Molecular Evolution Hot Paper

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Dynamic Exchange of Substituents in a Prebiotic Organocatalyst: Initial Steps towards an Evolutionary System

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Abstract: *All evolutionary biological processes lead to a change in heritable traits over successive generations. The responsible genetic information encoded in DNA is altered, selected, and inherited by mutation of the base sequence. While this is well known at the biological level, an evolutionary change at the molecular level of small organic molecules is unknown but represents an important prerequisite for the emergence of life. Here, we present a class of prebiotic imidazolidine-4-thione organocatalysts able to dynamically change their constitution and potentially capable to form an evolutionary system. These catalysts functionalize their building blocks and dynamically adapt to their (self-modified) environment by mutation of their own structure. Depending on the surrounding conditions, they show pronounced and opposing selectivity in their formation. Remarkably, the preferentially formed species can be associated with different catalytic properties, which enable multiple pathways for the transition from abiotic matter to functional biomolecules.*

The question of how highly functionalized organic structures, forming the basis of life, emerged from simple abiotic matter is linked to the probability of chemical evolution, which can produce and propagate new properties through alterations in the molecular encoding. But at which molecular level did a process of structural change, selection and continuous formation occur? The structural change and bias in product formation of a catalyst affects the molecular function and provides potentially new selective catalytic reaction pathways, if successful. The emergence of such a system represents an important molecular step towards the biomolecular machinery we know today. The focus of synthetic prebiotic chemistry is to identify possible reaction pathways to the key building blocks of our current biosystem—sugars, amino acids, and RNA/DNA nucleosides.[1–8] Although enormous achievements have been made in this context providing plausible synthetic scenarios for essential biomolecules, these are usually considered separately making the realization of compatible conditions a major challenge. Focusing on the molecular successors of billion years of biological evolution, chemistry that pre- or co-existed and thus played an important role in the pre-Darwinian world remains in the dark.[9–11] Therefore, origin of life research has been increasingly oriented towards a more holistic and cooperative bottom-up approach,[12–14] which considers the relevance and assistance of prebiotic but non-biological molecules.[15–17]

Instead of directed synthesis, the exploration of interconnecting networks that naturally assemble from the heterogeneous prebiotic feedstock could lead to unexpected and unique structures and functions.[18–20] Dynamic combinatorial libraries (DCL), consisting of numerous components that continuously form and interconvert by reversible processes, represent such chemical systems. Typically controlled by their thermodynamic equilibrium, the mixtures can react to changing conditions, resulting in amplification or selective formation of molecules.[21–22] The power of this reversible self-assembly and intermolecular recognition in complex mixtures has been demonstrated *inter alia* for self-replication[23–25] and folding of protein like structures.[26] Besides this natural selection of oligomeric compounds, it is as relevant to adapt such a conceptual strategy also to the beginning of complexity. The inherent characteristics of such dynamic reaction systems, namely variation, bias in formation (selection) and repetitive formation can thus represent a first form towards an evolutionary system on a molecular level. In a prebiotic system, this concept could create structures whose functions defy prediction and represent an important piece of the puzzle to the emergence of chemical complexity.

Here, we show that the prebiotically plausible imidazolidine-4-thione (photoredox) organocatalysts (ITO) are able to dynamically change their structures and able to potentially form an evolutionary system.

We recently identified imidazolidine-4-thiones as a class of prebiotically plausible thio-MacMillan-type organocatalysts, which are easily accessible in high yield (up to 76%) and selectivity in two steps from aldehydes or ketones in the presence of ammonia, cyanide and hydrogen sulphide under mild reaction conditions.[27–28] During this study on their catalytic properties in the α-alkylation of aldehydes, we observed a dynamic behaviour that aroused our interest

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regarding the potential of such organocatalysts for molecular evolution (Figure 1).

