

Influence of Rare-Earth-Elements on Prebiotic Reaction Networks Resembling the Biologically Relevant Krebs Cycle

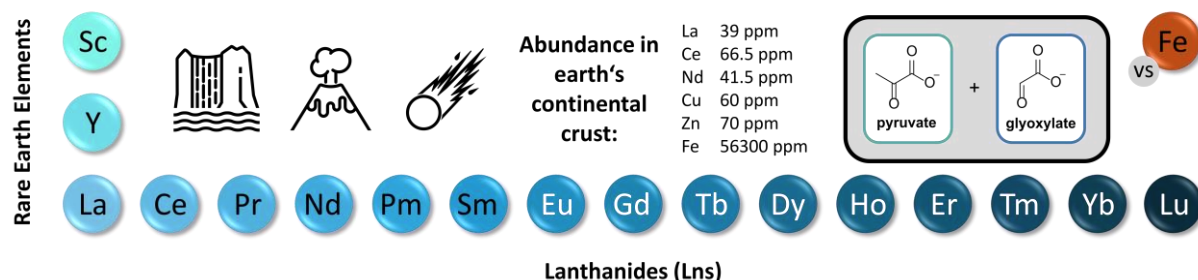
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Abstract

Rare earth elements (REEs) are not rare, but rather abundant in the earth's crust and excellent catalysts for a multitude of organic reactions. They have been recently shown to be used in the active sites of bacterial enzymes and thus essential for metabolic processes. However, these elements have so far been entirely overlooked in their possible contributions to the emergence of complex molecules. Here, we investigate the potential of REEs to act as catalysts in a prebiotic reaction network resembling the biological Krebs cycle starting from glyoxylate and pyruvate. Special focus is put on a comparison between trivalent REEs and ferrous iron. Reaction products were analyzed by gas chromatography–mass spectrometry (GC–MS) and nuclear magnetic resonance (NMR) spectroscopy. Contrary to Fe^{2+} , the formation of the reduced starting materials seems to be a major pathway when REEs are involved. Their high coordination numbers, flexible coordination spheres and their hard Lewis acidic properties make REEs excellent catalysts in abiotic chemical reaction networks resembling conserved biochemical pathways.

Introduction

A main goal of Origin of Life (OoL) research is to narrow the gaps between prebiotic chemical reactions on the early Earth and modern biochemistry. To this end it is necessary to get a complete picture of prebiotically plausible reactions covering all possible environments and conditions. However, one class of elements has so far been underrepresented with regards to OoL research – the rare-earth elements (REEs) consisting of the lanthanides (Lns), scandium and yttrium. These elements are, contrary to what their name might suggest, omnipresent in earth's continental crust today with abundances comparable to other common metals, such as

copper and zinc (La: 39 ppm, Ce: 66.5 ppm, Cu: 60 ppm, Zn: 70 ppm).^[1] In a prebiotic context, REEs are often used to investigate the nature and formation processes of the oldest terrestrial rocks^[2,3] and are in part represented in minerals most likely to be found on Hadean Earth.^[4,5] Additionally, they are incorporated in carbonaceous chondrite meteors in combination with a complex mixture of organic substances.^[6,7] From a reactivity stand point their weak ligand field stabilization^[8] and subsequent high water exchange rate coupled with a low hydrolysis constant makes them efficient Lewis acid catalysts in water.^[9,10] This fact alone makes it even more surprising that REEs have so far been neglected in OoL research. In contrast, the transition metal iron plays an integral part in the research into the abiotic generation of biological building blocks,^[11] given its important role in many different biological processes.^[12] Members of the REEs are the most recent addition to the group of biologically relevant metals.^[13] Methylophilic bacteria can use lanthanides instead of calcium in the active sites of methanol dehydrogenase enzymes (MDH), an essential component in their C1 metabolism.^[14–18] The gene *xoxF* encodes for this Ln-dependent MDH and as Keltjens *et al.* conclude, XoxF represents an evolutionary older prototype of the calcium-dependent MDH (MxaF-type).^[19] This fact, in combination with the likely existence of many more Ln-dependent enzymes, hints towards an essential role of rare-earth metals in life from its very beginning on.

In this study we investigate the potential of REEs as catalysts in a prebiotic reaction network resembling the biological Krebs cycle starting from glyoxylate and pyruvate. A special focus is put on a comparison between trivalent REEs and ferrous iron.

