 1).

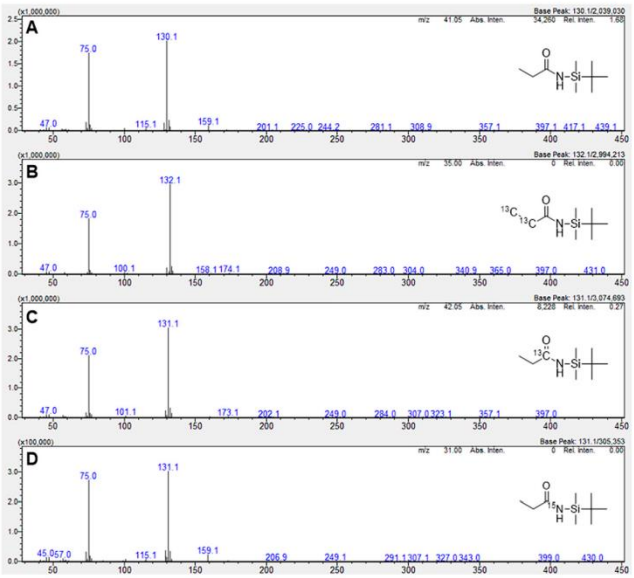



**Figure 1.** Different mass spectra of propionamide. (**A**) is from run 1 (Table S2), (**B**) is from a run with $^{13}C_2H_2$, (**C**) is from a run with $^{13}CO$ and (**D**) is from a run with $^{15}NH_4Cl$. The typical mass of $m/z$ = 130 results from the loss of the t-butyl group from TBDMS.

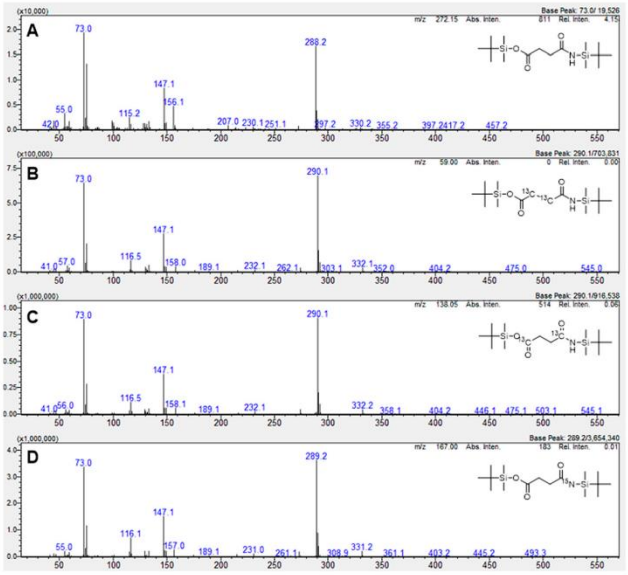



**Figure 2.** Different mass spectra of succinamic acid. (**A**) is from run 1 (Table S2), (**B**) is from a run with $^{13}C_2H_2$, (**C**) is from a run with $^{13}CO$ and (**D**) is from a run with $^{15}NH_4Cl$. The typical mass of $m/z$ = 288 results from the loss of the t-butyl group from the TBDMS.

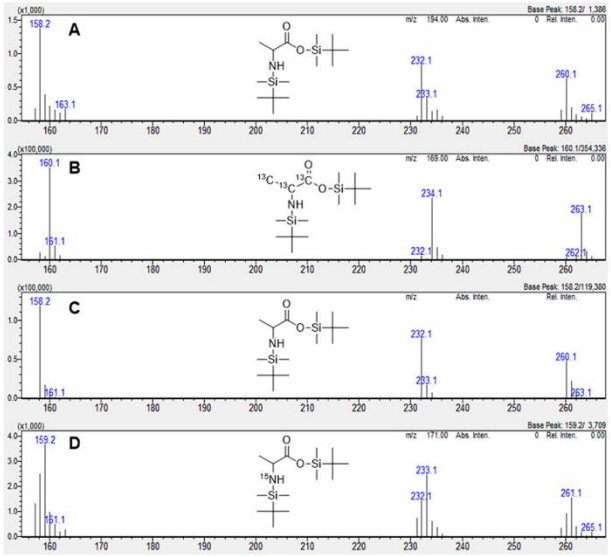



**Figure 3.** Different mass spectra of alanine. Measurement was performed in single ion monitoring (SIM) mode. Shown are the masses of typical fragments of TBDMS-derivatized amino acids. In the case of alanine these are $m/z$ = 260, $m/z$ = 232 and $m/z$ = 158. (**A**) is from run 1 (Table S2), (**B**) is from a run with $^{13}C_2H_2$, (**C**) is from a run with $^{13}CO$ and (**D**) is from a run with $^{15}NH_4Cl$.

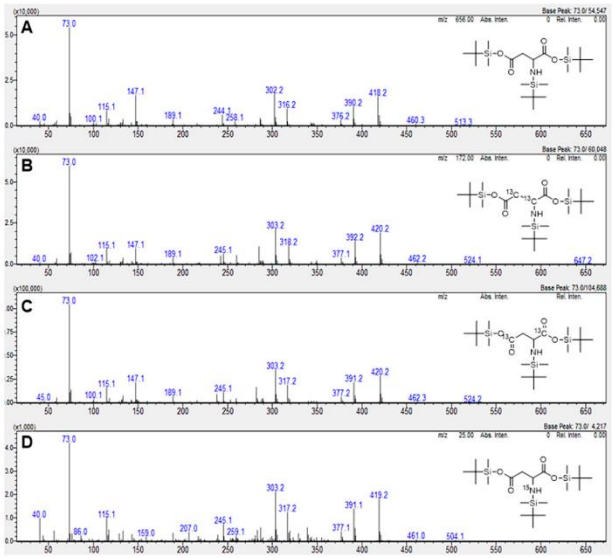
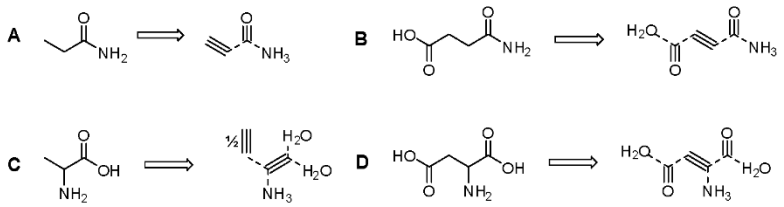



**Figure 4.** Different mass spectra of aspartic acid. (**A**) is from run 1 (Table S2), (**B**) is from a run with $^{13}C_2H_2$, (**C**) is from a run with $^{13}CO$ and (**D**) is from a run with $^{15}NH_4Cl$. Typical mass fragments of TBDMS-derivatized aspartic acid are $m/z$ = 418, $m/z$ = 390 and $m/z$ = 302.




**Scheme 2.** Retrosynthesis of propionamide from one molecule of ammonia, acetylene and carbon monoxide (**A**); succinamic acid from one molecule of ammonia, one molecule of acetylene, two molecules of carbon monoxide and one molecule of water (**B**); alanine from one molecule of ammonia, one and a half molecules of acetylene and two molecules of water (**C**); and aspartic acid from one molecule of ammonia and acetylene and two molecules of carbon monoxide and water (**D**).

Different reaction parameters were investigated. Tables S2–S5 show all of the reactions which were performed to elucidate the influence of the metal ion catalysts, pH value, reaction time and the amount of $Na_2S$ in the reaction mixture.

As shown in previous works [41], only metal sulfides, which were freshly formed in situ from metal sulfates and sodium sulfide, were catalytically active in our reaction setup. To analyze the role of the metal sulfide catalysts, we used $NiSO_4$, $CoSO_4$ and $FeSO_4$ as well as 50/50 (mol%) mixtures of two of them and a 33/33/33 (mol%) mixture of all three transition metals to form the respective sulfides. In previous works [41], nickel sulfide showed the best yields in product formation. However, in the case of propionamide and succinamic acid, cobalt sulfide or mixtures of the sulfides gave the best yields. In the case of alanine and aspartic acid, nickel sulfide or sulfide mixtures were superior catalysts (Figure 5).

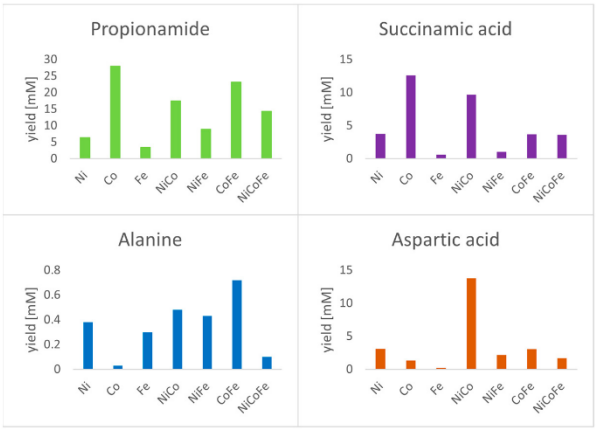



**Figure 5.** Formation of propionamide, succinamic acid, alanine and aspartic acid with different metal ions as metal sulfide catalysts. Mixtures of transition metal catalysts contain 50/50 (mol%) or 33/33/33 (mol%) of the corresponding metal-sulfides, respectively.