As long as aldehydes and ketones are available, ITOs could be continuously formed out of a single reactant pool (Figure 1a). In view of two types of variation mechanisms, a mutation of the catalyst's core structure can be imagined: If the pool of available building blocks is extended, either by external feeding (e.g. impact or catalytic formation) or by catalytic modification of the ITO itself, a new 2nd generation of catalysts can be formed (Figure 1b). Structural modification can also result from dynamic exchange of the carbonyl moiety in ring position 2. By incorporating new building blocks from their environment, the ITOs can develop and adapt to changing conditions without going through the entire formation process (Figure 1b′). Different ambient conditions can then favour certain catalysts, which leads to a bias in the formation, their stability or function (Figure 1c).

Here, we experimentally show that ITOs can, indeed, realize evolution on a molecular level consisting of repetitive formation, structural mutation by different ways of variation, and bias in formation of certain molecular structures.

To form the ITOs **3**, we initially synthesized α-aminonitriles **2** by a Strecker type synthesis and subsequently added the second carbonyl moiety **1** together with hydrogen sulphide and ammonia. To further adapt these reactions to a realistic prebiotic scenario, we realized the formation of catalysts as a one-pot transformation (Scheme 1a).

The respective catalyst **3aa** was formed by stirring the carbonyl component propionaldehyde **1a** in aqueous ammonia in the presence of KCN and H_2S. Instead of a complex mixture that would be expected considering the number of components, the desired catalyst was formed in high selectivity and only intermediates and a few by-products were detected (Scheme 1b) Changing the reaction medium to pure water, concomitant with the addition of NH_4Cl, or varying the ratio of reactants did not affect product formation, proving its robustness to changes in the chemical environment (isolated yield: 6 % in NH_3 (aq), 19 % in H_2O). Only the ratio and nature of by-products differed slightly over time (see Supporting Information Figures 1–4).

To investigate the process of formation, we examined the reaction mixtures in defined time periods using high