Results and Discussion

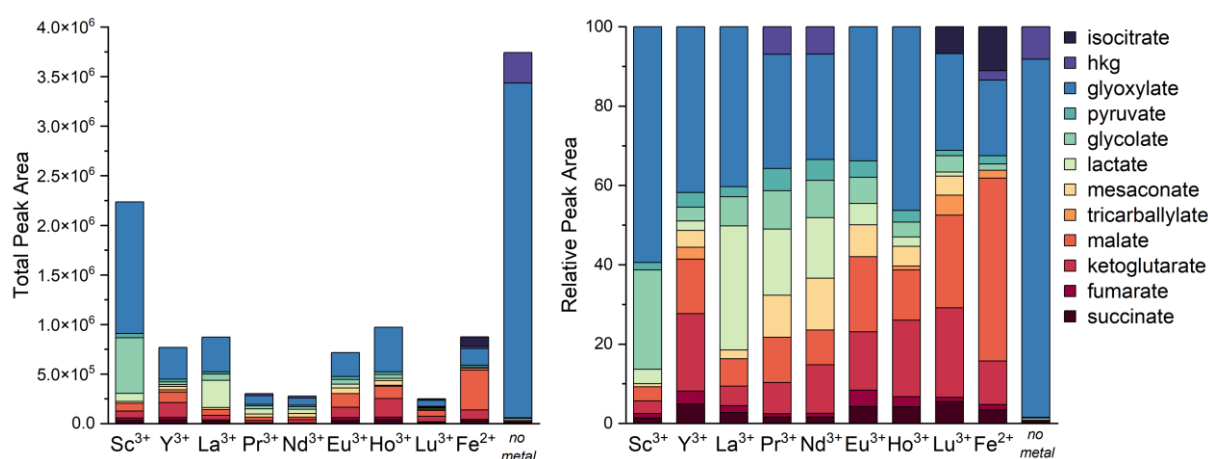


Figure 1. Absolute and relative peak areas of reaction product gas chromatograms of glyoxylate and pyruvate in the presence of different REE chlorides or ferrous chloride after 3 h at 70 °C (hkg = α -hydroxyketoglutarate).

Based on the seminal work of Moran and coworkers,^[20] we initially focused on a direct comparison of different REE chloride salts and ferrous chloride to survey their ability to catalyze reactions in a prebiotic reaction network resembling the metabolic Krebs cycle. To that end, sodium glyoxylate and sodium pyruvate were reacted with the respective salts for 3 h in mildly

acidic (pH 5 to 6) water at 70 °C in a sealed vessel purged with argon (molar ratios: 2:1:2, glyoxylate:pyruvate:metal salt) simulating the environment of a hydrothermal vent. After subsequent basification and derivatization according to a literature-known procedure,^[20] the reaction products were investigated by gas chromatography coupled with mass spectrometry (GC-MS) and the results are shown in Figure 1. The REEs that were chosen represent metal cations over the whole series, with different sizes and thus charge densities, possibly resulting in altered Lewis acidity and coordination numbers.

Notably, very similar products were obtained using REE or Fe²⁺, but there is a readily observable difference in the relative product distributions between the REE salts and FeCl₂. Whereas α-ketoglutarate is a major product of the reaction with Fe²⁺, the starting material glyoxylate is still present in significant amounts in the reactions containing REE salts. Another key difference between iron and the REEs is the observed formation of mesaconate in the REE-containing reactions. The pathway to this compound starts with a condensation of two molecules of pyruvate and subsequent dehydration and decarboxylation. With Fe²⁺, mesaconate could not be detected. While the formation of isocitrate is only observed with Fe²⁺ and Lu³⁺, lactate, the product of a reduction of the starting material pyruvate, can only be detected in the REE-containing reactions with La³⁺ showing the highest amount.

