How to cite: *Angew. Chem. Int. Ed.* **2023**, *62*, e202307591
doi.org/10.1002/anie.202307591

Prebiotic Chemistry | Hot Paper

Prolinyl Nucleotides Drive Enzyme-Free Genetic Copying of RNA

*Franziska Welsch, Eric Kervio, Peter Tremmel, and Clemens Richert**

Abstract: Proline is one of the proteinogenic amino acids. It is found in all kingdoms of life. It also has remarkable activity as an organocatalyst and is of structural importance in many folded polypeptides. Here, we show that prolinyl nucleotides with a phosphoramidate linkage are active building blocks in enzyme- and ribozyme-free copying of RNA in the presence of monosubstituted imidazoles as organocatalysts. Both dinucleotides and mononucleotides are incorporated at the terminus of RNA primers in aqueous buffer, as instructed by the template sequence, in up to eight consecutive extension steps. Our results show that condensation products of amino acids and ribonucleotides can act like nucleoside triphosphates in media devoid of enzymes or ribozymes. Prolinyl nucleotides are metastable building blocks, readily activated by catalysts, helping to explain why the combination of α-amino acids and nucleic acids was selected in molecular evolution.

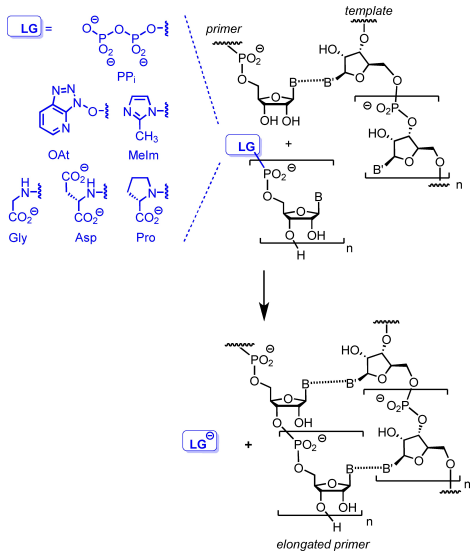
Introduction

Life relies on activated phosphates as metastable building blocks for energy-consuming processes.[1] Activated phosphates, such as nucleoside triphosphates, have functions ranging from energy currency of the cell and building blocks for cofactors to phosphorylating reagents and monomers for transcription and replication.[2] However, nucleoside triphosphates are made in enzymatically catalyzed processes, up to the ATPases of the respiratory chain, i.e. intricate molecular machines that could not have formed spontaneously from small building blocks.[3] Instead, evolution must have relied on simple processes and easy-to-form versions of activated nucleotides in the early stages, leading up to the first protocell, but this is not easy to extrapolate from biochemistry.[4] What these processes were and what mode of activation they involved is currently unclear.[5–7]

[*] M.Sc. F. Welsch, Dr. E. Kervio, Dr. P. Tremmel, Prof. C. Richert
Institute of Organic Chemistry, University of Stuttgart
70569 Stuttgart (Germany)
E-mail: lehrstuhl-2@oc.uni-stuttgart.de

The replication of genetic information is one of the processes that are pivotal for life and that require activated nucleoside phosphates. Nucleoside triphosphates are the monomers used by the DNA-dependent RNA polymerases of extant biology, as well as the RNA-dependent RNA polymerases of RNA viruses.[8] What drove the earliest, most probably enzyme-free replication processes is unclear, and triphosphates are unlikely candidates, as they are too unreactive in the absence of enzymes. Experimental evidence suggests that nucleotide species with organic leaving groups, which can be activated by Brønsted or Lewis acids, are better suited for the task.[9] Imidazolides, oxyazabenzotriazolides and 1-substituted imidazolium phosphates are the best known organic leaving groups in enzyme-free genetic copying.[10–14] Further, in situ activation with carbodiimides and organocatalysts has been achieved,[15,16] but again, the phosphodiester-forming extension reaction was driven by organic leaving groups not found in extant biology.

One seemingly unlikely candidate for the leaving group of activated NMPs are amino acids. Nature uses amino acids extensively as building blocks for protein biosynthesis, and amino acids are readily identified as components of organic matter on meteorites or the product mixtures of Miller-type experiments,[17,18] but amino acids are not usually thought of as leaving groups in organic synthesis. Two types of linkages between amino acids and the phosphates to be activated are reasonable



Scheme 1. Known forms of genetic copying with activated mono ($n=1$) or dinucleotides ($n=2$), driven by the release of a leaving group (LG).

choices for activation, though: mixed anhydrides and phosphoramidates (Scheme 1). Mixed anhydrides are intermediates of the enzymatic aminoacylation of tRNAs, and can be observed in condensation reactions in organic solvents,[19,20] but are labile outside the confinements of enzymes or organic solvents. In the other hand, phosphoramidates are the spontaneous products of condensation reactions between amino acids and ribonucleoside 5′-monophosphates in aqueous buffer.[21] Their formation can be driven by different chemical fuels, including carbodiimides, carbonyl diimidazole and cyanamide.[21] They are stable enough to persist for months in water in the cold.