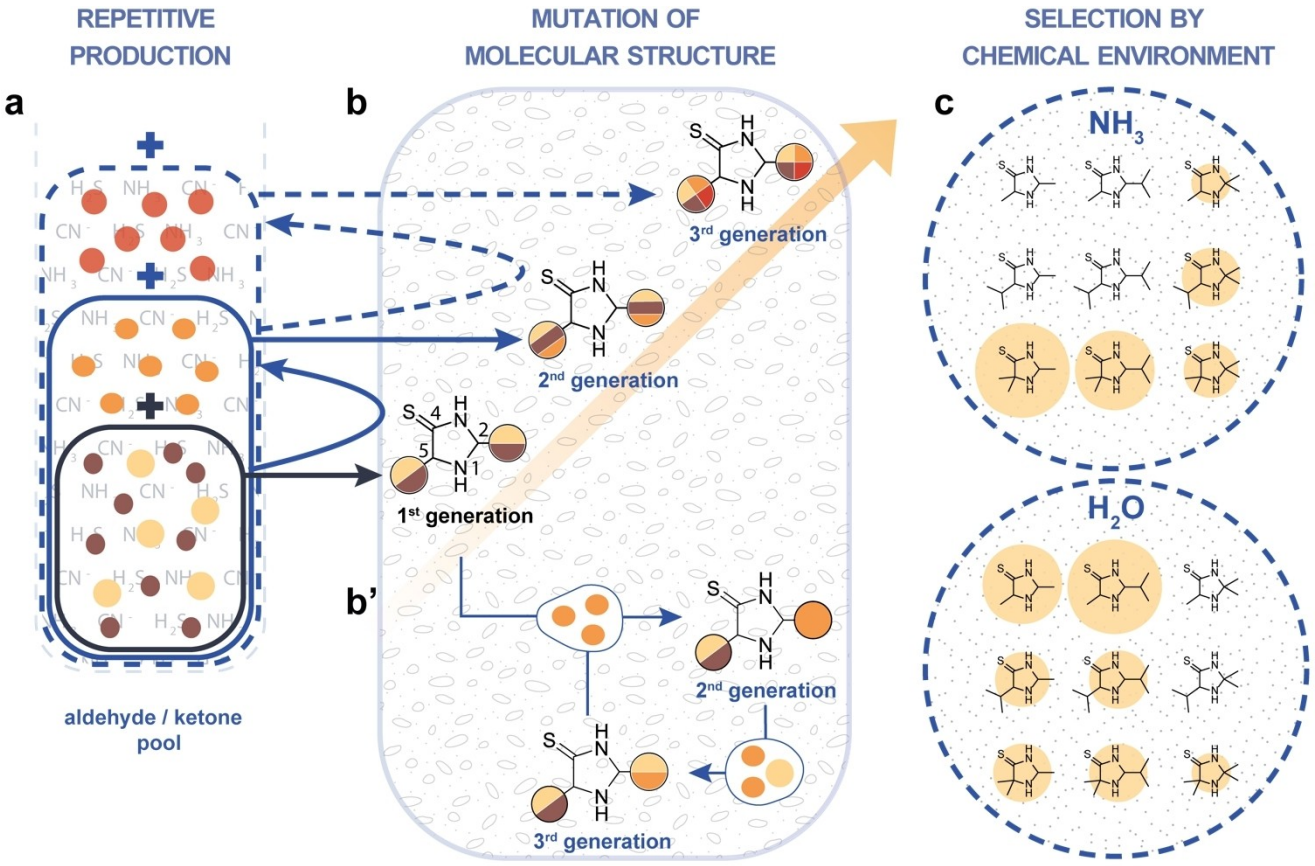
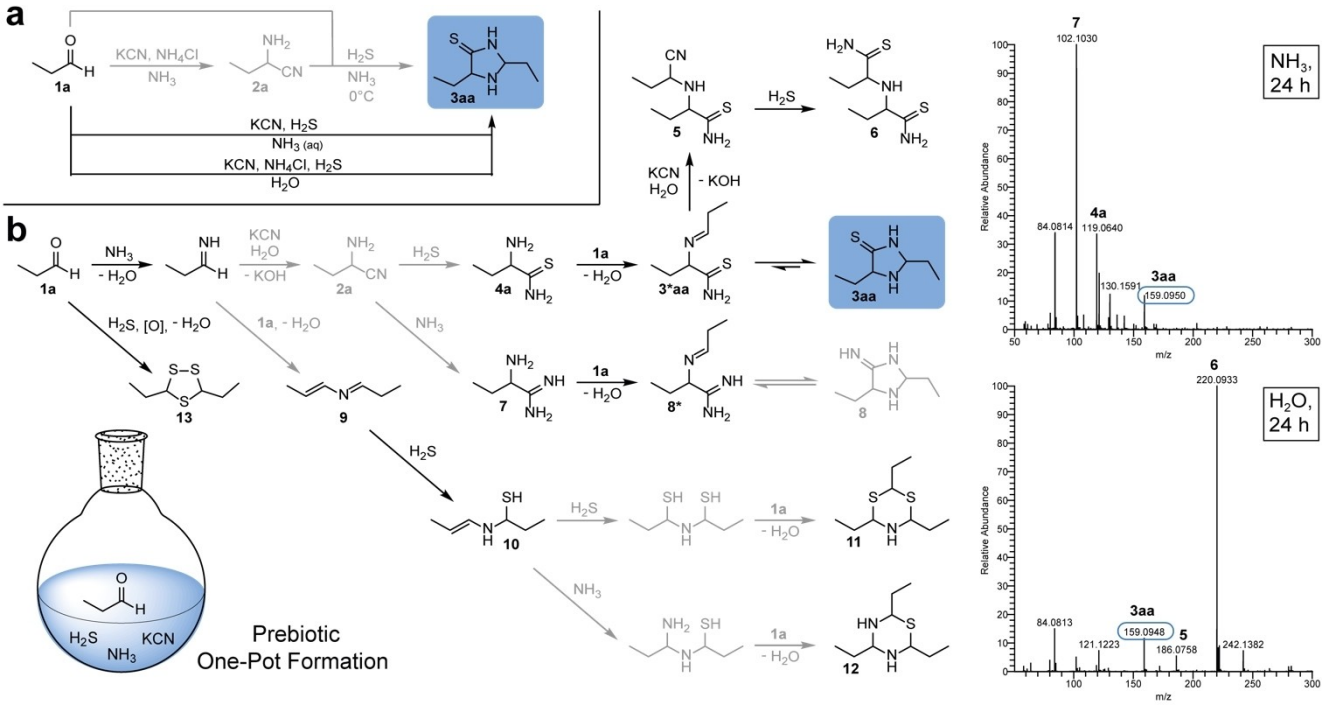


