Biomarkers of Puga Hot Spring, Ladakh, India: Tracing plausible Prebiotic Pathways and Biogenic Metabolites

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Abstract

The search to unravel the origins of life on Earth pivots on recognizing environments capable of synthesizing and preserving prebiotic organic molecules (chemical precursors to biological systems). Prebiotic chemistry plays a crucial role in understanding the origin of life on Earth, and terrestrial hydrothermal systems may offer the suitable environment for its emergence. In this study, we investigated the travertine deposit of the Puga hot spring in Ladakh, India- a site that not only mimics early Earth environments but also shares similarities with the hydrothermal conditions proposed for early and modern-day Mars. The Puga hot spring is situated in extreme cold location settings of Ladakh, India which is characterized by near-neutral to alkaline waters, enriched in boron, and exhibiting rapid calcium carbonate precipitation. Such features are conducive to capturing and preserving organic molecules that serve as molecular fossils of prebiotic processes.

We employed gas chromatography-tandem mass spectrometry (GC-MS/MS) for the first time to analyse sample scraped from both the inner and outer surfaces of the travertine deposit of Puga hot spring. Key compounds identified include traces of β-alanine derivatives (amino propanoic acid), pyran-2-thione, TMS-derivatized formamide, cyclooctasulphur (S_8), and hexadecenoic methyl ester (fatty acid). These findings suggest that both biogenic and abiotic mechanisms may contribute to the synthesis and preservation of these molecules. We hypothesised reaction pathways for molecules identified, which supports possibility of the dynamic interplay between high-temperature geothermal fluids and rapid cooling in a high UV, low-pressure environment facilitating the formation of complex prebiotic molecules.

The detection of these compounds in the Puga travertine not only supports the hypothesis that hot spring environments could have been frameworks of early life on Earth but also highlights their potential as analogues for extraterrestrial settings. Current study highlights the pivotal role of hydrothermal systems in chemical evolution associated with origin of life. Our findings provide substantial support for the hypothesis of a natural "prebiotic soup" like conditions in Puga hot-spring, Ladakh, India, revealing that cold environmental conditions may possibly work in synergy with hot spring conditions, playing a crucial role in effectively preserving organic moieties within calcic matrices.

Keywords: Origin of Life; Hot-Springs; Biomarkers; Martian analogue; Gas chromatography-Mass Spectrometry; prebiotic chemistry

1. Introduction

The origin of life on Earth remains one of the most captivating questions in science *(Pross and Pascal, 2013)*. It's possible that the right conditions existed in places such as deep-sea hydrothermal vents, geothermal hot springs, or even in hidden underground areas where water flows steadily *(Schaible et al., 2024)*. Recent studies increasingly suggest that natural settings like hot springs and other hydrothermal areas might have offered the ideal mix of energy, reactive chemicals, and mineral structures necessary to kickstart prebiotic chemistry *(Colín-García et al., 2019; Damer and Deamer, 2020; Deamer et al., 2019; Villafañe-Barajas and Colín-García, 2021)*. Hot springs found on land provided the right conditions for the early chemical reactions that sparked life. They were originally proposed as likely places for life's beginnings because these springs can accumulate organic materials and use natural cycles of wet and dry periods, driven by evaporation, to solve the "water problem" associated with deep hydrothermal vents *(Damer and Deamer, 2020; Schaible et al., 2024)*.

Hydrothermal environments are dynamic places where hot water colliding with the Earth's crust creates pronounced temperature and chemical differences. These variations initiate the process for the conversion of simple inorganic compounds into more complex organic molecules. It is reported that blending hydrothermal fluids with other natural waters could lead to the formation of diverse organic compounds such as amino acids and nucleobases that are central to the idea of prebiotic chemistry *(Shock and Schulte, 1998)*. This buildup of chemical complexity is seen as a key precursor to life on early Earth.

Travertine formations that develop around hot springs and in rapid calcium carbonate precipitation zones within hydrothermal systems serve as natural archives of ancient geochemical processes while effectively preserving them against degradation over geological timescales *(Garcia et al., 2021; Ricketts et al., 2019)*. Recent studies have focused on these deposits as potential insights into the chemical pathways that preceded biological life *(Luo et al., 2022; Martin et al., 2008)*. In hot spring system like Puga the precipitation is driven by the cooling and degassing of mineral-rich geothermal waters. This process can capture transient organic species that may have been generated by prebiotic reactions, thereby preserving a molecular record that is accessible through modern analytical techniques.

The Puga hot spring in Ladakh, India, is one of the notable modern analogues of early Earth conditions, drawing considerable astrobiological interest. Located high in the Indian Himalayas, this site presents a unique combination of characteristics that resembles the environments theorized to have sparked life on our planet *(Chaddha et al., 2024a, 2024b; Shukla et al., 2023)*. The Puga hot spring features waters that range from neutral to alkaline pH (pH ; 7-10) and discharge at roughly 70°C, with elevated boron levels and swift travertine formation *(Dutta et al., 2023)*. Boron, abundant in these geothermal waters, has been associated with stabilizing ribose a crucial RNA building block *(Ricardo et al., 2004)*. The combination of boron and other essential ions thus creates an environment conducive to the synthesis of life's fundamental molecules. Recent findings also highlight Puga's importance as a prebiotic analogue *(Sánchez-García et al., 2024)*.

In this study, we focus on detecting and characterizing the biomarkers and organic molecules preserved within the travertine deposits of the Puga hot spring, which serve as molecular fossils offering crucial insights into the metabolic pathways and environmental conditions that existed during their formation *(Summons et al., 2008)*. Moreover, the selection of Puga is supported by its striking resemblance to extraterrestrial environments, particularly Mars, where evidence suggests that hydrothermal systems once thrived during the planet's early evolution *(Boulesteix et al., 2024; Brakenridge et al., 1985; Ruff et al., 2020)*. Consequently, finding similar organic compounds in Earth's hot springs, such as those at Puga, not only reinforces the hypothesis of prebiotic chemistry on early Earth but also broadens our prospects for detecting signs of life on other planets. By analysing these biomarkers, our aim is to trace the chemical evolution that may have set the stage for the emergence of life. If these hydrothermal systems can promote the synthesis and preservation of organic molecules under harsh conditions such as high UV radiation, extreme cold environments, and low atmospheric pressure similar to those on modern Mars, then sites like Puga in Ladakh, India, become crucial for guiding future astrobiological explorations towards origin of life.

2. Study area and Sample collection

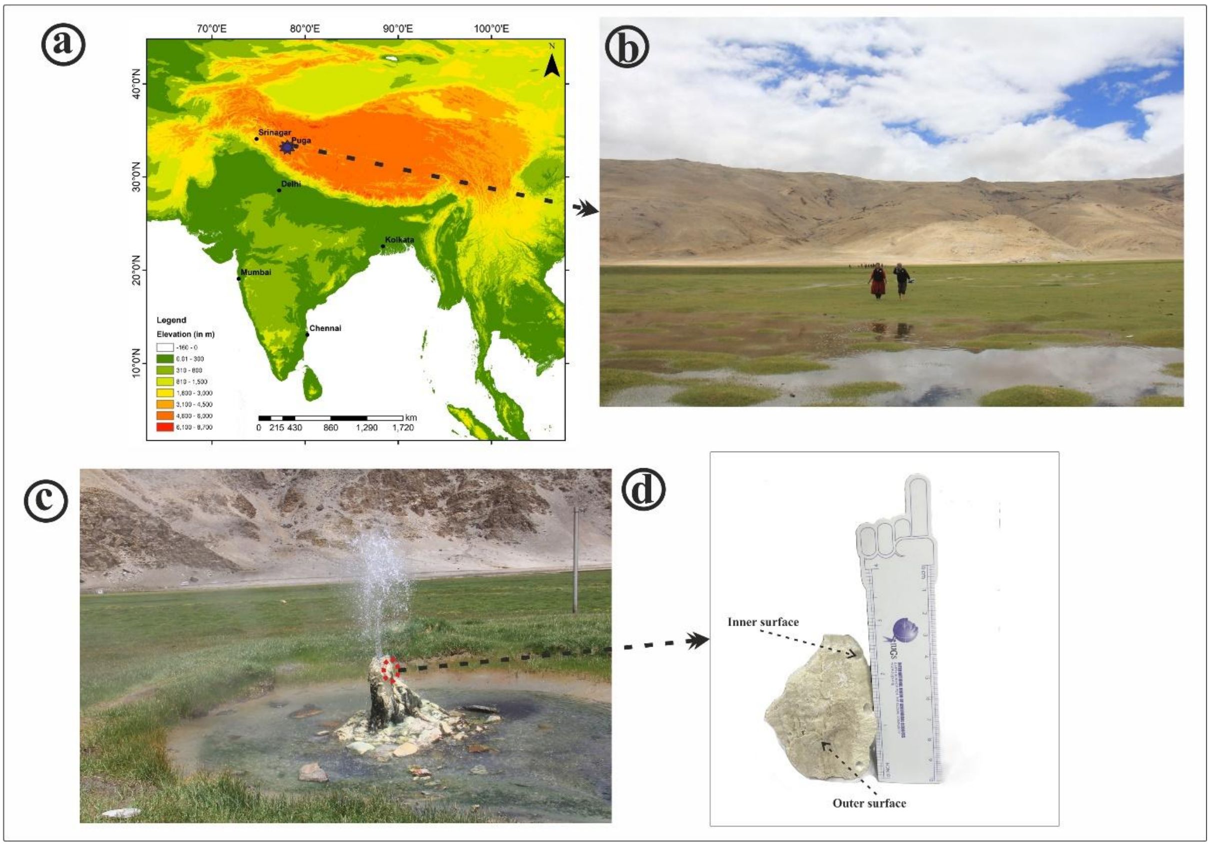

Fig.1 (a) A DEM map pinpoints the Puga hot spring site in northern India's Ladakh region, marked by a purple star. (b, c) Panoramic views reveal the sample collection area at Puga, highlighting the hot spring vent alongside a calcic-rich sinter (travertine deposit) near the vent's mouth, indicated by a dotted red ellipse. (d) A digital photograph of the travertine sample clearly shows two distinct zones the inner and outer surfaces from which samples were extracted for biomarker analysis.