Different pH values were achieved by adding NaOH to the reaction mixtures (Table S5). Propionamide, succinamic acid, aspartic acid and alanine showed maximum yields at about pH 8.4 (Figure 6). At acidic pH values, no formation of amides and amino acids could be detected. At pH values > 9.0 the formation of amides and amino acids both rapidly decreased.

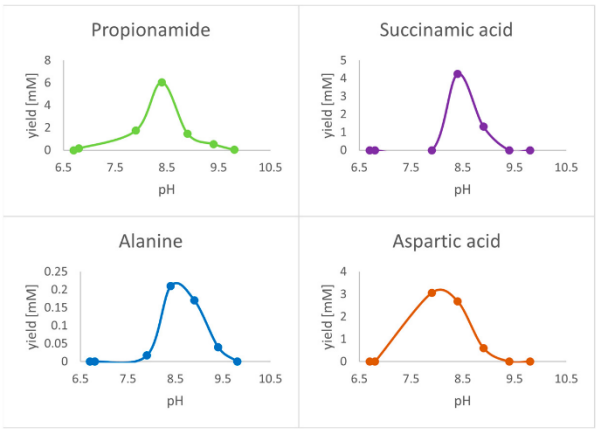



**Figure 6.** Formation of ***propionamide (green), succinamic acid (violet), alanine (blue) and aspartic acid (orange)*** in the presence of NiS at different pH values. pH values were measured at the end of the reaction time.

The formation of amides and amino acids was detected in reaction times ranging from 0 min to 7 days (Figure 7). However, product formation started comparatively slowly and constantly increased until the reaction was stopped at 7 days. According to our data, it seems promising to increase the reaction time beyond 7 days. However, all of the gases in the serum bottle were consumed after this time. Filling up the missing gas volume with CO and acetylene after that point showed no increase in product formation. This can probably be explained by a deactivation of the catalyst during the reaction period.

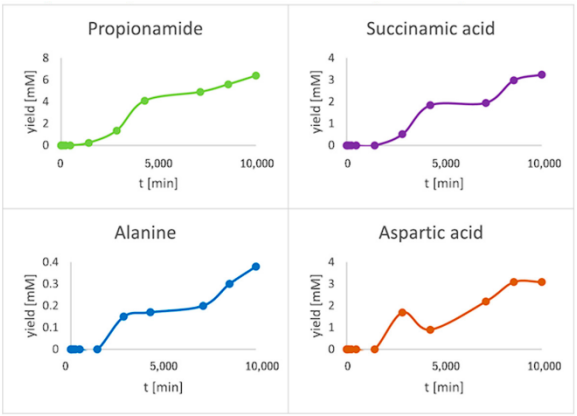



**Figure 7.** Time-dependent NiS catalyzed formation of ***propionamide (green), succinamic acid (violet), alanine (blue) and aspartic acid (orange)*** from acetylene, carbon monoxide and ammonia.

As was shown before [41], metal sulfate alone was not catalytically active in our setup. Therefore, the concentration of catalyst in the reaction can be controlled by changing the amount of $Na_2S$. As expected, an equimolar concentration of metal sulfate and sodium sulfide showed the best yields of amino acids and amides (Figure 8). Lesser concentrations of sulfide resulted in lesser amounts of active catalysts. Higher concentrations also had a negative effect, which was probably due to their effect on the pH value.

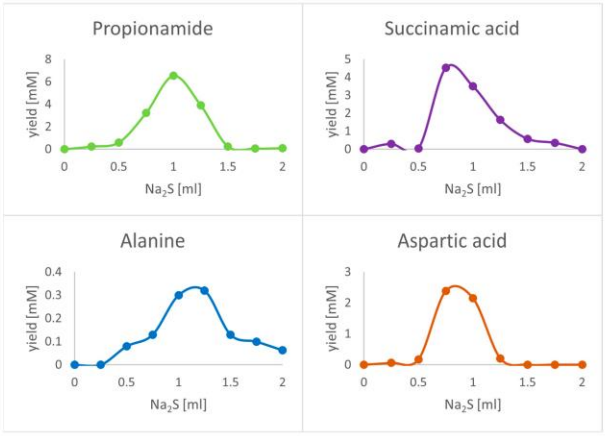



**Figure 8.** Formation of ***propionamide (green), succinamic acid (violet), alanine (blue) and aspartic acid (orange)*** with different concentration of $Na_2S$ and therefore different concentration of active catalysts.

## 4. Discussion

The synthesis of peptide bonds in non-biological systems is, despite recent success [49,50], a challenging issue [51]. These problems are even more serious considering that, in an origin of life scenario, complex protecting groups and appropriate solvents are not available.

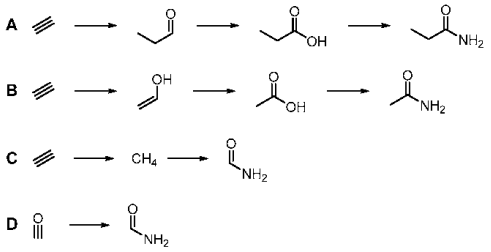
Supported by stable isotope labelling, here, we provided proof of the formation of simple amides, potential synthons for more complex peptides, in abiotic reactions starting from acetylene, ammonium chloride, carbon monoxide and metal sulfides under aqueous conditions at 105 °C. The best yields were achieved at a pH of 8.4, equimolar concentrations of metal sulfates and sodium sulfide and seven days of reaction time. Different metal sulfides and mixtures thereof showed catalytic potential in the formation of amino acids and simple amides. The conditions of our experiments were chosen to fit an Hadean scenario, where life possibly emerged near volcanic exhalations [52] or hydrothermal vents [53].

Based on the $^{13}C$ label distribution in the detected products, three mechanisms of amide formation from acetylene, carbon monoxide and ammonia can be postulated (Scheme 3). The dominant mechanism is the carbonylation or double carbonylation of acetylene, followed by amide formation leading to, e.g., propionamide and succinamic acid (Scheme 3A). A second pathway includes the hydration of acetylene, followed by oxidation and amination, leading to, e.g., acetic acid amide (Scheme 3B). In a third reaction pathway, the cleavage of acetylene is involved, as reflected by the about 66% $^{13}C$ enrichment in formic acid amide when $^{13}C$ labeled acetylene was used as starting material (Scheme 3C). The residual 33% represents a formic acid amide derived from CO (Scheme 3D). We hypothesize that in our experiments, all of the reaction steps took place in the reaction sphere of the metal sulfide precipitates.

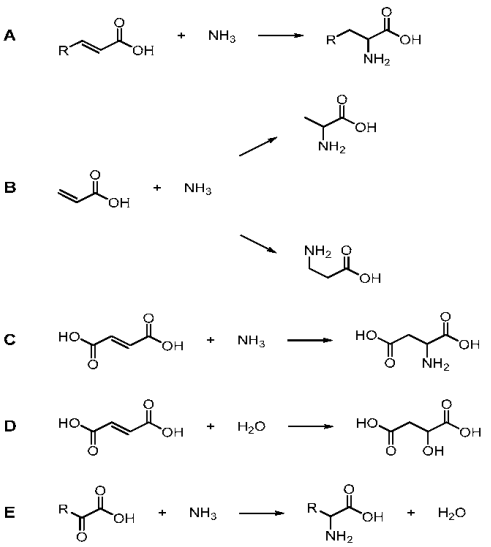
Amino acids can be formed from α, β unsaturated carboxylic acids through the addition of $NH_3$ (Scheme 4A), leading to α and β alanine (Scheme 4B) and aspartic acid (Scheme 4C), a reaction which has to compete with the addition of $H_2O$ (Scheme 4D) or the reductive amination of α-ketoacids (Scheme 4E) [54].

As mentioned before, the difference in the synthesis of alanine and β-alanine is particularly significant. The carbon atoms of β-alanine originate from one molecule of acetylene and one molecule of carbon monoxide. In contrast, all of the carbon atoms in α-alanine are from acetylene. Interestingly, evolution did not choose the more available anti-*Markownikow* product, β-alanine, as one of its basic building blocks, but rather preferred an

alternative route to the *Markownikow* product, α-alanine. This could indicate an early form of control over life processes through the preference for a less present reaction product.




**Scheme 3.** Formation of amides from acetylene as starting material. Shown is the formation of propionamide (**A**), acetamide (**B**) and formamide (**C**). (**D**) shows the simultaneously occurring reaction of CO into formamide.




**Scheme 4.** Possible mechanisms of the formation of amino acids in our setup. (**A**): proposed mechanism of $NH_3$ addition to α, β unsaturated carboxylic acids. (**B**,**C**): mechanism for the example of α, β alanine and aspartic acid. (**D**): competing reaction with the addition of water and (**E**): another possible mechanism via reductive amination of α-ketoacids.