Figure 1. Molecular evolution of the prebiotically plausible imidazolidine-4-thione organocatalysts (ITO). a) Pool of aldehyde/ ketone building blocks (coloured circles) is extended successively as the evolution proceeds (black to bright blue dashed lines). b) Starting from the initial feedstock molecules, a 1st generation of organocatalysts is formed (black arrow), which catalyses the modification of its own starting material (blue curved arrow). Modified higher-generation catalysts are formed from the extended starting material available (blue arrow), which in turn could enable further functionalization processes (dashed blue curved line). b′) ITOs are able to dynamically and selectively exchange their substituent at ring position 2 in water to enable ongoing structural mutation even without the presence of all reagents. c) The catalyst formation is influenced by the reaction medium. Preferred formation is indicated by enlarged circles. Solid reaction arrows refer to the pathways leading to a 2nd generation organocatalysts, dashed lines represent consecutive molecular mutations to the next generation.

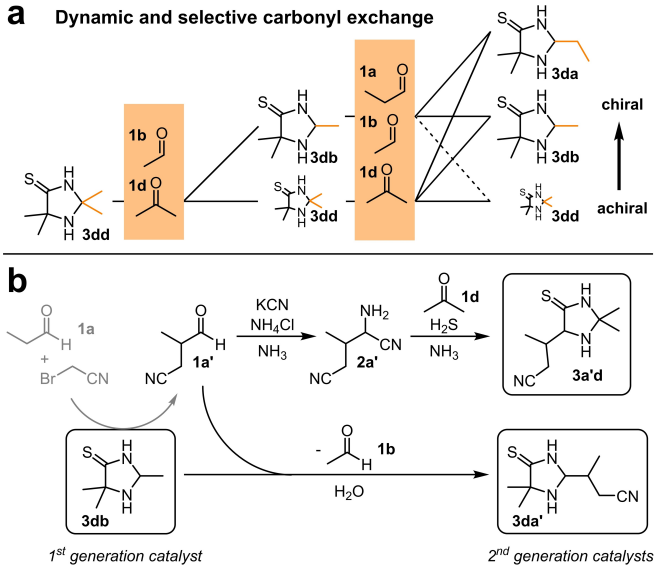


Scheme 1. Overview of the one-pot formation of ITOs and occurring intermediates and side-products. a) Comparison between the two-step procedure (grey) towards ITOs and the respective one-pot approach in concentrated aqueous ammonia and water (black). b) Reaction network of intermediates and side-products formed under different conditions from prebiotic building blocks towards ITOs. The structures coloured in black were identified by HR-MS (right) or NMR spectroscopy, whereas structures coloured in grey are likely intermediates or tautomers. The desired catalyst **3aa** is highlighted in blue. Peaks at *m/z* 84.0813 and 121.1223 were also detected in the blank measurements and thus not part of the samples.

resolution mass spectrometry (HR-MS). The six-membered sulphur compounds dithiazinane **11** and thiadiazinane **12**, known in food chemistry as odours or flavours formed due to degradation of sulphur-containing amino acids,[29–30] are mainly present in the beginning of the reaction and only remain if an excess of aldehyde is used (Supporting Information Figures 5 and 6). The ratio between ITO **3aa** and the nitrogen analogue imidazolidine-4-imine **8** can be changed by varying the reaction medium. Reducing the amount of ammonia leads to a lower content of imidazolidine-4-imine **8** and vice versa. The aminodithioamide **6** is formed from the iminothioamide **3*aa** that is in tautomeric equilibrium with the catalyst **3aa** and primarily occurs in water. After several days, this side-product precipitates independent of the reaction conditions applied. Considering the highly plausible existence of all the simple reactants on an early Earth as well as the robustness towards changing conditions, the continuous self-assembly of ITOs is highly likely.

