leaving group into the second epoxide allows a third dithiane–epoxide coupling to be achieved. The new epoxide **41** which is formed from the doubly alkylated product **40** by intramolecular S_N reaction, is opened by unreacted Brook rearrangement product **39**. Thus a C_{11}-polyol chain (**42**) with five free or protected secondary alcohol functions is formed from five components in a one pot reaction. However, this remarkable efficiency is achieved only if the complete stereochemical information of the polyol chain has already been introduced by the chiral epoxides.

Although the absolute configuration has only been established for a few polyene macrolide antibiotics, the search for new, efficient, and selective strategies for the synthesis of their polyol structures is in full swing. A number of the synthetic procedures presented here will certainly be used in future syntheses of this class of natural products.

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Keywords: aldol reactions · asymmetric syntheses · macrolides · polyenes · polyols

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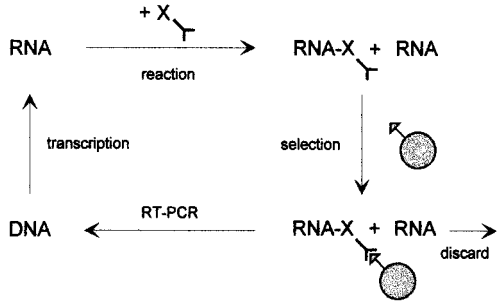
Catalysis of Organic Reactions by RNA

Christian Frauendorf and Andres Jäschke*

It has been known since the early 1980s that ribonucleic acids (RNAs) do not only participate in the flow of genetic information, but may also have catalytic properties.[1] This discovery, which was honored with the Nobel Prize in 1989, stimulated earlier hypotheses about the existence of a prebiotic "RNA world" in which both the storage of genetic information and the control of chemical reactions were carried out by RNA.[2] While the search for other naturally occurring ribozymes has not led to substantial discoveries in recent years, the use of in vitro selection and evolution techniques has provided significant contributions to the exploration of the catalytic potential of RNA.[3]

To isolate catalytically active RNA molecules, catalysis must be coupled with some other event that allows a selection. The most successful method for the selection of RNA catalysts is direct selection in which those members are selected from RNA libraries that can modify themselves in the absence of an external catalyst (Scheme 1). A combinatorial RNA pool is incubated with a substrate X, which contains an anchor group that is not present in unmodified RNA (e.g., a thiol group or biotin). After incubation, those RNAs are isolated by affinity chromatography that have been covalently linked to the anchor group. These RNA molecules are reverse transcribed into DNA and then amplified by polymerase chain reaction (PCR). The double-stranded DNA is subsequently used to generate RNA by transcription, and the next cycle starts with the incubation step. This cycle is repeated until the active RNA molecules dominate the pool.



Scheme 1. General principle of direct selection with a combinatorial RNA library.

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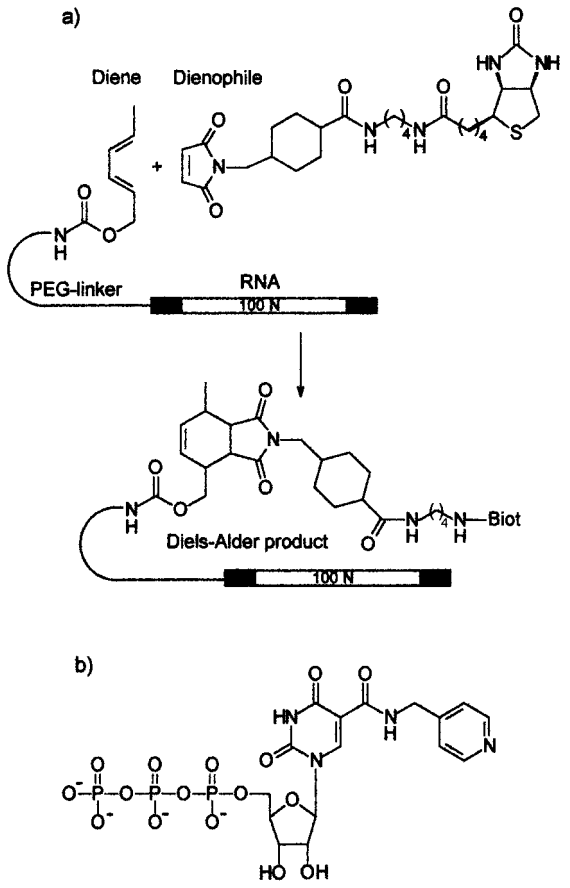
While the exploration of the catalytic potential of RNA initially focussed on reactions at the functional groups present in RNA (acylations, alkylations, phosphorylations of RNA molecules[4]), several studies have recently been published in which the limitation on self-modifying reactions has been overcome by using linker-coupled reactants. Herein we will discuss studies that deal with reactions central to the synthetic chemist—the Diels–Alder reaction, the synthesis of amides and esters, and peptide bond formation.