Ladakh, a region in the Trans-Himalayan area typically found above 3000 meters and often called the "cold desert of India" was chosen as the study site *(Juyal, 2014)*. This region endures scarce rainfall, very low temperatures, and wide daily temperature fluctuations *(Blöthe et al., 2014; Schmidt and Nüsser, 2017)*, with summer highs reaching 34.8°C and winter temperatures dipping to -27.9°C *(Chevuturi et al., 2018)*. Its sparse vegetation, rugged terrain, and high altitude expose it to UV-A radiation levels up to ten times those at sea level *(Chaddha et al., 2021, 2024b; Sharma and Phartiyal, 2018)*. These conditions, which include low atmospheric pressure and intense UV exposure, mirror aspects of the current Martian environment, making the area a valuable terrestrial analogue *(Cockell, 2000)*. The landscape also boasts diverse, near-pristine extreme environments like glaciers, arid terrains, sand dunes, interdunal lakes, geothermal springs, saline lakes, and manganese-rich varnish-coated rocks that further emphasize its similarity to Martian conditions *(Chaddha et al., 2024a, 2024b, 2024c ; Chaddha et al., 2023; Chaddha et al.,2021; Pandey et al., 2020)*. Samples were collected from a site near Ladakh's Puga Valley, a prominent geothermal energy location in the eastern Indus Valley within the North-Western Himalayas. Puga, situated south of the Indus Suture Zone in the Tso Morari area, is renowned for its geothermal features, such as hot springs, mud pools, and mineral deposits rich in sulphur and borax *(Craig et al., 2013; Dutta et al., 2023)*. At this site, hot spring water temperatures can reach up to 84°C at the vent, while nearby areas register around 5°C *(Craig et al., 2013)*. The underlying geology includes a variety of rock types, such as crystalline limestone, marble, carbon-rich shale, green chert, and mafic to ultramafic formations *(Dutta et al., 2023)*.

Samples were collected and processed for a range of analytical measurements as described by *(Chaddha et al., 2024c; Shukla et al., 2023)*. In summary, a whitish sinter deposit was retrieved from the mouth of the Puga hot spring in Ladakh, India (33°13′39.38″N, 78°18′22.98″E, 4414 m a.s.l.) using a pre-sterilized chisel. The sample was then wrapped in aluminium foil and placed in clean plastic boxes for transport to the laboratory. To minimize contamination, both the inner and outer layers of the travertine sample were carefully removed using a Dremel Micro drill (Model 800) equipped with a diamond-coated, sterilized coarse bit. These extracted fragments were subsequently stored in small Eppendorf tubes for organic biomarker analysis.

3. Material method

GC-MS-MS Analysis

The Trace GC Ultra TSQ 8000 Evo Mass Spectrometer (Thermo, USA) equipped with a TG-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness, 5% phenyl and 95% dimethyl polysiloxane) analyses the travertine samples. The inner and outer surface powders undergo extraction with 80% methanol using ultrasonication. After extraction, nitrogen evaporation removes the solvent to dryness, preparing the samples for Silylation.

The Silylation process derivatizes the dried extracts with 100 µL of methoxy amine hydrochloride and 100 µL of BSTFA (N, O-Bis(trimethylsilyl)trifluoroacetamide) containing 1% TMCS (trimethylchlorosilane). A diluent adjusts the volume of the derivatized samples, which then proceed to GC-MS/MS analysis. The GC instrument operates in split injection mode with a 1:10 split ratio, maintaining an injector port temperature of 80 °C. Helium (99.999% purity) flows as the carrier gas at a rate of 1.0 mL min^{-1}.

The GC oven follows a program starting at 60 °C for 3 minutes, increasing to 200 °C at 8 °C min^{-1} (held for 5 minutes), then rising to 230 °C at 6 °C min^{-1} (held for 5 minutes), and finally reaching 290 °C at 10 °C min^{-1} (held for 20 minutes), completing a total run time of 60 minutes. The system sets the ion source temperature to 230 °C and the interface temperature to 300 °C. The mass spectrometer employs 70 eV electron energy in full scan mode, covering a mass range of 50–850 m/z. The method injects 1 µL of the derivatized sample into the GC-MS/MS system *(Chaddha et al., 2023)*. XCalibur software (Thermo Fisher Scientific) processes the data and identifies peaks qualitatively using the NIST library database.

4. Result

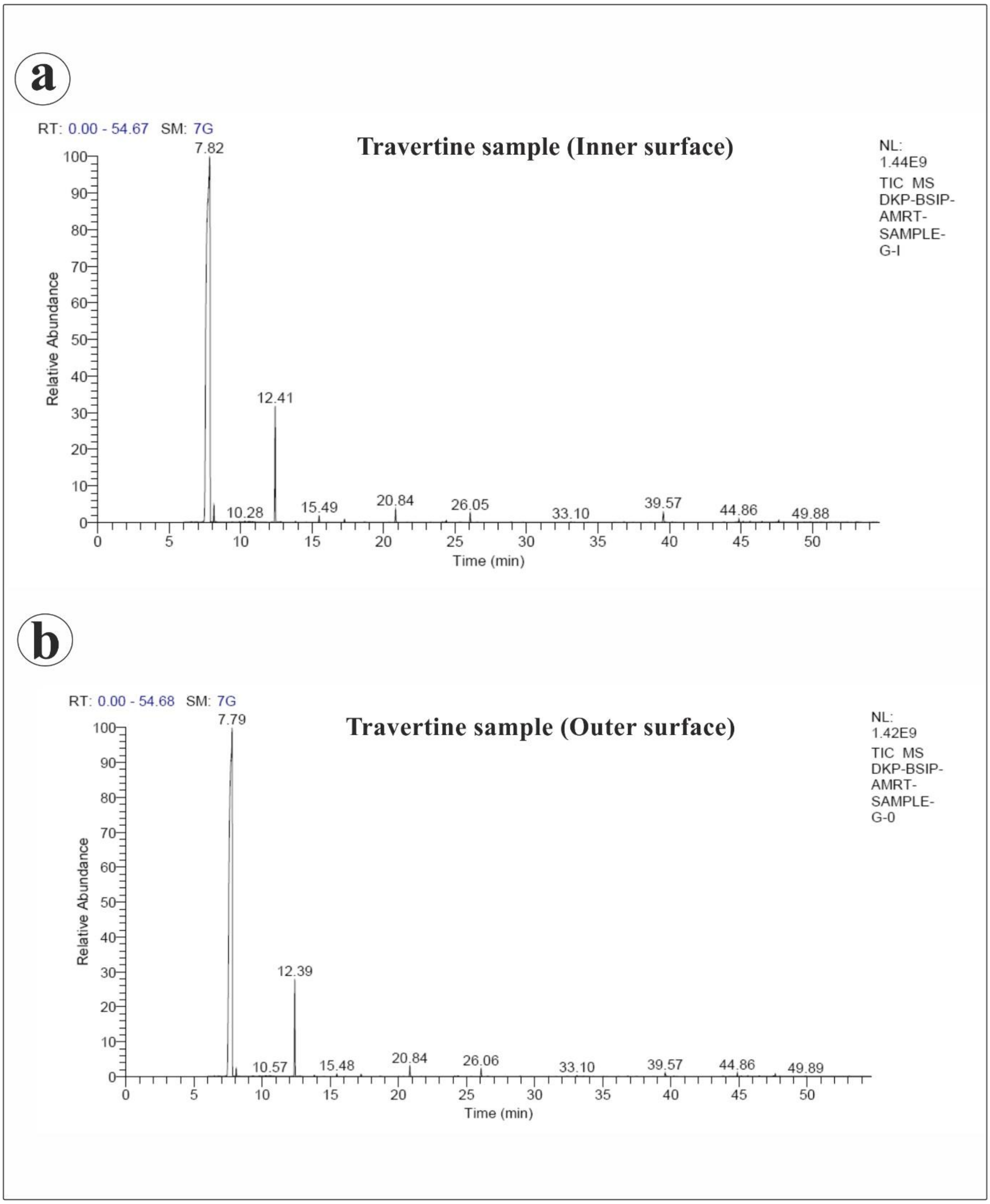

Fig.2 (a, b) Gas chromatography Tandem mass spectrometry (GC/MS-MS) chromatograms of travertine samples of Puga hot spring (full-scan mode).

GC-MS-MS analysis of the travertine sample collected from mouth of the Puga hot spring has enabled the identification of organic biosignatures within travertine samples. Inner and outer surface of the travertine sample (Fig.1d) reveals chromatograms with almost similar peaks (Fig. 2a, b). The resulting chromatograms showed nearly identical peak profiles, demonstrating a homogeneous distribution of organic compounds throughout the formation. Both inner and outer surface presents chromatograms at similar retention time, with relative high abundance peaks at 7.78 min (I), 7.82 min (O), 12.39 min (I), 12.41min (O). There are other minor peaks available as marked in respective chromatograms (Fig.2 a, b). On, investigating the individual peaks various organic compounds were obtained with a TMS (trimethyl silane) group connected to the molecule seen in the spectrometer as a result of trimethyl Silylation carried out on the sample before injecting it into the GC-MS/MS from inner and outer surface of the travertine sample (Fig.3). Both inner and outer surface of the travertine sample contains similar compounds, therefore it's evident that homogeneity persists throughout the course of formation of travertine while preserving organic compounds in it.