We would like to mention that our analytical setup did not discern between the D and L forms of amino acids. We assume, in the first instance, the formation of racemic mixtures which, in consecutive steps, are selected by different binding constants on the catalytic surfaces [19]. The formation of homochiral peptides and their stability in the Earth's crust was recently discussed by S. Toxvaerd [55].

The carbon sources in our reaction network were acetylene and carbon monoxide. Acetylene would have been commonly available in an early world scenario as it can be formed by volcanic processes [56] and/or from $CaC_2$ in a reaction with water [57]. Acetylene is also considered an important reagent for the formation of smaller molecules containing carbon atoms in interstellar chemistry [58,59]. Acetylene can form smaller hydrocarbons, polyenes and benzenes and therefore lead to a variety of possible carbon

precursors for early biochemistry [60]. In our experiments the labelling patterns of the products show the utilization of acetylene as the main carbon source.

Here, we show that all the transition metals, nickel, cobalt and iron, and even mixtures, can serve as potential catalysts in prebiotic reactions. All three can catalyze different reactions to different degrees, which is an indication that different biochemical compounds may have developed from them. These are used for different reactions in modern biochemistry. The natural availability of these metals on early Earth supports this hypothesis. Iron, nickel and cobalt are commonly found in the crust of the Earth [61,62].

Iron, as the most abundant mineral out of these three transition metals, was shown to have the best catalytic properties in earlier studies on reductive amination [54] and $CO_2$ reduction [63]. In this study, we report enhanced results for amide and amino acid formation by using nickel, cobalt and mixtures containing cobalt as one constituent. To date, only a limited number of studies have dealt with the electrochemical and catalytic properties of transition metals in an origin of life context and predictions are hard to give. A summary of recent studies is given by de Graaf and Li [64,65]. However, the relevance of all three transition metals as catalysts is supported by extant biochemistry. For example, iron in FeS clusters and hemoglobin enable redox reactions and oxygen transport, respectively. Nickel is important for hydrogen activation and in cofactor F430 for Methanogenesis. Cobalt is contained in coenzyme B12, a cofactor in enzymes that are necessary in the metabolism of amino acids.

As mentioned before, the amino acids are most likely synthesized via a Strecker reaction in a prebiotic environment. We show here that the formation of amino acids is also possible using ammonia only without cyanide, which is necessary for the Strecker reaction. Ammonia, however, does not fit in a relatively oxidized atmosphere, which is assumed for early Earth [66,67]. But, ammonia could be formed out of $NO_3^-$ due to reduction driven by $FeS/H_2S$ [68]. Nitrate, in this scenario, could be formed from atmospheric $N_2$ and $CO_2$ through electric discharges under oxygen free conditions [69] and subsequently be dissolved in the ancient ocean.

The formation of peptides is an endergonic process, which means that it does not occur spontaneously under aqueous conditions. This reaction occurs with a decrease in entropy and is so energetically unfavorable that the equilibrium constant $K_{syn}$ for the combination of two amino acids is $<10^{-5}$ [34]. Therefore, in modern synthetic pathways, amino acids must first be activated. To make matters worse, in a prebiotic scenario, an aqueous environment is essential, concentrations are low and activating agents are not available. This makes the condensation reaction even more difficult. Here, we can show the formation of amides, carrying the functionality of dipeptides, in water. In an origin of life context, this finding is important, because we now demonstrate that amides/peptides are not necessarily formed by the condensation of a carboxy group with an amine, but could also be synthesized directly from carbon monoxide, acetylene and ammonium. This pathway avoids the adverse circumstances of peptide formation from individual amino acids and underlines the importance of acetylene in origin of life syntheses.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life14060719/s1, Table S1. Retention time and typical fragment mass of amino acids and amides, Table S2. Propionamide, succinamic acid, alanine and aspartic acid formation based on different metal catalysts, Table S3. Propionamide, succinamic acid, alanine and aspartic acid formation based on different reaction times, Table S4. Propionamide, succinamic acid, alanine and aspartic acid formation based on different amounts of metal sulfide catalysts, Table S5. Propionamide, succinamic acid, alanine and aspartic acid formation based on different pH values, Figure S1. Typical fragments of TBDMS- amino acids in GC/MS experiments using the example of alanine.

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# 4 Conclusion and Outlook

## 4.1 Expanding the Acetyleno Network

As mentioned above, Huber *et al.* had outstanding success with the introduction of acetylene into reactions under Wächtershäuser conditions. At that time, the focus was on reactions with acetylene alone or with an additional carbon source in the form of CO. Now the reaction network is being expanded to include nitrogen in the form of ammonia.

### 4.1.1 Formation of Amino Acids

Amino acids are essential building blocks in extant biochemistry. As a result, there are numerous examples of prebiotic amino acid synthesis. Most of them have in common that they are based on cyanide, according to the Strecker scheme. We have now demonstrated a milder amino acid synthesis by using ammonia, thus avoiding the negative aspects that come with cyanide. We were able to demonstrate the formation of five amino acids, using reaction conditions that simulate a hydrothermal environment. The amount of amino acid obtained varies greatly between the different amino acids, covering two orders of magnitude in the millimolar range.

### 4.1.2 Formation of Short Fatty Acid Amides

After amino acids, the next step on the path to modern biochemistry is the polymerisation into peptides and then proteins. However, the formation of peptide bonds, which is used in extant biochemistry to form those polymeres, is almost impossible to perform in an aqueous environment without catalysis. In current biochemistry, enzymes catalyse the formation of peptide bonds. With the discovery of amides in our reaction network, this unfavourable reaction could be bypassed by building the peptide bond on top of a precursor instead of linking existing units together. The group of amides, in which the oxygen atom of a carboxy group is replaced by nitrogen, is the simplest representative of this process. We found a total of 13 amides in our reaction networks. Again, concentrations in the millimolar range could be achieved.

### 4.1.3 Formation of Pyrrole

At first glance, the role of pyrrole as a biomolecule is not very obvious. However, the full potential of pyrrole is revealed when four of them come together to form tetrapyrrole structures. These, especially porphyrin and corrins, play an important role in modern biochemistry. Their ability to hold and stabilise metal ions within their ring structure allows them to perform specific functions. For example, with iron as the central metal ion, haemoglobin is responsible for binding and transporting oxygen, which is needed for energy balance through respiration. With cobalt as the central ion, the corresponding complex is an essential cofactor known as vitamin $B_{12}$ or cobalamin. It

is required by animals, which use it as a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism. Pyrrole and dimethylpyrrole have been obtained in yields of up to 13% based on the conversion of ammonia.

### 4.1.4 Nucleobases

In the case of the nucleobases, uracil was found and confirmed by stable isotope labelling. However, the formation of the other nucleobases from acetylene and various other reactant molecules should be possible. Since previous experiments have shown a strong dependence on pH, this seems to be a possible explanation for the actual absence of the other nucleobases.

## 4.2 The Show Must Go On: Polymerisation

As mentioned above, the polymerisation into certain biomolecules such as proteins, DNA and RNA is an important prerequisite for the origin of life. So far, this work has only shown the formation of simple precursor molecules such as amino acids and pyrroles. There are several reasons why no large biomolecules have emerged from our reaction system, a fact which also has its benefits. Assuming again that the precursors produced would continue to react under the same conditions that led to their own formation, life would not have had a chance to emerge. For example, there would be no way of creating certain enzymes if amino acids reacted with any other amino acid directly at the point of their formation. Assuming that the formation of the various precursor molecules is very pH dependent, it is reasonable to assume that a different pH would also promote polymerisation reactions. It should also be noted that the concentrations of the molecules are quite low. An accumulation of these molecules in a different reaction environment could also have a positive effect on further reactions.

## 4.3 Possible Early Control Mechanisms of Life

As already mentioned, life is absolutely dependent on avoiding the state of chemical equilibrium, which is why the formation of compartments is essential. In modern biochemistry, one of the reasons for this is that reactions do not take place under normal conditions, but only under catalysis. By placing the catalyst where the reaction is needed, the time and place of the reaction can be determined. It also ensures that only the reactions that are actually needed take place. An interesting fact that we have found in this work is that there is evidence of an early control mechanism even before biochemistry began.

### 4.3.1 β-Alanine vs. α-Alanine

In modern biochemistry, the amino acid alanine is one of the most important building blocks of life. Its role in the formation of proteins has already been pointed out. In our experiments, however, we discovered an interesting fact. Assuming that α-alanines and β-alanines are formed from the same precursor molecules, they are in direct competition with each other. The product is formed that passes through the more stable transition state, in this case the anti-Markownikow product β-alanine. Heavy isotope labelling also showed that β-alanine is formed from one molecule each of $NH_3$, acetylene, CO and water, whereas α-alanine is formed from two molecules of water, one and a half molecules of acetylene and one molecule of ammonia.