In the condensation of amino acids and ribonucleotides, phosphodiesters were found as minor products, produced by a competing reaction channel to peptido RNA formation.[22] There was no indication that they are formed via amino acidyl phosphoramidates. However, elegant work by Herdewijn and co-workers had shown that phosphoramidate-linked amino acidyl nucleotides with aspartic acid residues are accepted as substrates by HIV reverse transcriptase.[23,24] Also, a histidinyl deoxyadenosine monophosphate with a phosphoramidate bond to the α-amino group was shown to be a substrate for the same enzyme,[25] again confirming that amino acids can act as leaving groups in primer extension, at least when a powerful polymerase enzyme catalyzes the reaction.

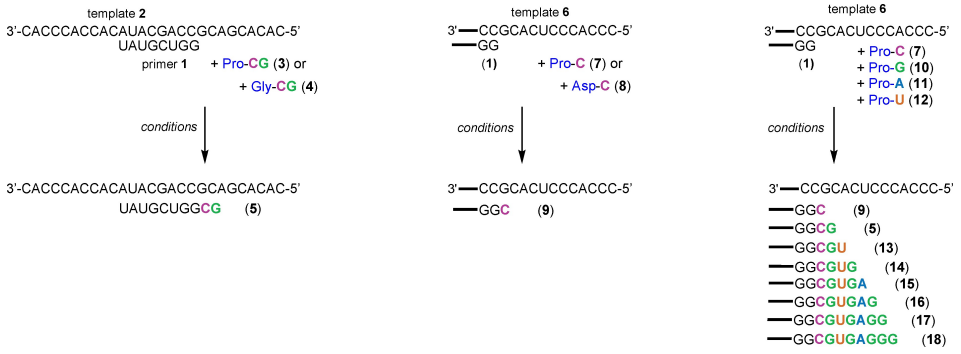
Another piece of evidence motivating the current study came from our systematic evaluation of the properties of different amino acidyl nucleotides.[22] One amino acid stuck out as giving a high percentage of amidates when reacting with ribonucleotides. In what may or may not be a coincidence, this was the very amino acid known as an organocatalyst in stereoselective addition reactions forming carbon-carbon bonds, namely proline.[26] Furthermore, prolinyl nucleotides proved more reactive than the phosphoramidates of other amino acids, or short peptides, when amino acidyl nucleotides relevant as intermediates of prodrug metabolism[27] were allowed to hydrolyze in buffer,[28] again confirming the special properties of this cyclic α-amino acid. Finally, a 5′-prolinyl phosphoramidate of AMP was found to undergo transamidation from the *N*α- to the imidazoyl amidate when reacting with histidine, indicating that transamidation to imidazolides is a valid pathway for such species.[28]

Together, these findings led to the question whether prolinyl nucleotides could be what may loosely be called a "missing link" between protein and RNA reaction networks. To provide one answer to this question, we decided to study the reactivity of prolinyl nucleic acids in genetic copying reactions, building on the recently described RNA copying scheme with dinucleotides.[29] Here we report that prolinyl nucleotides do indeed act as monomers in genetic copying, with both dimers and monomers being incorporated at the terminus of a primer, as directed by RNA templates. Products of up to eight incorporation steps were found in assays performed in dilute aqueous buffer in the cold.

Results and Discussion

Scheme 2 shows the RNA sequences employed in our study. The initial screen for activity of prolinyl nucleotides was performed with the most advanced system for enzyme-free copying, recently found to achieve copying of up to 12 bases in a template.[29] This system employs dinucleotides as building blocks and the strongly pairing bases (G and C) as the only bases in the templating region of the template. First, the extension of primer **1** on template **2** by prolinyl dinucleotide Pro-CG (**3**) was monitored by MALDI-TOF mass spectrometry, using conditions of quantitative detection.[30] Reactions were allowed to proceed in buffered solution at 4 °C. In the absence of an organocatalyst, no detectable conversion was observed with **3** within 21 d (Figure 1A). Upon addition of 1-ethylimidazole, the organocatalyst employed in "condensation buffer",[16] 52 % extended primer was found after 21 d. Optimization of pH, structure and concentration of organocatalyst and buffer then improved the conversion to 65 % after 14 d (Table 1 and Figure 1B).