The formation of imidazolidine-4-thiones **3** is highly robust and a broad variety of aldehydes and ketones is tolerated as building blocks. Therefore, as the prebiotic pool of aldehydes and ketones most likely changed over time or due to local differences, the organocatalysts can adapt and structurally change by building up their skeleton from the carbonyls present. In addition, the extension of the substrate pool and thus catalyst modification is not dependant on external feeding, e.g. meteorite impacts. As we have previously shown, ITOs are able to catalyse the functionalisation of aldehydes.[27] Thereby, they modify their own building blocks into new carbonyl starting material and can initiate self-mutation.

In an early Earth scenario, ubiquitous and especially simultaneous presence of multiple reagents over a long period of time cannot be guaranteed. Thus, the temporary drying up of the cyanide, ammonia or hydrogen sulphide source would risk this process of ongoing evolution of the catalyst. Intriguingly, we observed that the ITO core is dynamic under prebiotic conditions (Scheme 2a, Supporting Information Figure 7). In water, the carbonyl moiety in ring position 2 (orange) exchanged with carbonyl compounds present in solution, leading to their incorporation and release of the initial carbonyl moiety. Besides the newly formed ITOs, no exchange in ring position 5 or other products that might indicate further decomposition were detected. In conclusion, a mechanism can be proposed in which the heterocycle is hydrolysed to give α-aminothioamide **4**, which immediately condensates with another carbonyl compound followed by in-situ cyclisation. The irreversible hydrolysis to α-aminothioamides is known when treating ITOs with strongly acidic reagents[31] and some examples of a following carbonyl exchange were observed under acidic and refluxing conditions in toluene.[32] Here, the amount of exchange corresponded to the existing ratio of carbonyl compounds (see Supporting Information Figure 10) with an important exception: acetone **1d** was released but



Scheme 2. Structural mutation of ITOs (3). a) Dynamic exchange of carbonyl moieties at ring position 2 in water with carbonyl compounds present in solution. Over time, the achiral catalyst **3dd** is depleted by selective removal of acetone **1d**. b) Exemplary formation of 2nd generation catalysts. A functionalized aldehyde **1a′** is incorporated in ring position 5 via aminonitrile **2a′** and successive ring formation (top). The functionalized aldehyde **1a′** is incorporated into the catalyst at position 2 by dynamic exchange starting from a 1st generation catalyst **3db** (bottom). The bromoacetonitrile employed could result from radical recombination of interstellar or atmospheric bromine and acetonitrile.

not incorporated into the catalyst structure and thus selectively removed as the respective side chain of the ITO (Scheme 2a, Supporting Information Figure 8). Consequently, in water, acetone **1d** is exchanged with an aldehyde over time, which not only provides a process of structural mutation but also a mode of selective formation of a certain catalyst. Here, aqueous reaction conditions between pH 7.0 and 10.0 enabled the dynamic interconversion of different catalysts but excluded the irreversible degradation by hydrolysis, observed under strongly acidic conditions. Above pH 10.0, carbonyl exchange was observed neither by NMR spectroscopy nor mass spectrometry.

To exemplarily verify both structural mutation processes also for (self-) functionalized carbonyl compounds, we incorporated **1a′**, the product of our reported prebiotically plausible α-alkylation,[27] into the catalyst structure (Scheme 2b). Despite the cyano group, α-aminonitrile **2a′** was formed and transformed into ITO **3a′d**, a 2nd generation catalyst. Also, mutated 2nd generation catalyst **3da′** was successfully formed by dissolving ITO **3db** with the cyanomethylated propionaldehyde **1a′** in water, continuously yielding 2nd generation catalyst **3da′** over time (2 % in 12 h, see Supporting Information). It can be envisioned that recursive cycles can lead to a modulation of the exchange steering the reaction down different trajectories, as recently shown by Cronin and co-workers.[33] In addition to this capacity for self-modification, the dynamic carbonyl exchange provides a first form of self-optimization. By selective exchange of acetone **1d** with an aldehyde in ring position 2, the ITO automatically creates a chiral centre over time, which guarantees the chirality of the catalyst regardless of its remaining structure. This feature is of particular interest in view of their shown ability to transfer chirality during the catalysis.[27] In combination with their enantiopure crystallization (conglomerate), the ITO organocatalysts could be a powerful tool to propagate chirality towards our homochiral life.