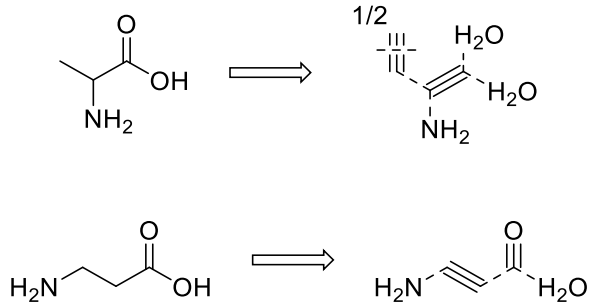


**Figure 19:** Retrosynthesis of α- and β-alanine. Adapted from Seitz *et al.* 2024.

The evolution of life chose the less favourable α-alanine, which is present in smaller quantities, as one of its building blocks.

### 4.3.2 Iron — Nickel — Cobalt

As mentioned above, catalysts play an important role in the origin of life. However, it is not only the presence of a catalyst that matters; we can also show that the type of catalyst plays a crucial role. In our experiments, we could show that Ni, Co, Fe and their mixtures can catalyse different reactions with varying degrees of effectiveness. Iron shows excellent catalytic properties in reductive amination. No results could be achieved in this reaction using either NiS or CoS as catalysts (Huber & Wächtershäuser 2003). The picture was different in the experiments on the formation of amino acids and fatty acid amides, where Cobalt proved to be the strongest central atom in the formation of propionamide and succinammic acid. NiS, on the other hand, has shown catalytic solid properties in previous experiments. However, in the experiments on the formation of amino acids, mixtures of metals proved to be more advantageous catalysts (Figure 20). It seems that the different metal sulphides (or mixtures of them) can catalyse different reactions. This again leads to a possible control mechanism.

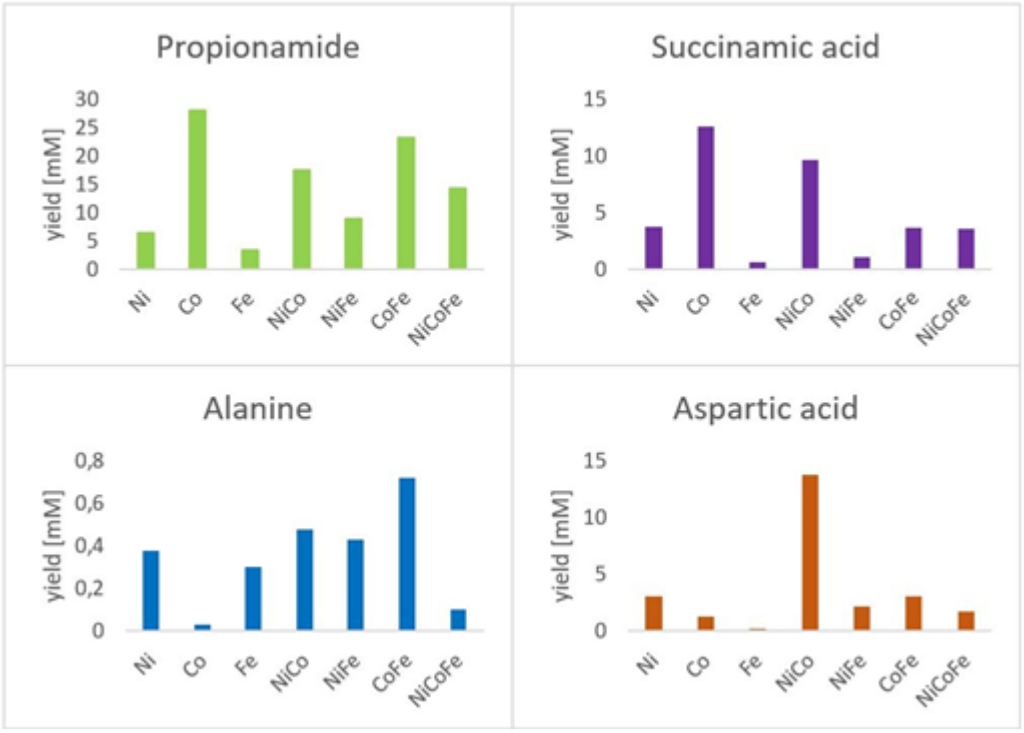


**Figure 20:** Different amounts of products achieved by using different metal sulphide catalysts. Adapted from Seitz *et al.* 2024.

In this work, some important precursors to modern biomolecules were synthesized which makes a further contribution to the importance of Wächtershäuser's iron sulphur-world-theory in connection with acetylene. As described above, it is a long way from simple biomolecules to the diversity of life, but this work may help us get a little closer to the mystery of the origin of life.

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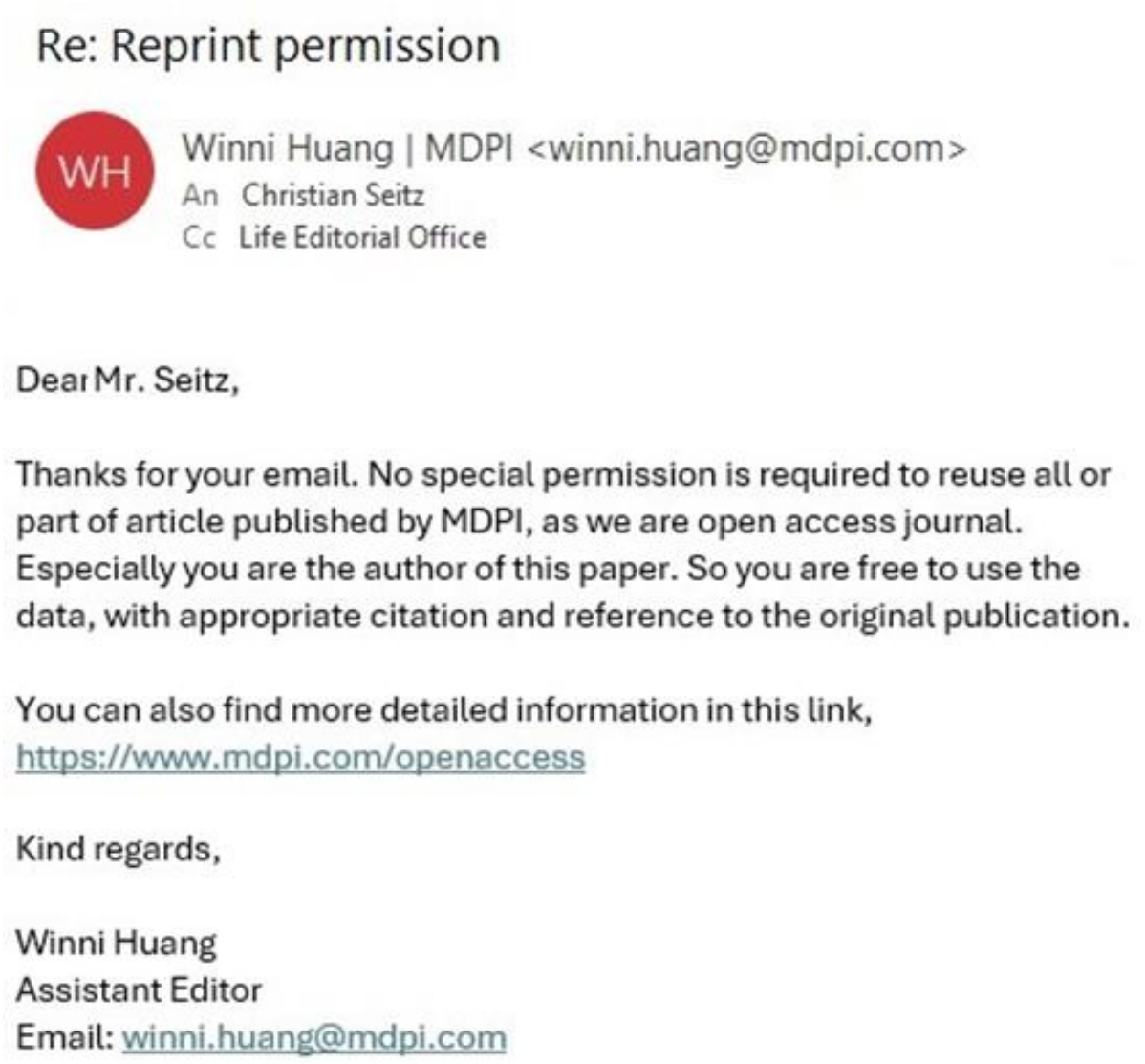

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# 7 SUPPORTING MATERIAL

## 7.1 Supplemental Material: “The Abiotic Formation of Pyrrole under Volcanic, Hydrothermal Conditions – An Initial Step towards Life’s First Breath?”