This level of conversion was obtained at 2 mM concentration of Pro-CG (**3**) with 1-(2-hydroxyethyl)imidazole as catalyst in 10 mM MOPS buffer, pH 7.5. The sequence Pro-GG gave 39 % after the same time span (see Figure S17 in the Supporting Information). No conversion was detected, however, when



Scheme 2. Genetic copying reactions studied. See Scheme 1 for amino acidyl (di)nucleotides and Table 1 and Figure legends for further details.

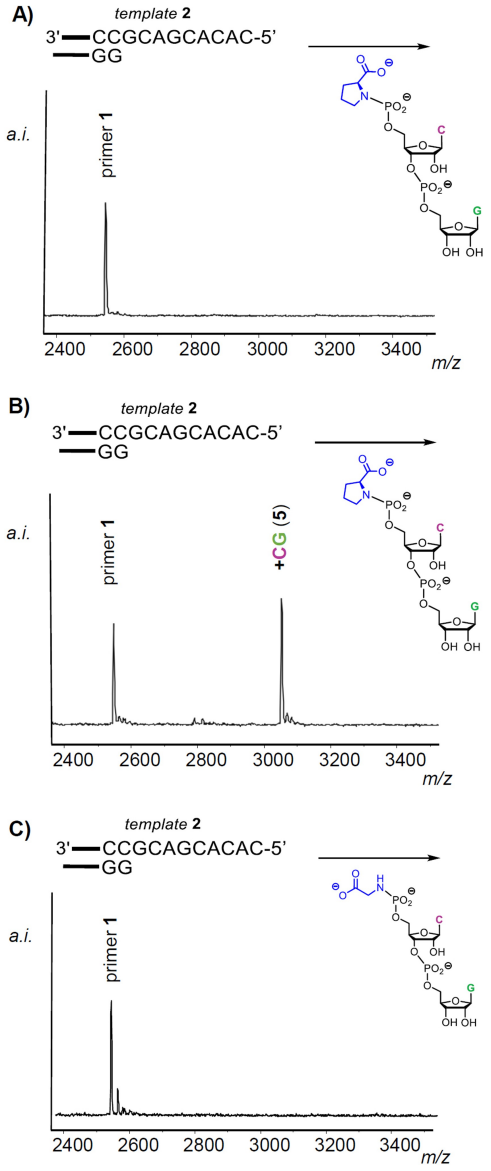


Figure 1. MALDI-TOF mass spectra from primer extension assays with dinucleotide CG after 14 d. A) Pro-CG (**3**); B) Pro-CG (**3**), 150 mM HyIm, 10 mM MOPS; C) 2 mM Gly-CG (**4**), 150 mM HyIm, 10 mM MOPS. Conditions: 2 mM building block, 40 μM primer, 60 μM template, 80 mM $MgCl_2$, pH 7.5, 4 °C.

glycine instead of proline was used as leaving group (Gly-CG, **4**), as shown in Figure 1C.

We then asked what limited the yields of primer extensions with the prolinyl building blocks. For this, a systems chemistry analysis was performed (Figure 2). Rate constants for the activation chemistry, including formation of the organocatalytic intermediate and the competing hydrolysis reaction were obtained using prolinyl cytidine 5′-monophosphate Pro-C (**7**) as model substrate and NMR spectroscopy as monitoring technique. Kinetics of the primer extension and the competing cyclization of the dimer were determined by MALDI-TOF MS. Binding constants for the activated and hydrolyzed forms of Pro-CG (**3**) were estimated based on our

Table 1: Results of the optimization study for extension of primer **1** on template **2** with Pro-CG (**3**).[a]

pH	Organocatalyst[b]	Conversion (%)[c]
7.5	–	<1
6	150 mM EtIm	4
7.5	150 mM EtIm	52
8.9	150 mM EtIm	45
7.5	10 mM EtIm	43
7.5	600 mM EtIm	19
7.5	150 mM HyIm	57
7.5	150 mM 2-AIm	4
7.5	150 mM MeIm	22
7.5	150 mM HyIm[d]	65

[a] Conditions: 40 μM primer, 60 μM template, 2 mM Pro-CG, 80 mM $MgCl_2$, 4 °C, 21 d. [b] EtIm = 1-ethylimidazole, 2-AIm = 2-aminoimidazole, HyIm = 1-(2-hydroxyethyl)imidazole. [c] As determined by quantitative MALDI-TOF MS. [d] Plus 10 mM MOPS, 14 d.

earlier work on this dimer in enzyme-free copying of RNA.[29] Figure 2 shows plots of the kinetics, and Figure 2D shows a flux diagram for key chemical steps, as obtained by integrating the coupled differential equations for the reactions involved.