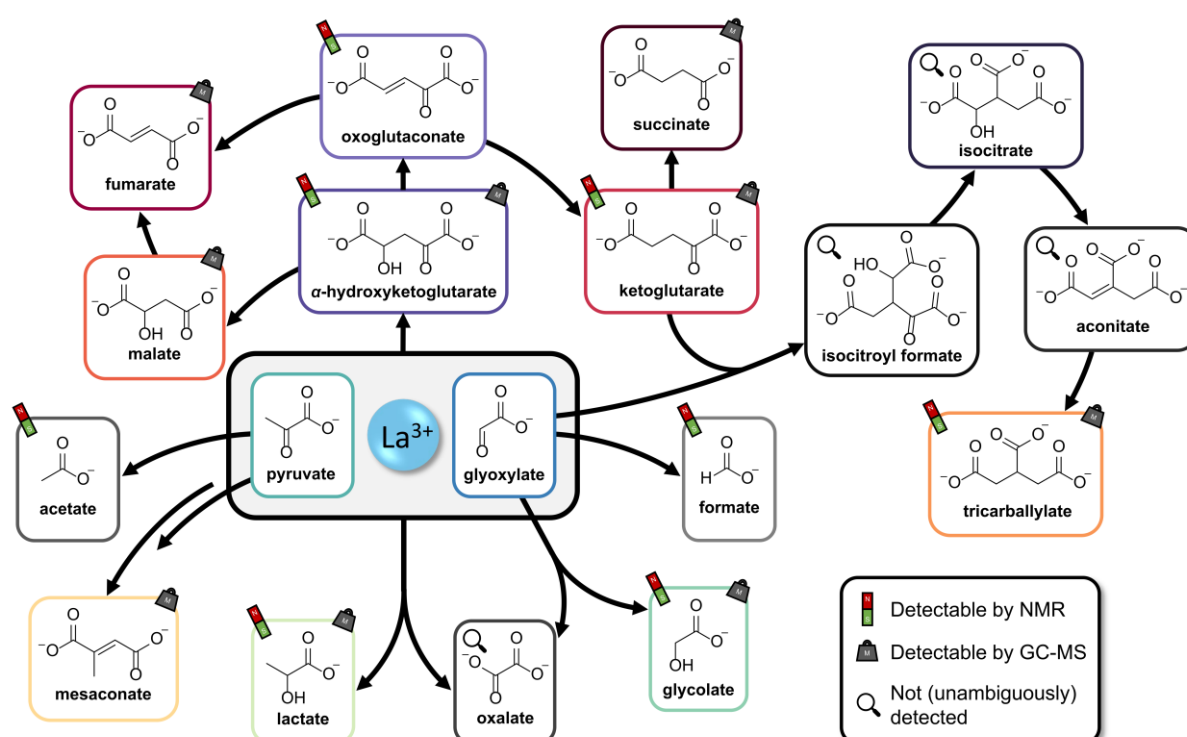


Figure 2. Reaction network arising from the La³⁺-catalyzed reaction of glyoxylate and pyruvate, including non-detected intermediates with the most probable routes represented by arrows requiring either one (direct arrow) or both of the reactants (arrow starting from grey box). The frame colors are consistent with respective substance coloring in the analytical data (see Figure 1 and SI).

Next, we wanted to track the variation in the product spectrum over time. The two metal salts FeSO₄ and La₂(SO₄)₃ were chosen, as preliminary experiments showed a higher reactivity for

$\text{La}_2(\text{SO}_4)_3$. This might be explained by the presence of the weakly basic sulfate anion. The stoichiometry was changed to 2:1:1 (glyoxylate:pyruvate: $\text{Fe}^{2+}/\text{La}^{3+}$). The reaction mixture was analyzed before metal salt addition (0 h) and after 1 h, 3 h, 5 h, 24 h, 48 h and 72 h, respectively. As the sample preparation in the aforementioned reactions under the influence of metal chlorides involved a harsh basification step to precipitate all present metal cations, which in itself could influence the product spectrum, we were looking for an alternative, milder protocol. To separate dissolved metal ions Chelex® 100 resin was used instead. Additional to the GC-MS analysis, ^1H NMR spectra were recorded for each step and the pH was determined.