## Supplement Materials



## Figures

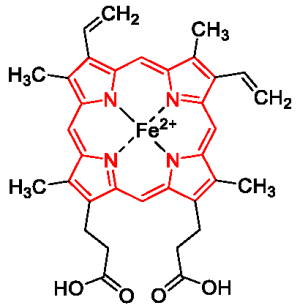



**Figure S1.** Structural formula of heme b. Tetrapyrrole is indicated in red

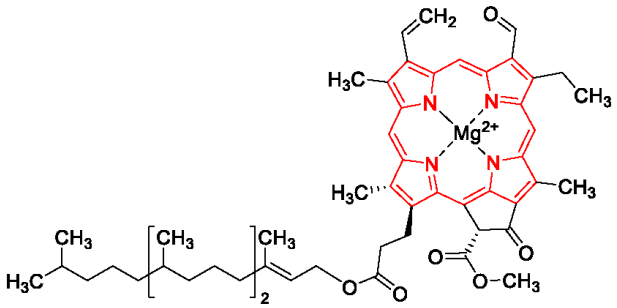


**Figure S2.** Structural formula of chlorophyll b. Tetrapyrrole is indicated in red

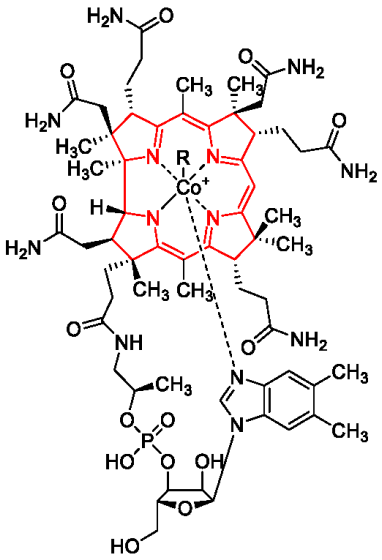


**Figure S3**. Structural formula of cobalamin (vitamin B12). Tetrapyrrole is indicated in red

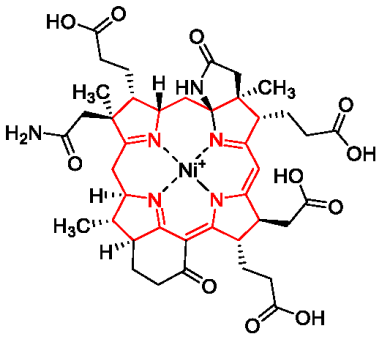



**Figure S4.** Structural formula of coenzyme F430. Tetrapyrrole is indicated in red

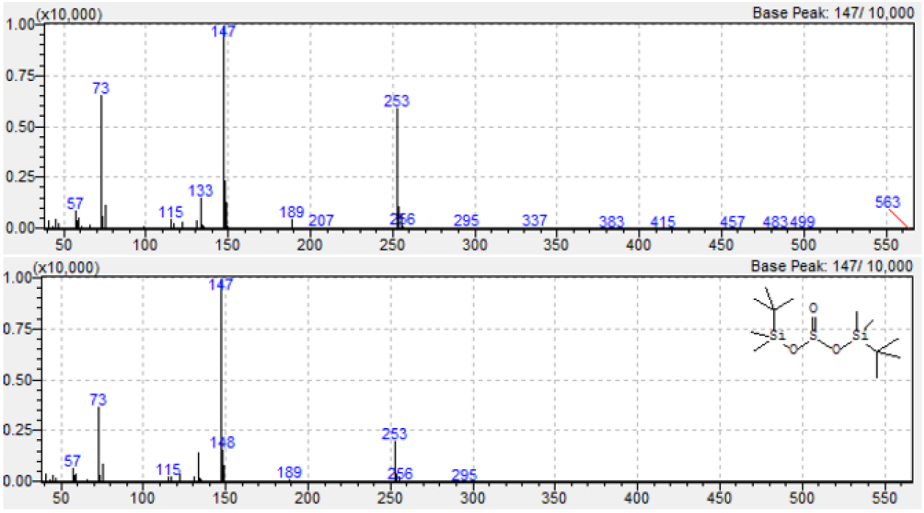



**Figure S5.** Mass-spectra of Bis(tert-butyldimethylsilyl)-sulfite. First spectrum is of a typical run, second spectrum is from NIST.

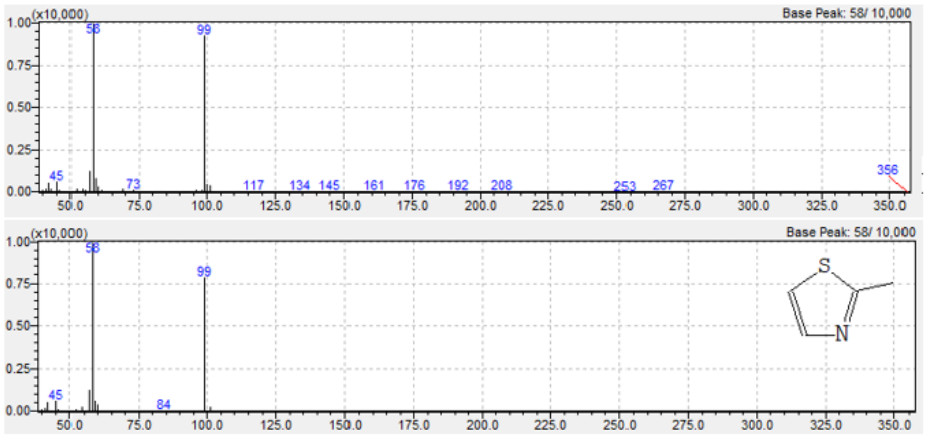



**Figure S6.** Mass-spectra of 2-methylthiazol. First spectrum is of a typical run, second spectrum is from NIST.

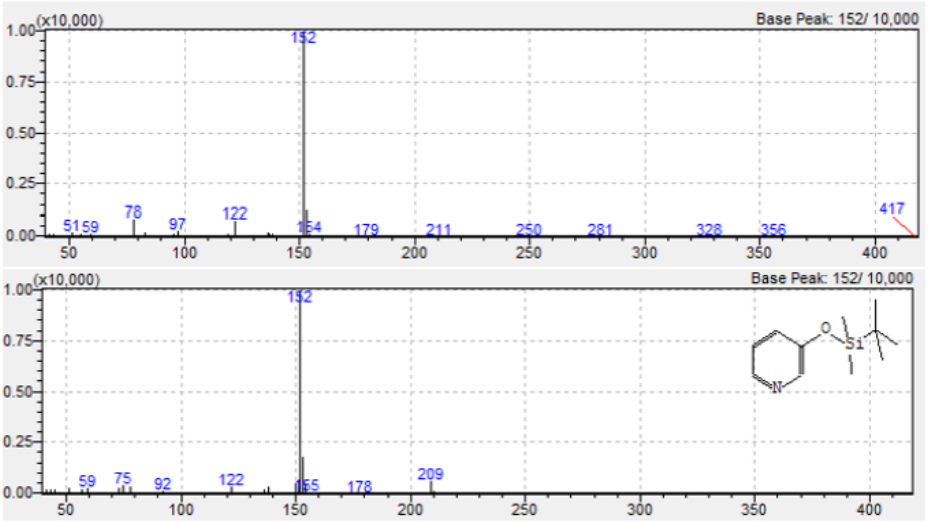
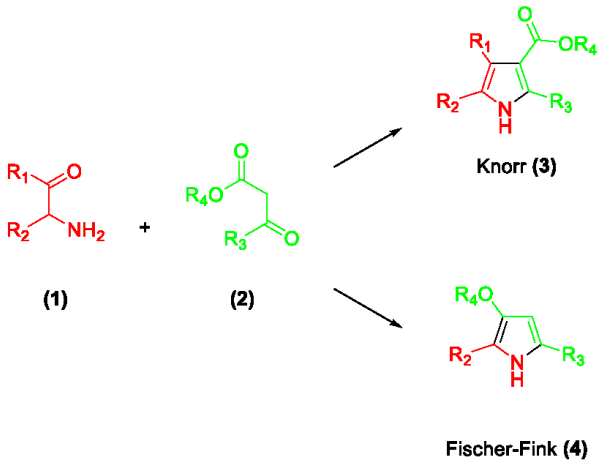
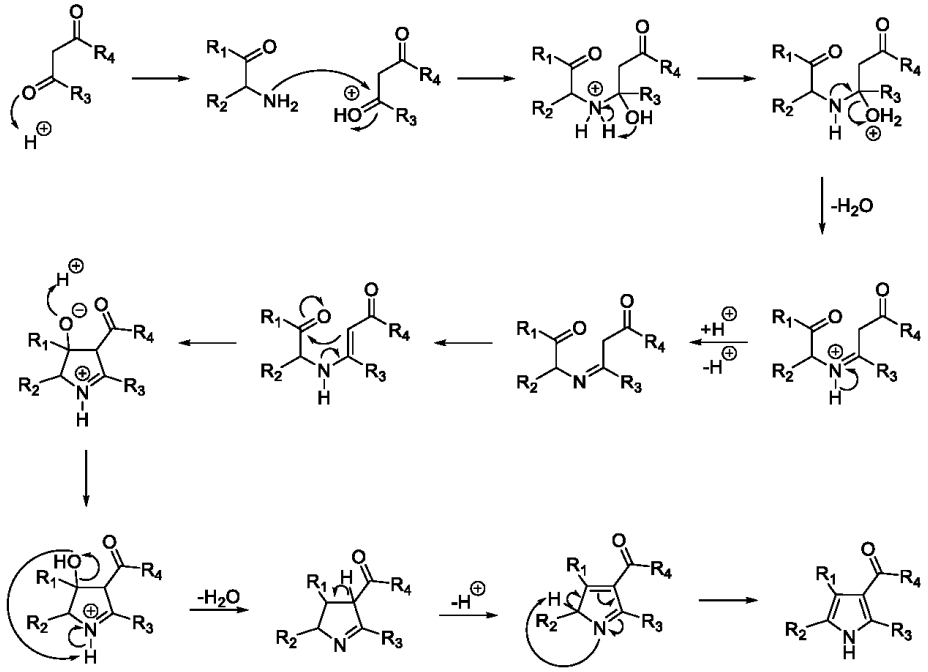



**Figure S7.** Mass-spectra of 3-tert-Butyldimethylsilyloxypyridine. First spectrum is of a typical run, second spectrum is from NIST.