The rate of the primer extension reaction was found to drop on the same time scale as the hydrolysis of the prolinyl nucleotide was found to occur. Because it is used in excess, most of the activated species suffer hydrolysis during the course of the assay. As hydrolysis sets in, the active building block is converted into a competitive inhibitor that increasingly blocks the extension site at the terminus of the primer, one of the known scenarios leading to incomplete copying.[31] When a simulation was run for a hypothetical reaction system without hydrolysis, a higher yield was predicted (broken line in Figure 2C).

If competitive inhibition was indeed the predominant cause of incomplete conversion, in situ (re−)activation should overcome the tapering off of reactivity in the second phase of our assays. This was indeed found to be the case. Figure 3 shows the kinetics of primer extension when EDC, a water-soluble carbodiimide, was added after 11 d, i.e. at a time point when spent building blocks begin to dominate in the reaction mixture. Upon addition of EDC, primer extension resumed, leading to an overall conversion of 85 % after 21 d (Figure 3). This result also indicates that the amino acid-based activation chemistry is compatible with in situ re-activation, and that cycles of activation/hydrolysis/re-activation may occur. This is important, as it suggests a pathway for sustained "metabolic activity" of such a system with repeated input of a chemical "fuel",[32] a feature that is favorable for molecular evolution.[33] Control experiments with free proline confirmed that the amino acid does not suppress primer extension, and that in situ activation to the prolinyl nucleotide does occur to a significant extent (see Chapter 8, SI).

Next, we performed exploratory experiments on the scope of the amino acidyl activation chemistry. This included preparing prolinyl phosphoramidates of all four

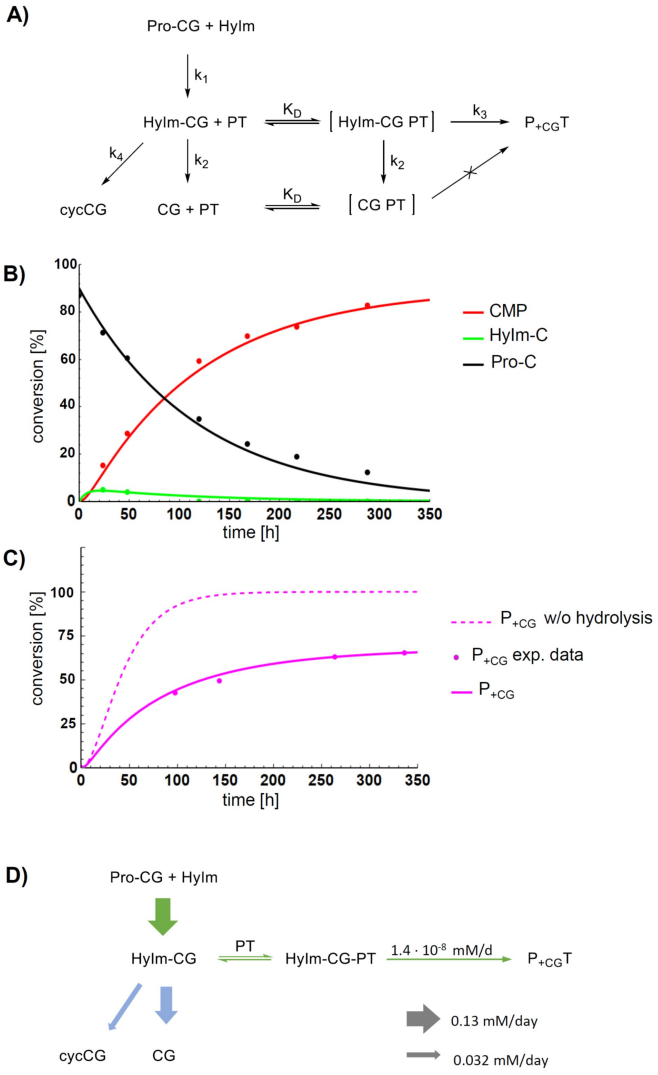


Figure 2. Systems chemistry analysis of activation and extension reaction. A) Reactions of the model; B) kinetics of activation and hydrolysis of Pro-C (5 mM) in 80 mM $MgCl_2$, 150 mM HyIm, 10 mM MOPS buffer, at pH 7.5, 4 °C; C) kinetics of primer extension under the same buffer conditions with 40 μM primer, 60 μM template, 2 mM Pro-CG and hypothetical kinetics without inhibition by hydrolysis product CG (broken line); D) flux diagram for relevant steps in activation and primer extension, as obtained by integrating the coupled differential equations of the model. Gray arrows show the correlation of arrow width with flux, except for the extension reaction, for which the rate constant is plotted on the arrow. See Chapter 7 of the Supporting Information for further details.