The selective release of acetone from the ITO structure in water was contrary to our initial observation of preferred acetone incorporation in the two-step catalyst synthesis in concentrated aqueous ammonia.[27] To further investigate this potential mechanism of selectivity and control thereof, we studied the ratio of ITOs formed in one-pot reactions of acetaldehyde **1b**, isobutyraldehyde **1c**, and acetone **1d** over time in water and aqueous ammonia solution. Intriguingly, these reaction media had a strong and opposing influence on the selectivity of catalyst formation (Figure 2).

In concentrated aqueous ammonia, the product distribution resembled the results of the two-step procedure, with a selective incorporation of acetone **1d** for both carbonyl moieties (Figure 2, Supporting Information Figures 13 and 14). As secondary aminonitrile (ring position 5), the combination with the smallest acetaldehyde **1a** was preferred, and if it was inserted in ring position 2, it mainly reacted with the largest aminonitrile of isobutyraldehyde **1d**. Counterintuitively, the dominant presence of acetone can be explained by the higher reactivity of aldehydes. Due to the basic conditions and the presence of ammonia, these aldehydes react in side-reactions, e.g. yielding the previously mentioned dithiazinanes **11** and thiadiazinanes **12** (Scheme 1b), which are not formed from acetone. The by far most pronounced catalyst was **3db** with a selectivity of over 50 % after 24 h. As **3db** also represents the most active chiral catalyst in our studied α-alkylation system, a connection between preferred formation and activity is shown.[27]

Whereas a strong bias in the formation of single species was observed in aqueous ammonia solution, water led to a more uniform distribution over time, except for a negligible amount of acetone incorporated in ring position 2. Directly at the beginning of the reaction, the ITO mixture resembled the expected kinetic distribution. The carbonyl moiety in ring position 2 was chosen based on its reactivity and steric hindering of the resulting imine, leading to a decreasing selectivity going from acetaldehyde **1b** to isobutyraldehyde **1c** to acetone **1d**. The reverse order was obtained for ring position 5. Instead of resulting from a preferred aminonitrile **2** formation (see Supporting Information Figure 15), this ratio can be explained by the following cyclisation rate. In line with the Thorpe–Ingold effect, large substituents (**2c**) and especially quaternary carbons (**2d**) kinetically favour ring formation. Over time, this kinetic composition changed due to the above-mentioned dynamic exchange in water and an increasing number of the sterically more demanding isobutyraldehyde **1c** was incorporated in ring position 2. As observed before, acetone remained in solution and was selectively excluded as the corresponding moiety. For the aldehydes, the equilibrium reflected approximately the ratio