Travertine sample revealed traces of organic molecules such derivative of β-alanine (3-Amino-3-(3-bromo-phenyl)-propionic acid) which is structurally related to alanine or amino acids, pyran -2 thione, TMS derivative formamide, cyclo-octane S_8 sulphur, fatty acid of methyl ester (hexadecenoic acid) (Fig.3). The EI mass spectrum of these specific compounds present in the travertine deposit sample from the Puga hot spring is also provided (Fig.4, Fig.5)

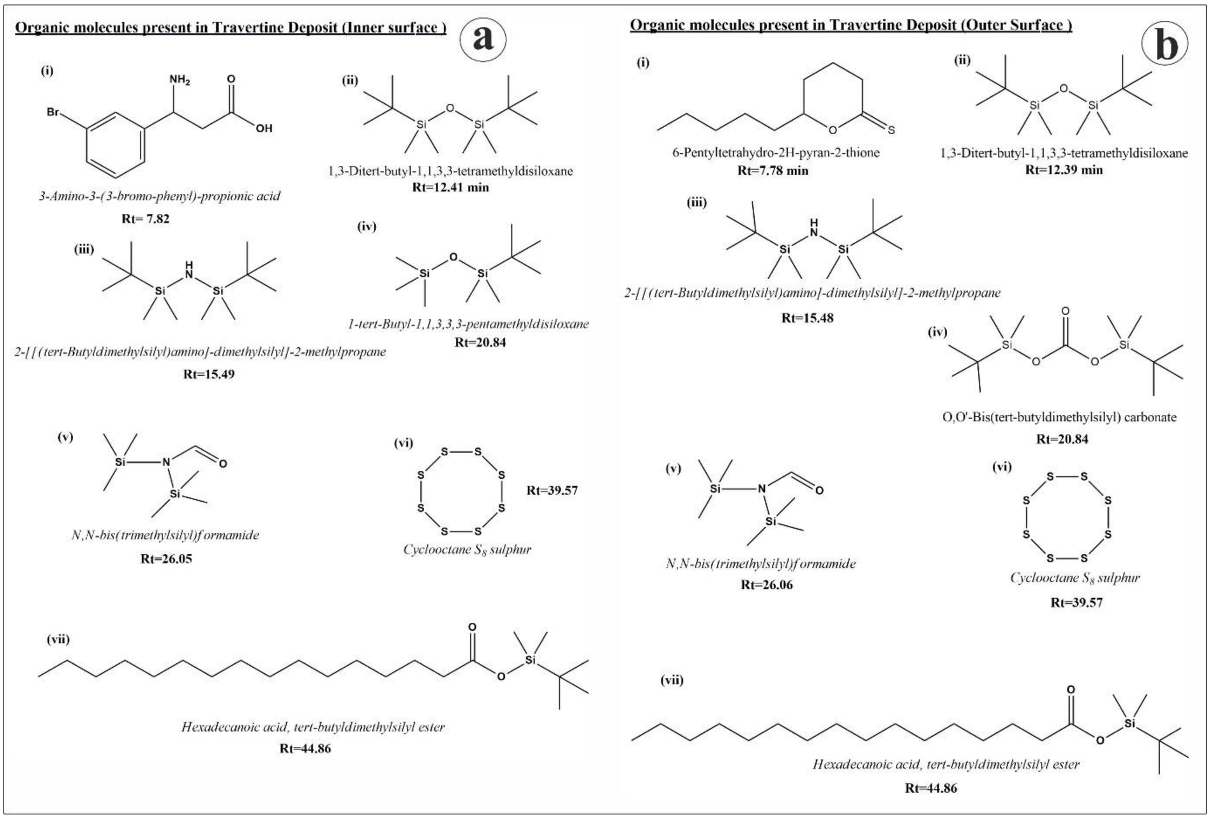

Fig.3 Presence of biomolecules in the travertine sample of Puga Hot Spring. Different type of organic compounds detected in the (a) inner and (b) outer scrapped layer of the travertine sample with their respective retention time in (min).

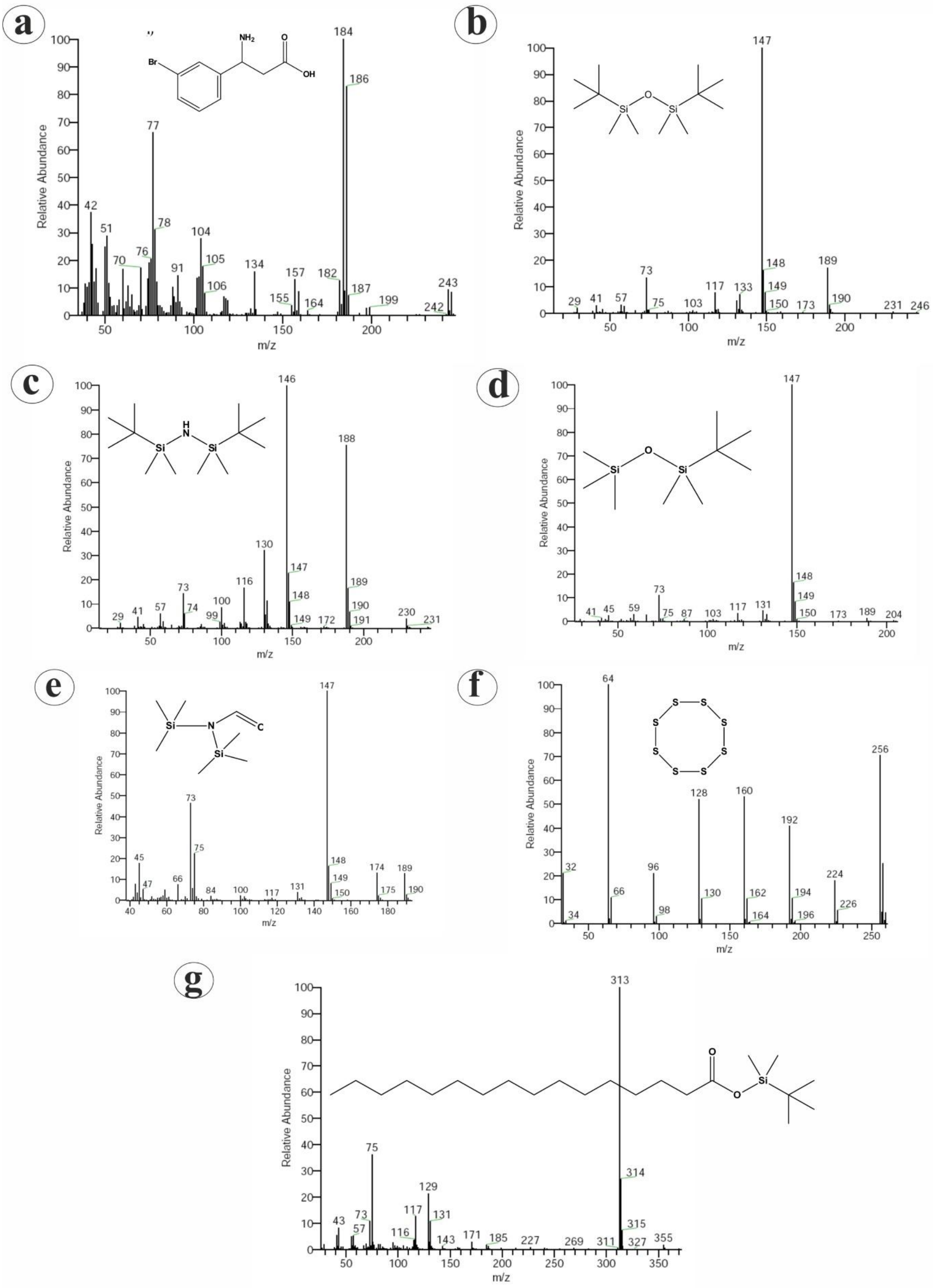

Fig.4 EI mass spectrum of the organic molecules present in the travertine sample (Inner surface) of the Puga hot spring.

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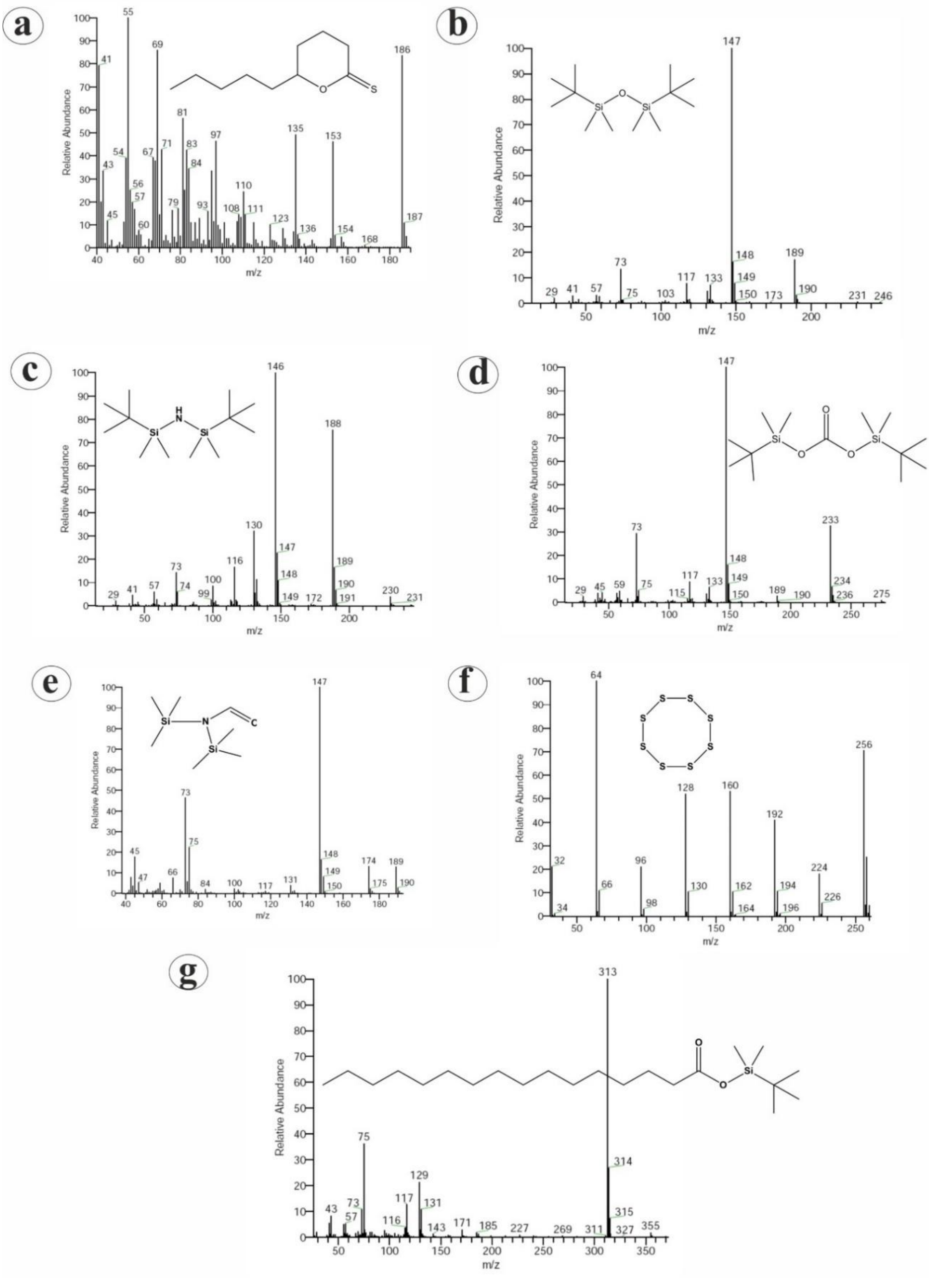