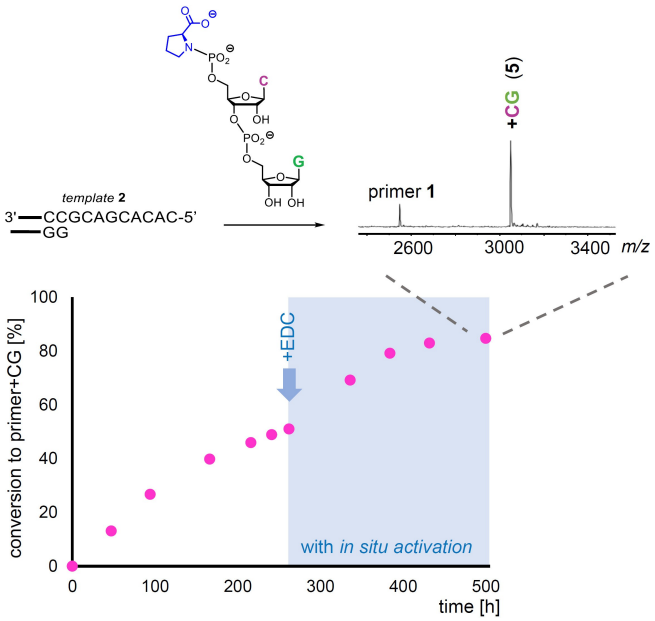


Figure 3. Primer extension with in situ activation after the initial phase, driven by preactivation to the prolinyl dimer (**3**) had levelled off. The addition of carbodiimide EDC is indicated by the blue arrow and the light-blue shaded area of the kinetics shown in the lower part. The MALDI-TOF mass spectrum shown above the last data point is for the extension with dimer CG after 21 d total reaction time. Conditions: 2 mM dimer, 40 μM primer, 60 μM template, 80 mM $MgCl_2$, and 150 mM HyIm, 10 mM MOPS buffer, 400 mM EDC after 11 d, at pH 7.5 and 4 °C.

canonical ribonucleotides (AMP, CMP, GMP and UMP). Further, aspartic acid was tested as alternative leaving group on the monomer level. Finally, we performed assays with another organocatalyst, namely 2-aminoimidazole, which has been tested extensively as leaving group,[34] hoping that transamidation equilibria would produce a productive intermediate and thus induce extension. Figures 4 and 5 show results from the corresponding assays. Additional results, including primer extensions on other templates and with hydroxyethylimidazole as organocatalyst, are shown in Figure S19 of the Supporting Information.

The data obtained show that Asp-NMPs have modest reactivity in our system, giving detectable extension after 12 d reaction time, with about just one-tenth of the conversion found for the prolinyl counterpart. After 40 d, 14 % conversion was found with Asp-C versus 57 % with Pro-C (Figure 4). However, 2-aminoimidazole, an organocatalyst for which little conversion had been observed in our initial screen with dimer Pro-CG (Table 1), gave surprisingly strong signals for multistep extension (Figure 5) when allowed to react for several months. Up to five base-specific incorporations were detectable after two months, including the incorporation of the weakly pairing bases A and U. After six months, product peaks for up to eight-fold extended primer were discernible, with the correct mass as the dominating peak for each extension step.

On a chemical level, our data suggest that seemingly unreactive phosphoramidates of α-amino acids[35] can act as building blocks in RNA synthesis. Their activity depends strongly on the structure of the amino acid and requires organocatalysis. Organocatalysts other than imidazoles have previously been shown to accelerate reactions of activated nucleotides, including pyridine[36] and *N*-methyladenines.[32] For both proline and aspartic acid, the carboxylate may induce an anchimeric effect, either by forming a mixed anhydride with the phosphate