By using direct selection with linker-coupled reactants, Eaton and co-workers identified catalysts for the formation of carbon–carbon bonds by the Diels–Alder reaction.[5] Despite its minor biochemical significance,[6] this key reaction of organic synthesis was often chosen for innovative approaches in catalysis research, and several laboratories have attempted, but with little success so far, to generate RNA catalysts for Diels–Alder reactions by selecting RNA molecules with high affinity to transition state analogues.[7]

The selection scheme employed by Eaton started with a pool of 10^{14} different RNA molecules; all of them containing a region of 100 completely randomized positions and flanked by two constant sequences (Scheme 2a). Hexadienol was coupled through a carbamate linkage to poly(ethylene glycol) (PEG), average molecular weight 2000, which was enzymatically linked to the pool RNA molecules. The highly flexible PEG linker made it possible for the diene to act like a free species in solution and thus interact with with all parts of a folded RNA molecule. This pool of RNA-linker-diene conjugates was incubated with the dienophile, a biotinylated maleimide, in the presence of various metal ions. RNA-coupled biotinylated cycloaddition products were separated from unmodified RNA by streptavidin (a biotin-binding protein) and enzymatically amplified. This enriched pool was then subjected to further rounds of selection and amplification.



Scheme 2. a) Reaction step in the selection of a Diels–Alderase from a combinatorial RNA library. Biot–OH = biotin. b) 5-[(4-Pyridylmethyl)-carbamoyl]-UTP, which was used instead of uridine triphosphate (UTP) in enzymatic RNA synthesis.

The introduction of additional functionalities into RNA was important for catalysis.[8] Instead of uridine-5′-triphosphate (UTP), the modified analogue 5-[(4-pyridylmethyl)carbamoyl]-UTP (Scheme 2b) was used in enzymatic RNA synthesis, allowing additional interactions like hydrogen bonding, hydrophobic and dipolar interactions, and metal ion coordination.

After 12 rounds of selection and amplification, functionally active molecules were enriched and eight sequence families were identified that were capable of accelerating the reaction up to 800-fold with respect to the uncatalyzed background reaction. The catalytic activity was shown to depend on the presence of the pyridyl modification; as expected, unmodified RNA molecules of the same nucleotide sequence had no activity, since they fold into different three-dimensional structures and lack the additional interaction sites. The most thoroughly characterized catalyst had an absolute requirement for Cu^{2+} ions, and the authors discuss that Diels–Alder reactions are known to be catalyzed by Lewis acids,[9] and RNA may coordinate these copper ions, presumably by the pyridyl moieties.

Eaton and co-workers have also recently described the catalysis of another important reaction by RNA that uses a similar selection scheme.[10] This time, a primary aliphatic amine was coupled to the end of the PEG linker, and the resulting RNA pool was allowed to react with the asymmetric anhydride of biotin and adenosine monophosphate (AMP, Scheme 3a). Again, modified nucleotides were used; the 5-position of uracil was derivatized with imidazole, which was expected to help improve properties in general acid/base catalysis and metal ion coordination (Scheme 3b). To select the most active RNA species, the selection pressure was gradually increased from round to round by reducing the concentration of reactants and the reaction time. After 16 rounds, the kinetic characterization of individual selected species revealed rate accelerations of up to 110000-fold. Again, the RNA species chosen for detailed characterization showed a strong dependence on the presence of the modified nucleotides and on the concentration of different metal ions. Remarkably, the adenosine moiety present in the free reactant biotin-AMP was not required for substrate recognition. The kinetic constants measured with the analogues uridine monophosphate-biotin and ribose-5-phosphate-biotin were almost identical to that measured with biotin-AMP.

These two studies demonstrate the successful application of direct selection to synthetically relevant reactions. A number of interesting questions raised by this work indicate that more detailed studies are still required. For instance: Can the selected RNAs act as true catalysts with two free reactants