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266 **Fig.5** EI mass spectrum of the organic molecules present in the travertine sample (outer
267 surface) of the Puga hot spring

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5. Discussion

The presence of various organic identified through GC-MS-MS analysis such as β-alanine derivatives, pyran-2-thione, TMS derivatives of formamide, cyclo-octane S_8 sulphur, fatty acid methyl esters, and siloxane derivatives in hot spring travertine deposits suggests a complex interplay of biogenic and abiotic processes. These molecules may form through microbial metabolic activities or abiotic geochemical pathways under specific environmental conditions we propose plausible biogenic and abiotic formation reaction routes and provides suitable putative hypothesis for the same.

5.1 Biomolecules from the hot spring and their traceability towards biogenic origin.

The presence of various organic compounds (Fig.3) identified substantiates that the hot spring associated travertine deposit hosts the ingredients for early life to be triggered. These molecules can form through microbial metabolic activities routes. The presence of these molecules in hot spring travertine deposits also raises intriguing possibilities about their biogenic origins.

To streamline the description of the samples, we divided the organic compounds into groups based on their chemical function/structure and their biogenic potential

(i) ***β-alanine derivatives:*** β-Alanine also known as 3-amino propanoic acid is a naturally occurring amino acid and a key metabolite in various biochemical pathways *(Yang et al., 2023)*. Its derivatives in travertine deposits may suggest the involvement of microbial or algal processes. β-Alanine is often associated as a part of microbial metabolic byproducts *(Song et al., 2023)*. Its presence in the travertine sample supports the possibility of biogenic activity, as microbes in hot springs often produce or metabolize amino acids *(Zhang et al., 2023)*.

(ii) ***Pyran-2-thione:*** Sulphur-containing heterocyclic compounds like pyran-2-thione can be linked through microbial sulphur metabolism. Certain bacteria involved in sulphur cycling (e.g., sulphate-reducing bacteria) might produce such compounds as intermediates or byproducts. For instance, cyanobacteria, sulphur-oxidizing bacteria, and other microbial communities found in hot springs contribute to biomineralization and organic compound synthesis through their metabolic processes *(Runge et al., 2023)*.

(iii) ***Formamide:*** Formamide is a simple organic compound and a precursor in abiotic synthesis pathways for nucleic acids under prebiotic conditions. While suggestive of prebiotic chemistry, it could also be a biogenic metabolite, although very rare. In some microorganisms, formamide occurs as a degradation product of histidine and cyanide *(Ferber et al., 1988; Kunz et al., 1994; Wachsman and Barker, 1955)*.

(iv) ***Cyclooctane S_8 sulphur:*** Elemental sulphur is a significant intermediate in microbial sulphur cycling, utilized or produced by sulphur-reducing and sulphur-oxidizing microbes *(Cosmidis et al., 2019)*. Microbial metabolism can involve the transformation of sulphur into various forms, including cyclooctasulphur(S_8) *(Wang et al., 2022)*. There are unique site where sulphur cycling significantly involves microbial metabolisms, where transient cold springs are often covered in bright sulphate and carbonate precipitates. The environment is characterized by a strong hydrogen sulphide Odor, indicating active sulphur metabolism and microbial processes. Similarly, presence of cyclooctane S8 sulphur in the travertine deposit from the mouth of the vent of Puga hot spring where extreme cold conditions are persistent throughout the year may strongly indicate microbial intervention. Generally, solubility of cyclooctasulphur in aqueous system increases with increase in temperature *(Kamyshny, 2009)*. As Ladakh hosts wide diurnal variation in temperature, thermal gradient is created near the mouth of the vent and across the hot spring. Therefore, when hot water gushes out of from the vent it becomes cold and makes the cyclooctane molecules to be precipitated in the travertine matrix. So, presence of cyclooctane sulphur points toward cooling associated formation mechanism, which drives the travertine formation not actual "hot" hot spring water. Clearly the role of microbial involvement associated with cyclooctane sulphur molecule needs to be explored further as abiotic chemical pathways can also be responsible for formation of this molecule and ultimately its presence in the hot spring deposit which will be discussed further in separate section (5.2) dedicated to abiotic pathways.

(v) **Hexadecanoic acid methyl ester (Palmitate):** Fatty acids are integral to microbial membranes. Their methyl esters may result from diagenesis or derivatization during analysis. The presence of FAMEs is often interpreted as a direct biomarker for microbial life, as they are highly suggestive of biogenic origin, especially if the chain-length distribution corresponds to known microbial lipid profiles**.** Short-chain fatty

acids, specifically palmitic acid (C16), identified in the sample , indicating the creation of organic material through bacterial action *(Malherbe et al., 2017)*. These saturated fatty acids are commonly present in the lipid composition of cell membranes and are essential components of glyco and phospholipids in bacteria *(Jetter et al., 2006)*. Typically, bacteria contain saturated fatty acids ranging from C-14 to C-18, while algae contain saturated fatty acids ranging from C-16 to C-26, and plants contain saturated fatty acids ranging from C-28 to C-36 respectively. The absence of long-chain fatty acids in the travertine sample suggests that the bioindicators are representation of metabolite derived from cyanobacterial and algae classes as reported from previous studies *(Hewelt-Belka et al., 2020; Kumari et al., 2013; Passos et al., 2023)*.

The compounds discussed above may also form through inorganic geochemical reactions driven by the high temperatures of hot spring water and the large thermal gradients induced by extreme cold conditions. Additionally, high UV radiation, along with the mineral and water chemistry in these environments, facilitates these reactions. They are influenced by factors such as pH, temperature, and the presence of dissolved inorganic carbon and sulphur species, which will be discussed and hypothesised with plausible reaction routes in the next section (5.2).

5.2 Exploration of biomolecules as Prebiotic Molecules in Calcium-Rich Travertine Hot Springs in Cold Conditions

Travertine deposits, formed in hot spring environments, present a mineralogical context conducive to prebiotic chemistry. Their calcium carbonate-rich composition provides a reactive interface for the concentration, stabilization, and catalysis of organic molecules. Below is an intriguing possibility and assessment of the role of the given molecules (beta-alanine derivative, pyran-2-thione, formamide, cyclooctasulfur (S_8), and hexadecanoic methyl ester) found in the Puga hot spring travertine sample as potential origin of life agents. We attempted to provide general possible reaction route(s) by which these molecules may form in hot spring conditions in the extreme cold environment of Ladakh.

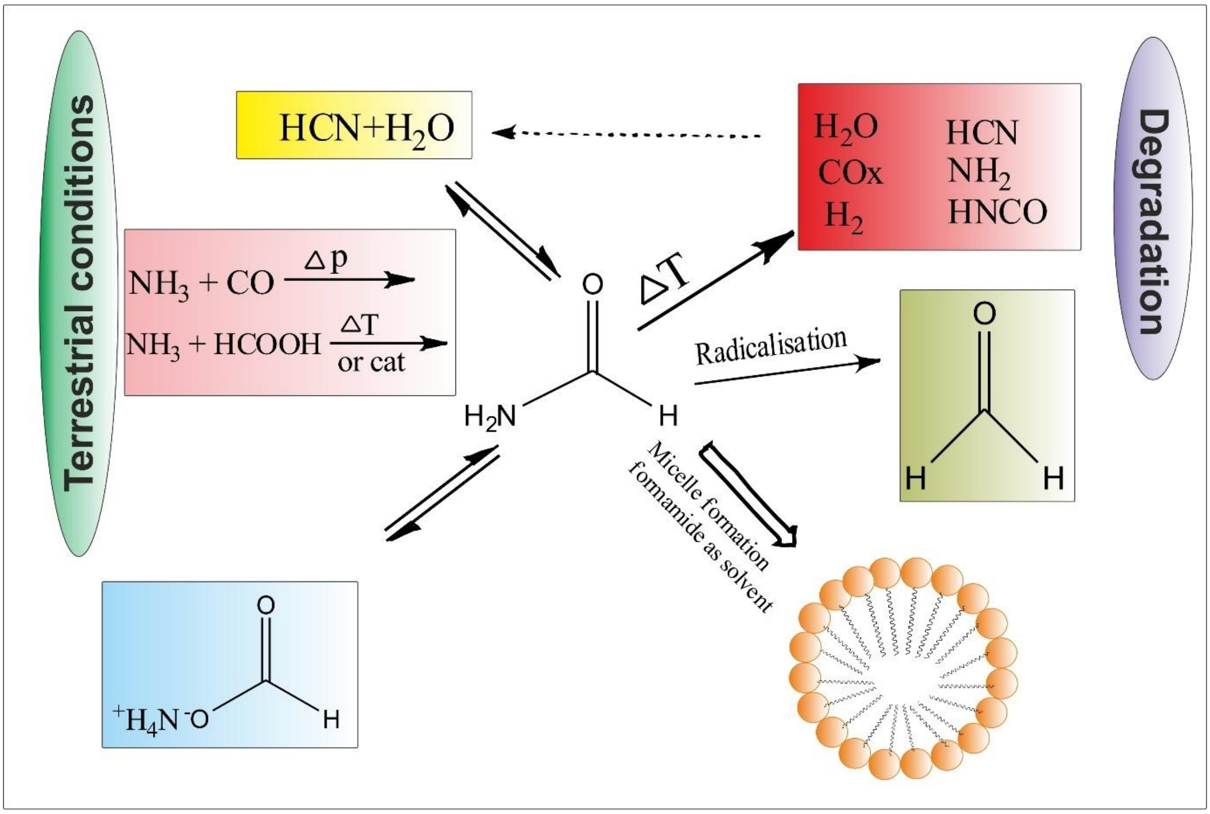

Fig.6 The basic prebiotic chemistry reactions layout of the formamide *(Saladino et al., 2012b)*; ΔP/ΔT are high pressure and high temperature conditions; cat is mineral/metal catalyst. "Modified from Physics of Life Reviews, 9, Saladino et.al, Formamide and the origin of life, 84-104, Copyright (2012), with permission from Elsevier.