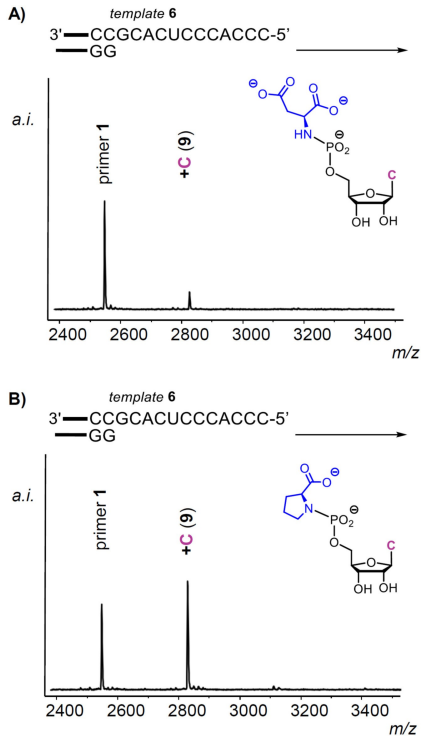


Figure 4. Representative MALDI-TOF mass spectra of primer extension assays with amino acidyl mononucleotides. A) with 20 mM Asp-C (**8**); B) with 20 mM Pro-C (**7**). Conditions: 40 μM primer, 60 μM template, 80 mM $MgCl_2$, and 150 mM HyIm, 10 mM MOPS, pH 7.5. 4 °C, 40 d.

or by acting as general acid in the activation reaction.[22] Having the carboxylate positioned at the correct angle through the preorganizing effect of the pyrrolidine ring of proline appears to favors such effects. For aspartic acid, with its less rigid structure, but an additional carboxylate in the side chain, a similar, but weaker effect is observed. Transamidation equilibria to the organocatalytic imidazolium phosphate (HyIm) or imidazolide (2-AIm) then probably produce the kinetically relevant activated species for phosphodiester formation.

The transamidation appears to be slow, explaining why long reaction times are required for successful primer extension. Further, there appear to be different optima for mono- and dinucleotides, probably because cyclization and formation of imidazolium bisphosphates as competing reactions occur on different levels for different backbone lengths. Finally, the transamidation appears to give amidates with a more favorable ratio between chain extension and hydrolysis, a phenomenon reminiscent of the what has been called "organocapture" in pathways leading to peptido RNAs.[32]

On what may be called a biochemical or systems chemistry level, our data suggest that prolinyl nucleotides and dinucleotides can act as a reservoir for active building blocks for RNA synthesis, metastable in aqueous medium, but readily activated by small organic heterocycles that act as catalysts. This is an important additional role, as amino acidyl nucleotides are already known to accelerate peptide chain growth over the background reaction[21] and to act as primers for single-nucleotide translation.[37] If a reservoir that can be replenished during times of activation, when condensation reactions occur, can later be drawn upon under conditions that liberate imidazole organocatalysts, a more sustainable scenario for molecular evolution emerges than those that rely on "direct use" activated nucleotides only. That re-activation is compatible with our system, as shown in Figure 3 and Chapter 8 of the SI, bodes well for such "multiple cycle" scenarios.

Without kinetically stable, but readily activated building blocks reminiscent of NTPs, a primitive living system cannot decouple activation from genetic copying, a trait that is found in all biological cells. Without cyclic processes, it cannot reuse valuable building blocks. With a xenobiotic leaving group rather than an amino acid, it needs an additional source of starting materials. So, taken together, there are several advantages for systems with prolinyl nucleotides, even when one does not take the ability to drive multi-step extensions into account (Figure 5).

Independent of more hypothetical scenarios, our results underline how two fundamental classes of biomo-