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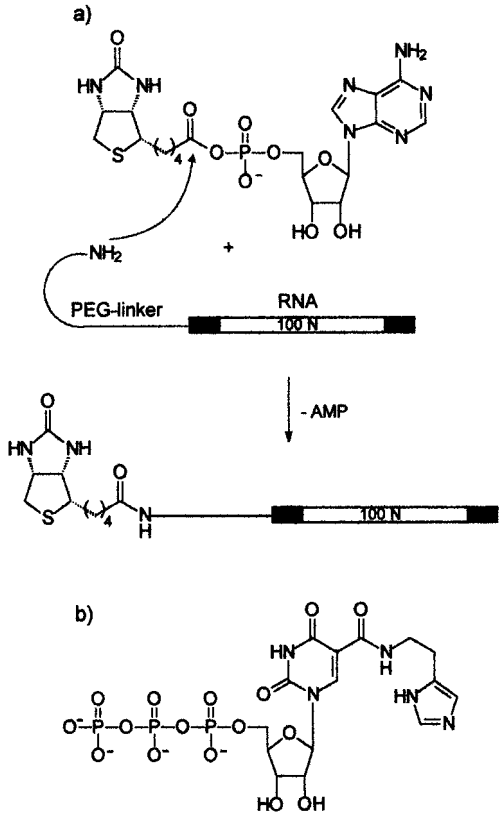
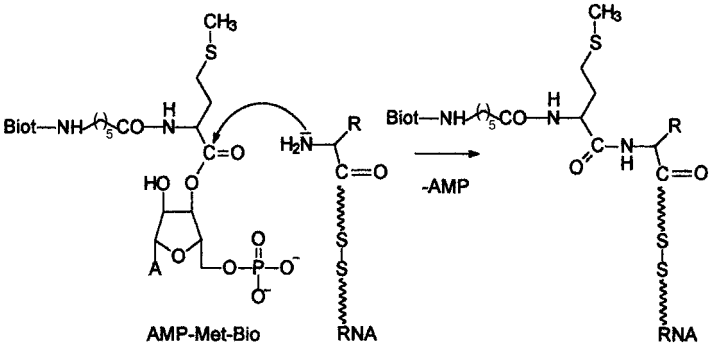


Scheme 3. a) Reaction step in the selection of an amide synthase. b) 5-[(5-Imidazolylethyl)carbamoyl]-UTP, which was used instead of uridine triphosphate (UTP) in enzymatic RNA synthesis.

and not only towards linker-coupled reactants? Are these molecules able to work with multiple turnover? What is the influence on the stereochemistry, especially in the case of the Diels–Alder reaction? Do different RNA catalysts lead to different products?

Zhang and Cech, too, used linker-coupled reactants in their search for RNA molecules that catalyze peptide bond formation.[11] RNA had already been shown to aminoacylate its own 3′-end and to transfer an RNA-coupled amino acid to another RNA molecule,[4a,b] now studies have been undertaken to establish whether RNA can catalyze the formation of a peptide bond similar to the ribosome.[12] The pool molecules were coupled to phenylalanine through a short linker harboring a disulfide bridge (Scheme 4; $R = CH_2C_6H_5$). The free amino group of phenylalanine was supposed to act as aminoacyl acceptor, and 2′(3′)-[6-(biotinylamido)caproyl-L-methionyl]adenosine-5′-monophosphate (AMP-Met-Bio), a simple model of natural *N*-formylmethionyl-tRNA, was used as free reactant and aminoacyl donor. Biotinylated RNA molecules were isolated on streptavidin matrices and subsequently released by reductive cleavage of the disulfide bridge. With this step, the products of side reactions were removed in which biotin got attached to internal positions of the RNA, since these species remained immobilized after cleavage of the disulfide bridge. After 19 rounds of selection, at least two classes of sequences were identified. The kinetic investigation of one RNA species revealed a rate acceleration of about 10^6 with respect to the uncatalyzed reaction and the requirement of high magnesium ion concentrations. The presence of the linker was essential for rate acceleration, presumably due to specific contacts between linker and RNA which were important for the exact positioning of the coupled reactant. In the free reactant, AMP but not biotin or the amino acid was involved in recognition. Therefore, other amino acids could be transferred to the acceptor from their respective AMP derivatives, too.



Scheme 4. RNA-catalyzed peptide bond formation. Biot–H = biotin.

Interestingly, Jenne and Famulok came to a completely different result using a very similar approach.[13] In their case, the linker-coupled amino acid was ignored: Instead of the desired peptidyl transferase activity only ribozymes were selected that catalyzed the transfer of the biotinylated amino acid from the acyl donor molecule to a certain internal 2′-hydroxyl group of the RNA. Presumably, the selection conditions used facilitated the enrichment of aminoacyl transferases, while the population of peptide-forming ribozymes was eliminated in the course of the selection. During the kinetic characterization, the originally selected ribozyme could be transformed into a true catalyst, catalyzing the aminoacylation of an external oligonucleotide in the presence of the acyl donor.

The described examples demonstrate that the catalytic capabilities of ribonucleic acids by far exceed modifying reactions at the functional groups present in RNA. The possibility of generating catalysts with controllable catalytic properties and definable specificity makes these techniques attractive beyond the field of prebiotic chemistry, especially since it is common practice to use a selected active sequence motif as starting point for other selections. It is therefore conceivable to use ribozymes in bioorganic or combinatorial syntheses. However, further studies are needed for the practical utilization of RNA catalysis for these purposes. The strategies described here but also alternative methods[14] may play an important role in this area.

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