(i) ***Why do we care for formamide:*** Of all the organic molecules discovered in space so far, formamide (NH_2CHO) appears to be particularly promising when it comes to addressing the question of the emergence of life on Earth, for several reasons. First, it has an amide functional group (-N−C(=O)-), which is necessary to form chains of amino acids and build up proteins. Second, it has been identified as a key precursor of a large variety of prebiotic molecules *(Saitta and Saija, 2014; Saladino et al., 2012a)*. Formamide is also considered a plausible origin-of-life molecule under cold conditions *(López-Sepulcre et al., 2019)*, expanding its relevance beyond high-temperature environments like hydrothermal vents *(Ferus et al., 2022)*. There are plausible ways by which formamide can be synthesised in-situ and near hot spring conditions (Terrestrial conditions) and may react or degrade further via different mechanisms to form other associated molecules (Fig.6).

Hydrothermal veins and a variety of pools and springs could mobilize and accumulate many of the elements vital for prebiotic chemistry, such as C, H, N, O, P, S, B, Zn, Mn, K, and others *(Van Kranendonk et al., 2021)*. Presence of gases such as CO_2,H_2 CO, CH_4, N_2, H_2S are commonly present in thermal springs in varied concentrations *(Hao et al., 2020; Rinehart, 1980; Yu et al., 2022)*. In geothermal settings, nitrogen is mainly present in its gaseous forms dinitrogen (N_2) and ammonia ($NH_{3(g)}$) while in water it appears as both ammonia ($NH_{3(aq)}$) and ammonium ions ($NH_4^+{}_{(aq)}$) *(Holloway et al., 2011)*. Presence of CN^- ions are also reported in certain hot springs *(Jawadi et al., 2021)*. Hot springs have all the ingredients (elements) and recipe (temperature and pressure) to make prebiotic molecules.

The temperature inside the Puga hot spring in Ladakh, India, can reach up to 160°C at depths around 450 meters, with the surface temperature of the springs typically ranging between 37°C and 90°C; the pressure within the reservoir is estimated to be around 2-3 kg/sq.cm^2 *(Craig et al., 2013)*. Although concentration of formamide found in Puga travertine sample is less it can be attributed due less contact time available for reactants to react due to continues recycling of the water. The water is not stagnant, but instead circulates and undergoes multiple cycles of evaporation to a dry state, followed by rehydration from precipitation or periodic hot spring and geyser activity. The cycling frequency is as short as minutes when caused by geyser splashing, hours due to fluctuating levels of pools fed by pulses from hot springs, or days to weeks in cycles of evaporation followed by precipitation. Therefore, formation of formamide may be possibly route through two mechanism(s):

(a) Hydration of HCN to Formamide

$$HCN + H_2O \rightarrow HCONH_{2\,(;\Delta P\ or\ \Delta T or\ Catalyst)}\ldots.(i)$$

(b) CO based synthesis:

$$CO + NH_3 +\ H_2O \rightarrow HCONH_{2\,(CaCO3\ Catalyst)}\ldots.(ii)$$

As hot water vents and cools, CO reacts with water in presence on ammonia/ammonium ions on $CaCO_3$ surfaces to form formamide. In this process cold stabilization prevents excess hydrolysis, maintaining detectable formamide concentrations. Rapid cooling prevents formamide breakdown and $CaCO_3$ surfaces catalyse CO hydration efficiently.

Therefore, above proposed reactions for the formation of formamide generally entail the presence of minerals, temperatures higher than 100 °C, and relatively high pH values, which are compatible with hydrothermal vent conditions *(Saladino et al., 2012b)*.

Once formed formamide can act as a potential precursor to various prebiotic molecules *(Costanzo et al., 2007)*. Formamide serves as a versatile agent in prebiotic chemistry; when it undergoes condensation and breakdown in the presence of minerals and metal oxides, it produces biologically significant compounds like amino acids, cofactors, nucleobases, and carboxylic acids *(Costanzo et al., 2007; Saladino et al., 2012b; Wang et al., 2013)*. Under certain conditions, formamide yields nucleosides such as adenine, purine, hypoxanthine, cytosine, thymine, and uracil *(Saladino et al., 2015)*. Interaction of formamide with the hydrothermal vent mineral pyrite (FeS_2) yields purine and adenine which are basic components of nucleic acids *(Saladino et al., 2008)*. Studies also indicates that formamide can act as a solvent that facilitates the phosphorylation of nucleosides into nucleotides *(Furukawa et al., 2015)* . Furthermore, degradation products of formamide, formic acid, formaldehyde, HCN, ammonia, and CO_x serve as substrates for the synthesis of other intermediates in prebiotic chemistry including sugars *(Civiš et al., 2016; Nguyen et al., 2011)*. Formamide is not only a parent molecule but also an intermediate in a series of reactions from very reactive small radicals to biologically significant molecules as implicated in Miller's classical experiment *(Miller, 1953)*. Overall, formamide is a versatile compound in prebiotic chemistry that can generate a range of monomers, from amino acids to nucleic acids.

(ii) ***β-alanine derivatives:*** Beta-alanine (3-amino propanoic acid) is a fundamental amino acid that acts as a precursor for peptides and a range of bioactive compounds. It can participate in non-enzymatic peptide bond formation under prebiotic conditions *(Frenkel-Pinter et al., 2020)*.

Within travertine, calcium ions may function as catalysts, promoting the condensation of beta-alanine into oligopeptides. Furthermore, Beta-alanine may reacts with formamide to yield dipeptides under mineral-catalysed conditions, mimicking processes in icy or cold thermal gradients *(Elmasry et al., 2021)*.

Hot spring water in Ladakh is often alkaline and rich in dissolved CO_2, NH_3, and trace cyanide (HCN) species from subsurface geochemistry. As the water vents into the cold atmosphere, rapid cooling stabilizes Strecker intermediates, preventing their hydrolysis *(Kitadai and Maruyama, 2018)*. Calcium carbonate ($CaCO_3$) deposits act as a catalytic surface, enhancing imine formation and promoting amino acid synthesis. Rapid cooling prevents excess hydrolysis, allowing beta-alanine to accumulate on $CaCO_3$ surfaces. Alkaline pH enhances the reaction by keeping NH_3 in its reactive form.

General plausible reaction pathway could be as follows:

Formation of an imine intermediate

$$CH_3CHO + NH_3 \rightarrow CH_3CH = NH + H_2O \ldots (i)$$

Nucleophilic addition of cyanide

$$CH_3CH = NH + HCN \rightarrow CH_3CH(NH_2)CN \ldots (ii)$$

Hydrolysis to beta alanine (Catalysed by $CaCO_3$)

$$CH_3CH(NH_2)CN + H_2O \rightarrow NH_2CH_2\,CH_2\,COOH + NH_3 \ldots (iii)$$

(iii) ***Pyran-2-thione:*** Sulphur-containing heterocycles like pyran-2-thione provide thiol groups, essential for early metabolic pathways and redox reactions. Pyran-2-thione can acts as a sulphur donor, forming thioesters with organic acids, as thioesters are intermediates in energy transfer and peptide synthesis *(Frenkel-Pinter et al., 2022; Patel et al., 2015; Sanden et al., 2020)*. Geyserite and calcium minerals may stabilize pyran-2-thione intermediates, enhancing reaction rates even at low temperatures.

Hot spring water in Ladakh contains dissolved sulphur species (H_2S, HS^-) from geothermal sources. Upon venting, exposure to oxygen and UV radiation promotes oxidation and polymerization, forming sulphur-based heterocycles. Cold conditions allow for slower hydrolysis and higher stabilization of pyran-2-thione intermediates. Cold temperatures slow down competing side reactions, allowing thiolation to proceed selectively. High UV exposure in Ladakh catalyses oxidation without extreme heat degradation. Plausible reaction pathway may be follows as:

Oxidation of H_2S to Polysulfides

$$H_2S + O_2 \rightarrow S_n + H_2O \ldots (i)$$

Reaction with α, β-Unsaturated Carbonyls

$$R - CH = CH - CO + S_n \rightarrow Pyran - 2 - thione \ldots (ii)$$

(iv) **Cyclo-octasulphur (S_8):** S_8 serves as a reservoir of elemental sulphur, participating in sulphur cycling and redox reactions that are foundational for prebiotic chemistry. S_8 reacts with organic compounds in cold, mineral-rich conditions, producing thiols and disulfides. These thiols can further form thioesters or participate in electron transfer reactions *(Cody et al., 2000; Patel et al., 2015)*. Calcium carbonate surfaces adsorb and stabilize sulphur intermediates, facilitating redox processes *(Baltrusaitis et al., 2007; Claesson et al., 2024)*.