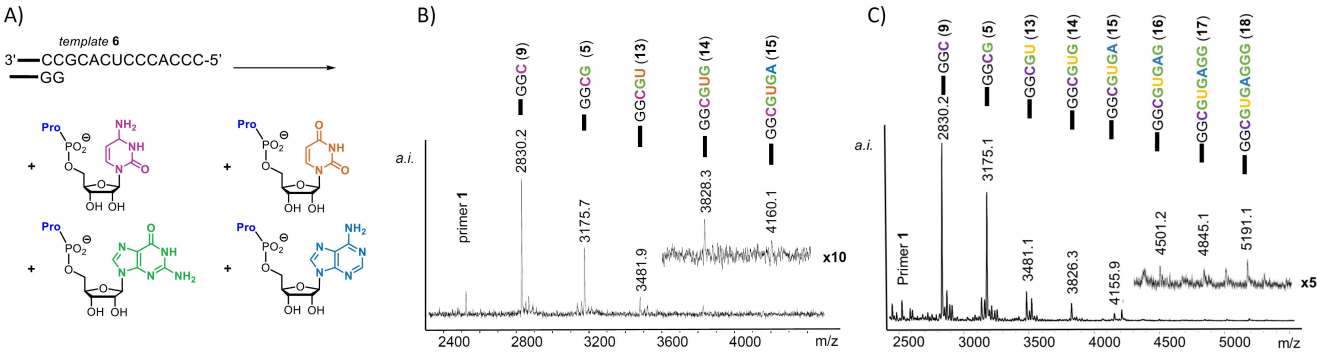


Figure 5. Assay system and MALDI-TOF MS spectra of desalted samples from primer extension with all four amino acidyl mononucleotides. A) Templating region and building blocks, B) mass spectrum after 2 months, C) mass spectrum after 6 months. Conditions: 40 μM primer, 60 μM template, Pro-C (**7**), Pro-G (**10**), Pro-A (**11**) and Pro-U (**12**) 20 mM each, and 2-aminoimidazole (150 mM) at pH 7.5 in 80 mM $MgCl_2$ at 4 °C; desalting used ZipTips and elution with CH_3CN.

lecules, amino acids and ribonucleotides, can act synergistically to set up a reaction system suitable for molecular evolution.[38] Our data thus provides an important piece in the puzzle of how genetically and metabolically productive reaction networks may have formed under prebiotic conditions. It also helps with a systems chemistry understanding of why amino acids and nucleotides were chosen as privileged biomolecules, found in all kingdoms of life, and why proline is found in the collection of molecules of life.

Conclusion

These results show that proline and, to a lesser extent, aspartic acid can act as leaving groups for ribonucleotides. Substitution of the leaving group in the phosphodiester-forming reaction drives the extension of RNA primers, as directed by a template. Further, our results show how small heterocycles acting as organocatalysts can enable reactions that are not observed in their absence, playing the role of enzymes in present-day biology in a more primitive way. Our data also shows that some reactions occurring in aqueous solution happen on time scales that are not typically studied in experimental work on prebiotic chemistry, producing seemingly labile RNA chains in exceedingly slow, but steady and productive processes. It seems likely that the typical approach of performing assays on the timescale of days or weeks has affected what activation chemistry has been considered effective thus far, and thus our view on which putative prebiotic processes are considered plausible and which ones are not. Our results confirm that the yield of reactions forming RNA is determined predominantly by the ratio of the rates for the desired reaction and all side reactions, not necessarily the absolute value of the rate constants. Further experimental work on the fascinating interplay of amino acid and nucleotide chemistry described here and the surprisingly slow transformations is under way in our laboratories.

Acknowledgements

The authors thank Dr. D. Pfeffer and D. Jovanovic for performing exploratory experiments during the early phase of this project, Dr. B. Claasen for discussions and help with data, and D. Göhringer for technical assistance. Supported by DFG, German Research Foundation, Project-ID 364653263—CRC 235 and Project-ID 513030456-INST 41/1175-1 FUGG. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Amino Acids · Genetic Copying · Prebiotic Chemistry · RNA · Template Effect