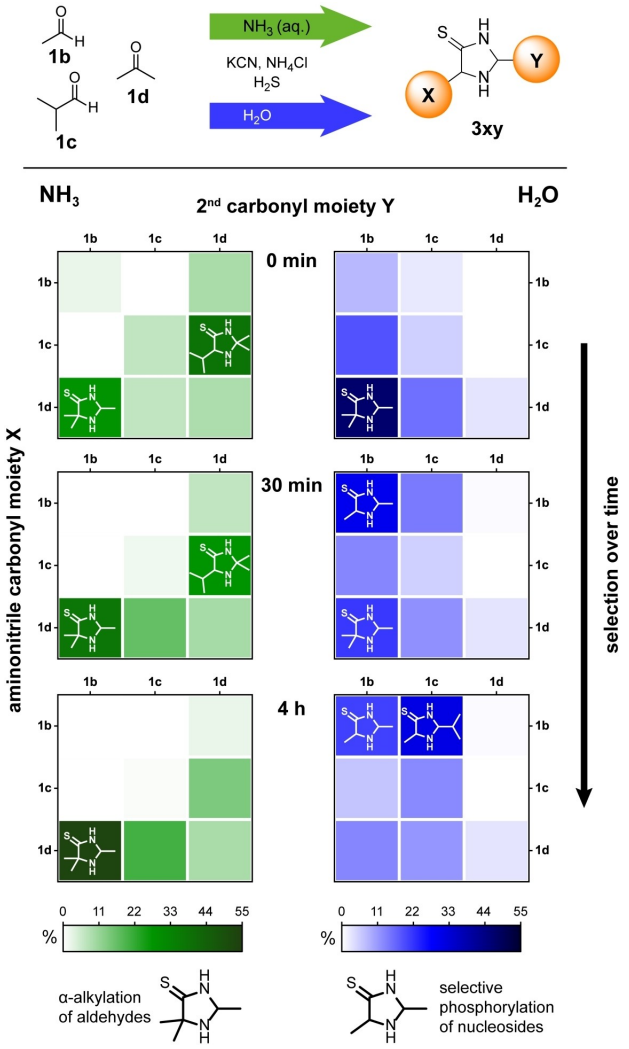


Figure 2. Selectivity of ITO (3) formation. Relative formation of ITOs from one-pot mixtures of acetaldehyde **1b**, isobutyraldehyde **1c** and acetone **1d** in concentrated aqueous ammonia (green) and water (blue). The ratio was determined by HPLC peak integration at 270 nm analysing reaction samples taken after 0 min (top), 30 min (middle) and 4 h (bottom). The colour intensity indicates the relative amount of formation and dominating structures are included into the plots. The formed structures at the bottom represent highly active catalysts for the transformations shown.

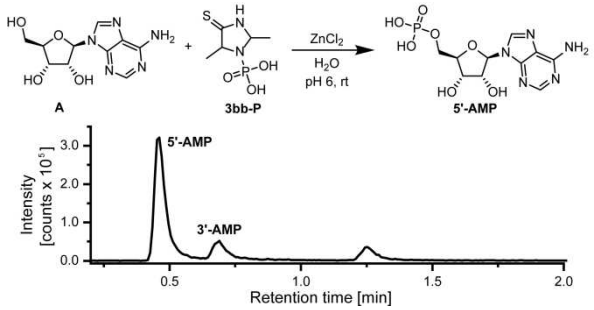


Figure 3. Selective nucleoside phosphorylation with ITO 3bb. The reaction of adenosine **A** with preformed **3bb-P** leads to phosphorylation of **A**. The products were analysed with UHPLC-QTOF MS (mass-filter: *m/z* 346.0541–346.0575) and clearly identified by comparison with reference compounds.

in solution, indicating only a small energetic influence of the side chain on the resulting catalyst structure.

With this opposing formation of ITO species in water, also catalysts formed only from aldehydes can be accessed. This is of high relevance, because preliminary investigations in our group indicate that these ITOs with lower steric hindrance (**3bb**) can function as catalyst or activating reagent in the phosphorylation of nucleosides, similar to the imidazole activated nucleotide strategy to achieve a non-enzymatic template copying (Figure 3).[34–36]

Considering a possible switch between the surrounding conditions simply by a change in pH or ammonia availability, a mechanism to bias the formation of a certain catalyst controlled by the environment is feasible. In combination with the already revealed differences in reactivity depending on the substitution pattern, the interconversion of catalysts can be associated with a change in function.

Concluding, we presented a class of prebiotically plausible ITO organocatalysts **3** that are capable of molecular evolution by dynamic modification of the catalyst structure. As the catalysts are able to functionalize their own building blocks, ongoing (self-) mutation is not dependant on external impacts. Readily forming out of prebiotic one-pot mixtures, different catalyst species, associated with different catalytic properties, are formed depending on the surrounding conditions. A change in the environment can thus initiate a change in function. With the selective removal of acetone in water, also a first irreversible mutation pathway is shown that provides catalyst chirality and thus optimization. As the catalytic activity of ITOs is certainly not exhausted yet, further transformations and structural mutations will be studied to reveal the influence of higher generations. These non-biological ITOs not only conceptually represent the possibility of natural evolution on the early Earth but might have actually played a role in the pre-Darwinian world, assisting and catalysing the pathways to our homochiral biosystem.

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Conflict of Interest

The authors declare no conflict of interest.

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