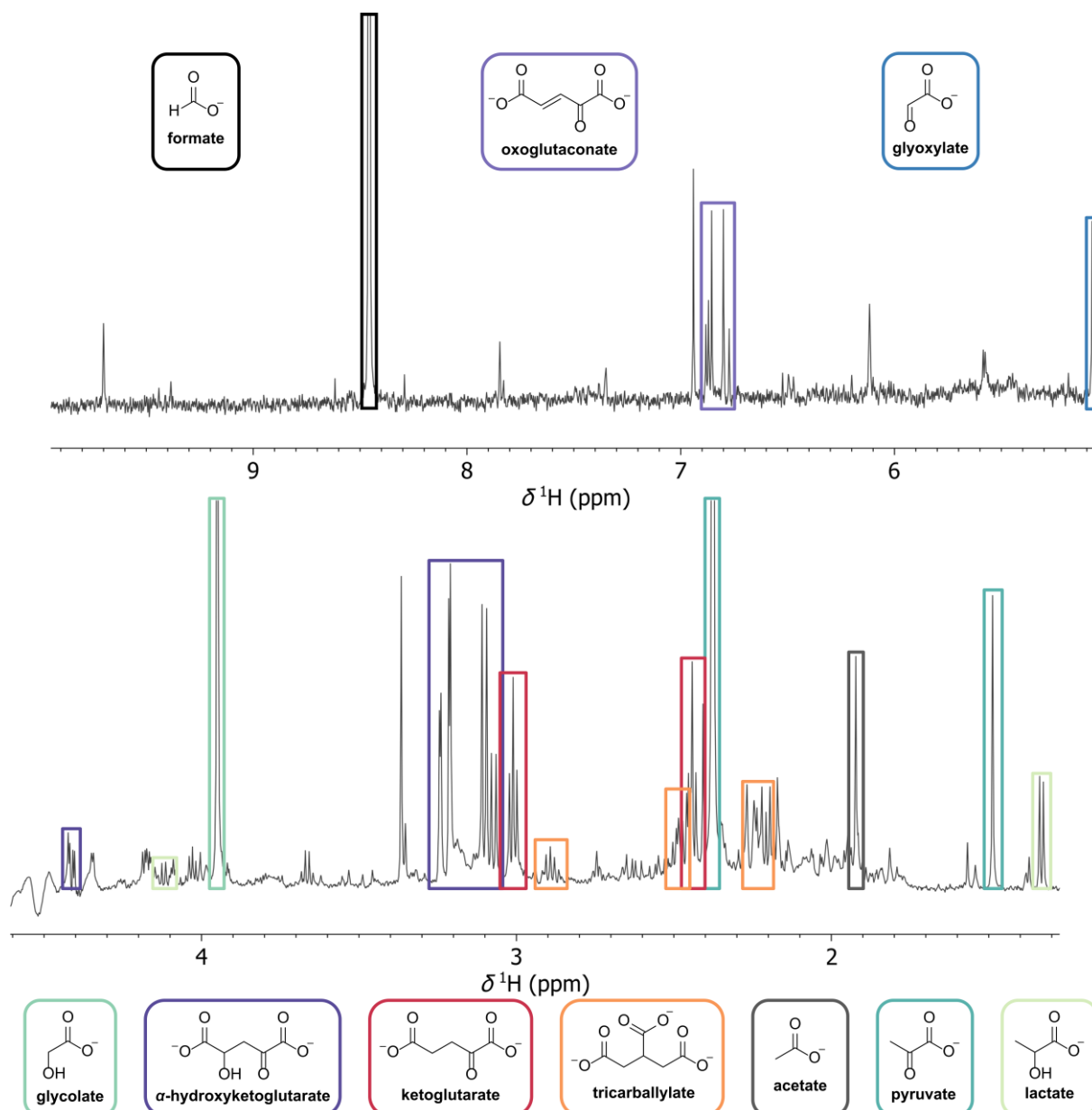


Figure 3. Representative ^1H NMR of the reaction mixture containing $\text{La}_2(\text{SO}_4)_3$ after 72 h with the assignable signals marked in colored boxes and the respective structures marked with the same color.

Gas chromatograms of the reaction containing Fe^{2+} show no significant changes after 24 h. The NMR spectra paint a similar picture with only slight changes after 24 h (see Figures S4

and S5). Especially in the NMR spectra after 1 h and 3 h the presence of paramagnetic iron in solution is yielding broad peaks making reliable integration impossible. This is an indication that the removal of metal ions is insufficient with the chosen exchange resin. After 5 h and beyond, the improved resolution of the spectra coincides with the observed formation of significant amounts of precipitate in the reaction vessel (see Figure S12). Overall, the formation of α -ketoglutarate and tricarballoylate as main products is verifiable by NMR and GC-MS. In the low-field region of the spectra the formation of oxoglutaconate and formate can clearly be observed, both of which could not be detected by GC-MS. The pH of the reaction mixture starts at 4.7 after metal salt addition and settles at about 6.5 after 24 h (see Figure S12).