Cyclooctasulphur (S_8) Formation may occur via Oxidation of H_2S. Elemental sulphur (S_8) forms through oxidation of H_2S as hot water meets oxygen. In colder conditions, sulphur accumulates as stable rings rather than reactive chains. Cold water prevents over-oxidation to sulphates, maintaining S_8. Surface deposition on $CaCO_3$ enables sulphur cycling. Plausible reaction pathway as follows:

H_2S Oxidation

$$8H_2S + 4O_2 \rightarrow S_8 + 8H_2O \ldots (i)$$

(v) **Hexadecanoic methyl ester**: This fatty acid methyl ester can be a precursor to simple membrane-like structures or micelles, essential for compartmentalization in early life. The self-assembly of lipid bilayer membranes to enclose functional biomolecules, thus defining a “protocell,” was a seminal moment in the emergence of life on Earth and likely occurred at the micro-environment of the mineral-water interface *(Sahai et al., 2017)*. Vesicles composed of single-chain amphiphiles, like fatty acids, are thought to have been key contributors to the emergence of life. Vesicles form most readily at temperatures of ~70 °C and require salinity and strongly alkaline conditions to self-assemble *(Geisberger et al., 2023; Williams et al., 2021)*. Thus, alkaline hydrothermal conditions not only permit protocell formation at the origin of life but actively favour it *(Jordan et al., 2019; Purvis et al., 2024)*. Hexadecanoic methyl ester self-assembles into micelles or vesicles in cold aqueous environments, especially when trapped in travertine micropores *(Deamer, 2024; Monnard and Deamer, 2002)*. Calcium ions in travertine stabilize the ester, facilitating interactions with other organic molecules and promoting lipid bilayer formation *(Balantič et al., 2022; Melcrová et al., 2016)*. CO and H_2 from hydrothermal sources undergo catalytic polymerization in the travertine. Cold exposure may favours condensation into long-chain fatty acid esters and promotes

lipid self-assembly into vesicles, aiding plausible protocell formation *(Kudella et al., 2019)*. $CaCO_3$ stabilizes fatty acids against degradation.

Plausible reaction pathway mechanism as follows:

CO Hydrogenation (minerals as catalyst)

$$CO + 2H_2 \rightarrow CH_3(CH_2)_{14}COOH \ldots (i)$$

Esterification with alcohols

$$CH_3(CH_2)_{14}COOH + CH_3OH \rightarrow Hexadecanoic\ Methyl\ Ester \ldots (ii)$$

Overall, we hypothesise that in a calcium-rich travertine system generated from an alkaline hot spring environment in cold environment like Ladakh where there is large thermal gradient created due to diurnal temperature variation along with high UV radiations at such high altitude. The interplay between these molecules may yield complex prebiotic products. This alanine (amino propanoic acids) and Formamide may form peptides or nucleotide precursors via mineral-catalysed condensation. Pyran-2-Thione and Cyclooctasulphur may Contribute sulphur atoms to thioesters, driving energy transfer reactions and hexadecanoic methyl ester may encapsulates reaction products, forming protocellular structures.

6. Conclusion

We conclude that the travertine deposits from the Puga hot spring actively preserve a diverse suite of organic compounds that hold significant implications for prebiotic chemistry. Our GC-MS/MS analysis revealed a homogeneous distribution of key molecules including β-alanine (amino propanoic acid) derivatives, formamide, pyran-2-thione, cyclooctasulphur, and hexadecanoic methyl ester suggesting that plausibly both biotic and abiotic processes operate in tandem under these conditions. Our work demonstrates that the rapid calcium carbonate precipitation in this hydrothermal setting creates a stable matrix capable of capturing and retaining molecular fingerprints, thereby supporting the hypothesis that similar environments could have nurtured the chemical evolution necessary for life on early Earth. We further establish that the dynamic interplay between high-temperature geothermal reactions and rapid cooling in a high UV, low-pressure atmosphere may promote complex prebiotic pathways similar to that of Puga hot spring, Ladakh, India. These findings highlight the potential of terrestrial hot springs as frameworks for early life and expand our understanding of similar processes on other planetary bodies, ultimately laying a new perspective for future astrobiological investigations into life's origins in extreme environments.

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References:

Balantič, K., Weiss, V.U., Allmaier, G., Kramar, P., 2022. Calcium ion effect on phospholipid bilayers as cell membrane analogues. Bioelectrochemistry 143, 107988. https://doi.org/10.1016/j.bioelechem.2021.107988

Baltrusaitis, J., Usher, C.R., Grassian, V.H., 2007. Reactions of sulfur dioxide on calcium carbonate single crystal and particle surfaces at the adsorbed water carbonate interface. Phys. Chem. Chem. Phys. 9, 3011. https://doi.org/10.1039/b617697f

Blöthe, J.H., Munack, H., Korup, O., Fülling, A., Garzanti, E., Resentini, A., Kubik, P.W., 2014. Late Quaternary valley infill and dissection in the Indus River, western Tibetan Plateau margin. Quaternary Science Reviews 94, 102–119. https://doi.org/10.1016/j.quascirev.2014.04.011

Boulesteix, D., Buch, A., Masson, G., Kivrak, L.L., Havig, J.R., Hamilton, T.L., Teece, B.L., He, Y., Freissinet, C., Huang, Y., Santos, E., Szopa, C., Williams, A.J., 2024. Environmental analogs from yellowstone hot springs on geochemical and microbial diversity with implications for the search for life on Mars. Planetary and Space Science 250, 105953. https://doi.org/10.1016/j.pss.2024.105953

Brakenridge, G.R., Newsom, H.E., Baker, V.R., 1985. Ancient hot springs on Mars: Origins and paleoenvironmental significance of small Martian valleys. Geol 13, 859. https://doi.org/10.1130/0091-7613(1985)13<859:AHSOMO>2.0.CO;2

Chaddha, A.S., Mathews, R.P., Kumar, K., Ali, S.N., Phartiyal, B., Manoj, M.C., Sharma, A., 2021. Caves as interim-refugia: Chemical signatures of human habitation under extreme environments of Ladakh, NW India. Journal of Archaeological Science: Reports 36, 102799. https://doi.org/10.1016/j.jasrep.2021.102799

Chaddha, A.S., Sharma, A., Singh, N.K., Ali, S.N., Das, P.K., Pandey, S.K., Phartiyal, B., Kumar, S., 2024a. Exploring the astrobiological potential of rock varnish from a mars analogue field site of Ladakh, India. Planetary and Space Science 248, 105932. https://doi.org/10.1016/j.pss.2024.105932

Chaddha, A.S., Sharma, A., Singh, N.K., Patel, D.K., Satyanarayana, G.N.V., 2023. Rock Varnish: Nature's Shield. ACS Earth Space Chem. 7, 1516–1527. https://doi.org/10.1021/acsearthspacechem.3c00071

Chaddha, A.S., Sharma, A., Singh, N.K., Shamsad, A., Banerjee, M., 2024b. Biotic-abiotic mingle in rock varnish formation: A new perspective. Chemical Geology 648, 121961. https://doi.org/10.1016/j.chemgeo.2024.121961

Chaddha, A.S., Shukla, S.K., Kumar, K., Sharma, A., Kapur, V.V., Phartiyal, B., Thakkar, M.G., 2024c. "Clues of Life" through Mineral Lattice Investigation in the Extreme Cold Conditions of

Ladakh, India: An Astrobiological Perspective Using the Geyser Travertine Deposit of Puga Hot Spring. https://doi.org/10.21203/rs.3.rs-5522737/v1

Chevuturi, A., Dimri, A.P., Thayyen, R.J., 2018. Climate change over Leh (Ladakh), India. Theor Appl Climatol 131, 531–545. https://doi.org/10.1007/s00704-016-1989-1

Civiš, S., Szabla, R., Szyja, B.M., Smykowski, D., Ivanek, O., Knížek, A., Kubelík, P., Šponer, J., Ferus, M., Šponer, J.E., 2016. TiO2-catalyzed synthesis of sugars from formaldehyde in extraterrestrial impacts on the early Earth. Sci Rep 6, 23199. https://doi.org/10.1038/srep23199

Claesson, P.M., Wojas, N.A., Corkery, R., Dedinaite, A., Schoelkopf, J., Tyrode, E., 2024. The dynamic nature of natural and fatty acid modified calcite surfaces. Phys. Chem. Chem. Phys. 26, 2780–2805. https://doi.org/10.1039/D3CP04432G

Cockell, C., 2000. The Ultraviolet Environment of Mars: Biological Implications Past, Present, and Future. Icarus 146, 343–359. https://doi.org/10.1006/icar.2000.6393

Cody, G.D., Boctor, N.Z., Filley, T.R., Hazen, R.M., Scott, J.H., Sharma, A., Yoder, H.S., 2000. Primordial Carbonylated Iron-Sulfur Compounds and the Synthesis of Pyruvate. Science 289, 1337–1340. https://doi.org/10.1126/science.289.5483.1337

Colín-García, M., Villafañe-Barajas, S., Camprubí, A., Ortega-Gutiérrez, F., Colás, V., Negrón-Mendoza, A., 2019. Prebiotic Chemistry in Hydrothermal Vent Systems, in: Handbook of Astrobiology. CRC Press.