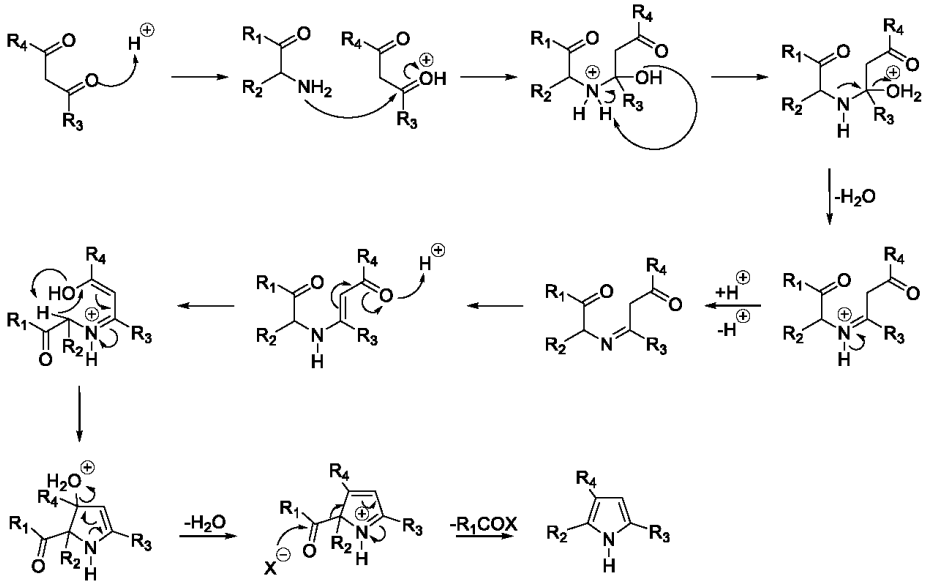
## Schemes



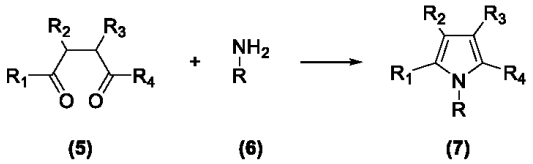

**Scheme S1.** Knorr-pyrrole synthesis. *Knorr*-product (4) as well as *Fischer-Fink*-product (5) can be observed in this reaction



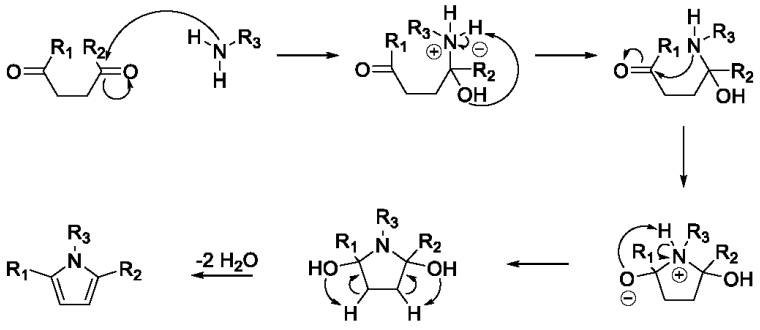

**Scheme S2.** Mechanism of the *Knorr*-pyrrole-synthesis



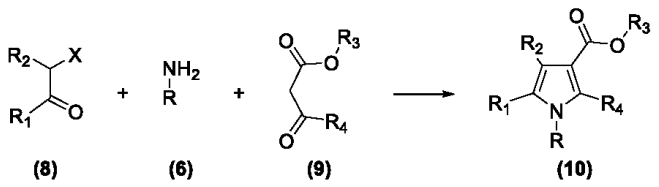
**Scheme S3.** Mechanism of the *Fischer-Fink*-pyrrole-synthesis



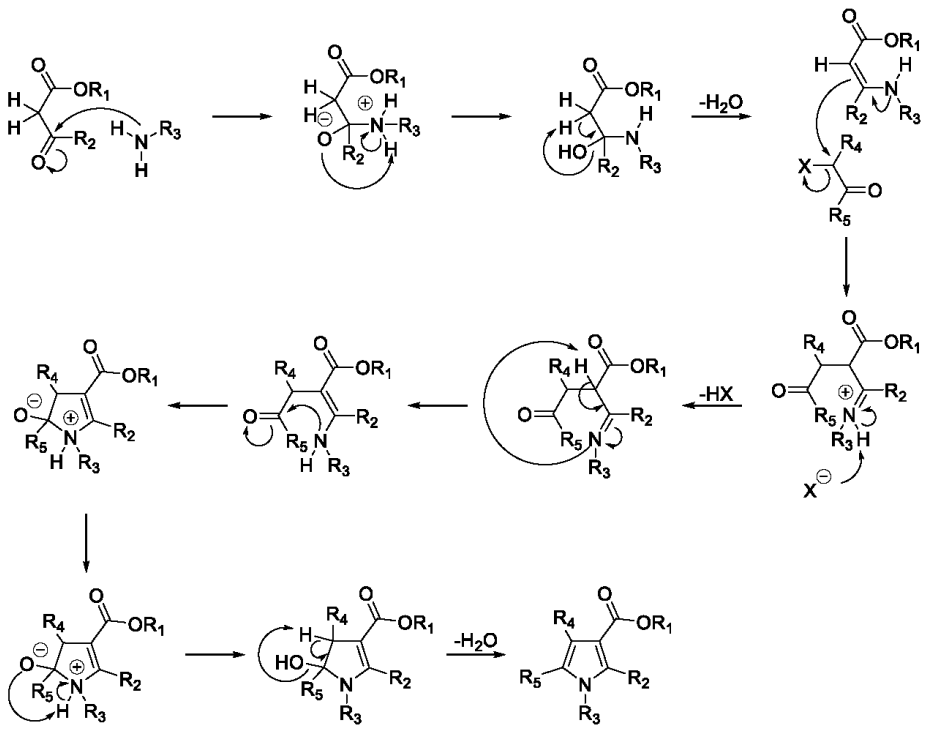
**Scheme S4.** *Paal-Knorr*-reaction.



**Scheme S5.** Mechanism of the *Paal-Knorr*-pyrrole-synthesis




**Scheme S6.** Pyrrole synthesis according to *Hantzsch*.




**Scheme S7.** Mechanism of the *Hantzsch'sche*-pyrrole-synthesis

## Tables

If not stated otherwise, all runs were performed with 1.00 mmol $NiSO_4$, 1.00 mmol $Na_2S$, 1 mL NaOH, 60 mL CO and 60 mL acetylene for one day at 105 °C. A total reaction volume of 5 mL was achieved by adding argon-saturated water. Yields are given at mol% conversion based on $NH_4Cl$. pH was measured at the end of the reaction time.