Similar to the reaction containing Fe^{2+} , in the reaction involving $\text{La}_2(\text{SO}_4)_3$ the product spectrum and distribution stays relatively constant after 24 h (see Figure S9) according to GC-MS. However, just as in the reactions involving the REE chlorides and contrary to Fe^{2+} , the formation of the reduced starting materials seems to be a major pathway which can also be confirmed by NMR spectroscopy (see Figures 3 and S2). While the quantity of lactate is comparatively stable over time, the quantity of glycolate is increasing continuously.

At the start of the reaction, $\text{La}_2(\text{SO}_4)_3$ never fully dissolves and a small amount of colorless precipitate is observable in the reaction vessel (see Figure S11). Already after 3 h a substantial increase in the amount of precipitate can be detected. The precipitate is still present after the treatment with the Chelex® resin. This prompted us to investigate the lanthanum concentration in solution at this point. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) gave a value of $330 \mu\text{g}\cdot\text{mL}^{-1}$ which corresponds to 2.4% of the initially added amount of La^{3+} . An elemental analysis of the precipitate after 3 h revealed a very low sulfur content in combination with a relatively low hydrogen content (C: 15.49, H: 2.56, S: 1.01). The precipitate after 3 h with one equivalent of La^{3+} was also investigated by IR spectroscopy (see Figure S16). As a classical Cannizzaro reaction of glyoxylate yields oxalate as a product and as metal oxalates are usually poorly soluble, this might point to the possible nature of the precipitate. The IR spectra indicate the presence of an absorption band of the precipitate at 1313 cm^{-1} which would correspond to the C=O stretch of oxalate.^[21] Thus, the resulting precipitate is most likely a mixture of La^{3+} with oxalate, molecules of higher carbon content, such as pyruvate, and small amounts of sulfate.

The fact, that the reaction continues at very low values of dissolved La^{3+} might indicate catalytic activity of La^{3+} species present in solution or in a heterogenous fashion and in turn prompted us to investigate the influence of La^{3+} concentration at the start of the reaction. To this end, the reaction was repeated with 0.5, 0.2, 0.1 and 0 equivalents of La^{3+} in the form of $\text{La}_2(\text{SO}_4)_3$ and the reaction mixtures were investigated *via* ^1H NMR after 3 h. The NMR spectra (see Figures S6 and S7) show an increase in reaction dynamics at higher La^{3+} concentration detectable by

a more pronounced decrease in concentration of the starting materials. However, the overall product spectrum and concentrations in all reactions containing La^{3+} are highly similar. The reaction containing the starting materials without La^{3+} doesn't show the formation of oxoglutaconate, ketoglutarate or lactate. These results are an indication of catalytic activity of La^{3+} in this reaction network.

As the most notable difference between the REE-containing reactions and those with Fe^{2+} is the formation of significant amounts of glycolate and lactate, the reduced forms of the two starting materials, these reactions justify a more detailed discussion. While Fe^{2+} could in principle act as a reducing agent,^[22] this is not the case for the used REEs. The reduction, however, could be explained by the intrinsic reactivity of aldehydes. A disproportionation of aldehydes is known as the Cannizzaro reaction^[23] and involves the hydride transfer of a reactive diol anion to the carbonyl function of a second aldehyde yielding the respective alcohol and carboxylate after a proton transfer.^[24] When a different carbonyl-containing molecule is present as hydride acceptor the reaction is generally called crossed Cannizzaro reaction. These reactions usually occur under strongly basic conditions as the first step involves the nucleophilic attack of a hydroxide anion to the aldehyde. In the case of aldehydes with another carbonyl in the α -position, a different mechanism comes into play when metal ions are involved. The coordination of the metal center by the 1,2-dicarbonyl-containing molecule allows for an intramolecular 1,2-hydride shift.^[25] This reaction has been reported with different metal ions including the REEs scandium and ytterbium.^[26] In the case of lactate formation in the context of this work, a possible mechanism is shown in Figure 4. In a first step, the glyoxylate hydrate loses a proton and forms the reactive diol anion. Subsequently, a hydride is transferred to the carbonyl of an adjacent pyruvate molecule, with a proton transfer yielding the products oxalate and lactate. The role of La^{3+} in this reaction may be the activation of the carbonyl function, as well as bringing both substrates in close proximity to one another. In the latter case, the more flexible coordination sphere of La^{3+} in comparison to transition metals might be advantageous.