Cosmidis, J., Nims, C.W., Diercks, D., Templeton, A.S., 2019. Formation and stabilization of elemental sulfur through organomineralization. Geochimica et Cosmochimica Acta 247, 59–82. https://doi.org/10.1016/j.gca.2018.12.025

Costanzo, G., Saladino, R., Crestini, C., Ciciriello, F., Di Mauro, E., 2007. Formamide as the main building block in the origin of nucleic acids. BMC Evol Biol 7, S1. https://doi.org/10.1186/1471-2148-7-S2-S1

Craig, J., Absar, A., Bhat, G., Cadel, G., Hafiz, M., Hakhoo, N., Kashkari, R., Moore, J., Ricchiuto, T.E., Thurow, J., Thusu, B., 2013. Hot springs and the geothermal energy potential of Jammu & Kashmir State, N.W. Himalaya, India. Earth-Science Reviews 126, 156–177. https://doi.org/10.1016/j.earscirev.2013.05.004

Damer, B., Deamer, D., 2020. The Hot Spring Hypothesis for an Origin of Life. Astrobiology 20, 429–452. https://doi.org/10.1089/ast.2019.2045

Deamer, D., 2024. Perspective: Protocells and the Path to Minimal Life. J Mol Evol 92, 530–538. https://doi.org/10.1007/s00239-024-10197-6

Deamer, D., Damer, B., Kompanichenko, V., 2019. Hydrothermal Chemistry and the Origin of Cellular Life. Astrobiology 19, 1523–1537. https://doi.org/10.1089/ast.2018.1979

Dutta, A., Mishra, P., Absar, A., Malviya, V.P., Singh, P.K., Srivastava, A., Ray, B., Kumar, A., Nitnaware, N.V., 2023a. Tracing hydrothermal mineral thenardite in geysers/hot springs of North-western Himalayan belt, Ladakh Geothermal Province, India by hydrogeochemistry, fluid-mineral equilibria and isotopic studies. Geochemistry 83, 125973. https://doi.org/10.1016/j.chemer.2023.125973

Elmasry, W., Kebukawa, Y., Kobayashi, K., 2021. Synthesis of Organic Matter in Aqueous Environments Simulating Small Bodies in the Solar System and the Effects of Minerals on Amino Acid Formation. Life 11, 32. https://doi.org/10.3390/life11010032

Ferber, D.M., Khambaty, F., Ely, B., 1988. Utilization of Histidine by Caulobacter crescentus. Microbiology 134, 2149–2154. https://doi.org/10.1099/00221287-134-8-2149

Ferus, M., Knížek, A., Petera, L., Pastorek, A., Hrnčířová, J., Jankovič, L., Ivanek, O., Šponer, J., Křivková, A., Saeidfirozeh, H., Civiš, S., Chatzitheodoridis, E., Mráziková, K., Nejdl, L., Saija, F., Šponer, J.E., Cassone, G., 2022. Formamide-Based Post-impact Thermal Prebiotic Synthesis in Simulated Craters: Intermediates, Products and Mechanism. Front. Astron. Space Sci. 9, 882145. https://doi.org/10.3389/fspas.2022.882145

Frenkel-Pinter, M., Bouza, M., Fernández, F.M., Leman, L.J., Williams, L.D., Hud, N.V., Guzman-Martinez, A., 2022. Thioesters provide a plausible prebiotic path to proto-peptides. Nat Commun 13, 2569. https://doi.org/10.1038/s41467-022-30191-0

Frenkel-Pinter, M., Samanta, M., Ashkenasy, G., Leman, L.J., 2020. Prebiotic Peptides: Molecular Hubs in the Origin of Life. Chem. Rev. 120, 4707–4765. https://doi.org/10.1021/acs.chemrev.9b00664

Furukawa, Y., Kim, H.-J., Hutter, D., Benner, S.A., 2015. Abiotic Regioselective Phosphorylation of Adenosine with Borate in Formamide. Astrobiology 15, 259–267. https://doi.org/10.1089/ast.2014.1209

Garcia, V.H., Ma, L., Ricketts, J.W., Dosseto, A., 2021. Record of Neotectonics and Deep Crustal Fluid Circulation Along the Santa Fe Fault Zone in Travertine Deposits of the Lucero Uplift, New Mexico, USA. Geochem Geophys Geosyst 22, e2020GC009454. https://doi.org/10.1029/2020GC009454

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

Hao, Y., Pang, Z., Tian, J., Wang, Y., Li, Z., Li, L., Xing, L., 2020. Origin and evolution of hydrogen-rich gas discharges from a hot spring in the eastern coastal area of China. Chemical Geology 538, 119477. https://doi.org/10.1016/j.chemgeo.2020.119477

Hewelt-Belka, W., Kot-Wasik, Á., Tamagnini, P., Oliveira, P., 2020. Untargeted Lipidomics Analysis of the Cyanobacterium Synechocystis sp. PCC 6803: Lipid Composition Variation in Response to Alternative Cultivation Setups and to Gene Deletion. IJMS 21, 8883. https://doi.org/10.3390/ijms21238883

Holloway, J.M., Nordstrom, D.K., Böhlke, J.K., McCleskey, R.B., Ball, J.W., 2011. Ammonium in thermal waters of Yellowstone National Park: Processes affecting speciation and isotope fractionation. Geochimica et Cosmochimica Acta 75, 4611–4636. https://doi.org/10.1016/j.gca.2011.05.036

Jawadi, H.A., Malistani, H.A., Moheghy, M.A., Sagin, J., 2021. Essential Trace Elements and Arsenic in Thermal Springs, Afghanistan. Water 13, 134. https://doi.org/10.3390/w13020134

Jetter, R., Kunst, L., Samuels, AL., 2006. Biology of the Plant Cuticle. eds Rieder M, Muller , Clackwell Publishing, Oxford, UK, pp. 145–181.

Jordan, S.F., Rammu, H., Zheludev, I.N., Hartley, A.M., Maréchal, A., Lane, N., 2019. Promotion of protocell self-assembly from mixed amphiphiles at the origin of life. Nat Ecol Evol 3, 1705–1714. https://doi.org/10.1038/s41559-019-1015-y

Juyal, N., 2014. Ladakh: The High-Altitude Indian Cold Desert, in: Kale, V.S. (Ed.), Landscapes and Landforms of India, World Geomorphological Landscapes. Springer Netherlands, Dordrecht, pp. 115–124. https://doi.org/10.1007/978-94-017-8029-2_10

Kamyshny, A., 2009. Solubility of cyclooctasulfur in pure water and sea water at different temperatures. Geochimica et Cosmochimica Acta 73, 6022–6028. https://doi.org/10.1016/j.gca.2009.07.003

Kitadai, N., Maruyama, S., 2018. Origins of building blocks of life: A review. Geoscience Frontiers 9, 1117–1153. https://doi.org/10.1016/j.gsf.2017.07.007

Kudella, P.W., Preißinger, K., Morasch, M., Dirscherl, C.F., Braun, D., Wixforth, A., Westerhausen, C., 2019. Fission of Lipid-Vesicles by Membrane Phase Transitions in Thermal Convection. Sci Rep 9, 18808. https://doi.org/10.1038/s41598-019-55110-0

Kumari, P., Kumar, M., Reddy, C.R.K., Jha, B., 2013. Algal lipids, fatty acids and sterols, in: Functional Ingredients from Algae for Foods and Nutraceuticals. Elsevier, pp. 87–134. https://doi.org/10.1533/9780857098689.1.87

Kunz, D.A., Wang, C.-S., Chen, J.-L., 1994. Alternative routes of enzymic cyanide metabolism in Pseudomonas fluorescens NCIMB 11764. Microbiology 140, 1705–1712. https://doi.org/10.1099/13500872-140-7-1705

López-Sepulcre, A., Balucani, N., Ceccarelli, C., Codella, C., Dulieu, F., Theulé, P., 2019. Interstellar Formamide (NH_2 CHO), a Key Prebiotic Precursor. ACS Earth Space Chem. 3, 2122–2137. https://doi.org/10.1021/acsearthspacechem.9b00154

Luo, L., Capezzuoli, E., Rogerson, M., Vaselli, O., Wen, H., Lu, Z., 2022. Precipitation of carbonate minerals in travertine-depositing hot springs: Driving forces, microenvironments, and mechanisms. Sedimentary Geology 438, 106207. https://doi.org/10.1016/j.sedgeo.2022.106207

Malherbe, C., Hutchinson, I.B., Ingley, R., Boom, A., Carr, A.S., Edwards, H., Vertruyen, B., Gilbert, B., Eppe, G., 2017. On the Habitability of Desert Varnish: A Combined Study by Micro-Raman Spectroscopy, X-ray Diffraction, and Methylated Pyrolysis–Gas Chromatography–Mass Spectrometry. Astrobiology 17, 1123–1137. https://doi.org/10.1089/ast.2016.1512

Martin, W., Baross, J., Kelley, D., Russell, M.J., 2008. Hydrothermal vents and the origin of life. Nat Rev Microbiol 6, 805–814. https://doi.org/10.1038/nrmicro1991

Melcrová, A., Pokorna, S., Pullanchery, S., Kohagen, M., Jurkiewicz, P., Hof, M., Jungwirth, P., Cremer, P.S., Cwiklik, L., 2016. The complex nature of calcium cation interactions with phospholipid bilayers. Sci Rep 6, 38035. https://doi.org/10.1038/srep38035

Miller, S.L., 1953. A Production of Amino Acids Under Possible Primitive Earth Conditions. Science 117, 528–529. https://doi.org/10.1126/science.117.3046.528

Monnard, P., Deamer, D.W., 2002. Membrane self-assembly processes: Steps toward the first cellular life. Anat. Rec. 268, 196–207. https://doi.org/10.1002/ar.10154

Nguyen, V.S., Abbott, H.L., Dawley, M.M., Orlando, T.M., Leszczynski, J., Nguyen, M.T., 2011. Theoretical Study of Formamide Decomposition Pathways. J. Phys. Chem. A 115, 841–851. https://doi.org/10.1021/jp109143j

Pandey, S., Clarke, J., Nema, P., Bonaccorsi, R., Som, S., Sharma, M., Phartiyal, B., Rajamani, S., Mogul, R., Martin-Torres, J., Vaishampayan, P., Blank, J., Steller, L., Srivastava, A., Singh, R., McGuirk, S., Zorzano, M.-P., Güttler, J.M., Mendaza, T., Soria-Salinas, A., Ahmad, S., Ansari, A., Singh, V.K., Mungi, C., Bapat, N., 2020. Ladakh: diverse, high-altitude extreme environments for off-earth analogue and astrobiology research. International Journal of Astrobiology 19, 78–98. https://doi.org/10.1017/S1473550419000119

Passos, L.S., De Freitas, P.N.N., Menezes, R.B., De Souza, A.O., Silva, M.F.D., Converti, A., Pinto, E., 2023. Content of Lipids, Fatty Acids, Carbohydrates, and Proteins in Continental Cyanobacteria: A Systematic Analysis and Database Application. Applied Sciences 13, 3162. https://doi.org/10.3390/app13053162

Patel, B.H., Percivalle, C., Ritson, D.J., Duffy, C.D., Sutherland, J.D., 2015. Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism. Nature Chem 7, 301–307. https://doi.org/10.1038/nchem.2202