**Table S1.** Formation of pyrrole depending on pH. Different values were achieved by adding different amounts of 1 M NaOH (run 5, 7, 8, 10, 11, 12, 14), $Ca(OH)_2$ (run 6, 9, 10), 1 M $H_2SO_4$ (run 1-3) or none of them (run 4). Other parameters as stated above. The defined standard run is run 8 in this table (1 mL NaOH (1M)).

| Run | pH | NaOH (1 M) | $Ca(OH)_2$ | $H_2SO_4$ (1 M) | pyrrole | yield |
|---|---|---|---|---|---|---|
| | | [mmol] | [mmol] | [mmol] | [µM] | [%] |
| 1 | 1.8 | - | - | 1 | <0.1 | <0.01 |
| 2 | 2.4 | - | - | 0.5 | <0.1 | <0.01 |
| 3 | 3.8 | - | - | 0.1 | <0.1 | <0.01 |
| 4 | 6.8 | - | - | - | 0.71 | 0.071 |
| 5 | 7.1 | 0.1 | - | - | 0.65 | 0.065 |
| 6 | 7.6 | - | 0.3 | - | 1.25 | 0.546 |
| 7 | 7.8 | 0.5 | - | - | 2.53 | 0.253 |
| 8 | 9.1 | 1.0 | - | - | 11.38 | 1.138 |
| 9 | 9.4 | - | 1.3 | - | 1.70 | 0.17 |
| 10 | 9.6 | 1.5 | - | - | 1.70 | 0.17 |
| 11 | 10.3 | 2.0 | - | - | 0.75 | 0.075 |
| 12 | 10.9 | 3.0 | - | - | 0.40 | 0.04 |
| 13 | 10.9 | - | 2.6 | - | 0.18 | 0.018 |
| 14 | 11.3 | 4.0 | - | - | 0.16 | 0.016 |

**Table S2.** Formation of pyrrole depending on reaction time. Other parameters as stated above. The defined standard run is run 19 in this table (1 d).

| Run | $t_{reac}$ | pH | pyrrole | yield |
|---|---|---|---|---|
| | [h] | | [µM] | [%] |
| 15 | 0.00 | 8.7 | <0. 1 | <0.01 |
| 16 | 0.08 | 8.8 | <0. 1 | <0.01 |
| 17 | 0.17 | 8.8 | <0. 1 | <0.01 |
| 18 | 0.50 | 8.7 | <0. 1 | <0.01 |
| 19 | 1.00 | 8.8 | 0.82 | 0.08 |
| 20 | 2.00 | 8.9 | 1.58 | 0.16 |
| 21 | 4.00 | 8.7 | 2.20 | 0.22 |
| 22 | 8.00 | 8.5 | 3.40 | 0.34 |

| | | | | |
|---|---|---|---|---|
| 23 | 24.0 | 9.1 | 11.377 | 1.138 |
| 24 | 48.0 | 8.2 | 3.66 | 0.37 |
| 25 | 72.0 | 8.6 | 2.85 | 0.29 |
| 26 | 96.0 | 8.5 | 1.88 | 0.19 |
| 27 | 120 | 8.2 | 1.52 | 0.15 |
| 28 | 144 | 8.4 | 1.00 | 0.10 |
| 29 | 168 | 8.3 | 0.92 | 0.09 |

**Table S3.** Formation of pyrrole depending on concentration of catalyst. Different concentrations of NiS were achieved by adding different amounts of $Na_2S$ to a constant amount of $NiSO_4$ (1 mmol). NiS, the actual catalyst is formed *in situ*. Other parameters as stated above. The defined standard run is run 34 in this table (1.00 mmol $Na_2S$).

| Run | Na2S | pH | pyrrole | yield |
|---|---|---|---|---|
| | [mmol] | | [µM] | [%] |
| 30 | 0.00 | 6.9 | <0.1 | <0.01 |
| 31 | 0.25 | 7.2 | <0.1 | <0.01 |
| 32 | 0.50 | 7.5 | 0.76 | 0.08 |
| 33 | 0.75 | 7.9 | 0.62 | 0.06 |
| 34 | 1.00 | 9.1 | 11.377 | 1.138 |
| 35 | 1.25 | 9.0 | 4.78 | 0.48 |
| 36 | 1.50 | 9.4 | 2.63 | 0.26 |
| 37 | 1.75 | 9.8 | 0.57 | 0.06 |
| 38 | 2.00 | 11.1 | 0.32 | 0.03 |

## 7.2 Supplemental Material: “From Zero to Hero: The Cyanide-Free Formation of Amino Acids and Amides from Acetylene, Ammonia and Carbon Monoxide in Aqueous Environments in a Simulated Hadean Scenario.”

# Supplemental Materials

## Tables

**Table S 1.** Retention times and typical fragment masses of amino acids and amides. Amino acids and amides were formed from acetylene (2.71 mmol), ammonia (1.00 mmol) and carbon monoxide (2.68 mmol) in the presence of NiS (1.00 mmol) in 5 ml H2O at 105°C after 7 days. Retention times are given for the method described in materials and methods. M-57 is the characteristic mass of each compound after fragmentation of the tert-butyl group from the silylated form.

| Compound | Rt [min] | $[M-57^{+\cdot}]$ |
|---|---|---|
| Amino acids | | |
| Glycine | 13.2 | 246 |
| Alanine | 12.9 | 260 |
| β-Alanine | 14.5 | 260 |
| Aspartic acid | 16.7 | 418 |
| β-Homoserine | 19.1 | 404 |
| Amides | | |
| Formamide | 7.1 | 102 |
| Urea | 14.6 | 231 |
| Acetamide | 11.0 | 116 |
| Acrylamide | 8.3 | 128 |
| Propionamide | 8.4 | 130 |
| β-Alaninamide | 14.5 | 259 |
| Succinamic acid | 16.1 | 288 |
| Fumaramic acid | 17.9 | 286 |
| Pentenoic amides | 9.7/10.5/10.6 | 156 |
| Pentanoic amide | 10.6 | 158 |
| 2-Aminobenzamide | 10.2 | 307 |
| 2,4-Heptadienoic amide | 13.1 | 182 |
| Benzamide | 14.4 | 178 |

If not stated otherwise, all runs were performed with 1.00 mmol $NiSO_4$, 1.00 mmol $Na_2S$, 1 ml NaOH, 60 ml CO (2.68 mmol) and 60 ml acetylene (2.71 mmol) for seven days at 105 °C. A total reaction volume of 5 ml was achieved by adding argon-saturated water. pH

values were measured at the end of the reaction time. Concentrations are given for the total liquid reaction system of 5 ml. Yields are based on the conversion of $NH_4Cl$.

**Table S 2.** Propionamide, succinamic acid, alanine and aspartic acid formation based on different metal catalysts. Ni, Co, Fe and mixture of them were used. Metal sulfides were formed *in situ* from metal sulfates and $Na_2S$. Other parameters as stated above. Defined standard run is marked in red (1mmol NiS catalyst).

| | NiS | CoS | FeS | pH | Conc. Prop | Conc. Succ | Conc. Ala | Conc. Asp | Yield Prop | Yield Succ | Yield Ala | Yield Asp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mmol] | [mmol] | [mmol] | | [mM] | [mM] | [mM] | [mM] | [%] | [%] | [‰] | [%] |
| **1** | 1 | - | - | 8.8 | 7.67 | 0.75 | 0.05 | 0.62 | 3.84 | 0.37 | 0.25 | 0.31 |
| **2** | - | 1 | - | 7.6 | 36.01 | 3.27 | <0.01 | 0.86 | 18.01 | 1.63 | 0.02 | 0.43 |
| **3** | - | - | 1 | 9.2 | 4.08 | 0.12 | 0.04 | 0.04 | 2.04 | 0.06 | 0.22 | 0.02 |
| **4** | 0.5 | 0.5 | - | 8.7 | 11.72 | 11.03 | 0.06 | 0.43 | 5.86 | 5.51 | 0.29 | 0.22 |
| **5** | 0.5 | - | 0.5 | 8.8 | 27.85 | 1.19 | 0.1 | 0.61 | 13.92 | 0.59 | 0.48 | 0.31 |
| **6** | - | 0.5 | 0.5 | 8.4 | 20.94 | 10.1 | 0.06 | 2.76 | 10.47 | 5.05 | 0.32 | 1.38 |
| **7** | 0.3 | 0.3 | 0.3 | 8.7 | 17.21 | 4.07 | 0.01 | 0.47 | 8.6 | 2.04 | 0.07 | 0.23 |

**Table S 3.** Propionamide, succinamic acid, alanine and aspartic acid formation based on different reaction times. Other parameters as stated above. Defined standard run is marked in red (7 days, 10080 minutes).

| | time | pH | Conc. Prop | Conc. Succ | Conc. Ala | Conc. Asp | Yield Prop | Yield Succ | Yield Ala | Yield Asp |
|---|---|---|---|---|---|---|---|---|---|---|
| | [min] | | [mM] | [mM] | [mM] | [mM] | [%] | [%] | [‰] | [%] |
| **8** | 0 | 9.3 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.03 | <0.01 |
| **9** | 5 | 9.3 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.01 | <0.01 |
| **10** | 10 | 9.3 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.02 | <0.01 |
| **11** | 30 | 9.5 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.02 | <0.01 |
| **12** | 60 | 9.3 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.03 | <0.01 |
| **13** | 120 | 9.3 | <0.01 | <0.01 | 0.01 | 0.02 | <0.01 | <0.01 | 0.05 | <0.01 |
| **14** | 240 | 9.3 | <0.01 | <0.01 | 0.02 | <0.01 | <0.01 | <0.01 | 0.09 | <0.01 |
| **15** | 480 | 9.3 | <0.01 | <0.01 | 0.02 | <0.01 | <0.01 | <0.01 | 0.12 | <0.01 |
| **16** | 1440 | 9.2 | 0.27 | 0.02 | 0.03 | 0.01 | 0.14 | <0.01 | 0.15 | <0.01 |
| **17** | 2880 | 9.1 | 1.64 | 0.1 | 0.03 | 0.34 | 0.82 | 0.05 | 0.16 | 0.17 |
| **18** | 4320 | 9 | 4.91 | 0.37 | 0.03 | 0.18 | 2.46 | 0.18 | 0.17 | 0.09 |
| **19** | 5760 | 8.6 | <0.01 | 0.39 | 0.04 | 0.45 | <0.01 | 0.19 | 0.2 | 0.22 |
| **20** | 7200 | 8.6 | 5.88 | 0.39 | 0.04 | 0.44 | 2.94 | 0.2 | 0.21 | 0.22 |
| **21** | 8640 | 8.6 | 6.71 | 0.6 | 0.06 | 0.62 | 3.36 | 0.3 | 0.3 | 0.31 |
| **22** | 10080 | 8.8 | 7.4 | 0.65 | 0.06 | 0.62 | 3.7 | 0.32 | 0.31 | 0.31 |