[1] F. H. Westheimer, *Science* **1987**, *235*, 1173–1178.
[2] J. R. Knowles, *Annu. Rev. Biochem.* **1980**, *49*, 877–919.
[3] J. P. Abrahams, A. G. Leslie, R. Lutter, J. A. Walker, *Nature* **1994**, *370*, 621–628.
[4] S. A. Benner, A. D. Ellington, A. Tauer, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 7054–7058.
[5] J. D. Ibanez, A. P. Kimball, J. Oro, *Science* **1971**, *173*, 444–446.
[6] A. C. Fahrenbach, C. Giurgiu, C. P. Tam, L. Li, Y. Hongo, M. Aono, J. W. Szostak, *J. Am. Chem. Soc.* **2017**, *139*, 8780–8783.
[7] A. Mariani, D. A. Russell, T. Javelle, J. D. Sutherland, *J. Am. Chem. Soc.* **2018**, *140*, 8657–8661.
[8] Y. Gao, L. Yan, Y. Huang, F. Liu, Y. Zhao, L. Cao, T. Wang, Q. Sun, Z. Ming, L. Zhang, J. Ge, L. Zheng, Y. Zhang, H. Wang, Y. Zhu, C. Zhu, T. Hu, T. Hua, B. Zhang, X. Yang, J. Li, H. Yang, Z. Liu, W. Xu, L. W. Guddat, Q. Wang, Z. Lou, Z. Rao, *Science* **2020**, *368*, 779–782.
[9] I. A. Kozlov, L. E. Orgel, *Mol. Biol.* **2000**, *34*, 781–789.
[10] R. Lohrmann, L. E. Orgel, *Nature* **1976**, *261*, 342–344.
[11] M. Kurz, K. Göbel, C. Hartel, M. W. Göbel, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 842–845.
[12] S. R. Vogel, C. Deck, C. Richert, *Chem. Commun.* **2005**, 4922–4924.
[13] J. W. Szostak, *Angew. Chem. Int. Ed.* **2017**, *56*, 11037–11043.
[14] S. J. Zhang, D. Duzdevich, D. Dinga, J. W. Szostak, *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2116429119.
[15] D. Sievers, G. von Kiedrowski, *Nature* **1994**, *369*, 221–224.
[16] M. Jauker, H. Griesser, C. Richert, *Angew. Chem. Int. Ed.* **2015**, *54*, 14559–14563.
[17] A. S. Burton, J. C. Stern, J. E. Elsila, D. P. Glavin, J. P. Dworkin, *Chem. Soc. Rev.* **2012**, *41*, 5459–5472.
[18] A. P. Johnson, H. J. Cleaves, J. P. Dworkin, D. P. Glavin, A. Lazcano, J. L. Bada, *Science* **2008**, *322*, 404.
[19] P. Berg, *J. Biol. Chem.* **1958**, *233*, 608–611.
[20] T. Moriguchi, T. Yanagi, T. Wada, M. Sekine, *J. Chem. Soc.-Perkin Trans.* **1999**, 1859–1865.
[21] H. Griesser, P. Tremmel, E. Kervio, C. Pfeffer, U. E. Steiner, C. Richert, *Angew. Chem. Int. Ed.* **2017**, *56*, 1219–1223.
[22] H. Griesser, M. Bechthold, P. Tremmel, E. Kervio, C. Richert, *Angew. Chem. Int. Ed.* **2017**, *56*, 1224–1228.
[23] O. Adelfinskaya, P. Herdewijn, *Angew. Chem. Int. Ed.* **2007**, *46*, 4356–4358.
[24] M. Maiti, S. Michielssens, N. Dyubankova, M. Maiti, E. Lescrinier, A. Ceulemans, P. Herdewijn, *Chem. Eur. J.* **2012**, *18*, 857–868.
[25] A. Giraut, P. Herdewijn, *ChemBioChem* **2010**, *11*, 1399–1403.
[26] B. List, *Synlett* **2001**, *26*, 1675–1686.
[27] J. Balzarini, A. Karlsson, S. Aquaro, C.-F. Perno, D. Cahard, L. Naesens, E. De Clerq, C. McGuigan, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7295–7299.
[28] D. Jovanovic, P. Tremmel, P. S. Pallan, M. Egli, C. Richert, *Angew. Chem. Int. Ed.* **2020**, *59*, 20154–20160.
[29] G. Leveau, D. Pfeffer, B. Altaner, E. Kervio, F. Welsch, U. Gerland, C. Richert, *Angew. Chem. Int. Ed.* **2022**, *61*, e202203067.
[30] D. Sarracino, C. Richert, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2543–2548.

[31] E. Kervio, B. Claasen, U. E. Steiner, C. Richert, *Nucleic Acids Res.* **2014**, *42*, 7409–7420.
[32] P. Tremmel, H. Griesser, U. E. Steiner, C. Richert, *Angew. Chem. Int. Ed.* **2019**, *58*, 13087–13092.
[33] P. Schwarz, S. Laha, J. Janssen, T. Huss, J. Boekhoven, C. Weber, *Chem. Sci.* **2021**, *12*, 7554–7560.
[34] T. Walton, W. Zhang, L. Li, C. P. Tam, J. Szostak, *Angew. Chem. Int. Ed.* **2019**, *58*, 10812–10819.
[35] Z. Liu, G. Ajram, J. C. Rossi, R. Pascal, *J. Mol. Evol.* **2019**, *87*, 83–92.
[36] M. Röthlingshöfer, E. Kervio, T. Lommel, U. Plutowski, A. Hochgesand, C. Richert, *Angew. Chem. Int. Ed.* **2008**, *47*, 6065–606.
[37] B. Jash, P. Tremmel, D. Jovanovic, C. Richert, *Nat. Chem.* **2021**, *13*, 751–757.
[38] C. De Duve, *Nature* **2005**, *433*, 581–582.

Manuscript received: May 30, 2023
Accepted manuscript online: June 29, 2023
Version of record online: July 11, 2023