Pross, A., Pascal, R., 2013. The origin of life: what we know, what we can know and what we will never know. Open Biol. 3, 120190. https://doi.org/10.1098/rsob.120190

Purvis, G., Šiller, L., Crosskey, A., Vincent, J., Wills, C., Sheriff, J., Xavier, C., Telling, J., 2024. Generation of long-chain fatty acids by hydrogen-driven bicarbonate reduction in ancient alkaline hydrothermal vents. Commun Earth Environ 5, 30. https://doi.org/10.1038/s43247-023-01196-4

Ricardo, A., Carrigan, M.A., Olcott, A.N., Benner, S.A., 2004. Borate Minerals Stabilize Ribose. Science 303, 196–196. https://doi.org/10.1126/science.1092464

Ricketts, J.W., Ma, L., Wagler, A.E., Garcia, V.H., 2019. Global travertine deposition modulated by oscillations in climate. J Quaternary Science 34, 558–568. https://doi.org/10.1002/jqs.3144

Rinehart, J.S., 1980. The Role of Gases in Geysers, in: Geysers and Geothermal Energy. Springer New York, New York, NY, pp. 78–91. https://doi.org/10.1007/978-1-4612-6084-4_4

Ruff, S.W., Campbell, K.A., Van Kranendonk, M.J., Rice, M.S., Farmer, J.D., 2020. The Case for Ancient Hot Springs in Gusev Crater, Mars. Astrobiology 20, 475–499. https://doi.org/10.1089/ast.2019.2044

Runge, E.A., Mansor, M., Kappler, A., Duda, J., 2023. Microbial biosignatures in ancient deep-sea hydrothermal sulfides. Geobiology 21, 355–377. https://doi.org/10.1111/gbi.12539

Sahai, N., Kaddour, H., Dalai, P., Wang, Z., Bass, G., Gao, M., 2017. Mineral Surface Chemistry and Nanoparticle-aggregation Control Membrane Self-Assembly. Sci Rep 7, 43418. https://doi.org/10.1038/srep43418

Saitta, A.M., Saija, F., 2014. Miller experiments in atomistic computer simulations. Proc. Natl. Acad. Sci. U.S.A. 111, 13768–13773. https://doi.org/10.1073/pnas.1402894111

Saladino, R., Botta, G., Pino, S., Costanzo, G., Di Mauro, E., 2012a. Genetics first or metabolism first? The formamide clue. Chem. Soc. Rev. 41, 5526. https://doi.org/10.1039/c2cs35066a

Saladino, R., Carota, E., Botta, G., Kapralov, M., Timoshenko, G.N., Rozanov, A.Y., Krasavin, E., Di Mauro, E., 2015. Meteorite-catalyzed syntheses of nucleosides and of other prebiotic compounds from formamide under proton irradiation. Proc. Natl. Acad. Sci. U.S.A. 112. https://doi.org/10.1073/pnas.1422225112

Saladino, R., Crestini, C., Pino, S., Costanzo, G., Di Mauro, E., 2012b. Formamide and the origin of life. Physics of Life Reviews 9, 84–104. https://doi.org/10.1016/j.plrev.2011.12.002

Saladino, R., Neri, V., Crestini, C., Costanzo, G., Graciotti, M., Di Mauro, E., 2008. Synthesis and Degradation of Nucleic Acid Components by Formamide and Iron Sulfur Minerals. J. Am. Chem. Soc. 130, 15512–15518. https://doi.org/10.1021/ja804782e

Sánchez-García, L., Carrizo, D., Parro, V., 2024. Contrasting lipid biomarkers profiles in two prebiotic-astrobiological analogs from Ladakh (Indian Himalayas): a boron-rich geothermal system (Puga) and an alkaline brine lake (Tso Kar). https://doi.org/10.5194/epsc2024-212

Sanden, S.A., Yi, R., Hara, M., McGlynn, S.E., 2020. Simultaneous synthesis of thioesters and iron–sulfur clusters in water: two universal components of energy metabolism. Chem. Commun. 56, 11989–11992. https://doi.org/10.1039/D0CC04078A

Schaible, M.J., Szeinbaum, N., Bozdag, G.O., Chou, L., Grefenstette, N., Colón-Santos, S., Rodriguez, L.E., Styczinski, M.J., Thweatt, J.L., Todd, Z.R., Vázquez-Salazar, A., Adams, A., Araújo, M.N., Altair, T., Borges, S., Burton, D., Campillo-Balderas, J.A., Cangi, E.M., Caro, T., Catalano, E., Chen, K., Conlin, P.L., Cooper, Z.S., Fisher, T.M., Fos, S.M., Garcia, A., Glaser, D.M., Harman, C.E., Hermis, N.Y., Hooks, M., Johnson-Finn, K., Lehmer, O., Hernández-Morales, R., Hughson, K.H.G., Jácome, R., Jia, T.Z., Marlow, J.J., McKaig, J., Mierzejewski, V., Muñoz-Velasco, I., Nural, C., Oliver, G.C., Penev, P.I., Raj, C.G., Roche, T.P., Sabuda, M.C., Schaible, G.A., Sevgen, S., Sinhadc, P., Steller, L.H., Stelmach, K., Tarnas, J., Tavares, F., Trubl, G., Vidaurri, M., Vincent, L., Weber, J.M., Weng, M.M., Wilpiszeki, R.L., Young, A., 2024. Chapter 1: The Astrobiology Primer 3.0. Astrobiology 24, S-4-S-39. https://doi.org/10.1089/ast.2021.0129

Schmidt, S., Nüsser, M., 2017. Changes of High Altitude Glaciers in the Trans-Himalaya of Ladakh over the Past Five Decades (1969–2016). Geosciences 7, 27. https://doi.org/10.3390/geosciences7020027

Sharma, A., Phartiyal, B., 2018. Late Quaternary Palaeoclimate and Contemporary Moisture Source to Extreme NW India: A Review on Present Understanding and Future Perspectives. Frontiers in Earth Science 6.

Shock, E.L., Schulte, M.D., 1998. Organic synthesis during fluid mixing in hydrothermal systems. J. Geophys. Res. 103, 28513–28527. https://doi.org/10.1029/98JE02142

Shukla, S.K., Chaddha, A.S., Kumar, K., Sharma, A., Pandey, S.K., Kapur, V.V., Phartiyal, B., Shivam, A., Dabhi, A., Bhushan, R., 2023. Hot spring diatoms are linked to extreme cold conditions: A new perspective for astrobiological implication from the sinter deposit of Puga hot spring, Ladakh, India. https://doi.org/10.22541/essoar.170158324.46307742/v1

Song, P., Zhang, X., Wang, S., Xu, W., Wei, F., 2023. Advances in the synthesis of β-alanine. Front. Bioeng. Biotechnol. 11, 1283129. https://doi.org/10.3389/fbioe.2023.1283129

Summons, R.E., Albrecht, P., McDonald, G., Moldowan, J.M., 2008. Molecular Biosignatures. Space Sci Rev 135, 133–159. https://doi.org/10.1007/s11214-007-9256-5

Van Kranendonk, M.J., Baumgartner, R., Djokic, T., Ota, T., Steller, L., Garbe, U., Nakamura, E., 2021. Elements for the Origin of Life on Land: A Deep-Time Perspective from the Pilbara Craton of Western Australia. Astrobiology 21, 39–59. https://doi.org/10.1089/ast.2019.2107

Villafañe-Barajas, S.A., Colín-García, M., 2021. Submarine hydrothermal vent systems: the relevance of dynamic systems in chemical evolution and prebiotic chemistry experiments. International Journal of Astrobiology 20, 427–434. https://doi.org/10.1017/S1473550421000331

Wachsman, J.T., Barker, H.A., 1955. THE ACCUMULATION OF FORMAMIDE DURING THE FERMENTATION OF HISTIDINE BY CLOSTRIDIUM TETANOMORPHUM. J Bacteriol 69, 83–88. https://doi.org/10.1128/jb.69.1.83-88.1955

Wang, J., Gu, J., Nguyen, M.T., Springsteen, G., Leszczynski, J., 2013. From Formamide to Purine: An Energetically Viable Mechanistic Reaction Pathway. J. Phys. Chem. B 117, 2314–2320. https://doi.org/10.1021/jp311423q

Wang, T., Ran, M., Li, X., Liu, Y., Xin, Y., Liu, Honglei, Liu, Huaiwei, Xia, Y., Xun, L., 2022. The Pathway of Sulfide Oxidation to Octasulfur Globules in the Cytoplasm of Aerobic Bacteria. Appl Environ Microbiol 88, e01941-21. https://doi.org/10.1128/aem.01941-21

Williams, A.J., Craft, K.L., Millan, M., Johnson, S.S., Knudson, C.A., Juarez Rivera, M., McAdam, A.C., Tobler, D., Skok, J.R., 2021. Fatty Acid Preservation in Modern and Relict Hot-Spring Deposits in Iceland, with Implications for Organics Detection on Mars. Astrobiology 21, 60–82. https://doi.org/10.1089/ast.2019.2115

Yang, S., Li, J., Meng, R., Yu, T., Wang, Z., Xiong, P., Gao, Z., 2023. Screening and identification of genes involved in β-alanine biosynthesis in Bacillus subtilis. Archives of Biochemistry and Biophysics 743, 109664. https://doi.org/10.1016/j.abb.2023.109664

Yu, X., Wei, Z., Wang, G., Ma, X., Zhang, T., Yang, H., Li, L., Zhou, S., Wang, X., 2022. Hot Spring Gas Geochemical Characteristics and Geological Implications of the Northern Yadong-Gulu Rift in the Tibetan Plateau. Front. Earth Sci. 10, 863559. https://doi.org/10.3389/feart.2022.863559

Zhang, Y., Liu, T., Li, M.-M., Hua, Z.-S., Evans, P., Qu, Y., Tan, S., Zheng, M., Lu, H., Jiao, J.-Y., Lücker, S., Daims, H., Li, W.-J., Guo, J., 2023. Hot spring distribution and survival mechanisms of thermophilic comammox *Nitrospira*. The ISME Journal 17, 993–1003. https://doi.org/10.1038/s41396-023-01409-w