**Table S 4.** Propionamide, succinamic acid, alanine and aspartic acid formation based on different amounts of the metal sulfide catalysts, which were achieved by adding different amounts of $Na_2S$. Other parameters as stated above. Defined standard run is marked in red (1 mmol $Na_sS$).

| | NaS | pH | Conc. Prop | Conc. Succ | Conc. Ala | Conc. Asp | Yield Prop | Yield Succ | Yield Ala | Yield Asp |
|---|---|---|---|---|---|---|---|---|---|---|
| | [mmol] | | [mM] | [mM] | [mM] | [mM] | [%] | [%] | [‰] | [%] |
| **23** | 0 | 6.8 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **24** | 0.25 | 7 | 0.21 | 0.06 | <0.01 | 0.01 | 0.11 | 0.03 | <0.01 | <0.01 |
| **25** | 0.5 | 7.1 | 0.55 | <0.01 | 0.02 | 0.04 | 0.27 | <0.01 | 0.08 | 0.02 |
| **26** | 0.75 | 8 | 2.92 | 0.91 | 0.03 | 0.48 | 1.46 | 0.45 | 0.14 | 0.24 |
| **27** | 1 | 8.6 | 5.88 | 0.7 | 0.06 | 0.62 | 2.94 | 0.35 | 0.31 | 0.31 |
| **28** | 1.25 | 9.1 | 3.53 | 0.21 | 0.06 | 0.4 | 1.76 | 0.11 | 0.32 | 0.2 |
| **29** | 1.5 | 9 | 0.22 | 0.12 | 0.03 | <0.01 | 0.11 | 0.06 | 0.13 | <0.01 |
| **30** | 1.75 | 9.3 | 0.05 | 0.07 | 0.02 | 0.04 | 0.03 | 0.04 | 0.11 | 0.02 |
| **31** | 2 | 9.6 | 0.08 | <0.01 | 0.01 | <0.01 | 0.04 | <0.01 | 0.06 | <0.01 |

**Table S 5.** Propionamide, succinamic acid, alanine and aspartic acid formation based on different pH values, which were achieved by adding different volumes of NaOH solution. Other parameters as stated above. Defined standard run is marked in red (1 mL NaOH).

| | NaOH | pH | Conc. Prop | Conc. Succ | Conc. Ala | Conc. Asp | Yield Prop | Yield Succ | Yield Ala | Yield Asp |
|---|---|---|---|---|---|---|---|---|---|---|
| | [ml] | | [mM] | [mM] | [mM] | [mM] | [%] | [%] | [‰] | [%] |
| **32** | 0 | 6.7 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **33** | 0.25 | 6.8 | 0.2 | <0.01 | <0.01 | <0.01 | 0.1 | <0.01 | <0.01 | <0.01 |
| **34** | 0.75 | 7.9 | 2.11 | <0.01 | <0.01 | <0.01 | 1.06 | <0.01 | 0.02 | <0.01 |
| **35** | 1 | 8.4 | 7.26 | 0.85 | 0.04 | 0.61 | 3.63 | 0.43 | 0.22 | 0.31 |
| **36** | 1.25 | 8.9 | 1.76 | 0.26 | 0.03 | 0.54 | 0.88 | 0.13 | 0.17 | 0.27 |
| **37** | 1.5 | 9.4 | 0.66 | <0.01 | <0.01 | 0.12 | 0.33 | <0.01 | 0.05 | 0.06 |
| **38** | 1.75 | 9.6 | 0.05 | <0.01 | <0.01 | <0.01 | 0.02 | <0.01 | <0.01 | <0.01 |
| **39** | 2 | 9.8 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

## Figures

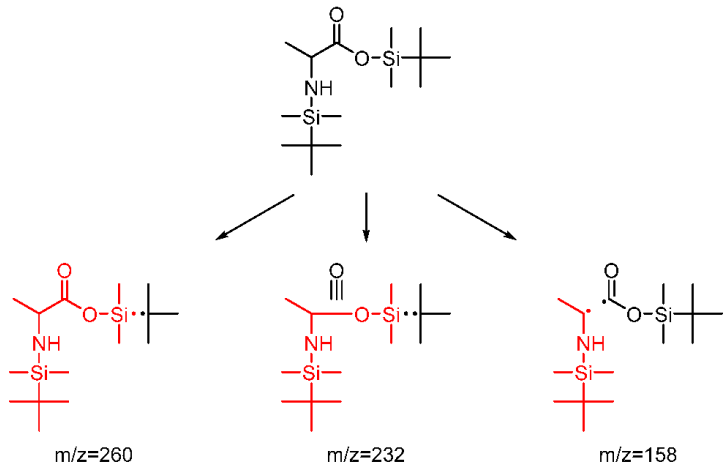



**Figure S 1.** Typical fragments of TBDMS- amino acids in GC/MS experiments at the example of alanine. Shown in red are the fragments *m/z*=260 ([M-57$^{+\cdot}$], left), *m/z*=232 ([M-85$^{+\cdot}$], middle) and *m/z*=158 ([M-159$^{+\cdot}$], right).

# 8 Publications and Conference Contributions

## 8.1 Journal Articles

Heker, I.; **Seitz, C.;** Voskuhl, L.; Kong, Y; Erdmann, I.; Huber, C.; Eisenreich, W.; Meckenstock, R. U. Chemoorganoautotrophic growth with polycyclic aromatic organic substrates, *Nat. Commun.* **2024** (under revision).

**Seitz, C.;** Geisberger, T.; West, A. R.; Fertl, J.; Eisenreich, W.; Huber, C. From Zero to Hero: The Cyanide-Free Formation of Amino Acids and Amides from Acetylene, Ammonia and Carbon Monoxide in Aqueous Environments in a Simulated Hadean Scenario. *Life* **2024**, *14,* 719. DOI: 10.3390/life14060719.

Meckenstock, R. U.; Heker, I.; **Seitz, C.;** Voskuhl, L.; Eisenreich, W. Chemo-organo-autotrophic degradation of aromatic hydrocarbons indicates a new type of bacterial metabolism. *ARPHA Conference Abstracts* **2023** 6: e111950. DOI: 10.3897/aca.6.e111950.

Diederich P.; Ruf, A.; Weidner, L.; Geisberger, T.; **Seitz, C.;** Eisenreich, W.; Huber, C.; Schmitt-Kopplin, P. C2-addition patterns emerging from acetylene in a prebiotic sulfur mineral setup under hydrothermal volcanic conditions. *Commun Chem* **2023,** *6,* 220. DOI: 10.1038/s42004-023-01021-1.

Geisberger, T.; Diederich, P.; Kaiser, C. J. O.; Vogele, K.; Ruf, A.; **Seitz, C.;** Simmel, F.; Eisenreich, W.; Schmitt-Kopplin, P.; Huber, C. Formation of vesicular structures from fatty acids formed under simulated volcanic hydrothermal conditions. *Sci Rep.* **2023,** 13(1):15227. DOI: 10.1038/s41598-023-42552.

Diederich, P.; Geisberger, T.; Yan, Y.; **Seitz, C.;** Ruf, A.; Huber, C.; Hertkorn, N.; Schmitt-Kopplin, P. Formation, stabilization and fate of acetaldehyde and higher aldehydes in an autonomously changing prebiotic system emerging from acetylene. *Commun Chem.* **2023,** 6(1):38. DOI: 10.1038/s42004-023-00833-5.

**Seitz, C;** Eisenreich, W; Huber, C. The Abiotic Formation of Pyrrole under Volcanic, Hydrothermal Conditions — An Initial Step towards Life's First Breath? *Life* **2021,** *11,* 980. DOI: 10.3390/life11090980.

## 8.2 Posters

07/2020 Molecular Origins of Life, Munich. Poster on "Chemical evolution of biomolecules formed under volcanic hydrothermal conditions".

06/2022 Molecular Origins of Life, Munich. Poster on "Prebiotic synthesis of pyrrole under volcanic hydrothermal conditions".

07/2024 Molecular Origins of Life, Munich. Poster on “Prebiotic synthesis of amino acids and amides under volcanic hydrothermal conditions”.