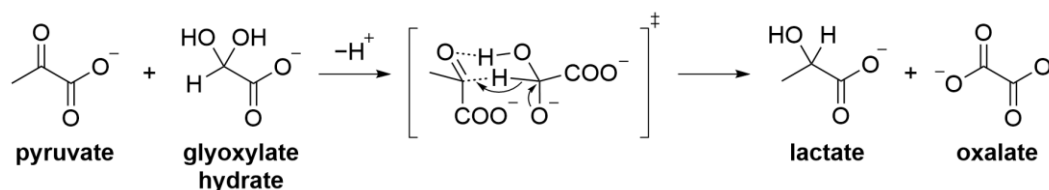


Figure 4. Proposed concerted mechanism of a La^{3+} -mediated crossed Cannizzaro reaction of glyoxylate hydrate with pyruvate. Coordination to the metal center is not shown for clarity.

As the Cannizzaro reaction is highly dependent on the pH of the reaction medium, we tested the effect of different starting pH values. To suppress the reaction of glyoxylate with itself, the stoichiometry was changed to 1:1:1 (glyoxylate:pyruvate: $\text{La}_2(\text{SO}_4)_3$) and the pH after metal salt addition was adjusted to 6, 7 and 8, respectively. NMR spectra of the reaction mixtures after

3 h paint a clear picture (see Figure S8). While the product spectrum of the reaction with a pH starting value of 6 shows almost no lactate and glycolate, the lactate signals become more pronounced at pH 7. At pH 8, the ratio of lactate to pyruvate is 1.9:1 with no other significant product peaks, apart from a small amount of glycolate. The IR spectra of the respective precipitates isolated after the reactions show significant similarities with lanthanum(III) oxalate at pH 7 and pH 8 (see Figure S17). At pH 6, the spectrum of the precipitate looks almost identical to the corresponding IR spectrum of the aforementioned 2:1 reaction after 3 h. Thus, it can be concluded that at pH 7 and 8 the reaction becomes much more selective towards the crossed Cannizzaro reaction with oxalate as a byproduct. At lower pH, aldol condensation reactions are more prominent. This also highlights the importance of controlling and testing pH for such reaction networks, also during the workup of samples. With the omission of the basification step during derivatization for GC-MS analysis and additional analysis *via* NMR, we could suppress possible unwanted reactions and better observe molecules actually present in the reaction mixture. For example, α -hydroxyketoglutarate is no longer detectable with either GC-MS or *via* NMR after addition of base.

Conclusion

The important role of metals in prebiotic reactions is undisputed.^[32,33] However, the potential of REEs in this respect has so far been overlooked. In this work we have shown that REEs have a substantially different reactivity compared to iron in the framework of a prebiotic reaction network based on the biological Krebs cycle starting from the prebiotically plausible glyoxylate and pyruvate. Especially the formation of the reduced starting materials as a major reaction pathway at neutral pH might represent an intriguing niche for REEs in Origins research. Also, the observation of La^{3+} reactivity in catalytic amounts indicates the ability of REEs to catalyze these types of reactions in a prebiotic setting, especially where a flexible coordination sphere involving multiple reactants and strong Lewis acids are needed. Furthermore, the hard Lewis acid character is especially relevant in the context of carbonyl/oxygen-donor ligand containing molecules. Although many reaction pathways shown here are hard-wired into the starting materials,^[34] the abundance of REEs and their favorable properties put these elements in a new light as potent catalysts in an OoL scenario.

Supporting Information

Additional information on experimental procedures, used methods and materials, as well as data supporting the findings in this manuscript can be found in the Supporting Information. A zip folder with raw NMR data and reference spectra has been deposited along with this manuscript.

Author Contributions

J.G.-T. and L.J.D designed the study and wrote the initial draft of the manuscript. J.G.-T. and C.G.H. performed the experiments and did the analytical work. L.J.D. provided the necessary infrastructure. All authors were involved in the review of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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